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Novel Rhein Analogues as Potential Anicancer Agents and a Novel Metal Free Synthesis of 6H-ISOINDOLO[2,1-A]INDOL-6-ONE

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NOVEL RHEIN ANALOGUES AS POTENTIAL ANICANCER AGENTS

AND

A NOVEL METAL FREE SYNTHESIS OF 6H-ISOINDOLO[2,1-A]INDOL-6-ONE

by

ALEXANDER BORYANOV DRAGANOV

Under the Direction of Professor Binghe Wang

ABSTRACT

The first section of this work describes the synthesis of a library of novel rhein analogues that are potential anticancer agents. The design of these compounds takes advantage of the ability for rhein to intercalate into DNA and as the incorporation of an alkylating agent, which serves to covalently modify DNA. In three cell lines, these compounds showed potent cytotoxicity with IC_{50} in the low to mid-μM range. The second project was focused on the development of an efficient synthesis of 6H-Isoindolo[2,1-α]indol-6-one (**24**), a core structure for a number of biologically active compounds. The approach is metal-free and uses a Beckmann rearrangement followed by an intramolecular cyclization.

INDEX WORDS: Anti- cancer agents, Rhein, DNA intercalators, Alkylation, IC_{50}, Beckmann rearrangement

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DEDICATION

To my loving parents Temenuzhka Marinova Draganova and Boryan Asenov Draganov.

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1) NOVEL RHEIN ANALOGUES AS POTENTIAL ANTICANCER AGENTS

1.1) Introduction

Rhein is a natural product having an anthraquinone scaffold. It was isolated from the ground plant Rhubarb, which belongs to the *Rheum* family. A number of anthraquinone compounds isolated form Rheum such as emodin has been studied and found to have good biological activities (*Figure 1.1*).[1] Compounds belonging to the same family of anthraquinones, such as doxorubicin and mitoxanthrone, have been on the market as anticancer drugs (*Figure 1.1*).[1-3] These compounds have shown great anticancer activity; however, they have shown serious cardiotoxicity side effects.[1,3] Studies have shown that rhein is well tolerated by the human body when used as a laxative, and it has anticancer activities against some tumor cells.[4-7] The anticancer activity of rhein against number of cancer cells has been found to be relatively low with IC_{50} in the range of 12~120 μM.[4-6,8,9] The potential that the rhein molecule and its analogues carry in the anticancer drug discovery, inspired the work that is described in this paper.[4,7,10] Comparing the rhein core structure to other known anthraquinone anticancer drugs, which have already been approved for clinical use (*Figure 1.1*), brings promise for improvement of the anticancer activity by designing new analogues. The compounds mentioned above are believed to be non-covalent DNA binding drugs.[1,7] In general DNA intercalators have common structural features, such as a planar polycyclic aromatic system with different side chains that can vary from simple amines to different sugars.[5,6,10,11] Intercalators can bind within the minor or the major grove of the DNA duplex. Upon binding they may induce conformational changes or even rupture of the DNA helix, which can cause cell apoptosis. Another possible mechanism of action is the inhibition of enzymes that bind to the DNA, which may disrupt DNA replication, transcription, etc. Modification of the planar structure of the molecule is a strategy that can

increase the activity of the molecule. Another strategy for improved potency of a compound is by varying the side chains. It has been reported that addition of sugars, heterocyclic moieties, and amines of different length has improved the activity of some anthraquinones from the *Rheum* family.[10,11] Herein we describe the design and synthesis of some novel rhein analogues that have alkylators on the side chains at position 3 of the planar polycyclic aromatic system. The idea behind the development of these analogues is to use the planar polycyclic aromatic system of rhein as an intercalating agent. In addition to the cytotoxycity effects of the intercalating agent on carcinoma cells; attachment of an alkylating agent to the rhein core structure is designed to interact with nucleophillic species of the DNA and further improve cytotoxicity. We have designed analogues that combine two mechanistic strategies for targeting DNA: intercalation and covalent DNA modification.

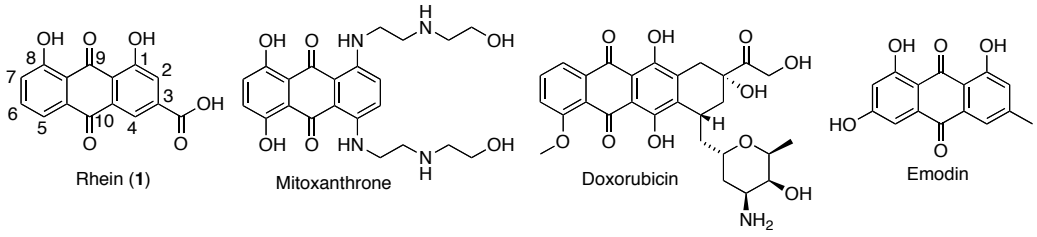


Figure 1.1. Rhein and other potent anthraquinones.

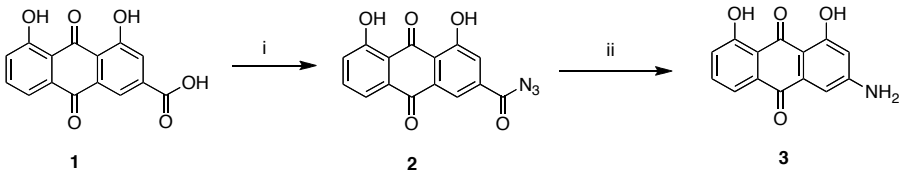
1.2) Results and Discussion

1.2.1) Chemical Synthesis

Previous studies by Dr. Xiaochuan Yang have shown that attaching an alkylating agent to the flat ring system of the rhein core structure at position 3 has the potential for improving the cytotoxicity effects of the molecule. A generally accepted mechanism of action of the anthraquinone compounds is that they are capable of non-covalent binding to the DNA duplex, possibly by intercalation.[1,11-14] The combination of a planar polycyclic aromatic system as an intercalation moiety and an alkylating agent has been proposed to generate synergy for improved cytotoxicity effects.[11] Based on Dr. Yang's observations, a plan was devised to synthesize a library of compounds that have an alkylating agents attached to position 3 of the rhein core structure with variations in the length and chemical nature of the linker. Two main approaches were considered when building this series of analogues. The first approach involves the direct replacement of the carboxylic acid group at position 3 of the core structure with an amino group. The second approach in creating novel rhein analogues involves the use of commercially available rhein (**1**) and the attachment of amine linkers of various sizes and chemical properties to position 3 of the polycyclic aromatic system before the addition of alkylating agents. Two different sized linkers were chosen to be attached to the Rhein (**1**) compound: a four-carbon diamine linker and a long diamine with ethylene glycol moieties for improved solubility.

The synthesis of the first series of compounds started with rhein (**1**). The first step involved the formation of acyl azide at position 3. The formation of the azide was done by addition of diphenylphosphoryl azide (DPPA) to (**1**) in anhydrous dimethylformamide (DMF). Triethyl amine (TEA) was used in the reaction as a base, and the yield of the reaction was 40%. The second step of the synthesis used Curtis rearrangement through heating at reflux in 1,4-dioxane for 2 hours and then hydrolysis with NaOH solution, giving a reaction yield of 30%. The

synthesized compound **3** was used as a starting material for three analogues, each containing different alkylating agent (*Scheme 1.1*). The amine at position 3 of compound **3** was acylated using three agents, leading to three different compounds. Chloroacetyl chloride was used to form compound **5**, bromoacetyl bromide was used to result in compound **6**, and iodoacetyl chloride was used for the formation of compound **7** (*Figure 1.3*).



Reagents and conditions: i) DPPA, Et_3N, DMF, 30 min., room temperature ii) dioxane, NaOH, reflux, 4 h, 31 % yield.

Scheme 1.1. Synthetic route to compound **3**.

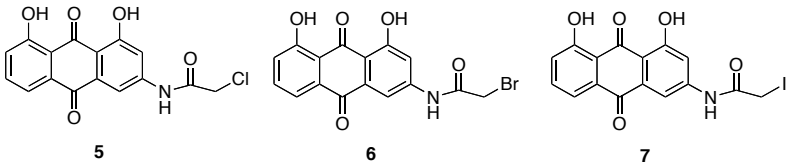
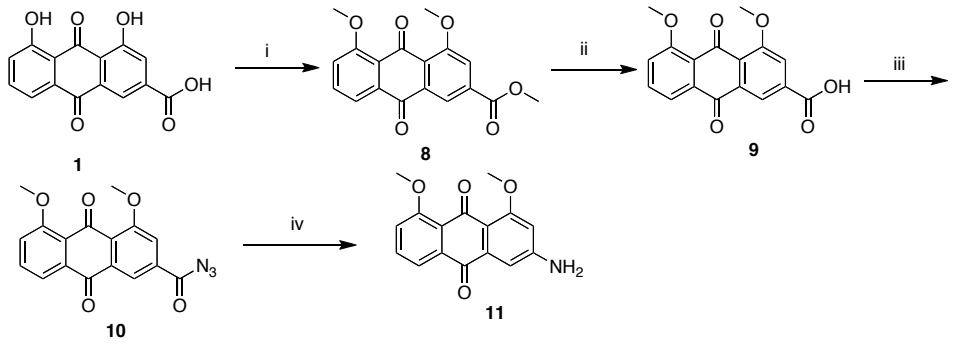


Figure 1.2. Analogues derived from compound **3.**

A similar approach was used to synthesize three more rhein analogues using amine (**11**) as a stating material (*Scheme 1.2*). Compound **11** was acylated using chloroacetyl chloride, bromoacetyl bromide, and iodoacetyl chloride, to give compounds **12**, **13**, and **14** respectively (*Figure 1.3*). The reaction yields were in the range between 30-40 %. The low yields for these reactions are due to the extremely poor solubility of the anthraquinone compounds being synthesized. The starting material **1** itself has poor solubility in dichloromethane (DCM).

Increased amount of the alkylating agent, up to two equivalent, to the free amine did not improve the reaction yields. Addition of one equivalent of TEA did not affect the reaction outcome as well. In an attempt to improve the yields the temperature of the reaction mixture was increased to 60^0 C; no significant increase in yield was observed.



Reagents and conditions i)NaH, MeI, DMF, ii) NaOH, EtOH/H_2O, iii) DPPA, Et_3N, DMF, iv) dioxane, NaOH

Scheme 1.2. Synthetic route to compound **11**.

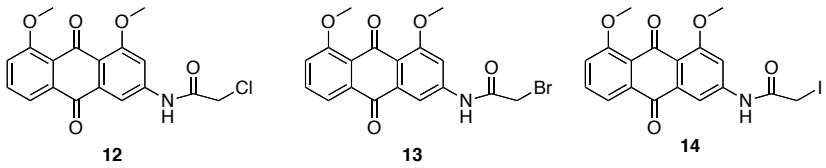
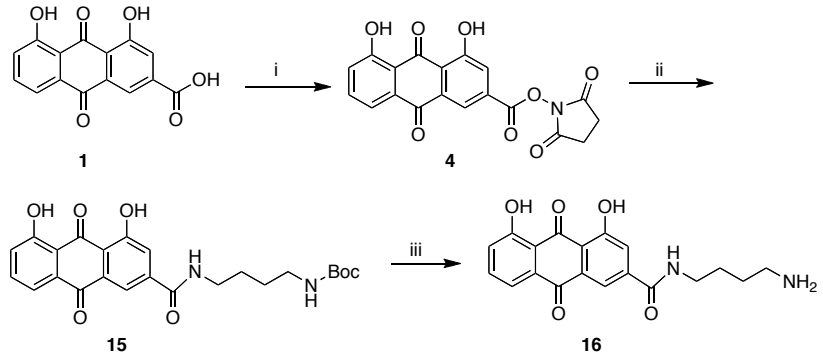


Figure 1.3. Analogues derived from compound **11**.

The same approach was used for the synthesis of the rhein-amine compounds **17** and **18** (*Figure 1.4*). The carboxylic group attached at the 3 position of compound **1** was converted to an activated ester by using *N*-hydroxysuccinimide (NHS) and coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI). Without further purification, by reacting the product with *N*-(3-minopropyl)carbamic acid *tert*-butyl ester (**34**) (*Figure 1.6*), the desired Boc-

protected amine **15** was synthesized in quantitative yield (*Scheme 1.3*). The next step in the formation of compounds **17**, and **18** was the de-protection of the Boc protecting group. The de-protection was carried out by stirring the protected amine in trifluoroacetic acid (TFA). Once the free amine **16** was obtained it was reacted with chloroacetyl chloride and bromoacetyl *N*-hydroxy succinimide to give compounds **17** and **18** in 36% and 32% yield respectively.



Reagents and conditions: i)NHS, EDCI, DCM, 0^0C to room temperature, overnight ii) **34**, Et_3N, DCM, room temperature, 2 h. iii) TFA, DCM, room temperature 2 h, quantitative yield.

Scheme 1.3. Synthetic route to compound **16**.

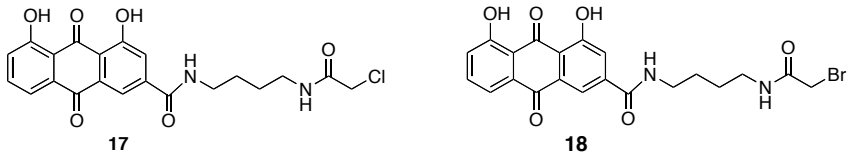
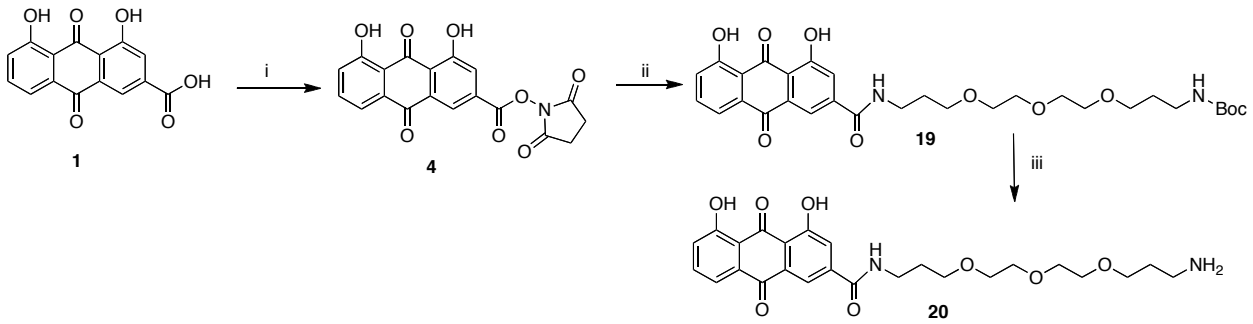


Figure 1.4. Analogues derived from compound **16**.

In an attempt to improve solubility, a long chain linker containing the ethylene glycol moiety was used in the synthesis of three more rhein analogues. *N*-[(*tert*-Butoxycarbonyl]-4,7,10-trioxa-1,13-tridecanediamine (**35**) (*Figure 1.6*) was directly added to the activated ester **4** generated *in situ* from **1** using EDCI and NHS to give the Boc- protected amine **19** in 98% yield (*Scheme*

1.4). This was followed by the de-protection of the Boc-group in TFA, giving the free amine **20** in quantitative yield. Amine **20** was reacted with chloroacetyl chloride, bromoacetyl *N*-hydroxy succinimide, and iodoacetyl chloride to give analogues **21**, **22**, and **23** respectively (*Figure 1.5*). Despite the addition of liker **35** containing the ethylene glycol moiety, the solubility of the synthesized compounds did not improve and the yields ranged from 30% to 39%. All of the eleven compounds were examined for their *in vitro* cytotoxicity using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-- diphenyltetrazolium bromide) assay.[15] The results are reported and discussed in the next section of this paper.



Reagents and conditions i)NHS, EDCI, DCM, 0^0C to room temperature, overnight ii) **35**, Et_3N, DCM, room temperature, 2 h. iii) TFA, DCM, room temperature 2 h., quantitative yield

Scheme 1.4. Synthetic route to compound **20**.

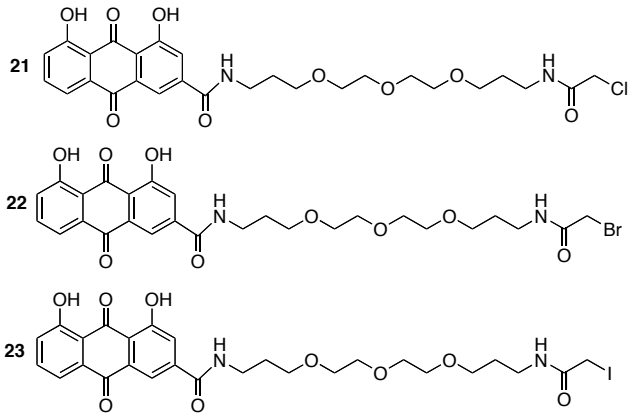


Figure 1.5. Analogues derived from compound **20**.

1.2.2) Biological Activities

The *in vitro* cytotoxicity studies of the synthesized compounds were performed in three different cell lines (HeLa, Hek, and KB). The activities of the herein described analogues were compared to that of rhein itself and doxorubicin. Doxorubicin is a marketed anticancer drug derived from anthraquinones from the *Rheum* family as well (*Figure 1.1*).[1] The quantitative evaluation of the cytotoxicity screenings for the synthesized compounds was done by performing MTT assay. The measurement of survival or proliferation of mammalian cells is a key factor in determining the effects of novel drug compounds. MTT assay is used to measure the viability of the cells. It is a colorimetric assay that utilizes a yellow tetrazolium dye. This dye is converted into a deeply colored product in live cells and is easy to be quantified. In this method, the yellow colored MTT is converted to purple colored formazan product. By dissolving in a solvent such as DMSO or isopropanol, formazan gives a "blue" colored product. The absorbance of this colored solution is quantified and analyzed by using a spectrophotometer at wavelengths generally

between 500 nm and 600 nm. The conversion of tetrazolium dye to the formazan product occurs in the presence of mitochondrial dehydrogenase enzyme. The mitochondrial dehydrogenase is produced only in active and viable cells. Thus the conversion can be directly related to the number of viable cells and inversely related to the cytotoxicity of the compounds tested. Due to the poor solubility of the compounds, all of the solutions used for biological testing were prepared by using DMSO as solvent. The cytotoxicity studies against HeLa cell line reveal that the compounds' activities are in the μM range and vary from IC_{50} = 40 μM to IC_{50} = 1.3 μM (*Table 1.1*). The least potent rhein analogue (**6**) (**(**IC_{50} = 40 μM) is 2.5 times more potent that rhein itself (IC_{50} = 100 μM). The most potent compound **12** (**(**IC_{50} = 1.3 μM) is about 100-fold more potent than rhein (IC_{50} = 100 μM) against the cervical cancer cell HeLa. Compound **12** (**(**IC_{50} = 1.3 μM) appears to have similar cytotoxicity against HeLa when compared with doxorubicin (**(**IC_{50} = 1.4 μM). All of the synthesized compounds showed significant improvement of the activity compared to rhein (*Table 1.1*). After analyzing the results obtained by the cytotoxicity assay against Hek cell lines, an observation can be made that all of the rhein analogues showed dramatically improved activity with IC_{50} values in the single digit μM range (*Table 1.1*). However, all of the analogues showed activity that is ten times lower than doxorubicin (**(**IC_{50} = 0.14 μM) (*Table 1.1*). The most potent compound against Hek cells is compound **18** (IC_{50} = 1.4 μM), and the least potent are compounds **6** and **17** (IC_{50} = 8 μM) (*Table 1.*1). The described rhein analogues have been tested against a third cell line, KB. Once again all of the synthesized compounds showed great improvement in their activity compared to rhein. However, the most potent compound against this cell line, **18** (**(**IC_{50} = 4.8 μM)**,** showed activity that is ten times lower than that of doxorubicin (**(**IC_{50} = 0.4 μM). The least potent

compound **13** (IC$_{50}$ = 38 μM) against the KB cells showed two and a half times increase in activity compared to rhein (IC$_{50}$ = 1100 μM). On the other hand it showed one hundred fold lower activity compared to doxorubicin (IC$_{50}$ = 0.4 μM) (Table 1.1). From the analysis of the data obtained from the cytotoxicity assays one can see that there are four compounds that carry great potential against the studied carcinoma cells. Rhein analogues **5**, **12**, **14**, and **18** showed low micro molar IC$_{50}$ values against all three cell lines assayed (*Table 1.1*). Two of the compounds that posses the highest activities **12** and **14**, have the two hydroxyl groups on the anthraquinone moiety protected using a methoxy group. The fact that the above-mentioned compounds have the lowest IC$_{50}$ values implies that the methoxy protection at positions 1 and 8 on the anthraquinone moiety plays a crucial role in the increased activity of these compounds. On the other hand, it has been reported that rhein analogues having the methoxy groups at positions 1 and 8 do not show any cytotoxicity against L1210 leukemic cells, which suggest that there might be other structural factors that might result in the increased cytotoxicity.[11] Considering the fact that in previous studies the methoxy protected compounds did not show cytotoxic activity against carcinoma cells one can speculate that in fact the key factor for the improved activity is the addition of alkylating agent to position 3 of the polycyclic aromatic system. The intercalating power of the 1, 8-methoxy protected compounds might not be as good as the one of the free 1, 8-hydroxy compounds, but one can assume that interactions of the planar part of these molecules with the the DNA base pairs can bring the alkylator in close proximity to the highly nuclephillic DNA backbone. Having the alkylating agent in close proximity to the DNA backbone or a nucleophilllic group of neighboring base can promote covalent interactions. Intercalation as well as covalent interactions with the DNA can cause conformational changes

and even rupture of the DNA helix, which can induce apoptosis. Two more important structural factors can play an important role; the type of alkylator and the length of the linker. In the case of **12** and **14,** the alkylating agent is directly attached to the amine at position 3. Compounds **12** and **14** have shown great improvement in cytotoxycity compared to rhein. These compounds can be used as leads for future development because they carry the potential for further improvement of their activity. The use of a longer linker between the polycyclic aromatic system and the alkylating agent might have positive effects on the compounds' cytotoxicities. One can notice that other three compounds that have a short linker at position 3 did not show high potency. On the contrary, some of them, **6**, **7**, and **13**, have the highest IC_{50} values in the *in vitro* studies (*Table 1.1*). The type of leaving group on the alkylating agent could play an important role in increasing the activity of the compounds. Initially a hypothesis was made based on the atomic size and the chemical properties of the leaving groups of the alkylating agents; compounds containing iodide and bromide should have exhibited better activity than compounds containing chloride. Strong evidence supporting that hypothesis was not obtained from the performed studies. Two of the four most potent compounds contain chlorides as leaving groups after a potential alkylation reaction. One of them has iodide, and one has bromide. Clearly the most potent compound against all of the three cell lines studies is compound **12** that carries the combination of methoxy protected hydroxy groups and a chloride as leaving group after potential alkylation reaction. This compound can be used as a starting point for further development of a successful DNA intercalating agent. Clearly the attempt to increase solubility by the addition of a long linker containing ethylene glycol did not result in the expected increase of the biological activity that can be significant enough for further development. A potentially good method for

structurally developing compound **12** in the pursuit of increased biological activity is the use of computational software that can help in explaining the intercalation mechanism of this compound and the ideal positioning of the alkylating moiety. Knowing the way of action of compound **12** and the way it interacts with its target can greatly help in the design of more potent DNA intercalators.

Table 1.1. Cytotoxicity of all synthesized compounds against three different cell lines.

Compounds	IC_{50} (μM) against HeLa	IC_{50} (μM) against Hek	IC_{50} (μM) against KB
Rhein	>100	>100	>100
5	4	4	4.5
6	40	8	15
7	10	2	9
12	1.3	1.9	5.2
13	8	2.3	38
14	2.5	1.4	10
17	16	8	19
18	3	1.4	4.8
21	14	8	21
22	7	2.2	9
23	5.8	4	8
Doxorubicin	1.5	0.15	0.4

1.3) Conclusions

In conclusion, eleven new rhein analogues have been synthesized by linking an alkylating agent to position 3 of the core structure. In addition, attempts were made to place three different alkylating agents. The synthesized analogues were tested *in vitro* by using the MTT assay against

three different cell lines: HeLa, Hek, and KB. All of the compounds tested showed improved cytotoxicity compared to rhein with IC_{50} values in the μM range against cancer cells. Four of the compounds **5**, **12**, **14**, and **18**, showed significant improvement, one of them stands out with an impressive IC_{50} value of 1.3 μM against HeLa cells. The improvement of cytotoxycity at such leves is evidence that the combination between an intercalating moiety and an alkylating agent can be a successful strategy for designing DNA targeting anticancer drugs.

1.4) Experimental Section

Rhein (**1**) was purchased from Nanjing ZeLang Medical Technology Co. LTD, China, and directly used without further purification. Other starting materials and solvents were purchased from Aldrich and Acros. Some starting materials such as **11** and the Boc- protected amine of compounds **15**, and **35** were obtained from past synthesis done by other group member working on the same project. ^1H spectra were obtained on a Bruker 400 NMR spectrometer in a deuterated solvent with TMS (δ = 0.00 ppm). For all reactions, analytical grade solvents were used. Anhydrous solvents were used for all moisture-sensitive reactions.

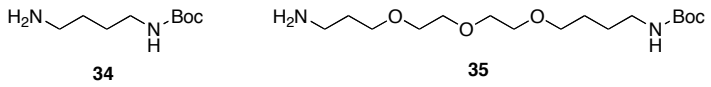


Figure 1.6. Uncommon reagents used.

1,8-Dihydroxy-3-amino-anthraquinone (3).

Compound **1** (500 mg, 1.7 mmol) was suspended in anhydrous DMF (6 mL) in a dry round bottom flask and cooled to 0 ^{o}C. TEA (490 µL, 3.5 mmol) was added at 0 ^{o}C. DPPA (400 µL, 1.8 mmol) was added drop wise after **1** was completely dissolved. The reaction mixture was stirred at room temperature for 20 minutes. TLC (hexanes: ethyl acetate = 3:1) showed complete consumption of the starting material. The solvent was evaporated *in vacuo* and the obtained oil was purified by silica gel column chromatography using a mixture of hexanes and ethyl acetate in a 2:1 ratio as the eluent. 200 mg brown- yellow powder were obtained. Compound **2** was dissolved in 1,4- dioxane (4 mL) and stirred under reflux for 2 hours. After the color of the solution turned from brown to bright red and TLC (hexanes: ethyl acetate = 2:1) showed product formation, the 1,2-dioxane volume was reduced by half using a rotavapor. 2N NaOH (4 mL) was added to the solution and a fine precipitate formed. The suspension was stirred under reflux for 2 additional hours. The solution was cooled and neutralized by addition of 1N HCl. The precipitate was too fine to be filtered so it was extracted by using DCM (3 × 10 mL) and washed with water (2 × 5 mL) and brine (1 × 5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo.* The obtained residue is bright red powder that was purified using silica gel column chromatography, with mixture of eluent hexanes and ethyl acetate in 10:1 ratio (50 mg, 31% yield). ^{1}H NMR (acetone - d_6): δ 12.04 (s, 1H), 12.27 (s, 1H), 7.67 (d, 1H, J = 1.2 Hz), 7.56- 7.50 (m, 2H), 7.27- 7.23 (m, 1H), 7.14-(d, 1H, J = 2.0 Hz), 6.37 (d, 1H, J = 2.0 Hz); MS: m/z $[M-H]^-$ calculated $C_{14}H_9NO_4$ 254.01, found 254.04.

1,8-Dihydroxy-3-(2'-chloro-acetamido)-anthraquinone (5).

Chloroacetyl chloride (23 µL, 0.3 mmol) was injected slowly into the solution of (**3**) (50 mg, 0.2 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was followed by TLC (DCM: MeOH = 10:1) and diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.*. The residue was purified with silica gel column chromatography eluting with DCM: MeOH (60:1) to give an orange colored solid product (20 mg, 30% yield). ^{1}H NMR (DMSO - d_6): δ 12.02 (s, 1H), 11.06 (s, 1H), 7.87 (s, 1H), 7.809- 7.79 (m, 2H), 7.74- 7.72 (m, 1H), 7.33- 7.31 (m, 1H), 4.36 (d, 2H, J = 1.2 Hz); MS: m/z $[M-H]^-$ calculated $C_{16}H_{10}ClNO_5$ 330.00, found 330.10.

1,8-Dihydroxy-3-(2'-bromo-acetamido)-anthraquinone (6).

Bromoacetyl bromide (26 µL, 0.3 mmol) was injected slowly into the solution of (**3**) (50 mg, 0.2 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was followed by TLC (DCM: MeOH = 10:1) and diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.* The residue was purified with silica gel column chromatography. The product was eluted with DCM: ethyl acetate (20:1). Orange color solid was obtained as product (18 mg, 30 % yield). ^{1}H NMR (DMSO - d_6): δ 12.05 (s, 1H), 10.18 (s, 1H), 7.88 (d, 1H, J = 2.0 Hz), 7.82- 7.80 (m, 2H), 7.78-7.76 (m, 1H), 7.35 (d, 1H, J = 8.4 Hz), 4.14 (s, 2H); MS: m/z $[M-H]^-$ calculated $C_{16}H_{10}BrNO_5$ 392.9, found 392.1.

1,8-Dihydroxy-3-(2'-iodo-acetamido)-anthraquinone (7).

Iodoacetyl chloride (27 µL, 0.3 mmol) was injected slowly into the solution of (**3**) (50 mg, 0.2 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room temperature for 2 h. The reaction was followed by TLC (DCM: MeOH = 10:1), and after completion was diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified with silica gel column chromatography. The compound was eluted with DCM: MeOH (1:0, 60:1). Orange color solid was obtained as product (24 mg, 33% yield). ^{1}H NMR (DMSO - d_6): δ 11.96 (s, 1H), 11.85 (s, 1H), 7.80 (d, 1H, J = 2.1 Hz), 7.75 (d, 1H, J = 2.0 Hz), 7.60-7.58 (m, 2H), 7.38 (d, 1H, J = 8.4 Hz), 3.87 (s, 2H); MS: m/z $[M-H]^+$ calculated $C_{16}H_{10}INO_5$ 422.9, found 422.1.

1,8-Methoxy-3-(2'-chloro-acetamido)-anthraquinone (12).

Chloroacetyl chloride (21µL, 0.2 mmol) was injected slowly into the solution of (**11**) (50 mg, 0.17 mmol) in anhydrous 1,4-dioxane (5 mL). The mixture was stirred at room temperature for 2 h. The reaction was followed by TLC (DCM: MeOH = 10:1), and after completion was diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified with silica gel column chromatography. It was eluted with DCM: MeOH (60:1). Orange color solid was obtained as product (8 mg, 30% yield). ^{1}H NMR (DMSO – d_6): δ 10.81 (s, 1H), 7.93 (d, 1H, J = 2.0 Hz), 7.82 (d, 1H, J = 2.0 Hz), 7.76- 7.682 (m, 2H), 7.55-7.529 (m, 1H), 4.34 (s, 2H), 3.93 (d, 6H, J = 7.6 Hz); MS: m/z $[M+H]^+$ calculated $C_{18}H_{14}ClNO_5$ 360.0, found 360.2.

1,8-Methoxy-3-(2'-bromo-acetamido)-anthraquinone (13).

Bromoacetyl bromide (23 μL, 0.26 mmol) was injected slowly into the solution of (**11**) (50 mg, 0.17 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room temperature for 2 h. The reaction was followed by TLC (DCM: MeOH = 10:1), and after completion was diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified with silica gel column chromatography. The compound was eluted with DCM: MeOH (1:0, 60:1). Orange color solid was obtained as product (13 mg 20% yield). ^{1}H NMR (DMSO - d_6): δ 10.95 (s, 1H), 7.91 (d, 1H, J = 2.0 Hz), 7.82 (d, 1H, J = 2.0 Hz), 7.76- 7.68 (m, 2H), 7.55-7.53 (m, 1H), 4.10 (s, 2H), 3.95 (d, 6H, J = 7.2 Hz); MS: m/z $[M+H]^+$ calculated $C_{18}H_{14}BrNO_5$ 405.2, found 405.2.

1,8-Methoxy-3-(2'-iodo-acetamido)-anthraquinone (14).

Iodoacetyl chloride (24 μL, 0.26 mmol) was injected slowly into the solution of (**11**) (50 mg, 0.17 mmol) in anhydrous 1,4-dioxane (5 mL). The mixture was stirred at room temperature for 2 h. The reaction was followed by TLC (DCM: MeOH = 10:1), and after completion was diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified with silica gel column chromatography. The residue was eluted with DCM: MeOH (1:0: 60:1). Orange color solid was obtained as product (27 mg, 35% yield). ^{1}H NMR (DMSO - d_6): δ 7.92 (d, 1H, J = 2.0 Hz), 7.78 (d, 1H, J = 2.0 Hz), 7.76- 7.66 (m, 2H), 7.48-7.46 (m, 1H), 3.93 (d, 6H, J = 5.2 Hz), 3.87 (s, 2H); MS: m/z $[M+H]^+$ calculated $C_{18}H_{14}INO_5$ 451.9, found 452.0.

1,8-Dihydroxy-3-(2'-amido-7'-amino)-anthraquinone (16).

The Boc- protected amine of (**15**) (200 mg, 0.4 mmol) was suspended in DCM (5 mL). Trifluoroacetic acid was added (5 mL) and the solution was stirred at room temperature and followed by TLC (DCM: MeOH = 10:1), until full de- protection was achieved. The organic layer was concentrated *in vacuo*. The residue was purified with silica gel column chromatography. The residue was eluted with DCM: MeOH (10:1, 8:1, 1:1). Orange color solid was obtained as product (142 mg, quantitative yield). ^{1}H NMR (DMSO - d_6): δ 8.96 (t, 1H, J = 5.6 Hz), 8.14 (d, 1H, J = 1.6 Hz), 7.81- 7.74 (m, 1H), 7.73-7.71 (m, 2H), 7.40- 7.38 (m, 1H), 3.38 (d, 2H, J = 5.6 Hz), 2.80 (s, 2H), 2.45 (s, 2), 1.57 (s, 4H);

1,8-Dihydroxy-3-(9'-chloro-aceta-2',7'diamido)-anthraquinone (17).

Chloroacetyl chloride (24 μL, 0.21 mmol) was injected slowly into the solution of **16** (50 mg, 0.14 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room temperature for 2 h. The reaction was followed by TLC (DCM: MeOH = 10:1), and after completion was diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.* The residue was purified with silica gel column chromatography. The residue was eluted with DCM: methanol (60:1). Orange color solid was obtained as product (22 mg, 36% yield). ^{1}H NMR (DMSO - d_6): δ 11.92 (s, 2H), 8.23 (s, 1H), 7.86- 7.84 9(m, 1H), 7.77 (d, 2H, J = 8.4 Hz), 7.43 (d, 1H, J = 8.4 Hz), 4.03 (s, 2H), 3.24- 3.20 (m, 2H), 3.25- 3.18 (m, 1H), 1.54- 1.50 (m, 2H), 1.49- 1.45 (m, 2H); MS: m/z $[M+H]^+$ calculated $C_{21}H_{19}ClN_2O_6$ 431.1, found 431.2.

1,8-Dihydroxy-3-(9'-bromo-aceta-2',7'diamido)-anthraquinone (18).

A solution of bromoacetyl *N*-hydroxy succinimide (39.4 mg, 0.17 mmol) in 1 mL of anhydrous 1,4- dioxane was injected slowly into the solution of **16** (50 mg, 0.14 mmol) in anhydrous dioxane (5 mL). The mixture was stirred at room temperature for 2 hours. The reaction was followed by TLC (DCM: MeOH = 10:1), and after completion was diluted by H_2O (20 mL) and the suspension was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.* The residue was purified with silica gel column chromatography. The compound was eluted with DCM: methanol (60:1). Orange color solid was obtained as product (21mg, 32% yield). ^{1}H NMR (DMSO - d_6): δ 11.9 (s, 2H), 8.91 (t, 1H, J = 5.6 Hz), 8.24 (t, 1H, J = 5.6 Hz), 8.16 (d, 1H, J = 1.6 Hz), 7.84- 7.80 (m, 1H), 7.76-7.63 (m, 2H), 7.41- 7.35 (m, 1H), 4.0 (s, 1H), 3.15- 3.10 (m, 2H), 2.52 (m, 2H), 1.51- 1.43 (m, 4H); MS $[M+H]^+$ calculated $C_{21}H_{19}BrN_2O_6$ 475.04, found 475.1

1,8-Dihydroxy-3-(6',9',12'-trioxa-2',16'-tridecanediamino)-anthraquinone (20).

Rhein (**1**) (500 mg, 1.8 mmol) was dissolved in dry DCM and cooled to 0 ^{o}C. NHS (202 mg, 1.9 mmol) was added and the reaction mixture was stirred for 5 minutes followed by addition of EDCI (278 mg, 1.8 mmol). The reaction was monitored by TLC (DCM: MeOH = 8:1) and stirred for 4 hours. To the reaction mixture, ***35*** (624 mg, 1.9 mmol) was added followed by TEA (183 μL, 1.3 mmol) and the mixture was stirred overnight at room temperature. After TLC showed consumption of the starting material, the organic solvent was concentrated *in vacuo*. The residue was purified using silica gel column chromatography with an eluent consisting of mixture between DCM: MeOH = 10:1. 940 mg brown oil was obtained. The Boc- protected amine **19** (500 mg, 0.9 mmol) was suspended in DCM (7 mL). TFA (7 mL) was added and the

solution was stirred at room temperature overnight. The reaction was followed by TLC (DCM: MeOH = 8:1). The organic layer was concentrated *in vacuo*. The residue was purified with silica gel column chromatography. The residue was eluted with DCM: MeOH (10:1, 8:1, 1:1). Orange color solid was obtained as product (438 mg, quantitative yield). ^{1}H NMR (DMSO - d_6): δ 7.9(s, 1H), 7.66- 7.60 (m, 2H), 7.54 (s, 1H), 7.28 (d, 1H, J = 8.0 Hz), 3.61-3.52 (m, 13H), 3.43 (t, 2H, J = 6.4 Hz), 3.22 (s, 2H), 3.08 (t, 2H, J = 6.3 Hz), 1.91- 1.84 (m, 4H);

1,8-Dihydroxy-3-(18'-chloro-aceta-6',9',12'-trioxa-2',16'-tridecanediamido)-anthraquinone (21).

Chloroacetyl chloride (24 μL, 0.3 mmol) was injected slowly into the solution of **20** (90 mg, 0.2 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room temperature for 30 minutes. The reaction was followed by TLC (DCM: MeOH = 10:1). The organic layer was concentrated *in vacuo* . The residue was purified using silica gel column chromatography. The compound was eluted with DCM: methanol (60:1). Orange color solid was obtained as product (11 mg, 30% yield). ^{1}H NMR (DMSO - d_6): δ 8.23 (s, 1H), 7.86- 7.81 (m, 1H), 7.79-7.73 (m, 2H), 7.45- 7.41 (m, 1H), 4.01 (s, 2H), 3.42- 3.51 (m, 14H), 3.40 (t, 2H, J = 6.0 Hz), 3.15- 3.10 (m, 2H), 1.73 (t, 2H, J = 6.2 Hz), 1.67 (t, 2H, J = 6.2 Hz); MS: m/z $[M-H]^+$ calculated $C_{27}H_{31}ClN_2O_9$ 563.1, found 563.3.

1,8-Dihydroxy-3-(18'-bromo-aceta-6',9',12'-trioxa-2',16'-tridecanediamido)-anthraquinone (22).

Bromoacetyl N-hydroxy succinimide (79 mg, 0.3 mmol) was injected slowly into the solution of (**20**) (80 mg, 0.2 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room

temperature for 1 hour. The reaction was followed by TLC (DCM: MeOH = 10:1), and 0.5 equivalent of TEA was added. The reaction was diluted by H_2O (2 mL) and the suspension was extracted with CH_2Cl_2 (2 × 20 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.* The residue was purified using silica gel column chromatography. The compound was eluted with DCM: methanol (1:0, 60:1). Orange color solid was obtained as product (47 mg, 39% yield). ^{1}H NMR (DMSO - d_6): δ 11.90 (s, 2H), 8.18 (s, 1H), 7.83 (t, 1H, J = 8.4 Hz), 7.61-7.45 (m, 2H), 7.42- 7.40 (m, 1H), 4.02 (s, 2H), 3.52- 3.45 (m, 14H), 3.39 (t, 2H, J = 6.2 Hz), 3.17- 3.08 (m, 2H), 1.82- 1.75 (m, 2H), 1.67- 1.61 (m, 2H); MS: m/z $[M-H]^-$ calculated $C_{27}H_{31}BrN_2O_9$ 607.1, found 606.3.

1,8-Dihydroxy-3-(18'-iodo-aceta-6',9',12'-trioxa-2',16'-tridecanediamido)-anthraquinone (23).

Iodoacetyl chloride (25 μL, 0.3 mmol) was injected slowly into the solution of **20** (90 mg, 0.2 mmol) in anhydrous 1,4- dioxane (5 mL). The mixture was stirred at room temperature for 1 hour. The reaction was followed by TLC (DCM: MeOH = 10:1). The organic layer was concentrated *in vacuo*. The residue was purified using silica gel column chromatography. The product was eluted with DCM: methanol (60:1). Orange color solid was obtained as product (26 mg, 20% yield). ^{1}H NMR (DMSO - d_6): δ 8.12 (d, 1H, J = 1.6), 7.79- 7.73 (m, 2H), 7.68-7.67 (m, 1H), 7.33- 7.31 (m, 1H), 3.68- 3.60 (m, 11H), 3.57- 3.47 (m, 7H), 3.36- 3.31 (m, 1H,), 3.26-3.21 (m, 1H), 1.94- 1.88 (m, 2H), 1.80- 1.71 (m, 2H); MS: m/z $[M-H]^+$ calculated $C_{27}H_{31}IN_2O_9$ 655.1, found 655.3.

MTT assay. HeLa, Hek, and KB cell lines were purchased from ATCC. All of the cell lines were cultured in RPMI-1640 medium. The medium was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. For the cytotoxicity assays, cells were seeded into 96-well plate (2.5×10^4 in 100 μL per well for HeLa, 3.0×10^4 for KB, and 5×10^4 for Hek). The compounds were dissolved or suspended in DMSO to make 10 mM stock solutions. The stock solution was diluted using DMSO to various concentrations. 1μL of each concentration was diluted 100 fold with medium into the well plate keeping the DMSO < 1% throughout the experiment. Addition of compounds was performed after adherent cells reached 40-50% confluence. After incubation for 48 h. at 37 ^{o}C in humidified atmosphere with 5% CO_2, 10 μL of MTT (5 mg/mL in PBS) was added. After addition of MTT the cells were incubated for another 4 h. The culture medium was then aspirated and 100 μL of DMSO was added to each well. The 96-well plate was read by microarray reader for optical density at 490 nm. All tests were performed in triplicates and IC_{50} values were estimated from the averaged response curves. For compounds with $IC_{50} < 100$ μM, the MTT assay was repeated.

2) A NOVEL METAL FREE SYNTHESIS OF 6H-ISOINDOLO[2,1-α]INDOLO-6-ONE

2.1) *Introduction*

6H-Isoindolo[2,1-α]indol-6-one (**24**) is a core structure for a number biologically active compounds.[16-19] Indole-based structures derived from heterocyclic systems resembling 6H-isoindolo[2,1-α]indol-6-one (**24**) have been used as melatonin MT_3 ligands **26**[17] and potential anti-tumor agents **25** [18,20] as well as intermediate **27** in the synthesis of bacterial NorA efflux pump inhibitors [16,19] (Figure 2.1). Melatonin is an indole derived neurohormone synthesized in the pineal gland in all mammalians.[17,21] Two melatonin receptors MT_1 and MT_2 are known and have been widely studied in the human body.[17,21] However, a third MT_3 receptor has been found, that needs to be closely studied.[21] It has been reporter that isoindolo compounds that have a core structure resembling compound **24** bind to this receptor and give the opportunity for further binding studies of the MT_3 receptor.[17] The importance of these isoindolo compounds to the study of the melatonin MT_3 receptor brings the need for a short and efficient synthetic route towards their core synthesis. Another important area of application in which isoindolo compounds have found place is the design of anticancer drugs. Targeting DNA, synthesizing intercalating agents, and developing topoisomerase II poisons is strategy that carries great potential in the anticancer drug design process. A number of compounds resembling the structure of 6H-isoindolo[2,1-α]indol-6-one (**24**) has shown great potential and cytotoxicity against some carcinoma cells in the low micromolar concentrations.[18,20] Being able to apply a short and efficient synthetic route to the synthesis of the core structure will bring ease to the development of libraries of potential anticancer agents. A convenient synthetic route to isoindolo compounds carrying similar

structures as compound **24** can also be applied for the development of bacterial NorA efflux pump inhibitors. It has been reported that compound **24** is an intermediate in the synthetic rout towards number of Nor A efflux inhibitors.[16] The here in described route is not just time efficient but also cost efficient. Route consisting of reactions with relatively cheap commercially available starting material and giving high yields can greatly contribute to the development of more novel Nor A efflux pump inhibitors. A number of routes for the synthesis of 6H-isoindolo[2,1-α]indol-6-one (**24**) and its analogues have been reported. [22-32]

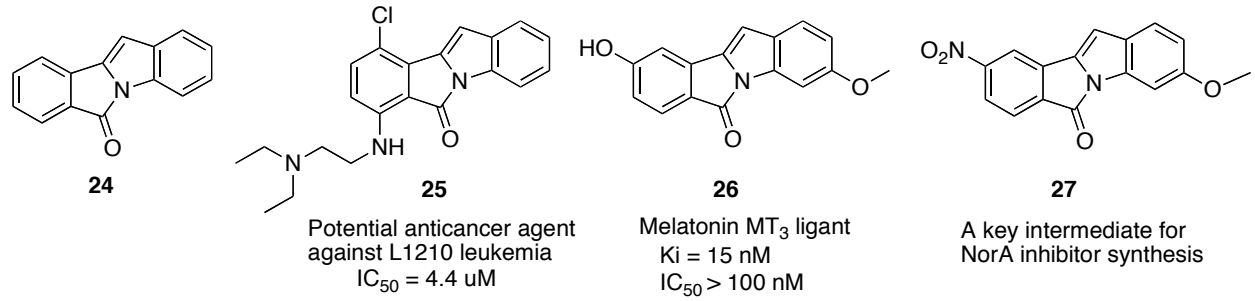
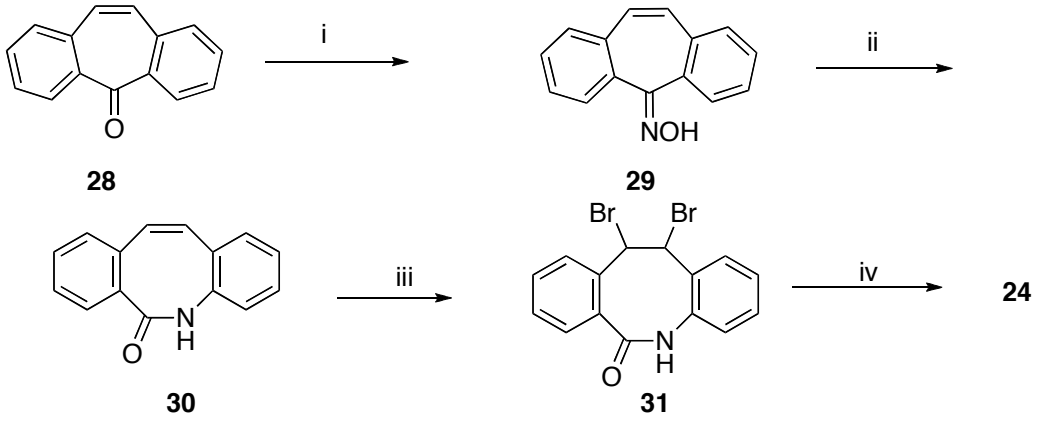


Figure 2.1.Chemical structures of 6H-Isoindolo[2,1-α]indol-6-one and some biologically relevant analogues.

To our best knowledge, most of the strategies described in the literature involve the use of indole-based compounds as the starting materials and expensive metal catalysts.[18,20] We herein report an efficient method that does not rely on an indole starting material and costly metal catalysts

2.2) *Results and Discussion*

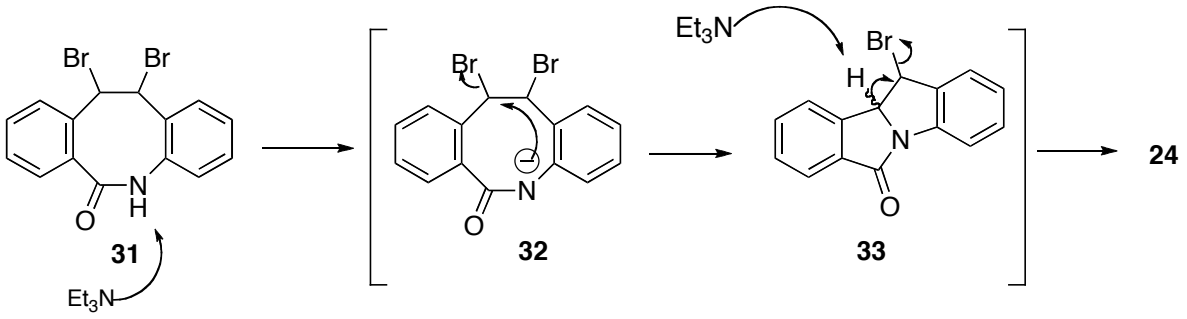
The synthesis began with a relatively cheap, commercially available starting material dibenzocyclohepten-5-one (**28**). Through reaction with hydroxylamine, oxime **29** was formed in 93% yield following standard procedures using dry pyridine as the solvent (*Scheme 2.1*). [33]



Scheme 2.1. Synthetic route to the desired 6H-Isoindolo[2,1-α]indol-6-one; Reagents and conditions: i) NH_2OH-HCl, Pyridine, reflux, 20 h., 93 % yield, ii) TFA, DCM, reflux, overnight, 95 % yield, iii) Br_2, DCM, room temperature, 3 h., 67 % yield, iv) TEA, THF, room temperature, overnight, 70 % yield.

The true challenge of this route was to establish the Beckmann rearrangement reaction conditions that would provide a convenient purification method due to the extremely poor solubility of lactam **30**. The standard Beckmann rearrangement conditions, heating the oxime in polyphosphoric acid, yielded a black solid that was insoluble in number of organic solvents including acetone, methanol, ethyl acetate, and hexanes, and had poor solubility in DMF and DMSO as well.[33] As a solution, we discovered that the Beckmann rearrangement could be

achieved by refluxing the oxime intermediate **29** in TFA, which led to the desired lactam **30** in 95% yield.[34] The work up procedure consisted of simple evaporation of the TFA followed by silica gel column chromatography. Subsequent bromination through addition of molecular bromine to a suspension of lactam **30** in DCM led to 9,10-dibromodibenzo[b,f]azocin-6-one (**31**) in 67% yield. [35] The last step involves intramolecular cyclization under basic conditions and elimination of hydrogen bromide (*Scheme 2.2*). This was accomplished by dissolving compound **31** in THF followed by the slow addition of an excess amount of a base. A number of bases were studied to optimize this intramolecular cyclization/elimination reaction: *t*-BuOK, LDA, KHMDS, and TEA. The base that gave the best yields was TEA (70% yield). The yields for the reactions using other bases ranged from 0 – 47%.



Scheme 2.2. Proposed mechanism for the intramolecular cyclization/elimination of **31**

2.3) ***Conclusions***

We have developed a new four-step synthetic route (Scheme 2.1) to isoindolo[2,1-α]indol-6-one (**24**). The overall yield of the route is 42 %. The described route is efficient and metal free, and allowed for the *de novo* construction of the indole and isoindole rings that are important in the synthesis of number biologically relevant compounds.

2.4) *Experimental Section*

Dibenzocyclohepten-5-one (**24**) was purchased form TCI America, and directly used without further purification. Other compounds and solvents were purchased from Acros and Aldrich. Analytical grade solvents were used for all reactions. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 NMR spectrometer in a deuterated solvent with TMS (δ = 0.00 ppm) or the NMR solvent as the internal reference. The deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories, Inc. Mass spectra were recorded on an API 3200 LC/MS/MS system.

Dibenzocyclohepten-5-one oxime (29):

A mixture of dibenzocyclohepten-5-one (**28**) (2.0 g, 9.65 mmol), hydroxylamine hydrochloride (1.0 g, 14.47 mmol), and dry pyridine (12 mL) was heated under reflux for 20 hrs. The pyridine in the reaction mixture was removed through evaporation in vacuum and the residue was poured into hexanes followed by acidification with 1 M hydrochloric acid. The two layers were placed in an ice bath for 2 hrs, which lead to precipitation. The solid was filtered and

re-crystallized in hexanes to give a white powder product (1.9 g, 93 % yield). The compound was characterized by ^{1}H NMR and MS.[33]

Dibenzo[b,f]azocin-6-one (30):

A round-bottom flask was charged with dibenzocyclohepten-5-one oxime (**29**) (100 mg, 0.45 mmol) and TFA (5 mL). The reaction mixture was heated under reflux overnight. The TFA was evaporated and the resulting product was purified via silica gel column chromatography, using a mixture of hexanes and ethyl acetate in a 4:1 ratio to give a grey powder product (92 mg, 95% yield). The compound was characterized by ^{1}H NMR and MS.[33]

9, 10-Dibromodibenzo[b,f]azocin-6-one (31):

Dibenzo[b,f]azocin-6-one (**7**) (100 mg, 0.45 mmol) was suspended in dry DCM (3 mL) and cooled to 0 ^{o}C. Br_2 (8.6 µL, 0.167 mmol) was slowly added in and the reaction mixture was stirred for 3 hrs at room temperature. Reaction was quenched with the addition of saturated sodium sulfite aqueous solution (1 mL). The organic and the aqueous layers were separated and the aqueous solution was extracted using DCM (3 × 5 mL). The combined organic layers was washed with brine, dried over $MgSO_4$, filtered and concentrated in vaccuo. The product was eluted from silica gel column chromatography with a solvent consisting of hexanes and ethyl acetate in a 6:1 ratio to give a light-brown powder product (116 mg, 67% yield). At this point the product consists of a mixture of stereoisomers, and was used in the next step without further purification. ^{1}H NMR (DMSO–d_6): δ 10.28 (s, 1H), 7.75 - 7.69 (m, 1H), 7.68 - 7.41 (m, 1H),

7.40 – 7.02 (m, 5H), 5.98 – 5.81(m, 2H). MS (ESI); m/z calculated $C_{15}H_{11}Br_2NO$ $[M+H]^+$: 382.06; found: 381.90.

Isoindolo[2,1-a]indolo-6–one (24):

9,10-Dibromodibenzo[b,f]azocin-6-one (**31**) (50 mg, 0.131 mmol) was dissolved in dry THF (5 mL). TEA (2.7 mL) was slowly added to the reaction mixture. After stirring at room temperature overnight, water (10 mL) was added and the organic layer was separated. The aqueous solution was extracted using DCM (3 × 10 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vaccuo. The residue was purified by silica gel column chromatography with an eluent consisting of hexanes and ethyl acetate in a 5:1 ratio to give a bright yellow-green solid (20 mg, 70 % yield). ^1H NMR ($CDCl_3$): δ 7.82 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 7.6 Hz, 1H), 7.44- 7.40 (m, 2 H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.26-7.18 (m, 2 H), 7.09 (m, 1H), 6.65 (s, 1 H); ^{13}C NMR ($CDCl_3$): δ 162.6, 138.9, 134.7, 134.5, 133.9, 133.7, 133.5, 128.8, 126.3, 125.3, 123.9, 122.3, 121.3, 113.6, 103.5. MS (ESI); m/z calculated $C_{15}H_9NO$ $[M+ H]^+$: 219.07; found 219.7.

References

(1) Tan, J. H.; Zhang, Q. X.; Huang, Z. S.; Chen, Y.; Wang, X. D.; Gu, L. Q.; Wu, J. Y. *Eur J Med Chem* **2006**, *41*, 1041.

(2) Katzhendler, J.; Gean, K.-F.; Bar-ad, G.; Tashma, Z.; Ben-shoshan, R.; Ringel, I.; Bachrach, U.; Ramu, A. *Eur J Med Chem* **1989**, *24*, 23.

(3) Huang, H. S.; Chin, H. F.; Yeh, P. F.; Yuan, C. L. *Helv Chim Acta* **2004**, *87*, 999.

(4) Ip, S. W.; Weng, Y. S.; Lin, S. Y.; Mei-Dueyang; Tang, N. Y.; Su, C. C.; Chung, J. G. *Anticancer Res* **2007**, *27*, 379.

(5) van Gorkom, B. A. P.; Timmer-Bosscha, H.; de Jong, S.; van der Kolk, D. M.; Kleibeuker, J. H.; de Vries, E. G. E. *Brit J Cancer* **2002**, *86*, 1494.

(6) Cui, X. R.; Tsukada, M.; Suzuki, N.; Shimamura, T.; Gao, L.; Koyanagi, J.; Komada, F.; Saito, S. *Eur J Med Chem* **2008**, *43*, 1206.

(7) Zhou, X.; Song, B.; Jin, L. H.; Hu, D. Y.; Diao, C. L.; Xu, G. F.; Zou, Z. H.; Yang, S. *Bioorg Med Chem Lett* **2006**, *16*, 563.

(8) Cichewicz, R. H.; Zhang, Y. J.; Seeram, N. P.; Nair, M. G. *Life Sci* **2004**, *74*, 1791.

(9) Shi, P.; Huang, Z. W.; Chen, G. C. *Am J Chinese Med* **2008**, *36*, 805.

(10) Monneret, C. *Eur J Med Chem* **2001**, *36*, 483.

(11) Koyama, M.; Takahashi, K.; Chou, T. C.; Darzynkiewicz, Z.; Kapuscinski, J.; Kelly, T. R.; Watanabe, K. A. *J. Med. Chem.* **1989**, *32*, 1594.

(12) Mueller, S. O.; Stopper, H. *Bba-Gen Subjects* **1999**, *1428*, 406.

(13) Lin, S. G.; Fujii, M.; Hou, D. X. *Arch. Biochem. Biophys.* **2003**, *418*, 99.

(14) Zagotto, G.; Uriarte, E.; Antonello, C.; Conconi, M. T.; Magno, S. M.; Palumbo, M. *Bioorg Med Chem Lett* **1992**, *2*, 659.

(15) Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.

(16) Ambrus, J. I.; Kelso, M. J.; Bremner, J. B.; Ball, A. R.; Casadei, G.; Lewis, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4294.

(17) Boussard, M. F.; Truche, S.; Rousseau-Rojas, A.; Briss, S.; Descamps, S.; Droual, M.; Wierzbicki, M.; Ferry, G.; Audinot, V.; Delagrange, P.; Boutin, J. A. *Eur. J. Med. Chem.* **2006**, *41*, 306.

(18) Guillaumel, J.; Leonce, S.; Pierre, A.; Renard, P.; Pfeiffer, B.; Arimondo, P. B.; Monneret, C. *Eur. J. Med. Chem.* **2006**, *41*, 379.

(19) Samosorn, S.; Bremner, J. B.; Ball, A.; Lewis, K. *Bioorg. Med. Chem.* **2006**, *14*, 857.

(20) Guillaumel, J.; Pierre, L.; Renard, P.; Pfeiffer, B.; Peruchon, L.; Arimondo, P.; Monneret, C. *Onc. Res.* **2003**, *13*, 537.

(21) P. Delagrange, J. A., y J. A. Boutin, L. Casteilla, D. Lesieur, R. Misslin, S. Pellissier, L. Pe´ nicaud, P. Renard *J. Neuroendocrin.* **2003**, *15*, 442.

(22) Kozikowski, A. P.; Ma, D. *Tetrahedron Lett.* **1991**, *32*, 3317.

(23) Lie´gault, B.; Lee, D.; Huestis, M.; Stuart, D.; Fagnou, K. *J. Org. Chem.* **2008**, *73*, 5022.

(24) Carruthers, W.; Evans, N. *J. Chem. Soc.* **1974**, 1523.

(25) Crawford, L. A.; Clemence, N. C.; McNab, H.; Tyas, R. G. *Org. Biomol. Chem.* **2008**, *6*, 2334.

(26) Dalton, L.; Humphrey, G. L.; Cooper, M. M.; Joule, J. A. *J. Chem. Soc.* **1983**, 2417.

(27) Dwight, T. A.; Rue, N. R.; Charyk, D.; Josselyn, R.; DeBoef, B. *Org. Lett.* **2007**, *9*, 3137.

(28) Garcia, A.; Rodriguez, D.; Castedo, L.; Saa, C.; Dominguez, D. *Tetrahedron Lett.* **2001**, *42*, 1903.

(29) Hooper, M.; Imam, S. H. *J. Chem. Soc.* **1985**, 1583.

(30) Itahara, T. *Chem. Soc. Jap.* **1981**, *54*, 305.

(31) Kim, G.; Kim, J. H.; Kim, W. J.; Kim, Y. A. *Tetrahedron Lett.* **2003**, *44*, 8207.

(32) Tierney, M. T.; Grinstaff, M. W. *J. Org. Chem.* **2000**, *65*, 5355.

(33) Yale, H. L.; Sowinski, F. A.; (E. R. Squibb and Sons, Inc.). Application: US 3448102, 1969, p 5 pp.

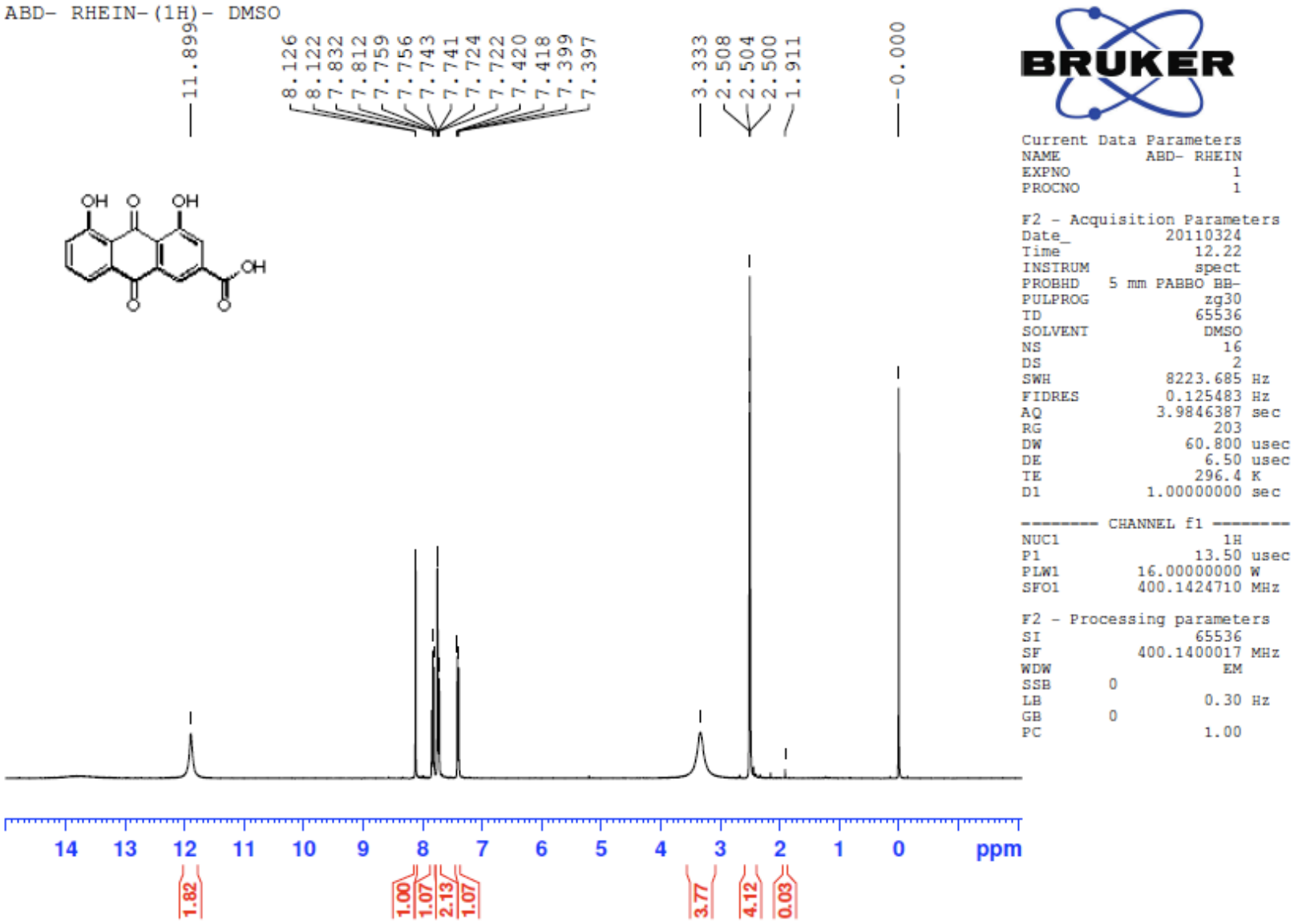
(34) Ronchin, L.; Vavasori, A. *J. Mol. Catal. A: Chem.* **2009**, *313*, 22.

(35) Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F.; van Hest, J. C. M.; van Delft, F. L. *Chem. Comm.* **2010**, *46*, 97.

APPENDIX

The contents of this appendix consist of NMR and MS spectra of the compounds which synthesis is described above.

Rhein



BRUKER

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PROCNO                 1

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Time               12.22
INSTRUM            spect
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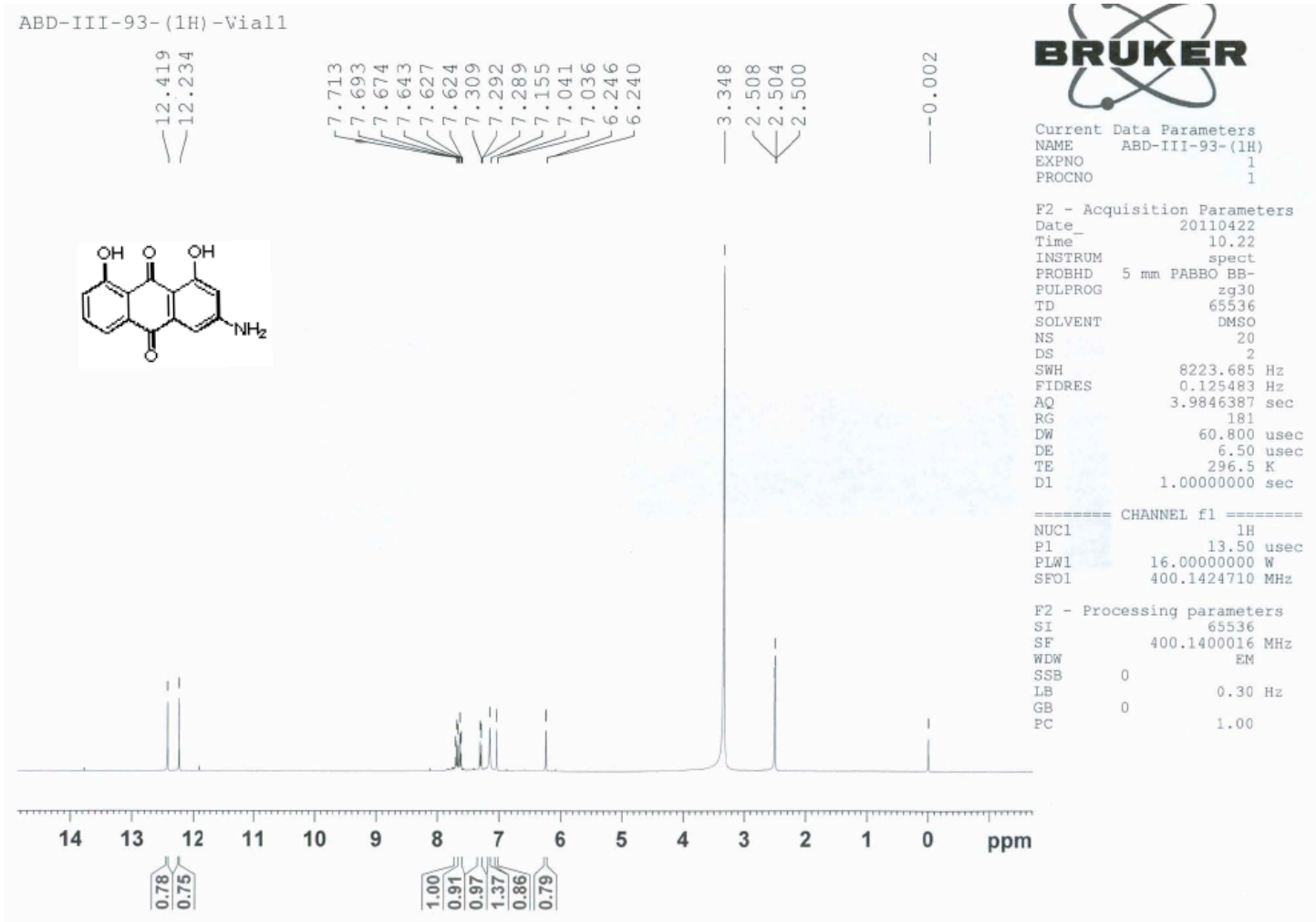
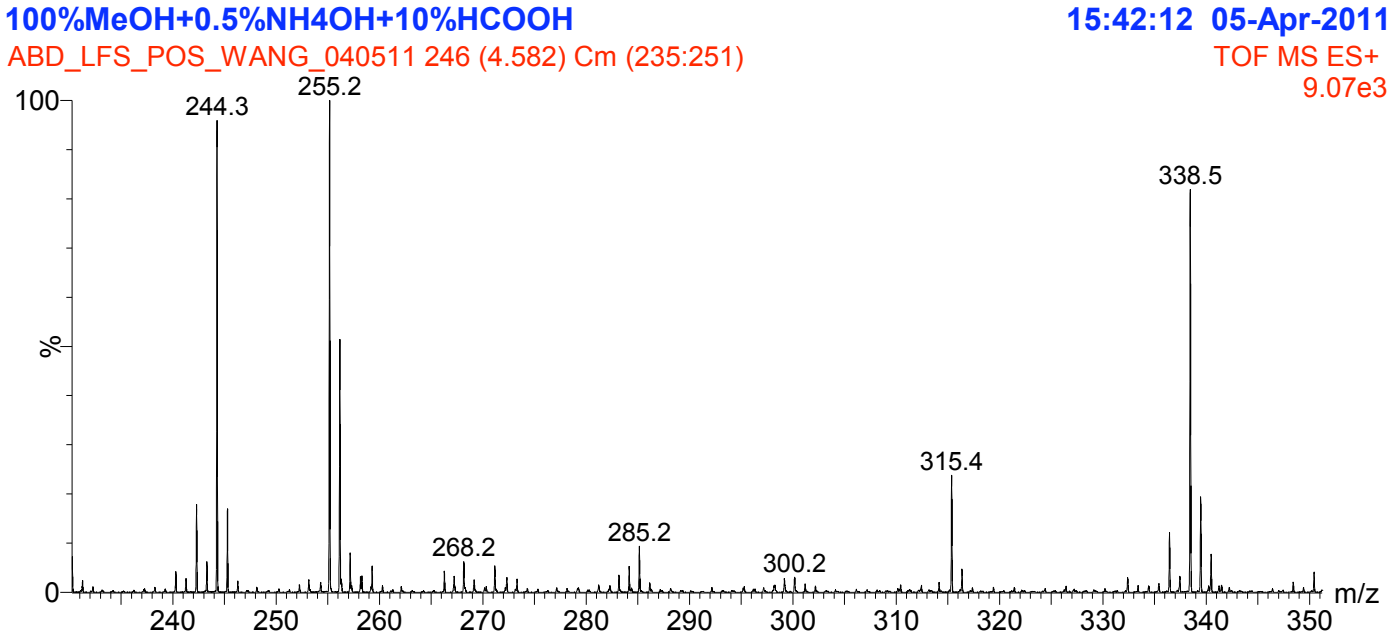
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ              3.9846387 sec
RG                   203
DW                60.800 usec
DE                 6.50 usec
TE                296.4 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 13.50 usec
PLW1         16.00000000 W
SFO1        400.1424710 MHz

F2 - Processing parameters
SI                 65536
SF          400.1400017 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

1,8-Dihydroxy-3-amino-anthraquinone (3).

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15:42:12 05-Apr-2011
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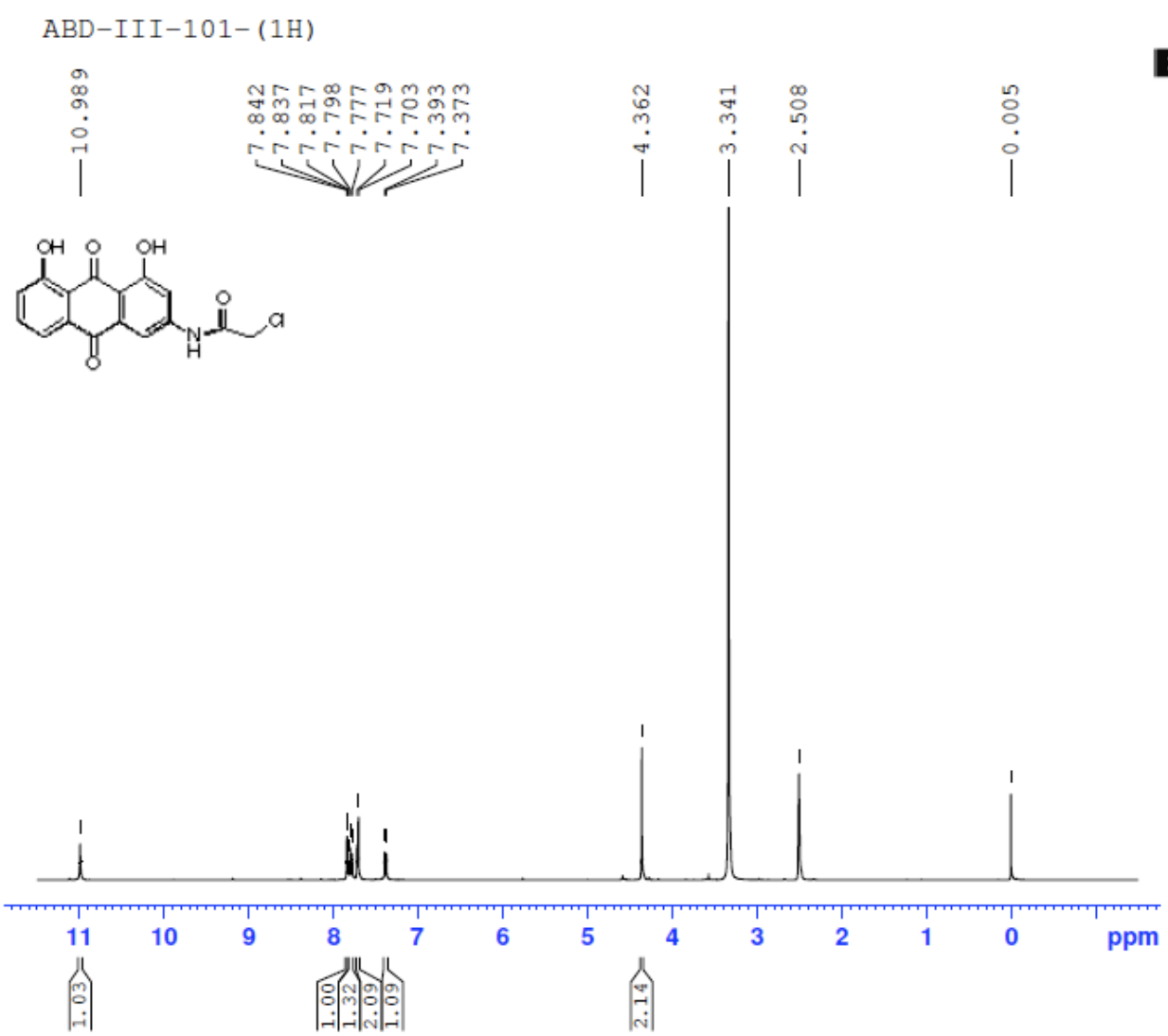
TOF MS ES+
9.07e3
100
%
0
244.3
255.2
268.2
285.2
300.2
315.4
338.5
240
250
260
270
280
290
300
310
320
330
340
350
m/z

1,8-Dihydroxy-3-(2'-chloro-acetamido)-anthraquinone (5).



BRUKER

```
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PROCNO                  1

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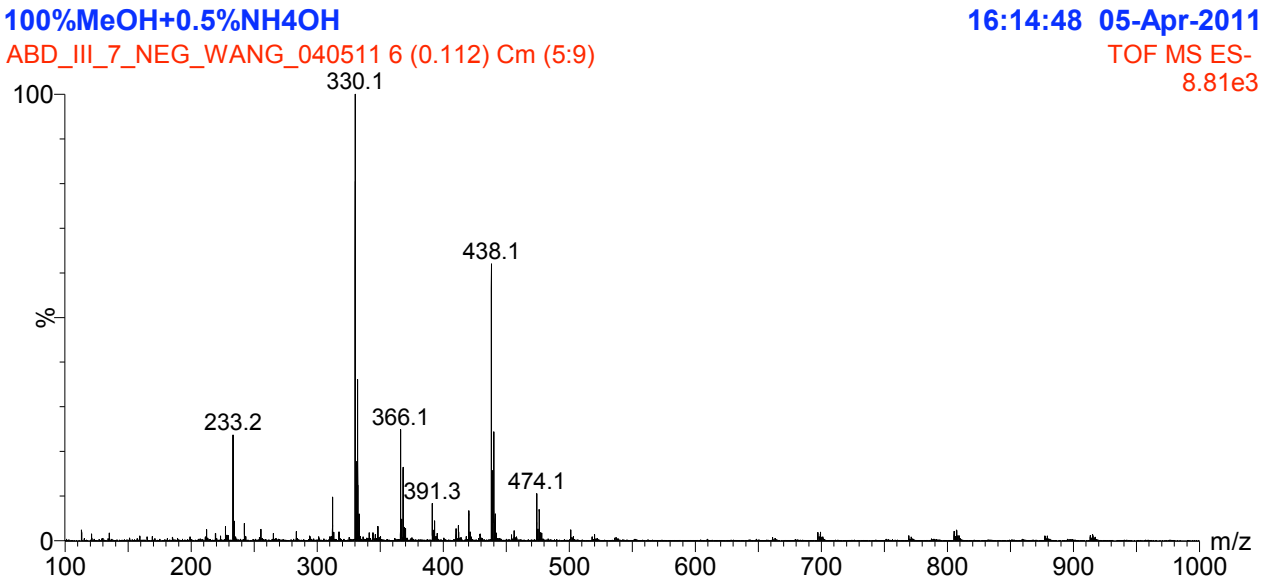
Date_           20110429
Time               13.20
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                32768
SOLVENT            DMSO
NS                    16
DS                     2
SWH             5197.505 Hz
FIDRES          0.158615 Hz
AQ             3.1523316 sec
RG                   101
DW                96.200 use
DE                 6.50 use
TE                296.5 K
D1           2.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 13.50 use
PLW1         12.21800041 W
SFO1         400.1420007 MHz

F2 - Processing parameters
SI                 32768
SF          400.1400000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```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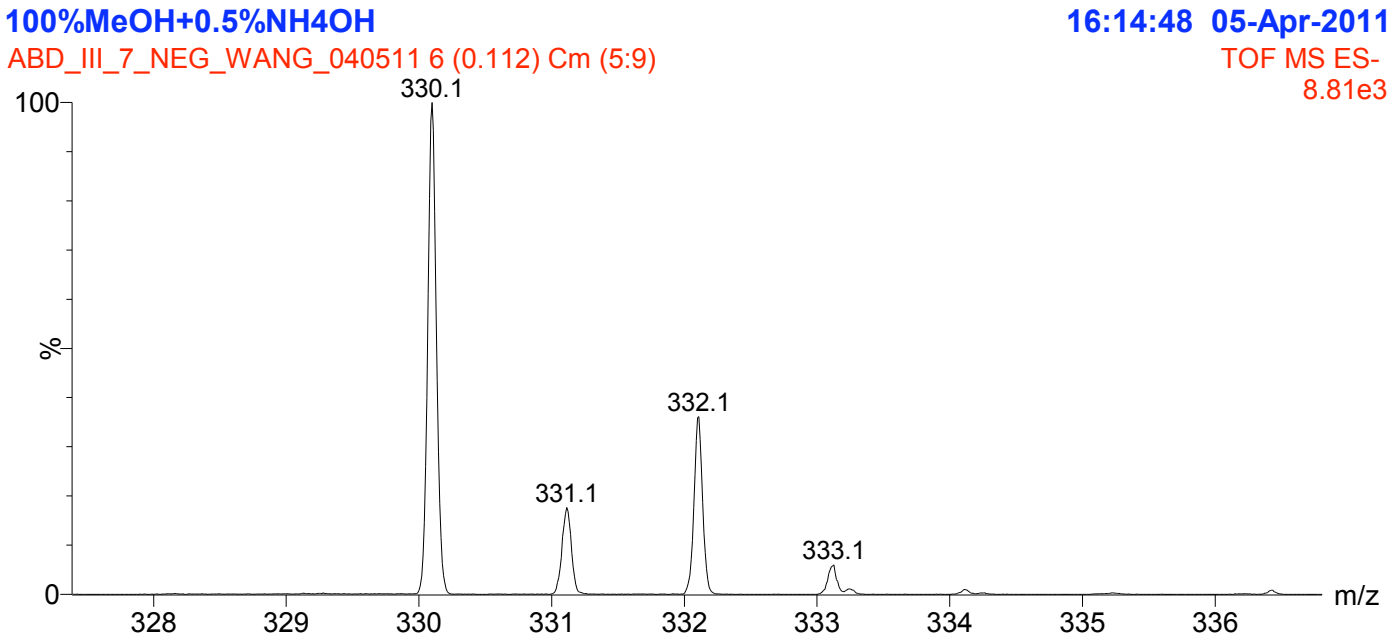
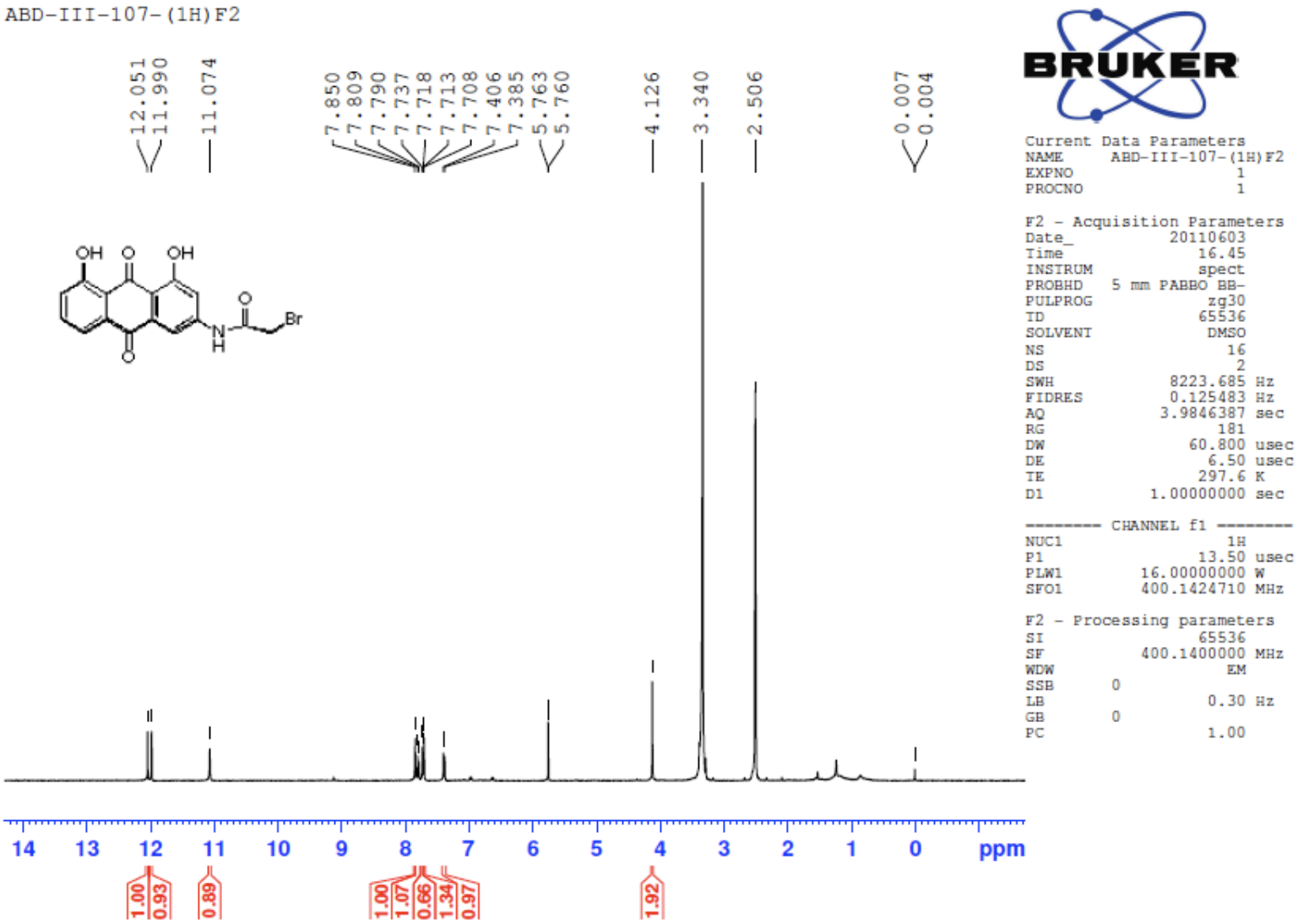
TOF MS ES-
8.81e3
330.1
438.1
233.2
366.1
391.3
474.1
100
%
0
100
200
300
400
500
600
700
800
900
1000
m/z


100%MeOH+0.5%NH4OH
16:14:48 05-Apr-2011
ABD_III_7_NEG_WANG_040511 6 (0.112) Cm (5:9)
TOF MS ES-
8.81e3
330.1
332.1
331.1
333.1
100
%
0
328
329
330
331
332
333
334
335
336
m/z

1,8-Dihydroxy-3-(2'-bromo-acetamido)-anthraquinone (6).

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PROCNO                     1

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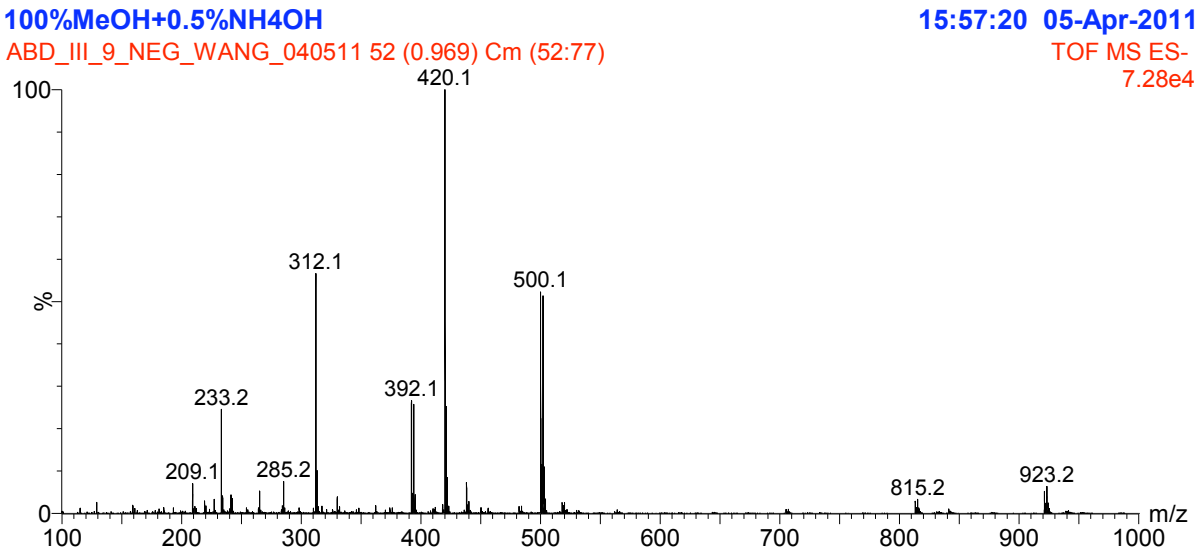
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                     16
DS                      2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ               3.9846387 sec
RG                    181
DW                 60.800 usec
DE                  6.50 usec
TE                 297.6 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  13.50 usec
PLW1          16.00000000 W
SFO1          400.1424710 MHz

F2 - Processing parameters
SI                  65536
SF            400.1400000 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```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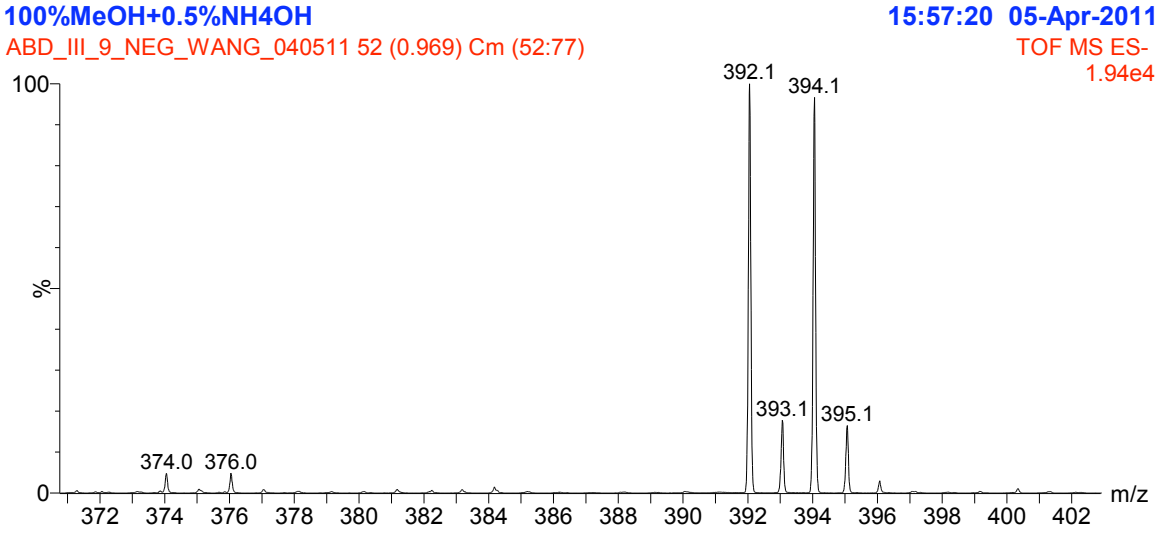
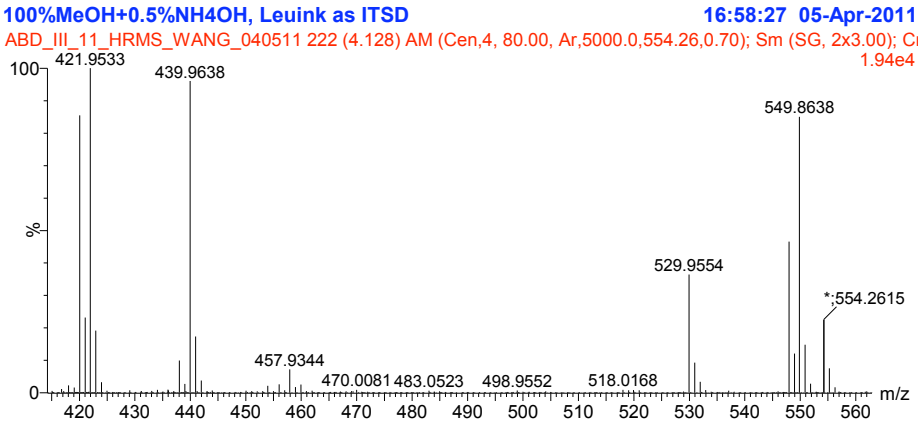
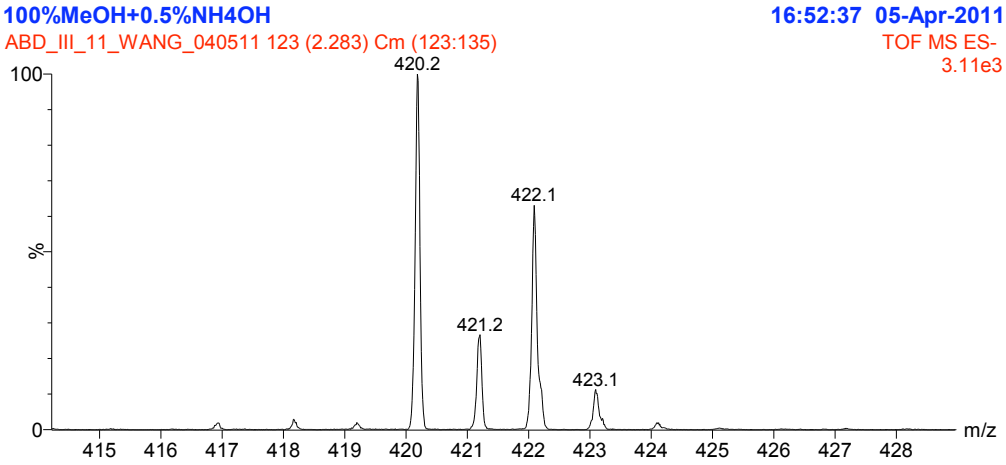
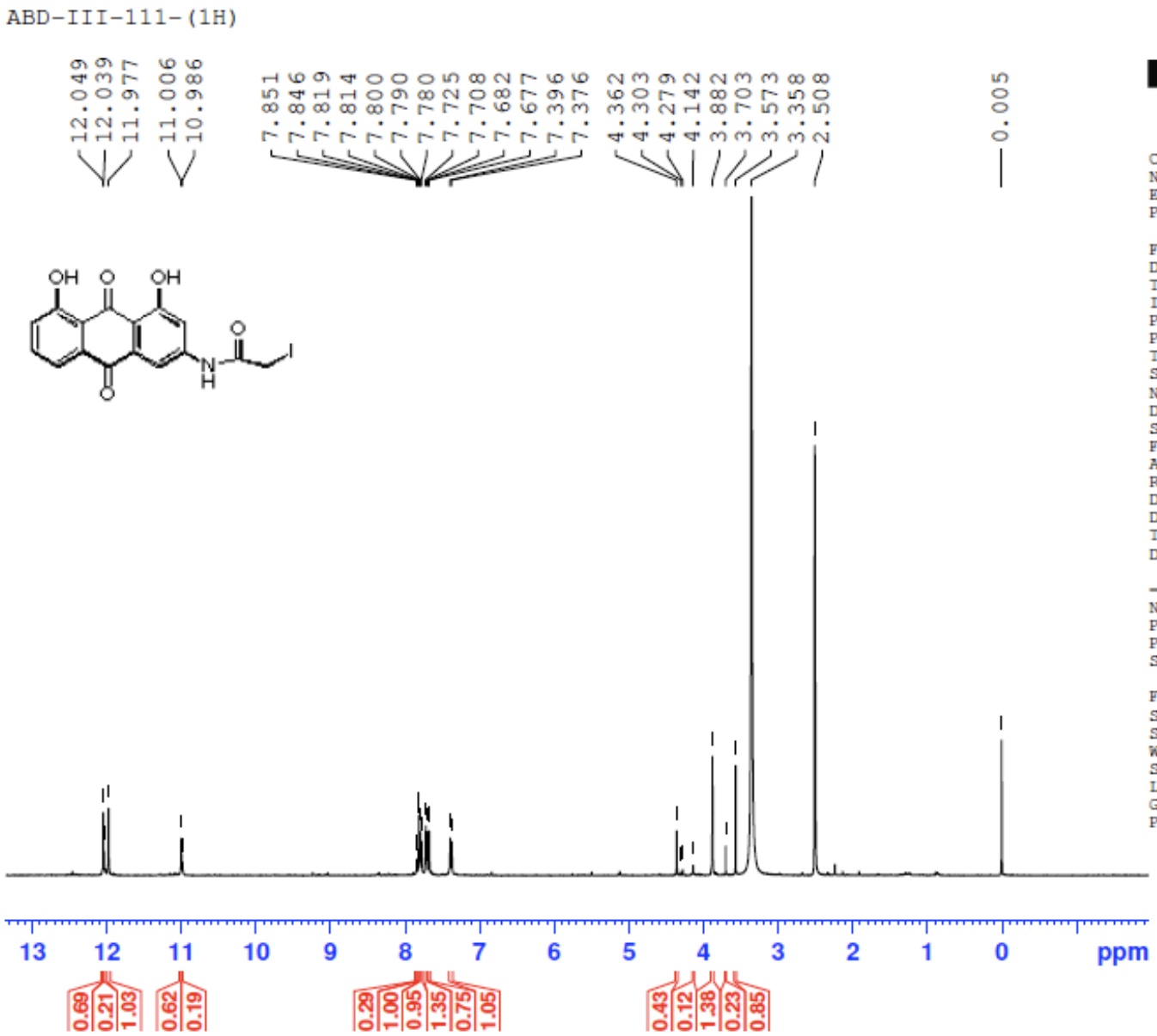
TOF MS ES-
7.28e4
100
%
0
420.1
312.1
500.1
233.2
392.1
209.1
285.2
815.2
923.2
m/z
100
200
300
400
500
600
700
800
900
1000


100%MeOH+0.5%NH4OH
15:57:20 05-Apr-2011
ABD_III_9_NEG_WANG_040511 52 (0.969) Cm (52:77)
TOF MS ES-
1.94e4
100
%
0
392.1
394.1
393.1
395.1
374.0
376.0
m/z
372
374
376
378
380
382
384
386
388
390
392
394
396
398
400
402

1,8-Dihydroxy-3-(2'-iodo-acetamido)-anthraquinone (7)







BRUKER

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EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
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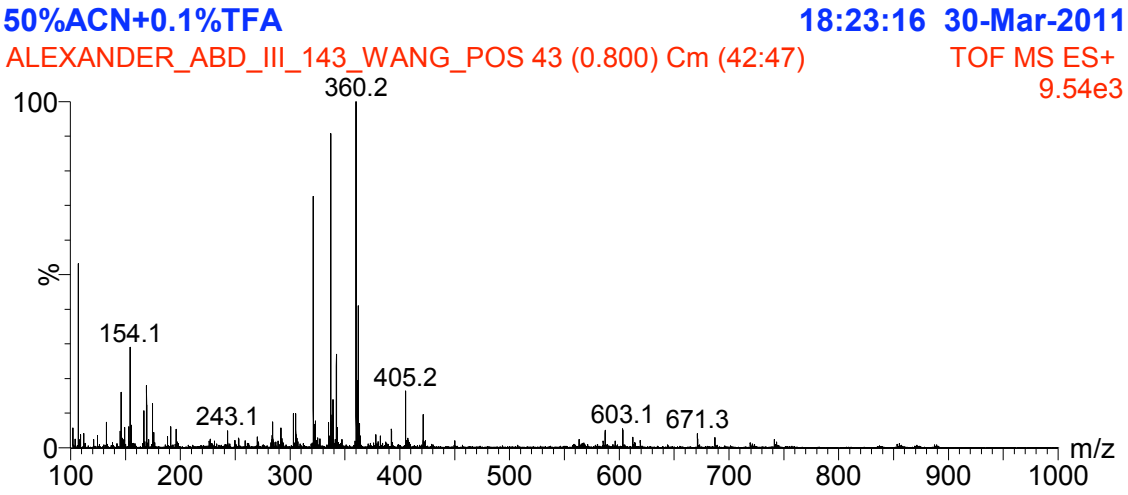
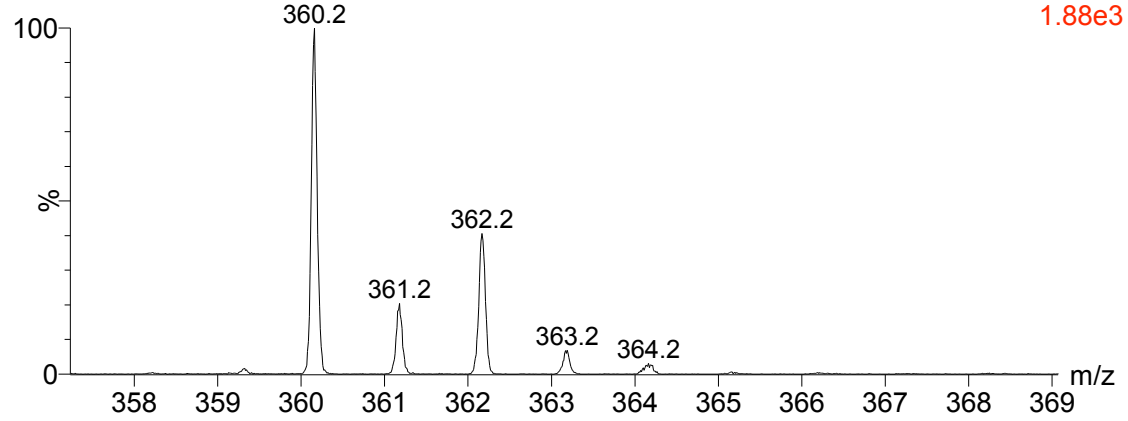
Time 14.17
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 203
DW 60.800 usec
DE 6.50 usec
TE 297.2 K
D1 1.00000000 sec

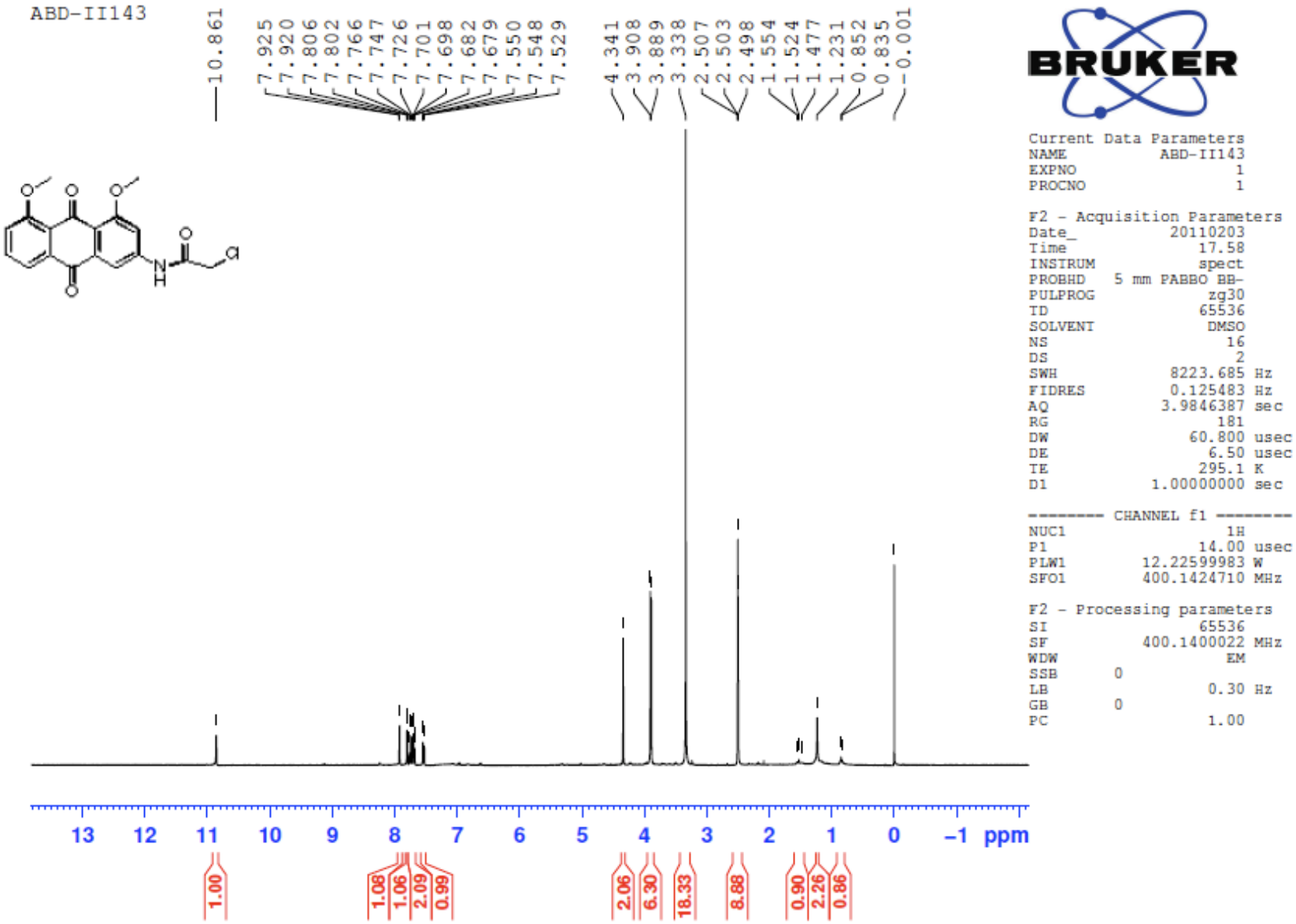
======== CHANNEL f1 ========
NUC1 1H
P1 13.50 usec
PLW1 16.00000000 W
SFO1 400.1424710 MHz

F2 - Processing parameters
SI 65536
SF 400.1400000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1,8-Methoxy-3-(2'-chloro-acetamido)-anthraquinone (12).







BRUKER

```
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EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
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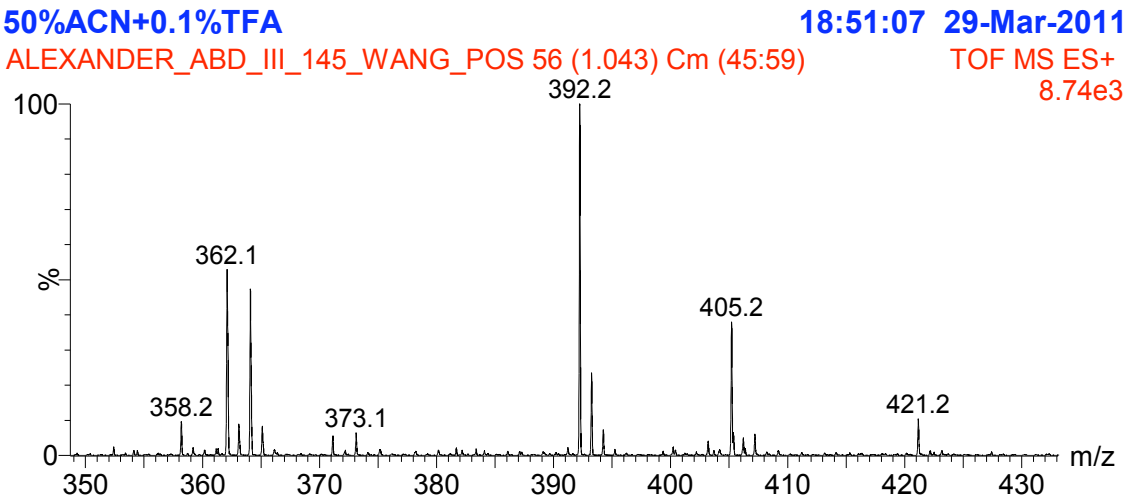
Time              17.58
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                    16
DS                     2
SWH            8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                   181
DW               60.800 usec
DE                6.50 usec
TE                295.1 K
D1           1.00000000 sec

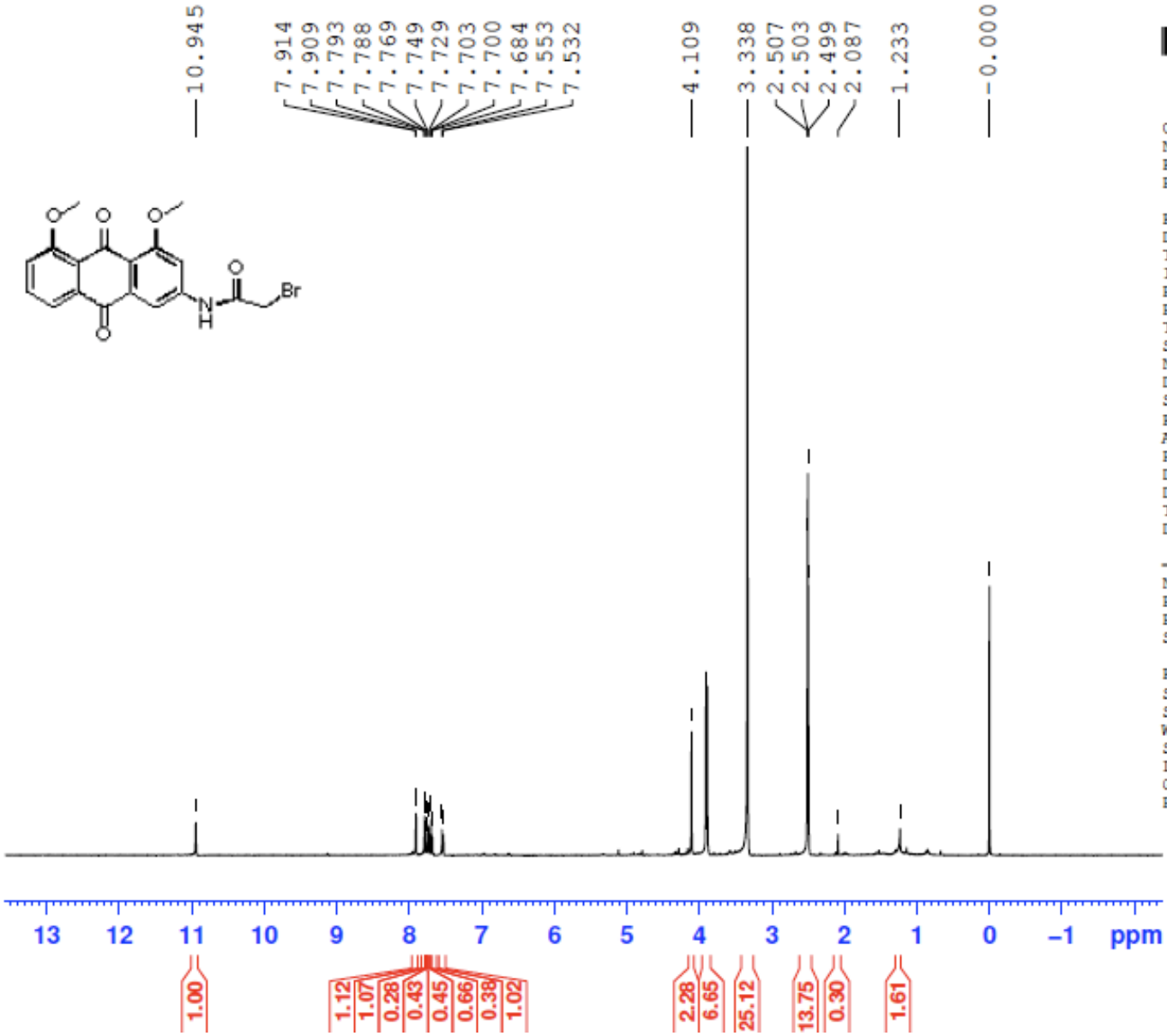
======== CHANNEL f1 ========
NUC1                 1H
P1               14.00 usec
PLW1        12.22599983 W
SFO1        400.1424710 MHz

F2 - Processing parameters
SI                65536
SF          400.1400022 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

1,8-Methoxy-3-(2'-bromo-acetamido)-anthraquinone (13)



ABD-II-145pure



BRUKER

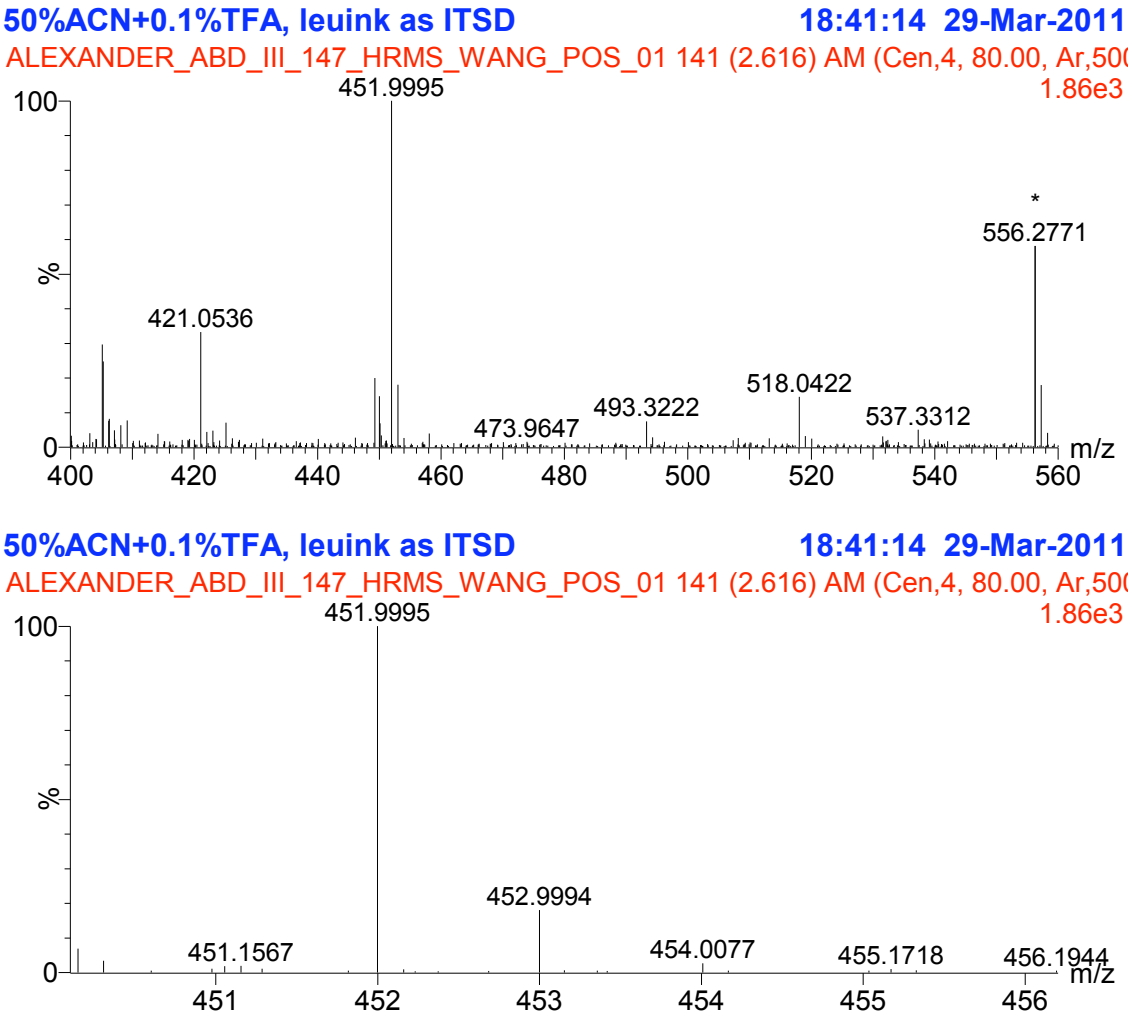
```
Current Data Parameters
NAME      ABD-II-145pure
EXPNO                  1
PROCNO                 1

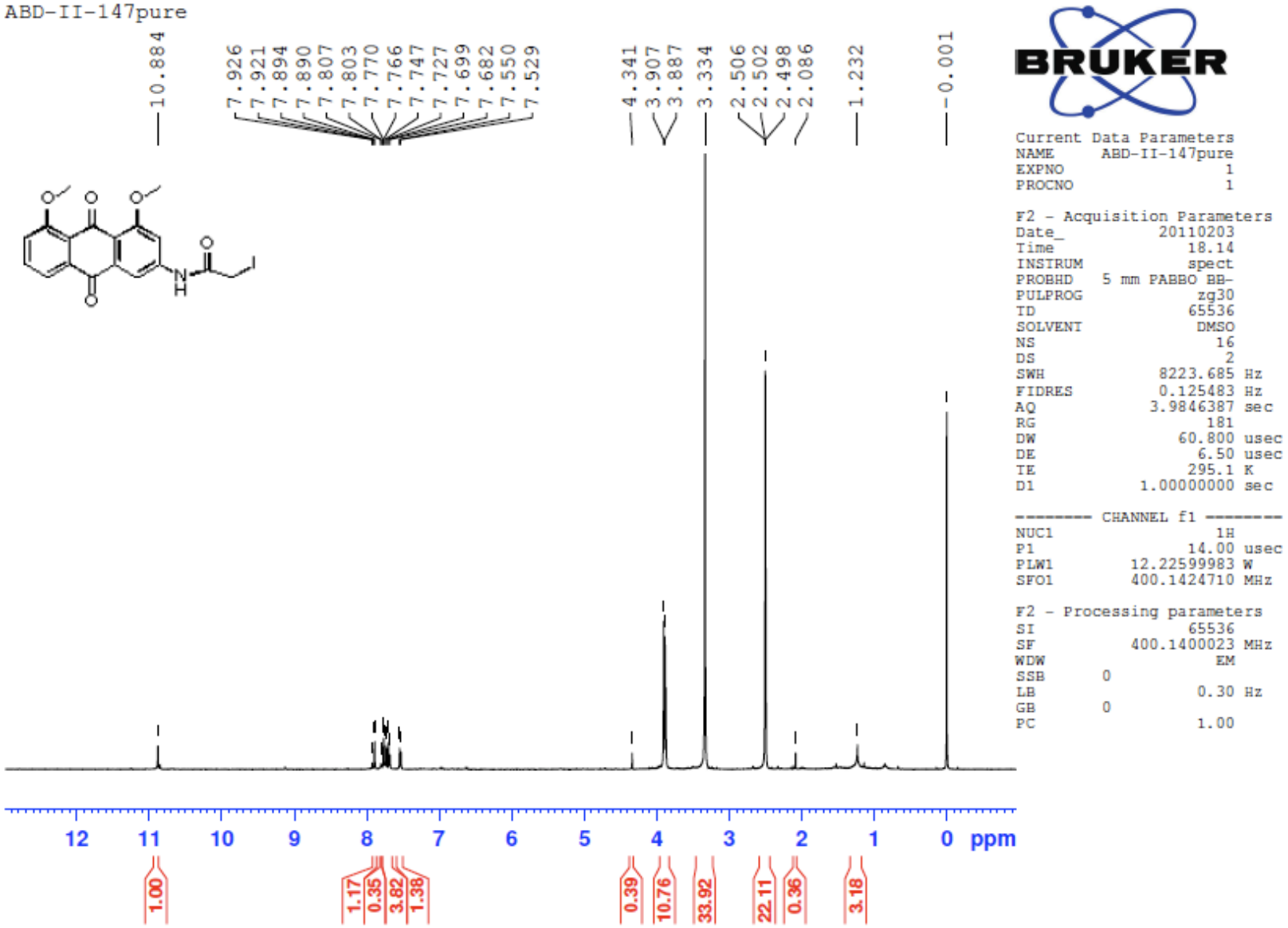
F2 - Acquisition Parameters
Date_           20110203
Time               17.21
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ              3.9846387 sec
RG                   203
DW                60.800 usec
DE                 6.50 usec
TE                295.0 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 14.00 usec
PLW1         12.22599983 W
SFO1         400.1424710 MHz

F2 - Processing parameters
SI                 65536
SF          400.1400017 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

1,8-Methoxy-3-(2'-iodo-acetamido)-anthraquinone (14)





BRUKER

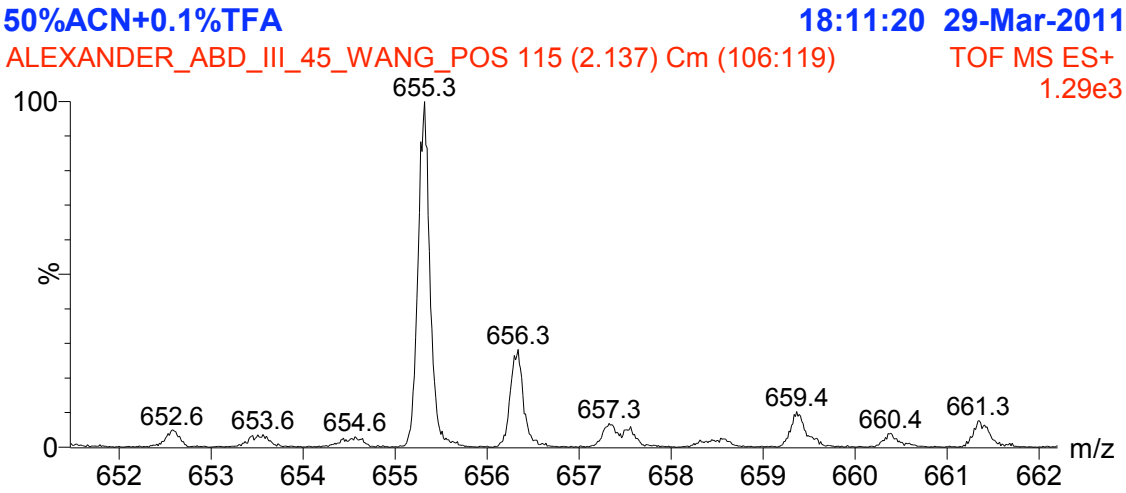
Current Data Parameters
NAME ABD-II-147pure
EXPNO 1
PROCNO 1

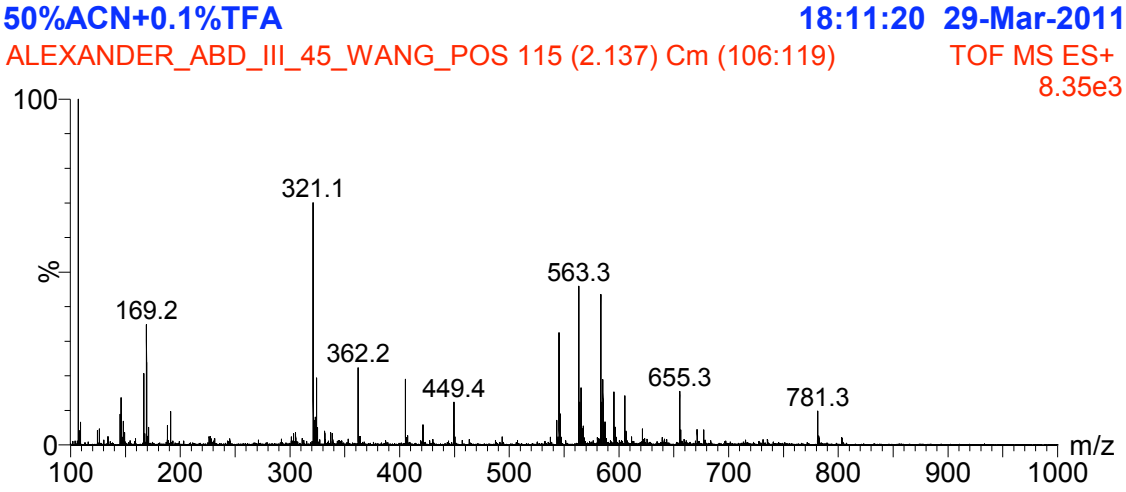
F2 - Acquisition Parameters
Date_ 20110203
Time 18.14
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 181
DW 60.800 usec
DE 6.50 usec
TE 295.1 K
D1 1.00000000 sec

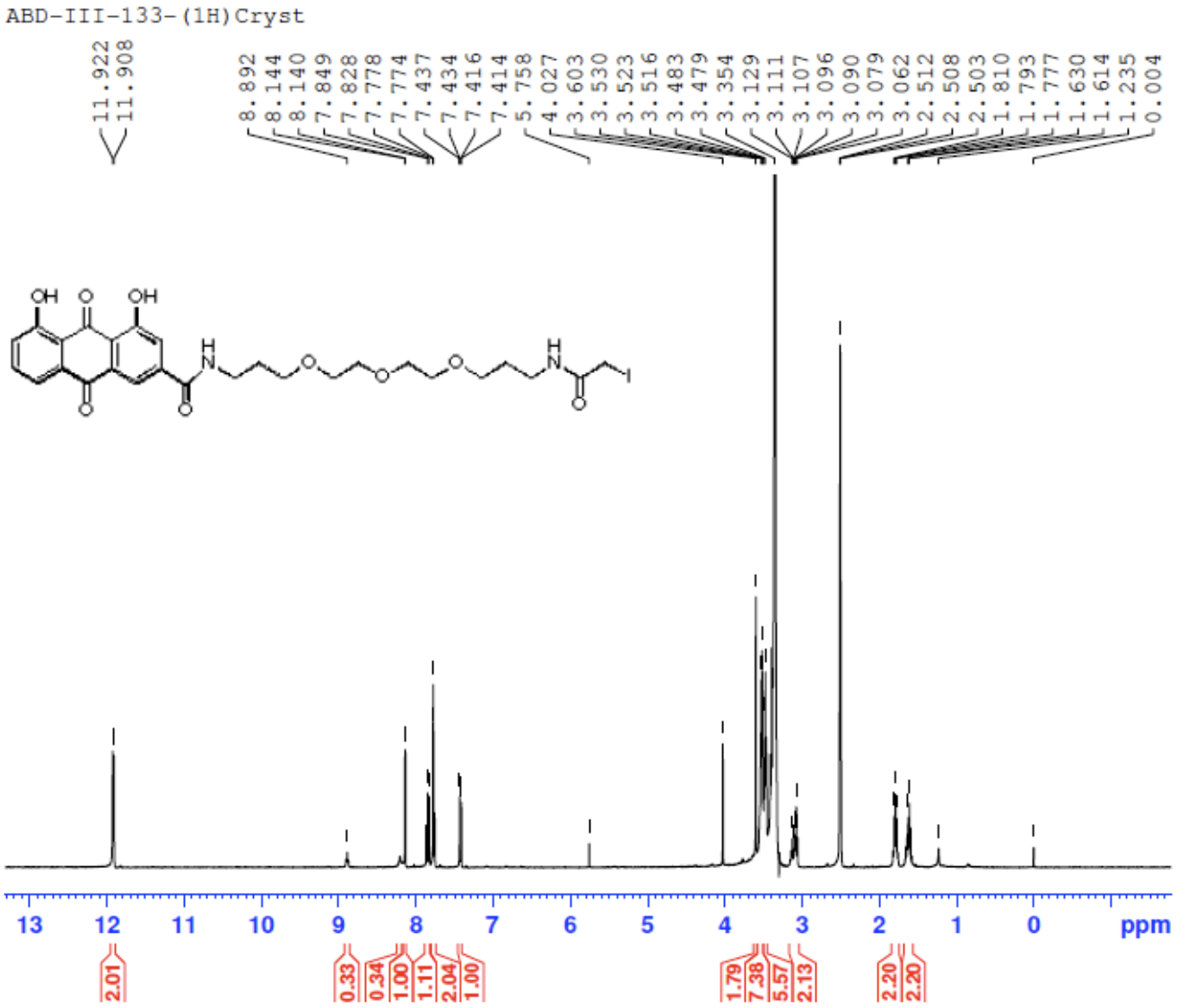
-------- CHANNEL f1 --------
NUC1 1H
P1 14.00 usec
PLW1 12.22599983 W
SFO1 400.1424710 MHz

F2 - Processing parameters
SI 65536
SF 400.1400023 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1,8-Dihydroxy-3-(18'-iodo-aceta-6',9',12'-trioxa-2',16'-tridecanediamido)-anthraquinone (23).







BRUKER

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PROCNO                 1

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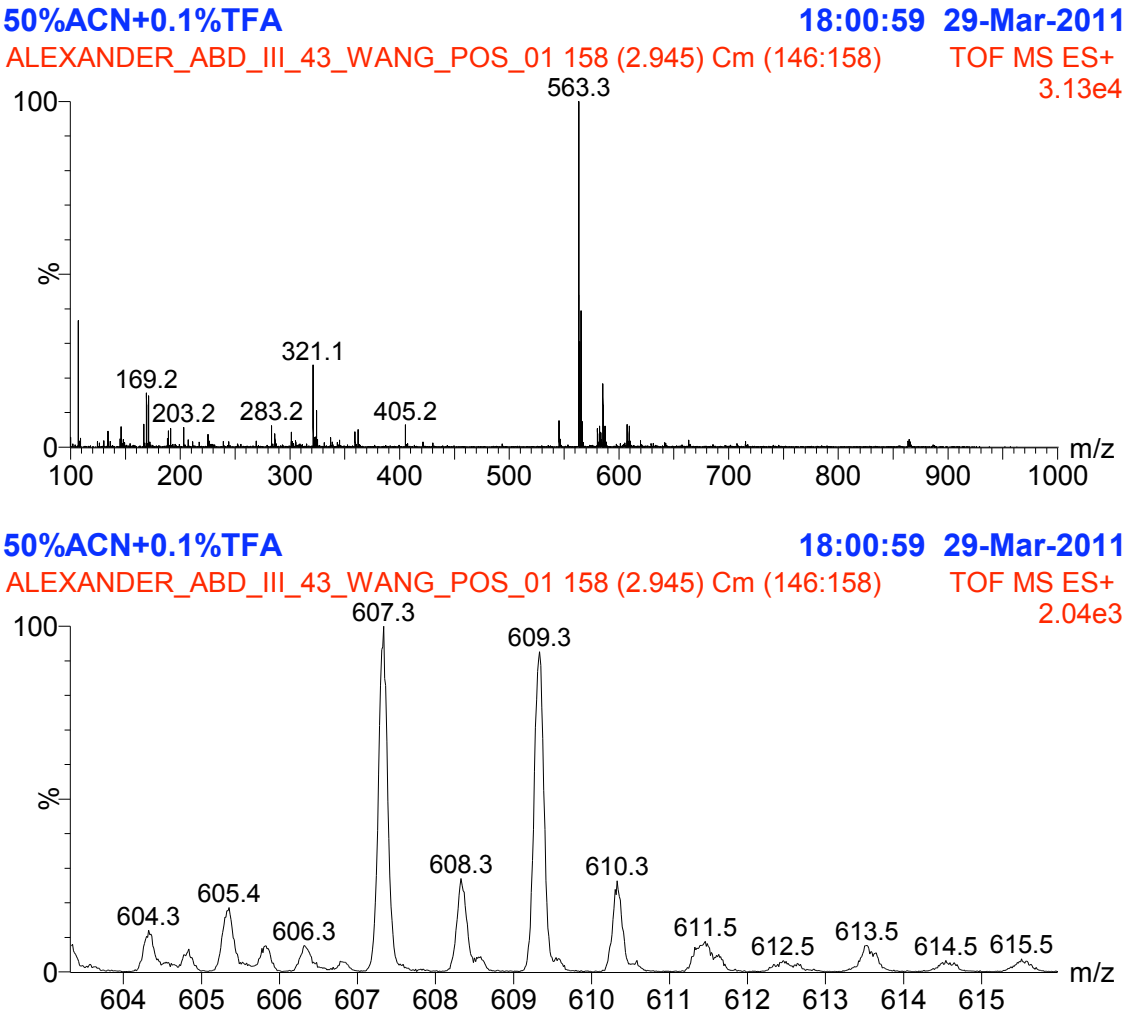
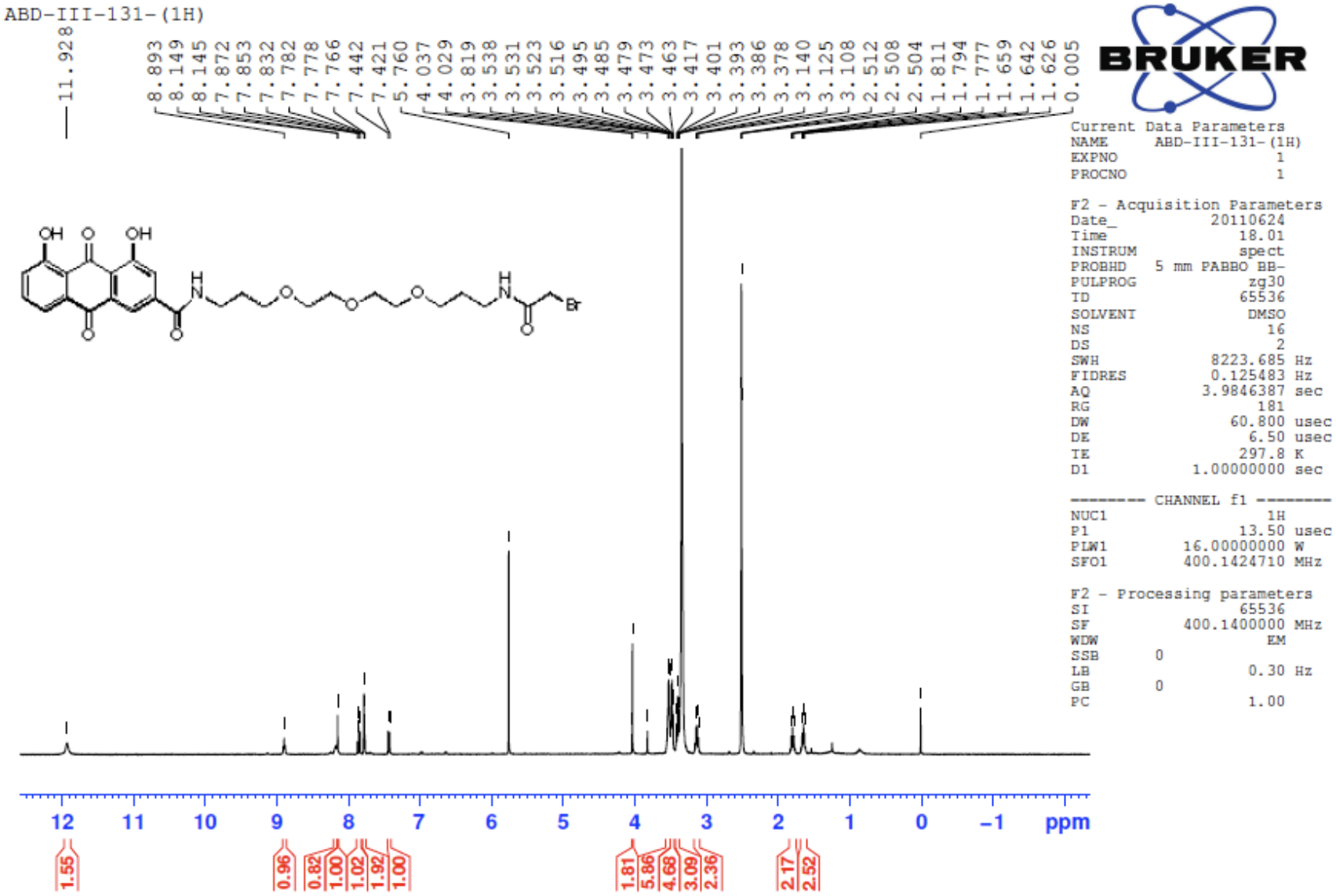
Time              15.37
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD              65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH            8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                  144
DW               60.800 usec
DE                6.50 usec
TE               297.8 K
D1          1.00000000 sec

====== CHANNEL f1 ======
NUC1                1H
P1               13.50 usec
PLW1        16.00000000 W
SFO1        400.1424710 MHz

P2 - Processing parameters
SI                65536
SF          400.1400000 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

1,8-Dihydroxy-3-(18'-bromo-aceta-6',9',12'-trioxa-2',16'-tridecanediamido)-anthraquinone (22).





BRUKER

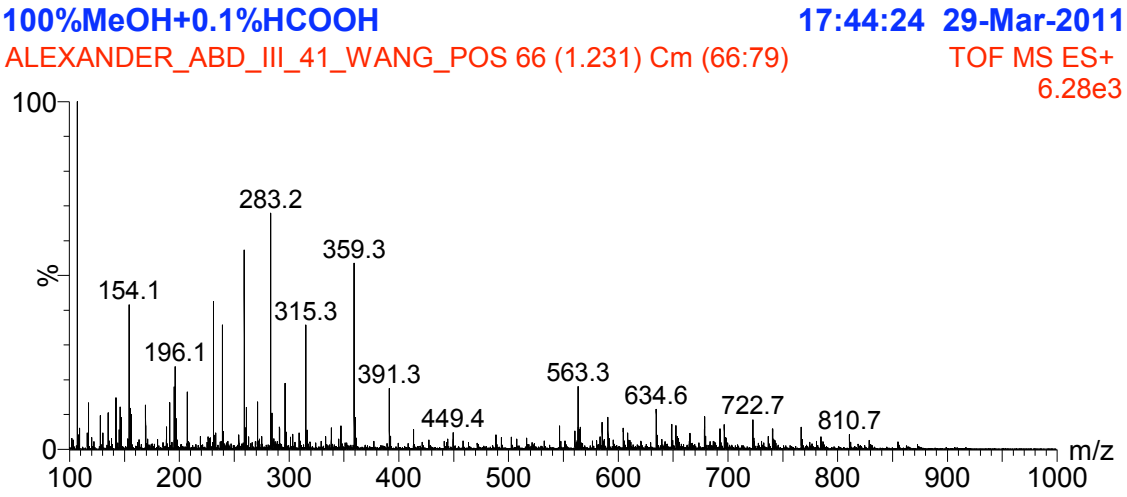
Current Data Parameters
NAME ABD-III-131-(1H)
EXPNO 1
PROCNO 1

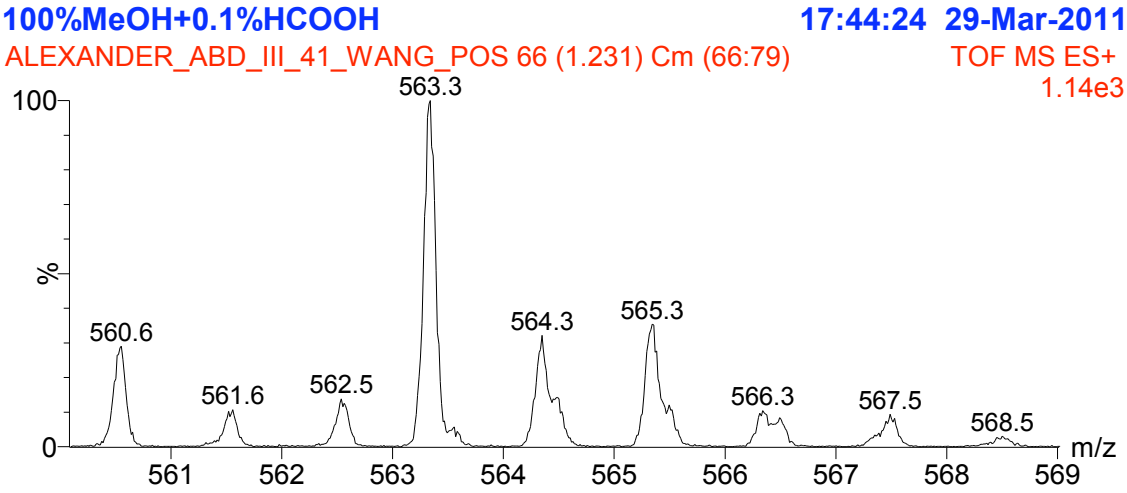
F2 - Acquisition Parameters
Date_ 20110624
Time 18.01
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 181
DW 60.800 usec
DE 6.50 usec
TE 297.8 K
D1 1.00000000 sec

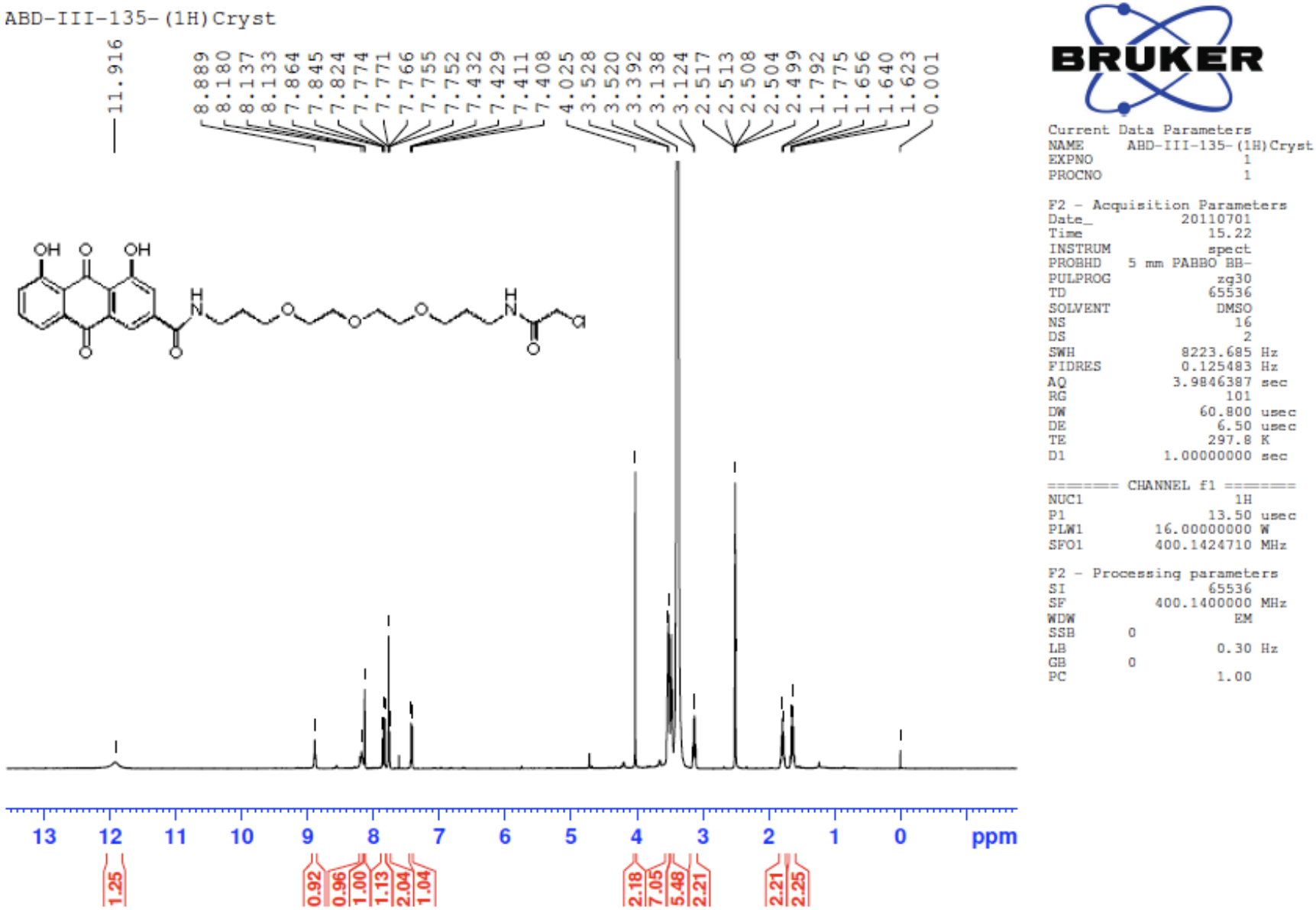
-------- CHANNEL f1 --------
NUC1 1H
P1 13.50 usec
PLW1 16.00000000 W
SFO1 400.1424710 MHz

F2 - Processing parameters
SI 65536
SF 400.1400000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1,8-Dihydroxy-3-(18'-chloro-aceta-6',9',12'-trioxa-2',16'-tridecanediamido)-anthraquinone (21).







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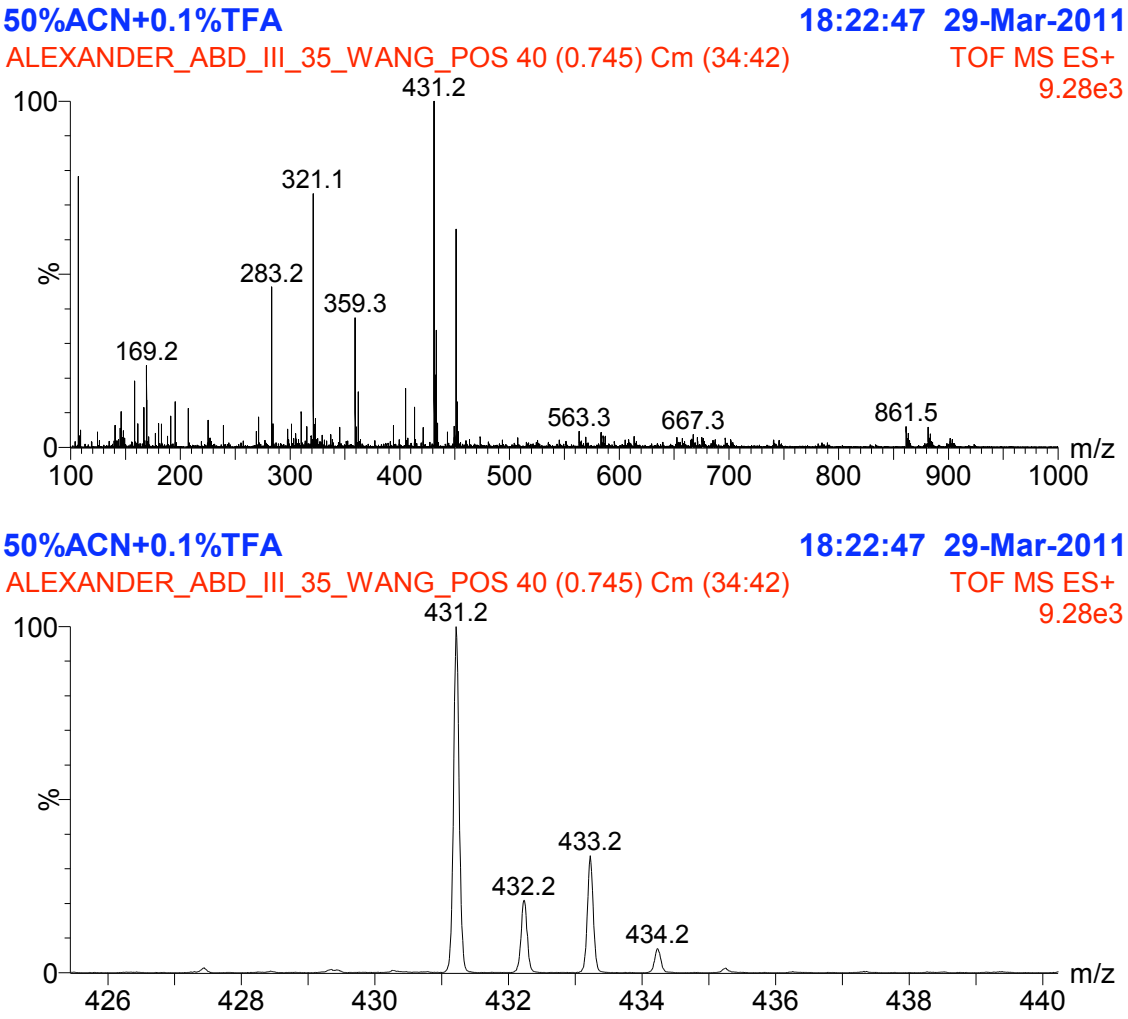
Current Data Parameters
NAME ABD-III-135-(1H)Cryst
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20110701
Time 15.22
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 101
DW 60.800 usec
DE 6.50 usec
TE 297.8 K
D1 1.00000000 sec

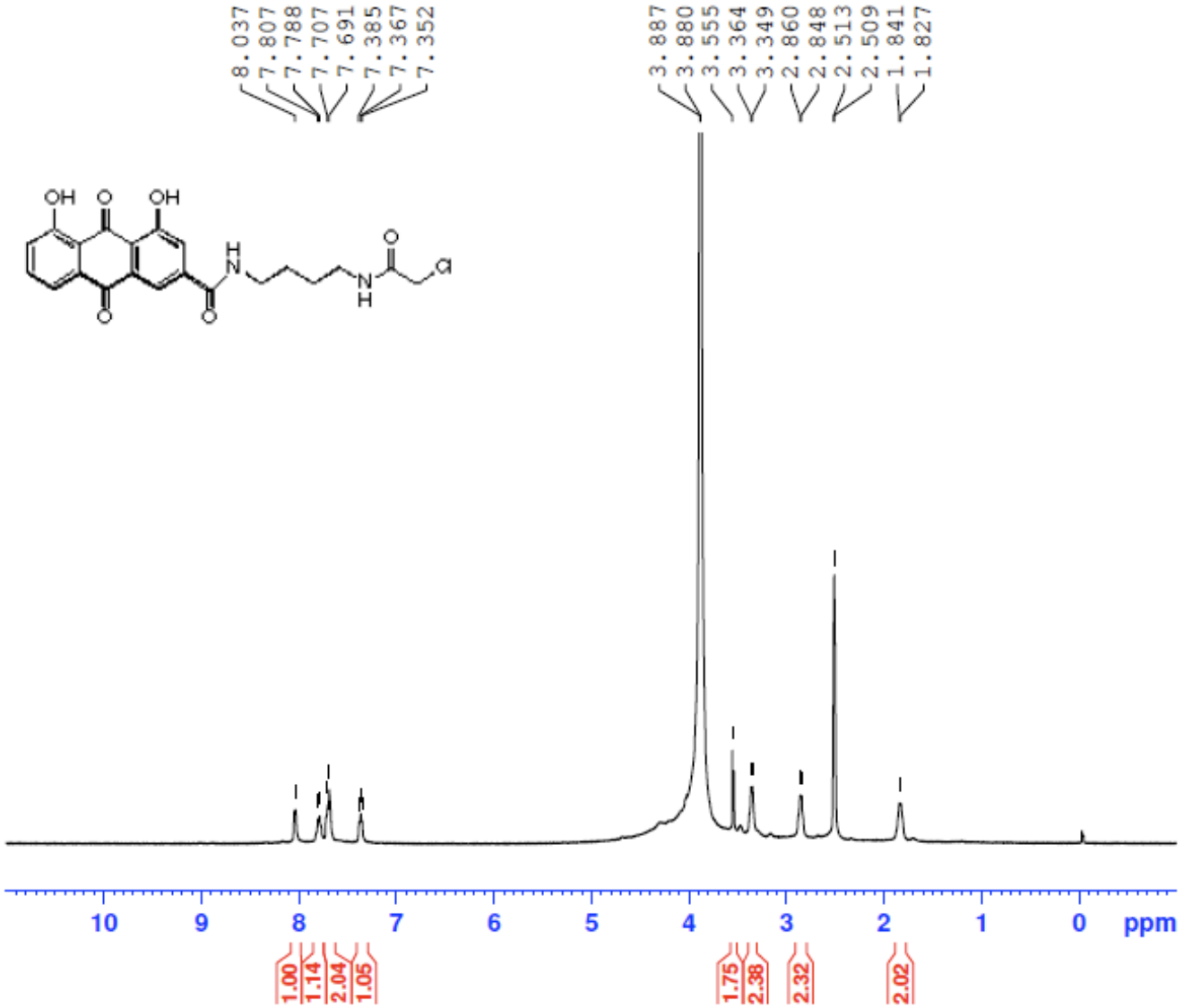
======= CHANNEL f1 =======
NUC1 1H
P1 13.50 usec
PLW1 16.00000000 W
SFO1 400.1424710 MHz

F2 - Processing parameters
SI 65536
SF 400.1400000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1,8-Dihydroxy-3-(9'-chloro-aceta-2',7'diamido)-anthraquinone (17).



ABD-III-113-(1H)crystalizedD2O



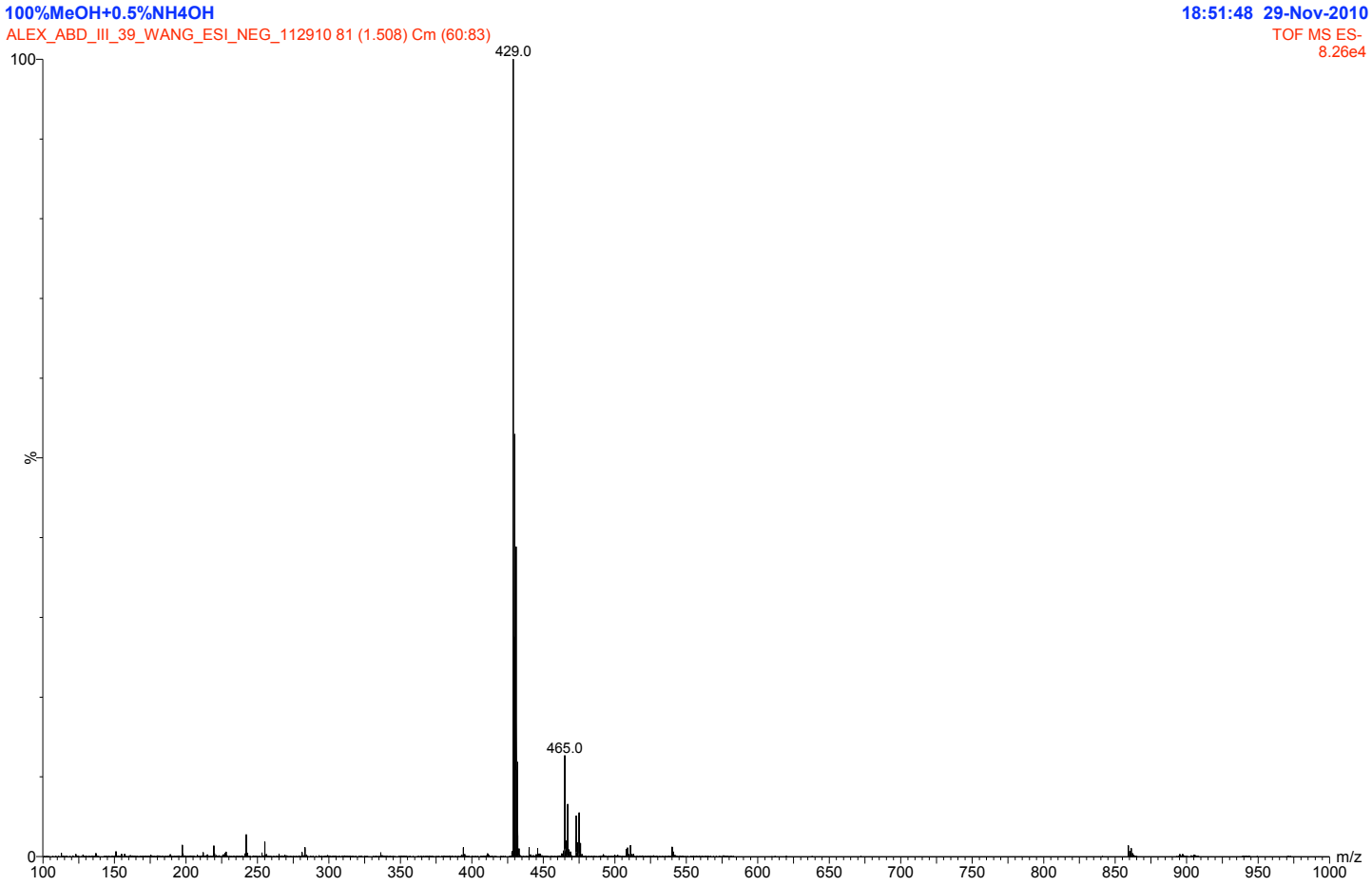

```
Current Data Parameters
NAME      ABD-III-113-(1H)crystalized
EXPNO                 2
PROCNO                1

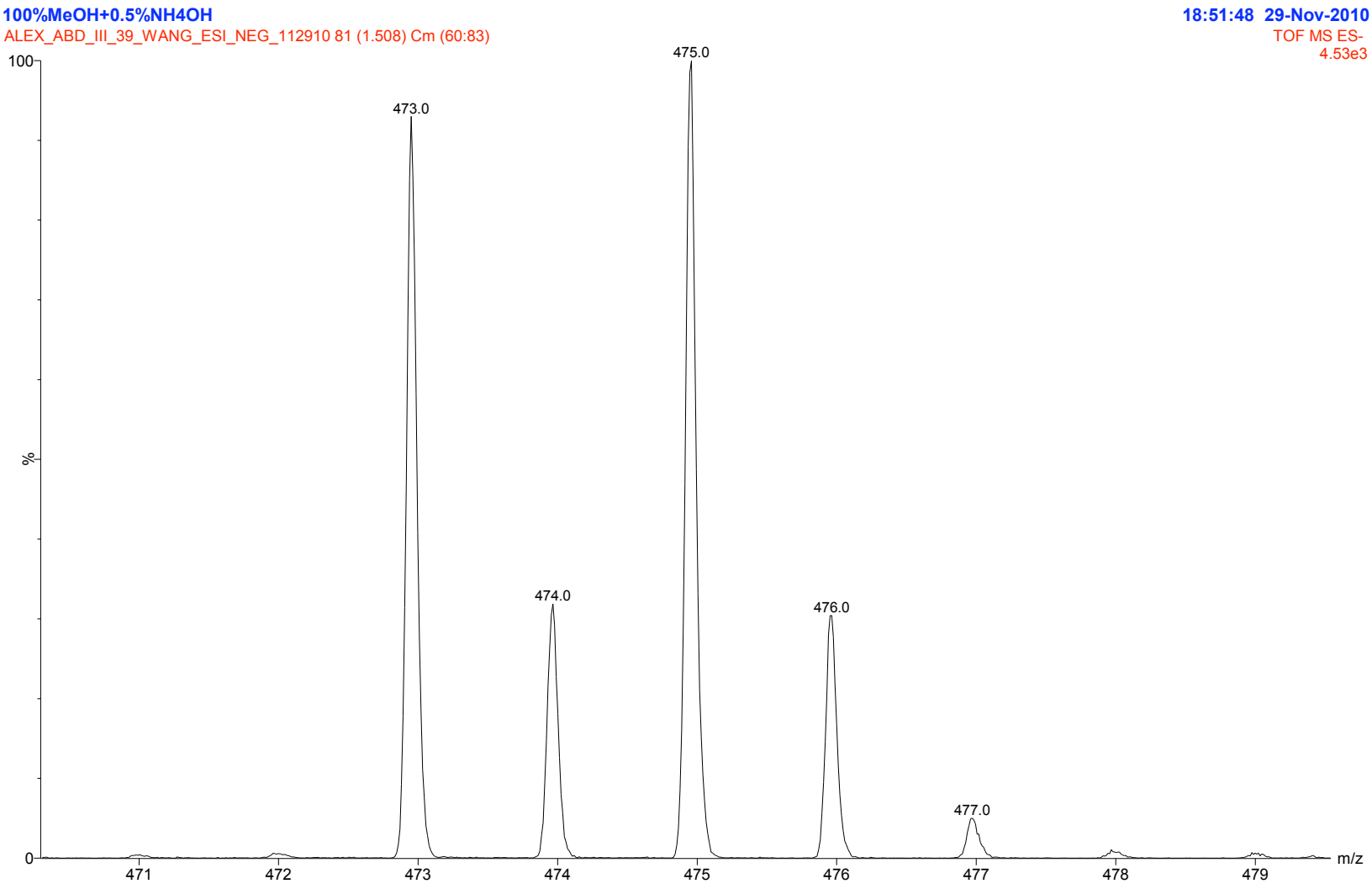
F2 - Acquisition Parameters
Data_           20110523
Time              10.13
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG           zg30
TD               65536
SOLVENT           DMSO
NS                  16
DS                   2
SWH            8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                 144
DW              60.800 usec
DE               6.50 usec
TE              296.8 K
D1           1.00000000 sec

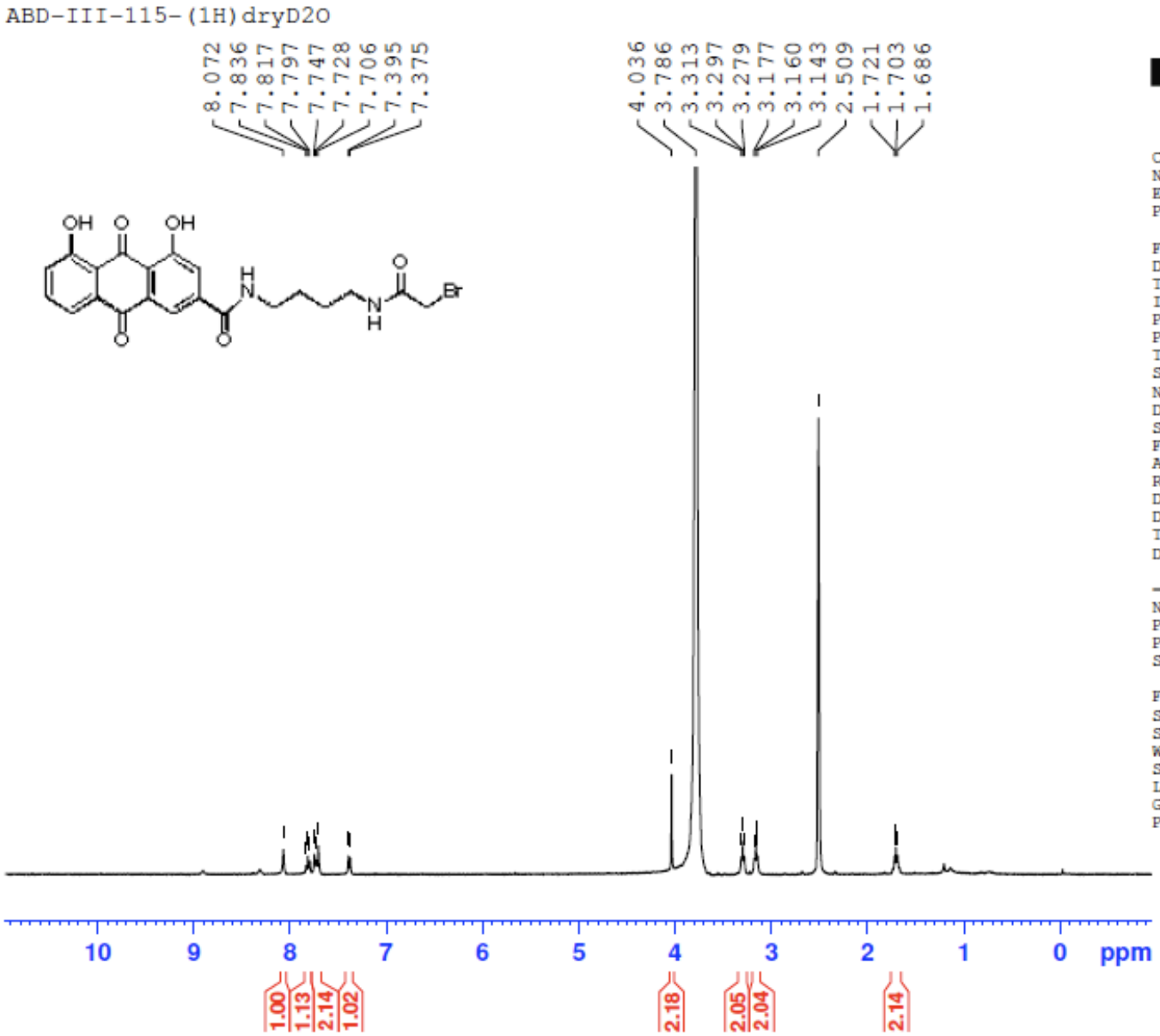
======== CHANNEL f1 ========
NUC1                1H
P1               13.50 usec
PLW1        16.00000000 W
SFO1       400.1420007 MHz

F2 - Processing parameters
SI               65536
SF         400.1400000 MHz
WDW                 EM
SSB       0
LB               0.30 Hz
GB        0
PC               1.00
```

1,8-Dihydroxy-3-(9'-bromo-aceta-2',7'diamido)-anthraquinone (17).




100%MeOH+0.5%NH4OH
ALEX_ABD_III_39_WANG_ESI_NEG_112910 81 (1.508) Cm (60:83)
18:51:48 29-Nov-2010
TOF MS ES-
4.53e3
100
%
0
473.0
474.0
475.0
476.0
477.0
471
472
473
474
475
476
477
478
479
m/z



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```
Current Data Parameters
NAME      ABD-III-115-(1H)dry
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_              20110523
Time                 10.22
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                      16
DS                       2
SWH               8223.685 Hz
FIDRES            0.125483 Hz
AQ                3.9846387 sec
RG                     161
DW                  60.800 usec
DE                   6.50 usec
TE                  296.8 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   13.50 usec
PLW1          16.00000000 W
SFO1          400.1420007 MHz

F2 - Processing parameters
SI                   65536
SF            400.1400000 MHz
WDW                     EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```