Natural Products

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Total Synthesis and Biological Evaluation of Jerantinine E**

*Reto Frei, Davide Staedler, Aruna Raja, Raimo Franke, Florenz Sasse, Sandrine Gerber-Lemaire and Jérôme Waser**

Indole alkaloids have attracted the attention of synthetic chemists because of their intriguing structural features and remarkable bioactivities. This interest is perhaps best represented by the *Vinca* alkaloid vinblastine (**1**), isolated from *Catharanthus roseus*, which is currently among the foremost drugs used to combat cancer.[1] However, *de novo* synthesis of novel vinblastine analogues remains a daunting task due to its highly complex structure. Consequently, the discovery of simpler bioactive *Aspidosperma* alkaloids is of high interest to facilitate the discovery of new anti-cancer agents. Not surprisingly, intensive synthetic efforts have been directed towards the synthesis of vindoline (**2**),[2] or simpler *Aspidosperma* alkaloids such as aspidospermidine (**3**)[3] and vincadifformine (**4**).[4]

In 2008, Kam and co-workers reported the isolation of seven new *Aspidosperma* indole alkaloids, jerantinine A-G, from a leaf extract of the Malayan plant *Tabernaemontana corymbosa*.[5] Among them was jerantinine E (**5**), which is structurally related to vincadifformine (**4**) but has a more oxidized indole core. The jerantinines displayed significant cytotoxic activity (half maximal inhibitory concentration; IC_{50} = 0.27-0.96 μg/L (0.70-2.50 μM)) against human KB cells, which is rare among simple *Aspidosperma* alkaloids. Nevertheless, the mode of action for the cytotoxicity of the jerantinines is currently unknown. Furthermore, the highly oxygenated core of the jerantinines renders them challenging synthetic targets and a total synthesis of jerantinines A-G has not yet been reported.

Herein, we describe the first total synthesis of (±)-jerantinine E (**5**) in 17 steps and 15.7% overall yield from δ-valerolactam (**10**).

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

The availability of a significant amount of the natural product permitted the separation of the enantiomers, in-depth biological evaluation of the cytotoxic activity in various human cancer cell lines, as well as a first investigation on the origin of the observed cytotoxicity.

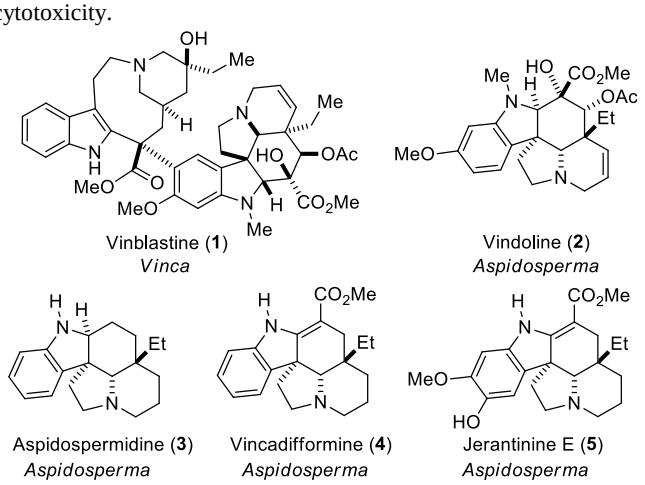


Figure 1. *Vinca* and *Aspidosperma* alkaloids.

As shown in our retrosynthesis (Scheme 1, **A**), we envisaged that the free hydroxyl group of jerantinine E (**5**) could be generated *via* selective demethylation during the last step of the total synthesis. This strategy would simplify the synthesis by avoiding a multi-step hydroxyl group protecting/deprotecting sequence. We expected this selective methyl group removal to proceed under oxidative conditions due to the presence of the electron-rich nitrogen in the *para* position.[6] As such, the indole core could be oxidized to the corresponding iminoquinone followed by *in situ* reduction to give **5**. We next planned to build-up the **e**-ring system by a bis-alkylation and to install the methyl ester group on the **c**-ring *via* acylation using Mander's reagent. This ring formation/acylation sequence was developed during the synthesis of *Aspidosperma* alkaloids by Rawal and co-workers, albeit with a less oxygenated indole core.[3d] The requisite starting material for this sequence could be generated through *N*-protecting group removal and deoxygenation of intermediate **6**. Tetracyclic product **6** was envisaged to be accessed by a selective cyclization (formal homo-Nazarov reaction) of aminocyclopropane **7**, previously developed in our group,[7] forming the central ring system **c**. Cyclization precursor **7** would finally be obtained by adding an organometallic reagent derived from commercially available indole **9** onto Weinreb amide **8**.

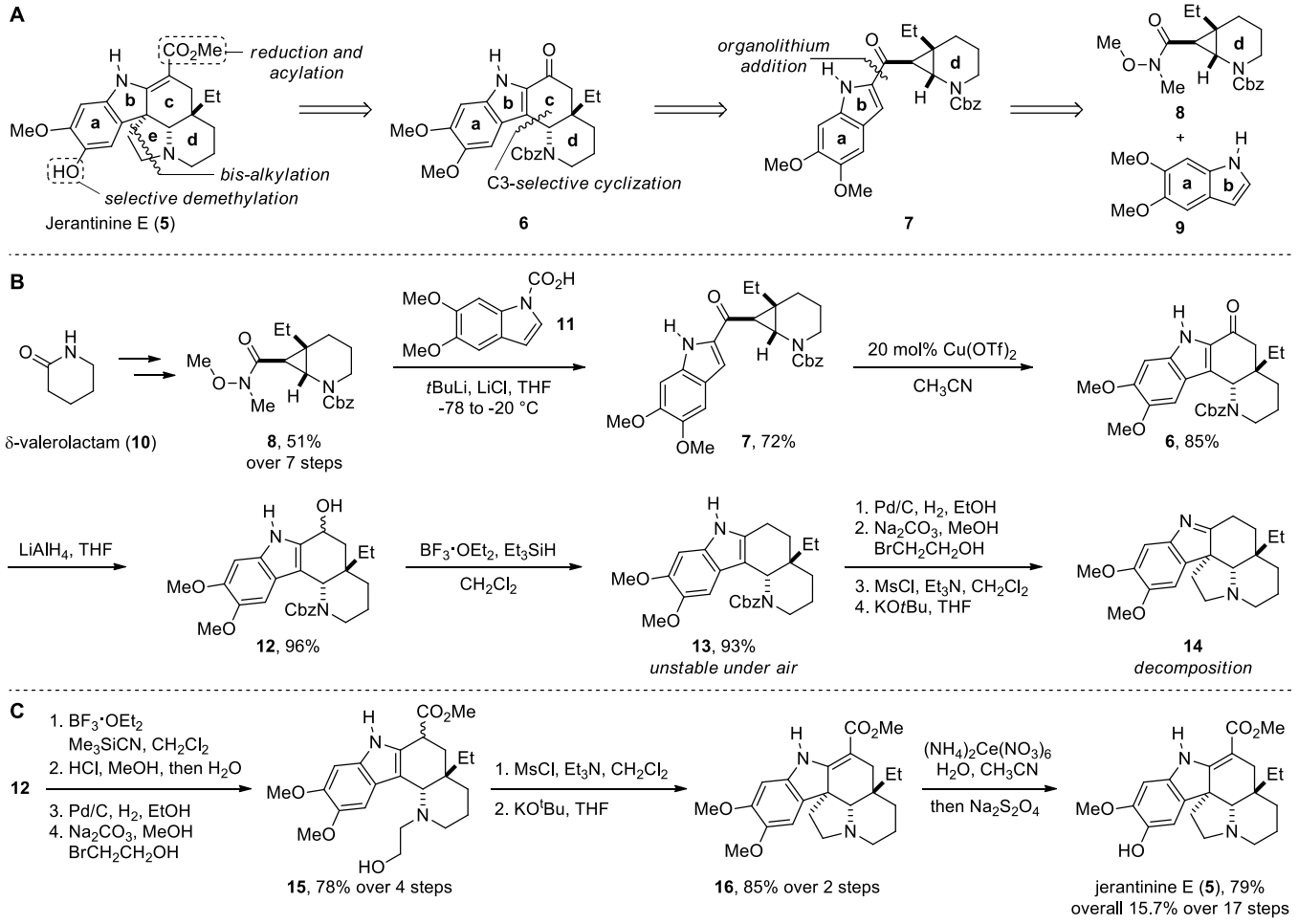
Our synthesis commenced with the coupling of Weinreb amide **8**, obtained in seven steps and 51% overall yield from commercially available δ-valerolactam (**10**),[7] and the bis-lithiated organolithium reagent derived from *N*-carboxy indole **11** (Scheme 1, **B**). Selecting the carboxylate as *N*-indole protecting group had a dual purpose, as it directed the lithiation to the C-2 indole position and was readily removed during the aqueous work-up. The desired aminocyclopropane **7** was obtained in 72% yield. The formal homo-

Nazarov cyclization of **7** afforded the *cis*-diastereoisomer of **6** exclusively in 85% yield with high C-3 regioselectivity.[8]

At this point of the synthesis, four of the five ring systems of jerantinine E (**5**) were installed and we turned our attention to the formation of the **e**-ring. Deoxygenation of the ketone was unexpectedly challenging however, and the reported one-step reduction by Wenkert and co-workers led only to decomposition of the starting material.[9] Thus, a two-step procedure was devised to remove the ketone functionality. Alcohol **12** was obtained in 96% yield employing lithium aluminium hydride under mild conditions. We reasoned that an ionic reduction could be ideal at this stage to remove the hydroxyl group, as a facile carbocation formation can be envisaged due to the highly electron-rich adjacent indole ring system. Indeed, treatment of alcohol **12** with boron trifluoride etherate at low temperature led to carbocation formation, which upon triethylsilane mediated hydride transfer gave intermediate **13** in 93% yield. Compound **13** was highly unstable under air and the subsequent transformations, including Cbz-removal and Rawal's three-step procedure,[3d] had to be completed in one-pot while carefully using Schlenk techniques. Although liquid chromatography-mass spectrometry analysis of the reaction progress indicated that Cbz-deprotection, alkylation, mesylation and base-mediated ring-closure were working, we were unable to isolate the desired pentacyclic compound **14** as it immediately decomposed into several unidentifiable products during work-up.

At this point, it became clear that a new approach avoiding highly electron-rich intermediates and reactive imines, such as **14**, had to be designed to gain access to jerantinine E (**5**). We speculated that these objectives could be realized by introducing the electron-withdrawing ester group present on the natural product prior to the instalment of the last ring system (Scheme 1, **C**). Thus, the presumably reactive imine double bond would migrate into conjugation with the ester group upon closure of the fifth ring system.

To test this hypothesis, we made use of the facile carbocation formation through the treatment of alcohol **12** with boron trifluoride etherate. The carbocation intermediate was reacted with trimethylsilylcyanide and the resulting nitrile product was transformed into ester **15** *via* Pinner methanolysis, Cbz-deprotection and alkylation with 2-bromoethanol. Gratifyingly, intermediate **15** successfully underwent the ring-closing procedure, generating pentacyclic product **16** in 85% yield. As postulated, the presence of the ester group facilitated double bond migration into conjugation, producing a perfectly stable compound. Finally, selective demethylation of **16** was possible *via* cerium ammonium nitrate mediated oxidation followed by *in situ* reduction with sodium dithionite to give jerantinine E (**5**) in 79% yield. Thus, the first total synthesis of jerantinine E (**5**) was accomplished in 17 steps and a 15.7% overall yield starting from δ-valerolactam (**10**).



Scheme 1. Retrosynthetic analysis (**A**), initial approach (**B**) and final (**C**) synthetic route for the total synthesis of jerantinine E (**5**).

With significant amounts of jerantinine E (**5**) in hand, we focused our attention on exploring its potential as an anticancer agent. Considering that breast and lung cancers are among the most frequent and fatal types of this disease,[10] two human-derived breast- and lung-cancer cell lines were selected to investigate the cytotoxic activity of jerantinine E (**5**). A first moderately invasive cell line (MCF-7) was selected from breast ductal carcinoma in conjunction with a second highly invasive breast cancer cell line (MDA-MB-231).[11] The human adenocarcinoma cell line (A549) derived from alveolar epithelial cells and adenosquamous carcinoma cell line (HTB-178)[12] were chosen to serve as lung cancer model cell lines. The two enantiomers of jerantinine E (**5**) were first separated by chiral chromatography and their cytotoxic activity examined separately. The cellular assays showed that only naturally occurring (-)-jerantinine E (**5**) was significantly bio-active (Figure 2).[13]

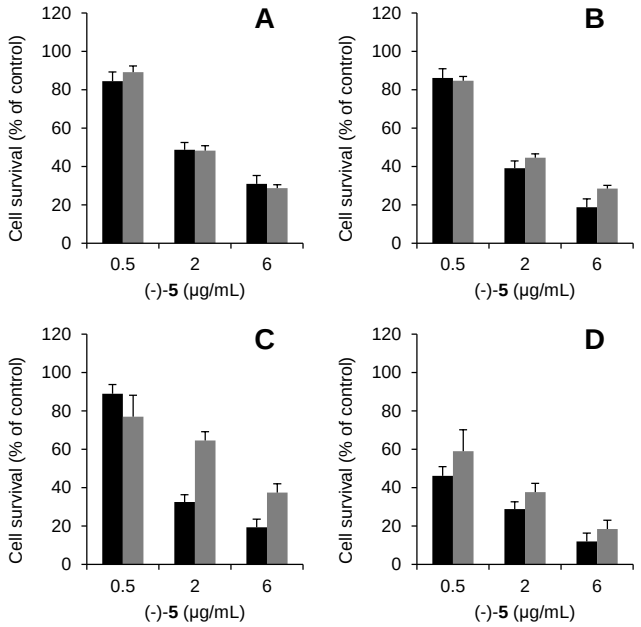


Figure 2. Effect of (-)-jerantinine E (**5**) on the survival of breast cancer cells (**A** (24 h exposure) and **B** (72 h exposure) black bar: MDA-MB-231, grey bar: MCF-7) and lung cancer cells, (**C** (24 h exposure) and **D** (72 h exposure) black bar: A549, grey bar: HTB-178). Please see the Supporting Information for additional experimental details.

Both breast cancer cell lines gave comparable inhibition values for cell growth after 24 and 72 hours (Figure 2A and 2B) having IC_{50}-values between 1.7 to 2.3 µg/mL (4.4 to 6.0 µM). The screening assay in the two lung cancer cell lines revealed that (-)-jerantinine E (**5**) exhibits greater cytotoxicity against the adenocarcinoma A549 cell line compared to adenosquamous HTB-178 cells at both, 24 and 72 hours (Figure 2C and 2D). In the four tested human cancer cell lines, (-)-jerantinine E (**5**) displayed the highest cytotoxic effect in the A549 cell line with an IC_{50} after 24 and 72 hours of exposure of 1.6 and 0.4 µg/mL (4.2 and 1.0 µM), respectively (Figure 2C and 2D).

After studying the cytotoxic activity profile for jerantinine E (**5**), we set out to elucidate possible mechanisms for the observed cell growth inhibition. First, a cell migratory assay was carried out with the above described human-derived cancer cell lines. Inhibition of cell migration ranging between 50 and 90% was observed in all four cancer cell lines after 15 hours of exposure to 2 and 6 µg/mL of jerantinine E (**5**).[14] Second, an impedance study of jerantinine E (**5**) against the L-929 (mouse fibroblast) cell line was carried out. In this assay, cells are placed on a gold electrode plate and treated with the test compound at their IC_{90} concentration. During a five day time period, the change in impedance, due to changes in the morphological state of the cells upon compound exposure, is measured. Through cluster analysis, the obtained data can then be matched to the data of a reference compound with a known target.[15] Using this approach, we found that jerantinine E (**5**) displayed an impedance profile in accordance with reference compounds, such as colchicine, which affects the microtubule network. An immunofluorescent staining assay was then carried out (Figure 3) with PtK2 kidney cells to confirm this possible mode of action. As shown in Figure 3B, multi-lobed nuclei were observed after exposing PtK2 kidney cells to 2 µg/mL jerantinine E (**5**). Furthermore, disruption of the microtubule network already became apparent at this stage and is nearly complete at higher concentration (Figure 3C and 3D). Such observations further suggested microtubule disruption as a plausible mode of action. To confirm this hypothesis, the ability of both enantiomers and racemic jerantinine E (**5**) to inhibit tubulin polymerization was studied on the purified tubulin. The assay showed (-)-jerantinine E (**5**) to be a potent inhibitor of tubulin polymerization with an IC_{50}-value of 0.17 µg/mL (0.45 µM).[14] It is noteworthy that (-)-jerantinine E (**5**) proved to be even slightly more active than the known microtubule skeleton disruptor colchicine.

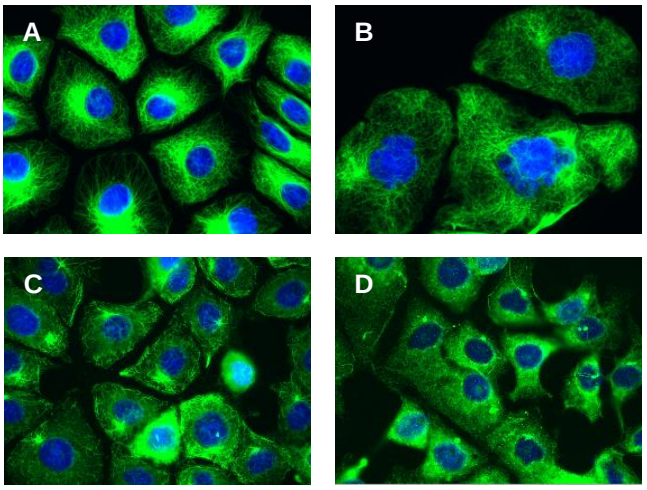


Figure 3. Immunofluorescent staining of untreated PtK2 kidney cells (**A**) and cells exposed to 2 µg/mL (**B**), 6 µg/mL (**C**) and 10 µg/mL (**D**) jerantinine E (**5**) showing the effect on microtubules (green) and the cell nucleus (blue). Please see the Supporting Information for additional experimental details.

In conclusion, we have described the first total synthesis of the indole alkaloid jerantinine E (**5**) in 17 steps and 15.7% overall yield starting from δ-valerolactam (**10**). An efficient and highly selective formal homo-Nazarov cyclization of an aminocyclopropane was used to give access to four of the five rings contained in the complex polycyclic core of the natural product. A novel strategy to install the ester group onto the highly sensitive indole alkaloid core was devised, addressing the challenges represented by the highly oxygenated and reactive core of the molecule. Lastly, a late-stage selective demethylation led to an efficient synthetic sequence. The total synthesis enabled us to investigate the biological profile of jerantinine E (**5**). The cytotoxic activity of the natural product was determined in several human-derived breast and lung cancer cell lines, displaying for instance an IC_{50} value of 0.4 µg/mL (1.0 µM) in

the A549 lung cancer cell line. Investigations into the mode of action suggested that jerantinine E (**5**) acts *via* disruption of the microtubule network, as indicated by its potent inhibitory activity displayed in tubulin polymerization. Future work will focus on the synthesis of analogues for a detailed structure-activity relationship study with the goal of finding compounds with improved activity and potency. Additional experiments to elucidate the exact origin of the observed microtubule disruption will also be considered to further understand the source of the observed cytotoxicity.

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[13] Please see the Supporting Information for exact IC_{50}-values, including error range, for both enantiomers and racemic jerantinine E (**5**) in the four described cancer cell lines.

[14] Please see the Supporting Information for more details.

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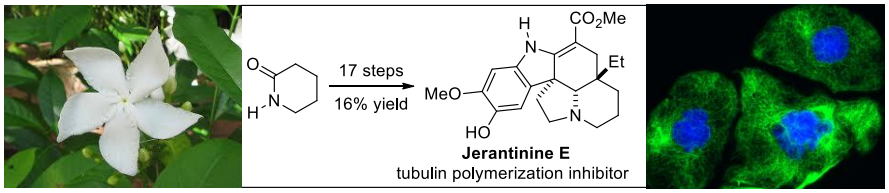


Nature's beauty: The first total synthesis of the alkaloid natural product jerantinine E is reported. Based on a selective cyclization of an aminocyclopropane, the natural product could be accessed in 17 steps and 15.7% overall yield from commercially available δ-valerolactam. Preliminary investigations into the bioactivity of jerantinine E demonstrated that it inhibits the polymerization of tubulin, displaying significant cytotoxicity and antimigratory activity against both breast and lung cancer cell lines.

Table of Contents

1. List of Abbreviations

EtOAc	Ethyl acetate
DMTMM	Dimethoxytriazin-*N*-methylmorpholinium chloride
DCM	Dichloromethane
NMM	*N*-Methylmorpholine
THF	Tetrahydrofuran
Cbz	Carboxybenzyl
Ultra	CH_2Cl_2:MeOH 3:1 (v/v) with 5% NH_3 (aq., 25%)
GHP	General hydrogenolysis procedure

2. General Methods

Technical grade solvents were used for quantitative flash chromatography. HPLC grade solvents purchased from Sigma-Aldrich or freshly distilled solvents were used for flash chromatography for compounds undergoing full characterization. Reaction solvents were dried by passage over activated alumina under nitrogen atmosphere (H_2O content < 30 ppm, Karl-Fischer titration). Commercially available reagents were purchased from Acros, Aldrich, Fluka, VWR, Aplichem or Merck and used without any further purification. Chromatographic purification was performed as flash chromatography using Macherey-Nagel silica 40-63, 60 Å, using the solvents indicated as eluent with 0.1-0.5 bar pressure. TLC was performed on Merck silica gel 60 F254 TLC plates and visualized with UV light and permanganate stain. Melting points were measured on a calibrated Büchi B-540 melting point apparatus using open glass capillaries. ^{1}H NMR spectra were measured on a Brucker DPX-400 400 MHz spectrometer, all signals are reported in ppm with the corresponding internal solvent peak or TMS as standard. The data is being reported as (s = singlet, d = doublet, t = triplet, q = quadruplet, qi = quintet, m = multiplet or unresolved, br = broad signal, coupling constant(s) in Hz, integration; interpretation). ^{13}C NMR spectra were carried out with 1H-decoupling on a Brucker DPX-400 100 MHz. All signals are reported in ppm with the corresponding internal solvent signal or TMS as standard. Infrared spectra were obtained on a JASCO FT-IR B4100 spectrophotometer with an ATR PRO410-S and a ZnSe prisma and are reported as cm^{-1} (w = weak, m = medium, s = strong, sh = shoulder). High resolution mass spectrometric measurements were performed by the mass spectrometry service of ISIC at the EPFL on a MICROMASS (ESI) Q-TOF Ultima API. Optical rotatory measurements were performed on a JASCO P-2000 Polarimeter.

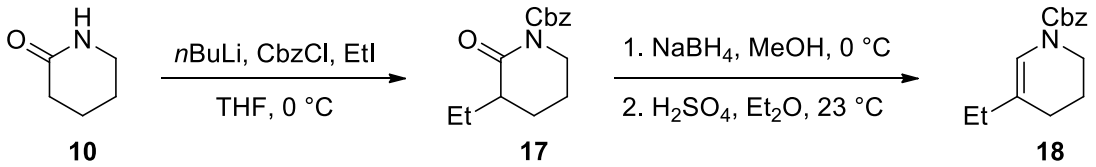
3. General Hydrogenolysis Procedure (GHP)

A solution of of Cbz-protected amine (0.02 M in ethanol) was charged at room temperature with Pd/C (0.1 eq.) and hydrogen gas was bubbled through the mixture until full conversion of the starting material was observed by TLC.[1] The suspension was filtered through a short plug of celite (prewashed with DCM) using additional amounts of DCM and EtOAc to ensure that complete product elution form the plug was achieved. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. No further purification was necessary.

[1] A batch to batch Pd/C dependency for the reaction time was observed. It is consequently important to carefully monitor the reaction *via* TLC analysis.

4. Total Synthesis of Jerantinine E

Benzyl 5-ethyl-3,4-dihydropyridine-1(2*H*)-carboxylate (18)

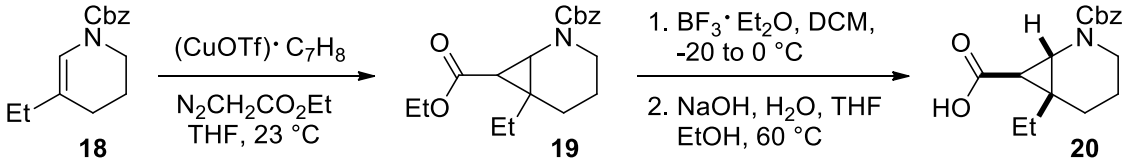


Following a slight modification of a reported procedure,[2] a 1.6 M solution of *n*BuLi in hexane (90.0 mL, 144 mmol, 2.20 eq.) was added dropwise to a solution of δ-valerolactam (**10**, 6.50 g, 65.6 mmol, 1.00 eq.) in dry THF (100 mL) at 0 °C. The resulting yellow solution was stirred at 0 °C for 30 minutes, after which freshly distilled ethyl iodide (7.91 mL, 98.0 mmol, 1.50 eq.) was added. The reaction mixture was stirred for an additional 20 minutes at 0 °C before benzyl chloroformate (9.83 mL, 68.8 mmol, 1.05 eq.) was added. The reaction mixture was stirred for 30 minutes at 0 °C, diluted with diethyl ether (170 mL) and washed with brine (2 x 70 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (pentane:EtOAc 5:1) to yield lactam **17** (12.4 g, 47.5 mmol, 72%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46-7.39 (m, 2 H, Ar*H*), 7.39-7.28 (m, 3 H, Ar*H*), 5.27 (s, 2 H, OCH_2), 3.85-3.76 (m, 1 H, NCH_2), 3.73-3.64 (m, 1 H, NCH_2), 2.40-2.29 (m, 1 H, C*H*CO), 2.07-1.73 (m, 4 H; CH_2CH_2), 1.58-1.44 (m, 2 H, CH_2), 0.96 (t, 3 H, J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.3, 154.4, 135.6, 126.8, 128.3, 128.1, 68.4, 46.2, 45.4, 25.5, 24.2, 21.7, 11.5. The values of the NMR spectra are in accordance with reported literature data.[3]

Following a reported procedure,[4] sodium borohydride (1.27 g, 34.0 mmol, 1.20 eq.) was added portionwise to a solution consisting of Cbz-protected lactam (**17**, 7.40 g, 28.3 mmol, 1.00 eq.) and methanol (150 mL) at 0 °C. The reaction mixture was stirred at 0 °C for two hours and then quenched with ice water (10 mL). The mixture was diluted with water (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting colorless oil was dissolved in dry diethyl ether (75 mL) and concentrated sulfuric acid (30 µL) was added at room temperature. The reaction mixture was stirred at room temperature for 30 minutes, then quenched with solid K_2CO_3 and dried with $MgSO_4$. The suspension was filtered and concentrated *in vacuo* to afford **18** (6.82 g, 27.8 mmol, 98%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42-7.28 (m, 5 H, Ar*H*), 6.70 (s, 0.45 H, alkene-*H* rotamer A), 6.60 (s, 0.55 H, alkene-*H* rotamer B), 5.19 (s, 1.1 H, OCH_2 rotamer B), 5.17 (s, 0.9 H, OCH_2 rotamer A), 3.57 (m, 2 H, NCH_2), 2.07-1.94 (m, 4 H), 1.82 (m, 2 H), 1.06-0.97 (m, 3 H; CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) (rotamers!) δ 153.4, 153.0, 136.5, 136.4, 128.3, 127.9, 127.8, 121.0, 120.6, 119.1, 118.7, 67.1, 67.0, 41.9, 41.8, 28.1, 28.1, 24.8, 24.7, 21.6, 21.6, 12.6, 12.4. The values of the NMR spectra are in accordance with reported literature data.[2]

[2] P. A. Grieco, M. D. Kaufman, *J. Org. Chem.* **1999**, *64*, 7586-7593.
[3] F. De Simone, J. Gertsch, J. Waser, *Angew. Chem. Int. Ed.* **2010**, *49*, 5767-5770.
[4] Y. Takeuchi, K. Azuma, M. Oshige, H. Abe, H. Nishioka, K. Sasaki, T. Harayama, *Tetrahedron* **2003**, *59*, 1639-1646.

2-((Benzyloxy)carbonyl)-6-ethyl-2-azabicyclo[4.1.0]heptane-7-carboxylic acid (**20**)

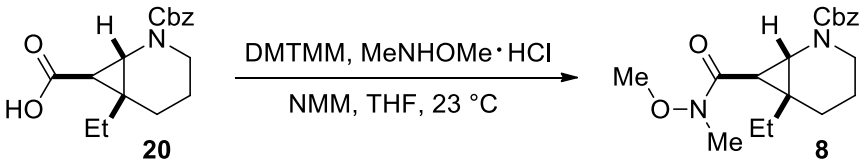


Following a slight modification of a reported procedure,[5] a solution of ethyl diazoacetate (6.05 mL, 57.1 mmol, 4.00 eq.) in dry DCM (14 mL) was added to a solution of enamine **18** (3.50 g, 14.3 mmol, 1.00 eq.) and copper triflate (I) toluene complex (148 mg, 0.286 mmol, 0.0200 eq.) in DCM (14 mL) over 18 h (1.3 mL/h) *via* syringe pump. After the addition was complete, the reaction was concentrated *in vacuo* and purified by flash column chromatography (pentane:EtOAc 15:1 to pentane:EtOAc 10:1) to yield **19** (3.67 g, 11.3 mmol, 78%) as colorless oil.

Following a reported procedure,[1] the mixture of exo and endo esters (**19**, 2.13 g, 6.43 mmol, 1.00 eq.) in DCM (25 mL) was treated at -20 °C with freshly distilled BF_3-etherate (210 µL, 0.964 mmol, 0.150 eq.). The reaction was allowed to warm up to 0 °C over a two hour time period during which the isomerization went to completion. The reaction was quenched with triethylamine (1.5 mL), diluted with diethyl ether (75 mL) and extracted with water (50 mL). The aqueous layer was extracted with additional portions of diethyl ether (2 x 30 mL) and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a pale yellow oil (2.13 g, 6.43 mmol, quant.), which was used directly in the next step. Thus, the ester-intermediate (2.13 g, 6.43 mmol, 1.00 eq.) was dissolved in a mixture of H_2O:THF:EtOH 1:1:3 (total volume = 35 mL) and cooled to 0 °C. Next, NaOH (2.31 g, 57.8 mmol, 9.00 eq.) was added portionwise and the resulting solution was heated at 60 °C for 90 minutes. The mixture was diluted with water (30 mL) and extracted with diethyl ether (3 x 30 mL). The combined organic layers were washed with water (2 x 30 mL). The combined aqueous layers were acidified with 1.0 M aq. HCl to pH = 2, followed by extraction with DCM (3 x 40 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **20** (1.87 g, 6.17 mmol, 96% over two steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39-7.21 (m, 5 H, Ar*H*), 5.23-5.09 (m, 2 H, OCH_2), 3.86 (dt, 0.7 H, *J* = 12.5, 3.1 Hz, NCH_2 rotamer A), 3.73 (m, 0.3 H, NCH_2 rotamer B), 3.55 (d, 0.3 H, *J* = 3.5 Hz, NC*H* rotamer B), 3.49 (d, 0.7 H, *J* = 3.6 Hz, NC*H* rotamer A), 2.77 (dt, 0.3 H. *J* = 12.5, 1.7 Hz, NCH_2 rotamer B), 2.66 (dt, 0.7 H, *J* = 12.5, 3.4 Hz, NCH_2 rotamer A), 2.05 (m, 1 H, C*H*CO), 1.93-1.12 (m, 6 H, CH_2), 0.99 (t, 1 H, *J* = 7.2 Hz, CH_3 rotamer B), 0.93 (t, 2 H, *J* = 7.4 Hz, CH_3 rotamer A). ^{13}C NMR ($CDCl_3$, 100 MHz) (rotamers!) δ 177.2, 176.9, 156.2, 136.5, 128.5, 128.4, 128.1, 127.7, 127.2, 67.1, 67.0, 44.9, 44.8, 41.5, 41.1, 34.7, 34.0, 31.1, 30.6, 25.9, 25.7, 21.5, 21.3, 20.9, 10.3, 9.9. The values of the NMR spectra are in accordance with reported literature data.[2]

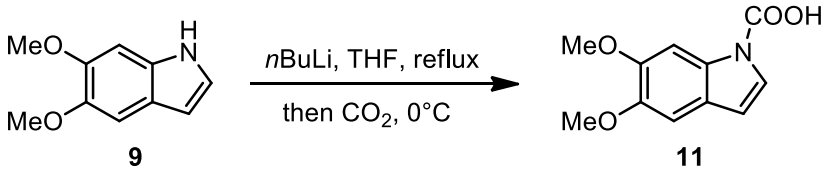
[5] R. Beumer, C. Bubert, C. Cabrele, O. Vielhauer, M. Pietzsch, O. Reiser, *J. Org. Chem.* **2000**, *65*, 8960-8969.

Benzyl 6-ethyl-7-(methoxy(methyl)carbamoyl)-2-azabicyclo[4.1.0]heptane-2-carboxylate (8)



Freshly prepared dimethoxytriazin-*N*-methylmorpholinium chloride[6] (DMTMM, 2.43 g, 8.79 mmol, 1.50 eq.) was added to a solution consisting of carboxylic acid **20** (1.78 g, 5.86 mmol, 1.00 eq.) and dry THF (20 mL). The resulting white suspension was stirred at room temperature for one hour, after which *N*,*O*-dimethylhydroxylamine hydrochloride (572 mg, 5.86 mmol, 1.00 eq.) and *N*-methylmorpholine (NMM, 1.29 mL, 11.7 mmol, 2.00 eq.). The reaction mixture was stirred for 48 hours at room temperature followed by quenching with 5% aq. citric acid (30 mL). The mixture was extracted with diethyl ether (3 x 30 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude oil was purified by flash column chromatography (hexane:EtOAc 4:1) to yield Weinreb-amide **8** (1.97 g, 5.68 mmol, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35-7.19 (m, 5 H, Ar*H*), 5.23 (d, 0.8 H, *J* = 12.9 Hz, OCH_2 rotamer A), 5.14 (d, 0.2 H, *J* = 12.5 Hz, OCH_2 rotamer B), 5.07 (d, 1 H, *J* = 13.0 Hz, OCH_2), 3.84 (dt, 0.8 H, *J* = 12.5, 3.4 Hz, NCH_2 rotamer A), 3.71 (m, 0.2 H, NCH_2, rotamer B), 3.64 (s, 0.6 H, OCH_3 rotamer B), 3.58-3.52 (m, 2.4 H, OCH_3 rotamer A and NC*H* rotamer B), 3.49 (d, 0.8 H, *J* = 3.7 Hz, NC*H* rotamer A), 3.15 (s, 0.6 H, NCH_3 rotamer B), 3.12 (s, 2.4 H, NCH_3 rotamer A), 2.73 (t, 0.2 H, *J* = 11.7 Hz, NCH_2 rotamer B), 2.64 (dt, 0.8 H, *J* = 12.5, 2.2 Hz, NCH_2 rotamer A), 2.09-1.87 (m, 2 H, C*H*CO and CH_2), 1.77-1.54 (m, 4 H, CH_2), 1.36 (m, 1 H, CH_2), 0.85 (t, 3 H, *J* = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (rotamers!) 171.2, 156.2, 136.8, 128.2, 128.1, 127.8, 127.8, 127.4, 127.0, 66.8, 66.5, 61.2, 42.9, 42.7, 41.5, 41.0, 33.0, 32.4, 29.1, 28.5, 26.0, 25.9, 25.2, 21.6, 10.3. The values of the NMR spectra are in accordance with reported literature data.[2]

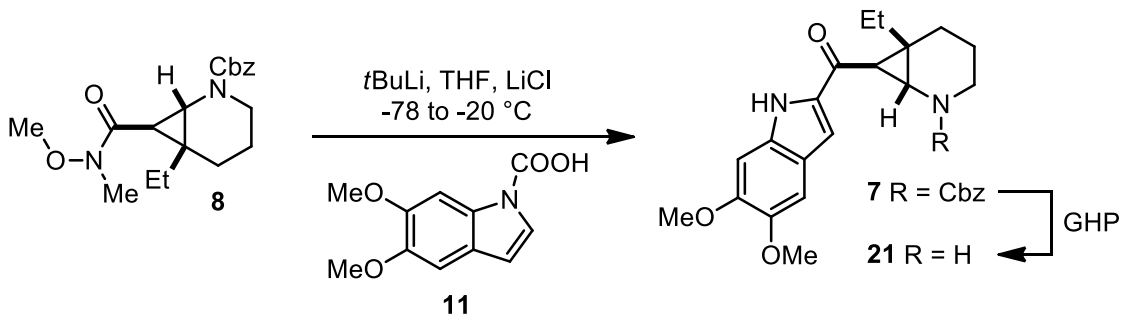
Benzyl 7-(5,6-dimethoxy-1*H*-indole-2-carbonyl)-6-ethyl-2-azabicyclo[4.1.0]heptane-2-carboxylate (7)



Following a slight modification of a reported procedure,[7] commercially available 5,6-dimethoxyindole (**9**, 180 mg, 1.02 mmol, 1.00 eq.) was dissolved in dry THF (7.0 mL). The colorless solution was cooled to 0 °C and a 1.6 M solution of *n*BuLi in hexane (760 µL, 1.22 mmol, 1.10 eq.) was added dropwise. The reaction mixture was heated at reflux for 2 hours and then cooled back to 0 °C to bubble CO_2 into the reaction mixture for 45 minutes. The resulting suspension was quenched with water (10 mL) at 0 °C and extracted with diethyl ether (3 x 10 mL). The combined organic layers were washed with water (20 mL). The

[6] M. Kunishima, C. Kawachi, F. Iwasaki, K. Terao, S. Tani, *Tetrahedron Lett.* **1999**, *40*, 5327-5330.
[7] D. A. Shirley, P. A. Roussel, *J. Am. Chem. Soc.* **1953**, *75*, 375-378.

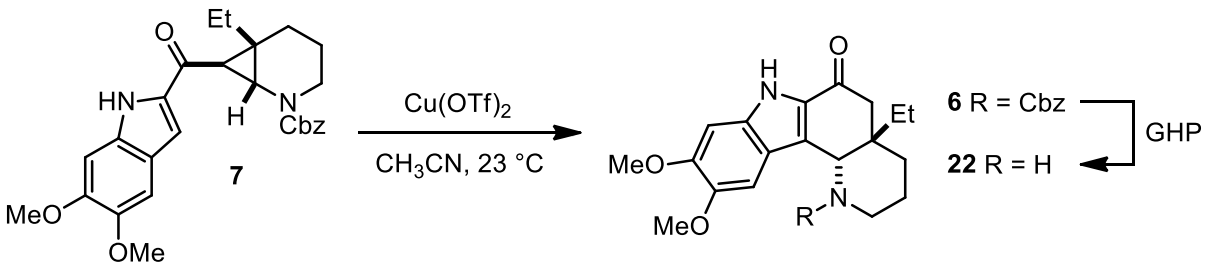
combined aqueous layers were acidified with 1.0 M aq. HCl to pH = 2, followed by extraction with diethyl ether (3 x 15 mL). The three combined diethyl ether layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* (for only five minutes to avoid potential decarboxylation) to yield carboxylic acid protected indole **11** (224 mg, 1.01 mmol, quant.) as a white solid, which was used in the next step without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (s, 1 H, Ar*H*), 7.53 (d, 1 H, *J* = 3.7 Hz, Ar*H*), 7.04 (s, 1 H, Ar*H*), 6.57 (d, 1 H, *J* = 3.6 Hz, Ar*H*), 4.00 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.7, 148.2, 147.1, 129.5, 124.2, 123.6, 109.5, 102.9, 99.5, 56.5, 56.3.



A 1.6 M solution of *t*BuLi in pentane (6.95 mL, 11.1 mmol, 2.20 eq.) was added dropwise to a solution of freshly prepared carboxylic acid protected indole **11** (1.23 g, 5.56 mmol, 1.10 eq.) and lithium chloride[8] (236 mg, 5.56 mmol, 1.10 eq.) in dry THF (26 mL) at -78 °C. The mixture was stirred for 3 hours at -78 °C and then transferred *via* cannula into a, to 78 °C pre-cooled, solution of Weinreb-amide **8** (1.77 g, 5.11 mmol, 1.00 eq.) dissolved in dry THF (13 mL). The reaction mixture was slowly allowed to warm up to -20 °C over a 3.5 hour time period and then kept at -20 °C for 30 minutes. The reaction was quenched by transferring it *via* cannula to saturated aq. $NaHCO_3$ (75 mL) pre-cooled to 0 °C. The mixture was stirred for 10 minutes at 0 °C, after which the ice/water bath was removed and stirring was continued for an additional 20 minutes. The aqueous mixture was extracted with diethyl ether (4 x 50 mL) and the combined organic layers were dried over $MgSO_4$, filtered and treated with deactivated silica gel prior to removing the solvent *in vacuo*. The crude product was purified by flash column chromatography (pentane:EtOAc 4:1 to 3:1), dry loading the crude, affording **7** (1.69 g, 3.65 mmol, 72%) as a colorless oil (R_f in pentane:EtOAc 7:3 = 0.64). As NMR analysis of **7** was difficult due to the rotamers caused by the Cbz-protecting group, the above described general hydrogenolysis procedure (GHP) was utilized on a small aliquot of **7** to yield free amine **21** for a simplified compound analysis of the resulting light yellow oil. R_f (DCM:Ultra 4:1) = 0.57. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 7.10 (bs, 1 H, N*H*), 7.07 (s, 1 H, Ar*H*), 6.79 (s, 1 H, Ar*H*), 4.87 (s, 1 H, Ar*H*), 3.94 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 3.27 (d, 1 H, *J* = 17.0 Hz, NHC*H*), 3.16-3.08 (m, 1 H, NHCH_2), 2.90 (td, 1 H, *J* = 11.8, 2.7 Hz, NHCH_2), 2.21 (d, 1 H, *J* = 17.2 Hz, COC*H*), 1.82-1.55 (m, 4 H) 1.42-1.21 (m, 3 H), 0.81 (t, 3 H, *J* = 7.5 Hz, CH_2CH_3). ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 189.9, 151.6, 147.3, 132.6, 132.0, 120.9, 105.8, 103.7, 92.4, 71.3, 56.6, 56.5, 46.0, 41.0, 39.7, 33.6, 30.9, 22.4, 7.5. IR ν 2935 (w), 2856 (w), 1658 (m), 1527 (s), 1493 (w), 1464 (w), 1408 (w), 1350 (w), 1318 (w), 1224 (s), 1051 (w). HRMS (ESI) $C_{19}H_{25}N_2O_3^+$ $[M+H]^+$ calc. = 329.1860; $[M+H]^+$ obs. = 329.1867.

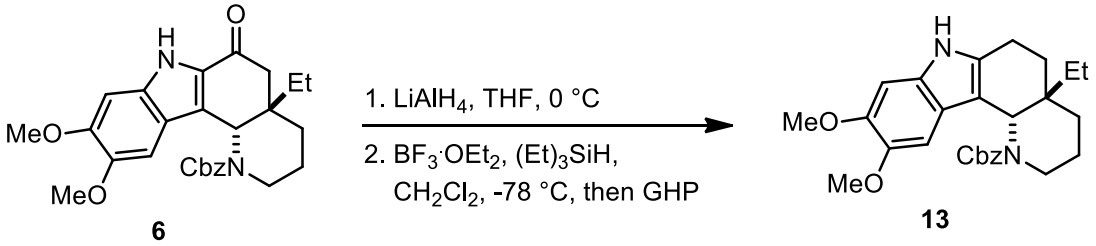
[8] LiCl was dried under careful flame heating and high vacuum (<0.05 Torr) before it was dissolved in dry THF.

Benzyl 4-ethyl-9,10-dimethoxy-6-oxo-2,3,4,4,5,6,7,11-octahydro-1*H*-pyrido[3,2]carbazole-1-carboxylate (**6**)



A 0.1 M solution of copper(II) triflate in acetonitrile (4.93 mL, 0.493 mmol, 0.200 eq.) was slowly added at room temperature to a solution consisting of vinyl cyclopropyl ketone **7** (1.14 g, 2.47 mmol, 1.00 eq.) and dry acetonitrile (120 mL). The reaction mixture was stirred at room temperature for 10 minutes and then quenched by adding saturated aq. $NaHCO_3$ (150 mL). The mixture was extracted with diethyl ether (3 x 50 mL). The combined organic layers were washed with brine (75 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (pentane:EtOAc 3:1 to 2:1) to afford **6** (971 mg, 2.10 mmol, 85%) as a light yellow solid (R_f in pentane:EtOAc 1:1 = 0.50). As NMR analysis of **6** was difficult due to the rotamers caused by the Cbz-protecting group, the above described general hydrogenolysis procedure (GHP) was utilized on a small aliquot of **6** to yield free amine **22** for a simplified compound analysis resulting light yellow oil. R_f (DCM:Ultra 3:2) = 0.54. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 9.24 (bs, 1 H, N*H* indole), 7.15 (s, 1 H, Ar*H*), 6.83 (s, 1 H, Ar*H*), 3.98 (s, 1 H, NC*H*), 3.88 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 3.07 (d, 1 H, *J* = 16.7 Hz, COCH_2), 3.02-2.93 (m, 1 H, NHCH_2), 2.86-2.74 (m, 1 H, NHCH_2), 2.21 (d, 1 H, *J* = 16.6 Hz, COCH_2), 1.77-1.57 (m, 4 H), 1.56-1.45 (m, 2 H), 1.40-1.29 (m, 1 H), 0.81 (t, 3 H, *J* = 7.5 Hz, CH_2CH_3). ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 190.3, 151.8, 146.9, 134.3, 130.1, 129.1, 119.2, 101.9, 94.8, 57.0, 56.6, 56.4, 45.8, 43.3, 41.2, 33.4, 31.4, 23.3, 8.0. IR ν 3271 (w), 2934 (w), 1644 (s), 1627 (s), 1539 (m), 1488 (s), 1463 (m), 1296 (w), 1266 (s), 1220 (s), 1162 (w), 1012 (w). HRMS (ESI) $C_{19}H_{25}N_2O_3^+$ $[M+H]^+$ calc. = 329.1860; $[M+H]^+$ obs. = 329.1865.

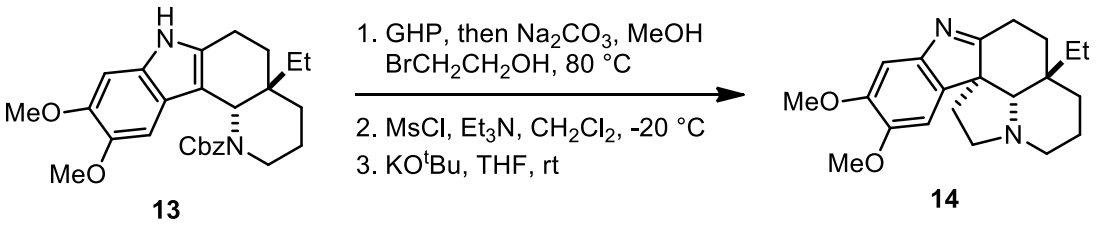
(4,11)-benzyl 4-ethyl-9,10-dimethoxy-2,3,4,5,6,7,11-octahydro-1*H*-pyrido[3,2-]carbazole-1-carboxylate (**13**)



The Cbz-protected ketone starting material **6** (200 mg, 0.432 mmol, 1.00 eq.) was dissolved in dry THF (7 mL), cooled to -78 °C and slowly transferred *via* cannula to a suspension of lithium aluminum hydride (32.8 mg, 0.865 mmol, 2.00 eq.) at -78 °C. The reaction mixture was stirred for 2 hours at -78 °C, after which the dry ice/acetone bath was removed to stir the reaction for an additional two hours at room temperature. The mixture was cooled to 0 °C and quenched by carefully adding a few drops of water. The resulting mixture was filtered through

a, with THF prewashed, pad of celite and concentrated *in vacuo* to yield the corresponding hydroxy derivative **12** as a white solid.[9] Intermediate **12** was dissolved in dry DCM (10 mL) and cooled to -78 °C. To the clear colorless solution was added dropwise freshly distilled boron trifluoride diethyl etherate (137 µL, 0.519 mmol, 1.2 eq.) to produce a dark red solution, which was quickly treated with excess triethylsilane (5.0 mL). The reaction mixture was stirred at -78 °C for 10 minutes and then quenched by transferring it *via* cannula to saturated aq. $NaHCO_3$ (20 mL) pre-cooled to 0 °C. The mixture was stirred for 10 minutes at 0 °C, after which the ice/water bath was removed and stirring was continued for an additional 20 minutes. The aqueous mixture was extracted with DCM (3 x 20 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (pentane:EtOAc 3:1 to 2:1) affording **13** (180 mg, 0.402 mmol, 93%) as a light beige solid.[10] R_f (pentane:EtOAc 3:2) = 0.36. ^{1}H NMR ($CDCl_3$, 400 MHz): 7.63 (bs, 0.6 H, N*H* rotamer A), 7.61 (bs, 0.4 H, N*H* rotamer B), 7.47-7.26 (m, 5 H, Ar*H*), 6.81 (s, 0.6 H, Ar*H* rotamer A), 6.80 (s, 0.4 H, Ar*H* rotamer B), 6.70 (s, 0.4 H, Ar*H* rotamer B), 6.68 (s, 0.6 H, Ar*H* rotamer A), 5.41-5.16 (m, 3 H, OC*H*$_2$ and NC*H*), 4.15-4.07 (m, 0.6 H rotamer A), 4.07-3.99 (m, 0.4 H rotamer B), 3.88 (s, 1.8 H, OC*H*$_3$ rotamer A), 3.87 (s, 1.2 H, OC*H*$_3$ rotamer B), 3.64 (s, 1.8 H, OC*H*$_3$ rotamer A), 3.59 (s, 1.2 H, OC*H*$_3$ rotamer B), 2.81-2.69 (m, 1 H), 2.69-2.58 (m, 1 H), 2.57-2.46 (m, 1 H), 1.86-1.24 (m, 8 H), 0.92 (t, 1.2 H, *J* = 7.5 Hz, OC*H*$_3$ rotamer B), 0.86 (t, 1.8 H, *J* = 7.5 Hz, OC*H*$_3$ rotamer A). HRMS (ESI) $C_{27}H_{33}N_2O_4^+$ $[M+H]^+$ calc. = 449.2435; $[M+H]^+$ obs. = 449.2437.

3-Ethyl-8,9-dimethoxy-2,3,3,4,5,11,12-octahydro-1*H*-indolizino[8,1]carbazole (**14**)



A Schlenk tube was charged under nitrogen with intermediate **13** (20.0 mg, 0.0446 mmol, 1.00 eq.) and the above described GHP was carried out for Cbz-group removal. The reaction was monitored by HPLC-MS, which indicated full deprotection ($[M+H]^+$ calc. = 315.2607; $[M+H]^+$ obs. = 315.2603) after 15 minutes of stirring at room temperature. The Pd-catalyst was quickly removed *via* filtration through a CHROMAFIL® Xtra RC-20/25 syringe filter. After washing the filter several times with dry ethanol, the solvent was removed *in vacuo*. Following a slight modification of a reported procedure,[11] the free amine was immediately dissolved in dry methanol (2.0 mL) in a Schlenk tube under nitrogen. To the resulting solution was added 2-bromoethanol (31.6 µL, 0.446 mmol, 10.0 eq.) and dry sodium carbonate (47.0 mg, 0.446 mmol, 10.0 eq.). The sealed Schlenk tube was stirred at reflux and the reaction was monitored by HPLC-MS. The free amine was consumed after 4 hours and HPLC-MS analysis indicated the desired alkylation as the main product (HRMS: $[M+H]^+$ calc. = 359.2329; $[M+H]^+$ obs. = 359.2338). Thus, the solvent was removed *in vacuo* and the resulting light yellow solid (still in the Schlenk tube) was dissolved in dry DCM (2.0 mL) and cooled to -20

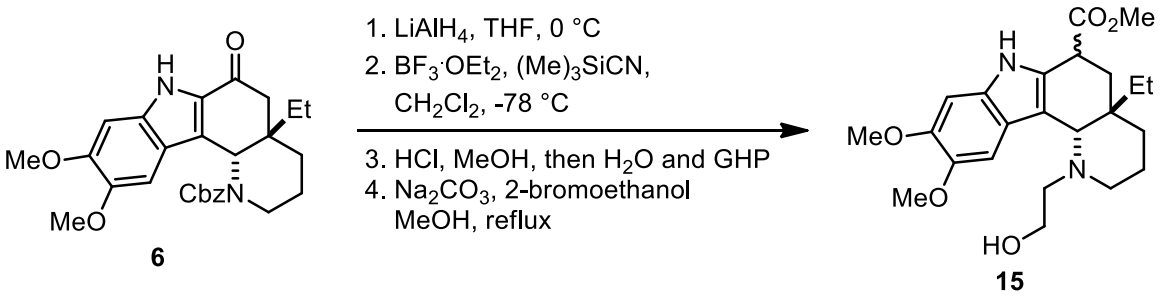
[9] The full characterization of one of the two formed hydroxy diastereoisomer (**12a**) is shown below for the transformation **6** to **15**.

[10] We observed that intermediate **13** decomposed quickly, even at -20 °C and under argon, and should be used immediately for a subsequent transformation.

[11] S. A. Kozmin, T. Iwama, Y. Huang, V. H. Rawal, *J. Am. Chem. Soc.* **2002**, *124*, 4628-4641.

°C. The solution was treated with methanesulfonyl chloride (6.90 µL, 0.0891 mmol, 2.00 eq.), triethylamine (18.7 µL, 0.134 mmol, 3.00 eq.) and then slowly let come to room temperature over a 3 hour time period. HPLC-MS analysis indicated formation of the 2-chloroethylamine[12] alkylation product (HRMS: $[M+H]^+$ calc. = 377.1990; $[M+H]^+$ obs. = 377.2009) as well as some already cyclized product **14** (HRMS: $[M+H]^+$ calc. = 341.2224; $[M+H]^+$ obs. = 341.2238). In order to cyclize the remaining chloroethylamine intermediate, the mixture was concentrated *in vacuo*, the resulting yellow solid suspended in dry THF (4.0 mL) and cooled to 0 °C. Next was added dropwise at 0 °C a 0.3 M potassium *tert*.-butoxide solution in THF (0.300 mL, 0.0892 mmol, 2.00 eq.) and the mixture was stirred overnight for 16 hours, letting the reaction temperature slowly come to room temperature. HPLC-MS analysis indicated full consumption of the remaining chloroethylamine intermediate. Thus, the reaction mixture was quenched by adding a few drops of water, diluted with THF (5.0 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Attempts to purify the crude product by column chromatography or crystallization were unsuccessful as the product quickly decomposed into numerous unidentifiable products during these purification efforts.

Methyl 4-ethyl-1-(2-hydroxyethyl)-9,10-dimethoxy-2,3,4,5,6,7,11-octahydro-1*H*-pyrido[3,2]carbazole-6-carboxylate (**15**)



The Cbz-protected ketone starting material (**6**, 435 mg, 0.940 mmol, 1.00 eq.) was dissolved in dry THF (43 mL), cooled to 0 °C and slowly transferred *via* cannula to a suspension of lithium aluminum hydride (71.4 mg, 1.88 mmol, 2.00 eq.) at 0 °C. The reaction mixture was stirred overnight for 11 hours at 0 °C and then quenched by carefully adding a few drops of water. The mixture was filtered through a, with THF prewashed, pad of celite and concentrated *in vacuo* to yield the two corresponding hydroxy diastereoisomers **12** (420 mg, 0.904 mmol, 96%, R_f in pentane:EtOAc 1:1 = 0.48 for **12a** and 0.44 for **12b**) as a white solid. Intermediate **12** was stored under argon at -20 °C and aliquots of it were used to investigate further transformations. We note that highly pure solvents, reagents and clean glassware are required for subsequent reactions utilizing **12**, as in certain cases rapid decomposition of this sensitive intermediate was observed. It was possible to obtain a pure sample of hydroxy diastereoisomer **12a** for characterization purposes by flash column chromatography (pentante:EtOAc 2:1). Partial degradation of sensitive hydroxy intermediate **12** was however observed during flash column chromatography purification.

Hydroxy diastereoisomers **12a**: R_f (pentane:EtOAc 1:1) = 0.48. ^{1}H NMR (CD_2Cl_2, 400 MHz): 8.55 (bs, 0.4 H, N*H* rotamer B), 8.53 (bs, 0.6 H, N*H* rotamer A), 7.47-7.23 (m, 5 H, Ar*H*),

[12] The 2-chloroethylamine derivative is presumably formed by displacement of the initially formed mesylate by the proximate nucleophilic nitrogen atom of the piperidine ring, producing an aziridinium ion, which can be opened by the chloride ion.

6.80 (s, 1 H, Ar*H*), 6.65 (s, 0.4 H, Ar*H* rotamer B), 6.63 (s, 0.6 H, Ar*H* rotamer A), 5.34-5.16 (m, 3 H), 4.95-4.88 (m, 0.4 H, NC*H* rotamer B), 4.88-4.80 (m, 0.6 H, rotamer A), 4.11-4.02 (m, 0.4 H, NC*H* rotamer B), 4.02-3.93 (m, 0.6 H, rotamer A), 3.78 (s, 3 H, OCH_3), 3.61 (s, 1.3 H, OCH_3 rotamer B), 3.55 (s, 1.7 H, OCH_3 rotamer A), 2.52-2.37 (m, 1 H), 2.18-2.01 (m, 1 H), 1.70-1.33 (m, 6 H), 1.31-1.18 (m, 2 H), 0.88 (t, 1.3 H, J = 7.5 Hz, OCH_3 rotamer B), 0.78 (t, 1.3 H, J = 7.5 Hz, OCH_3 rotamer A). ^{13}C NMR (CD_2Cl_2, 100 MHz) δ (rotamers!) 156.6, 147.6, 147.5, 145.5, 145.4, 137.7, 135.8, 135.2, 129.0, 128.9, 128.5, 128.4, 119.1, 110.1, 109.8, 101.6, 101.5, 95.4, 67.6, 64.1, 64.0, 56.5, 56.4, 56.3, 55.5, 43.3, 43.2, 40.1, 40.0, 39.9, 39.8, 28.7, 28.5, 27.1, 26.7, 21.6, 21.2, 8.1, 8.0. IR ν 3368 (w), 2940 (m), 2864 (w), 1676 (s), 1485 (m), 1463 (m), 1434 (s), 1320 (m), 1210 (s), 1157 (m), 1113 (m), 1027 (w), 757 (w). HRMS (ESI) $C_{27}H_{33}N_2O_5^+$ $[M+H]^+$ calc. = 465.2384; $[M+H]^+$ obs. = 465.2387.

Hydroxy intermediate **12** (23.0 mg, 0.0495 mmol, 1.00 eq.) was dissolved in freshly distilled DCM (2.0 mL) and cooled to -78 °C. To the clear colorless solution was added dropwise freshly distilled boron trifluoride diethyl etherate[13] (8 µL, 0.06 mmol, 1.2 eq.) to produce a dark red solution, which was quickly treated with freshly distilled trimethylsilyl cyanide (20 µL, 0.15 mmol, 3.0 eq.). The reaction mixture was stirred at -78 °C for 5 minutes and then quickly transferred *via* cannula to saturated aq. $NaHCO_3$ (10 mL) pre-cooled to 0 °C. The mixture was extracted with DCM (3 x 10 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexane:EtOAc 3:2) to afford the cyano intermediate (22.2 mg, 0.0469 mmol, 95%, R_f in EtOAc:Hexane 1:1 = 0.38, HRMS: $[M+H]^+$ calc. = 474.2387; $[M+H]^+$ obs. = 474.2382) as a colorless oil, which was immediately used for subsequent transformations.

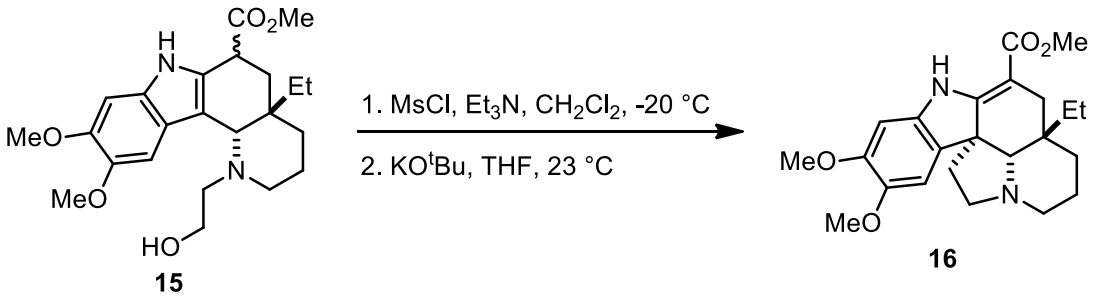
The cyano intermediate (15.6 mg, 0.0329 mmol, 1.00 eq.) was dissolved in freshly distilled methanol (70 µL) and cooled to 0 °C at which 4.0 M HCl in cyclopentyl methyl ether (830 µL) was added dropwise. The reaction mixture was stirred overnight for 12 hours at 0 °C, followed by hydrolyzing the corresponding Pinner salt by slowly adding water (10 mL) at 0 °C. The resulting mixture was stirred for 60 minutes at room temperature and extracted with DCM (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexane:EtOAc 3:1) to afford the pure Cbz-protected methyl ester intermediate (R_f in EtOAc:Hexane 3:2 = 0.53, HRMS: $[M+H]^+$ calc. = 507.2490; $[M+H]^+$ obs. = 507.2494), which was subsequently subjected to the general hydrogenolysis procedure giving the highly reactive free secondary amine (10.8 mg, 0.0290 mmol, 88%, HRMS: $[M+H]^+$ calc. = 373.2122; $[M+H]^+$ obs. = 373.2119) as a light yellow oil.[14]

Following a slight modification of a reported procedure,[11] the free secondary amine (40.6 mg, 0.109 mmol, 1.00 eq.) was dissolved in freshly distilled methanol (9.0 mL) in a Schlenk tube under nitrogen. The resulting solution was charged at room temperature under nitrogen with freshly distilled 2-bromoethanol (77.0 µL, 1.09 mmol, 10.0 eq.) and dry sodium carbonate (115 mg, 1.09 mmol, 10.0 eq.). The reaction mixture was stirred at reflux overnight for 12 hours in the sealed Schlenk tube, after which the solvent was removed *in vacuo*. The crude was extracted with water (10 mL) and DCM (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (DCM:Ultra 97:3) to afford alkylation product **15** (42.2 mg, 0.101 mmol, 93%, 78% overall from **12**) as a light yellow oil. R_f (DCM:Ultra 7:3) = 0.51. ^{1}H

[13] $InCl_3$ can be used as an alternative as it produced comparable results.
[14] NMR analysis was unfeasible as the compound readily decomposed as solution in CD_2Cl_2.

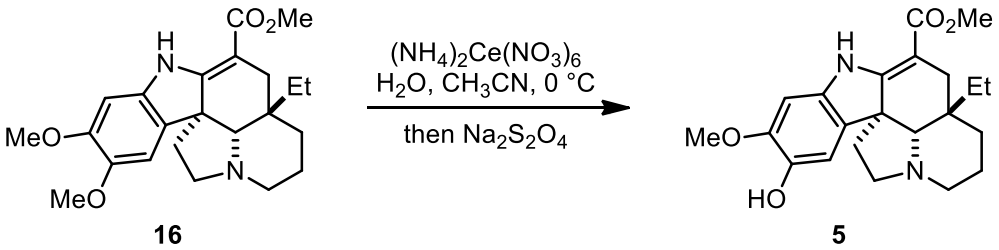
NMR (CD_2Cl_2, 400 MHz): 8.31 (bs, 1 H, N*H*), 6.90 (s, 1 H, Ar*H*), 6.87 (s, 1 H, Ar*H*), 3.85 (s, 3 H, OC*H*$_3$), 3.84 (s, 3 H, OC*H*$_3$), 3.83-3.78 (m, 1 H), 3.77 (s, 3 H, CO_2C*H*$_3$), 3.45 (td, 1 H, *J* = 10.3, 3.1 Hz), 3.17 (s, 1 H, NC*H*), 3.14-2.93 (m, 5 H), 2.26-2.15 (m, 2 H), 1.97-1.82 (m, 1 H), 1.81-1.72 (m, 1 H), 1.68-1.55 (m, 2 H), 1.45 (td, 1 H, *J* = 13.6, 4.8 Hz), 1.19-1.08 (m, 1 H), 1.01-0.90 (m, 1 H), 0.77 (t, 3 H, *J* = 7.5 Hz, CH_2C*H*$_3$). ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 174.0, 147.6, 145.9, 130.9, 129.5, 122.5, 113.0, 101.4, 95.6, 63.2, 58.7, 57.0, 56.7, 55.1, 52.9, 52.9, 39.3, 38.6, 34.9, 30.4, 28.1, 22.4, 8.1. IR ν 3352 (w), 2936 (w), 2831 (w), 1733 (s), 1486 (s), 1464 (m), 1295 (m), 1235 (m), 1201 (s), 1161 (s), 1121 (m), 1055 (w), 1034 (m), 956 (w). HRMS (ESI) $C_{23}H_{33}N_2O_5^+$ $[M+H]^+$ calc. = 417.2384; $[M+H]^+$ obs. = 417.2381.

Methyl 3-ethyl-8,9-dimethoxy-2,3,4,6,11,12-octahydro-1*H*-indolizino[8,1]carbazole-5-carboxylate (**16**)



Following a slight modification of a reported procedure,[11] primary alcohol **15** (40.1 mg, 0.0963 mmol, 1.00 eq.) was dissolved in dry DCM (4.0 mL) and cooled to -20 °C. The solution was treated with methanesulfonyl chloride (15.0 µL, 0.193 mmol, 2.00 eq.), triethylamine (40.3 µL, 0.289 mmol, 3.00 eq.) and stirred at -20 °C for 60 minutes. The reaction mixture was diluted with DCM (10 mL) and extracted with saturated aq. $NaHCO_3$ (10 mL). The aqueous layer was extracted with additional portions of DCM (2 x 10 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting yellow oil was dissolved in dry THF (4.0 mL) and cooled to 0 °C. Next was added dopwise at 0 °C a 0.3 M potassium *tert*.-butoxide solution in THF (1.60 mL, 0.481 mmol, 5.00 eq.) and the mixture was stirred overnight for 14 hours, letting the reaction temperature slowly come to room temperature. The reaction mixture was quenched by adding a few drops of water, diluted with THF (5.0 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (DCM:Ultra 98:2) to afford pentacyclic product **16** (32.8 mg, 0.0823 mmol, 85%) as a light yellow oil. R_f (DCM:Ultra 4:1) = 0.56. ^{1}H NMR (CD_2Cl_2, 400 MHz): 8.75 (bs, 1 H, N*H*), 6.81 (s, 1 H, Ar*H*), 6.49 (s, 1 H, Ar*H*), 3.79 (s, 6 H, OC*H*$_3$), 3.72 (s, 3 H, CO_2C*H*$_3$), 3.14-3.06 (m, 1 H), 2.93-2.86 (m, 1 H), 2.69 (d, 1 H, *J* = 14.9 Hz), 2.59-2.50 (m, 1 H), 2.45-2.36 (m, 2 H), 2.23 (dd, 1 H, *J* = 15.1, 2.0 Hz), 1.99 (td, 1 H, *J* = 11.3, 6.5 Hz), 1.88–1.74 (m, 2 H), 1.64 (dd, 1 H, *J* = 11.3, 4.8, Hz), 1.56–1.49 (m, 1 H), 1.32–1.20 (m, 1 H), 1.02-0.93 (m, 1 H), 0.69–0.54 (m, 4 H). ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 169.4, 169.2, 149.8, 144.3, 137.7, 129.5, 108.0, 96.2, 93.0, 73.2, 57.6, 56.6, 56.3, 52.2, 51.3, 51.1, 45.7, 38.8, 33.5, 29.7, 25.9, 22.8, 7.5. IR ν 3362 (w), 2934 (m), 2774 (w), 1674 (m), 1615 (s), 1498 (s), 1461 (m), 1291 (m), 1264 (s), 1204 (s), 1191 (s), 1164 (s), 1137 (s), 1115 (m), 1048 (m). HRMS (ESI) $C_{23}H_{31}N_2O_4^+$ $[M+H]^+$ calc. = 399.2278; $[M+H]^+$ obs. = 399.2286.

Jerantinine E (**5**)



Dimethoxy starting material **16** (15.0 mg, 0.0376 mmol, 1.00 eq.) was dissolved in acetonitrile (1.5 mL) and cooled to 0 °C. To the mixture was slowly added a solution consisting of ceric ammonium nitrate (45.4 mg, 0.0830 mmol, 2.20 eq.) dissolved in water (1.0 mL) at 0 °C. The reaction was stirred for 5 minutes at 0 °C and then poured into a 1.0 M aq. sodium dithionite solution (10 mL) at 0 °C. The reaction mixture was stirred for 10 minutes at 0 °C, diluted with DCM (10 mL) and extracted. The aqueous layer was washed with additional portions of DCM (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (DCM:Ultra 98:2) to afford jerantinine E (**5**, 11.4 mg, 0.0297 mmol, 79%) as a yellow oil. R_f (DCM:Ultra 4:1) = 0.48. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.76 (bs, 1 H, N*H*), 6.83 (s, 1 H, Ar*H*), 6.43 (s, 1 H, Ar*H*), 5.27 (bs, 1 H, O*H*), 3.86 (s, 3 H, OCH_3), 3.75 (s, 3 H, CO_2CH_3), 3.14-3.05 (m, 1 H), 2.94-2.85 (m, 1 H), 2.70 (d, 1 H, *J* = 15.0 Hz), 2.58-2.47 (m, 1 H), 2.43-2.32 (m, 2 H), 2.24 (dd, 1 H, *J* = 15.1, 1.9 Hz), 2.03 (td, 1 H. *J* = 11.4, 6.4 Hz), 1.88–1.74 (m, 2 H), 1.66 (dd, 1 H, *J* = 11.5, 4.6, Hz), 1.57–1.48 (m, 1H), 1.31–1.18 (m, 1 H), 1.02-0.92 (m, 1 H), 0.68–0.54 (m, 4 H). ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 169.5, 169.2, 146.4, 140.5, 136.8, 130.5, 108.9, 95.2, 92.9, 73.3, 56.9, 56.1, 52.1, 51.3, 51.2, 45.8, 38.9, 33.4, 29.7, 25.9, 22.9, 7.5. IR ν 3370 (w), 2934 (w), 2776 (w), 1671 (m), 1606 (s), 1494 (s), 1441 (m), 1266 (s), 1196 (s), 1165 (s), 1136 (s), 1114 (m), 1047 (w). HRMS (ESI) $C_{22}H_{29}N_2O_4^+$ $[M+H]^+$ calc. = 385.2122; $[M+H]^+$ obs. = 385.2113.

As shown below in **Figure S1**, the ^{1}H NMR spectrum of synthesized jerantinine E is identical to the ^{1}H NMR spectrum of jerantinine E isolated from the Malayan plant *Tabernaemontana corymbosa*.[15]

[15] The isolation ^{1}H NMR spectrum of jerantinine E was kindly provided by Prof. T. S. Kam through a personal communication. The NMR data of isolated jerantinine E is summarized in the manuscript by Prof. Kam and co-workers: K. H. Lim, O. Hiraku, K. Komiyama, T. S. Kam, *J. Nat. Prod.* **2008**, *71*, 1591-1594.

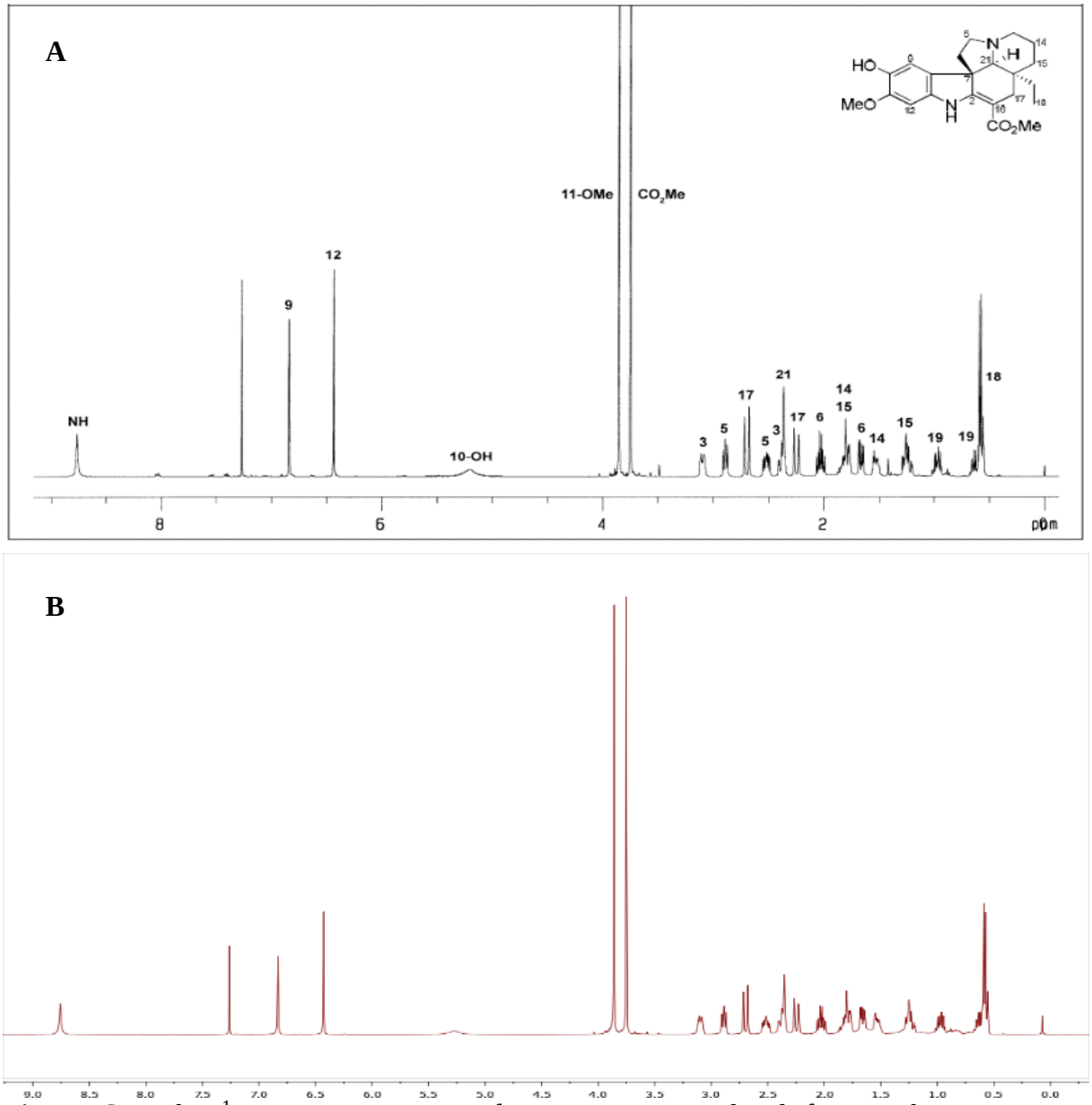


Figure S1. The ^{1}H NMR spectrum of jerantinine E isolated from *Tabernaemontana corymbosa* (**A**) and the synthesized jerantinine E ^{1}H NMR spectrum (**B**).

Separation of the Enantiomers by Chiral HPLC

The two enantiomers of jerantinine E were separated by chiral HPLC on a JASCO HPLC system with an AS2055 Autosampler, a PU 2089 Pump, a UV 2075 detector and a SEDEX 85 (SEDERE) detector using an analytical CHIRALPAK IB column from DAICEL Chemical Industries Ltd. A mixture of HPLC grade hexane:isopropanol 98:2 (no additives) was used as mobile phase. The flow rate was set to 2.0 mL per minute with an acquisition time of 20 minutes per run. Under the optimized separation conditions, 50 µL of a mixture consisting of approximately 2 mg jerantinine E dissolved in 1.5 mL hexane:isopropanol 9:1 were injected per run. Shown below in **Figure S2** is a representative HPLC trace of a separation run (**A**), as well as traces of the two purified enantiomers (**B** and **C**).

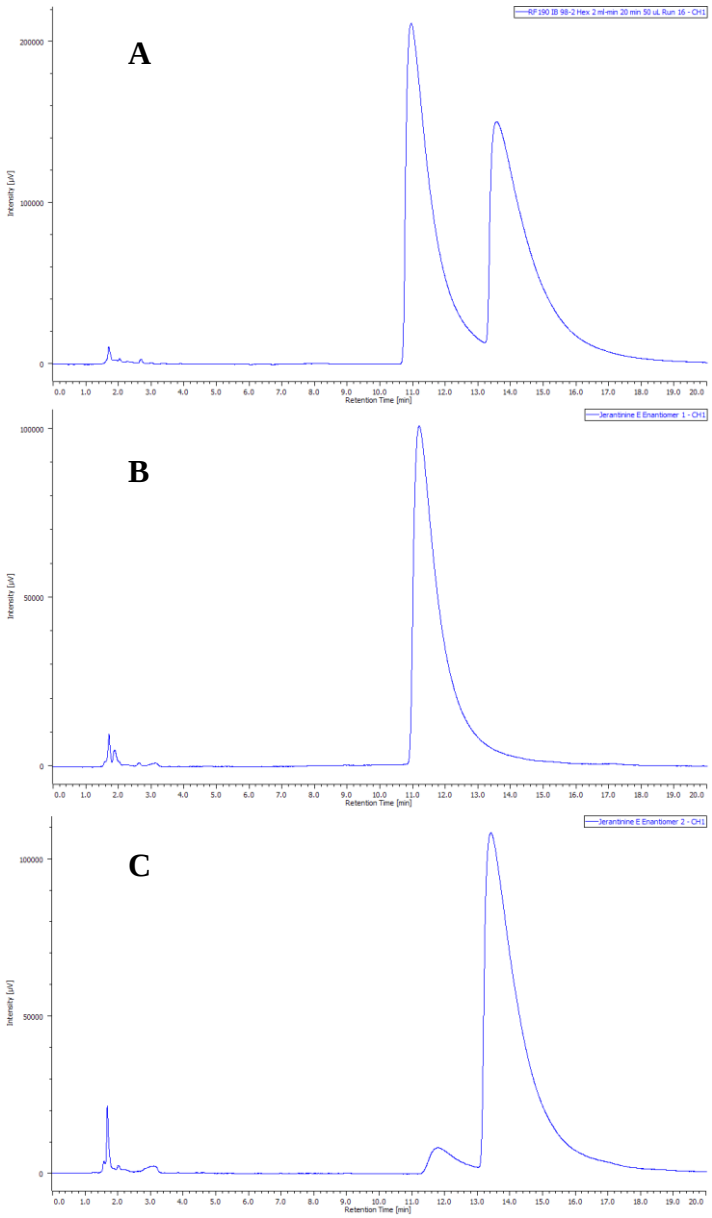


Figure S2. HPLC traces of racemic jerantinine E (**A**) and the two enantiomers (+)-jerantinine E (**B**) and (-)-jerantinine E (**C**).

Both enantiomers were analyzed for their optical rotatory power, which indicated that the first enantiomer (**Figure S2**, **B**) is non-natural, (+)-jerantinine E (>95%ee; $[\alpha]_D^{25.0}$ +229 at c 0.05 in $CHCl_3$) and the second enantiomer (**Figure S3**, **C**) is naturally occurring (-)-jerantinine E (>90%ee; $[\alpha]_D^{25.0}$ -237 at c 0.06 in $CHCl_3$).[16]

We note that the small contamination (approximately 5%) of (+)-jerantinine E in the sample of (-)-jerantinine E is negligible due to its minimal bio-activity.

[16] The manuscript describing the isolation of jerantinine E reports that the $[\alpha]_D^{25.0}$ is -357 at c 0.10 in $CHCl_3$: K. H. Lim, O. Hiraku, K. Komiyama, T. S. Kam, *J. Nat. Prod.* **2008**, *71*, 1591-1594.

5. Biological Evaluation of Jerantinine E (5)

5.1 Evaluation of Jerantinine E (5) in Human Cancer Cell Lines[17]

General Information for the Evaluation of Jerantinine E (5) in Cellular Assays

The cellular assays were performed on two human-derived breast cancer cell lines and two human-derived lung cancer cell lines. One moderately invasive cell line was selected from an invasive breast ductal carcinoma (MCF-7), whereas the second breast cancer cell line was highly invasive (MDA-MB-231).[18] A human adenocarcinoma cell line (A549) derived from alveolar epithelial cells and an adenosquamous carcinoma cell line (HTB-178) were selected as lung cancer models.[19]

Dose-Dependent Cytotoxic Effect of Jerantinine E (5)

Cell lines A549, HTB-178, MCF-7 and MDA-MB-231 were obtained through the American Tissue Culture Collection (Manassas, VA, USA) and cell culture reagents and materials were purchased from Invitrogen. Cell lines A549, MCF-7 and MDA-MB-231 were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L glucose, 10% heat-inactivated fetal calf serum (FCS) and penicillin/streptomycin. HTB-178 cells were grown in complete Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 20% FCS and penicillin/streptomycin.

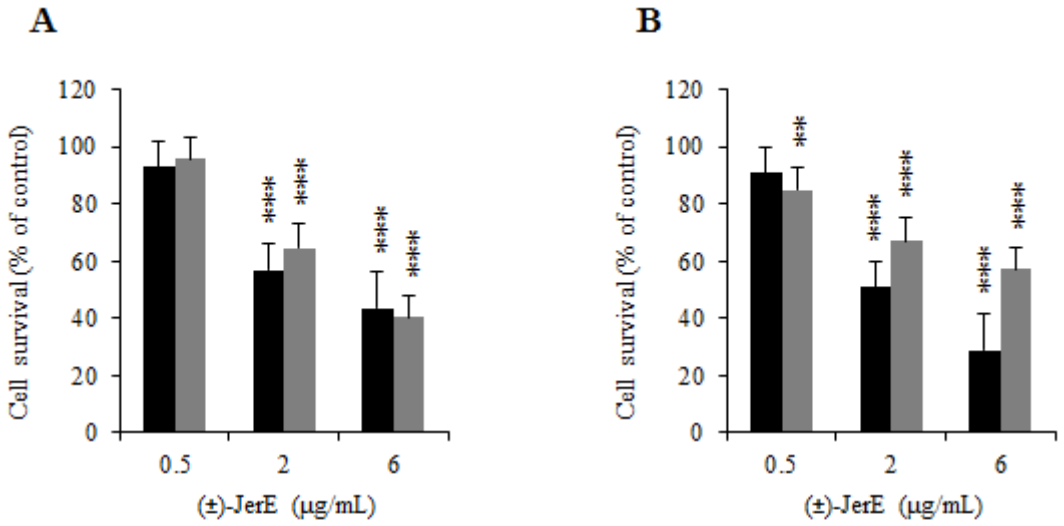
Cells were grown for 24 hours in 48 well plates (250 µL/well), after which the medium was exchanged with jerantinine E (**5**) containing medium at the indicated concentrations (prepared from stock solutions of either 1900 mg/µL or 770 µg/mL jerantinine E (**5**)), followed by an incubation for 24 or 72 hours. At the end of corresponding incubation periods, cell viability was evaluated using the MTT assay. As such, 10 µL aliquots of a solution consisting of 5 mg/mL MTT (3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide) in PBS were added to each well, followed by incubation for a 90 minutes. The cell culture supernatants were removed and the cells were treated with 250 µL of 2-propanol/0.04 N HCl (5 mL of 4 M HCl were diluted in 2-propanol until a final volume of 500 mL) and incubated for 15 minutes, after which 200 µL were transferred into a fresh 96 well plate. Absorbance measurements were carried out at 540 nm in a multi-well plate spectrophotometer (Synergy HT, Biotek) and compared with the values of control wells (*i.e.* no jerantinine E (**5**) present in the cell medium). All experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation.

[17] Performed by Mr. Davide Staedler under the supervision of Dr. MER Sandrine Gerber-Lemaire at the Laboratory of Synthesis and Natural Products at EPFL.

[18] D. Staedler, E. Idrizi, B. H. Kenzaoui, L. Juillerat-Jeanneret, *Cancer Chemoth. Pharm.* **2011**, *68*, 1161-1172.

[19] D. Staedler, T. Magouroux, R. Hadji, C. Joulaud, J. Extermann, S. Schwungi, S. Passemard, C. Kasparian, G. Clarke, M. Gerrmann, R. Le Dantec, Y. Mugnier, D. Rytz, D. Ciepielewski, C. Galez, S. Gerber-Lemaire, L. Juillerat-Jeanneret, L. Bonacina, J. P. Wolf, *Acs Nano* **2012**, *6*, 2542-2549.

Racemic Jerantinine E (5) in Human-Derived Breast Cancer Cell Lines

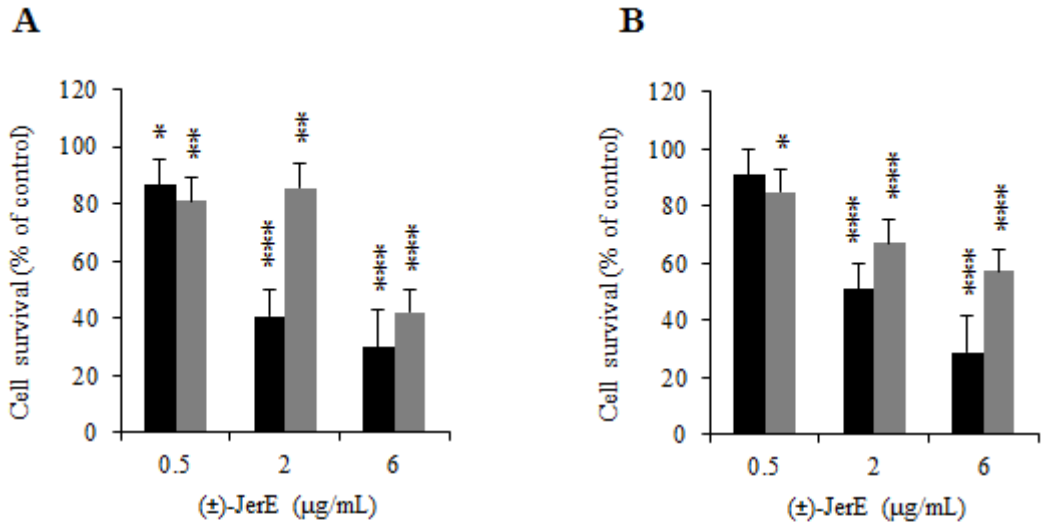


C

Cell line	IC_{50} after 24h exposure (µg/mL)	IC_{50} after 72h exposure (µg/mL)
MDA-MB-231	3.5 ± 0.27	2.1 ± 0.15
MCF-7	3.8 ± 0.23	6.3 ± 0.34

Figure S3. Cytotoxic effect of (±)-jerantinine E (**5**) on the survival of human-derived breast cancer cell lines. Human MDA-MB-231 (black bars) and MCF-7 (grey bars) breast cancer cells were exposed for 24 (**A**) or 72 hours (**B**) to increasing concentrations (0.5, 2, and 6 µg/mL) of (±)-jerantinine E (**5**). The experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation. Cells treated with (±)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test: ** $p < 0.01$; *** $p < 0.001$. The obtained IC_{50}-values after 24 and 72 hours are tabulated in part **C**.

Racemic Jerantinine E (5) in Human-Derived Lung Cancer Cell Lines



C

Cell line	IC_{50} after 24h exposure (µg/mL)	IC_{50} after 72h exposure (µg/mL)
A549	0.7 ± 0.05	1.9 ± 0.14
HTB-178	5.4 ± 0.43	6.9 ± 0.36

Figure S4. Cytotoxic effect of (±)-jerantinine E (**5**) on the survival of human-derived lung cancer cell lines. Human A549 (black bars) and HTB-178 (grey bars) lung cancer cells were exposed for 24 (**A**) or 72 hours (**B**) to increasing concentrations (0.5, 2, and 6 µg/mL) of (±)-jerantinine E (**5**). The experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation. Cells treated with (±)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The obtained IC_{50} values after 24 and 72 hours are tabulated in part **C**.

Non-Natural (+)-Jerantinine E (5) in Human-Derived Breast Cancer Cell Lines

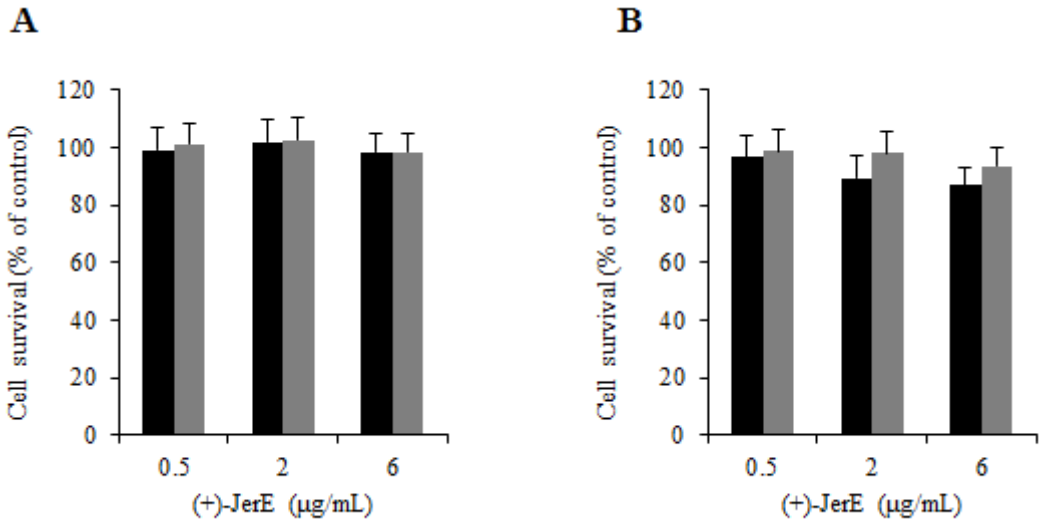


Figure S5. Cytotoxic effect of (+)-jerantinine E (**5**) on the survival of human-derived breast cancer cells.

Human MDA-MB-231 (black bars) and MCF-7 (grey bars) breast cancer cells were exposed for 24h (**A**) or 72h (**B**) to increasing concentrations (0.5, 2 and 6 µg/mL) of (+)-jerantinine E (**5**). The experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation. Cells treated with (+)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test and showed no significant differences.

Non-Natural (+)-Jerantinine E (5) in Human-Derived Lung Cancer Cell Lines

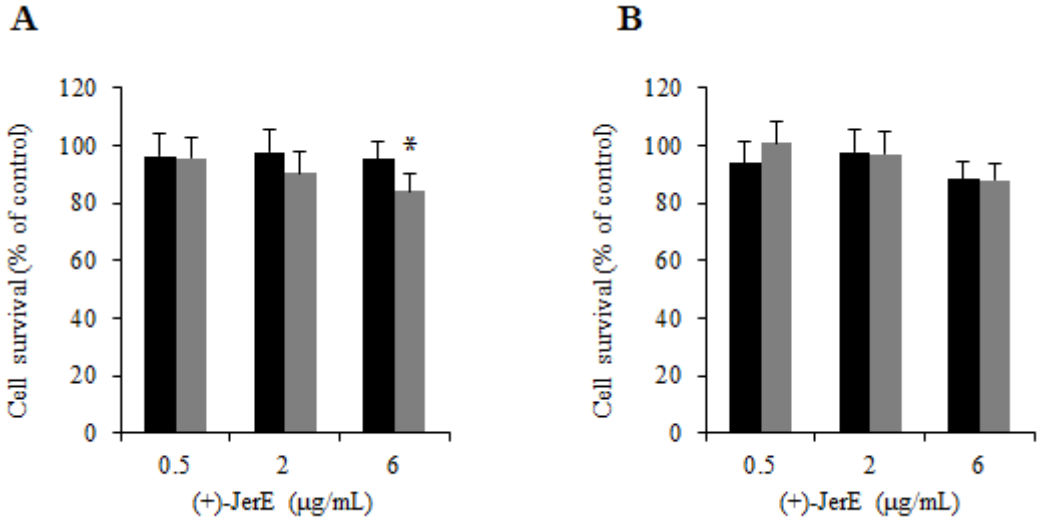
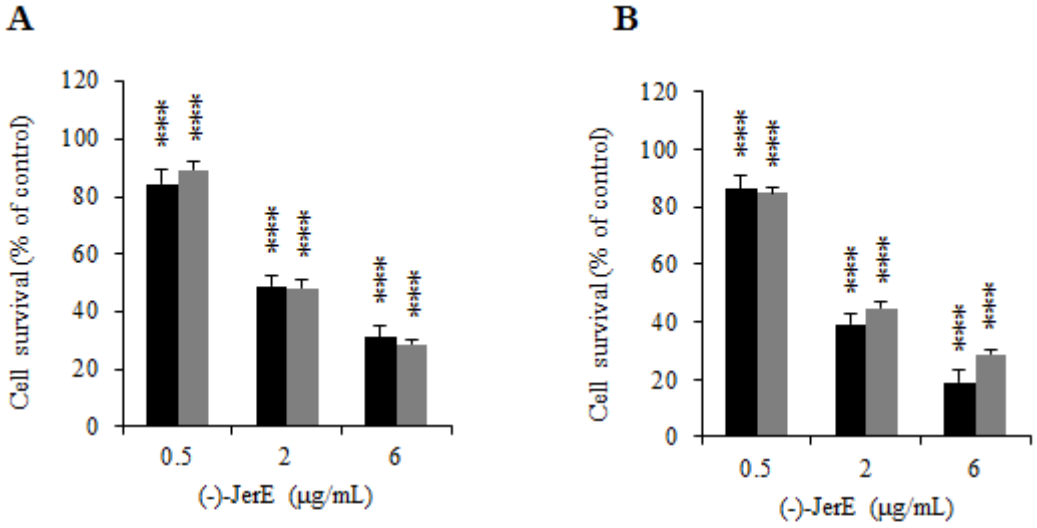


Figure S6. Cytotoxic effect of (+)-jerantinine E (**5**) on the survival of human-derived breast cancer cells.

Human A549 (black bars) and HTB-178 (grey bars) breast cancer cells were exposed for 24h (**A**) or 72h (**B**) to increasing concentrations (0.5, 2 and 6 µg/mL) of (+)-jerantinine E (**5**). The experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation. Cells treated with (+)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test: * $p < 0.05$.

Natural (-)-Jerantinine E (5) in Human-Derived Breast Cancer Cell Lines

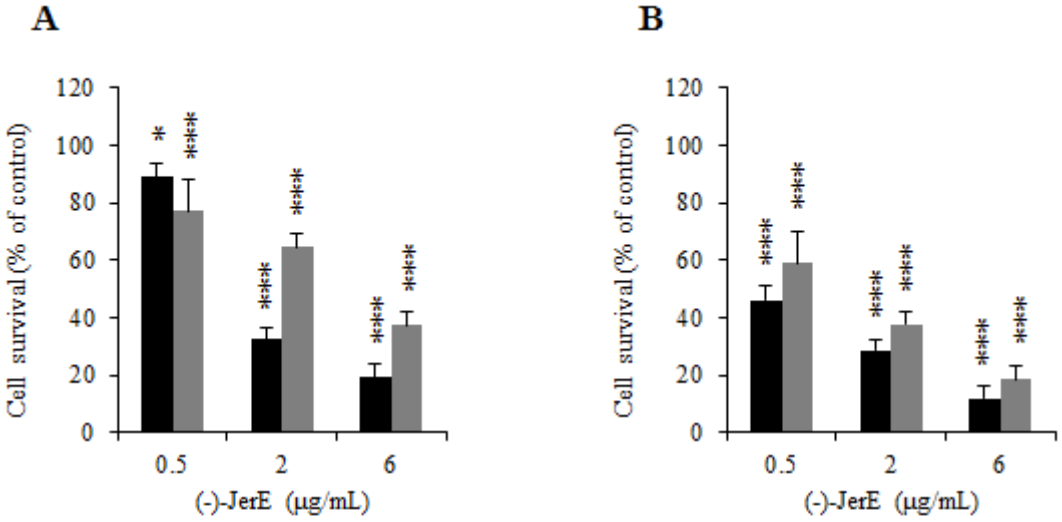


C

Cell line	IC_{50} after 24h exposure (µg/mL)	IC_{50} after 72h exposure (µg/mL)
MDA-MB-231	2.2 ± 0.12	1.7 ± 0.09
MCF-7	2.3 ± 0.14	2.0 ± 0.10

Figure S7. Cytotoxic effect of (-)-jerantinine E (**5**) on the survival of human-derived breast cancer cell lines. Human MDA-MB-231 (black bars) and MCF-7 (grey bars) breast cancer cells were exposed for 24 (**A**) or 72 hours (**B**) to increasing concentrations (0.5, 2, and 6 µg/mL) of (±)-jerantinine E (**5**). The experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation. Cells treated with (-)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test: *** $p < 0.001$. The obtained IC_{50}-values after 24 and 72 hours are tabulated in part **C**.

Natural (-)-Jerantinine E (5) in Human-Derived Lung Cancer Cell Lines



C

Cell line	IC_{50} after 24h exposure (µg/mL)	IC_{50} after 72h exposure (µg/mL)
A549	1.6 ± 0.10	0.4 ± 0.02
HTB-182	3.4 ± 0.24	0.9 ± 0.05

Figure S8. Cytotoxic effect of (-)-jerantinine E (**5**) on the survival of human-derived lung cancer cell lines. Human A549 (black bars) and HTB-178 (grey bars) lung cancer cells were exposed for 24 (**A**) or 72 hours (**B**) to increasing concentrations (0.5, 2, and 6 µg/mL) of (-)-jerantinine E (**5**). The experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation. Cells treated with (±)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test: * $p < 0.05$; *** $p < 0.001$. The obtained IC_{50} values after 24 and 72 hours are tabulated in part **C**.

Cell Migration Assays

The A549, HTB-178, MCF-7 and MDA-MB-231 cell lines were grown as described above. The cells were diluted in 100 µL of corresponding cell culture medium (300'000 cells/mL) containing jerantinine E (**5**) at the desired concentration and added to the upper chamber of a two-chamber Transwell device (6.5 mm diameter, 8.0 µm pore size polyethylene membrane, Falcon, BD). The lower Transwell chamber contained 600 µL of the corresponding cell culture medium and jerantinine E (**5**) at the desired concentration. After 15 hours of incubation, the cell layers were fixed in 4% formaldehyde in PBS and cells on the upper side of the membrane were removed by swiping with a damp cotton swab. The membrane was rinsed with PBS and stained for 10 minutes with 0.05% crystal violet in 1.5% glacial acetic acid. After washing with PBS, cells of one field of the lower side of the membrane were counted under a microscope at a 200x magnification. Experiments were conducted in duplicate and repeated twice to report the mean ± the standard deviation.

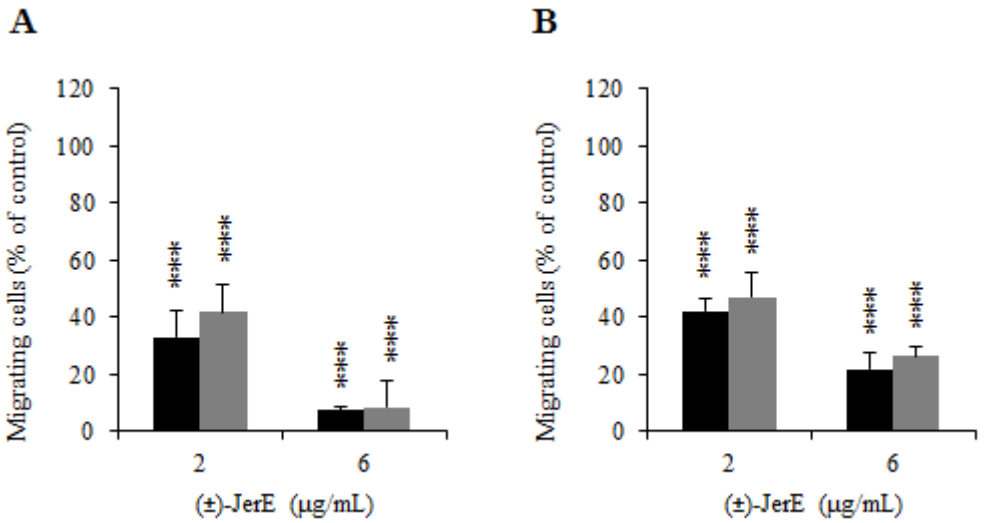


Figure S9. Migratory effect of (±)-jerantinine E (**5**) on the migration of human-derived cancer cell lines. Human-derived MDA-MB-231 (black bars) and MCF-7 (grey bars) breast cancer cells (**A**) and human-derived A549 (black bars) and HTB-178 (grey bars) lung cancer cells (**B**) were exposed for 15 hours to 2 or 6 µg/mL of (±)-jerantinine E (**5**). The number of migrating cells through a transwell device was determined through two independent experiments to report the mean ± the standard deviation. Migrating cells treated with (±)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test (we note that all comparisons were statistically significant with ***p <0.001).

Inhibition of cell migration was observed for all four cancer cell lines after 15 h exposure at 2 and 6 µg/mL concentrations of (±)-jerantinine E (**5**) (**Figure S9**). The inhibition was dose-dependent and particularly marked in breast cancer cells (**Figure S9**, **A**). The cell survival after 15 hours of (±)-jerantinine E (**5**) exposure to the corresponding cell lines was determined to investigate if the antimigratory effect is simply due to cytotoxicity. The results of this control experiment are summarized below in **Figure S10** and indicated that the antimigratory effect is not solely due to cytotoxicity as the observed inhibition of cell migration was much more significant than the observed cytotoxic effect after 15 hours.

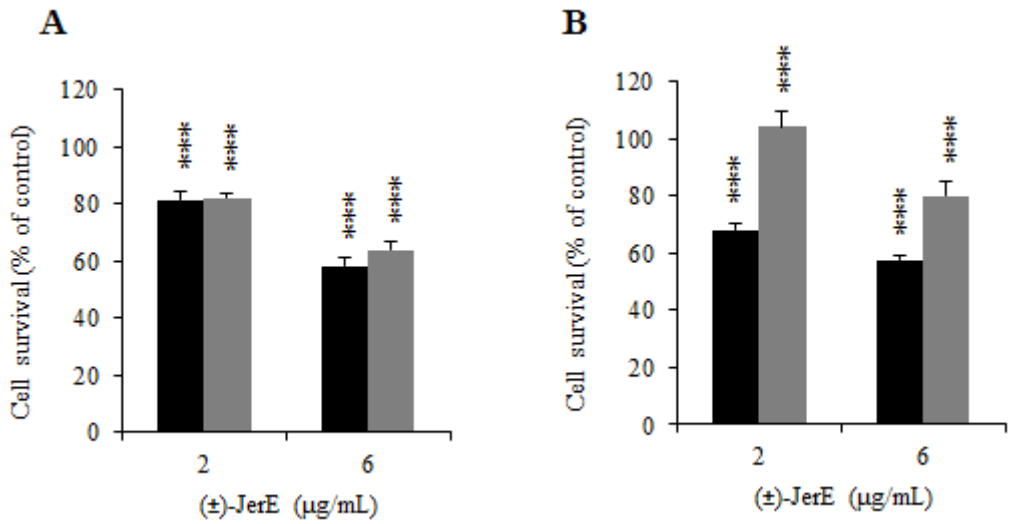


Figure S10. Cytotoxic effect of (±)-jerantinine E (**5**) on the survival of human-derived breast and lung cancer cell lines. Human-derived MDA-MB-231 (black bars) and MCF-7 (grey bars) breast cancer cells (**A**) and human-derived A549 (black bars) and HTB-178 (grey bars) lung cancer cells (**B**) were exposed for 15 hours to 2 or 6 µg/mL of (±)-jerantinine E (**5**). The experiments were conducted in triplicate wells and repeated twice to report the mean ± the standard deviation. Cells treated with (±)-jerantinine E (**5**) were compared to untreated cells using the student's *t*-test: *** $p < 0.001$.

5.2 Impedance and Cell Staining Assays[20]

Cytotoxicity assay: A MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to determine the IC_{50} of (±)-jerantinine E (**5**) with L-929 (DSMZ ACC 2) mouse fibroblast and PtK2 (ATCC CCL-56) potoroo cell lines (**Figure S11**). As such, 60 µL of serial dilutions of (±)-jerantinine E (**5**) (stock concentration 1 mg/mL in MeOH) were added to 120 µL aliquots of a cell suspension (50 000 mL^{-1}) in 96-well microplates. Blank and solvent controls were incubated under identical conditions. After 5 days, 20 µL MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg mL^{-1}. After 2 h, the resulting precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed with 100 µL PBS and dissolved in 100 µL isopropanol containing 0.4% hydrochloric acid. The microplates were gently shaken for 20 min to ensure a complete dissolution of the formazan and finally measured at 595 nm using an ELISA plate reader. All experiments were carried out in two parallels. Activity values were calculated as the mean with respect to the controls set to 100%. Cell lines were grown in media recommended by the supplier (DSMZ or ATCC).

[20] Performed by Ms. Aruna Raja, Dr. Raimo Franke and Dr. Florenz Sasse at the Helmholtz Centre for Infection Research in Braunschweig.

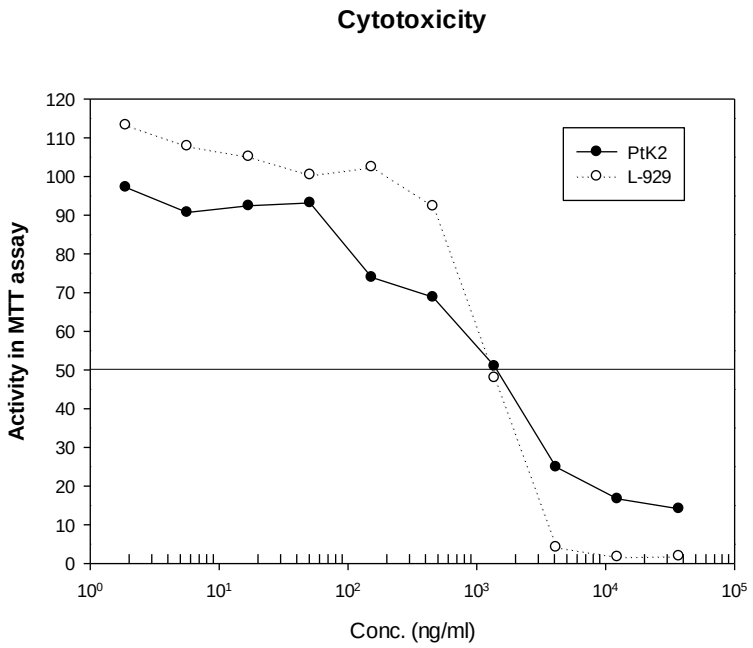


Figure S11: Cytotoxicity of (±)-jerantinine E (**5**) with mouse fibroblast cells (L-929) and rat kangaroo kidney cells (PtK2) indicating an IC_{50} of 14 µg/ml.

Impedance profiling

The impedance measurements were performed with small modifications on a RT-CES system (xCelligence) from Acea Biosciences (Roche), which has been described previously.[21] For time-dependent cell response profiling, 60 µL of Dulbecco modified Eagle medium (DMEM) were added to 96-well E-Plate to obtain background readings followed by the addition of 120 µL cell suspension of L-929 cells. After each step, the E-Plates were incubated for 30 minutes at room temperature and then placed on the reader in the incubator for continuous recording of impedance as reflected by the cell index. After 24 hours of incubation the cells were treated with the compounds. To prepare (±)-jerantinine E (**5**) for screening, the stock solution (10 mM in DMSO) was diluted with DMEM to get a final test concentration of the IC_{90} and less than 0.1 % DMSO. 1 µL of each prepared solution was then transferred into the 96-well E-Plate. Each E-plate also contained wells with DMSO only as a solvent control. All measurements were performed in triplicates and run for 5 days. The time-dependent cellular response profiles (TCRP) were recorded by the Roche RTCA Software, Version 1.2. Data processing and mining workflow was implemented in the statistical programming language R, Version 2.12.2 (R Development Core Team, 2011). The following additional R packages were used in addition: class[22], gplots[23] and MASS.[21] For the development of the R code the integrated development environment R Studio, Version 0.94.92, was used. The workflow

[21] a) K. Solly, X. Wang, X. Xu, B. Strulovici, W. Zheng, *Assay Drug Dev. Technol.* **2004**, *2*, 363; b) J. M. Atienza, N. Yu, S. L. Kirstein, B. Xi, X. Wang, X. Xu, Y. A. Abassi, *Assay Drug Dev. Technol.* **2006**, *4*, 597; c) Y. A. Abassi, B. Xi, W. Zhang, P. Ye, S. L. Kirstein, M. R. Gaylord, S. C. Feinstein, X. Wang, X. Xu, *Chem. Biol*. **2009**, *16*, 712.

[22] W. N. Venables, B. D. Ripley, *Modern Applied Statistics with S* (4th ed.) 2002, Springer New York.

[23] G. R. Warnes gplots: Various R programming tools for plotting data. 2010. Retrieved from http://cran.rproject.org/package=gplots.

starts by importing the raw impedance data which is provided by the RTCA software as cell index (CI) data. The CI is already background corrected and is calculated as follows (Eq. 1):[24]

Eq. 1: $$CI(t)=\frac{R_t-R_b}{Z_n}$$

where CI(t) is the cell index at time point t, R_t is defined as measured electrode impedance of the well with the cells in the medium at a certain time point and R_b as measured background impedance of the well with the cell medium alone. Z_n is a frequency factor which corrects for different frequencies of the alternating voltage the xCelligence system can use, with the standard setting being Z_n=15. The raw data were imported into R and normalized as suggested by Abassi and colleagues by dividing the cell indices for each time point after compound addition by the cell index at a reference time point (Eq. 2).[23] As reference time point, the last measurement before compound addition was taken. For subsequent analysis, only the measurements starting at the reference time point (with normalized cell index = 1) and later were considered.

Eq. 2: $$NCI=\frac{CI_t}{CI_{t(reference)}}$$

The reference compounds and (±)-jerantinine E (**5**) with unknown mode of action were measured as triplicates which were randomly distributed over the microtiter plates (using sampling without replacement in R) to avoid batch effects.

Detection and removal of outliers was carried out using the median polishing procedure. The central idea of the data mining concept is to use cubic smoothing splines for the approximation of the impedance data and as dimension reduction technique. This approach has the benefit of avoiding the curse of dimensionality and the Runge phenomenon that occurs for high polynomials, while keeping the complexity of the data set. The smooth.spline function of R was used for TCRP approximation. As set of descriptors the spline basis coefficients were extracted to construct a distance matrix that was used for hierarchical cluster analysis. A heatmap was constructed that displays the Z-transformed values of the 22 descriptors (= basis spline coefficients). Hierarchical cluster analysis of the reference compounds together with the compound of unknown mode of action was carried out. Co-clustering of the compound of unknown mode of action with reference compounds with known activity class label is used to predict the mode of action. The results of the impedance profiling are shown below in **Figure S12**.

[24] N. Ke, B. Xi, P. Ye, W. Xu, M. Zheng, L. Mao, M. J. Wu, J. Zhu, J. Wu, W. Zhang, J. Zhang, J. Irelan, X. Wang, X. Xu, Y. A. Abassi, *Anal. Chem.* **2010**, *82*, 6495.

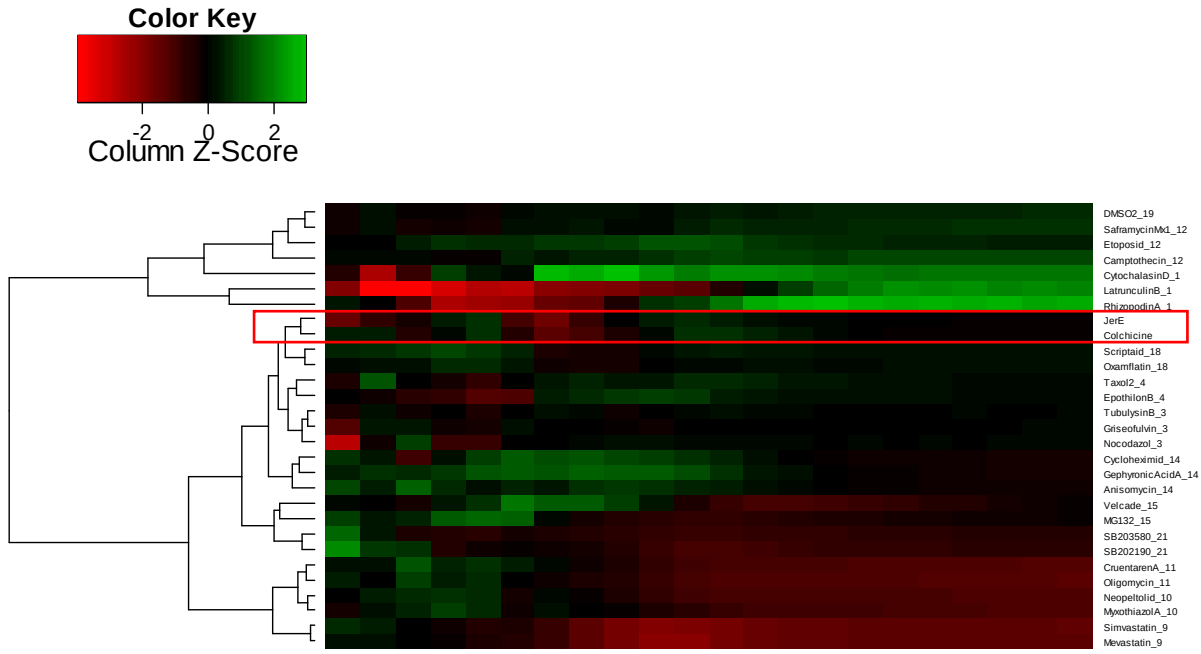


Figure S12. Cluster analysis based on impedance monitoring curves shows that the closest neighbour of (±)-jerantinine E (**5**, JerE) is colchicin, a known tubulin disrupting agents.

Cell Staining: PtK2 cells (ATCC CCL-56) were grown in 750 µL medium in 4-well plates (Nunc) on glass coverslips, and incubated with (±)-jerantinine E (**5,** 2 µg mL–1) overnight. For α-tubulin staining, cells were fixed with cold (–20 °C) MeOH/acetone (1:1) for 10 min and incubated with a mouse anti α-tubulin antibody (1:100; Sigma) followed by a secondary Alexa Fluor 488 labelled goat anti-mouse IgG antibody (1:200; Molecular Probes) and mounted in ProLong Antifade Gold (Molecular Probes), which included DAPI (4',6-diamidino-2-phenylindole) to stain the nuclei. The results of the immunofluorescent staining are depicted below in **Figure S13**.

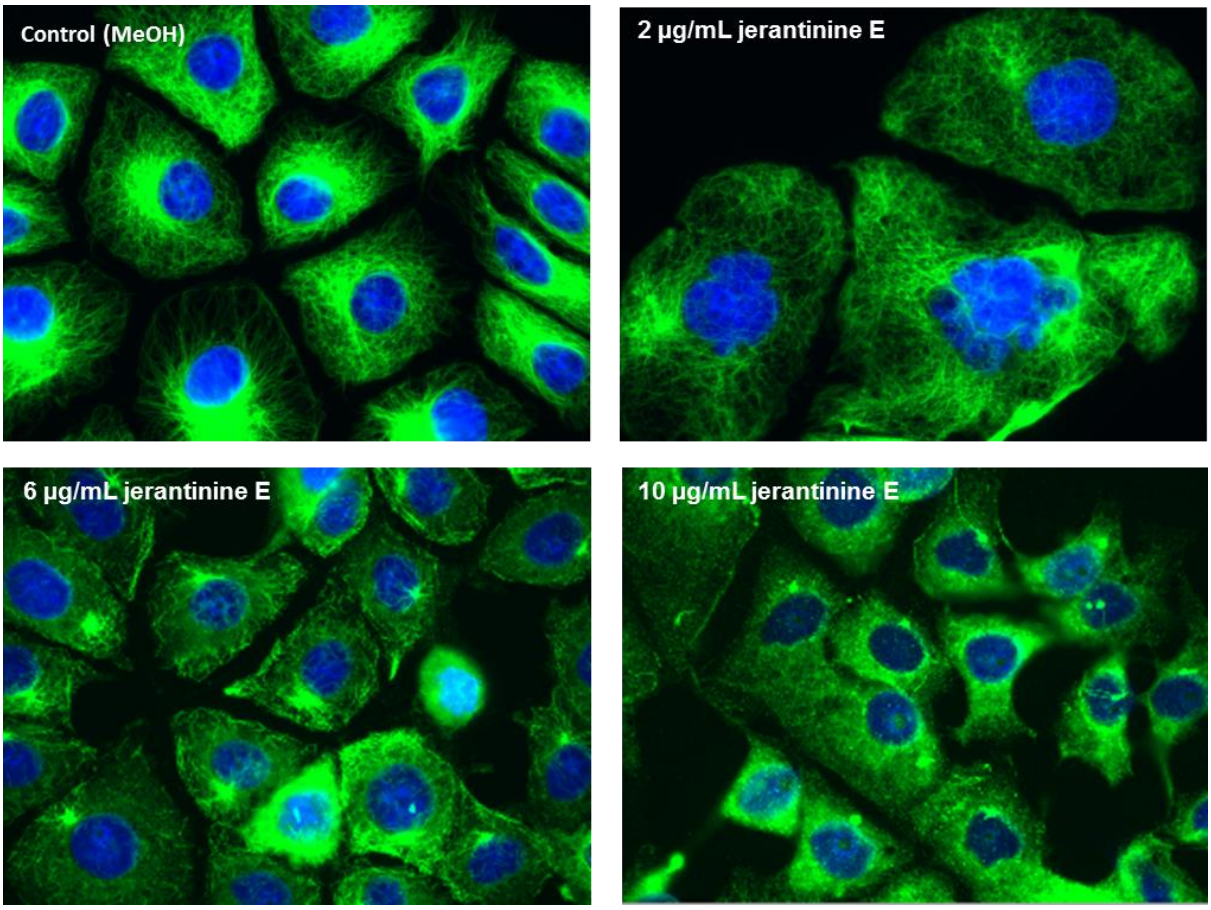


Figure S13. Immunofluorescent staining of untreated PtK2 kidney cells (control/MeOH) and cells exposed to 2 µg/mL, 6 µg/mL and 10 µg/mL (±)-jerantinine E (**5**) showing the effect on microtubules (green) and the cell nucleus (blue).

5.3 Tubulin Polymerization Assay[17]

Effects on tubulin polymerization were assessed at 0.5, 1, 3, 5 and 10 µM for both enantiomers and racemic jerantinine E (**5**) using the Tubulin Polymerization Assay Kit purchased from Cytoskeleton (cat. no. BK011P). The assay was carried out according to the corresponding protocol instructions. The compounds paclitaxel and colchicine (at 3 µM; two different stocks of colchicine, purchased from Sigma-Aldrich, were freshly prepared and tested in two independent duplicates) were used as tubulin polymerization enhancer and inhibitor controls, respectively. The assay was performed applying the conditions for tubulin polymerization inhibition detection. Stock solutions of test compounds were prepared at 2 mM in DMSO. Measurements were obtained in duplicate form and repeated twice. The IC_{50}–values were calculated form the dose-response curve during the growth phase. Summarized below are the IC_{50}–values of both enantiomers and racemic jerantinine E (**5**) as well as the inhibition of tubulin polymerization values at 3 µM (including colchicine).

IC_{50}–values of (±)-jerantinine E, (-)-jerantinine E and (+)-jerantinine E:

Compound	**IC_{50} (μg/mL)**	**IC_{50} (μM)**
(±)-Jerantinine E	3.89 ± 0.11	10.1 ± 0.28
(+)-Jerantinine E	>> 4	>> 10
(-)-Jerantinine E	0.171 ± 0.01	0.446 ± 0.02

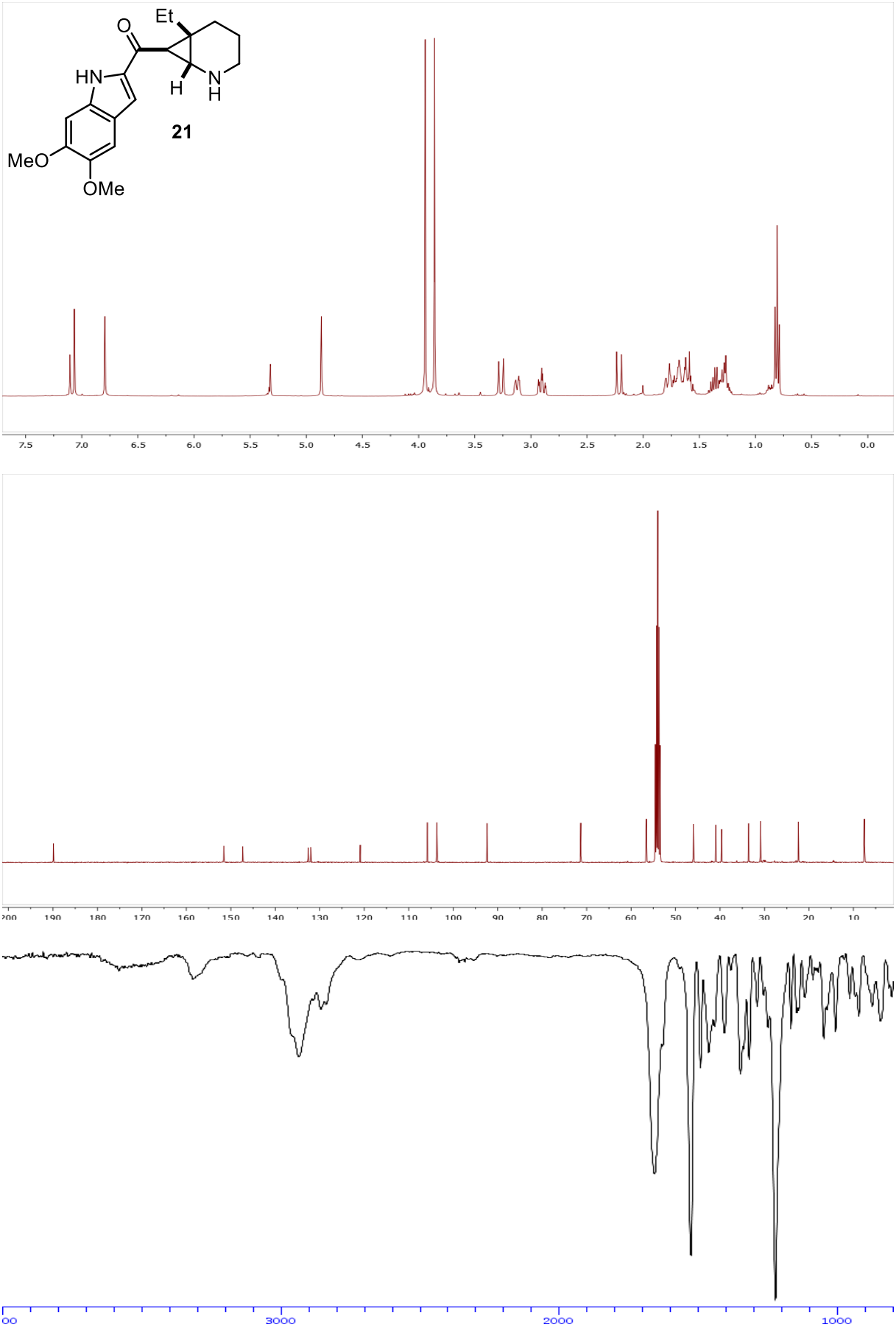
Inhibition of tubulin polymerization at 3 μM: comparison between jerantinine E, (-)-jerantinine E, (+)-jerantinine E and colchicine.

Compound	**% of inhibition**
(±)-Jerantinine E	18.4% ± 0.7%
(+)-Jerantinine E	11.8% ± 0.5%
(-)-Jerantinine E	79.1% ± 2.3%
Colchicine	75.0% ± 2.5%

We note that (-)-jerantinine E (**5**) was a more effective inhibitor of tubulin polymerization than colchicine (the difference was statistically significant with *$p<0.05$ (Student *t* test)).

6. Spectra of New Compounds

Shown below are the corresponding ^{1}H, ^{13}C NMR and IR spectra of the above fully characterized new compounds. All ^{1}H NMR spectra were obtained at 400 MHz and all ^{13}C NMR spectra were obtained 101 MHz. Deuterated chloroform or dichloromethane, as indicated above in the tabulated NMR data for each fully characterized compound, were used as solvent to carry out the corresponding NMR characterization.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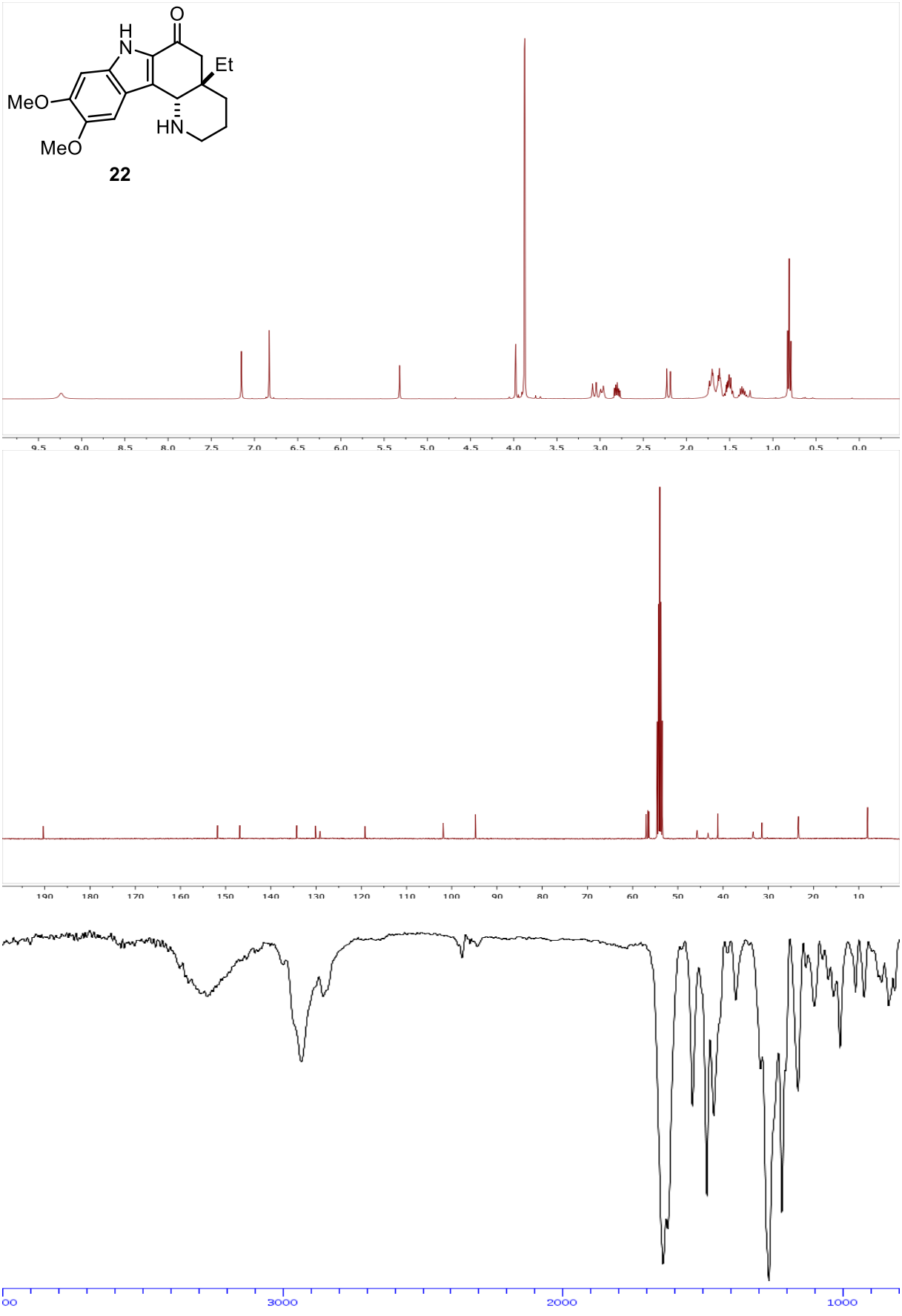
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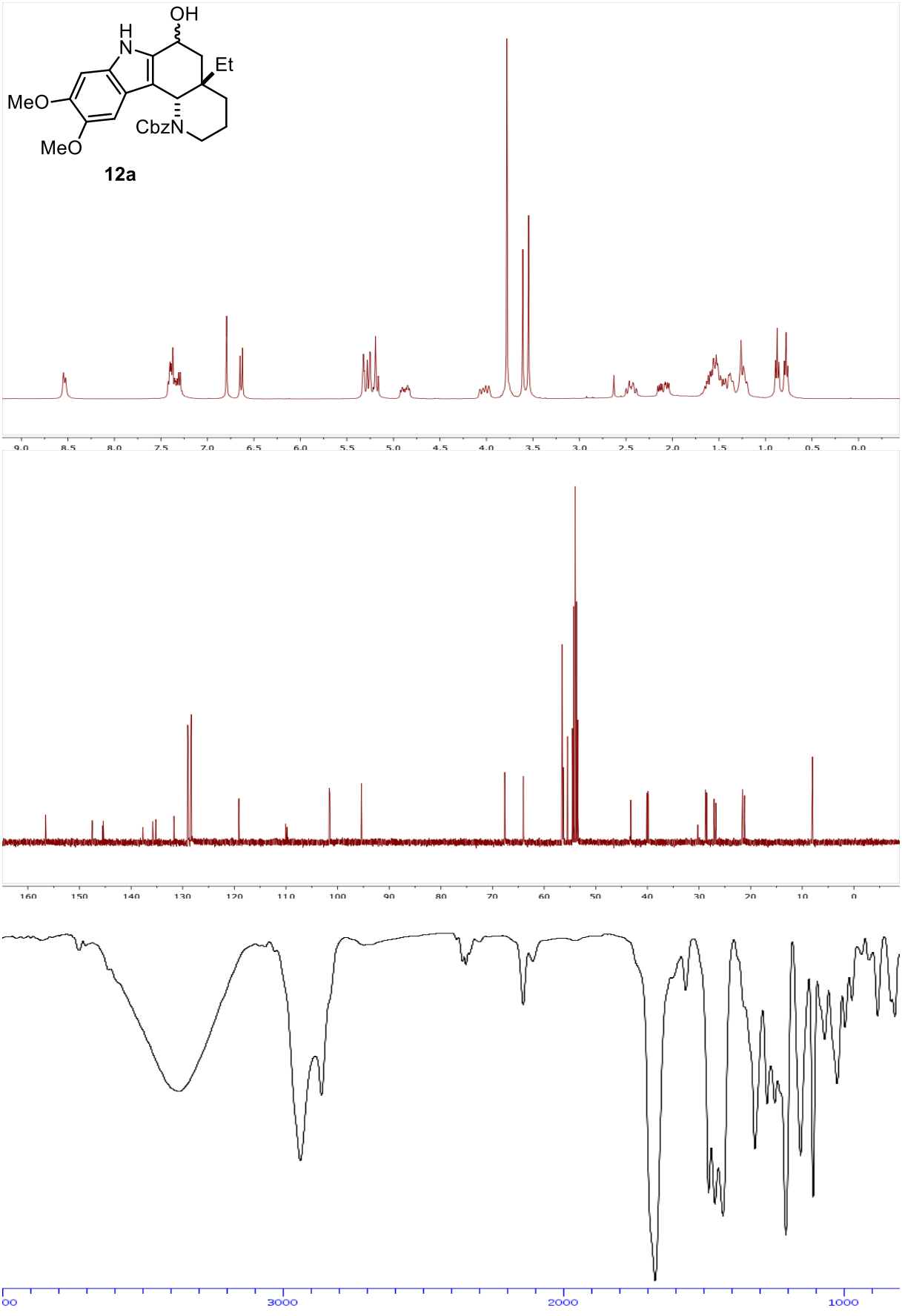
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CbzN
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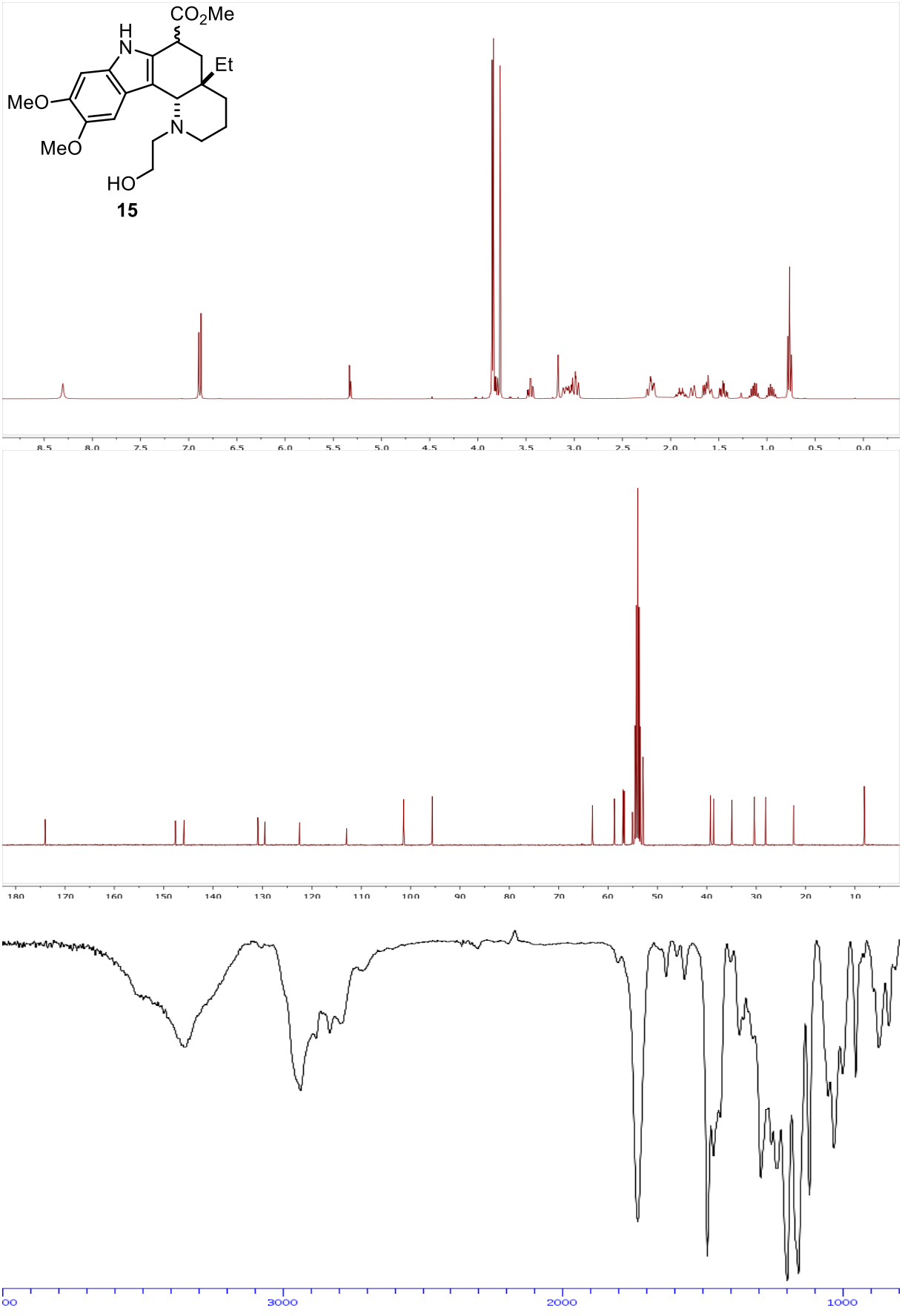
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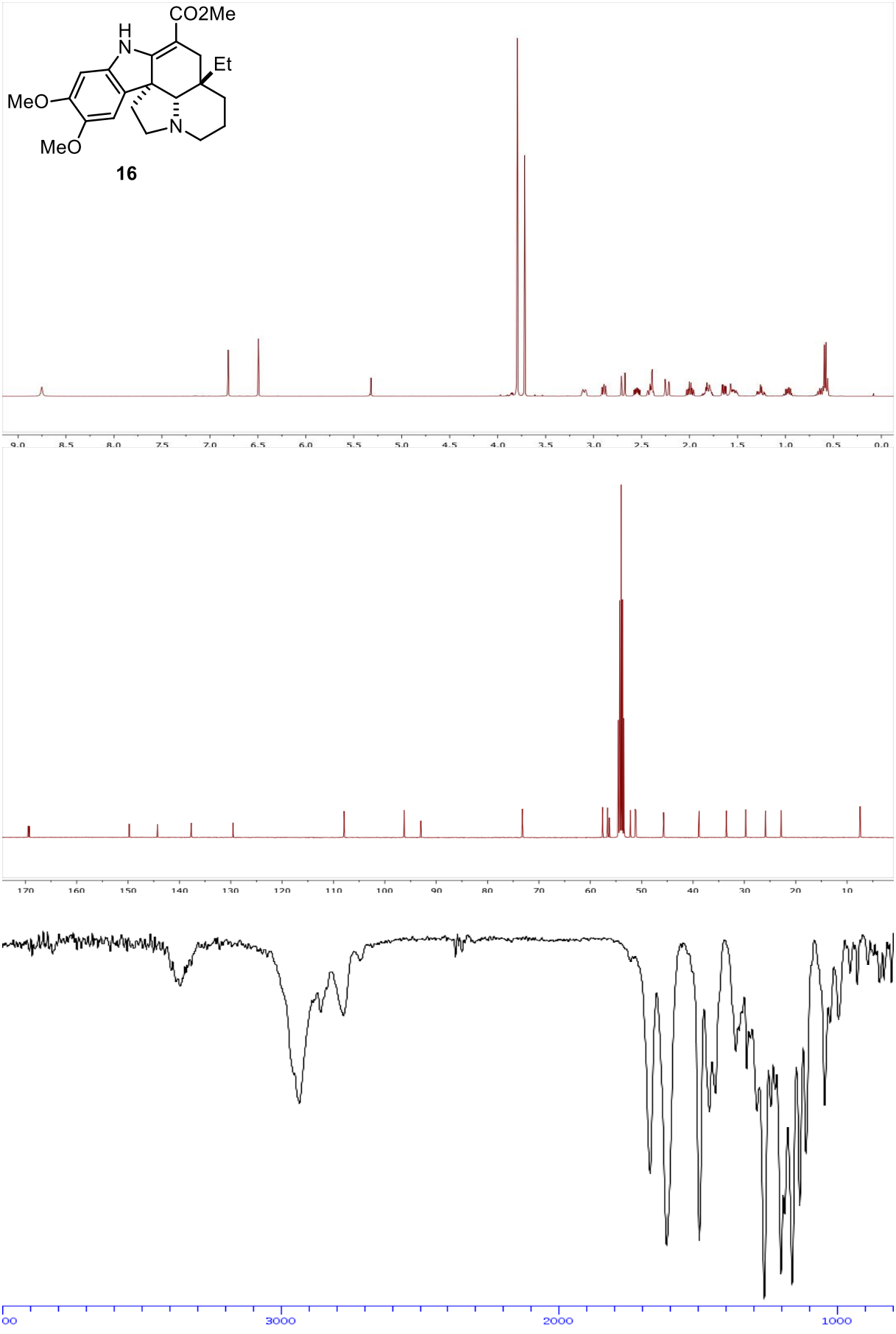
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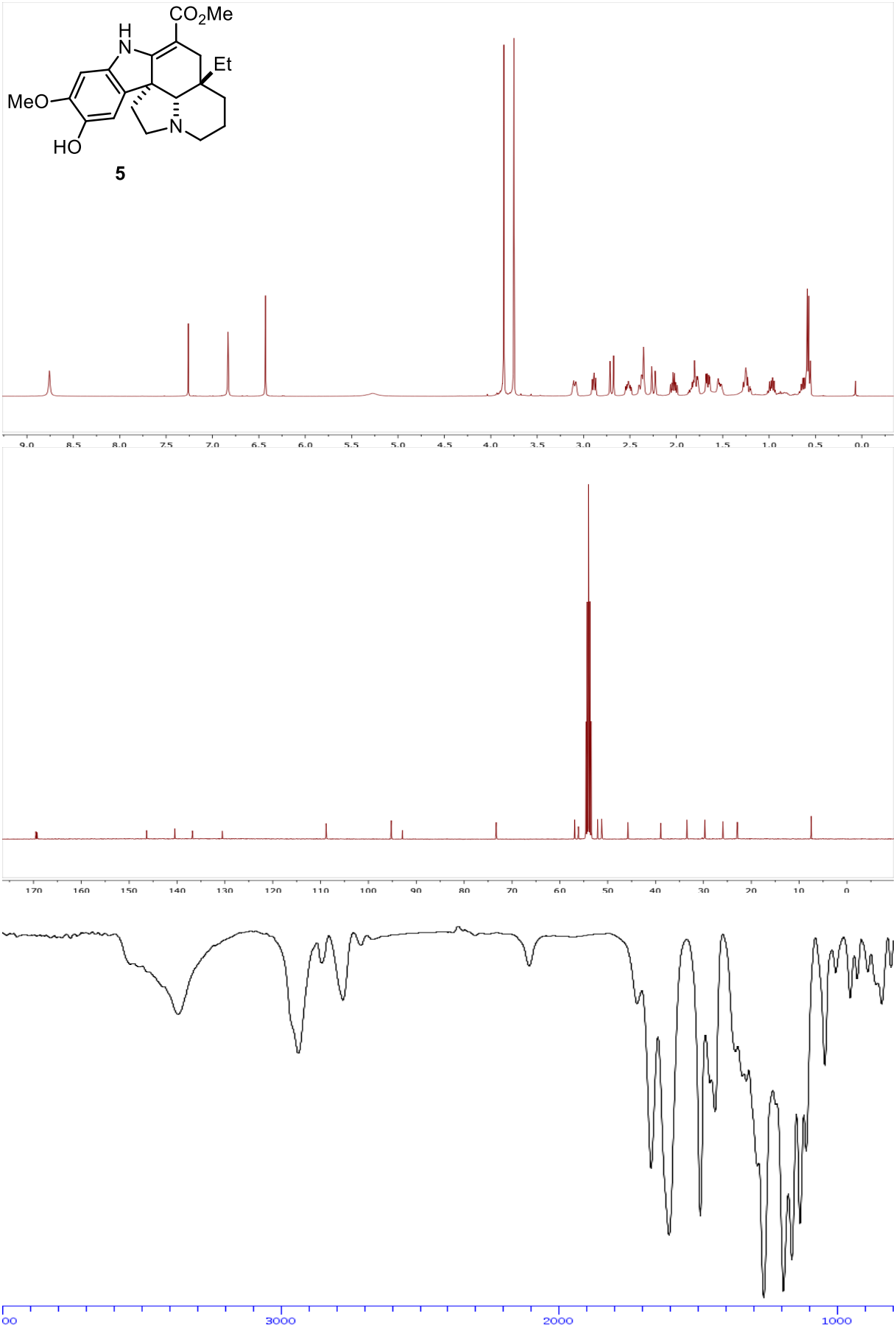
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