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A Scalable Total Synthesis of Halofuginone

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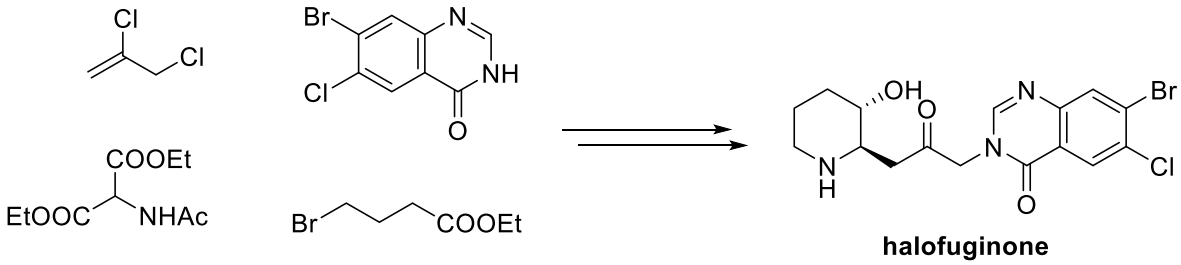
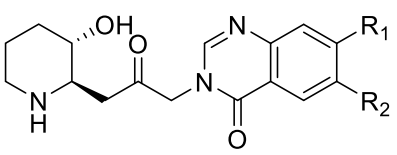


halofuginone

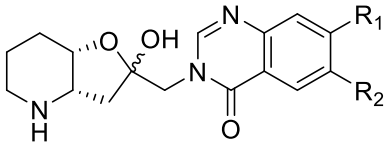
ABSTRACT: A scalable total synthesis of halofuginone has been accomplished. This synthetic route features a total of 12 steps of highly efficient reactions, without any chromatographic purification. Halofuginone was obtained in 17% overall yield and over 98.5% HPLC purity. All the reaction conditions are mild and reliable. In addition, no hazardous materials were used or produced. All reagents are commercially available and inexpensive. This route is safe, robust, scalable, cost-effective and environmentally benign.

KEYWORDS: Halofuginone, Total Synthesis, Dieckmann Condensation, Isomerization, Quinazolinone

Halofuginone **1**, a synthetic analogue of the quinazolinone alkaloid febrifugine **2** originally isolated from the Chinese herb *Dichroa febrifuga*,[1] displayed extremely rich biological activities. Halofuginone hydrobromide (racemic) has been used as an anticoccidial feed additive for broilers and turkeys for several decades under the trade name Stenorol®,[2] while its lactate (racemic) named Halocur® has been used for the prevention of diarrhoea caused by diagnosed *Cryptosporidium parvum* in new born calves.[3] In addition to veterinary applications, halofuginone has also demonstrated great potential in the development of human medicine. For examples, it reportedly inhibits the development of proinflammatory Th17 cells, procollagen type I gene expression and extracellular matrix deposition, thus may be effective for the treatment of autoimmune disorders, fibrosis or cancer.[4] As a result, the total synthesis of halofuginone has attracted the attention of many chemists.[5] A detailed summary about the synthetic work of febrifugine and its analogues (including halofuginone) has been reported by Evans *et al*.[5a, 5b] From a practical point of view, we have developed a scalable synthetic route which may provide kilograms of high purity halofuginone.



R_1 = Br, R_2 = Cl, **halofuginone 1**
R_1 = R_2 = H, **febrifugine 2**



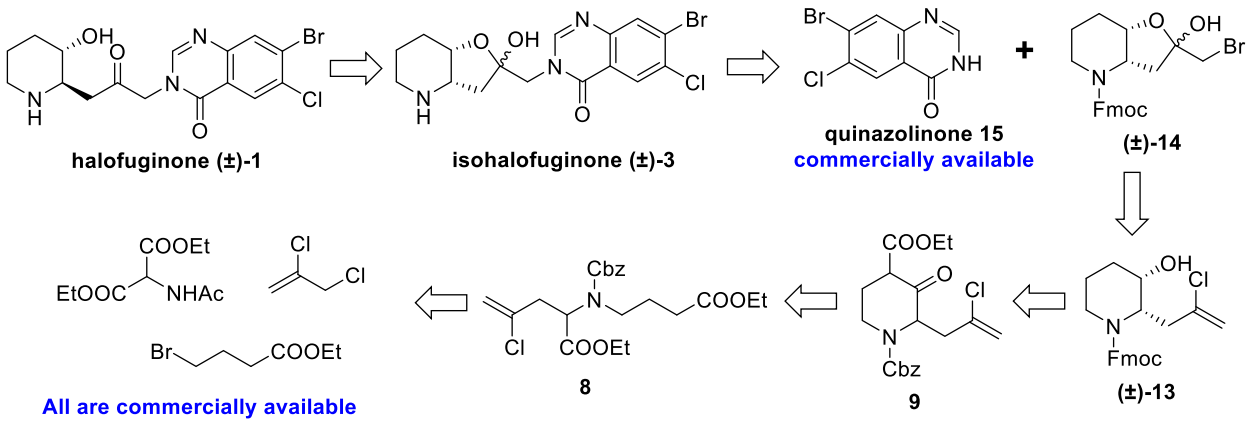
R_1 = Br, R_2 = Cl, **isohalofuginone 3**
R_1 = R_2 = H, **isofebrifugine 4**

Figure 1. Selected examples of halofuginone family compounds.

Our retrosynthetic analysis is shown in **Scheme 1**. Halofuginone (±)-**1** may be obtained through the isomerization of isohalofuginone **3** inspired by a similar transformation from isofebrifugine **4** to febrifugine **2** reported by Takeuchi *et al*.[5f] and applied to the synthesis of halofuginone by Li *et*

al.[5h] Compound **3** may be assembled from bromohemiacetal **14** and commercially available quinazolinone **15** under basic conditions. Bromination of compound **13**, which may be derived from the β-keto-ester **9** through a series of transformations, may lead to compound **14** under aqueous conditions, while compound **9** may be obtained from compound **8**, an intermediate that may be assembled with commercially available materials (including diethyl acetamidomalonate, 2,3-dichloropropene and ethyl 4-bromobutyrate) through a series of conventional reactions, by using the Dieckmann condensation.

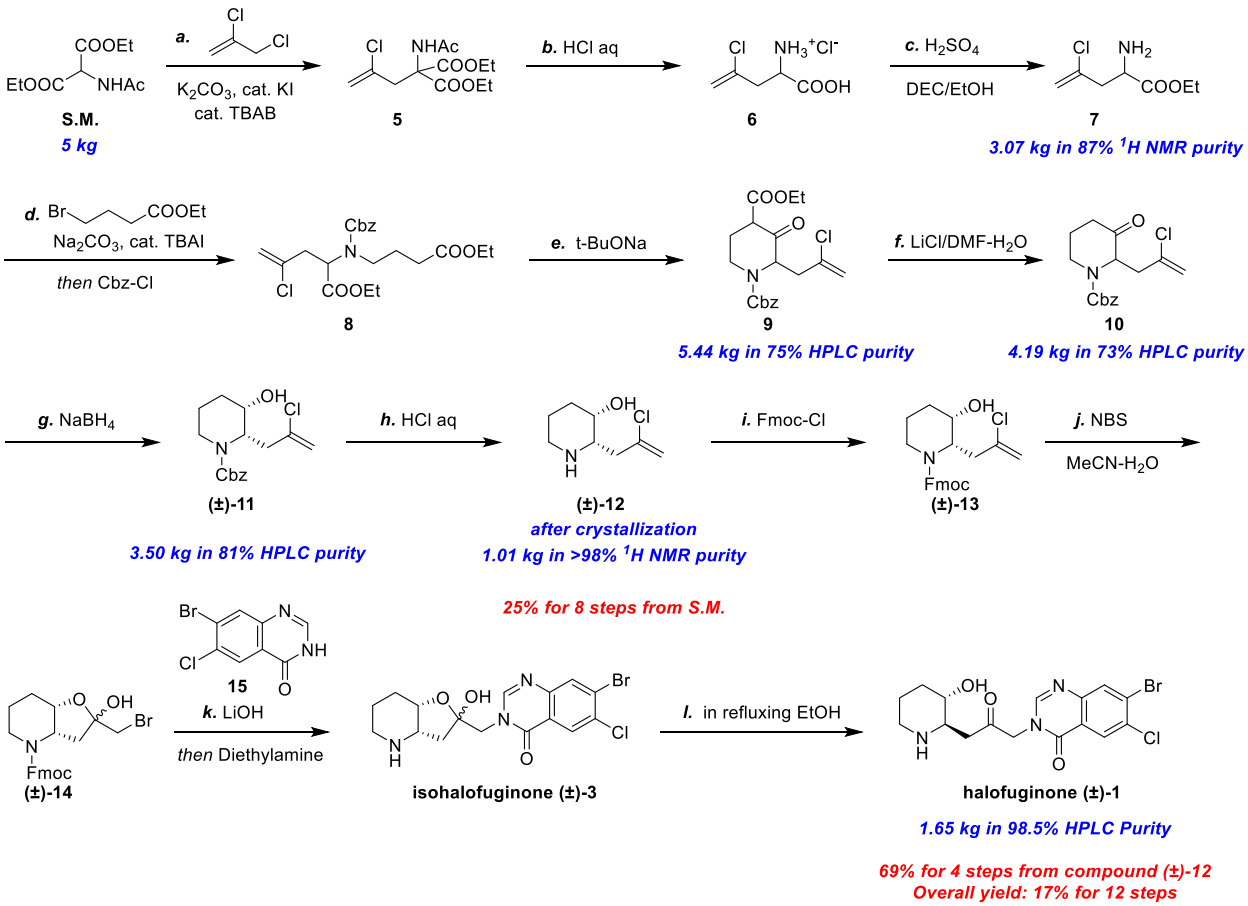
Scheme 1. Retrosynthetic Analysis of Halofuginone.



Based on the above analysis, the total synthesis of halofuginone is shown in **Scheme 2**. Compound **5** was prepared from diethyl acetamidomalonate via alkylation with 2,3-dichloropropene. Decarboxylation of one ester group of compound **5** in refluxing aqueous HCl solution afforded α-amino acid hydrochloride **6**. After esterification, compound **7** was obtained, the purity of which was determined to be 87% using ^{1}H NMR. With compound **7** in hand, we next carried out its N-substitution reaction with ethyl 4-bromobutyrate. While the reaction did not work well when an organic base (Et_3N, DIPEA or DBU) was used in an organic solvent (acetonitrile or toluene), a high conversion rate (92%) was observed when anhydrous Na_2CO_3 was used in the

presence of a catalytic amount of TBAI in toluene. After the completion of the N-substitution reaction, Cbz-Cl was added dropwise directly to the reaction mixture to afford compound **8**.[6]

Scheme 2. Synthesis of Halofuginone.

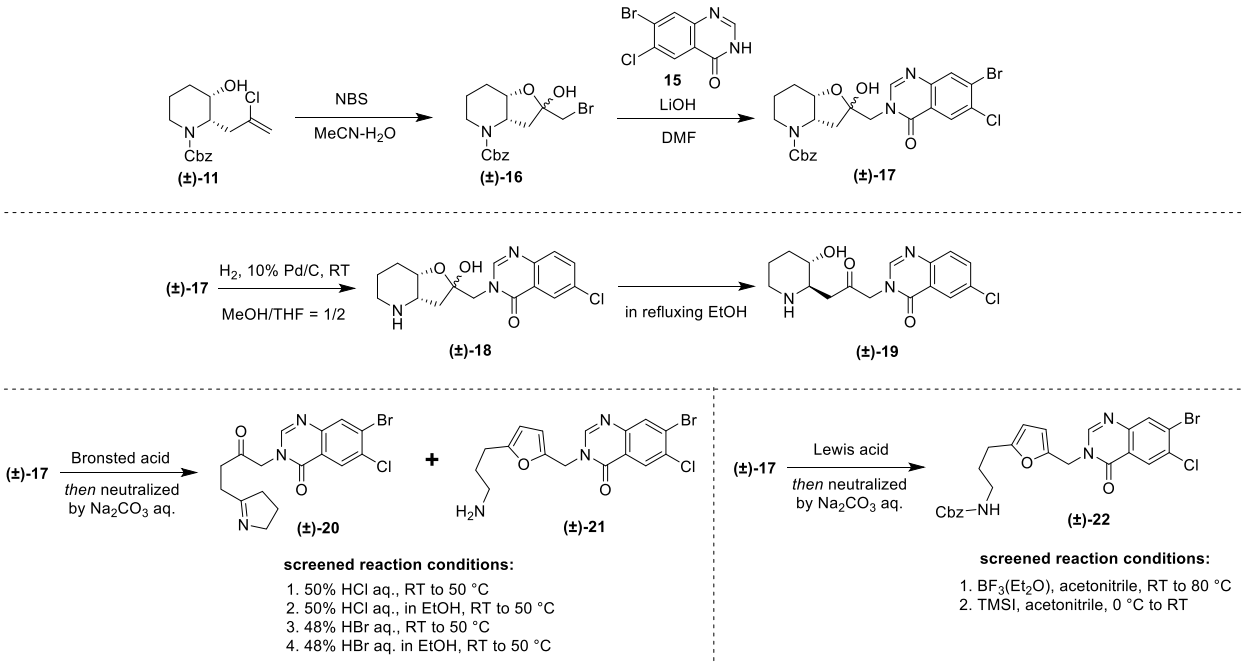


Reagents and conditions: (***a***) 2,3-Dichloropropene, K_2CO_3, *cat.* KI, *cat.* TBAB, acetonitrile, 85-90 °C; (***b***) 6M HCl(aq), refluxing; (***c***) conc. H_2SO_4, DEC/EtOH, refluxing; DEC = diethyl carbonate; (***d***) Ethyl 4-bromobutyrate, Na_2CO_3, *cat.* TBAI, toluene, 75-85 °C, *then* Cbz-Cl, 25 °C; (***e***) *t*-BuONa, THF, 0±5 °C; (***f***) LiCl, DMF, H_2O, 120 °C; (***g***) $NaBH_4$, EtOH, 5-10 °C; (***h***) 6M HCl(aq)/EtOH, refluxing; (***i***) Na_2CO_3, dioxane, H_2O, Fmoc-Cl, 20 °C; (***j***) NBS, acetonitrile, H_2O, 0-5 °C; (***k***) quinazolinone **15**, LiOH, DMF, 0-5 °C, *then* diethylamine, 0-5 °C; (***l***) EtOH, refluxing.

It was surprising that the seemingly straightforward Dieckmann condensation turned out to be problematic since no condensation product was observed when compound **8** was treated with NaOMe in MeOH or with NaOEt in EtOH. However, treating compound **8** (5g scale) with either NaOMe or NaOEt in either THF or toluene afforded the desired product **9** in less than 50% yield.

Inspired by this result, we thought that a polar aprotic solvent may be a better choice for the Dieckmann condensation of compound **8**. After a brief screening, we found that *t*-BuONa in THF gave 89% yield (15g scale compound **8**). Thus, *t*-BuONa was used as the base in the following scale-up experiments, in which 5.44 kg of compound **9** was obtained in a single batch in 75% HPLC purity.[7] The removal of the ethoxycarbonyl functionality of compound **9** was achieved by Krapcho decarboxylation to afford 4.19 kg of compound **10** in 73% HPLC purity. Reduction of the carbonyl group with $NaBH_4$ with the hydride attacking the carbonyl group from the sterically less congested side led to the *cis* isomer, and 3.50 kg of compound **11** was obtained in 81% HPLC purity.[5c, 5d] The Cbz group was removed by using aqueous HCl and compound **12** was obtained in over 98% ^{1}H NMR purity after one crystallization from acetonitrile.

Scheme 3. Side Reactions of the Cbz Removal Reaction of Compound **17**

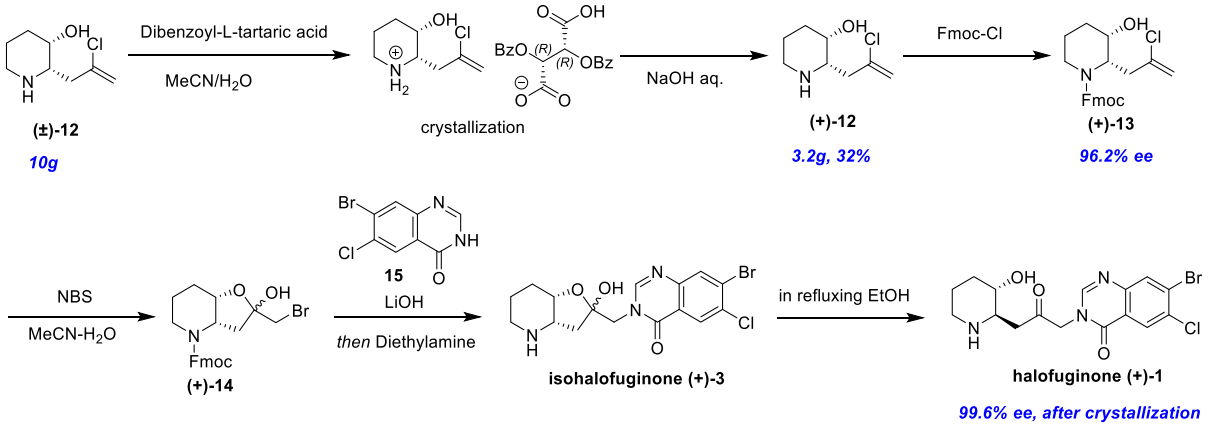


It was necessary for us to switch the protecting group from Cbz to Fmoc, the rationale for which is illustrated in Scheme **3**. Compound **17** was initially prepared via a two-step transformation from

11, but the removal of the Cbz group of **17** was problematic. Hydrogenolysis of the Cbz of **17** was accompanied by debromination leading to **18**, which was isomerized to product **19** in refluxing EtOH. Treating **17** with a Bronsted acid afforded compound **20** and **21** as the two major products, while treating **17** with Lewis acids afforded compound **22** as the major product. The results obtained from the reaction of Cbz-protected isohalofuginone **17** with acid were in agreement with those previously reported.[5k, 8] Based on the above observations, it was realized that the protecting group of the piperidine nitrogen played an extremely important role in the last few steps of the entire synthetic route. An ideal protecting group here should not only tolerate the reaction conditions of the next two steps, i.e., NBS bromination and base-promoted substitution with quinazolinone **15**, but also be easily removable without affecting the piperidine ring. Fortunately, this purpose was served when Fmoc was used to protect the piperidine nitrogen. Protection of the N atom of **12** with Fmoc-Cl afforded compound **13**, which was treated with NBS in the presence of water to provide **14**.[5h] Slow addition of the DMF solution of **14** to the preformed quinazolinone **15** lithium salt in DMF produced the coupling product, which upon deprotection of the Fmoc group with diethylamine afforded isohalofuginone **3** containing a small amount of halofuginone **1** (< 5%). It was found that **3** was isomerized to **1** during the solvent evaporation process because the ratio of these two compounds gradually changed (e.g., isohalofuginone **3**/halofuginone **1** = 2/1). The total weight of the resulting mixture was 1.84 kg (77% over 3 steps), of which 98% is isohalofuginone **3** and halofuginone **1**. Isohalofuginone **3** was further isomerized to halofuginone **1** in refluxing EtOH in 94% conversion. After crystallization, 1.65 kg of halofuginone **1** (> 98.5% HPLC purity) was obtained in 90% yield. Starting from 5 kg diethyl acetamidomalonate, 1.65 kg halofuginone was obtained in high purity (> 98.5% HPLC purity), which corresponds to an overall yield of 17% over 12 steps.

This synthetic route may also enable the large scale production of enantiomerically pure halofuginone **1**. As shown in **Scheme 4**, resolution of racemic-**12** using dibenzoyl-L-tartaric acid provided (+)-**12** in 32% yield, and its ee value was determined to be 96.2% in the form of its Fmoc derivative (**13**).

Scheme 4. Synthesis of (+)-halofuginone **1**.



In conclusion, we have accomplished a kilogram-scale total synthesis of halofuginone in high purity over 12 steps. No column chromatographic purification was involved in the entire process. This synthetic route may also be used for the preparation of enantiomerically pure halofuginone. The reaction conditions are mild, safe and environmentally benign. The production operations are quite simple and convenient. All the materials in this route are commercially available and inexpensive. The success of this approach is dependent on a series of key chemical transformations, including (1) Dieckmann condensation to efficiently set up the piperidine ring; (2) Fmoc was chosen as a key protecting group for the piperidine nitrogen-atom at the late stage; (3) high conversion rate of isomerization of isohalofuginone in ethanol to halofuginone; and (4) resolution of racemic-**12** by using dibenzoyl-L-tartaric acid to provide (+)-**12** in 96.2% ee value.

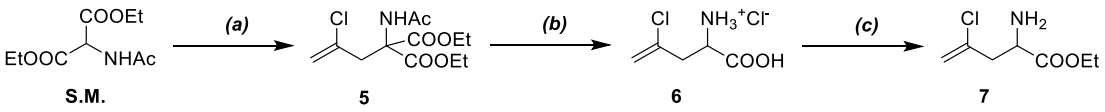
EXPERIMENTAL SECTION

General Procedures:

^{1}H NMR spectra were recorded on 400 MHz or/and 500 MHz (126 MHz for ^{13}C NMR) Bruker FT-NMR spectrometers and calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$ @ δ 7.26 ppm ^{1}H NMR, δ 77.16 ppm ^{13}C NMR; DMSO @ δ 2.50 ppm ^{1}H NMR, δ 39.52 ppm ^{13}C NMR). The following abbreviations (or combinations thereof) were used to explain ^{1}H NMR multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet, br = broad. High Resolution Mass (MS) analysis was obtained using on an Agilent 6210 LC/MSD TOF spectrometer system with Electrospray Ionization (ESI).

Experimental procedure:

Note: compound **5**, **6**, **7**, **8**, **9**, **10**, **11**, **13**, **14** were prepared as crude materials in the entire process. However, it was counted as 100% purity when calculating the stoichiometery.



S.M. **5** **6** **7**

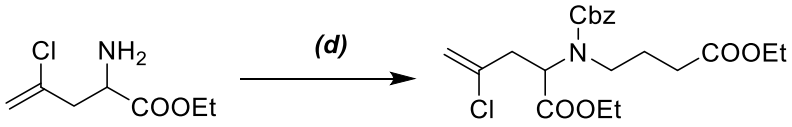
*Synthesis of **5***. A 50 L glass reactor was charged with diethyl acetamidomalonate (5.00 kg, 23.02 mol), anhydrous K_2CO_3 (6.35 kg, 46.04 mol), KI (0.76 kg, 4.58 mol), TBAB (0.37 kg, 1.15 mol), and acetonitrile (25 L). After being stirred for 20 min, 2,3-dichloropropene (3.07 kg, 27.62 mol) was added. The reaction mixture was stirred at 85–90 °C for about 10 h, at which time the starting material was consumed over 95% (HPLC). After being cooled to 25 °C, dilute $HCl_{(aq)}$ (1 M) was

slowly added to neutralize the reaction mixture to pH 7–7.5. The reaction mixture was allowed to stand at r.t. for a few minutes . The layers were separated. The organic layer was concentrated under reduced pressure at 50 °C to provide a crude slurry that was dissolved in EtOH–H_2O (1:10, 20 L). The mixture was stirred at 25 °C for 1 h and compound **5** crystallized out of the solution. After filtration through a Buchner funnel, the crystals were washed with water (2 × 5 L). The final product weighted 10.50 kg (wet weight), which was used directly in the next step. Data for compound **5**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.28 (d, *J* = 1.2 Hz, 1H), 5.17 (d, *J* = 1.1 Hz, 1H), 4.26 (qd, *J* = 7.1, 2.4 Hz, 4H), 3.47 (s, 2H), 2.03 (s, 3H), 1.55 – 1.51 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 169.3, 167.3, 136.5, 117.8, 65.2, 63.0, 41.6, 23.0, 14.0. HRMS (m/z): calc. for $C_{12}H_{19}ClNO_5$ $[M+H]^+$ = 292.0952; found, 292.0954.

*Synthesis of **6***. Compound **5 [**10.50 kg (wet weight)] was charged to a 100 L glass reactor. $HCl_{(aq)}$ (6 M, 56.5 L) was added. The mixture was refluxed for about 8–12 h at 100 °C. After completion of the reaction, activated charcoal (650 g) was added. After being cooled to below 50 °C, the reaction mixture was filtered through a Buchner funnel. The filter cake was washed with water, and the combined filtrates were concentrated under reduced pressure at 80–85 °C to provide crude **6** (4.05 kg) as a yellowish solid, which was used directly in the next step.

*Synthesis of **7***. A 50 L glass reactor was charged with crude **6** (4.05 kg), diethyl carbonate (12 L), and anhydrous ethanol (4 L). To the stirred mixture, was added H_2SO_4 (1.13 kg, 98%, 11.51 mol) in 20–30 min. The reaction mixture was heated for about 20–25 h at 85–95 °C. After completion of the reaction, the mixture was concentrated under reduced pressure at 50–60 °C. The residual liquid was diluted with water. After being cooled to below 10 °C, $NaOH_{(aq)}$ (30%wt, 4.88kg) was slowly added to neutralize the mixture to pH 8–9. After being warmed to 25 °C, the mixture was extracted with DCM (3 × 10 L).The combined organic layers were dried over

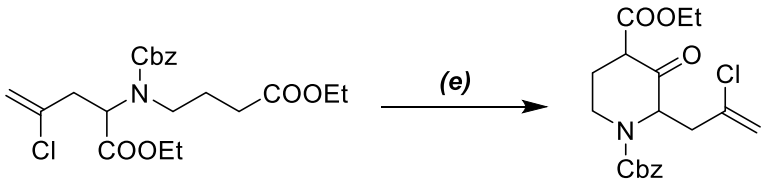
anhydrous Na_2SO_4, and filtered through a Buchner funnel. The filtrate was concentrated under reduced pressure at 40 °C to provide crude **7** as a brownish oil (3.07 kg, in 87% ^{1}H-NMR purity), which was used directly in the next step. Data for compound **7**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.30 (d, *J* = 1.3 Hz, 1H), 5.26 (q, *J* = 1.1 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.78 (dd, *J* = 8.6, 4.8 Hz, 1H), 2.81 (ddd, *J* = 14.2, 4.8, 1.1 Hz, 1H), 2.56 (dd, *J* = 14.2, 8.6 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 174.5, 138.5, 115.9, 61.4, 52.3, 44.6, 14.3. HRMS (m/z): calc. for $C_7H_{13}ClNO_2$ $[M+H]^+$ = 178.0635; found, 178.0628.



7 **8**

*Synthesis of **8***. A 50 L glass reactor was charged with the crude compound **7** (3.07 kg, 87% by ^{1}H-NMR), anhydrous Na_2CO_3 (5.49 kg, 51.79 mol), TBAI (0.64 kg, 1.73 mol), and toluene (9 L). After being stirred for 20 min, ethyl 4-bromobutyrate (3.37 kg, 17.26 mol) dissolved in toluene (6 L) was added. The reaction mixture was stirred at 75–80 °C for about 72 h, at which time compound **7** was consumed over 90% (monitored by HPLC). After being cooled to 20–25 °C, water (9 L) was added. After being stirred for 10–15 min, benzyl chloroformate (2.94 kg, 17.26 mol) was added dropwise in 2–3 h. The reaction mixture was stirred at 25 °C for about 2 h before addition of water (10 L) and toluene (10 L). The layers were separated. The organic layer was washed with $NaOH_{(aq)}$ (5%wt, 15 L), water (20 L), $HCl_{(aq)}$ (5%wt, 15 L), and water (20 L) respectively. After the organic layer was stirred at 25 °C for 1 h with activated charcoal (250 g), it was filtered through a Buchner funnel. The filtrate was concentrated under reduced pressure at 60 °C to give crude **8** (8.10 kg) as a brownish oil, which was used directly in the next step. Data for compound **8**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.39 – 7.27 (m, 5H), 5.19 (d, *J* = 1.3 Hz,

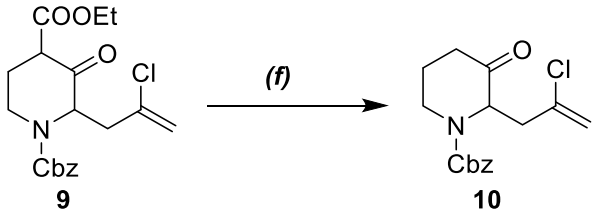
1H), 5.17 – 5.07 (m, 2.6H), 5.02 (s, 0.4H), 4.27 – 3.89 (m, 5H), 3.68 – 3.56 (m, 1H), 3.23 – 3.10 (m, 1.6H), 3.02 – 2.90 (m, 1.4H), 2.47 – 2.26 (m, 2H), 1.99 – 1.84 (m, 2H), 1.27 – 1.17 (m, 4.8H), 1.13 (t, J = 7.2 Hz, 1.2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.3, 173.2, 170.3, 170.2, 155.6, 138.8, 138.4, 136.7, 136.2, 128.7, 128.6, 128.4, 128.2, 127.9, 116.3, 116.0, 67.6, 67.4, 61.7, 60.5, 59.6, 58.7, 49.2, 48.9, 40.5, 39.4, 31.6, 31.4, 24.2, 23.7, 14.4, 14.14, 14.07. HRMS (m/z): calc. for $C_{21}H_{29}ClNO_6$ $[M+H]^+$ = 426.1683; found, 426.1678.



8 **9**

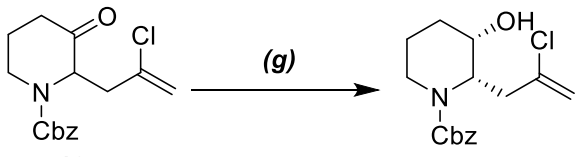
Synthesis of ***9***. A 100 L glass reactor was charged with *t*-BuONa (3.31 kg, 34.53 mol) and anhydrous THF (38 L). After being cooled to −5 °C, crude compound **8** (8.10 kg) dissolved in THF (15 L) was added dropwise in 4–5 h while keeping the temperature below 0°C. The reaction mixture was stirred at 0–5 °C for about 3 h. After completion of the reaction, $HCl_{(aq)}$ (1 M) was slowly added to neutralize the reaction mixture to pH 5–6. After addition of EA (10 L), the reaction mixture was stirred at 25 °C for a few minutes. The layers were separated and the organic layer was washed with saturated $NaCl_{(aq)}$ (2 × 20 L), while the aqueous layer was extracted with EA (10 L). After addition of activated charcoal (500 g), the combined organic layers were stirred at 25 °C for 1 h, and then filtered through a Buchner funnel. The filtrate was concentrated under reduced pressure to give crude **9** (5.44 kg) as a light brown oil, which was used directly in the next step. Data for compound **9**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 12.23 (s, 1H), 7.41 – 7.28 (m, 5H), 5.27 – 5.05 (m, 4H), 5.01 (s, 0.4H), 4.94 – 4.82 (m, 0.6H), 4.30 (dd, J = 13.6, 5.7 Hz, 0.6H), 4.23 (q, J = 7.1 Hz, 2H), 4.19 – 4.12 (m, 0.4H), 3.09 – 2.66 (m, 3H), 2.46 – 2.23 (m, 2H), 1.30 (t, J =

7.1 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.0, 171.9, 168.8, 168.2, 155.2, 155.1, 138.4, 138.3, 136.7, 136.3, 128.5, 128.2, 128.0, 115.8, 115.6, 97.7, 97.4, 67.7, 67.5, 61.0, 52.7, 52.5, 41.5, 40.7, 38.2, 37.3, 22.8, 22.3, 14.3. HRMS (m/z): calc. for $C_{19}H_{23}ClNO_5$ $[M+H]^+$ = 380.1265; found, 380.1266.



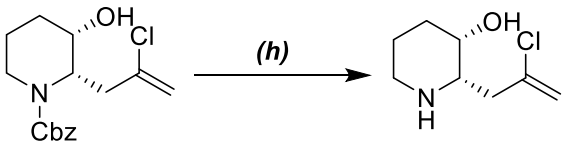
9 **10**

*Synthesis of **10***. A 50 L glass reactor was charged with crude **9** (5.44 kg), LiCl (0.61 kg, 14.33 mol), H_2O (2.7 L), and DMF (16.3 L). The reaction mixture was stirred at 120 °C for about 14 h. After completion of the reaction, the mixture was cooled to 25 °C. After addition of H_2O (25 L) and MTBE (30 L), the layers were separated. The organic layer was washed with water (2 × 25 L). The combined aqueous layers were extracted with MTBE (25 L). Then the combined organic layers were concentrated under reduced pressure at 45 °C to give crude **10** (4.19 kg) as a brownish oil, which was used directly in the next step. Data for compound **10**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.40 – 7.29 (m, 5H), 5.32 – 5.03 (m, 4H), 4.86 (s, 1H), 4.34 – 3.98 (m, 1H), 3.23 (s, 1H), 2.94 – 2.59 (m, 2H), 2.59 – 2.39 (m, 2H), 2.05 (s, 1H), 2.01 – 1.92 (m, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 206.7, 155.5, 128.6, 128.3, 116.1, 67.8, 61.8, 37.1, 22.5. HRMS (m/z): calc. for $C_{16}H_{19}ClNO_3$ $[M+H]^+$ = 308.1053 ; found, 308.1057.



10 **(±)-11**

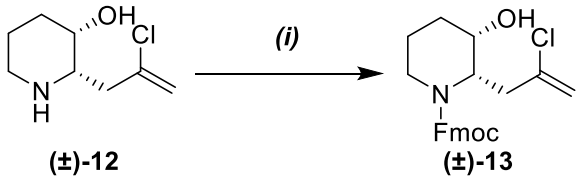
*Synthesis of **11***. A 50 L glass reactor was charged with EtOH (18 L). After being cooled to 5–10 °C, $NaBH_4$ (0.51 kg, 13.61 mol) was added and crude **10** (4.19 kg) dissolved in EtOH (9 L) was added dropwise while keeping the temperature below 10 °C. The reaction mixture was stirred at 5–10 °C for about 1 h. After completion of the reaction, H_2O (20 L) was slowly added followed by MTBE (25 L). After standing at r.t. for a few minutes, the layers were separated. The organic layer was washed with $NaOH_{(aq)}$ (10%wt, 10L), $HCl_{(aq)}$ (5%wt, 2 × 10 L), and water (10 L), respectively. The combined aqueous layers were extracted with MTBE (25 L). After addition of activated charcoal (500 g), the combined organic layers were stirred at 25 °C for 1 h before filtration through a Buchner funnel. The filtrate was concentrated under reduced pressure at 45 °C to give crude **11** (3.50 kg) as a brown oil, which was used directly in the next step. Data for compound **11**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.42 – 7.27 (m, 5H), 5.21 – 5.06 (m, 4H), 4.76 (s, 1H), 4.05 (d, *J* = 14.0 Hz, 1H), 3.92 – 3.79 (m, 1H), 2.84 – 2.60 (m, 3H), 1.87 – 1.76 (m, 1H), 1.76 – 1.65 (m, 1H), 1.58 – 1.42 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 155.8, 139.8, 136.7, 128.5, 128.2, 128.1, 114.5, 68.7, 67.5, 54.2, 37.9, 33.5, 27.9, 24.3. HRMS (m/z): calc. for $C_{16}H_{21}ClNO_3$ $[M+H]^+$ = 310.1210; found, 310.1208.



(±)-11 **(±)-12**

*Synthesis of **12***. A 50 L glass reactor was charged with crude **11** (3.50 kg), $HCl_{(aq)}$ (6 M, 18.83 L) and EtOH (20 L). The reaction mixture was refluxed for about 30 h. After completion of the reaction, the mixture was concentrated under reduced pressure at 50–55 °C to remove EtOH. To the residual liquid was added with MTBE (2 × 20 L), and resulting mixture was stirred for a few

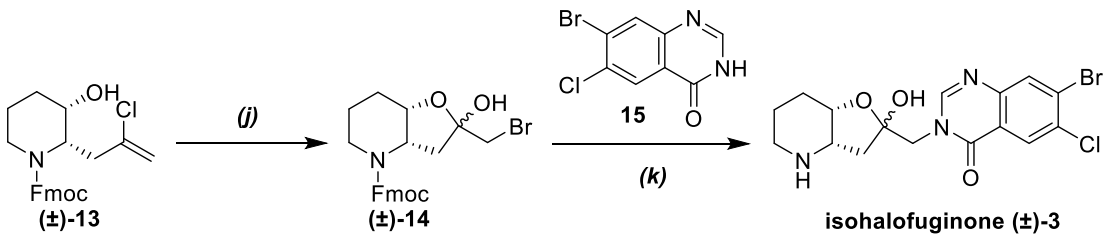
minutes. The layers were separated. The aqueous layer was neutralized with $NaOH_{(aq)}$ (40%wt) to pH >11, and then extracted with EA (2 × 20 L). The combined organic layer was dried over anhydrous $MgSO_4$ before filtration through a Buchner funnel. The filtrate was concentrated under reduced pressure to give crude **12** that was dissolved in acetonitrile (5 L) at 70 °C. After standing at 25 °C, compound **12** crystallized out of the solution. After filtration through a Buchner funnel, the collected crystals were dried in vacuum. The final product weighted 1.01 kg (> 98% ^{1}H NMR purity, 25% yield for 8 steps from diethyl acetamidomalonate). Data for compound **12**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.25 (d, *J* = 1.1 Hz, 1H), 5.23 (t, *J* = 1.0 Hz, 1H), 3.65 (s, 1H), 3.03 (ddt, *J* = 11.5, 4.3, 2.0 Hz, 1H), 2.88 (ddd, *J* = 7.5, 6.3, 1.4 Hz, 1H), 2.65 (td, *J* = 11.9, 2.9 Hz, 1H), 2.49 (d, *J* = 6.8 Hz, 2H)., 1.91 (dtt, *J* = 13.4, 4.0, 2.0 Hz, 1H), 1.73 (qt, *J* = 13.1, 4.3 Hz, 1H), 1.54 (tdd, *J* = 13.3, 4.7, 2.5 Hz, 1H), 1.45 (ddq, *J* = 12.9, 4.9, 2.6 Hz, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 139.8, 115.2, 67.1, 57.7, 47.2, 43.0, 32.2, 20.4. HRMS (m/z): calc. for $C_8H_{15}ClNO$ $[M+H]^+$ = 176.0842; found, 176.0837.



(±)-12 **(±)-13**

*Synthesis of **13***. A 50 L glass reactor was charged with compound **12** (1.00 kg, 5.69 mol), anhydrous Na_2CO_3 (0.91 kg, 8.54 mol), H_2O (5 L), and dioxane (5 L). After being cooled to 5–10 °C, Fmoc-Cl (1.47 kg, 5.69 mol) dissolved in dioxane (2 L) was added dropwise while keeping the temperature below 20 °C. The reaction mixture was stirred at 25 °C for about 1 h. After completion of the reaction, the reaction mixture was added with EA (20 L) and water (20 L). The layers were separated. The organic layer was washed with saturated $NaCl_{(aq)}$ (2 × 5 L), while the aqueous layer was extracted with EA (10 L). To the combined organic layers were added activated

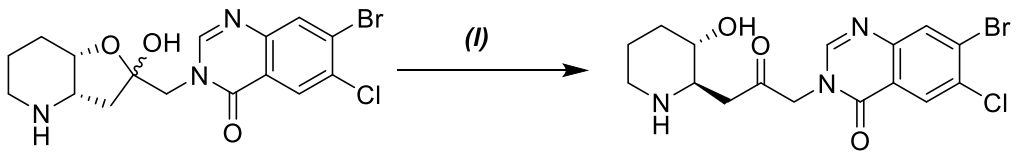
charcoal (500 g) and then stirred at 25 °C for 1 h. After filtration through a Buchner funnel, the organic layer was concentrated under reduced pressure to give crude **13** (2.81 kg) as a yellow slurry, which was used directly in the next step. Data for compound **13**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.81 – 7.71 (m, 2H), 7.65 – 7.53 (m, 2H), 7.40 (td, *J* = 7.5, 2.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.17 (s, 1H), 5.14 (s, 1H), 4.93 – 4.51 (m, 1H), 4.49 (dd, *J* = 10.7, 6.7 Hz, 1H), 4.41 (dd, *J* = 10.7, 6.5 Hz, 1H), 4.25 (t, *J* = 6.5 Hz, 1H), 3.92 (s, 1H), 3.76 (s, 1H), 2.85 – 2.54 (m, 3H), 1.87 – 1.74 (m, 1H), 1.73 – 1.58 (m, 1H), 1.57 – 1.33 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 155.8, 144.1, 141.5, 141.4, 139.8, 127.73, 127.70, 127.15, 127.10, 125.09, 125.06, 120.02, 119.99, 114.4, 68.4, 67.5, 54.3, 47.5, 37.9, 33.3, 27.7, 24.2. HRMS (m/z): calc. for $C_{23}H_{25}ClNO_3$ $[M+H]^+$ = 398.1523; found, 398.1530



*Synthesis of **14***. A 50 L glass reactor was charged with compound **13** (2.81 kg), H_2O (5 L), and acetonitrile (10 L). After being cooled to 0–5 °C, NBS (1.01 kg, 5.69 mol) was added in portions while keeping the temperature below 5 °C. The reaction mixture was stirred at 0–5 °C for about 0.5 h. After completion of the reaction, $Na_2SO_{3(aq)}$ (10%wt, 10 L) was added. The mixture was stirred for 0.5 h before extraction with EA (2 × 10 L). The combined organic layers were washed with saturated $NaHCO_{3(aq)}$ (5 L) and saturated $NaCl_{(aq)}$ (2 × 5 L), respectively. After addition of activated charcoal (320 g), the organic layer was stirred at 25 °C for 1 h. After addition of $MgSO_4$ (1.00 kg) the mixture was stirred for another 0.5 h. The mixture was filtered through a Buchner

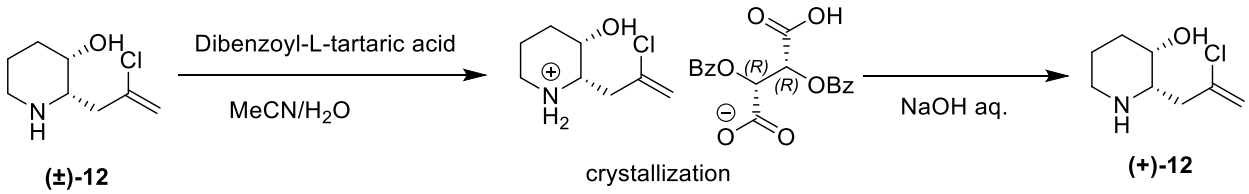
funnel, and the filtrate was concentrated under reduced pressure to give crude **14** (2.85 kg) as a slurry, which was used directly in the next step.

*Synthesis of **3***. A 100 L glass reactor was charged with compound **15** (1.40 kg, 5.41 mol), LiOH (0.15 kg, 6.26 mol) , and DMF (28 L). The reaction mixture was stirred at 25 °C for 1 h before being cooled to 0–5 °C. To the stirred mixture, crude compound **14** (2.85 kg) dissolved in DMF (2.8 L) was added dropwise in 4–5 h, while keeping the temperature below 5°C. The reaction mixture was stirred at 0–5 °C for 24 h before diethylamine (1 L) was added. The reaction mixture was stirred at 0–5 °C for another 12 h. After addition of H_2O (25 L) and EA (30 L), the layers were separated. The aqueous layer was extracted with EA (3 × 20 L). The combined organic layers were concentrated under reduced pressure. To the residual product was added Lactic $acid_{(aq)}$ (85%wt, 1.5 kg). The mixture was stirred at 25 °C for 1 h before being extracted with MTBE (2 × 10 L). The aqueous layer (containing compound **3**) was neutralized to pH 8–9 with K_2CO_3 before extraction with EA (3 × 15 L). The combined organic layers (containing compound **3**) were concentrated under reduced pressure to give crude **3**, to which EA (8 L) was added. The mixture was stirred at 25 °C for 0.5 h before being filtered through a Buchner funnel. The filter cake was dried in vacuum. The final product weighted 1.84 kg as a white solid. Data for compound **3**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (s, 1H), 8.26 (s, 1H), 7.98 (s, 1H), 4.34 (d, *J* = 13.9 Hz, 1H), 4.16 (d, *J* = 13.9 Hz, 1H), 3.88 (t, *J* = 3.1 Hz, 1H), 3.29 (t, *J* = 3.4 Hz, 1H), 2.97 (d, *J* = 10.9 Hz, 1H), 2.52 (t, *J* = 11.8 Hz, 1H), 2.10 (d, *J* = 15.1 Hz, 1H), 2.03 (dd, *J* = 13.1, 3.7 Hz, 2H), 1.83 (d, *J* = 13.2 Hz, 1H), 1.81 – 1.72 (m, 1H), 1.54 (ddt, *J* = 15.0, 12.0, 3.4 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 160.1, 149.5, 147.2, 133.4, 132.7, 129.4, 127.9, 122.1, 105.3, 78.0, 55.8, 50.3, 44.67, 43.7, 26.9, 20.2. HRMS (m/z): calc. for $C_{16}H_{18}BrClN_3O_3$ $[M+H]^+$ = 414.0220/416.0200; found, 414.0216/416.0197.



isohalofuginone (±)-3 **halofuginone (±)-1**

*Synthesis of **1***. A 50 L glass reactor was charged with above obtained white solid **3** (1.84 kg) and EtOH (20 L). The mixture was refluxed for about 24 h, at which time the conversion to halofuginone was over 94% (HPLC). After being cooled to 55–60 °C, the reaction mixture was filtered through a Buchner funnel. The filter cake was washed with EtOH and then dried in vacuum. The final product weighted 1.65 kg as a white solid in 98.5% HPLC purity (69% yield for 4 steps from compound **12**, and 17% overall yield for 12 steps). Data for compound **1**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (s, 1H), 8.22 (s, 1H), 8.15 (s, 1H), 4.99 (d, J = 2.8 Hz, 2H), 4.79 (d, J = 5.8 Hz, 1H), 2.98 (dt, J = 15.3, 4.7 Hz, 2H), 2.78 (d, J = 12.3 Hz, 1H), 2.64 (td, J = 8.9, 3.8 Hz, 1H), 2.44 (dd, J = 15.5, 8.7 Hz, 1H), 2.36 (td, J = 12.1, 2.7 Hz, 1H), 1.95 – 1.83 (m, 1H), 1.56 (dt, J = 13.3, 3.2 Hz, 1H), 1.34 (qt, J = 12.4, 3.7 Hz, 1H), 1.28 – 1.13 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 200.7, 158.6, 149.5, 147.2, 132.4, 131.8, 128.4, 126.8, 121.7, 66.7, 56.2, 54.4, 43.0, 30.5, 20.1. HRMS (m/z): calc. for $C_{16}H_{18}BrClN_3O_3$ $[M+H]^+$ = 414.0220/416.0200; found, 414.0214/416.0195.



(±)-12 crystallization **(+)-12**

*Synthesis of (+)-**12***. A 500 mL glass reactor was charged with compound (±)-**12** (10.0 g, 0.057 mol) and acetonitrile (100 mL). After being warmed to 60 °C, dibenzoyl-L-tartaric acid (21.4, 0.06 mol) dissolved in acetonitrile (60 mL) was added dropwise in 10 min. After being stirred at 60 °C for 20–30 min, the reaction mixture was cooled to 25 °C and stirred for 2 h. The white solid

crystallized out of the solution. After filtration through a Buchner funnel, the collected crude solid was washed with acetonitrile (20 ml) and then dried in vacuum. This salt was then mixed with H_2O–acetonitrile (1:4, 150 mL). The stirred mixture was warmed to 80 °C until most of the solid was dissolved. After filtration through a Buchner funnel, the filtrate was cooled to 25 °C and stirred for 2 h. The white solid crystallized out of the solution. After filtration through a Buchner funnel, the white solid was washed with acetonitrile (50 ml), and then dried in vacuum. The collected product weighted 9.7 g as a white solid. A 500 mL glass reactor was charged with the above obtained solid (9.7 g), H_2O (50 mL), and EA (50 mL). $NaOH_{(aq)}$ (1 M) was slowly added to neutralize the stirred mixture to pH 12–14. After standing at r.t. for a few minutes, the layers were separated. The aqueous layer was extracted with EA (2 × 50 mL). The combined organic layers were dried on anhydrous Mg_2SO_4. After filtration through a Buchner funnel, the filtrate was concentrated under reduced pressure at 50 °C to provide compound (+)-**12** (3.2 g, 32%) as a white solid.

*Following the procedures for the synthesis of racemic halofuginone, the preparations of compound (+)-**13**, (+)-**14**, (+)-**3**, and (+)-**1** starting from compound (+)-**12** were completed.*

*Optical rotation values of compound (+)-**12**, (+)-**13**, (+)-**14**, (+)-**3**, and (+)-**1** (Optical rotation were obtained at 20 °C, measured at 589 nm) are as follows:*

compound (+)-**12**: $[\alpha]_D$= +8.4° (*c* = 0.46, MeOH);

compound (+)-**13**: $[\alpha]_D$= +25.4° (*c* = 0.35, $CHCl_3$);

compound (+)-**14**: $[\alpha]_D$= +36.7° (*c* = 0.30, $CHCl_3$);

compound (+)-**3**: $[\alpha]_D$= +81.2° (*c* = 0.53, $CHCl_3$);

compound (+)-**1**: $[\alpha]_D$= +18.5° (*c* = 0.53, DMSO).

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, ^{13}C NMR, HPLC analysis

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Author Contributions

‡H.X. and W.Y. contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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