A Dissertation for the Degree of Doctor of Philosophy in Pharmacy

Part A. Solid-supported Fluorogenic Chemical Assay System for the Identification and Isolation of Terminal Alkyne Natural Products

Part B. Formal Synthesis of Cephalotaxine via Chirality Transfer and [2,3]-Sigmatropic Rearrangement

Part A. Chemodosimetric fluorescent probe를 이용한 **chemical assay**기법의 개발과 천연물 분리에의 응용
Part B. Chirality transfer 및 **[2,3]-rearrangement**를 이용한 천연물 **cephalotaxine**의 전합성

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Abstract

Part A. Solid-supported Fluorogenic Chemical Assay System for the Identification and Isolation of Terminal Alkyne Natural Products

As a novel prototype of chemical assay-guided natural product isolation, a reliable and competent chemical assay system was devised. The identification and isolation of naturally occurring terminal alkynes was realized with this system developed. This chemical assay system is based on the fluorescent chemodosimeter which is immobilized on solid support. It offers diverse advantages in identifying the desired natural compounds in complex natural product mixtures. For the isolation of only compounds with the specific functional groups, the click chemistry was utilized. Especially, copper(I) catalyzed azide–alkyne cycloaddition (CuAAC) reaction was adopted to develop this system for the isolation of terminal alkyne-containing natural products. With the sensing system in hand, a naturally occurring terminal alkyne was identified, quantified, and isolated from plant extracts. This method offers the following advantages: 1) easy handling without any special techniques or equipment, 2) reliable target compound identification without interference from other components, and 3) spectroscopic visualization of the presence of target compounds.

Key word: Click chemistry, Fluorescent probe, Solid support, Terminal alkyne, Natural product

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Abstract

Part B. Formal Synthesis of Cephalotaxine via Chirality Transfer and [2,3]-Sigmatropic Rearrangement

The Cephalotaxus alkaloid family consists of natural products isolated from eight known species of the genus *Cephalotaxus* (Cephalotaxaceae). Their novel chemical structures and biological activities have attracted attention from synthetic chemists. Cephalotaxine, the parent structure of the Cephalotaxus alkaloid family, has a rare 1-azaspiro[4,4]nonane skeleton fused to a benzazepine. We developed an efficient asymmetric synthesis of cephalotaxine using C to N to C chirality transfer and [2,3]-sigmatropic rearrangement to construct the C-quaternary center. After the [2,3]-rearrangement product was obtained, the formal synthesis of cephalotaxine was completed via aziridinium salt formation, ring closing metathesis, and an acid-catalyzed Friedel-Crafts reaction

Key word: Cephalotaxine, Chirality transfer, [2,3]-Sigmatropic rearrangement, Formal synthesis

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Part A. Solid-supported Fluorogenic Chemical Assay System for the Identification and Isolation of Terminal Alkyne Natural Products

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Part A.

Solid-supported Fluorogenic Chemical Assay System for the Identification and Isolation of Terminal Alkyne Natural Products

I. Introduction

Natural products are still a major source of new drugs[1] in spite of the advent of drug discovery technologies. In addition, they have been used as an valuable tool with which to study biology.[2] Among the assays to facilitate the natural product research, bioassay-guided isolation is the most common procedure for identifying of new natural substances.[3] Although the development of modern analytical techniques have made it considerably competitive,[4] bioassay-guided isolation still has some limitations caused by false assay signals rising from compounds with unspecific activities.[5] Thus, complementary and alternative approaches are required to advance natural product research.

The isolation of natural products can be also guided by the identification of compounds that possess a chemical functional group of interest.[6] This method utilizes chemical reactions to detect specific chemical functional groups in the natural products. For example, the phenolic group of tannins was detected by $FeCl_3$, and the tertiary amino group in alkaloids was identified by Dragendorff's reagent. The application of chemical reactions in the natural product isolation process has a very long history and received considerable attention before the advent of modern analytical tools.[7] However, this method is not considered useful, primarily because of the low sensitivity and specificity of the chemical tests on extracts. If efficient

chemical assay systems are available that can reliably isolate a compound with a specific functional group from extracts, the systematic exploration of the natural product chemical space would be greatly facilitated.

Numerous reactions are known to be thermodynamically favorable and to reliably lead to a single product under mild reaction conditions. Some typical examples are nucleophilic ring-opening reactions of epoxides and aziridines, non-aldol-type carbonyl reactions, hydrazine formation, additions to carbon-carbon multiple bonds, Michael additions, alkene hydrothiolation, and cycloaddition reactions. These reactions are commonly referred to as "click chemistry".[8] We anticipated that the click chemistry would guarantee the reliability and specificity of the chemical assay system. The copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction is the most popular click reaction.[9] It produces a 1,4-substituted triazole from azide and a terminal alkyne in generally excellent yield and with a short reaction time. Herein, we present a chemical assay system using CuAAC to assess the potential of click chemistry-based chemical assay-guided natural product isolation.

II. Results and Discussion

The present system is designed for the identification of terminal alkynes, which exhibit various biological properties.[10] As shown in Figure 1, the designed alkyne sensing chemical assay system consists of a fluorescent chemodosimetric probe, a solid support, and a linker. The fluorescent chemodosimetric probe[11] is the key moiety of our sensing system. It captures a terminal alkyne group selectively through CuAAC reaction and produces a fluorescence signal. Fluorescence detection is highly sensitive, thus enabling the identification of target components present even in low concentrations. Immobilization of a fluorescent probe onto a solid support was adopted for easy tracking and handling. It enables to recognize the presence of terminal alkyne compounds through fluorescent responses from the solid bead. Additionally, a cleavable linker allows the captured target component to be detached from the bead as a click product for the spectroscopic analysis

The proper choice of solid support and linker is critical to the successful solid-phase chemistry. As a proper solid support, TentaGel™ MB amino resin was selected because of its high swelling capacity in various solvents and its relatively large particle size (140–170 μm), which is suitable for even single-bead assays. As shown in Figure 2a, the linker chosen was (2-phenyl-2-trimethylsilyl)ethyl-(PTMSEL)-linker, which can be readily cleaved by fluoride anion under almost

neutral conditions.[12] The fluorescent chemodosimetric probe should be able to detect a terminal alkyne group and produce a visually apparent signal through a selective chemical reaction. The profluorophore 3-azidocoumarin moiety was chosen for this role because it gives highly fluorescent triazolylcoumarin products after the chemoselective CuAAC reaction with terminal alkynes.[13] The chemodosimetric probe was indirectly attached to the PTMSEL linker through glycolic acid, which enables facile analysis by reverse-phase LC/MS after cleavage from the linker.

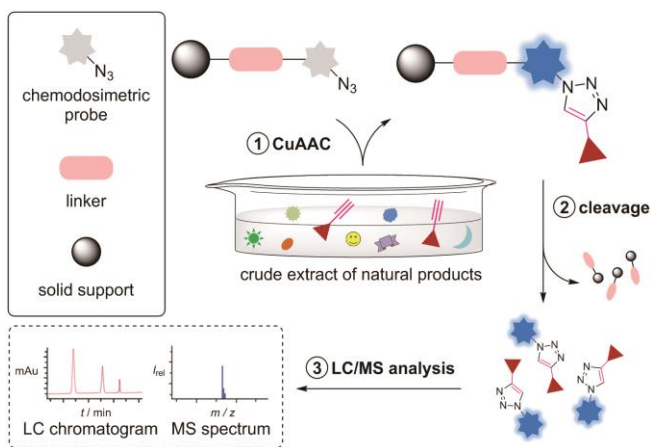


Figure 1. A solid-supported fluorogenic chemical assay strategy for the identification of terminal alkynes in natural product extracts.

The alkyne sensing bead **1** was prepared with a loading level of 0.3 mmol/g using solid-phase synthesis techniques (See SI). The optimal conditions for CuAAC was explored with this sensing bead **1**. 1-Pentyne (0.9 μmol) was used as a model

alkyne substrate. After the investigation of many reaction conditions, the optimal condition was observed as follows: Treatment of the sensing bead **1** (0.3 μmol, 1 mg) with $CuSO_4 \cdot 5H_2O$ (1.5 μmol), ascorbic acid (7.5 μmol), *N*,*N*-diisopropylethylamine (15.0 μmol), acetic acid (30.0 μmol), and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (3.0 μmol) in DMF (ca. 50 μL) at room temperature produced the triazolylcoumarin product without side reactions such as the reduction of azide to amine.[14,15] When 1-pentyne was reacted with bead **1** under the aforementioned conditions, fluorescence emission was observed from the beads with conventional fluorescence microscopy (Figure 2b). However, in a control experiment with 1-pentene, no fluorescence activation was exhibited.

The fluorescent product on the resins could be cleaved from the solid support by treatment with tetrabutylammonium fluoride trihydrate ($TBAF \cdot 3H_2O$) and subsequently analyzed by LC/MS. The analysis was readily achieved using a conventional LC/MS system equipped with a diode-array UV detector and a reverse-phase column, because the detached compound has a UV-active coumarin functionality and a hydrophilic carboxylic acid group, as exemplified by compound **3** (Figure 2e). In the LC chromatogram of the analyte released from the fluorescent beads (analyte **B**), only one peak was shown which was identified by the molecular ions $[M–H]^-$ at *m*/*z* 328 in the ESI negative ionization mode (Figure 2c, d, and SI). The molecular mass (329 Daltons) of this CuAAC product matched well with the

theoretical value for triazolylcoumarin **3**. The LC chromatogram of the control experiment (analyte **A**) also showed only one peak with a molecular ion at *m*/*z* 260 ([M–H]$^-$), which matched well with the theoretical molecular weight of unreacted 3-azidocoumarin **2**.

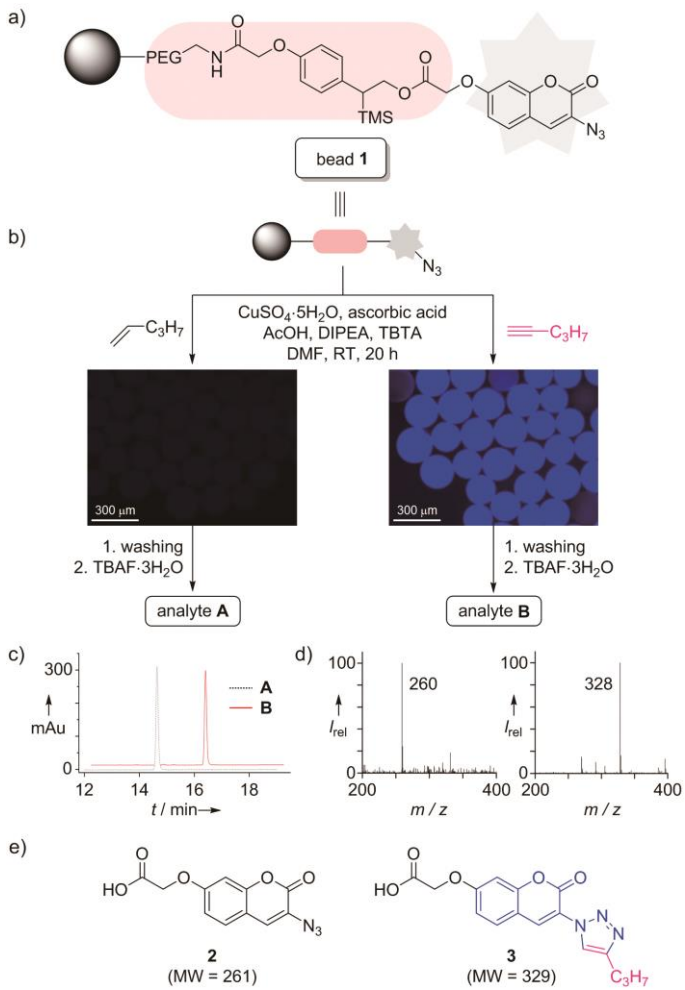


Figure 2. The model study of alkyne sensing system. a) The structure of alkyne sensing bead **1**; b) Schematic representation of the reaction procedure and the fluorescence images

of the beads. (excitation/emission filters of 387 nm/434±9 nm and a 10× objective lens); c) The overlaid LC chromatogram of analytes **A** and **B** (at 345 nm); d) The ESI negative ionization mode mass spectrum of the peaks at 12.6 min in analyte **A** and 14.2 min in analyte **B**; e) The structures of **2** and **3**. DIPEA = *N*,*N*-diisopropylethylamine, TBTA = tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] amine, MW = molecular weight.

As another model study, a mixture of different terminal alkynes was assayed. The mixture of equimolar amounts of four terminal alkynes **4a**–**d** (0.2 equiv. each to a maximum loading level of beads) was treated with the sensing bead **1** (Figure 3a). The resulting fluorescent beads were treated with TBAF·$3H_2O$ in dichloromethane, and the mixture of detached compounds was analyzed by LC/MS. Total five peaks were observed in the LC/MS chromatogram (Figure 3b). The molecular masses of the peaks matched the theoretical values of triazolylcoumarins **5a**–**d** and 3-azidocoumarin **2**. Furthermore, the integration values of the five peaks were very similar, corresponding to the theoretical ratio. This result revealed that the relative quantification of terminal alkynes is possible with this sensing system (see SI).

To demonstrate the utility of the alkyne sensing bead **1** in the natural product isolation process, the methanol extract of the leaves of *Litsea japonica* (Lauraceae) was tested because two independent groups have already isolated the four terminal alkyne compounds **6a**–**d** from the leaves of *L. japonica* (Figure 4a).[16]

As expected, fluorescence activation was exhibited in the leaf methanol extract (3 mg) after the click reaction with our sensing bead **1** (0.5 mg). In the LC/MS

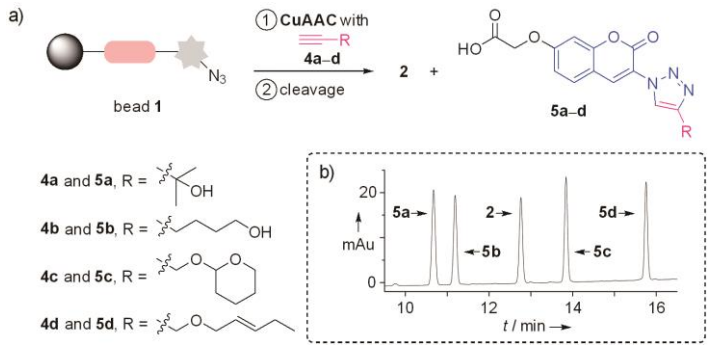


Figure 3. Validation of the system with a set of terminal alkynes. a) Schematic representation of the chemical assay with bead **1** and terminal alkynes **4a**–**d**; b) The LC chromatogram of the bead-released sample (at 345 nm).

analysis of the off-bead mixture, five peaks were observed in the LC chromatogram with peak of **2** (Figure 4b). The deduced molecular mass (521 Daltons) of the major peak (retention time: 18.65 min) matched well with the theoretical molecular weight of **7a**, the resulting triazolylcoumarin product from the CuAAC reaction of **6a**. The other four peaks exhibited the same *m*/*z* value of 538 ($[M–H]^-$). Their deduced molecular masses (539 Daltons) are in good agreement with those of triazolylcoumarins **7b**–**d**. These results revealed the presence of **6a**–**d** in the leaves of *L. japonica* and suggested the presence of one additional, yet-unidentified terminal alkyne compound with a molecular weight of 278.

To confirm this result with the leaves of *L. japonica*, methanol extract of the stem heartwood of the same plant (3 mg) was treated to the sensing bead **1**. It also

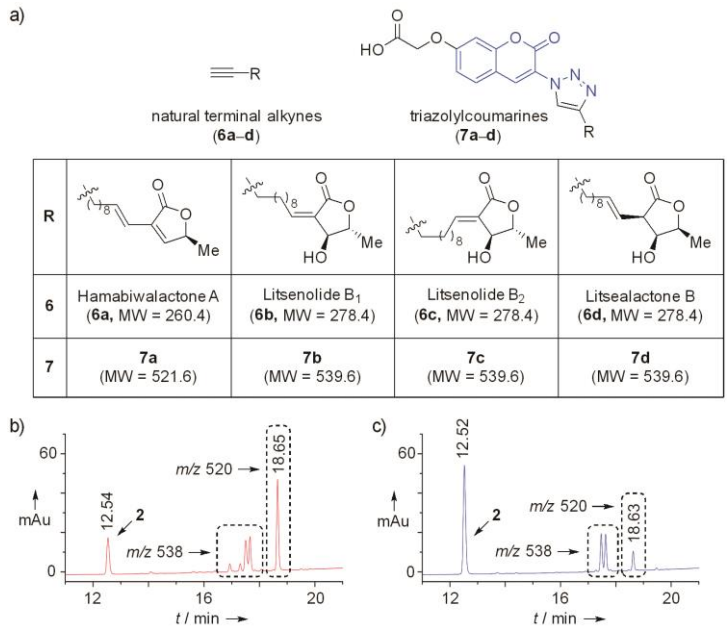


Figure 4. Validation of the sensing system with the crude methanol extracts of *L. japonica*. a) The chemical structures and molecular weights of the reported terminal alkyne-containing natural products **6a**–**d** in the leaves of *L. japonica* and the corresponding triazolylcoumarins **7a**–**d**; b) The LC chromatogram (at 345 nm) of the bead-released sample in the methanol extract of the leaves of *L. japonica*; c) The LC chromatogram (at 345 nm) of the bead-released sample in the methanol extract of the stem heartwood of *L. japonica*.

showed the fluorescence from the beads. However, compared to the leaf extracts, somewhat different pattern of peaks was observed in the LC/MS chromatogram with the cleaved products. This result suggests that the levels of specific terminal alkyne natural products **6a**–**d** varied among different parts of the plant (Figure 4c).

Triazolylcoumarin moiety has the characteristic and intense UV absorption, thus

the quantification of individual terminal alkyne compounds was possible by the standard curve method. For example, on the basis of the respective integration values in the LC chromatogram, compound **6a** is estimated to exist in the methanol extract of leaves at a concentration of approximately 1.0 mg/g, whereas it is present at a concentration of approximately 0.2 mg/g in the extract of stem heartwood (see SI).

Encouraged by the successful results with the crude extract of *L. japonica*, we performed further investigations to isolate terminal alkyne compounds from the extracts of natural products. Among the tested natural plant extracts of various origins, the methanol extract of the whole plant of *Chrysanthemum morifolium* (Compositae) showed the fluorescence activation (Figure 5a and SI). However, no terminal alkyne compounds have been previously isolated yet in *Chrysanthemum morifolium*.

Analysis of the LC/MS chromatogram after resin cleavage indicated the presence of three terminal alkyne compounds. To isolate the terminal alkyne component corresponding to the major peak, 30 g of the methanol extract was suspended in water and successively extracted with hexane, chloroform, ethyl acetate, and *n*-butanol (Figure 5b). A small portion of each fraction was subjected to the click reaction with the sensing bead **1**. Only hexane fraction showed the fluorescence activation. The hexane extract was fractionated on normal phase silica gel to obtain

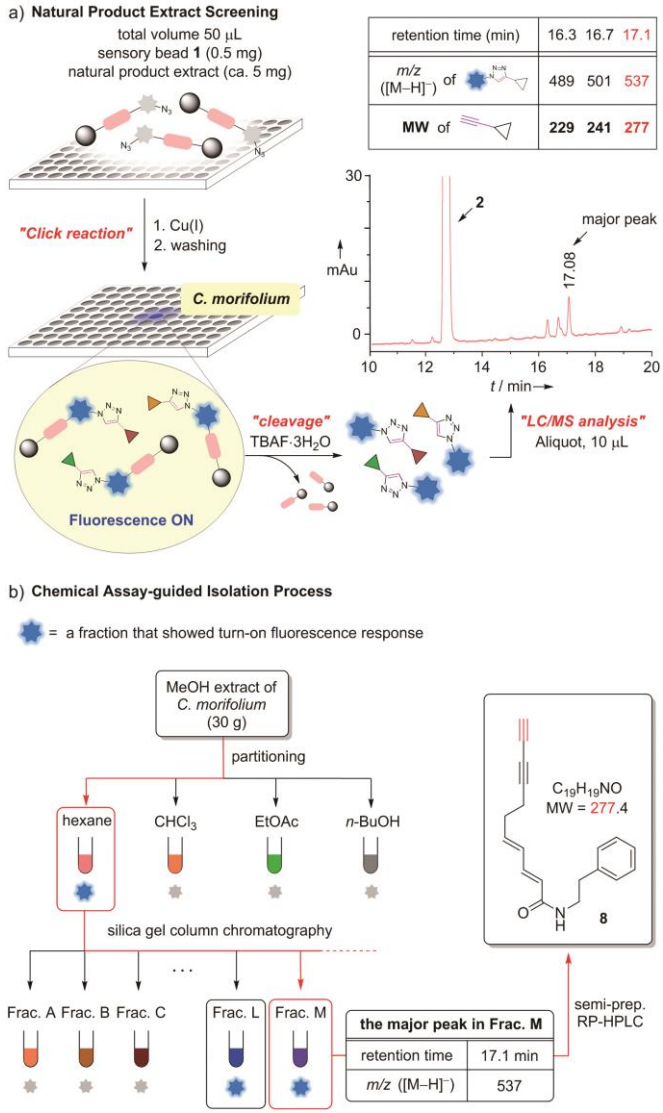


Figure 5. Overall workflow of the natural product isolation process with sensing bead **1**.

fifteen different fractions (A–O). The chemical assay and LC/MS analysis indicated that the desired component was present primarily in fraction M. Fraction M was purified by semi-preparative reverse-phase HPLC to afford a pure

compound as a white solid (*m*/*z* value: 278 $[M+H]^+$). Through analysis of the spectroscopic data and literature search, the isolated compound was determined to be terminal diyne **8**. Although the compound is a previously reported natural product,[17] this is the first report of the known natural compound **8** being isolated in the extract of *C. morifolium*.

III. Conclusions

In conclusion, we developed a novel prototype chemical assay-guided natural product isolation method. We devised a chemical assay system that can reliably isolate a compound with a specific functional group from extracts. Click chemistry utilized in this assay system enables to detect and identify the target compounds with the specific functional group from the crude mixture of natural products. Fluorescence-sensing platform was employed to permit visualization of target compounds at even low concentrations. The chemodosimetric probe was immobilized onto a solid support for easy handling and tracking. Through the application of CuAAC reaction and the fluorescence activation system, the designed alkyne sensing bead **1** can quantitatively identify terminal alkyne natural products. With the guidance of the alkyne sensing bead, we were able to isolate the terminal alkyne natural product from the plant extract. This result demonstrates the prospect of chemical assay-guided isolation of natural products. We believe that ‘click reaction/fluorescent chemodosimetric probe/solid support’ chemical assay system could also be applicable to numerous other fields, such as metabolomics and food science, that require the analysis of complex mixtures of compounds.

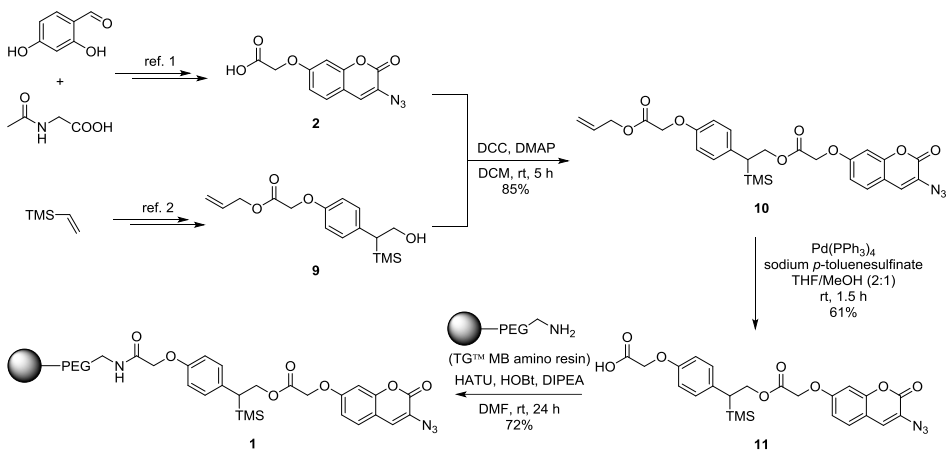
IV. Experimental

IV-1. General.

All chemicals were reagent grade and were used as received. The reactions were monitored by TLC analysis using silica-gel 60 F-254 TLC plates. Flash column chromatography was performed on silica gel (230-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in δ units relative to the non-deuterated solvent as an internal reference. The IR spectra were measured on a Fourier-transform infrared spectrometer. High-resolution mass spectra (HRMS) were recorded using fast atom bombardment (FAB). UV absorption and fluorescence emission spectra were recorded using a UV-Vis (Hitachi U-3010) and a fluorescence spectrophotometer (JASCO FP-6500), respectively. Fluorescence images were acquired using a fluorescence microscope (Nikon Eclipse Ti-U, 10× objective lens).

IV-2. Synthesis of solid-supported alkyne sensing bead 1 and related compounds

(1) Preparation of alkyne sensing bead **1**



3-Azido-7-(carboxymethoxy)-chromen-2-one (2).

Synthesis of the 3-azido-7-(carboxymethoxy)-chromen-2-one (**2**) was performed using the procedure reported in the literature.[1] IR (neat) υ_{max} 3081, 2919, 2781, 2151, 2128, 1726, 1615 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$/MeOD (1:1)): δ 7.40 (d, *J* = 8.7 Hz, 1H), 7.29 (s, 1H), 6.93 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.85 (d, *J* = 2.3 Hz, 1H), 4.67 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$/MeOD (1:1)): δ 171.84, 161.51, 159.42, 154.07, 129.87, 128.26, 124.88, 114.88, 114.79, 102.92, 66.58; HRMS (FAB): calcd. for $C_{11}H_6N_3O_5$ ($[M–H]^-$) 260.0307, found 260.0301.

Allyl 4-[2-hydroxy-1-(trimethylsilyl)-ethyl]-phenoxyacetate (9).

Allyl 4-[2-hydroxy-1-(trimethylsilyl)-ethyl]-phenoxyacetate (**9**) was performed using the procedure reported in the literature.[2] IR ($CHCl_3$) υ_{max} 3426, 2955, 1760, 1740, 1508 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.98–7.04 (m, 2H), 6.81–6.87 (m, 2H), 5.90 (tdd, *J* = 17.3, 10.4, 5.9 Hz, 1H), 5.31 (qd, *J* = 17.3, 1.4 Hz, 1H), 5.24 (qd, *J* = 10.5, 1.2 Hz, 1H), 4.69 (td, *J* = 5.7, 1.4 Hz, 2H), 4.61 (s, 1H), 4.06 (t, *J* = 11.3 Hz, 1H), 3.93 (dd, *J* = 11.3, 4.4 Hz, 1H), 2.37 (dd, *J* = 11.4, 4.5 Hz, 1H), –0.06 (s, 9H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 186.76, 155.69, 133.55, 131.42, 128.78, 119.00, 114.99, 65.78, 65.58, 63.19, 40.81, –2.65; HRMS (FAB): calcd. for $C_{16}H_{25}O_4Si$ ($[M+H]^+$) 309.1522, found 309.1525.

Allyl 2-(4-(2-(2-((3-azido-2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)-1-(trimethyl silyl)ethyl)phenoxy) acetate (10).

To a solution of **9** (970 mg, 3.14 mmol, 1.0 eq.) in CH_2Cl_2 (16 mL) were added 3-azidocoumarin **2** (904 mg, 3.46 mmol, 1.1 eq.), *N*,*N'*-dicyclohexylcarbodiimide (973 mg, 4.72 mmol, 1.5 eq.) and 4-dimethylaminopyridine (38 mg, 0.31 mmol, 0.1 eq.). The reaction mixture was stirred at room temperature for 5 h under nitrogen, filtered through Celite 545, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (20% EtOAc/hexane) to give **10** (1.5 g, 2.7 mmol, 85% yield) as a clear oil. IR (neat) υ_{max} 2957, 2118, 1756, 1727, 1613, 1511 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.24 (d, *J* = 8.7 Hz, 1H), 7.15 (s, 1H), 6.85–6.92 (m, 2H), 6.73–6.79 (m, 2H), 6.66 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.61 (d, *J* = 2.4 Hz, 1H), 5.91 (tdd, *J* = 17.1, 10.2, 5.7, 1H), 5.31 (qd, *J* = 17.3, 1.4 Hz, 1H),

5.24 (qd, *J* = 10.5, 1.2 Hz, 1H), 4.65–4.74 (m, 3H), 4.61 (s, 2H), 4.47–4.55 (m, 3H), 2.51 (dd, *J* = 12.0, 4.5 Hz, 1H), –0.04 (s, 9H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.74, 168.32, 159.59, 157.50, 155.56, 152.57, 132.90, 131.44, 128.22, 126.07, 123.80, 118.98, 114.65, 113.38, 113.04, 101.56, 66.61, 65.78, 65.47, 65.27, 36.43, –2.72; HRMS (FAB): calcd. for $C_{27}H_{30}N_3O_8Si$ ($[M+H]^+$) 552.1802, found 552.1804.

2-(4-(2-(2-((3-Azido-2-oxo-2*H*-chromen-7-yl)oxy)acetoxy)-1-(trimethylsilyl) ethyl)phenoxy)acetic acid (11).

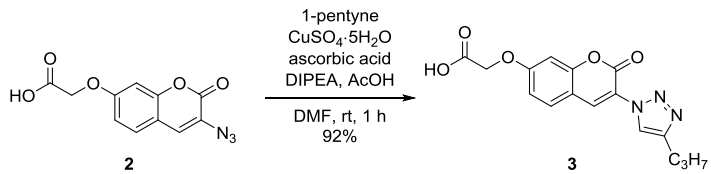
A solution of **10** (723 mg, 1.31 mmol, 1.0 eq.) in THF (18 mL) and MeOH (9 mL) was degassed by bubbling nitrogen for 15 min. Then, sodium *p*-toluenesulfinate (382 mg, 1.97 mmol, 1.5 eq.) and tetrakis(triphenylphosphine)palladium(0) (91 mg, 0.08 mmol, 0.06 eq.) were added, and the reaction mixture was stirred at room temperature for 1.5 h in the dark. After completion of the reaction, the reaction mixture was evaporated *in vacuo* and purified by silica-gel column chromatography (10% MeOH/CH_2Cl_2 + 0.2% acetic acid) to afford acid **11** (409 mg, 0.80 mmol, 61% yield) as a light-yellow oil. IR ($CHCl_3$) υ_{max} 2951, 2119, 1734, 1712, 1620, 1507 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): 12.95 (br s, 1H), 7.62 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 2H), 4.79 (s, 2H), 4.59 (s, 2H), 4.48–4.65 (m, 2H), 2.50–2.58 (m, 1H), –0.05 (s, 9H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 170.29, 168.39, 159.49, 157.10, 155.23, 152.25, 132.64, 128.76, 128.12,

127.02, 122.54, 114.08, 113.03, 101.33, 66.05, 64.95, 64.43, 35.42, –2.70; HRMS (FAB): calcd. for $C_{24}H_{24}N_3O_8Si$ ($[M–H]^-$) 510.1333, found 510.1324.

Preparation of sensing bead 1.

In a 20 mL vial, TentaGel™ MB-NH_2 resin (Sigma–Aldrich, 140–170 μm beads, ~0.40 mmol/g, 200 mg, 0.08 mmol) was pre-swollen in DMF (7 mL) for 1 h. To this solution were added acid **11** (49 mg, 0.10 mmol, 1.2 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexa fluorophosphate (91 mg, 0.24 mmol, 3.0 eq.), 1-hydroxybenzotriazole hydrate (32 mg, 0.24 mmol, 3.0 eq.), and *N*,*N*-diisopropylethylamine (42 μL, 0.24 mmol, 3.0 eq.). The vial was shaken at room temperature for 24 h in the dark and then filtered. The resin was washed two times each with DMF (10 mL), H_2O (10 mL), MeOH (10 mL) and CH_2Cl_2 (10 mL). Pyridine/acetic anhydride (3:1; 10 mL) was added, and the mixture was shaken for 3 h. The resin was subsequently washed three times with DMF (10 mL), H_2O (10 mL), MeOH (10 mL), and CH_2Cl_2 (10 mL). The resin was dried *in vacuo* to afford the loaded sensing bead **1** (230 mg). The loading was determined by UV absorption of the 3-azidocoumarin **2** obtained by treatment of the loaded resin (5 mg) in CH_2Cl_2 (900 μL) with TBAF·$3H_2O$ solution (100 μL, 10 mg/mL in CH_2Cl_2) and subsequent shaking of the reaction mixture at room temperature for 15 min. Loading: c = 0.30 mmol/g (which corresponds to a coupling yield of 72%).

(2) Preparation of triazolylcoumarin **3**

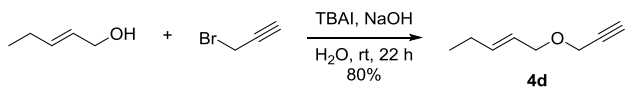


2-((2-Oxo-3-(4-propyl-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-7-yl)oxy)acetic acid (3).

To a solution of 3-azidocoumarin **2** (55 mg, 0.21 mmol, 1 eq.) in DMF (2 mL) were added 1-pentyne (104 μL, 1.06 mmol, 5 eq.), *N*,*N*-diisopropylethylamine (37 μL, 0.21 mmol, 1 eq.), acetic acid (12 μL 0.21 mmol, 1 eq.), copper(I) sulfate pentahydrate (10 mg, 0.04 mmol, 0.2 eq.), and ascorbic acid (15 mg, 0.08 mmol, 0.4 eq.). The reaction mixture was stirred at room temperature under nitrogen. After 1 h, the mixture was diluted with EtOAc (10 mL) and water (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica-gel column chromatography (10% MeOH/CH_2Cl_2 + 0.2% acetic acid) to afford triazolylcoumarin **3** (64 mg, 0.19 mmol, 92% yield) as a white solid. IR (neat) υ_{max} 3180, 3040, 2922, 2875, 1730, 1713, 1610, 1513 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 1H), 8.33 (s, 1H), 7.83 (d, *J* = 8.6 Hz), 7.10–7.13 (m, 1H), 7.07 (dd, *J* = 8.6, 2.3 Hz, 1H), 4.84 (s, 2H), 2.69 (t, *J* = 7.4 Hz, 2H), 1.62–1.73 (m,

2H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 169.40, 161.73, 156.08, 154.10, 146.92, 135.16, 130.41, 122.66, 120.50, 113.59, 111.81, 101.38, 65.18, 26.78, 22.06, 13.48; HRMS (FAB): calcd. for $C_{16}H_{14}N_3O_5$ ([M–H]$^-$) 328.0933, found 328.0939.

(3) Preparation of alkyne **4d**



(*E*)-1-(Prop-2-yn-1-yloxy)pent-2-ene (4d).

Synthesis of (*E*)-1-(prop-2-yn-1-yloxy)pent-2-ene (**4d**) was achieved using almost the same procedure as that reported in the literature.[3] To a vigorously stirring suspension of *trans*-2-penten-1-ol (1.9 mL, 18.80 mmol, 1 eq.), tetrabutylammonium iodide (TBAI) (69 mg, 0.19 mmol, 0.01 eq.), and NaOH (2.3 g, 56.40 mmol, 3 eq.) in H_2O (5 mL), propargyl bromide solution (80 wt.% in toluene, 2.1 mL, 18.80 mmol, 1 eq.) was added slowly at 0 ℃. The reaction mixture was allowed to equilibrate to room temperature and stirred for 22 h. After completion of the reaction, the reaction mixture was poured into water, extracted with ether, and washed with brine and saturated aqueous $Na_2S_2O_3$ solution. The organic layer was dried with $MgSO_4$ and filtered. The residue was partially evaporated to remove ether under reduced pressure. The crude mixture was then

purified using a Kugelrohr distillation apparatus under reduced pressure to afford the alkyne **4d** (1.9 g, 15.04 mmol, 80% yield) as a clear liquid. IR (neat) υ_{max} 3297, 2964, 2935, 2874, 2853, 2116, 1670, 1458, 1441, 1355 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.72–5.82 (m, 1H), 5.44–5.57 (m, 1H), 4.09 (d, *J* = 2.4 Hz, 2H), 3.95–4.00 (m, 2H), 2.38 (t, *J* = 2.4 Hz, 1H), 1.98–2.50 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 137.39, 124.25, 79.82, 74.14, 70.35, 56.66, 25.24, 13.21; HRMS (FAB, *m/z*): calcd. for $C_8H_{11}O$ ($[M–H]^-$) 123.0810, found 123.0807.

IV-3. Absorption and fluorescence emission spectra of compounds 2 and 3

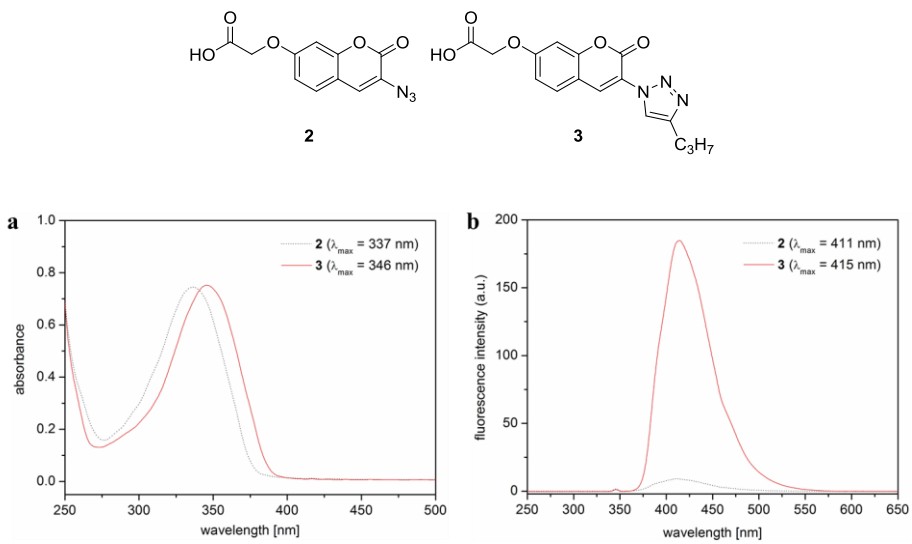


Figure S1. a) Comparison of the UV/Vis absorption spectra of compounds **2** (black dotted line) and **3** (red solid line) (30 μM in $CHCl_3$); b) Comparison of the fluorescence emission spectra (λ_{ex} = 345 nm) of compounds **2** (black dotted line) and **3** (red solid line) (30 μM in $CHCl_3$).

IV-4. Standard curve of triazolylcoumarin 3

The standard curve of triazolylcoumarin **3** was generated by the injection of 5 μL of stock standard solutions of **3** to an Agilent 1260 Infinity LC (Agilent Technologies, Palo Alto, CA, USA). The stock standard solutions of **3** were prepared at concentrations of 1, 2, 5, 10, 20, 50, 100, and 200 μM in methanol. The *x*-axis is the concentration of the stock solutions, and the *y*-axis is the area of peaks in the LC chromatograms (at 345 nm). The linearity of the standard curve was determined by linear regression analysis, which revealed a coefficient of determination (r^2) greater than 0.999.

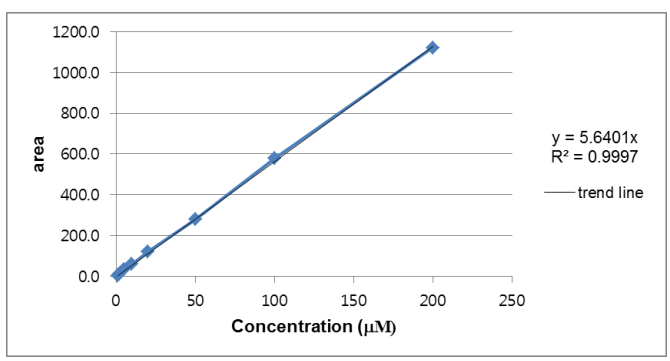


Figure S2. Standard curve of triazolylcoumarin **3**

IV-5. Representative procedure for the chemical assay with sensing bead 1

Representative procedure for the chemical assay with the sensing bead 1: The sensing beads **1** (0.5 or 1 mg, 0.15 or 0.30 μmol) were pre-swollen in 50 μL DMF in a 96-well round-bottom, polypropylene plate at room temperature for 1 h. The DMF was removed with a multichannel pipette, and a solution of terminal alkynes or natural product extracts in DMF (ca. 30 μL) was added. To the suspension were added tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (300 mM in DMF, 10 μL), acetic acid (1.6 μL), *N*,*N*-diisopropylethylamine (2.5 μL), copper(I) sulfate pentahydrate (300 mM in H_2O, 5 μL), and ascorbic acid (1500 mM in DMF, 5 μL). The resulting suspensions were shaken on a horizontal shaker (IKA HS 260 control, Janke & Kunkel & Co. IKA Labortechnik, Staufen, Germany) at 250 rpm at room temperature in the dark. After 20 h, the resulting beads were washed with DMF (200 μL), H_2O (200 μL), MeOH (200 μL), and CH_2Cl_2 (200 μL) three times each. For the cleavage procedure, the beads were treated with a solution of tetrabutylammonium fluoride trihydrate (12 μL, 10 mg/mL in CH_2Cl_2) in CH_2Cl_2 (288 μL) and shaken at room temperature for 15 min. An aliquot (5 or 10 μL) of the reaction solution was analyzed by LC/MS.

LC/MS analysis: LC/MS analysis was performed by using Agilent 6100 Series Single Quad LC/MS systems (Agilent Technologies, Palo Alto, CA, USA). Mobile phase A consisted of 0.1% formic acid in HPLC-grade water. HPLC analysis was performed using a reverse-phase Agilent Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm) at a flow rate of 0.7 mL/min (20–100% aqueous MeOH with 0.1% formic acid over 20 min and 100% MeOH with 0.1% formic acid from 20 to 25 min). Mass spectra were acquired in both positive and negative ion modes with the capillary voltage set at 4000 eV.

IV-6. LC/MS spectra for validation of the sensing system

(a) A solution of only TBAF·3H_2O in CH_2Cl_2

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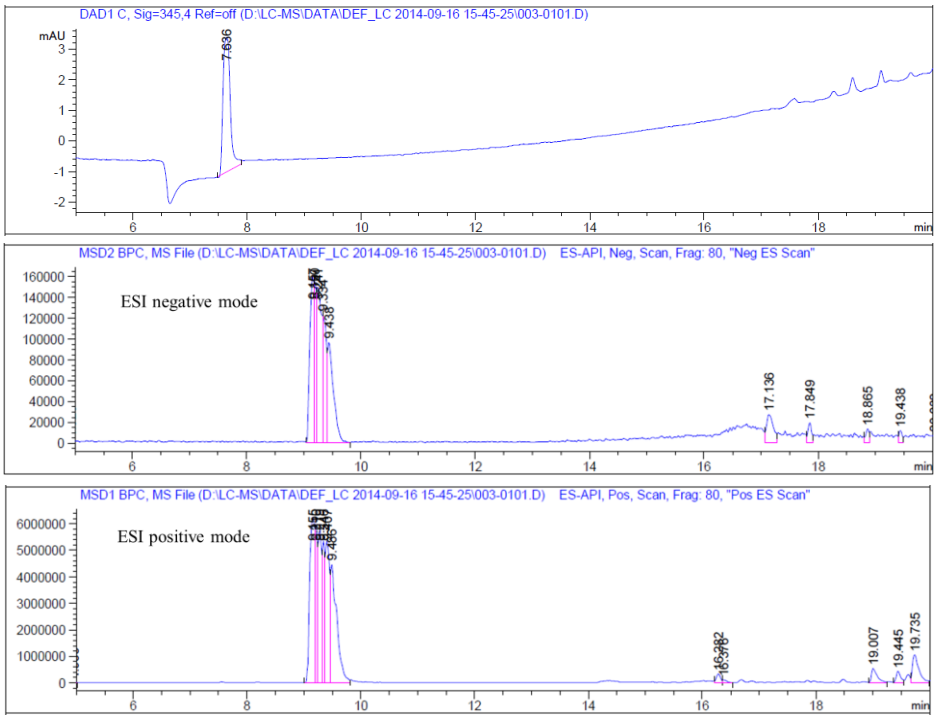

(b) Reaction with 1-pentyne

- LC/MS spectrum of analyte A

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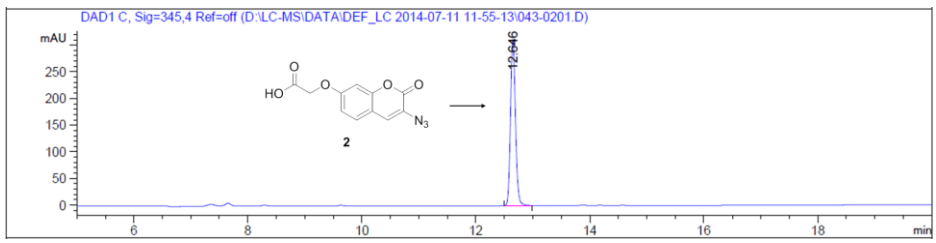

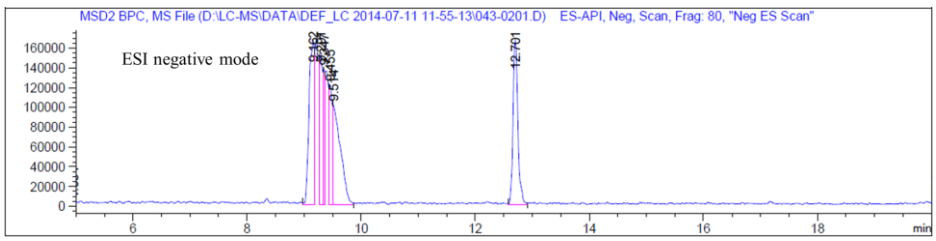


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                             260.10 I
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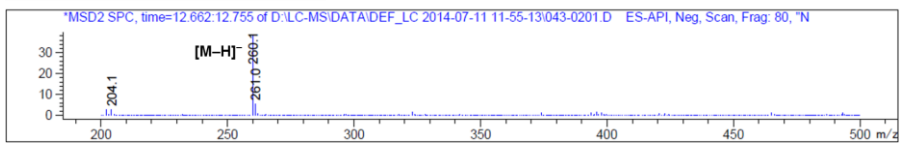

- LC/MS spectrum of analyte B

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Last changed    : 11/07/2014 10:43:29 AM by Y. Kwon
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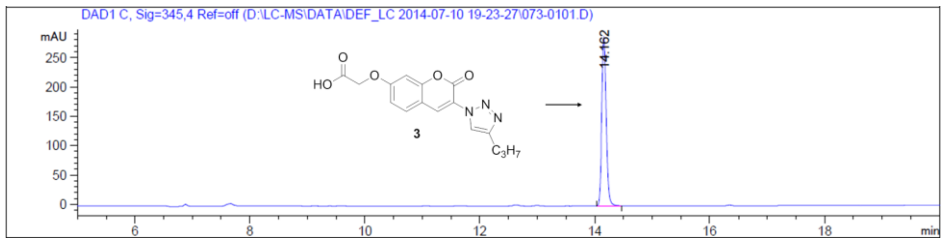

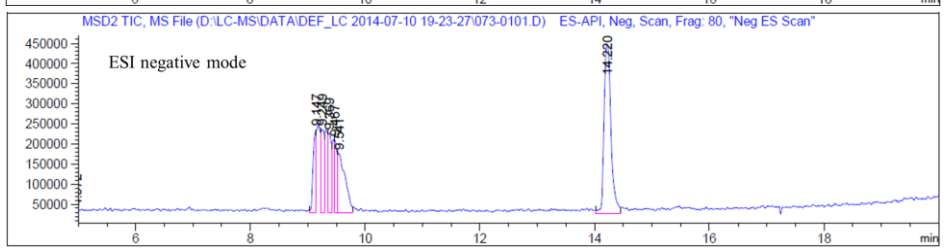


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                             270.05 I
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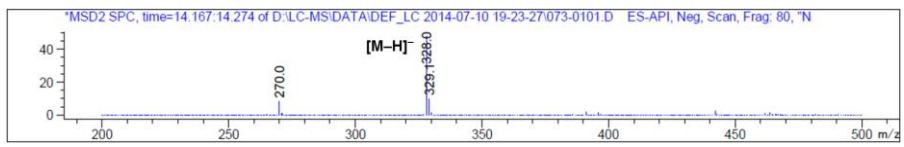

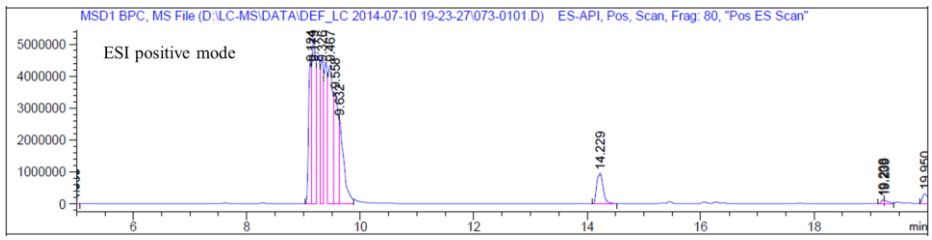

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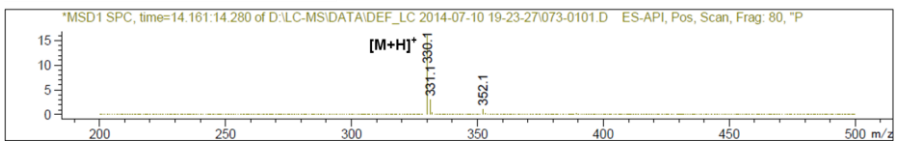
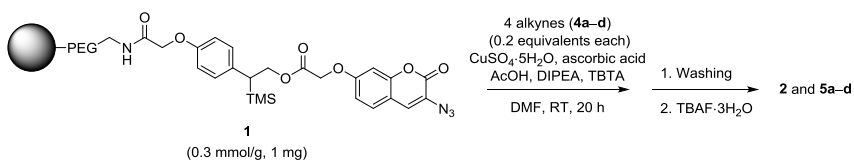
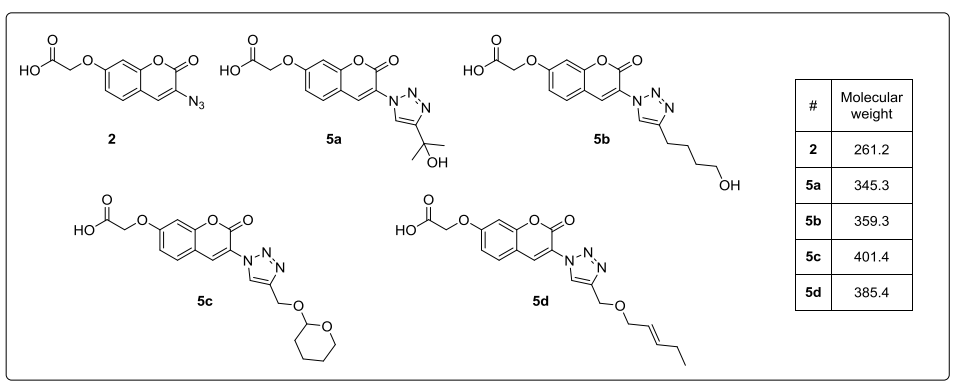
330.10 I



(c) Reaction with a set of terminal alkynes





#	Molecular weight
2	261.2
5a	345.3
5b	359.3
5c	401.4
5d	385.4

Figure S3. The designed chemical assay with a set of alkynes (**4a**–**d**). The structures of 3-azidocoumarin **2** and triazolylcoumarins **5a**–**d** corresponding to alkynes **4a**–**d** are listed, along with their molecular weights.

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4	11.190	BB	0.0860	109.35860	19.92295	15.0006
5	12.762	BB	0.0860	104.79527	19.09686	14.3747
6	13.843	BB	0.0794	119.36368	23.45429	16.3730
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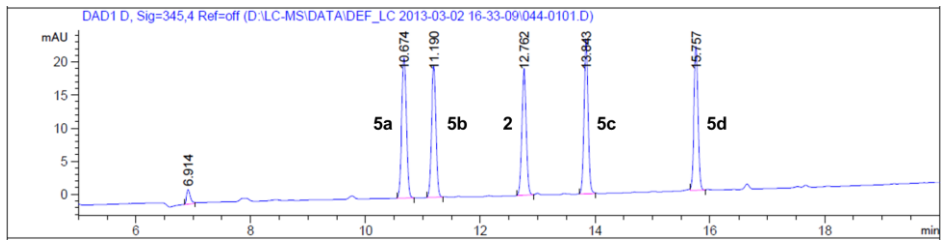

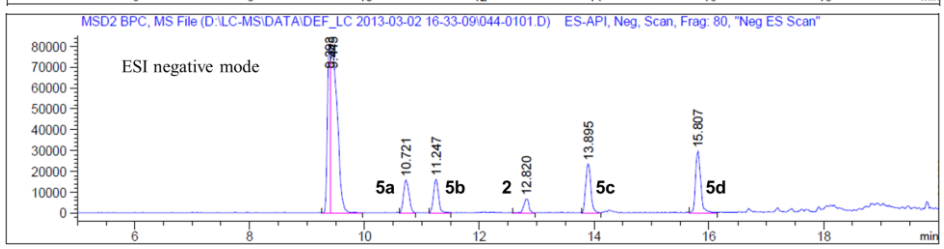

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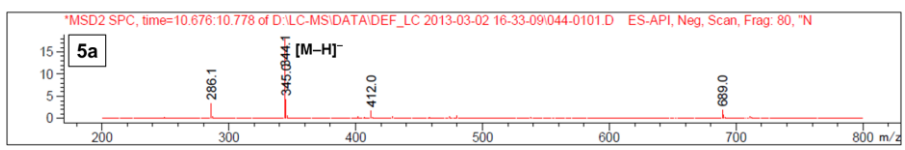
		344.15 I
		286.10 I



11.252 347807 426.15 I
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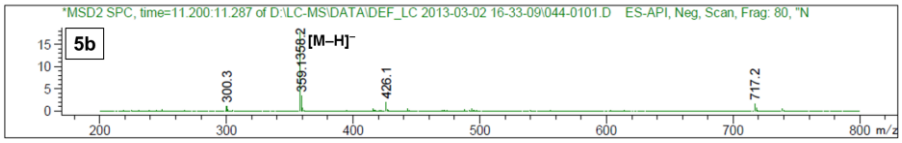
358.20 I



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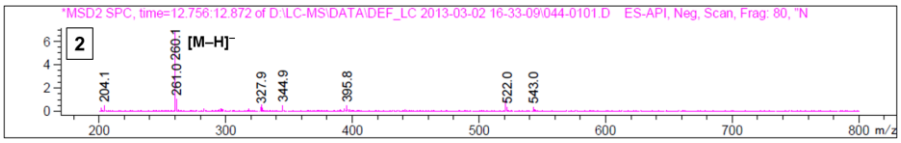
261.00 I
260.10 I



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400.15 I
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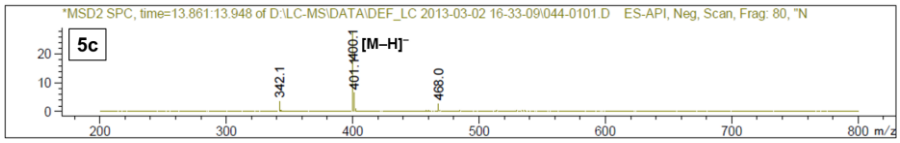

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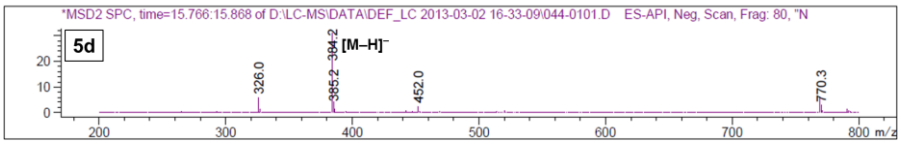
384.20 I
326.05 I



(d) Validation of the sensing chemical assay system with the methanol extract of *Litsea japonica*

- Methanol extract of leaves of *L. japonica* (3 mg)

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3	7.582	BB	0.1249	40.45161	4.41418	6.6246
4	12.539	BB	0.1018	120.78407	18.57892	19.7804
5	16.932	BB	0.0769	20.97437	4.15377	3.4349
6	17.306	BV	0.0761	20.29920	4.07687	3.3243
7	17.504	VV	0.0853	89.26628	15.93954	14.6189
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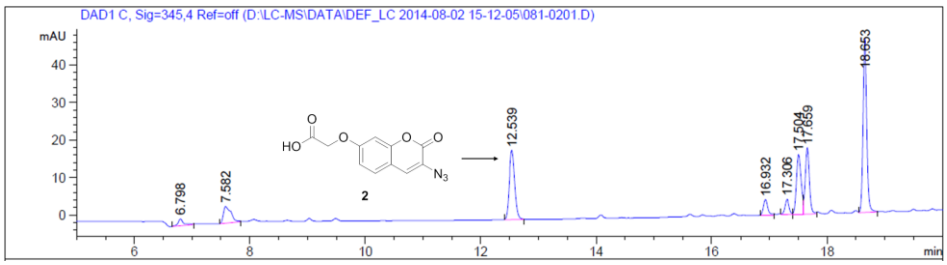

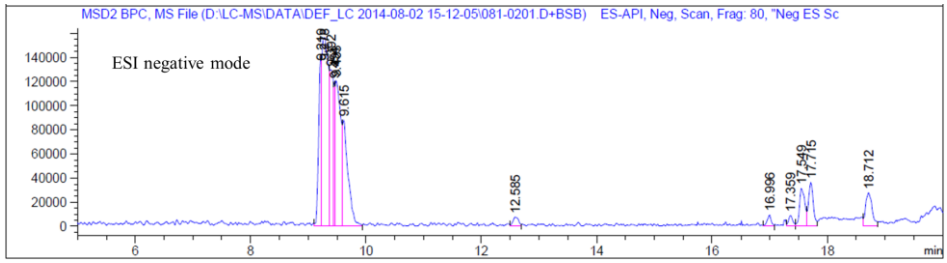

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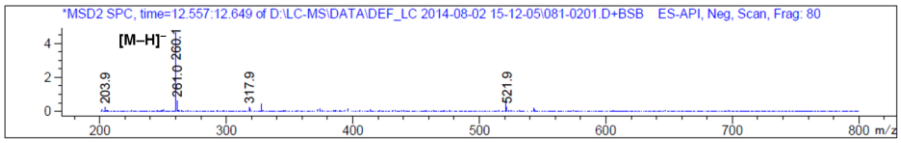
260.10 I



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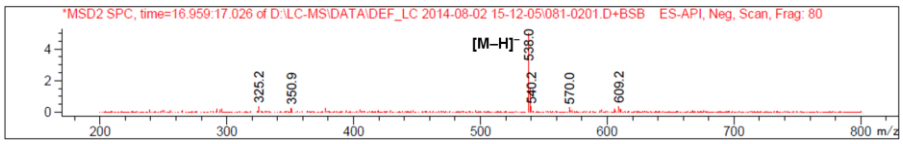
538.05 I



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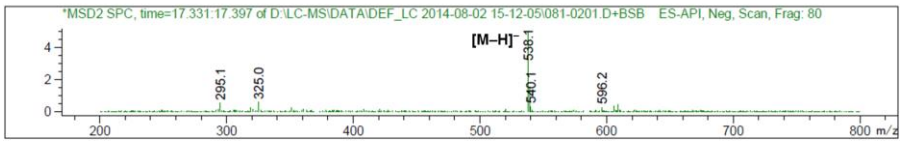
295.15 I



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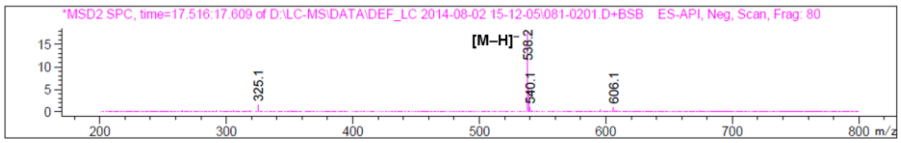
538.15 I



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325.15 I
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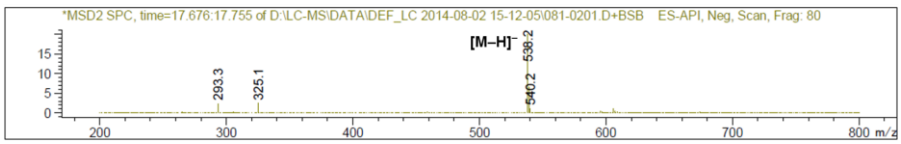

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520.10 I
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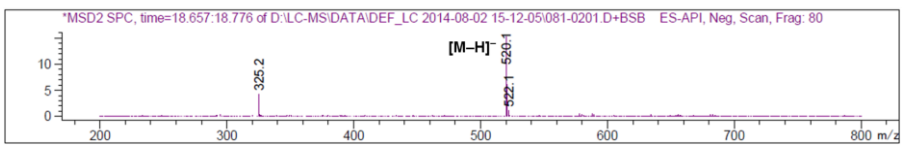

- Methanol extract of stem heartwood of *L. japonica* (3 mg)

```
==================================================================
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                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\DEFAULT.M
Last changed    : 30/09/2014 9:45:08 PM by Y. Kwon
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Method Info     : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                  ESI Positive Ion Sensitivity Test
```

Signal 3: DAD1 C, Sig=345,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
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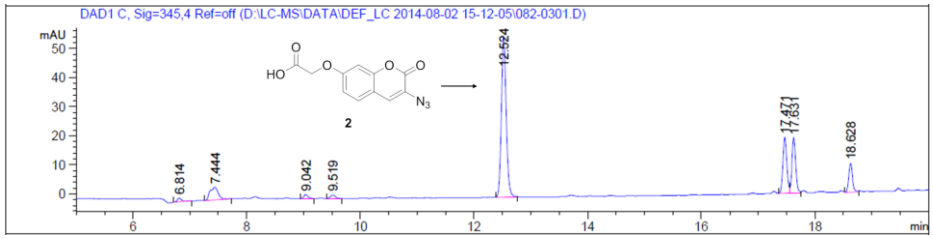
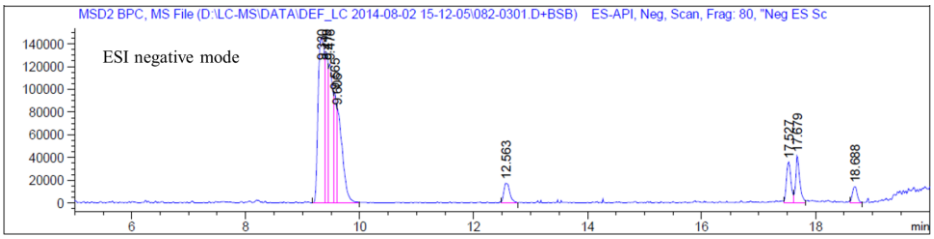
2	7.444	BB	0.1416	44.36143	4.32897	7.0205
3	9.042	BB	0.0826	7.29431	1.35872	1.1544
4	9.519	BB	0.0953	7.09844	1.16102	1.1234
5	12.524	BB	0.0956	339.85944	55.40146	53.7850
6	17.471	VV	0.0726	90.49281	19.34243	14.3211
7	17.631	VV	0.0729	89.53394	19.01467	14.1693
8	18.628	VB	0.0720	45.91363	9.92785	7.2661





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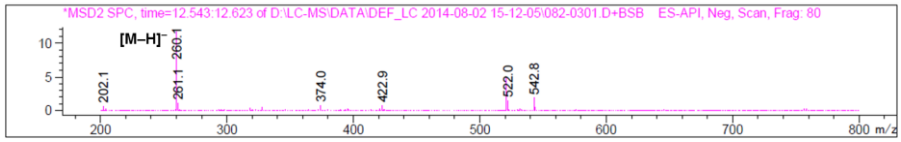

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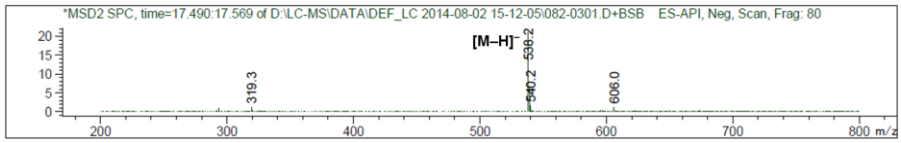

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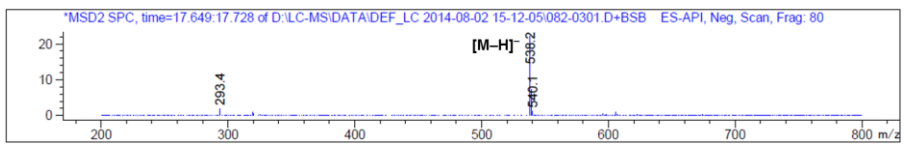
538.20 I



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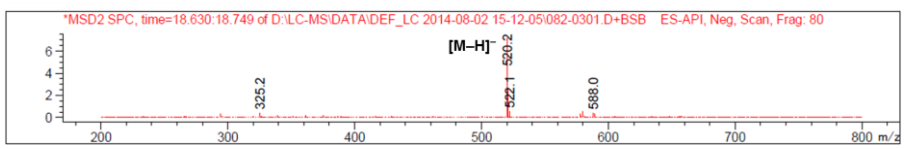

(e) Application of the sensing chemical assay system to *Chrysanthemum morifolium*

- Methanol extract of whole plants of *C. morifolium* (10 mg)

```
==================================================================
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Injection Date  : 4/09/2014 8:35:20 PM                Inj :   1
                                                 Inj Volume : 10.000 µl
Acq. Method     : D:\LC-MS\DATA\DEF_LC 2014-09-04 20-34-09\JUN (4 UV, 20PERCEN
Last changed    : 25/06/2014 4:00:39 PM by Y. Kwon
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Last changed    : 6/09/2014 3:49:37 PM by Y. Kwon
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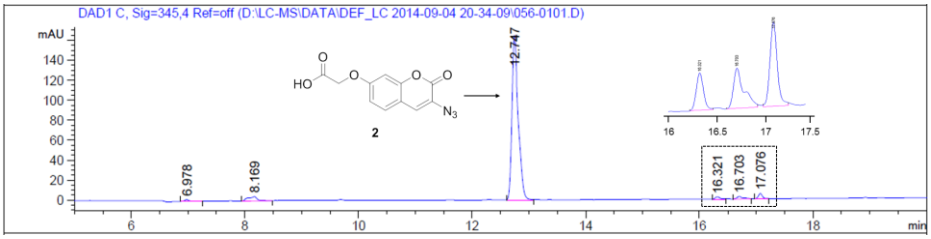

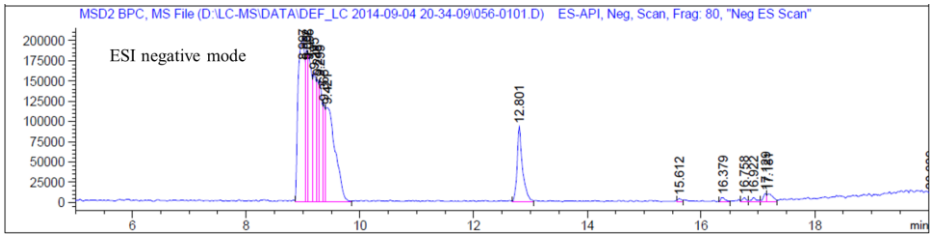


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                             521.95 I
                             521.05 I
                             260.15 I
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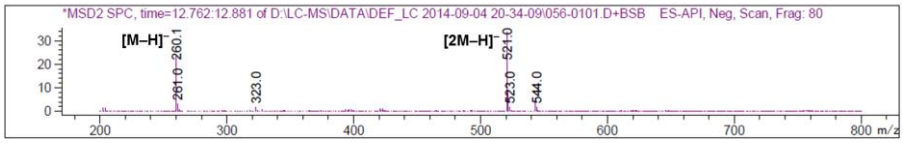

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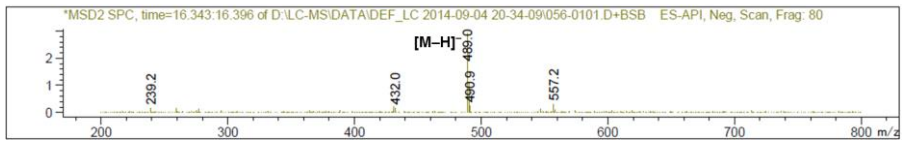
490.10 I
489.05 I



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501.05 I
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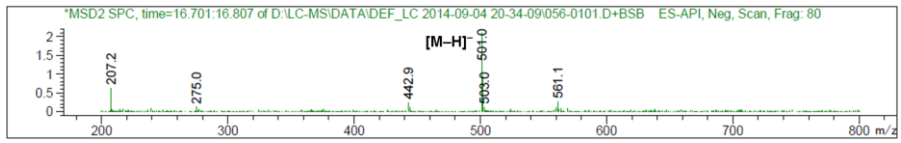

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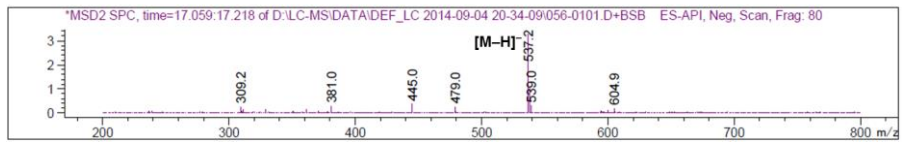
537.15 I
445.00 I



- Hexane extract (fractionated from methanol extract) of whole plants of *C. morifolium* (10 mg)

```
======================================================================
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Injection Date  : 7/07/2014 12:09:23 PM               Inj :   1
                                                Inj Volume : 10.000 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5.000 µl
Acq. Method     : D:\LC-MS\DATA\DEF_LC 2014-07-07 10-04-11\JUN (4 UV, 20PERCEN
Last changed    : 7/07/2014 12:08:29 PM by Y. Kwon
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Analysis Method : C:\CHEM32\1\METHODS\JUN (4 UV, 20PERCENTMEOH).M
Last changed    : 7/07/2014 3:19:55 PM by Y. Kwon
                  (modified after loading)
Method Info     : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                  ESI Positive Ion Sensitivity Test
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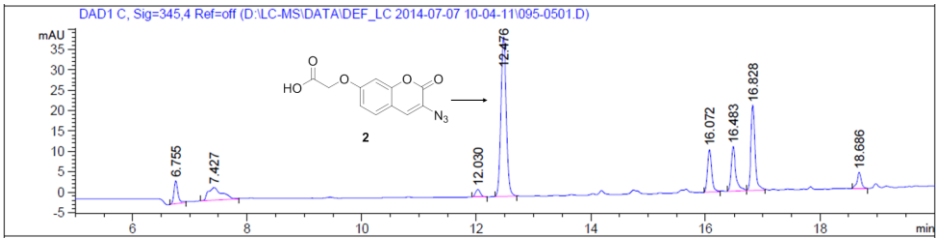

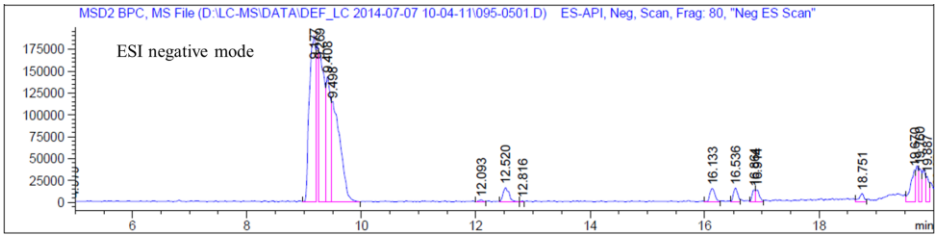

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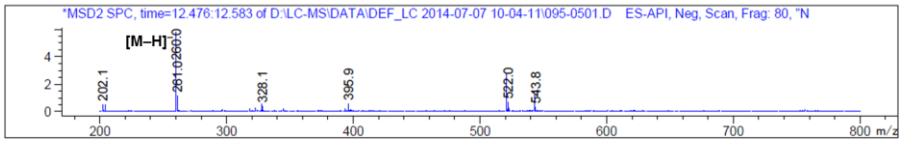
520.90 I
261.05 I
260.15 I
259.95 I



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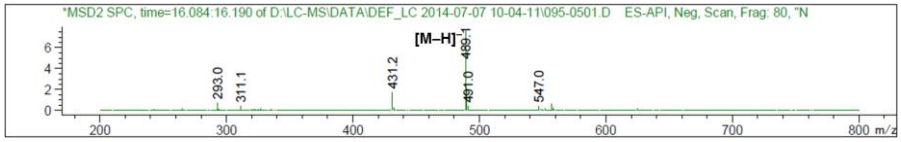
489.15 I
431.20 I



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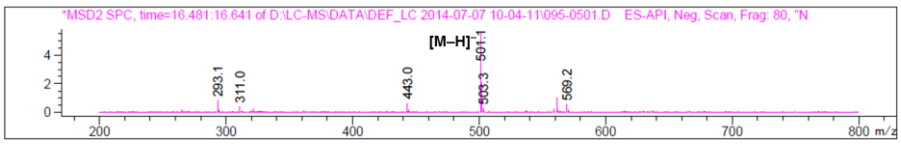
561.10 I
502.10 I
501.10 I
443.05 I
293.10 I



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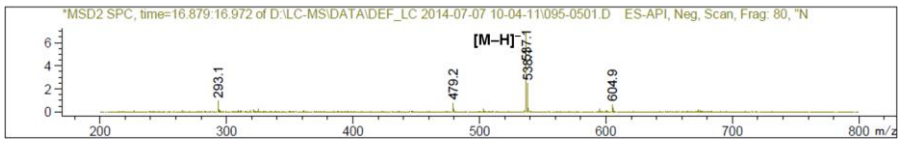
537.10 I
479.20 I
293.10 I



IV-7. The list of the screened extracts of various natural plants

The methanol extracts of the tested plants were purchased from the Plant Extract Bank of Korea (Daejeon, Korea).

Entry	Scientific name	Part	Family	Assayed amount	Fluorescence response
1	*Achillea sibirica*	whole plant	Compositae	5 mg	–
2	*Actinodaphne lancifolia*	leaves	Lauraceae	5 mg	–
3	*Ainsliaea acerifolia*	whole plant	Compositae	5 mg	–
4	*Angelica dahurica*	whole plant	Umbelliferae	5 mg	–
5	*Artemisia iwayomogi*	whole plant	Compositae	5 mg	–
6	*Artemisia princeps* var. *orientalis*	whole plant	Compositae	10 mg	–
7	*Betula chinensis*	leaves	Betulaceae	5 mg	–
8	*Calendula arvensis*	whole plant	Compositae	5 mg	–
9	*Callistephus chinensis*	whole plant	Compositae	5 mg	–
10	*Camellia sinensis*	stems, leaves	Theaceae	5 mg	–
11	*Centaurea cyanus*	whole plant	Compositae	5 mg	–
12	*Cephalonoplos segetum*	whole plant	Compositae	5 mg	–
13	*Chamaecyparis obtusa*	leaves	Cupressaceae	10 mg	–
14	*Chamaecyparis pisifera*	leaves, stems	Cupressaceae	10 mg	–
15	*Chrysanthemum boreale*	whole plant	Compositae	5 mg	–
16	*Chrysanthemum indicum*	whole plant	Compositae	5 mg	–
17	*Chrysanthemum morifolium*	whole plant	Compositae	10 mg	**O**
18	*Cinnamomum camphora*	leaves	Lauraceae	5 mg	–
19	*Dahlia pinnata*	whole plant	Compositae	10 mg	–
20	*Dendropanax morbifera*	whole plant	Dendropanax	5 mg	–
21	*Eclipta prostrata*	whole plant	Compositae	10 mg	–
22	*Erigeron annuus*	whole plant	Compositae	5 mg	–
23	*Humulus japonicus*	whole plant	Cannabinaceae	5 mg	–
24	*Hypolepis punctata*	whole plant	Pteridaceae	5 mg	–
25	*Ixeris polycephala*	whole plant	Compositae	5 mg	–
26	*Ligularia taquetii*	whole plant	Compositae	5 mg	–
27	*Lindera glauca*	leaves	Lauraceae	10 mg	–
28	*Lindera sericea*	leaves, stems, fruits	Lauraceae	5 mg	–
29	*Lonicera insularis*	leaves	Caprifoliaceae	5 mg	–
30	*Lythrum salicaria*	seeds	Lythraceae	10 mg	–
31	*Machilus japonica*	leaves, stems	Lauraceae	5 mg	–
32	*Machilus thunbergii*	stem bark	Lauraceae	5 mg	–
33	*Neolitsea sericea*	stem bark	Lauraceae	5 mg	–
34	*Oenanthe javanica*	whole plant	Umbelliferae	5 mg	–
35	*Oplopanax elatus*	leaves	Araliaceae	5 mg	–
36	*Saussurea ussuriensis*	whole plant	Compositae	5 mg	–
37	*Senecio pseudo-sonchus*	whole plant	Compositae	5 mg	–
38	*Sonchus asper*	whole plant	Compositae	5 mg	–
39	*Trillium kamtschaticum*	whole plant	Liliaceae	5 mg	–
40	*Vaccinium bracteatum*	leaves	Ericaceae	5 mg	–

IV-8. Isolation procedure of the natural compound 8 from the extract of *C. morifolium*

The methanol extract (30 g) of the whole plant of *Chrysanthemum morifolium* was suspended in H_2O (300 mL) and successively extracted with hexane (3 × 200 mL), $CHCl_3$ (3 × 200 mL), EtOAc (3 × 200 mL), and *n*-BuOH (3 × 200 mL) to give dried hexane (4.2 g), $CHCl_3$ (1.8 g), EtOAc (2.2 g), and *n*-BuOH (4.6 g) extracts. Among the extracts, only the hexane portion exhibited fluorescence emission after the click reaction with sensing bead **1**. The hexane fraction was subjected to silica-gel column chromatography and eluted with a gradient mixture of hexane–EtOAc (12:1 to 1:1) and CH_2Cl_2–MeOH (10:1 to 6:1) to give fifteen fractions (A–O). After click reactions with each fraction, the fluorescence response was observed in fractions L and M. After the cleavage step, LC/MS analysis showed that the peak with a molecular ion of *m/z* 537 ($[M–H]^-$) was predominant in fraction M. This finding indicates that fraction M contains a larger amount of the target compound (real molecular mass of 277 Daltons) compared to fraction L. Subsequently, fraction M (61 mg) was purified by semi-preparative reverse-phase HPLC conducted with a Phenomenex Luna 10 µm C18(2) column (250 × 10.00 mm) at a flow rate of 5 mL/min (30–100% aqueous MeCN with 0.1% formic acid over 20 min and 100% MeCN with 0.1% formic acid from 20 to 25 min) to give pure diyne

8 (1.2 mg) as a white solid. IR ($CHCl_3$) υ_{max} 3291, 3060, 3030, 2917, 2226, 1653, 1625, 1610, 1544, 1260; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.10–7.34 (m, 5H), 6.18 (dd, *J* = 15.0, 11.0 Hz, 1H), 6.01–6.09 (m, 1H), 5.73 (d, *J* = 14.5 Hz, 1H), 5.45 (br s, 1H), 3.61 (q, *J* = 6.5 Hz, 2H), 2.85 (t, *J* = 6.8 Hz, 2H), 2.37–2.45 (m, 4H), 1.98 (s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 165.96, 140.57, 139.20, 138.90, 129.74, 128.79, 128.67, 126.53, 122.93, 68.22, 65.49, 65.03, 40.66, 35.67, 31.25, 18.80; HRMS (FAB, *m/z*): calcd. for $C_{19}H_{20}NO$ ($[M+H]^+$) 278.1545, found 278.1543.

V. References

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V-2. References for Supporting Information

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(3) K. Mikami, S. Kataoka, Y. Yusa, K. Aikawa, *Org. Lett.* **2004**, *6*, 3699–3701.

VI. Acknowledgements

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Part B.

Formal Synthesis of Cephalotaxine via Chirality Transfer and [2,3]-Sigmatropic Rearrangement

I. Introduction

The Cephalotaxus alkaloid family consists of natural products isolated from eight known species of the genus *Cephalotaxus* (Cephalotaxaceae).[1] They have novel chemical structures (Figure 1) and significant biological activities.[1,2] One member of this family, the C-3 α-hydroxysuccinate ester derivative homoharringtonine **1**, was approved by the FDA in 2012 as an adult orphan-drug for the treatment of chronic myeloid leukemia resistant to tyrosine kinase inhibitors.[3]

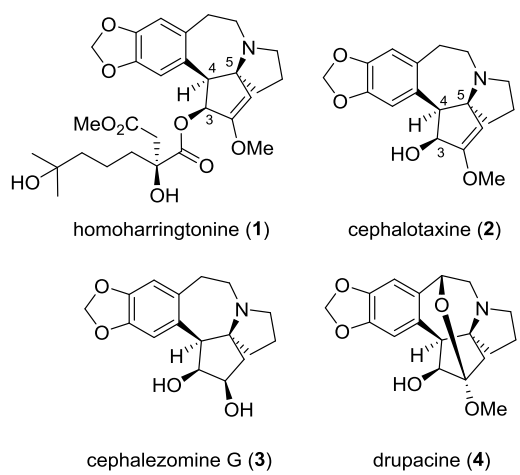


Figure 1. Representative examples of *Cephalotaxus* alkaloids (**1**–**4**)

Cephalotaxine **2**, the parent structure of homoharringtonine **1**, has a rare 1-azaspiro[4,4]nonane skeleton fused to a benzazepine. Various approaches have been reported for the synthesis of this novel structure.[4] The first total synthesis of cephalotaxine was achieved by Weinreb and Semmelhack in 1972.[4a,4b] Since then,

over thirty racemic, formal, or enantioselective syntheses have been reported. However, the efficient asymmetric construction of the quaternary carbon center in 1-azaspiro[4,4]nonane has proven to be one of the most intractable tasks in total synthesis.[5] Mori et al. showed that the azaspirocycle could be constructed from D-(+)-proline using the procedure developed by Seebach.[4f] Alternatively, Tietze et al. utilized asymmetric reduction using a CBS catalyst and Pd-catalyzed spiro-cyclization.[4g] However, the substrate-controlled asymmetric synthesis of cephalotaxine without using an external chiral source has not been extensively studied.

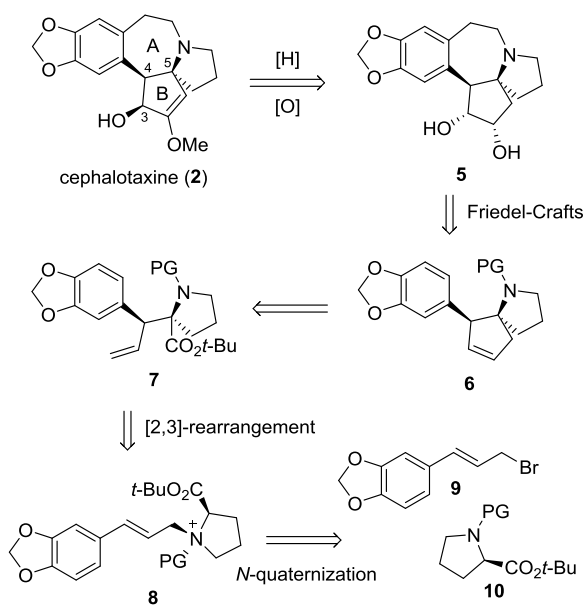
A number of strategies have been proposed for the asymmetric synthesis of quaternary proline derivatives.[6] We focused on two of these approaches for the introduction of an asymmetric Cα-quaternary center into proline derivatives without using an external chiral source: self-reproduction of chirality[7] and C to N to C chirality transfer.[8] The self-reproduction of chirality approach has been thoroughly researched by Seebach et al. It is a common approach to introducing a new chiral quaternary carbon center, through the formation of an additional ring. Using this method, the original chirality of an optically active proline could be transferred to an adjacent carbon center, then transferred back to the Cα-center to reproduce a chiral Cα-quaternary center. C to N to C chirality transfer is a similar approach. This method could be used to introduce a new chiral Cα-quaternary

center through the indigenous nitrogen atom in proline, rather than though an external atom. This methodology may be an alternative or complementary to the self-reproduction of chirality approach. However, it has not yet been investigated as thoroughly.

West et al. investigated C to N to C chirality transfer using *N*-benzyl proline ester.[8b] They found that direct *N*-quaternization (C to N chirality transfer) was achieved in an approximately 4:1 diastereomeric ratio, using alkylating reagents such as methyl iodide or prenyl bromide. Structural determination using X-ray crystallography suggested that the alkyl halide approached from the same face as the ester group, because of a preferred *N*-pyramidal form in which the benzyl and carbomethoxy groups are *trans*-disposed. Although the resultant diastereomeric mixture was purified by repetitive recrystallization, the low degree of C to N chirality transfer encouraged us to improve this methodology and apply it to the synthesis of cephalotaxine **2**.

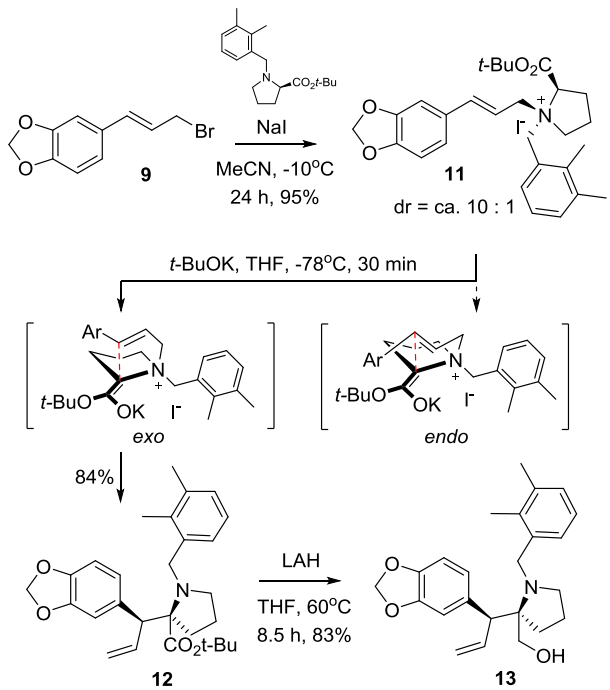
II. Results and Discussion

As shown in Scheme 1, we anticipated that the diol **5** (Mori's intermediate)[4f] could be obtained from olefin **6** through facial selective dihydroxylation and an acid catalyzed Friedel-Crafts reaction. Olefin **6** could be synthesized from *t*-butyl ester **7** by B-ring construction via the addition of a vinyl group and ring closing metathesis (RCM). We proposed that the stereoselective preparation of **7** could be accomplished by [2,3]-Stevens rearrangement of ammonium salt **8**. This salt could be generated in a diastereoselective manner through the *N*-quaternization of cinnamyl bromide **9** and *N*-protected D-proline ester **10** using C to N to C chirality transfer.



Scheme 1. Retrosynthetic analysis of cephalotaxine (**2**)

To achieve a high degree of C to N chirality transfer, we hypothesized that a bulky *N*-substituted proline ester would improve the diastereomeric ratio of the *N*-quaternization step, because of the increased proportion of the preferred invertomer. Substituted *N*-benzyl auxiliaries deemed to be the most suitable, because they can be easily removed by various common methods.

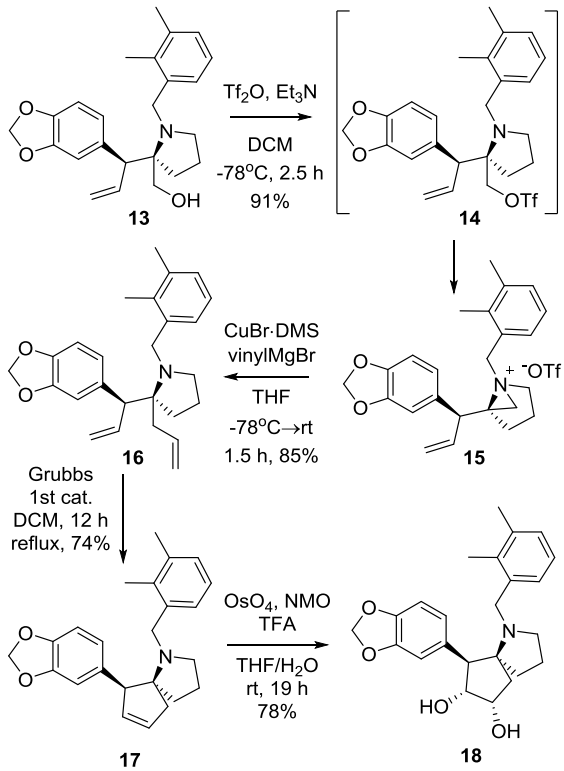


Scheme 2. *N*-Quaternization and transition state of [2,3]-Stevens rearrangement

We screened a variety of substituted *N*-benzyl groups to improve the diastereomeric ratio of the *N*-quaternization step. After optimizing the reaction conditions (data not shown), ammonium iodide salt **11** was obtained in 84% yield with a diastereomeric ratio of 10:1 (Scheme 2). This reaction was conducted using

2,3-methylenedioxy cinnamyl bromide (1 equiv.), 2,3-dimethylbenzyl D-proline *t*-butyl ester (1 equiv.), and sodium iodide (1.5 equiv.) in acetonitrile (1 M) at −10℃ for 24 h. We hypothesized that the two methyl substituents of the *N*-benzyl group increased the proportion of the *trans*-configured invertomer of the proline ester.

The optimal reaction conditions for the [2,3]-Stevens rearrangement of the *N*-allylic ammonium salt **11** of the proline ester were found to be *t*-BuOK in THF at −78℃, as in similar reactions.[8b,8c] Under these optimized reaction conditions, the ammonium salt **11** gave γ,δ-unsaturated α-quaternary proline *t*-butyl ester **12** in 84% yield. This reaction was almost isomerically pure. The products of other possible rearrangements, such as the Sommelet–Hauser[9] and [1,2]-Stevens[10] rearrangements, were not detected in the reaction mixture. The relative stereochemistry of the rearrangement product **12** was determined by considering the transition state. When *t*-BuOK was added to the ammonium salt **11**, the enolate (with arbitrary enolate geometry) could be generated via either an *endo* or *exo* transition state, depending on the location of the cinnamyl group. Because of the unfavorable steric interaction between the *t*-butyl ester and the aromatic ring in the *endo* transition state, the [2,3]-rearrangement via an *exo* transition state may be favored. The *t*-butyl ester **12** was easily reduced to the corresponding *neo*-pentyl alcohol **13**.



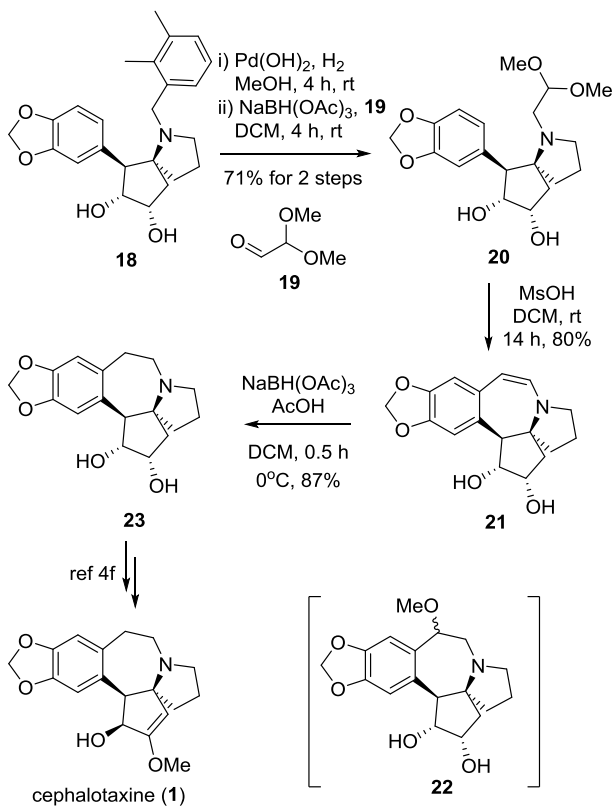
Scheme 3. *N*-Quaternization and transition state of [2,3]-Stevens rearrangement

Scheme 3 shows the synthesis of the B-ring of the cephalotaxine. Initially, we attempted to determine the optimal alcoholic activation conditions for the addition of a vinyl group. We screened halogenating reagents such as iodine, CBr_4, and PBr_3. However, we failed to obtain a good yield of a halogenated product. When *neo*-pentyl alcohol **13** was treated with trifluoromethanesulfonic anhydride and trimethylamine in DCM at −78℃, aziridinium **15** was afforded as a triflate salt. We proposed that the adjacent tertiary alkyl amine may attack the *neo*-pentyl carbon center immediately after the formation of triflate **14**. The relative

stereochemistry of **14** was determined by X-ray crystallography (see SI), which indicated that the [2,3]-rearrangement occurred via an *exo* transition state. Vinyl cuprate, generated *in situ* from a copper(I) bromide–dimethyl sulfide complex and magnesium vinyl bromide, was added to the aziridinium **15** in THF to give diene **16** in 85% yield. In contrast, addition of the aziridinium **15** to vinyl cuprate only produced the brominated product with moderate yield. The subsequent RCM reaction of **16** was catalyzed by Grubbs-II catalyst (5 mol%) and proceeded in dichloromethane or toluene to give the olefin **17** and an inseparable side product. Grubbs-I catalyst (20 mol%) was more effective; producing olefin **17** with a trace amount of an unidentified side product. Smaller amounts of Grubbs-I catalyst and lower temperatures both prevented completion of the RCM reaction. Treatment of **17** with osmium tetroxide, in the presence of *N*-methylmorpholine *N*-oxide (NMO) and trifluoroacetic acid, formed diol **18**. Without trifluoroacetic acid diol **18** was obtained with very low yield.

Prior to installing the A-ring via an acid-catalyzed Friedel-Crafts reaction, debenzylation of **18** was performed using $Pd(OH)_2$ and H_2 gas, followed by reductive amination with 2,2-dimethoxyacetaldehyde **19** and sodium triacetoxyborohydride to yield the acetal **20** in 71% yield in two steps (Scheme 4). After determining the optimal conditions for the acid-catalyzed Friedel-Crafts reaction, the reaction of **20** with an excess of methanesulfonic acid in

dichloromethane at room temperature afforded the enamine **21** in 87% yield. Enamine **21** may be formed through the benzyl methyl ether **22** intermediate (not isolated). Reduction of enamine **21** was performed with $NaBH(OAc)_3$ in acidic conditions to give the diol **23** (Mori's intermediate),[4f] which enabled the completion of the formal synthesis of cephalotaxine. Additionally, diol **23** could be directly synthesized from acetal **20** with 82% yield in a one-pot reaction using methanesulfonic acid and a borane *t*-butylamine complex.[11]



Scheme 4. Completion of the formal synthesis of cephalotaxine

III. Conclusion

We have developed a concise route for the formal synthesis of cephalotaxine. C to N to C chirality transfer and [2,3]-Stevens rearrangement were used to establish the main stereochemistry of cephalotaxine. To increase the degree of C to N chirality transfer, 2,3-dimethyl-*N*-benzyl proline ester was used as an *N*-quaternization substrate, which increased the proportion of the favored invertomer of the *N*-substituted proline ester. B-ring formation was achieved by vinyl addition and RCM, followed by facial selective dihydroxylation. Two-carbon insertion for A-ring formation was achieved by debenzylation and reductive amination. Finally, the formal synthesis of cephalotaxine was accomplished via an acid-catalyzed Friedel-Crafts reaction. This study demonstrates the successful synthesis of a natural product in a substrate-controlled manner without an external chiral source.

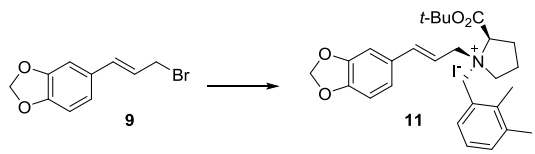
IV. Experimental

IV-1. General.

All chemicals were of reagent grade and used as received. All reactions were performed under an inert atmosphere of dry nitrogen using distilled dry solvents. Reactions were monitored by TLC analysis using silica gel 60 F-254 thin layer chromatography plates. Flash column chromatography was carried out on silica gel (230-400 mesh). Optical rotations were measured using sodium light (D line 589.3 nm). ^{1}H NMR (800 MHz) and ^{13}C NMR (200 MHz) spectra were recorded in δ units relative to the non-deuterated solvent as the internal reference. IR spectra were measured on a Fourier Transform Infrared spectrometer. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded using fast atom bombardment (FAB).

IV-2. Experimental procedure and spectroscopic data analysis

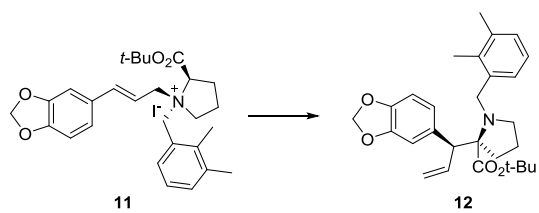
(1*S*,2*R*)-1-((*E*)-3-(benzo[*d*][1,3]dioxol-5-yl)allyl)-2-(*tert*-butoxycarbonyl)-1-(2,3-dimethylbenzyl)pyrrolidin-1-ium iodide (11).



To a solution of 2,3-methylenedioxy cinnamyl bromide **9** (10.0 g, 41.5 mmol) in MeCN (41 mL) was added 2,3-dimethylbenzyl proline *t*-butyl ester (12.0 g, 41.5 mmol) and sodium iodide (9.3 g, 62.3 mmol). This reaction mixture was stirred vigorously at –10℃ for 24 h. The reaction mixture was concentrated *in vacuo*, followed by purification using silica gel column chromatography (CH_2Cl_2/MeOH, 30:1) to afford quaternary ammonium salt **11** (22.8 g, 95%) as a light yellow wax. ^{1}H NMR (**Major diastereomer**, $CDCl_3$, 800 MHz) δ 7.35 (d, *J* = 7.7 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.81 (d, *J* = 15.5 Hz, 1H), 6.72 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.70 (d, *J* = 1.5 Hz, 1H), 6.63 (d, *J* = 8.0 Hz, 1H), 5.86 (s, 2H), 5.56 (ddd, *J* = 15.5, 8.9, 6.6 Hz, 1H), 5.23 (d, *J* = 13.4 Hz, 1H), 5.13 (d, *J* = 13.4 Hz, 1H), 4.93 (t, *J* = 10.2 Hz, 1H), 4.30 (dd, *J* = 13.5, 6.4 Hz, 1H), 4.08–4.12 (m, 1H), 3.74–3.76 (m, 1H), 2.51–2.56 (m, 1H), 2.31–2.35 (m, 1H), 2.31 (s, 3H), 2.23 (s, 3H), 2.13–2.18 (m, 1H), 2.01–2.07 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 165.3, 148.4, 148.0, 141.7, 138.8, 138.2, 132.3, 131.1, 128.8, 128.8, 126.4,

126.2, 122.5, 112.8, 108.2, 105.6, 101.2, 85.5, 77.2, 71.4, 63.0, 60.0, 59.8, 27.8, 25.0, 20.8, 19.3, 16.8; IR (neat) υ_{max} = 2974, 2901, 1727, 1645, 1488, 1444, 1368, 1249, 1149, 1034, 929, 747 cm^{-1}; $[\alpha]_D$ = –9.06 (c = 1.0; $CHCl_3$); HRMS (FAB): calcd. for $C_{28}H_{36}NO_4$ ($[M]^+$) 450.2644, found 450.2644.

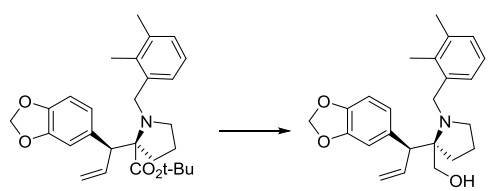
tert-butyl (_R_)-2-((_S_)-1-(benzo[_d_][1,3]dioxol-5-yl)allyl)-1-(2,3-dimethylbenzyl) pyrrolidine-2-carboxylate (12).



Anhydrous THF (346 mL) was added to the ammonium iodide **11** (10 g, 17.3 mmol) and cooled to -78 ℃. *t*-BuOK in 1 M THF (26 mL, 26.0 mmol) was added dropwise to the reaction mixture. After stirring for 1 h at -78 ℃, the reaction mixture was quenched with aqueous NH_4Cl solution and warmed to room temperature. The mixture was extracted twice with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 20:1) to afford *t*-butyl ester **12** (6.5 g, 84%) as a colorless oil. ^1H NMR ($CDCl_3$, 800 MHz) δ 7.32 (d, *J* = 7.6 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 1H), 6.78 (d, *J* = 1.4 Hz, 1H), 6.73 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.37 (ddd, *J* =

17.1, 10.7, 6.2 Hz, 1H), 5.14 (td, *J* = 1.6, 10.5 Hz, 1H), 4.85 (td, *J* = 1.6, 17.3 Hz, 1H), 4.19 (d, *J* = 14.6 Hz, 1H), 4.05 (d, *J* = 5.9 Hz, 1H), 3.48 (d, *J* = 14.5 Hz, 1H), 3.01 (dt, *J* = 8.8, 2.6 Hz, 1H), 2.29–2.34 (m, 2H), 2.30 (s, 3H), 2.22 (s, 3H), 2.04–2.08 (m, 1H), 1.78–1.83 (m, 1H), 1.68–1.74 (m, 1H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 170.7, 147.1, 145.9, 138.4, 137.7, 136.4, 134.4, 134.4, 138.4, 137.7, 136.4, 134.4, 134.4, 128.0, 126.1, 125.2, 123.1, 117.1, 110.1, 107.7, 100.7, 80.9, 74.4, 51.0, 50.7, 30.3, 28.2, 21.6, 20.5, 14.7; IR (neat) υ_{max} = 2970, 2924, 1710, 1487, 1440, 1365, 1230, 1154, 1039, 914, 753 cm^{-1}; $[\alpha]_D$ = +50.35 (c = 1.0; $CHCl_3$); HRMS (FAB): calcd. for $C_{28}H_{36}NO_4$ ($[M+H]^+$) 450.2644, found 450.2637.

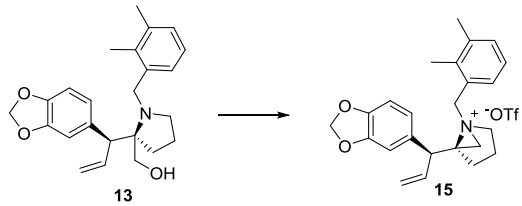
((*R*)-2-((*S*)-1-(benzo[d][1,3]dioxol-5-yl)allyl)-1-(2,3-dimethylbenzyl) pyrrolidin-2-yl)methanol (13).



To a solution of *t*-butyl ester **12** (5 g, 11.1 mmol) in THF (56 mL) was added lithium aluminum hydride in 1 M THF (16.7 mL, 16.7 mmol). This reaction mixture was stirred for 8.5 h at 60℃. After the reaction mixture was cooled to 0℃, H_2O (2 mL), 1 N NaOH (2 mL), and H_2O (6 mL) were added to the pot dropwise, subsequently. The mixture was warmed to room temperature and stirred for 30 min,

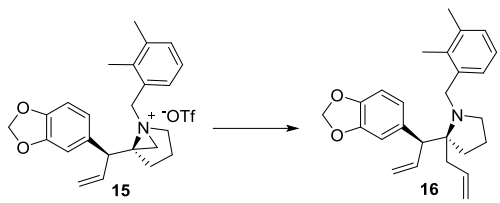
filtered by celite and washed with EtOAc. The filterate was concentrated in vacuo, followed by purification using silica gel column chromatography (hexane/EtOAc 3:1) to give *neo*-pentyl alcohol **10** (3.8 g, 90%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 800 MHz) δ 7.19-7.20 (m, 1H), 7.08–7.11 (m, 2H), 6.79 (d, *J* = 1.7 Hz, 1H), 6.75 (d, *J* = 7.9 Hz, 1H), 6.72 (dd, *J* = 8.0, 1.7 Hz, 1H), 6.37 (ddd, *J* = 17.3, 10.2, 7.2 Hz, 1H), 5.93 (s, 2H), 5.24 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.10 (d, *J* = 17.1 Hz, 1H), 4.03 (d, *J* = 13.3 Hz, 1H), 3.96 (d, *J* = 13.3 Hz, 1H), 3.81 (d, *J* = 7.4 Hz, 1H), 3.39 (d, *J* = 10.7 Hz, 1H), 3.14 (d, *J* = 10.7 Hz, 1H), 2.91–2.94 (m, 1H), 2.86 (br s, 1H), 2.83 (dt, *J* = 3.6, 8.2 Hz, 1H), 2.36–2.40 (m, 1H), 2.31 (s, 3H), 2.27 (s, 3H), 1.84 (ddd, *J* = 13.4, 9.6, 4.1 Hz, 1H), 1.72–1.78 (m, 1H), 1.67–1.73 (m, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 147.5, 146.2, 139.3, 137.5, 136.8, 134.9, 134.5, 128.5, 127.2, 125.3, 122.2, 117.2, 109.1, 108.0, 100.8, 68.7, 65.4, 53.5, 52.0, 48.7, 30.8, 23.1, 20.6, 14.8; IR (neat) υ_{max} = 3422, 2943, 2875, 1502, 1484, 1439, 1231, 1037, 917, 750 cm^{-1}; $[\alpha]_D$ = +53.52 (c = 1.0; $CHCl_3$); HRMS (FAB): calcd. for $C_{24}H_{30}NO_3$ ($[M+H]^+$) 380.2226, found 380.2219.

(1*S*,5*R*)-5-((*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)allyl)-1-(2,3-dimethylbenzyl)-1-azabicyclo[3.1.0]hexan-1-ium trifluoromethanesulfonate (15).



To a solution of *neo*-pentyl alcohol **13** (3.0 g, 7.9 mmol) in CH_2Cl_2 (158 mL) was added triethylamine (2.2 mL, 15.8 mmol) and trifluoromethanesulfonic anhydride (2.4 mL, 15.8 mmol) dropwise at -78℃. This reaction mixture was stirred for 2.5 h at -78℃ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 15:1) to afford aziridinium salt **15** (3.7 g, 91%) as a white solid. ^{1}H NMR ($CDCl_3$:MeOD = 10:1, 800 MHz) δ 7.18 (d, *J* = 7.6 Hz, 1H), 7.14 (d, *J* = 7.4 Hz, 1H), 7.06–7.11 (m, 1H), 6.73–6.76 (m, 2H), 6.70–6.72 (m, 1H), 6.25–6.31 (m, 1H), 5.86 (s, 2H), 5.45 (d, *J* = 10.4 Hz, 1H), 5.36 (d, *J* = 17.1 Hz, 1H), 4.70 (d, *J* = 13.9 Hz, 1H), 4.33 (d, *J* = 13.9 Hz, 1H), 4.08 (d, *J* = 8.0 Hz, 1H), 3.45 (d, *J* = 4.4 Hz, 1H), 3.39 (dt, *J* = 8.1, 11.3 Hz, 1H), 3.14–3.17 (m, 1H), 3.10 (d, *J* = 4.6 Hz, 1H), 2.54–2.59 (m, 1H), 2.22 (s, 3H), 2.19 (s, 3H), 2.05–2.08 (m, 1H), 1.93–1.98 (m, 1H), 1.51–1.59 (m, 1H); ^{13}C NMR ($CDCl_3$:MeOD = 10:1, 200 MHz) δ 148.2, 147.3, 138.5, 135.9, 133.4, 131.7, 130.7, 128.4, 128.1, 126.5, 121.7, 120.9, 108.8, 108.3, 101.2, 66.5, 56.2, 56.0, 48.6, 42.2, 26.7, 20.5, 18.0, 15.7; IR (neat) υ_{max} = 2887, 1499, 1442, 1252, 1149, 1028, 935, 761 cm^{-1}; $[\alpha]_D$ = +8.68 (c = 1.0; DMSO); HRMS (FAB): calcd. for $C_{24}H_{28}NO_2$ ($[M]^+$) 362.2120, found 362.2126.

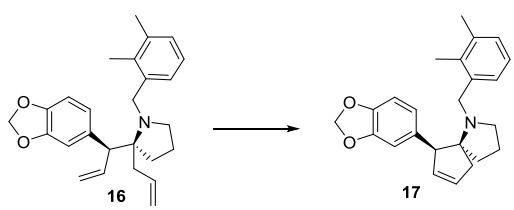
(*S*)-2-allyl-2-((*S*)-1-(benzo[*d*][1,3]dioxol-5-yl)allyl)-1-(2,3-dimethylbenzyl) pyrrolidine (16).



To a suspension of copper bromide dimethyl sulfide complex (3.6 g, 17.6 mmol) in THF (53 mL) at −78℃ was added vinylmagnesium bromide (1 M THF solution, 35.2 mL, 35.2 mmol). The suspension was stirred vigorously while the temperature was slowly warmed to −15℃ for 1 h. The stirring was stopped and the clear solution of vinyl cuprate solution (ca. 50 mL) was added to the suspension of aziridinium **15** (3.0 g, 5.9 mmol) in THF (12 mL) at −78℃ via cannula. The reaction pot was warmed to room temperature for 1.5 h and cooled to 0℃, quenched with saturated aqueous NH_4Cl solution and warmed to room temperature. The mixture was extracted twice with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 15:1) to afford diene **16** (2.0 g, 85%) as a light yellow oil. ^{1}H NMR ($CDCl_3$, 800 MHz) δ 7.39 (d, *J* = 7.6 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 1H), 6.88 (d, *J* = 1.6 Hz, 1H), 6.75 (dd, *J* = 7.9, 1.6 Hz, 1H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.34 (ddd, *J* = 17.3, 10.3, 7.1 Hz, 1H), 5.92 (dd, *J* = 8.2, 1.6 Hz, 1H), 5.74–5.80 (m, 1H), 5.07 (td, *J* = 10.5, 1.5 Hz, 1H), 5.00–

5.04 (m, 2H), 4.89 (td, *J* = 17.3, 1.6 Hz, 1H), 4.03 (d, *J* = 14.6 Hz, 1H), 3.73 (d, *J* = 14.5 Hz, 1H), 3.45 (d, *J* = 7.3 Hz, 1H), 2.97 (dt, *J* = 8.3, 3.1 Hz, 1H), 2.45–2.48 (m, 1H), 2.36 (dd, *J* = 14.0, 6.8 Hz, 1H), 2.29 (s, 3H), 2.25 (dd, *J* = 14.0, 7.6 Hz, 1H), 2.19 (s, 3H), 2.01–2.05 (m, 1H), 1.76–1.80 (m, 1H), 1.57–1.61 (m, 1H), 1.42–1.47 (m, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 147.2, 145.9, 140.0, 138.3, 136.4, 136.2, 136.1, 134.4, 127.9, 126.1, 125.2, 123.1, 117.3, 116.1, 110.0, 107.8, 100.7, 67.8, 57.0, 52.5, 50.4, 41.1, 33.0, 22.7, 20.6, 14.7; IR (neat) υ_{max} = 2969, 2905, 1485, 1440, 1232, 1039, 753 cm^{-1}; $[\alpha]_D$ = +38.35 (c = 1.0; $CHCl_3$); HRMS (FAB): calcd. for $C_{26}H_{32}NO_2$ ($[M+H]^+$) 390.2433, found 390.2430.

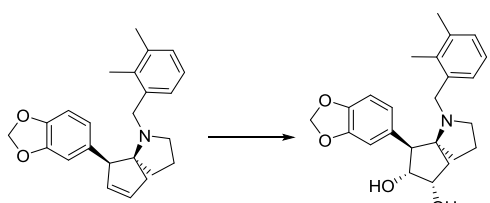
(5*S*,6*S*)-6-(benzo[*d*][1,3]dioxol-5-yl)-1-(2,3-dimethylbenzyl)-1-azaspiro[4.4] non-7-ene (17).



To a solution of diene **16** (1.5 g, 3.9 mmol) in CH_2Cl_2 (77 mL) was added 20 mol% Grubbs-I catalyst (642 mg, 0.78 mmol). The reaction mixture was refluxed for 12 h, cooled to room temperature, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc, 15:1) to give olefin **17** (1.0 g, 74%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 800 MHz) δ 6.88–6.91 (m, 2H),

6.79–6.80 (m, 1H), 6.70 (d, *J* = 1.6 Hz, 1H), 6.67 (d, *J* = 8.0, 1.6 Hz, 1H), 6.63 (d, *J* = 8.0 Hz, 1H), 6.00–6.02 (m, 1H), 5.82–5.84 (m, 1H), 5.80 (d, *J* = 1.6 Hz, 1H), 5.77 (d, *J* = 1.6 Hz, 1H), 3.69 (d, *J* = 1.6 Hz, 1H), 3.27 (d, *J* = 14.8 Hz, 1H), 3.08 (d, *J* = 14.8 Hz, 1H), 2.79–2.82 (m, 2H), 2.46–2.49 (m, 1H), 2.17 (s, 1H), 2.15–2.18 (m, 1H), 2.10–2.12 (m, 1H), 1.84–1.92 (m, 2H), 1.90 (s, 3H), 1.76–1.80 (m, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 147.3, 146.0, 138.6, 135.7, 134.8, 134.0, 133.2, 130.8, 127.4, 125.9, 124.7, 121.8, 109.8, 107.6, 100.6, 74.8, 58.9, 51.7, 51.3, 42.6, 38.6, 21.2, 20.4, 14.1; IR ($CHCl_3$) υ_{max} = 2879, 1482, 1438, 1227, 1037, 932, 756 cm^{-1}; $[\alpha]_D$ = –99.04 (c = 1.0; $CHCl_3$); HRMS (FAB): calcd. for $C_{24}H_{28}NO_2$ ($[M+H]^+$) 361.2042, found 361.2043.

(5*S*,6*S*,7*R*,8*S*)-6-(benzo[*d*][1,3]dioxol-5-yl)-1-(2,3-dimethylbenzyl)-1-azaspiro[4.4]nonane-7,8-diol (18).

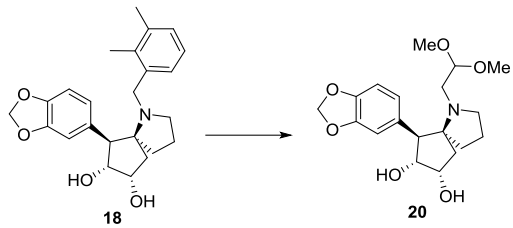


17 18

To a solution of olefin **17** (800 mg, 2.2 mmol) in THF (11 mL) was added trifluoroacetic acid (842 μL, 11 mmol) at 0℃. The reaction mixture was stirred at room temperature for 10 min before OsO_4 in H_2O (4 wt.%, 672 μL, 0.11 mmol) and *N*-methylmorpholine *N*-oxide (387 mg, 3.3 mmol) in H_2O (2 mL) were added.

After 19 h, the mixture was quenched with saturated aqueous NH_4Cl solution at 0℃ and warmed to room temperature, and stirred for 10 min. It was poured to water and extracted twice with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 1:1) to yield diol **18** (679 mg, 78%) as a light yellow oil. ^1H NMR ($CDCl_3$, 800 MHz) δ 6.94 (d, *J* = 7.4 Hz, 1H), 6.90 (t, *J* = 7.5 Hz, 1H), 6.84 (s, 1H), 6.76 (d, *J* = 7.8 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 5.85 (d, *J* = 1.7 Hz, 1H), 5.81 (d, *J* = 1.6 Hz, 1H), 4.44 (dd, *J* = 4.7, 11.4 Hz, 1H), 4.27–4.29 (m, 1H), 3.52 (d, *J* = 13.9 Hz, 1H), 3.36 (d, *J* = 13.9 Hz, 1H), 3.28 (d, *J* = 11.4 Hz, 1H), 2.66 (dt, *J* = 4.4, 9.0 Hz, 1H), 2.39 (dd, *J* = 5.6, 15.1 Hz, 1H), 2.23 (s, 1H), 2.16–2.21 (m, 1H), 2.12–2.16 (m, 1H), 2.03 (s, 3H), 1.75 (d, *J* = 15.1 Hz, 1H), 1.61–1.67 (m, 1H), 1.52–1.58 (m, 1H), xxx (d, *J* = 7.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 147.5, 146.1, 137.4, 135.9, 133.9, 131.5, 127.7, 126.2, 124.8, 122.0, 109.7, 107.9, 100.7, 76.4, 71.4, 71.0, 56.6, 52.4, 52.2, 42.0, 37.2, 21.4, 20.4, 14.4; IR (neat) υ_{max} = 3363, 2932, 2878, 1488, 1439, 1231, 1034, 926 cm^{-1}; $[\alpha]_D$ = +71.86 (c = 1.0; $CHCl_3$); HRMS (FAB): calcd. for $C_{24}H_{30}NO_4$ ($[M+H]^+$) 396.2175, found 396.2180.

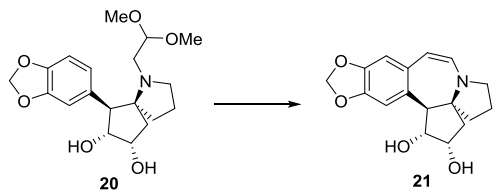
(5*S*,6*S*,7*R*,8*S*)-6-(benzo[*d*][1,3]dioxol-5-yl)-1-(2,2-dimethoxyethyl)-1-azaspiro[4.4]nonane-7,8-diol (20).



To a solution of diol **18** (500 mg, 1.3 mmol) in MeOH (7 mL) was added 20 wt.% $Pd(OH)_2$ (100 mg). The reaction mixture was stirred under H_2 balloon for 4 h and filtered by celite. The filterate was concentrated *in vacuo* to give the debenzylated product. To a solution of the crude mixture in DCM was added 2,2-dimethoxyacetaldehyde solution (60 wt.% in H_2O, 392 μL, 2.6 mmol) and $NaBH(OAc)_3$ (551 mg, 2.6 mmol). The mixture was stirred for 4 h at room temperature, poured to the saturated aqueous $NaHCO_3$, extracted twice with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 1:1) to yield acetal **20** (337 mg, 71% for 2 steps) as a colorless oil. ^{1}H NMR ($CDCl_3$, 800 MHz) δ 7.01 (d, *J* = 1.5 Hz, 1H), 6.76 (d, *J* = 8.0, 1.4 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 5.89 (d, *J* = 1.5 Hz, 1H), 5.88 (d, *J* = 1.5 Hz, 1H), 4.34–4.36 (m, 1H), 4.19–4.20 (m, 1H), 3.76 (t, *J* = 5.1 Hz, 1H), 3.26 (s, 3H), 3.24 (s, 3H), 3.04 (d, *J* = 11.6 Hz, 1H), 2.96 (dt, *J* = 8.6, 3.2 Hz, 1H), 2.78 (br s, 1H), 2.50 (br s, 1H), 2.33 (dd, *J* = 12.8, 5.3 Hz, 1H), 2.26 (td, *J* = 8.5, 8.5 Hz, 1H), 2.13 (dd, *J* = 15.2, 5.9 Hz, 1H), 1.96–1.99 (m, 1H), 1.86–1.89 (m, 1H), 1.69 (dd, *J* = 15.1, 1.9 Hz, 1H), 1.52–1.56 (m, 1H), 1.28–1.34 (m, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz)

δ 147.3, 146.1, 132.0, 122.3, 110.0, 107.6, 105.5, 100.7, 76.4, 71.6, 70.4, 57.5, 54.9 , 54.7, 54.2, 53.8, 41.2, 38.6, 22.2; IR (neat) υ_{max} = 3391, 2931, 2881, 1490, 1439, 1249, 1035, 928, 750 cm^{-1}; $[\alpha]_D$ = +68.81 (c = 0.35; $CHCl_3$); HRMS (FAB): calcd. for $C_{19}H_{28}NO_6$ ($[M+H]^+$) 366.1917, found 366.1921.

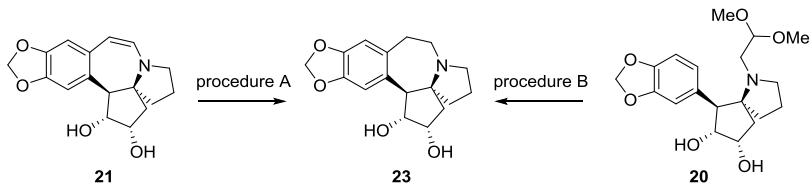
(11b*S*,12*R*,13*S*,14a*S*)-2,3,11b,12,13,14-hexahydro-1H-[1,3]dioxolo[4',5':4,5] benzo[1,2-*d*]cyclopenta[*b*]pyrrolo[1,2-*a*]azepine-12,13-diol (21).



To a solution of acetal **20** (150 mg, 0.41 mmol) in DCM (8 mL) was added methanesulfonic acid (666 μL, 10.3 mmol) at 0℃. The reaction mixture was stirred at room temperature for 14 h. The mixture was quenched with solid $NaHCO_3$, stirred at room temperature for 15 min. It was poured to water and extracted twice with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 1:1) to yield enamine **21** (99 mg, 80%) as a colorless oil. ^1H NMR ($CDCl_3$, 800 MHz) δ 6.66 (s, 1H), 6.54 (s, 1H), 5.93 (d, *J* = 8.2 Hz, 1H), 5.88 (d, *J* = 1.4 Hz, 1H), 5.85 (d, *J* = 1.4 Hz, 1H), 5.60 (d, *J* = 8.2 Hz, 1H), 4.22 (t, *J* = 4.0 Hz, 1H), 4.06 (dd, *J* = 3.9, 10.0 Hz, 1H), 3.11 (dt, *J* = 4.5, 9.3 Hz, 1H), 2.95 (d, *J* =

10.0 Hz, 1H), 2.87 (dt, *J* = 6.1, 9.9 Hz, 1H), 2.39 (br s, 1H), 2.25 (q, *J* = 10.5 Hz, 1H), 2.04–2.07 (m, 1H), 2.01 (dd, *J* = 4.8, 14.6 Hz, 1H), 1.88–1.94 (m, 1H), 1.80–1.86 (m, 1H), 1.79 (d, *J* = 14.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 146.3, 145.2, 136.5, 132.5, 131.5, 113.3, 111.8, 109.5, 100.8, 87.0, 72.7, 57.8, 50.3, 41.2, 38.3, 21.1; IR (neat) υ_{max} = 3368, 2918, 1634, 1483, 1232, 1034, 746 cm^{-1}; $[\alpha]_D$ = –208.20 (c = 1.0; $CHCl_3$); HRMS (FAB): calcd. for $C_{17}H_{20}NO_4$ ($[M+H]^+$) 302.1392, found 302.1398.

(11b*S*,12*R*,13*S*,14a*S*)-2,3,5,6,11b,12,13,14-octahydro-1*H*-[1,3]dioxolo [4',5':4,5]benzo[1,2-*d*]cyclopenta[*b*]pyrrolo[1,2-*a*]azepine-12,13-diol (23).



Procedure A: To a solution of enamine **21** (50 mg, 0.17 mmol) in DCM (3 mL) was added acetic acid (28 μL, 0.51 mmol) and $NaBH(OAc)_3$ (90 mg, 0.51 mmol) at 0^{o}C. After the reaction mixture was stirred for 0.5 h, it was poured to water and extracted twice with EtOAc and DCM, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 8:1) to yield diol **23** (45 mg, 87%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 800 MHz) δ 6.70 (s, 1H), 6.66 (s, 1H), 5.87 (d, *J* = 1.3 Hz, 1H), 5.86 (d, *J* = 1.3 Hz, 1H), 4.27 (dd, *J* = 4.1, 9.8 Hz, 1H), 4.16 (t, *J* =

4.1 Hz, 1H), 3.10 (d, *J* = 9.8 Hz, 1H), 3.01–3.06 (m, 1H), 2.91–2.96 (m, 2H), 2.64–2.68 (m, 1H), 2.58 (dd, *J* = 6.8, 14.8 Hz, 1H), 2.44–2.48 (m, 1H), 2.41–2.44 (m, 1H), 2.08 (dd, *J* = 4.0, 14.2 Hz, 1H), 5.87 (d, *J* = 1.3 Hz, 1H), 1.71–1.78 (m, 1H), 1.66–1.71 (m, 1H), 1.65 (d, *J* = 14.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ; 148.7, 148.4, 134.4, 132.2, 114.2, 112.3, 102.9, 80.2, 74.0, 60.9, 55.2, 50.6, 49.4, 44.7, 33.2, 32.2, 20.6; IR (neat) υ_{max} = 3340, 2942, 1502, 1485 cm^{-1}; $[\alpha]_D$ = –11.61 (c = 0.5; MeOH); HRMS (FAB): calcd. for $C_{17}H_{22}NO_4$ ($[M+H]^+$) 304.1549, found 304.1554.

Procedure B: To a solution of acetal **20** (50 mg, 0.14 mmol) in DCM (3 mL) was added methanesulfonic acid (227 μL, 3.5 mmol) at 0℃. The reaction mixture was stirred at room temperature for 14 h. Borane *t*-butylamine complex (37 mg, 0.42 mmol) was added to the reaction pot, stirred for 15 min at room temperature. It was poured to water and extracted twice with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) to yield diol **23** (36 mg, 82%) as a colorless oil.

- **X ray Crystallographic data for *ent*-15**

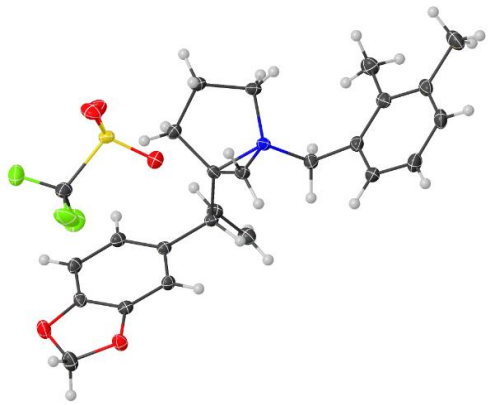


Figure S1. X-ray crysatallographic structure of ***ent*-15**

```
Bond precision:  C-C = 0.0030 A          Wavelength=1.54184

Cell:           a=8.50693(7)    b=10.99492(9)       c=13.44063(11)
                alpha=90        beta=105.0772(8)    gamma=90
Temperature:    100 K

                Calculated                  Reported
Volume          1213.867(18)                1213.867(18)
Space group     P 21                        P 1 21 1
Hall group      P 2yb                       P 2yb
Moiety formula  C24 H28 N O2, C F3 O3 S     C F3 O3 S, C24 H28 N O2
Sum formula     C25 H28 F3 N O5 S           C25 H28 F3 N O5 S
Mr              511.54                      511.54
Dx,g cm-3       1.400                       1.400
Z               2                           2
Mu (mm-1)       1.717                       1.717
F000            536.0                       536.0
F000'           538.50
h,k,lmax        10,13,16                    10,13,16
Nref            5103[ 2689]                 4968
Tmin,Tmax       0.745,0.966                 0.759,1.000
Tmin'           0.676

Correction method= # Reported T Limits: Tmin=0.759 Tmax=1.000
AbsCorr = MULTI-SCAN

Data completeness= 1.85/0.97      Theta(max)= 76.362

R(reflections)= 0.0276( 4933)     wR2(reflections)= 0.0734( 4968)

S = 1.035                 Npar= 318
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V. References

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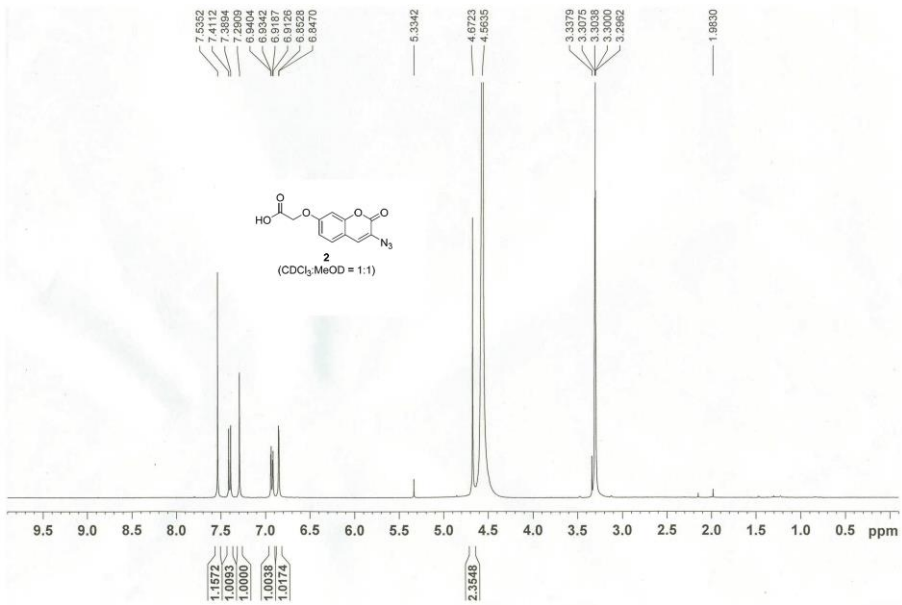
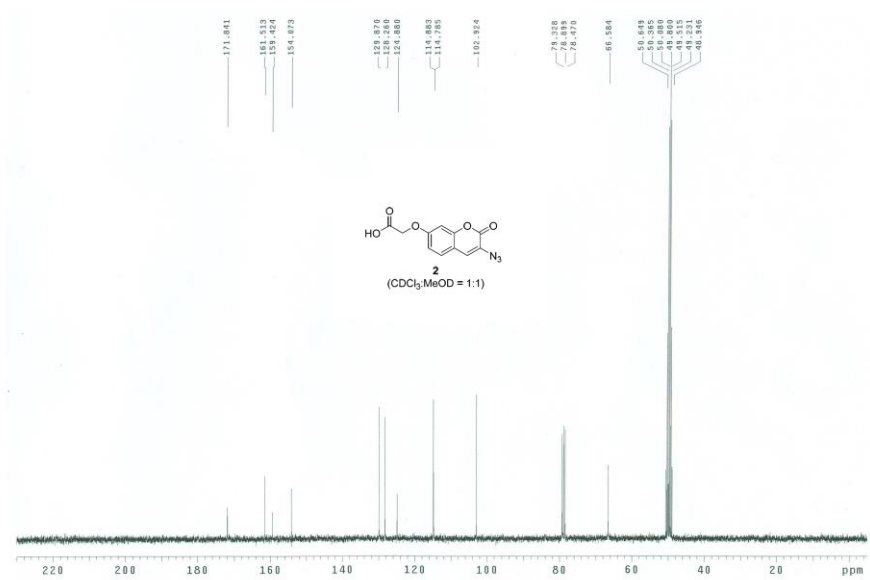
Appendix I

Spectra of Compounds

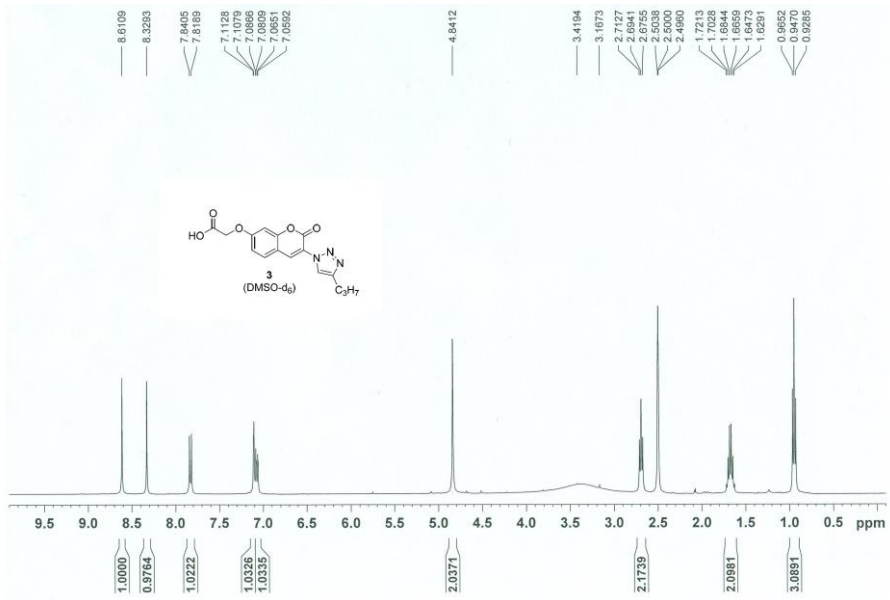
(Part A)

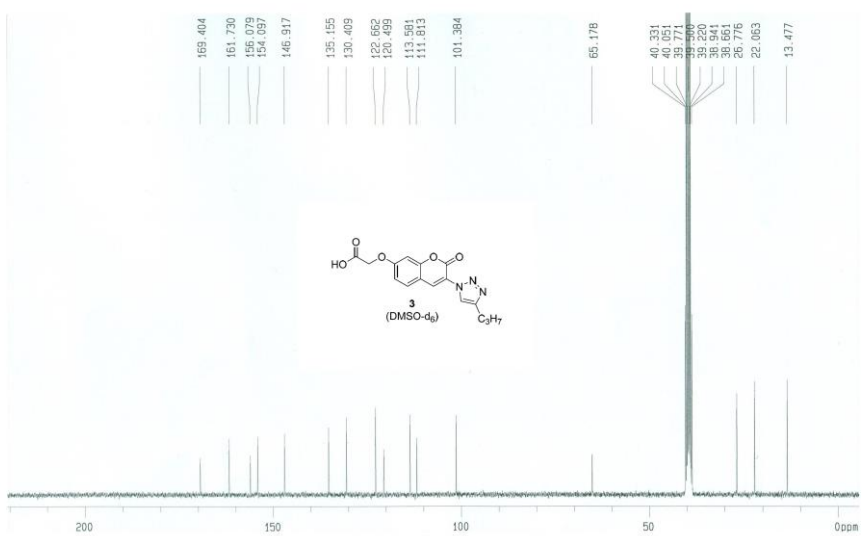
^{1}H NMR and ^{13}C NMR Spectra of 2





^{1}H NMR and ^{13}C NMR Spectra of 3





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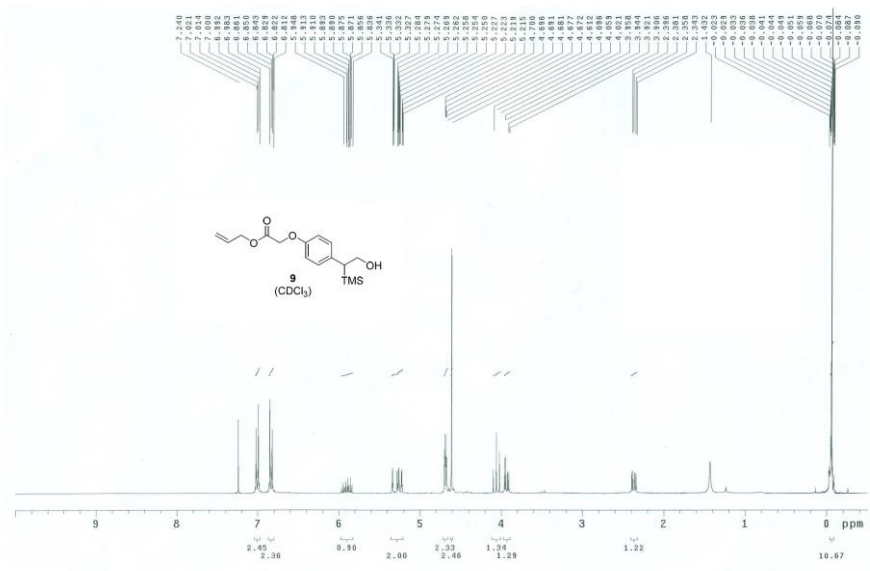

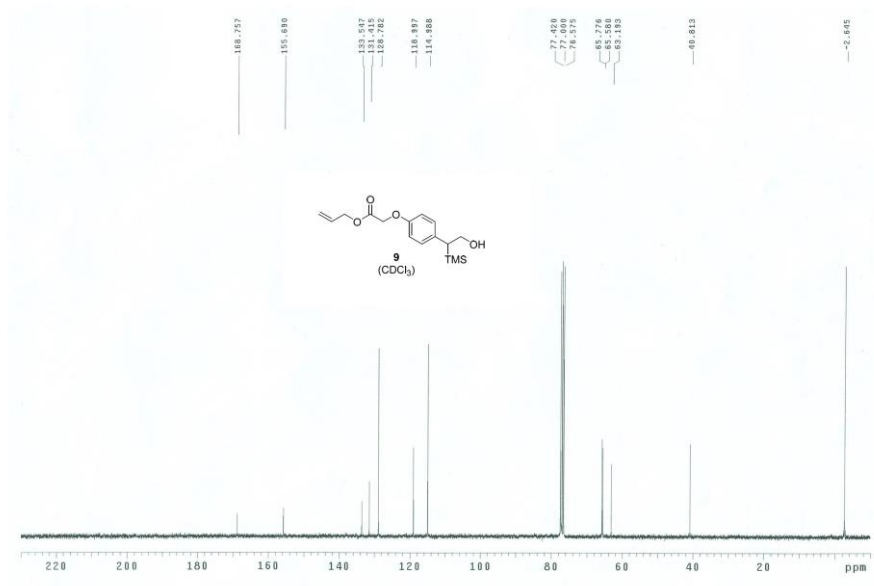

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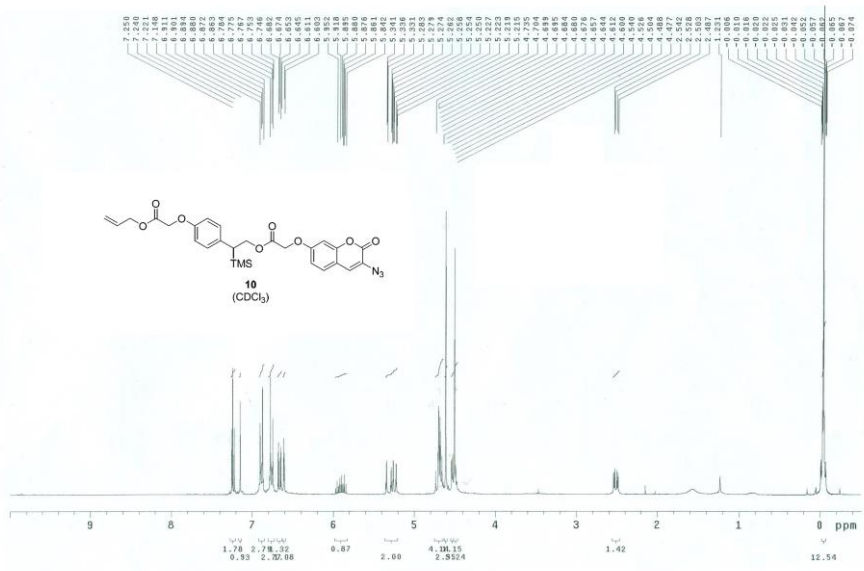

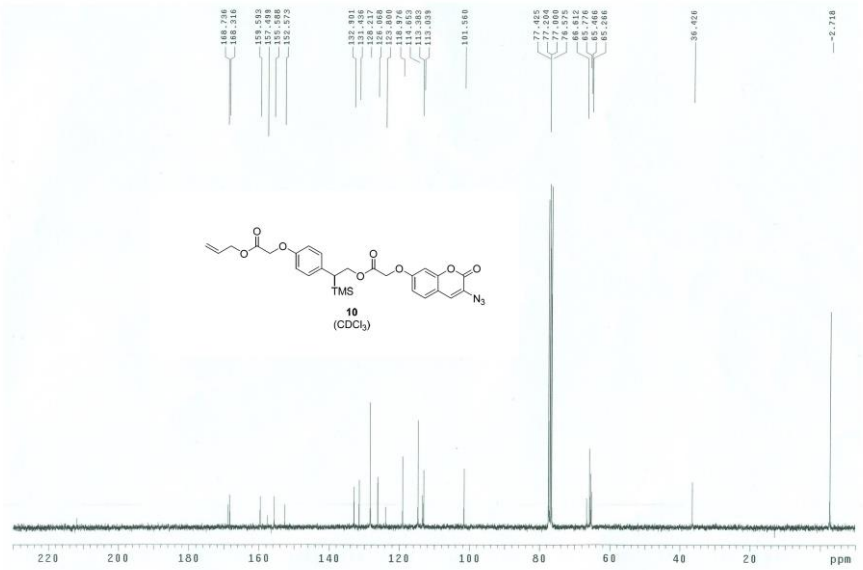

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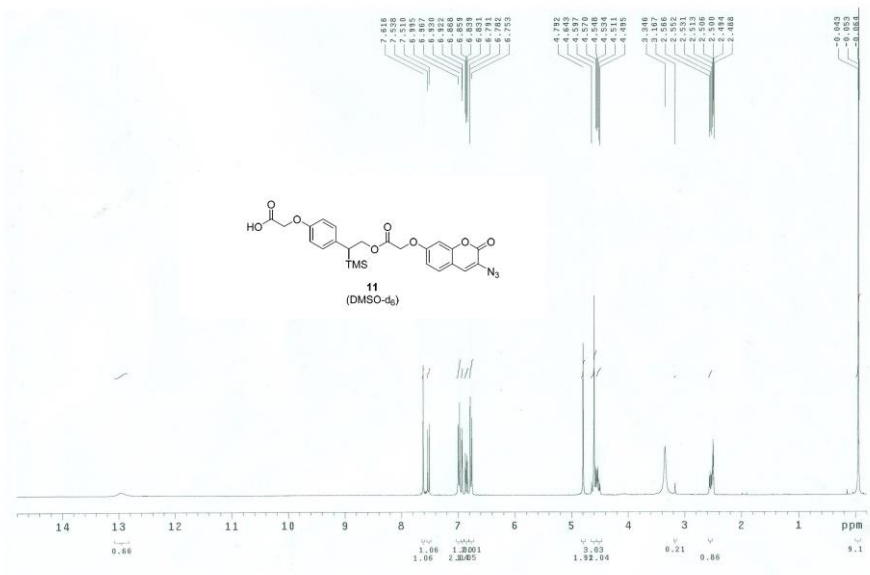
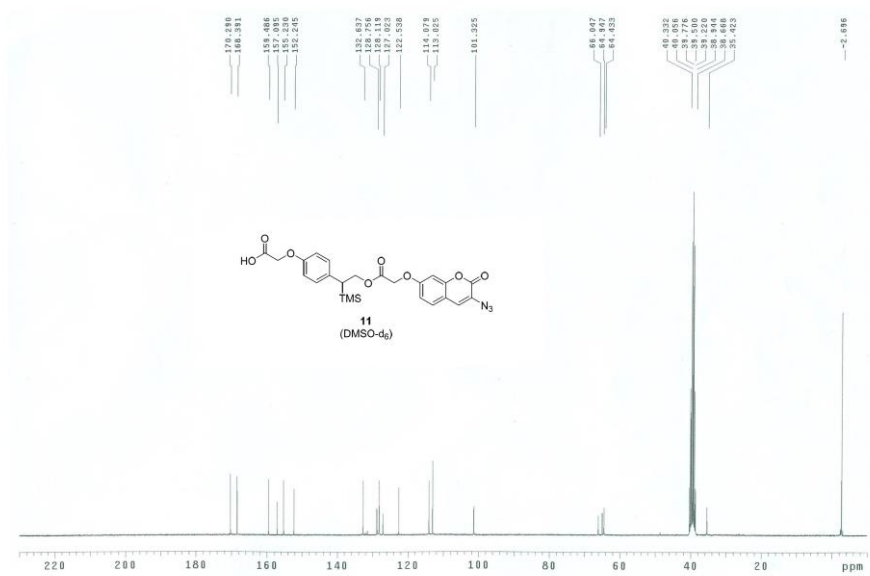
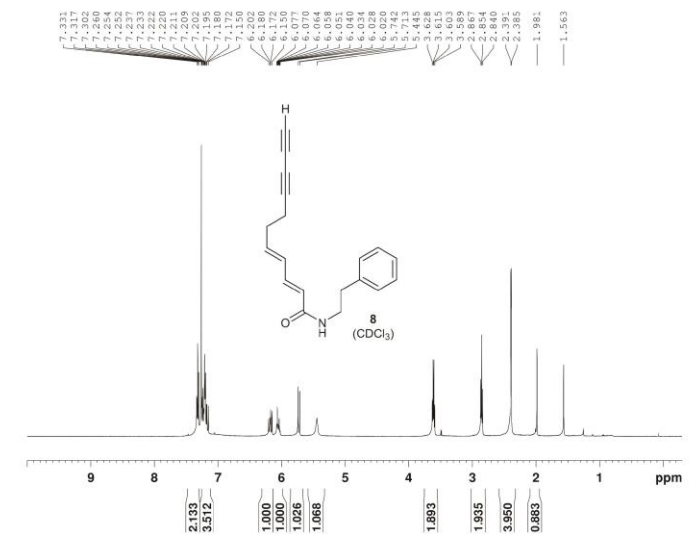




^{1}H NMR and ^{13}C NMR Spectra of 8



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SEOUL NATIONAL UNIVERSITY
기초과학공동기기원
핵자기공명연구실

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PROCNO                  1

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TE                298.0 K
D1              1.00000000 se
TD0                     1

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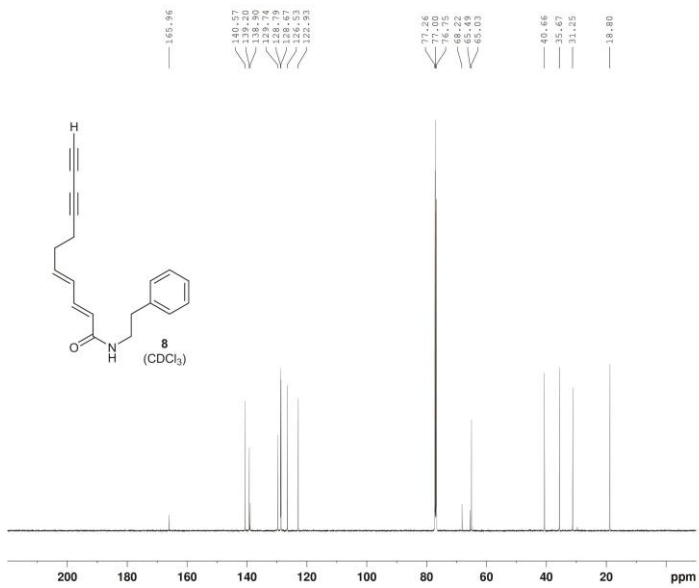

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기초과학공동기기원
핵자기공명연구실

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D1              2.00000000 sec
D11             0.03000000 sec
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CPDPRG[2            waltz16
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PLW2           19.00000000 W
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Appendix II

Spectra of Compounds

(Part B)

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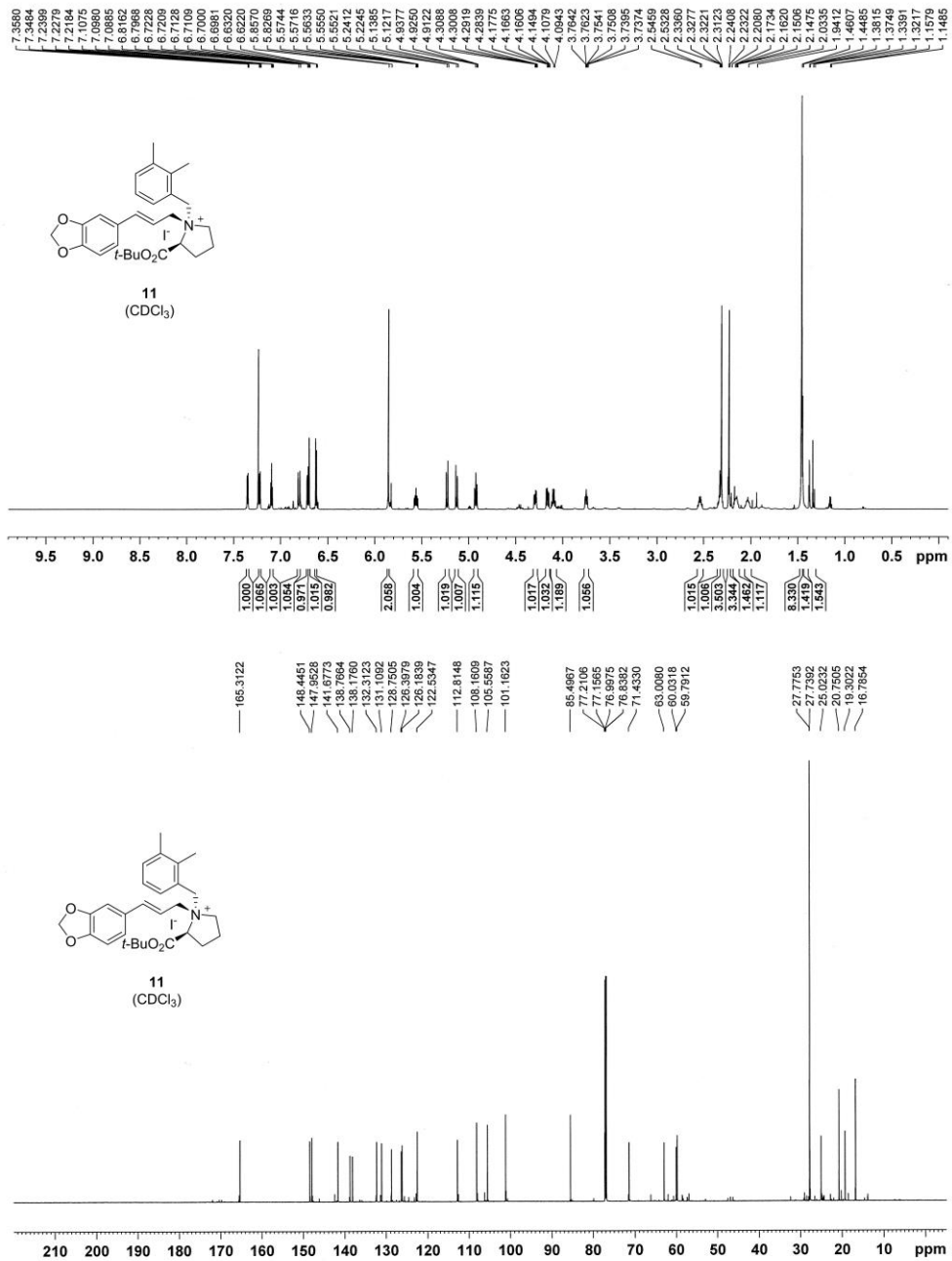
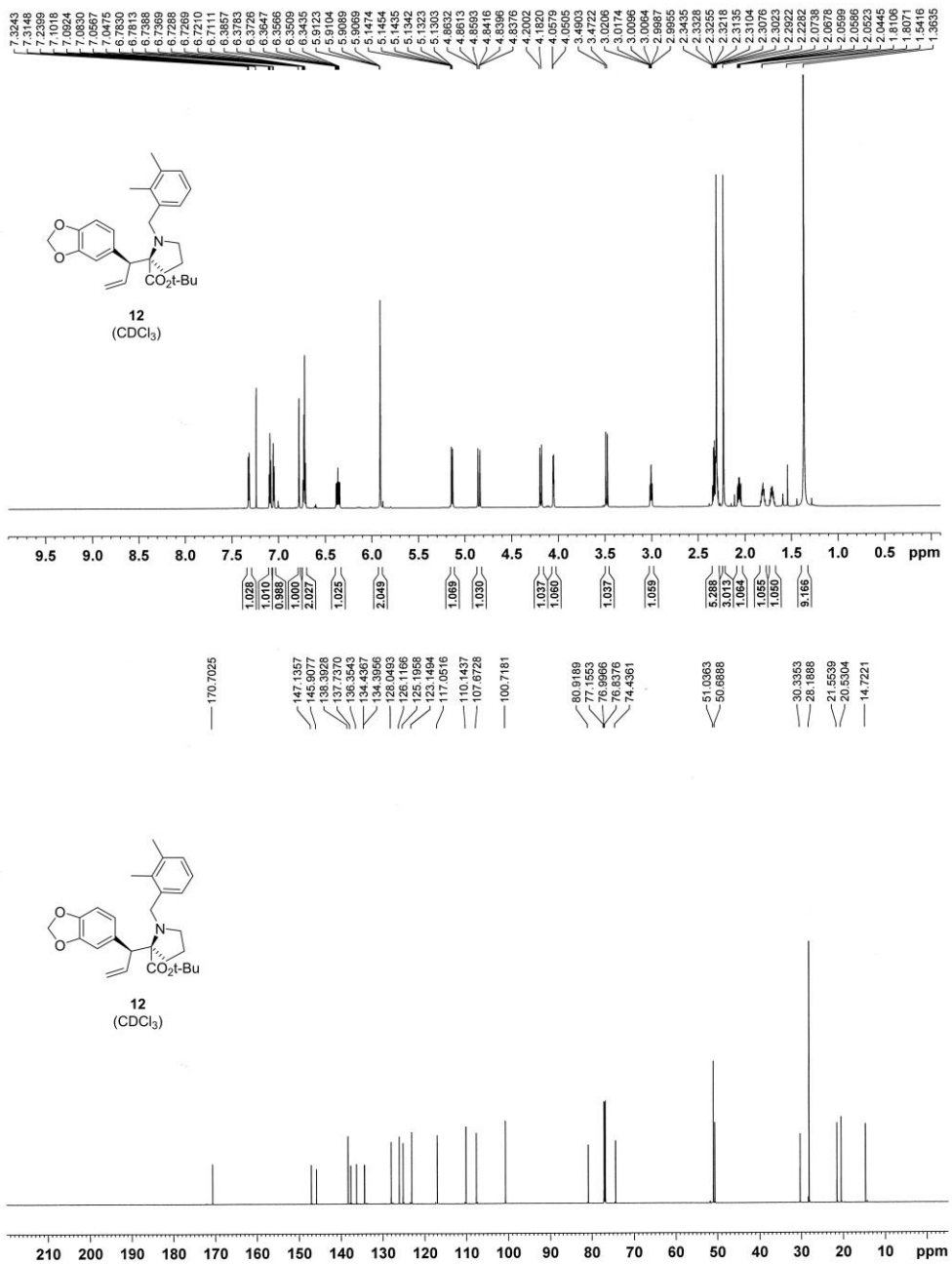


^{1}H NMR and ^{13}C NMR Spectra of 12



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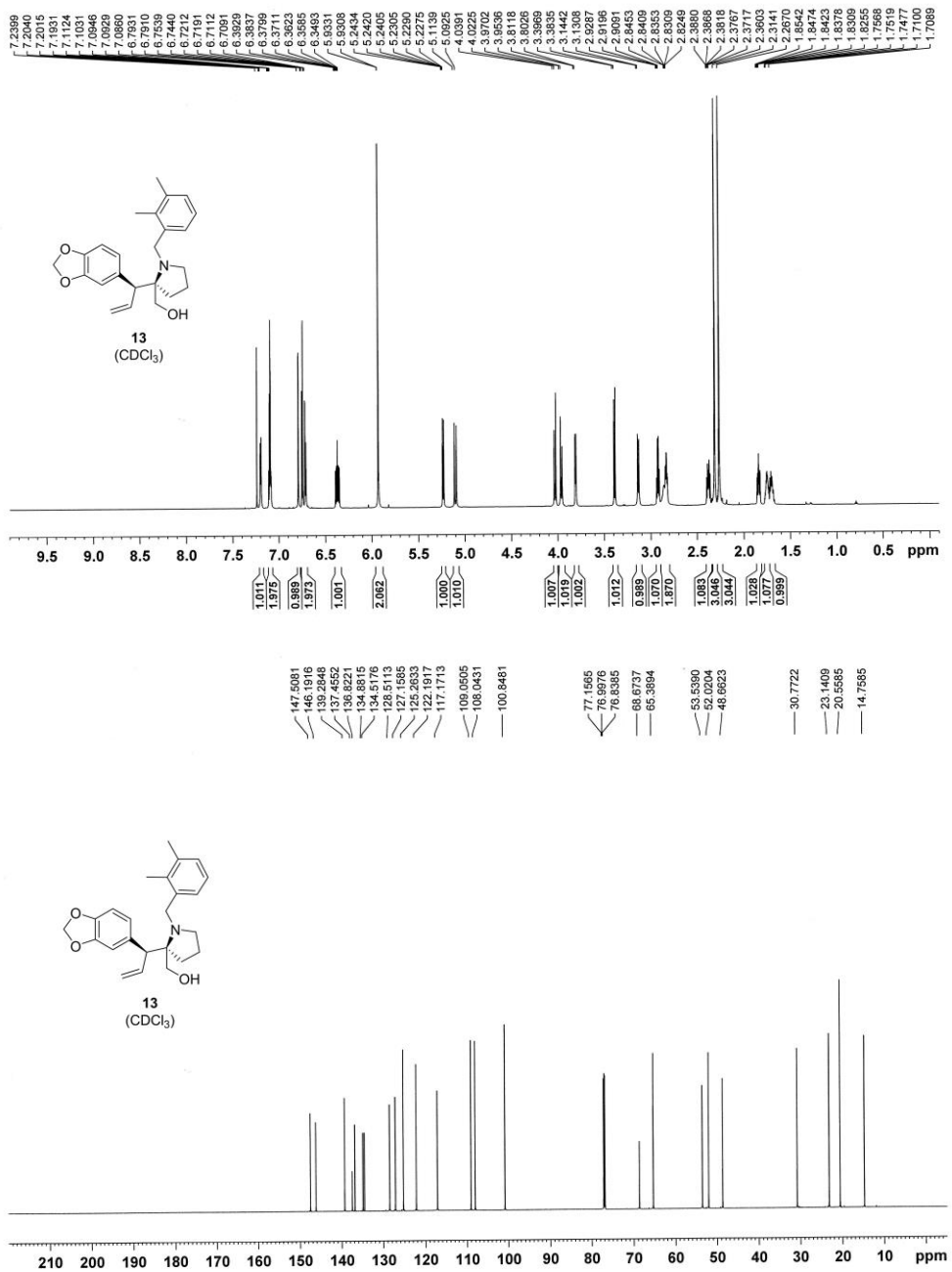
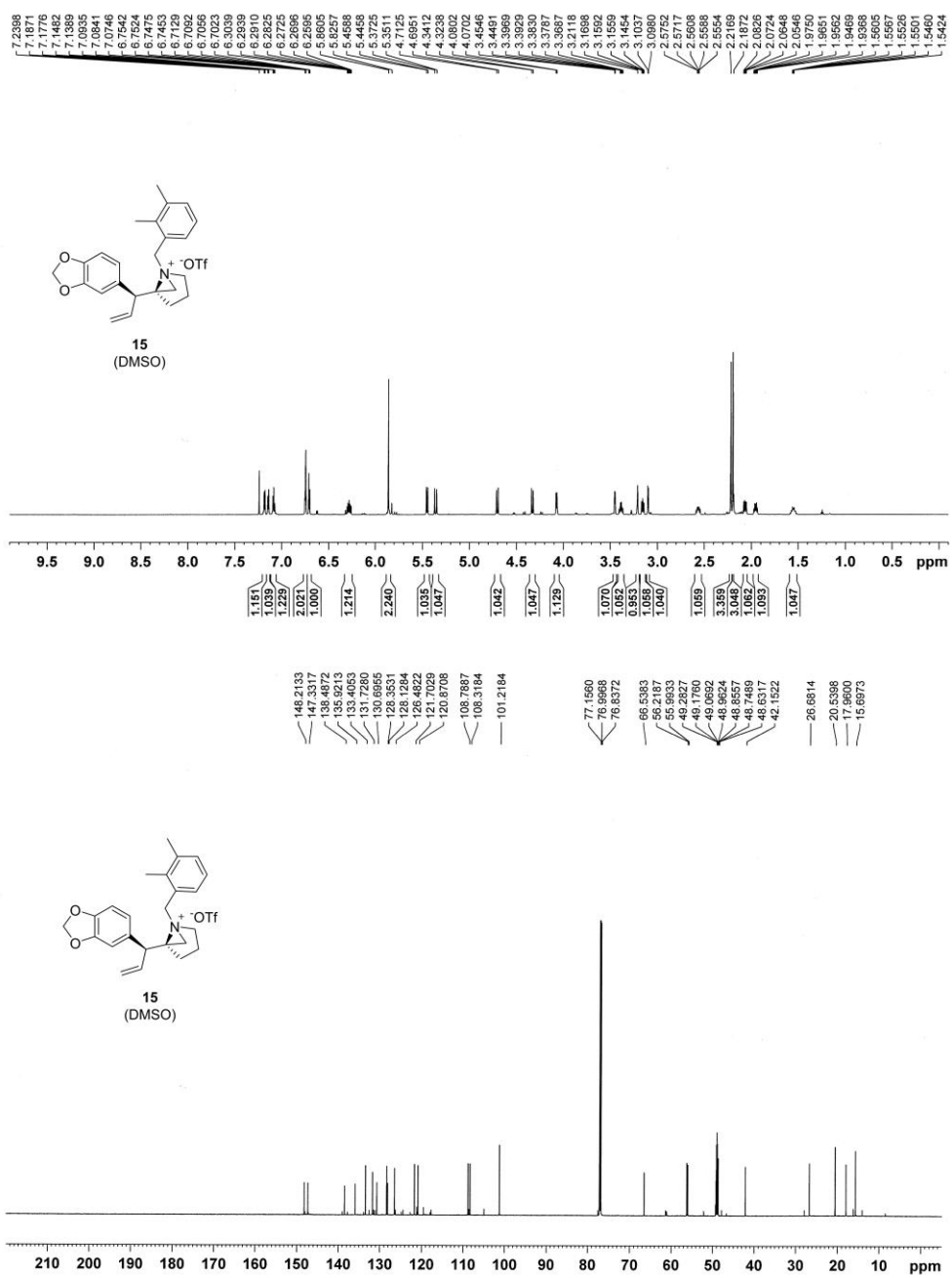


^{1}H NMR and ^{13}C NMR Spectra of 15



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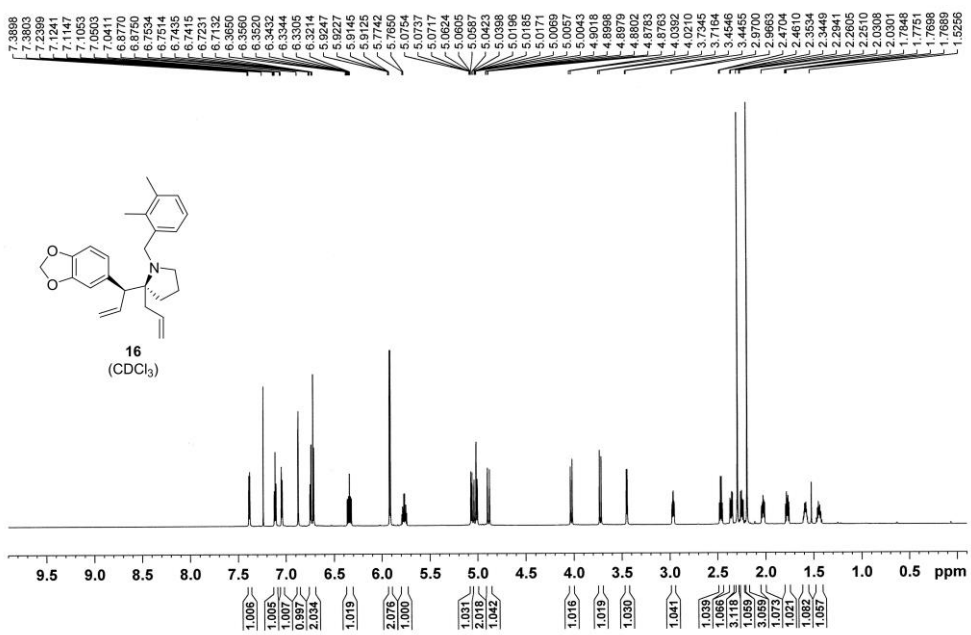
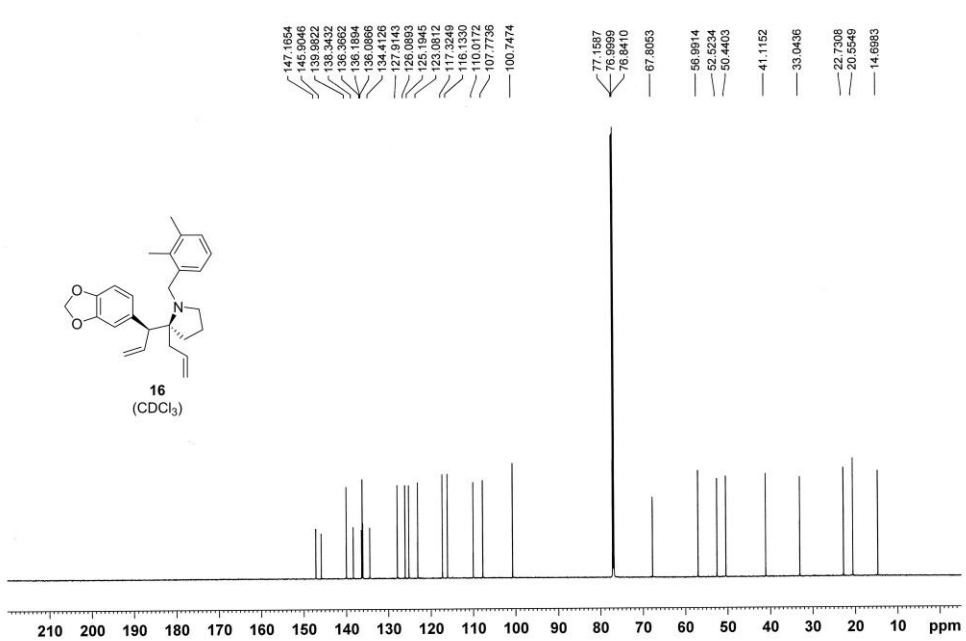
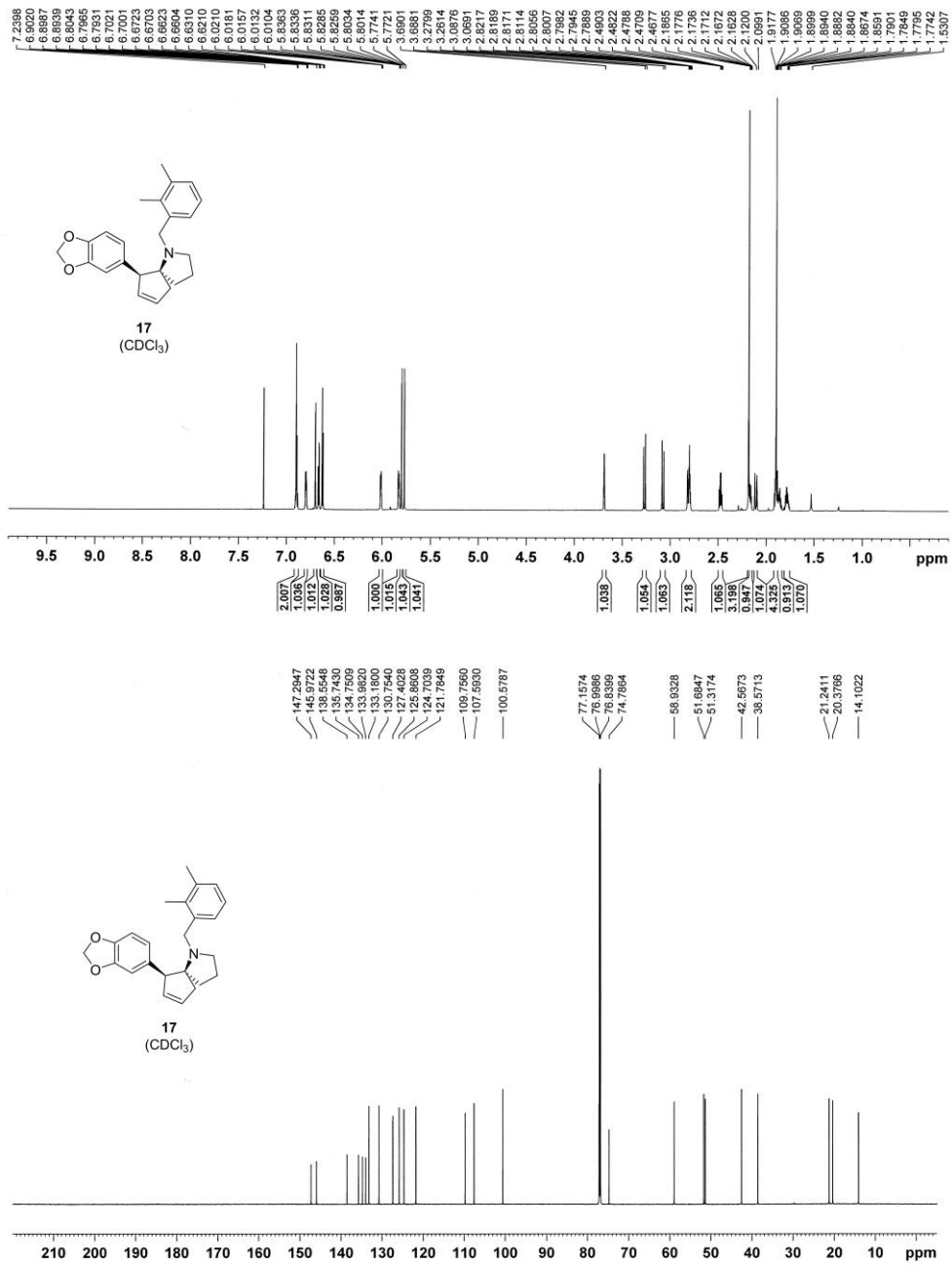




^{1}H NMR and ^{13}C NMR Spectra of 17



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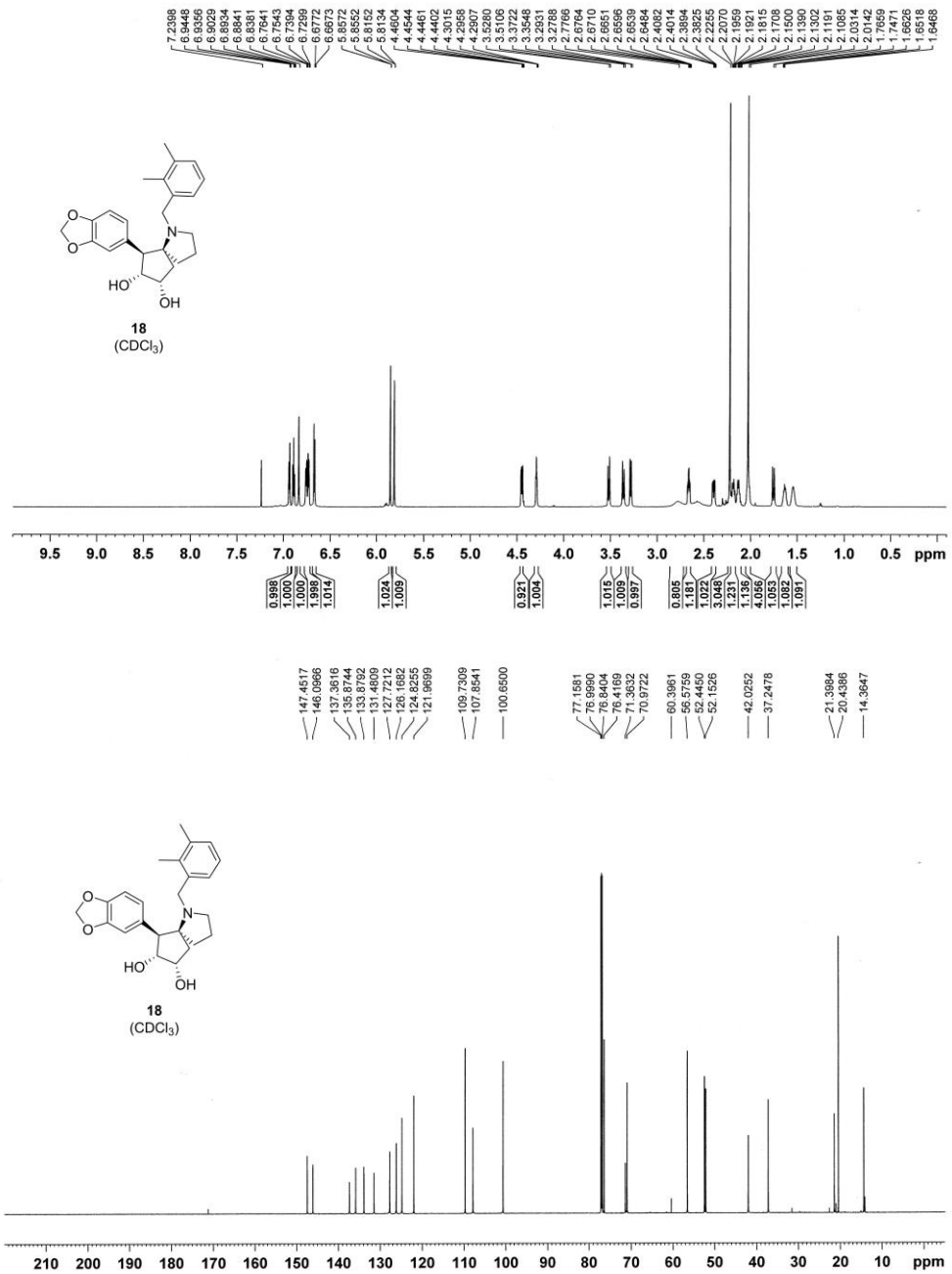

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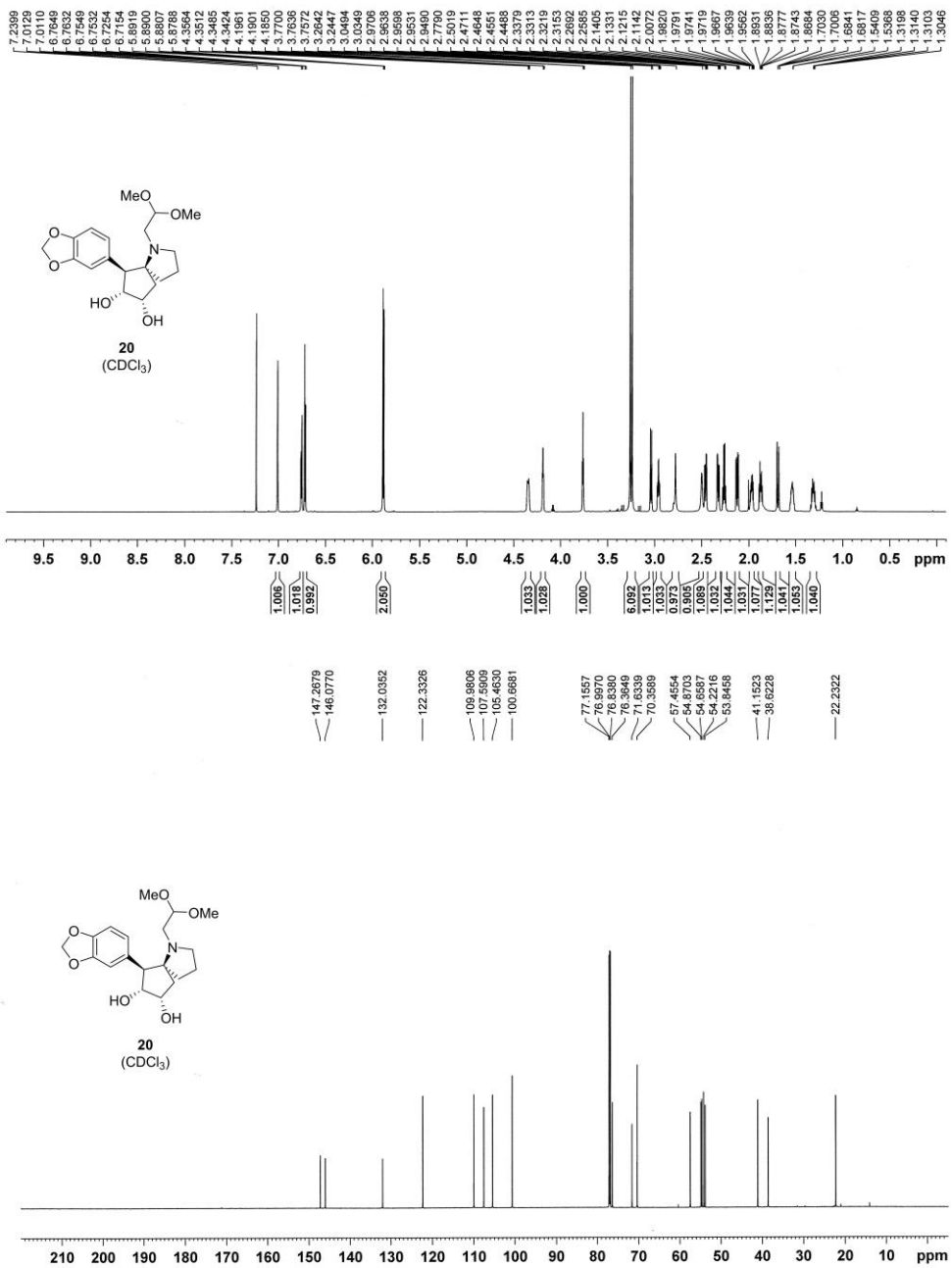
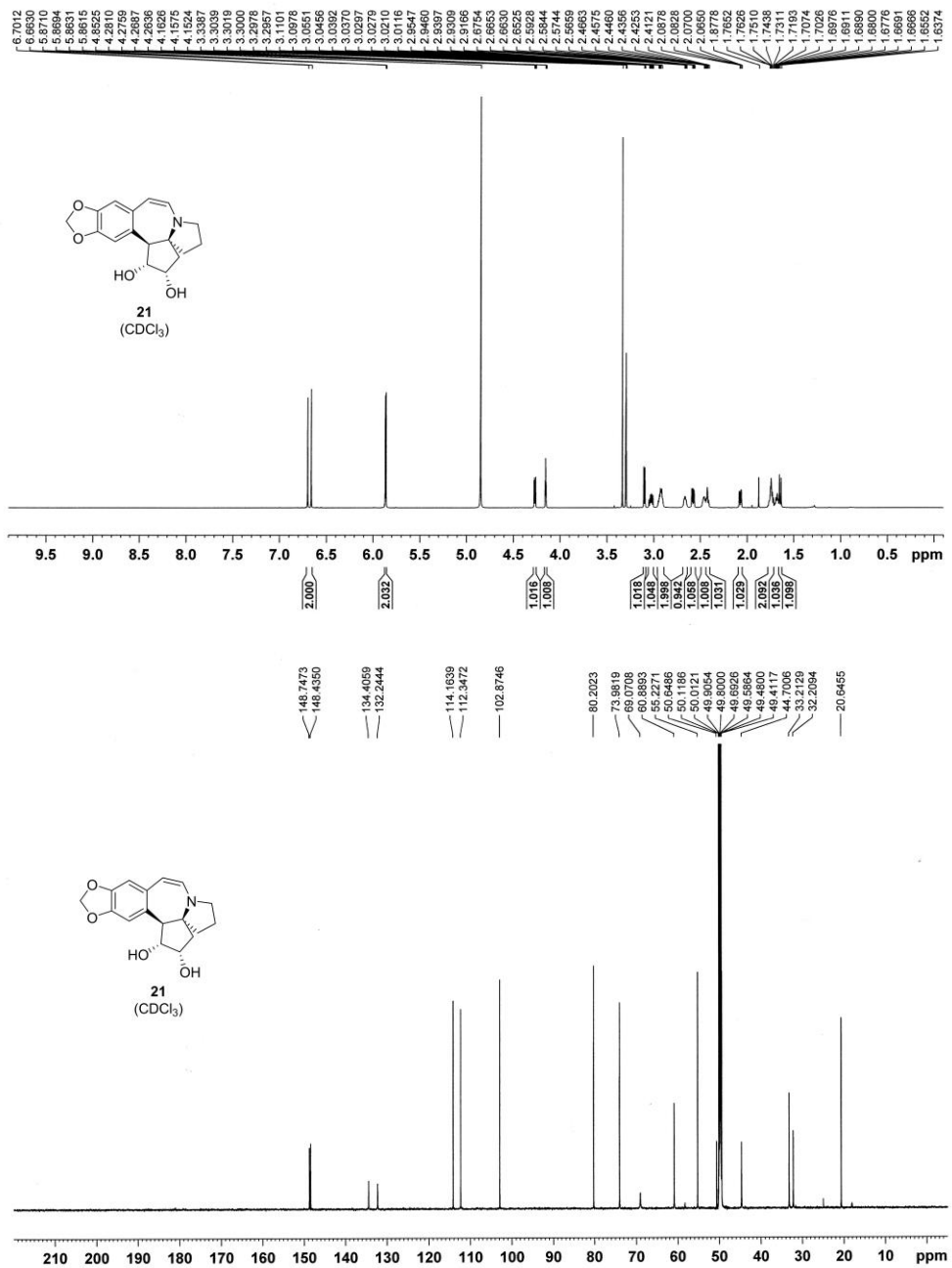
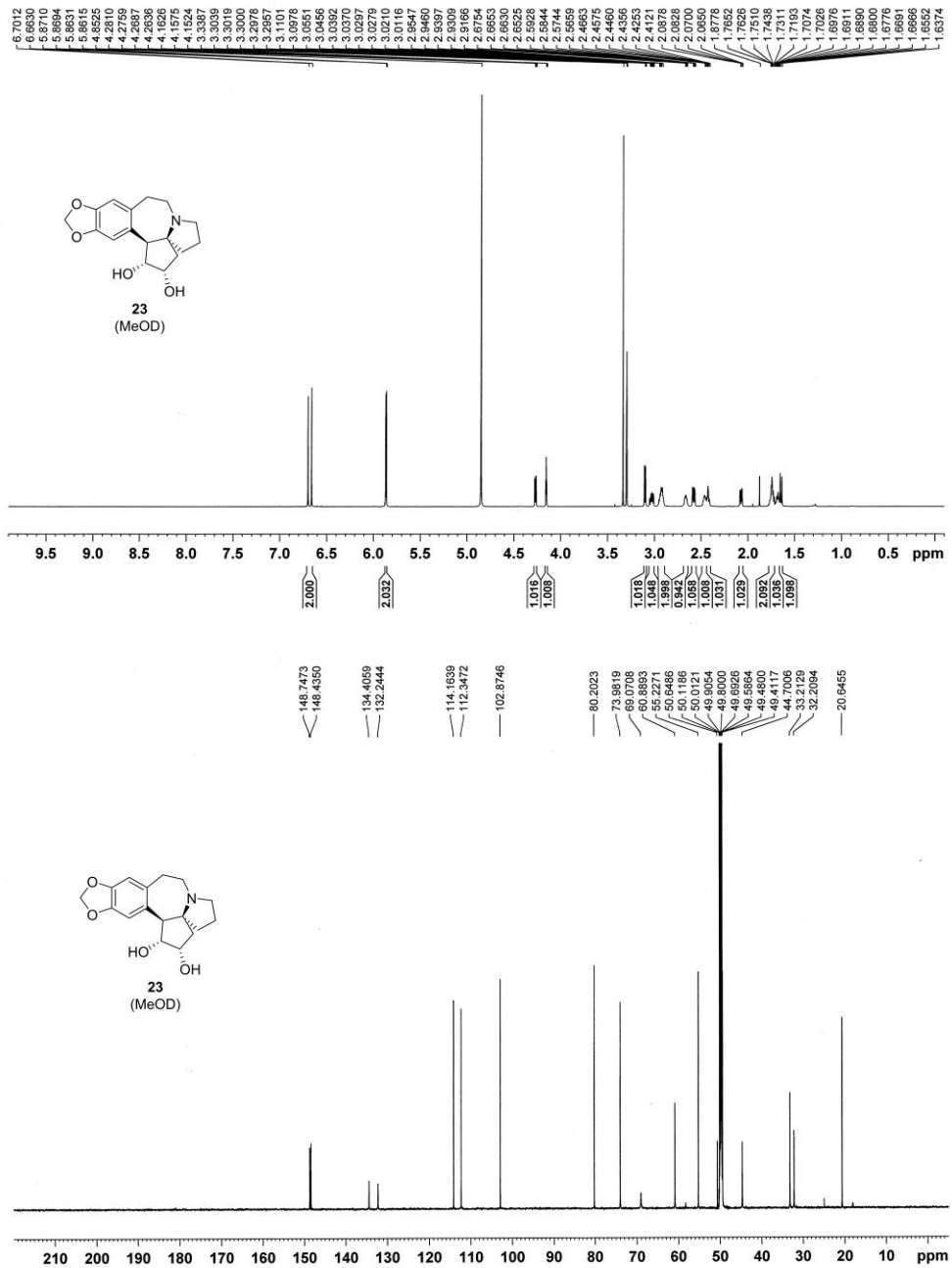


^{1}H NMR and ^{13}C NMR Spectra of 21



^{1}H NMR and ^{13}C NMR Spectra of 23



국 문 초 록

Part A. Chemodosimetric fluorescent probe를 이용한 chemical assay 기법의 개발과 천연물 분리에의 응용

많은 신약 개발 기술들이 발전하고 있는 가운데, 천연물은 아직도 신약개발의 기본 tool 혹은 source로서 중요한 역할을 하고 있으며 천연물을 분리 혹은 동정하는 기술들도 많은 발전을 거듭하고 있다. 그 중 chemical assay 기법은 특정한 작용기를 가지는 천연물 군을 검출해 내는 분리 및 동정 기법이지만, 여러 한계점 때문에 널리 응용되고 있지 않다.

본 연구자는 fluorescent chemodosimetric probe를 응용하여 천연물 분리 모델로서의 chemical assay 시스템을 새로이 고안하였다. 고체 지지체 (solid support)에 형광을 발현할 수 있는 chemodosimeter를 부착하여 특정 작용기를 가지는 천연물을 검출할 수 있다. 특정 작용기와 chemodosimeter 간의 화학적 반응으로 “click reaction”을 선택하였다. 그 중, 말단 삼중 결합 (terminal alkyne)과 azide 그룹이 구리 촉매 하에 반응하여 triazole 구조를 형성하는 copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC)을 본 연구에 응용하였다. CuAAC를 이용하여 천연 추출물 내에 있는 terminal alkyne

구조를 가지는 특정 천연물만 선택적으로 형광 신호를 통해 검출해 낼 수 있다. 이후 solid support로부터의 linker cleavage로 얻어낸 천연물 유도체를 이용해 분석 기기를 통한 천연물의 동정 과정을 진행할 수 있다.

본 연구자는 실제로 *Chrysanthemum morifolium* (국화)의 메탄올 추출물에서 현재까지 해당 식물에서는 발견되지 않았던 terminal alkyne을 가지는 천연물을 새로이 발굴해 낼 수 있었다. 'Click reaction/fluorescent chemodosimetric probe/solid support'의 조합으로 개발한 해당 chemical assay 기법은 천연물뿐만 아니라 다른 특정 화합물도 검출해 낼 가능성을 가지고 있다. 이는 해당 기법이 천연물 화학뿐만 아니라 식품 과학 혹은 대사체학에도 응용이 가능함을 시사한다.

주요어: Click chemistry, Fluorescent probe, 고체 지지체, 말단 삼중 결합, 천연물

학번: 2010-21727

국 문 초 록

Part B. Chirality transfer 및 [2,3]-rearrangement를 이용한 천연물 cephalotaxine의 전합성

천연물 cephalotaxine은 *Cephalotaxus harringtonii*로부터 분리/보고된 pentacyclic alkaloid로서 benzazepine구조를 특징으로 하는 천연물이다. Cephalotaxine의 ester analogues인 homoharringtonine이 chronic myeloid leukemia에 대한 신약으로서 2012년 FDA의 승인받은 바가 있을 정도로 cephalotaxine은 연구 가치가 높은 천연물이라 할 수 있다. 이와 더불어 cephalotaxine의 흥미로운 구조적 특징으로 이제까지 많은 합성적 노력이 이어져 왔다.

Cephalotaxine의 합성에 있어 가장 주목해야 할 점은 quaternary carbon center가 높은 chirality를 가지도록 구축하는 방법이라 할 수 있다. 본 논문은 C to N to C chirality transfer를 통해 높은 chirality를 도모하며 [2,3]-Stevens rearrangement를 이용해 효율적으로 quaternary carbon center를 구축하는 것을 골자로 하고 있다.

합성 방법이 알려진 cinnamyl bromide와 benzyl proline ester 간의 chirality transfer를 이용한 *N*-quaternization을 통해 chiral ammonium salt를 합성한 뒤, 염기 조건에서 [2.3]-Stevens rearrangement를

진행하여 chiral quaternary carbon center를 가지는 핵심 중간체를 효율적으로 합성하였다. 이후, aziridinium salt formation과 vinyl group addition, ring closing metathesis를 통해 B ring의 골격을 형성하였다. Substrate-controlled facial selective dihydroxylation을 통해 diol을 합성한 후, two carbon insertion과 acid-catalyzed Friedel-Crafts 반응을 통해 A ring을 구축하여 cephalotaxine의 formal synthesis를 완료하였다.

주요어: Cephalotaxine, Chirality transfer, [2,3]-Stevens rearrangement, Formal synthesis

학번: 2010-21727