Supporting Information (Experimental Procedures)

Precursor-Directed Biosynthesis of New Phenylbenzoisoquinolindione Alkaloids and the Discovery of a Phenylphenalenone-Based Plant Defense Mechanism

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Contents

1. General Experimental Methods

1.1. General experimental details

Solvents and reagents were purchased from commercial sources (Sigma-Aldrich, Deisenhofen, Germany) and used without further purification. All NMR spectra (^{1}H NMR, SELTOCSY, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{1}H ROESY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) were acquired either on a Bruker Avance III HD 500 NMR spectrometer (operating at 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C; 5 mm TCI cryoprobe) or on a Bruker Avance III HD 700 NMR spectrometer (operating at 700.13 MHz for ^{1}H and 175.75 MHz for ^{13}C; 1.7 mm TCI microcryoprobe) (Bruker Biospin, Karlsruhe, Germany), controlled by Bruker TopSpin ver. 3.5 and ver. 3.2, respectively. Data processing was accomplished using Bruker TopSpin. Standard Bruker pulse sequences as implemented in Bruker TopSpin were used. Solvent signals of acetone-d_6 (δ 2.09, C$\underline{H_3}$COC$\underline{H_3}$; δ 29.92, $\underline{C}H_3CO\underline{C}H_3$) were used for referencing spectra in the ^{1}H and ^{13}C dimensions of the new PBIQs (**7 - 16**).

Chromatographic analyses and separations were conducted on an Agilent Infinity 1260 HPLC consisting of a quaternary pump (G1311B), autosampler (G1367E), column oven (G1316A), and photodiode array detector (G1315D) (Agilent, Waldbronn, Germany). The devices were controlled using Bruker Hystar ver. 3.2 (Bruker Biospin, Karlsruhe, Germany). Semi-preparative HPLC separations were carried out using a Shimadzu Prominence HPLC system, controlled by the Shimadzu LCSolution ver. 1.21, and consisting of a DGU-20As degasser, LC-20AT gradient pump, SIL-10AP autosampler, CTO-20A column oven, SPD-20A UV detector, FRC-10A fraction collector and CBM-20A system controller (Shimadzu, Duisburg, Germany). Furthermore, semi-preparative HPLC separations were performed on an Agilent 1100 system

(Agilent, Waldbronn, Germany) consisting of a degasser G1322A, binary pump G1312A, autosampler G1367A and photodiode array detector G1315A. The detector outlet was coupled to an Advantec CHF 122SB fraction collector (Jasco, Gross-Umstadt, Germany). For conducting micro-preparative separations (HPLC-SPE), the Agilent Infinity 1260 HPLC was coupled to a Bruker /Spark Holland Prospekt 2 SPE unit (Bruker Biospin, Karlsruhe, Germany / Spark Holland, Emmen, The Netherlands) equipped with HySphere resin GP cartridges (10 µm; 10 × 2 mm) to trap selected peaks. Depending on the flow rate used for chromatographic separations, a four-fold flow of water was added post-column by a make-up pump (Knauer, Berlin, Germany) in order to reduce the eluotropic capacity of the eluent. After chromatographic loading, cartridges were dried using a stream of nitrogen for 15 min before being eluted with MeCN into 2 mL Eppendorf micro-reaction vessels (Eppendorf, Wesseling-Berzdorf, Germany) for further by MS or NMR analysis.

The following columns and gradient conditions were used for HPLC chromatographic separations:

A) LC-HRESIMS measurements. Zorbax C18 column (3.5 µm; 150 × 4.6 mm; Agilent, St Louis, MO, USA) with a constant flow rate of 500 µL min^{-1} at 25 °C, binary solvent system of H_2O (solvent A) and MeCN (solvent B), both containing 0.1% (v/v) formic acid (FA). Linear binary gradient: 0-2 min 5% B, 40 min 100% B, 40-55 min 100% B, and 55-60 min 5% B.

B) LC-ESIMS measurements. Nucleodur C-18 EC Isis column (5 µm; 250 × 4.6 mm; Macherey-Nagel, Düren, Germany) with a guard column (4 × 4 mm) at 30 °C with a flow rate of 0.8 ml min^{-1}, binary solvent system of 0.1% (v/v) FA in H_2O (solvent A) and 0.1%

(v/v) FA in MeCN (solvent B). Linear binary gradient: 0-50 min 5-65% B, 50-55 min 65-100% B, 55-60 min 100% B, and 60-65 min 5% B.

C) Semipreparative separations by a Shimadzu Prominence HPLC system. MN Nucleodur C-18 HTEC column (5 µm; 250 × 10 mm; Macherey-Nagel, Düren, Germany) at 25 °C with a flow rate of 3.5 mL min^{-1}. Binary solvent system of 0.05% FA (v/v) in H_2O (solvent A) and 0.05% FA (v/v) in MeOH (solvent B). Linear binary gradient: 0-15 min 45-65% B, 15-30 min 65% B, 40-45 min 100% B, and 45-50 min 45% B.

D) Semipreparative separations by an Agilent 1100 system. Merck Purospher STAR RP-18e (5 µm; 250 × 4.6 mm; Merck, Darmstadt, Germany) using water (0.1% (v/v) FA, solvent A) and MeCN (0.1% (v/v) FA, solvent B) at 25 °C with a flow rate of 0.8 ml min^{-1}. Linear binary gradient: 0-5 min 18-25% B, 5-35 min 25% B, 35-40 min 25-30% B, 40-48 min 100% B, and 48-55 min 18% B.

For HRESIMS measurements, an Agilent Infinity 1260 HPLC was coupled to a Bruker Compact OTOF mass spectrometer (Bruker Daltonics, Bremen, Germany), controlled by the Bruker Compass control suite (ver. 1.9) together with Bruker OTOFControl ver. 4.0 (Bruker Daltonics, Bremen, Germany). The samples were analyzed in the positive ionization mode in the mass range *m/z* 50 to 1300 using 30,000 m/Δm resolving power. Low-resolution ESIMS data were recorded using a Bruker Esquire 3000 ion trap mass spectrometer operated by EsquireControl ver. 3.5 (Bruker Daltonics, Bremen, Germany). Samples were measured in the positive ionization mode in the range *m/z* 150–1500. Optical rotations were obtained on a Jasco P-2000 polarimeter (Jasco, Gross-Umstadt, Germany) at the Na wavelength 589 nm.

1.2. Plant material

Plants of *Xiphidium caeruleum* Aubl. were obtained from the Ruhr University of Bochum (Botanical Institute), vegetatively propagated, and grown in 1 L pots in the greenhouse of the Max Planck Institute for Chemical Ecology under the following conditions: day 22–24 °C; night 20–22 °C; relative air humidity 60–70%. The natural daily photoperiod was supported by 16 h illumination from Philips Sun-T Agro 400 W sodium lights.

2. Spontaneous Formation of PBIQs

2.1. ^{13}C-Labeled compounds 5 and 6 obtained by incubating flowers with ^{13}C-labeled phenylalanine and leucine

The buds of *X. caeruleum* were excised at the base of the receptacle before the onset of darkness and incubated in a 1.7 mM aqueous solution of (3-^{13}C)-L-phenylalanine or a 3.5 mM aqueous solution of (1-^{13}C)-L-leucine in conical flasks (volume 100 mL) on a gyratory shaker (100 rpm) for one night. A volume of approximately 20 mL of amino acid solution to cover just the buds was used (Figure S1).

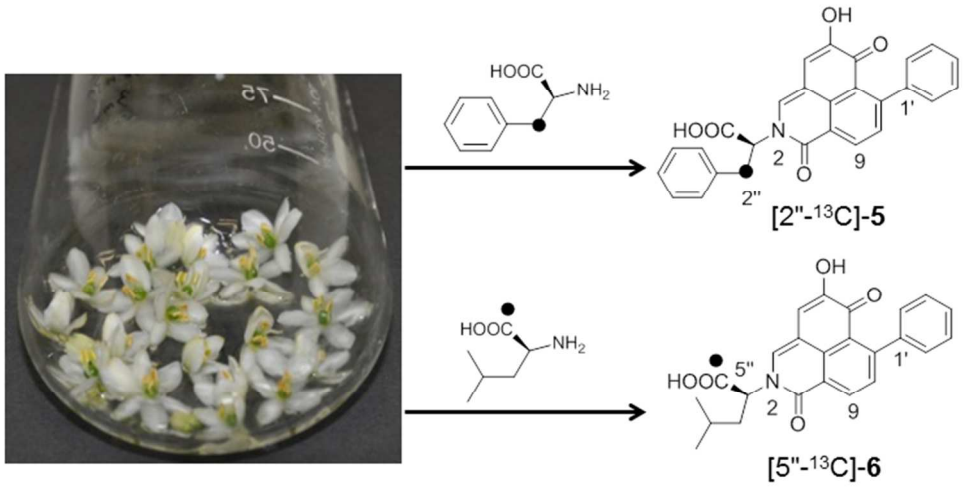


Figure S1. Incubation of (3-^{13}C)-L-phenylalanine and (1-^{13}C)-L-leucine with buds of *X. caeruleum* to obtain labeled PBIQs [2"-^{13}C]-**5** and [5"-^{13}C]-**6**.

By next morning, the buds had opened and were harvested from the incubation solution, washed with distilled water, frozen in liquid nitrogen and stored at -80 °C. Flowers incubated with (3-^{13}C)-L-phenylalanine were ground in liquid N_2 and extracted with acetone (150 mL) three times. The combined acetone extract was then diluted with 750 mL distilled water and passed through a preconditioned Chromabond HRX SPE cartridge (500 mg, 6 mL, Macherey–Nagel, Düren, Germany). The loaded cartridge was eluted with acetone. The acetone eluate was evaporated using a stream of nitrogen, reconstituted in acetone and used to isolate [2"-^{13}C]-**5** (1.5 mg, from 230 flowers) by HPLC-SPE (method B). ^{13}C-Labeled **6** (1.4 mg, from 200 flowers) was obtained by the same manner from incubation with (1-^{13}C)-L-leucine. The incorporation of (3-^{13}C)-L-phenylalanine into [2"-^{13}C]-**5** and (1-^{13}C)-L-leucine into [5"-^{13}C]-**6** was calculated from the integral ratio of the ^{13}C NMR signals of C-2" of **5** and C-5" of **6** in the spectra of the labeled versus the unlabeled compound. Signals of naturally abundant ^{13}C atoms corresponding to 1.11% ^{13}C were used as a reference (Figure S2).

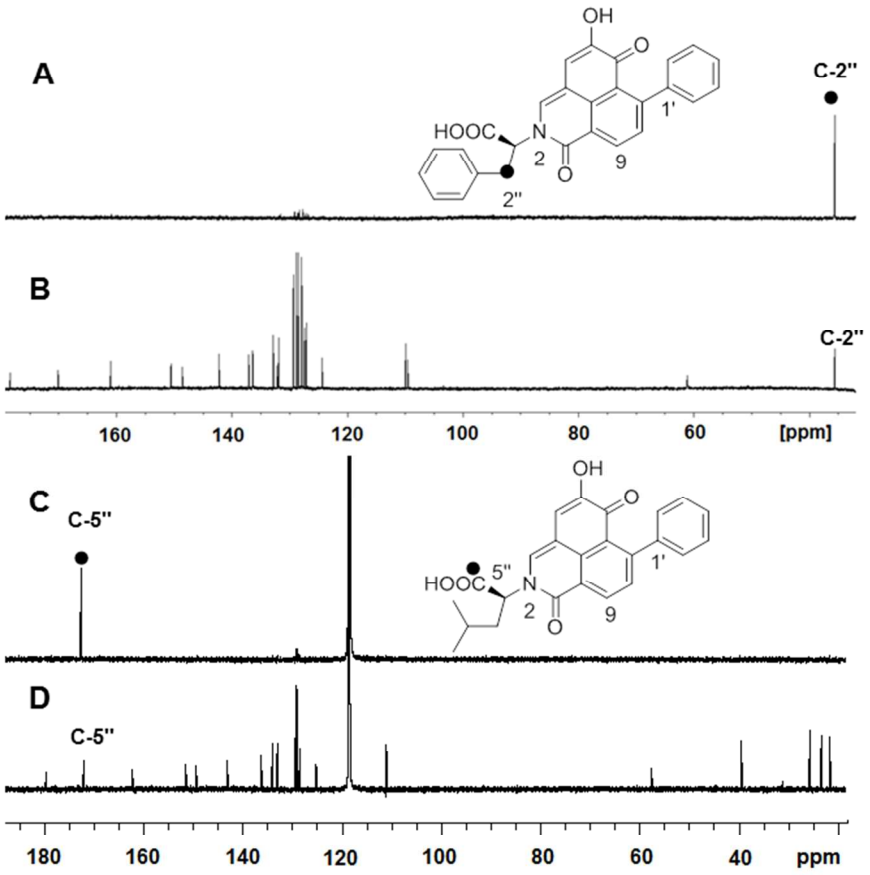


Figure S2. ^{13}C NMR spectra of the PBIQs **5** (125 MHz, acetone-d_6) and **6** (125 MHz, MeCN-d_6). A) ^{13}C-Labeled [2''-^{13}C]-**5** from *X. caeruleum* isolated after the incorporation of (3-^{13}C)-L-phenylalanine. B) Unlabeled reference of **5**. C) ^{13}C-Labeled [5''-^{13}C]-**6** from *X. caeruleum* isolated after the incorporation of (1-^{13}C)-L-leucine. D) Unlabeled reference of **6**. The black dots (●) indicate enrichment with ^{13}C from (3-^{13}C)-L-phenylalanine and (1-^{13}C)-L-leucine.

2.2. PBIQs obtained by incubating fresh plant materials with various amino acids

Preparation of PBIQs

Eleven proteinogenic L-amino acids and two D-amino acids were used for incubation experiments as follows: fresh plant material of *X. caeruleum* (flowers or 1 cm snippets of

leaves) was suspended in acetone in an Erlenmeyer flask covered with perforated aluminum foil to allow exposure to air. To the suspension, amino acids (see Table S1) in 50% acetone-water (10–50 mL, depending on the solubility of the amino acids) were added one at a time. After being shaken for 2 d at ambient temperature, the suspension was filtered through degreased cotton. The filtrate was diluted with the 5-fold volume of distilled water and passed through a preconditioned HRX SPE cartridge. After being washed with water, the loaded cartridge was eluted with MeCN. The MeCN eluate was evaporated using a stream of nitrogen, reconstituted with acetone and subjected to semi-preparative HPLC in order to isolate the PBIQs (method C). Nine reported PBIQs were isolated: 2-[(1″*S*)-1″-carboxy-2″-phenyl-ethyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**5**), 2-[(1″*S*)-1″-carboxy-3"-methyl-*n*-butyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**6**), *N*-(2-hydroxyethyl)-lachnanthopyridone (**17**), 2-[(1″*S*)-1″-carboxy-2″-(4‴-hydroxyphenyl)-ethyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**27**), 2-[(1″*S*)-1″-carboxyethyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**28**), 2-[(1″*S*)-1″-carboxy-2″-(1‴*H*-imidazol-5‴-yl)-ethyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**29**), 2-[(1″*S*)-1″-carboxy-4″-diaminomethylideneamino-*n*-butyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**30**), 2-[(1″*S*)-1″-carboxy-2″-methyl-*n*-butyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**31**), and 2-[(1″*S*)-1″-carboxy-2″-(1*H*-indol-3‴-yl)-ethyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**32**).[1] Furthermore, ten new PBIQs were discovered (Table S1): 2-[(1″*R*)-1″-carboxy-2″-phenyl-ethyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**7**), 2-[(1″*R*)-1″-carboxy-3″-methyl-*n*-butyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**8**), 2-[(1″*R*)-1″-carboxy-2″-phenyl-ethyl]-5-methoxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**9**), 2-[(1″*R*)-1″-carboxy-3″-methyl-*n*-

butyl]-5-methoxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**10**), 2-[(1″*S*)-1″-carboxy-2″-phenyl-ethyl]-5-methoxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**11**), 2-[(1″*S*)-1″-carboxy-2″-(4‴-hydroxyphenyl)-ethyl]-5-methoxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**12**), 2-[(1″*S*)-1″-carboxy-2″-(1*H*-indol-3‴-yl)-ethyl]-5-methoxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**13**), 2-[(1″*S*)-1″-carboxy-3″-methylthio-propyl]-5-hydroxy-7-phenyl-2*H*-benzo[*de*]isoquinoline-1,6-dione (**14**), 2-[(1″*S*)-1″-carboxy-hexacyl]-5,5‴-hydroxy-7,7‴-phenyl-1″,5″-di-[2*H*-benzo[*de*]isoquinoline-1,6-dione] (**15**), and (3*R*)-3-carboxy-8-phenyl-10-hydroxy-2*H*-benzo[*de*]5*H*-thiazolo[3,2-*b*]isoquinoline-5,9-dione (**16**). When flowers were utilized for incubation, 5-hydroxy PBIQs were obtained, whereas other aerial green parts produced both 5-hydroxy PBIQs and 5-methoxy PBIQs. The structures of all PBIQs were established by NMR (Tables S2-5) and MS.

Structure elucidation of new PBIQs

The NMR data of the tricyclic $C_{18}N$ core structure of the new PBIQs **7** - **14** closely resembled those of reported aza-phenylphenalenones and the previously employed assignment strategy[1a,1b] was employed to establish the structure of the new compounds. ^{1}H NMR data (Table S2) and ^{13}C NMR chemical shifts (Table S3) extracted from HSQC and HMBC spectra confirmed that compounds **7** – **14** have the same PBIQ skeleton, but different amino acid-derived side chains. HMBC correlations of H-1″ with C-1 and C-3 showed that the side chain is attached to position 2 of the $C_{18}N$ skeleton. Compared to compounds **7**, **8**, and **14**, the NMR spectra of compounds **9** - **13** (Table S2) showed signals of an additional OCH_3 group. For each of the methoxy compounds, HMBC correlations assigned the OCH_3 group to position 5.

Compound **15**, obtained by incubating flowers of *X. caeruleum* with lysine (Table S1), showed signals of two $C_{18}N$ units, but only one lysine unit, in the ^{1}H-, ^{13}C- and heterocorrelation NMR spectra (Table S4, for spectra see section 8 of Supporting information). This signal pattern suggested linkage of the two amino groups of lysine with each one phenylphenalenone unit. HMBC correlations of H-1″ (δ 5.65) with C-1 (δ 161.9) and C-3 (δ 136.4) and of H-3 (δ 8.07) with C-1″ (δ 59.2) indicated that the α-amino group of lysine has been incorporated into position 2 of a PBIQ ring system. Further HMBC correlations, namely of H-5″α (δ 4.18 - 4.24) and H-5″β (δ 4.09 - 4.14) with C-1‴ (δ 161.7) and C-3‴ (δ 138.4) and of H-3‴ (δ 8.01) with C-5″ (δ 49.3) substantiated that the terminal amino group of lysine has become part of the second PBIQ ring system (position 2‴).

ESIMS and HRESIMS data (see below) were consistent with the suggested structures of PBIQs **7** - **15**. The absolute configuration of the chiral center at C-1″ was concluded from the configuration of the corresponding amino acids (Table S1), which were incubated with plant material of *X. caeruleum* to generate the PBIQs.

The ESIMS spectrum (positive ionization mode) of compound **16**, obtained by incubating flowers of *X. caeruleum* with cysteine (Table S1), indicated a molecular ion of *m/z* 392 $[M + H]^{+}$, two mass units less than expected for the hypothetical PBIQ with a cysteine side chain. The HRESIMS data, from which a molecular formula of $C_{21}H_{14}NO_5S$ was established, suggested a ring closure between the thiol and the $C_{18}N$ ring, resulting in a thiazole ring annulated to the PBIQ. The missing singlet of H-3 in the ^{1}H NMR spectrum and HMBC correlation between one of the methylene protons (H-2α, δ 3.94) of the cysteine side chain and C-12 (δ 150.6) confirmed this assumption. Thus, compound **16** was identified as (3*R*)-3-carboxy-8-phenyl-10-hydroxy-2*H*-

benzo[*de*]5*H*-thiazolo[*3,2-b*]isoquinoline-5,9-dione. The *R*-configuration of the chiral center at C-3 was concluded from the configuration of L-cysteine used to generate compound **16**.

Table S1. PBIQs obtained by incubating plant material of *X. caeruleum* with amino acids and an amine

Compounds	Fresh plant materials, mass	Amino acids / Amine, mass	Products, mass
5	Flowers, 2.5 g	L-Phenylalanine, 4.5 mg	2.3 mg
6	Flowers, 3.5 g	L-Leucine, 9.2 mg	2.1 mg
14	Flowers, 4.6 g	L-Methionine, 25.7 mg	2.8 mg
15	Flowers, 4.6 g	L-Lysine, 12.7 mg	1.0 mg
16	Flowers, 4.8 g	L-Cysteine, 40.0 mg	1.8 mg
17	Flowers, 2.5 g	Ethanolamine, 15.1mg	1.6 mg
27	Flowers, 4.4 g	L-Tyrosine, 10.0 mg	3.6 mg
28	Flowers, 3.7 g	L-Alanine, 14.1 mg	2.8 mg
29	Flowers, 2.9 g	L-Histidine, 16.2 mg	1.4 mg
30	Flowers, 5.0 g	L-Arginine, 40.0 mg	2.7 mg
31	Flowers, 1.9 g	L-Isoleucine, 4.1 mg	1.0 mg
5/11	Aerial green parts, 51 g	L-Phenylalanine, 127 mg	**5**, 8.0 mg
			11, 1.7 mg
7/9	Aerial green parts, 45 g	D-Phenylalanine, 100 mg	**7**, 3.7 mg
			9, 1.9 mg
8/10	Aerial green parts, 45 g	D-Leucine, 80 mg	**8,** 3.5 mg
			10, 1.9 mg
12/27	Aerial green parts, 61 g	L-Tyrosine, 140 mg	**27**, 4 mg
			12, 0.6 mg
13/32	Aerial green parts, 60 g	L-Tryptophan, 150 mg	**32**, 8.4 mg
			13, 2,7 mg

Table S2. ^{1}H NMR data of compounds **7** to **14** (500 MHz, acetone-d_6, δ values, J in Hz)

Position	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	δ_H (mult., J)	δ_H (mult., J)	δ_H (mult., J)	δ_H (mult., J)	δ_H (mult., J)	δ_H (mult., J)	δ_H (mult., J)	δ_H (mult., J)
3	7.96 (s)	8.12 (s)	7.93 (s)	8.07 (s)	7.93 (s)	7.93 (s)	7.93 (s)	8.13 (s)
4	6.94 (s)	7.08 (s)	6.92 (s)	7.05 (s)	6.92 (s)	6.96 (s)	6.87 (s)	7.07 (s)
8	7.56 (d, 8.0)	7.61 (d, 8.0)	7.53 (d, 8.0)	7.58 (d, 8.1)	7.52 (d, 8.0)	7.54 (d, 8.1)	7.49 (d, 8.1)	7.61 (d, 8.1)
9	8.60 (d, 8.0)	8.69 (d, 8.0)	8.56 (d, 8.0)	8.64 (d, 8.1)	8.56 (d, 8.0)	8.57 (d, 8.1)	8.55 (d, 8.1)	8.68 (d, 8.1)
2'/6'	7.34 (dd, 7.9, 1.6)	7.36 (dd, 7.9, 1.6)	7.29 - 7.32 (m)	7.32 - 7.34 (m)	7.30 (dd, 8.0, 1.6)	7.32 (d, 8.1, 1.8)	7.27 - 7.29 (m)	7.36 (dd, 8.0, 1.9)
3'/4'/5'	7.37 - 7.43 (m)	7.38 - 7.45 (m)	7.35 - 7.41 (m)	7.35 - 7.42 (m)	7.34 - 7.40 (m)	7.35 - 7.42 (m)	7.34 - 7.40 (m)	7.39 - 7.45 (m)
1''	5.82 (dd, 11.1, 4.8)	5.80 (dd, 11.2, 4.6)	5.82 (dd, 11.0, 4.8)	5.82 (dd, 11.1, 4.6)	5.82 (dd, 10.9, 5.0)	5.78 (dd, 11.0, 4.9)	5.91 (dd, 9.9, 5.0)	5.68 (m)
2''a	3.73 (dd, 14.6, 5.0)	2.27 - 2.33 (m)	3.72 (dd, 14.8, 5.0)	2.24 - 2.31 (m)	3.72 (dd, 14.7, 5.0)	3.62 (dd, 14.7, 4.9)	3.76 - 3.85 (m)	2.50 - 2.70 (m)
2''b	3.61 (dd, 14.6, 11.3)	2.11 - 2.16 (m)	3.59 (dd, 14.5, 11.0)	2.11 - 2.16 (m)	3.59 (dd, 14.5, 11.2)	3.51 (dd, 14.7, 11.1)	3.76 - 3.85 (m)	2.50 - 2.70 (m)
3''		1.56 - 1.63 (m)		1.55 - 1.63 (m)				2.50 - 2.70 (m)
4''		1.01 (d, 6.6)		1.02 (d, 6.6)				
5''		0.98 (d, 6.6)		0.98 (d, 6.6)				
2'''	7.29 (d, 7.4)		7.27 - 7.29 (m)		7.28 (d, 7.6)	7.11 (d, 8.5)	7.14 (d, 2.2)	
3'''	7.21 (t-like, 7.3)		7.21 (t-like, 7.3)		7.21 (t-like, 7.2)	6.69 (d, 8.5)		
4'''	7.14 (t-like, 7.3)		7.14 (t-like, 7.4)		7.14 (t-like, 7.5)		7.67 (d, 7.9)	
5'''	7.21 (t-like, 7.3)		7.21 (t-like, 7.3)		7.21 (t-like, 7.2)	6.69 (d, 8.5)	7.06 (ddd, 7.9, 6.9, 1.0)	
6'''	7.29 (d, 7.4)		7.27 - 7.29 (m)		7.28 (d, 7.6)	7.11 (d, 8.5)	6.99 (ddd, 7.9, 6.9, 1.0)	
7'''							7.31 (dd, 7.9)	
5-OCH_3			3.74 (s)	3.77 (s)	3.73 (s)	3.76 (s)	3.76 (s)	
3''-SCH_3								2.11 (s)

Table S3. ^{13}C NMR data of compounds **7** to **14** (125 MHz, acetone-d_6, δ)

Position	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	161.7	161.9	161.6	162.4	161.9	161.5	161.7	162.2
3	137.0	136.1	135.8	134.9	135.5	136.1	135.9	137.1
3a	110.6	110.7	110.2	109.2	110.4	109.0	109.9	111.1
4	110.3	110.8	110.1	110.2	109.5	110.7	110.2	111.1
5	149.4	149.4	152.5	153.2	152.9	152.2	152.5	149.6
6	179.2	179.2	178.0	178.7	178.5	178.0	178.1	179.4
6a	125.0	125.1	124.7	125.5	125.2	124.7	124.9	125.6
7	151.3	151.3	151.0	151.4	151.2	150.9	150.8	151.3
8	132.6	132.6	132.6	132.7	132.3	132.5	132.6	132.9
9	133.5	133.7	132.8	132.9	132.4	132.6	132.8	133.9
9a	125.0	125.1	126.7	127.8	125.6	126.9	126.9	125.4
9b	132.9	132.9	132.9	133.4	133.0	132.5	132.8	133.3
1'	142.9	142.9	143.3	144.2	143.7	143.4	143.4	143.3
2'/6'	129.2	129.2	129.2	129.2	128.6	129.0	129.0	129.4
3'/5'	128.6	128.6	128.5	128.6	128.2	128.4	128.6	128.9
4'	128.1	128.1	127.7	128.1	127.4	127.4	127.8	128.4
1''	61.8	57.0	61.7	56.9	61.2	61.6	60.7	59.9
2''	36.2	39.7	36.5	39.8	36.0	35.4	26.6	30.3
3''	170.9	25.6	170.9	26.2	171.2	170.8	171.4	31.3
4''		21.6		21.6				171.4
5''		23.4		23.4				
6''		171.9		172.6				
1'''	137.7		137.7		138.1	128.0		
2'''	130.0		129.7		129.6	130.7	124.6	
3'''	129.5		129.6		129.2	116.1	110.5	
3a'''							128.2	
4'''	127.8		128.0		127.4	157.0	119.1	
5'''	129.5		129.6		129.2	116.1	122.4	
6'''	130.0		129.7		129.6	130.7	119.8	
7'''							112.4	
7a'''							137.5	
5-OCH_3			55.9	55.8	55.5	55.6	55.7	
3''-SCH_3								15.4

Table S4. NMR data (500 MHz, acetone-d_6, δ values, J in Hz) of compound **15**

Position	δ_H (mult., J)	δ_C	HMBC
1		161.9	
3	8.07 (s)	136.4	C-1, 4, 9b, 1''
3a		110.8	
4	7.03 (s)	110.7	C-3, 5, 6, 9b
5		149.3	
6		179.0	
6a		124.9	
7		151.2	
8	7.52 (d, 8.1)	132.4	C-6a, 9a, 1'
9	8.56 (d, 8.1)	133.6	C-1, 7, 9a, 9b
9a		124.8	
9b		132.8	
1'		142.9	
2'/6'	7.29 - 7.33 (m)	129.2	C-7, 4'
4'	7.38 - 7.42 (m)	128.1	C-1', 2', 6'
3'/5'	7.38 - 7.42 (m)	128.6	C-1', 2', 6'
1''	5.65 (dd, 9.4, 6.0)	59.2	C-1, 3, 2'', 3'', 6''
2''	2.42 - 2.47 (m)	30.2	C-1'', 3'', 6''
3''	1.51 – 1.57 (m)	23.6	C-2'', 4'', 5''
4''	2.09 – 2.07 (m)	29.1	C-2'', 3'', 5''
	1.91 – 1.98 (m)		C-2'', 3'', 5''
5''	4.18 – 4.24 (m)	49.3	C-3'', 4'', 1''', 3'''
	4.09 – 4.14 (m)		C-3'', 4'', 1''', 3'''
6''		171.4	
1'''		161.7	
3'''	8.01 (s)	138.4	C-1''', 4''', 9b''', 5''
3a'''		110.1	
4'''	6.91 (s)	110.6	C-3''', 5''', 6''', 9b'''
5'''		149.2	
6'''		179.0	
6a'''		125.2	
7'''		150.8	
8'''	7.49 (d, 8.1)	132.2	C-6a''', 9a''', 1''''
9'''	8.54 (d, 8.1)	133.4	C-1''', 7''', 9a''', 9b'''
9a'''		124.9	
9b'''		132.8	
1''''		142.9	
2''''/6''''	7.29 - 7.33 (m)	129.2	C-7''', 4''''
4''''	7.38 - 7.42 (m)	128.1	C-1'''', 2'''', 6''''
3''''/5''''	7.38 - 7.42 (m)	128.6	C-1'''', 2'''', 6''''

Table S5. NMR data (500 MHz, acetone-d_6, δ values, J in Hz) of compound **16**

Position	δ_H (mult., J)	δ_C	HMBC
2	4.19 (dd, 11.8, 8.7)	33.3	C-3, 1'
	3.94 (dd, 11.8, 1.5)		C-3, 12, 1'
3	5.87 (dd, 8.7, 1.5)	64.3	C-2, 5, 12, 1'
5		160.8	
5a		124.5	
6	8.55 (d, 8.0)	133.3	C-5, 5a, 8, 11b
7	7.47 (d, 8.0)	131.5	C-6, 8, 8a, 1''
8		151.8	
8a		124.1	
9		178.8	
10		149.2	
11	6.88 (s)	109.4	C-9, 10,11b, 12
11a		104.6	
12		150.6	
1'		169.7	
1''		143.5	
2''/6''	7.34 (dd, 8.0, 2.0)	126.2	C-8, 4''
4''	7.35 - 7.42 (m)	128.3	C-1'', 2'', 6''
3''/5''	7.35 - 7.42 (m)	128.8	C-1'', 2'', 6''

*2-[(1''R)-1''-Carboxy-2''-phenyl-ethyl]-5-hydroxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**7**):* Yellow powder. LC-ESIMS (method B), t_R 46.4 min; UV (MeCN-H_2O) λ_{max} 205, 235, 324, 438 nm; $[\alpha]_D^{22}$ +70.6 (c 0.10, CH_3OH); ^{1}H NMR data, see Table S2; ^{13}C NMR data, see Table S3; ESIMS *m/z* 438 $[M + H]^+$ (100), 897 $[2M + Na]^+$ (19); HRESIMS *m/z* 438.1333 $[M + H]^+$ (calcd for $C_{27}H_{20}NO_5$, 438.1341).

*2-[(1''R)-1''-Carboxy-3''-methyl-n-butyl]-5-hydroxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**8**):*Yellow powder. LC-ESIMS (method B), t_R 48.4 min; UV (MeCN-H_2O) λ_{max} 206, 237, 323, 438 nm; $[\alpha]_D^{22}$ -2.1 (c 0.09, CH_3OH); ^{1}H NMR data, see Table S2; ^{13}C NMR data, see Table

S3; ESIMS *m/z* 404 $[M + H]^+$ (100), 829 $[2M + Na]^+$ (4); HRESIMS *m/z* 404.1487 $[M + H]^+$ (calcd for $C_{24}H_{22}NO_5$, 404.1498).

*2-[(1''R)-1''-Carboxy-2''-phenyl-ethyl]-5-methoxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**9**):* Yellow powder. LC-ESIMS (method B), t_R 44.8 min; UV (MeCN-H_2O) λ_{max} 219, 322, 430 nm; $[\alpha]_D^{22}$ +31.3 (c 0.07, CH_3OH); ^1H NMR data, see Table S2; ^{13}C NMR data, see Table S3; ESIMS *m/z* 452 $[M + H]^+$ (100), 925 $[2M + Na]^+$ (76); HRESIMS *m/z* 452.1492 $[M + H]^+$ (calcd for $C_{28}H_{22}NO_5$, 452.1498).

*2-[(1''R)-1''-Carboxy-3''-methyl-n-butyl]-5-methoxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**10**):*Yellow powder. LC-ESIMS (method B), t_R 47.0 min; UV (MeCN-H_2O) λ_{max} 221, 319, 423 nm; $[\alpha]_D^{22}$ -2.0 (c 0.09, CH_3OH); ^1H NMR data, see Table S2; ^{13}C NMR data, see Table S3; ESIMS *m/z* 418 $[M + H]^+$ (100), 857 $[2M + Na]^+$ (81); HRESIMS *m/z* 418.1642 $[M + H]^+$ (calcd for $C_{25}H_{24}NO_5$, 418.1654).

*2-[(1''S)-1''-Carboxy-2''-phenyl-ethyl]-5-methoxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**11**):*Yellow powder. LC-ESIMS (method B), t_R 43.9 min; UV (MeCN-H_2O) λ_{max} 206, 235, 323, 428 nm; $[\alpha]_D^{22}$ -18.6 (c 0.06, CH_3OH); ^1H NMR data, see Table S2; ^{13}C NMR data, see Table S3; ESIMS *m/z* 452 $[M + H]^+$ (100), 825 $[2M + Na]^+$ (81); HRESIMS *m/z* 452.1495 $[M + H]^+$ (calcd for $C_{25}H_{24}NO_5$, 452.1498).

*2-[(1''S)-1''-Carboxy-2''-(4'''-hydroxyphenyl)-ethyl]-5-methoxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**12**):* Orange powder. LC-ESIMS (method B), t_R 34.9 min; UV (MeCN-H_2O) λ_{max} 206, 231, 321, 429 nm; $[\alpha]_D^{22}$ +4.6 (c 0.09, CH_3OH); ^1H NMR data, see Table S2; ^{13}C NMR data, see Table S3; ESIMS *m/z* 468 $[M + H]^+$ (100), 957 $[2M + Na]^+$ (26); HRESIMS *m/z* 468.1438 $[M + H]^+$ (calcd for $C_{28}H_{22}NO_6$, 468.1447).

*2-[(1''S)-1''-Carboxy-2''-(1H-indol-3'''-yl)-ethyl]-5-methoxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**13**):* Orange powder. LC-ESIMS (method B), t_R 41.4 min; UV (MeCN-H_2O) λ_{max} 205, 220, 241, 279, 321, 431 nm; $[\alpha]_D^{22}$ -4.5 (c 0.06, CH_3OH); ^1H NMR data, see Table S2; ^{13}C NMR data, see Table S3; ESIMS *m/z* 491 $[M + H]^+$ (100), 1003 $[2M + Na]^+$ (35); HRESIMS *m/z* 491.1591 $[M + H]^+$ (calcd for $C_{30}H_{23}N_2O_5$, 491.1607).

*2-[(1''S)-1''-Carboxy-3''-methylthio-propyl]-5-hydroxy-7-phenyl-2H-benzo[de]isoquinoline-1,6-dione (**14**):* Orange powder. LC-ESIMS (method B), t_R 42.4 min; UV (MeCN-H_2O) λ_{max} 207, 233, 322, 436 nm; $[\alpha]_D^{22}$ -2.4 (c 0.04, CH_3OH); ^1H NMR data, see Table S2; ^{13}C NMR data, see Table S3; ESIMS *m/z* 422 $[M + H]^+$ (100), 865 $[2M + Na]^+$ (13); HRESIMS *m/z* 422.1059 $[M + H]^+$ (calcd for $C_{23}H_{20}NO_5S$, 422.1062).

*2-[(1''S)-1''-Carboxy-hexacyl]-5,5'''-hydroxy-7,7'''-phenyl-1'',5''-di-[2H-benzo[de]isoquinoline-1,6-dione] (**15**):*Orange powder. LC-ESIMS (method B), t_R 47.9 min; UV (MeCN-H_2O) λ_{max} 206, 233, 268, 325, 437 nm; $[\alpha]_D^{22}$ -93.9 (c 0.04, CH_3OH); NMR data, see Table S4; ESIMS *m/z* 691 $[M + H]^+$ (100), 404 (4); HRESIMS *m/z* 691.2063 $[M + H]^+$ (calcd for $C_{42}H_{31}N_2O_8$, 691.2080).

(3*R*)*-3-Carboxy-8-phenyl-10-hydroxy-2H-benzo[de]5H-thiazolo[3,2-b]isoquinoline-5,9-dione (**16**):* Orange powder. LC-ESIMS (method B), t_R 41.7 min; UV (MeCN-H_2O) λ_{max} 216, 275, 334, 464 nm; $[\alpha]_D^{22}$ -91.9 (c 0.04, CH_3OH); NMR data, see Table S5; ESIMS *m/z* 392 $[M + H]^+$ (100), 805 $[2M + Na]^+$ (7); HRESIMS *m/z* 392.0584 $[M + H]^+$ (calcd for $C_{21}H_{14}NO_5S$, 392.0593).

3. Phytochemical Re-Investigation of *X. caeruleum*

3.1. HPLC-ESIMS analysis of different tissues

Before being freeze-dried, greenhouse-grown plants were harvested; separated into flowers, aerial green parts, and roots; washed with distilled water; and frozen in liquid nitrogen. Lyophilized material (30 mg) of each part of the plant (flowers, aerial green parts, and roots) was transferred into 2 mL homogenization tubes containing 1.4 mm zirconium dioxide beads and homogenized in 80% acetone-water and acetone, respectively. The six samples, each of which was suspended in 1.5 mL solvent, were homogenized for 1 min at 5.500 rpm in a Minilys cell disruptor (Bertin Technologies, Montigny-le-Bretonneux, France). Subsequently, the homogenate was centrifuged at 13,000 rpm using an Eppendorf centrifuge 5415R (Eppendorf, Wesseling-Berzdorf, Germany) for 10 min at 4 °C. Supernatants were collected and the remaining debris was extracted two more times with each solvent (1.5 mL). The pooled supernatants were dried using a stream of nitrogen gas, dissolved in 100 µL extract solvent, filtered and subjected to HPLC-ESIMS analysis (method B). The chromatograms showed significant differences between the aerial parts and the roots. However, the profiles shared major peaks of glucosides (**1a** - **1c**, **18**, **19**) and weak peaks of aglycones for each part of the plant (Figure S3).

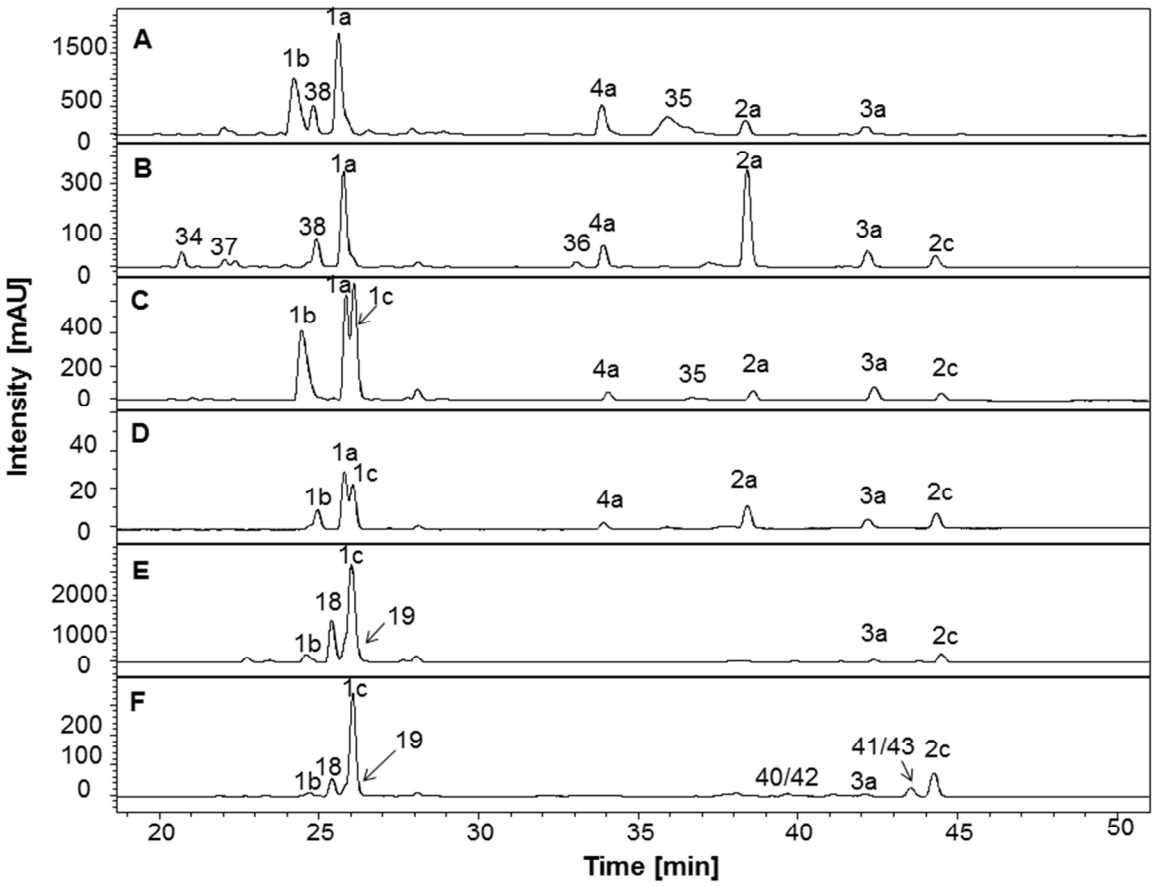


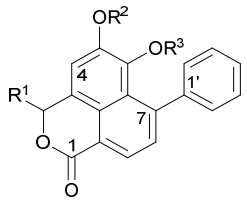
Figure S3. HPLC-ESIMS profile (λ = 254 nm) of acetone and 80% acetone-water extracts of different tissues of *X. caeruleum*. Equal amounts (30 mg) of dried plant material were used in each case to produce 100 µL of the extracts. A) 80% Acetone-water extract of flowers. B) Acetone extract of flowers. C) 80% Acetone-water extract of leaves. D) Acetone extract of leaves. E) 80% Acetone-water extract of roots. F) Acetone extract of roots.

3.2. Isolation of the glucosides and intermediates

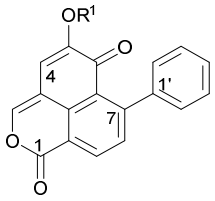
To identify the structures of the compounds in the chromatograms in Figure S3, 3 g of the respective lyophilized plants part (flowers, aerial green parts, and roots) was transferred into a 50 mL polypropylene centrifuge tube and powdered by shaking in the presence of steel balls (2 mm

diameter) in a paint shaker SO-10 m (Fluid Management, Sassenheim, The Netherlands) for 1 - 2 min. The powdered material was suspended in 50 mL 80% acetone-water, shaken on a gyratory shaker (150 rpm) for 3 h at room temperature and then treated in an ultrasonic bath for 3 min. After removal of the solvent, the remaining debris was extracted two more times with 80% acetone-water (50 mL). The pooled extracts were filtered and diluted with 1350 mL (9-fold volume) distilled water. The diluted extract was passed through a preconditioned MN HRX SPE cartridge (500 mg, 6 ml, Macherey–Nagel, Düren, Germany) using a MN PTFE tube adaptor. The loaded cartridges were eluted with MeCN. The three MeCN eluates of flowers, aerial green parts and roots were evaporated using a stream of nitrogen gas to yield residues of 136 mg, 81 mg and 77 mg, respectively, which were subsequently reconstituted in MeCN and subjected to separation using HPLC-SPE or HPLC coupled with a fraction collector (method B). The following compounds were obtained from the flower extracts: 6-*O*-[(6″-*O*-malonyl)-*β*-D-glucopyranosyl]-5-hydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**1a**, 3.4 mg),[2] 3-carboxy-5-hydroxy-6-*O*-*β*-D-glucopyranosyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**1b**, 4.5 mg) (for the isolation of compound **1b**, see 3.3.), 5,6-dihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**2a**, 0.8 mg),[2] 5-hydroxy-7-phenylbenzo[*de*]isochromene-1,6-dione (**3a**, lachnanthopyrone, 0.6 mg),[3] 3,5,6-trihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**4a**, 1.1 mg), 5,6-dihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1,3-dione (**22**, 0.4 mg),[1b] 5-hydroxy-6-*O*-*β*-D-glucopyranosyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**33**, 0.5 mg), 3,5-dihydroxy-6-*O*-*β*-D-glucopyranosyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**34**, 0.6 mg), 3-carboxy-5-hydroxy-6-*O*-malonyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**35**, 0.5 mg), apigenin (**36**, 0.5 mg), 7-*O*-*β*-D-glucopyranosylapigenin (**37**, 0.8 mg), and 7-*O*-(6″-*O*-malonyl-*β*-D-glucopyranosyl)-apigenin (**38**, 1.7 mg).[1b] Furthermore, compound **1a** (1.5 mg), compound **1b** (1.8 mg), 6-*O*-[(6″-*O*-

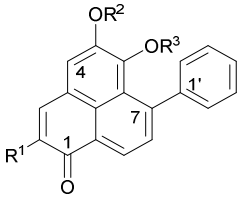
malonyl)-*β*-D-glucopyranosyl]-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**1c**, 2.5 mg),[1b] compound **2a** (0.4 mg), compound **2c** (0.6 mg),[4] compound **3a** (0.4 mg), 5-methoxy-7-phenylbenzo[*de*]isochromene-1,6-dione (**3b**, 1.1 mg),[1b] compound **4a** (0.4 mg), 3,6-dihydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**4b**, 0.5 mg),[1b] compound **33** (0.4 mg), compound **34** (0.7 mg), compound **35** (0.8 mg), and 5-methoxy-6-*O*-*β*-D-glucopyranosyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**39**, 0.7 mg) were isolated from extracts of the aerial green parts.[2] In addition, compound **1b** (0.5 mg), compound **1c** (1.0 mg), compound **2c** (0.5 mg), compound **3a** (0.4 mg), 6-*O*-[(6″-*O*-malonyl)-*β*-D-glucopyranosyl]-2,5-dihydroxy-7-phenylphenalen-1-one (**18**, 0.8 mg)[2], 6-*O*-[(6″-*O*-malonyl)-*β*-glucopyranosyl]-2-methoxy-5-hydroxy-7-phenylphenalen-1-one (**19**, 0.9 mg)[2], a mixture (0.7 mg) of 2,6-dihydroxy-5-methoxy-7-phenylphenalen-1-one (**40**, haemodorin)[5] and 2-methoxy-5,6-dihydroxy-9-phenylphenalen-1-one (**42**)[2], and a mixture (0.6 mg) of 2-methoxy-5,6-dihydroxy-7-phenylphenalen-1-one (**41**)[2] and 2,6-dihydroxy-5-methoxy-9-phenylphenalen-1-one (**43**)[2] were isolated from extracts of the roots. 3-Methoxy-5,6-dihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**20**, 5.1 mg)[2] and 3,5-dimethoxy-6-hydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**21**, 4.3 mg)[2] were obtained from an MeOH extract of 100.0 g of fresh leaves by using HPLC-SPE (method B) (Scheme S1). The structures were elucidated by interpretation of the NMR and MS data.



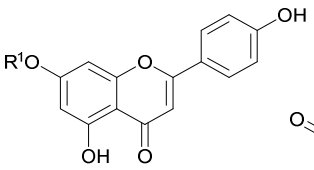
1a R^1 = H, R^2 = H, R^3 = mal-glc
1b R^1 = COOH, R^2 = H, R^3 = glc
1c R^1 = H, R^2 = CH_3, R^3 = mal-glc
2a R^1 = H, R^2 = H, R^3 = H
2c R^1 = H, R^2 = CH_3, R^3 = H
4a R^1 = OH, R^2 = H, R^3 = H
4b R^1 = OH, R^2 = CH_3, R^3 = H
20 R^1 = OCH_3, R^2 = H, R^3 = H
21 R^1 = OCH_3, R^2 = CH_3, R^3 = H
33 R^1 = H, R^2 = H, R^3 = glc
34 R^1 = OH, R^2 = H, R^3 = glc
35 R^1 = COOH, R^2 = H, R^3 = mal
39 R^1 = H, R^2 = CH_3, R^3 = glc



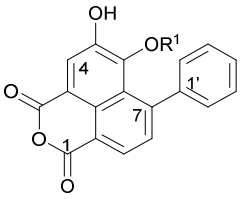
3a R^1 = H
3b R^1 = CH_3



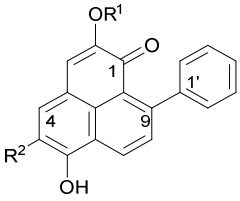
18 R^1 = OH, R^2 = H, R^3 = mal-glc
19 R^1 = OCH_3, R^2 = H, R^3 = mal-glc
40 R^1 = OH, R^2 = CH_3, R^3 = H
41 R^1 = OCH_3, R^2 = H, R^3 = H



36 R^1 = H
37 R^1 = glc
38 R^1 = mal-glc



22 R^1 = H



42 R^1 = CH_3, R^2 = OH
43 R^1 = H, R^2 = OCH_3

	Flowers	Leaves	Roots
Phenylphenalenones (C_{19} skeleton)			**18, 19, 40, 41, 42, 43**
Oxa-Phenylphenalenones ($C_{18}O$ skeleton)	**1a, 1b, 2a, 3a, 4a, 22, 33, 34, 35**	**1a, 1b, 1c, 2a, 2c, 3a, 3b, 4a, 4b, 20, 21, 33, 34, 35, 39**	**1b, 1c, 2c, 3a**
Flavonoids	**36, 37, 38**		

Scheme S1. Structures of compounds **1a** to **43** isolated from *X. caeruleum* and their occurrence in different plant parts. glc, *ß*-glucopyranosyl; mal-glc, 6-malonyl-*ß*-glucopyranosyl.

3.3. Purification of 1b using $(NH_4)_2SO_3$

During the isolation experiments described above (3.2.), we failed to obtain pure compound **1b** using air-exposed open vessels to collect HPLC fractions. HPLC analysis of the fraction collected in this way indicated a mixture of compound **1b** with four additional constituents. We realized that compound **1b** was oxidized either by O_2 from the solvent or the atmosphere.

Therefore, we attempted to use $(NH_4)_2SO_3$ to prevent oxidation. Eventually, compound **1b** was purified when the respective fraction was collected in vials containing 10 mM aqueous $(NH_4)_2SO_3$ (pH 4) (method D). To remove $(NH_4)_2SO_3$, the solution was passed through a HRX SPE cartridge, washed with degassed water and eluted with MeCN. The MeCN eluate was immediately dried using a stream of nitrogen to give pure compound **1b** (Figure S4), which showed analytical data identical with those reported by Opitz.[6]

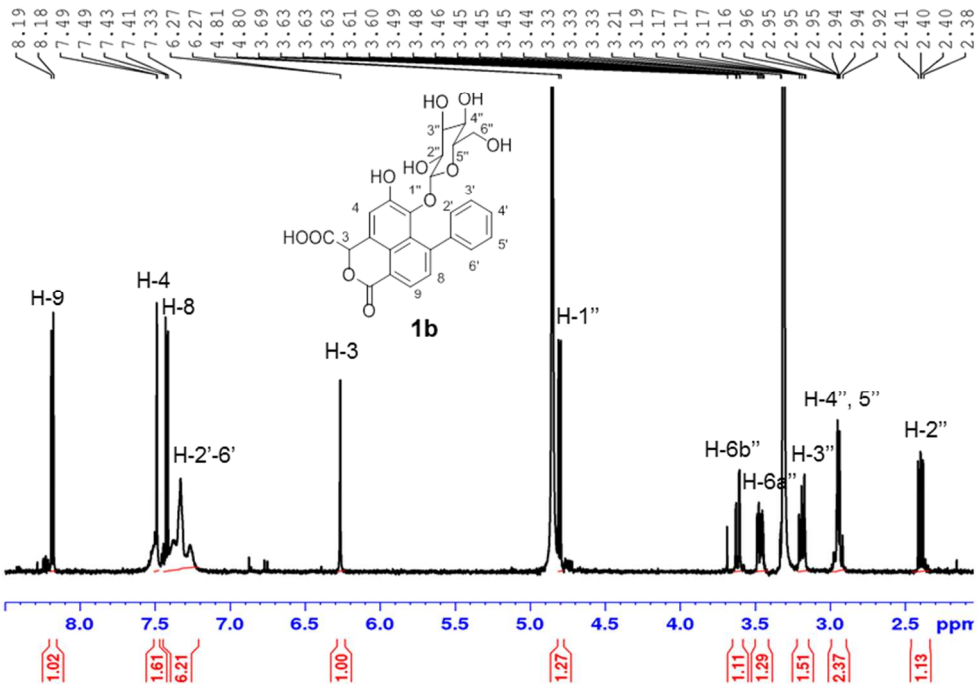


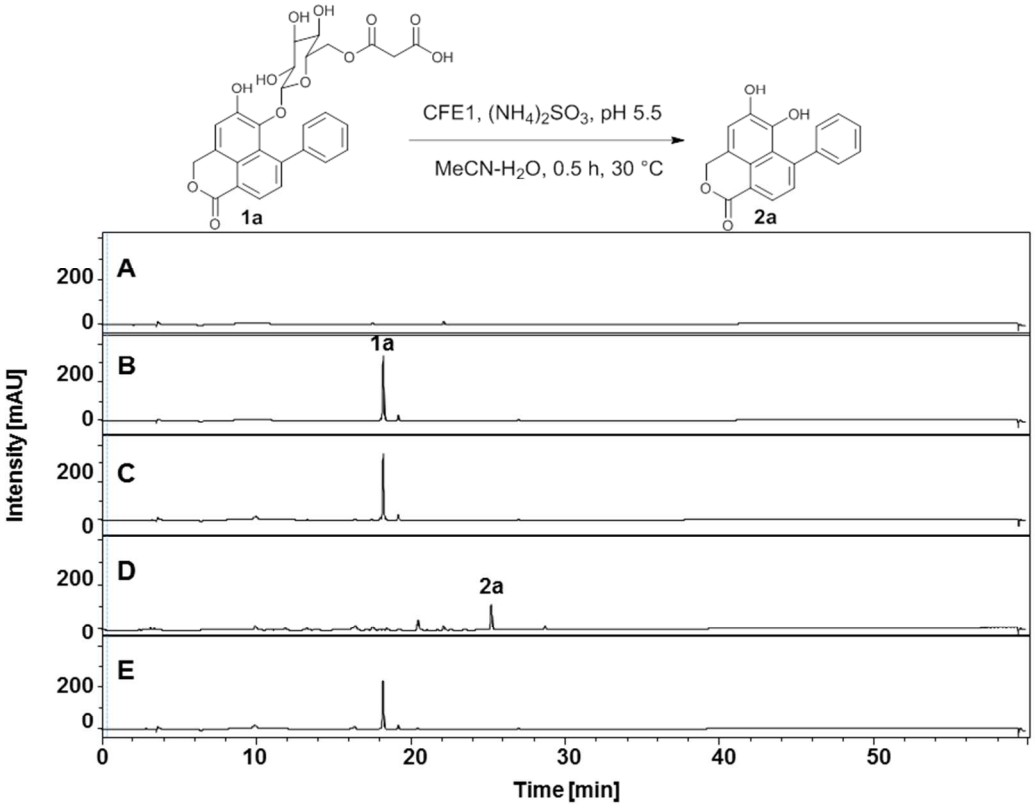
Figure S4. ^{1}H-NMR spectrum of pure compound **1b**.

4. Incubation of Cell-Free Extracts with Isolated Glucosides

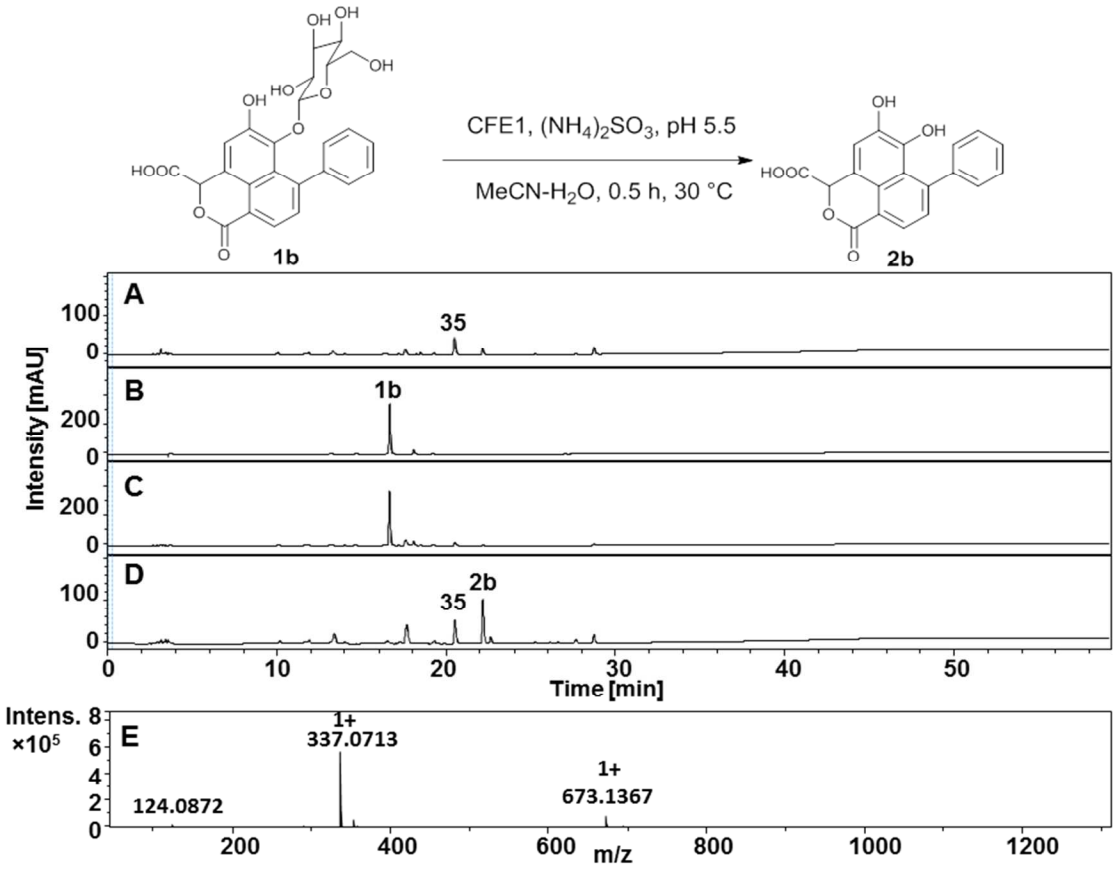
4.1. Enzymatic conversion of the glucosides 1a - 1c to their aglycones by cell-free extracts with oxidation suppression

Fresh *X. caeruleum* leaf material (300 mg) was transferred to a 2 mL homogenization tube containing 1.4 mm zirconium dioxide beads (Bertin Technologies, Montigny-le-Bretonneux, France) in 1.5 mL 0.1 M $(NH_4)_2SO_3$ aqueous solution (containing 0.4% FA, pH 5.5) and was homogenized for 1 min at 5.500 rpm in a Minilys cell disruptor (Bertin Technologies, Montigny-le-Bretonneux, France). Then the homogenate was centrifuged at 16,000 rpm for 20 min at 0 °C (Centrifuge 5415R, Eppendorf, Wesseling-Berzdorf, Germany). The supernatant was used as a cell-free enzyme extract (CFE1). Solutions of glucosides **1a - 1c** (10 µL, 0.8 µg μL^{-1}, in MeCN), 0.1 M $(NH_4)_2SO_3$ (30 µL) and CFE1 (40 µL) were mixed in 2 mL homogenization tubes. The mixture was incubated in a water bath at 30 °C for 30 min. The reaction was terminated by immersing the tube in liquid nitrogen followed by freeze-drying. The same mixture without a glucoside substrate served as a blank. The glucoside substrate in buffer solution and in buffer solution mixed with heat-inactivated CFE1 served as controls. The freeze-dried reaction mixtures of the incubation experiments (blank, control and complete reaction mixtures) were extracted by adding 50 µL 95% MeCN-water and sonicating at room temperature for 5 min. Afterwards, the mixtures were centrifuged and the supernatants were subjected to HPLC-HRESIMS analysis. As shown in Schemes S2.1-S2.3 (D), the chromatograms of the reaction mixture obtained after incubation revealed the presence of the new peaks of the aglycones **2a - 2c**. No such peak was detected in the crude extract blanks (Scheme S2.1-S2.3 (A)), in the buffer solution controls (Scheme S2.1-S2.3 (B)) or in the heat-inactivated controls (Scheme S2.1-S2.3 (C)). When castanospermine (9 mM), an inhibitor of glucosidase, was added to the CFE1, neither aglycones

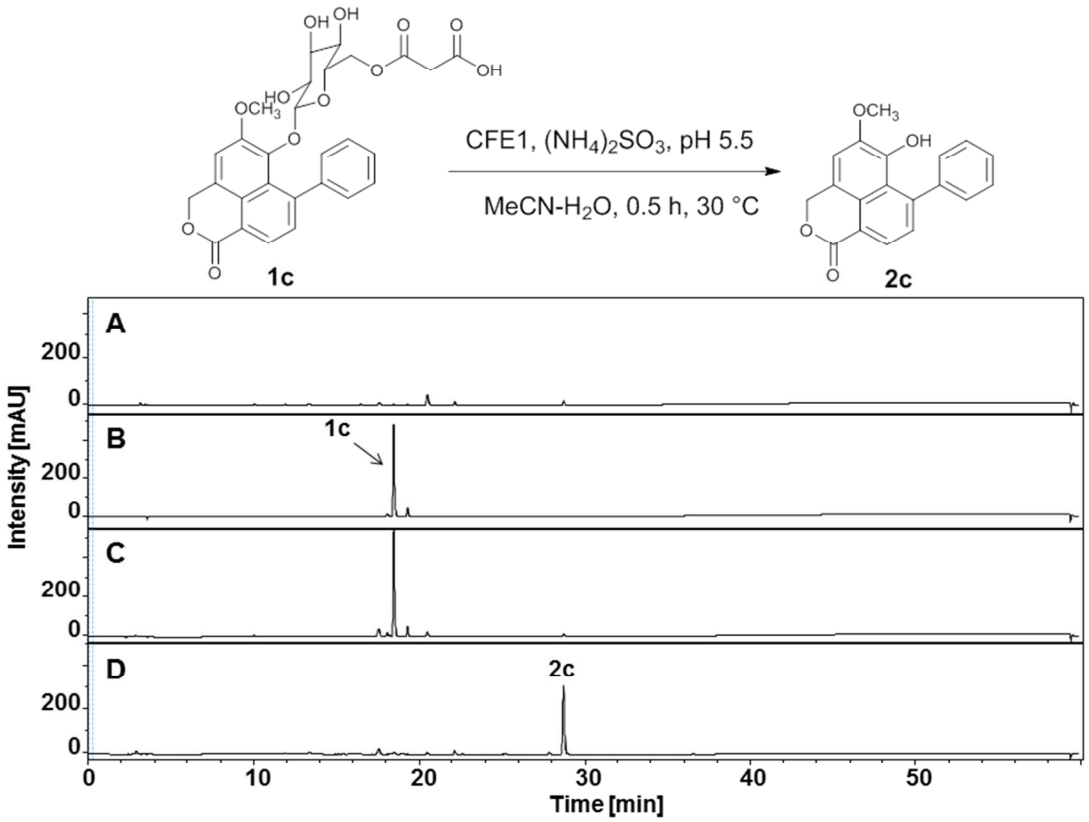
nor other transformates were detected (Scheme S2.1 (E)). The products **2a** and **2c** were identified by comparing their spectroscopic data with those of isolated reference compounds. Compound **2b** was identified by its HRESIMS *m/z* 337.0713 $[M + H]^+$ (calcd for $C_{19}H_{13}O_6$, 337.0707) (Scheme S2.2 (E)). These results clearly indicated that, under conditions preventing oxidation by $(NH_4)_2SO_3$, the glucosides **1a - 1c** were hydrolyzed to their aglycones **2a - 2c** by the CFE1 from the green leaves of *X. caeruleum*. The conversion could be blocked by the action of the glucosidase inhibitor castanospermine.



Scheme S2.1. UV-chromatogram (λ = 254 nm) monitoring the conversion of glucoside **1a** to its aglycone **2a** by cell-free extract 1 (CFE1) from *X. caeruleum* leaves with oxidation suppression by $(NH_4)_2SO_3$. A) CFE1 blank. B) Compound **1a** in buffer solution (control). C) Compound **1a** in heat-inactivated CFE1 (control). D) Complete reaction mixture of compound **1a** in CFE1 solution. E) Compound **1a** in CFE1 solution with castanospermine.



Scheme S2.2. UV-chromatogram (λ = 254 nm) monitoring the conversion of glucoside **1b** to its aglycone **2b** by cell-free extract 1 (CFE1) from *X. caeruleum* leaves with oxidation suppression by $(NH_4)_2SO_3$. A) CFE1 blank. B) Compound **1b** in buffer solution (control). C) Compound **1b** in heat-inactivated CFE1 (control). D) Complete reaction mixture of compound **1b** in CFE1 solution. E) HRESIMS spectrum of compound **2b**.

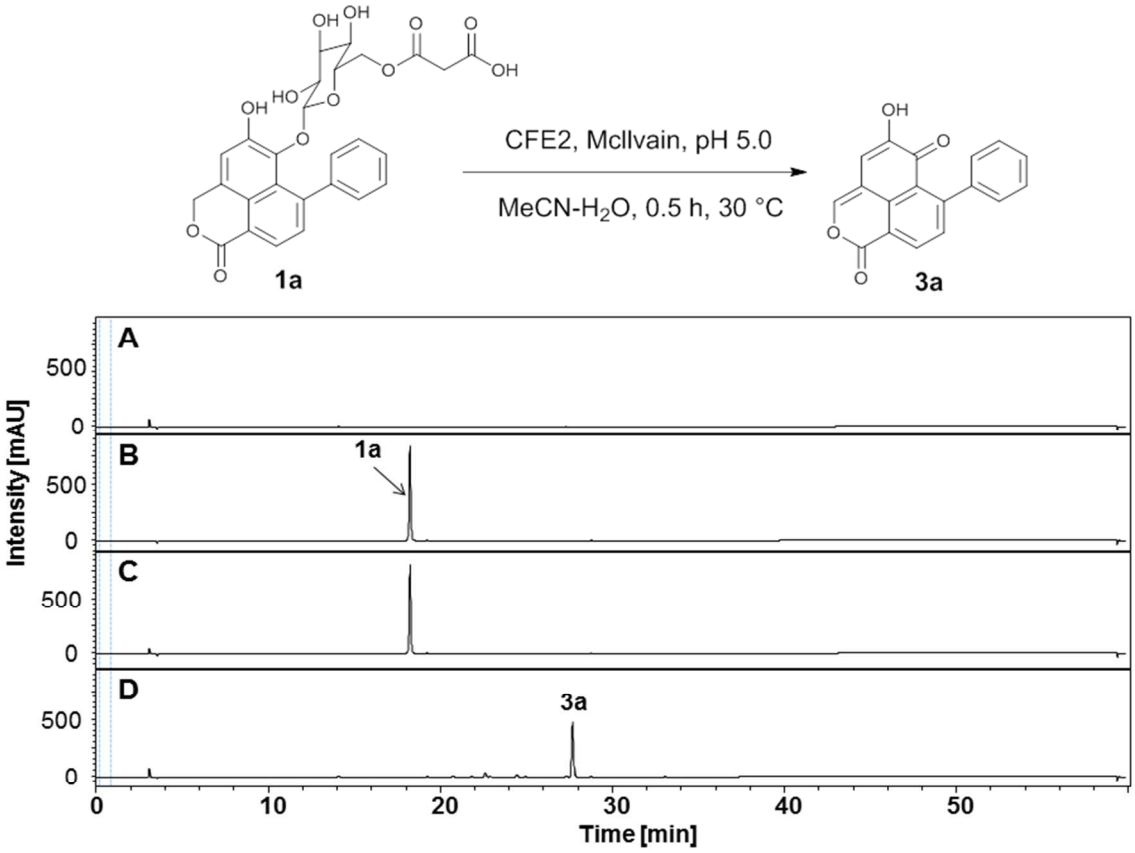


Scheme S2.3. UV-chromatogram (λ = 254 nm) monitoring the conversion of glucoside **1c** to its aglycone **2c** by cell-free extract 1 (CFE1) from *X. caeruleum* leaves with oxidation suppression by $(NH_4)_2SO_3$. A) CFE1 blank. B) Compound **1c** in buffer solution (control). C) Compound **1c** in heat-inactivated CFE1 (control). D) Complete reaction mixture of compound **1c** in CFE1 solution.

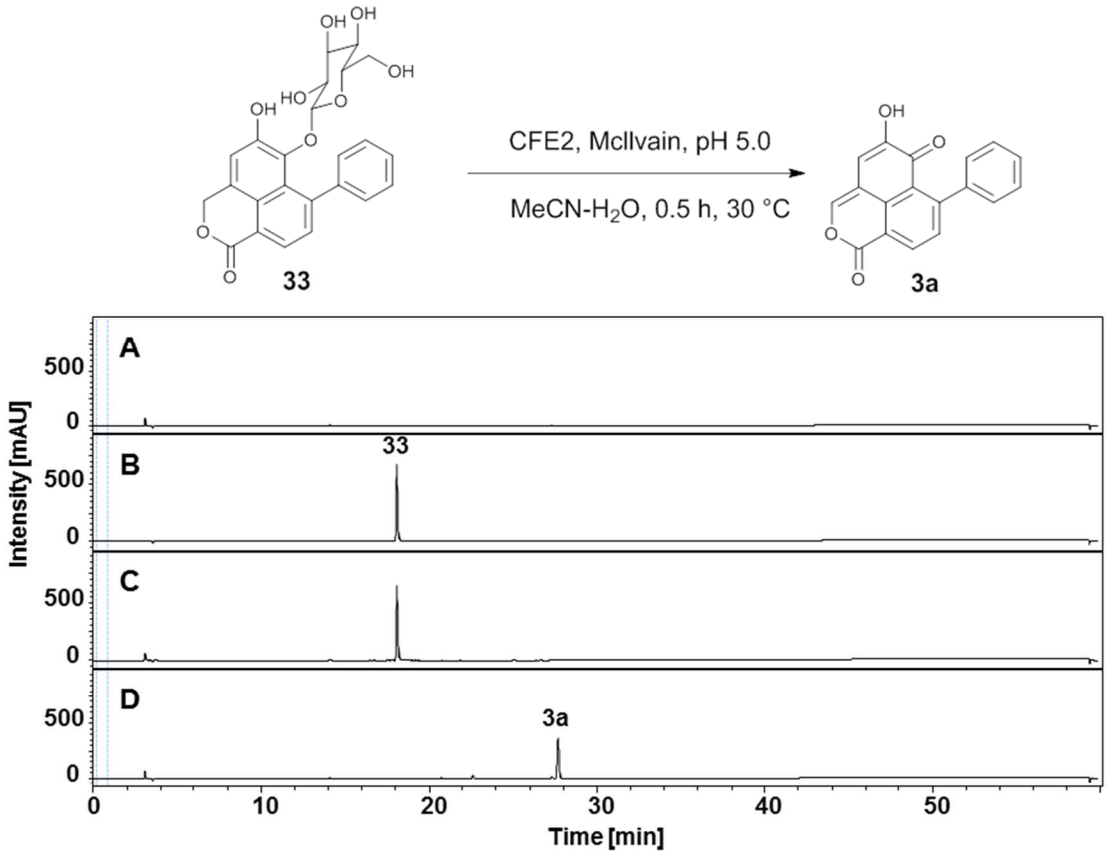
4.2. Enzymatic conversion of the glucosides 1a - 1c, 33, 39 to 3a or 3b by cell-free extracts

As in the method described in 4.1., incubations with cell-free extract incubations were conducted, except that McIlvain buffer (0.1 M, pH 5.0) was used instead of aqueous $(NH_4)_2SO_3$ solution (CFE2). Compound **1a** (1.6 µg µL^{-1}), compound **1b** (0.8 µg µL^{-1}), compound **1c** (0.8 µg µL^{-1}), compound **33** (1.6 µg µL^{-1}) and compound **39** (0.5 µg µL^{-1}) in MeCN were used as

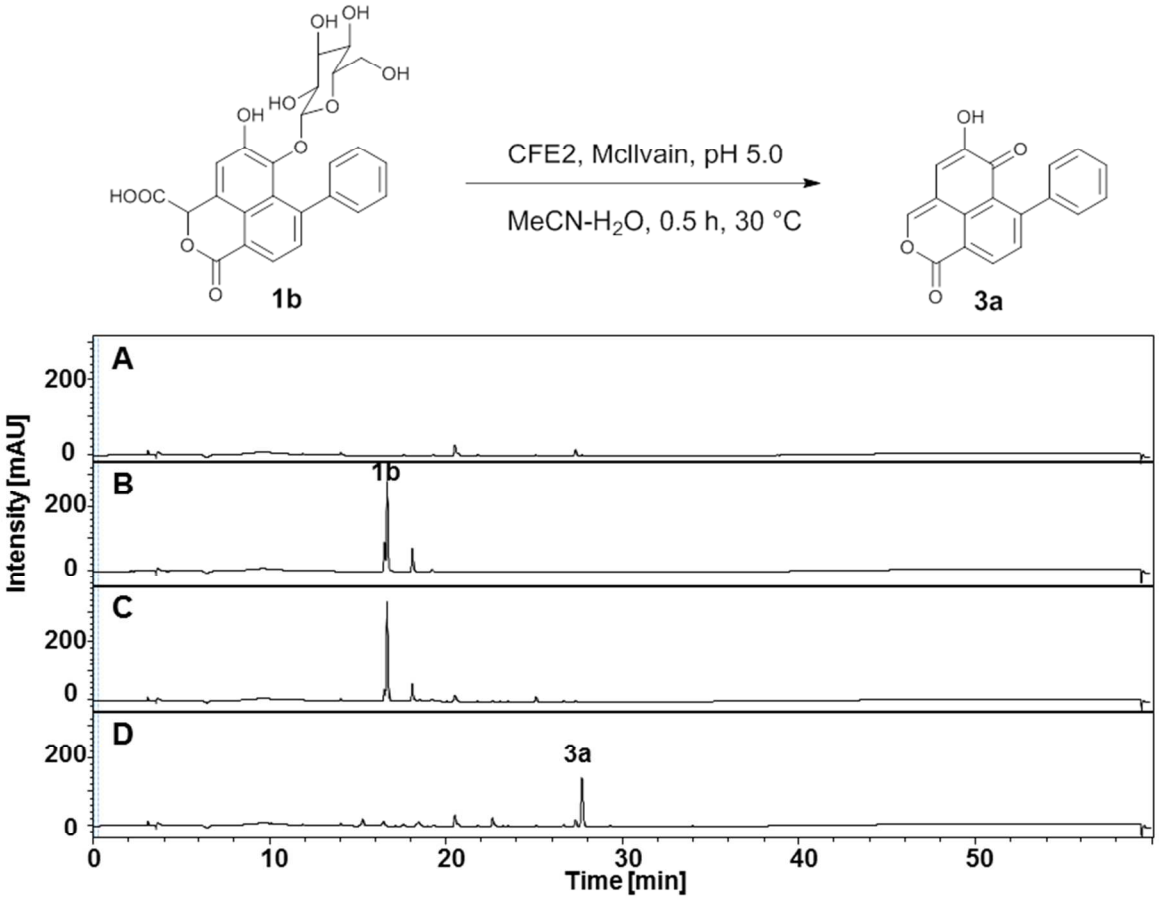
substrates. The products lachnanthopyrone (**3a**) and 5-methoxy-lachnanthopyrone (**3b**) were identified by comparing their spectroscopic data with those of isolated reference compounds. As shown in Scheme S3.1-S3.5, the 5-hydroxy-PBIC glucosides **1a**, **1b** and **33** were converted to compound **3a**, while the 5-methoxy-PBIC glucosides **1c** and **39** were converted to compound **3b**.



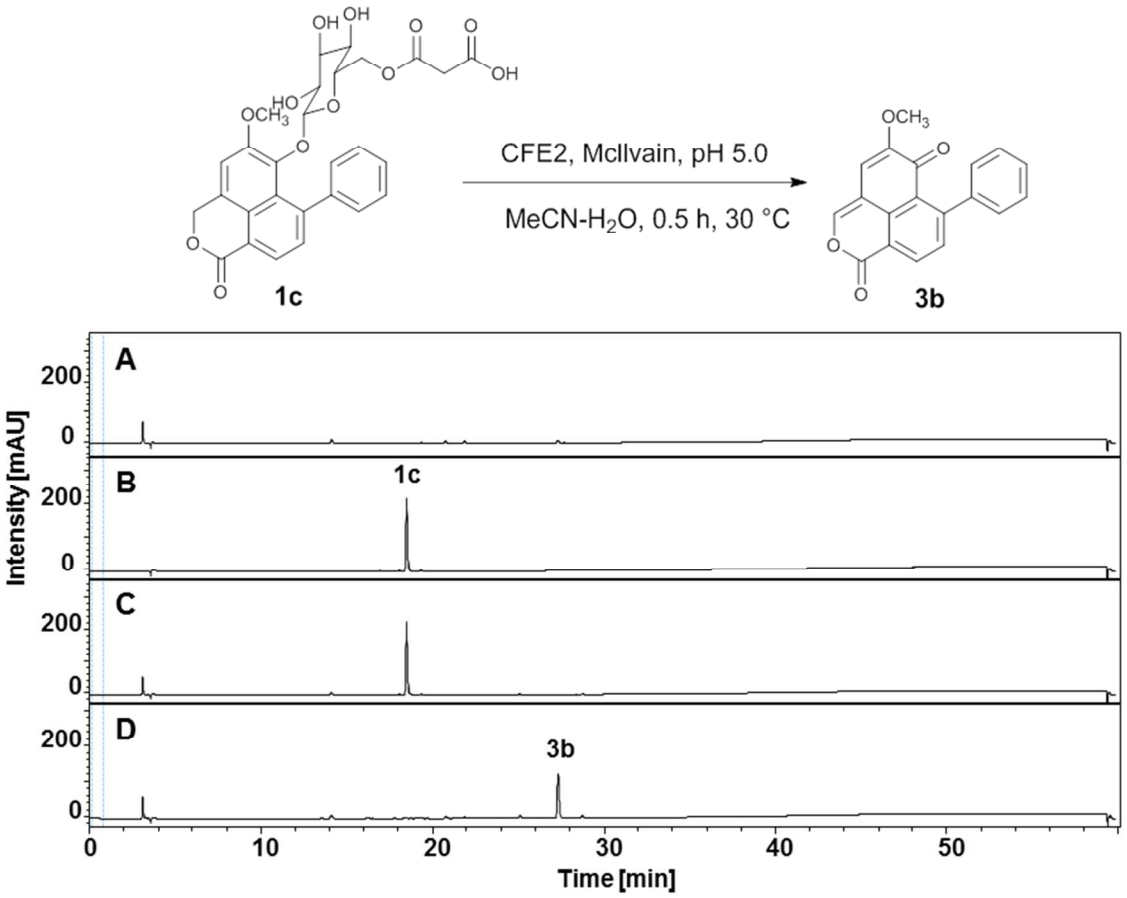
Scheme S3.1. UV-chromatogram (λ = 254 nm) monitoring the conversion of glucoside **1a** to compound **3a** by the cell-free extract 2 (CFE2). A) CFE2 blank. B) Compound **1a** in buffer solution (control). C) Compound **1a** in heat-inactivated CFE2 (control). D) Complete reaction mixture of compound **1a** in CFE2 solution.



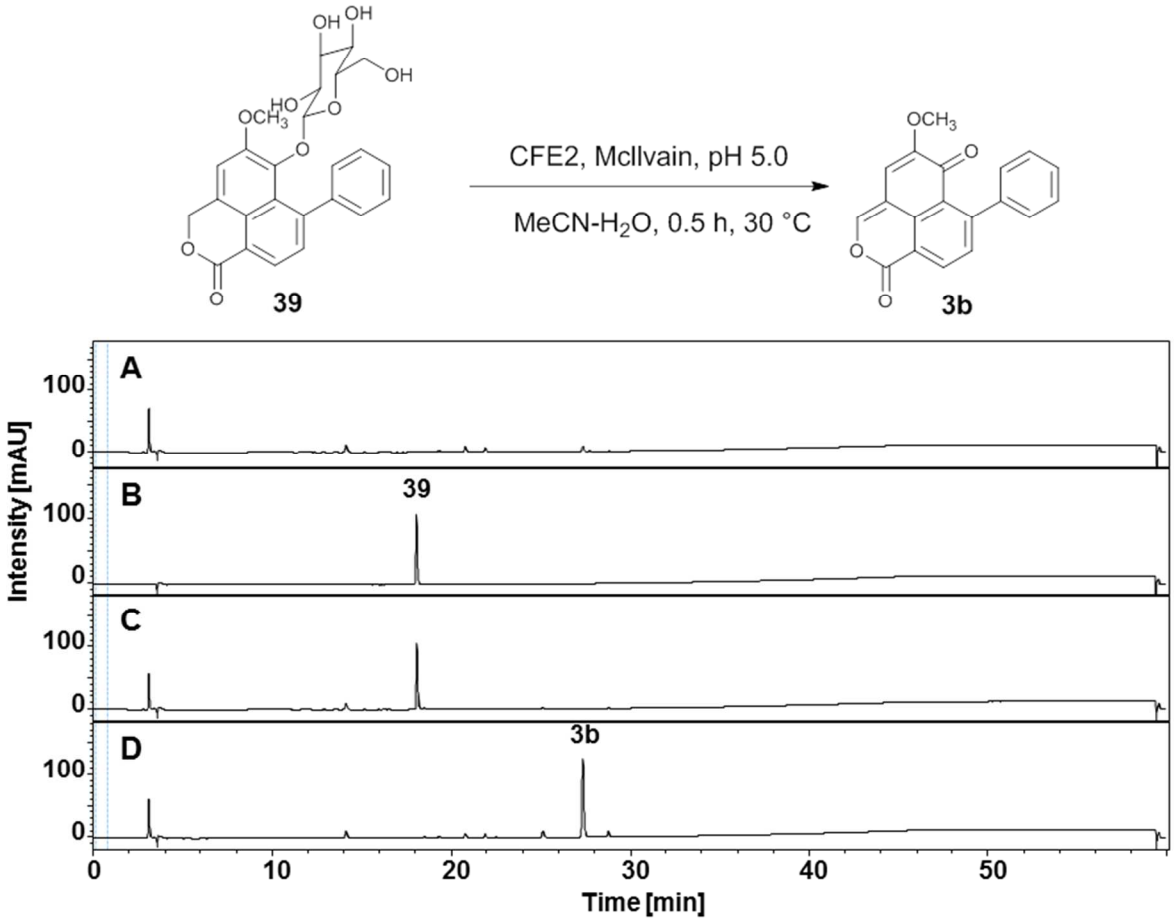
Scheme S3.2. UV-chromatogram (λ = 254 nm) monitoring of the conversion of glucoside **33** to compound **3a** by cell-free extract 2 (CFE2). A) CFE2 blank. B) Compound **33** in buffer solution (control). C) Compound **33** in heat-inactivated CFE2 (control). D) Complete reaction mixture of compound **33** in CFE2 solution.



Scheme S3.3. UV-chromatogram (λ = 254 nm) monitoring of the conversion of glucoside **1b** to compound **3a** by cell-free extract 2 (CFE2). A) CFE2 blank. B) Compound **1b** in buffer solution (control). C) Compound **1b** in heat-inactivated CFE2 (control). D) Complete reaction mixture of compound **1b** in CFE2 solution.



Scheme S3.4. UV-chromatogram (λ = 254 nm) monitoring of the conversion of glucoside **1c** to compound **3b** by cell-free extract 2 (CFE2). A) CFE2 blank. B) Compound **1c** in buffer solution (control). C) Compound **1c** in heat-inactivated CFE2 (control). D) Complete reaction mixture of compound **1c** in CFE2 reaction solution.

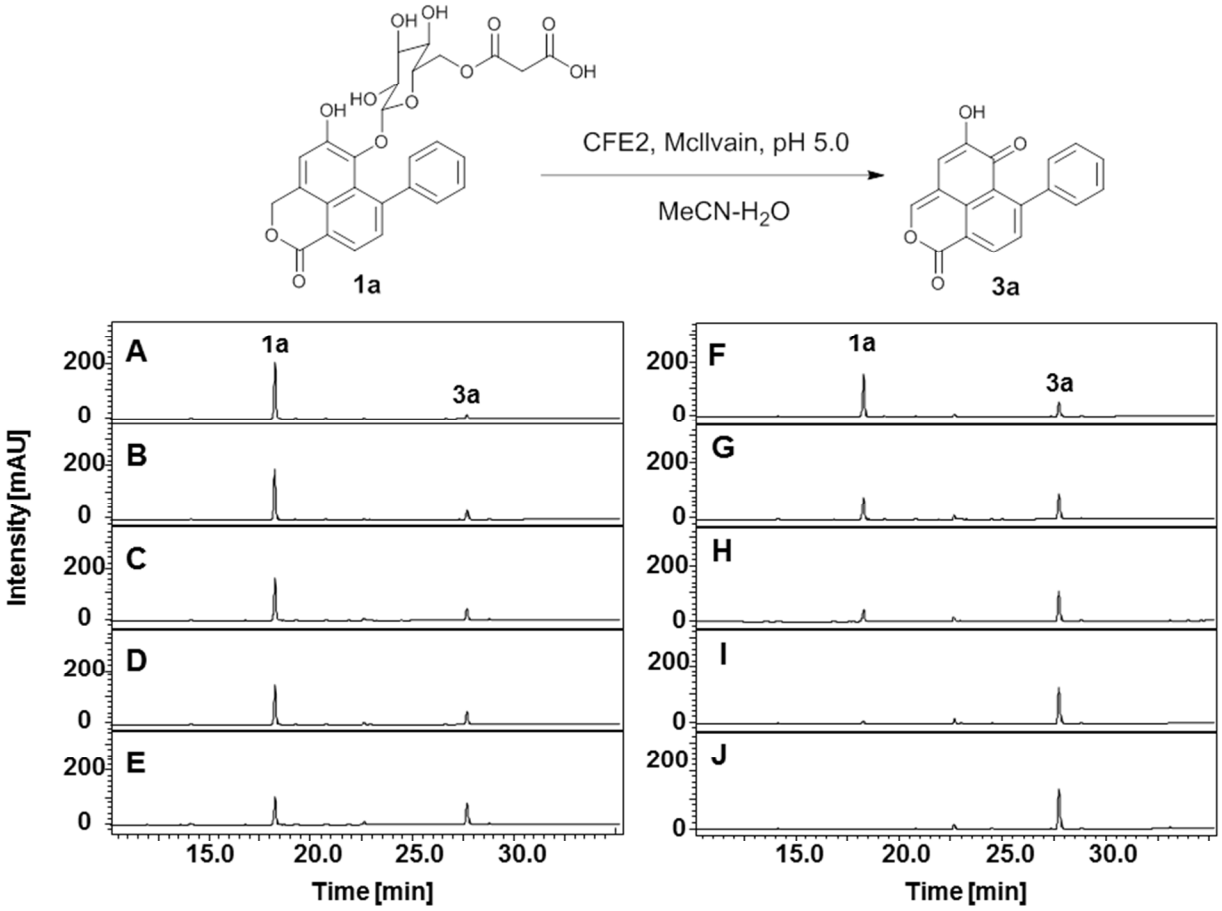


Scheme S3.5. UV-chromatogram (λ = 254 nm) monitoring of the conversion of glucoside **39** to compound **3b** by cell-free extract 2 (CFE2). A) CFE2 blank. B) Compound **39** in buffer solution (control). C) Compound **39** in heat-inactivated CFE2 (control). D) Complete reaction mixture of compound **39** in CFE2 solution.

4.3. The conversion of glucoside 1a to compound 3a is time- and temperature-dependent

The conversion of glucoside **1a** (0.6 μg μL^{-1}, in MeCN) to compound **3a** by CFE2 was repeated at 10°C (Scheme S4 A-E) and 20 °C (Scheme S4 F-J) for different periods of time (3, 6, 9, 18,

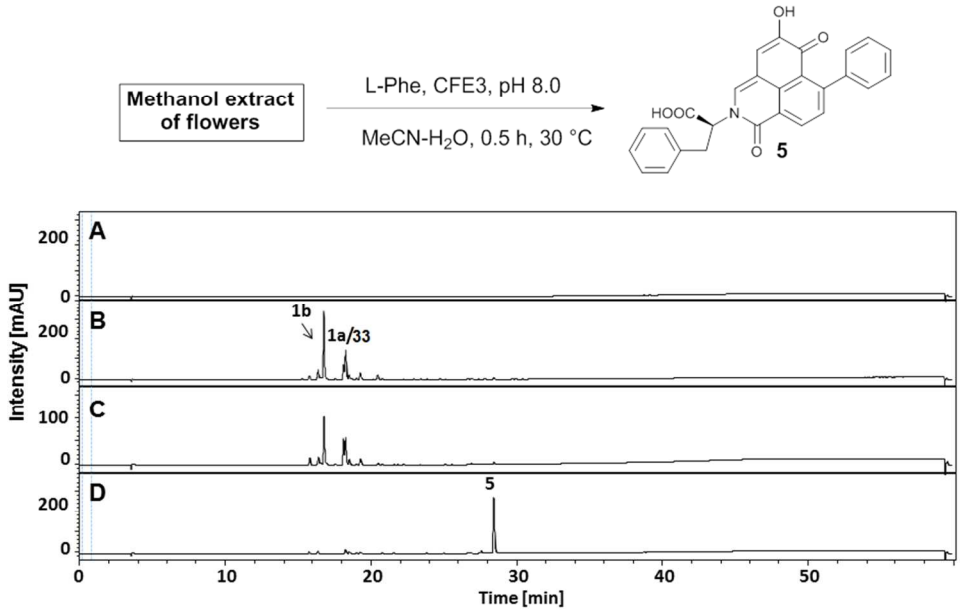
30 min). The peak areas of compound **3a** observed under different incubation conditions indicated direct conversion and yield improving with increasing time and rising temperature.



Scheme S4. UV-chromatogram (λ = 254 nm) monitoring of the conversion of glucoside **1a** to compound **3a** by the cell-free extract 2 (CFE2) at different temperatures and different reaction times. A) 10 °C, 3 min. B) 10 °C, 6 min. C) 10 °C, 9 min. D) 10 °C, 18 min. E) 10 °C, 30 min. F) 20 °C, 3 min. G) 20 °C, 6 min. H) 20 °C, 9 min. I) 20 °C, 18 min. J) 20 °C, 30 min.

4.4. Conversion of the glucosides 1a, 1b, and 33 to PBIQ 5 by cell-free extracts

For the preparation of cell-free extract 3 (CFE3), experimental conditions of incubation and HPLC-HRESIMS analysis was the same as described above (4.1.), except that McIlvain buffer (0.1 M) with pH 8.0 was used. An MeOH extract, containing glucosides **1a**, **1b** and **33** as the major constituents, was obtained by extracting lyophilized flowers of *X. caeruleum*. The sample was prepared using the method described in **3.1**. A 50% MeCN–water solution of the substrate mixture containing a total amount of glucosides **1a**, **1b** and **33** of 2 µg μL^{-1} was used. The product PBIQ **5** was identified by comparing its spectroscopic data with data of **5** isolated from the plant. As shown in Scheme S5, all of the 5-hydroxy-PBIC glucosides in the extract, **1a**, **1b** and **33**, were converted to PBIQ **5**.

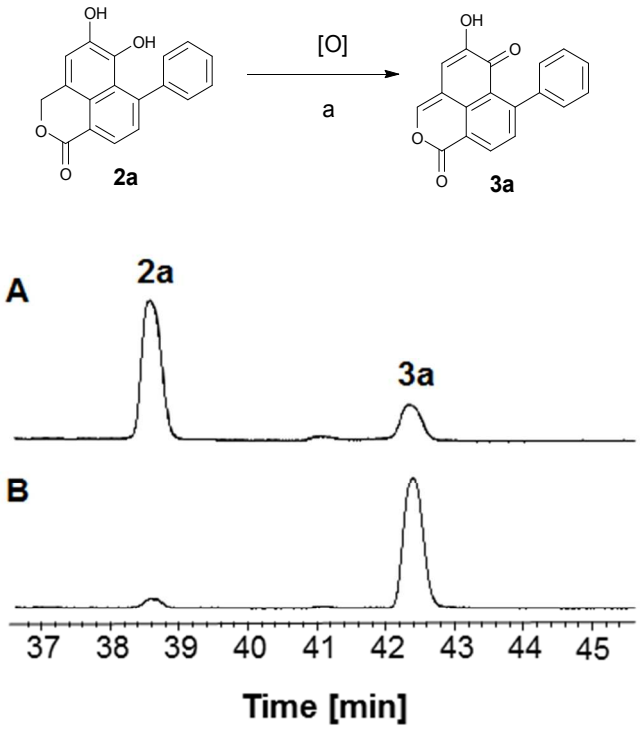


Scheme S5. UV-chromatogram (λ = 254 nm) monitoring of the conversion of compounds **1a**, **1b** and **33** to compound **5** by cell-free extract (CFE3) in pH 8.0 McIlvain buffer. A) CFE3 blank. B) Substrates in buffer solution (control). C) Substrates in heat-inactivated CFE3 (control). D) Complete reaction mixture in CFE3 solution.

5. Non-Enzymatic Conversions of Isolated Metabolites

5.1. Conversion of compound 2a to compound 3a.

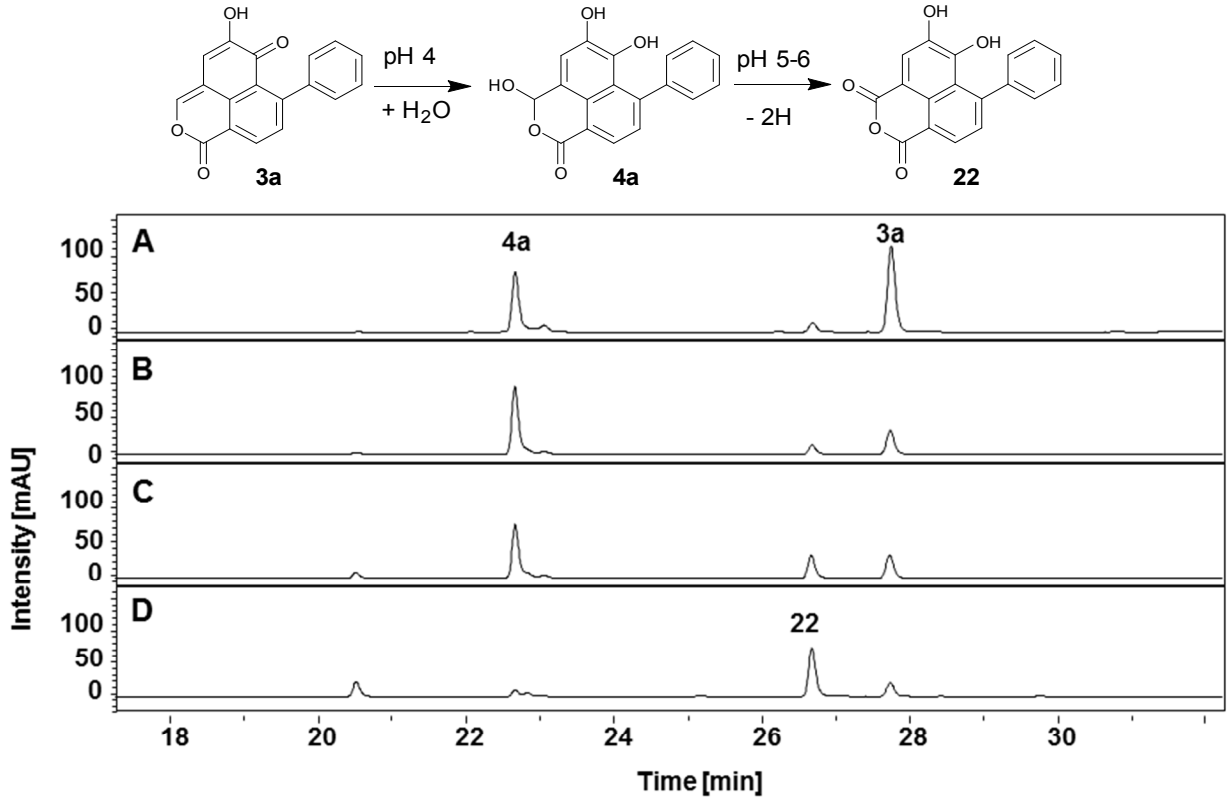
A solution of compound **2a** (70 µg, containing a small amount of compound **3a**; Scheme S6A) in 70 µL MeCN was kept in a stream of dried air at room temperature. After 20 h, HPLC-ESIMS analysis indicated that most of compound **2a** had been oxidized to compound **3a** (Scheme S6B).



Scheme S6. UV-chromatogram (λ = 254 nm) monitoring of the oxidation of compound **2a** in dried air to compound **3a**. Reaction condition: a) dried air, room temperature, 20 h. A) Solution of reactant **2a** containing a small amount of compound **3a**. B) Product mixture after oxidation.

5.2. Conversion of compound 3a to compounds 4a and 22

Four substrate solutions (compounds **3a** and **4a** in a 2:3 ratio, total amount of 1 μg μL^{-1} in 10 μL MeCN) were individually diluted with McIlvain buffer solutions of different pH (0.1 M, pH 4.0, 5.0, and 6.0, 40 μL) or, for a control, with 40 μL MeCN. After being maintained at room temperature, the mixtures were subjected to HPLC-HRESIMS analysis at 0.5, 1.5, 3.5, and 5.5 h. The analyses indicated that most of compound **3a** was transformed to compound **4a** by a hydration reaction at pH 4.0 - 5.0 (Scheme S7 A-C). After increasing the pH value of the buffer to pH 5.0-6.0, compound **4a** was oxidized to compound **22** (Scheme S7 D), a compound first reported from *Lachnanthes tinctoria*[7] and later from *X. caeruleum*[1b] and *Wachendorfia thyrsiflora*[2].

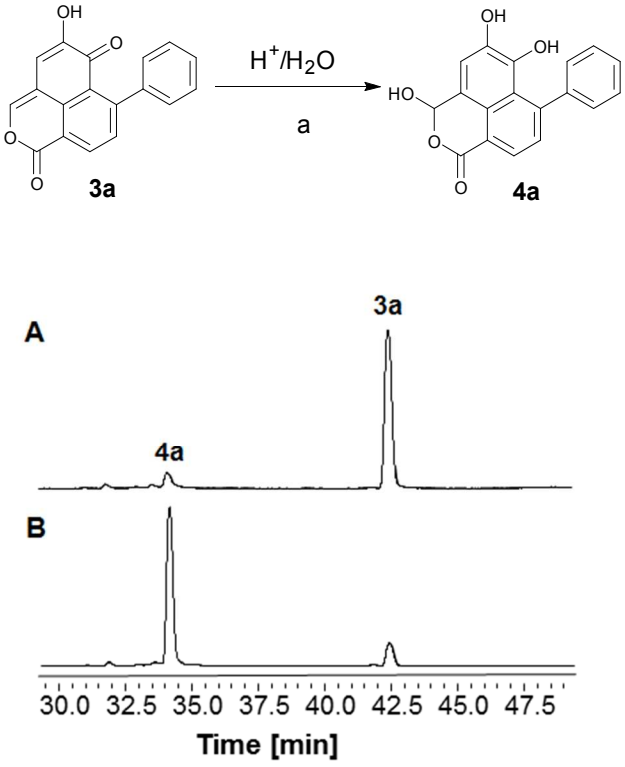


Scheme S7. UV-chromatogram (280 nm) monitoring the conversion of compound **3a** to compounds **4a** and **22** in buffer of different pH values at room temperature. A) Initial reaction

mixture of compounds **3a** and **4a**. B) Reaction mixture, pH 4.0, after 1.5 h. C) Reaction mixture, pH 5.0, after 3.5 h. D) Reaction mixture, pH 6.0, after 5.5 h.

5.3. Hydration of compound 3a to compound 4a

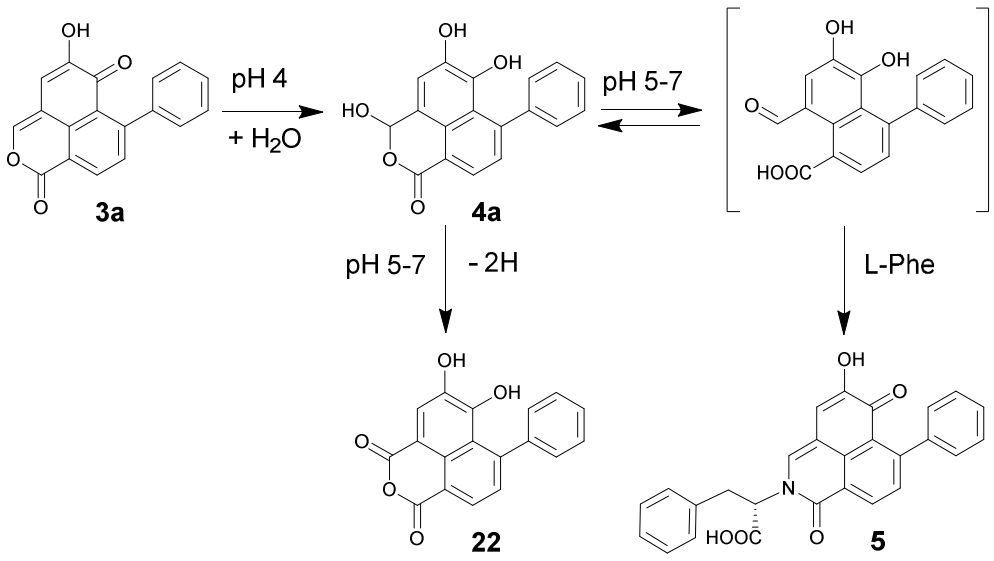
In order to substantiate conversion of compound **3a** to compound **4a**, compound **3a** (15 μg) was dissolved in 60 μL 25% MeCN-water (0.075% FA), sealed and left standing at room temperature for 4 h. HPLC-ESIMS analysis indicated that most of **3a** was hydrated to **4a** (Scheme S8).

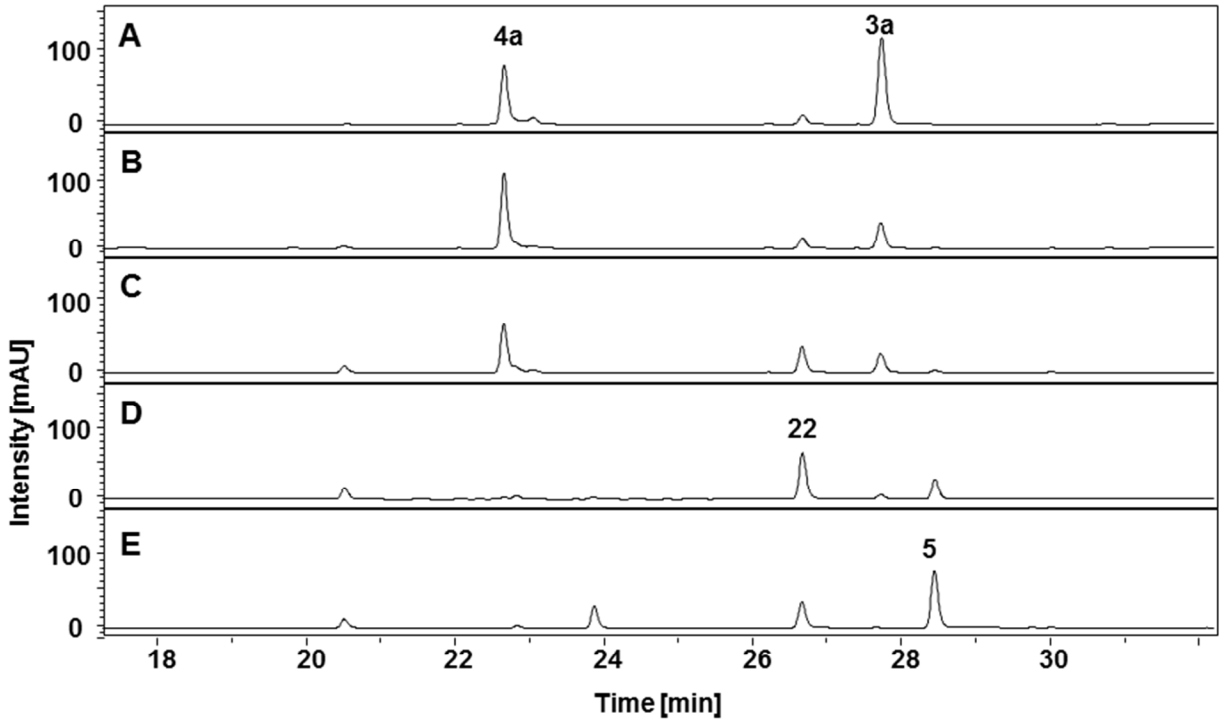


Scheme S8. UV-chromatogram (λ = 254 nm) monitoring the hydration of compound **3a** in weakly acidic solution to give compound **4a**. Reaction condition: a) 25% MeCN-water (0.075% FA), room temperature, 4 h. A) Reactant **3a** solution containing a small amount of compound **4a**. B) Product mixture after hydration.

5.4. Conversion of compound 3a to PBIQ 5

The experiments demonstrating conversion of compound **3a** to compound **22** described above (5.2.) were modified by adding 1.8 equiv. L-phenylalanine (1 μg μL^{-1} in McIlvain buffer pH 4.0, 5.0, 6.0, and 7.0, 10 μL) to each reaction mixture. As shown in Scheme S9 A-B, most of compound **3a** was transformed to compound **4a** at pH 4.0. PBIQ **5** started to appear at pH 5.0 (Scheme S9 C), and its yield increased with increasing pH (Scheme S9 D-E). Based on the gradual disappearance of compound **4a** between pH 5.0 and 6.0 and the simultaneous emergence of PBIQ **5** (Scheme S9 C-D), a precursor-product relationship was suggested between the two compounds. Opening the hydroxylactone ring of compound **4a** generates the intermediate 1-carboxy-5,6-dihydroxy-4-phenylnaphthalene-8-aldehyde, which forms a Schiff base with the amino acid and finally closes the ring to form PBIQ **5**. As shown above (Scheme S7 D), compound **4a** is also the precursor of the anhydride **22**, which was observed as a minor product.



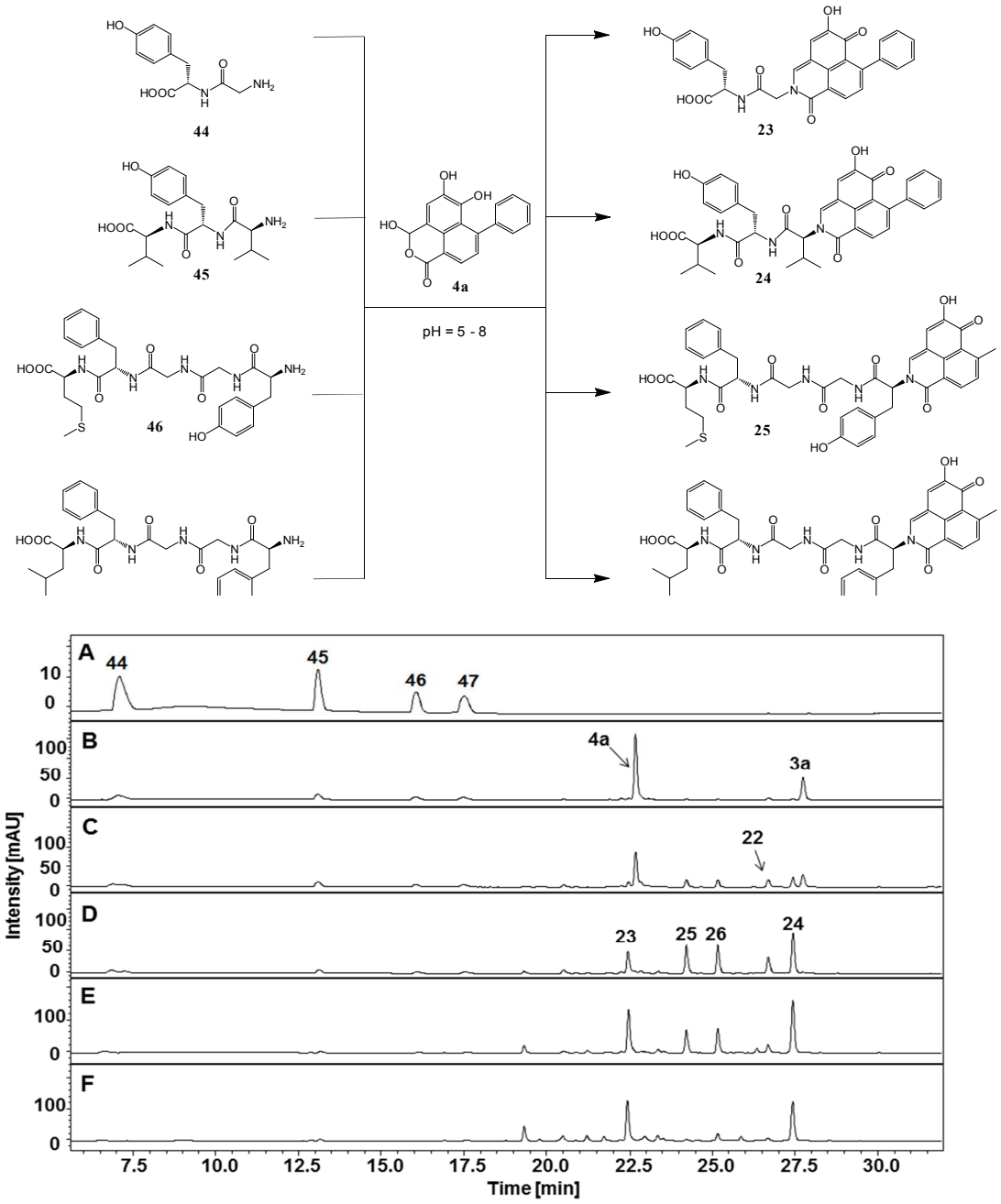


Scheme S9. UV-chromatogram (λ = 280 nm) monitoring the conversion of compound **3a** to PBIQ **5** in the presence of L-phenylalanine in buffer of different pH at room temperature. A) Substrate mixture of compounds **3a** and **4a**. B) Reaction mixture, pH 4.0, after 2.5 h. C) Reaction mixture, pH 5.0, after 4.5 h. D) Reaction mixture, pH 6.0, after 6.5 h. E) Reaction mixture, pH 7.0, after 8.5 h.

6. *N*-terminal Modification of Peptides with PBIC 4a

A HPLC peptide standard mixture (Sigma-Aldrich, H2016-1VL) containing 0.5 mg TYR-GLY (**44**), 0.5 mg VAL-TYR-VAL (**45**), 0.5 mg MET-PHE-GLY-GLY-TYR (methionine enkephalin, **46**) and 0.5 mg LEU-PHE-GLY-GLY-TYR (leucine enkephalin, **47**) was dissolved in 2 mL ultrapure water and used as a peptide substrate solution (0.25 μg μL^{-1} for each peptide). Solutions of compounds **4a** and **3a** (7:3 ratio, 1 μg μL^{-1} in MeCN, 10 μL) were individually mixed with 20

μL of the peptide solution in 2.0 mL Eppendorf micro-reaction vessels, and McIlvain buffer (30 μL, 0.1 M) of different pH (4.0, 5.0, 6.0, 7.0, and 8.0, 30 μL) was added. The reaction mixtures were incubated in a water bath (30 °C) for 2 h and centrifuged at 13,000 rpm using an Eppendorf centrifuge 5415R (Eppendorf, Wesseling-Berzdorf, Germany) for 3 min at 20 °C. Then the centrifuged solutions were subjected to HPLC-HRESIMS analysis (method A). The initial peptide solution served as a reference (Scheme S10 A). The results indicated that four new peaks corresponding to compounds **23** – **26** started to form at pH 5.0 (Scheme S10 C). With the consumption of PBICs **4a** and **3a** and the peptides, the yield of products **23** - **26** increased with the basicity of the buffer (Scheme S10 D-E). HRESIMS spectra indicated a $C_{18}H_8O_3$ fragment corresponding to the phenylphenalenone moiety in each of compounds **23** - **26** (Table S6). The $C_{18}H_8O_3$ fragment also existed in the PBIQs produced from amino acids by incubating extracts from *X. caeruleum* plant material (Table S1). The results suggested that the free amino group of the peptides reacted with compound **4a** to yield peptides modified at the *N*-terminus. In the UV spectra, an enhanced absorbance at λ_{max} 320-325 and 435-440 nm, which are characteristic of the PBIQs, also confirmed that each peptide had reacted with compound **4a**. At pH 8, the derivatives **23** and **24** with short chain peptide were the major products (Scheme S10 F).



Scheme S10. UV-chromatogram (λ = 280 nm) monitoring *N*-terminal modification of peptides by reaction with compound **4a** in buffers of different pH value at 30 °C after 2 h incubation. A) Peptide substrate, a mixture of compounds **44** – **47**. B) Reaction mixture, pH 4.0. C) Reaction

mixture, pH 5.0. D) Reaction mixture, pH 6.0. E) Reaction mixture, pH 7.0. F) Reaction mixture, pH 8.0.

Table S6. HPLC-HRESIMS data of the peptide adducts **23** - **26** and their corresponding substrate peptides **44 – 47**.

Corresponding Peptide Substrates			Adducts						Increased Fragment
No.	t_R (min)	Molecular Formula	No.	t_R (min)	$[M + H]^+$ (*m/z*)	Molecular Formula	$[M + H]^+$ (Cal.)	Err. (ppm)	
44	7.1	$C_{11}H_{14}N_2O_4$	**23**	22.5	511.1511	$C_{29}H_{22}N_2O_7$	511.1500	-2.1	$C_{18}H_8O_3$
45	13.1	$C_{19}H_{29}N_3O_5$	**24**	27.5	652.2666	$C_{37}H_{37}N_3O_8$	652.2653	-2.0	$C_{18}H_8O_3$
46	16.1	$C_{27}H_{35}N_5O_7S$	**25**	24.2	846.2807	$C_{45}H_{43}N_5O_{10}S$	846.2803	-0.4	$C_{18}H_8O_3$
47	17.5	$C_{28}H_{37}N_5O_7$	**26**	25.2	828.3241	$C_{46}H_{45}N_5O_{10}$	828.3239	-0.2	$C_{18}H_8O_3$

7. Antimicrobial tests

Bioassays were conducted at 37 °C using bacterial and fungal cultures (Table S7). *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *M. vaccae* were cultivated on standard I nutrient agar (NA I) in Petri dishes. *S. salmonicolor* and *P. notatum* were cultivated on malt agar (MA). *C. albicans* was cultivated on yeast morphology agar (YMA). Fungus assays were conducted at 30 °C. After inoculation, a disc (9 mm in diameter) was removed from the center of the Petri dish and 50 µl of

the test solution was added to the cavity. After 18 h of incubation at the respective temperatures, the inhibiting areola was measured (Table S8).

Table S7: Microorganisms used for bioassays and media used for cultivating microorganisms

Strain	Collection/strain number
Bacillus subtilis	JMRC:STI:10880
Escherichia coli	JMRC:ST:33699
Mycobacterium vaccae	JMRC:STI:10670
Pseudomonas aeruginosa	JMRC:ST:33771
Pseudomonas aeruginosa	JMRC:ST:33772
Staphylococcus aureus	JMRC:STI:10760
Staphylococcus aureus (MRSA)	JMRC:ST:33793
Candida albicans	JMRC:STI:50163
Penicillium notatum	JMRC:STI:50164

NA1 Standard I nutrient agar (Merck Millipore, order no. 107881)

YMA Yeast morphology agar, Wickerham formula (BD Difco™, order no. 239320)

MA Malt agar: 40 g l^{-1} standard malt extract (Carl Roth, Karlsruhe, Germany), 4 g l^{-1} yeast (Ohly, Hamburg, Germany), 1 l aqua dest., 15 g l^{-1} agar-agar (Carl Roth, Karlsruhe, Germany), pH 5.7-6.0.

Table S8: Results of the antimicrobial tests. Numbers represent the diameter of the inhibiting areola in mm. Ciprofloxacin and amphotericin B were used as standards.

Compounds	Concentration	Solvent	*Bacillus subtilis* 6633 B1	*Staph. aureus* 511 B3	*E. coli* 458 B4	*Pseud. aeruginosa* SG 137 B7	*Pseud. aeruginosa* K 799/61 B9	MRSA *Staph. aureus* 134/93 R9	VRSA *Enteroc. faecalis* 1528 R10	*Mycobact. vaccae* 10670 M4	*Sporobolo. salmonicolor* 549 H4	*Candida albicans* C.alb. H8	*Penicillium notatum* JP36 P1
1a	1000 µg/ml	DMSO	0	0	16p	0	0	0	0	0	20	0	0
1c	1000 µg/ml	DMSO	0	0	15p	14P	0	0	0	0	23	0	0
3b	1000 µg/ml	DMSO	n.t.	11	15p	n.t.	0	0/A	12/15p	17	13/19p	0	14
18	1000 µg/ml	DMSO	0	0	16p	0	0	0	0	0	20	0	0
19	1000 µg/ml	DMSO	10EK	0	15p	0	0	0	0	0	25	12p	0
20	1000 µg/ml	DMSO	12EK	12	14P	0	0	10	14p	19	14/18p	13	15
21	1000 µg/ml	DMSO	12EK	0	13p	15P	0	12P	0	18	25	13p	16
34	1000 µg/ml	DMSO	0	0	14P	15P	0	0	0	0	24	0	0
Ciprofloxacin	5 µg/ml	A.dest./A.dest.	31	19	24/32p	26	28/33p	0	17F	21p			
LM		DMSO	12P	13P	12P	13P	20p	0	12p	12p	11p/17P	0	12p
AmphotericinB	10 µg/ml	DMSO/MeOH									17p	21	18p

p = colonies in inhibition zone
P = many colonies in inhibition zone
A = indication of inhibition
F = enhanced growth
n.t. = not tested
MRSA = Methicillin-resistent *Staphylococcus aureus*
VRSA = vancomycin-resistent *Staphylococcus aureus*

Compounds	Concentration	Solvent	*Bacillus subtilis* 6633 B1	*Staph. aureus* 511 B3	*E. coli* 458 B4	*Pseud. aeruginosa* SG 137 B7	*Pseud. aeruginosa* K 799/61 B9	MRSA *Staph. aureus* 134/93 R9	VRSA *Enteroc. faecalis* 1528 R10	*Mycobact. vaccae* 10670 M4	*Sporobolo. salmonicolor* 549 H4	*Candida albicans* C.alb. H8	*Penicillium notatum* JP36 P1
2a	1000 µg/ml	DMSO	11EK/14p	0	14P	11/15P	0	0	13p	0	20p	14	18
2c	1000 µg/ml	DMSO	11EK/14p	0	14P	10/15P	0	0	13p	17p	18p	13	16/22p
3a	1000 µg/ml	DMSO	11EK	0	15P	11/15P	0	10	13p	13p	18p	14	17
4a	1000 µg/ml	DMSO	11EK/18p	n.t.	15P	n.t.	0	10	14p	0	19p	13	15
38	1000 µg/ml	DMSO	0	0	14P	0	0	0	0	0	22p	0	0
Ciprofloxacin	5 µg/ml	A.dest./A.dest.	30EK	19	24/31p	25	28/34p	0	16F	22p			
LM		DMSO	12P	13P	13P	12P	20P	0	11p	12p	12p/17P	0	12p
AmphotericinB	10 µg/ml	DMSO/MeOH									18p	20	18p

p = colonies in inhibition zone
P = many colonies in inhibition zone
A = indication of inhibition
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MRSA = Methicillin-resistent *Staphylococcus aureus*
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Compounds	Concentration	Solvent	*Bacillus subtilis* 6633 B1	*Staph. aureus* 511 B3	*E. coli* 458 B4	*Pseud. aeruginosa* SG 137 B7	*Pseud. aeruginosa* K 799/61 B9	MRSA *Staph. aureus* 134/93 R9	VRSA *Enteroc. faecalis* 1528 R10	*Mycobact. vaccae* 10670 M4	*Sporobolo. salmonicolor* 549 H4	*Candida albicans* C.alb. H8	*Penicillium notatum* JP36 P1
5	1000 µg/ml	MeOH	10	10	10	0	10	0	12p	14	0	0	11
14	1000 µg/ml	MeOH	0	0	0	0	0	0	0	0	0	0	0
16	1000 µg/ml	MeOH	10	10	0	0	10	0	10	0	0	0	0
28	1000 µg/ml	MeOH	0	0	0	0	0	0	0	0	0	0	0
30	1000 µg/ml	MeOH	10	0	0	0	12P	0	0	0	0	0	0
32	1000 µg/ml	MeOH	0	0	0	0	0	0	0	0	0	0	0
Ciprofloxacin	5 µg/ml	A.dest./A.dest.	32	19	24/33p	24	28/36p	0	17F	21p			
LM		MeOH	0	0	0	0	0	0	0	0	10	0	10
AmphotericinB	10 µg/ml	DMSO/MeOH									18p	20	17p

p = colonies in inhibition zone
P = many colonies in inhibition zone
A = indication of inhibition
F = enhanced growth
n.t. = not tested
MRSA = Methicillin-resistent *Staphylococcus aureus*
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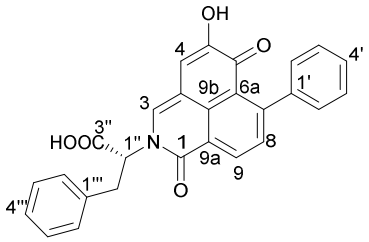
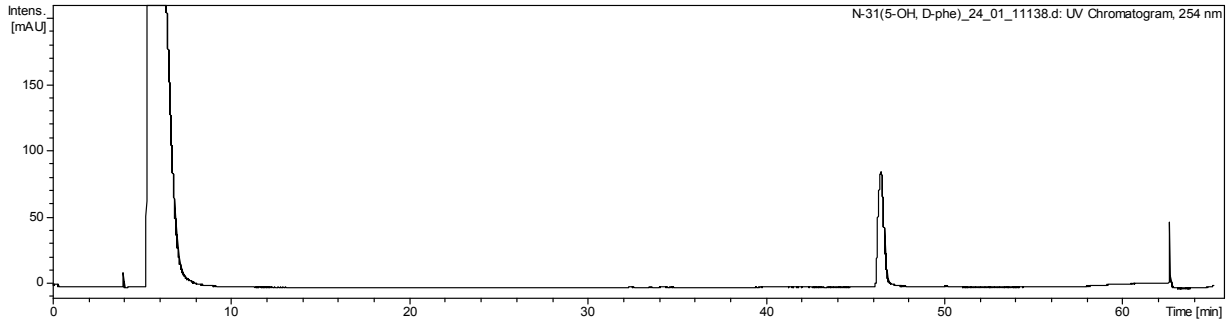
8. Spectra of the New PBIQs Generated by Incubating Plant Extracts with Amino Acids

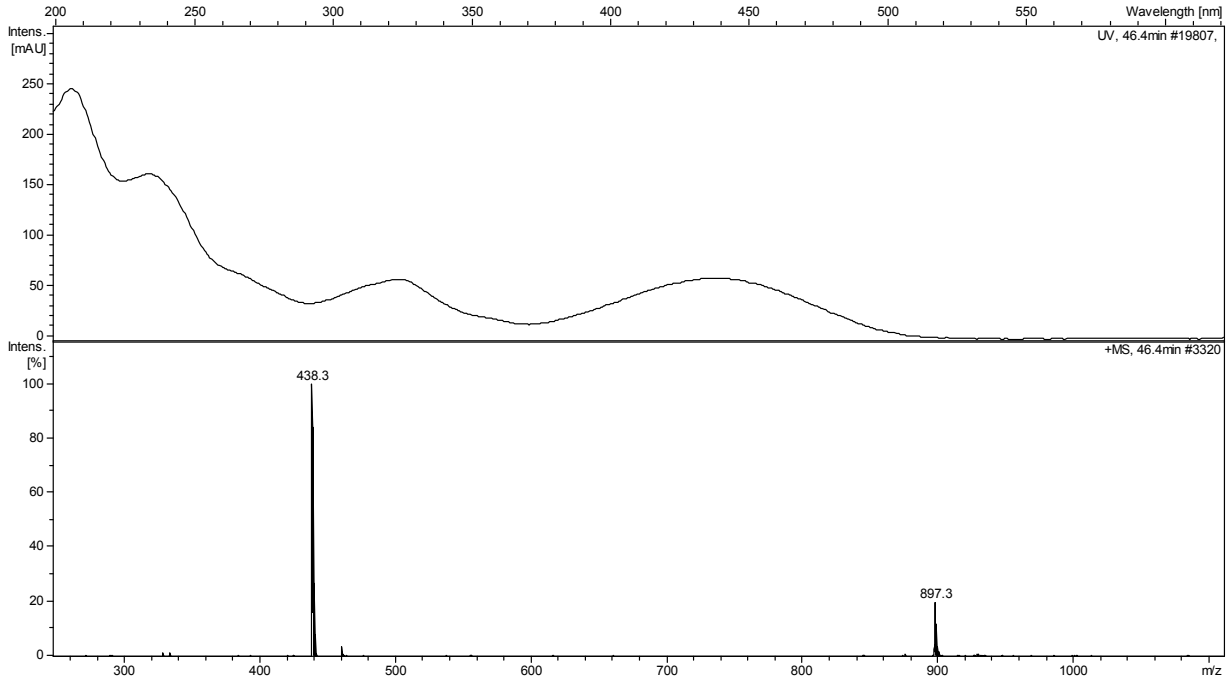


Structure of compound **7**

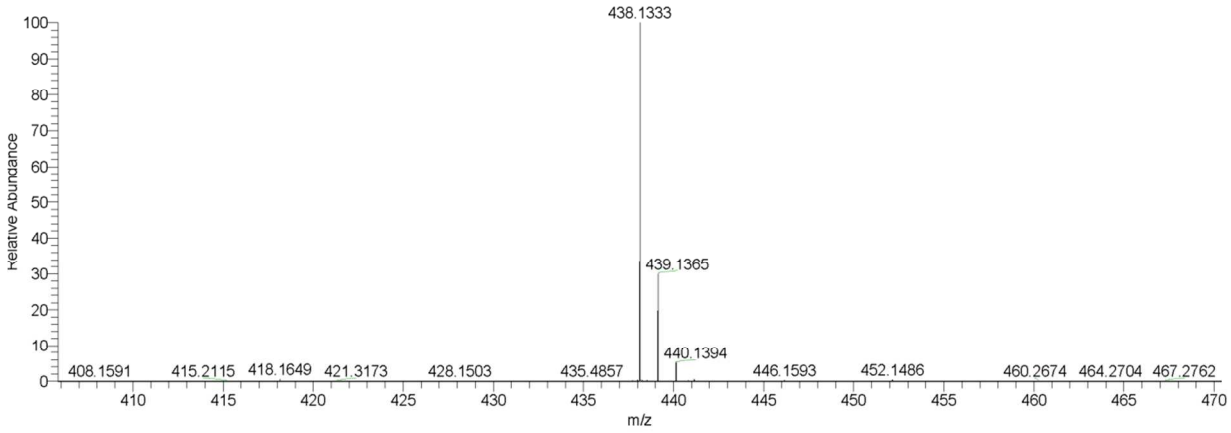
HPLC-ESIMS profile of compound **7** (UV 254 nm)



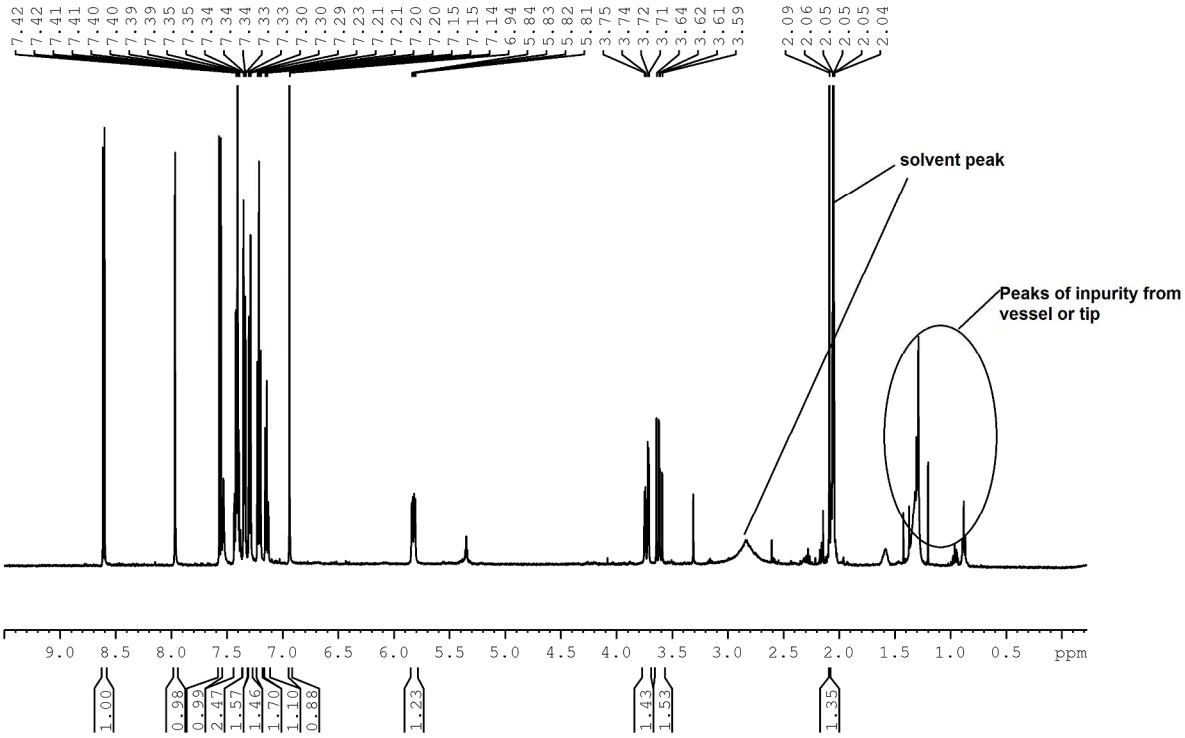
UV and ESIMS of **7**



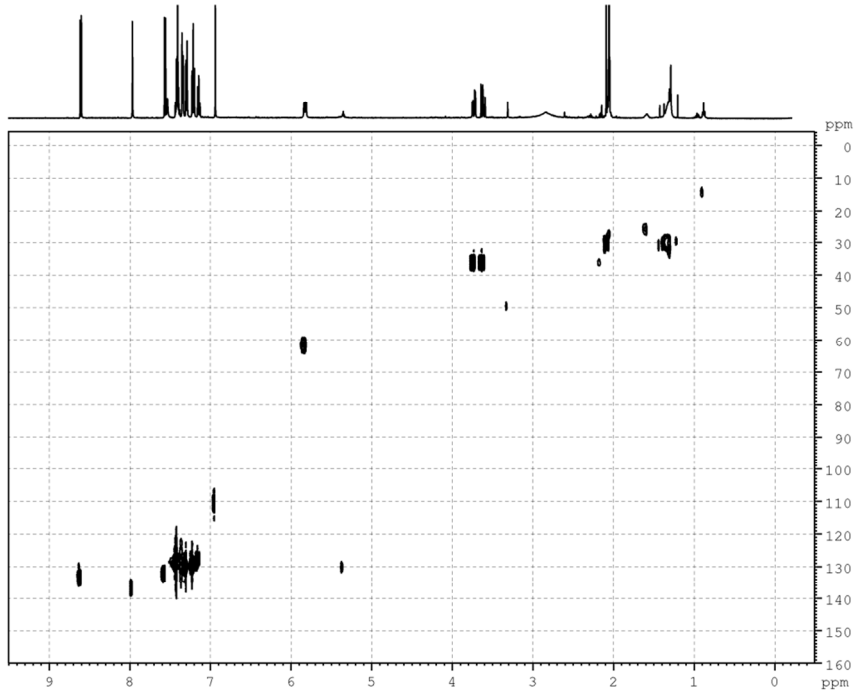
HRMS of **7**



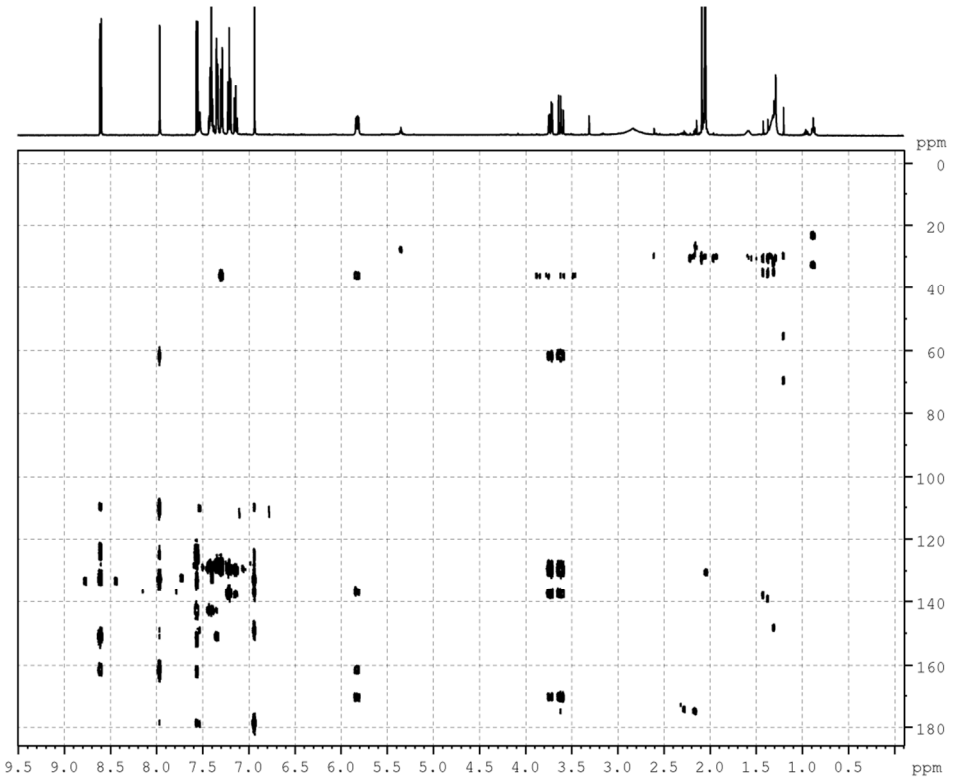
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **7**

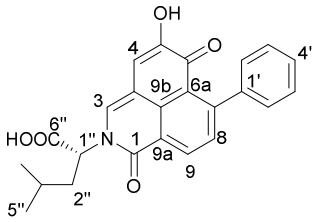


HSQC spectrum (500 MHz, acetone-d_6) of **7**



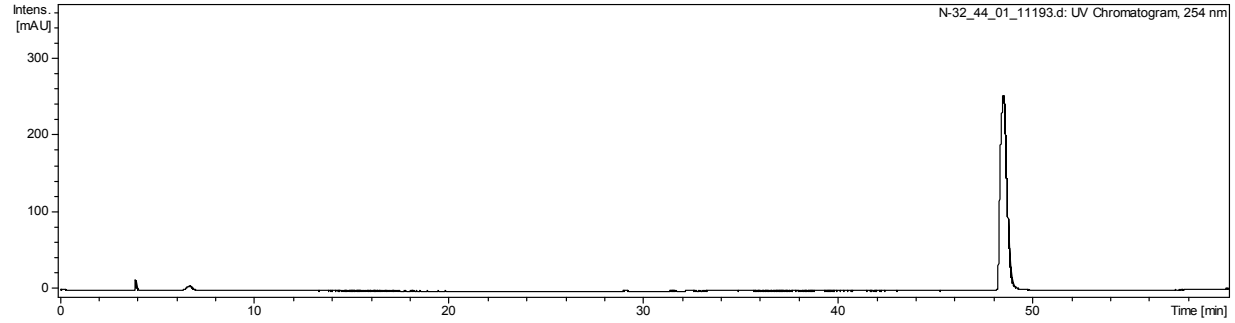
HMBC spectrum (500 MHz, acetone-d_6) of **7**



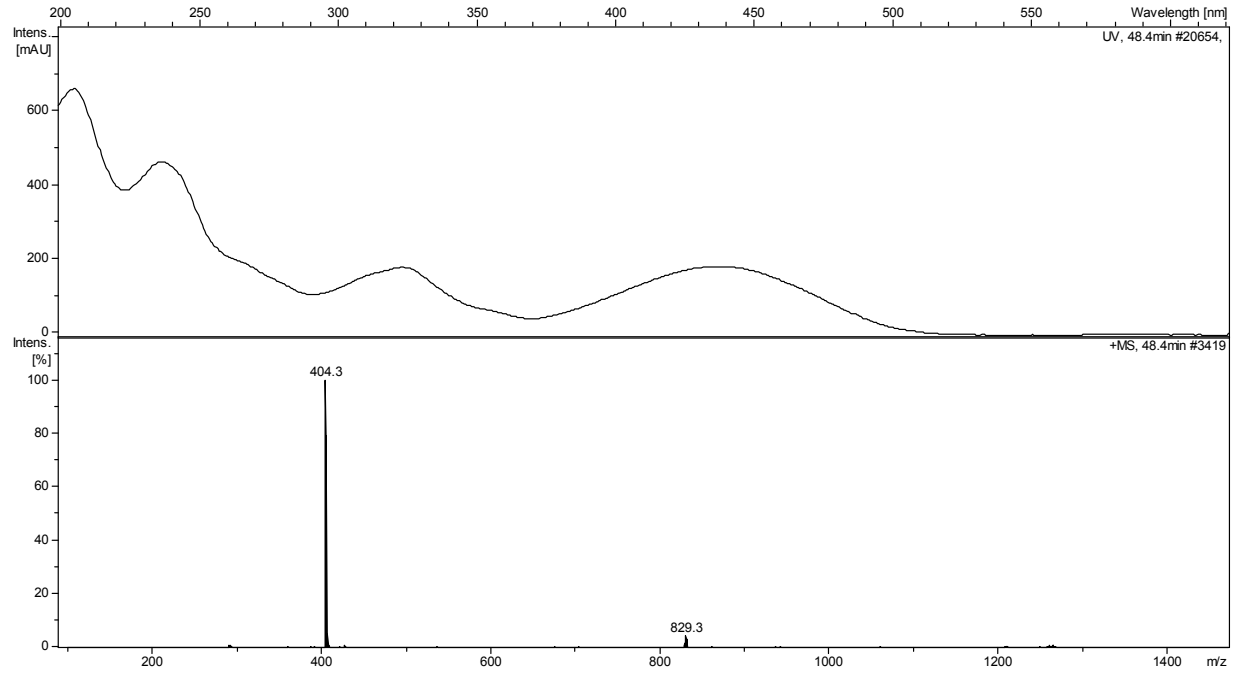


Structure of compound **8**

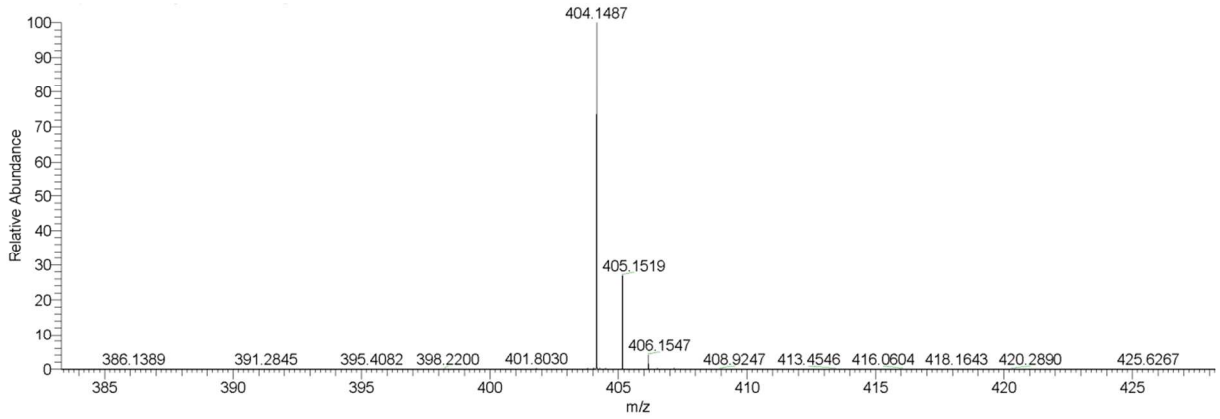
HPLC-ESIMS profile of **8** (UV 254 nm)



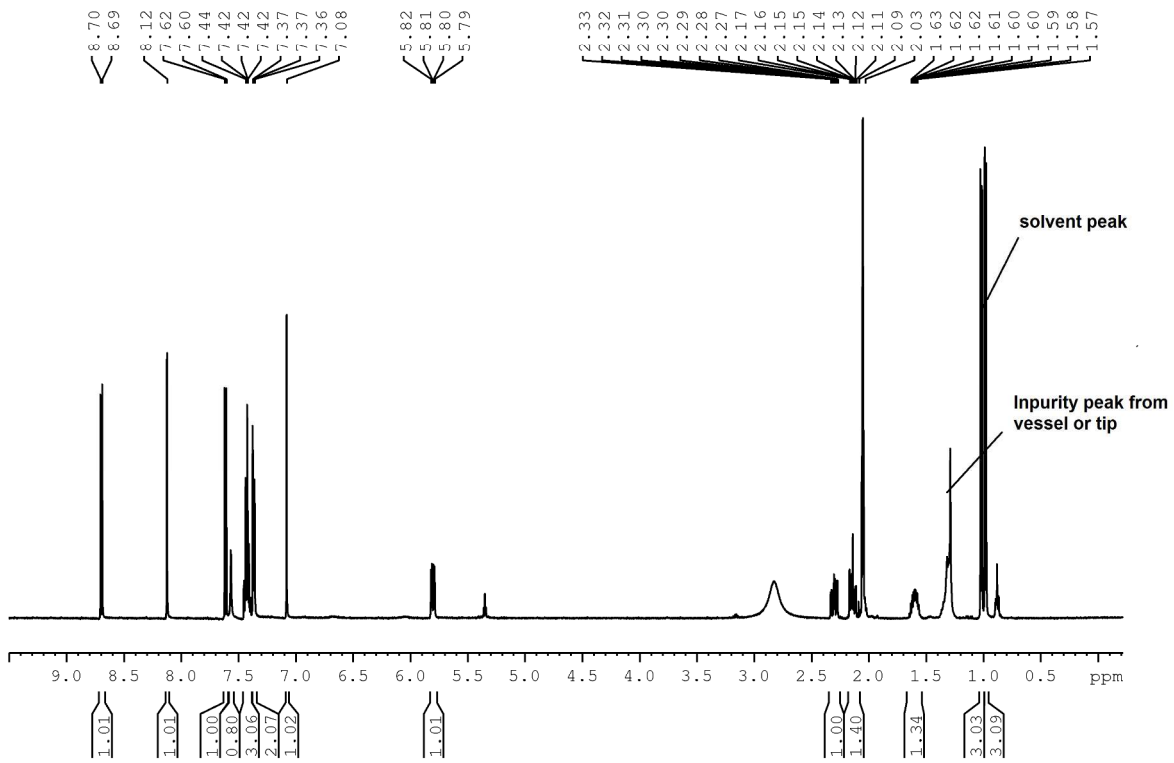
UV and ESIMS of **8**



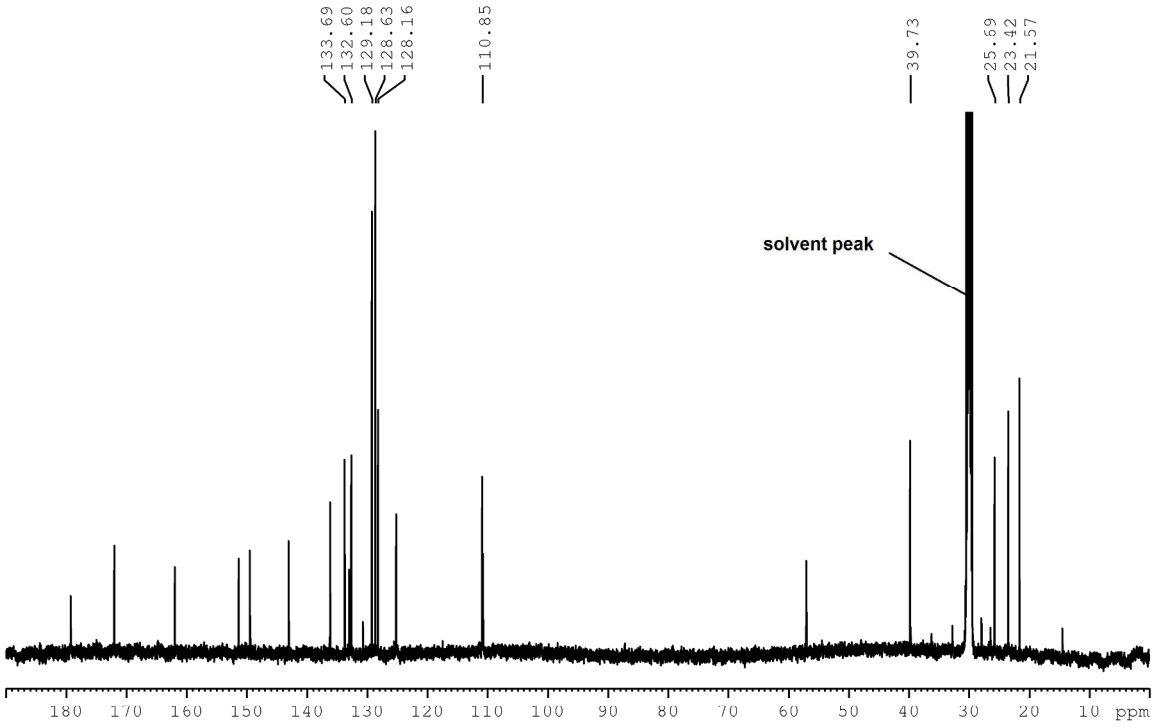
HRMS of **8**



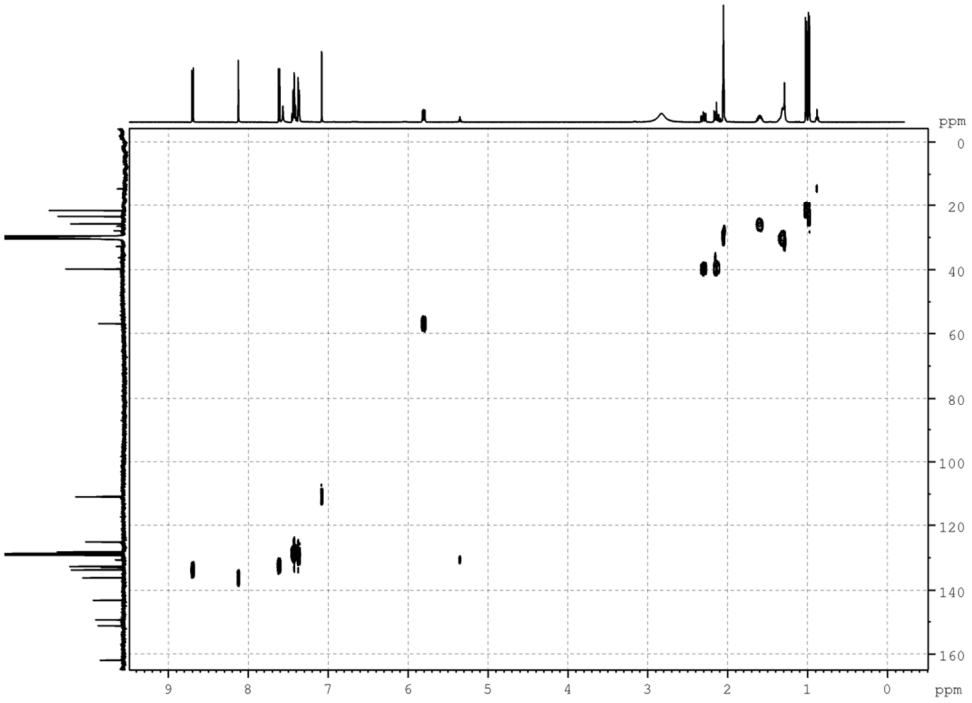
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **8**



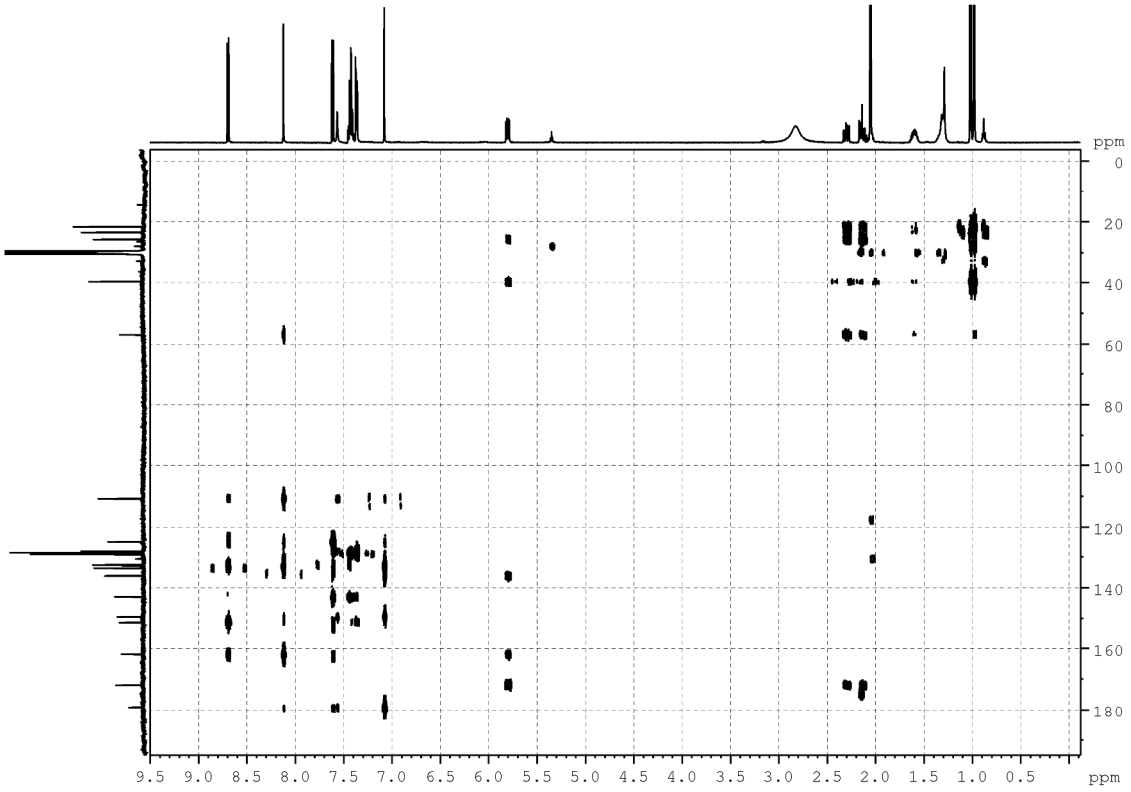
^{13}C NMR spectrum (125 MHz, acetone-d_6) of **8**

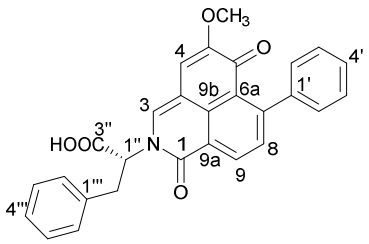


HSQC spectrum (500 MHz, acetone-d_6) of **8**



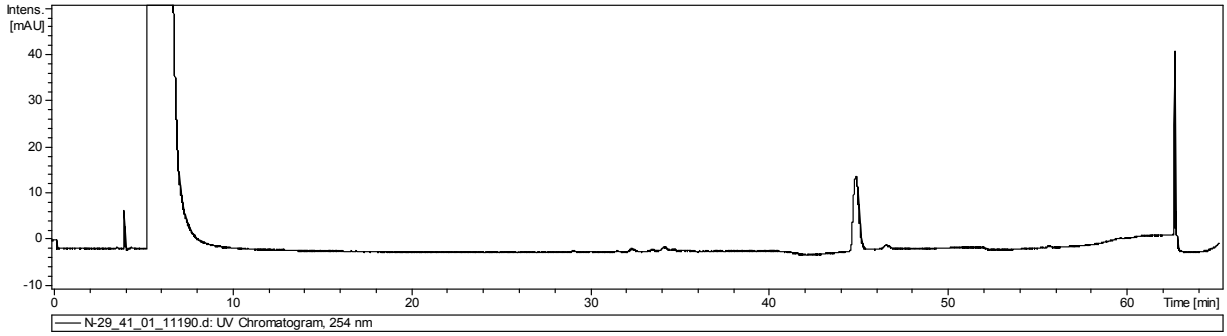
HMBC spectrum (500 MHz, acetone-d_6) of **8**



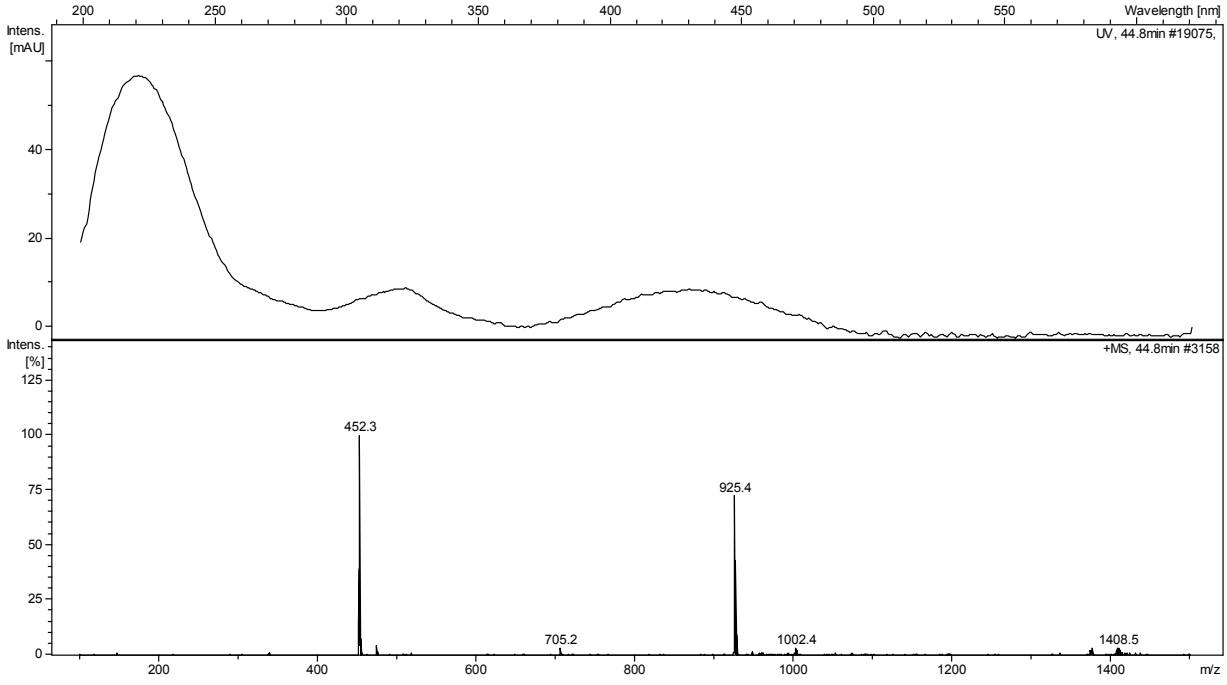


Structure of compound **9**

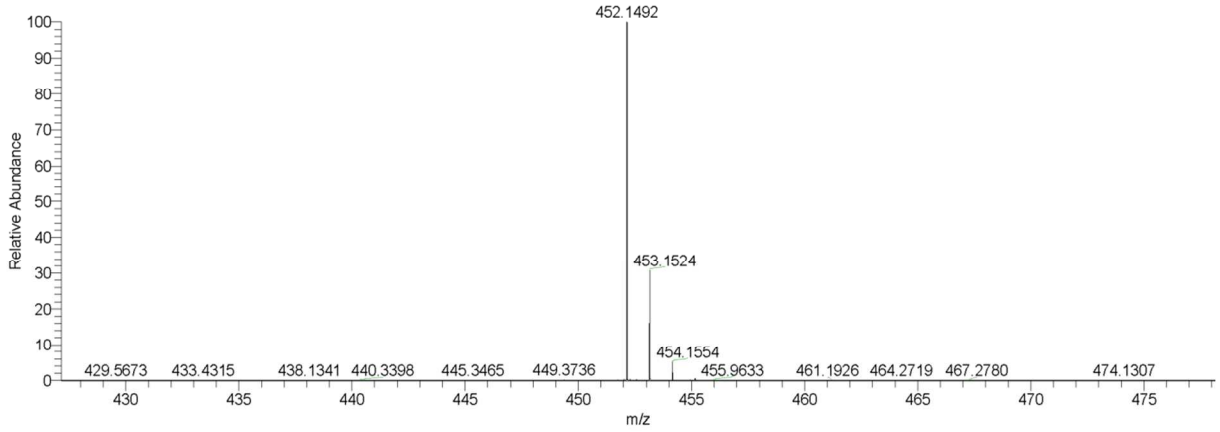
HPLC-ESIMS profile of **9** (UV 254 nm)



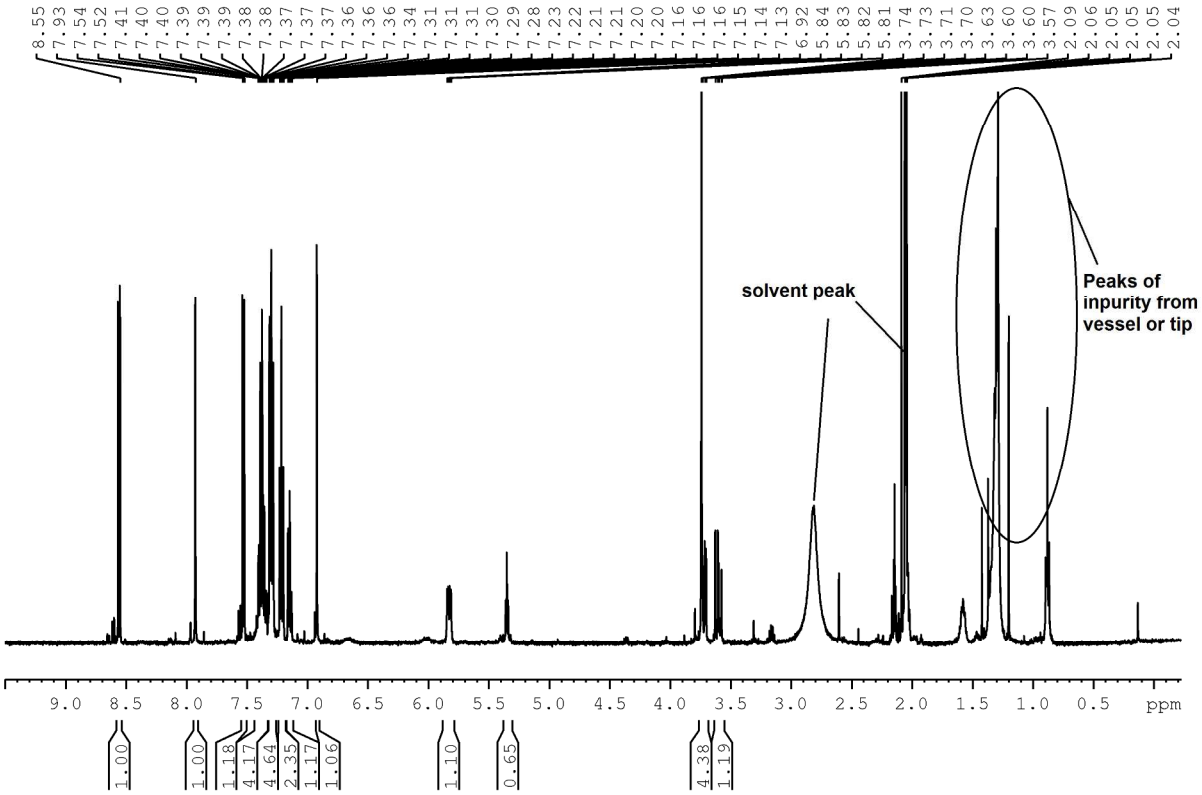
UV and ESIMS of **9**



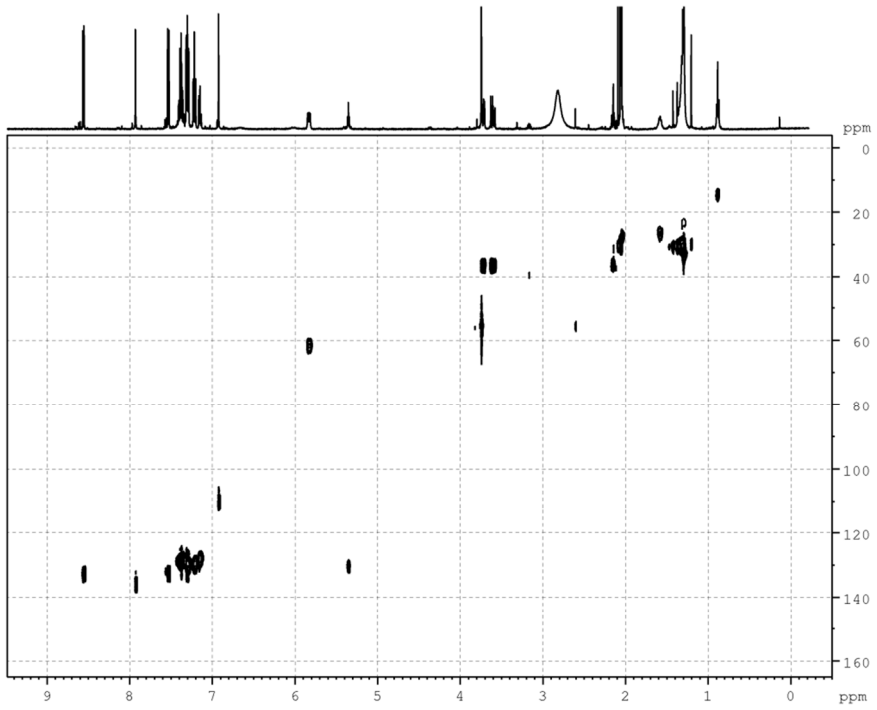
HRMS of **9**



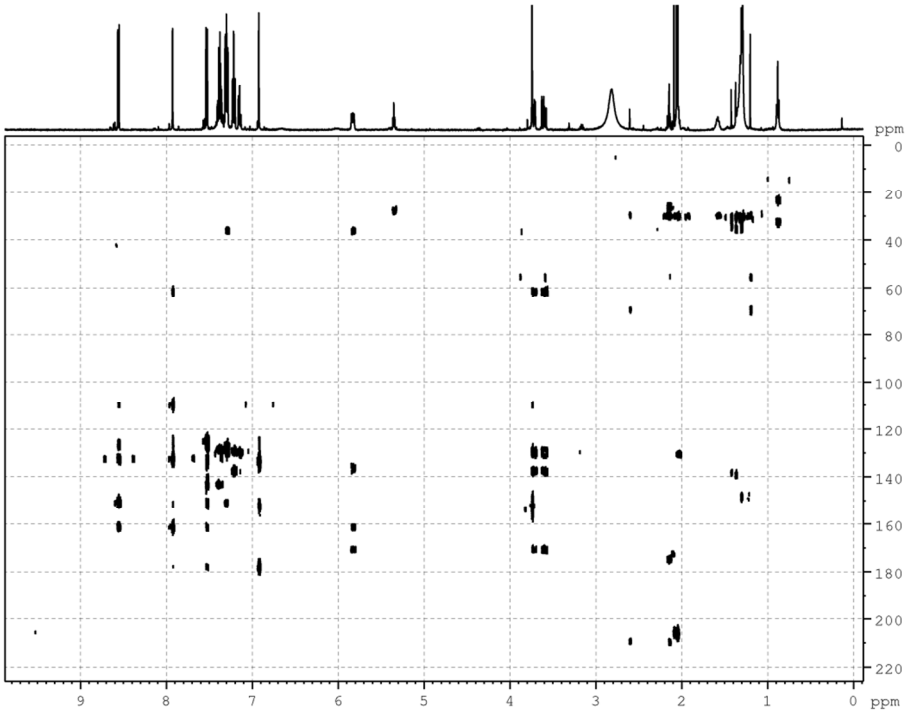
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **9**

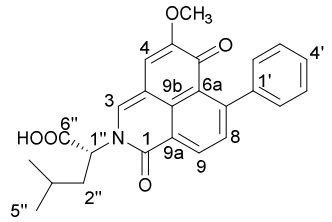


HSQC spectrum (500 MHz, acetone-d_6) of **9**



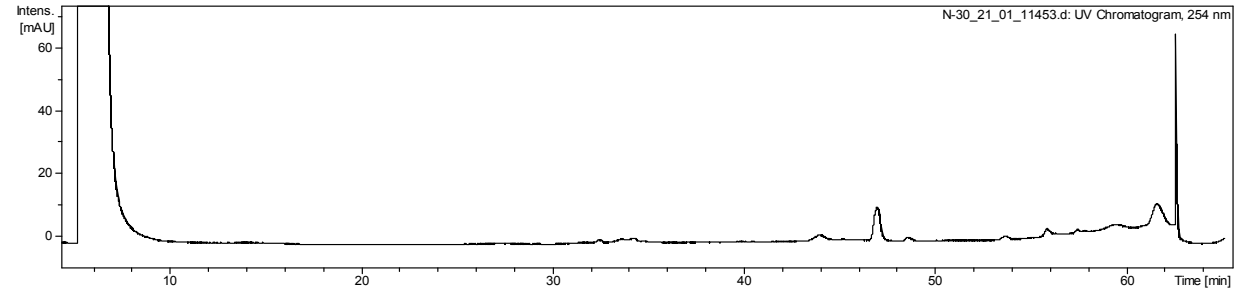
HMBC spectrum (500 MHz, acetone-d_6) of **9**



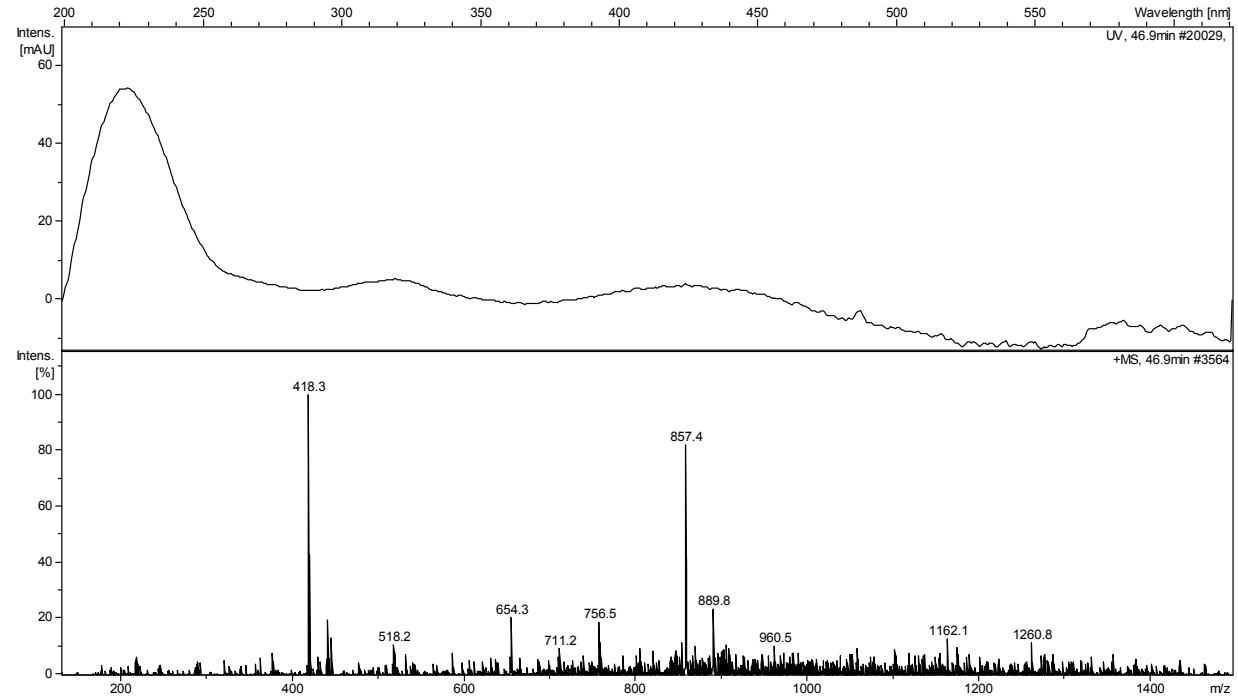


Structure of compound **10**

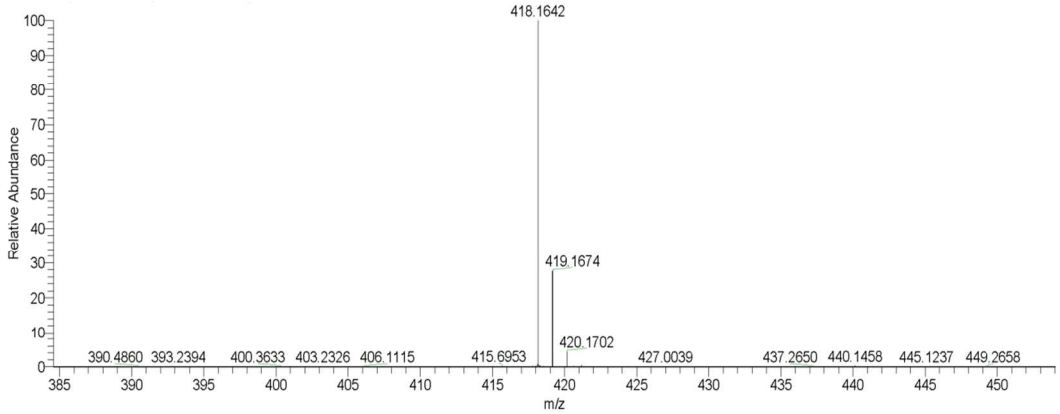
HPLC-ESIMS profile of **10** (UV 254 nm)



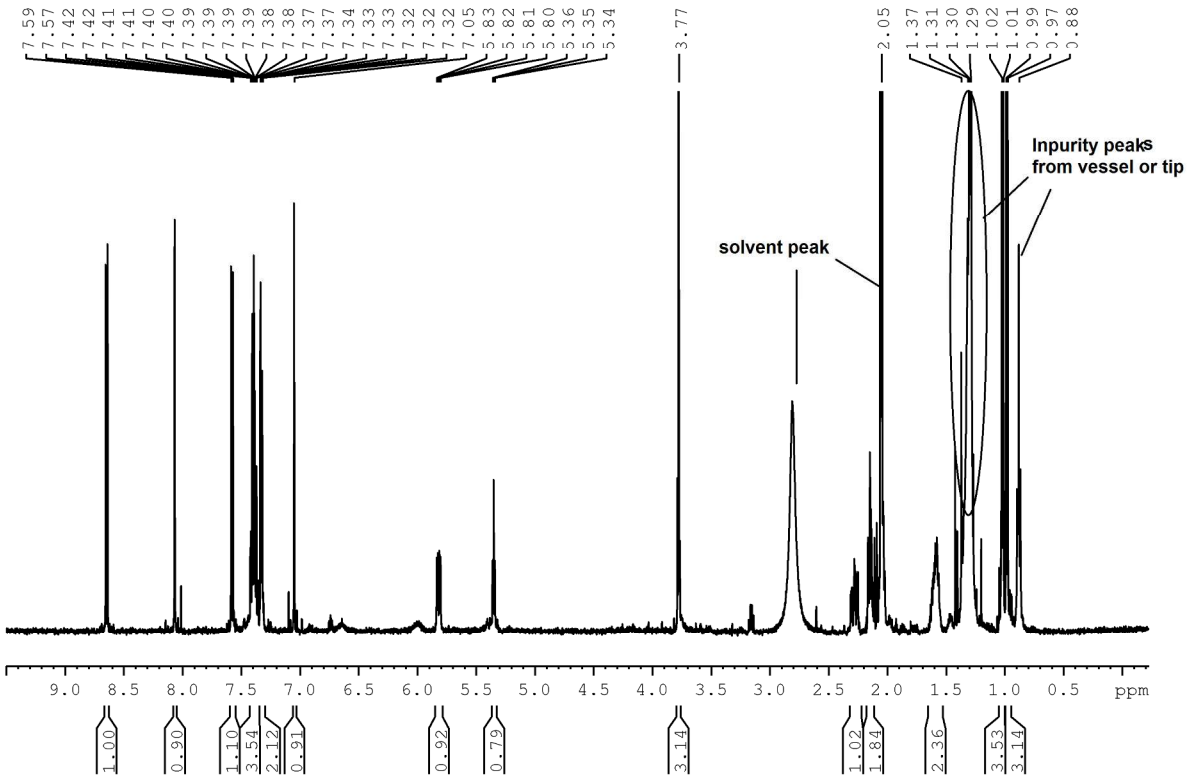
UV and ESIMS of **10**



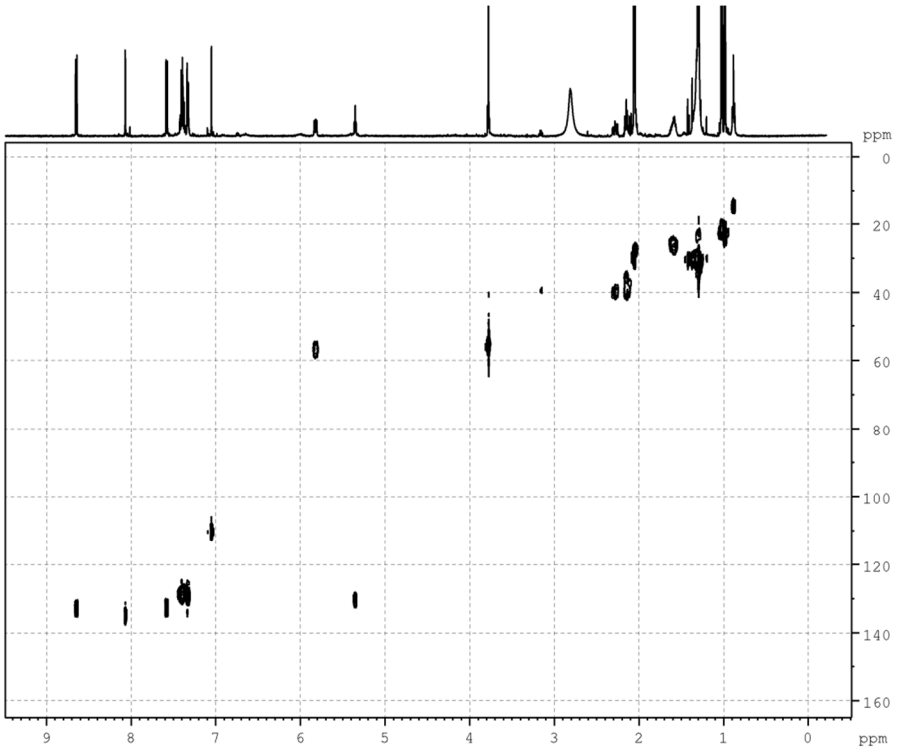
HRMS of **10**



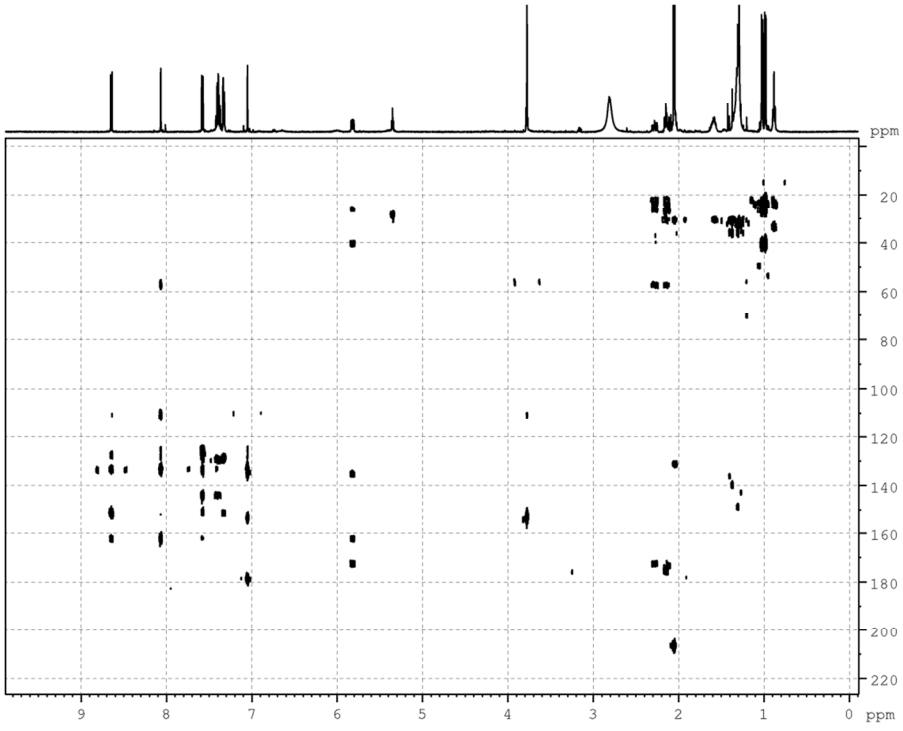
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **10**

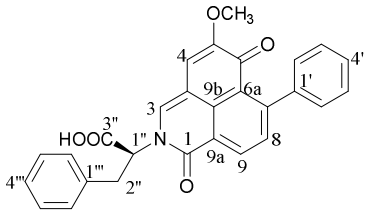


HSQC spectrum (500 MHz, acetone-d_6) of **10**



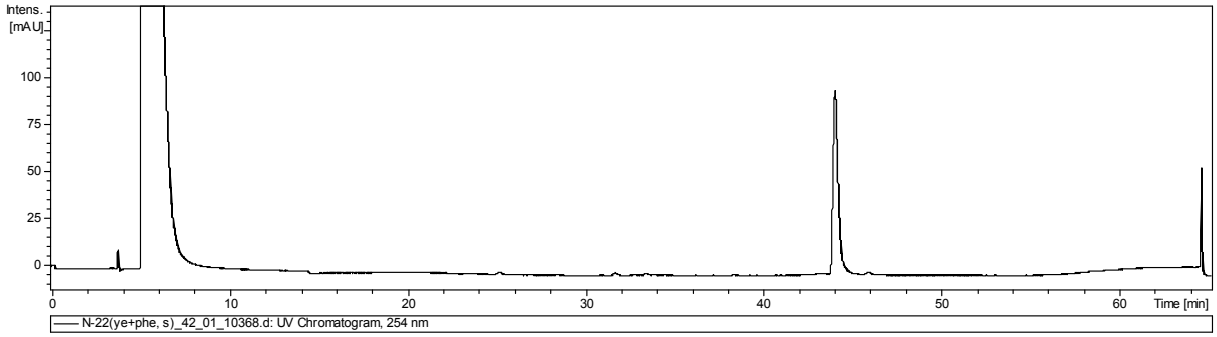
HMBC spectrum (500 MHz, acetone-d_6) of **10**



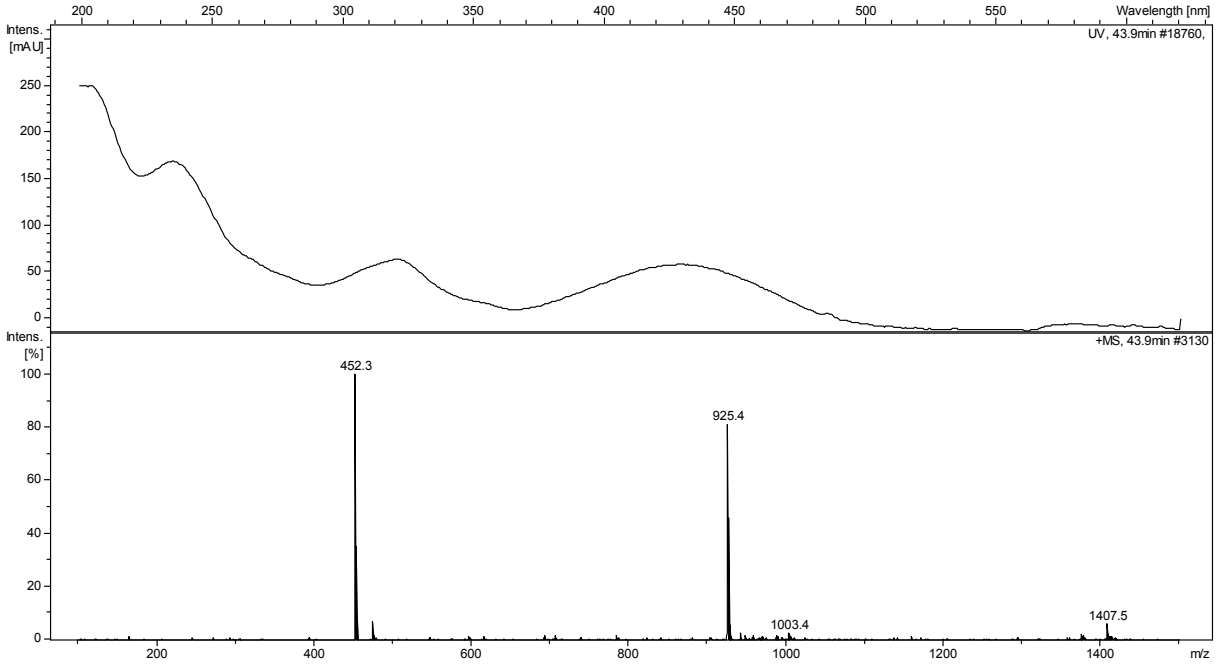


Structure of compound **11**

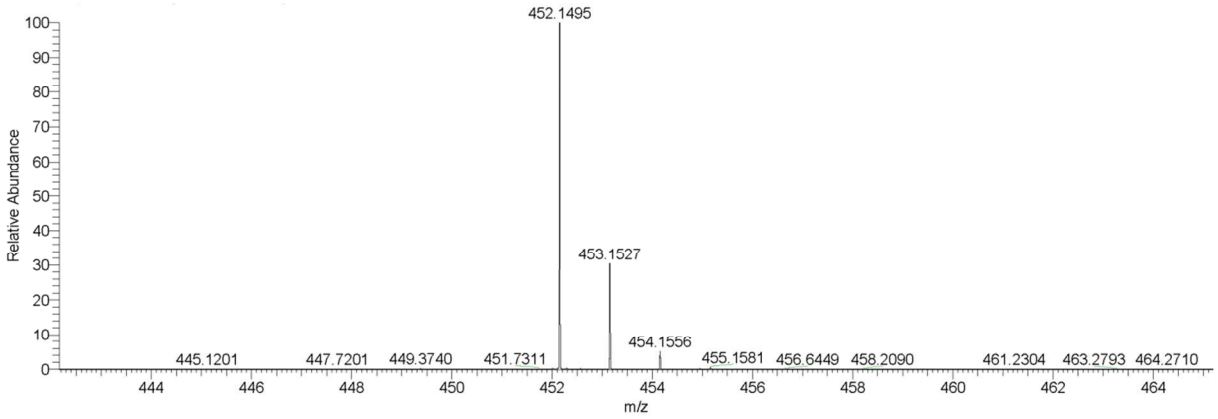
HPLC-ESIMS profile of **11** (UV 254 nm)



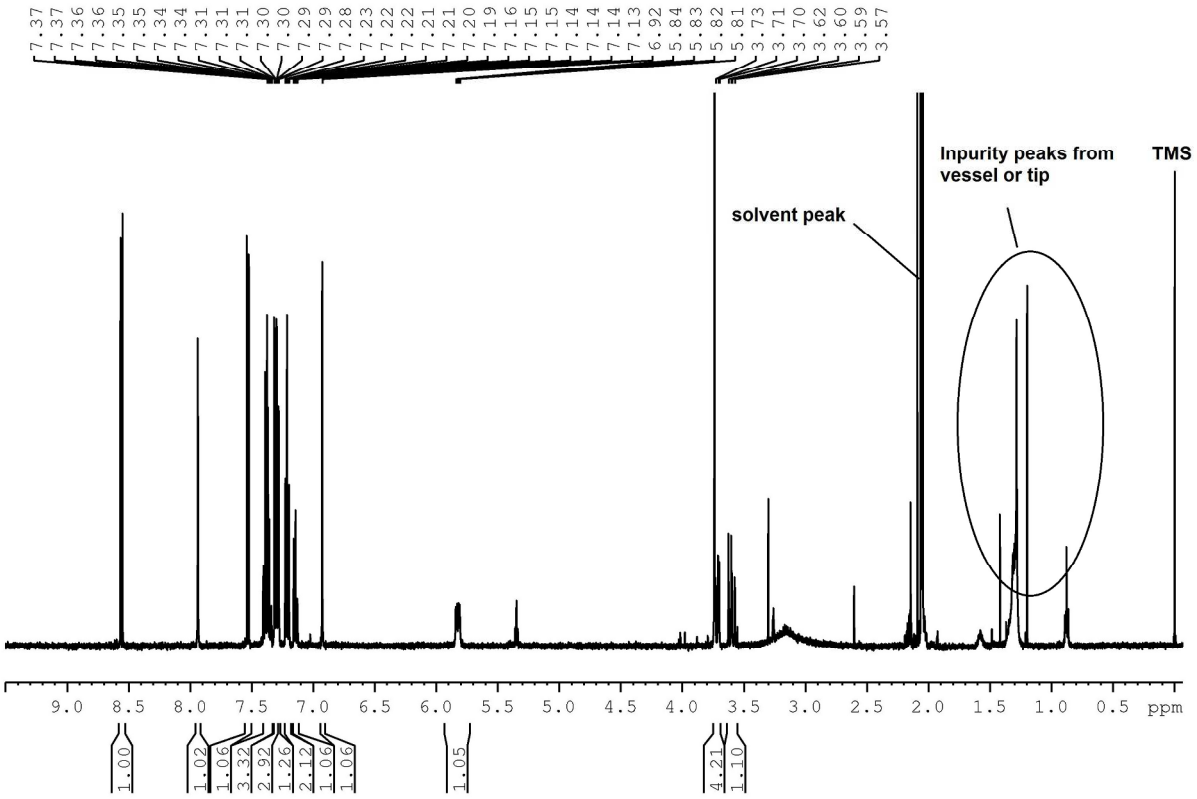
UV and ESIMS of **11**



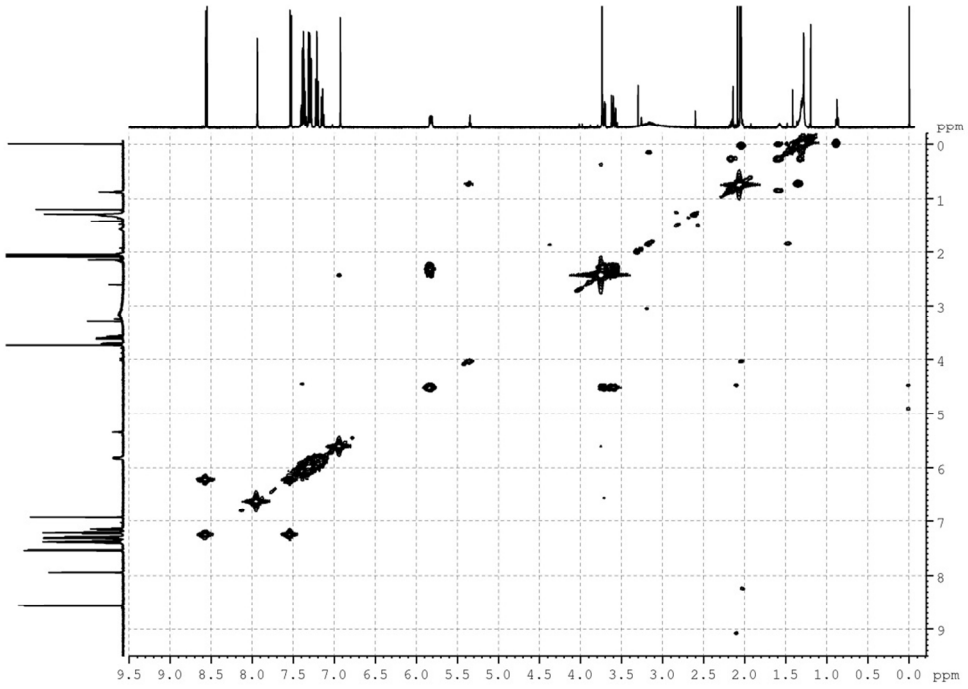
HRMS of **11**



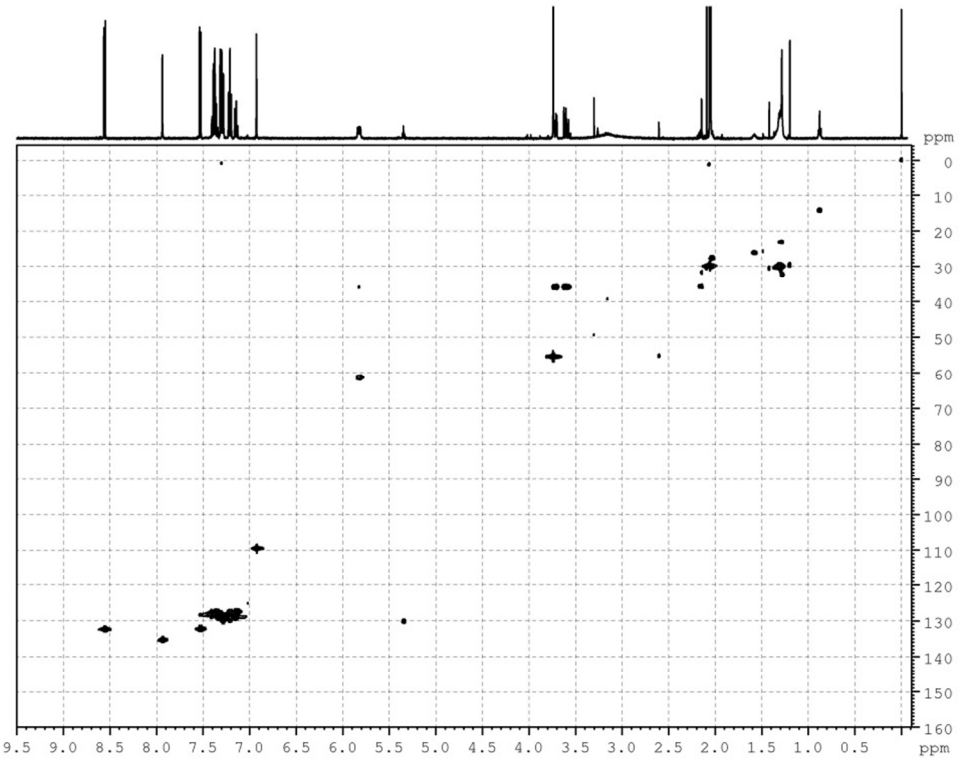
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **11**



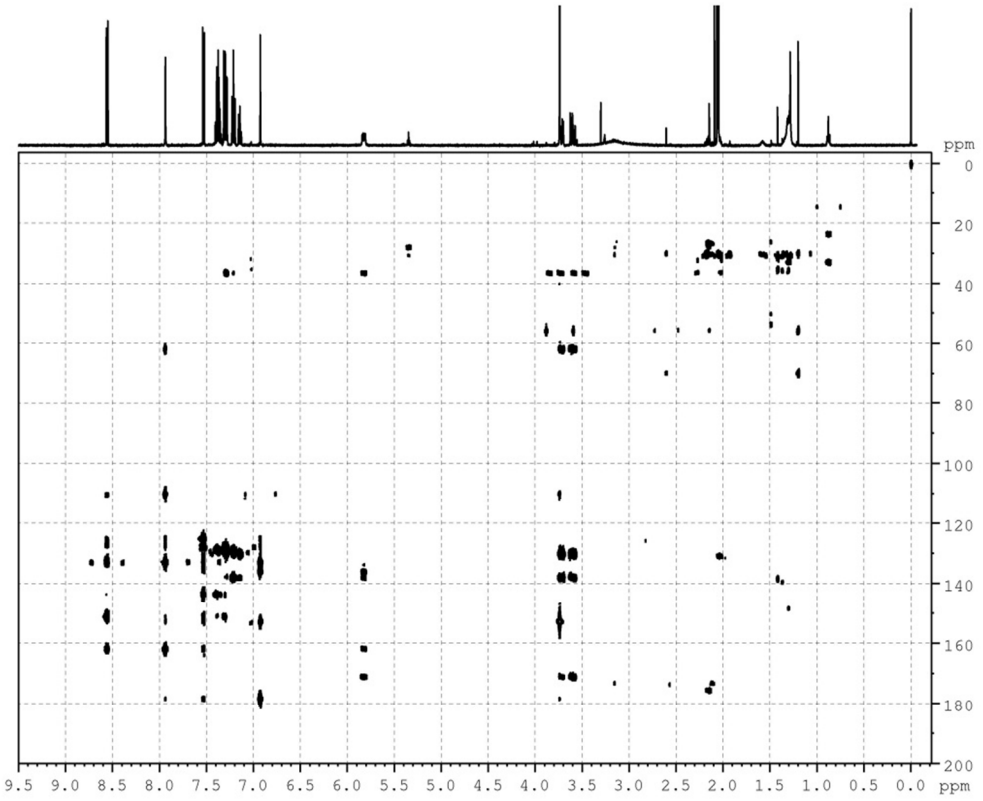
COSY spectrum (500 MHz, acetone-d_6) of **11**

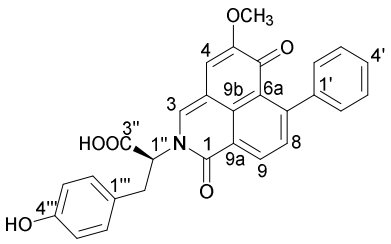


HSQC spectrum (500 MHz, acetone-d_6) of **11**



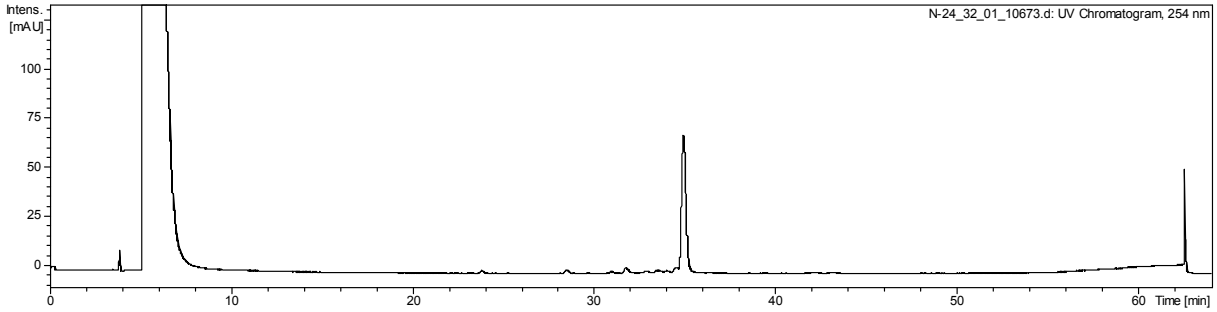
HMBC spectrum (500 MHz, acetone-d_6) of **11**



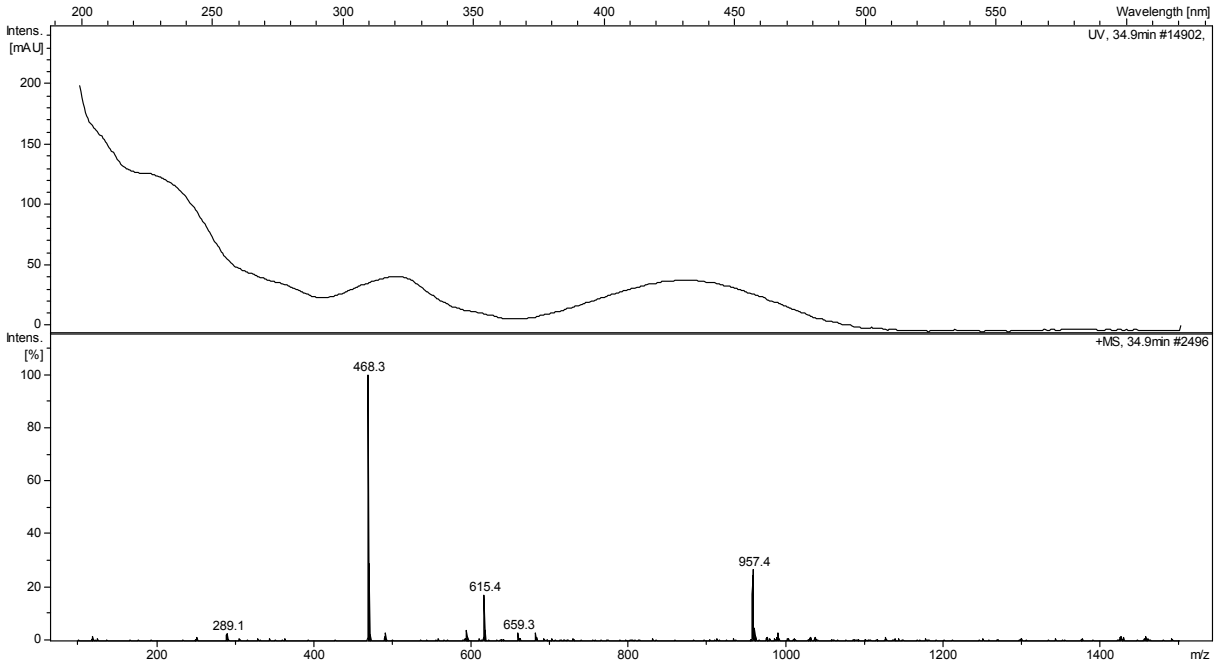


Structure of compound **12**

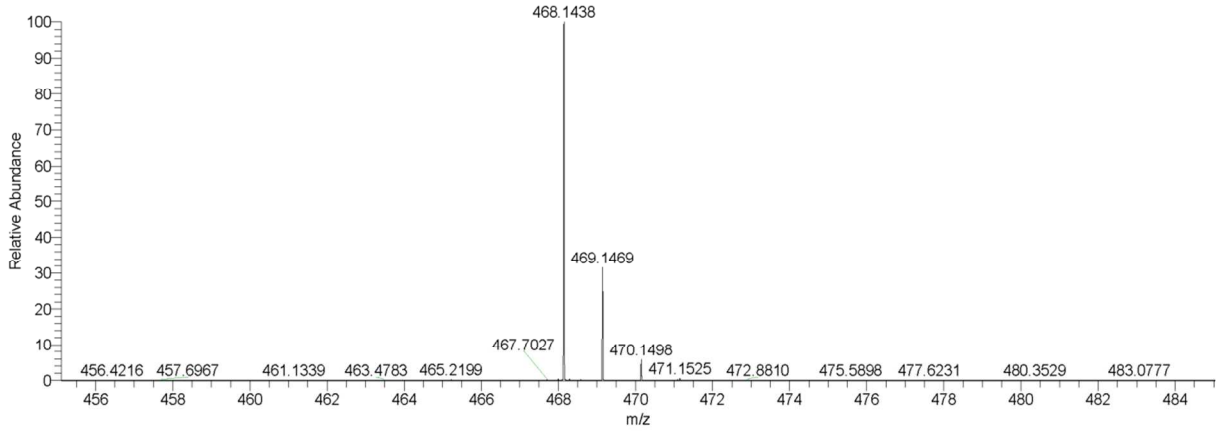
HPLC-ESIMS profile of **12** (UV 254 nm)



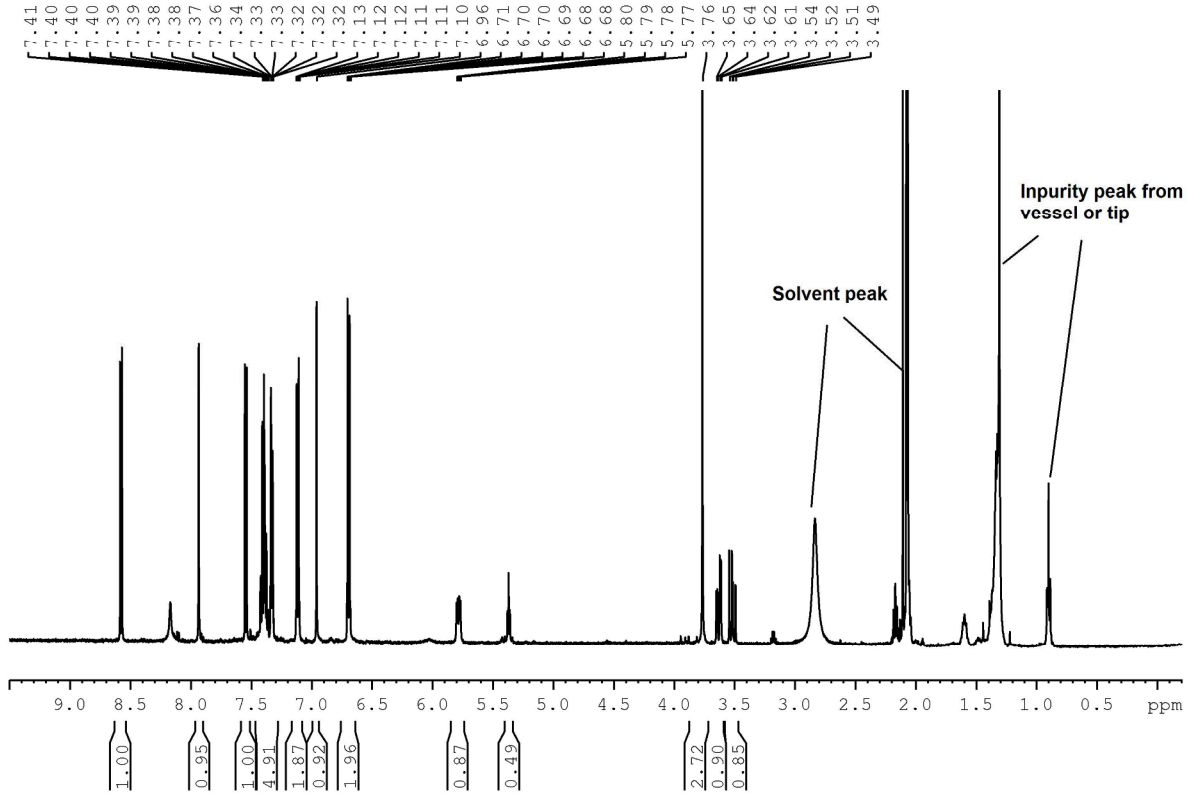
UV and ESIMS of **12**



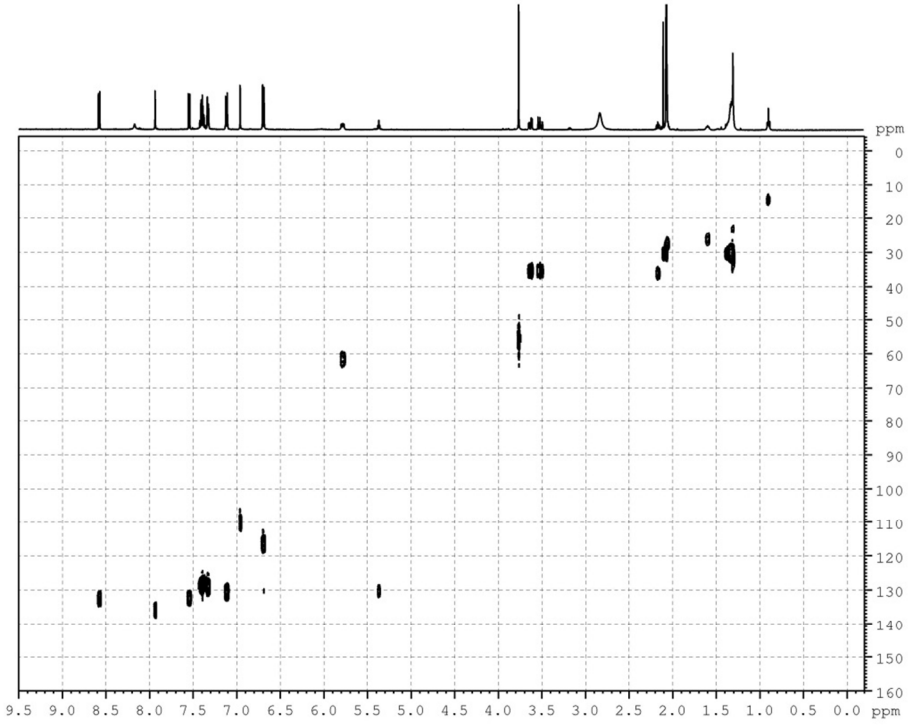
HRMS of **12**



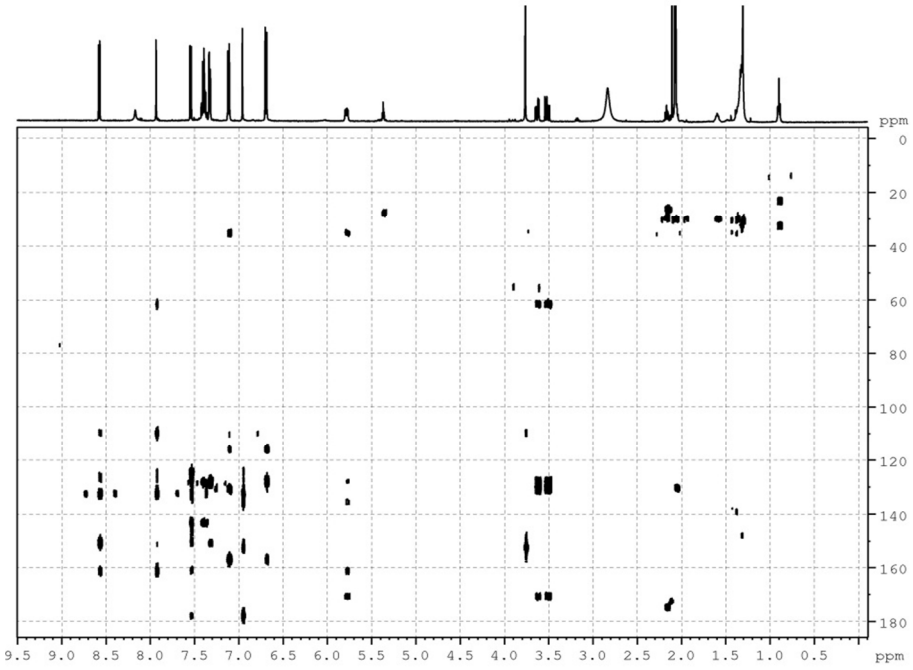
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **12**

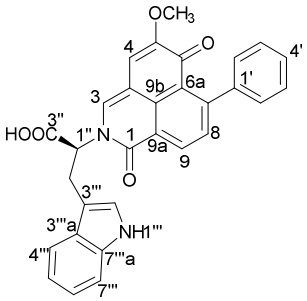


HSQC spectrum (500 MHz, acetone-d_6) of **12**



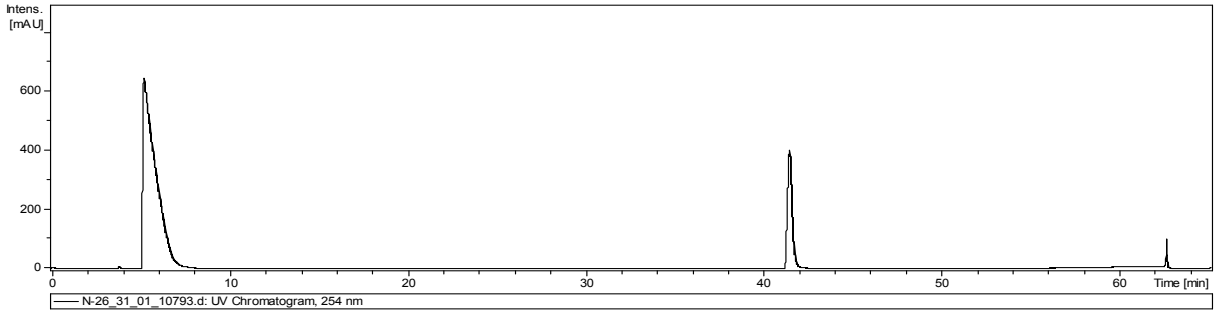
HMBC spectrum (500 MHz, acetone-d_6) of **12**



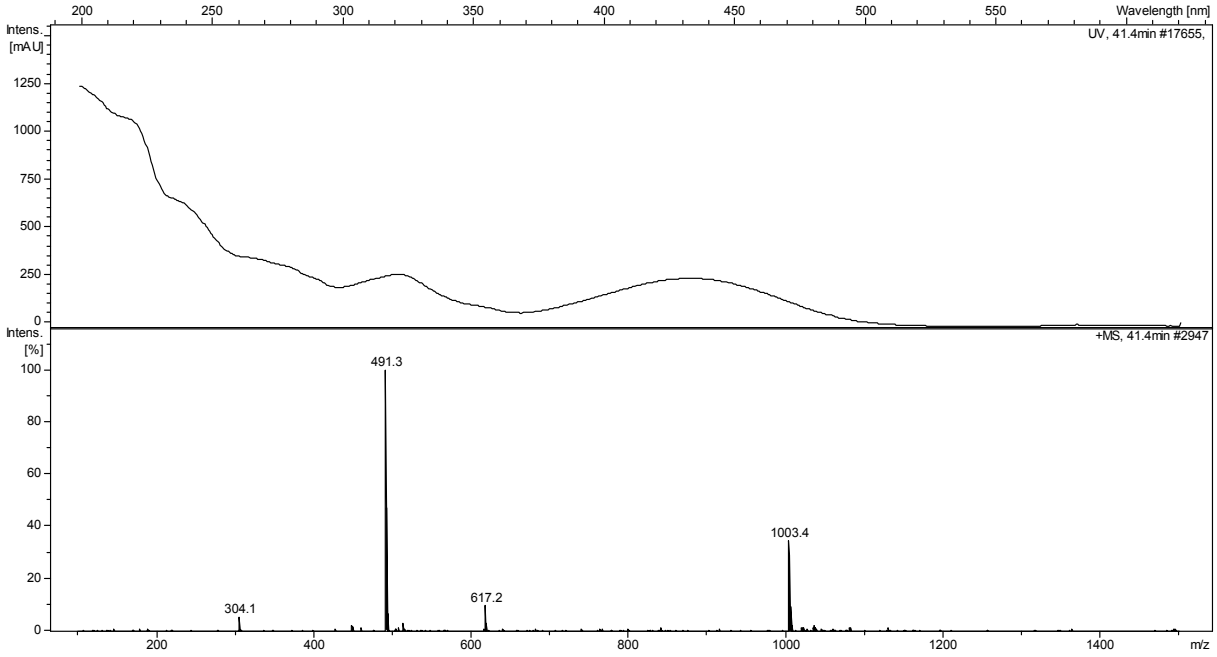


Structure of compound **13**

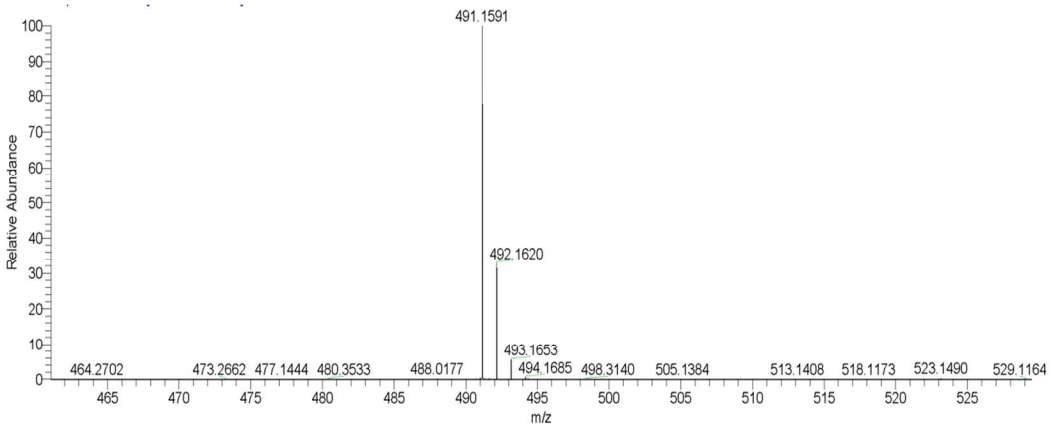
HPLC-ESIMS profile of **13** (UV 254 nm)



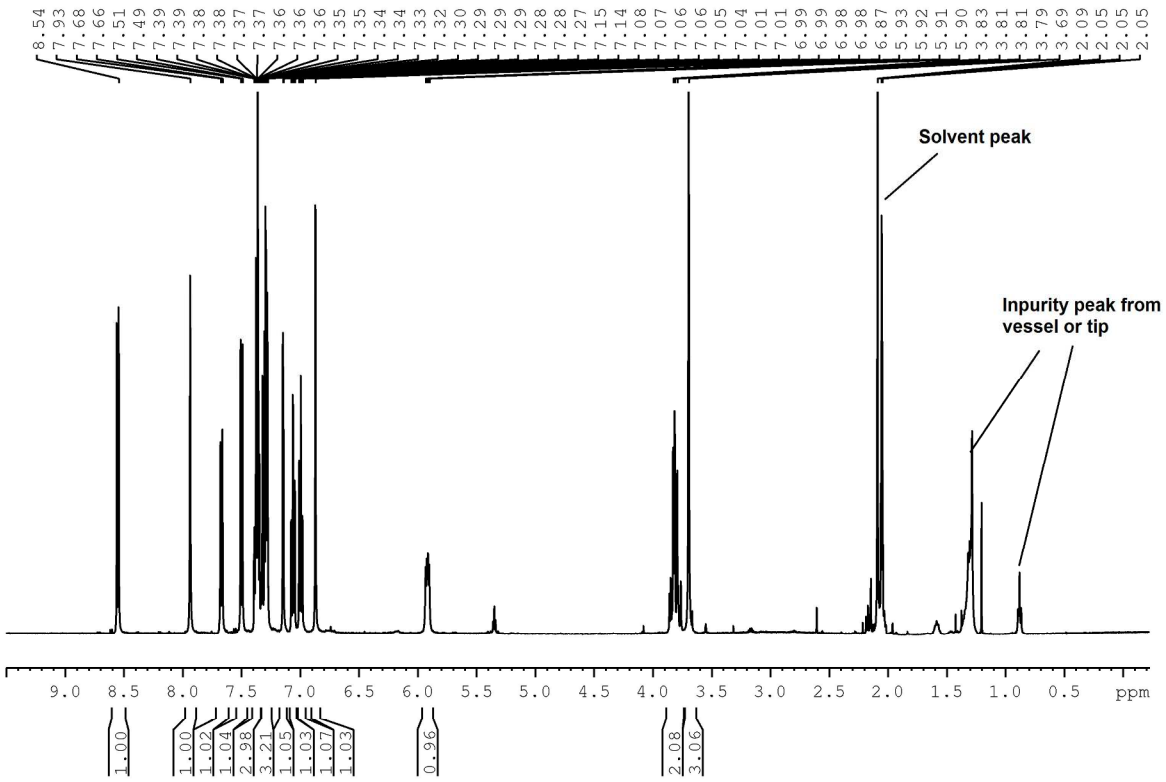
UV and ESIMS of **13**



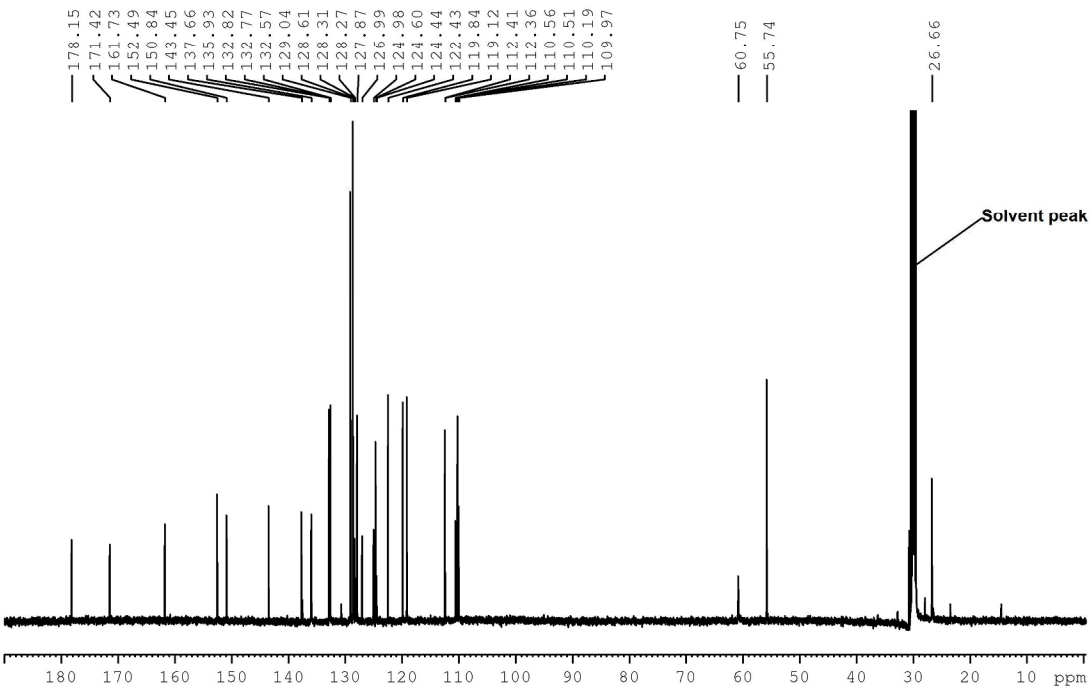
HRMS of **13**



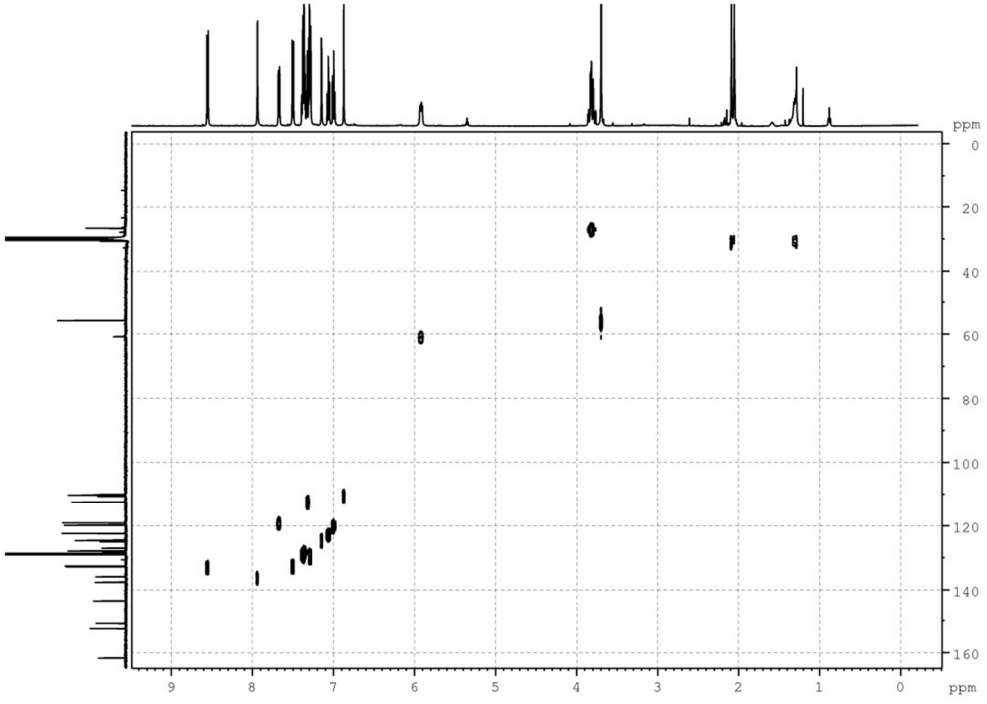
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **13**



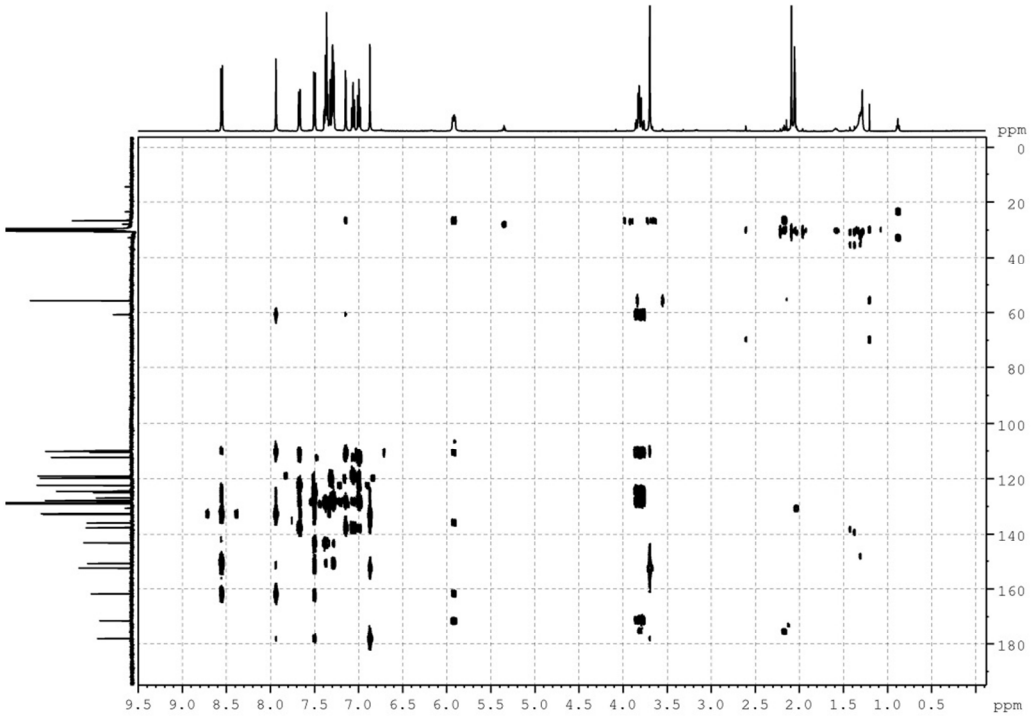
^{13}C NMR spectrum (125 MHz, acetone-d_6) of **13**

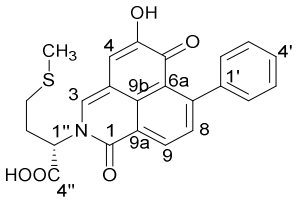


HSQC spectrum (500 MHz, acetone-d_6) of **13**



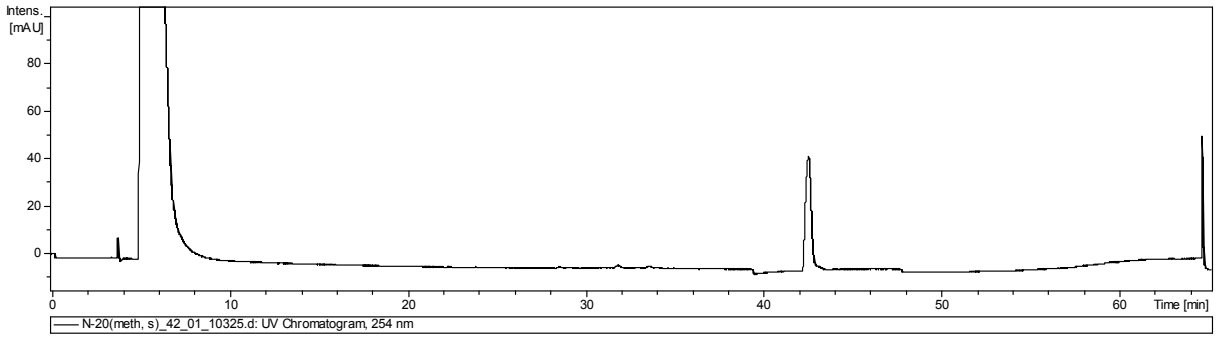
HMBC spectrum (500 MHz, acetone-d_6) of **13**



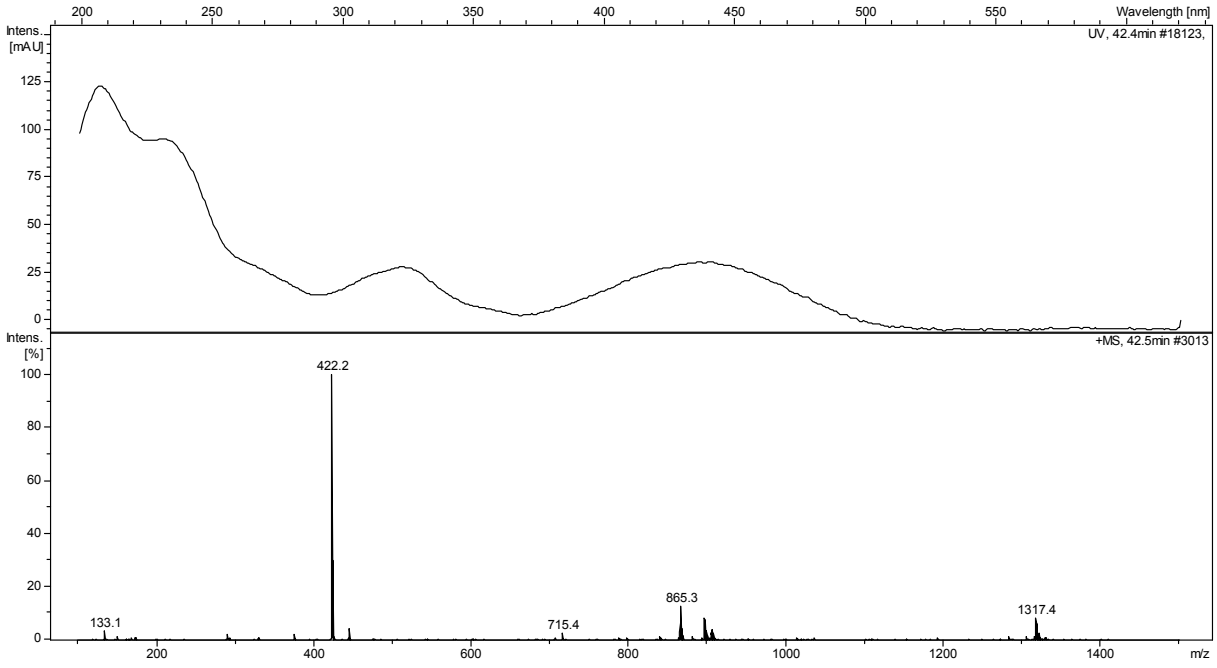


Structure of compound **14**

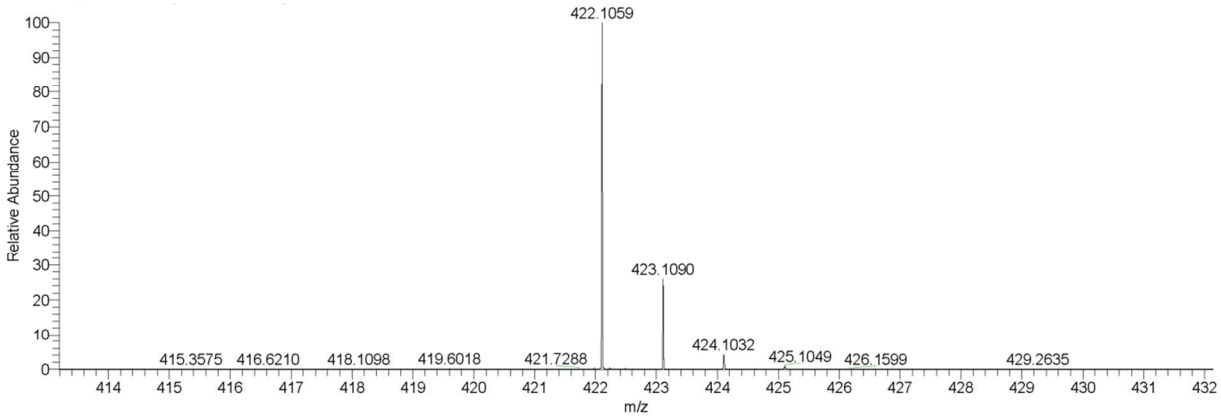
HPLC-ESIMS profile of **14** (UV 254 nm)



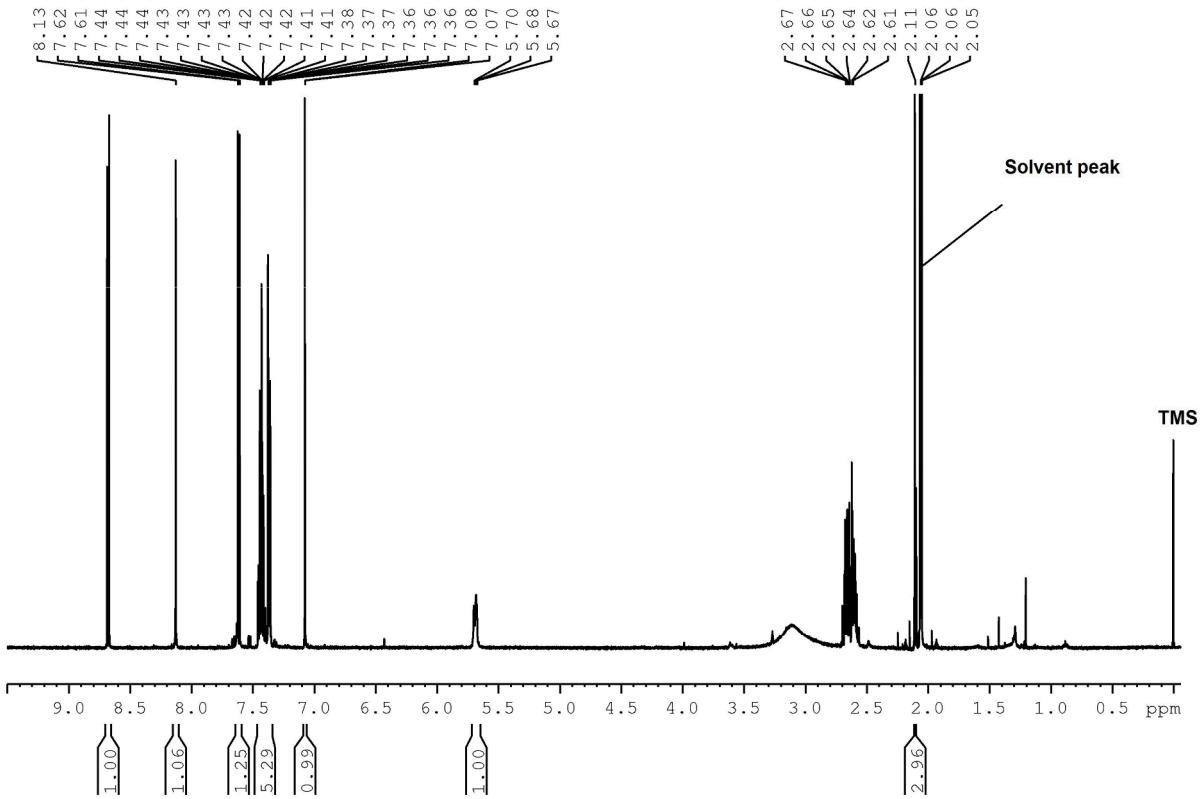
UV and ESIMS of **14**



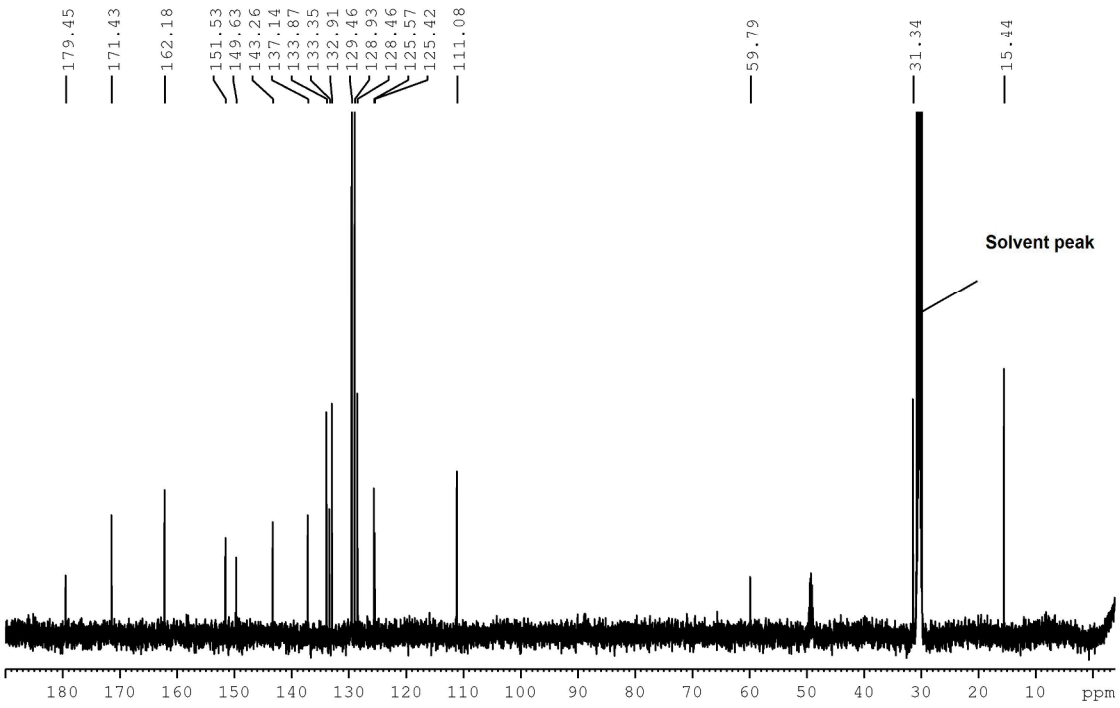
HRMS of **14**



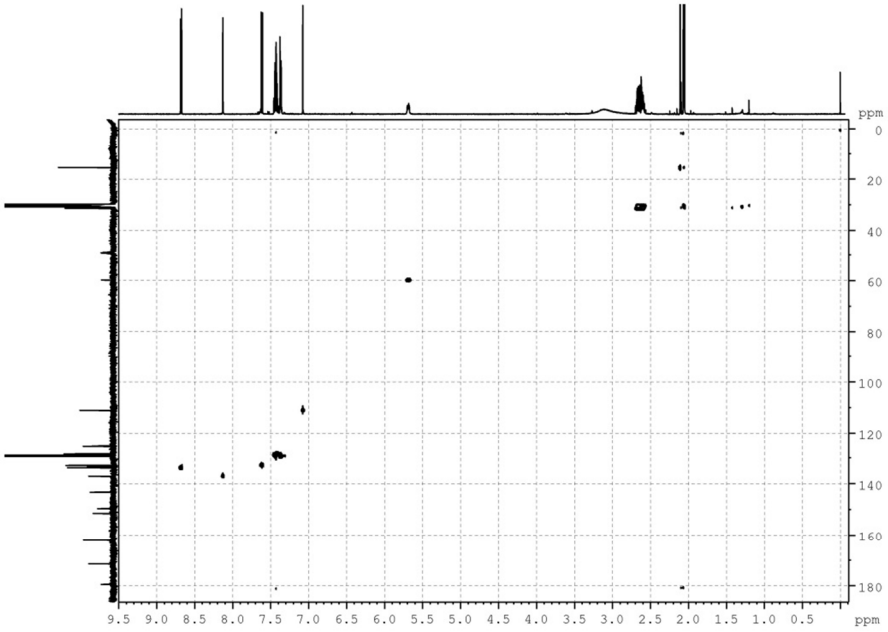
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **14**



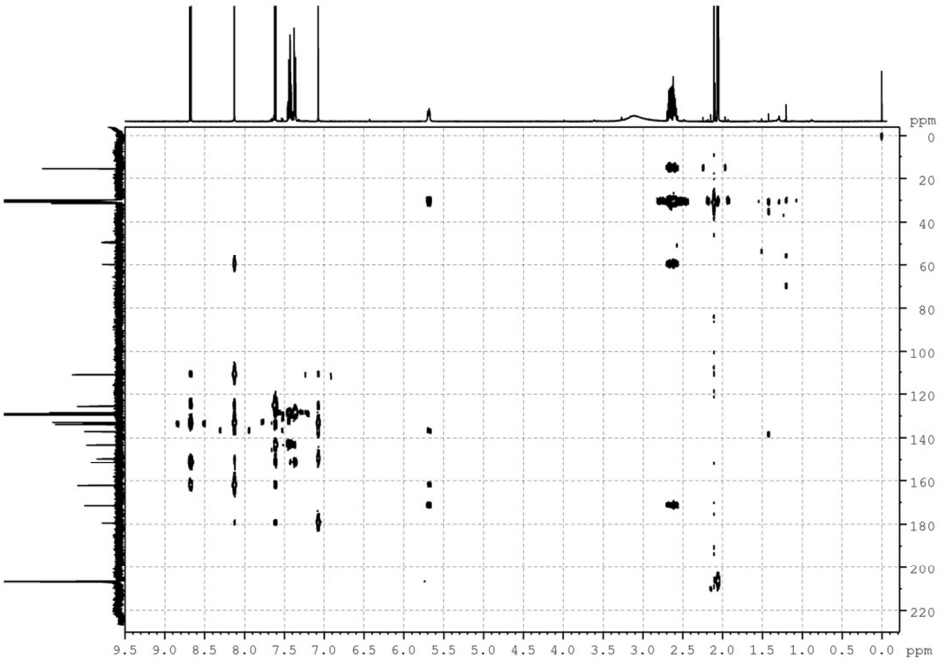
^{13}C NMR spectrum (125 MHz, acetone-d_6) of **14**

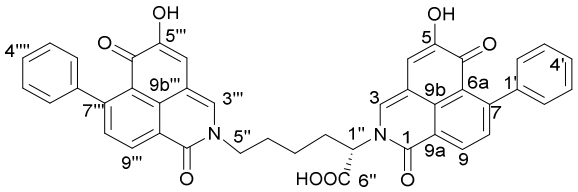


HSQC spectrum (500 MHz, acetone-d_6) of **14**



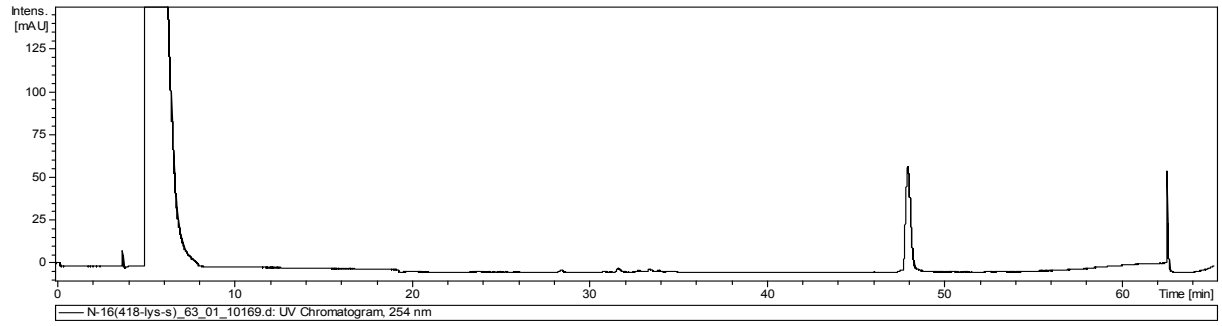
HMBC spectrum (500 MHz, acetone-d_6) of **14**



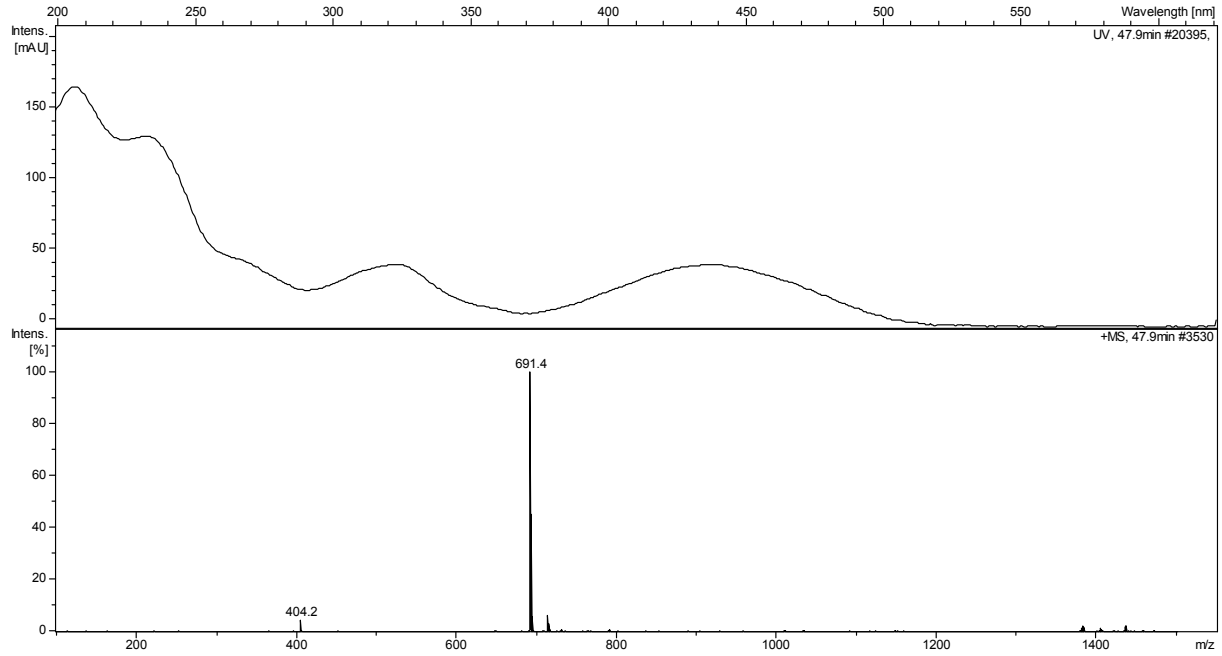


Structure of compound **15**

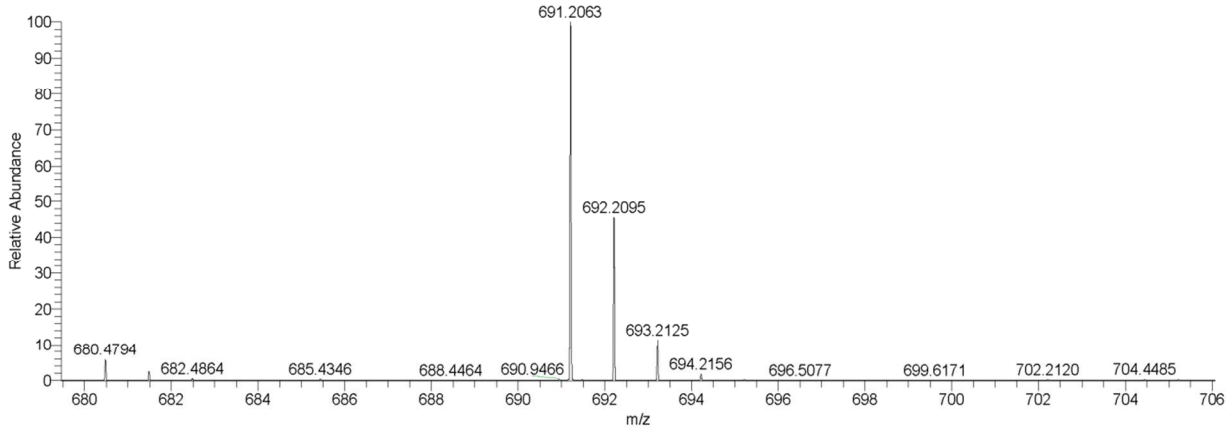
HPLC-ESIMS profile of **15** (UV 254 nm)



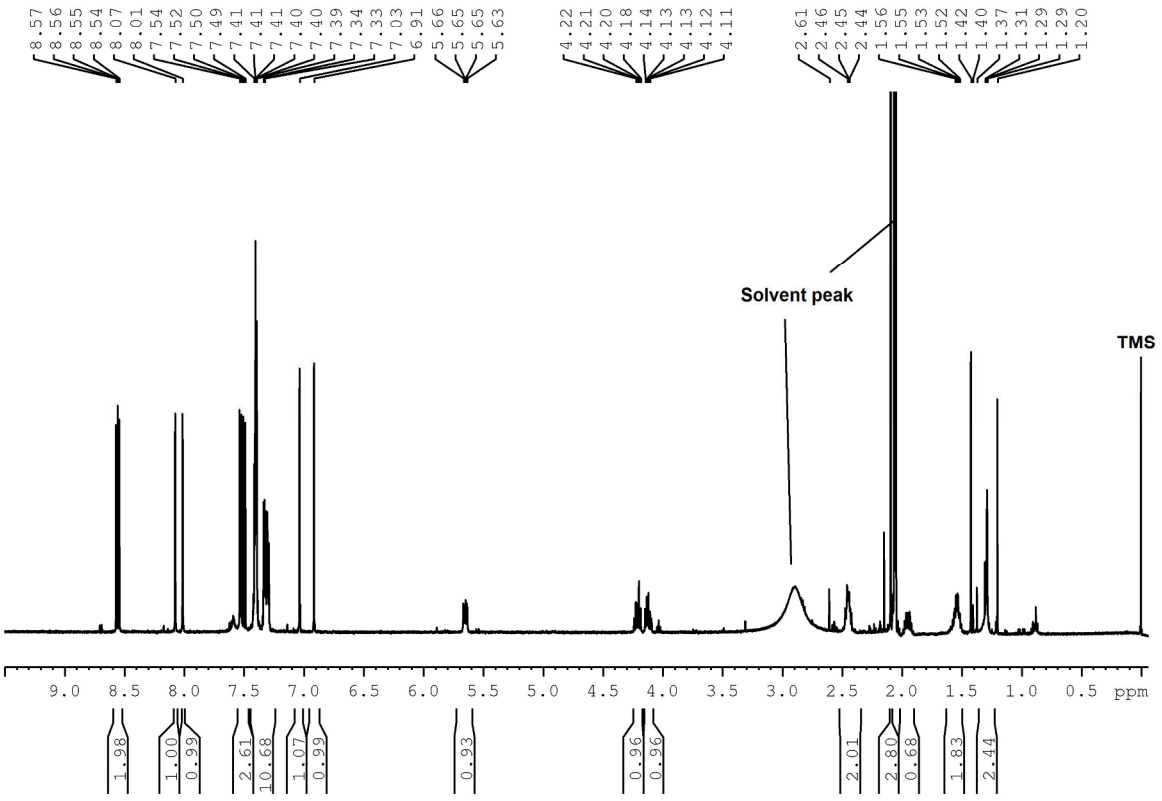
UV and ESIMS of **15**



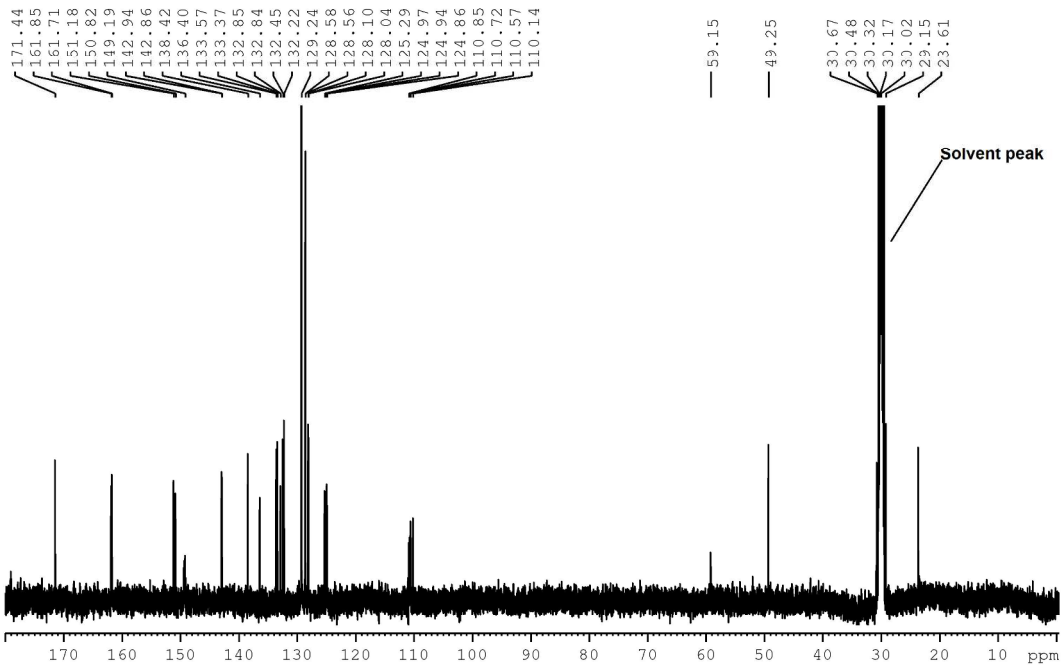
HRMS of **15**



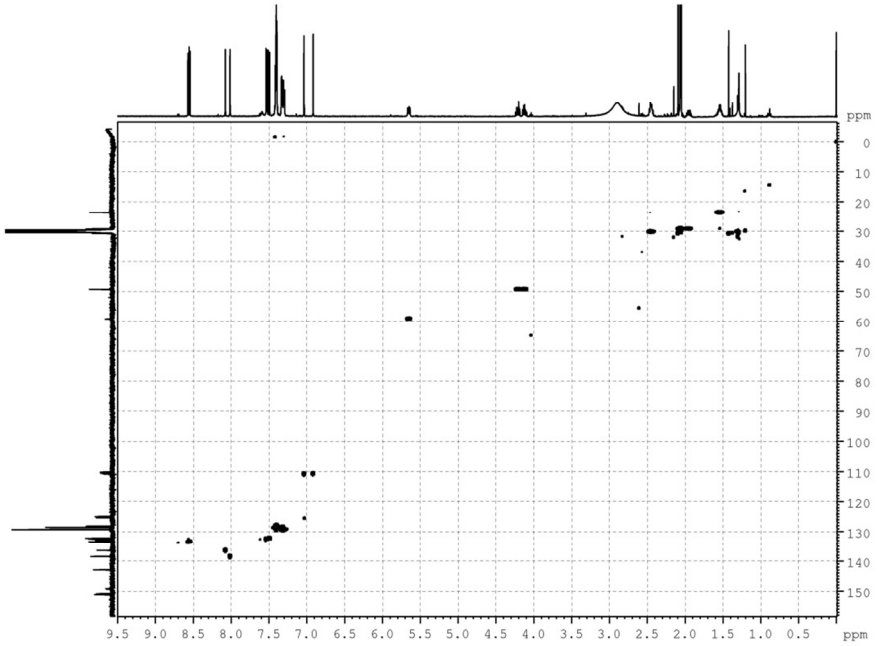
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **15**



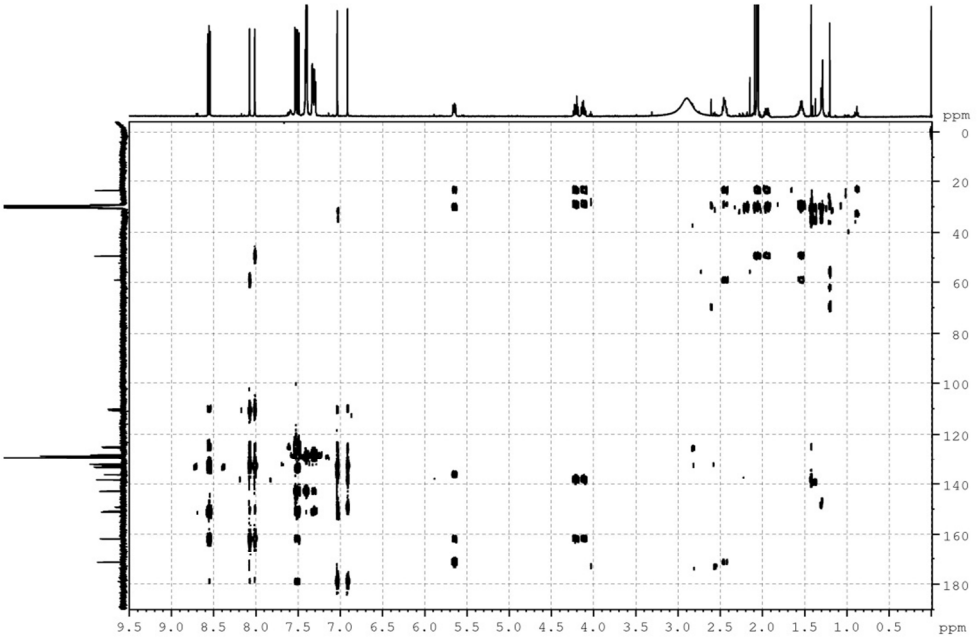
^{13}C NMR spectrum (500 MHz, acetone-d_6) of **15**

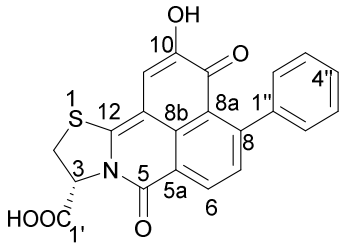


HSQC spectrum (500 MHz, acetone-d_6) of **15**



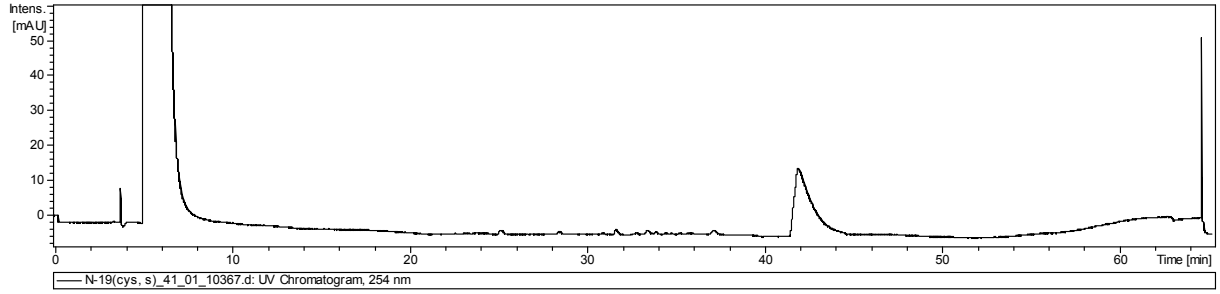
HMBC spectrum (500 MHz, acetone-d_6) of **15**



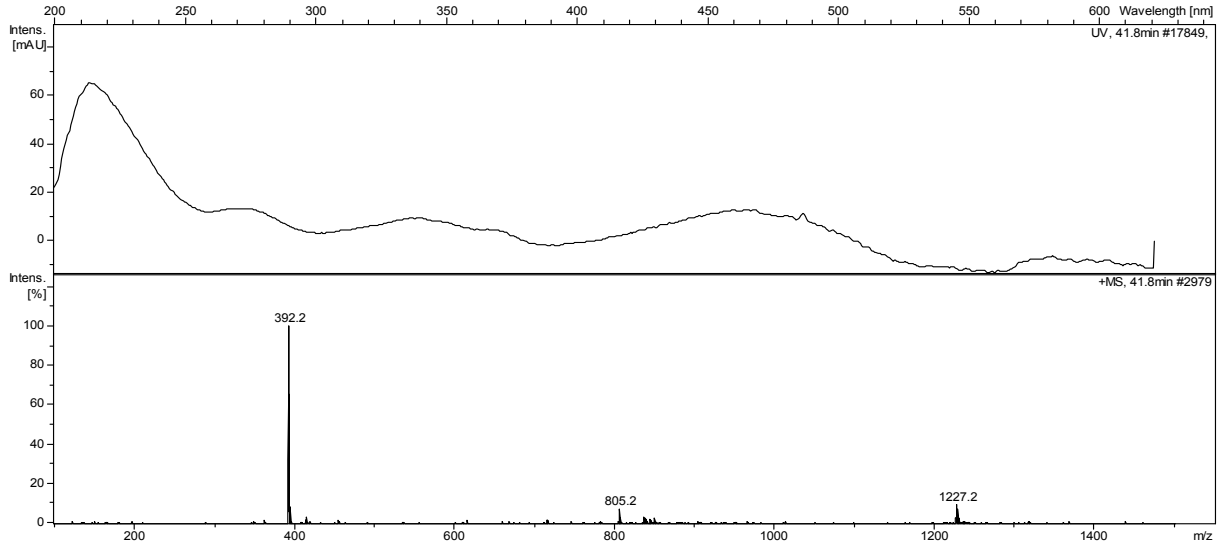


Structure of compound **16**

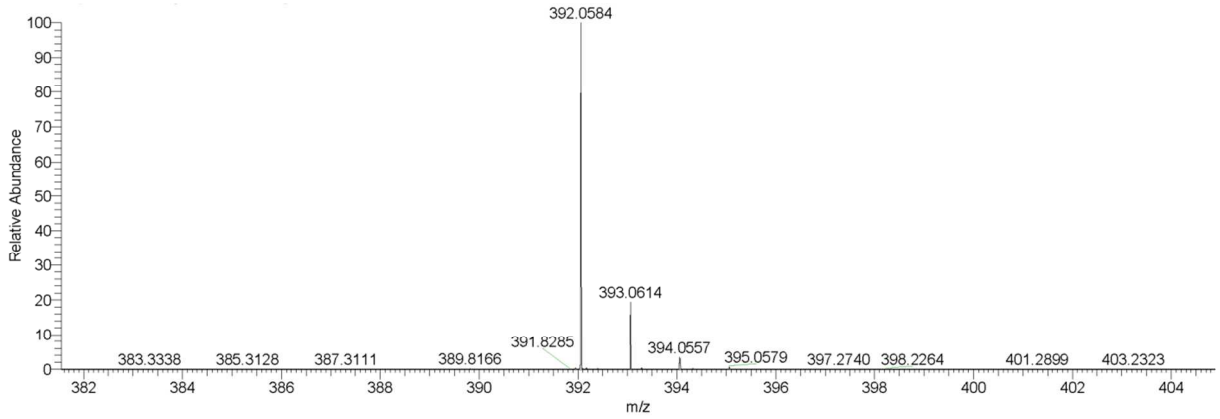
HPLC-ESIMS profile of **16** (UV 254 nm)



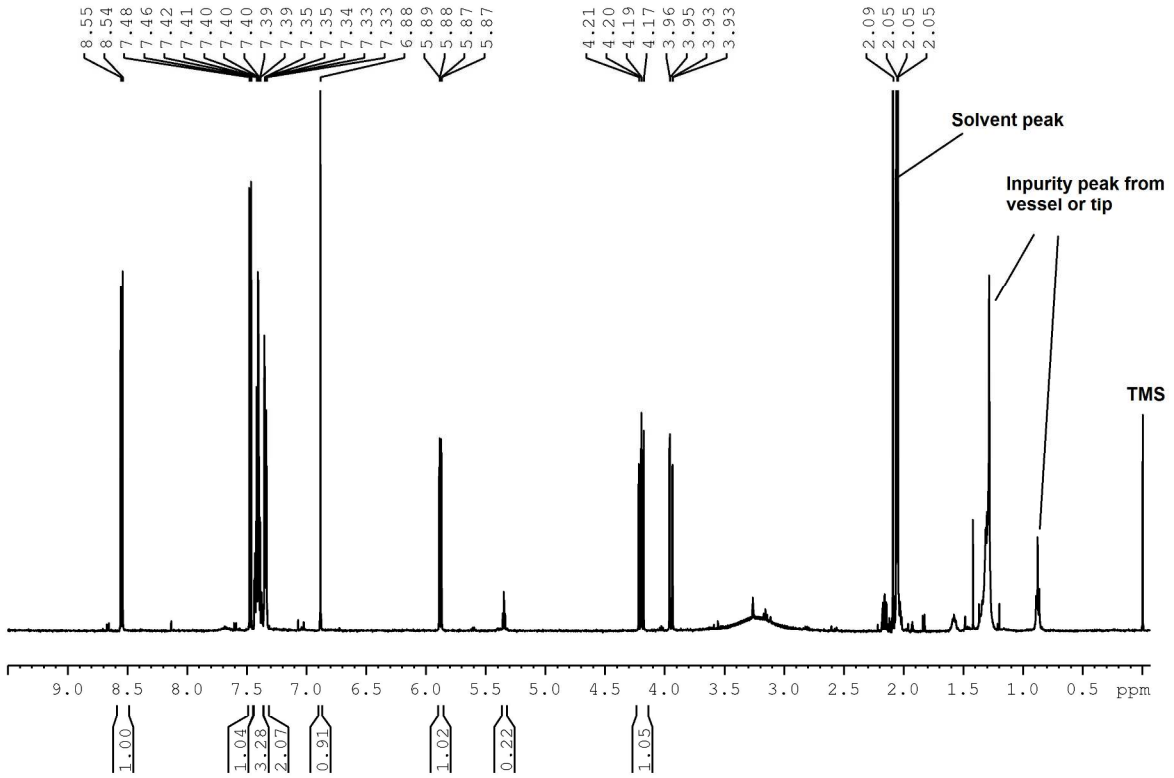
UV and ESIMS of **16**



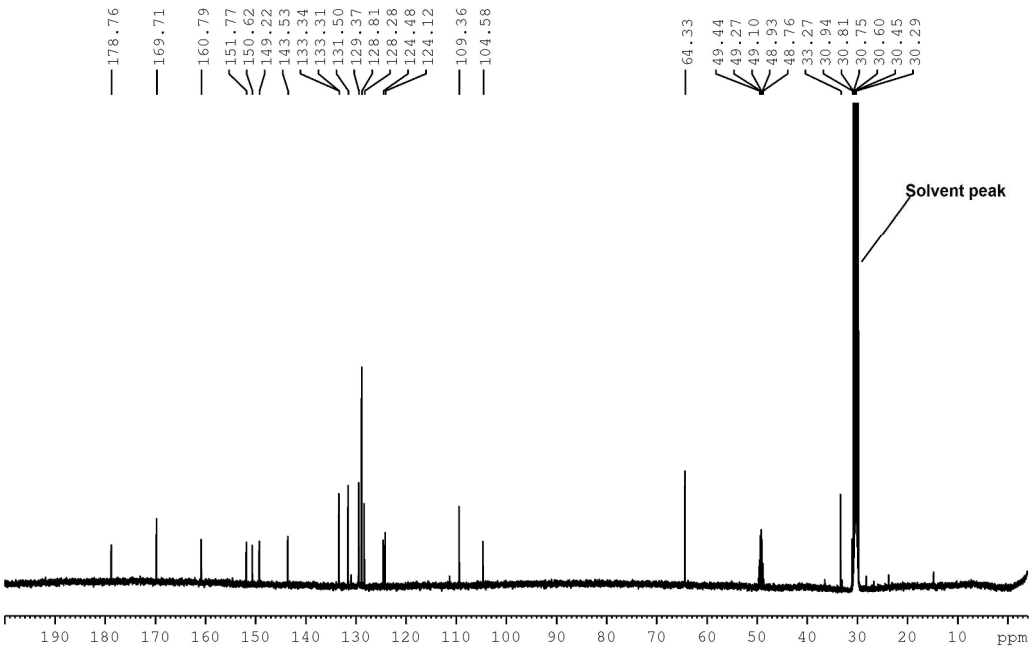
HRMS of **16**



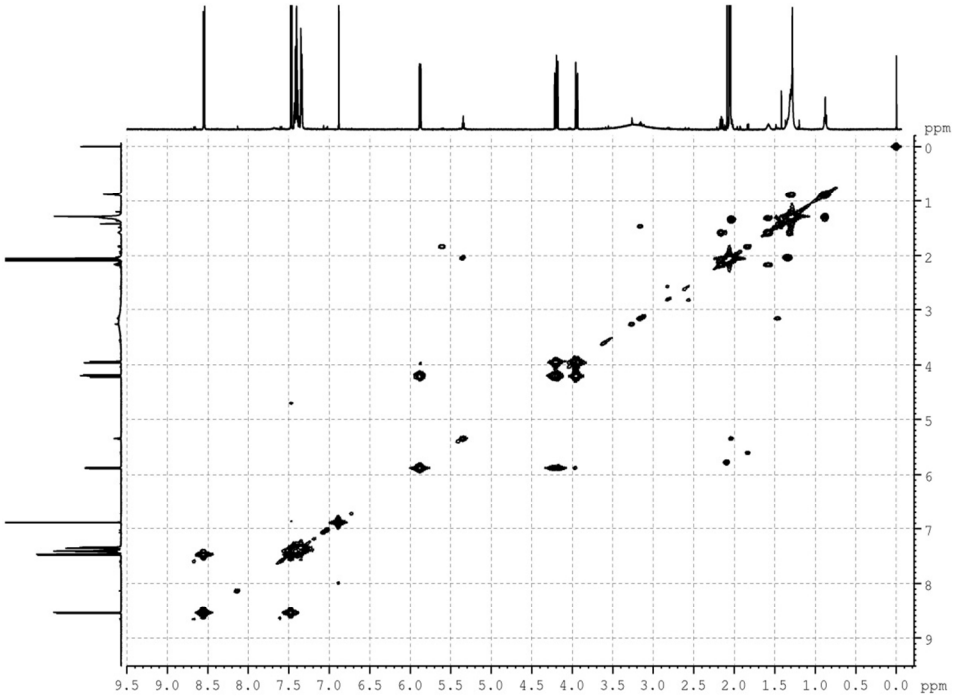
^{1}H NMR spectrum (500 MHz, acetone-d_6) of **16**



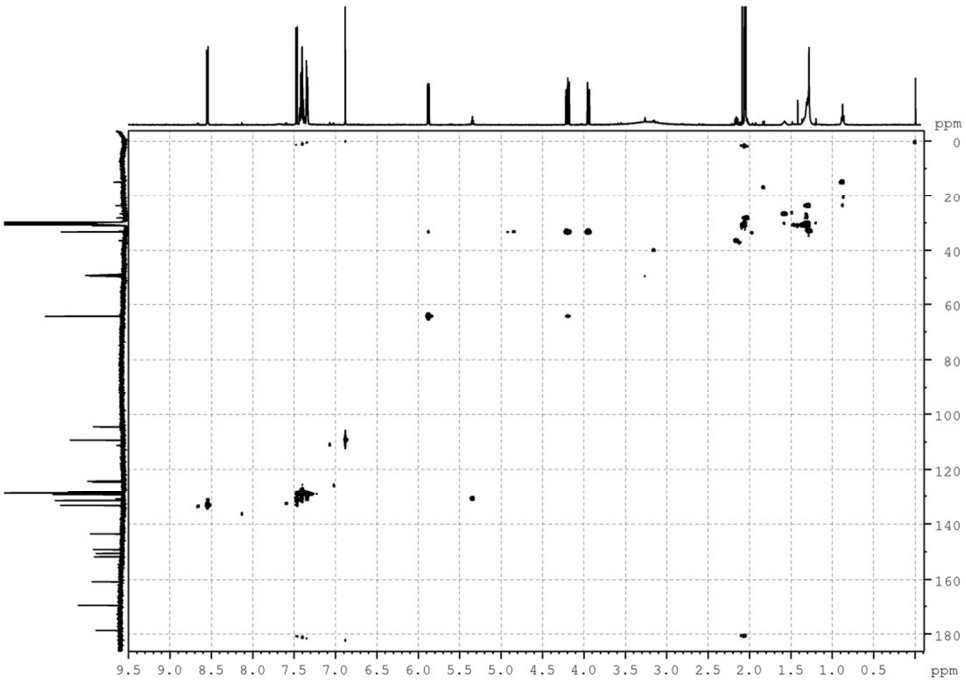
^{13}C NMR spectrum (500 MHz, acetone-d_6) of **16**



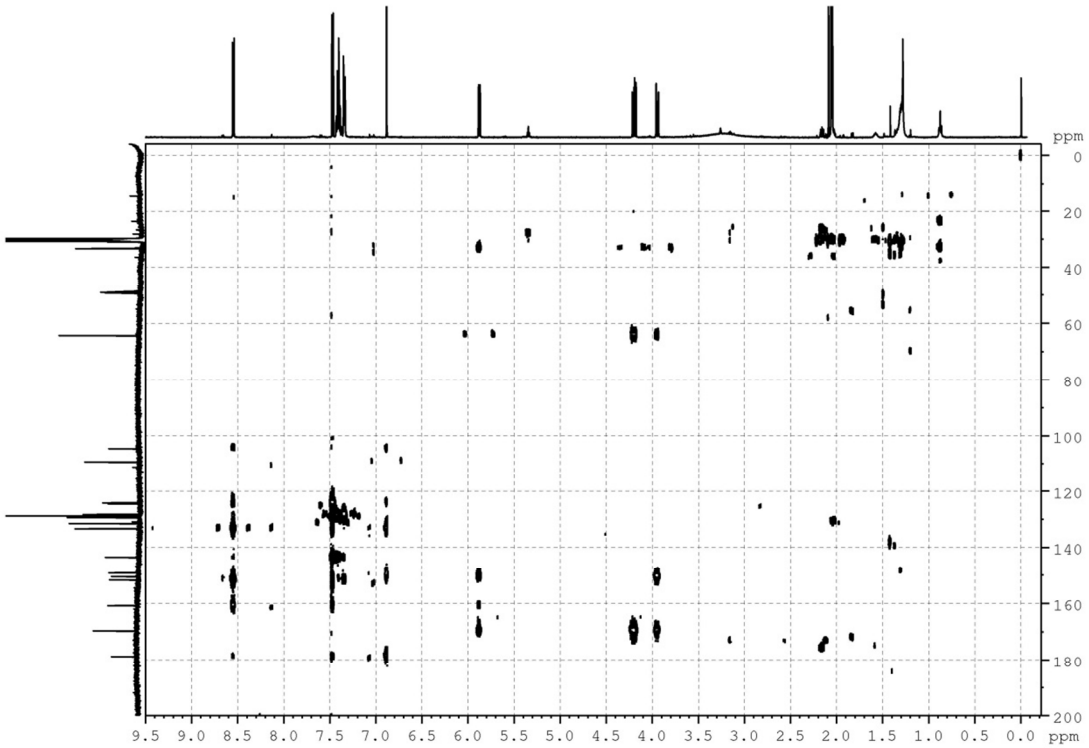
COSY spectrum (500 MHz, acetone-d_6) of **16**



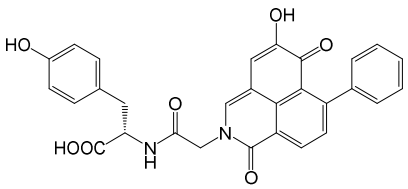
HSQC spectrum (500 MHz, acetone-d_6) of **16**



HMBC spectrum (500 MHz, acetone-d_6) of **16**

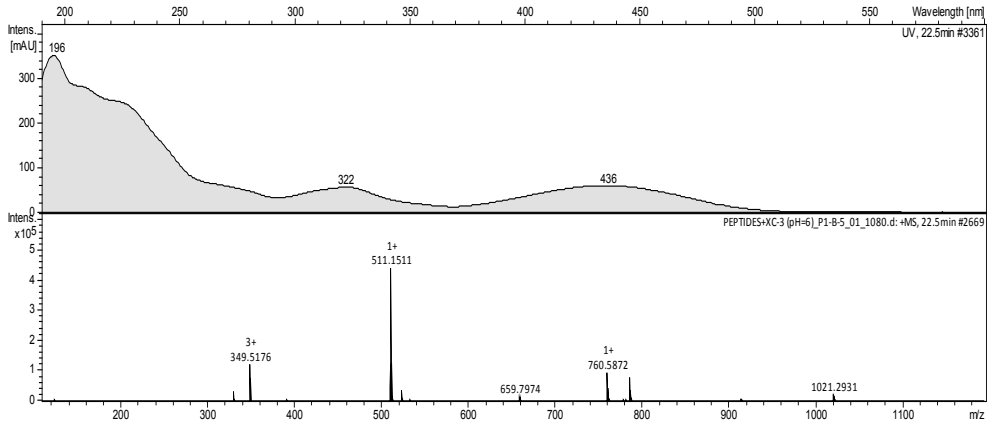


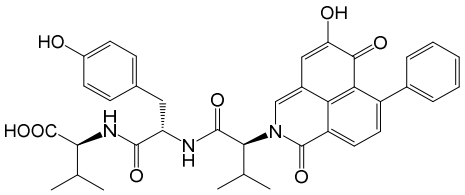
9. Spectra of the *N*-Terminal-Modified Peptides



Structure of compound **23**

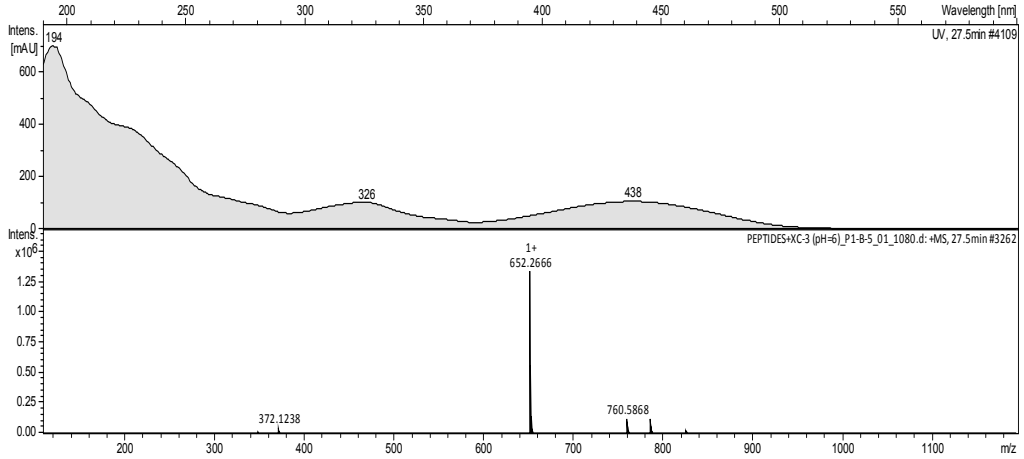
UV and HRESIMS of **23**

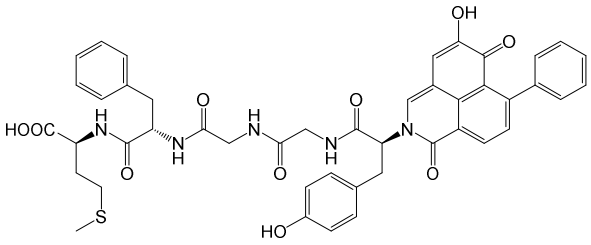




Structure of compound **24**

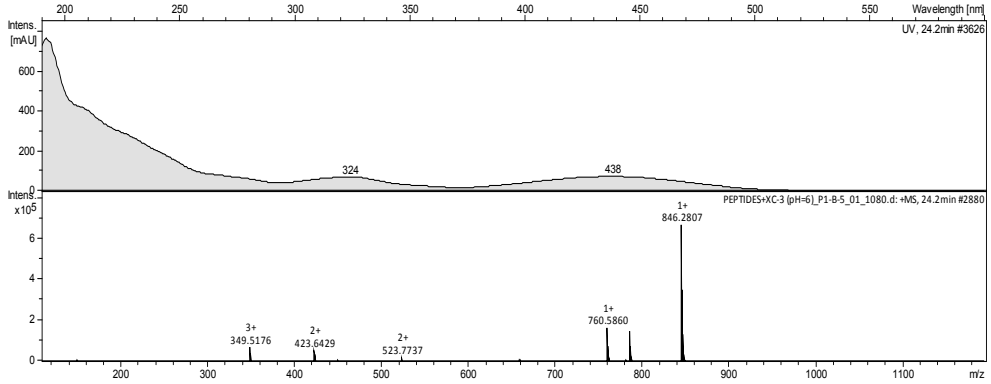
UV and HRESIMS of **24**

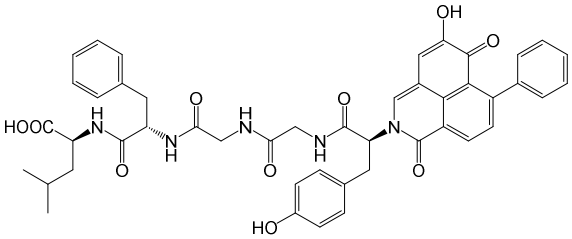




Structure of compound **25**

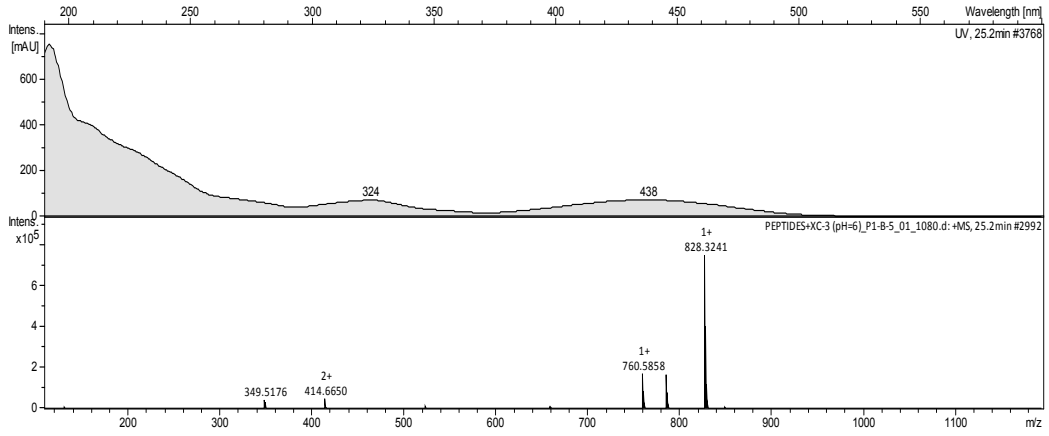
UV and HRESIMS of **25**





Structure of compound **26**

UV and HRESIMS of **26**



REFERENCES

(1) a) Chen, Y.; Paetz, C.; Menezes, R. C.; Schneider, B. *Phytochemistry* **2016**, *128*, 95-101;

b) Fang, J.-J.; Hölscher, D.; Schneider, B. *Phytochemistry* **2012**, *82*, 143-148.

(2) Fang, J.-J.; Kai, M.; Schneider, B. *Phytochemistry* **2012**, *81*, 144-152.

(3) Edwards, J.; Weiss, U. *Tetrahedron Lett.* **1972**, *13*, 1631-1634

(4) Edwards, J.; Weiss, U. *Phytochemistry* **1974**, *13*, 1597-1602.

(5) Bick, I.; Blackman, A. *Aust. J. Chem*. **1973**, *26*, 1377-1380.

(6) Opitz, S.; Hölscher, D.; Oldham, N. J.; Bartram, S.; Schneider, B. *J. Nat. Prod.* **2002**, *65*, 1122-1130.

(7) Bazan, A. C.; Edwards, J. *Phytochemistry* **1976**, *15*, 1413-1415.