Experimental Section and Spectra

General information

Antibiotics were purchased from Sigma Aldrich (Germany), DLD Scientific (South Africa) and Hangzhou Dayangchem Co., Ltd (China). Reagents and solvents were purchased from Sigma Aldrich and Merck. All solvents were dried by means of standard procedures. Thin Layer Chromatography (TLC) was performed using Merck Kieselgel 60 F254 plates. All the synthetic steps were monitored using LC-MS (Shimadzu 2020 UFLC-MS, Japan). The LC-MS method used a gradient of 5% ACN: H_2O (0.1% formic acid) to 95% ACN:H_2O (0.1% formic acid) over 9 minutes on an XBridgeTM C18 5µm 4.6x150mm column, where the flow rate is 1 mL/min. Purification of the intermediates was done by either gravity column chromatography (mesh particle size, 40-63 µm) and preparatory supercritical fluid chromatography performed on a Sepiatec Prep SFC basic/basic 30 (Germany). High resolution mass spectrometric (HRMS) data were obtained with a Bruker micrOTOF-Q II instrument that operated at ambient temperatures and at a sample concentration of 2 µg/ml. Infrared spectrometric (IR) data were recorded on a Perkin Elmer Spectrum 100 instrument with a Universal ATR Sampling Accessory. NMR data were recorded using a Bruker AVANCE III 400 MHz at room temperature. Chemical shifts are expressed in ppm and coupling constants are reported in Hz. Purity of the final compounds were measured by LC-MS.

Note for the NMR spectra of the final compounds

The NMR spectra of the final compounds appeared with much overlapping. This was attributed to the presence of rotamers and/or the ability of the chelator moieties to appear bent due to the 3D structure of the lactam (four membered ring adjacent to five/six membered ring).[1] The floppy nature of the molecule is likely to result in the broadening of the signals leading to the poor resolution of the multiplets. This is further complicated by overlapping signals arising from the many protons in similar environments. The integration corresponded to the number of protons on the products however the spectra appeared ‘messy’ due to this overlap. After consulting with NMR experts (collaborators in Sweden), by subjecting the samples in their labs to temperature variation as well as complexing the chelators with either Zn or Cu, no significant changes in the spectra were observed.[2] Hence the NMR spectra of all starting material was recorded and confirmed. The NMR spectra of all chelators on its own also displayed much overlap. For these reasons, the

final compounds were further treated and characterized as per peptides in organic synthesis in which the NMR spectra is not recorded however supported by other means of characterization such LC-MS trace and HRMS spectra.[3]

Note for optical purity of the final compounds

All final compounds were obtained as a racemate as revealed by optical rotation measurements.

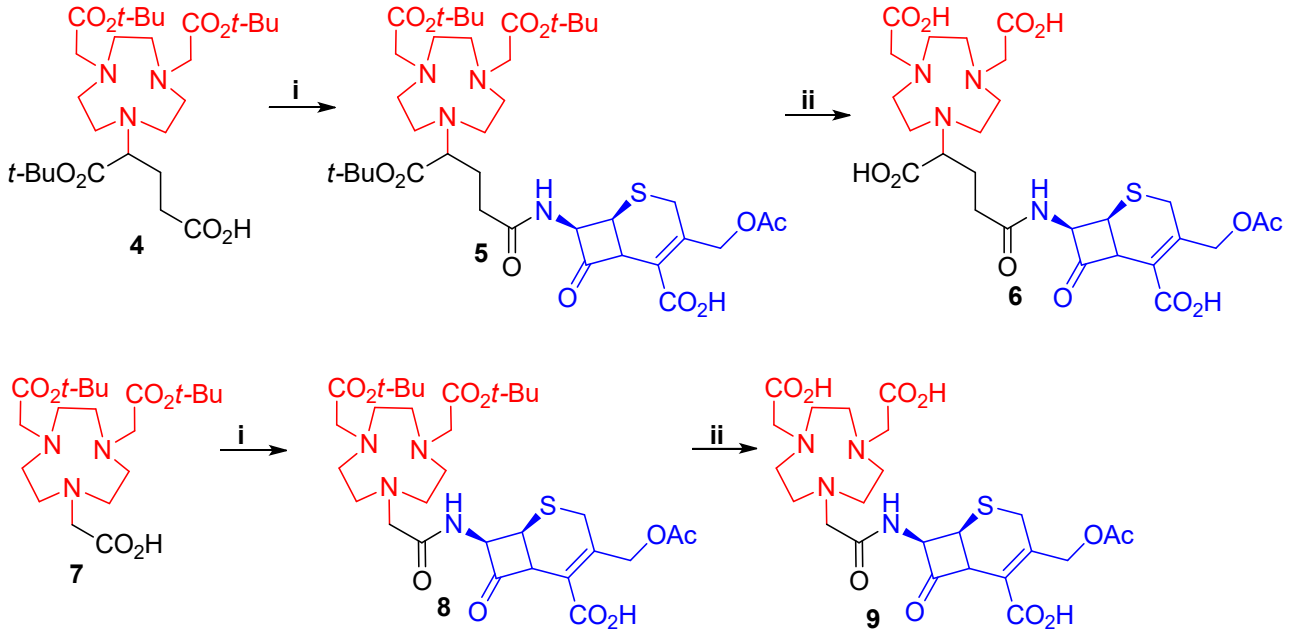
Method A

Prep SFC purification was done using a Sepia tec Prep SFC basic/30. All were compounds were purified the following parameters: sample concentration = 10-20 mg/mL in acetonitrile or methanol, injection volume = 100-200 µL, column = Ethylpyridine (250x10 mm, 5 Å) at 40 °C, mobile phase = 10 – 50% MeOH: ACN (1:1) spike with 0.3% DIEA (diisopropyl ethylamine) or 0.1% TFA as the modifier (Pump A) with tech grade-wet CO_2 the balance of the flow (Pump B), in 10 min, flow = 10 mL/min, BPR setting = 150 bar, monitoring and collection at 220 nm. All samples were injected in a multiply circle between 2-100 and the product fraction was collected and concentrated.

Method B

Method protocol similar to **Method A**, the exception, the mobile phase = 10 – 30% MeOH: ACN (1:1) spike with 0.3% DIEA or 0.1% TFA as the modifier (Pump A) with tech grade-wet CO_2 the balance of the flow (Pump B), in 5 min, then washed with 50% Modifier for 2 min, flow = 10 mL/min, BPR setting = 150 bar, monitoring and collection at 220 nm. All samples were injected in a multiply circle between 2-100 and the product fraction was collected and concentrated.

Scheme 1: Synthesis of compound 6 and 9



i = HATU, DIEA, ACN, 7-ACA (dissolved in mixture of $NaHCO_3$, H_2O, Acetone, ACN), -20 °C, 1 hr. **ii** = Thioanisole, TFA, rt, 3 hrs.

Compounds 5 and 8

7-Aminocephalosporanic acid (7-ACA) (30.0 mg, 0.110 mmol, 1.2 equiv.) for the synthesis of coumpound **5** or (39.2 mg, 0.144 mmol, 1.2 equiv.) for the synthesis of compound **8** was dissolved in a water:acetone mixture (2:1 mL/mmol) with $NaHCO_3$ (3.6 equiv.) and stirred at -20 °C. A stock solution of compound **4** (50 mg, 0.092 mmol, 1.0 equiv.) or **7** (50 mg, 0.120 mmol, 1.0 equiv.) in DMF (250 mg/mL) was diluted with 0.5 mL ACN, added DIEA (2.2 equiv.) and HATU (1.1 equiv.). Thereafter the mixture was vortexed, cooled to about -20 °C within 2 minutes, and added to compound 7-ACA mixture in a single shot. The reaction was monitored by LCMS (typically complete after an hour). The solvent was removed under reduced pressure and the crude reaction mixture was purified by prep SFC following Method A. The sample was dissolved to 10 mg/mL in EtOH:MeOH (2:1), Modifier mixture used was MeOH: ACN (1:1) with 0.1% TFA.

6*S*,7*S*)-3-(acetoxymethyl)-7-(4-(4,7-*bis*(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)-5-(*tert*-butoxy)-5-oxopentanamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (5)

Compound **5** was obtained in a 40 % yield. LCMS: *m/z* = 798. I.R. (υ_{max}/cm^{-1}) 2978, 2934, 1723, 1659, 1602, 1455, 1370, 1367, 1246, 1227, 1148, 1068, 1034, 843, 747, 717. ([M+H]). HRMS (ESI): *m/z* [M+H] calcd for $C_{37}H_{60}N_5O_{12}S$: 798.3954; Found: 798.3892.

(6*S*,7*S*)-3-(acetoxymethyl)-7-(2-(4,7-*bis*(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (8)

Compound **8** was obtained in a 25% yield. LCMS: *m/z* = 670 ([M+H]). I.R. (υ_{max}/cm^{-1}) 2977, 2933, 2872, 1771, 1723, 1662, 1457, 1387, 1368, 1224, 1150, 1066, 1034, 846, 784, 744, 659. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{48}N_5O_{10}S$: 670.3116; Found: 670.3048

Compound **6** and **9**

To compounds **5** or **8** (1.0 equiv.) and thioanisole (8.0 equiv.) was added TFA (44 mL/mmol) at room temperature. Reaction progress was monitored using LCMS. After 3 hours of reaction (typical time to completion), the volatiles were removed by passing a gentle stream of nitrogen over the reaction until a pale-yellow residue remained. The residues were wash with Et_2O (5-10 mL x 3) using a sonic bath (10 minutes) and the washing decanted and then dried to afford pure **6** or **9** as off white solids.

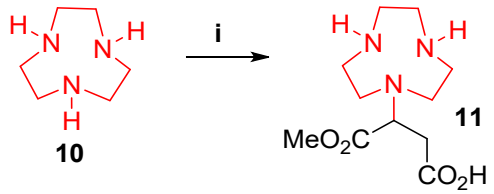
2,2'-(7-(2-(((6*S*,7*S*)-3-(acetoxymethyl)-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-2-oxoethyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (6)

Compound **6** was obtained in a 60% yield (99% purity). LCMS *m/z* = 558 ([M+H]). I.R. (υ_{max}/cm^{-1}) 2933, 2871, 1721, 1660, 1493, 1385, 1345, 1180, 1131, 1071, 1033, 798, 719, 683. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{31}N_5O_{10}S$: 558.1865; Found: 558.1792.

2,2'-(7-(5-(((6*S*,7*S*)-3-(acetoxymethyl)-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-1-(*tert*-butoxy)-1,5-dioxopentan-2-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid (9)

Compound **9** was obtained in a 70 % yield (99% purity). LCMS: *m/z* = 630 ([M+H]$^+$). I.R. (υ_{max}/cm^{-1}) 2916, 2868, 1774, 1722, 1666, 1459, 1383, 1346, 1224, 1178, 1129, 1067, 1027, 981, 798, 718, 677, 599. HRMS (ESI): *m/z* [M+H] calcd for $C_{29}H_{44}N_5O_{12}S_4$: 630.6441; Found: 630.1987.

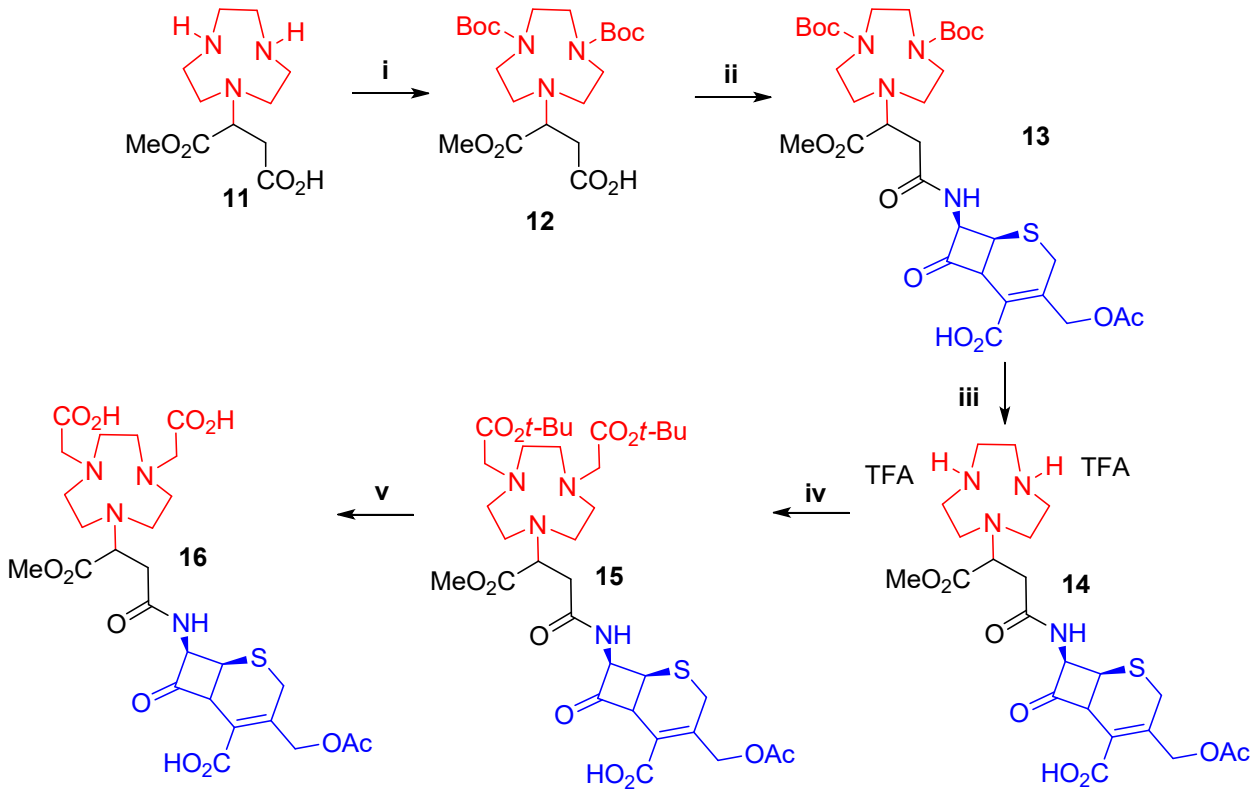
Scheme 2: Synthesis of 4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanoic acid (11)



i = *Mono*-methyl fumarate, DIEA, DCM, rt, 4 hrs.

1,4,7-triazononane (**10**) (748 mg, 5.79 mmol, 1.5 equiv.) was dissolved in 67 mL DCM with DIEA(1000 µL, 1.6 equiv.) in a round bottom flask and stirred in an ice bath. *Mono*-methyl fumarate (500 mg, 3.84 mmol 1.0 equiv.) was dissolved with the same equivalence of DCM with DIEA added to **10**. The *mono*-methyl fumarate solution was added dropwise slowly using a separatory funnel over 15-30 minutes to 1,4,7-triazononane (**10**) solution stirred in an ice bath at 0°C. Thereafter, reaction was allowed to gently warm up to room temperature. LCMS was used to monitor the progress of the reaction and after 4 hours the solvent was removed to afford slurry-DIEA layers. The DIEA was carefully removed and the recrystallized with 3 mL of DMF at -10 °C overnight. The recrystallized product was washed toluene (5-10 mL x 3) using a sonic bath and centrifuge then dried under high vacuum to afford compound **11** in qualitative yield. LCMS: *m/z* = 260 [M+H]. ^{1}H NMR (400 MHz, MeOD): δ 3.36 (1H, t), 2.93 (2H, m), 2.74 (2H, m), 2.61-2.70 (2H, m), 2.58 (2H, m), 2.56 (3H, s), 2.36-2.51 (5H, m), 2.24 (1H, m). ^{13}C NMR (100 MHz, MeOD): δ 179.3, 175.1, 64.4, 52.2 (2), 50.2, 46.8 (2), 45.8 (2), 35.1. I.R. (υ_{max}/cm^{-1}) 3377, 2946, 2920, 2850, 2797, 1729, 1656, 1579, 1352, 1324, 1195, 864, 683, 525.

Scheme 3: Synthesis of compound 16



i = Boc_2O, DIEA, DCM, rt, 4 hrs. **ii** = HATU, DIEA, ACN, 7-ACA (dissolved in mixture of $NaHCO_3$, H_2O, Acetone , ACN), -20 C, 1 hrs. **iii** = Anisole, TFA, DCE , rt, 20 min. **iv** = *tert*-butyl bromoacetate, DIEA, DCE, 0-25 °C, 1 hrs. **v** = Thioansole, TFA, rt, 3 hrs.

3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanoic acid (12)

To compound **11** (500 mg, 1.93 mmol 1.0 equiv.), was added 9.8 mL DCM (5 mL/mmol) and DIEA (1000 µL, 3.0 equiv.) at room temperature with stirring. Thereafter, Boc_2O (2.05 equiv.) was added to the reaction mixture. The reaction was monitored using LCMS and was completed after 4 hours. The solvent was removed under vacuo, and target **12** was purified through a SiO_2 gravity column: dry load with DCM: Hexane (1:1) and eluted using DCM: MeOH: AcOH (98: 1.8: 0.2) to afford compound **12** in 85-96% Yield. LCMS: *m/z* = 460 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3269, 2973, 2929, 2866, 1733, 1687, 1365, 1245, 1144, 856, 773. HRMS (ESI) *m/z* calculated for $C_{21}H_{38}N_3O_8$: 460.2653, Found: [M+H] 460.2764.

(6*R*,7*R*)-3-(acetoxymethyl)-7-(3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (13)

Compound **13** was prepared following an adjusted protocol for the preparation of compound **5** and **8,** whereby compound **12** (200 mg, 0.44 mmol, 1.0 equiv.) was used in place of the stock solutions of compound **4** or **7**. After purification, compound **13** was obtained as a yellow oil in 50% (155 mg) yield. Confirmed by LCMS: 714 *m/z* ([M+H]). I.R. (υ_{max}/cm^{-1}) 3621, 2981, 2720, 1778, 1670.7, 1600, 1465, 1404, 1366, 1243, 1160, 1160, 1144, 1069, 1032, 833, 556. HRMS (ESI) *m/z* calculated for $C_{31}H_{48}N_5O_{12}S$: 714.3015, Found [M+H] 714.3101.

(6*R*,7*R*)-3-(acetoxymethyl)-7-(4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetic acid salt (14)

The isolated compound **13** from the previous step (155 mg, 0.22 mmol,1.0 equiv.) was dissolved in DCE (8.0 mL/mmol) and anisole (8.0 equiv.) was added, followed by TFA (6.0 mL/mmol) at room temperature. The reaction was monitored using LCMS. After reaction completion, the volatiles were removed by passing a gentle stream of nitrogen over the reaction until a pale-yellow residue remained. The residues were washed with Et_2O (5-10 mL x 3) using a sonicator bath (10 minutes) and the washing was decanted to afford pure compound **14** as a precipitate in yield a of 95% (155 mg). LCMS: *m/z* = 514 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3031, 1772, 1725, 1671, 1440, 1287, 1229, 1198, 1134, 1026, 837, 721, 557, 498. HRMS (ESI) *m/z* calculated for $C_{21}H_{32}N_5O_8S$: 514.1966, Found: [M+H] 514.2034.

(6*R*,7*R*)-3-(acetoxymethyl)-7-(3-(4,7-bis(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (15)

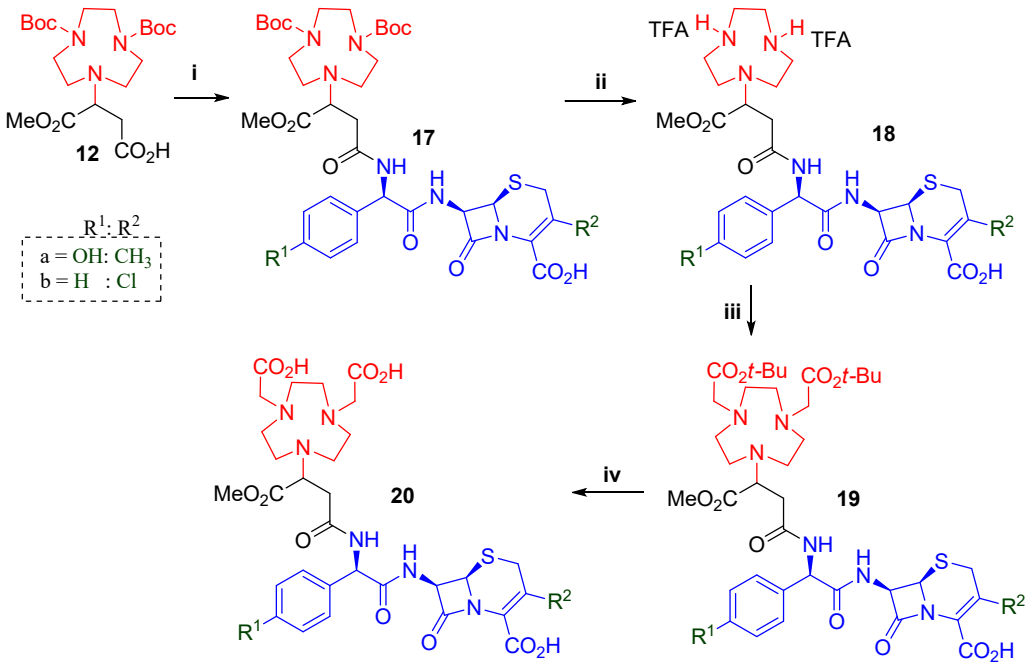
The isolated compound **14** from the previous step (155 mg, 0.21 mmol, 1.0 equiv.) was dissolved in DCE (5.0 mL/mmol) and DIEA (20.0 equiv.) was added and stirred at 0 ºC. After, *tert*-butylbromoacetate (2.05 equiv.) was added in a single shot. The reaction was gently warmed up to room temperature and monitored using LCMS. The reaction was complete in an hour. The crude was concentrated under reduced pressure and residual washed in a sonicator bath (10 minutes) with Et_2O (5-10 mL x 3) to afford a precipitate. The precipitate was further purified using Method A to afford pure compound **15** in 46% yield (71.5 mg). Confirmed by LCMS: *m/z* =742 ([M+H]).

I.R. (υ_{max}/cm^{-1}) 2979, 2951, 2864, 2844, 2361, 2342, 1731, 1657, 1436, 1200, 1152, 1055, 1019, 836, 799, 719. HRMS (ESI) *m/z* calculated for $C_{33}H_{52}N_5O_{12}S$: 742.3328, Found: [M+H] 742.3416.

2,2'-(7-(4-(((6*R*,7*R*)-3-(acetoxymethyl)-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-1-methoxy-1,4-dioxobutan-2-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid (16)

The isolated compound **15** from the previous step (71.5 mg, 0.097 mmol, 1.0 equiv.) was mixed with thioanisole (8.0 equiv.), followed by TFA (44 mL/mmol) at room temperature. Reaction progress was monitored using LCMS. The reaction was complete in 3 hours, thereafter, volatiles were removed by passing a gentle stream of nitrogen over the reaction until a pale-yellow residue remained. The residues were washed with Et_2O (5-10 mL x 3) using a sonicator bath (10 minutes) and the washing decanted to afford pure compound **18** as a precipitate in 65% yield (39.6 mg) (98% purity). Confirmed by LCMS: *m/z* = 630 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3401, 2955, 2865, 1723, 1667, 1379, 1227, 1196, 1128, 1058, 1032, 833, 798, 720. HRMS (ESI) *m/z* calculated for $C_{25}H_{36}N_5O_{12}S$: 630.2076, Found [M+H] 630.2245.

Scheme 4: Synthesis route of compounds 20a-b



i = β-lactam, HOBt, EDC.HCl, DMF/ DMSO , rt, 6-12 hrs or COMU, DIEA, ACN, rt, 2 hrs. **ii** = Anisole, TFA, DCE, rt, 2-6 hrs. **iii** = *tert*-butyl bromoacetate, DIEA, DCE , 0-25 °C, 2-6 hrs. **iv** = TFA, thioanisole, rt, 3-6 hrs. or 4M HCl in Dioxane, rt, 3-hrs.

Compounds **17a-b**

Compound **12** (200 mg, 0.44 mmol, 1.0 equiv.) was dissolved in dry DMF or DMSO, activated with HOBt (1.1 equiv.) and EDC.HCl (1.1 equiv.) and stirred at room temperature. After 10 min, 1.0 equiv. *β*-lactam (cefadroxin (**a**) or Cefaclor (**b**) was added in one shot. The reaction was completed overnight and then quenched with 5 mL of deionized water and extracted with 5 mL CH_2Cl_2. The organic extract was concentrated then purified using method B.

(6*R*,7*R*)-7-((2*R*)-2-(3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)-2-(4-hydroxyphenyl)acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (17a),

Compound **17a** was prepared using cefadroxin (**a**)(Scheme 4) and afforded a yellow oil with 55% yield (195 mg). Confirmed by LCMS: *m/z* = 805 ([M+H]). I.R. (υ_{max}/cm^{-1}) 2975, 2916, 2367, 1766, 1513, 1461, 1411, 1365, 1245, 1151, 988, 838, 773, 525. HRMS (ESI) *m/z* calculated for $C_{37}H_{52}N_6O_{12}S$: 805.3437, Found [M+H] 805.3535.

(6*R*,7*R*)-7-((2*R*)-2-(3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)-2-phenylacetamido)-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (17b),

Compound **17b** was prepared using cefaclor(**b**)(Scheme 4) affording a yellow oil in 70% yield (249 mg). Confirmed by LCMS: *m/z* = 809 *m/z* ([M+H]). I.R. (υ_{max}/cm^{-1}) 3369, 2978, 2931, 1773, 1659, 1477, 1461, 1411 1365, 1247, 1146, 1097, 1030, 990. HRMS (ESI) *m/z* calculated for $C_{36}H_{49}ClN_6O_{11}S$: 809.2941, Found [M+H] 809.2916

Compound 18a-b

Compound **18a-c** was prepared similarly to **14** varying only in reaction time.

(6*R*,7*R*)-7-((2*R*)-2-(4-hydroxyphenyl)-2-(4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanamido)acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetic acid salt (18a)

Compound **18a** reaction was complete after 3 hours, thereafter, washed to give a yellow precipitate in 96% yield (193 mg). Confirmed by LCMS: *m/z* = 605 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3271, 3031,

1763, 1663, 1536, 1535, 1515, 1439, 1368, 1179, 1131, 837, 798, 720, 557. HRMS (ESI) *m/z* calculated for $C_{27}H_{36}N_6O_8S$: 605.2388, Found [M+H] 605.2453

(6*R*,7*R*)-3-chloro-7-((2*R*)-2-(4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanamido)-2-phenylacetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetic acid salt (18b)

Compound **18b** reaction was complete after 1 hours, thereafter washed to give a pale-yellow precipitate in 92% yield (234 mg) . Confirmed by LC-MS, 609 *m/z* (positive mode). I.R. (υ_{max}/cm^{-1}) 2925, 2853, 2361, 1718, 1655, 1138, 797, 719, 701. HRMS (ESI) *m/z* calculated for $C_{26}H_{33}ClN_6O_7S$: 609.1893, Found [M+H]: 609.1859

Compound 19a-b

19a-b was prepared similarly to **15** with different in reaction time.

(6*R*,7*R*)-7-((2*R*)-2-(3-(4,7-*bis*(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)-2-(4-hydroxyphenyl)acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (19a)

Compound **19a** reaction was complete after 3 hours. The prep sample was prepared similar to **17** and purified using Method A using MeOH:ACN with DIEA as a modifier to afford pure compound **19a** as a yellow oil with 30% yield (58.0 mg). Confirmed by LC-MS, *m/z* = 832 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3279, 2980, 2866, 1725, 1657, 1514, 1455, 1438, 1369, 1248, 1129, 838, 718. HRMS (ESI) *m/z* calculated for $C_{39}H_{57}N_6O_{12}S$: 833.3750, Found [M+H]: 833.3859

(6*R*,7*R*)-7-((2*R*)-2-(3-(4,7-*bis*(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)-2-phenylacetamido)-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (19b)

Compound 19b reaction was complete after 3 hours. The prep sample was prepared similar to **17** and purified using Method B using MeOH:ACN with DIEA as a modifier to afford pure compound **19b** as a yellow oil with 40% yield (94.6 mg). Confirmed by LC-MS, 837 *m/z* (positive mode). I.R. (υ_{max}/cm^{-1}) 3294, 3009, 2967, 2865, 2843, 1776, 1656, 1535, 1497, 1438, 1345, 834, 720, 514. HRMS (ESI) *m/z* calculated for $C_{38}H_{53}ClN_6O_{11}S$: 837.3254, Found [M+H]: 837.3233

Compound 20a-b

Compound **20a-b** was prepared from compound **21a-b** using an identical protocol employed for compound **16**. Reaction progress was monitored using LCMS.

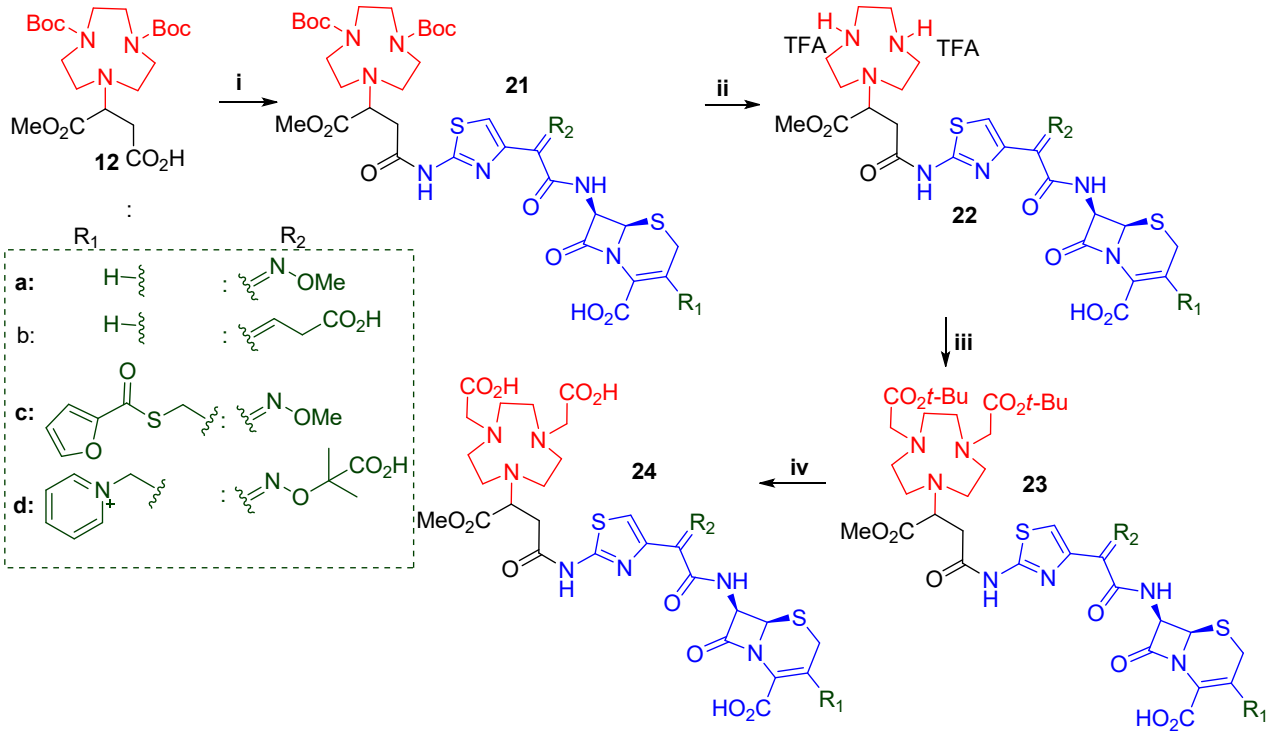
2,2'-(7-(4-(((*R*)-2-(((6*R*,7*R*)-2-carboxy-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-1-(4-hydroxyphenyl)-2-oxoethyl)amino)-1-methoxy-1,4-dioxobutan-2-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid (**20a**)

Compound **20a** reaction was complete after 3 hours, thereafter washed to give a yellow precipitate 85% yield (42.8 mg) with 99% purity. Confirmed by LCMS: *m/z* = 721 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3278, 2980, 2666, 1725, 1657, 1514, 1455, 1438, 1369, 1129, 838 718 HRMS (ESI) *m/z* calculated for $C_{31}H_{40}N_6O_{12}S$: 721.2498, Found [M+H] 721.2585.

2,2'-(7-(4-(((*R*)-2-(((6*R*,7*R*)-2-carboxy-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-2-oxo-1-phenylethyl)amino)-1-methoxy-1,4-dioxobutan-2-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid (**20b**)

Compound **20b** reaction was complete after 1 hours, thereafter washed to give a white precipitate 90% yield (77.0 mg) with 98% purity. Confirmed by LCMS: *m/z* = 725 ([M+H]). I.R. (υ_{max}/cm^{-1}) 2924.7, 2853, 2361, 1718, 1654, 1138, 797, 719, 701. HRMS (ESI) *m/z* calculated for $C_{30}H_{37}ClN_6O_{11}S$: 725.2002, Found [M+H] 725.2019

Scheme 5: Synthesis route of compounds 24a-d



i = β-lactam, HOBt, EDC.HCl, DMF or DMSO , rt, 6-12 hrs. **ii** = Anisole, TFA, DCE, rt, 2-6 hrs. **iii** = *tert*-butyl bromoacetate, DIEA, DCE , 0-25 °C, 2-6 hrs. **iv** = Thioansole, TFA, rt, 3-8 hrs.

Synthesis of compounds 21a-d

Compounds **21a-d** were prepared similar compounds **17a-b**. Compound **12** (200 mg, 0.44 mmol, 1.0 equiv.) was dissolved in dry DMF or DMSO (1.0 mL/mmol) was activated with HOBt (1.1 equiv.) and EDC.HCl (1.1 equiv.). This solution was stirred for 10 minutes to allow for the activation of the carboxylic acid group. Thereafter, 1.0 equiv. of the appropriate antibiotic (ceftizoxime **(a)**, ceftibuten **(b)**, ceftiofur **(c)** and ceftazidime**(d)**) was added. The reaction was stirred at room temperature for about 12 hours. The reaction was quenched with 5 mL of deionized H_2O and extracted the aqueous phase with DCM (5 mL x 3). The organic extract was concentrated then purified using method A or Method B.

(6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (21a)

Compound **21a** was obtained as a brown thick oil in 60% yield (218 mg). LCMS: *m/z* = 825 ([M+H]). I.R. (υ_{max}/cm^{-1}) 2974, 2939, 2867, 1671, 1545, 1410, 1368, 1199, 1139, 1044, 1034, 721. HRMS (ESI) *m/z* calculated for $C_{34}H_{48}N_8O_{12}S_2$: 825.2906 Found: [M+H] 825.2931.

(6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-((carboxymethyl)imino)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (21b)

Compound **21b** was obtained as a yellow, thick oil after prep SFC purification in 65 % yield (244 mg). LC-MS, *m/z* = 852 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3013, 2978, 2859, 1662, 1551, 1427, 1283, 1179, 1129, 835, 797, 720. HRMS (ESI) *m/z* calculated for $C_{36}H_{49}N_7O_{13}S_2$: 852.2903 Found: [M+H] 852.2990.

(6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-3-(((furan-2-carbonyl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (21c)

Compound **21c** was obtained as a dark brown, thick oil in 53% yield (225 mg). Confirmed with LCMS: *m/z* = 966 $[M+H]^+$. IR (υ_{max}/cm^{-1}) 3696, 3663, 2972, 2938, 2865, 2843, 1765, 1713, 1664, 1461, 1365, 1248, 1143, 1054, 1033, 847, 760. HRMS (ESI+) *m/z*: calculated for $C_{40}H_{52}N_8O_{14}S_3$: C40H53N8O14S3 Found[M+H]: 965.2798.

1-(((6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-(((2-carboxypropan-2-yl)oxy)imino)acetamido)-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridin-1-ium chloride salt (21d)

Compound **21d** was obtained as a brown, thick oil was obtained 55% yield (247 mg) . Confirmed with LC-MS: *m/z* = 989 [M+H] IR (υ_{max}/cm^{-1}) 2974, 2926, 2362, 2342, 1729, 1665, 1541, 1479, 1439, 1414, 1366, 1250, 1141, 1984, 855, 772, 718, 681. HRMS (ESI+) *m/z*: calculated for $C_{43}H_{58}N_9O_{14}S_2$: 988.3539, Found [M]: 988.3633

Synthesis of compounds 22a-d

The compound was synthesized following the synthesis protocol of compound **16** monitored by LC-MS until reaction was complete.

(6*R*,7*R*)-7-((*Z*)-2-(2-(4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetic acid salt (22a)

Compound **22a** was obtained as pale-yellow powder was obtained in 95% yield (213 mg). LCMS: *m/z* = 625 *m/z* ([M+H]). I.R. (υ_{max}/cm^{-1}) 2981, 2951, 2844, 1764, 1720, 1546, 1438, 1868, 1281, 1198, 1035, 989, 834, 799, 721. HRMS (ESI) *m/z* calculated for $C_{24}H_{32}N_8O_8S_2$: 625.1857, Found: [M+H] 625.1905.

(6*R*,7*R*)-7-((*Z*)-2-((carboxymethyl)imino)-2-(2-(4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanamido)thiazol-4-yl)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetic acid salt (22b)

Compound **22b** was obtained as a pale-yellow powder was obtained in 96% yield (241 mg). LCMS: *m/z* = 652 ([M+H]). I.R. (υ_{max}/cm^{-1}) 2981, 2913, 1725, 1667, 1549, 1437, 1362, 1278, 1196, 1129, 909, 833, 720. HRMS (ESI) *m/z* calculated for $C_{26}H_{33}N_7O_9S_2$: 652.1854, Found: [M+H] 652.1714.

(6*R*,7*R*)-3-(((furan-2-carbonyl)thio)methyl)-7-((*Z*)-2-(2-(4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetic acid salt (22c)

Compound **22c** was obtained as pale-yellow, powder was obtained in 80% yield (185 mg). Confirmed with LCMS: *m/z* = 765 $[M+H]^+$. IR (υ_{max}/cm^{-1}) 2973,2945, 1728, 1667, 1552, 1462, 1440, 1384, 1333, 1256, 1195, 1178, 1130, 1046, 1032, 954, 844, 798. HRMS (ESI+) *m/z*: calculated for $C_{30}H_{37}N_8O_{10}S_3$: 765.8568, Found [M+H]: 765.1765

1-(((6*R*,7*R*)-2-carboxy-7-((*Z*)-2-(((2-carboxypropan-2-yl)oxy)imino)-2-(2-(4-methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanamido)thiazol-4-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridin-1-ium trifluoroacetic acid salt (22d)

Compound **22d** was obtained as pale-yellow, powder was obtained 93% yield (256 mg). Confirmed with LCMS: *m/z* = 789 $[M+H]^+$. IR (υ_{max}/cm^{-1}) 1769, 1724, 1667, 1548, 1438, 1364, 1276, 1176, 1129, 983, 908, 832, 797, 719, 680 HRMS (ESI+) *m/z* calculated for $C_{33}H_{43}N_9O_{10}S_2$: 788.2491, Found [M]: 788.2456

Synthesis of compounds 23a-d

The compounds were synthesized following the synthesis protocol of compound **17**. Crude reaction mixtures were purified using SFC.

(7*S*)-7-((*E*)-2-(2-(3-(4-(2-(*tert*-butoxy)-2-oxoethyl)-7-(carboxymethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (23a)

Compound **23a** was purified using Method B with basic modifier (TEA) as a yellow oil in 30% yield (63.9 mg). LCMS: *m/z* = 853 *m/z* ([M+H]). I.R. (υ_{max}/cm^{-1}) 2980, 2940, 2867, 1727, 1671, 1544, 1438, 1310, 1251, 1198, 1151, 1034, 893, 831, 798, 718. HRMS (ESI) *m/z* calculated for $C_{36}H_{53}N_8O_{12}S_2$: 853.3219, Found: [M+H] 853.3277.

(6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-((carboxymethyl)imino)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (23b)

Compound **25b** was purified using Method B with acidic modifier (TFA) as a yellow oil in 34% yield (82.2 mg). LCMS, *m/z* = 880 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3701, 3667, 2981, 2939, 2922, 2866, 2844, 1728, 1688, 1542, 1455, 1370, 1250, 1152, 1053, 1033, 1015, 799, 718. HRMS (ESI) *m/z* calculated for $C_{38}H_{54}N_7O_{13}S_2$: 880.3216, Found: [M+H] 880.3198

(6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-3-(((furan-2-carbonyl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (23c)

Compound **23c** was purified using Method A with basic modifier (TEA) as a yellow oil in 40% yield (73.5 mg). Confirmed with LCMS: *m/z* = 994 ([M+H]). IR (υ_{max}/cm^{-1}) 2978, 2929, 2680, 2343, 1730, 1671, 1542, 1459, 1369, 1251, 1200, 1129, 1039, 845, 798, 719. HRMS (ESI+) *m/z*: calculated for $C_{42}H_{57}N_8O_{14}S_3$: 993.3151, Found [M+H]: 994.3137

1-(((6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-(((2-carboxypropan-2-yl)oxy)imino)acetamido)-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridin-1-ium (23d) TFA salt

Compound **23d** was purified using Method A with acid modifier (TFA) as a yellow oil in 37% yield (94 mg). Confirmed with LCMS: *m/z* = 1017 ([M+H). IR (υ_{max}/cm^{-1}) 2978, 2936, 2865, 2360, 2343, 1730, 1671, 1544, 1393, 1364, 1057, 983, 833, 798, 746, 681. HRMS (ESI+) *m/z*: calculated for $C_{45}H_{61}N_9O_{10}S_2$: 1016.3852, Found [M]: 1016.3797

Synthesis of compounds 24a-d

The compounds were synthesized following the synthesis protocol of compound **18**.

2,2'-(7-(4-((4-((*Z*)-2-(((6*R*,7*R*)-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-1-(methoxyimino)-2-oxoethyl)thiazol-2-yl)amino)-1-methoxy-1,4-dioxobutan-2-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid (24a)

Compound **24a** was as a pale-yellow power in 96% yield (61 mg) with 99% purity. LCMS, *m/z* = 853 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3402, 2304, 2951, 2666, 1721, 1670, 1544, 1458, 1370.6, 1278, 1174, 1124, 1036, 894, 799, 720. HRMS (ESI) *m/z* calculated for $C_{28}H_{37}N_8O_{12}S_2$: 741.1967, Found: [M+H]: 741.1992

2,2'-(7-(4-((4-((*Z*)-2-(((6*R*,7*R*)-2-carboxy-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-1-((carboxymethyl)imino)-2-oxoethyl)thiazol-2-yl)amino)-1-methoxy-1,4-dioxobutan-2-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid (26b)

Compound **24b** was as a pale-yellow power in 89% yield (67 mg) with 99% purity. LCMS, *m/z* = 798 ([M+H]). I.R. (υ_{max}/cm^{-1}) 3711, 3680, 2953, 2922, 2868, 2845, 1668, 1459, 1377, 1055, 1014, 799, 720. HRMS (ESI) *m/z* calculated for $C_{38}H_{53}N_7O_{13}S_2$: 768.1964, Found: [M+H] 768.1784.

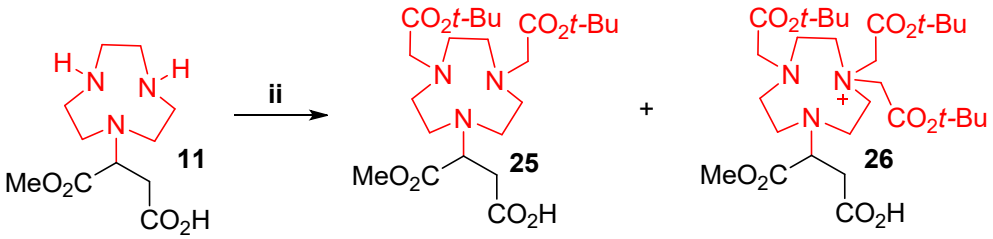
2,2'-(7-(4-((4-((*Z*)-2-(((6*R*,7*R*)-2-carboxy-3-(((furan-2-carbonyl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl)amino)-1-(methoxyimino)-2-oxoethyl)thiazol-2-yl)amino)-1-methoxy-1,4-dioxobutan-2-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid (24c)

Compound **24c** was as a pale-yellow power in 93% yield (60.6 mg) with 99% purity. Confirmed with LCMS: *m/z* = 881 ([M+H]). IR (υ_{max}/cm^{-1}) 3393, 2975, 2938, 2737, 2674, 2490, 1729, 1670, 1544, 1463, 1395, 1367, 1251, 1202, 1154, 1035, 847. HRMS (ESI+) *m/z*: calculated for $C_{34}H_{41}N8O_{14}S_3$: 881.1899, Found [M+H]: 881.1867

1-(((6*R*,7*R*)-7-((*Z*)-2-(2-(3-(4,7-*bis*(carboxymethyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanamido)thiazol-4-yl)-2-(((2-carboxypropan-2-yl)oxy)imino)acetamido)-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridin-1-ium (24d)

Compound **24d** was as a pale-yellow power in 95% yield (94 mg) with 99% purity. Confirmed with LCMS: *m/z* = 904 ([M+H]). IR (υ_{max}/cm^{-1}) 3253, 2988, 2950, 1724, 1666, 1547, 1370, 1270, 1172, 1131, 981. HRMS (ESI+) *m/z*: calculated for $C_{37}H_{46}N9O_{14}S_2$: 452.6337, Found [M+H]: 452.6445

Scheme 6: Attempted synthesis of protectected NODASA(25)



i = *tert*-butyl bromoacetate, DIEA, DCE or ACN, 0-25 °C

Since protected NODASA chelator (**25**) has not commercially available, we attempted to synthesize the compound via compound **11**. However, the reaction resulted in a mixture of the desired compound **25** and the over-alkylated side product, **26** (Scheme 6). Various reaction conditions, such as solvents, temperature, ratios of the reagents, and efforts at purification, were explored but were unsuccessfully.

Synthesis of NOTA-Zn complex

The complexation was performed as previously reported in literature[4]. Zinc perchlorate hexahydrate (12.3 mg, 0.033 mmol, 1.0 equiv.) was added to a solution of NOTA (10 mg, 0.033 mmol, 1.0 equiv.) in methanol (0.50 mL) The mixture was heated then cooled, and taken to pH 7, using a concentrated solution of NaOH in methanol. The mixture was kept at – 20 °C overnight. The supernatant was discarded, and the white precipitate was isolated and characterized using LC-MS.

Biological Testing

Cytotoxicity assay

Cell culture

HepG2 cells were cultured in 25 ml flasks using Eagle's minimum essentials medium (EMEM) supplemented with 10% fetal bovine serum, 1% pen-strep-fungizone and 1% L-glutamine, maintained in a humidified incubator (37 °C, 5% CO_2) until approximately 80% confluent.

Methyl Thiazol Tetrazolium Assay

The MTT assay was used to determine *in vitro* cell viability of each chelator on HepG2 cells. HepG2 cells (15,000 cells/well) were seeded into a 96-well microtiter plate and allowed to adhere overnight (37 °C, 5% CO_2). Thereafter, the cells were incubated (37 °C, 5% CO_2) with a range of chelator concentrations (0, 1, 8, 10, 50, 100 and 200 µg/ml) in triplicate for 6 h. After the 6 h incubation, the cells were washed with 0.1 M phosphate buffered saline (PBS) and incubated with MTT salt solution (5 mg/ml in 0.1 M PBS) and 100 µl CCM for 4 h (37 °C, 5% CO_2). The MTT salt solution was removed and DMSO (100 µl/well) was added and incubated for 1 h. The optical density was measured using a spectrophotometer (Bio-Tek µQuant) at 570/690 nm. Results are expressed as % cell viability versus chelator concentration (µg/ml).

Lactate Dehydrogenase Assay

The LDH assay was used to assess membrane damage of HepG2 cells. Supernatant collected from the control and chelator treated cells were centrifuged (400 x g, 24°C, 10 minutes) and dispensed

(100 µl/well) in triplicate into a 96-well microtiter plate. LDH reagent (100 µl, 11644793001, Sigma Aldrich) was added to each well. The plate was incubated for 30 minutes at room temperature in the dark. Absorbance was read using a spectrophotometer (Bio-Tek µQuant,) at 500 nM. Results are represented as relative fold change compared to untreated control.

Antibacterial assay

The chelator-cephalosporin derivatives were tested for the Minimum Inhibitory Concentrations (MIC) utilizing the checkerboard assay under the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[5] Serial dilutions of meropenem (32-0 mg/L) were made in Mueller-Hinton broth (MHB). Each chelator investigated was added using a concentration range of 64-0 mg/L. Finally, a 0.5 McFarland standardized inoculum of either *Escherichia coli* NDM-1 or *Klebsiella pneumoniae* NDM, was added to all wells. The microtitre plate was incubated at 35 °C for 18-20 hours. Post incubation, the resazurin dye was added to all wells of the plate, to aid in the visualization of the MIC. The MIC was recorded within 2 hours of adding the dye. The MIC of combination therapy was noted by the concentration of inhibitor that corresponds to the lowest meropenem concentration ($\leq$ 2 mg/L), in which there was an absence of bacterial growth. The assay was done in triplicate.

Time kill studies

Time-kill studies were performed according to previously published methods [6], including those described by CLSI document M26-A [7]. An overnight culture of *K. pneumoniae* NDM was diluted to a 0.5 McFarland standard, that correlated to approximately 10^6 cfu/ml. The prepared bacterial suspensions were added to 96 plate wells containing a fixed dose of 32 mg/L of each chelator and meropenem in concentrations of 0.5, 1 or 2 mg/L. Plates were incubated at 35^{o}C and 100 rpm shaking. A bacterial control without the addition of any drugs was included as well as a meropenem only control, employing similar conditions. Viability counts were performed at 0, 2, 4, 6, 8 and 24 h by sampling 0.1 mL, diluting as appropriate, and spreading onto Mueller Hinton agar (MHA). These plates were incubated at 35^{o}C for at least 18 h. Colonies were enumerated as cfu/mL.

Enzyme kinetic assay

Nitrocefin, a chromogenic cephalosporin with a conjugated dinitrostyrene group, was used to determine the enzymatic activity of IMP-1 and VIM-2. This substance undergoes a colour change from yellow to red and a change in absorbance when the C-N bond of a β-lactam has been hydrolysed. The working absorbance for the nitrocefin hydrolysis is 490 nm, and upon hydrolysis, a corresponding decreased in absorbance is observed. The nitrocefin hydrolysis assay was performed in a 96 well plate, in triplicate and the properties of the catalytic reaction involving IMP-1 and VIM-2 i.e., k_{cat}, K_m and k_{cat}/K_m were determined. A final volume of 100 μL was used for each well, to which 0.5 μL of nitrocefin (0.1 mM for K_m) and (0 μM – 5 μM for k_{cat}) IMP-1 and VIM-2 were added, respectively. The enzyme activity assay was performed on a Jasco V-630 spectrophotometer at 37 °C. Lineweaver-Burk plots were used to calculate the enzyme parameters which were derived from the Michaelis-Menten conditions as per equation 1.

$$V = V_{max}[S] / (K_m + [S]) \quad \textbf{(1)}$$

Enzyme inhibition assay

24b-c were evaluated against IMP-1 and VIM-2 by measuring the rate at which the substrate nitrocefin was hydrolysed. A concentration of 100 nM of purified IMP-1 and VIM-2 was used for each assay at 37 °C. The initial stock solutions of each inhibitor were made up to a concentration of 1000 μM in 50 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES, pH 7.0), and eight serial dilutions were made between 0 and 500 μM, for the inhibition assay. In each well, was a final concentration of 0.1 mM nitrocefin, NOTA-based inhibitor (0 – 500 μM), 100 nM sample of either IMP-1 or VIM-2 and the difference was made up with the HEPES buffer. The kinetics parameters were deduced using GraphPad Prism v9.3.1.

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Enzyme Assay Results

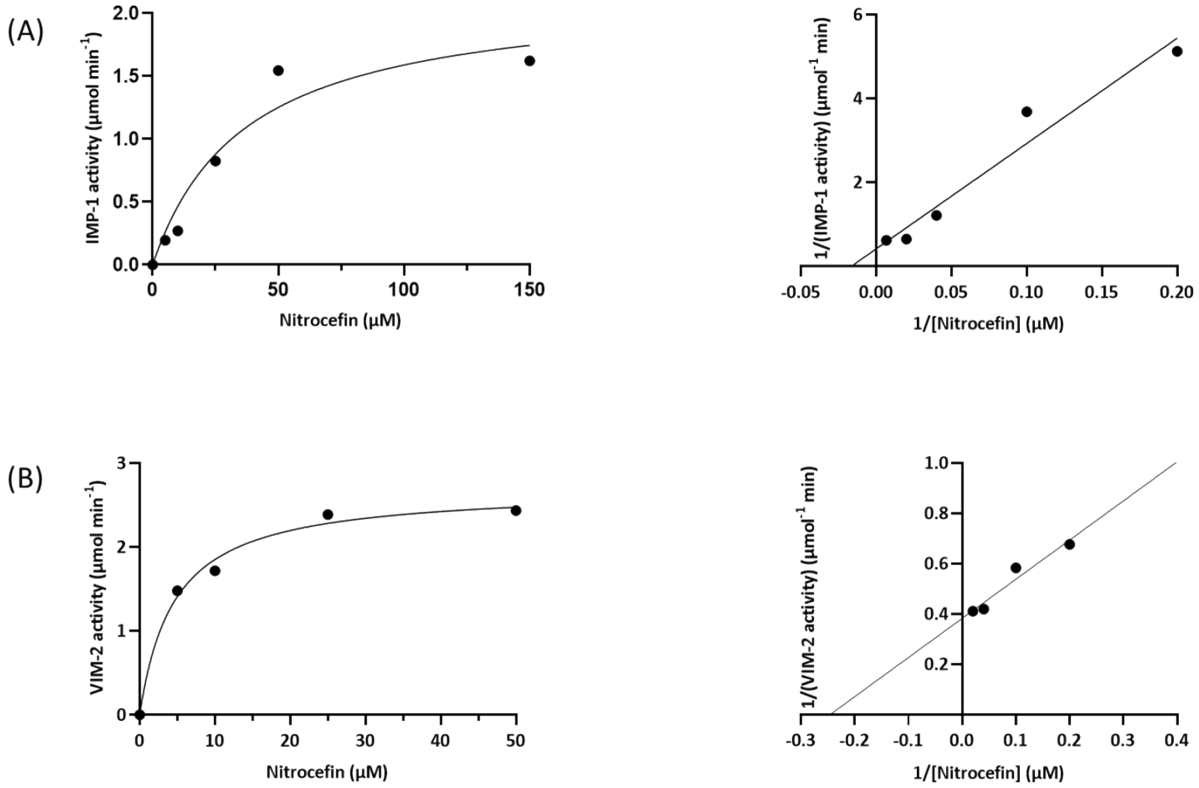
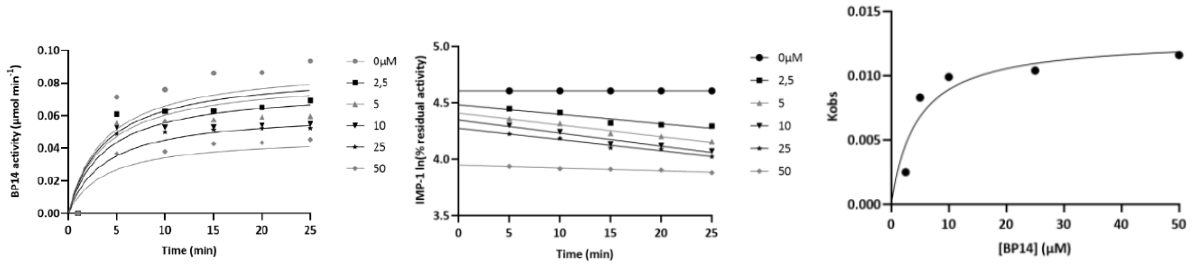


Figure S1: Michaelis-Menten and Lineweaver-Burk plots of (A) IMP-1 and (B) VIM-2. Experiments were conducted in triplicate.

A



B

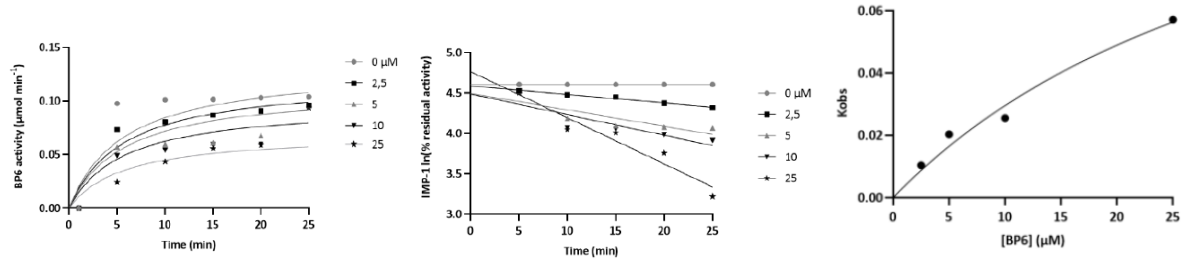
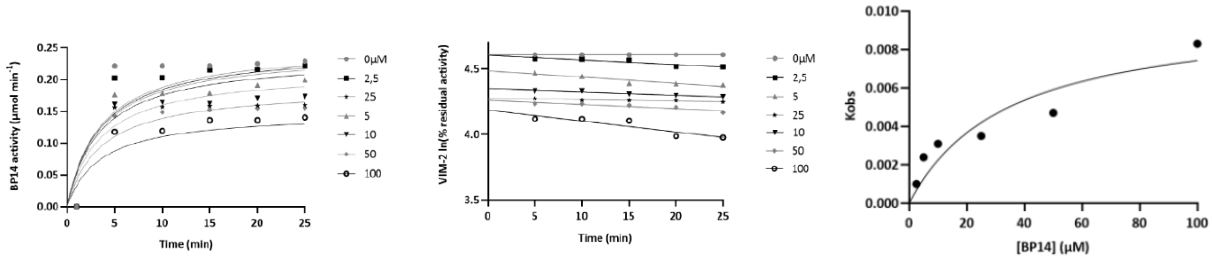


Figure S2: Inhibition targeted against IMP-1 utilizing inhibitor A) 24b and B) 24c.

Experiments were conducted in triplicate.

A



B

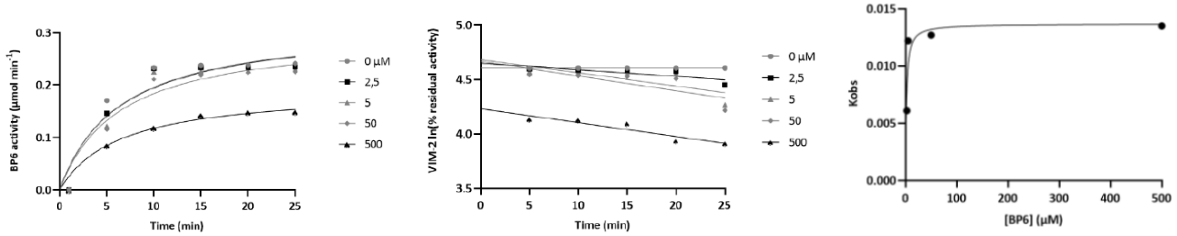
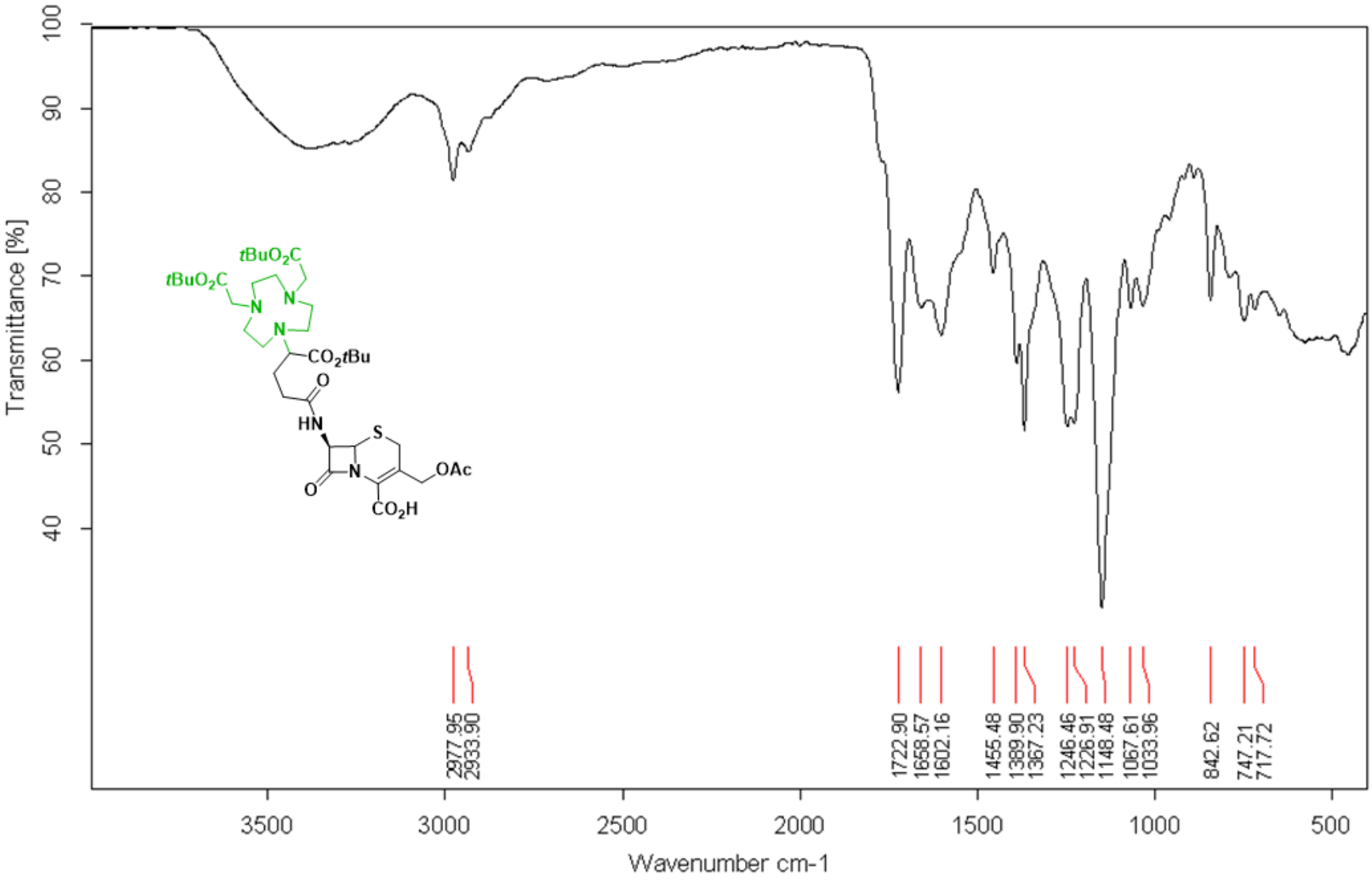


Figure S3: Inhibition targeted against VIM-2 utilizing inhibitor A) 24b and B) 24c.

Experiments were conducted in triplicate.

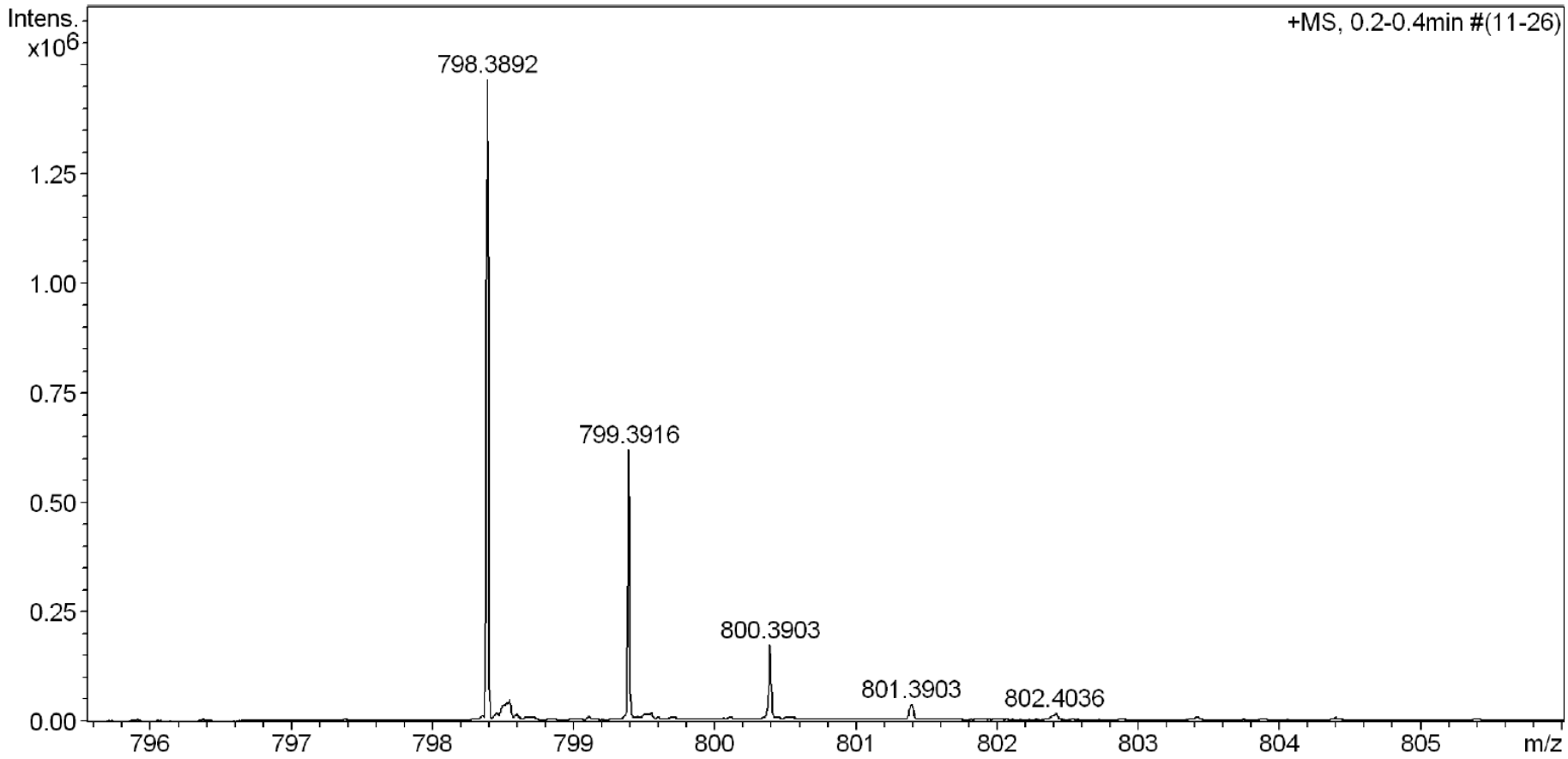
IR spectrum of **5**



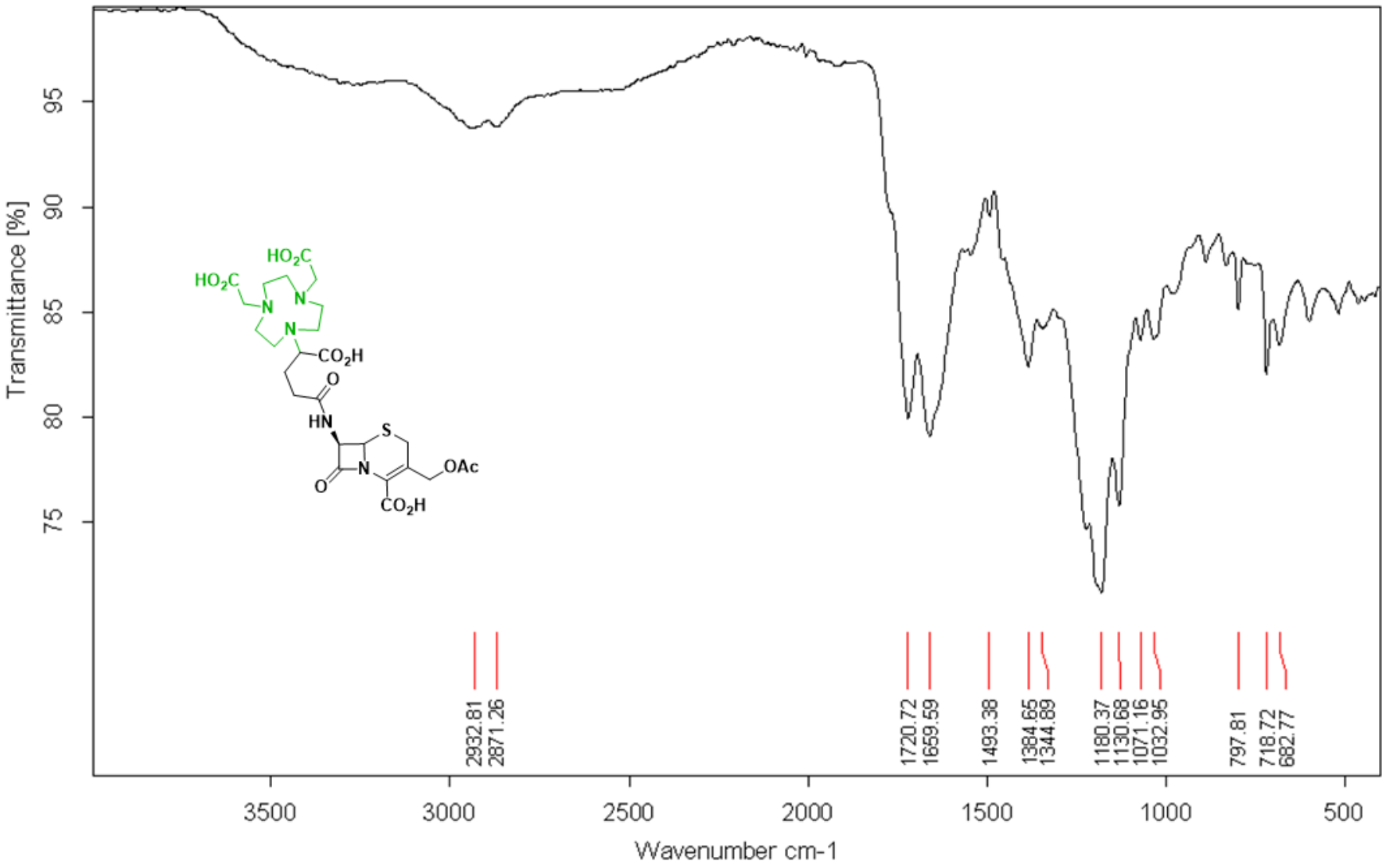
HRMS of **5**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



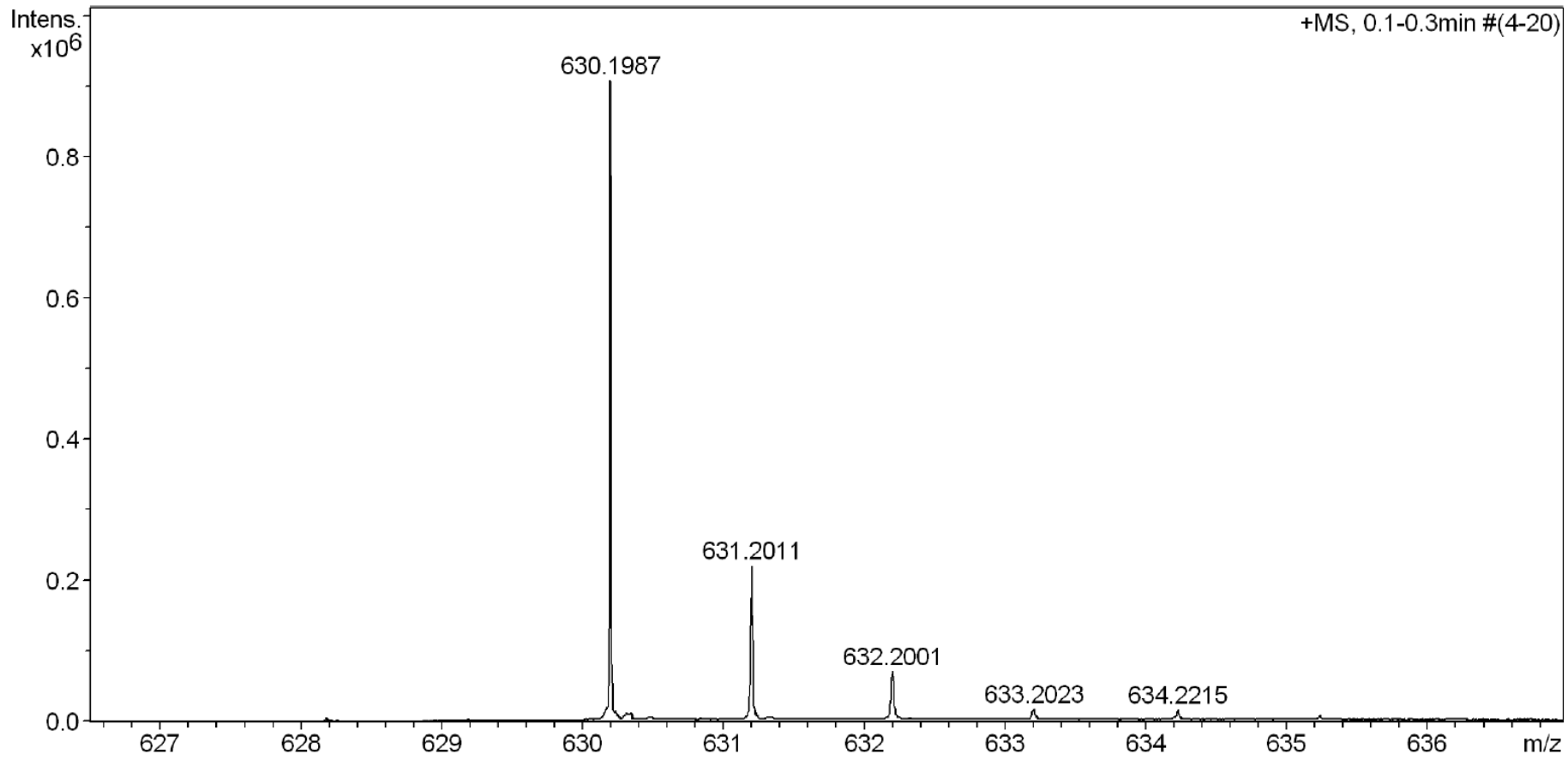
IR spectrum of **6**



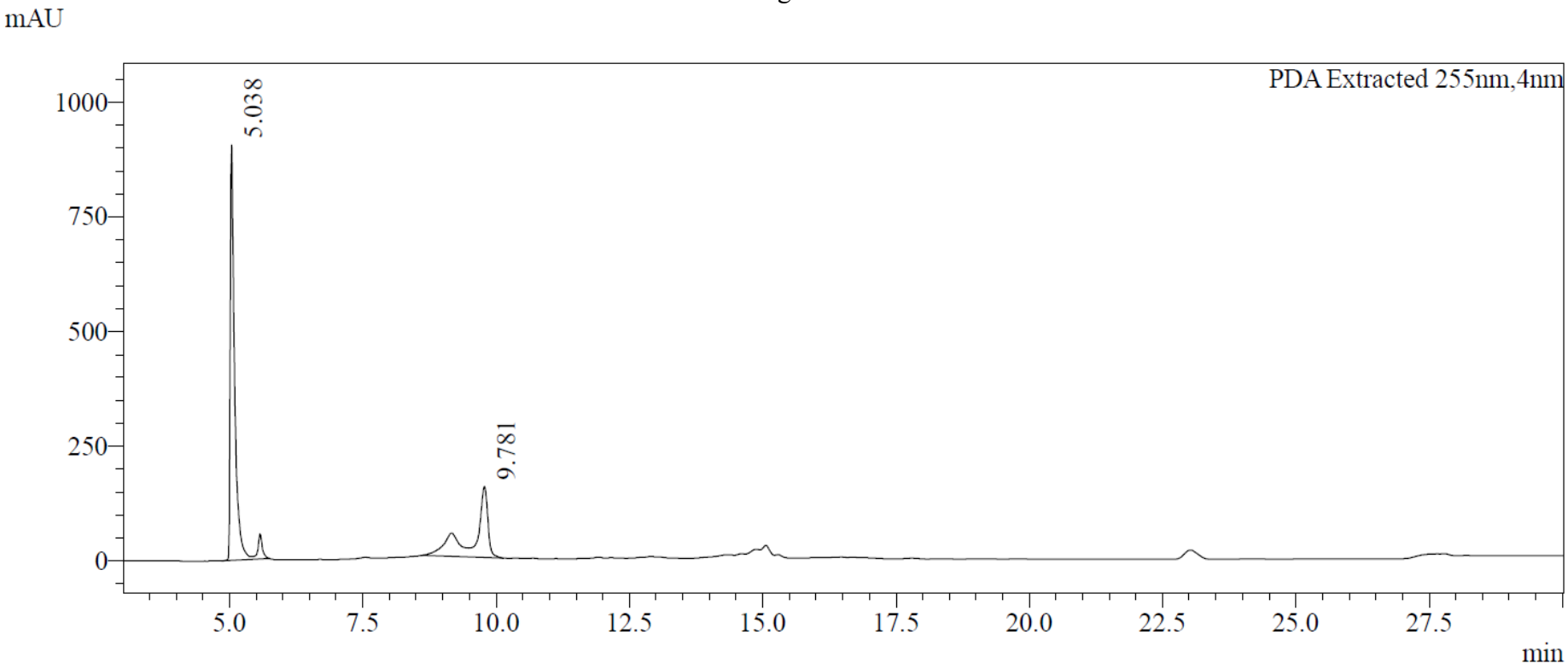
HRMS of **6**

Acquisition Parameter

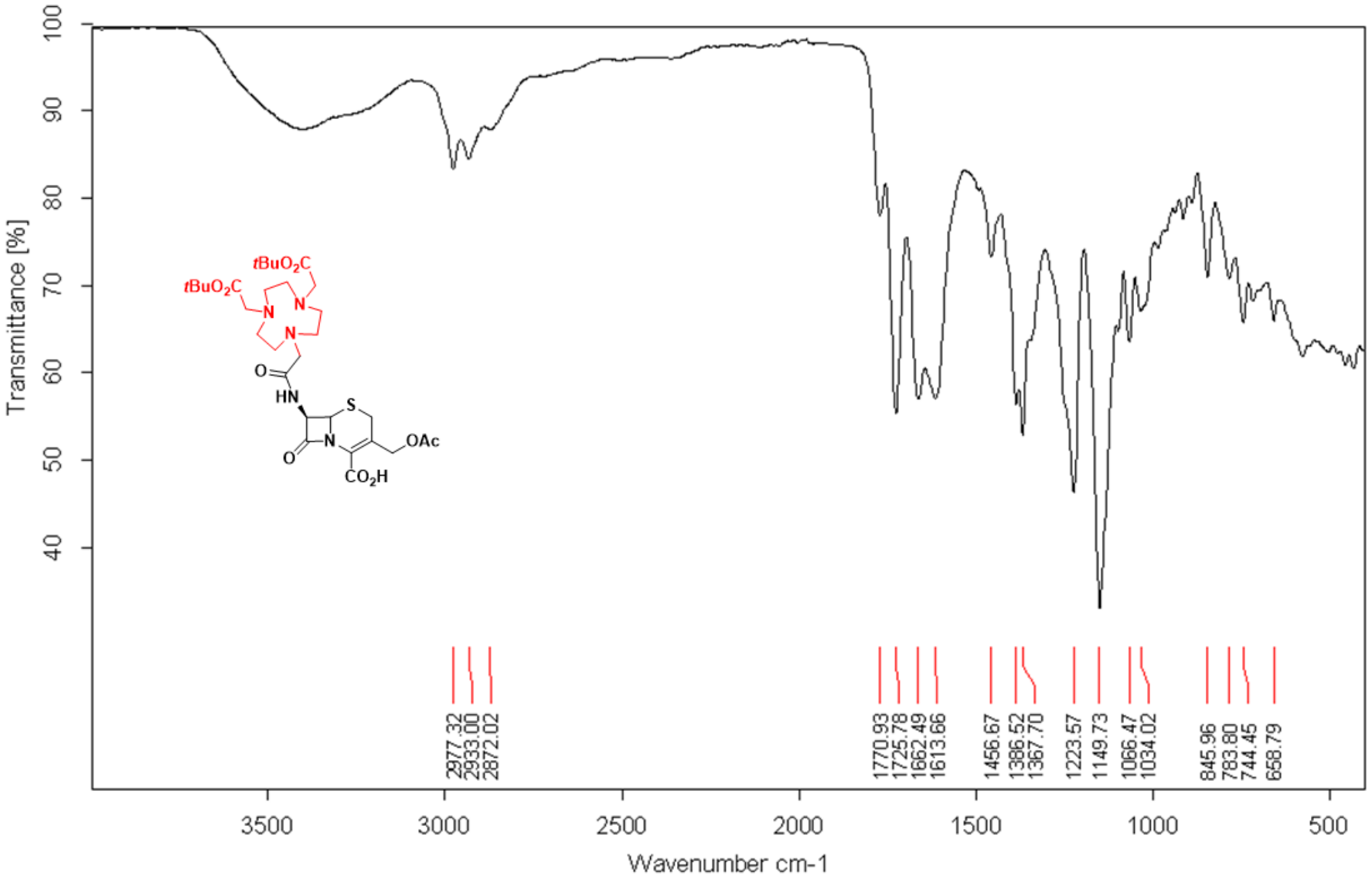
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **6**



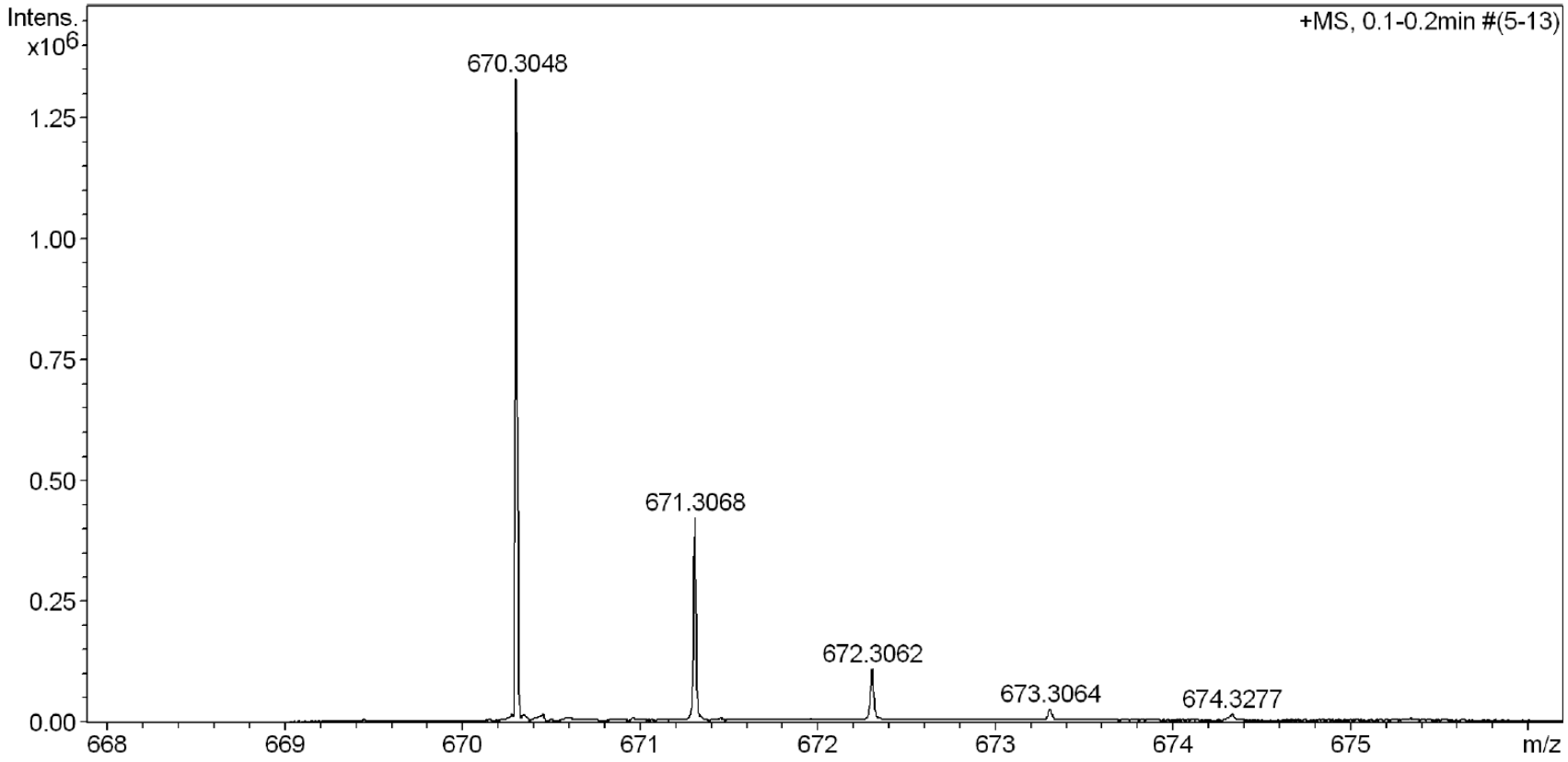
IR spectrum of **8**



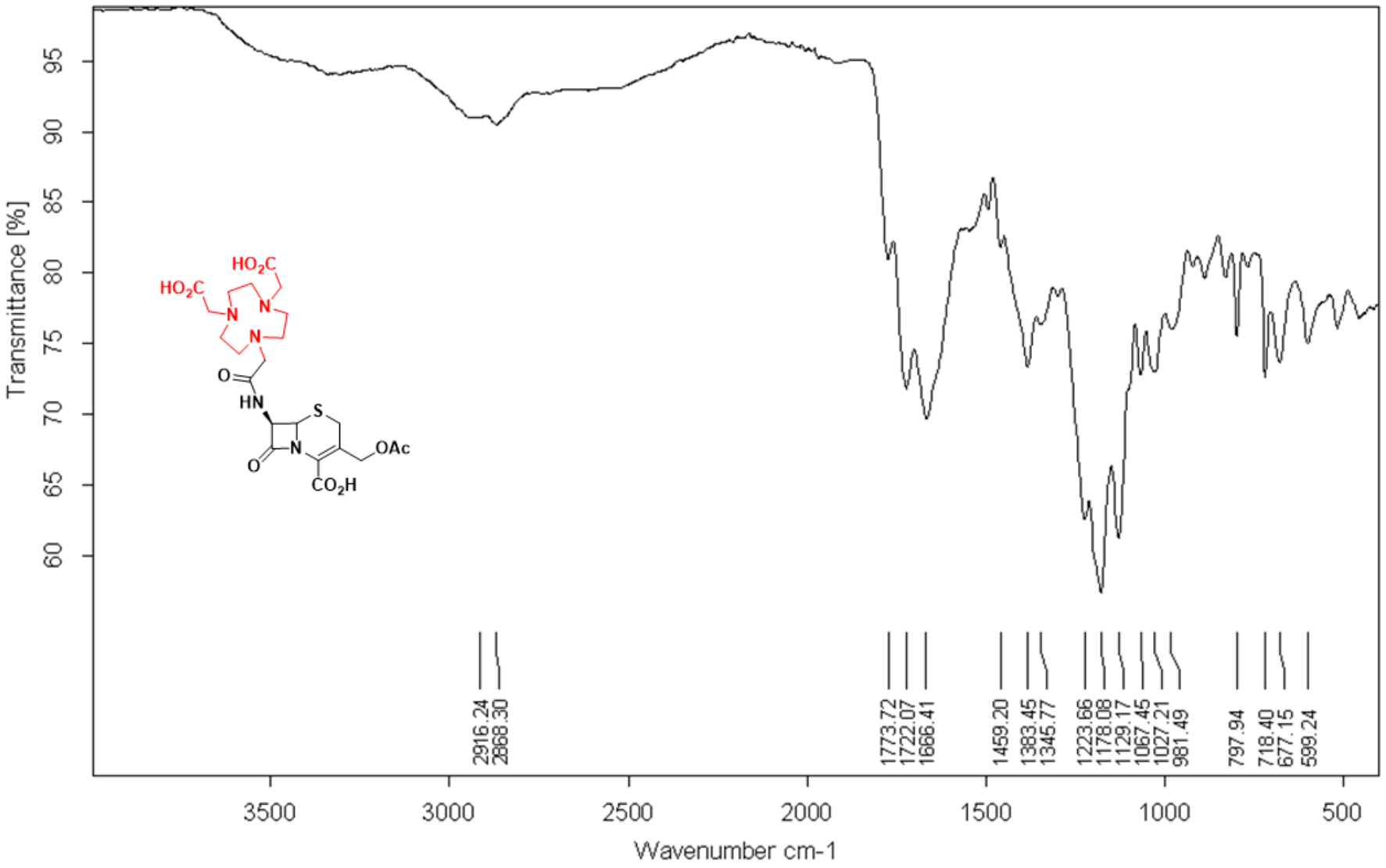
HRMS of **8**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



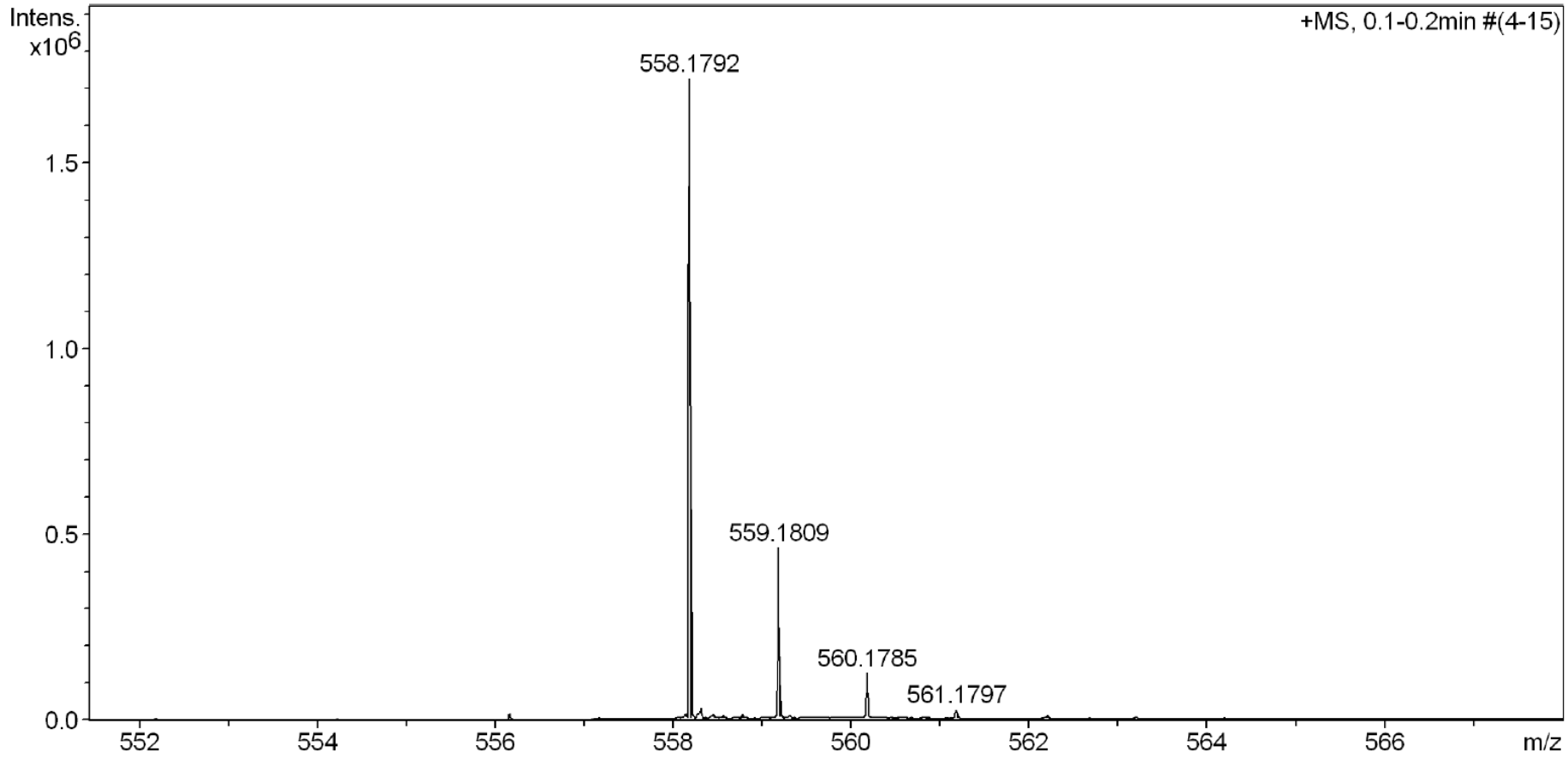
IR spectrum of **9**



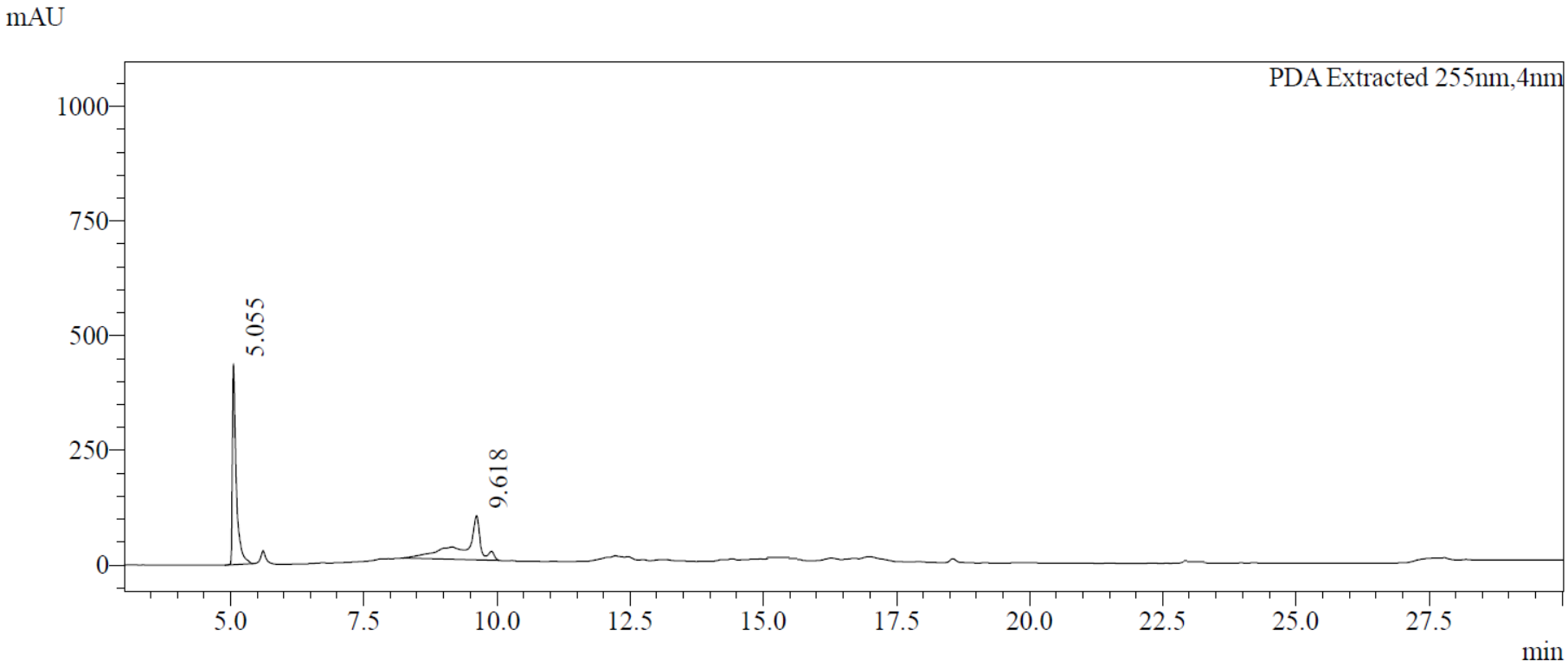
HRMS of **9**

Acquisition Parameter

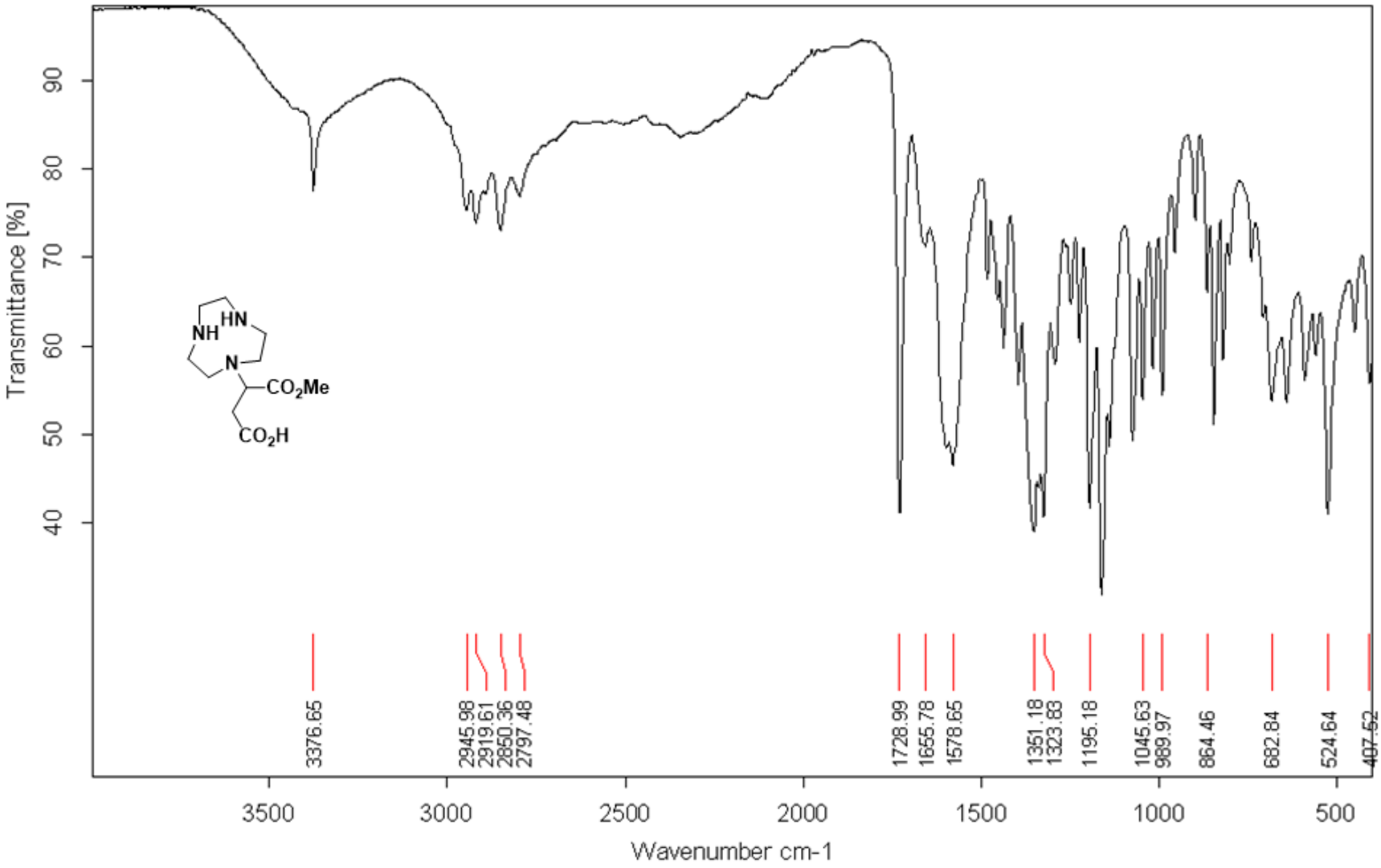
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



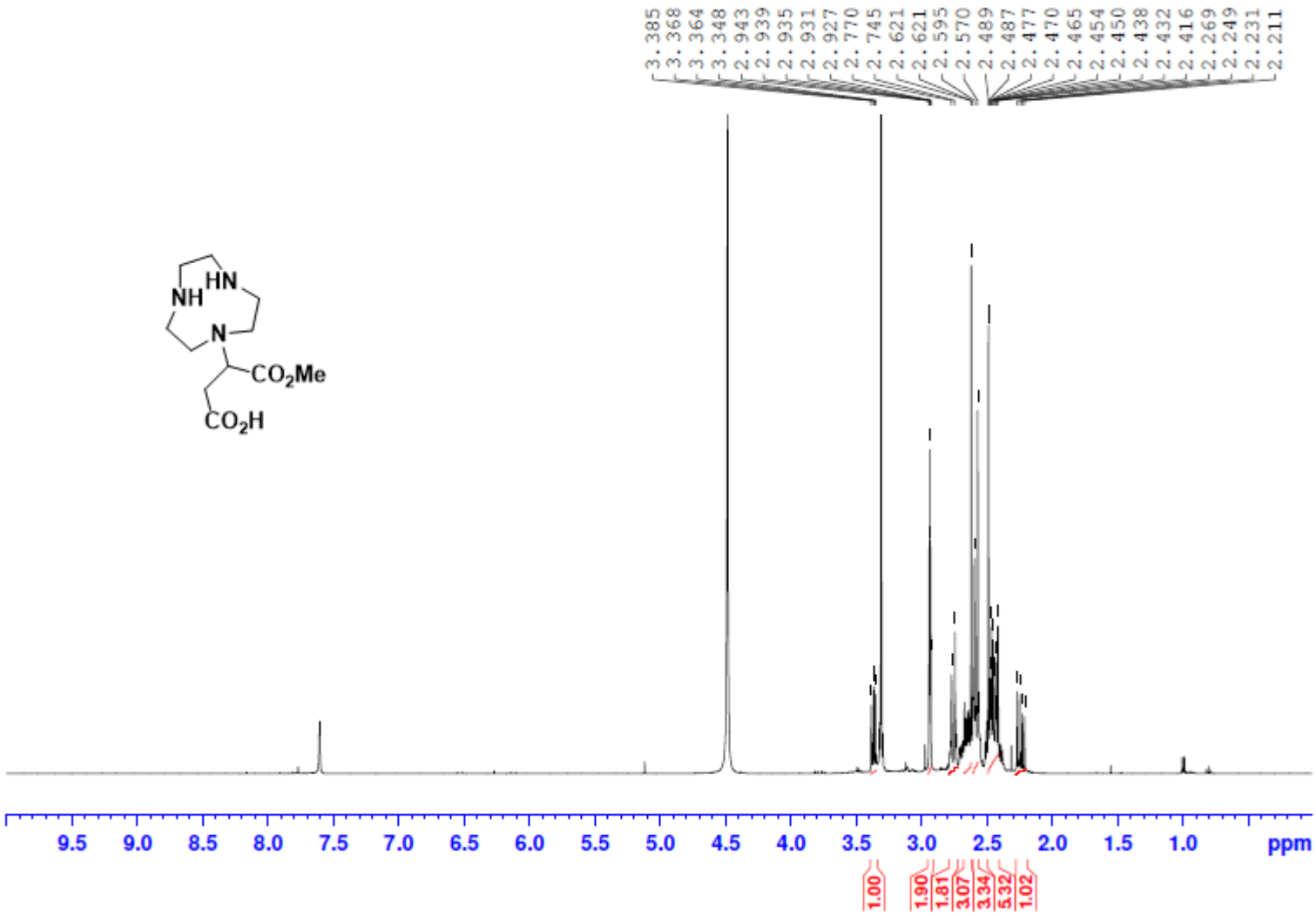
LC-MS chromatogram of **9**

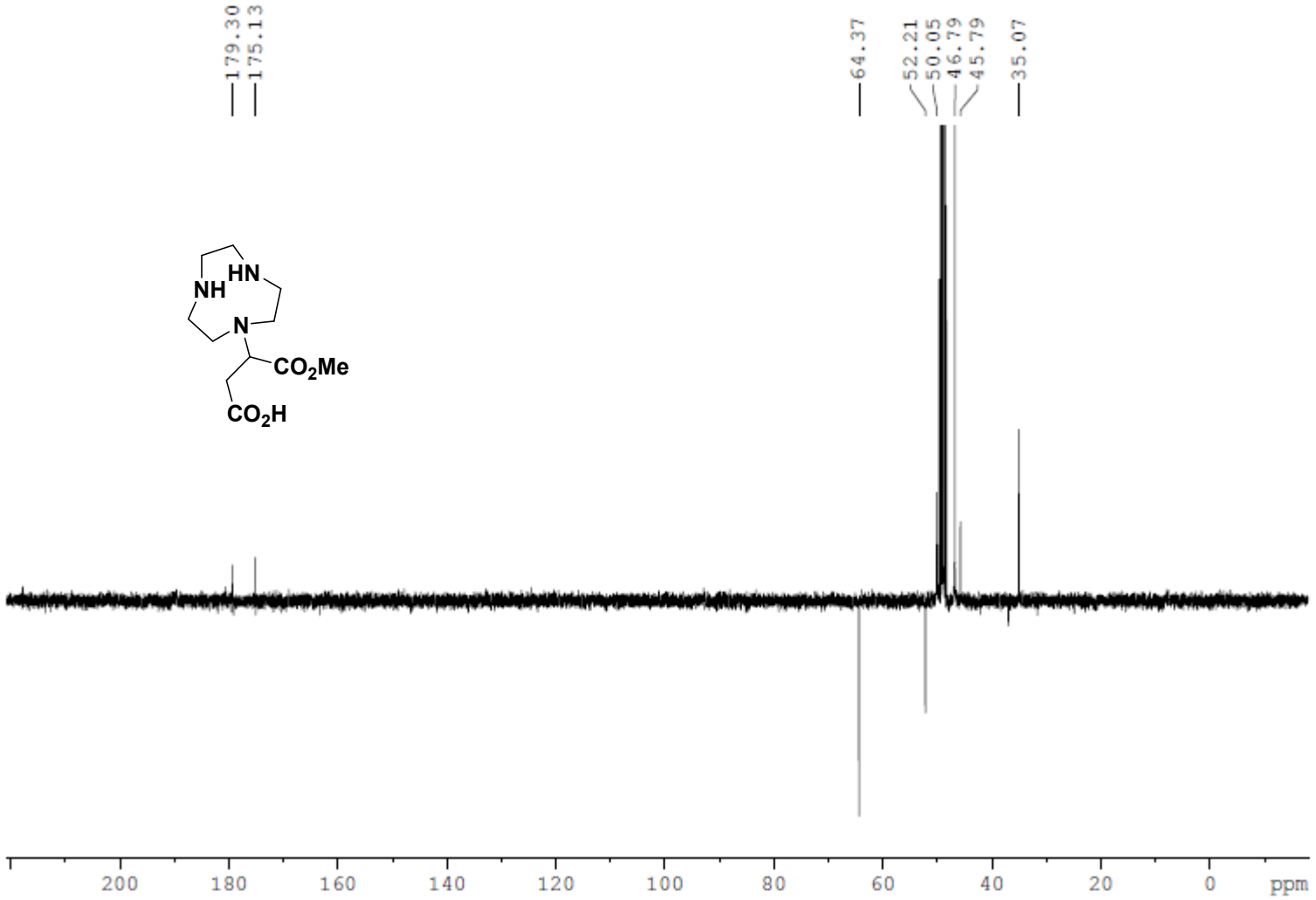


IR spectrum of (**11**)



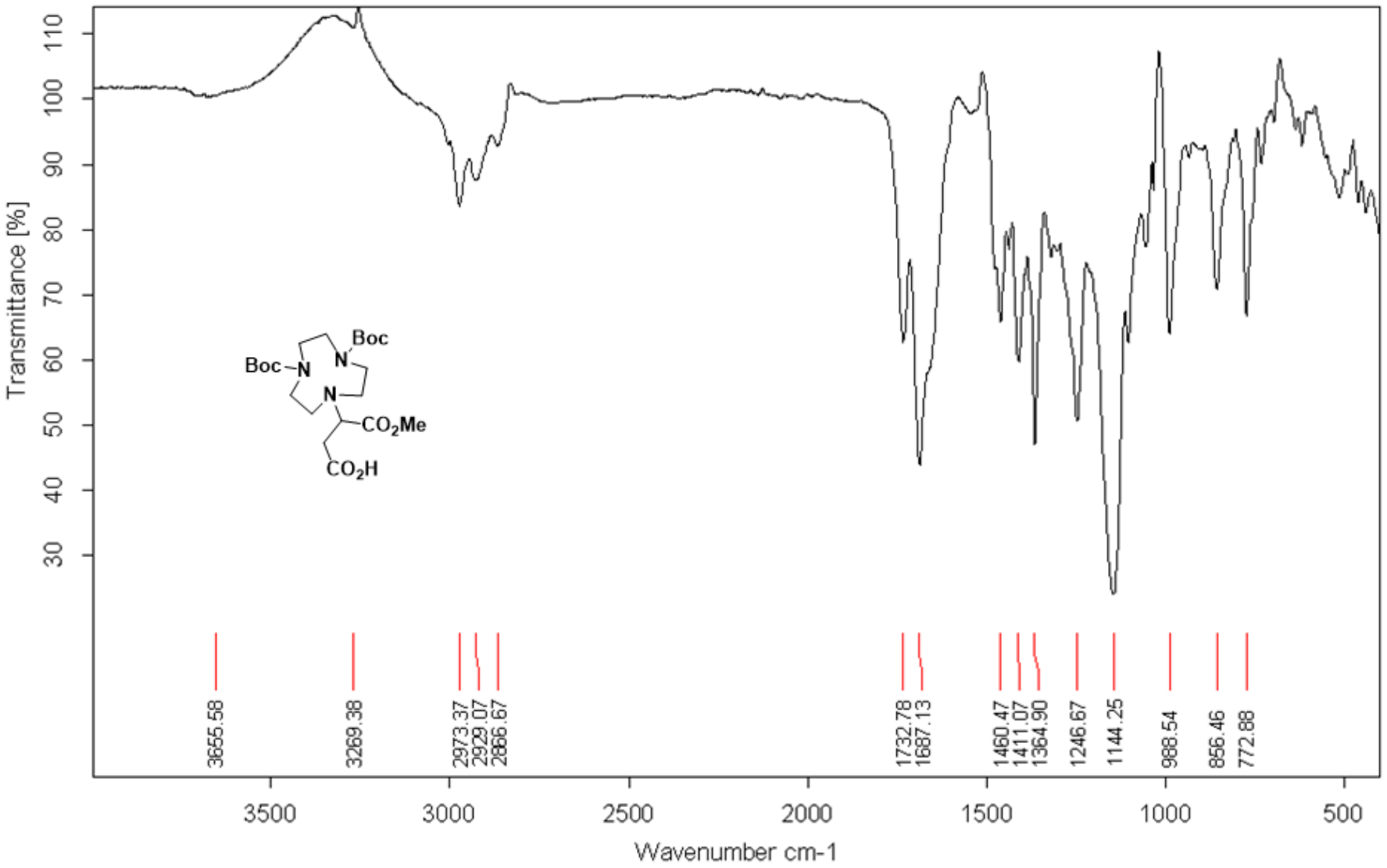
^{1}H NMR of (**5**) in MeOD


3.385
3.368
3.364
3.348
2.943
2.939
2.935
2.931
2.927
2.770
2.745
2.621
2.621
2.595
2.570
2.489
2.487
2.477
2.470
2.465
2.454
2.450
2.438
2.432
2.416
2.269
2.249
2.231
2.211
NH
HN
N
CO2Me
CO2H
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
1.90
1.81
3.07
3.34
5.32
1.02



^{13}C NMR of (**11**) in MeOD

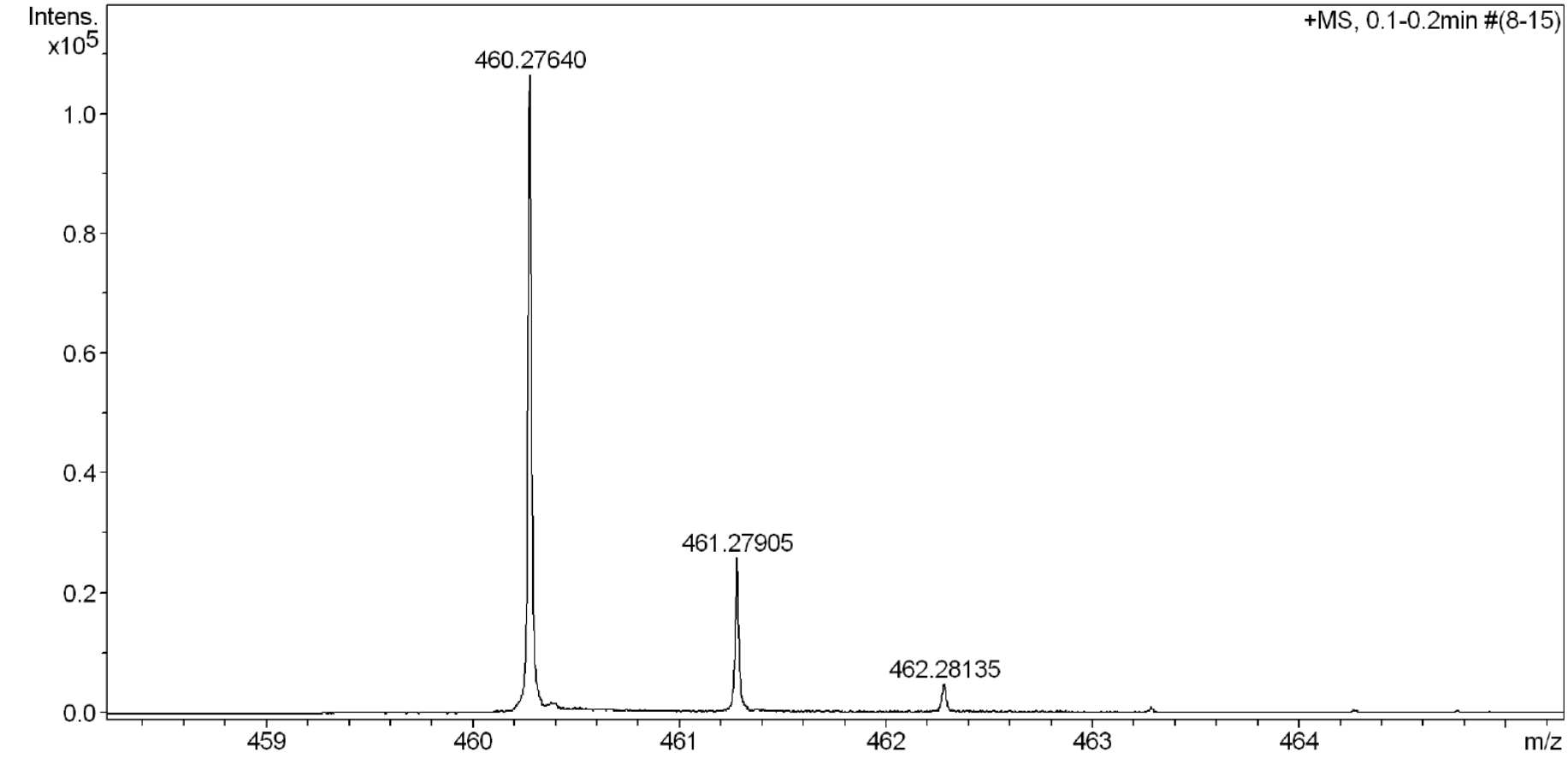
IR spectrum of **12**



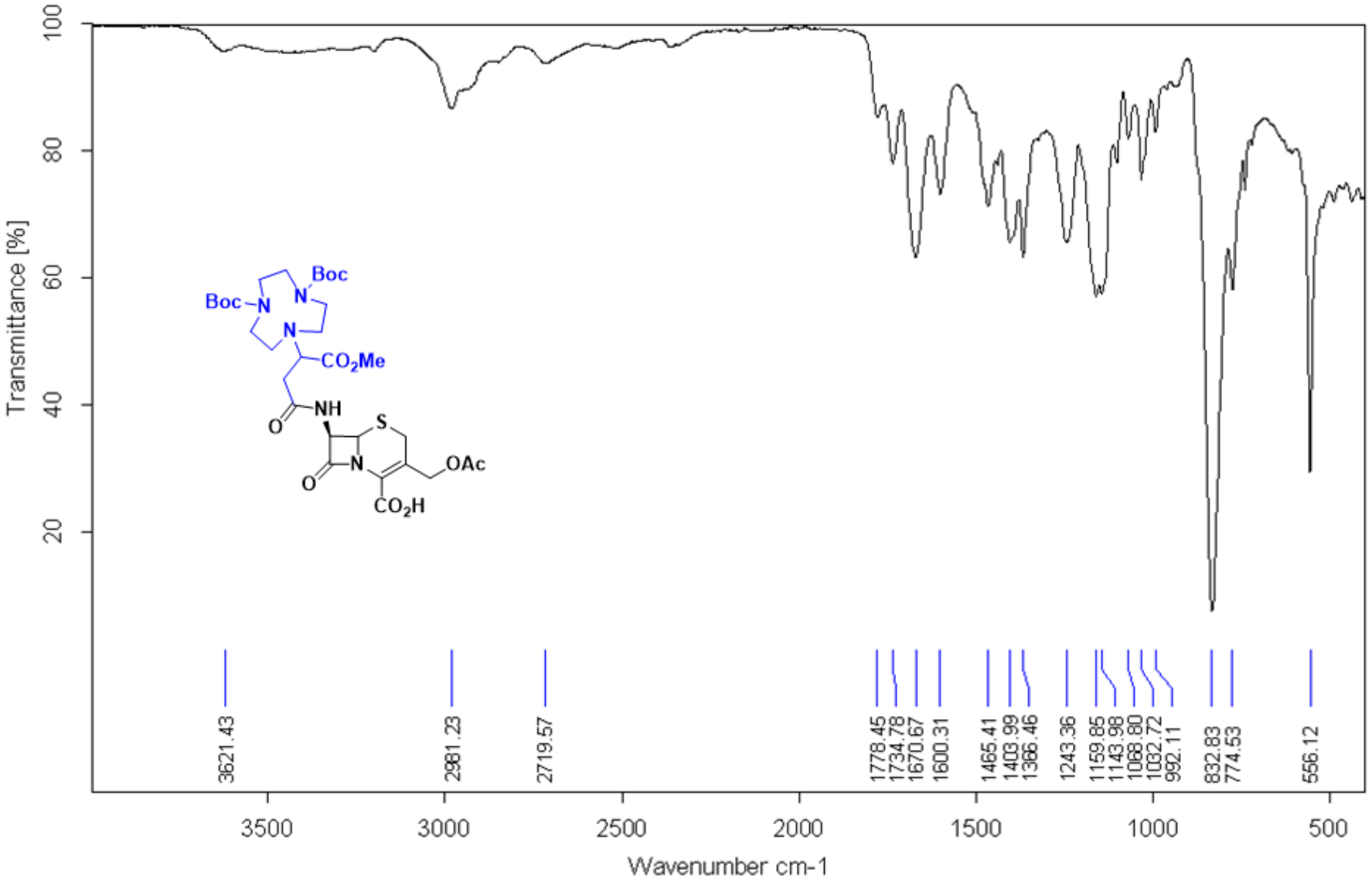
HRMS of **12**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



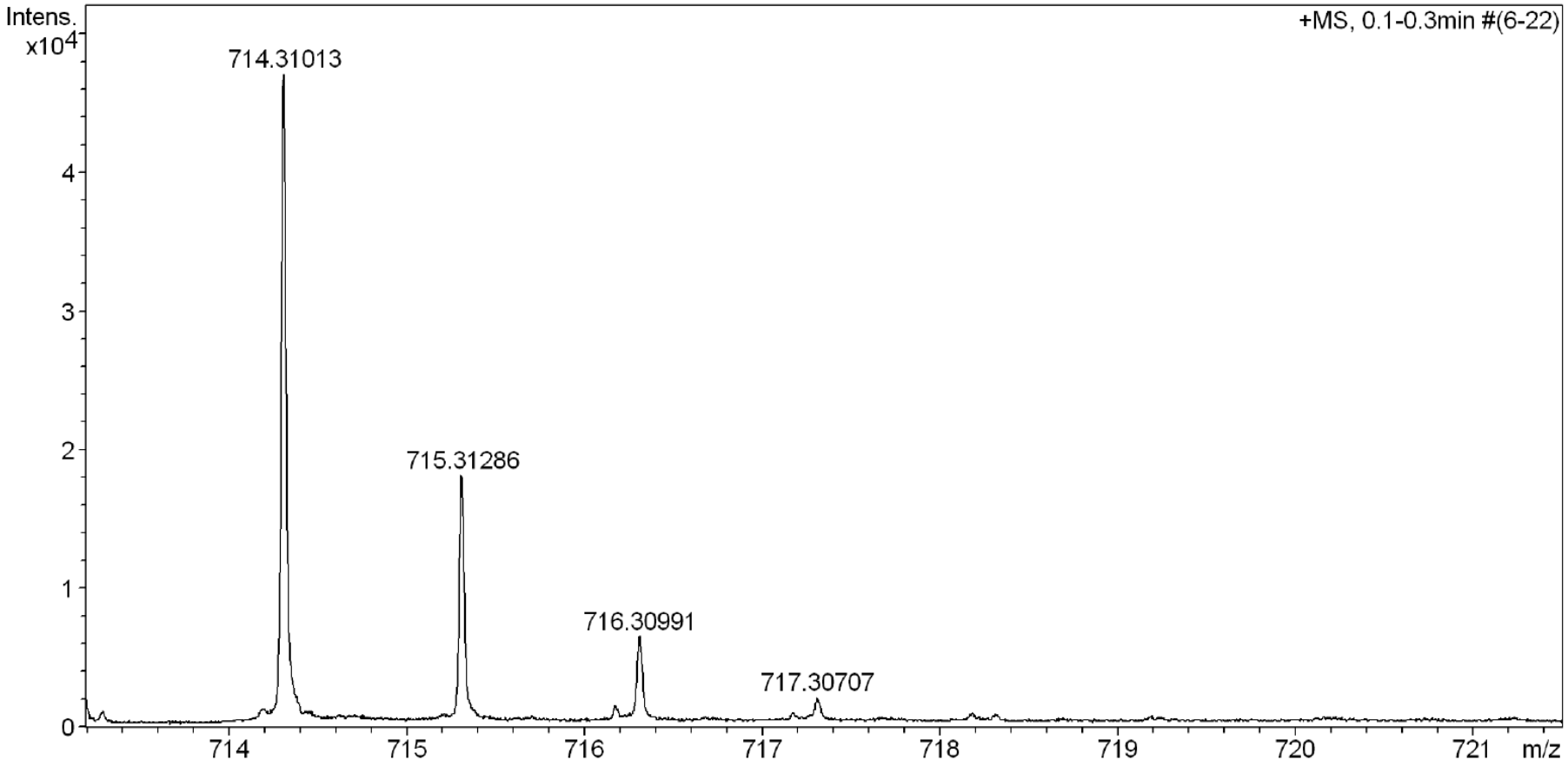
IR spectrum of (**13**)



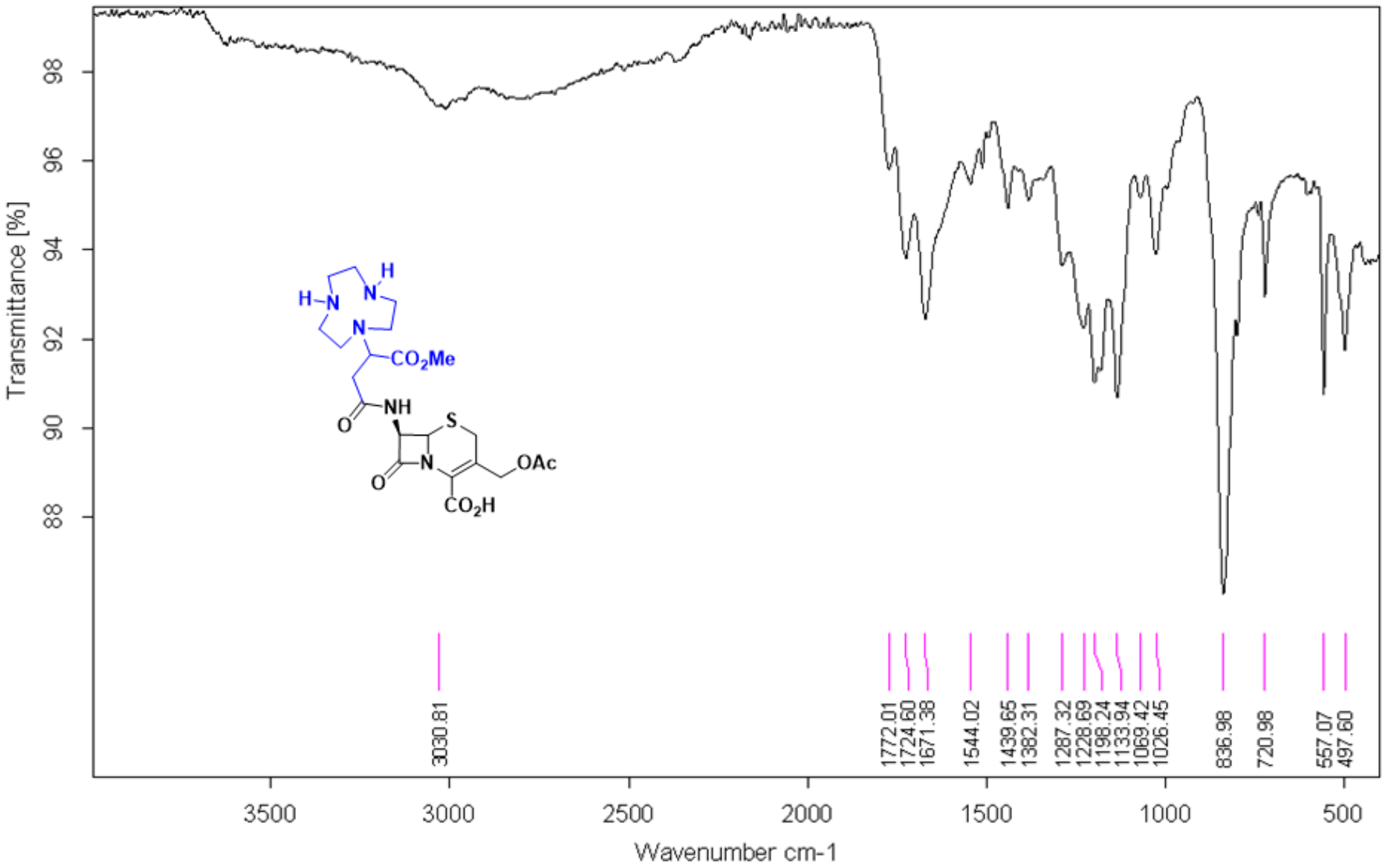
HRMS of **13**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



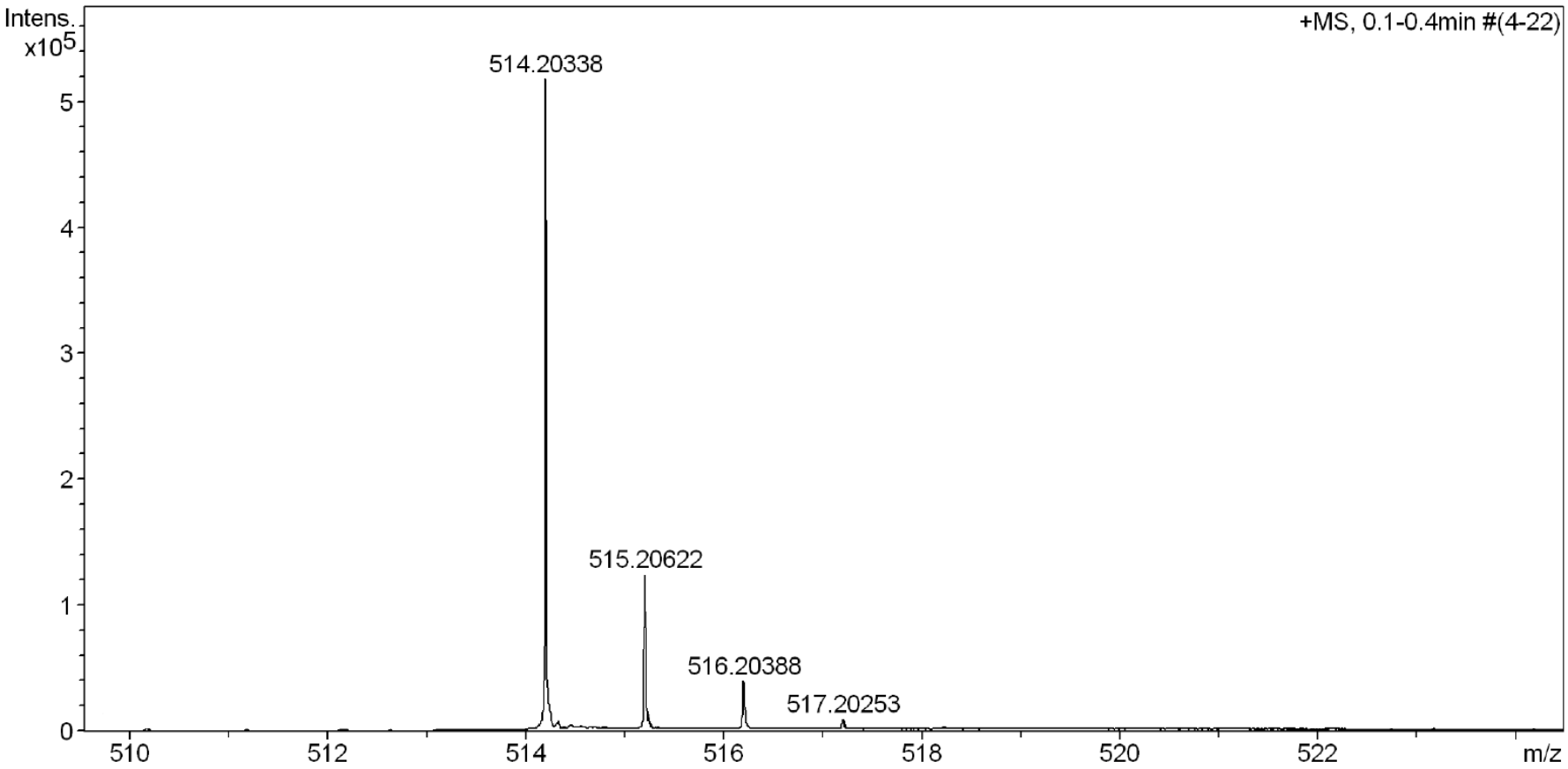
IR spectrum of **14**



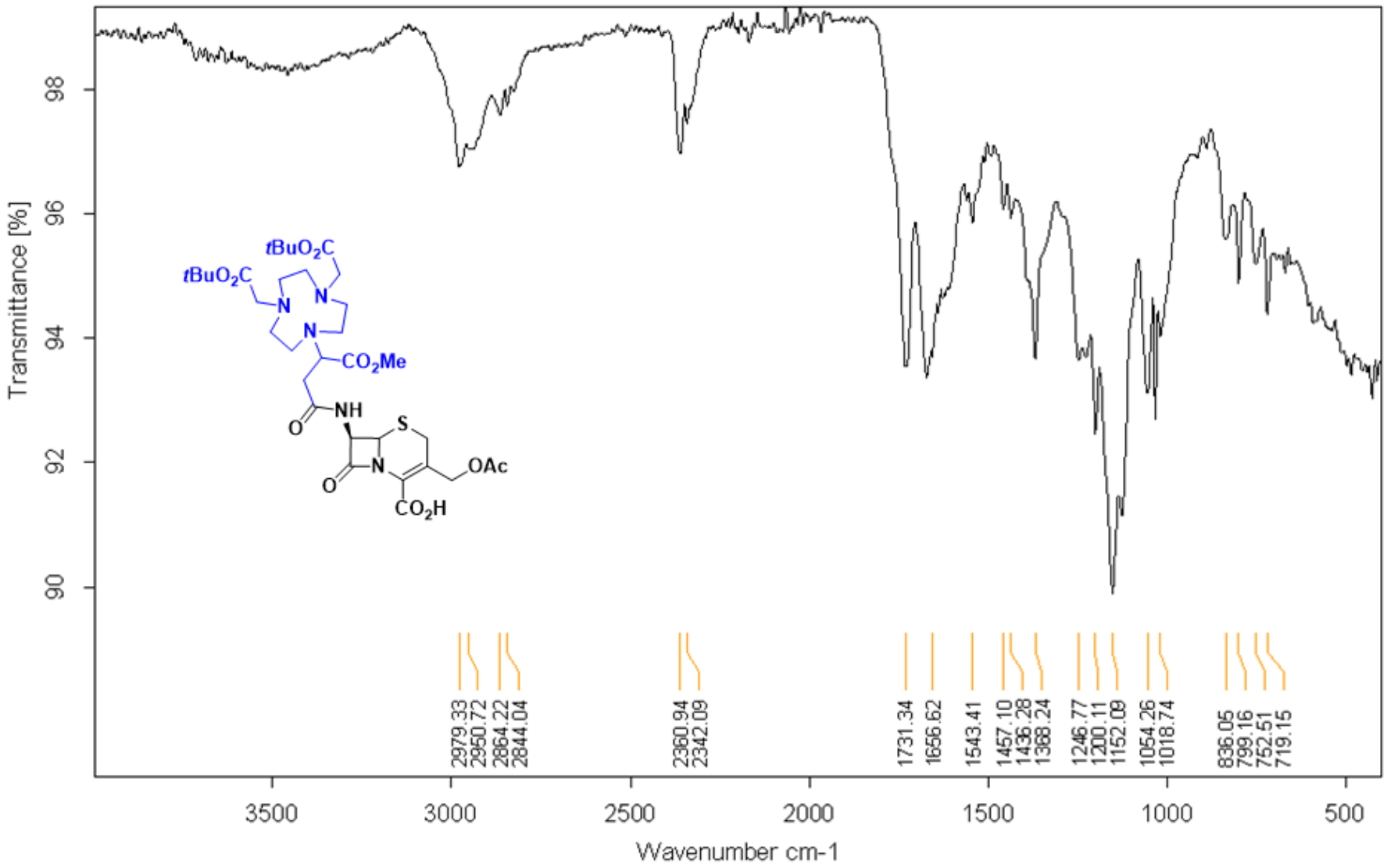
HRMS of **14**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



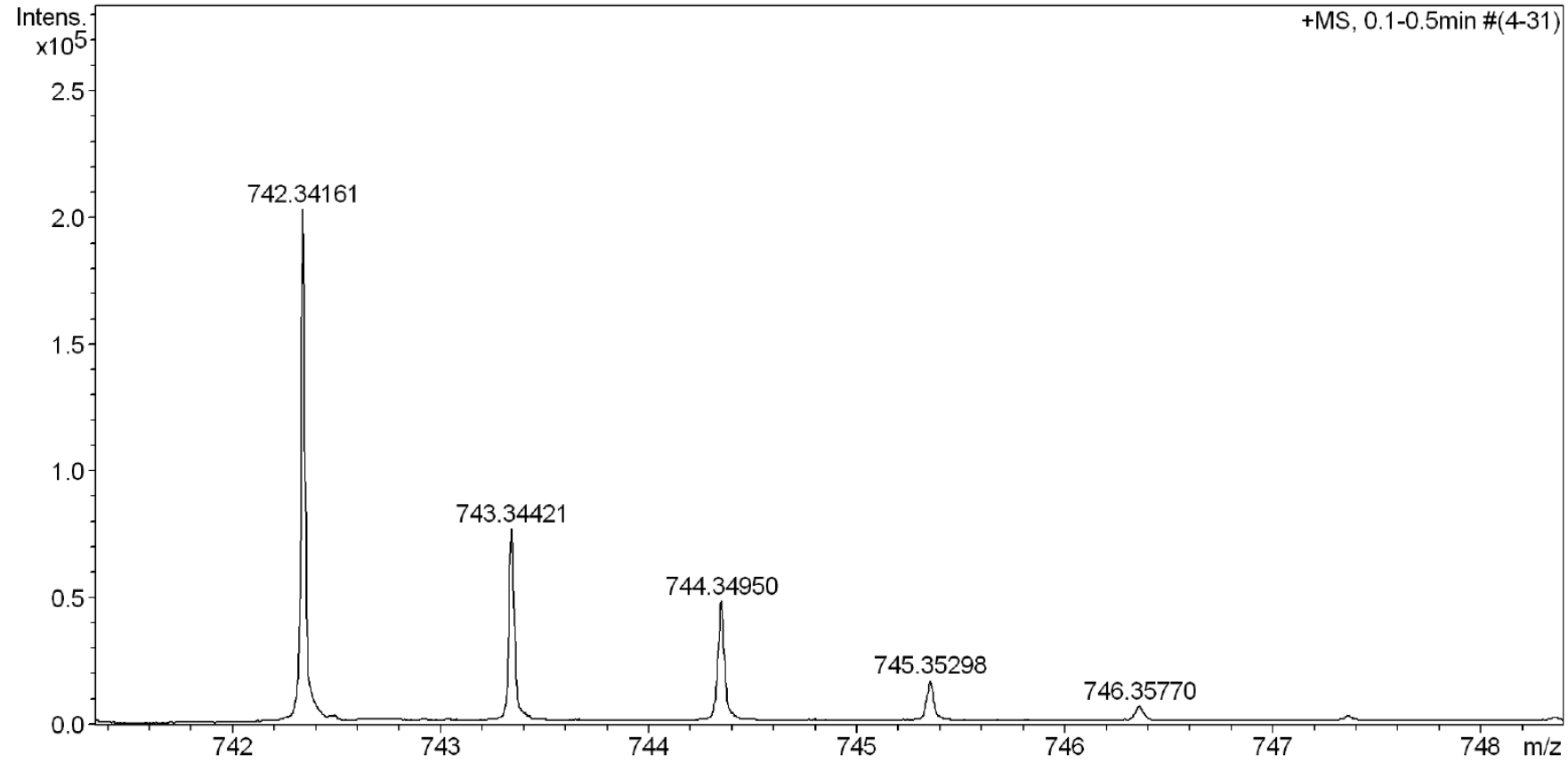
IR spectrum of **15**



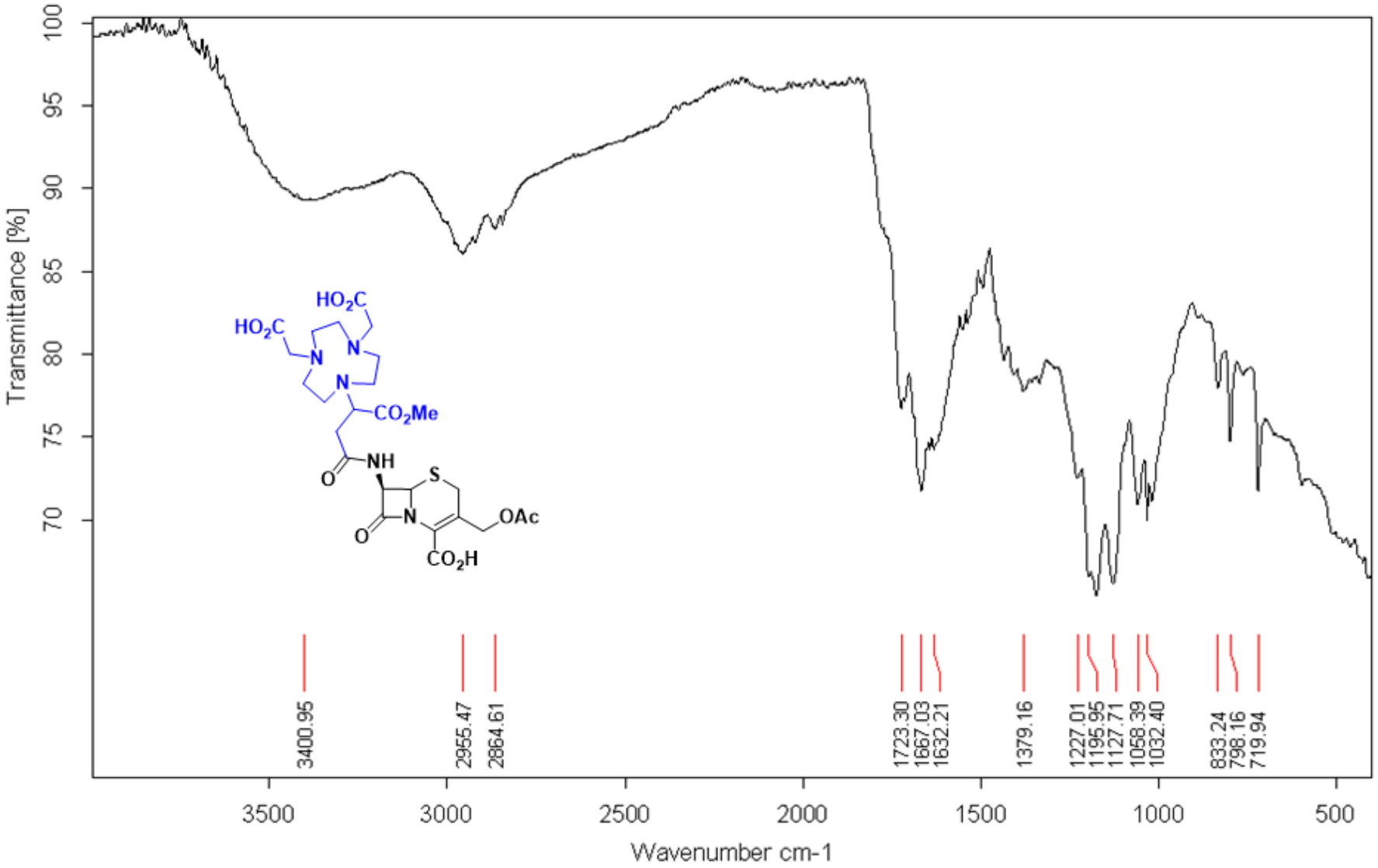
HRMS of **15**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



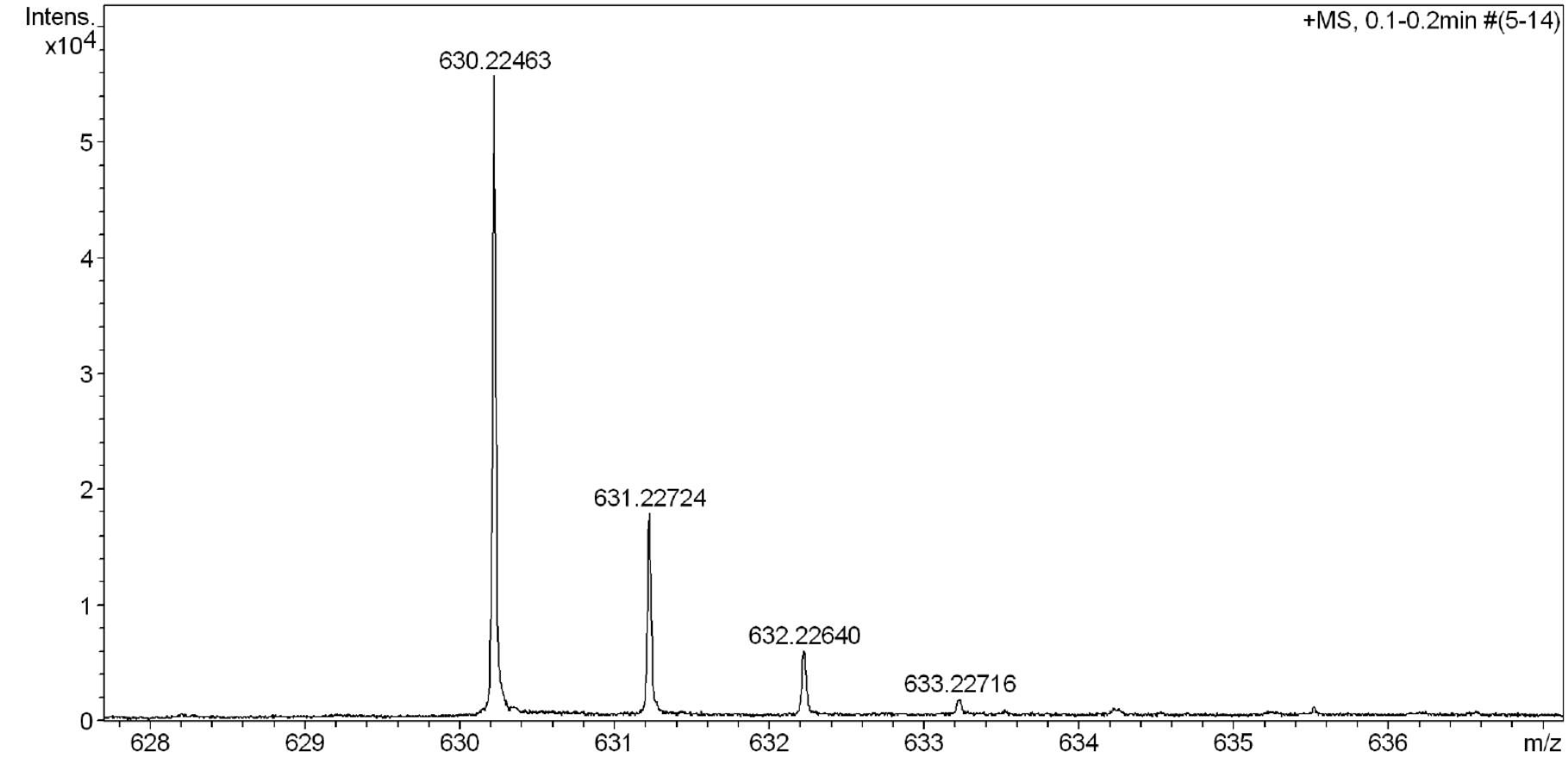
IR spectrum of **16**



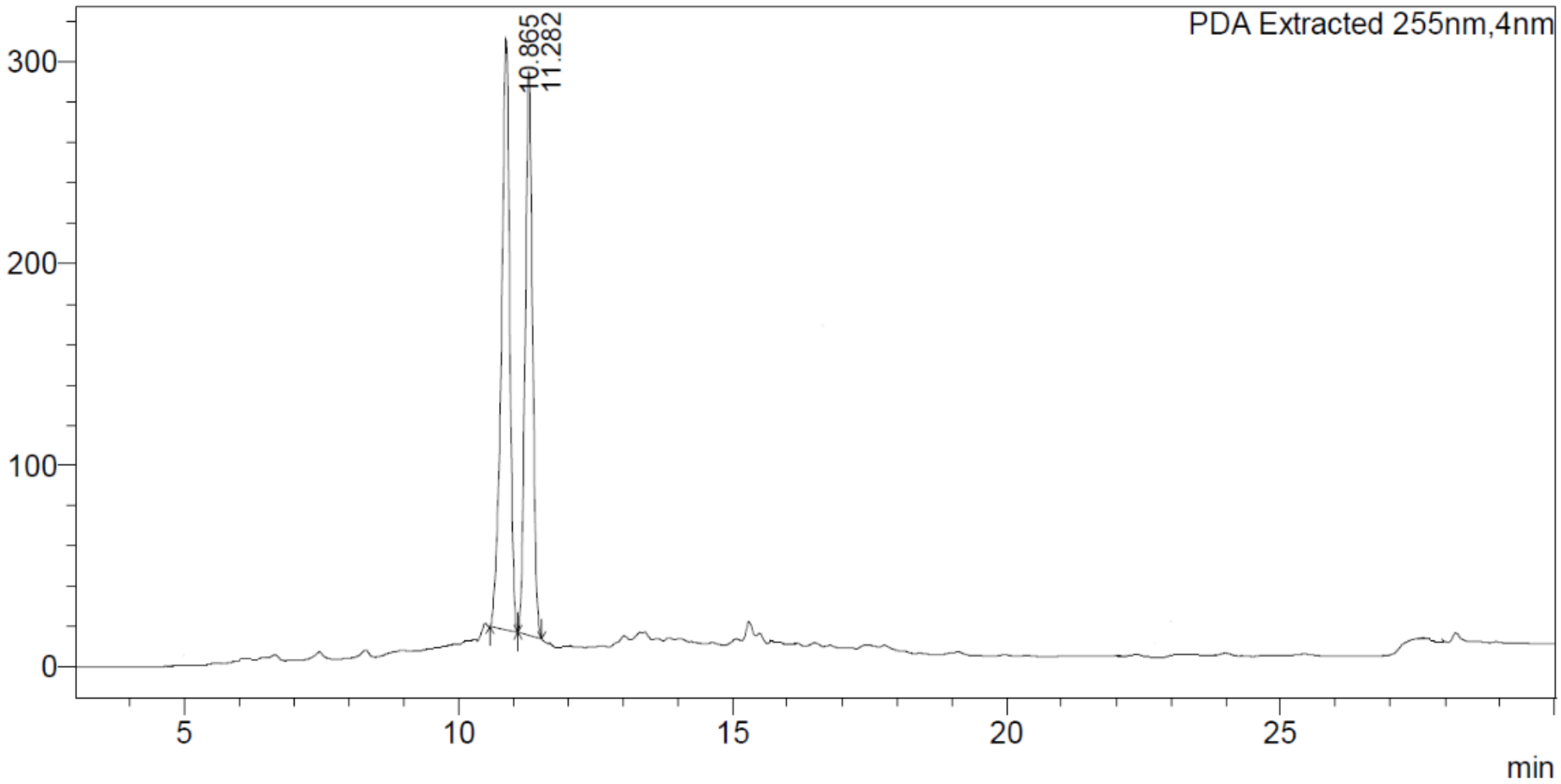
HRMS of **16**

Acquisition Parameter

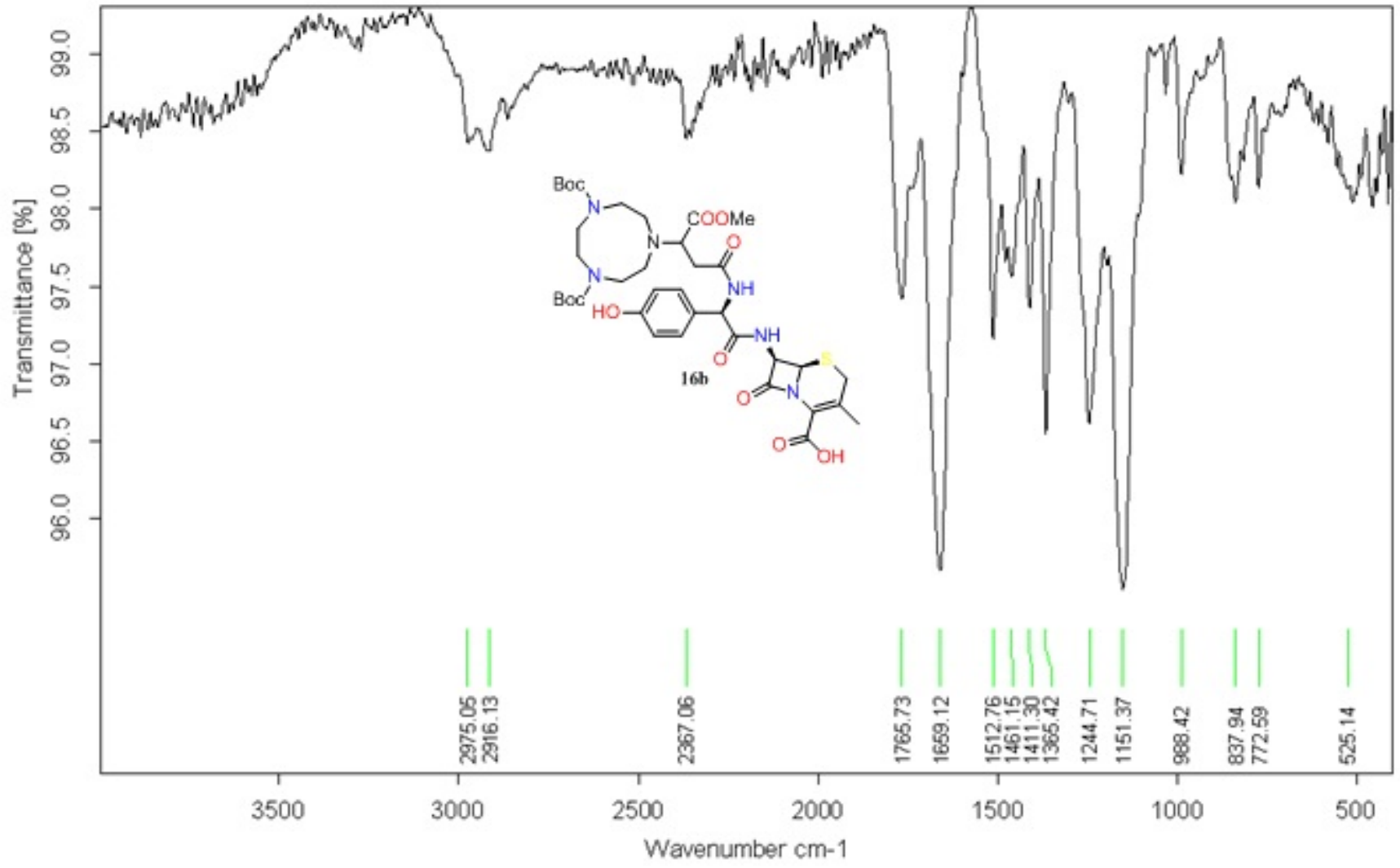
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **16**



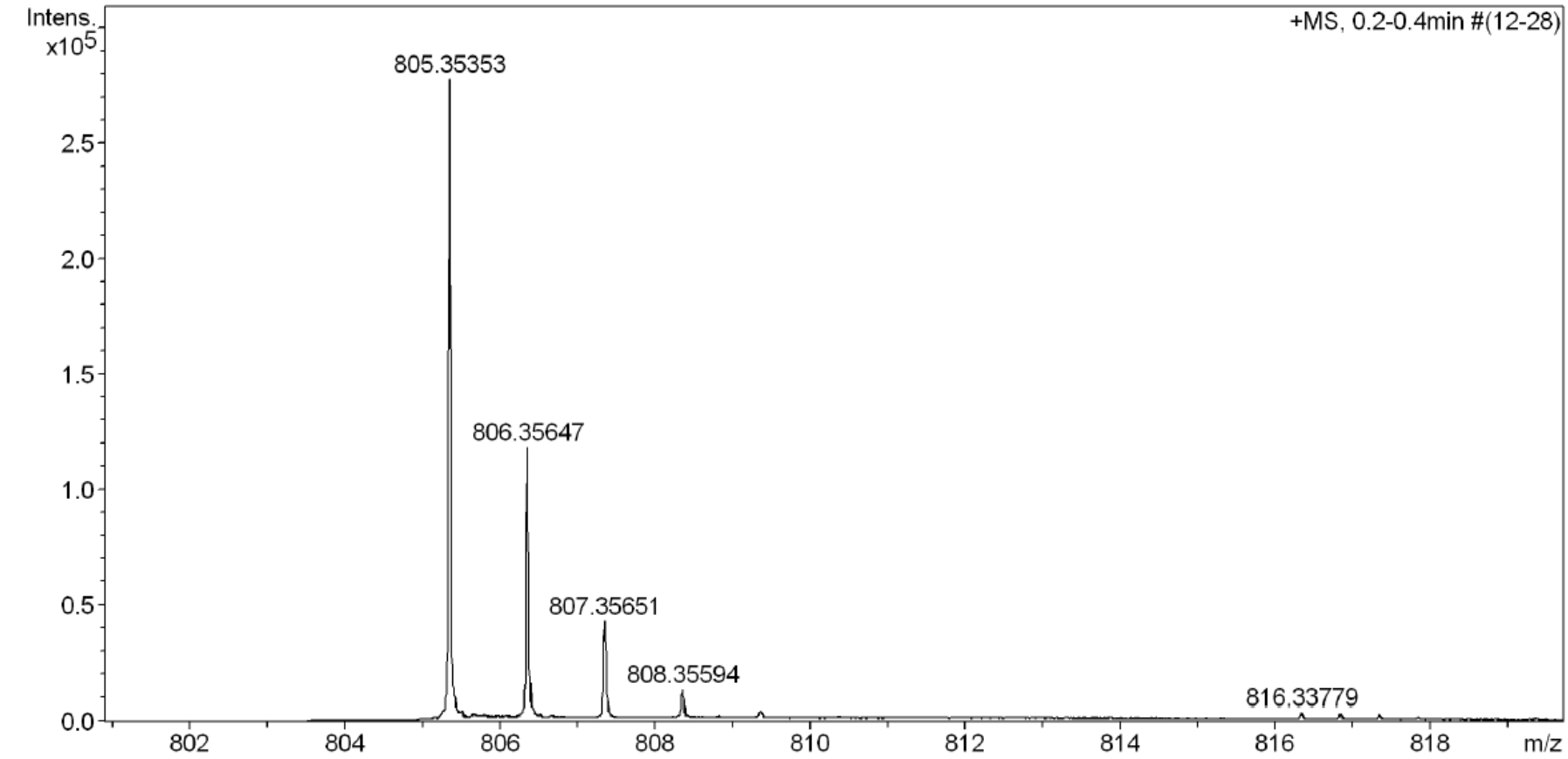
IR spectrum of **17a**



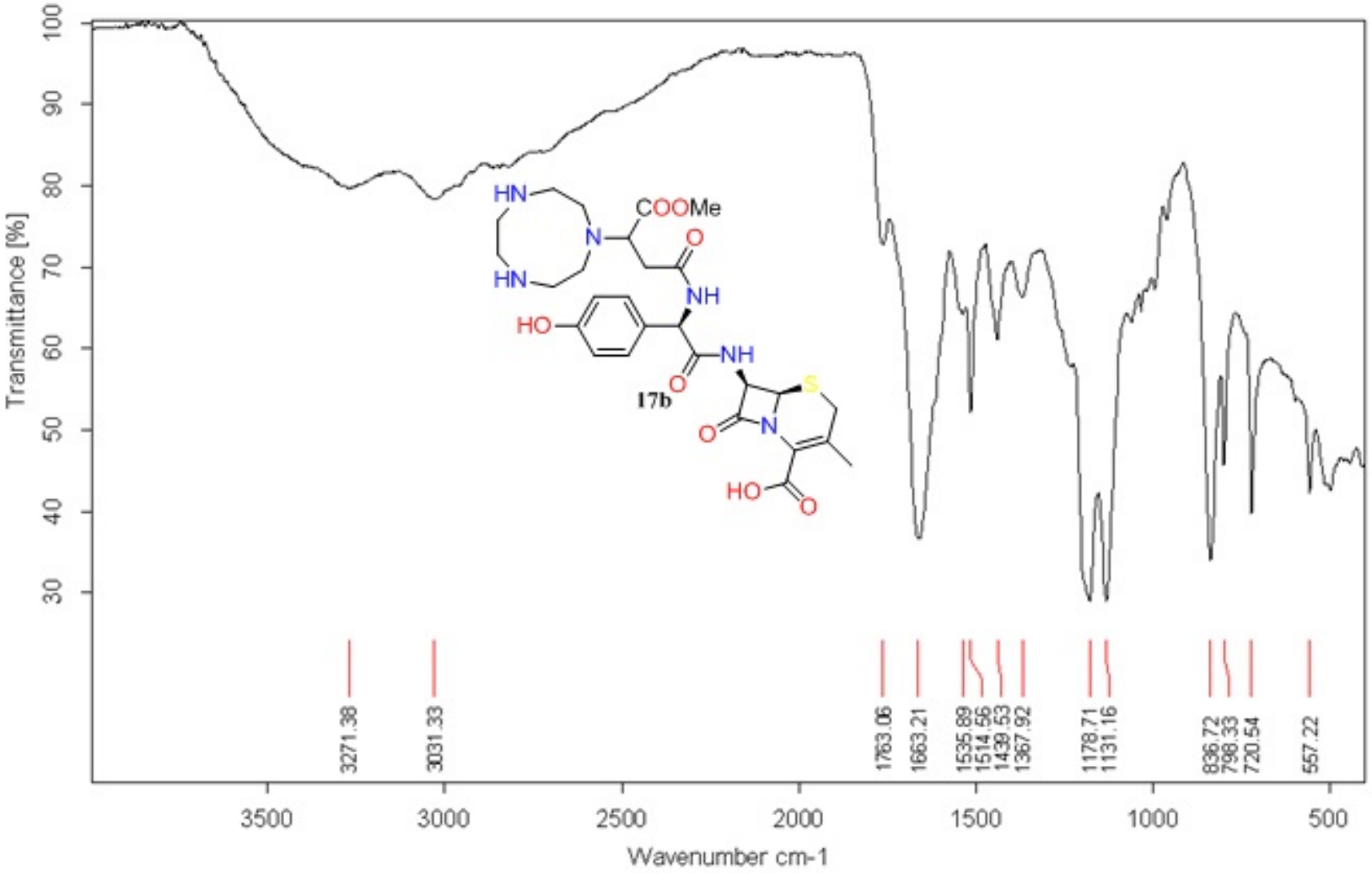
HRMS of **17a**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



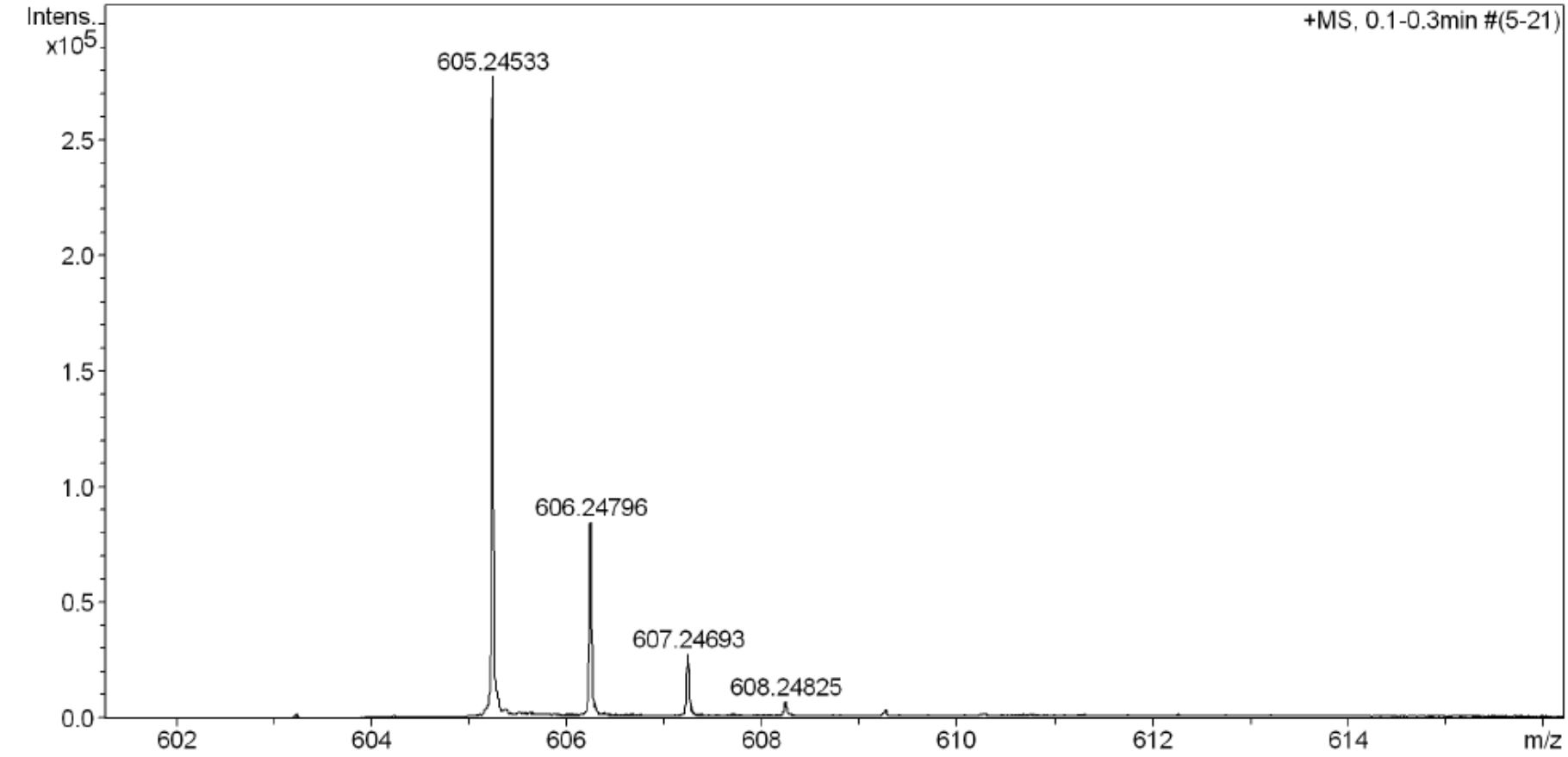
IR spectrum of **18a**



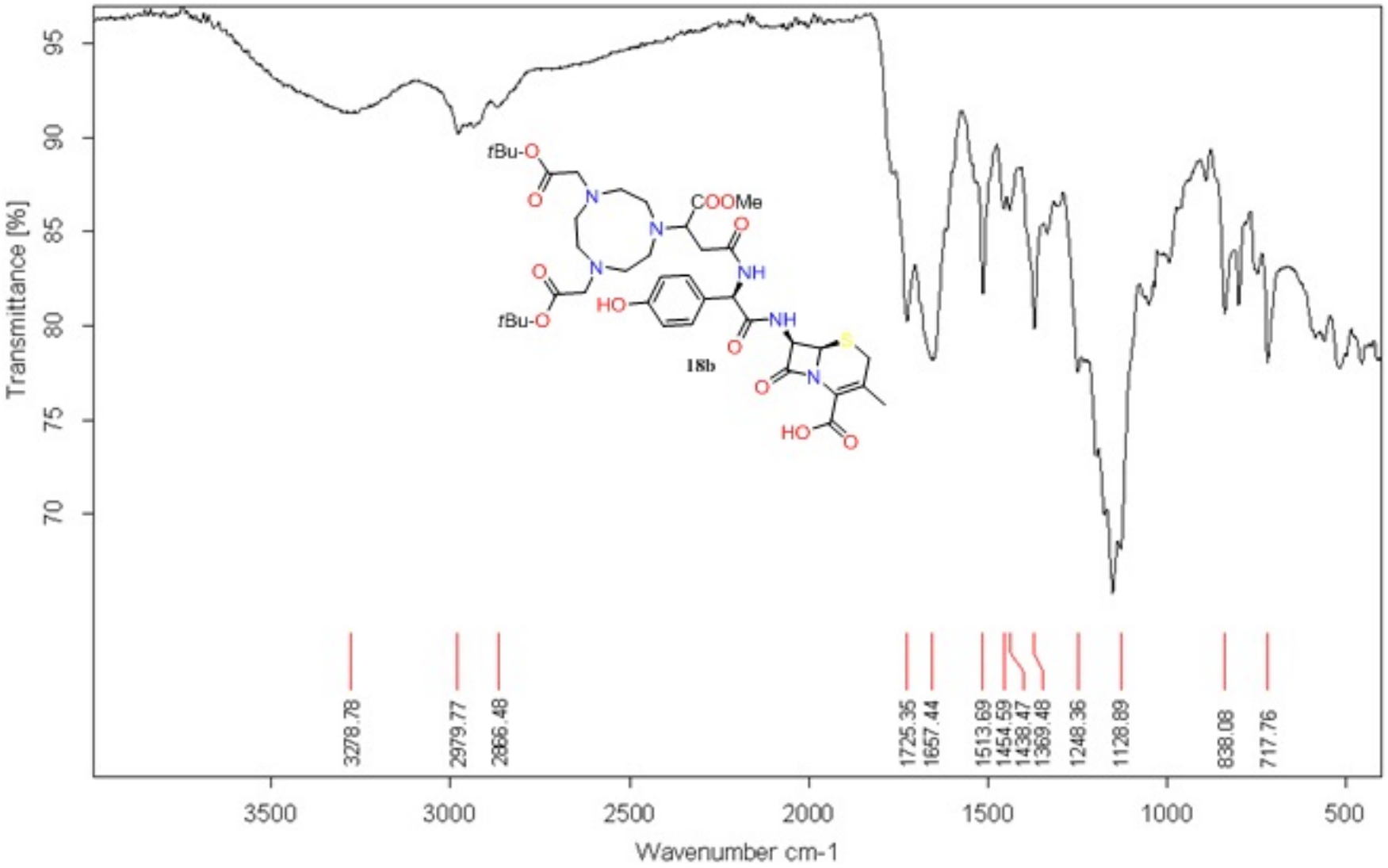
HRMS of **18a**

Acquisition Parameter

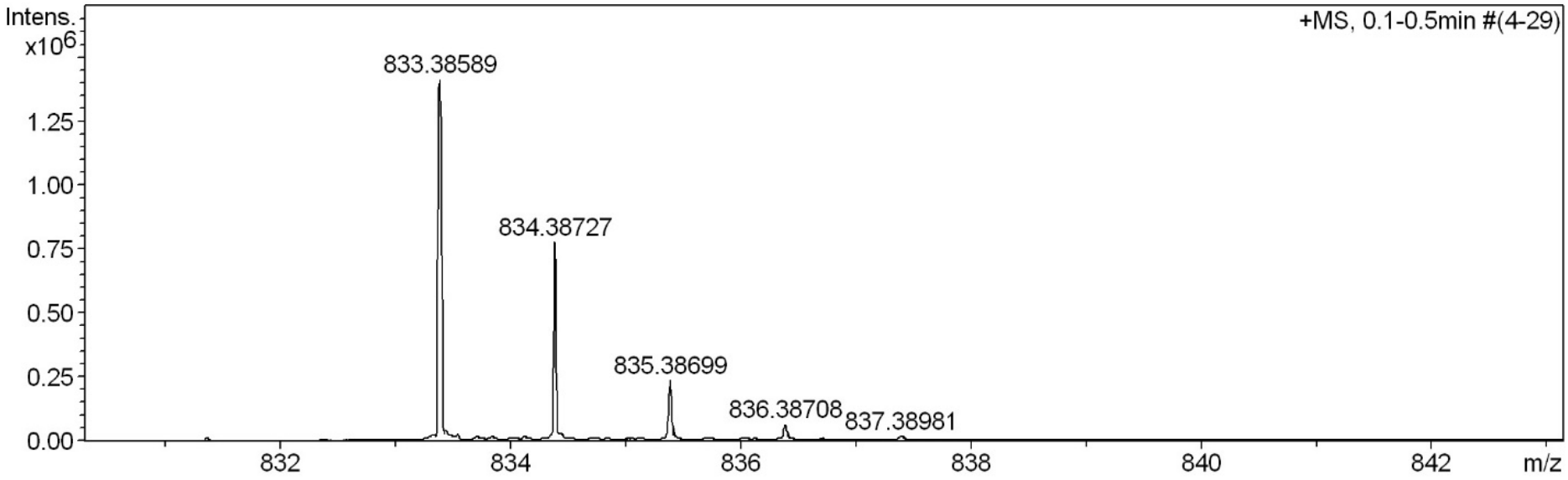
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



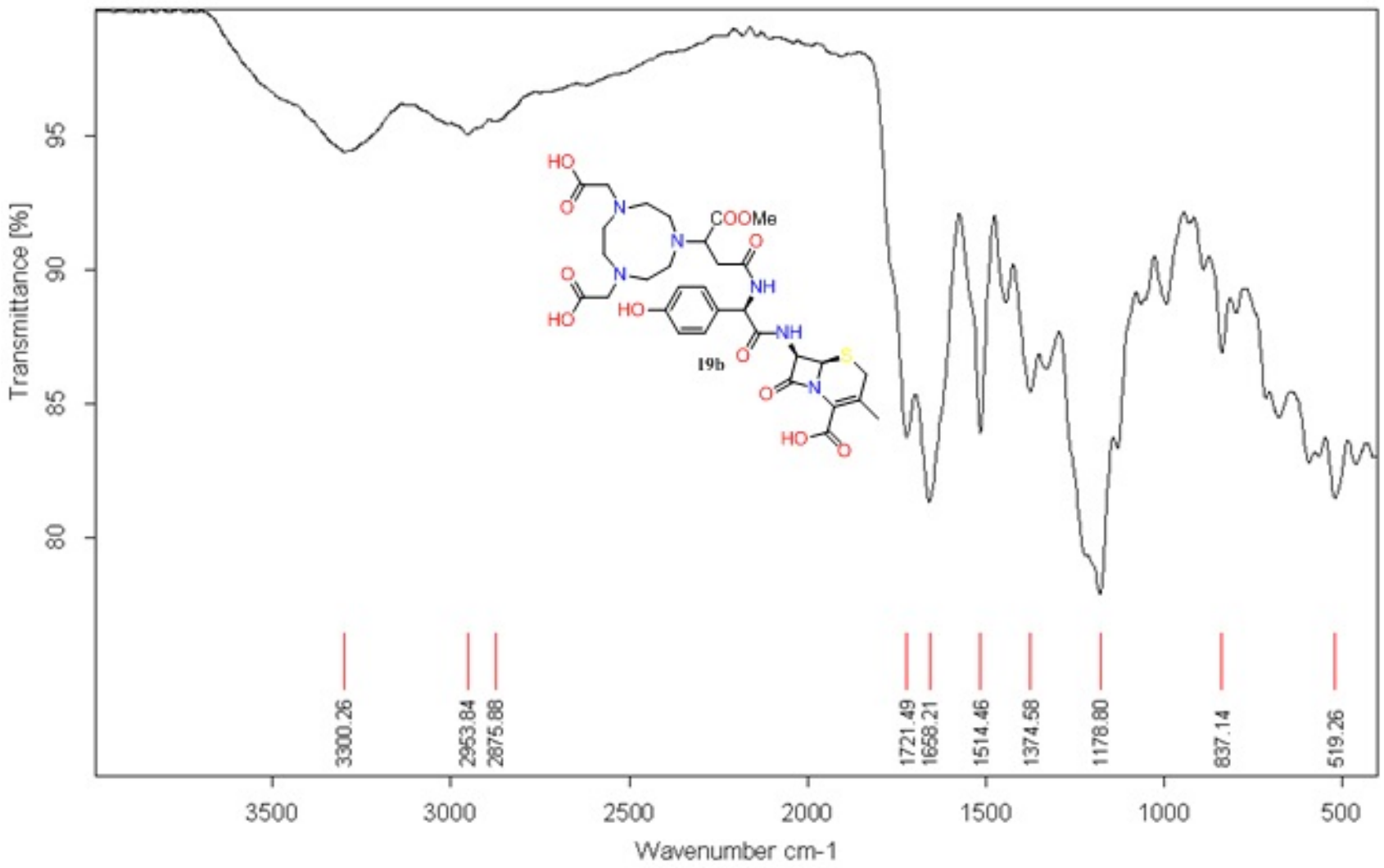
IR spectrum of **19a**



HRMS of **19a**


Intens.
x10^6
1.25
1.00
0.75
0.50
0.25
0.00
833.38589
834.38727
835.38699
836.38708
837.38981
+MS, 0.1-0.5min #(4-29)
832
834
836
838
840
842
m/z

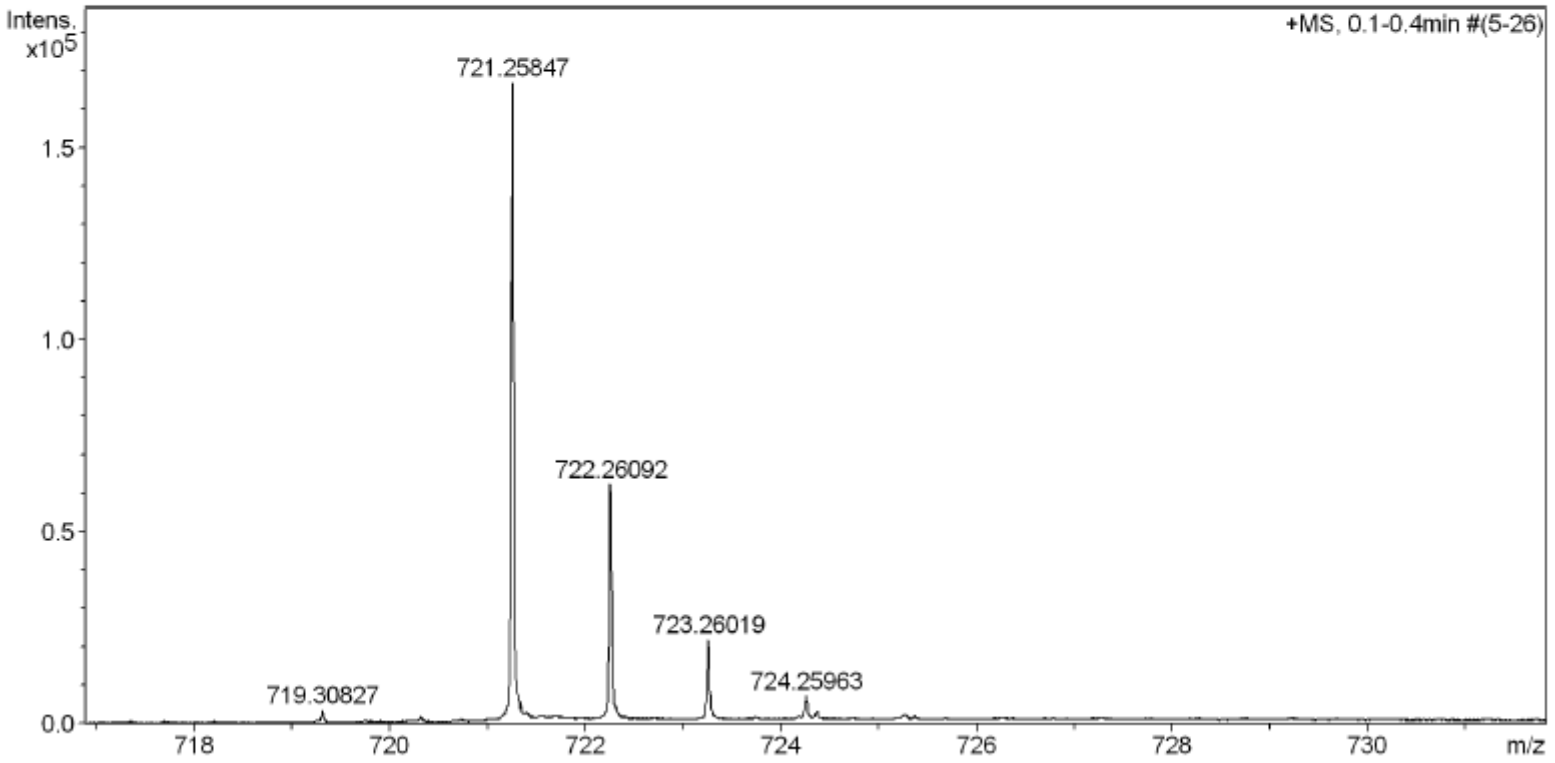
IR spectrum of (**20a**)



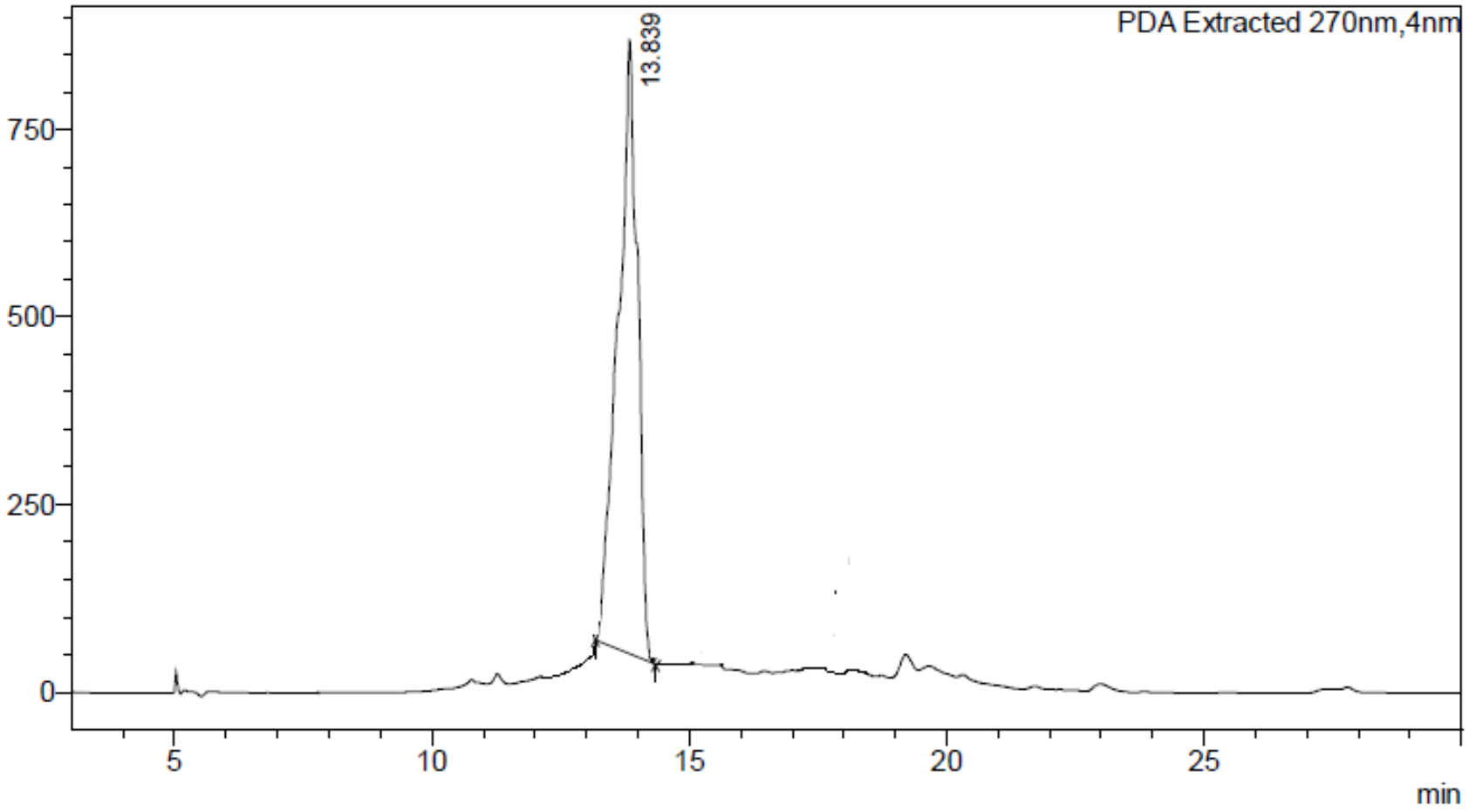
HRMS of **20a**

Acquisition Parameter

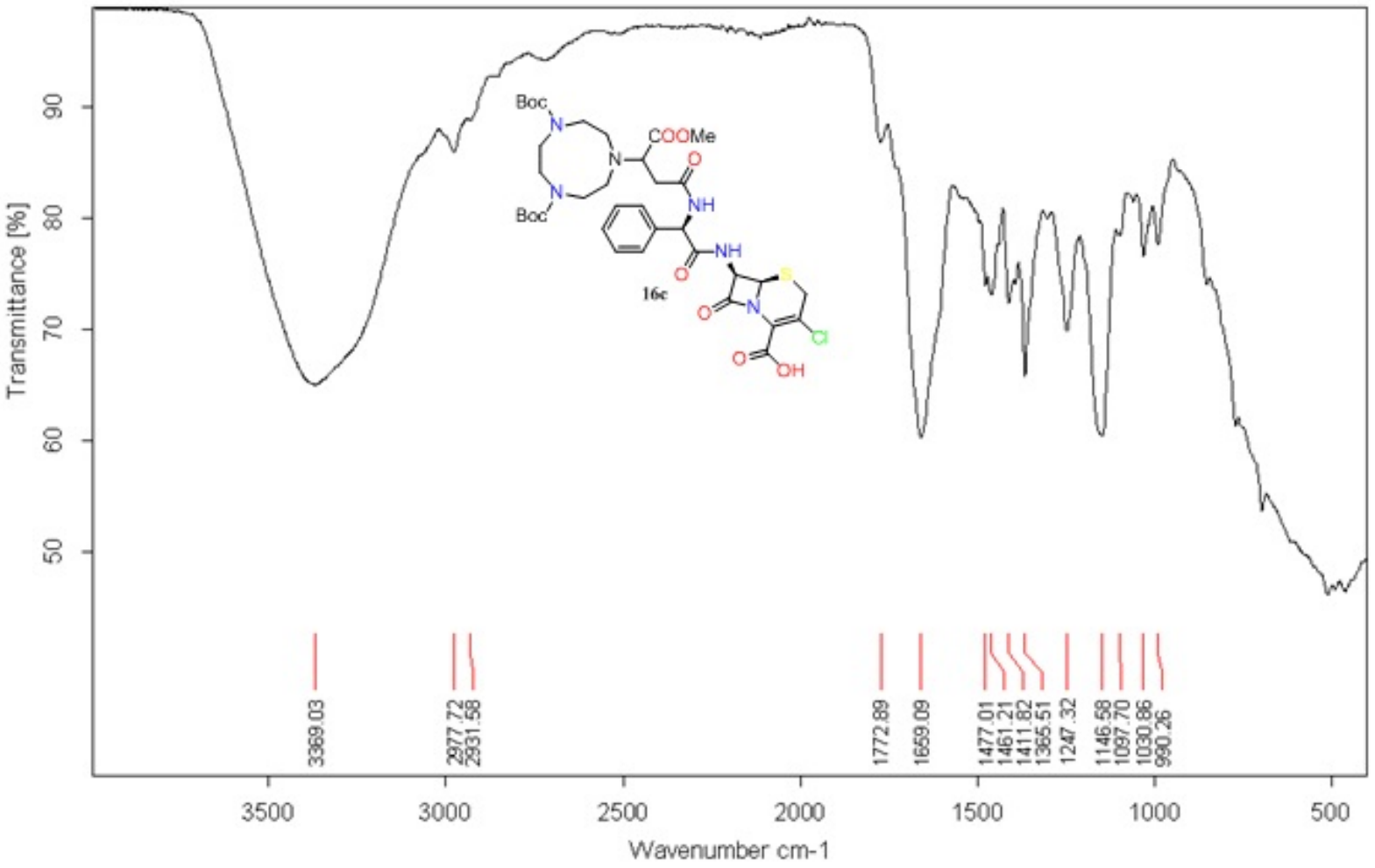
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **20a**



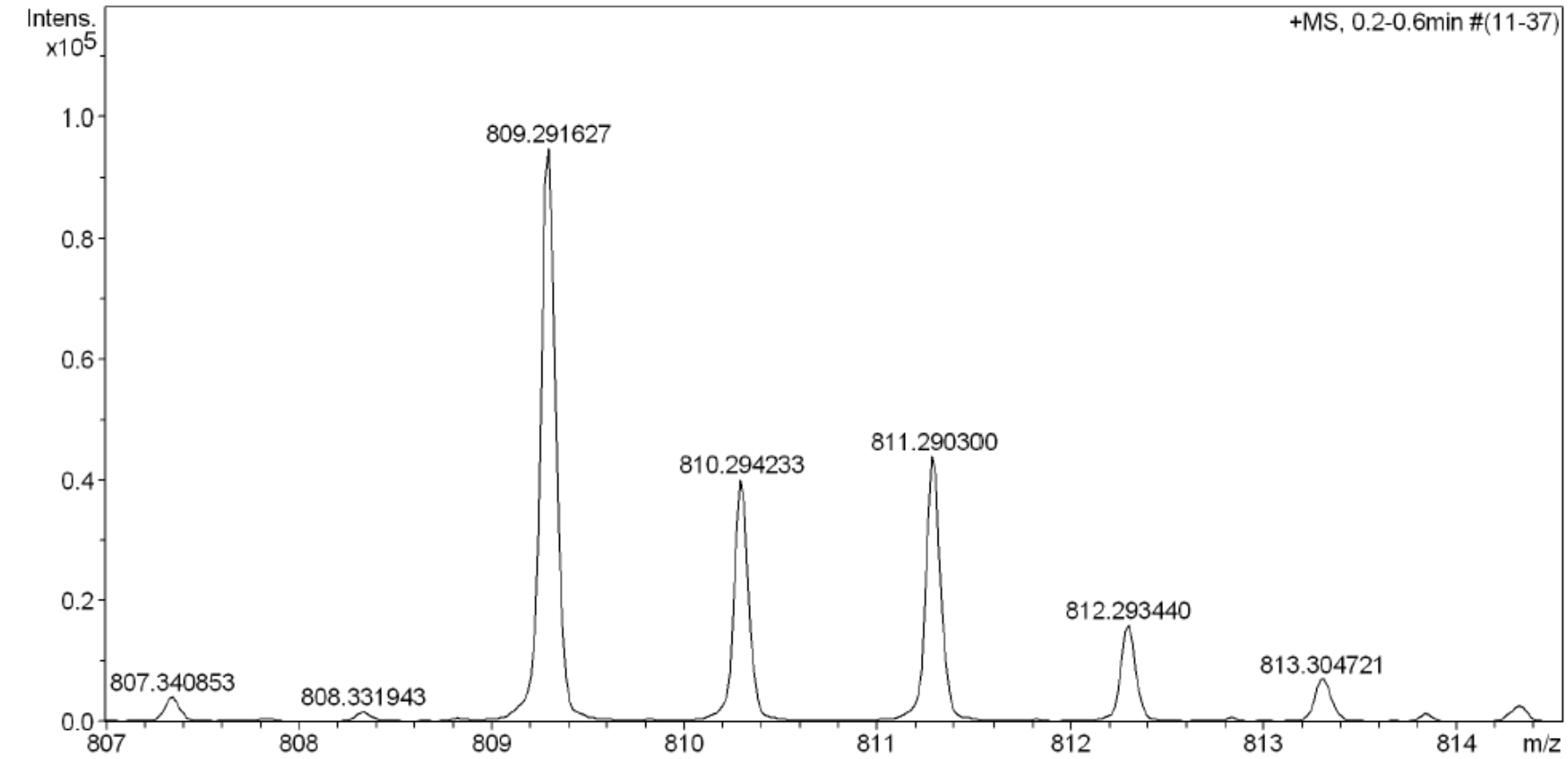
IR spectrum of **17b**



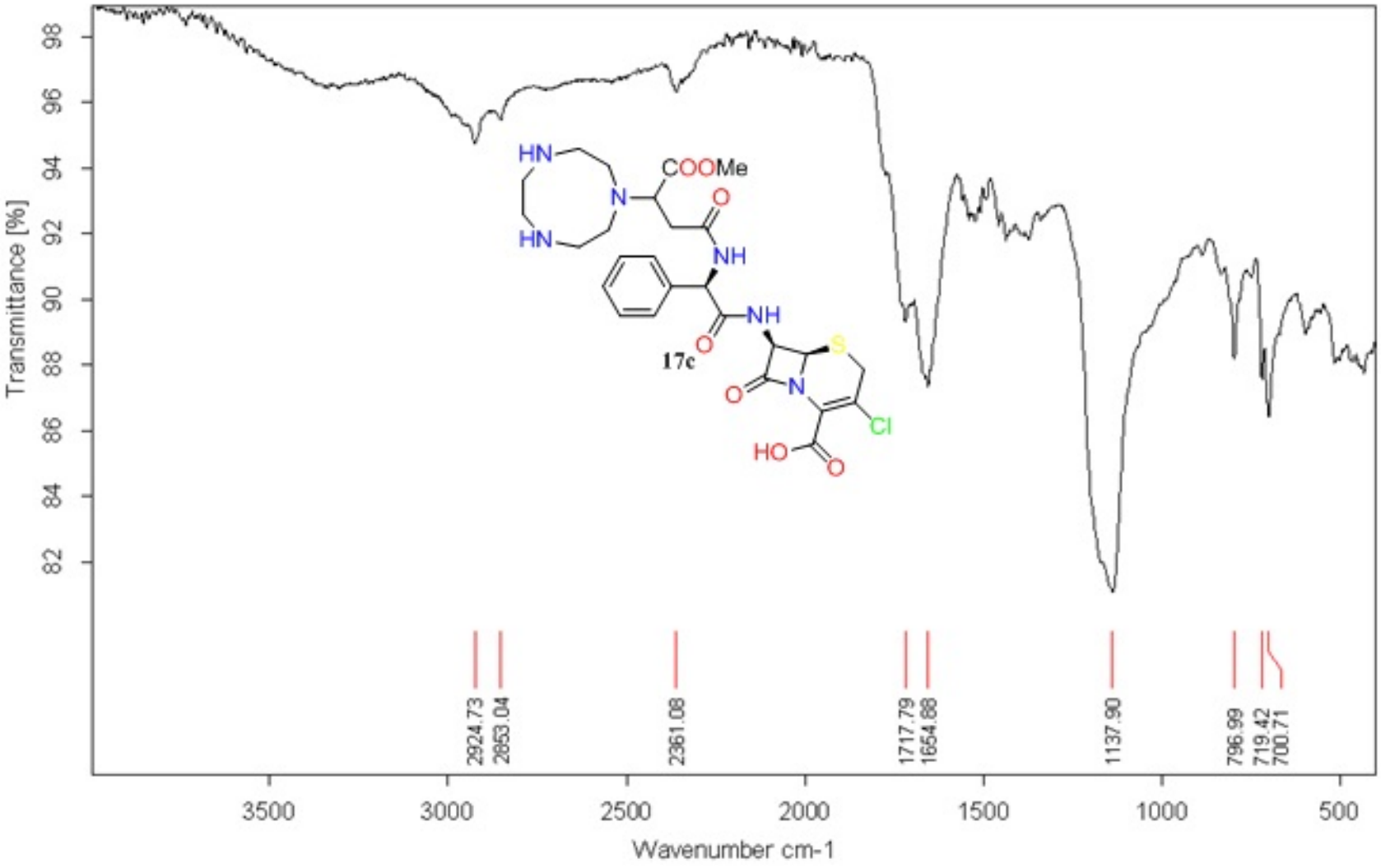
HRMS of **17b**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



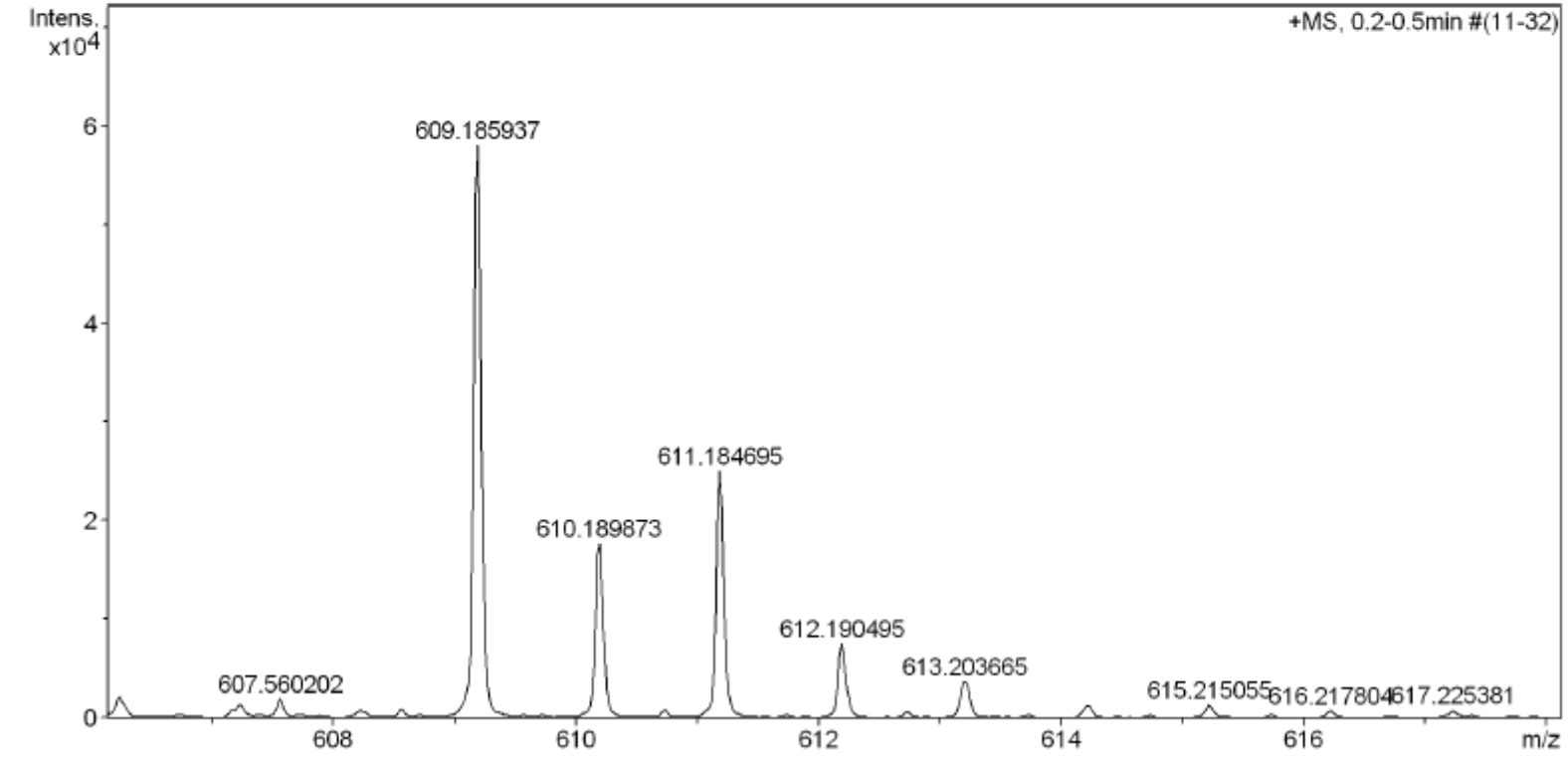
IR spectrum of **18b**



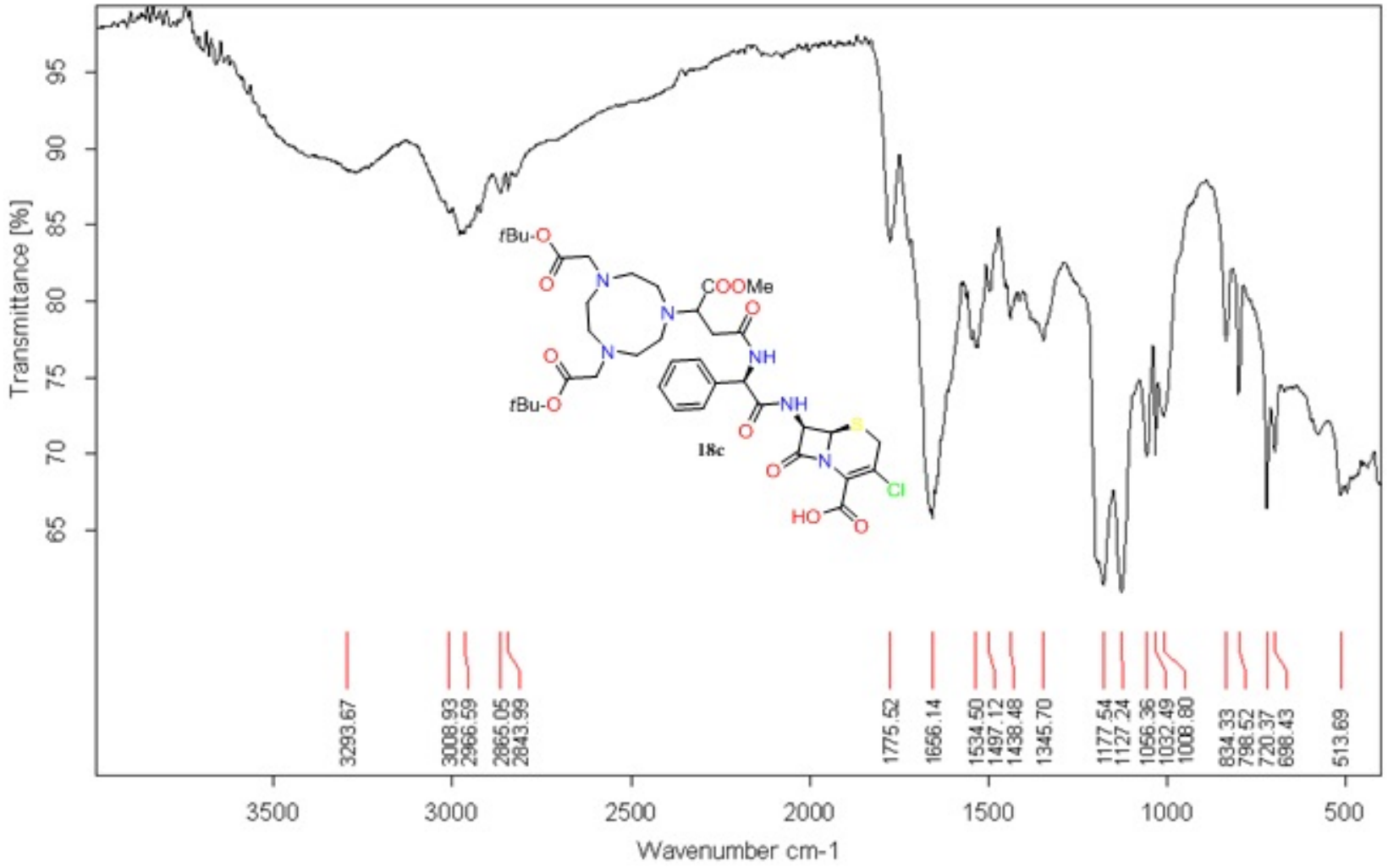
HRMS of **18b**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



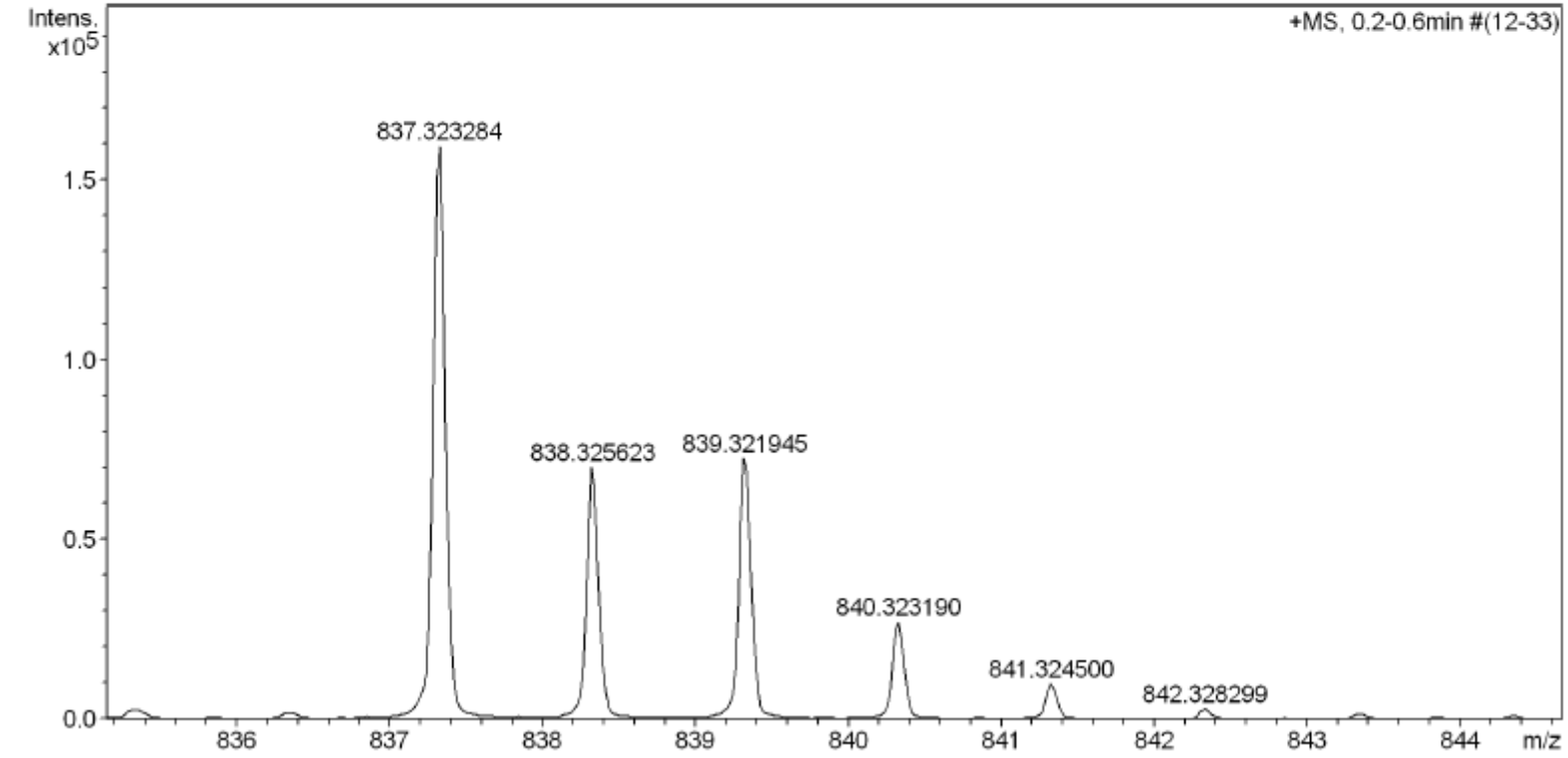
IR spectrum of **19b**



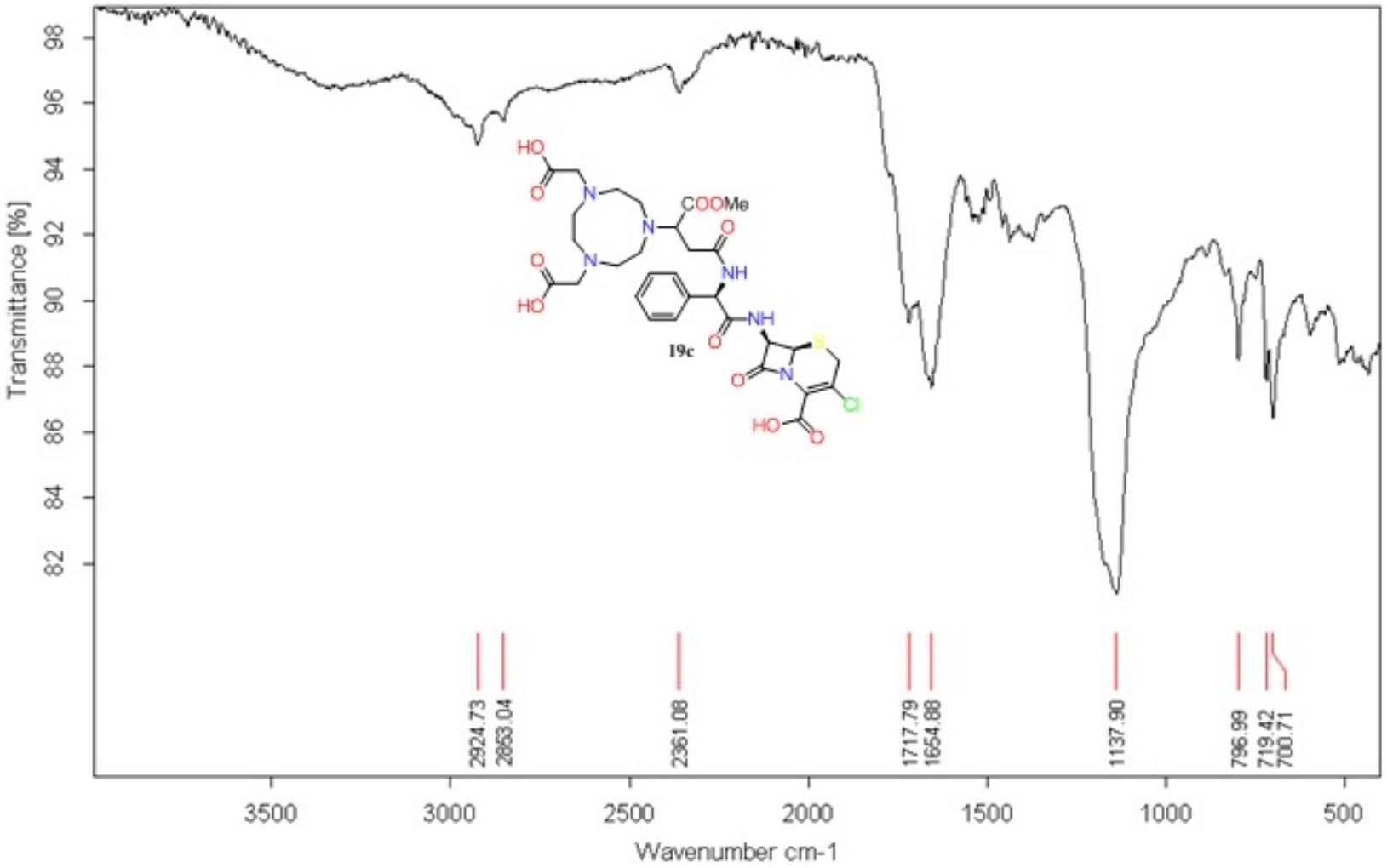
HRMS of **19b**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



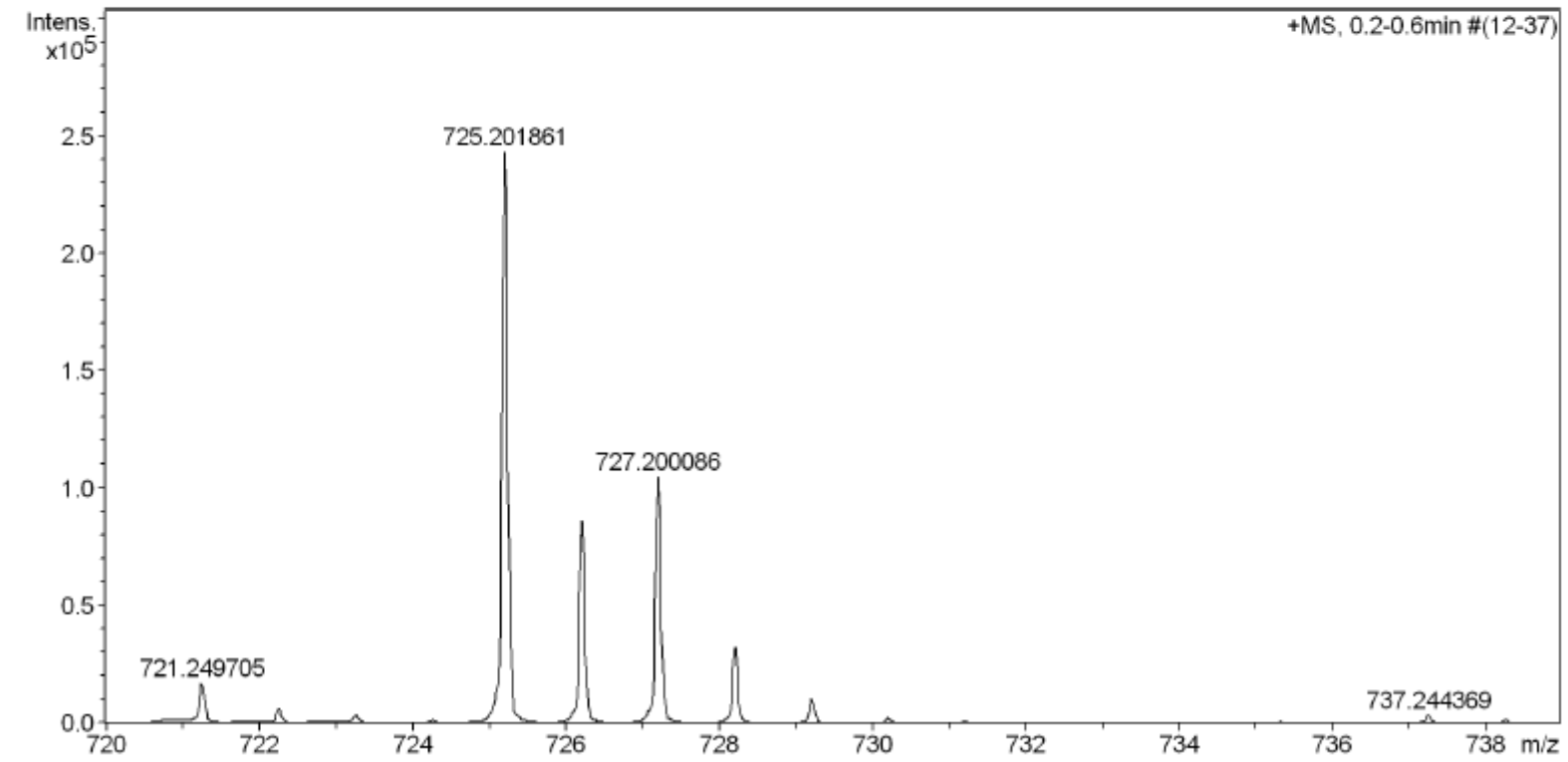
IR spectrum of **20b**



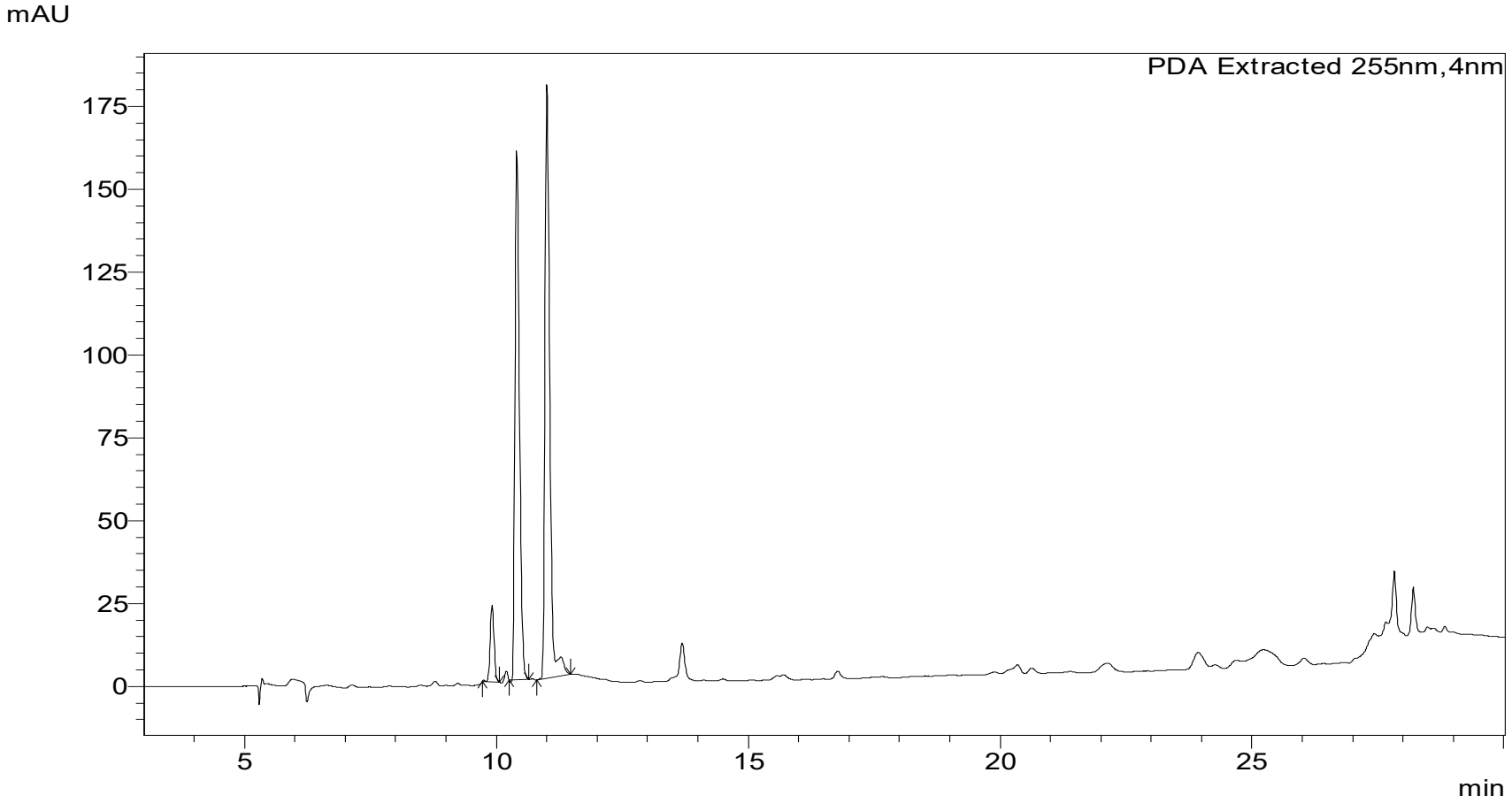
HRMS of **20b**

Acquisition Parameter

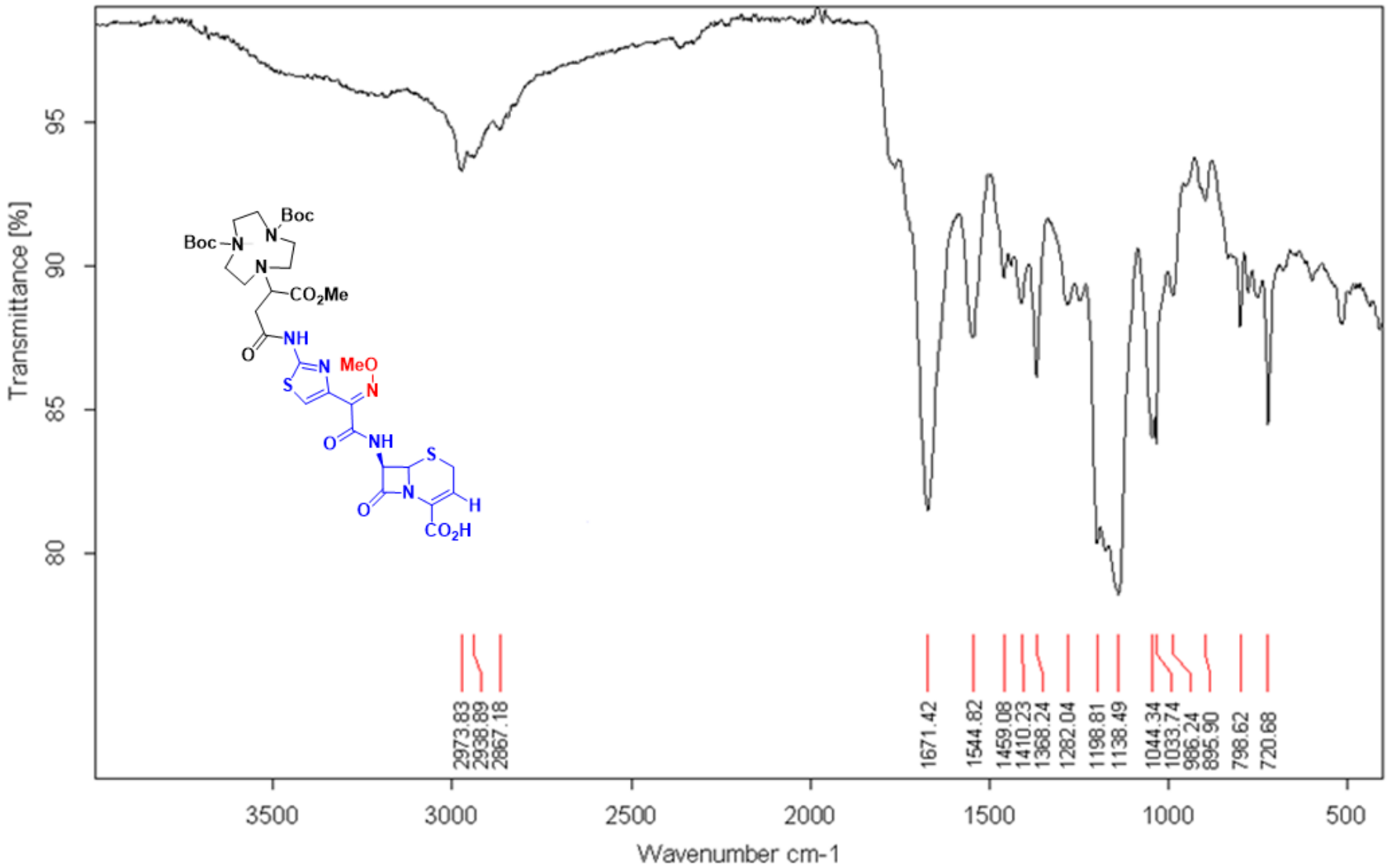
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **20b**



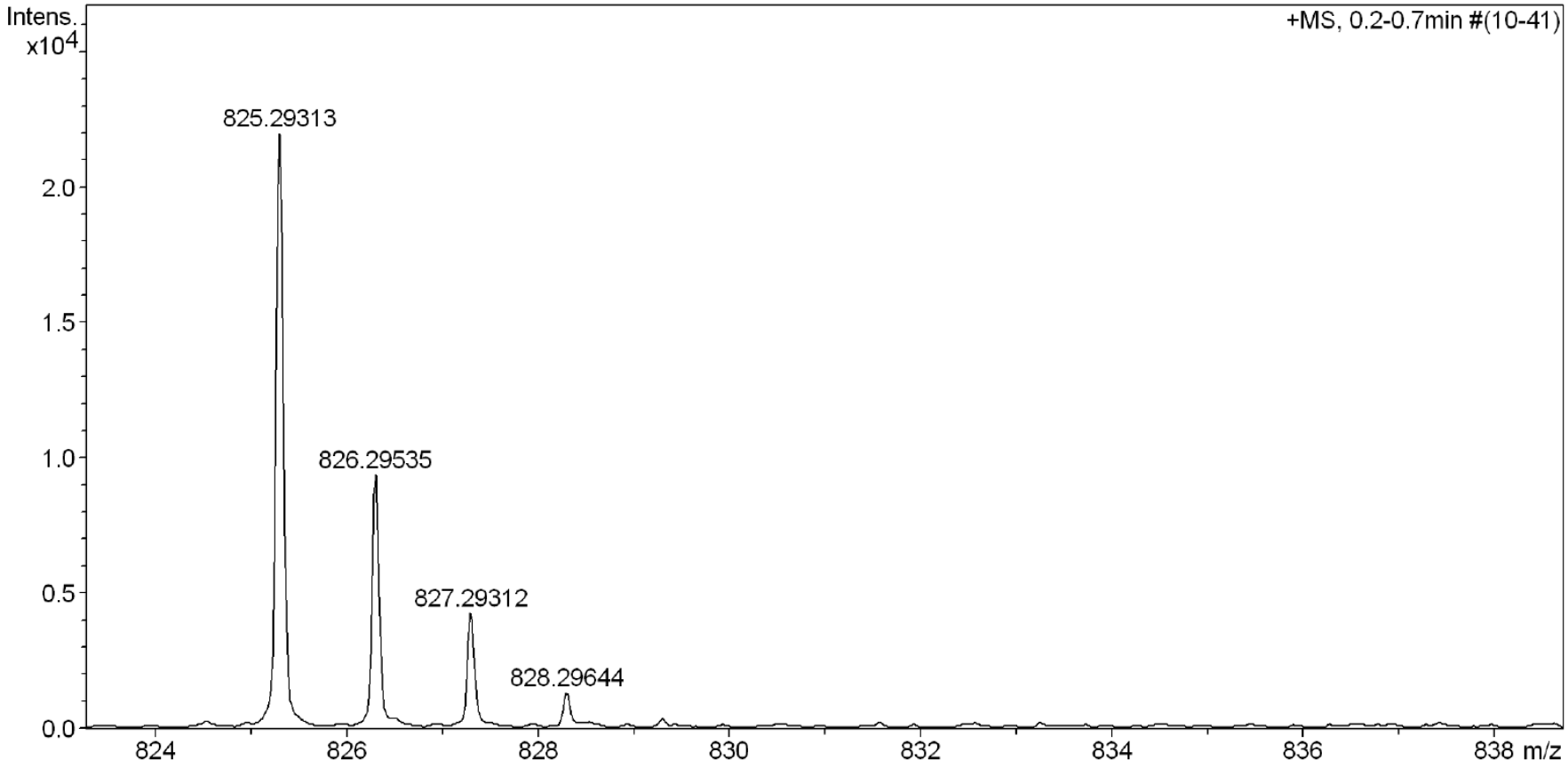
IR spectrum of **21a**



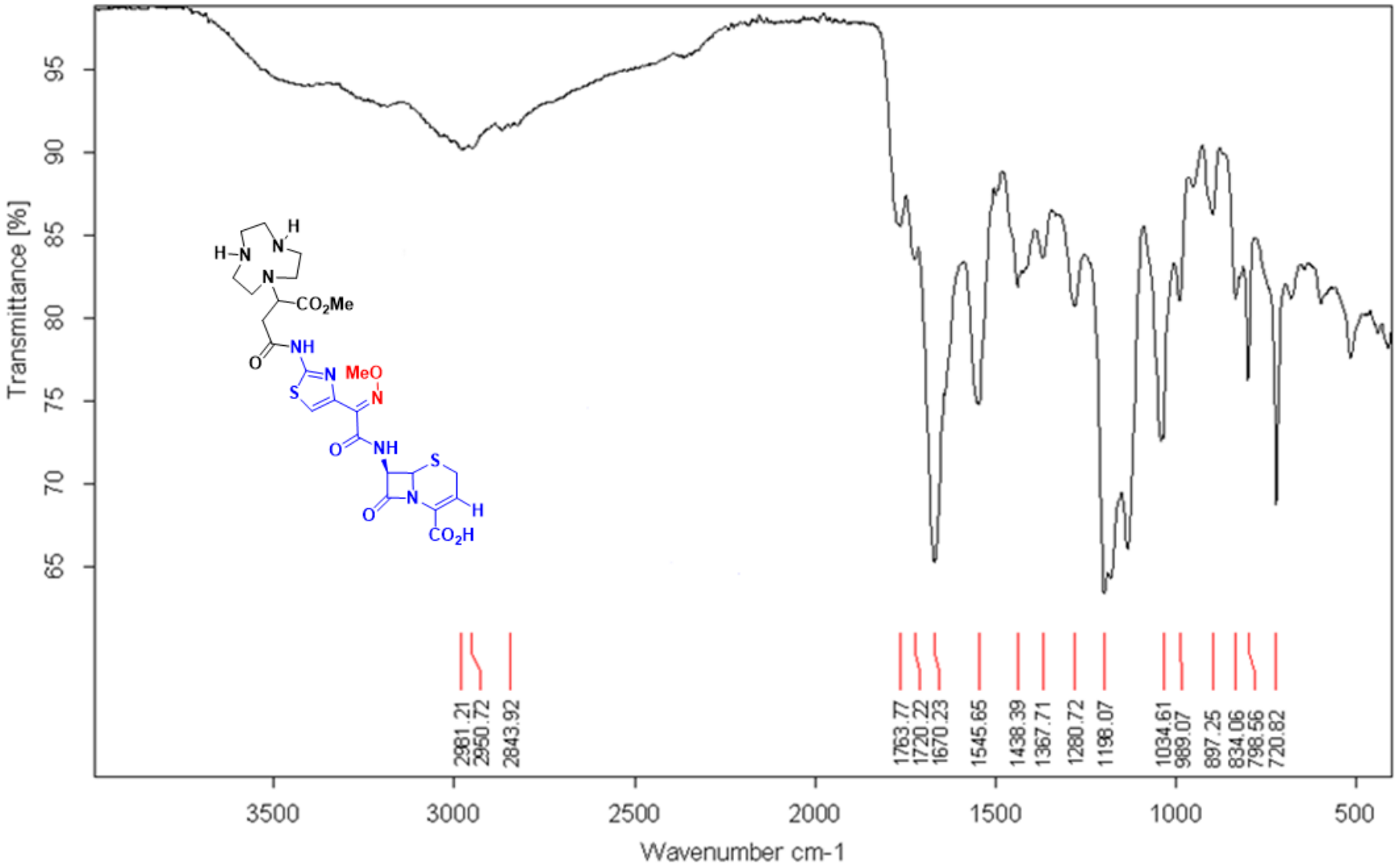
HRMS of **21a**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



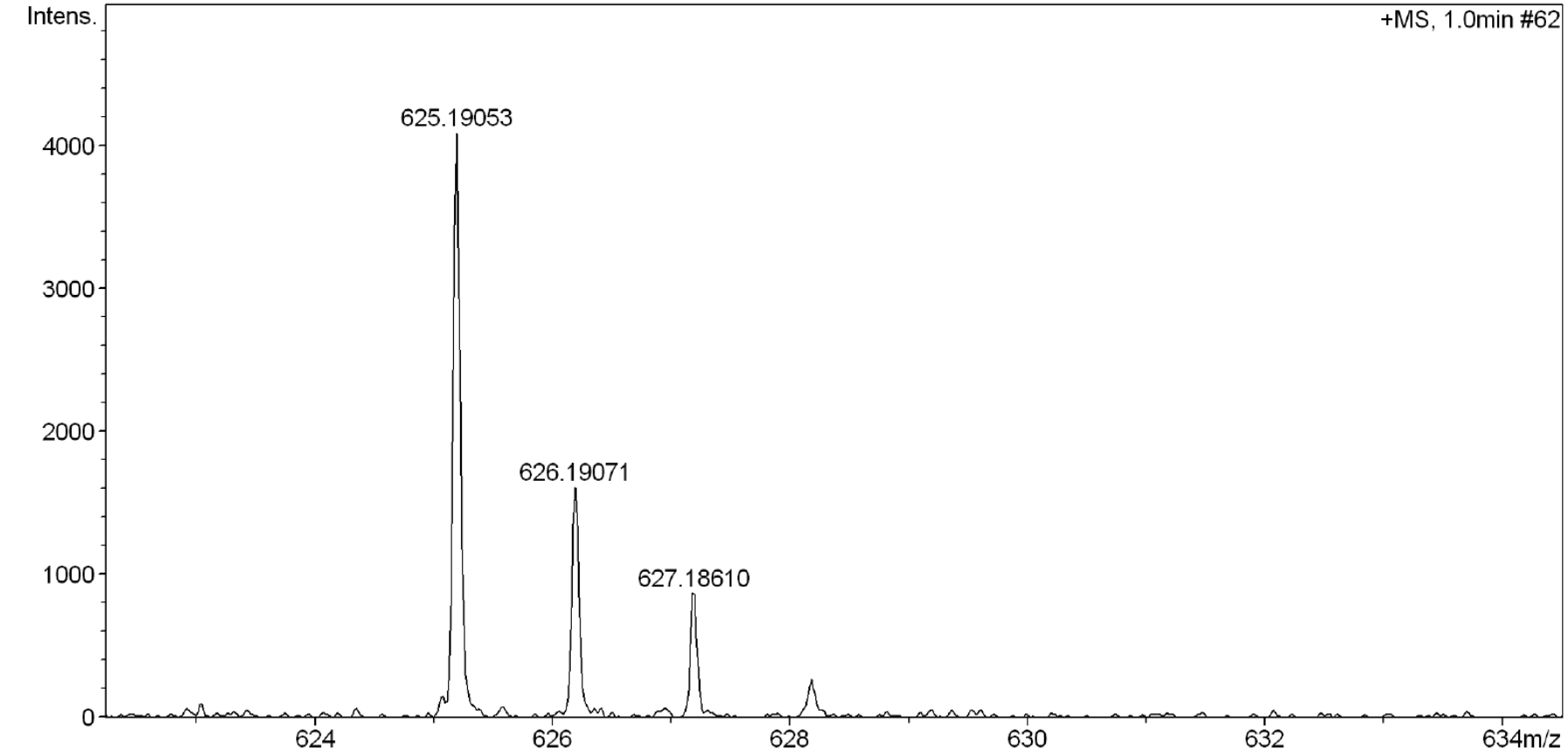
IR spectrum of **22a**



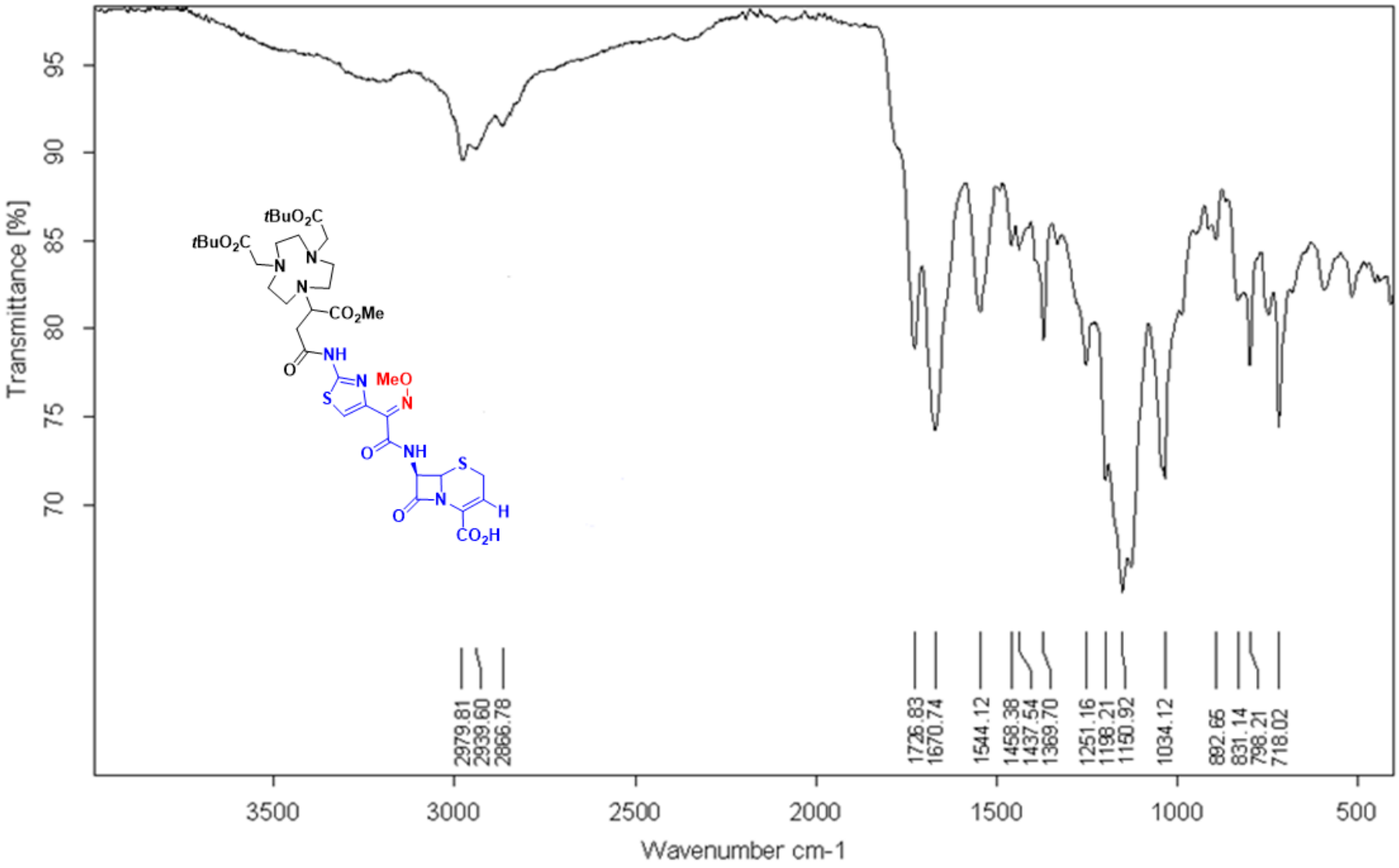
HRMS of **22a**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



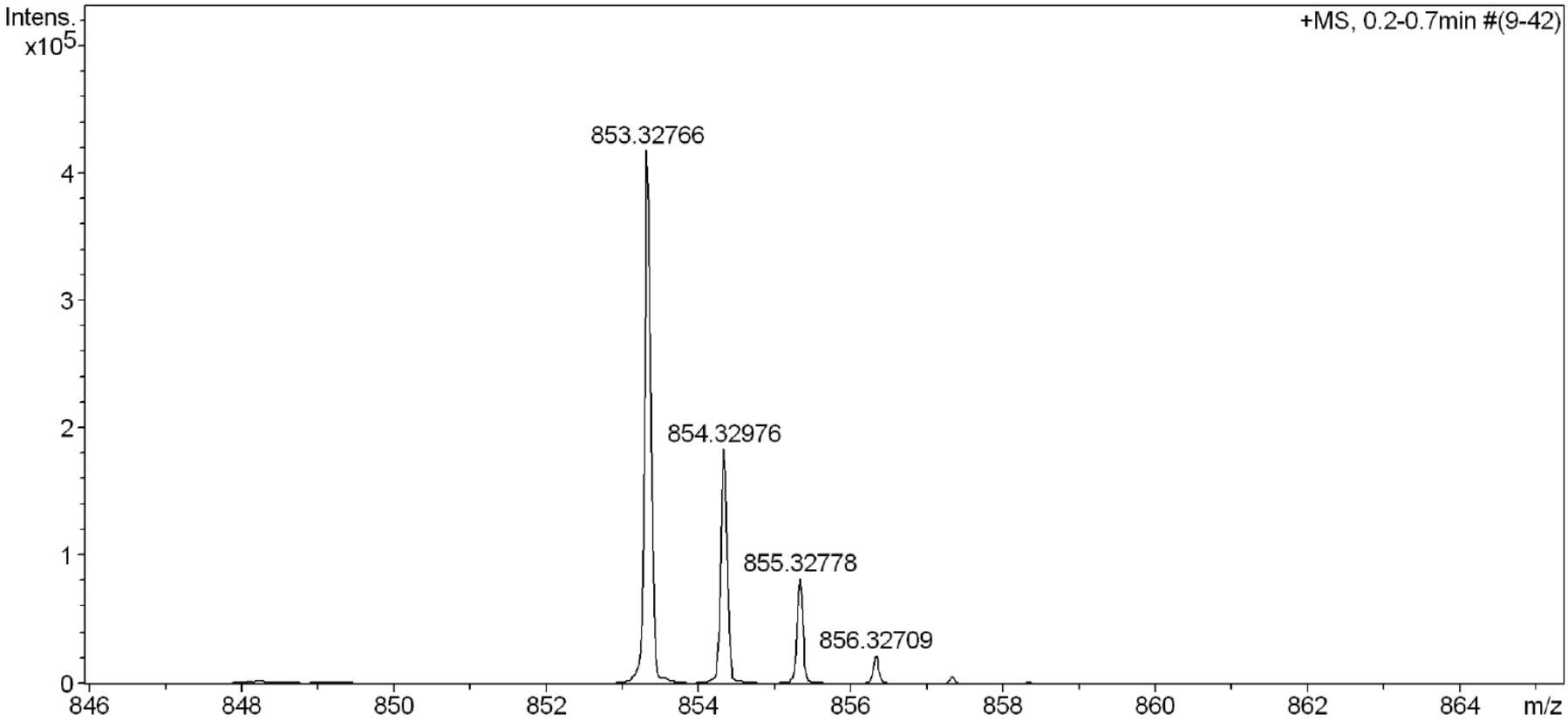
IR spectrum of **23a**)



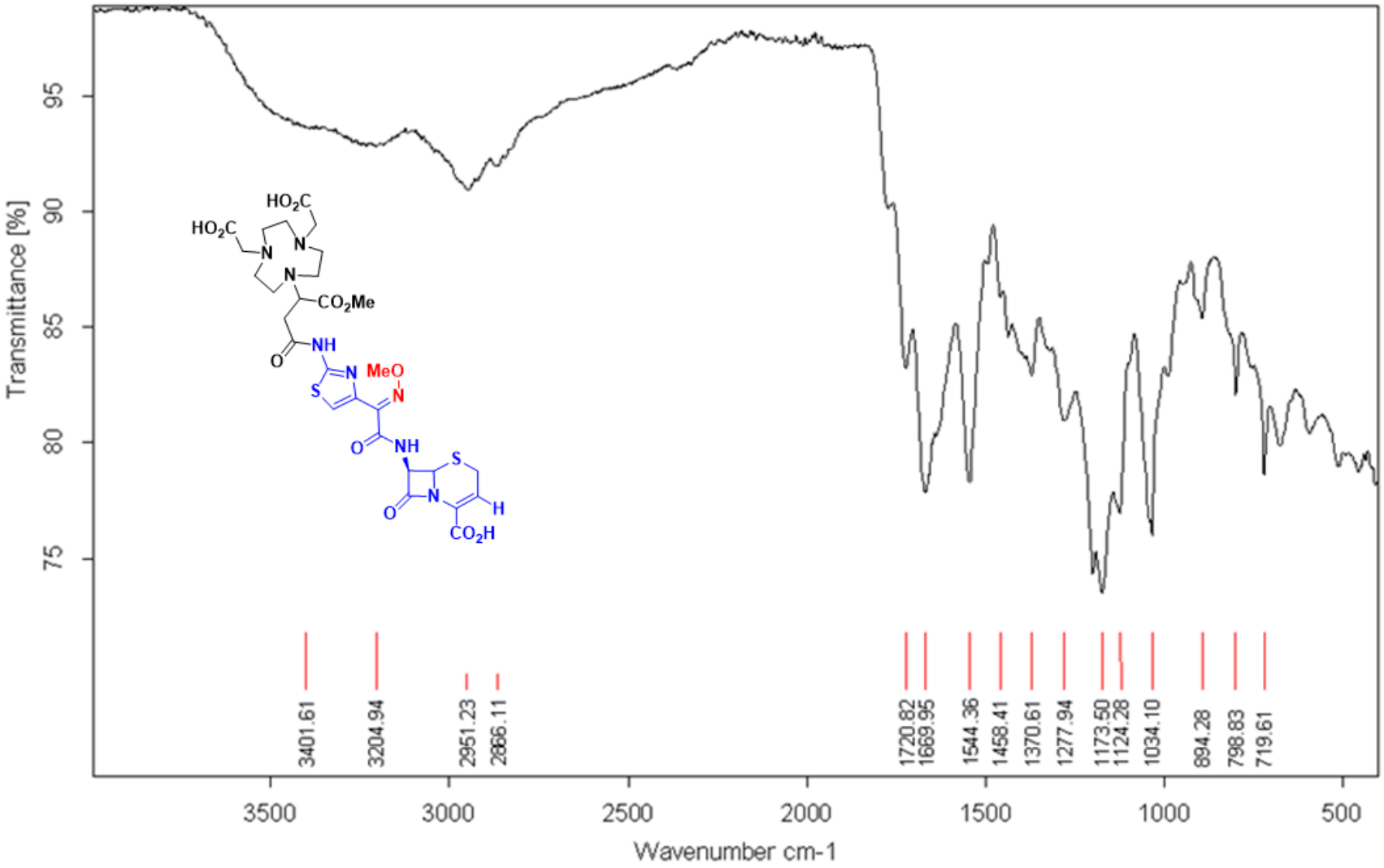
HRMS of **23a**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



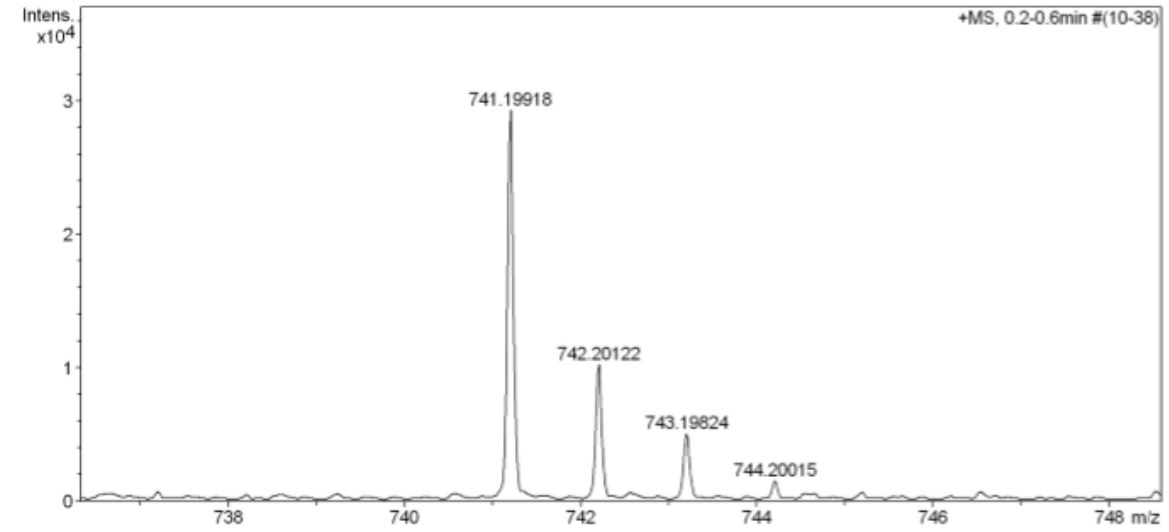
IR spectrum of **24a**)



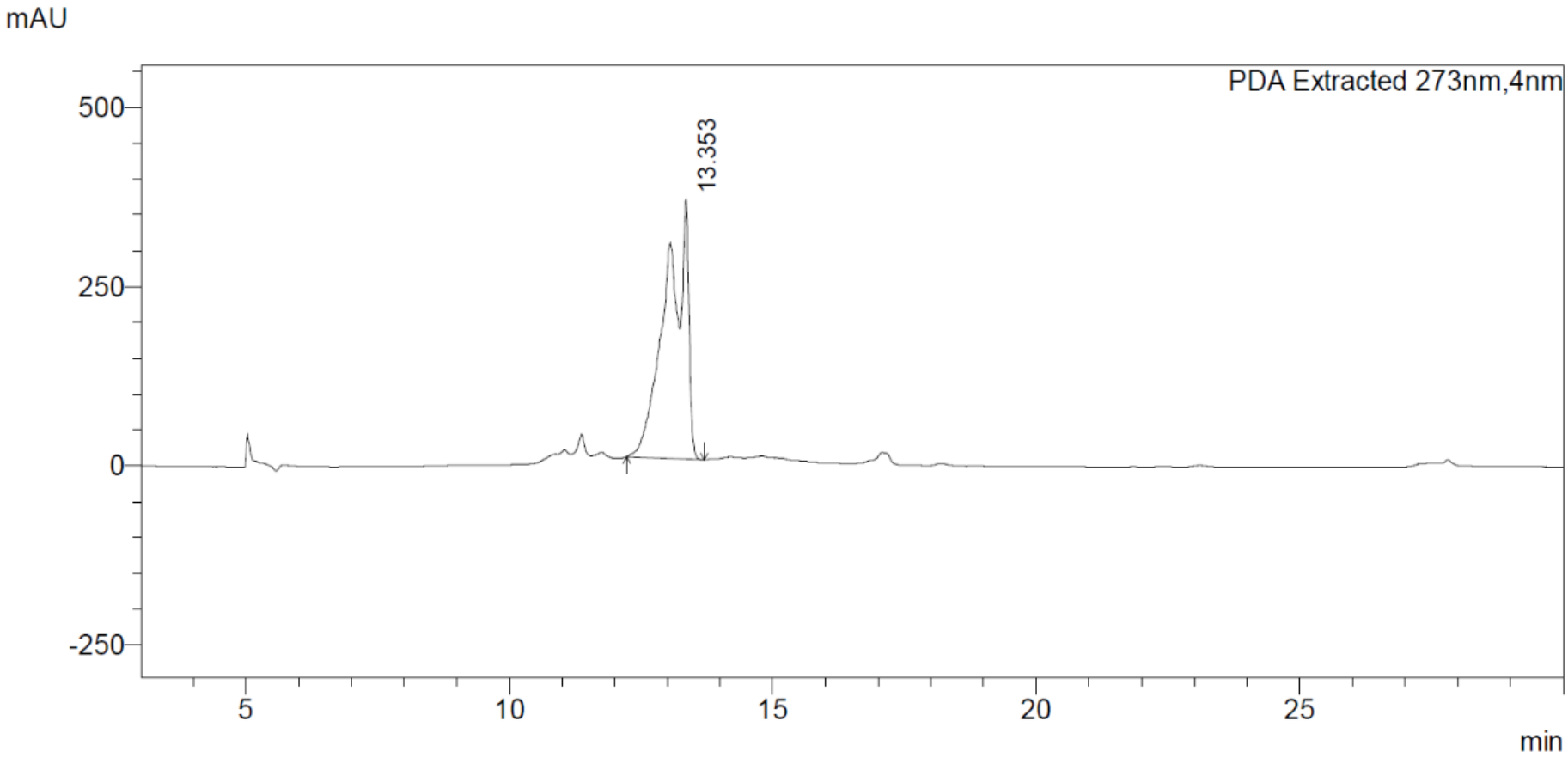
HRMS of **24a**

Acquisition Parameter

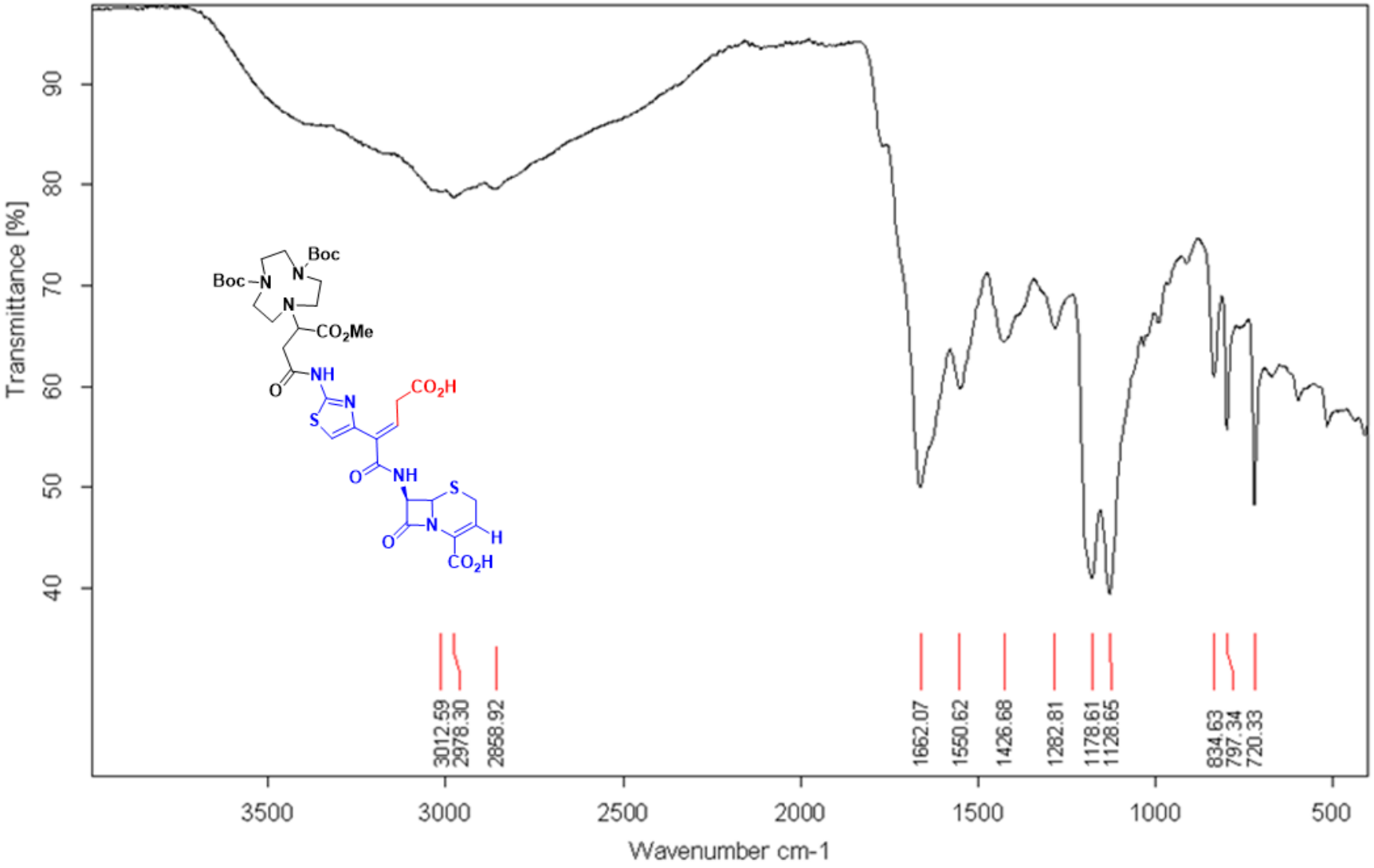
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **24a**



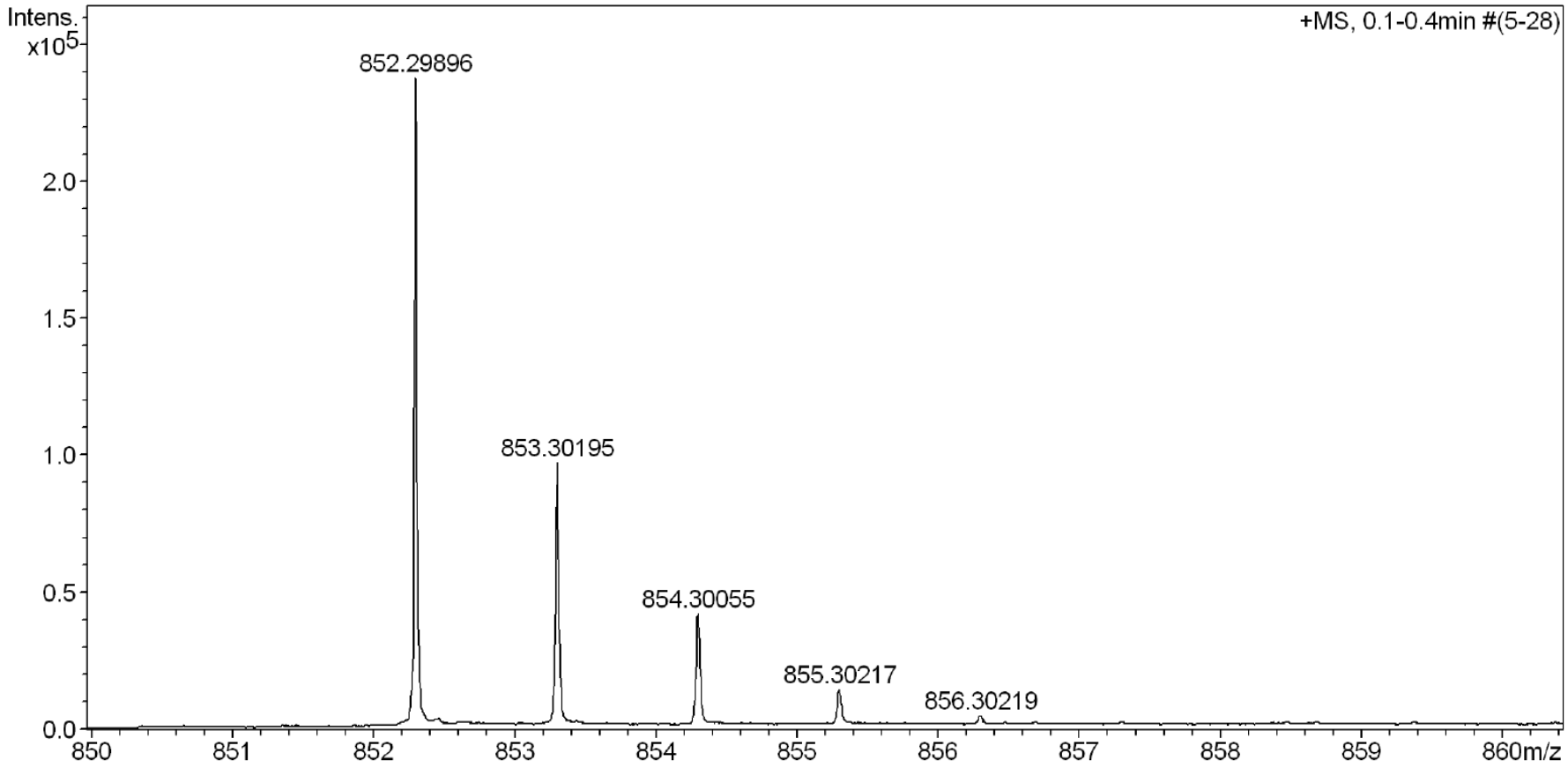
IR spectrum of **21b**



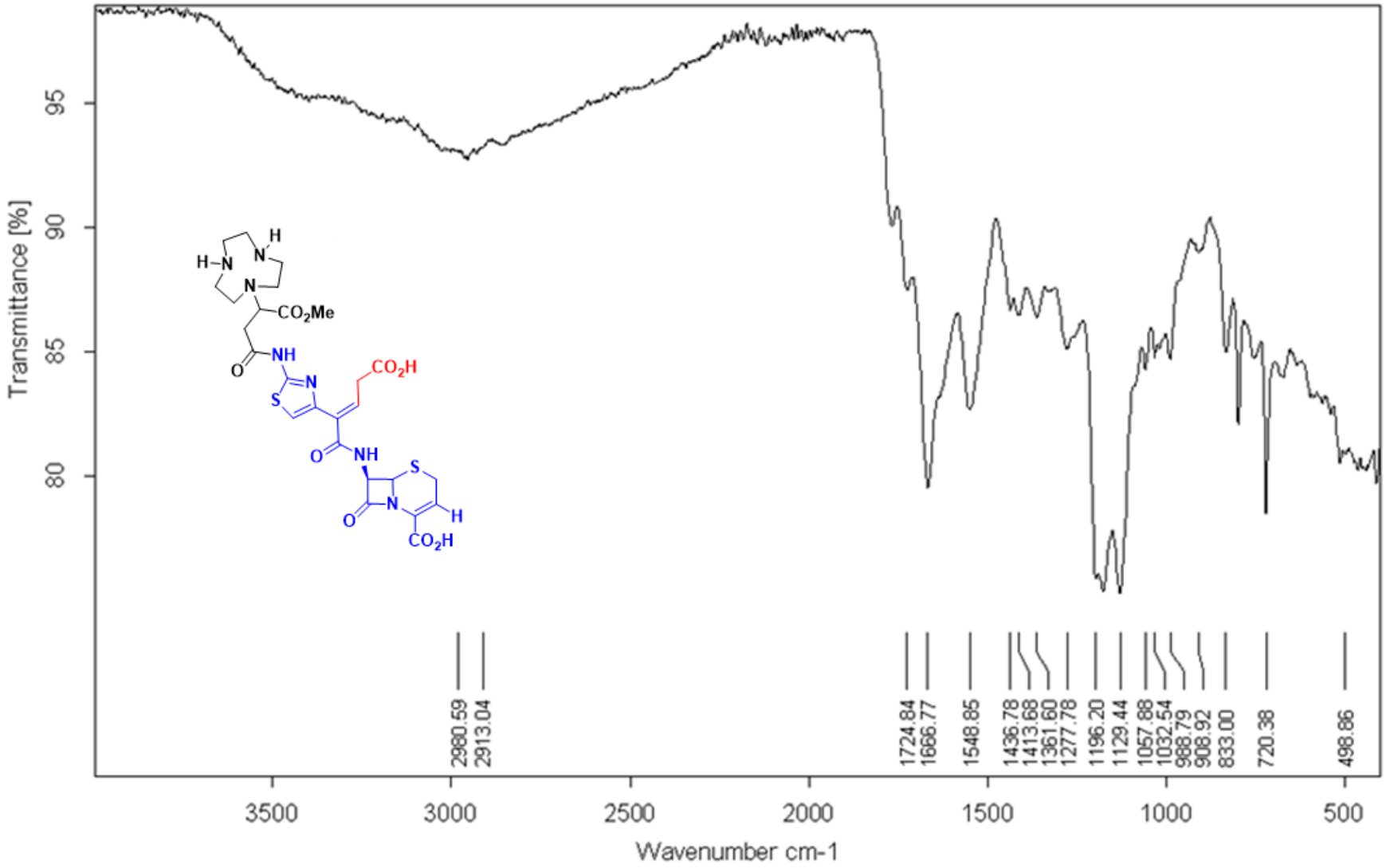
HRMS of (**21b**)

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



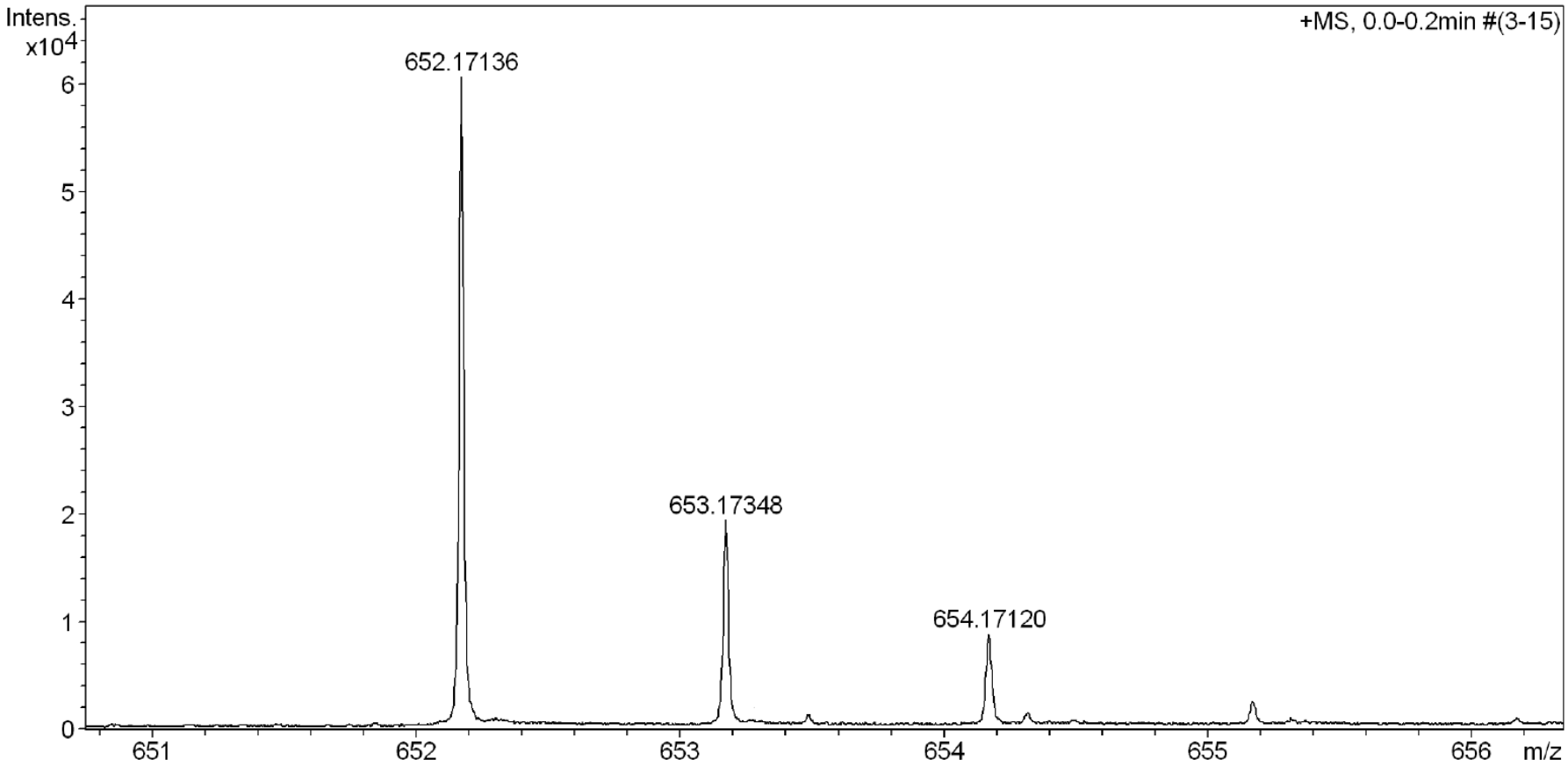
IR spectrum of **22b**



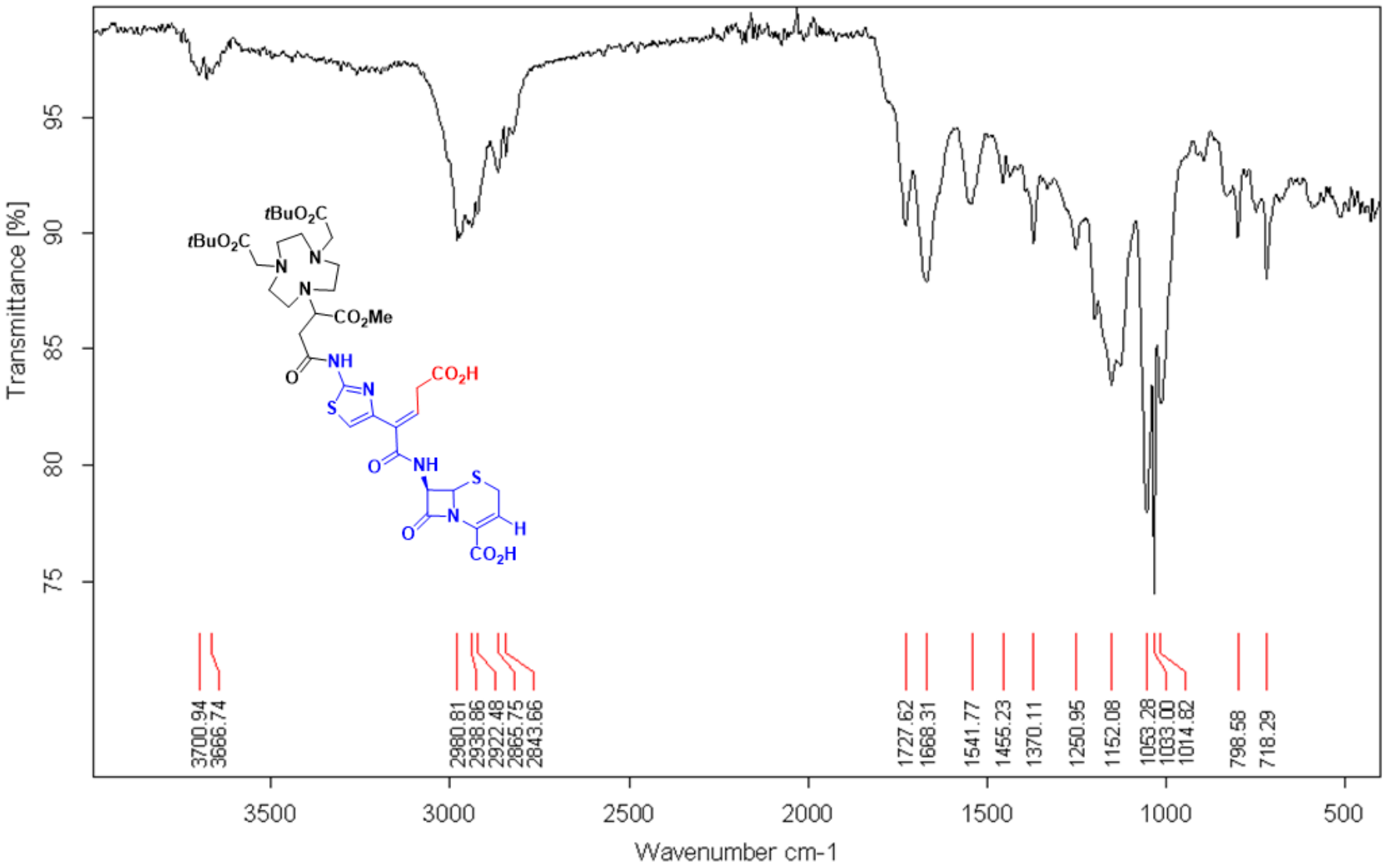
HRMS of **22b**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2500 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



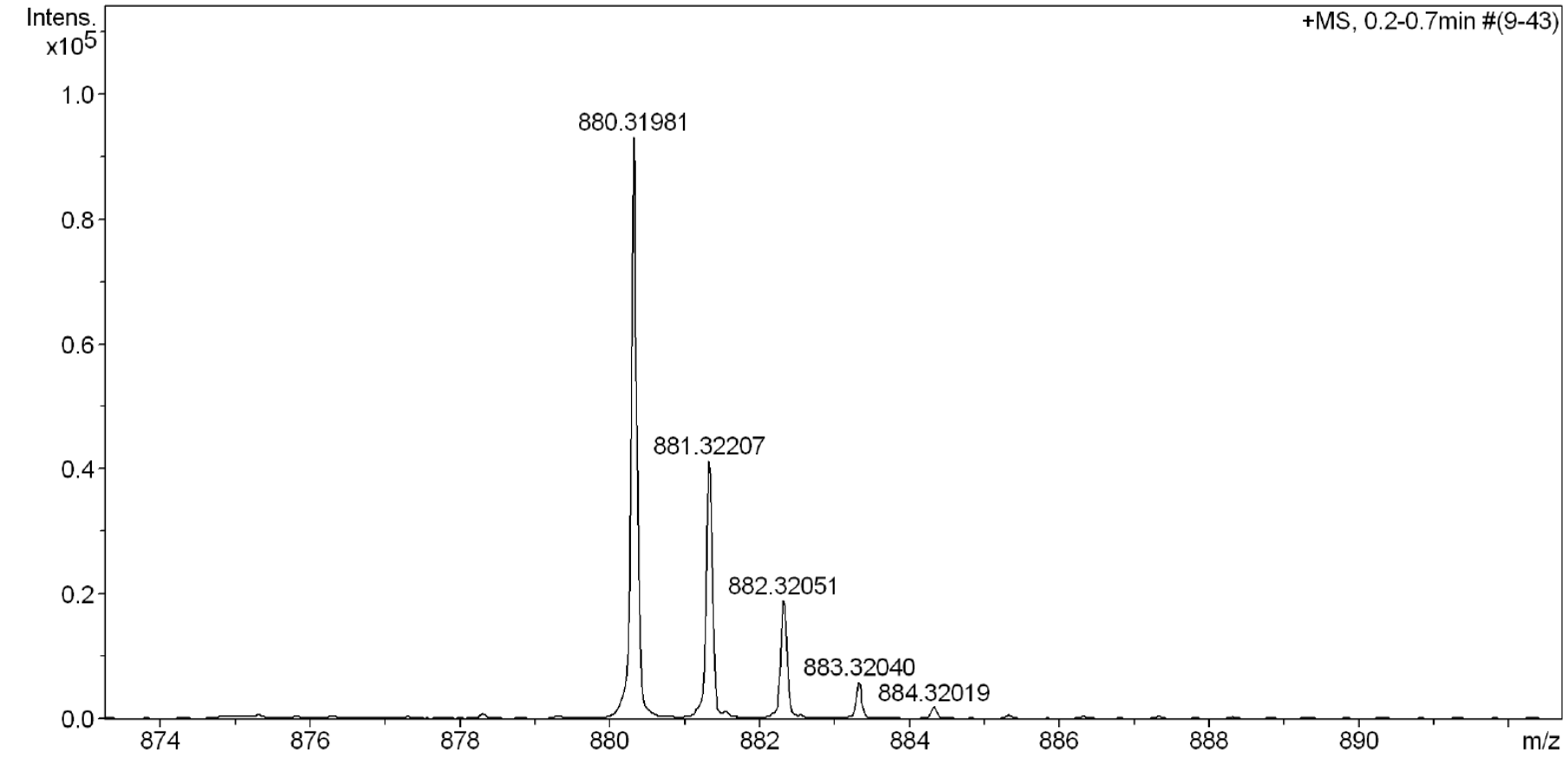
IR spectrum of **23b**



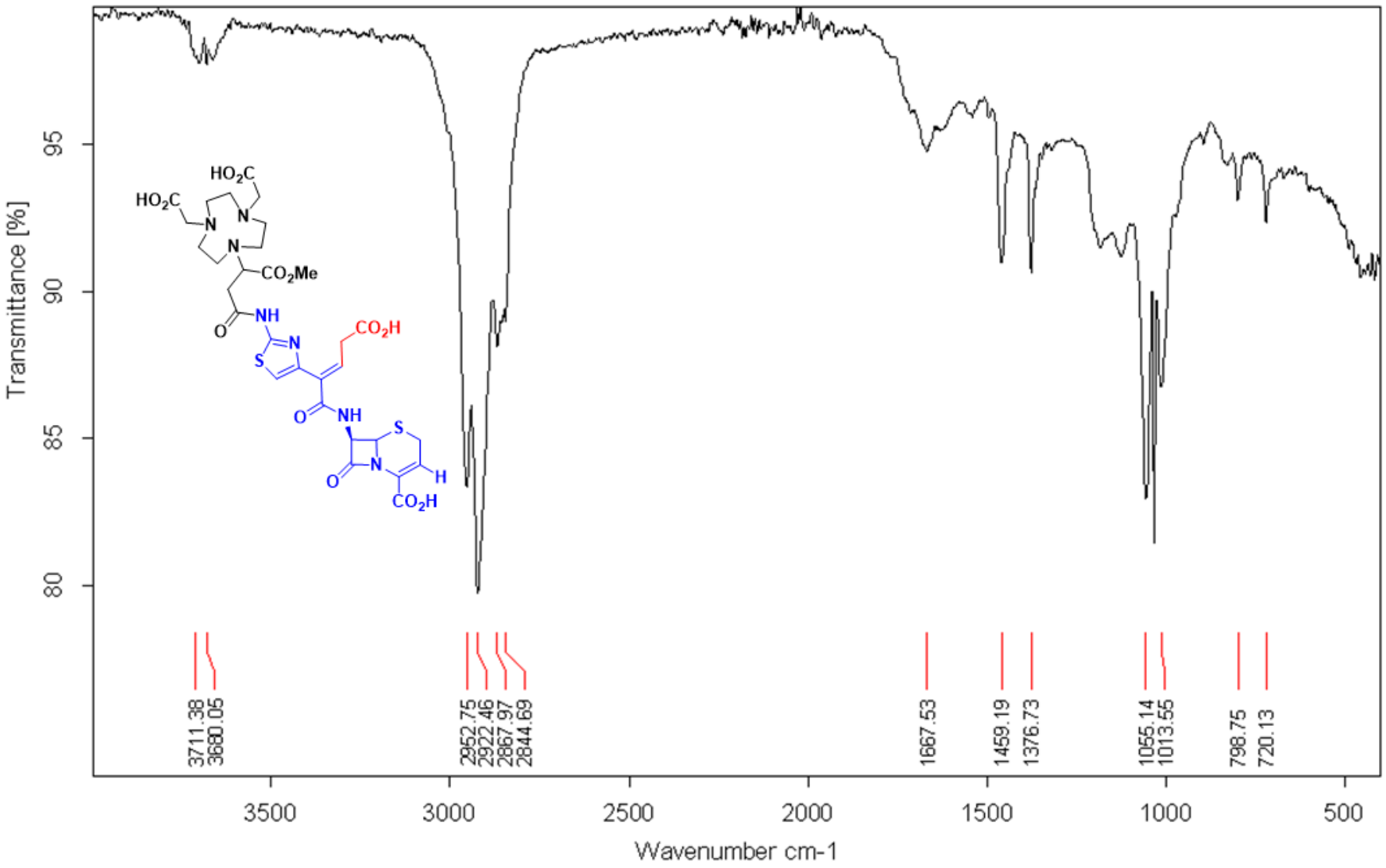
HRMS of **23b**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



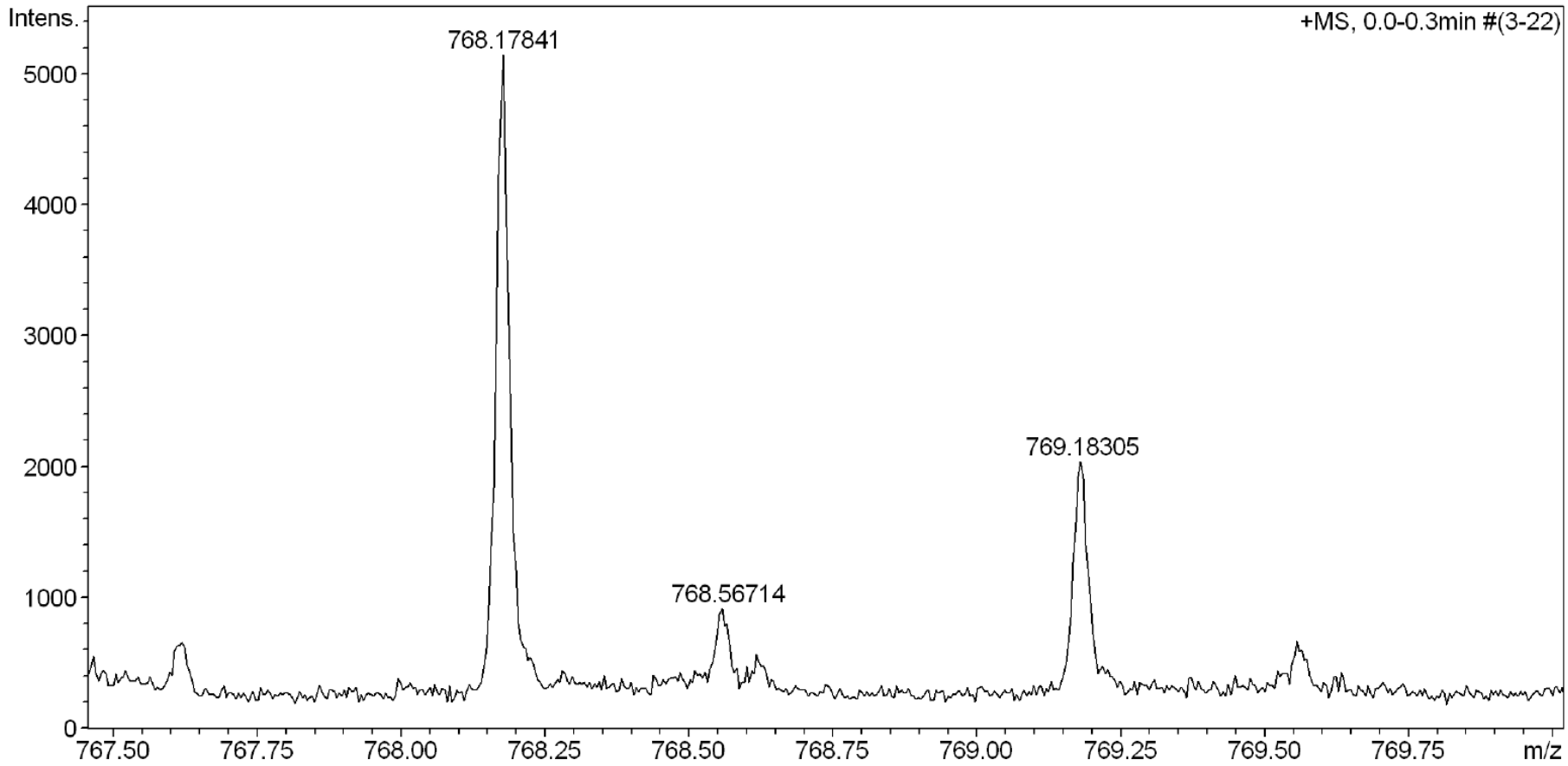
IR spectrum of **24b**



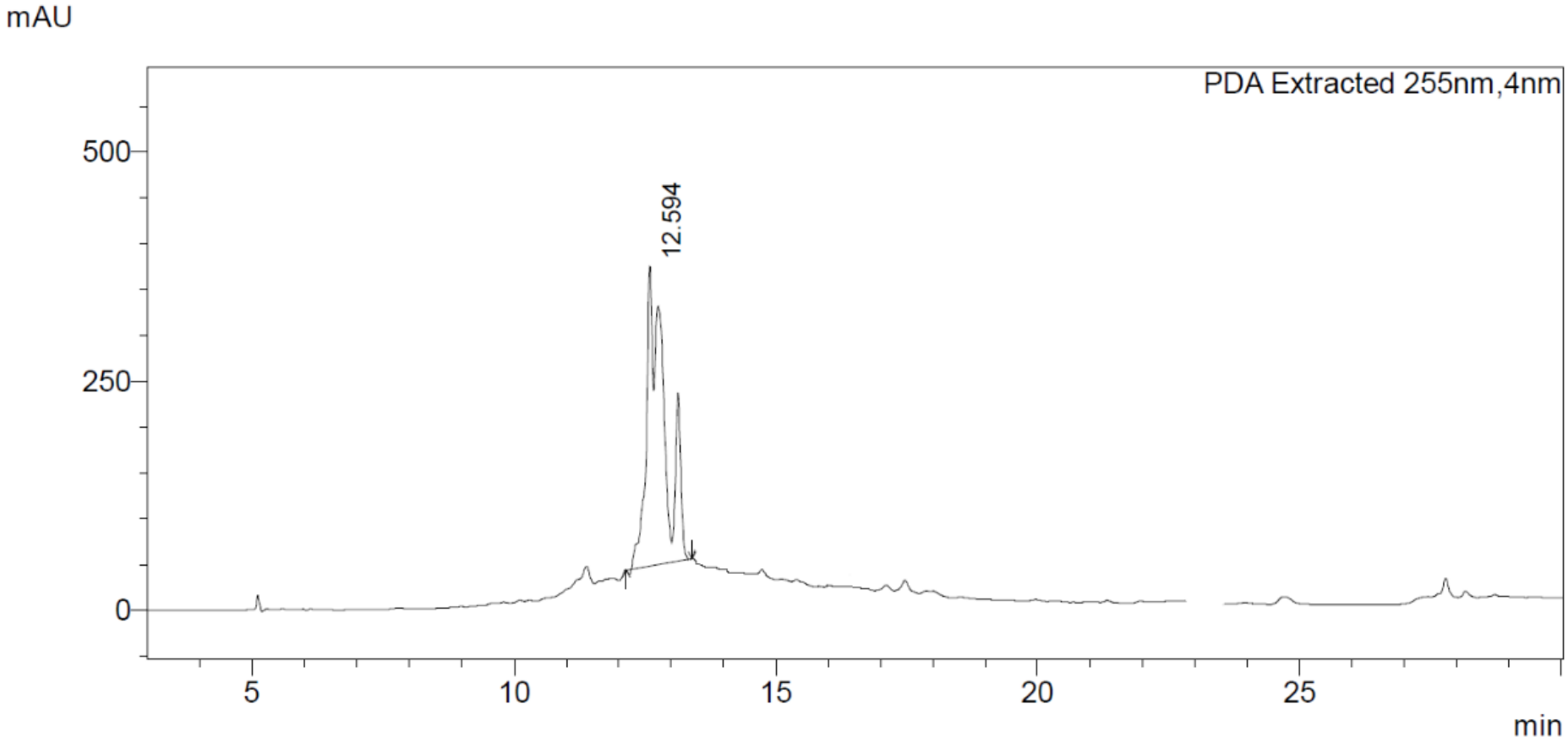
HRMS of 2**4b**

Acquisition Parameter

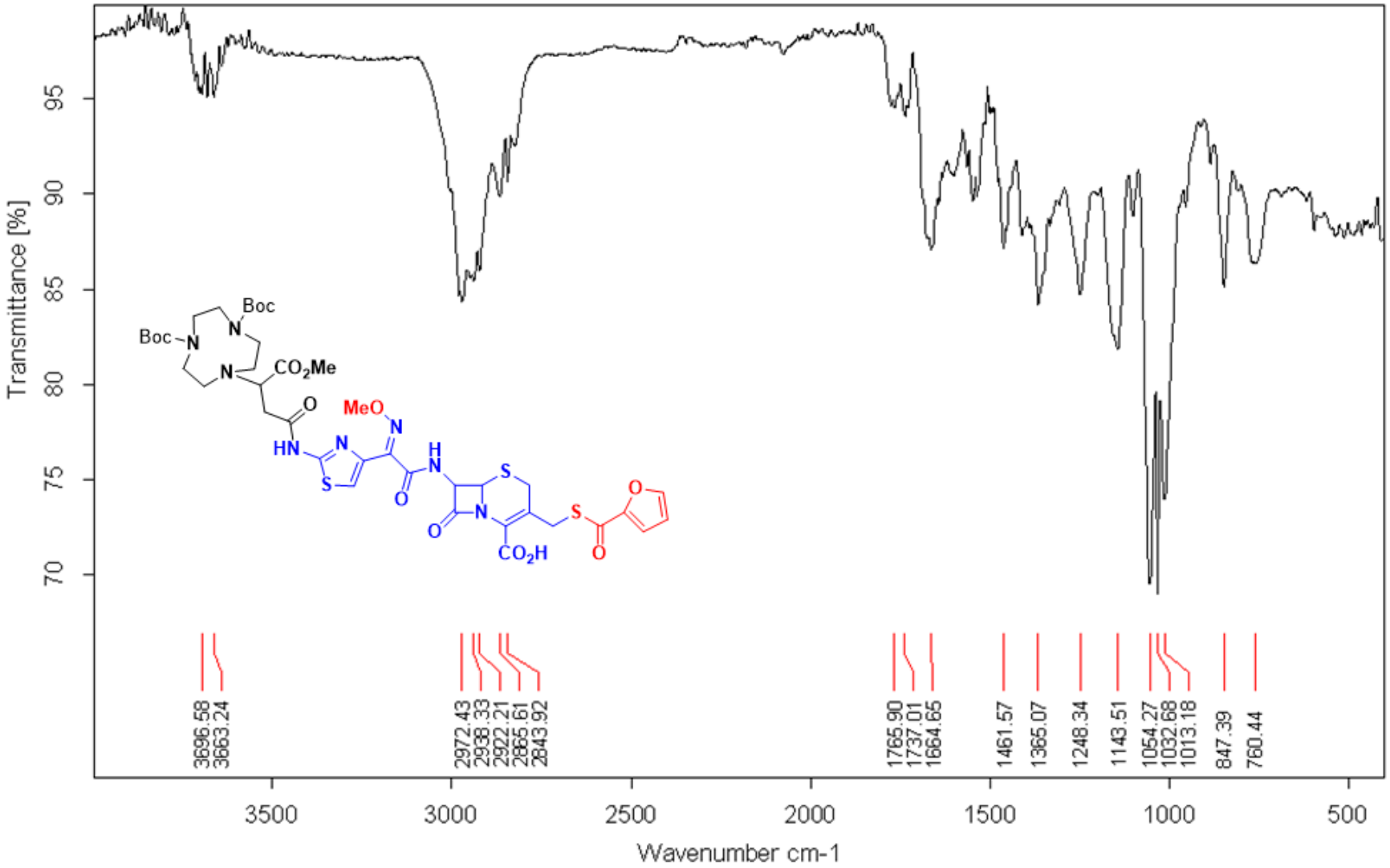
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2500 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **24b**



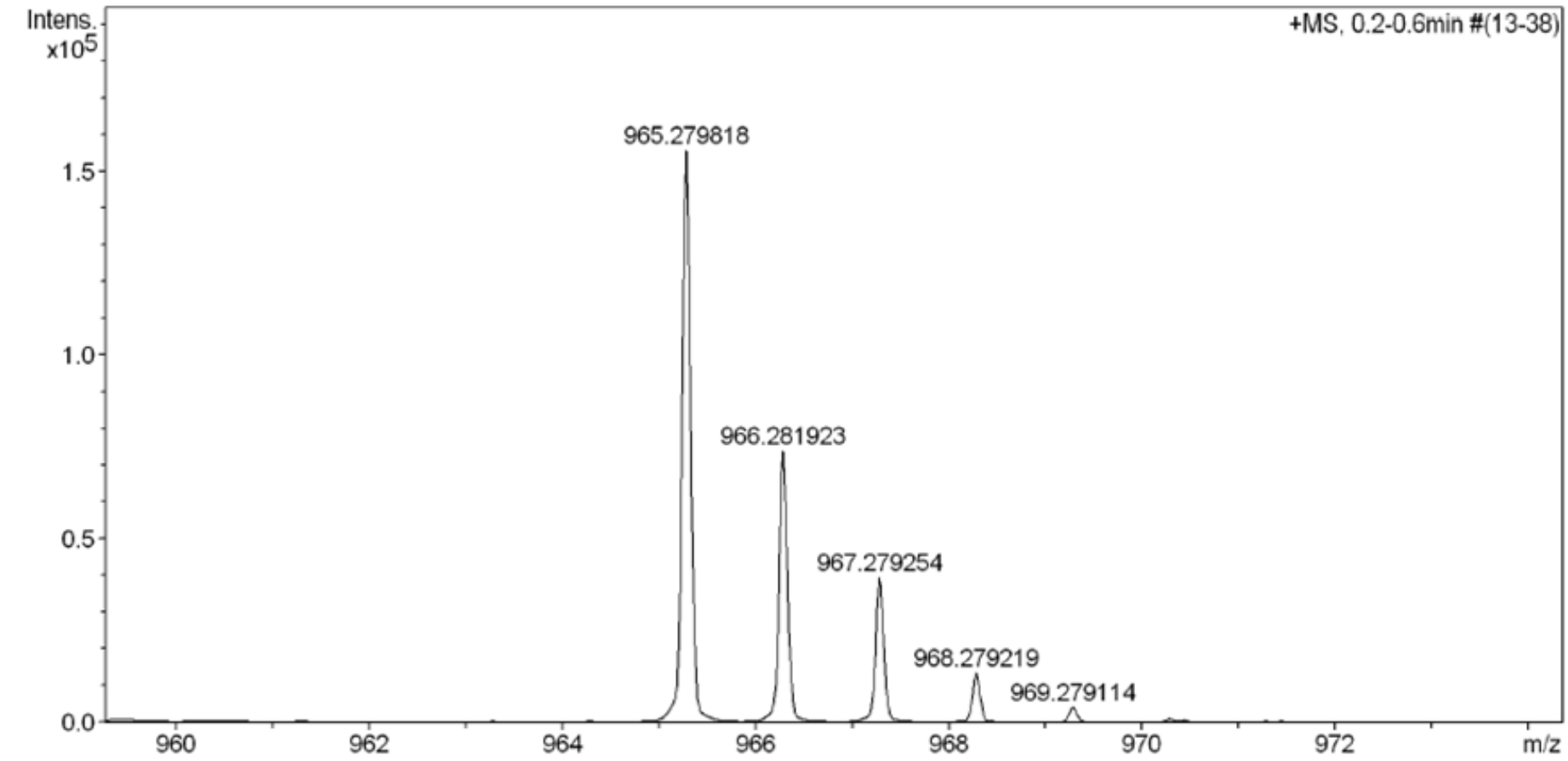
IR spectrum of **21c**



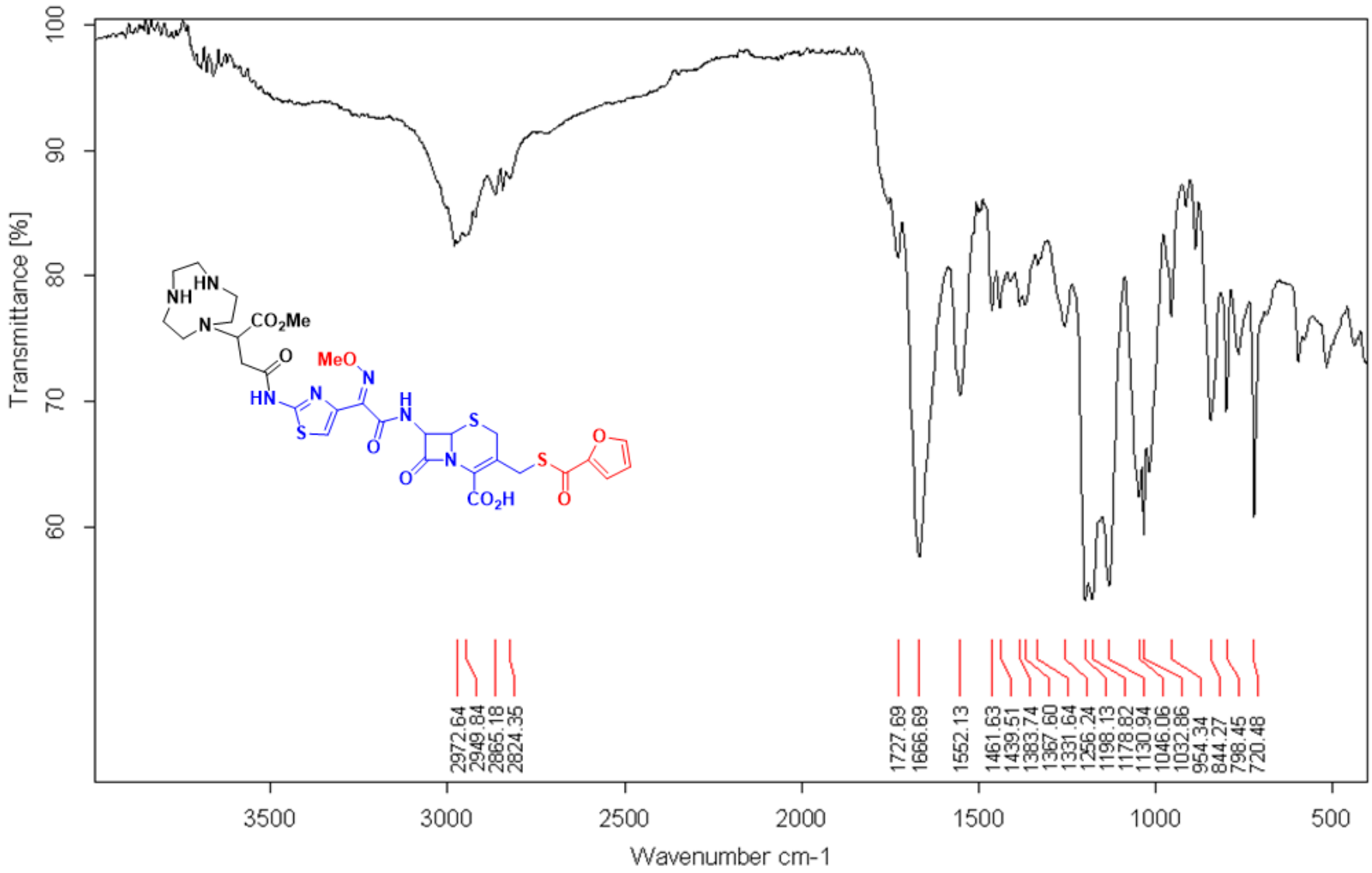
HRMS chromatogram of **21c**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



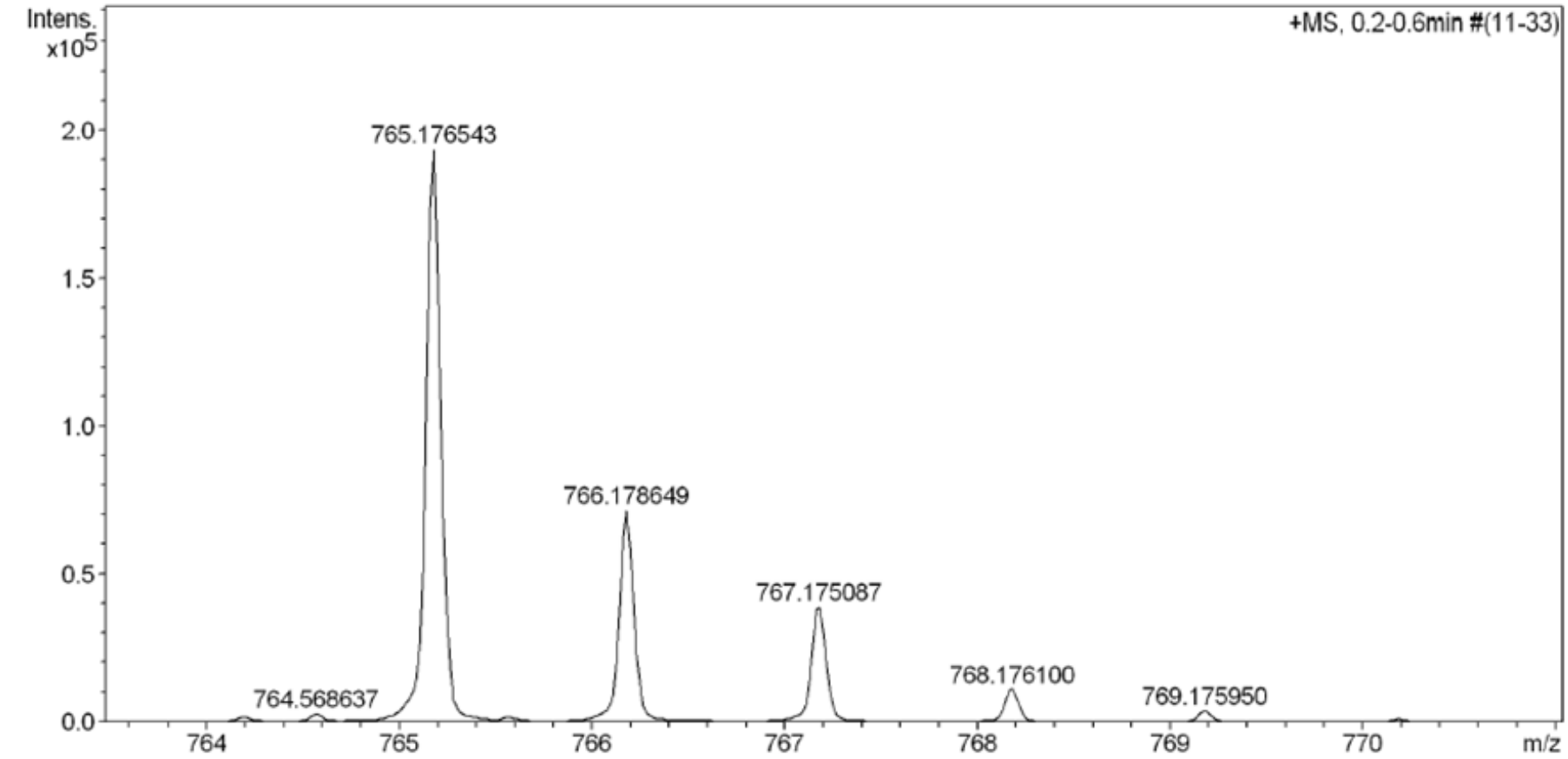
IR spectrum of **22c**



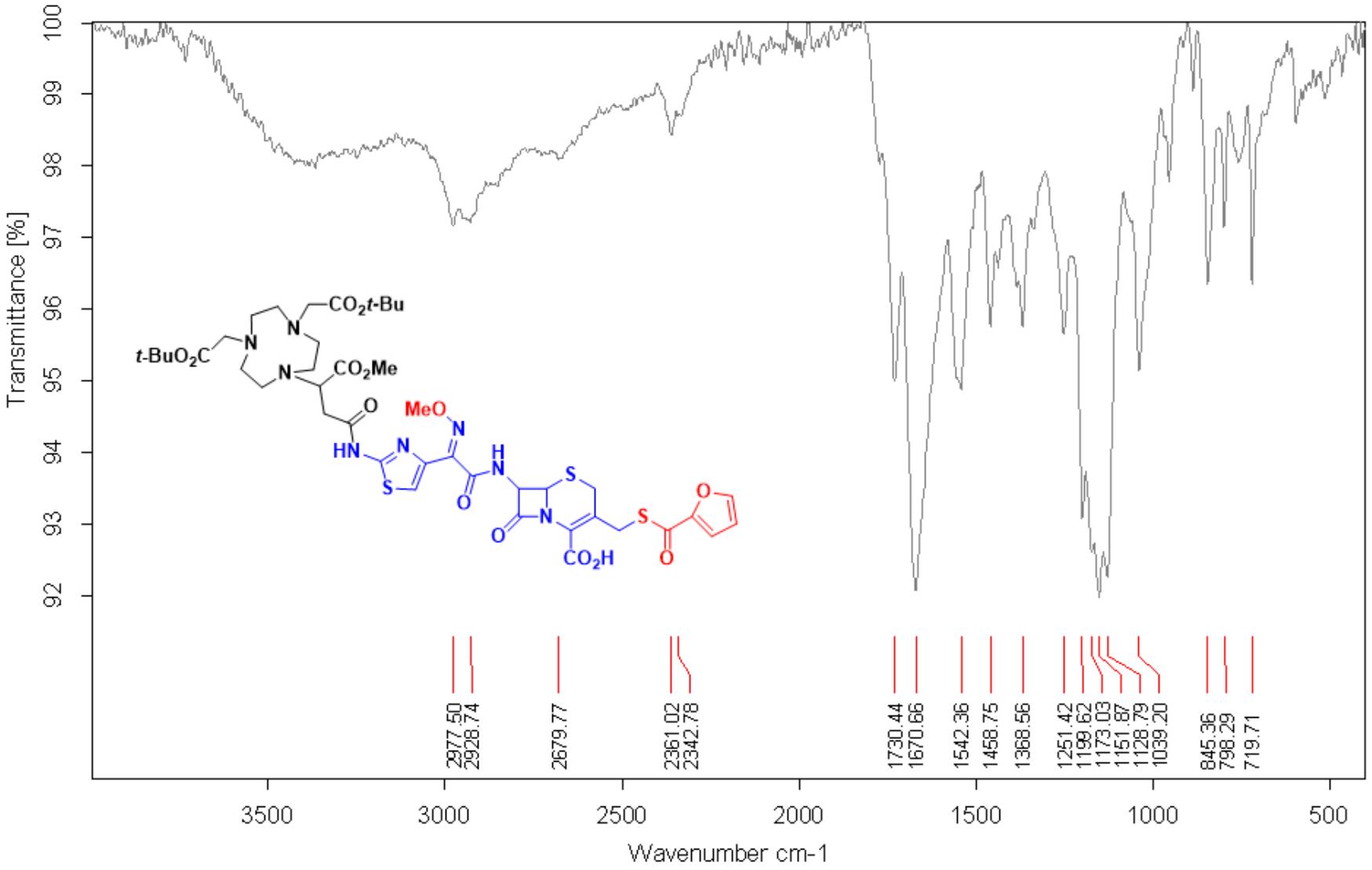
HRMS chromatogram of **22c**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



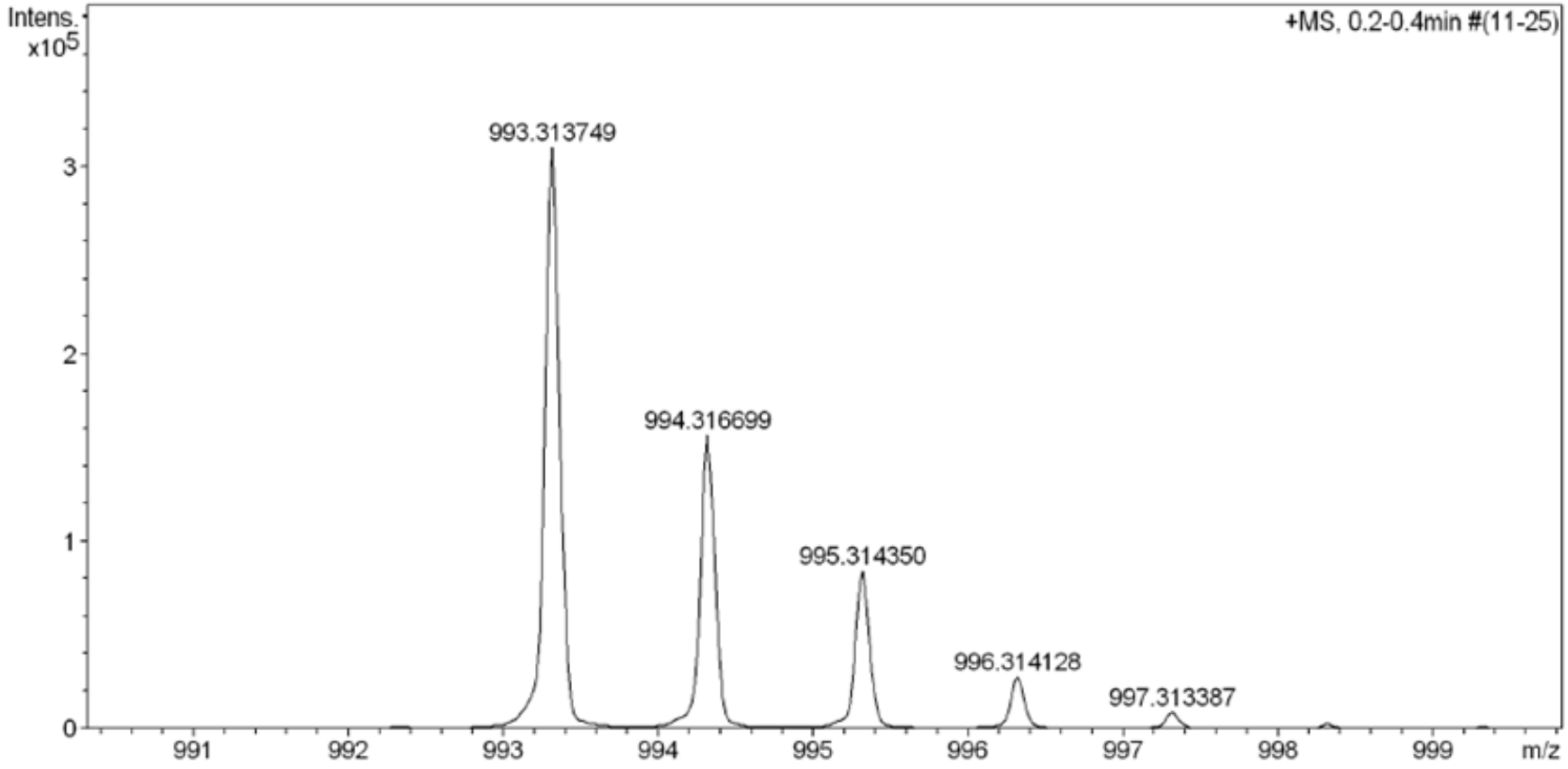
IR spectrum of **23c**



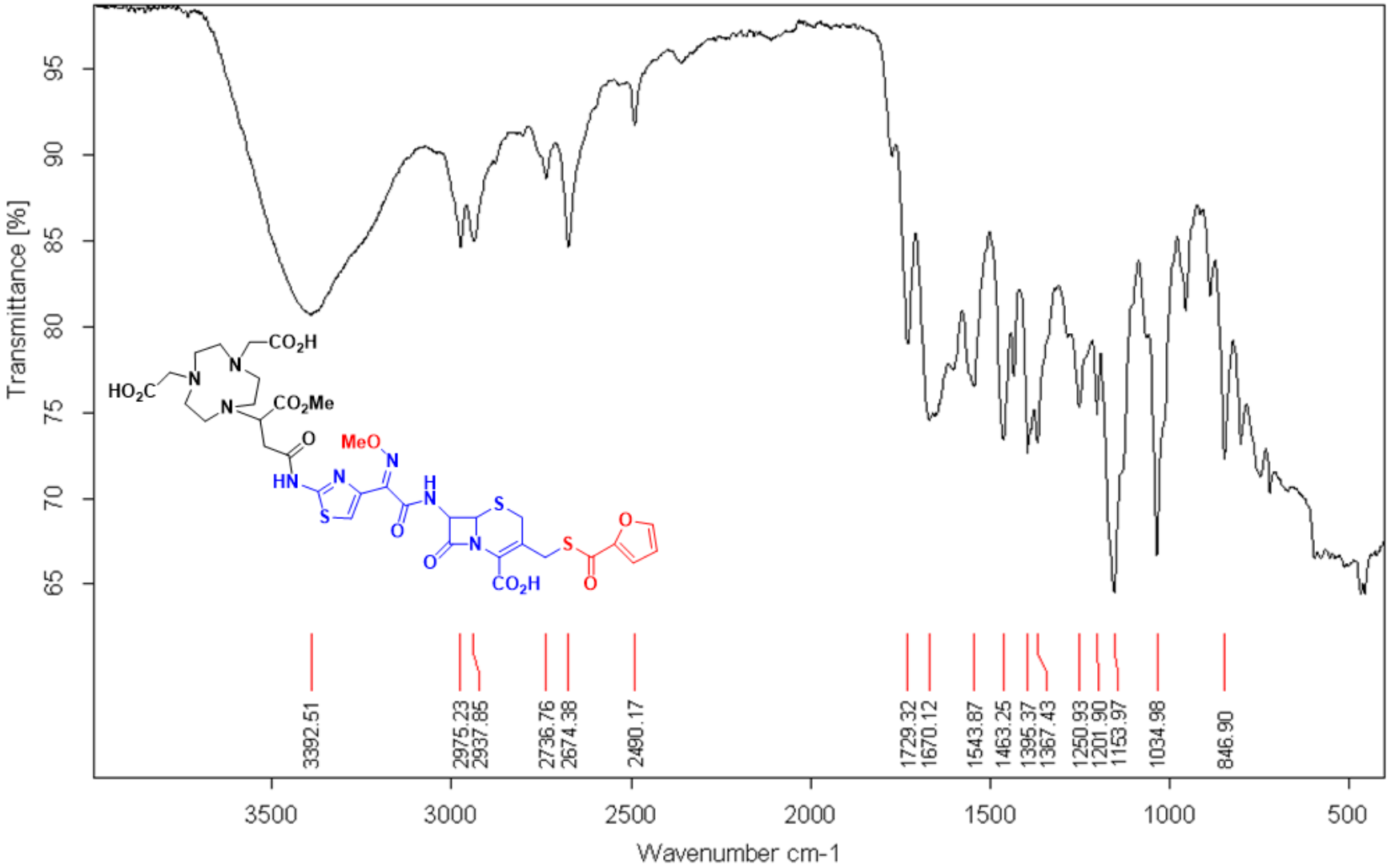
HRMS chromatogram of **23c**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



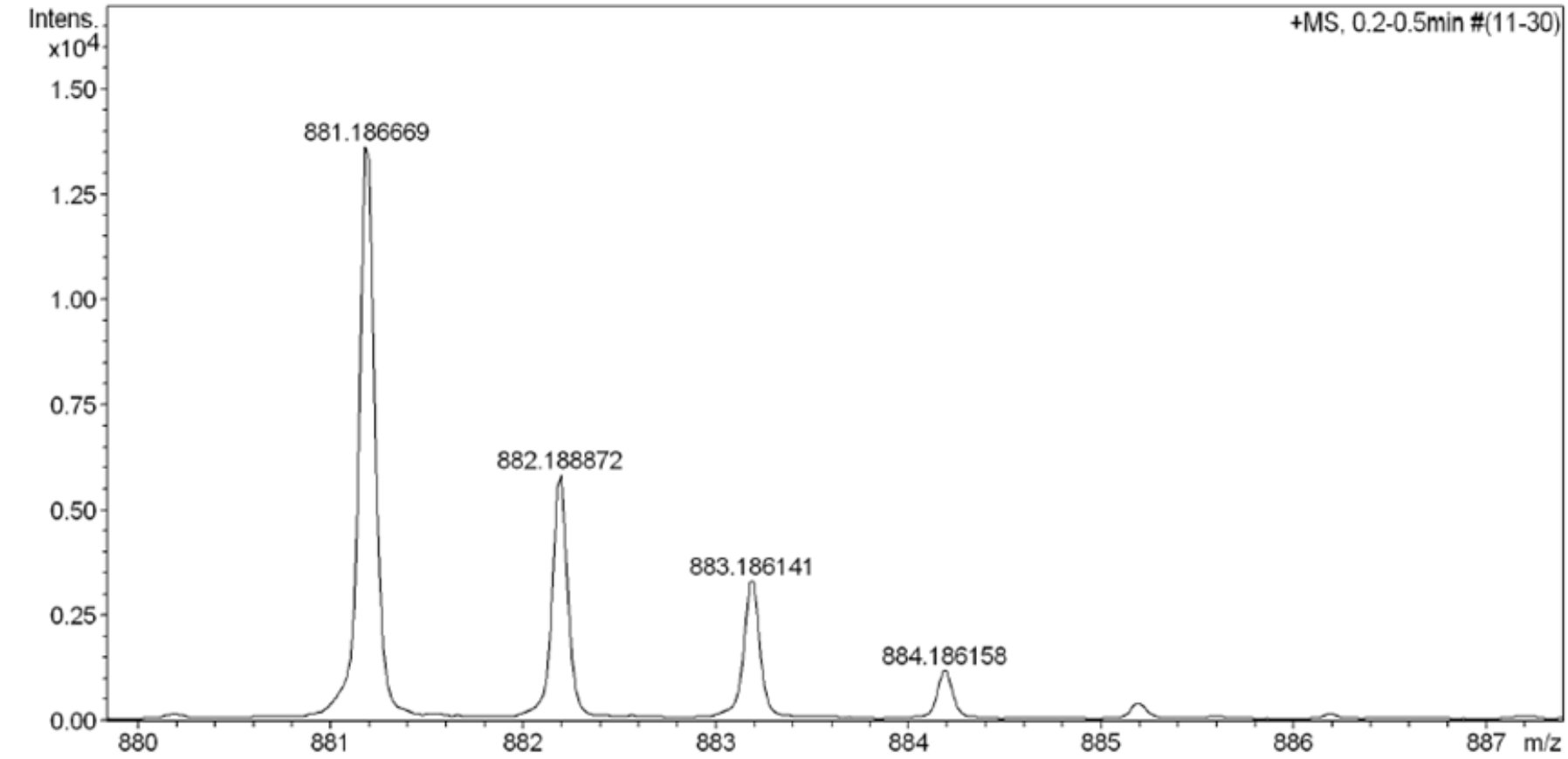
IR spectrum of **24c**



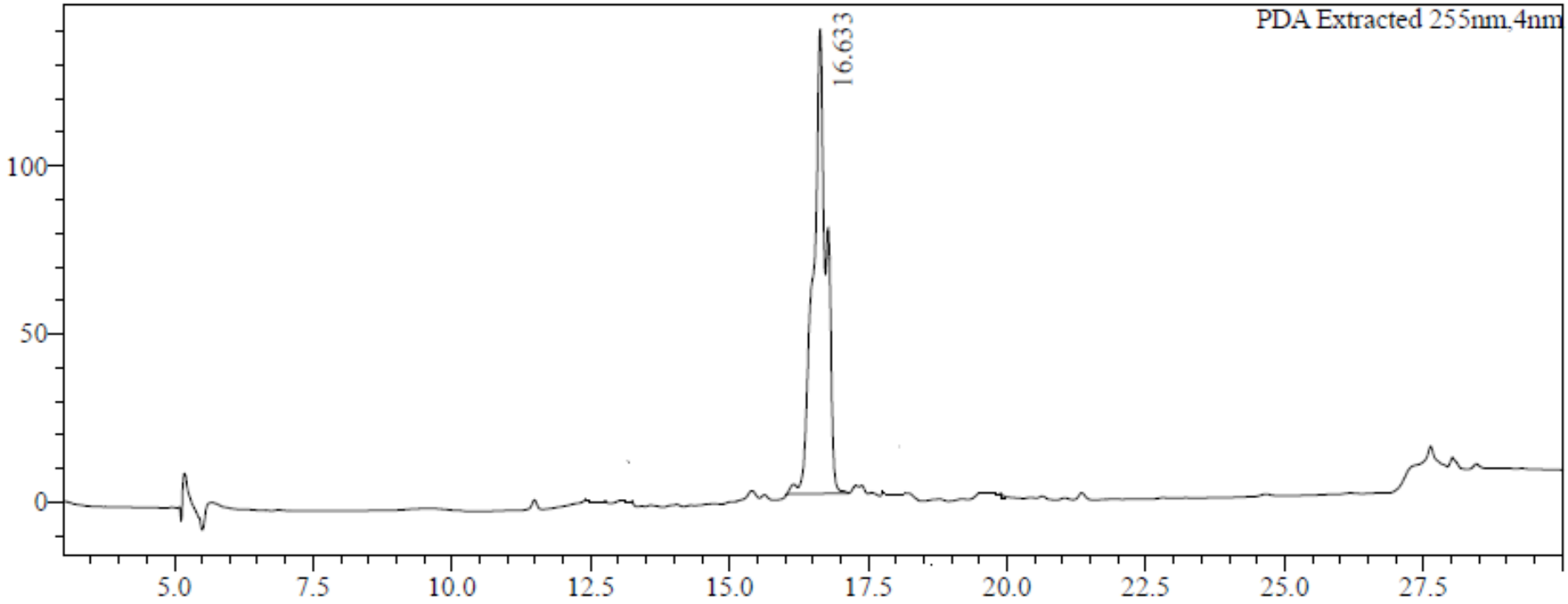
HRMS chromatogram of **24c**

Acquisition Parameter

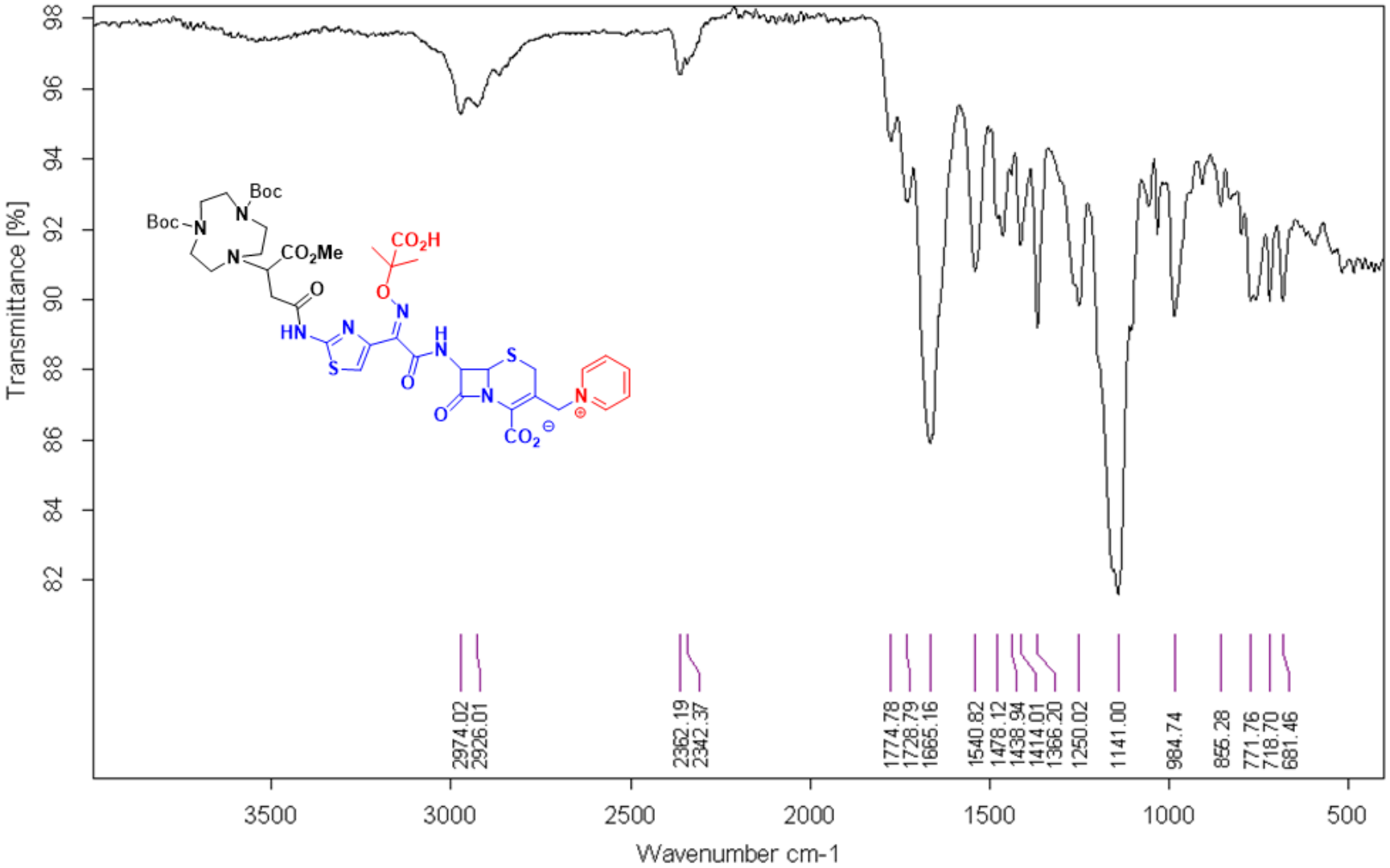
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **24c**



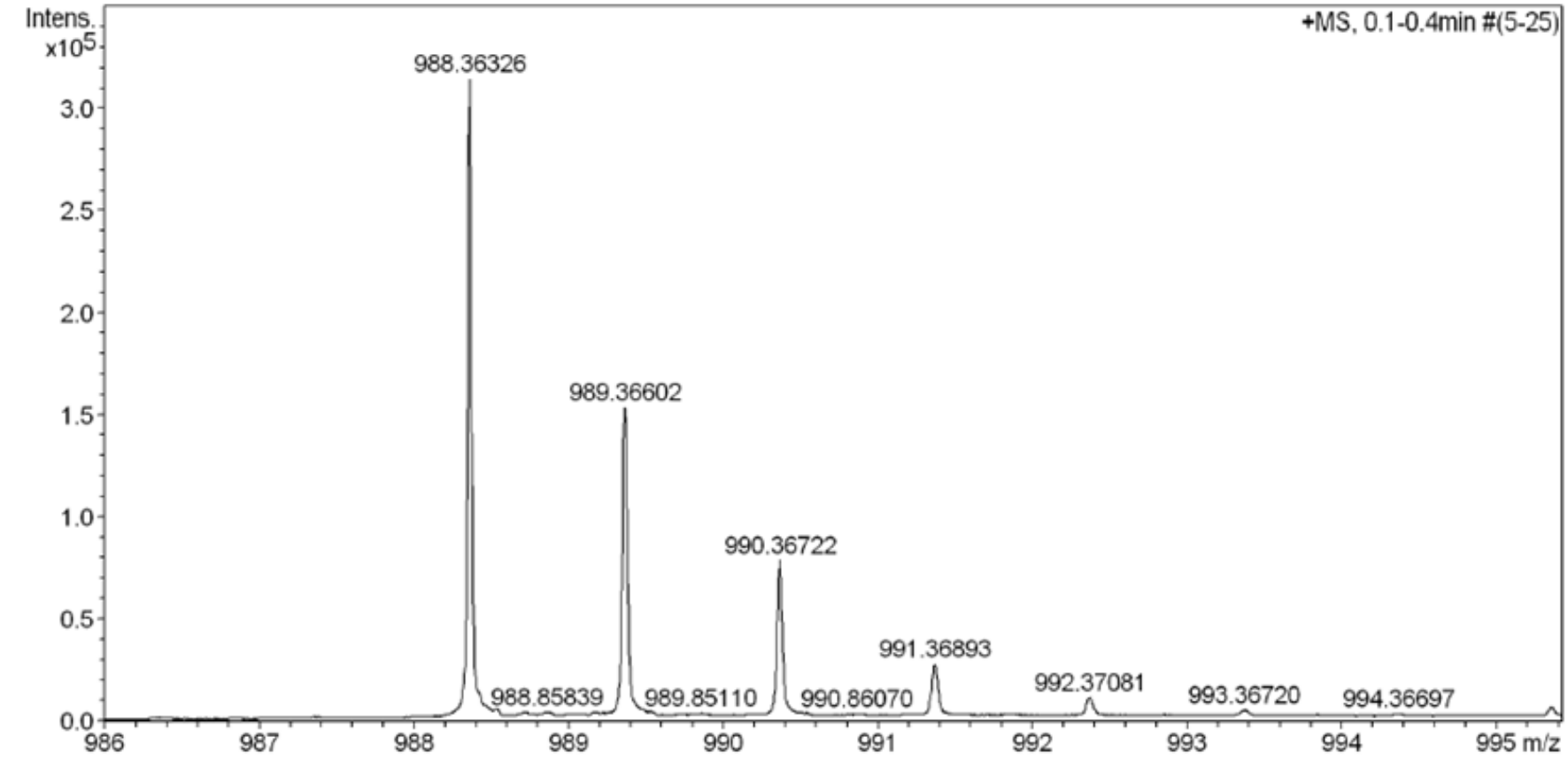
IR spectrum of **21d**



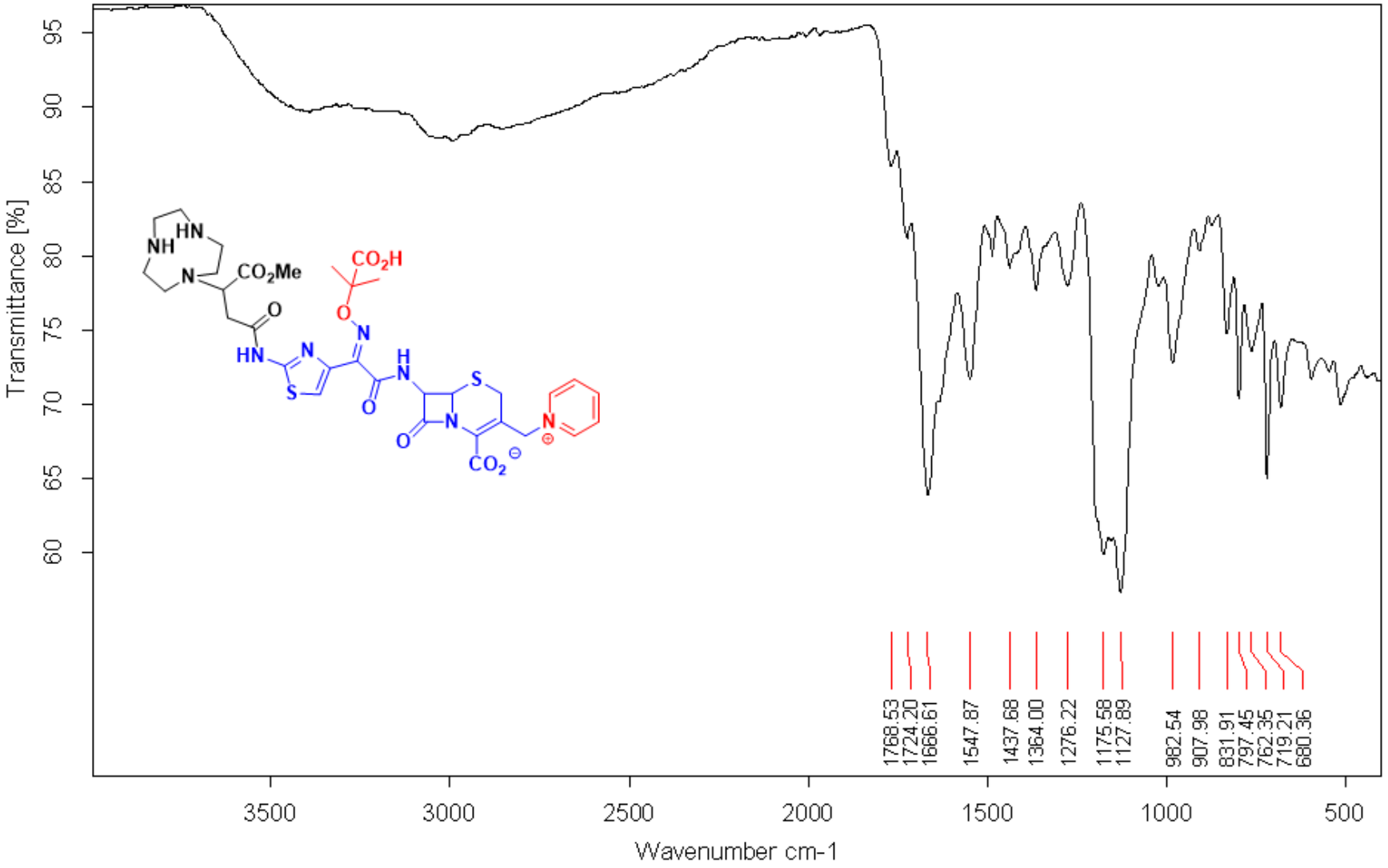
HRMS chromatogram of **21d**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



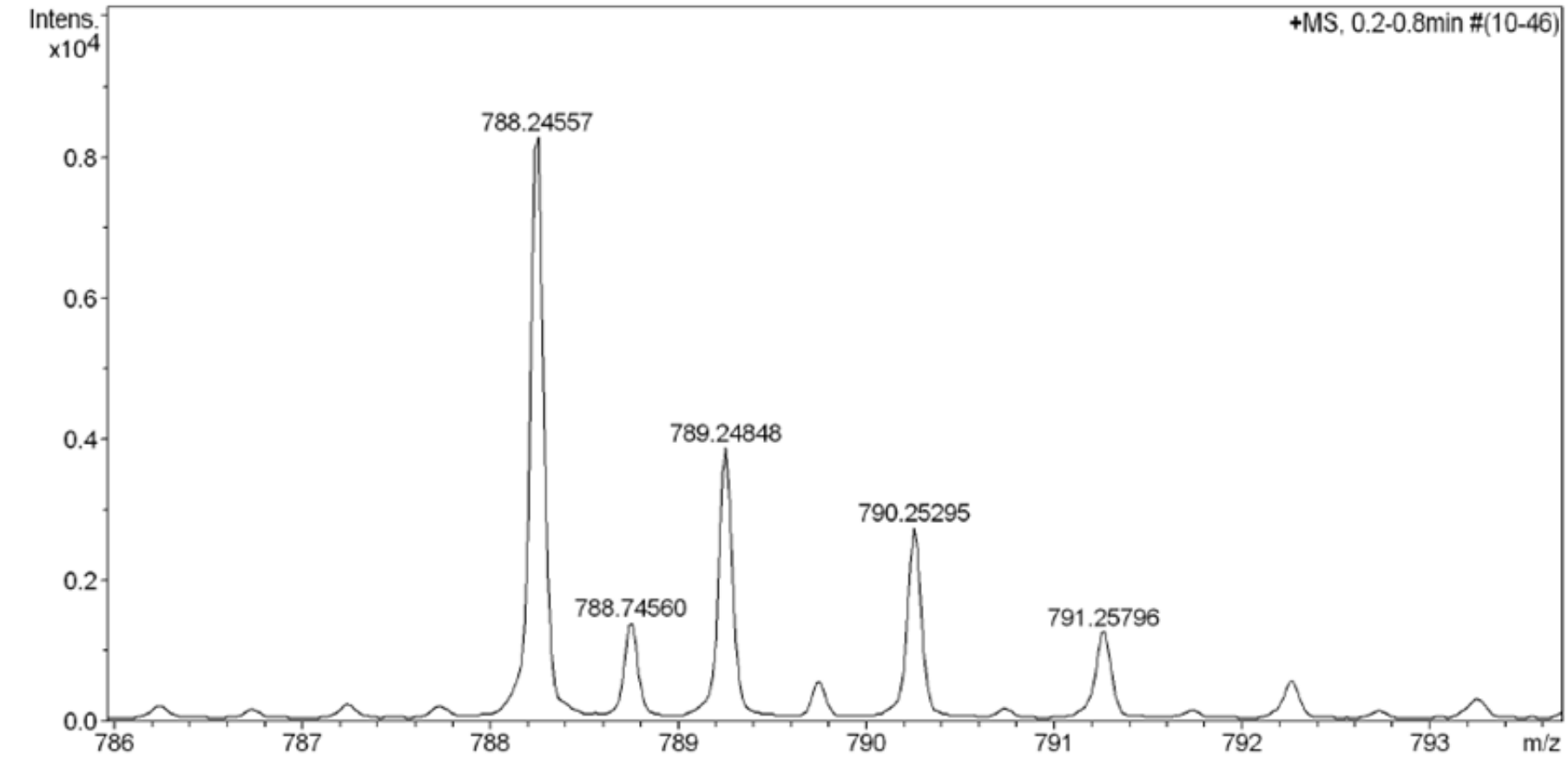
IR spectrum of LC-MS chromatogram of **22d**



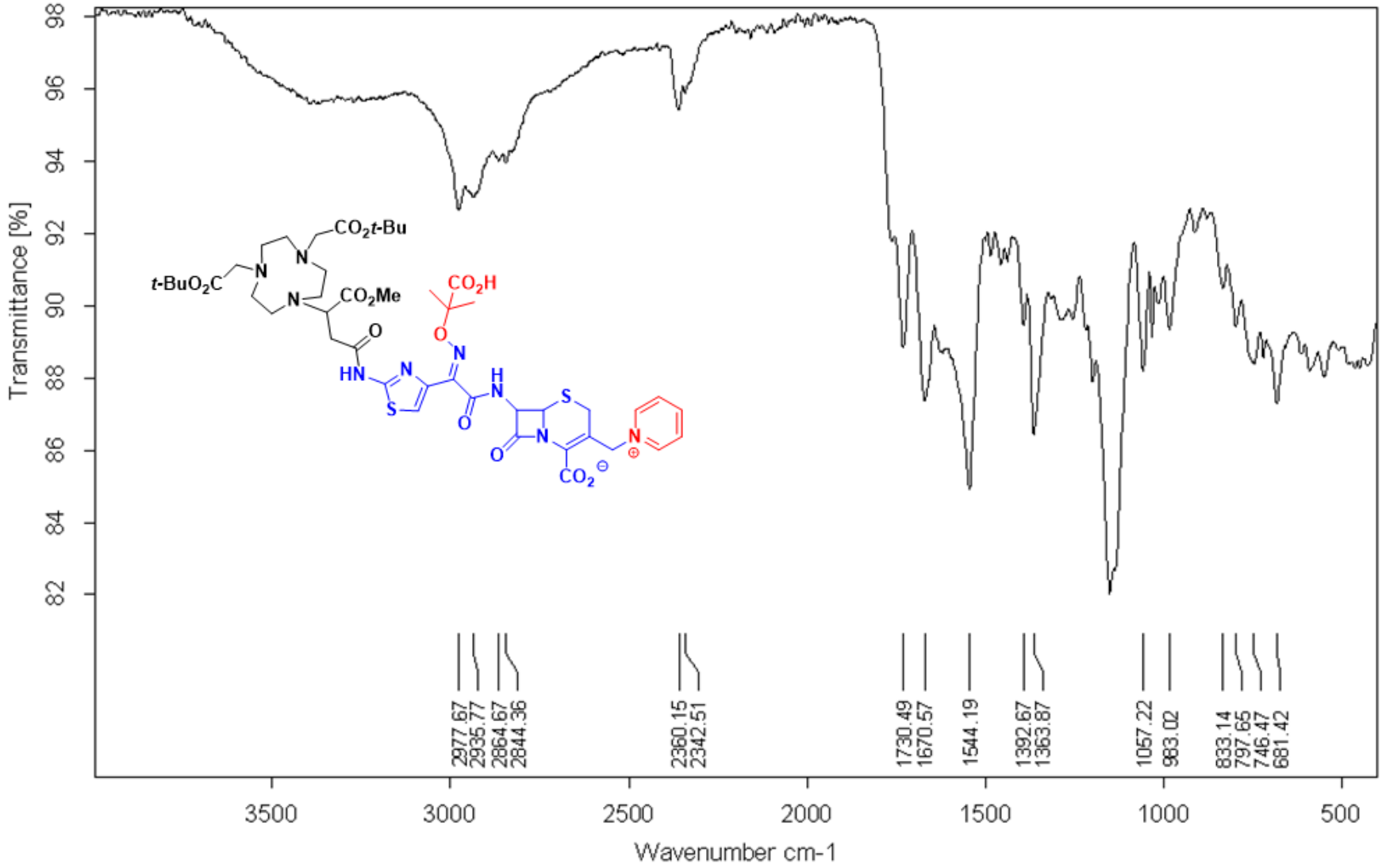
HRMS chromatogram of **22d**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



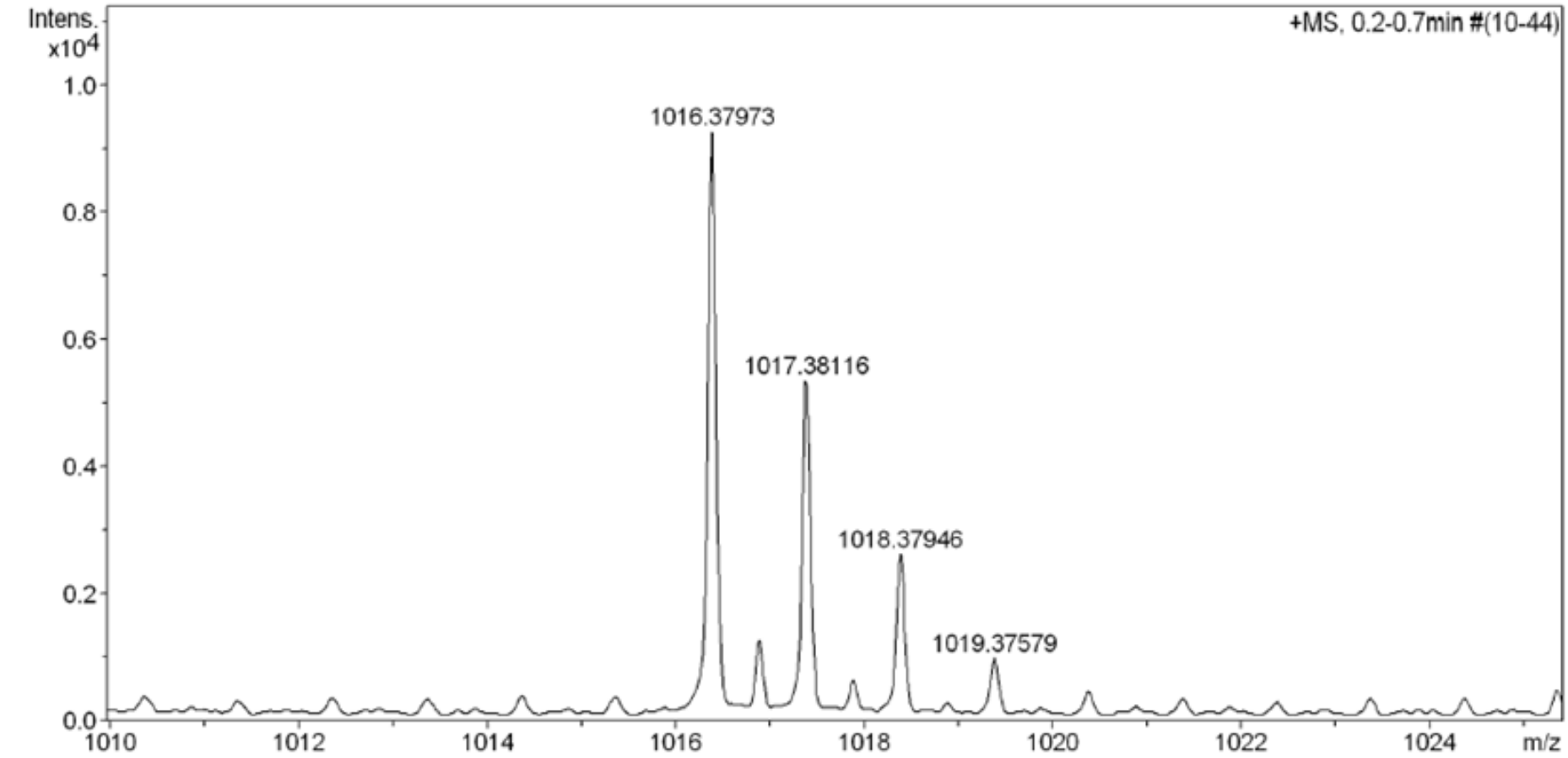
IR spectrum of **23d**



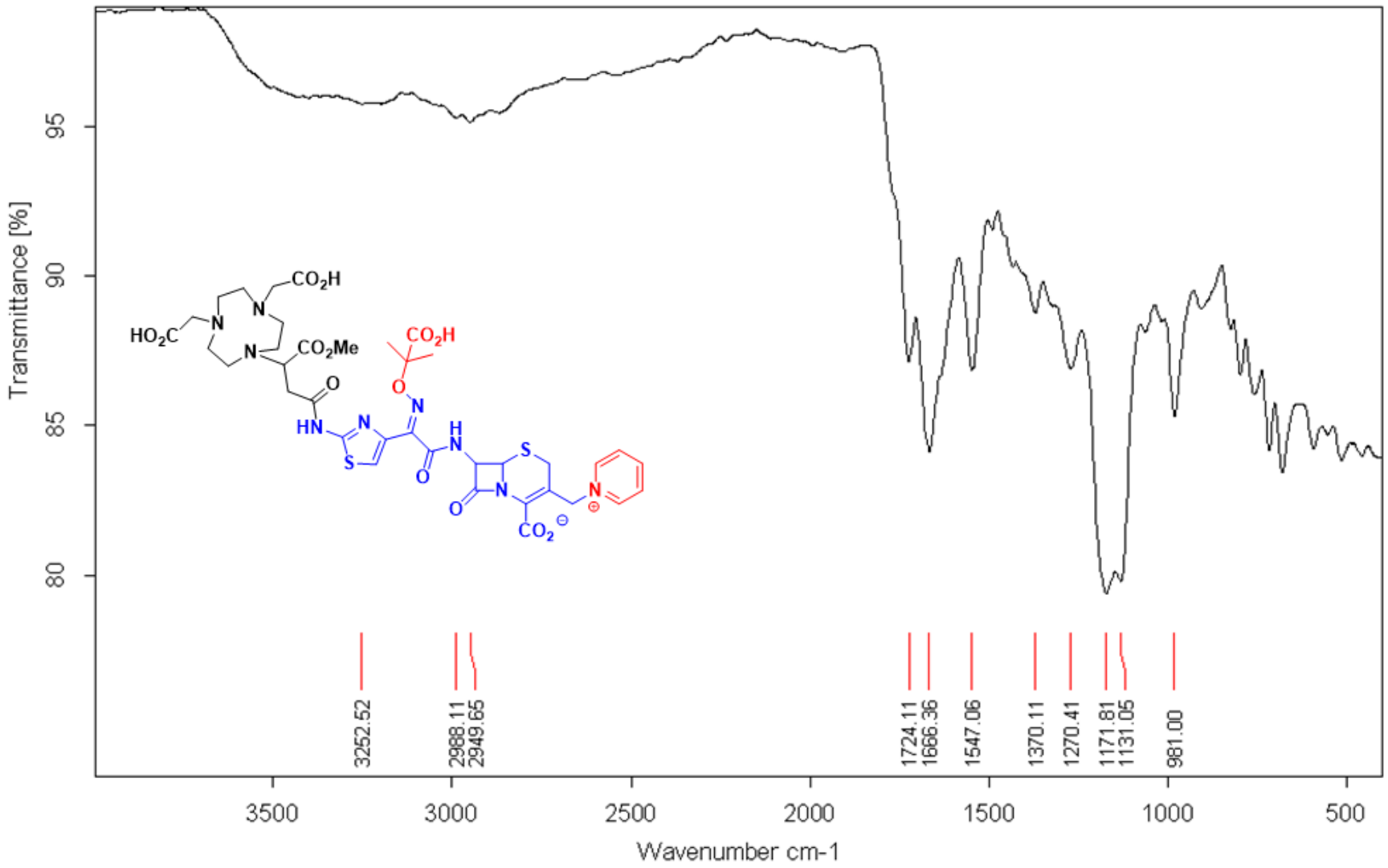
HRMS chromatogram of **23d**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Not active	Set Capillary	5500 V	Set Dry Heater	220 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



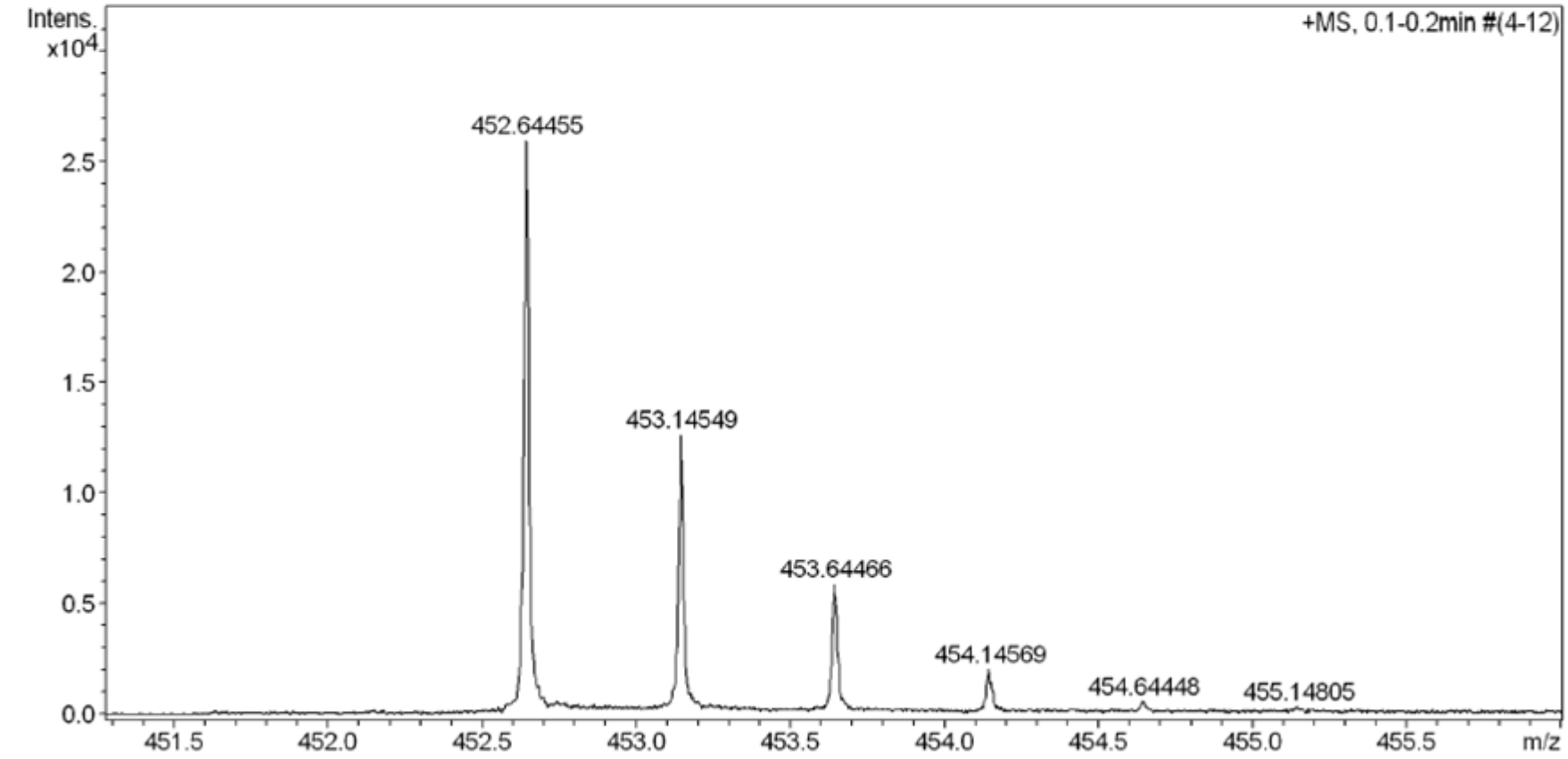
IR spectrum of **24d**



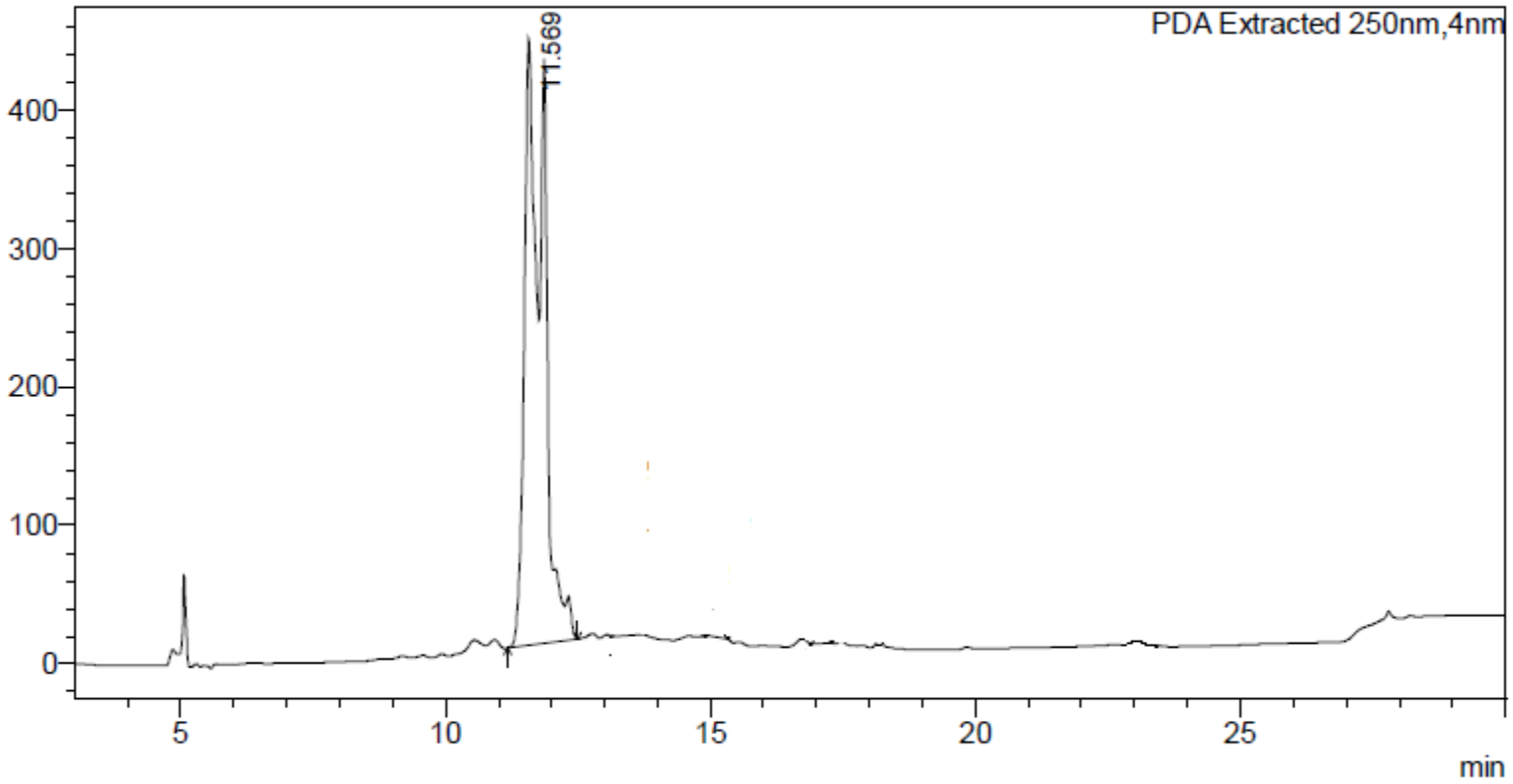
HRMS chromatogram **24d**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	5500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	2600 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



LC-MS chromatogram of **24d**



LC-MS chromatogram of NOTA-Zn complex