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Graphical Abstract

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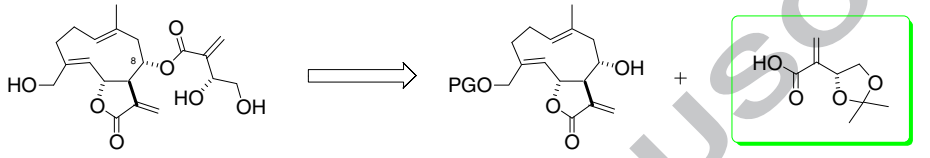

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Synthetic study of cnicin: Synthesis of the side chain and its esterification

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ABSTRACT

Cnicin is a germacranolide sesquiterpene lactone that possesses potent inhibitory activity against the protozoan parasite *Trypanosoma brucei*, which causes human African trypanosomiasis (HAT). Although cnicin has an interesting structure and attractive biological activity, synthetic studies of cnicin have not yet been reported. This report describes the synthesis of the protected side chain carboxylic acid moiety at C8 of cnicin *via* two routes starting from L-ascorbic acid. In addition, esterification between the synthetic side chain and salonitenolide derivative, which can be achieved *via* hydrolysis of cnicin and protection of the primary alcohol, was conducted. Thus, a semi-synthesis of cnicin was achieved.

1. Introduction

Cnicin (**1**, Figure 1) was first isolated from the leaves and stalks of *Cnicus benedictus* by Šorm and co-workers in 1959.[1] As a germacranolide sesquiterpene lactone, **1** has a 10-membered ring diene and a fused five-membered lactone with an ester side chain at the C8 position. Cnicin **1** possesses a variety of biological and pharmacological activities, such as allelopathy,[2] cytotoxicity, anti-bacterial properties,[3] anti-myeloma activity,[4] and cytostatic activity.[5] Despite the useful properties of **1**, synthetic studies have not yet been reported.

Figure 1. Structure of cnicin **1** (carbon numbering of **1** adapted from Ref. 6).

In 2014, cnicin **1** was reported to exhibit inhibitory activity against *Trypanosoma brucei* (IC_{50} = 0.4 μM),[7,8] the parasite responsible for human African trypanosomiasis (HAT).[9] HAT, also known as sleeping sickness, is transmitted by blood-feeding tsetse flies in sub-Saharan Africa, and is fatal if left untreated. The disease occurs in two stages: an initial hemolymphatic stage, followed by an encephalitic second stage characterized by invasion of the parasite into the central nervous system, which causes breakdown of neurological functions.[10] The arsenic-based melarsoprol is the only drug available for the treatment of second-stage East African HAT, and is associated with potentially lethal side effects.[11]

Although the antitrypanosomal activity of cnicin **1** is less potent than that of melarsoprol (IC_{50} = 0.01 μM),[6] the total synthesis and a structure-activity relationship (SAR) study of **1** are necessary to aid the development of a more effective drug for HAT. The present report describes the synthesis of the chiral side chain moiety, which may serve as an advanced intermediate in the subsequent total synthesis, and esterification of the 10-membered ring main core and the synthetic side chain for the semi-synthesis of **1**.

2. Results and discussion

As illustrated in Scheme 1, cnicin **1** could be retrosynthetically divided into two parts through esterification. The main core **2** could be obtained *via* hydrolysis of **1**, isolated from *Cnicus benedictus*, followed by triisopropylsilyl (TIPS) protection of the primary alcohol. The side chain moiety **3** with a protecting group would be prepared *via* methyl ester **4**, which can be led from the starting material L-ascorbic acid **5**.[12]

Our synthesis commenced with the preparation of the methyl ester **4** (Scheme 2). Protection of the alkyl diol in **5** with acetonide was achieved by treatment with acetyl chloride (AcCl) in acetone *via* acetonide formation to give **6** in 91% yield.[13] Compound **6** was then converted into the methyl ester **4** in 67% yield in one pot.[14] In the first step, the lactone moiety of **6** was hydrolyzed using 30% NaOH solution, followed by oxidative cleavage using 30% hydrogen peroxide (H_2O_2) and sodium hydrogen carbonate ($NaHCO_3$) to afford carboxylate intermediate **6'**. Subsequent methylation of **6'** using dimethyl sulfate (Me_2SO_4), sodium sulfite (Na_2SO_3), and $NaHCO_3$ gave alcohol **4**.

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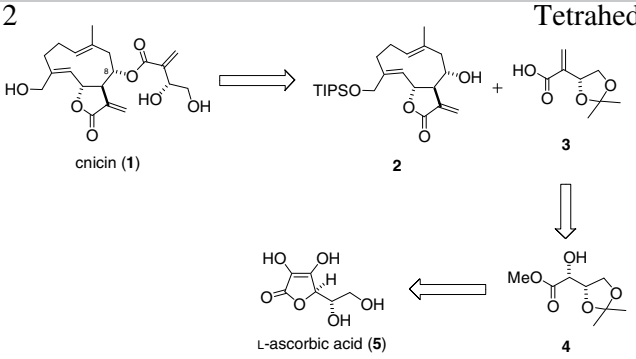
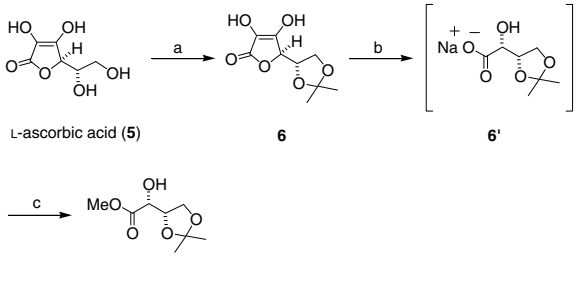
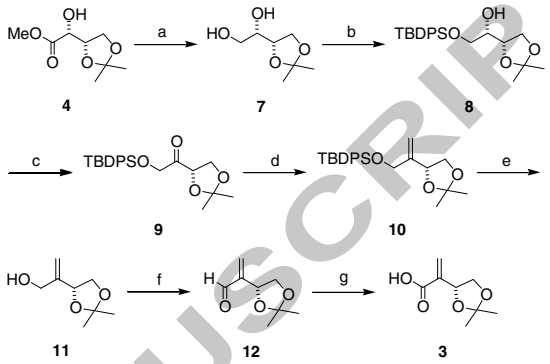


Scheme 1. Retrosynthesis of **1**.



Scheme 2. Synthesis of methyl ester **4**. Reagents and conditions: (a) acetone, AcCl, rt to 3.5 h, then 5 ℃, 17 h, 91%; (b) 30% NaOH, 30% H_2O_2, $NaHCO_3$, H_2O, rt, 1 h; (c) Me_2SO_4, Na_2SO_3, $NaHCO_3$, 40 ℃, 5 h, 67%.

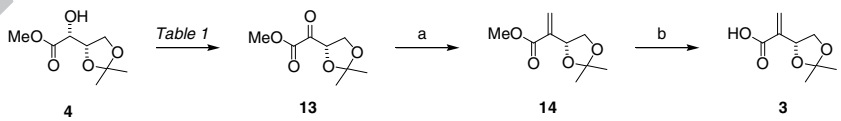
Scheme 3 shows the synthesis of **3** starting from methyl ester **4**. Compound **4** was converted into diol **7** in 62% yield *via* reduction with lithium aluminum hydride (LAH) in tetrahydrofuran (THF). Subsequently, selective protection of the primary alcohol of **7** with *t*-butyldiphenylsilyl chloride (TBDPSCl) in *N*,*N*-dimethylformamide (DMF) gave alcohol **8** in 83% yield. Oxidation of **8** with 2-azaadamantane-*N*-oxyl (AZADO), sodium hypochlorite (NaOCl), potassium bromide (KBr), and tetrabutylammonium bromide (TBAB) then afforded ketone **9** in 96% yield.[15] The resulting ketone **9** was then converted into exo-olefin **10** in 90% yield *via* Wittig reaction with methyltriphenylphosphonium bromide (Ph_3PCH_3Br) and *n*-butyllithium (*n*BuLi). Removal of the TBDPS group of **10** was achieved using tetrabutylammonium fluoride (TBAF) in THF to produce alcohol **11** in 84% yield. Compound **11** was then oxidized with manganese dioxide (MnO_2) to give aldehyde **12**. Kraus-Pinnick oxidation[16] of **12** was conducted using 1-methyl-2-butene, sodium dihydrogenphosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), and sodium perchlorate ($NaClO_4$) in a 3:2 mixture of *t*-butanol (*t*BuOH) and H_2O to give the desired carboxylic acid **3** in 69% yield over two steps.[17] Thus, a protected side chain was prepared from L-ascorbic acid **5** over nine steps in 12% overall yield using this synthesis.



Scheme 3. Synthesis of carboxylic acid **3**. Reagents and conditions: (a) $LiAlH_4$, THF, 0 ℃ to rt, 1 h, 62%; (b) TBDPSCl, imidazole, DMF, rt, 17 h, 83%; (c) AZADO, NaOCl, KBr, TBAB, CH_2Cl_2, $NaHCO_3$ aq, 0 ℃, 30 min, 96%; (d) Ph_3PCH_3Br, *n*BuLi, THF, -78 ℃ to 0 ℃, 2 h, then rt, 2.5 h, 90%; (e) TBAF, THF, rt, 45 min, 84%; (f) MnO_2, CH_2Cl_2, rt, 6.5 h; (g) 2-methyl-2-butene, $NaH_2PO_4{\cdot}2H_2O$, $NaClO_2$, *t*BuOH/H_2O (3:2), rt, 3 h, 69% (two steps).

While these results were satisfactory, obtaining the desired carboxylic acid **3** in greater yield and fewer steps, starting from methyl ester 4, was of interest. In this revised synthetic strategy, direct oxidation of 4 was conducted using Swern oxidation (entry 1), tetrapropylammonium perruthenate (TPAP, entry 2), Dess-Martin periodinane (DMP, entry 3), AZADO (entry 4), and pyridinium chlorochromate (PCC, entry 5), as shown in Table 1. However, a satisfactory yield of ketone 13 with satisfactory purity could not be obtained in any of these cases. Therefore, Wittig olefination of crude ketoester **13** was conducted to afford the desired ester **14**. In addition, oxidation of methyl ester **4** by PCC, followed by Wittig reaction, gave **14** in 27% yield over two steps. Despite the effort of the oxidation, the use of PCC gave the best yield. The low yield of this oxidation was probably due to the steric effects caused by the αβ-diol with both the acetonide and methyl ester.

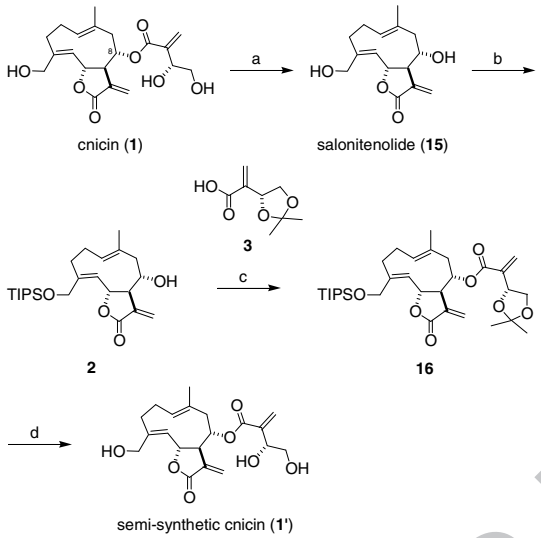
Table 1. Synthesis of **3** via **13** and **14** from methyl ester **4**. Reagents and conditions: (a) Ph_3PCH_3Br, nBuLi, THF, –78 ℃ to rt, 1.5 h, then rt, 3 h; (b) LiOH, THF/H_2O (1:1), rt, 24 h, 95%.



Entry	Conditions	Yield from **4** to **14** (two steps, %)
1	DMSO, $(COCl)_2$, Et_3N, –78 ℃ to 0 ℃, 1.5 h	0
2	TPAP, NMO, MS 4Å, CH_2Cl_2, 0 ℃ to rt, 18 h	0
3	DMP, CH_2Cl_2, rt, 5.5 h	8
4	AZADO, NaOCl, KBr, TBAB, CH_2Cl_2, $NaHCO_3$ aq., 0 ℃, 20 min	11
5	PCC, MS 4Å, CH_2Cl_2, rt, 16 h	27

The obtained **14** was then hydrolyzed with lithium hydroxide (LiOH) to give the desired **3** in 95% yield. For this route, the protected side chain was prepared from L-ascorbic acid over five steps in 16% overall yield, which involved fewer steps and slightly better yield than the previous synthetic route.

Next, esterification between the synthetic side chain moiety and the salonitenolide derivative was investigated (Scheme 4). For the preparation of the salonitenolide derivative, isolation of cnicin **1** was carried out from *Cnicus benedictus* according to the literature procedure.[8a,18] Hydrolysis of **1** using 0.1 N sodium carbonate (Na_2CO_3) was then conducted at room temperature for 16 h to afford salonitenolide[19] **15** in 51% yield.[20] Then, treatment of **15** with TIPSCl, imidazole, and DMAP at 0 ℃ for 2 h afforded TIPS-protected salonitenolide derivative **2** in 63% yield.



Scheme 4. Synthesis of semi-synthetic cnicin (**1'**). Reagents and conditions: (a) 0.1 N Na_2CO_3 aq, dioxane/H_2O (2:1), rt, 16 h, 51%; (b) TIPSCl, imidazole, DMAP, CH_2Cl_2, 0 ℃, 2 h, 63%. (c) EDC, DMAP, CH_2Cl_2, rt, 24 h, 10%; (d) 1N HCl, CH_2Cl_2, rt, 28 h, 33%.

Segments for semi-synthesis of **1** were prepared successfully. Esterification of TIPS-protected salonitenolide derivative **2** and the synthetic side chain **3** using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) was conducted at room temperature for 24 h to afford **16** in 10% yield. The low yield was probably due to an unexpected side reaction and/or steric hindrance around C8. Removal of TIPS and acetonide protection by 1 N HCl at room temperature for 28 h afforded semi-synthetic cnicin **1'** in 33% yield. The optical rotation of **1'** {$[\alpha]_D^{20}$ +159.9 (*c* 0.1, MeOH)} was good agreement with that of the isolated natural product **1** ($[\alpha]_D^{20}$ +169.6 (*c* 0.1, MeOH)).

3. Conclusion

In summary, the protected side chain of antitrypanosomal cnicin **1** was prepared *via* two routes starting from L-ascorbic acid **5**. In the initial route, synthesis of **3** was achieved in nine steps in 12% overall yield. Subsequently, a more effective synthesis eliminating placement of an unnecessary protecting group was developed. The synthesis involved PCC oxidation and Wittig reaction and yielded **3** in 16% overall yield in five steps. Finally, esterification of synthetic side chain **3** and TIPS-protected salonitenolide derivative **2** was conducted to achieve the semi-synthesis of cnicin **1'**. This is the first reported synthetic study of cnicin **1**. The results are expected to aid further efforts toward developing the first total synthesis of cnicin **1** for SAR studies.

Acknowledgments

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Supplementary Material

Experimental procedures, NMR spectra. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/

*Highlights

- This is the first synthetic study of sesquiterpene lactone cnicin.
- The side chain of cnicin was synthesized via two routes.
- A semi-synthesis of cnicin was acheived.