รายงานวิจัยฉบับสมบูรณ์

โครงการ การสังเคราะห์สารต้านมะเร็ง melotenine A

โดย ผศ.ดร. ไพบูลย์ เงินมีศรี และคณะ

กันยายน 2557

สัญญาเลขที่ DBG5480018

รายงานวิจัยฉบับสมบูรณ์

โครงการ การสังเคราะห์สารต้านมะเร็ง melotenine A

ผู้วิจัย	สังกัด
1. ผศ.ดร.ไพบูลย์ เงินมีศรี	มหาวิทยาลัยเกษตรศาสตร์
2. นางสาวหัสยาพร รัตนรัตน์	มหาวิทยาลัยเกษตรศาสตร์
3. นางสุดาพร อารีย์	มหาวิทยาลัยเกษตรศาสตร์
4. นางสาวสโรชา ซุ้นกิจ	มหาวิทยาลัยเกษตรศาสตร์

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

สารบัญ

บทคัดย่อ

รหัสโครงการ: DBG5480018

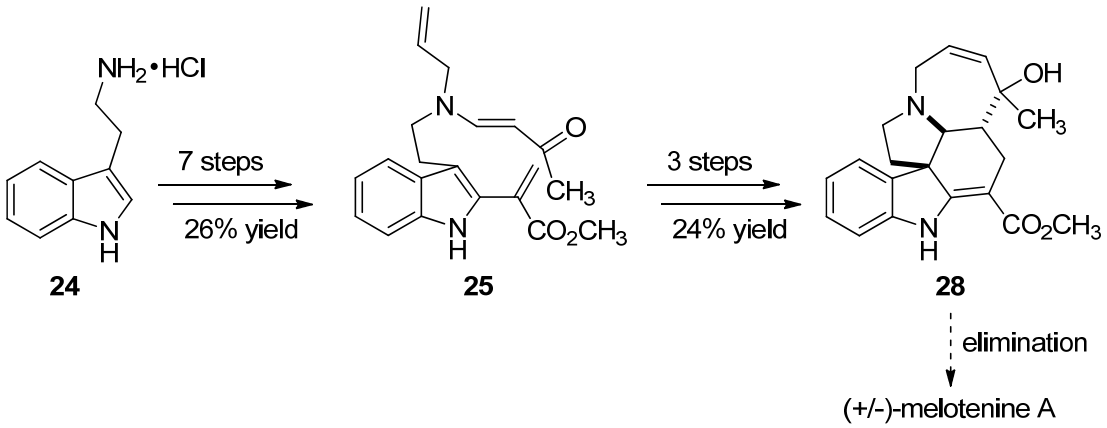
ชื่อโครงการ: การสังเคราะห์สารต้านมะเร็ง melotenine A

ชื่อนักวิจัย และสถาบัน: ผศ.ดร.ไพบูลย์ เงินมีศรี มหาวิทยาลัยเกษตรศาสตร์

อีเมล์: fscipbn@ku.ac.th

ระยะเวลาโครงการ: 30 กันยายน 2554 ถึง 29 กันยายน 2557

บทคัดย่อ:



Melotenine A ซึ่งแยกได้จากต้น *Melodinus tenuicaudatus* ที่พบในประเทศจีนเป็นสารผลิตภัณฑ์ธรรมชาติประเภท monoterpene indole alkaloid โดยมีโครงสร้างเป็นวง 5 วงแบบ 6/5/5/6/7 ซึ่งไม่เคยมีการพบมาก่อน นอกจากนี้สารนี้ยังมีสมบัติต้านเซลล์มะเร็งในมนุษย์ถึง 5 ชนิด กลยุทธ์ของเราในการสังเคราะห์สารนี้คือการใช้ Diels-Alder ในการสร้างวงห้าเหลี่ยมและหกเหลี่ยมและปฏิกิริยา ring-closing metathesis ในการสร้างวงเจ็ดเหลี่ยม ในตอนนี้เราได้สังเคราะห์สารตั้งต้น (**25**) สำหรับทำปฏิกิริยา Diels-Alder เสร็จเรียบร้อยแล้ว โดยมีทั้งหมด 7 ขั้นตอนและร้อยละผลิตภัณฑ์รวมเป็น 26% จากนั้นสารนี้ถูกเปลี่ยนเป็นสารที่ **28** ใน 3 ขั้นตอน คือปฏิกิริยา Diels-Alder, ปฏิกิริยา Grignard และปฏิกิริยา ring-closing metathesis ตามลำดับ โดยมีร้อยละผลิตภัณฑ์รวมเป็น 24% ขณะนี้เรากำลังศึกษาขั้นตอนสุดท้ายซึ่งเป็นปฏิกิริยาการขจัดเพื่อที่สังเคราะห์ melotenine A ให้เสร็จสมบูรณ์

คำหลัก : จำนวน 3-5 คำ

การสังเคราะห์สารผลิตภัณฑ์ธรรมชาติ, Melotenine A, สารต้านมะเร็ง, ปฏิกิริยา Diels-Alder, ปฏิกิริยา Ring-closing Metathesis

Abstract

Project Code: DBG5480018

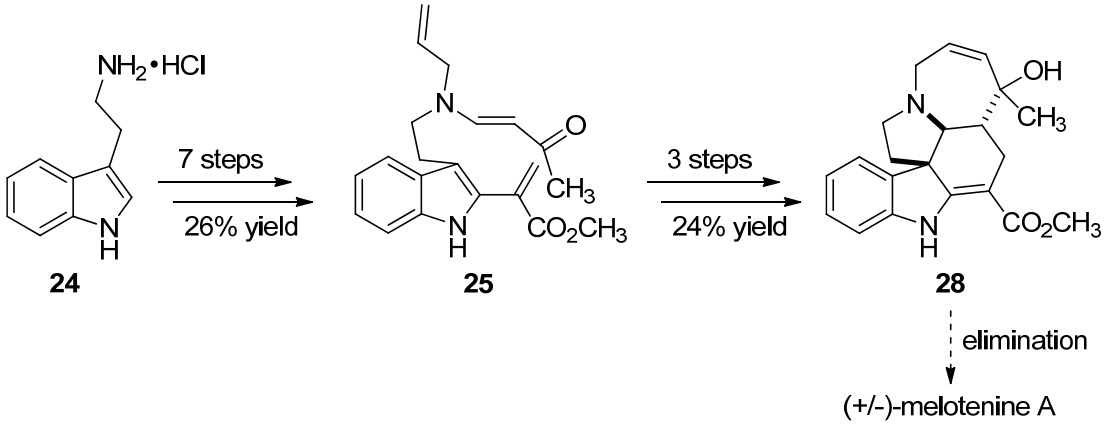
Project Title: Total Synthesis of Anticancer Melotenine A

Investigator: Asst. Prof. Dr. Paiboon Ngernmeesri

E-mail Address: fscipbn@ku.ac.th

Project Period: September 30, 2011 to September 29, 2014

Abstract:



Melotenine A isolated from of *Melodinus tenuicaudatus* found in China is a monoterpene indole alkaloid bearing an unprecedented skeleton with a 6/5/5/6/7 pentacyclic ring system. This natural product has been found to be an inhibitor against five human cancer cell lines. Our strategy to synthesize this natural product employs a Diels-Alder reaction to construct five- and six-membered rings, and ring-closing metathesis to construct a seven-membered ring. Up to now, precursor **25** for the Diels-Alder reaction has been prepared in seven steps with 26% overall yield. This compound was converted to pentacycle **28** in three steps with 24% overall yield via Diels-Alder reaction, Grignard reaction and ring-closing metathesis, respectively. Currently, we are investigating the last step, which is an elimination reaction, to complete the synthesis.

Keywords : 3-5 words

Natural Product Synthesis, Melotenine A, Anticancer, Diels-Alder Reaction, Ring-closing Metathesis

Executive summary

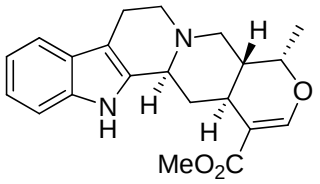
งานวิจัยนี้เป็นการศึกษาการสังเคราะห์สารต้านมะเร็ง melotenine A โดยสารนี้มีสมบัติต้านเซลล์มะเร็งในมนุษย์ถึง 5 ชนิดด้วยกันได้แก่มะเร็งเต้านม (SK-BR-3 breast), มะเร็งเซลล์ตับ (SMMC-7721 hepatocellular carcinoma), มะเร็งเม็ดเลือดขาว (HL-60 myeloid leukemia), มะเร็งตับอ่อน (PANC-1 pancreatic cancer) และมะเร็งปอด (A-549 lung cancer) และเมื่อเทียบสมบัตินี้กับยารักษามะเร็ง cisplatin พบว่าสารตัวใหม่นี้มีสมบัติต้านเซลล์มะเร็งได้ดีกว่ามาก เราวางแผนการสังเคราะห์สาร melotenine A ไว้ทั้งหมด 11 ขั้นตอน โดยในตอนนี้ทำเสร็จไปแล้ว 10 ขั้นตอน โดยมีร้อยละผลิตภัณฑ์รวมเป็น 6.4% เรามีขั้นตอนที่เหลือคือปฏิกิริยาการขจัดซึ่งเป็นขั้นตอนสุดท้ายเพียงขั้นตอนเดียวเท่านั้นที่จะทำให้การสังเคราะห์สารเสร็จสมบูรณ์และสามารถนำผลงานไปตีพิมพ์ในวารสารนานาชาติได้

วัตถุประสงค์

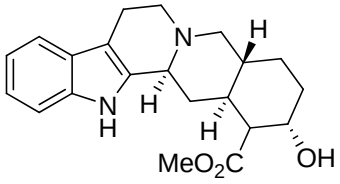
1) เพื่อสังเคราะห์สารต้านมะเร็ง Melotenine A และทดสอบฤทธิ์ทางยาของสารประกอบตัวอื่นๆที่สังเคราะห์ขึ้นจากการสังเคราะห์สารต้านมะเร็งนี้
2) เพื่อตีพิมพ์ผลงานทางวิชาการ
3) เพื่อผลิตบัณฑิตระดับปริญญาโท และ/หรือ ปริญญาเอกที่มีความเชี่ยวชาญทางด้านเคมีอินทรีย์สังเคราะห์

คำนำ

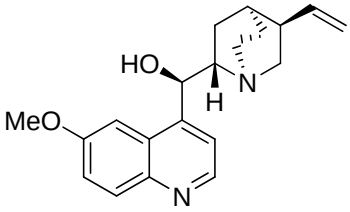
Terpene indole alkaloids are a large group of natural products. Some of them have complicated and interesting structures, and possess a wide range of biological activities, including anticancer, anti-malarial and anti-arrhythmic (Figure 1).[1]



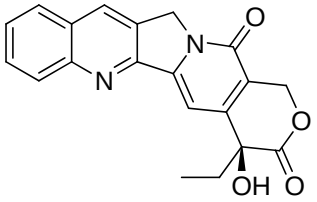
ajmalicine (**1**)
(hypertension)
Catharanthus roseus,
Rauwolfia serpentina



yohimbine (**2**)
(adrenergic receptor blocker)
Rauwolfia serpentina



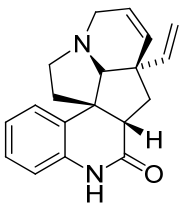
quinine (**3**)
(anti-malarial)
Cinchona



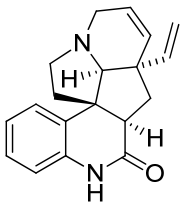
camptothecin (**4**)
(anti-tumor)
Ophiorrhiza pumila,
Camptotheca acuminata

Figure 1 Representative terpene indole alkaloids, with corresponding biological function and species of plant from which they are isolated.

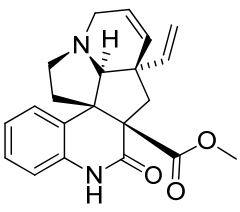
It was reported that more than 80 compounds have been isolated from the genus *Melodinus* (Apocynaceae). Most of these compounds are monomeric and dimeric monoterpenoid indole as well as quinoline alkaloids, which are considered to be biosynthesized from the condensation of tryptophan with secologanin. Examples of these natural products are meloscine (**5**), epimeloscine (**6**), scandine (**7**), and deoxoapodine (**8**) as shown in Figure 2.[2]



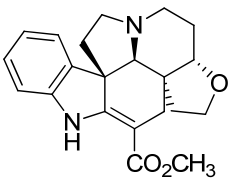
meloscine (**5**)



epimeloscine (**6**)



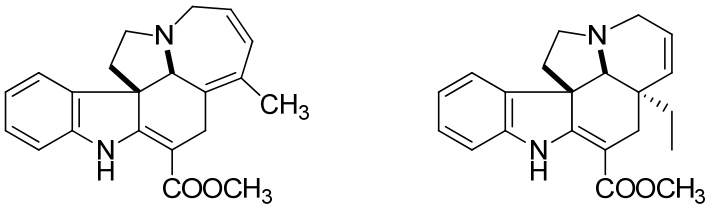
scandine (**7**)



deoxoapodine (**8**)

Figure 2 Many characteristic *Melodinus* alkaloids

In 2010, Luo and co-workers isolated two natural products from the plant *Melodinus tenuicaudatus*. These natural products are melotenine A (**9**) and tabersonine (**10**) as shown in Figure 3. Melotenine A is a monoterpene indole alkaloid and has an unprecedented skeleton with a 6/5/5/6/7 pentacyclic ring system whereas tabersonine is a known apidospermane alkaloid considered to be the precursor of melotenine A.



melotenine A (**9**) tabersonine (**10**)

Figure 3 General structure of melotenine A (**9**) and tabersonine (**10**).

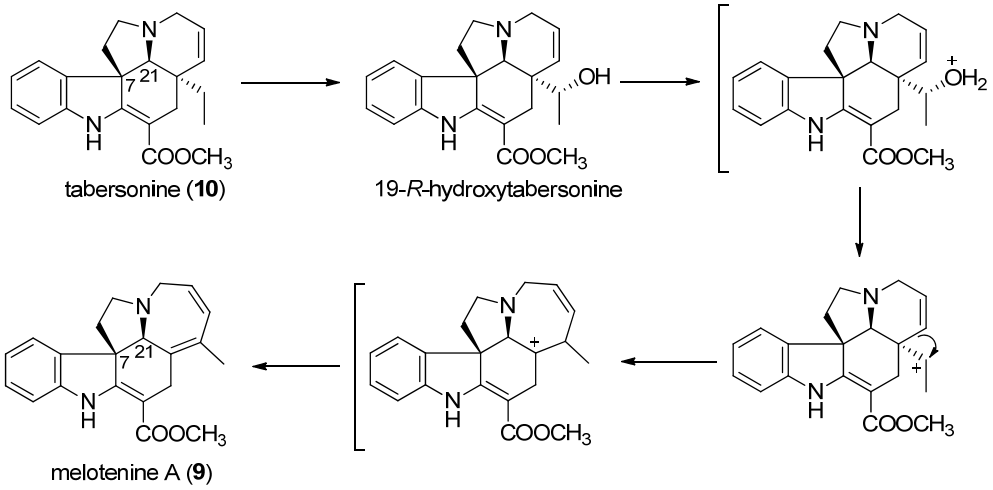
Melotenine A has been found to be a potent cytotoxicity against five human cancer cell lines, including SK-BR-3 breast, SMMC-7721 hepatocellular carcinoma, HL-60 myeloid leukemia, PANC-1 pancreatic cancer, and A-549 lung cancer. Table 1 shows that melotenine A exhibits stronger inhibitory activity with low IC_{50} values than that of cisplatin, which is an anticancer drug. Unfortunately, further pharmacological investigation on melotenine A could not be performed due to the limited amount available.

Table 1 Cytotoxicity of melotenine A, tabersonine and cisplatin.

	IC_{50} (μM)		
cells	melotenine A	tabersonine	cisplatin
SK-BR-3	2.8	11.7	21.7
SMMC-7721	5.2	18.8	18.1
HL-60	0.9	5.4	2.6
PANC-1	3.6	30.5	24.8
A-549	10.7	25.9	15.8

Luo has proposed a plausible biogenetic pathway that melotenine A (**9**) might be derived from tabersonine (**10**) (Scheme 1). In this pathway, tabersonine was oxidized to produce 19-*R*-hydrotabersonine, which might undergo a Wagner-Meerwein rearrangement. This rearrangement involves the formation of 3° carbocation intermediate from 2° carbocation and then a 1,2-alkyl shift occurs to produce the novel

skeleton of melotenine A. Since it has been known that the absolute configurations at C-7 and C-21 of tabersonine are *R* and *S*, respectively, the absolute configuration at stereogenic centers of **10** could be inferred as 7*R*, 21*S*.

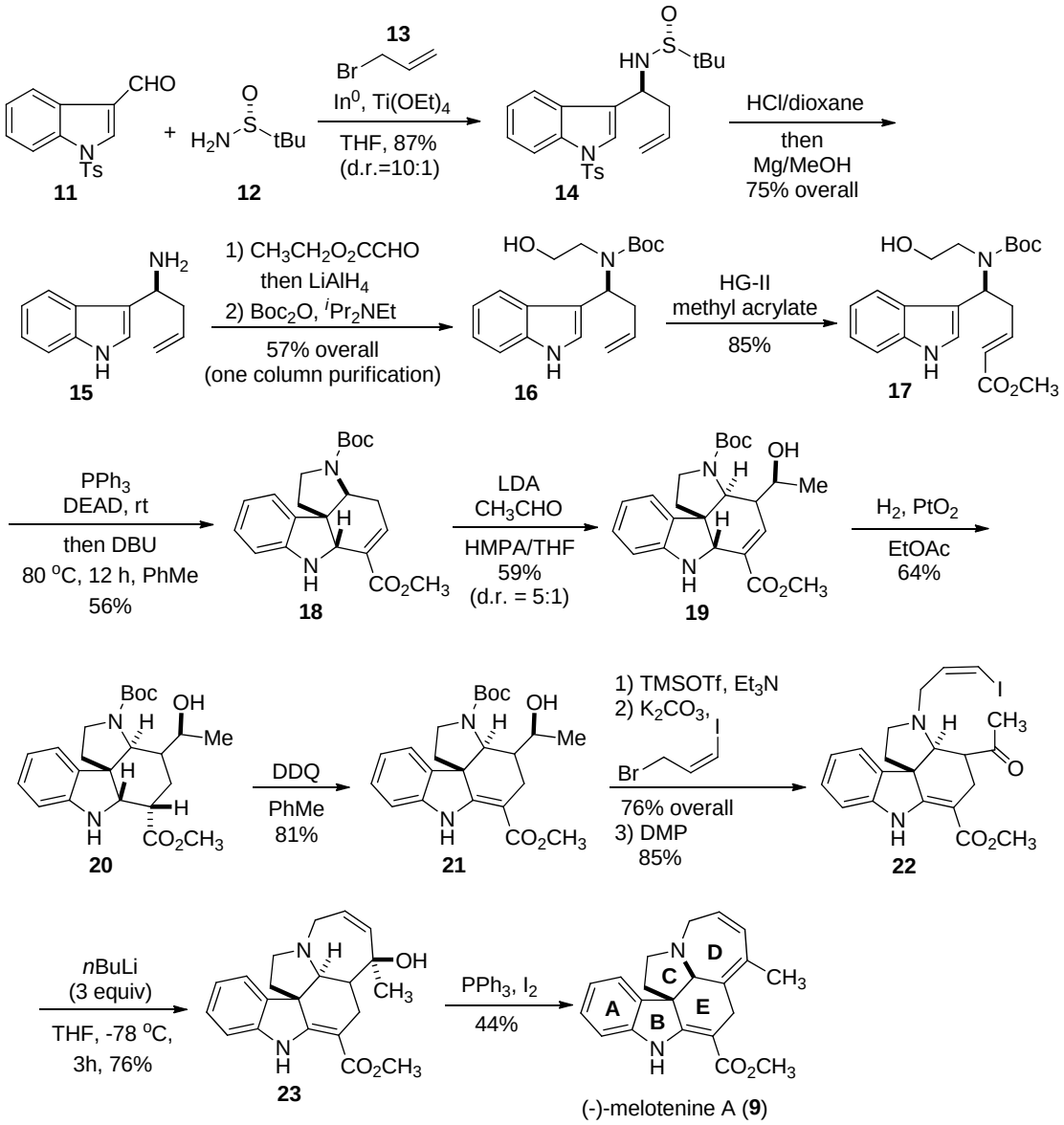


Scheme 1 Plausible biogenetic pathway to melotenine A.

Up to date, there has been only one synthesis of (-)-melotenine A, which was reported by Andrade *et al*. in 2013.[3] Their asymmetric synthesis consists of fourteen steps with 1% overall yield from commercially available *N*-tosylindole-3-carboxaldehyde. Key steps include a Piers annulation, an intermolecular vinylogous aldol reaction, and a novel one-pot sequence to prepare the ABCE (Scheme 2).

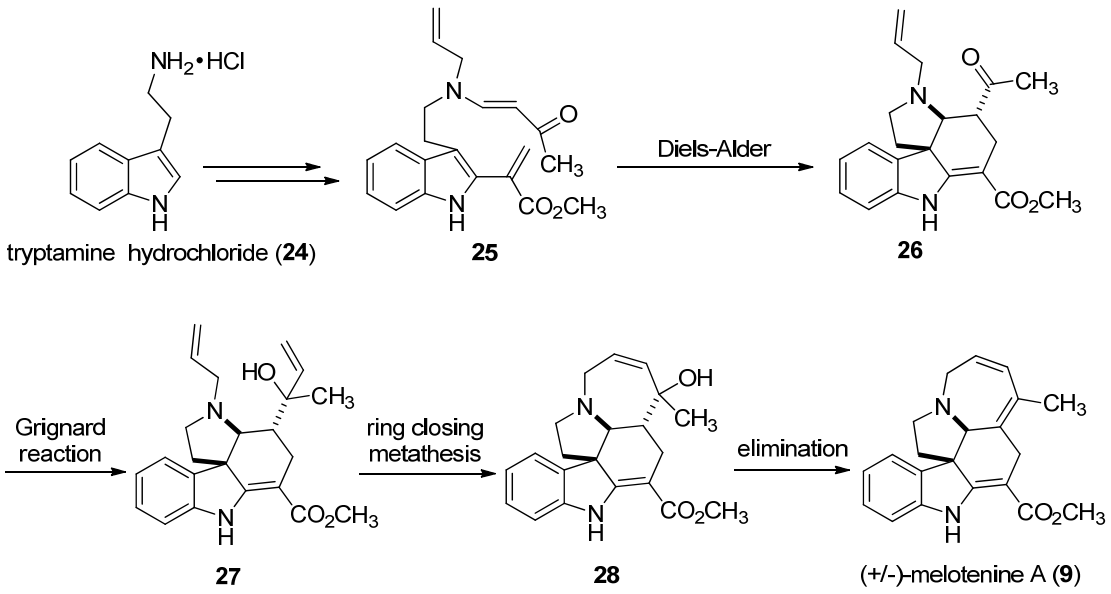
The synthesis started with the preparation of homoallylic sulfonamide **14** from *N*-tosylindole-3-carboxaldehyde (**11**), (*R*)-*N*-*tert*-butanesulfinamide (**12**) and allyl bromide (**13**) to afford homoallylic sulfinamide **14** in 87% yield. Compound **14** was converted to **17** in five steps, which are the removals of the chiral auxiliary and the protecting group, hydroxyethylation, reduction, protection and cross-metathesis. Treatment of a bis-cyclization precursor **17** with DEAD and PPh_3 in toluene resulted in the formation of the pyrrolidine C ring, and this compound was subsequently treated with DBU to give compound **18** in 56% yield. Compound **18** was deprotonated with LDA and the resulting enolate reacted with acetaldehyde to furnish alcohol **19** as a major diastereomer in relatively good yield. Isomerization of the olefin in **19** with platinum-catalyzed hydrogenation gave compound **20** and then oxidation with DDQ generated compound **21** in 81% yield. Preparation of the Piers annulation precursor **22** was accomplished in three steps, which are the removal of the protecting group, chemoselective alkylation and oxidation.

The D ring of the natural product was constructed by intramolecular nucleophilic addition to the carbonyl ketone of **22** to afford tetrahydroazepinol **23** in 76% yield. Then elimination of the hydroxyl group of compound **23** with PPh_3 and I_2 furnished (-)-melotenine A, $[\alpha]_D^{20}$ -373.9º (c 0.218, $CHCl_3$), in 44% yield.



Scheme 2 Andrade's synthesis of (-)-melotenine A (**9**).

Since melotenine A is a novel compound with very interesting biological activities and a challenging framework skeleton, it would be worthwhile to explore a different strategy to synthesize this compound. Our strategy is to use Diels-Alder reaction and ring-closing metathesis as key transformations to construct five-, six- and seven-membered rings of melotenine A from commercially available tryptamine hydrochloride (**24**) as shown in Scheme 3.

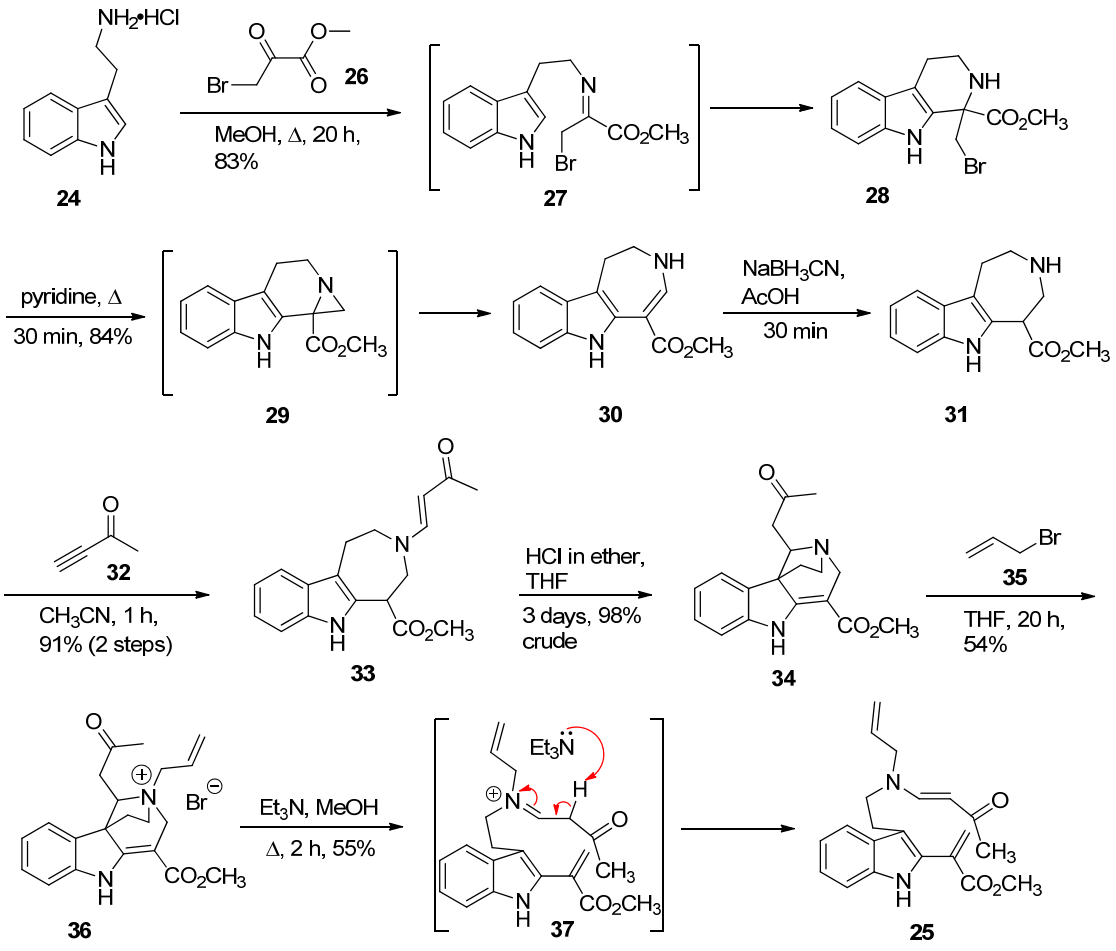


Scheme 3 Proposed synthesis of (+/-)-melotenine A

ผลการทดลอง สรุปและวิจารณ์ผลการทดลอง

Preparation of Diels-Alder Precursor **25**

The precursor for the Diels-Alder reaction was prepared in seven steps from commercially available tryptamine hydrochloride (**24**) as shown in Scheme 4. The synthesis in the first five steps followed closely the methods described by Kuehne[4] with some modifications. In the first step, methyl bromopyruvate (**26**) was employed instead of methyl chloropyruvate since the latter was not commercially available. In addition, it was reported by Li that methyl bromopyruvate provided a slightly better yield than the corresponding chloropyruvate[5] (78% vs 74%). We also did not add decolorizing charcoal to the reaction mixture as reported by both Kuehne and Li since it seemed to lower the reaction yield. In this reaction, tryptamine hydrochloride reacted with methyl bromopyruvate to give imine **27**, which then collapsed to provide amine **28** in 83% yield. It should also be noted that the use of much cheaper tryptamine instead of tryptamine hydrochloride resulted in a complex mixture and no desired product was obtained.

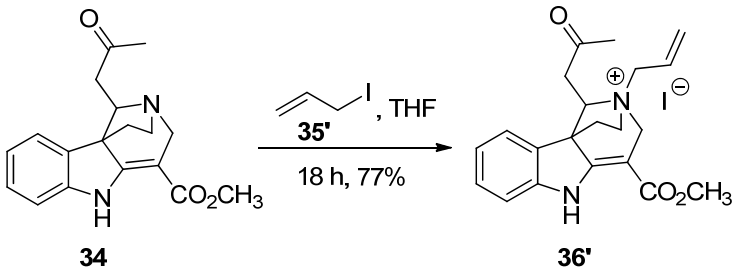


Scheme 4 Preparation of the precursor for the Diels-Alder reaction

The use of methyl bromopyruvate also provided a better leaving group in the second step, which was the ring expansion to form dihydroazepine **30**. When compound **28** was heated at reflux in pyridine for 30 minutes, the desired product was obtained in 84% yield, which was slightly lower that reported by Li (87%). The double bond of compound **30** was then reduced by sodium cyanoborohydride to form tetrahydroazepine **31** exclusively in almost quantitive yield. This crude product was subsequently added to 3-butyne-2-one (**32**) to give enone **33** in 91% (2 steps).

When compound **33** was dissolved in THF and a saturated HCl solution in diethyl ether was added, a precipitate took place. After stirring the reaction mixture vigorously for three days, intramolecular Michael addition of C3 of the indole on the enone occurred to generate bridged compound **34** in 98% (crude). This compound was relatively unstable as it slowly converted back to the starting material. The conversion was accelerated if the crude product was purified by chromatography on silica gel. Attempts to deactivate silica gel with a base such as Et_3N failed to solve the problem. Therefore, this crude product was carried on to the next step without further purification.

When Kuhne performed the next step with benzyl bromide, the desired *N*-benzylated product was obtained in 76%. However, when we employed allyl bromide instead of benzyl bromide, the reaction became slower and not a lot of precipitate formed even after the reaction mixture was stirred overnight. The yield of ammonium salt **36** was low and usually did not exceed 54% yield since most of compound **34** converted back to enone precursor **33**. This enone could be recovered and subjected to the same reaction conditions but the yield of the desired ammonium salt obtained the second time was even lower. We attempted to improve the yield by adding a few drops of HCl/ether to the reaction mixture since compound **34** was unlikely to convert to its precursor under acidic conditions. Unfortunately, the reaction yield did not significantly increase. We also attempted to purify compound **34** by chromatography on silica gel in order to obtain cleaner starting material although some of **34** was lost in the process. However, the yield of **36** still did not improve. Fortunately, when we switched from allyl bromide to allyl iodide (**35´**), the corresponding ammonium salt (**36´**) was obtained in good yield (77%) (Scheme 5). This improvement might be a result of the fact that iodide is a better leaving group than bromide. Therefore, the conversion of **34** to **36´** took place faster and less of **34** converted back to its precursor.

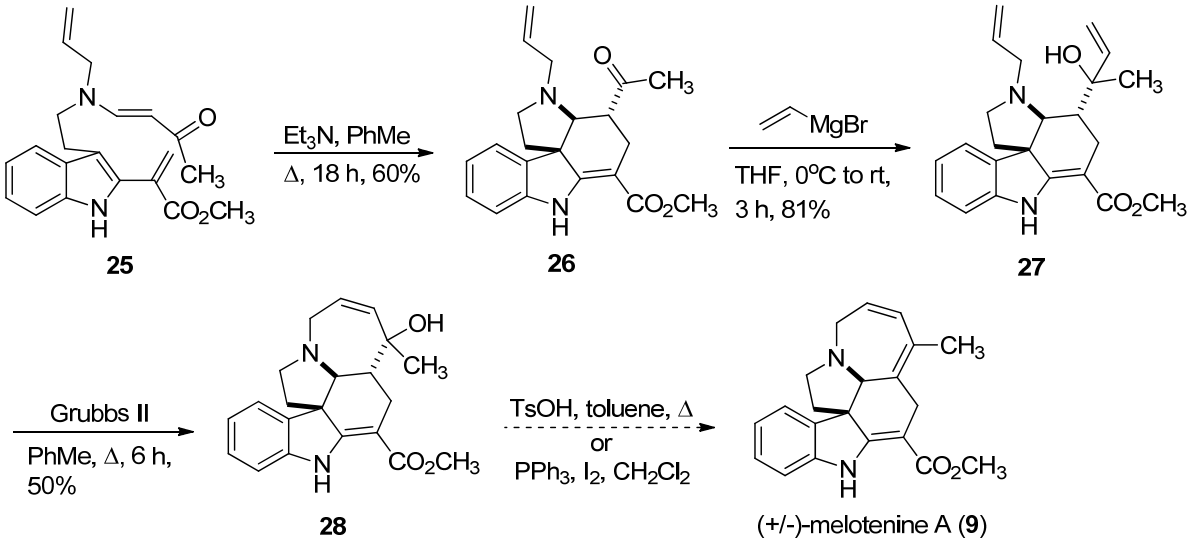


Scheme 5 Improvement of the yield of ammonium salt

Finally, retro Diels-Alder reaction of **36** (or **36´**) was carried out in refluxing methanol with catalytic amount of triethylamine to give intermediate **37**, which was depretonated by Et_3N to give indoloacrylate **25** in moderate yield (55%). Overall, we were able to prepare precursor **25** from tryptamine hydrochloride and allyl iodide in seven steps with 26% overall yield.

Preparation of Precursor **28**

When indoloacrylate **25** was heated at reflux in toluene in the presence of Et_3N as a catalyst (Scheme 6), Diels-Alder reaction occurred to give tetracycle **26** in moderate yield (60%). When compound **26** was subsequently treated with vinyl magnesiumbromide, alcohol **27** (dr ~10:1) was obtained in 81% yield (the major diastereomer was only isolated for the purpose of characterization). In this step, excess vinyl magnesiumbromide (3 eq.) was used so that the NH group could be deprotonated to generate an ester enolate, which was less electrophilic than the original ester group. Therefore, the C=O of the ester group could be prevented from participating in the Grignard reaction.



Scheme 6 Key transformations to complete the synthesis of (+/-)-melotenine A

It should be noted that the yields of this reaction were quite inconsistent with each run since factors such as temperature, reaction time, reaction scale, the purity of starting material, and the concentration of vinyl magnesiumbromide solution seemed to affect the reaction yields. For example, when we performed this reaction on a bigger scale, more reaction time was required and when the reaction mixture was stirred for too long, the product also started to decompose. The concentration of vinyl magnesiumbromide solution stored in a bottle also deteriorated over time, and that made it difficult to calculate the exact volume needed. If not enough vinyl magnesiumbromide was added, more reagent would be added later to make the reaction go to completion but additional time was also required, which means more decomposition could also take place. However, if too much vinyl magnesiumbromide was added, the product also decomposed more quickly.

In the next step, we predicted that the hydroxyl group of **27** might coordinate to the Grubbs catalyst and deactivate it. Therefore, we initially performed ring closing metathesis of **27** in the presence of TsOH. However, no desired product was obtained when the reaction was performed in toluene or CH_2Cl_2 either at room temperature or elevated temperatures. We also attempted to protect this tertiary alcohol, but we were not successful. Finally, we performed the ring closing metathesis of **27** without any protecting groups, and pentacycle **28** was obtained in moderate yield (50%). Similar to the previous step, we had a problem with reproducibility with this step since decomposition seemed to easily occur. Although we started this reaction with a mixture of diastereomers, only one isomer of the desired product could be detected and isolated due to the decomposition of the other isomer. It should also be noted that both the ^{1}H and ^{13}C NMR spectra of compound **28** are slightly different from those of compound **23**, which was synthesized by Andrade and coworkers,[3] suggesting that these two compounds were diastereomers.

To complete the synthesis, we attempted to perform an elimination reaction under acidic conditions (e.g. TsOH) but no desired product took place. If the reaction mixture was heated for a few hours, no reaction occurred. If it was heated for too long, decomposition took place. In addition, we performed this step using the Appel protocol as reported by Andrade and co-workers in the synthesis of (-)-melotenine A.[3] In this step, we expected that a tertiary carbocation would be generated and then attacked by I^- to form an alkyl iodide. Then this resulting alkyl iodide would undergo the elimination reaction to form metotenine A in one pot. Thus far, our attempts were unsuccessful due to decomposition although $Ph_3P{=}O$, which was the by-product, could be isolated. We

also tried to perform this Appel protocol in two steps by attempting to isolate the alkyl iodide first, and the resulting product would be treated with a base to generate an alkene. However, the alkyl iodide could not be detected and isolated due to decomposition.

In summary, we were able to synthesize pentacycle **28** in ten steps with 6.4% overall yield from tryptamine hydrochloride. This starting material already contained two rings and additional rings were constructed by the Diels-Alder reaction and ring-closing metathesis. The former was used to build the five- and six-membered rings and the latter was used to form the seven-membered ring. Our final step, which is the elimination reaction of alcohol **28** to furnish melotenine A, is currently being investigated.

วิธีทดลอง

Instrument

^{1}H NMR and ^{13}C NMR) spectra were recorded on a VARIAN INOVA 400 spectrometer. Chemical shifts of ^{1}H NMR spectra are reported in ppm relative to $CDCl_3$ (δ 7.26). Data are presented as follows: multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = mutiplet, dd = doublet of doublets, td = triplet of doublets, ddd = doublet of doublet of doublets, br = broad), coupling constant (J/Hz) and integration. Chemical shifts of ^{13}C NMR spectra are reported in ppm relative to $CDCl_3$ (δ 77.0).

Infrared (IR) spectra were recorded in cm^{-1} on a Perkin-Elmer 2000 Fourier transform infrared spectrophotometer at the Chemistry Department, Faculty of Science, Kasetsart University.

Low resolution mass spectra were obtained on an Agilent Technology 1100 series LL/MSD Trap and on a GCMS-QP-5050QA spectrometer in electrospray ionization mode (ESI^{+}) at the Scientific Equipment Center, Faculty of Science, Kasetsart University. The first number denotes *m/z* value, and the abundance and ion assignment are given in parentheses.

Melting points (m.p.) were determined on a Mel-Temp electrothermal apparatus at the Chemistry Department, Kasetsart University and are uncorrected.

Chromatographic system

Analytical thin-layer chromatography (TLC) was conducted on aluminum-backed 0.2 mm thick silica gel 60 F_{254} plates (Merck) and the chromatograms were visualized under a 254 nm UV lamp and/or by spraying with a solution of vanillin (3% in ethanol with 3% sulfuric acid) followed by heating.

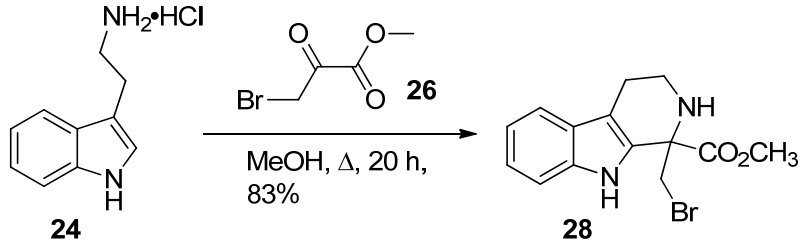
Flash column chromatography was performed on silica gel 60 (mesh size 0.040-0.063 mm).

Chemical reagents

Tetrahydrofuran (THF), diethyl ether and toluene were distilled from sodium and benzophenone under a nitrogen atmosphere. Dichloromethane and acetonitrile were distilled from calcium hydride under a nitrogen atmosphere. Methanol was distilled from magnesium under a nitrogen atmosphere. All other commercially obtained chemicals were used as received.

Methods

Methyl 1-(Bromomethyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-1-carboxylate (28).



A mixture of tryptamine hydrochloride (**24)** (5 g, 25.40 mmol) and methyl bromopyruvate (3.14 mL, 29.46 mmol) in anhydrous methanol (60 mL) was heated at reflux for 20 h under a nitrogen atmosphere. This reaction mixture was allowed to cool to room temperature, concentrated under vacuum to about 5 mL, and then diluted with about 30 mL of water. Concentrated ammonium hydroxide was slowly added until the aqueous phase became strongly basic. The resulting precipitate was collected by filtration and washed with ether to give compound **28** as a brown solid (6.85 g, 83%). ^{1}H NMR data are consistent with those reported in the literature.[5]

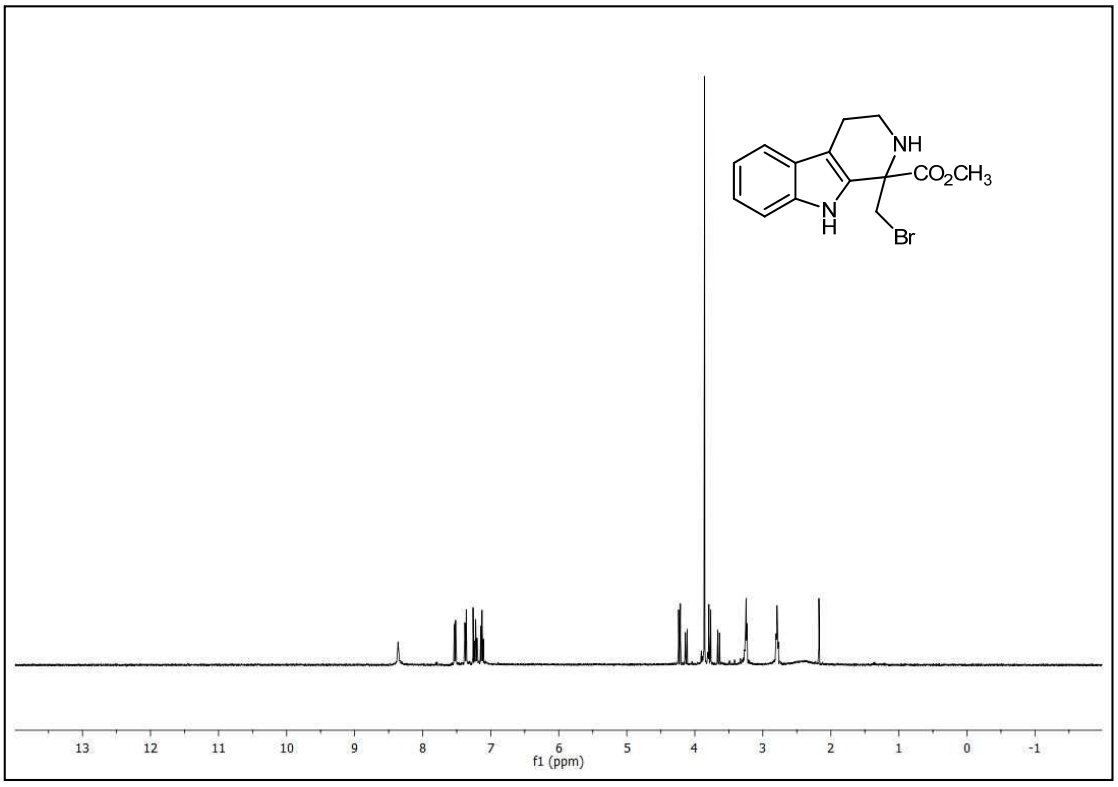
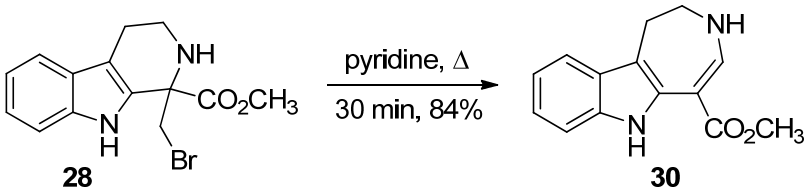


Figure 4 400 MHz ^{1}H NMR spectrum of methyl 1-(bromomethyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4*b*]indole-1-carboxylate (**47**)

(*E*)-Methyl 1,2,3,6-Tetrahydroazepino[4,5-*b*]indole-5-carboxylate (30).



A solution of **28** (6.00 g, 18.56 mmol) in pyridine (20 mL) was heated at reflux under a nitrogen atmosphere for 30 min. The solution was concentrated to dryness under vacuum. The crude residue was dissolved in dichloromethane (100 mL) and washed with water (3 x 30mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness under vacuum. The crude residue was purified by flash chromatography on SiO_2 (1:2 EtOAc/hexane) to give compound **30** as a yellow solid (3.78 g, 84%). ^{1}H NMR data are consistent with those reported in the literature.[4]

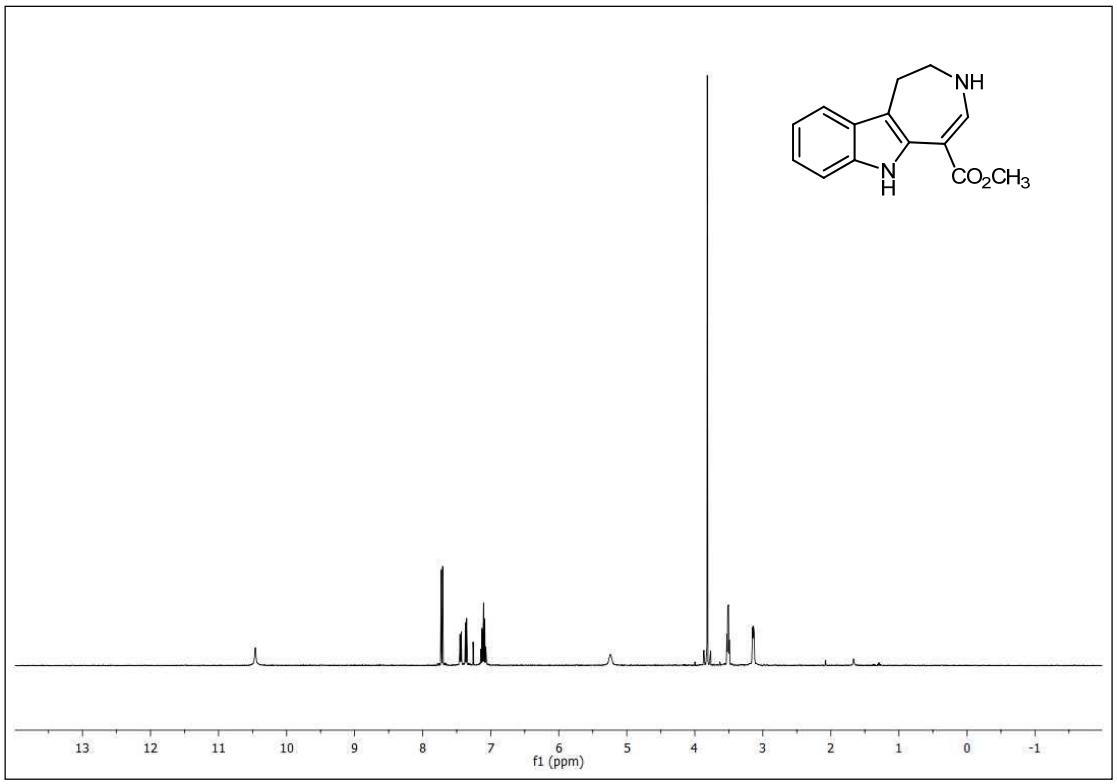
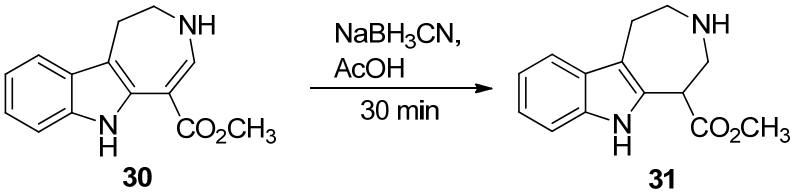


Figure 5 400 MHz ^{1}H NMR spectrum of (*E*)-methyl 1,2,3,6-tetrahydroazepino[4,5*b*] indole-5-carboxylate (**30**).

Methyl 1,2,3,4,5,6-Hexahydroazepino[4,5-*b*]indole-5-carboxylate (31).



30 **31**

To a stirred slurry of unsaturated azepine **30** (3.00 g,12.38 mmol) in glacial acetic acid (25 mL) was added sodium cyanoborohydride (2.18 g, 34.66 mmol) in small portions. The reaction mixture was stirred at room temperature for 2 h under a nitrogen atmosphere, then cooled in an ice bath and 1 mL of concentrated hydrochloric acid was slowly added, with cooling, until gas evolution ceased. Then, a solution of NH_4OH was slowly added until the solution turned basic. This mixture was then extracted with dichloromethane. The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under vacuum to give compound **31** as a light yellow foamy solid (3.00 g, 99% crude). ^{1}H NMR data are consistent with those reported in the literature.[4]

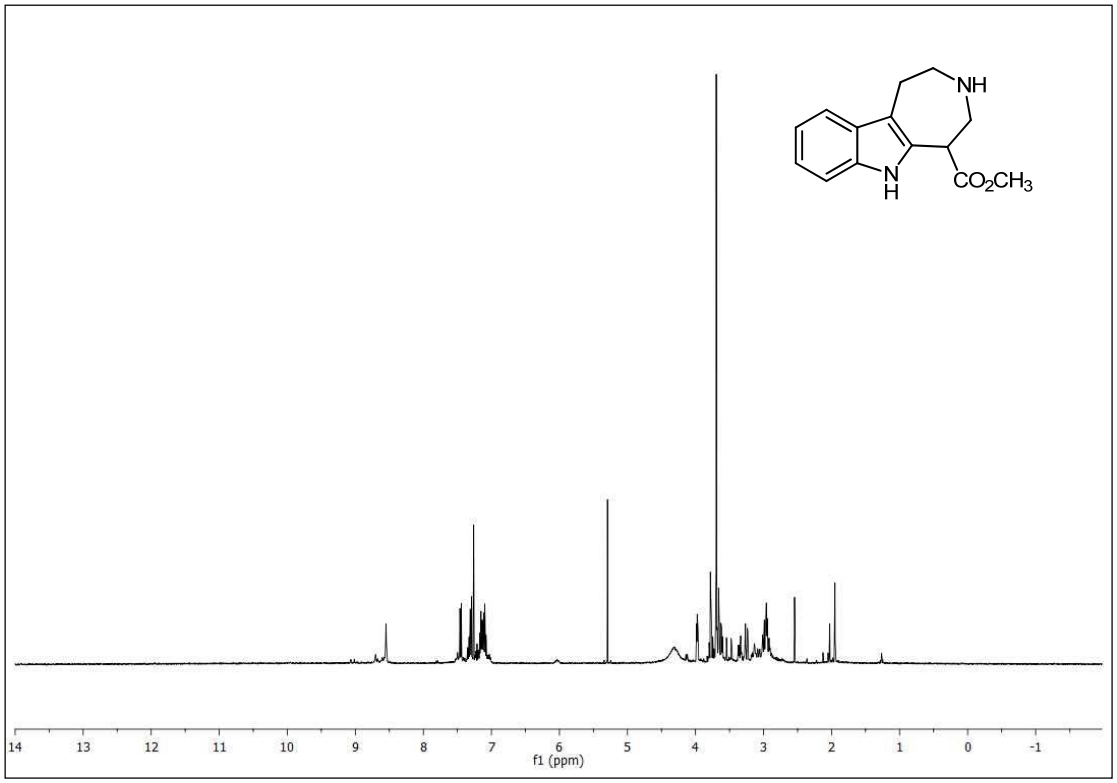
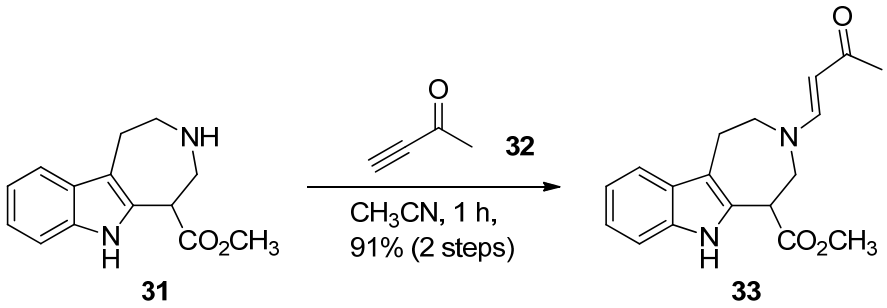


Figure 6 400 MHz ^{1}H NMR spectrum methyl 1,2,3,4,5,6-hexahydroazepino[4,5*b*] indole-5-carboxylate (**31**).

Methyl 3-(4-But-3-ene-2-one-yl)-1,2,3,4,5,6-hexahydroazepino(4,5-*b*)indole-5-carboxylate (33).



To a suspension of **31** (3.00 g, 12.28 mmol) in acetronitrile (37 mL) was added 3-butyne-2-one (**32**) (1.37 mL, 17.56 mmol). The reaction mixture was stirred at room temperature for 3 h under a nitrogen atmosphere, and then it was concentrated to dryness under vacuum. The crude residue was purified by flash chromatography on SiO_2 (4:1: EtOAc/hexane) to give compound **33** as a yellow solid (3.55 g, 91% over 2 steps). ^{1}H NMR data are consistent with those reported in the literature.[6]

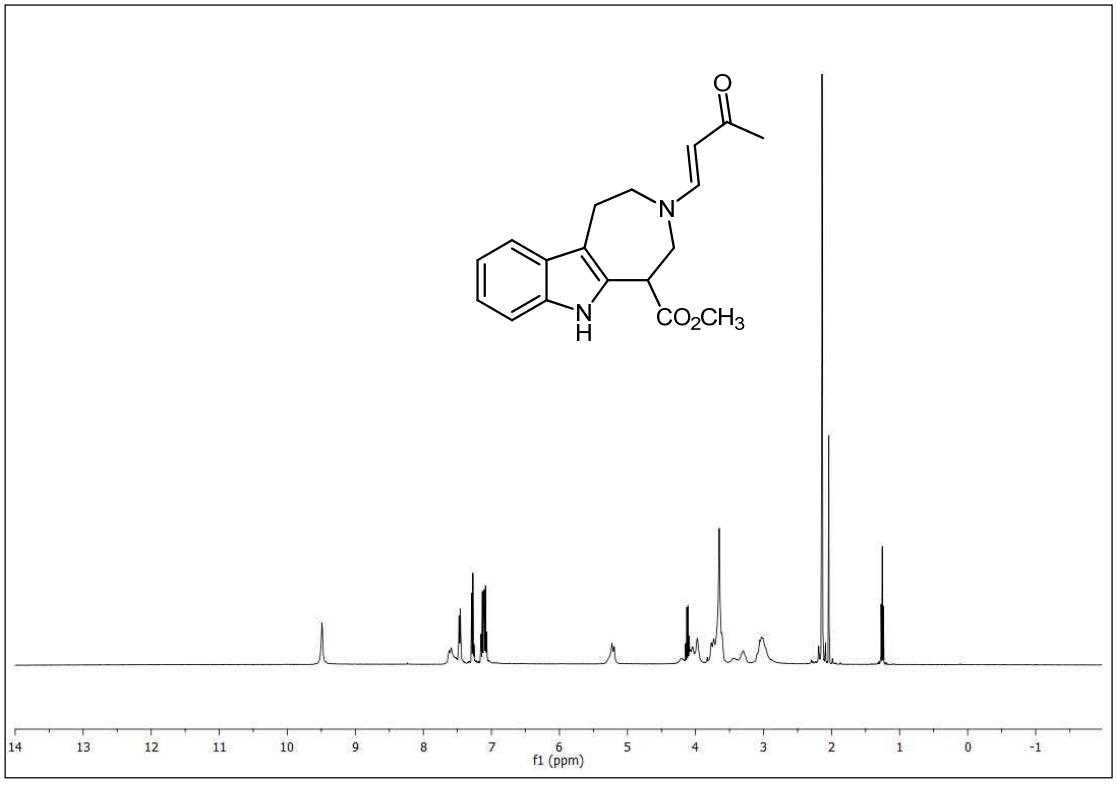
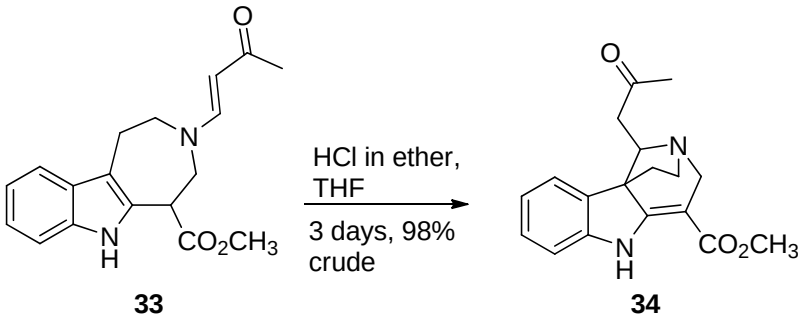


Figure 7 400 MHz ^{1}H NMR spectrum of methyl 3-(4-but-3-ene-2-one-yl)-1,2,3,4,5,6-hexahydroazepino (4,5*b*)indole-5-carboxylate (**33**)

Methyl 1,2,4,6-Tetrahydro-11-(3-propan-2-one-yl)-3,5*b*-methanoazepino(4,5-*b*)-indole-5-carboxylate (34).



33 **34**

To a solution of **33** (2.00 g, 6.40 mmol) in 50 mL of THF was added ether saturated with HCl gas (1.80 mL). A precipitate formed immediately. The reaction mixture was stirred vigorously to break down the precipitate. The resulting yellow suspension was stirred for 3 days. The solvent was removed under vacuum to give a solid residue. To this residue was added CH_2Cl_2 (60 mL) followed by 1 M NaOH (60 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under vacuum to give compound **34** as a light brown solid (1.96 g, 98%). ^{1}H NMR data are consistent with those reported in the literature.[6]

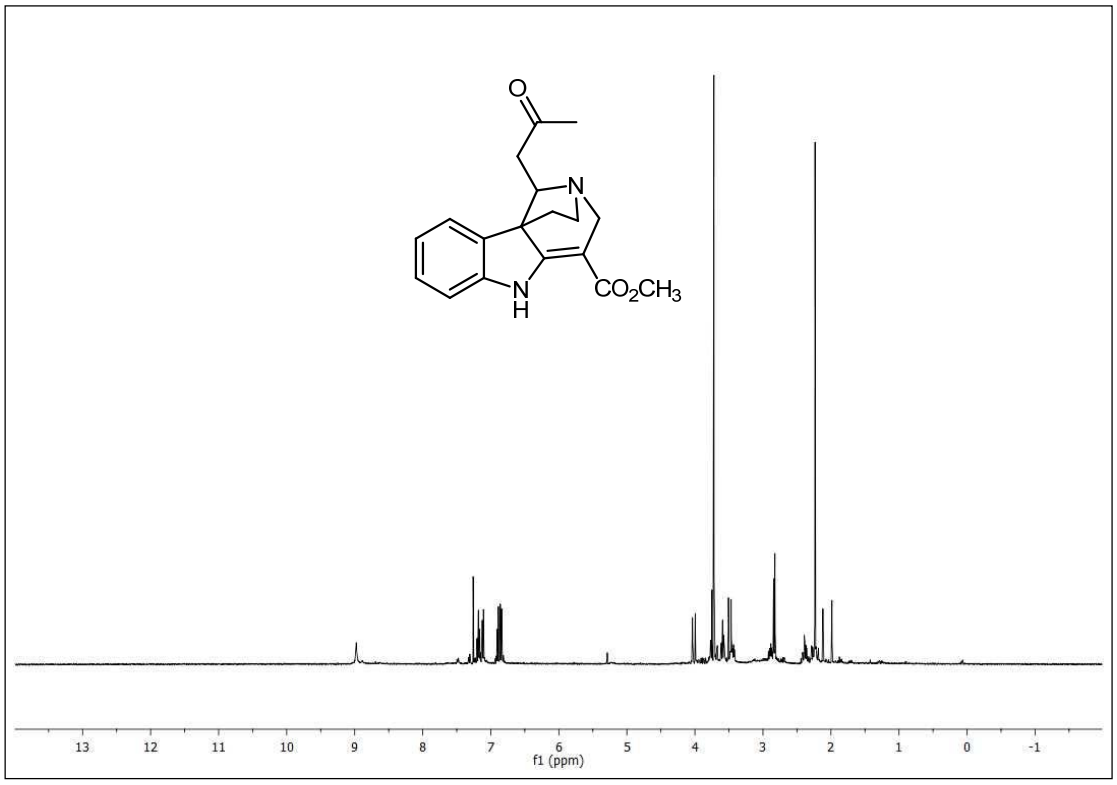
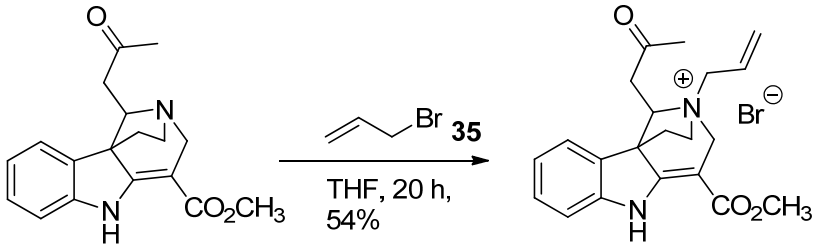


Figure 8 400 MHz ^{1}H NMR spectrum of methyl 1,2,4,6-tetrahydro-11-(3-propan-2-one-yl)-3,5*b*-methanoazepino(4,5*b*)indole-5-carboxylate (**34**)

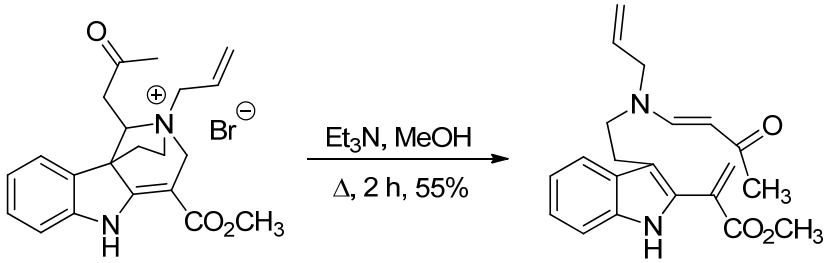
***N*-allyl ammonium bromide 36.**



34 **36**

To a solution of **34** (3.34 g, 10.69 mmol) in THF (50 mL) was added allyl bromide (2.12 mL, 24.59 mmol). The reaction mixture was stirred at room temperature for 20 h under a nitrogen atmosphere. A light yellow precipitate formed. The reaction mixture was cooled in an ice bath and the liquid phase was carefully removed with a Pasteur pipet. The solid residue was washed with EtOAc (2 x 20 mL) to give compound **36** as a light brown solid (2.54 g, 54% crude).

Indoloacrylate 25.



36 **25**

A mixture of **36** (1.00 g, 2.31 mmol), Et_3N (16 drops) and MeOH (25 mL) was heated at reflux for 2 h under a nitrogen atmosphere. The reaction mixture was concentrated to dryness under vacuum. The crude residue was purified by flash chromatography on SiO_2 (4:5:1 EtOAc/hexane/Et_3N) to give compound **25** as a yellow solid (455 mg, 55%); m.p. 139-141 ^{o}C.

IR (film) 3448, 1719, 1655 cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.18 (br s, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.42 (br s,1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.4 Hz, 1H), 6.53 (s, 1H), 6.02 (br s, 1H), 5.70 (ddd, *J* = 5.5, 10.5, 22.1 Hz, 1H), 5.19 (d, *J* = 10.1 Hz, 1H), 5.11 (d, *J* = 16.1 Hz, 1H), 3.87 (s, 3H), 3.36 (d, *J* = 4.1 Hz, 2H), 3.43 (br s, 2H), 3.09 (t, *J* = 7.2 Hz, 2H), 2.09 (br s, 3H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.7, 167.2, 151.2, 155.6, 131.8, 128.0, 123.3, 120.0, 118.7, 111.5, 52.8

LRMS(ESI) m/z (relative intensity) 353.4 (100%, $M+H^+$)

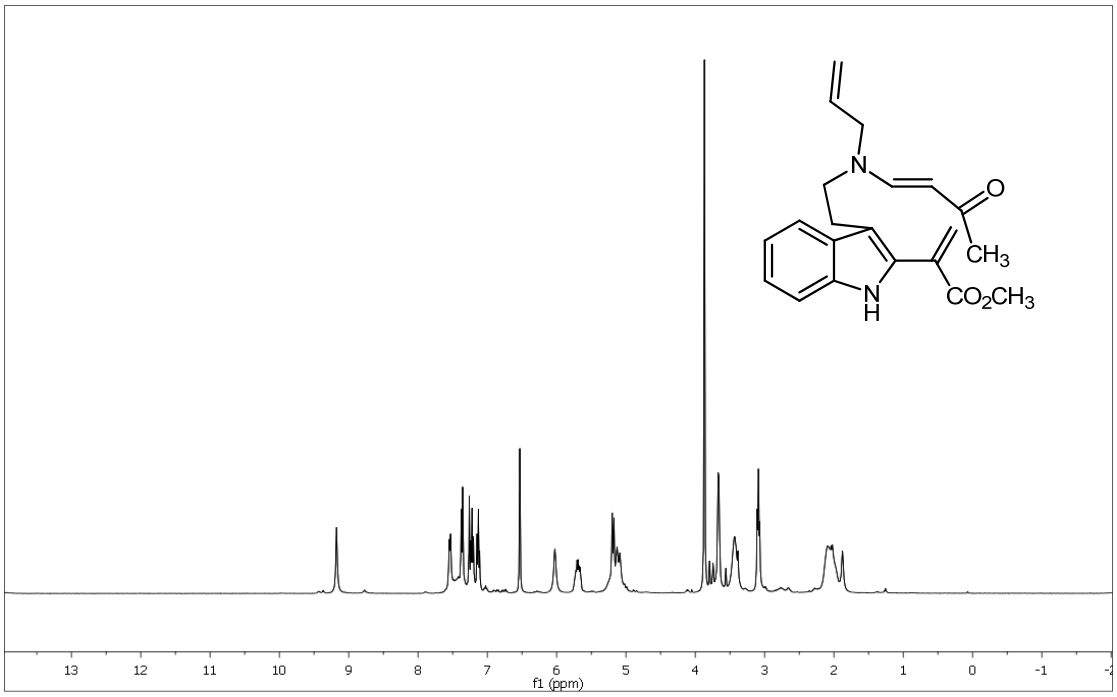


Figure 9 400 MHz ^{1}H NMR spectrum of indoloacrylate **25**

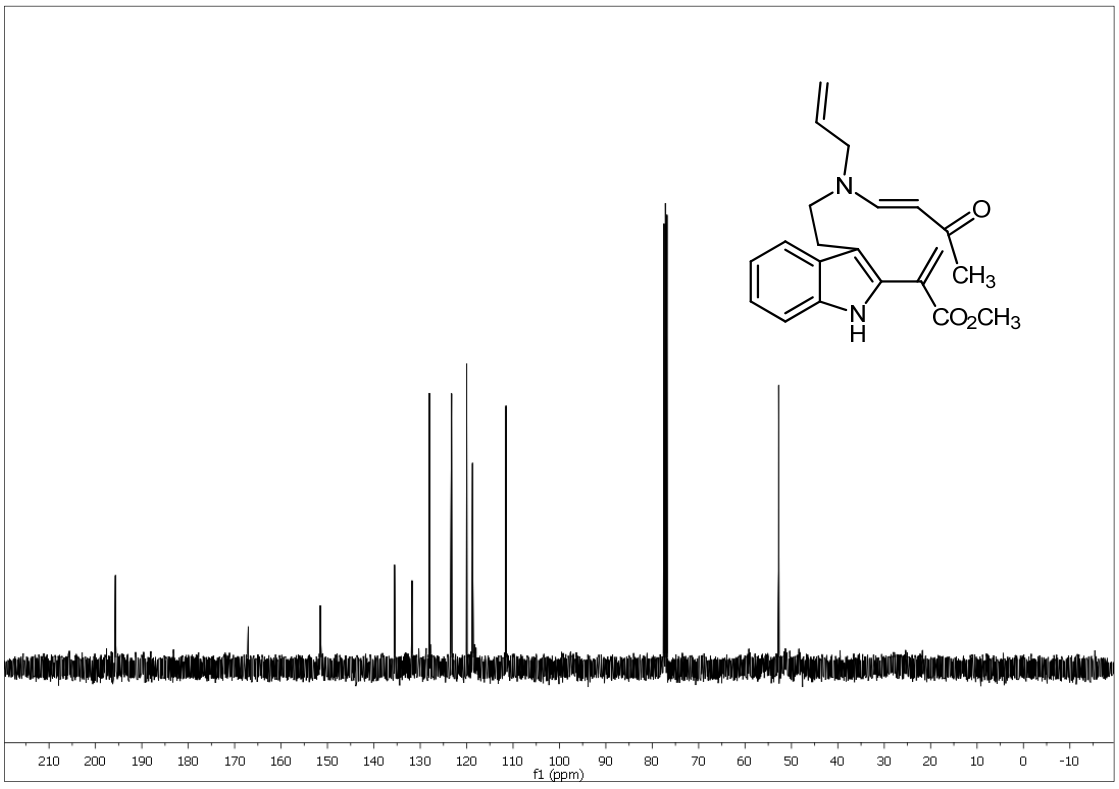
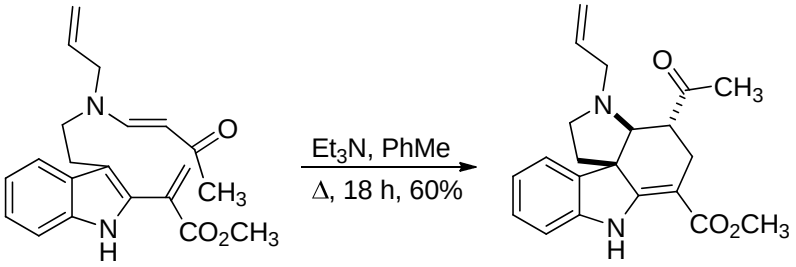


Figure 10 75 MHz ^{13}C NMR spectrum of indoloacrylate **25**

Tetracyclic Ketone 26.



25 **26**

A solution of **37** (580 mg, 1.64 mmol) and Et_3N (5 drops) in toluene (18 mL) was heated at reflux for 21 h under a nitrogen atmosphere. The reaction mixture was concentrated to dryness under vacuum. The crude residue was purified by flash chromatography on SiO_2 (1:2 EtOAc/hexane) to give compound **25** as a yellow solid (353 mg, 61%); m.p. 117-119 ^{o}C.

IR (film) 3391, 1701, 1671 cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.81 (br s, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.15 (td, *J* = 0.9, 7.6 Hz, 1H), 6.91 (td, *J* = 1.0, 7.5 Hz, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 5.98 (dddd, *J* = 6.2, 6.9, 10.6, 16.7 Hz, 1H), 5.29 (d, *J* = 17.2 Hz, 1H), 5.17 (d, *J* = 9.9 Hz, 1H), 3.76 (s, 3H), 3.62 (s, 1H), 3.47 (dd, *J* = 6.1, 13.4 Hz, 1H), 3.33 (dd, *J* = 7.0, 13.2 Hz, 1H), 3.20 (d, *J* = 15.7 Hz, 1H), 3.07 (t, *J* = 9.2 Hz, 1H), 2.83 (s, 1H), 2.78-2.70 (m, 1H), 2.67 (d, *J* = 4.36 Hz, 1H), 2.03 (s, 3H), 1.99 (dd, *J* = 6.3, 12.2 Hz, 1H), 1.73 (dd, *J* = 4.8, 12.0 Hz, 1H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.8, 165.8, 142.5, 137.3, 135.5, 127.6, 121.8, 120.7, 117.3, 109.2, 89.1, 66.4, 57.5, 55.7, 53.2, 50.9, 50.8, 41.6, 27.9, 21.3

HRMS (ESI) calculation for $C_{21}H_{24}N_2O_3$+H = 353.1787, found 353.1874

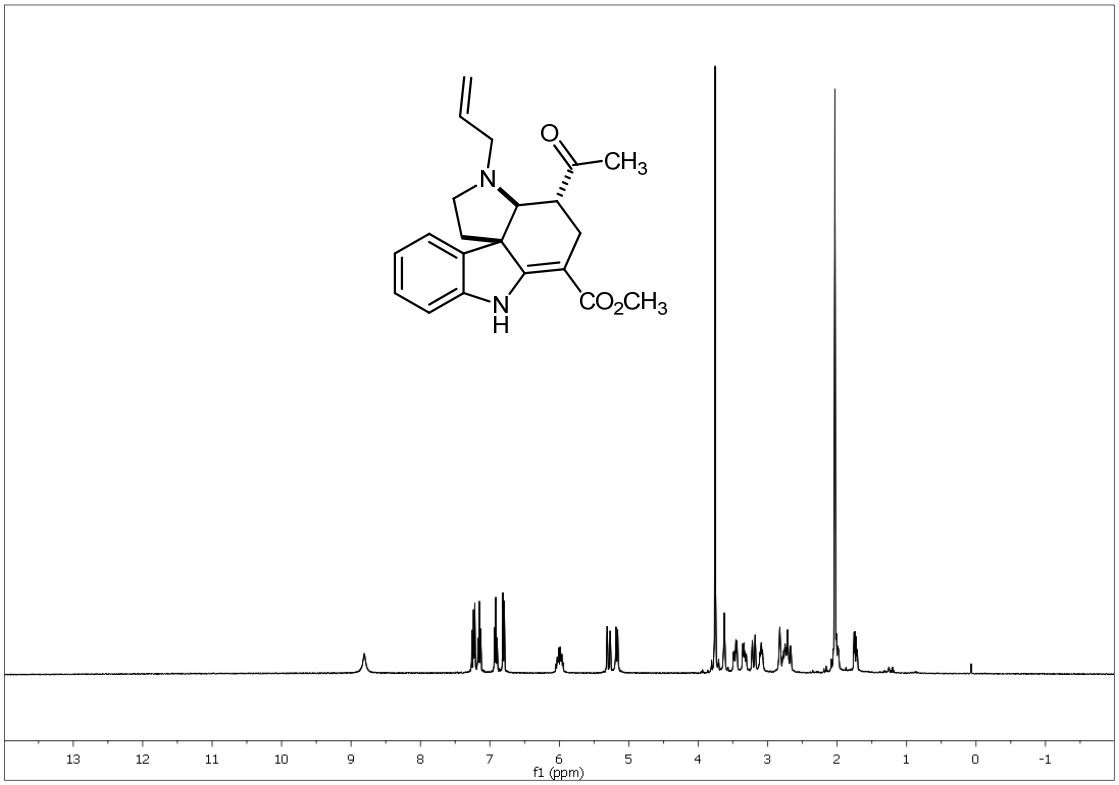


Figure 11 400 MHz ^{1}H NMR spectrum of tetracyclic ketone **26**

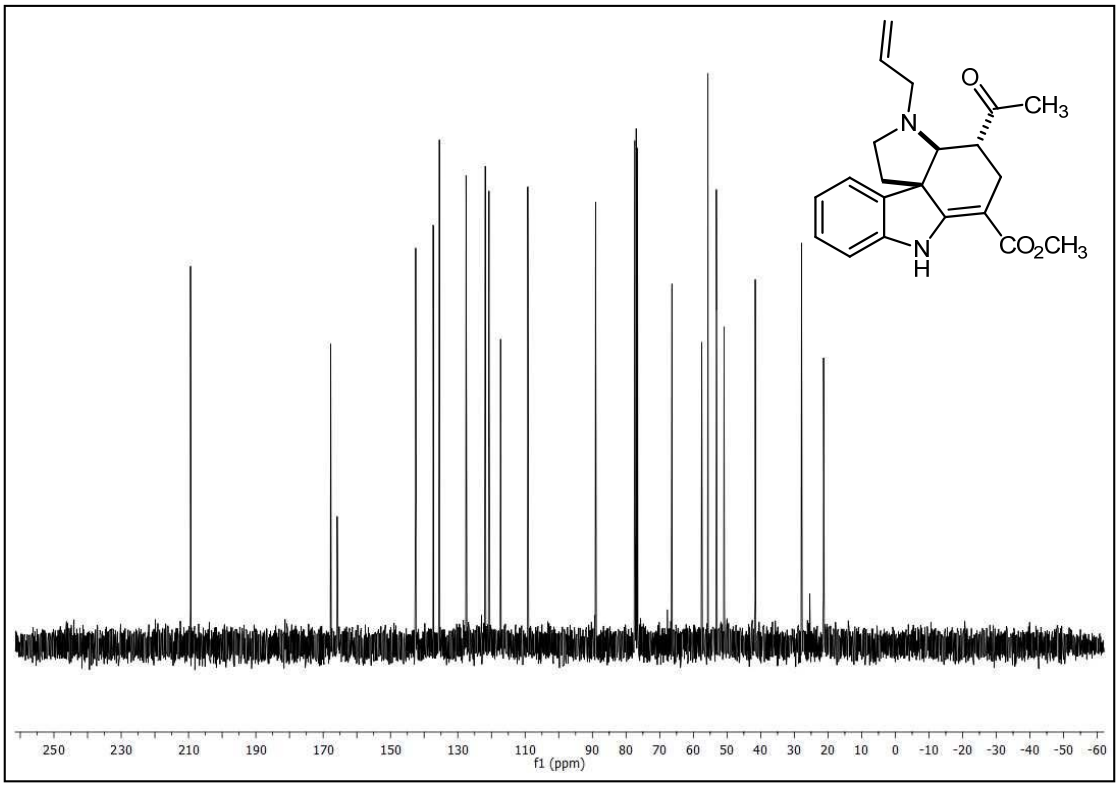
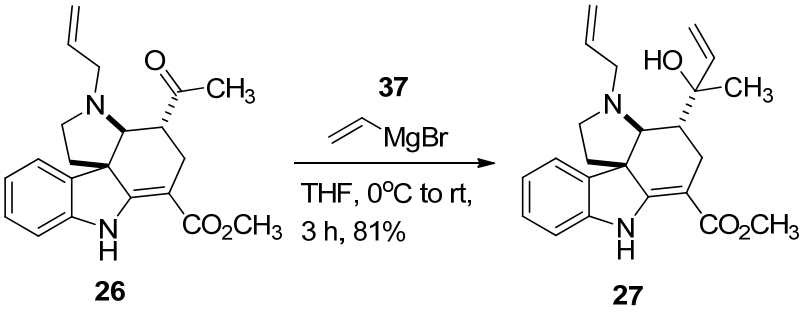


Figure 12 75 MHz ^{13}C NMR spectrum of tetracyclic ketone **26**

Alcohol 27.



26 **27**

To an ice-cooled solution of tetracyclic ketone **26** (621 mg, 1.76 mmol) in THF (8 mL) was added a solution of 1 M vinylmagnesium bromide (**37**) in THF (5.3 mL, 5.28 mmol). The reaction mixture was stirred (0 ^{o}C to room temperature) for 3 h under a nitrogen atmosphere. To this reaction mixture was added 5 mL of saturated NH_4Cl, and diluted with EtOAc (60 mL) and water (50 mL). The organic phase was separated and the aqueous phase was extracted with EtOAc (2 x 30 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated to dryness under vacuum. The crude residue was purified by flash chromatography on SiO_2 (1:4 and 1:2 EtOAc/hexanes) to give compound **27** as a white solid (547 mg, 81%); m.p. 63-65 ^{o}C.

Major diastereomer:

IR (film) 3392, 1670 cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.95 (br s, 1H), 7.19 (d, *J* = 7.3 Hz, 1H), 7.15 (td, *J* = 1.1, 7.7 Hz, 1H), 6.88 (td, *J* = 0.8, 7.5 Hz, 1H), 6.80 (d, *J* = 7.4 Hz, 1H), 6.03 (dddd, *J* = 5.4, 7.6, 10.1, 15.6 Hz, 1H), 5.58 (dd, *J* = 10.7, 17.2 Hz, 1H), 5.31 (dd, *J* = 1.0, 17.1 Hz, 1H), 5.20 (d, *J* = 10.1 Hz, 1H), 4.92 (dd, *J* = 1.2, 17.2 Hz, 1H), 4.84 (dd, *J* = 1.1, 10.7 Hz, 1H), 3.77 (s, 3H), 3.61 (dd, *J* = 5.3, 13.8 Hz, 1H), 3.26 (s, 1H), 3.23 (d, *J* = 7.6 Hz, 1H), 3.03 (dd, *J* = 6.3, 9.0 Hz, 1H), 2.93 (dt, *J* = 2.1, 4.3, 16.0 Hz, 1H), 2.73 (ddd, *J* = 4.8, 9.2, 12.5 Hz, 1H), 2.45 (dd, *J* = 4.4, 15.9 Hz, 1H), 2.06 (td, *J* = 6.3, 12.0 Hz, 1H), 1.86 (t, *J* = 3.2 Hz, 1H), 1.70 (dd, *J* = 4.6, 11.8 Hz, 1H), 1.34 (br s, 1H), 1.0 (s, 3H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.2, 165.5, 145.0, 142.9, 137.5, 135.1, 127.7, 122.1, 120.5, 117.8, 111.2, 109.0, 91.0, 74.3, 67.1, 56.0, 55.7, 50.9, 50.0, 49.3, 41.1, 26.9, 19.8

HRMS (ESI) calculation for $C_{23}H_{28}N_2O_3$+H = 380.2100, found 381.2191

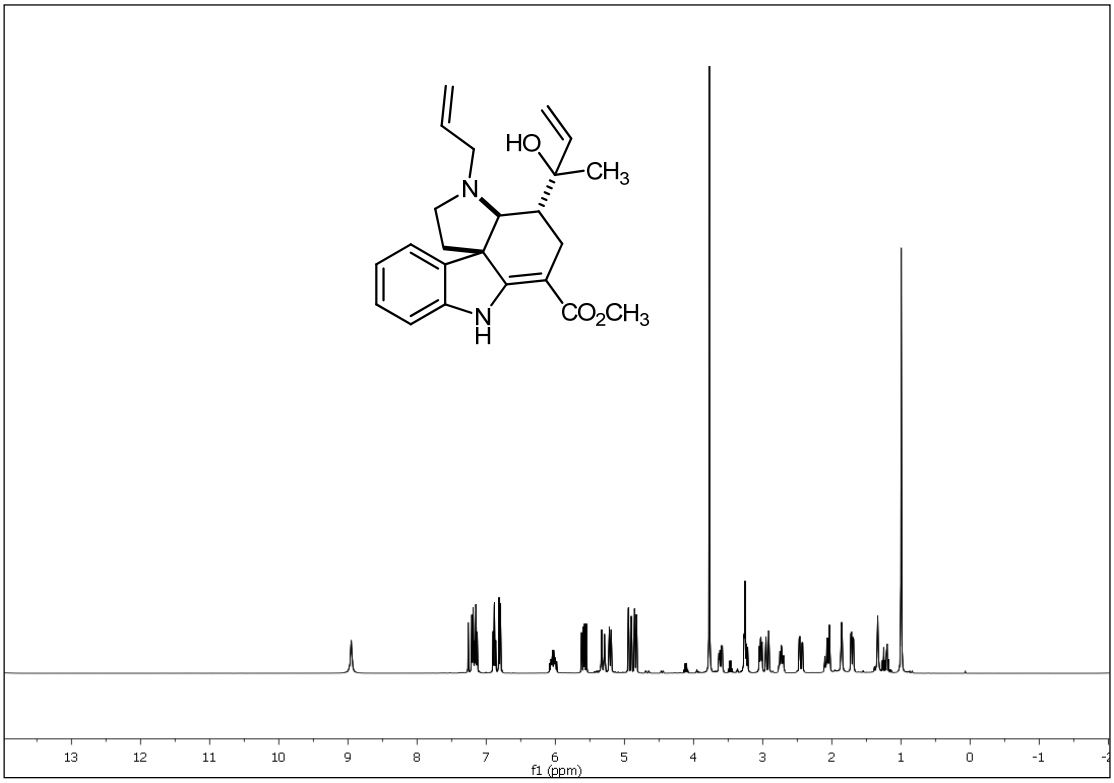


Figure 13 400 MHz ^{1}H NMR spectrum of alcohol **27**

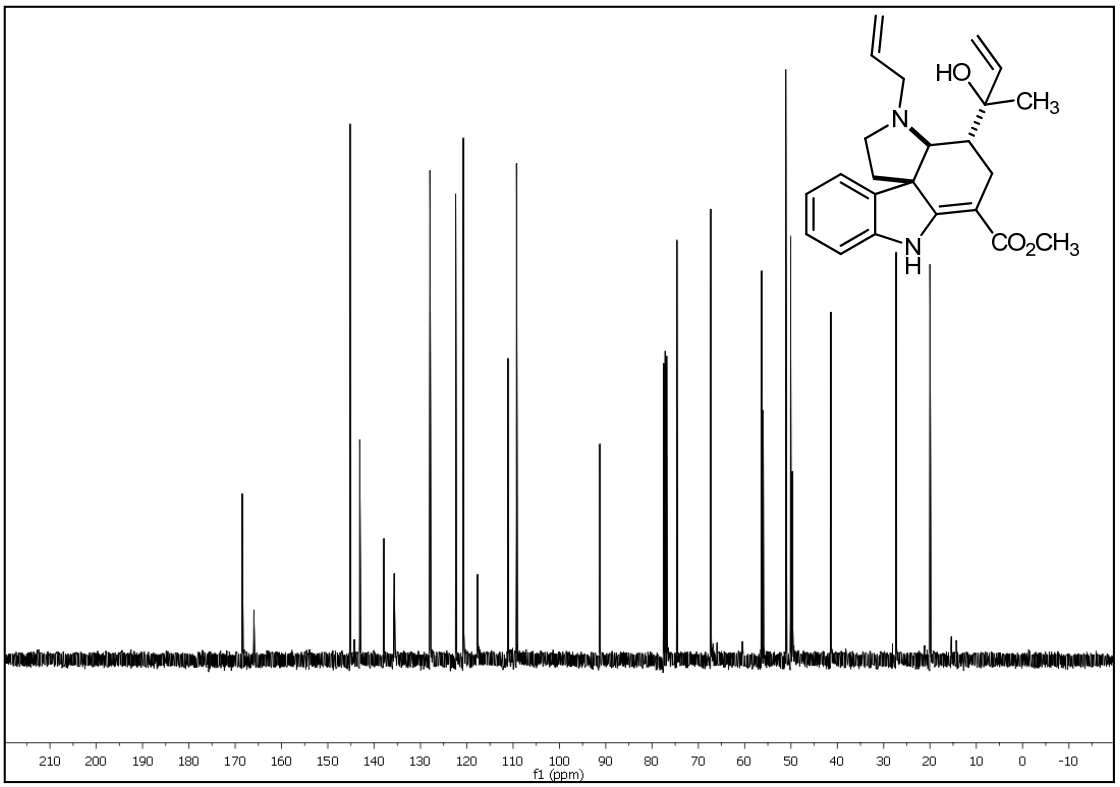
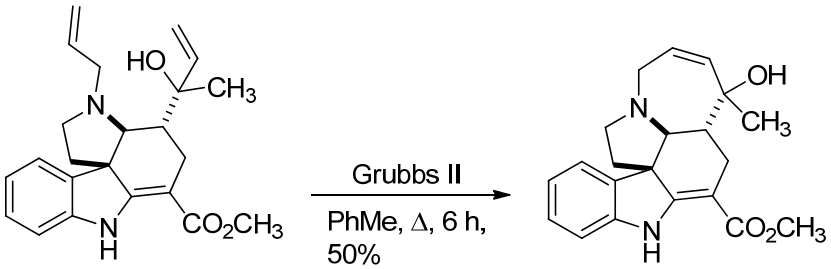


Figure 14 75 MHz ^{13}C NMR spectrum of alcohol **27**

Pentacyclic Ring System 28.



27 **28**

A solution of alcohol **27** (100 mg, 0.26 mmol) in a toluene (8 mL) was heated at 80 ºC under a nitrogen atmosphere. Then a solution of Grubbs second generation catalyst (15 mg, 0.018 mmol) in a toluene (4 mL) was added. The reaction mixture was kept at 80 ºC for 6 h, and then allowed to cool down to room temperature. The solvent was remove under reduced pressure and the crude residue was purified by flash chromatography on SiO_2 (1:4 and 1:2 EtOAc:hexane) to provided compound **28** as a yellow solid (46 mg, 50%)

IR (film) 3416, 1679 cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.09 (br s, 1H), 7.62 (d, *J* = 6.6 Hz, 1H), 7.13 (td, *J* = 7.7, 1.3 Hz, 1H), 6.87 (td, *J* = 7.5, 1.0 Hz, 1H), 6.78 (d, *J* = 7.7 Hz, 1H), 5.71(ddd, *J* = 11.9, 7.1, 4.8 Hz, 1H), 5.63 (dd, *J* = 11.7, 1.6 Hz, 1H), 3.76 (s, 3H), 3.61 (dd, *J* = 13.2, 7.2 Hz, 1H), 3.53 (d, *J* = 6.9 Hz, 1H), 3.39 (dd, *J* = 17.5, 8.9 Hz, 1H), 3.25 (dd, *J* = 13.4, 5.0 Hz, 1H), 2.88 (dd, *J* = 16.8, 4.0 Hz, 1H), 2.59 (dd, *J* = 16.6, 10.0 Hz, 1H), 2.59 (dd, *J* = 16.6, 10.0 Hz, 1H), 2.41-2.33 (m, 2H), 1.84-1.78 (m, 1H), 1.67 (br s, 1H), 1.40 (s, 3H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.9, 161.5, 143.5, 141.2, 136.7, 127.8, 124.0, 123.2, 120.9, 108.5, 91.6, 76.3, 65.6, 55.1, 51.3, 50.9, 48.9, 36.1, 35.9, 27.9, 21.8

HRMS (ESI) calculation for $C_{21}H_{25}N_2O_3$+H = 353.1887, found 353.1877

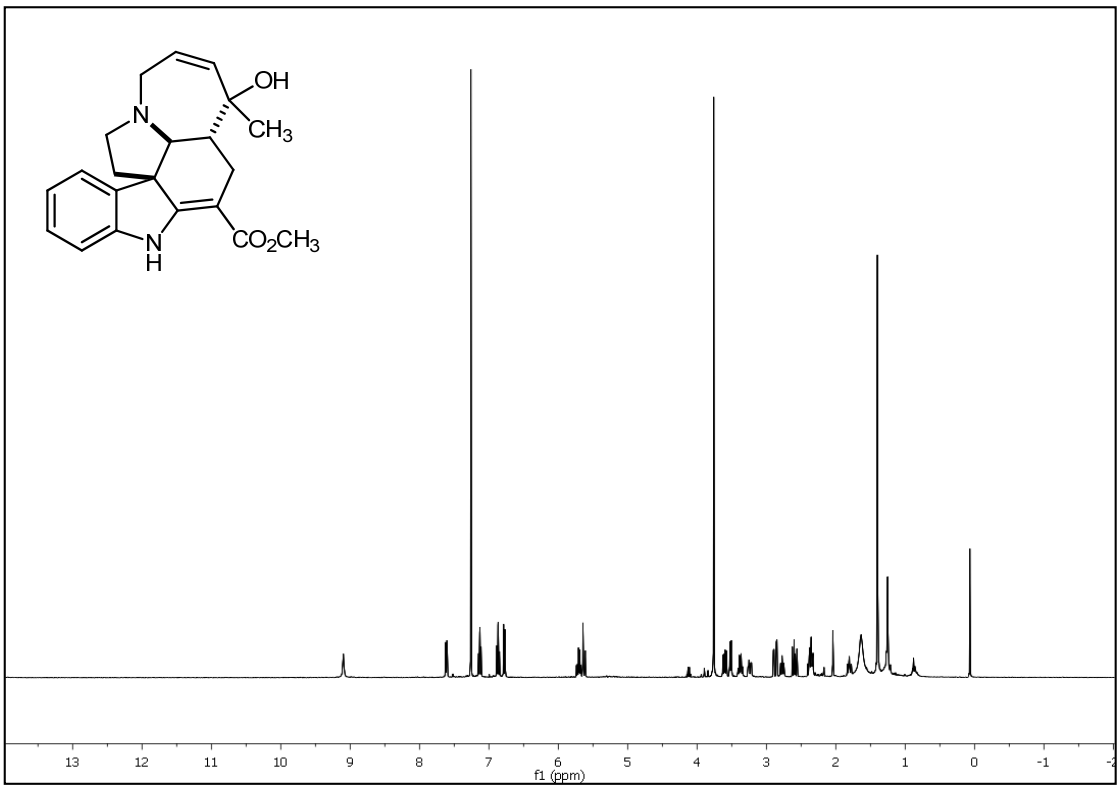


Figure 15 400 MHz ^{1}H NMR spectrum of compound **28**

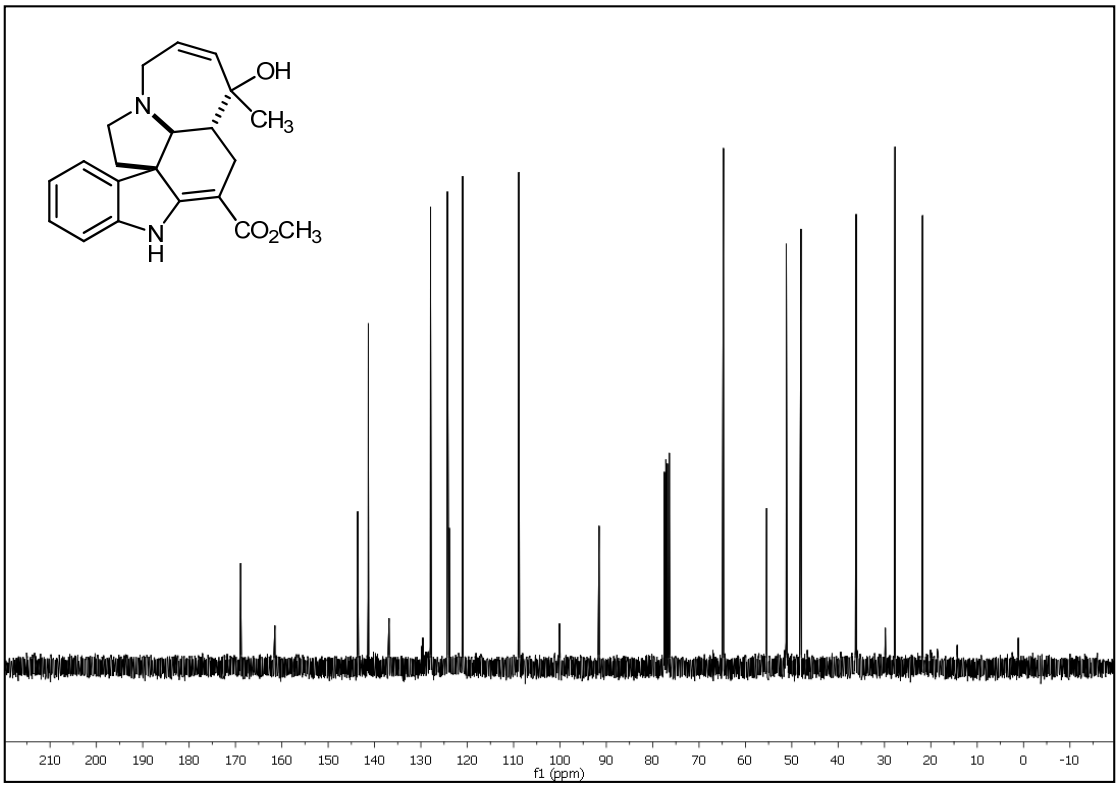


Figure 16 75 MHz ^{13}C NMR spectrum of compound **28**

เอกสารอ้างอิง

1. O'Connor, E. S.; Maresh, J. 2006. *J. Nat. Prod. Rep.* **2006**, *23*, 532–547.
2. Feng, T.; Li, Y.; Liu, Y.-P.; Cai, X.-H.; Wang, Y.-Y.; Luo, X.-D *Org. Lett.* **2010**, *12*, 968-971.
3. Zhao, S.; Sirasani, G.; Vaddypally, S.; M. J. Zdilla, M. J.; Andrade, R. B. *Angew. Chem. Int. Ed.* **2013**, *52*, 8309-8311.
4. Kuehne, M. E.; Bohnert, J. C.; Bornmann, W. G.; Kirkemo, C. L.; Kuehne, M. E.; Seaton P. J.; Zebovitz, T. C. *J. Org. Chem.* **1985**, *50*, 919-924.
5. Chen, P.; Cao, L.; Li, C. *J. Org. Chem.* **2009**, *74*, 7533-7535.
6. Kuehne, M. E.; Earley, W. G. *Tetrahedron* **1983**, *39*, 3707-3714.

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ

   การสังเคราะห์สารยังไม่เสร็จสมบูรณ์และยังไม่ได้ตีพิมพ์ผลงานในวารสารวิชาการนานาชาติ

2. การนำผลงานวิจัยไปใช้ประโยชน์
   - เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)

   2.1 มีการสร้างนักวิจัยใหม่ โดยมีนิสิตระดับปริญญาโทที่ร่วมทำโครงการวิจัยนี้ 3 คนและจบการศึกษาไปแล้ว 2 คน

   2.2 มีการนำความรู้ที่ได้จากโครงการวิจัยนี้ไปใช้ในการเรียนการสอนวิชา 01403423 (Modern Synthesis and Reactions of Organic Compounds), 01403524 (Advanced Organic Reactions I) และ 01403621 (Advanced Organic Reactions and Synthesis)

3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

   3.1 Proceeding เรื่อง Toward the Synthesis of Anticancer (+/-)-Melotenine A ในงานประชุมวิชาการ Pure and Applied Chemistry International Conference 2014 (PACCON 2014), January 8-10, 2014, Khon Kaen, Thailand

   3.2 นำเสนอผลงานปากเปล่าเรื่อง Studies toward the Synthesis of Anticancer Melotenine A ในงานประชุมวิชาการ The 5th Chinese-Thailand Workshop on Natural Products and Drug Discovery, December 11-16, 2014, Shanghai, China

   3.3 นำเสนอผลงานปากเปล่าเรื่อง Studies toward the Synthesis of Anticancer Melotenine A ในงานประชุมวิชาการ 2015 Mini-Symposium on Chemistry for Creative Economy, January 15-18, 2015, National Tsing Hua University, Taiwan

ภาคผนวก

Proceeding เรื่อง Toward the Synthesis of Anticancer (+/-)-Melotenine A ในงานประชุมวิชาการ Pure and Applied Chemistry International Conference 2014 (PACCON 2014), January 8-10, 2014, Khon Kaen, Thailand

TOWARD THE SYNTHESIS OF ANTICANCER (±) MELOTENINE A

Sudaporn Aree, Paiboon Ngernmeesri*

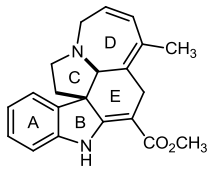
Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

* Author for correspondence; E-mail: fscipbn@ku.ac.th, Tel. +66 2 5625555 ext 2202, Fax. 66 2 5793955

Abstract: (-)-Melotenine A, which was isolated from cultures of *Melodinus tenuicaudatus* found in China, is a monoterpene indole alkaloid bearing an unprecedented skeleton with a 6/5/5/6/7 pentacyclic ring system. This natural product has been found to be an inhibitor against five human cancer cell lines, including SK-BR-3 breast, SMMC-7721 hepatocellular carcinoma, HL-60 myeloid leukemia, PANC-1 pancreatic cancer, and A-549 lung cancer. Herein, we report a progress toward the synthesis of (±) melotenine A. Our strategy employed a Diels-Alder reaction to construct a tetracyclic ring system of this compound. Up to now, the synthesis of tetracyclic ring system 2 has been achieved in nine steps (1.4% overall yield) from tryptamine hydrochloride.

1. Introduction

(-)-Melotenine A is a monoterpene indole alkaloid isolated from cultures of *Melodinus tenuicaudatus* found in China. This natural product has been found to be an inhibitor against five human cancer cell lines, including SK-BR-3 breast (IC_{50} = 2.8 µM), SMMC-7721 hepatocellular carcinoma (5.2 µM), HL-60 myeloid leukemia (0.9 µM), PANC-1 pancreatic cancer (3.6 µM), and A-549 lung cancer (10.7 µM)[1]. This interesting biological activity, along with its unprecedented skeleton with a 6/5/5/6/7 pentacyclic ring system, has drawn our attention to synthesize this compound. Recently, the first asymmetric total synthesis of (-)-melotenine A has been reported by Andrade and co-workers in fourteen steps with 1% overall yield using their one-pot, bis-cyclization method to build the ABCE tetracycle[2].



(-)-melotenine A

2. Materials and Methods

2.1 General

All reactions were carried out in dried glassware under a nitrogen atmosphere employing standard techniques in handling air-sensitive materials with stirring by a magnetic stir bar. Tetrahydrofuran (THF) and toluene were dried by distillation from sodium with benzophenone ketyl as an indicator under a nitrogen atmosphere. Acetonitrile was dried by distillation from calcium hydride under a nitrogen atmosphere. Methanol was dried by distillation from magnesium under a nitrogen atmosphere. All other commercially obtained reagents were used as received. Flash chromatography was performed on 32-63 µm silica gel or basic alumina (particle size 0.05-0.15 mm, pH 9.5 ± 0.5). ^{1}H-NMR spectrum was recorded on a VARIAN INOVA 400 spectrometer. Chemical shifts of ^{1}H NMR spectra are reported in ppm relative to $CDCl_3$ (δ 7.26). Data are presented as follows: multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad), coupling constant (J/Hz) and integration. Mass spectra were obtained on an Agilent Technology 1100 series LL/MSD Trap and on a GCMS-QP-5050QA spectrometer in electrospray ionization mode (ESI^+); the first number denoted *m/z* value and the ion assignment and abundance are given in parentheses.

2.1.1 Methyl 1,2,4,6-Tetrahydro-11-(3-propan-2-one-yl)-3,5b-methanoazepino(4,5b)indole-5-carboxylate (**9**)

To a solution of **8**[3] (2.00 g, 6.4 mmol) in 50 mL of THF was added ether saturated with HCl gas (1.80 mL). A precipitate formed immediately. The reaction mixture was stirred vigorously to break down the precipitate. The resulting yellow suspension was stirred at room temperature for 3 days. The solvent was removed under reduced pressure to give a solid residue. To this residue was added CH_2Cl_2 (60 mL) followed by 1 M NaOH (60 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness to give compound **9** as a light brown solid (1.96 g, 98%), m.p. 146-148 °C. ^{1}H NMR data are consistent with those reported in literature.[3] This compound was used in the next step without further purification.

2.1.2 *N*-Allyl Ammonium Bromide 10

To a solution of **9** (2.00 g, 6.4 mmol) in THF (32 mL) was added allyl bromide (1.27 mL, 14.72 mL). The reaction mixture was stirred at room temperature for 20 h. A light yellow precipitate formed. The reaction mixture was cooled in an ice bath and the liquid phase was carefully removed with a pipet. The solid residue was washed with ether (2 x 10 mL) to give compound **10** as a light brown solid (1.53 g, 55%), m.p. 119-122 °C. This compound was used in the next step without further purification.

2.1.3 Indoloacrylate 4

A mixture of **10** (1.20 g, 2.77 mmol)**,** Et_3N (16 drops) and MeOH (30 mL) was heated under reflux for 2 h. The reaction mixture was concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography on silica gel (4:5:1 EtOAc/hexanes/Et_3N as eluent) to give compound **4** as a yellow solid (397 mg, 41%), m.p. 139-141 °C.

2.1.4 Tetracyclic Amino Ketone 3

A solution of **4** (740 mg, 2.10 mmol) and a catalytic amount of Et_3N (4 drops) in toluene (18 mL) was heated under reflux for 18 h. The reaction mixture was concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography on silica gel (1:2 EtOAc/hexanes as eluent) to give a compound **3** as a yellow solid (375 mg, 51%), m.p. 103-105 °C. ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.81 (br s, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.15 (dt, *J* = 1.2, 7.7 Hz, 1H), 6.91 (dt, *J* = 1.0, 7.5 Hz, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 5.98 (dddd, *J* = 6.2, 6.9, 10.6, 16.7 Hz, 1H), 5.28 (dd, *J* = 1.5, 17.1 Hz, 1H), 5.16 (d, *J* = 10.3 Hz, 1H), 3.76 (s, 3H), 3.60 (s, 1H), 3.45 (dd, *J* = 6.0, 13.6 Hz, 1 H), 3.31 (dd, *J* = 7.0, 13.5 Hz, 1H), 3.19 (ddd, *J* = 2.0, 2.9, 15.4 Hz, 1H), 3.07 (dd, *J* = 6.2, 9.2 Hz, 1H), 2.81 (t, *J* = 3.6 Hz, 1H), 2.73 (ddd, *J* = 4.7, 9.1, 12.6 Hz, 1H), 2.65 (dd, *J* = 4.3, 15.6 Hz, 1H), 2.03 (s, 3H), 1.99 (dd, *J* = 6.3, 12.2 Hz, 1H), 1.73 (dd, *J* = 4.8, 12.0 Hz), 1H); LRMS(ESI) m/z (relative intensity) 353.4 (100%, $M+H^+$).

2.1.5 Alcohol 2

To an ice-cooled solution of tetracyclic ketone **3** (34 mg, 0.096 mmol) in THF (3 mL) was added a solution of 1 M vinylmagnesium bromide in THF (210 μL, 0.21 mmol) . The reaction mixture was stirred at 0 °C for 2 h. Water (10 mL) was addedd to the mixture and then extracted with EtOAc (3 x 10 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography on silica gel (1:4 EtOAc/hexanes as eluent) to give compound **2** as a white solid (8 mg, 22%). LRMS(ESI) m/z (relative intensity) 381.5 (100%, $M+H^+$).

3. Results and Discussion

3.1 Retrosynthetic analysis of of melotenine A

The retrosynthesis is outlined in Figure 1. Melotenine A could be formed by dehydration of compound **1**, which could in turn be derived from compound **2** by ring closing metathesis. Compound **2** could be generated from a Grignard reaction of compound **3** with vinylmagmesium bromide. Compound **3** could then be prepared from precursor **4** by a known method.[3]

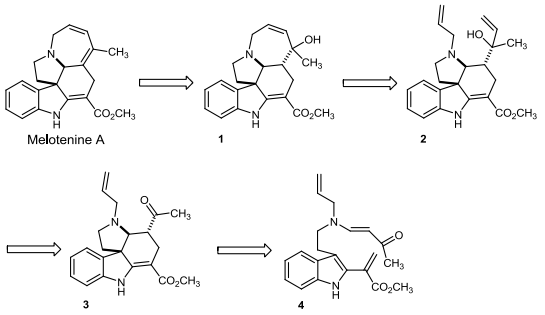


Figure 1. Retrosynthetic analysis of (±)-melotenine A.

3.2 Preparation of the Precursor

The precursor **4** for the Diels-Alder reaction was prepared according to the method described by Kuehne.[3] In the first step, the Pictet-Spengler condensation of tryptamine hydrochloride with methyl bromopyruvate afforded the bromomethylated compound **5** in good yield (Figure 2). In this reaction, Kuehne originally used methyl chloropyruvate but this compound is not commercially available. In addition, it was reported by Li[4] that methyl bromopyruvate provided a starting material with a better leaving in the second step. When **5** was heated at reflux in pyridine, nucleophilic substitution of the bromide occurred followed by ring opening to give olefinic indoloazepine **6** in 84% yield, and this yield was comparable with that reported by Li (87%). Then the double bond of **6** was reduced with $NaBH_3CN$ to provide amine **7**. This compound is very polar, and ^{1}H NMR spectrum of the crude product showed that this desired product formed almost exclusively. Therefore, it was used in the next step without further purification on silica gel. Alkylation of **7** with 3-butyn-2-one in CH_3CN provided the desired β-amino enone **8** in 83% over 2 steps.

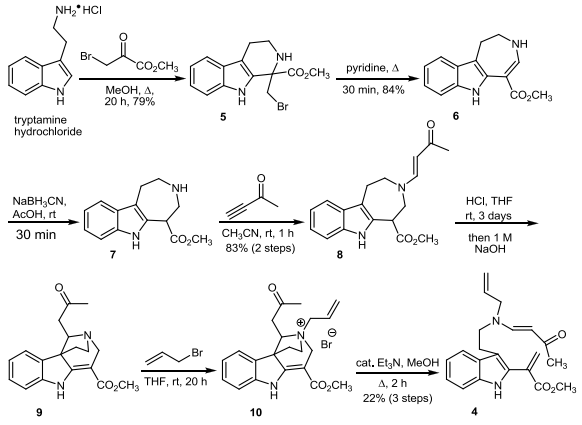


Figure 2. Preparation of precursor **4**

Treatment of compound **8** with HCl in THF gave the bridge indoloazepine **9**. Our experimental time for the rearrangement was longer than that by Kuehne [3] (3 d vs 1 h). Stirring of the precipitate, which immediately formed after an addition of acid in THF,

for 3 days gave **9** as confirmed by ^{1}H NMR of the crude product. Attempted purification of **9** by column chromatography (silica gel) was problematic as it partially converted back to the starting material **8**. In addition, deactivating silica gel with a base such as Et_3N could not solve this problem. Therefore, the crude product was used in the next step without further purification.

Treatment of **9** with allyl bromide in THF provided ammonium salt **10** (55% crude from **9**) as a precipitate, which was easily collected by removal of the solvent and washing with ether. Finally, retro Diels-Alder reaction of **10** was carried out in refluxing methanol with catalytic amount of triethylamine to give precursor **4** in moderate yield (41%). Attempts to improve the reaction yield of this step are in progress. Overall, we were able to prepare precusor **4** from tryptamine hydrochloride in seven steps with 12% overall yield.

3.3 Synthesis of the tetracyclic ring system

When indoloacrylate **4** was heated at reflux in toluene in the presence of Et_3N as a catalyst (Figure 3), Diels-Alder reaction occurred to give tetracycle **3** in moderate yield (51%). In addition, we were able to successfully perform the Grignard reaction[5] of compound **3** with vinyl magnesiumbromide in the absence of a protecting group on the amine. Although we have not obtained a very clean ^{1}H NMR spectrum, it was obvious that this compound contained olefinic protons from both the allyl and vinyl groups. In addition, both the methyl groups were still intact, suggesting that the carbonyl of the ester group did not participate in the Grignard reaction. This might be caused by the fact that the Grignard reagent deprotonated the amine to generate an ester enolate, and thus lowering the electrophilicity of the carbonyl. Although TLC analysis of this reaction showed almost exclusively the desired product, only 22% yield of alcohol **2** was obtained since this reaction was performed on a small scale and some of the desired product seemed to be lost on silica gel during purification. Further improvements of these reactions are in progress.

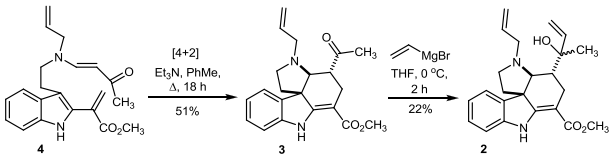


Figure 3. Synthesis of tetracyclic ring system **2**.

4. Conclusion

The synthesis of tetracyclic ring system **2**, a precursor for the synthesis of (±)-melotenine A, was accomplished in nine steps with 1.4% overall yield from commercially available tryptamine hydrochloride. Efforts to complete the synthesis of this natural product are underway.

Acknowledgements

This work was supported by the Thailand Research Fund (DBG5480018), the Center of Excellence for Innovation in Chemistry (PERCH-CIC) and the Faculty of Science, Kasetsart University.

References

[1] T. Feng, Y. Li, Y. Liu, X. Cai, Y. Wang and X. Luo, *Org. Lett.* **12** (2010) 968–971.
[2] S. Zhao, G. Sirasani, S. Vaddypally, M.J. Zdilla and R. B. Andrade, *Angew. Chem. Int. Ed.* **52** (2013) 8309–8311.
[3] M.E. Kuehne and W.G. Earley, *Tetrahedron* **39** (1983) 3707–3714.
[4] P. Chen, L. Lidong and C. Li, *J. Org. Chem.* **74** (2009) 7533–7535.
[5] C.L. Martin, L.E. Overman and J.M. Rohde, *J. Am. Chem. Soc.* **130** (2008) 7568–7569.