Bromotyrosines from the Sponges *Acanthodendrilla* sp. and *Pseudoceratina* cf. *purpurea*

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A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Pharmaceutical Sciences

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Thesis Title	Bromotyrosines from the sponges *Acanthodendrilla* sp. and *Pseudoceratina* cf. *purpurea*
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Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

……Signature

(Mr. Opeyemi Joshua Olatunji)

Candidate

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Major Program Pharmaceutical Sciences

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ABSTRACT

The preliminary investigation of the CH_2Cl_2- and CH_3OH-extracts from the sponge *Acanthodendrilla* sp., showed potent acetylcholinesterase-inhibiting activity (79.5% at 0.1 mg/mL), and the $CHCl_3$- and *n*-BuOH-extracts from the sponge *Pseudoceratina* cf. *purpurea* showed potent acetylcholinesterase-inhibiting activity (92.0% and 94.0% inhibition, respectively at 0.1 mg/mL). Further investigation led to the isolation of five bromotyrosine-derived alkaloids; aerothionin (**1**), homoaerothionin (**2**), 11,19-dideoxyfistularin 3 (**3**), 11-oxoaerothionin (**4**), and 11-deoxyfistularin 3 (**5**), from the sponge *Acanthodendrilla* sp., and 15 alkaloids of the same class; purealidin Q (**6**), aplysamine 2 (**7**), aeroplysinin 1 (**8**), purpureamine J (**9**), aerophobin 1 (**10**), fistularin 3 (**11**), hemifistularin 3 (**12**), purealidin R (**13**), aplyzanzine A (**14**), iso-anomoian (**15**), purealidin B (**16**), purealidin J (**17**), aerophobin 2 (**18**), araplysillin I (**19**), and araplysillin II (**20**) from the sponge *P. purpurea*. All the isolated compounds were assayed for their acetylcholinesterase-inhibiting and cytotoxic activities. Compounds **6**, **7**, **14**, and **15** showed inhibitory activity against the acetylcholinesterase enzyme at IC_{50} values of 1.2, 1.3, 106.8, and 70.4 μM, respectively, with a non-competitive mode of inhibition. Structural similarities among the active compounds suggested that the *N,N*-dimethylaminopropyloxy moiety may exert an importance in the enzyme inhibition. On the other hand, compound **3** was cytotoxic against MCF-7 and human fibroblast cell lines at IC_{50} values of 0.53 and 1.46 μM respectively, while **10** showed cytotoxic activity against MCF-7, KB, HT-29, and human fibroblast cell lines at IC_{50} values of 0.79, 0.53, 0.91, and 4.2 μM, respectively.

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To the only wise God be all glory, majesty, dominion and power both now and forever, to Him be all the glory for the grace, favor, strength and ability to go through this work. I thank you Lord.

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Opeyemi Joshua Olatunji

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CHAPTER 1

INTRODUCTION

1.1 General introduction

Nature is an ancient pharmacy that used to be the solitary source of therapeutics for the early years. For thousands of years, nature has played a very important role in health care and prevention of diseases. The ancient civilization of the Chinese, Indians, and North Africans provided written evidences for the uses of natural sources for curing various diseases (Phillipson, 2001). However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants, beginning with the isolation of morphine by Friedrich Serturner from *Papaver somniferum* in 1806. Since then, natural products have been extensively exploited for their medicinal purposes.

According to recent studies conducted by World Health Organization, about 80% of the world's population relies on traditional medicine (WHO news, 2002). One hundred and twenty one drugs prescribed in the USA today are from natural sources, 90 of which are either directly obtained or indirectly derived from plant sources (Benowitz, 1996). Thus, a natural source makes a very significant contribution to the health care system.

Approximately 70% of the earth surface is covered by oceans, providing significant biodiversity for exploration for drug sources. The early days of marine natural products discovery efforts focused mostly on the conspicuous and easy-to-collect fauna and flora. Before the advent of SCUBA, exploration of the marine world lacked behind that of the terrestrial world because of the inability to access marine lives beyond the intertidal. By the middle of the twentieth century, marine natural products had become an established sub-discipline of natural products chemistry, and several thousand compounds had been described (Faulkner, 2002). Since then, marine natural products have emerged as an alternative and promising source of compounds with unprecedented chemical structures and novel pharmacological properties. Structurally, marine-derived secondary metabolites possess unique functional groups and chemical moieties, which in turn provide a possible unusual pharmacophores. This is due to the extreme and harsh

conditions in which marine environment provides. Many marine organisms have sedentary lifestyle and are under a persistent threat from predators. In response, they have engineered complex and extremely potent chemicals as their means of defense against predators (Haefner, 2003). These chemicals can serve as possible remedies for various ailments. Thus, the ocean represents a very rich and still largely untapped resources for biologically active compounds.

The first report on the active compound isolated from marine species was in the 1950's by Bergman and Feeney through their discovery of the nucleosides spongothymidine and spongouridine from the Caribbean sponge *Cryptotethya crypta* (Bergman and Feeney, 1951). The compounds became the basis for the syntheses of ara-A and ara-C, the first marine derived anticancer agents for the treatment of acute myelocytic leukemia and non-Hodgkin's lymphoma (Bodey et al., 1969). This has become an inspiration, leading to an extensive research to identify novel drug candidates from marine sources. To date, six marine-derived natural products have been approved to be used clinically with several others in clinical trials. Along with ara-C and ara-A mentioned above, a series of anticancer drugs derived from marine invertebrates have been approved. Trabectedin (Yondelis®) was first isolated from the Caribbean sea squirt *Ecteinascidia turbinata* (Rinehart et al., 1990), and now is approved as the first marine-derived anticancer drug for the treatment of advanced soft tissue sarcoma (Molinski et al., 2009). Another notable drug from marine invertebrates is eribulin mesylate (Halaven®), which is used for the treatment of metastatic breast cancer (Huyck et al., 2011). Eribulin mesylate is a structurally simplified macrocyclic analog of the potent cytotoxic compound halichondrin B, which was first isolated from the marine sponge *Halichondria okadai* (Hirata and Uemura, 1986).

The most recent addition to the list of the approved anti-cancer agents is brentuximab vedotin (Adcetris®), an achimeric antibody attached through a protease cleavable linker to a derivative of the potent antitubulin agent dolastatin 10 (Katz et al., 2011), for the treatment of Hodgkin's lymphoma and systemic anaplastic large cell lymphoma. Dolastatin 10 was first reported from the sea hare *Dolabella auricularia* (Pettit et al., 1987). The medicinal uses of marine natural products are not limited to only anticancer agents, but also extends to other classes of medicines. The most prominent one is ziconotide (Prialt®), a synthetic form of ω-conotoxin peptide isolated from the cone snail *Conus magnus*, used as an intrathecal analgesic for terminal state AIDS and cancer patients (Myers et al., 1993). Ziconotide acts as a selective N-type

voltage gated calcium channel blocker. This action inhibits the release of pro-nociceptive neurochemicals in the brain and spinal cord resulting in pain relief.

In addition to the currently approved drugs listed above, several other marine-derived compounds are now in their advanced stages of clinical trials and are listed in Table 1.

Table 1. Current clinical pipeline of marine natural products inspired drugs*

Compound name	Source	Disease area
Approved		
cytarabine, Ara-C (Cytosar-U®)	*Cryptotethia crypta* (sponge)	acute leukemia
vidarabine, Ara-A (Vira-A®)	*Cryptotethia crypta* (sponge)	antiviral
ziconotide (Prialt®)	*Conus magus* (cone snail)	chronic pain
eribulin mesylate (Halaven®)	*Halichondria okadai* (sponge)	breast cancer
trabectedin (Yondelis®)	*Ecteinascidia turbinata* (tunicate)	soft tissue sarcoma
brentuximab vedotin (Adcetris®)	*Dolabella auricularia* (sea hare)	Hodgkin's lymphoma
Phase III		
plitidepsin (Aplidine®)	*Aplidium albicans* (tunicate)	acute lymphoblastic leukemia
Phase II		
PM00104 (Zalypsis®)	*Jorunna funebris* (nudibranch)	cervical cancer
elisidepsin (Irvalec®)	*Elysia rufescens* (nudibranch)	gastric cancer
plinabulin	*Halimeda lacrimosa* (green algae)	non-small cell lung cancer
TZT 1027 (Soblidotin)	*Dolabella auricularia* (sea hare)	metastatic soft tissue sarcoma
ILX-651 (Synthadotin)	*Dolabella auricularia* (sea hare)	prostate cancer

Table 1. (cont.)

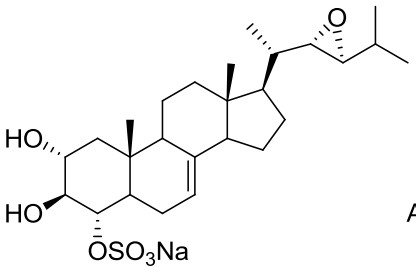
Compound name	Source	Disease area
Phase I		
pseudopterosins	*Pseudopterogorgia elisabethae* (soft coral)	wound healing
hemiasterlin	*Hemiasterella minor* (sponge)	cancer
bryostatin 1	*Bugula neritina* (bryozoan)	cancer
marizomib (salinosporamide A)	*Salinispora tropica* (marine bacteria)	cancer

*Adapted from Gerwick and Moore (2012), and Yonghong (2012).

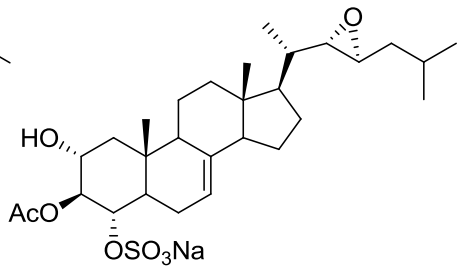
1.2 Chemical constituents from the sponge *Acanthodendrilla* sp.

The genus *Acanthodendrilla* belongs to the family Dictyodendrillidae (order Dendroceratida), in which 108 species have been taxonomically identified and reported (van Soest et al., 2011), and is distributed around the Pacific Ocean and Mediterranean Sea (Uriz and Maldonado, 2000). The sponge is a foliaceous to massive erect sponge. The holotype is 9.5 cm high, 8 cm wide erect thin fan-like sheet, with some lateral projections perpendicular to the general sponge plane.

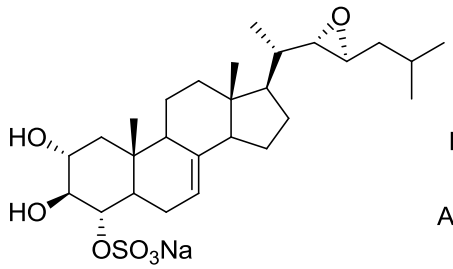
To date, there are very few publications in regard to the chemical study on the sponges of this genus. Acanthosterols A-J were first isolated from the Japanese specimen of *Acanthodendrilla* (Tsukamoto et al., 1998). Acanthosterols I and J inhibited *Saccharomyces cerevisiae* A364A and its mutant at 0.1 mg/disk (Tsukamoto et al., 1998).



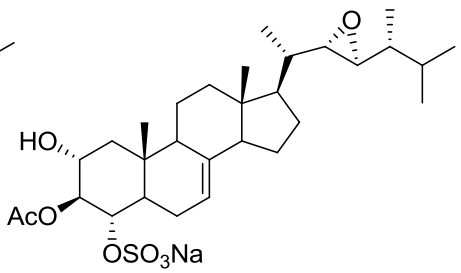
acanthosterol A



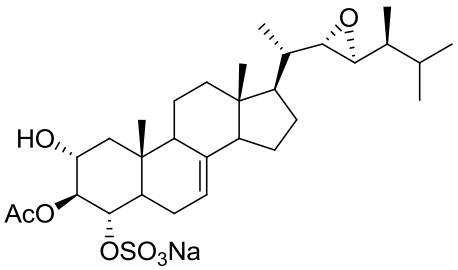
acanthosterol B



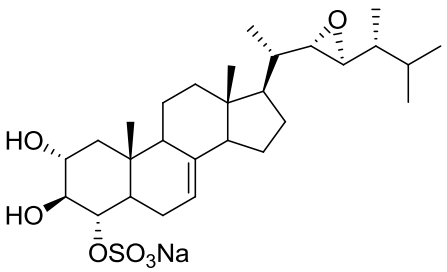
acanthosterol C



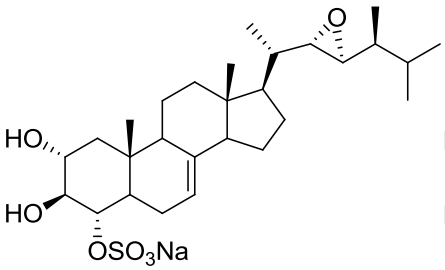
acanthosterol D



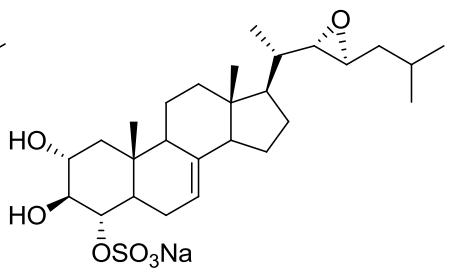
acanthosterol E



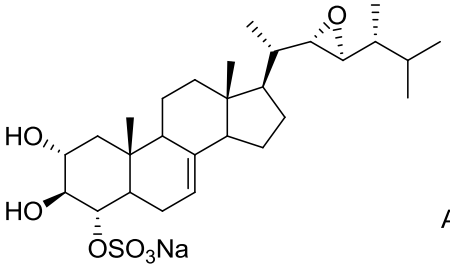
acanthosterol F



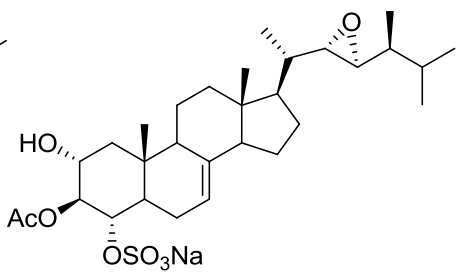
acanthosterol G



acanthosterol H

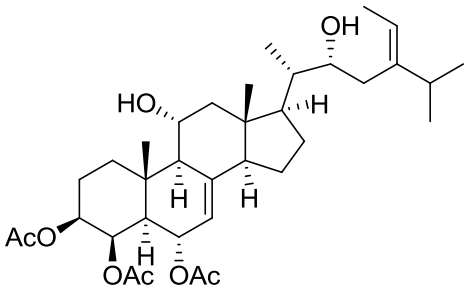


acanthosterol I

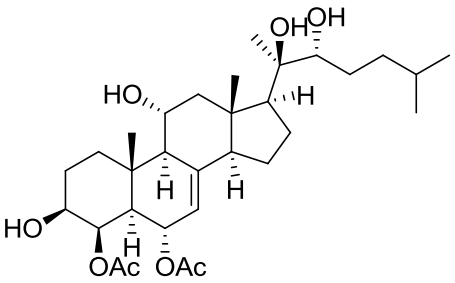


acanthosterol J

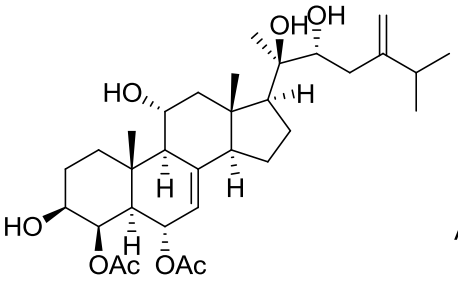
Tsukamoto et al. (2003) reported seven agosterol congeners from the Japanese specimen of *Acanthodendrilla* sp. Agosterols E and E_3 were the first 9α-hydroxy agosterol congeners. Agosterol C showed a potent proteasome inhibitory activity with an IC_{50} value of 10 μg/mL.



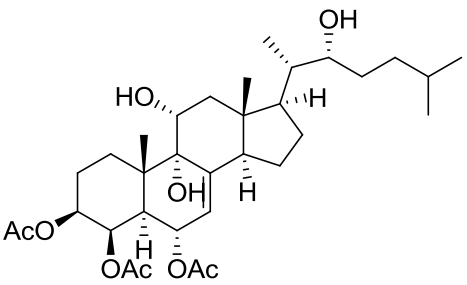
agosterol A_3



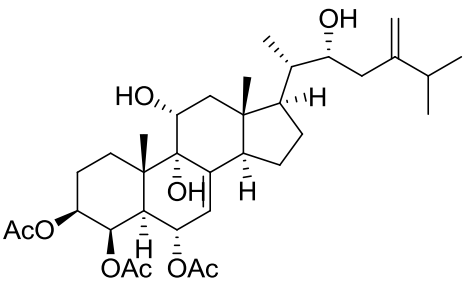
agosterol C_2



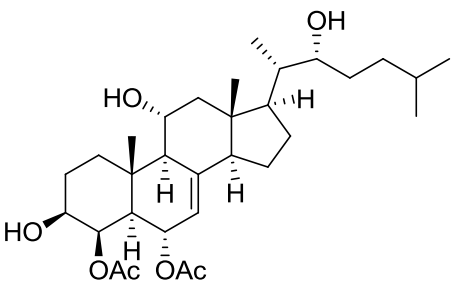
agosterol C_7



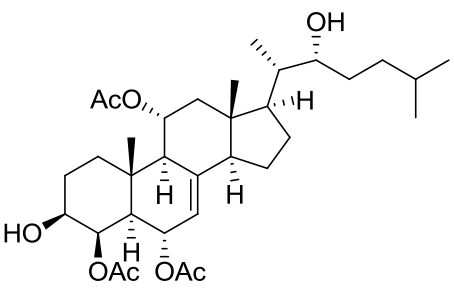
agosterol E



agosterol E_3

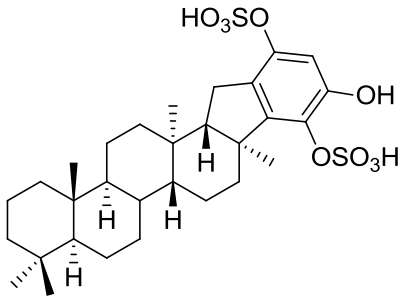


agosterol F



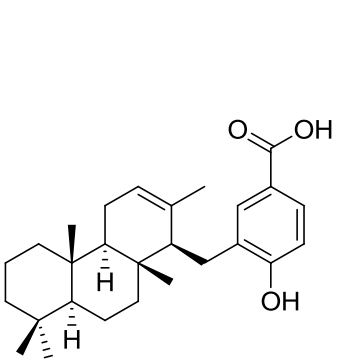
agosterol G

Recently, acanthosulfate, a disulfated hydroxyhydroquinone having a scalarane type carbon skeleton, has also been reported from a specimen of sponge *Acanthodendrilla* sp. gotten from Boracay Island, Philippines. It is the first reported merosesterterpene with such configuration. It inhibited proteasome function with an IC_{50} value of 4.5 μM (West and Faulkner, 2008).

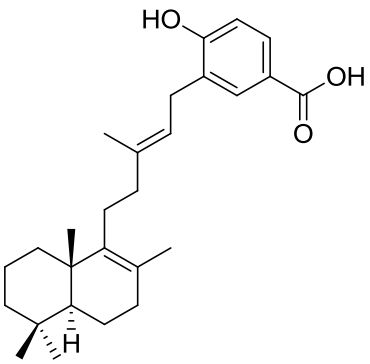


acanthosulfate

Various classes of terpenoid derivatives, including meroterpenoid and monocyclic sesterterpenes were also reported from *Acanthodendrilla* sponges. (+)-Makassaric and (+)-suberic acids were reported from an Indonesian specimen of the sponge *Acanthodendrilla* sp., and showed inhibitory activity against protein kinase MK2 at 20 and 9.6 μM, respectively (Williams et al., 2004).



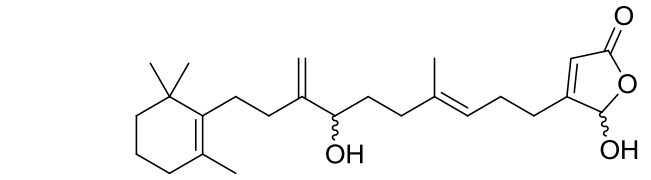
(+)-makassaric acid



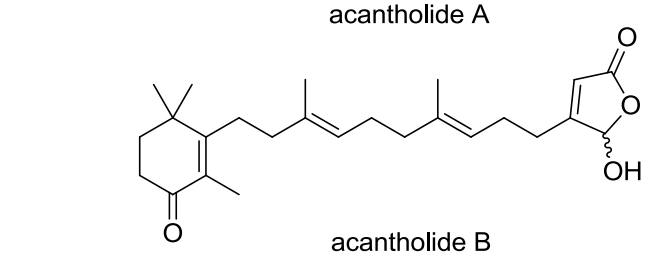
(+)-suberic acid

Elkhayat et al. (2004) reported a series of luffariellolide-related sesterterpenes, acantholides A-E, along with luffariellolide and its 25-*O*-methyl and 25-*O*-ethyl derivatives from an Indonesian specimen of *Acanthodendrilla*. Acantholide E, luffariellolide, and 25-*O*-methylluffariellolide were cytotoxic against the mouse lymphoma L5187Y cell line at ED_{50}

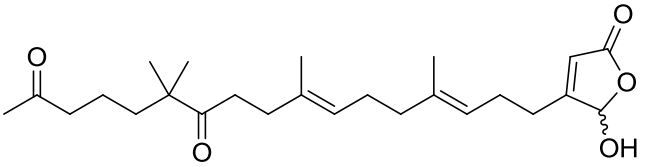
values of 7 µg/mL, 3.3 µg/mL, and 0.7 µg/mL, respectively. Luffariellolide also inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* at concentrations of 5 µg and 10 µg/disk. Acantholide B and 25-*O*-methylluffariellolide were active against the plant pathogenic fungus *Cladosporium herbarum* at a concentration of 10 µg/mL.



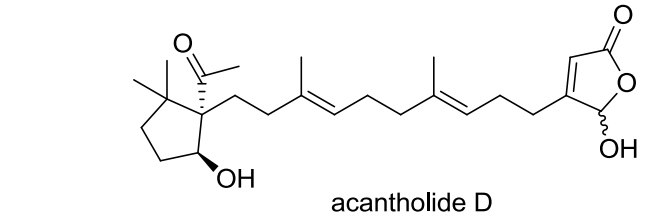
acantholide A



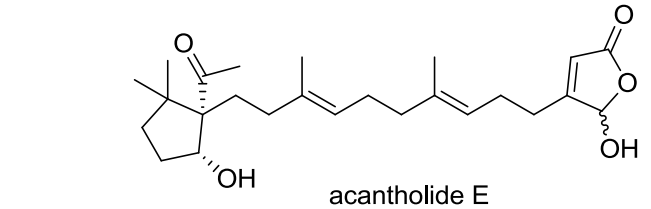
acantholide B



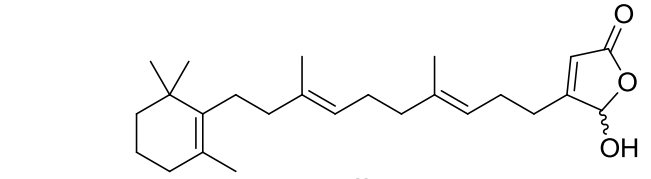
acantholide C



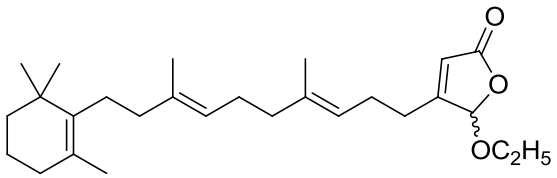
acantholide D



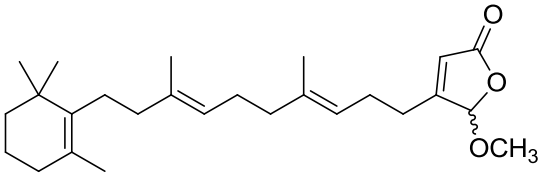
acantholide E



luffariellolide



25-*O*-ethylluffariellolide

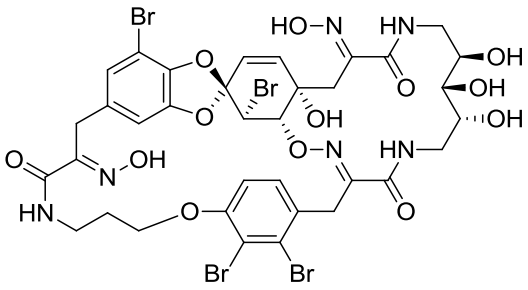


25-O-methyllluffariellolide

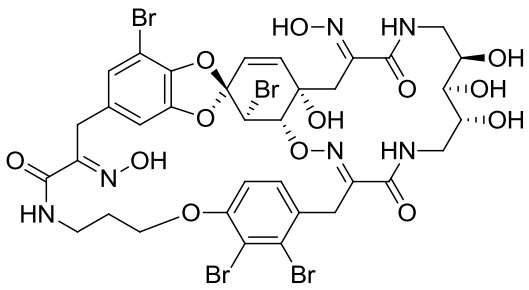
1.3 Chemical constituents from the sponge *Pseudoceratina purpurea*

The genus *Pseudoceratina* belongs to the family Pseudoceratinidae (order Verongida) in which five species have been taxonomically identified, namely *P. purpurea*, *P. durissima*, *P. arabica*, *P. verrucosa*, and *P. droogeri*. All the sponges in the genus *Pseudoceratina* have been chemotaxonomically characterized by the presence of secondary metabolites that are derived from bromotyrosine or from chlorotyrosine (D'Ambrosio et al., 1984).

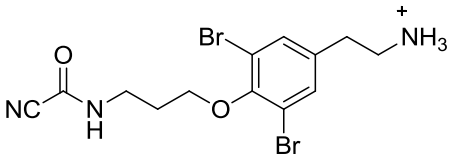
The sponge *Pseudoceratina purpurea,* which is the focal point in this thesis, is extremely varied in shapes from knobs and lobes, to slender branching stems emerging from an encrusting base. The sponge can cover large areas of coral rubbles or small stones. The colors range from bright yellow or greenish yellow to dark or olive green with yellowish tips. Jang et al. (2007) reported two antifungal bicyclic pseudoceratins A and B from a specimen of *P. purpurea* collected at Oshima-shinsone in southern Japan. Pseudoceratins A and B showed a growth inhibitory activity against the erg6 mutant of *Saccharomyces cerevisiae* at 6.5 μg/disk. Ceratinamine and moloka'iamine were also reported from the sponge *P. purpurea* collected off Hachijo-jima Island, Japan. Ceratinamine and moloka'iamine showed antifouling activity against *Balanus amphitrite* cyprids with EC_{50} values of 5.0 and 4.3 μg/mL, respectively, and were also cytotoxic against P388 murine leukemia cells with IC_{50} values of 3.4 and 2.1 μg/mL (Tsukamoto et. al, 1996[a]).



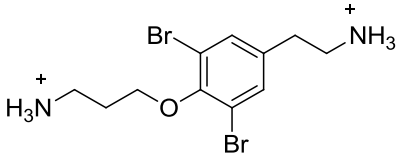
pseudoceratin A



pseudoceratin B

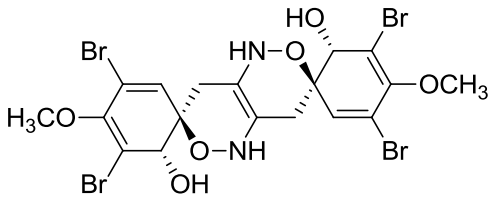


ceratinamine

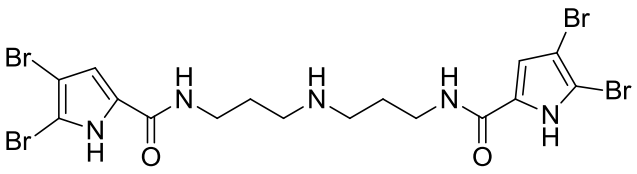


moloka'iamine

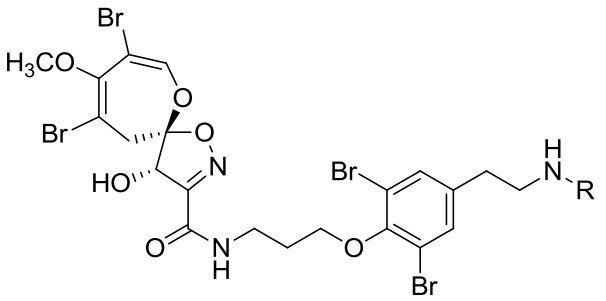
Zamamistatin was reported from the Okinawan specimen of *P. purpurea* (Takada et al., 2001; Kita et al., 2008). It exhibited antibacterial activity against the marine bacteria *Rhodospirillum salexigens*. An antifouling pseudoceratidine was isolated from the Japanese specimen of *P. purpurea.* It inhibited larva settlement and metamorphosis of the barnacle *Balanus amphitrite* with an ED_{50} value of 8.0 µg/mL and was lethal to larvae at the concentrations greater than 30 µg/mL (Tsukamoto et al., 1996[b]). Two antifouling agents, ceratinamides A and B, along with the known psammaplysins A and E, were reported isolated from a specimen of *P. purpurea* collected off Hachijo-jima Island, Japan (Tsukamoto et al., 1996[c]).



zamamistatin



pseudoceratidine

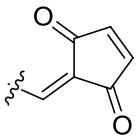


R

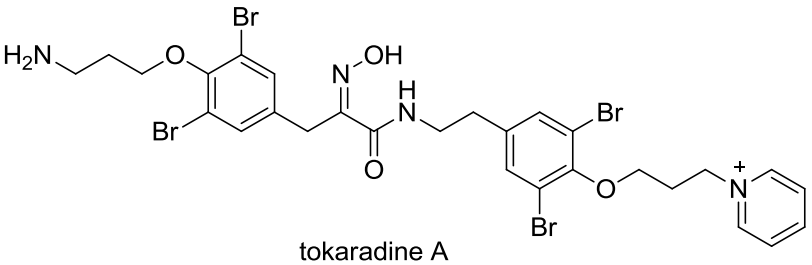
ceratinamide A; CHO

ceratinamide B; $CO(CH_2)_{11}CH(CH_3)_2$

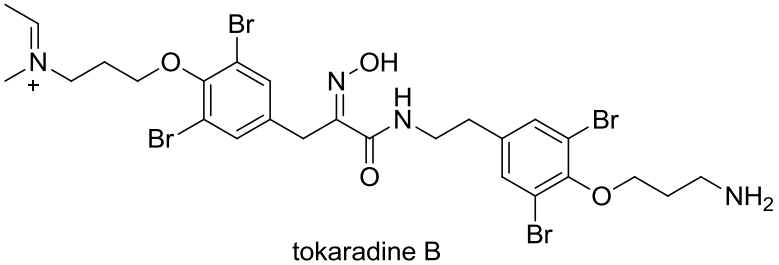
psammaplysin A; H

psammaplysin E; 

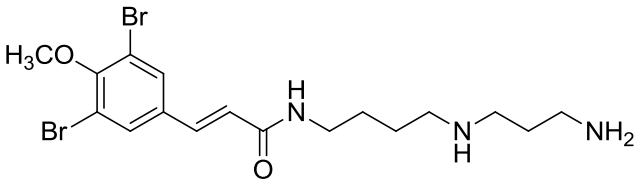
Fusetani et al. (2001) reported the isolation of tokaradines A, B, and C, along with the known purealidins A and C from *P. purpurea* collected off Nakaroshima Island, Japan. Tokaradines A and B were lethal to the crab *Hemigrapsus sanguineus* at 50 and 20 μg/g, respectively.



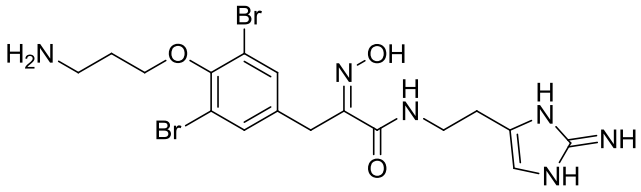
tokaradine A



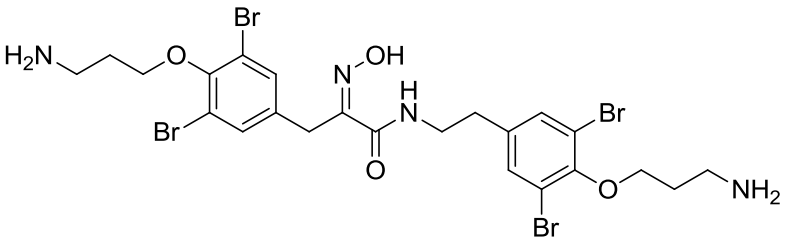
tokaradine B



tokaradine C



tokaradine A

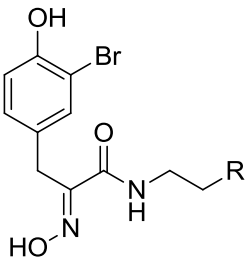


purealidin C

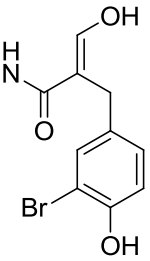
Ten bisulfide bromotyrosine derivatives, psammaplins A-J and bisaprasin were reported from two specimens of *P. purpurea* from Bagabag and Hermit Islands, Papua New Guinea. Psammaplins A, F, and bisaprasin were potent histone deacetylase inhibitors at IC_{50} values of 4.2, 8.6, and 10.7 nM, respectively. Psammaplins A, G, and bisaprasin were also potent DNA methyltransferase inhibitors at 18.6, 12.8, and 3.4 nM, respectively (Pina et al., 2003).

1.4 Bromotyrosine derived natural products

Bromotyrosine-derived natural products are alkaloids produced mainly by sponges belonging to the order Verongida. Over 90% of the reported bromotyrosines are derived from four families in the order Verongida, namely: Aplysinidae, Aplysinellidae, Ianthellidae, and Pseudoceratinidae (Tran et al., 2013). These marine derived alkaloids are well known for displaying a wide range of biological activities including antibacterial, antifouling, antimalarial

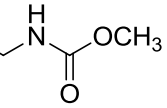


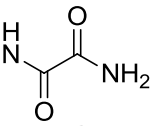
psammaplin A; R= S—S

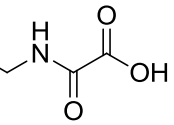


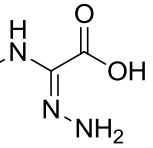
psammaplin B; R= SCN

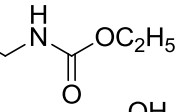
psammaplin C; R= SO_2NH_2

psammaplin D; R= S—S 

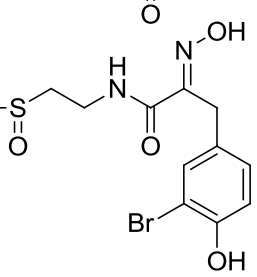
psammaplin E; R= S—S 

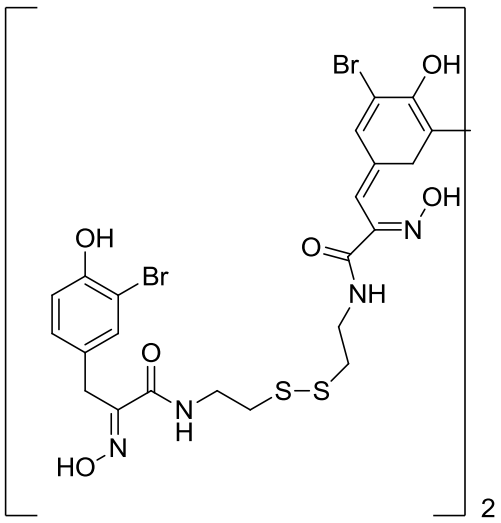
psammaplin F; R= S—S 

psammaplin G; R= S—S 

psammaplin H; R= S—S 

psammaplin I; R= SO_2CH_3

psammaplin J; R= S—S 



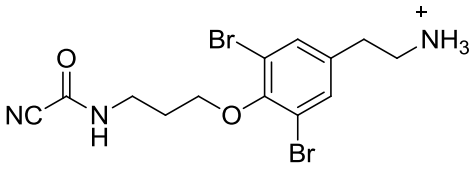
bisaprasin

cytotoxic, antiangiogenic, histamine H_2 and H_3 receptor antagonists, anti-human immuno-deficiency virus 1 (HIV-1), and mycothiol-*S*-conjugate amidase inhibitory activities (Tran et al., 2013).

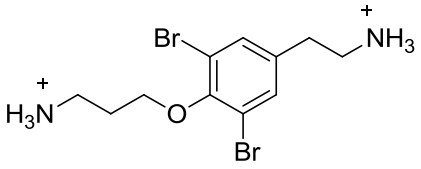
To date there have been over 350 bromotyrosine-derived natural products reported. These alkaloids exhibit a wide range of chemical structures. Categorization has been made to classify the alkaloids systematically (Peng et al., 2005). However, in line with the findings in this study, the categorization of bromotyrosine alkaloids might as well be organized as follows;

- Single-unit bromotyrosine alkaloids
- Binary bromotyrosine alkaloids
- Multi-order bromotyrosine conjugates
- Bromotyrosines with other amino acids

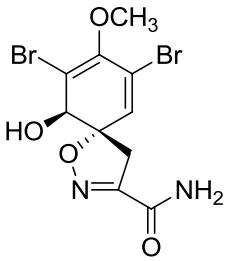
As stated by the name, single-unit bromotyrosine alkaloids are derived solely from one tyrosine residue. The structural features may range from those in which the tyrosine unit is fully intact with very little transformation as seen in moloka'iamine and ceratinamine, to a highly derived one as in the cyclized-dearomatized analogs, particularly as a 1-oxa-2-azaspiro[4.5]deca-2,6,8-triene, as seen in purealidin R.



ceratinamine

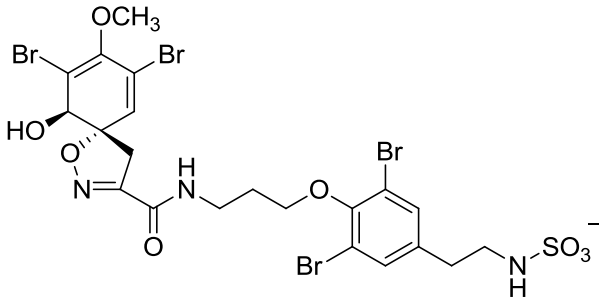


moloka'iamine

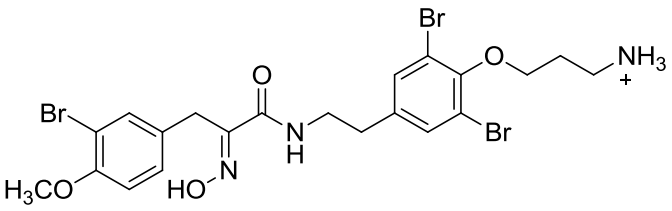


purealidin R

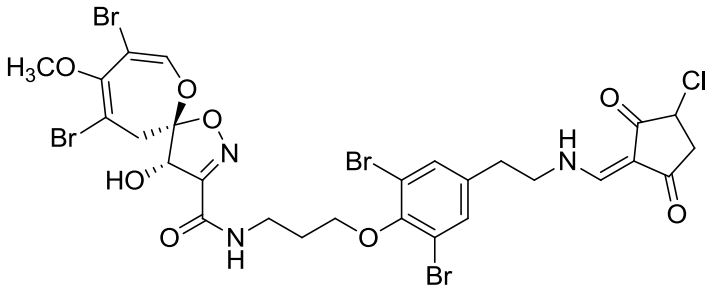
The binary bromotyrosine alkaloids constitute the largest group of bromotyrosine alkaloids. In general, the binary alkaloids consist of two units, very often non-parallelly derived, joining through either an amide, amino, or ether linkages, depended on the orientation of the linking moieties. Structural variations take place in two major features. The first one is the nature of such linkages as a direct linkage between functional group directly derived from the original bromotyrosine residue, or through an additional linker such as putrescine and related polyamines. Another featural variation is the cyclization and the oxidation state of the amino acid functionalities. Cyclization-dearomatization towards oxa,azaspirodecatriene similar to those described for single unit bromotyrosine is common, although other forms of cyclization have been reported. The amino acid functionalities can be found either as a saturated amine or the higher order nitrogenated functionalities as in imine and oxime. Some examples of compounds in this category include ianthesine E, psammaplysin X, and aplysamine 3.



ianthesine E

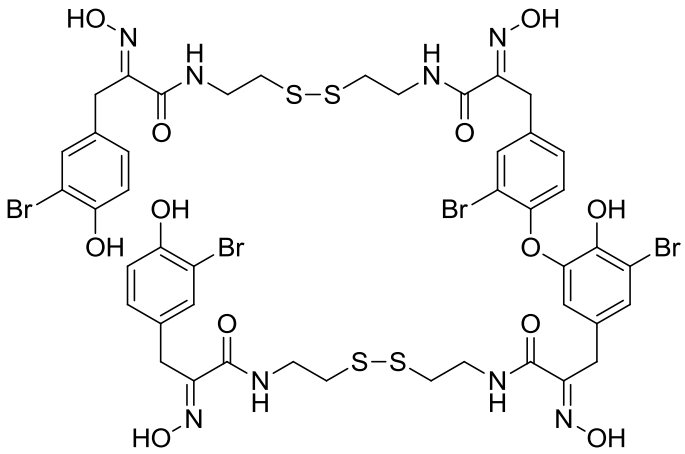


aplysamine 3

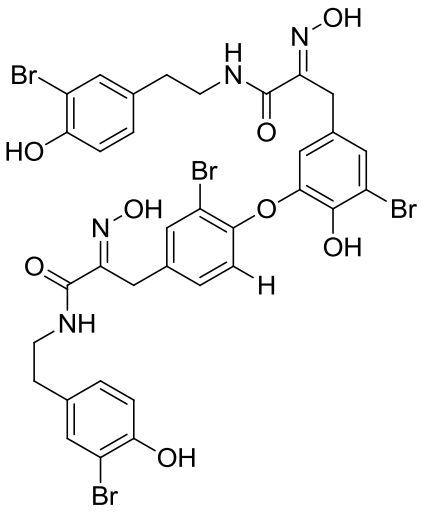


psammaplysin X

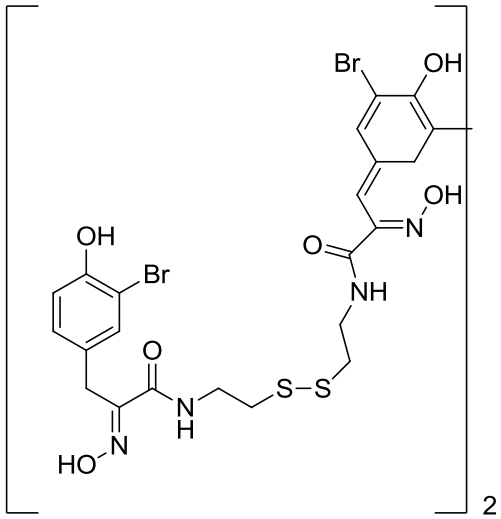
Although less encountered, bromotyrosines with multiple-unit conjugation are widely known. The most well known one may be exemplified by the bastadins, in which four units of bromotyrosines conjugate and linked by either a disulphide, ether or amide linkages. Examples include bastadin 1, bisaprasin, and bispsammaplin A



bispsammaplin A

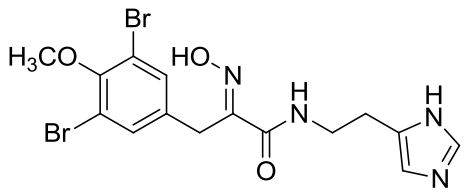


bastadin 1

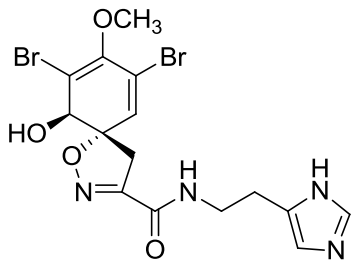


bisaprasin

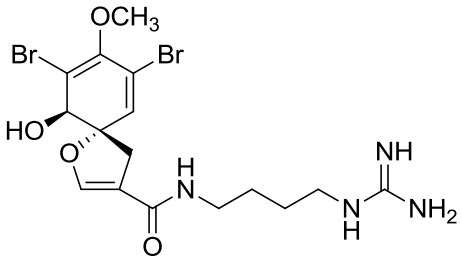
Apart from binary alkaloids of two bromotyrosines, conjugated alkaloids between bromotyrosine and other amino acids have been reported. The most common amino acid found conjugating with bromotyrosines is histidine, from which residues of imidazole and 2-aminoimidazole are introduced. Some examples include aerophobin 1, purealidin L, and 5-bromoverongamine.



5-bromoverongamine



aerophobin 1



purealidin L

Listed in Table 2 is the compilation of bromotyrosine alkaloids, their sources, and biological activities reported to date. Organization of the compounds as shown in the table is according to the categorized order stated above.

1.5 Rationale and objectives

The uniqueness of marine natural products as described previously has established a well designed rationale for the drug discovery targeting the utilization of marine-derived chemical moieties as possible research targets. Based on this rationale, the research described in this dissertation is composed of two separated but closely related projects. The first part involves the isolation and characterization of bioactive components from the sponge *Acanthodendrilla* sp. The CH_2Cl_2- and CH_3OH-extracts of the sponge were preliminarily tested to show potent acetylcholinesterase-inhibiting activity (79.5% inhibition at 0.1 mg/mL). Along with the isolation and structure determination, the biological activities, namely acetylcholinesterase-inhibiting and cytotoxic activities, were also investigated.

The second part involves the isolation and identification of bioactive components from the sponge *Pseudoceratina* cf. *purpurea*. The $CHCl_3$- and *n*-BuOH-extracts from the sponge *P.* cf. *purpurea* were screened for their acetylcholinesterase-inhibiting (92.0% and 94.0% inhibition at 0.1 mg/mL) and cytotoxic activities (> 80% inhibition). Based on the aforementioned preliminary screening, the extensive investigation on the chemical entities in both sponges were pursued. The objectives of this work include

i) the isolation and structural characterization of the chemical constituents from the sponges *Acanthodendrilla* sp. and *P.* cf. *purpurea*.

ii) the determination for the acetylcholinesterase-inhibiting and cytotoxic activities of the isolated compounds.

CHAPTER 2

EXPERIMENTAL

2.1 General experimental procedures

Unless stated otherwise, all chemicals and chromatographic packing materials were used as purchased. Solvents for chromatographic and general purposes were commercial grade and were re-distilled prior to use. All HPLC solvents were HPLC grade, and were filtered through a 0.45 μm membrane filter and degassed in an ultrasonic bath. Thin layer chromatography (TLC) was performed on SiO_2 60 F 254 on aluminum supports (0.02 mm layer thickness; Merck®). Visualization was done under UV light (254 nm), and with Dragendorff spraying reagent. Vacuum and flash chromatographies were performed on SiO_2 60 (particle size 0.04-0.06 mm; Scharlau®). Size exclusion chromatography was conducted on Sephadex™ LH-20 (GE Healthcare®), which was saturated in eluting solvents for 24 hours before using. Semi-preparative HPLC was performed either on a Waters 600E System Controller equipped with a Rheodyne 7125 injector port, a Waters 484 tunable absorbance detector, and a Jasco 807-IT integrator, or on a Thermo Finigan Spectra System Controller 1000 equipped with a P 4000 quaternary pump, a Rheodyne 7725i injector port, and a UV 6000 LP diode array detector, operated under ChromQuest 4.2.34 version 3.1.6 software.

Optical rotations were measured on a Perkin Elmer Polarimeter 341 (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University). UV spectra were obtained from a Hewlett Packard 8452A diode array (Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University). IR spectra were recorded on a Jasco 810 IR spectrophotometer. Mass spectra were measured on a Micromass LCT spectrometer (Scientific Equipment Center, Prince of Songkla University). NMR spectra were recorded on a Varian Unity Inova 500 MHz NMR spectrometer (500 MHz for ^{1}H; 125 MHz for ^{13}C; Scientific Equipment Center, Prince of Songkla University). The operating solvent was DMSO-d_6 (2.50 ppm of residual CD_2HSOCD_3 for ^{1}H and

39.5 ppm for ^{13}C NMR). The spectra were referred to solvent signals as stated for internal standards.

2.2 Chemical constituents of the sponge *Acanthodendrilla* sp.

2.2.1 Animal material

The sponge *Acanthodendrilla* sp. (family Dictyodendrillidae, order Dendroceratida) was collected using SCUBA at the depth of 15-20 m, from Koh Ha Islets, Krabi Province (7° 25.467' N, 98° 53.415' E), in March 2007. The specimens were preserved in an ice chest (0 °C) upon surfacing, and at -20 °C on arrival to the laboratory until the time of extraction. The sponge was identified by Dr. Sumaitt Putchakarn, Institute of Marine Science, Burapha University. The sponge had an irregular shape with several hollows on the surface. The texture of the sponge is coarse, rough, slippery and compressible. The sponge appears creamy white under water and turns light brown upon surfacing (Figure 1). A voucher specimen (AP07-003-01) was deposited at Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

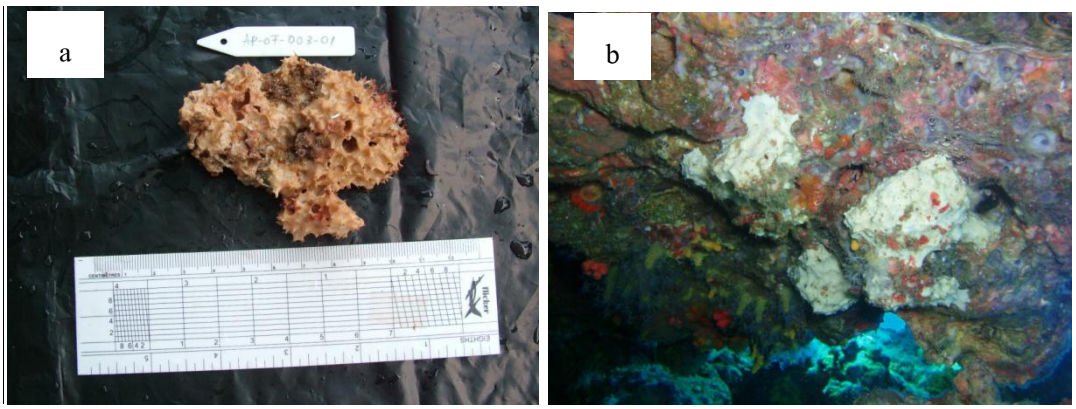


Figure 1 The sponge *Acanthodendrilla* sp.: surface (a) underwater (b)

(Courtesy Dr. Khanit Suwanborirux)

2.2.2 Extraction and isolation

The freeze-dried sponge (118 g) was chopped and macerated consecutively and exhaustively (500 mL × 5) in hexane, CH_2Cl_2 and CH_3OH to yield hexane- (0.22 g), CH_2Cl_2- (1.98 g) and CH_3OH- (7.03 g) extracts after evaporation to dryness. The CH_2Cl_2- and CH_3OH-extracts were selected for the further purification due to their acetylcholinesterase-inhibiting activity (79.5% at 0.1 mg/mL). The CH_2Cl_2-extract was fractionated over a SiO_2 column, (5% CH_3OH in CH_2Cl_2), and fractions with similar chromatographic patterns were combined to yield two major fractions. The first fraction (1.1 g) was chromatographed over Sephadex™ LH-20 (CH_3OH), then with RP-C18 HPLC (VertiSep™, 10 μm, 250 × 10 mm; H_2O/CH_3OH 35:65, flow rate 2.5 mL/min) to yield **1** (59 mg) and **2** (70 mg). The second fraction (418 mg) was purified using Sephadex™ LH-20 (CH_3OH), then RP-C18 HPLC (VertiSep™, 10 μm, 250 × 10 mm; H_2O/CH_3OH 45:55, flow rate 2.5 mL/min) to yield **3** (15 mg), and additional **1** (10 mg) and **2** (15 mg).

The CH_3OH-extract was fractionated over SiO_2 (EtOAc/CH_2Cl_2 1:1), and fractions with similar chromatographic patterns were combined to afford two major fractions. The first fraction (480 mg) was purified with RP-C18 HPLC (VertiSep™, 10 μm, 250 × 10 mm; H_2O/CH_3CN 50:50, flow rate 2.0 mL/min) to afford **4** (10 mg) along with additional **1** (22 mg), **2** (15 mg), and **3** (8 mg). The second fraction (255 mg) was purified using RP-C18 HPLC (VertiSep™, 10 μm, 250 × 10 mm; H_2O/CH_3CN 55:45, flow rate 2.0 mL/min) to yield **5** (5.0 mg).

Aerothionin (1). Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +277 (*c* 0.09 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.12) nm; IR (KBr) ν 3372 (br), 2935, 2858, 1660, 1597, 1547, 1440 cm^{-1}; ^{1}H and ^{13}C NMR see Table 3; ESI-MS *m/z* (% relative intensity) 845 (4), 843 (45), 841 (100), 839 (55), 837 (5) $[M+Na]^+$; HRESI-MS *m/z* 838.8359 (calcd for $C_{24}H_{26}N_4O_8{}^{79}Br_3{}^{81}BrNa$ 838.8361).

Homoaerothionin (2). Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +282 (*c* 0.29 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.38) nm; IR (KBr) ν 3398 (br), 2929, 2854, 1656, 1596, 1545, 1436 cm^{-1}; ^{1}H and ^{13}C NMR see Table 4; ESI-MS *m/z* (% relative intensity) 859 (5), 857 (45), 855 (100), 853 (60), 851 (5) $[M+Na]^+$; HRESI-MS *m/z* 852.8489 (calcd for $C_{25}H_{28}N_4O_8{}^{79}Br_3{}^{81}BrNa$ 852.8518).

11,19-Dideoxyfistularin 3 (3). Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +146 (*c* 0.28 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.28) nm; IR (KBr) ν 3407 (br), 2934, 1660, 1587, 1550 cm^{-1}; ^{1}H and ^{13}C NMR see Table 6; ESI-MS *m/z* (% relative intensity) 1111 (0.4), 1109 (9), 1107 (52), 1105 (100), 1103 (62), 1101 (9), 1099 (0.2) $[M+Na]^{+}$; HRESI-MS *m/z* 1102.6995 (calcd for $C_{31}H_{30}N_4O_9{}^{79}Br_4{}^{81}Br_2Na$ 1102.6970).

11-Oxoaerothionin (4). Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +275 (*c* 0.04 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 230 (4.34) nm; IR (KBr) ν 3436 (br), 2924, 1734, 1663, 1543 cm^{-1}; ^{1}H and ^{13}C NMR see Table 5; ESI-MS *m/z* (% relative intensity) 859 (5), 857 (45), 855 (100), 853 (50), 851 (5) $[M+Na]^{+}$.

11-Deoxyfistularin 3 (5). White amorphous solid; $[\alpha]^{20}_{D}$ +180 (*c* 0.1 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.79) nm; IR (KBr) ν 3435 (br), 2342, 1658, 1542, 1456 cm^{-1}; ^{1}H and ^{13}C NMR see Table 7; ESI-MS *m/z* (% relative intensity) 1127 (0.5), 1125 (10), 1123 (55), 1121 (100), 1119 (68), 1117 (12), 1115 (0.2) $[M+Na]^{+}$.

2.3 Chemical constituents of the sponge *Pseudoceratina* cf. *purpurea*

2.3.1 Animal material

The sponge *Pseudoceratina* cf. *purpurea* (family Pseudoceratinidae, order Verongida) was collected using SCUBA at the depth of 15-20 m, from Koh Ha Islets, Krabi Province (7° 25.467' N, 98° 53.415' E), in February 2010. The specimens are preserved in an ice chest (0°C) upon surfacing, and at -20°C on arrival to the laboratory until the time of extraction. The sponge was identified by Dr. Sumaitt Putchakarn, Institute of Marine Science, Burapha University. It is a branching or encrusting yellow to golden purple sponge. It is tough and slimy in texture. Under water it appears as a yellow sponge but rapidly changes to dark brown upon surfacing (Figure 2). A voucher specimen (AP10-008-01K) was deposited at Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

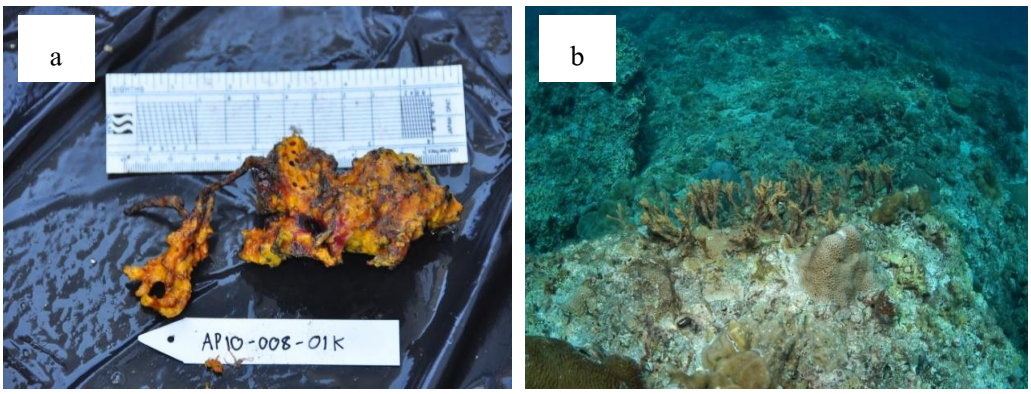


Figure 2 The sponge *Pseudoceratina* cf. *purpurea*: surface (a) underwater (b)

(Courtesy Dr. Khanit Suwanborirux)

2.3.2 Extraction and isolation

The sponge (1.2 kg, wet weight), was macerated exhaustively (2 L × 5) in CH_3OH/EtOAc (1:1). The dried extract was partitioned with series of solvents to yield hexane-, C_2Cl_4-, $CHCl_3$-, and *n*-BuOH-extracts (4.52, 1.74, 1.44 and 3.55 g, respectively). The $CHCl_3$- and *n*-BuOH-extracts showed potent acetylcholinesterase-inhibiting activity (92.0% and 94.0% inhibition at 0.1 mg/mL, respectively).

The $CHCl_3$-extract was fractionated over a SiO_2 column, (10% CH_3OH in CH_2Cl_2), and fractions with similar chromatographic patterns were combined to yield four major fractions. The first fraction (200 mg) was subjected to a RP-C18 HPLC (VertiSep™, 10 µm, 250 × 10 mm; 0.1% aqueous TFA/CH_3OH 40:60, flow rate 2.0 mL/min) to yield **6** (15 mg) and **7 (**28 mg). The second fraction (45 mg) was purified using RP-C18 HPLC (VertiSep™, 10 µm, 250 × 10 mm; 0.1% aqueous TFA/CH_3OH 40:60, flow rate 1.5 mL/min) to afford **8** (18 mg), **9** (11 mg), and additional **7** (3 mg). The third fraction (90 mg) was also purified using RP-C18 HPLC (VertiSep™, 10 µm, 250 × 10 mm; 0.1% aqueous TFA/CH_3OH 40:60, flow rate 1.5 mL/min) to afford **10** (5 mg), and additional **6** (5 mg), **7** (10 mg), and **9** (12 mg). The fourth fraction (220 mg) was purified using Sephadex™ LH-20 (CH_3OH) and RP-C18 HPLC (VertiSep™, 10 µm, 250 × 10 mm; 0.1% aqueous TFA/CH_3OH 40:60, flow rate 1.5 mL/min) to yield **11** (17 mg), **12** (5 mg), and **13** (5 mg).

The *n*-BuOH-extract was separated using a SiO_2 column ($H_2O/CH_3OH/CH_2Cl_2$, 1:4:15) to yield two major fractions. The first fraction (150 mg) was purified using Sephadex™ LH-20 (CH_3OH) and RP-C18 HPLC (VertiSep™, 10 μm, 250 × 10 mm; 0.1% aqueous TFA/CH_3OH 50:50, flow rate 2.0 mL/min) to yield **14** (10 mg), **15** (7 mg), and **16** (10 mg). The second fraction (85 mg) was purified using RP-C18 HPLC (VertiSep™, 10 μm, 250 × 10 mm; 0.1% aqueous TFA/CH_3OH 50:50, flow rate 1.5 mL/min) to obtain **17** (4 mg), **18** (10 mg), **19** (24 mg), and additional **9** (7 mg).

The C_2Cl_4-extract was fractionated using Sephadex™ LH-20 (CH_3OH) and RP-C18 HPLC (VertiSep™, 10 μm, 250 × 10 mm; CH_3CN/*i*-PrOH/H_2O 45:40:15, flow rate 2.5 mL/min) to afford **20** (20 mg).

Purealidin Q (6). Yellow amorphous solid; $[\alpha]^{20}_D$ -23 (*c* 0.53 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.69) nm; IR (KBr) ν 3422 (br), 1681, 1542, 1456, 1312, 1260, 1205, 1136 cm^{-1}; ^1H and ^{13}C NMR see Table 11; ESI-MS *m/z* (% relative intensity) 750 (8), 748 (60), 746 (100), 744 (80), 742 (15) $[M]^+$.

Aplysamine 2 (7). Pale yellow amorphous solid; UV (CH_3OH) λ_{max} (log ε) 210 (4.73) nm; IR (KBr) ν 3421 (br), 2342, 1682, 1544, 1496, 1440, 1284, 1256, 1205, 1136 cm^{-1}; ^1H and ^{13}C NMR see Table 18; ESI-MS *m/z* (% relative intensity) 654 (28), 652 (85), 650 (100), 648 (45) $[M]^+$.

Aeroplysinin 1 (8). Pale yellow amorphous solid; $[\alpha]^{20}_D$ +164 (*c* 0.89 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 268 (4.05), 210 (4.26) nm; IR (KBr) ν 3444 (br), 2220, 1682, 1633, 1584, 1406, 1315, 1205, 1141, 1205, 1023 cm^{-1}; ^1H and ^{13}C NMR see Table 9; ESI-MS *m/z* (% relative intensity) 364 (32), 362 (100), 360 (38) $[M+Na]^+$.

Purpureamine J (9). Pale yellow amorphous solid; UV (CH_3OH) λ_{max} (log ε) 208 (3.72) nm; IR (KBr) ν 3434 (br), 2931, 1675, 1563, 1204, 1128 cm^{-1}; ^1H and ^{13}C NMR see Table 19; ESIMS *m/z* (% relative intensity) 670 (20), 668 (100), 666 (100), 664 (35) $[M]^+$.

Aerophobin 1 (10). Pale yellow amorphous solid; $[\alpha]^{20}_D$ +32 (*c* 0.34 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (3.93) nm; IR (KBr) ν 3437 (br), 1681, 1436, 1206, 1139 cm^{-1}; ^1H and ^{13}C NMR see Table 20; ESI-MS *m/z* (% relative intensity) 479 (48), 477 (100), 475 (52) $[MH]^+$.

Fistularin 3 (11). Pale yellow amorphous solid; $[\alpha]^{20}_D$ +220 (*c* 0.2 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.78) nm; IR (KBr) ν 3429 (br), 2924, 1675, 1542, 1435, 1206, 1139 cm^{-1}; ^1H and

^{13}C NMR see Table 15; ESI-MS *m/z* (% relative intensity) 1143 (0.6), 1141 (8), 1139 (50), 1137 (100), 1135 (60), 1133 (10), 1131 (0.3) $[M+Na]^+$.

Hemifistularin 3 (12). Yellow amorphous solid; $[\alpha]^{20}_D$ +93 (*c* 0.14 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.57), 248 (3.91) nm; IR (KBr) ν 3436 (br), 2924, 1681, 1207, 1141 cm^{-1}; ^{1}H and ^{13}C NMR see Table 10; ESI-MS *m/z* (% relative intensity) 703 (5), 701 (45), 699 (100), 697 (50), 695 (5) $[M+Na]^+$.

Purealidin R (13). Yellow amorphous solid; $[\alpha]^{20}_D$ +100 (*c* 0.11 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 214 (3.71) nm; IR (KBr) ν 3437 (br), 2927, 1682, 1436, 1206, 1140 cm^{-1}; ^{1}H and ^{13}C NMR see Table 8; ESI-MS *m/z* (% relative intensity) 407 (6), 405 (22), 403 (8) $[M+Na]^+$.

Aplyzanzine A (14). Yellow amorphous solid; $[\alpha]^{20}_D$ +26 (*c* 0.65 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.38) nm; IR (KBr) ν 3400 (br), 2928, 2857, 2340, 1646, 1558, 1470, 1454, 1259, 1042, 735 cm^{-1}; ^{1}H and ^{13}C NMR see Table 16; ESI-MS m/z (% relative intensity) 748 (5), 746 (50), 744 (100), 742 (55), 740 (5) $[MH]^+$.

Iso-anomoian A (15). Pale yellow amorphous solid; $[\alpha]^{20}_D$ +11 (*c* 0.92 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.15) nm; IR (KBr) ν 3395 (br), 2923, 2851, 1657, 1643, 1558, 1470, 1451, 1256, 1045 cm^{-1}; ^{1}H and ^{13}C NMR see Table 17; ESI-MS *m/z* (% relative intensity) 734 (5), 732 (50), 730 (100), 728 (60), 726 (5) $[MH]^+$.

Purealidin B (16). Yellow amorphous solid; $[\alpha]^{20}_D$ -37 (*c* 0.72 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.69) nm; IR (KBr) ν 3422 (br), 1681, 1542, 1456, 1312, 1260, 1205, 1136 cm^{-1}; ^{1}H and ^{13}C NMR see Table 12; ESI-MS *m/z* (% relative intensity) 764 (5), 762 (62), 760 (100), 758 (80), 756 (10) $[M]^+$.

Purealidin J (17). Pale yellow amorphous solid; $[\alpha]^{20}_D$ +38 (*c* 0.6 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 236 (5.31), 212 (5.26) nm; IR (KBr) ν 3436 (br), 2347, 1681, 1436, 1205, 1138, 1025 cm^{-1}; ^{1}H and ^{13}C NMR see Table 21; ESI-MS *m/z* (% relative intensity) 494 (25), 492 (100), 490 (35) $[M]^+$.

Aerophobin 2 (18). Pale yellow amorphous solid; $[\alpha]^{20}_D$ +55 (*c* 0.32 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 236 (5.31), 212 (5.26) nm; IR (KBr) ν 3401 (br), 2345, 1682, 1542, 1437, 1204, 1137, 1048, 1025, 992 cm^{-1}; ^{1}H and ^{13}C NMR see Table 22; ESI-MS *m/z* (% relative intensity) 508 (48), 506 (100), 504 (52) $[M]^+$.

Araplysillin I (19). Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +46 (*c* 0.22 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.44) nm; IR (KBr) ν 3436 (br), 2347, 1675, 1205, 1138 cm^{-1}; ^{1}H and ^{13}C NMR see Table 13; ESI-MS *m/z* (% relative intensity) 722 (5), 720 (42), 718 (100), 716 (60), 714 (5) $[MH]^{+}$.

Araplysillin II (20). Yellow amorphous solid; $[\alpha]^{20}_{D}$ -48 (*c* 0.5 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210 (4.66) nm; IR (KBr) ν 3410 (br), 2925, 2853, 1651, 1542, 1457, 1470, 1257 cm^{-1}; ^{1}H and ^{13}C NMR see Table 14; ESI-MS *m/z* (% relative intensity) 968 (5), 966 (30), 964 (50), 962 (25), 960 (4) $[M+Na]^{+}$.

2.4 Biological activity

2.4.1 Acetylcholinesterase-inhibiting activity

The assay for acetylcholinesterase-inhibiting activity was serviced by Bioscreening Laboratory, Faculty of Pharmaceutical Sciences, Naresuan University, Thailand. The acetylcholinesterase inhibitory activity was assayed using the Ellman method (Ellman et al., 1961, Ingkaninan et al., 2003). In brief, 125 µL of 3 mM 5,5'-dithiobis(2-nitrobenzoic acid), 25 µL of 15 mM acetylthiocholine iodide, and 50 µL Tris-HCl buffer (pH 8.0) were added to each well of the micro-plate, followed by 25 µL of tested sample dissolved in the buffer containing not more than 10% methanol, and 25 µL of 0.28 U/mL acetylcholinesterase in Tris-HCl buffer (pH 8.0). The micro-plate (Beckman Coulter) was then read spectroscopically at 405 nm every 5 s for 2 min. Enzyme activity is calculated as a percentage of the reaction velocities compared to that of the assay using the buffer as a negative control. Inhibitory activity is calculated as a percentage of enzyme activity and referred to that of galantamine as the positive standard.

The enzyme-inhibition kinetics of the active compounds (compounds **6**, **7**, **14** and **15**) was determined in the same manner as described above, except for the concentration of acetylthiocholine iodide as substrate which was varied from 25 to 10000 µM. Michaelis-Menten plot for each compound were obtained in a comparison between the exposure to each tested sample at the concentration of its IC_{50} and blank, in which bare buffer was used.

2.4.2 Cytotoxic activity

The cytotoxic activity based on sulphorrhodamine B (SRB) method (Skehan et al., 1990) was supported by Assist. Prof. Dr. Supreeya Yuenyongsawad, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The human breast carcinoma (MCF-7), human cervical carcinoma (KB), human colon adenocarcinoma (HT-29), and human fibroblast cell lines were targeted. In brief, the monolayer culture of each targeted cell line in a 96-well microplate was exposed to a serial dilution of tested samples in an EMEM medium doped with 2 mM glutamine, 10% heat-inactivated new born calf serum, 50 IU/mL penicillin G sodium, 50 µg/mL streptomycin sulphate, and 0.125 µg/mL amphotericin B. The plate was incubated at 37 °C (5% CO_2, 95% humidity) for 6 days, at the middle of which time the medium was refreshed. Cells were fixed with 100 µL of iced-cold 40% trichloroacetic acid. The fixed cells were washed and stained with 0.4% SRB in 1% acetic acid. The excess dye was removed and the plate allowed to dry overnight. Stained dye was dissolved with Tris base for the measurements. The survival percentage of the cells was determined on a microplate reader (Biotek PowerWaveX) at 492 nm using camptothecin as a reference standard.

CHAPTER 3

RESULTS AND DISCUSSION

The preliminary screening of the acetylcholinesterase-inhibiting activity of the CH_2Cl_2-, and CH_3OH-extracts from the sponge *Acanthodendrilla* sp., showed 79.5% inhibition at 0.1 mg/mL, and of the $CHCl_3$- and *n*-BuOH-extracts from the sponge *Pseudoceratina* cf. *purpurea* showed 92.0 and 94.0% inhibition, respectively at a concentration of 0.1 mg/mL. Chemical investigation for the active components from the two sponges was conducted, leading to the isolation of a total of 20 bromotyrosine alkaloids, among which five were isolated from the sponge *Acanthodendrilla* sp. and 15 were from the sponge *P. purpurea*.

3.1 Bromotyrosine alkaloids from the sponge *Acanthodendrilla* sp.

3.1.1 Isolation of chemical constituents

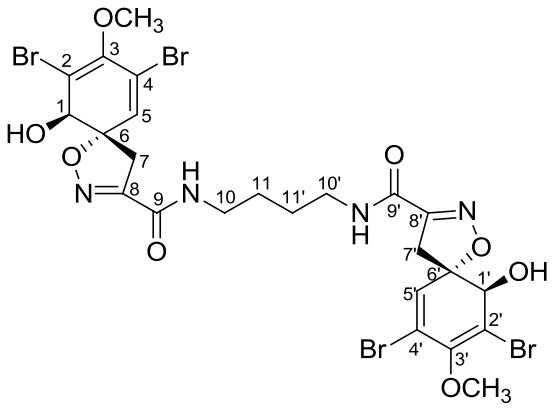
The sponge *Acanthodendrilla* sp. was collected at the depth of 15-20 m in March 2007. The freeze-dried sponge (118 g), was extracted consecutively and exhaustively (500 mL × 5, each) in hexane, CH_2Cl_2 and CH_3OH to yield extract from each solvent weighed 0.22, 1.98, and 7.03 g, respectively. The CH_2Cl_2-extract was fractionated to yield compounds **1-3** (69, 85, and 15 mg, respectively), and the CH_3OH-extract yielded compounds **4** and **5** (15 and 5mg respectively), along with additional **1** (22 mg), **2** (15 mg), and **3** (8 mg).

3.1.2 The structure elucidation

The structure determination of compounds isolated from the sponge *Acanthodendrilla* sp. revealed that all five compounds were binary-bromotyrosine derivatives, possessing two oxa,azaspirodecatriene units connected with a variety of linkers. The discussion hereafter is therefore organized according to the increasing complexity of such linkers.

Compound **1** was obtained as a yellow solid from the CH_2Cl_2 fraction using series of chromatographic steps (SiO_2, 5% CH_3OH in CH_2Cl_2; Sephadex LH-20, CH_3OH; and RP-C18 HPLC, H_2O/CH_3OH 35:65). The molecular formula of **1** was proposed to be $C_{24}H_{26}N_4O_8Br_4$ according to a pseudomolecular peak in the ESI mass spectrum at *m/z* 836.7 $[M+Na]^+$. This was further confirmed by the HR-ESI mass at *m/z* 838.8359 (calcd for $C_{24}H_{26}N_4O_8{}^{79}Br_3{}^{81}BrNa$ 838.8361). The presence of four bromine atoms is strongly indicated by the characteristic cluster of five isotopic peaks at *m/z* 837/839/841/843/845 (1:4:6:4:1). The molecular formula yielded an unsaturation degree of 12, comprising four olefinic bonds, two carbonyls, two imines, and four rings.

Despite the large molecular formula, only nine resonances of ^1H and 12 of ^{13}C were observed (Table 3, Figures 3 and 4), thus indicating a symmetry element within the structure. As for the ^{13}C resonances, six sp^2 (five Cs and one CH), and six sp^3 (one C, one CH, three CH_2s, and one CH_3) carbons were observed. Each signal belongs to two carbon atoms, therefore confirming the symmetry as described above. In the HMBC spectrum, the singlet olefinic proton at δ 6.57 (H-5) coupled extensively to its surrounding carbons at δ_C 73.7 (C-1), 147.2 (C-3), 120.9 (C-4), and 90.2 (C-6). This combined with the correlations from H-1 (δ 3.91, s) to C-2, C-3, C-5, C-6, and C-7 (δ 39.5), and from H-7 (δ 3.60, d, J = 18.4 Hz; 3.21, d, J = 18.4 Hz) to C-1, C-5, C-6, and C-8 (δ 154.6); hence a spin system from C-1 to C-8 was proposed. The characteristic chemical shifts of C-6 (δ 90.2) and C-8 (δ 154.6) allow the placement of an oxygen and a nitrogen onto C-6 and C-8, respectively. Also based on the chemical shifts and the HMBC correlations, a hydroxy group was placed on C-1 (δ 73.7), a methoxy on C-3 (δ 147.2) and two bromo groups on C-2 (δ 113.2) and C-4 (δ 120.9). The described HMBC correlations also indicated a cyclic structural unit of two symmetrized oxa,azaspirodecatriene comprising the above spin system. The two oxa,azaspirodecatriene residues connected to each other over a butyrodiamide linker as indicated by two methylenes at δ_H 3.15 (dt, J = 5.9, 5.7 Hz, H-10/10') and 1.44 (m, H-11/11') and an amide carbonyl (δ 158.9, C-9/9'). The structure of **1** was therefore proposed to be a bis-oxa,azaspirodecatriene amide aerothionin. The compound was first reported by Moody et al. (1972) from *Verongia thiona* and *V. aerophoba*.



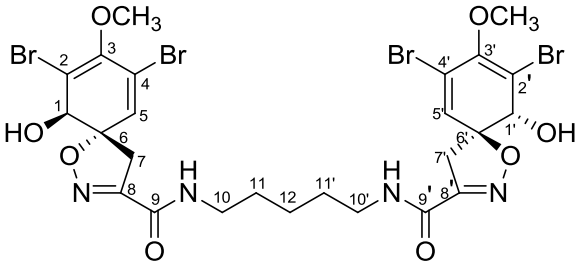
1

Table 3. NMR chemical shifts of **1** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1/1'	3.91, s	73.7, CH	2/2', 3/3', 5/5', 6/6',7/7'
2/2'	-	113.2, C	
3/3'	-	147.2, C	
4/4'	-	120.9, C	
5/5'	6.57, s	131.4, CH	1/1', 3/3', 4/4', 6/6', 7/7'
6/6'	-	90.2, C	
7/7'	3.60, d (18.4)	39.5, CH_2	1/1', 5/5', 6/6', 8/8'
	3.21, d (18.4)		1/1', 5/5', 6/6', 8/8'
8/8'	-	154.6, C	
9/9'	-	158.9, C	
10/10'	3.13, dt (5.9, 5.7), 4H	38.5, CH_2	11/11'
11/11'	1.44, m, 4H	26.4, CH_2	10/10'
3/3'-OCH_3	3.63, s, 6H	59.7, CH_3	3/3'
1/1'-OH	6.34, s	-	
9/9'-NH	8.64, t (5.9)	-	9/9'

Compound **2** was isolated as a yellow solid. The molecular formula of $C_{25}H_{28}N_4O_8Br_4$ was deduced from the mass of $[M+Na]^+$ at *m/z* 851 and was confirmed by the HR-ESI mass at *m/z* 852.8489 (calcd for $C_{25}H_{28}N_4O_8{}^{79}Br_3{}^{81}BrNa$ 852.8518). Similar to **1**, the isotopic

cluster for four bromines was observed at *m/z* 851/853/855/857/859 (1:4:6:4:1). The resemblance in the NMR spectra between **1** and **2** was remarkable (Table 4, Figures 5 and 6), and indicated that both possess a closely related structure. With one additional methylene residue at δ_H 1.24 (quintet, J = 7.0 Hz, H-12); δ_C 23.6 (C-12), **2** was proposed to be a homolog of **1** named homoaerothionin (Moody et al., 1972). The configurations of **1** and **2** as shown were drawn due to the comparable specific rotation (+277, *c* 0.09, CH_3OH, and +282, *c* 0.29, CH_3OH, respectively; lit. +210, *c* 1.7, CH_3OH, +191.5, $CHCl_3$, respectively) (McMillan et al., 1981).

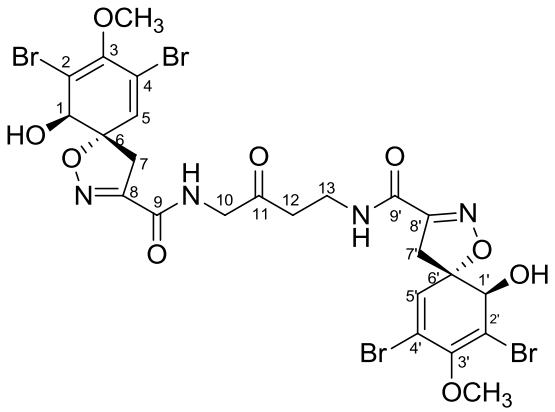


2

Table 4. NMR chemical shifts of **2** (500 MHz for ^1H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1/1'	3.91, s	73.7, CH	2/2', 3/3', 5/5', 6/6',7/7'
2/2'	-	113.2, C	
3/3'	-	147.2, C	
4/4'	-	120.8, C	
5/5'	6.57, s	131.4, CH	1/1', 3/3', 4/4', 6/6', 7/7'
6/6'	-	90.2, C	
7/7'	3.60, d (18.1)	39.6, CH_2	1/1', 5/5', 6/6', 8/8'
	3.21, d (18.1)		1/1', 5/5', 6/6', 8/8'
8/8'	-	154.6, C	
9/9'	-	158.9, C	
10/10'	3.11, dt (5.7, 6.2), 4H	38.8, CH_2	11/11', 12
11/11'	1.45, tt (6.2, 7.0), 4H	28.6, CH_2	10/10', 12
12	1.24, quintet (7.0), 2H	23.6, CH_2	10/10', 11/11'
3/3'-OCH_3	3.64, s, 6H	59.7, CH_3	3/3'
1/1'-OH	6.35, s	-	
9/9'-NH	8.47, t (5.7)	-	9/9'

Compound **4** was obtained as a yellow solid from the CH_3OH-extract. The molecular formula of $C_{24}H_{24}N_4O_9Br_4$ was deduced based on the ESI mass spectrum of $[M+Na]^+$ at *m/z* 851, with an isotopic cluster for four bromines at *m/z* 851, 853, 855, 857, 859. The NMR spectra of **4** (Table 5, Figures 7 and 8) were almost identical to those of **1** and **2**, indicating the structural similarity. Compared with **1**, **4** has one additional oxygen and two less hydrogens, hence indicating the replacement of a methylene with an oxo functionality. This was evident as a carbonyl was observed at δ_C 204.5 (C-11). This keto was part of the oxo-butyryl bridge resonating at δ_H 4.00 (d, J = 5.9 Hz, H-10), 2.70 (t, J = 7.1 Hz, H-12), and 3.33 (dt, J = 5.9, 7.1 Hz, H-13). **4** was therefore proposed to be 11-oxoaerothionin, an oxo analog of **1**, first reported from the sponge *Aplysina lacunosa* (Acosta and Rodriguez, 1992). The configuration of **4** as shown was proposed due to the comparable specific rotation (+275, *c* 0.04, CH_3OH; lit. +181, *c* 2.17, DMSO) (Acosta and Rodriguez, 1992).



4

Table 5. NMR chemical shifts of **4** (500 MHz for ^1H and 125 MHz for ^{13}C; DMSO-d_6)

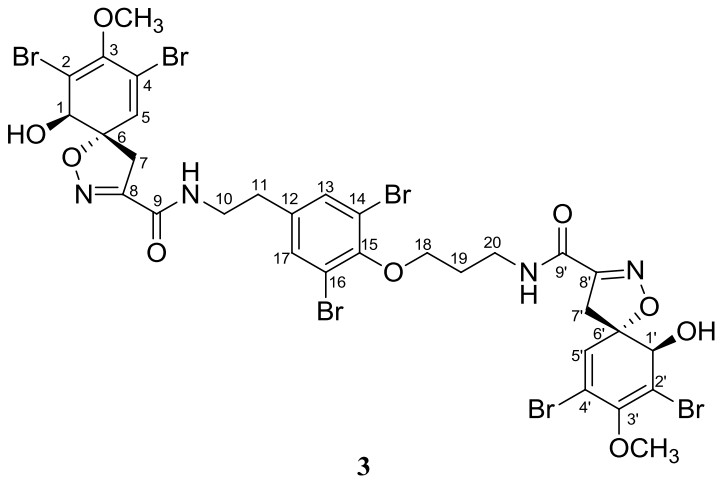
Position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.93, d (7.6)	73.7, CH	2, 3, 5, 6
2	-	113.2, C	
3	-	147.2, C	
4	-	121.0, C	
5	6.59, s	131.4, CH	1, 3, 4. 7
6	-	90.6, C	
7	3.62, d (18.3)	39.2, CH_2	1, 5, 6, 8

Table 5. (cont.)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
	3.22, d (18.3)		1, 5, 6, 8
8	-	154.5, C	
9	-	159.2, C	
10	4.00, d (5.9), 2H	48.6, CH_2	9, 11
11	-	204.5, C	
12	2.70, t (7.1), 2H	38.7, CH_2	11, 13
13	3.33, dt (5.9, 7.1), 2H	33.9, CH_2	11. 12
1'	3.91, d (7.6)	73.7, CH	2', 3', 5', 6'
2'	-	113.2, C	
3'	-	147.2, C	
4'	-	120.9, C	
5'	6.56, s	131.3, CH	1', 3', 4', 7'
6'	-	90.4, C	
7'	3.58, d (18.3)	39.4, CH_2	1', 5', 6', 8'
	3.18, d (18.3)		1', 5', 6', 8'
8'	-	154.1, C	
9'	-	159.0, C	
3-OCH_3	3.63, s, 3H	59.8, CH_3	3
3'-OCH_3	3.64, s, 3H	59.6, CH_3	3'
1-OH	6.36, d (7.6)	-	
1'-OH	6.38, d (7.6)	-	
9-NH	8.61, t (5.9)	-	9
9'-NH	8.43, t (5.9)	-	9'

Whereas the molecular masses of **1**, **2**, and **4** were in the ranges of 837 to 851 mu, the molecular mass of **3** obtained both from the CH_2Cl_2- and from the CH_3OH- extracts was much larger. An isotopic cluster at *m/z* 1099 $[M+Na]^+$, 1101, 1103, 1105, 1107, 1109, 1111 observed in the ESI mass spectrum and was coherent with the molecular formula of $C_{31}H_{30}N_4O_9Br_6$. This was further confirmed by HR-ESI mass spectrum at *m/z* 1102.6995 (calcd

for $C_{31}H_{30}N_4O_9{}^{79}Br_4{}^{81}Br_2Na$ 1102.6970). The presence of both oxa,azaspirodecatriene units was observed at δ_C 73.7 (C-1), 73.6 (C-1'), 113.2 (C-2/2'), 147.2 (C-3/3'), 121.0 (C-4), 120.9 (C-4'), 131.4 (C-5), 131.3 (C-5'), 90.3 (C-6/6'), 39.4 (C-7/7'), 154.6 (C-8), and 154.5 (C-8'); δ_H 3.92 (H-1, s), 3.91 (H-1', s), 6.57 (H-5, s), 6.56 (H-5', s), 3.62 (H-7/7', d, J = 18.1 Hz) and 3.22 (H-7/7', d, J = 18.1 Hz). Both residues however were linked with a propyloxydibromotyramine bridge. The bromotyramine unit was observed at δ_C 39.8 (C-10), 33.2 (C-11), 138.9 (C-12), 133.1 (C-13/17), 117.4 (C-14/16), 150.8 (C-15); δ_H 3.36 (dt, J = 5.7, 6.7 Hz, H-10), 2.74 (t, J = 6.7 Hz, H-11), and 7.51 (s, H-13/17), and the propyloxy moiety was at δ_C 71.3 (C-18), 29.5 (C-19) and 36.3 (C-20); δ_H 3.95 (t, J = 6.2 Hz, H-18), 1.99 (quintet, J = 6.2 Hz, H-19), and 3.40 (t, J = 6.2 Hz, H-20). The orientation of the two bromo substitution was deduced to be *ortho* to the oxypropyl moiety as indicated by the HMBC correlations from H-13/17 (δ 7.51) to C-11 (δ 33.2), C-14/16 (δ 117.4) and C-15 (δ 150.8), and from H-11 (δ 2.74, t, J = 6.7 Hz) to C-12 (δ 138.9) and C-13/17 (δ 133.1). **3** was therefore identified as 11,19-dideoxyfistularin 3, first isolated from the sponge *Pseudoceratina durrisima* (Kernan et al., 1990). The configuration of **3** was proposed to be as shown due to the comparable specific rotation (+146, *c* 0.28, CH_3OH; lit. +98.5, *c* 0.10, CH_3OH) (Kernan et al., 1990).



3

Table 6. NMR chemical shifts of **3** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, s	73.7, CH	2, 3, 5, 6
2	-	113.2, C	
3	-	147.2, C	
4	-	121.0, C	
5	6.57, s	131.4, CH	1, 3, 4, 6, 7
6	-	90.3, C	
7	3.62, d (18.1)	39.4, CH_2	1, 5, 6, 8
	3.22, d (18.1)		1, 5, 6, 8
8	-	154.6, C	
9	-	159.0, C	
10	3.36, dt (5.7, 6.7), 2H	39.8, CH_2	9, 11, 12
11	2.74, t (6.7), 2H	33.2, CH_2	10, 12, 13/17
12	-	138.9, C	
13/17	7.51, s, 2H	133.1, CH	11, 14/16, 15
14/16	-	117.4, C	
15	-	150.8, C	
18	3.95, t (6.2), 2H	71.3, CH_2	19, 20
19	1.99, quintet (6.2), 2H	29.5, CH_2	18, 20
20	3.40, t (6.2), 2H	36.3, CH_2	18, 19
1'	3.91, s	73.6, CH	2', 3', 5', 6'
2'	-	113.2, C	
3'	-	147.2, C	
4'	-	120.9, C	
5'	6.56, s	131.3, CH	1', 3', 4', 6', 7'
6'	-	90.3, C	
7'	3.62, d (18.1)	39.4, CH_2	1', 5', 6', 8'
	3.22, d (18.1)		1', 5', 6', 8'
8'	-	154.5, C	
9'	-	159.0, C	
3-OCH_3	3.64, s, 3H	59.7, CH_3	3

Table 6. (cont.)

Position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
3'-OCH_3	3.64, s, 3H	59.7, CH_3	3'
1-OH	6.40, s	-	
1'-OH	6.40, s	-	
9-NH	8.58, t (5.7)	-	9
9'-NH	8.54, t (5.7)	-	9'

Compound **5** possesses a molecular formula of $C_{31}H_{30}N_4O_{10}Br_6$ as indicated by the molecular mass of $[M+Na]^+$ at *m/z* 1115, and the cluster for six bromines at *m/z* 1115, 1117, 1119, 1121, 1123, 1125, 1127, observed in the ESI mass spectrum. The closely related NMR spectra (Table 7, Figures 11 and 12) between **3** and **5** indicated that both also have a similar skeleton. With an additional oxygen **5** was proposed to be a hydroxy derivative of **3**. This was supported by the resonances of C-19 (δ68.1), and H-19 (δ4.05, m). **5** was therefore identified to be 11-deoxyfistularin 3. The compound was first isolated from the sponge *Aplysina fistularis insularis* (Compagnone et al., 1999). The configuration of **5** as shown were deduced in the same manner as those of **1-4;** i.e., $[\alpha]_D$ of **5** was +180 (*c* 0.10, CH_3OH), comparable to the reported one at +194 (*c* 4.12, CH_3OH) (Compagnone et al., 1999).

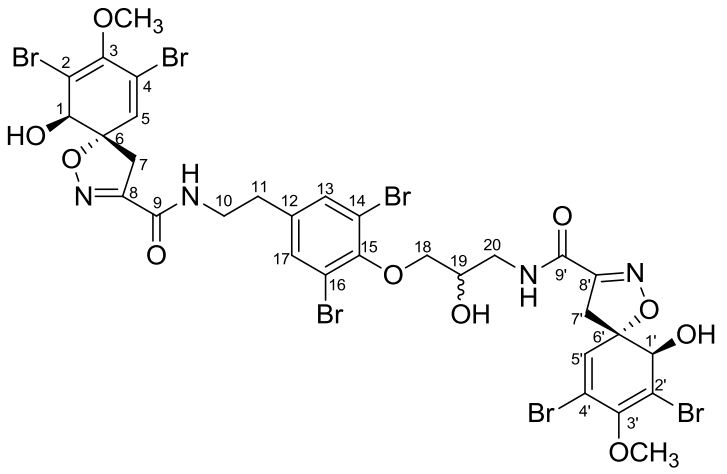


Table 7. NMR chemical shifts of **5** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, s	73.7, CH	2, 3, 5, 6, 7
2	-	113.2, C	
3	-	147.2, C	
4	-	121.0, C	
5	6.56, s	131.3, CH	3, 4, 6, 7
6	-	90.4, C	
7	3.62, d (18.1)	39.4, CH_2	1, 5, 6, 8
	3.22, d (18.1)		1, 5, 6, 8
8	-	154.5, C	
9	-	159.2, C	
10	3.36, overlap, 2H	39.9, CH_2	9, 11, 12
11	2.74, t (6.7), 2H	33.2, CH_2	10, 12, 13/17
12	-	138.0, C	
13/17	7.51, s, 2H	133.1, CH	11, 14/16, 15
14/16	-	117.3, C	
15	-	150.8, C	
18	3.88, dd (9.0, 5.6)	75.4, CH_2	20
	3.80, dd (9.0, 5.6)		20
19	4.05, m	68.1, CH	
20	3.48, ddd (10.9, 6.6, 5.6)	42.7, CH_2	
	3.30, overlap		19
1'	3.91, s	73.7, CH	2', 3', 5', 6', 7'
2'	-	113.2, C	
3'	-	147.2, C	
4'	-	121.0, C	
5'	6.55, s	131.3, CH	3', 4', 6', 7'
6'	-	90.3, C	
7'	3.62, d (18.1)	39.4, CH_2	1', 5', 6', 8'
	3.22, d (18.1)		1', 5', 6', 8'
8'	-	154.5, C	

Table 7. (cont.)

Position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
9'	-	159.0, C	
3-OCH_3	3.64, s, 3H	59.7, CH_3	3
3'-OCH_3	3.64, s, 3H	59.7, CH_3	3'
1-OH	6.38, br s	-	
1'-OH	6.38, br s	-	
9-NH	8.58, t (5.6)	-	9
9'-NH	8.35, t (5.6)	-	9'

3.2 Bromotyrosine alkaloids from the sponge *Pseudoceratina* cf. *purpurea*.

3.2.1 Isolation of chemical constituents

The sponge was collected at the depth of 15-20 m in February 2010. The sponge (1.2 kg, wet weight), was macerated exhaustively (2 L × 5, each) in CH_3OH/EtOAc (1:1) to yield a crude extract. Solvent portioning yielded hexane-, C_2Cl_4-, $CHCl_3$-, and *n*-BuOH-extracts (4.52, 1.74, 1.44, and 3.55 g, respectively). The $CHCl_3$- and *n*-BuOH-extracts showed potent acetylcholinesterase-inhibiting activity (92.0% and 94.0% inhibition at 0.1 mg/mL, respectively). The $CHCl_3$-extract was fractionated over SiO_2, (10% CH_3OH in CH_2Cl_2), Sephadex LH-20 (CH_3OH), and RP-C18 HPLC column (0.1% aqueous TFA/CH_3OH 40:60, flow rate 1.5 mL/min) to yield **6** (15 mg), **7** (28 mg)**, 8** (18 mg), **9 (**11 mg), **10** (5mg), **11** (17 mg), **12** (5 mg), and **13** (5 mg).

The *n*-BuOH-extract was fractionated using SiO_2 (H_2O/CH_3OH/CH_2Cl_2 1:4:15), Sephadex LH-20 (CH_3OH), and RP-C18 HPLC column (0.1% aqueous TFA/CH_3OH 50:50, flow rate 1.5 mL/min) to yeild **14** (10 mg), **15** (7 mg), **16** (10 mg), **17** (4 mg), **18** (16 mg), and **19** (24 mg).

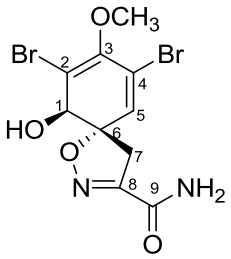
The C_2Cl_4-extract was fractionated using Sephadex LH-20 (CH_3OH) and RP-C18 HPLC column (CH_3CN/*i*-PrOH/H_2O 45:40:15, flow rate 2.5mL/min) to yield **20** (20 mg).

3.2.2 The structure elucidation

Similar to the compounds isolated from the sponge *Acanthodendrilla* sp. described in section 3.1, all the compounds obtained from *P. purpurea* showed the distinctive clusters of brominated masses in the mass spectra and the prominent signals of either phenyl or oxa,azaspirodecatriene moieties characteristic to the bromotyrosine derivatives. Here, the discussion on the structure determination is organized according to the nature of the brominated phenyl/oxa,azaspirodecatriene moieties and the alkyl/aryl extension on the amide functionality, leading to five distinctive structural genres. The five groups are; (**A**) simple bromotyrosine derivatives (compounds **13** and **8**); (**B**) binary-oxa,azaspirodecatriene bromotyramine amides (compounds **12** and **6**); (**C**) binary-oxa,azaspirodecatriene bromotyramines with propyloxyamine linker (compounds **16**, **19**, **20** and **11**); (**D**) binary-bromotyramine amides (compounds **7**, **9**, **14**, and **15**); (**E**) bromotyrosine-imidazoyl alkyl amides (compounds **10**, **17**, and **18**).

Group A; Simple bromotyrosine derivatives

Two simple bromotyrosine derivatives, compounds **13** and **8**, are categorized into this group. Compound **13** possesses a molecular formula of $C_{10}H_{10}N_2O_4Br_2$ due to the pseudo-molecular peaks at *m/z* 403 $[M+Na]^+$, with an isotopic cluster for two bromines at *m/z* 403, 405, 407. Compared with all the bromotyrosine analogs isolated from *Acanthodendrilla* sp. reported earlier, the characteristic NMR signals (Table 8, Figures 13 and 14) of oxa,azaspirodecatriene moiety at δ_C 73.8 (C-1), 113.2 (C-2), 147.2 (C-3), 120.8 (C-4), 131.5 (C-5), 90.4 (C-6), 39.4 (C-7), 154.6 (C-8), and 160.9 (C-9); δ_H 3.91 (d, J = 7.1 Hz, H-1), 6.57 (s, H-5), 3.57 (d, J = 18.1 Hz, H-7), 3.14 (d, J = 18.1 Hz, H-7), are immediately recognizable. However, a molecular formula less than half of those for **1**-**5**, and with the absence of signals belonging to the alkyl linkers, **13** was therefore identified as an amido-bromotyrosine derivative with only one single unit of the oxa,azaspirodecatriene, named purealidin R. The compound was first isolated from the sponge *Psammaplysilla purea* (Kobayashi et al., 1995). The configuration of **13** was proposed as shown due to the comparable specific rotation to that reported by Kobayashi et al. (1995) (+100, *c* 0.11, CH_3OH; lit. +86, *c* 0.19, CH_3OH).



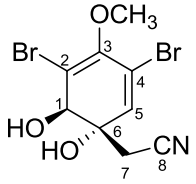
13

Table 8. NMR chemical shifts of **13** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, d (7.1)	73.8, CH	2, 3, 5, 6
2	-	113.2, C	
2	-	147.2, C	
4	-	120.8, C	
5	6.57, s	131.5, CH	1, 3, 4, 6, 7
6	-	90.4, C	
7	3.57, d (18.1)	39.4, CH_2	1, 5, 6, 8
	3.14, d (18.1)		1, 5, 6, 8
8	-	154.6, C	
9	-	160.9, C	
3-OCH_3	3.64, s, 3H	59.6, CH_3	3
1-OH	6.35, d (7.1)	-	1, 2, 6
9-NH	7.81, s	-	9
9-NH	7.57, s	-	8

Compound **8** possesses a molecular formula of $C_9H_9NO_3Br_2$ according to the mass of $[M+Na]^+$ at *m/z* 360, and the dibromo cluster at *m/z* 360, 362, 364 in the ESI mass spectrum. Coherent to **13** and also to the bromotyrosine derivatives presented earlier, the spin systems characteristic to the dibromo oxa,azaspirodecatriene moiety (Table 9, Figures 15 and 16) were immediately recognizable at δ_H 3.91 (d, J = 7.8 Hz, H-1), 6.30 (s, H-5), and δ_C 77.1 (C-1), 113.4 (C-2), 146.7 (C-3), 119.0 (C-4), 133.4 (C-5), and 73.2 (C-6). However, the carbon

resonances that was previously assigned for C-8 and C-9 of **13** (δ_C 154.6 and 160.9, Figure 14) were replaced by a nitrile carbon at δ 118.1 (C-8). This was supported by the IR absorption at 2220 cm^{-1}. Compound **8** was therefore identified as aeroplysinin 1, first reported by Fattorusso et al. (1972) from the sponge *Verongia aerophoba*. The configuration of **8** as shown was proposed based on the comparable specific rotation to the previously reported by Fattorusso et al (1972) (+164, *c* 0.89, CH_3OH; lit. +186, *c* 1.0, CH_3OH).



8

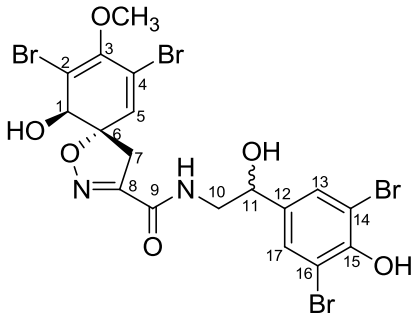
Table 9. NMR chemical shifts of **8** (500 MHz for ^1H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, d (7.8)	77.1, CH	2, 3, 5, 6, 7
2	-	113.4, C	
3	-	146.7, C	
4	-	119.0, C	
5	6.30, s	133.4, CH	1, 3, 4, 6, 7
6	-	73.2, C	
7	2.77, s, 2H	26.1, CH_2	1, 5, 6, 8
8	-	118.1, C	
3-OCH_3	3.64, s, 3H	59.5, CH_3	3
1-OH	6.14, d (7.8)	-	2, 6, 5
6-OH	6.10, s	-	1, 5, 6, 7

Group B; Oxa,azaspirodecatriene-bromotyramide

Extended from a single unit of an oxa,azaspirodecatriene as seen in group **A**, the bromotyrosines in group **B** (compounds **12** and **6**) have additional bromotyramine unit linked to the residue of oxa,azaspirodecatriene through an amide linkage.

Compound **12** has a molecular formula of $C_{18}H_{16}N_2O_6Br_4$ coherent with the mass cluster at *m/z* 695, 697, 699, 701, 703 as observed in the ESI mass spectrum. The NMR spectra of **12** (Table 10, Figures 17 and 18) showed the signals that can be assigned for the oxa,azaspirodecatriene unit closely identical to the spectra of **13** at δ_C 73.7 (C-1), 113.2 (C-2), 147.2 (C-3), 121.0 (C-4), 131.3 (C-5), 90.4 (C-6), 39.4 (C-7), and 154.5 (C-8); δ_H 3.91 (d, J = 7.3 Hz, H-1), 6.55 (s, H-5), 3.58 (d, J = 18.3 Hz, H-7), and 3.13 (d, J = 18.3 Hz, H-7) . The additional aromatic signals at δ 7.42 (s, H-13/17), a methylene at δ 3.30 (H-10), and a carbinol methine at δ 4.60 (dt, J = 6.3, 4.2 Hz, H-11) indicated the presence of a bromotyramine subunit, on which C-11 was hydroxylated. The linkage between the two bromotyrosine-derived unit took place over an amide moiety and was confirmed by the HMBC correlations from H-10 (δ 3.30) to C-9 (δ 159.1), C-11 (δ 69.5) and C-12 (δ 137.9), and from H-11 (δ 4.60, dt, J = 6.3, 4.2 Hz) to C-10 (δ 46.5) and C-13/17 (δ 130.0). Compound **12** was therefore elucidated as hemifistularin 3. The compound was previously reported from an unidentified sponge by Mancini et al. (1993). The configuration of **12** was proposed to be as shown due to the comparable specific rotation (+93, *c* 0.14, CH_3OH; lit. +110, *c* 0.2, CH_3COCH_3) (Mancini et al., 1993).



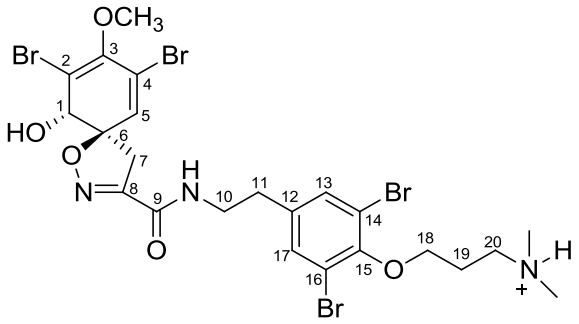
12

Table 10. NMR chemical shifts of **12** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, d (7.3)	73.7, CH	2, 3, 5, 6
2	-	113.2, C	
3	-	147.2, C	
4	-	121.0, C	
5	6.55, s	131.3, CH	1, 3, 4, 6, 7
6	-	90.4, C	
7	3.58, d (18.3)	39.4, CH_2	1, 5, 6, 8
	3.13, d (18.3)		1, 5, 6, 8
8	-	154.5, C	
9	-	159.1, C	
10	3.30, overlap, 2H	46.5, CH_2	9, 11, 12
11	4.60, dt (6.3, 4.2), 2H	69.5, CH	10, 13, 17
12	-	137.9, C	
13/17	7.42, s, 2H	130.0, CH	11, 12, 14/16, 15
14/16	-	111.8, C	
15	-	149.7, C	
OCH_3	3.64, s, 3H	59.8, CH_3	3
9-NH	8.32, t (5.6)	-	9
1-OH	6.33, d (7.3)	-	1, 2, 3, 6
11-OH	5.58, d (4.2)	-	10
15-OH	9.80, s	-	15

Compound **6** was isolated as a solid. Its molecular formula was deduced to be $C_{23}H_{28}N_3O_5Br_4$ on the basis of mass cluster in the ESI mass spectrum at *m/z* 742, 744, 746, 748, 750. Notice that the molecular formula was proposed with an extra hydrogen atom, indicating a pre-existing protonated chemical structure as a quaternary ammonium compound. The presence of oxa,azaspirodecatriene bromotyramide unit similar to that of **12** was recognized at δ_C 73.7 (C-1), 113.2 (C-2), 147.2 (C-3), 121.0 (C-4), 131.3 (C-5), 90.3 (C-6), 39.6 (C-7), and 154.6 (C-8); δ_H 3.91 (d, J = 7.8 Hz, H-1), 6.56 (s, H-5), 3.60 (d, J = 18.1 Hz, H-7), and 3.16 (d, J = 18.1 Hz, H-7)

(Table 11, Figures 19 and 20). The additional moiety of $C_5H_{12}N$ was proposed to be a unit of *N*,*N*-dimethylpropyloxyammonium moiety, comprising two methyls at δ_H 2.83 [s, 20-N$(CH_3)_2$] and three methylenes at δ_H 3.98 (t, *J* = 5.7 Hz, H-18), 2.16 (tt, *J* = 5.7, 5.6 Hz, H-19), 3.32 (H-20). This substituted the hydroxyl group of the bromotyramine terminal. The presence of the quaternary ammonium moiety was supported by the characteristic chemical shift of the two methyls at δ 2.83 (s) and the methylene δ 3.32 (H-20). **6** was identified to be purealidin Q, first isolated from the sponge *Psammaplysilla purea* (Kobayashi et al., 1995). The specific rotation of the sample isolated in this work is different from that previously reported by Kobayashi et al. (1995) (+9.1, *c* 0.39, CH_3OH). Here, the specific rotation of **6** was -23 (*c* 0.53, CH_3OH), comparable in magnitude but opposite in sign. The configuration of **6** is therefore proposed here as an enantiomer of purealidin Q reported by Kobayashi et al. (1991).



6

Table 11. NMR chemical shifts of **6** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, d (7.8)	73.7, CH	2, 3, 5, 6
2	-	113.2, C	
3	-	147.2, C	
4	-	121.0, C	
5	6.56, s	131.3, CH	1, 3, 4
6	-	90.3, C	
7	3.60, d (18.1)	39.4, CH_2	1, 5, 6, 8

Table 11. (cont.)

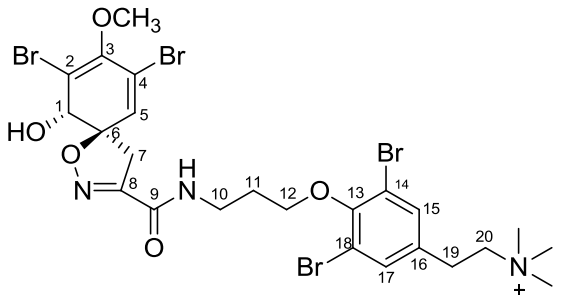
position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
	3.16, d (18.1)		1, 5, 6, 8
8	-	154.6, C	
9	-	159.0, C	
10	3.36, overlap, 2H	39.6, CH_2	9, 11, 12
11	2.75, t (6.8), 2H	33.2, CH_2	10, 12, 13
12	-	139.2, C	
13/17	7.53, s, 2H	131.1, CH	11, 14/16, 15
14/16	-	117.3, C	
15	-	150.4, C	
18	3.98, t (5.7), 2H	70.3, CH_2	19, 20
19	2.16, tt (5.7, 5.6), 2H	24.9, CH_2	20
20	3.32, overlap, 2H	54.5, CH_2	19, 21/22
20-$N(CH_3)_2$	2.83, s, 6H	42.5, CH_3	20
3-OCH_3	3.64, s, 3H	59.7, CH_3	3
1-OH	6.32, d (7.8)	-	
9-NH	8.57, t (5.6)	-	9
20-$^+$NH	9.61, br s	-	

Group C; Binary oxa,azaspirodecatriene-bromotyrosine amides

The characteristic feature of bromotyrosine derivatives in this group is the presence of an oxa,azaspirodecatriene coupling to a bromotyramine unit, not directly as seen with group **B** alkaloids, but through a propyloxyamide linker. Four compounds, **16**, **19**, **20** and **11,** were isolated in this study.

Compound **16** was isolated as a solid. The molecular formula of $C_{24}H_{30}N_3O_5Br_4$ was proposed based on the ESI mass spectrum at *m/z* 756 $[M]^+$, with an isotopic cluster for four bromines at *m/z* 756, 758, 760, 762, 764. Similar to compound **6**, notice here the extra hydrogen atom present in the molecular formula, indicating that the compound is indeed a quaternary ammonium. The presence of the oxa,azaspirodecatriene unit similar to those discussed

previously was observable at δ_H 3.91 (d, J = 7.8 Hz, H-1), 6.56 (s, H-5), 3.60 (d, J = 18.1 Hz, H-7), 3.16 (d, J = 18.1 Hz, H-7), and δ_C 73.7 (C-1), 113.2 (C-2), 147.2 (C-3), 121.0 (C-4), 131.3 (C-5), 90.3 (C-6), 39.4 (C-7), 154.6 (C-8) (Table 12, Figures 21 and 22). The dibromotyramine residue similar to that observed with compounds **3** and **5** can be identified through the resonances of the aromatic protons at δ 7.53 (s, H-15/17) and the ethylene bridge at δ_H 3.02 (tt, J = 6.7, 5.6 Hz, H-19), 3.50 (tt, J = 6.7, 5.9 Hz, H-20); δ_C 133.5 (C-15/17), 27.1 (C-19), 65.4 (C-20). A trimethyl ammonium moiety (δ_H 3.10, s; δ_C 52.5) was placed on the other end of the ethylene bridge as indicated by the lower-field chemical shift of H-20 (δ 3.50, J = tt, 6.7, 5.9 Hz) and C-20 (δ 65.4). H-20 also showed ^{14}N-^{1}H coupling (t, J = 5.9 Hz) characteristic to the quaternary ammonium moiety. The two bromotyrosine-derived units were linked through a propyloxyamide bridge at δ_H 3.40 (dt, J = 6.1, 6.3 Hz, H-10), 1.99 (quintet, J = 6.3 Hz, H-11), 3.96 (t, J = 6.3 Hz, H-12); δ_C 159.0 (C-9), 36.3 (C-10), 29.5 (C-11), 71.4 (C-12). The orientation of the propyloxyamide as shown was indicated by the HMBC correlations from H-10 (δ 3.40) to C-9 (δ 159.0), C-11 (δ 29.5), and C-12, (δ 71.4), from H-11 (δ 1.99) to C-10 (δ 36.3), and C-12, and from H-12 (δ 3.96) to C-10, C-11, and C-13 (δ 151.5). Compound **16** was therefore identified to be purealidin B. The compound was first isolated from *Psammaplysilla purea* (Kobayashi et al., 1991). The configuration of **16** was proposed to be as shown due to the comparable specific rotation (-37, c 0.72, CH_3OH; lit. -4.5, c 1.3, CH_3OH).



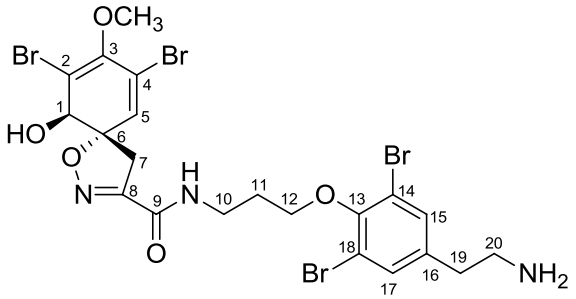
16

Table 12. NMR chemical shifts of **16** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, s	73.7, CH	2, 3, 5, 7
2	-	113.2, C	
3	-	147.2, C	
4	-	120.9, C	
5	6.57, s	131.3, CH	1, 3, 4, 7
6	-	90.3, C	
7	3.60, d (18.3)	39.4. CH_2	1, 5, 6, 8
	3.18, d (18.3)		1, 5, 6, 8
8	-	154.6, C	
9	-	159.0, C	
10	3.40, dt (6.1, 6.3), 2H	36.3, CH_2	9, 11, 12
11	1.99, quintet (6.3), 2H	29.5, CH_2	10, 12
12	3.96, t (6.3), 2H	71.4, CH_2	10, 11, 13
13	-	151.5, C	
14/18	-	117.7, C	
15/17	7.68, s, 2H	133.5, CH	13, 14/18, 16, 19
16	-	136.0, C	
19	3.02, tt (6.7, 5.6), 2H	27.1, CH_2	15/17, 16, 20
20	3.50, tt (6.7, 5.9), 2H	65.4, CH_2	19, 21, 22
20-$N(CH_3)_3$	3.10, s, 9H	52.5, CH_3	
3-OCH_3	3.64, s, 3H	59.7, CH_3	3
9-NH	8.55, t (6.1)	-	9

Compound **19** was isolated as a solid. Its molecular formula of $C_{21}H_{23}N_3O_5Br_4$ was deduced as indicated in the ESI mass spectrum at *m/z* 714 $[MH]^+$, with an isotopic cluster for four bromines at *m/z* 714, 716, 718, 720, 722. Compared with **16**, the spin systems coherent with the two bromotyrosine-derived units and the propyloxyamide linker were all intact (Table 13, Figures 23 and 24). However, the trimethyl quaternary ammonium moiety of **16** was missing in **19** and was replaced by a rather simple primary amino group (δ 7.76, 20-NH_2). **19** was therefore

identified as a primary amine analog of **16**, named araplysillin I. It was first isolated from the sponge *Psammaplysilla arabica* (Longeon et al., 1990). The specific rotation of **19** (+46, *c* 0.22, CH_3OH) is different from that previously reported by Longeon et al. (1990) (-70, *c* 0.70, CH_3OH), comparable in magnitude but opposite in sign. The configuration of **19** was therefore proposed as an enantioner of that reported by Longeon et al. (1990).



19

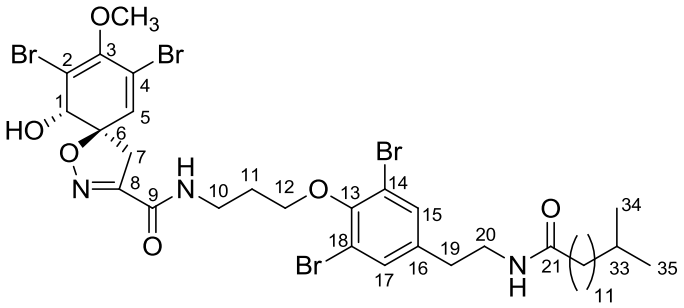
Table 13. NMR chemical shifts of **19** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
1	3.90, d (7.9)	73.7, CH	2, 3, 5, 6
2	-	113.1, C	
3	-	147.2, C	
4	-	120.9, C	
5	6.57, s	131.3, CH	1, 3, 4, 6, 7
6	-	90.3, C	
7	3.60, d (18.3)	39.5, CH_2	1, 5, 6, 8
	3.22, d (18.3)		1, 5, 6, 8
8	-	154.6, C	
9	-	159.0, C	
10	3.40, dt (6.2, 7.3), 2H	33.6, CH_2	9, 11, 12
11	1.99, tt (7.3, 6.4), 2H	29.5, CH_2	10, 12
12	3.95, t (6.4), 2H	71.3, CH_2	10, 11, 13
13	-	151.4, C	
14/18	-	117.7, C	
15/17	7.59, s, 2H	133.3, CH	13, 14/18, 16, 19

Table 13. (cont.)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
16	-	136.8, C	
19	2.81, t (6.3), 2H	31.6, CH_2	16, 20
20	3.07, t (6.3), 2H	39.4, CH_2	19
3-OCH_3	3.64, s, 3H	59.7, CH_3	3
1-OH	6.35, d (7.9)	-	1, 2
9-NH	8.54, t (6.2)	-	9
NH_2	7.76, br s, 2H	-	

Compound **20** was isolated as a solid. The molecular formula of $C_{36}H_{51}N_3O_6Br_4$ was deduced on the basis of the mass of $[M+Na]^+$ in the ESI mass spectrum at *m/z* 960, and the isotopic cluster at *m/z* 960, 962, 964, 966, 968 indicates the presence of four bromines. The NMR spectra of **20** (Table 14, Figures 25 and 26) were almost identical to those of **16** and **19**, indicating a similar binary unit of oxa,azaspirodecatriene-bromotyramine linked to a propyloxyamide moiety as seen in **16** and **19**. The mass of 224 mu higher than that of **20,** however, indicated the additional moiety of $C_{15}H_{28}O$, and was coherent to the cluster of methylene protons resonating in a range of δ_H 1.21 - 1.97 (H-22 - H-33), and δ_C 25.4 - 38.6 (C-22 - C-33). The terminal dimethyl at δ_H 0.82 (d, *J* = 6.42 Hz, H-34/35) and δ_C 22.6 (C-34/35) and an amide carbonyl at δ 172.2 (C-21) furnished this alkyl terminal as an iso-decapentanoyl amide moiety. **20** was identified as araplysillin II, first isolated from *Psammaplysilla arabica* (Longeon et al., 1990). The configuration of **20** was proposed in the same manner as those previously described i.e., the specific rotation of **20** was -48 (*c* 0.5, CH_3OH), [lit. -38, *c* 0.73, $CHCl_3$] (Longeon et al., 1990).



20

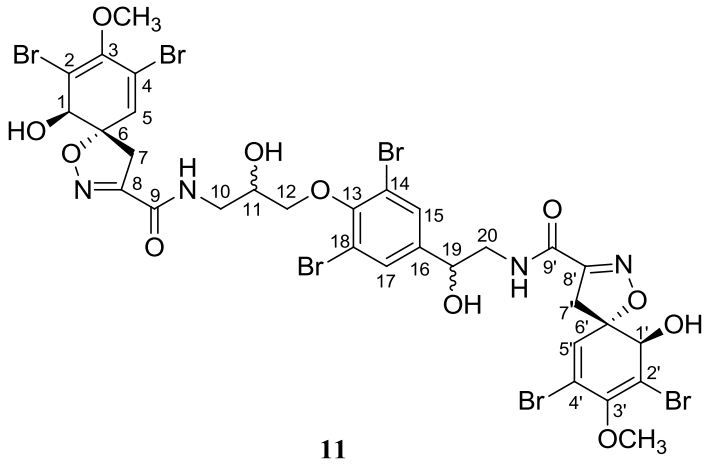
Table 14. NMR chemical shifts of **20** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.92, s	73.7, CH	2, 3, 4, 5, 6, 7
2	-	113.2, C	
3	-	147.2, C	
4	-	120.9, C	
5	6.56, s	131.4, CH	1, 3, 4, 6, 7
6	-	90.3, C	
7	3.60, d (18.3)	39.4, CH_2	1, 5, 6, 8
	3.18, d (18.3)		1, 5, 6, 8
8	-	154.6, C	
9	-	159.0, C	
10	3.30, overlap, 2H	36.3, CH_2	9, 11
11	1.98, overlap, 2H	29.0, CH_2	10
12	3.92, t (6.4), 2H	71.3, CH_2	10, 11
13	-	150.7, C	
14/18	-	117.3, C	
15/17	7.46, s, 2H	133.1, CH	13, 14/18, 16, 19
16	-	139.3, C	
19	2.64, t (6.6), 2H	33.6, CH_2	16, 15/17, 20
20	3.23, dt (5.8, 6.6), 2H	39.9, CH_2	19, 21
21	-	172.2, C	
22	1.97, t (7.3), 2H	35.5, CH_2	
23	1.41, tt (7.3, 6.6), 2H	25.4, CH_2	

Table 14. (cont.)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
24-32	1.21, m, 18H	38.6 – 29.1, CH_2	
33	1.45, septet (6.6)	27.5, CH	
34/35	0.82, d (6.6), 6H	22.6, CH_3	33
3-OCH_3	3.64, s, 3H	59.7, CH_3	3
1-OH	6.33, br s	-	
9-NH	8.51, t (5.9)	-	9
21-NH	7.75, t (5.8)	-	21

Compound **11** was isolated as a solid and has a molecular formula of $C_{31}H_{30}N_4O_{11}Br_6$ as indicated in the ESI mass spectrum at *m/z* 1131 $[M+Na]^+$, and the isotopic cluster at *m/z* 1131, 1133, 1135, 1137, 1139, 1141, 1143, indicative of six bromine atoms. Whereas the NMR spectra of **11** (Table 15, Figures 27 and 28) showed the spin systems belonging to the oxa,azaspirodecatriene-bromotyramine unit similar to those of **16**, **19**, and **20**, compound **11** in fact bears a close resemblance to compound **5** previously described in section 3.1, with two oxa,azaspirodecatriene units connected by a bromotyramine and a propyloxyamide linkers. The additional hydroxy group was placed on C-11, based on the characteristic chemical shifts of H-11 at δ 4.06 (m) and C-11 at δ 68.2. **11** was elucidated as fistularin 3, first reported from *Aplysina fistularis* (Gopichand and Schmitz, 1979). The configuration of **11** was proposed as shown due to the comparable specific rotation to that reported by Gopichand and Schmitz (1979) (+220, *c* 0.2, CH_3OH; lit. +104.2, *c* 1.67, CH_3OH).



11

Table 15. NMR chemical shifts of **11** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.92, overlap	73.7, CH	2, 3, 5, 6, 7
2	-	113.2, C	
3	-	147.2, C	
4	-	121.0, C	
5	6.56, s	131.2, CH	1, 3, 4, 6, 7
6	-	90.4, C	
7	3.62, d (18.3)	39.4, CH_2	1, 5, 6, 8
	3.22, d (18.3)		1, 5, 6, 8
8	-	154.5, C	
9	-	159.2, C	
10	3.48, ddd (10.7, 5.9, 5.6)	42.7, CH_2	9
	3.30, overlap		9, 11, 12
11	4.06, m	68.2, CH	
12	3.89, overlap	75.2, CH_2	10
	3.82, dd, (9.3, 5.4)		10
13	-	151.4, C	
14/18	-	117.2, C	
15/17	7.51, s, 2H	130.6, CH	13, 14/18, 19
16	-	142.7, C	
19	4.67, t (5.9)	69.5, CH	
20	3.33, overlap	46.4, CH_2	
	3.48, m		
1'	3.92, s	73.6, CH	
2'	-	113.2, C	
3'	-	147.2, C	
4'	-	121.1, C	
5'	6.58, s	131.3, CH	1', 3', 4', 6', 7'
6'	-	90.4, C	
7'	3.59, d (18.3)	39.5, CH_2	1', 5', 6', 8'
	3.18, d (18.3)		1', 5', 6', 8'

Table 15. (cont.)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
8'	-	154.6, C	
9'	-	159.1, C	
3-OCH_3	3.64, s, 3H	59.8, CH_3	3
3'-OCH_3	3.64, s, 3H	59.7, CH_3	3'
1-OH	6.35, t (6.1)	-	
1'-OH	6.35, t, (6.1)	-	
9-NH	8.35, t (5.6)	-	9
9'-NH	8.38, t (5.9)	-	9'
11-OH	5.24, br s	-	
20-OH	5.71, br s	-	

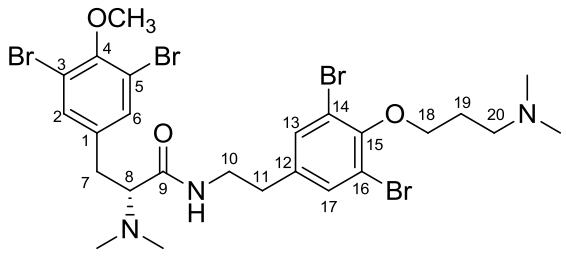
Group D; Binary-bromotyramine amides

Along with the oxa,azaspirodecatriene derivatives, a series of binary-bromotyramine amides alkaloids were also isolated from the sponge *P. purpurea*. The alkaloids in this group include compounds **14**, **15**, **7**, and **9**, all of which are characterized by the bromotyrosine residue linked to a unit of bromotyramine through an amide linkage.

Compound **14** was obtained as a solid, and its molecular formula was deduced to be $C_{25}H_{33}N_3O_3Br_4$ based on the mass cluster at *m/z* 740 $[MH]^+$, and the isotopic cluster at *m/z* 740, 742, 744, 746, 748 (1:4:6:4:1), indicative of four bromine atoms. From the ^{13}C-NMR (Table 16, Figure 30), two phenyl moieties were detected through the resonances at δ_C 151.6, 151.0, 139.2, 138.9, 133.4, 131.1, 117.3, and 116.9. The first spin system comprises the phenyl resonances at δ 138.9 (C-1), 133.4 (C-2/6), 116.9 (C-3/5), 151.6 (C-4), which is substituted by a methoxy [δ_C (60.4), δ_H (3.74, s)], two bromo, and an alkyl side chain based on the characteristic chemical shifts and the HMBC correlations from 4-OCH_3 (δ 3.74, s) to C-4 (δ 151.6), from H-7 (δ 2.75) to C-1 (δ 138.9) and C-8 (δ 68.6), and from H-8 (δ 3.11, t, J = 6.6 Hz) to C-1 (δ 138.9) and C-7 (δ 32.7). A dimethyl amino group [δ 41.4 and δ 2.14, s, 8-$N(CH_3)_2$] was placed on C-8 according to the HMBC correlations from H-8 (δ 3.11) to 8-*N* methyl carbon, and from the 8-*N*

methyl protons to C-8, allowing a fragment of *N,N*-dimethyl amino-dibromo-*O*-methyl tyrosine to be constructed.

The other phenyl moiety also was substituted by two bromine, an ethylene bridge, and an alkoxy group as indicated by the HMBC correlations from H-10 (δ 3.20) to C-11 (δ 33.7) and C-12 (δ 139.2); from H-11 (δ 2.60, m) to C-10 (δ 39.2), C-12 (δ 139.2), and C-13/17 (δ 133.1). The alkoxy side chain was in fact an *N,N*-dimethylaminopropyloxy moiety as indicated by the characteristic chemical shifts of δ_C 71.8 (C-18), 27.8 (C-19), 55.7 (C-20), 45.3 [20-N(CH_3)$_2$] and δ_H 3.91 (t, J = 6.6 Hz, H-18), 1.88 (tt, J = 6.6, 6.9 Hz, H-19), 2.40 (t, J = 6.9 Hz, H-20), 2.13 [s, 20-N(CH_3)$_2$], and by the HMBC correlations from H-18 (δ 3.91, t, J = 6.6 Hz) to C-19 (δ 27.8) and C-20 (δ 55.7), from H-19 (δ 1.88, tt, J = 6.6, 6.9 Hz) to C-18 (δ 71.8) and C-20 (δ 55.7), from H-20 (δ 2.40, t, J = 6.9 Hz) to C-18 (δ 71.8), C-19 (δ 27.8), 20-*N*-methyl carbon (δ 45.3) and from 20-*N*-methyl protons (δ 2.13, s) to C-20 (δ 55.7). The second subunit of dibromotyramine analog coupled to the first dibromotyrosine subunit through an amide linkage as indicated by the HMBC correlations from H-7 (δ 2.75) to C-9 (δ 170.0), from H-8 (δ 3.11, t, J = 6.6 Hz) to C-9 (δ 170.0), and from H-10 (δ 3.22) to C-9 (δ 170.0). Compound **14** was therefore identified as aplyzanzine A, first reported from an unidentified Verongid sponge (Evan et al., 2001). The configuration of **14** was confirmed as shown based on the comparable specific rotation with that reported by Kottakota et al. (2012) (+26, *c* 0.65, CH_3OH; lit. +30, *c* 5.0, CH_3OH).



14

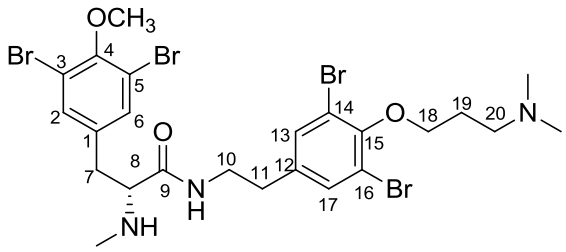
Table 16. NMR chemical shifts of **14** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	-	138.9, C	
2/6	7.45, s, 2H	133.4, CH	3, 4, 5, 7
3/5	-	116.9, C	

Table 16. (cont.)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
4	-	151.6, C	
7	2.75, t (6.6), 2H	32.7, CH_2	1, 8, 9
8	3.11, t (6.6)	68.6, CH	1, 7, 9, 10, 21/22
9	-	170.0, C	
10	3.20, overlap, 2H	39.2, CH_2	9, 11, 12
11	2.60, m, 2H	33.7, CH_2	10, 12, 13/17
12	-	139.2, C	
13/17	7.42, s, 2H	131.1, CH	11, 14/16, 15
14/16	-	117.3, C	
15	-	151.0, C	
18	3.91, t (6.6), 2H	71.8, CH_2	19, 20
19	1.88, tt (6.6, 6.9), 2H	27.8, CH_2	18, 20
20	2.40, t (6.9), 2H	55.7, CH_2	18, 19, 23/24
8-$N(CH_3)_2$	2.14, s, 6H	41.4, CH_3	8
20-$N(CH_3)_2$	2.13, s, 6H	45.3, CH_3	20
4-OCH_3	3.74, s, 3H	60.4, CH_3	4
9-NH	7.83, t (5.7)	-	9, 10

Compound **15** was also isolated as a solid and possesses a molecular formula of $C_{24}H_{31}N_3O_3Br_4$ on the basis of the cluster at *m/z* 726 $[MH]^+$, and the isotopic cluster at *m/z* 726, 728, 730, 732, 734 in the ESI mass spectrum. The NMR spectra of **14** and **15** (Tables 16 and 17, Figures 29, 30, 31, and 32) were almost identical except for one *N*-methyl group that was missing in compound **15**. This is coherent with the 14 mu less in the mass spectrum of **15**, indicating that **15** is a demethyl derivative of **14**. The *N,N*-dimethyl group on C-9 of **14** was replaced by *N*-methyl group as indicated by the chemical shifts of 8-*N* methyl carbon and proton resonances at (δ_C 34.1, δ_H 2.04, s). **15** was therefore identified as iso-anomoian A (Kottakota et al., 2012). The configuration of **15** was proposed as shown based on the comparable specific rotation with that reported by Kottakota et al. (2012). (+11, *c* 0.92, CH_3OH; lit. +4.5, *c* 1.0, CH_3OH).

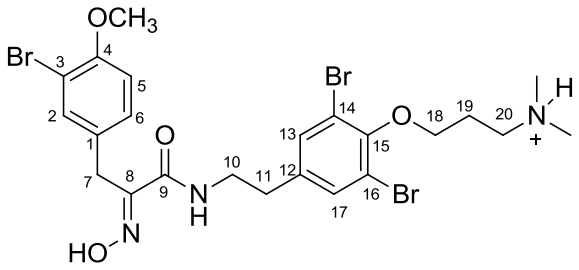


15

Table 17. NMR chemical shifts of **15** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	-	138.7, C	
2/6	7.44, s, 2H	135.5, CH	3, 4, 5
3/5	-	116.8, C	
4	-	151.7, C	
7	2.56, dd (13.2, 7.9), 2H	38.9, CH_2	1, 2/6, 8, 9
8	2.99, dd (7.9, 5.6)	64.9, CH	1, 9, 10, 22
9	-	173.0, C	
10	3.26, dt (5.5, 6.2), 2H	37.7, CH_2	9, 11, 12
11	2.64, t (6.2), 2H	33.7, CH_2	10, 12
12	-	139.0, C	
13/17	7.45, s, 2H	133.2, CH	11, 14/16, 15
14/16	-	117.4, C	
15	-	151.0, C	
18	3.90, t (6.6), 2H	71.9, CH_2	19, 20
19	1.89, tt (6.6, 6.9), 2H	27.8, CH_2	18, 20
20	2.38, t (6.9), 2H	55.7, CH_2	18, 19, 23/24
8-N-CH_3	2.04, s, 3H	34.1, CH_3	8
20-N$(CH_3)_2$	2.12, s, 6H	45.3, CH_3	20
4-OCH_3	3.74, s, 3H	60.4, CH_3	4
9-NH	7.89, t (5.5)	-	9

Compound **7** was isolated as a solid. The molecular formula of $C_{23}H_{29}N_3O_4Br_3$ as deduced from the mass cluster at *m/z* 648 $[M]^+$, 650, 652, 654 in the ESI mass spectrum, which indicated a pre-existing charged species as a quaternary ammonium similar to **6** and **16** discussed previously. Compared with **14** and **15**, despite similar carbon framework as indicated in the ^{13}C NMR spectra (Table 18, Figure 34), a series of distinguishable features can be recognized. First, the characteristic tri-substituted phenyl protons at δ_H 7.37 (d, *J* = 2.0 Hz, H-2), 6.97 (d, *J* = 8.5 Hz, H-5), and 7.10 (dd, *J* = 8.5, 2.0 Hz, H-6), indicated the replacement of the tetrasubstituted phenyl ring with dibromo substitution of **14** and **15** with a trisubstituted phenyl ring with a single bromo substitution. Another prominently different feature was the down-field shift of the two methyl groups at [δ_C 42.5, δ_H 2.82 (d, *J* = 2.9 Hz), 20-N$(CH_3)_2$] and the methylene at δ_C 54.5 (C-20) and δ_H 3.32 (H-20), which strongly indicated the presence of the quaternary ammonium moiety. The last and most distinctive feature of **7** was the oxime functionality indicated by the characteristic chemical shifts at δ_C 151.8 (C-8) and δ_H 11.87. The oxime group replaced the methyl amino groups previously described for **14** and **15**, as supported by the HMBC correlations from H-7 (δ3.70, s) to C-8 (δ151.8), C-9 (δ163.3) and *N*-OH (δ11.84, s) to C-8 (δ 151.8). **7** was therefore proposed to be aplysamine 2. The compound was previously reported from the sponge *Aplysina* sp. (Xynas and Capon, 1989). The *E* geometry of the C-8 oxime was determined by the diagnostic carbon chemical shift of the benzylic methylene (δ_C 27.9, C-7). The benzylic methylene corresponding to *Z* oximes are known to resonate greater than 35 ppm (Arabshahi and Schmitz, 1987).



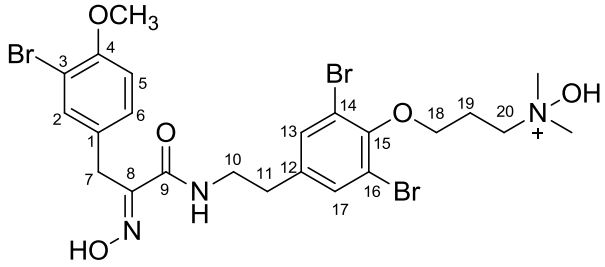
7

Table 18. NMR chemical shifts of **7** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	-	130.5, C	
2	7.37, d (2.0)	133.0, CH	3, 4, 6, 7
3	-	110.3, C	
4	-	153.9, C	
5	6.97, d (8.5)	112.7, CH	1, 3, 4, 6
6	7.10, dd (8.5, 2.0)	129.3, CH	2, 4, 7
7	3.70, s, 2H	27.9, CH_2	6, 8, 9
8	-	151.8, C	
9	-	163.9, C	
10	3.36, overlap, 2H	39.6, CH_2	9, 11, 12
11	2.72, t (7.1), 2H	33.4, CH_2	12, 13/17
12	-	139.4, C	
13/17	7.48, s, 2H	133.0, CH	11, 14/16, 15
14/16	-	117.2, C	
15	-	150.3, C	
18	3.95, t (5.9), 2H	70.2, CH_2	19, 20
19	2.13, tt (5.9, 5.6), 2H	24.9, CH_2	18, 20
20	3.34, overlap, 2H	54.5, CH_2	19
20-$N(CH_3)_2$	2.82, d (2.9), 6H	42.5, CH_3	20
4-OCH_3	3.78, s, 3H	56.3, CH_3	4
8-N-OH	11.87, s	-	8
9-NH	8.05, t (5.9)	-	9, 10
20-$^{+}$NH	9.64, br s	-	

Compound **9** has a molecular formula of $C_{23}H_{29}N_3O_5Br_3$ as deduced from the mass cluster at *m/z* 664 $[M]^+$, 666, 668, 670 in the ESI mass spectrum. Whereas the NMR spectra of **9** (Table 19, Figures 35 and 36) were identical to that of **7**, suggesting the similar carbon and proton framework, the 16 mu higher than that of **7** indicated the presence of an additional oxygen atom. The downfield shifts of H-20 at δ_H 3.90 (tt, J = 5.9, 5.7, Hz, H-20), and of the methyl

groups at δ_H 3.48 [s, 20-N(CH_3)$_2$] allowed the placement of this additional oxygen as an *N*-oxide moiety. The solution of **9** in DMSO-d_6 showed that the compound existed as a fully protonated species, with the 20-*N*-OH group resonating at δ 12.62 (br s). Compound **9** was proposed here as purpuramine J (Tabudravu and Jaspars, 2002). The *E* geometry of C-8 oxime of **9** was determined by the diagnostic carbon chemical shift of the benzylic methylene at C-7 (δ_C 27.9) (Arabshahi and Schmitz, 1987).



9

Table 19. NMR chemical shifts of **9** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
1	-	130.5, C	
2	7.37, d (2.2)	133.08, CH	3, 4, 6, 7
3	-	110.3, C	
4	-	153.9, C	
5	6.97, d (8.5)	112.7, CH	1, 3
6	7.10, dd (8.5, 2.2)	129.3, CH	2, 3, 7
7	3.71, s, 2H	27.9, CH_2	1, 2, 6, 8, 9
8	-	151.8, C	
9	-	163.3, C	
10	3.34, overlap, 2H	39.1, CH_2	9, 11, 12
11	2.73, t (7.0), 2H	33.5, CH_2	10, 12, 13/17
12	-	139.5, C	
13/17	7.48, s, 2H	133.09, CH	11, 14/16, 15
14/16	-	117.2, C	
15	-	150.3, C	
18	4.00, t (5.9), 2H	70.0, CH_2	19, 20

Table 19. (cont.)

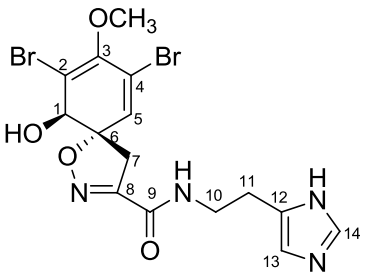
position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
19	2.31, pentet (5.9), 2H	23.6, CH_2	18, 20
20	3.90, tt (5.9, 5.7), 2H	66.2, CH_2	19, 21/22
20-N$(CH_3)_2$	3.48, s, 6H	55.8, CH_3	20
4-OCH_3	3.78, s, 3H	56.3, CH_3	4
8-N-OH	11.84, s	-	8
9-NH	8.02, t (5.9)	-	9
20-$^+$N-OH	12.62, br s	-	

Group E; Bromotyrosine-imidazole alkyl amides

The highly diverse chemical structures of bromotyrosine alkaloids are expressed in the alkaloids isolated in this investigation. Three alkaloids, **10**, **17**, and **18,** have bromotyrosine subunit coupled to a histidine-derived imidazole alkylamine through an amide linkage.

Compound **10** was isolated as a solid and has a molecular formula of $C_{15}H_{16}N_4O_4Br_2$ according to the ESI mass spectrum, showing the cluster of isotopic peaks at *m/z* 475 $[M+H]^+$, 477, 479. Compared with alkaloids in groups **A–D,** and those from the *Acanthodendrilla* sponge, the NMR signals at δ_C 73.7 (C-1), 113.2 (C-2), 147.2 (C-3) 120.9 (C-4), 131.3 (C-5), 90.4 (C-6), 39.3 (C-7), 154.8 (C-8), and at δ_H 3.91 (s, H-1), 6.65 (s, H-5), 3.58 (d, *J* = 18.3 Hz, H-7), 3.15 (d, *J* = 18.3 Hz, H-7), were conventionally elucidated for the oxa,azaspirodecatriene moiety (Table 20, Figures 37 and 38). Two aromatic protons at δ 7.44 (s, H-13) and 8.97 (s, H-14) were characteristic to an imidazole unit. Long range correlations from H-10 (δ 3.45, dt, *J* = 5.9, 6.8 Hz) to C-12 (δ 130.9), from H-11 (δ 2.83, t, *J* = 6.8 Hz) to C-12 (δ 130.9) and C-13 (δ 116.3) allowed the connection of the imidazole with an ethylamide moiety. This coupled further to the oxa,azaspirodecatriene residue through an amide linkage through HMBC correlation from H-10 (δ 3.45, dt, *J* = 5.9, 6.8 Hz) to C-9 (δ 159.2). Compound **10** was therefore identified as aerophobin 1 (Cimino et al., 1983). The configuration of **10** as shown was

proposed due to the comparable specific rotation (+32, *c* 0.34, CH_3OH; lit. +107.8, *c* 0.6, CH_3OH) (Gunasekera and Gunasekera, 1989).

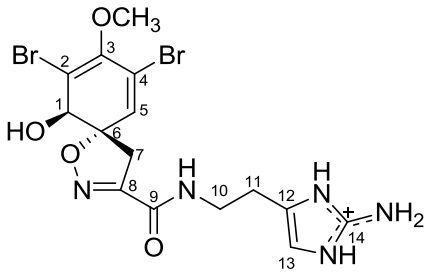


10

Table 20. NMR chemical shifts of **10** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, s	73.7, CH	2, 3, 5, 6
2	-	113.2, C	
3	-	147.2, C	
4	-	120.9, C	
5	6.65, s	131.3, CH	1, 3, 4, 7
6	-	90.4, C	
7	3.62, d (18.3)	39.3, CH_2	1, 5, 6, 8
	3.18, d (18.3)		1, 5, 6, 8
8	-	154.8, C	
9	-	159.2, C	
10	3.45, dt (5.9, 6.8), 2H	37.7, CH_2	9, 11, 12
11	2.83, t (6.8), 2H	24.2, CH_2	10, 12, 13
12	-	130.9, C	
13	7.44, s	116.3, CH	12, 14
14	8.97, s	133.9, CH	13
3-OCH_3	3.64, s, 3H	59.7, CH_3	3
1-OH	6.34, br s	-	
9-NH	8.64, t (5.9)	-	9

Compound **17** possess a molecular formula of $C_{15}H_{18}N_5O_4Br_2$ based on the mass cluster at *m/z* 490 $[M]^+$, 492, 494 in the ESI mass spectrum. Compared with **10,** both compounds share a similar framework of oxa,azaspirodecatriene coupled to an imidazole ethylamine. However, the proton previously resonating at δ 8.97, which was identified for H-14 in compound **10,** was missing. On the other hand, two protons resonating at δ 7.32 (s) were observed, belonging to an amino group substituting on C-14 (δ 146.9) of the imidazole subunit. **17** was proposed as a 2-aminoimidazole analog of **10** named purealidin J (Kobayashi et al., 1995). The configuratuion of **10** was proposed as shown based on the comparable specific rotation (+38, *c* 0.60, CH_3OH; lit. +24, *c* 0.98, CH_3OH) (Kobayashi et al., 1995).



17

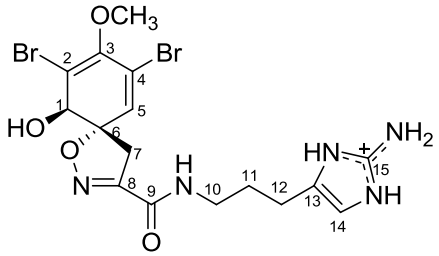
Table 21. NMR chemical shifts of **17** (500 MHz for ^1H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
1	3.90, (7.8)	73.7, CH	2, 3, 5, 6
2	-	113.1, C	
3	-	147.2, C	
4	-	120.9, C	
5	6.56, s	131.6, CH	1, 3, 4, 6, 7
6	-	90.4, C	
7	3.60, d (18.3)	39.4, CH_2	1, 5, 6, 8
	3.16, d (18.3)		1, 5, 6, 8
8	-	154.5, C	
9	-	159.1, C	
10	3.37, overlap, 2H	37.5, CH_2	9, 11, 12
11	2.61, t (7.1), 2H	24.4, CH_2	10, 12

Table 21. (cont.)

position	δ_H (*J* in Hz)	δ_C	HMBC (H ⟶ C)
12	-	124.3, C	
13	6.60, s	109.6, CH	14
14	-	146.9, C	
3-OCH_3	3.64, s, 3H	59.7, CH_3	3
9-NH	8.58, t (5.1)	-	9
1-OH	6.32, d (7.8)	-	1, 2
12-NH	11.59, br s	-	
13-NH	12.02, br s	-	
14-NH_2	7.32, s, 2H	-	

Compound **18** has a molecular formula of $C_{16}H_{20}N_5O_4Br_2$ corresponding to the mass cluster at *m/z* 504 $[M]^+$, 506, 508 from the ESI mass spectrum. The NMR spectra of **18** (Table 22, Figures 41 and 42) were identical to that of **17**, with an exception of the additional methylene protons indicated by the additional 14 mu in the mass spectra, and by the resonance at δ 1.69 (tt, *J* = 6.3, 6.8 Hz, H-11). The coupling constants and the HMBC correlations allowed the placement of this methylene as part of a propylene bridge. Hence **18** was proposed here as a homolog of **17**, named aerophobin 2 (Cimino et al., 1983). The configuration as shown was proposed based on the comparable specific rotation to the previously reported one by cimino et al. (1993) (+55, *c* 0.32, CH_3OH; lit. +139, *c* 1.9, CH_3OH).



18

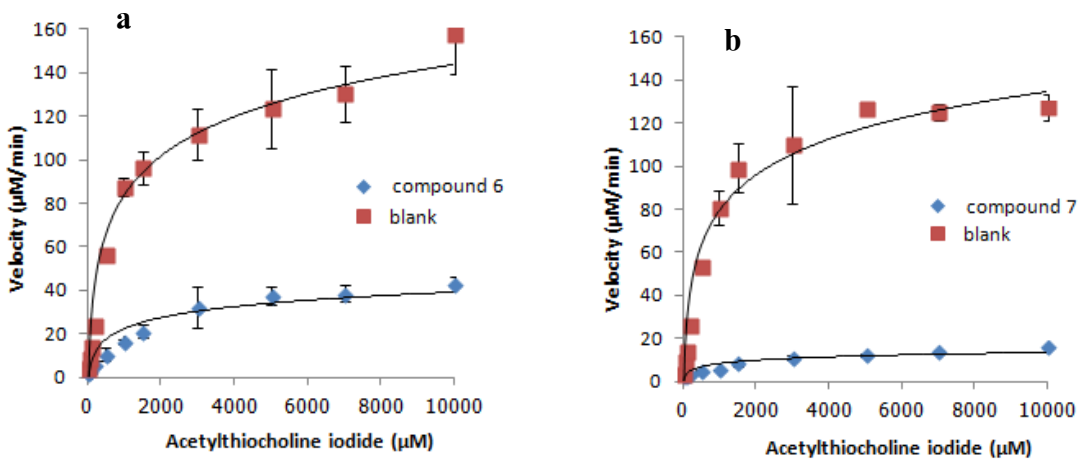
Table 22. NMR chemical shifts of **18** (500 MHz for ^{1}H and 125 MHz for ^{13}C; DMSO-d_6)

position	δ_H (J in Hz)	δ_C	HMBC (H ⟶ C)
1	3.91, (7.1)	73.6, CH	2, 3, 5, 6
2	-	113.2, C	
3	-	147.2, C	
4	-	120.9, C	
5	6.57, s	131.3, CH	1, 3, 4, 6, 7
6	-	90.3, C	
7	3.60, d (18.0)	39.4, CH_2	1, 5, 6, 8
	3.16, d (18.0)		1, 5, 6, 8
8	-	154.6, C	
9	-	159.1, C	
10	3.17, overlap, 2H	38.1, CH_2	9, 11, 12
11	1.70, tt (6.3, 6.8), 2H	27.4, CH_2	10, 12, 13
12	2.40, t (6.8), 2H	21.6, CH_2	10, 11, 14
13	-	126.4, C	
14	6.58, s	108.9, CH	13
15	-	146.9, C	
3-OCH_3	3.64, s, 3H	59.4, CH_3	3
9-NH	8.56, t (5.9)	-	9, 10
1-OH	6.34, d (7.1)	-	
13-NH	11.68, br s	-	
14-NH	12.09, br s	-	
15-NH_2	7.36, s, 2H	-	

3.3 Biological activity

All the isolated compounds obtained from both sponges in this investigation were assessed for their acetylcholinesterase-inhibiting activity using the Ellman method (Ellman et al., 1961, Ingkaninan et al., 2003), and for the cytotoxic activity against four cancer cell lines (MCF-7, KB, HT-29, and human fibroblast) using the SBR assay (Skehan et al., 1990). Compounds **6**, **7**, **14**, and **15** showed inhibitory activity against the acetylcholinesterase enzyme at IC_{50} values of 1.2, 1.3, 106.8, and 70.4 µM respectively, while that of the positive standard galantamine was 0.35 µM. Compound **3** was cytotoxic against MCF-7 and human fibroblast cell lines at the IC_{50}s of 0.53 and 1.46 µM, respectively, while **10** showed cytotoxic activity against MCF-7, KB, HT-29, and human fibroblast cell lines at the IC_{50}s of 0.79, 0.53, 0.91, and 4.2 µM, respectively. Camptothecin, which was the reference standard, showed IC_{50} values of 1.2×10^{-3}, 5.7×10^{-3}, 2.6×10^{-4}, and 459.3×10^{-3} µM against MCF-7, KB, HT-21, and human fibroblast, respectively. The acetylcholinesterase-inhibiting activity of bromotyrosine-derived alkaloids is reported here for the first time to show a moderate-to-good potency against the enzyme.

The effect of compounds **6, 7**, **14**, and **15** on the enzyme kinetics is shown in Figure 43. The Michaelis-Menten plots showed the decreasing V_{max} with no significant change in K_m upon the exposure to the tested compounds. This strongly indicated that all four compounds act as reversible non-competitive inhibitors.



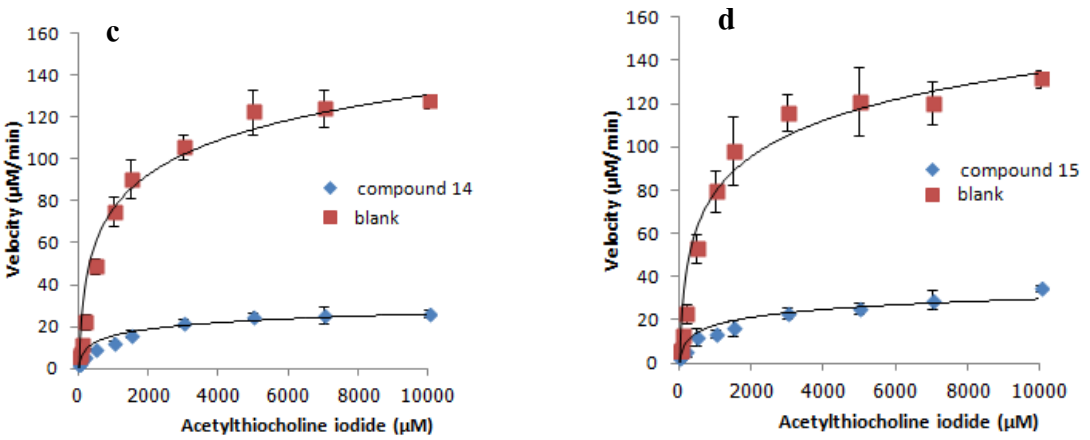
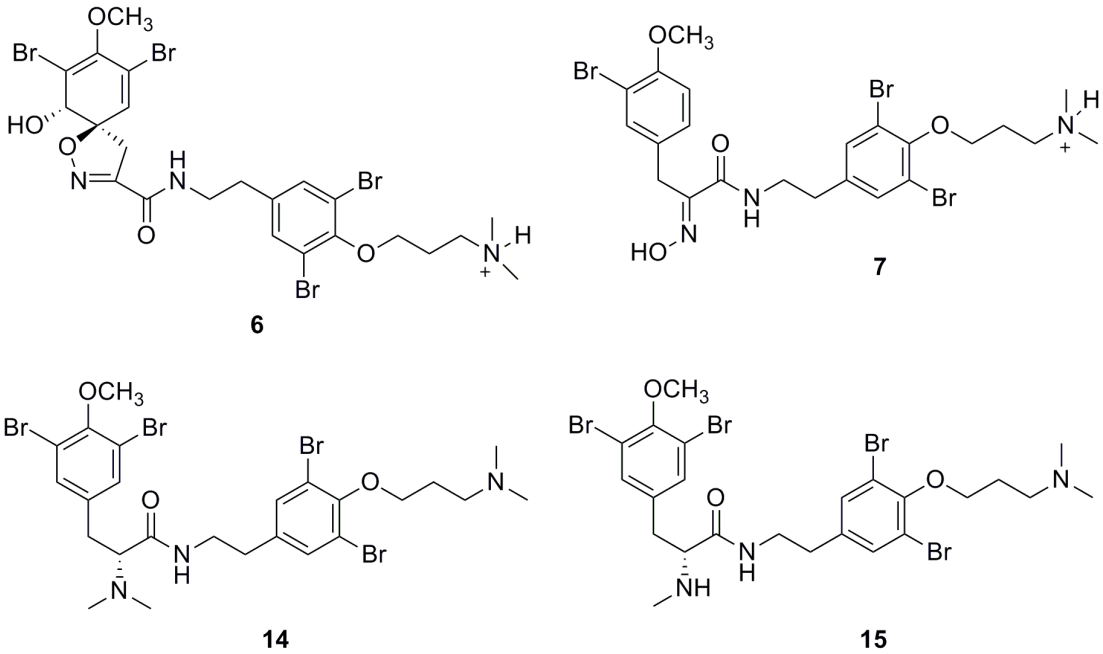
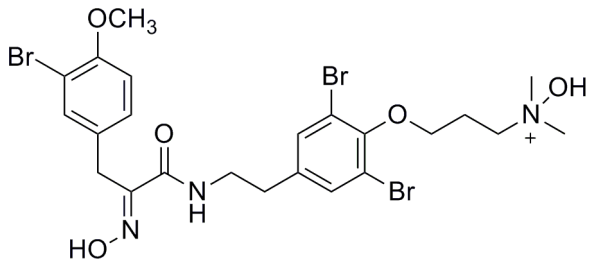


Figure 43. Michaelis-Menten plots for acetylcholinesterase inhibition upon exposure to compounds **6** (a), **7** (b), **14** (c), and **15** (d).

The similarity among the chemical structures of all the active compounds (**6**, **7**, **14**, and **15**) suggest the necessity of the *N,N*-dimethylaminopropyloxydibromoyramine residue for the enzyme inhibiting activity.



Whereas the influence of the amino group is ambiguous whether as a tertiary amine or as a quaternary ammonium could be more effective, the electronic state of the *N*-oxide as seen in compound **9** clearly casts the negative effect on the activity.



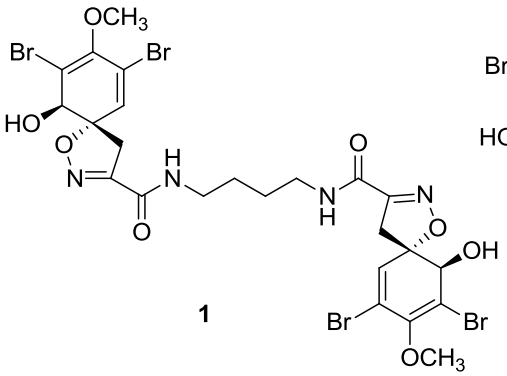
9

It has been widely known that the sites inside the binding pocket of acetylcholinesterase include at least two binding loci; the peripheral anionic site and the active site consisting of the anionic and esteratic subsites. The amino group of the active bromotyrosines can be proposed to bind to the anionic subsite, whereas the aromatic residue binds to the conserved aromatic residues that line the gorge leading to the active site. This suggestion agrees well with the non-competitive mode of enzyme inhibition, caused by the change in enzyme conformation upon biding to the allosteric site acetylcholinesterase enzyme by the aromatic moiety.

CHAPTER 4

CONCLUSION

This dissertation was focused on the investigation of the chemical constituents responsible for the acetylcholinesterase-inhibiting activity in the sponges *Acanthodendrilla* sp. and *Pseudoceratina* cf. *purpurea*. The investigation led to the isolation of 20 bromotyrosine-derived alkaloids; aerothionin (**1**), homoaerothionin (**2**), 11,19-dideoxyfistularin 3 (**3**), 11-oxoaerothionin (**4**), and 11-deoxyfistularin 3 (**5**) were isolated from the sponge *Acanthodendrilla* sp., while purealidin Q (**6**), aplysamine 2 (**7**), aeroplysinin 1 (**8**), purpureamine J (**9**), aerophobin 1 (**10**), fistularin 3 (**11**), hemifistularin 3 (**12**), purealidin R (**13**), aplyzanzine A (**14**), iso-anomoian A (**15**), purealidin B (**16**), purealidin J (**17**), aerophobin 2 (**18**), araplysillin I (**19**), and araplysillin II (**20**), were from the sponge *P. purpurea*. The structures of all isolated compounds are summarized below;



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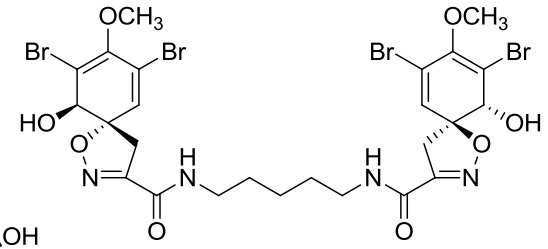

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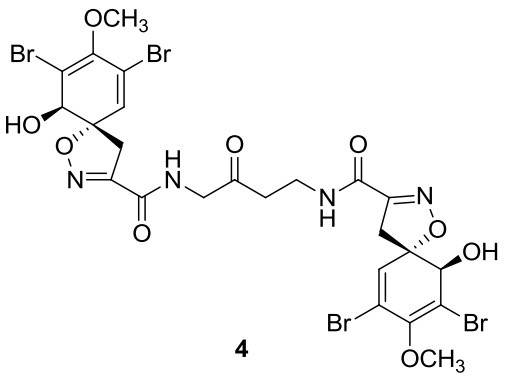

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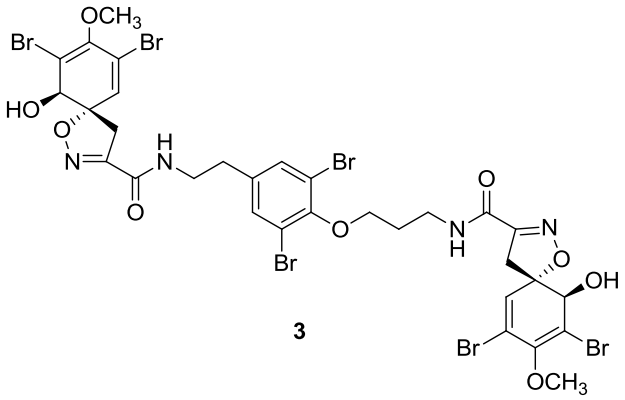

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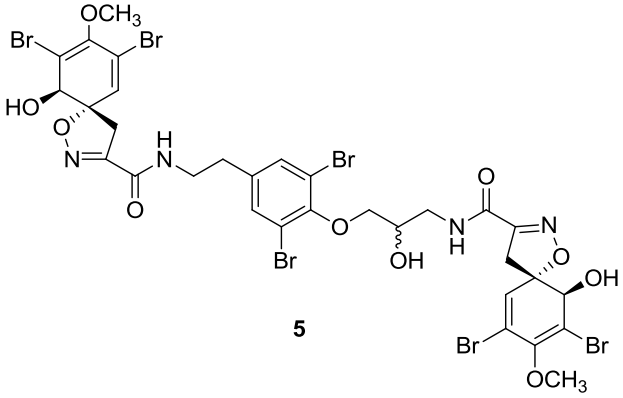

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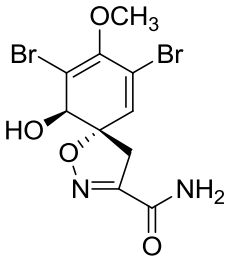

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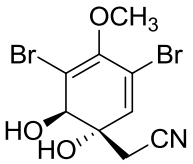

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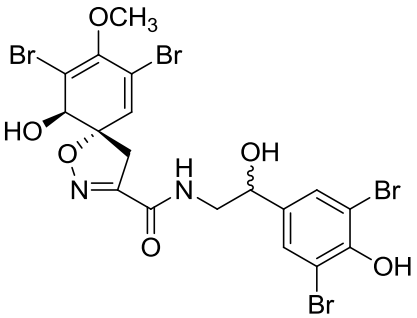

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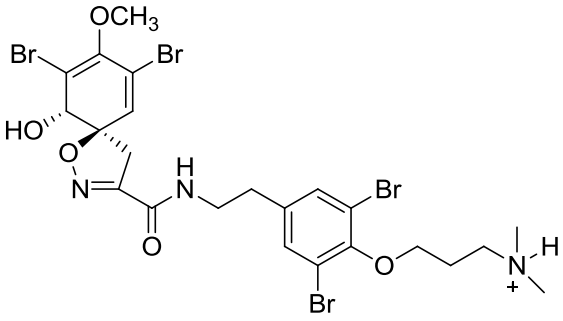

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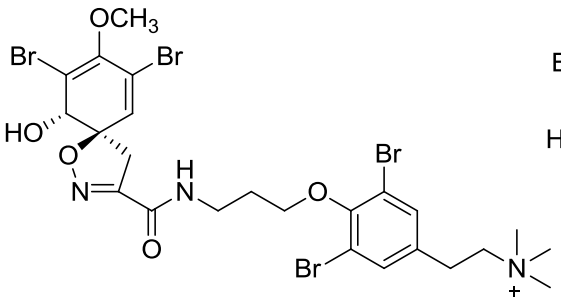

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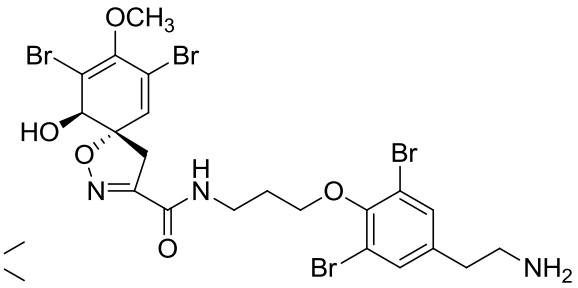

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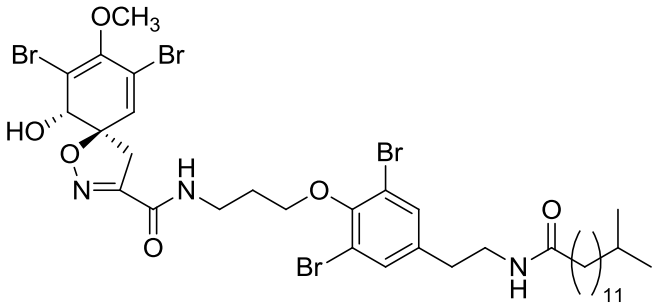

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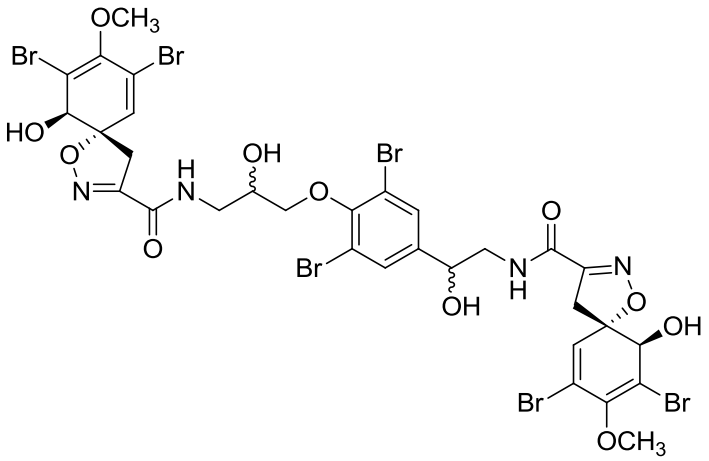

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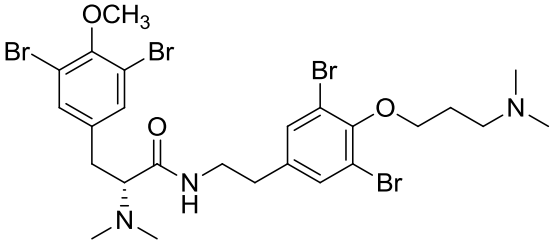

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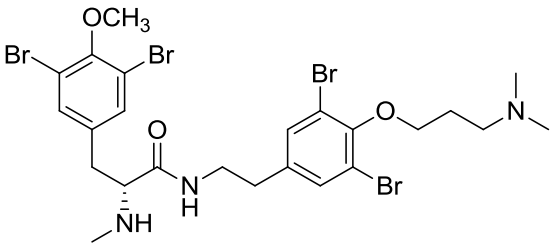

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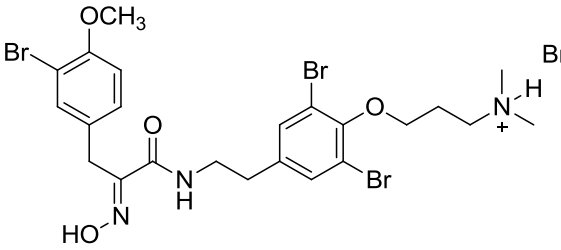

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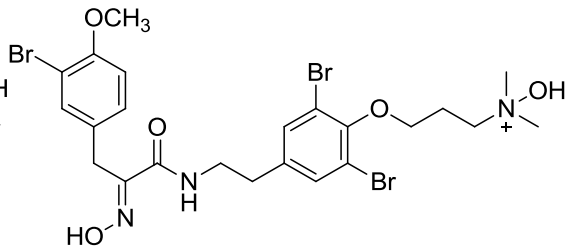

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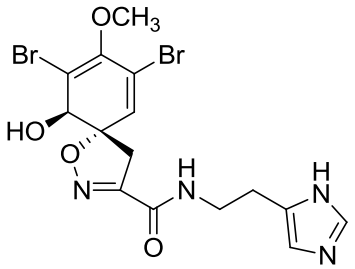

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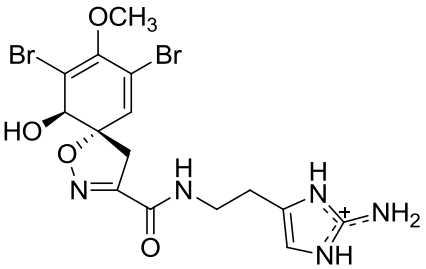

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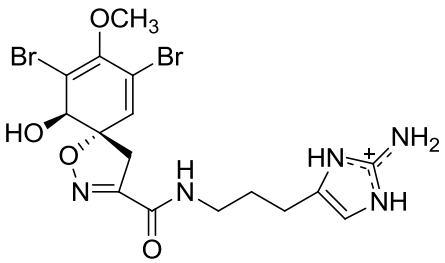

18

All the isolated compounds were assayed for their acetylcholinesterase-inhibiting and cytotoxic activities. Compounds **6**, **7**, **14**, and **15** showed inhibitory activity against the acetylcholinesterase enzyme at IC_{50} values of 1.2, 1.3, 106.8, and 70.4 µM, respectively. Compound **3** was cytotoxic against MCF-7 and human fibroblast cell lines at 0.53 and 1.46 µM respectively, while **10** showed cytotoxic activity against MCF-7, KB, HT-29, and human fibroblast cell lines at 0.79, 0.53, 0.91, and 4.2 µM, respectively. The degree of acetylcholinesterase inhibition depends largely on the presence of the *N,N*-dimethylaminopropyloxydibromotyramide as observed in compounds **6, 7**, **14**, and **15**. The

enzyme kinetics assay suggests that all four compounds (**6**, **7**, **14**, and **15**) act as non-competitive inhibitors.

It is acknowledged that marine natural products continue to be a source of inspiration and innovation in many areas of biomedical science, result of which has been applied in the development and treatment of various ailments. The modern and advanced technologies have allowed researchers to investigate issues on production, utility, and function of marine natural products in highly insightful ways. Prospect for the field of marine natural products appears very bright because the field has embraced the involvement of many disciples and technologies, thus ensuring high level of innovation and vitality.

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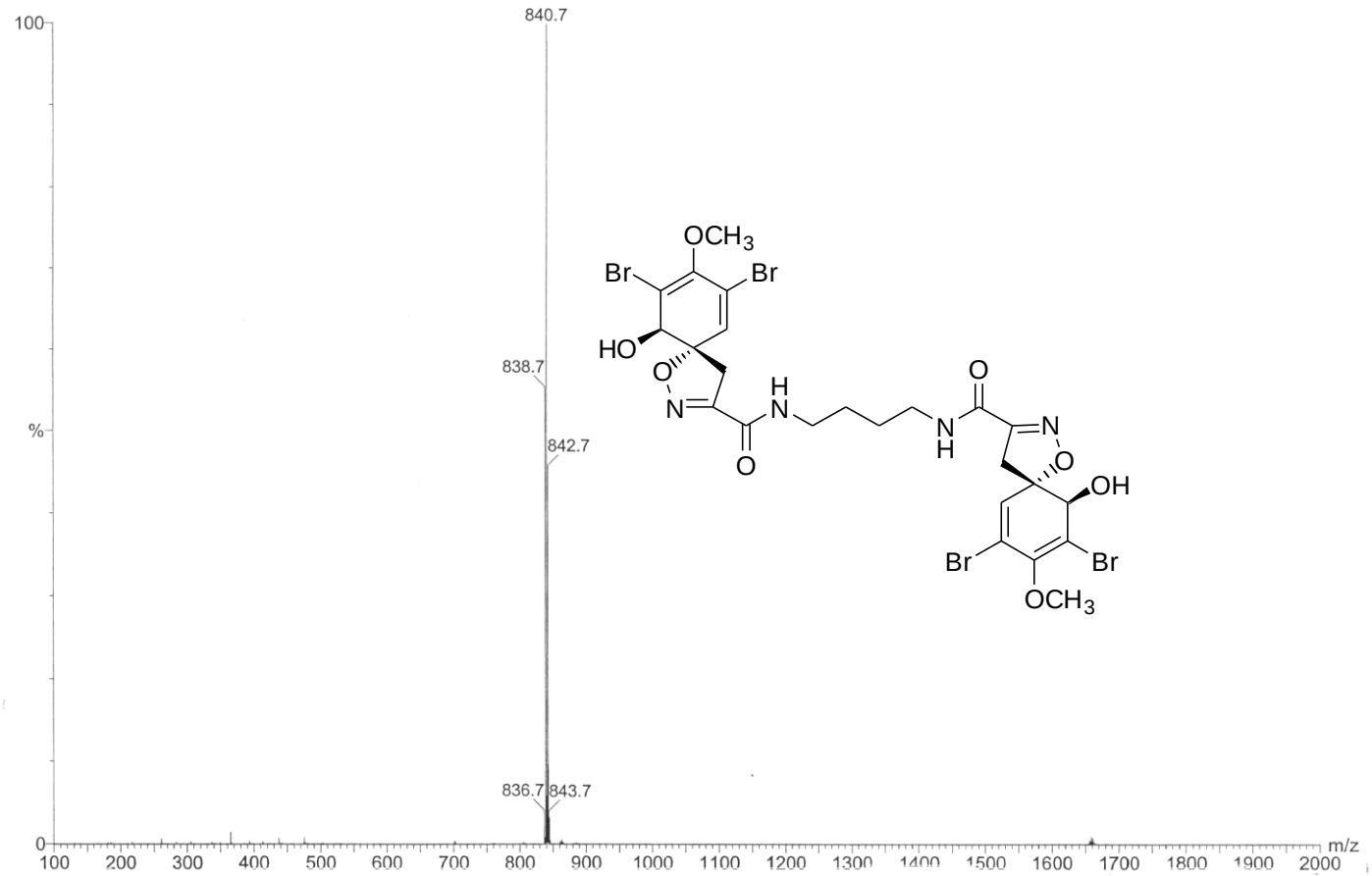
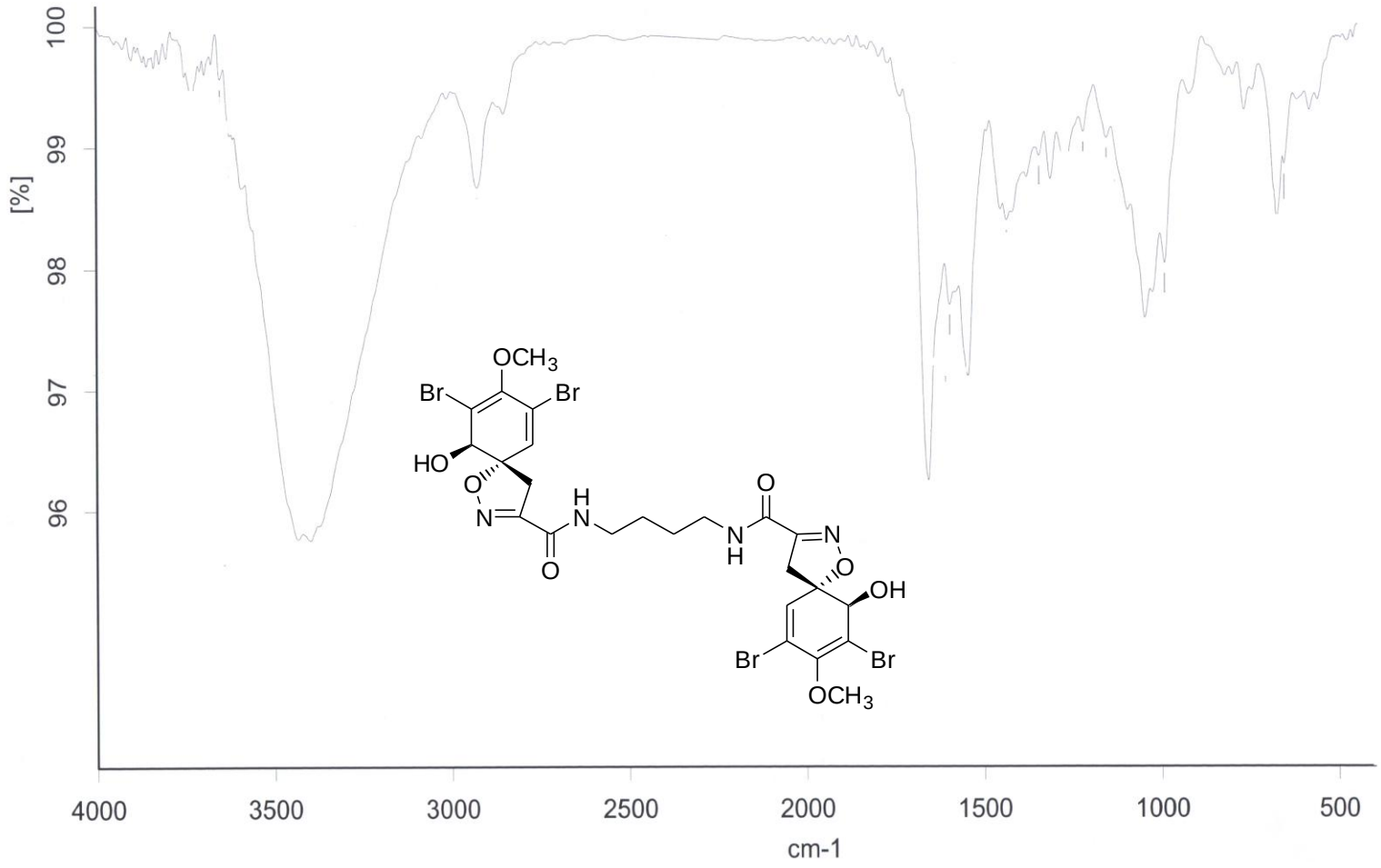
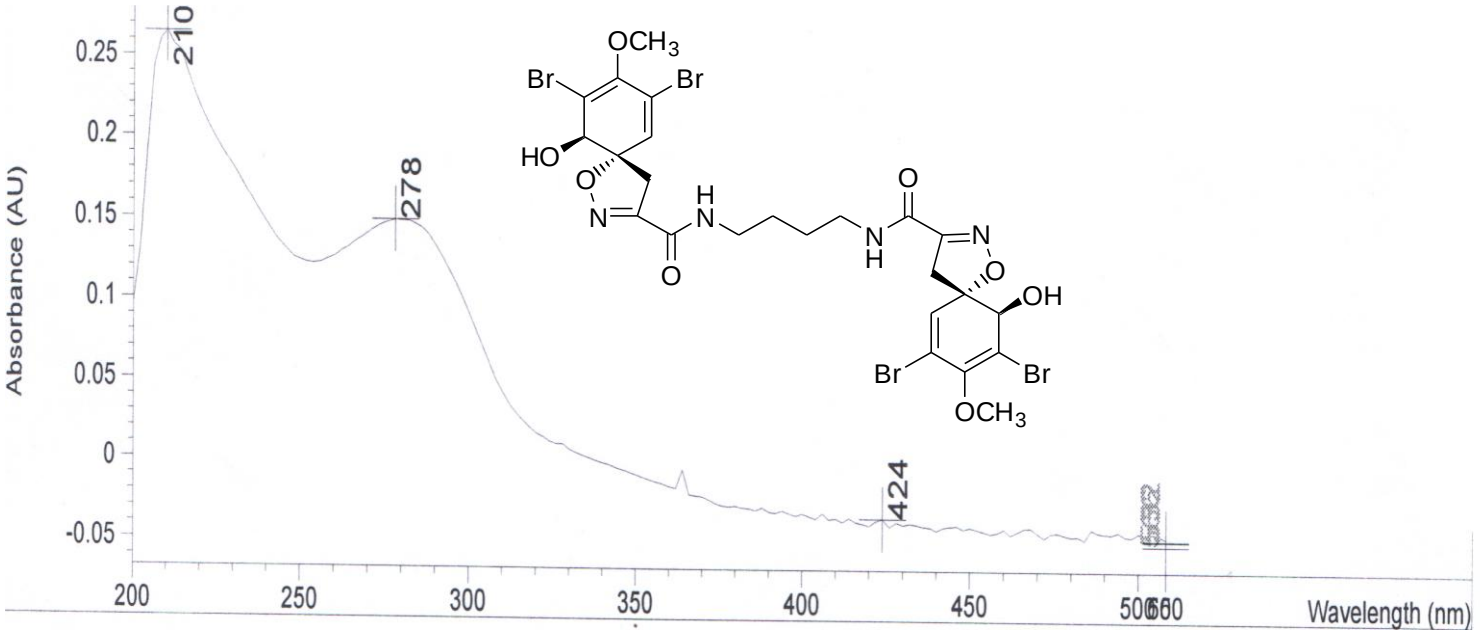


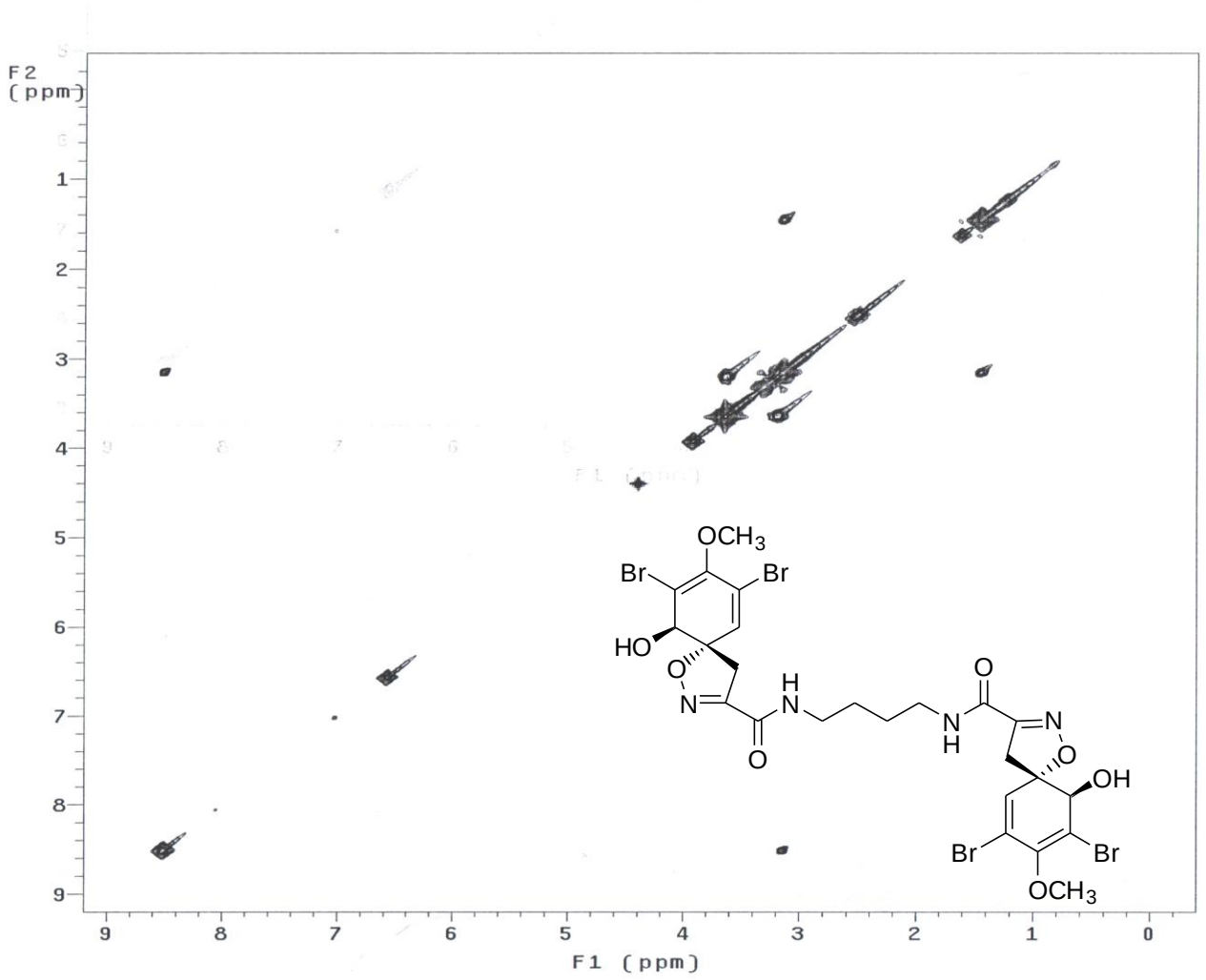
ESI mass spectrum of **1**



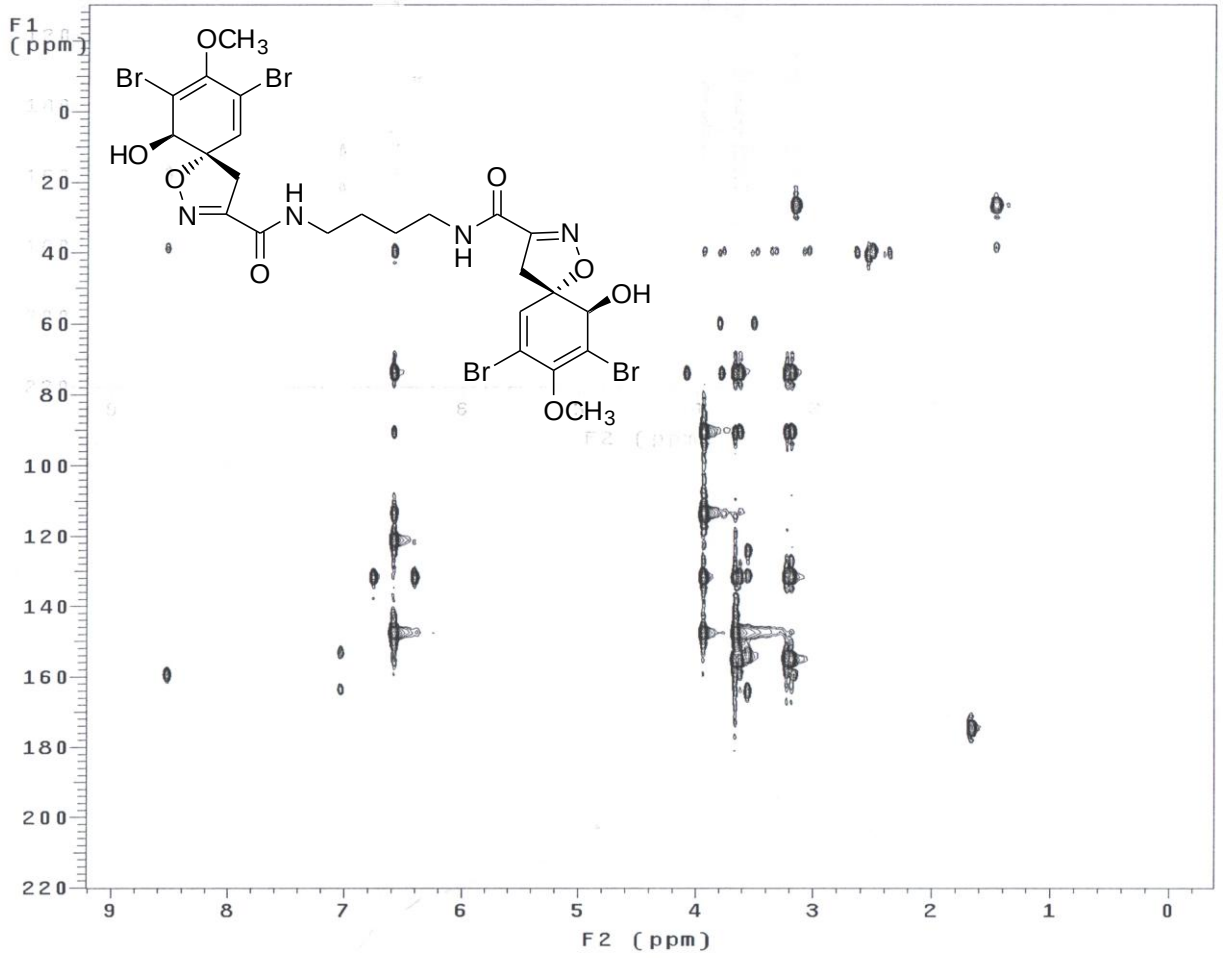
IR spectrum of **1** (KBr)



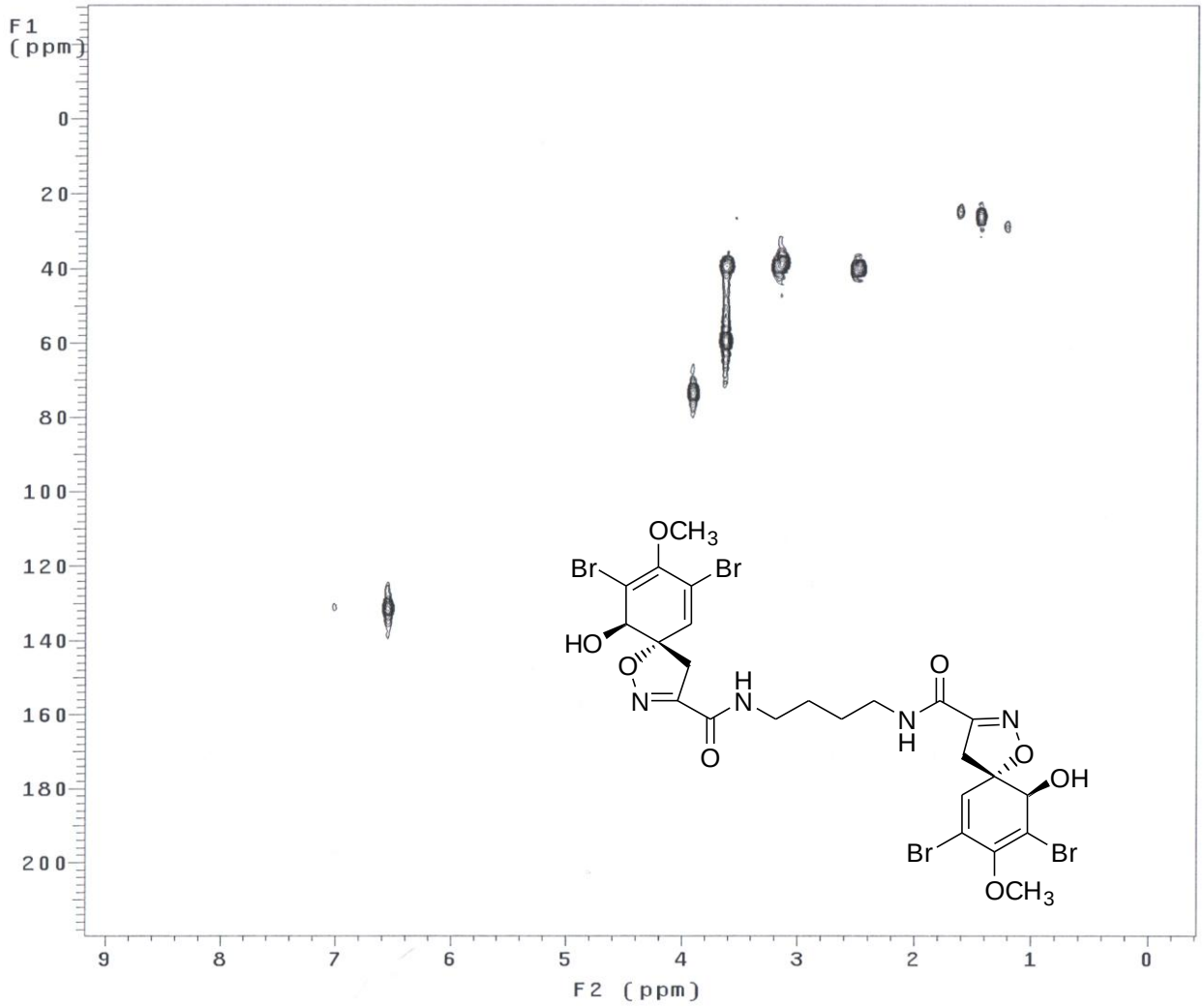
UV spectrum of **1** (CH_3OH)



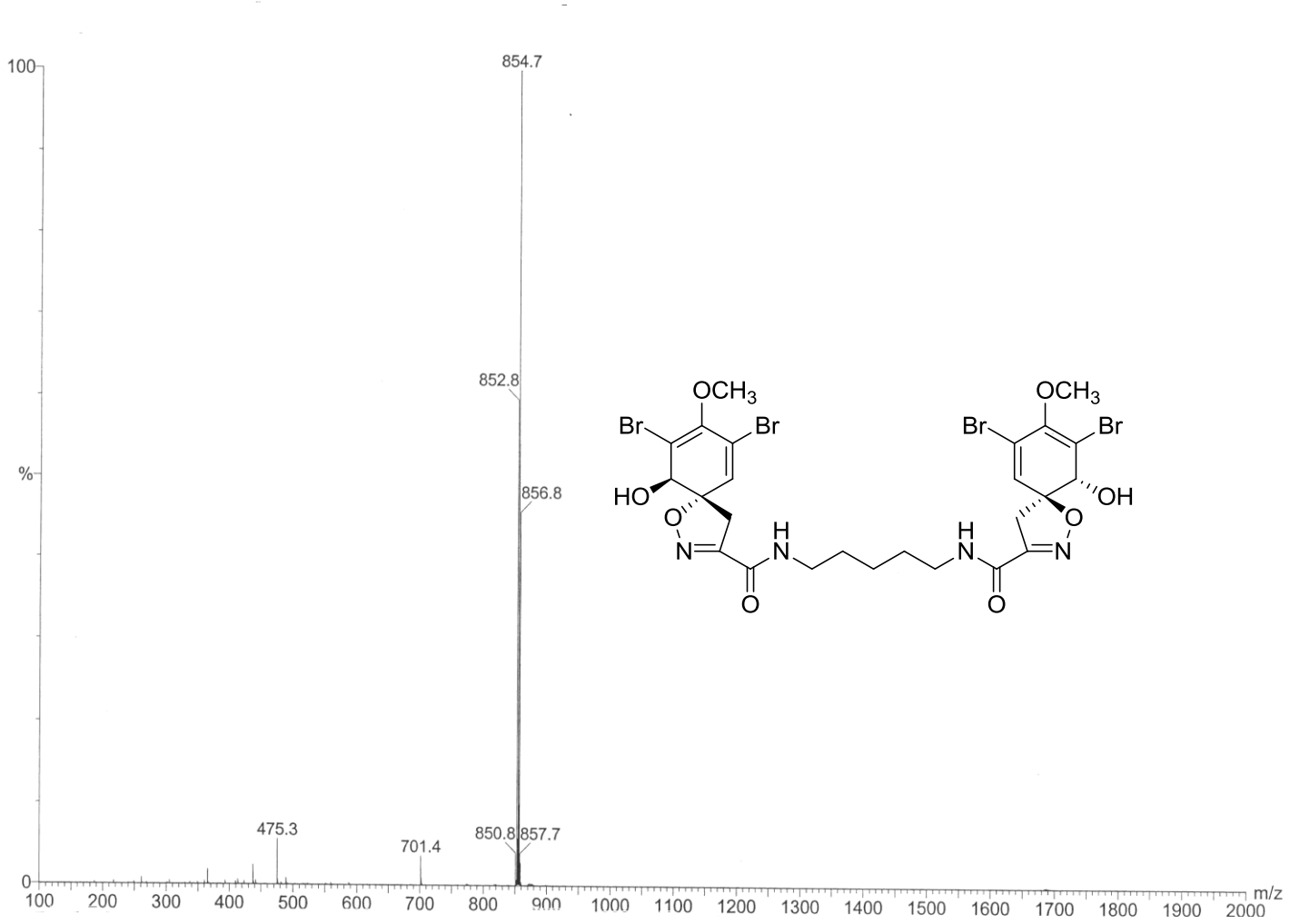
^{1}H-^{1}H COSY spectrum of **1** (500 MHz, DMSO-d_6)



HMBC spectrum of **1** (500 MHz, DMSO-d_6)



HMQC spectrum of **1** (500 MHz, DMSO-d_6)



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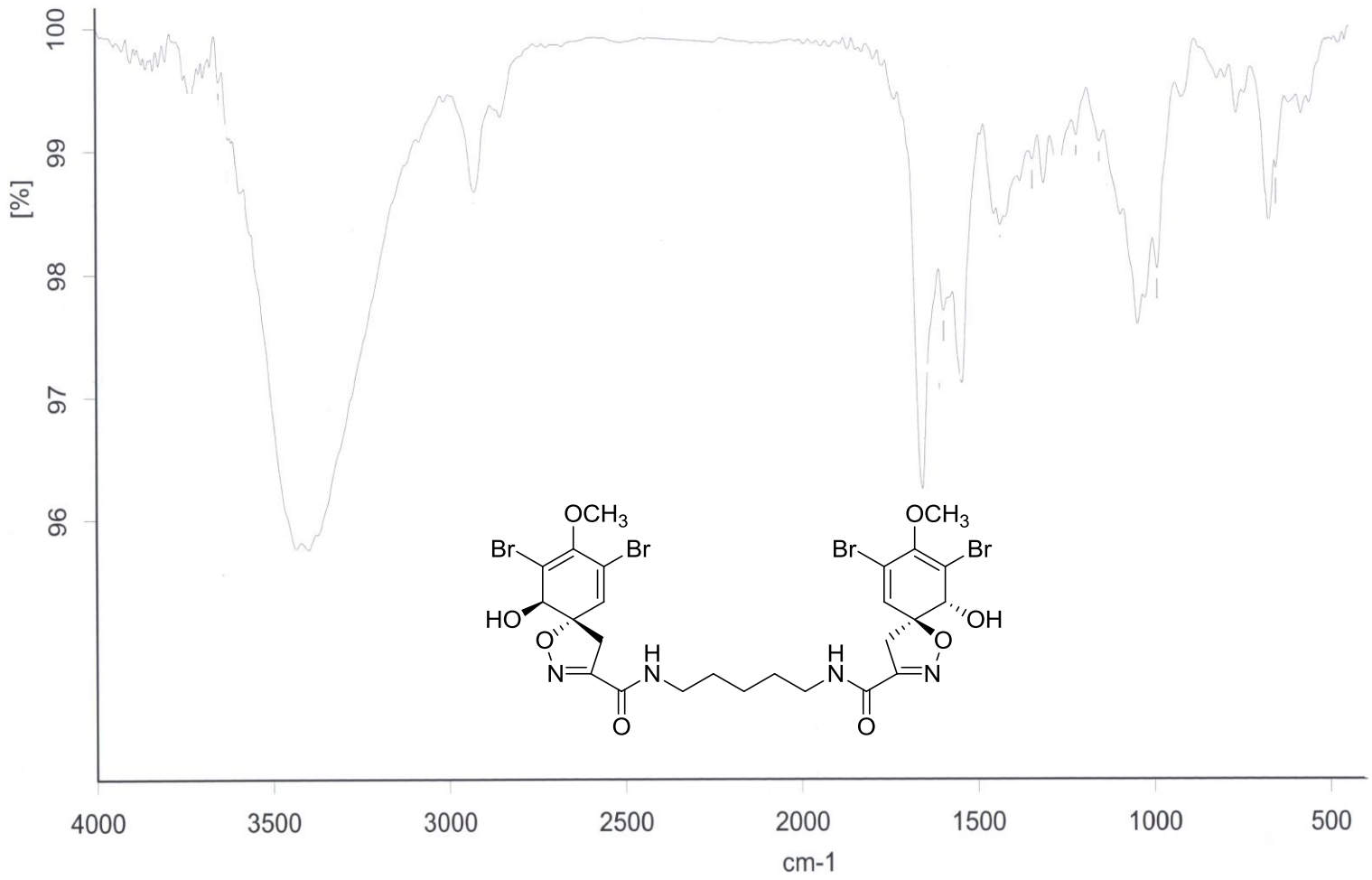
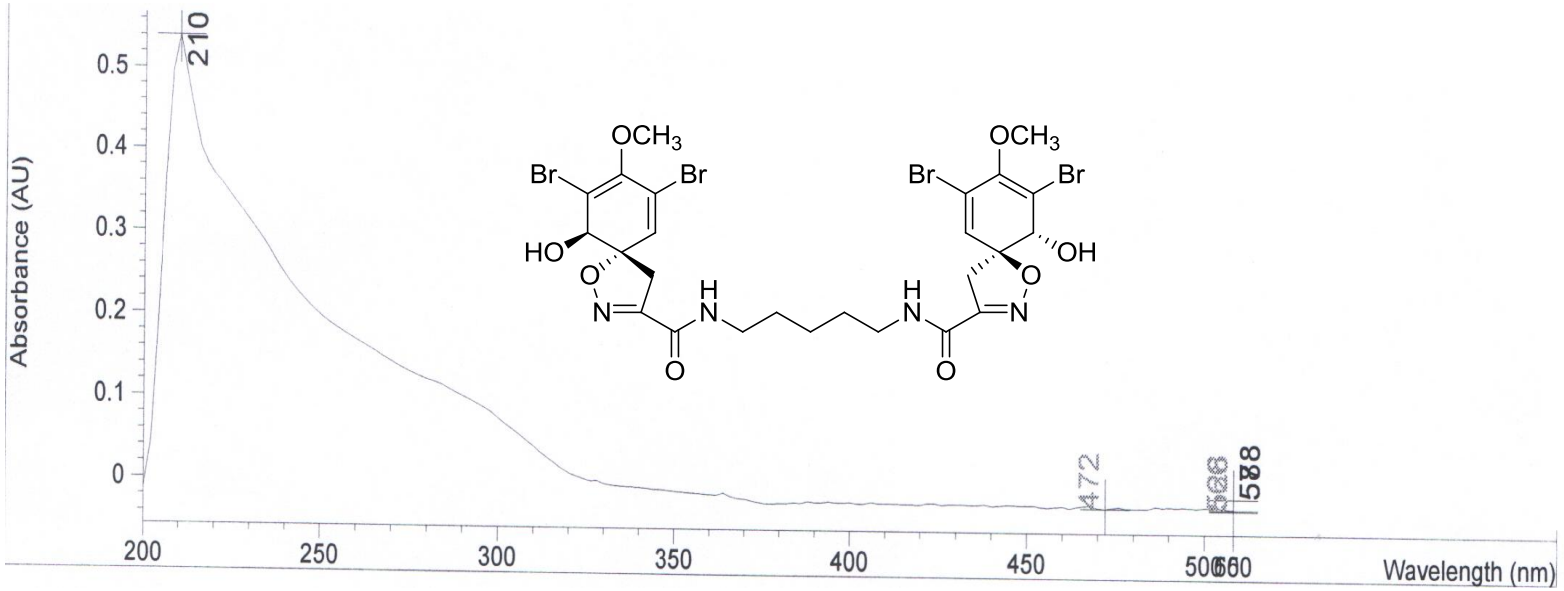
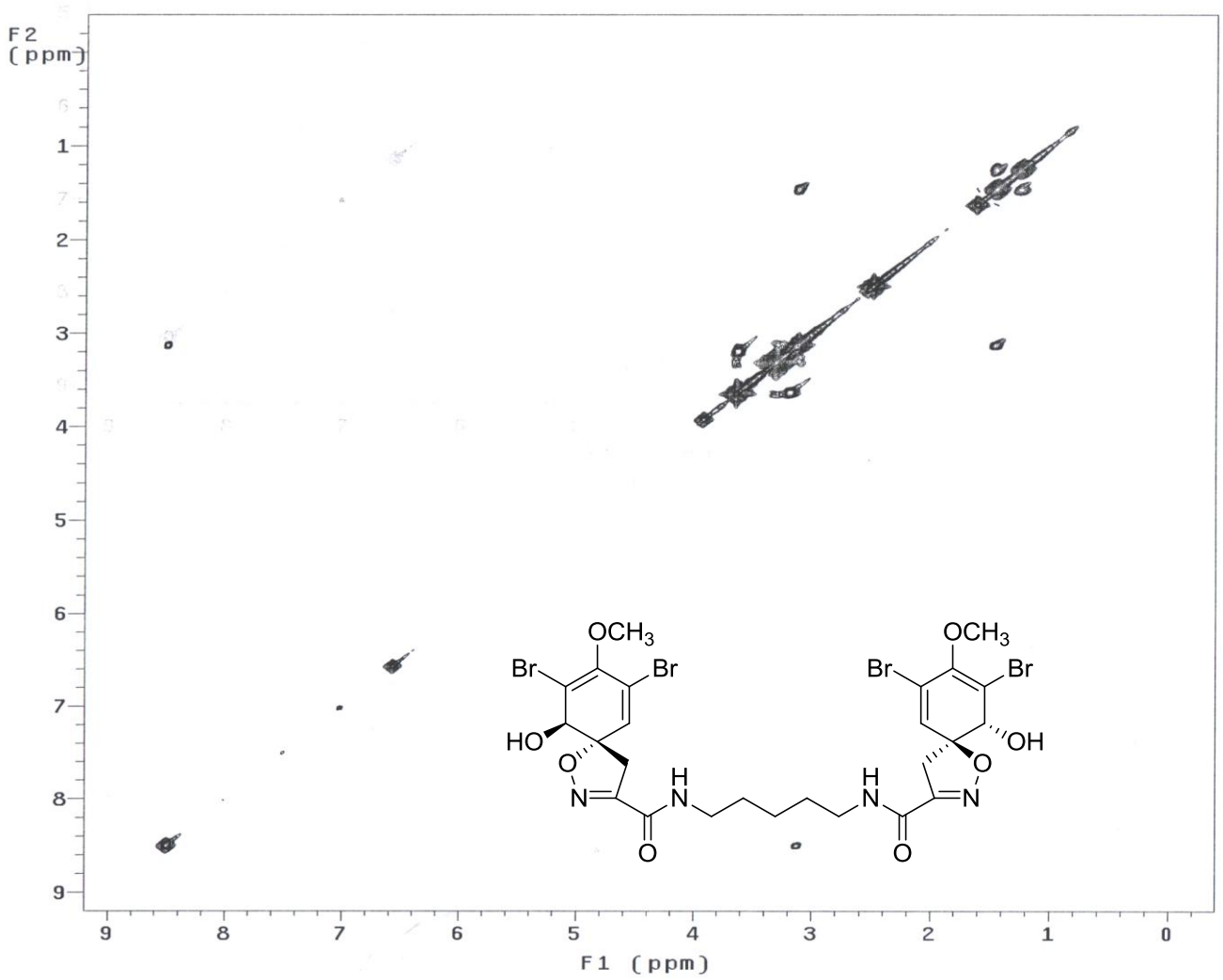


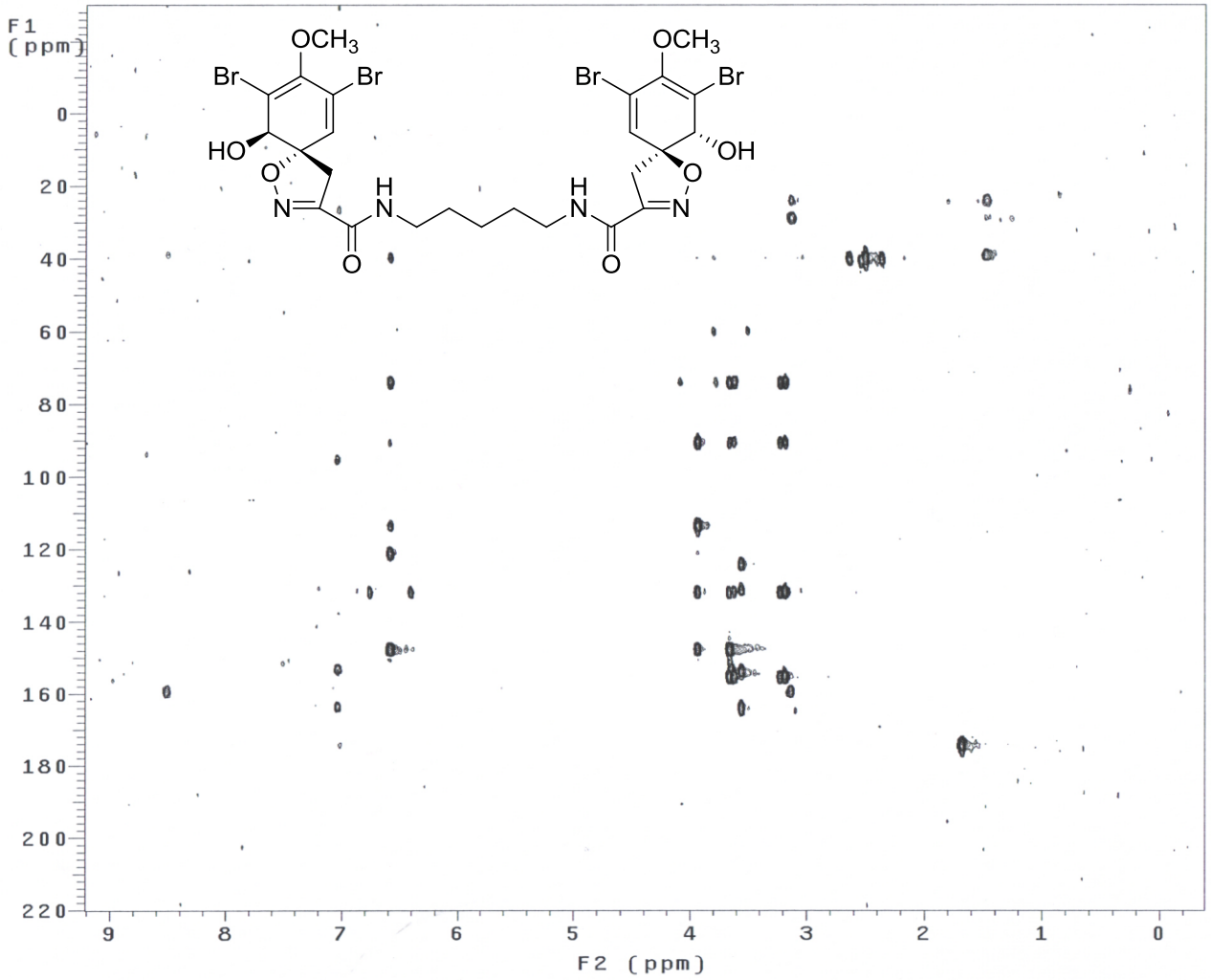
IR spectrum of **2** (KBr)



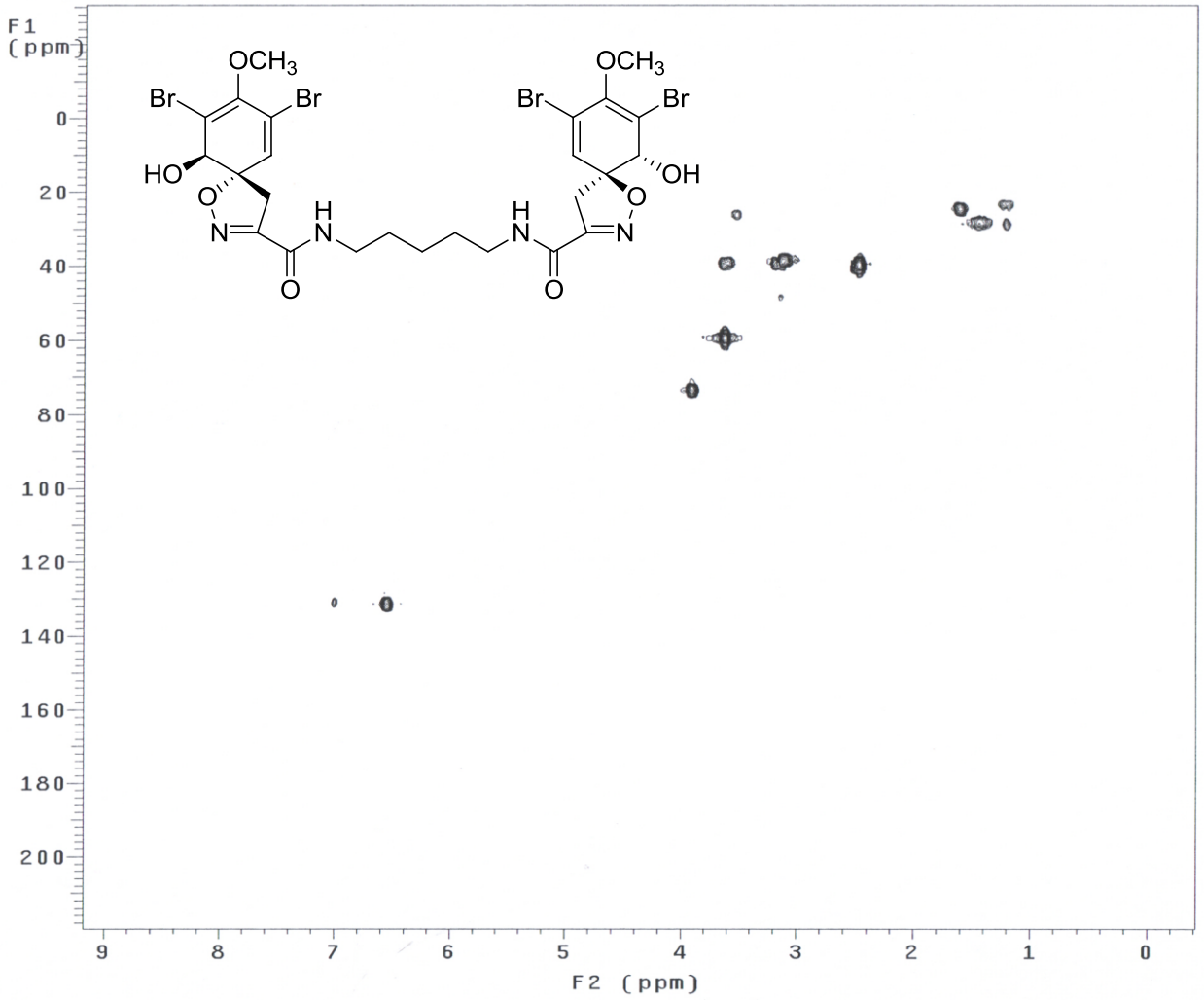
UV spectrum of **2** (CH_3OH)



^{1}H-^{1}H COSY spectrum of **2** (500 MHz, DMSO-d_6)



HMBC spectrum of **2** (500 MHz, DMSO-d_6)



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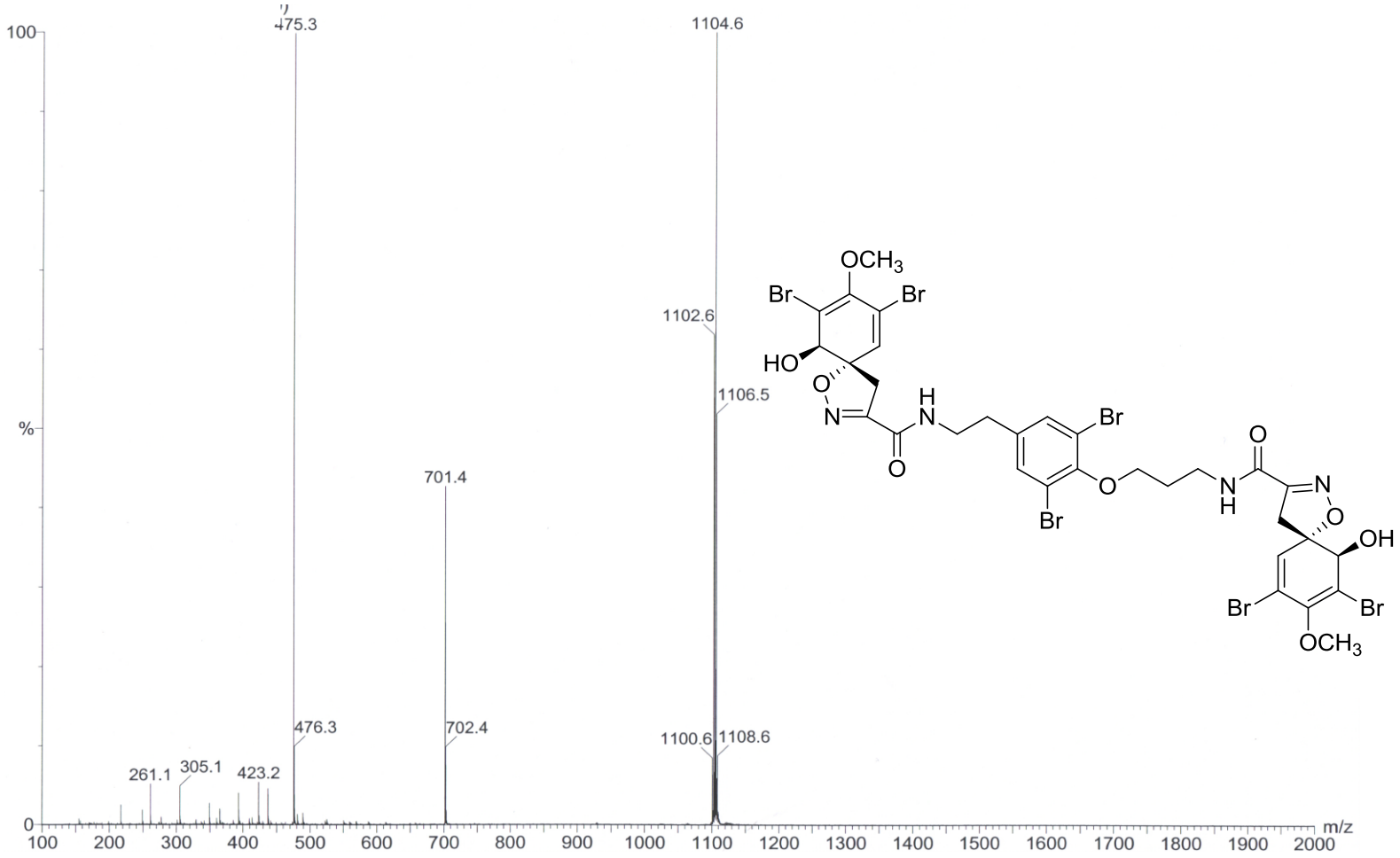

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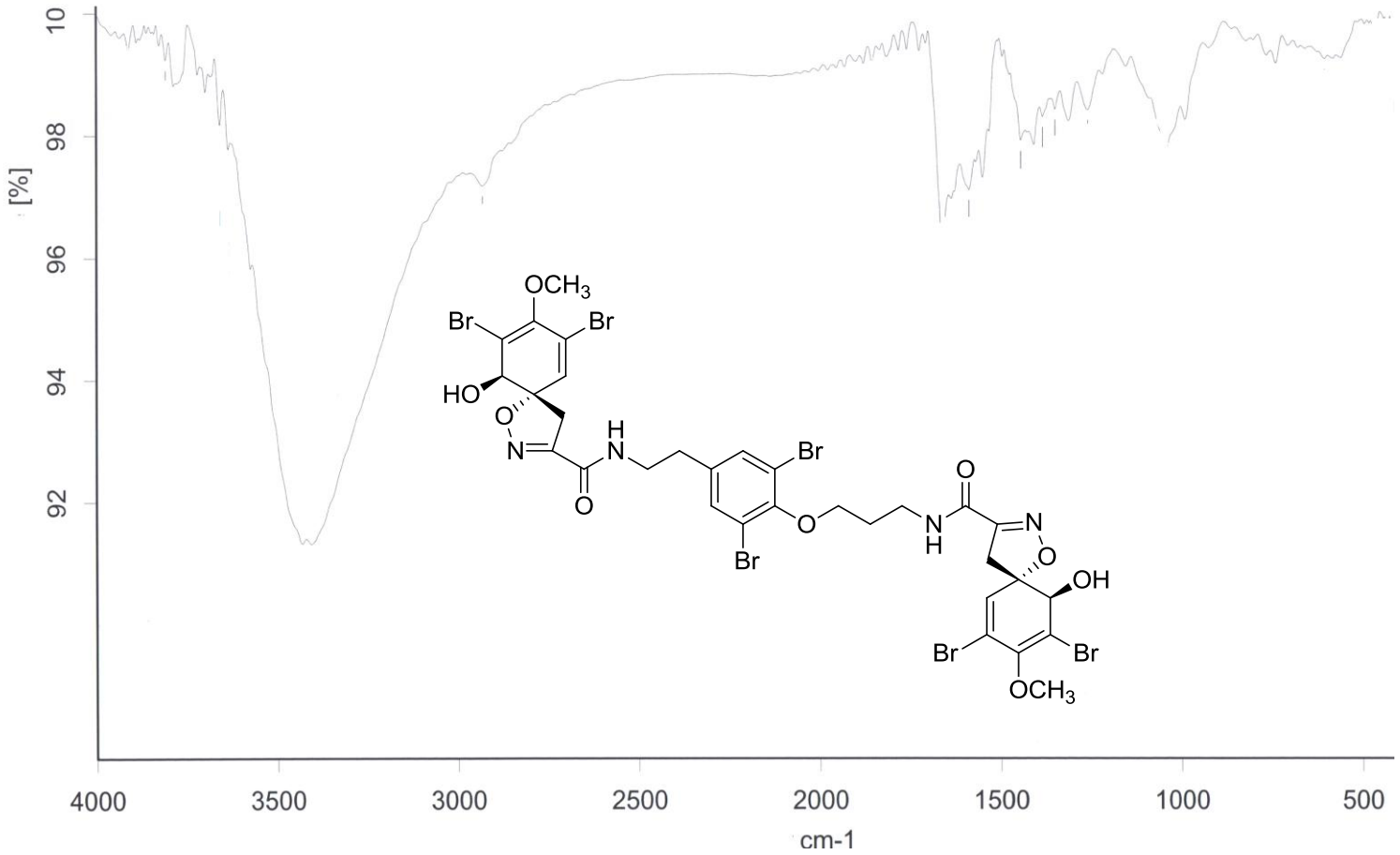
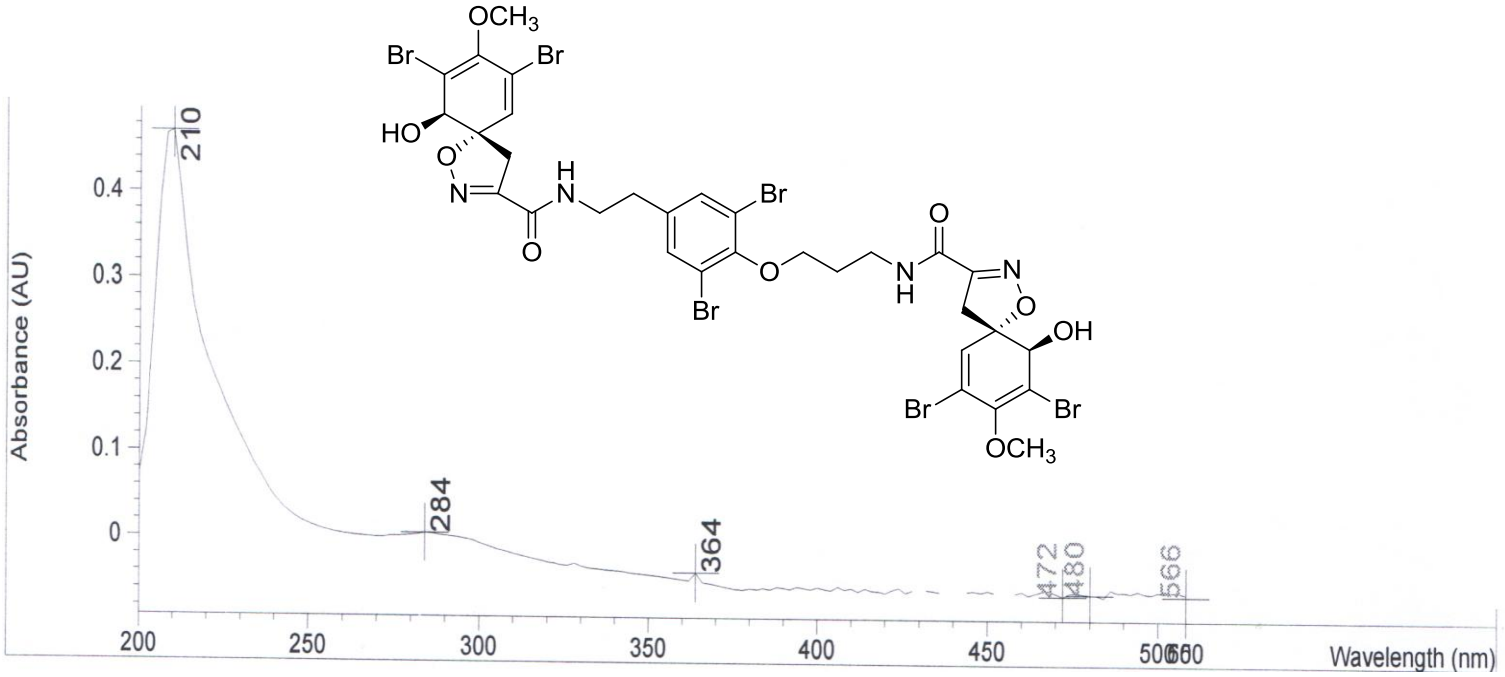
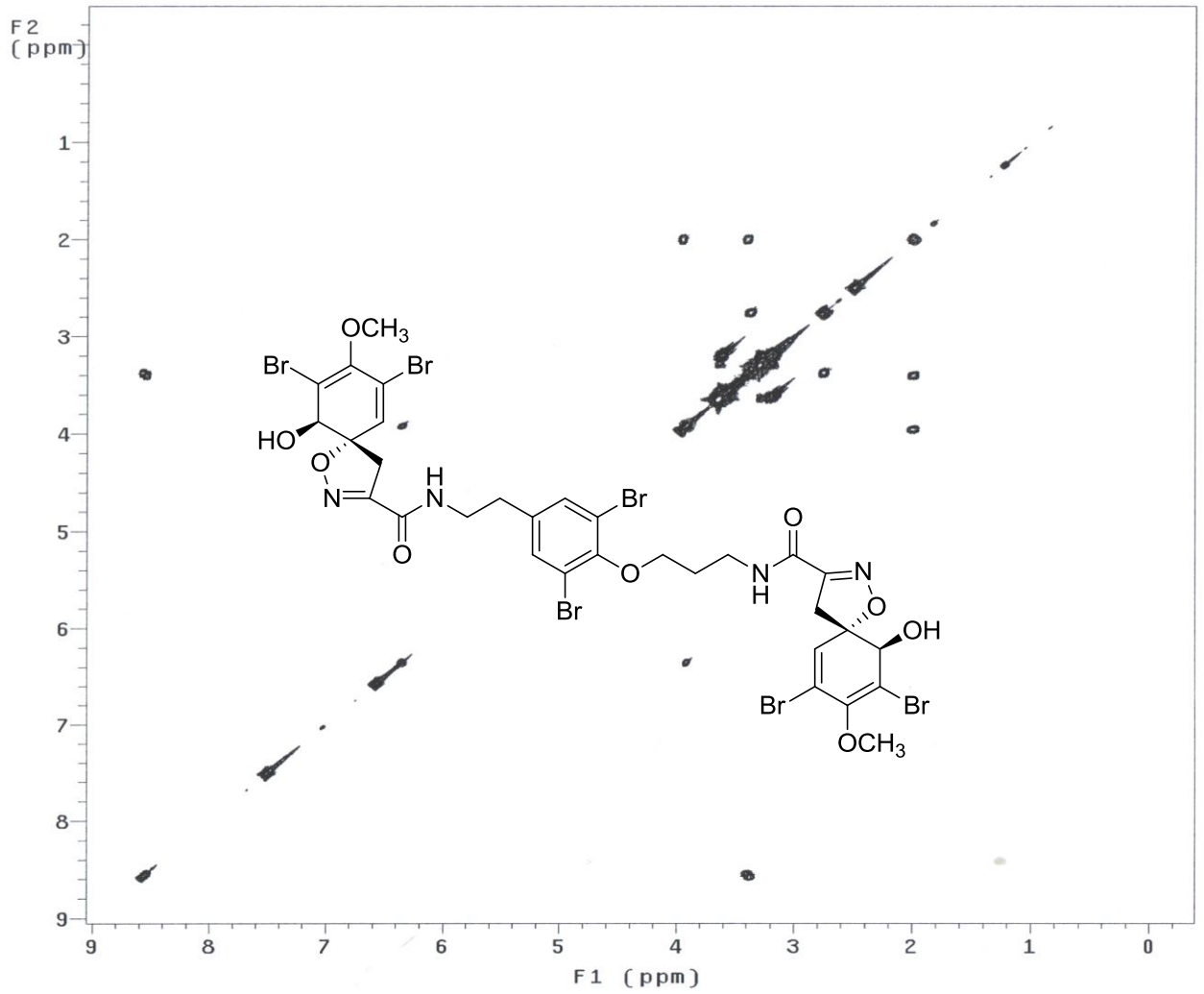


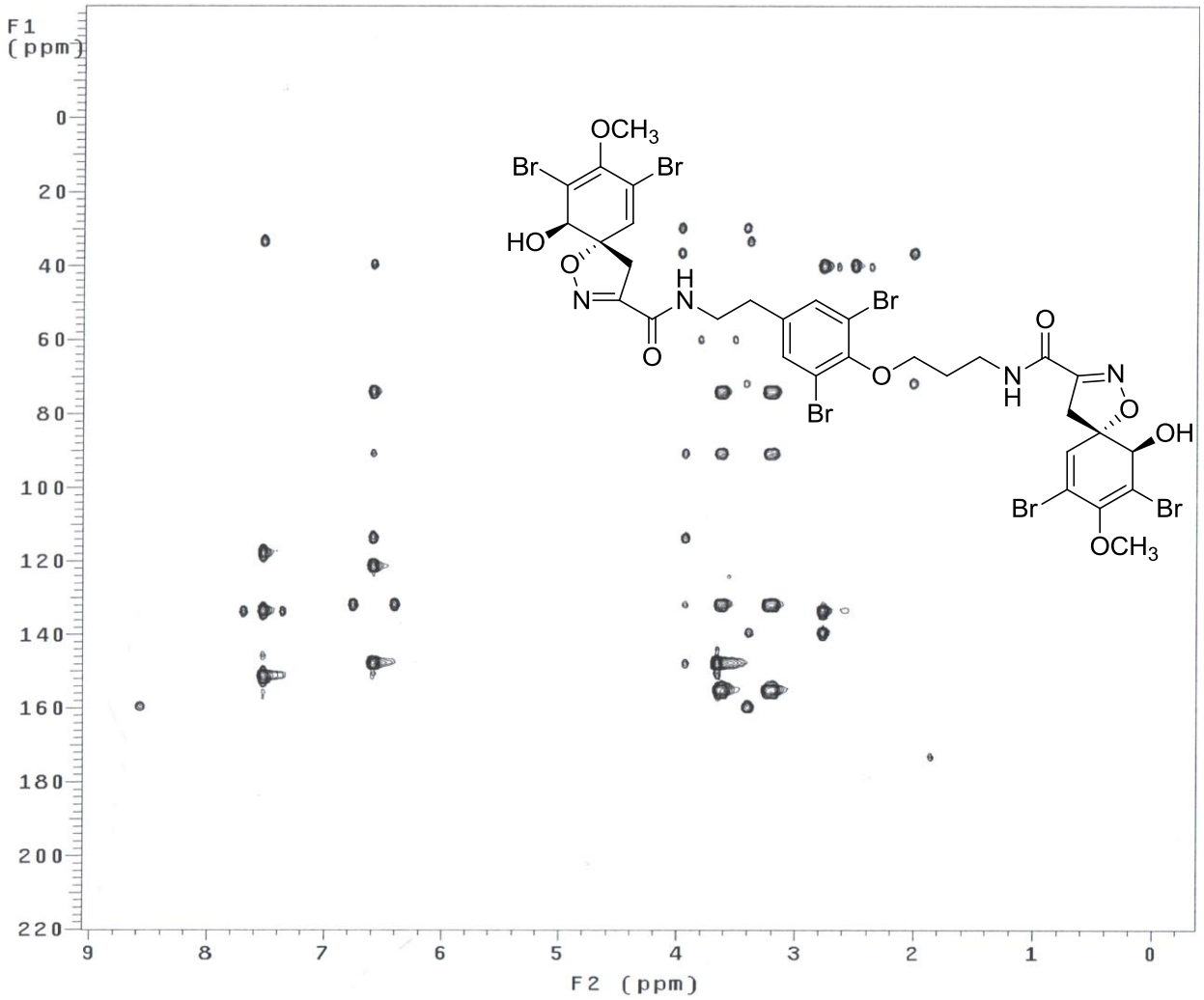
IR spectrum of **3** (KBr)



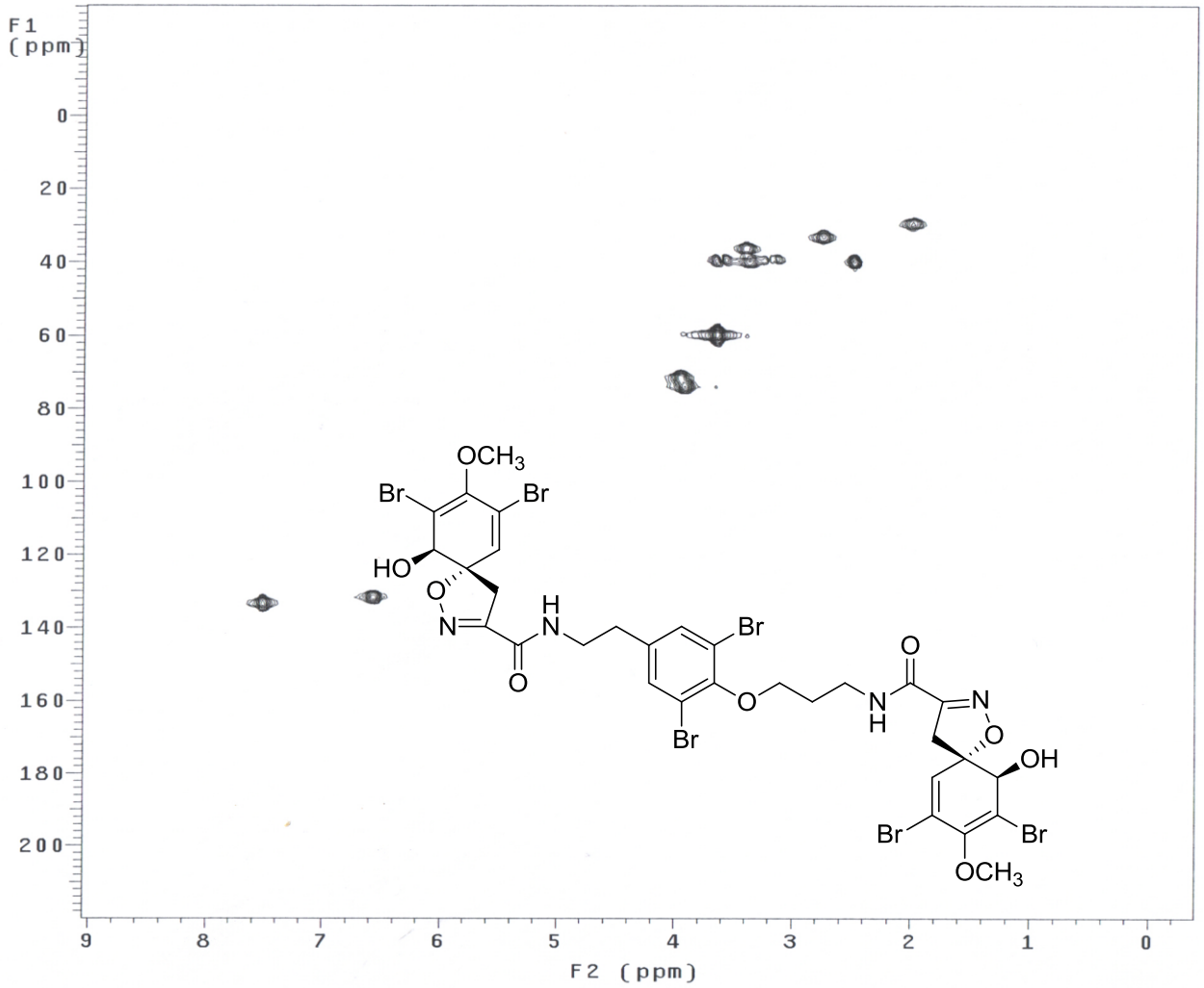
UV spectrum of **3** (CH_3OH)



^{1}H-^{1}H COSY spectrum of **3** (500 MHz, DMSO-d_6)



HMBC spectrum of **3** (500 MHz, DMSO-d_6)



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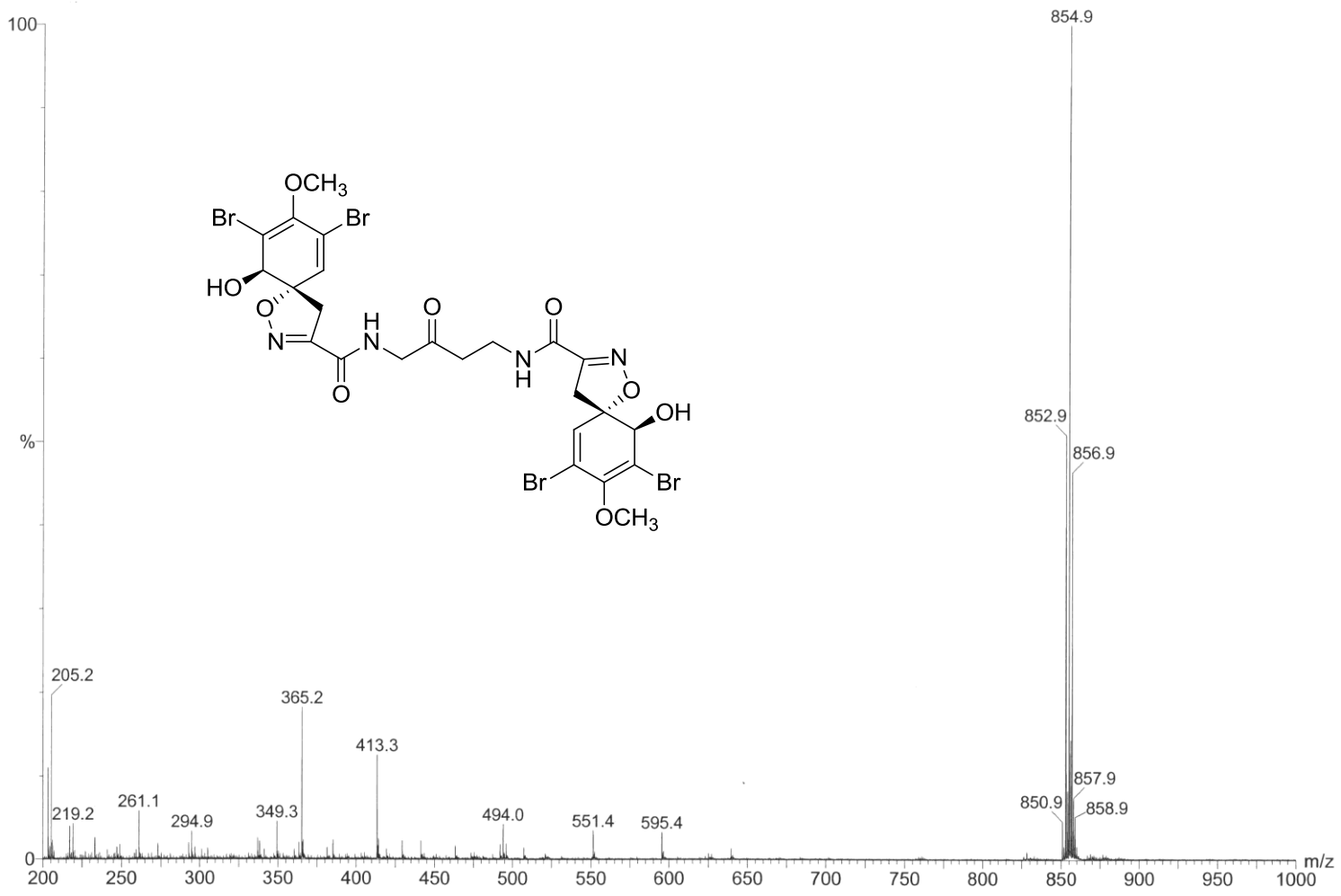
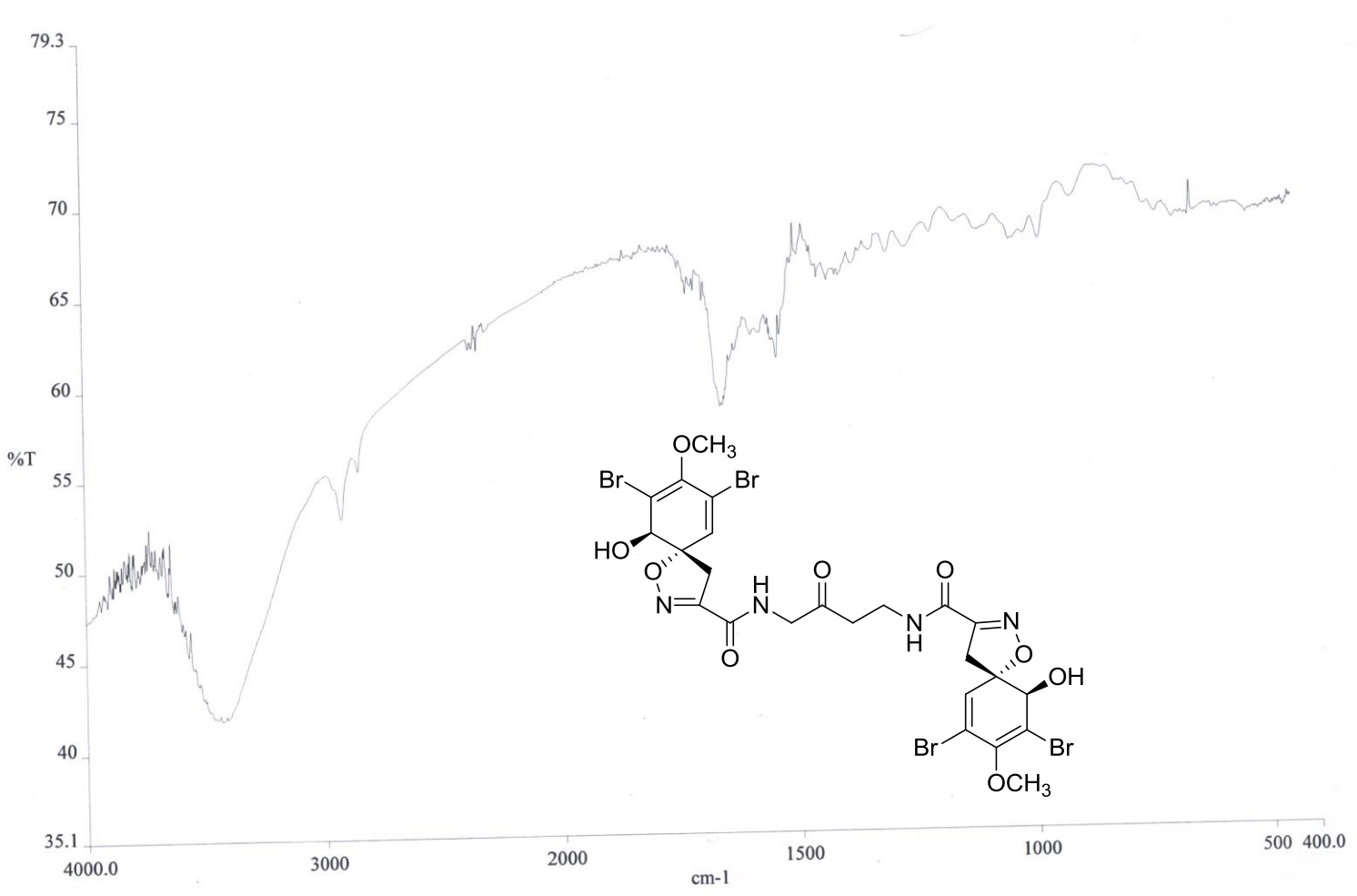
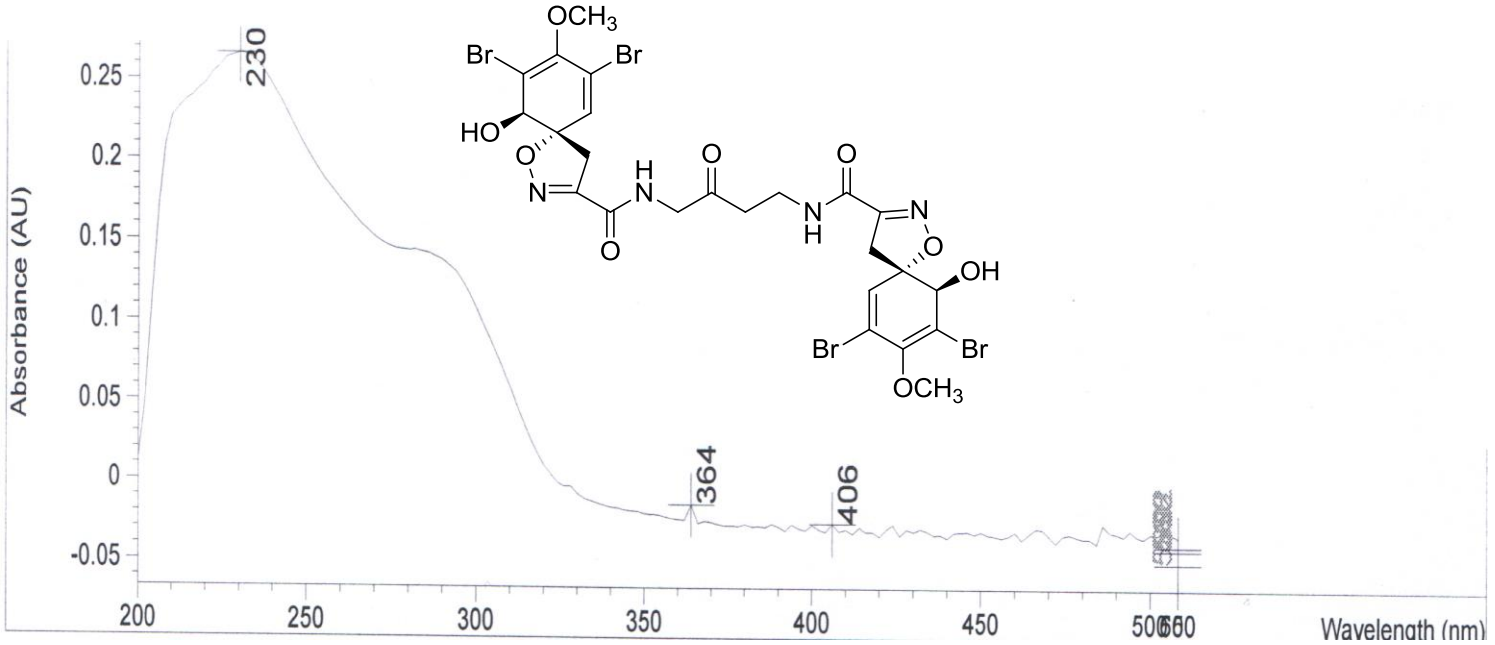


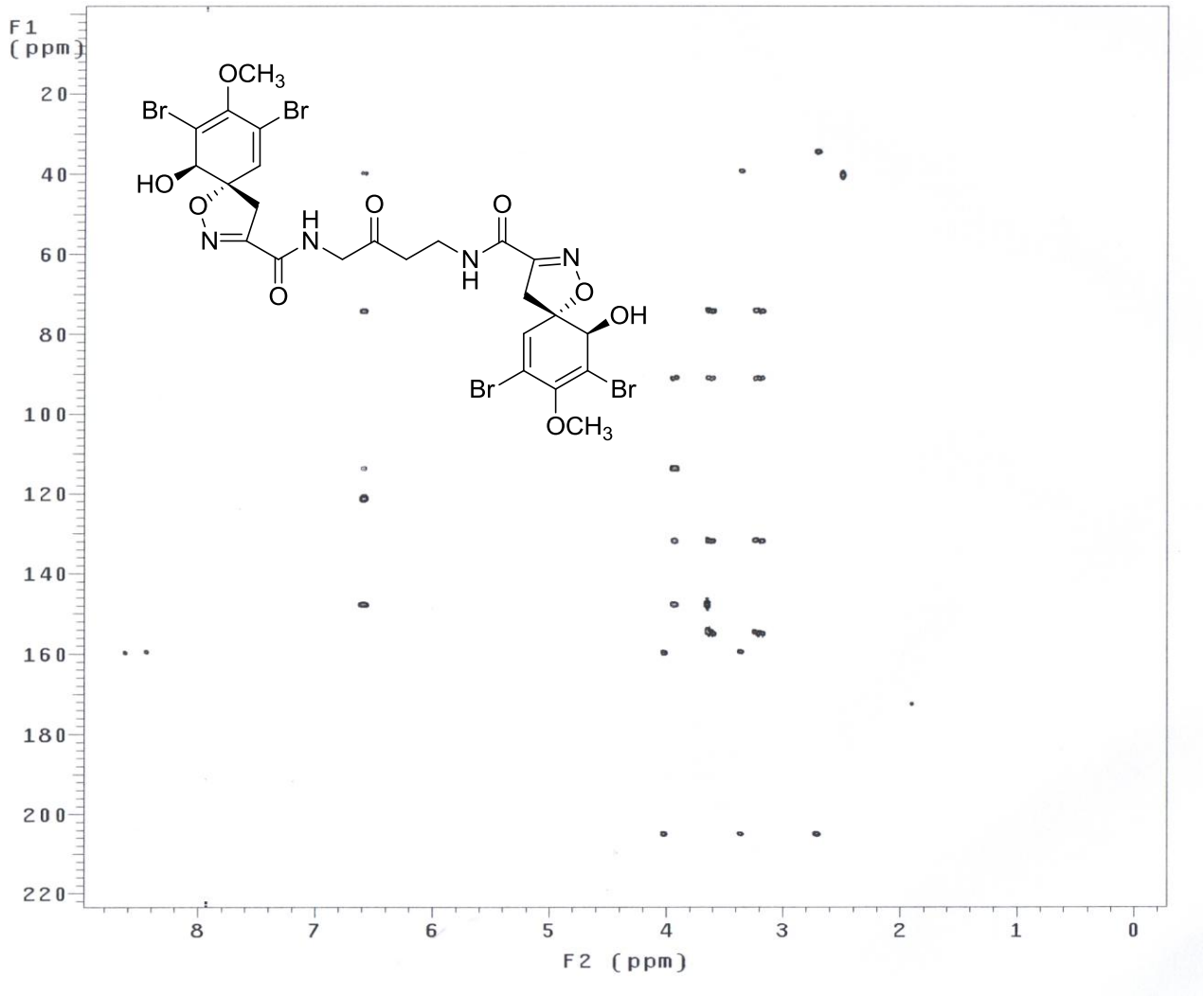
ESI mass spectrum of **4**



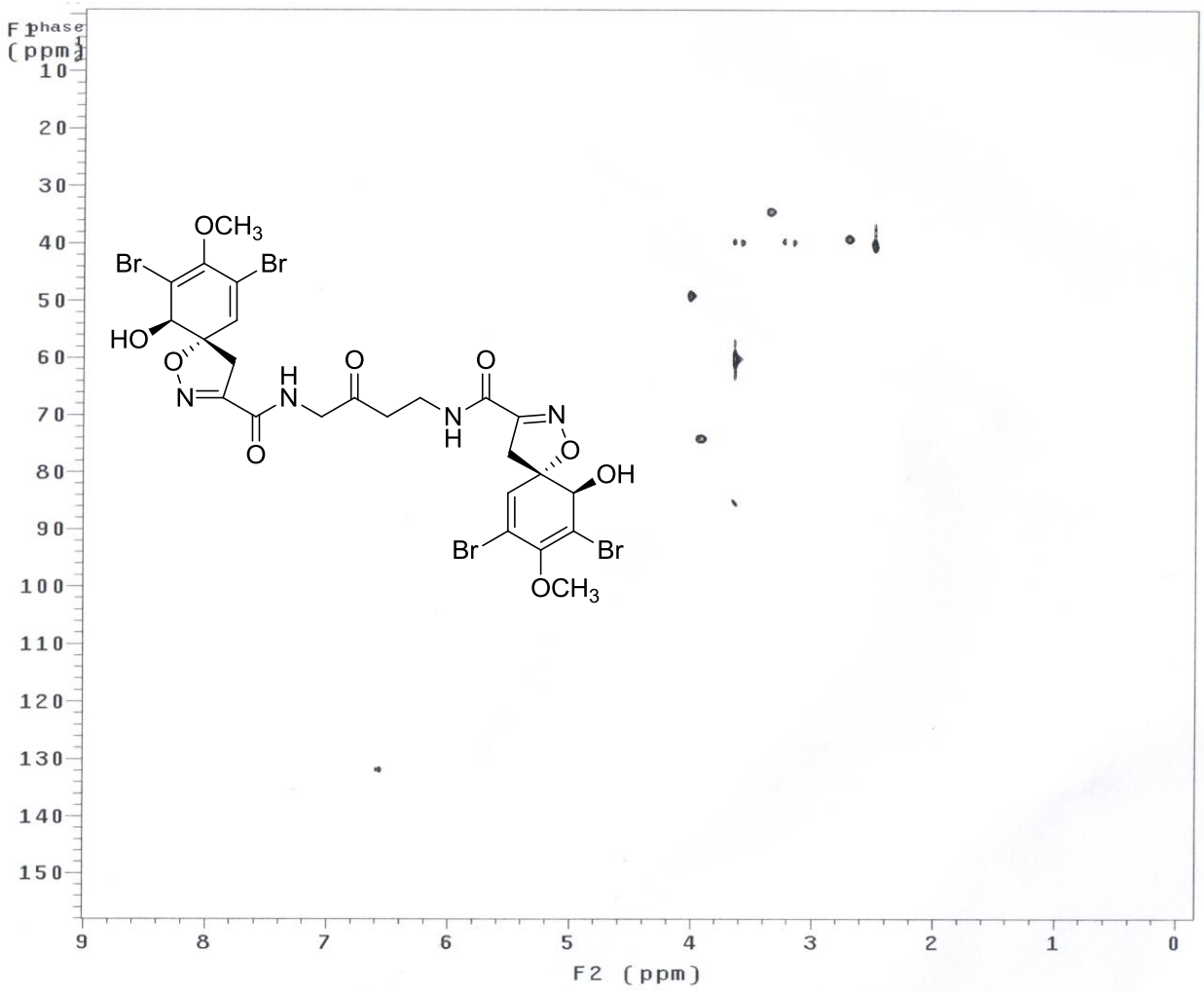
IR spectrum of **4** (KBr)



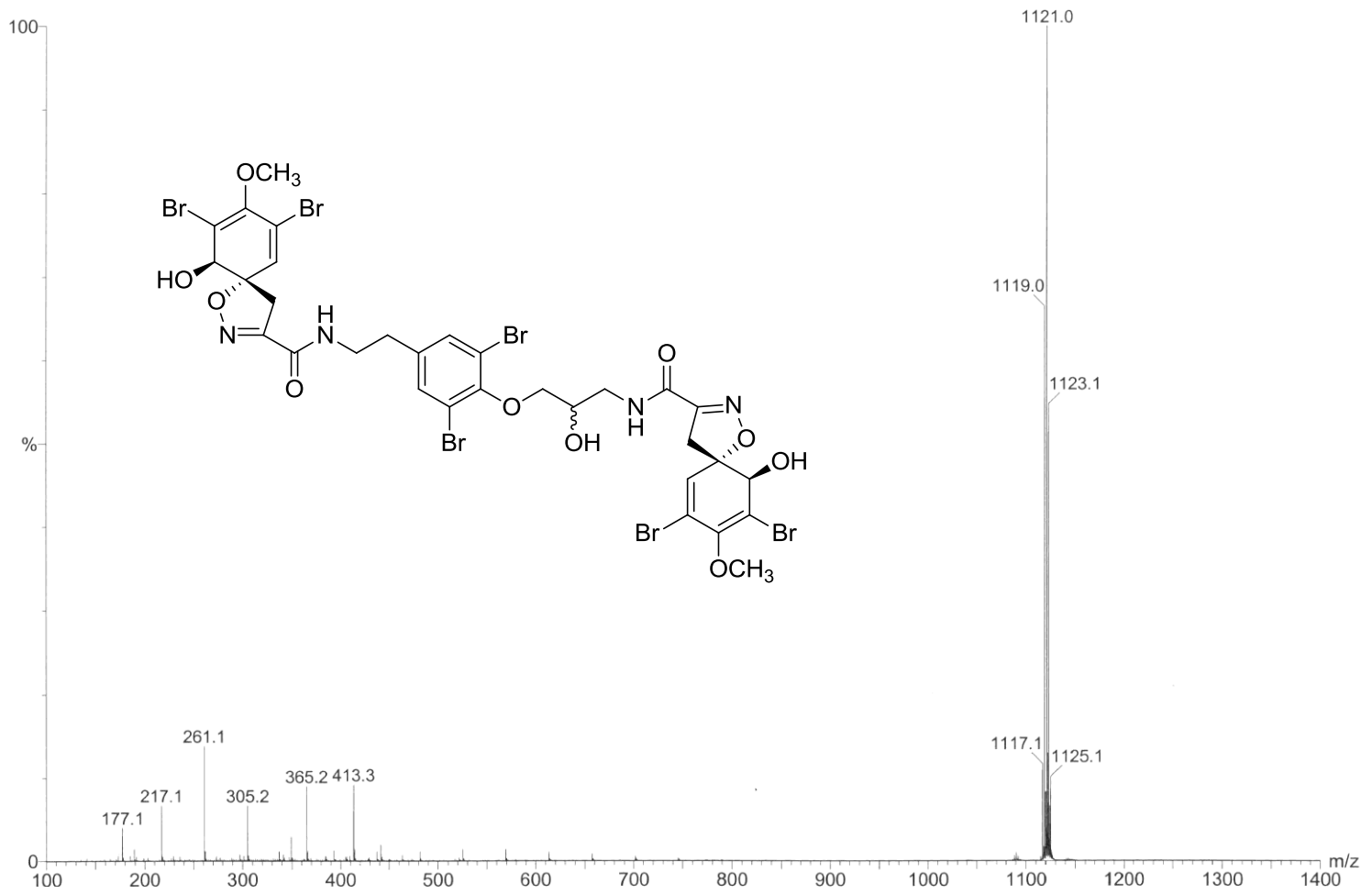
UV spectrum of **4** (CH_3OH)



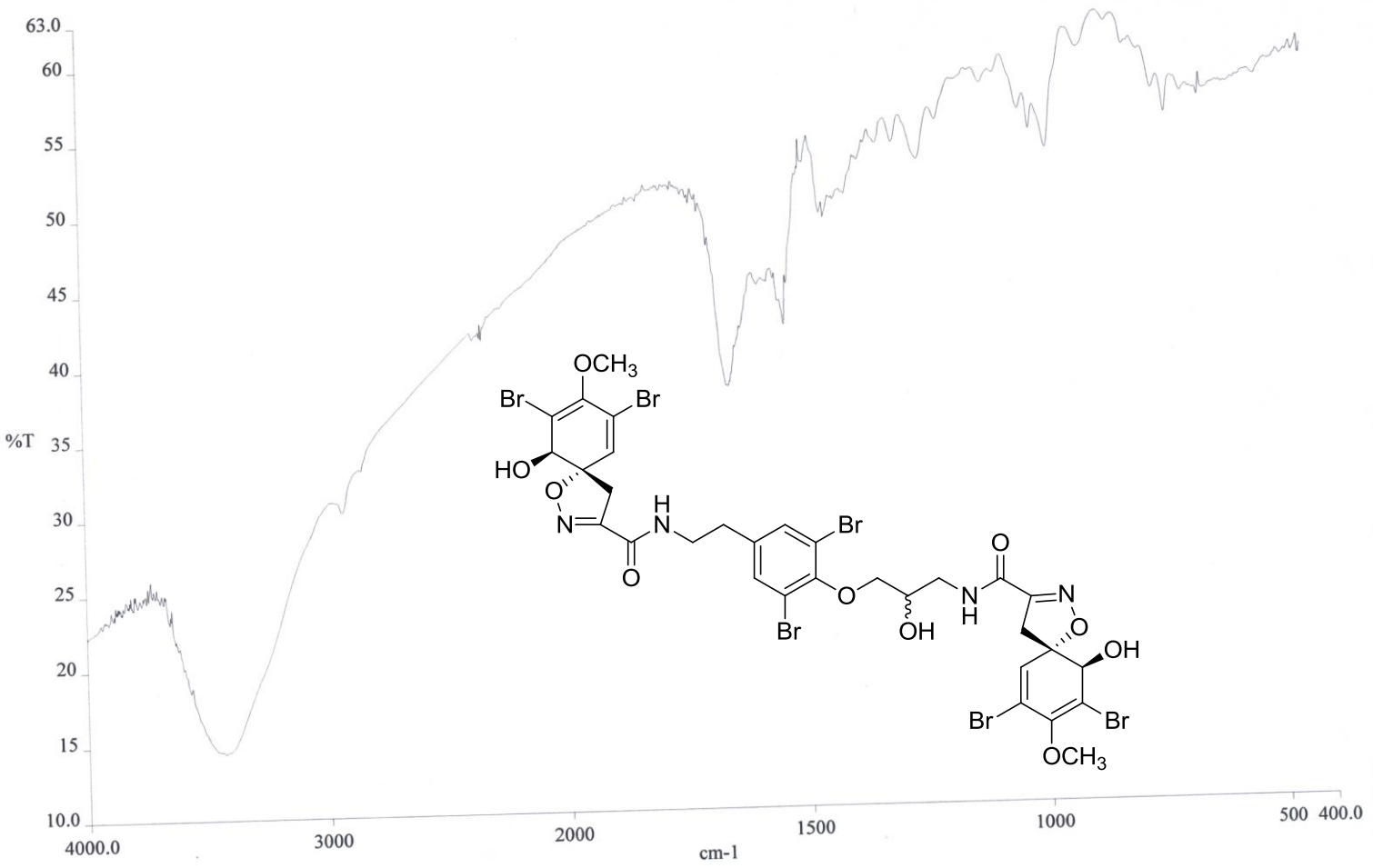
HMBC spectrum of **4** (500 MHz, DMSO-d_6)



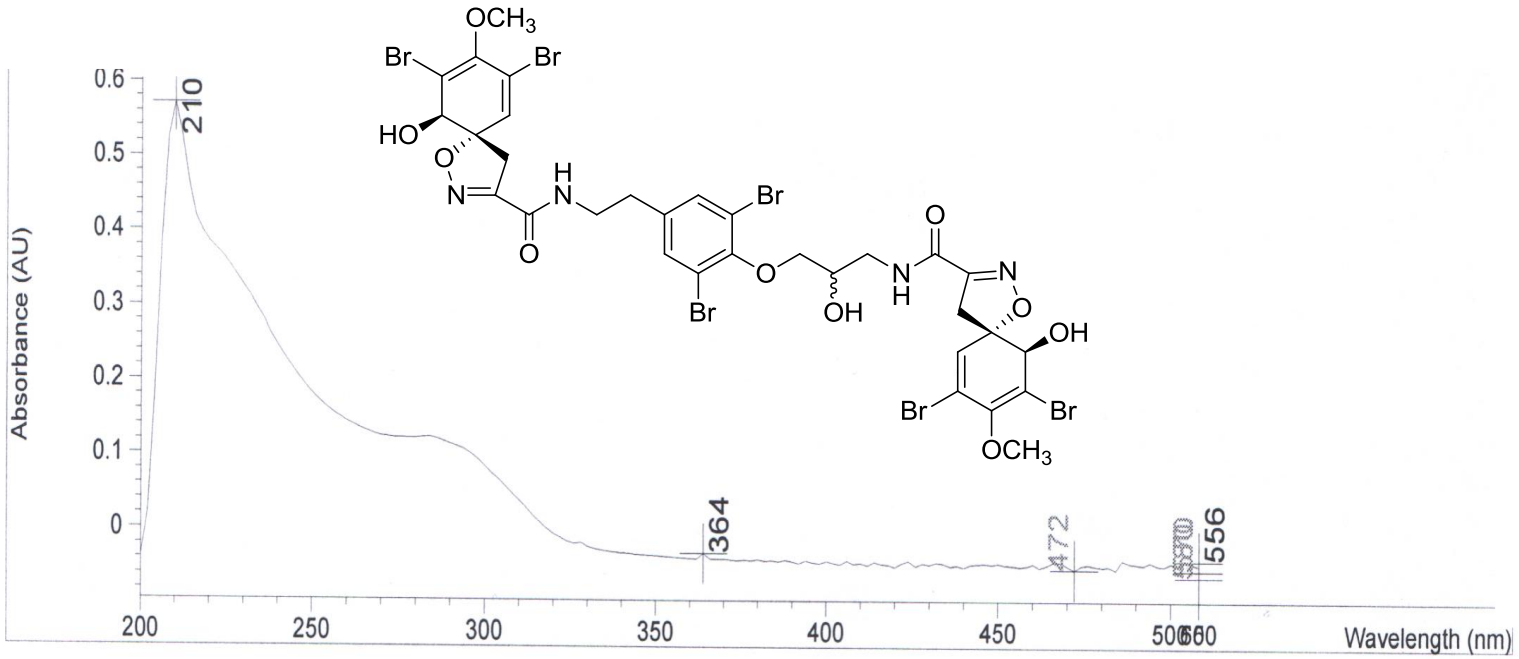
HMQC spectrum of **4** (500 MHz, DMSO-d_6)



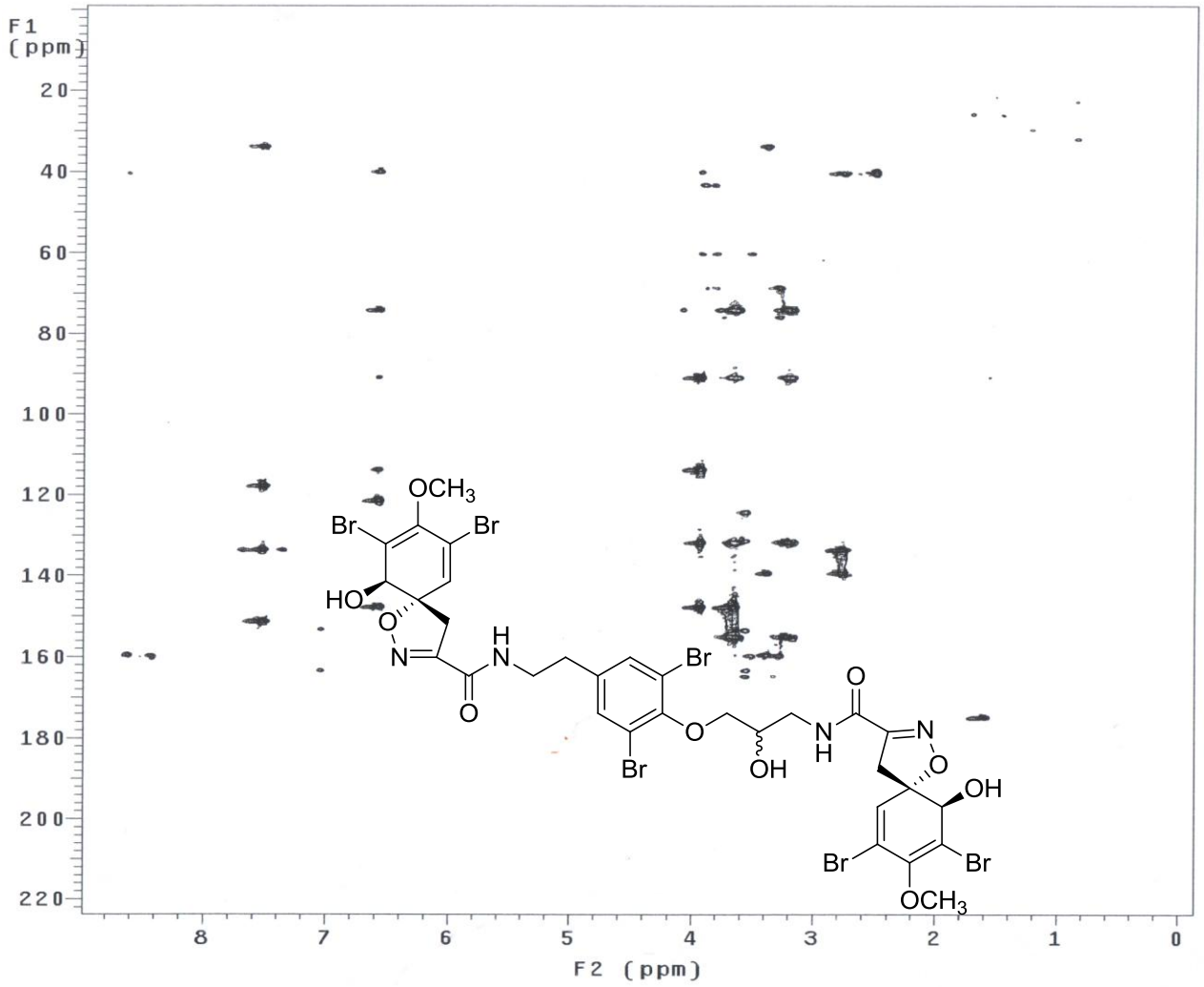
ESI mass spectrum of **5**



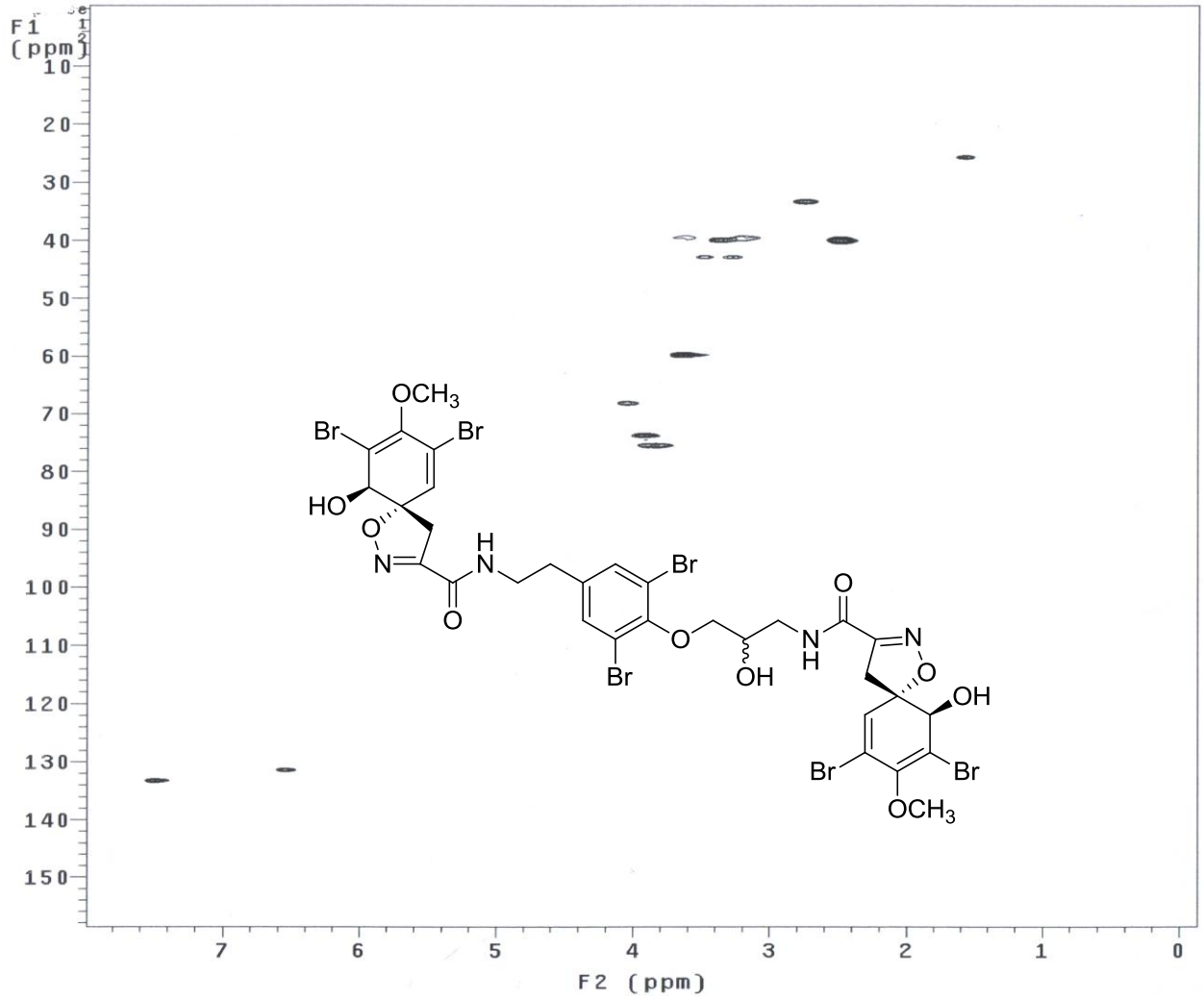
IR spectrum of **5** (KBr)



UV spectrum of **5** (CH_3OH)



HMBC spectrum of **5** (500 MHz, DMSO-d_6)



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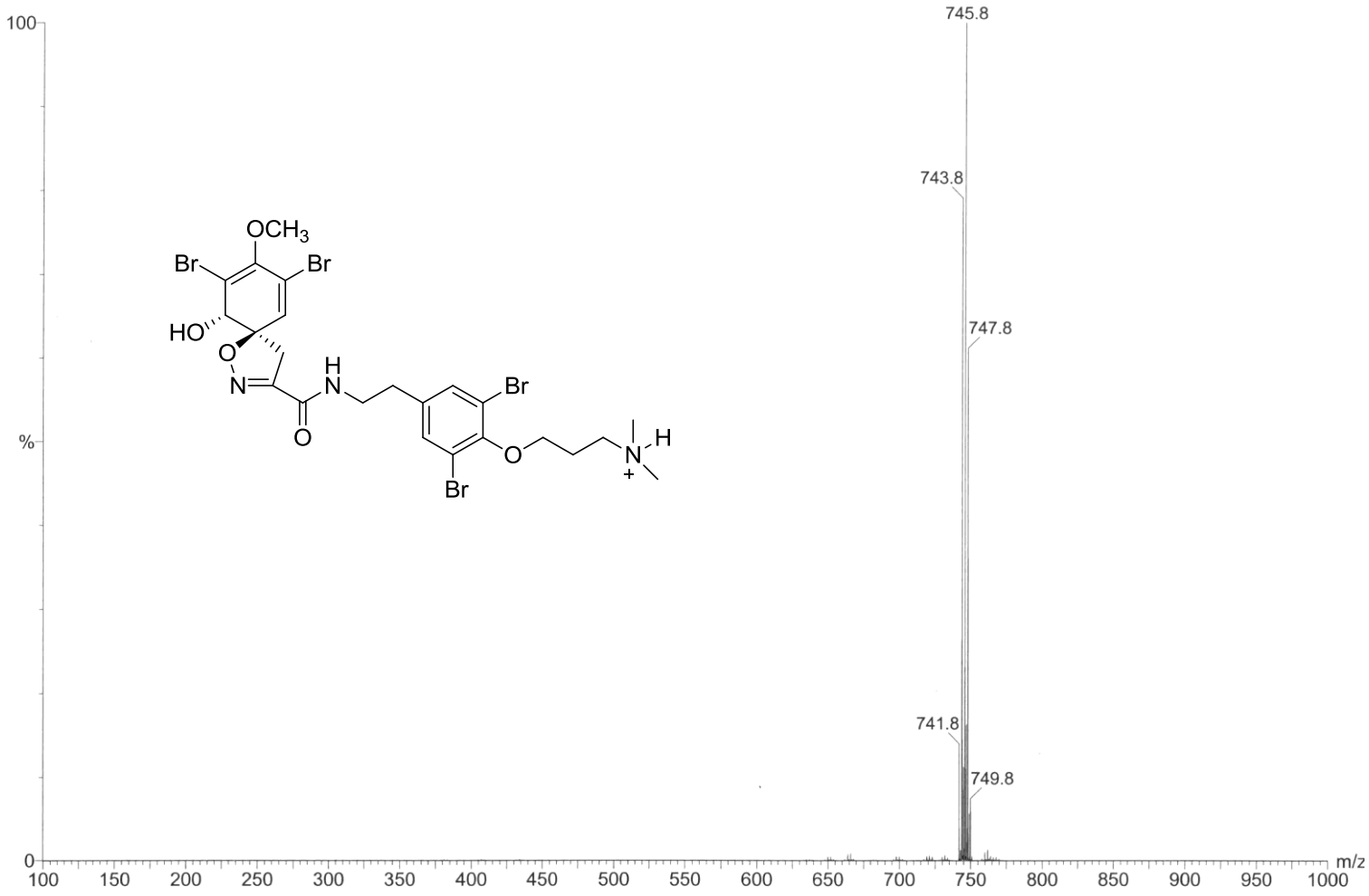

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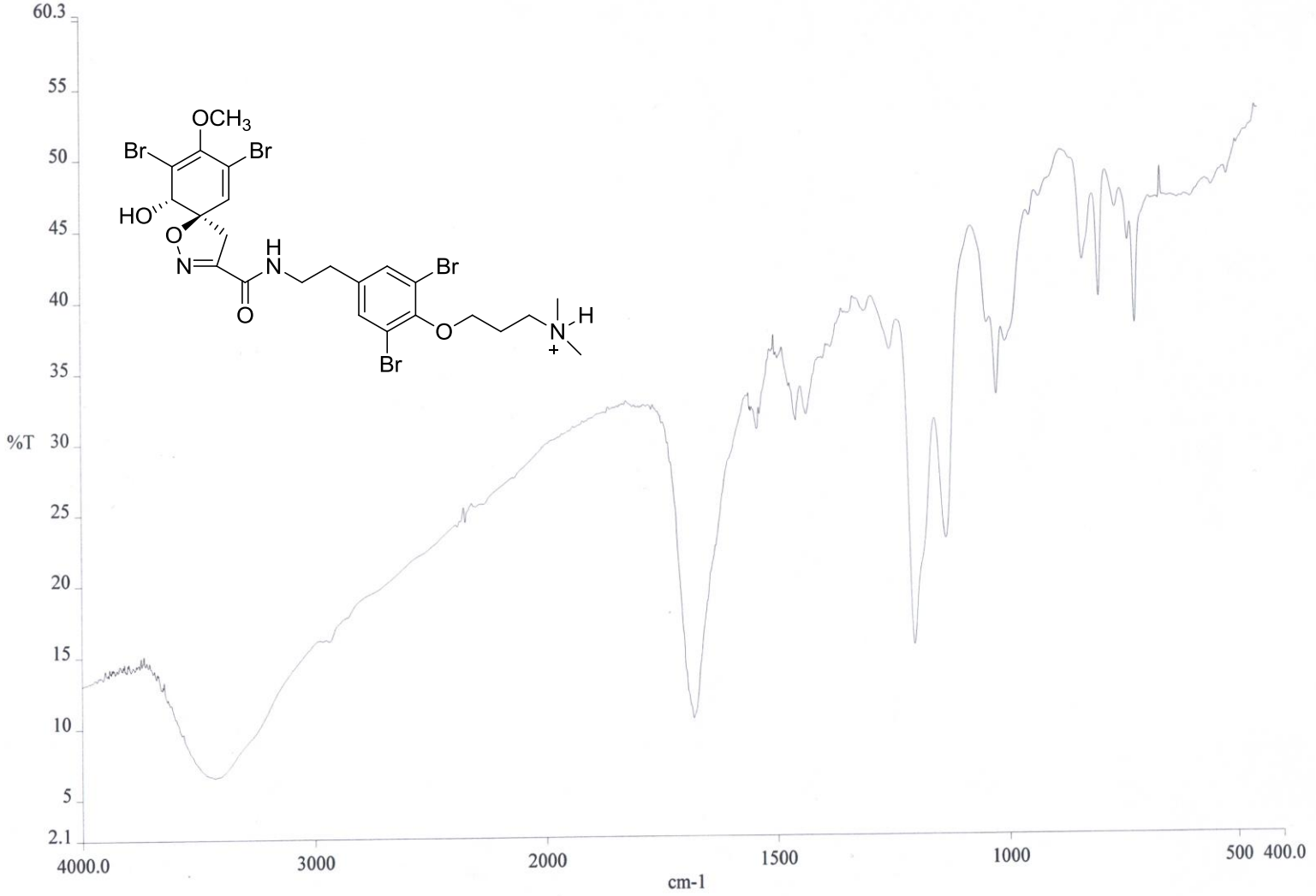
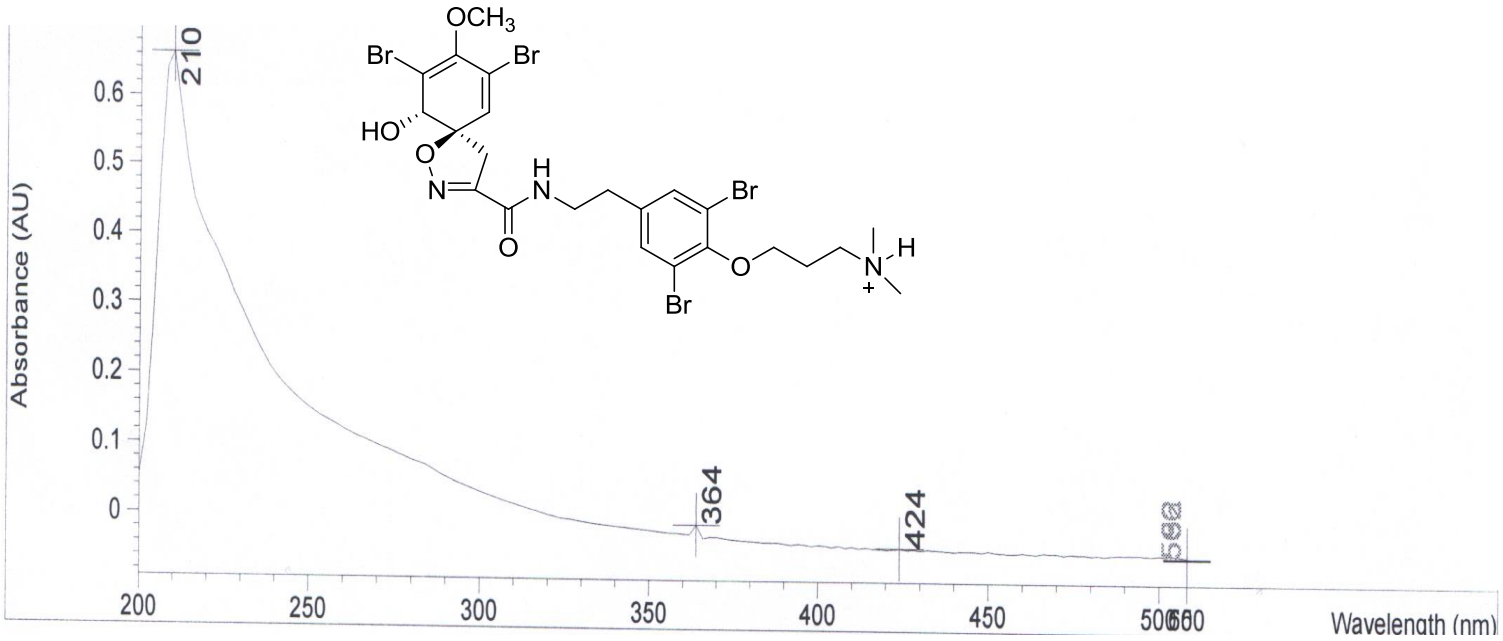
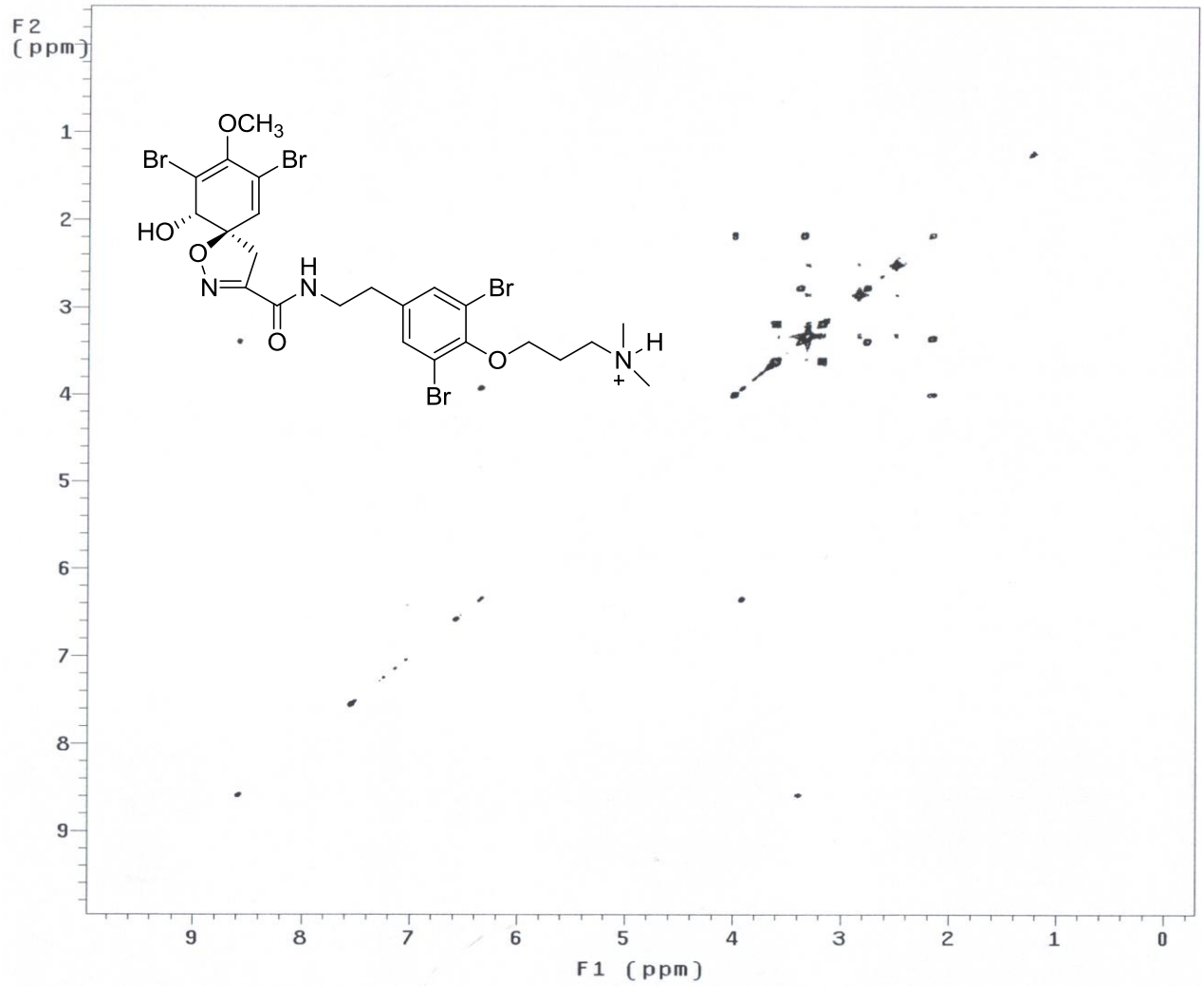


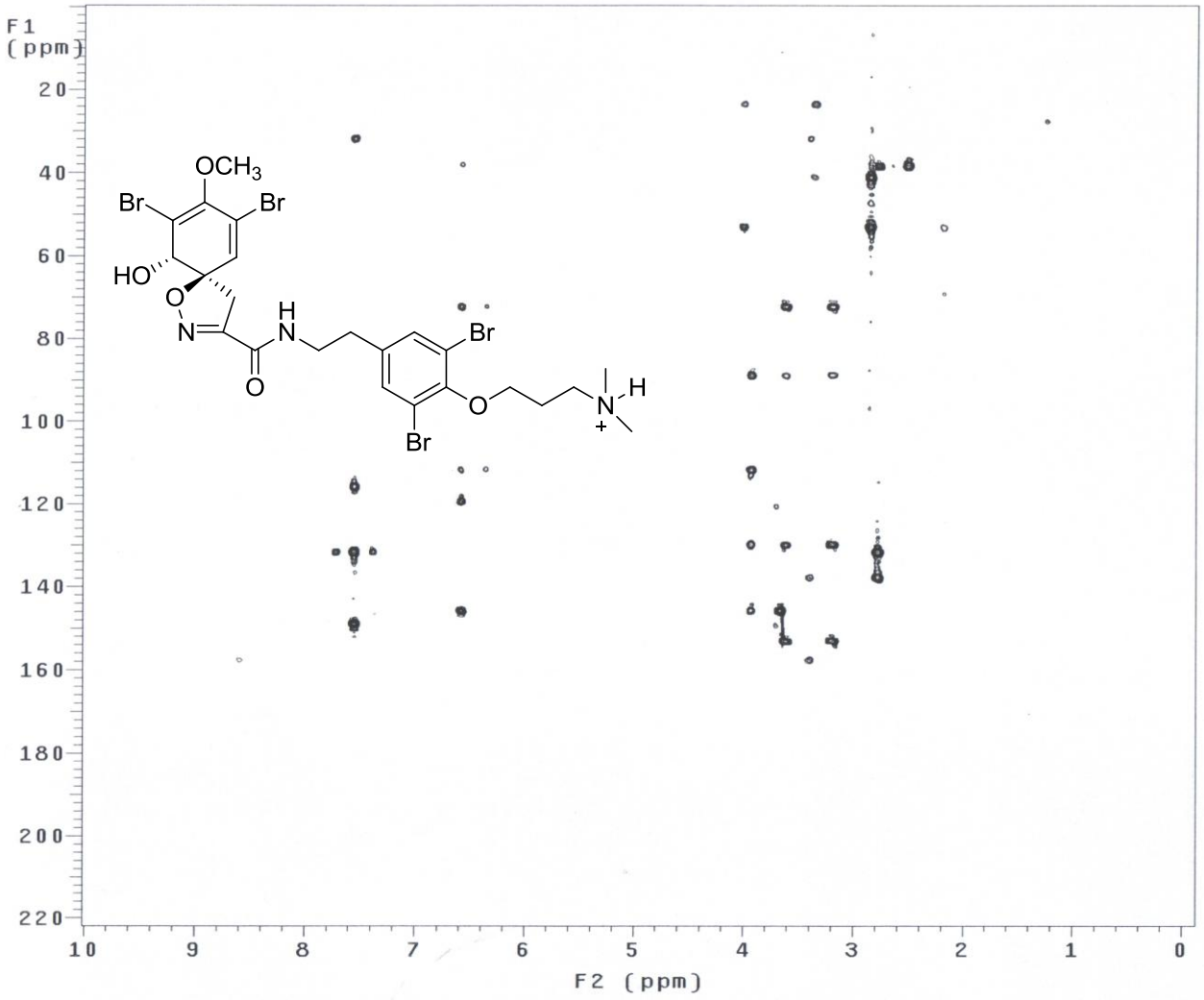
IR spectrum of **6**



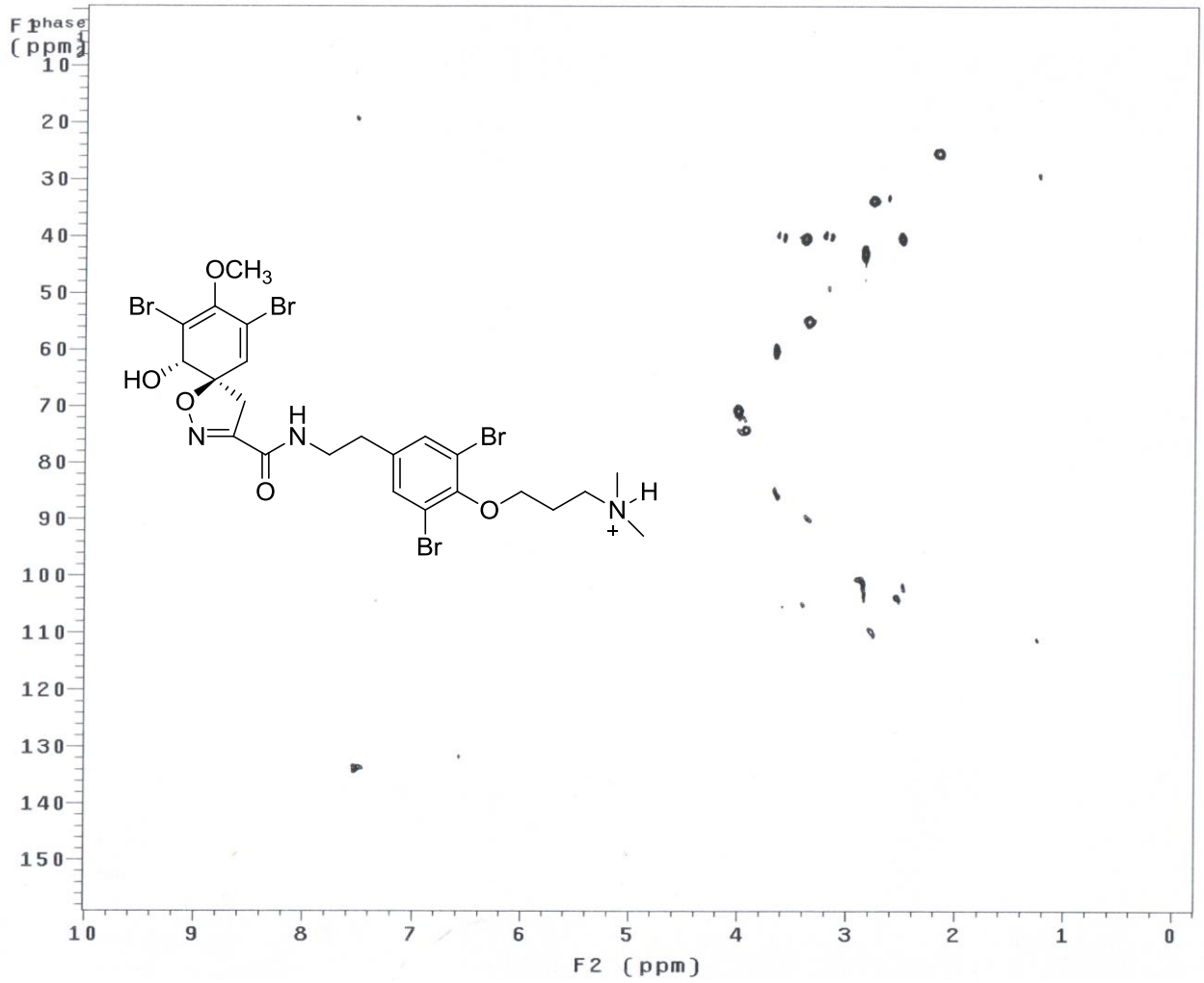
UV spectrum of **6** (CH_3OH)



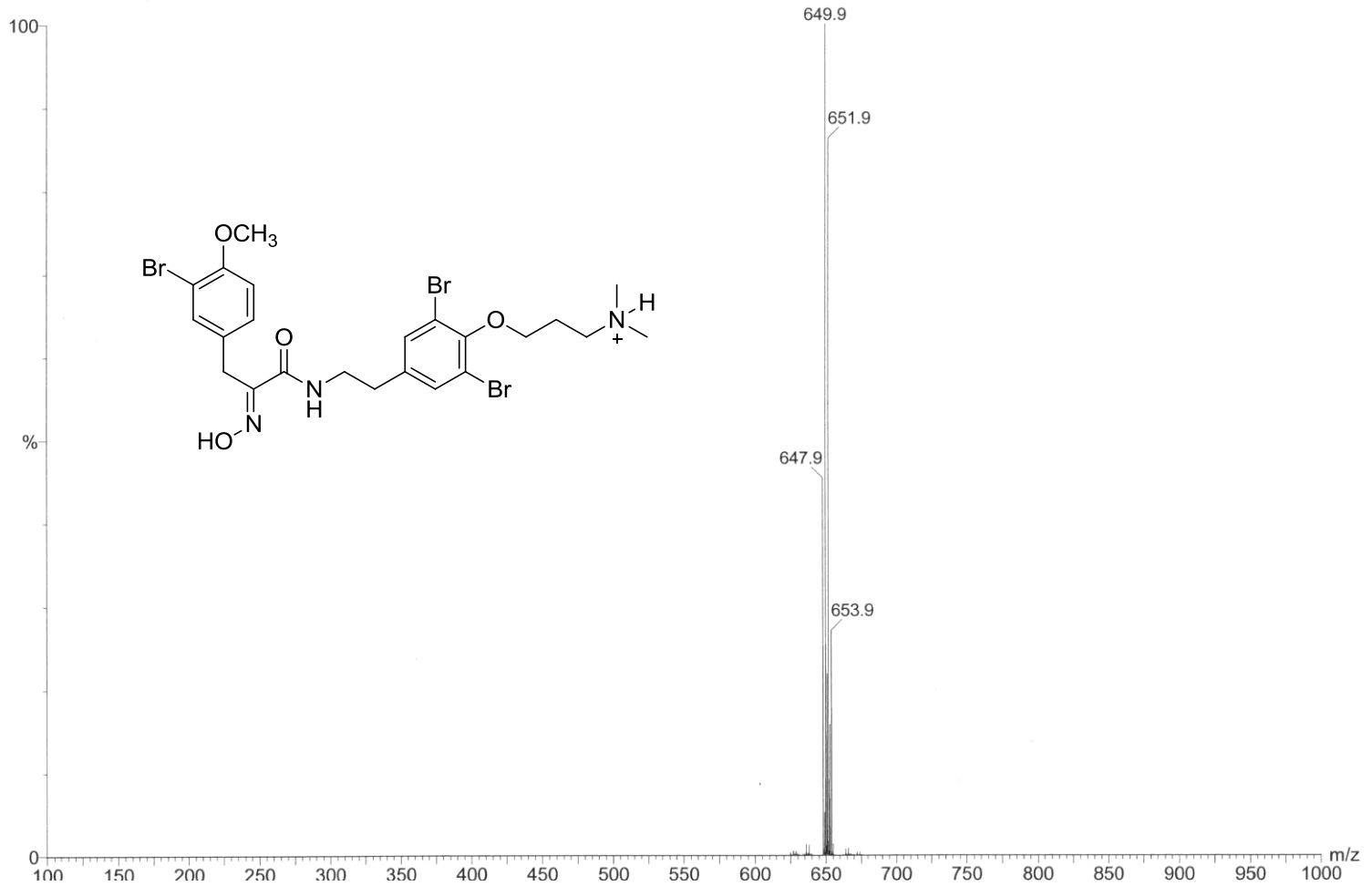
^{1}H-^{1}H COSY spectrum of **6** (500 MHz, DMSO-d_6)



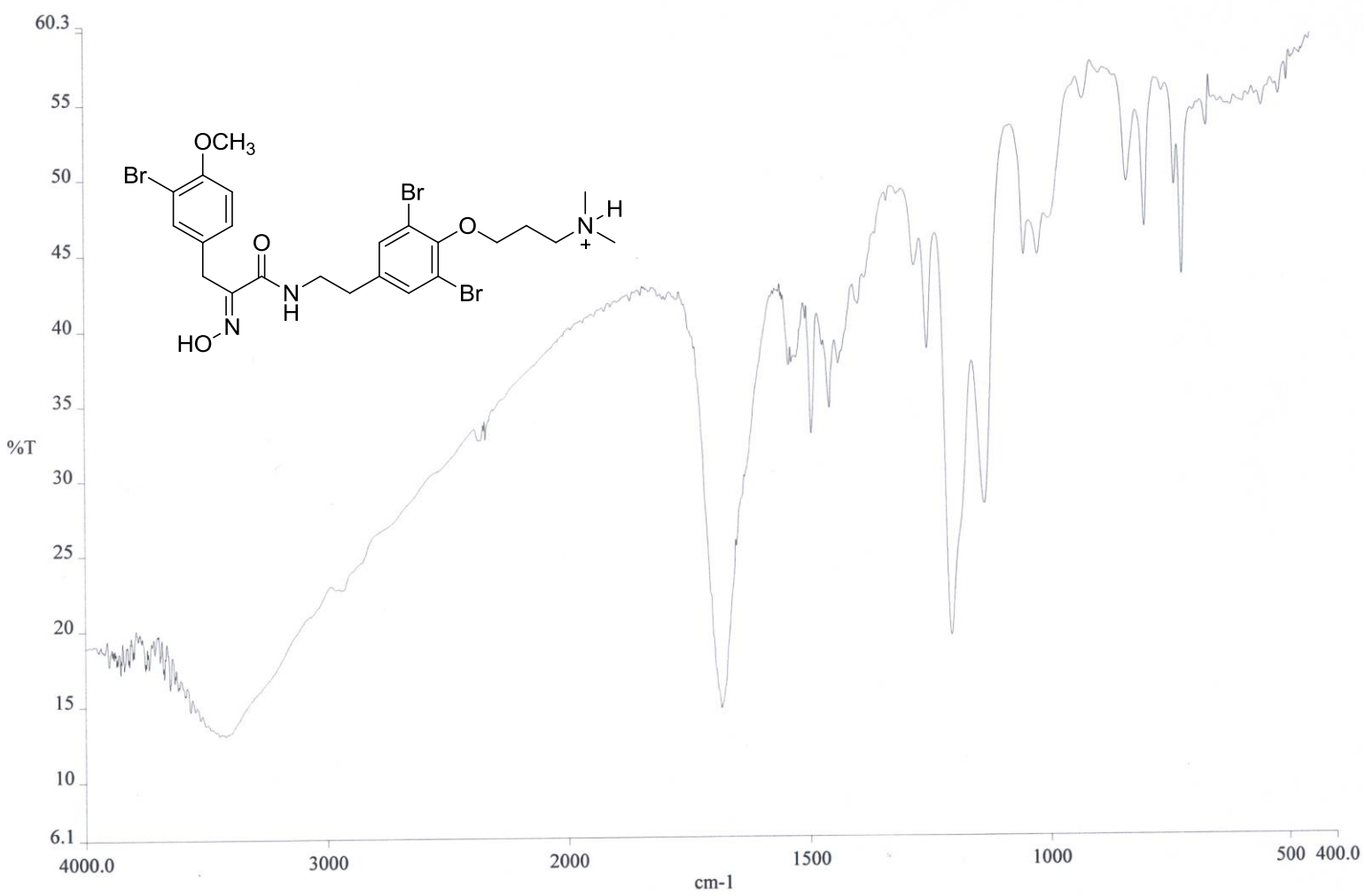
HMBC spectrum of **6** (500 MHz, DMSO-d_6)



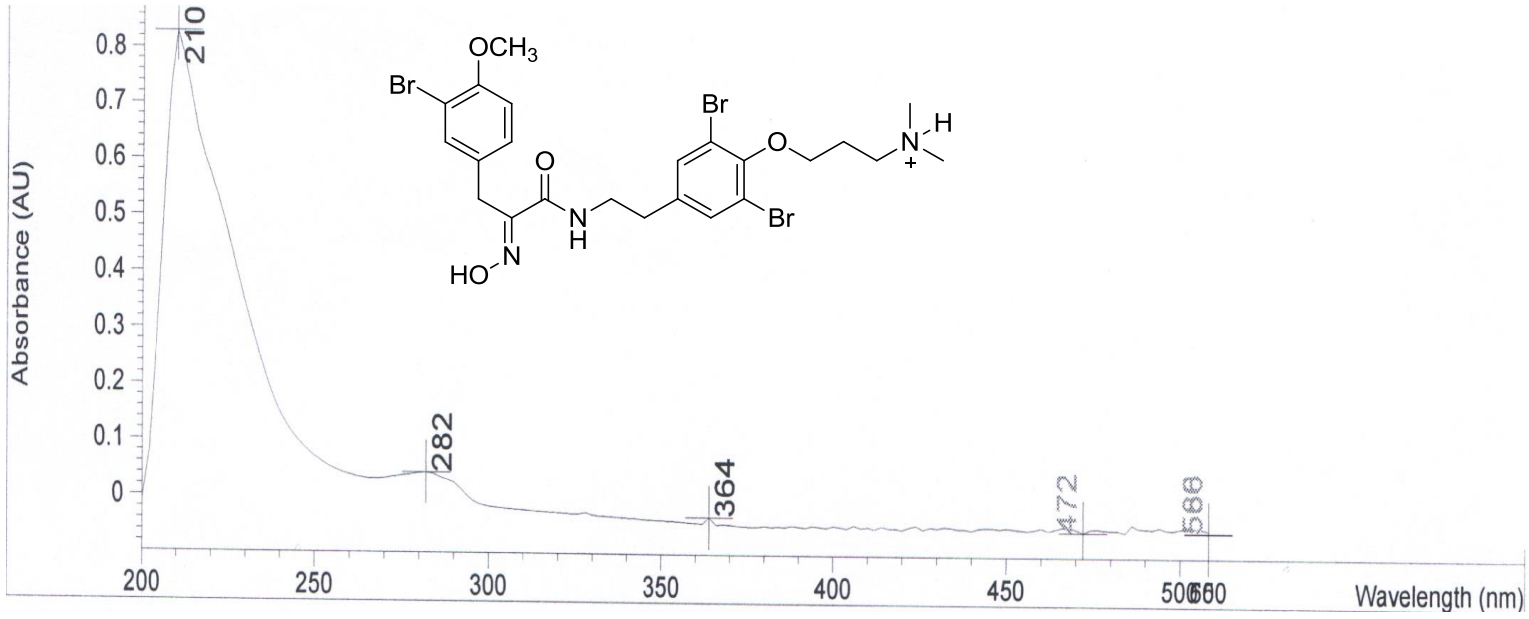
HMQC spectrum of **6** (500 MHz, DMSO-d_6)



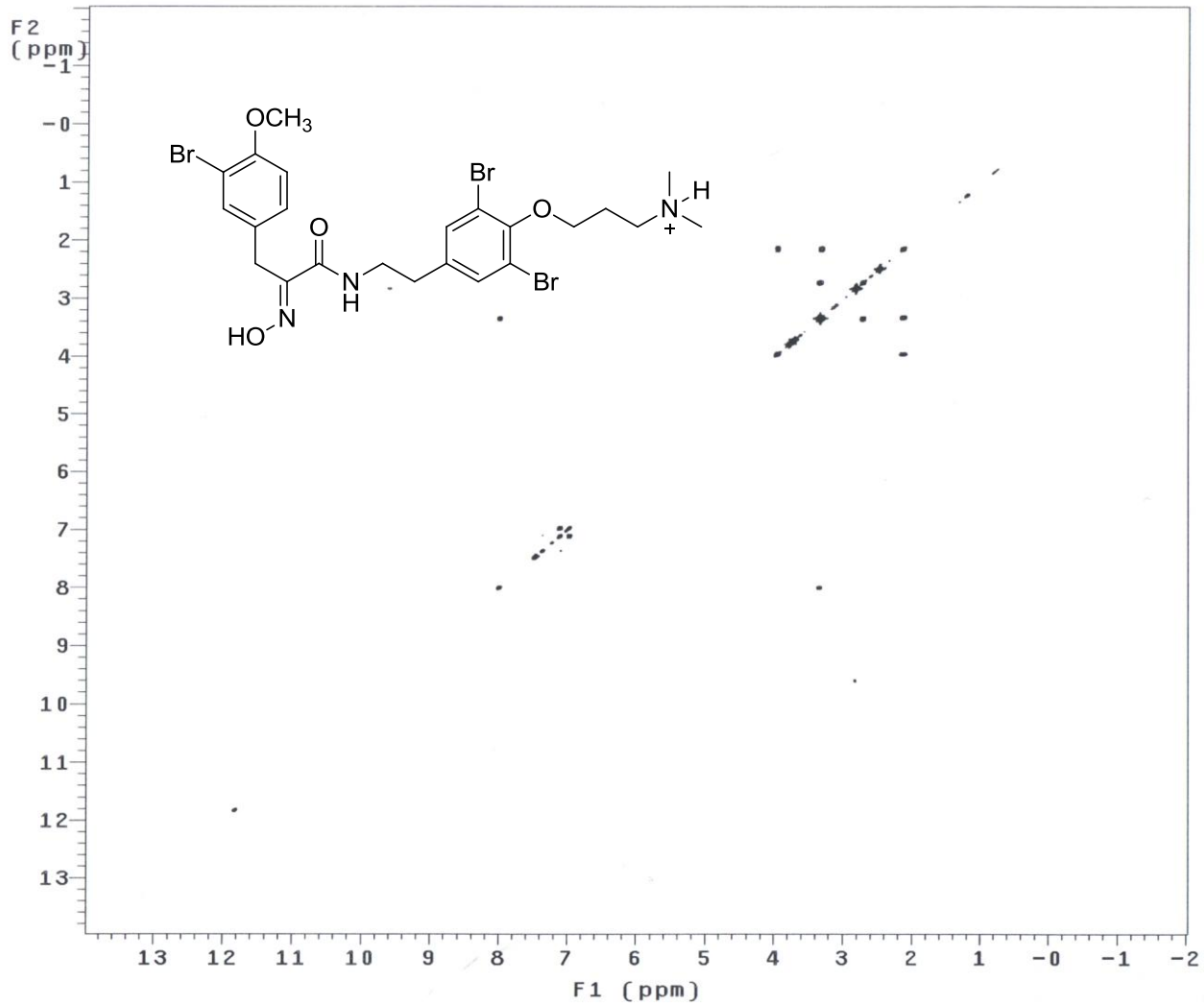
ESI mass spectrum of **7**



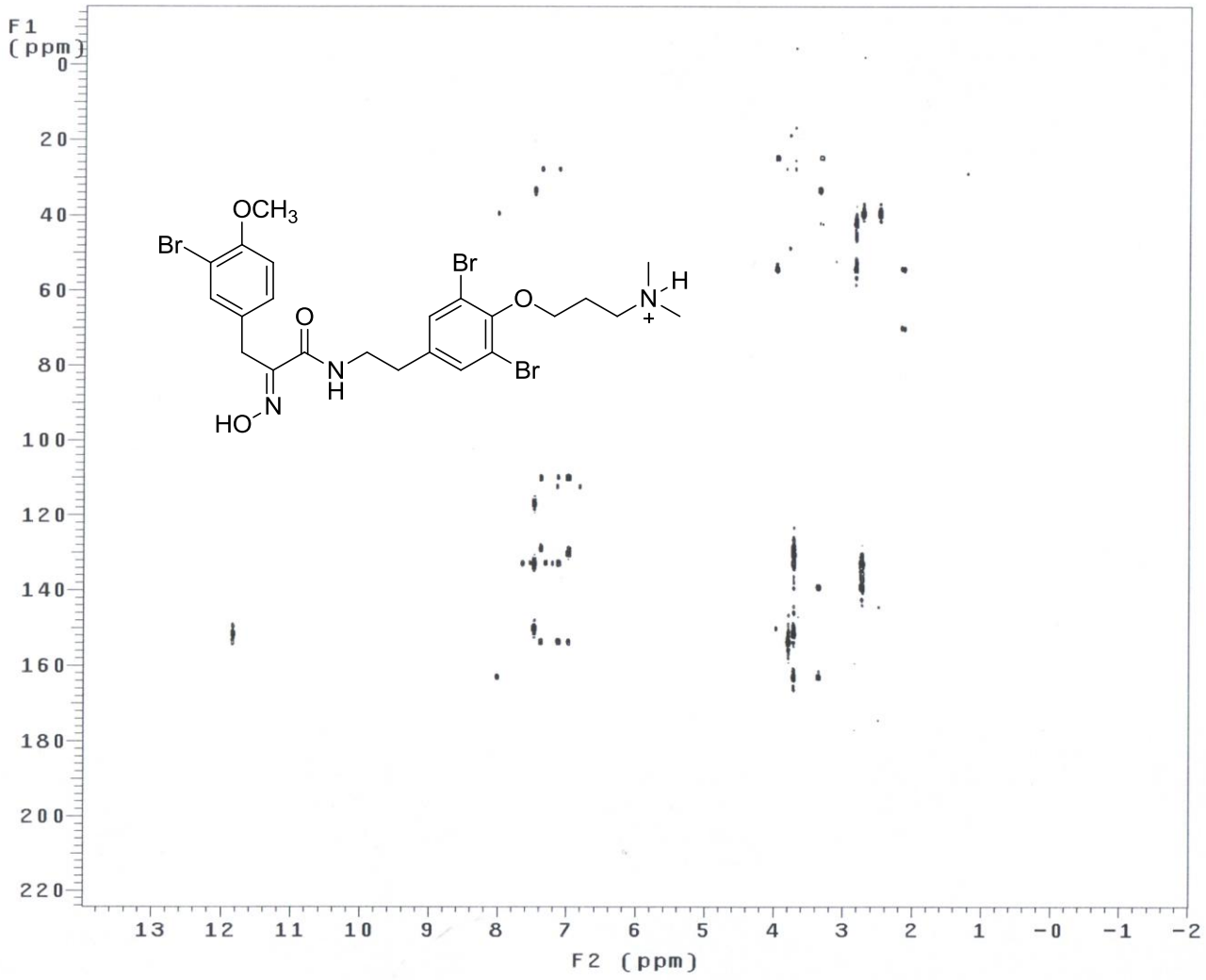
IR spectrum of **7** (KBr)



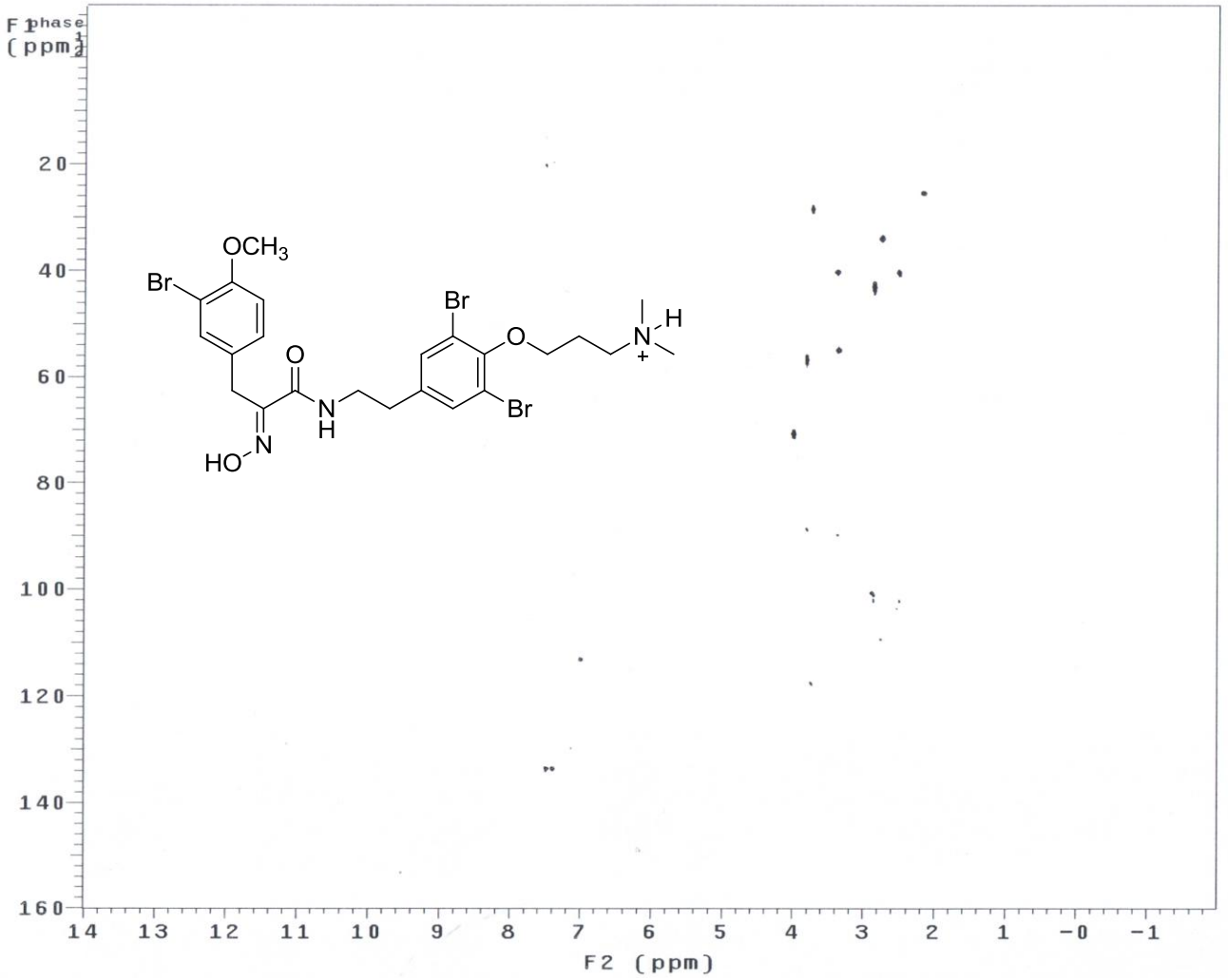
UV spectrum of **7** (CH_3OH)



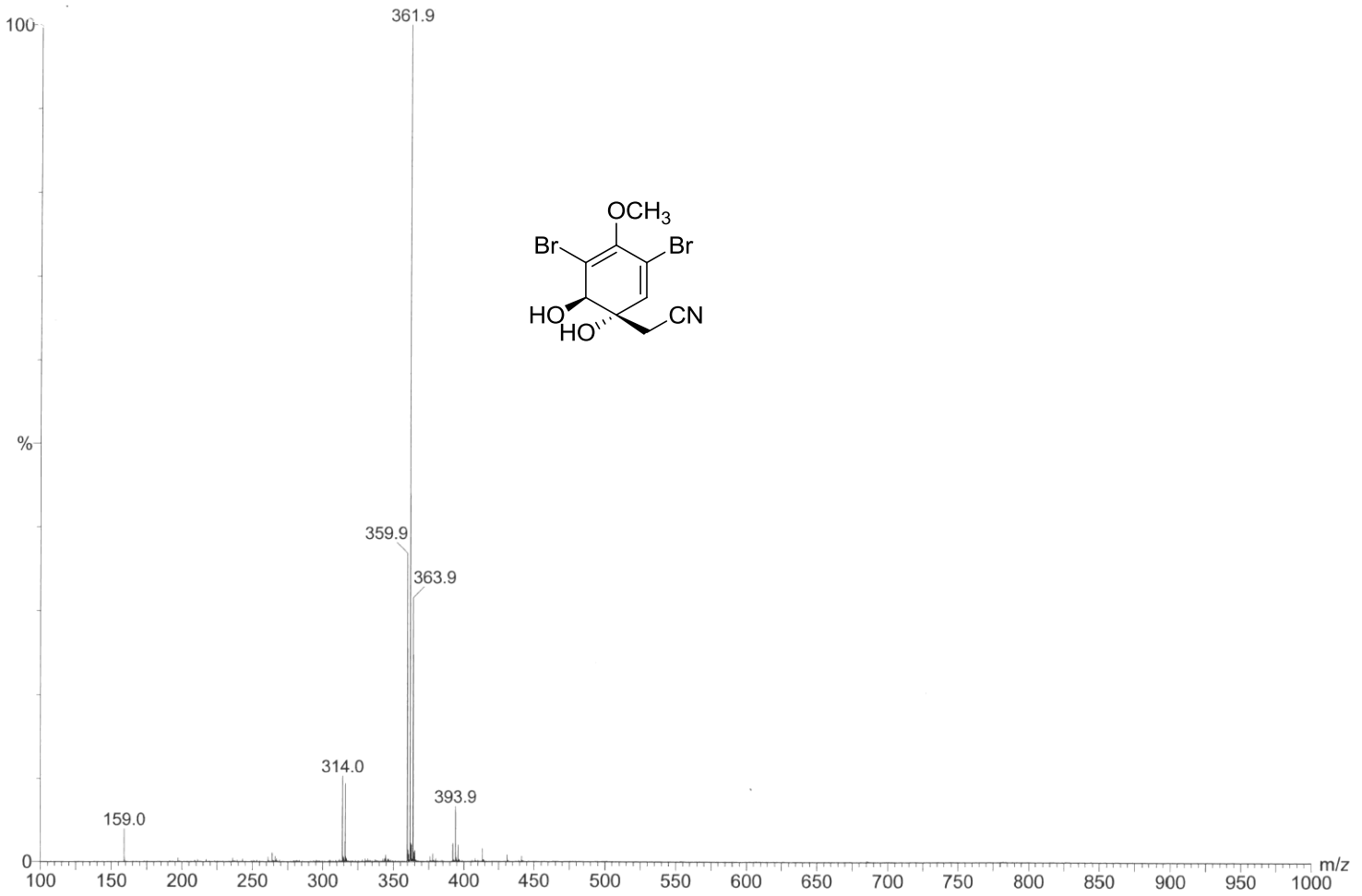
^{1}H-H^{1} COSY spectrum of **7** (500 MHz, DMSO-d_6)



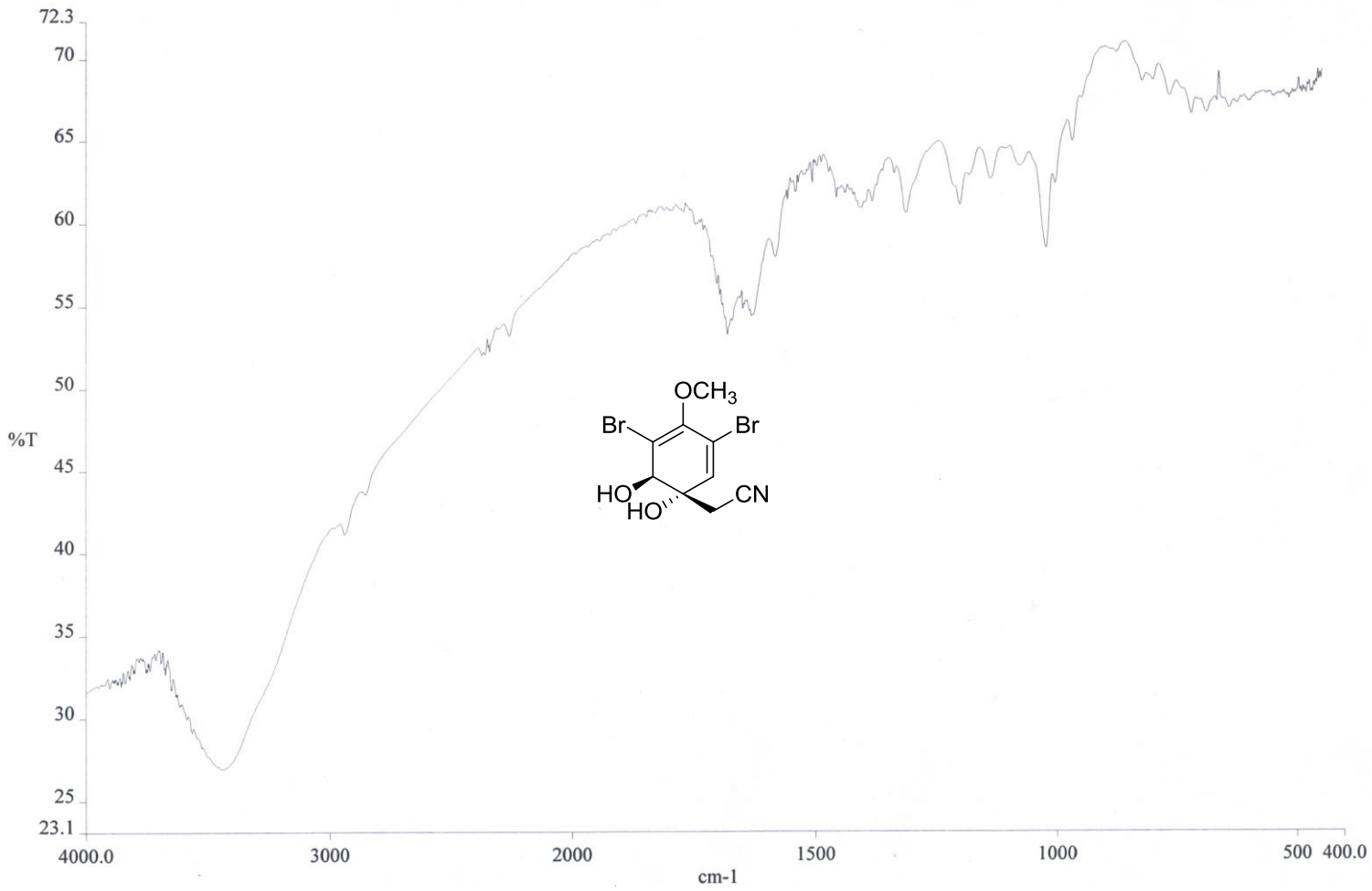
HMBC spectrum of **7** (500 MHz, DMSO-d_6)



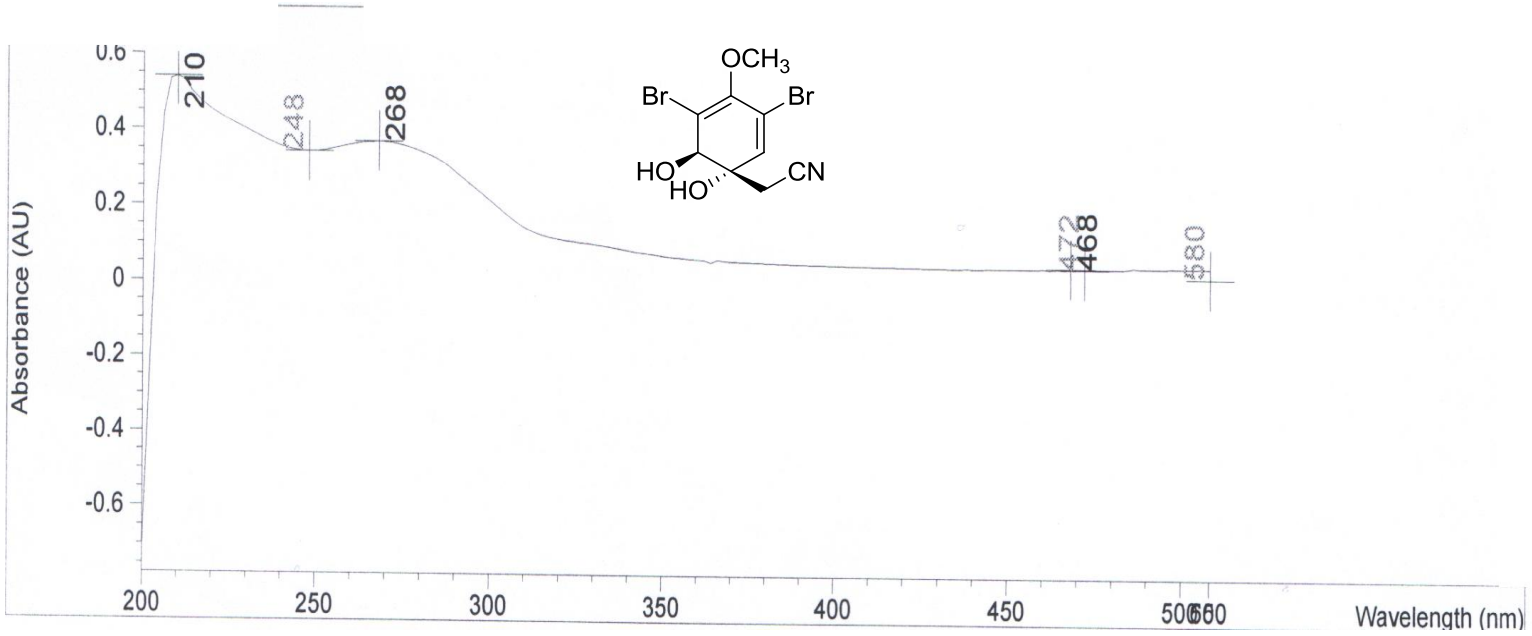
HMQC spectrum of **7** (500 MHz, DMSO-d_6)



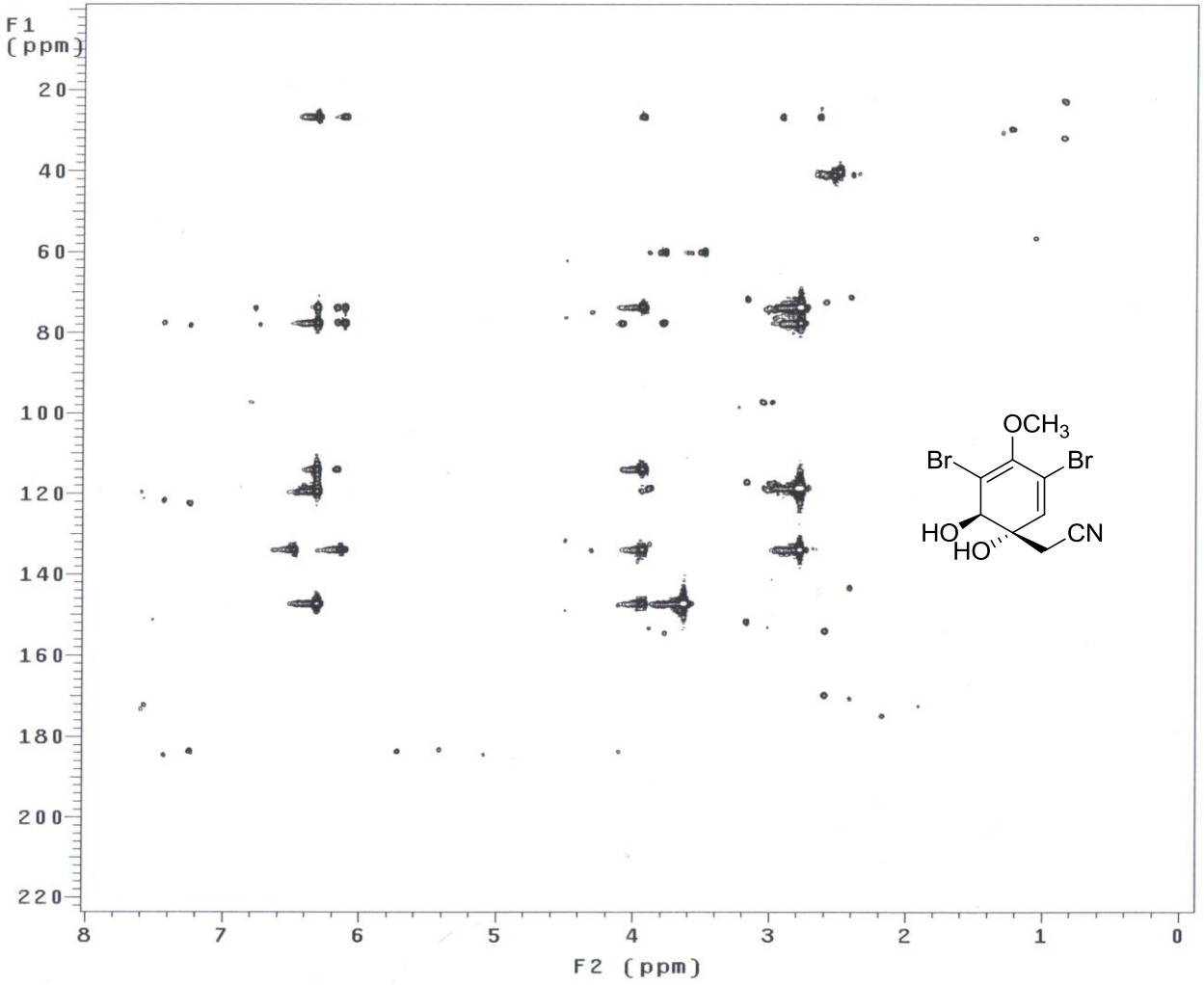
ESI mass spectrum of **8**



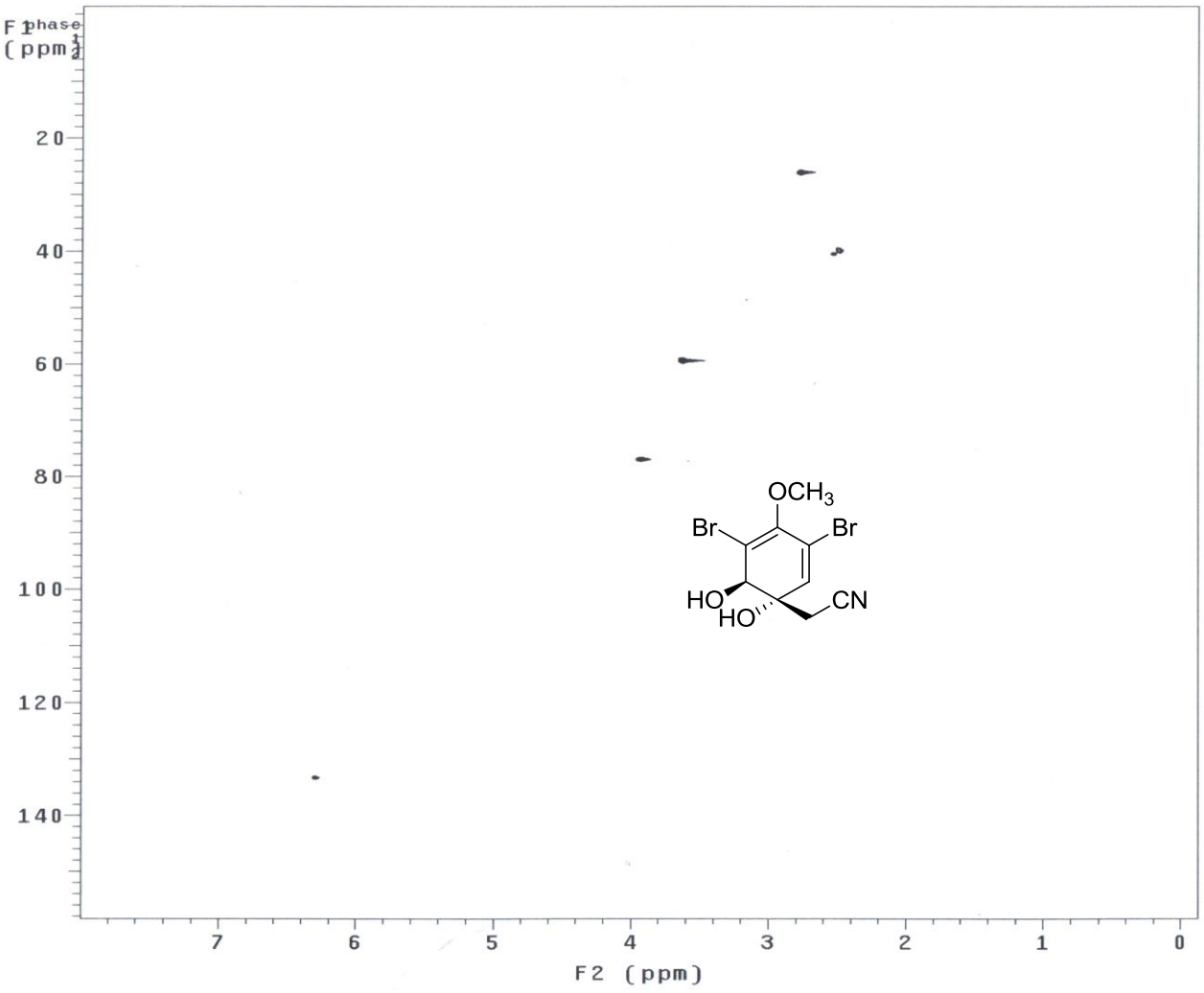
IR spectrum of **8** (KBr)



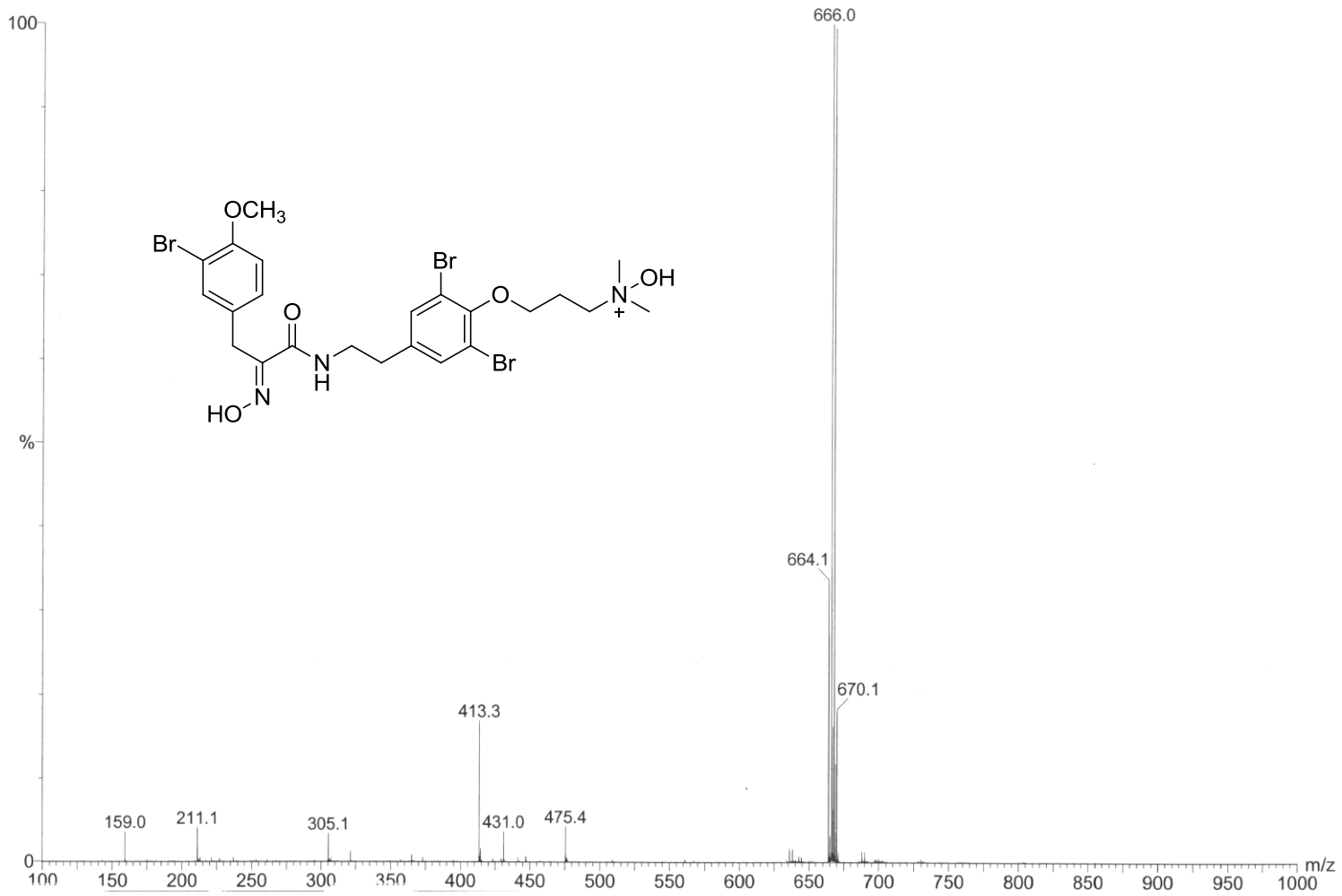
UV spectrum of **8** (CH_3OH)



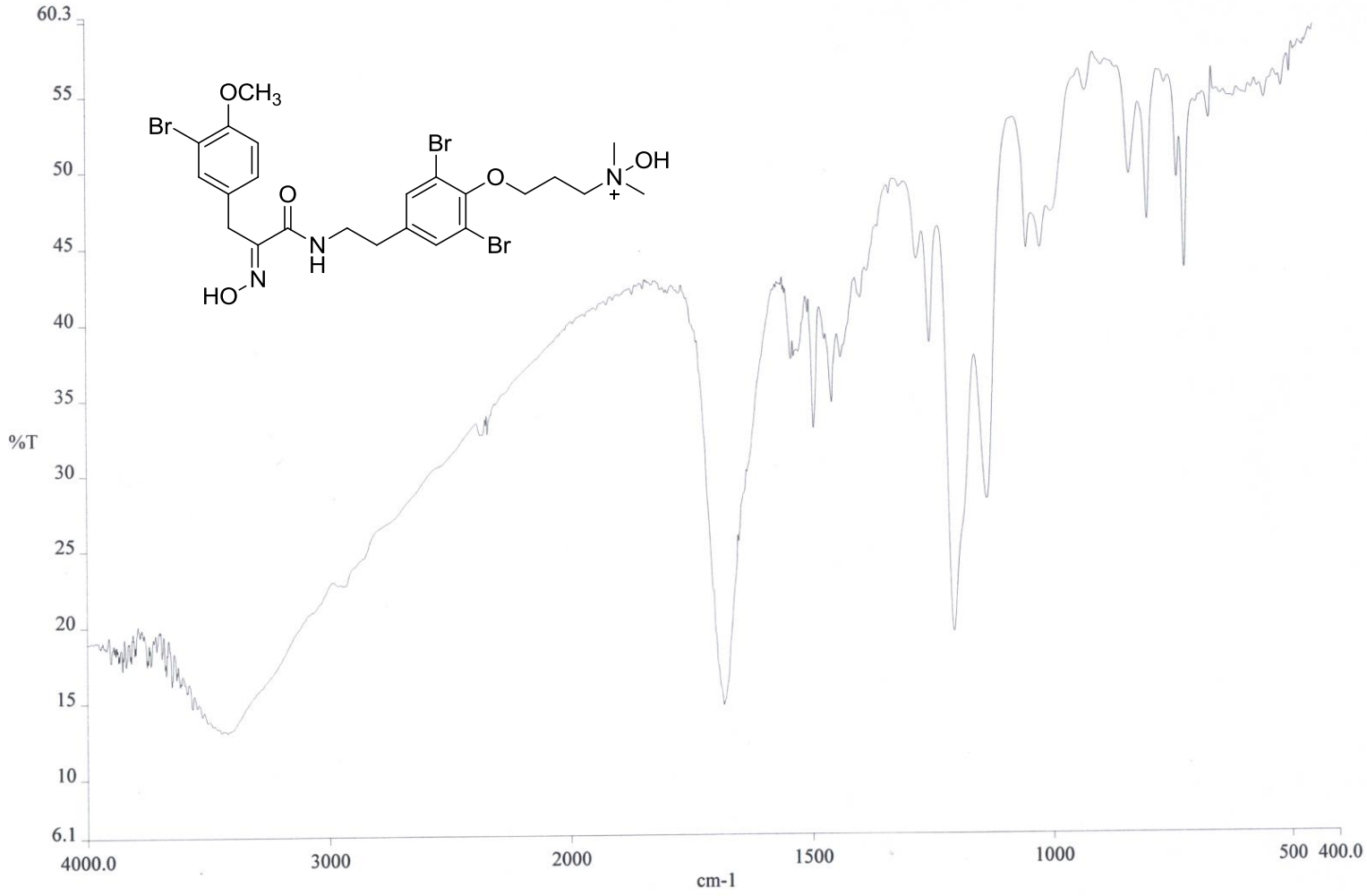
HMBC spectrum of **8** (DMSO-d_6)



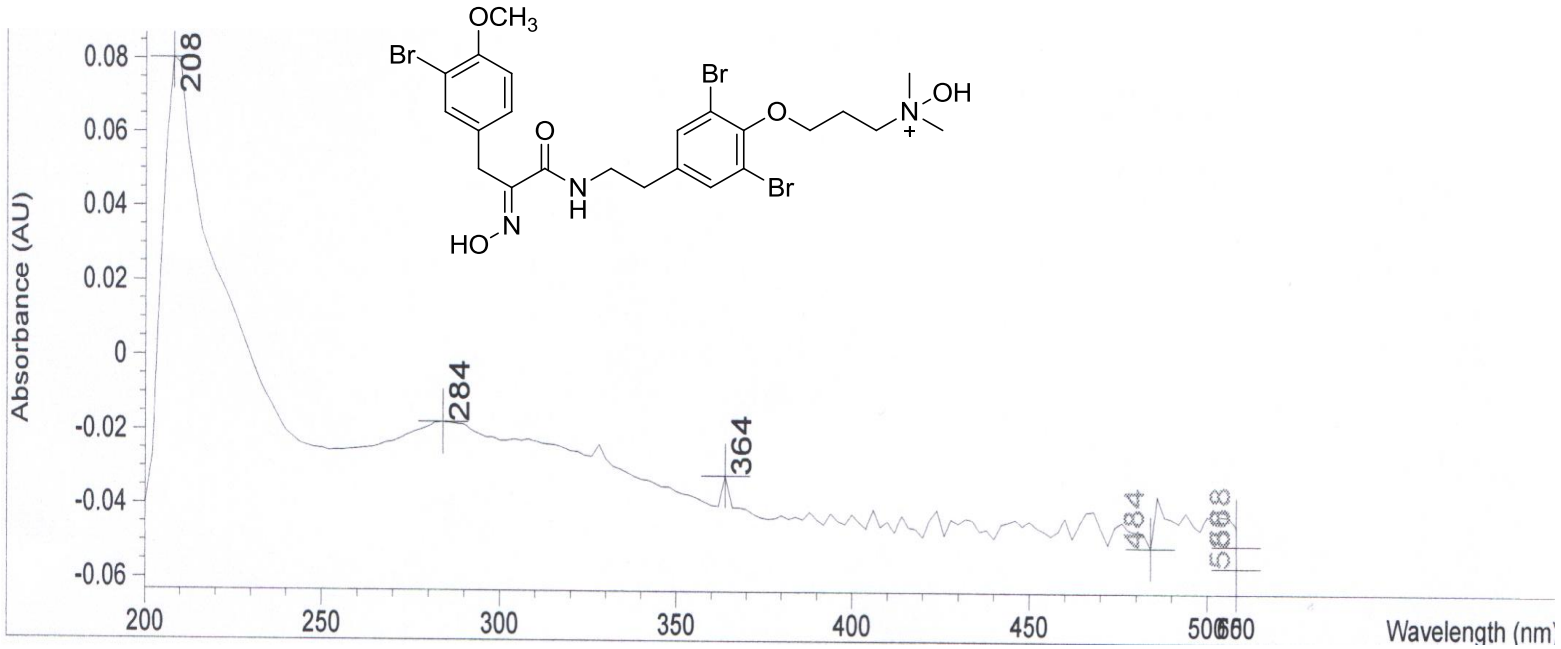
HMQC spectrum of **8** (DMSO-d_6)



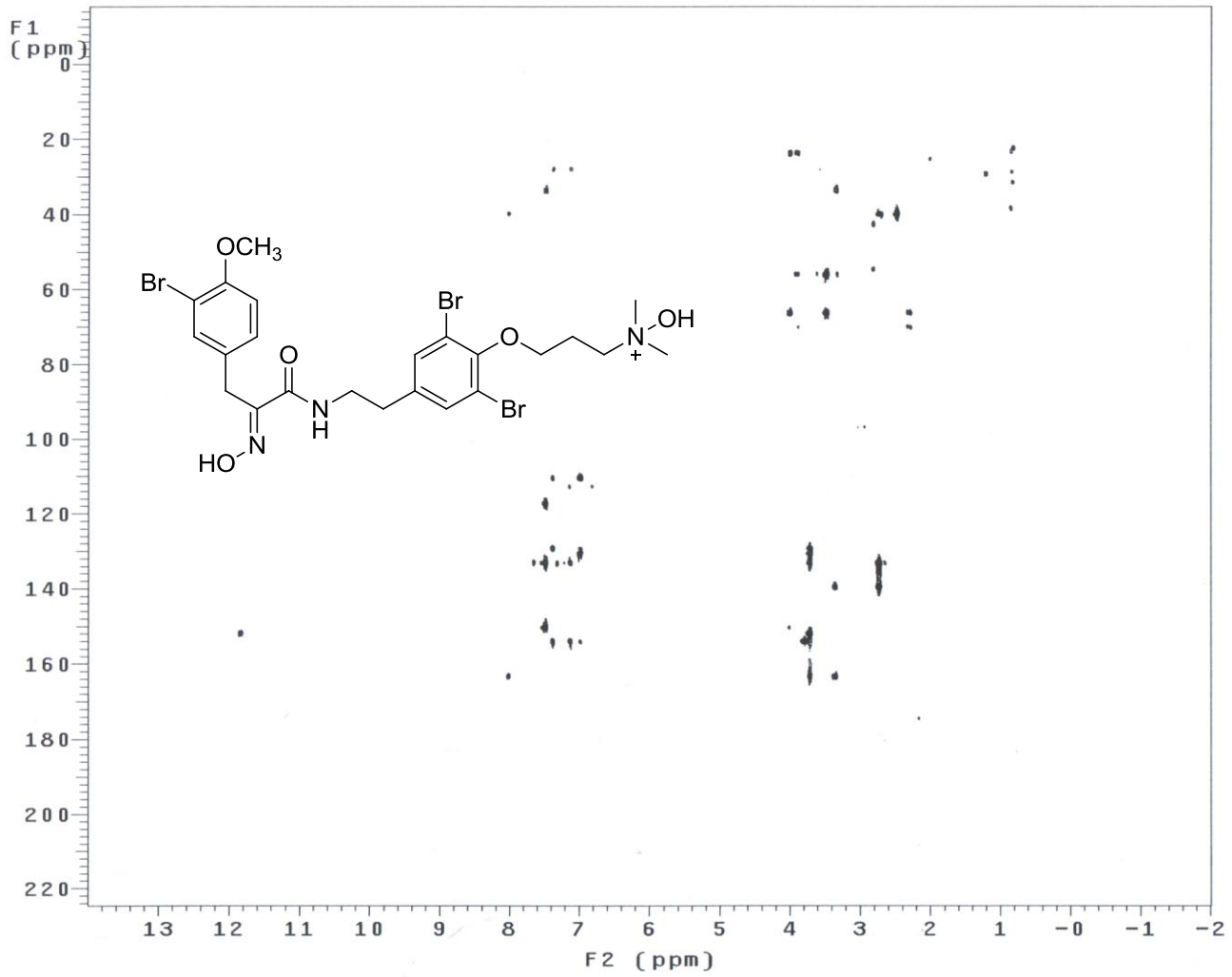
ESI mass spectrum of **9**



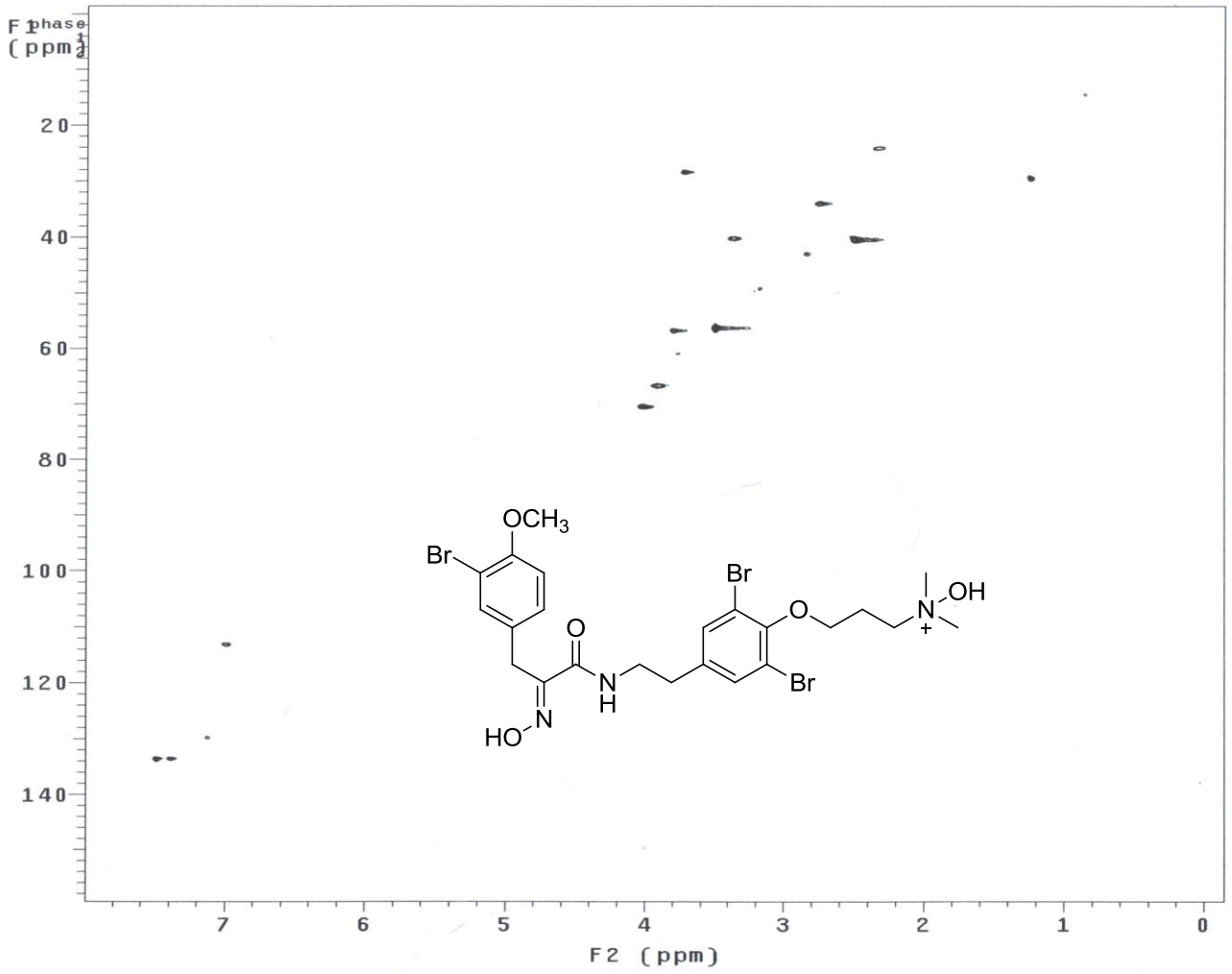
IR spectrum of **9** (KBr)



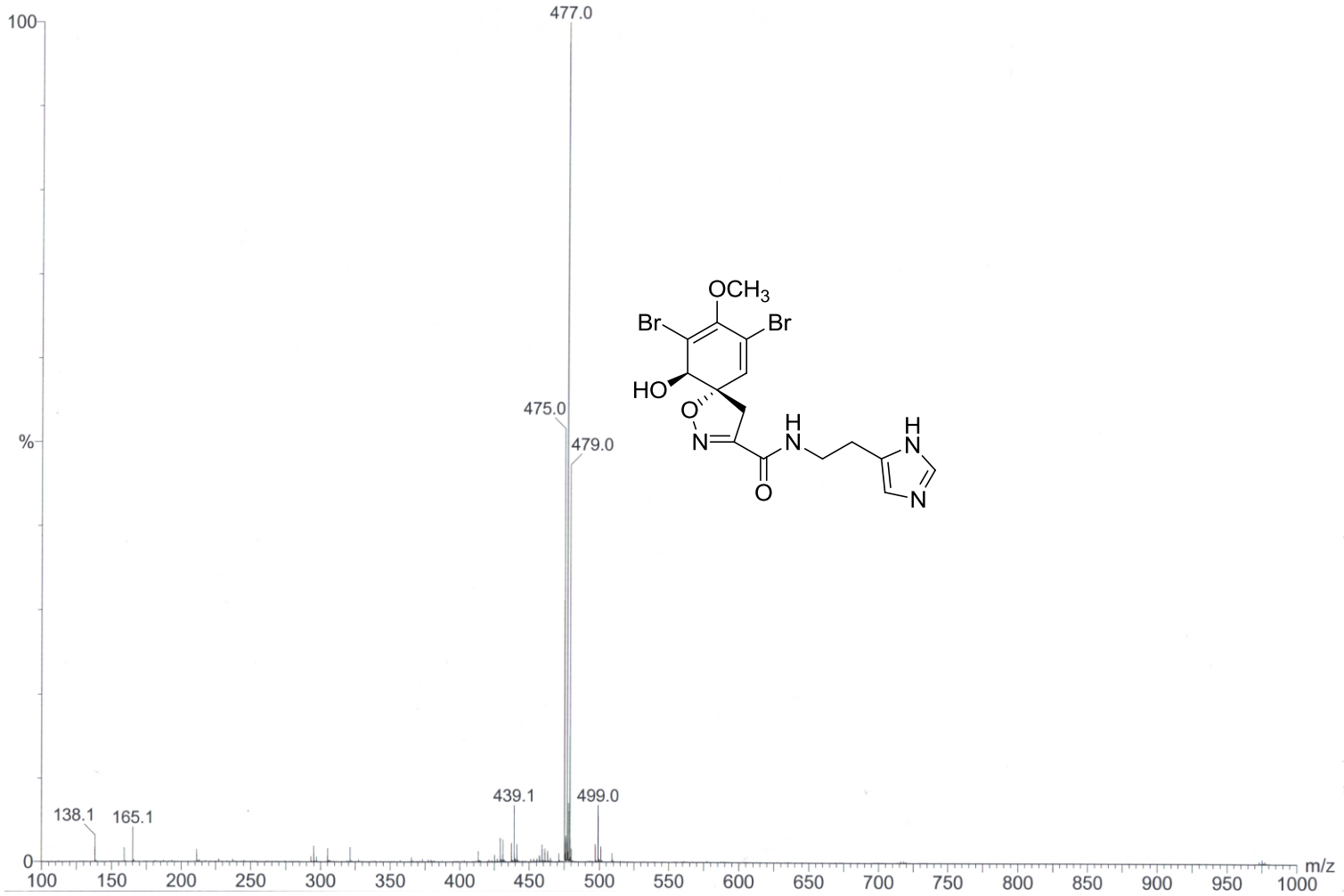
UV spectrum of **9** (CH_3OH)



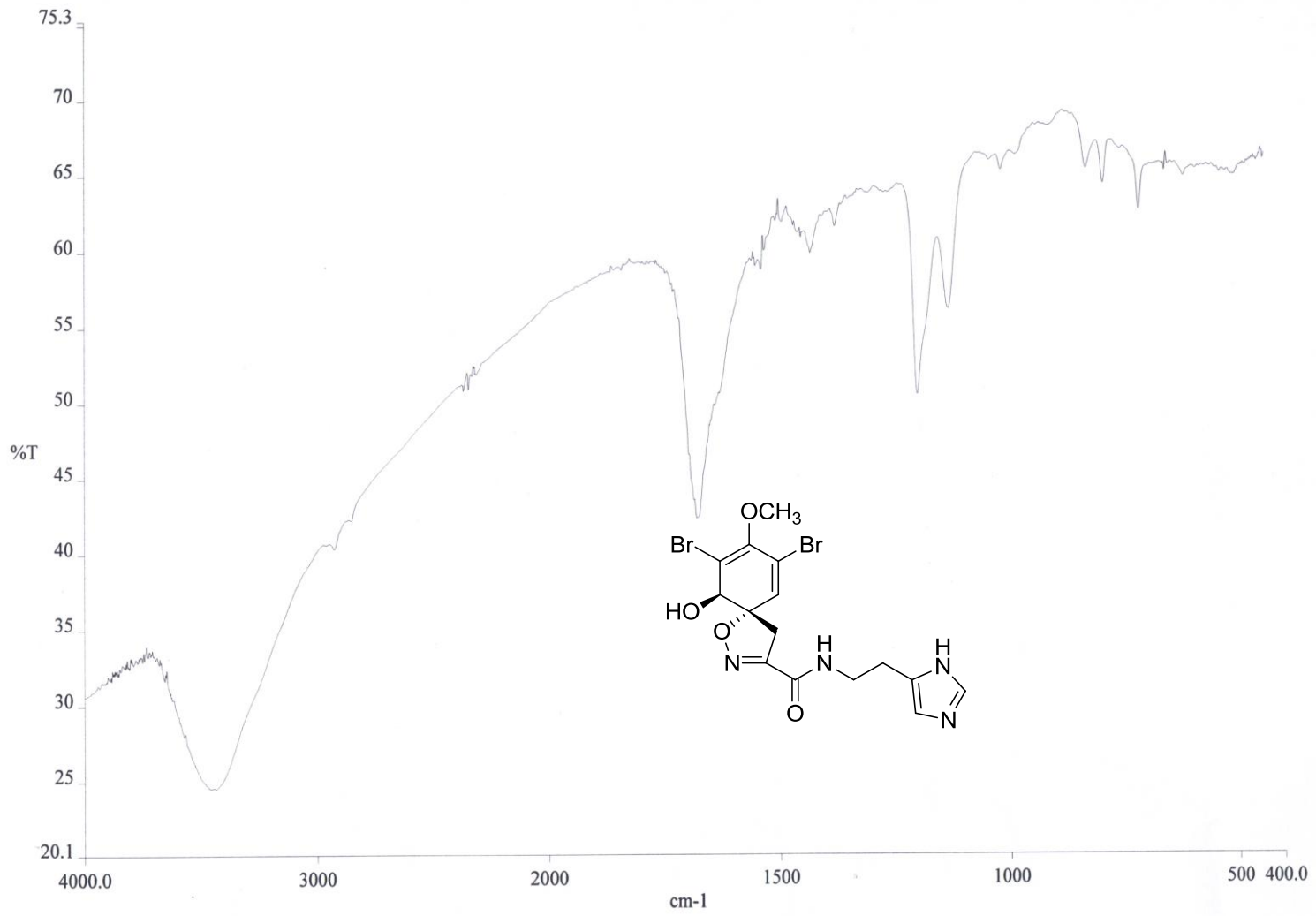
HMBC spectrum of **9** (500 MHz, DMSO-d_6)



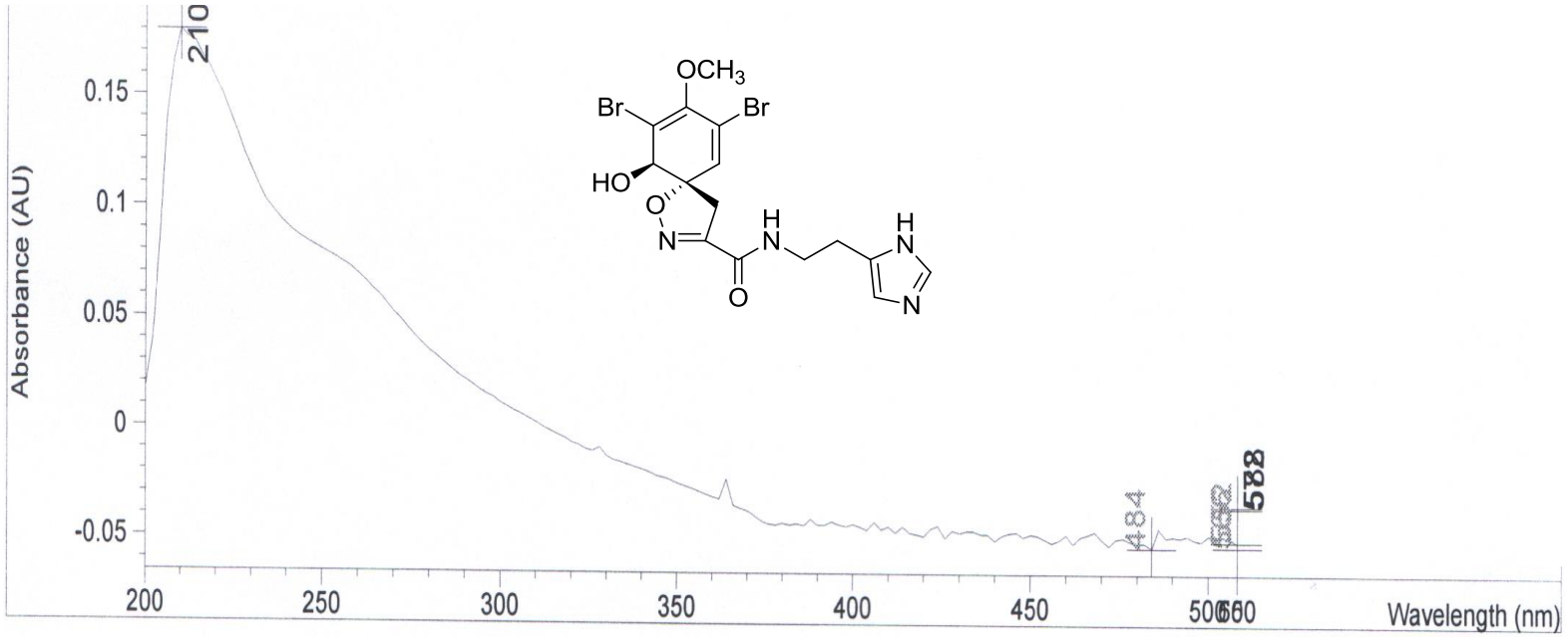
HMQC spectrum of **9** (500 MHz, DMSO-d_6)



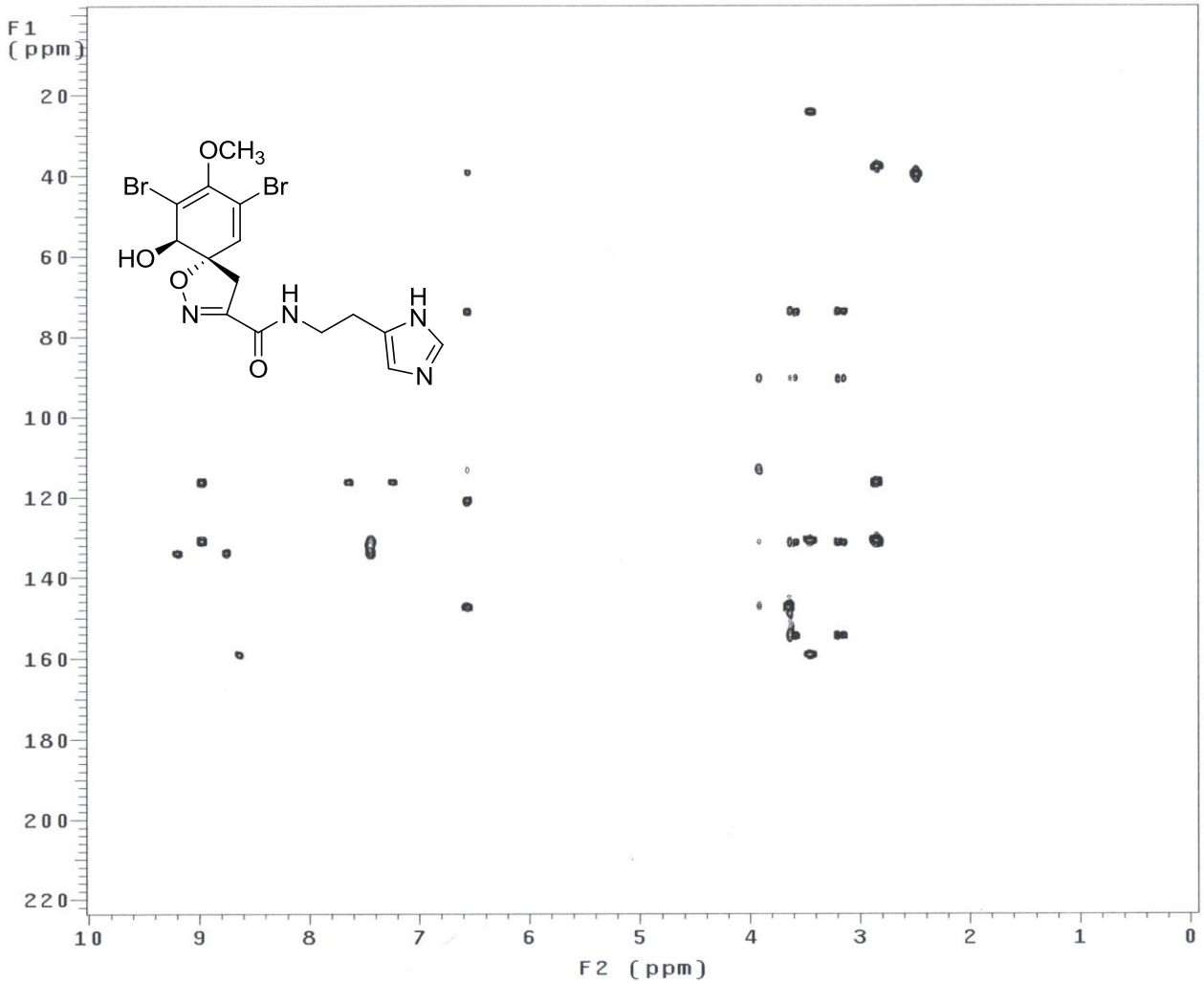
ESI mass spectrum of **10**



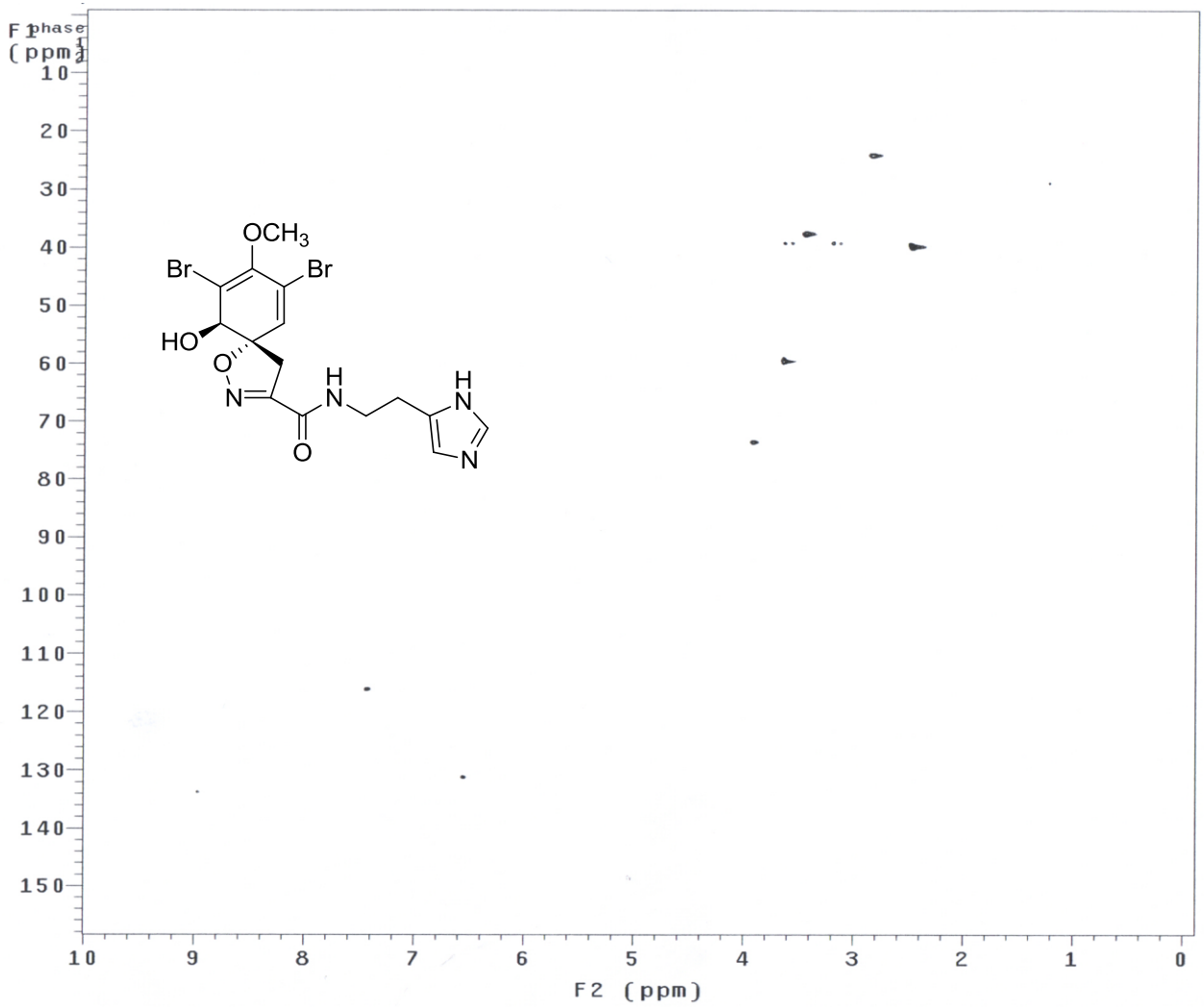
IR spectrum of **10** (KBr)



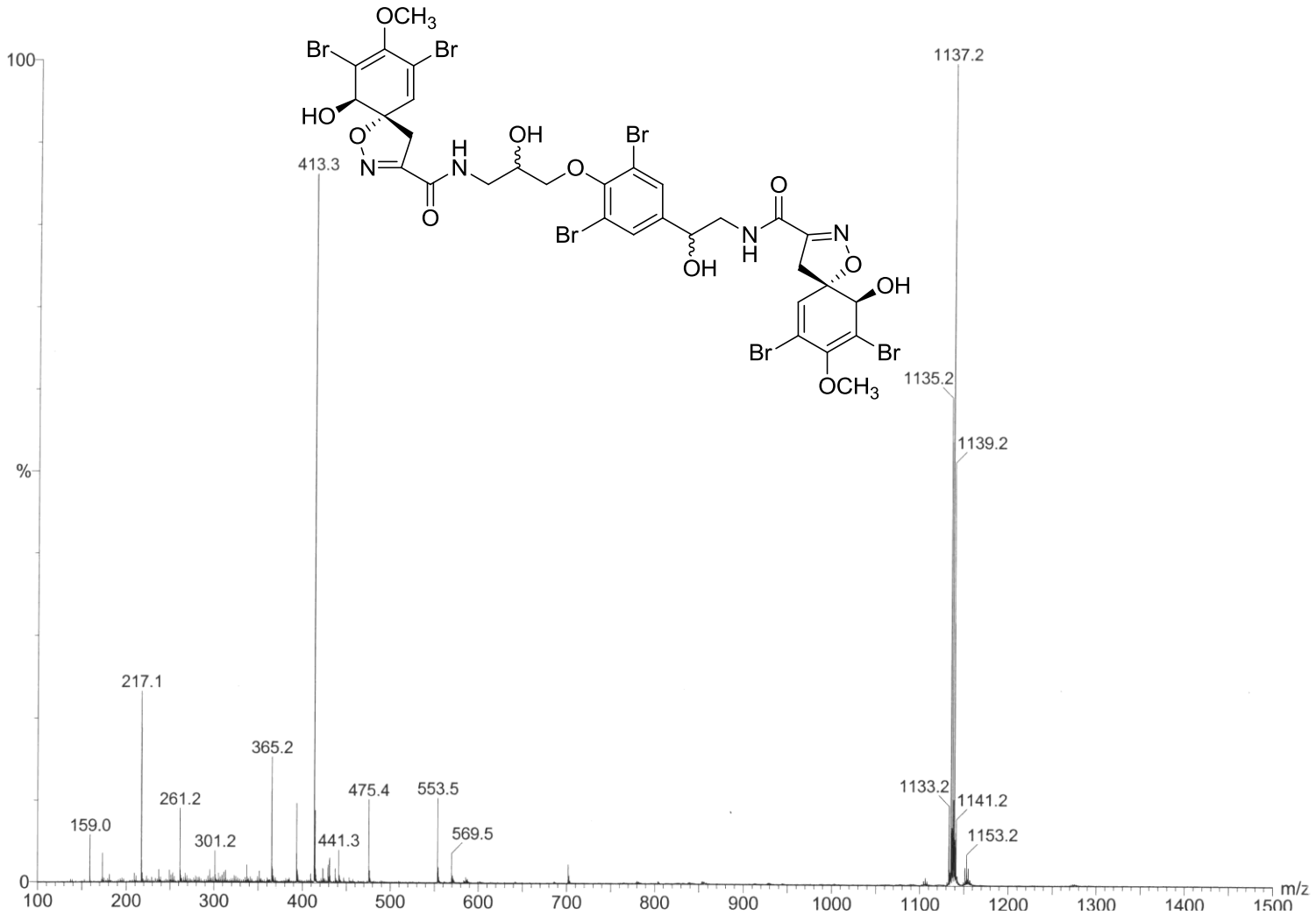
UV spectrum of **10** (CH_3OH)



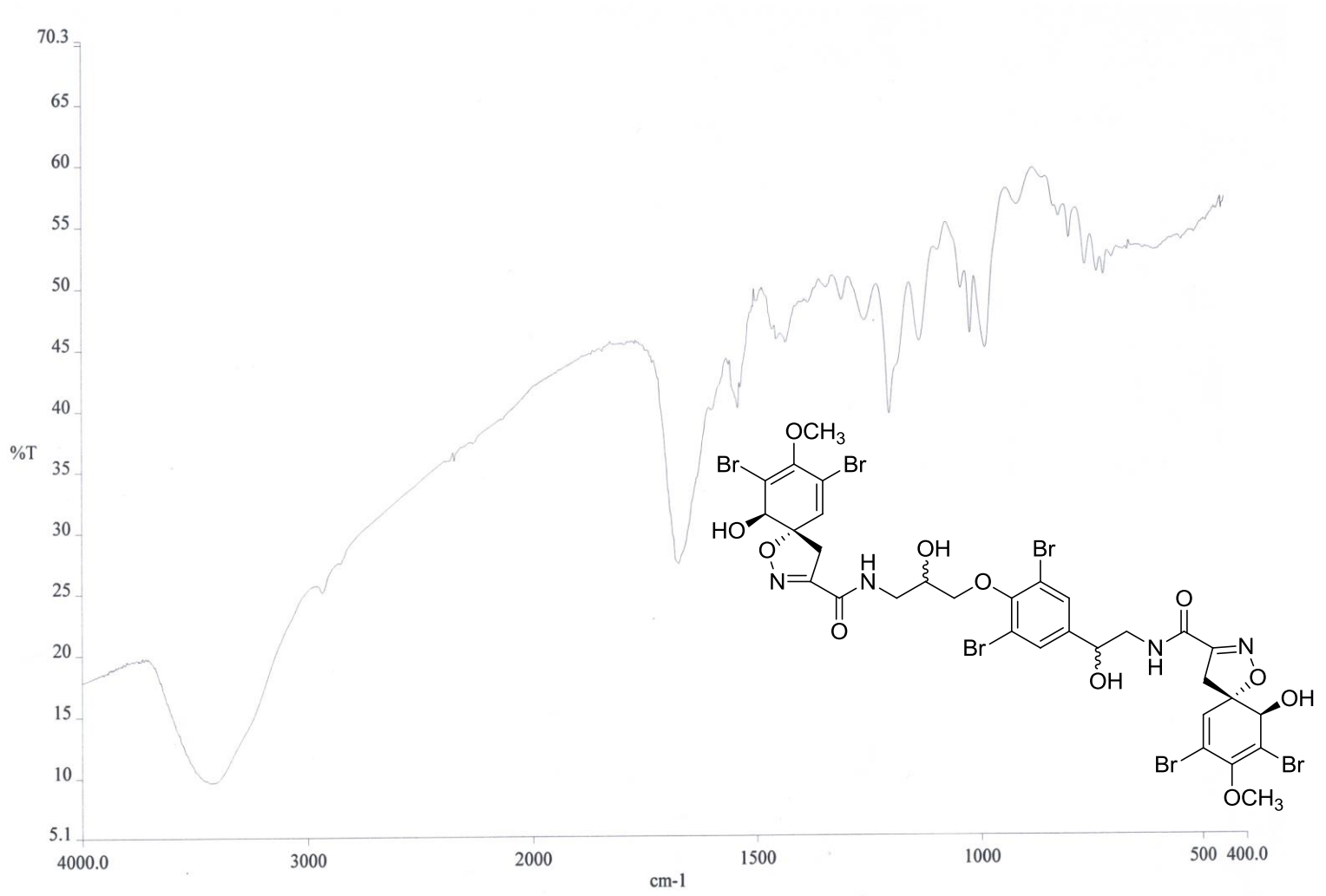
HMBC spectrum of **10** (500 MHz, DMSO-d_6)



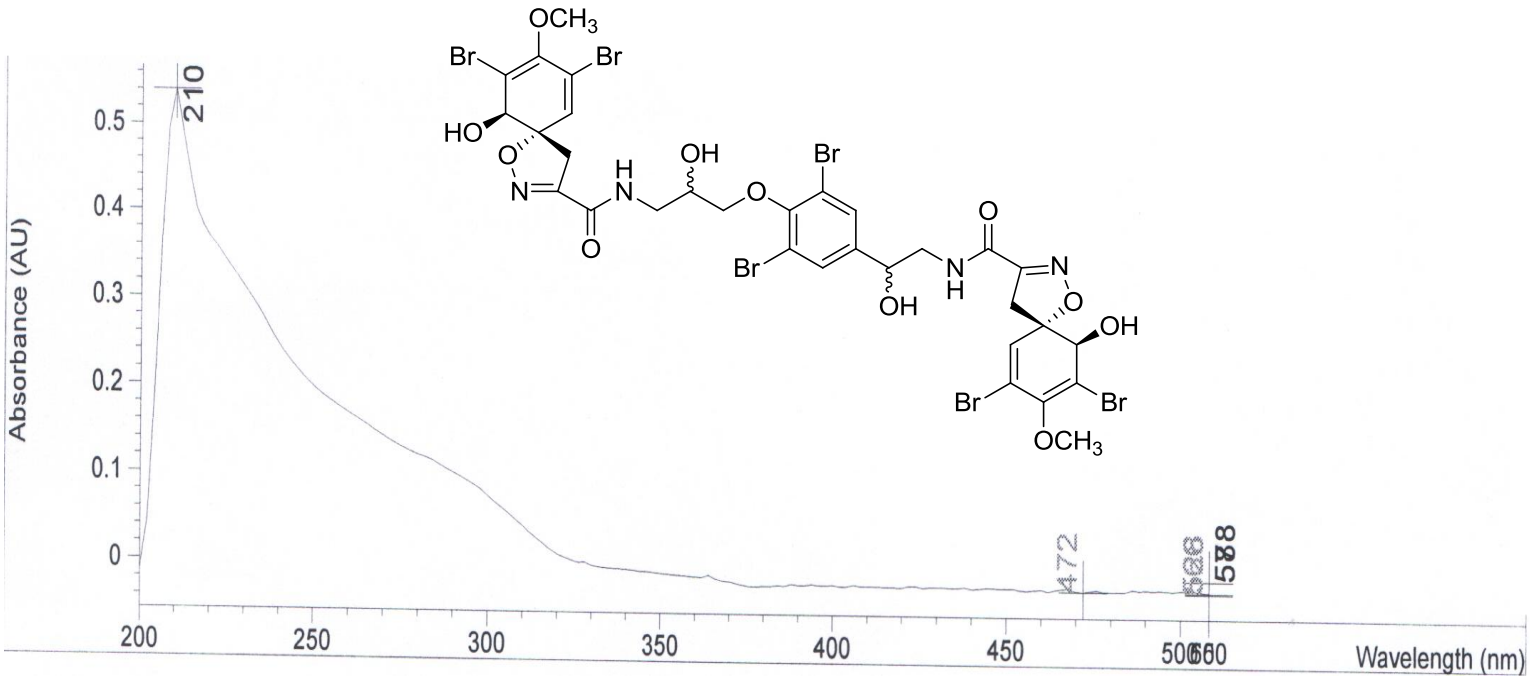
HMQC spectrum of **10** (500 MHz, DMSO-d_6)



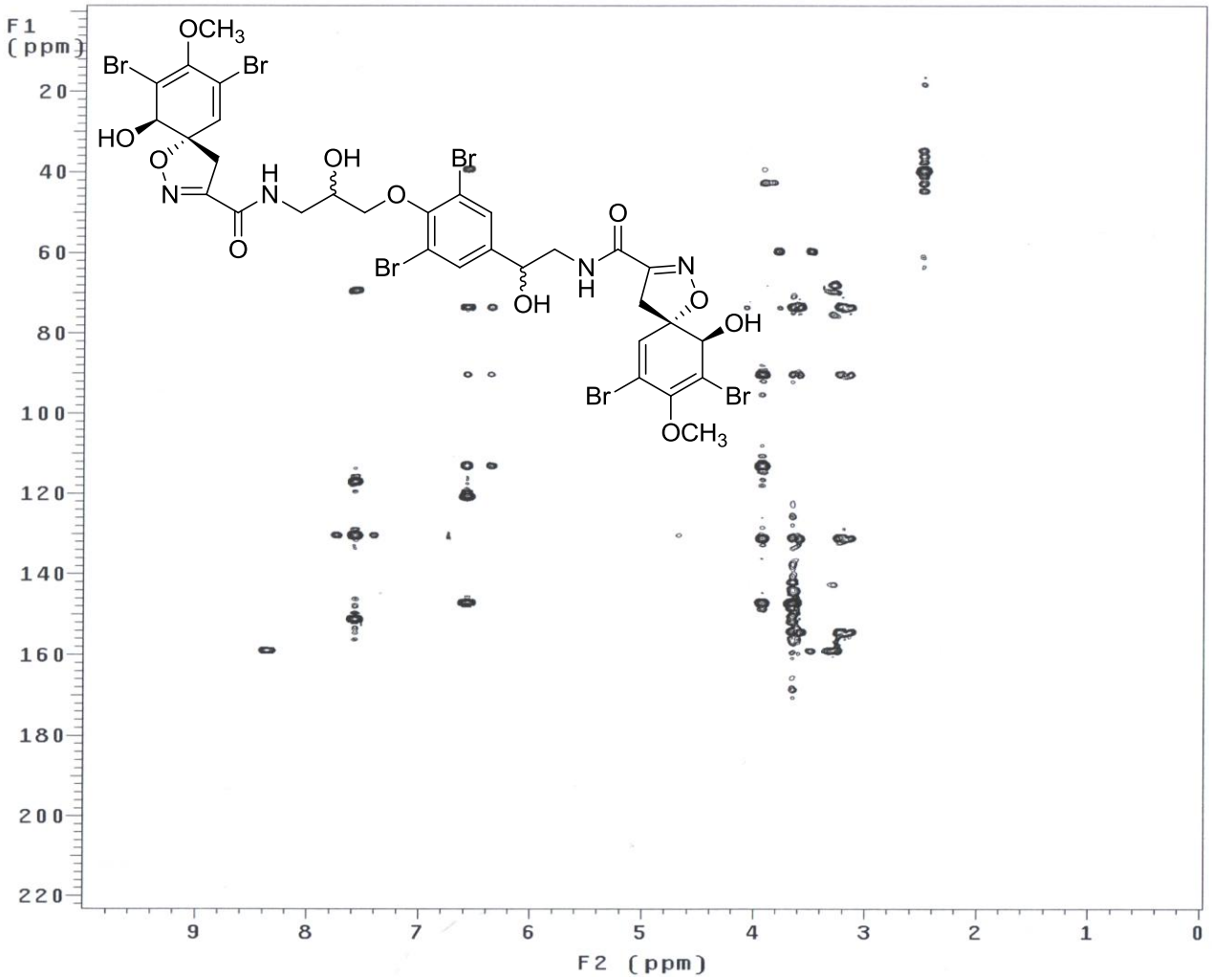
ESI mass spectrum of **11**



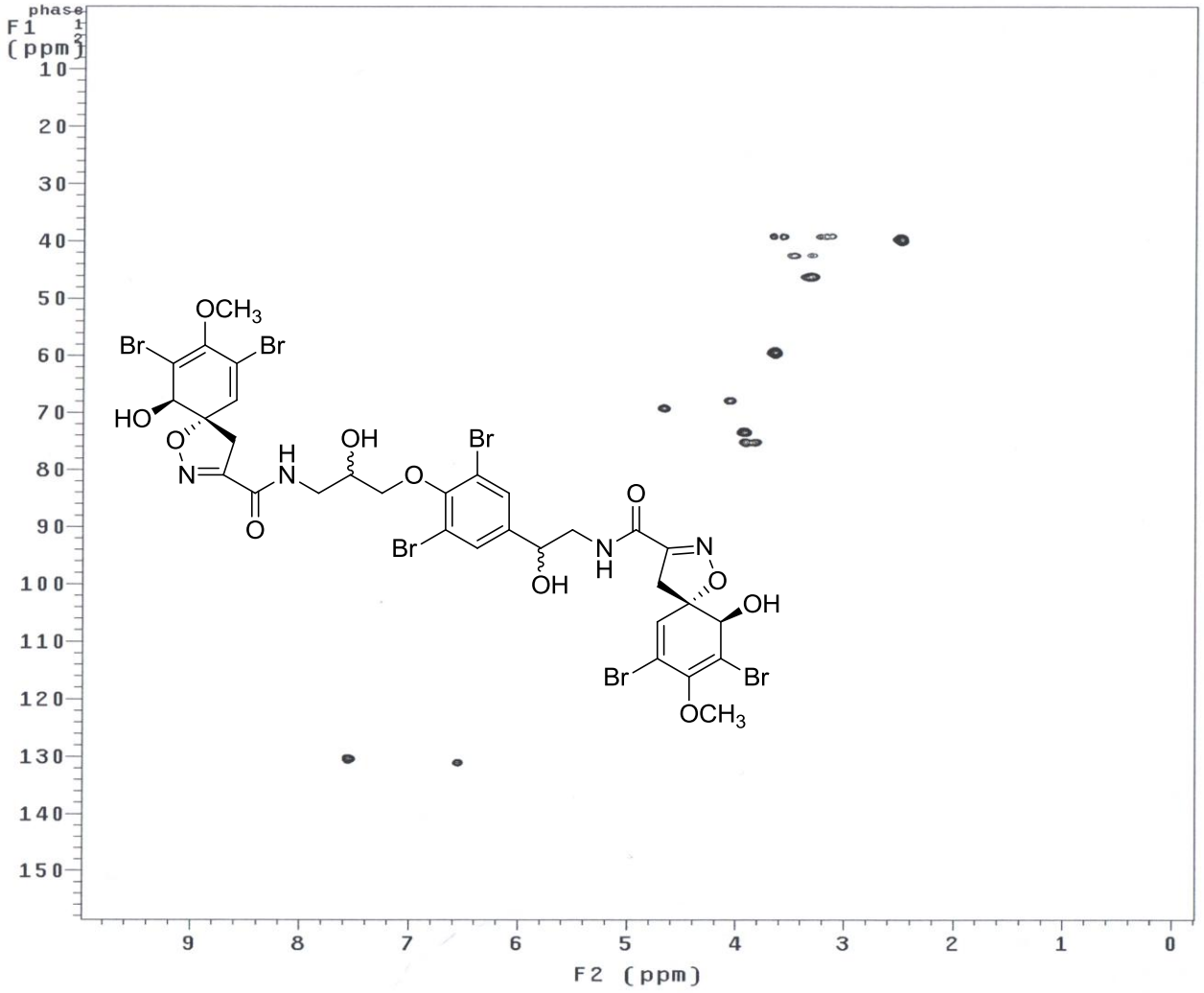
IR spectrum of **11** (KBr)



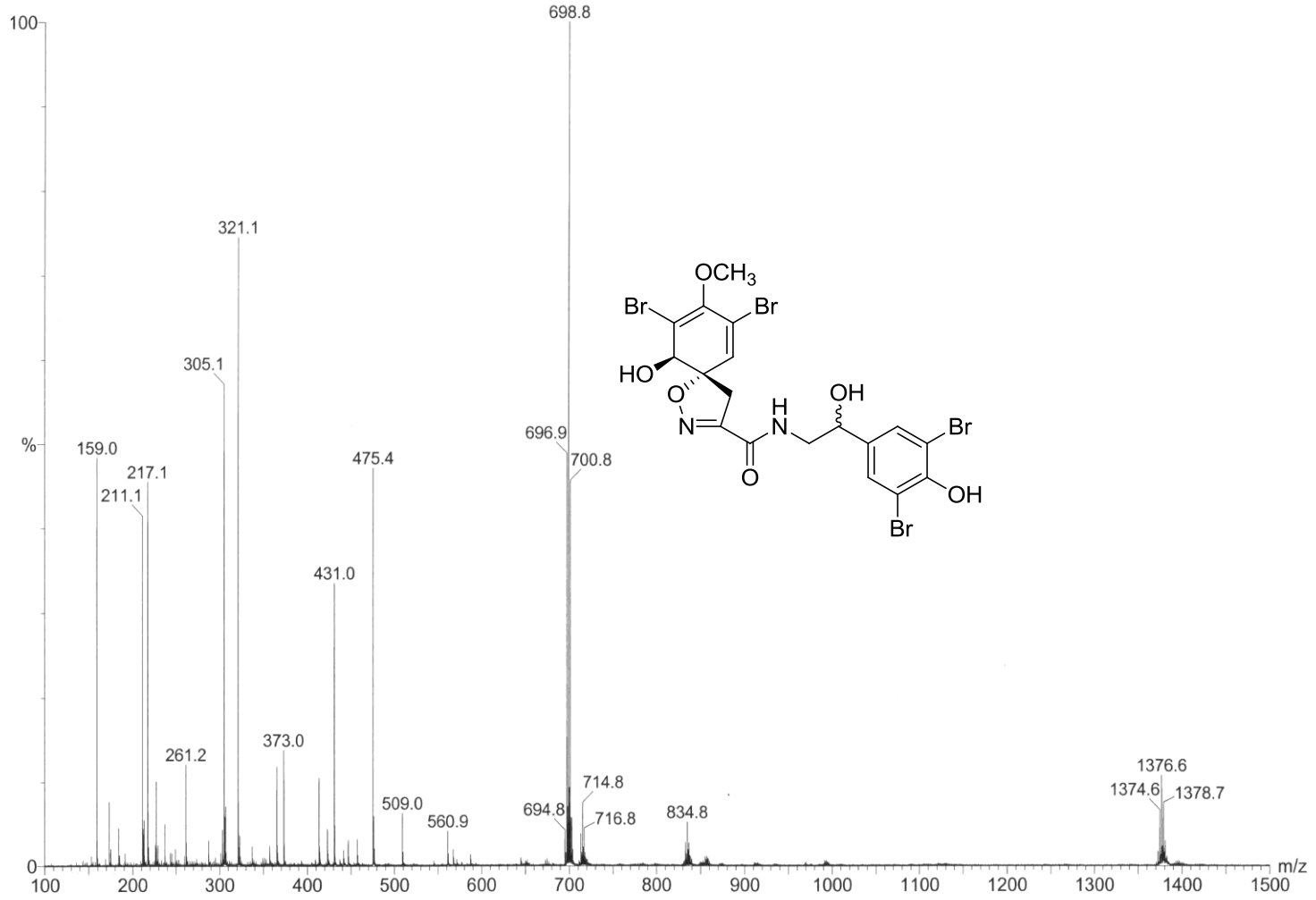
UV spectrum of **11** (CH_3OH)



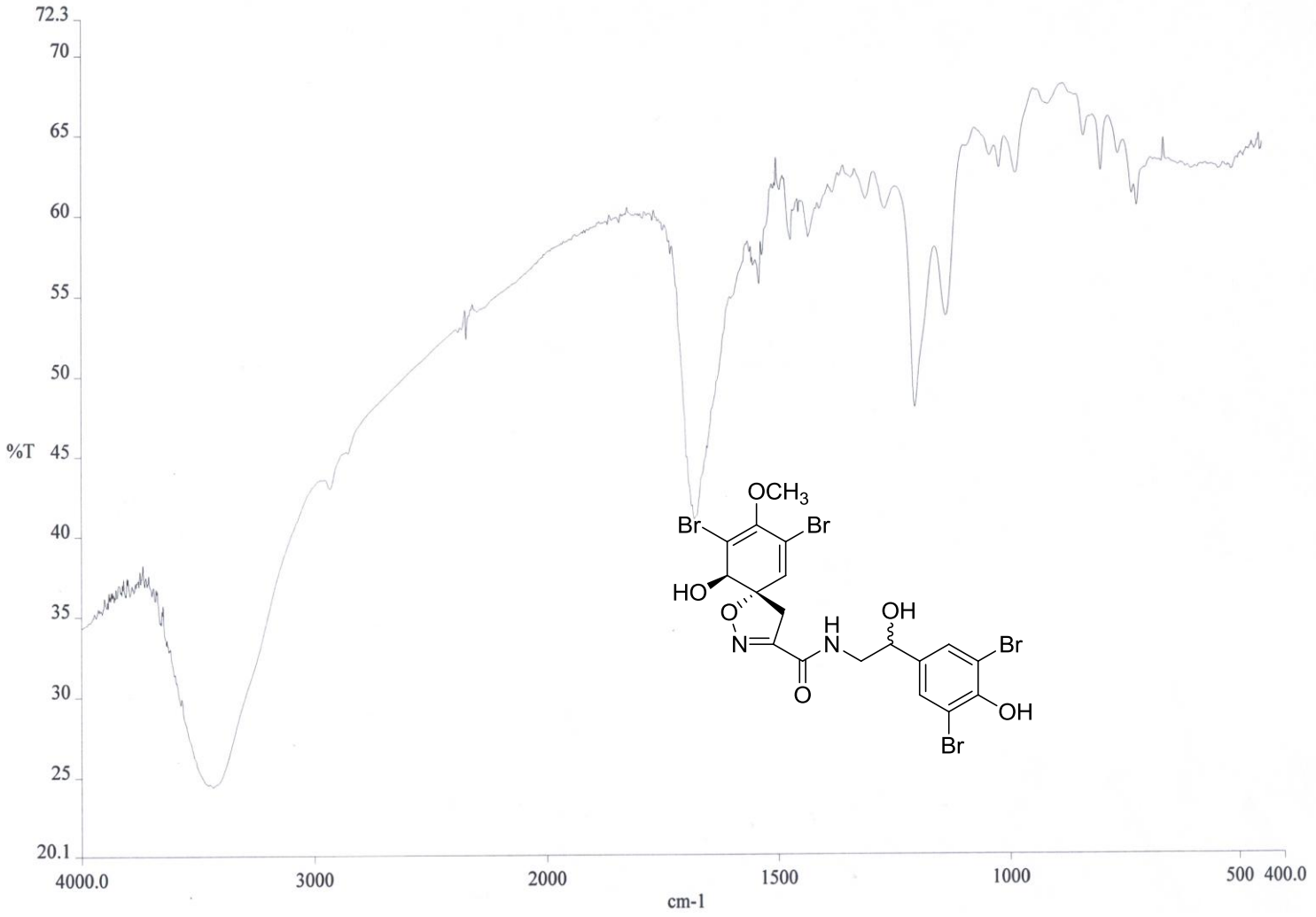
HMBC spectrum of **11** (500 MHz, DMSO-d_6)



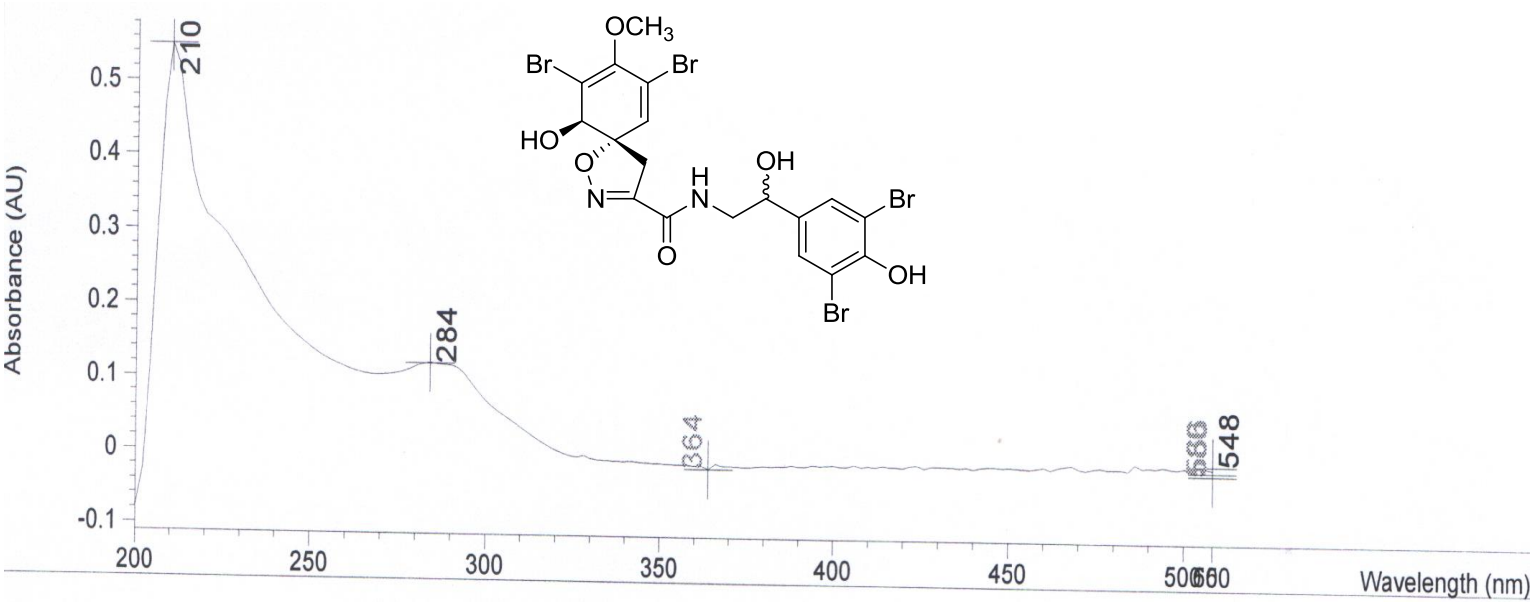
HMQC spectrum of **11** (500 MHz, DMSO-d_6)



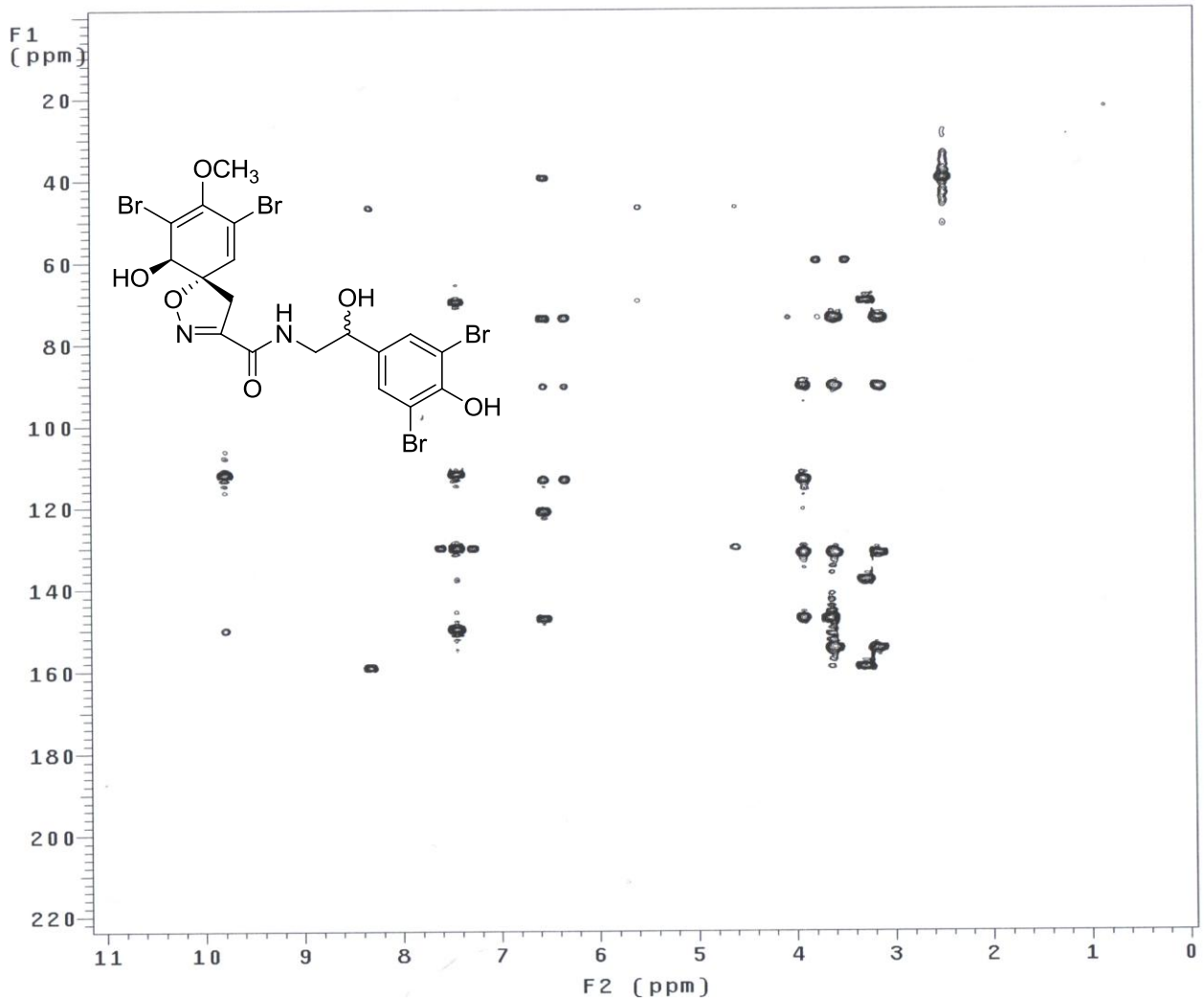
ESI mass spectrum of **12**



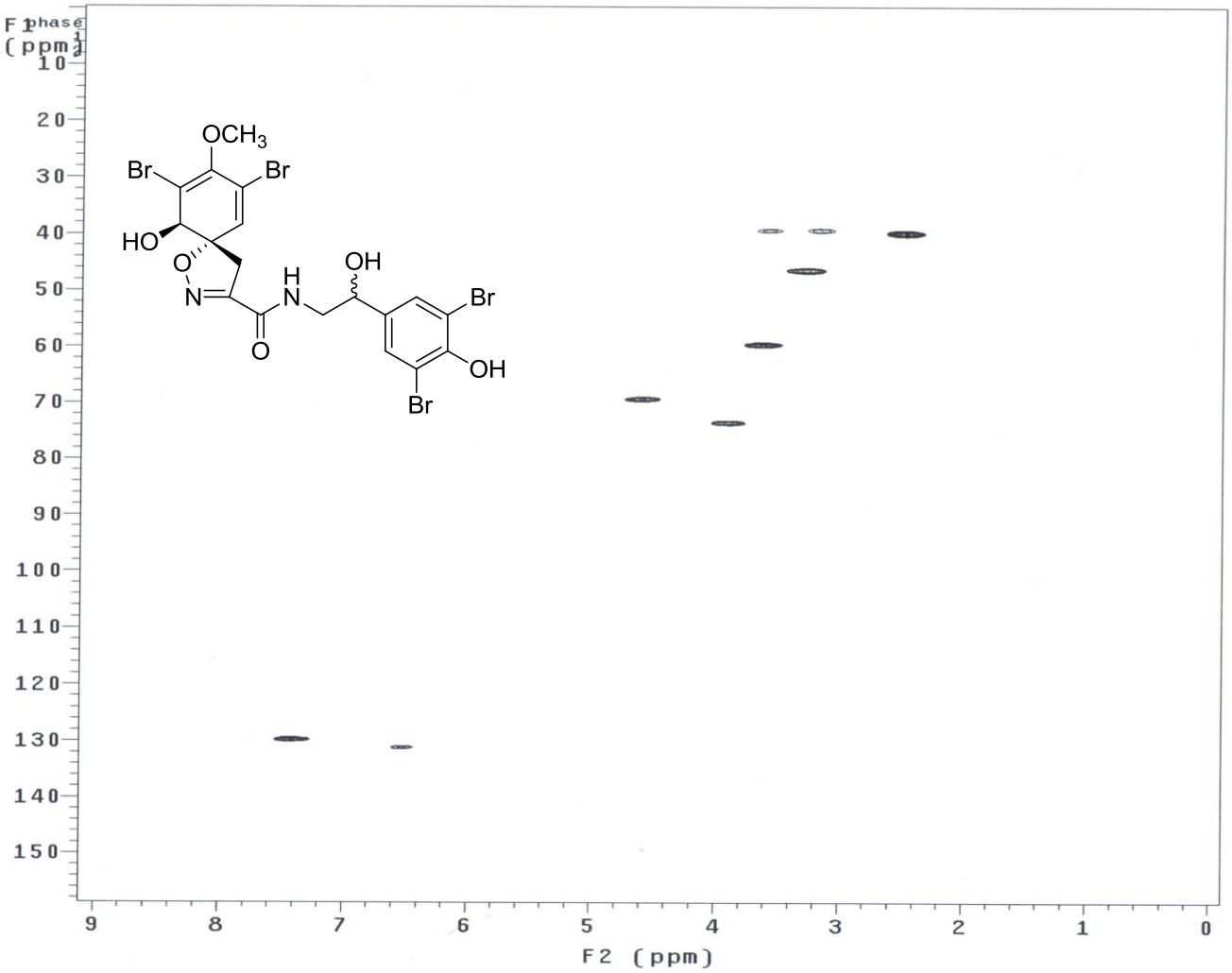
IR spectrum of **12** (KBr)



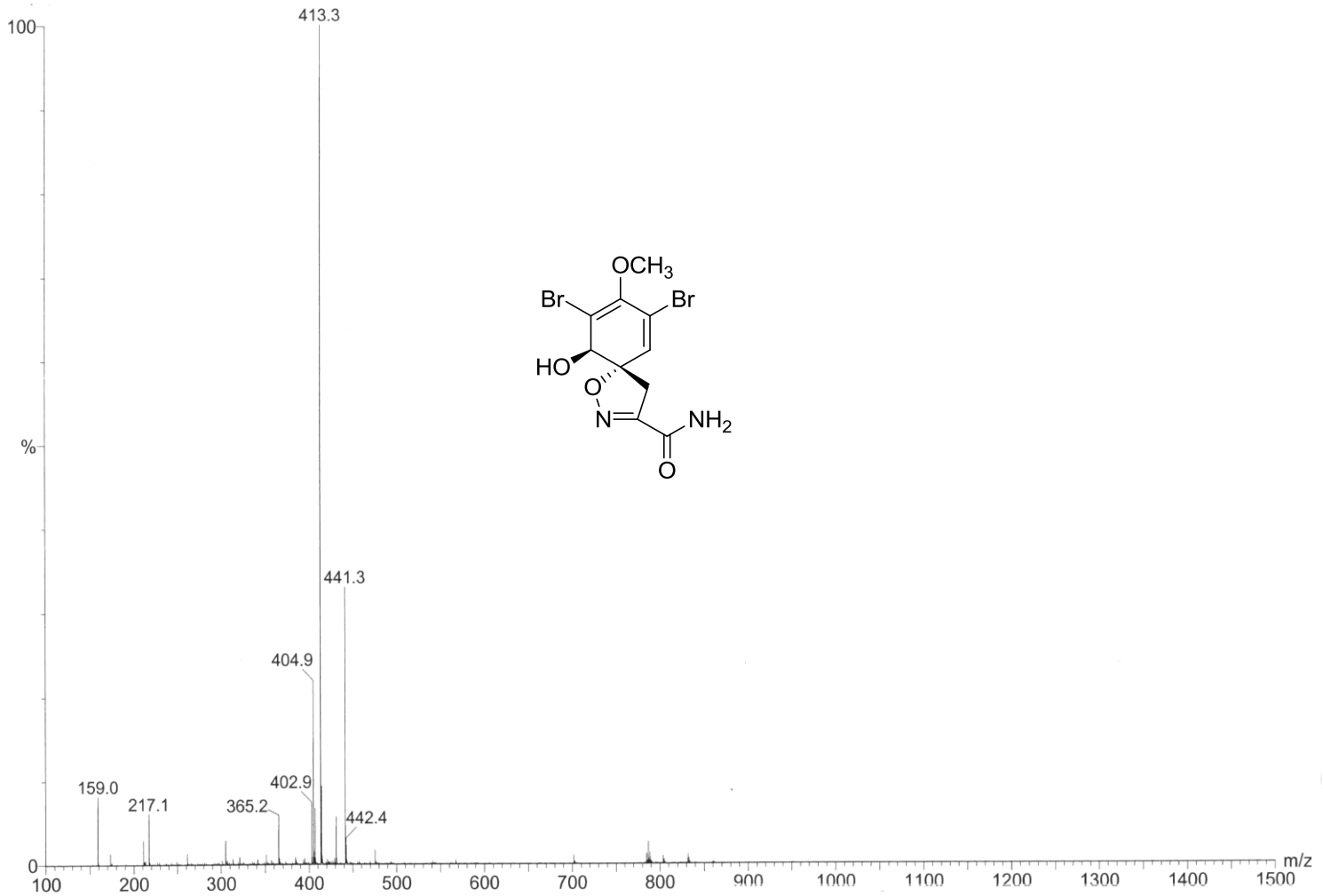
UV spectrum of **12** (CH_3OH)



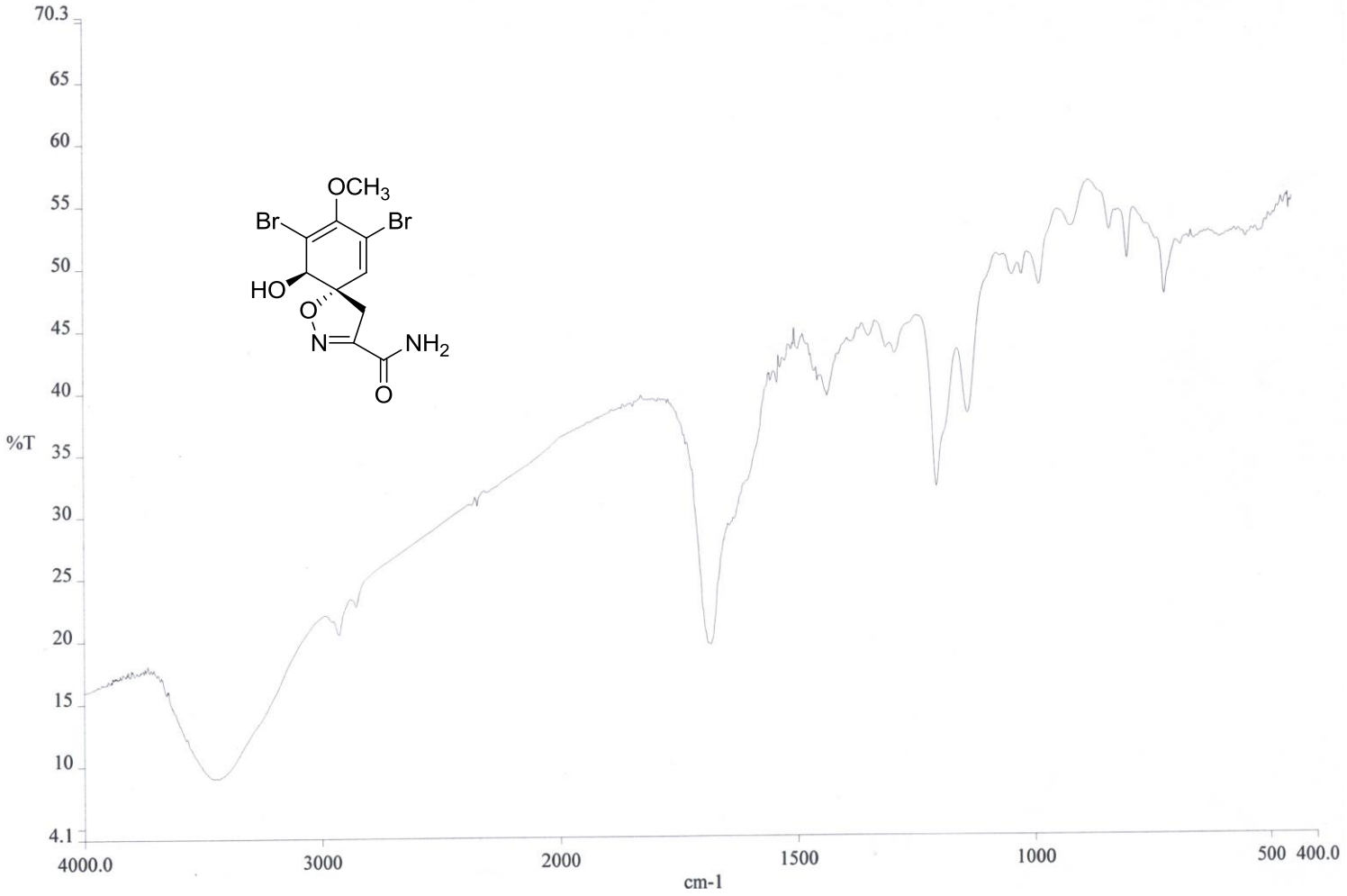
HMBC spectrum of **12** (DMSO-d_6)



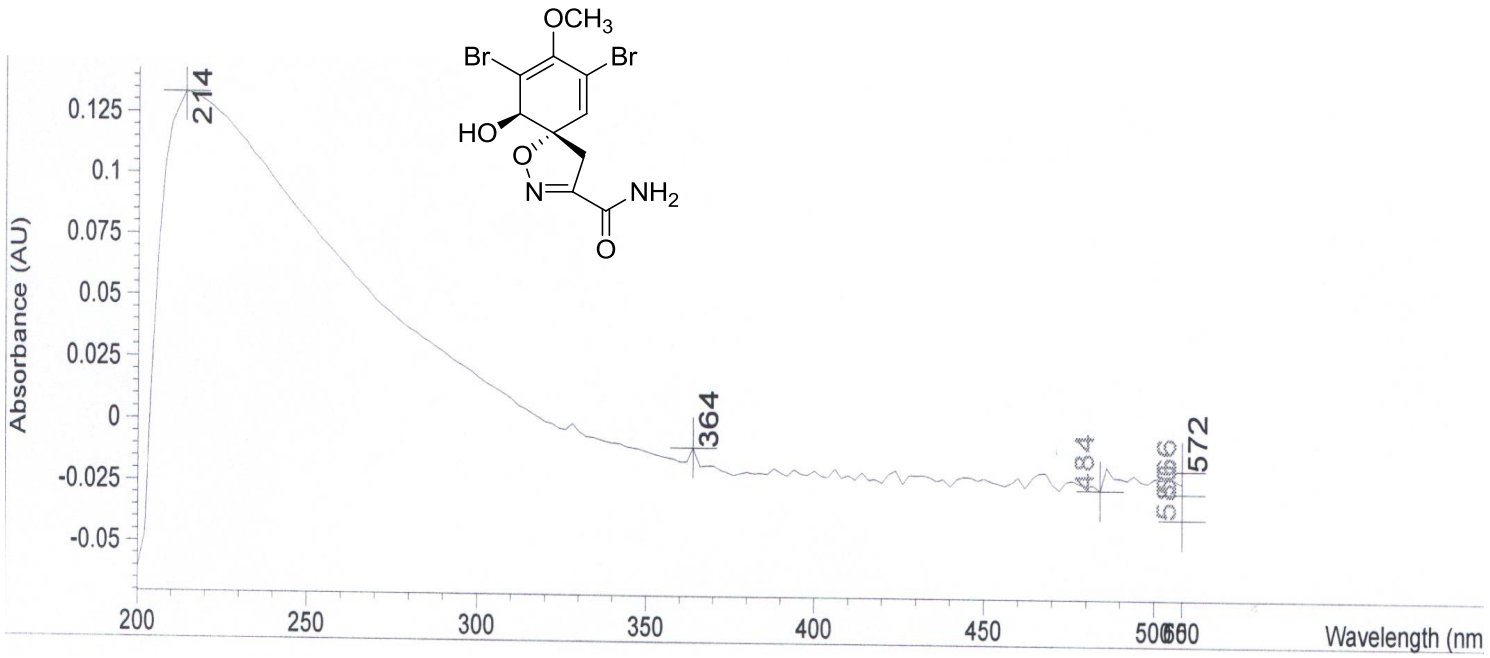
HMQC spectrum of **12** (DMSO-d_6)



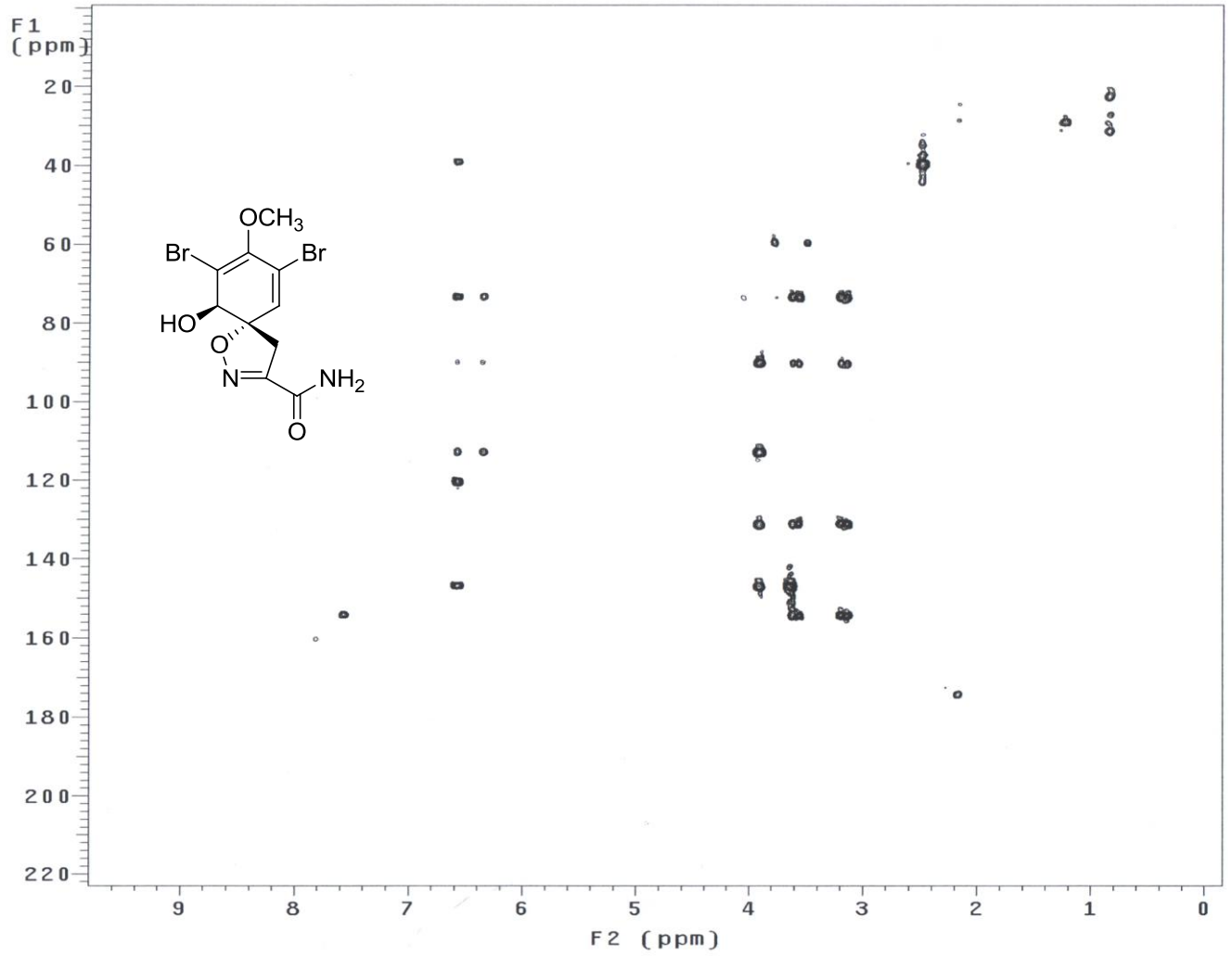
ESI mass spectrum of **13**



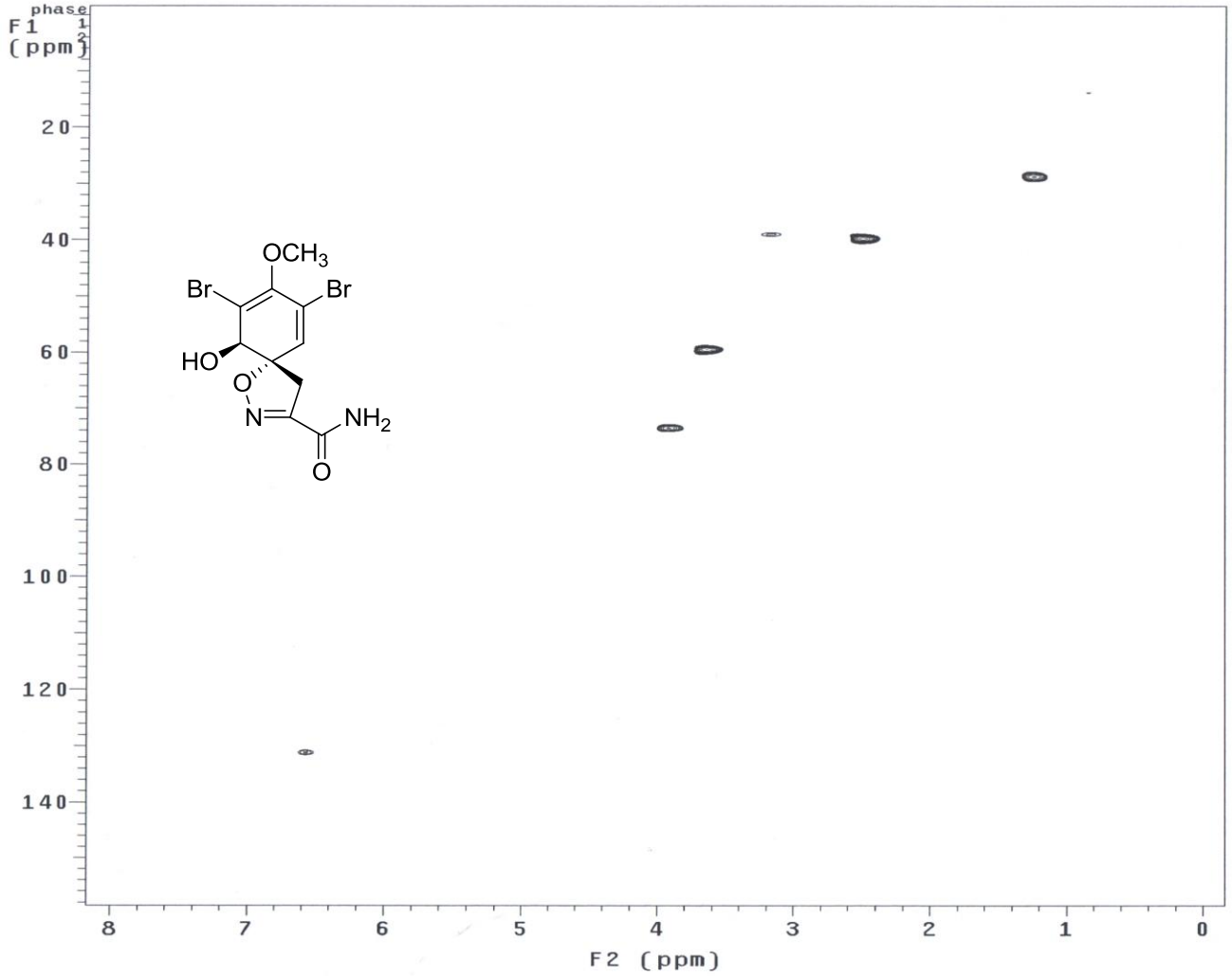
IR spectrum of **13** (KBr)



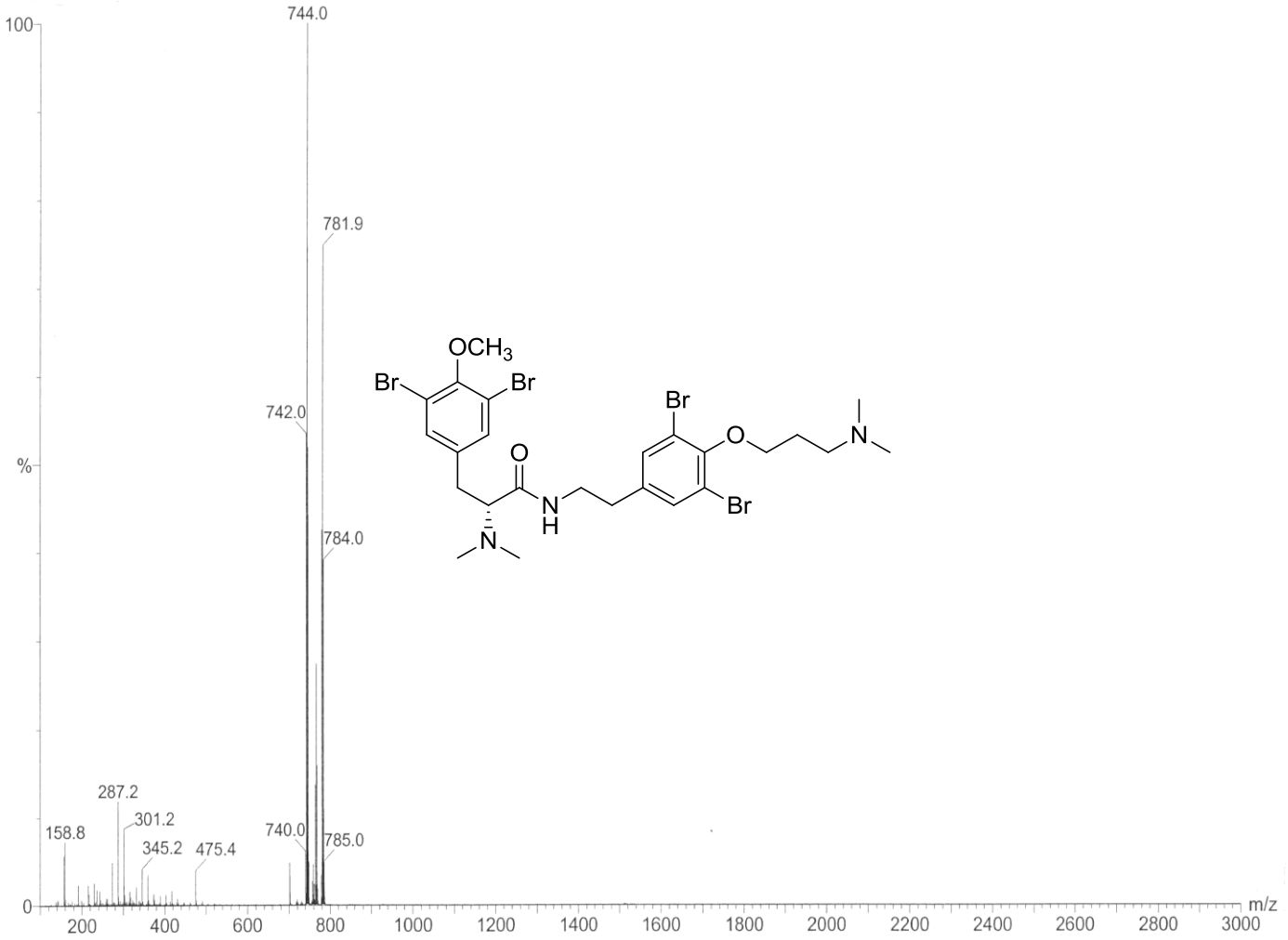
UV spectrum of **13** (CH_3OH)



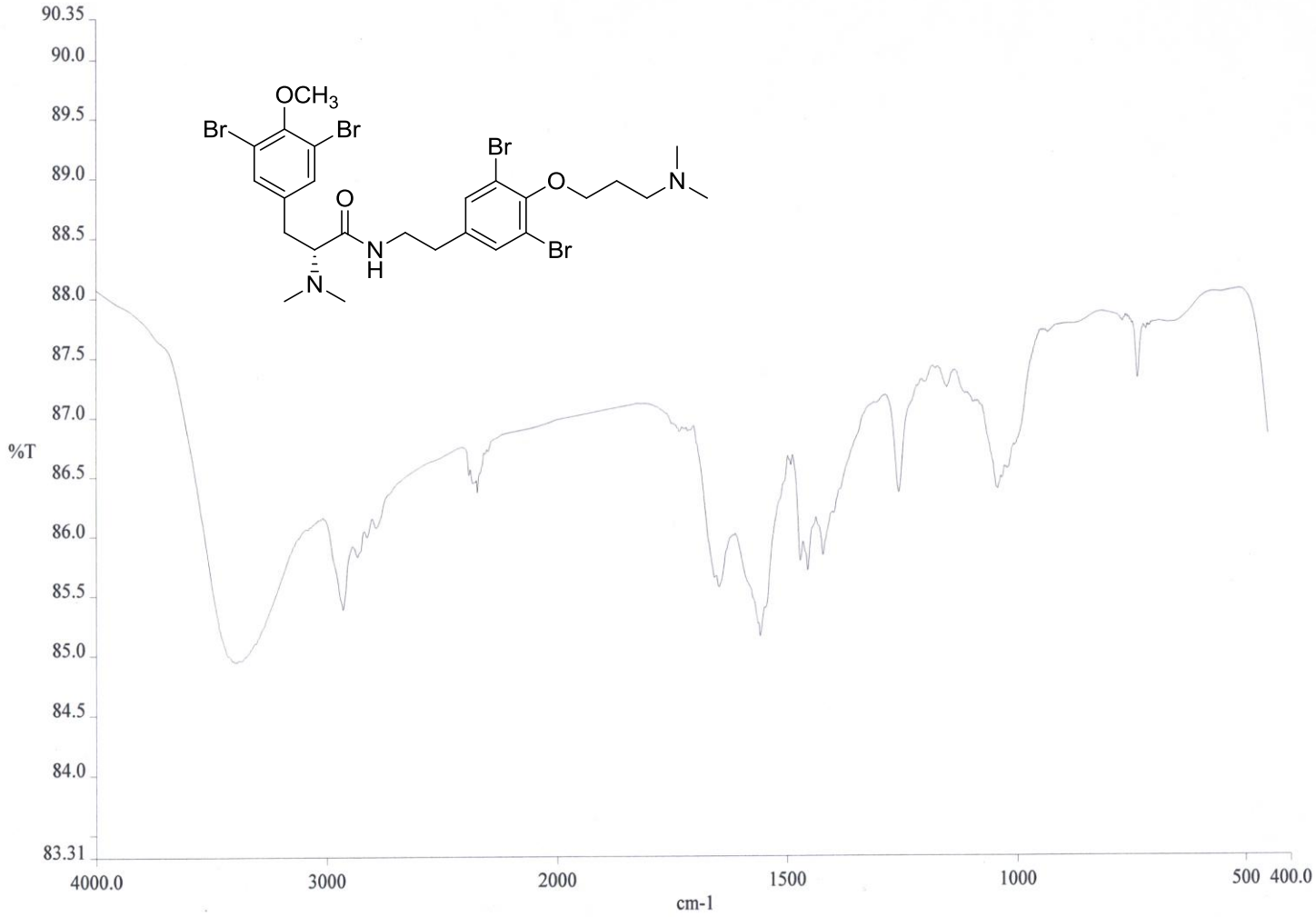
HMBC spectrum of **13** (DMSO-d_6)



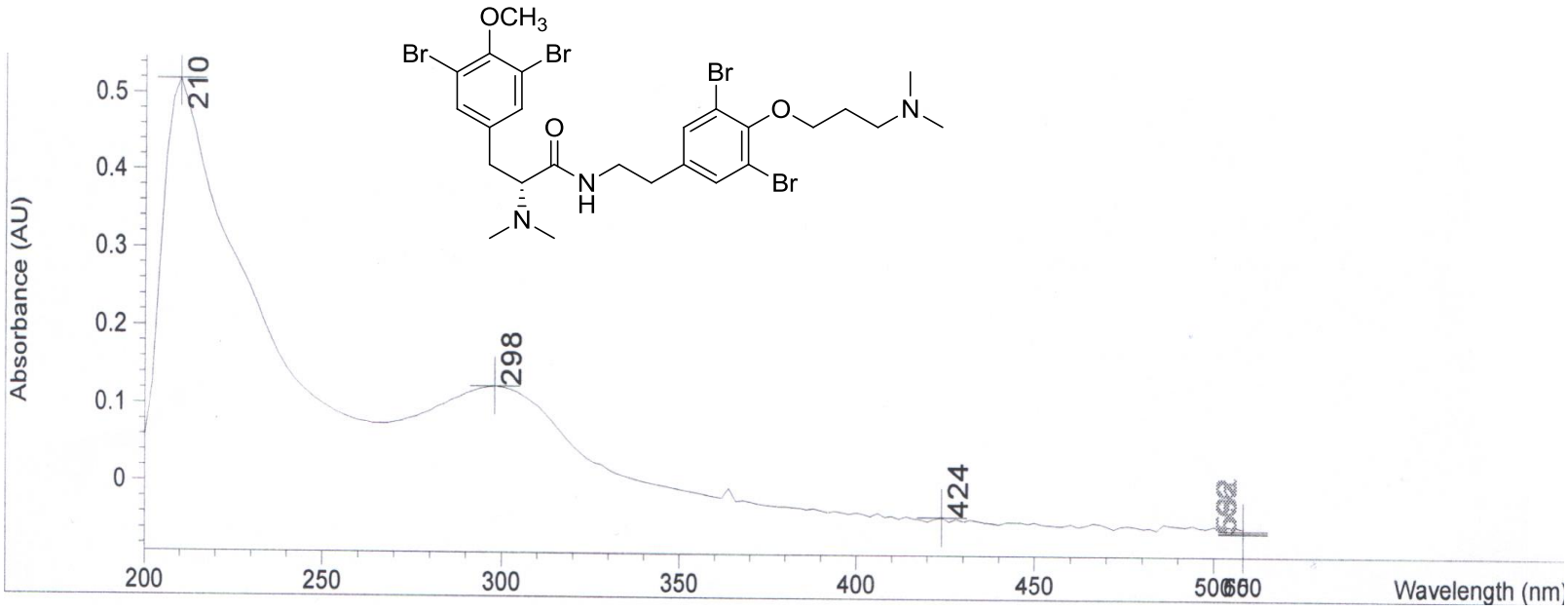
HMQC spectrum of **13** (DMSO-d_6)



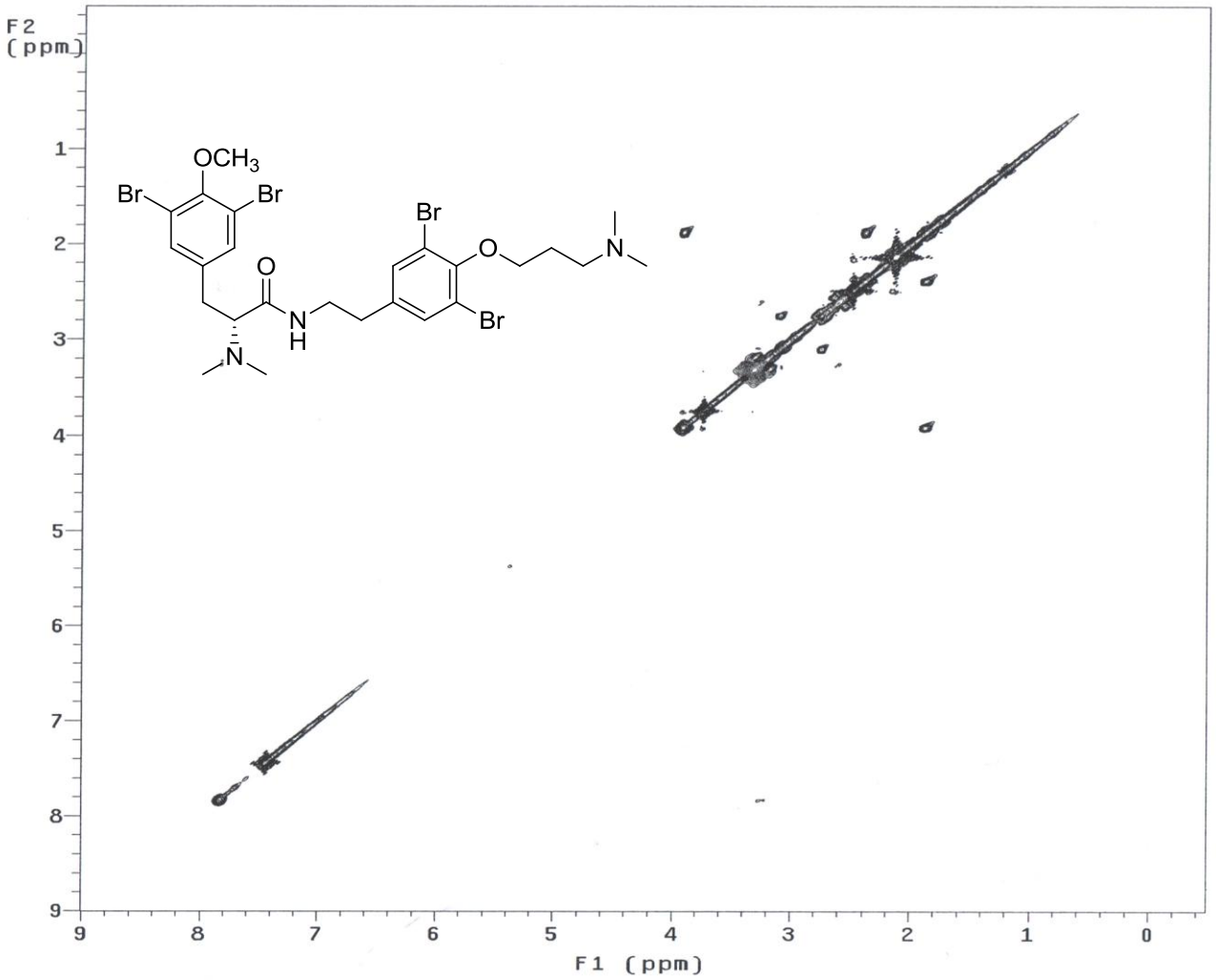
ESI mass spectrum of **14**



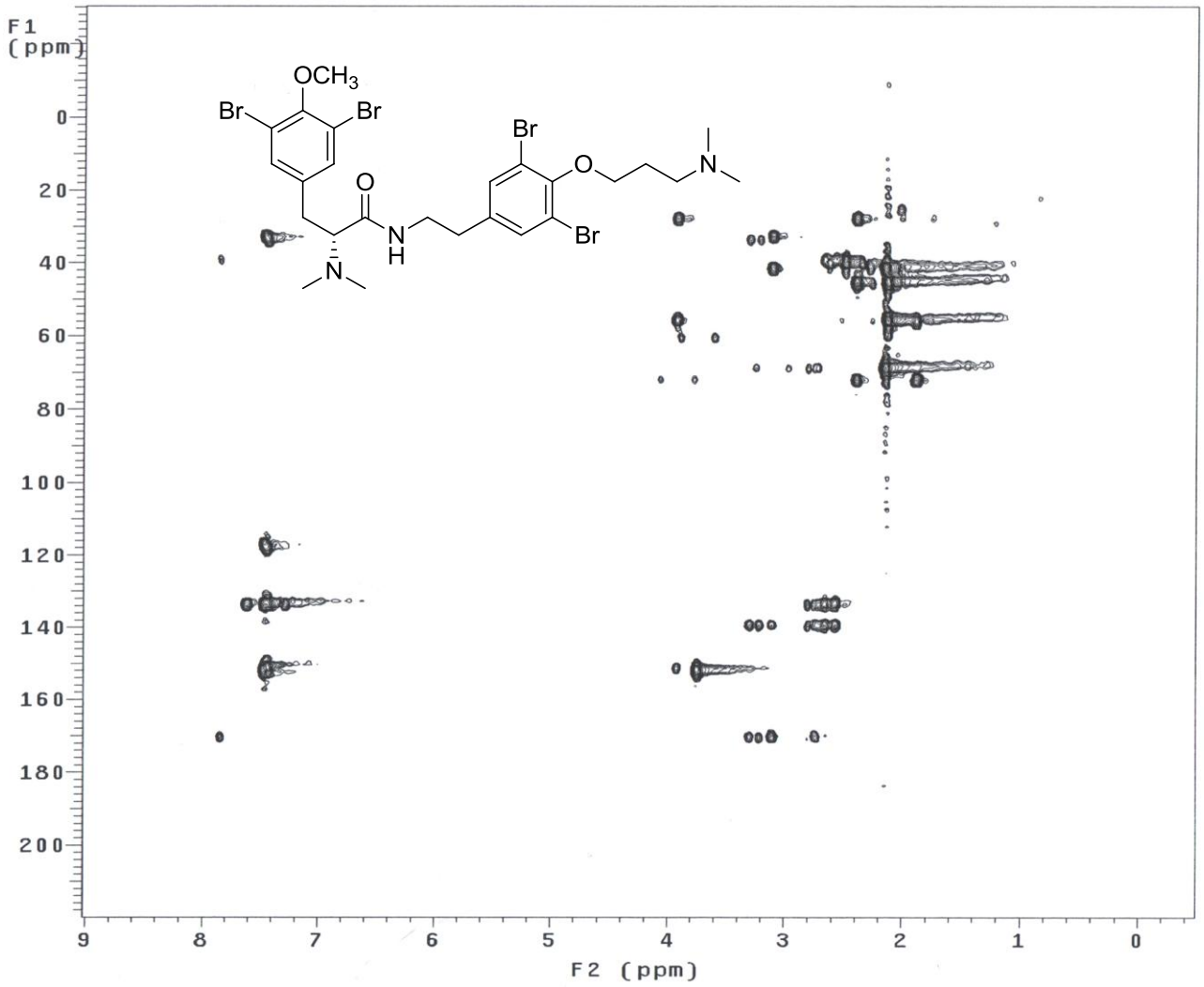
IR spectrum of **14** (KBr)



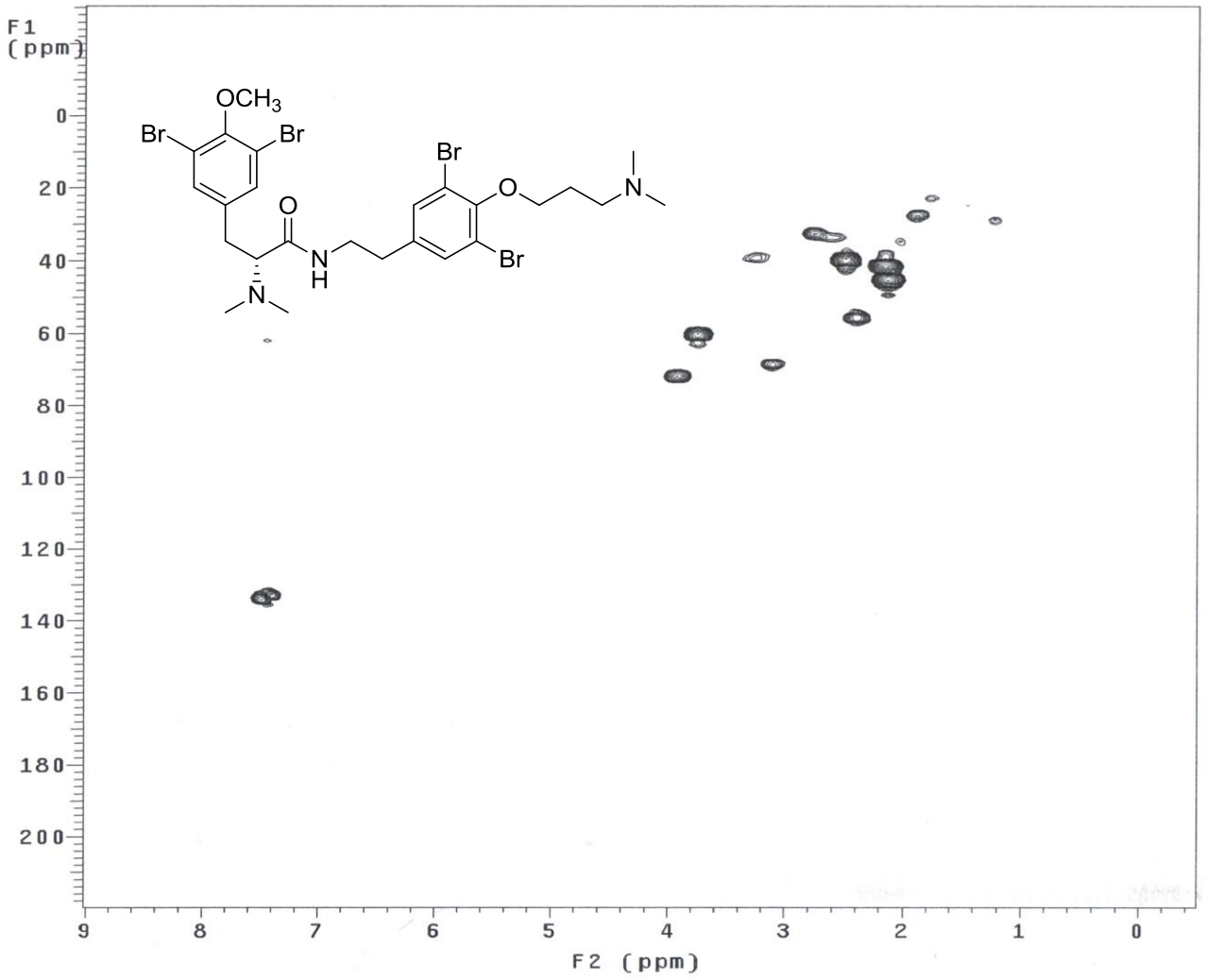
UV spectrum of **14** (CH_3OH)



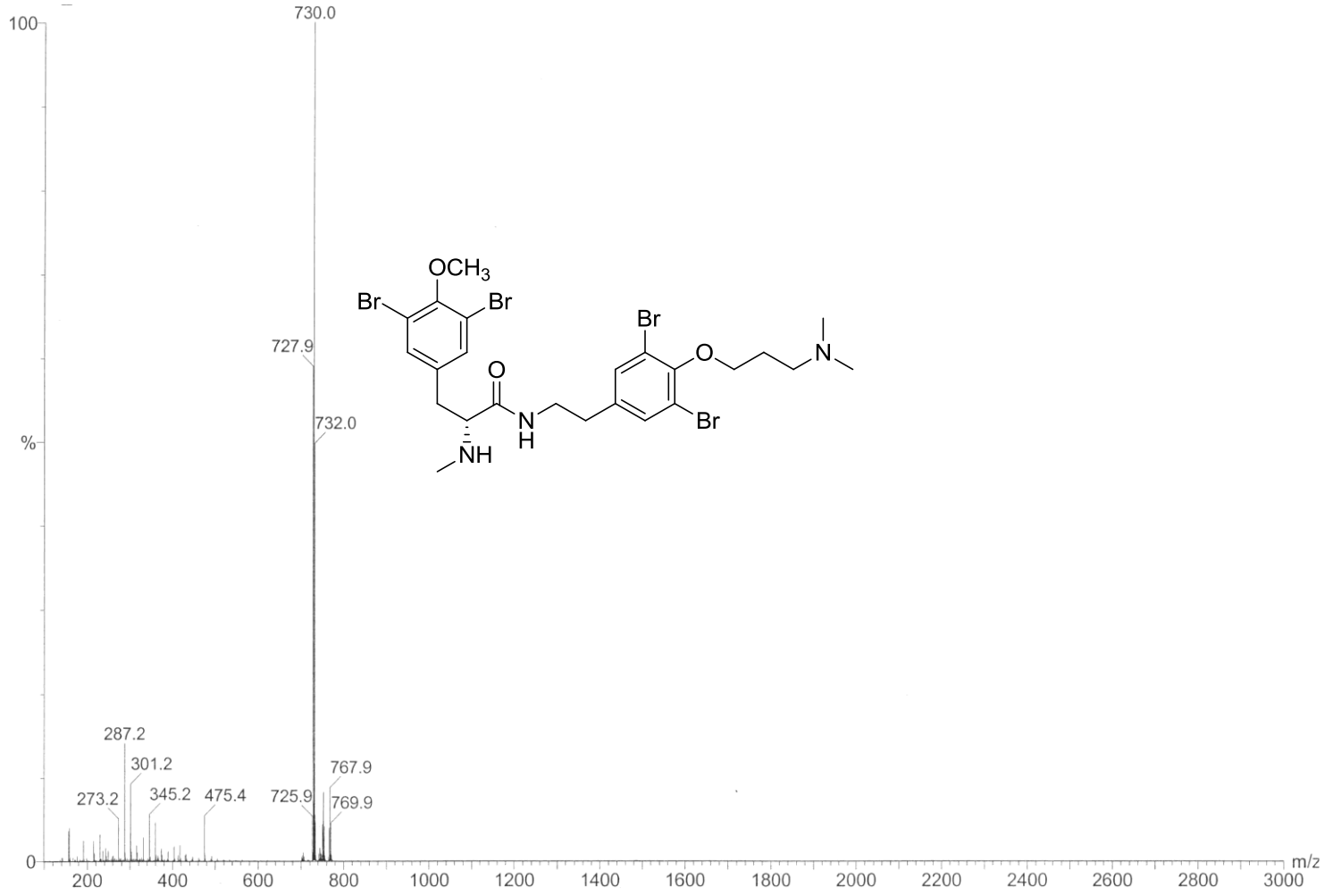
^{1}H-^{1}H COSY spectrum of **14** (500 MHz, DMSO-d_6)



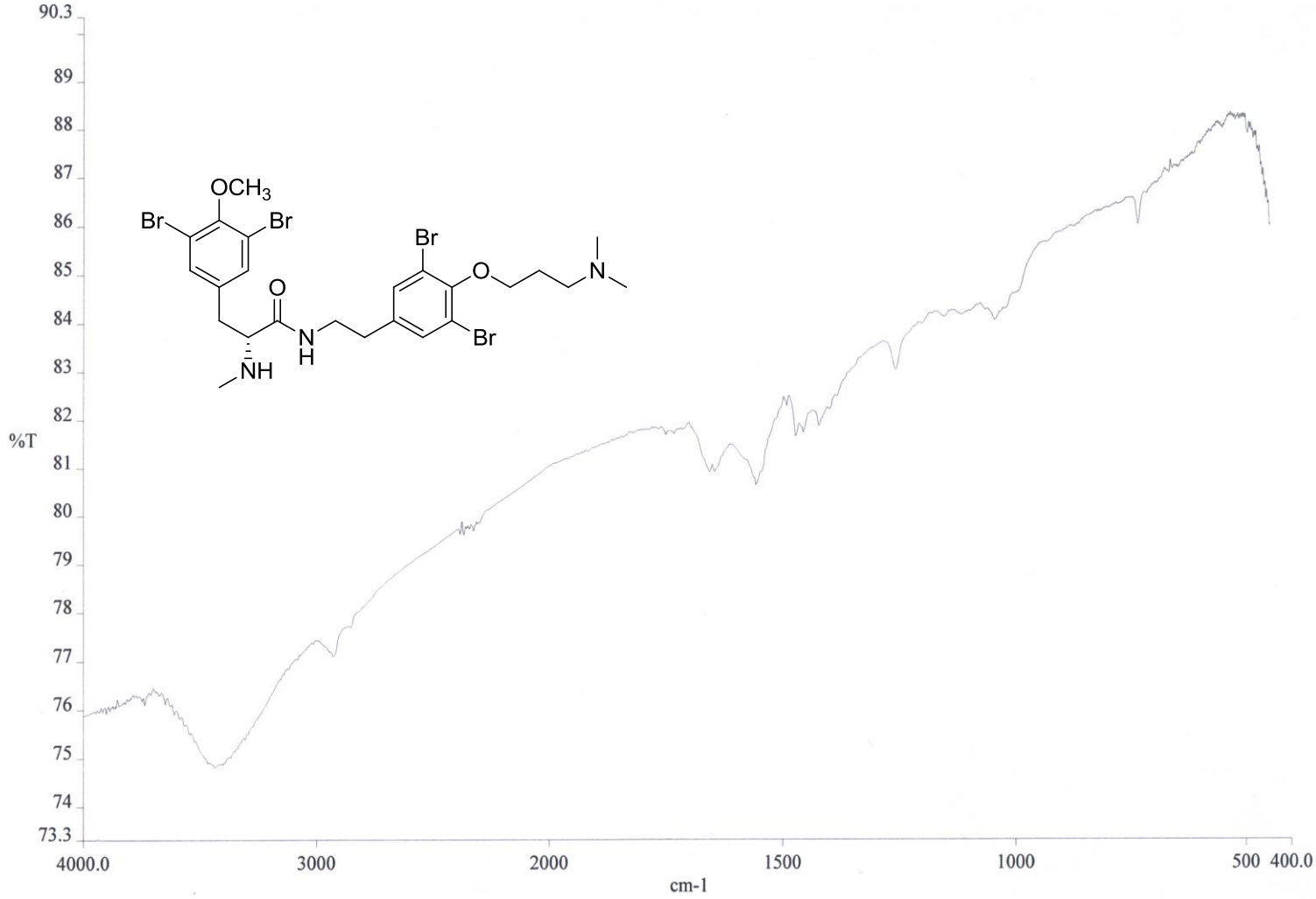
HMBC spectrum of **14** (500 MHz, DMSO-d_6)



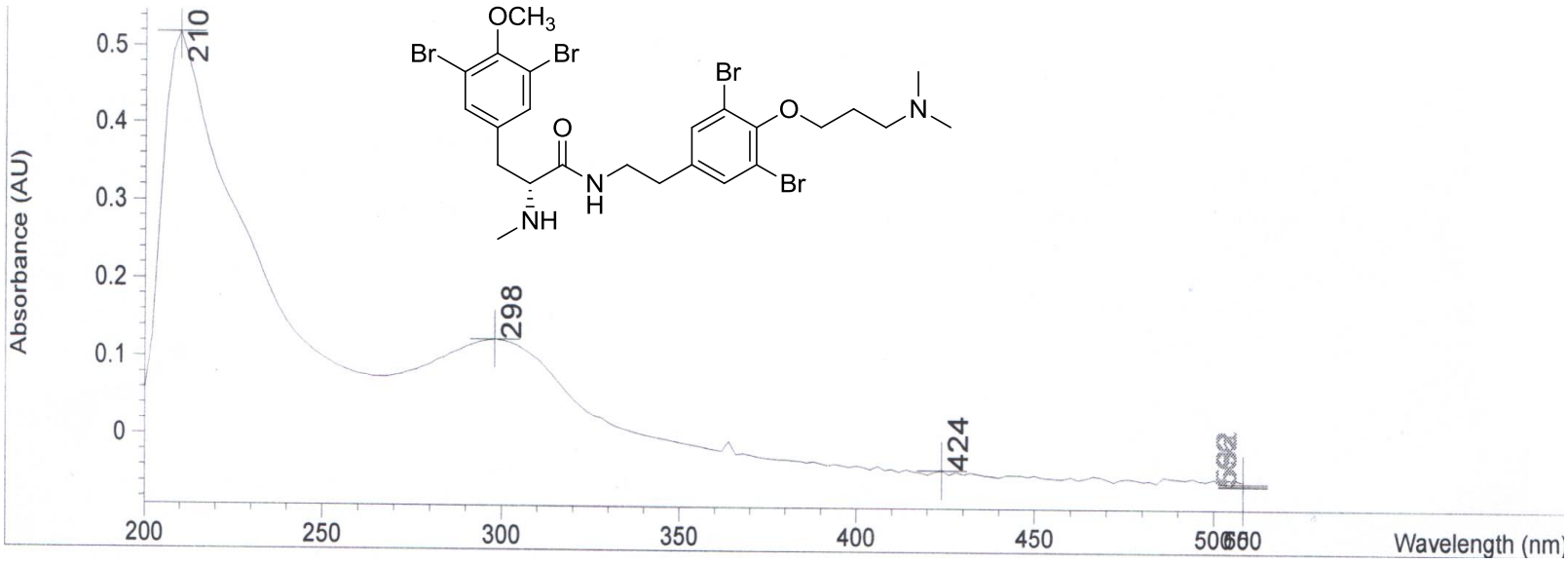
HMQC spectrum of **14** (500 MHz, DMSO-d_6)



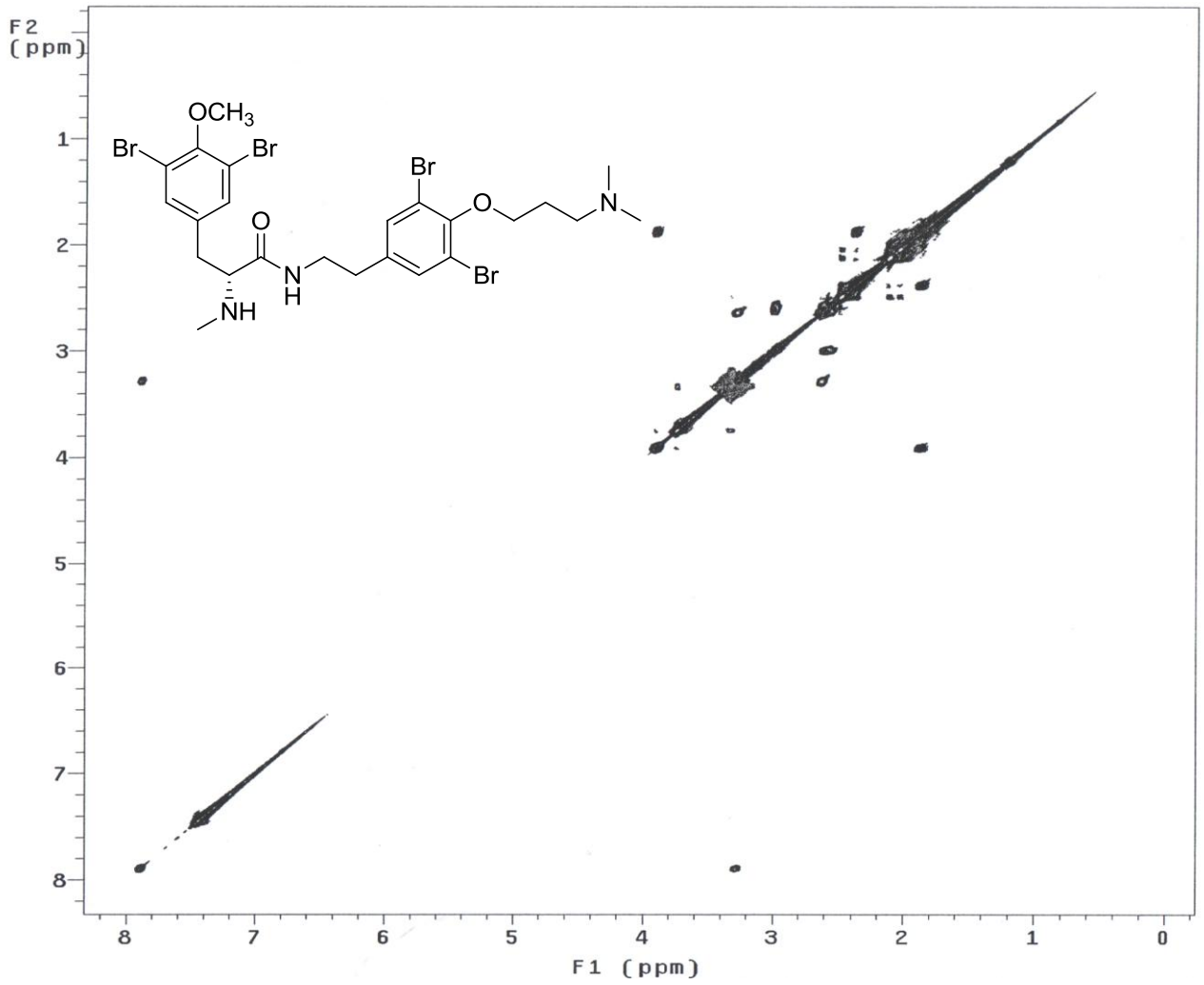
ESI mass spectrum of **15**



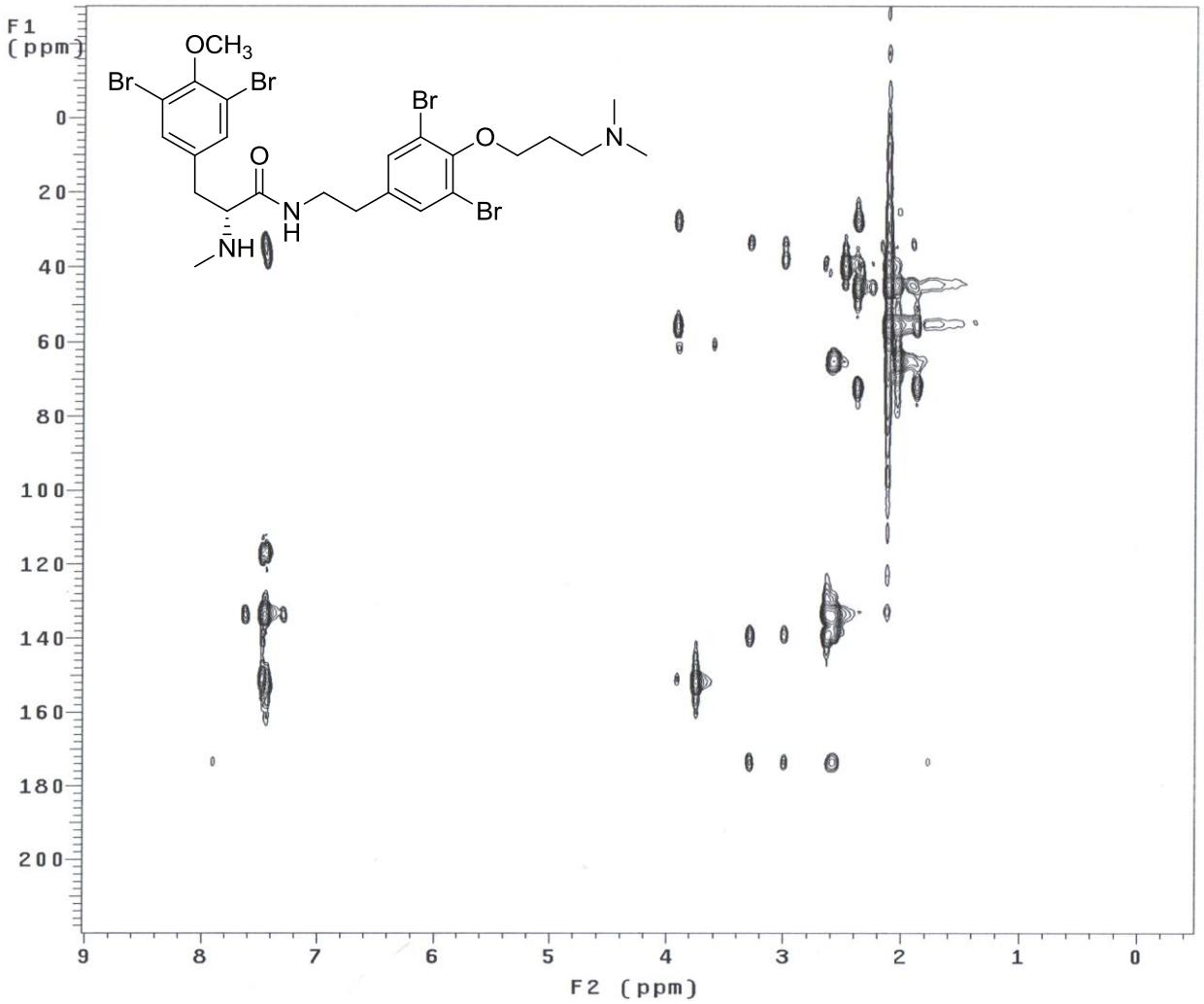
IR spectrum of **15** (KBr)



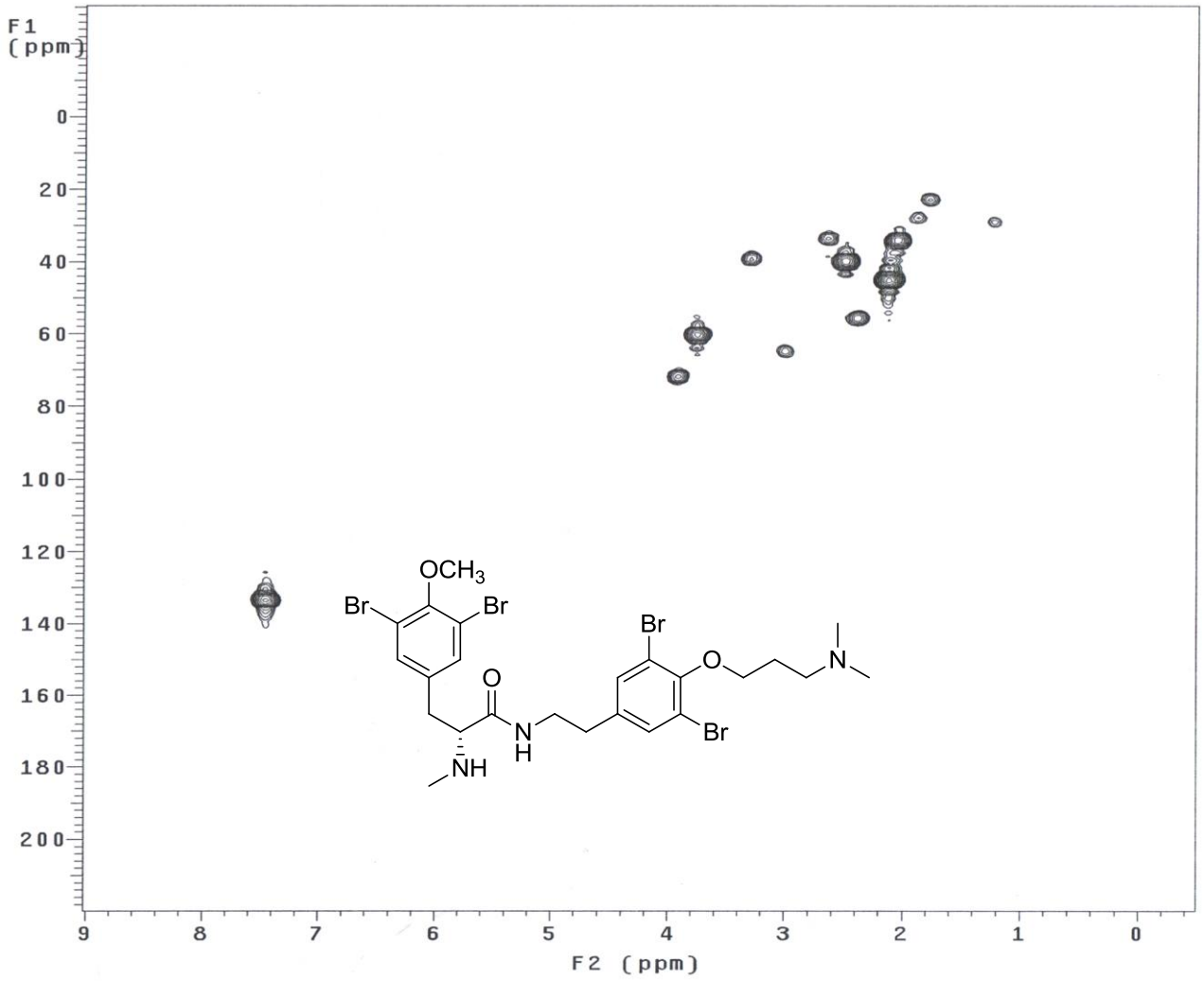
UV spectrum of **15** (CH_3OH)



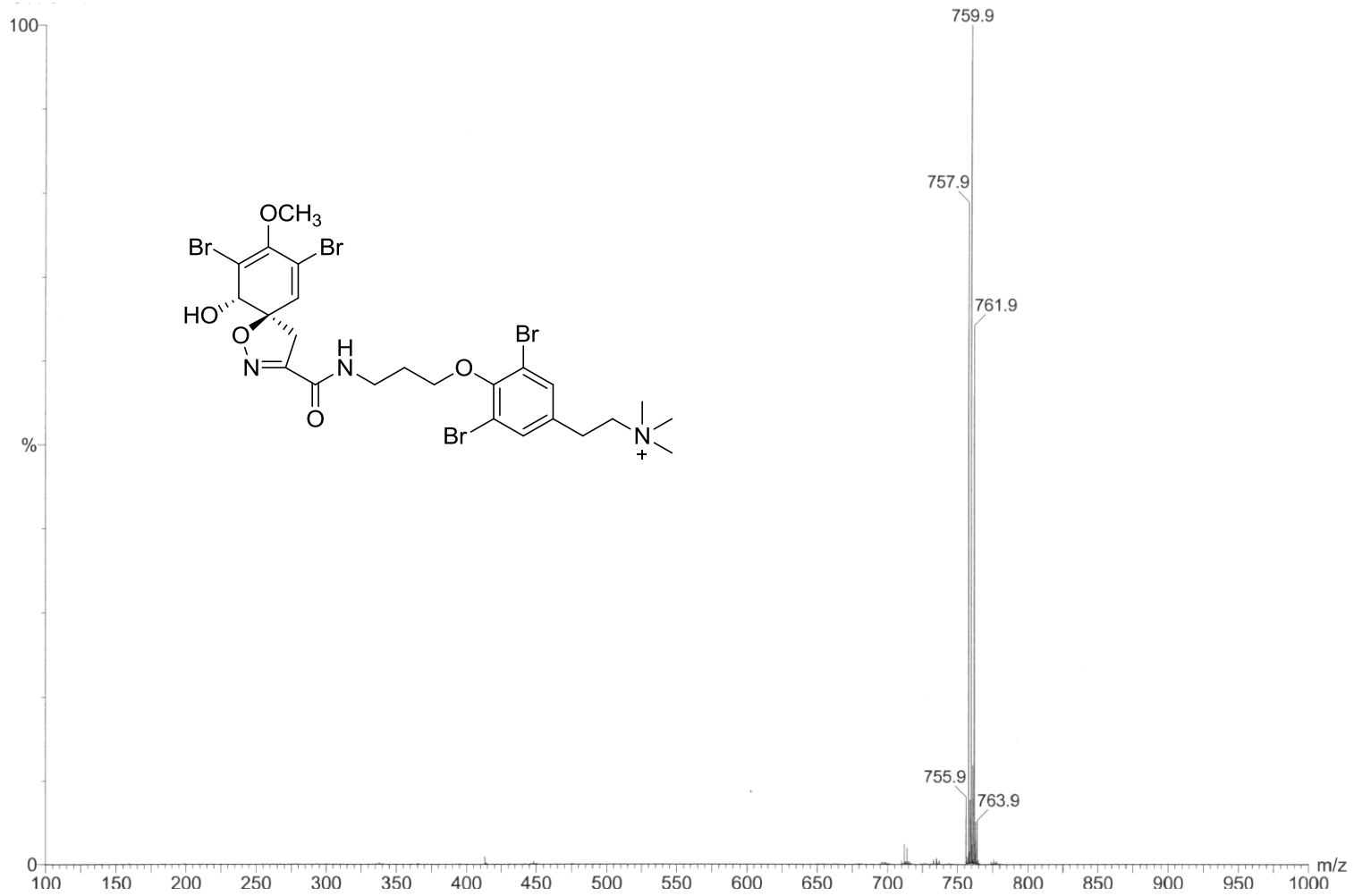
^{1}H-^{1}H COSY spectrum of **15** (500 MHz, DMSO-d_6)



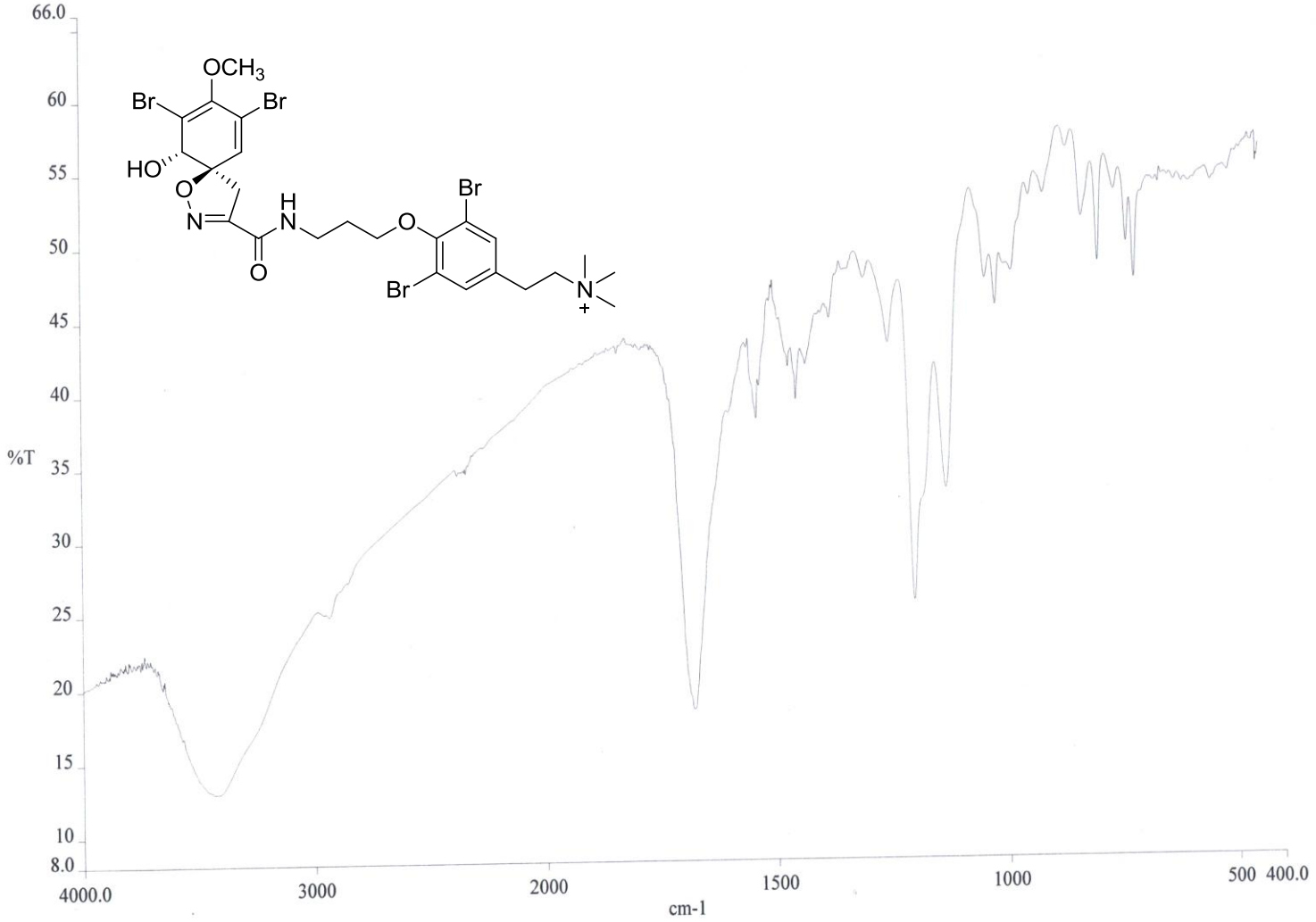
HMBC spectrum of **15** (500 MHz, DMSO-d_6)



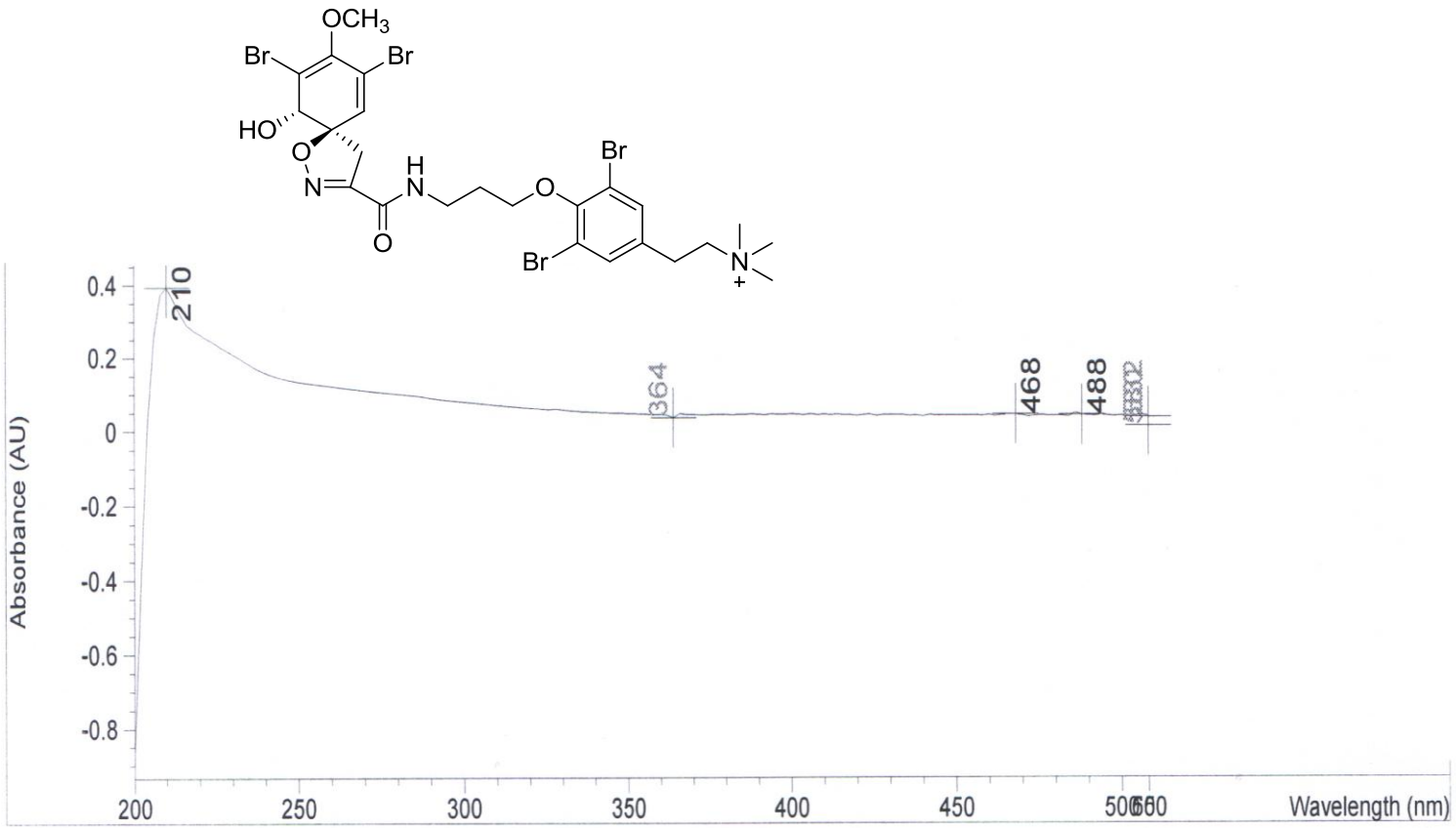
HMQC spectrum of **15** (500 MHz, DMSO-d_6)



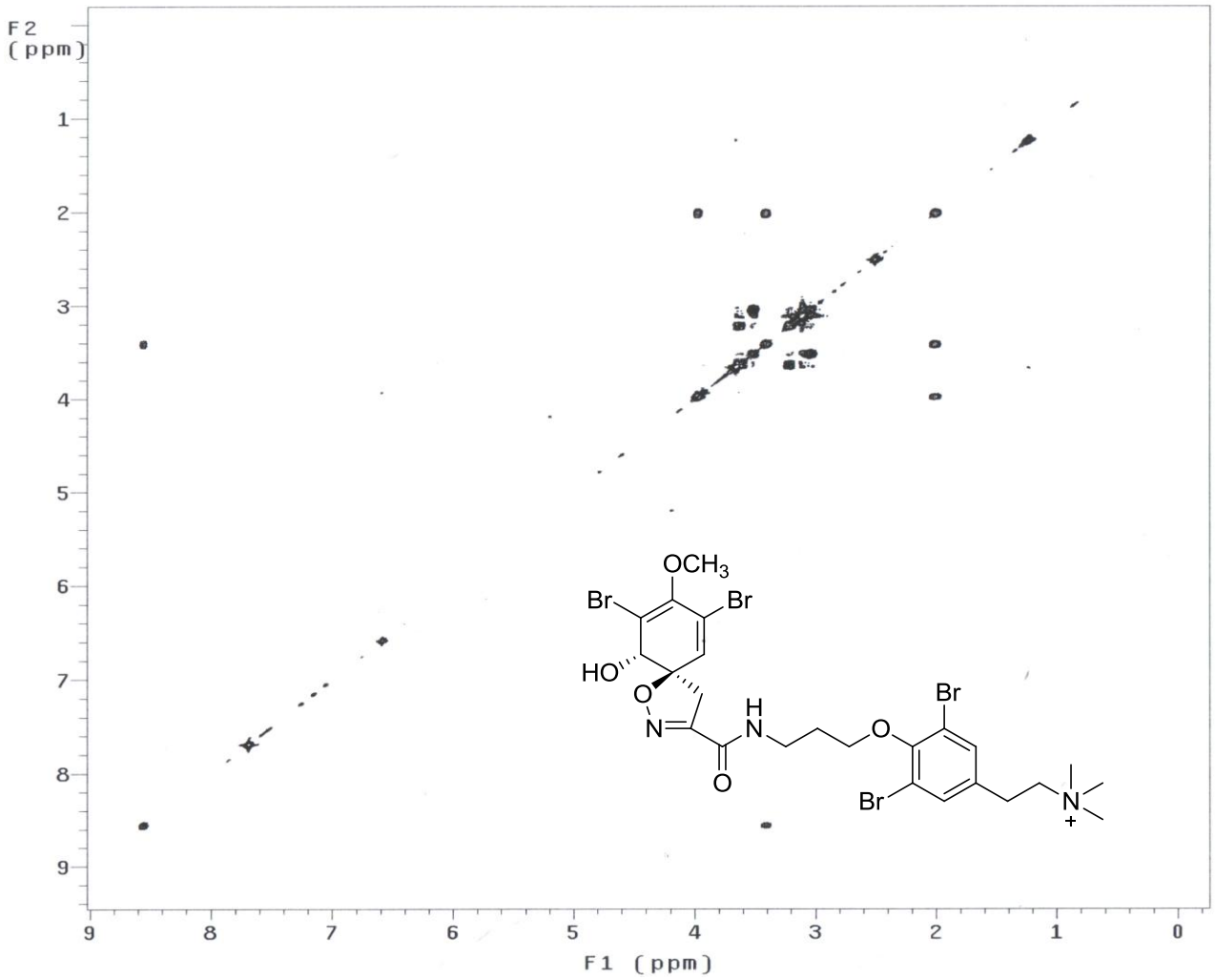
ESI mass spectrum of **16**



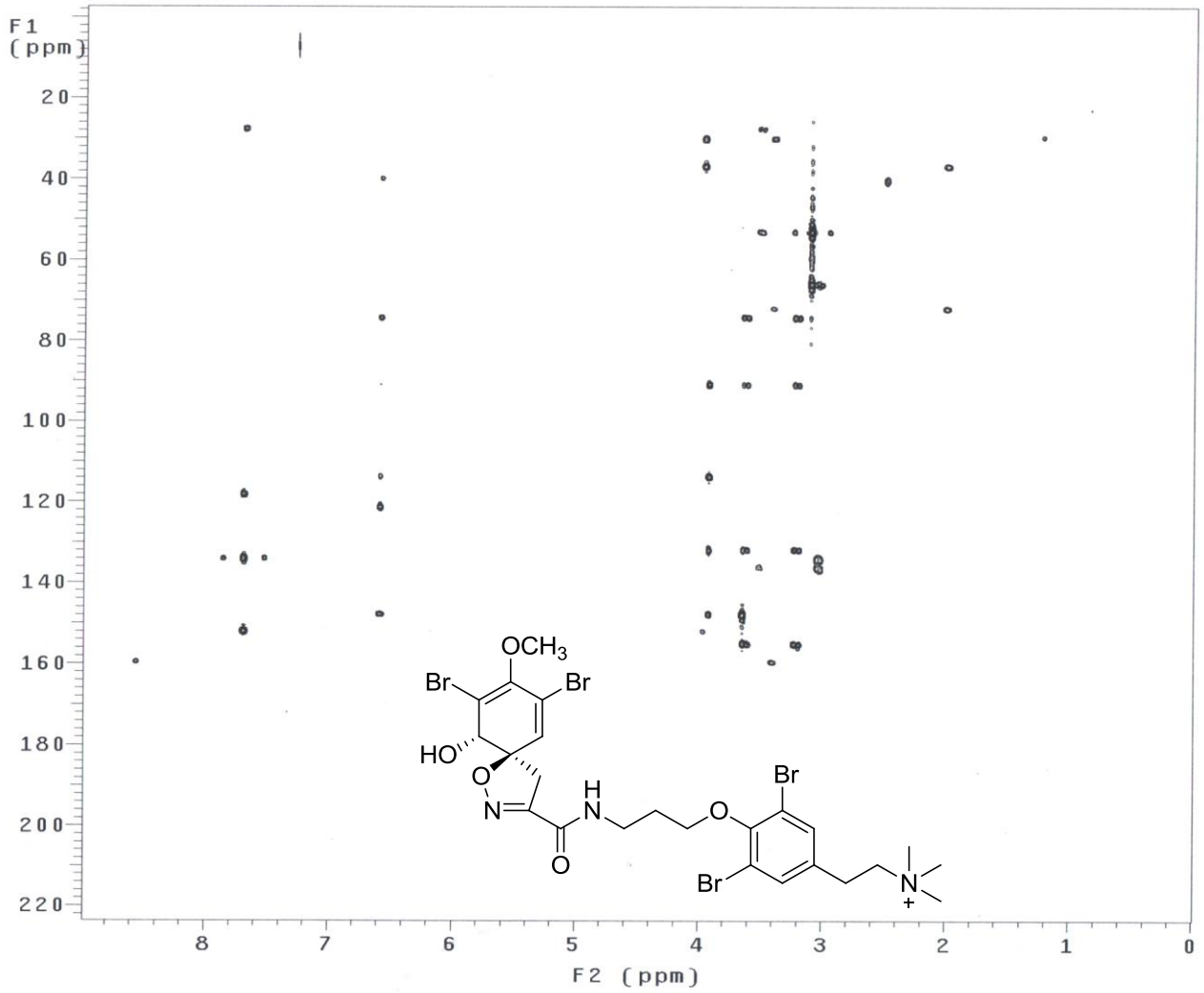
IR spectrum of **16** (KBr)



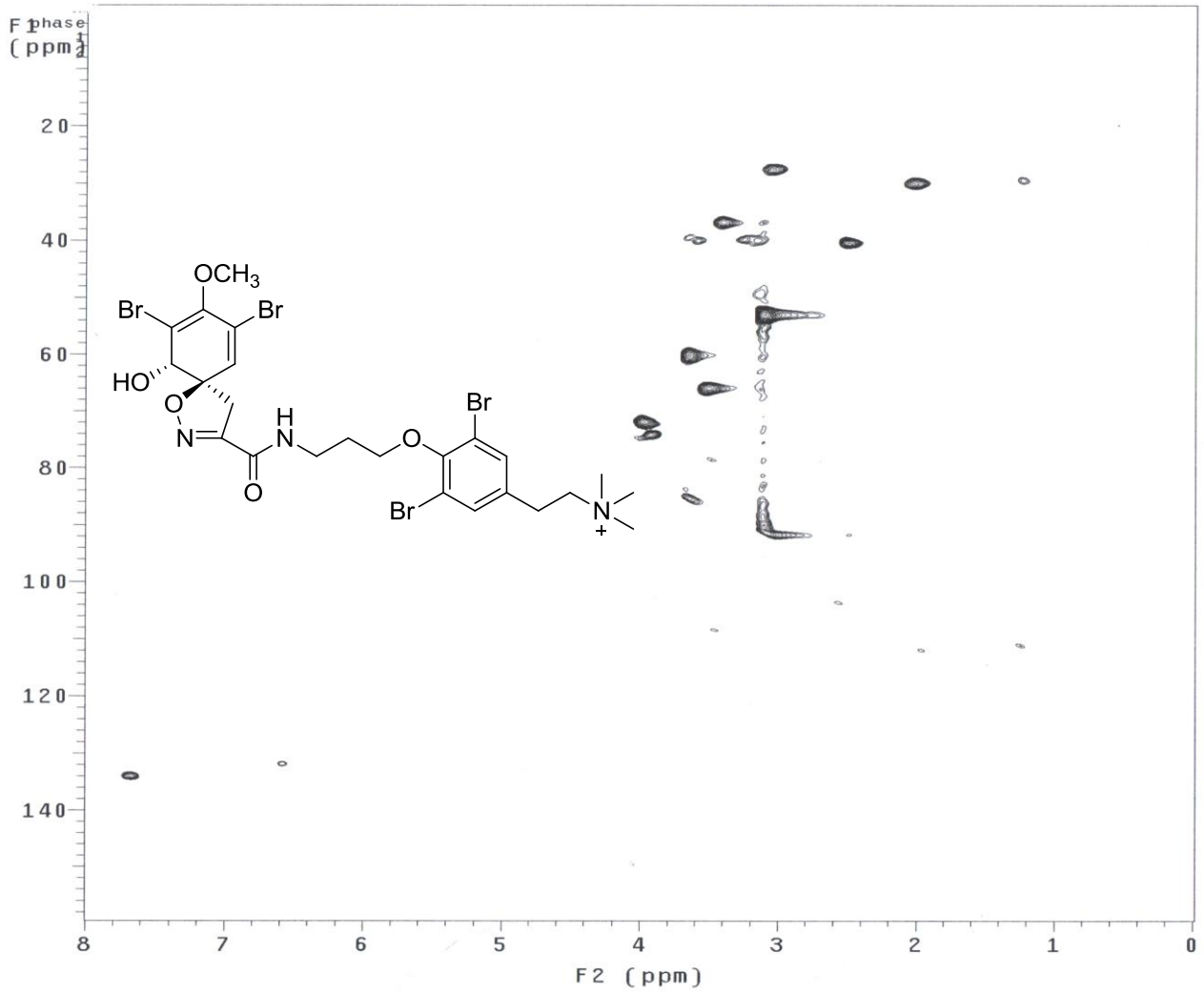
UV spectrum of **16** (CH_3OH)



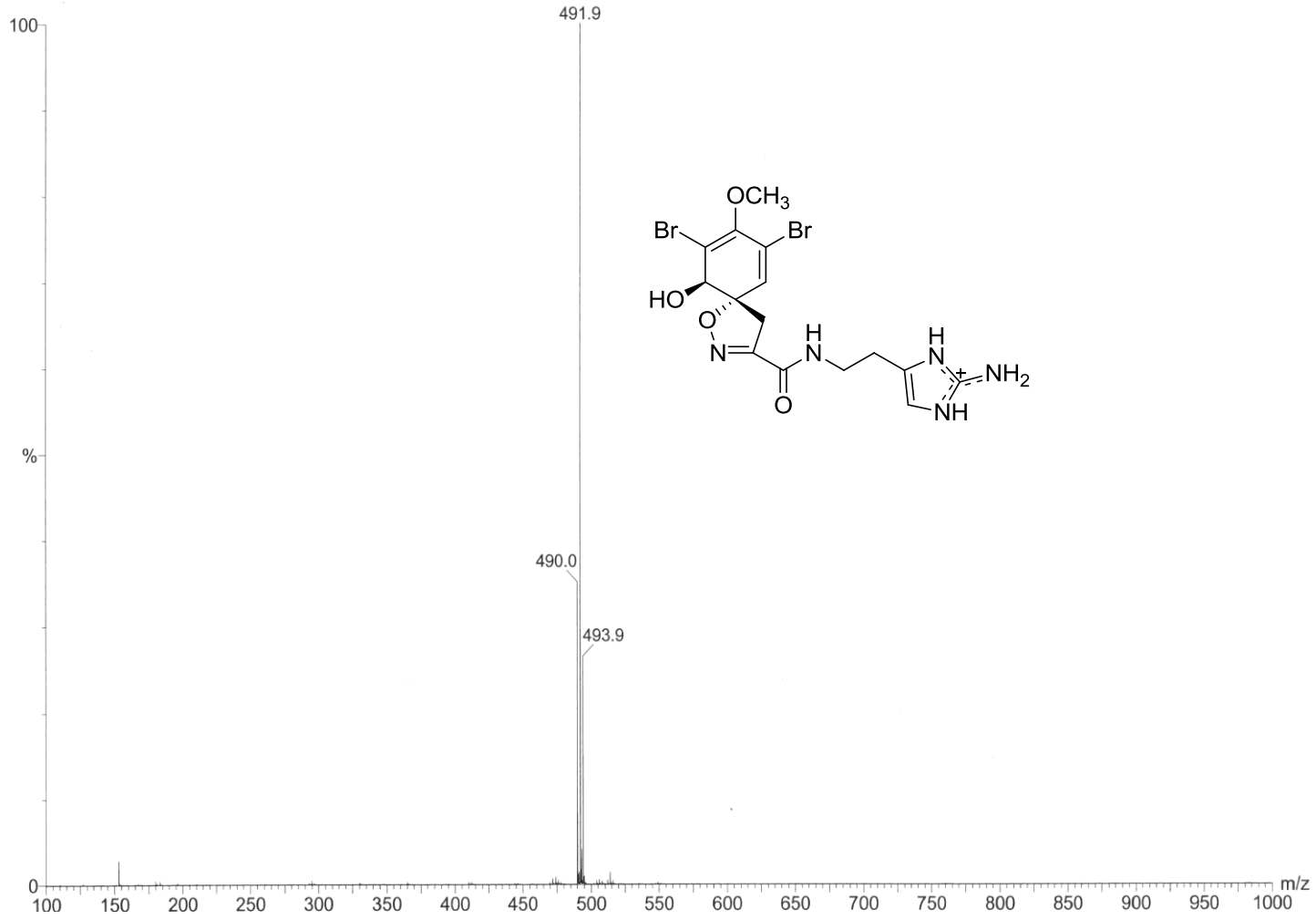
^{1}H-^{1}H COSY spectrum of **16** (500 MHz, DMSO-d_6)



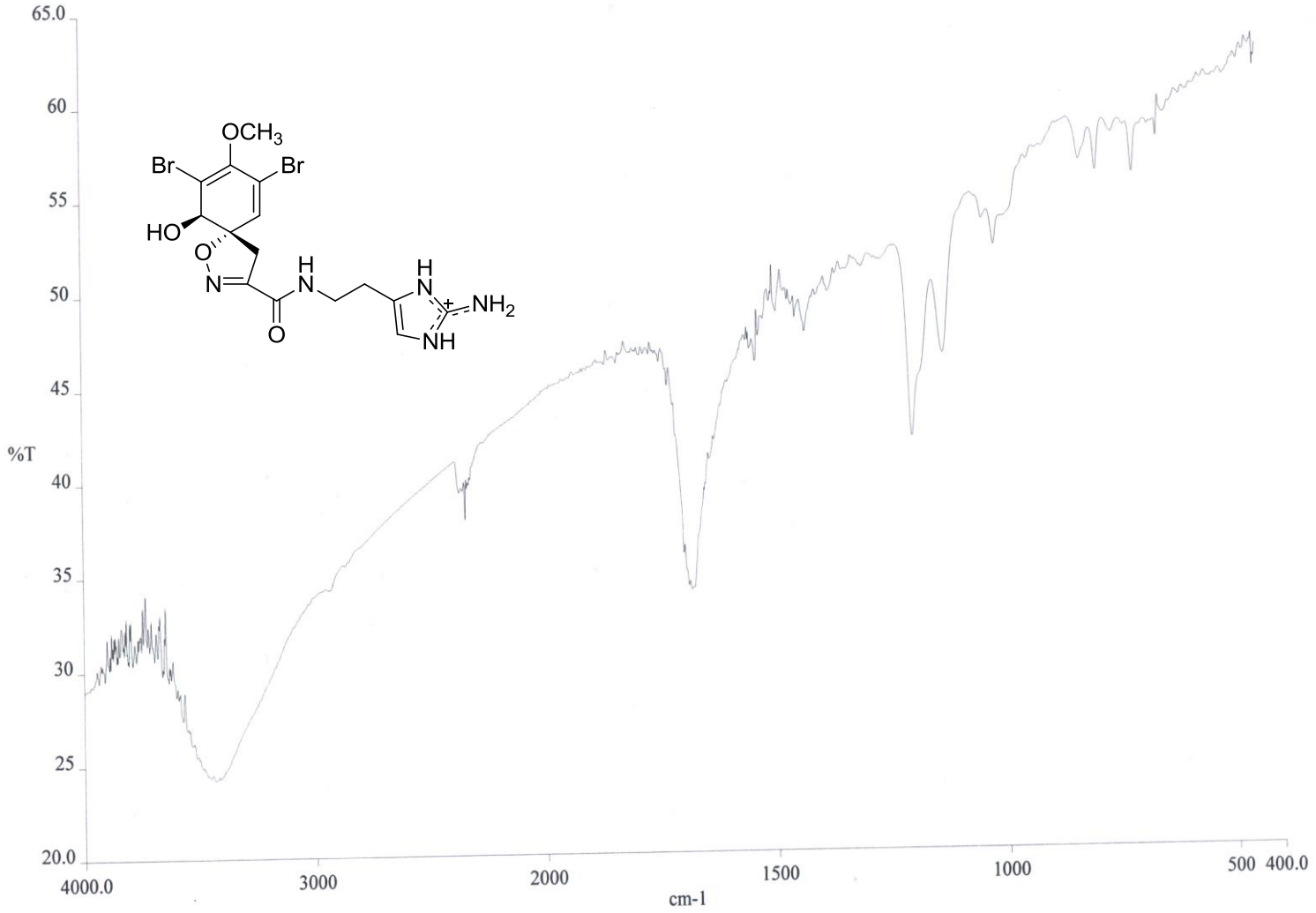
HMBC spectrum of **16** (500 MHz, DMSO-d_6)



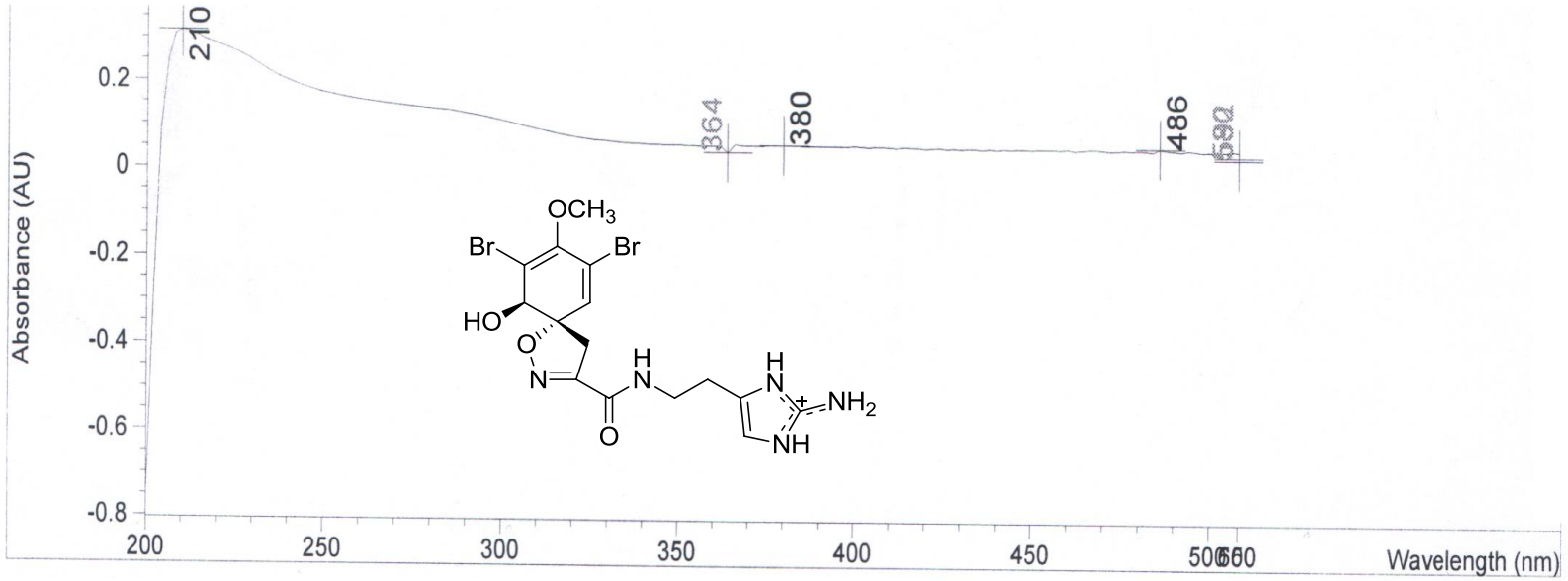
HMQC spectrum of **16** (500 MHz, DMSO-d_6)



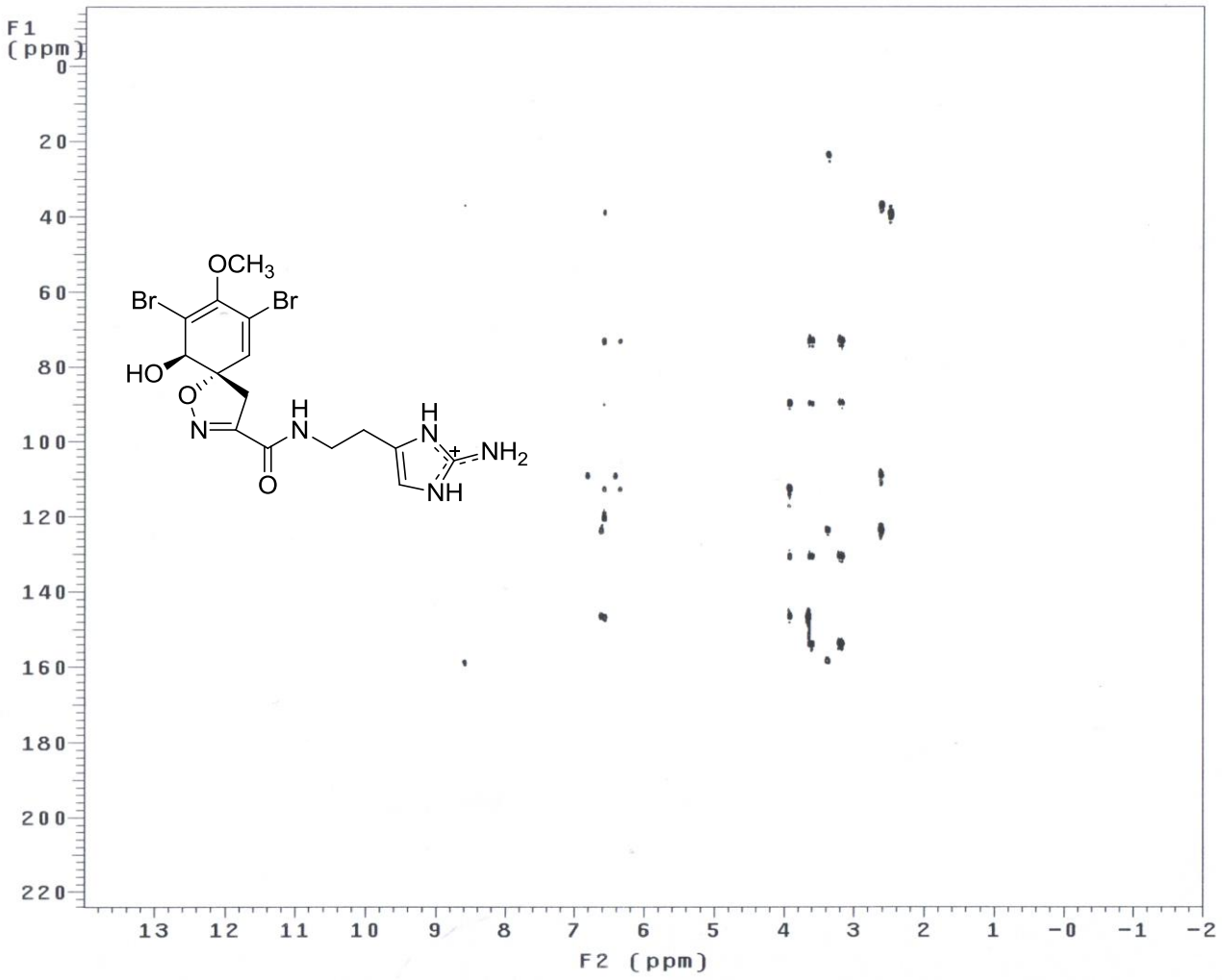
ESI mass spectrum of **17**



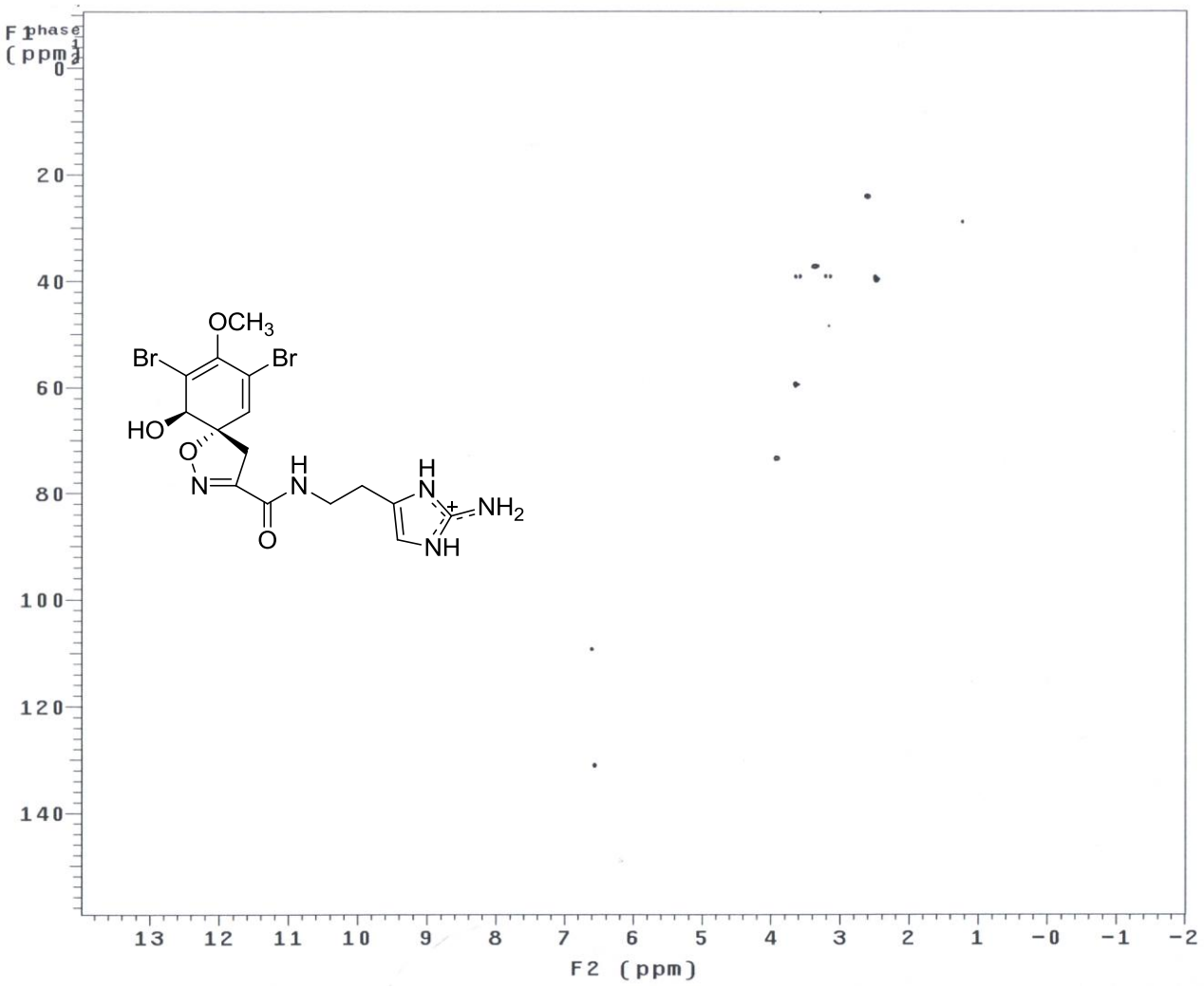
IR spectrum of **17** (KBr)



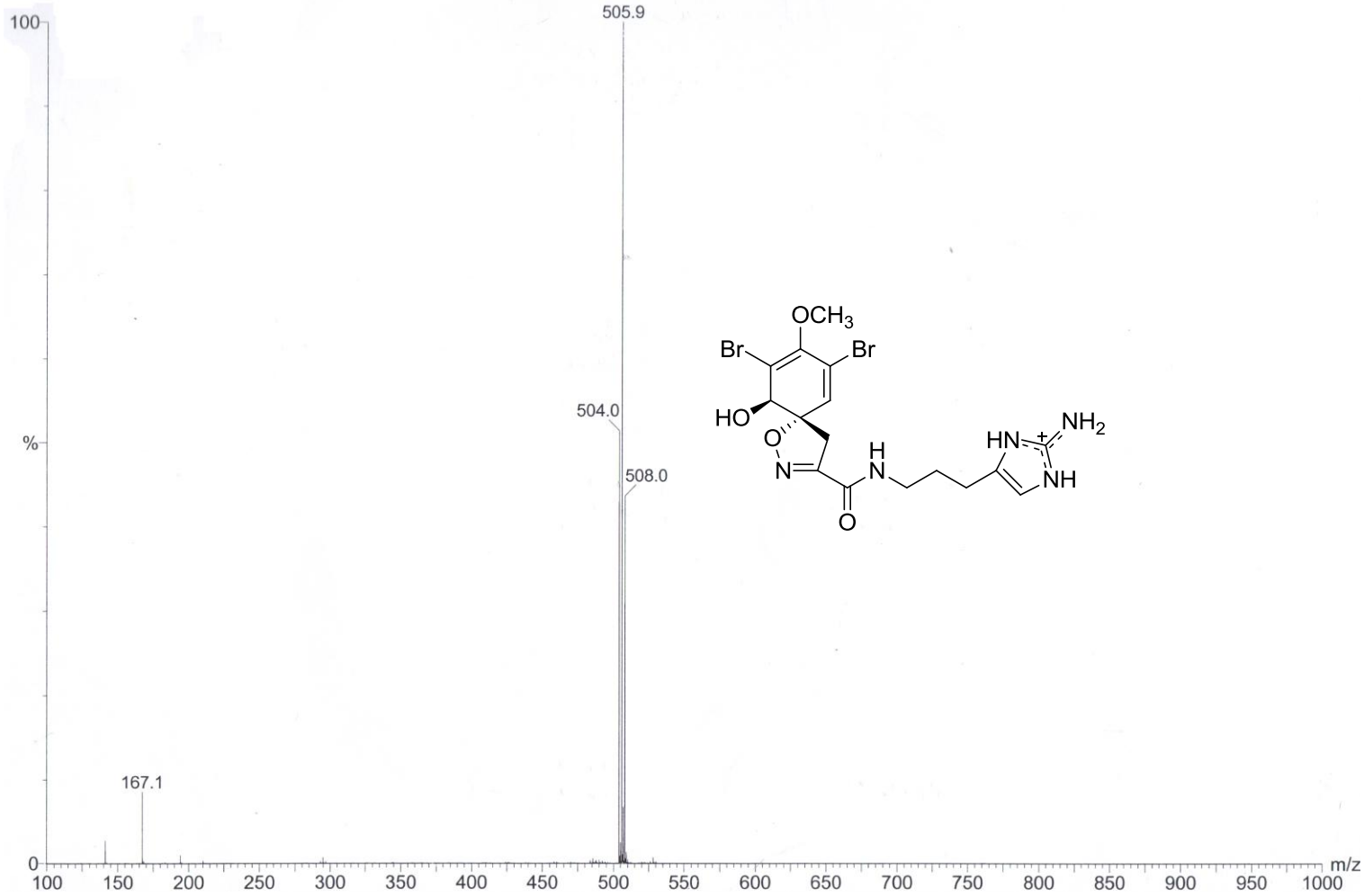
UV spectrum of **17** (CH_3OH)



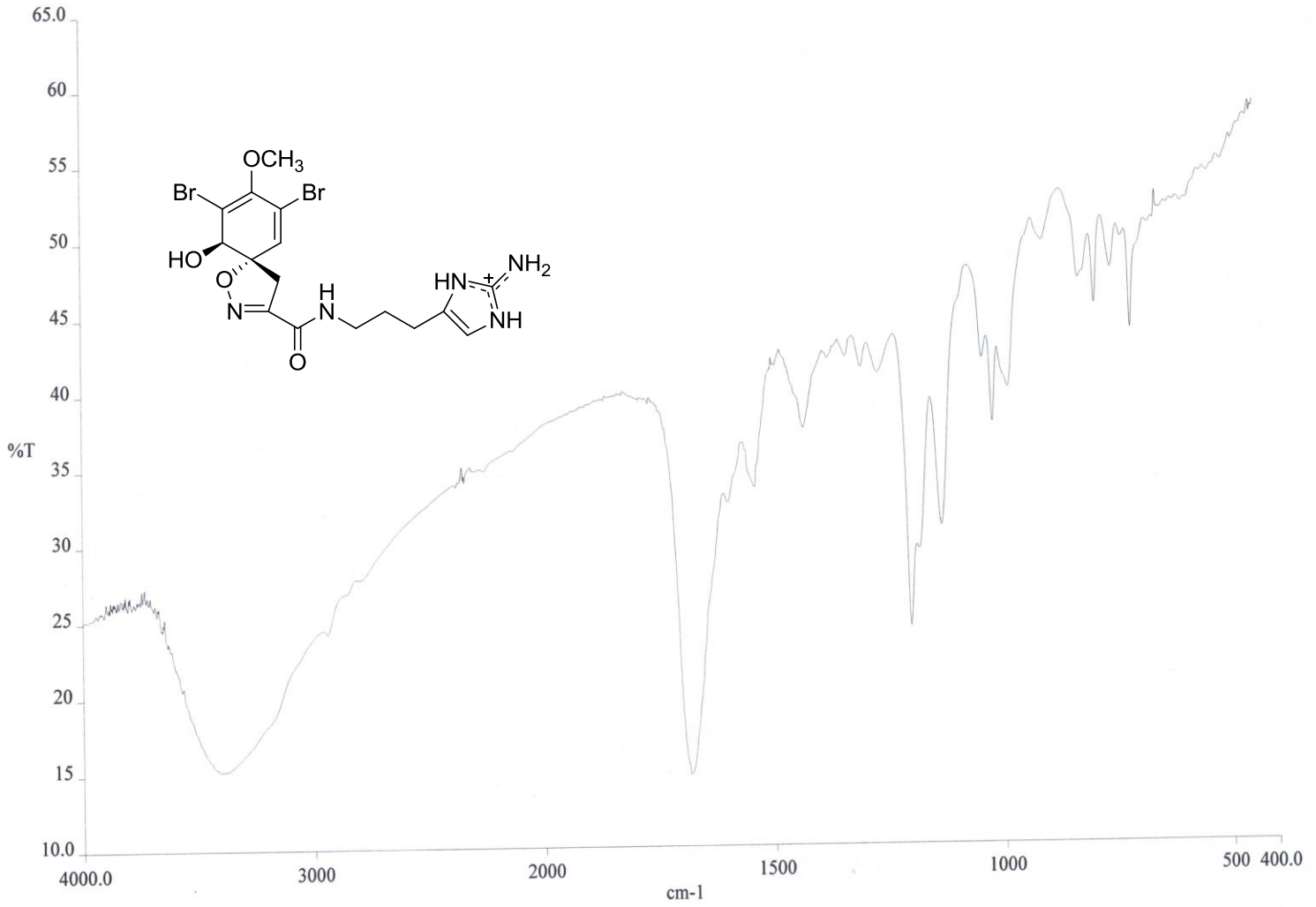
HMBC spectrum of **17** (500 MHz, DMSO-d_6)



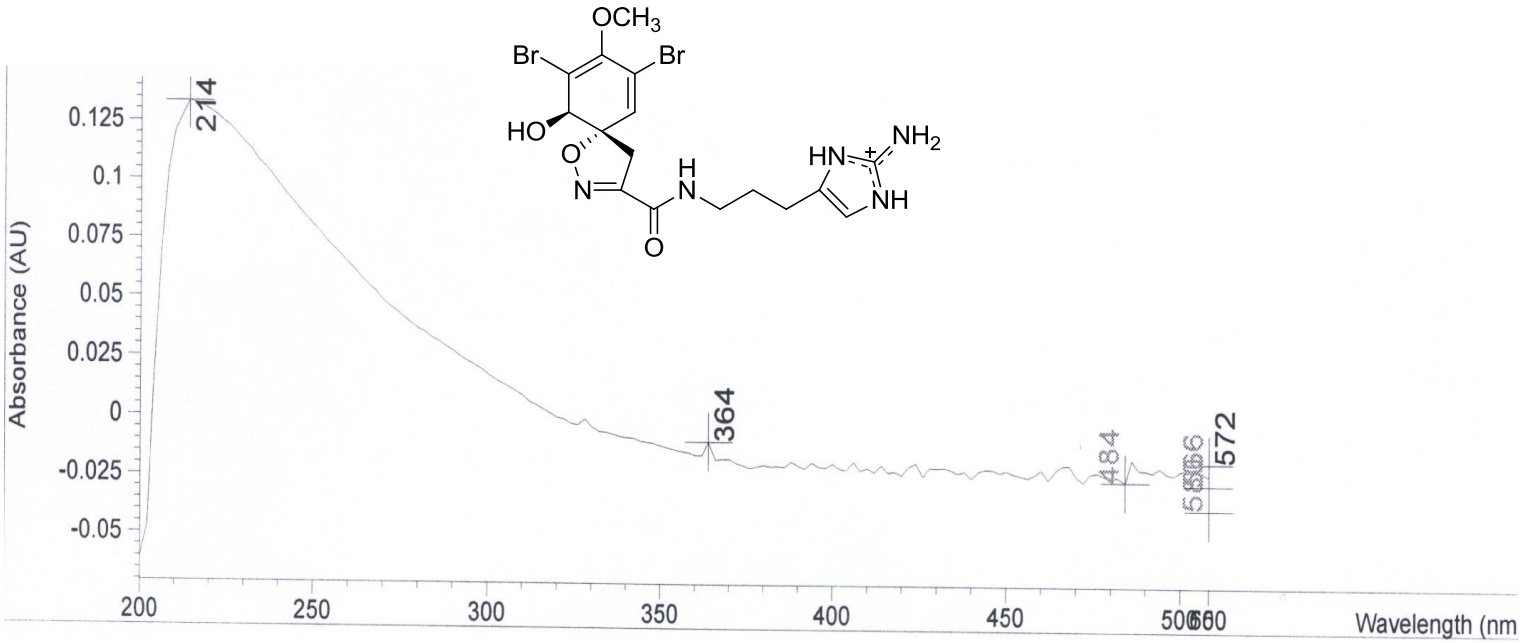
HMQC spectrum of **17** (500 MHz, DMSO-d_6)



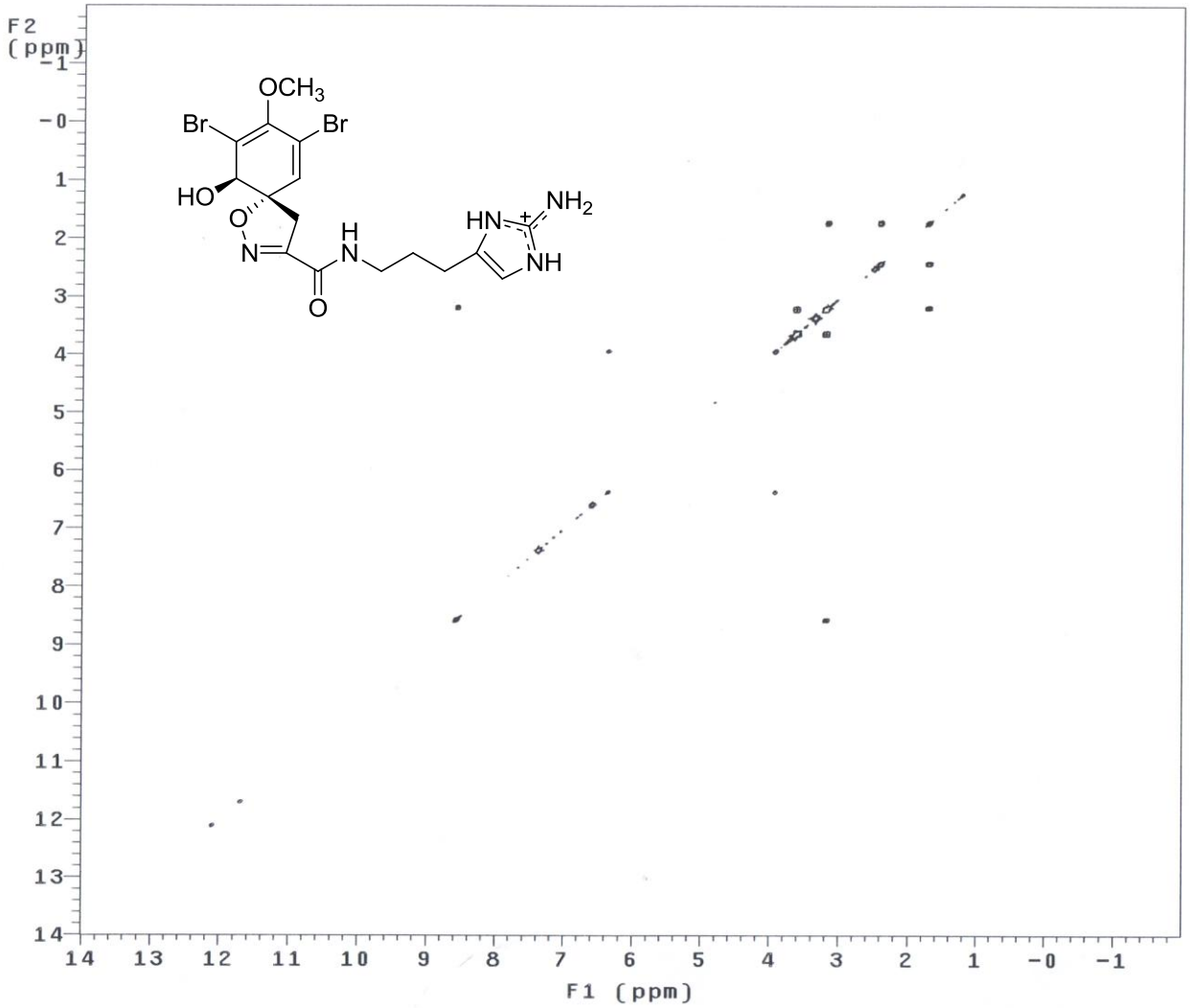
ESI mass spectrum of **18**



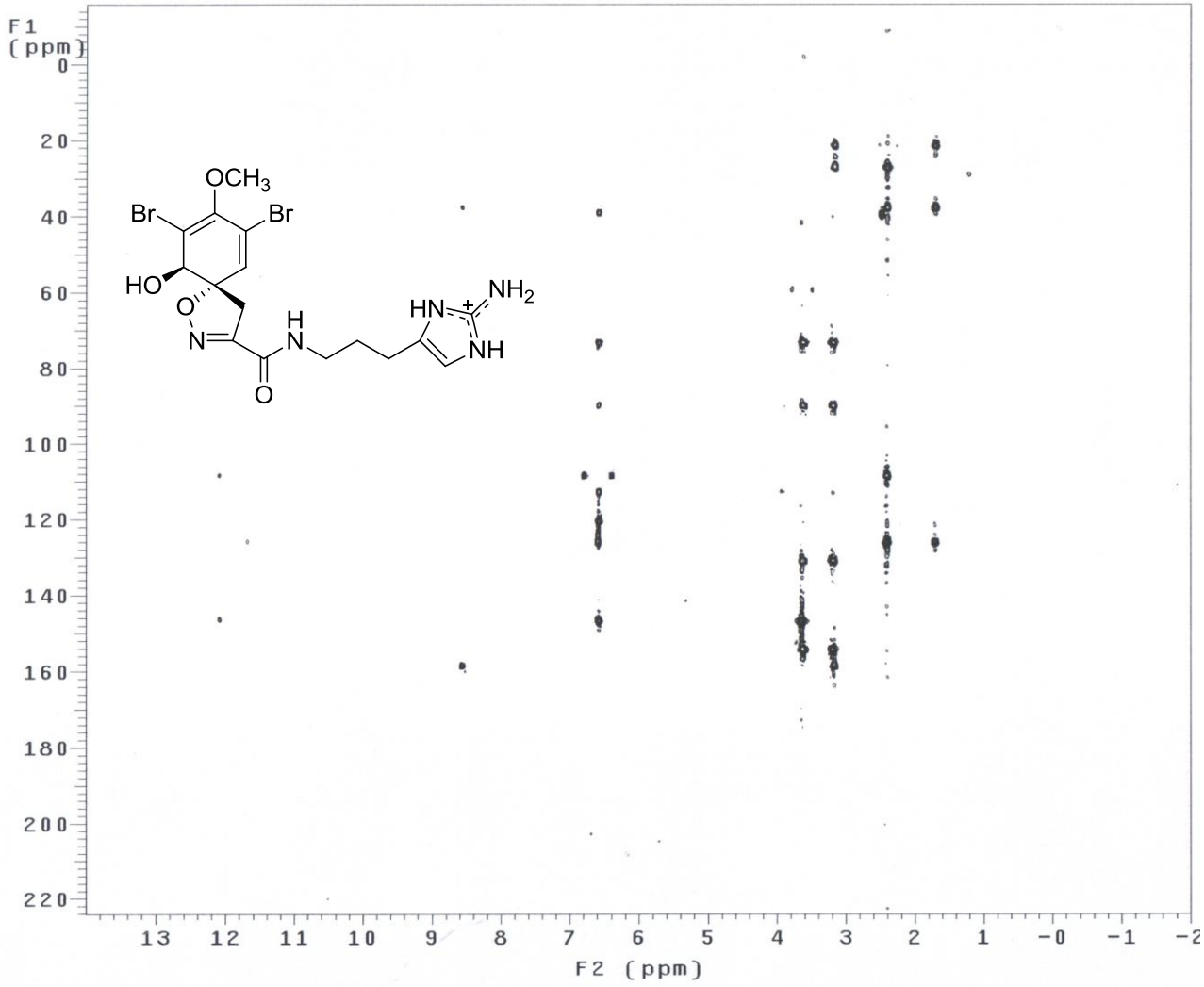
IR spectrum of **18** (KBr)



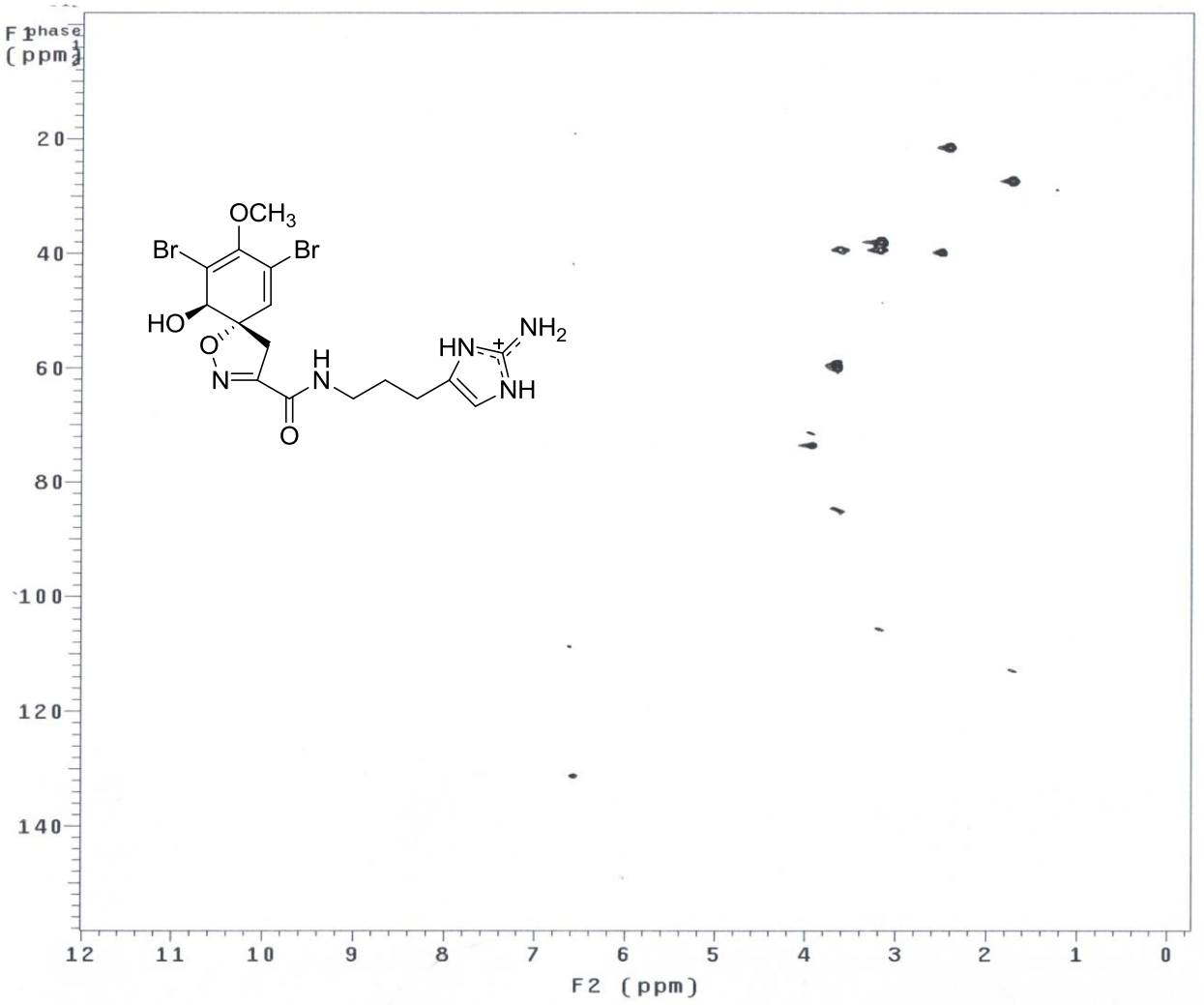
UV spectrum of **18** (CH_3OH)



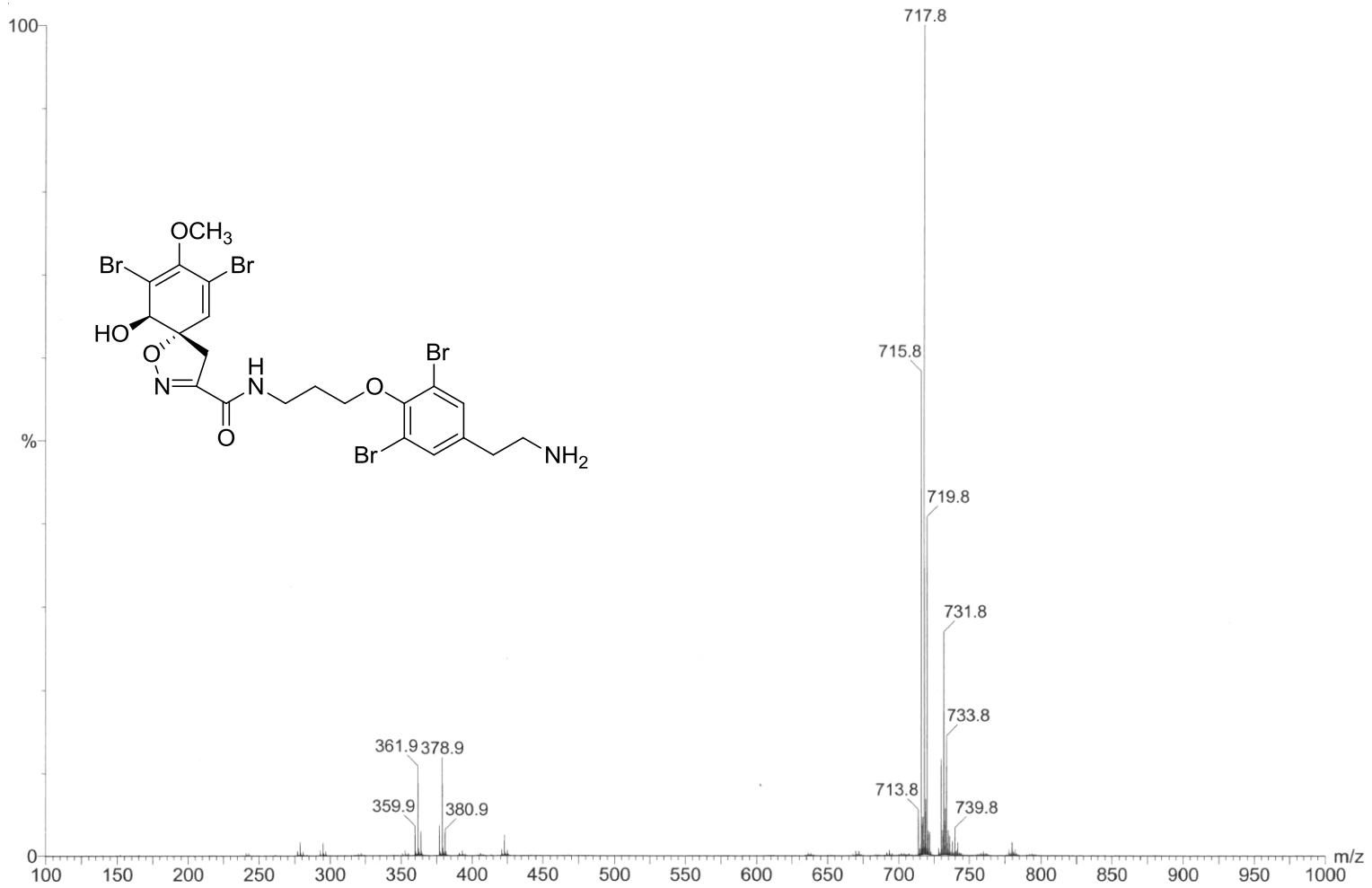
^{1}H-^{1}H COSY spectrum of **18** (500 MHz, DMSO-d_6)



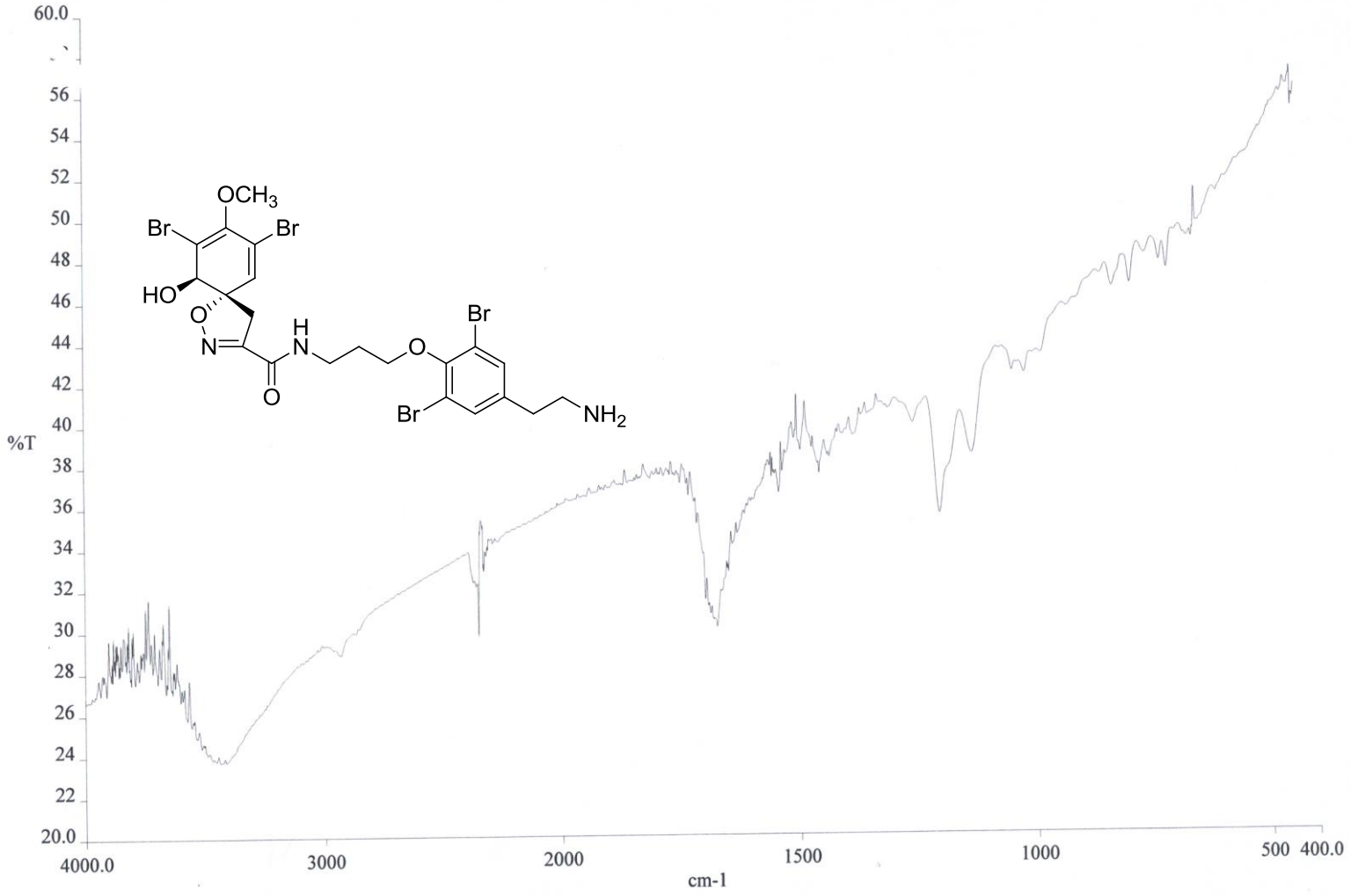
HMBC spectrum of **18** (500 MHz, DMSO-d_6)



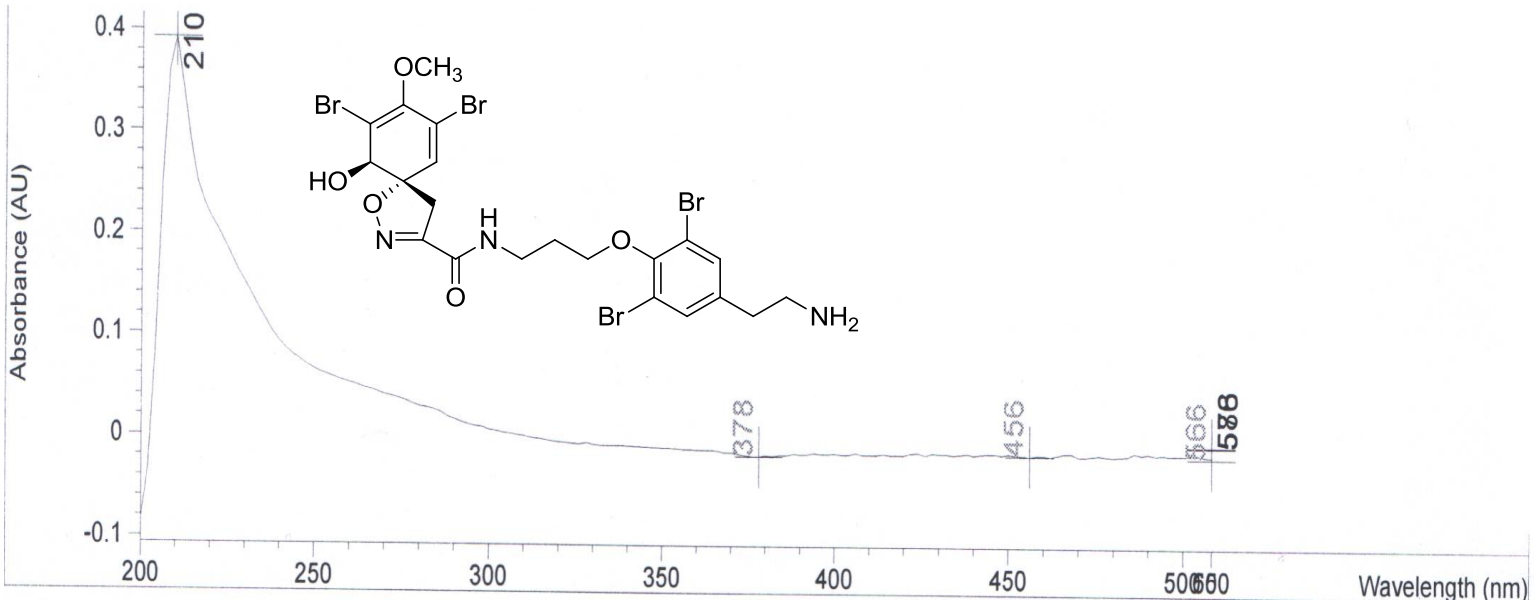
HMQC spectrum of **18** (500 MHz, DMSO-d_6)



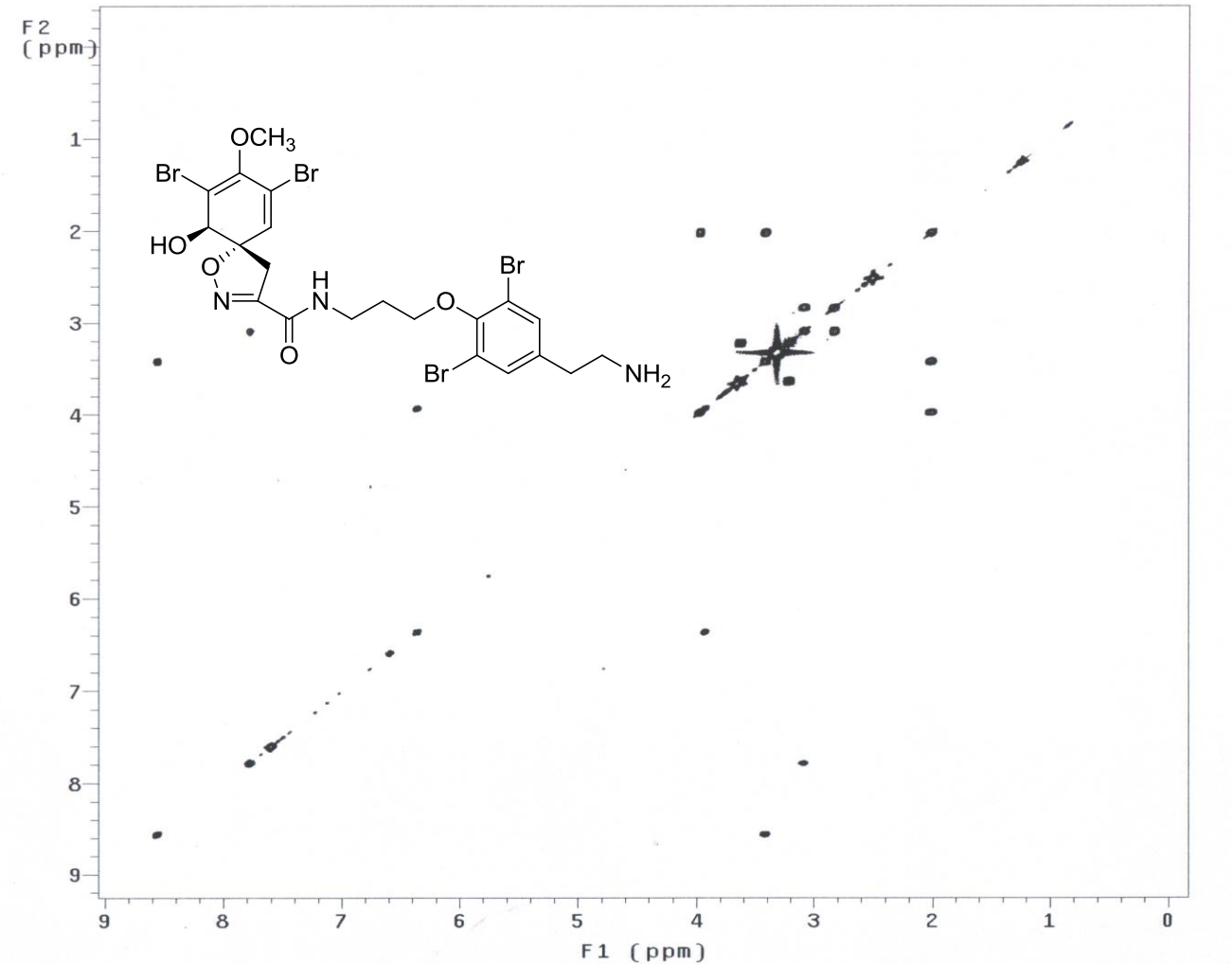
ESI mass spectrum of **19**



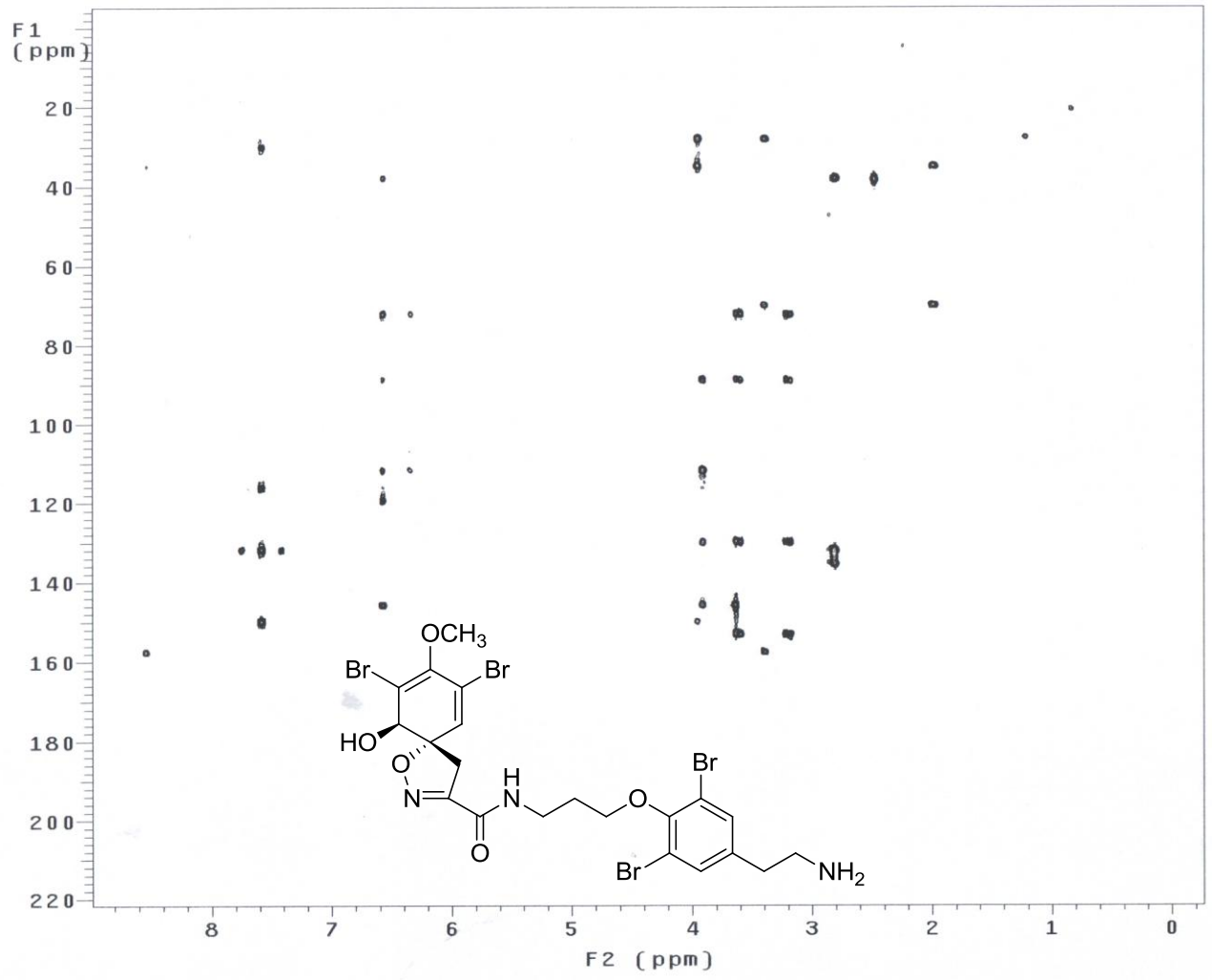
IR spectrum of **19** (KBr)



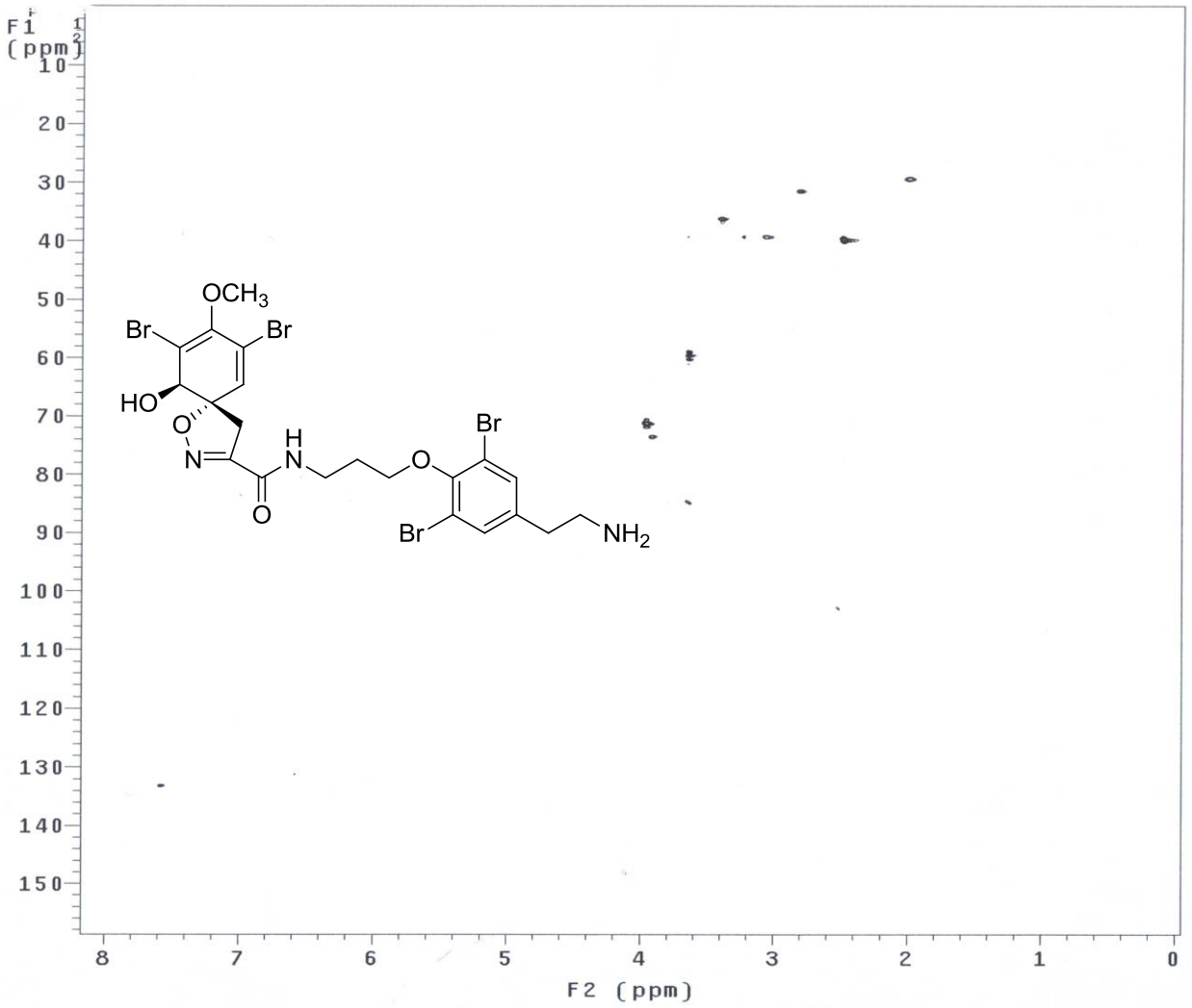
UV spectrum of **19** (CH_3OH)



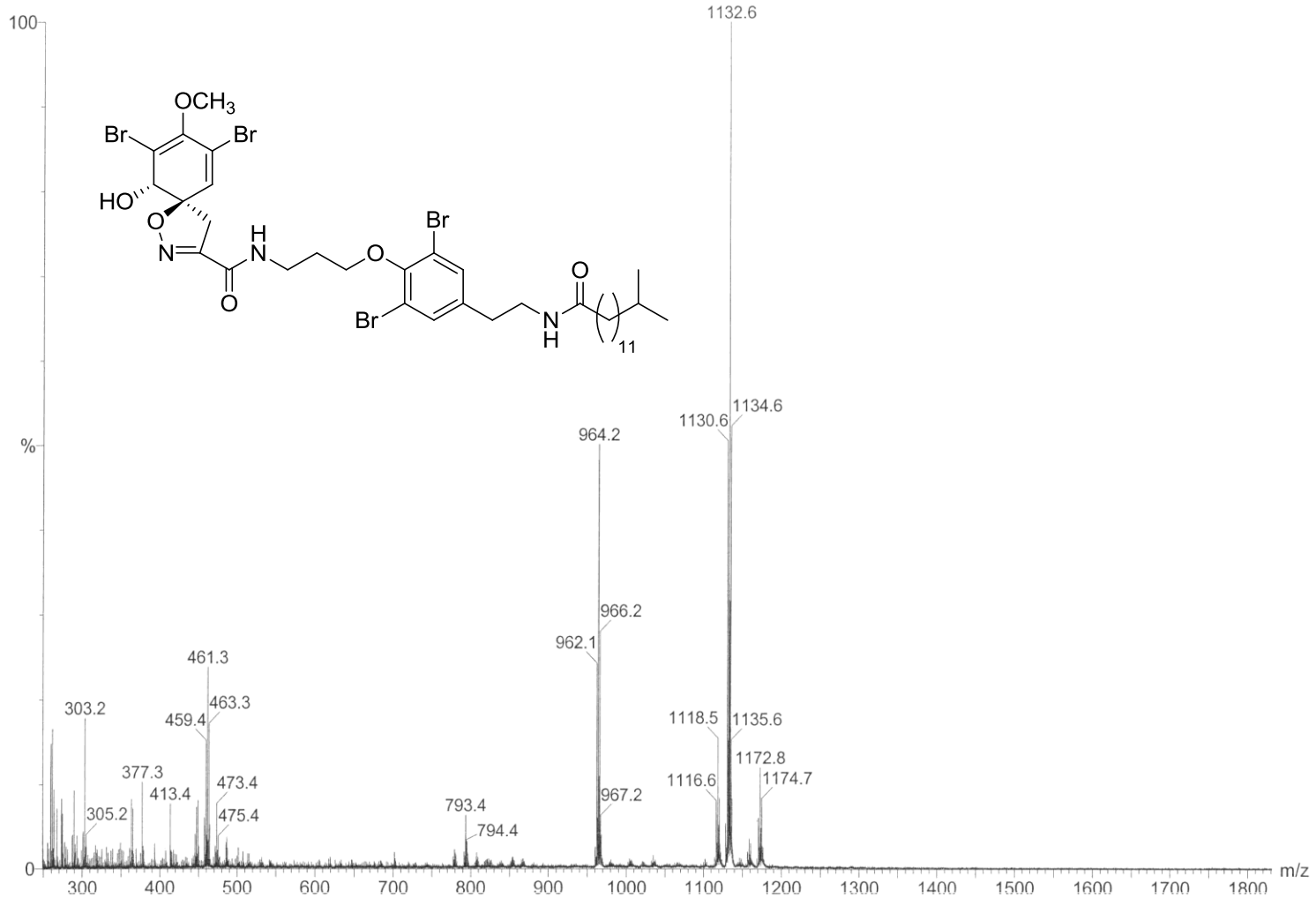
^{1}H-^{1}H COSY spectrum of **19** (500 MHz, DMSO-d_6)



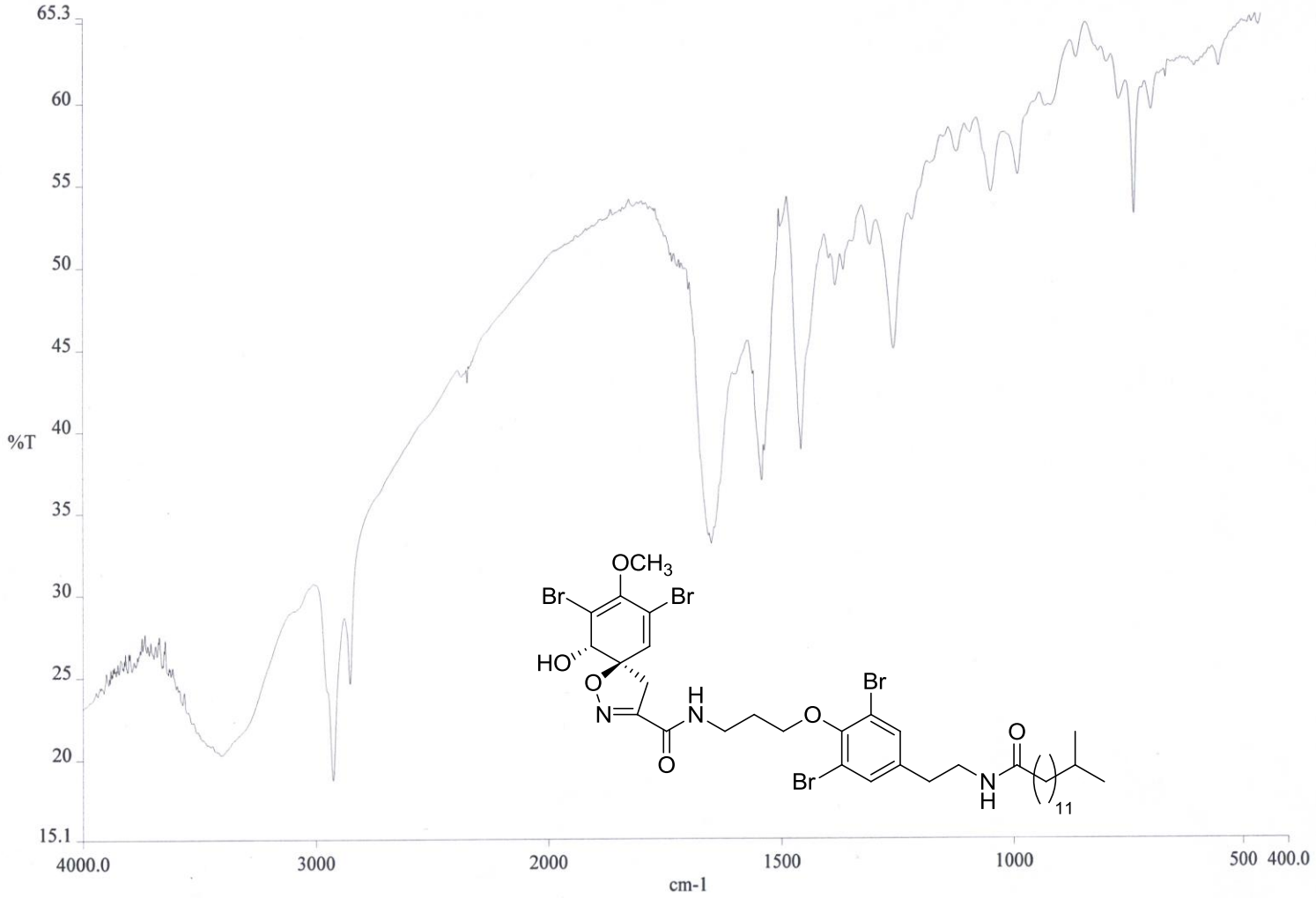
HMBC spectrum of **19** (500 MHz, DMSO-d_6)



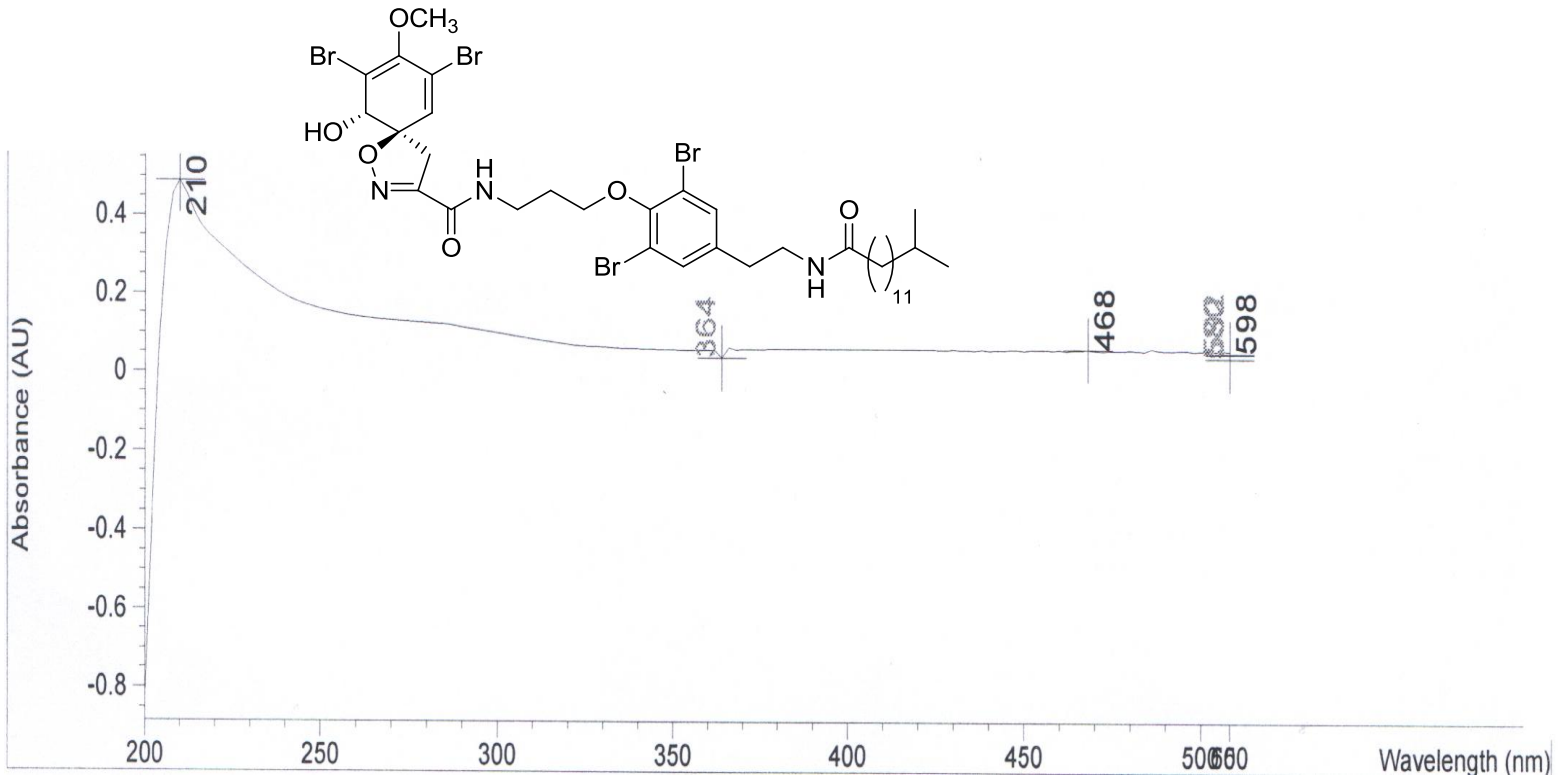
HMQC spectrum of **19** (500 MHz, DMSO-d_6)



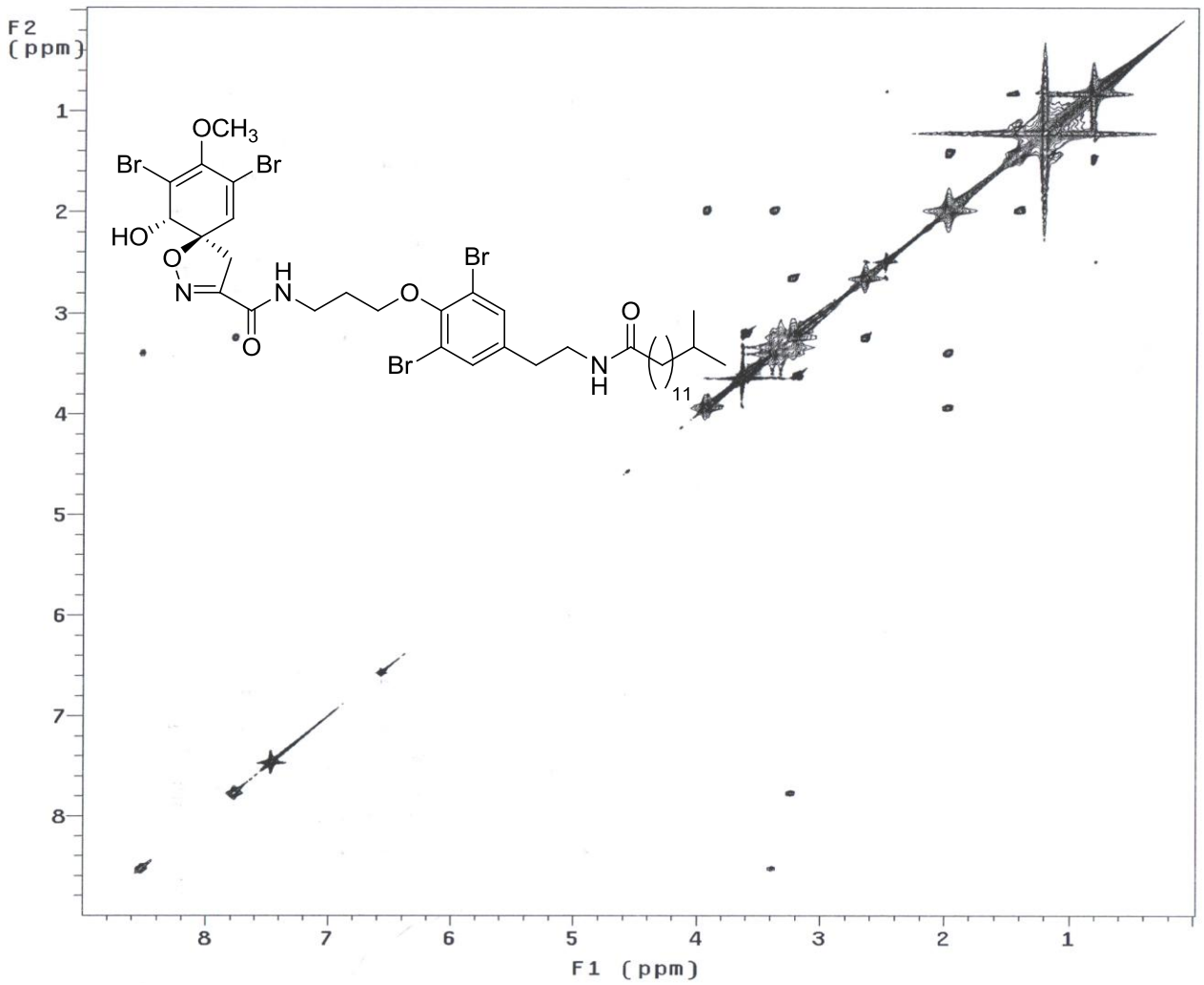
ESI mass spectrum of **20**



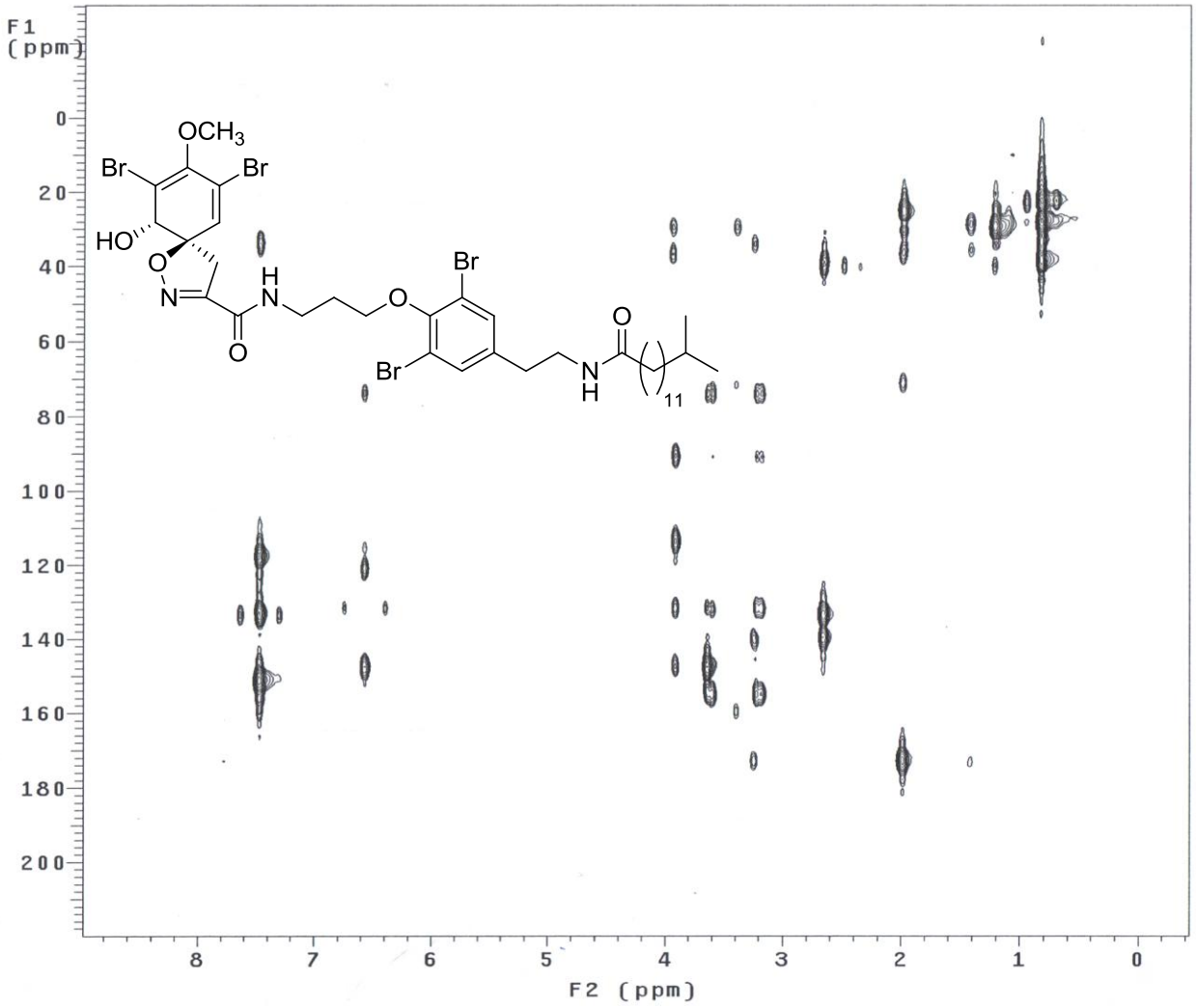
IR spectrum of **20** (KBr)



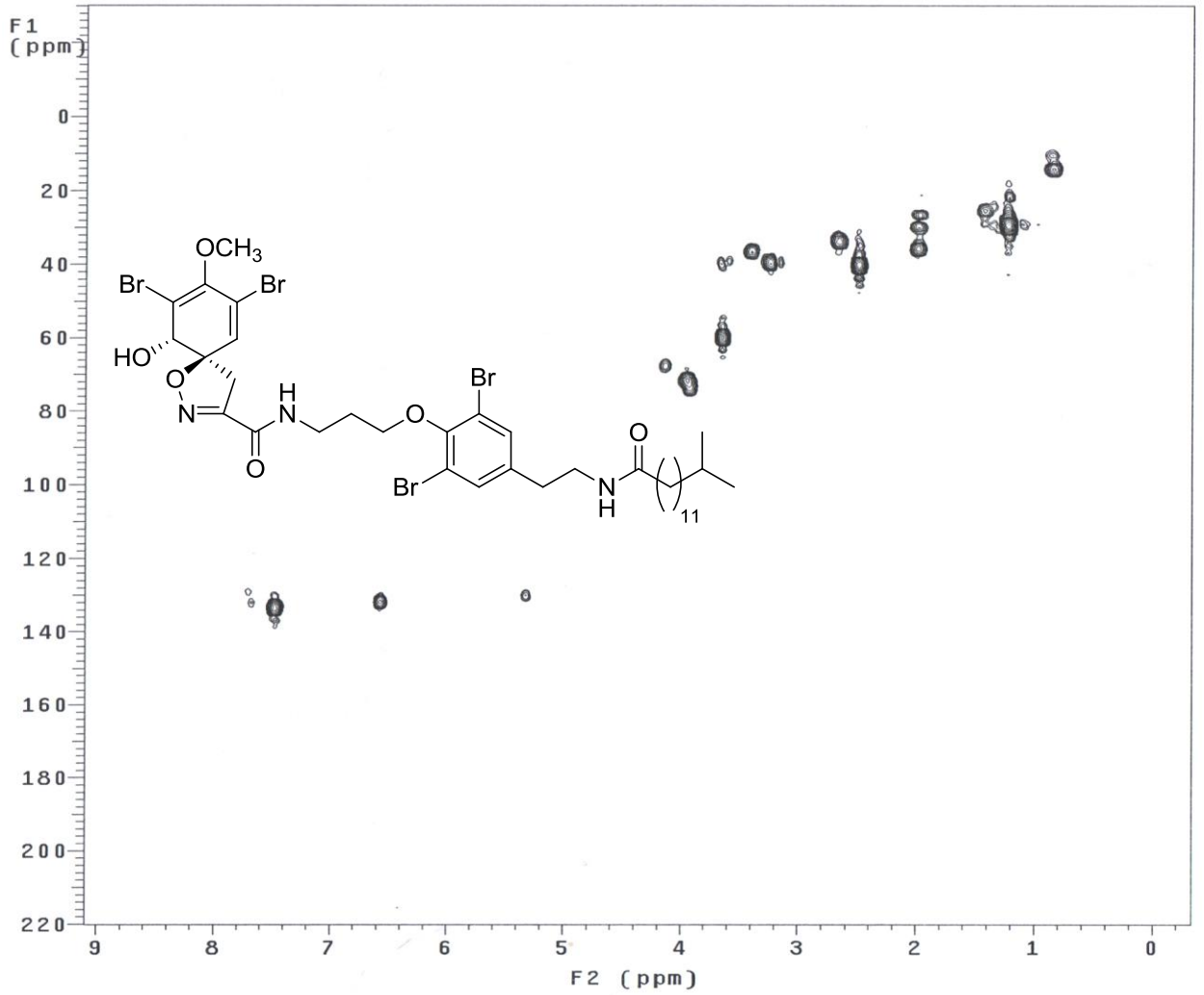
UV spectrum of **20** (CH_3OH)



^{1}H-^{1}H COSY spectrum of **20** (500 MHz, DMSO-d_6)



HMBC spectrum of **20** (500 MHz, DMSO-d_6)



HMQC spectrum of **20** (500 MHz, DMSO-d_6)

VITAE

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Student ID 5210730011

Educational Attainment

Degree	Name of Institution	Year of Graduation
Masters of Science	University of Lagos	2006
Bachelor of Science	University of Ilorin	2003

Scholarship Awards during Enrolment

2009 Prince of Songkla University Graduate Studies Grant.

2010 National Research University Project of Thailand.

2012 Graduate School Thesis Supporting Grant, Prince of Songkla University.

2013 Faculty of Pharmaceutical Sciences Thesis Supporting Grant, Prince of Songkla University.

List of Publication and Proceeding

Opeyemi Olatunji.; Anuchit Plubrukarn. Bromotyrosine derived alkaloids from the sponge *Acanthodendrill*a sp. In proceeding of International Conference of Natural Products 2013. Shah Alam, Selangor, Malaysia, 4-6 March 2013.

Opeyemi Olatunji.; Anuchit Plubrukarn. Bromotyrosine derivatives from the sponge *Acanthodendrilla* sp. In proceeding of 2nd Current Drug Development International Conference 2012. Phuket, Thailand, 2-4 May 2012.

Sirirak, T.; Plubrukarn, A. Antimalarial trisoxazole macrolides from the Thai sponge, *Plakinastrella* sp. In proceeding of 6th Regional IMT-GT UNINET Conference 2008. Penang, Malaysia, 28-30 August, 2008.