ELECTRONIC SUPPLEMENTARY INFORMATION

Amide and ester derivatives of chlorido[4-carboxy-1,2-disalicylidene-aminobenzene]iron(III) as necroptosis and ferroptosis inducers

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General procedure for the synthesis of the 3,4-diaminobenzamides. 1 eq of 3,4-dinitrobenzoic acid was dissolved in dry toluene (0.25 mM) and reacted with 6 eq of thionyl chloride under ice-cooling. It was heated to reflux for 4 h, allowed to cool down and the solvent as well as the excess of thionyl chloride were removed *in vacuo*. The obtained acid chloride was washed 3x with toluene, followed by evaporation of the solvent, and used without further purification. The mixture was ice-cooled and a solution containing 1.3 eq of triethylamine and 1.3 eq of the respective amine dissolved in toluene was added to obtain **6** and **7**. For the synthesis of **5**, 4.3 eq of dried and ground ethylamine hydrochloride was added to the solution. The reaction was performed in suspension. 3 eq of triethylamine in dry toluene were added under ice-cooling and stirred overnight at room temperature. For compound **5**, the precipitate was 3x extracted with toluene, later being combined with the organic layer.

The precipitates were filtered off and the organic layer was washed with hydrochloric acid (5%, m/m), with saturated sodium hydrogen carbonate solution, and with deionized water until the organic layer appeared clear and finally with saturated sodium chloride solution. It was dried over sodium sulfate and the solvent was removed *in vacuo* yielding an intensively orange to red coloured solid of the respective 3,4-dinitrobenzamides. The extracts of **5** from formerly insoluble solids of the reaction and organic layer were united and treated equally.

The solid was dissolved in methanol (250 mL) in a Parr flask for hydration, which was flushed with argon and evacuated 3x. An amount of 8% (m/m) palladium on activated charcoal, proportional to the deployed precursor, was added to the solution and 50 psi hydrogen were added to react at room temperature for 2 h. The catalyst was removed by filtration and the crude was obtained after evaporation of the solvent. Finally, **5-7** were purified via column chromatography on silica gel with ethyl acetate, methanol and triethylamine (90:9:1) as eluent.

*N-Ethyl-3,4-diaminobenzamide (**2**)*. From 3,4-dinitrobenzoic acid (14.1 mmol, 3.0 g), thionyl chloride (85 mmol, 10.1 g) in toluene (0.25 mM). Via assumed 100% obtained 3,4-dinitrobenzoyl chloride (14.1 mmol) without further purification and continuous reaction with dried ethylamine hydrochloride (60.8 mmol, 4.96 g) and triethylamine (42.4 mmol, 4.29 g) in presence of toluene (0.25 mM). *N*-Ethyl-3,4-dinitrobenzamide was purified, orange powder, yield 2.89 g (12.1 mmol, 86%), subsequently reduction in methanol solution with palladium on activated charcoal in presence of hydrogen. Yellow powder, yield 1.46 g (8.19 mmol, 68%). ^{1}H-NMR (DMSO-d_6): δ = 7.89-7.83 (t, 1H, CONH), 7.04-7.03 (d, 1H, Ar-H), 6.96- 6.91 (dd, 1H, Ar-H), 6.48-6.44 (d, 1H, Ar-H), 4.91 (s, 2H, NH_2), 4.52 (s, 2H, NH_2), 3.26-3.13 (q, 2H, CH_2), 1.10-1.03 (t, 3H, CH_3).

*N-Propyl-3,4-diaminobenzamide (**3**).* From 3,4-dinitrobenzoic acid (23.6 mmol, 5.0 g), thionyl chloride (141 mmol, 16.8 g) in toluene (0.25 mM). Via assumed 100% obtained 3,4-dinitrobenzoyl chloride (23.6 mmol) without further purification and continuous reaction with propylamine (30.6 mmol, 1.81 g) and triethylamine (30.6 mmol, 3.10 g) in toluene (0.3 mM). *N*-Propyl-3,4-dinitrobenzamide is purified by liquid-liquid extraction with saturated sodium hydrogen carbonate solution to yield an orange powder, 5.30 g (20.9 mmol, 89%), followed by reduction in methanol solution with palladium on activated charcoal in presence of hydrogen. Yellow powder, yield 0.99 g (5.11 mmol, 26%). ^{1}H-NMR (DMSO-d_6): δ = 7.87-7.82 (t, 1H, CONH), 7.04-7.03 (d, 1H, Ar-H), 6.97-6.92 (dd, 1H, Ar-H), 6.48-6.44 (d, 1H, Ar-H), 4.90 (s, 2H, NH_2), 4.52 (s, 2H, NH_2), 3.18-3.08 (q, 2H, CH_2), 1.53-1.42 (q, 2H, CH_2), 0.89-0.82 (t, 3H, CH_3).

*N-Butyl-3,4-diaminobenzamide (**4**).* From 3,4-dinitrobenzoic acid (14.1 mmol, 3.0 g), thionyl chloride (85 mmol, 10.1 g) in toluene (0.25 mM). Via assumed 100% obtained 3,4-dinitrobenzoyl chloride (14.1 mmol) without further purification and continuous reaction with butylamine (18.4 mmol, 1.34 g) and triethylamine (18.4 mmol, 1.86 g) in toluene (25 mM). *N*-Propyl-3,4-dinitrobenzamide is purified by liquid-liquid extraction with saturated sodium hydrogen carbonate solution, to yield an orange powder, 2.50 g (9.35 mmol, 66%), followed by reduction in methanol solution with palladium on activated charcoal in presence of hydrogen. Yellow powder, yield 0.30 g (1.45 mmol, 15%). ^{1}H-NMR (DMSO-d_6): δ = 7.86-7.80 (t, 1H, CONH), 7.03-7.02 (d, 1H, Ar-H), 6.96-6.91 (dd, 1H, Ar-H), 6.43 (d, 1H, Ar-H), 4.89 (s, 2H, NH_2), 4.52 (s, 2H, NH_2), 3.22-3.12 (q, 2H, CH_2), 1.48-1.38 (q, 2H, Ar-H), 1.34-1.23 (q, 2H, CH_2), 0.92-0.85 (t, 3H, CH_3).

General procedure for the synthesis of the 3,4-diaminobenzoates. 1 eq of 3,4-diaminobenzoic acid was dissolved in an excess of the respective alcohol. Esterification was catalyzed by sulfuric acid and conducted under reflux overnight. The solvent was removed *in vacuo*, a solution of NaOH (1 M) was added followed by 3x extraction with dichloromethane. The organic layer was neutralized with HCl (0.05 M), washed with saturated sodium hydrogen carbonate solution, deionized water until it appeared clear, and 3x with saturated sodium chloride solution, then dried over sodium sulfate. A highly viscous solution was obtained after evaporation of the solvent. Fine crystalline needles grew over time.

*Ethyl 3,4-diaminobenzoate (**5**).* From 3,4-diaminobenzoic acid (10 mmol, 1.52 g), sulfuric acid (40 mmol, 3.92 g) in 50 ml ethanol. Light brown needles, yield 1.19 g (6.63 mmol, 66%). ^{1}H-NMR (DMSO-d6): δ = 7.15-7.15 (d, 1H, Ar-H), 7.10-7.10 (d, 1H, Ar-H), 7.08-7.08 (d, 1H, Ar-H), 6.15-6.49 (d, 1H, Ar-H), 5.26 (s, 2H, $ArNH_2$), 4.65 (s, 2H, $ArNH_2$), 4.20-4.15 (q4, 2H, CH_2), 1.28-1.24 (t, 3H, CH_3).

*Propyl 3,4-diaminobenzoate (**6**).* From 3,4-diaminobenzoic acid (10 mmol, 1.55 g), sulfuric acid (40 mmol, 3.92 g) in 50 ml n-propanol. Light brown needles, yield 1.04 g (5.36 mmol, 54%). ^{1}H-NMR (DMSO-d_6): δ = 7.16-7.06 (m, 2H, Ar-H), 6.52-6.47 (d, 1H, Ar-H), 5.24 (s, 2H, Ar-NH_2), 4.68 (s, 2H, Ar-NH_2), 4.12-4.05 (t, 2H, CH_2), 1.71-1.61 (q5, 2H, CH_2), 0.98-0.91 (t, 3H, CH_3).

*Butyl 3,4-diaminobenzoate (**7**).* From 3,4-diaminobenzoic acid (10 mmol, 1.51 g), sulfuric acid (40 mmol, 3.92 g) in 50 ml n-butanol. Brown needles, yield 1.24 g (5.96 mmol, 60%). ^{1}H-NMR (DMSO-d6): δ = 7.14-7.14 (d, 1H, Ar-H), 7.10-7.06 (dd, 1H, Ar-H), 6.52-6.48 (d, 1H, Ar-H), 5.27 (s, 2H, Ar-NH_2), 4.66 (s, 2H, Ar-NH_2), 4.16-4.10 (t, 2H, CH_2), 1.70-1.56 (q5, 2H, CH_2), 1.48-1.30 (s6, 2H, CH_2), 1.00-0.88 (t, 3H, CH_3).

Analytical methods applied for structure characterization and purity check

^{1}H-NMR spectroscopy

All samples were dissolved in deuterated DMSO with 0.03% TMS as internal standard and measured immediately on either a Varian Gemini VX 200 (200 MHz) or a Bruker Advance 4 Neo spectrometer (400 MHz).

HR MS

The compounds were solved in either MeOH or MeCN and high-resolution mass spectrometry (HR MS) was performed on an Orbitrap Elite (Thermo Fisher Scientific, Waltham, USA) via direct infusion and electrospray ionization (ESI) in positive mode at 150 °C.

FT IR spectroscopy

Infrared (IR) spectroscopy was carried out employing an Alpha FT IR Spectrometer (Bruker, Billerica, USA). The compounds were measured pure on an ATR unit.

HPLC chromatography

All samples were prepared in MeCN gradient grade (100 µM) and LC purity investigations were performed on a Chromolith® HighResolution RP-18e (100×4.6mm, Merck) column. For the purity investigation of **16–21** MeCN/phosphate buffer 25mM, pH 3 (25/75, v/v) was used as mobile phase. For the stability examination of the complexes **15** and **19** the mobile phase was optimized to MeCN/TFA 0.1% (22/78 v/v) to warrant a baseline separation of **19** and the possible hydrolysis product **15**. This modified method was also used for the purity check of **15** and **19** for better comparison.

An isocratic flow rate of 1 mL/min was used throughout the LC experiments. Detection was performed by DAD at 310 nm and the injection volume was 8 µL. Compounds were monitored at 310 nm. Peak identification was performed by UV scan using the DAD detector.

Figure S1: [1]H-NMR spectrum of precursor *N-ethyl-3,4-diaminobenzamide* (**2**)

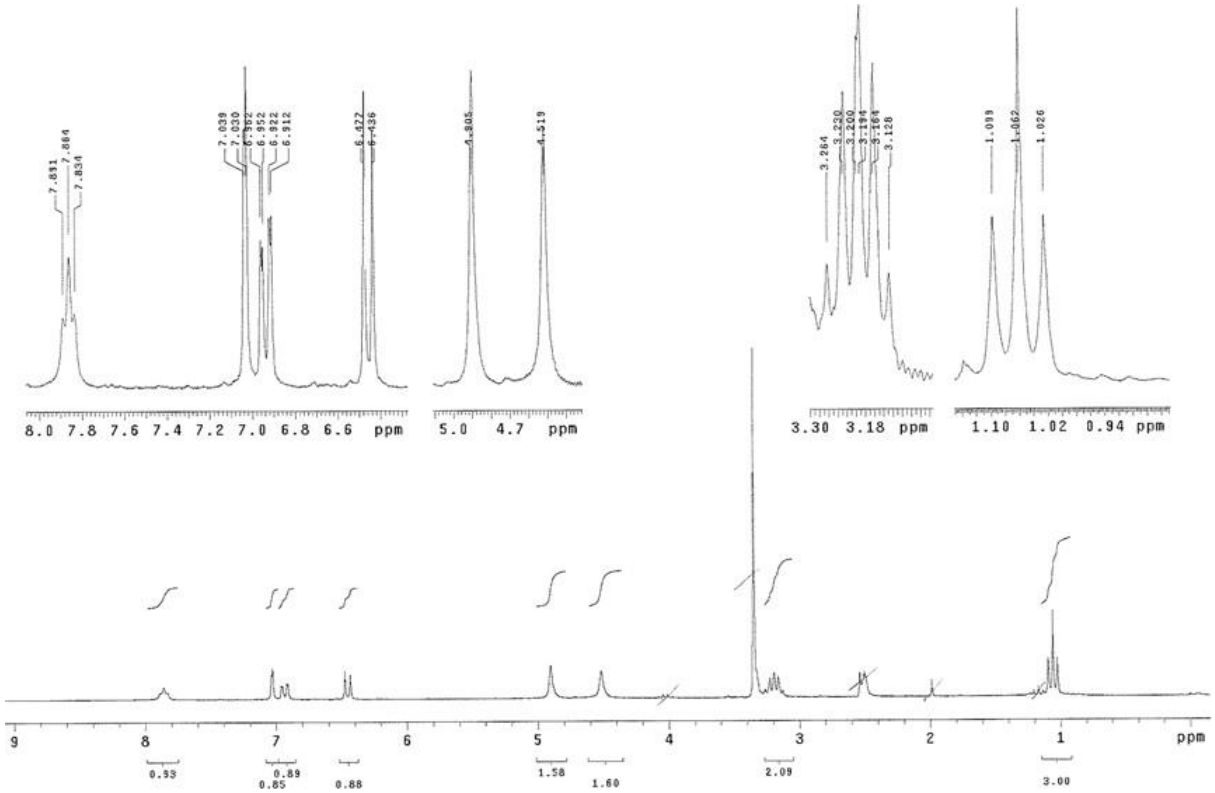


Figure S2: [1]H-NMR spectrum of precursor *N-propyl-3,4-diaminobenzamide* (**3**)

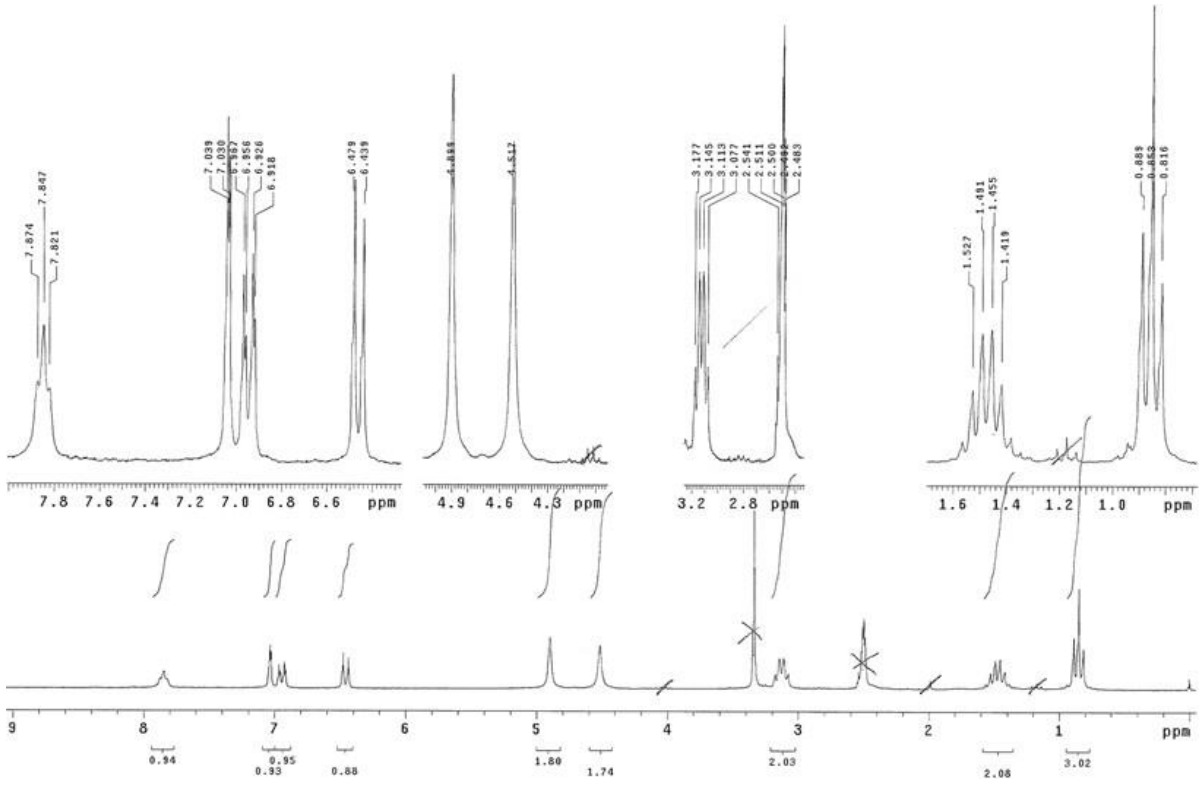


Figure S3: ^{1}H-NMR spectrum of precursor *N-butyl-3,4-diaminobenzamide* (**4**)

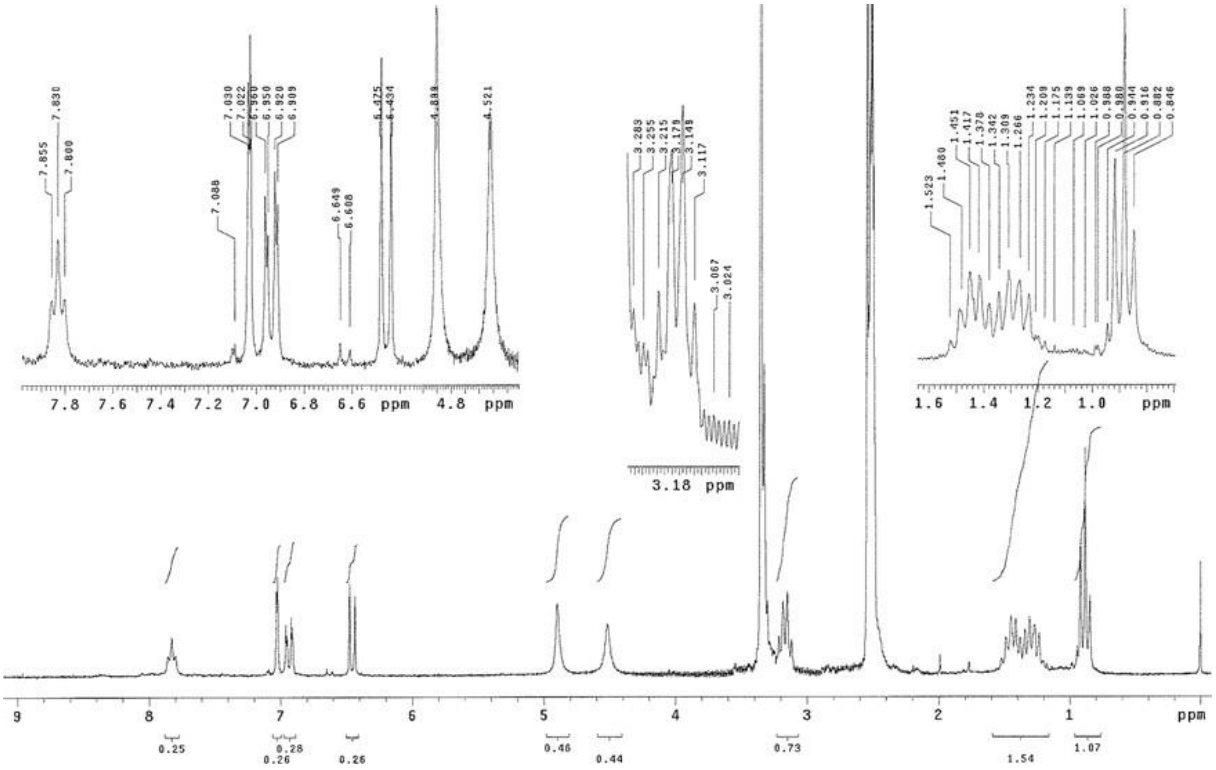


Figure S4: ^{1}H-NMR spectrum of precursor *ethyl 3,4-diaminobenzoate* (**5**)

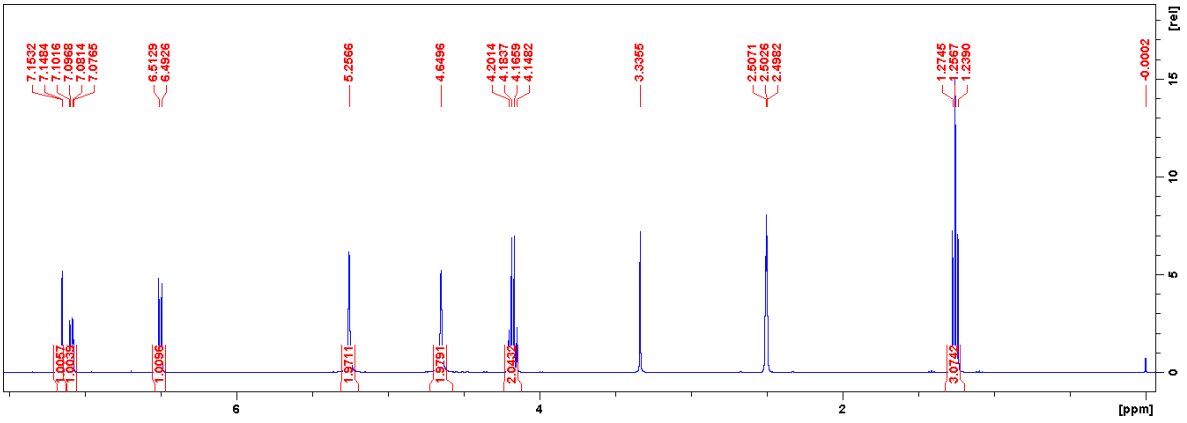


Figure S5: ^{1}H-NMR spectrum of precursor *propyl 3,4-diaminobenzoate* (**6**)

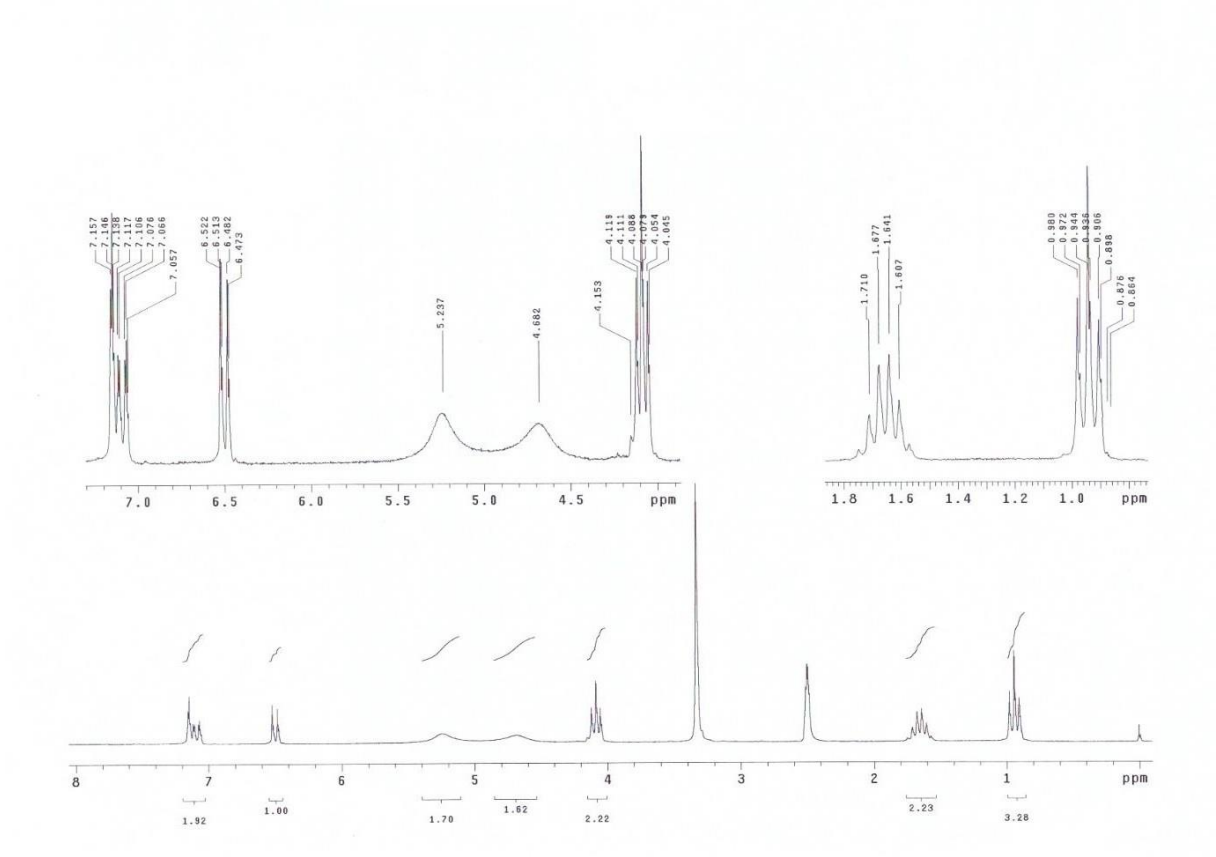


Figure S6: ^{1}H-NMR spectrum of precursor *butyl 3,4-diaminobenzoate* (**7**)

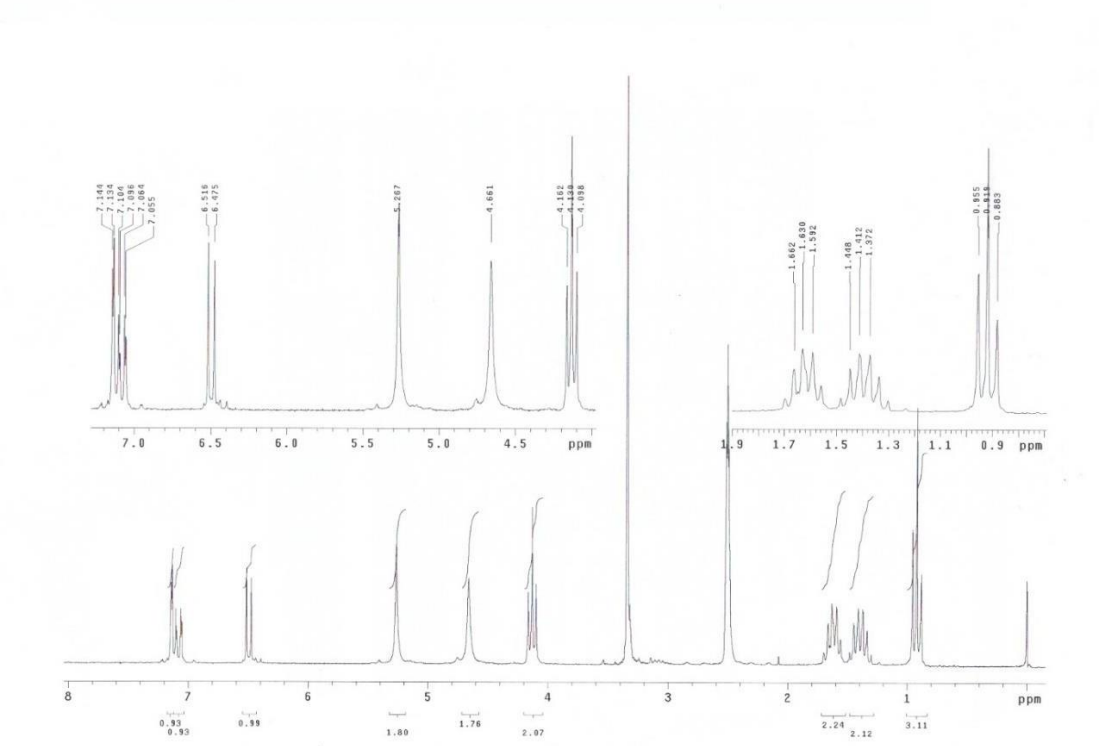
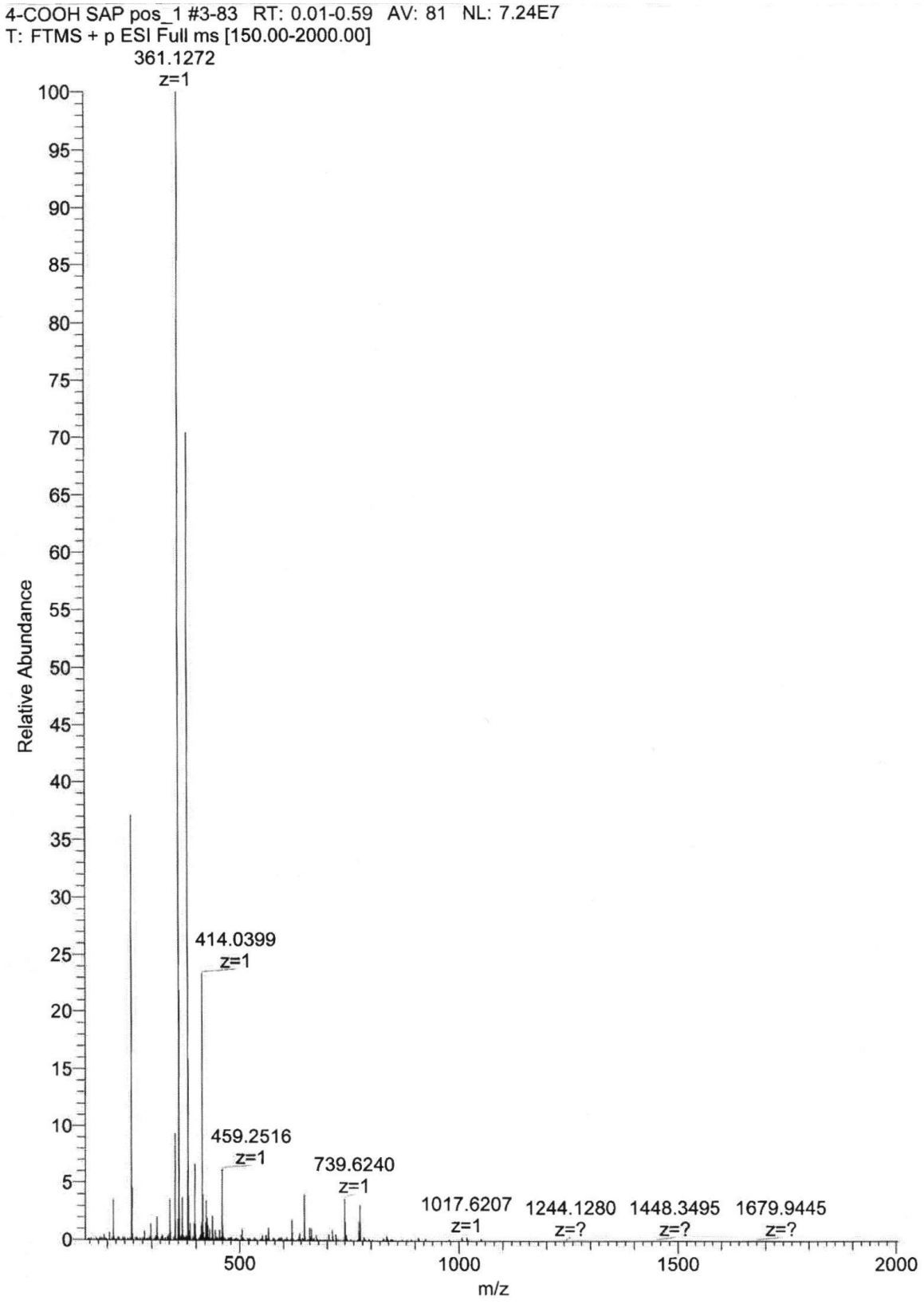
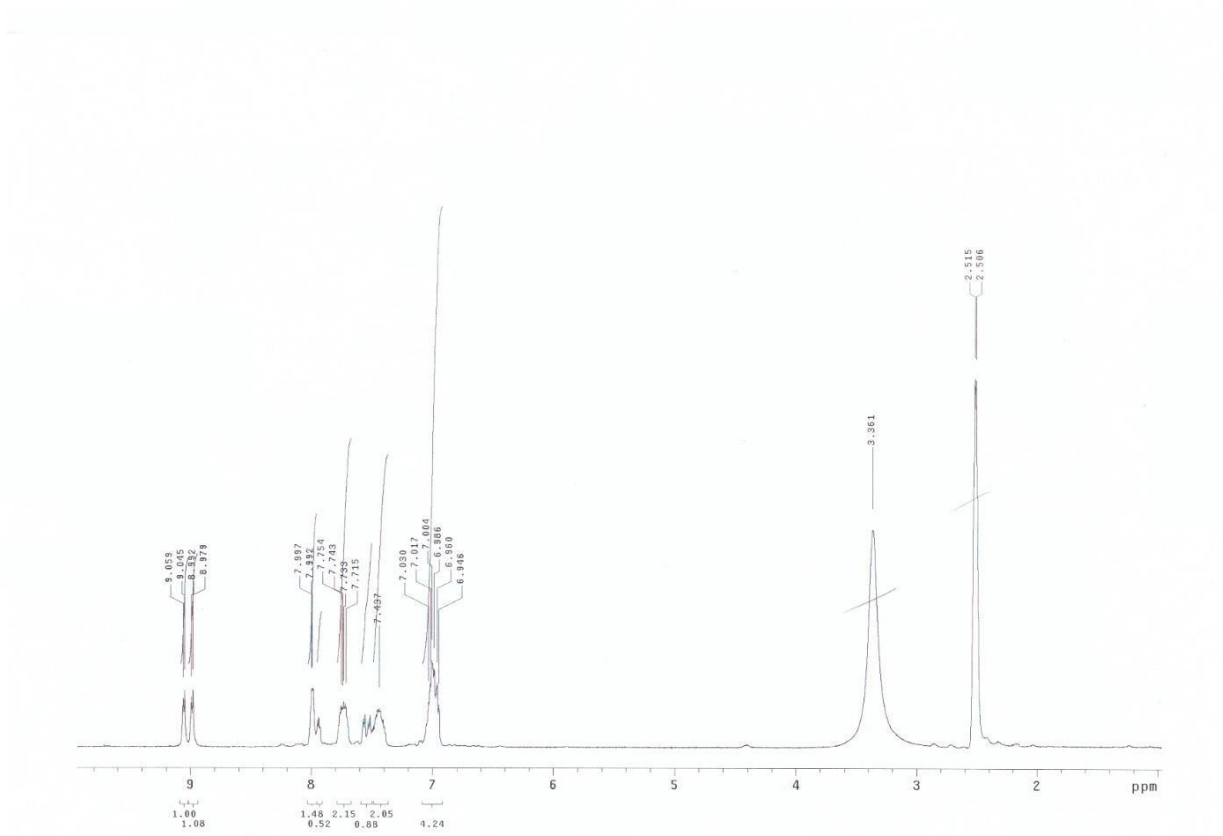


Figure S7: HR MS, ^{1}H-NMR and IR-spectrum of ligand *3,4-disalicylideneaminobenzoic acid* (**8**)

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6.946
3.361
2.515
2.506
9
8
7
6
5
4
3
2
ppm
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1.08
1.48
0.52
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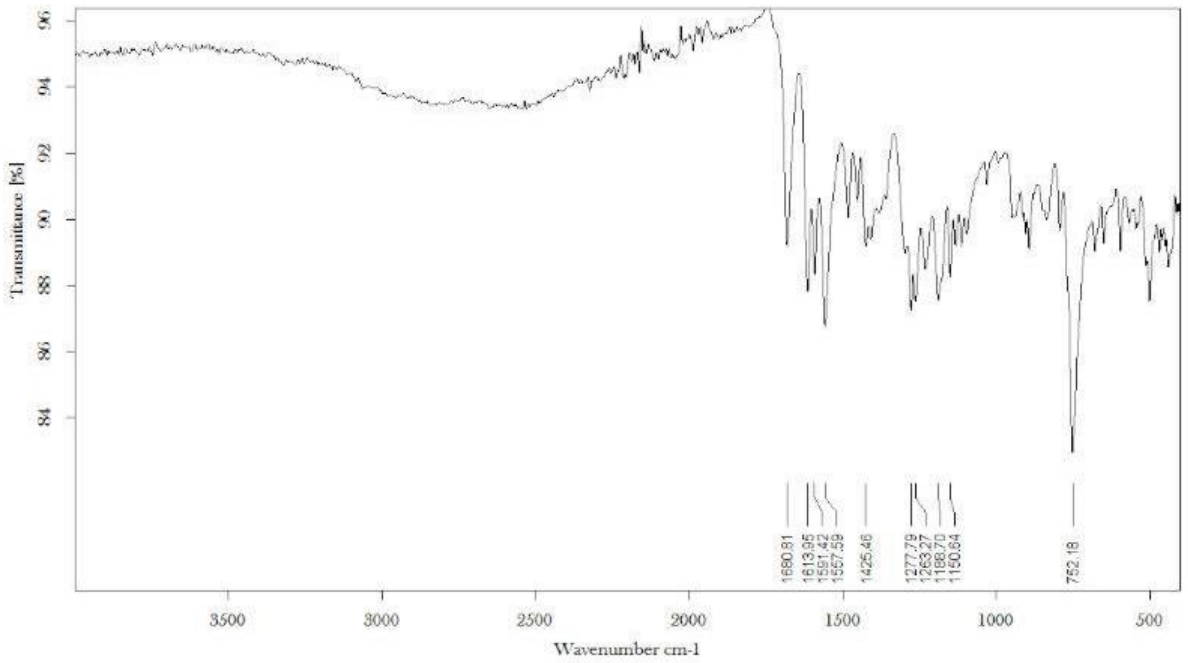
0.88
2.05
4.24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90
88
86
84
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1557.59
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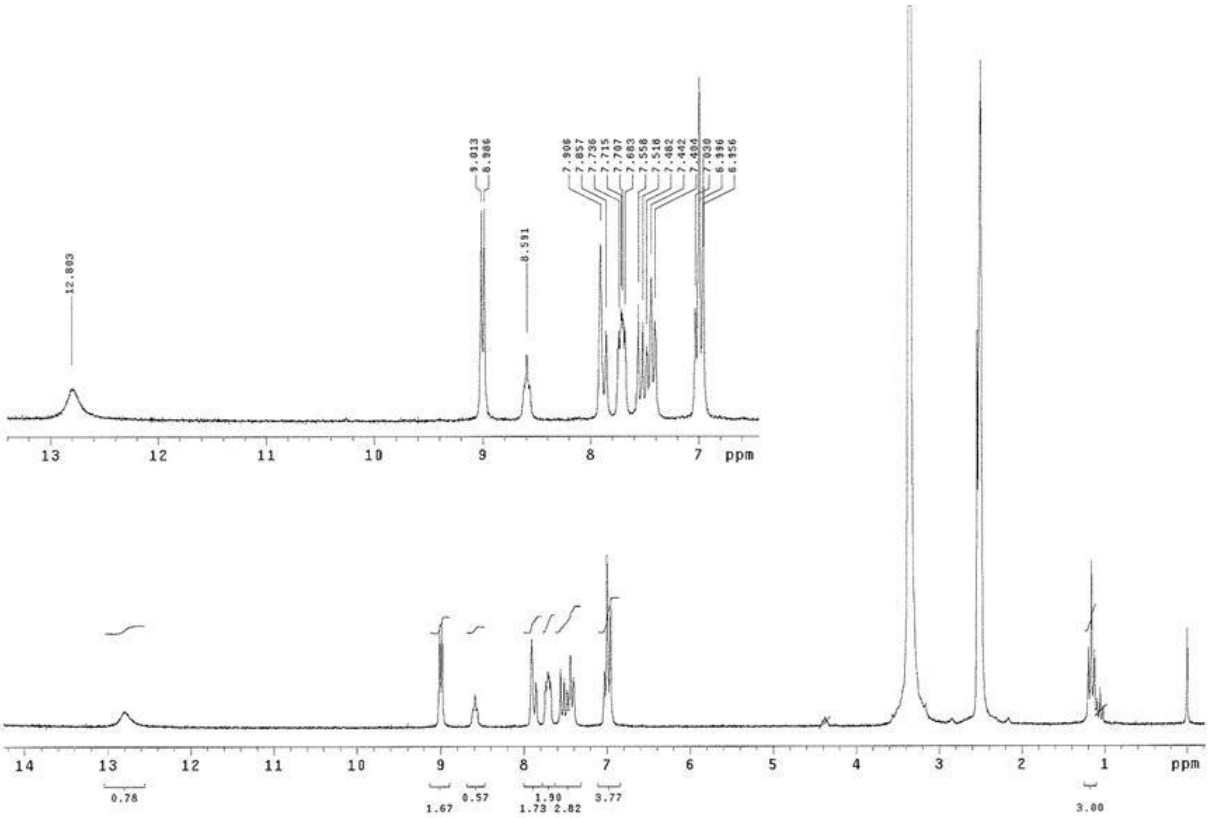
1263.27
1188.70
1150.64
752.18
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

Figure S8: [1]H-NMR and IR-spectrum of ligand *N-ethyl-3,4-disalicylidenediaminobenzamide* (**9**)



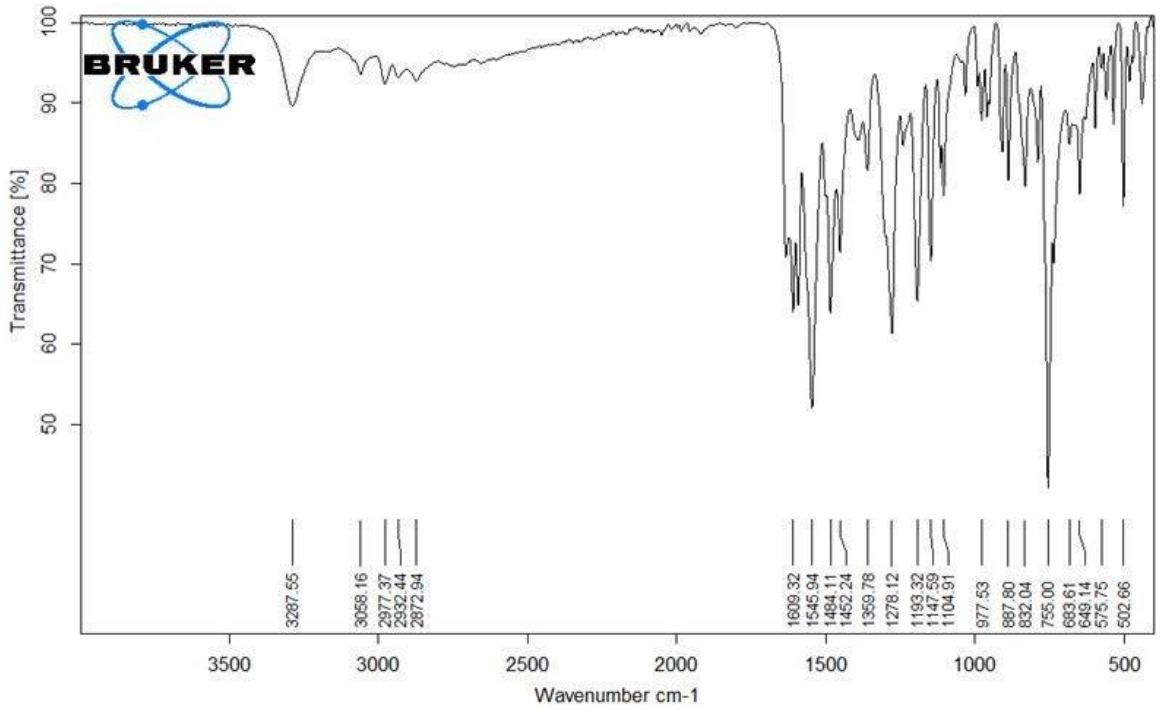
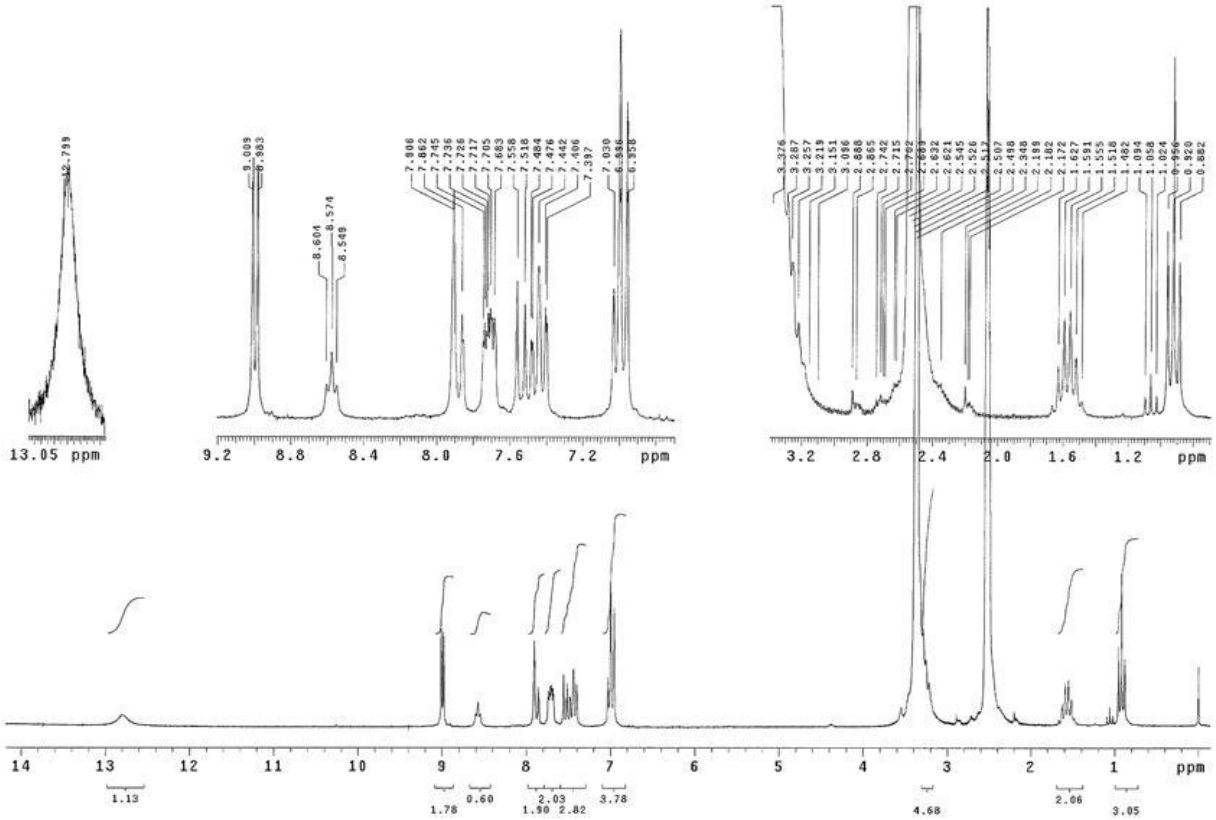


Figure S9: [1]H-NMR and IR-spectrum of ligand *N-propyl-disalicylidene-3,4-diaminobenzamide* (**10**)



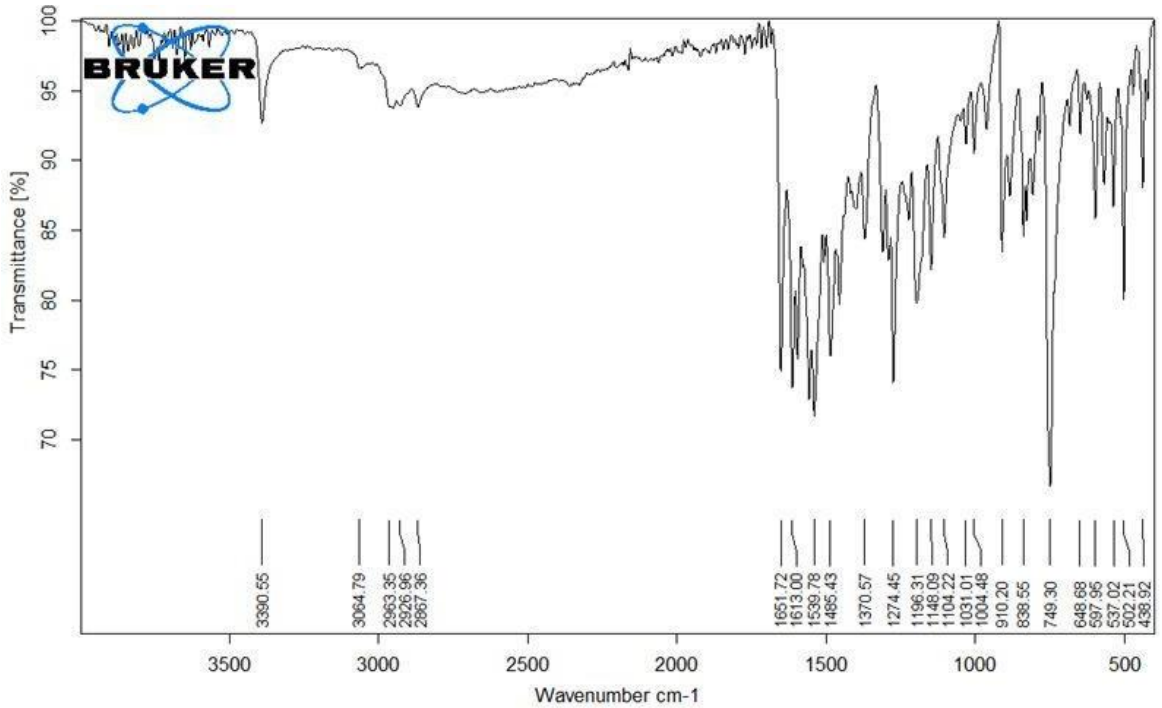
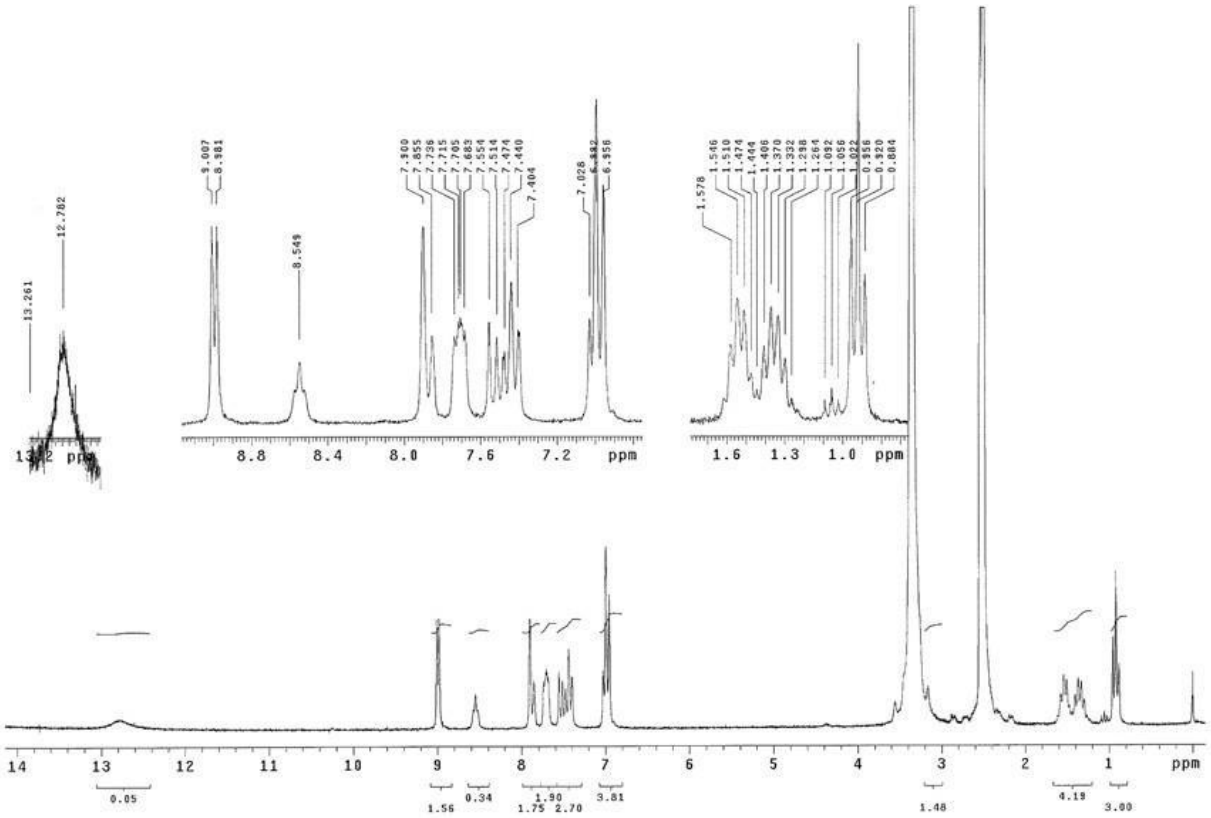


Figure S10: ^{1}H-NMR and IR-spectrum of ligand *N-butyl-3,4-disalicylidenediaminobenzamide* (**11**)



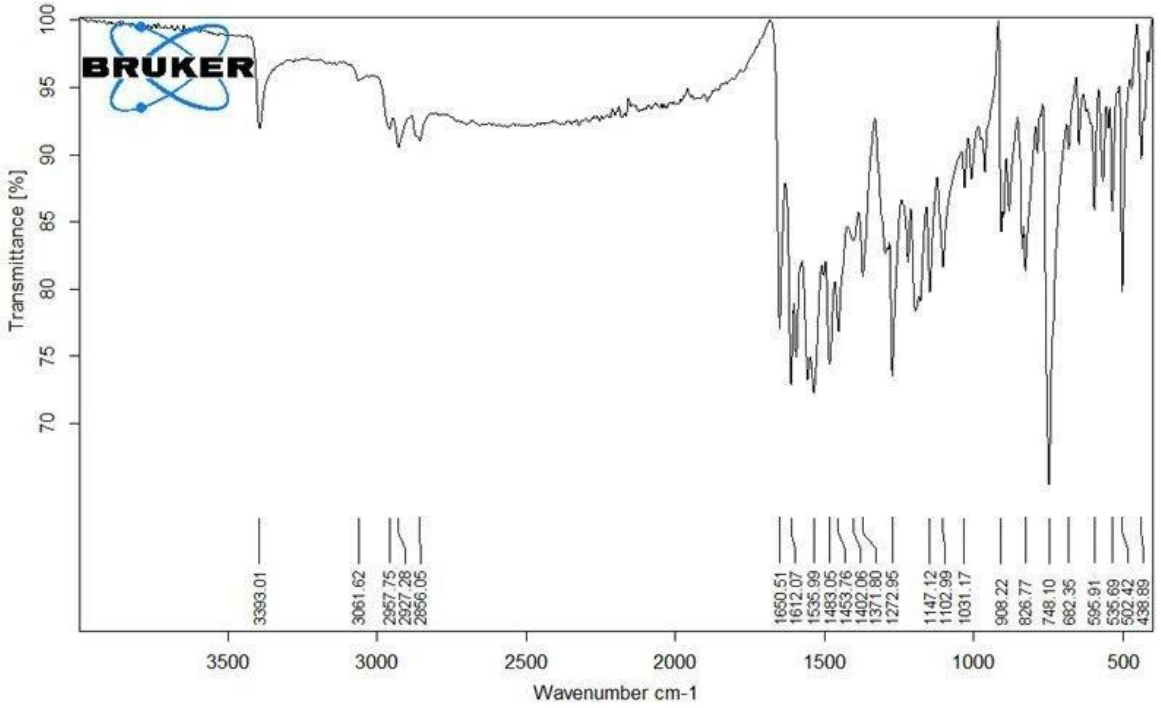


Figure S11: ^{1}H-NMR and IR-spectrum of ligand *ethyl-3,4-disalicylidenediaminobenzoate* (**12**)

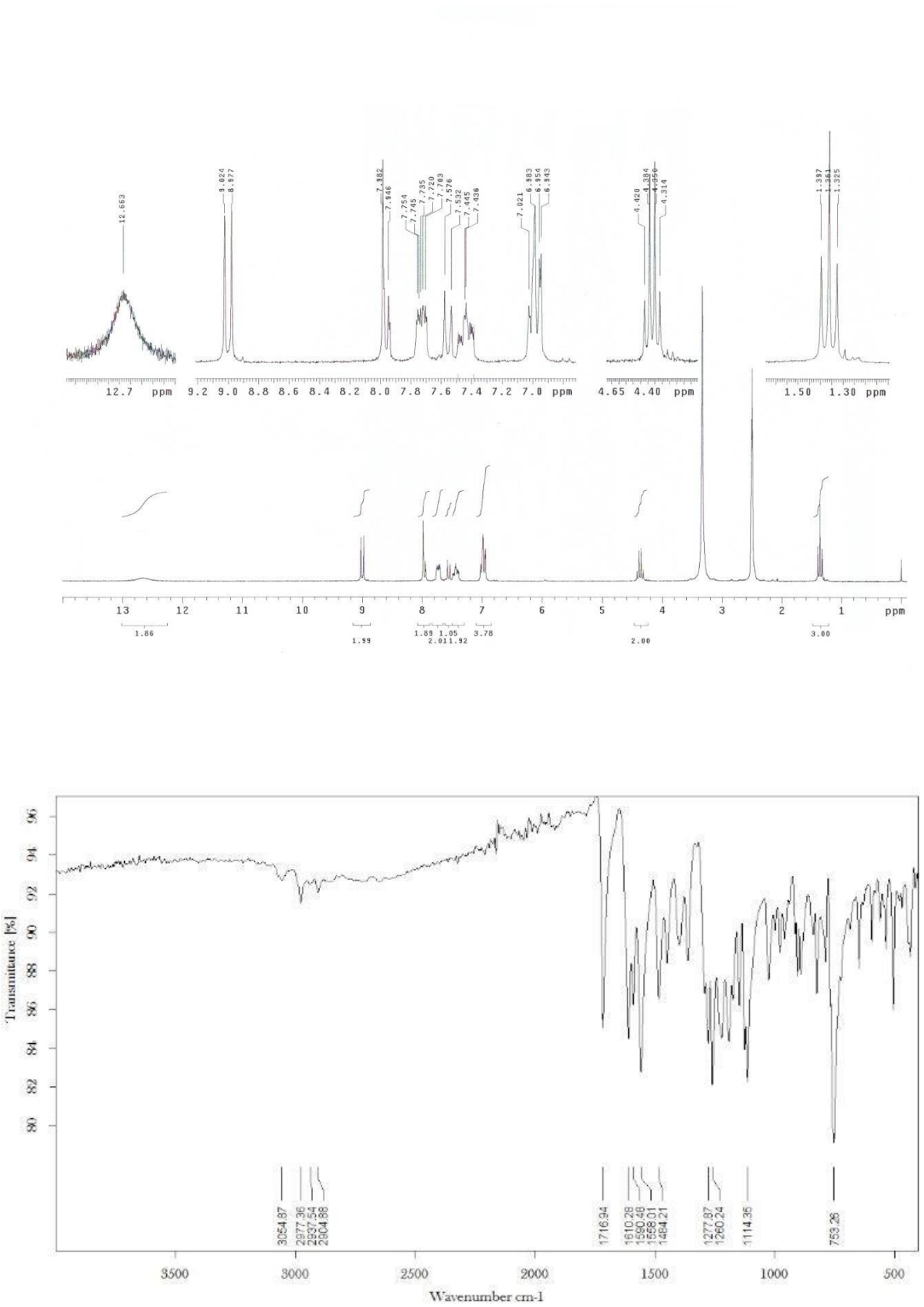
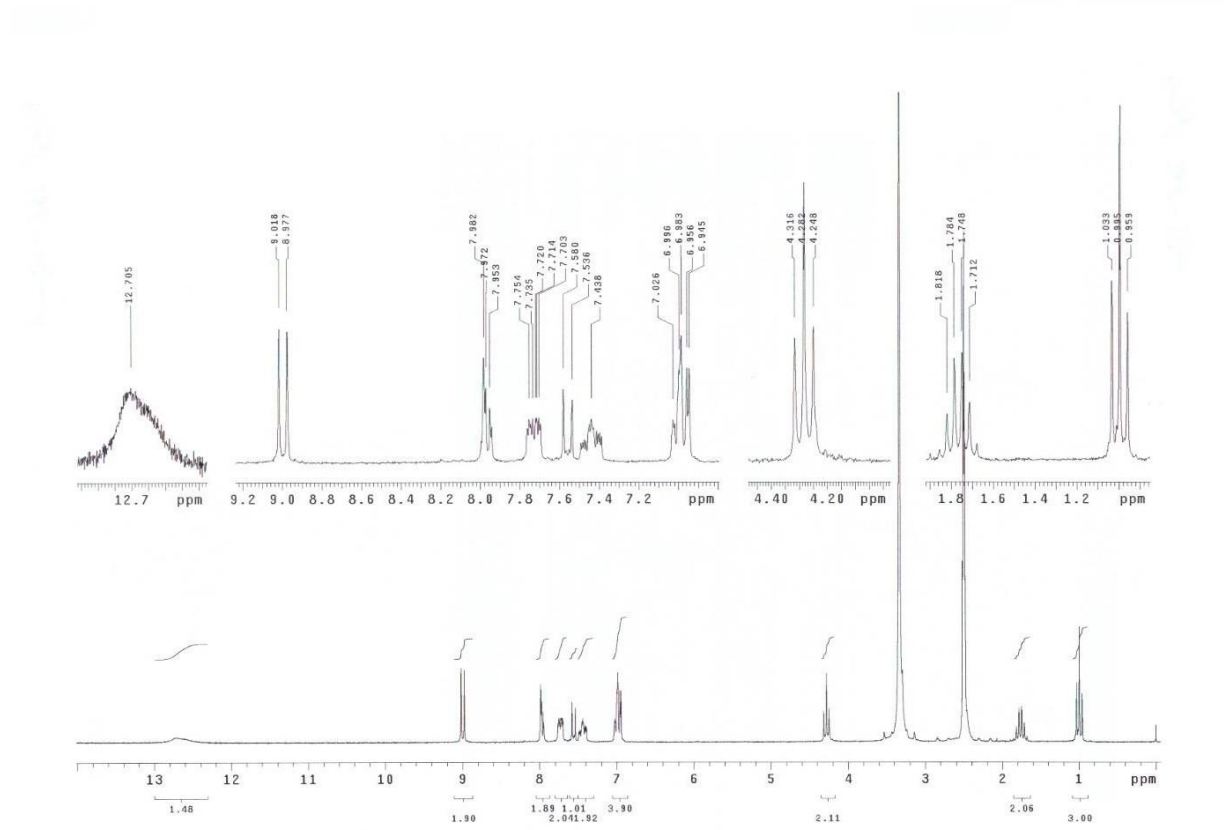


Figure S12: ^{1}H-NMR and IR-spectrum of ligand *propyl-3,4-disalicylidenediaminobenzoate* (**13**)



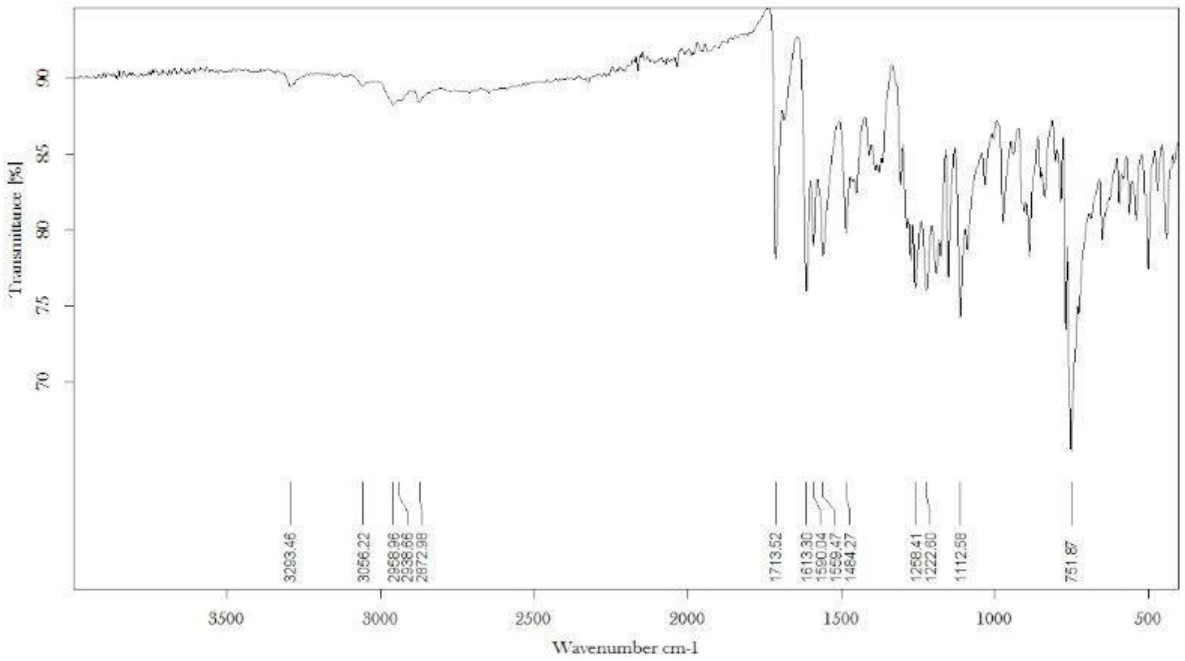
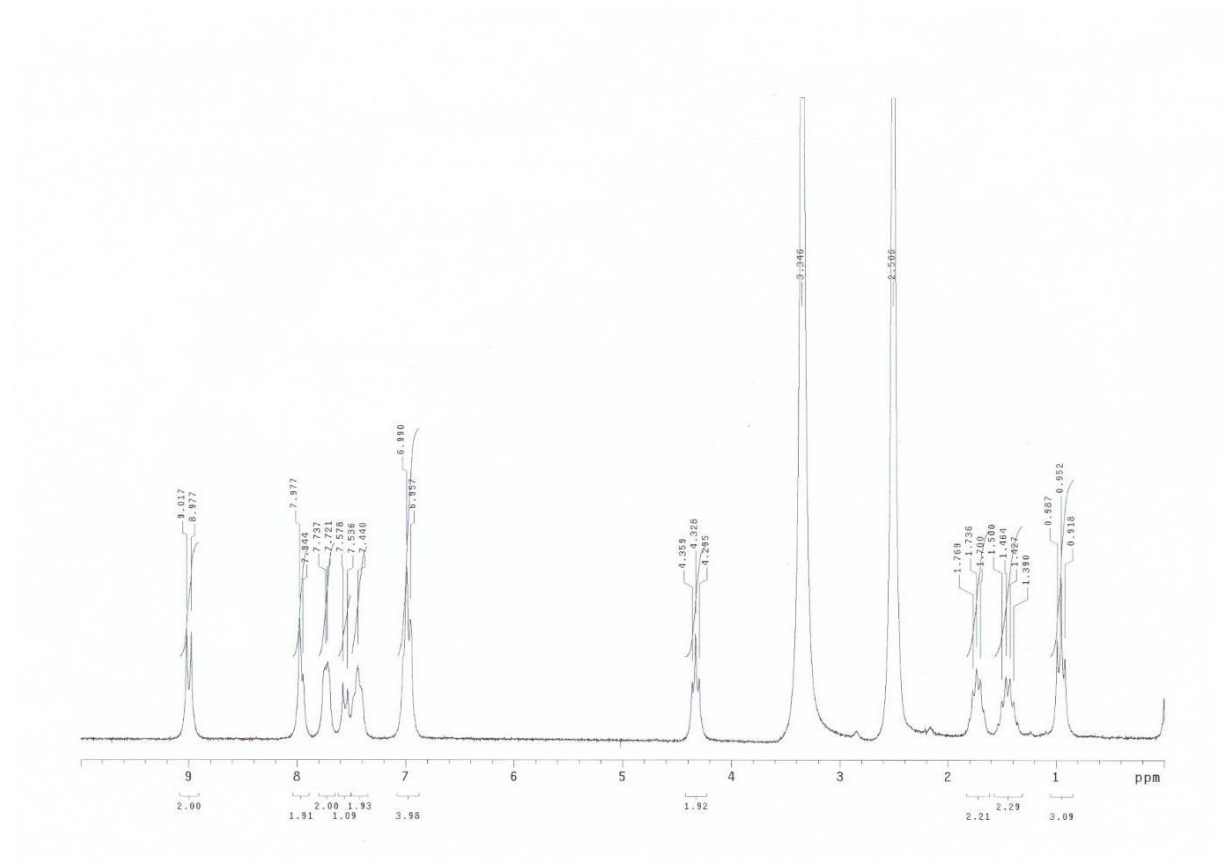


Figure S13: ^{1}H-NMR and IR-spectrum of ligand *butyl-3,4-disalicylidenediaminobenzoate* (**14**)



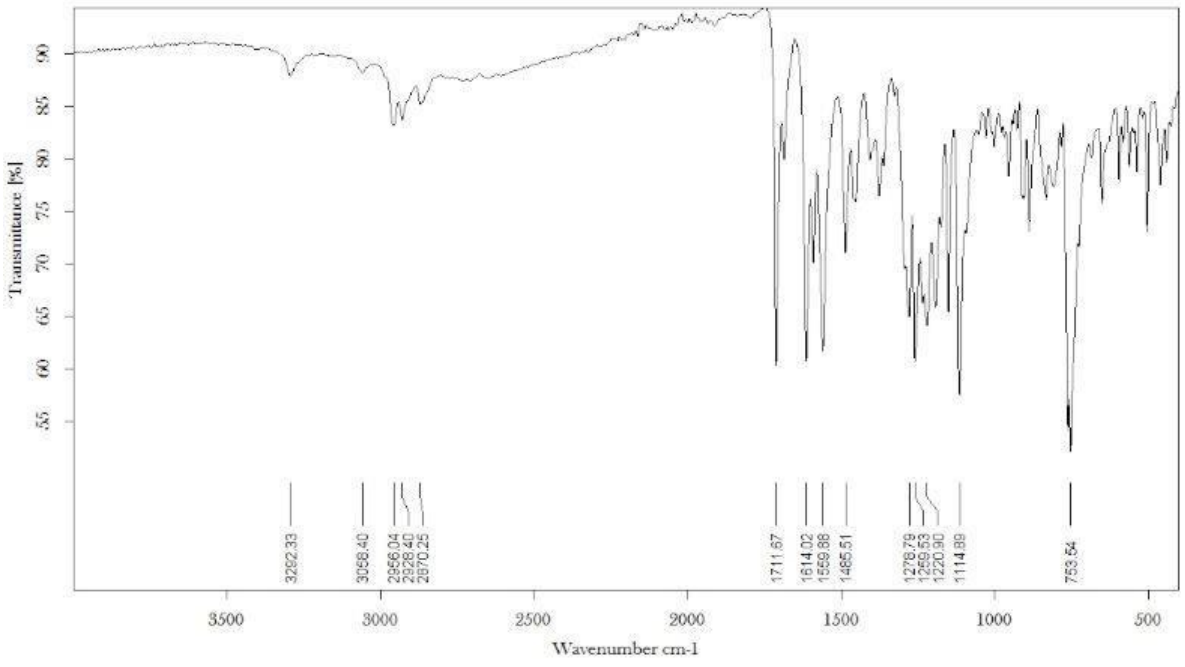
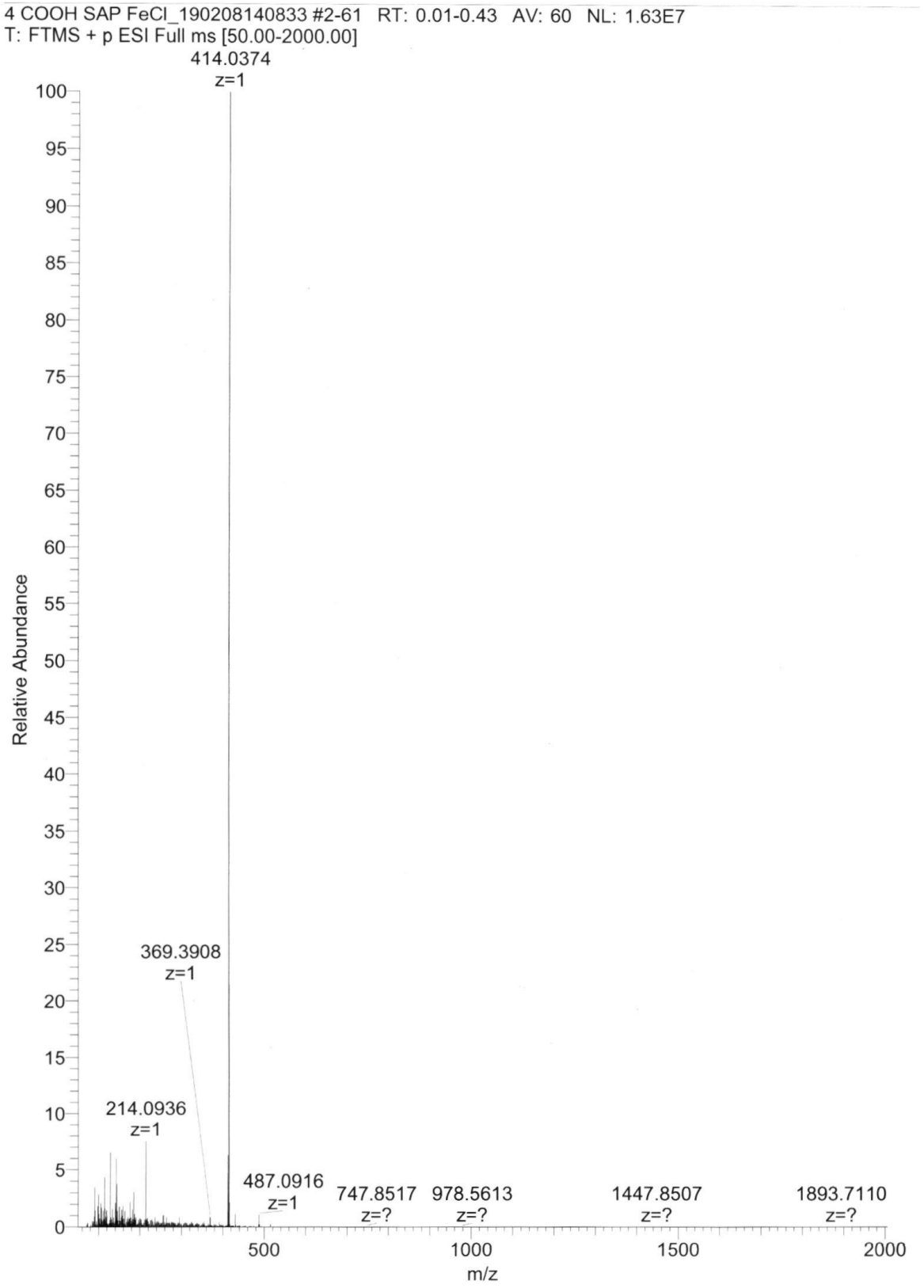
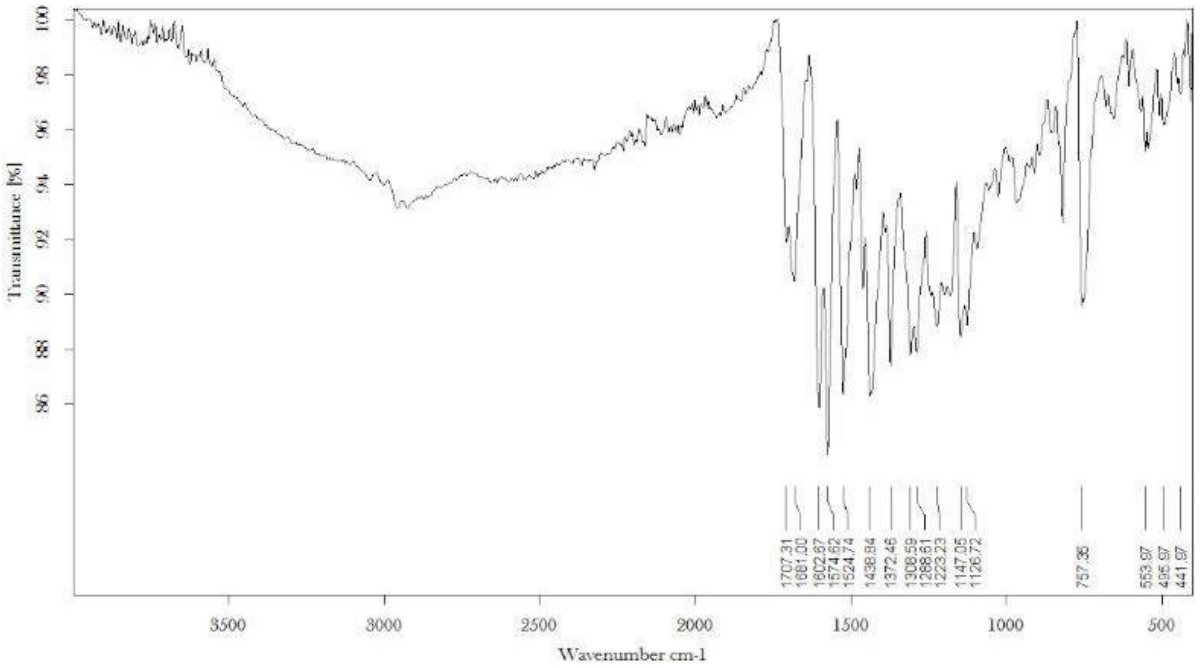


Figure S14: HR MS, IR-spectrum and HPLC chromatogram of complex *chlorido[4-carboxy-1,2-disalicylideneaminobenzene]iron(III)* (**15**)





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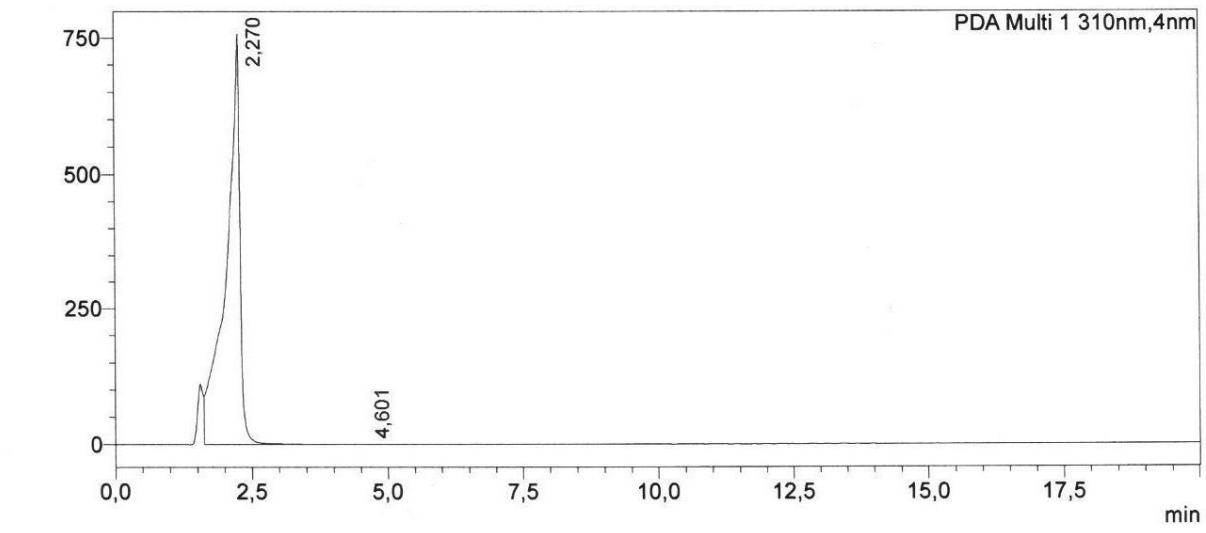

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HPLC chromatogram with mobile phase MeCN/phosphate buffer pH 3 (25/75, v/v)

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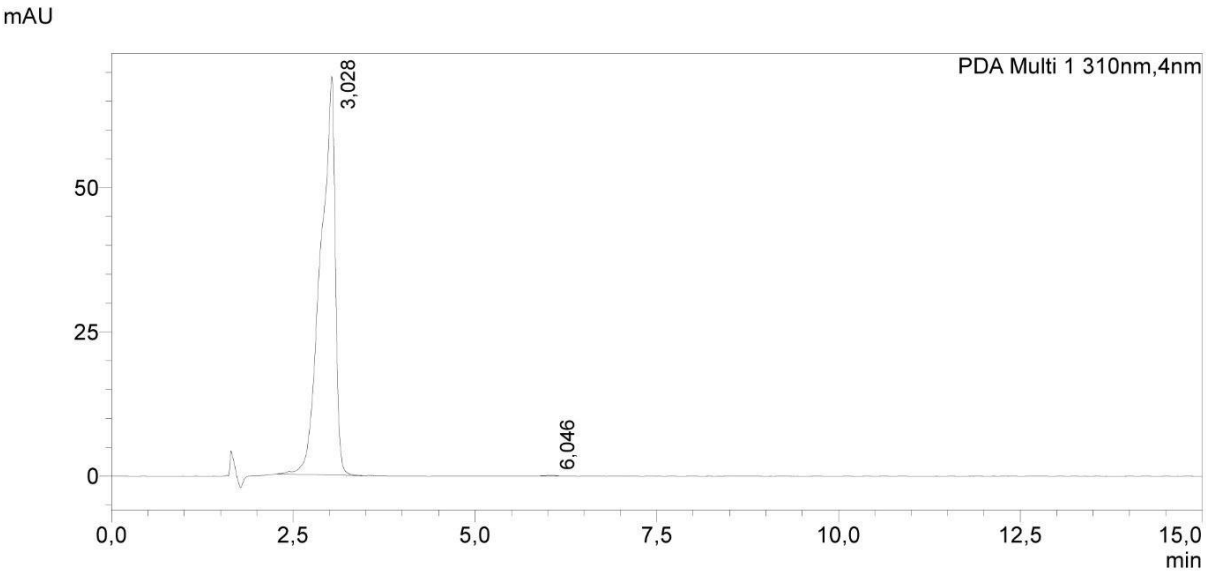

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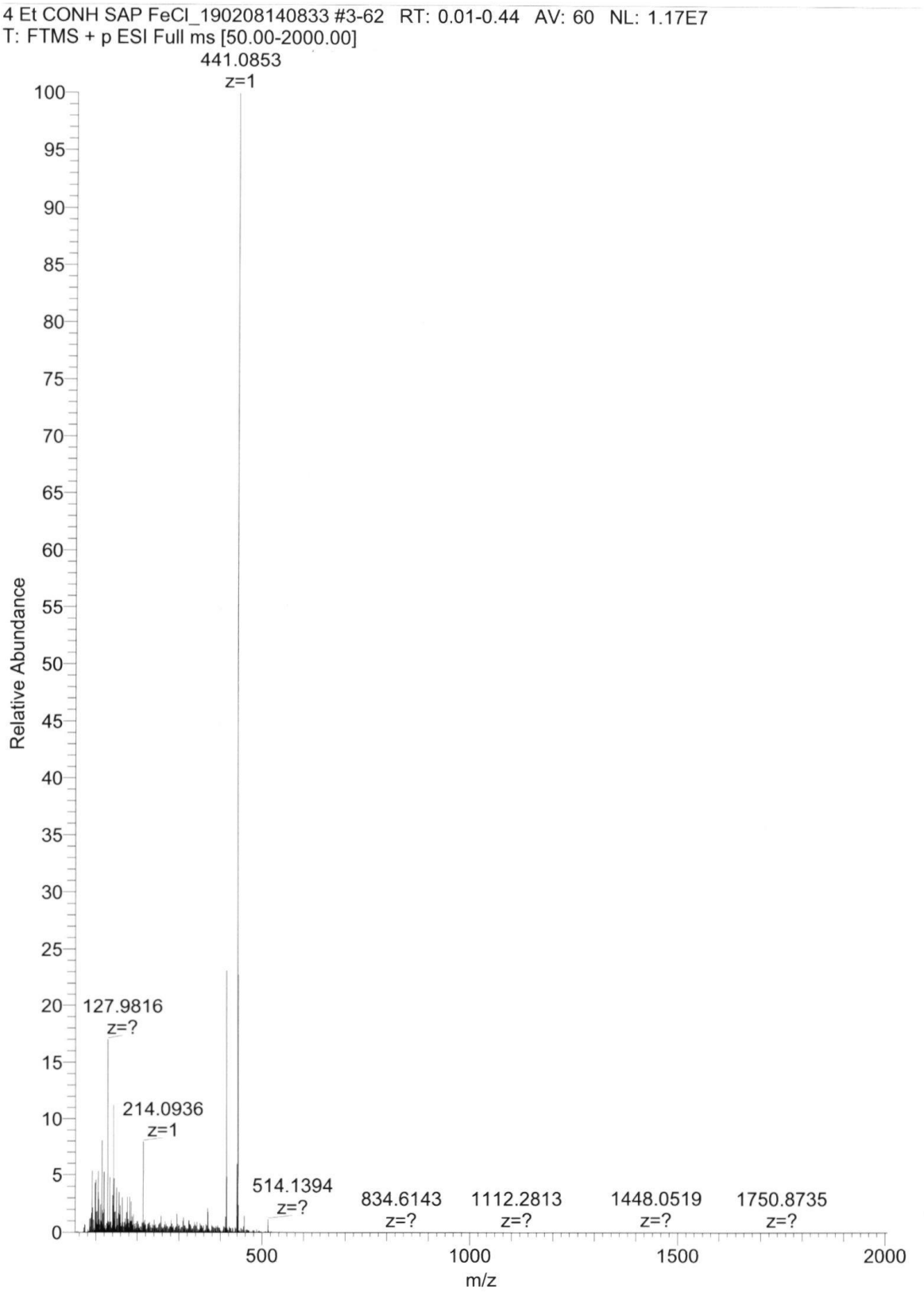
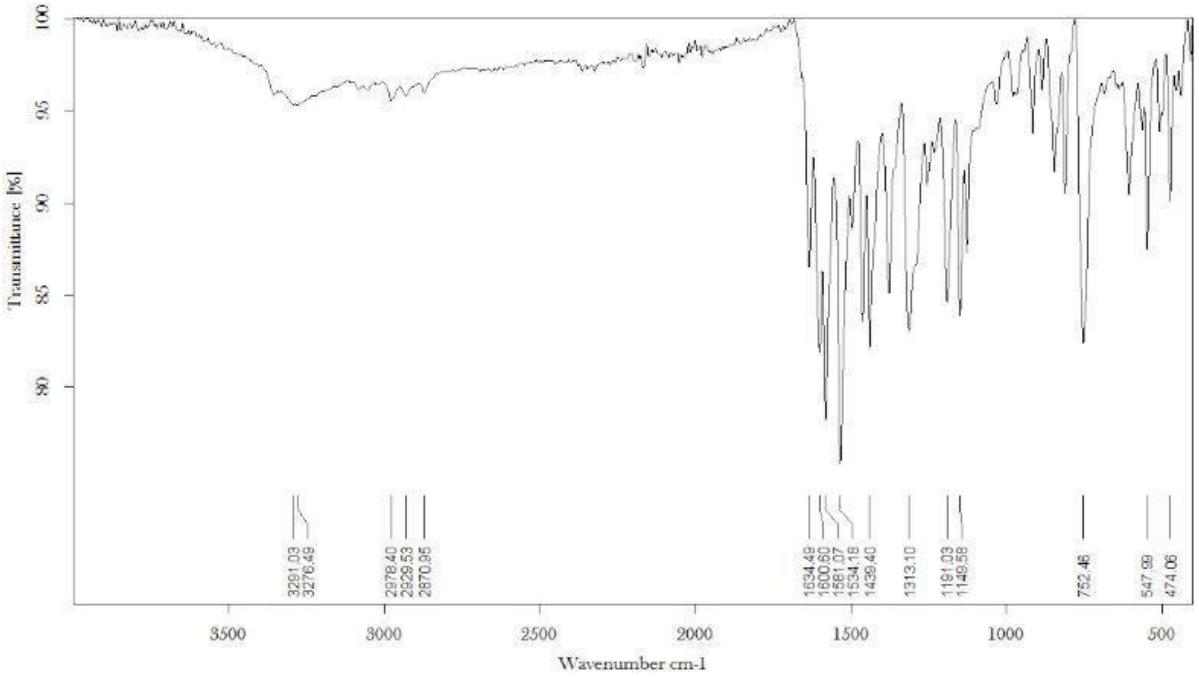
2	6,046	1371	130	0,141		M	
Total		970565	69161				

HPLC chromatogram with mobile phase MeCN/TFA 0.1% (22/78, v/v)

Figure S15: HR MS, IR-spectrum and HPLC chromatogram of complex *chlorido[4-ethylaminocarbonyl-1,2-disalicylideneaminobenzene]iron(III)* (**16**)





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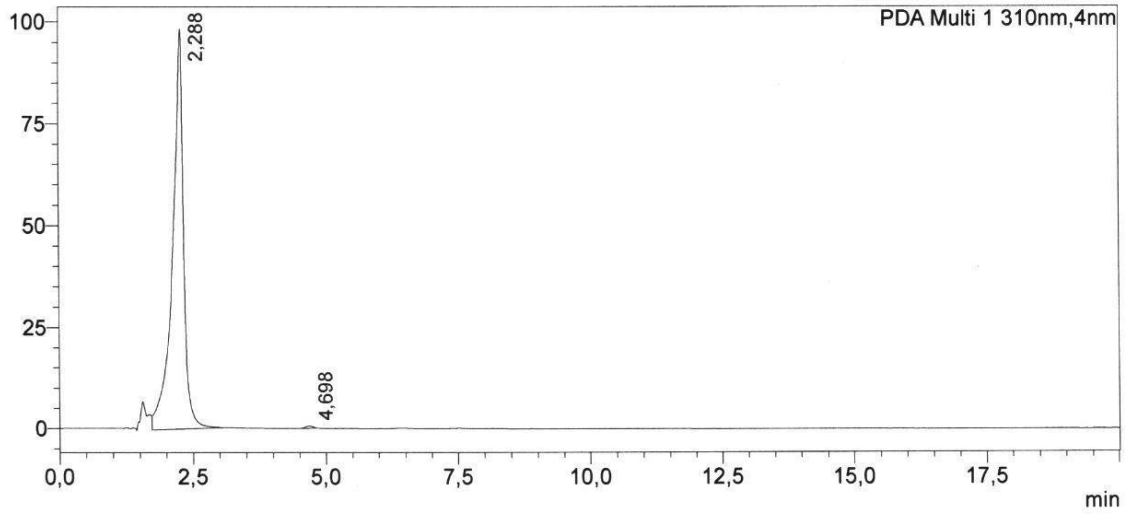

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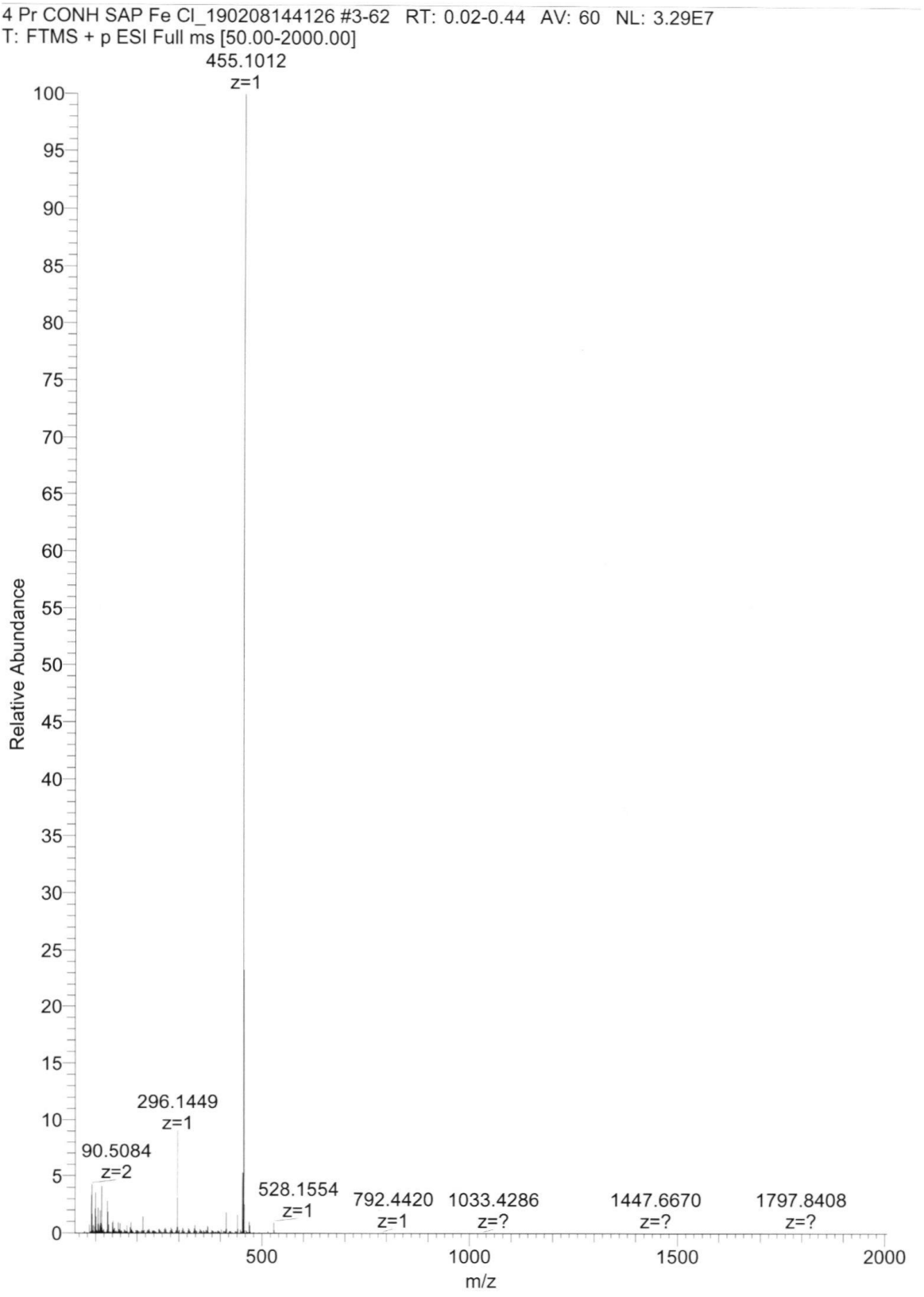
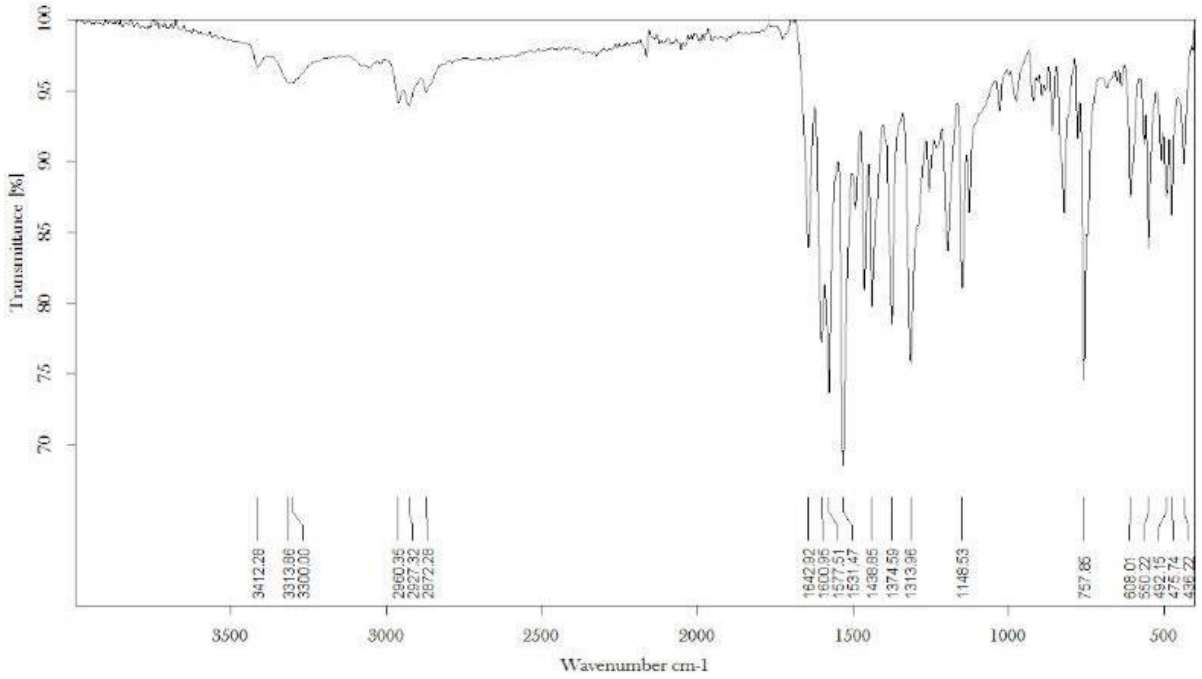
Total		1418780	98693				

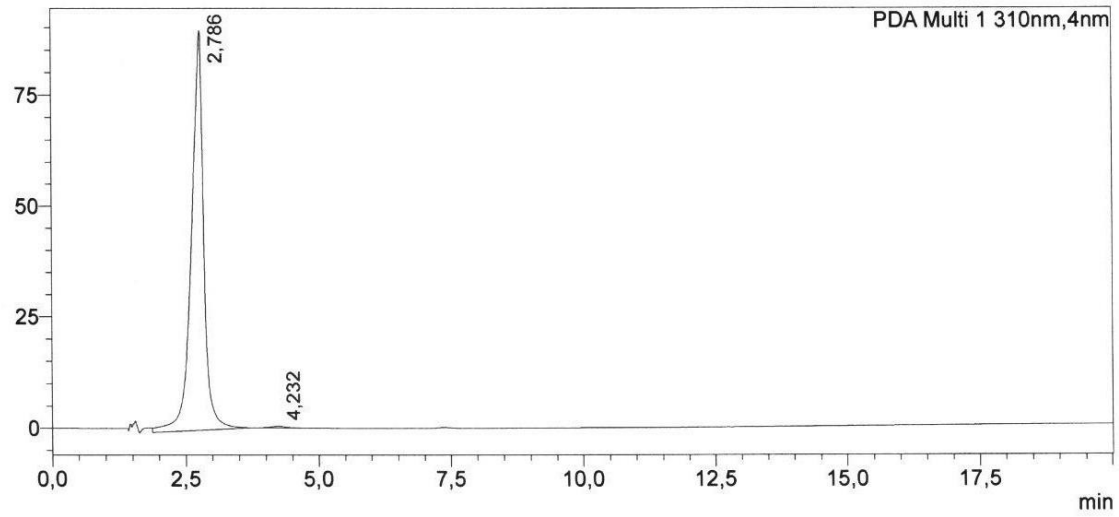
Figure S16: HR MS, IR-spectrum and HPLC chromatogram of complex *chlorido[4-propylaminocarbonyl-1,2-disalicylideneaminobenzene)]iron(III)* (**17**)





<Chromatogram>

mAU

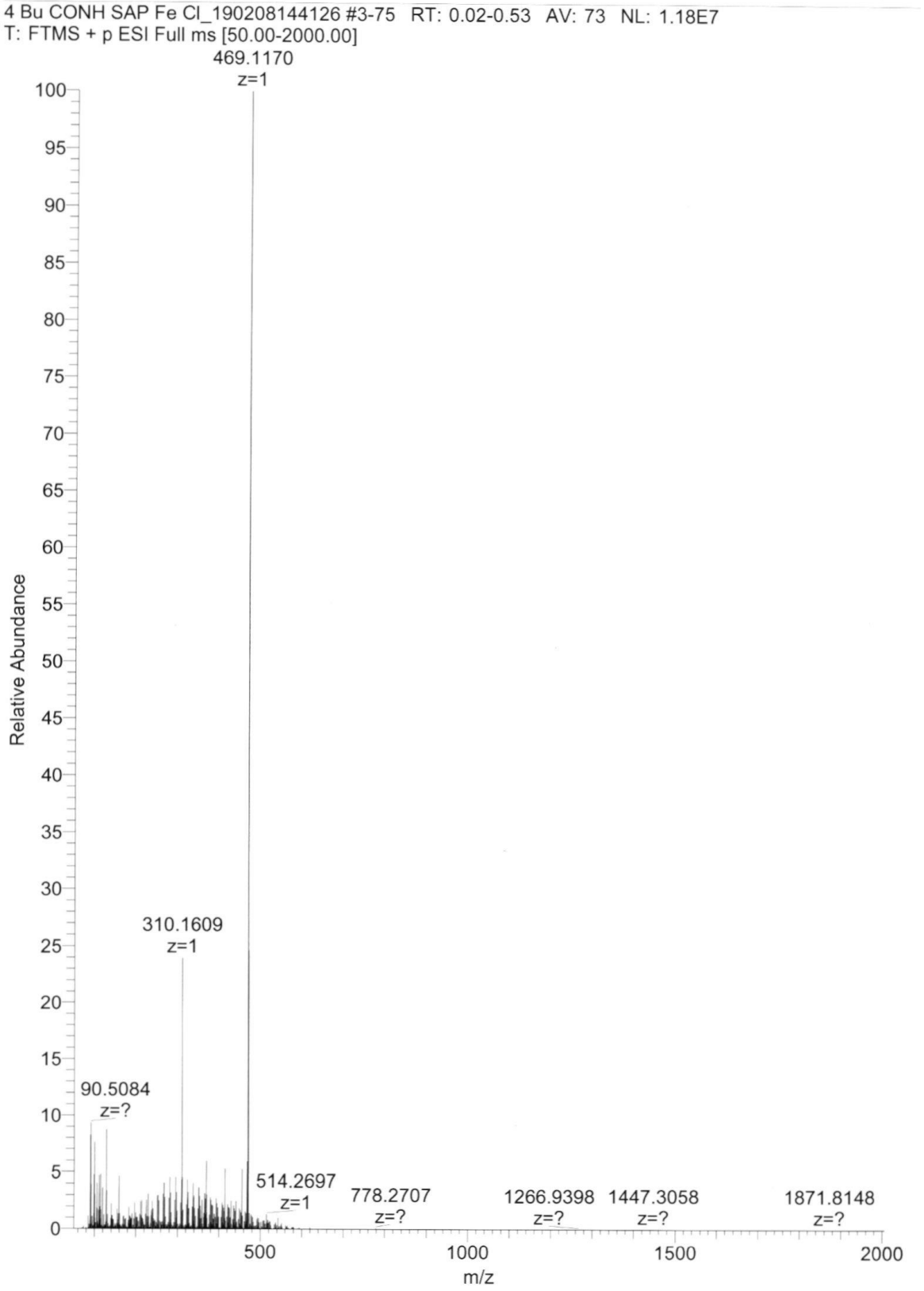


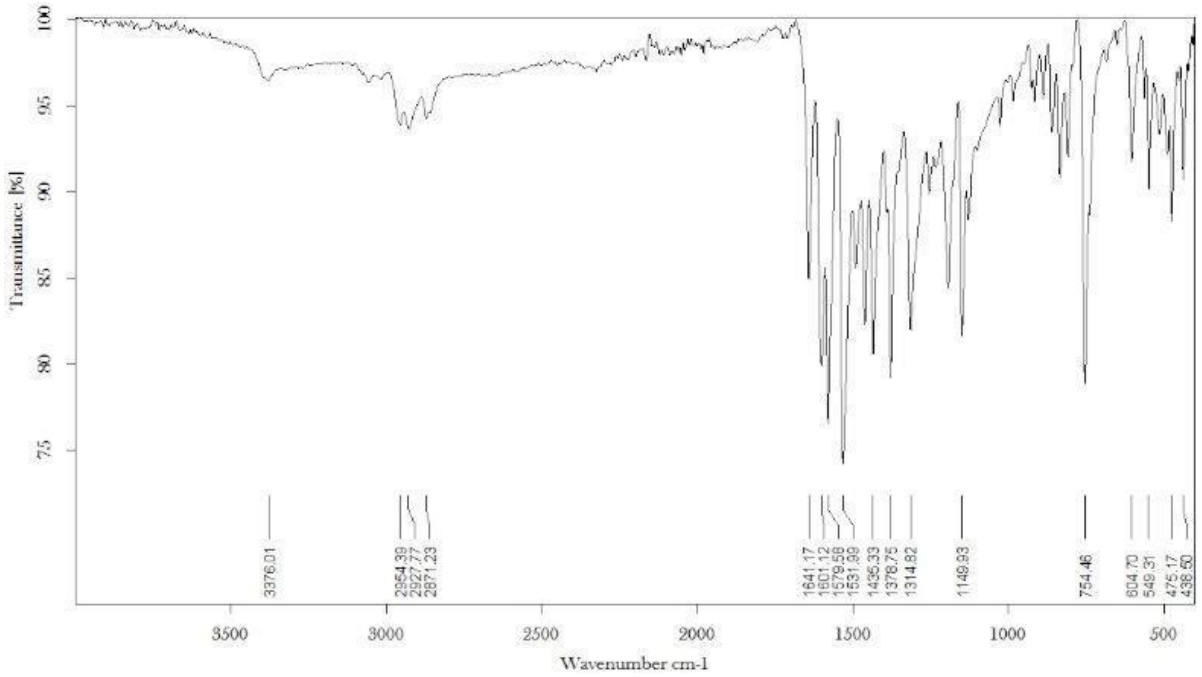
<Peak Table>

PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2,786	1448432	89512	99,469			
2	4,232	7733	427	0,531			
Total		1456166	89938				

Figure S17: HR MS, IR-spectrum and HPLC chromatogram of complex *chlorido[4-butylaminocarbonyl-1,2-disalicylideneaminobenzene)]iron(III)* (**18**)





<Chromatogram>

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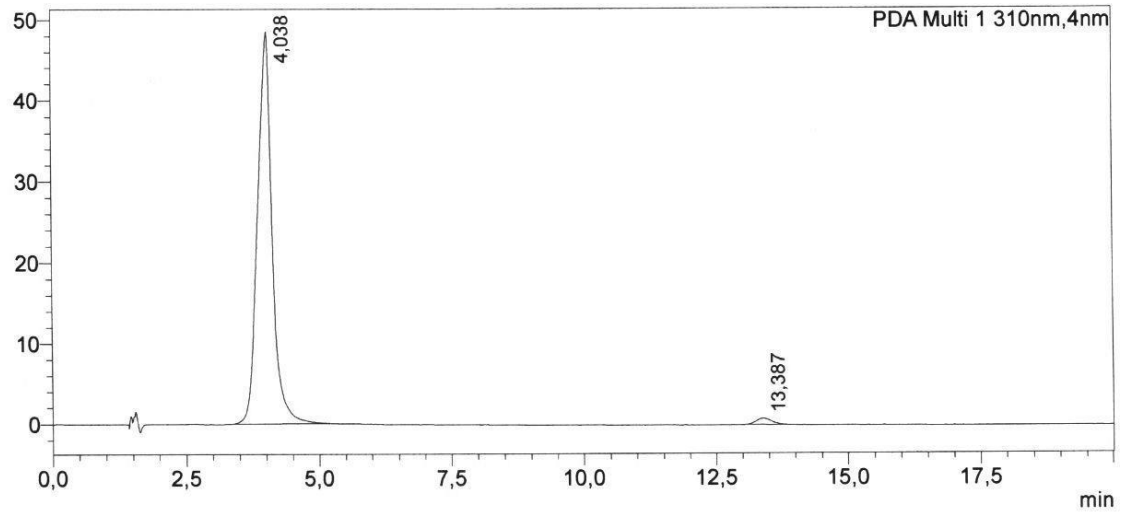
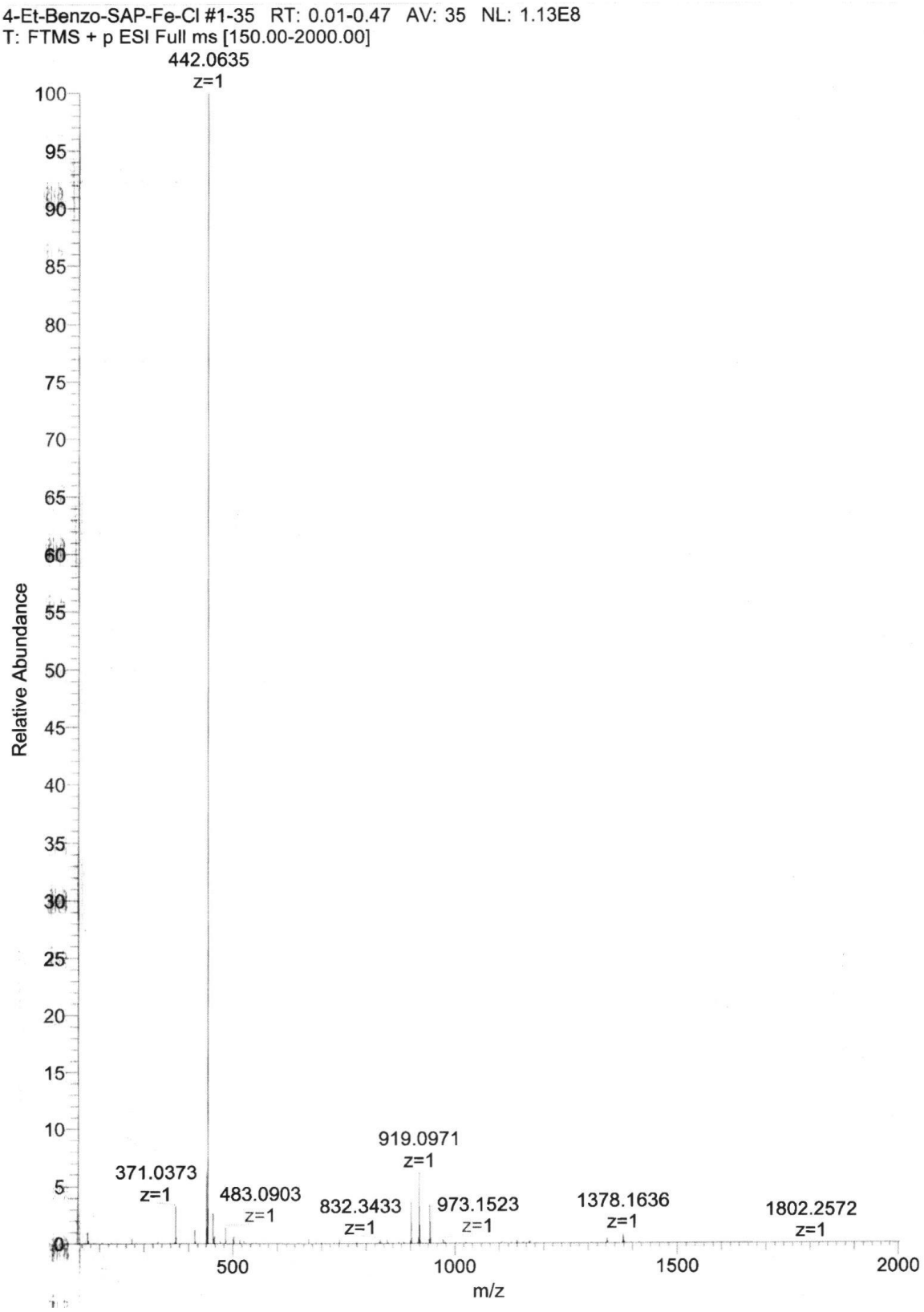


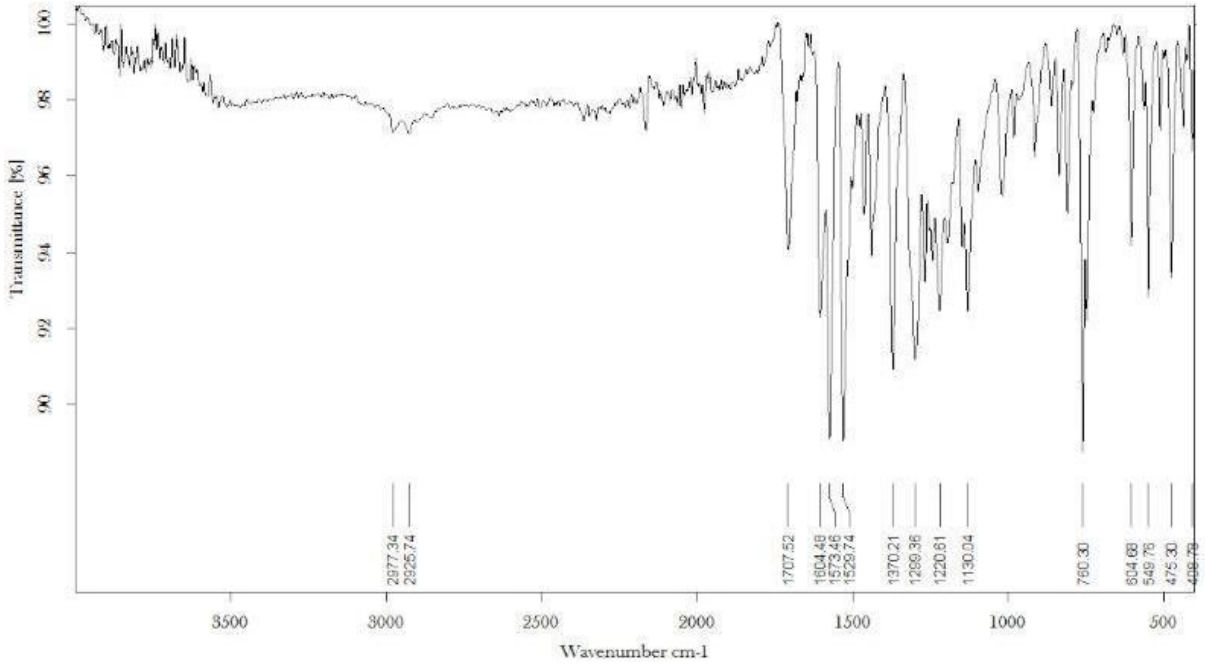
<Peak Table>

PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4,038	922119	48512	98,378			
2	13,387	15204	739	1,622			
Total		937322	49251				

Figure S18: HR MS, IR-spectrum and HPLC chromatogram of complex *chlorido[4-ethoxycarbonyl-1,2-disalicylideneaminobenzene]iron(III)* (**19**)





<Chromatogram>

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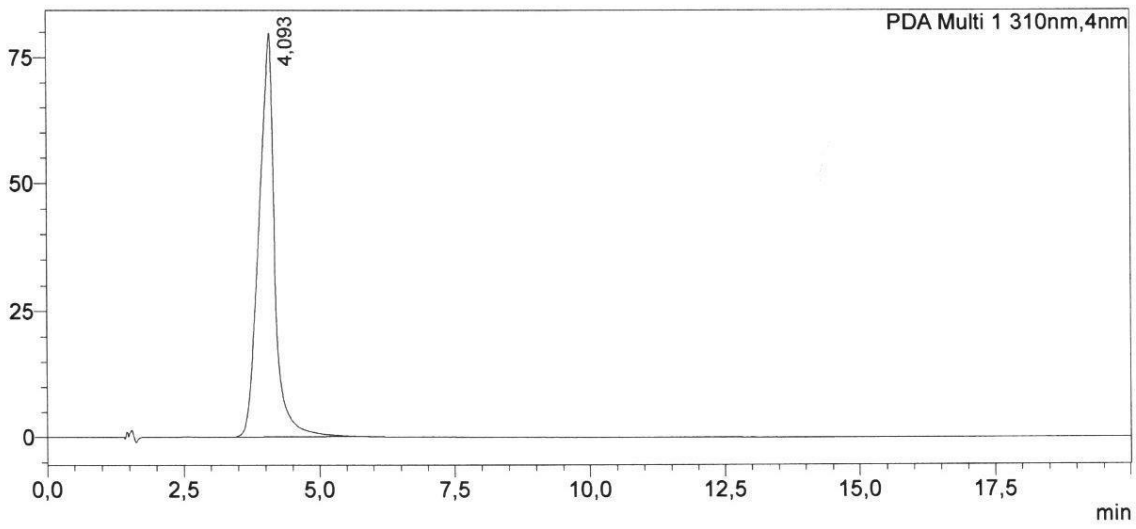


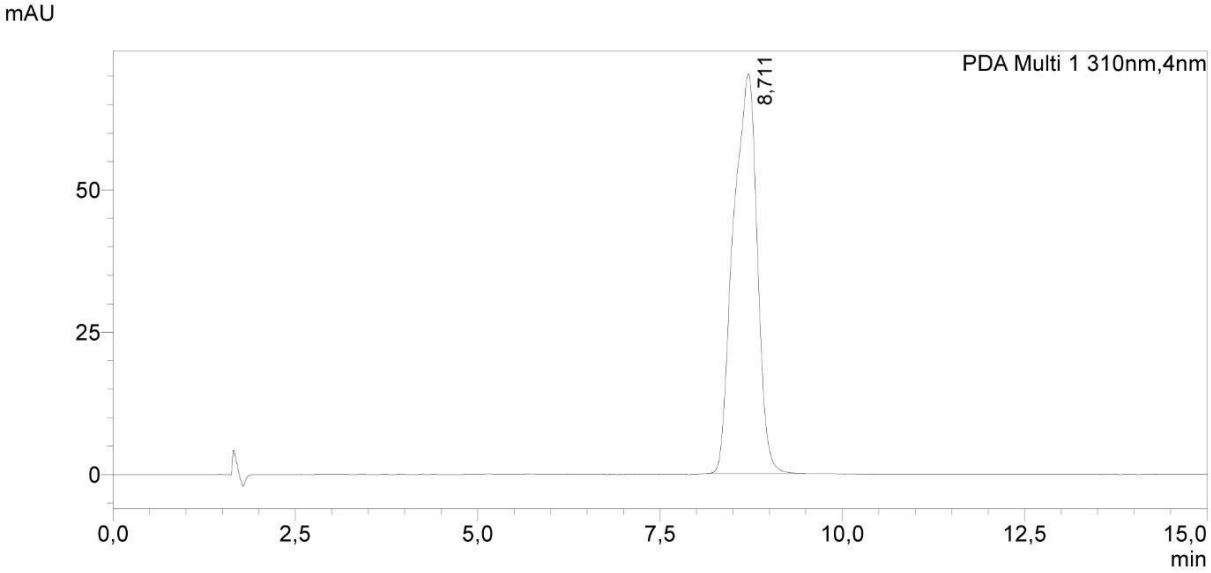
<Peak Table>

PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4,093	1608295	79677	100,000			
Total		1608295	79677				

HPLC chromatogram with mobile phase MeCN/phosphate buffer pH 3 (25/75, v/v)

<Chromatogram>



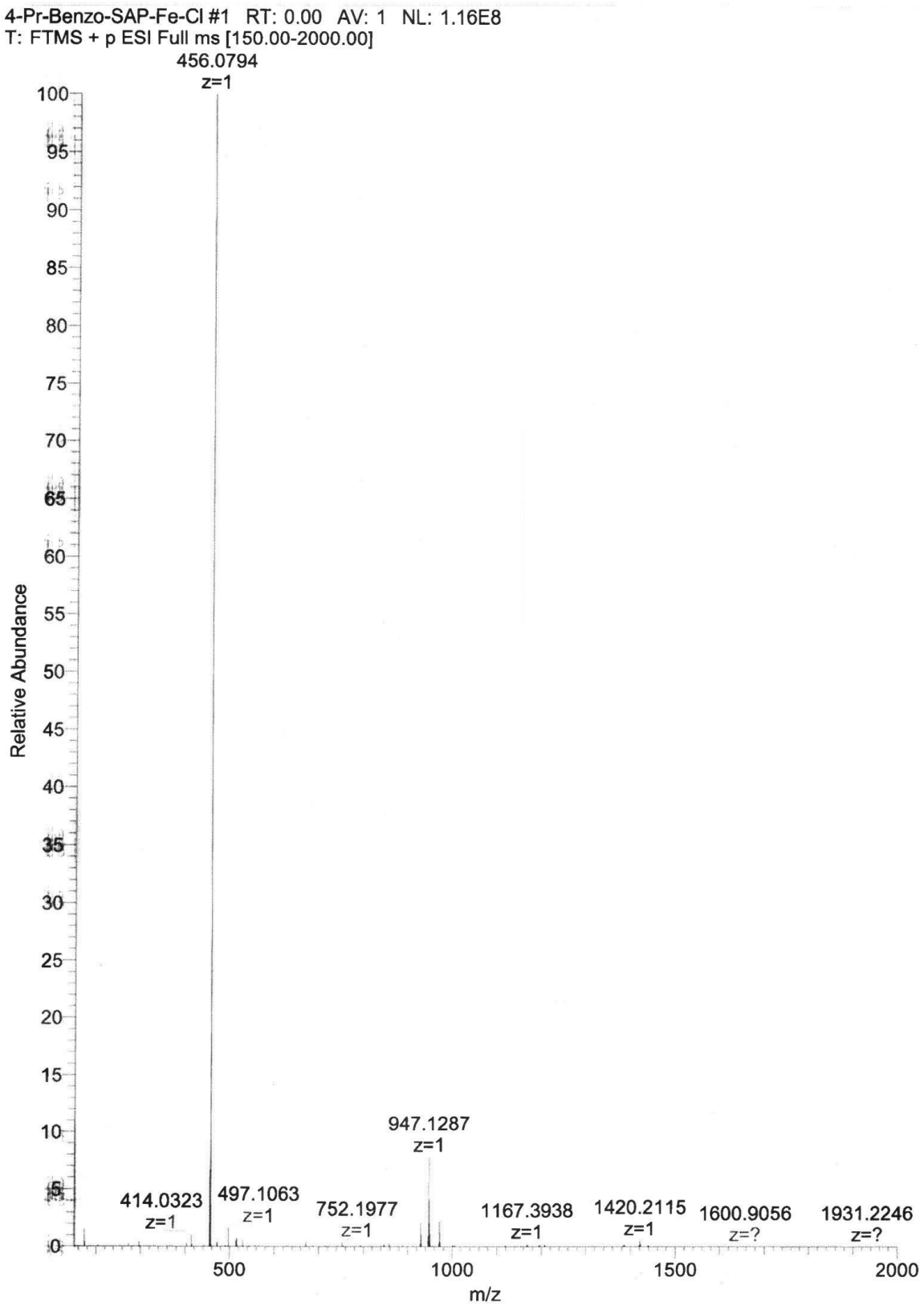
<Peak Table>

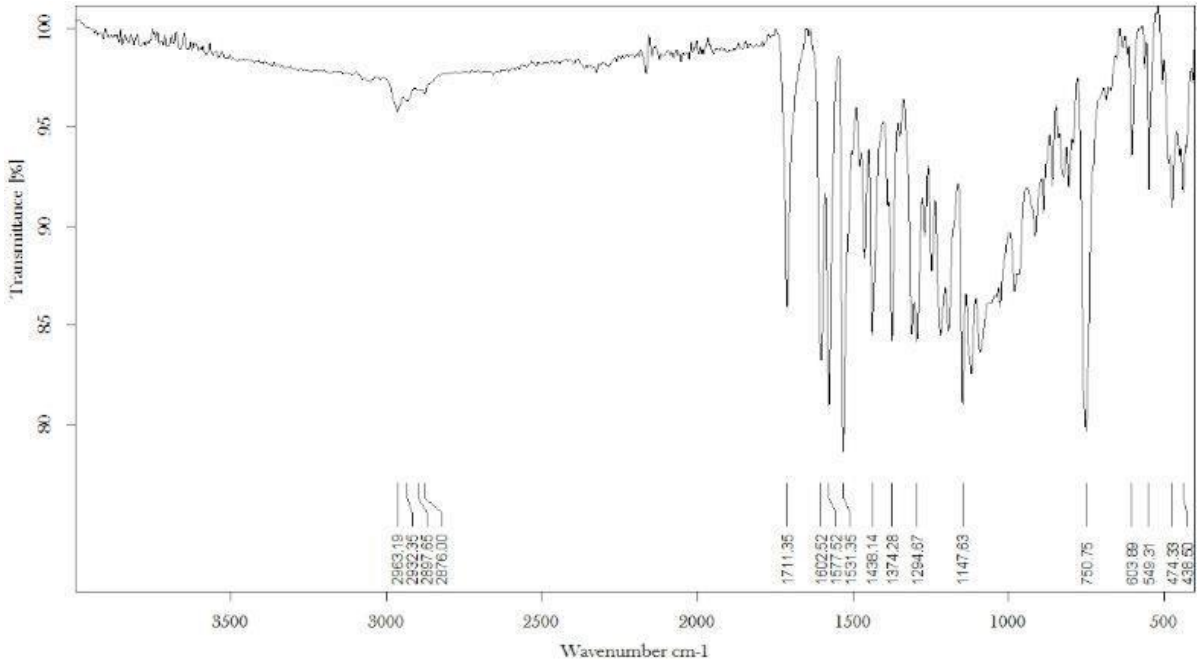
PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8,711	1603122	70293	100,000			
Total		1603122	70293				

HPLC chromatogram with mobile phase MeCN/TFA 0.1% (22/78, v/v)

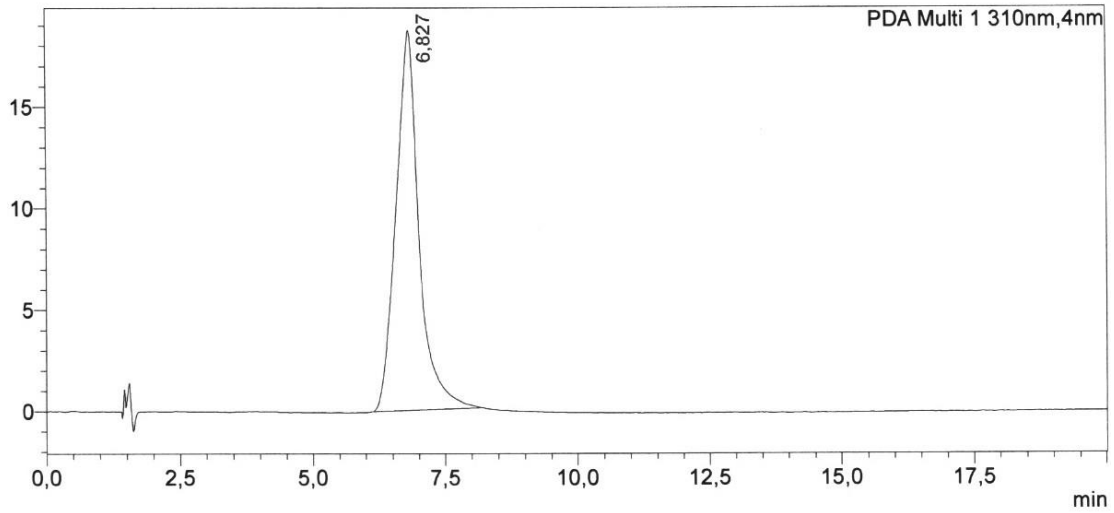
Figure S19: HR MS, IR-spectrum and HPLC chromatogram of complex *chlorido[4-propoxycarbonyl-1,2-disalicylideneaminobenzene]iron(III)* (**20**)





<Chromatogram>

mAU

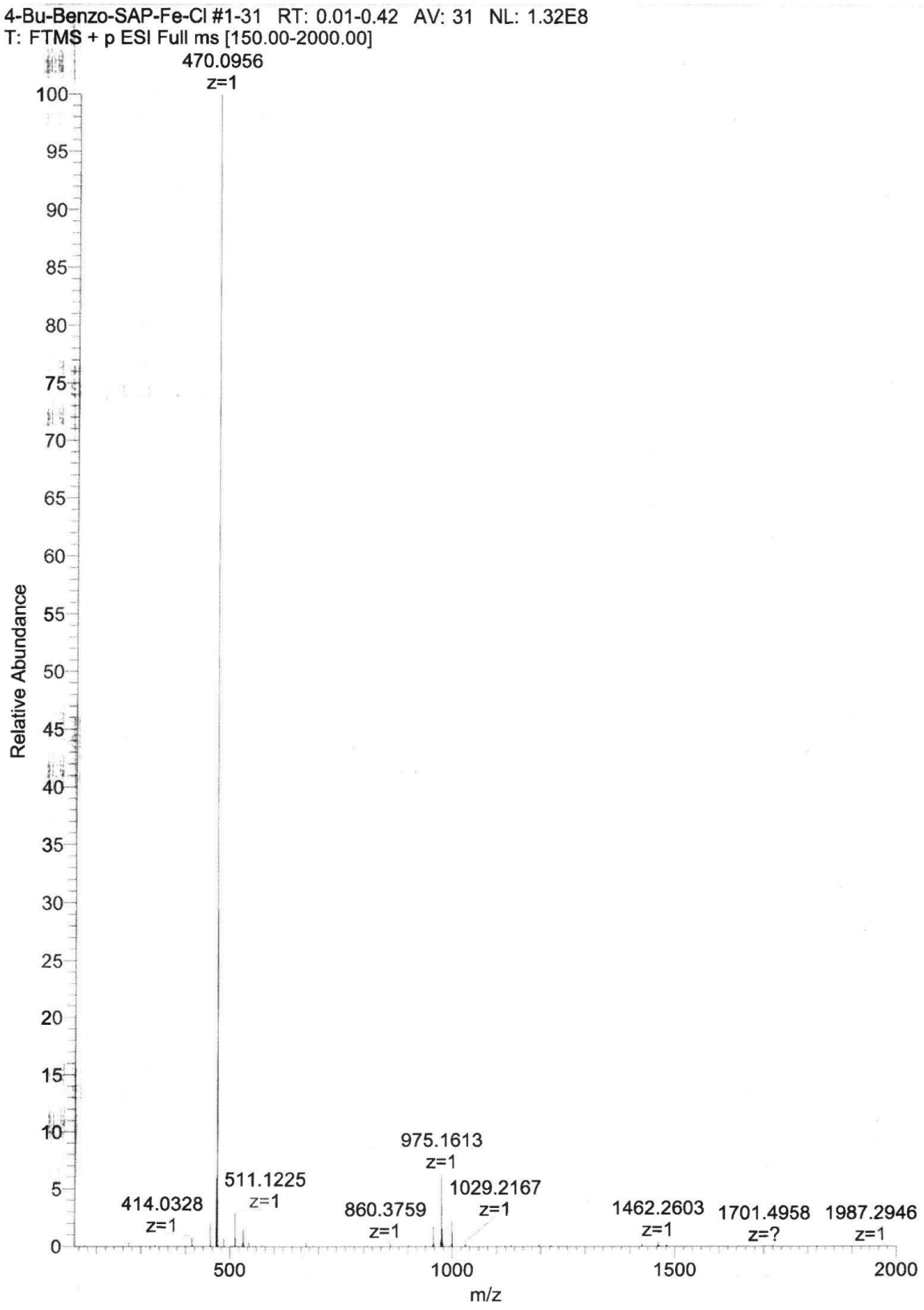


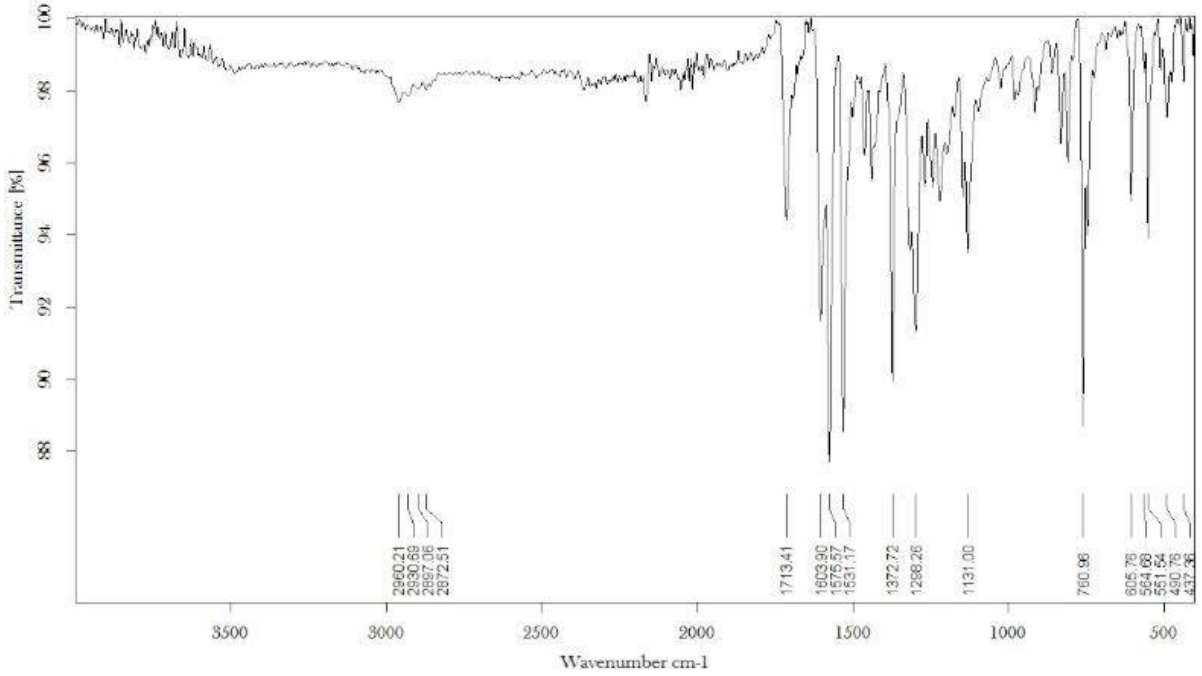
<Peak Table>

PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	6,827	567646	18726	100,000			
Total		567646	18726				

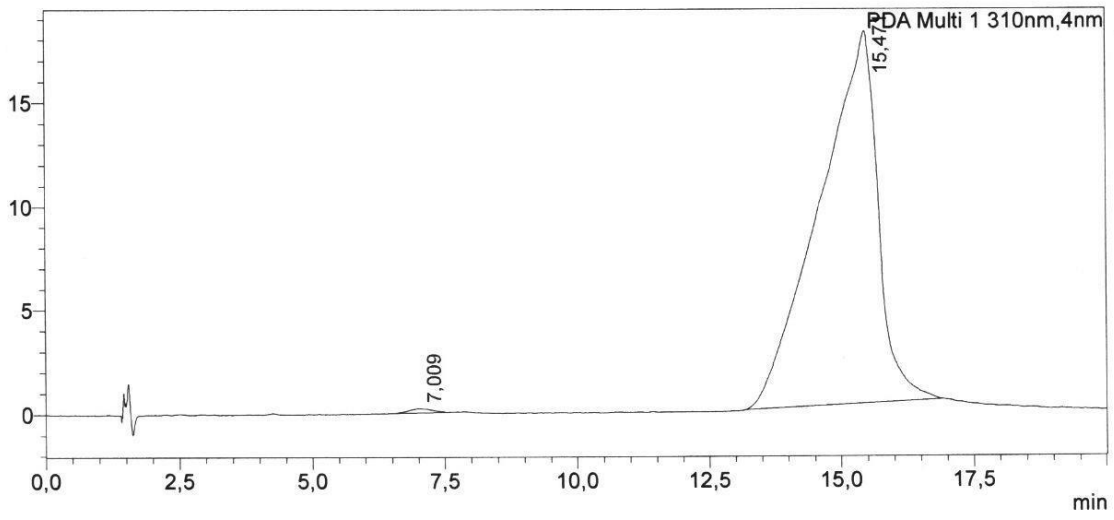
Figure S20: HR MS, IR-spectrum and HPLC chromatogram of complex *chlorido[4-butoxycarbonyl-1,2-disalicylideneaminobenzene]iron(III)* (**21**)





<Chromatogram>

mAU



<Peak Table>

PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	7,009	5948	192	0,431		M	
2	15,471	1375107	17909	99,569			
Total		1381055	18101				

Figure S21: Antimetabolic activity of **15** plus Fer-1 or Nec-1

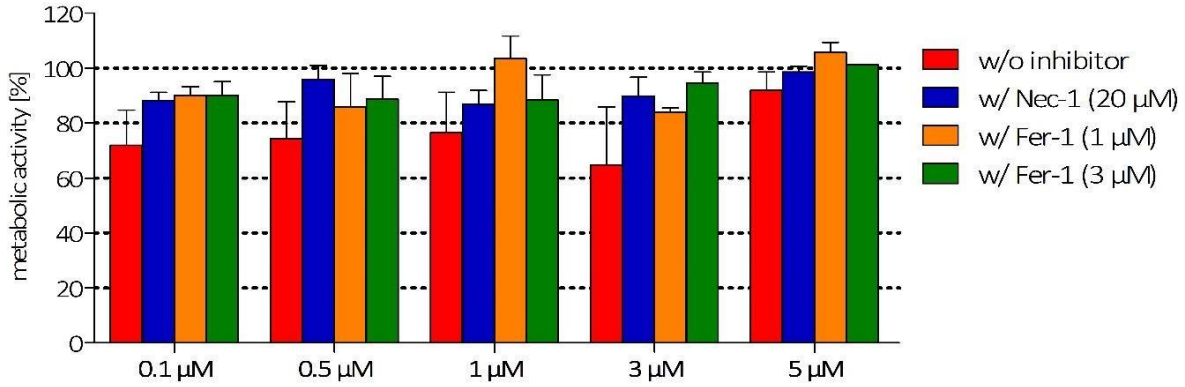


Figure S22: Antimetabolic activity of **16** plus Fer-1 or Nec-1

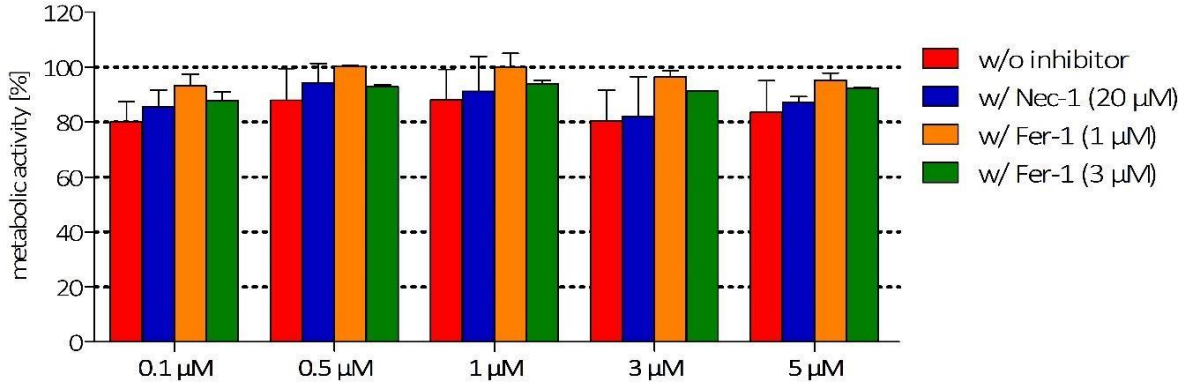


Figure S23: Antimetabolic activity of **17** plus Fer-1 or Nec-1

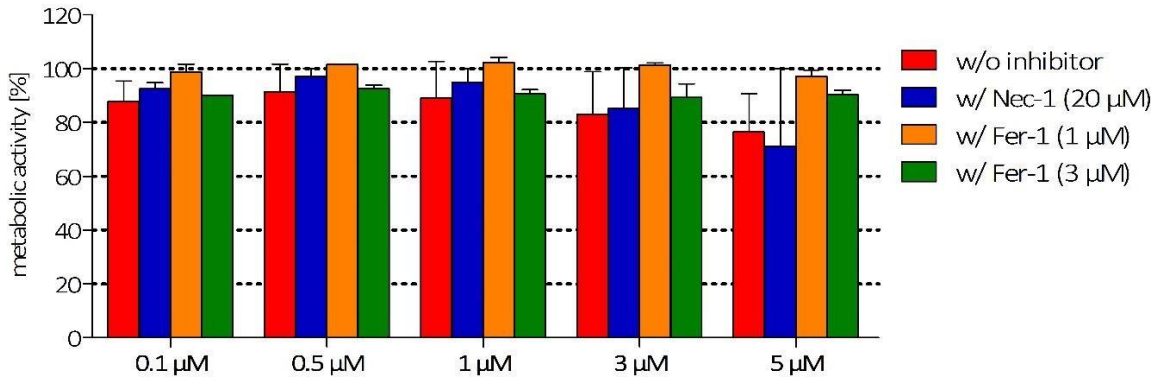


Figure S24: Antimetabolic activity of **20** plus Fer-1 or Nec-1

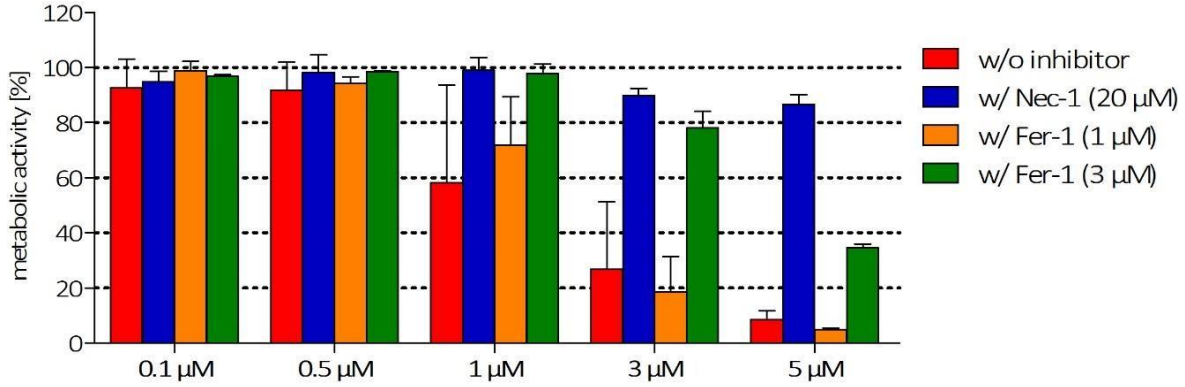


Figure S25: Antimetabolic activity of **21** plus Fer-1 or Nec-1

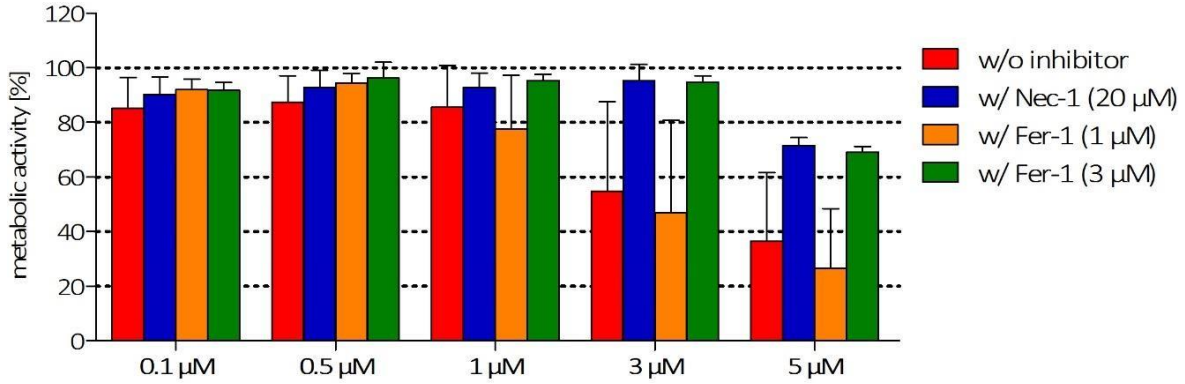


Figure S26: Antimetabolic activity of **15, 16–18** (amides) plus Fer-1 and Nec-1

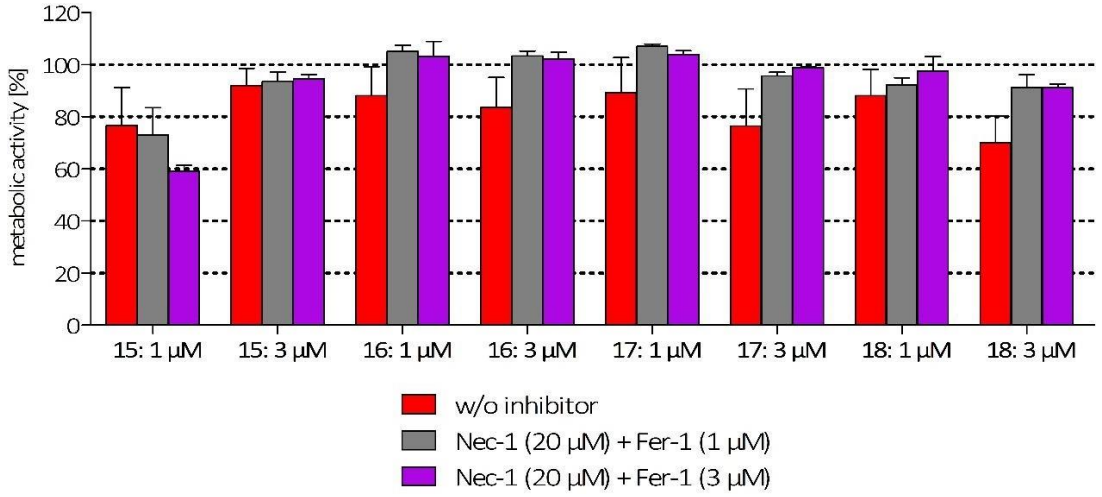


Figure S27: Antimetabolic activity of **15, 19–21** (esters) plus Fer-1 and Nec-1

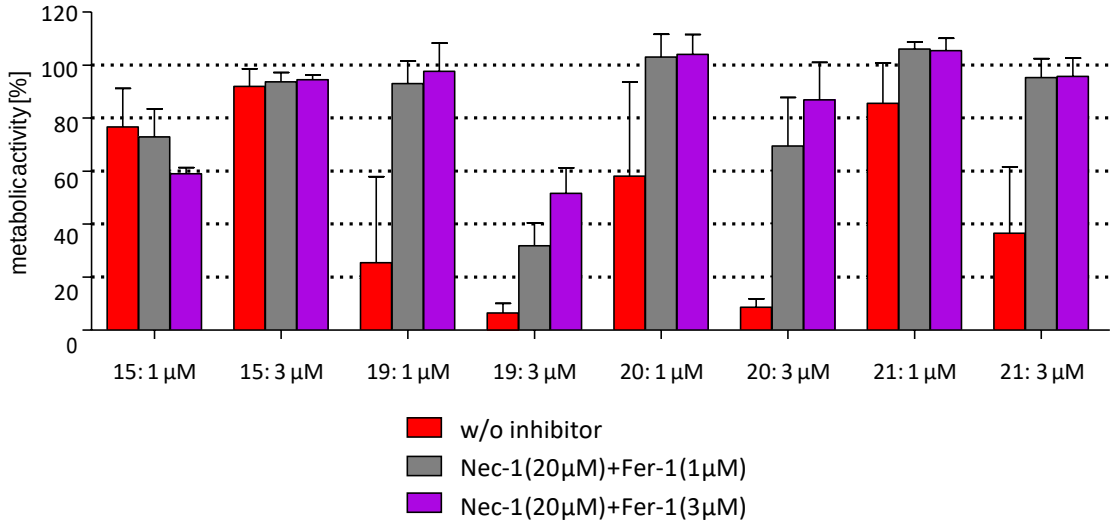


Figure S28: Antimetabolic activity of **15–21** on fibroblasts

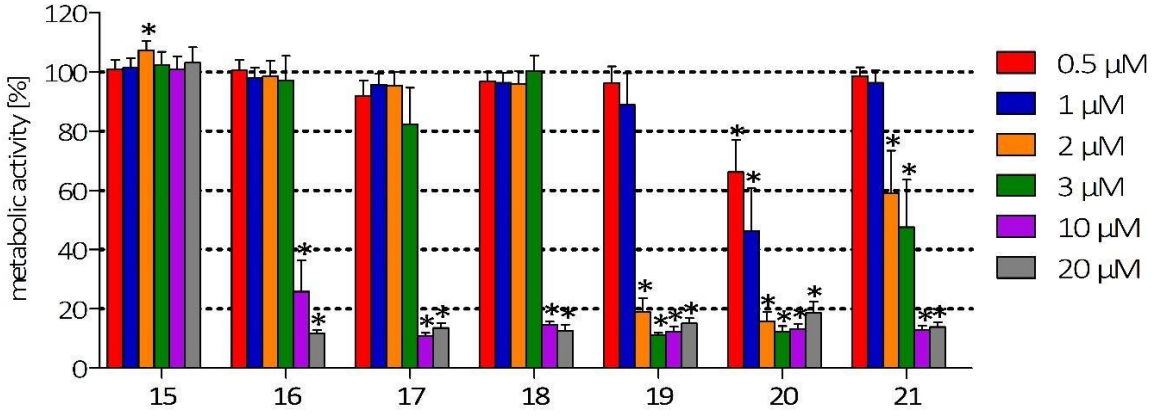


Figure S29: HPLC identification of **15** and **19** with MeCN/TFA 0.1% (22/78) as mobile phase

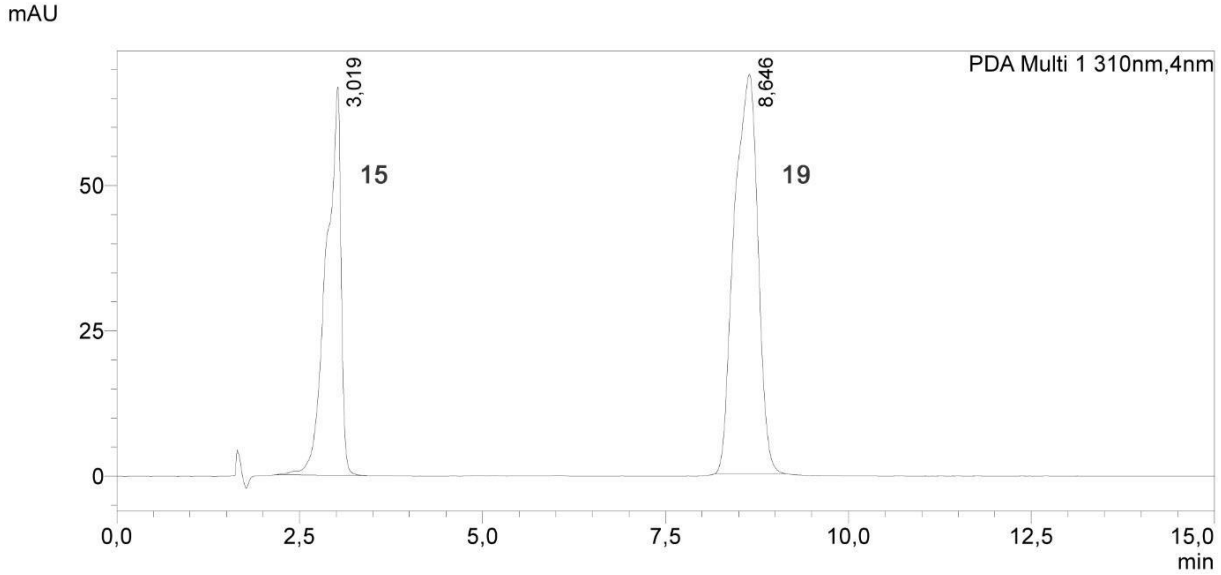


Figure S30: HPLC identification of DMSO blank in medium containing FBS (10%) with MeCN/TFA 0.1% (22/78) as mobile phase

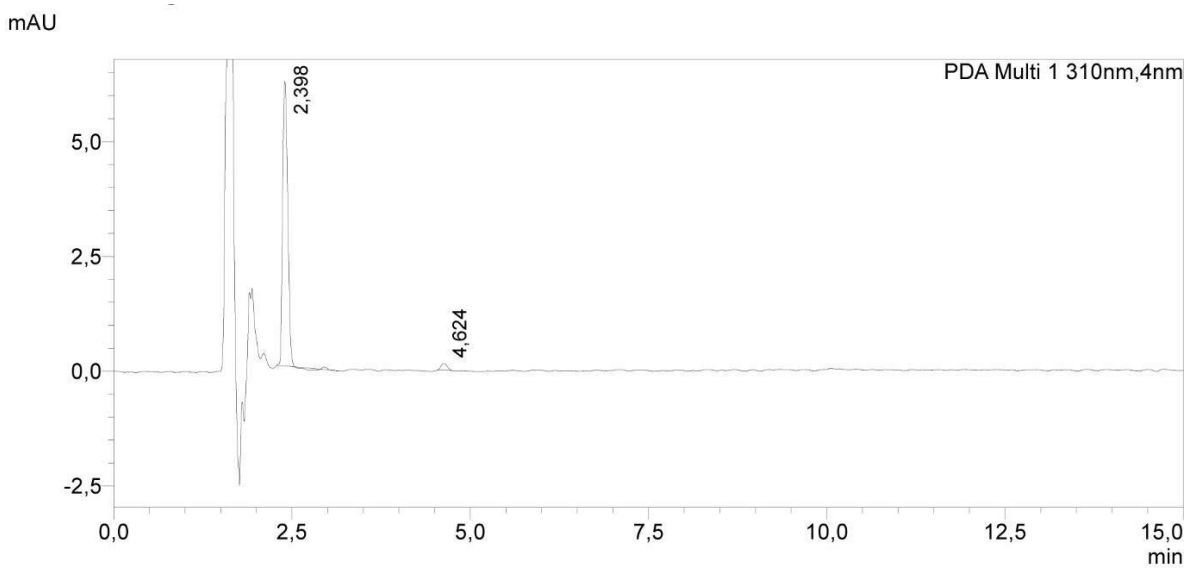
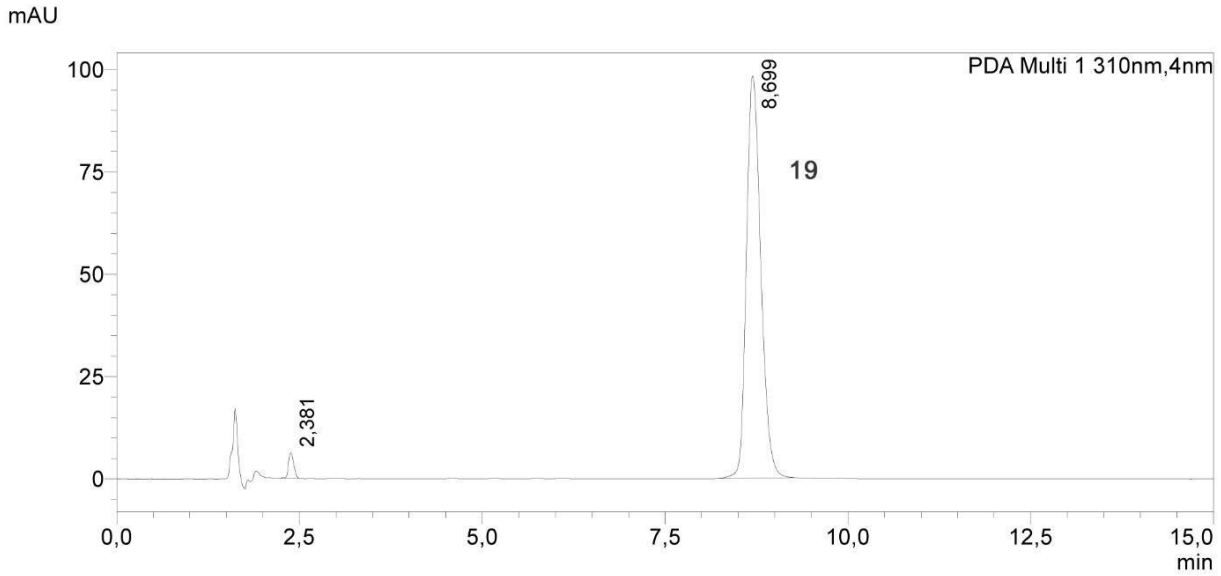


Figure S31: HPLC stability of **19** at 0 h of incubation at 37 °C in medium containing FBS (10%) mimicking esterases, stopped instantly with EtOH (96%), MeCN/TFA 0.1% (22/78) as mobile phase. Peak at 2.38 min shows a component of medium and was not considered in further measurements.

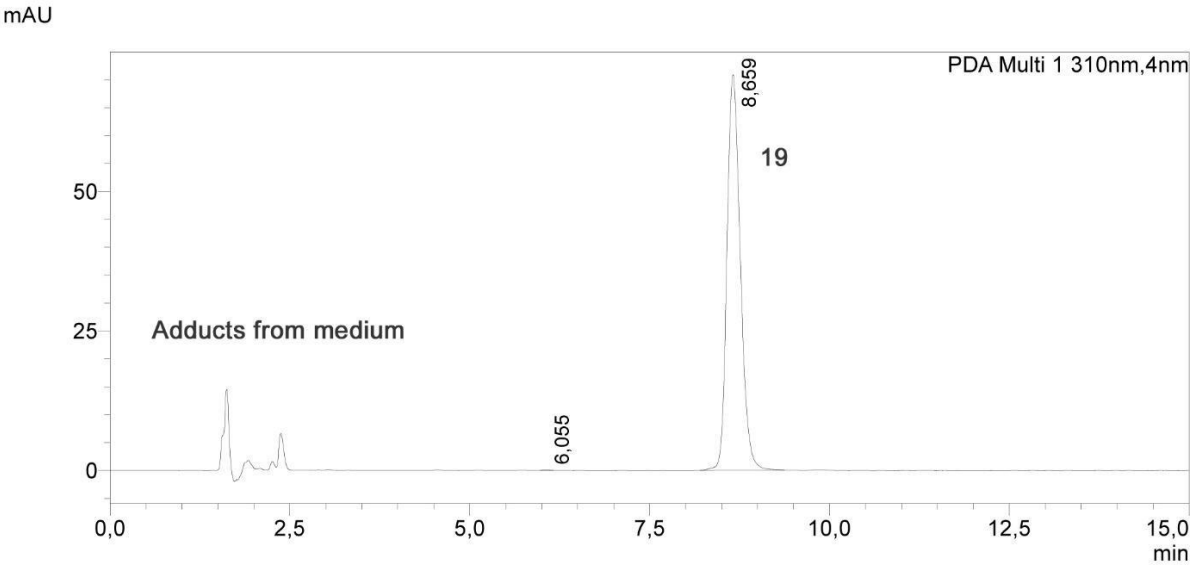


<Peak Table>

PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2,381	30901	6178	2,244		M	
2	8,699	1346215	98119	97,756		M	
Total		1377116	104296				

Figure S32: HPLC stability of **19** after 18 h of incubation at 37 °C in medium containing FBS (10%) mimicking esterases, stopped with EtOH (96%), MeCN/TFA 0.1% (22/78) as mobile phase.



<Peak Table>

PDA Ch1 310nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	6,055	1231	128	0,134			
2	8,659	919554	70794	99,866			
Total		920785	70923				