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Organocatalyzed Mannich Reactions on Minocycline: Towards Novel Tetracycline Antibiotics

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ABSTRACT

Herein, we report the development of a mild synthetic route towards novel minocycline derivatives using the proline-catalyzed three-component Mannich reaction. The reaction conditions were optimized and was then screened for its tolerance to the other popular organocatalysts as well as variation of the ketone and aldehyde substrates. The Mannich adducts were evaluated for their *in vitro* antibacterial efficacies against Gram-negative and Gram-positive bacteria.

KEYWORDS

Minocycline,tetracycline, antibiotics, Mannich reaction, organocatalysis.

1. Introduction

The first class of tetracyclines was introduced in the 1950s as safe and effective broad-spectrum antibiotics. These agents have been important medical products for the last 50 years;[1] however, the misuse of antibiotics has accelerated the natural inevitable phenomenon of resistant strain evolution. Tetracyclines inhibit bacterial growth by interfering with protein synthesis. They attach themselves to the bacterial 30S subunit of the rRNA (A site) which results in the prevention of the binding of amino-acyl-tRNA.[2] The emergence of resistant bacteria to many of the current antibiotics is a major worldwide threat to public health.

There is an urgent need for the identification and development of novel antibiotics with new modes of action and significant therapeutic efficacies against these pathogens.[3] Modification of existing antibiotic classes to new derivatives is an option to overcome this rapid drug resistance development. The urgent need to revive the tetracycline has brought about renewed interest to this class of compounds.

Most of the early tetracyclines were naturally occurring molecules (first generation), e.g. from *Streptomyces aureofaciens* and *Streptomyces rimosus*, or a product of semi-sythethic methods (second generation), e.g. minocycline and doxycycline (Fig. 1). Despite the great success of the early tetracyclines, improvements had to be made to better the pharmacokinetic properties, antimicrobial potency and also to decrease toxicity. This prompted the development of the third generation,which has led to many more derivatives and to the discovery of tigecycline, used in the fight against resistant strains.[4] Although tigecycline is the first tetracycline drug to be brought to the market in the last half century, two more tetracyclines (omadacycline and eravacycline) are following in phase III clinical trials.[5]

Structure–activity relationship (SAR) studies[6] on the tetracycline backbone have shown that it can tolerate structural modifications on positions C4 to C9 (Fig. 1).[7] These chemical modifications often result in the change of chemical–physical properties and improved ability to overcome drug efflux. Recently K.C. Nicolaou *et al.* (2014), Cuixiang Sun *et al.* (2015) and Hrvoje Petković *et al.* (2015) reported novel tetracycline derivatives with extensions on the C and D rings.[8] These compounds showed antibacterial activity against a wide range of clinically important bacterial isolates, including multidrug-resistant, Gram-negative pathogens.[8a,8b]

Given the recent need and synthetic reports on the tetracycline backbone to yield more potent derivatives such as tigecycline and omadacycline, we envisioned an easy entry to new derivatives based on the commercially available minocycline scaffold through milder synthetic routes.

Biocatalysts and metal complexes are amongst the most popular ways to synthesize optically pure molecules. Asymmetric organocatalysis was developed, in the past decade, as the need for alternatives that are less costly, use of less toxic reagents and more environmental friendly conditions.[9] The last few years have witnessed a spectacular advancement in this field for the synthesis of medicinally important chiral.[9] A substantial number of reactions using organocatalysis have been developed that were equivalent or better than conventional synthetic methods. Amongst the approaches that benefited are the well-known Aldol[10], Mannich[11] and Michael[12] reactions, which are powerful strategies in synthetic organic chemistry, as they allow the formation of new C-C bonds.[13] Recently there is a steady increase in the number of reports that are applying organocatalysis in the field of medicinal chemistry due to its mild and relatively benign approach to asymmetric synthesis.[14]

We have recently reported on the application of organocatalysis in the synthesis of novel β-lactam derivatives.[13b,15] Herein we report the first organocatalyzed Mannich reactions, to make the D-ring substituent modification, on the minocycline scaffold to increase substituent bulk that can mimic the steric hindrance achieved by tigecycline. The end products of the Mannich reactions are also known as beta-amino ketones.[16] These compounds have shown the ability to increase the hydrophilic properties of drugs through the introduction of a polar function in their structure.[17] In other studies, they have been shown to act as prodrugs, releasing the active substance under controlled hydrolytic conditions *via* deaminomethylation[18] or

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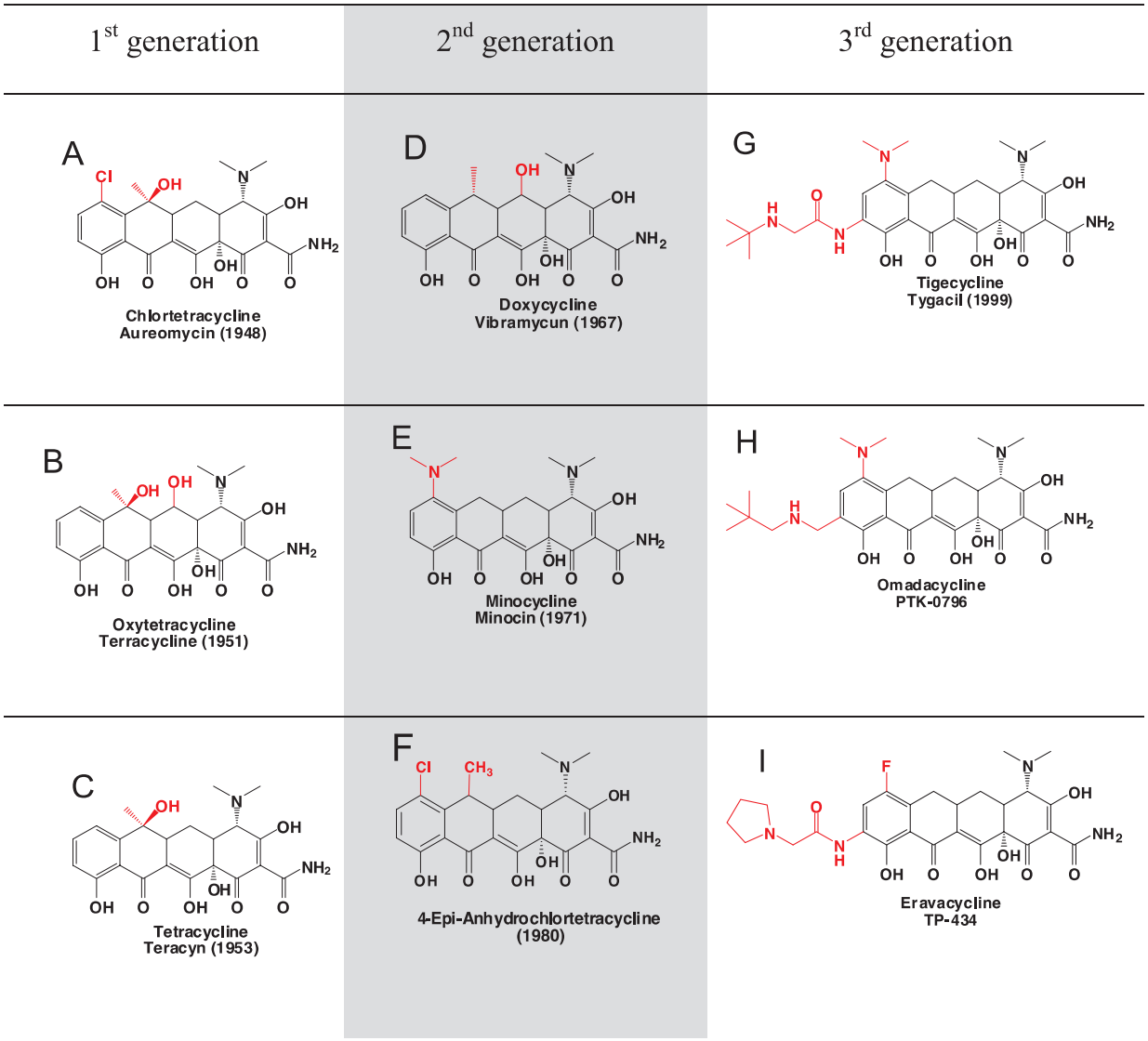


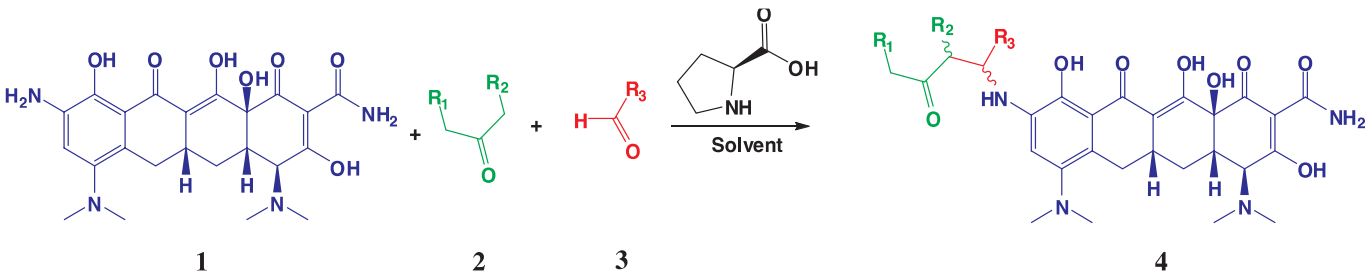
Figure 1 Timeline of tetracycline derivatives.[5c] Chemical structure of first generation (A–C), second generation tetracyclines (D–F) and third generation (G–I). The numbers in the brackets show the year the drug was discovered.

deamination.[19] In addition to the organocatalyzed synthesis of new minocycline derivatives, preliminary biological evaluation of these compounds is also outlined.

2. Results and Discussion

We successfully carried out a proline catalyzed three component Mannich reaction on the commercially available 9-amino minocycline core witrious aldehydes and ketones (Scheme 1).

The reaction was optimized using a 9-amino minocycline (**1**), hydroxyl acetone (**2**) and nitro-benzaldehyde (**3**) in a one-pot reaction (Table 1). Proline was initially chosen to optimize the reaction as it has been well established to catalyze similar transformations.[13a,20] Solvent screening began with dimethylsulphoxide (DMSO) since it is the most commonly reported solvent for Mannich reactions. However, in this case it gave poor conversion (41 %), which was probably due to the partial solubility of **1**. Other polar solvents such as ethanol, methanol, 2-isopropanol and dimethylformamide (DMF) in which the amine **1** was more soluble was attempted. DMF gave poor conversion (57 %) despite being able to fully solubilize **1**. The alcoholic solvents exhibited much improved results in the range of 76–96 %, with methanol as the best solvent (96 % conversion) under room



Scheme 1
Synthesis of tetracycline derivatives *via* a three-component Mannich reaction.

Table 1 Optimization of the catalytic asymmetric three-component Mannich reaction.

Entry	Solvent	Catalyst	Mole %	Conversion %
1	DMSO	–	–	Trace
2	DMSO	L-proline	30	41
3	DMF	L-proline	30	57
4	2-Isopropanol	L-proline	30	76
5	Ethanol	L-proline	30	82
6	Methanol	L-proline	30	96
7	Methanol	Tetrazol	30	96
8	Methanol	Jørgensen	30	94
9	Methanol	Pyrrolidine	30	84
10	Methanol	D-proline	30	95
11	Methanol	L-proline	20	94
12	Methanol	L-proline	10	90

temperature conditions and 8 hours of reaction time.

In order to examine further catalyst effects, we selected a few popular amine catalysts (proline, tetrazol, pyrrolidine and Jørgensen catalyst) that are known to facilitate the progression of Mannich reactions[21]. All of the catalysts showed good activities with conversions in the range of 84–96 %; in alignment with past reports proline typically gave superior results.[11a,20,22] The amount of proline could also be effectively reduced to 10 mol % while still obtaining good product conversions (>90 %) in a reasonable reaction time (<24 h).

As previously shown by B. List *et al.* (2001)[6a], that a variety of ketones can be used in such proline-catalyzed Mannich reactions. Reacting four different ketones (butanone, methoxyacetone, ethyl methyl ketone, acetone and hydroxyacetone) with 9-amino minocycline and benzaldehyde furnished the desired products with high conversions 80–98 % (Fig. 2). The aliphatic (**1a**–**1c**) and cyclic ketones (**1d**) generated the desired products while aromatic ketones only produced trace amounts on the HPLC-MS.

We next performed an aldehyde screen for the three-component Mannich reactions (Fig. 3). Among the aldehydes screened the α-branched aromatic aldehydes (**2a**–**2h**) provided the Mannich products in reasonable yields. Not only benzaldehyde derivatives but also heteroaromatic aldehydes (**2f**, **2g** and **2h**) worked well. Typically α-branched aldehydes are more efficient substrates than the α-unbranched aldehydes[11a,23] but in our case; α-unbranched aldehydes (**2i** and **2j)** only afforded trace amounts of the corresponding Mannich adducts.

The proton and especially the ^{13}C NMR data of the starting material and related products appeared to be problematic. Upon a literature search it was found that the majority of reports did not provide ^{13}C spectral data.[8b,24] It appears that specialized automated triple broadband (ATB) probes are required for the generation of quality ^{13}C spectra.[8c,25]

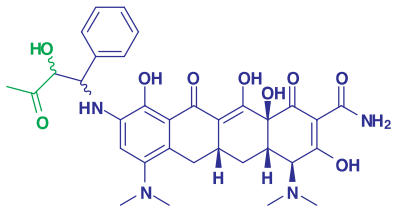
Similarly, the proton spectra were broad and it was difficult to deduce much about the diastereomeric details of the products. Based on the proton spectra as well as HPLC results, it appears that the products may be formed largely as one diastereomer.

The activities of the above new tetracycline derivatives were determined by the agar dilution method following the recommendations of the National Committee for Clinical Laboratory Standards[26]. The analogues tested exhibited antibacterial efficacies against both Gram-positive and Gram-negative bacteria (Table 2).

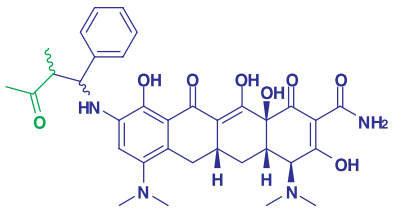
2.1. Antibacterial Activity

Tigecycline and Doxycycline were used as control drugs and their MIC values were in agreement with the Clinical Laboratory Standards Institute (CLSI).[40] The Mannich adducts in general showed good activities against Gram-positive bacteria, with the best giving a MIC of 1.0 μg mL^{-1} against *B. subtilis*. However, these compounds were always less active against Gram-negative bacteria; none of them showed activity against *P. aeruginosa* and the MICs were >16 μg mL^{-1} against *E.coli* with only a couple of exceptions. The poor activities of these compounds against these strains could be as a result of low permeationof the Gram-negative cell wall.

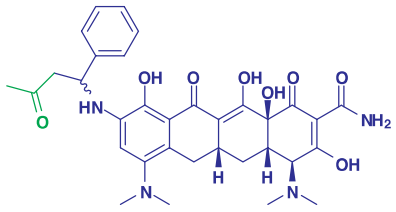
Compounds **1a, 1b, 1c** and **1d** showed the effect of changing the ketone on the adducts, with the most polar exhibitiing the better activity. The aldehydes were then varied and the benzaldehyde derivatives demonstrated a mentionable structural–activity interrelation. Addition of a substitution group onto the benzene ring proved to be crucial for the antibacterial activity. Compounds **2a**, **2b** and **2d** have electron-withdrawing substituents on the para position of the benzene ring and exhibited significant improvements compared to the non-substituted



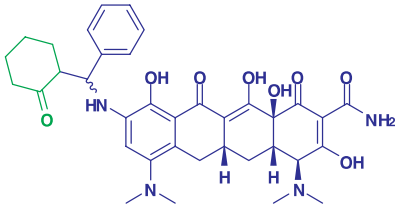
1a, 97%, 30%



1b, 96%, 64%



1c, 80%, 35%



1d, 98%, 30%

Figure 2 Three-component Mannich reaction with various ketones. The compound number is followed by the conversion and isolated yield.

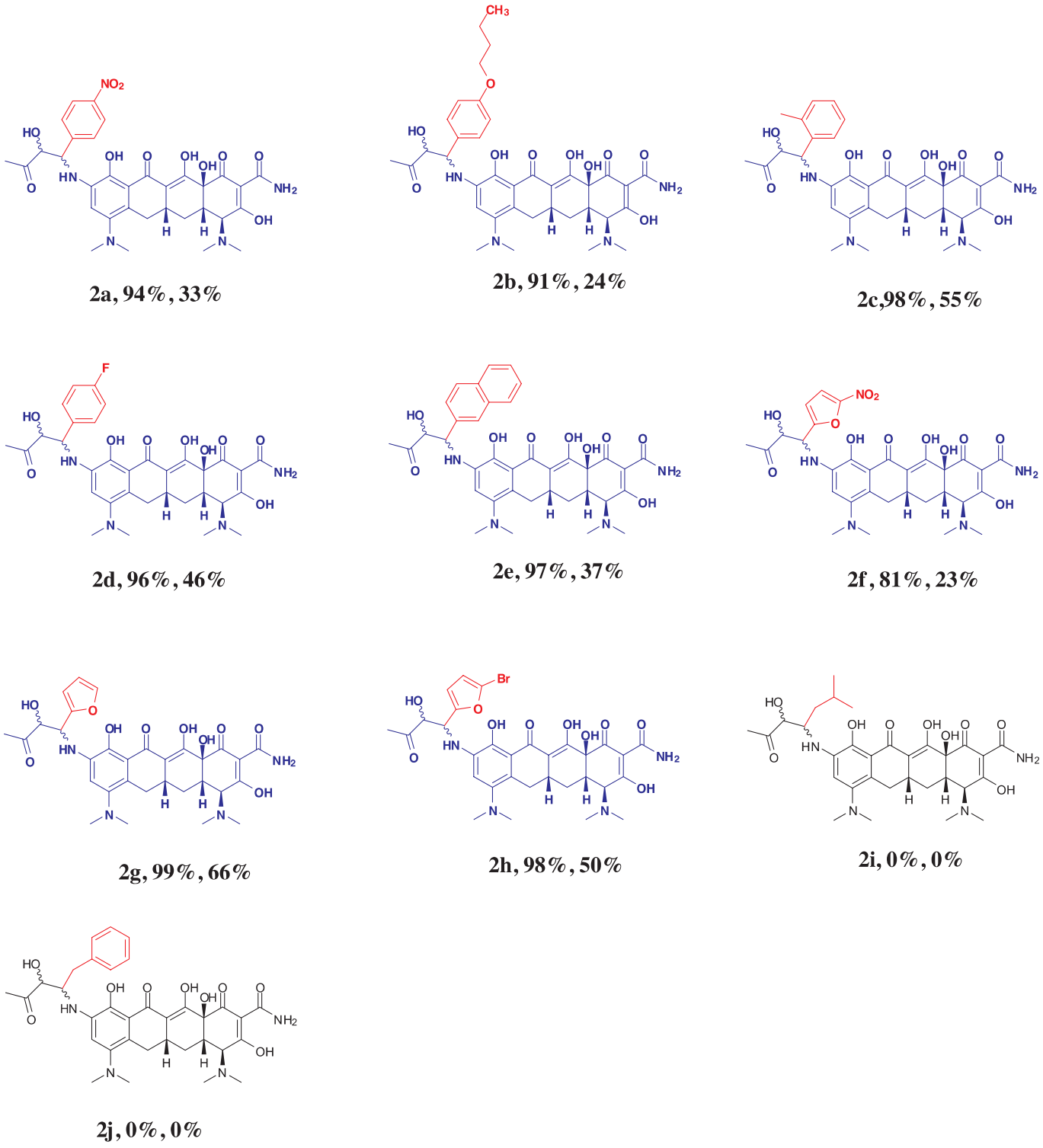


Figure 3 Three-component Mannich reaction with various aldehydes. The compound number is followed by the conversion and isolated yield.

1a. Compound **2b** exhibited an enormous drop in potency for the Gram-negative bacteria. This could be due to the hydrophobicity of the extended carbon chain, which makes it difficult for the compound to pass through the peptidoglycan layer of the cell due to increased hydrophobic interactions. Increasing the bulkiness of the benzene ring had a less significant effect on the potency of this compound as seen by compounds **2c**(methyl group) and **2e**(fused benzene ring). The furfuraldehyde derivatives gave poor structural-activity correlation, as the addition of a substitution group to the furfural ring (**2f**, **2g** and **2h**) did not have much influence on the MICs.

3. Conclusions

In summary, we have shown for the first time that organocatalysis can be employed to synthesize tetracycline derivatives. Although, comparsion to the parent minocycline showed lack of benefit from the addition of the alkylamino side chains at the C9 position, this methodology shows a new and mild synthetic route to derivatize this family of compounds and could be a starting point for future analogues with better biological activity. The Mannich adducts were more active against Gram-positive bacteria than they are with Gram-negatives. The structure–activity relationship studies of these compounds provided use-

Table 2 Antibacterial activity of Mannich adducts ($n = 2$).

Product (see Fig. 1)	Gram-positive bacteria /μg mL^{-1}		Gram-negative bacteria /μg mL^{-1}	
	S. aureus ATCC 25923	*B. subtilis* ATCC 6051	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
Tigecycline	0.25	0.06	0.25	16
Doxycycline	0.25	0.06	0.25	16
1a	32	8	32	NI
1b	32	8	128	NI
1c	16	16	32	NI
1d	128	16	NI	NI
2a	8	2	4	NI
2b	4	2	128	NI
2c	16	4	32	NI
2d	32	4	16	NI
2e	32	4	16	NI
2f	8	2	16	NI
2g	8	1	16	NI
2h	4	2	8	NI

ful information on the structural requirements for activity against Gram-negative bacteria. It also indicated that it is possible to design compounds with selectivity just against Gram-positive bacteria. The promising *in vitro* activity of some of the compounds (e.g. **2a** and **2g**) makes them potential candidates for the development of new antibiotics selectively targeting Gram-positive pathogens.

4. Experimental Section

4.1. General Experimental Procedures

Reagents and solvents were purchased from Sigma-Aldrich and were used without purification unless otherwise stated. High resolution mass spectrometric data were obtained with a Bruker micrOTOF-Q II instrument that operated at ambient temperatures and at a sample concentration of *ca.* 1.0 ppm. NMR spectra were recorded with Bruker AVANCE III 400 or 600 MHz instruments at room temperature. Chemical shifts are expressed ppm, the deuterated solvent reference peak were used as an internal standard for autocalibration.

4.2. General Experimental Procedure for the Synthesis of Mannich Products from Hydroxyacetone

To a suspension of L-proline (4.2 mg, 0.3 mmol) in methanol (2.0 mL), 9-amino minocycline hydrochloride (60 mg, 1.1 mmol) was added. To this mixture, the aldehyde (1.0 mmol) followed by the ketone (1.0 mL) was added and stirred at room temperature for overnight. The reactions were then directly purified straight using semi-preparative reverse-phase HPLC.

4.3. Chromatography Method

Semi-preparative reverse-phase HPLC (Shimadzu, Japan) was conducted using a ACE C18 preparative column (150 × 21.2 mm) using gradient of 0 % B for 5 min, 0 % B to 85 % B over 55 min, 85 % B to 95 % B over 1 min, 95 % B for 5 min, 95 % B to 0 % B over 1 min, 0 % B for 5 min (A = dH_2O with 0.1 % FA; B = Methanol with 0.1 % FA; flow rate =15 mL min^{-1}; A_{254} nm). The fractions were characterized by LCMS (Shimadzu, Japan). Fractions which showed desired mass were collected and concentrated under reduced pressure and lyophilized to give the products as dark red powders. The products are hygroscopic and also decompose upon heating, therefor melting points were not obtained.

4.4. Antibacterial Assays

MICs of the tested compounds were determined by broth microdilution, according to CLSI guidelines. Briefly, two fold dilutions of each inhibitor was made in cation adjusted Mueller-Hinton Broth (CAMHB) in a microtitre plate. A 10 mL of 0.5 McFarland bacteria inoculum was added to make a total volume of 100ml in each microtitre well. Plates were then incubated at 37 ° for 18–22 h under aerobic conditions. MICs were defined at the lowest concentration of antimicrobial agent inhibiting visible growth using the Alamar blue assay. *S. aureus* ATCC 25923, *B. subtilis* ATCC 6051, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used in this study. Control wells of the amount of DMSO used were also done. Assays were done in duplicate to confirm results.[26]

4.5. NMR Data

OH and NH protons did not show on the NMR spectra.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)
^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.45–7.36 (m, 2H), 7.33–7.28 (m, 1H), 7.28–7.23 (m, 1H), 7.23–7.18 (m, 1H), 6.88–6.75 (m, 1H), 4.89–4.74 (m, 1H), 4.52–4.21 (m, 1H), 3.23–2.82 (m, 14H), 2.37–2.21 (m, 3H), 2.21–2.13 (m, 1H), 2.08–1.91 (m, 2H), 1.66–147 (m, 1H), 1.32–1.24 (m, 1H), (OH's, 5, NH's, 3) . HRMS (ESI$^+$): calcd. for $C_{33}H_{39}N_4O_9$ $[M + H]^+$ 635.2712; found 635.2724.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)
^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.45–7.35 (m, 2H), 7.34–7.26 (m, 2H), 7.24–7.18 (m, 1H), 6.75–6.68 (m, 1H), 4.85–4.78 (m, 1H), 4.11–3.97 (m, 1H), 3.17–2.80 (m, 17H), 2.52–2.23 (m, 2H), 2.20–2.16 (m, 1H), 2.00–1.96 (m, 1H), 1.64–1.48 (m, 1H), 1.28–1.22 (m, 1H), 1.08–1.00 (m, 1H), 1.00–0.906 (m, 1H), (OH's, 4, NH's, 3). HRMS (ESI$^+$): calcd. for $C_{34}H_{41}N_4O_8$ $[M + H]^+$ 633.2919; found 633.2906.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-(3-oxo-1-phenylbutylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1c)
^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.63–7.59 (m, 2H), 7.41–7.38 (m, 2H), 7.33–7.27 (m, 1H), 6.77–6.70 (m, 1H), 4.867–4.75 (m, 1H), 3.25–3.19 (m, 8H), 3.08–2.97 (m, 12H), 2.48–2.38 (m, 1H), 2.37–2.32 (m, 1H), 2.19–2.10 (m, 2H), (OH's, 4, NH's, 3). HRMS (ESI$^+$): calcd. for $C_{33}H_{39}N_4O_8$ $[M + H]^+$ 619.2762; found 6.19.2751.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-((1R)-(2-oxocyclohexyl)(phenyl)methylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1d)
^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.47–7.40 (m, 2H), 7.36–7.26 (m, 2H), 7.24–7.17 (m, 1H), 6.82–6.75 (m, 1H), 4.90–4.78 (m, 1H), 4.12–4.04 (m, 1H), 3.11–2.96 (m, 14H), 2.36–2.28 (m, 4H), 1.73–1.56 (m, 9H), (OH's, 4, NH's, 3). HRMS (ESI$^+$): calcd. for $C_{36}H_{43}N_4O_8$ $[M + H]^+$ 659.3362; found 659.3352.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(4-nitrophenyl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2a)
^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 8.20–8.15 (m, 2H), 7.72–7.66 (m, 2H), 6.94–6.88 (m, 1H), 4.49–4.45 (m, 1H), 4.11–4.03 (m, 1H), 3.13–2.98 (m, 14H), 2.37–2.29 (m, 3H), 2.14–2.08 (m, 2H), 1.64–1.53 (m, 1H), 1.33–1.27 (m, 2H), (OH's, 5, NH's, 3). HRMS

(ESI$^+$): calcd. for $C_{33}H_{38}N_5O_{11}$ $[M + H]^+$ 680.2562; found 680.2554.

(4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)

^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.36–7.28 (m, 2H), 6.87–6.83 (m, 2H), 6.82–6.77 (m, 1H), 4.43–4.38 (m, 1H), 4.11–4.04 (m, 1H), 3.93–3.86 (m, 2H), 3.15–2.96 (m, 15H), 2.30–2.23 (m, 3H), 2.21–2.15(m, 1H), 2.08–2.03 (m, 1H), 1.71–1.65 (m, 2H), 1.63–1.56 (m, 1H), 1.47–1.41 (m, 2H), 1.39–1.36 (m, 1H), 0.95–0.90 (m, 3H), (OH's, 5, NH's, 3). HRMS (ESI$^+$): calcd. for $C_{37}H_{47}N_4O_{10}$ $[M + H]^+$ 707.3287; found 707.3270.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-3-oxo-1-o-tolylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2c)

^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.23–7.17 (m, 2H), 7.14–7.11 (m, 1H), 7.09–7.05 (m, 1H), 6.51–6.48 (m, 1H), 4.90–4.74 (m, 1H), 4.392–4.35 (m, 1H), 3.05–2.96 (m, 15H), 2.59–2.56 (m, 3H), 2.51–2.47 (m, 1H), 2.35–2.32 (m, 3H), 1.99–1.97 (m, 1H), 1.63–1.53 (m, 2H), (OH's, 5, NH's, 3) . HRMS (ESI$^+$): calcd. for $C_{34}H_{41}N_4O_9$ $[M + H]^+$ 649.2868; found 649.2866.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)

^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.52–7.41 (m, 2H), 7.10–6.97 (m, 2H), 6.91–6.81 (m, 1H), 4.47–4.37 (m, 1H), 4.18–4.01 (m, 1H), 3.21–2.92 (m, 17H), 2.35–2.29 (m, 3H), 2.10–2.06 (m, 1H), 1.68–1.55 (m, 1H), (OH's, 5, NH's, 3). HRMS (ESI$^+$): calcd. for $C_{33}H_{38}FN_4O_9$ $[M + H]^+$ 653.2617; found 653.2592.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(naphthalen-2-yl)-3-oxobutyl amino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2e)

1H NMR (600 MHz, CD3OD + 0.1 % TFA) 7.93–7.90 (m, 1H), 7.80–7.78 (m, 2H), 7.61–7.58 (m, 1H), 7.45–7.40 (m, 3H), 6.91–6.88 (m, 1H), 4.85–4.79 (m, 1H), 4.56–4.52 (m, 1H), 3.04–2.95 (m, 14H), 2.35–2.32 (m, 3H), 2.25–2.22 (m, 1H), 2.19–2.16 (m, 1H), 2.078–2.036 (m, 1H), 2.02–2.00 (m, 1H), 1.59–1.53 (m, 1H), (OH's, 5, NH's, 3) . HRMS (ESI$^+$): calcd. for $C_{37}H_{41}N_4O_9$ $[M + H]^+$ 685.2868; found 685.2865.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1R,2R)-2-hydroxy-1-(5-nitrofuran-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2f)

^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.41–7.27 (m, 2H), 6.73–6.65 (m, 1H), 4.90–4.80 (m, 1H), 4.74–4.65 (m, 1H), 3.28–3.18 (m, 10H), 3.08–3.00 (m, 7H), 2.34–2.30 (m, 3H), 1.72–1.58 (m, 2H), (OH's, 5, NH's, 3) . HRMS (ESI$^+$): calcd. for $C_{31}H_{36}N_5O_{12}$ $[M + H]^+$ 670.2355; found 670.2322.

(4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1R,2R)-1-(fura-2-yl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2g)

^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.51–7.35 (m, 1H), 7.17–7.05 (m, 1H), 6.38–6.23 (m, 2H), 4.86–4.80 (m, 1H), 4.65–4.56 (m, 1H), 3.22–3.15 (m, 10H), 3.09–2.98 (m, 7H), 2.41–2.32 (m, 1H), 2.30–2.24 (m, 2H), 2.22–2.19 (m, 1H), 1.63–1.57 (m, 1H), (OH's, 4, NH's, 3) . HRMS (ESI$^+$): calcd. for $C_{31}H_{37}N_4O_{10}$ $[M + H]^+$ 625.2504; found 625.2486.

(4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)

^{1}H NMR (600 MHz, CD_3OD + 0.1 % TFA) 7.22–7.08 (m, 1H), 6.39–6.20 (m, 2H), 4.87–4.78 (m, 1H), 4.61–4.52 (m, 1H), 3.27–3.11 (m, 10H) 3.09–2.92 (m, 7H), 2.44–2.33 (m, 1H), 2.30–2.21 (m, 3H), 1.67–1.54 (m, 1H), (OH's, 5, NH's, 3). HRMS (ESI$^+$): calcd. for $C_{31}H_{36}Br\ N_4O_{10}$ $[M + H]^+$ 703.1609; found 703.1588.

Acknowledgements

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Supplementary Material

For copies of proton NMR spectra, HPLC purity chromatographs and high-resolution mass spectra.

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Organocatalyzed Mannich Reactions on Minocycline: Towards Novel Tetracycline Antibiotics

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SUPPLEMETARY DATA

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1. Copies of HRMS spectra for products

HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)

Display Report

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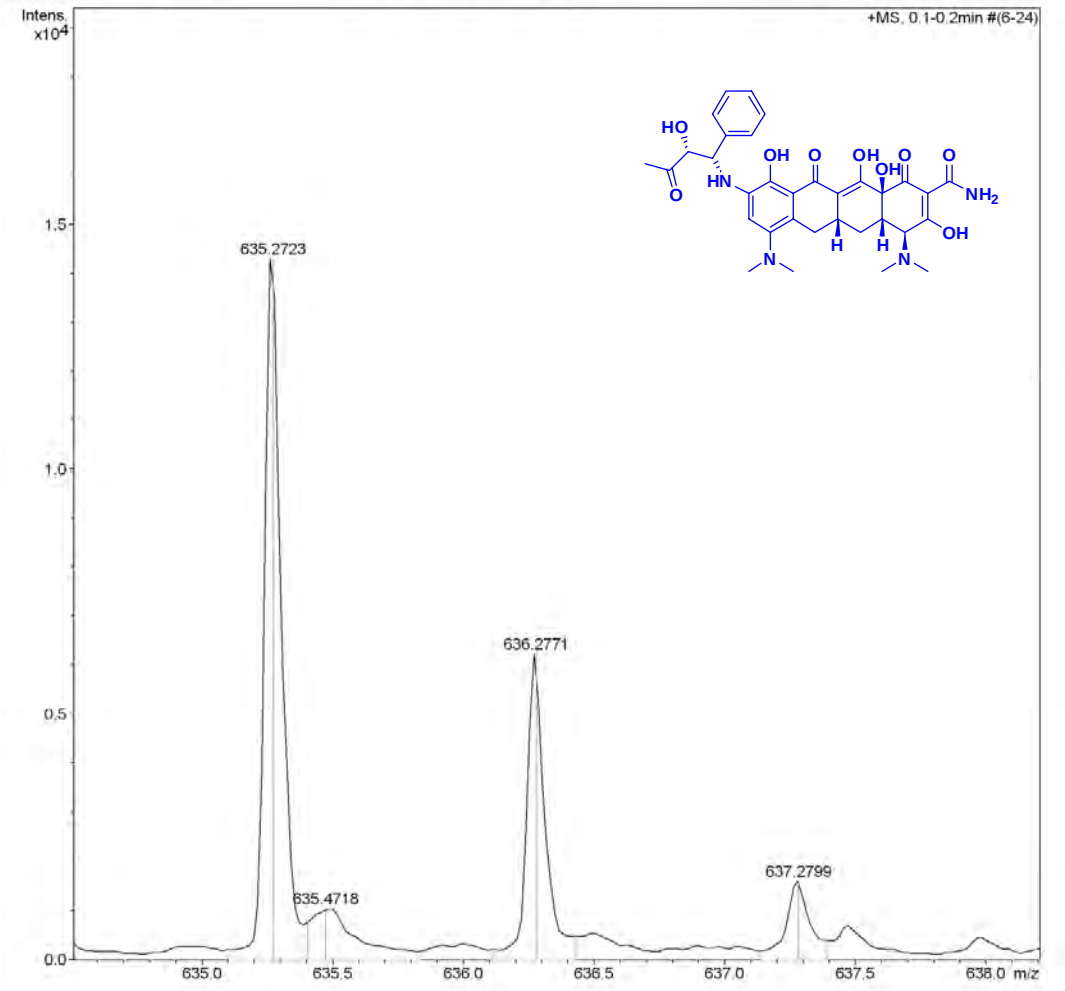

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Display Report

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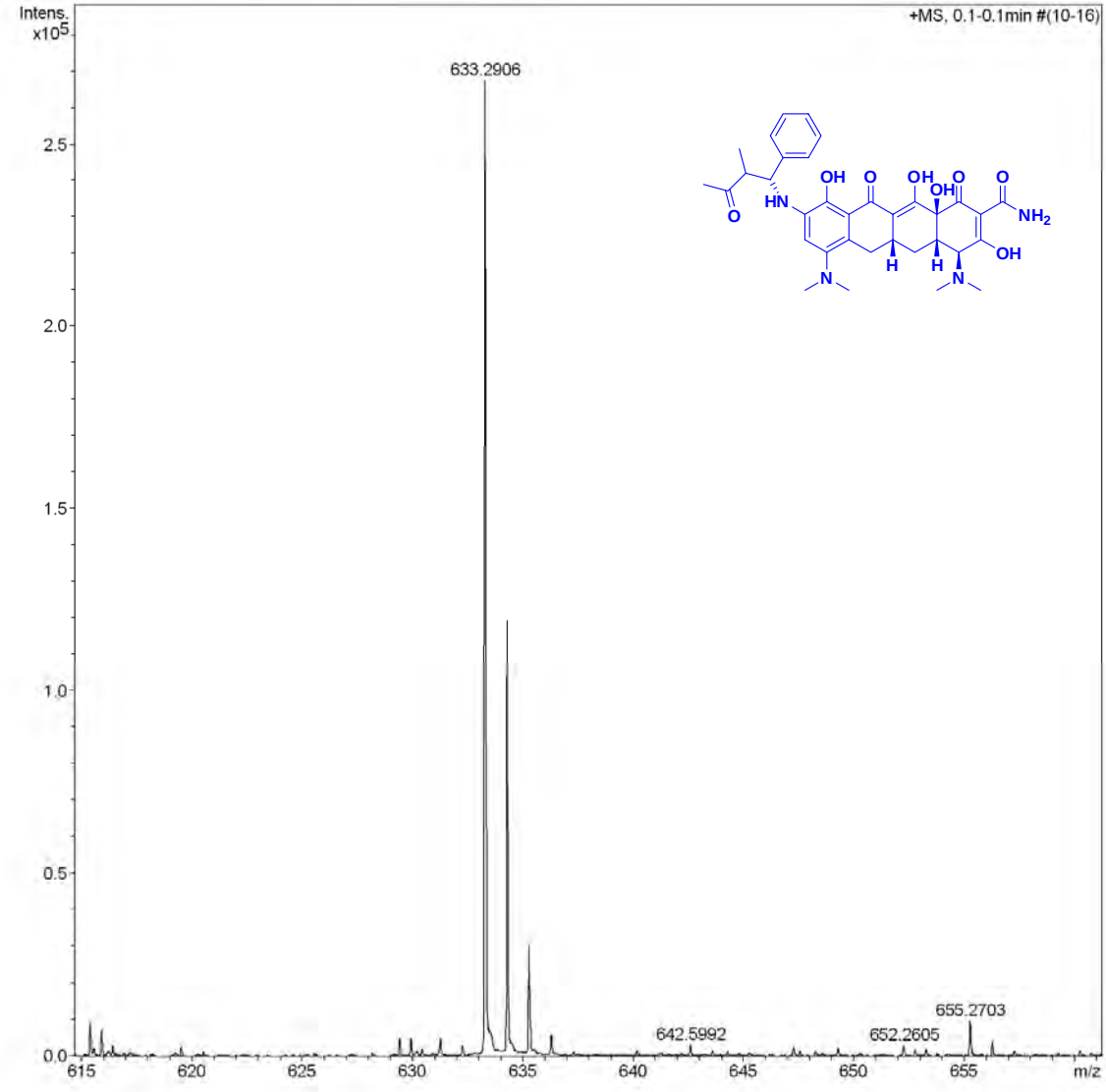

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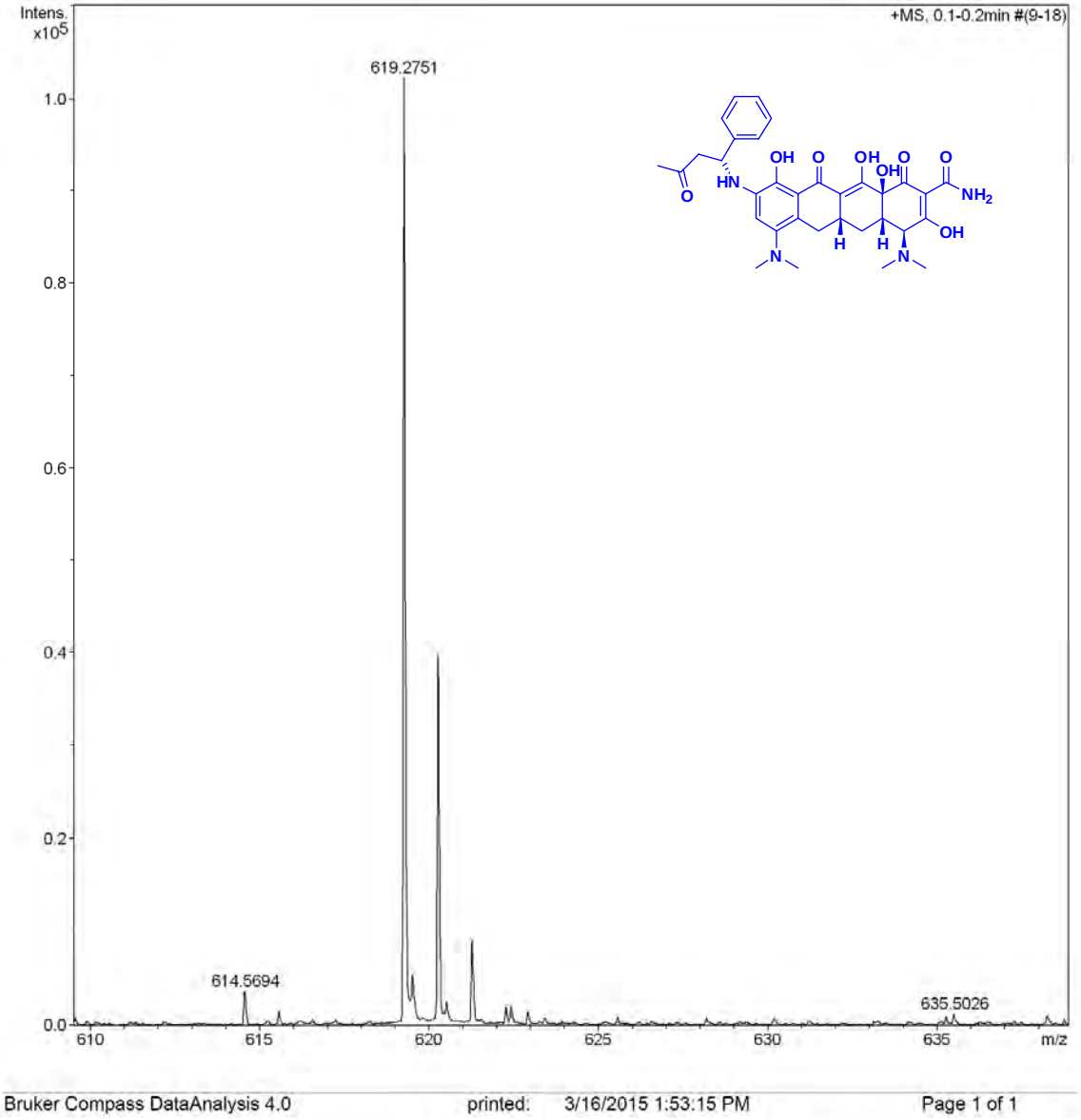

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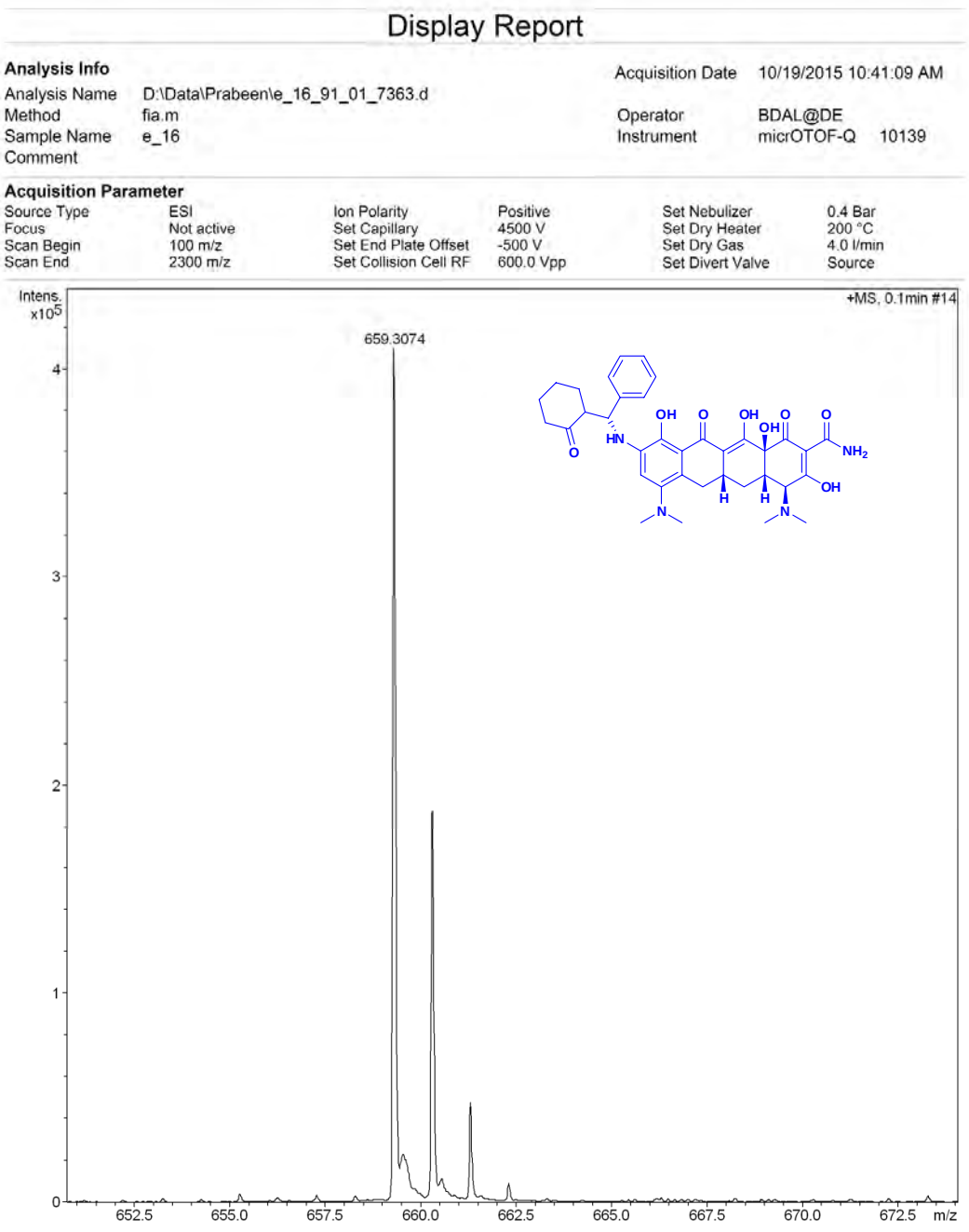

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Display Report

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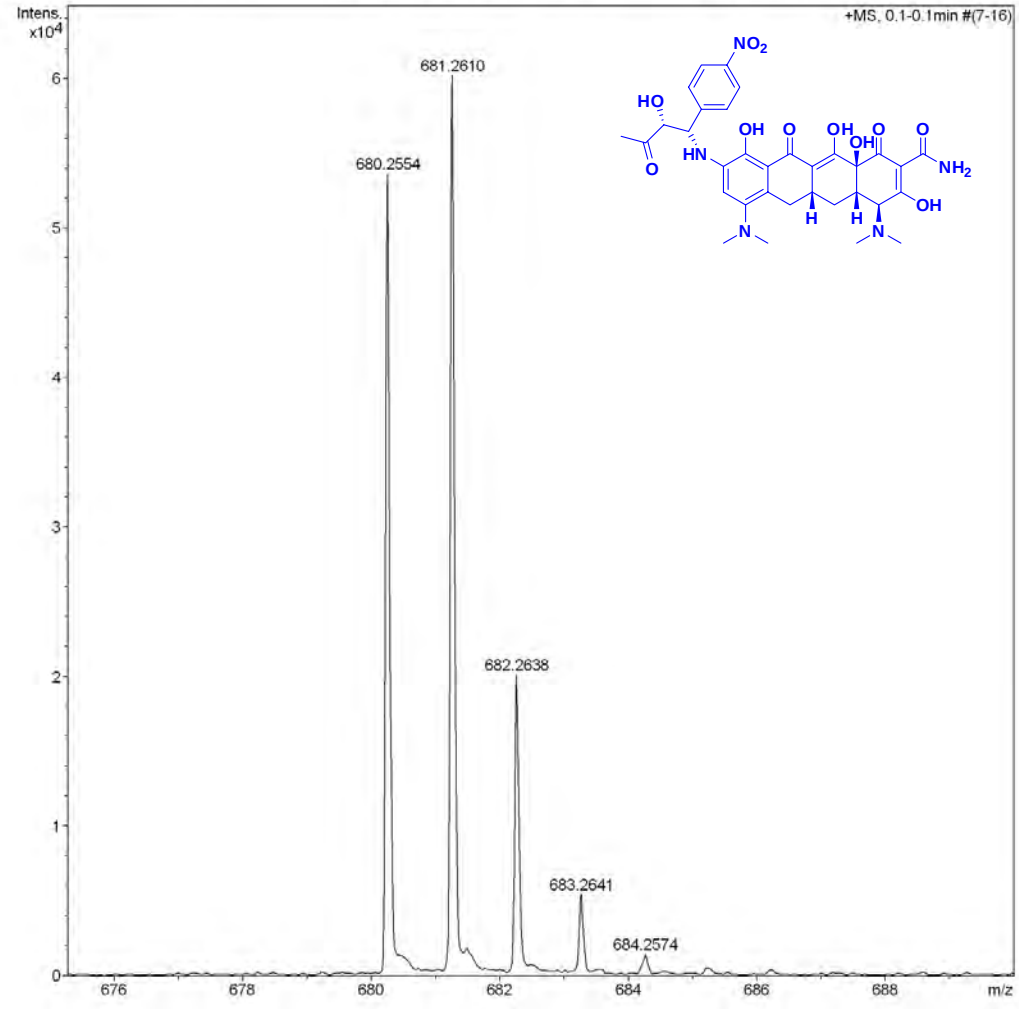

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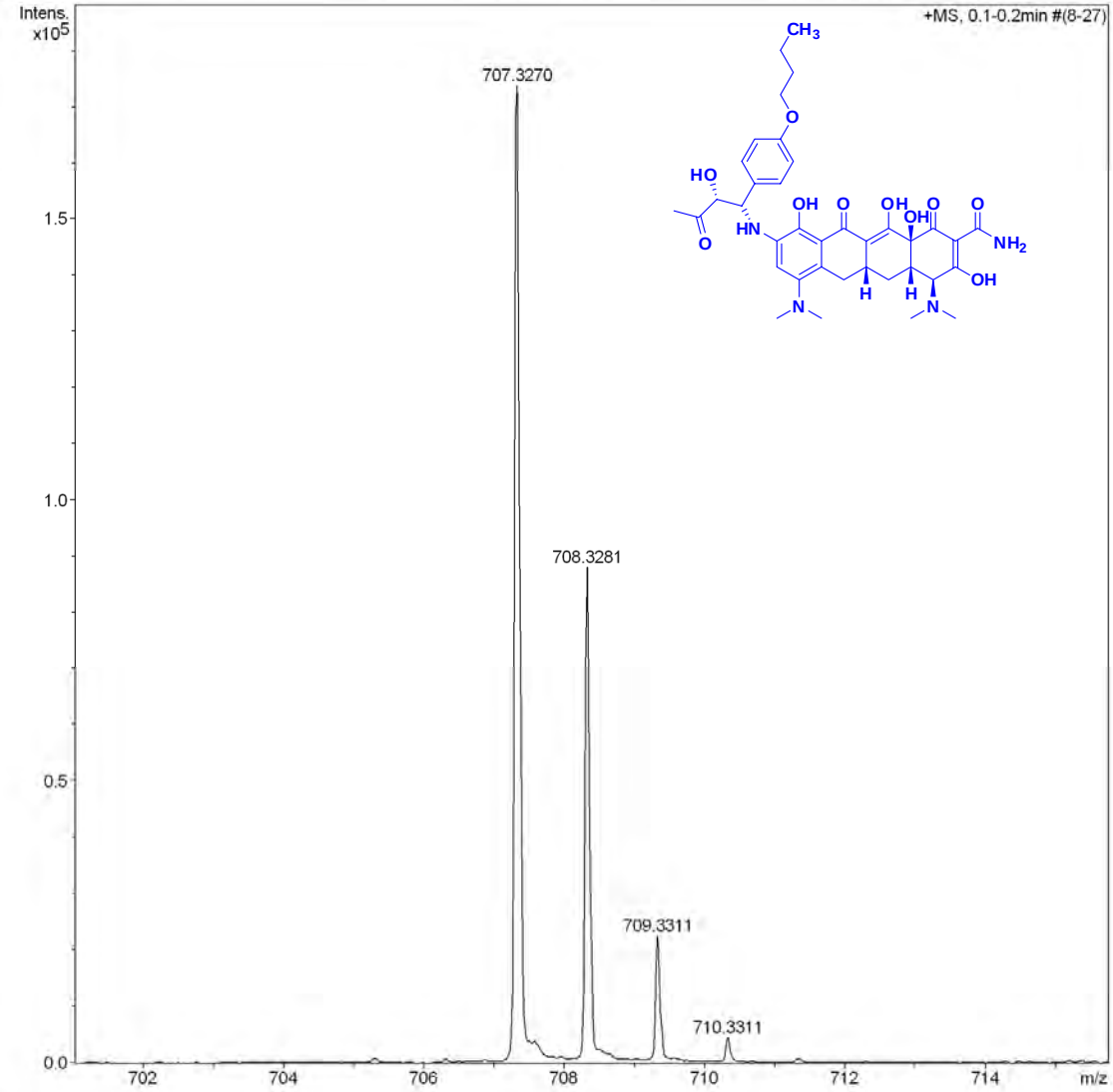

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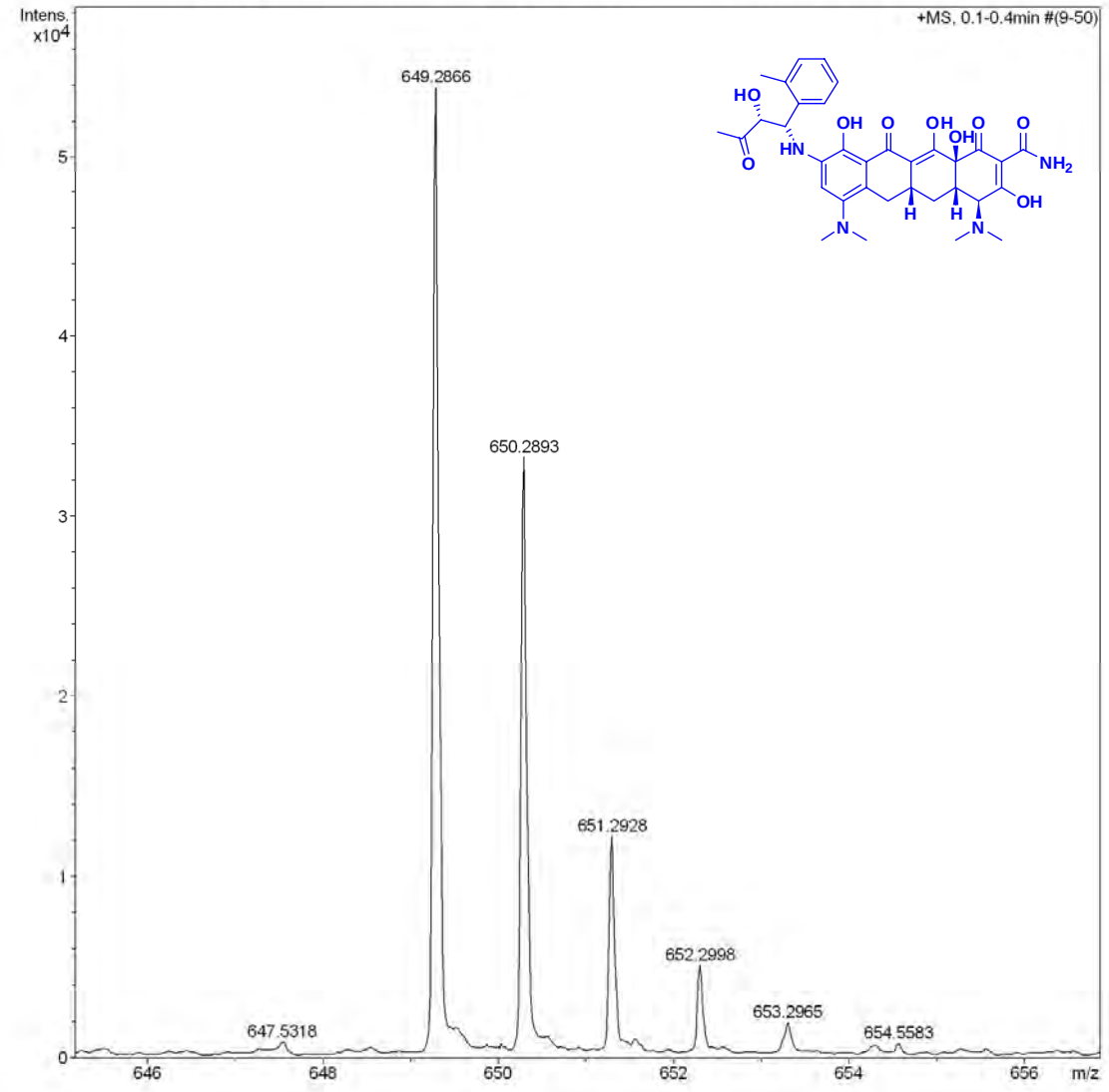

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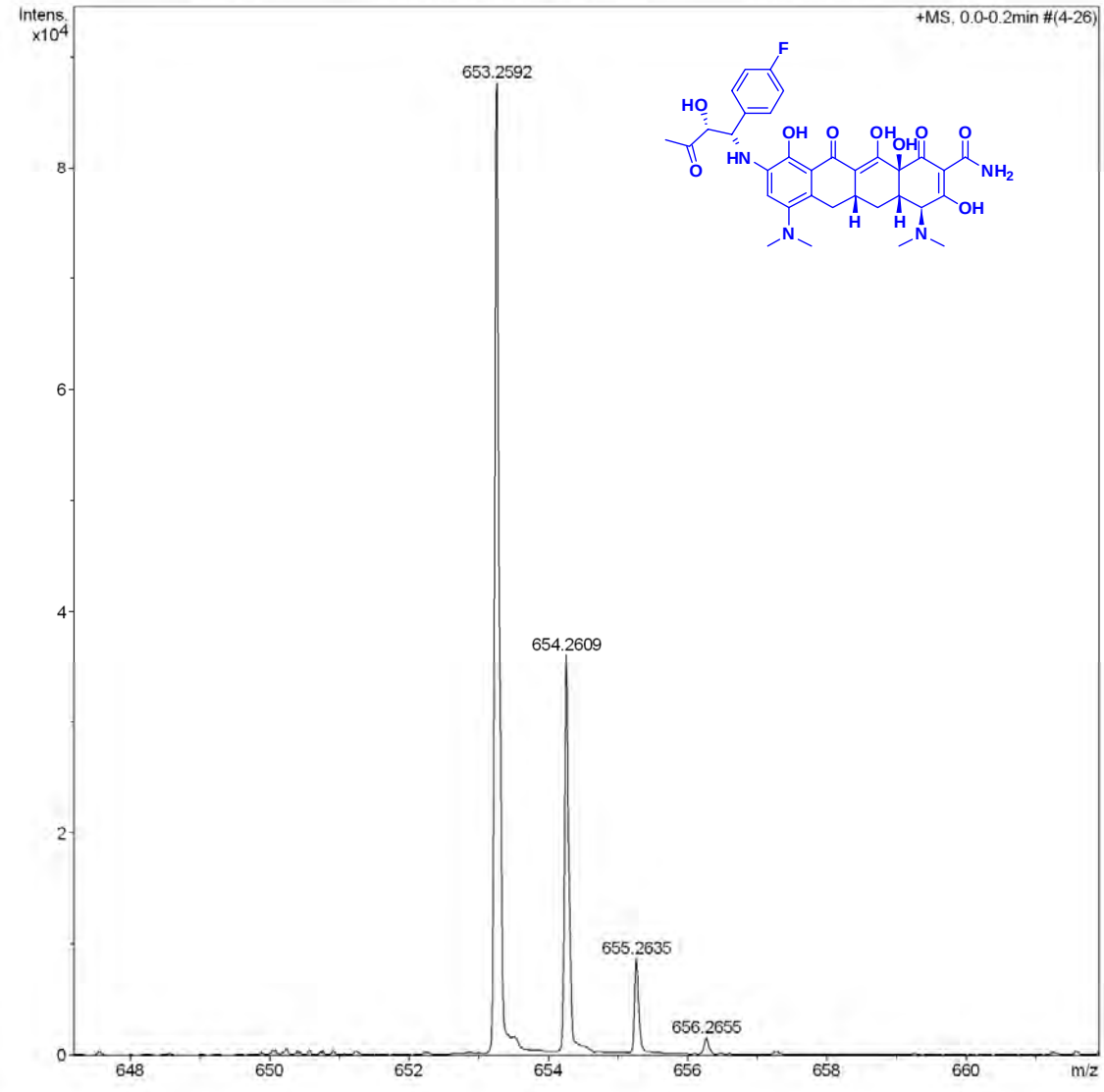

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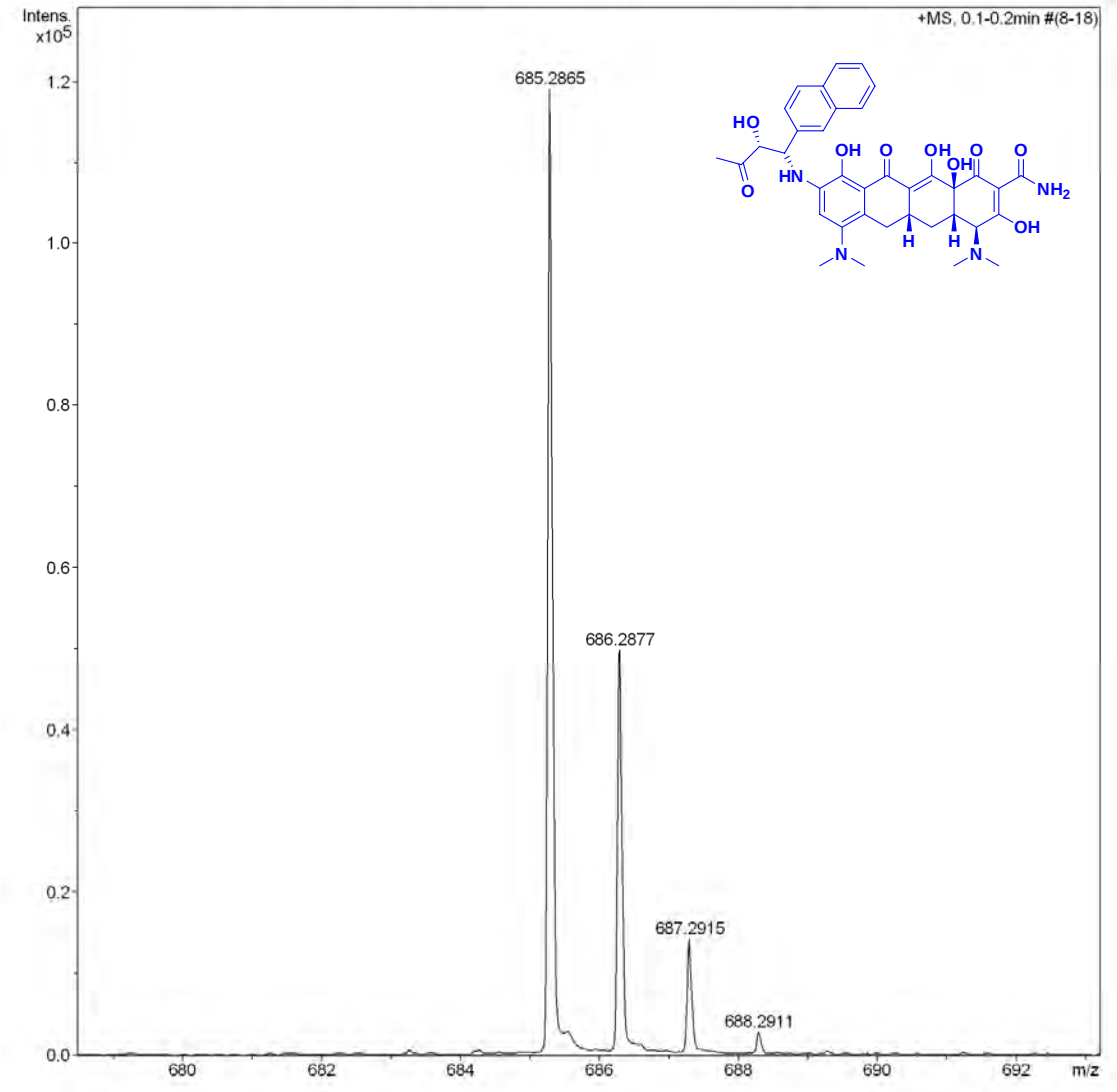
Method	fia.m	Operator	BDAL@DE
Sample Name	E9	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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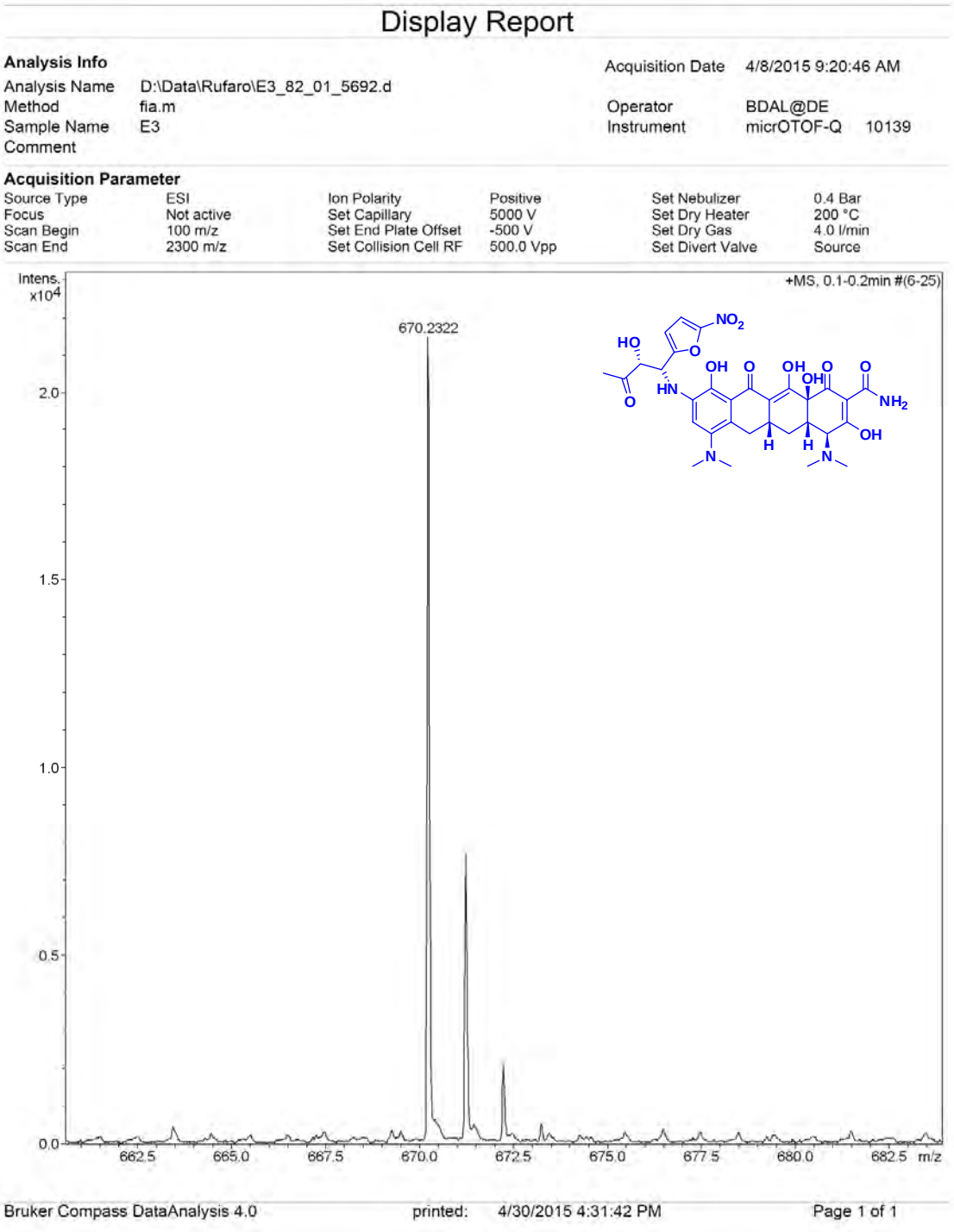
HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1R,2R)-2-hydroxy-1-(5-nitrofuran-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12 octahydrotetracene-2-carboxamide (2f)



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Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E7_52_01_5461.d	Acquisition Date	3/16/2015 1:19:08 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E7	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
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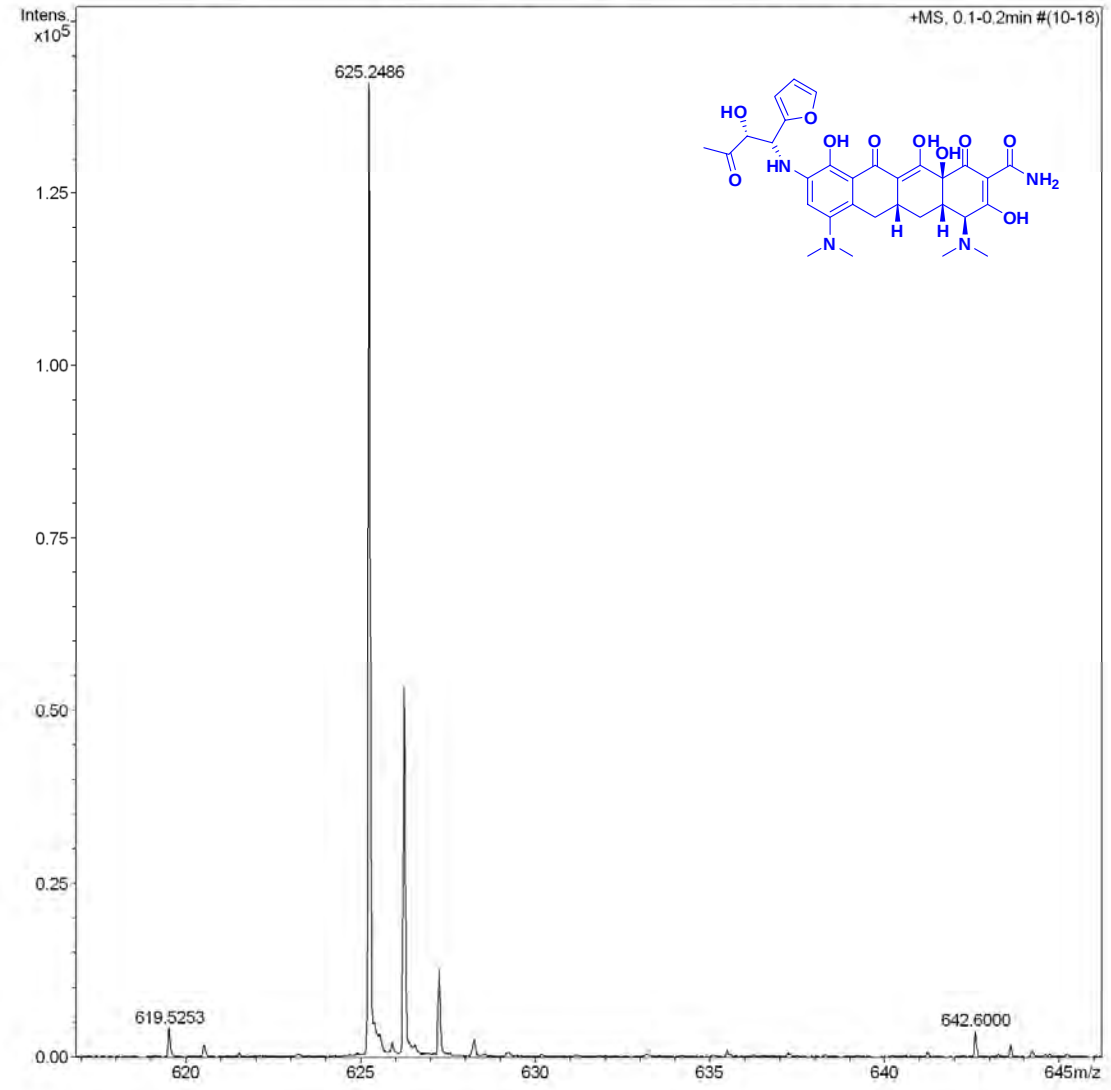
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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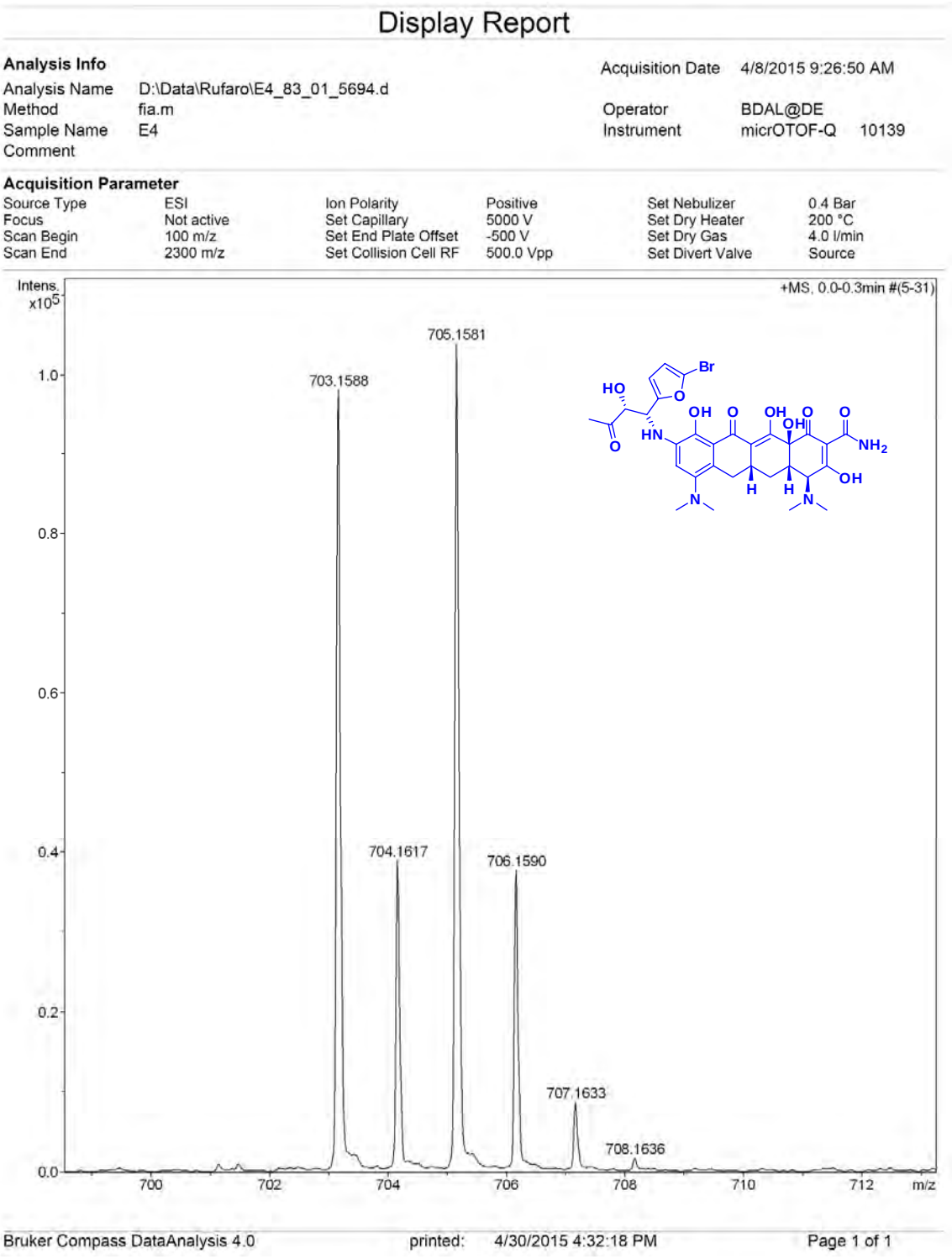
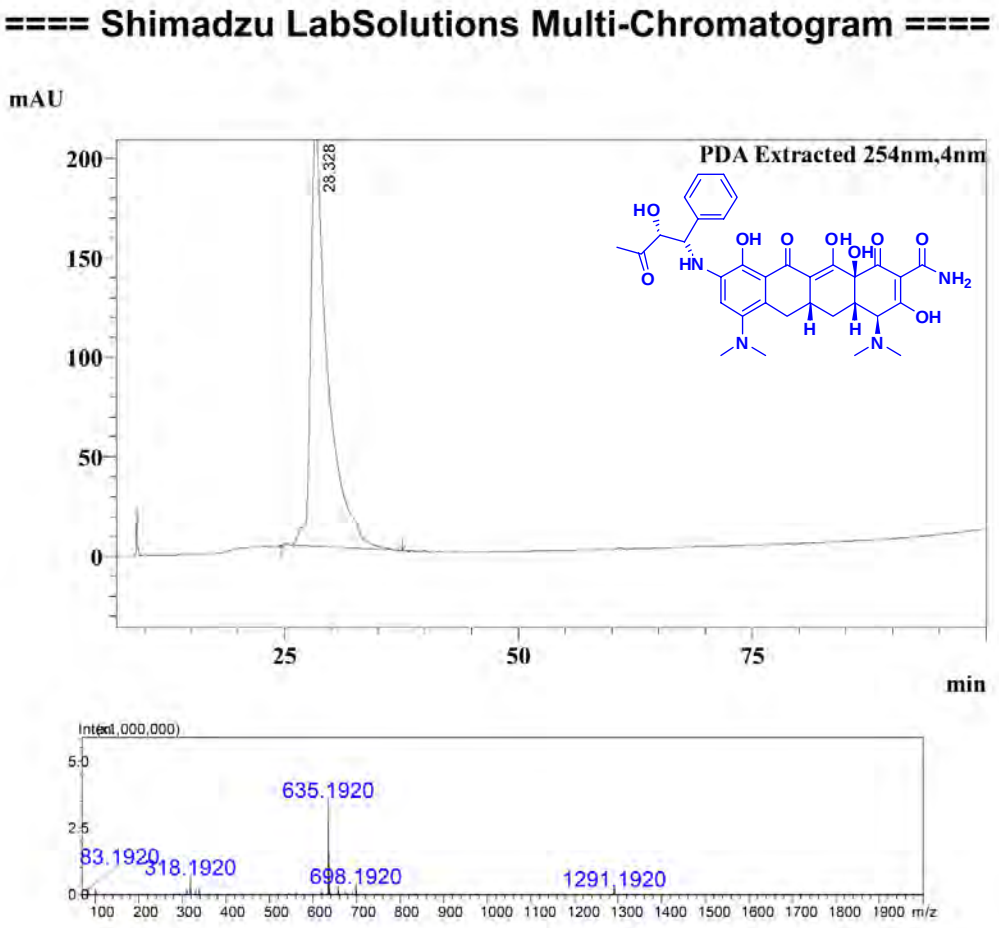
HRMS of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)



2. Copies of LC-MS spectra for products

LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)



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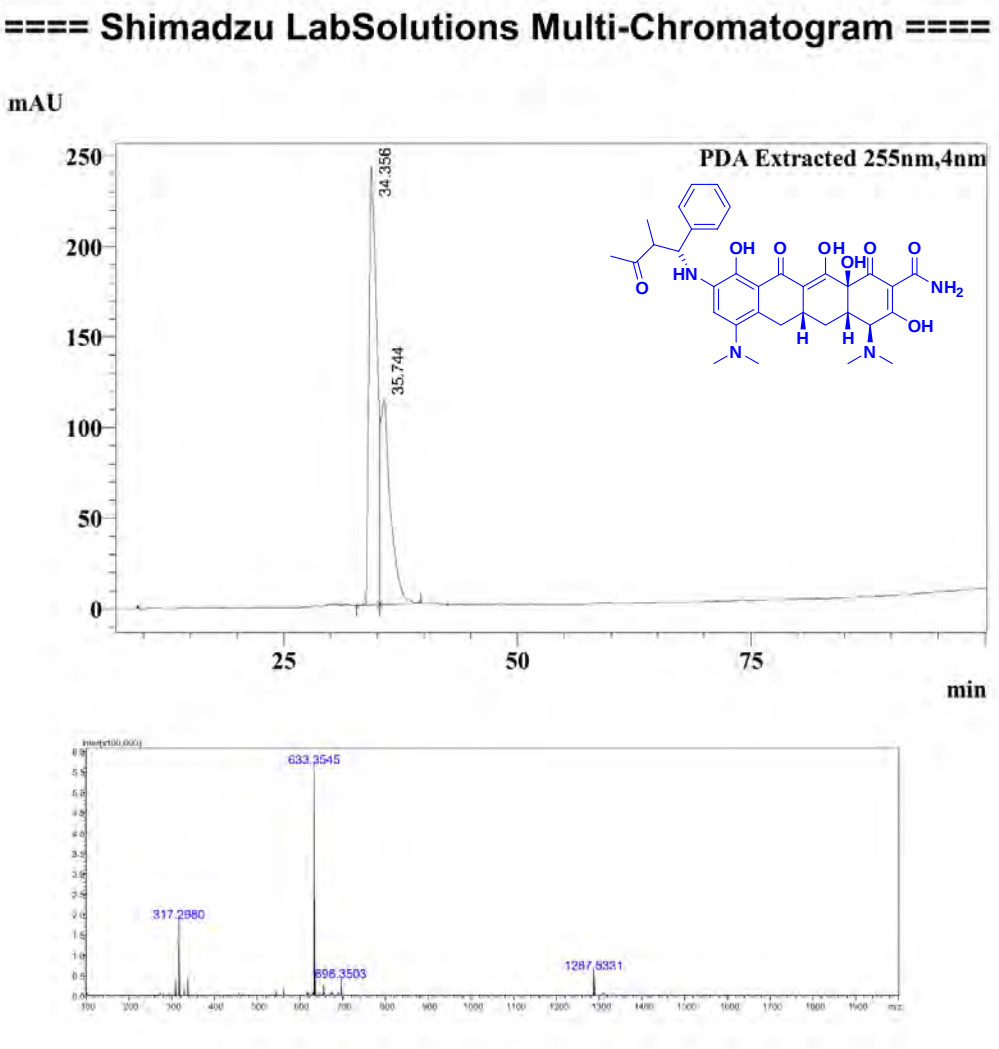

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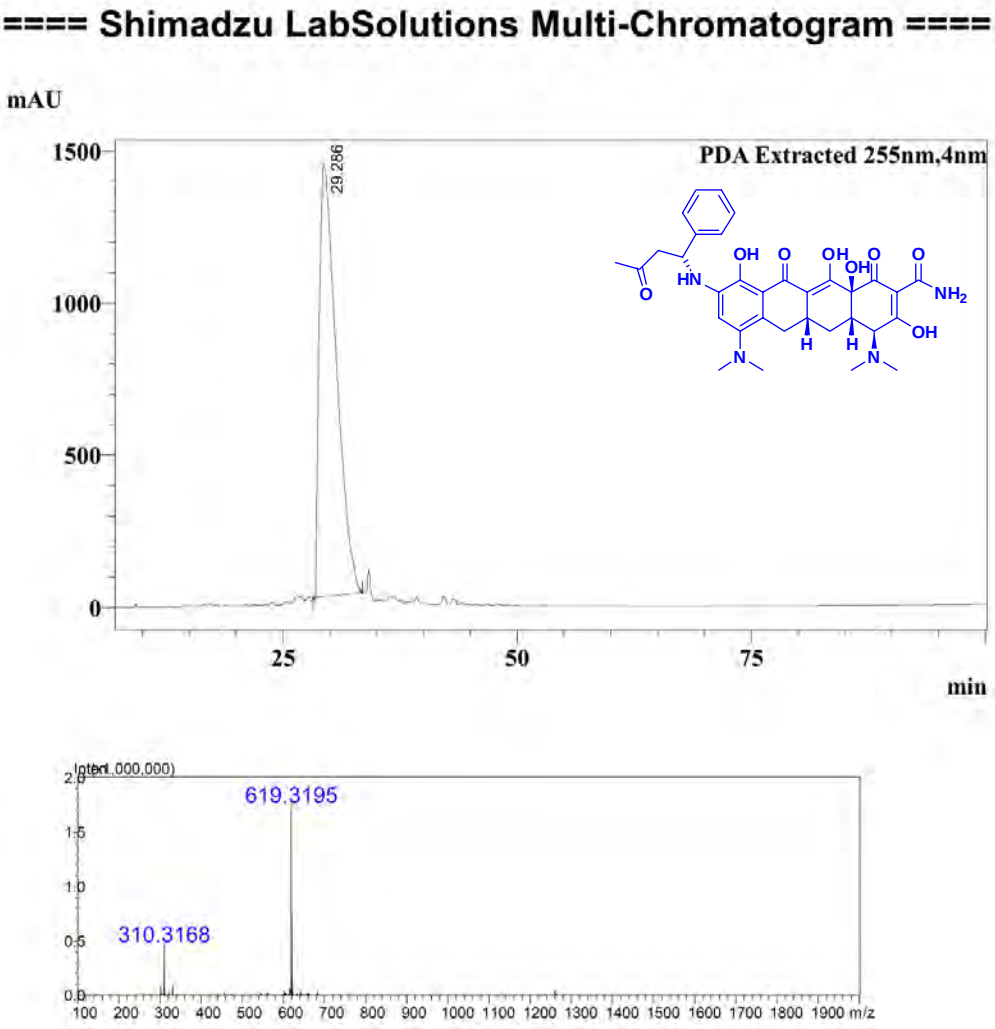

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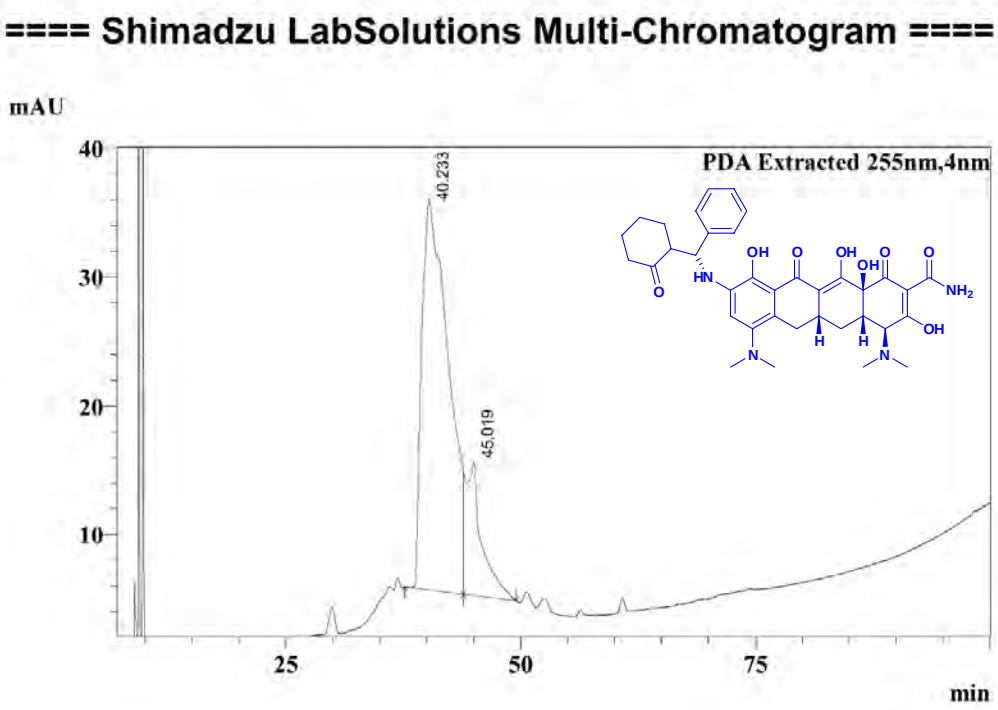
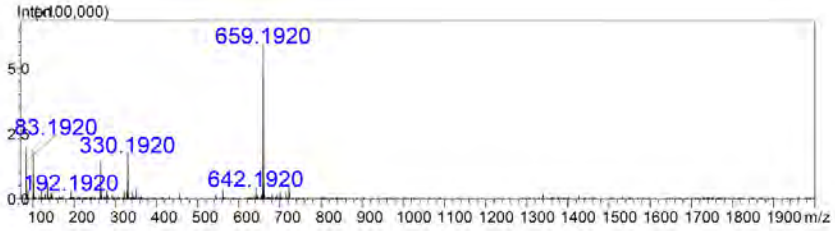
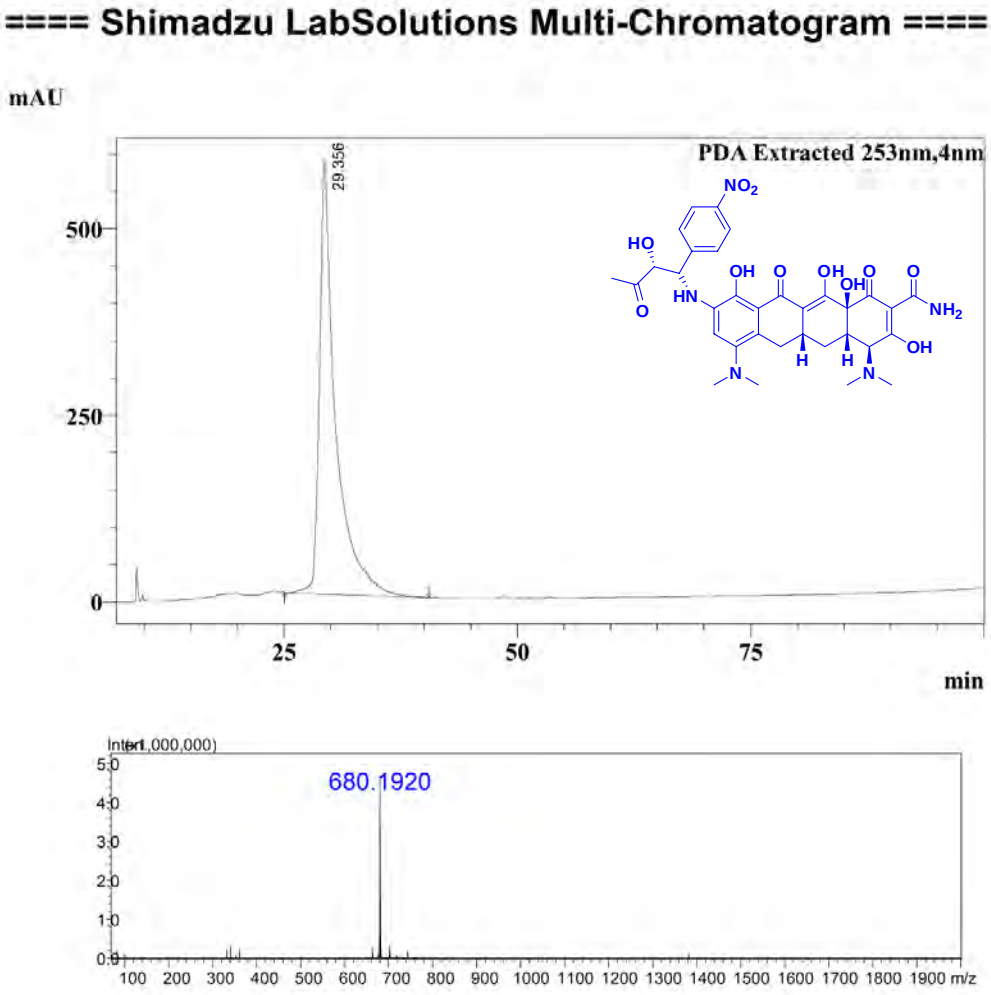
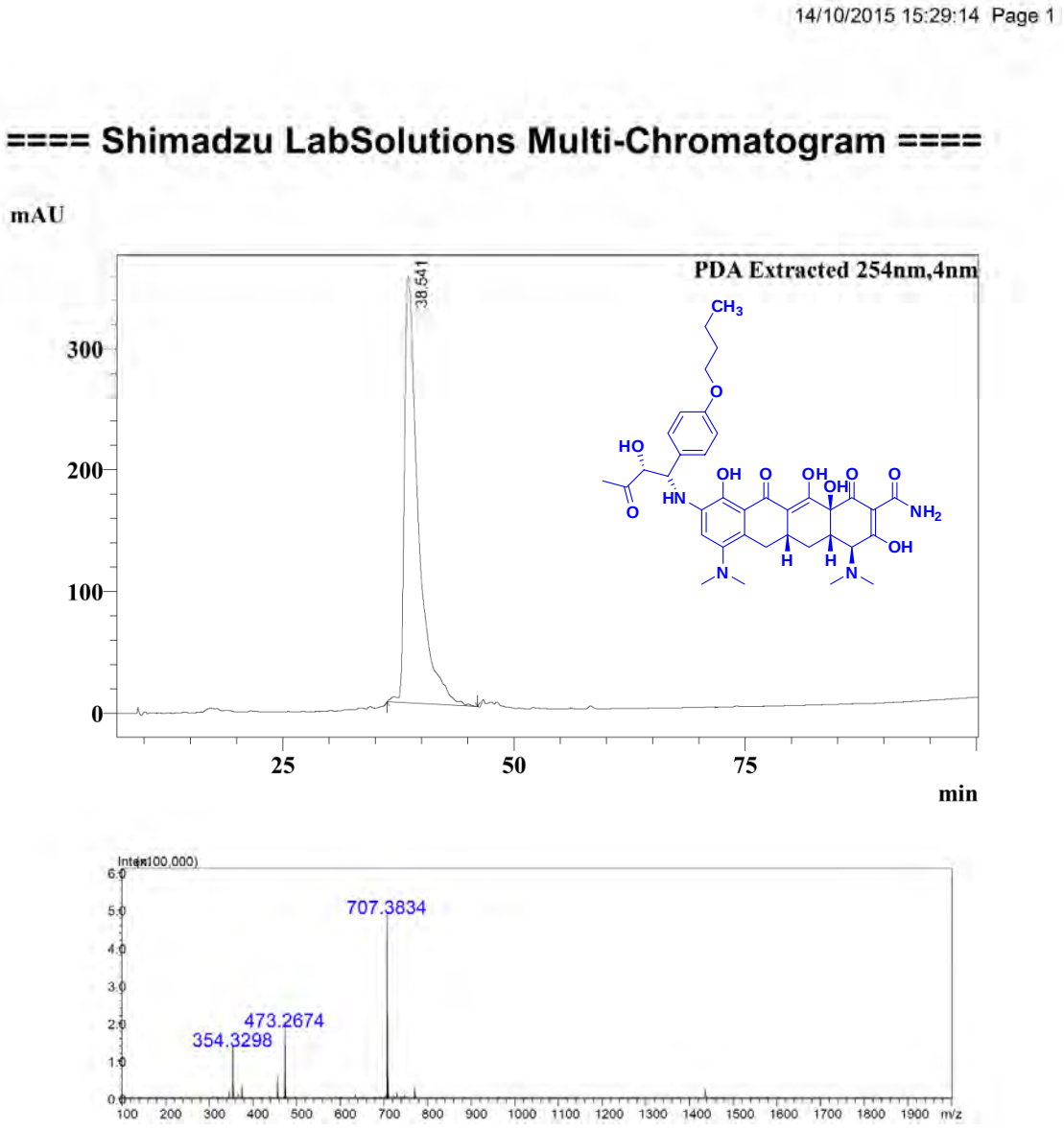




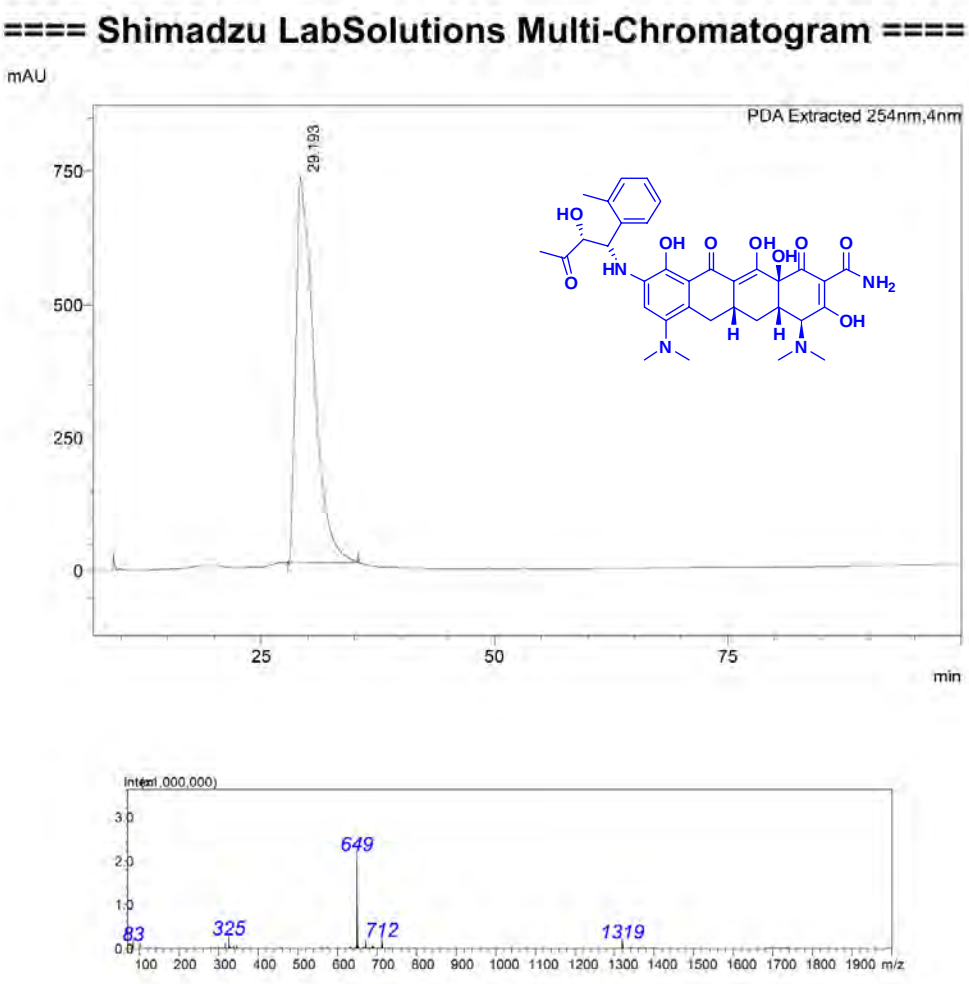
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(4-nitrophenyl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2a)



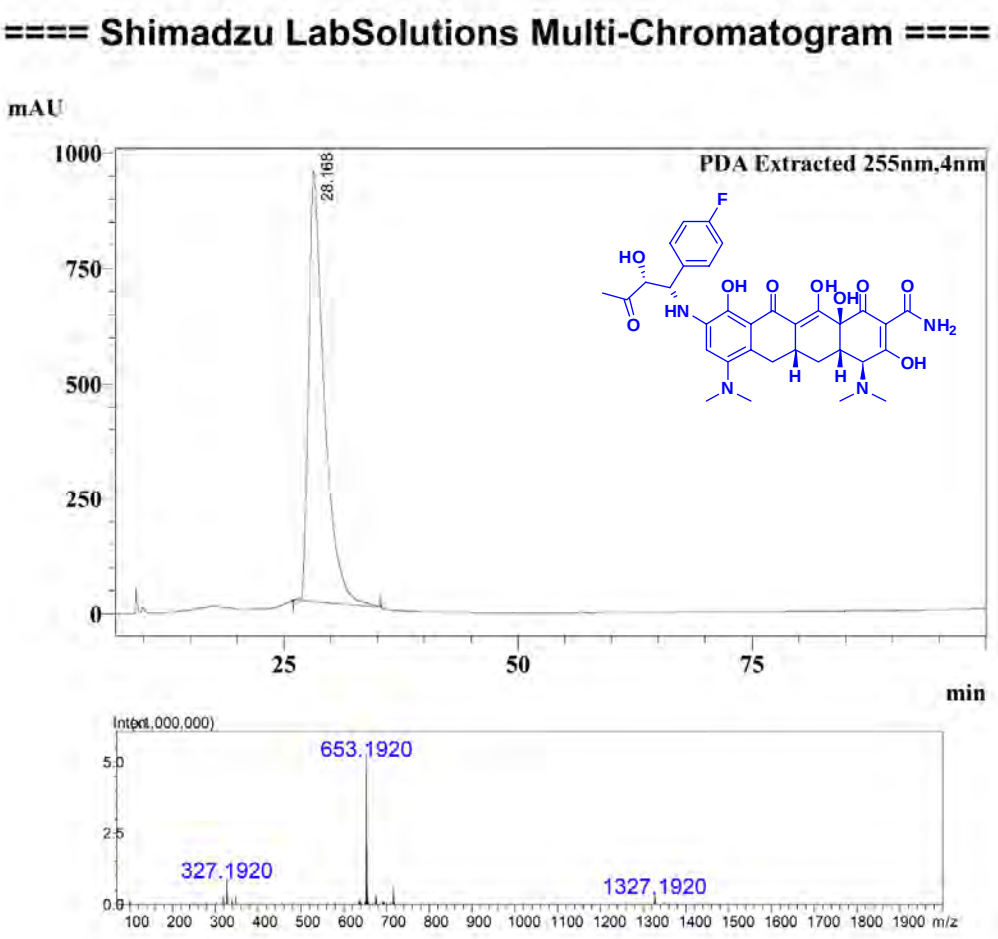
LC-MS of (4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)



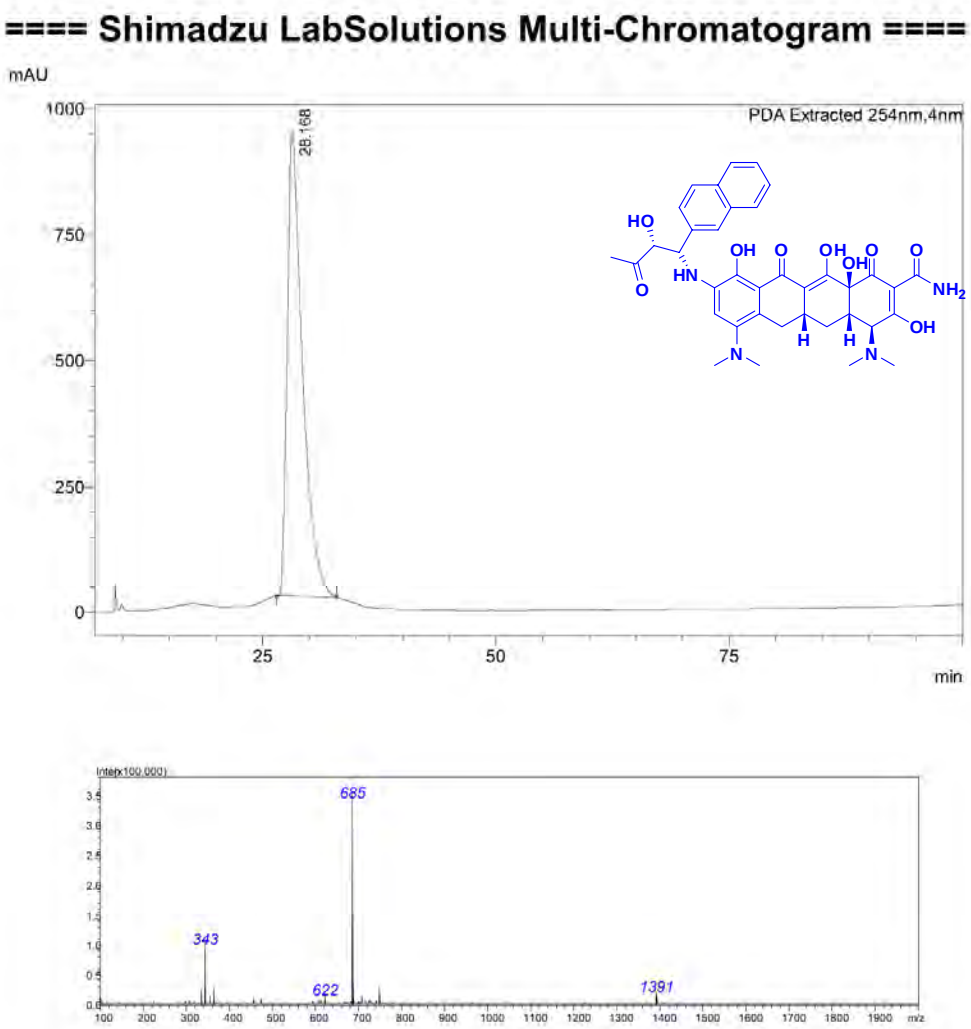
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-3-oxo-1-o-tolylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2c)



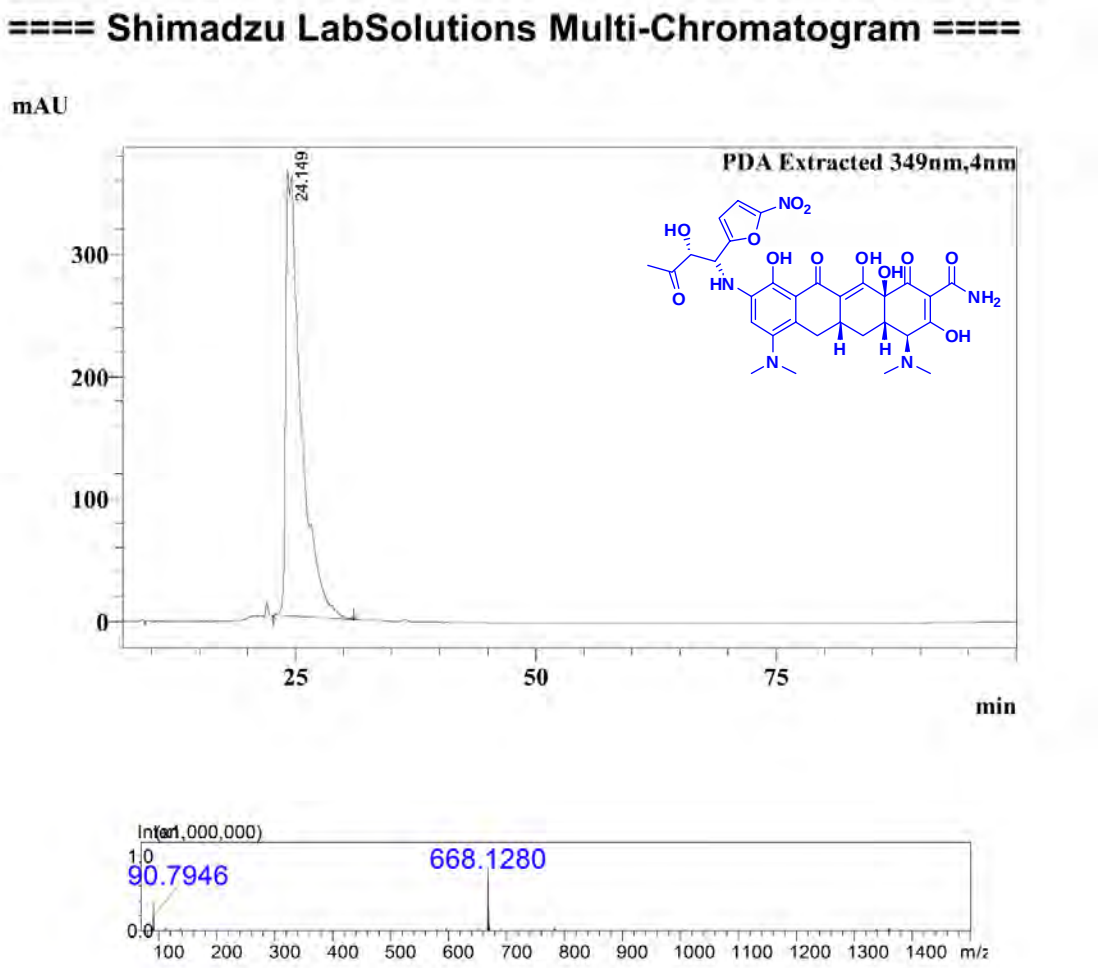
LC-MS SPECTRUM of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)



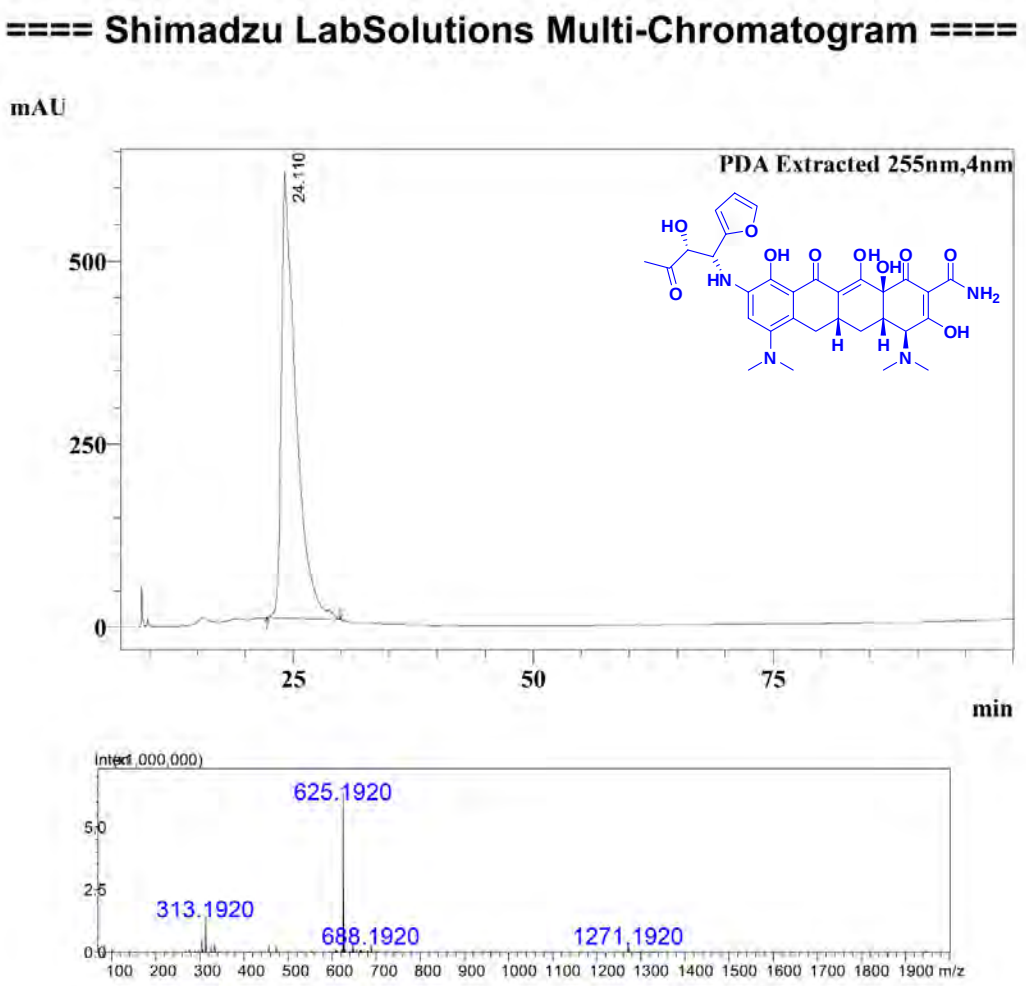
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(naphthalen-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2e)



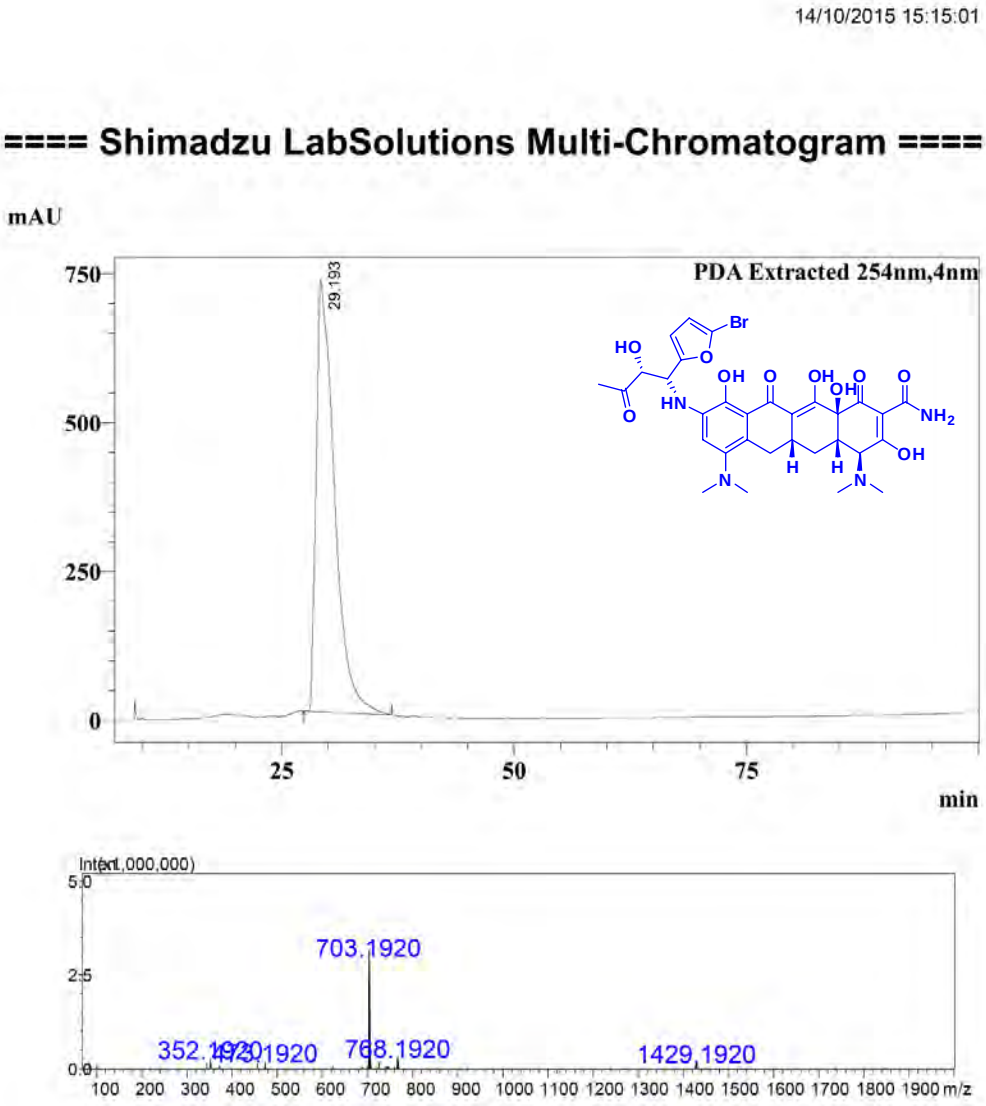
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1R,2R)-2-hydroxy-1-(5-nitrofuran-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12 octahydrotetracene-2-carboxamide (2f)



LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1R,2R)-1-(furan-2-yl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2g)

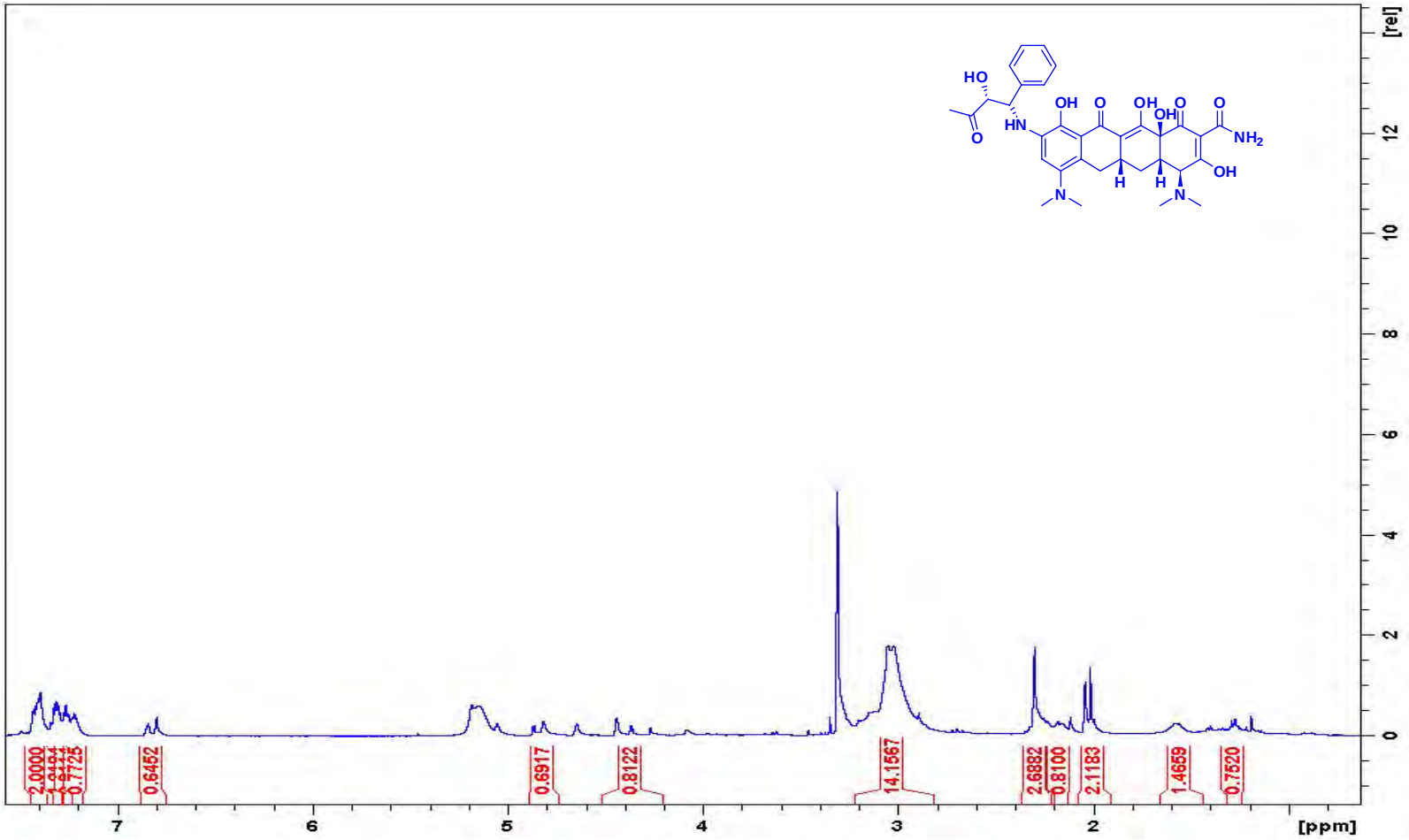


LC-MS of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)

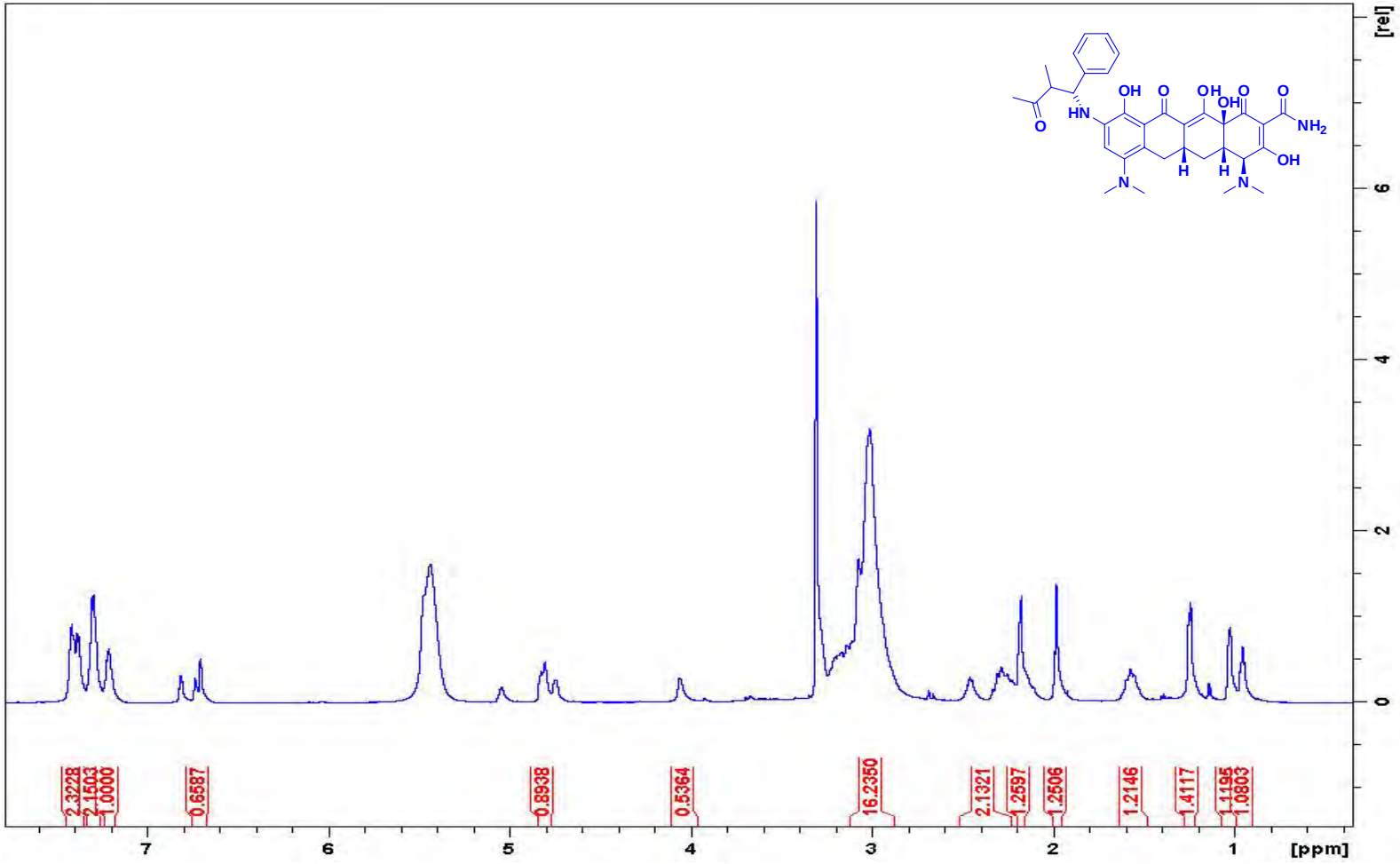


3. Copies of NMR spectra for products

^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)



^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)



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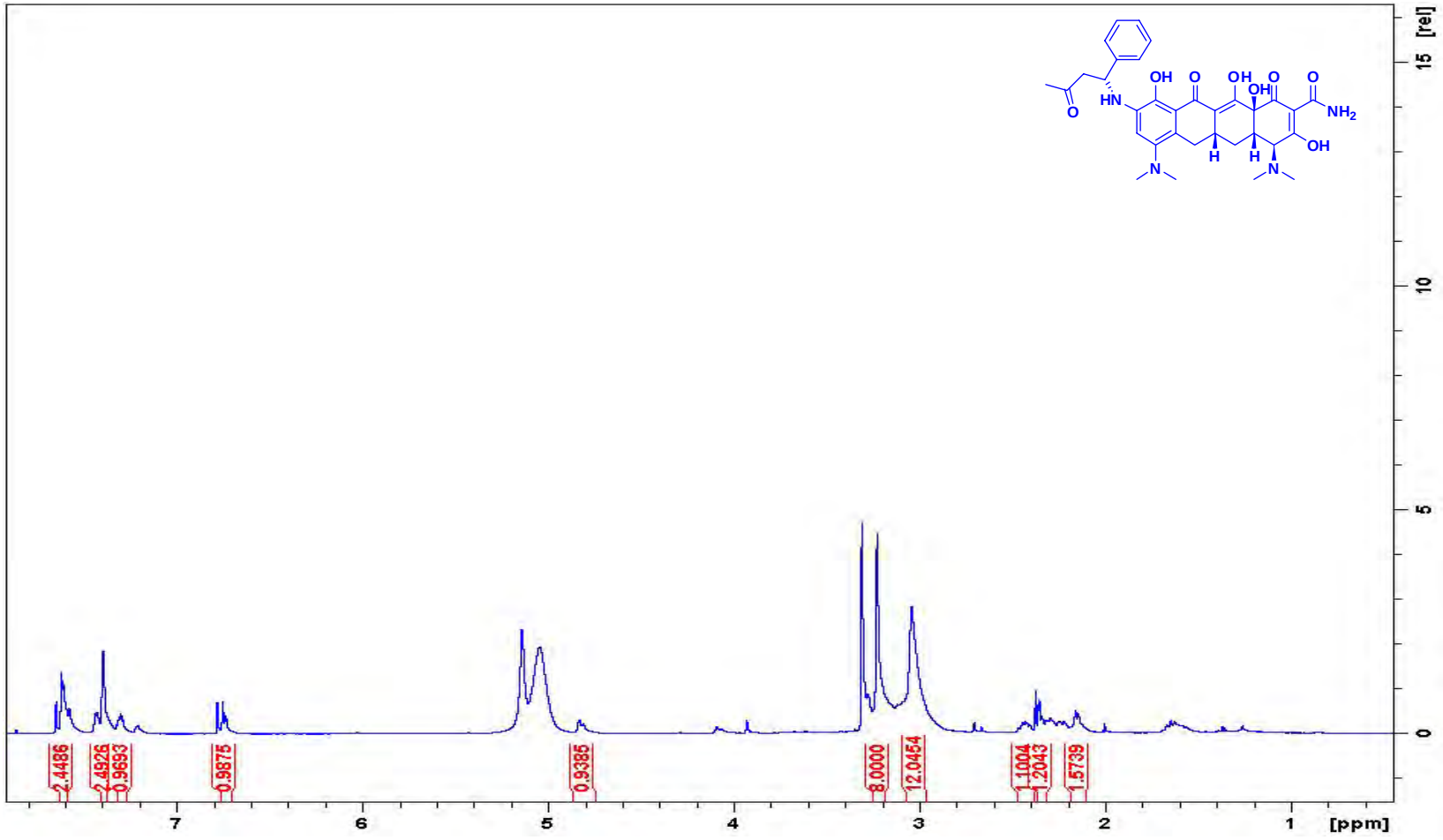

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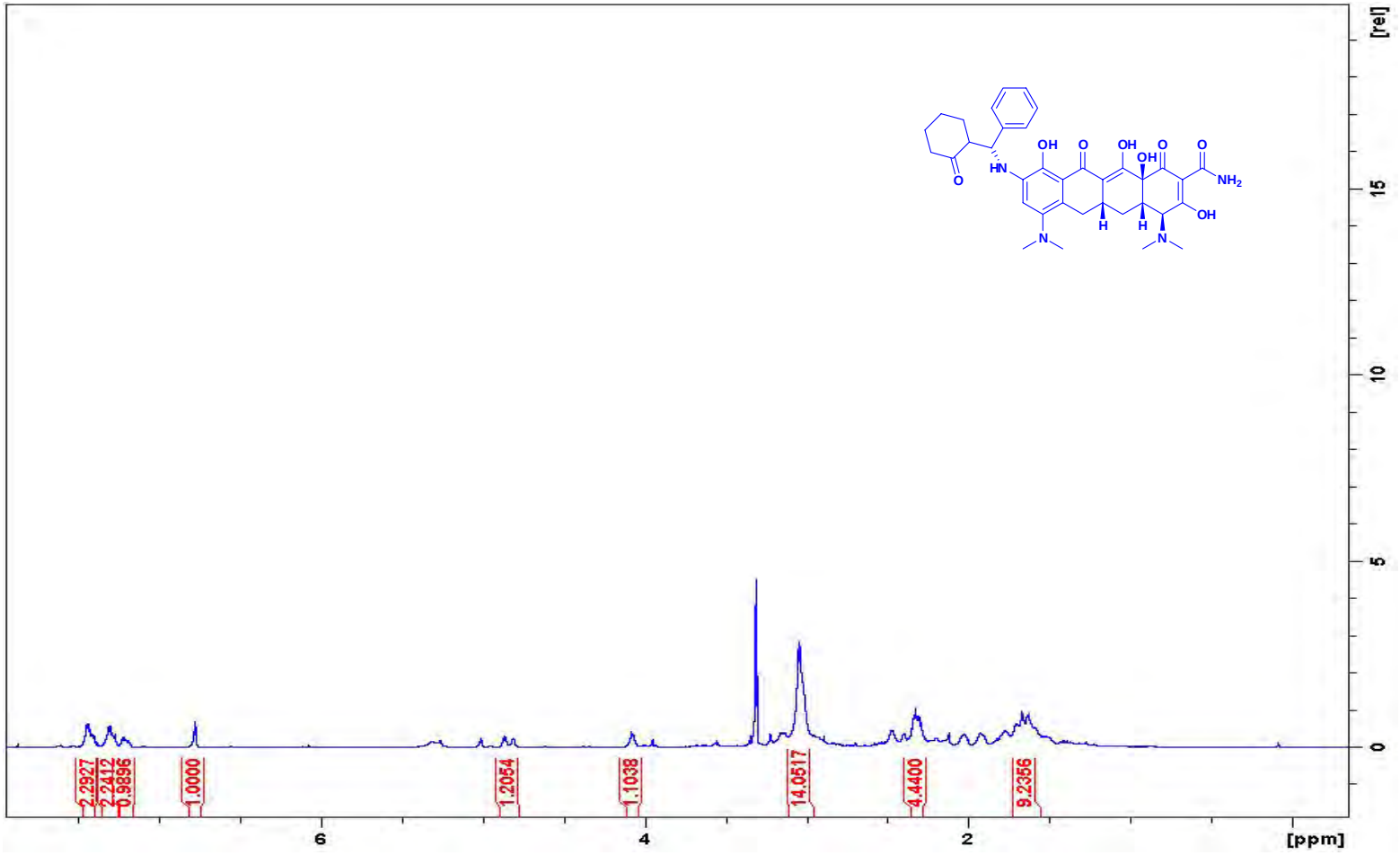

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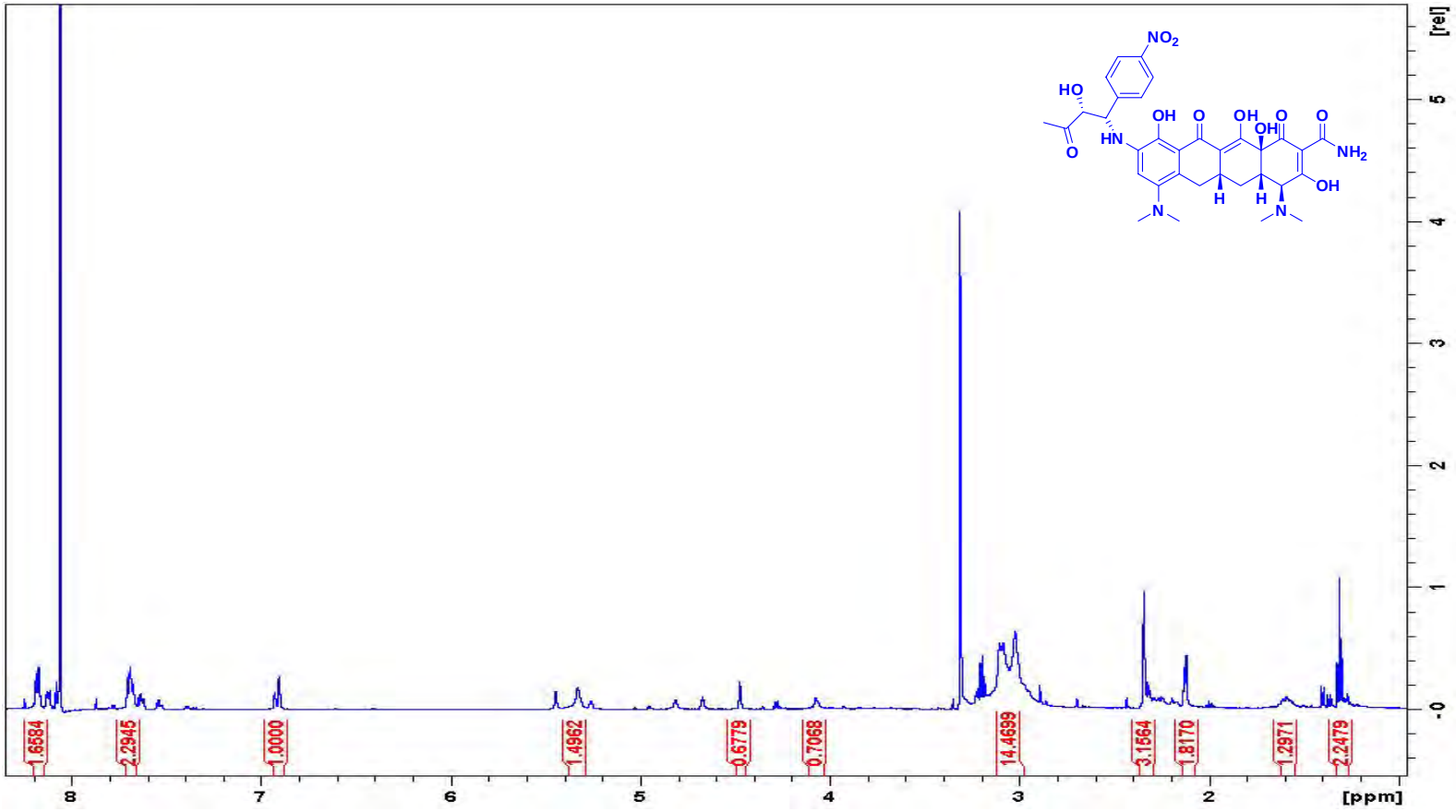

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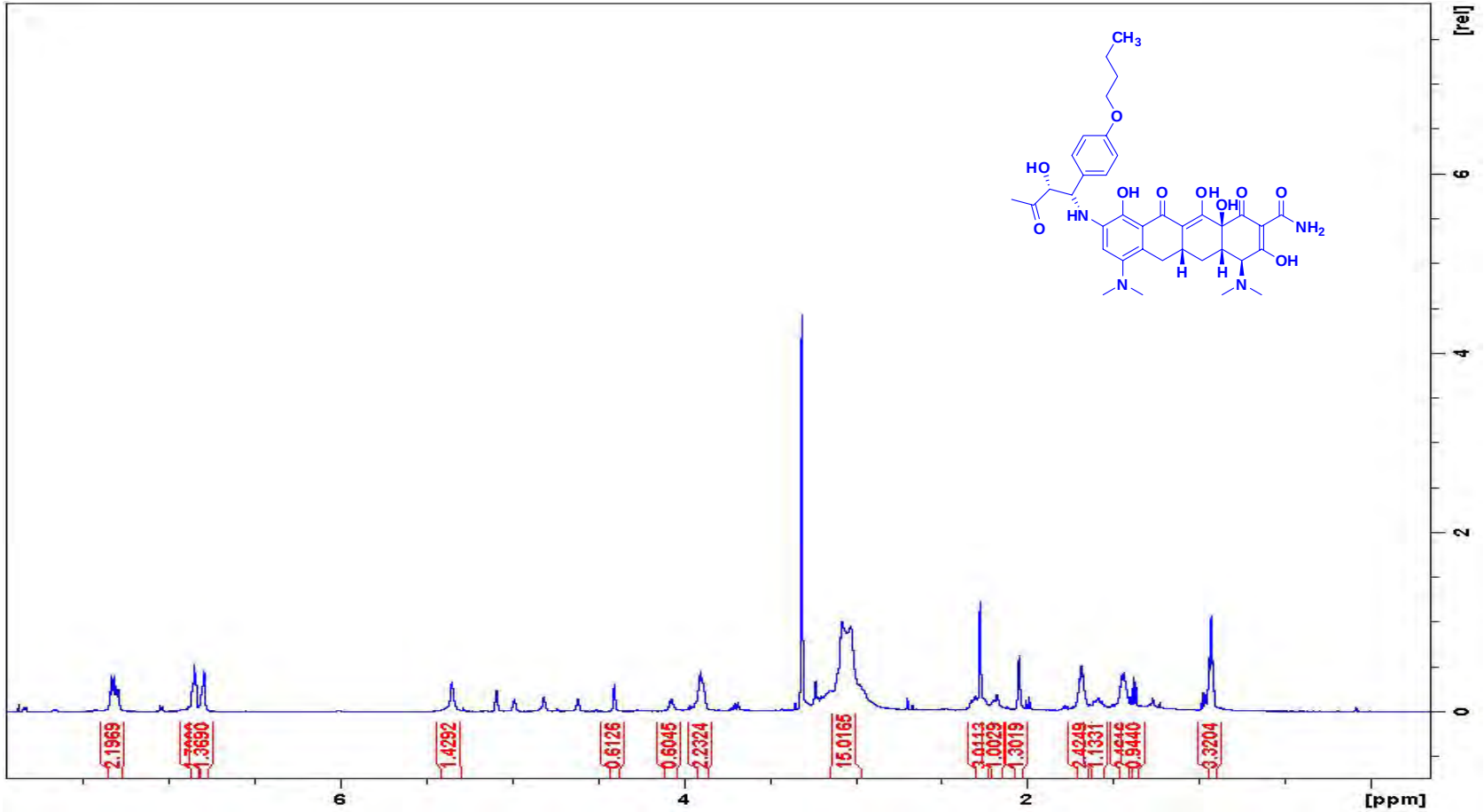
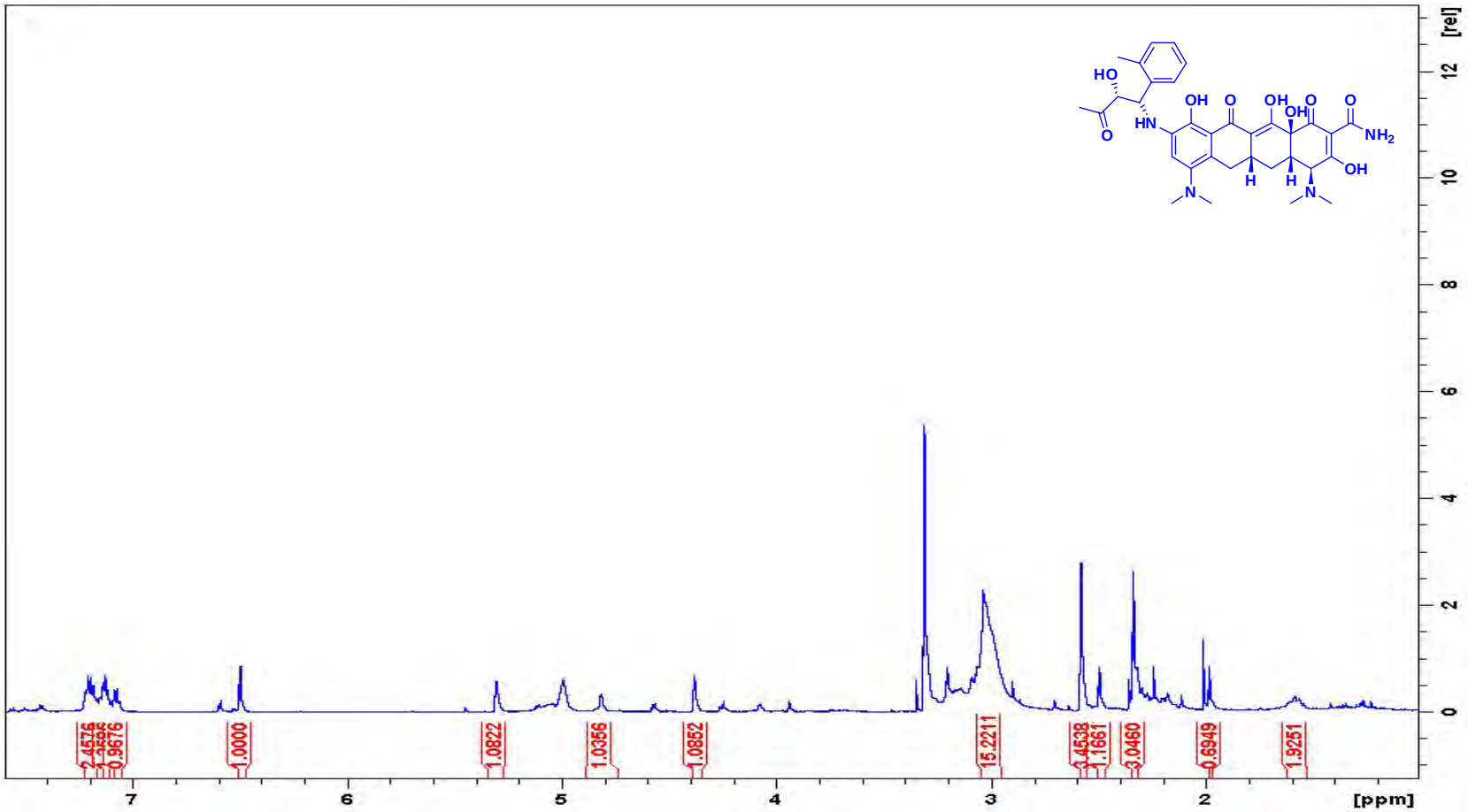


^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-3-oxo-1-o-tolylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2c)



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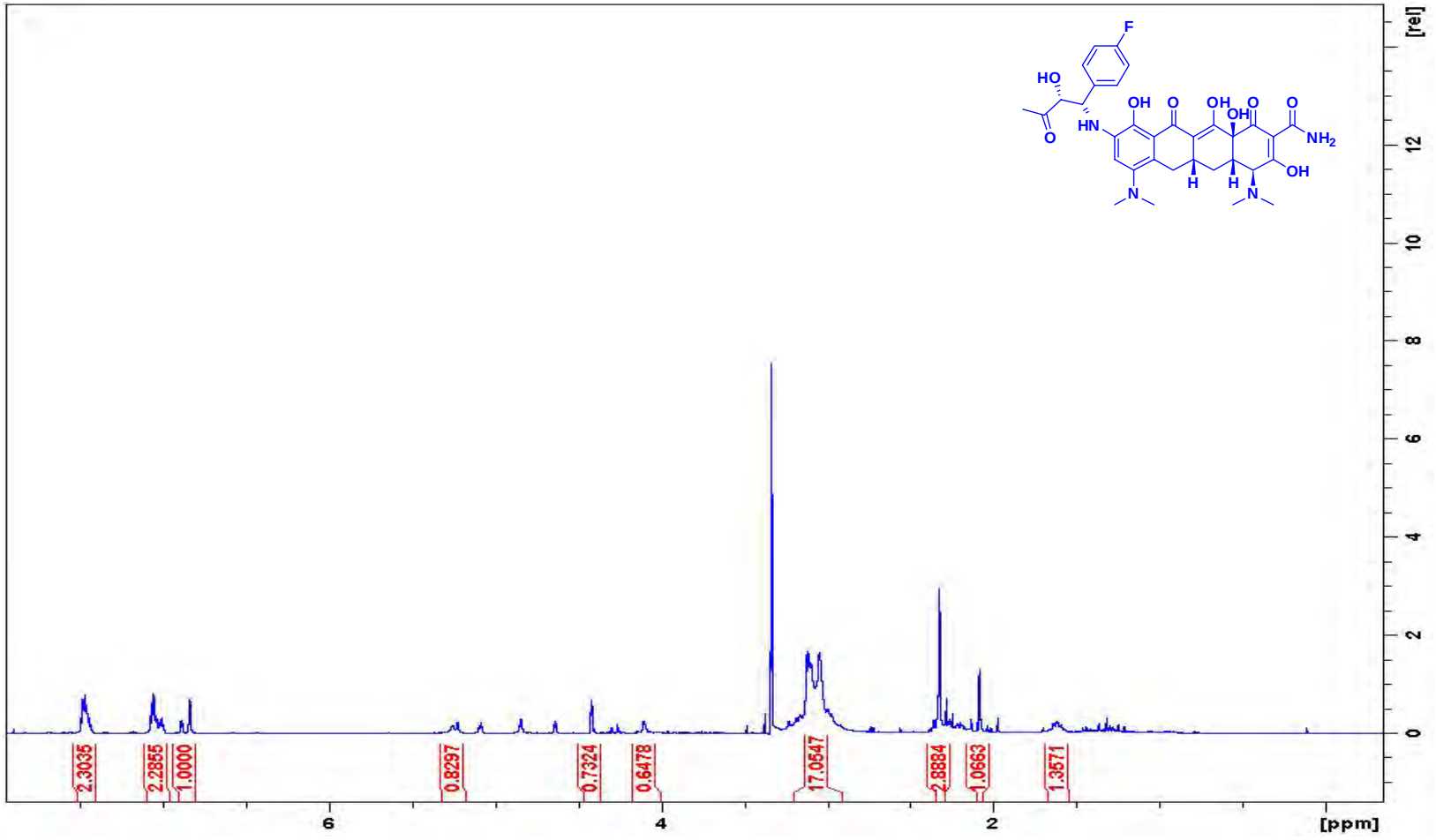

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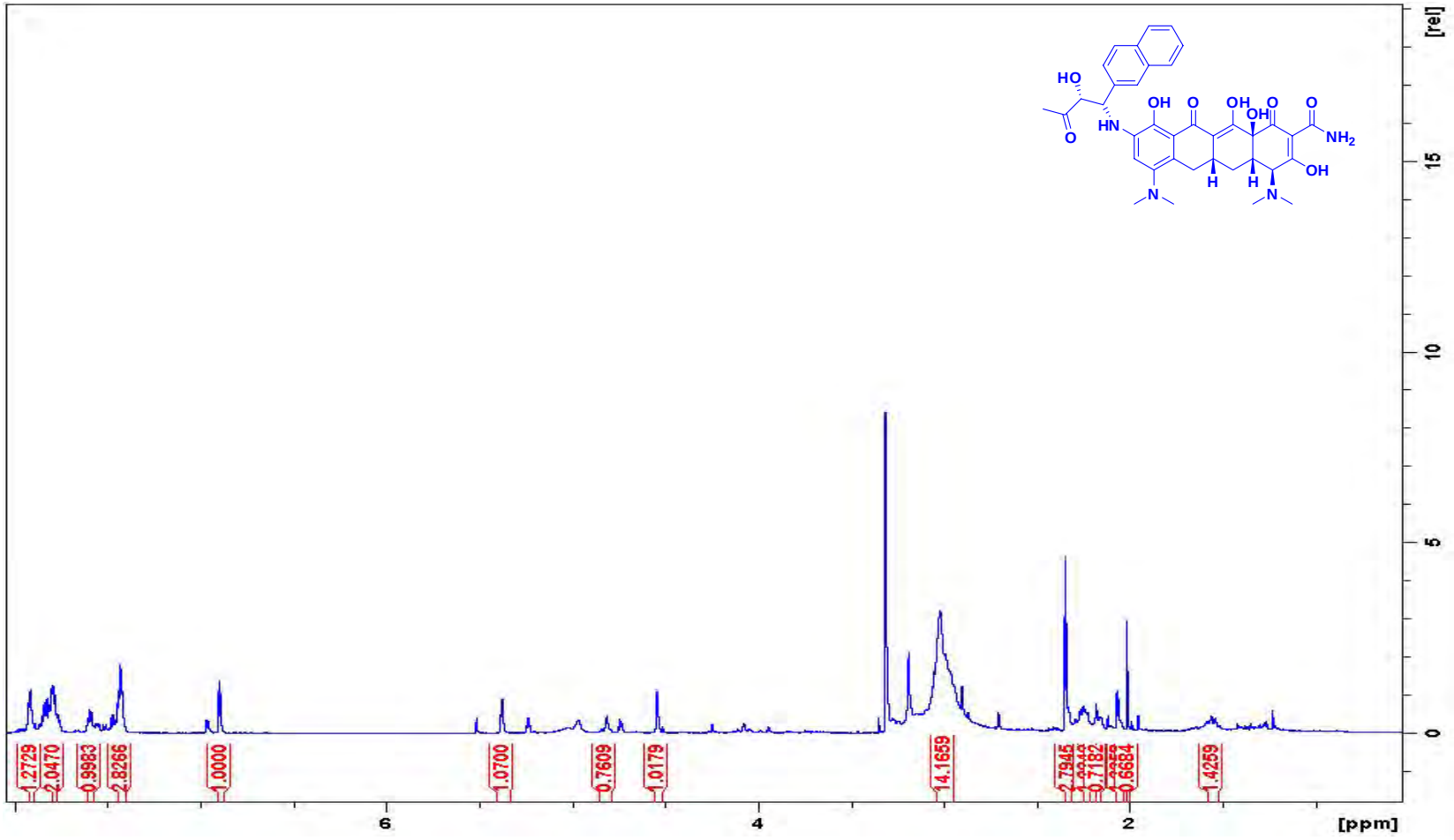

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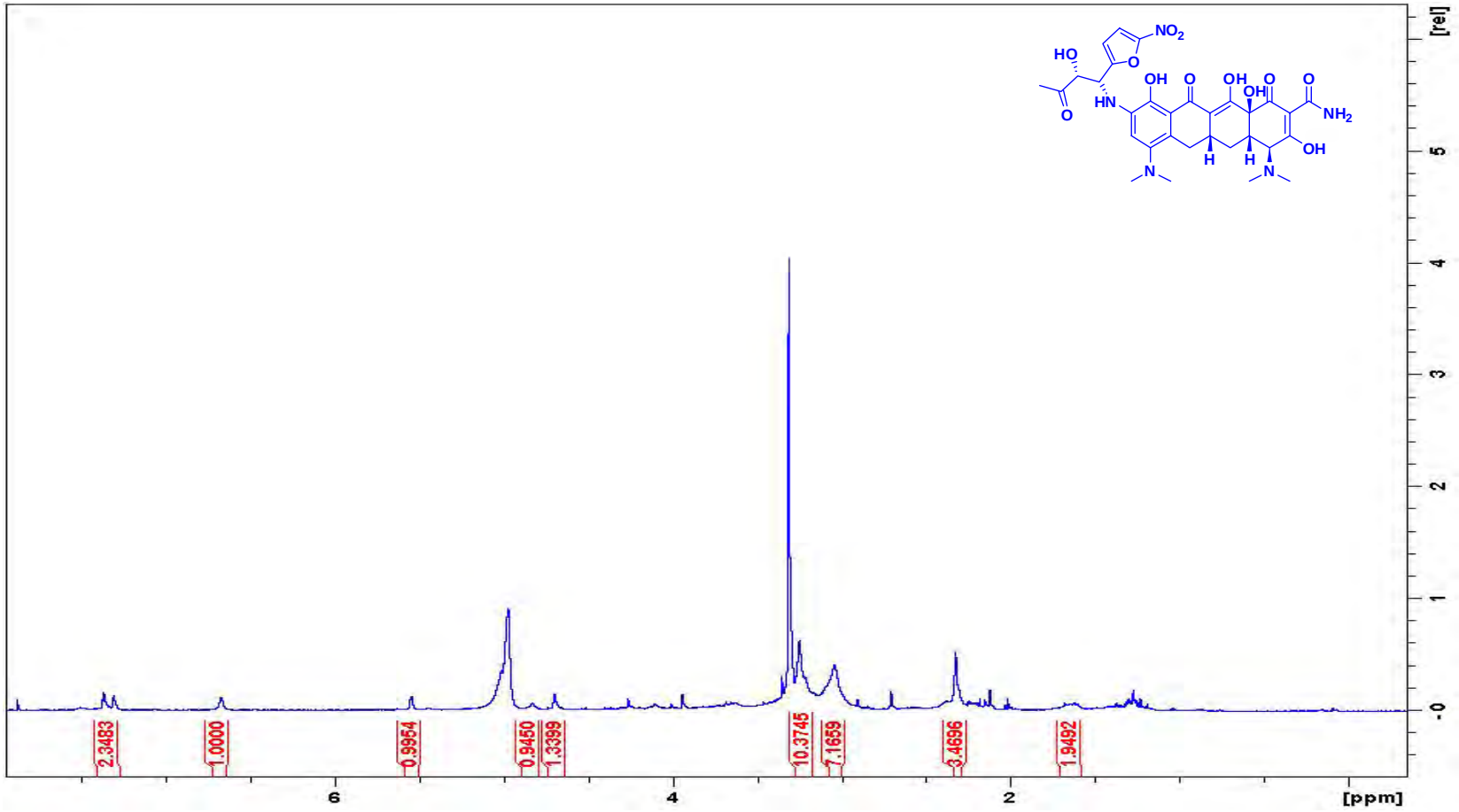

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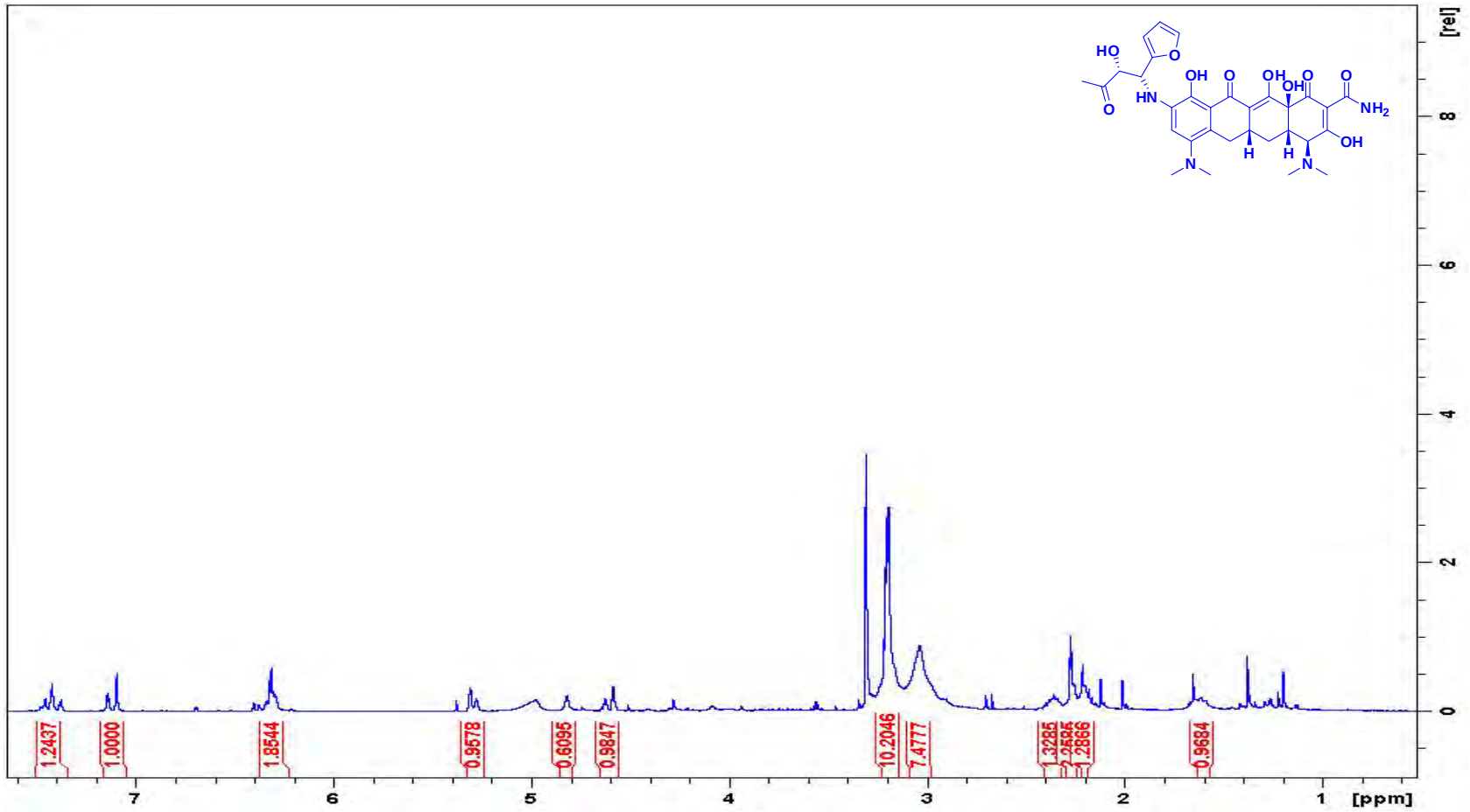

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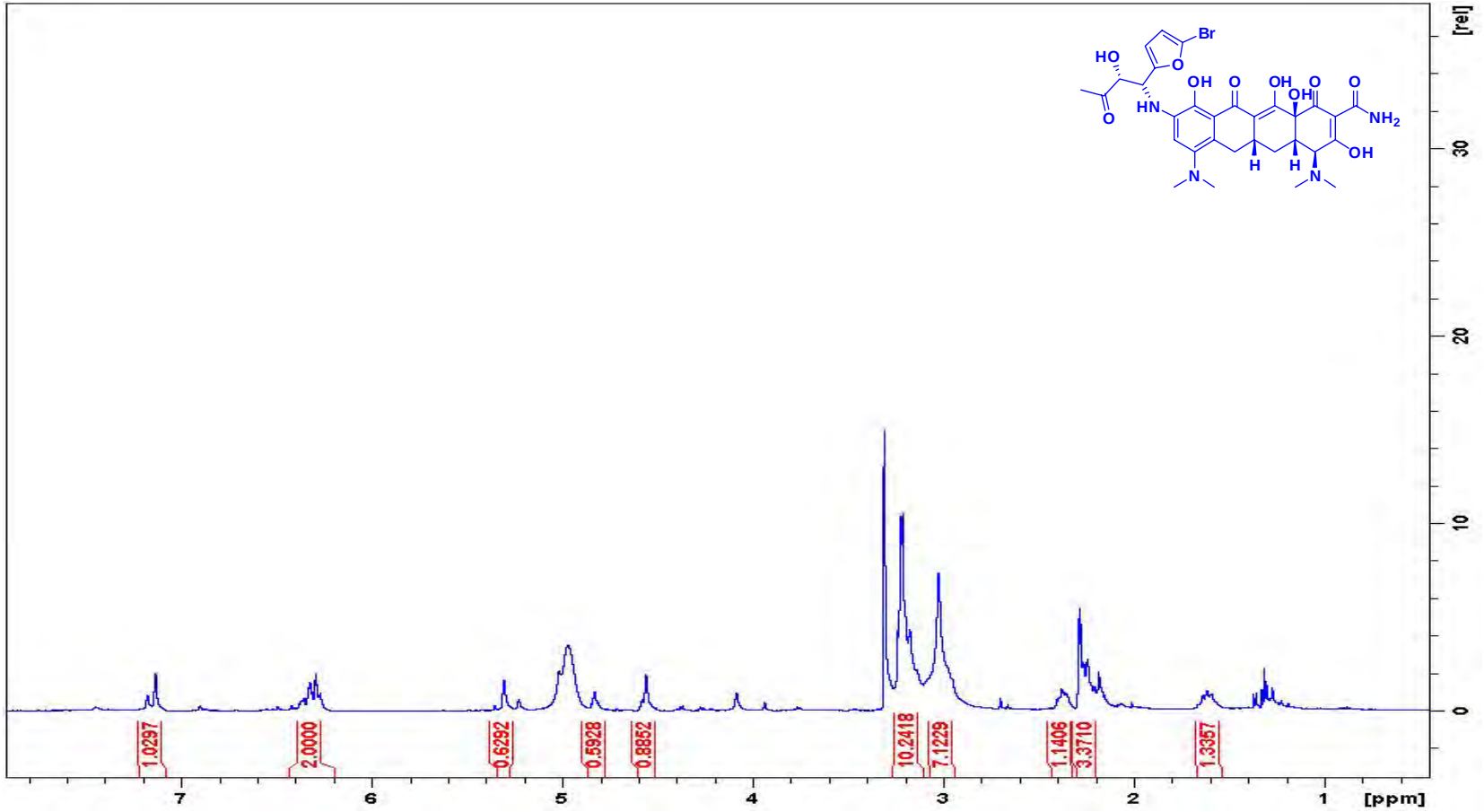

Organocatalyzed Mannich Reactions on Minocycline: Towards Novel Tetracycline Antibiotics

Tirivashe E. Chiwunze[a], Rafiatu Azumah[a], Melissa Ramtahal[a], Per I. Arvidsson[b], Sabiha Y. Essack [c], Hendrik G. Kruger[a], Thavendran Govender[a], Tricia Naicker[a]

[a] School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.

*e-mail: Naickert1@ukzn.ac.za>

[b] Science for Life Laboratory, Drug Discovery and Development Platform, and Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

[c] School of Chemistry and Physics, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

SUPPLEMETARY DATA

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1. Copies of HRMS spectra for products.....3

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3. Copies of NMR spectra for products.....27

1. Copies of HRMS spectra for products

HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)

Display Report

Analysis Info

Analysis Name	D:\Data\New Folder\E2_63_01_5473.d	Acquisition Date	3/20/2015 10:48:44 AM
Method	fia.m	Operator	BDAL@DE
Sample Name	E2	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

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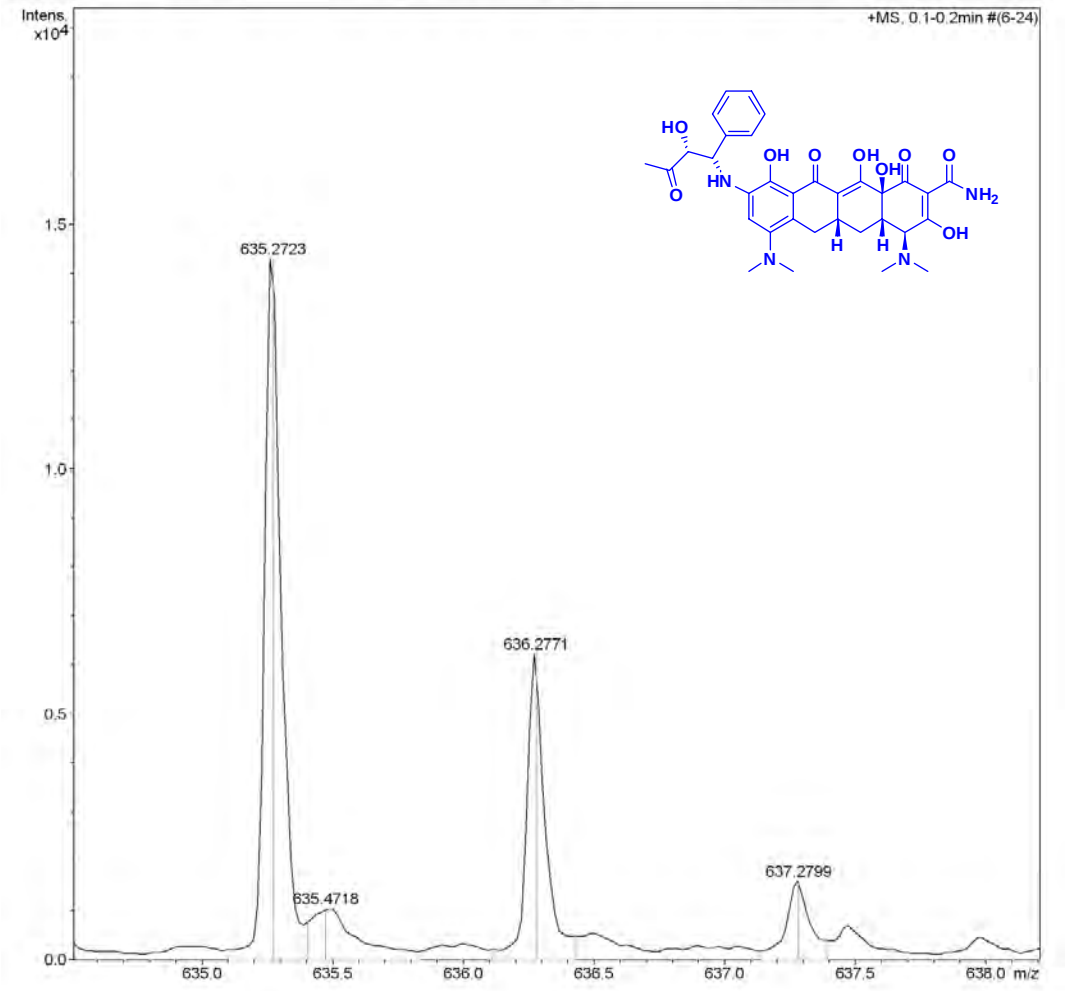
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)

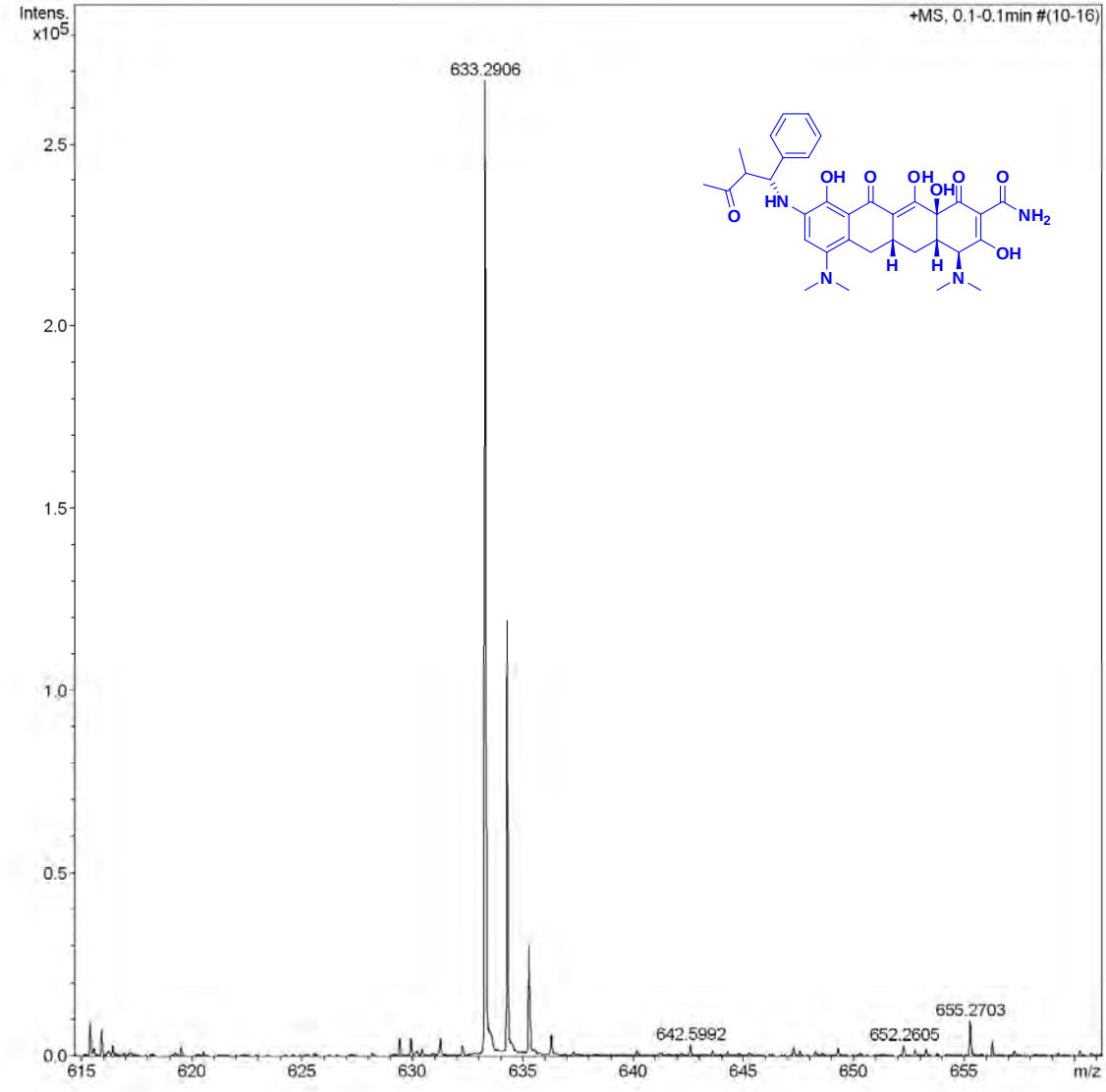
Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E12_54_01_5465.d	Acquisition Date	3/16/2015 1:31:16 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E12	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Bruker Compass DataAnalysis 4.0 printed: 3/16/2015 1:54:05 PM Page 1 of 1

HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-(3-oxo-1-phenylbutylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1c)

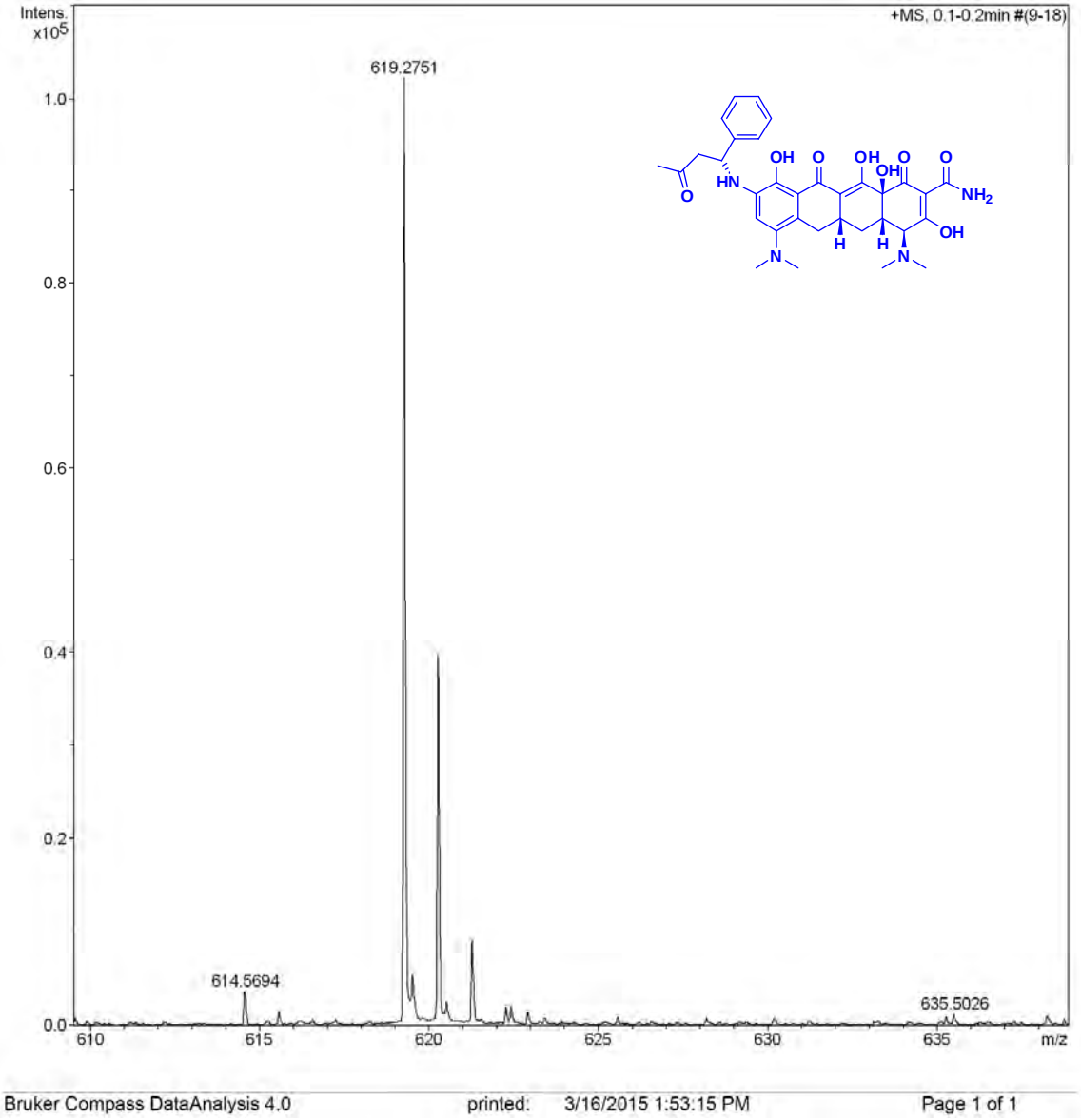
Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E13_55_01_5467.d	Acquisition Date	3/16/2015 1:37:20 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E13	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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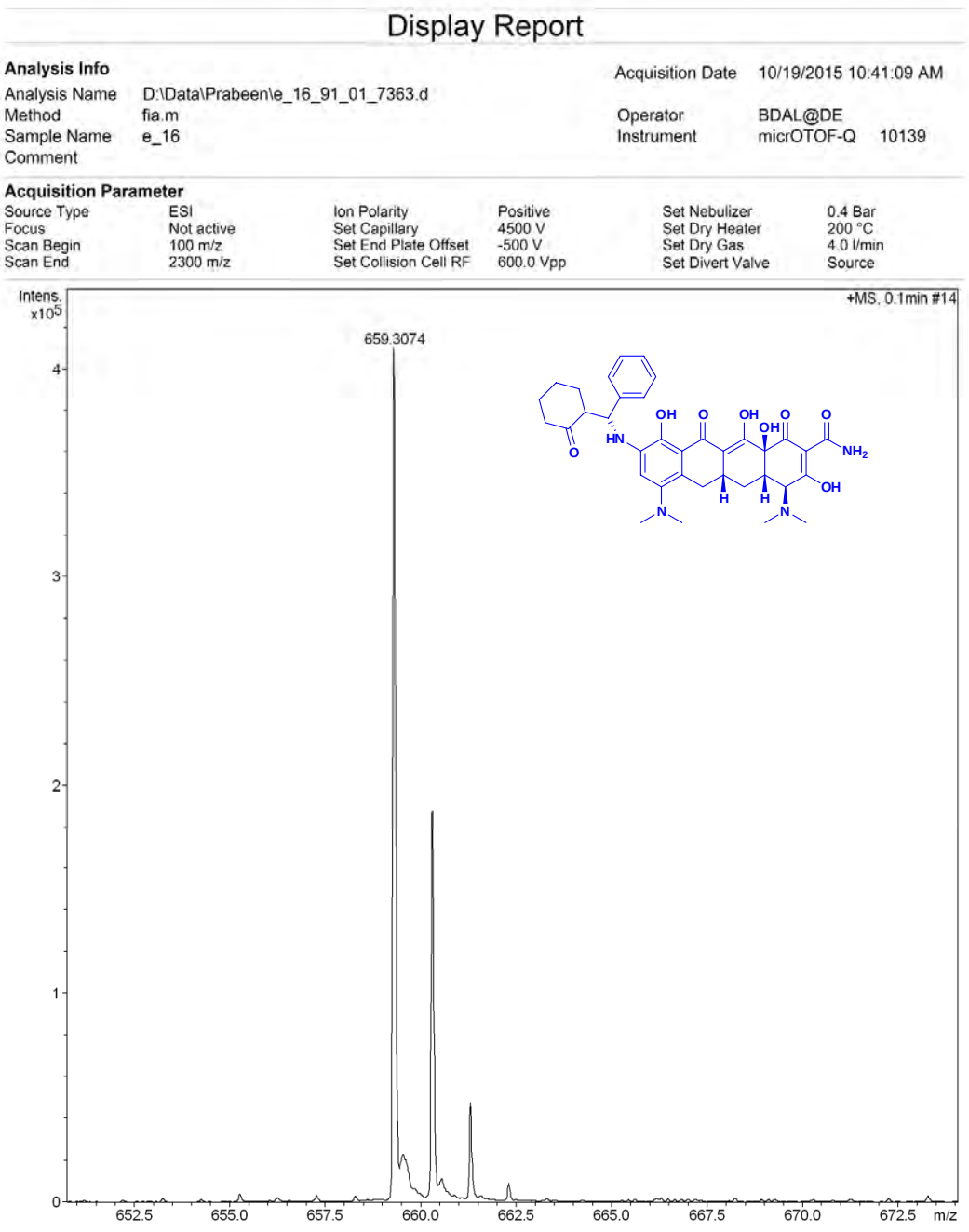
HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-((1R)-(2-oxocyclohexyl)(phenyl)methylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1d)



HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(4-nitrophenyl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2a)

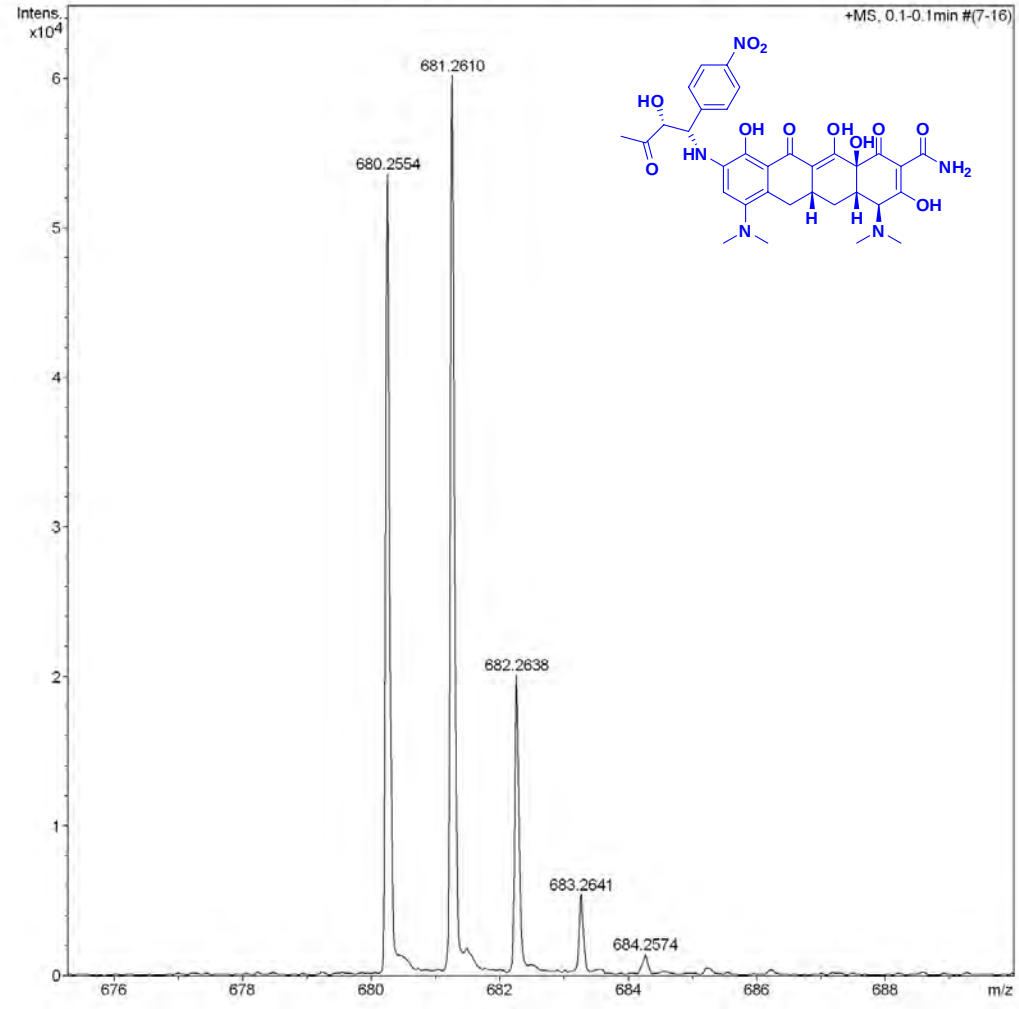
Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E1_51_01_5459.d	Acquisition Date	3/16/2015 1:13:05 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E1	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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HRMS of (4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)

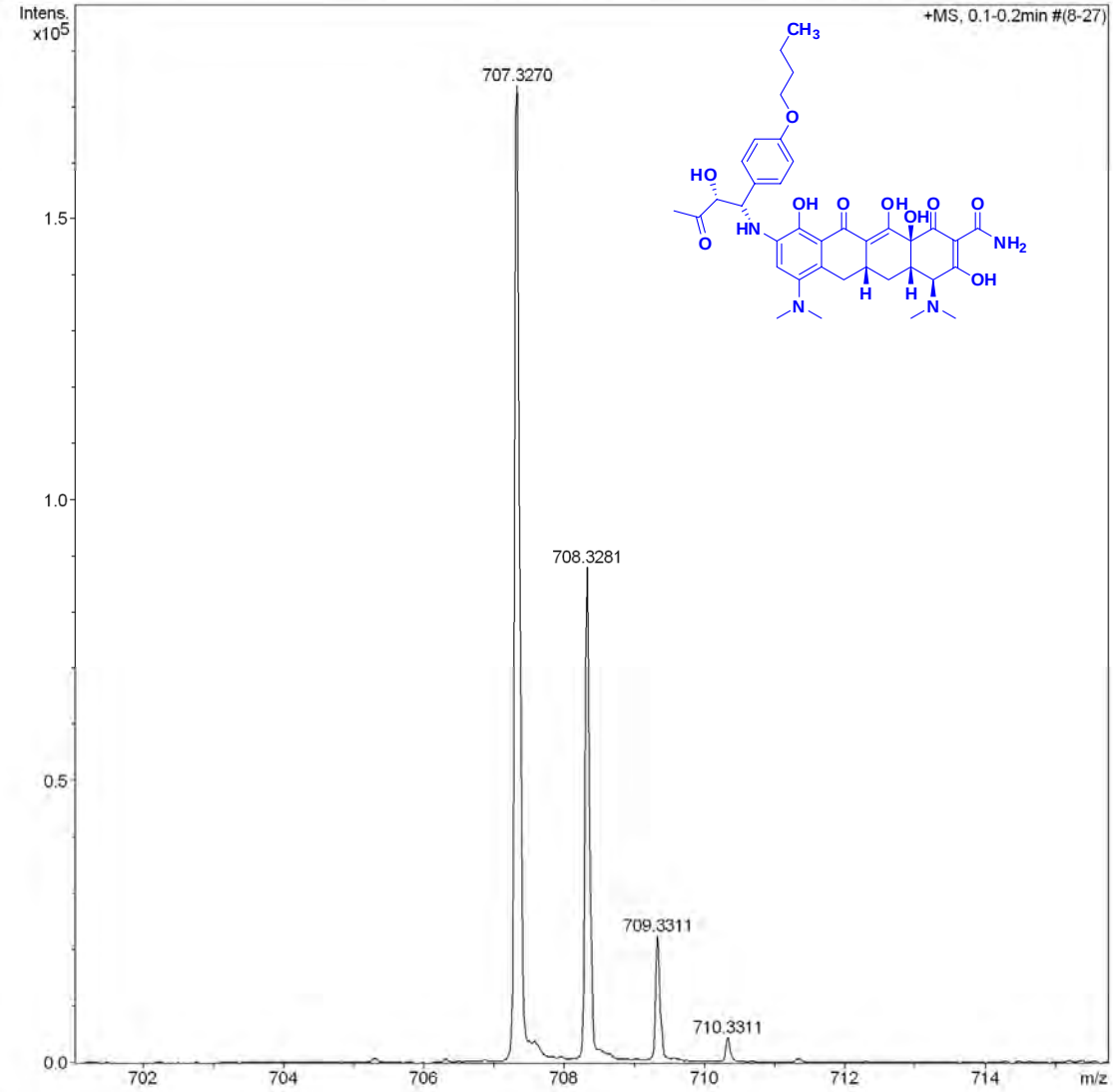
Display Report

Analysis Info

Analysis Name	D:\Data\Rufaro\E5_84_01_5696.d	Acquisition Date	4/8/2015 9:32:50 AM
Method	fia.m	Operator	BDAL@DE
Sample Name	E5	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Bruker Compass DataAnalysis 4.0 printed: 4/30/2015 4:32:43 PM Page 1 of 1

HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-3-oxo-1-o-tolylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2c)

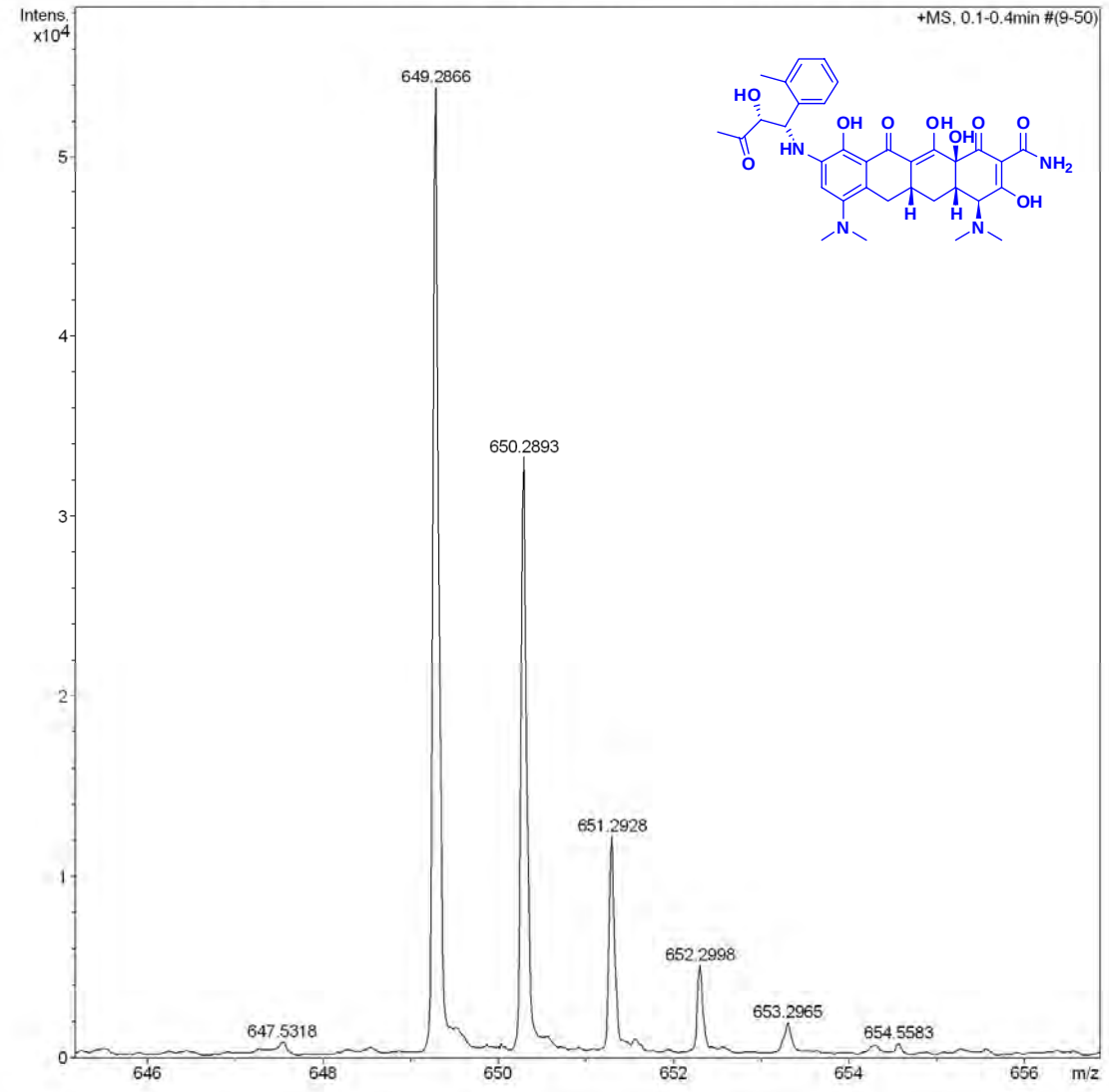
Display Report

Analysis Info

Analysis Name	D:\Data\New Folder\E6_62_01_5470.d	Acquisition Date	3/19/2015 9:07:55 AM
Method	fia.m	Operator	BDAL@DE
Sample Name	E6	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Bruker Compass DataAnalysis 4.0 printed: 6/1/2015 11:44:13 AM Page 1 of 1

HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)

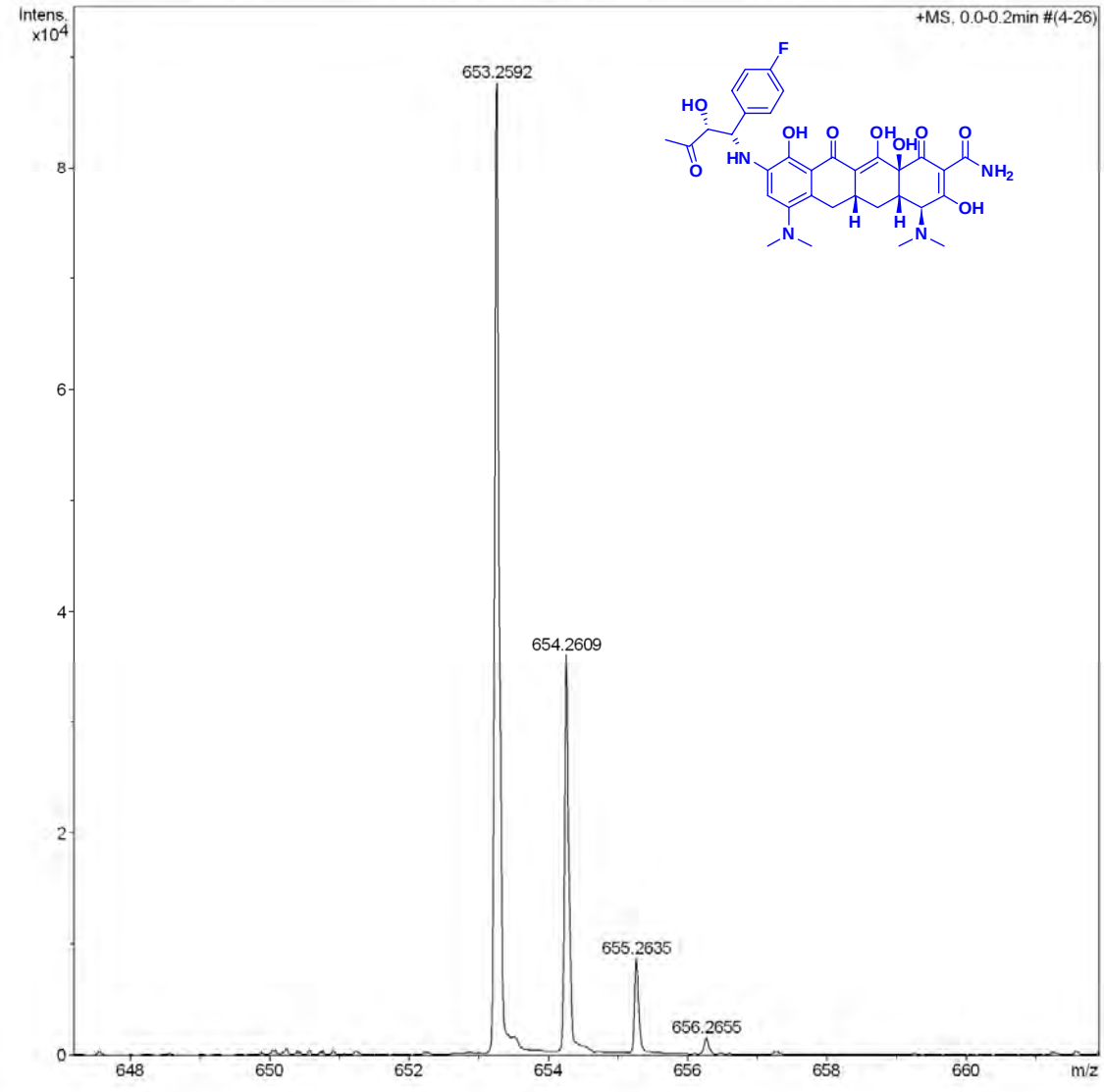
Display Report

Analysis Info

Analysis Name	D:\Data\Rufaro\E8_85_01_5698.d	Acquisition Date	4/8/2015 9:38:51 AM
Method	fia.m	Operator	BDAL@DE
Sample Name	E8	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Bruker Compass DataAnalysis 4.0 printed: 4/30/2015 4:33:18 PM Page 1 of 1

HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(naphthalen-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2e)

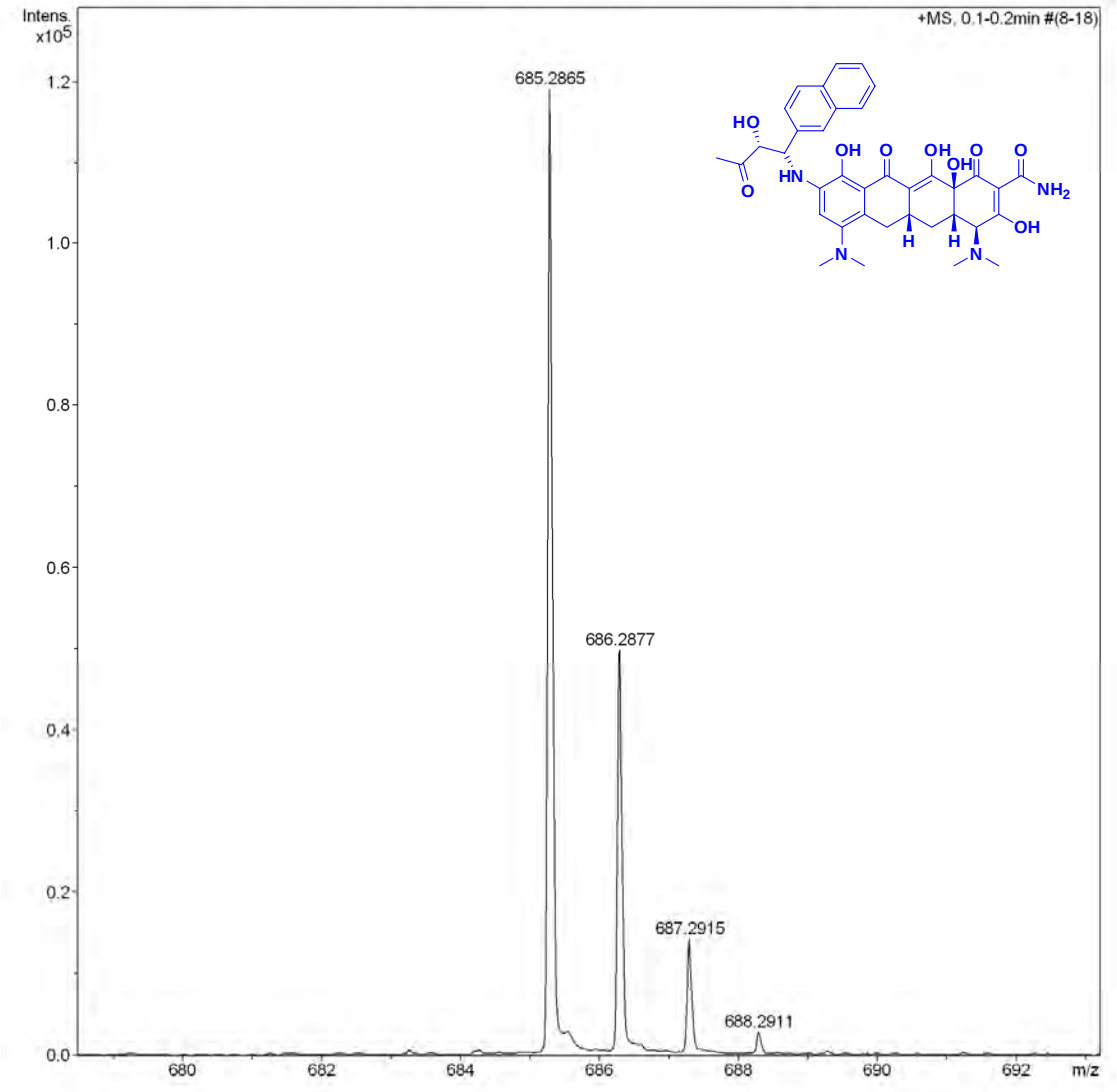
Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E9_53_01_5463.d	Acquisition Date	3/16/2015 1:25:13 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E9	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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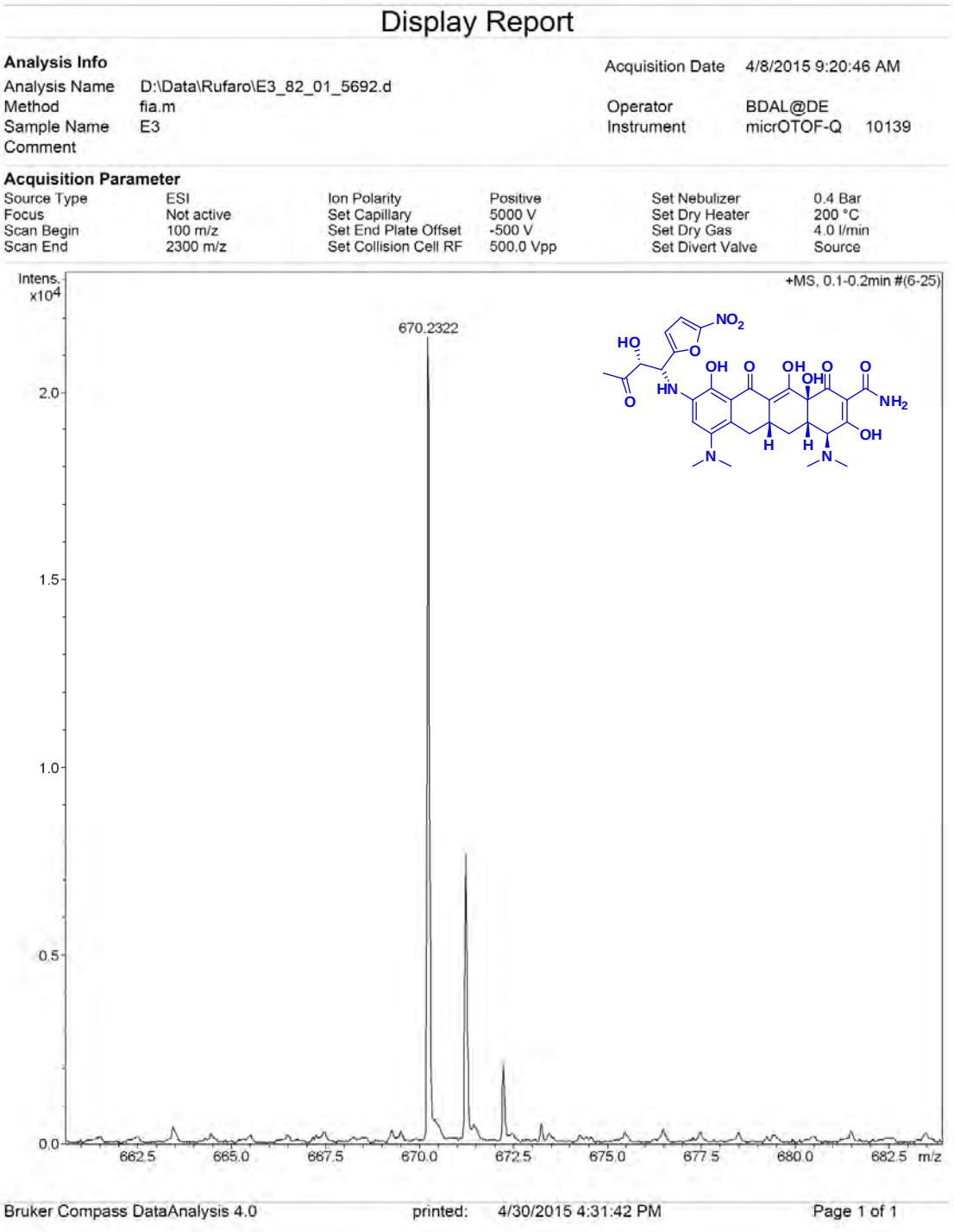
HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1R,2R)-2-hydroxy-1-(5-nitrofuran-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12 octahydrotetracene-2-carboxamide (2f)



HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1R,2R)-1-(furan-2-yl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2g)

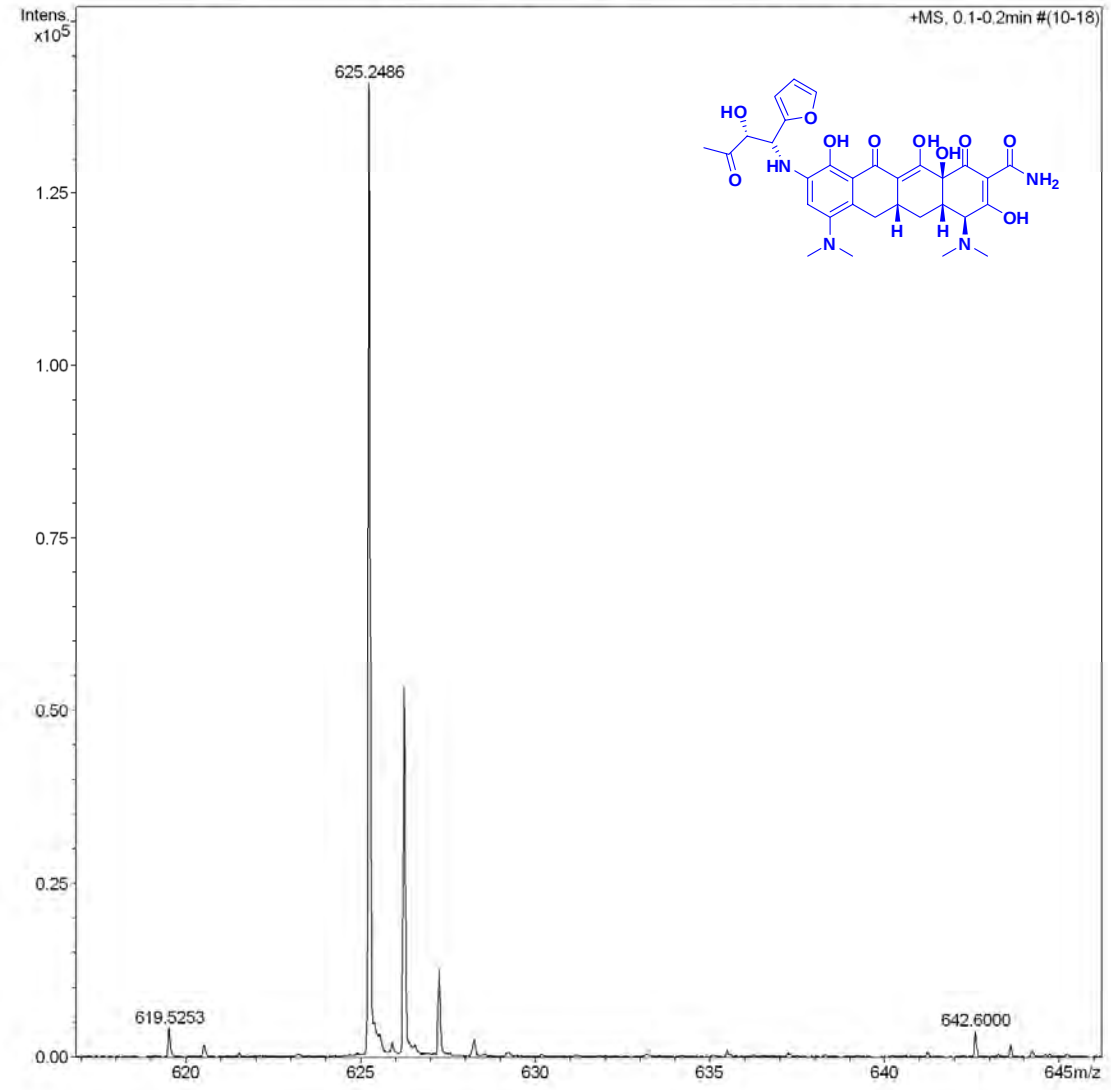
Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E7_52_01_5461.d	Acquisition Date	3/16/2015 1:19:08 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E7	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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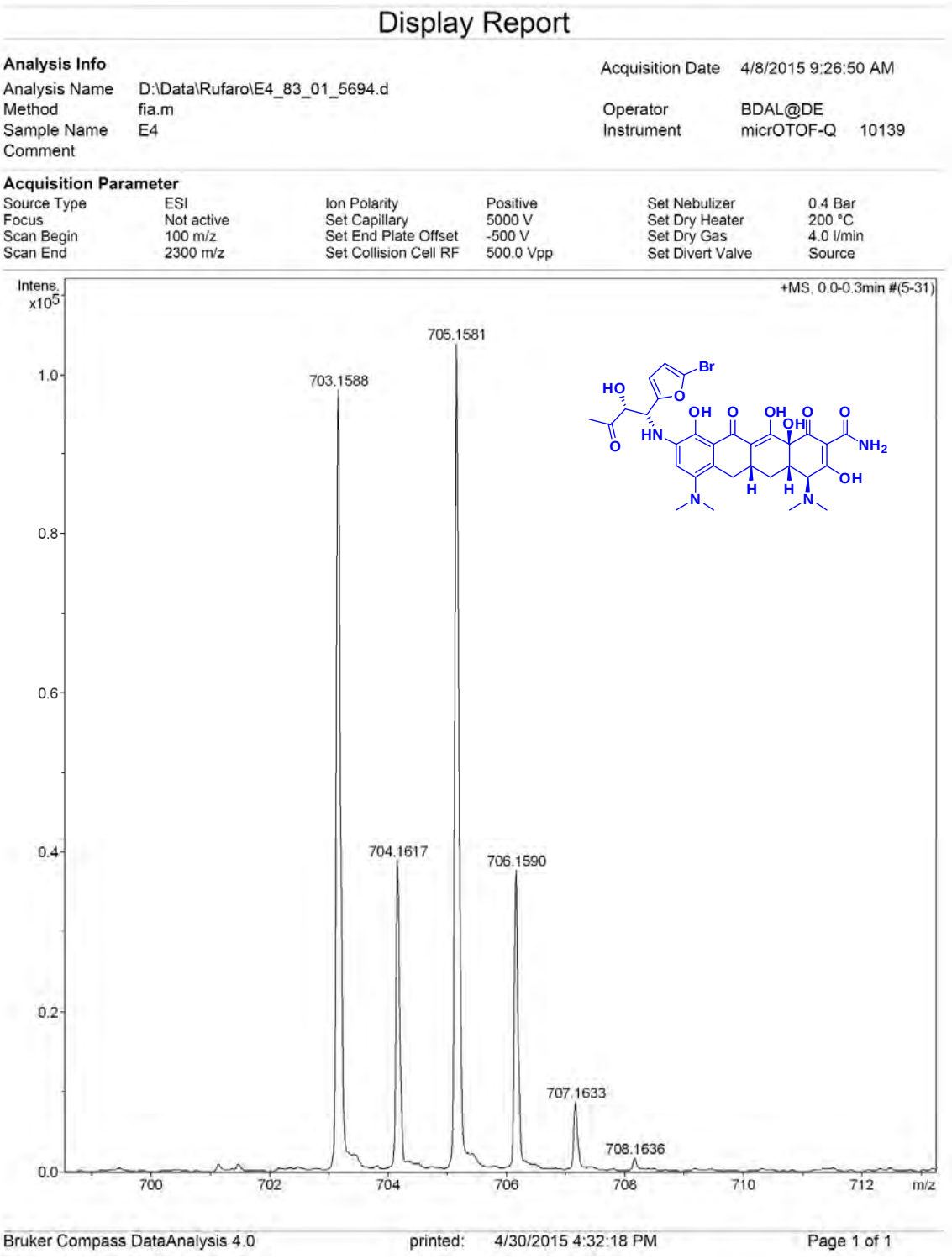
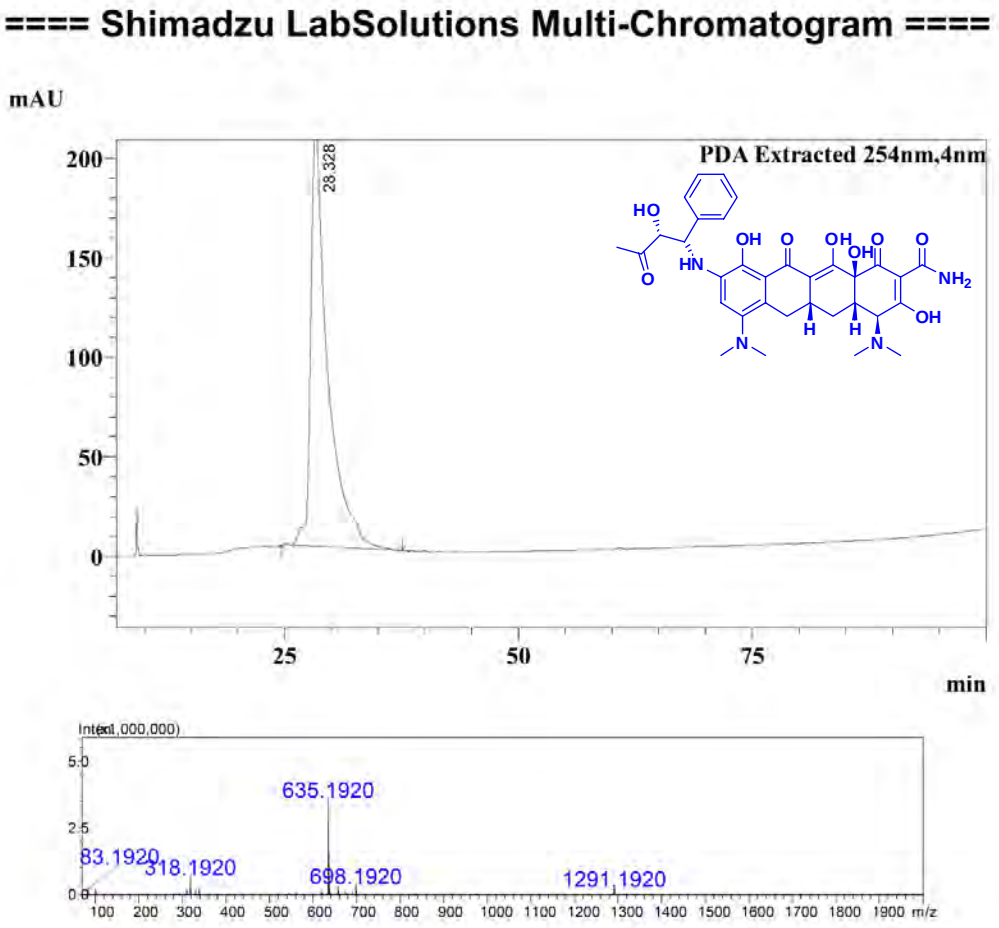
HRMS of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)

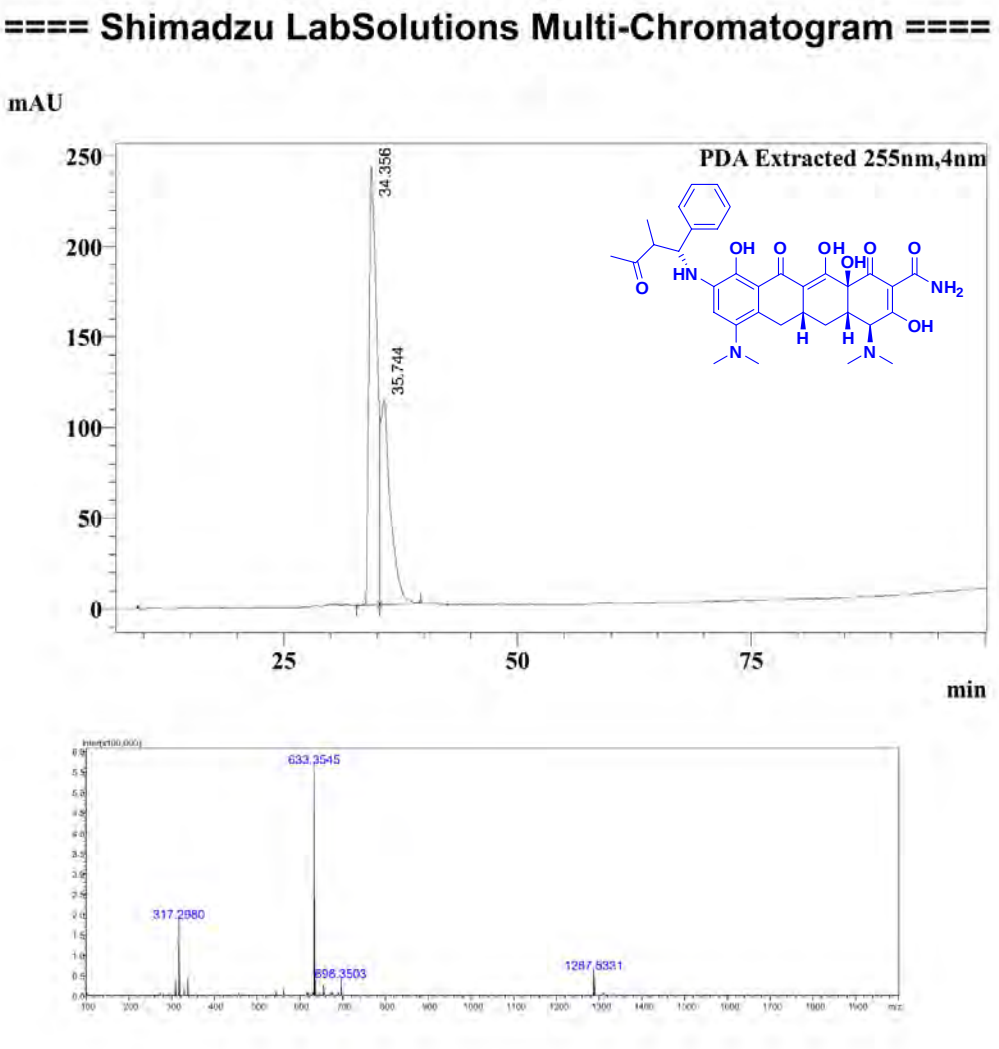


2. Copies of LC-MS spectra for products

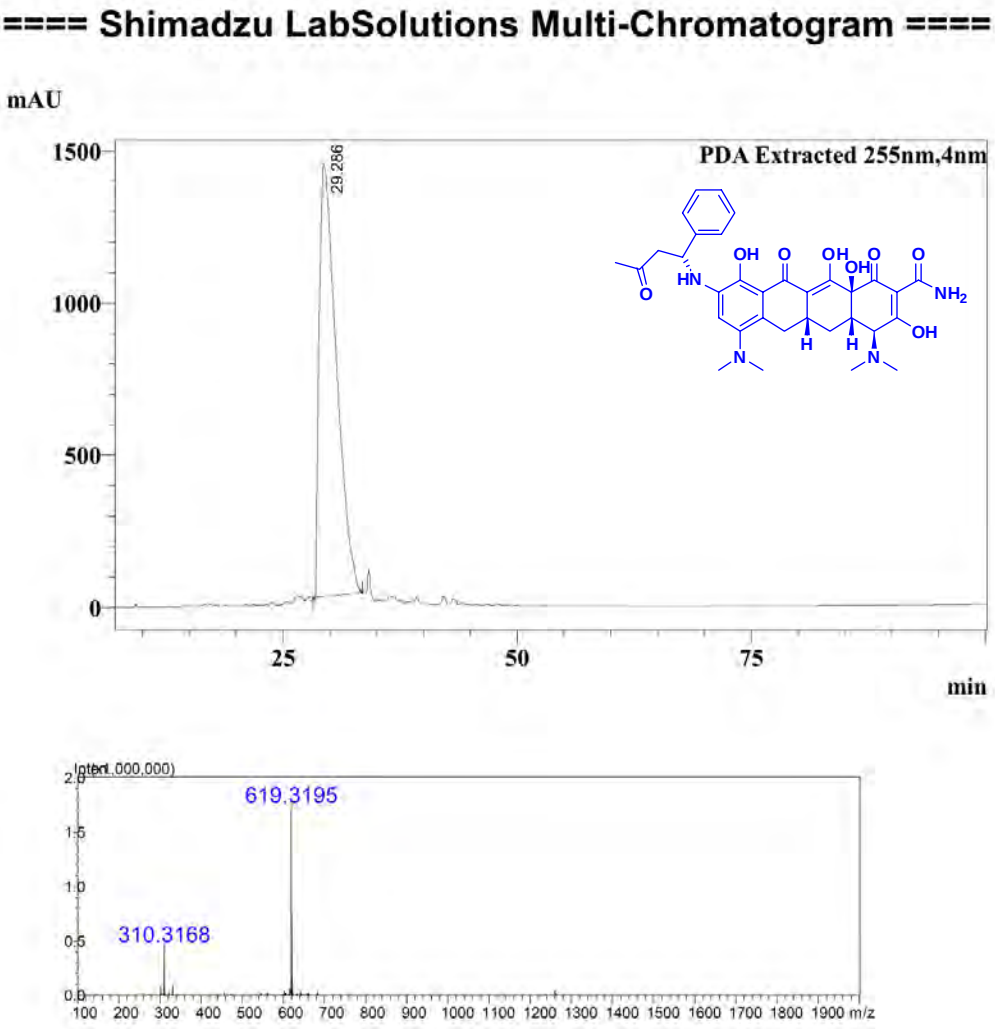
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)



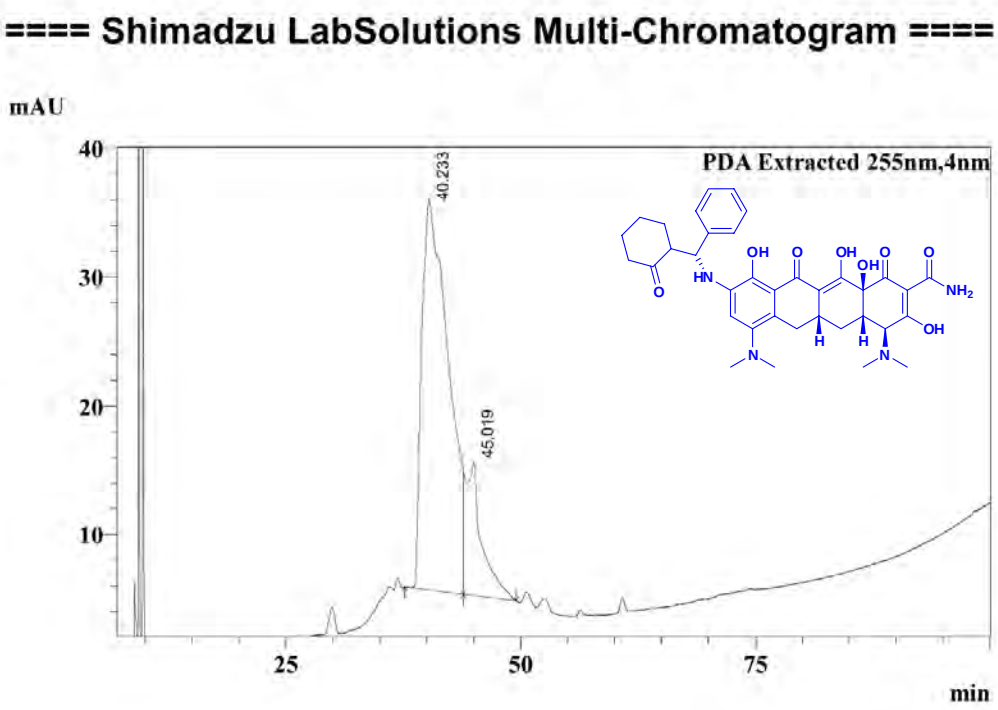
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)

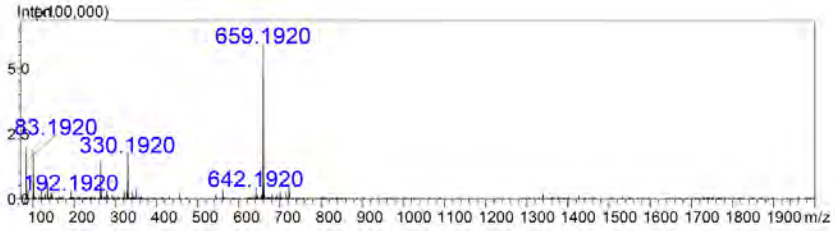


LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-(3-oxo-1-phenylbutylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1c)

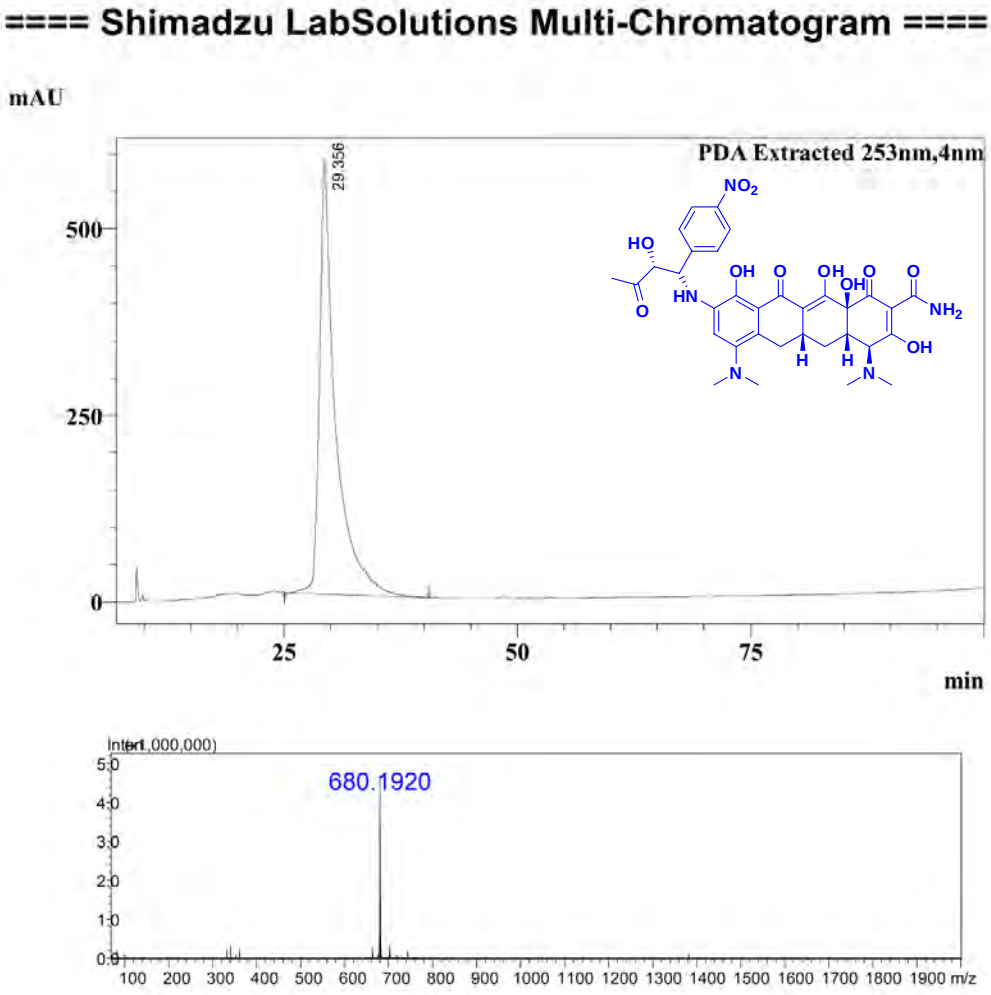


LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-((1R)-(2-oxocyclohexyl)(phenyl)methylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1d)

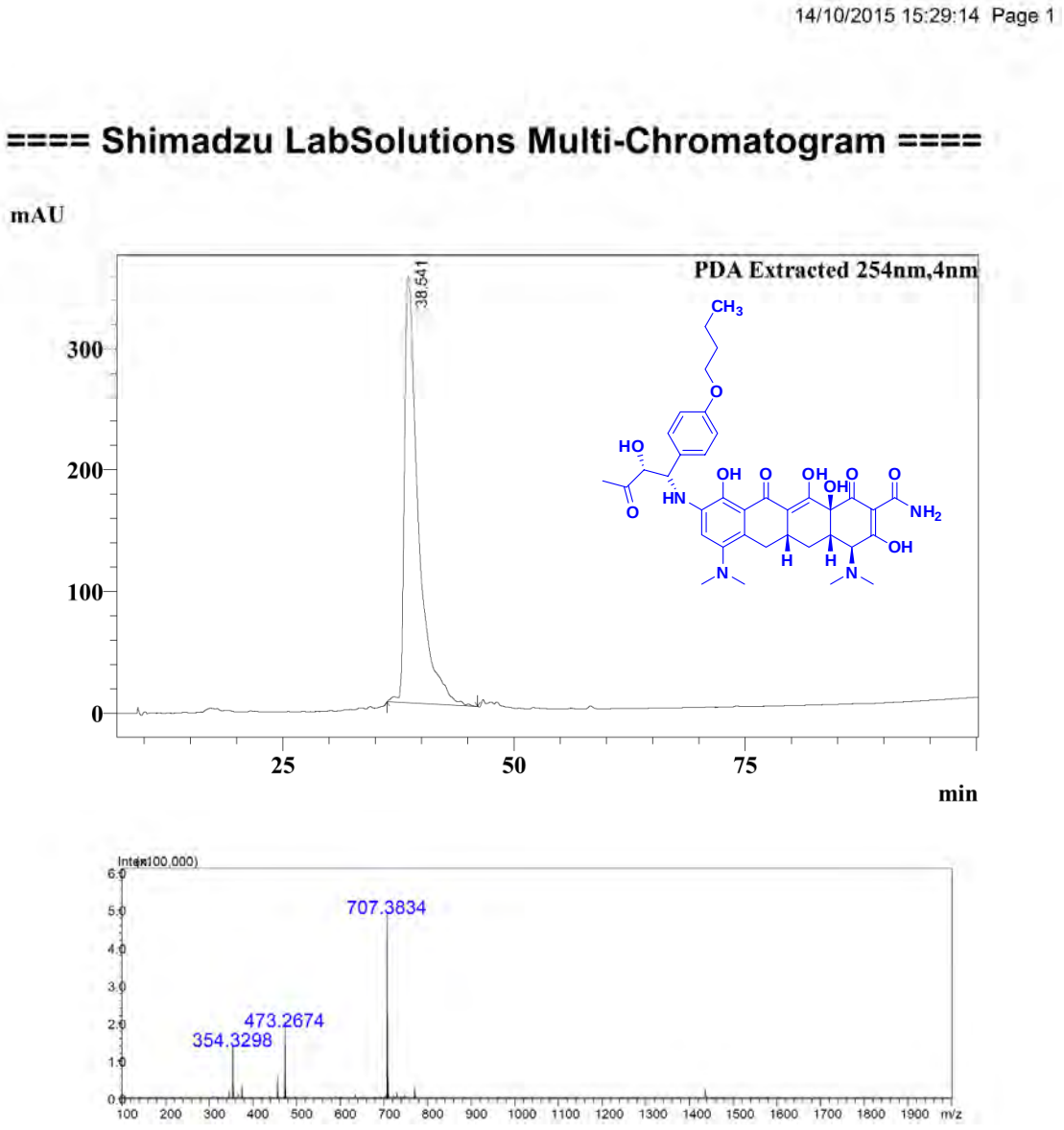




LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(4-nitrophenyl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2a)



LC-MS of (4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)



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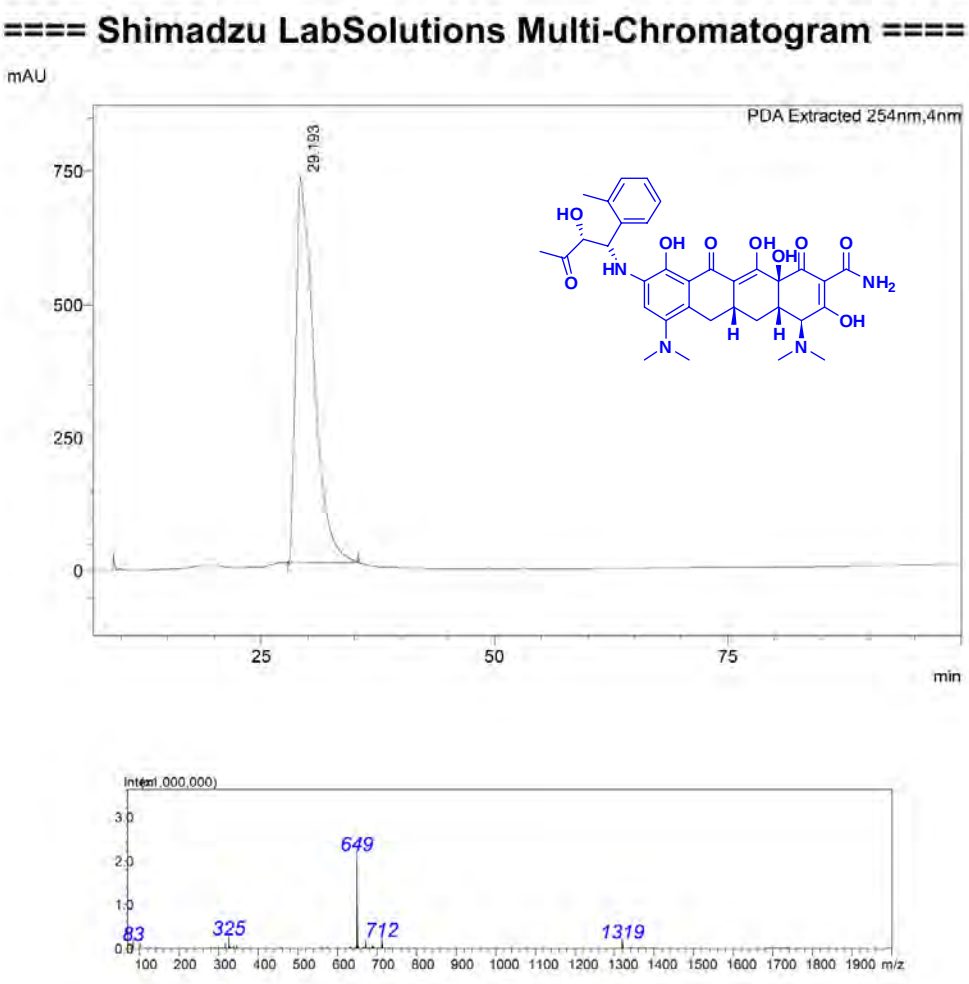
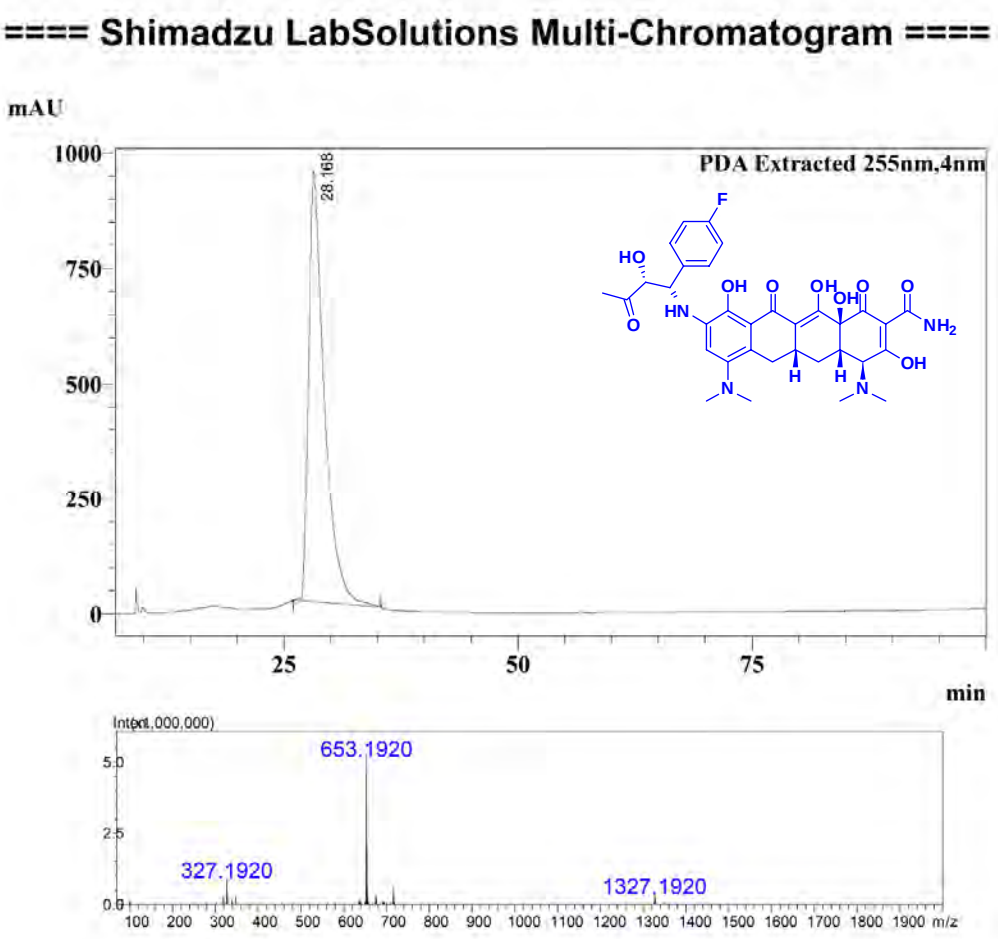
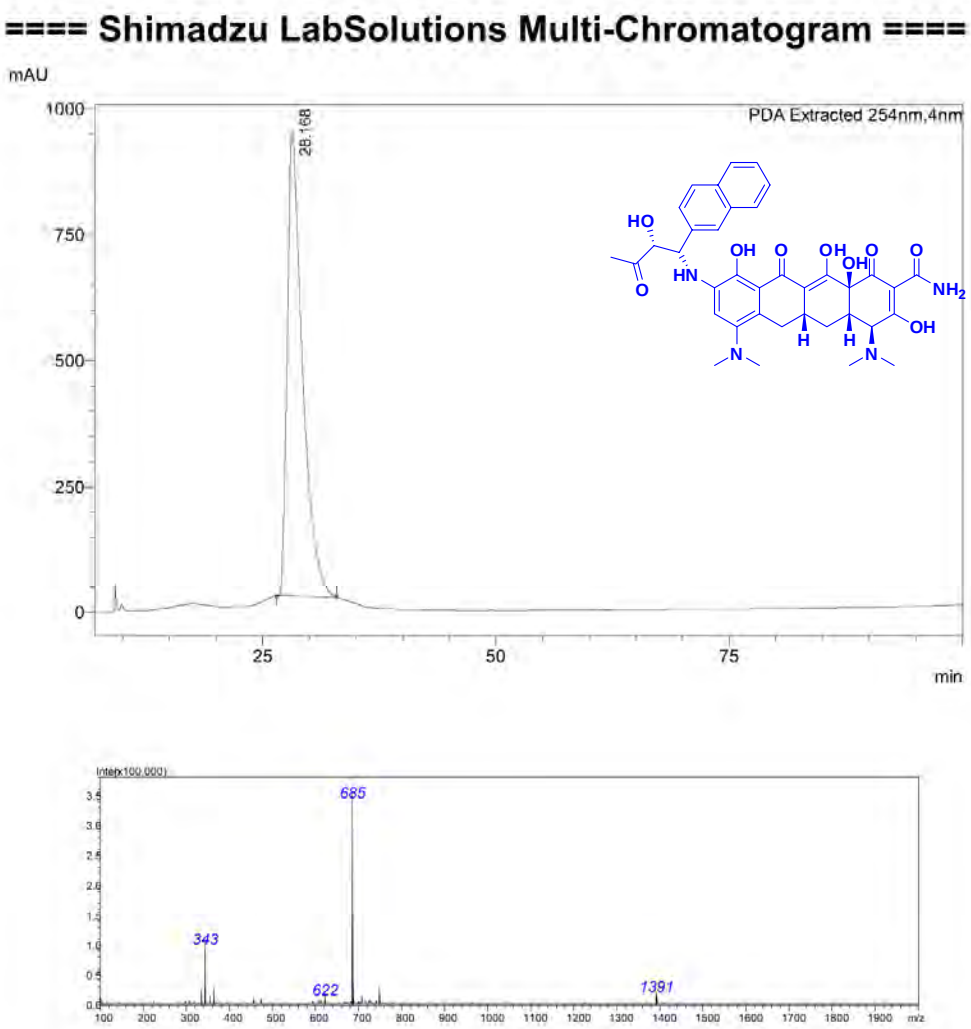


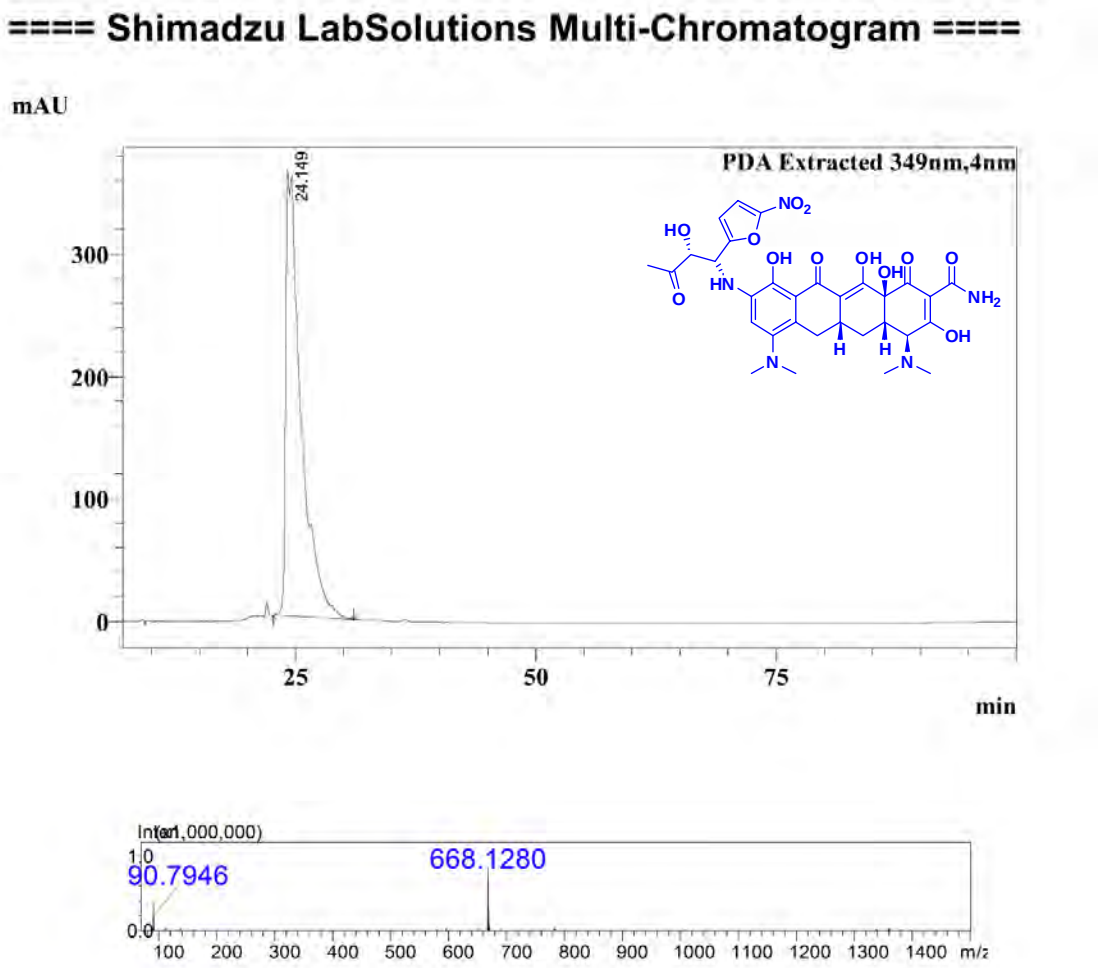
LC-MS SPECTRUM of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)



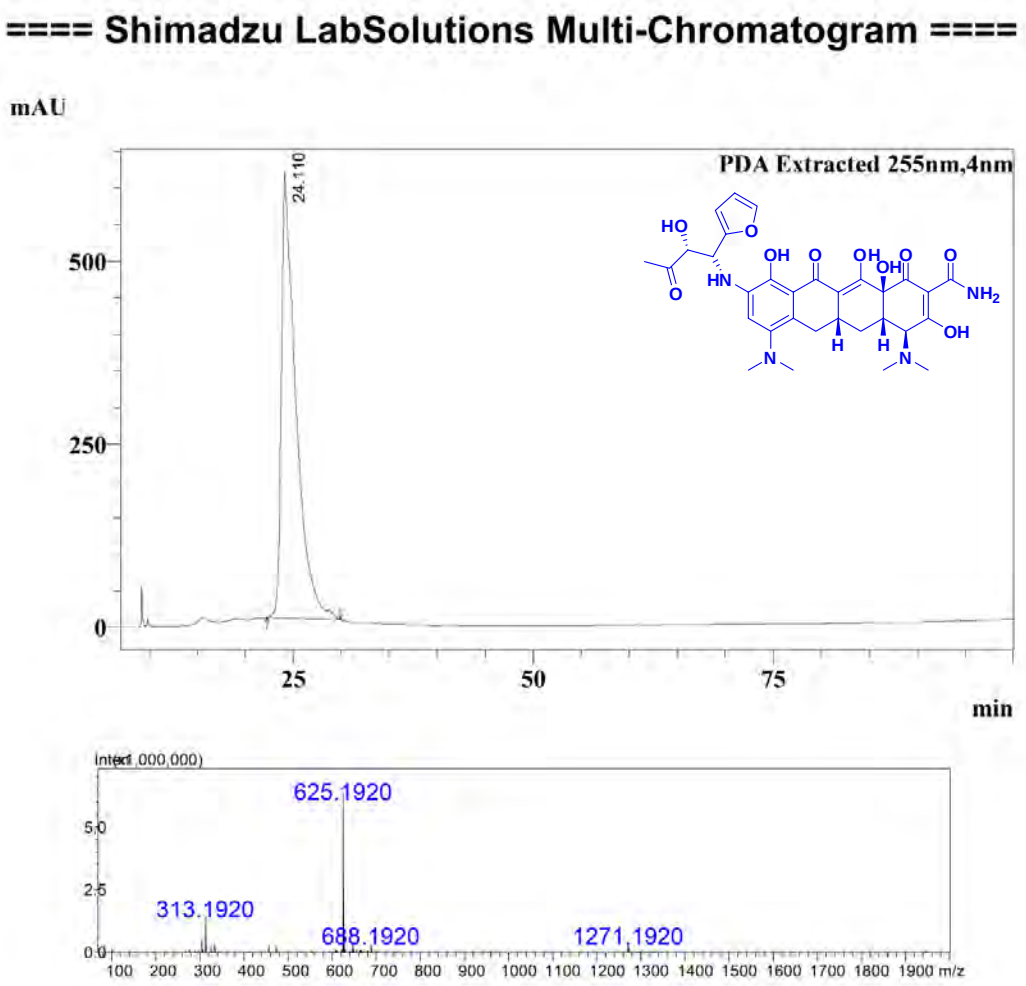
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(naphthalen-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2e)



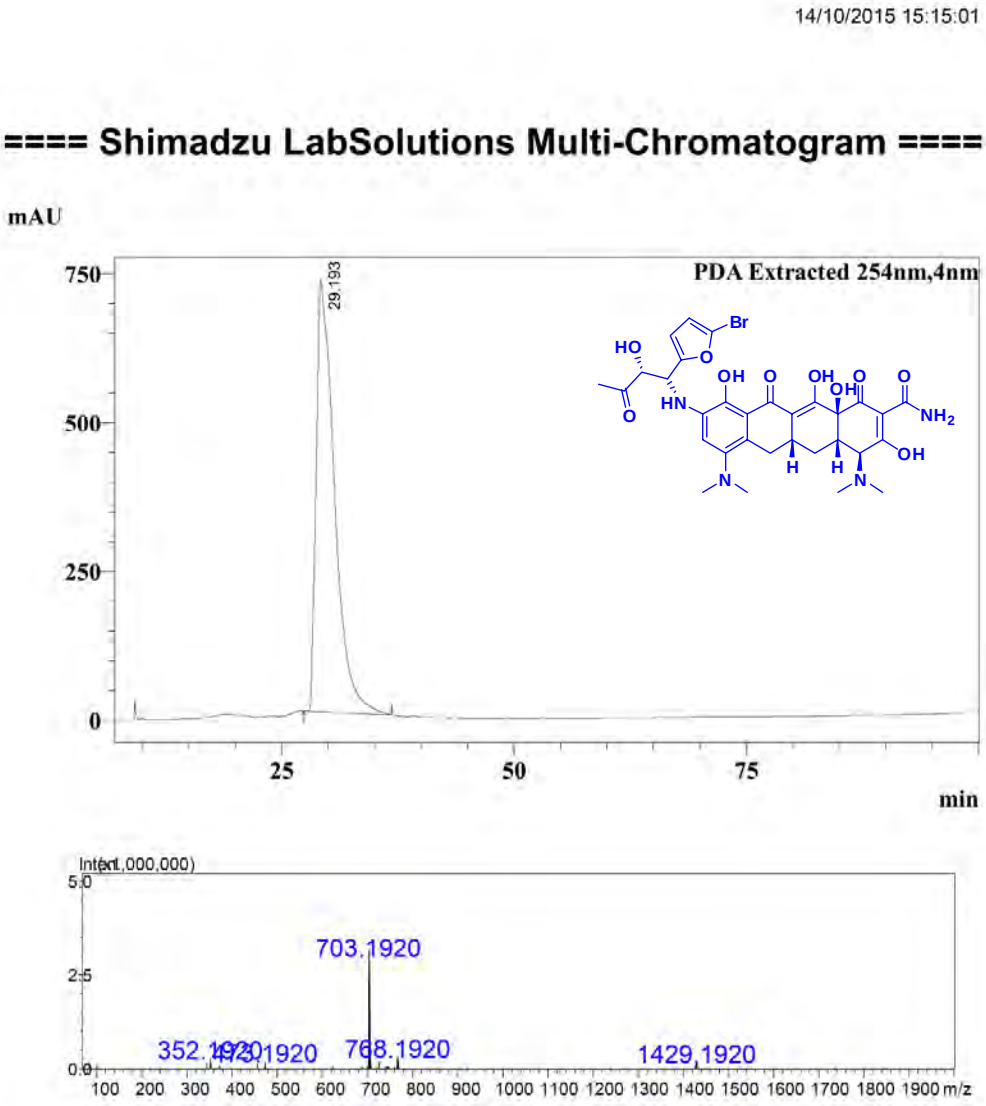
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1R,2R)-2-hydroxy-1-(5-nitrofuran-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12 octahydrotetracene-2-carboxamide (2f)



LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1R,2R)-1-(furan-2-yl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2g)

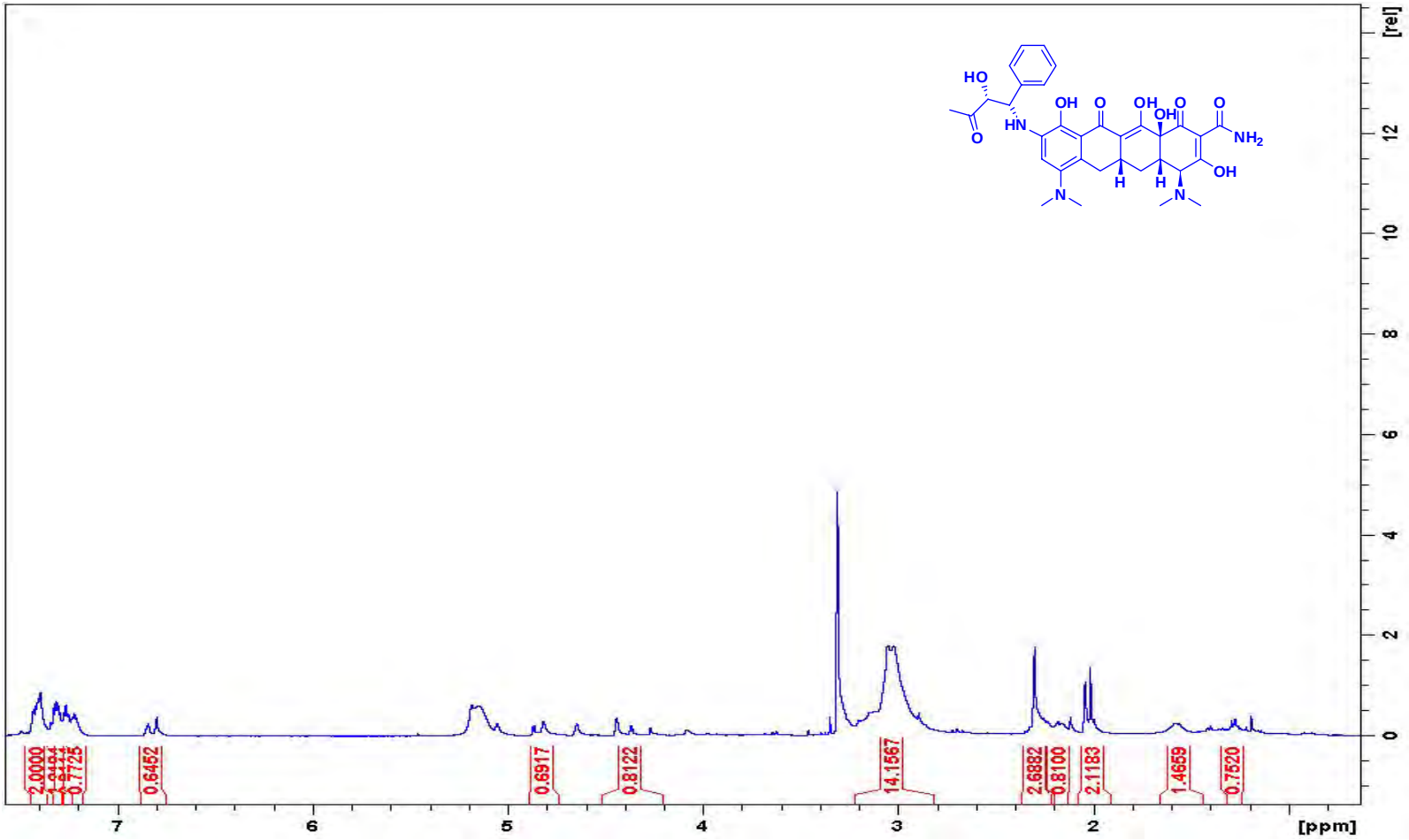


LC-MS of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)

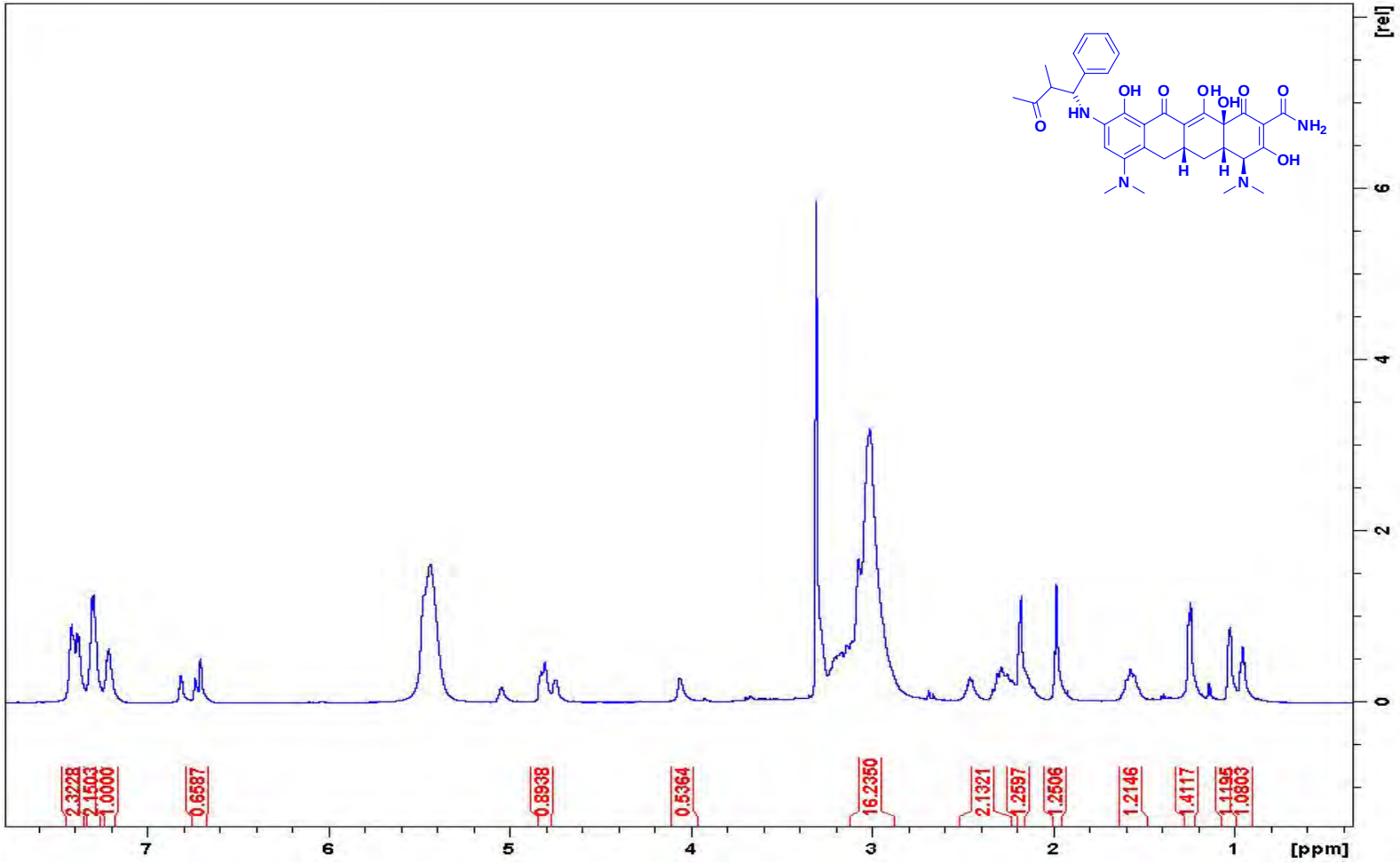


3. Copies of NMR spectra for products

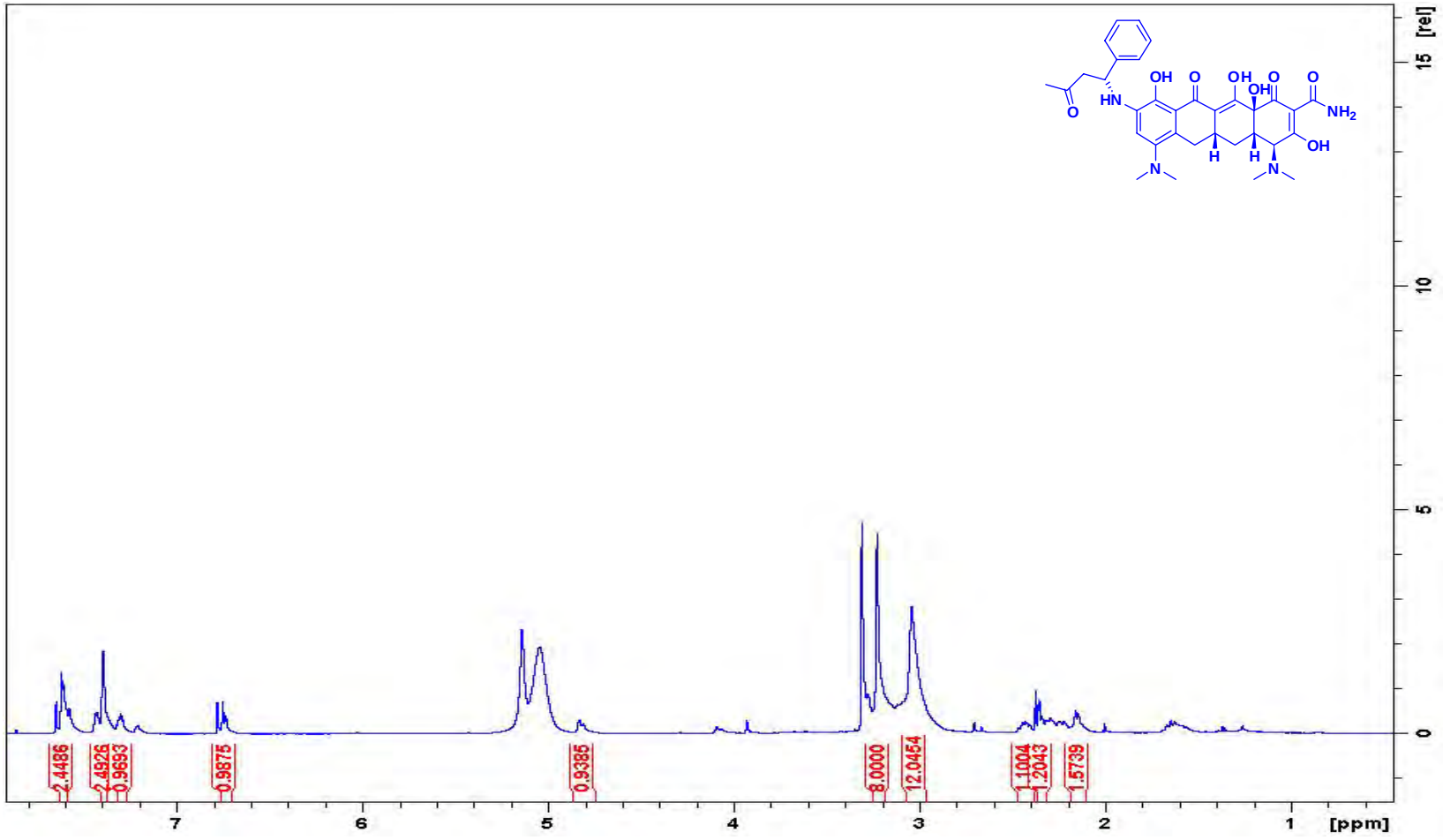
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)



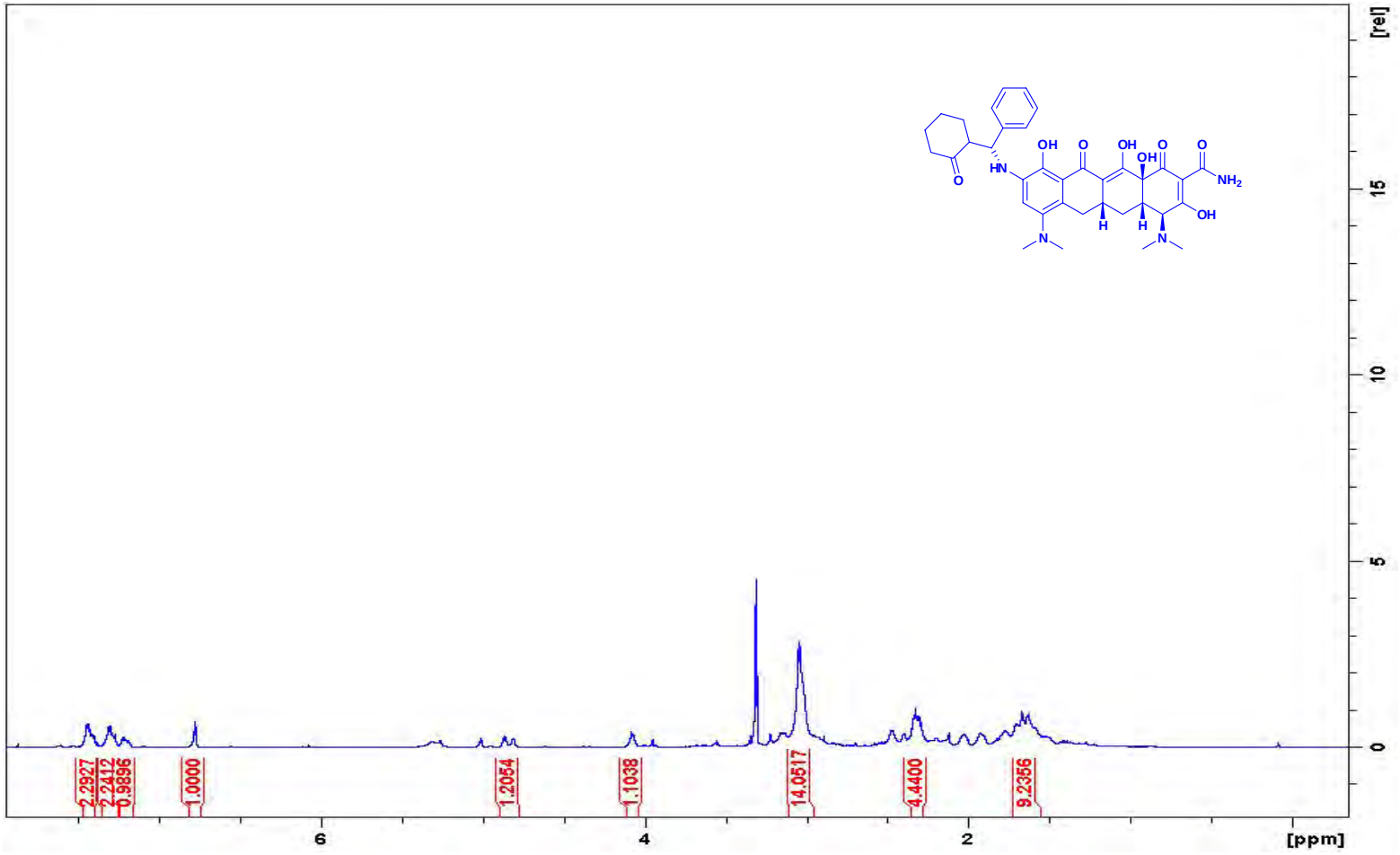
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)



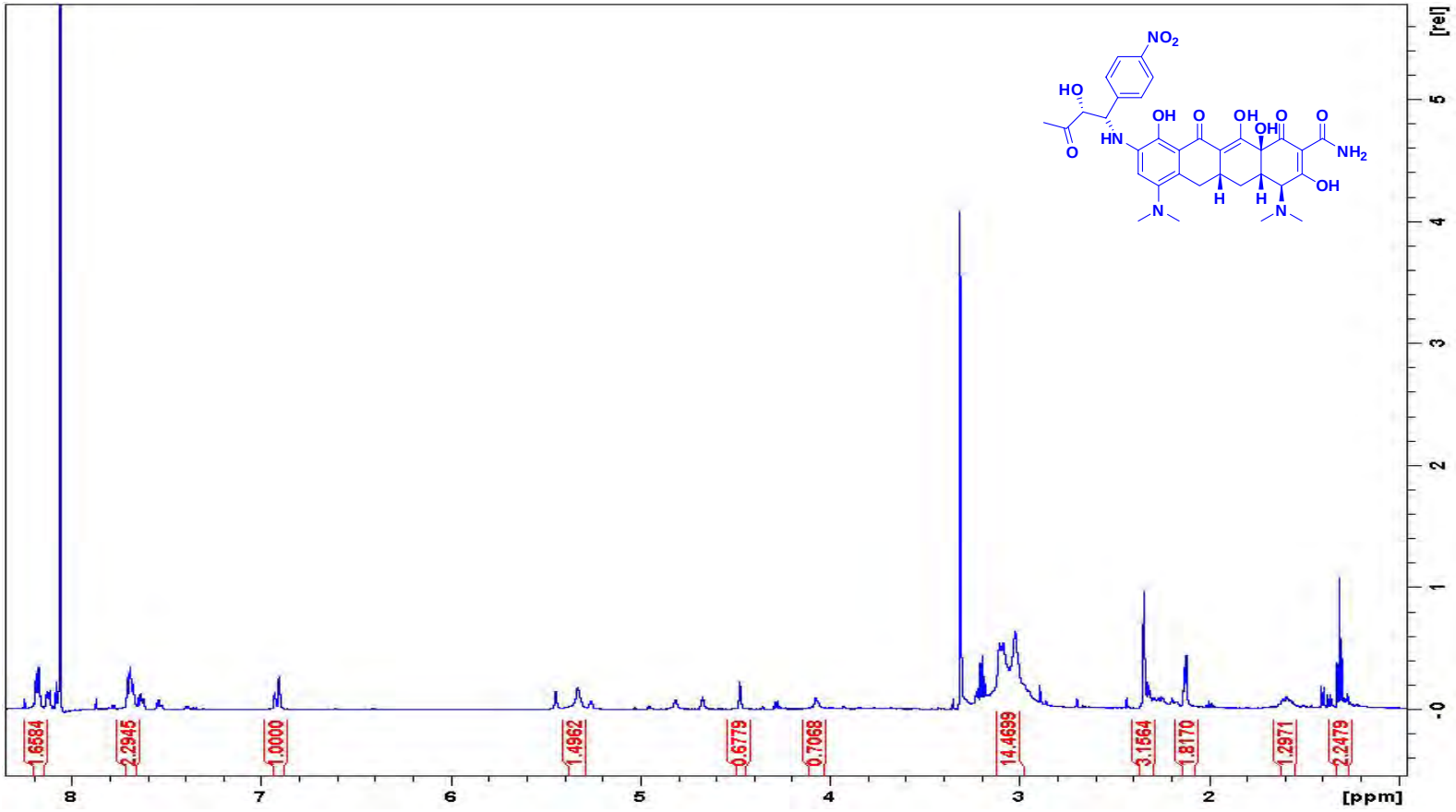
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-(3-oxo-1-phenylbutylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1c)



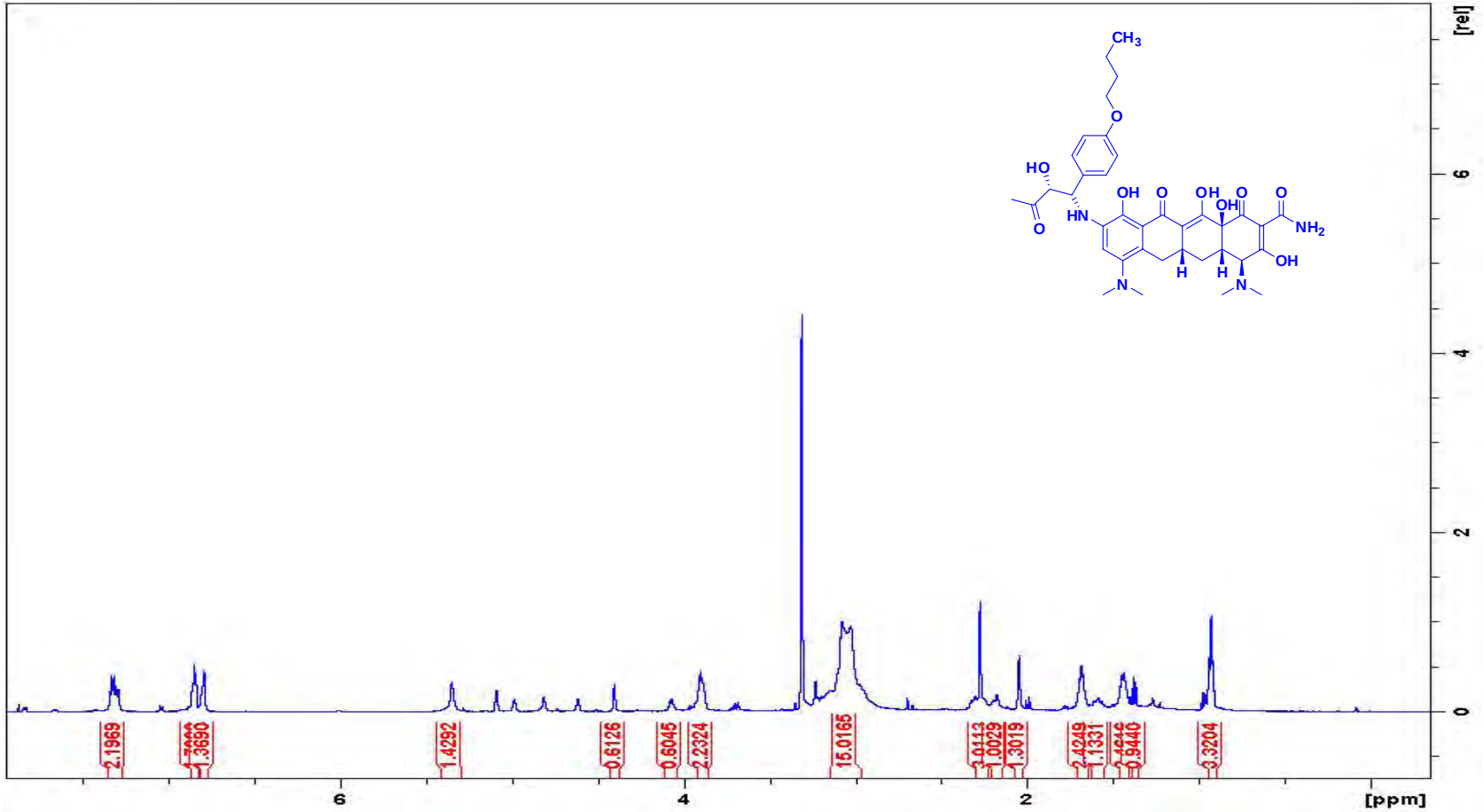
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-((1R)-(2-oxocyclohexyl)(phenyl)methylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1d)



^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(4-nitrophenyl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2a)



^{1}H NMR of (4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)



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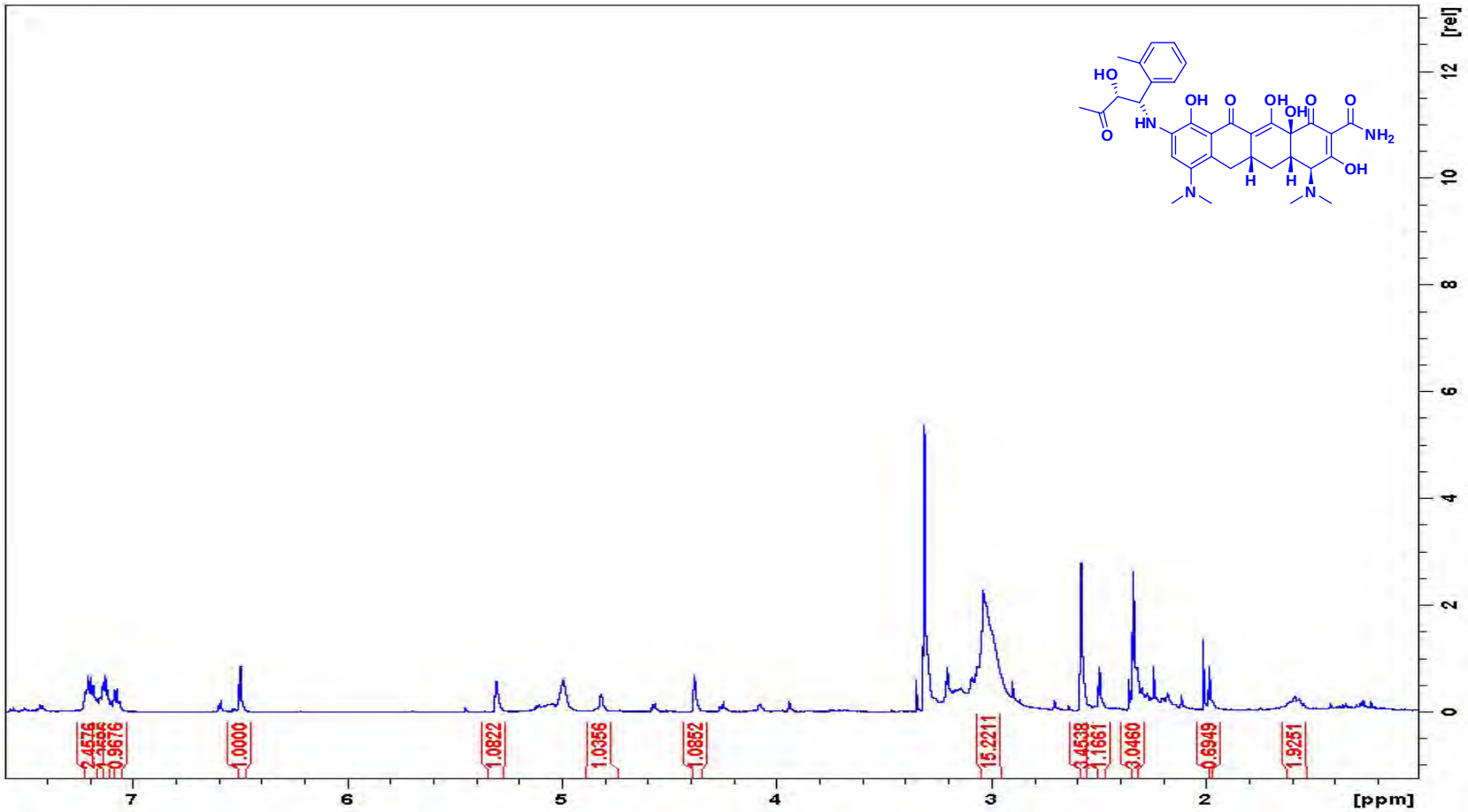
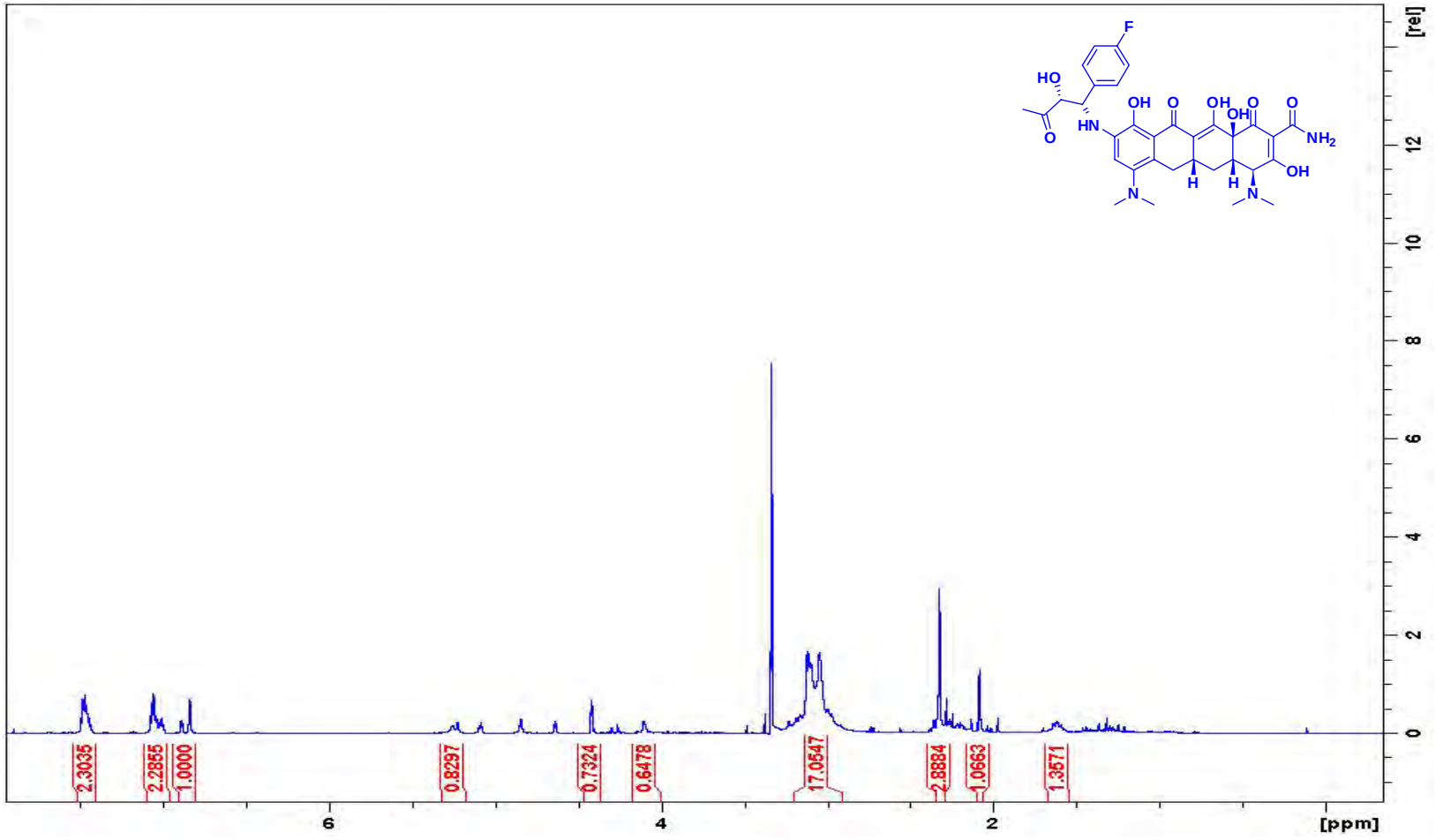


^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)



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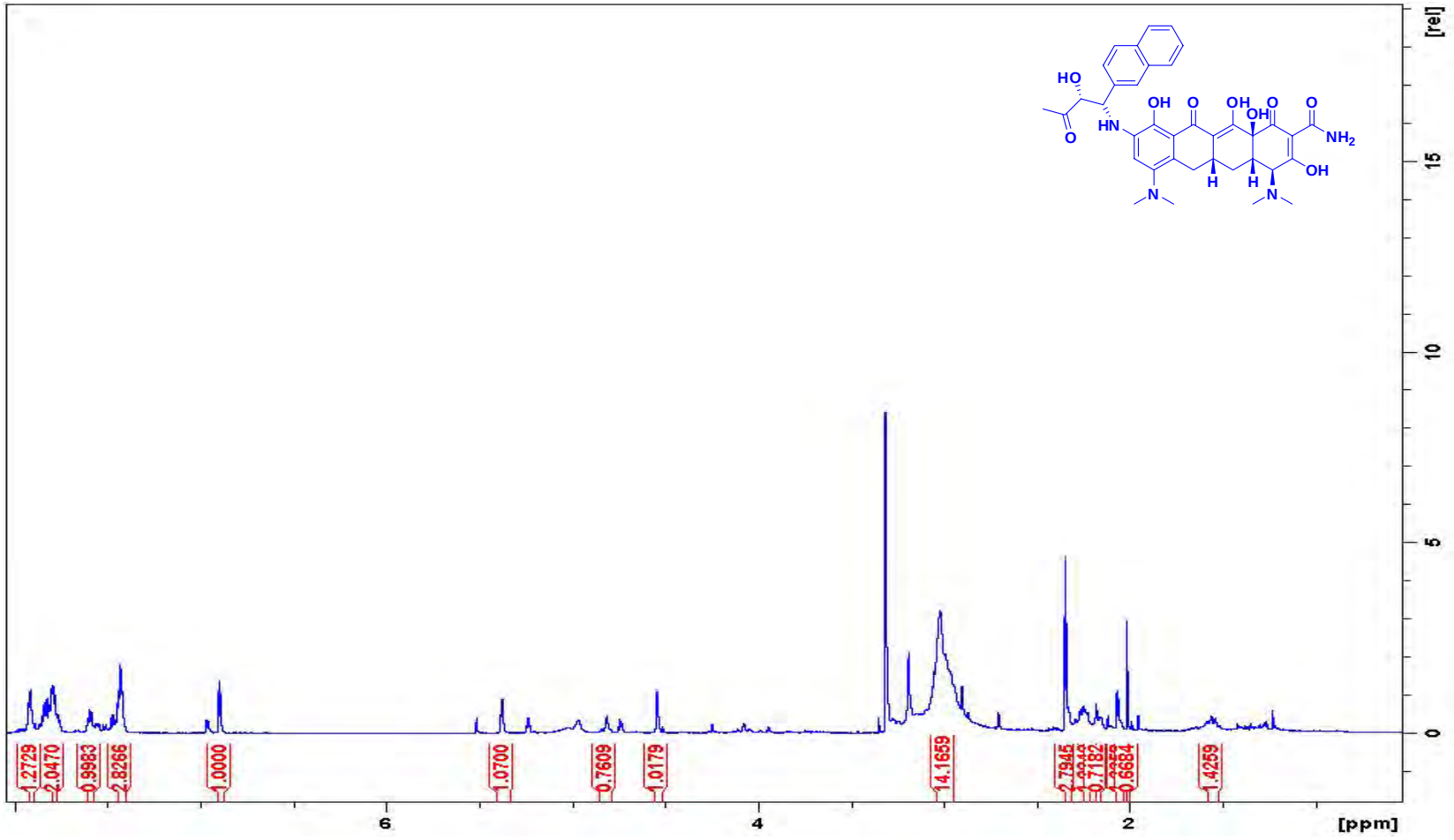

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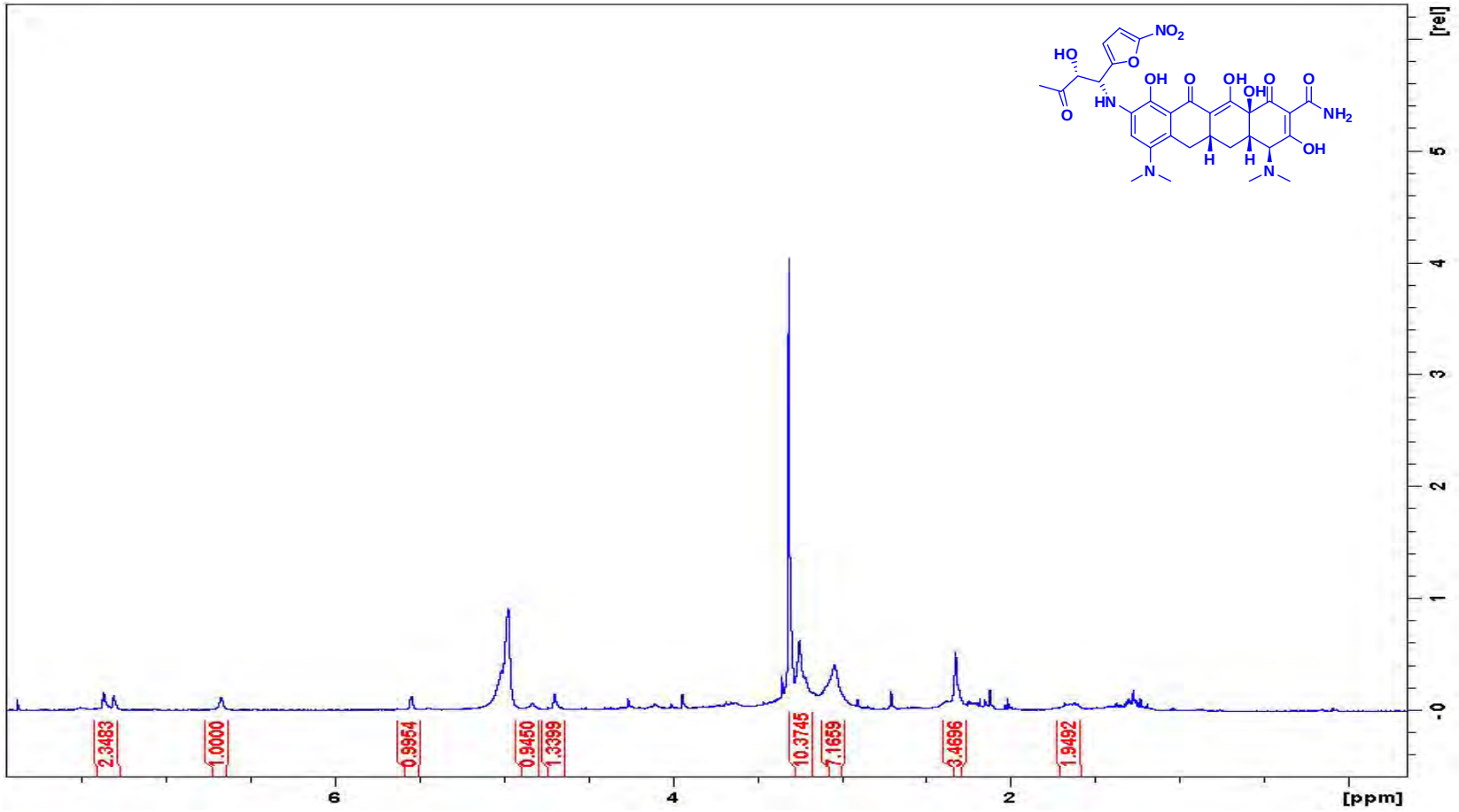

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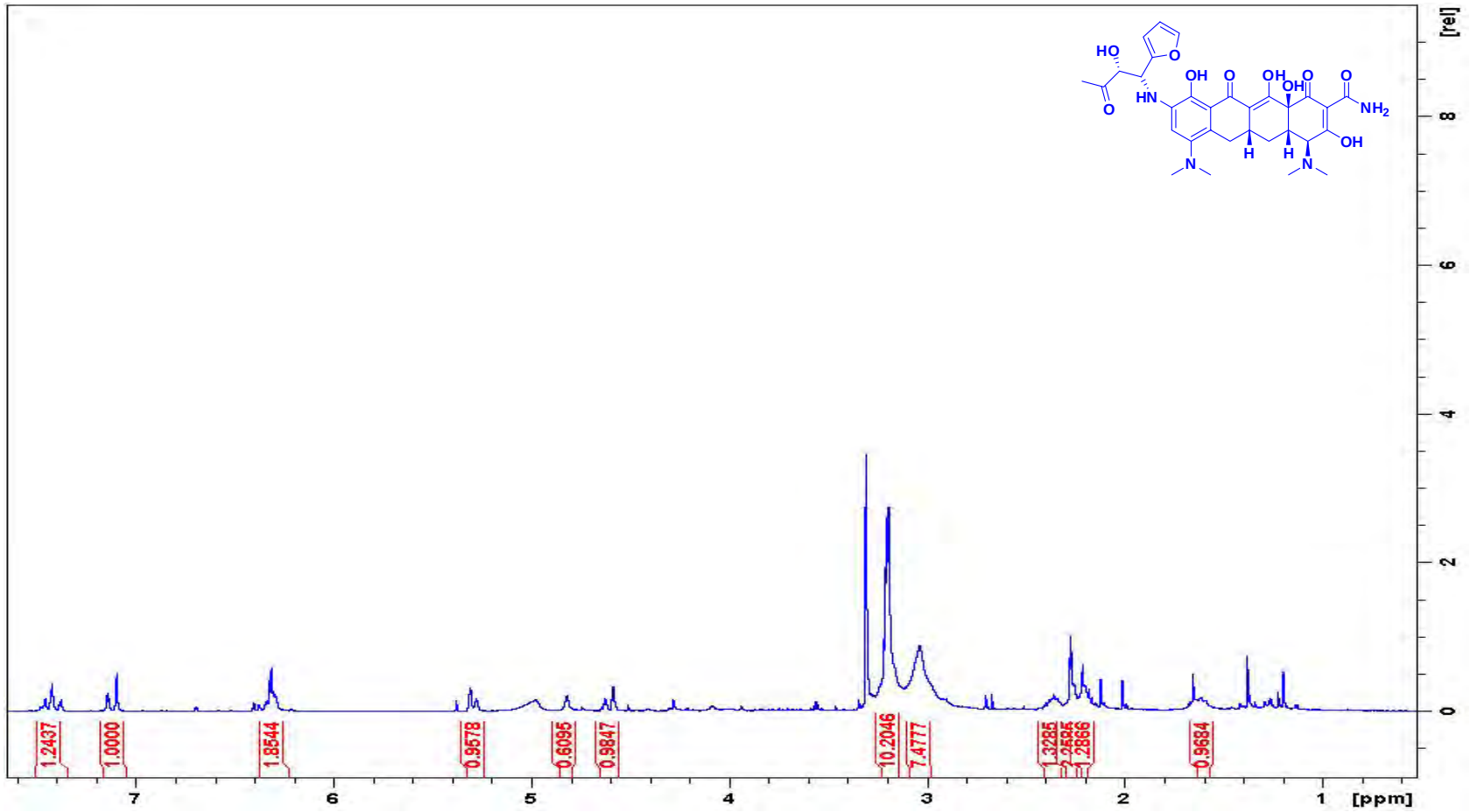
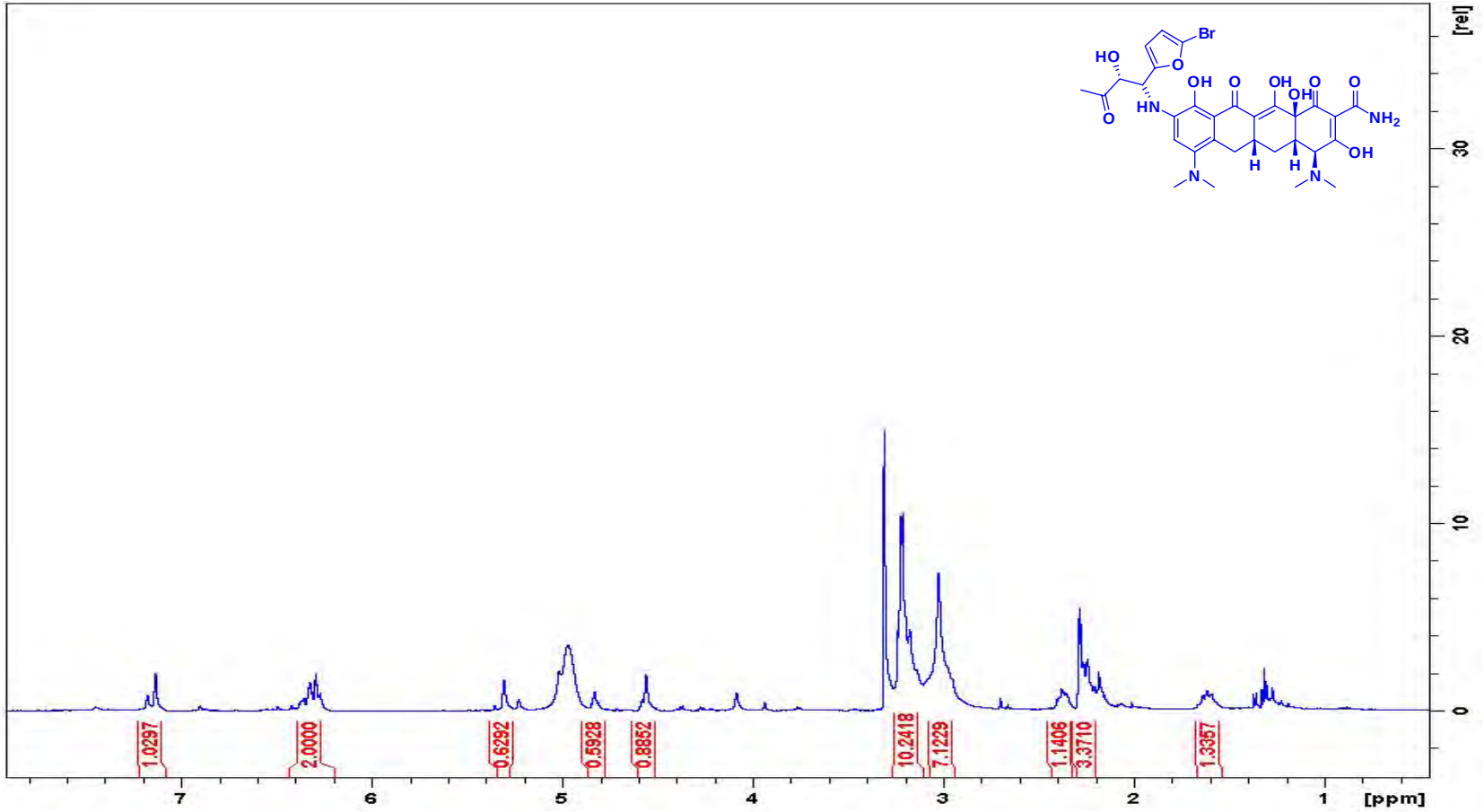


^{1}H NMR of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)



Organocatalyzed Mannich Reactions on Minocycline: Towards Novel Tetracycline Antibiotics

Tirivashe E. Chiwunze[a], Rafiatu Azumah[a], Melissa Ramtahal[a], Per I. Arvidsson[b], Sabiha Y. Essack [c], Hendrik G. Kruger[a], Thavendran Govender[a], Tricia Naicker[a]

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SUPPLEMETARY DATA

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1. Copies of HRMS spectra for products

HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)

Display Report

Analysis Info

Analysis Name	D:\Data\New Folder\E2_63_01_5473.d	Acquisition Date	3/20/2015 10:48:44 AM
Method	fia.m	Operator	BDAL@DE
Sample Name	E2	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	5000 V	Set Dry Heater	200 °C
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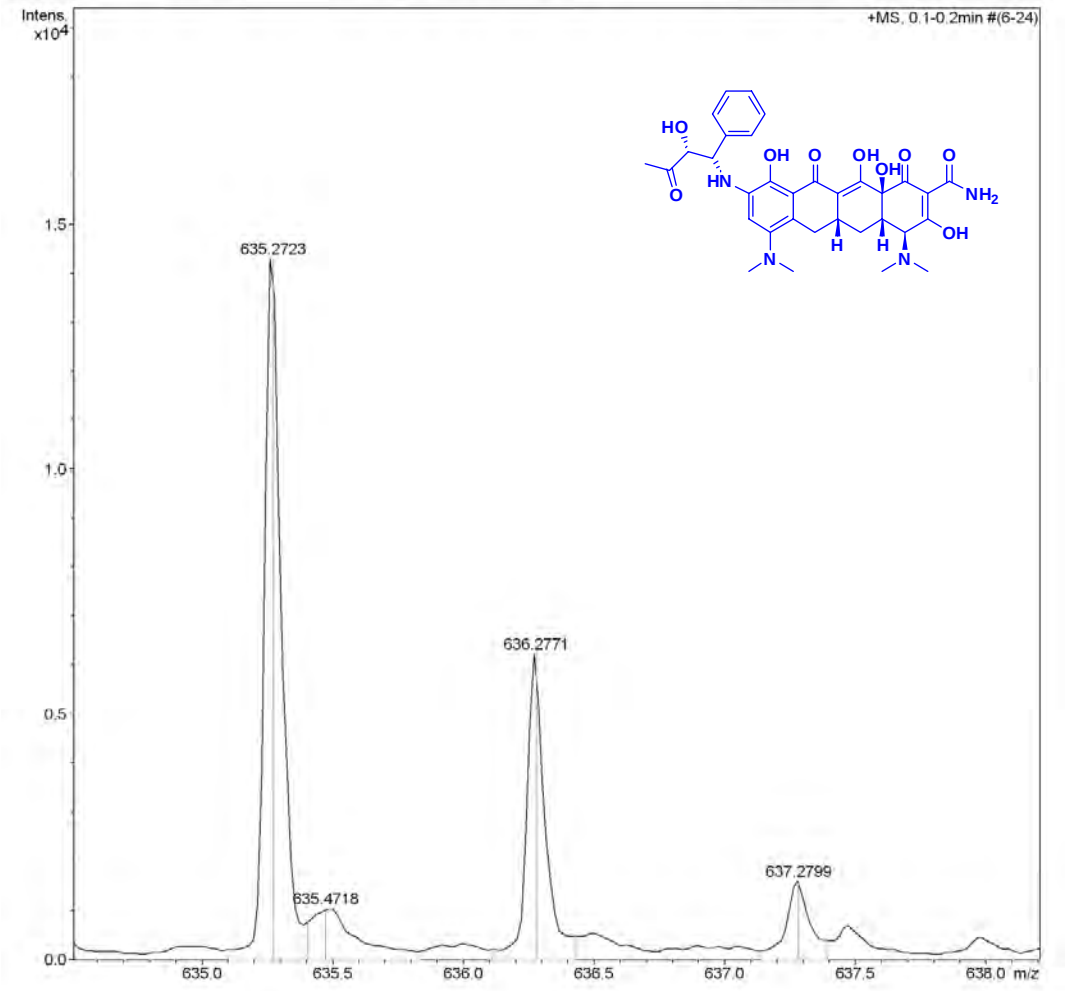
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)

Display Report

Analysis Info

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Sample Name	E12	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

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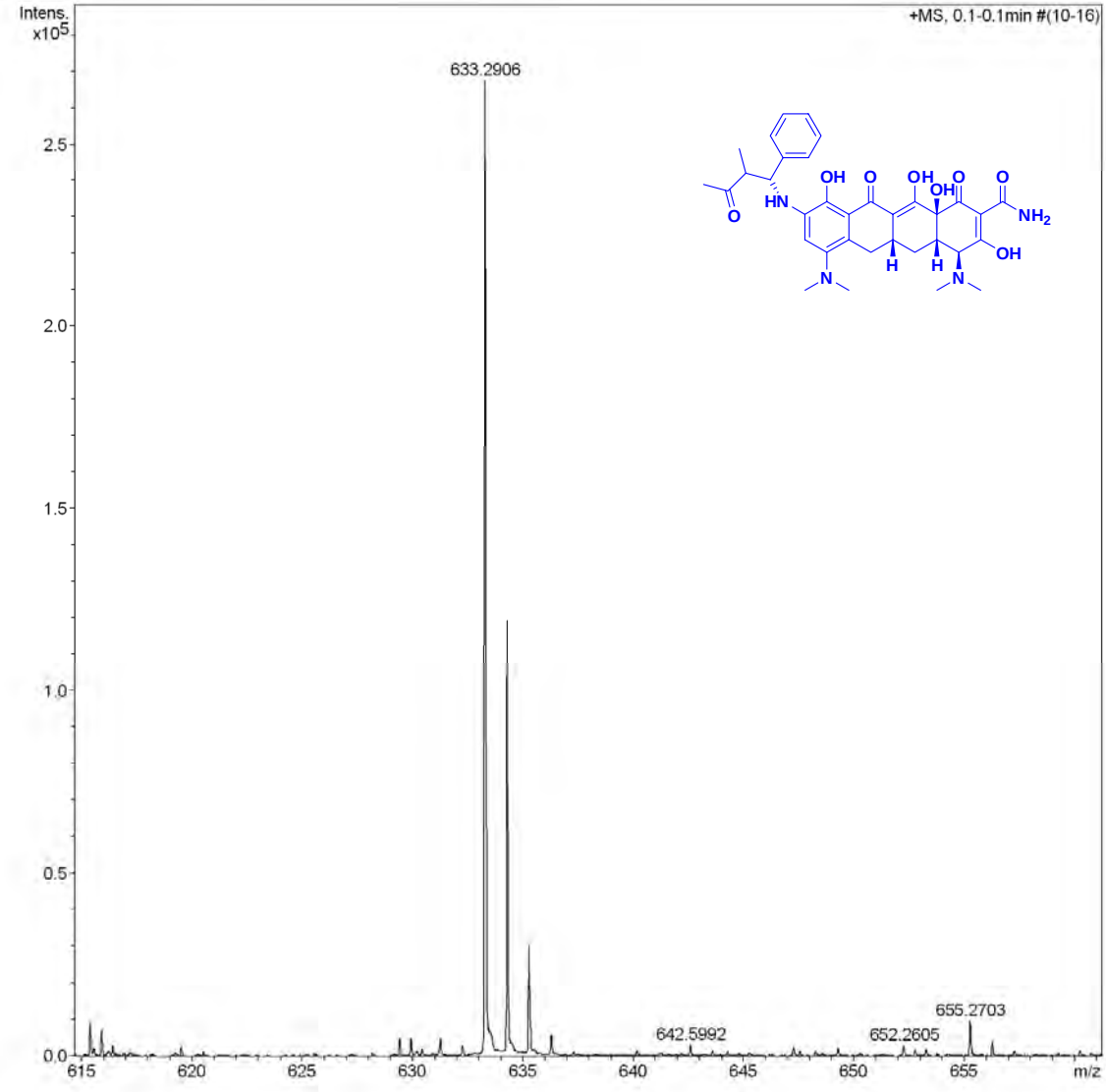

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Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E13_55_01_5467.d	Acquisition Date	3/16/2015 1:37:20 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E13	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

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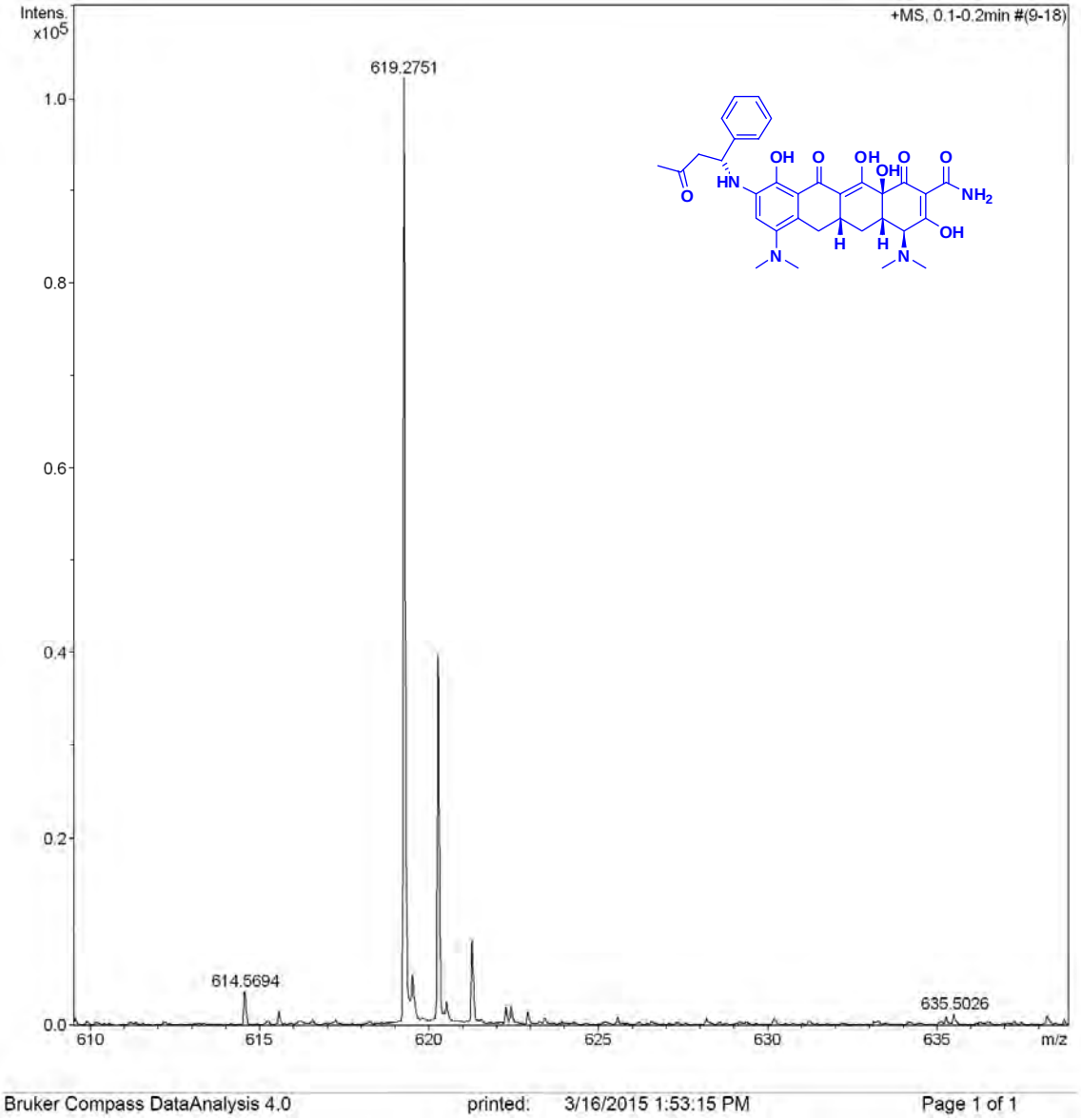
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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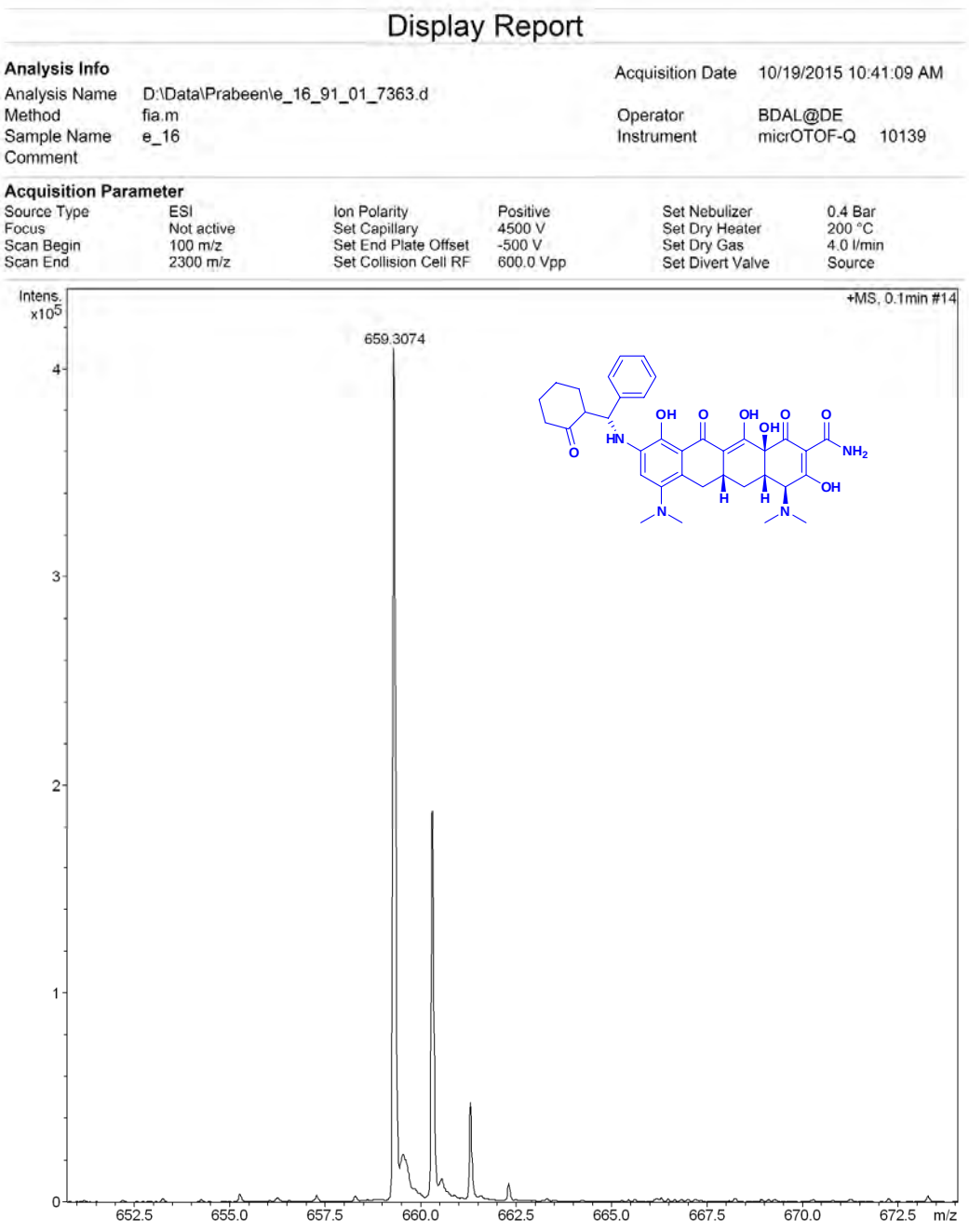

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Display Report

Analysis Info

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Method	fia.m	Operator	BDAL@DE
Sample Name	E1	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

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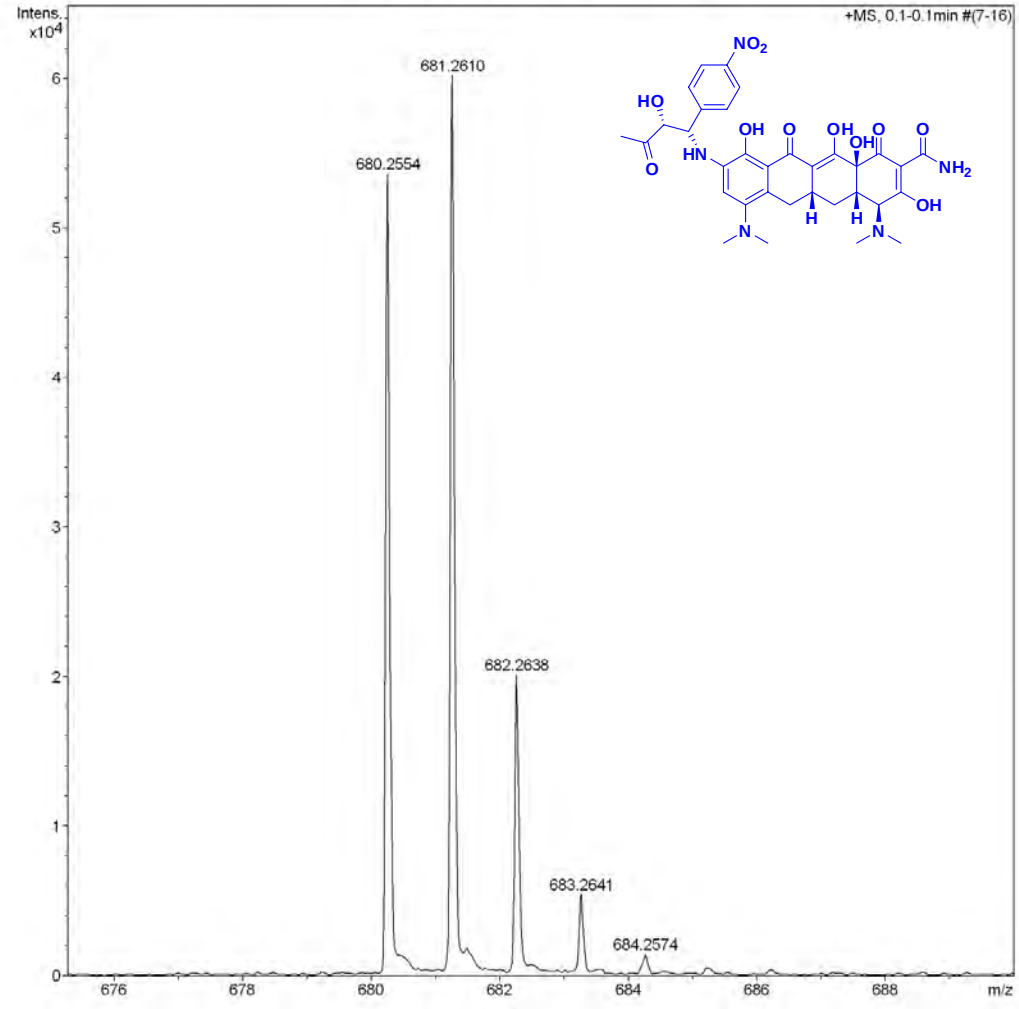

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HRMS of (4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)

Display Report

Analysis Info

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Method	fia.m	Operator	BDAL@DE
Sample Name	E5	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
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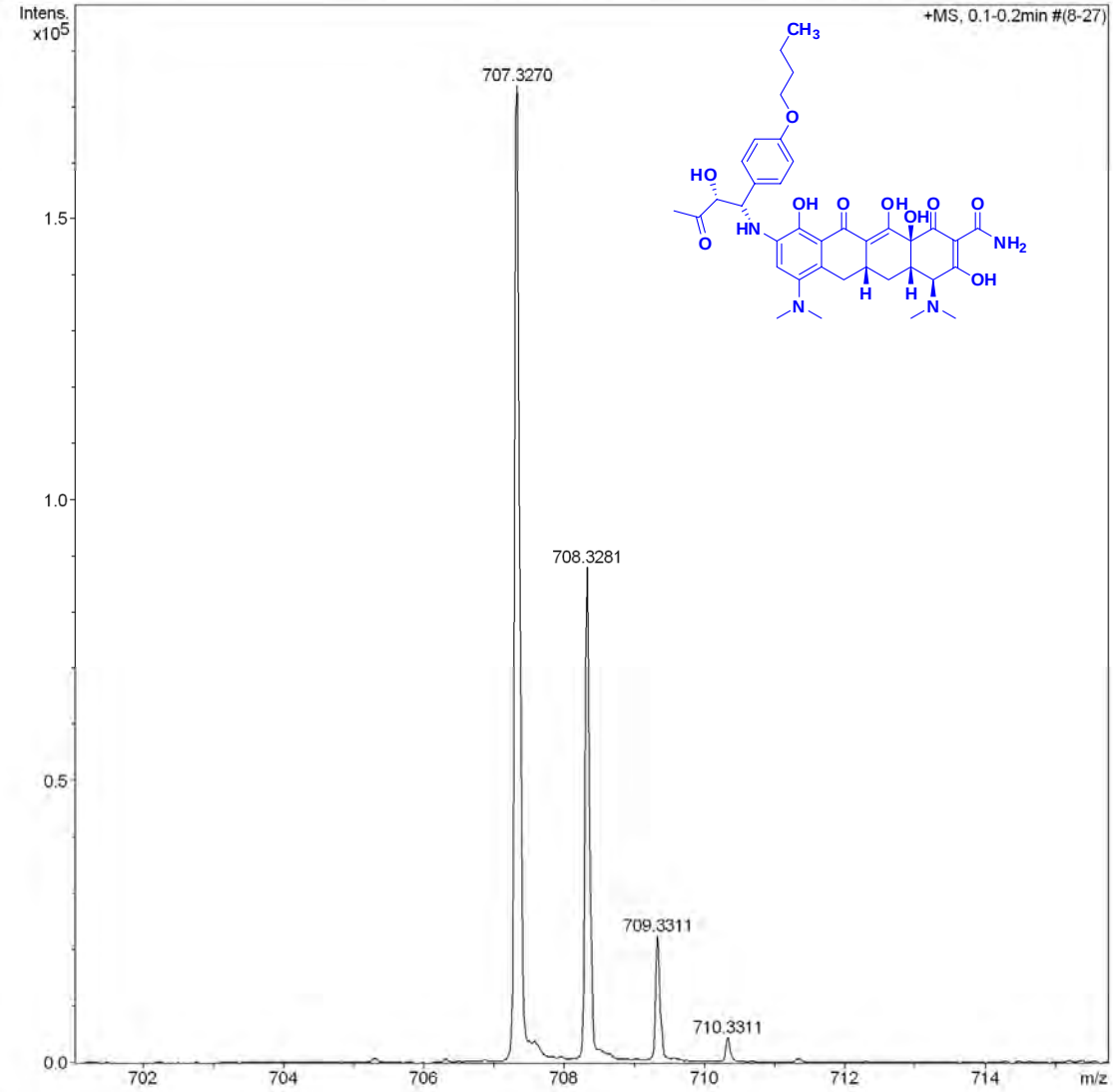
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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Display Report

Analysis Info

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Method	fia.m	Operator	BDAL@DE
Sample Name	E6	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

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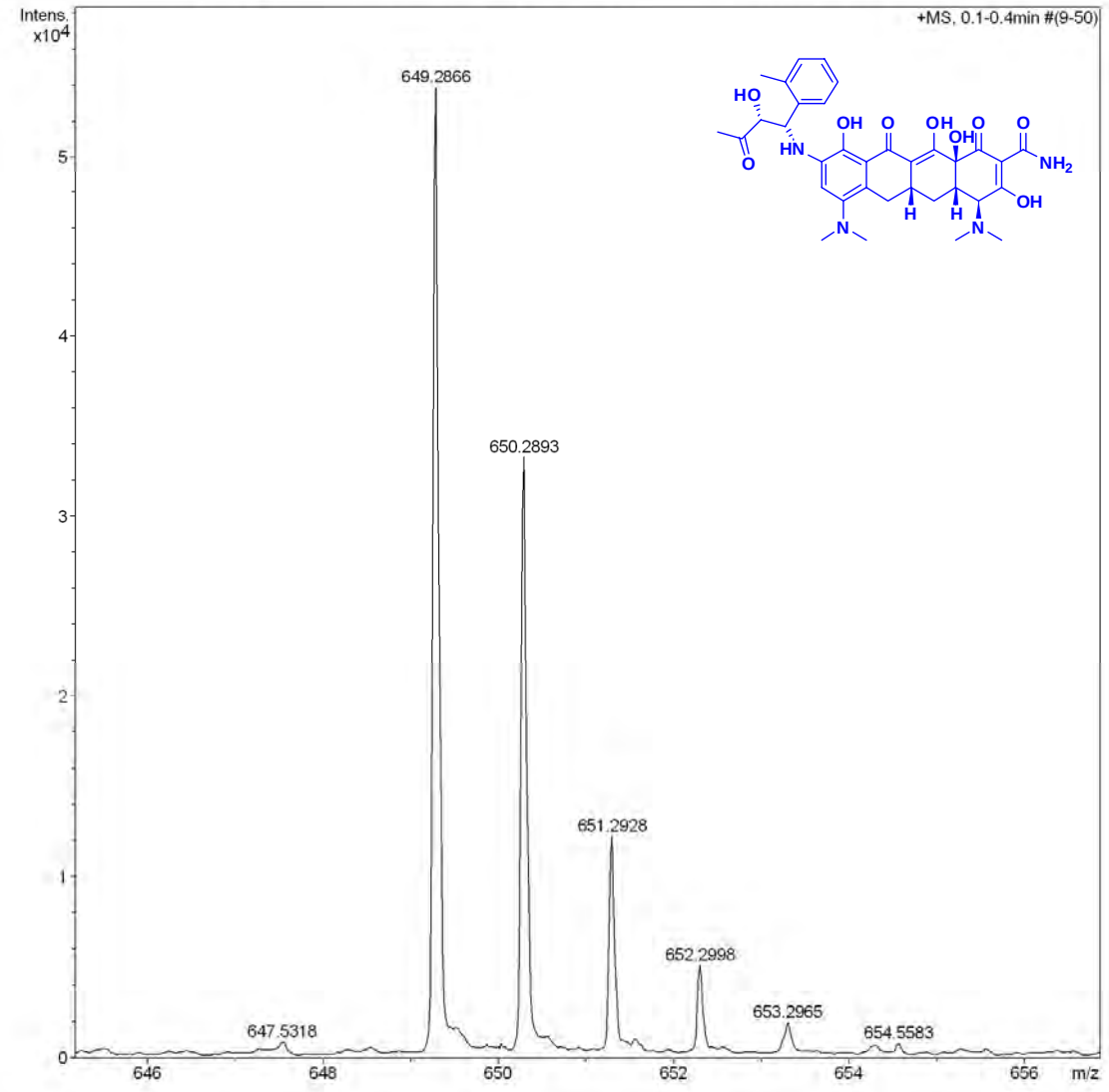
Scan End	2300 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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HRMS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)

Display Report

Analysis Info

Analysis Name	D:\Data\Rufaro\E8_85_01_5698.d	Acquisition Date	4/8/2015 9:38:51 AM
Method	fia.m	Operator	BDAL@DE
Sample Name	E8	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

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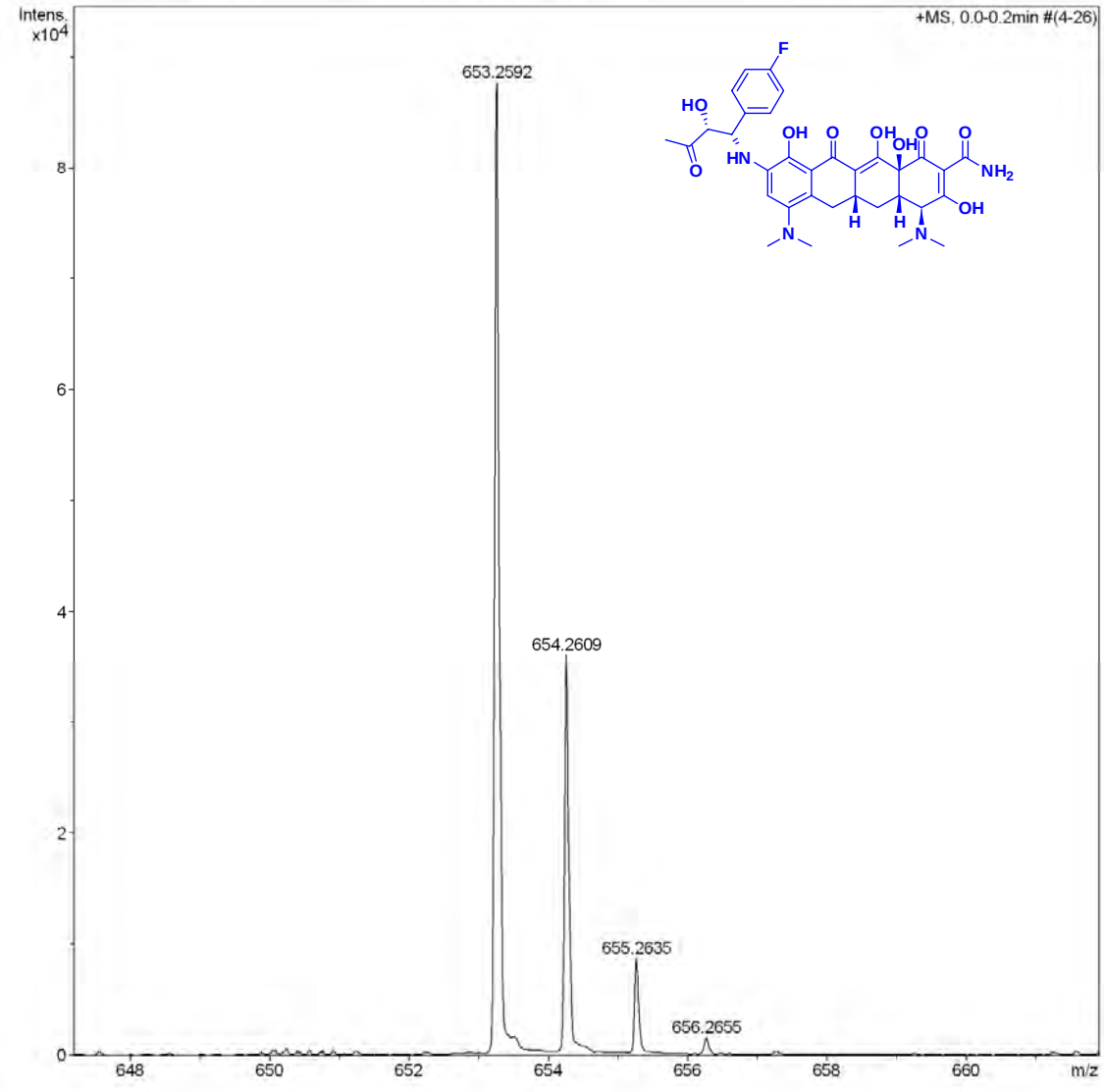

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Display Report

Analysis Info

Analysis Name	D:\Data\Elton\E9_53_01_5463.d	Acquisition Date	3/16/2015 1:25:13 PM
Method	fia.m	Operator	BDAL@DE
Sample Name	E9	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
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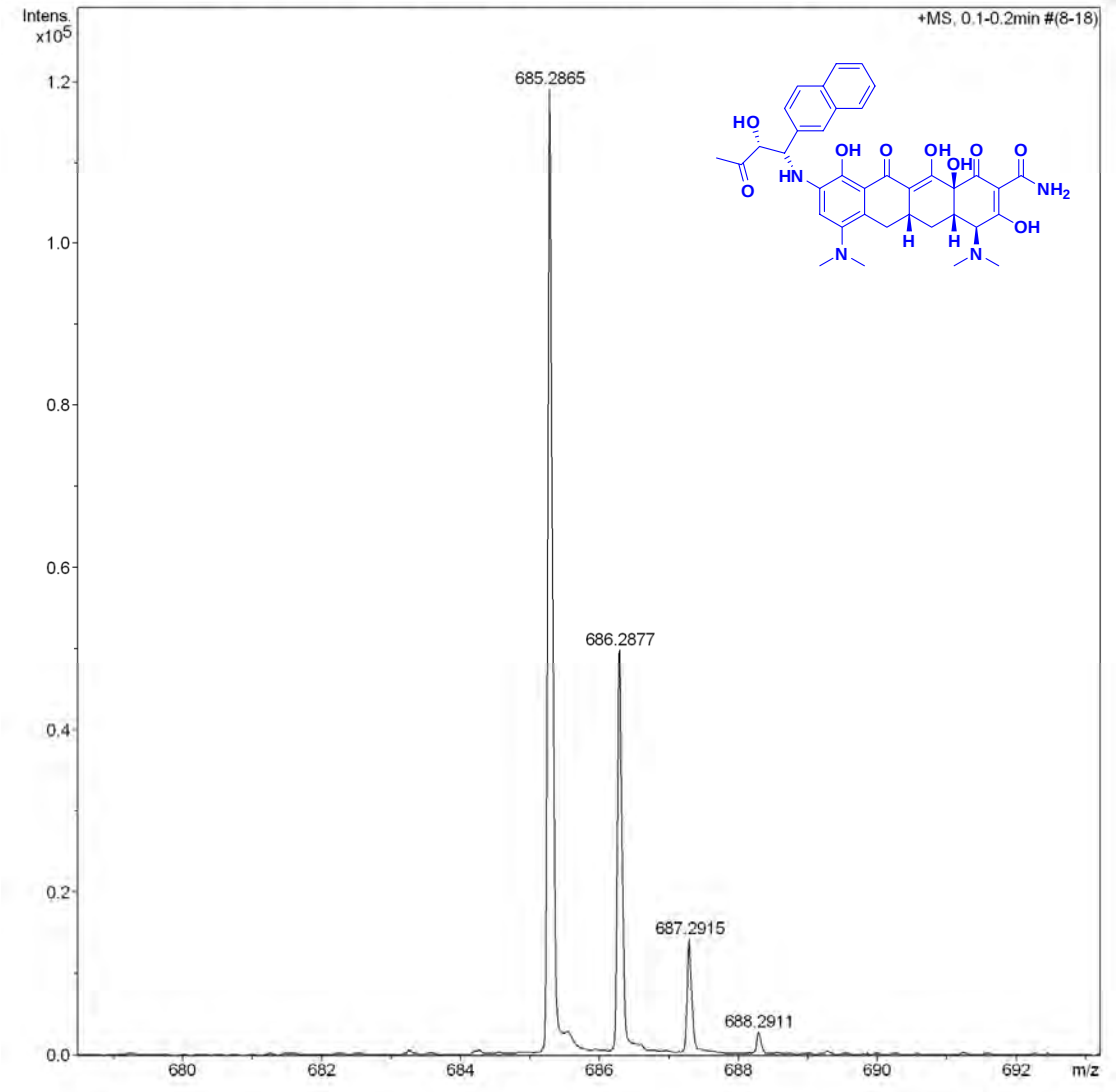

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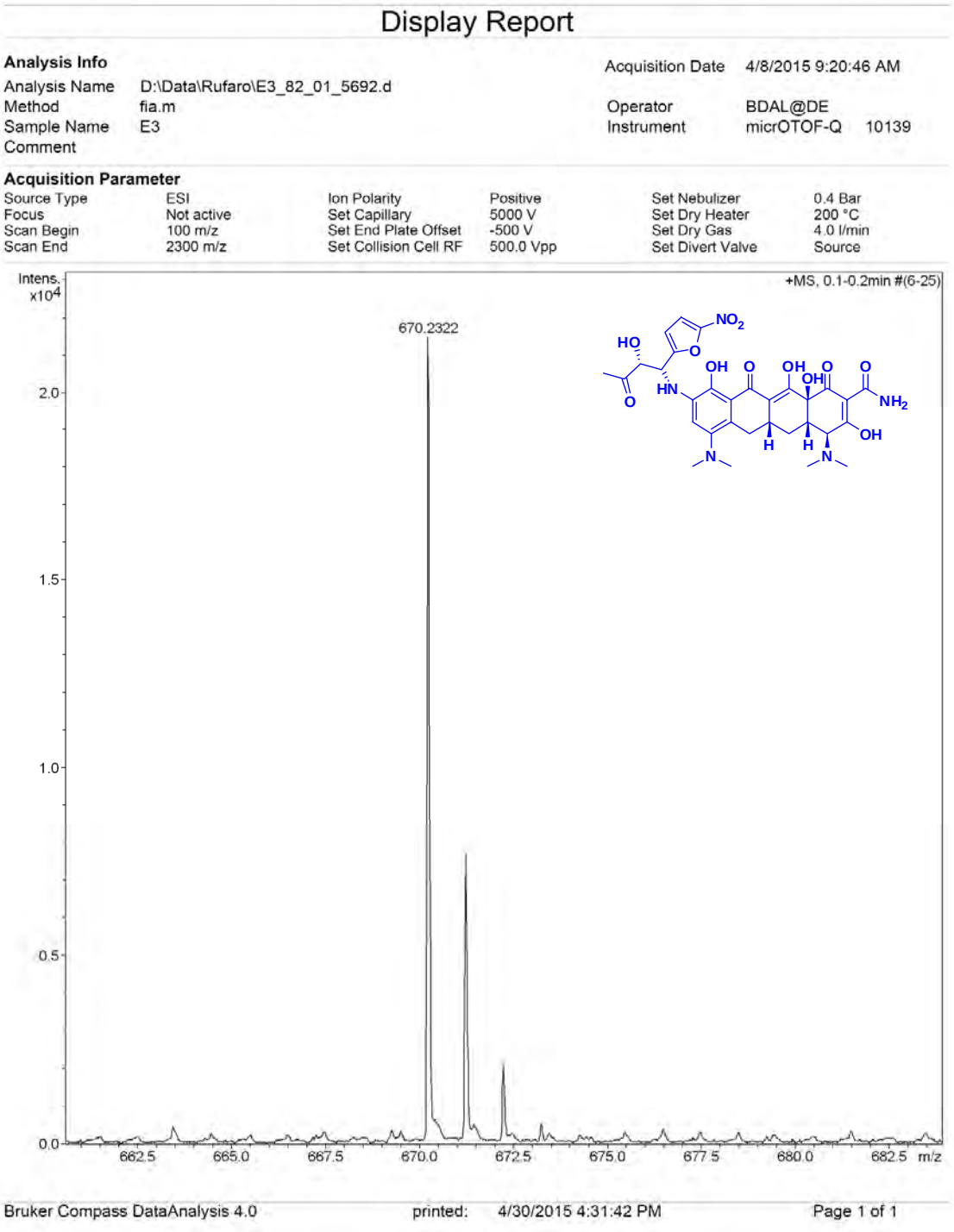

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Display Report

Analysis Info

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Comment			

Acquisition Parameter

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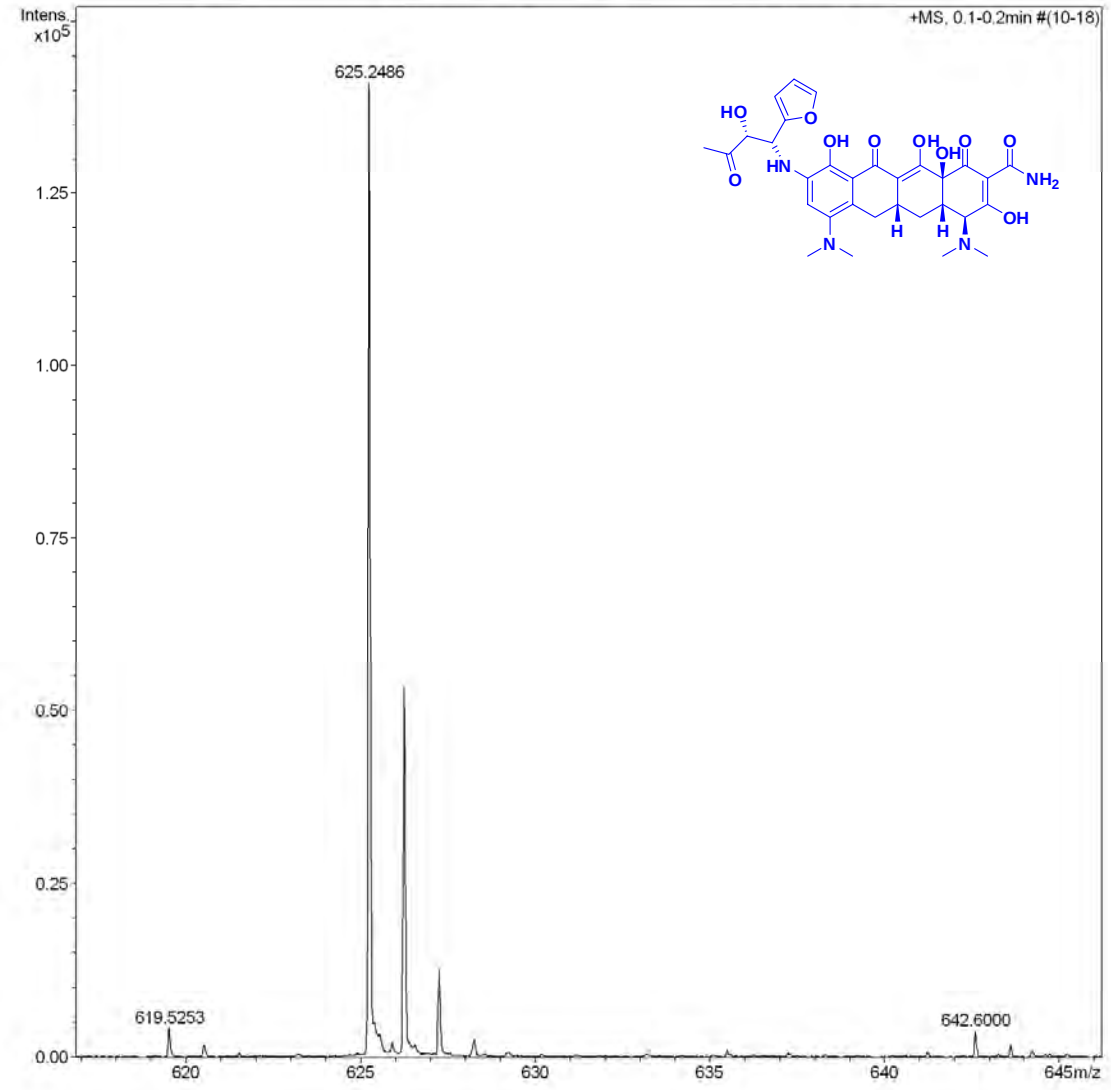

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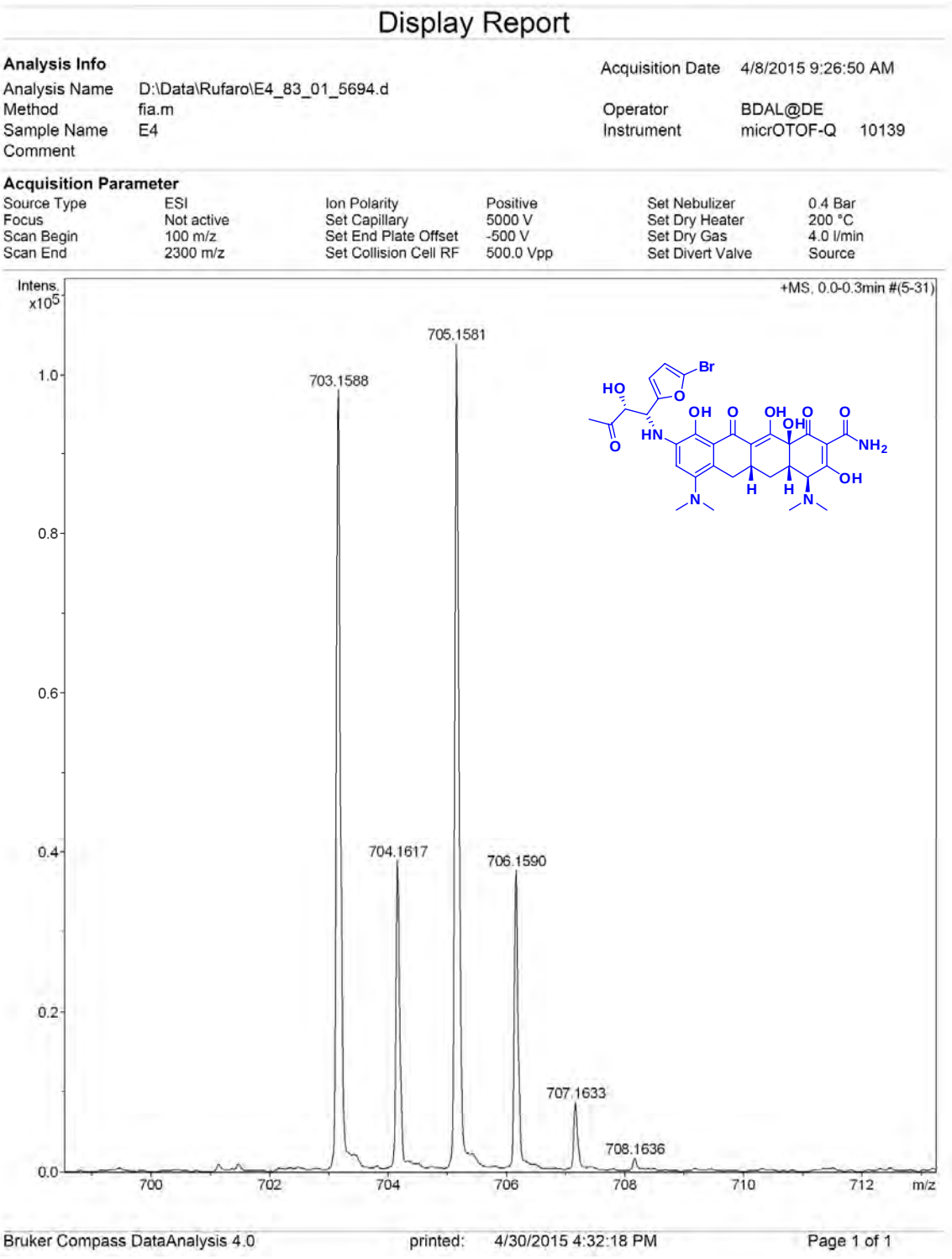
HRMS of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)



2. Copies of LC-MS spectra for products

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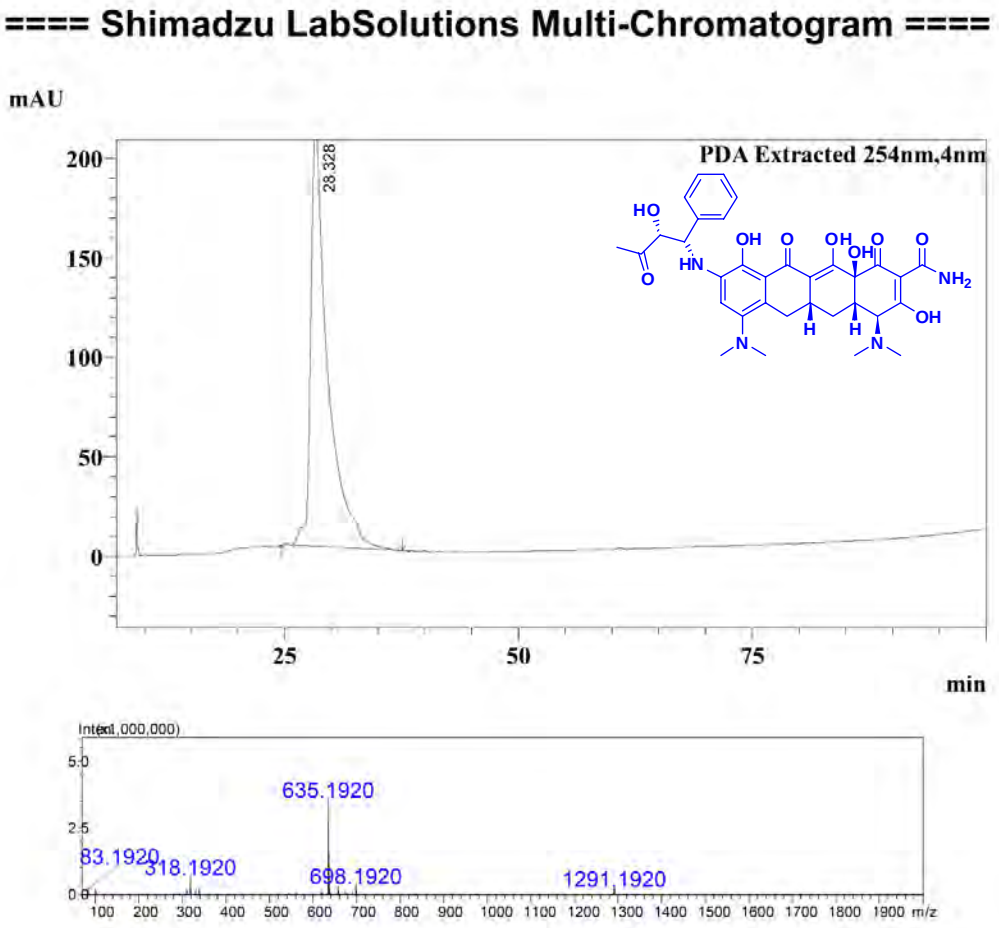
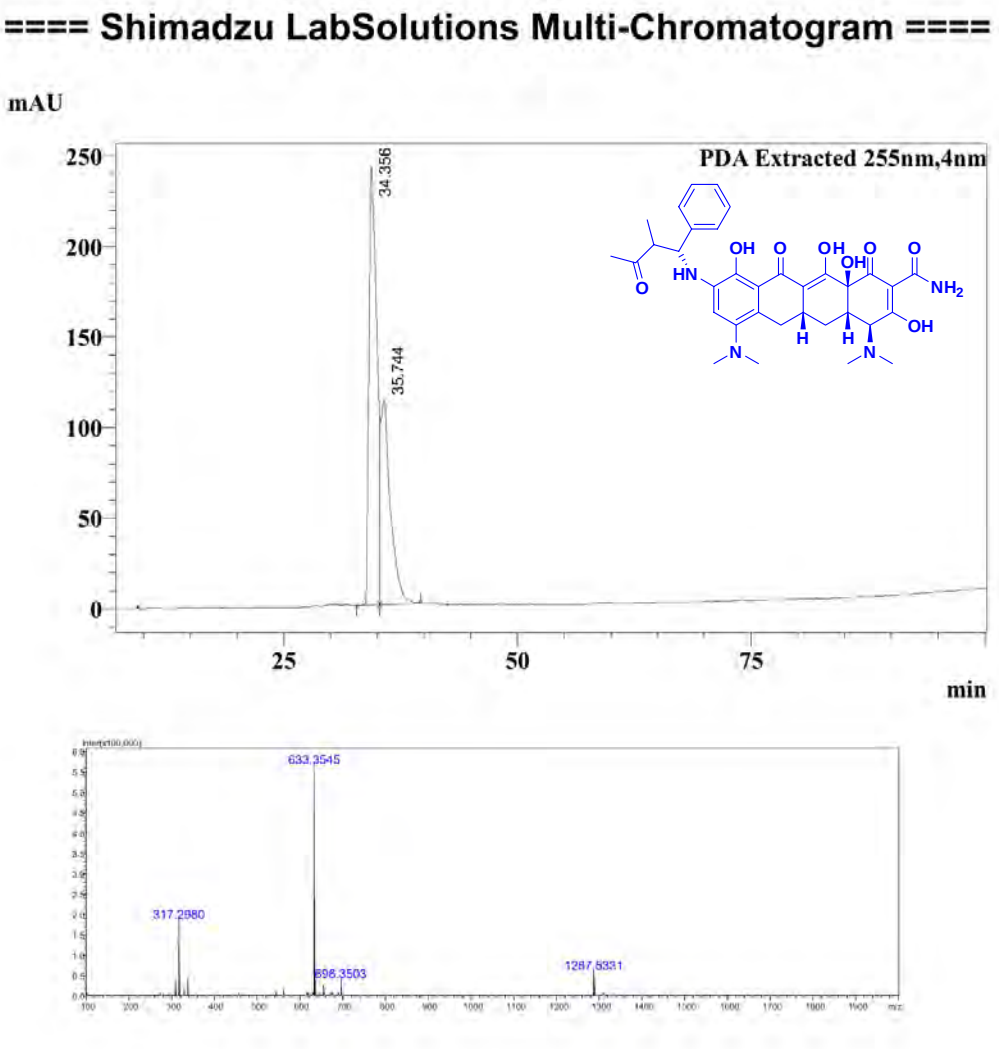
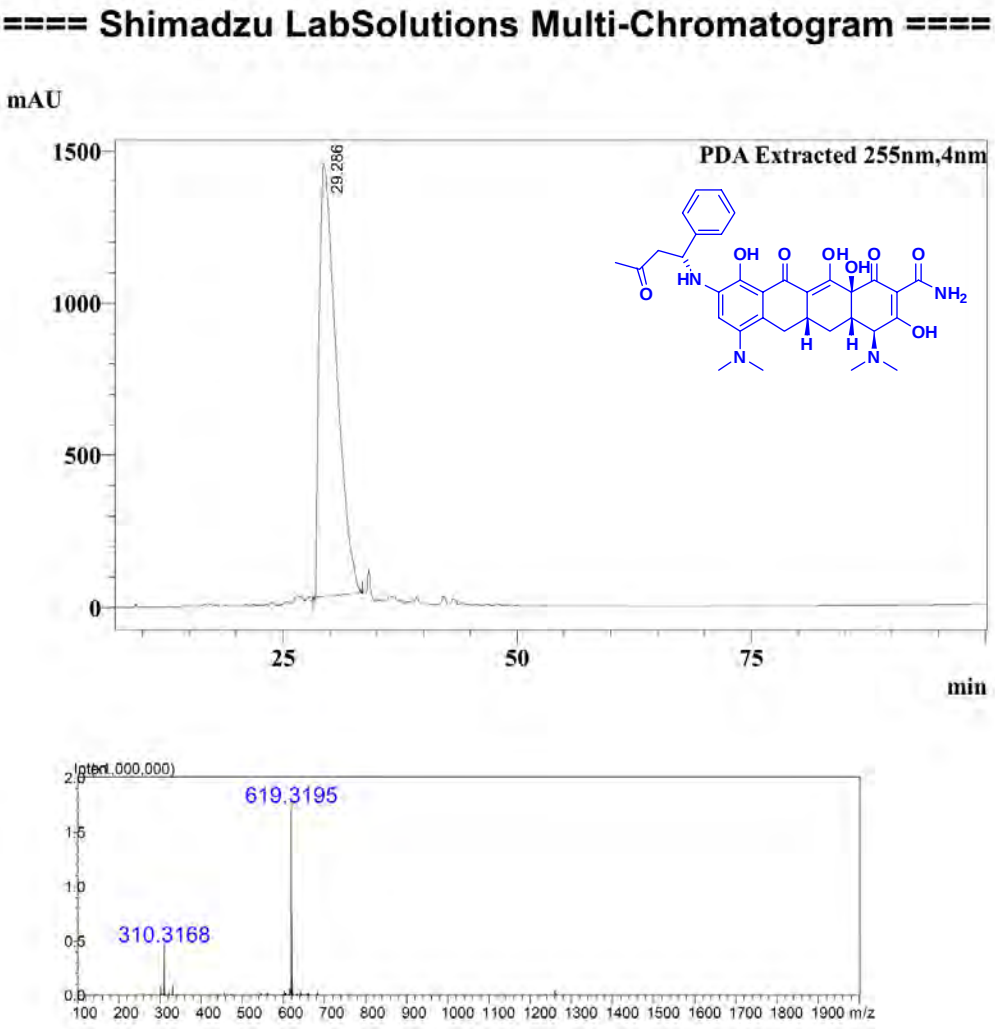


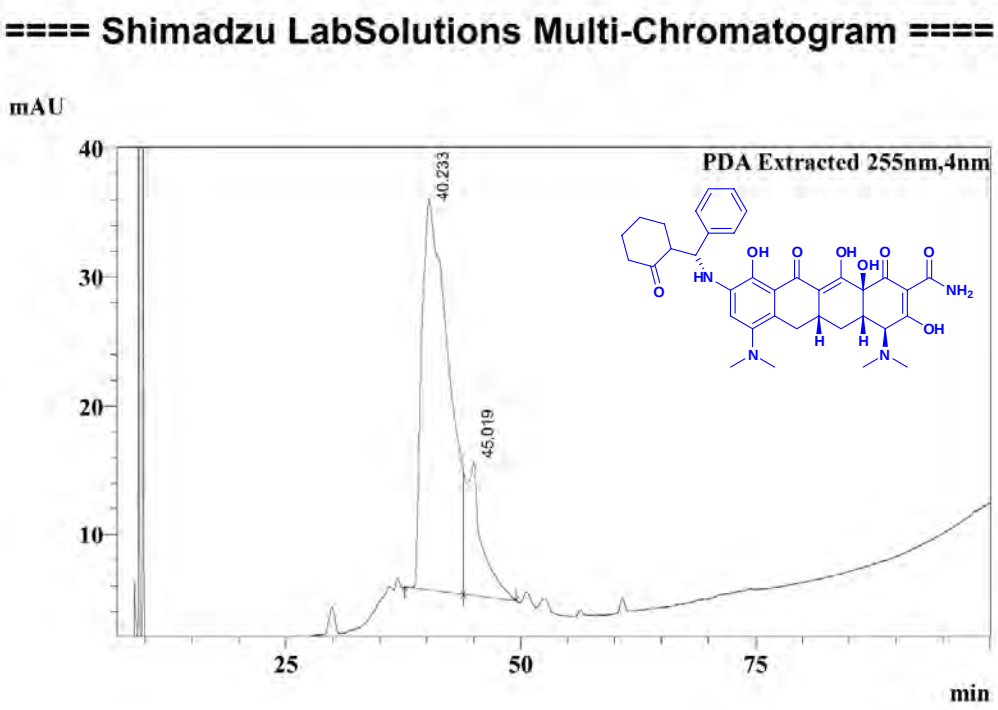
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)

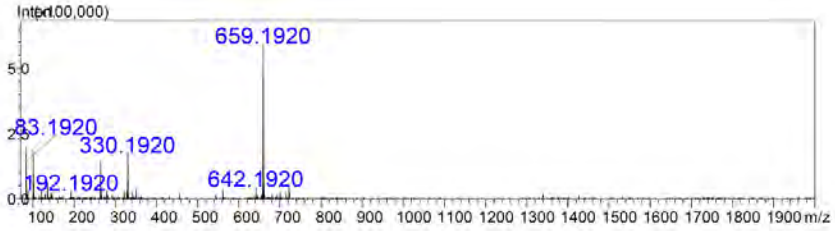


LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-(3-oxo-1-phenylbutylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1c)

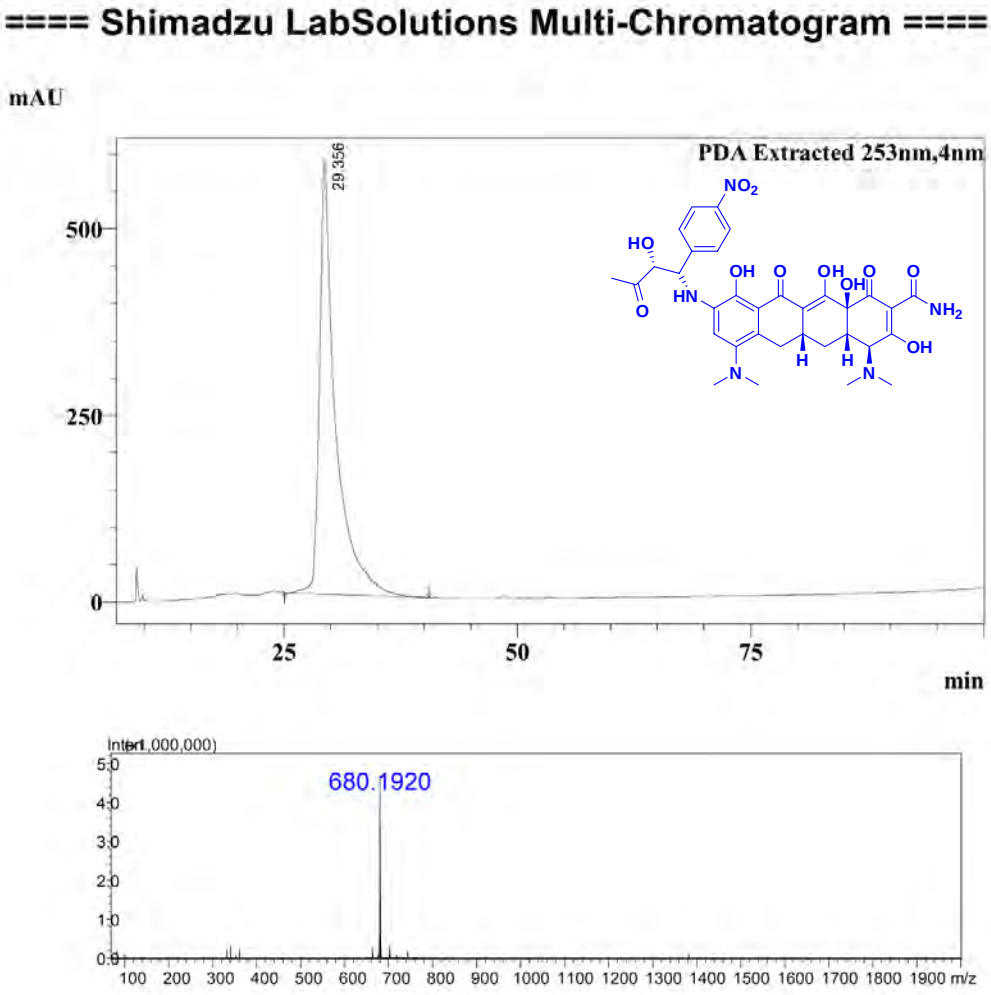


LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-((1R)-(2-oxocyclohexyl)(phenyl)methylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1d)

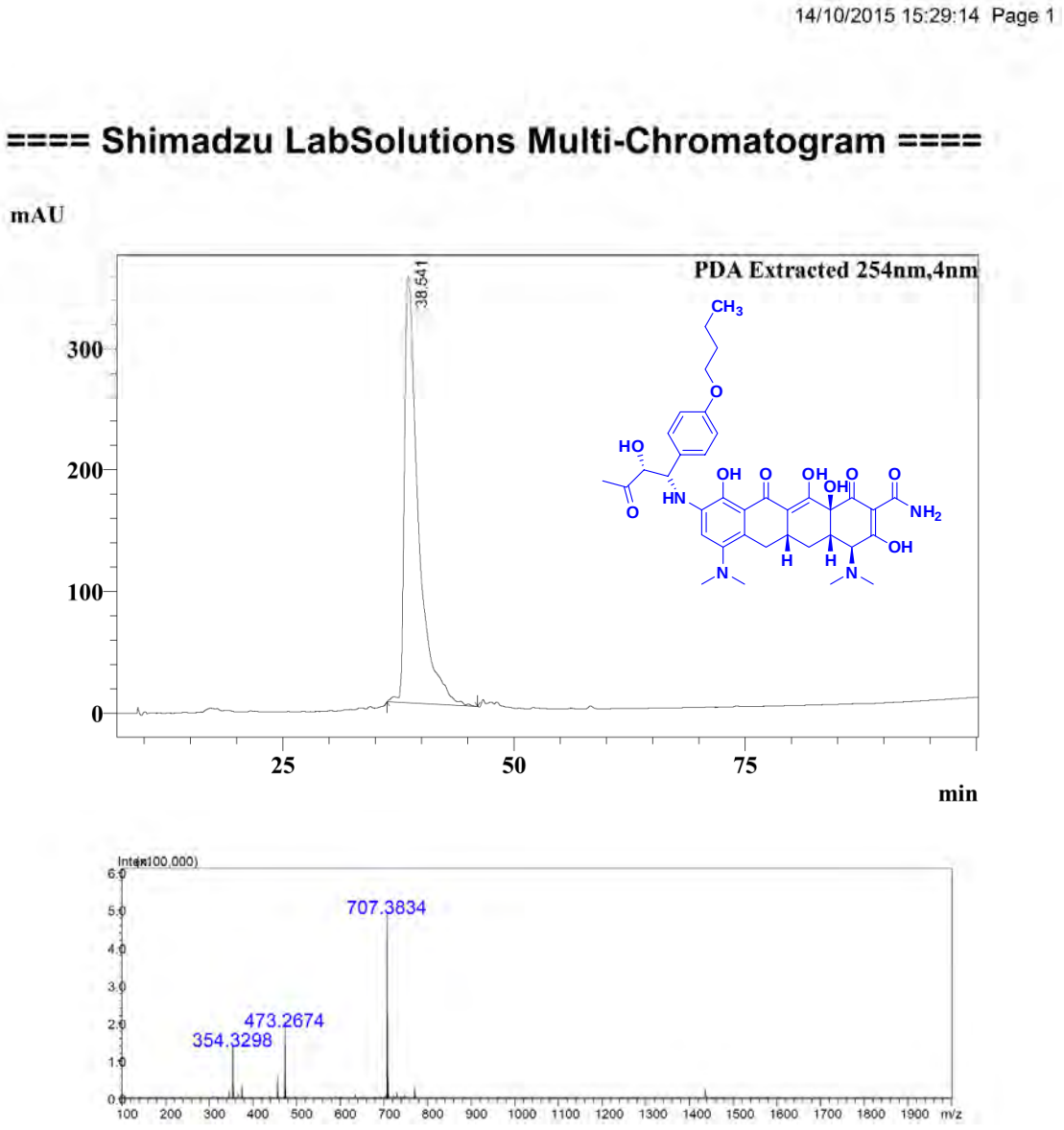




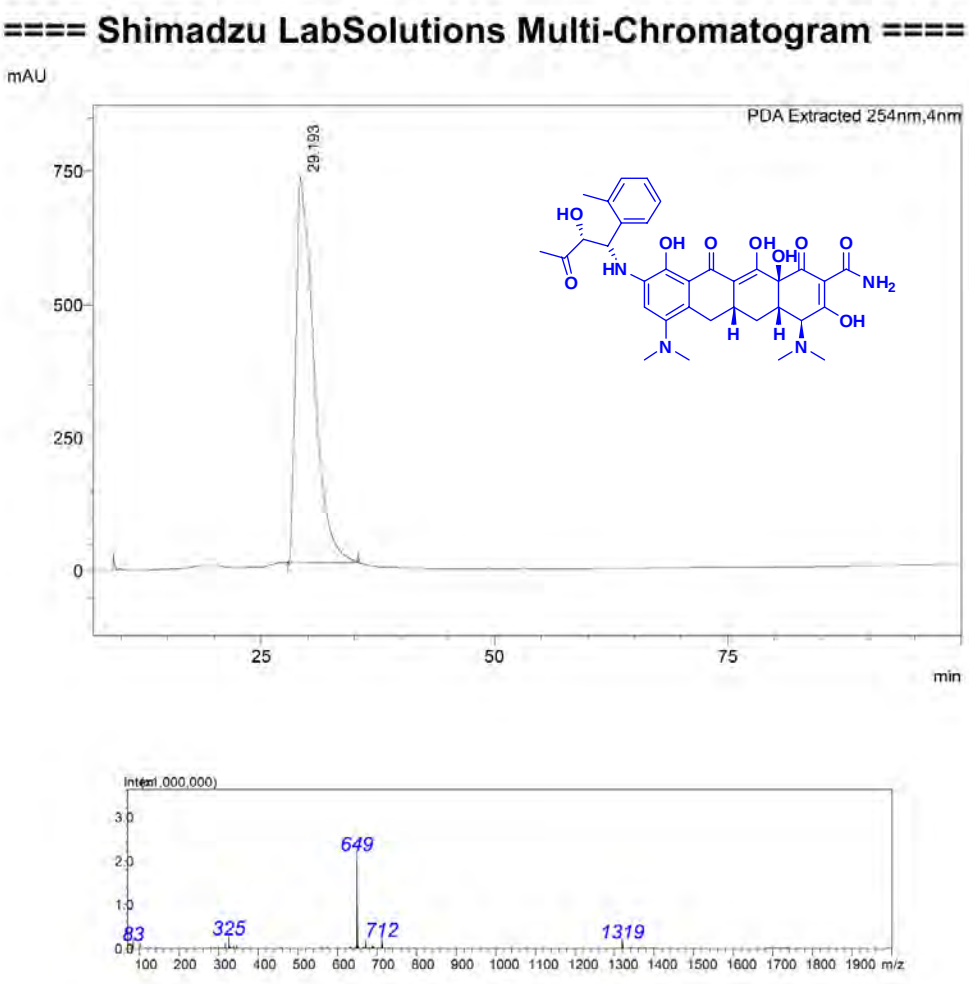
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(4-nitrophenyl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2a)



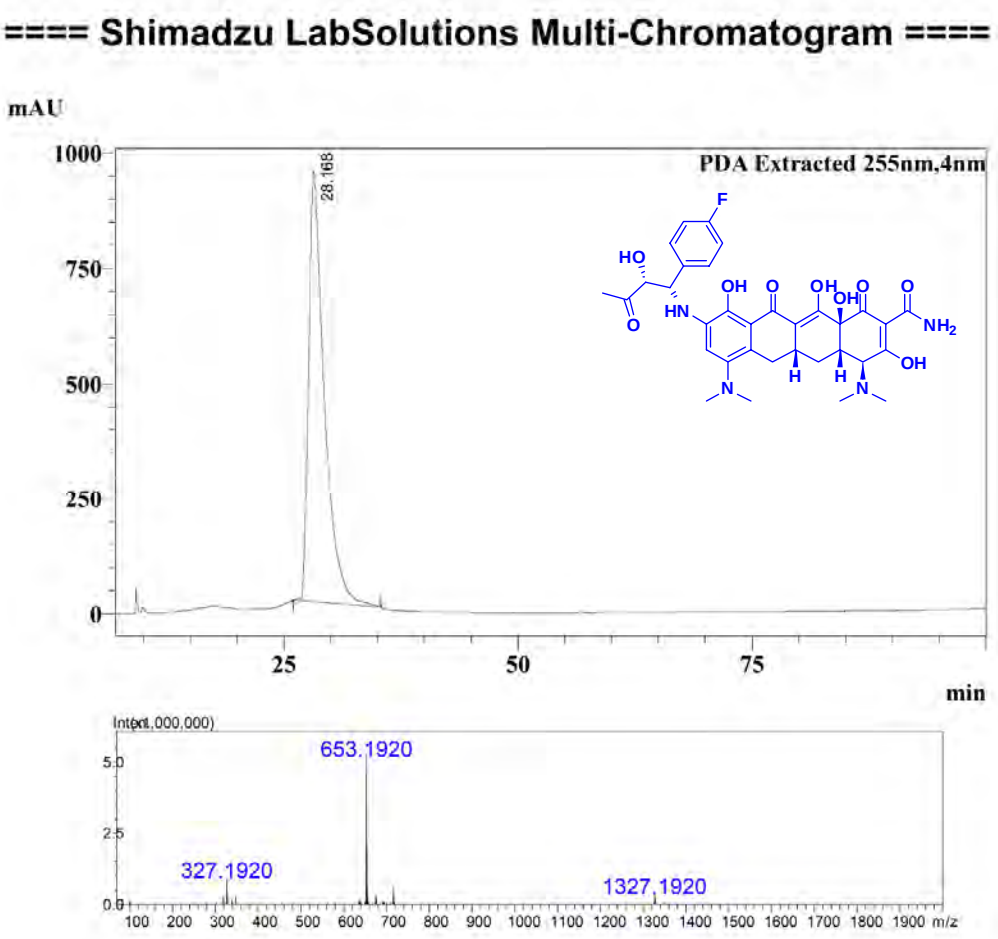
LC-MS of (4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)



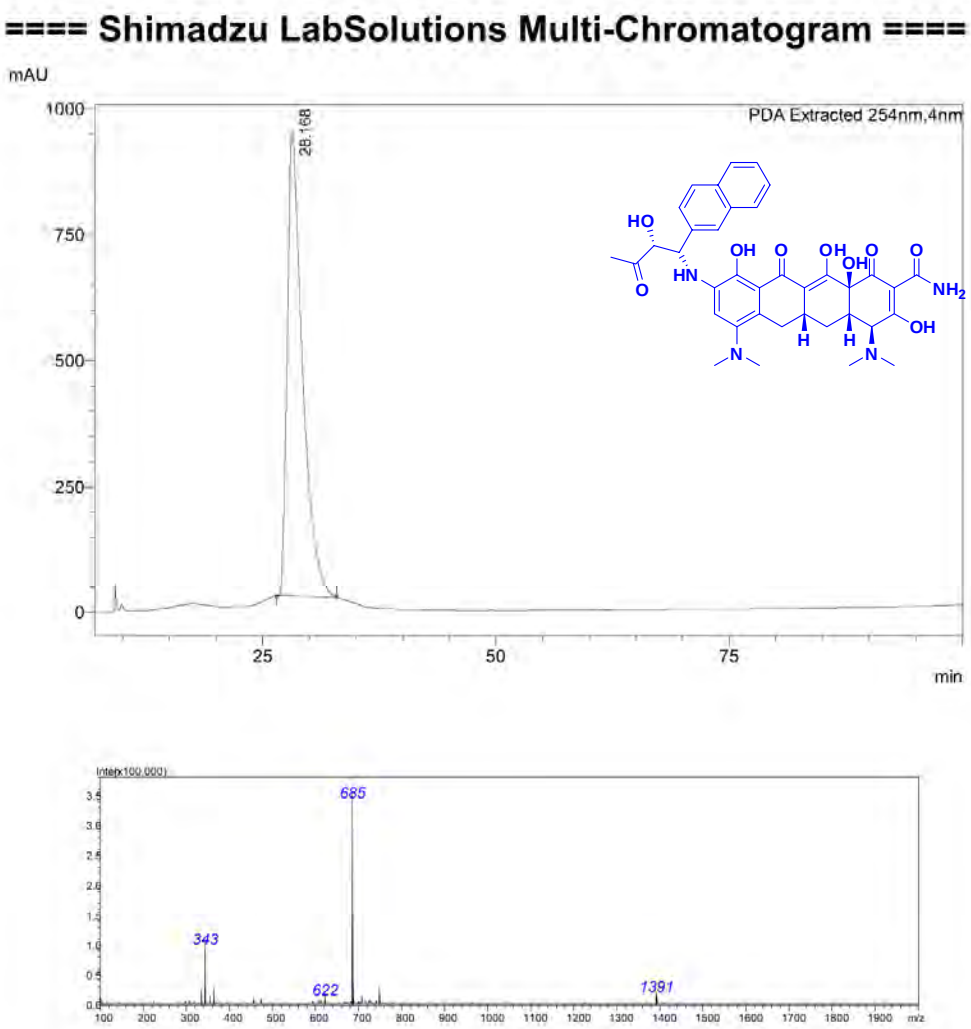
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-3-oxo-1-o-tolylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2c)



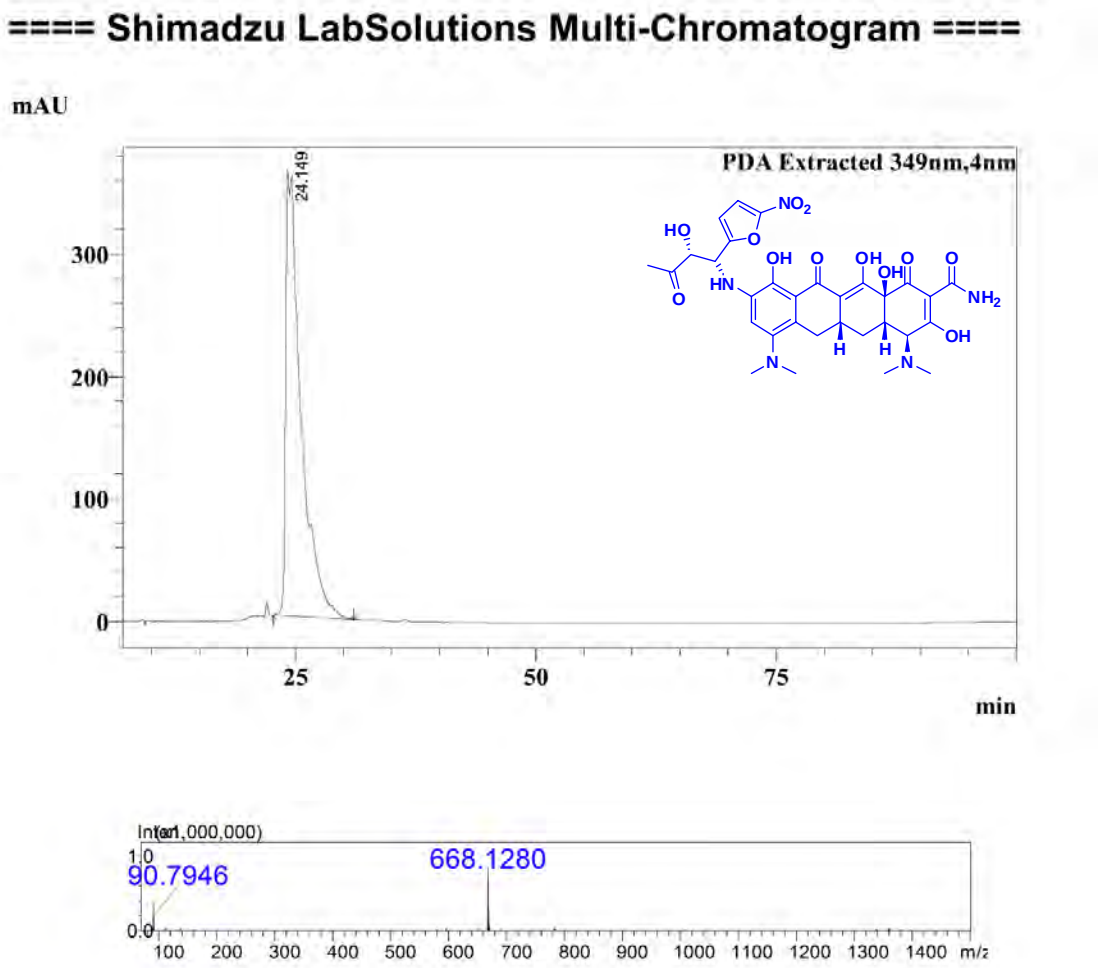
LC-MS SPECTRUM of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)



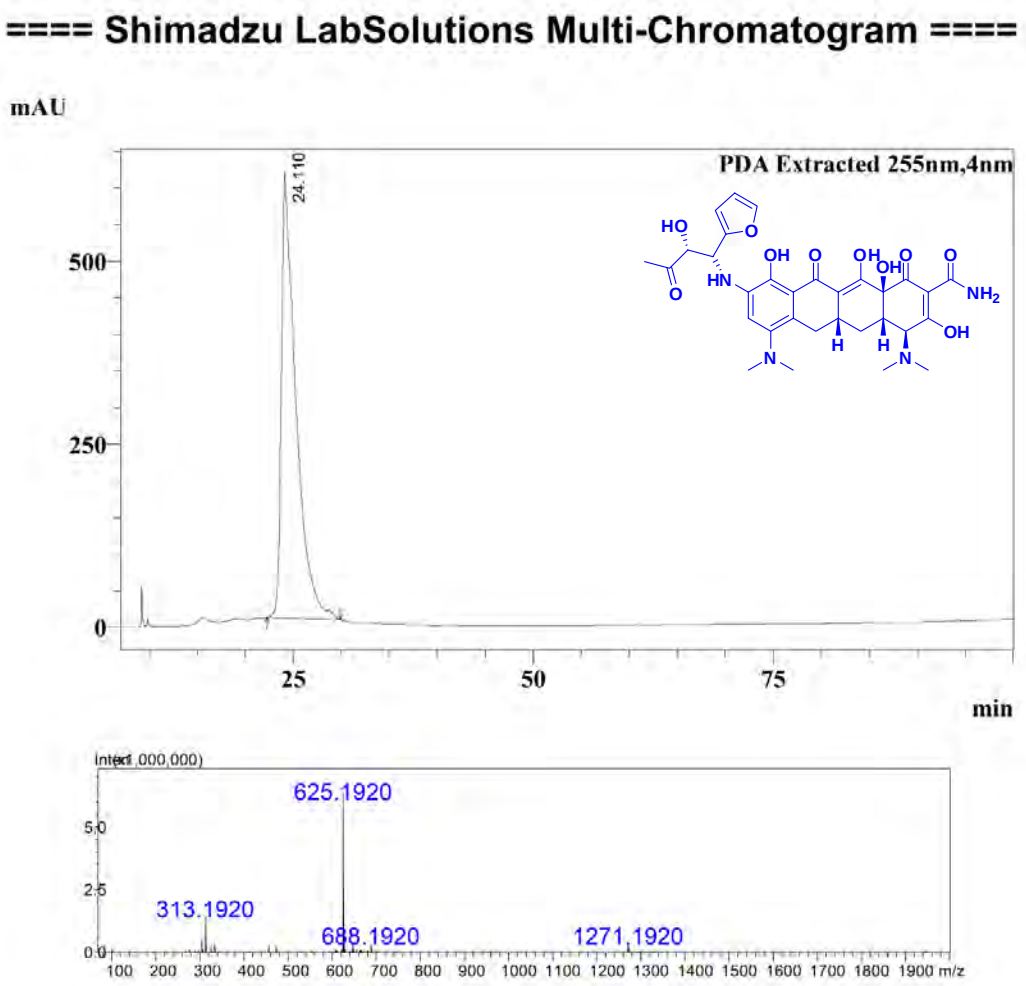
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(naphthalen-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2e)



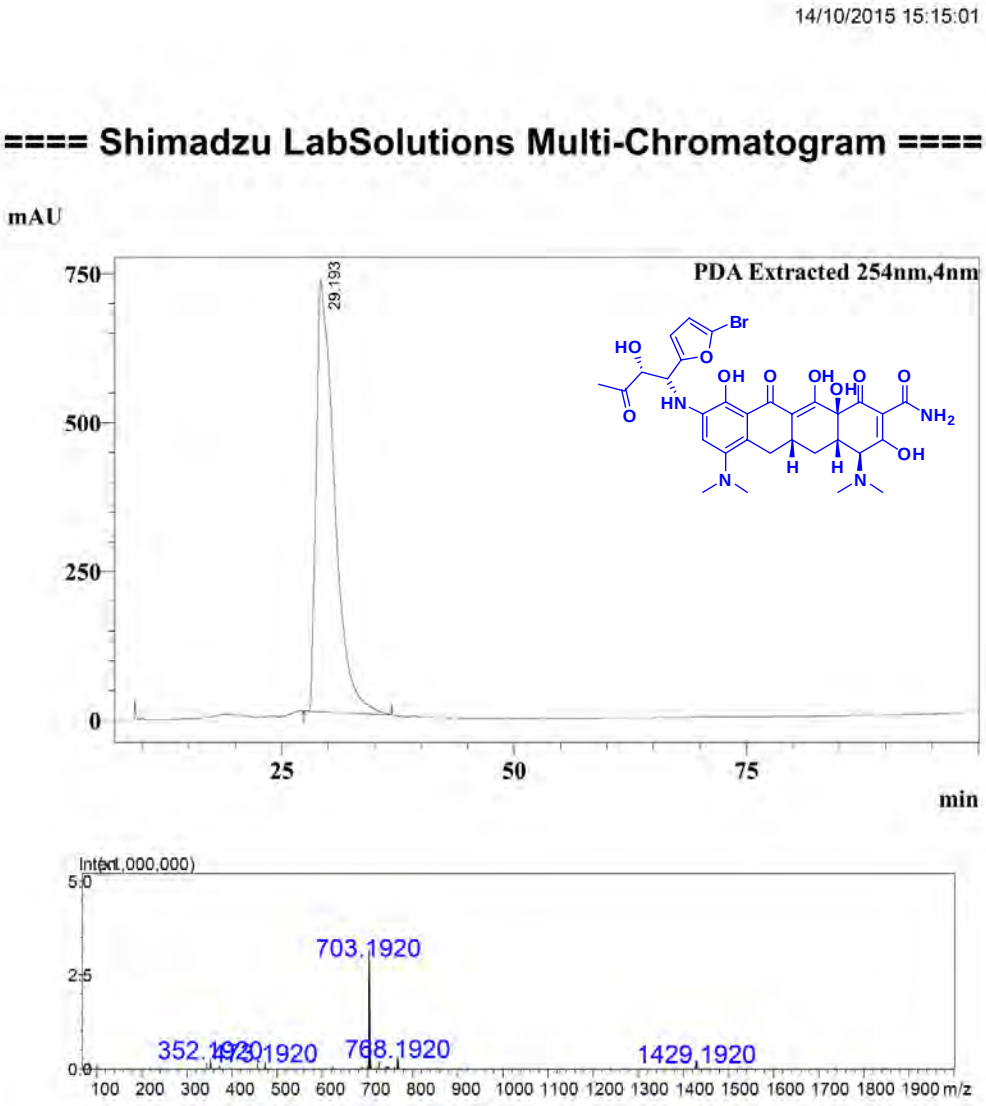
LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1R,2R)-2-hydroxy-1-(5-nitrofuran-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12 octahydrotetracene-2-carboxamide (2f)



LC-MS of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1R,2R)-1-(furan-2-yl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2g)

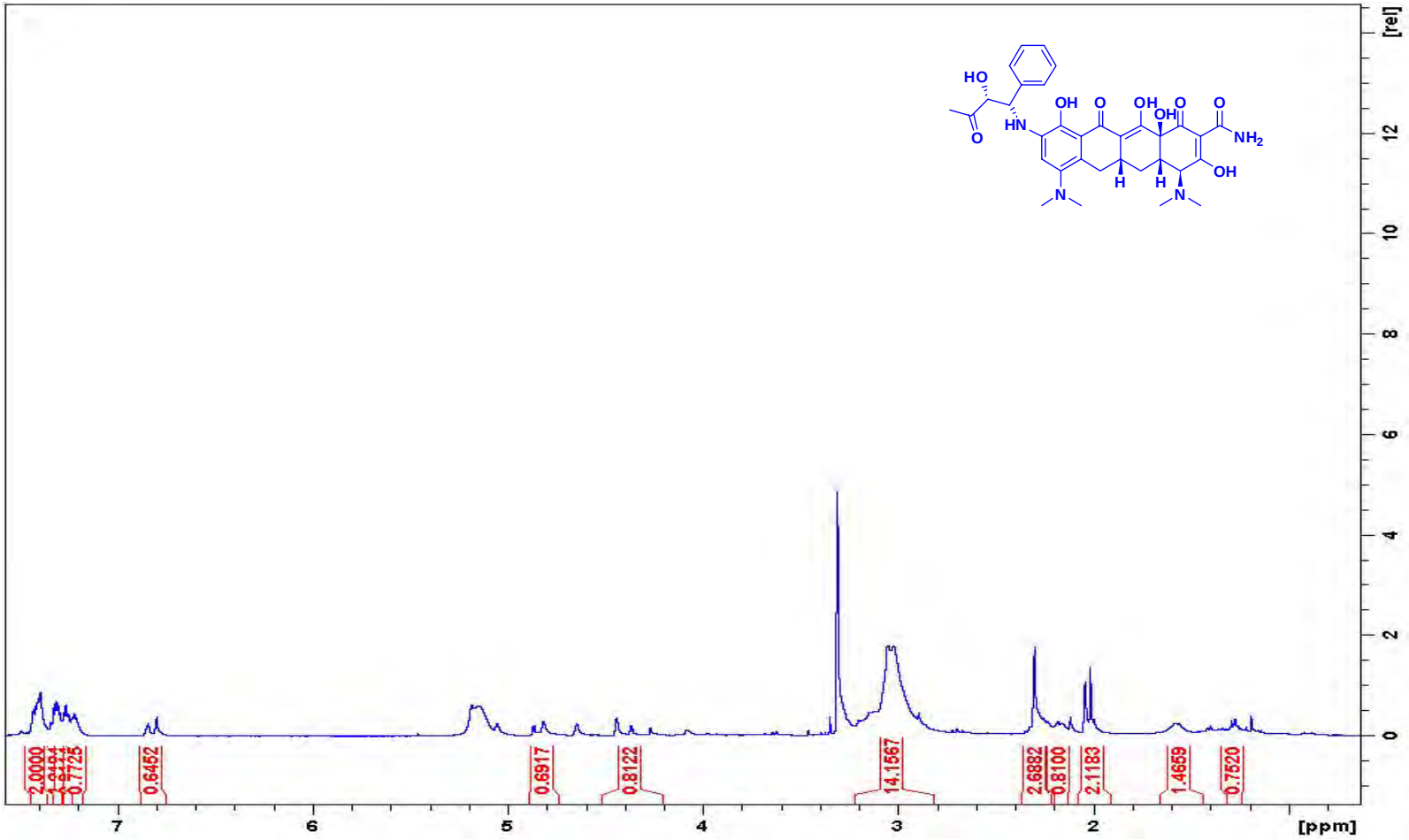


LC-MS of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)

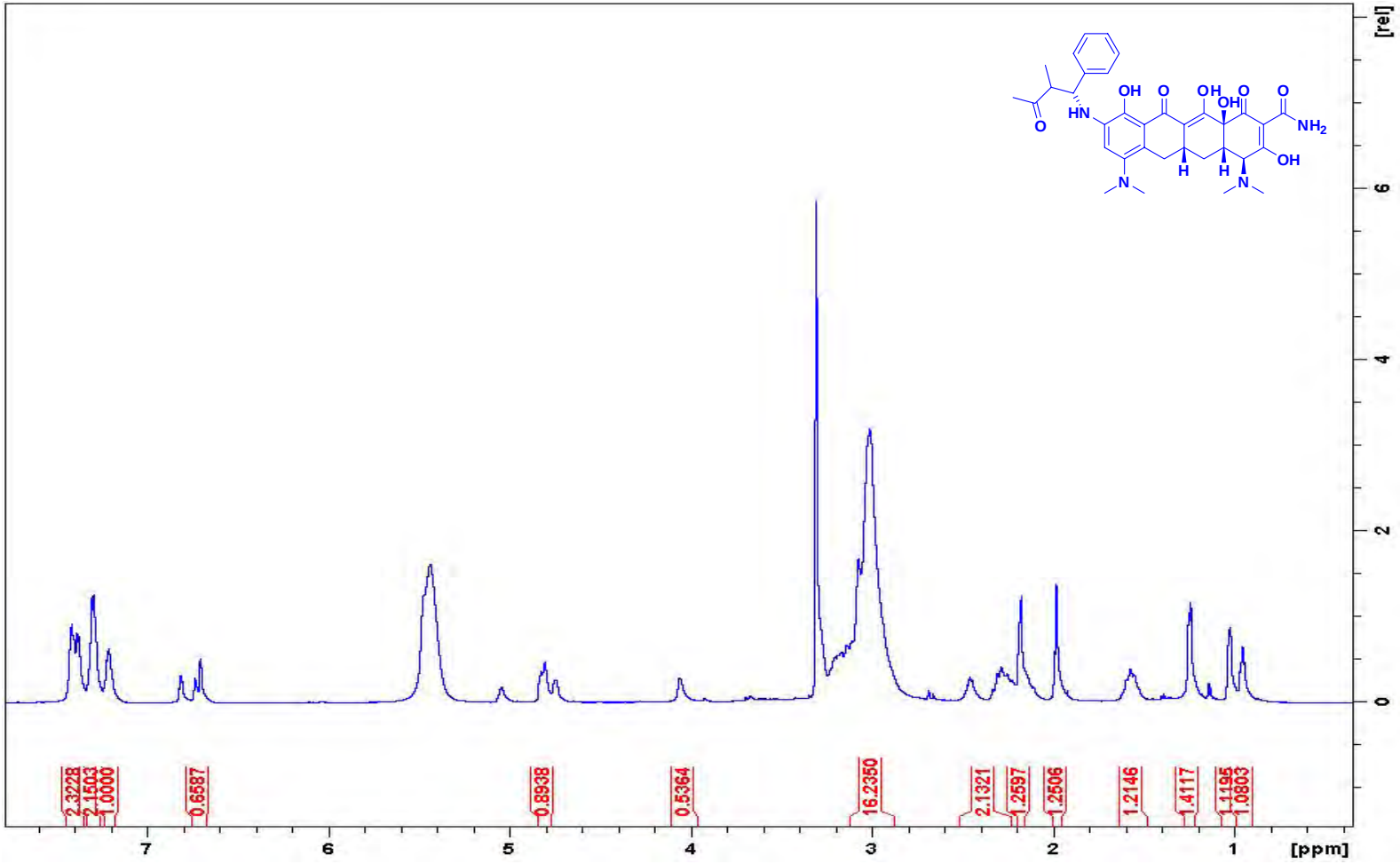


3. Copies of NMR spectra for products

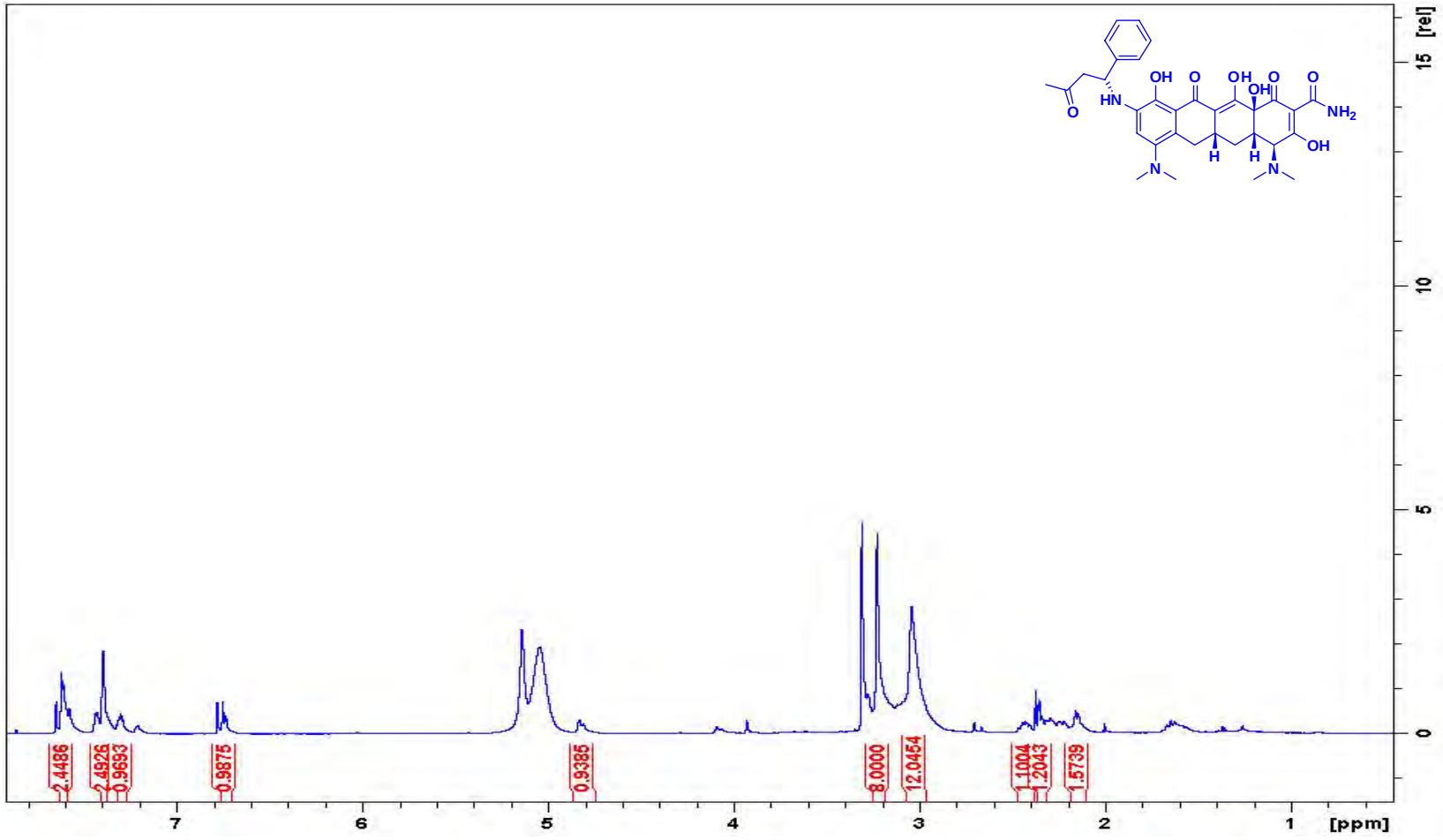
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-hydroxy-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1a)



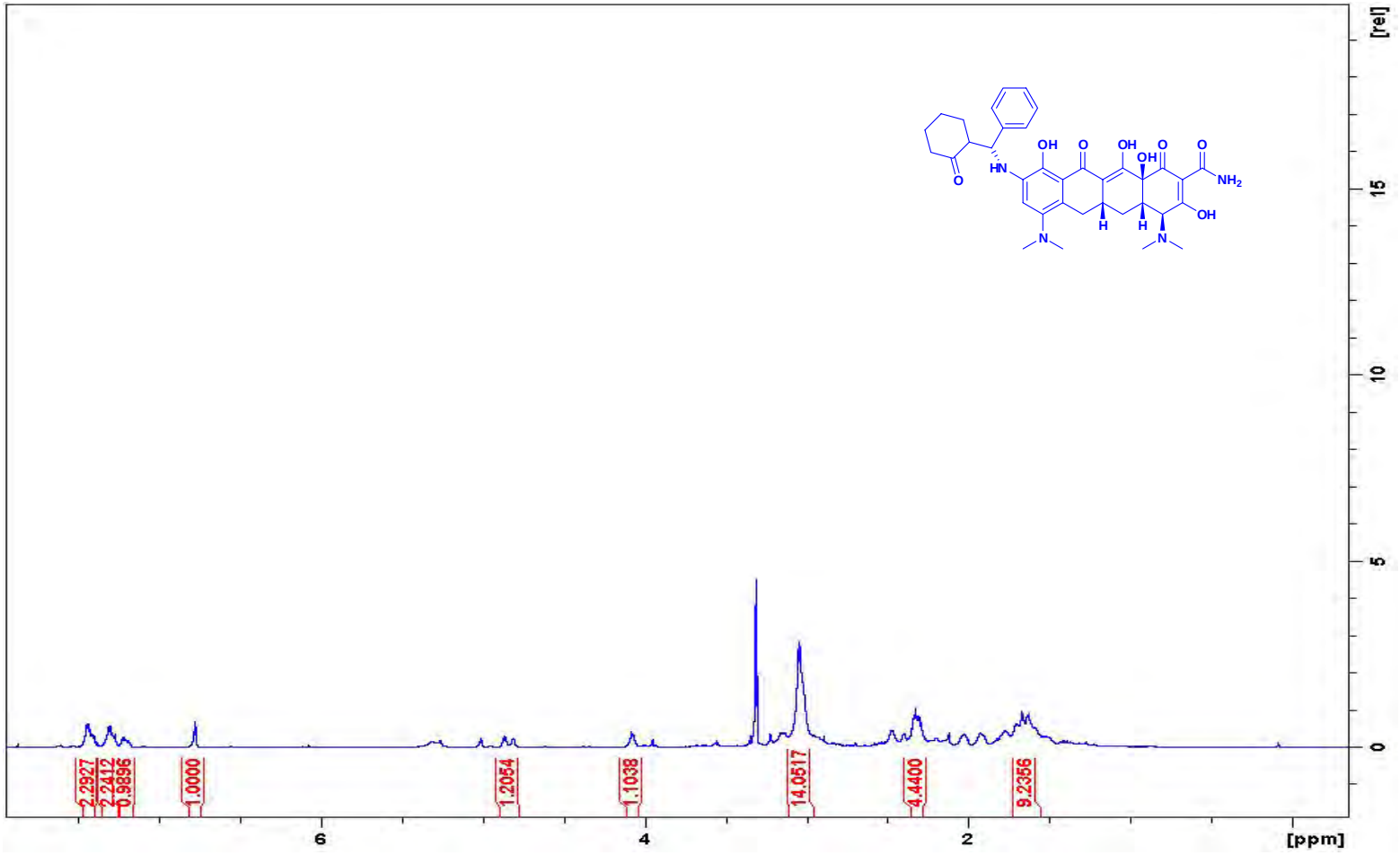
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(2-methyl-3-oxo-1-phenylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1b)



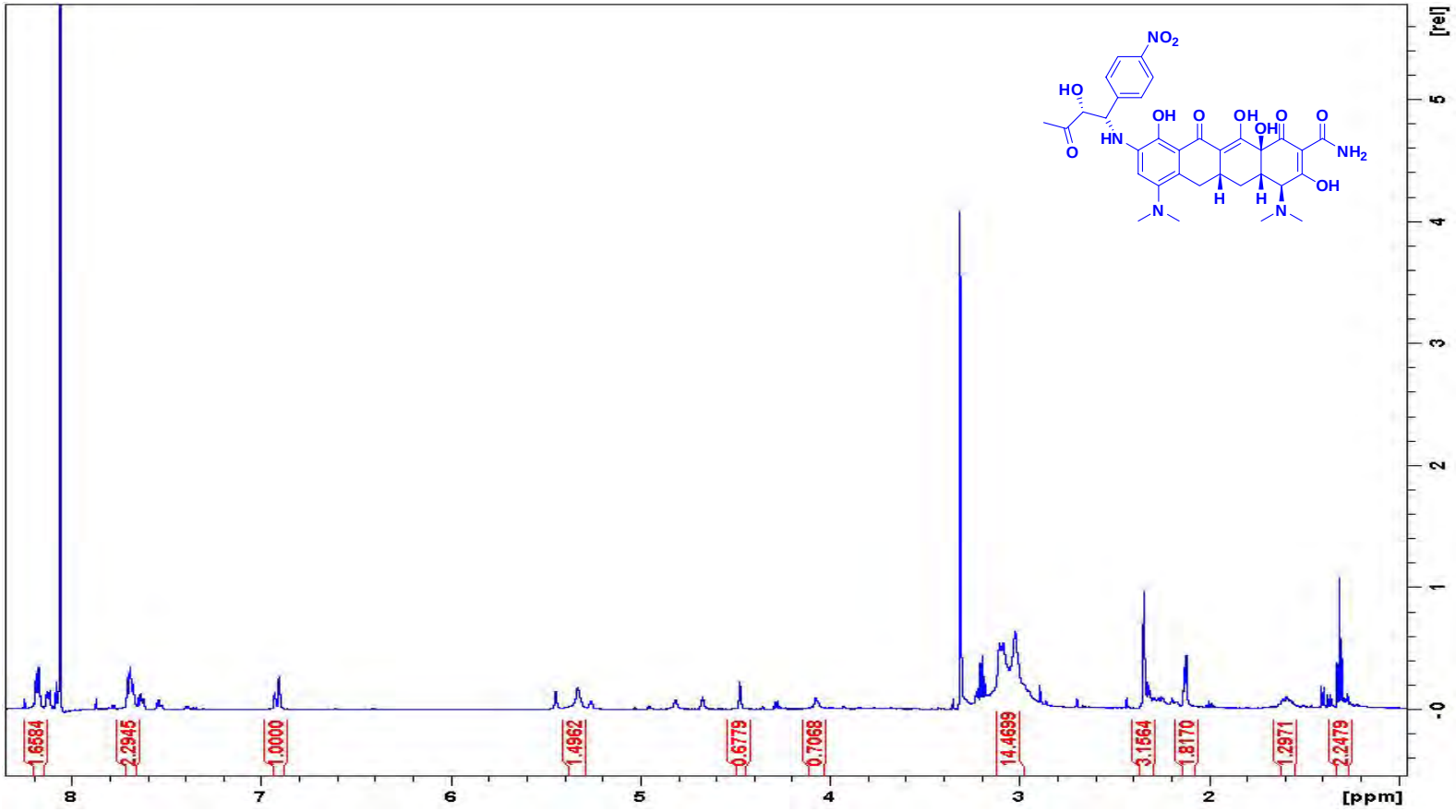
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-(3-oxo-1-phenylbutylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1c)



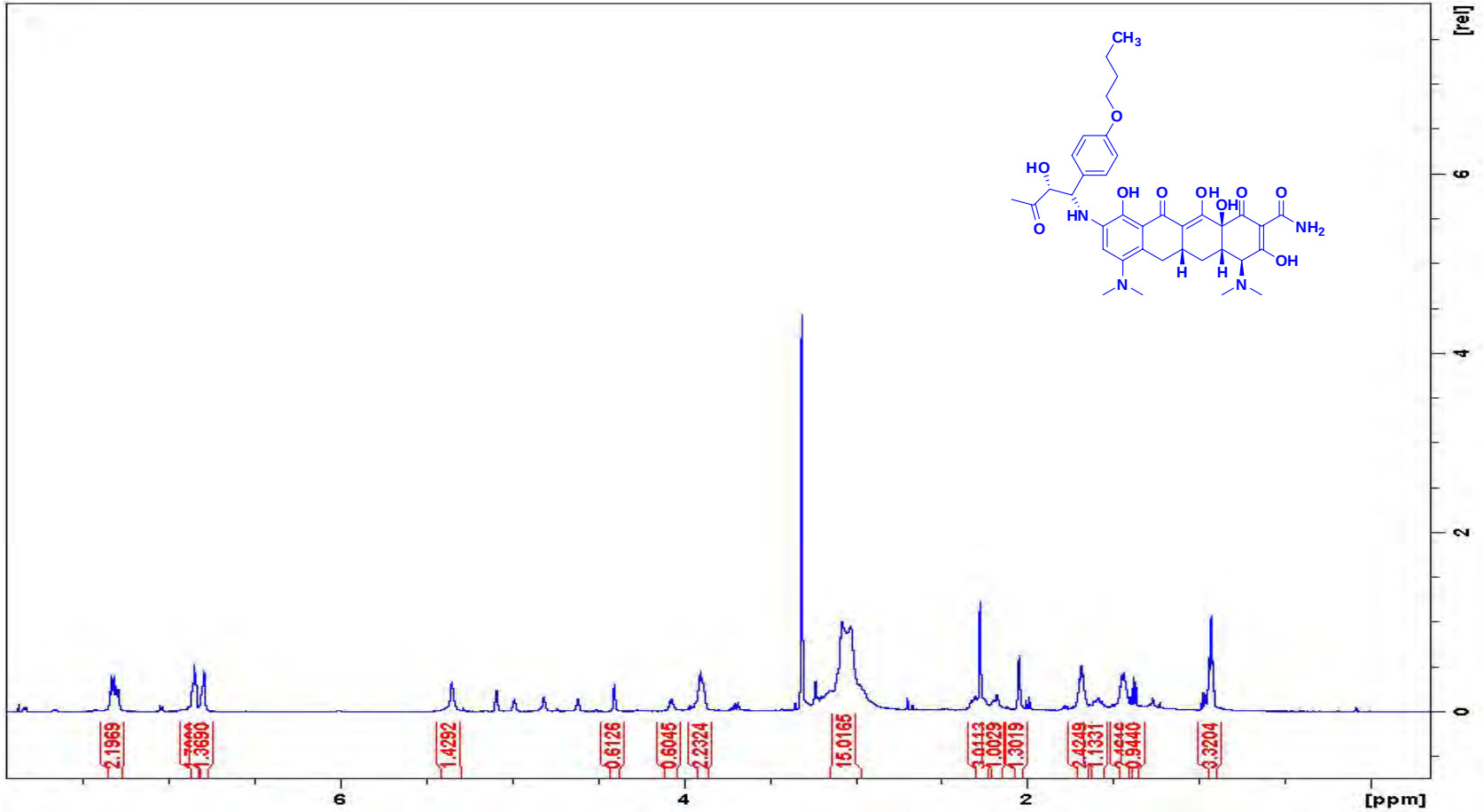
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-((1R)-(2-oxocyclohexyl)(phenyl)methylamino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (1d)



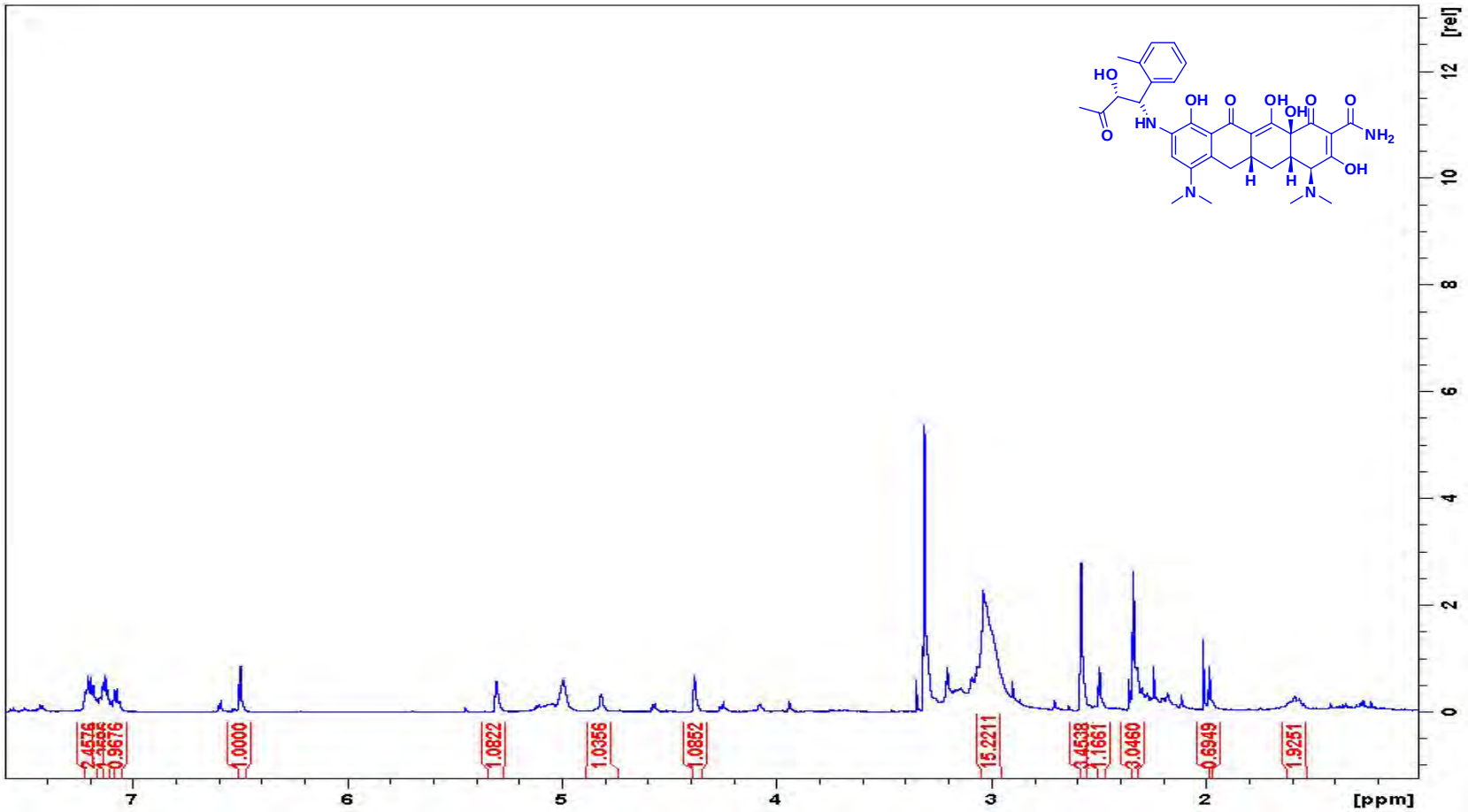
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(4-nitrophenyl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2a)



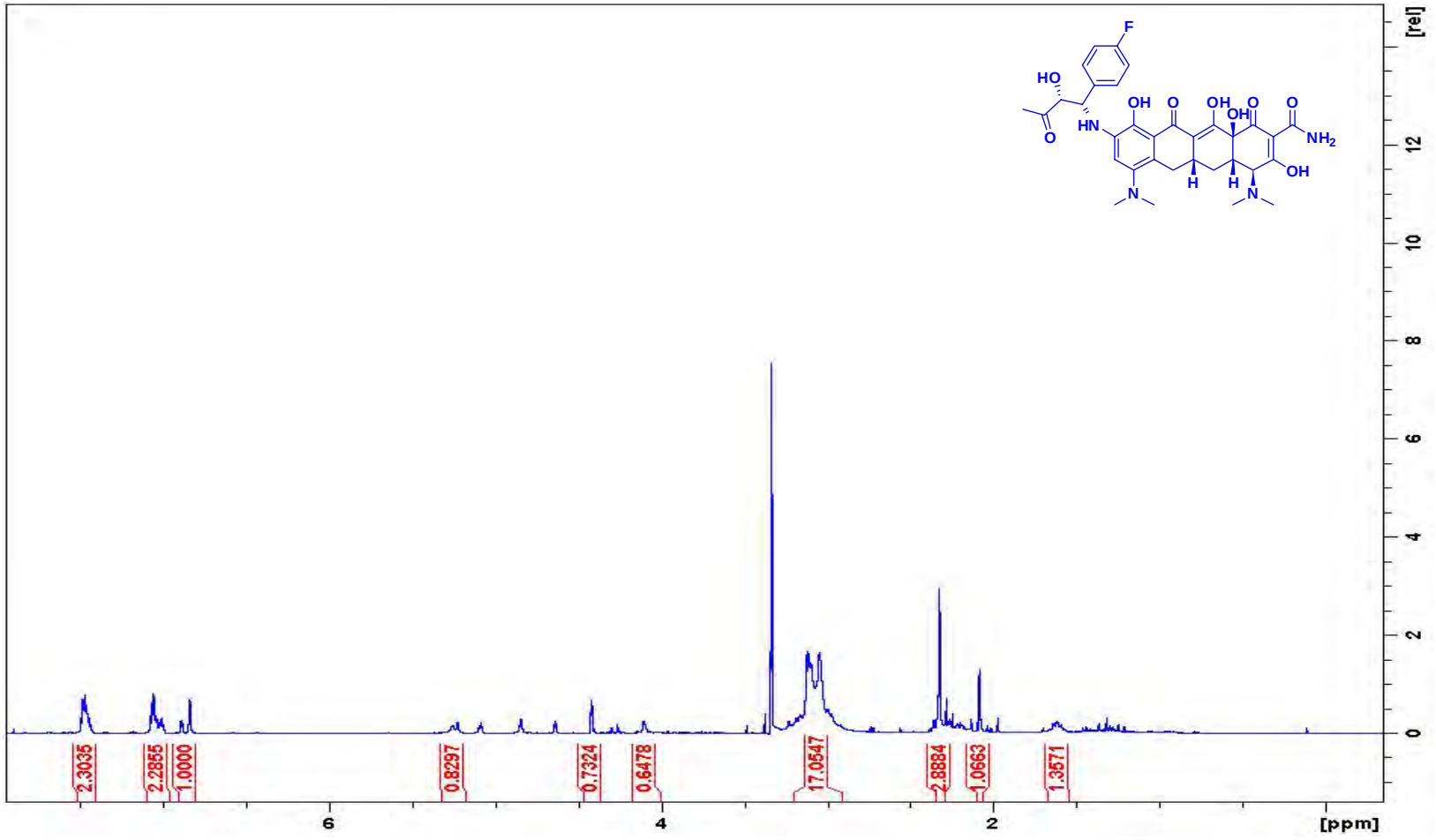
^{1}H NMR of (4S,4aS,5aR,12aS)-9-((1S,2R)-1-(4-butoxyphenyl)-2-hydroxy-3-oxobutylamino)-4,7 bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2b)



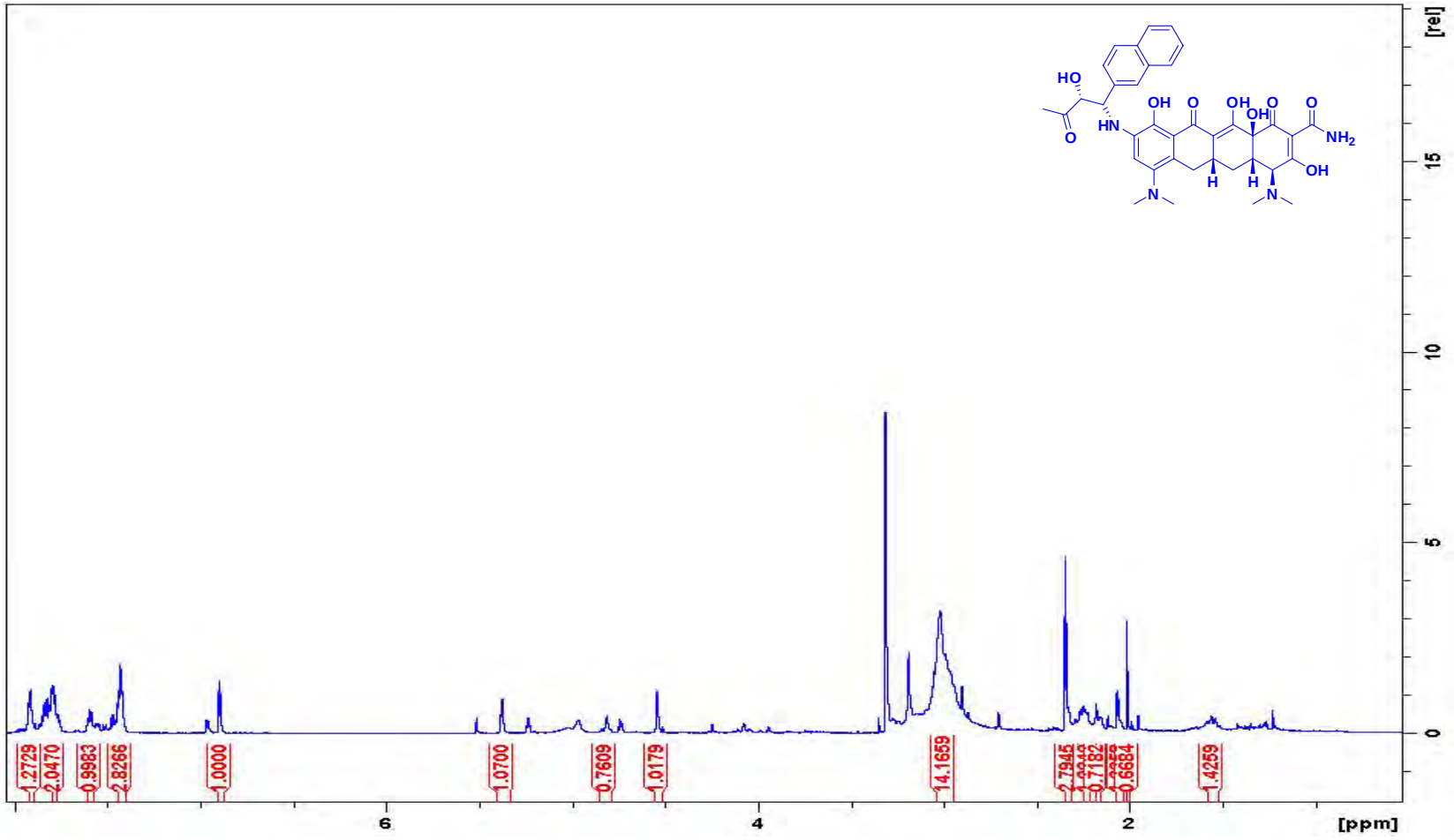
^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-3-oxo-1-o-tolylbutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2c)



^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1S,2R)-1-(4-fluorophenyl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2d)



^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-((1S,2R)-2-hydroxy-1-(naphthalen-2-yl)-3-oxobutylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2e)



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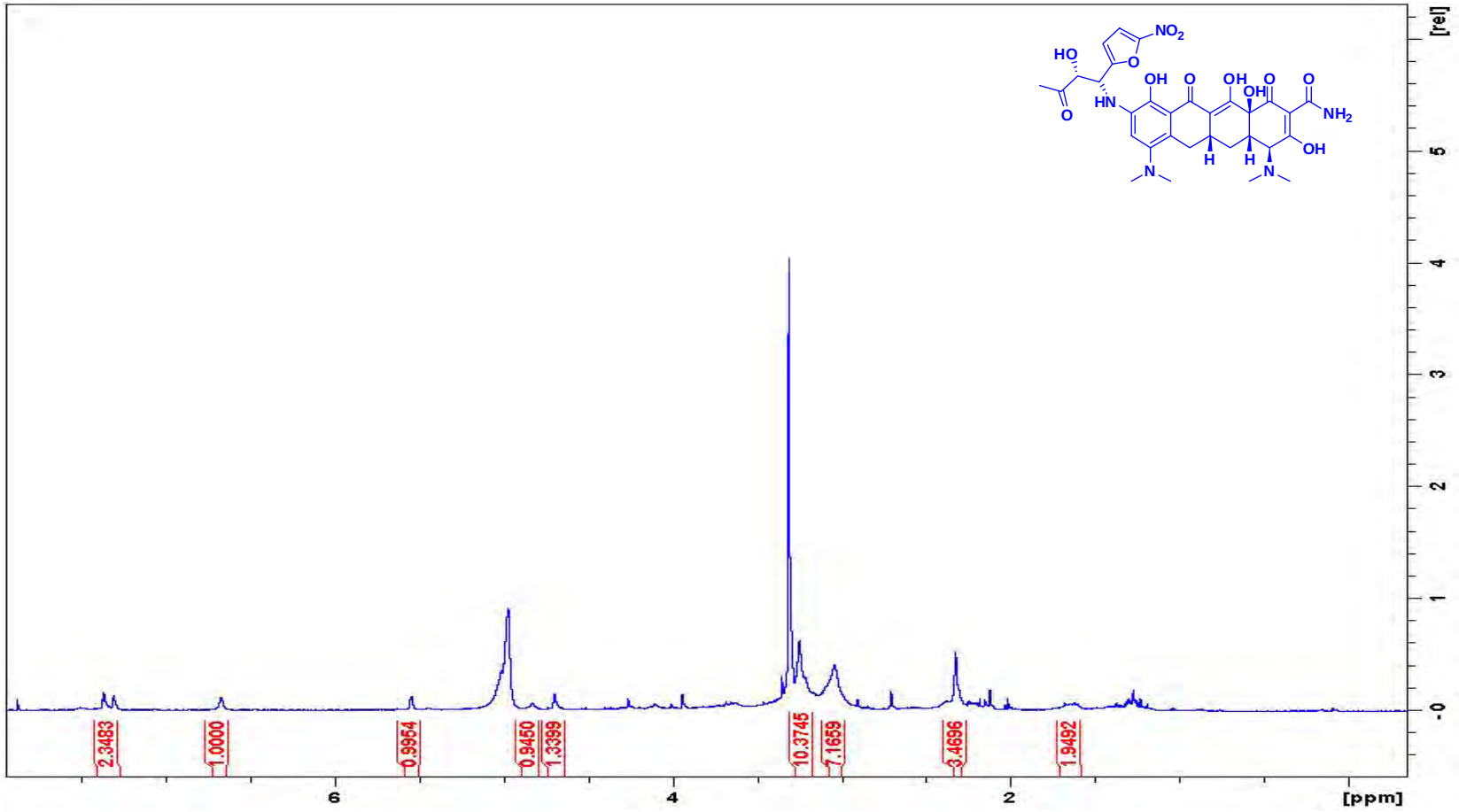
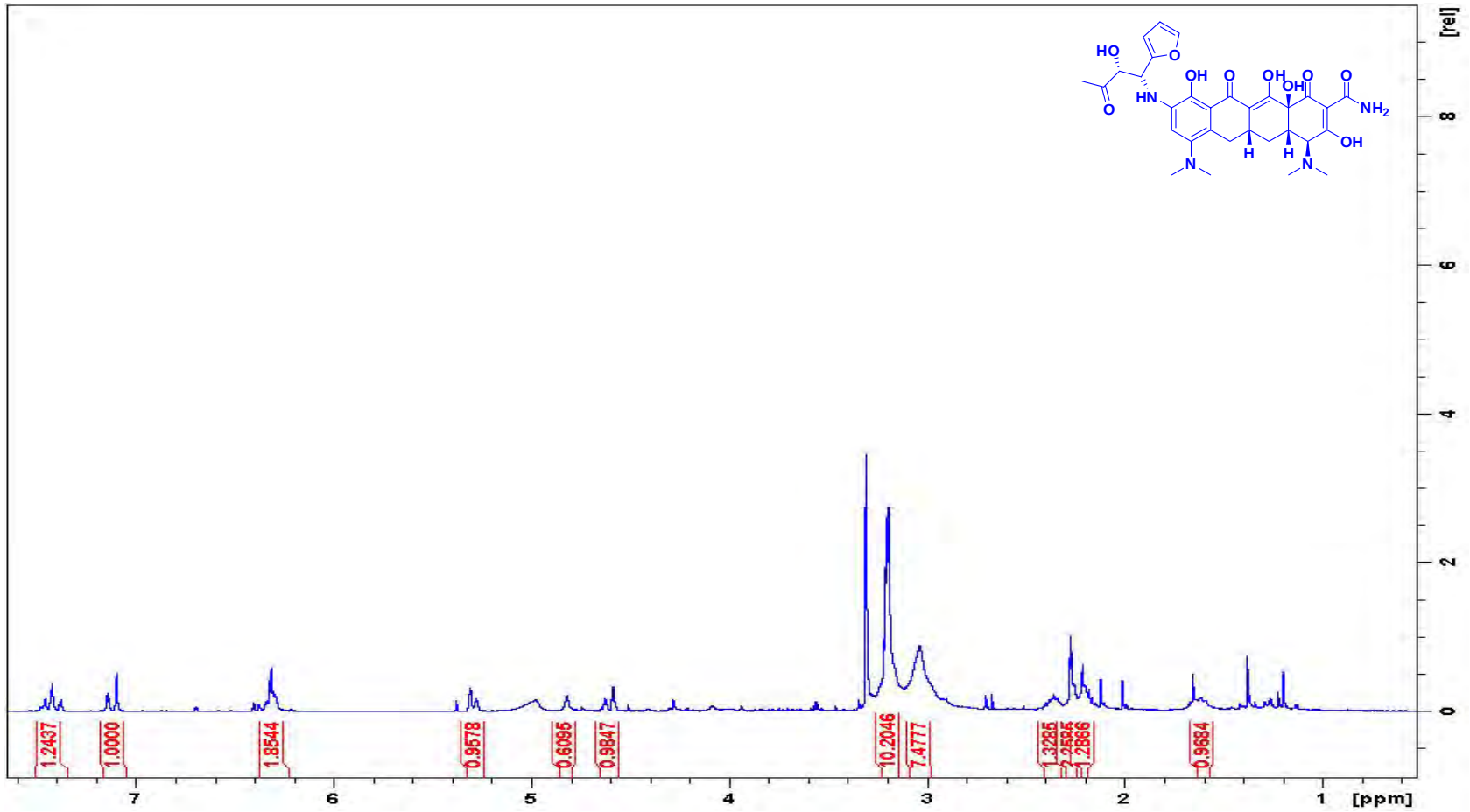


^{1}H NMR of (4S,4aS,5aR,12aS)-4,7-bis(dimethylamino)-9-((1R,2R)-1-(furan-2-yl)-2-hydroxy-3-oxobutylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2g)



^{1}H NMR of (4S,4aS,5aR,12aS)-9-(1-(5-bromofuran-2-yl)-2-hydroxy-3-oxobutylamino)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2h)