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Biomimetic Synthesis of Macahydantoins A and B from *Lepidium meyenii*, and Structure Revision of Macahydantoin B as a Class of Thiohydantoin with a 4-Methyl-hexahydropyrrolo[1,2-*c*]imidazole Skeleton

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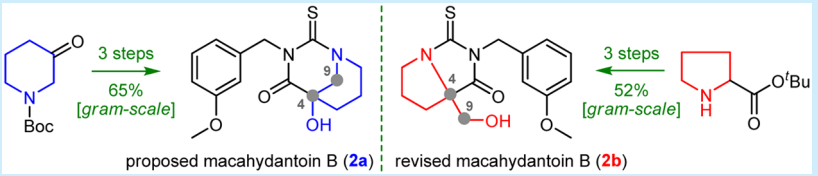
Supporting Information



ABSTRACT: Phytochemical investigation on *Lepidium meyenii* led to the discovery of macahydantoin C (**3**), a new thiohydantoin with a 1,3-diazabicyclo[3.3.1]nonane core, the spectral properties of which indicate a potential structural misassignment of its previously reported analogue, macahydantoin B (**2a**). To probe this hypothesis, a concise, scalable, and biomimetic synthesis of the originally proposed **2a** and its revised structure (**2b**) was efficiently accomplished using the modified Edman degradation as the key step from commercially available materials in 65% (three steps) and 52% (three steps) overall yields, respectively. These synthetic endeavors undoubtedly reassigned the structure of macahydantoin B as an unreported type of thiohydantoin featuring a 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole scaffold.

Recently, (±)-macahydantoins A (**1**) and B (reported **2a**), a novel class of thiohydantoins with a 1,3-diazabicyclo[3.3.1]-nonane core linking to a benzyl moiety, were both isolated as racemic mixtures from the roots of *Lepidium meyenii* by Qiu and co-workers (Figure 1).[1] Their structures, including absolute

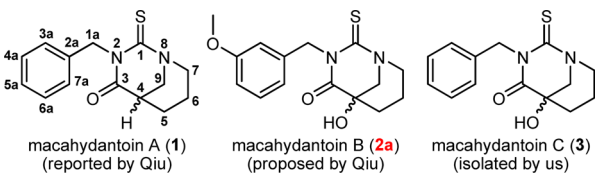


Figure 1. Structures of macahydantoins **1**, **2a**, and **3**.

configurations, were elucidated using a combination of NMR spectroscopy and ECD calculations. Moreover, macahydantoin A was further confirmed by a five-step synthesis route with an overall yield of 23% from benzylamine. According to the close spectroscopic data, Qiu's group suggested that the structure of **2a** was similar to that of **1**, except for the presence of an additional methoxy group located at C-4a and one more hydroxy group located at C-4, respectively. Interestingly, in our ongoing research for bioactive sulfur-containing derivatives from the roots of the title plant collected from the Yunnan province of China,[2] we also obtained a new analogue, macahydantoin C (**3**) (Figure 1). The only structural difference between the proposed **2a** with **3** is that **3** is lacking the additional methoxy group at C-4a.

However, the respective ^{13}C NMR signals belonging to their 1,3-diazabicyclo[3.3.1]nonane nucleus of **2a** and **3** were distinctly different, indicating a potential problem with one of the structures. Herein, we disclose the concise biomimetic synthesis of the originally proposed structure for macahydantoin B (**2a**) reported by Qiu and its revised structure (**2b**) (Figure 2). On the basis of these findings, the published **2a** with the 1,3-diazabicyclo[3.3.1]nonane skeleton has been revised to **2b** possessing an unreported 4-methyl-hexahydropyrrolo[1,2-*c*]-imidazole framework.

In this study, macahydantoin C (**3**), a new thiohydantoin with a previously reported 1,3-diazabicyclo[3.3.1]nonane framework,[1] was isolated as a racemic mixture from the lipidic fraction

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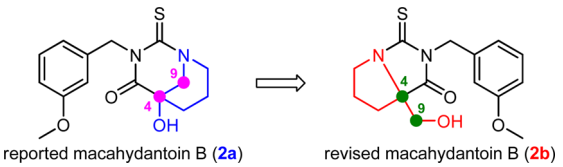
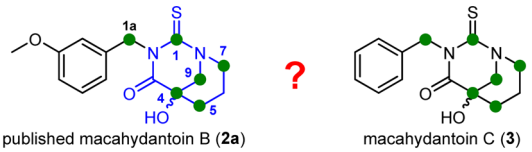


Figure 2. Structures of the published **2a** and revised **2b**.

of *Lepidium meyenii*. The absolute configurations of (+)-**3** and (−)-**3** were elucidated as 4*S* and 4*R* by comparison of their experimental and calculated ECD spectra, respectively (see Supporting Information, Figure S1). The only structural difference between **3** and **2a** was the lacking of a methoxy group at C-4a of the benzyl moiety of **3**. However, except for the signals of the 3-methoxybenzyl moiety and benzyl moiety in their ^{13}C NMR spectra, the chemical shifts belonging to the heterocyclic moiety were significantly different (chemical shift differences up to 9.6 ppm): especially for C-1 ($\Delta\delta$ = −3.9 ppm), C-4 ($\Delta\delta$ = +5.5 ppm), C-5 ($\Delta\delta$ = −6.7 ppm), C-7 ($\Delta\delta$ = −8.7 ppm), and C-9 ($\Delta\delta$ = +9.6 ppm) (Table 1). Thus, we concluded

Table 1. Comparison of the ^{13}C NMR Data of the Reported Structure 2a by Qiu and Our Isolated 3 in $CDCl_3$ (δ ppm)



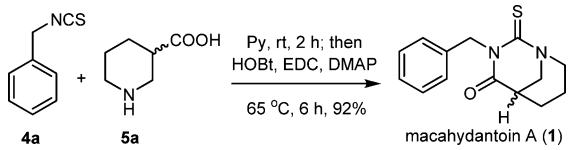
no.	2a	3	Δδ	no.	2a	3	Δδ
1	188.1 s	192.0 s	− 3.9	2a	137.0 s	136.6 s	+ 0.4
3	175.6 s	174.5 s	+ 1.1	3a	113.5 d	128.0 d	− 14.5
4	74.8 s	69.3 s	+ 5.5	4a	159.6 s	128.6 d	+ 31.0
5	27.8 t	34.5 t	− 6.7	5a	113.4 d	127.6 d	− 14.2
6	25.4 t	23.0 t	+ 2.4	6a	129.5 d	128.6 d	+ 0.9
7	48.6 t	57.3 t	− 8.7	7a	120.3 d	128.0 d	− 7.7
9	63.9 t	54.3 t	+ 9.6	OMe	55.2 q	-	-
1a	45.2 t	48.7 t	− 3.5				

that one of their structures could be misassigned. To tackle this problem, we first proceeded to design a concise biomimetic strategy to construct the 1,3-diazabicyclo[3.3.1]nonane nucleus.

Previously, Qiu's laboratory suggested that macahydantoins A (**1**) and B (reported structure, **2a**) might be biosynthetically derived from nicotinamide and benzoic acid derivative.[1] This hypothetical pathway involved a series of condensation, hydrogenation, thionation, and oxidation reactions. Considering the fact that natural isothiocyanates occur commonly in cruciferous plants,[3] we hypothesized that the 2-thioxo-1,3-diazabicyclo[3.3.1]nonan-4-one core of macahydantoins A−C could be more smoothly constructed by a simple Edman degradation reaction[2,4] between related isothiocyanates (**4a** or **4b**) with piperidine-3-carboxylic acid (**5a**) or 4-hydroxy-piperidine-3-carboxylic acid (**5b**) (Scheme 1).

Guided by this biosynthetic consideration, we have developed a gram-scale one-pot synthetic method (a single-step process, 92% yield) for the synthesis of macahydantoin A (**1**) using readily available starting materials, benzyl isothiocyanate (**4a**) and piperidine-3-carboxylic acid (**5a**) (Scheme 2). Briefly,

Scheme 2. One-Pot Method for Synthesizing Macahydantoin A



exposure of **4a** to **5a** in pyridine at room temperature for 2 h, followed by condensation with HOBt, EDC, and DMAP at 65 °C for an additional 6 h, provided the desired macahydantoin A (**1**), whose spectral data were identical in all respects to those for the natural substance.[1] This structure was further confirmed by a single crystal X-ray diffracton analysis (CCDC 1566874) (Figure 3).

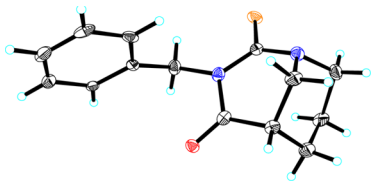
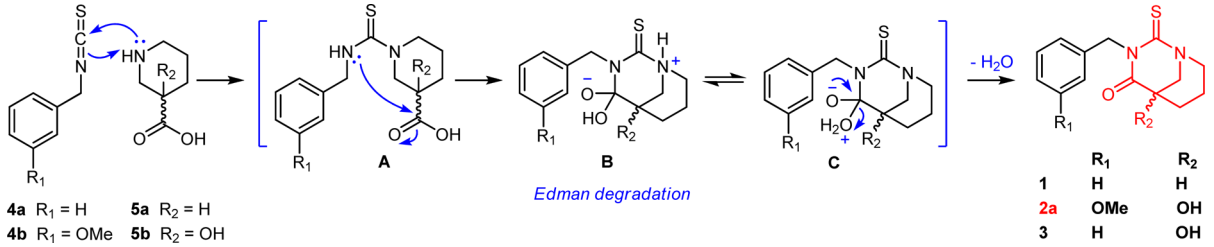


Figure 3. ORTEP diagram of synthetic **1**.

With the above-mentioned strategy in mind, we envisioned that the proposed **2a** would be incorporated from 3-methoxybenzyl isothiocyanate (**4b**) and 4-hydroxy-piperidine-3-carboxylic acid (**5b**) in a similar manner, as depicted in our retrosynthetic plan (Scheme 3). Further analysis indicated that the key intermediate **5b** could be readily accessible by acid hydrolysis of the cyano group from cyanohydrin **6**, which would in turn arise by nucleophilic addition of cyanide from commercially available Boc-protected piperidine-3-carboxylic acid (**7**).[5]

The preparation of the published **2a** is summarized in Scheme 4. Lewis acid-catalyzed nucleophilic addition of starting material **7** with trimethylsilyl cyanide (TMSCN) in DCM afforded cyanohydrin **6**, which was hydrolyzed in refluxing concentrated HCl at 85 °C for 12 h to give the desired hydroxy acid hydrochloride **5b**.[5] With the key intermediate **5b** in hand, attention was turned to the key condensation reaction. After

Scheme 1. Putative Biogenetic Pathway of Macahydantoins A (1), B (Reported Structure, 2a) and C (3)



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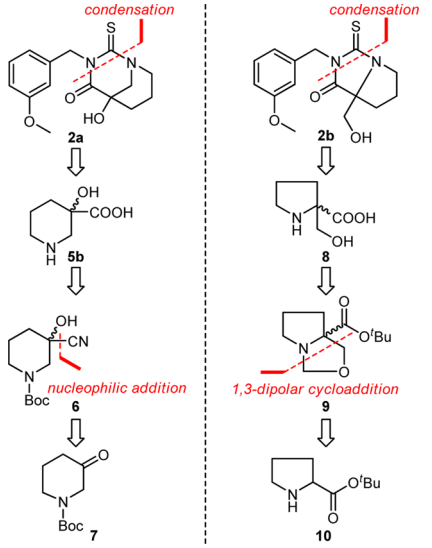
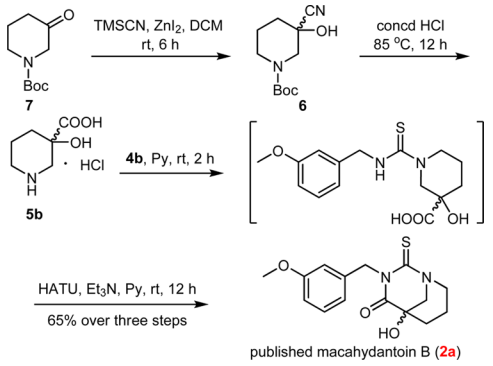
Scheme 3. Retrosynthetic Plan for the Reported 2a and Revised 2b



Scheme 4. Concise Biomimetic Synthesis of the Reported 2a



extensive optimization of the reaction conditions, we found that **5b** was successfully coupled with 3-methoxybenzyl isothiocyanate (**4b**) in the presence of HATU and Et_3N to provide the target compound **2a** on a gram scale (a three-step route, an overall yield of 65%).

As expected, the ^{13}C NMR spectrum of the synthetic **2a** exhibited noticeable similarity to those of the naturally occurring macahydantoin C (**3**). However, the synthetic **2a** has some odd inconsistent ^{13}C NMR data (chemical shift differences up to 9.6 ppm) compared to those of the published **2a** assigned by Qiu's group,[1] particularly for C-1 ($\Delta\delta$ = −3.9 ppm), C-4 ($\Delta\delta$ = +5.5 ppm), C-5 ($\Delta\delta$ = −6.6 ppm), C-7 ($\Delta\delta$ = −8.6 ppm), C-9 ($\Delta\delta$ = +9.6 ppm), and C-1a ($\Delta\delta$ = −3.6 ppm). Additionally, we further carefully reexamined the 2D NMR data of the published **2a** in the literature,[1] and observed the appearance of an unreasonable HMBC correlation from H_2-7 to C-4 (a four-bond correlation) and the absence of the key HMBC correlation from H_2-9 to C-1 (a three-bond correlation) (Figure 4). These observations led us to consider a radical structural revision for macahydantoin B as **2b**. Instead of the 1,3-diazabicyclo[3.3.1]nonane core as originally proposed, we hypothesized that a novel 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole skeleton (**2b**) was in fact the correct structure of the natural macahydantoin B.

To add further evidence in favor of this assumption, we set out to synthesize the postulated structure **2b**, the retrosynthetic analysis of which is shown in Scheme 3. We rationalized that the

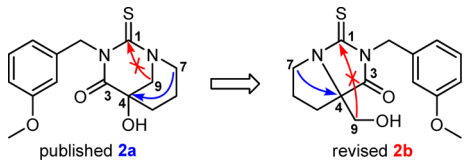
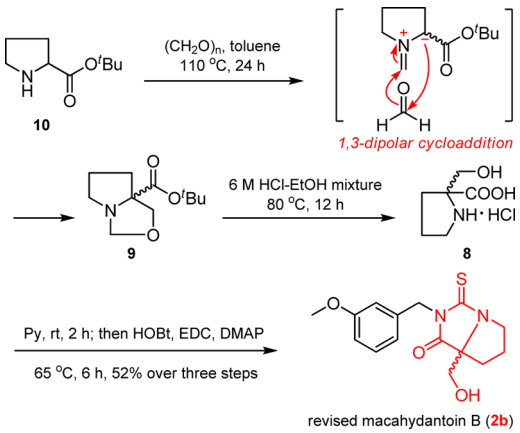


Figure 4. Reexamination of the key HMBC correlations in the published structure **2a** and the reassigned structure **2b**.

hexahydropyrrolo[1,2-*c*]imidazole core in **2b** would be available from α-hydroxymethylproline hydrochloride (**8**) and 3-methoxybenzyl isothiocyanate (**4b**) by means of modified Edman degradation.[2] In turn, intermediate **8** could be constructed from bicyclic hexahydropyrrolo[1,2-c]oxazole derivative (**9**), which might be readily prepared by a 1,3-dipolar cycloaddition between commercially available proline *t*-butyl ester (**10**) and paraformaldehyde.[6]

Initially, a racemic mixture of proline *t*-butyl ester (**10**) was treated with paraformaldehyde in toluene at 110 °C to afford bicyclic hexahydropyrrolo[1,2-c]oxazole derivative (**9**) in approximately 90% isolated yield, which was subsequently transformed into α-hydroxymethylproline hydrochloride **8** by acid hydrolysis in a refluxing 6 M HCl−EtOH mixture (Scheme 5).[5] Without further purification, HOBt/EDC/DMAP coupling

Scheme 5. Concise Biomimetic Synthesis of the Revised 2b



of the key intermediate **8** with 3-methoxybenzyl isothiocyanate **4b** under mild conditions furnished the presumed (±)-macahydantoin B (**2b**) on a gram scale (a three-step route, an overall yield of 52%) (Scheme 5).

To our delight, the NMR spectral data of the revised structure **2b** were in full agreement with those reported for the natural (±)-macahydantoin B (Figure 5).[1] On the basis of this positive result, the absolute configurations of its enantiomers (+)-macahydantoin B and (−)-macahydantoin B were unambiguously established as 4*S* and 4*R* by computational evidence, respectively (see Supporting Information, Figure S1). This evidence strongly corroborates the hypothesis that the natural (±)-macahydantoin B represents a new class of thiohydantoins with an unique 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole skeleton. Biosynthetically, this novel nucleus might be formed by a combination of Edman degradation[4] and Aldol condensation[7] from the related proline (**11**) and isothiocyanate **4b** (Scheme 6).

Compounds **1**, **2a**, **2b**, and **3** were tested for their cytotoxic activity against a panel of human cancer cell lines using the MTT method.[8] However, no significant activities were detected for these compounds at the concentrations up to 40 μM.

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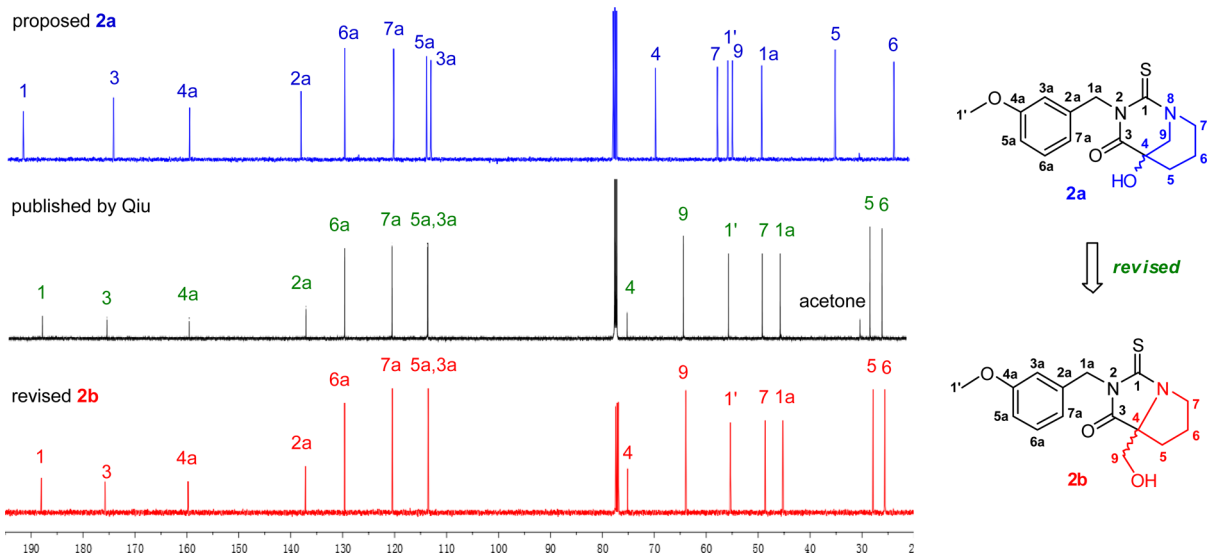
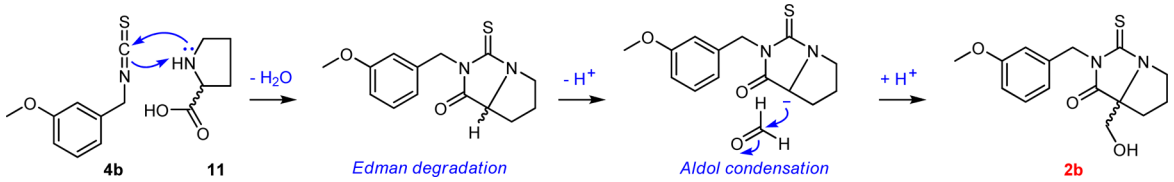


Figure 5. Comparison of the ^{13}C NMR spectra of the synthetic **2a**, the published **2**, and the synthetic **2b** in $CDCl_3$ (δ ppm).

Scheme 6. Putative Biogenetic Pathway of Macahydantoin B (Revised Structure, 2b)



In summary, a concise, scalable, and biomimetic synthesis of the originally proposed structure for macahydantoin B (**2a**) and its revised structure **2b** has been efficiently achieved using commercially available materials in 65% (three steps) and 52% (three steps) overall yields, respectively. The key transformations include a 1,3-dipolar cycloaddition and an modified Edman degradation process. On the basis of their spectral properties, we strongly suggest that the published **2a** with a 1,3-diazabicyclo[3.3.1]nonane core should be revised as **2b** possessing a novel 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole scaffold. We are investigating the biological function of the novel molecules. These results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02433.

Experimental procedures, spectral and other characterization data of isolated or synthetic compounds (PDF)

X-ray data for **1** (CIF)

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Notes

The authors declare no competing financial interest.

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