

Supporting Information

Synthesis and Evaluation of Small Molecule Drug Conjugates

Harnessing Thioester-linked Maytansinoids

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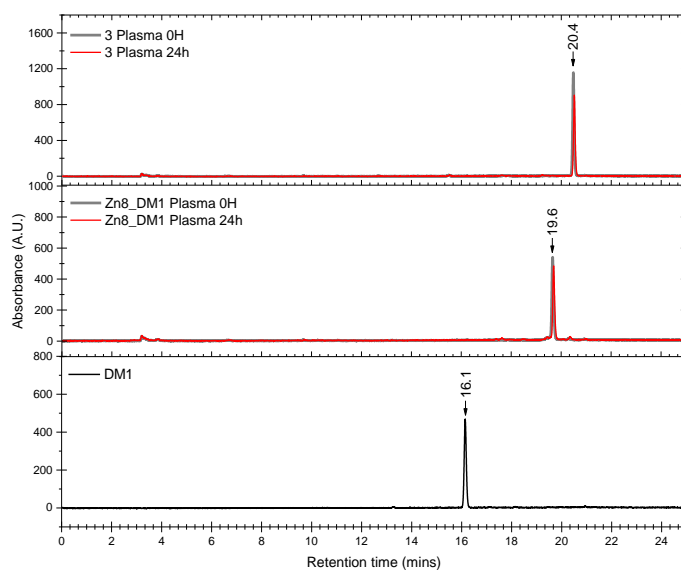
² Institute of Molecular and Genomic Medicine, National Health Research Institutes, Miaoli 35053, Taiwan, ROC

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(kelvintsou@nhri.edu.tw)

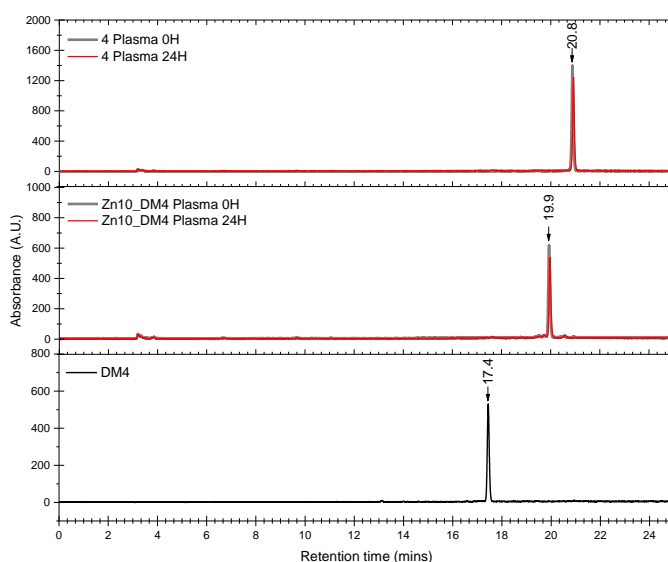
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Zn8_DM1: 0H VS. 24H in plasma.



Zn10_DM4: 0H VS. 24H in plasma.



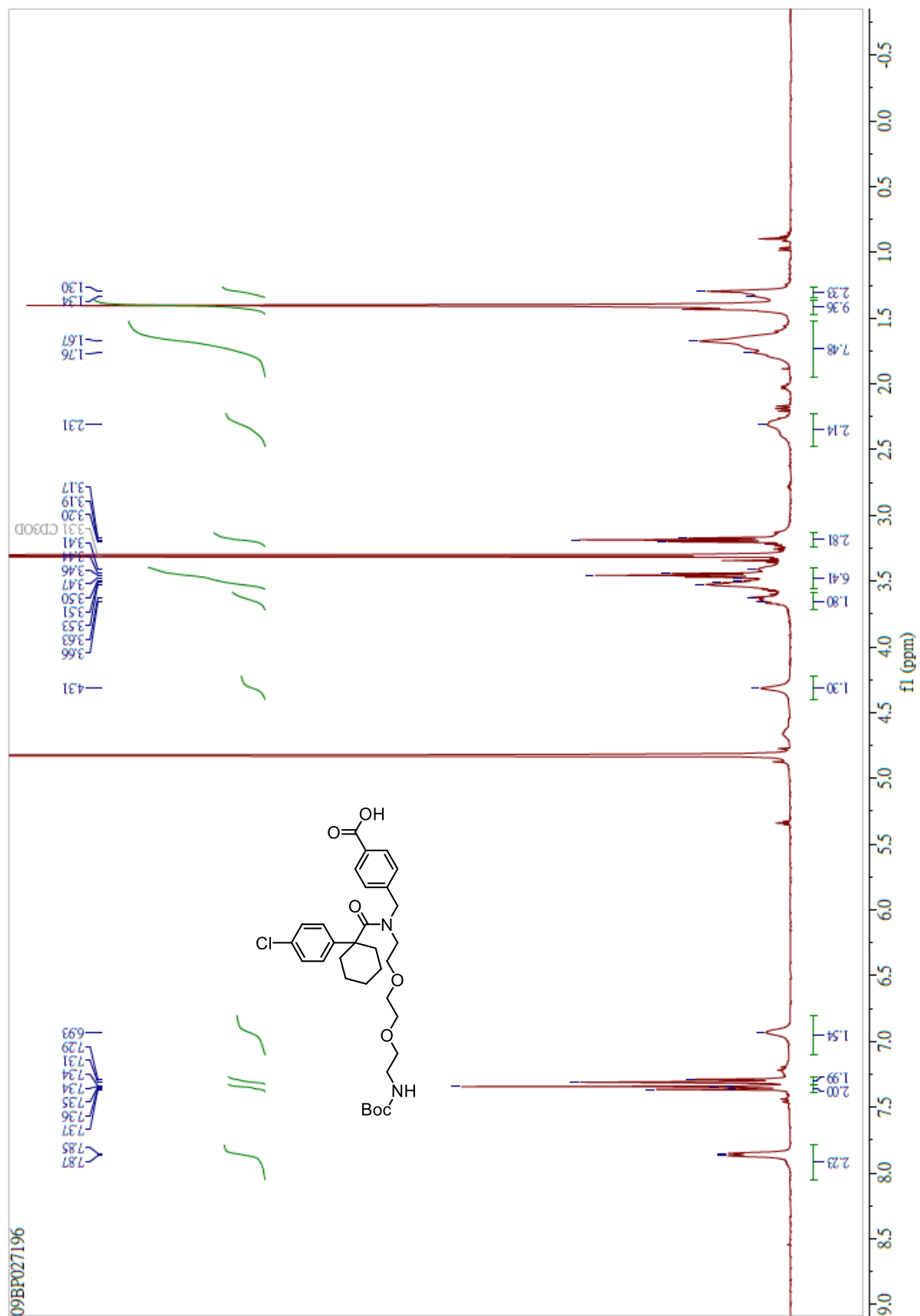
Supporting Figure S1. Incubation of Linker-drug 3 and 4, conjugates Zn8_DM1 and Zn10_DM4, DM1 and DM4 with plasma for 24 hrs. HPLC condition is listed below.

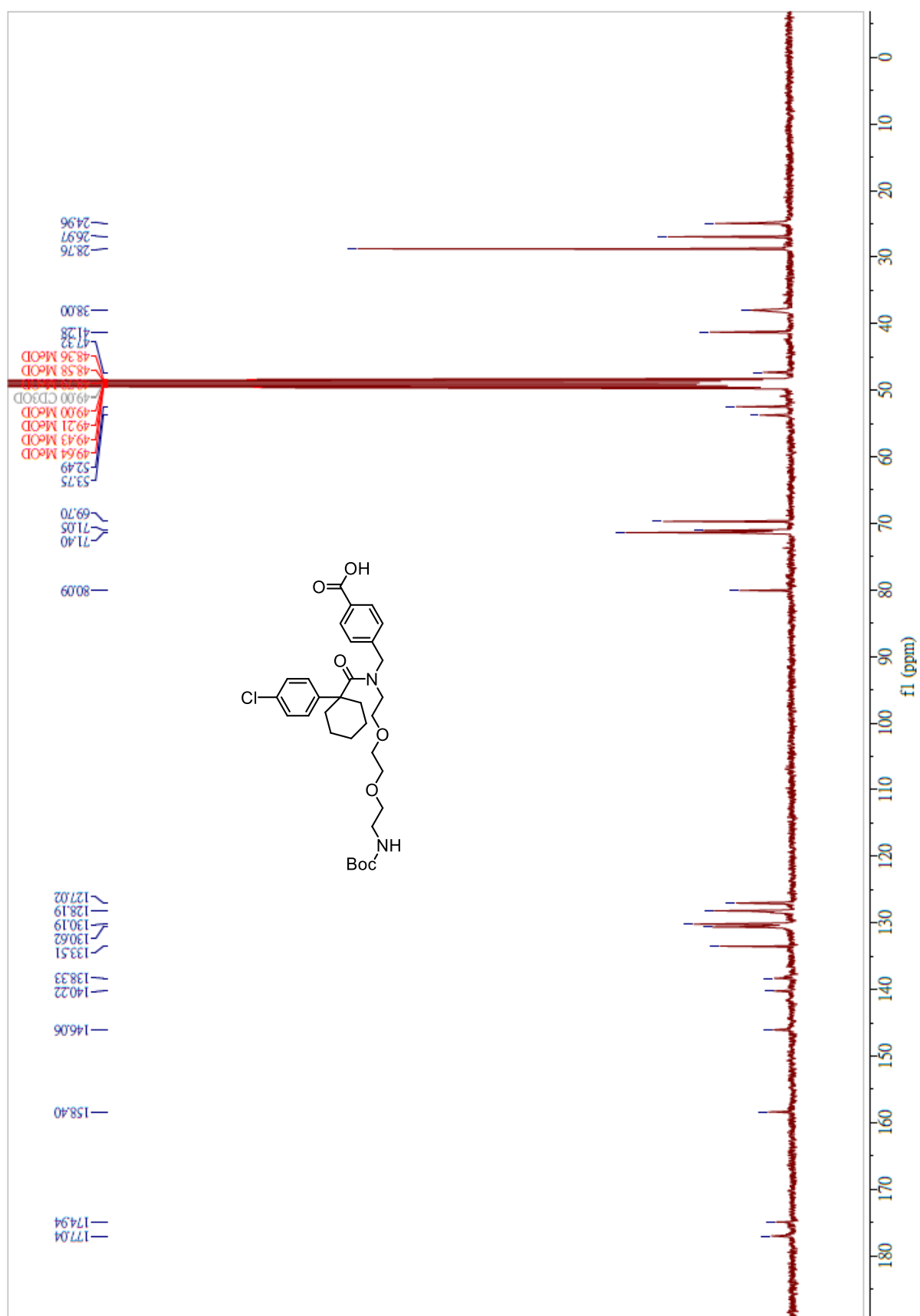
HPLC Information

Agilent 1100 HPLC was utilized in this project. Detailed chromatographic conditions used for purity analysis are listed in Table S1.

Table S1. Chromatographic conditions and parameters

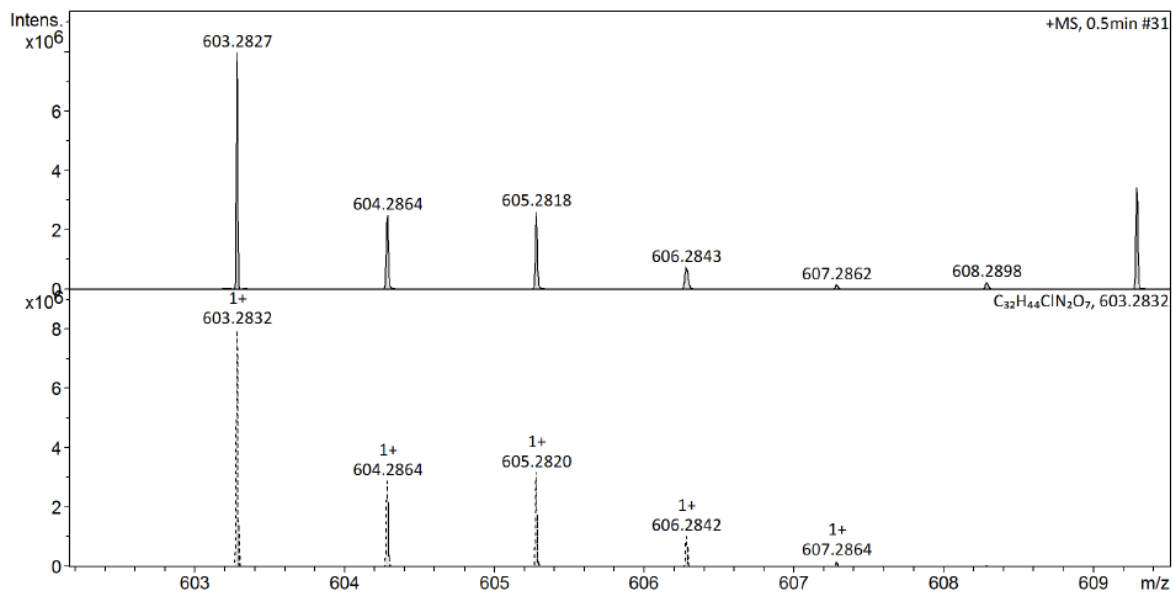
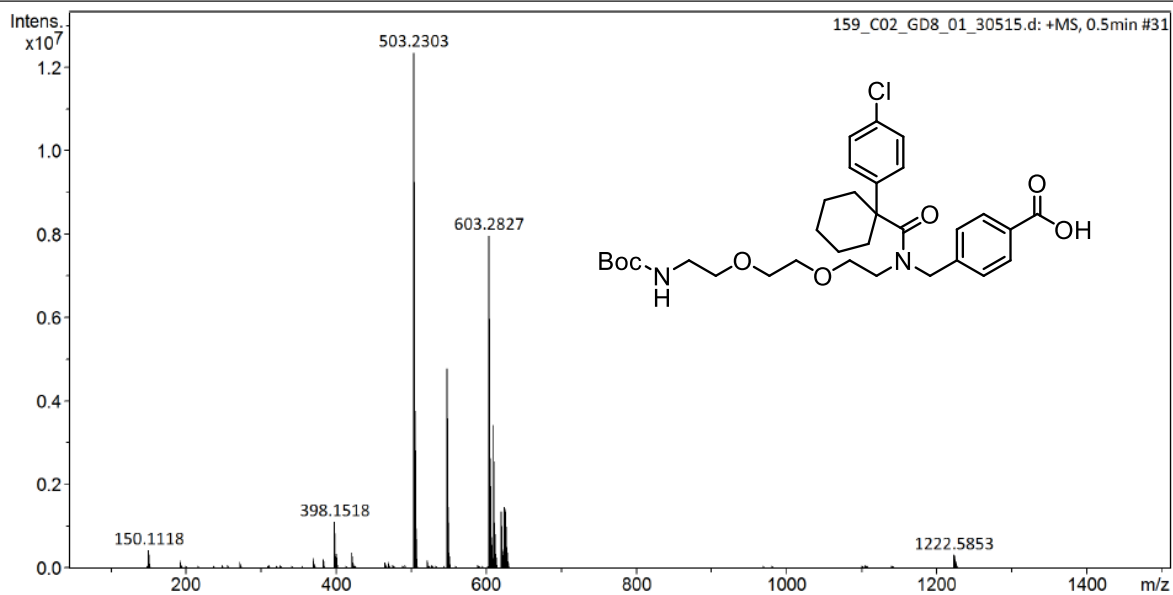
Parameters	Value
Column	Thermo hypersil GOLD 250*4.6 particle: 5µm
Mobile Phase	A: 0.1% TFA in H ₂ O ; B: MeOH
Gradient table	Time(min) % B
	0 10
	2 10
	6 50
	16 90
	17 90
Flow rate	0.5 ml/min
Injection volume	10µL
Detector wavelength	UV at 254 nm
Run time	25 min
Post time	6 min
Column temperature	RT
Sample temperature	RT





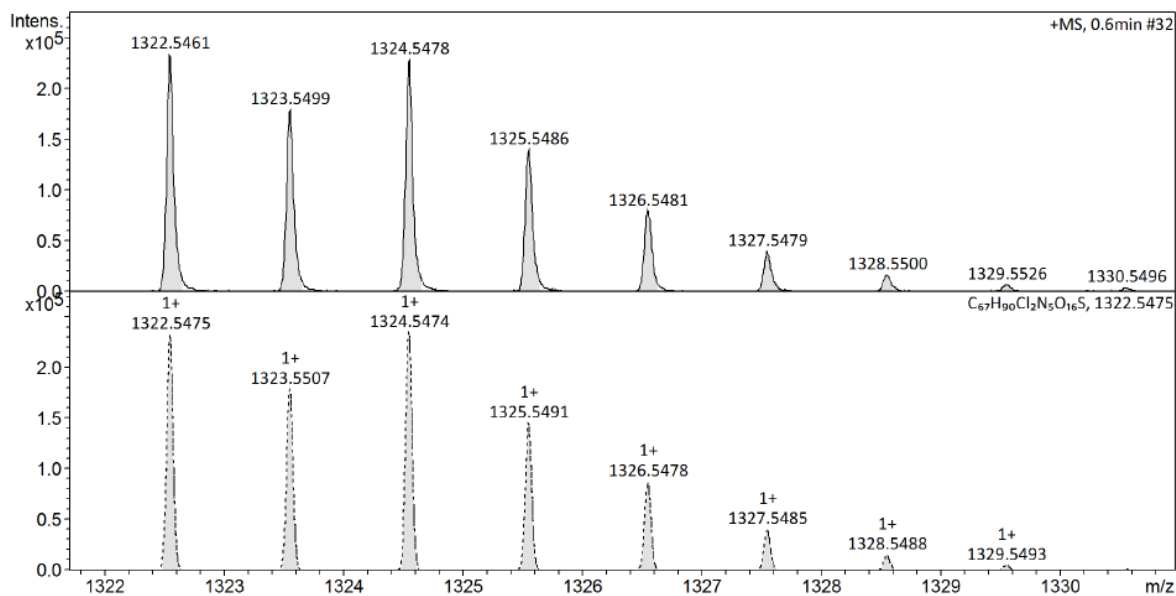
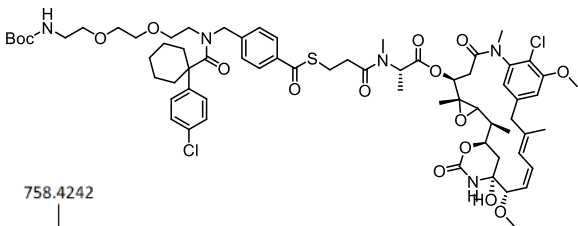
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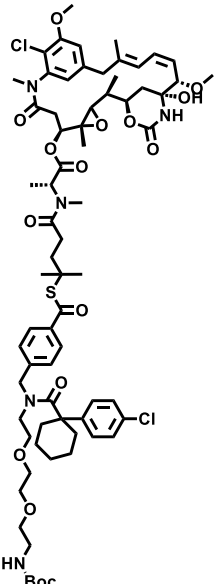
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Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

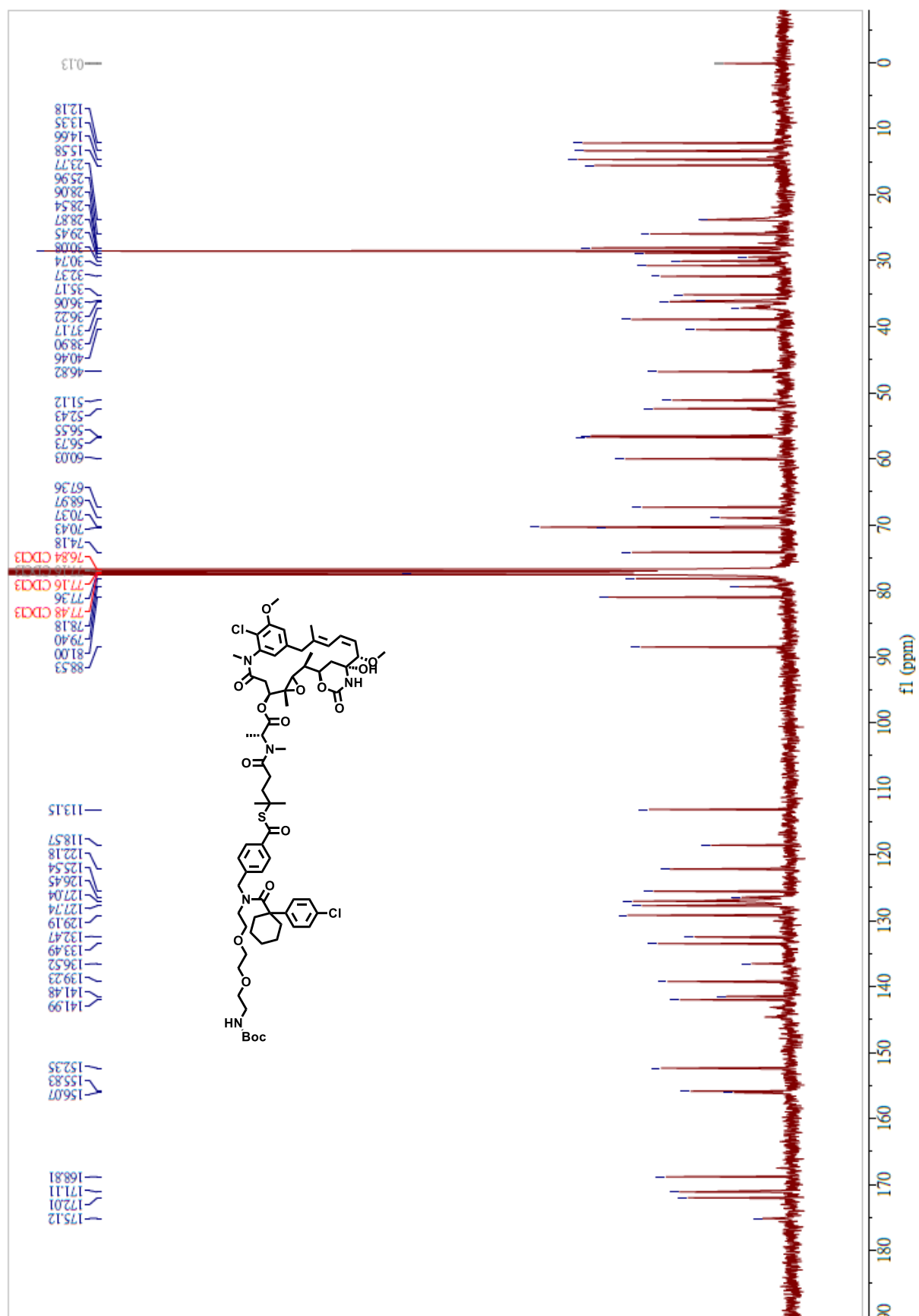




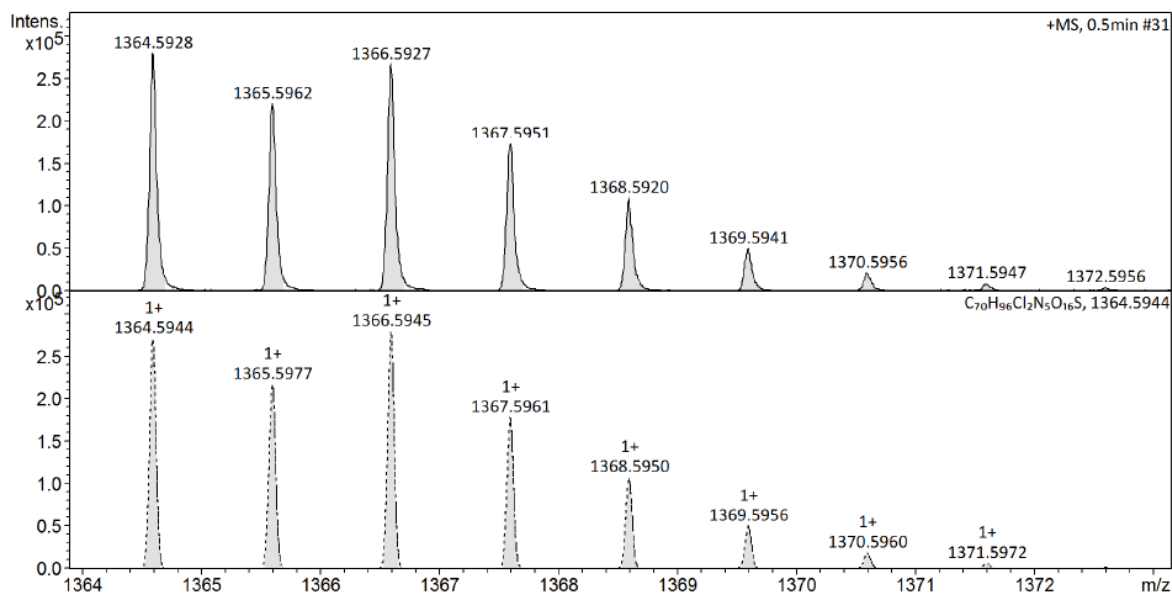
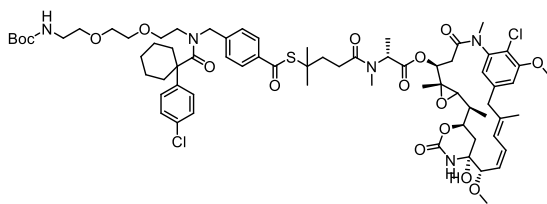
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Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

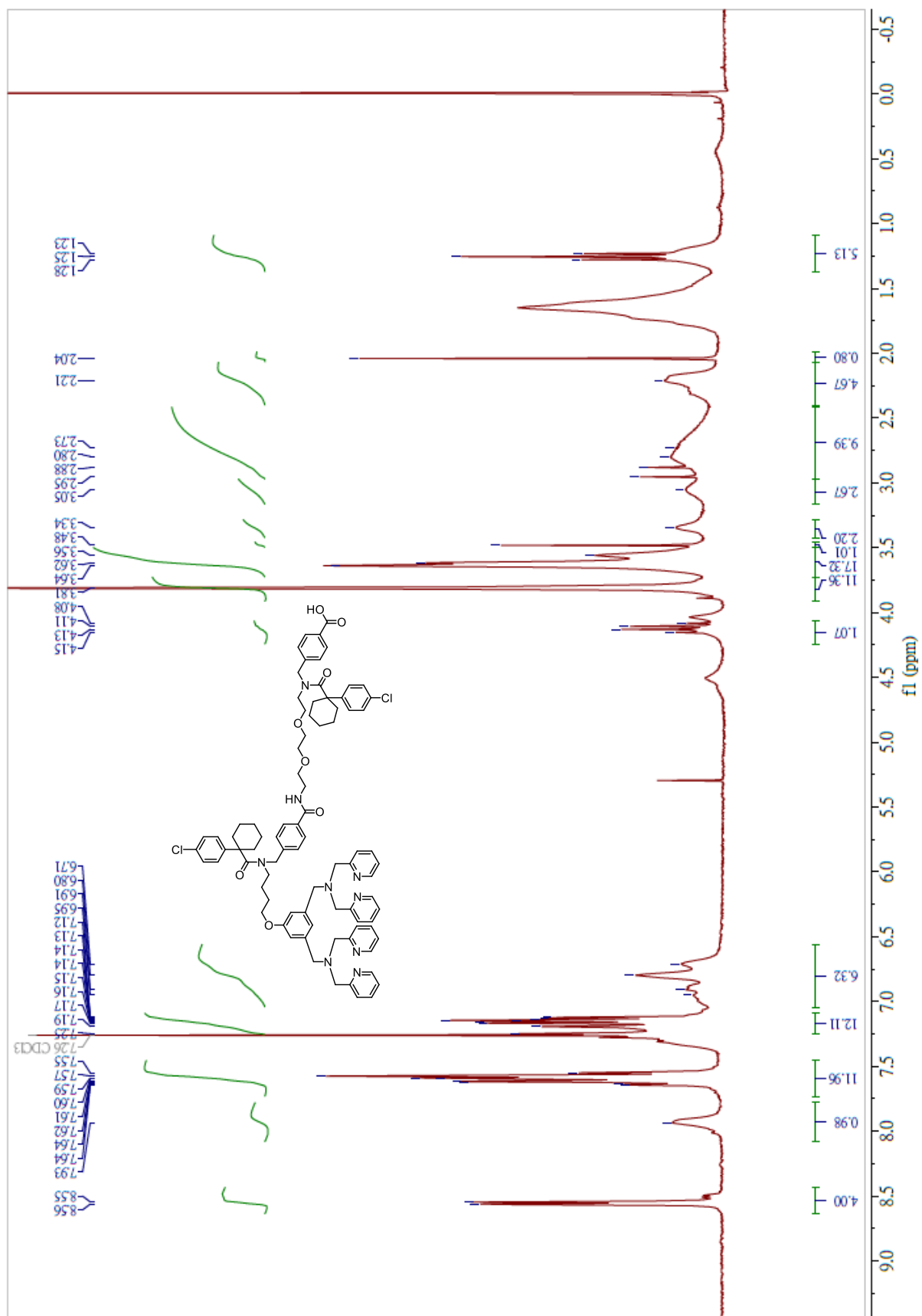


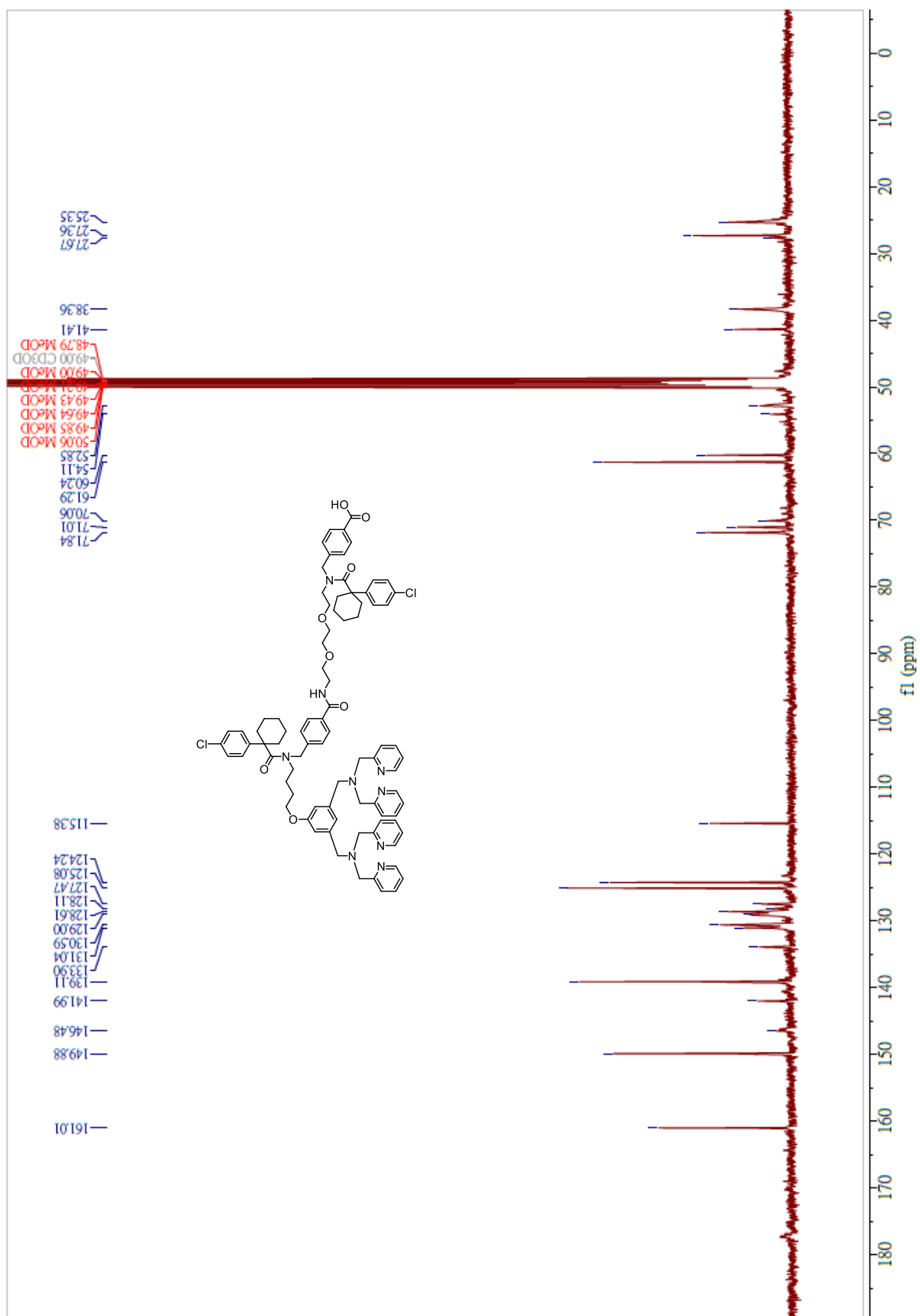




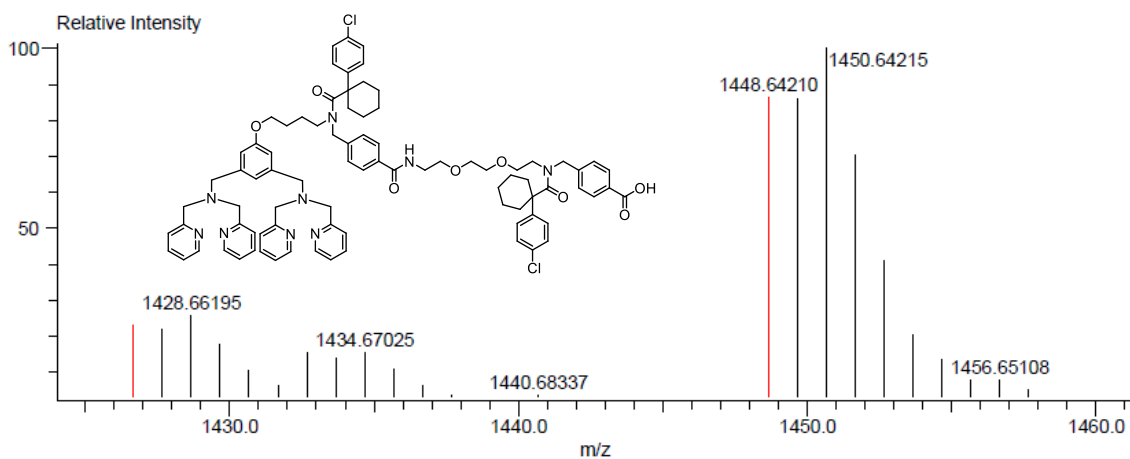
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Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C







Charge number:1 Tolerance:150.00[ppm], 100.00 .. 100.... Unsaturation Number:-200.5 .. 300.0 (...
 Element:¹²C:84 .. 84, ¹H:0 .. 94, ³⁵Cl:2 .. 2, ¹⁴N:9 .. 9, ²³Na:0 .. 2, ¹⁶O:8 .. 8



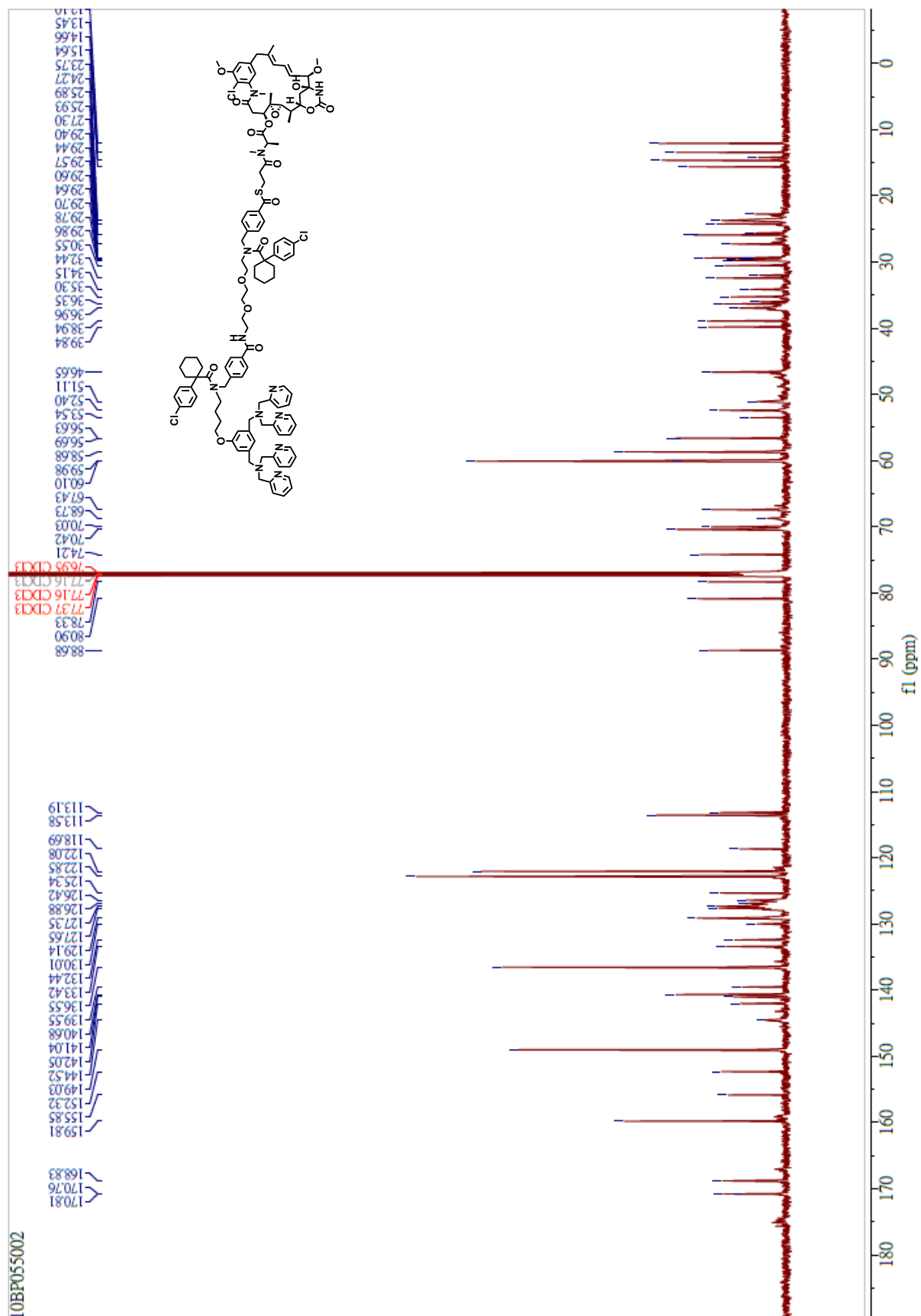
Mass	Intensity	Calc. Mass	Mass Difference [ppm]	Possible Formula
1426.66139	28257.29	1426.66024	0.81	¹² C ₈₄ ¹ H ₉₄ ³⁵ Cl ₂ ¹⁴ N ₉ ¹⁶ O ₈
1448.64210	106156.39	1448.64218	-0.06	¹² C ₈₄ ¹ H ₉₃ ³⁵ Cl ₂ ¹⁴ N ₉ ²³ Na ₁ ¹⁶ O ₈

10BP055002

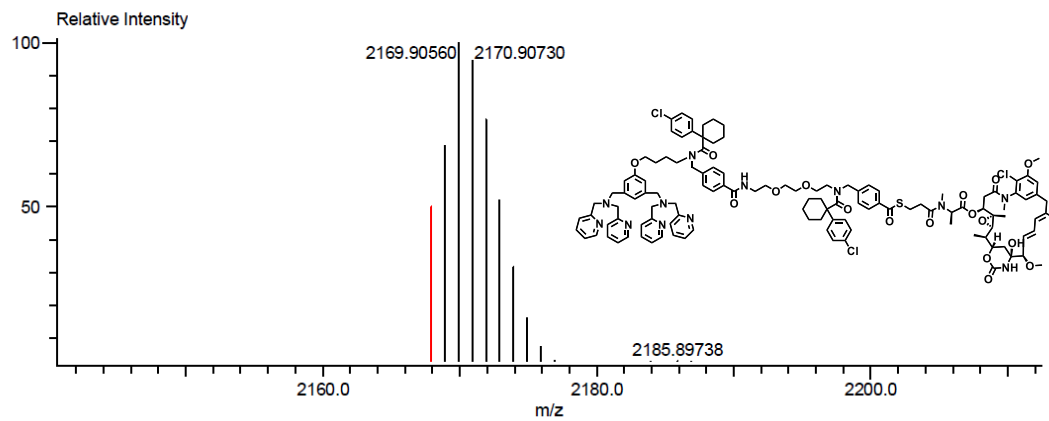
¹H NMR spectrum (CDCl₃) of compound 10BP055002. The x-axis represents the chemical shift in ppm, ranging from 0.78 to 8.49. The spectrum shows several peaks, with integration values indicated below the baseline. A broad peak at 1.62 ppm is labeled H₂O. A sharp peak at 7.26 ppm is labeled CDCl₃. The chemical structure of the compound is shown above the spectrum.

Chemical structure of 10BP055002:

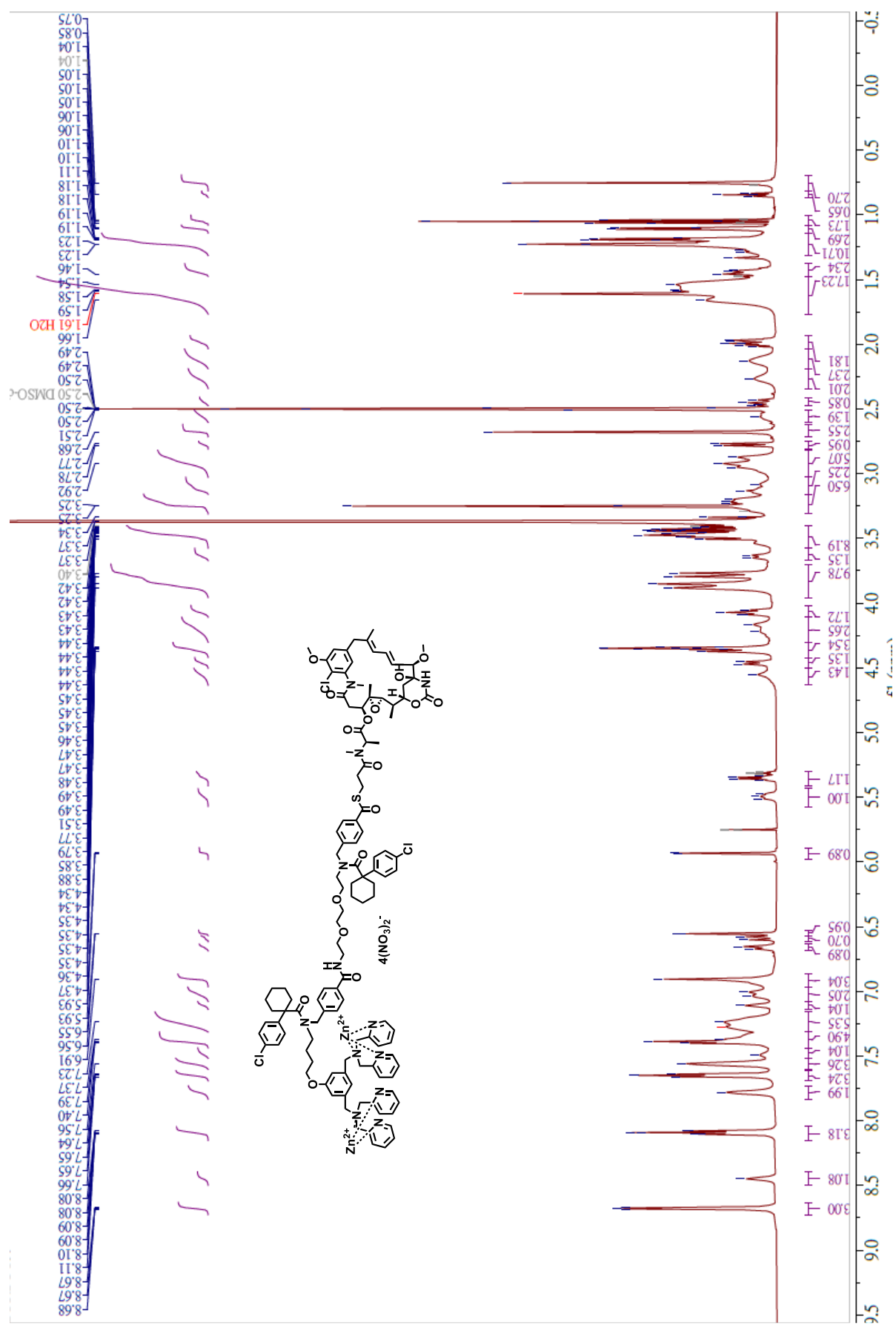
CC(=O)N[C@@H]1C[C@H](C(=O)N[C@@H]2C[C@H](C(=O)N[C@@H]3C[C@H](C(=O)N[C@@H]4C[C@H](C(=O)N[C@@H]5C[C@H](C(=O)N[C@@H]6C[C@H](C(=O)N[C@@H]7C[C@H](C(=O)N[C@@H]8C[C@H](C(=O)N[C@@H]9C[C@H](C(=O)N[C@@H]10C[C@H](C(=O)N[C@@H]11C[C@H](C(=O)N[C@@H]12C[C@H](C(=O)N[C@@H]13C[C@H](C(=O)N[C@@H]14C[C@H](C(=O)N[C@@H]15C[C@H](C(=O)N[C@@H]16C[C@H](C(=O)N[C@@H]17C[C@H](C(=O)N[C@@H]18C[C@H](C(=O)N[C@@H]19C[C@H](C(=O)N[C@@H]20C[C@H](C(=O)N[C@@H]21C[C@H](C(=O)N[C@@H]22C[C@H](C(=O)N[C@@H]23C[C@H](C(=O)N[C@@H]24C[C@H](C(=O)N[C@@H]25C[C@H](C(=O)N[C@@H]26C[C@H](C(=O)N[C@@H]27C[C@H](C(=O)N[C@@H]28C[C@H](C(=O)N[C@@H]29C[C@H](C(=O)N[C@@H]30C[C@H](C(=O)N[C@@H]31C[C@H](C(=O)N[C@@H]32C[C@H](C(=O)N[C@@H]33C[C@H](C(=O)N[C@@H]34C[C@H](C(=O)N[C@@H]35C[C@H](C(=O)N[C@@H]36C[C@H](C(=O)N[C@@H]37C[C@H](C(=O)N[C@@H]38C[C@H](C(=O)N[C@@H]39C[C@H](C(=O)N[C@@H]40C[C@H](C(=O)N[C@@H]41C[C@H](C(=O)N[C@@H]42C[C@H](C(=O)N[C@@H]43C[C@H](C(=O)N[C@@H]44C[C@H](C(=O)N[C@@H]45C[C@H](C(=O)N[C@@H]46C[C@H](C(=O)N[C@@H]47C[C@H](C(=O)N[C@@H]48C[C@H](C(=O)N[C@@H]49C[C@H](C(=O)N[C@@H]50C[C@H](C(=O)N[C@@H]51C[C@H](C(=O)N[C@@H]52C[C@H](C(=O)N[C@@H]53C[C@H](C(=O)N[C@@H]54C[C@H](C(=O)N[C@@H]55C[C@H](C(=O)N[C@@H]56C[C@H](C(=O)N[C@@H]57C[C@H](C(=O)N[C@@H]58C[C@H](C(=O)N[C@@H]59C[C@H](C(=O)N[C@@H]60C[C@H](C(=O)N[C@@H]61C[C@H](C(=O)N[C@@H]62C[C@H](C(=O)N[C@@H]63C[C@H](C(=O)N[C@@H]64C[C@H](C(=O)N[C@@H]65C[C@H](C(=O)N[C@@H]66C[C@H](C(=O)N[C@@H]67C[C@H](C(=O)N[C@@H]68C[C@H](C(=O)N[C@@H]69C[C@H](C(=O)N[C@@H]70C[C@H](C(=O)N[C@@H]71C[C@H](C(=O)N[C@@H]72C[C@H](C(=O)N[C@@H]73C[C@H](C(=O)N[C@@H]74C[C@H](C(=O)N[C@@H]75C[C@H](C(=O)N[C@@H]76C[C@H](C(=O)N[C@@H]77C[C@H](C(=O)N[C@@H]78C[C@H](C(=O)N[C@@H]79C[C@H](C(=O)N[C@@H]80C[C@H](C(=O)N[C@@H]81C[C@H](C(=O)N[C@@H]82C[C@H](C(=O)N[C@@H]83C[C@H](C(=O)N[C@@H]84C[C@H](C(=O)N[C@@H]85C[C@H](C(=O)N[C@@H]86C[C@H](C(=O)N[C@@H]87C[C@H](C(=O)N[C@@H]88C[C@H](C(=O)N[C@@H]89C[C@H](C(=O)N[C@@H]90C[C@H](C(=O)N[C@@H]91C[C@H](C(=O)N[C@@H]92C[C@H](C(=O)N[C@@H]93C[C@H](C(=O)N[C@@H]94C[C@H](C(=O)N[C@@H]95C[C@H](C(=O)N[C@@H]96C[C@H](C(=O)N[C@@H]97C[C@H](C(=O)N[C@@H]98C[C@H](C(=O)N[C@@H]99C[C@H](C(=O)N[C@@H]100C[C@H](C(=O)N[C@@H]101C[C@H](C(=O)N[C@@H]102C[C@H](C(=O)N[C@@H]103C[C@H](C(=O)N[C@@H]104C[C@H](C(=O)N[C@@H]105C[C@H](C(=O)N[C@@H]106C[C@H](C(=O)N[C@@H]107C[C@H](C(=O)N[C@@H]108C[C@H](C(=O)N[C@@H]109C[C@H](C(=O)N[C@@H]110C[C@H](C(=O)N[C@@H]111C[C@H](C(=O)N[C@@H]112C[C@H](C(=O)N[C@@H]113C[C@H](C(=O)N[C@@H]114C[C@H](C(=O)N[C@@H]115C[C@H](C(=O)N[C@@H]116C[C@H](C(=O)N[C@@H]117C[C@H](C(=O)N[C@@H]118C[C@H](C(=O)N[C@@H]119C[C@H](C(=O)N[C@@H]120C[C@H](C(=O)N[C@@H]121C[C@H](C(=O)N[C@@H]122C[C@H](C(=O)N[C@@H]123C[C@H](C(=O)N[C@@H]124C[C@H](C(=O)N[C@@H]125C[C@H](C(=O)N[C@@H]126C[C@H](C(=O)N[C@@H]127C[C@H](C(=O)N[C@@H]128C[C@H](C(=O)N[C@@H]129C[C@H](C(=O)N[C@@H]130C[C@H](C(=O)N[C@@H]131C[C@H](C(=O)N[C@@H]132C[C@H](C(=O)N[C@@H]133C[C@H](C(=O)N[C@@H]134C[C@H](C(=O)N[C@@H]135C[C@H](C(=O)N[C@@H]136C[C@H](C(=O)N[C@@H]137C[C@H](C(=O)N[C@@H]138C[C@H](C(=O)N[C@@H]139C[C@H](C(=O)N[C@@H]140C[C@H](C(=O)N[C@@H]141C[C@H](C(=O)N[C@@H]142C[C@H](C(=O)N[C@@H]143C[C@H](C(=O)N[C@@H]144C[C@H](C(=O)N[C@@H]145C[C@H](C(=O)N[C@@H]146C[C@H](C(=O)N[C@@H]147C[C@H](C(=O)N[C@@H]148C[C@H](C(=O)N[C@@H]149C[C@H](C(=O)N[C@@H]150C[C@H](C(=O)N[C@@H]151C[C@H](C(=O)N[C@@H]152C[C@H](C(=O)N[C@@H]153C[C@H](C(=O)N[C@@H]154C[C@H](C(=O)N[C@@H]155C[C@H](C(=O)N[C@@H]156C[C@H](C(=O)N[C@@H]157C[C@H](C(=O)N[C@@H]158C[C@H](C(=O)N[C@@H]159C[C@H](C(=O)N[C@@H]160C[C@H](C(=O)N[C@@H]161C[C@H](C(=O)N[C@@H]162C[C@H](C(=O)N[C@@H]163C[C@H](C(=O)N[C@@H]164C[C@H](C(=O)N[C@@H]165C[C@H](C(=O)N[C@@H]166C[C@H](C(=O)N[C@@H]167C[C@H](C(=O)N[C@@H]168C[C@H](C(=O)N[C@@H]169C[C@H](C(=O)N[C@@H]170C[C@H](C(=O)N[C@@H]171C[C@H](C(=O)N[C@@H]172C[C@H](C(=O)N[C@@H]173C[C@H](C(=O)N[C@@H]174C[C@H](C(=O)N[C@@H]175C[C@H](C(=O)N[C@@H]176C[C@H](C(=O)N[C@@H]177C[C@H](C(=O)N[C@@H]178C[C@H](C(=O)N[C@@H]179C[C@H](C(=O)N[C@@H]180C[C@H](C(=O)N[C@@H]181C[C@H](C(=O)N[C@@H]182C[C@H](C(=O)N[C@@H]183C[C@H](C(=O)N[C@@H]184C[C@H](C(=O)N[C@@H]185C[C@H](C(=O)N[C@@H]186C[C@H](C(=O)N[C@@H]187C[C@H](C(=O)N[C@@H]188C[C@H](C(=O)N[C@@H]189C[C@H](C(=O)N[C@@H]190C[C@H](C(=O)N[C@@H]191C[C@H](C(=O)N[C@@H]192C[C@H](C(=O)N[C@@H]193C[C@H](C(=O)N[C@@H]194C[C@H](C(=O)N[C@@H]195C[C@H](C(=O)N[C@@H]196C[C@H](C(=O)N[C@@H]197C[C@H](C(=O)N[C@@H]198C[C@H](C(=O)N[C@@H]199C[C@H](C(=O)N[C@@H]200C[C@H](C(=O)N[C@@H]201C[C@H](C(=O)N[C@@H]202C[C@H](C(=O)N[C@@H]203C[C@H](C(=O)N[C@@H]204C[C@H](C(=O)N[C@@H]205C[C@H](C(=O)N[C@@H]206C[C@H](C(=O)N[C@@H]207C[C@H](C(=O)N[C@@H]208C[C@H](C(=O)N[C@@H]209C[C@H](C(=O)N[C@@H]210C[C@H](C(=O)N[C@@H]211C[C@H](C(=O)N[C@@H]212C[C@H](C(=O)N[C@@H]213C[C@H](C(=O)N[C@@H]214C[C@H](C(=O)N[C@@H]215C[C@H](C(=O)N[C@@H]216C[C@H](C(=O)N[C@@H]217C[C@H](C(=O)N[C@@H]218C[C@H](C(=O)N[C@@H]219C[C



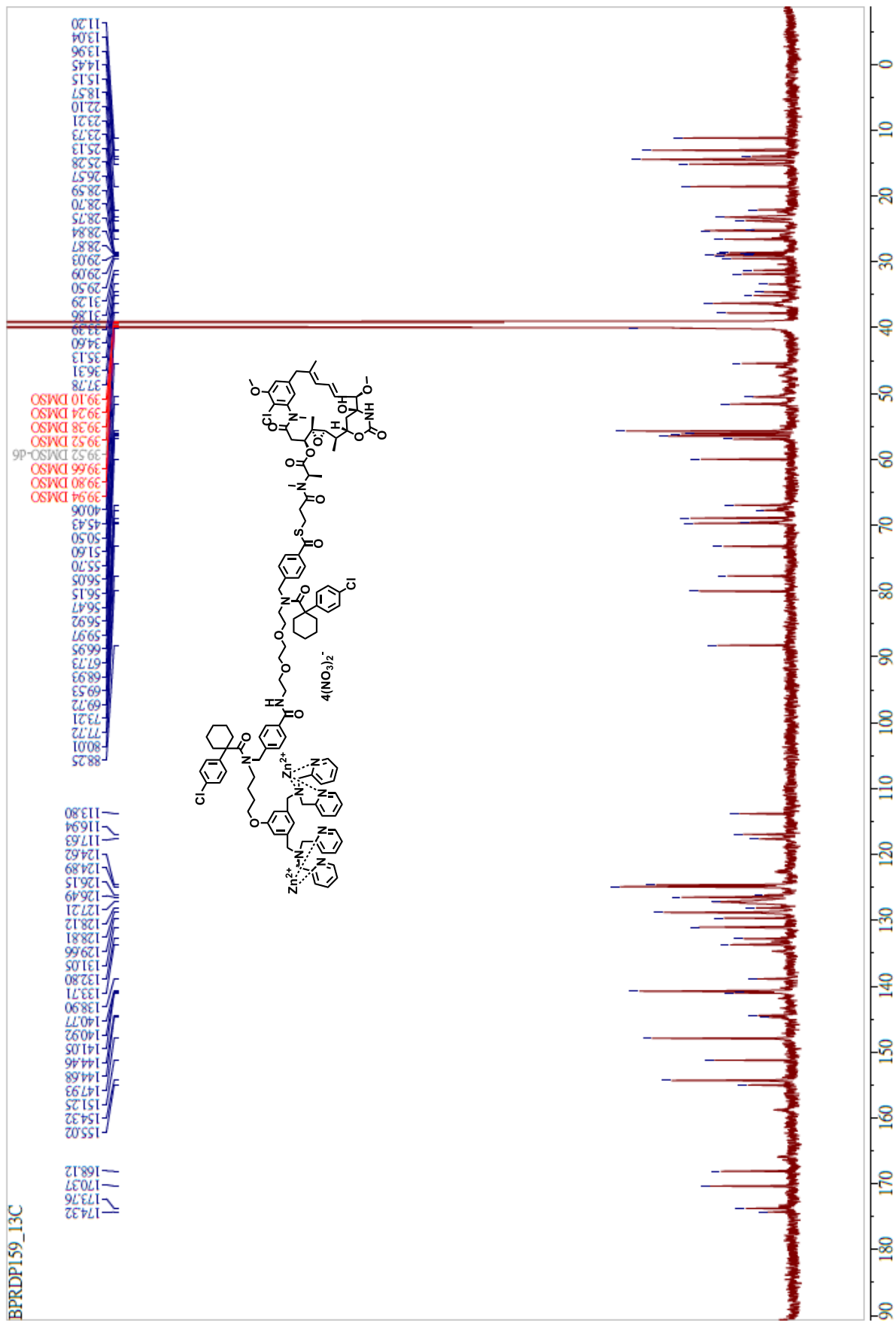
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 Element:¹²C:119 .. 119, ¹H:0 .. 140, ³⁵Cl:3 .. 3, ¹⁴N:12 .. 12, ²³Na:0 .. 1, ¹⁶O:17 .. 17, ³²S:1 .. 1



Mass	Intensity	Calc. Mass	Mass Difference [mDa]	Mass Difference [ppm]
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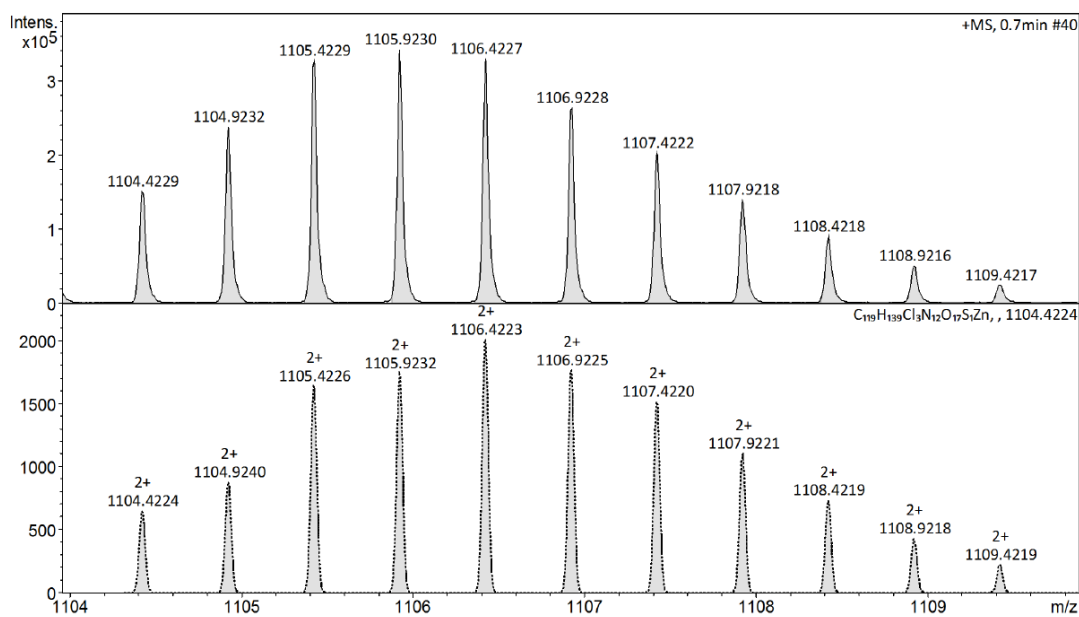
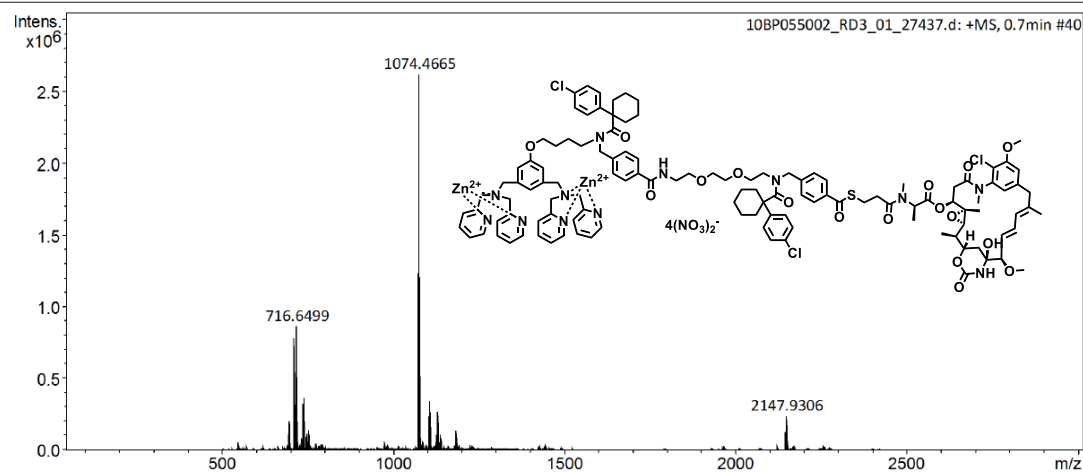


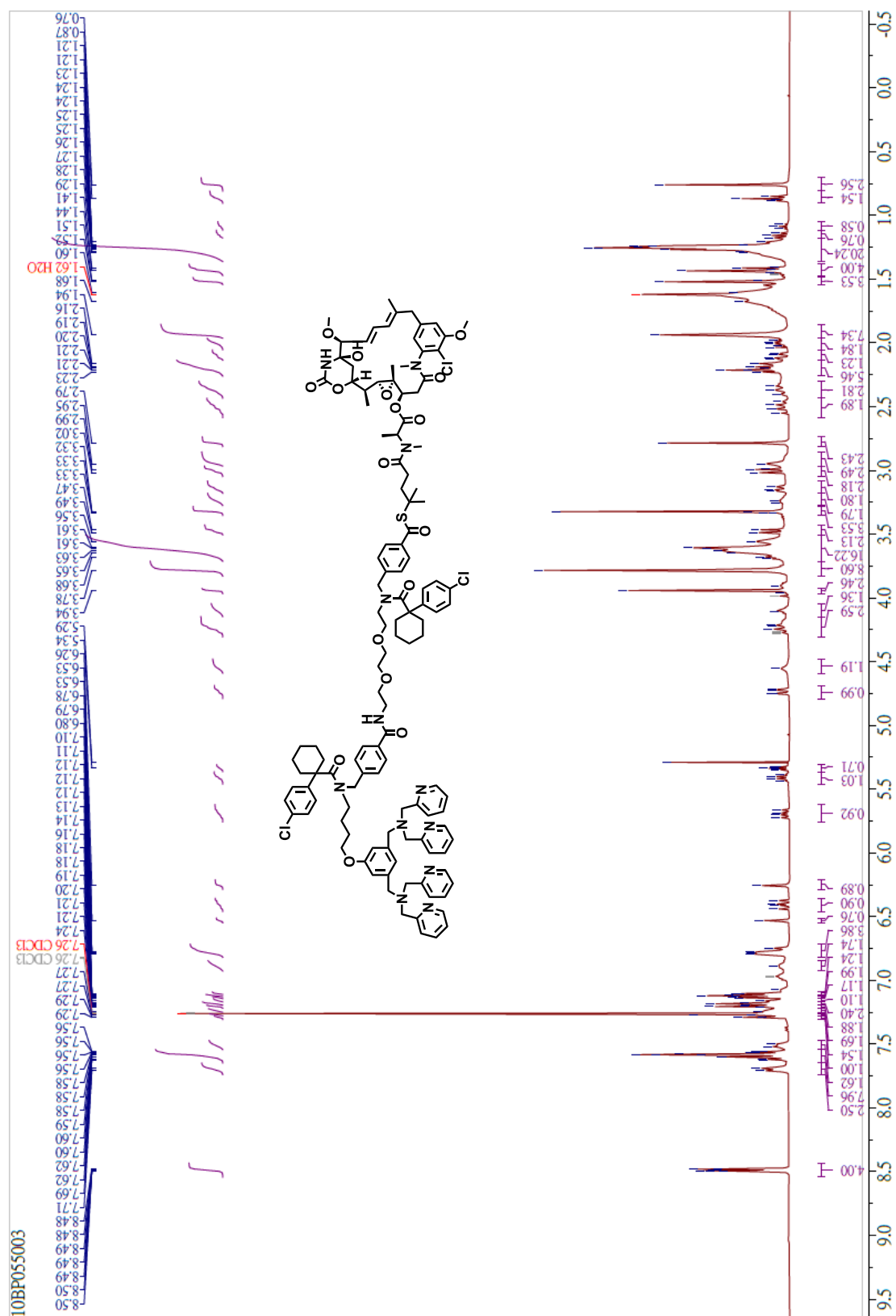
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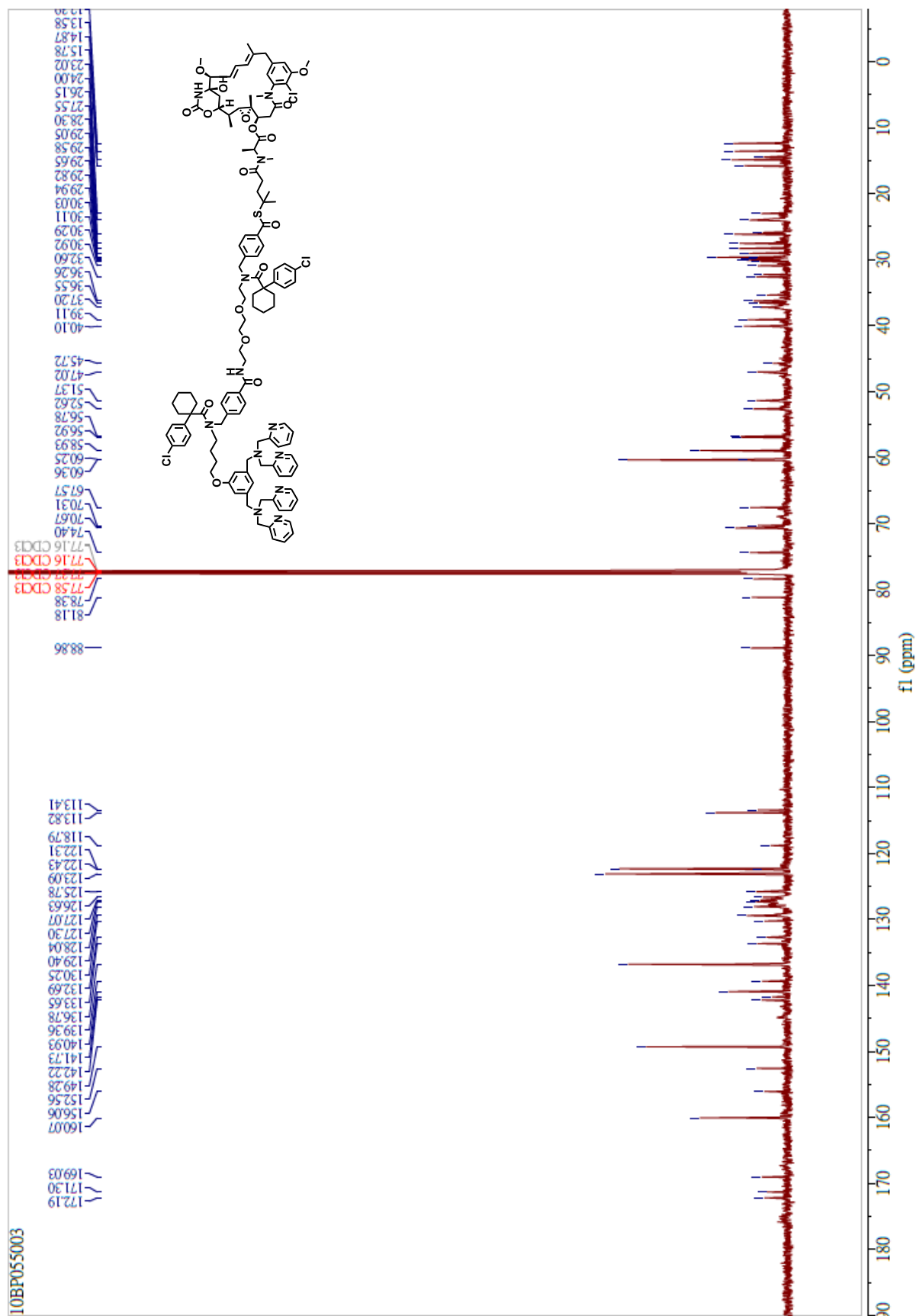


Acquisition Parameter

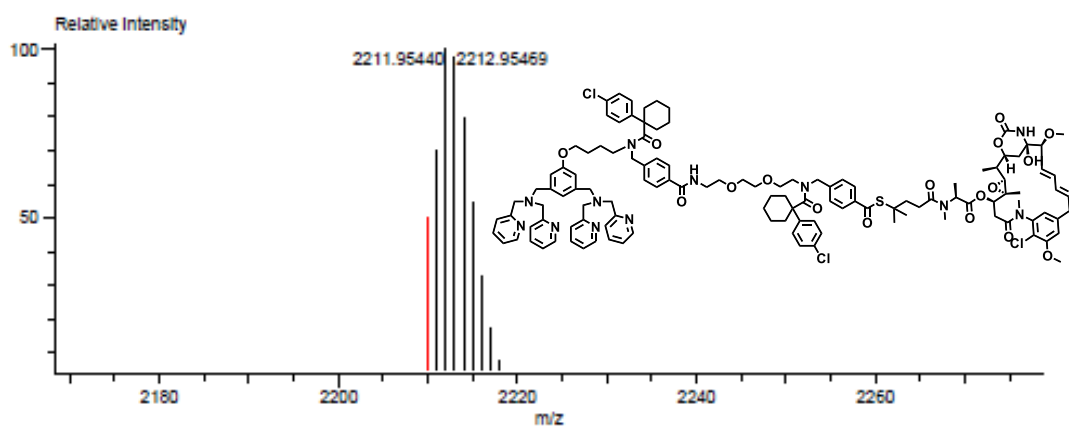
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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C



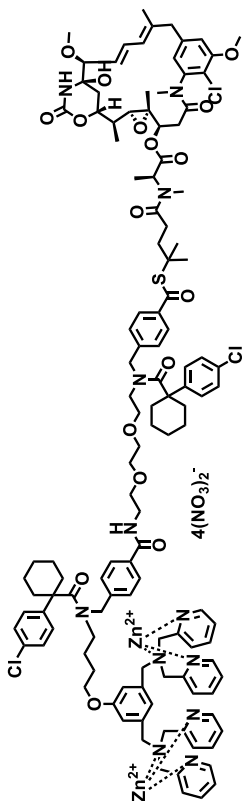


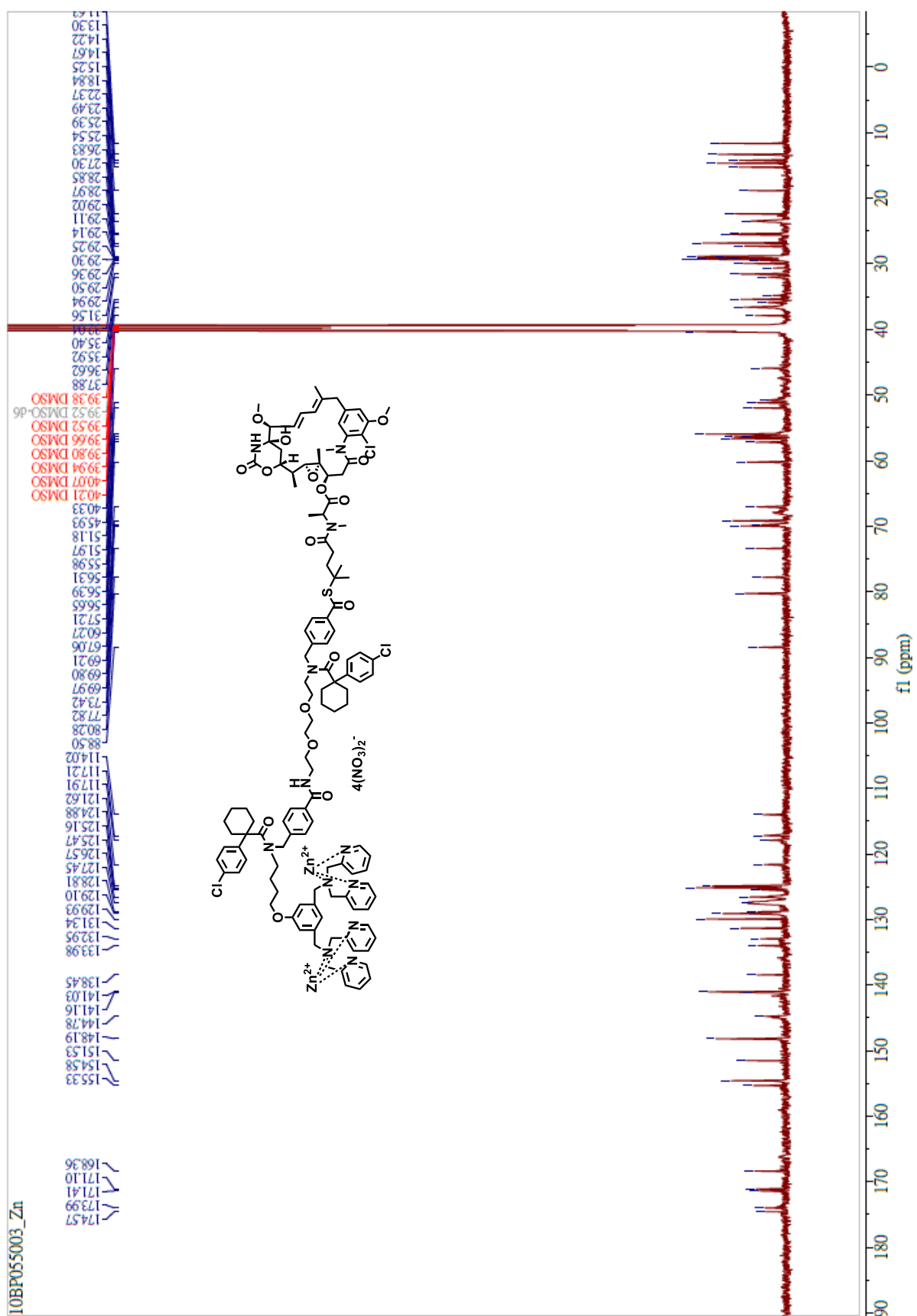


Charge number:1 Tolerance:300.00[ppm], 250.00 .. 250.... Unsaturation Number:-100.0 .. 200.0 (...
 Element:¹²C:122 .. 122, ¹H:0 .. 146, ³⁵Cl:3 .. 3, ¹⁴N:12 .. 12, ²³Na:0 .. 1, ¹⁶O:17 .. 17, ³²S:1 .. 1



Mass	Intensity	Calc. Mass	Mass Difference [mDa]	Mass Difference [ppm]
2209.95189	25511.96	2209.95348	-1.57	-0.71





Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

