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Synthesis of cynaropicrin-d_4

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ABSTRACT

Cynaropicrin is a guaianolide sesquiterpene lactone, which has potent in vitro and in vivo inhibitory activity against *Trypanosoma brucei*, the protozoan parasite that causes human African trypanosomiasis (HAT; sleeping sickness). Herein, we describe the synthesis of cynaropicrin's deuterated derivative, cynaropicrin-d_4, by the replacement of the side chain of natural cynaropicrin. The synthesized cynaropicrin-d_4 could be employed as an internal standard for liquid chromatography–mass spectrometry (LC–MS) analysis, in the pharmacokinetic study of cynaropicrin. This could potentially advance the study of this therapeutic lead.

Cynaropicrin (**1**, Fig. 1), the bitter ingredient of artichokes, was first isolated by Šorm and co-workers in 1960, from *Cynara scolymus* L.[1] The compound was later also found in other Asteraceae, such as *Centaurea solstitialis* L.,[2] *Hemisteptia lyrata* B.,[3] and *Saussurea calcicola*.[4] Categorized as a guaianolide sesquiterpene lactone, **1** has a 5-7-5 fused tricyclic framework with six carbon stereocenters, four exo-olefins, and two hydroxyl groups. Compound **1** exhibits a host of biological activity, such as anti-inflammatory properties,[5] suppression of NF-κB,[6] and activation of bitter sensory receptors.[7]

In 2012, **1** was reported as a compound with potent in vitro activity against the protozoan parasite *Trypanosoma brucei*,[8] the pathogen that causes human African trypanosomiasis (HAT).[9,10] HAT, also known as 'sleeping sickness', is an endemic disease in sub-Saharan Africa that is transmitted by blood-feeding tsetse flies, and is fatal when left untreated. Currently, there are only a few therapeutic agents to treat HAT. Melarsoprol, an arsenic-containing drug, still the only option for the treatment of second-stage East African HAT, is associated with lethal side effects.[11] The antitrypanosomal natural product **1** has been described as a prospective new drug lead.[8] We previously reported the chemical derivatization of cynaropicrin and the structure–activity-relationship (SAR) study against *Trypanosoma brucei*.[12] In addition, we also tested antitrypanosomal activity, for a set of 34 natural and semi-synthetic sesquiterpene lactones.[13]

In the present study, the deuterated cynaropicrin-d_4 (**2**, Fig. 1) was designed to conduct the pharmacokinetic studies of cynaropicrin, as the internal standard for LC–MS analysis. The pharmacokinetic study would provide critical information about the potential utility of cynaropicrin **1** as a drug lead for HAT. Thus, compound **2**, bearing four deuterium atoms on the cynaropicrin-d_4 side chain, was designed and synthesized.

Initially, synthesis of side-chain **6**, in which the hydroxyl group was protected by a triisopropylsilyl (TIPS) group, was carried out (Scheme 1).[14] The Morita–Baylis–Hillman reaction of methyl acrylate **3** with paraformaldehyde and 1,4-diazabicyclo[2.2.2]octane (DABCO) in 1,4-dioxane/H_2O (1:1), gave alcohol **4**.[15] This was followed by formation of **5**, via protection of the free hydroxyl by

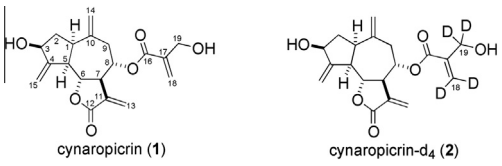
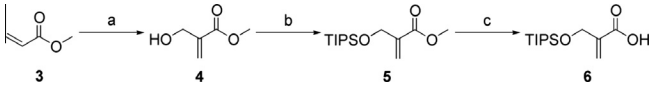


Figure 1. Structures of cynaropicrin (**1**) and cynaropicrin-d_4 (**2**). The carbon numbering of **1** and **2** was adapted from Ref. 2.



Scheme 1. Reagents and conditions: (a) paraformaldehyde, DABCO, 1,4-dioxane/H_2O (1:1), rt, 24 h; (b) TIPSCl, imidazole, DMAP, CH_2Cl_2, 0 °C–rt, 18 h, 52% (2 steps); (c) LiOH, THF/H_2O (1:1), rt, 25 h, 93%.

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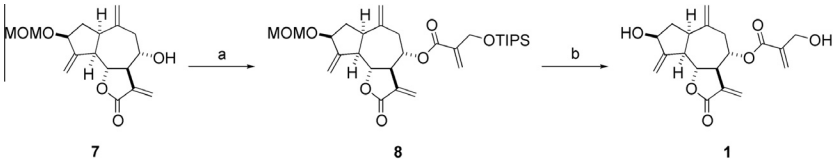
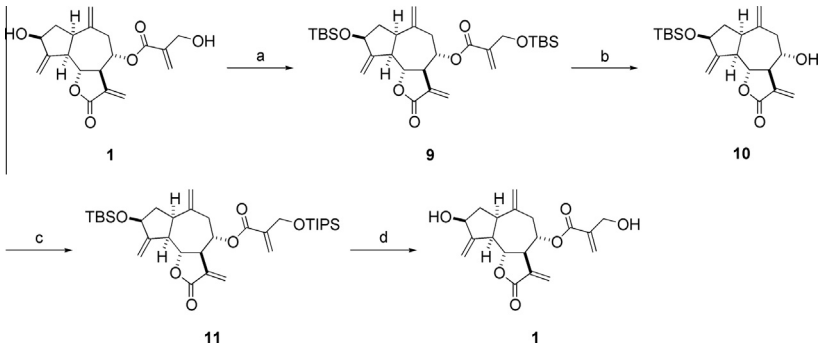
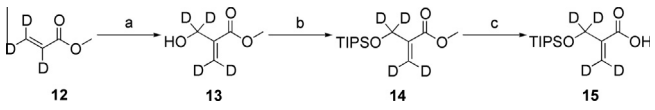


Scheme 2. Reagents and conditions: (a) **6**, 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, toluene, reflux, 23 h, 71%; (b) PPTS, *t*BuOH, reflux, 44 h, 23%.



Scheme 3. Reagents and conditions: (a) TBSCl, 2,4,6-trimethylpyridine, DMF, rt, 3 h, 91%; (b) 2 N NaOH, THF, rt, 28 h, 67%; (c) **6**, 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, toluene, reflux, 25 h, 66%; (d) TBAF, THF, rt, 4 h, 75%.



Scheme 4. Reagents and conditions: (a) paraformaldehyde-d_2, DABCO, 1,4-dioxane/H_2O (1:1), rt, 24 h; (b) TIPSCl, imidazole, DMAP, CH_2Cl_2, 0 °C–rt, 1 h; (c) LiOH, THF/H_2O (1:1), rt, 24 h, 12% (3 steps).

TIPSCl, using imidazole and *N*,*N*-dimethyl-4-aminopyridine (DMAP) in dichloromethane (CH_2Cl_2), in 52% yield over the two steps. Hydrolysis of **5** using lithium hydroxide (LiOH) in a 1:1 tetrahydrofuran (THF)–water (H_2O) mixture, led to carboxylic acid **6** in 93% yield.

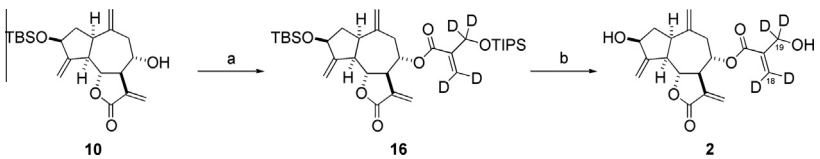
A gram-scale isolation of cynaropicrin **1** was carried out from *C. scolymus* L. (Asteraceae) according to the literature procedure.[8,16] In a previous study,[12] compound **7** was already obtained via MOM-protection of natural **1** and hydrolysis in two steps. Thus, we attempted the introduction of side chain **6** onto **7** (Scheme 2). Yamaguchi esterification of alcohol **7** using **6**, 2,4,6-trichlorobenzoyl chloride, triethylamine (Et_3N), and DMAP in toluene was carried out under reflux for 23 h to afford ester **8** in 71% yield.[17–19] Treatment of **8** with pyridinium *p*-toluenesulfonate (PPTS) in *tert*-butanol (*t*BuOH) under reflux for 44 h then afforded **1** in 23% yield.[20] The overall yield from natural **1** to synthetic **1** was 13% in four steps.

To further improve the previous synthetic sequence, 3,19-di-*tert*-butyldimethylsilyl (TBS) derivative **9** was also synthesized from **1** using TBSCl and 2,4,6-trimethylpyridine in *N*,*N*-dimethylformamide (DMF) at room temperature for 3 h in 91% yield from **1** (Scheme 3).[21] Hydrolysis of **9** was then conducted in aqueous NaOH and THF to give **10** in 67% yield.[22] Although we tried MeOH as a solvent, it was not possible to obtain the desired compound, probably due to the low solubility toward substrate **9**. Next, Yamaguchi esterification with **6** was performed under reflux for 25 h to give ester **11** in 66% yield.[17–19] Treatment of **11** with tetrabutylammonium fluoride (TBAF) in THF at room temperature for 4 h afforded **1** in 75% yield. The overall yield of this TBS-protection route (Scheme 3) was 30% in four steps, which was higher than the previous MOM-protection sequence (Scheme 2).

Synthesis of the TIPS-protected deuterated side-chain **15** was then carried out (Scheme 4).[14] Treatment of methyl acrylate-d_3 (**12**) with paraformaldehyde-d_2 and DABCO in 1,4-dioxane/H_2O (1:1) gave **13**, followed by formation of **14** using TIPSCl, imidazole, and DMAP in CH_2Cl_2. Finally, **14** was hydrolyzed to tetra-deuterated carboxylic acid **15**, in 12% yield over three steps. Compared to the yield using non-isotopically labeled starting materials, the overall yield of deuterated compound was decreased, probably due to the isotope effects.

Synthesis of cynaropicrin-d_4 was then carried out (Scheme 5). Esterification of **10** and **15** was performed at 40 °C for 2 h to give ester **16** in 70% yield.[17–19] Treatment of **16** with TBAF in THF at room temperature for 4 h afforded the desired product **2** in 43% yield. The synthesis of **2** was confirmed by the fact that the peaks of H18 and H19 were not observed in ^1H NMR compared to natural product **1**. In addition, the mass spectrum obtained in EI-HRMS of **2** showed a shift of m/z 4 compared to **1**.

In summary, the synthesis of cynaropicrin-d_4 **2** was achieved via esterification of hydrolyzed cynaropicrin and deuterated side-chain. Isotopic purity of compound **2** was high, as determined by ^1H NMR and EI-HRMS.[23] The synthesized cynaropicrin-d_4 **2** could serve as an internal standard for LC–MS analysis in the



Scheme 5. Reagents and conditions: (a) **15**, 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, toluene, 40 °C, 2 h, 70%; (b) TBAF, THF, rt, 4 h, 43%.

pharmacokinetic study of cynaropicrin. A high-yielding method for introducing the ester side chain was also established, which would be useful for the total synthesis of **1**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.10.065.

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23. *General:* All non-aqueous reactions were conducted under an atmosphere of nitrogen with magnetic stirring. CH_2Cl_2, *i*Pr_2NEt, and toluene were dried using activated molecular sieves. PPTS was prepared according to literature,[24] and DMAP was recrystallized from toluene. All reagents were obtained from commercial suppliers and used without further purification, unless otherwise stated. Paraformaldehyde-d_2 was purchased from Kanto Chemicals (Tokyo, Japan), and methyl acrylate (2,2,3-d_3) was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Analytical thin-layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates produced by Merck. Column chromatography was performed with acidic Silica gel 60 (spherical, 40–50 μm) produced by Kanto Chemicals.
Infrared (IR) spectra were recorded on a JASCO FT-IR 4100 spectrometer and reported in wavenumbers (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-EXC 300 spectrometer (300 MHz) or a JEOL JNM-ECA 500 spectrometer (500 MHz). ^1H NMR data are reported as follows: chemical shift (δ, ppm), integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constants (J) in Hz, assignments. ^{13}C NMR data are reported in terms of chemical shift (δ, ppm). EI-MS spectra were recorded on a JEOL JMS-700 instrument. ESI-MS spectra were recorded on a JEOL JMS-T100LC instrument.
Isolation of cynaropicrin (**1**): Extraction and isolation of cynaropicrin from artichoke leaves was performed by following the literature procedure.[8,16] Dried artichoke leaves (100 g) purchased from Dixa (Switzerland), were exhaustively extracted (three times) with ethyl acetate (900 mL), yielding 5.4 g of crude extract. The extracts were separated using silica-gel column chromatography, with ethyl acetate as the mobile phase. Fractions containing **1** were concentrated to give 1.7 g of impure cynaropicrin. Purification via silica-gel column chromatography (hexane/EtOAc = 1:2) afforded **1** as a yellow solid (1.4 g); R_f 0.62 (hexane/EtOAc = 1:3); IR (neat, cm^{-1}), 3419, 3080, 2931, 1765, 1712, 1643; ^1H NMR (500 MHz, $CDCl_3$) δ 6.29 (1H, s, H18), 6.17 (d, J = 2.3 Hz, H13), 5.94 (1H, s, H18), 5.59 (1H, 1.7 Hz, H13), 5.44 (1H, s, H15), 5.33 (1H, s, H15), 5.13–5.09 (2H, m, H8/14), 4.91 (1H, s, H14), 4.53 (1H, t, J = 6.9 Hz, H3), 4.35 (2H, s, H19), 4.25 (1H, t, J = 9.7 Hz, H6), 3.20–3.14 (1H, m, H7), 2.94 (1H, dd, J = 19.3, 8.4, H1), 2.81 (1H, t, J = 10.3 Hz, H5), 2.69 (1H, dd, J = 14.9, 4.6 Hz, H9), 2.36 (1H, dd, J = 14.9, 3.4 Hz, H9), 2.23–2.13 (1H, m, H2), 1.71 (1H, m, H2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4 (C12), 165.4 (C16), 152.2 (C4), 141.8 (C10), 139.4 (C17), 137.4 (C11), 126.8 (C18), 122.9 (C13), 118.3 (C14), 113.6 (C15), 78.6 (C6), 74.4 (C8), 73.7 (C3), 62.1 (C19), 51.4 (C5), 47.6 (C7), 45.4 (C1), 39.1 (C2), 37.0 (C9); EI-MS (m/z) calcd for $C_{19}H_{22}O_6$ $[M]^+$ 346.1, found 346.0.
Methyl 17-((triisopropylsilyloxy)methyl)acrylate (**5**): A solution of paraformaldehyde (901.3 mg, 29.1 mmol, 5.0 equiv) in 1,4-dioxane/H_2O (1:1, 10 mL) was stirred for 30 min at room temperature. To the mixture, methyl acrylate **3** (520 μL, 5.81 mmol, 1.0 equiv) and DABCO (3.26 g, 29.1 mmol, 5.0 equiv) were added. After stirring for 24 h, the mixture was diluted with ether and quenched with a saturated NaCl solution. The aqueous layer was then extracted with ether. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product **4** was used in the next reaction, without further purification.
To a solution of **4** (101.0 mg, 0.86 mmol, 1.0 equiv) and imidazole (349.5 g, 5.16 mmol, 6.0 equiv) in CH_2Cl_2, TIPSCl (400.4 μL, 1.89 mmol, 2.2 equiv) was added at room temperature. After stirring for 18 h, the mixture was diluted with CH_2Cl_2 and quenched with a saturated NH_4Cl solution. The aqueous layer was then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 40:1) afforded **5** as a yellow oil (123.3 mg, 0.45 mmol, 52%, over two steps). R_f 0.30 (hexane/EtOAc = 40:1); ^1H NMR (300 MHz, $CDCl_3$) δ 6.28 (1H, dd, J = 4.1, 2.1 Hz, H18), 6.00 (1H, dd, J = 4.5, 2.1 Hz, H18), 4.45 (2H, t, J = 2.0 Hz, H19), 3.75 (3H, s, Me), 1.21–1.05 (21H, m, TIPS).
17-((Triisopropylsilyloxy)methyl)acrylic acid (**6**): To a solution of **5** (223.9 mg, 0.82 mmol, 1.0 equiv) in THF/H_2O (1:1, 2.0 mL), LiOH (80.9 mg, 0.82 mmol, 4.0 equiv) was added at room temperature. After stirring for 25 h, the mixture was diluted with ether and quenched with 5% HCl solution. The aqueous layer was then extracted with ether. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 10:1) afforded **6** as a yellow oil (198.0 mg, 0.76 mmol, 93%); R_f 0.15 (hexane/EtOAc = 20:1); ^1H NMR (300 MHz, $CDCl_3$) δ 6.41 (1H, dd, J = 3.8, 2.1 Hz, H19), 6.09 (1H, dd, J = 3.8, 2.1 Hz, H19), 4.48 (2H, t, J = 2.0 Hz, H18), 1.30–1.04 (21H, m, TIPS).
(1R,3S,5R,6R,7R,8S)-3-(Methoxymethoxy)-13,14,15-trimethylene-12-oxododecahydroazuleno[6,7-β]furan-8-yl 17-((triisopropylsilyloxy)methyl)acrylate (**8**): To a solution of **6** (107.0 mg, 0.413 mmol, 10.9 equiv) in toluene (1 mL), 2,4,6-trichlorobenzoyl chloride (64.6 μL, 0.413 mmol, 10.9 equiv) and Et_3N (57.6 μL, 0.413 mmol, 10.9 equiv) were added. After stirring for 30 min at room temperature, a solution of **7** (11.6 mg, 0.0379 mmol, 1.0 equiv) and DMAP (9.26 mg, 0.0758 mmol, 2.0 equiv) in toluene (1.0 mL) was added to the reaction mixture. After stirring for 23 h under reflux, the mixture was diluted with Et_2O, and quenched with a $NaHCO_3$ solution. The aqueous layer was then extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 10:1) afforded **8** as a colorless oil (14.8 mg, 0.0271 mmol, 71%); R_f 0.34 (hexane/EtOAc = 4:1); ^1H NMR (300 MHz, $CDCl_3$) δ 6.33 (1H, s, H18), 6.20 (1H, d, J = 3.4 Hz, H13), 6.08 (1H, dd, J = 9.6, 2.0 Hz, H18), 5.60 (1H, d, J = 2.7 Hz, H13), 5.48 (1H, s, H15), 5.34 (1H, s, H15), 5.14–5.09 (2H, m, H8/14), 4.91 (1H, s, H14), 4.78 (1H, d, J = 6.9 Hz, CH_2), 4.65 (1H, d, J = 6.5 Hz, CH_2), 4.49–4.42 (3H, m, H3/19), 4.25 (1H, t, J = 9.8 Hz, H6), 3.38 (3H, s, Me), 3.20–3.14 (1H, m, H7), 3.01 (1H, q, J = 8.3 Hz, H1), 2.83–2.66 (2H, m, H5/9), 2.41–2.26 (2H, m, H2/9), 1.85–1.75 (1H, m, H2), 1.44–0.95 (21H, m, TIPS); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 165.1, 148.6, 142.1, 139.7, 137.7, 125.1, 122.6, 118.2, 115.3, 94.9, 78.42, 77.8, 74.1, 61.6, 55.6, 52.3, 47.5, 46.0, 37.3, 37.1, 28.9, 18.1, 28.8, 17.2, 13.4, 12.6, 12.19; ESI-HRMS (m/z) calcd for $C_{30}H_{46}NaO_7Si$ $[M+Na]^+$ 569.2911, found 569.2911.
*Cynaropicrin (**1**) (via MOM route):* To a solution of **8** (14.8 mg, 0.0271 mmol, 1.0 equiv) in *t*BuOH (2 mL) PPTS (68.1 mg, 0.271 mmol, 10.0 equiv) was added. After stirring for 44 h under reflux, the mixture was diluted with EtOAc, and quenched with a saturated $NaHCO_3$ solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 1:2) afforded **1** as a colorless oil (3.5 mg, 0.010 mmol, 23%); ^1H NMR (300 MHz, $CDCl_3$) δ 6.39 (1H, s, H18), 6.23 (1H, d, J = 3.4 Hz, H13), 5.96 (1H, s, H18), 5.62 (1H, d, J = 3.1 Hz, H13), 5.51 (1H, s, H15), 5.37 (1H, s, H15), 5.18–5.12 (2H, m, H14/H8), 4.95 (1H, s, H14), 4.57 (1H, t, J = 7.2 Hz, H3), 4.39 (2H, s, H19), 4.26 (1H, t, J = 9.4 Hz, H6), 3.24–3.17 (1H, m, H7), 3.01–2.95 (1H, m, H1), 2.85 (1H, t, J = 10.3 Hz, H5), 2.72 (1H, dd, J = 14.6, 5.4 Hz, H9), 2.41 (1H, dd, J = 14.4, 3.4 Hz, H9), 2.30–2.20 (1H, m, H2), 1.79–1.69 (1H, m, H2); ESI-MS (m/z) calcd for $C_{19}H_{22}NaO_6$ $[M+Na]^+$ 369.13, found 369.10.
(1R,3S,5R,6R,7R,8S)-3-(tert-Butyldimethylsilyloxy)-13,14,15-trimethylene-12-oxododecahydroazuleno[6,7-β]furan-4-yl 17-((tert-butyldimethylsilyloxy)methyl) acrylate (**9**): To a solution of **1** (70.0 mg, 0.202 mmol, 1.0 equiv) and TBSCl (140.0 mg, 0.929 mmol, 4.6 equiv) in dry DMF (0.7 mL), 2,4,6-trimethylpyridine (0.40 mL, 3.03 mmol, 15.0 equiv) was added. After stirring for 3 h at room temperature, the mixture was diluted with Et_2O, and quenched with water. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and then concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 10:1) afforded **9** as a yellow oil (105.1 mg, 0.18 mmol, 91%); R_f

0.67 (hexane/EtOAc = 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 1.8 Hz, H18), 6.21 (1H, d, J = 3.4 Hz, H13), 6.01 (1H, d, J = 2.0 Hz, H18), 5.61 (1H, d, J = 3.1 Hz, H13), 5.45 (1H, s, H15), 5.26 (1H, s, H15), 5.13–5.07 (2H, m, H8/14), 4.92 (1H, s, H14), 4.49 (1H, t, J = 7.6 Hz, H3), 4.39 (2H, t, J = 1.7 Hz, H19), 4.25 (1H, dd, J = 9.6 Hz, H6), 3.21 (1H, m, H7), 2.95 (1H, m, H1), 2.83 (1H, m, H5), 2.66 (1H, dd, J = 14.3 Hz, H9), 2.35 (1H, dd, J = 14.2 Hz, H9), 2.07 (1H, m, H2), 1.68 (1H, m, H2), 0.96–0.87 (18H, m, TBS), 0.12–0.09 (12H, m, TBS).

(1R,3S,5R,6R,7R,8S)-3-(tert-Butyldimethylsilyloxy)-8-hydroxy-13,14,15-trimethylene-decahydroazuleno[6,7-β]furan-12-one (**10**): To a solution of **9** (31.1 mg, 0.054 mmol, 1.0 equiv) in THF (0.05 mL), 2 N NaOH (0.5 mL) was added. After stirring for 28 h at room temperature, the mixture was diluted with EtOAc, and quenched with a saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 2:1) afforded **10** as a white solid (13.5 mg, 0.036 mmol, 67%); R_f 0.28 (hexane/EtOAc = 2:1); IR (neat, cm^{-1}), 3740, 3426, 2945, 2856, 2362, 1741, 1644, 1465, 1281, 1164, 1100, 983, 894, 832; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.27 (1H, d, J = 2.8 Hz, H13), 6.15 (1H, d, J = 3.1 Hz, H13), 5.44 (1H, s, H15), 5.24 (1H, s, H15), 5.11 (1H, s, H14), 4.95 (1H, s, H14), 4.47 (1H, m, H3), 4.16 (1H, t, J = 10.3, H6), 3.92 (1H, m, H8), 2.96 (1H, m, H1), 2.84–2.77 (2H, m, H5/7), 2.67 (1H, dd, J = 13.8 Hz, H9), 2.26 (1H, dd, J = 13.8 Hz, H9), 2.07 (1H, m, H2), 1.74 (1H, m, H2), 0.92–0.90 (9H, m, TBS), 0.09 (6H, m, TBS); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.8, 143.0, 137.7, 122.9, 116.3, 111.3, 79.2, 73.7, 71.7, 50.2, 49.6, 44.6, 41.4, 40.1, 25.6, 17.9; ESI-HRMS (m/z) calcd for $C_{21}H_{32}NaO_4Si$ [M+Na]$^+$ 399.1970, found 399.1968.

(1R,3S,5R,6R,7R,8S)-3-(tert-Butyldimethylsilyloxy)-13,14,15-trimethylene-12-oxododecahydroazuleno[6,7-β]furan-8-yl 17-((triisopropylsilyloxy)methyl)acrylate (**11**): To a solution of **6** (20.4 mg, 0.0791 mmol, 2.0 equiv) in toluene (0.5 mL), 2,4,6-trichlorobenzoyl chloride (13.6 μL, 0.0870 mmol, 2.2 equiv) and Et_3N (22.0 μL, 0.158 mmol, 4.0 equiv) were added. After stirring for 1 h at room temperature, a solution of **10** (14.9 mg, 0.0395 mmol, 1.0 equiv) and DMAP (12.6 mg, 0.103 mmol, 2.6 equiv) in toluene (1.5 mL) was added to the reaction mixture. After stirring for 25 h under reflux, the mixture was diluted with EtOAc, and quenched with saturated $NaHCO_3$ solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 10:1) afforded **11** as a yellow oil (16.0 mg, 0.0259 mmol, 66%); R_f 0.92 (hexane/EtOAc = 2:1); IR (neat, cm^{-1}), 2944, 2864, 1773, 1716, 1640, 1463, 1389, 1260, 1096, 1014, 962, 882, 835, 776, 683; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (1H, s, H18), 6.21 (1H, d, J = 3.4 Hz, H13), 6.09 (1H, s, H18), 5.61 (1H, d, J = 3.1 Hz, H13), 5.45 (1H, s, H15), 5.26 (1H, s, H15), 5.13–5.07 (2H, m, H8/14), 4.92 (1H, s, H14), 4.49 (3H, m, H3/19), 4.25 (1H, t, J = 9.8 Hz, H6), 3.21 (1H, m, H7), 2.96 (1H, m, H1), 2.84 (1H, m, H5), 2.67 (1H, dd, J = 14.1 Hz, H9), 2.35 (1H, dd, J = 14.1 Hz, H9), 2.10–2.06 (1H, m, H2), 1.71–1.64 (1H, m, H2), 1.44–0.95 (21H, m, TIPS), 0.91–0.89 (9H, m, TBS), 0.09–0.07 (6H, m, TBS); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 164.5, 151.4, 142.0, 139.4, 137.0, 124.7, 122.6, 117.1, 111.5, 79.0, 73.8, 61.3, 49.6, 46.8, 44.4, 39.6, 37.5, 25.6, 17.9, 11.7, 0.74, −5.0, −8.4; ESI-HRMS (m/z) calcd for $C_{34}H_{56}NaO_6Si_2$ [M+Na]$^+$ 639.3513, found 639.3491.

*Cynaropicrin (**1**) (via TBS route):* To a solution of **11** (3.7 mg, 0.006 mmol, 1.0 equiv) in THF (0.2 mL), was added TBAF (8.5 μL, 0.030 mmol, 5.0 equiv) at 0 °C. After stirring for 4 h at room temperature, the mixture was diluted with EtOAc and quenched with a saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 1:2) afforded **1** as a colorless oil (1.5 mg, 0.004 mmol, 75%); R_f 0.20 (hexane/EtOAc = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.39 (1H, s, H18), 6.23 (1H, d, J = 3.4 Hz, H13), 5.96 (1H, s, H18), 5.62 (1H, d, J = 3.1 Hz, H13), 5.51 (1H, s, H15), 5.37 (1H, s, H15), 5.18–5.12 (2H, m, H8/14), 4.95 (1H, s, H14), 4.57 (1H, t, J = 7.2 Hz, H3), 4.39 (2H, s, H19), 4.26 (1H, dd, J = 10.6 Hz, H6), 3.24–3.17 (1H, m, H7), 3.01–2.95 (1H, m, H1), 2.85 (1H, t, J = 10.3 Hz, H5), 2.72 (1H, dd, J = 14.8 Hz, H9), 2.41 (1H, dd, J = 14.4 Hz, H9), 2.30–2.20 (1H, m, H2), 1.79–1.69 (1H, m, H2).

Methyl 2-((triisopropylsilyloxy)methyl)acrylate-d_4 (**14**): A solution of paraformaldehyde-d_2 (916.9 mg, 28.1 mmol, 5.0 equiv) in a mixture of 1,4-dioxane/H_2O (1:1, 4 mL) was stirred for 30 min at room temperature. To the mixture, methyl acrylate-d_3 **12** (500.0 mg, 5.61 mmol, 1.0 equiv) and DABCO (3.1523 g, 28.1 mmol, 5.0 equiv) were added. After stirring for 24 h, the mixture was diluted with Et_2O, and quenched with a saturated NaCl solution. The aqueous layer was then extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was used in the next reaction, without further purification.

To a solution of **13** (674.0 mg, 5.61 mmol, 1.0 equiv) and imidazole (2.2943 g, 33.7 mmol, 6.0 equiv) in CH_2Cl_2, TIPSCl (2.61 mL, 12.3 mmol, 2.2 equiv) was added at 0 °C. After stirring for 30 min, the solution was warmed to room temperature and stirred for another 30 min. The mixture was diluted with CH_2Cl_2, and quenched with a saturated NH_4Cl solution. The aqueous layer was then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 40:1) afforded crude **14** as a colorless oil. The crude product was used in the next reaction without further purification; EI-HRMS (m/z) calcd for $C_{11}H_{17}D_4O_3Si$ [M−CH$(CH_3)_2$]$^+$ 233.1511, found 233.1504.

17-((Triisopropylsilyloxy)methyl)acrylic acid-d_4 (**15**): To a solution of **14** (609.7 mg, 2.20 mmol, 1.0 equiv) in THF/H_2O (1:1, 5.4 mL) LiOH (210.8 mg, 8.80 mmol, 4.0 equiv) was added at room temperature. After stirring for 24 h, the mixture was diluted with Et_2O, and quenched with 5% HCl solution. The aqueous layer was then extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 10:1) afforded **15** as a colorless oil (71.4 mg, 0.27 mmol, 12%, over three steps); R_f 0.15 (hexane/EtOAc = 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30–1.04 (21H, m, TIPS).

(1R,3S,5R,6R,7R,8S)-3-(tert-Butyldimethylsilyloxy)-13,14,15-trimethylene-12-oxododecahydroazuleno[6,7-β]furan-8-yl 17-((triisopropylsilyloxy)methyl)acrylate-d_4 (**16**): To a solution of **15** (67.7 mg, 0.258 mmol, 1.0 equiv) in toluene (0.6 mL), 2,4,6-trichlorobenzoyl chloride (44.2 μL, 0.283 mmol, 1.1 equiv) and Et_3N (71.9 μL, 0.516 mmol, 2.0 equiv) were added. After stirring for 1 h at room temperature, a solution of **10** (97.0 mg, 0.258 mmol, 1.0 equiv) and DMAP (40.9 mg, 0.335 mmol, 1.3 equiv) in toluene (2.0 mL) was added to the reaction mixture. After stirring for 2 h at 40 °C, the mixture was diluted with EtOAc, and quenched with saturated $NaHCO_3$ solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 10:1) afforded **16** as a colorless oil (112.6 mg, 0.181 mmol, 70%); R_f 0.92 (hexane/EtOAc = 2:1); IR (neat, cm^{-1}), 3747, 2944, 2892, 1773, 1715, 1462, 1385, 1258, 1104, 1013, 881, 834, 679; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.21 (1H, d, J = 3.4 Hz, H13), 5.61 (1H, d, J = 3.1 Hz, H13), 5.45 (1H, s, H15), 5.25 (1H, s, H15), 5.13–5.07 (2H, m, H14/H8), 4.91 (1H, s, H14), 4.49 (1H, t, J = 11.3 Hz, H3), 4.25 (1H, dd, J = 10.0 Hz, H6), 3.21 (1H, m, H7), 2.95 (1H, m, H1), 2.86 (1H, m, H5), 2.65 (1H, dd, J = 14.1 Hz, H9), 2.35 (1H, dd, J = 14.1 Hz, H9), 2.10–2.06 (1H, m, H2), 1.71–1.64 (1H, m, H2), 1.28–1.05 (21H, m, TIPS), 0.99–0.85 (9H, TBS), 0.11–0.06 (6H, TBS); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 165.1, 151.8, 142.2, 139.4, 137.4, 122.8, 117.8, 112.0, 79.3, 74.1, 50.0, 47.2, 44.8, 40.0, 37.8, 26.0, 18.0, 12.4, −4.6; ESI-HRMS (m/z) calcd for $C_{34}H_{52}D_4NaO_6Si_2$ [M+Na]$^+$ 643.3764, found 643.3737.

Cynaropicrin-d_4 (**2**): To a solution of **16** (56.8 mg, 0.091 mmol, 1.0 equiv) in THF (1.6 mL), TBAF (129.0 μL, 0.455 mmol, 5.0 equiv) was added at 0 °C. After stirring for 4 h at room temperature, the mixture was diluted with EtOAc, and quenched with a saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc = 1:2) afforded **2** as a colorless oil (13.6 mg, 0.039 mmol, 43%); R_f 0.20 (hexane/EtOAc = 1:1); IR (neat, cm^{-1}), 3742, 3413, 3081, 2931, 2362, 1765, 1712, 1647, 1456, 1272, 1174, 1046, 961, 910, 756, 634; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.23 (1H, d, J = 3.4 Hz, H13), 5.62 (1H, d, J = 3.1 Hz, H13), 5.49 (1H, s, H15), 5.36 (1H, s, H15), 5.17–5.11 (2H, m, H14/H8), 4.94 (1H, s, H14), 4.56 (1H, t, J = 7.2 Hz, H3), 4.26 (1H, dd, J = 10.3 Hz, H6), 3.23–3.16 (1H, m, H7), 3.00–2.94 (1H, m, H1), 2.85 (1H, t, J = 10.3 Hz, H5), 2.72 (1H, dd, J = 14.8 Hz, H9), 2.41 (1H, dd, J = 14.4 Hz, H9), 2.32–2.22 (1H, m, H2), 1.79–1.68 (1H, m, H2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.3, 141.9, 137.4, 118.4, 113.8, 78.6, 74.4, 73.9, 51.5, 47.7, 45.4, 39.2, 37.2; EI-HRMS (m/z) calcd for $C_{19}H_{18}D_4O_6$ [M]$^+$ 350.1667, found 350.1654.

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