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Unified Total Synthesis of Five Bufadienolides

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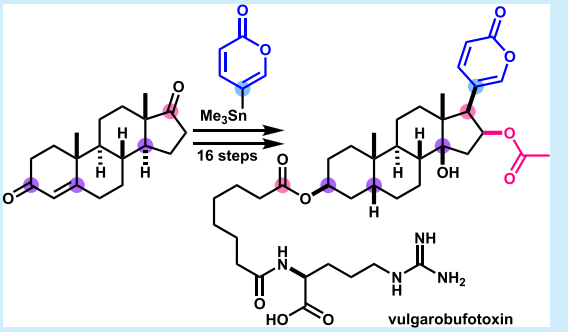
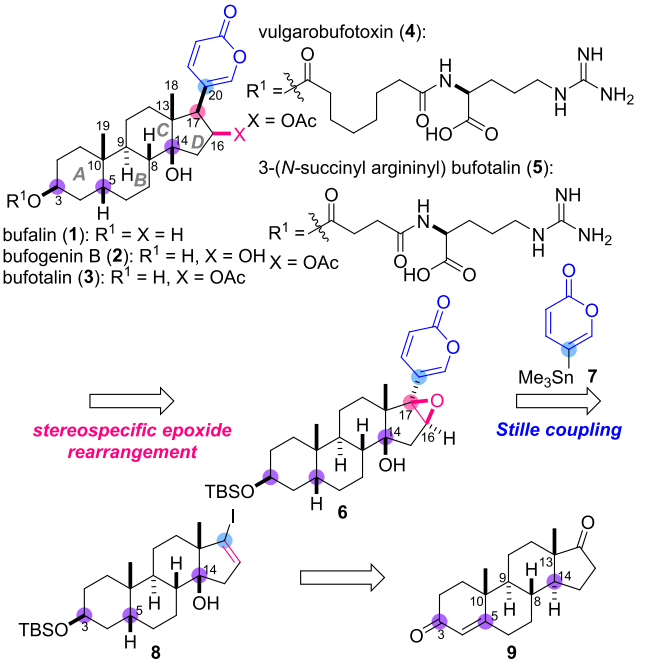
ABSTRACT: We report a unified total synthesis of five bufadienolides: bufalin (**1**), bufogenin B (**2**), bufotalin (**3**), vulgarobufotoxin (**4**), and 3-(*N*-succinyl argininyl) bufotalin (**5**). After the steroidal ABCD ring **8** was produced, the D ring was cross-coupled with a 2-pyrone moiety and stereoselectively epoxidized to generate **6**. TMSOTf promoted a stereospecific 1,2-hydride shift from **6** to establish the β-oriented 2-pyrone of **19**. Functional group manipulations from **19** furnished **1**−**5**, which potently inhibited cancer cell growth.



Bufadienolides have been identified in both animals and plants over the years, comprising a large group of steroidal natural products (e.g., **1**−**5**; Scheme 1).[1] These compounds are known as the bioactive ingredients of the traditional Chinese drug Chansu. Chansu is a dried skin gland secretion of toads and has been used for hundreds of years to treat various diseases, including heart failure, cancer, inflammation, and respiratory infections. Recent biological studies confirmed the significant anticancer activities of bufadienolides in vitro and in vivo.[2]

Scheme 1. Structures of Bufalin (1), Bufogenin B (2), Bufotalin (3), Vulgarobufotoxin (4), and 3-(*N*-Succinyl argininyl) Bufotalin (5), and the Synthetic Plan for the Five Bufadienolides



Bufadienolides are structurally related to cardenolides, another steroid family with potent cardiotonic effects.[3] Both of these steroid family members share atypical *cis*-fused A/B and C/D ring systems but differ in the β-oriented heterocyclic ring substituted at C17. A five-membered unsaturated γ-butyrolactone (butenolide) in cardenolides is replaced with a six-membered doubly unsaturated δ-valerolactone (2-pyrone) in bufadienolides. The complex structures of bufadienolides and their high potential as anticancer agents have attracted the widespread attention of synthetic organic chemists. However, while modern and efficient routes to cardenolides have been recently developed,[4,5] total syntheses of bufadienolides were reported only in the 1970s and 1980s.[6,7] Construction of the 2-pyrone ring in these syntheses involves tedious and harsh stepwise manipulations from a linear or saturated precursor. Direct introduction of the 2-pyrone moiety would streamline the synthesis, but this has yet to be achieved, reflecting the synthetic challenge posed by bufadienolide structures. Herein we devised a new method for installing the C17β-attached 2-pyrone employing direct Stille coupling of the pyrone, stereoselective epoxidation, and stereospecific epoxide rearrangement. These key transformations efficiently assembled a

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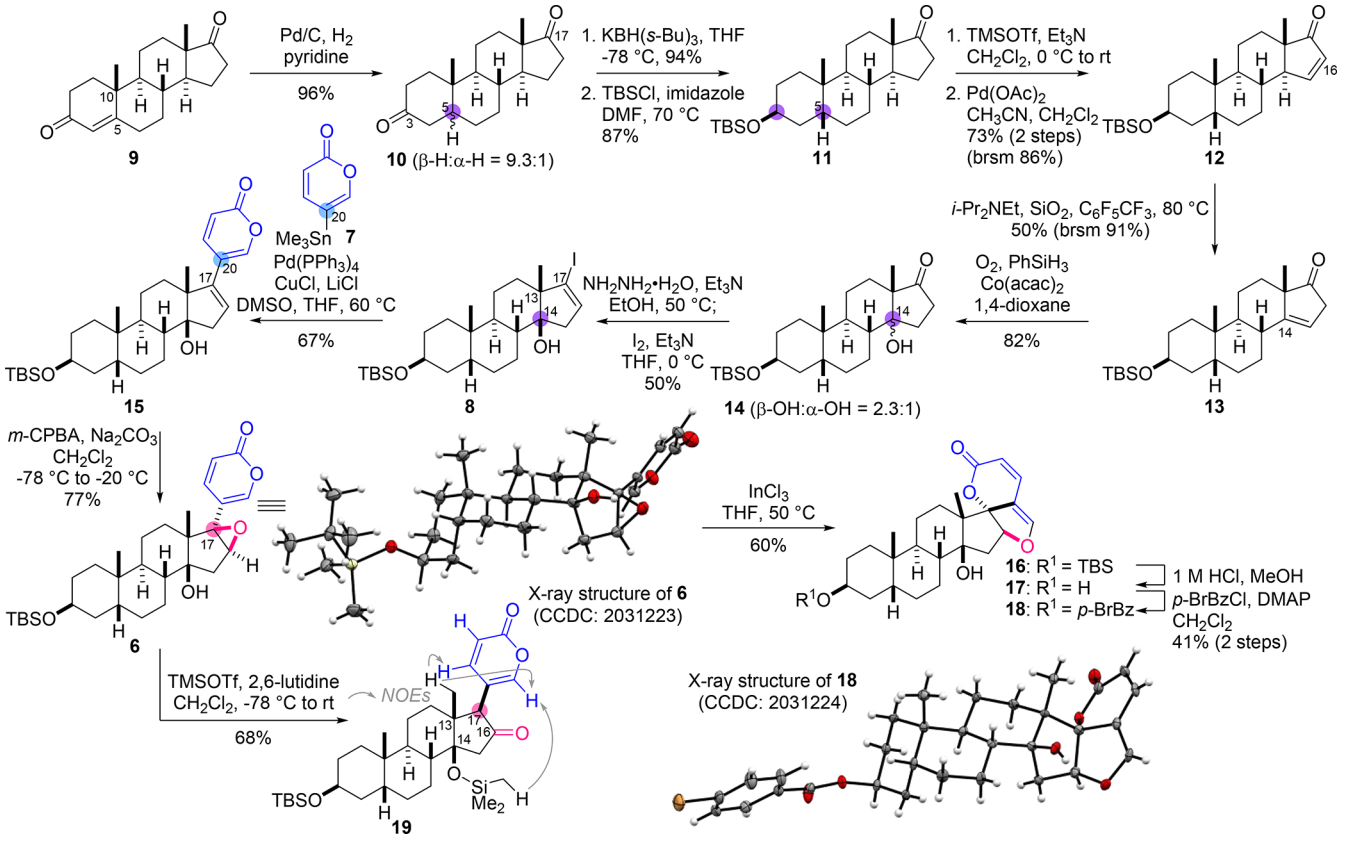
Scheme 2. Construction of C17β-2-Pyrone 19



highly functionalized intermediate that was utilized for a unified total synthesis of the five bufadienolides **1**−**5**.[8] The synthesized steroids were subjected to growth inhibition assays against MCF-7 human breast cancer cells to investigate their activities.[9]

Our synthetic plan for the chemical construction of **1**−**5** is illustrated in Scheme 1. Bufalin (**1**)[10] possesses C3- and C14-hydroxy groups, and bufogenin B (**2**)[11] has an additional C16-OH on the D ring. Bufotalin (**3**)[12] is C16O-acetylated **2**, while vulgarobufotoxin (**4**)[13] and 3-(*N*-succinyl argininyl) bufotalin (**5**)[14] are the *N*-suberyl arginine and *N*-succinyl arginine esters of **3**, respectively. Considering the structural relationship of the targets, we planned to prepare an appropriately protected form of **2** as a common intermediate. This intermediate would then be derivatized to **1** via C16-deoxygenation and to **3**−**5** through acylation. Protected **2** was in turn retrosynthetically converted to β-epoxide **6**. In the synthetic direction, a Lewis acid would be expected to promote a stereospecific 1,2-hydride shift[15] to control the β-orientation of the 2-pyrone structure. Compound **6** would be synthesized through Stille coupling[16] between the known 2-pyrone unit **7**[17] and tetracycle **8**, followed by stereoselective epoxidation. Thus, commercially available 4-androstene-3,17-dione (**9**) was selected as a starting material. As **9** has the four correct stereocenters of bufadienolides (C8,9,10,13), **9** would be readily altered into the AB-*cis*/CD-*cis* ring skeleton **8** by introducing C3β-OH, C5β-H, and C14β-OH.

First, the preparation of tetracycle **8** started with functionalization of the A ring of **9** (Scheme 2). The C5-double bond of **9** was stereoselectively hydrogenated from the opposite face of the C10-methyl group by catalysis of Pd/C in pyridine, providing the *cis*-fused AB ring **10** as a major diastereomer (β-H:α-H at C5 = 9.3:1).[18] The C3-ketone of **10** was stereoselectively reduced in the presence of the C17-ketone by the action of the bulky reductant KBH(*s*-Bu)$_3$.[19] The resultant C3-OH was protected as its *tert*-butyldimethylsilyl (TBS) ether to generate **11** after separating the diastereomers. The *cis*-fused CD ring was then built according to the protocol developed by Baran.[5b] Trimethylsilyl (TMS) enol ether formation and subsequent Ito−Saegusa oxidation[20] with Pd(OAc)$_2$ transformed ketone **11** to the α,β-unsaturated ketone **12**. The conjugated C16-olefin of **12** was isomerized to the more substituted nonconjugated C14-olefin of **13**[21] by employing *i*-Pr$_2$NEt and SiO$_2$ in $C_6F_5CF_3$. A Co(acac)$_2$-catalyzed Mukaiyama hydration[22] of olefin **13** using O$_2$ and PhSiH$_3$ stereoselectively introduced the requisite β-configured C14 tertiary alcohol of **14** (β-OH:α-OH at C14 = 2.3:1). To prepare for the Stille coupling reaction, the C17-ketone of **14** was changed to the corresponding vinyl iodide of **8** through sequential treatment with NH_2NH_2 and then with I_2 and Et_3N.[23]

A Stille reaction and subsequent epoxidation successfully functionalized the sterically shielded C17 position proximal to the C13,14-tetrasubstituted carbons. When **8** was treated with **7** (3.3 equiv) in the presence of Pd(PPh$_3$)$_4$ (0.5 equiv), CuCl (5.0 equiv), and LiCl (6.0 equiv) in dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) at 60 °C,[4,24] the C(sp^2)−C(sp^2) bond was formed to furnish adduct **15**, thereby directly introducing the 2-pyrone moiety to the ABCD ring. Upon using *m*-chloroperoxybenzoic acid (*m*-CPBA) with the acid scavenger Na_2CO_3 in CH_2Cl_2 at −20 °C, the most electron rich C17-double bond of **15** was epoxidized chemoselectively over the potentially more exposed diene of the 2-pyrone ring. The reaction proceeded from the convex β-

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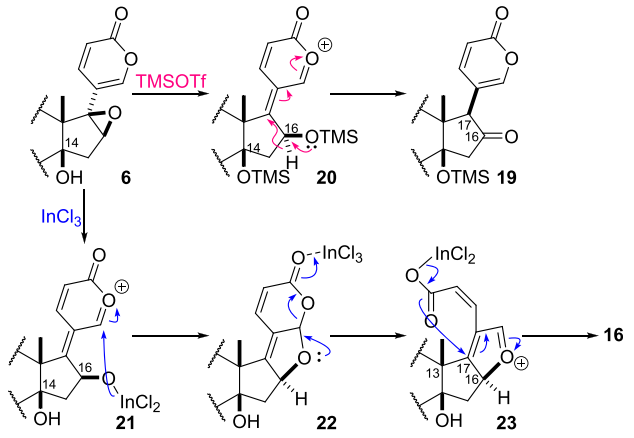
face of the *cis*-fused CD ring, giving rise to pyrone-attached epoxide **6** as a single diastereomer. The X-ray crystallographic analysis of **6** uncovered the entire stereostructure, showing the U shape of the steroidal skeleton and the α-configured 2-pyrone at the hindered C17 position.

The epoxide rearrangement from **6** to **19** was not easily accomplished because of the unusual reactivity of 2-pyrone. The conditions greatly affected the reaction outcome. For example, $InCl_3$ activated the epoxide of **6**, but the desired product **19** was not detected. The major compound was fused-hexacycle **16**, which was isolated in 60% yield. Compound **16** was derivatized into *p*-bromobenzoate **18**, whose structure was determined by X-ray crystallographic analysis. After the reagents were screened, the combination of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and 2,6-lutidine[25] was found to induce the requisite rearrangement. TMSOTf (2.4 equiv) and 2,6-lutidine (5.1 equiv) in CH_2Cl_2 promoted the C14O-TMS ether formation and the 1,2-hydride shift from **6**, leading to **19**. The β-orientation of all the substituents at C13, C14, and C17 of **19** was confirmed by nuclear Overhauser effect (NOE) correlations. Consequently, the three-step transformations under mild conditions constructed the densely substituted D ring structure without affecting the acid-sensitive C14-tertiary alcohol.

A plausible mechanism of the two distinct pathways is depicted in Scheme 3. The $InCl_3$-induced opening of the

Scheme 3. Plausible Mechanism of Lewis Acid-Mediated Reactions of Epoxide 6



oxirane ring is assisted by electron donation from the oxygen lone pair of the 2-pyrone ring, producing cation **21**. The nucleophilic indium alkoxide in **21** attacks the electrophilic oxocarbenium ion to afford the chemically unstable acetal **22**. Further activation of **22** with $InCl_3$ cleaves the acetal, and the ejected carboxylate of **23** adds to the C17 position from the opposite face of the C13-methyl group, providing the rigid hexacycle **16**. On the other hand, TMSOTf and 2,6-lutidine activate the oxirane ring and cap both C14- and C16-alcohols with TMS groups. Introduction of the bulky and electron-donating C16O-TMS group of **20** decelerates the nucleophilic attack on the oxocarbenium ion and accelerates the 1,2-hydride shift. Hence, the α-oriented C16-hydride stereospecifically adds to the C17 position to invert the C17 stereoconfiguration, thereby rendering the β-configured 2-pyrone of **19**.

The common intermediate **24** was prepared for the total synthesis of bufadienolides **1**−**5** (Scheme 4). Since the C14O-TMS group of **19** that was introduced at the prior step shielded the β-face, $NaBH_4$ reduction occurred from an opposite side, stereoselectively leading to C16β-alcohol **24**. The two silyl protecting groups of **24** were then removed using HF·pyridine in pyridine and THF to provide bufogenin B (**2**). Alternatively, the free C16-alcohol of **24** was acetylated before the HF·pyridine-mediated deprotection, giving rise to bufotalin (**3**).

Reductive removal of the C16-alcohol of **24** in turn produced bufalin (**1**). Application of the typical Barton−McCombie-type deoxygenation to **24** was unsuccessful, presumably due to the unwanted participation of 2-pyrone in the reaction. Accordingly, alcohol **24** was subjected to a neutral halogenation/dehalogenation process. CBr_4, PPh_3, and imidazole[26] transformed **24** to the desired bromide **26** and the undesired olefin **27** via an S_N2 reaction and an E2 elimination, respectively. Compounds **26** and **27** were together subjected to radical deoxygenation conditions using Et_3B and *n*-Bu_3SnH under an O_2 atmosphere. In one pot, aqueous HCl and MeOH were added for deprotection to afford **1** along with the byproduct **28**.[27]

Finally, esterification of the C3-hydroxy group of bufotalin (**3**) delivered vulgarobufotoxin (**4**) and 3-(*N*-succinyl argininyl) bufotalin (**5**). Argininyl suberic acid **29a** and argininyl succinic acid **29b** were prepared as the protected forms: the carboxylic acid and the guanidine were capped with the *t*-Bu group and the 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) group,[28] respectively. C3-alcohol **3** was conjugated with the carboxylic acid of **29a**/**29b** using *N*,*N*-dimethyl-4-aminopyridine (DMAP) and *N*,*N*′-diisopropylcarbodiimide (DIC) to form **30a**/**30b**. Removal of the *t*-Bu and Pbf groups was realized by applying a mixture of CF_3CO_2H, PhOMe, PhSMe, and $HSCH_2CH_2SH$ in CH_2Cl_2,[29] leading to **4**/**5**.[30]

The total synthesis of bufadienolides **1**−**5** permitted us to study their structure−activity relationship (SAR).[31] The cell growth inhibitory activities of **1**−**5** and the byproduct C17-olefin **28** were assessed against MCF-7 human breast cancer cells using the sulforhodamine B assay (Table 1).[4c,32] Among the six tested compounds, **1** with no C16-oxygen functional group exhibited the highest activity (50% growth inhibitory concentration (GI_{50}) = 13.3 nM). C16-OH **2** and C16-OAc **3** were 4.1-fold and 2.1-fold weaker than **1** while they retained two-digit nanomolar activity. The three data suggested that the C16-hydroxy group had an unfavorable effect on the potency. The GI_{50} values of C3O-acylated **4** and **5** were at least 10-fold larger than that of C3O-nonacylated **3**, corroborating that their C3O-acyl chains negatively influenced the activity. The least potent compound was **28**, which is the C16,17-didehydro analogue of the most potent compound **1**. The 180-fold weaker activity (2390 nM) of **28** in comparison with **1** confirmed the biological significance of the β-orientation of the 2-pyrone group.

In summary, we accomplished a unified total synthesis of the 5 bufadienolides **1**−**5** in 13−16 steps from the commercially available steroid **9**. The two sequences of transformations played crucial roles in the syntheses. C5-hydrogenation, C3-reduction, and C14-hydroxylation constructed the AB-*cis*/CD-*cis* ring system of **8**, while Stille coupling, stereoselective epoxidation, and a stereospecific TMSOTf-mediated 1,2-hydride shift installed the β-oriented 2-pyrone of **19**. The following stereoselective hydride addition produced **24**, which served as the common intermediate for the total synthesis of

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Scheme 4. Total Synthesis of Bufogenin B (2), Bufotalin (3), Bufalin (1), Vulgarobufotoxin (4), and 3-(*N*-Succinyl argininyl) Bufotalin (5)

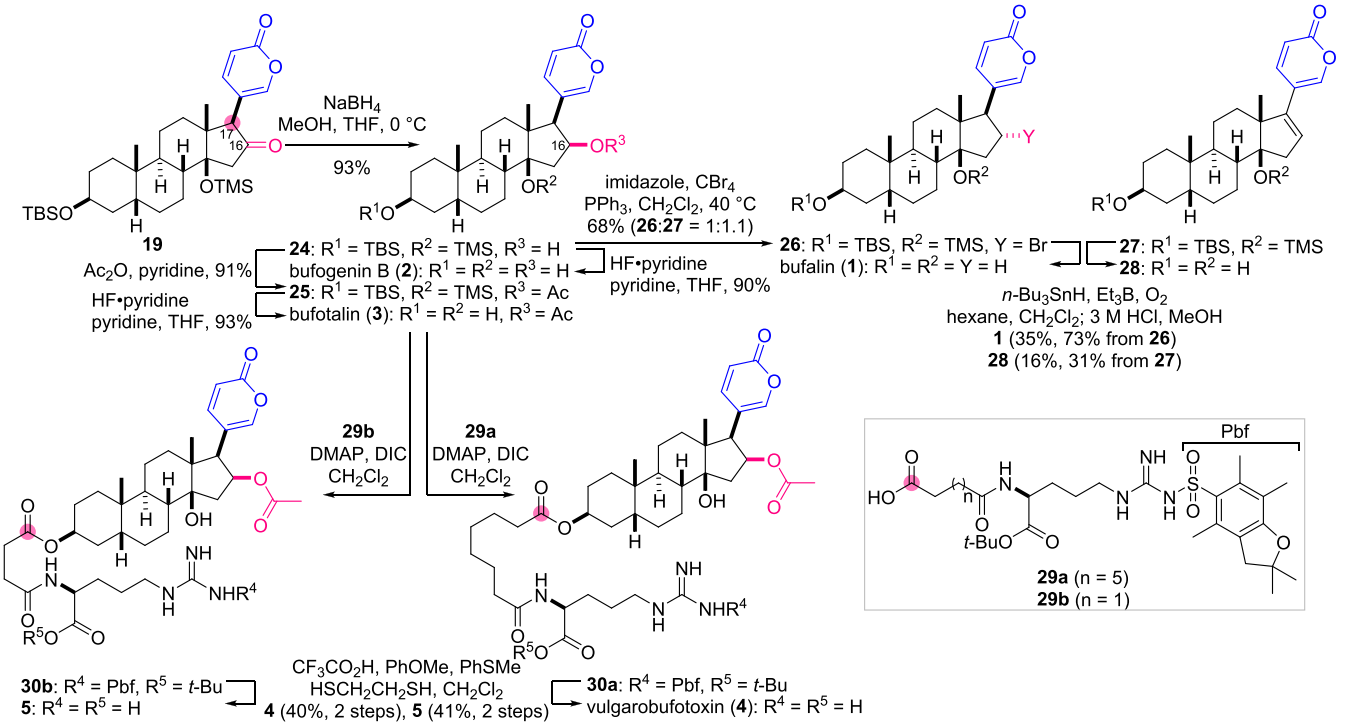

Table 1. Growth Inhibitory Activity of 1−5 and 28 against MCF-7 Human Breast Cancer Cells

compound	GI_{50} (nM)[a]
1	13.3 ± 1.8
2	55.0 ± 5.7
3	28.2 ± 3.9
4	301 ± 36
5	1190 ± 340
28	2390 ± 670

[a]Three independent experiments were performed (mean ± SD).

1−5. The SAR study of **1−5** and **28** revealed the importance of the hydrophobic functionality at C16, the free alcohol at C3, and the *β*-oriented 2-pyrone at C17 for the strong growth inhibitory activity against MCF-7 cells. As this newly developed method for attaching the 2-pyrone moiety is mild and efficient, it should be applicable to a wide range of structurally different bufadienolides with potent anticancer activities.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c03251.

Experimental procedures, characterization data, X-ray crystallographic data of **6** and **18**, and NMR data of all newly synthesized compounds (PDF)

Accession Codes

CCDC 2031223−2031224 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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providing us with ^{1}H and ^{13}C NMR spectra of 3-(*N*-succinyl argininyl) bufotalin (**5**).

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