Supporting Information

Biomimetic enterobactin analogue mediates iron-uptake and cargo transport into *E. coli* and *P. aeruginosa*

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Experimental details

General methods

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. (*N,N*-Dimethylformamid (DMF): *Acros Organics*, puriss., extra dry, over molesieve (water ≤ 0.005%), Ethanol (EtOH): *Acros Organics*, puriss., absolut, extra dry (water ≤ 0.005%), Pyridin (Pyr): *Acros Organics*, puriss., extra dry, over molesieve (water ≤ 0.005%), Dimethylsulfoxid (DMSO): *Acros Organics*, puriss., extra dry, over mol sieve (water ≤ 0.005%), Methanol (MeOH): *Acros Organics*, puriss., extra dry (water ≤ 0.005%)).

AD-mix-α was purchased from Merck (392758) containing 29.37w% K_2CO_3, 0.53w% $(DHQ)_2PHAL$, 69.97w% $K_3[Fe(CN)_6]$ and 0.13w% $K_2[OsO_2(OH)_4]$. Natural enterobactin (**Ent**) and human apo-transferrin were purchased from *Sigma-Aldrich*.

Moisture sensitive reactions were performed under argon atmosphere in dried glassware. Dry dichloromethane, diethyl ether, toluene and tetrahydrofuran for moisture sensitive reactions have been taken from a MB-SPS-800 (MBraun) solvent purifications system and stored under argon. All solvents used for workup and purification were of HPLC grade. Reactions were monitored by TLC, LCMS or NMR.

Solution of compounds in organic solvents were concentrated using rotary evaporators at a water bath temperature of max. 30°C. Solvent residues were removed in high vacuum at pressure of appr. 10^{-2} mbar. Unless otherwise noted solvents were degassed either by a continuous Argon flow over minimum of 15 min or using the Freeze-Pump-Thaw technique.[1]

Flash chromatography[2] was done using appropriate glass columns filled with silicagel (Merck Millipore, Geduran® Si60, 1.11567.9025, 40-63 µm) or using the Biotage Select® chromatography system with a DAD detector and cartridges packed with silicagel (Merck Millipore, Geduran® Si60, 1.11567.9025, 40-63 µm) using a Cartridger® C-670 from the company Büchi.

Preparative reversed phase high pressure liquid chromatography (prep. HPLC RP) was performed on either a Hypersil GOLD C18 RP-column (Part No. 25005-259270), 5 µm, 250 mm×21.2 mm (10 mL/min) or a Hypersil GOLD C18 RP-column (Part No. 25005-259070A), 5 µm, 250 mm×10.0 mm (5 mL/min) each equipped with a guard column of the same material using a Thermo Fisher Scientific Dionex Ultimate 3000 HPLC system. Eluents, gradients and additives are given in parentheses. As eluents HPLC grade acetonitrile and water (VWR Chemicals, HPLC grade) with or without 0.1% of TFA (Carl Roth, 6957.1, 99.9%) or buffer added were used. Appropriate reaction mixtures were filtered through CHROMAFIL® PET-45/15 MS filters (45 µm) before injected. Product containing fractions were

combined diluted with dest. H_2O (min. 1:1/solvent:H_2O), frozen and lyophilized using a VaCo2® Freeze dryer from Zirbus (-80°C, 0.05 mbar).

Thin-layer chromatography (TLC) was performed on pre-coated glass plates (Merck TLC Silicagel 60 F_{254}, 1.15341.0001, 2.5x7.5 cm) and components were visualized by observation under UV light (λ = 254 nm [UV^{254}] or λ = 366 nm [UV^{366}]) or visible light, treatment of developed plates in an iodine chamber or by treating the plates with TLC staining solutions (for preparation see list below) followed by heating. Eluent or eluent-mixtures used are reported in parentheses.

CAM staining solution [CAM]: 1 g $Ce(IV)(SO_4)_2$, 2.5 g $(NH_4)_6Mo_4O_7$ in 100 mL 10% H_2SO_4

Ninhydrin staining solution [Ninhydrin]: 1.5 g Ninhydrin in 100 mL *abs.* EtOH and 3.0 mL HOAc.

Preparative thin-phase chromatography was performed on pre-coated glass plates (Merck TLC Silicagel 60 F_{254}, 1.05715.0001, 20x20 cm, max. 10-15 mg/plate and Analtech Uniplate Silica gel GF Z51305-9, 20x20 cm x 2 mm, max 100-150 mg/plate). Eluent or eluent-mixtures used and number of developments are reported in parentheses. Compounds were visualized by observation under UV light (λ = 254 or 366 nm). Compound containing silica gel fractions were scratched from the plate with a scapell, crushed to small pieces and compounds were eluated by appropriate solvent mixtures.

NMR spectra were recorded on a Bruker AV-300, AVIII400 und AVIIIHD500 with cryoprobe system at 293.15 K. ^1H NMR spectra were recorded at 300 MHz, 400 MHz and 500 MHz. ^{13}C NMR spectra were recorded at 76 MHz, 100 MHz and 126 MHz. Chemical shifts are reported in ppm relative to solvent signal. Multiplicity is indicated as follows: s (singlet); bs (broad singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublets), etc.. For the processing of the raw data the software MestReNova (Version 9.0.1-13254) from MestreLab Research S.L. were utilized.

IR spectra were recorded on a Bruker Tensor 27 IR spectrometer with ATR-technique. Only the wave numbers of observed absorption peaks are given.

Low resolution mass spectrometry (LRMS) data were recorded using an LC-MS system consisting of an Accela HPLC (Thermo Scientific) equipped with an Accela photodiode array (PDA) Detector, Accela autosampler, and Accela 1250 pump which was coupled to an LTQ XL mass spectrometer (Thermo Scientific) for HPLC/HESI-MS analyses. Heated electrospray ionization was used with an enhanced scan range of 120 to 2000 amu. Gradient HPLC solvent programs consisted of LCMS-grade H_2O, CH_3CN, and 2% formic acid in H_2O. An Agilent Zorbax Eclipse Plus C18 (3.5µm, 2.1×150 mm) column was used, which was kept at 30°C. The PDA detector was set to a scanning range from 190 to 600 nm with 1 nm wavelength steps.

High resolution mass spectrometry (HRMS) data were recorded on a Finnigan MAT 95 (EI, 70eV) mass spectrometer and a Finnigan MAT 95 XL (ESI) mass spectrometer.

Optical rotation data were recorded on a Polarimeter MCP 150 (Anton Paar).

UV-Vis spectroscopy data were recorded on a Cary 100 Bio (Varian).

Fluorescence Emission Spectroscopy data were recorded on a Cary Eclipse (Varian).

Photochemical reactions were performed in a Chamber reactor Model RPR-200 (Rayonet).

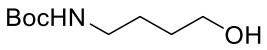
Hydrogenation reactions were performed in a laboratory high pressure autoclave HR-100 (Berghof).

Thin-layer chromatography mass spectrometry (TLC-MS) data were recorded on an Advion Expression compact mass spectrometer equipped with an Advion Plate Express automated TLC plate reader.

Imaging of bacteria (microscopy) was performed using a DMi8 inverted microscope equipped with a 40x/1.30 oil immersion objective. Images were captured in bright field mode (BF) or in fluorescence mode (F) using a GFP filter set (excitation filter: 480 nm, 20 nm bandwidth; emission filter: 527 nm, 15 nm)

Synthesis of the compounds

tert-Butyl (4-hydroxybutyl)carbamate (3)

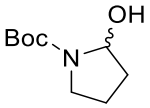


Chemical Formula: $C_9H_{19}NO_3$
Molecular Weight: 189,26

Following a slightly modified procedure of *Flack et al.*:[3] Triethyl amine 17.1 mL, 123.4 mmol, 1.1 equiv) and di-*tert*-butyl dicarbonate (26.4 mL, 123.4 mmol, 1.1 equiv) were added to a solution of 4-aminobutanol (**2**) (10.0 g, 56.1 mmol, 1.0 equiv) in dry CH_2Cl_2 (500 mL, 0.22 M) under Argon atm. and the mixture was stirred for 15 h at 23°C. The reaction mixture was quenched by addition of sat. aq. NH_4Cl solution (80 mL) and the phases were separated. The organic phase was washed with brine (80 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. *tert*-Butyl (4-hydroxybutyl)carbamate (**3**) was obtained as a white, amorph solid (23.4 g, 123.7 mmol, 100%) and used in the next reaction without further purification.

TLC (EtOAc:Hex/4:6) R_f: 0.35 [Ninhydrin]. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 3.63 (t, *J* = 6.0 Hz, 2H), 3.12 (t, *J* = 6.7 Hz, 2H), 1.59 – 1.52 (m, 4H), 1.42 (s, 9H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 156.30, 79.35, 62.39, 40.56, 29.79, 28.52, 26.68. The analytical data were in accordance with the literature.[3]

tert-Butyl 2-hydroxypyrrolidine-1-carboxylate (4)

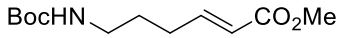


Chemical Formula: $C_9H_{17}NO_3$
Molecular Weight: 187,24

Following a slightly modified procedure of *Kokotos et al.*:[4] DMSO (26.2 mL, 369 mmol, 5.4 equiv) was added dropwise to a stirred solution of oxalyl chloride (7.0 mL, 82 mmol, 1.2 equiv) in dry CH_2Cl_2 (600 mL, 0.11 M) under Argon atm. at -78°C and stirred for 15 min at -78°C. A solution of *tert*-butyl (4-hydroxybutyl)carbamate (**3**) (12.9 g, 68 mmol, 1.0 equiv) in dry CH_2Cl_2 (20 mL, 2.8 M) was added at -78°C and the reaction mixture was stirred for 1 h at -78°C. DiPEA (34.0 mL, 205 mmol, 3.0 equiv) was added to the reaction mixture at -78°C and the solution was allowed to warm up to 0°C over a period of 20 min and stirred for 1 h at 0°C. The reaction mixture was quenched by the addition of water (100 mL) and the phases were separated. The organic phase was washed with water (2x 100 mL) and brine (80 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. *tert*-Butyl 2-hydroxypyrrolidine-1-carboxylate (**4**) was obtained as a colorless, viscous liquid (12.3 g, 65.6 mmol, 96%) and used in the next reaction without further purification.

TLC (EtOAc:Hex/4:6) R_f: 0.68 [Ninhydrin]. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 5.63 – 4.97 (m, 1H), 3.44 – 3.99 (m, 1H), 3.29 – 3.11 (m, 1H), 2.04 – 1.70 (m, 4H), 1.43 – 1.40 (m, 9H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 155.16, 153.67, 81.75, 81.51, 80.40, 80.06, 46.00, 41.04, 33.62, 32.82, 28.5 (6C), 22.81, 22.12. The analytical data were in accordance with the literature.[4]

Methyl (E)-6-((*tert*-butoxycarbonyl)amino)hex-2-enoate (6)

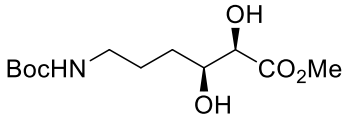


Chemical Formula: $C_{12}H_{21}NO_4$
Molecular Weight: 243,30

Methyl (triphenylphosphoranylidene) acetate (**5**) (11.1 g ,33.1 mmol, 1.05 equiv) was added to a solution of *tert*-butyl 2-hydroxypyrrolidine-1-carboxylate (**4**) (5.9 g, 31.5 mmol, 1.0 equiv) in dry toluene (125 mL, 0.25 M) and the mixture was heated to 100°C for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (EtOAc:Hex/1:9 → 3:7) yielding methyl (E)-6-((*tert*-butoxycarbonyl)amino)hex-2-enoate (**6**) (7.1 g, 29.3 mmol, 93%) as a white, amorphous solid.

TLC (EtOAc:Hex/3:7) R_f: 0.35 [UV^{254}, Ninhydrin]. **IR** (ATR) [cm^{-1}]: 3369, 2976, 2940, 1714, 1522, 1442, 1394, 1365, 1274, 1274, 1169, 1037, 984, 871, 781, 716, 663, 616, 592, 565. **HRMS** (ESI) [m/z]: 266.13630, calculated 266.13628 for $[C_{12}H_{21}NO_4Na]^+$, err [ppm] 0.08; 509.28346, calculated 509.28334 for $[C_{24}H_{42}N_2O_8Na]^+$, err [ppm] 0.24. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 6.88 (dt, *J* = 15.6, 6.9 Hz, 1H), 5.77 (dt, *J* = 15.6, 1.6 Hz, 1H), 4.50 (s, 1H), 3.66 (s, 3H), 3.07 (q, *J* = 6.9 Hz, 2H), 2.22 – 2.11 (m, 2H), 1.66 – 1.52 (m, 2H), 1.37 (s, 9H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 167.03, 156.03, 148.42 , 121.56, 79.39, 51.54, 40.13, 29.55, 28.66, 28.50.

Methyl (2*R*,3*S*)-6-((*tert*-butoxycarbonyl)amino)-2,3-dihydroxyhexanoate (7)



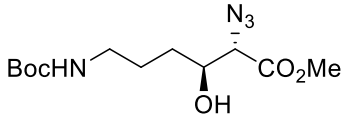
Chemical Formula: $C_{12}H_{23}NO_6$
Molecular Weight: 277,32

A solution of methyl (E)-6-((*tert*-butoxycarbonyl)amino)hex-2-enoate (**6**) (3.0 g, 12.3 mmol, 1.0 equiv) in THF (13 mL, 0.95 M) was added to a stirred mixture of AD-mix alpha (21.0 g ≈ 6.17 g, 44.64 mmol, 3.63 equiv K_2CO_3, 111.3 mg, 0.142 mmol, 0.012 equiv $(DHQ)_2PHAL$, 14.69 g, 44.63 mmol, 3.63 equiv $K_3[Fe(CN)_6]$, 27.3 mg, 0.074 mmol, 0.006 equiv $K_2[OsO_2(OH)_4]$) and methane sulfonamide (1.2 g, 12.4 mmol, 1.05 equiv) in tBuOH (54 mL, 0.23 M) and H_2O (74 mL, 0.17 M) at 23°C. The biphasic mixture was stirred vigorously for 24 h at 23°C. The mixture was quenched by addition of sat. aq.

$Na_2S_2O_3$ solution (50 mL) and extracted with CH_2Cl_2 (200 mL). The organic extract was washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/1:0 → 9:1) yielding methyl (2*R*,3*S*)-6-((*tert*-butoxycarbonyl)amino)-2,3-dihydroxyhexanoate (**7**) (2.8 g, 10.2 mmol, 83%) as a colorless, viscous liquid.

TLC (CH_2Cl_2:MeOH/95:5) R_f: 0.40 [Ninhydrin]. $[\alpha]_D^{23°C}$ = -6.3° (c =8.5 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3364, 2937, 1740, 1686, 1525, 1446, 1395, 1367, 1275, 1250, 1168, 1133, 1079, 1043, 984, 863, 781, 713, 633, 546. **HRMS** (ESI-IT) [m/z]: 300.14187, calculated 300.14176 for $[C_{12}H_{23}NO_6Na]^+$, err [ppm] 0.37; 577.29454, calculated 577.29430 for $[C_{24}H_{46}N_2O_{12}Na]^+$, err [ppm] 0.42. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 4.76 (s, 1H), 4.07 (d, *J* = 2.2 Hz, 1H), 3.89 (tt, *J* = 4.5, 2.2 Hz, 1H), 3.79 (s, 3H), 3.25 – 3.09 (m, 2H), 3.02 (s, 2H), 1.73 – 1.51 (m, 4H), 1.41 (s, 9H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 173.96, 156.4 , 79.44, 73.62, 72.38, 52.84, 40.45, 30.62, 28.51, 26.60.

Methyl (2*S*,3*S*)-2-azido-6-((*tert*-butoxycarbonyl)amino)-3-hydroxyhexanoate (8)



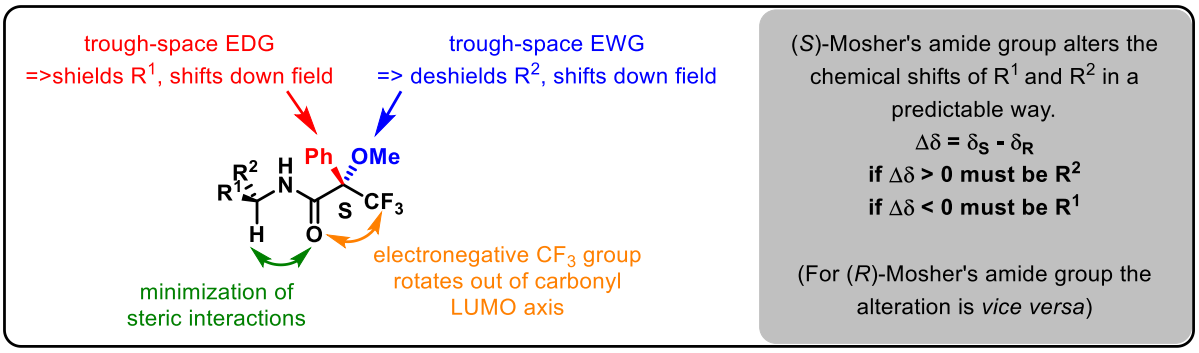
Chemical Formula: $C_{12}H_{22}N_4O_5$
Molecular Weight: 302,33

Thionyl chloride (1.45 mL, 20.2 mmol, 2.0 equiv) was added dropwise to a solution of methyl (2R,3S)-6-((*tert*-butoxycarbonyl)amino)-2,3-dihydroxyhexanoate (**7**) (2.8 g, 10.2 mmol, 1.0 equiv) and triethylamine (8.4 mL, 60.5 mmol, 6.0 equiv) in dry CH_2Cl_2 (340 mL, 0.03 M) under Argon atm. at 0 °C and the reaction mixture was stirred for 90 min at 0 °C. The reaction mixture was quenched by addition of sat. aq. $NaHCO_3$ solution (100 mL) and the phases were separated. The organic phase was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue containing the intermediate cyclic sulfite was dissolved in DMF (150 mL, 0.07 M) at 23°C, sodium azide (1.97 g, 30.3 mmol, 3.0 equiv) was added and the mixture was heated to 40°C for 12 h. The reaction mixture was diluted with EtOAc (1000 mL), washed with H_2O (3x 1000 mL) and brine (200 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (EtOAc:Hex/4:6) yielding methyl (2*S*,3*S*)-2-azido-6-((*tert*-butoxycarbonyl)amino)-3-hydroxyhexanoate (**8**) (2.0 g, 6.5 mmol, 64%) as a pale-yellow, viscous liquid.

TLC (EtOAc:Hex/4:6) R_f: 0.39 [Ninhydrin]. $[\alpha]_D^{23°C}$ = -37.0° (c =3.9 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3370, 2973, 2108, 1742, 1685, 1520, 1445, 1396, 1365, 1253, 1207, 1168, 1104, 1013, 922, 864, 780, 651, 577. **TLC-MS** (ESI) [m/z]: 325.6 $[C_{12}H_{22}N_4O_5Na]^+$. **HRMS** (ESI-IT) [m/z]: 325.14844, calculated 325.14824

for $[C_{12}H_{22}N_4O_5Na]^+$, err [ppm] 0.62; 627.30750, calculated 627.30726 for $[C_{24}H_{44}N_9O_{10}Na]^+$, err [ppm] 0.38. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 4.64 (s, 1H), 3.96 (s, 2H), 3.82 (s, 3H), 3.22 (s, 1H), 3.12 (s, 2H), 1.62 (m, 4H), 1.43 (s, 9H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 169.54, 156.49, 79.62, 71.91, 66.67, 52.90, 40.26, 29.63, 28.51, 26.65.

Determination of the absolute configuration of methyl (2S,3S)-2-azido-6-((*tert*-butoxycarbonyl)-amino)-3-hydroxyhexanoate (8)



	CH_3	CH_3	CH_2	H
S-Mosher ester	1.43 ppm	3.79 ppm	3.02 ppm	4.36 ppm
R-Mosher ester	1.44 ppm	3.75 ppm	3.10 ppm	4.26 ppm
Δδ	-0.01 ppm	+0.04 ppm	-0.08 ppm	+0.10 ppm

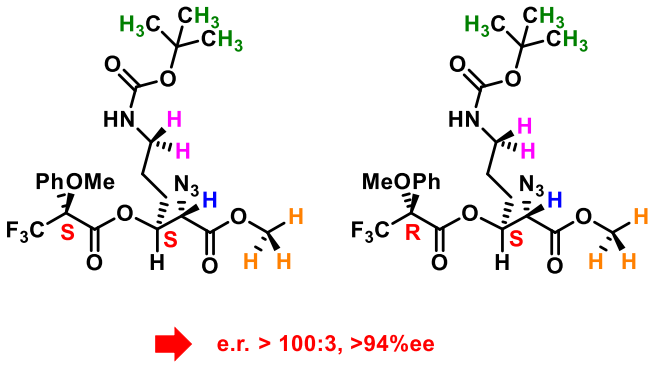
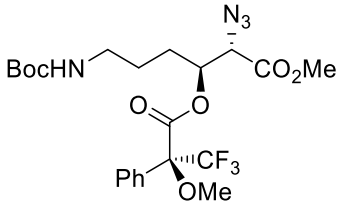


Figure S1: Determination of the absolute configuration of methyl (2*S*,3*S*)-2-azido-6-((tert-butoxycarbonyl)-amino)-3-hydroxyhexanoate (**8**)[5]

Methyl (2*S*,3*S*)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (*S*)-Mosher ester

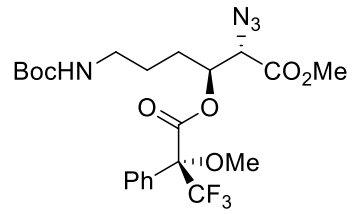


Chemical Formula: $C_{22}H_{29}F_3N_4O_7$
Molecular Weight: 518,49

DIC (8.0 µL, 52.0 µmol, 1.2 equiv) and DMAP (0.5 mg, 4.3 µmol, 0.1 equiv) were added to a solution of methyl (2S,3S)-2-azido-6-((*tert*-butoxycarbonyl)-amino)-3-hydroxyhexanoate (**8**) (13.0 mg, 43.0 µmol, 1.0 equiv), (S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (12.1 mg, 52.0 µmol, 1.2 equiv) and NMM (5.7 µL, 52.0 µmol, 1.2 equiv) in dry CH_2Cl_2 (300 µL, 0.14 M) at 0 °C and the mixture was stirred at 23°C for 16 h. The mixture was concentrated under reduced pressure and the residue was purified by preparative thin layer chromatography (EtOAc:Hex/3:7) yielding the methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (S)-Mosher ester (16.0 mg, 31.0 µmol, 72%) as a colorless, viscous liquid.

TLC (EtOAc:Hex/4:6) R_f: 0.60 [UV^{254}, Ninhydrin]. **IR** (ATR) [cm^{-1}]: 3360, 2963, 2858, 2114, 1751, 1706, 1514, 1448, 1395, 1366, 1250, 1170, 119, 1082, 1017, 865, 822, 771, 721, 645, 591, 552. **HRMS** (ESI-IT) [m/z]: 541.18827, calculated 541.18805 for $[C_{22}H_{29}F_3N_4O_7Na]^+$, err [ppm] 0.41.**^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 7.62 – 7.53 (m, 2H), 7.43 – 7.41 (m, 3H), 5.46 – 5.32 (m, 1H), 4.40 (s, 1H), 4.37 (d, *J* = 4.4 Hz, 1H), 3.79 (s, 3H), 3.56 (s, 3H), 3.03 – 3.00 (m, 2H), 1.87 – 1.52 (m, 4H), 1.43 (s, 9H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 167.67, 166.20, 155.99, 131.92, 129.95, 128.66, 127.40, 124.46, 122.17, 84.76, 79.45, 75.05, 63.73, 55.73, 53.26, 39.89, 28.52, 26.88, 25.53. **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: -71.61.

Methyl (2*S*,3*S*)-2-azido-6-((*tert*-butoxycarbonyl)amino)-3-(((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (*R*)-Mosher ester



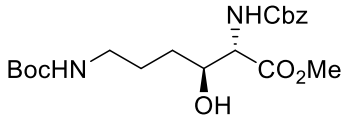
Chemical Formula: $C_{22}H_{29}F_3N_4O_7$
Molecular Weight: 518,49

DIC (8.0 µL, 52.0 µmol, 1.2 equiv) and DMAP (0.5 mg, 4.3 µmol, 0.1 equiv) were added to a solution of methyl (2S,3S)-2-azido-6-((*tert*-butoxycarbonyl)-amino)-3-hydroxyhexanoate (**8**) (13.0 mg, 43.0 µmol, 1.0 equiv) (R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (12.1 mg, 52.0 µmol, 1.2 equiv) and NMM (5.7 µL, 52.0 µmol, 1.2 equiv) in dry CH_2Cl_2 (300 µL, 0.14 M) at 0 °C and the mixture was stirred at 23°C for 16 h. The mixture was concentrated under reduced pressure and the residue was purified via preparative thin layer chromatography (EtOAc:Hex/3:7) yielding Methyl (2*S*,3*S*)-2-azido-6-((*tert*-butoxycarbonyl)amino)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (*R*)-Mosher ester (13.0 mg, 25.1 µmol, 61%) as a colorless, viscous liquid.

TLC (EtOAc:Hex/4:6) R_f: 0.60 [UV^{254}, Ninhydrin]. **IR** (ATR) [cm^{-1}]: 3357, 2927, 2856, 2114, 1750, 1705, 1514, 1448, 1395, 1366, 1344, 1250, 1169, 1118, 1089, 1017, 865, 822, 770, 720, 646, 594, 558. **HRMS**

(ESI-IT) [m/z]: 541.18828, calculated 541.18805 for $[C_{22}H_{29}F_3N_4O_7Na]^+$, err [ppm] 0.42. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ [ppm]: 7.54 – 7.51 (m, 2H), 7.46 – 7.39 (m, 3H), 5.50 – 5.33 (m, 1H), 4.52 (s, 1H), 4.25 (d, *J* = 4.7 Hz, 1H), 3.75 (s, 3H), 3.53 (s, 3H), 3.20 – 2.97 (m, 2H), 1.95 – 1.54 (m, 4H), 1.44 (s, 9H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 167.68, 166.16, 156.03, 131.68, 129.97, 128.71, 127.45, 84.87, 79.49, 74.99, 63.73, 55.67, 53.19, 40.01, 29.85, 28.52, 27.10, 25.85. **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: -71.8.

Methyl (2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)-3-hydroxy-hexanoate (9)

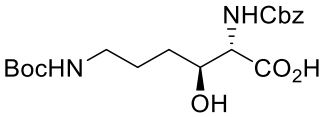


Chemical Formula: $C_{20}H_{30}N_2O_7$
Molecular Weight: 410,47

Triphenylphosphine (2.55 g, 9.72 mmol, 1.05 equiv) was added to a solution of methyl (2S,3S)-2-azido-6-((*tert*-butoxycarbonyl)amino)-3-hydroxyhexanoate (**8**) (1.9 g, 4.63 mmol, 1.0 equiv) in THF (60 mL, 0.07 M) and H_2O (6 mL, 0.07 M) and the mixture was stirred at 23°C for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by a fast flash column chromatography through silica gel (CH_2Cl_2:MeOH/1:0 → 8:2). The resulting methyl (2S,3S)-2-amino-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoate was immediately dissolved in dry CH_2Cl_2 (25 mL, 0.19 M) under Argon atm.. DiPEA (847 µL, 4.86 mmol, 1.05 equiv) and afterwards benzyl chloroformiate (653 µL, 4.86 mmol, 1.05 equiv) was added dropwise at 0°C and the mixture was stirred at 23°C for 13 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (EtOAc:Hex/1:1) yielding methyl (2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)-amino)-3-hydroxyhexanoate (**9**) (1.65 g, 4.03 mmol, 87%) as a white, amorphous solid.

TLC (EtOAc:Hex/1:1) R_f: 0.38 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = +9.3° (c =13.2 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3358, 2954, 1688, 1518, 1448, 1394, 1366, 1525, 1167, 1101, 1044, 914, 866, 780, 736, 698, 576. **TLC-MS** (ESI) [m/z]: 433.7 **HRMS** (ESI-IT) [m/z]: 433.19484, calculated 433.19452 for $[C_{20}H_{30}N_2O_7Na]^+$, err [ppm] 0.74; 843.40086, calculated 843.39982 for $[C_{40}H_{60}N_4O_{14}Na]^+$, err [ppm] 1.23. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 7.40 – 7.28 (m, 5H), 5.80 (s, 1H), 5.11 (s, 2H), 4.65 (s, 1H), 4.41 (m, 1H), 3.92 (s, 1H), 3.76 (s, 3H), 3.28 (s, 1H), 3.22 – 3.01 (m, 2H), 1.69 – 1.56 (m, 2H), 1.56 – 1.45 (m, 2H), 1.42 (s, 9H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 170.93, 156.54, 156.43, 136.13, 128.68, 128.40, 128.30, 79.47, 72.78, 67.43, 58.97, 52.71, 40.16, 30.11, 28.53, 26.82.

(2*S*,3*S*)-2-(((Benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)-3-hydroxyhexanoic acid (1)



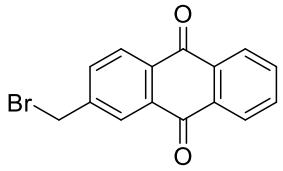
Chemical Formula: $C_{19}H_{28}N_2O_7$
Molecular Weight: 396,44

Following the protocol of *Nicoloau et al*.,[6] trimethyltin hydroxide (1.17 g, 6.51 mmol, 2.5 equiv) was added to a solution of methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)-amino)-3-hydroxyhexanoate (**9**) (1.07 g, 2.61 mmol, 1.0 equiv) in 1,2-$C_2H_4Cl_2$ (25 mL, 0.1 M) at 23 °C and the mixture was stirred for 2.5 h at 65°C. CH_2Cl_2 (25 mL) was added and the solution was washed with citric acid (10 wt%, 3x 25 mL) and brine (25 mL). The organic phase was dried over Na_2SO_4, filtered concentrated under reduced pressure to obtain (2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxy-carbonyl)amino)-3-hydroxyhexanoic acid (**10**) as colorless, viscous liquid (970 mg, 2.45 mmol, 94%) and used in the next reaction without further purification.

Alternatively, LiOH H_2O (122 mg, 2.93 mmol, 1.2 equiv) was added to a solution of methyl (2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)-amino)-3-hydroxyhexanoate (**9**) (1.0 g, 2.44 mmol, 1.0 equiv) in THF (10 mL, 0.19 M) and H_2O (3 mL, 0.19 M) at 0°C and the mixture was stirred for 70 min at 0°C. The reaction mixture was acidified with aq. citric acid solution (10wt%, 40 mL) and extracted with EtOAc (3x 40 mL). The combined organic phases were dried over Na_2SO_4, filtered concentrated under reduced pressure. (2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxy-carbonyl)amino)-3-hydroxyhexanoic acid (**1**) was obtained as a colorless, viscous liquid (870 mg, 2.20 mmol, 90%) and used in the next reaction without further purification.

TLC (CH_2Cl_2:MeOH/85:15) R_f: 045 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = +14.1° (c =3.2 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3340, 3066, 3035, 2974, 2937, 1692, 1521, 1454, 1412, 1366, 1251, 1168, 1104, 1048, 983, 913, 858, 780, 734, 698, 626, 582, 538. **HRMS** (ESI) [m/z]: 419.17930, calculated 419.17887 for $[C_{19}H_{28}N_2O_7Na]^+$, err [ppm] 0.08; 815.36937, calculated 815.36852 for $[C_{38}H_{56}N_4O_{14}Na]^+$, err [ppm] 1.04. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 7.32 – 7.27 (m, 5H), 6.12 (s, 1H), 5.09 (s, 2H), 4.85 (s, 1H), 4.40 – 4.36 (m, 1H), 3.93 – 3.94 (s, 1H), 3.21 – 3.01 (m, 2H), 1.59 – 1.57 (m, 4H), 1.41 (s, 9H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 172.79, 156.65, 136.03, 128.47, 128.15, 128.08, 79.74, 72.68, 67.21, 58.71, 29.96, 28.35, 26.58.

2-(Bromomethyl)anthracene-9,10-dione (10)

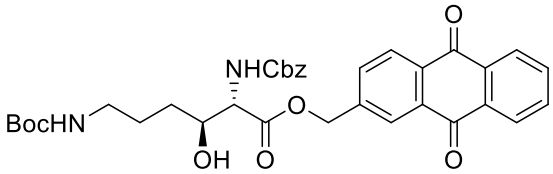


Chemical Formula: $C_{15}H_9BrO_2$
Molecular Weight: 301,14

N-Bromosuccinimide (3.8 g, 21.6 mmol, 1.2 equiv) was added to a mixture of 2-methylanthracene-9,10-dione (4.0 g, 18.0 mmol, 1.0 equiv) in dry benzene (200 mL, 0.09 M) under Argon atm. and the mixture was heated up to 90 °C. AIBN (296 mg, 1.8 mmol, 0.1 equiv) was added portionwise and the mixture was stirred for 24 h at 90°C. The reaction was quenched by addition of water (100 mL) and the phases were separated. The organic phase was washed with water (100 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. 2-(bromomethyl) anthracene-9,10-dione was obtained as a beige, amorph solid (5.0 g, 16.6 mmol, 92%) and used in the next step without further purification.

TLC (EtOAc:Hex/5:95) R_f: 0.85 [UV^{254}]. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 8.32 - 8.27 (m, 4H), 7.82 – 7.79 (m, 3H), 4.59 (s, 2H).**^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 182.79, 182.64, 144.31, 134.67, 134.43, 134.37, 133.96, 133.53, 133.23, 128.19, 127.68, 127.43, 31.62. The analytical data were in accordance with the literature.[7]

(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoate (11)

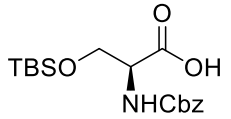


Chemical Formula: $C_{34}H_{36}N_2O_9$
Molecular Weight: 616,67

2-(Bromomethyl)anthraquinone (**10**) (881 mg, 2.93 mmol, 1.33 equiv) and DBU (432 µL, 2.93 mmol, 1.33 equiv) were added to a solution of (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoic acid (**1**) (870 mg, 2.20 mmol, 1.0 equiv) in dry THF (10 mL, 0.22 M) under Argon atm. and the mixture was stirred at 23°C for 24 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (EtOAc:Hex/1:1) yielding (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)-3-hydroxyhexanoate (**11**) (754 mg, 1.22 mmol, 55%) as a pale-yellow, amorphous solid.

TLC (EtOAc:Hex/1:1) R_f: 0.40 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = +10.6° (c =16.6 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3370, 2930, 1706, 1676, 1596, 1519, 1453, 1366, 1327, 1291, 1257, 1168, 1039, 925, 852, 710. **HRMS** (ESI-IT) [m/z]: 639.23144, calculated 639.23130 for $[C_{34}H_{36}N_2O_9Na]^+$, err [ppm] 0.22; 1255.47396, calculated 1255.47338 for $[C_{68}H_{72}N_4O_{18}Na]^+$, err [ppm] 0.46. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.45 – 8.15 (m, 4H), 7.82 – 7.73 (m, 3H), 7.33 – 7.26 (m, 5H), 5.82 (s, 1H), 5.35 (s, 2H), 5.12 (s, 2H), 4.74 (s, 1H), 4.52 – 4.48 (m, 2H), 4.00 (s, 1H), 3.28 (s, 1H), 3.20 – 3.05 (m, 2H), 1.63 – 1.57 (m, 4H), 1.39 (s, 9H).**^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 182.98, 182.76, 170.32, 156.52, 141.94, 136.10, 134.44, 134.35, 133.78, 133.56, 133.53, 133.31, 133.07, 128.68, 128.41, 128.32, 127.97, 127.45, 127.43, 126.16, 79.50, 72.87, 67.49, 66.19, 59.13, 40.08, 30.38, 29.84, 28.52, 26.91.

N-((benzyloxy)carbonyl)-O-(*tert*-butyldimethylsilyl)-L-serine - Cbz-Ser(OTBS)-OH (12)

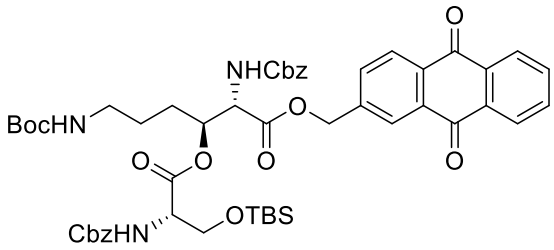


Chemical Formula: $C_{17}H_{27}NO_5Si$
Molecular Weight: 353,49

TBSCl (9.45 g, 62.7 mmol, 2.5 equiv) was added to a solution of Cbz-Ser-OH (6.0 g, 25.0 mmol, 1.0 equiv) and imidazole (5.12 g, 75.2 mmol, 3.0 equiv) in dry DMF (30 mL, 0.83 M) at 0°C and the mixture was stirred at 23°C for 13 h. The reaction was quenched by addition of aq. citric acid solution (10wt%, 100 mL) and extracted with EtOAc (300 mL). The organic extract was washed with H_2O (3x 300 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was suspended in a solution of NaOH (3.0 g, 75 mmol, 3.0 equiv) in H_2O (60 mL, 0.42 M) and the mixture was stirred at 23°C for 30 min. The mixture was acidified with aq. citric acid (10wt%, 100 mL) and extracted with EtOAc (3x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/1:0 → 9:1) yielding Cbz-Ser(OTBS)-OH (**12**) (6.1 g, 17.3 mmol, 69%) as a white, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.60 [UV^{254}, CAM]. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 9.49 (s, 1H), 7.36 – 7.33 (m, 5H), 5.67 (d, *J* = 8.2 Hz, 1H), 5.18 – 5.06 (m, 2H), 4.48 – 4.31 (m, 1H), 4.09 (dd, *J* = 10.1, 3.2 Hz, 1H), 3.86 (dd, *J* = 10.1, 3.2 Hz, 1H), 0.85 (s, 9H), 0.03 (s, 6H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 175.91, 156.40, 136.26, 128.64, 128.31, 128.29, 67.30, 63.46, 56.13, 25.88, 18.34, -5.41. The analytical data were in accordance with the literature.[8]

(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((*N*-((benzyloxy)carbonyl)-O-(*tert*-butyl-dimethylsilyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)hexan-oate **(**13**)**

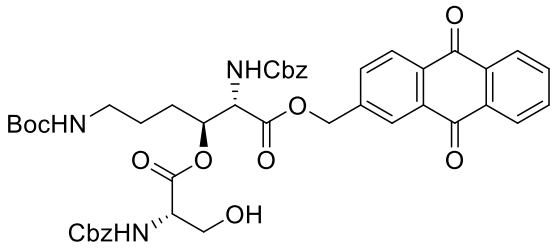


Chemical Formula: $C_{51}H_{61}N_3O_{13}Si$
Molecular Weight: 952,14

EDCI HCl (594 mg, 3.08 mmol, 2.5 equiv) and DMAP (25 mg, 0.26 mmol, 0.2 equiv) were added to a solution of (9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoate (**11**) (762 mg, 1.23 mmol, 1.0 equiv), Cbz-Ser(OTBS)-OH (**12**) (884 mg, 3.08 mmol, 2.5 equiv) and NMM (681 µL, 6.15 mmol, 5.0 equiv) in dry CH_2Cl_2 (6 mL, 0.21 M) under Argon atm. at 0°C. The reaction mixture was allowed to warm up to 23°C and stirred at 23°C for 13 h. The mixture was diluted with CH_2Cl_2 (50 mL) and sat. aq. NH_4Cl solution (30 mL). The phases were separated and the organic phase was washed with brine (30 mL) and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (EtOAc:Hex/3:7 → 1:1) yielding (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((*N*-((benzyloxy)carbonyl)-O-(*tert*-butyl-dimethylsilyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)hexan-oate (**13**) (981 mg, 1.03 mmol, 84%) as an pale-green, foamy solid.

TLC (EtOAc:Hex/1:1) R_f: 0.80 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = +3.4° (c =13.6 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3360, 3065, 3035, 2934, 2889, 2859, 1711, 1678, 1597, 1509, 1458, 1391, 1365, 1327, 1292, 1250, 1168, 1112, 1054, 982, 924, 840, 780, 734, 706, 668, 641, 576. **HRMS** (ESI-IT) [m/z]: 974.38725, calculated 974.38659 for $[C_{51}H_{61}N_3O_{13}Na]^+$, err [ppm] 0.68; 1926.78697, calculated 1926.78731 for $[C_{101}H_{122}N_6O_{26}Na]^+$, err [ppm] 0.18. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.44 – 8.04 (m, 4H), 7.89 – 7.68 (m, 3H), 7.46 – 7.01 (m, 10H), 6.00 (d, *J* = 9.0 Hz, 1H), 5.71 (d, *J* = 7.8 Hz, 1H), 5.47 – 5.22 (m, 3H), 5.19 – 4.97 (m, 4H), 4.71 – 4.68 (m, 2H), 4.34 (dt, *J* = 6.5, 3.0 Hz, 1H), 4.05 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.85 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.25 – 2.97 (m, 2H), 1.84 – 1.70 (m, 2H), 1.62 – 1.49 (m, 2H), 1.38 (s, 9H), 0.82 (s, 9H), 0.00 (s, 6H).**^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 182.99, 182.68, 170.11, 168.61, 156.40, 156.05, 141.75, 136.21, 134.43, 134.32, 133.85, 133.57, 133.53, 133.38, 133.01, 128.64, 128.57, 128.40, 128.32, 128.27, 127.98, 127.48, 127.41, 126.12, 79.22, 75.62, 67.35, 67.29, 66.33, 63.48, 60.52, 56.67, 56.50, 40.01, 28.62, 28.50, 26.26, 25.92, 25.89, 21.19, 18.46, 14.33, -5.40.

(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((((benzyloxy)carbonyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)hexanoate (14)

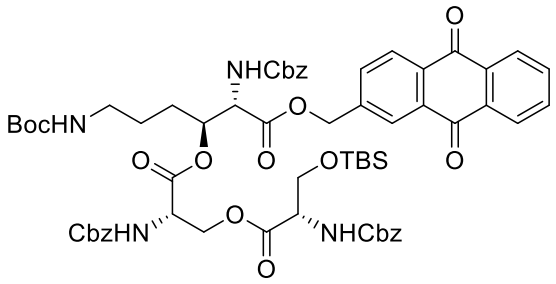


Chemical Formula: $C_{45}H_{47}N_3O_{13}$
Molecular Weight: 837,88

HF (48w% in H_2O) (57 µL, 1.94 mmol, 2.0 equiv) and TBAF (1 M in THF) (1.45 mL, 1.45 mmol, 1.5 equiv) were added dropwise to a solution of (9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(tert-butyl-dimethylsilyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexan-oate (**14**) (930 mg, 0.97 mmol, 1.0 equiv) in THF (11 mL, 0.09 M) at 0°C and the mixture was stirred at 23°C for 90 min. The reaction mixture was diluted with EtOAc (30 mL) and the excess of fluoride was precipitated by the addition of a aq. $Ca(OAc)_2$ solution (10wt%, 5 mL). The phases were separated and the organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (EtOAc:Hex/1:1) yielding (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((((benzyloxy)carbonyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)-hexanoate (**15**) (745 mg, 0.88 mmol, 92%) as a white foamy solid.

TLC (EtOAc:Hex/1:1) R_f: 0.39 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = +2.2° (c =12.5 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3368, 3067, 3036, 2971, 1706, 1676, 1596, 1519, 1454, 1398, 1367, 1328, 1290, 1215, 1167, 1058, 912, 851, 816, 779, 732, 643, 573. **HRMS** (ESI-IT) [m/z]: 860.30097, calculated 860.30011 for $[C_{45}H_{47}N_3O_{13}Na]^+$, err [ppm] 1.00; 1698.61526, calculated 1698.61435 for $[C_{90}H_{92}N_6O_{26}Na]^+$, err [ppm] 0.54. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.44 – 8.09 (m, 4H), 7.93 – 7.60 (m, 3H), 7.40 – 7.27 (m, 10H), 6.11 (d, *J* = 8.9 Hz, 1H), 5.87 (d, *J* = 8.1 Hz, 1H), 5.46 – 5.20 (m, 3H), 5.15 – 5.04(,m, 4H), 4.88 (dd, *J* = 9.0, 3.1 Hz, 1H), 4.71 (s, 1H), 4.36 (d, *J* = 10.4 Hz, 1H), 3.93 (d, *J* = 12.1 Hz, 1H), 3.74 (d, *J* = 12.1 Hz, 1H), 3.12 – 2.97 (m, 2H), 1.83 – 1.45 (m, 4H), 1.38 (s, 9H).**^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 182.93, 182.45, 170.37, 168.74, 156.62, 156.23, 156.03, 141.33, 136.13, 135.75, 134.34, 134.20, 133.66, 133.37, 133.29, 133.03, 128.49, 128.44, 128.26, 128.21, 128.13, 128.07, 127.96, 127.89, 127.32, 127.28, 125.96, 79.24, 74.94, 67.52, 67.03, 66.46, 62.46, 60.36, 39.83, 28.33, 26.92, 26.16, 21.02, 14.17.

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((*N*-((benzyloxy)carbonyl)-O-(N-((benz-yloxy)carbonyl)-O-(*tert*-butyldimethylsilyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)-amino)-6-((*tert*-butoxycarbonyl)amino)hexanoate (15)



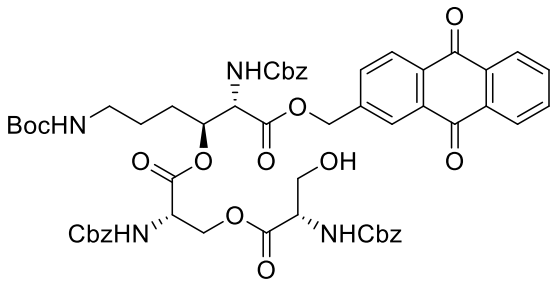
Chemical Formula: $C_{62}H_{72}N_4O_{17}Si$
Molecular Weight: 1173,35

EDCI HCl (65 mg, 0.34 mmol, 1.3 equiv) and DMAP (2.6 mg, 2.6 µmol, 0.1 equiv) were added to a solution of (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((((benzyloxy)carbonyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-hexanoate (**14**) (220 mg, 0.26 mmol, 1.0 equiv), Cbz-Ser(OTBS)-OH (**12**) (120 mg, 0.34 mmol, 1.3 equiv) and NMM (74 µL, 0.68 mmol, 2.6 equiv) in dry CH_2Cl_2 (1.7 mL, 0.15 M) under Argon atm. at 0°C and the reaction mixture was allowed to warm up to 23°C over a period of 30 min and stirred at 23°C for 4.5 h. The mixture was diluted with CH_2Cl_2 (20 mL) and the reaction was quenched by addition of sat. aq. NH_4Cl solution (5 mL). The phases were separated and the organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (EtOAc:Hex/4:6) yielding (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((*N*-((benzyloxy)carbonyl)-O-(*N*-((benzyloxy)-carbonyl)-O-(*tert*-butyldimethylsilyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)hexanoate (**15**) as a white foamy solid (286 mg, 0.244 mmol, 94%).

TLC (EtOAc:Hex/1:1) R_f: 0.65 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = +2.5° (c =10.0 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3352, 3065, 3035, 2955, 2934, 2886, 2858, 1716, 1595, 1515, 1456, 1392, 1369, 1328, 1295, 1253, 1211, 1168, 1113, 1068, 981, 931, 839, 780, 743, 702, 667, 637, 582, 540. **HRMS** (ESI-IT) [m/z]: 1195.45630, calculated 1195.45539 for $[C_{625}H_{727}N_4O_{17}SiNa]^+$, err [ppm] 0.76. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.39 – 8.20 (m, 4H), 7.86 – 7.70 (m, 3H), 7.38 – 7.27 (m, 15H), 5.96 (d, J = 8.1 Hz, 1H), 5.89 (d, J = 8.1 Hz, 1H), 5.65 (d, J = 8.1 Hz, 1H), 5.40 – 5.25 (m, 3H), 5.17 – 5.00 (m, 6H), 4.79 (s, 1H), 4.71 (dd, J = 8.1, 2.3 Hz, 1H), 4.64 – 4.53 (m, 2H), 4.43 – 4.32 (m, 2H), 3.93 (d, J = 12.6 Hz, 1H), 3.66 (d, J = 10.3 Hz, 1H), 3.10 – 3.07 (m, 2H), 1.82 – 1.69 (m, 2H), 1.61 – 1.53 (m, 2H), 1.38 (s, 9H), 0.79 (s, 9H), -0.06 (s, 6H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 183.07, 182.69, 170.18, 169.00, 168.44, 156.32, 156.20, 156.13, 155.87, 141.66, 136.31, 136.13, 136.10, 134.46, 134.36, 133.88, 133.59, 133.53, 133.45, 133.13, 128.62, 128.57, 128.46, 128.36, 128.30, 128.27, 128.03, 127.53, 127.43, 126.11, 79.26, 75.90,

67.51, 67.39, 67.30, 66.48, 64.80, 63.43, 56.85, 56.23, 53.78, 39.98, 29.84, 28.52, 28.33, 26.37, 25.89, 18.40, 14.27, 1.17, -5.51.

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((*N*-((benzyloxy)carbonyl)-O-(((benzyl-oxy)-carbonyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)-amino)hexanoate (16)



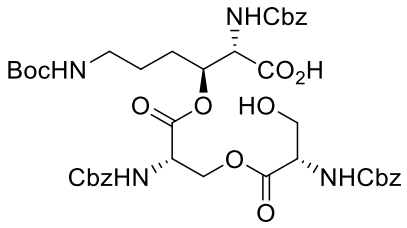
Chemical Formula: $C_{56}H_{58}N_4O_{17}$
Molecular Weight: 1059,09

HF (48% in H_2O) (29 µL, 0.96 mmol, 2.0 equiv) and TBAF (1 M in THF) (720 µL, 0.72 mmol, 1.5 equiv) were added dropwise to a solution of (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(N-((benzyloxy)-carbonyl)-O-(tert-butyldimethylsilyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoate (**15**) (565 mg, 0.48 mmol, 1.0 equiv) in THF (5.5 mL, 0.09 M) at 0°C and the mixture was stirred at 23°C for 90 min. The mixture was diluted with EtOAc (30 mL) and the excess of fluoride was precipitated by the addition of a aq. $Ca(OAc)_2$ solution (10 Wt%, 5 mL). The phases were separated and the organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (EtOAc:Hex/1:1) yielding (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((*N*-((benzyloxy)carbonyl)-O-(((benzyl-oxy)-carbonyl)-L-seryl)-L-seryl)oxy)-2-(((benzyl-oxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)-amino)hexanoate (**16**) (445 mg, 0.42 mmol, 88%) as a pale-green, viscous liquid.

TLC (EtOAc:Hex/1:1) R_f: 0.38 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = -5.7° (c = 19.9 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3361, 3065, 3035, 2969, 1707, 1678, 1598, 1519, 1455, 1394, 1372, 1328, 1292, 1249, 1167, 1059, 912, 851, 778, 734, 644, 580, 551. **HRMS** (ESI-IT) [m/z]: 1081.36958, calculated 1081.36892 for $[C_{56}H_{58}N_4O_{17}Na]^+$, err [ppm] 0.61. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 8.46 – 8.21 (m, 4H), 7.95 – 7.73 (m, 3H), 7.38 – 7.35 (m, 15H), 6.24 (d, *J* = 8.6 Hz, 1H), 6.11 (d, *J* = 8.5 Hz, 1H), 6.03 (d, *J* = 7.8 Hz, 1H), 5.51 – 5.32 (m, 3H), 5.26 – 5.08 (m, 6H), 4.96 (s, 1H), 4.90 – 4.66 (m, 3H), 4.48 – 4.25 (m, 2H), 4.05 (d, *J* = 12.0 Hz, 1H), 3.77 (d, *J* = 12.0 Hz, 1H), 3.33 (s, 1H), 3.20 – 3.12 (m, 2H), 1.95 – 1.74 (m, 2H), 1.72 – 1.57 (m, 2H), 1.44 (s, 9H).**^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 183.16, 182.61, 170.42, 169.22, 168.63, 156.70, 156.61, 156.38, 155.99, 141.50, 136.25, 136.12, 135.92, 134.51, 134.37, 133.80, 133.51, 133.43, 133.40, 133.22, 133.20, 133.12, 128.63, 128.56, 128.49, 128.41, 128.33, 128.28, 128.22,

128.16, 128.10, 128.01, 127.47, 127.42, 126.05, 79.45, 75.57, 67.59, 67.37, 67.29, 66.52, 65.05, 62.63, 56.79, 56.50, 53.64, 40.02, 28.47, 27.99, 26.33.

(5S,9S,12S,13S)-9,13-bis(((Benzyloxy)carbonyl)amino)-12-(3-((*tert*-butoxycarbonyl)amino)propyl)-5-(hydroxymethyl)-3,6,10-trioxo-1-phenyl-2,7,11-trioxa-4-azatetradecan-14-oic acid (17)

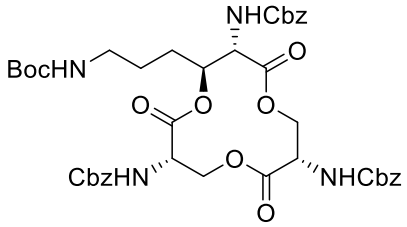


Chemical Formula: $C_{41}H_{50}N_4O_{15}$
Molecular Weight: 838,86

A solution of (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2*S*,3*S*)-3-((*N*-((benzyloxy)carbonyl)-O-(((benzyl-oxy)-carbonyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((*tert*-butoxycarbon-yl)-amino)hexanoate (**16**) (514 mg, 0.49 mmol, 1.0 equiv) and NMM (270 µL, 2.43 mmol, 5.0 equiv) in degassed $CHCl_3$ (12.0 mL, 0.016 M) and degassed *i*PrOH (19.0 mL, 0.016 M) was irradiated with light (Chamber reactor Model RPR-200 (Rayonet), 366 nm, 144 W) at 23°C for 2.5 h. The mixture was diluted with CH_2Cl_2 (50 mL), washed with aq. citric acid solution (10wt%, 30 mL) and brine (30 mL), over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5 → 85:15) yielding (5*S*,9*S*,12*S*,13*S*)-9,13-bis(((Benzyloxy)carbonyl)amino)-12-(3-((*tert*-butoxycarbonyl)amino)prop-yl)-5-(hydroxymeth-yl)-3,6,10-trioxo-1-phenyl-2,7,11-trioxa-4-azatetradecan-14-oic acid (**17**)) (265 mg, 0.32 mmol, 65%) as a white, amorphous solid.

TLC (CH_2Cl_2:MeOH /85:15) R_f: 0.50 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = -19.9° (c =7.8 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3367, 3065, 3035, 2971, 1697, 1605, 1515, 1455, 1411, 1337, 1248, 1206, 1167, 1056, 909, 850, 777, 729, 696, 645, 571. **HRMS** (ESI-IT) [m/z]: 861.31679, calculated 861.31649 for $[C_{41}H_{50}N_4O_{15}Na]^+$, err [ppm] 0.35. **^{1}H-NMR** (500 MHz, CD_3OD) δ [ppm]: 7.46 – 7.14 (m, 15H), 5.34 (d, *J* = 10.1 Hz, 1H), 5.21 – 4.95 (m, 6H), 4.65 – 4.50 (m, 3H), 4.32 (dt, *J* = 9.9, 4.6 Hz, 2H), 3.94 – 3.79 (m, 1H), 3.73 (dd, *J* = 11.5, 4.0 Hz, 1H), 3.03 – 2.96 m, 2H), 1.74 – 1.72 (m, 1H), 1.62 – 1.53 (m, 3H), 1.41 (s, 9H). [ppm]: **^{13}C-NMR** (126 MHz, CD_3OD) δ [ppm]: 173.99, 171.71, 170.44, 158.59, 158.46, 158.33, 138.14, 138.06, 138.00, 129.47, 129.45, 129.01, 128.98, 128.86, 79.90, 77.08, 67.90, 67.84, 65.41, 62.96, 58.59, 57.88, 54.84, 40.98, 28.81, 27.92, 27.04.

Tribenzyl ((3*S*,4*S*,7*S*,11*S*)-4-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,6,10-trioxo-1,5,9-trioxacyclododecane-3,7,11-triyl)tricarbamate (18)

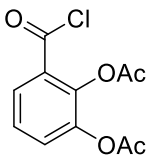


Chemical Formula: $C_{41}H_{48}N_4O_{14}$
Molecular Weight: 820,85

2,2'-Dithiodipyridine (13.0 mg, 59.0 µmol, 1.3 equiv) and triphenylphosphine (15.5 mg, 59.0 µmol, 1.3 equiv) was added to a solution of (5*S*,9*S*,12*S*,13*S*)-9,13-bis(((Benzyloxy)carbonyl)amino)-12-(3-((*tert*-butoxycarbonyl)amino)prop-yl)-5-(hydroxymethyl)-3,6,10-trioxo-1-phenyl-2,7,11-trioxa-4-aza-tetradecan-14-oic acid (**17**) (43.6 mg, 52.0 µmol, 1.0 equiv) in dry CH_2Cl_2 (400 µL, 0.13 M) under Argon atm. at 23°C and the mixture was stirred at 23°C for 90 min. Dry CH_2Cl_2 (400 µL, 0.07 M) and silver acetate (17.4 mg, 104.0 µmol, 2.0 equiv) was added and the mixture was stirred at 23°C for 13 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (EtOAc:Hex/1:1) tribenzyl ((3*S*,4*S*,7*S*,11*S*)-4-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,6,10-trioxo-1,5,9-trioxacyclodo-decane-3,7,11-triyl)tricarbamate (**18**) (14.5 mg, 17.6 µmol, 32%) as a white, amorphous solid.

TLC (EtOAc:Hex/1:1) R_f: 0.41 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = 3.0° (c = 8.0 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3322, 3064, 3035, 2968, 1698, 1521, 1454, 1393, 1367, 1333, 1249, 1205, 1171, 1049, 913, 848, 780, 735, 698, 646, 589, 535. **HRMS** (ESI-IT) [m/z]: 843.30621, calculated 843.30592 for $[C_{41}H_{48}N_4O_{14}Na]^+$, err [ppm] 0.34. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 7.48 – 7.22 (m, 15H), 6.03 (d, *J* = 9.1 Hz, 1H), 5.82 – 5.70 (m, 15H), 5.59 (d, *J* = 9.0 Hz, 1H), 5.16 – 5.08 (m, 3H), 4.66 – 4.51 (m, 5H), 4.27 – 4.17 (m, 2H), 3.15 – 3.05 (m, 2H), 1.86 – 1.82 (m, 2H), 1.51 – 1.49 (m, 2H), 1.41 (s, 9H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 169.92, 169.89, 168.52, 156.32, 155.87, 155.86, 155.67, 136.03, 135.96, 135.78, 128.73, 128.72, 128.68, 128.60, 128.53, 128.44, 128.42, 128.39, 128.30, 79.54, 67.72, 67.56, 67.55, 65.75, 65.34, 57.66, 53.94, 52.97, 39.58, 29.85, 29.07, 28.54, 25.89.

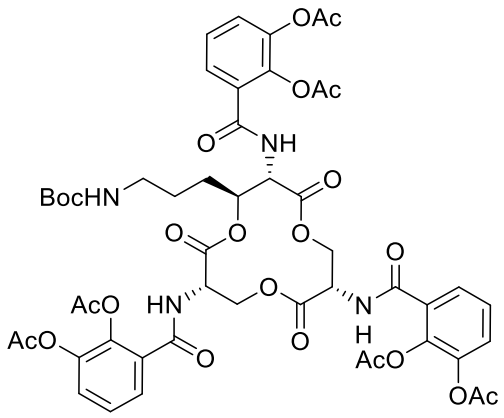
2,3-Diacetoxybenzoic acid chloride (19)



Chemical Formula: $C_{11}H_9ClO_5$
Molecular Weight: 256,64

According to the procedure of *Ji et al*.:[9] Oxalyl chloride (18 µL, 0.21 mmol, 2.0 equiv) and a drop of DMF were added to a slurry of 2,3-diacetoxybenzoic acid (25 mg, 0.10 mmol, 1.0 equiv) in dry CH_2Cl_2 (300 µL, 0.33 M) at 0°C and the mixture was stirred at 23°C for 1 h. The solution was concentrated under reduced pressure yielding 2,3-diacetoxybenzoic acid chloride (**20**) as a brownish, viscous liquid, which was used immediately in the next step without further purification or characterization.

(AcO)Ent$_{KL}$



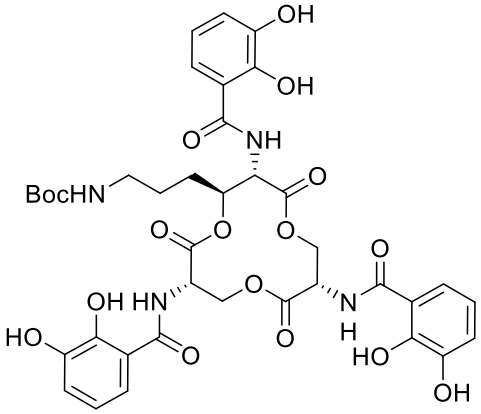
Chemical Formula: $C_{50}H_{54}N_4O_{23}$
Molecular Weight: 1078,99

Palladium on charcoal (10w% Pd, 12.0 mg ≈ 1.0 eq Pd) was added to a solution of tribenzyl ((3*S*,4*S*,7*S*,11*S*)-4-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,6,10-trioxo-1,5,9-trioxa-cyclododecane-3,7,11-triyl)tricar-bamate (**18**) (9.6 mg, 11.6 µmol, 1.0 equiv) in a mixture of MeOH (450 µL, 0.02 M), EtOAc (150 µL, 0.02 M) and TFA (17 µL, 0.02 M) under Argon atm. in an laboratory high pressure autoclave (HR-100, Berghof). A pressure of 20 bar H_2 atm. was applied and the mixture was stirred at 23°C for 2.5 h. The mixture was filtered through celite®, co-evaporated with toluene (5 mL) and concentrated under reduced pressure. The residue was dissolved in aq. $NaHCO_3$ solution (0.5 M, 200 µL, 0.03 M) at 0°C and a solution of 2,3-diacetoxybenzoic acid chloride (**19**)[9] (approx. 12 mg, 46.8 µmol, 4.0 equiv) in dry THF (200 µL, 0.03 M) was added over a period of 30 min via syringe pump at 0°C. The mixture was stirred for additional 30 min at 0 °C. The mixture was diluted with CH_2Cl_2 (10 mL) and brine (2 mL) and the phases were separated. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5 → 92.5:7.5) yielding **(AcO)Ent$_{KL}$** (9.2 mg, 8.5 µmol, 73%) as a white, amorphous solid.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.50 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = 9.7° (c =6.5 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3362, 2931, 2861, 1771, 1667, 1582, 1523, 1460, 1371, 1338, 1258, 1203, 1166, 1075, 1015, 906, 828, 801, 734, 672, 605, 579, 542. **LRMS** (ESI) [m/z]: 1101.1 $[C_{50}H_{54}N_4O_{23}Na]^+$, 979.1 $[C_{45}H_{47}N_4O_{21}]^+$. **HRMS** (ESI-IT) [m/z]: 1101.30862, calculated 1101.30738 for $[C_{50}H_{54}N_4O_{23}Na]^+$, err

[ppm] 1.13; 562.14851, calculated 562.14816 for $[C_{50}H_{54}N_4O_{23}Na_2]^{2+}$, err [ppm] 0.62. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 7.75 (d, *J* = 7.7 Hz, 1H), 7.71 (d, *J* = 7.9 Hz, 1H), 7.60 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.51 (dd, *J* = 7.1, 2.1 Hz, 1H), 7.45 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.32 – 7.26 (m, 4H), 7.25 (d, *J* = 1.3 Hz, 1H), 7.16 (t, *J* = 8.2 Hz, 1H), 5.24 – 5.09 (m, 2H), 4.96 – 4.85 (m, 3H), 4.76 (dd, *J* = 11.4, 4.2 Hz, 1H), 4.68 (s, 1H), 4.29 (dd, *J* = 11.6, 3.7 Hz, 1H), 4.19 (dd, *J* = 11.4, 4.6 Hz, 1H), 3.26 – 3.17 (m, 1H), 3.15 – 3.10 (m, 1H), 2.38 – 2.09 (m, 18H), 2.04 – 2.00 (m, 1H), 1.96 – 1.90 (m, 1H), 1.60 – 1.55 (m, 2H), 1.39 (s, 9H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 170.52, 168.90, 168.80, 168.75, 168.37, 168.23 (2C), 168.15, 168.14, 165.27, 165.12, 165.05, 156.26, 143.12, 143.03, 142.98, 140.69, 140.64, 140.42, 128.91, 128.78, 127.17, 126.79 – 126.83 (6C), 126.58, 126.52, 126.46, 79.51, 65.63, 65.25, 57.05, 53.04, 51.67, 39.64, 29.85, 29.62, 28.50 (3), 26.28, 20.76 (2C), 20.70 (2C), 20.51, 20.42.

Ent_{KL}

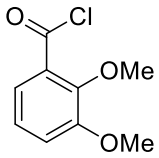


Chemical Formula: $C_{38}H_{42}N_4O_{17}$
Molecular Weight: 826,77

A methanolic solution of NH_3 (7 M in MeOH, 4.2 µL, 29 µmol, 12.0 equiv)) was added to a solution of **(AcO)Ent$_{KL}$** (2.6 mg, 2.4 µmol, 1.0 equiv) in MeOH (150 µL, 0.02 M) at 0 °C and the mixture was stirred for 45 min at 0 °C. The solvent was removed and the residue was dissolved in 500 µL of H_2O, filtered through a CHROMAFIL® 45 µm filter and purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (0.1% TFA):MeCN (0.1% TFA)/9:1 → 5:95), t_R = 22.8 min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **Ent$_{KL}$** (230 µg, 0.27 µmol, 11%) as a white, amorphous solid.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.00 [UV^{254}, Ninhydrin]. **HRMS** (ESI-IT) [m/z]: 825.24668, calculated 825.24722 for $[C_{34}H_{36}N_2O_9Na]^+$, err [ppm] -0.65.

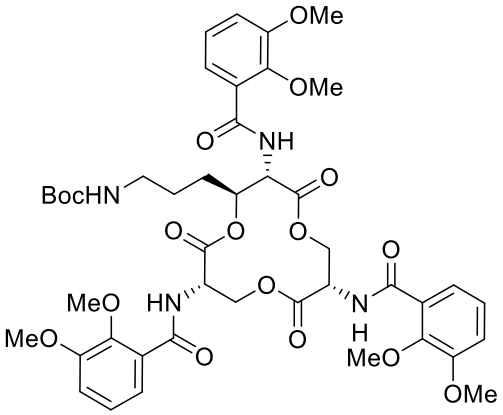
2,3-Dimethoxybenzoic acid chloride (20)



Chemical Formula: $C_9H_9ClO_3$
Molecular Weight: 200,62

Oxalyl chloride (23 µL, 0.26 mmol, 2.0 equiv) and a drop of DMF were added to a slurry of 2,3-dimethoxybenzoic acid (25 mg, 0.13 mmol, 1.0 equiv) in dry CH_2Cl_2 (350 µL, 0.37 M) at 0°C and the mixture was stirred at 23°C for 1 h. The solution was concentrated under reduced pressure yielding 2,3-dimethoxybenzoic acid chloride (**20**) as a brownish, viscous liquid, which was used immediately in the next step without further purification or characterization.

(MeO)Ent$_{KL}$



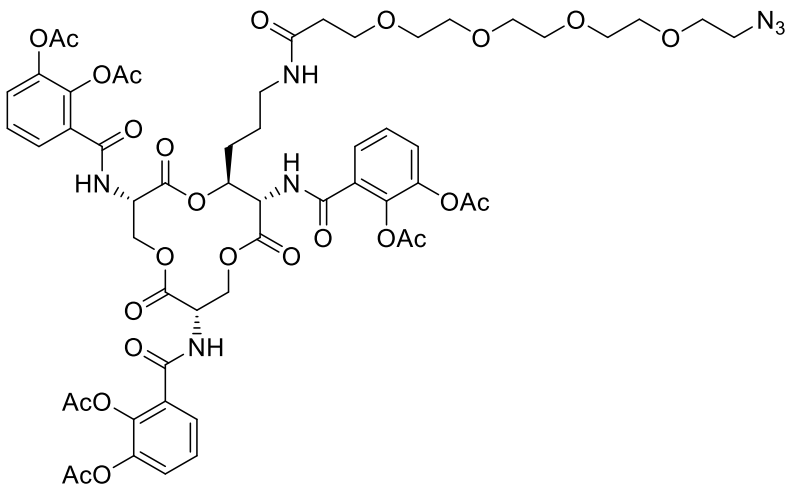
Chemical Formula: $C_{44}H_{54}N_4O_{17}$
Molecular Weight: 910,93

Palladium on charcoal (10w% Pd, 12.0 mg ≈ 1.0 eq Pd) was added to a solution of tribenzyl ((3*S*,4*S*,7*S*,11*S*)-4-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,6,10-trioxo-1,5,9-trioxa-cyclododecane-3,7,11-triyl)tricar-bamate (**18**) (9.0 mg, 10.6 µmol, 1.0 equiv) in a mixture of MeOH (450 µL, 0.02 M) EtOAc (150 µL, 0.02 M) and TFA (17 µL, 0.02 M) under Argon atm. in an laboratory high pressure autoclave (HR-100, Berghof). A pressure of 20 bar H_2 atm. was applied and the mixture was stirred at 23°C for 2.5 h. The mixture was filtered through celite®, co-evaporated with toluene (5 mL) and concentrated under reduced pressure. The residue was dissolved in aq. $NaHCO_3$ solution (0.5 M, 200 µL, 0.03 M) at 0°C and a solution of 2,3-dimethoxybenzoic acid chloride (**20**)[9] (approx. 9 mg, 44.9 µmol, 4.0 equiv) in dry THF (200 µL, 0.03 M) was added over a period of 30 min via syringe pump at 0°C. The mixture was stirred for additional 30 min at 0 °C. The mixture was diluted with CH_2Cl_2 (10 mL) and brine (2 mL) and the phases were separated. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via preparative thin layer

chromatography (CH_2Cl_2:MeOH/92.5:7.5) yielding **(MeO)Ent$_{KL}$** (8.9 mg, 9.7 µmol, 92%) as a white, amorphous solid.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.50 [UV^{254}, Ninhydrin]. $[\alpha]_D^{23°C}$ = 10.0° (c =3.8 mg/mL in $CHCl_3$). **IR** (ATR) [cm^{-1}]: 3354, 2935, 2861, 1750, 1709, 1657, 1579, 1519, 1470, 1430, 1391, 1367, 1339, 1311, 1265, 1233, 1199, 1169, 1132, 1063, 993, 804, 755, 620, 584, 545. **LRMS** (ESI) [m/z]: 1820.4 $[C_{88}H_{109}N_8O_{34}]^+$, 1720.7 $[C_{83}H_{101}N_8O_{32}]^+$, 910.6 $[C_{44}H_{55}N_4O_{17}]^+$, 810.2 $[C_{39}H_{47}N_4O_{15}]^+$. **HRMS** (ESI-IT) [m/z]: 933.33876, calculated 933.33762 for $[C_{44}H_{54}N_4O_{17}Na]^+$, err [ppm] 1.22; 1844.69002, calculated 1844.68937 for $[C_{88}H_{108}N_8O_{34}Na]^+$, err [ppm] 0.35. **^{1}H-NMR** (600 MHz, $CDCl_3$) δ [ppm]: 8.99 (d, *J* = 7.8 Hz, 2H), 8.90 (d, *J* = 7.1 Hz, 1H), 7.69 – 7.67 (m, 3H), 7.16 – 7.10 (m, 3H), 7.09 – 7.03 (m, 3H), 5.26 – 5.23 (m, 1H), 5.20 – 5.14 (m, 2H), 5.11 (dd, *J* = 8.3, 5.4 Hz, 1H), 4.77 (s, 1H), 4.64 (dd, *J* = 11.3, 4.3 Hz, 1H), 4.58 – 4.48 (m, 2H), 4.43 (dd, *J* = 10.9, 7.9 Hz, 1), 4.05 – 3.73 (m, 18H), 3.15 (d, *J* = 7.5 Hz, 2H), 2.04 – 1.88 (m, 2H), 1.70 – 1.66 m, 2H), 1.39 (s, 9H). **^{13}C-NMR** (151 MHz, $CDCl_3$) δ [ppm]: 170.26, 169.33, 169.05, 165.00, 164.90, 164.85, 155.99, 152.66, 152.65, 152.64, 148.24, 148.17, 148.12, 125.19, 125.14, 125.13, 124.34, 124.29, 124.21, 122.97, 122.90, 122.87, 116.24, 79.00, 65.17, 64.82, 61.54, 61.39, 61.38, 56.17, 56.16, 56.14, 55.66, 52.16, 51.31, 28.37.

(AcO)Ent$_{KL}$-PEG$_4$-N$_3$



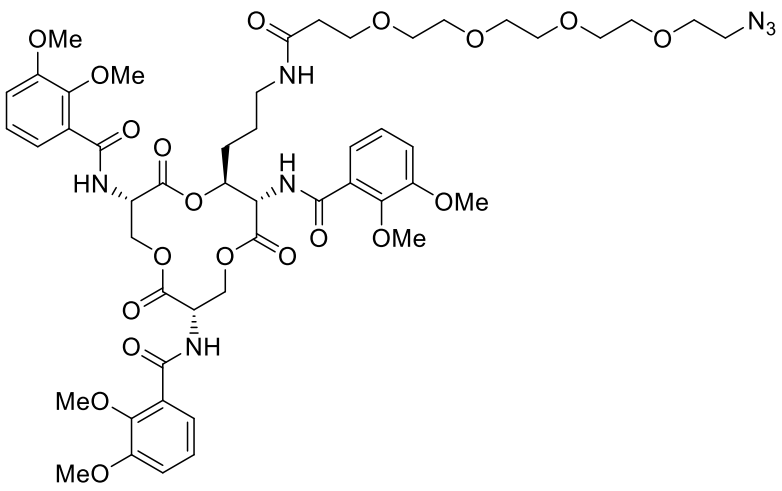
Chemical Formula: $C_{56}H_{65}N_7O_{26}$
Molecular Weight: 1252,16

TFA (120 µL, 1.57 mmol, 196 equiv) was added to a solution of **(AcO)Ent$_{KL}$** (8.7 mg, 8.1 µmol, 1.0 equiv) in dry CH_2Cl_2 (600 µL, 0.014 M) at 0°C and the solution was stirred at 23°C for 1 h. The mixture was co-evaporated with toluene (2 x 5 mL) and concentrated under reduced pressure. The residue was dissolved in dry THF (150 µL, 0.05 M) at 23°C and **N$_3$-PEG$_4$-NHS** (7.8 mg, 18.0 µmol, 2.5 equiv) was added. A solution of DiPEA (2.80 µL, 16.1 µmol, 2.0 equiv) in dry THF (150 µL, 0.11 M) was added at 23°C dropwise over a period of 90 min. The mixture was concentrated under reduced pressure and

the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5 → 92.5:7.5) yielding **(AcO)Ent$_{KL}$-PEG$_4$-N$_3$** (5.8 mg, 4.69 µmol, 58%) as a colorless oil.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.35 [UV^{254}, Ninhydrin]. **IR** (ATR) [cm^{-1}]: 3359, 3308, 3199, 2922, 2854, 2103, 1771, 1660, 1527, 1463, 1421, 1371, 1257, 1203, 1168, 1127, 1078, 1017, 902, 669, 643, 589, 570, 542. **HRMS** (ESI-IT) [m/z]: 1274.38766, calculated 1274.38715 for $[C_{56}H_{65}N_7O_{26}Na]^+$, err [ppm] 0.40; 648.68894, calculated 648.68818 for $[C_{56}H_{65}N_7O_{26}Na_2]^{2+}$, err [ppm] 1.17. **^{1}H-NMR** (700 MHz, $CDCl_3$) δ [ppm]: 7.98 (s, 1H), 7.75 (s, 1H), 7.66 – 7.58 (m, 2H), 7.51 – 7.51 (m, 1H), 7.35 – 7.25 (s, 5H), 7.18 (t, J = 7.9 Hz, 1H), 6.82 (s, 1H), 5.46 – 5.43 (m, 1H), 5.40 – 5.30 (m, 6H), 5.15 (dt, J = 8.1, 4.1 Hz, 1H), 4.96 (dt, J = 7.4, 3.7 Hz, 1H), 4.88 – 4.73 (m, 2H), 4.38 (dd, J = 11.7, 3.7 Hz, 1H), 4.27 – 4.21 (m, 1H), 3.72 – 3.54 (m, 14H), 3.38 (t, J = 5.0 Hz, 2H), 2.45 – 2.12 (m, 18H), 1.91 (q, J = 7.2 Hz, 2H), 1.60 – 1.57 (m, 4H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 169.06, 168.86, 168.63, 168.25, 168.21, 168.15, 166.86, 165.23, 165.16, 143.14, 143.08, 142.98, 140.81, 140.77, 140.73, 140.43, 128.81, 127.27, 127.19, 126.88, 126.85, 126.80, 126.77, 126.72, 126.67, 126.63, 126.59, 126.54, 126.51, 126.49, 126.45, 70.89, 70.86, 70.83, 70.81, 70.79, 70.66, 70.62, 70.39, 70.18, 70.15, 67.13, 65.87, 56.63, 53.58, 53.34, 50.85, 50.80, 32.31, 32.08, 29.85, 25.73, 22.85, 20.76, 20.45, 14.28.

(MeO)Ent$_{KL}$-PEG$_4$-N$_3$



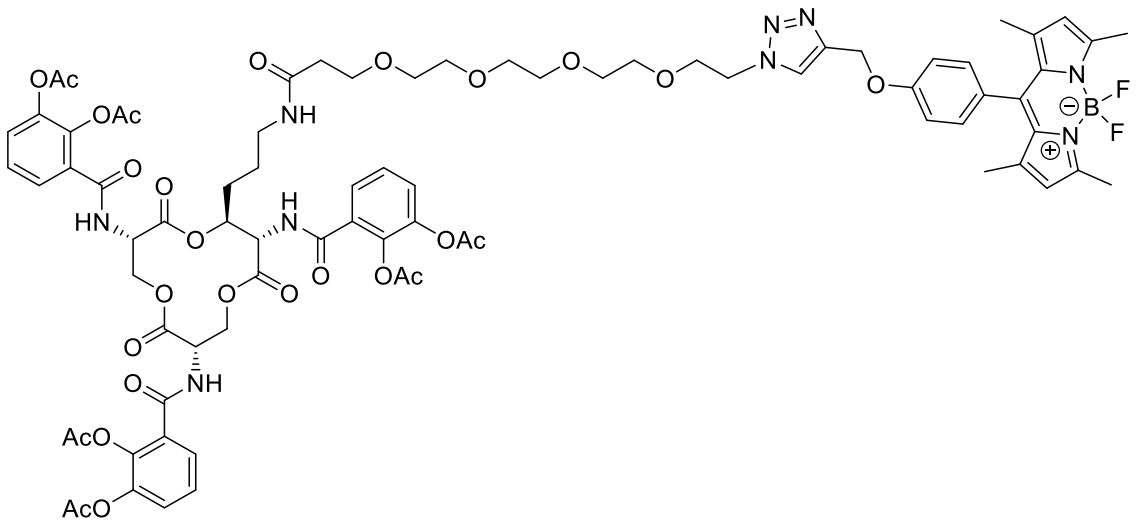
Chemical Formula: $C_{50}H_{65}N_7O_{20}$
Molecular Weight: 1084,10

TFA (100 µL, 1.32 mmol, 279 equiv) was added to a solution of **(MeO)Ent$_{KL}$** (4.3 mg, 4.7 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 0.009 M) at 0°C under Argon atm. and the solution was stirred at 23°C for 1 h. The mixture was co-evaporated with toluene (2 x 5 mL) and concentrated under reduced pressure. The residue was dissolved in dry THF (150 µL, 0.03 M) at 23°C and **N$_3$-PEG$_4$-NHS** (2.8 mg, 7.1 µmol, 1.5 equiv) was added. A solution of DiPEA (1.25 µL, 7.1 µmol, 1.5 equiv) in dry THF (150 µL,

0.02 M) was added at 23°C dropwise over a period of 10 min and stirred at 23°C for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5 → 92.5:7.5) yielding **(MeO)Ent$_{KL}$-PEG$_4$-N$_3$** (4.5 mg, 4.16 µmol, 89%) as a colorless oil.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.35 [UV^{254}, Ninhydrin]. **IR** (ATR) [cm^{-1}]: 3350, 2931, 2870, 2105, 1818, 1743, 1658, 1579, 1519, 1466, 1357, 1305, 1265, 1229, 1205, 1116, 1971, 993, 932, 812, 756, 647, 582. **HRMS** (ESI-IT) [m/z]: 1106.41827, calculated 1106.41766 for $[C_{50}H_{65}N_7O_{20}Na]^+$, err [ppm] 0.55; 564.70358, calculated 564.70344 for $[C_{50}H_{65}N_7O_{20}Na_2]^{2+}$, err [ppm] 0.25. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 9.00 – 8.86 (m, 2H), 8.84 (d, J = 7.1 Hz, 1H), 7.64 – 7.52 (m, 3H), 7.12 – 6.94 (m, 6H), 6.55 (s, 1H), 5.21- 5.18 (m, 1H), 5.12 – 5.05 (m, 2H), 5.02 (dd, J = 8.2, 5.4 Hz, 1H), 4.58 (dd, J = 11.3, 4.3 Hz, 1H), 4.49 (dd, J = 10.8, 4.3 Hz, 1H), 4.43 (dd, J = 11.3, 5.4 Hz, 1H), 4.36 (dd, J = 10.8, 7.7 Hz, 1H), 3.94 – 3.72 (m, 16H), 3.68 – 3.40 (m, 18H), 3.31 (t, J = 5.0 Hz, 2H), 3.25 – 3.11 (m, 2H), 1.94 – 1.89 (m, 2H), 1.65 – 1.63 (m, 4H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: δ 171.64, 169.27, 169.05, 165.01, 164.90, 164.84, 152.68, 152.65, 148.23, 148.16, 148.10, 125.11, 124.35, 124.27, 124.21, 122.87, 122.85, 122.84, 122.79, 122.79, 116.22, 116.20, 70.64, 70.56, 70.47, 70.31, 70.16, 70.00, 67.24, 61.55, 61.39, 56.15, 55.76, 51.32, 50.66, 33.46, 31.93, 29.74, 29.71, 29.67, 29.63, 29.42, 29.37, 23.29, 22.70, 18.42, 14.13.

(AcO)Ent$_{KL}$-PEG$_4$-BODIPY



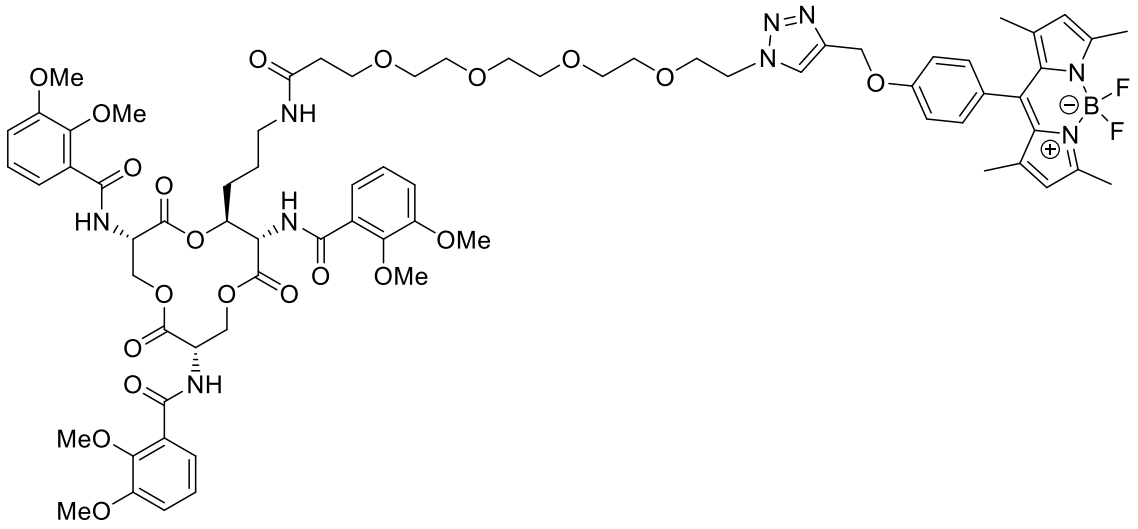
Chemical Formula: $C_{78}H_{86}BF_2N_9O_{27}$
Molecular Weight: 1630,39

An aliquot of a stock solution of TBTA (0.10 mg, 0.19 µmol, 0.1 equiv ≈4.0 µL) in DMSO (0.047 M), an aliquot of a stock solution of NaAsc (80 µg, 0.40 µmol, 0.2 quiv ≈3.3 µL) in H_2O (0.13 M) and an aliquot of a stock solution of $CuSO_4$ (16 µg, 0.10 µmol, 0.05 equiv ≈0.8 µL) in H_2O (0.13 M) were added to a solution of **(AcO)Ent$_{KL}$-PEG$_4$-N$_3$** (2.4 mg, 1.9 µmol, 1.0 equiv) and 5,5-difluoro-10-(4-(prop-2-yn-1-yloxy)phenyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**BODIPY-alkyne**) (0.9 mg, 2.3

µmol, 1.2 equiv) in a mixture of DMSO (150 µL, 0.02 M), H_2O (30 µL, 0.02 M), THF (30 µL, 0.02 M) and MeOH (30 µL, 0.02 M) and. The mixture was stirred at 23°C for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5 → 9:1) yielding **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY** (2.5 mg, 1.55 µmol, 82%) as a red, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.60 [UV^{254}, Ninhydrin]. **IR** (ATR) [cm^{-1}]: 3356, 2924, 2855, 1771, 1663, 1611, 1544, 1515, 1463, 1409, 1370, 1307, 1256, 1198, 1163, 1113, 1081, 1015, 979, 903, 827, 804, 760, 731, 703, 600, 544. **LRMS** (ESI) [m/z]: 1630.6 $[C_{81}H_{87}BF_2N_9O_{27}]^+$, 805.8 $[C_{76}H_{85}N_9O_{26}]^{2+}$. **HRMS** (ESI-IT) [m/z]: 1652.56007, calculated 1652.55865 for $[C_{78}H_{86}BF_2N_9O_{27}Na]^+$, err [ppm] 0.86; 837.77465, calculated 837.77393 for $[C_{78}H_{86}BF_2N_9O_{27}Na_2]^{2+}$, err [ppm] 0.86. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 7.92 – 7.90 (m, 1H), 7.68 – 7.42 (m, 2H), 7.42 – 7.26 (m, 4H), 7.21 – 6.98 (m, 5H), 5.97 (s, 2H), 5.48 – 5.30 (m, 1H), 5.24 – 5.21 (m, 2H), 5.15 – 4.92 (m, 1H), 4.87 – 4.72 (m, 2H), 4.60 – 4.53 (m, 2H), 4.38 – 4.19 (m, 2H), 3.91 – 3.81 (m, 2H), 3.67 – 3.47 (m, 16H), 2.55 (s, 6H), 2.40 – 2.16 (m, 18H), 1.42 (s, 6H), 1.36 – 1.27 (m, 4H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 168.72, 168.48, 168.04, 168.00, 167.92, 165.07, 165.05, 165.00, 155.33, 143.08, 142.98, 142.91, 142.83, 141.56, 140.61, 140.27, 133.97, 131.76, 130.01, 129.71, 129.29, 129.28, 129.22, 128.98, 128.13, 127.62, 127.04, 126.57, 126.53, 126.51, 126.49, 126.46, 126.43, 126.39, 126.35, 126.32, 126.30, 121.14, 115.39, 70.57, 70.52, 70.49, 70.47, 70.45, 70.44, 70.26, 70.04, 69.30, 66.98, 53.16, 51.54, 31.92, 29.76, 29.70, 29.66, 29.36, 29.32, 29.23, 27.22, 27.16, 25.50, 22.69, 20.60, 20.28, 14.58, 14.12. **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: -146.7 (q, *J* = 33.2 Hz). **^{11}B-NMR** (128 MHz, $CDCl_3$) δ [ppm]: 1.0 (t, *J* = 33.2 Hz). **UV-Vis/fluorescence emission:** λ_{Ex}= 498 nm, λ_{Em}= 509 nm (c = 0.102 mg/10 mL in MeOH).

(MeO)Ent$_{KL}$-PEG$_4$-BODIPY

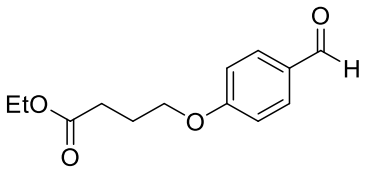


Chemical Formula: $C_{72}H_{86}BF_2N_9O_{21}$
Molecular Weight: 1462,33

An aliquot of a stock solution of TBTA (0.19 mg, 0.36 µmol, 0.1 equiv ≈7.6 µL) in DMSO (0.047 M), an aliquot of a stock solution of NaAsc (150 µg, 0.76 µmol, 0.2 equiv ≈6.3 µL) in H_2O (13 M) and an aliquot of a stock solution of $CuSO_4$ (30 µg, 0.19 µmol, 0.05 equiv ≈1.5 µL) in H_2O (13 M) were added to a solution of **(MeO)Ent$_{KL}$-PEG$_4$-N$_3$** (3.9 mg, 3.5 µmol, 1.0 equiv) and 5,5-difluoro-10-(4-(prop-2-yn-1-yloxy)phenyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (1.6 mg, 4.3 µmol, 1.3 equiv) (**BODIPY-alkyne**) in a mixture of DMSO (150 µL, 0.02 M), H_2O (30 µL, 0.02 M), THF (30 µL, 0.02 M) and MeOH (30 µL, 0.02 M) and. The mixture was stirred at 23°C for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/92.5:7.5) yielding **(MeO)Ent$_{KL}$-PEG$_4$-BODIPY** (4.4 mg, 3.0 µmol, 87%) as a red, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.60 [UV^{254}, Ninhydrin]. **IR** (ATR) [cm^{-1}]: 3362, 2931 2861, 1771, 1667, 1582, 1523, 1460, 1371, 1338, 1258, 1203, 1166, 1075, 1015, 906, 828, 801, 734, 672, 665, 579, 542. **LRMS** (ESI) [m/z]: 1462.6 $[C_{72}H_{87}BF_2N_9O_{21}]^+$**HRMS** (ESI-IT) [m/z]: 1484.59073, calculated 1484.58916 for $[C_{72}H_{86}BF_2N_9O_{21}Na]^+$, err [ppm] 1.15. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 9.12 – 8.78 (m, 2H), 7.83 (s, 1H), 7.70 – 7.52 (m, 3H), 7.30 (d, *J* = 5.1 Hz, 1H), 7.16 – 6.95 (m, 10H), 5.90 (s, 2H), 5.17 (s, 2H), 5.13 – 4.97 (m, 3H), 4.62 – 4.32 (m, 5H), 3.93 – 3.67 (m, 20H), 3.65 – 3.42 (m, 18H), 3.09 (d, *J* = 6.5 Hz, 2H), 2.47 (s, 6H), 1.52 – 1.47 (m, 4H), 1.35 (s, 6H). **^{13}C-NMR** (126 MHz, $CDCl_3$) δ [ppm]: 170.26, 169.21, 169.06, 165.00, 164.90, 164.83, 158.89, 155.28, 152.67, 152.64, 152.63, 148.20, 148.15, 148.08, 143.10, 141.61, 131.77, 129.26, 129.22, 128.12, 125.14, 125.05, 124.41, 124.35, 124.27, 124.22, 122.83, 122.79, 122.78, 122.73, 121.12, 121.11, 116.21, 116.20, 115.96, 115.40, 70.56, 70.53, 70.50, 70.45, 70.33, 70.17, 69.37, 67.20, 65.12, 64.79, 63.97, 62.55, 61.54, 61.38, 56.13, 55.76, 55.35, 52.74, 52.13, 51.31, 31.92, 29.71, 28.41, 26.64, 24.77, 22.69, 14.58, 14.12. **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: -146.7 (q, *J* = 33.2 Hz). **^{11}B-NMR** (128 MHz, $CDCl_3$) δ [ppm]: 1.0 (t, *J* = 33.2 Hz). **UV-Vis/fluorescence emission:** λ_{Ex}= 498 nm, λ_{Em}= 509 nm (c = 0.120 mg/10 mL in MeOH).

Ethyl 4-(4-formylphenoxy)butanoate



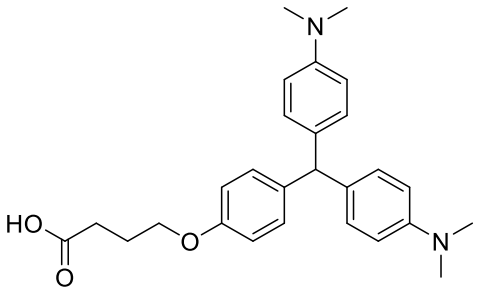
Chemical Formula: $C_{13}H_{16}O_4$
Molecular Weight: 236,27

4-Bromobutyric acid ethyl ester (427 µL, 3.29 mmol, 1.2 equiv) was added to a mixture of *p*-hydroxy benzaldehyde (300 mg, 2.46 mmol, 1.0 equiv) and K_2CO_3 (408 mg, 2.95 mmol, 1.2 equiv) in dry DMF (5.0 mL, 0.6 M) under Argon atm. at 0 °C. The mixture was stirred for 12 h at 23 °C. The mixture was

diluted with EtOAc (50 mL) and quenched by addition of water (50 mL). The phases were separated and the organic layer was washed with water (2x 50 mL) and brine (20 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (EtOAc:Hex/1:9) yielding ethyl 4-(4-formylphenoxy)butanoate (560 mg, 2.37 mmol, 96%) as a colorless oil.

TLC (EtOAc:Hex/1:9) R_f: 0.25 [UV^{254}, Ninhydrin]. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 9.87 (s, 1H), 7.90 – 7.74 (m, 2H), 6.98 (d, *J* = 8.7 Hz, 2H), 4.27 – 3.97 (m, 4H), 2.52 (t, *J* = 7.2 Hz, 2H), 2.27 – 2.04 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). **^{13}C-NMR** (76 MHz, CDCl3) δ [ppm]: 190.88, 173.07, 163.95, 132.07, 130.02, 114.82, 67.21, 60.63, 30.68, 24.49, 14.31. The analytical data were in accordance with the literature.[10]

4-(4-(Bis(4-(dimethylamino)phenyl)methyl)phenoxy)butanoic acid



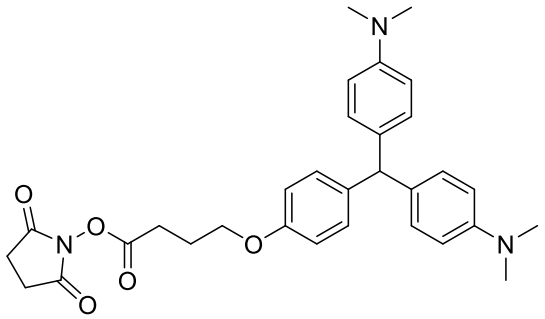
Chemical Formula: $C_{27}H_{32}N_2O_3$
Molecular Weight: 432,56

$ZnCl_2$ (173 mg, 1.23 mmol, 2.0 equiv) was added to a solution of ethyl 4-(4-formylphenoxy)butanoate (150 mg, 0.64 mmol, 1.0 equiv) and N,N-dimethylaniline (161 µL, 1.23 mmol, 2.0 equiv) in dry EtOH (6.0 mL, 0.1 M) under Argon atm. at 23 °C and the mixture was heated to 90°C for 2 d. The reaction was quenched by addition of water (30 mL) and extracted with EtOAc (2x 30 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in THF (3.0 mL, 0.22 M) and a solution of KOH (72 mg, 1.28 mmol, 2.0 equiv) in H_2O (0.5 mL, 2.56 M) was added. The mixture was heated to 50°C for 5 h, cooled down to 0°C and acidified with aqueous HCl solution (1.0 M, 20 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2x 15 mL), the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography trough silica gel (EtOAc:Hex/6:4-1:0) yielding 4-(4-(bis(4-(dimethylamino)phenyl)methyl)-phenoxy)butanoic acid (147 mg, 0.34 mmol, 53%) as pale blue oil.

TLC (EtOAc:Hex/6:4) R_f: 0.25 [UV^{254}, CAM]. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 7.99 (s, 1H), 7.04 – 6.97 (m, 6H), 6.78 (d, *J* = 8.6 Hz, 2H), 6.68 (d, *J* = 8.7 Hz, 4H), 5.33 (s, 1H), 3.94 (t, *J* = 6.1 Hz, 2H), 2.91 (s, 12H), 2.49 (t, *J* = 7.3 Hz, 2H), 2.10 - 2.03 (m, 2H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 179.19, 157.06,

148.97, 137.69, 133.58, 130.30, 129.96, 114.13, 112.90, 66.82, 54.26, 40.98, 31.46, 24.88. The analytical data were in accordance with the literature.[11]

2,5-Dioxopyrrolidin-1-yl 4-(4-(bis(4-(dimethylamino)phenyl)methyl)phenoxy)butanoate

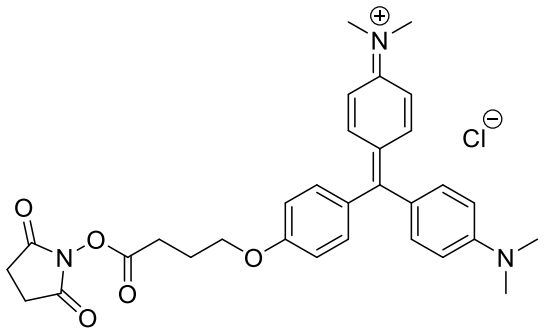


Chemical Formula: $C_{31}H_{35}N_3O_5$
Molecular Weight: 529,64

EDCI HCl (22 mg, 115 µmol, 2.0 equiv) and DMAP (0.7 mg, 5.7 µmol, 0.1 equiv) was added to a solution of 4-(4-(bis(4-(dimethylamino)phenyl)methyl)phenoxy)butanoic acid (25 mg, 58 µmol, 1.0 equiv), *N*-hydroxysuccinimide (13.5 mg, 117 µmol, 2.0 equiv) and DiPEA (30 µL, 173 µmol, 3.0 equiv) in dry CH_2Cl_2 (1.0 mL, 0.06 M) under Argon atm. at 23 °C and the mixture was stirred for 12 h at 23°C. The mixture was concentrated under reduced pressure and purified by flash column chromatography trough silica gel (EtOAc:Hex/6:4) yielding 2,5-dioxopyrrolidin-1-yl 4-(4-(bis(4-(dimethylamino)phenyl)-methyl)phenoxy)butanoate (19 mg, 36 µmol, 62%) as pale blue oil.

TLC (EtOAc:Hex/6:4) R_f: 0.30 [UV^{254}, CAM]. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: δ 7.08 – 6.92 (m, 6H), 6.85 – 6.77 (m, 2H), 6.75 – 6.60 (m, 4H), 5.33 (s, 1H), 4.02 (t, J = 5.9 Hz, 2H), 2.91 (s, 12H), 2.91 – 2.78 (m, 6H), 2.28 – 2.14 (m, 2H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 169.21, 168.53, 156.83, 148.97, 138.05, 133.35, 130.38, 129.99, 114.16, 112.70, 65.96, 54.26, 40.93, 27.95, 25.70, 24.63. The analytical data were in accordance with the literature.[12]

N-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)-methylene)cyclohexa-2,5-dien-1-ylidene)-*N*-methylmethanaminium chloride (MG-NHS)

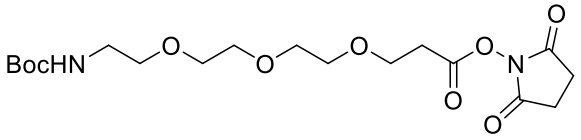


Molecular Weight: 564,08
Chemical Formula: $C_{31}H_{34}N_3O_5Cl$

p-Chloranil (22 mg, 91 µmol, 2.0 equiv) was added to a solution of 4-(4-(bis(4-(dimethylamino)-phenyl)methyl)phenoxy)butanoate (24 mg, 45 µmol, 1.0 equiv) in EtOAc (2 mL, 0.02 M) at 23°C. The mixture was stirred for 2.5 h at 23°C. The mixture was concentrated under reduced pressure and purified by flash column chromatography trough silica gel (CH_2Cl_2:MeOH/9:1) yielding *N*-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)methylene)cyclo-hexa-2,5-dien-1-ylidene)-*N*-methylmethanaminium chloride (**MG-NHS**) (5.0 mg, 8.9 µmol, 20%) as a dark green, amorph solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.20 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3398, 2928, 1812, 1781, 1735, 1585, 1479, 1423, 1365, 1299, 1260, 1173, 1128, 1069, 940, 910, 837, 801, 722, 646, 573. **HRMS** (ESI) [m/z]: 528.24945, calculated 528.24930 for $[C_{31}H_{34}N_3O_5]^+$, err [ppm] 0.28. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 7.52 – 7.21 (m, 6H), 7.07 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 9.1 Hz, 4H), 4.23 (t, J = 5.9 Hz, 2H), 3.35 (s, 12H), 2.90 – 2.86 (m, 6H), 2.34 – 2.25 (m, 2H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 178.16, 169.30, 168.29, 163.92, 156.81, 140.88, 137.83, 132.05, 127.18, 115.10, 113.56, 66.75, 41.15, 27.76, 25.77, 24.30.

2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (NHS-PEG$_3$-NHBoc)



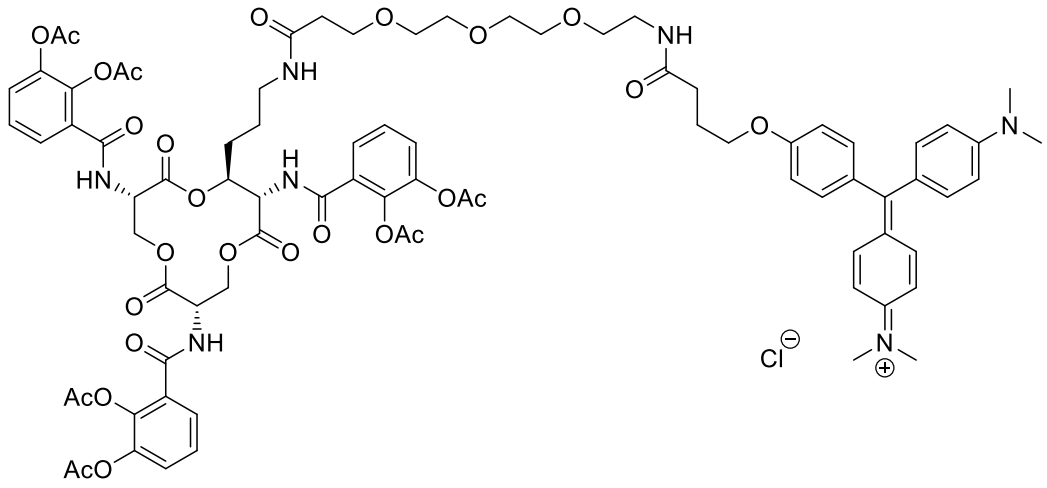
Chemical Formula: $C_{18}H_{30}N_2O_9$
Molecular Weight: 418,44

EDCI HCl (60 mg, 0.31 mol, 2.0 equiv) and DMAP (1.9 mg, 16 µmol, 0.1 equiv) were added to a solution of 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oic acid (50 mg, 0.155 mmol, 1.0 equiv), *N*-hydroxysuccinimide (36 mg, 0.31 µmol, 2.0 equiv) and DiPEA (82 µL, 0.47 mmol, 3.0 equiv) in dry THF (1.0 mL, 0.16 M) and dry DMF (0.5 mL, 0.31 M) under Argon atm. at 23 °C. The mixture was stirred for 6 h at 23 °C. The mixture was concentrated under reduced pressure and purified by flash column chromatography trough silica gel (CH_2Cl_2:MeOH/9:1) yielding 2,5-dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (**NHS-PEG$_3$-NHBoc**) (65 mg, 0.155 mmol, 100%) as a colorless oil.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.25 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3401, 2927, 1812, 1781, 1584, 1478, 1425, 1364, 1298, 1259, 1172, 1068, 942, 908, 836, 722, 650, 571, 542. **HRMS** (ESI) [m/z]: 441.18505, calculated 441.18435 for $[C_{18}H_{30}N_2O_9Na]^+$, err [ppm] 0.28. **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 5.03 (s, 1H), 3.81 (t, J = 6.4 Hz, 2H), 3.61 (s, 4H), 3.59 – 3.57 (m, 4H), 3.28 – 3.23 (m, 2H), 2.86 (t, J = 6.4 Hz,

2H), 2.81 – 2.77 (m, 4H), 1.39 (s, 9H). **^{13}C-NMR** (76 MHz, $CDCl_3$) δ [ppm]: 169.09, 166.78, 156.01, 79.14, 70.75, 70.57, 70.49, 70.24, 70.18, 65.73, 40.37, 32.16, 28.44, 25.60.

(AcO)Ent_{KL}-PEG_3-MG



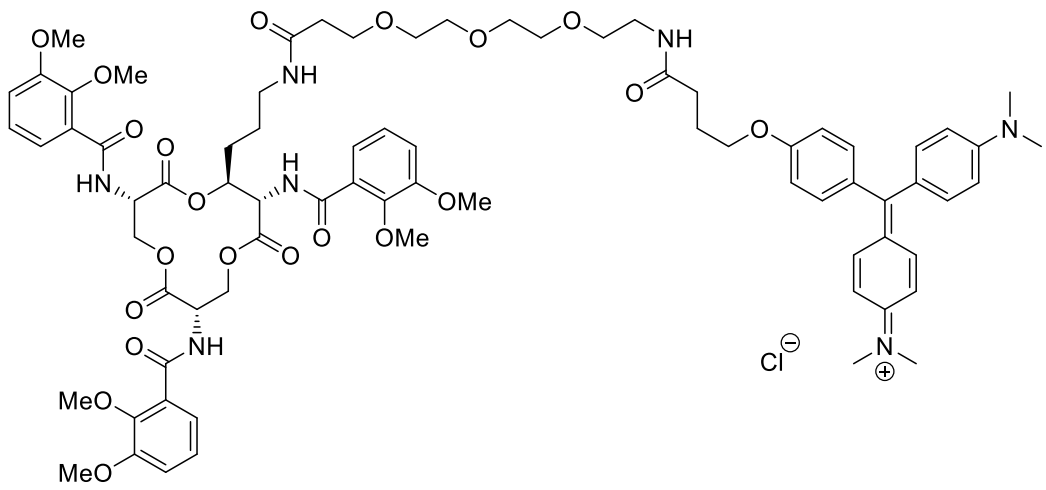
Chemical Formula: $C_{81}H_{92}N_7O_{27}Cl$
Molecular Weight: 1631,10

TFA (100 µL, 1.31 mmol, 215 equiv) was added to a solution of **(AcO)Ent_{KL}** (6.6 mg, 6.1 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 0.012 M) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min. The mixture was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (150 µL, 0.4 M) and PBS buffer (pH = 8.0, 50 µL, 0.12 M) and 2,5-dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (**NHS-PEG_3-NHBoc**) (3.3 mg, 7.8 µmol, 1.3 eq) was added at 23°C. The solution was stirred at 23°C for 8.5 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/92.5:7.5). The residue was dissolved in dry CH_2Cl_2 (500 µL, 0.012 M) under Argon atm. and TFA (100 µL, 1.31 mmol, 215 equiv) was added at 0°C. The solution was stirred at 23°C for 50 min, before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (150 µL, 0.04 M) and PBS buffer (pH = 8.0, 50 µL, 0.12 M) and *N*-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)methylene)-cyclohexa-2,5-dien-1-ylidene)-*N*-methylmethanaminium chloride (**MG-NHS**) (1.8 mg, 3.3 µmol, 0.54 eq) was added at 23°C. The solution was stirred at 23°C for 7 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography trough silica gel (CH_2Cl_2:MeOH/9:1) yielding **(AcO)Ent_{KL}-PEG_3-MG** (2.9 mg, 1.78 µmol, 29%) as a dark green, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.31 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 2925, 2857, 1773, 1739, 1690, 1661, 1586, 1473, 1425, 1366, 1300, 1260, 1172, 1122, 1068, 1022, 942, 910, 835, 801, 720, 621, 576. **LRMS** (ESI)

[m/z]: 1594.5 $[C_{81}H_{93}N_7O_{27}]^+$, 798.2 $[C_{81}H_{94}N_7O_{27}]^{2+}$. **HRMS** (ESI) [m/z]: 1594.60352, calculated 1594.60357 for $[C_{81}H_{92}N_7O_{27}]^+$, err [ppm] – 0.03, [m/z]: 797.80578, calculated 797.80542 for $[C_{81}H_{93}N_7O_{27}]^{2+}$, err [ppm] 0.45. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 7.49 – 7.29 (m, 10H), 7.24 – 6.98 (m, 10H), 6.98 – 6.88 (m, 4H), 5.40 – 5.36 (m, 1H), 5.36 – 5.33 (m, 2H), 5.19 – 4.76 (m, 3H), 4.31 – 4.11 (m, 4H), 3.74 – 3.52 (m, 14H), 3.36 – 3.19 (m, 12H), 3.16 – 3.11 (m, 2H), 2.46 (s, 2H), 2.38 – 2.09 (m, 18H), 2.08 – 1.94 (m, 4H), 1.60 – 1.44 (m, 4H). **UV-Vis** λ_{Ex}= 606 nm, 465 nm (c = 0.061 mg/10 mL in MeOH).

(MeO)Ent$_{KL}$-PEG$_3$-MG



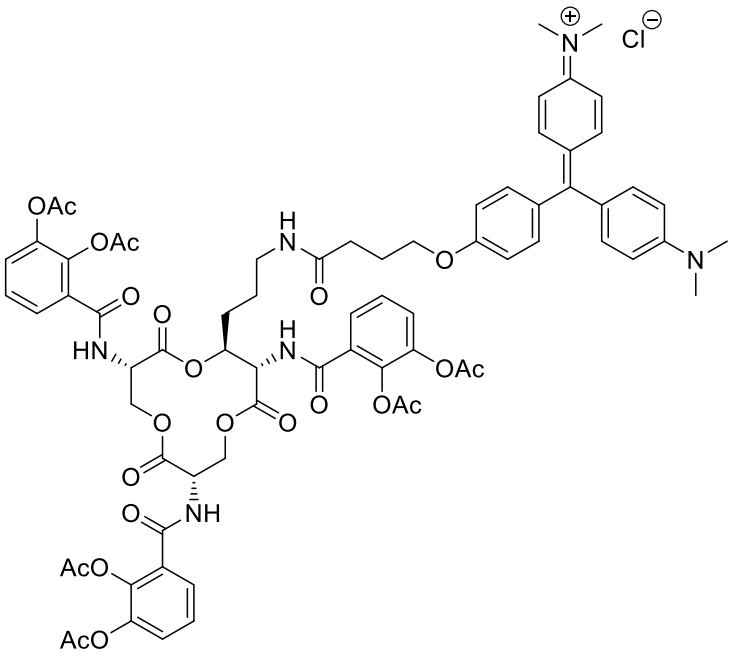
Chemical Formula: $C_{75}H_{92}N_7O_{21}Cl$
Molecular Weight: 1463,04

TFA (100 µL, 1.31 mmol, 291 equiv) was added to a solution of **(MeO)Ent$_{KL}$** (4.1 mg, 4.5 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 0.009 M) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min, before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (150 µL, 0.023 M) and PBS buffer (pH = 8.0, 50 µL, 0.07 M) and 2,5-dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (NHS-PEG$_3$-NHBoc) (2.4 mg, 5.8 µmol, 1.3 eq) was added at 23°C. The solution was stirred at 23°C for 10 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/92.5:7.5). The residue was dissolved in dry CH_2Cl_2 (500 µL, 0.009 M) and TFA (100 µL, 1.31 mmol, 291 equiv) was added at 0°C. The solution was stirred at 23 °C for 50 min, before it was co-evaporated with toluene (3 x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (150 µL, 0.4 M) and PBS buffer (pH = 8.0, 50 µL, 0.12 M) and *N*-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)methylene)-cyclohexa-2,5-dien-1-ylidene)-*N*-methylmethanaminium chloride (**MG-NHS**) (2.4 mg, 4.6 µmol, 1.0 eq) was added at 23°C. The solution was stirred at 23°C for 12 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column

chromatography trough silica gel (CH_2Cl_2:MeOH/9:1) yielding **(MeO)Ent$_{KL}$-PEG$_3$-MG** (1.9 mg, 1.30 µmol, 29%) as a dark green, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.31 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3346, 2926, 2859, 1746, 1694, 1657, 1585, 1516, 1470, 1426, 1366, 1302, 1262, 1226, 1172, 1122, 1061, 991, 941, 909, 832, 801, 755, 720, 664, 633, 600, 550. **LRMS** (ESI) [m/z]: 1426.6 $[C_{75}H_{93}N_7O_{21}]^+$, 713.8 $[C_{75}H_{94}N_7O_{21}]^{2+}$. **HRMS** (ESI) [m/z]: 1426.63410, calculated 1426.63408 for $[C_{75}H_{92}N_7O_{21}]^+$, err [ppm] 0.01, [m/z]: 724.81173, calculated 724.81165 for $[C_{75}H_{92}N_7O_{21}Na]^{2+}$, err [ppm] 0.11. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.97 (d, J = 8.1 Hz, 1H), 8.93 (d, J = 7.2 Hz, 1H), 8.87 (d, J = 7.0 Hz, 1H), 7.60 – 7.51 (m, 2H), 7.31 (dt, J = 9.0, 3.5 Hz, 5H), 7.27 – 7.21 (m, 2H), 7.11 – 6.97 (m, 8H), 6.89 – 6.81 (m, 6H), 5.30 – 4.93 (m, 3H), 4.64 – 4.32 (m, 3H), 4.25 – 4.07 (m, 3H), 3.93 – 3.72 (m, 18H), 3.68 – 3.48 (m, 14H), 3.43 – 3.38 (m, 2H), 3.26 (s, 12H), 2.41 (t, J = 7.2 Hz, 2H), 2.15 – 2.09 (m, 2H), 1.60 – 1.49 (m, 4H). **UV-Vis** λ_{Ex}= 605 nm, 464 nm (c = 0.054 mg/10 mL in MeOH).

(AcO)Ent$_{KL}$-MG



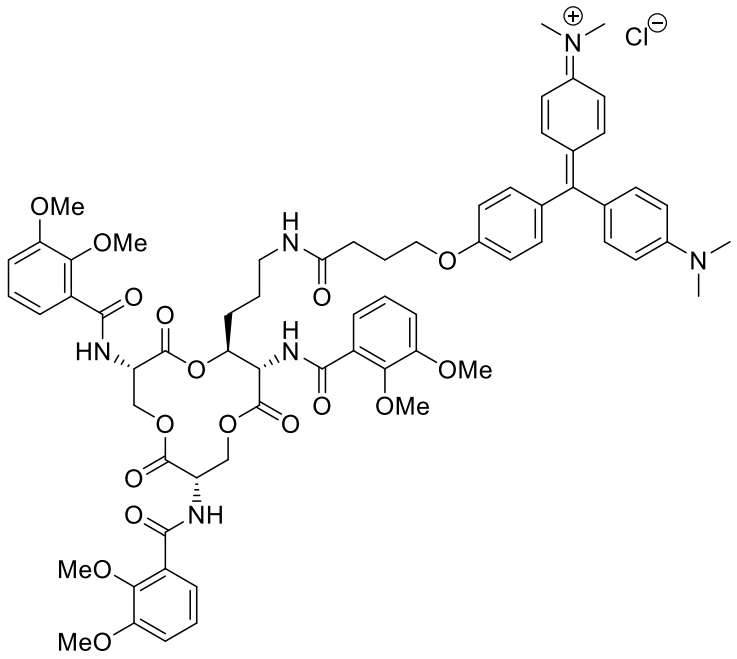
Chemical Formula: $C_{72}H_{75}N_6O_{23}Cl$
Molecular Weight: 1427,86

TFA (100 µL, 1.31 mmol, 689 equiv) was added to a solution of **(AcO)Ent$_{KL}$** (2.1 mg, 1.9 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 4 mM) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min, before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (80 µL, 0.023 M) and PBS buffer (pH = 8.0, 25 µL, 0.08 M) and *N*-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)methylene)-cyclohexa-2,5-dien-1-ylidene)-*N*-methylmethanaminium chloride (**MG-NHS**) (1.2 mg, 2.1 µmol, 1.1 eq) was added at 23°C. The solution was stirred at 23°C for 3 h, before the mixture was diluted

with 700 µL of H_2O/MeCN (7:3, 0.05% TFA), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (0.1% TFA):MeCN (0.1% TFA)/3:1 → 5:95), t_R = 18.5 min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(AcO)Ent$_{KL}$-MG** (1.1 mg, 0.8 µmol, 42%) as a dark green, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.35 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3398, 2926, 2859, 1769, 1666, 1586, 1528, 1464, 1368, 1260, 1171, 1128, 1017, 944, 906, 830, 802, 721, 693, 686, 676, 649, 595, 555, 528. **LRMS** (ESI) [m/z]: 1391.4 $[C_{72}H_{75}N_6O_{23}]^+$, 696.2 $[C_{72}H_{76}N_6O_{23}]^{2+}$ **HRMS** (ESI) [m/z]: 1391.48885, calculated 1391.48781 for $[C_{72}H_{75}N_6O_{23}]^+$, err [ppm] 0.7, [m/z]: 707.23935, calculated 707.23852 for $[C_{72}H_{75}N_6O_{23}Na]^{2+}$, err [ppm] 1.03. **^{1}H-NMR** (600 MHz, $CDCl_3$) δ [ppm]: 7.80 – 7.20 (m, 13H), 7.01 (s, 8H), 5.39 – 5.14 (m, 6H), 5.05 – 4.70 (m, 4H), 4.30 (m, 1H), 4.21 – 4.16 (m, 1H), 4.06 (m, 1H), 3.61 – 3.47 (m, 2H), 3.30 – 2.99 (m, 12H), 2.36 – 1.97 (m, 24H). **UV-Vis** λ_{Ex}= 606 nm, 463 nm (c = 0.041 mg/10 mL in MeOH).

(MeO)Ent$_{KL}$-MG



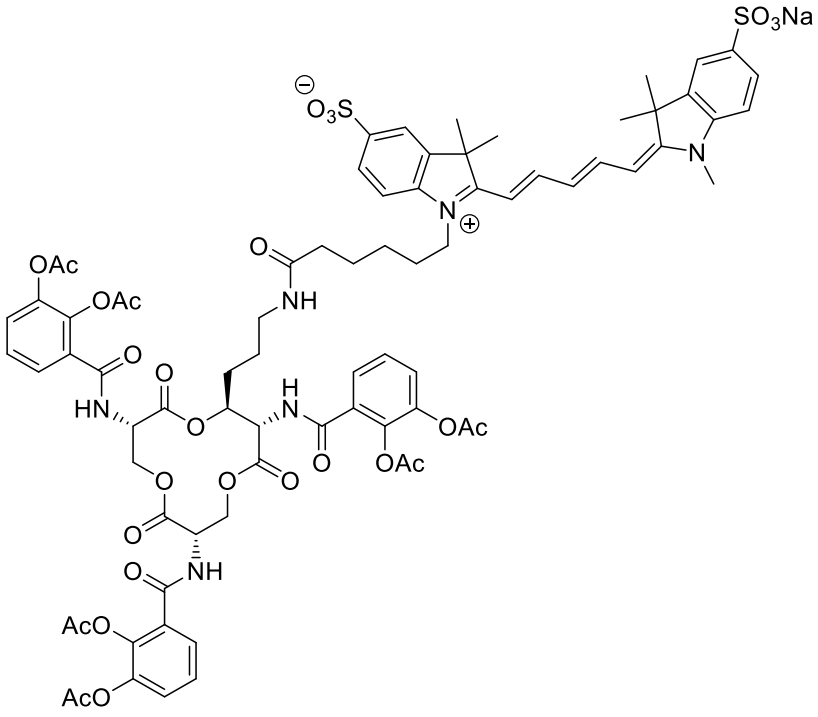
Chemical Formula: $C_{66}H_{75}N_6O_{17}Cl$
Molecular Weight: 1259,80

TFA (100 µL, 1.31 mmol, 569 equiv) was added to a solution of **(MeO)Ent$_{KL}$** (2.1 mg, 2.3 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 5 mM) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min, before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (110 µL, 0.021 M) and PBS buffer (pH = 8.0, 25 µL, 0.09 M) and *N*-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-

oxobutoxy)phenyl)methylene)-cyclohexa-2,5-dien-1-ylidene)-*N*-methylmethanaminium chloride (**MG-NHS**) (1.7 mg, 3.0 µmol, 1.3 eq) was added at 23°C. The solution was stirred at 23°C for 3 h, before the mixture was diluted 700 µL of H_2O/MeCN (7:3, 0.05% TFA), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (0.1% TFA):MeCN (0.1% TFA)/3:1 → 5:95), t_R = 19.4min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(MeO)Ent$_{KL}$-MG** (1.1 mg, 0.9 µmol, 38%) as a dark green, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.37 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3345, 2925, 2107, 1742, 1656, 1583, 1465, 1363, 1261, 1168, 910, 796, 723, 644, 571. **LRMS** (ESI) [m/z]: 1223.5 $[C_{66}H_{75}N_6O_{17}]^+$, 612.2 $[C_{66}H_{76}N_6O_{17}]^{2+}$ **HRMS** (ESI) [m/z]: 1223.51889, calculated 1223,51832 for $[C_{66}H_{75}N_6O_{17}]^+$, err [ppm] 0.5, [m/z]: 623.25393, calculated 623.25377 for $[C_{66}H_{75}N_6O_{17}Na]^{2+}$, err [ppm] 0.3. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 9.11 – 8.78 (m, 2H), 7.77 – 7.58 (m, 2H), 7.41 – 7.29 (m, 6H), 7.18 – 6.99 (m, 8H), 6.89 (m, 4H), 5.44 – 5.01 (m, 4H), 4.73 – 4.36 (m, 2H), 4.14 (t, *J* = 6.3 Hz, 2H), 3.97 – 3.75 (m, 18H), 3.72 – 3.59 (m, 4H), 3.31 (s, 12H), 2.48 – 2.30 (m, 2H), , 1.63 (m, 4H). **UV-Vis** λ_{Ex}= 606 nm, 465 nm (c = 0.074 mg/10 mL in MeOH).

(AcO)Ent$_{KL}$-SulfoCy5



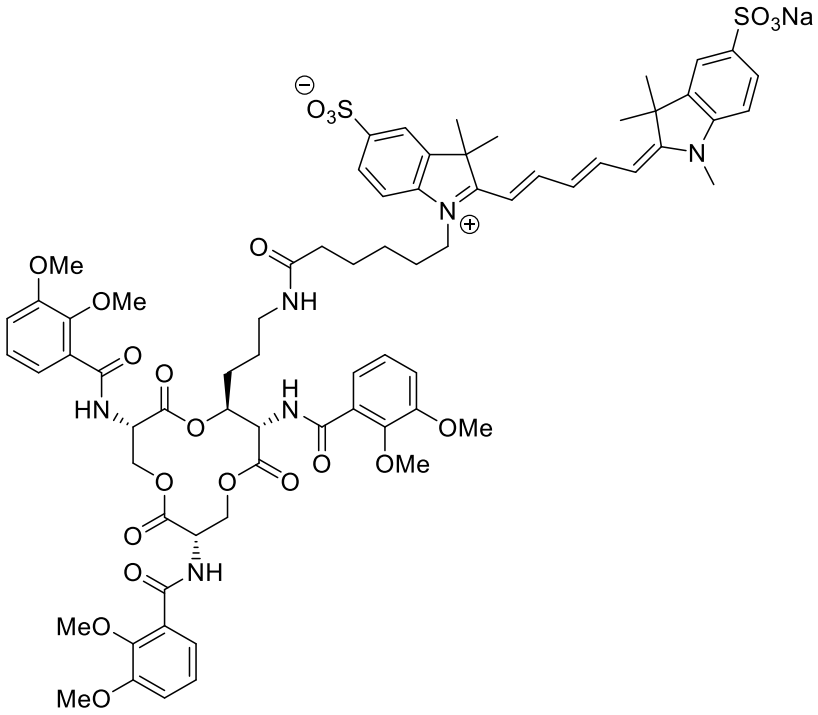
Chemical Formula: $C_{77}H_{81}N_6NaO_{28}S_2$
Molecular Weight: 1625,62

TFA (100 µL, 1.31 mmol, 524 equiv) was added to a solution of **(AcO)Ent$_{KL}$** (2.7 mg, 2.5 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 5 mM) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min,

before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (90 µL, 0.027 M) and PBS buffer (pH = 8.0, 30 µL, 0.08 M) and potassium 1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethyl-2-((1E,3E)-5-((E)-1,3,3-trimethyl-5-sulfonatoindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium-5-sulfonate (**SulfoCy5-NHS**) (2.1 mg, 2.8 µmol, 1.1 eq) was added at 23°C. The solution was stirred at 23°C for 3 h, before the mixture was diluted 500 µL of H_2O/MeCN (7:3, 0.05% TFA), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents:, H_2O (0.1% TFA):MeCN (0.1% TFA)/3:1 → 5:95), t_R = 15.5min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(AcO)Ent$_{KL}$-SulfoCy5** (1.4 mg, 0.8 µmol, 32%) as a dark blue, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.12 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3412, 2926, 2857, 1767, 1667, 1587, 1498, 1465, 1371, 1331, 1205, 1101, 1066, 1021, 927, 812, 726, 693, 686, 648, 625, 551, 542. **LRMS** (ESI) [m/z]: 1602.4 $[C_{77}H_{81}N_6O_{28}S_2]^-$ **HRMS** (ESI) [m/z]: 835.21119, calculated 835.21112 for $[C_{77}H_{81}N_6O_{28}Na_3]^{2+}$, err [ppm] 0.08, **^{1}H-NMR** (500 MHz, CD_3OD) δ [ppm]: 8.38 – 8.19 (m, 2H), 7.92 – 7.82 (m, 4H), 7.54 (ddd, *J* = 7.6, 1.6, 0.8 Hz, 1H), 7.47 (ddd, *J* = 14.6, 7.6, 1.6 Hz, 1H), 7.42 – 7.17 (m, 12H), 6.65 (t, *J* = 12.3 Hz, 1H), 6.31 (d, *J* = 13.6 Hz, 2H), 5.49 – 5.27 (m, 2H), 4.72 – 4.57 (m, 2H), 4.53 – 4.34 (m, 4H), 4.05 (t, *J* = 7.6 Hz, 1H), 3.59 – 3.53 (m, 3H), 2.96 (ddd, *J* = 22.1, 10.6, 4.8 Hz, 1H), 2.31 – 2.26 (m, 18H), 2.24 – 2.20 (m, 6H), 2.03 (d, *J* = 8.2 Hz, 1H), 2.00 – 1.95 (m, 1H), 1.87 – 1.80 (m, 2H), 1.73 (m, 6H), 1.63 (dq, *J* = 14.4, 7.0 Hz, 4H), 1.41 – 1.33 (m, 4H). **UV-Vis/fluorescence emission** λ_{Ex}= 640 nm, λ_{Em}= 670 nm (c = 0.044 mg/10 mL in MeOH).

(MeO)Ent$_{KL}$-SulfoCy5



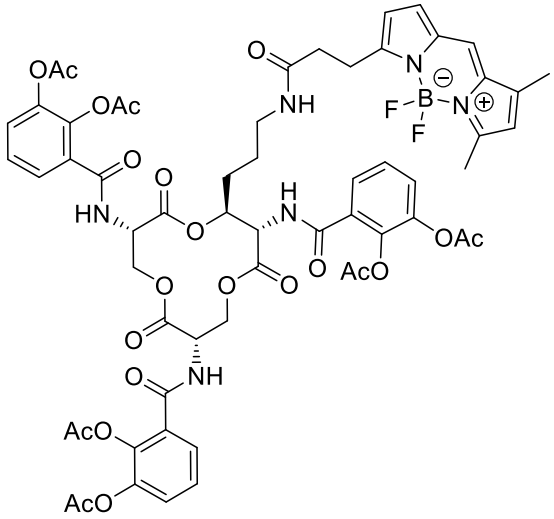
Chemical Formula: $C_{71}H_{81}N_6NaO_{22}S_2$
Molecular Weight: 1457,56

TFA (100 µL, 1.31 mmol, 451 equiv) was added to a solution of **(MeO)Ent$_{KL}$** (2.7 mg, 2.9 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 6 mM) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min, before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (90 µL, 0.032 M) and PBS buffer (pH = 8.0, 30 µL, 0.1 M) and potassium 1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethyl-2-((1E,3E)-5-((E)-1,3,3-trimethyl-5-sulfonatoindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium-5-sulfonate (**SulfoCy5-NHS**) (2.5 mg, 3.1 µmol, 1.1 eq) was added at 23°C. The solution was stirred at 23°C for 3 h, before the mixture was diluted with 500 µL of H_2O/MeCN (7:3, 0.05% TFA), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC ((Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (0.1% TFA):MeCN (0.1% TFA)/3:1 → 5:95), t_R = 16.0 min).Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(MeO)Ent$_{KL}$-SulfoCy5** (1.5 mg, 1.0 µmol, 35%) as a dark blue, amorphous solid.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.14 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3460, 2927, 2855, 1747, 1652, 1578, 1499, 1465, 1372, 1331, 1267, 1218, 1179, 1104, 1066, 1021, 928, 817, 757, 725, 693, 649, 642, 626, 569. **LRMS** (ESI) [m/z]: 1436.5 $[C_{71}H_{82}N_6O_{22}S_2]^+$, 718.3 $[C_{71}H_{83}N_6O_{22}S_2]^{2+}$ **HRMS** (ESI) [m/z]: 751.22659, calculated 751.22637 for $[C_{71}H_{81}N_6O_{22}Na_3]^{2+}$, err [ppm] 0.3 **^{1}H-NMR** (500 MHz, CD_3OD) δ [ppm]: 8.43 – 8.20 (m, 1H), 8.09 – 7.71 (m, 4H), 7.42 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.37 (ddd, *J* = 7.9, 4.7, 1.6 Hz, 2H), 7.27 (ddd, *J* = 14.6, 7.9, 1.0 Hz, 2H), 7.22 – 7.00 (m, 8H), 6.64 (t, *J* = 12.4 Hz, 1H), 6.30 (dd, *J* = 15.5, 13.7 Hz,

2H), 5.44 – 5.35 (m, 1H), 5.14 (dd, *J* = 5.5, 4.1 Hz, 1H), 4.97 – 4.94 (m, 4H), 4.89 (s, 1H), 4.70 (dd, *J* = 11.3, 4.1 Hz, 1H), 4.46 (dd, *J* = 11.6, 5.5 Hz, 1H), 4.38 (dd, *J* = 11.6, 3.7 Hz, 1H), 4.15 – 4.05 (m, 2H), 3.97 – 3.78 (m, 18H), 3.71 (d, *J* = 8.6 Hz, 6H), 3.57 (s, 3H), 2.19 (td, *J* = 7.3, 1.9 Hz, 2H), 2.13 – 1.99 (m, 2H), 1.79 (q, *J* = 7.3 Hz, 2H), 1.71 (d, *J* = 3.7 Hz, 4H), 1.69 (d, *J* = 3.4 Hz, 6H), 1.45 (q, *J* = 8.0 Hz, 2H), 1.33 (s, 2H). **UV-Vis/fluorescence emission** λ_{Ex}= 640 nm, λ_{Em}= 671 nm (c = 0.056 mg/10 mL in MeOH).

(AcO)Ent$_{KL}$-BODIPY$_{FL}$



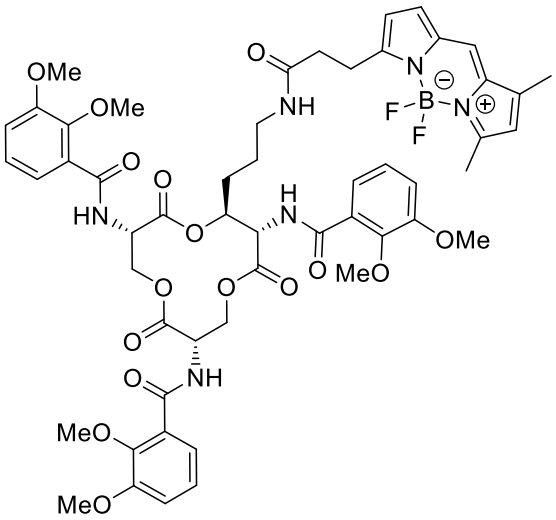
Chemical Formula: $C_{59}H_{59}BF_2N_6O_{22}$
Molecular Weight: 1252,95

TFA (100 µL, 1.31 mmol, 437 equiv) was added to a solution of **(AcO)Ent$_{KL}$** (3.2 mg, 3.0 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 0.006 M) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min, before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in DMSO (90 µL, 0.03 M) and PBS buffer (pH = 8.0, 30 µL, 0.1 M) and 2,5-dioxopyrrolidin-1-yl 3-(5,5-difluoro-7,9-dimethyl-5H-5l4,6l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diaza-borinin-3-yl)propanoate (**BODIPY$_{FL}$-NHS**) (1.4 mg, 3.6 µmol, 1.2 eq) was added at 23°C. The solution was stirred at 23°C for 3.5 h, before the mixture was diluted with 500 µL of H_2O/MeCN (1:1), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (10 mM NH_4OAc):MeCN/3:1 → 5:95), t_R = 20.3 min).Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** (1.4 mg, 1.1 µmol, 37%) as a red, amorphous solid.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.47 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3376, 2926, 2857, 1768, 1664, 1606, 1527, 1459, 1371, 1337, 1256, 1204, 1167, 1134, 1079, 1014, 975, 899, 804, 755, 669, 618, 590, 562, 543. **LRMS** (ESI) [m/z]: 1254.5[$C_{59}H_{60}BF_2N_6O_{22}$]$^+$ **HRMS** (ESI) [m/z]: 1275.36534, calculated 1275.36358 for [$C_{59}H_{59}BF_2N_6O_{22}Na$]$^+$, err [ppm] 1.38; 649.17715 calculated 649.17640 for [$C_{59}H_{59}BF_2N_6O_{22}Na_2$]$^{2+}$, err [ppm] 1.15, **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 7.82 (d, *J* = 7.7 Hz, 1H), 7.69 (dd, *J* = 6.3, 3.8 Hz,

1H), 7.62 (dd, *J* = 7.0, 2.4 Hz, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.54 – 7.43 (m, 2H), 7.36 – 7.26 (m, 3H), 7.21 – 7.11 (m, 2H), 6.89 (d, *J* = 4.0 Hz, 1H), 6.21 (d, *J* = 4.1 Hz, 1H), 6.19 – 6.08 (m, 1H), 5.97 (s, 1H), 5.38 – 5.22 (m, 2H), 5.16 – 4.89 (m, 2H), 4.86 – 4.68 (m, 3H), 4.34 – 4.19 (m, 1H), 3.41 (dq, *J* = 13.9, 6.9 Hz, 1H), 3.28 – 3.05 (m, 3H), 2.59 – 2.48 (m, 5H), 2.42 – 2.13 (m, 21H), 2.10 (s, 3H), 1.88 – 1.79 (m, 1H), 1.78 – 1.70 (m, 1H), 1.49 – 1.41 (m, 4H). **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: --144.4 – (-144.86) (m, 2F). **^{11}B-NMR** (128 MHz, $CDCl_3$) δ [ppm]: 1.2 (t, *J* = 33.7 Hz). **UV-Vis/fluorescence emission** λ_{Ex}= 504 nm, λ_{Em}= 510 nm (c = 0.073 mg/10 mL in MeOH).

(MeO)Ent$_{KL}$-BODIPY$_{FL}$



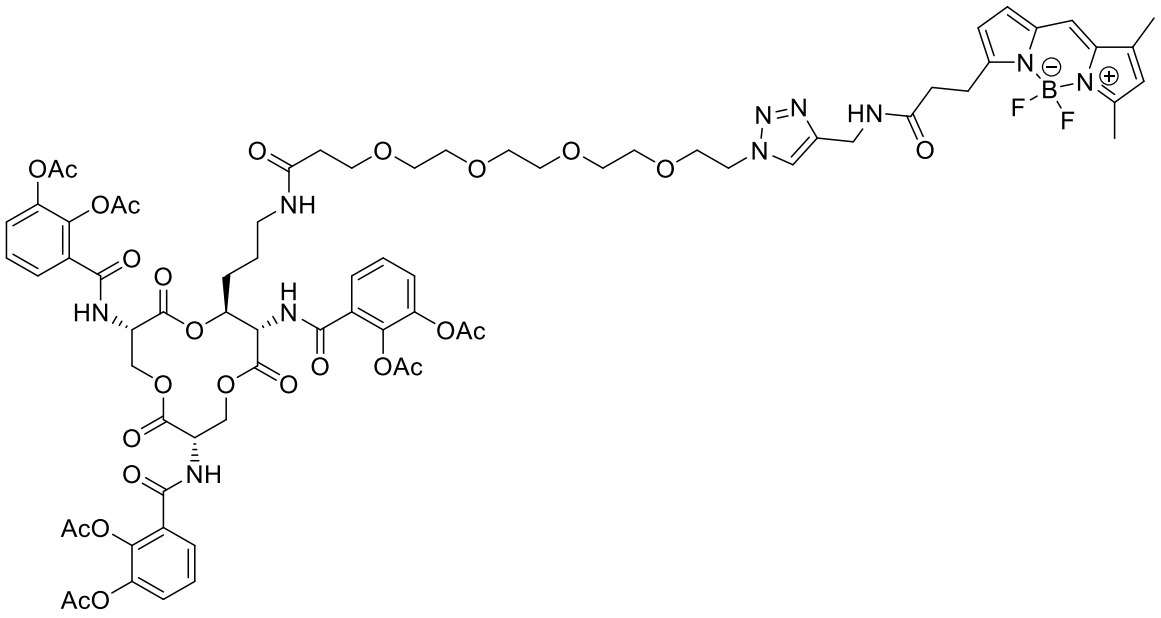
Chemical Formula: $C_{53}H_{59}BF_2N_6O_{16}$
Molecular Weight: 1084,89

TFA (100 µL, 1.31 mmol, 485 equiv) was added to a solution of **(MeO)Ent$_{KL}$** (2.5 mg, 2.7 µmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 6 mM) under Argon atm. at 0°C and the solution was stirred at 23°C for 35 min, before it was co-evaporated with toluene (3x 3 mL) and concentrated under reduced pressure. The residue was dissolved in THF (100 µL, 0.03 M) and 2,5-dioxopyrrolidin-1-yl 3-(5,5-difluoro-7,9-dimethyl-5H-5l4,6l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanoate (**BODIPY$_{FL}$-NHS**) (1.4 mg, 3.6 µmol, 1.2 eq) and NMM (0.5 µL, 4.1 µmol, 1.5 equiv) were added at 23°C. The solution was stirred at 23°C for 3 h, before the mixture was diluted with 500 µL of H_2O/MeCN (1:1), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (10 mM NH_4OAc):MeCN/4:1 → 5:95), t_R = 22.1 min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(MeO)Ent$_{KL}$-BODIPY$_{FL}$** (1.3 mg, 1.2 µmol, 44%) as a red, amorphous solid.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.51 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3342, 2927, 2853, 1749, 1657, 1606, 1520, 1467, 1315, 1263, 1184, 1135, 1073, 988, 929, 811, 754, 670, 639, 612, 574, 548. **HPLC-MS** (HESI)

[m/z]: 1085.7 $[C_{53}H_{60}BF_2N_6O_{16}]^+$ **HRMS** (ESI) [m/z]: 1107.39538, calculated 1107.39409 for $[C_{53}H_{59}BF_2N_6O_{16}Na]^+$, err [ppm] 1.16; 565.19216 calculated 565.19165 for $[C_{53}H_{59}BF_2N_6O_{16}Na_2]^{2+}$, err [ppm] 0.9, **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.99 (dd, *J* = 7.7, 3.1 Hz, 2H), 8.89 (d, *J* = 7.0 Hz, 1H), 7.68 – 7.65 (m, 3H), 7.18 – 7.01 (m, 7H), 6.87 (d, *J* = 4.0 Hz, 1H), 6.24 (d, *J* = 4.0 Hz, 1H), 6.14 – 6.07 (m, 1H), 6.05 (s, 1H), 5.20 (dt, *J* = 8.0, 5.0 Hz, 1H), 5.02 (dd, *J* = 8.0, 5.5 Hz, 2H), 4.63 (dd, *J* = 11.3, 4.3 Hz, 1H), 4.55 – 4.45 (m, 2H), 4.42 (dd, *J* = 10.8, 7.7 Hz, 1H), 4.09 – 3.63 (m, 20H), 3.34 – 3.12 (m, 4H), 2.59 (t, *J* = 7.3 Hz, 2H), 2.55 (s, 3H), 2.25 (s, 3H), 1.97 – 1.81 (m, 2H). **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: --144.5 – (-145.4) (m, 2F). **^{11}B-NMR** (128 MHz, $CDCl_3$) δ [ppm]: 1.2 (t, *J* = 33.7 Hz). **UV-Vis/fluorescence emission** λ_{Ex}= 504 nm, λ_{Em}= 511 nm (c = 0.123 mg/10 mL in MeOH).

(AcO)Ent_{KL}-PEG_4-$BODIPY_{FL}$

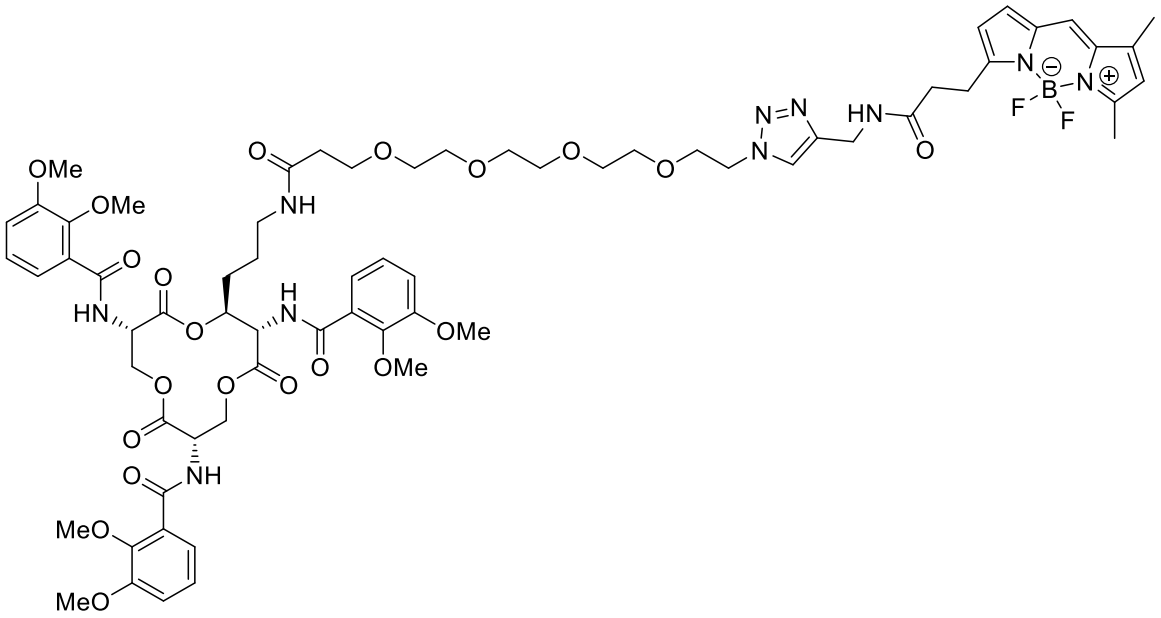


Chemical Formula: $C_{73}H_{83}BF_2N_{10}O_{27}$
Molecular Weight: 1581,32

10.0 µL (0.15 mg, 0.28 µmol, 0.2 equiv) of a stock solution of TBTA 15.0 mg 1.0 mL DMSO (0.028 M)), 4.0 µL (55 µg, 0.28 µmol, 0.2 equiv) of a stock solution of NaAsc (13.8 mg in 1.0 mL H_2O (0.07 M)) and 2.0 µL (22 µg, 0.14 µmol, 0.1 equiv)of a stock solution of $CuSO_4$ (11.0 mg in 1.0 mL H_2O (0.07 M)) were added to a solution of **(AcO)Ent_{KL}-PEG_4-N_3** (1.8 mg, 1.4 µmol, 1.0 equiv) and 3-(5,5-difluoro-7,9-dimethyl-5H-5l4,6l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)-N-(prop-2-yn-1-yl)propanamide (**$BODIPY_{FL}$-alkyne**) (0.6 mg, 1.9 µmol, 1.3 equiv) in DMSO (90 µL, 0.02 M) at 23 °C. The solution was stirred at 23°C for 2.5 h, before the mixture was diluted with 500 µL of H_2O/MeCN (1:1), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (10 mM NH_4OAc):MeCN/4:1 → 5:95), t_R = 18.4 min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(AcO)Ent_{KL}-PEG_4-$BODIPY_{FL}$** (0.7 mg, 0.44 µmol, 31%) as a red, amorphous solid.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.43 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3351, 2923, 2857, 1766, 1658, 1607, 1532, 1461, 1371, 1258, 1204, 1083, 1016, 908, 805, 668, 579. **LRMS** (ESI) [m/z]: 1581.5 $[C_{73}H_{84}BF_2N_{10}O_{27}]^+$; 791.2 $[C_{73}H_{85}BF_2N_{10}O_{27}]^{2+}$; **HRMS** (ESI) [m/z]: 1603.54020, calculated 1603.53825 for $[C_{73}H_{83}BF_2N_{10}O_{27}Na]^+$, err [ppm] 1.22; 813.26483 calculated 813.26373 for $[C_{73}H_{83}BF_2N_{10}O_{27}Na_2]^{2+}$, err [ppm] 1.35, **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.04 (d, *J* = 7.7 Hz, 1H), 7.87 – 7.67 (m, 2H), 7.63 – 7.28 (m, 4H), 7.21 – 6.81 (m, 5H), 6.29 – 6.23 (m, 1H), 6.10 (s, 1H), 5.49 – 5.43 (m, 1H), 5.40 – 5.34 (m, 3H), 5.00 – 4.71 (m, 3H), 4.44 (s, 3H), 4.38 – 4.21 (m, 1H), 4.07 – 3.98 (m, 1H), 3.82 (d, *J* = 5.3 Hz, 2H), 3.70 – 3.51 (m, 15H), 3.24 (q, *J* = 7.4 Hz, 2H), 2.66 – 2.57 (m, 2H), 2.53 (s, 3H), 2.36 – 2.18 (m, 21H), 2.09 (d, *J* = 1.8 Hz, 3H), 2.04 – 1.98 (m, 3H), 1.91 – 1.87 (m, 1H). **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: -144.1 (q, *J* = 33.8 Hz). **^{11}B-NMR** (128 MHz, $CDCl_3$) δ [ppm]: 1.1 (t, *J* = 33.8 Hz). **UV-Vis/fluorescence emission** λ_{Ex}= 505 nm, λ_{Em}= 512 nm (c = 0.102 mg/10 mL in MeOH).

(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$



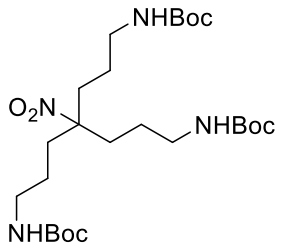
Chemical Formula: $C_{67}H_{83}BF_2N_{10}O_{21}$
Molecular Weight: 1413,26

22.0 µL (0.21 mg, 0.48 µmol, 0.2 equiv)of a stock solution of THPTA (9.6 mg in 1.0mL DMSO (0.022 M)), 5.7 µL (95 µg, 0.48 µmol, 0.2 equiv) of a stock solution of NaAsc (16.7 mgin 1.0 mL H_2O (0.08 M)) and 3.1 µL (38 µg, 0.24 µmol, 0.1 equiv) of a stock solution of $CuSO_4$ (12.3 mg in 1.0 mL H_2O (0.08 M)) were added to a solution of **(MeO)Ent$_{KL}$-PEG$_4$-N$_3$** (2.6 mg, 2.4 µmol, 1.0 equiv) and 3-(5,5-difluoro-7,9-dimethyl-5H-5l4,6l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)-N-(prop-2-yn-1-yl)-propanamide (**BODIPY$_{FL}$-alkyne**) (1.0 mg, 3.1 µmol, 1.3 equiv) in DMSO (90 µL, 0.02 M) at 23°C. The solution was stirred at 23°C for 2.5 h, before the mixture was diluted with 500 µL of H_2O/MeCN (1:1), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×10.0 mm (5 mL/min), Eluents: H_2O (10 mM

NH_4OAc):MeCN/4:1 → 5:95), t_R = 19.3 min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding **(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** (1.4 mg, 1.0 µmol, 42%) as a red, amorphous solid.

TLC (CH_2Cl_2:MeOH/92.5:7.5) R_f: 0.46 [UV-Vis]. **IR** (ATR) [cm^{-1}]: 3339, 2926, 2861, 1748, 1656, 1605, 1523, 1467, 1337, 1316, 1263, 1186, 1135, 1063, 989, 932, 814, 755, 666, 650, 642, 598, 584, 572, 546. **LRMS** (ESI) [m/z]: 1413.5 $[C_{67}H_{84}BF_2N_{10}O_{21}]^+$; 707.3 $[C_{67}H_{85}BF_2N_{10}O_{21}]^{2+}$; **HRMS** (ESI) [m/z]: 1435.57059, calculated 1435.56876 for $[C_{67}H_{83}BF_2N_{10}O_{21}Na]^+$, err [ppm] 1.28; 729.27971 calculated 729.27899 for $[C_{67}H_{83}BF_2N_{10}O_{21}Na_2]^{2+}$, err [ppm] 1.0, **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 9.00 (t, *J* = 7.9 Hz, 2H), 8.91 (d, *J* = 7.1 Hz, 1H), 7.84 (s, 1H), 7.70 – 7.61 (m, 3H), 7.16 – 7.03 (m, 7H), 6.85 (d, *J* = 4.0 Hz, 2H), 6.24 (d, *J* = 4.0 Hz, 1H), 6.10 (s, 1H), 5.26 (q, *J* = 6.0 Hz, 1H), 5.21 – 5.11 (m, 2H), 5.07 (dd, *J* = 8.2, 5.2 Hz, 1H), 4.65 (dd, *J* = 11.3, 4.3 Hz, 1H), 4.58 – 4.50 (m, 5H), 4.47 (dd, *J* = 11.3, 5.6 Hz, 1H), 4.43 – 4.36 (m, 1H), 3.98 – 3.78 (m, 20H), 3.66 (t, *J* = 6.0 Hz, 2H), 3.63 – 3.53 (m, 13H), 3.26 (t, *J* = 7.7 Hz, 4H), 2.65 (dd, *J* = 8.2, 7.0 Hz, 2H), 2.53 (s, 3H), 2.40 (t, *J* = 6.0 Hz, 2H), 2.24 (s, 3H), 2.00 – 1.95 (m, 2H) 1.69 – 1.62 (m, 4H). **^{19}F-NMR** (282 MHz, $CDCl_3$) δ [ppm]: -145.2 (q, *J* = 33.8 Hz). **^{11}B-NMR** (128 MHz, $CDCl_3$) δ [ppm]: 1.0 (t, *J* = 33.8 Hz). **UV-Vis/fluorescence emission** λ_{Ex}= 504 nm, λ_{Em}= 510 nm (c = 0.096 mg/10 mL in MeOH).

Di-*tert*-butyl (4-(3-((*tert*-butoxycarbonyl)amino)propyl)-4-nitroheptane-1,7-diyl)dicarbamate

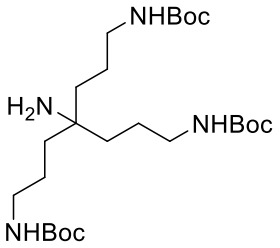


Chemical Formula: $C_{25}H_{48}N_4O_8$
Molecular Weight: 532,68

Following the procedure of *Ji et al*.:[9] BH_3 SMe_2 (2 M solution in THF) (17 mL, 34 mmol, 5.0 equiv) was added to a solution of 5-(3-cyanopropyl)-5-nitrononanedinitrile (1.5 g, 6.8 mmol, 1.0 equiv) in dry THF (30 mL, 0.23 M) and the slurry was heated up to 80°C for 16 h. The reaction mixture was cooled to 0°C, and adjusted to pH = 1 by addition of $HCl_{(aq.)}$ (5 M) and heated up to 50°C for 30 min. The solvent was removed and $NaOH_{(aq.)}$ (4 M, 50 mL) and EtOAc (50 mL) were added to the residue. The phases were separated and the aqueous phase was extracted with EtOAc (2x 75 mL). The combined organic layers were washed with brine (50 mL) and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and the residue was dissolved in dry methanol (18 mL, 0.38 M), triethylamine (3.1 mL, 22.4 mmol, 3.3 equiv) and Boc_2O (4.8 mL, 22.4 mmol, 3.3 equiv) were added and the mixture was heated up to 75°C for 5 h. The solution was concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL) and washed with citric acid (10 wt%, 50 mL) and brine (30 mL). The solution was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (EtOAc:Hex/1:1) yielding di-tert-butyl (4-(3-((tert-butoxycarbonyl)amino)propyl)-4-nitroheptane-1,7-diyl)dicarbamate (1.9 g, 3.87 mmol, 57%) as a pale yellow foam.

TLC (EtOAc:Hex/1:1) R_f: 0.52 [ninhydrin], **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: δ 4.65 (s, 3H), 3.10 (q, *J* = 6.5 Hz, 6H), 1.96 – 1.83 (m, 6H), 1.44 – 1.40 (m, 33H).**^{13}C-NMR:** (76 MHz, $CDCl_3$) δ [ppm]: δ 156.1, 94.0, 79.5, 40.3, 32.8, 28.5, 24.4. The analytical data were in accordance with the literature.[9]

Di-*tert*-butyl (4-amino-4-(3-((*tert*-butoxycarbonyl)amino)propyl)heptane-1,7-diyl)dicarbamate

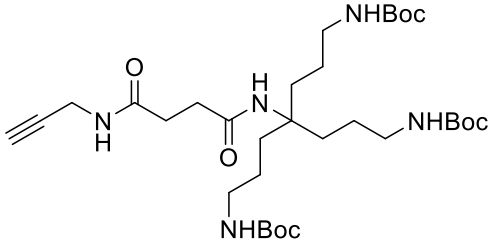


Chemical Formula: $C_{25}H_{50}N_4O_6$
Molecular Weight: 502,70

Following the procedure of *Ji et al.*:[9] $NaBH_4$ (57 mg ,1.5 mmol, 1.5 equiv) was added to a solution of $NiCl_2 \cdot 6\ H_2O$ (118 mg, 0.5 mmol, 0.5 equiv) in methanol (6.4 mL) and the mixture was stirred for 30 min at 23°C. A solution of di-tert-butyl (4-(3-((tert-butoxycarbonyl)amino)propyl)-4-nitroheptane-1,7-diyl)dicarbamate (530 mg, 1.0 mmol, 1.0 equiv) in methanol (6.4 mL, 0.16 M) and $NaBH_4$ (113 mg, 3.0 mmol, 3.0 equiv) were added to the reaction mixture and stirred for 30 min. $NaBH_4$ (113 mg, 3.0 mmol, 3.0 equiv) was added to the reaction mixture and stirred for 30 min. The mixture was filtered through a pad of celilte and the solution was concentrated under reduced pressure. H_2O (20 ml) and CH_2Cl_2 (40 mL) were added to the resulting residue and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2x 40 mL), the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure yielding di-*tert*-butyl (4-amino-4-(3-((*tert*-butoxycarbonyl)amino)propyl)heptane-1,7-diyl)dicarbamate (430 mg, 0.86 mmol, 86%) as a colorless oil.

TLC (EtOAc:Hex/6:4) R_f: 0.1 [ninhydrin], **^{1}H-NMR** (300 MHz, DMSO-d_6) δ [ppm]: δ 6.81 – 6.53 (m, 3H), 3.32 (s, 2H), 2.85 (q, *J* = 6.5 Hz, 6H), 1.36 (s, 39H). **HRMS** (ESI-IT) [m/z]: 503.37996, calculated 503.38031 for $C_{25}H_{51}N_4O_6$ $[M+H]^+$, -0.70 err [ppm]. The analytical data were in accordance with the literature.[9]

Di-*tert*-butyl (4-(3-((*tert*-butoxycarbonyl)amino)propyl)-4-(4-oxo-4-(prop-2-yn-1-ylamino)butanamido)heptane-1,7-diyl)dicarbamate

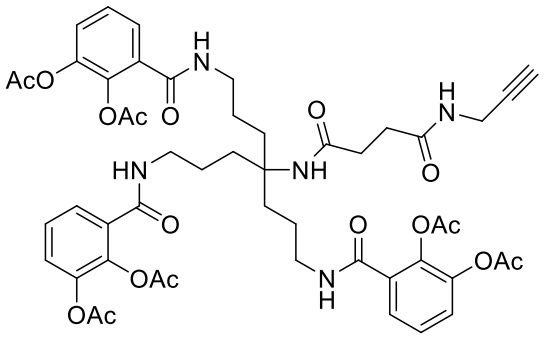


Chemical Formula: $C_{32}H_{57}N_5O_8$
Molecular Weight: 639,835

4-Oxo-4-(prop-2-yn-1-ylamino)butanoic acid (19 mg, 0.12 mmol, 1.2 equiv) and DCC (25 mg, 0.12 mmol, 1.2 equiv) were added to a solution of di-tert-butyl (4-amino-4-(3-((tert-butoxycarbonyl)amino)propyl)heptane-1,7-diyl)dicarbamate (50 mg, 0.1 mmol, 1.0 equiv) in dry CH_2Cl_2 (500 µL, 0.2 M) at 0°C and the mixture was stirred for 16 h at 23°C. The reaction mixture was filtered, concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (EtOAc:Hex/3:1) yielding di-tert-butyl (4-(3-((tert-butoxycarbonyl)amino)propyl)-4-(4-oxo-4-(prop-2-yn-1-ylamino)butanamido)heptane-1,7-diyl)di-carbamate (32 mg, 0.05 mmol, 50%) as a colorless oil.

TLC (EtOAc:Hex/3:1) R_f: 0.45 [CAM], **^{1}H-NMR** (300 MHz, $CDCl_3$) δ [ppm]: 4.86 (s, 2H), 4.05 (dd, *J* = 5.2, 2.6 Hz, 2H), 3.08 (q, *J* = 6.1 Hz, 6H), 2.68 (dd, *J* = 7.6, 5.2 Hz, 2H), 2.55 (dd, *J* = 7.6, 5.2 Hz, 2H), 2.22 (t, *J* = 2.6 Hz, 1H), 1.46 – 1.27 (m, 39H). **^{13}C-NMR:** (76 MHz, $CDCl_3$) δ [ppm]: 172.2, 170.9, 156.3, 79.3, 71.8, 61.6, 41.0, 31.2, 30.3, 29.8, 29.4, 28.6, 28.1, 23.6. **HRMS** (ESI-IT) [m/z]: 678.40520, calculated 678.40485 for $C_{32}H_{57}N_5O_9Na$ $[M+H]^+$, 0.52 err [ppm].

(AcO)Ent$_M$

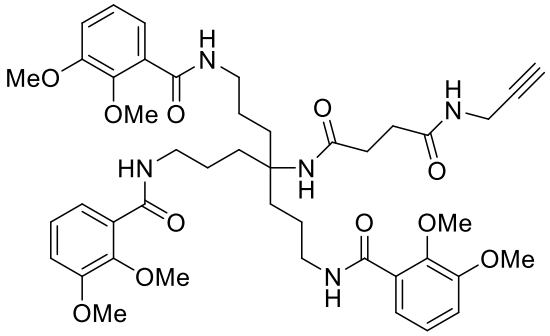


Chemical Formula: $C_{50}H_{57}N_5O_{17}$
Molecular Weight: 1000,024

TFA (400 µL) was added to a solution of di-tert-butyl (4-(3-((tert-butoxycarbonyl)amino)propyl)-4-(4-oxo-4-(prop-2-yn-1-ylamino)butanamido)heptane-1,7-diyl)dicarbamate (45 mg, 70 µmol, 1.0 equiv) in dry CH_2Cl_2 (2.0 mL, 0.035 M) at 0°C. The solution was stirred 2 h at 23°C, evaporated with toluene (2x 5 mL) and concentrated under reduced pressure. The residue was dissolved in dry DMF (500 µL, 0.14 M) and DiPEA (80 µL, 460 µmol, 6.5 equiv) was added. The solution was cooled down to 0°C, diacetoxybenzoic acid chloride (**19**) (64 mg, 250 µmol, 3.5 equiv) was added as a solution in dry THF (800 µL, 0.31 M) and stirred for 1 h at 23°C. EtOAc (20 mL) was added and the solution was quenched by addition of $NH_4Cl_{(aq.)}$ (5 mL), layers were separated and the organic layer was washed with water (2x 10 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/9:1) yielding **(AcO)Ent$_M$** (36 mg, 36 µmmol, 51%) as a colorless oil.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.3 [UV^{254},ninhydrin], **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 7.49 – 7.48 (m, 3H), 7.28 – 7.18 (m, 6H), 6.65 (t, *J* = 5.8 Hz, 3H), 6.30 (t, *J* = 5.3 Hz, 1H), 3.83 (dd, *J* = 5.3, 2.5 Hz, 2H), 3.38 – 3.35 (m, 6H), 2.50 (dd, *J* = 7.4, 5.7 Hz, 2H), 2.28 (s, 2H), 2.28 (s, 18H), 2.17 (t, *J* = 2.5 Hz, 1H), 1.57 – 1.52 m, 6H), 1.43 – 1.40 (m, 6H).**^{13}C-NMR:** (126 MHz, $CDCl_3$) δ [ppm]: δ 172.5, 170.9, 168.4, 168.4, 165.5, 143.1, 140.3, 130.8, 126.6, 126.4, 125.7, 79.8, 71.5, 62.0, 40.2, 31.0, 30.0, 29.2, 27.9, 23.1, 20.8, 20.8. **HRMS** (ESI-IT) [m/z]: 1038.35953, calculated 1038.35908 for $C_{50}H_{57}N_5O_{18}Na$ $[M+Na]^+$, 0.43 err [ppm].

(MeO)Ent$_M$



Chemical Formula: $C_{44}H_{57}N_5O_{11}$
Molecular Weight: 831,964

TFA (400 µL) was added to a solution di-tert-butyl (4-(3-((tert-butoxycarbonyl)amino)propyl)-4-(4-oxo-4-(prop-2-yn-1-ylamino)butanamido)heptane-1,7-diyl)dicarbamate (45 mg, 70 µmol, 1.0 equiv) in dry CH_2Cl_2 (2.0 mL, 0.035 M) at 0°C. The solution was stirred 2 h at 23°C, evaporated with toluene (2x 5 mL) and concentrated under reduced pressure. The residue was dissolved in dry DMF (500 µL, 0.14 M) and DiPEA (80 µL, 460 µmol, 6.5 equiv) was added. The solution was cooled down to 0 °C, dimethoxybenzoic acid chloride (50 mg, 250 µmol, 3.5 equiv) was added as a solution in dry THF (800 µL, 0.31 M) and stirred for 1 h at 23 °C. EtOAc (20 mL) was added and the solution was quenched by addition of $NH_4Cl_{(aq.)}$ (5 mL), layers were separated and the organic layer was washed with water (2x 10 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/9:1) yielding **(MeO)Ent$_M$** (33 mg, 40 µmmol, 57%) as a colorless oil.

TLC (CH_2Cl_2:MeOH/9:1) R_f: 0.3 [UV^{254},ninhydrin], **^{1}H-NMR** (500 MHz, $CDCl_3$) δ [ppm]: 8.02 (t, *J* = 5.9 Hz, 3H), 7.62 (dd, *J* = 7.9, 1.7 Hz, 3H), 7.11 (t, *J* = 8.0 Hz, 3H), 7.01 (dd, *J* = 8.2, 1.7 Hz, 4H), 3.98 (dd, *J* = 5.3, 2.6 Hz, 2H), 3.87 (d, *J* = 1.2 Hz, 18H), 3.42 (dd, *J* = 13.8, 7.5 Hz, 6H), 2.63 (td, *J* = 7.0, 1.1 Hz, 2H), 2.49 (t, *J* = 6.7 Hz, 2H), 2.17 (t, *J* = 2.6 Hz, 1H), 1.70 – 1.37 (m, 12H).**^{13}C-NMR:** (126 MHz, $CDCl_3$) δ [ppm]: δ 171.8, 170.4, 165.0, 152.2, 147.1, 126.5, 124.0, 122.3, 115.0, 79.5, 70.9, 61.1, 61.0, 55.7, 39.7, 31.2,

30.3, 28.8, 28.0, 23.0. **HRMS** (ESI-IT) [m/z]: 870.39009, calculated 870.38959 for $C_{44}H_{57}N_5O_{12}Na$ $[M+Na]^+$, 0.57 err [ppm].

Computational details

All calculations were done applying density functional theory (DFT) as implemented in Gaussian 09[13] program package, Revision D.01. All geometry optimizations and analytical second derivatives were done using Becke's three-parameter hybrid functional (B3LYP)[14] and a standard 6-31G (d,p) basis set. Additionally, Grimme's dispersion correction with the original D3 damping function[15] was applied. In order to evaluate the kinetic stabilities of all important Fe-O bonds, relaxed force constants were calculated applying our own Compliance 3.0.2. code and using the B3LYP cartesian force constants as input.[16,17] Simulating the DMSO environment, a Polarizable Continuum Model (PCM)[18] using the integral equation formalism variant (IEFPCM) was used consistently with a dielectric constant ε(DMSO) = 46.826. In order to obtain comparable values for stability and energy of the corresponding systems, the ΔG-values of the protocol of *Baramov et al*.[19] were used, which have been utilized for similar systems before. The bond energies and free enthalpies in solution G(sol), respectively, were calculated as following:

(1) $$G(sol) = G(gas) + G(solv)$$

(2) $$G(gas) = H(gas) - \ T \times S(gas)$$

(3) $$H(gas) = E(SCF) + ZPE$$

(4) $$G(solv) = [H(solv) - T \ \times S(solv)] - G(gas)$$

(5) $$H(solv) = \ E(SCF) + ZPE$$

(6) $$\Delta G(sol) = \ \sum G(sol) for\ reactants - \sum G(sol) for\ products$$

G(gas) is the free energy in the gas phase, G(solv) is the free energy of the solvatization, H(gas)/H(solv) is the enthalpy in gas phase/solution, T is the temperature (298.15 K), S(gas)/S(solv) is the entropy in the gas phase/solution, E(SCF) is the self-consistent field energy and ZPE is the zero-point energy.

For the enterobactin **Ent** system the formula shown in Equation 7 was set.

(7) $$\Delta G(sol) = \Delta G[FeEnt + 6\ H_2O + 6\ H^+] - \Delta G[Fe(H_2O)_6 + Ent]$$

Herein, the formed protons were not reacted with the released water to hydronium ions (quasi solvatization with explicit solvents), but were considered separately. Therefore, G(solv) = 273.3 kcal/mol was assumed as fixed value for the total solvatization energy in DMSO, based on studies by *Kelly et al*..[20] The translation entropy was assumed -S(298.15 K) = -7.7 kcal/mol based on calculations by *Ryu et al.* using the *Sackur-Tetrode* method.[21] This was a necessary to obtain absolute values with correct signs.

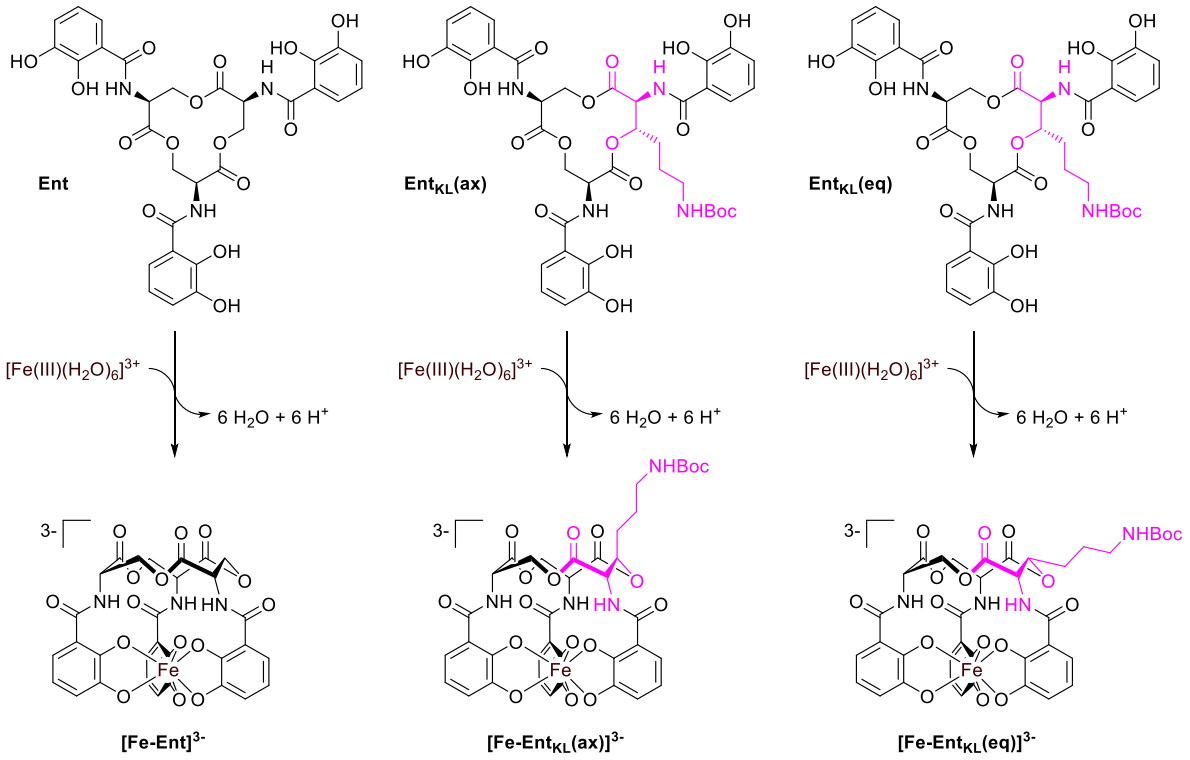
As literature references for the enterobactin **Ent** the bond constant of $K_d = 10^{49}$ the experimentally determined by *Raymond et al.* was used,[22,23] which leads following Equation 8 to a

ΔG(sol) = -66.8 kcal/mol. Only the catecholate binding mode and not the alternative salicylate binding mode was considered.

(8) $$\Delta G = R \times T \times ln\frac{K_d}{c}$$

R is the universal gas constant (1.987 10^{-3} kcal K^{-1} mol^{-1}), T is the temperature (298.1 K), K_d is the dissociation constant (K^{-1}) and c is the standard reference concentration (1 mol/L)

The values for **Ent_{KL}(ax)** and **Ent_{KL}(eq)** were calculated analogue to Equation 7 and as outlined in Scheme S1. Initial test calculations for the ferri-siderophore complexes revealed that the Fe(III)-high-spin state is energetically favored. Furthermore, the *trans*-configuration of the amide group bearing the substituents in α-position was identified as the energetically more stable isomer. The calculated values for ΔG(sol) are shown in Table S1.



Scheme S1: Complexation of Fe(III) ions by **Ent**, **Ent_{KL}(ax)** and **Ent_{KL}(eq)**.

Table S1: Calculated ΔG(sol) of **$[Fe\text{-}Ent]^{3-}$**, **$[Fe\text{-}Ent_{KL}(ax)]^{3-}$** and **$[Fe\text{-}Ent_{KL}(eq)]^{3-}$**.

DMSO	**ΔG(sol) $[Fe\text{-}Ent]^{3-}$**	**ΔG(sol) $[Fe\text{-}Ent_{KL}(ax)]^{3-}$**	**ΔG(sol) $[Fe\text{-}Ent_{KL}(eq)]^{3-}$**
[kcal/mol]	-64.5	-67.2	-70.7
[kJ/mol]	-269.9	-281.6	-296.0
[eV]	-2.80	-2.92	-3.07

The calculated value for ΔG(sol) **[Fe-Ent]$^{3-}$** is very close to the literature reference of -66.8 kcal/mol[22,23] positively validating the calculations as the experimentally determined absolute value for the Gibbs energy could be reproduced. Furthermore, the value for **[Fe-Ent$_{KL}$(eq)]$^{3-}$** was calculated to be by 3.5 kcal/mol energetically more favored as compared to **[Fe-Ent$_{KL}$(ax)]$^{3-}$**, whereas both artificial enterobactin analogues were calculated to be energetically lower than the unsubstituted **Ent**. Although, the absolute values need to be considered with caution, it can be assumed that both artificial enterobactin analogues **Ent$_{KL}$(eq)** and **Ent$_{KL}$(ax)** lead to complexes, which are at least of similar stability compared to **[Fe-Ent]$^{3-}$** and that there is no significant energetical advantage of one analogue over the other.

For the evaluation of the kinetic stabilities of the iron-oxygen bonds compliance matrices were used, which were generated with the Compliance 3.0.2. software.[16,17] In Table S2 the inverted and averaged values of the diagonal elements of the compliance matrices, which is the average of the relaxed force constant for the six iron-oxygen single bonds is shown.

Table S2: Averaged, calculated relaxed force constants of the iron-oxygen single bonds of the ferri-siderophore complexes in DMSO.

DMSO	**Fe-O bond [Fe-Ent]$^{3-}$**	**Fe-O bond [Fe-Ent$_{KL}$(ax)]$^{3-}$**	**Fe-O bond [Fe-Ent$_{KL}$(eq)]$^{3-}$**
[N/cm]	0.95	0.97	0.98

Values below 1 N/cm are indicating a very weak bond with less or none covalency.[16,24–27] Furthermore, the values differ only less, indicating that the total strength of the iron-oxygen bonds in all complexes in sum are approximately equal, thus all complexes are kinetically similarly labile, while being thermodynamically stable as found earlier for **[Fe-Ent]$^{3-}$** by *Raymond et al*..[28,29]

Biological evaluation of the compounds

Bacterial Strains

The microorganisms used within this study were obtained from the internal collection at Helmholtz Institute for Pharmaceutical Research (HIPS), KEIO collection or kindly provided by Mark Brönstrup from the department Chemical Biology at the Helmholtz Centre for Infection Research. All strains were handled according to standard protocols. The *E. coli* K-12 *ΔentA* strain was always cultivated in the presence of 50 µg/mL kanamycin (Merck KGaA, Germany) unless otherwise stated. Overnight cultures of microorganisms were prepared in 50% reduced (10 g/L) MHB II (17.5g/L acid hydrolysate of casein, 3 g/L beef extract, 1.5 g/L starch) (Merck KGaA, Germany) and back diluted (1:100) into fresh 50% reduced (10 g/L) MHB II (17.5g/L acid hydrolysate of casein, 3 g/L beef extract, 1.5 g/L starch) (Merck KGaA, Germany) or in 50% reduced (10 g/L) MHB II (17.5g/L acid hydrolysate of casein, 3 g/L beef extract, 1.5 g/L starch) (Merck KGaA, Germany) with the addition of 2,2-bipiridine (DP) (Merck KGaA, Germany) (200 µM DP for *E. coli* and 600 µM DP for *P. aeruginosa*) to obtain iron-limiting medium conditions.

Growth recovery assays

Overnight cultures of microorganisms were prepared and diluted to an OD_{600} value of 0.01 in 50% reduced (10 g/L) MHB II (17.5g/L acid hydrolysate of casein, 3 g/L beef extract, 1.5 g/L starch) (Merck KGaA, Germany). Different concentrations of compounds were added to a 96-well flat bottom plate (Corning™, USA). The corresponding bacterial culture was added and the plate was wrapped in parafilm and incubated for 24 h at 37°C with shaking at 150 rpm in a microplate shaker (Heidolph Instrument, Germany). After 24 h, growth recovery was assessed by measuring the OD_{600} with a TECAN Pro200 plate reader (Tecan Trading AG, Switzerland). Each plate contained two duplicates of each condition and the plates were repeated three times on different days. The mean of the OD_{600} measurements were reported and the error bars are the standard deviation of the mean obtained from the three replicates. Statistical significance was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1).

Further growth recovery assays were conducted on *P. aeruginosa* K648 ΔPvd/Pch with **(AcO)Ent$_{KL}$** and **(AcO)Ent$_{KL}$-SulfoCy5** in the presence of different concentrations of bovine albumin (Sigma-Aldrich, Germany) and human apo-transferrin (Sigma-Aldrich, Germany), respectively.

The growth of the *E. coli* and *P. aeruginosa* siderophore biosynthesis defect mutants were restored by the addition of the synthesized enterobactin derivatives **(AcO)Ent$_{KL}$**, **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY,** **(AcO)Ent$_{KL}$-BODIPY$_{FL}$,** **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$,** **(AcO)Ent$_{KL}$-SulfoCy5** and **(AcO)Ent$_M$** in a concentration-dependent manner (see Figures S2-S6 and S10), indicating their ability to be

internalized by the iron-uptake machinery of the utilized Gram-negative bacteria. To further support the obtained results, the corresponding permethylated enterobactin derivatives **(MeO)Ent$_{KL}$**, **(MeO)Ent$_{KL}$-PEG$_4$-BODIPY, (MeO)Ent$_{KL}$-BODIPY$_{FL}$, (MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$, (MeO)Ent$_{KL}$-SulfoCy5** and **(MeO)Ent$_M$** were not able to bind iron which prevented growth recovery to occur (see Figures S2-S6 and S10). The response to the added compounds of *P. aeruginosa,* compare to *E. coli*, was significantly higher. In addition, the **(AcO)Ent$_{KL}$-PEG$_3$-MG** and **(AcO)Ent$_{KL}$-MG** bearing a malachite green cargo were not able to restore growth of *E. coli*, while the growth of *P. aeruginosa* was restored by addition of these compounds in a concentration dependent manner (see Figures S7-S8). Consistently, **(MeO)Ent$_{KL}$-PEG$_3$-MG** did not show any growth recovery of *P. aeruginosa* (see Figures S7). These results are in line with finding of *Nolan* and co-workers[30] reporting that *P. aeruginosa* exhibits greater promiscuity for the uptake compared to *E. coli*.

Furthermore, growth of *P. aeruginosa* was restored by addition of **(AcO)Ent$_{KL}$** presence of all tested concentration of albumin, but with lower efficiency at higher concentrations of albumin (see Figure S9). In addition, growth of *P. aeruginosa* was restored by addition of **(AcO)Ent$_{KL}$-SulfoCy5** in the presence of all tested concentrations of apo-transferrin*,* however, with clearly reduced efficiency performance at higher concentrations of apo-transferrin (see Figure S9).

A comparision between **Ent, (AcO)Ent$_{KL}$** and **(AcO)Ent$_M$** at 10 μM concentration of revealed a similar efficacy in the growth recovery of *E. coli* as well as *P. aeruginosa*.

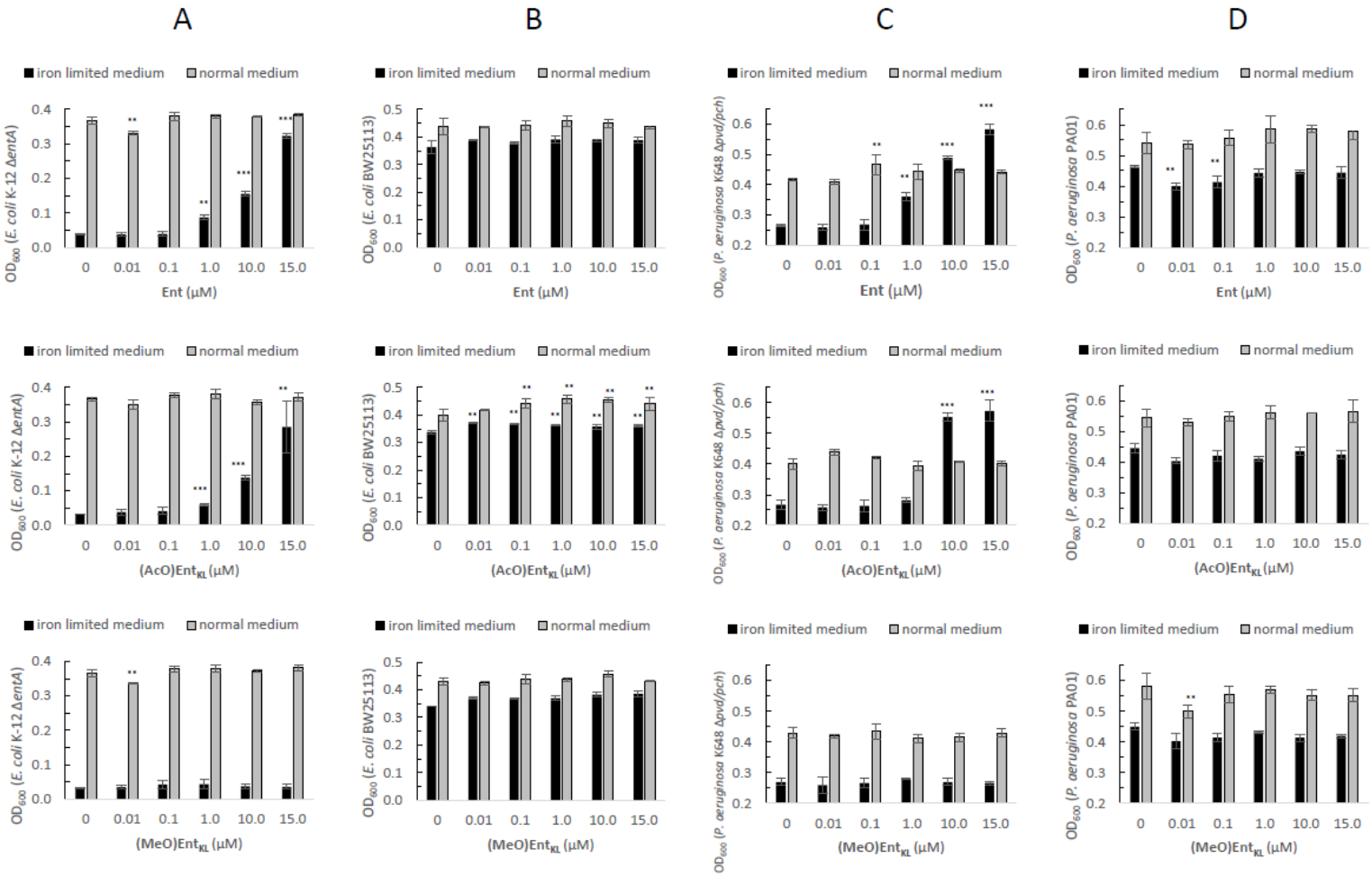


Figure S2: Growth recovery assays of *E. coli* K-12 Δ*entA* (A), and *P. aeruginosa* K648 Δ*pch/pvd* (C) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **Ent**, **(AcO)Ent$_{KL}$** and **(MeO)Ent$_{KL}$**, in comparison to *E. coli* BW25113 (B) and *P. aeruginosa* PA01 (D). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

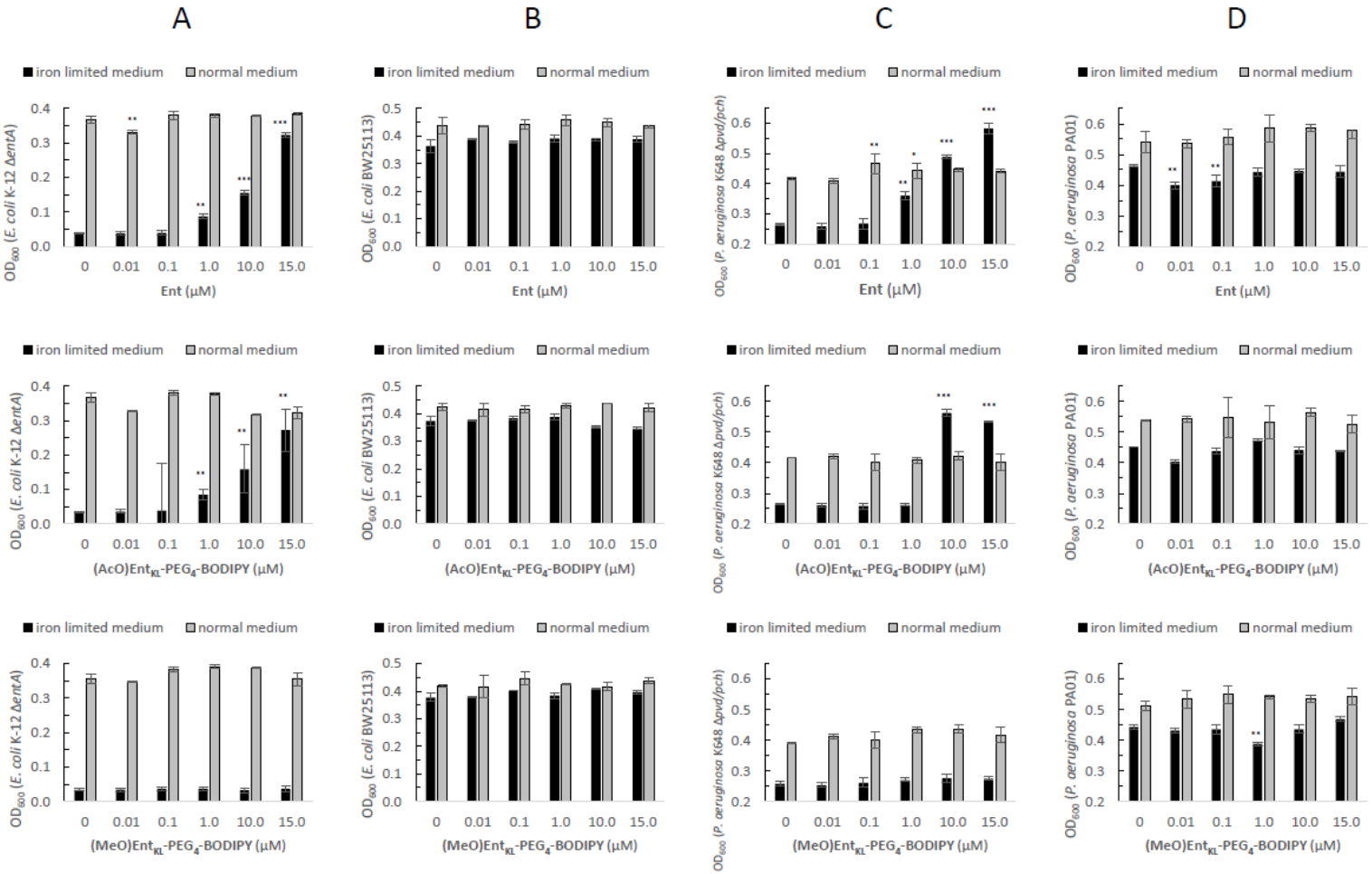


Figure S3: Growth recovery assays of *E. coli* K-12 Δ*entA* (A), and *P. aeruginosa* K648 Δ*pch/pvd* (C) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **Ent**, **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY** and **(MeO)Ent$_{KL}$-PEG$_4$-BODIPY** in comparison to *E. coli* BW25113 (B) and *P. aeruginosa* PA01 (D). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2 Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05..

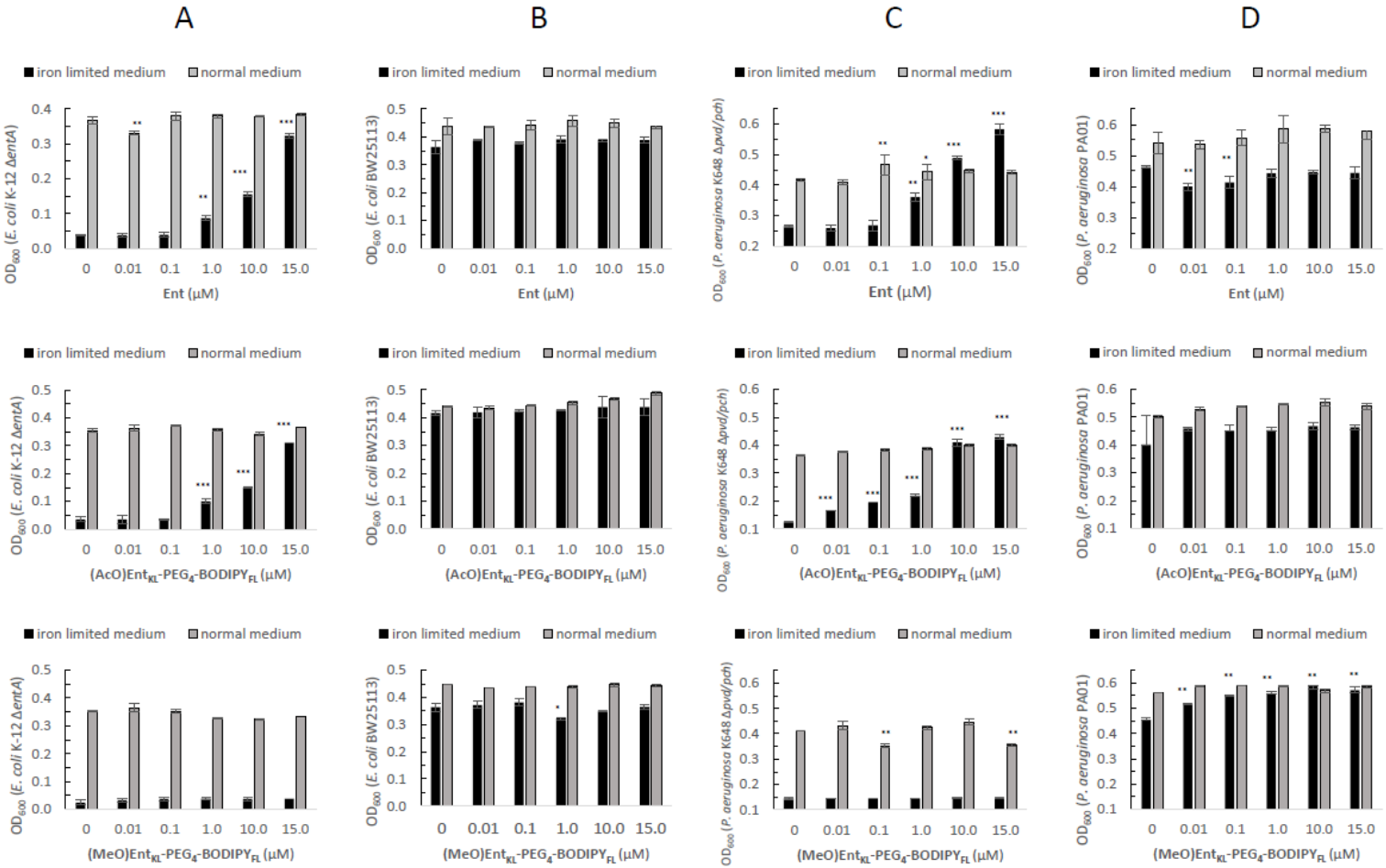


Figure S4: Growth recovery assays of *E. coli* K-12 Δ*entA* (A), and *P. aeruginosa* K648 Δ*pch/pvd* (C) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **Ent**, **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** and **(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** in comparison *E. coli* BW25113 (B) and *P. aeruginosa* PA01 (D). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

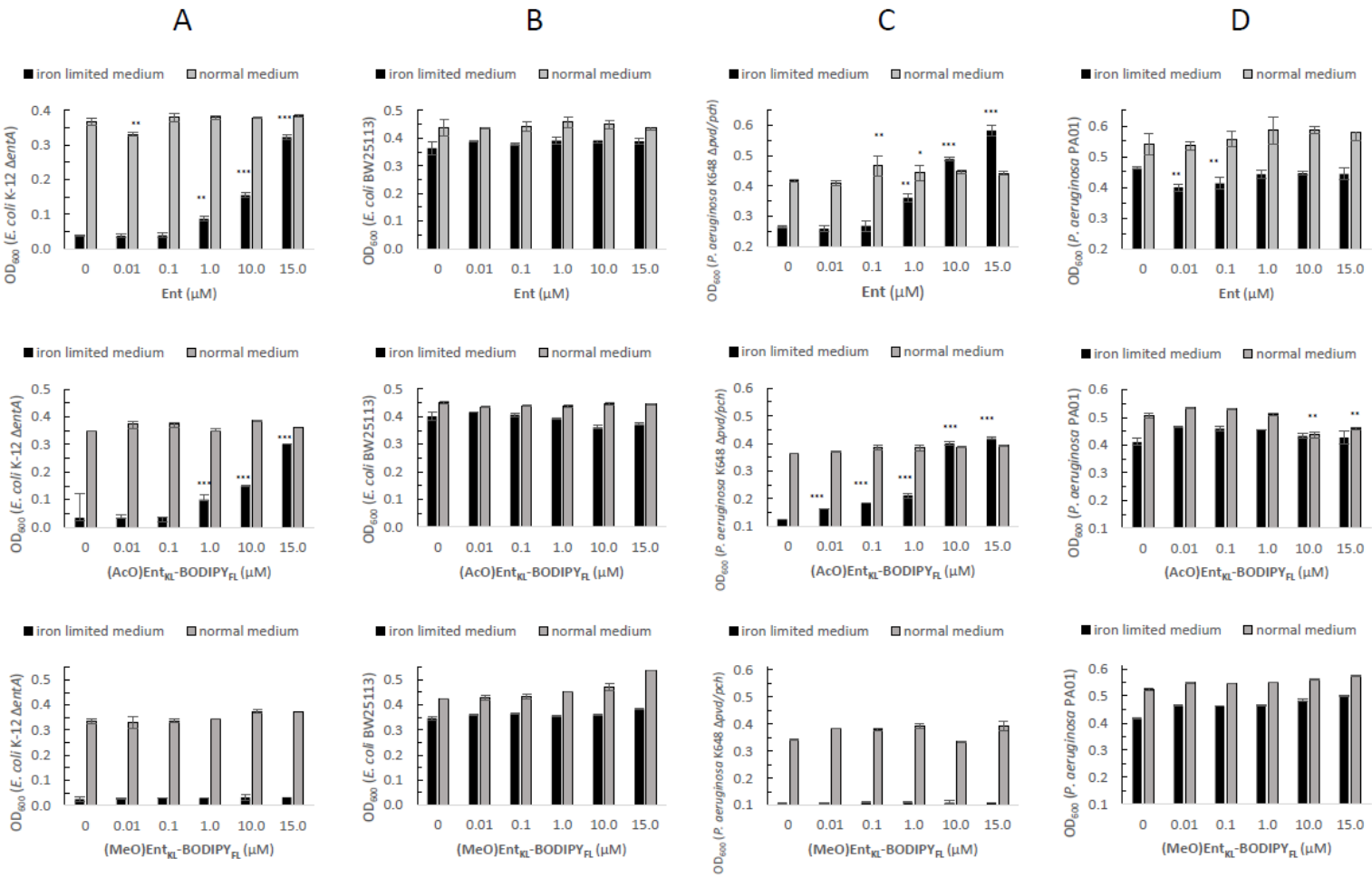


Figure S5: Growth recovery assays of *E. coli* K-12 Δ*entA* (A), and *P. aeruginosa* K648 Δ*pch/pvd* (C) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **Ent**, **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** and **(MeO)Ent$_{KL}$-BODIPY$_{FL}$** in comparison to *E. coli* BW25113 (B) and *P. aeruginosa* PA01 (D). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

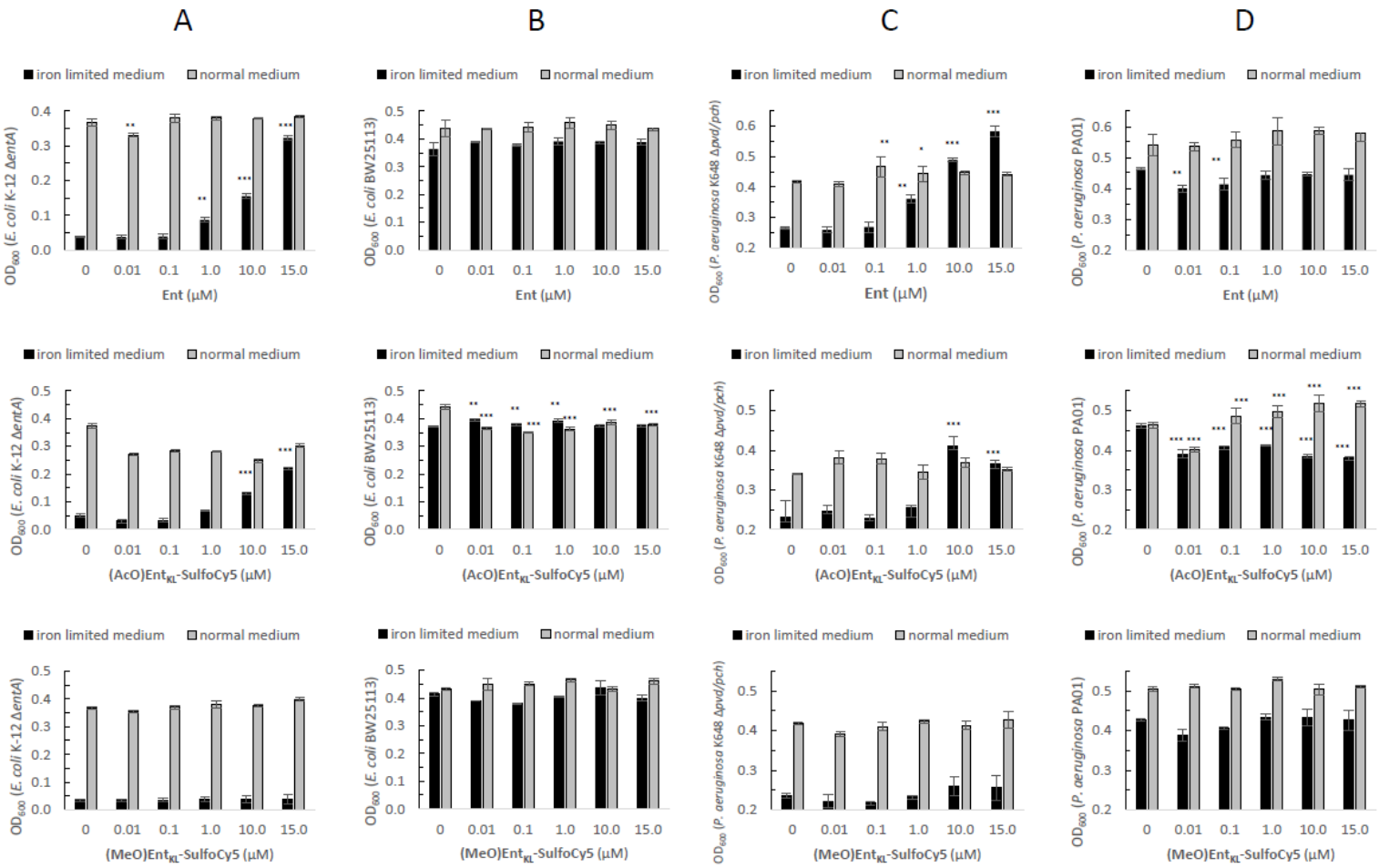


Figure S6: Growth recovery assays of *E. coli* K-12 Δ*entA* (A), and *P. aeruginosa* K648 Δ*pvd/pch* (C) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **Ent**, **(AcO)Ent$_{KL}$-SulfoCy5** and **(MeO)Ent$_{KL}$-SulfoCy5** in comparison to *E. coli* BW25113 (B) and *P. aeruginosa* PA01 (D). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

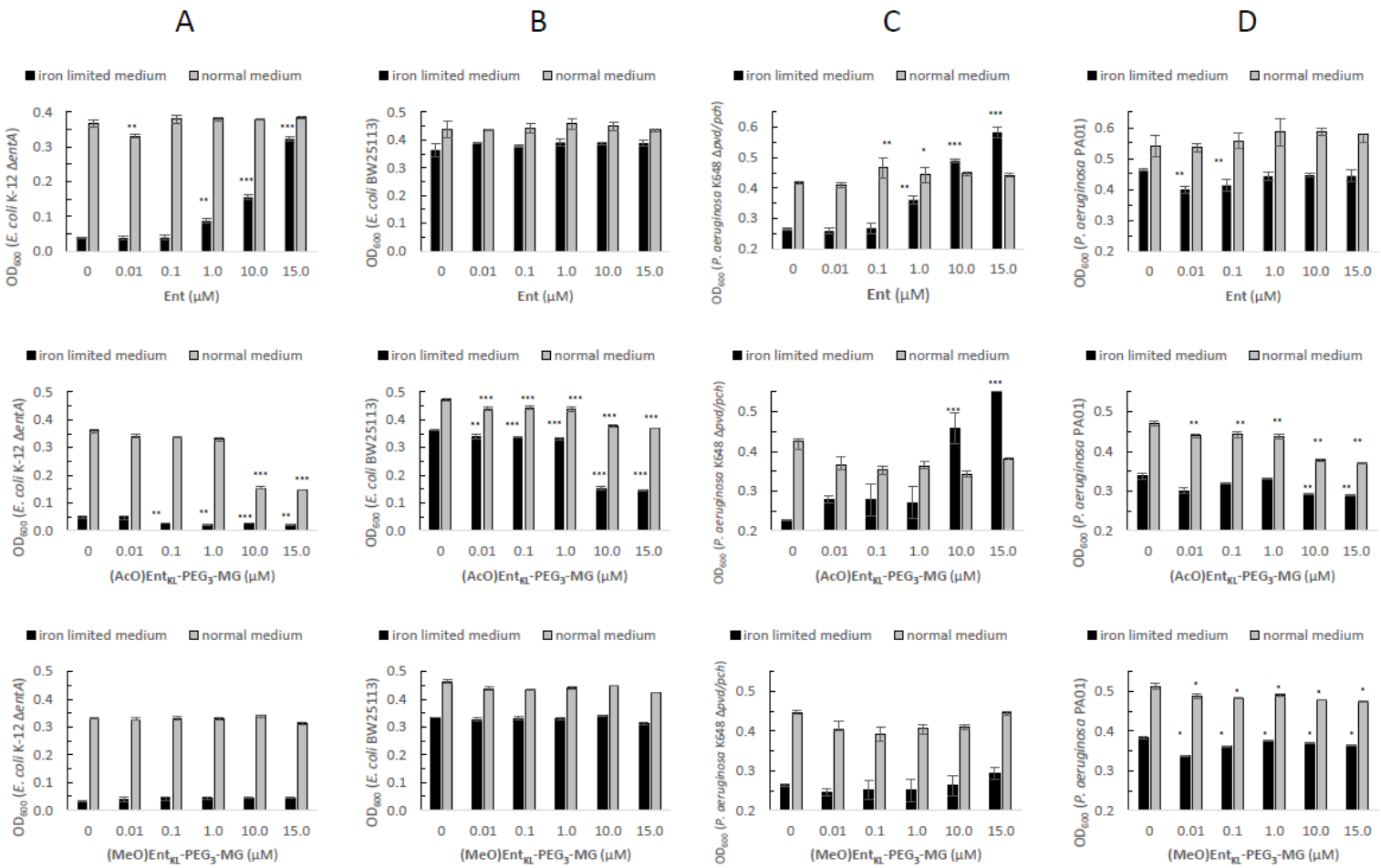


Figure S7: Growth recovery assays of *E. coli* K-12 ΔentA (A), and *P. aeruginosa* K648 Δpch/pvd (C) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **Ent**, **(AcO)Ent$_{KL}$-PEG$_3$-MG** and **(MeO)Ent$_{KL}$-PEG$_3$-MG** in comparison to *E. coli* BW25113 (B) and *P. aeruginosa* PA01 (D). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

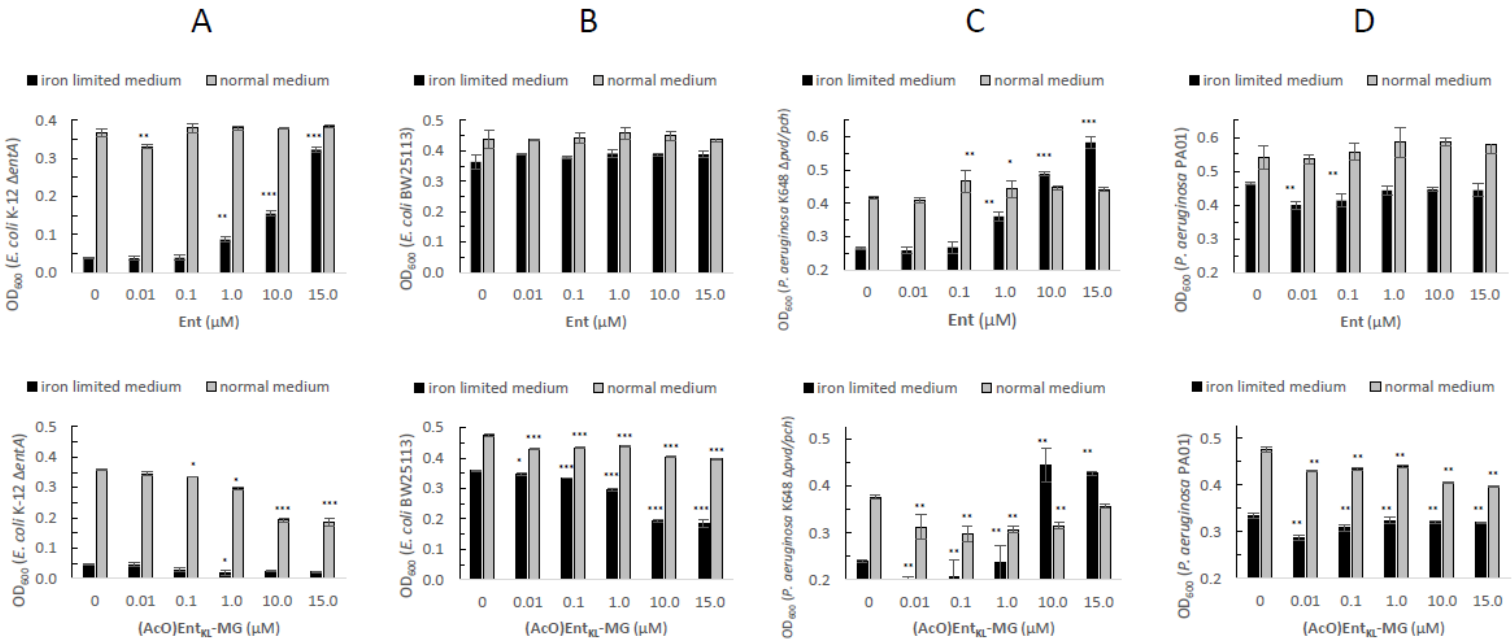


Figure S8: Growth recovery assays of *E. coli* K-12 Δ*entA* (A), and *P. aeruginosa* K648 Δ*pch/pvd* (C) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **Ent** and **(AcO)Ent$_{KL}$-MG** in comparison to *E. coli* BW25113 (B) and *P. aeruginosa* PA01 (D). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

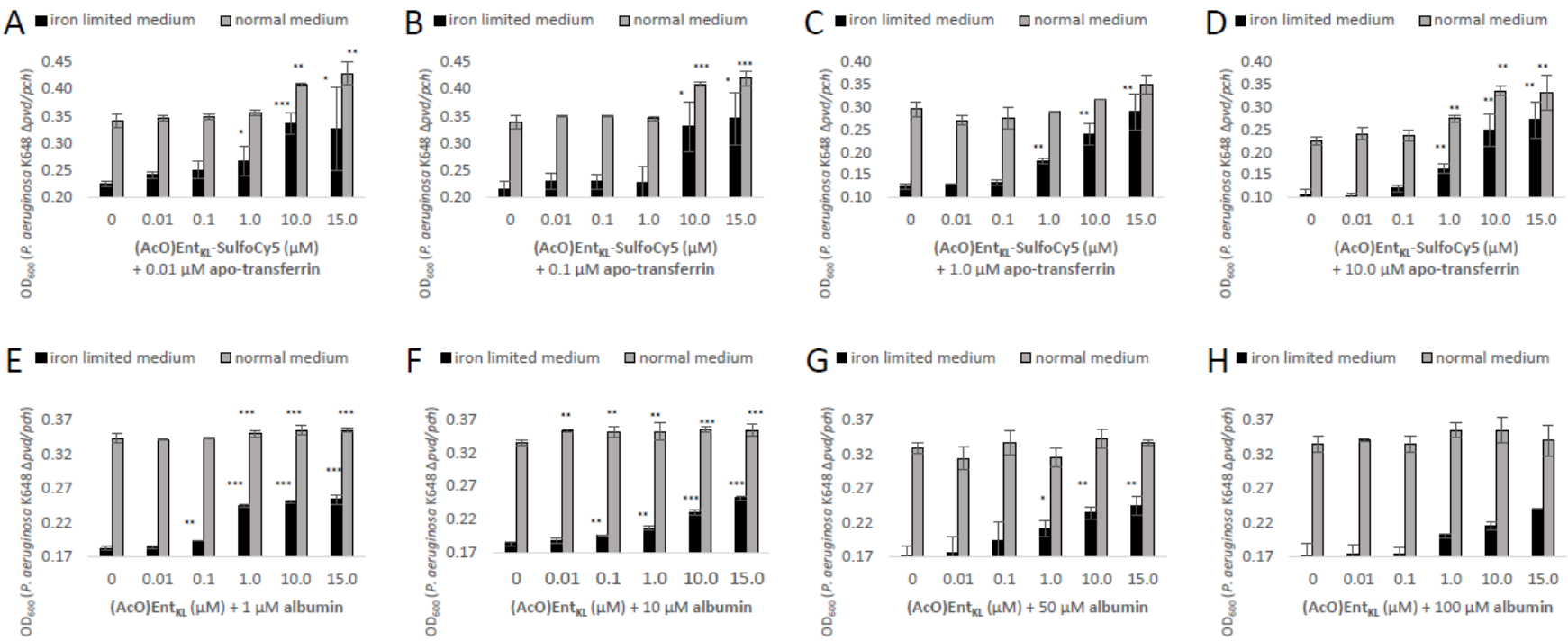


Figure S9: Growth recovery assays of *P. aeruginosa* K648 Δ*pch/pvd* under iron-limiting (black bars) and non-limiting (grey bars) media conditions after 24 h of incubation at different concentrations of **(AcO)Ent$_{KL}$-SulfoCy5** in presence of human **apo-transferrin** (0.01 µM (A), 0.1 µM (B), 1.0 µM (C) and 10.0 µM (D)) and **(AcO)Ent$_{KL}$** in the presence of bovin serum **albumin** (1.0 µM (E), 10.0 µM (F), 50.0 µM (G) and 100.0 µM (H)). Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

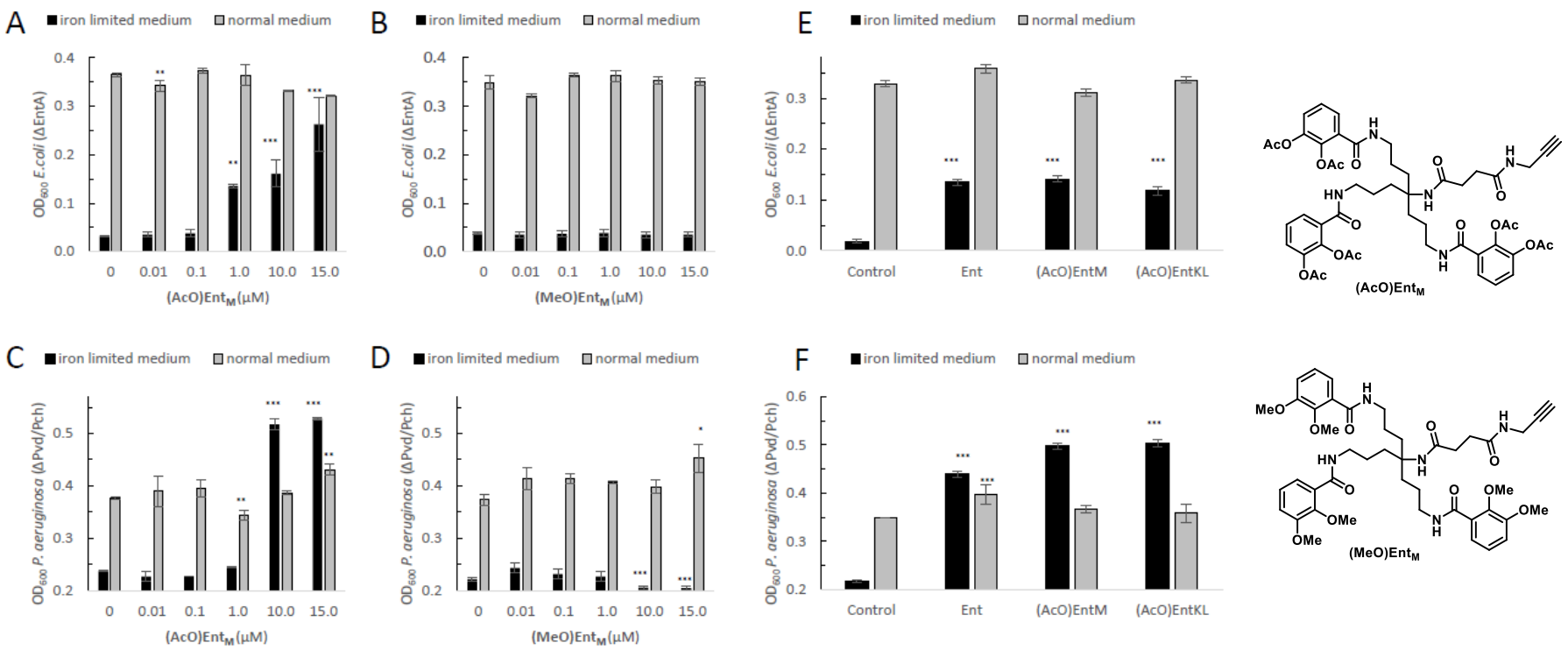


Figure S10: Growth recovery assays of *E. coli* K-12 Δ*entA* (A and B), and *P. aeruginosa* K648 Δ*pch/pvd* (C and D) under iron-limiting (black bars) and non-limiting (gray bars) media conditions after 24 h of incubation at different concentrations of **(AcO)Ent$_M$** (A and C) and **(MeO)Ent$_M$** (B and D) and comparison of the growth recovery of *E. coli* K-12 Δ*entA* (E), and *P. aeruginosa* K648 Δ*pch/pvd* (F) at 10 μM concentration of **Ent**, **(AcO)Ent$_{KL}$** and **(AcO)Ent$_M$**. Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean. As *P. aeruginosa* shows a growth of <0.25 OD in the absence of an external siderophore, Y-axis is shown starting at an OD_{600} of 0.2. Statistical significance is indicated as a p-value <0.05 and was determined by a unpaired t-test using GraphPad Prism 9 (version 9.1.1). *** p-value <0.001, ** p-value <0.005 and * p-value <0.05.

Fluorescence microscopy of bacterial cells

Overnight cultures were prepared and diluted as stated above. Different concentrations of compounds and/or different ratios were added to the diluted cell cultures. Microorganisms were incubated for 4 h at 37°C at 400 rpm on a microplate shaker (Heidolph Instrument, Germany). After the incubation period, cells were washed three times with PBS (Merck KGaA, Germany). The pellets were resuspended in PBS (Merck KGaA, Germany) and mixed with ProLong™ Glass Antifade mounting media (ThermoFisher Scientific, USA) and placed on glass slides for imaging.

The corrected total cell fluorescence (CTCF) of selected experiments was determined utilizing the software ImageJ Version 1.53a (Wayne Rashband. National Institute of Health, USA. Http://imagej.nih.gov/ij, for more information on method please see: https://theolb.readthedocs.io/en/latest/imaging/measuring-cell-fluorescence-using-imagej.html) as outlined in Table S3.

Table S3: Corrected total cell fluorescence of imaging experiments on *E. coli* BW25113, *E. coli* K-12 Δ*entA*, *P. aeruginosa* PA01 and *P. aeruginosa* K648 Δ*pch/pvd*.

Conjugate/Conditions	***E. coli* BW25113, CTCF[a]**	***P. aeruginosa* PA01, CTCF[a]**
1 µM **(AcO)Ent$_{KL}$-BODIPY$_{FL}$**	3038.17	555.80
10 µM **(AcO)Ent$_{KL}$-BODIPY$_{FL}$**	6858.12	1555.40
1 µM **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$**	3252.00	2688.20
10 µM **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$**	5636.00	3423.20
Conjugate/Conditions	***E. coli* K-12 Δ*entA*, CTCF[a]**	***P. aeruginosa* K648 Δ*pch/pvd*, CTCF[a]**
1 µM **(AcO)Ent$_{KL}$-BODIPY$_{FL}$**	2568.30	0
10 µM **(AcO)Ent$_{KL}$-BODIPY$_{FL}$**	7694.20	4773.00
1 µM **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** + 10 µM **Ent**	5135.00	2144.40
10 µM **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** + 10 µM **Ent**	8527.00	7207.00
1 µM **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$**	2563.80	0
10 µM **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$**	7464.00	6750.00

[a] number of cells, n = 5 for each experiment, each experiment has been reproduced at least 2 times.

With both, **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** (Figures S11 and S16) and **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** (Figures S13 and S18), weak to moderate fluorescence labelling of *E. coli* BW25113 and *E. coli* K-12 Δ*entA* as well as *P. aeruginosa* PA01 was achieved at a concentration of 1.0 µM, while almost no labelling was observed for *P. aeruginosa* K468 Δ*pvd/pch*. At 10.0 µM concentration a strong labelling for all bacterial strains tested was observed. In general, the fluorescence labelling with each compound was more pronounced for *E. coli*

strains compared to *P. aeruginosa*, and mutant strains were more efficiently labelled than wild type strains (Table S3). However, slight differences in the labelling performance of **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** and **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** were observed. While **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** led to more prominent labelling of *E. coli* BW25113 and *E. coli* K-12 Δ*entA* (Table S3), **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** led to more prominent labelling of *P. aeruginosa* PA01 and *P. aeruginosa* K468 Δ*pvd/pch* (Table S3).

The fluorescence labelling of *E. coli* K-12 Δ*entA* and *P. aeruginosa* K468 Δ*pvd/pch* was also observed treating the bacteria with mixtures of **(AcO)Ent$_{KL}$-BODIPY$_{FL}$:Ent**/1:10, 1:1, 10:1 or 10:10 (Figures S21 and S22). Interestingly, the fluorescence labelling is increased in the presence of **Ent** (Table S3). Furthermore, neither **(MeO)Ent$_{KL}$-BODIPY$_{FL}$, (MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$**, or **BODIPY$_{FL}$-alkyne** alone led to any fluorescence labelling of any of the bacteria (Figures S12, S14, S15, S17, S19 and S20).

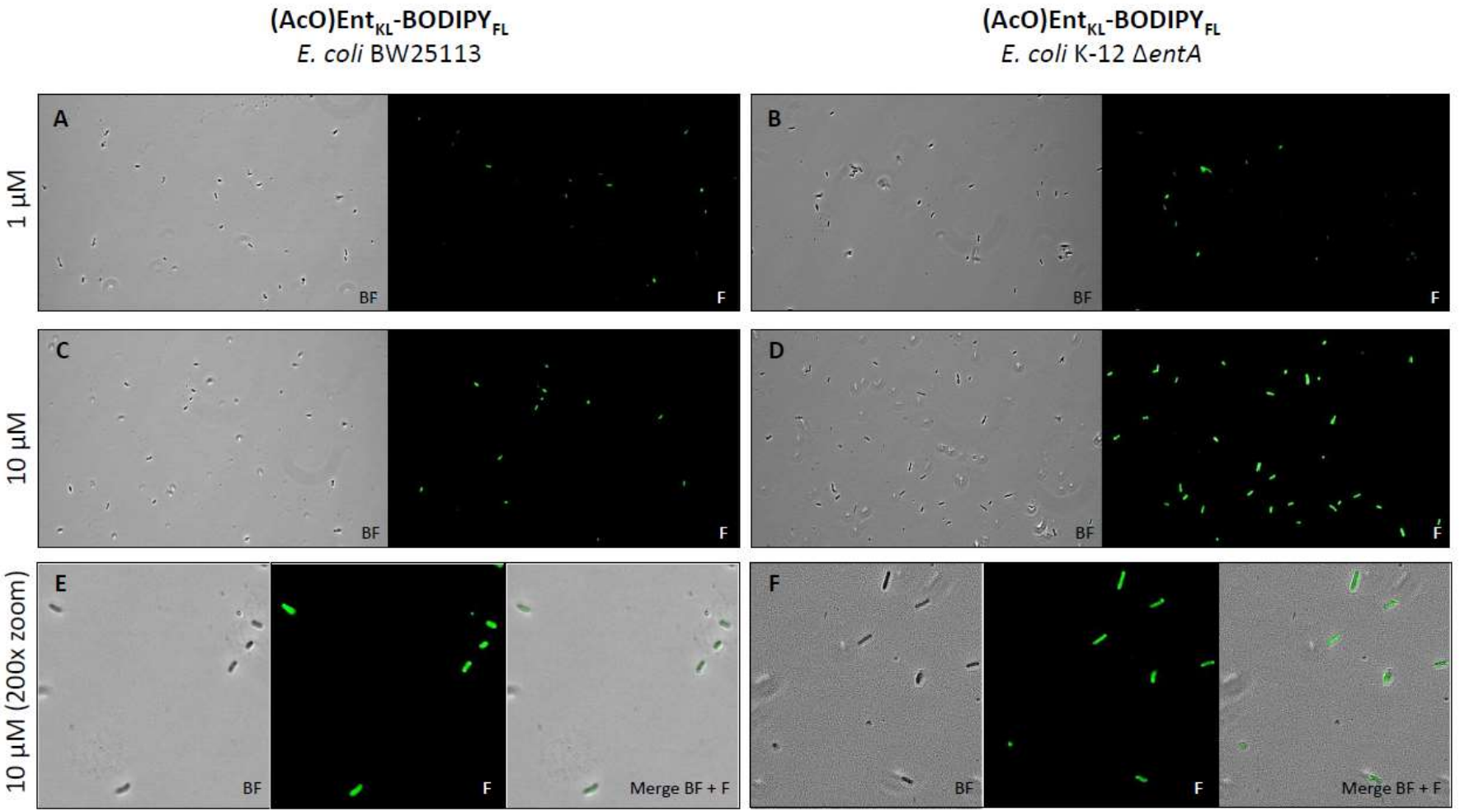


Figure S11: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *E. coli* BW25113 and *E. coli* K-12 Δ*entA* cultivated in 50% MHB II under iron-limiting conditions in the presence of 200 µM DP and for 4 h at 37°C treated with **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

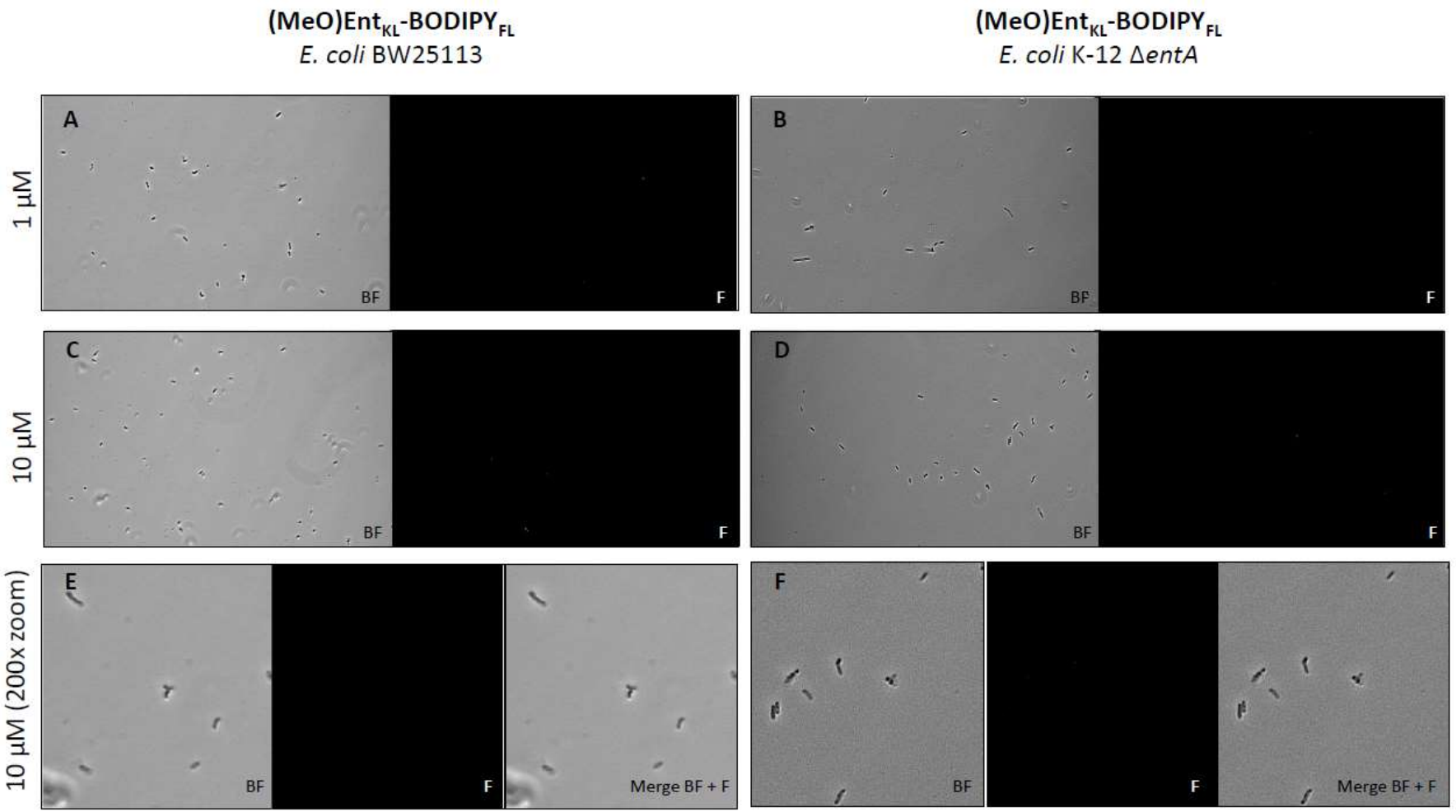


Figure S12: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *E. coli* BW25113 and *E. coli* K-12 Δ*entA* cultivated in 50% MHB II under iron limiting conditions in the presence of 200 µM DP and for 4 h at 37°C treated with **(MeO)Ent$_{KL}$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

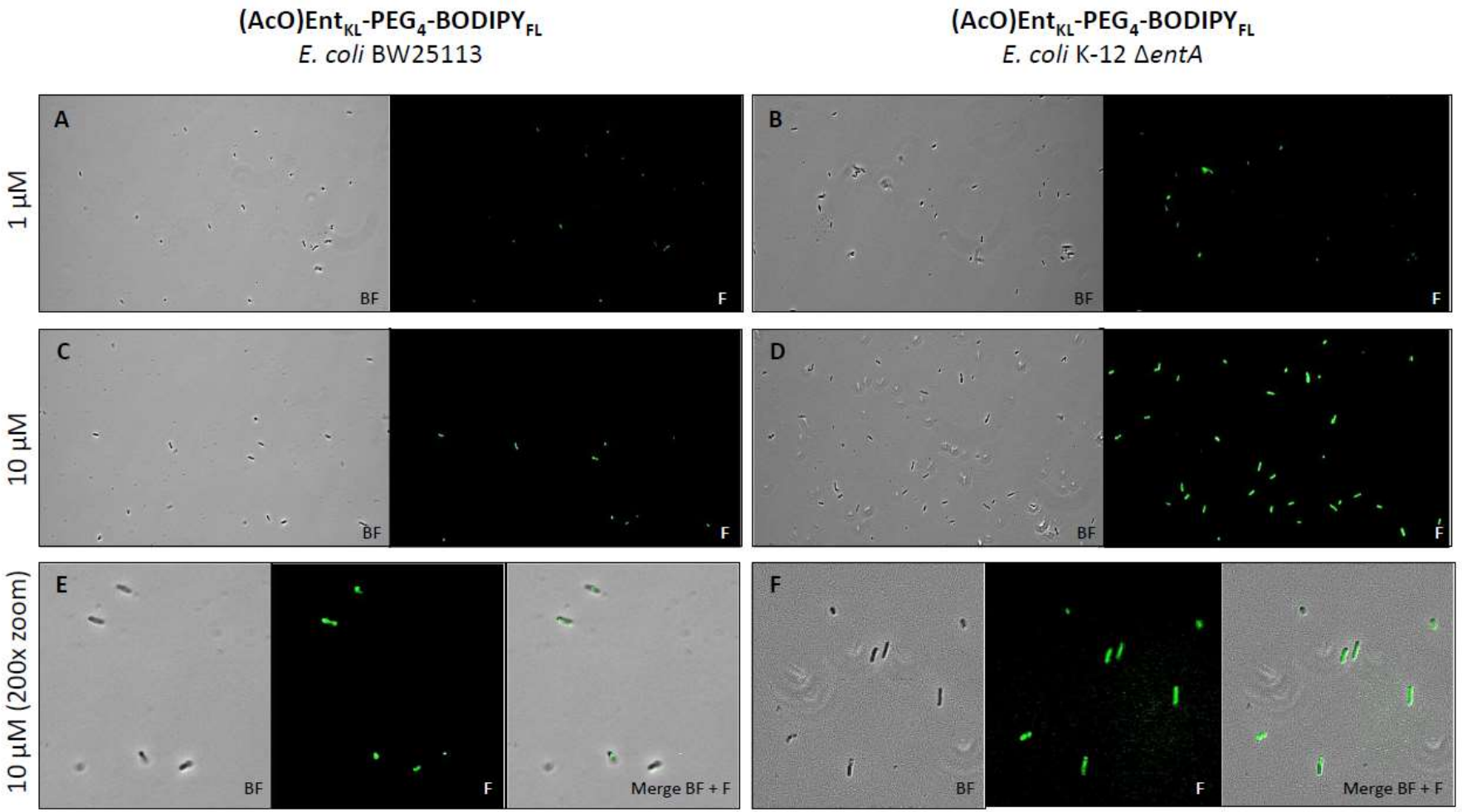


Figure S13: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *E. coli* BW25113 and *E. coli* K-12 Δ*entA* cultivated in 50% MHB II under iron-limiting conditions in the presence of 200 µM DP and for 4 h at 37°C treated with **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

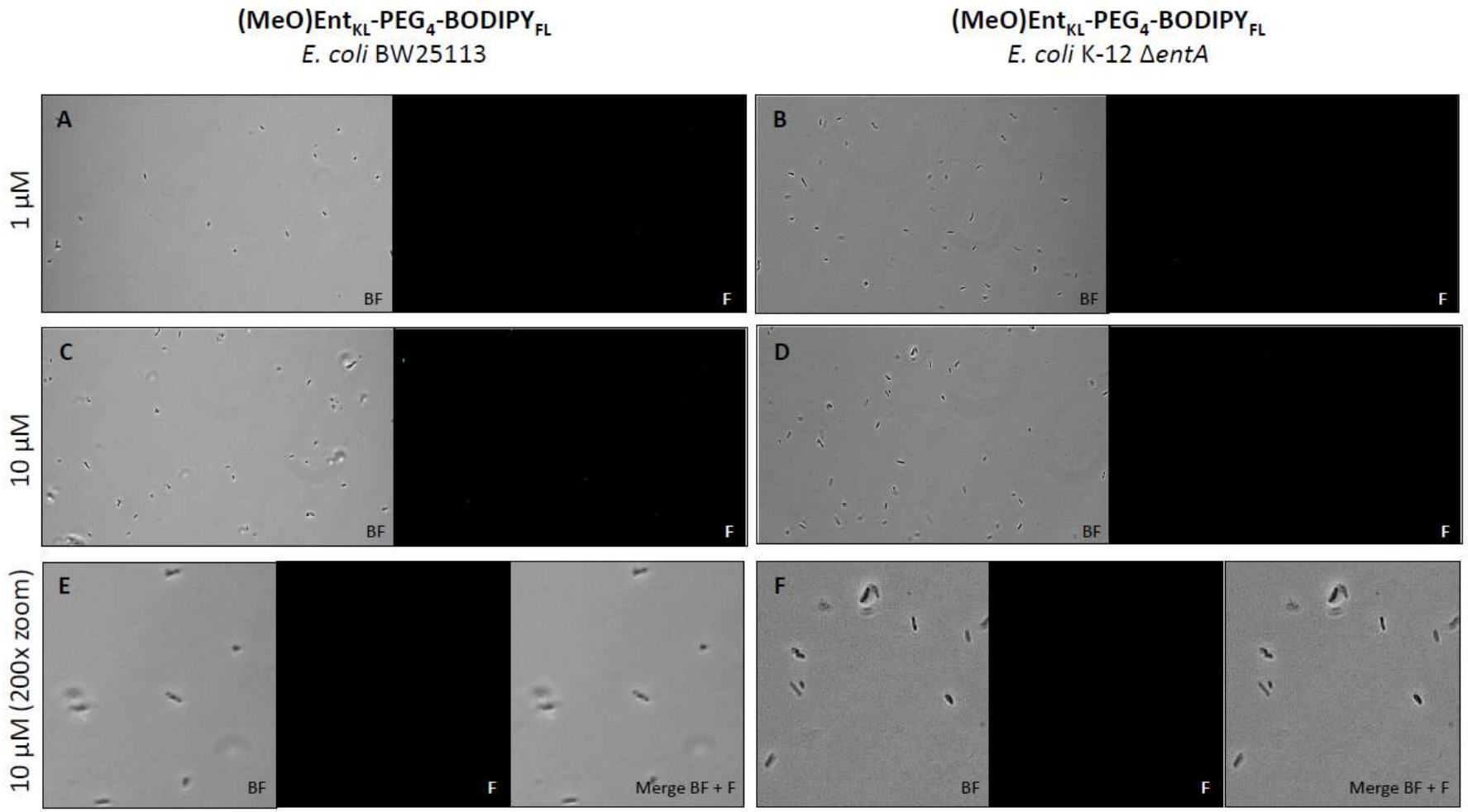


Figure S14: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *E. coli* BW25113 and *E. coli* K-12 Δ*entA* cultivated in 50% MHB II under iron-limiting conditions in the presence of 200 µM DP and for 4 h at 37°C treated with **(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

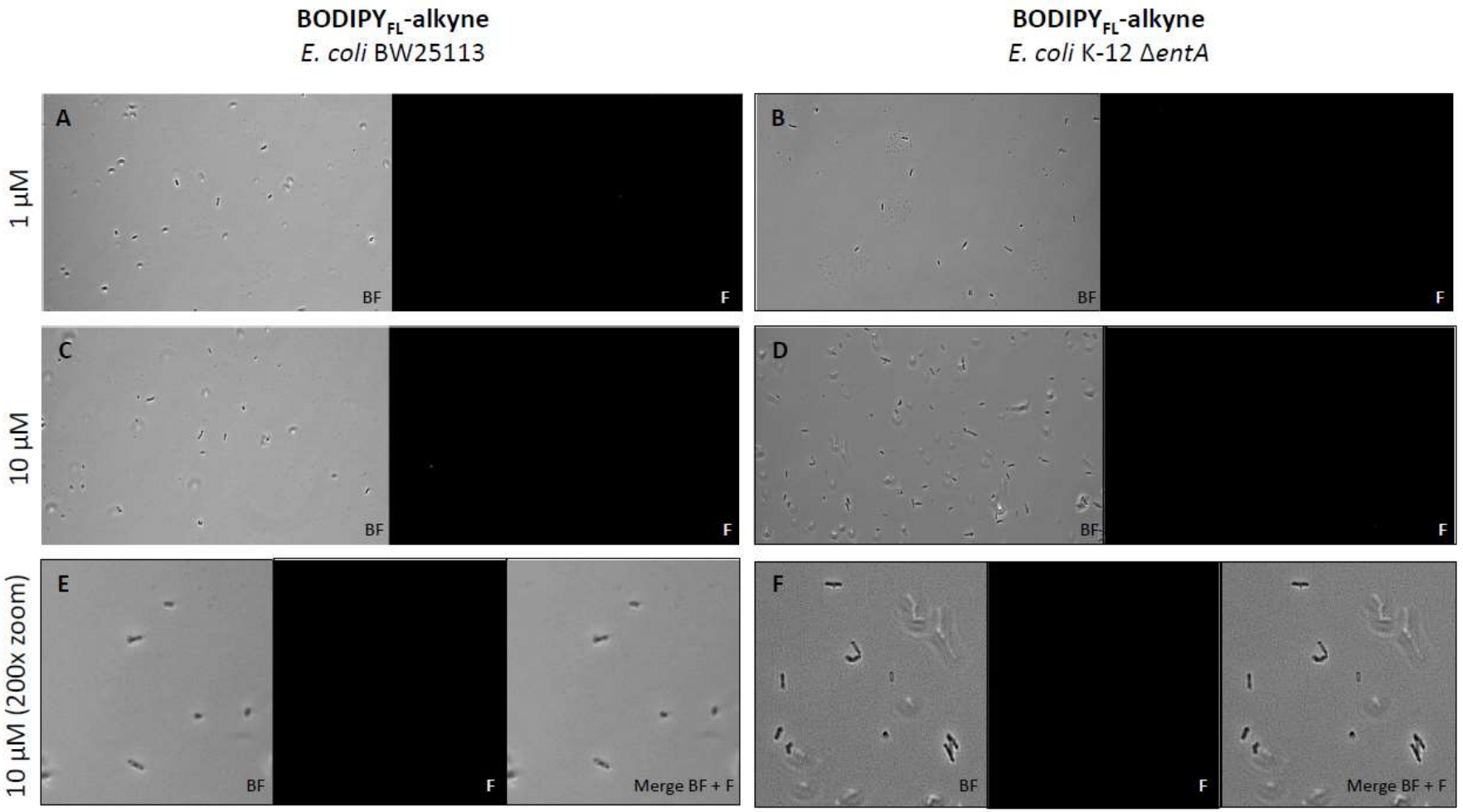


Figure S15: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *E. coli* BW25113 and *E. coli* K-12 Δ*entA* cultivated in 50% MHB II under iron-limiting conditions in the presence of 200 µM DP and for 4 h at 37°C treated with **BODIPY$_{FL}$-alkyne** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

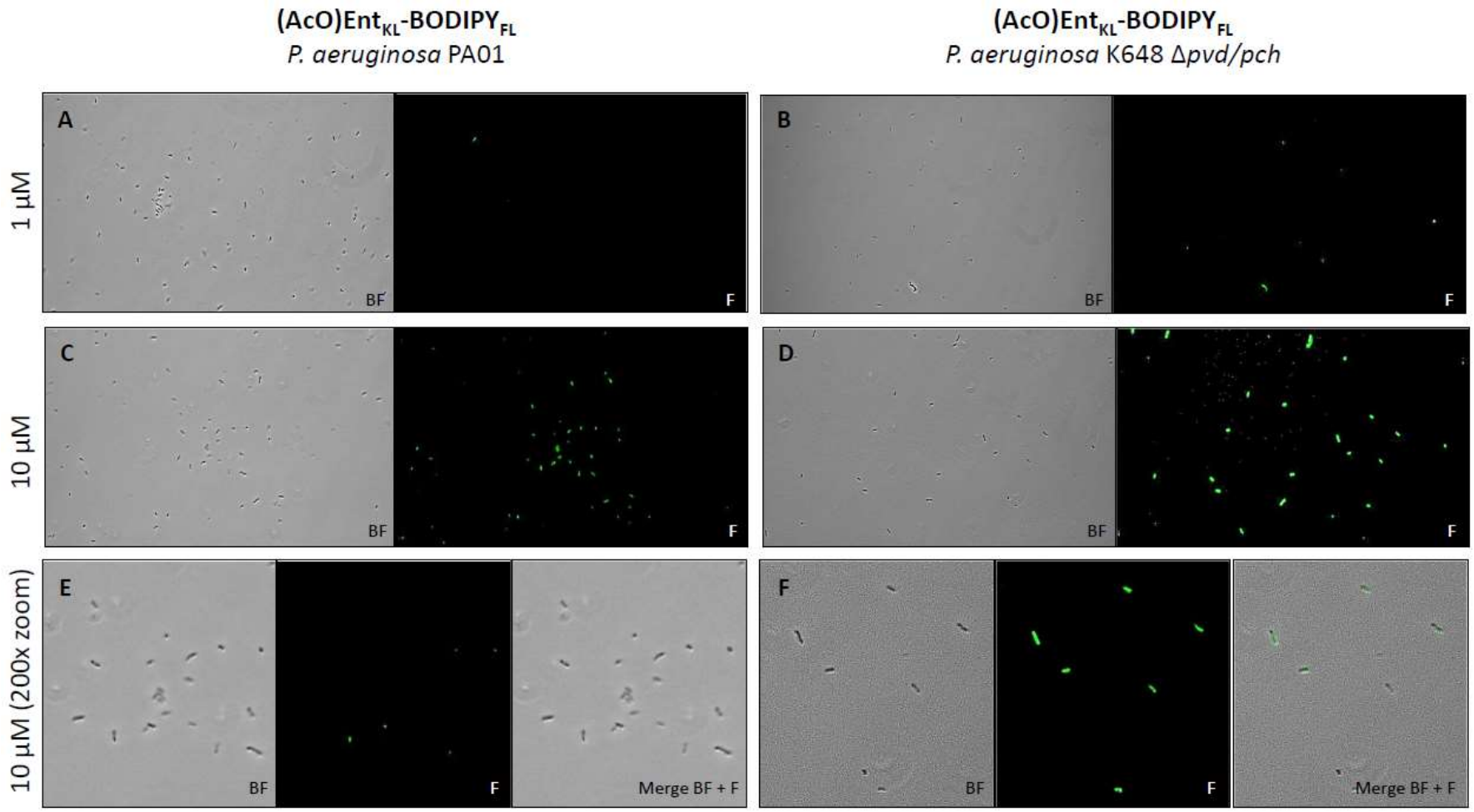


Figure S16: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *P. aeruginosa* PA01 and *P. aeruginosa* K468 Δ*pvd/pch* cultivated in 50% MHB II under iron-limiting conditions in the presence of 600 µM DP and for 4 h at 37°C treated with **(AcO)Ent$_{KL}$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

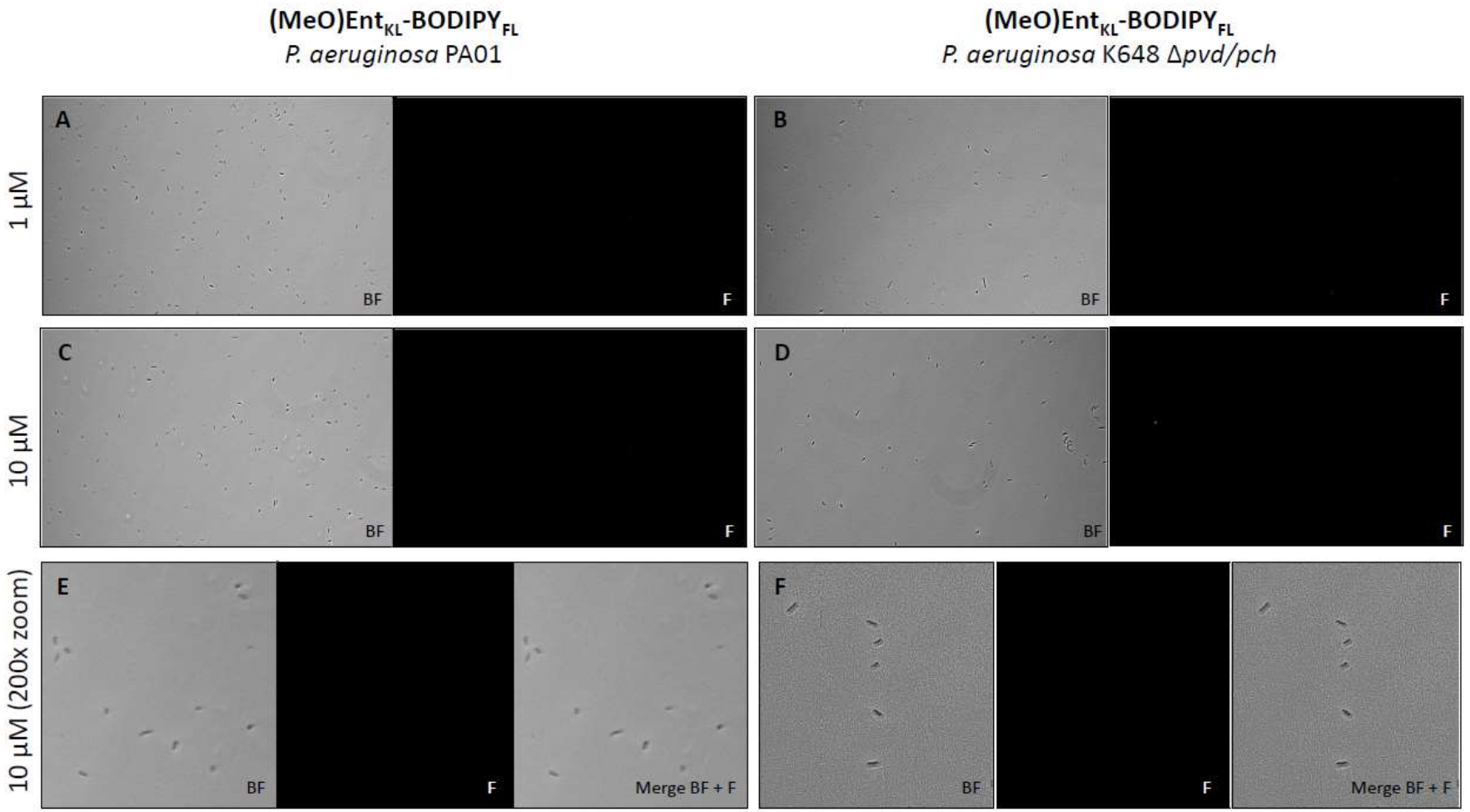


Figure S17: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *P. aeruginosa* PA01 and *P. aeruginosa* K468 Δ*pvd/pch* cultivated in 50% MHB II under iron-limiting conditions in the presence of 600 µM DP and for 4 h at 37°C treated with **(MeO)Ent$_{KL}$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

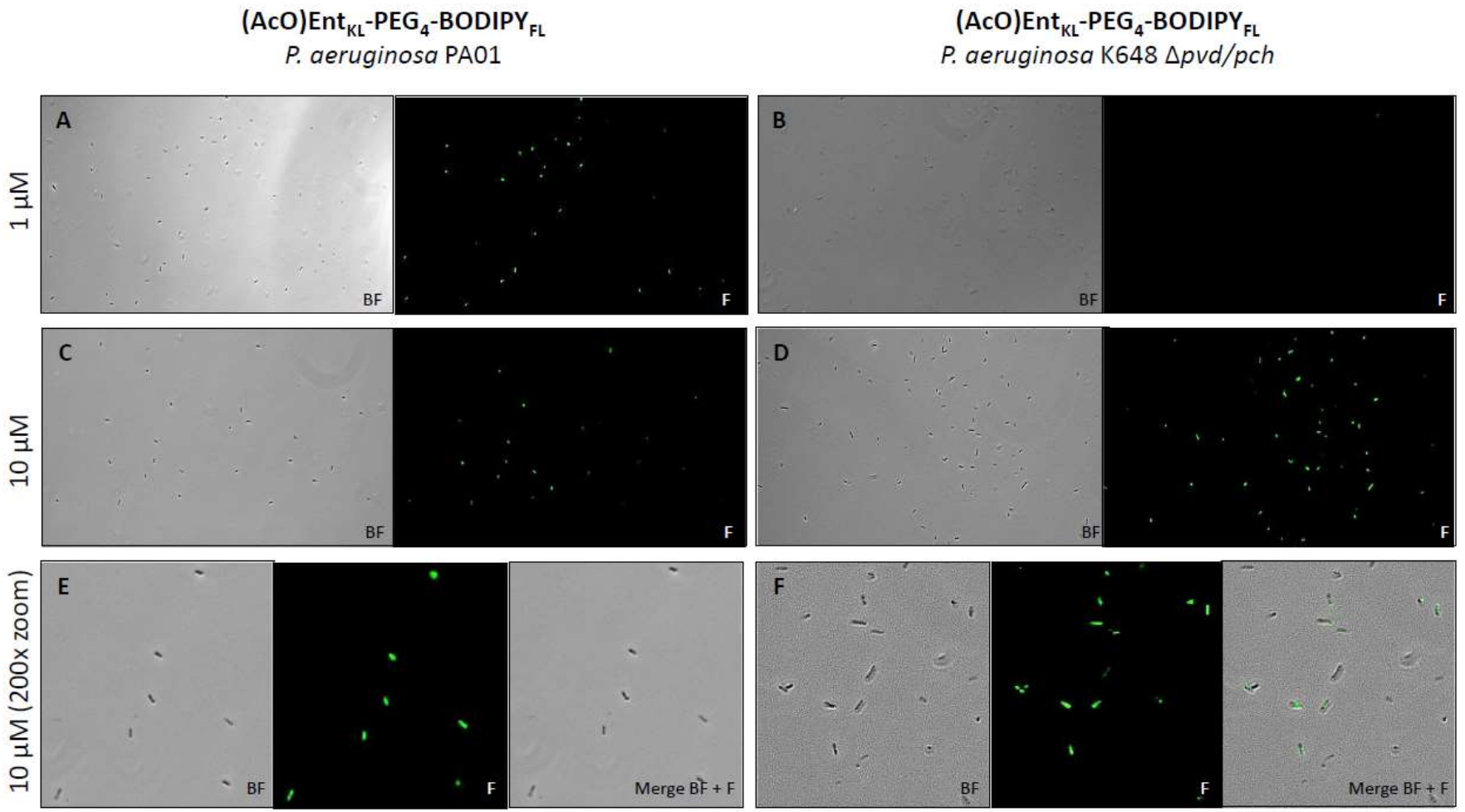


Figure S18: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *P. aeruginosa* PA01 and *P. aeruginosa* K468 Δ*pvd/pch* cultivated in 50% MHB II under iron-limiting conditions in the presence of 600 µM DP and for 4 h at 37°C treated with **(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

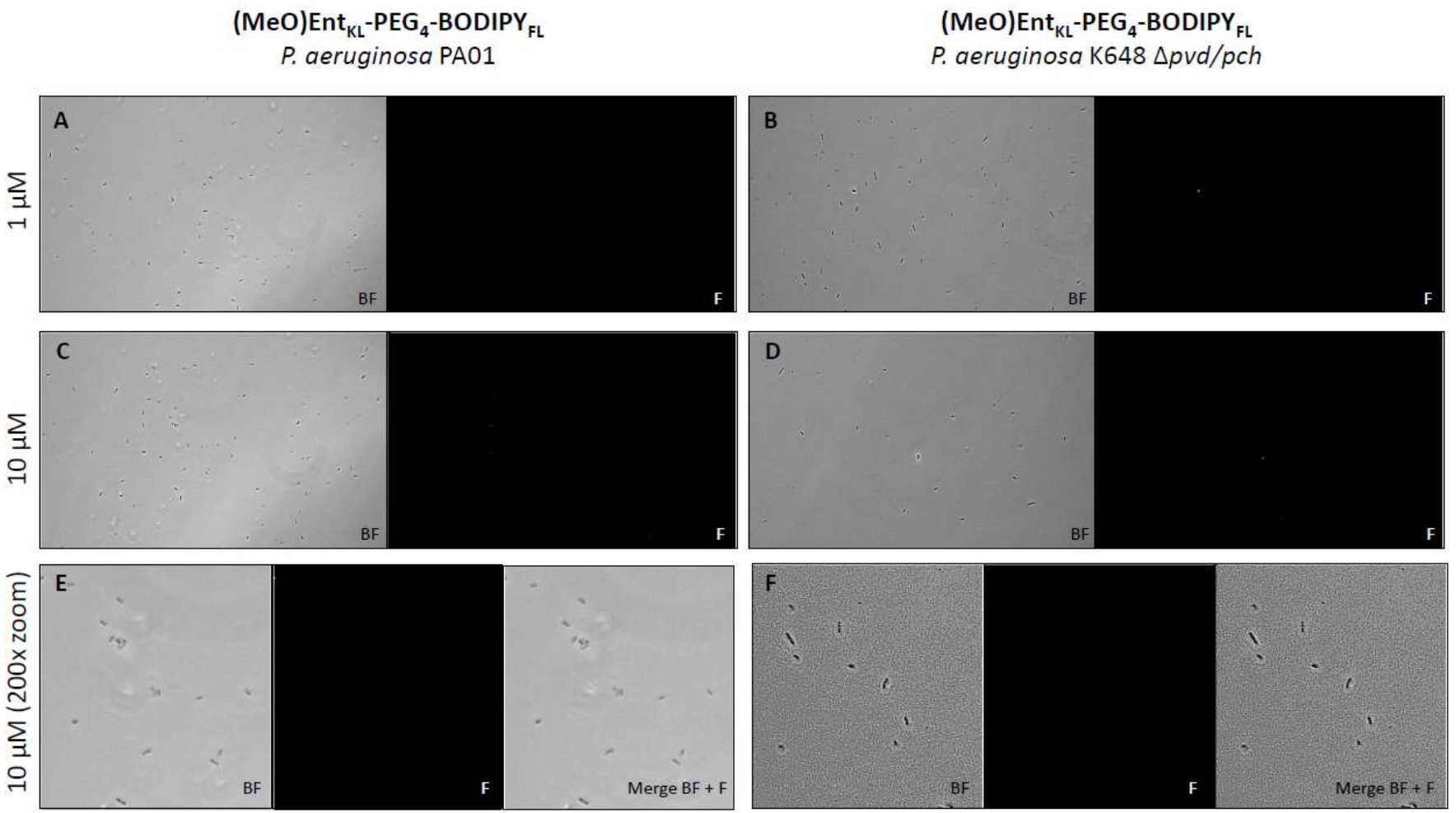


Figure S19: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *P. aeruginosa* PA01 and *P. aeruginosa* K468 Δ*pvd/pch* cultivated in 50% MHB II under iron-limiting conditions in the presence of 600 µM DP and for 4 h at 37°C treated with **(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

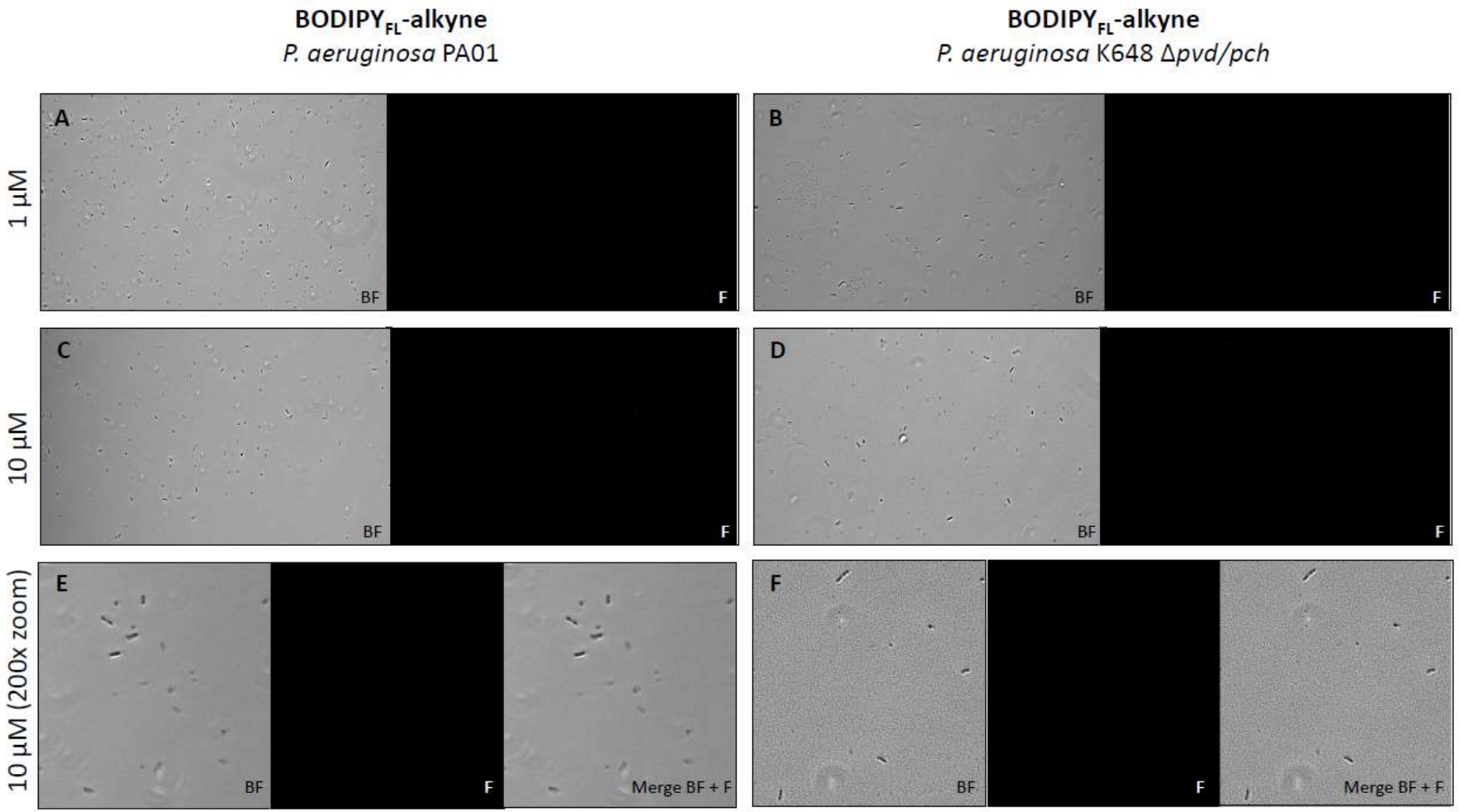


Figure S20: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *P. aeruginosa* PA01 and *P. aeruginosa* K468 Δ*pvd/pch* cultivated in 50% MHB II under iron-limiting conditions in the presence of 600 µM DP and for 4 h at 37°C treated with **BODIPY$_{FL}$-alkyne** at different concentrations (A and B: 1 µM, C and D: 10.0 µM, E and F: 10.0 µM, at 200x Zoom). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

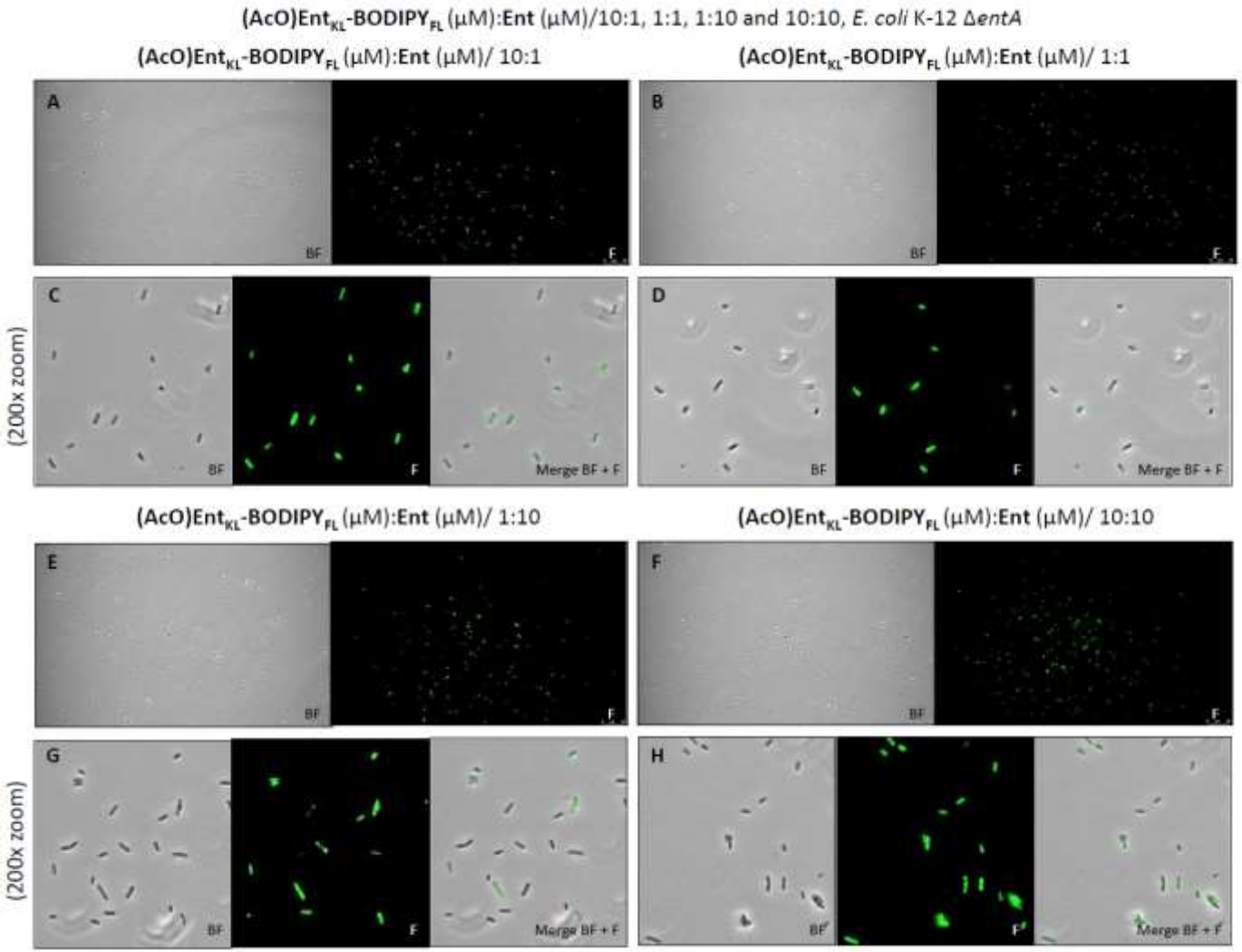


Figure S21: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *E. coli* K-12 Δ*entA* cultivated in 50% MHB II under iron limiting conditions in the presence of 200 µM DP and for 4 h at 37°C treated with different rations of **(AcO)-Ent$_{KL}$-BODIPY$_{FL}$** (µM): **Ent** (µM)/10:1 (A and C), 1:1 (B and D), 1:10 (E and G), 10:10 (F and H). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

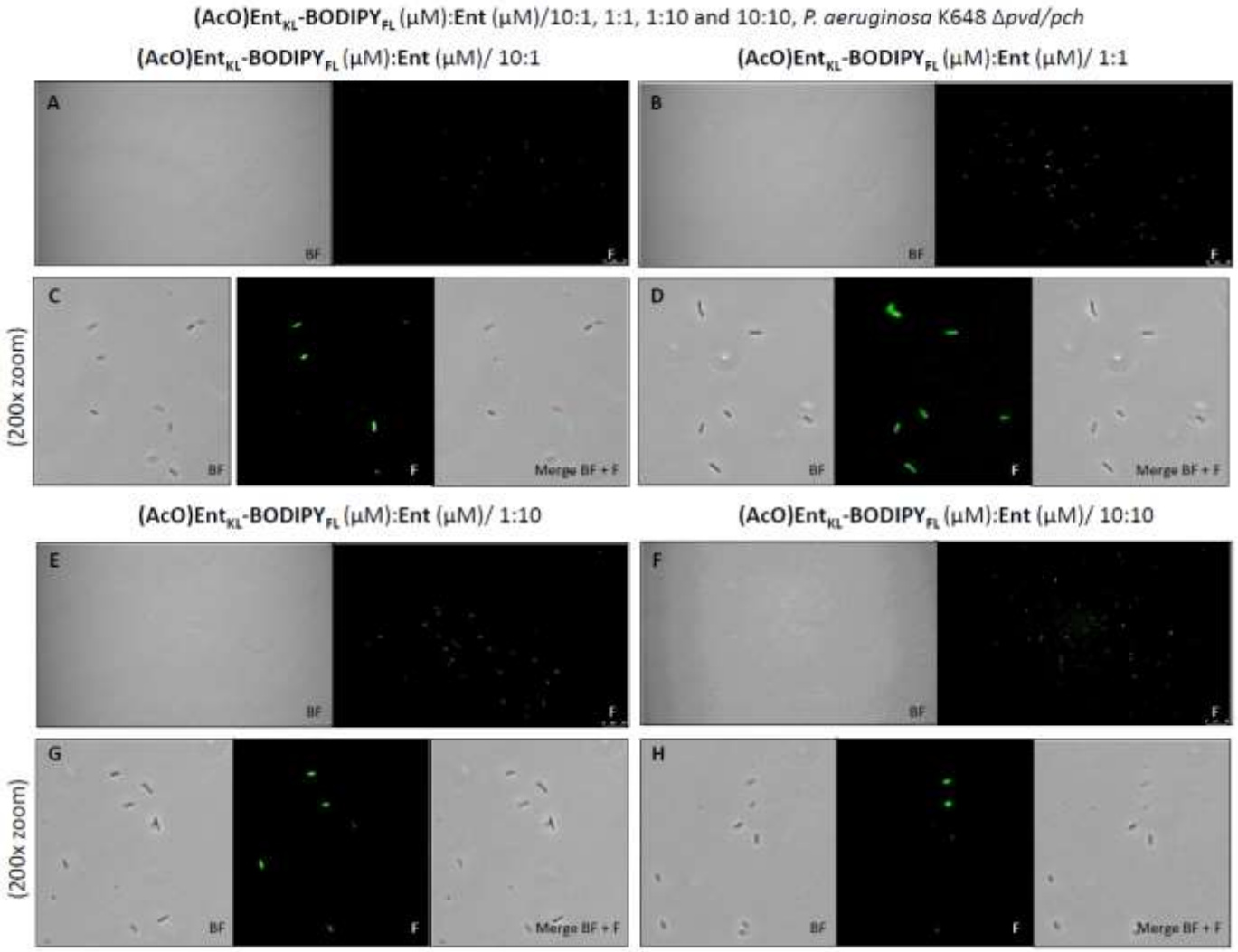


Figure S22: Fluorescence microscopy (80% Brightness, 5s Exposure time) of *P. aeruginosa* K468 Δ*pvd/pch* cultivated in 50% MHB II under iron-limiting conditions in the presence of 200 μM DP and for 4 h at 37°C treated with different rations of **(AcO)-Ent$_{KL}$-BODIPY$_{FL}$** (μM): **Ent** (μM)/10:1 (A and C), 1:1 (B and D), 1:10 (E and G), 10:10 (F and H). All images were captured using a 40x/1.30 objective (overall magnification of 400x). BF: Bright field, F: Fluorescence GFP filter set (excitation: 480 nm, 20 nm bandwidth; emission: 527 nm, 15 nm bandwidth). Each experiment has been reproduced at least 2 times.

Antibacterial minimal inhibitory concentrations (MIC) of the compounds

Overnight cultures were prepared and diluted as previously stated. Serial dilutions of compounds were prepared as duplicates in sterile U-bottom shaped 96-well plates (Corning™, USA)**.** The bacterial suspension was added and the plate was incubated at 37°C in static conditions. Growth inhibition was assessed after 24 h. None of the tested enterobactin derivatives showed an antibacterial activity at the tested concentrations

Cytotoxic activity (IC_{50}) of the compounds

The HepG2 cell line was obtained from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ). The cell line was cultured under conditions recommended by the depositor. Media were purchased from Sigma-Aldrich (Germany), fetal bovine serum (FBS Gold) from PAA (Germany), and other reagents from GIBCO (Invitrogen, USA).

Cells were seeded at 1 x 10^5 cells per well of 96-well Corning CellBind® well plates (Corning™, USA) in 120 µL Dulbecco's Modified Eagle's Medium (DMEM) (addition of 4.5 g/L glucose, 0.584 g/L L-Glutamine, 50 mL Fetal Bovine Serum (FBS Gold) and 5 mL Penicillin-Streptomycin, Merck KGaA, Germany). Cells were treated with compounds in serial dilution after two hours of equilibration. All compounds were tested in duplicate. Cells were incubated for 5 days and for the assessment of viability in comparison to the internal solvent control. After incubation, 20 µL of Thiazol Blue Tetrazolium Bromide in Phosphate-buffered saline (pH 7.4) (Merck KGaA, Germany) was added per well and it was further incubated for 2 hours. The medium was then discarded and cells were washed with PBS (pH 7.4) (Merck KGaA, Germany) before adding 2-propanol/10 N HCl (250:1) (Merck KGaA, Germany) in order to dissolve formazan granules. The absorbance was measured at 570 nm by a TECAN Pro200 plate reader (Tecan Trading AG, Switzerland) and cell viability was expressed as percentage relative to the respective methanol control. IC_{50} values were determined by sigmoidal curve fitting and values represent the average ± SD of two independent measurements. None of the enterobactin derivatives designed in this study showed a cytotoxic activity at the tested concentrations (see Table S4). However, **(AcO)MEnt$_{KL}$** and **(MeO)Ent$_{KL}$**, both derivatives of Miller's enterobactin analogue,[9] showed significant cytotoxic activity against HepG2 cells with an IC_{50} value of 12.17 µg/mL and 31.98 µg/mL, respectively.

Table S4: Cytotoxic activity and antibacterial activity of enterobactin derivatives.

Compound	IC_{50} [µg/mL]	MIC [µg/mL] in MHBII, iron-limiting conditions			
	HepG2	*E. coli* BW25331	*E. coli* K12 ΔEnt	*P. aeruginosa* PAO1	*P. aeruginosa* PAO1 ΔPvd/Pch
(AcO)Ent$_{KL}$	>37	>64	>64	>64	>64
(MeO)Ent$_{KL}$	>37	>64	>64	>64	>64
(AcO)Ent$_{KL}$-PEG$_4$-BODIPY	>37	>64	>64	>64	>64
(MeO)Ent$_{KL}$-PEG$_4$-BODIPY	>37	>64	>64	>64	>64
(AcO)Ent$_{KL}$-BODIPY$_{FL}$	>37	>64	>64	>64	>64
(MeO)Ent$_{KL}$-BODIPY$_{FL}$	>37	>64	>64	>64	>64
(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$	>37	>64	>64	>64	>64
(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$	>37	>64	>64	>64	>64
(AcO)Ent$_{KL}$-Sulfo-Cy5	>37	>64	>64	>64	>64
(MeO)Ent$_{KL}$-Sulfo-Cy5	>37	>64	>64	>64	>64
(AcO)Ent$_{KL}$-MG	>37	>64	>64	>64	>64
(MeO)Ent$_{KL}$-MG	>37	>64	>64	>64	>64
(AcO)Ent$_{KL}$-PEG$_3$-MG	>37	>64	>64	>64	>64
(MeO)Ent$_{KL}$-PEG$_3$-MG	>37	>64	>64	>64	>64
(AcO)Ent$_M$	12.17	>64	>64	>64	>64
(MeO)Ent$_M$	31.98	>64	>64	>64	>64

References

[1] N. Branda, *JoVE Science Education Database, Organic Chemistry* **2019**.

[2] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923–2925.

[3] T. Flack, C. Romain, A. J. P. White, P. R. Haycock, A. Barnard, *Org. Lett.* **2019**, *21*, 4433–4438.

[4] C. G. Kokotos, V. K. Aggarwal, *Chem. Commun.* **2006**, 2156–2158.

[5] J. A. Dale, D. L. Dull, H. S. Mosher, *J. Org. Chem.* **1969**, *34*, 2543–2549.

[6] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew. Chemie Int. Ed.* **2005**, *44*, 1378–1382.

[7] Y. Guo, Q. Song, J. Wang, J. Ma, X. Zhang, D. L. Phillips, *J. Org. Chem.* **2018**, *83*, 13454–13462.

[8] N. Kurokawa, Y. Ohfune, *J. Am. Chem. Soc.* **1986**, *108*, 6043–6045.

[9] C. Ji, P. A. Miller, M. J. Miller, *J. Am. Chem. Soc.* **2012**, *134*, 9898–9901.

[10] B. Rioux, C. Pouget, C. Fidanzi-Dugas, A. Gamond, A. Laurent, J. Semaan, A. Pinon, Y. Champavier, D. Y. Léger, B. Liagre, et al., *Bioorganic Med. Chem. Lett.* **2017**, *27*, 4354–4357.

[11] C. Szent-Gyorgyi, B. A. Schmidt, Y. Creeger, G. W. Fisher, K. L. Zakel, S. Adler, J. A. J. Fitzpatrick, C. A. Woolford, Q. Yan, K. V. Vasilev, et al., *Nat. Biotechnol.* **2008**, *26*, 235–240.

[12] M. P. Bruchez, B. F. Schmidt, *WO 2011/150079 A1*, **2011**.

[13] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, et al., *Gaussian-09 Revision E.01, gaussian Inc, Wallingford CT* **2009**.

[14] A. Becke, *J. Chem. Phys.* **1993**, *98*, 5648–5652.

[15] S. Grimme, J. Antony, S. Ehrlich, H. Krieg, *J. Chem. Phys.* **2010**, *132*, 154104.

[16] K. Brandhorst, J. Grunenberg, *J. Chem. Phys.* **2010**, *132*, 184101.

[17] K. Brandhorst, J. Grunenberg, *Chem. Soc. Rev.* **2008**, *37*, 1558–1567.

[18] J. Tomasi, *Chem. Phys.* **1981**, *55*, 117–129.

[19] T. Baramov, B. Schmid, H. Ryu, J. Jeong, K. Keijzer, L. von Eckardstein, M. H. Baik, R. D. Süssmuth,

Chem. - A Eur. J. **2019**, *25*, 6955–6962.

[20] C. P. Kelly, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem. B* **2007**, *111*, 408–422.

[21] H. Ryu, J. Park, H. K. Kim, J. Y. Park, S. T. Kim, M. H. Baik, *Organometallics* **2018**, *37*, 3228–3239.

[22] R. C. Scarrow, D. J. Ecker, C. Ng, S. Liu, K. N. Raymond, *Inorg. Chem.* **1991**, *30*, 900–906.

[23] L. D. Loomis, K. N. Raymond, *Inorg. Chem.* **1991**, *30*, 906–911.

[24] M. D. Walter, J. Grunenberg, P. S. White, *Chem. Sci.* **2011**, *2*, 2120–2130.

[25] J. Grunenberg, N. Goldberg, *J. Am. Chem. Soc.* **2000**, *122*, 6045–6047.

[26] J. Grunenberg, *Chem. Sci.* **2015**, *6*, 4086–4088.

[27] J. Grunenberg, *Angew. Chemie* **2017**, *129*, 7394–7397.

[28] S. S. Isied, G. Kuo, K. N. Raymond, *J. Am. Chem. Soc.* **1976**, *98*, 1763–1767.

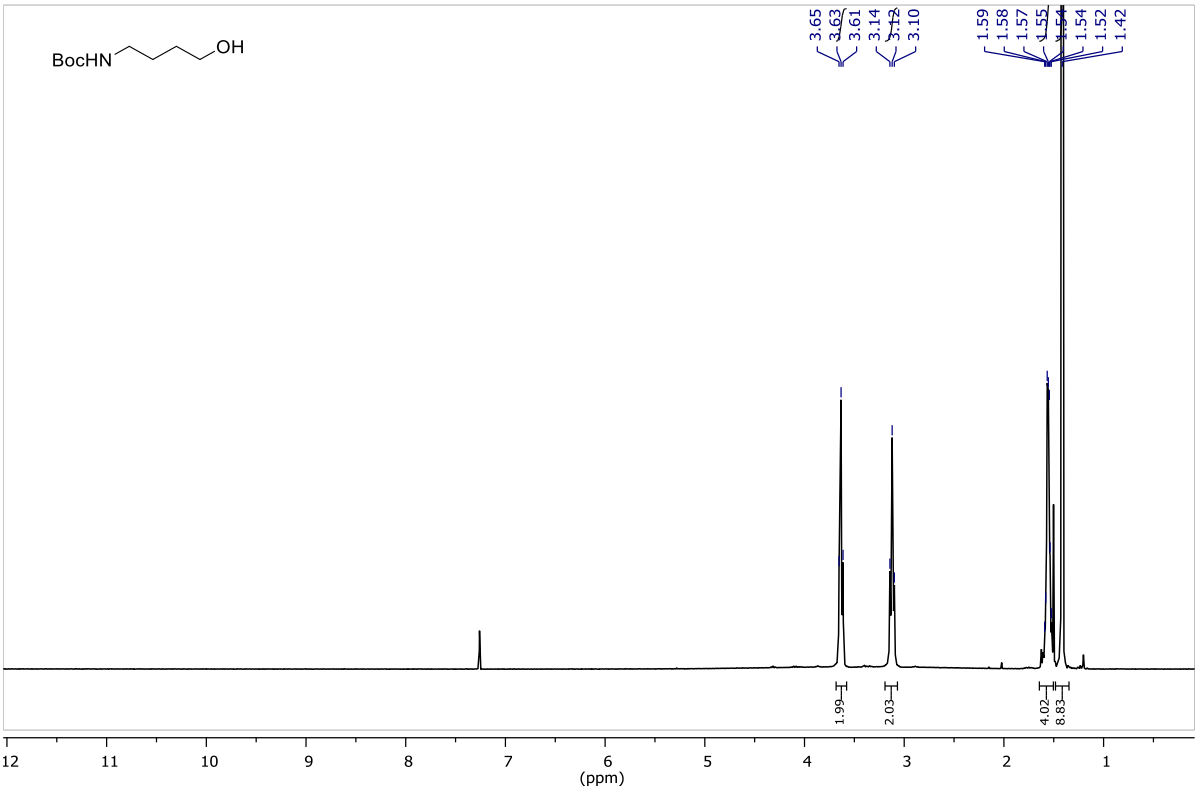
[29] A. Avdeef, S. R. Sofen, T. L. Bregante, K. N. Raymond, *J. Am. Chem. Soc.* **1978**, *100*, 5362–5370.

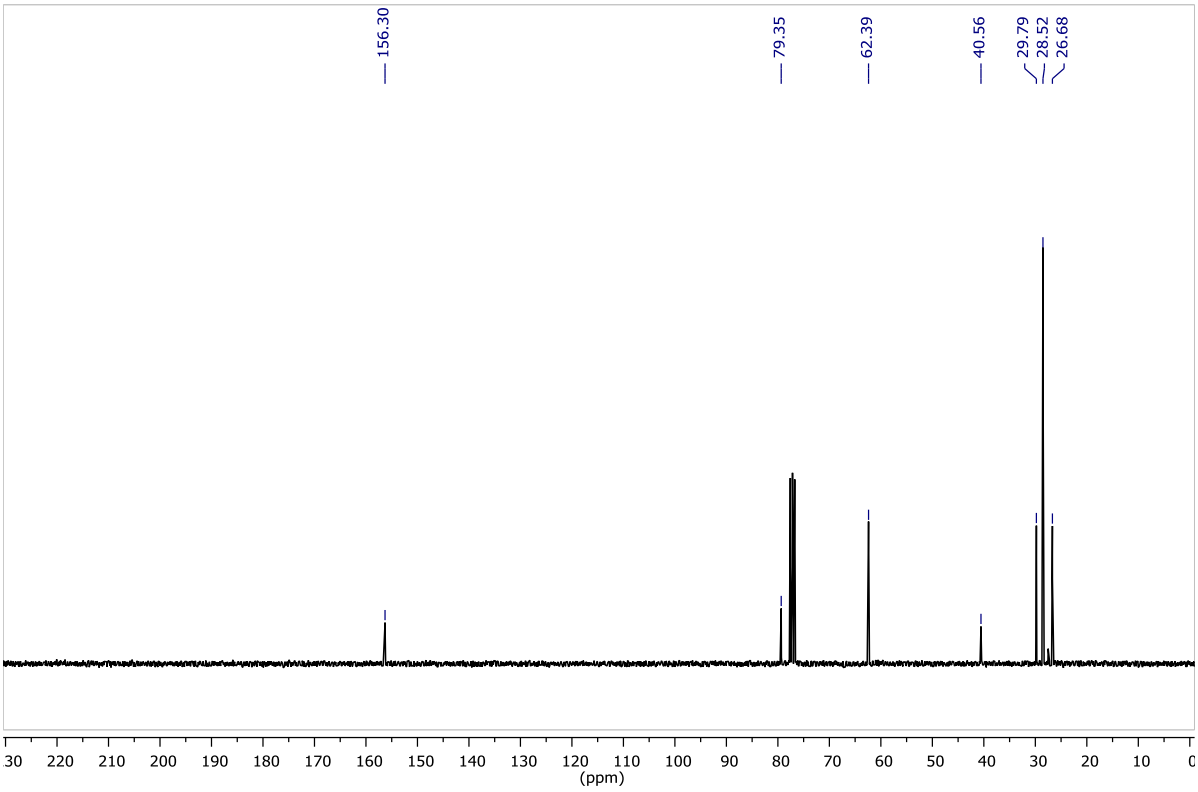
[30] T. Zheng, J. L. Bullock, E. M. Nolan, *J. Am. Chem. Soc.* **2012**, *134*, 18388–18400.

Appendix

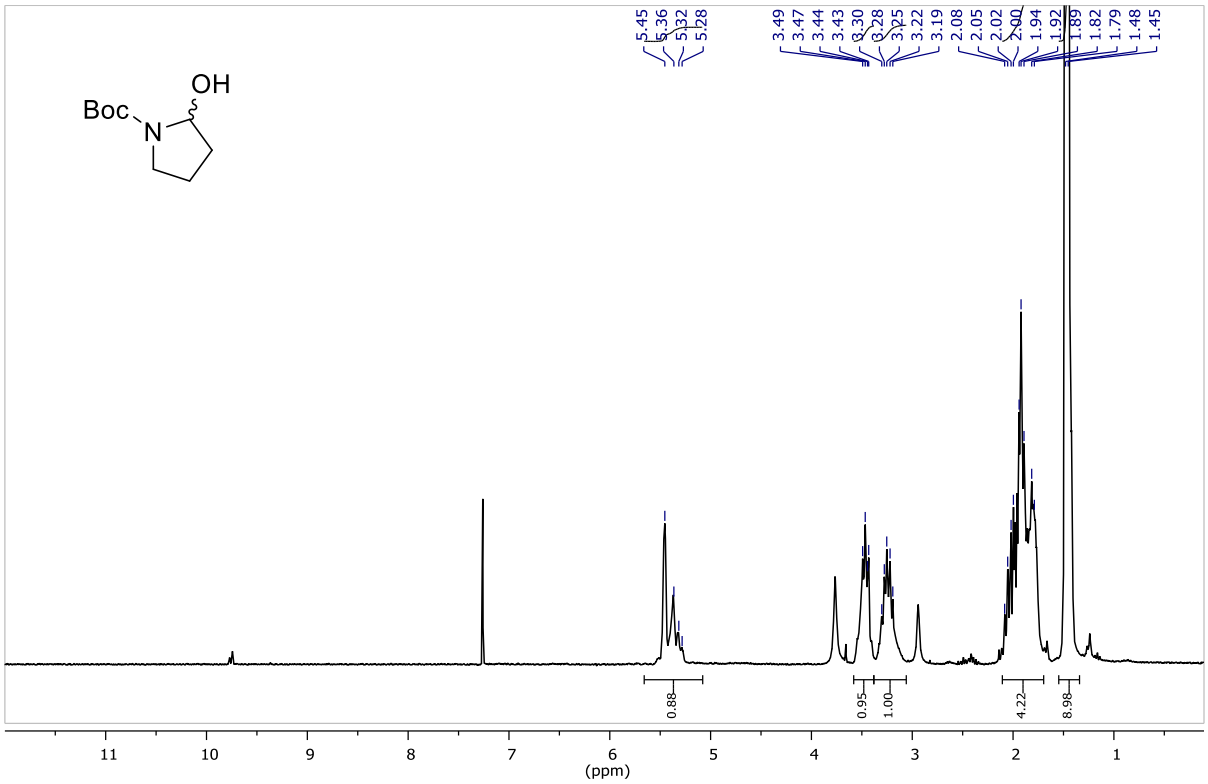
^{1}H-NMR spectra, ^{13}C-NMR spectra, ^{11}B-NMR spectra, ^{19}F-NMR spectra, IR spectra, LC-MS chromatograms, HRMS spectra and UV-Vis spectra and fluorescence emission spectra of novel compounds

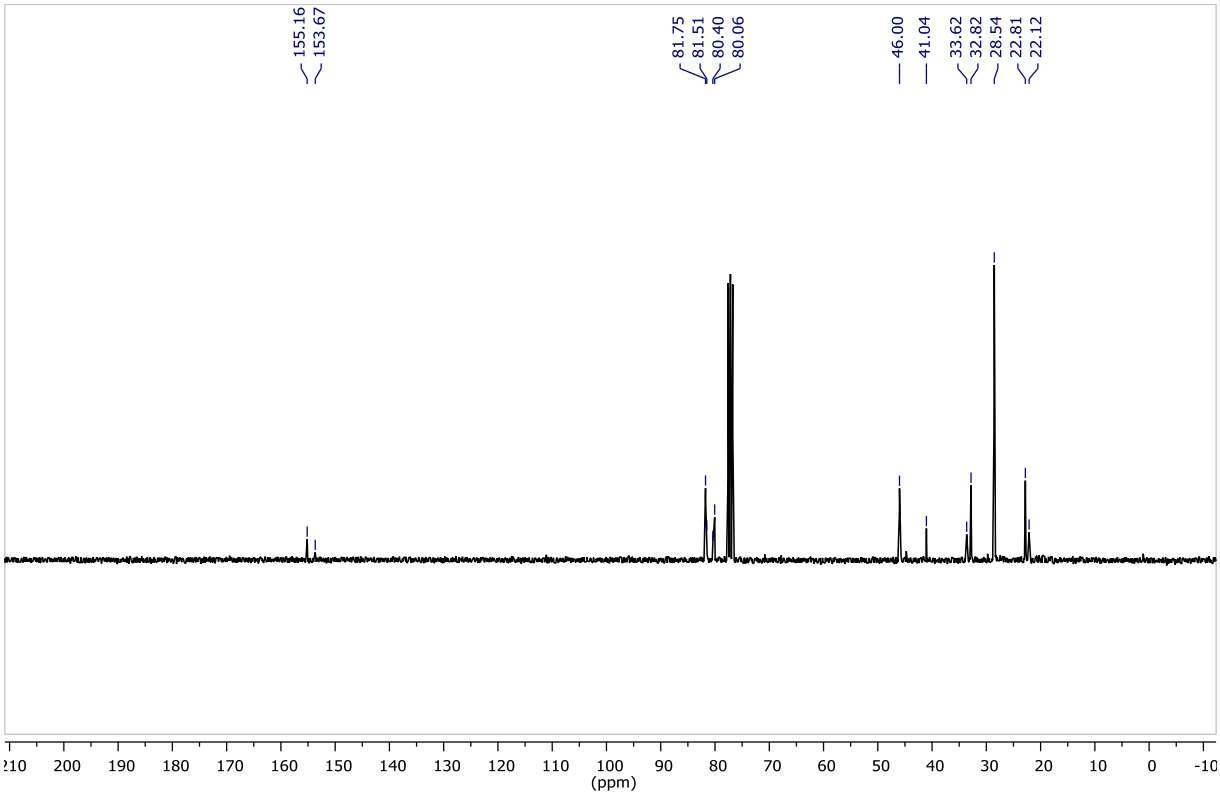
tert-Butyl (4-hydroxybutyl)carbamate (3) (^{1}H and ^{13}C NMR)



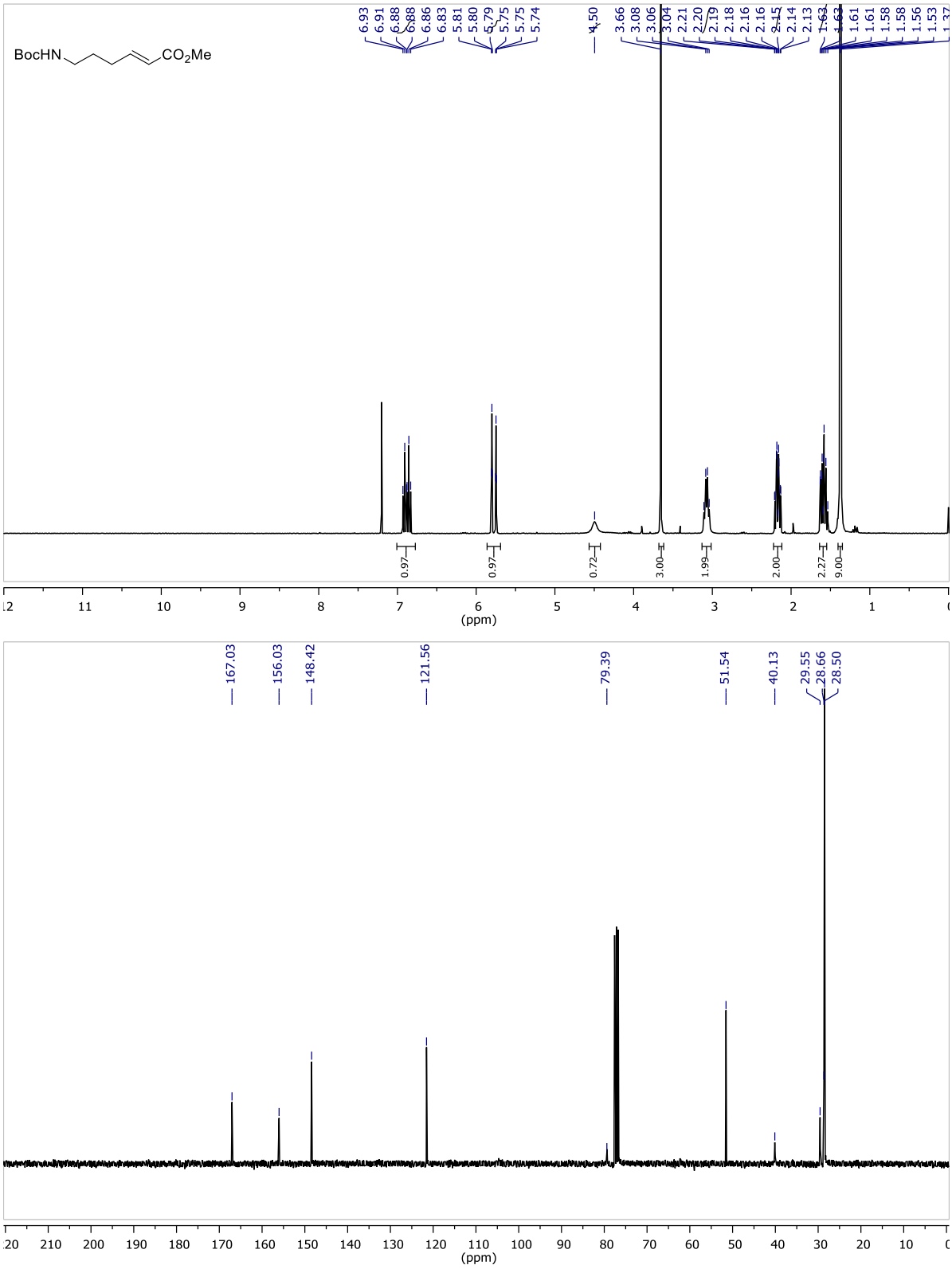


tert-Butyl 2-hydroxypyrrolidine-1-carboxylate (4) (^{1}H and ^{13}C NMR)





Methyl (E)-6-((*tert*-butoxycarbonyl)amino)hex-2-enoate (6) (^{1}H and ^{13}C NMR)



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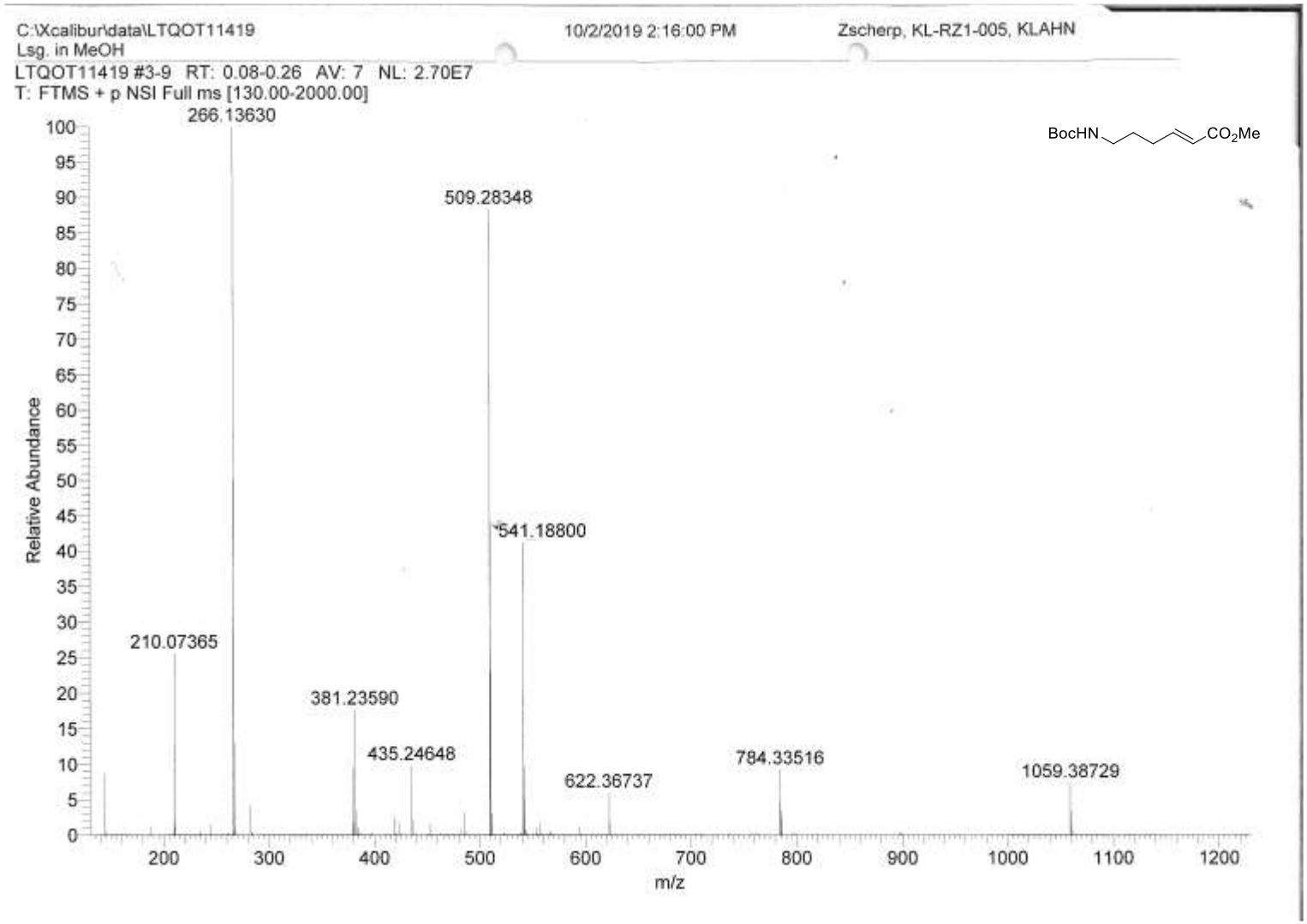
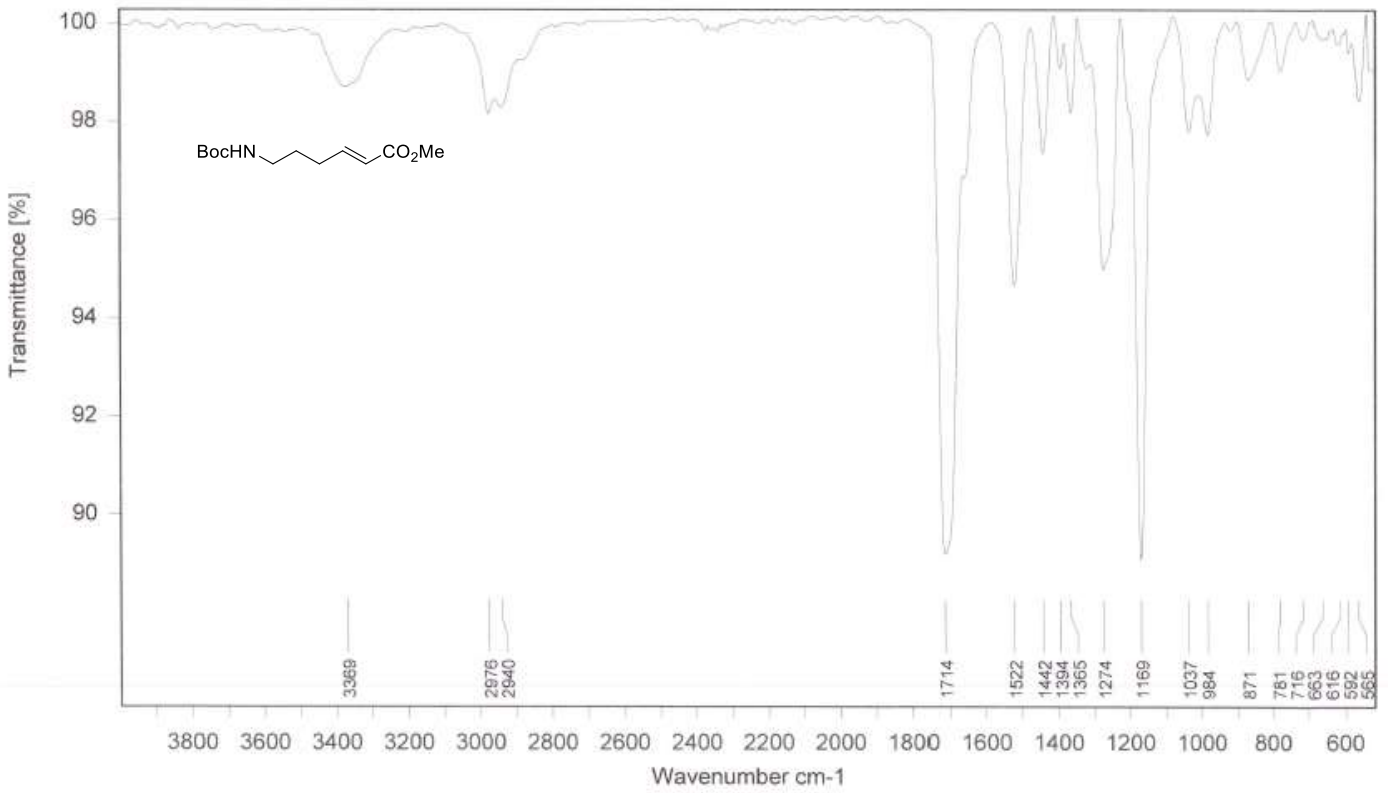


Methyl (E)-6-((*tert*-butoxycarbonyl)amino)hex-2-enoate (6) (ATR-IR)



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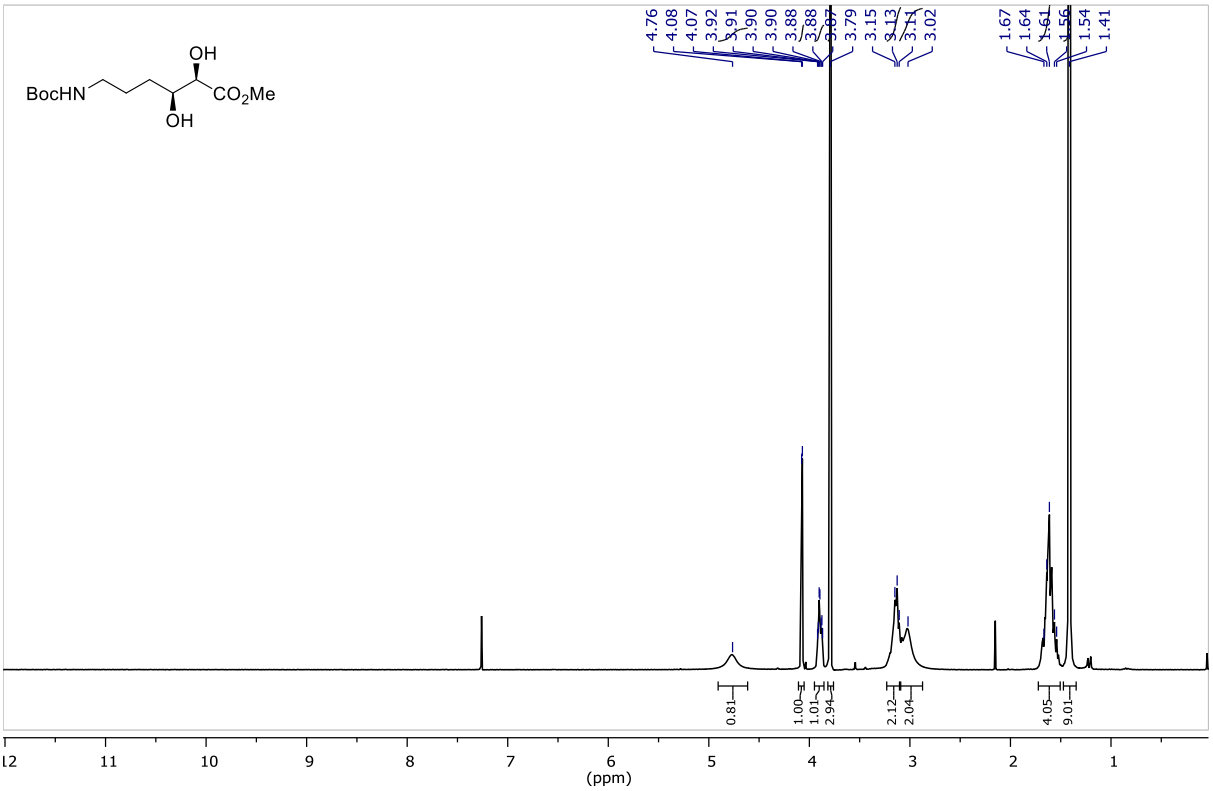
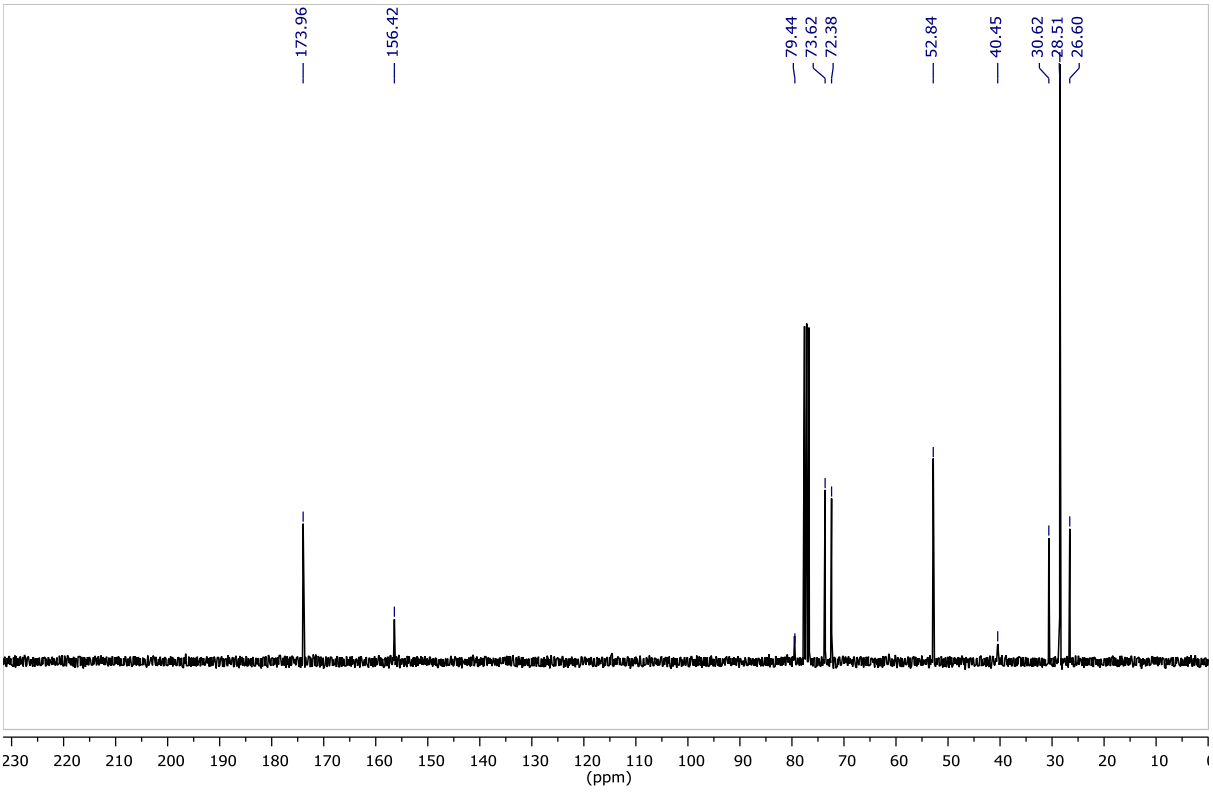
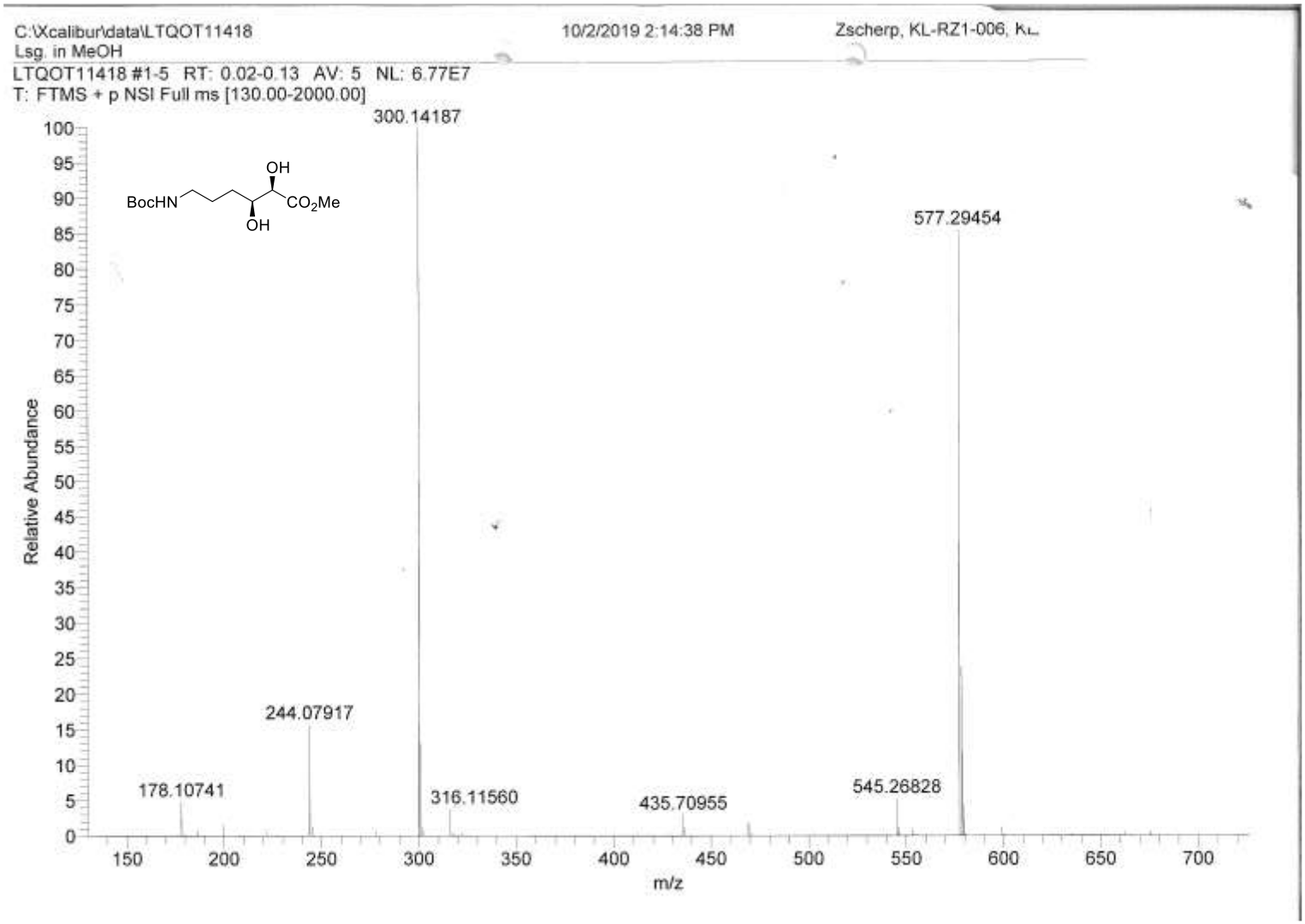
26.11.2020

Methyl (2R,3S)-6-((tert-butoxycarbonyl)amino)-2,3-dihydroxyhexanoate (7) (^{1}H and ^{13}C NMR)





Methyl (2R,3S)-6-((tert-butoxycarbonyl)amino)-2,3-dihydroxyhexanoate (7) (HRMS)



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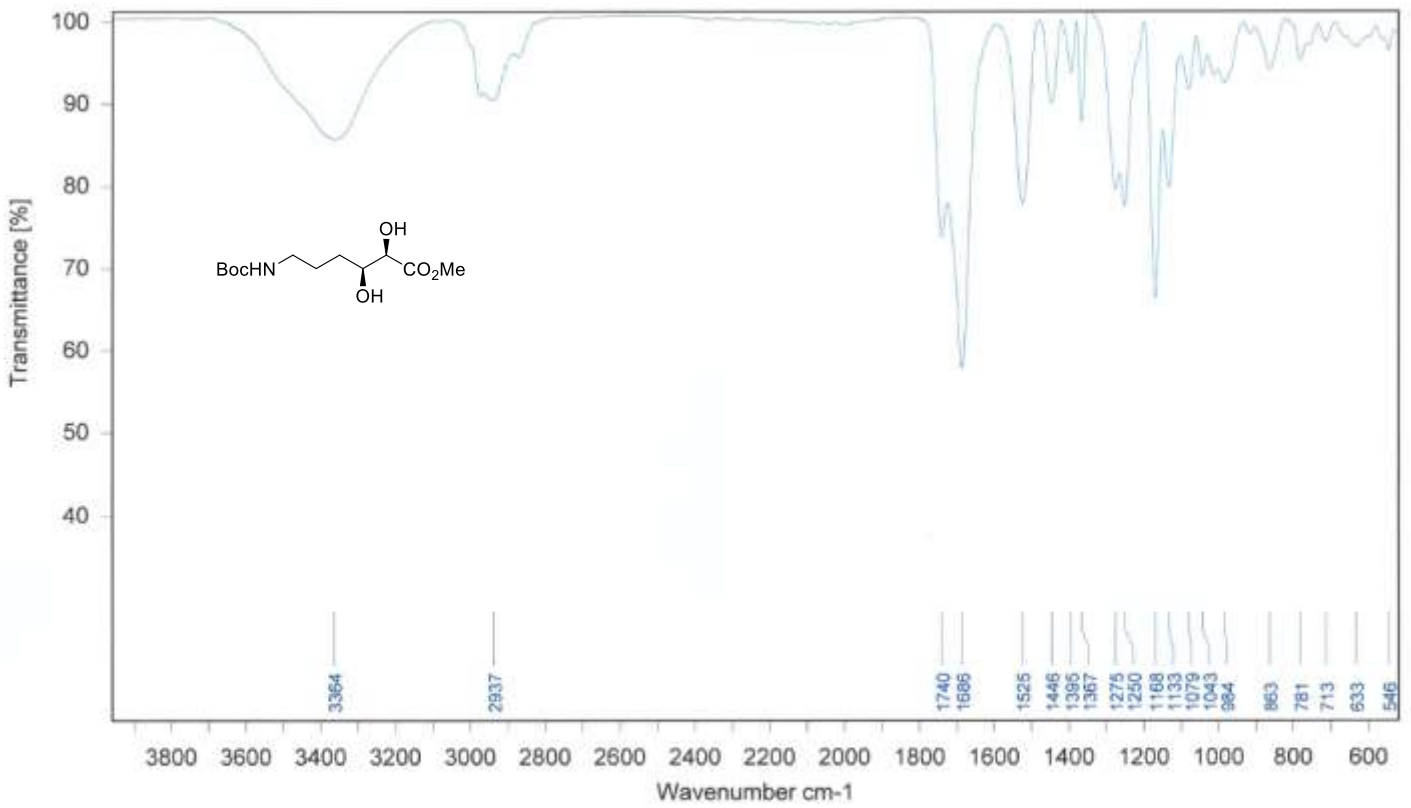

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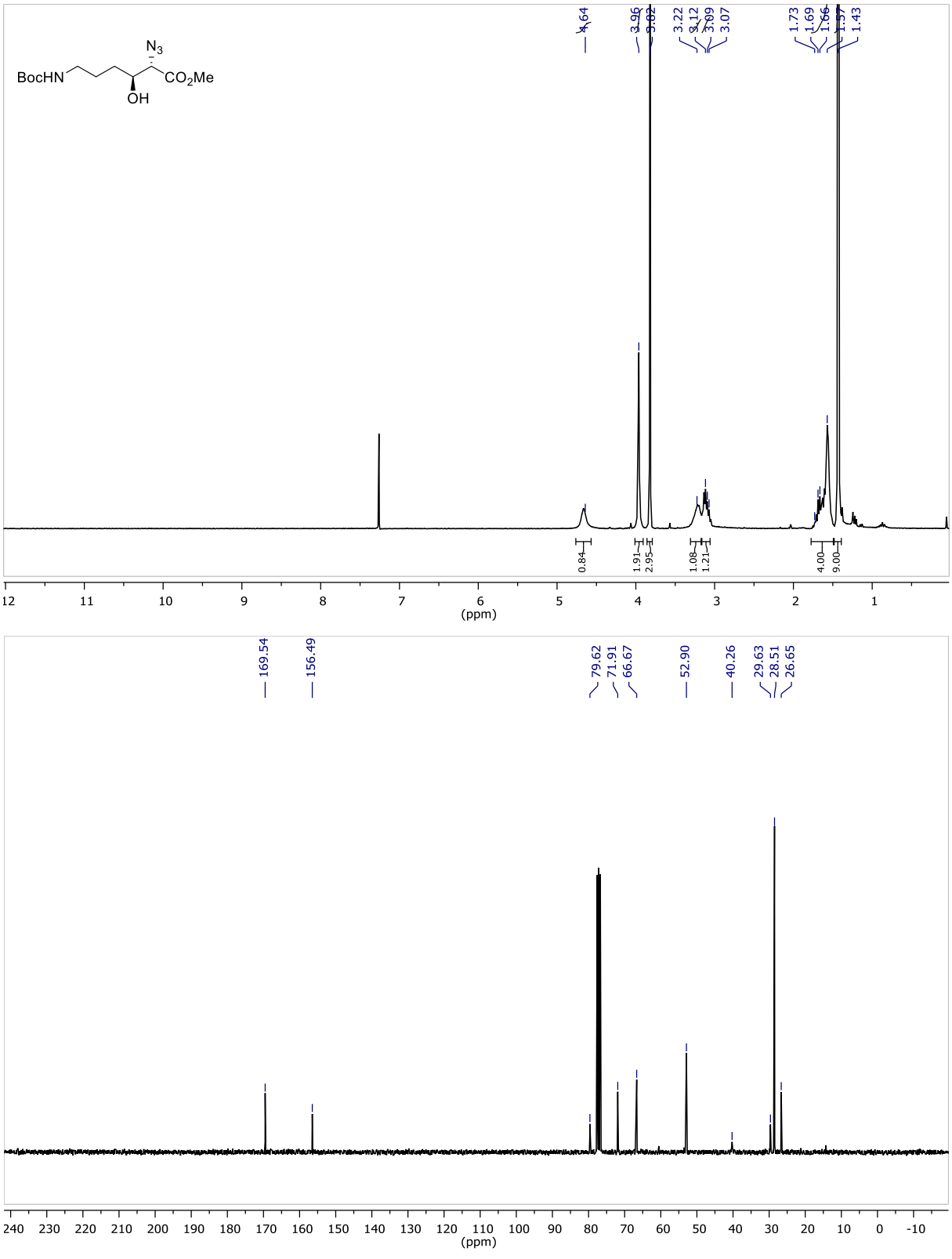
27.03.2020

Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoate (8) (^{1}H and ^{13}C NMR)



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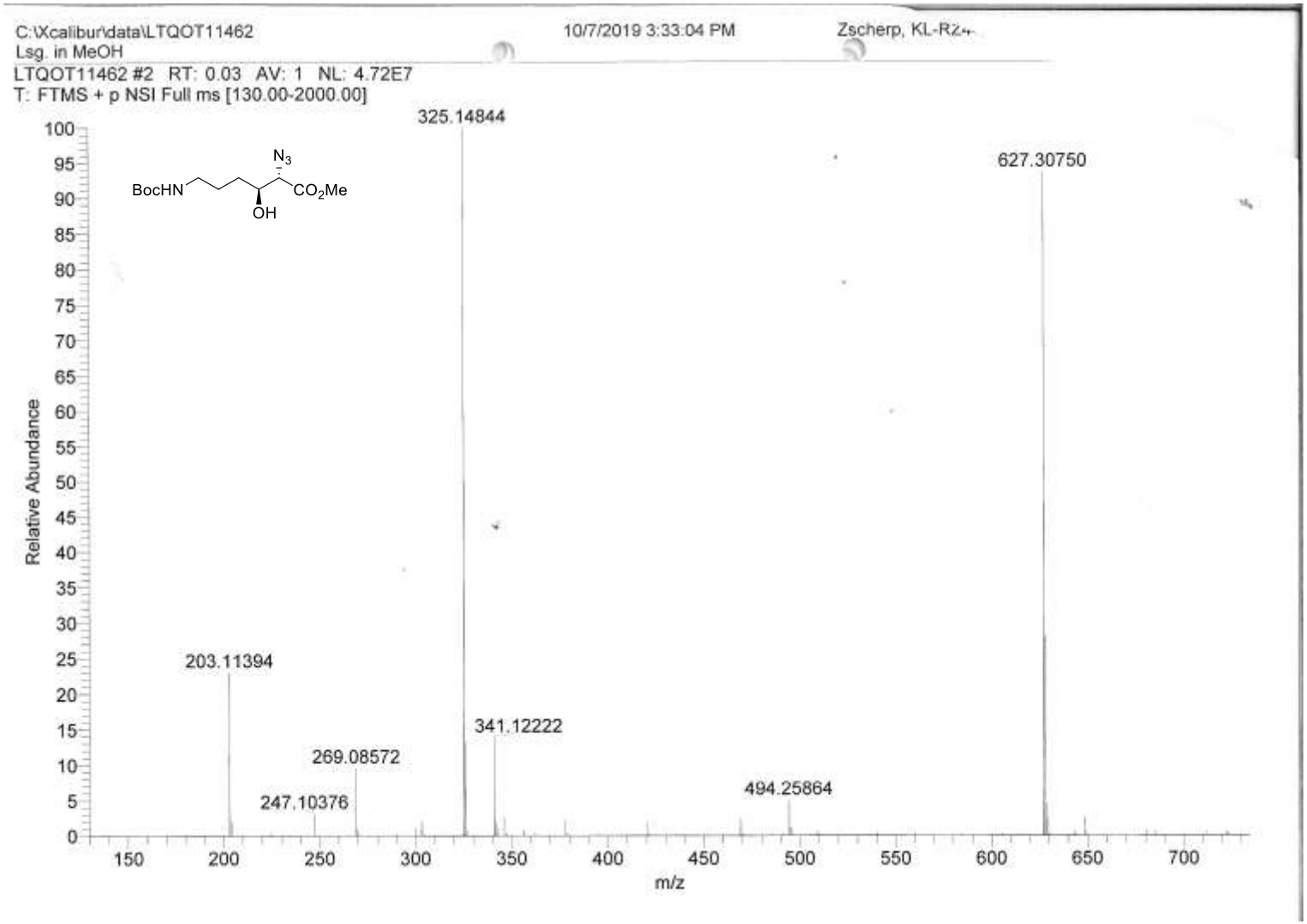

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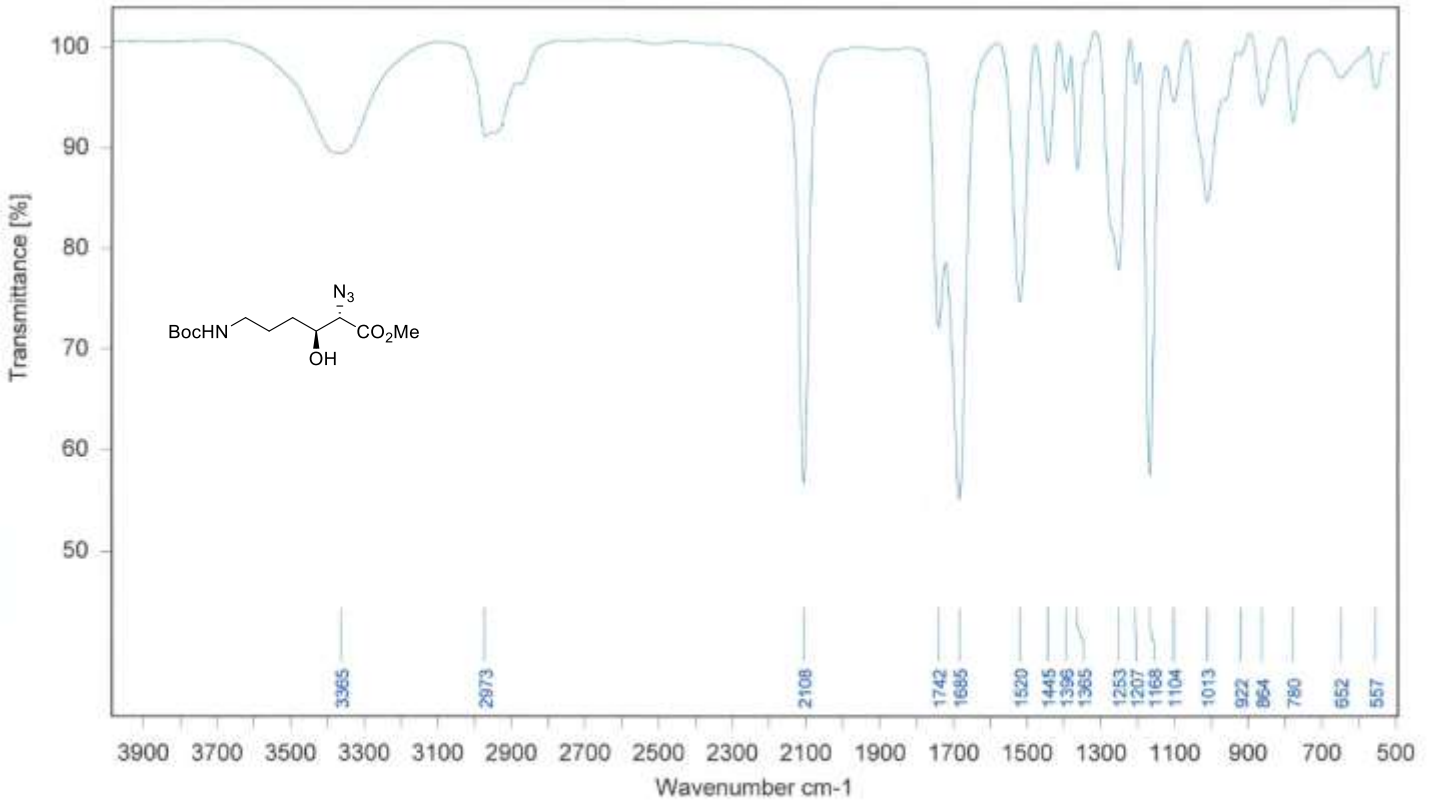

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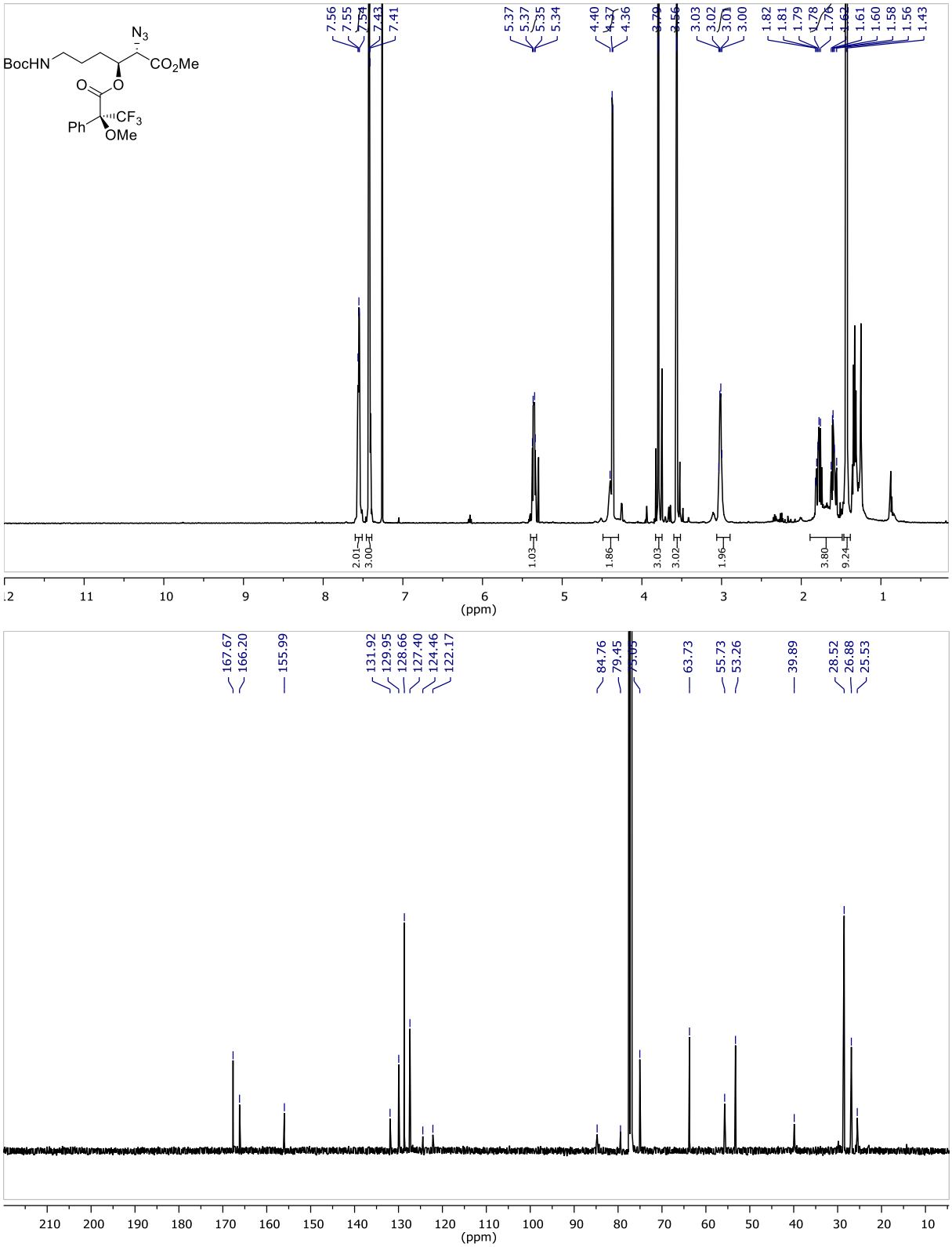
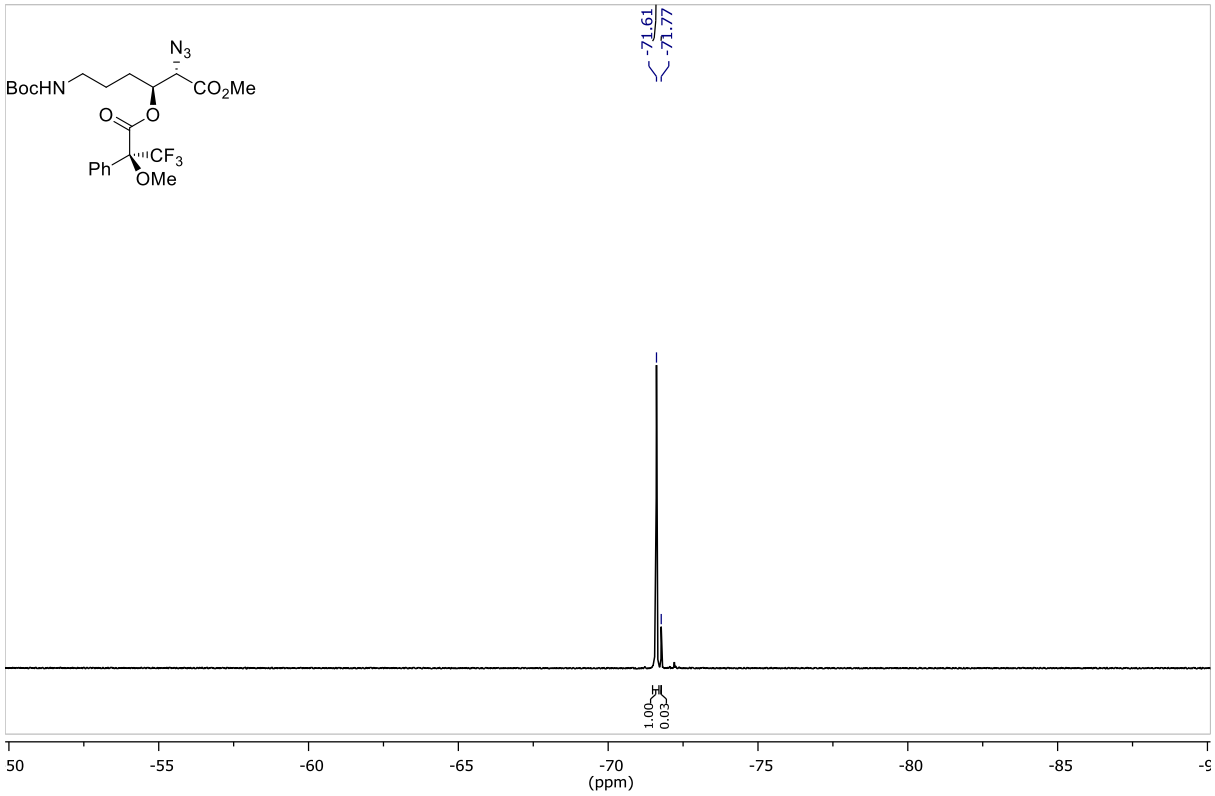
05.01.2020

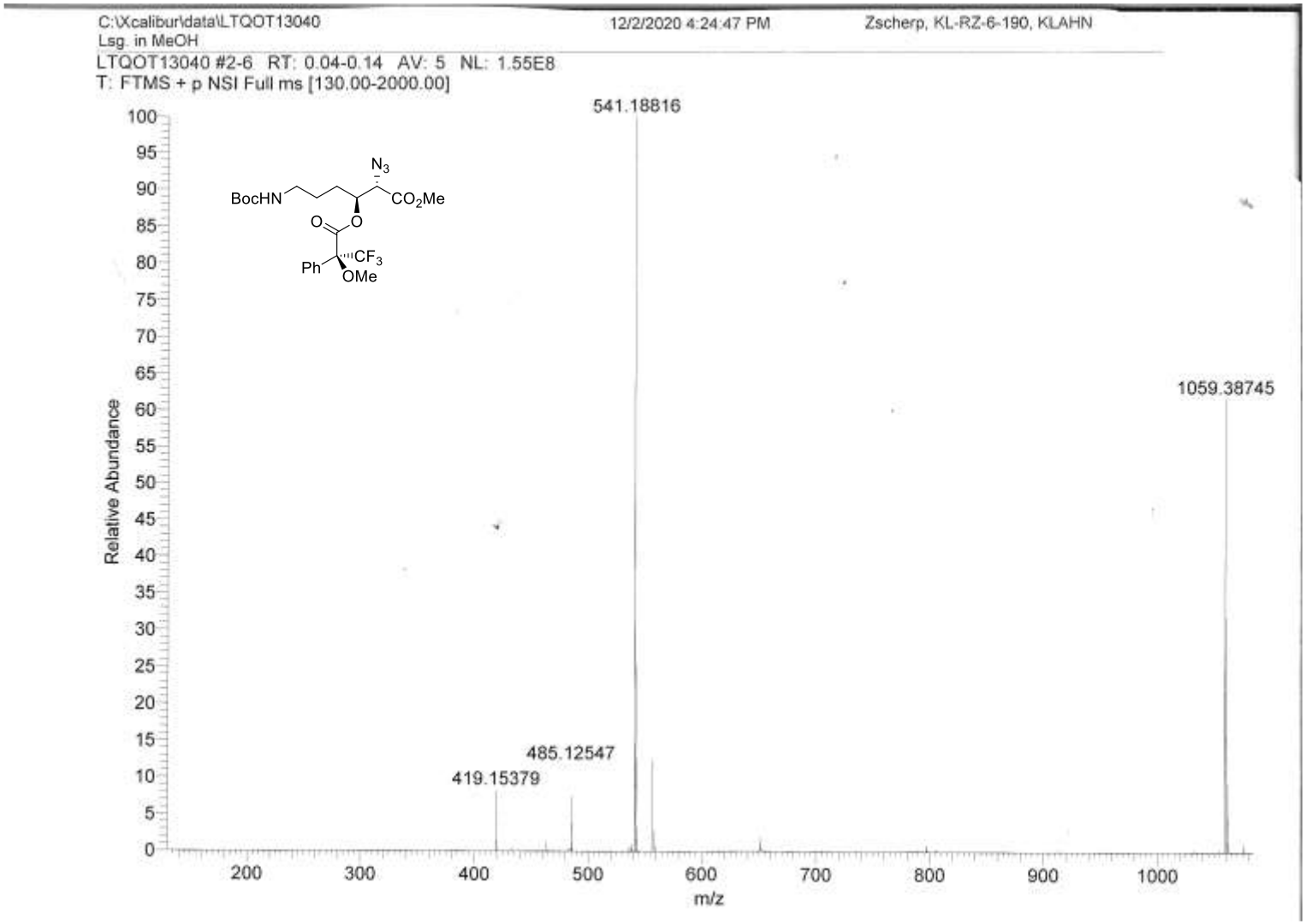
Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (S)-Mosher ester (^{1}H and ^{13}C NMR)



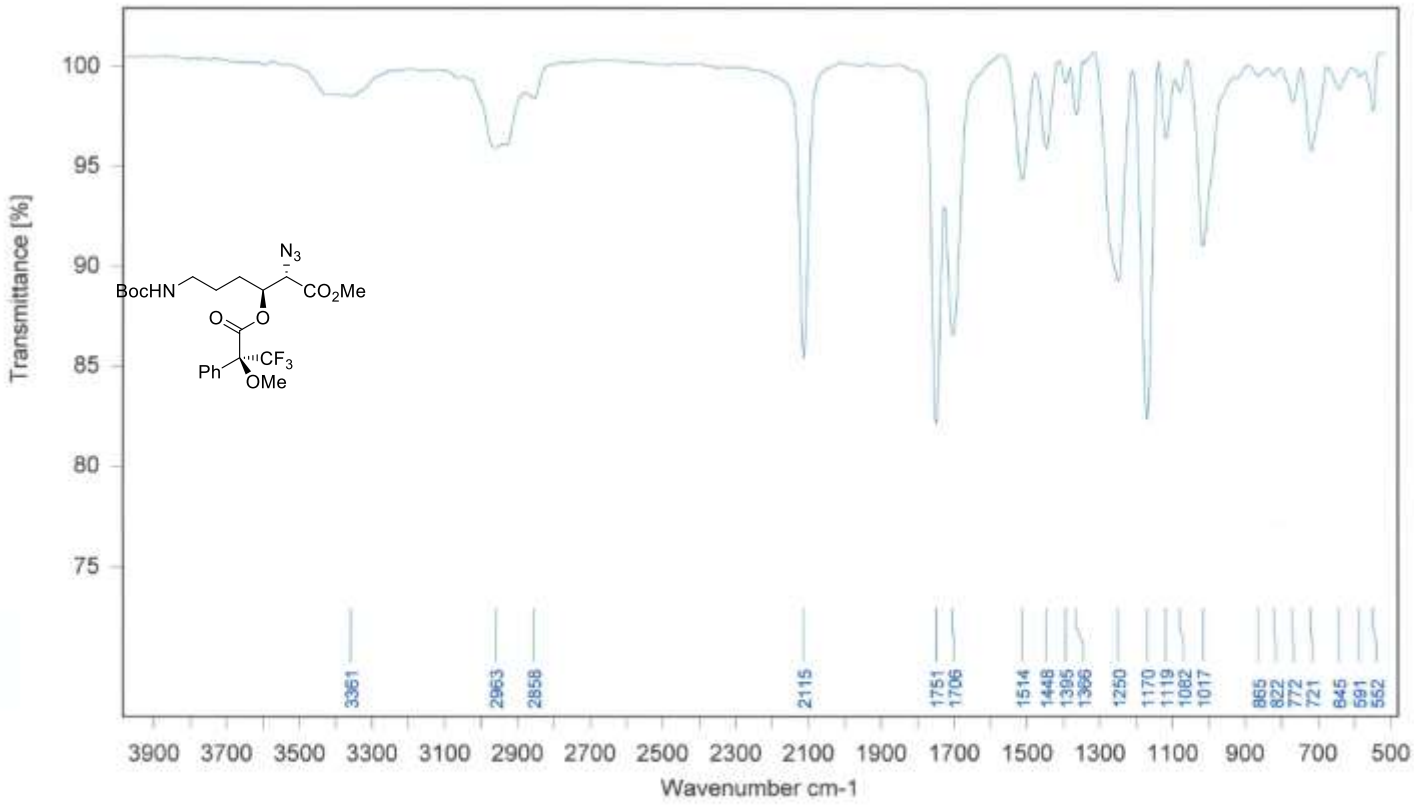
Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (S)-Mosher ester (^{19}F NMR)



Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (S)-Mosher ester (HRMS)



Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (S)-Mosher ester (ATR-IR)



Instrument: Bruker Tensor 27	
Filename: zsr28949.1	Number of Scans: 32
Sample Name: KL-RZ4-117	Operator Name: Default
Technique: Diam -ATR	Date & Time of Measurement: 18/10/2019 11:33:34

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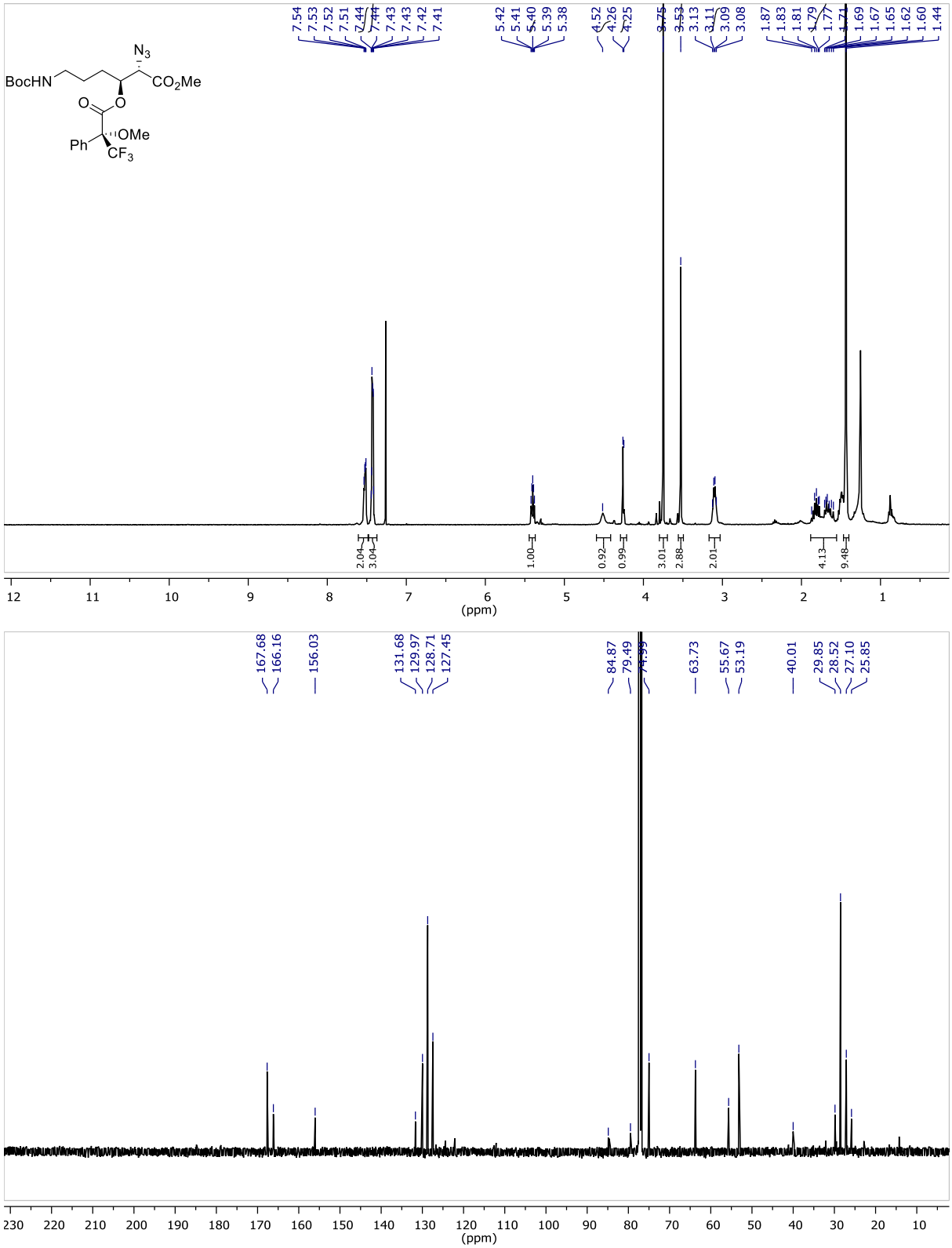
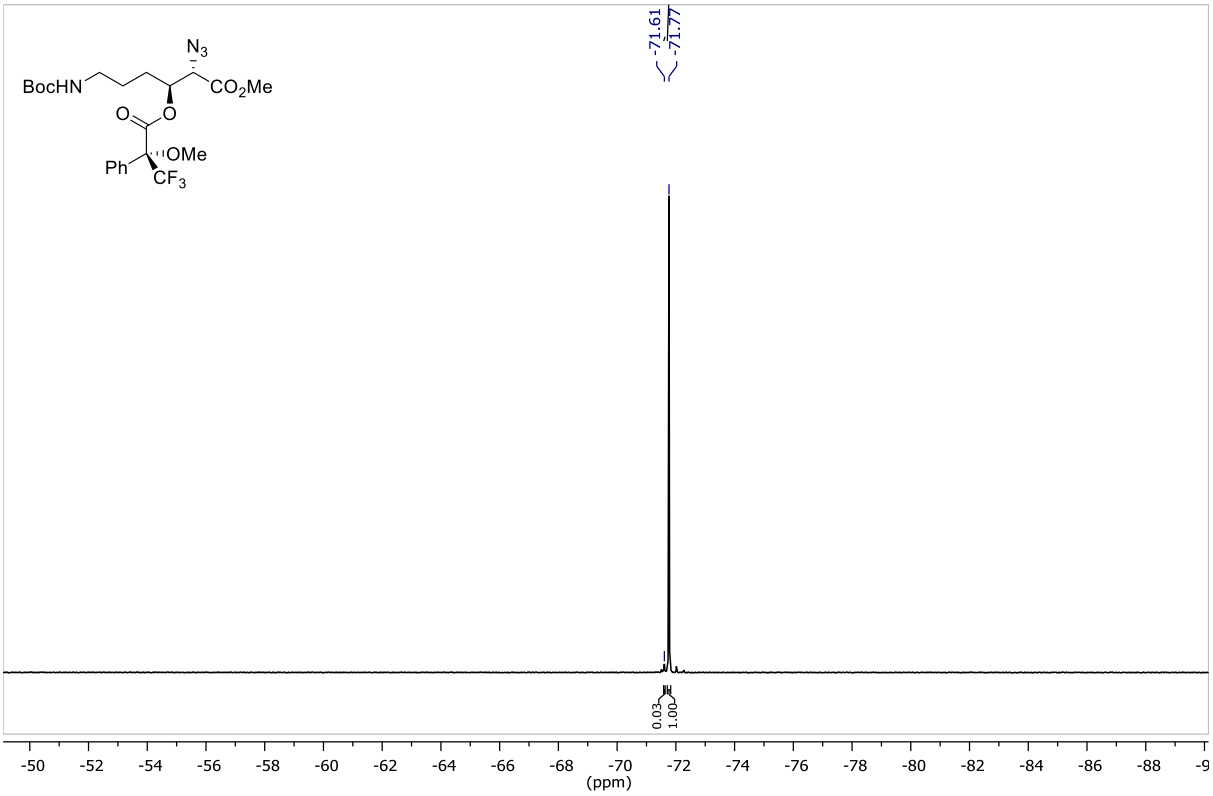
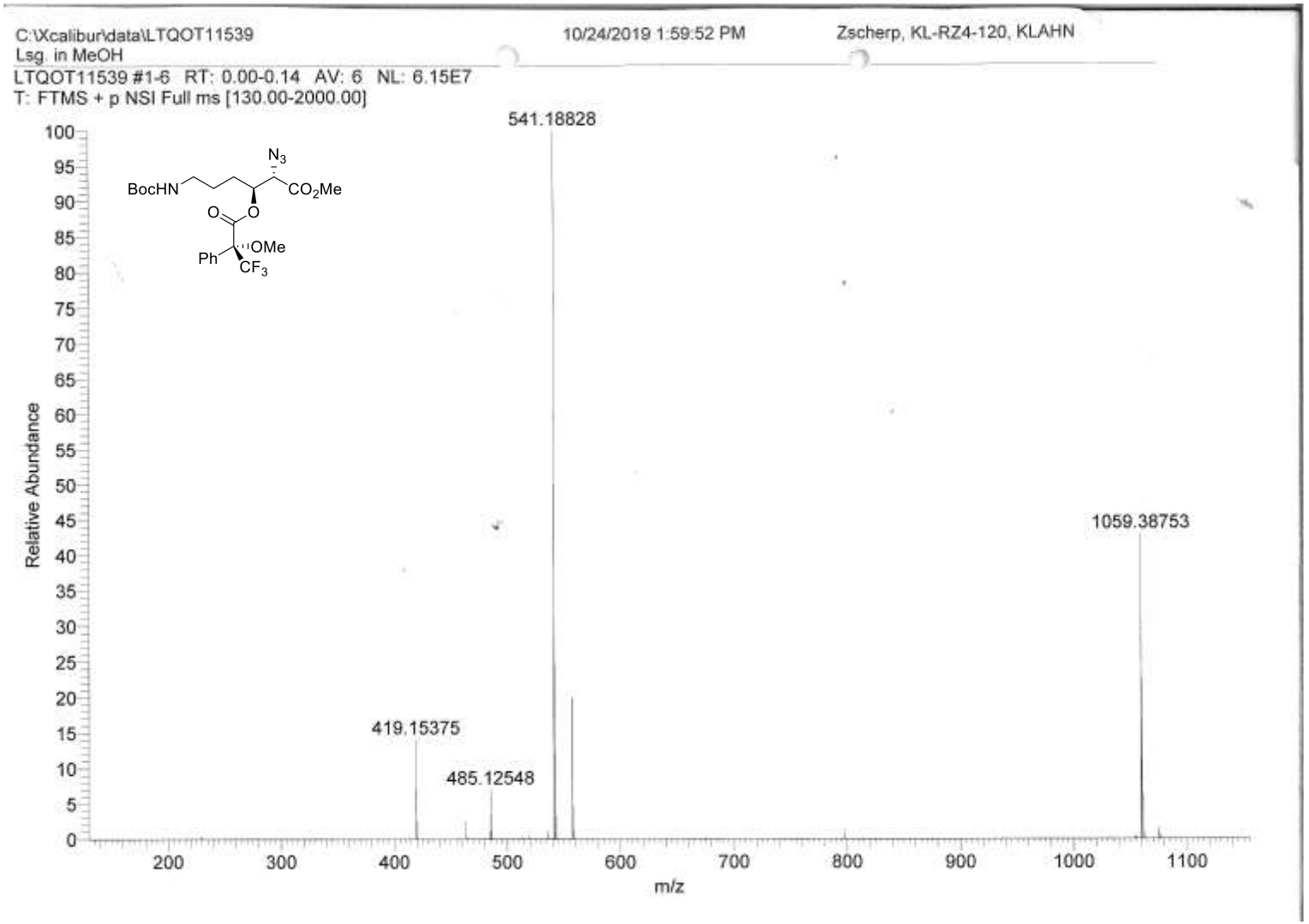
Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (R)-Mosher ester (^{1}H and ^{13}C NMR)



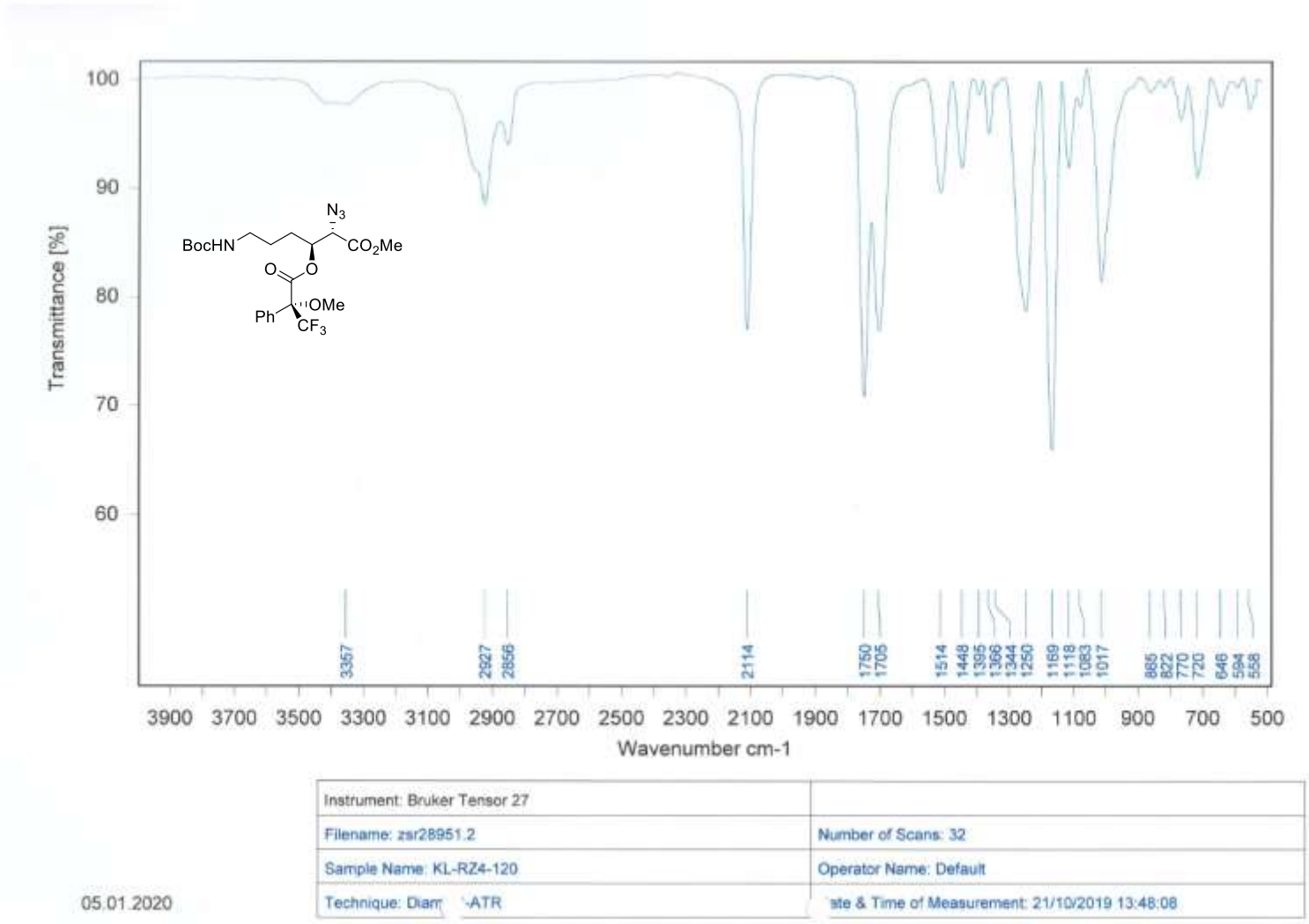
Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (R)-Mosher ester (^{19}F NMR)



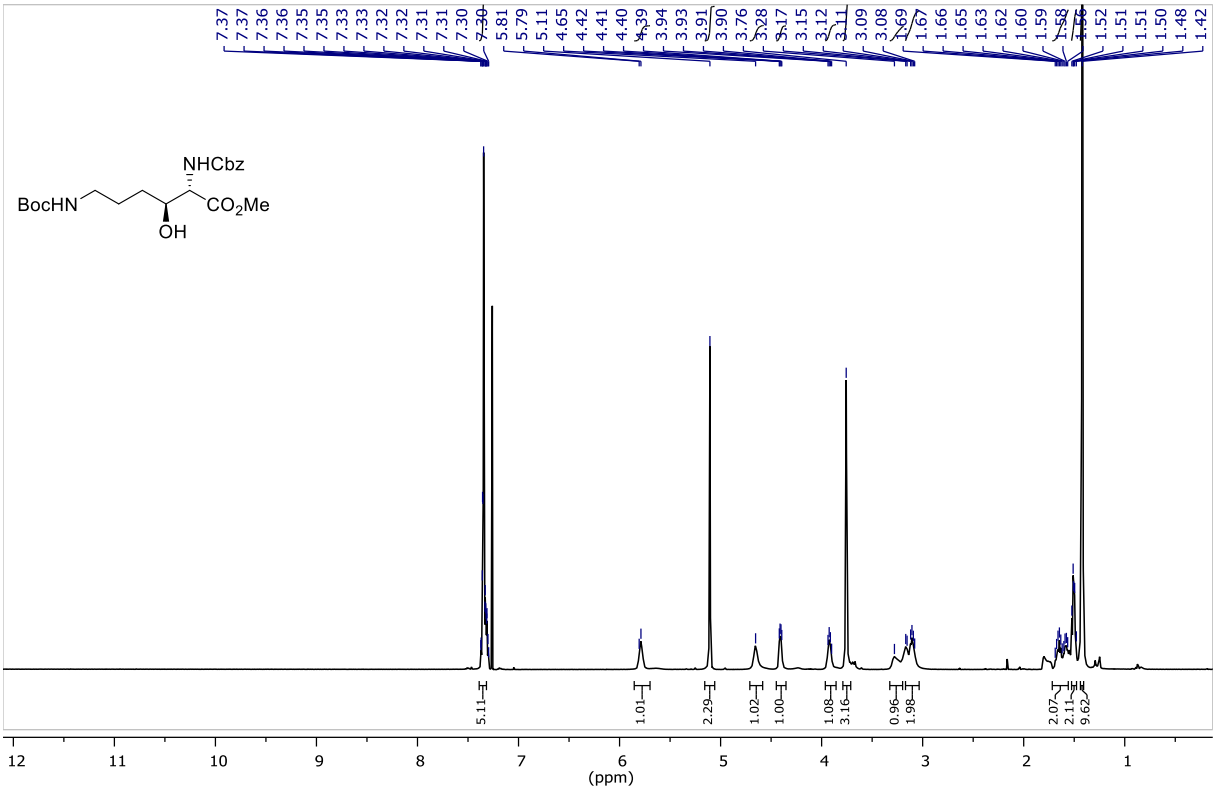
Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (R)-Mosher ester (HRMS)

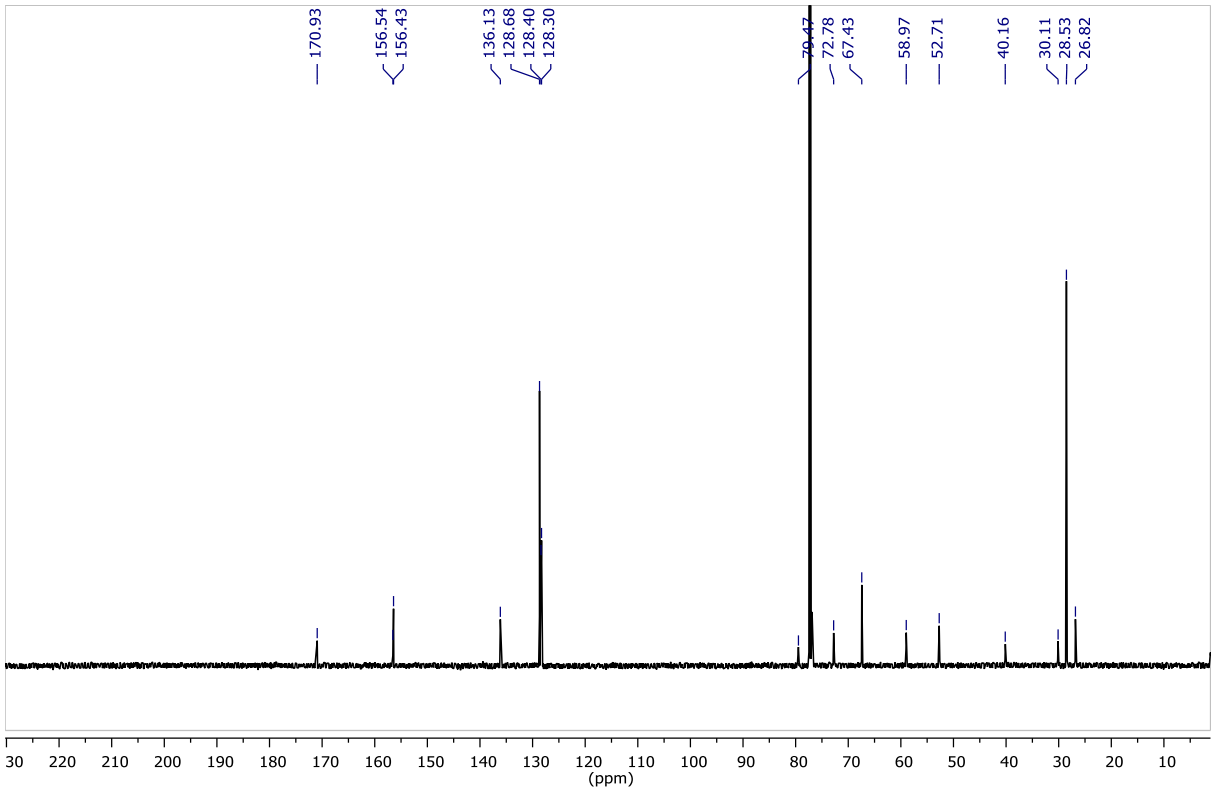


Methyl (2S,3S)-2-azido-6-((tert-butoxycarbonyl)amino)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-oyl)oxy)hexanoate – (R)-Mosher ester (ATR-IR)

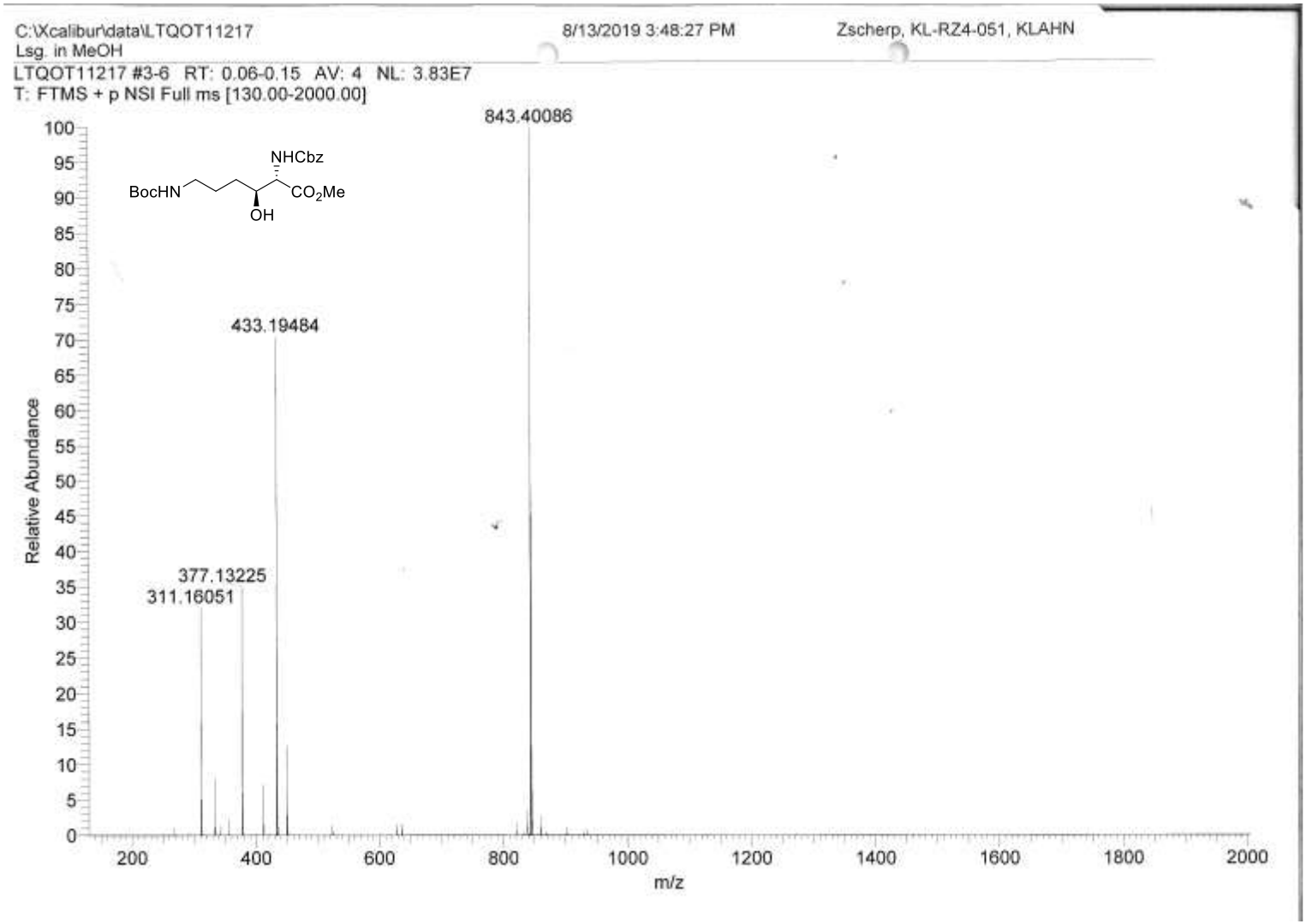


Methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxy-hexanoate (9) (^{1}H and ^{13}C NMR)

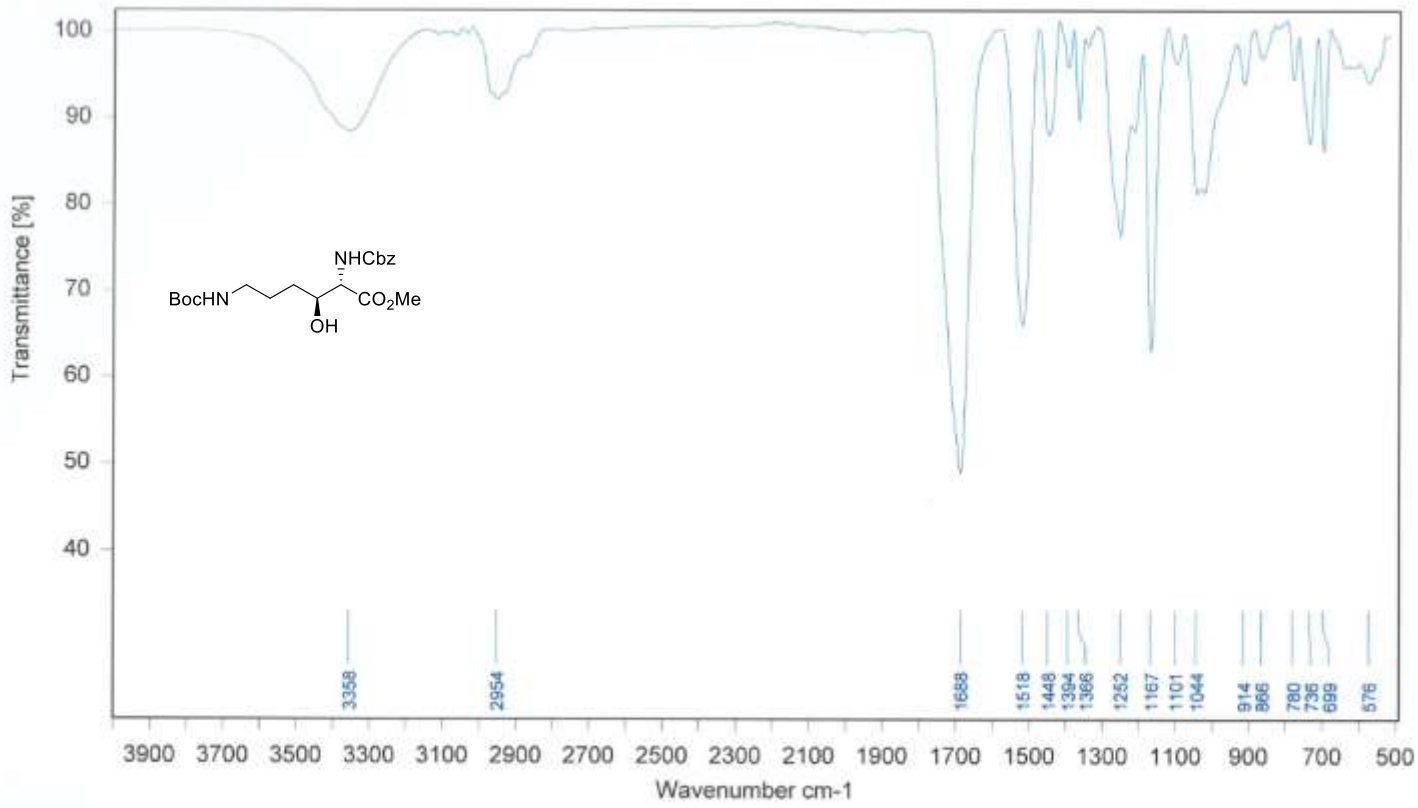




Methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxy-hexanoate (9) (HRMS)



Methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxy-hexanoate (9) (ATR-IR)



Instrument: Bruker Tensor 27	
Filename: zsr28650.1	Number of Scans: 32
Sample Name: KL-RZ4-051	Operator Name: Default
Technique: Diamal ATR	e & Time of Measurement: 13/08/2019 07:26:41

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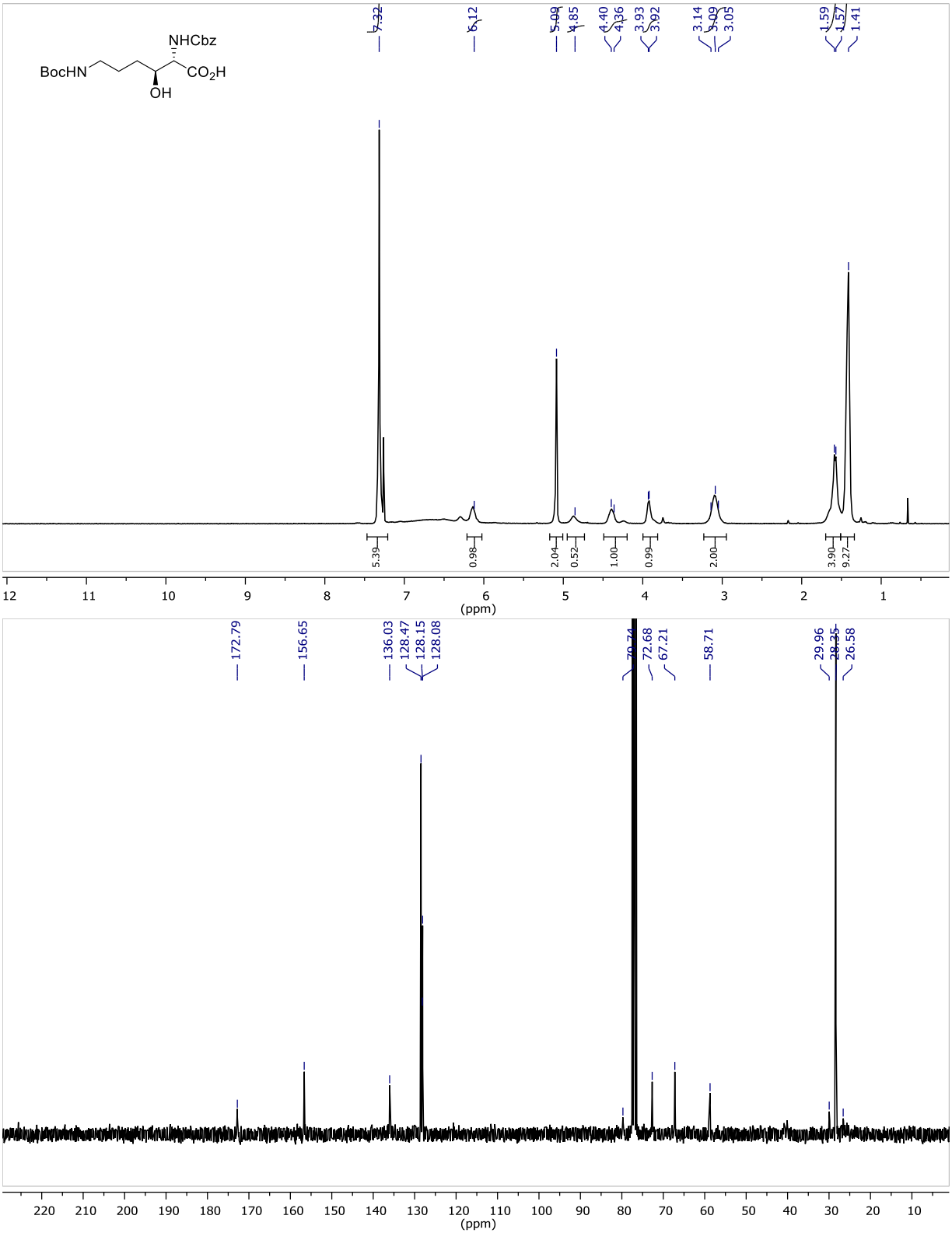
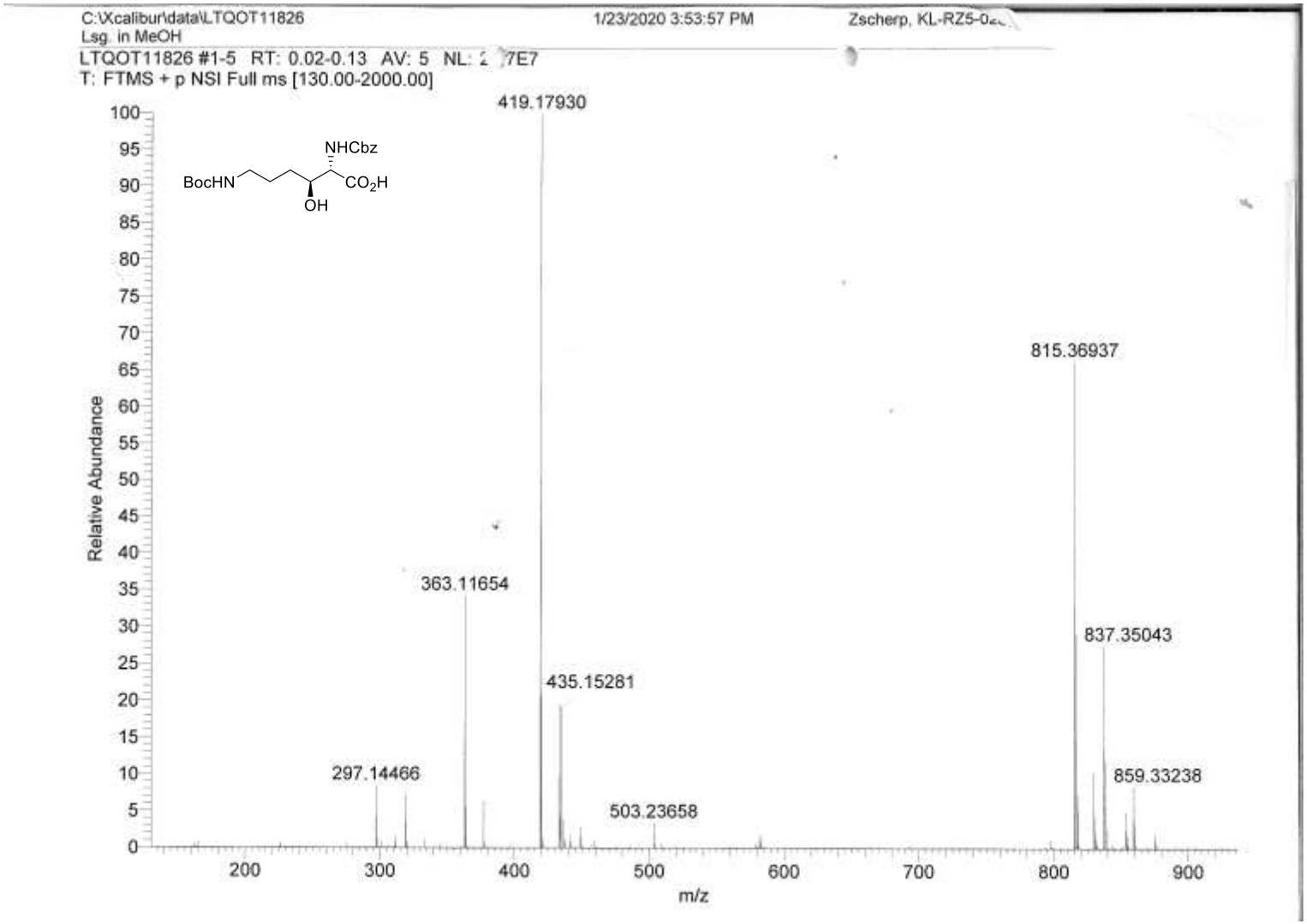
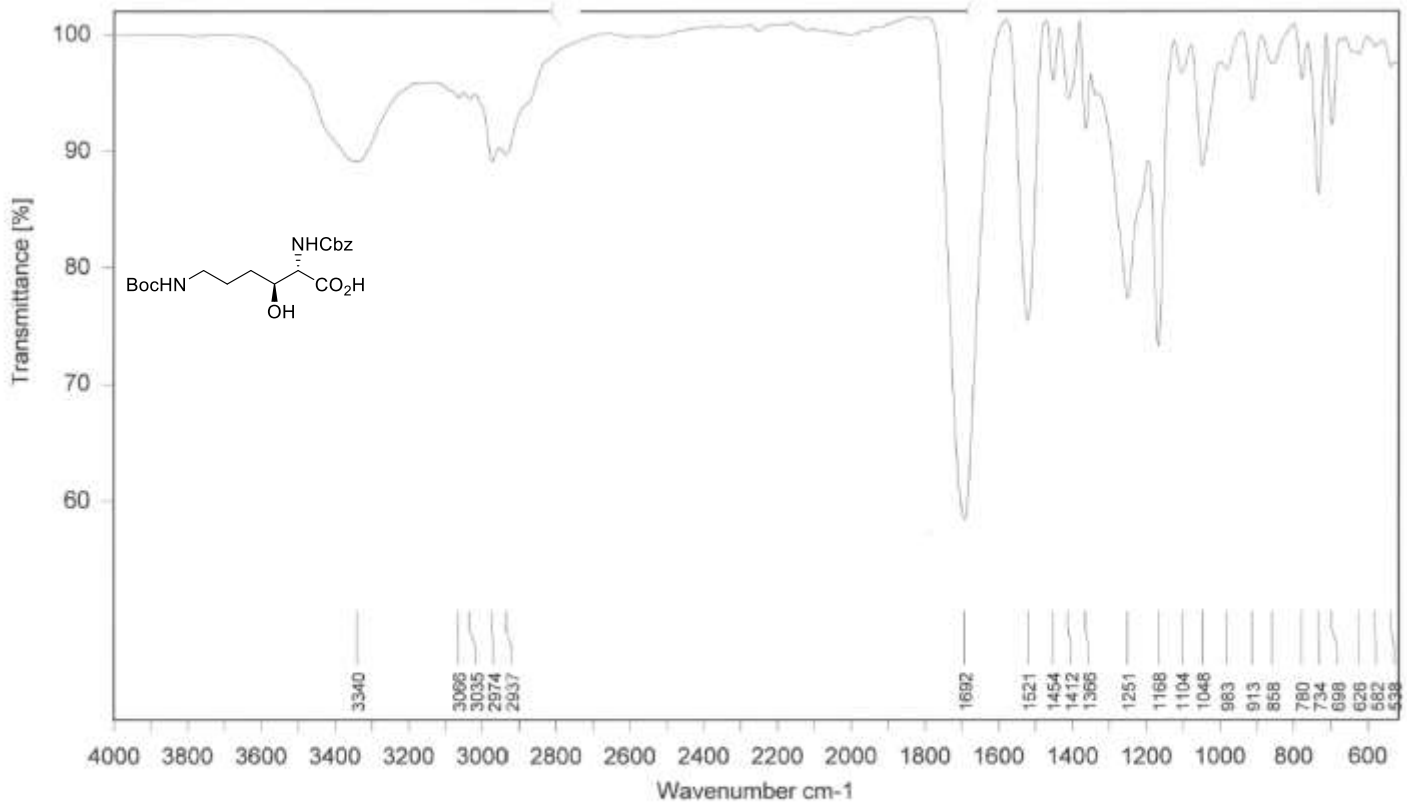
(2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoic acid (1) (^{1}H and ^{13}C NMR)



(2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoic acid (1) (HRMS)



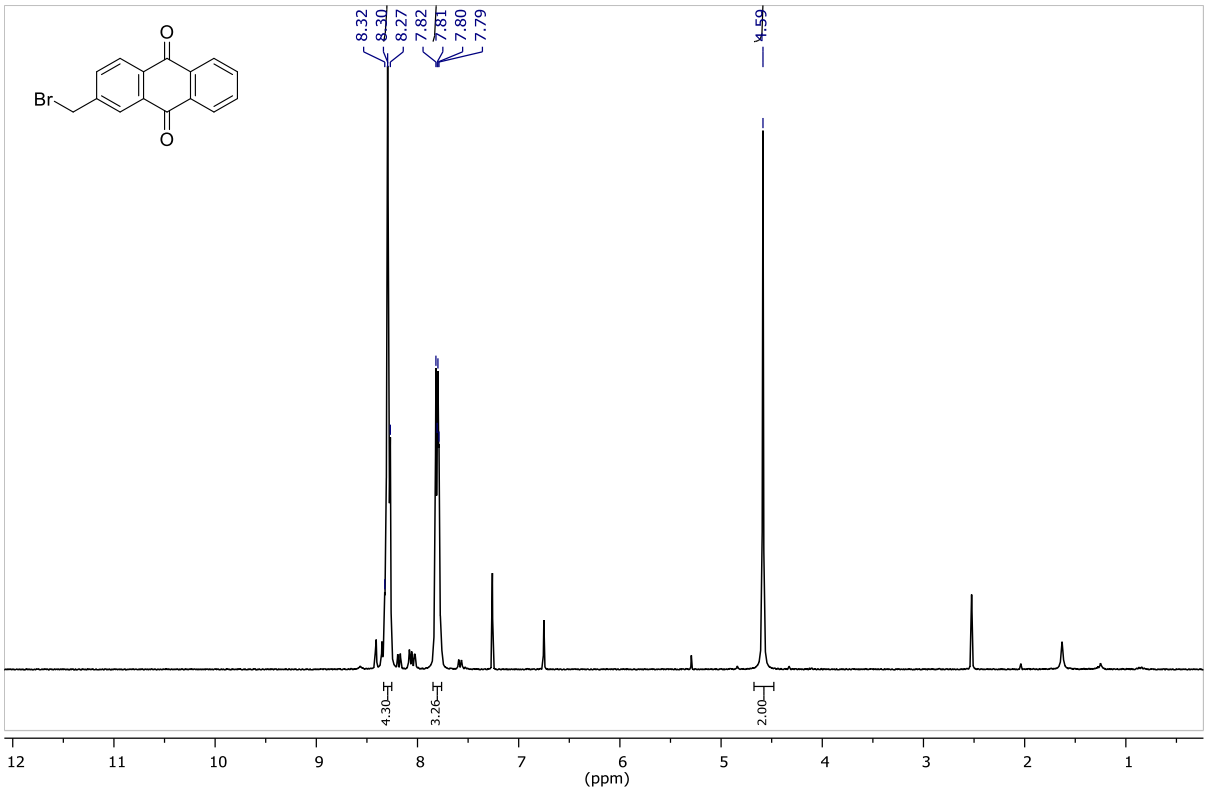
(2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoic acid (1) (ATR-IR)

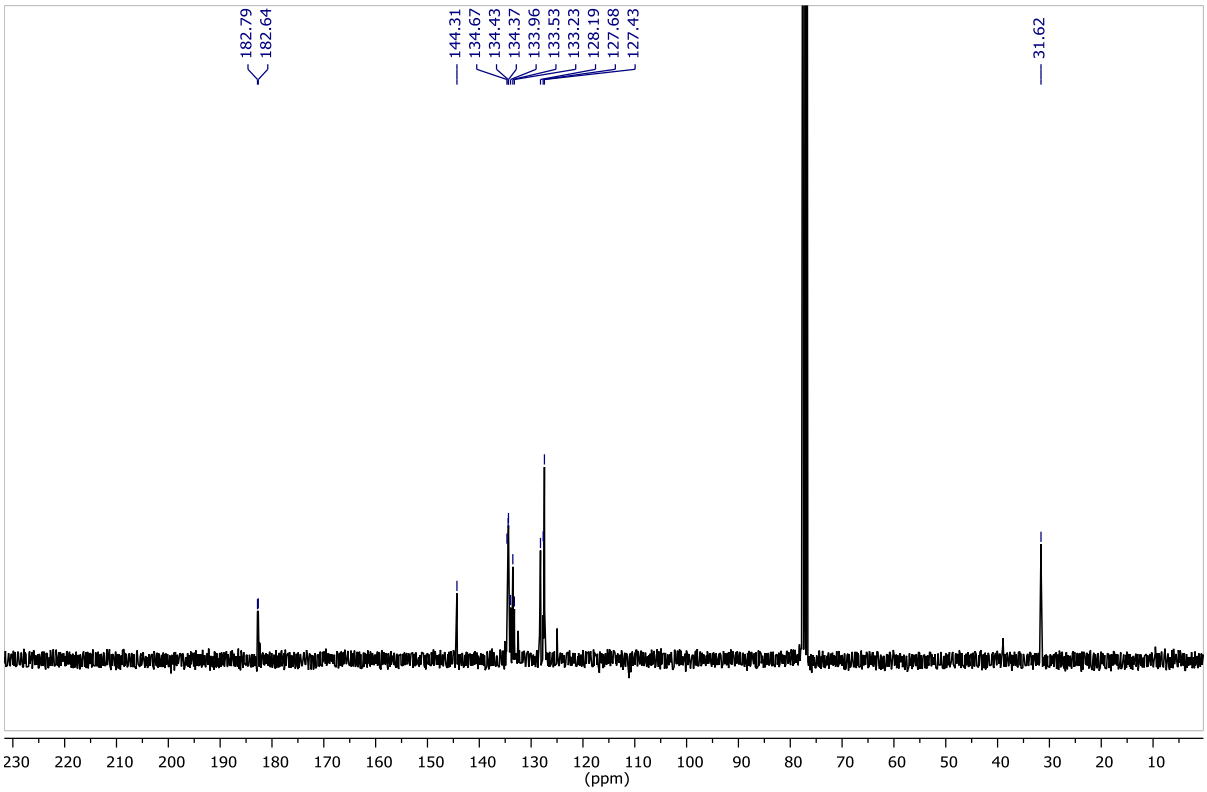


Instrument: Bruker Tensor 27	
Filename: zsr29208.1	Number of Scans: 32
Sample Name: KL-RZ5-038a	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 23.01.2020 08:20:51

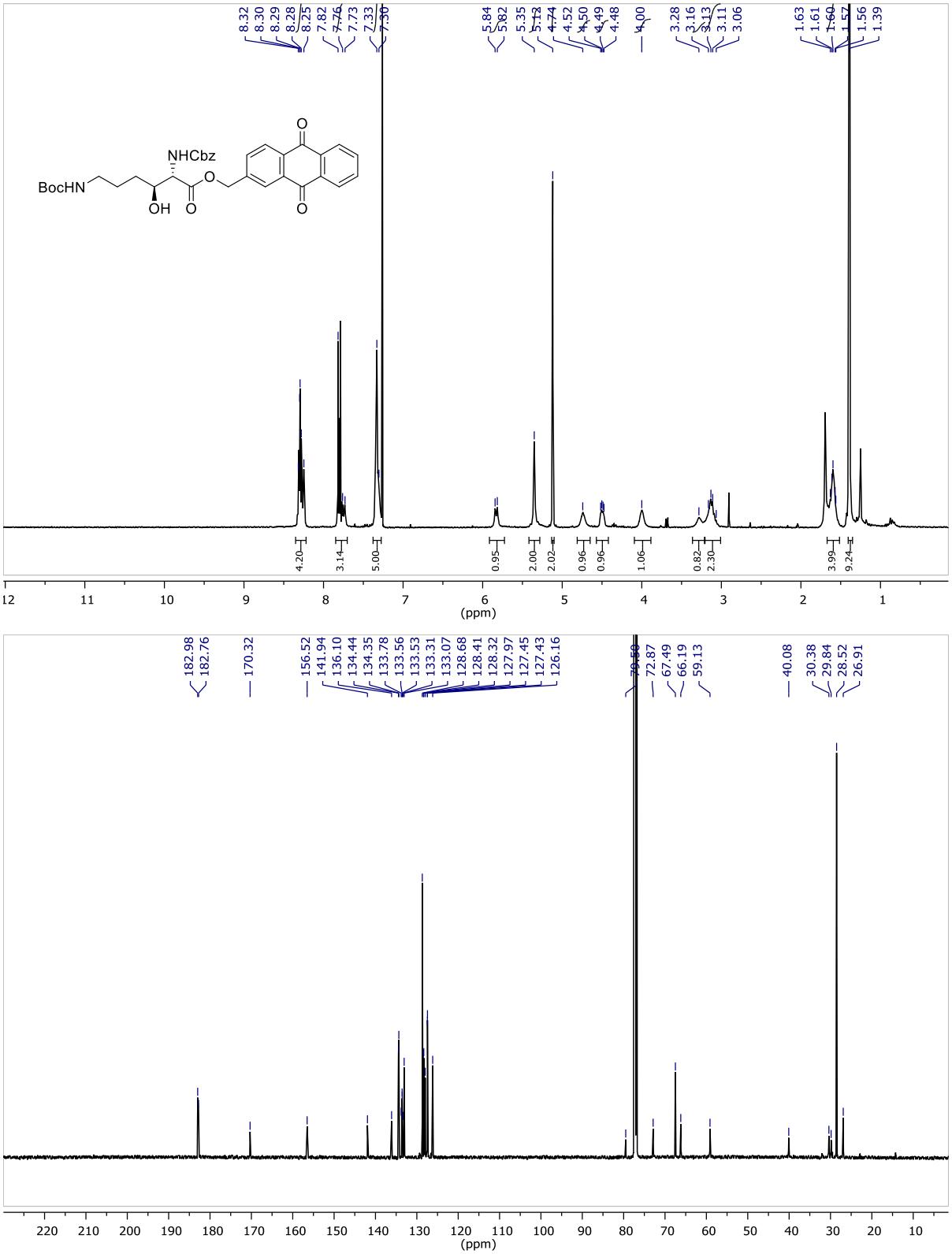
23.01.2020

2-(Bromomethyl)anthracene-9,10-dione (10) (^{1}H and ^{13}C NMR)

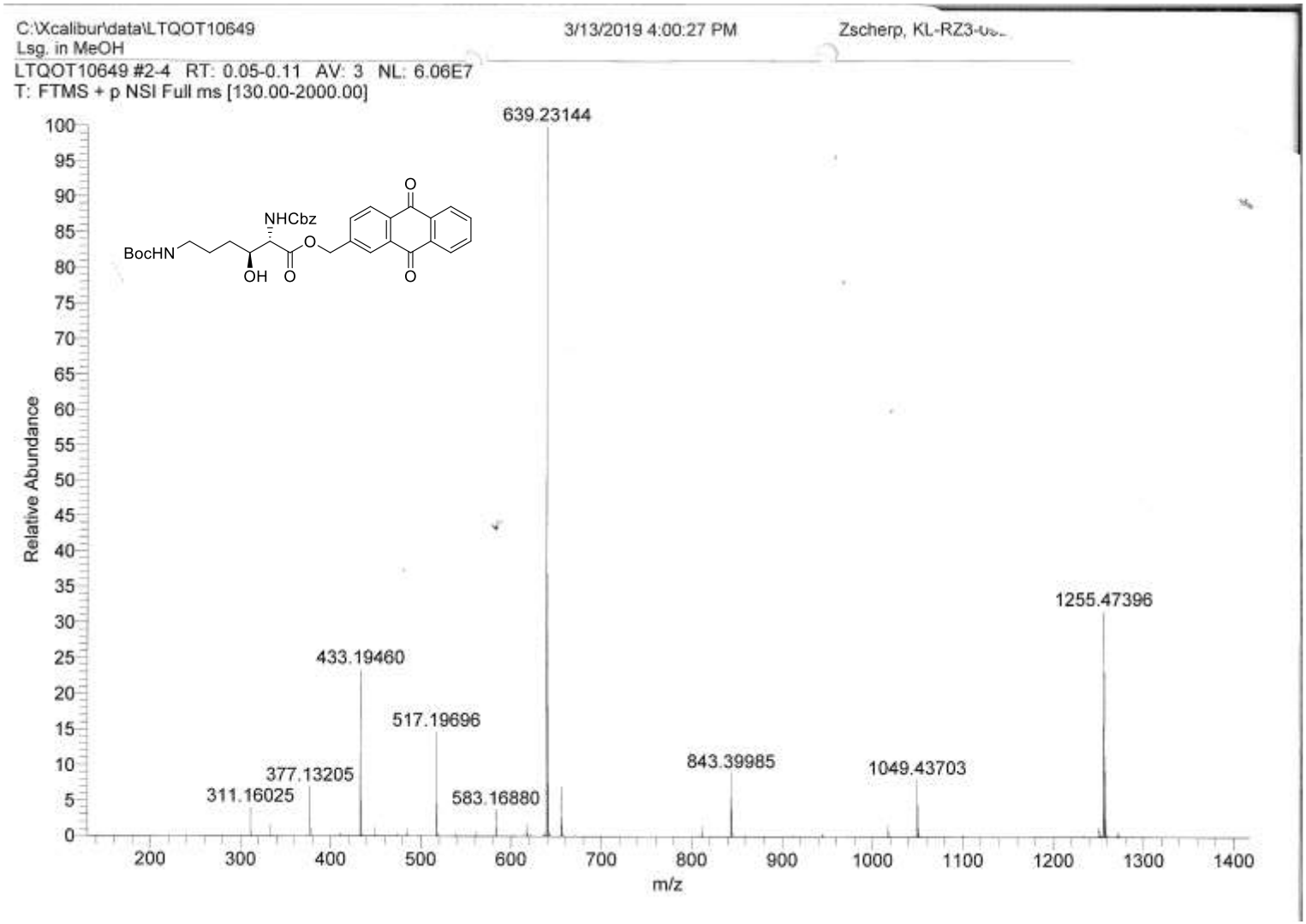




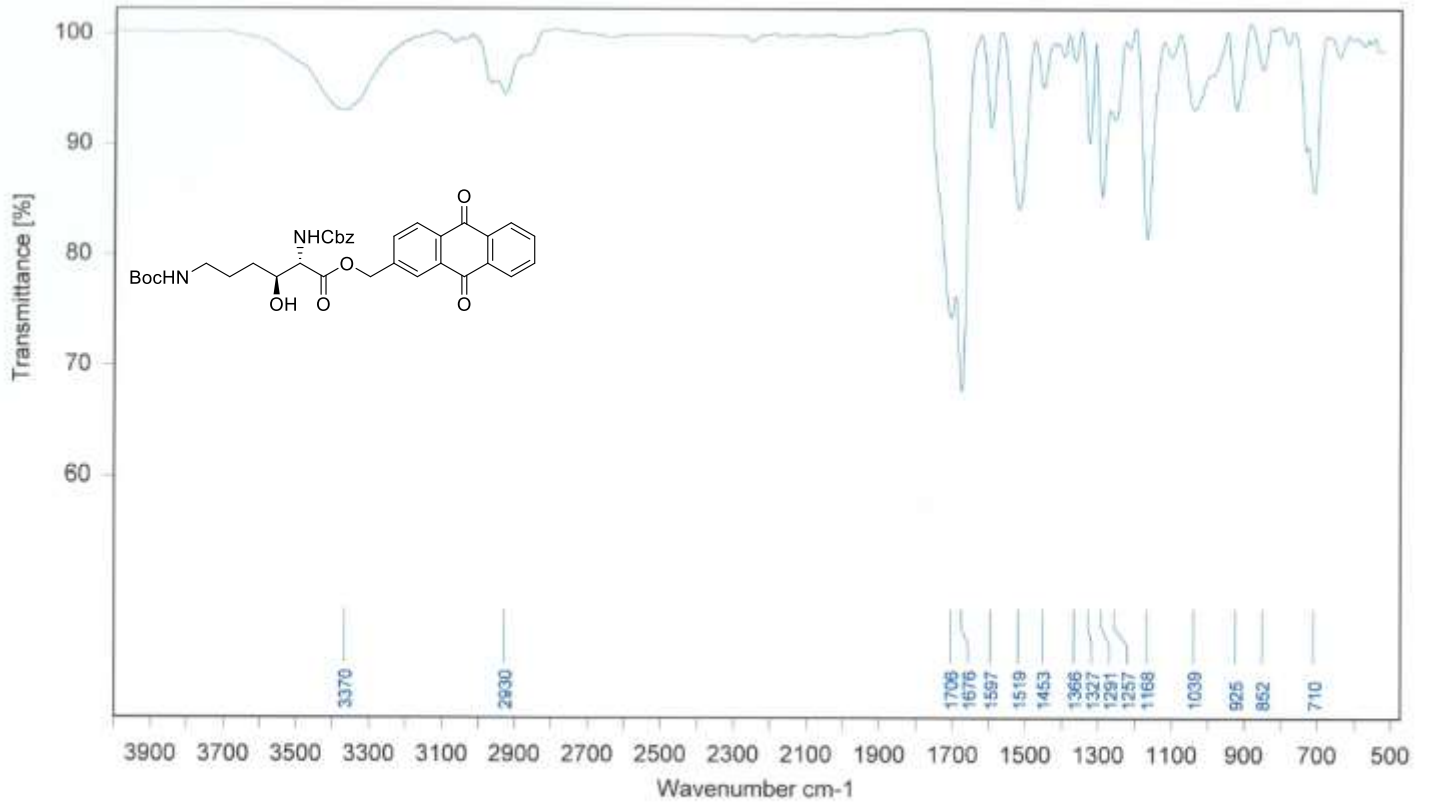
(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoate (11) (^{1}H and ^{13}C NMR)



(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoate (11) (HRMS)



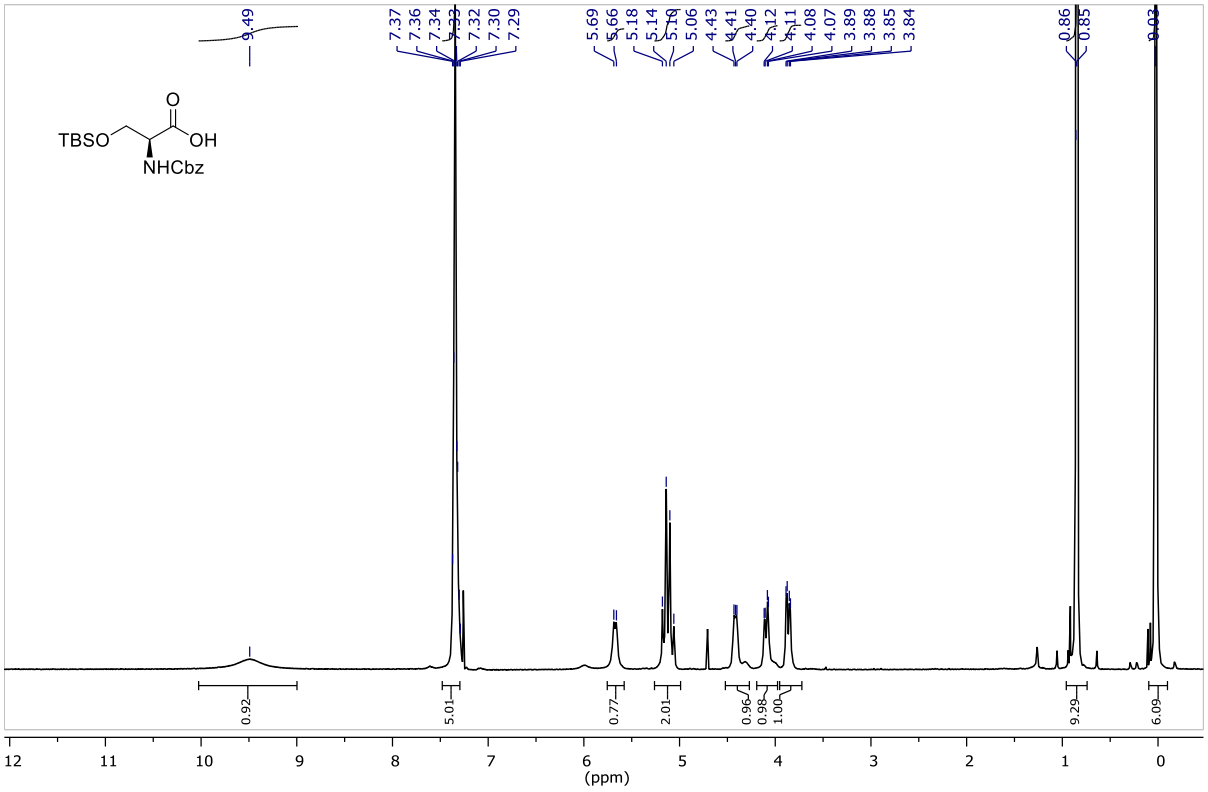
(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-3-hydroxyhexanoate (11)
(ATR-IR)

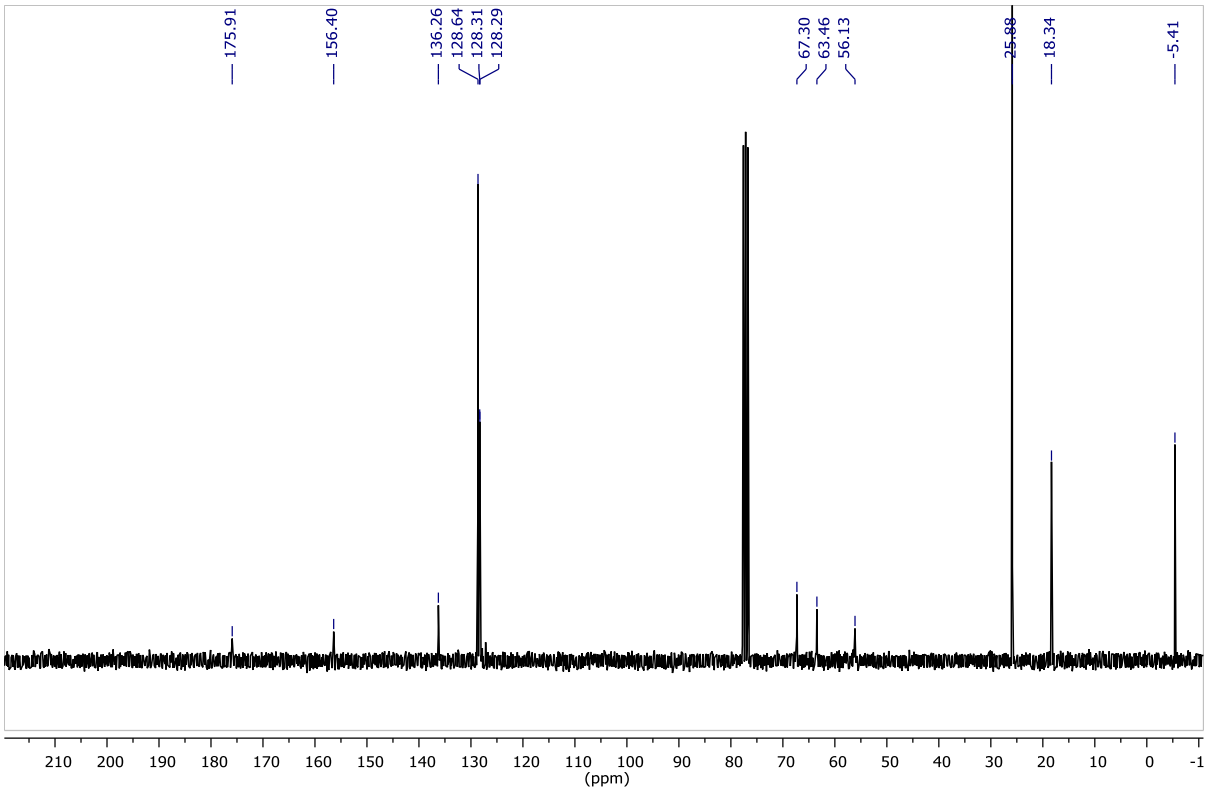


Instrument: Bruker Tensor 27	
Filename: zsr28651.1	Number of Scans: 32
Sample Name: KL-RZ4-053	Operator Name: Default
Technique: Diama. . ATR	. .te & Time of Measurement: 13/08/2019 07:35:31

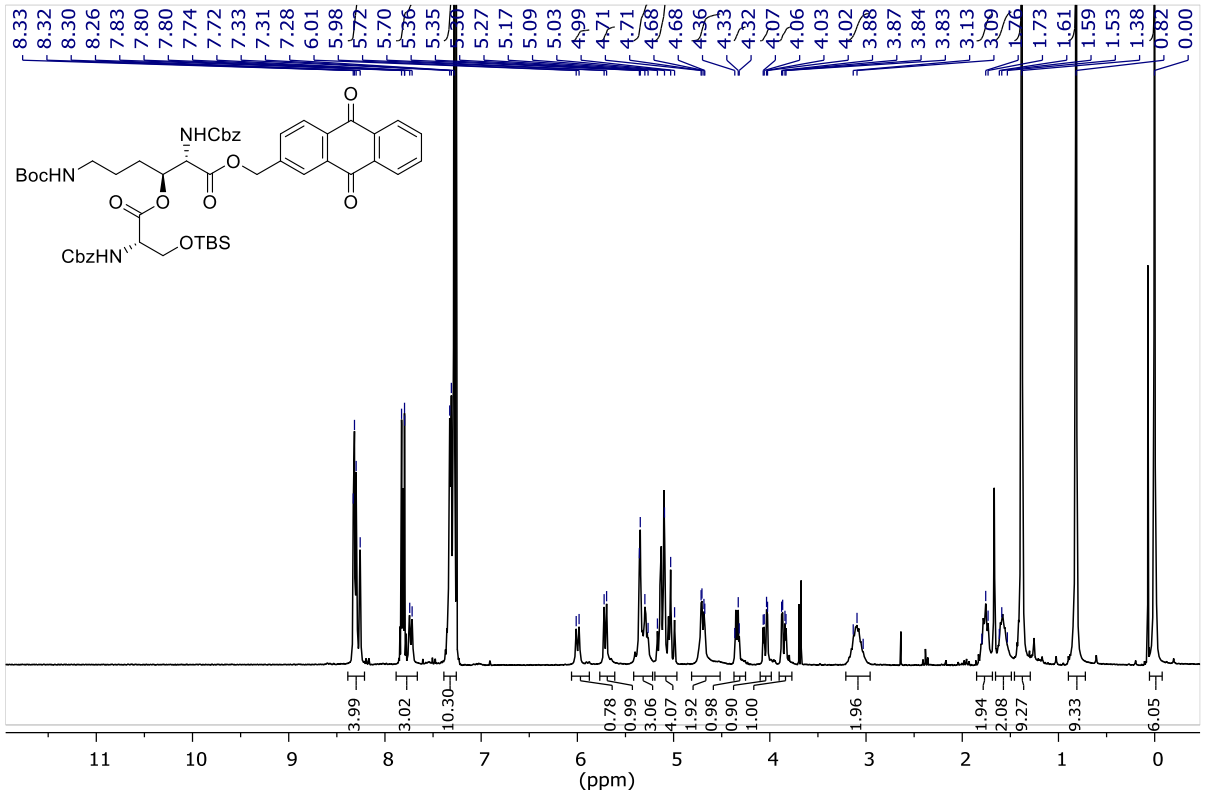
05.01.2020

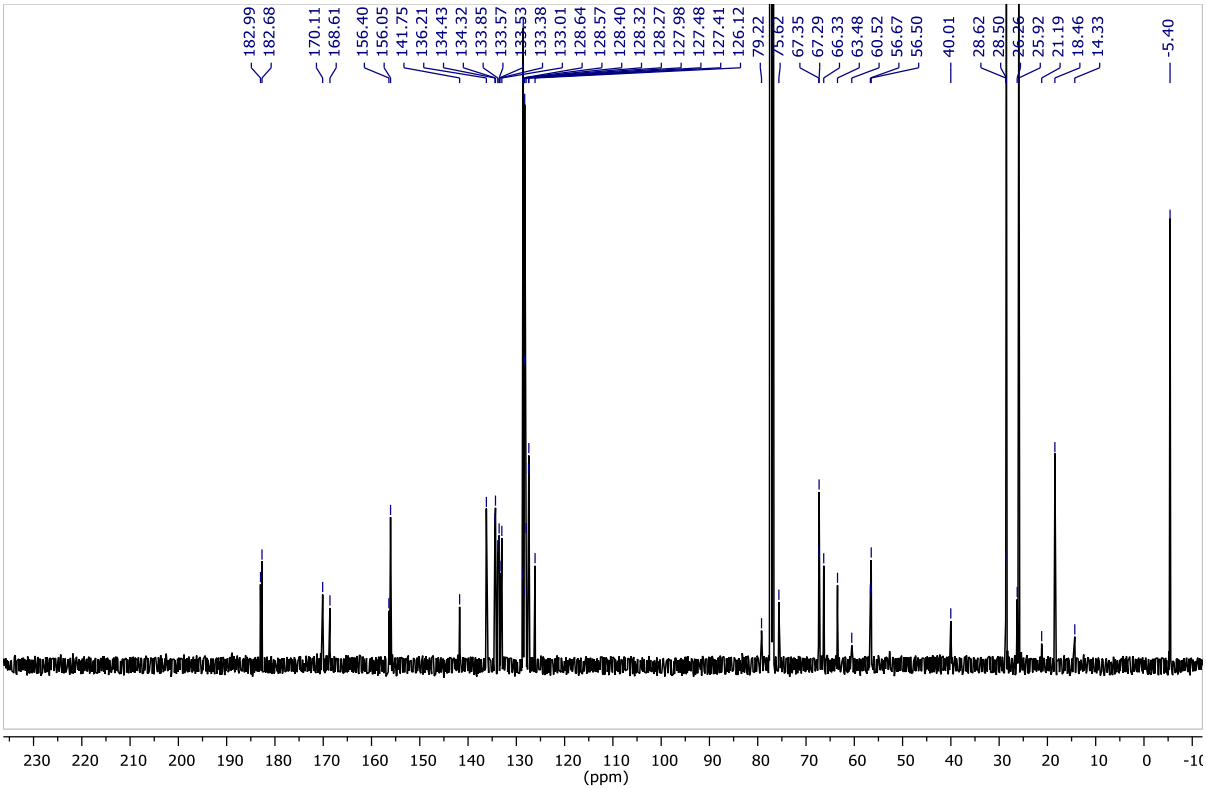
N-((benzyloxy)carbonyl)-O-(tert-butyldimethylsilyl)-L-serine - Cbz-Ser(OTBS)-OH (12) (^{1}H and ^{13}C NMR)



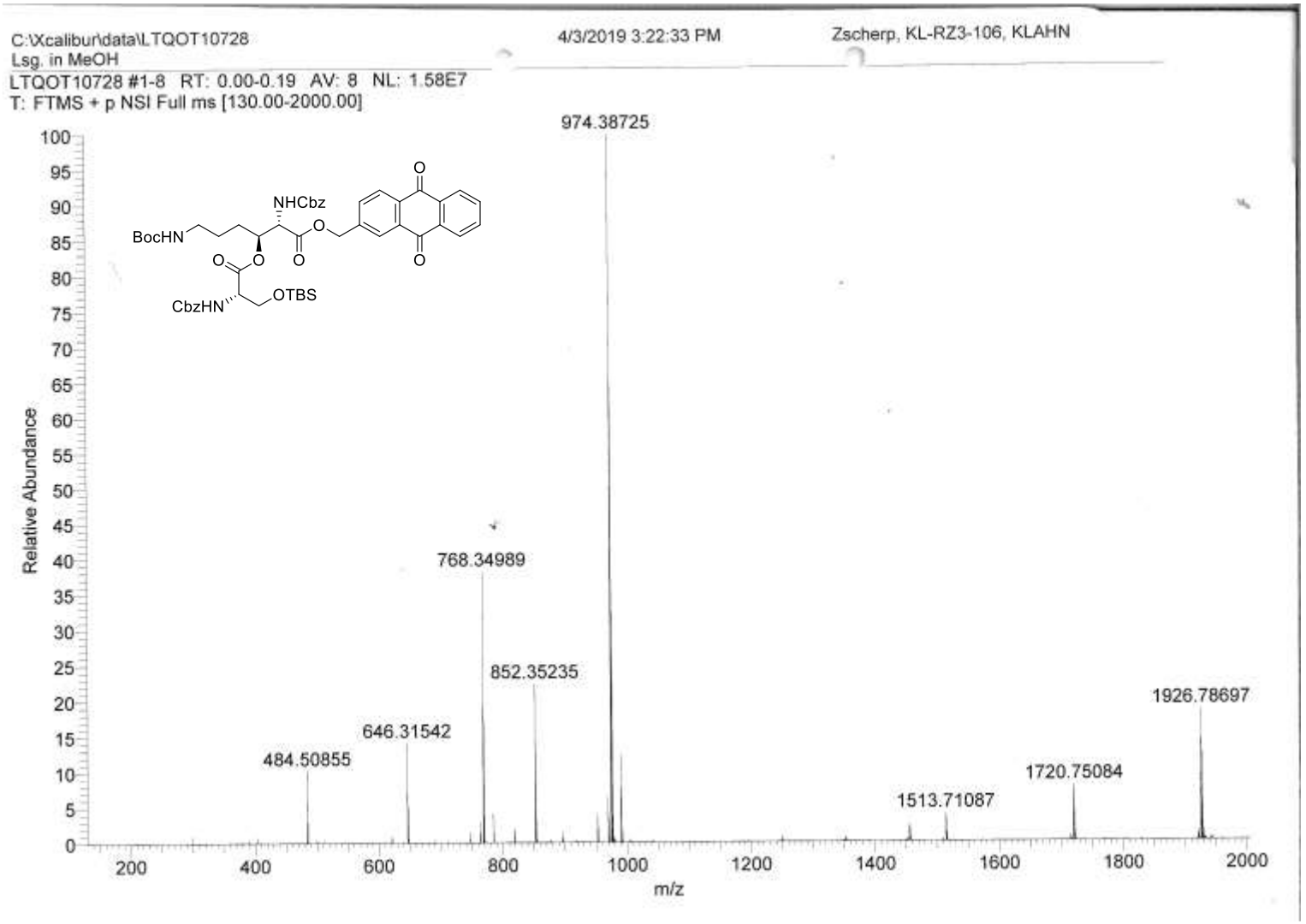


(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(tert-butyl-dime-thylsilyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hex-anoate **(13)** (^{1}H and ^{13}C NMR)

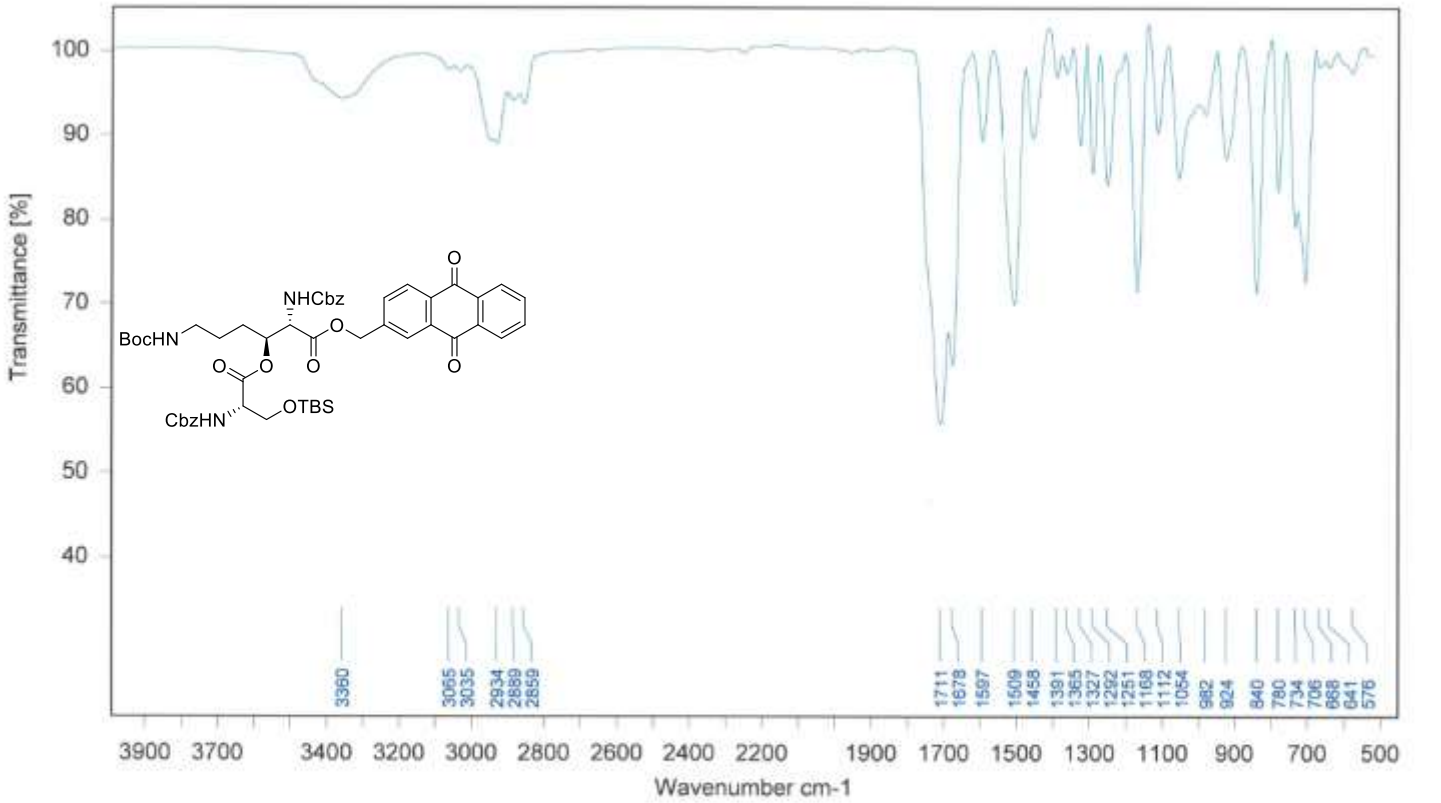




(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(tert-butyl-dime-thylsilyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hex-anoate **(13)** (HRMS)



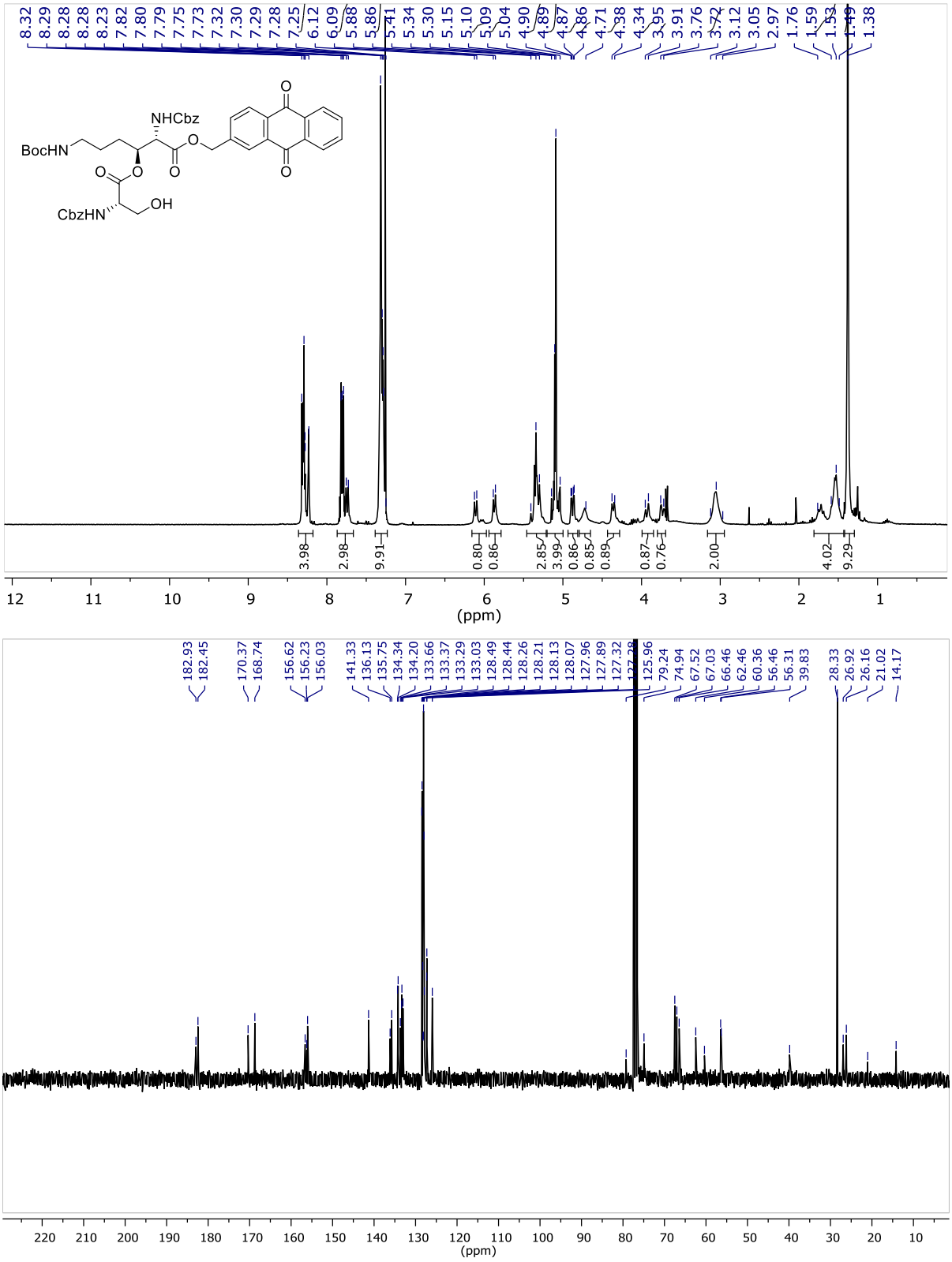
(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(tert-butyl-dime-thylsilyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hex-anoate (13) (ATR-IR)



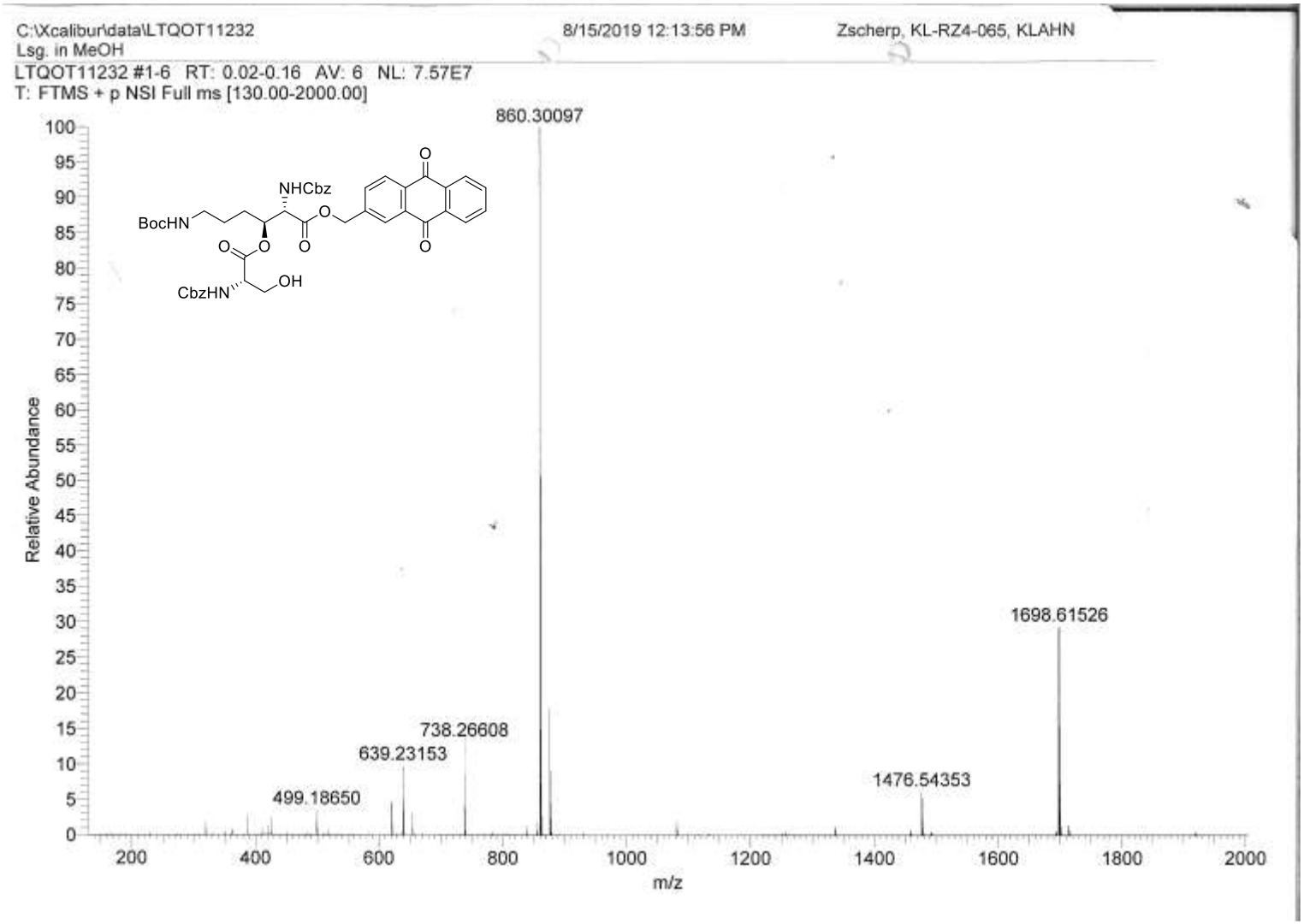
Instrument: Bruker Tensor 27	
Filename: zsr28652.1	Number of Scans: 32
Sample Name: KL-RZ4-061	Operator Name: Default
Technique: Diama. ATR	.e & Time of Measurement: 13/08/2019 07:40:14

05.01.2020

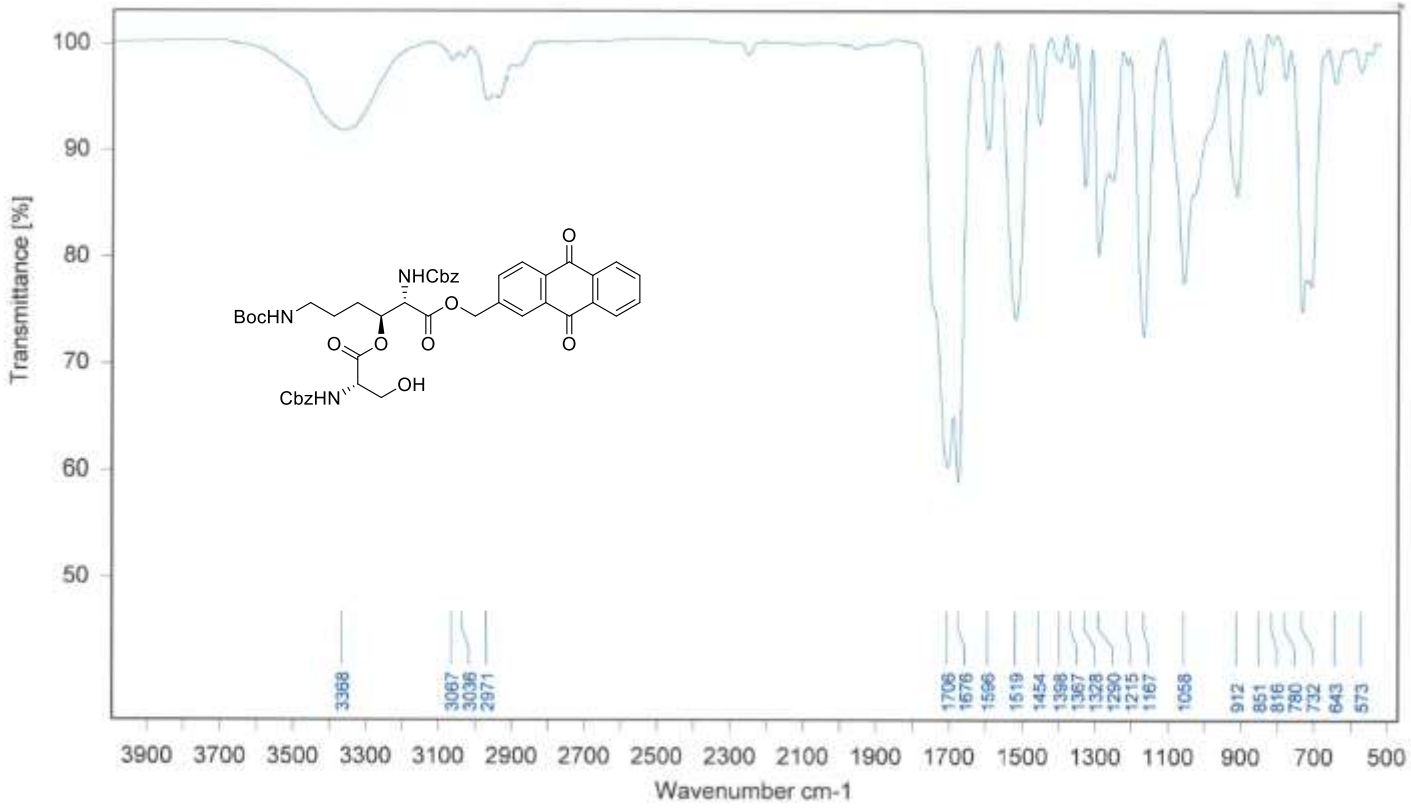
(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((((benzyloxy)carbonyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoate (14) (^{1}H and ^{13}C NMR)



(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((((benzyloxy)carbonyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoate (14) (HRMS)



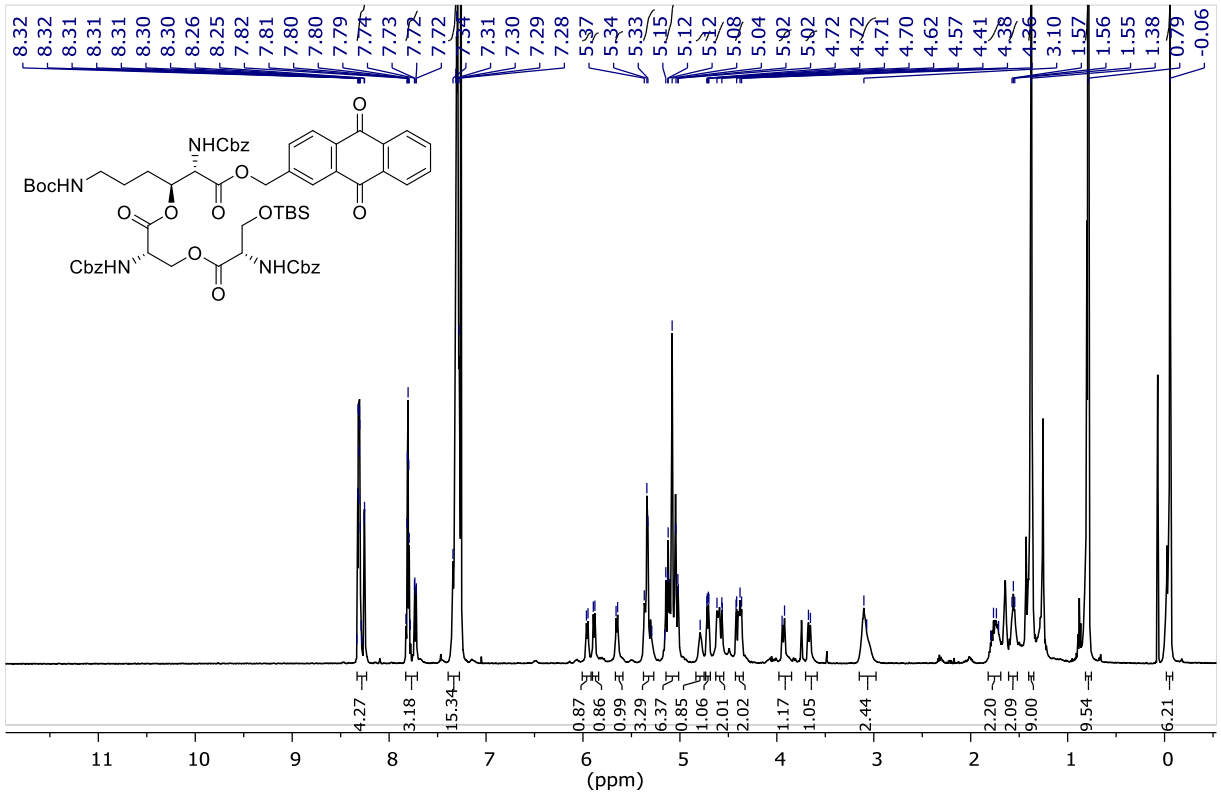
(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((((benzyloxy)carbonyl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoate (14) (ATR-IR)

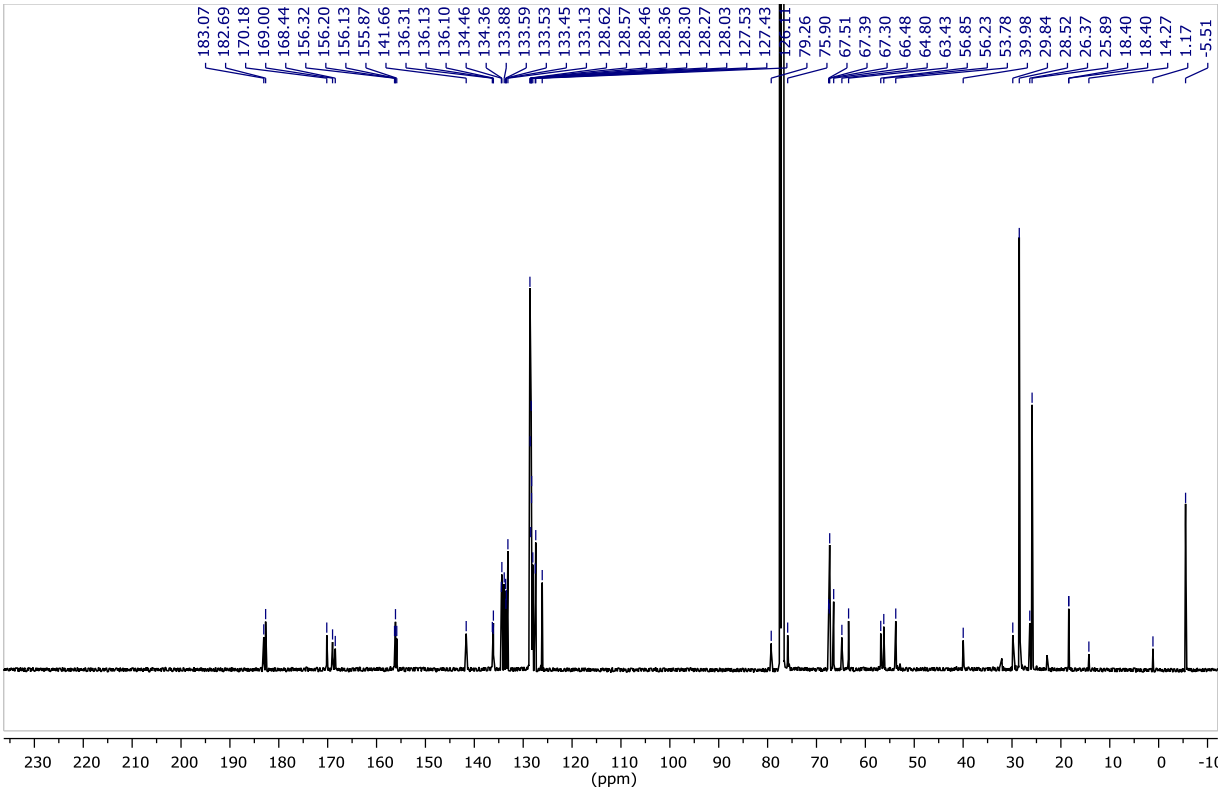


Instrument: Bruker Tensor 27	
Filename: zsr28656.1	Number of Scans: 32
Sample Name: KL-RZ4-065	Operator Name: Default
Technique: Diamá ATR	...te & Time of Measurement: 14/08/2019 09:15:09

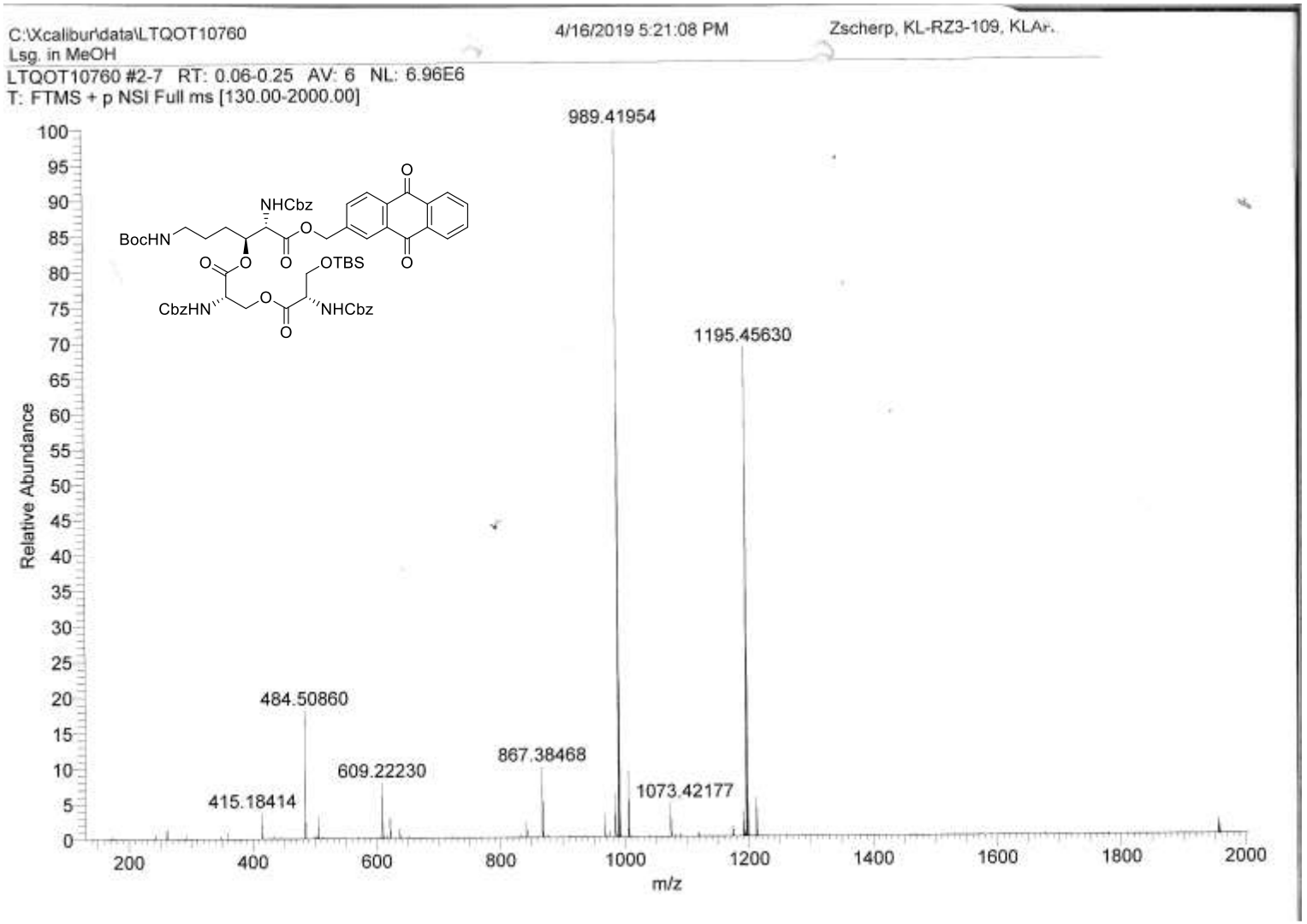
05.01.2020

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(N-((benzyloxy)carbonyl)-O-(tert-butyldimethylsilyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)-amino)-6-((tert-butoxycarbonyl)amino)hexanoate (15) (^{1}H and ^{13}C NMR)

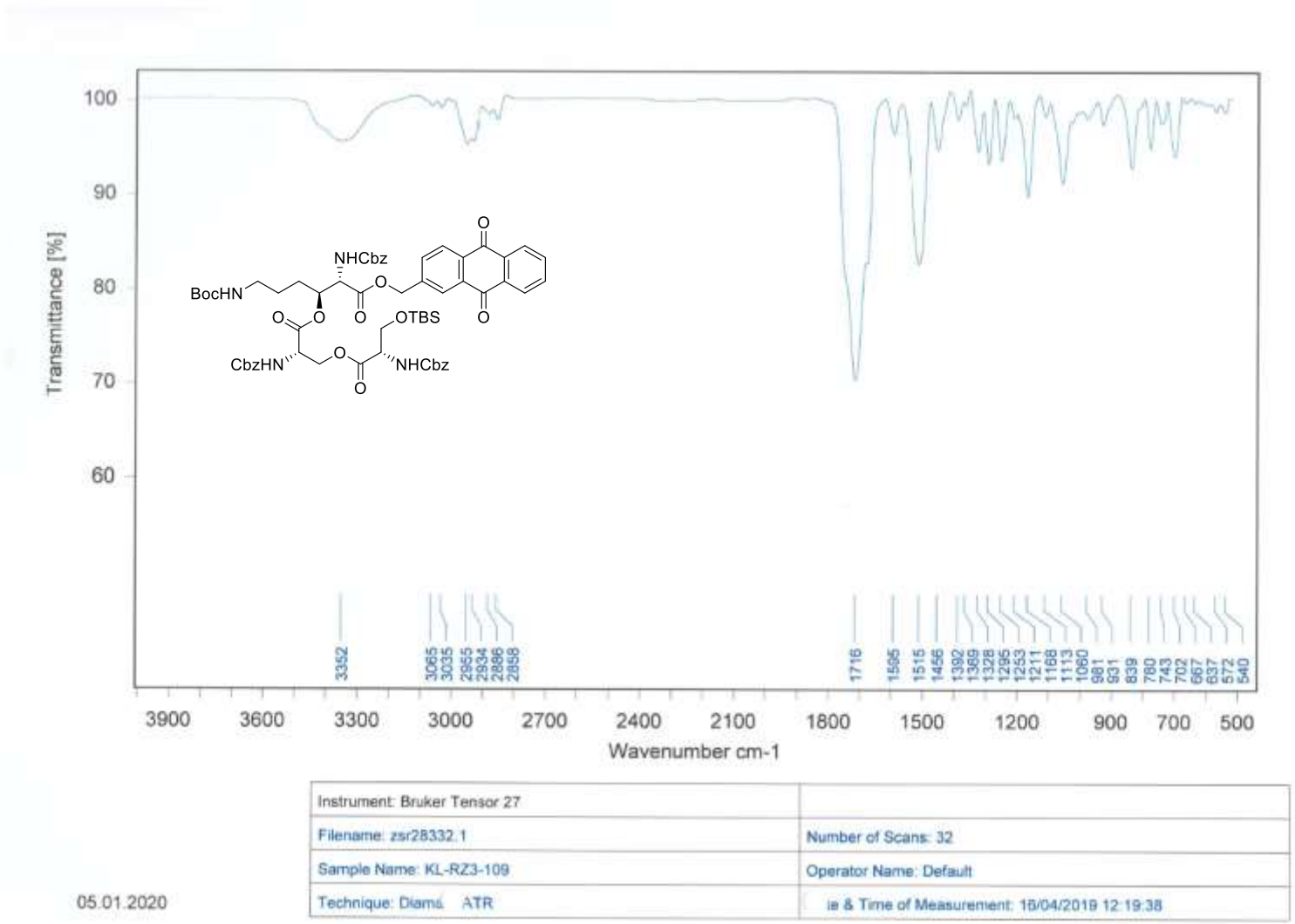




(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(N-((benz-yloxy)carbonyl)-O-(tert-butyldimethylsilyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)-amino)-6-((tert-butoxycarbonyl)amino)hexanoate (15) (HRMS)



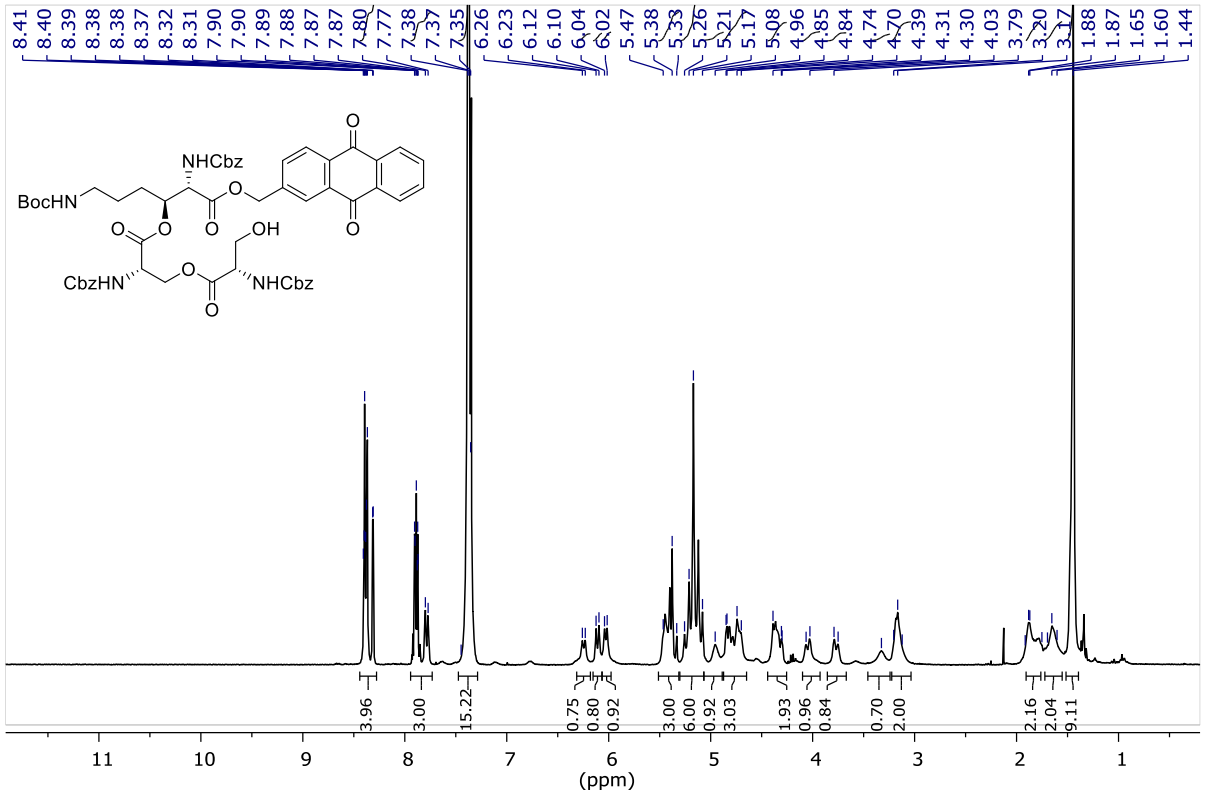
(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(N-((benz-yloxy)carbonyl)-O-(tert-butyldimethylsilyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)-amino)-6-((tert-butoxycarbonyl)amino)hexanoate (15) (ATR-IR)

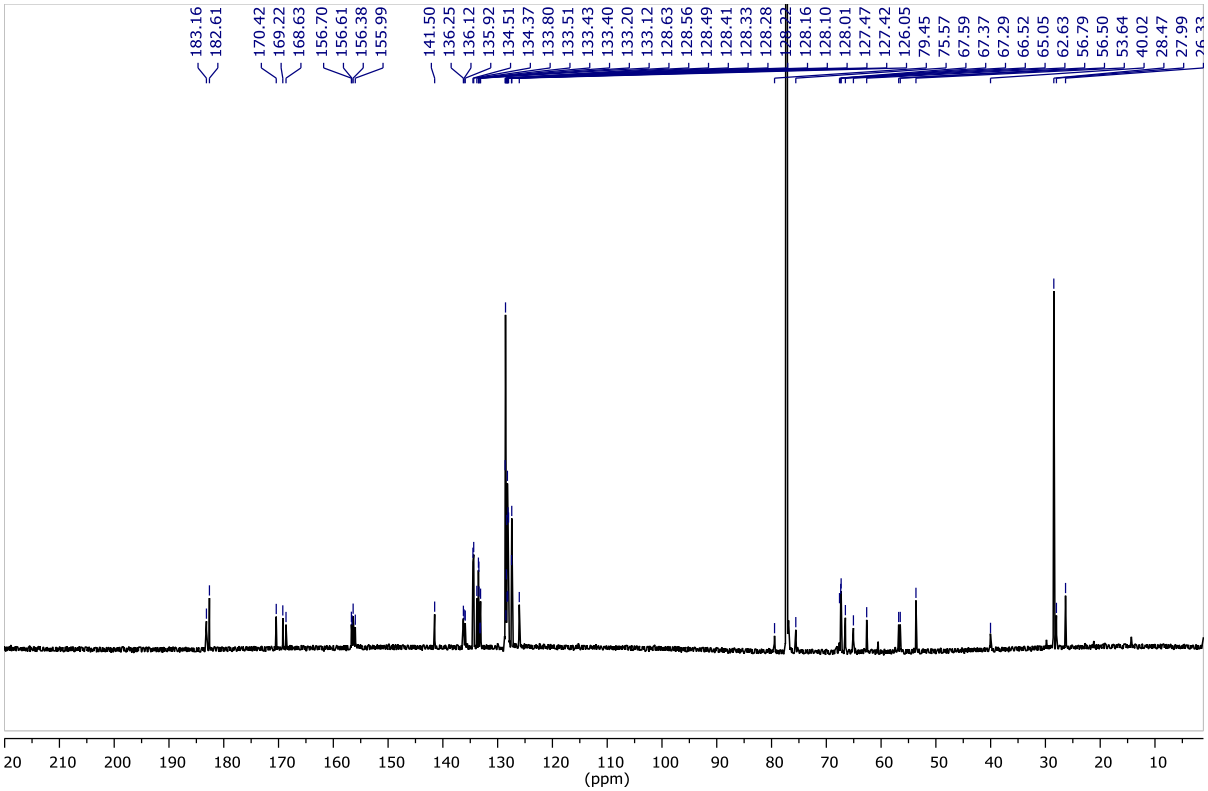


Instrument: Bruker Tensor 27	
Filename: zsr28332.1	Number of Scans: 32
Sample Name: KL-RZ3-109	Operator Name: Default
Technique: Diamd ATR	ie & Time of Measurement: 16/04/2019 12:19:38

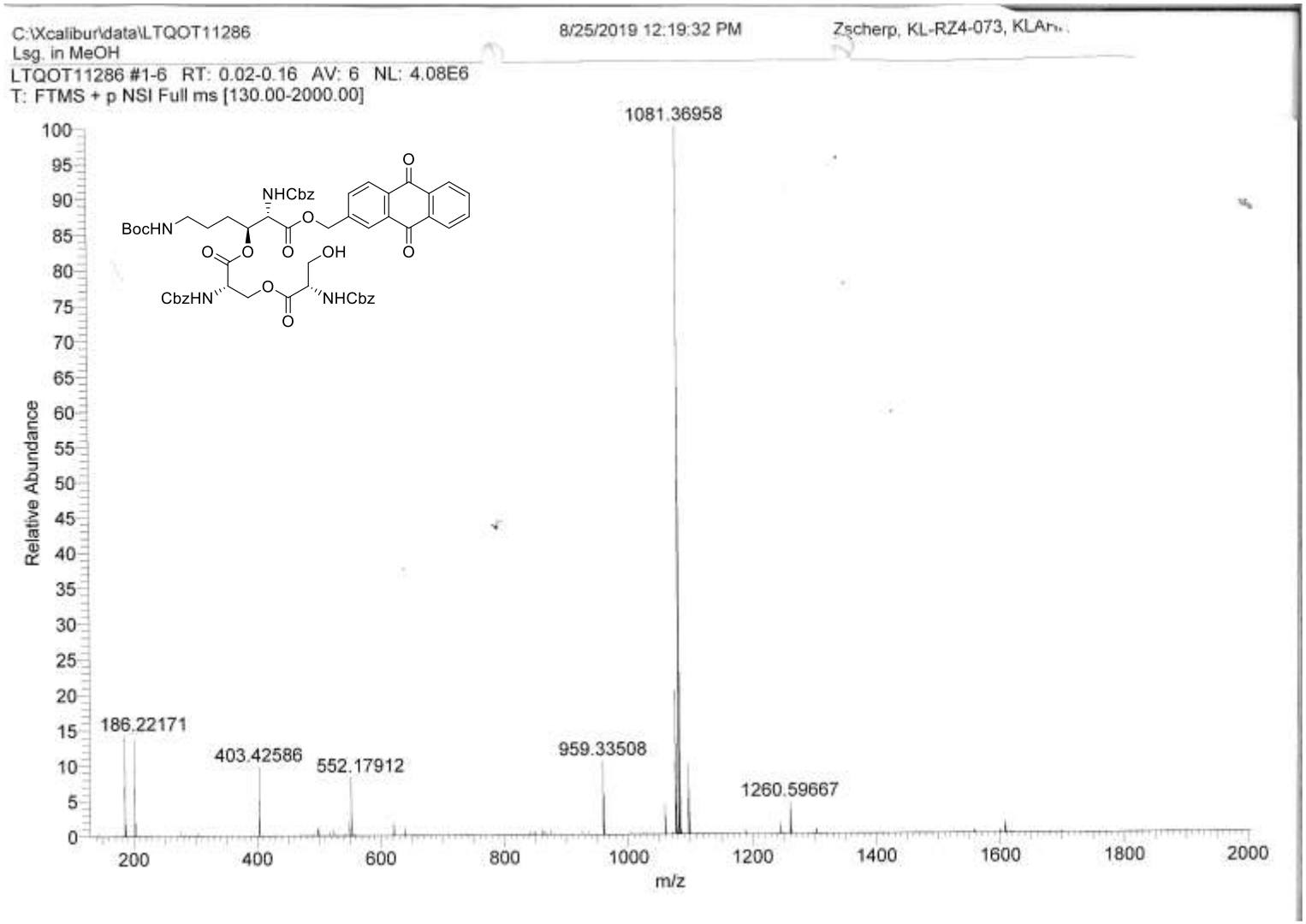
05.01.2020

(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(((benzyl-oxy)-carbonyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)-amino)hexanoate (16) (^{1}H and ^{13}C NMR)

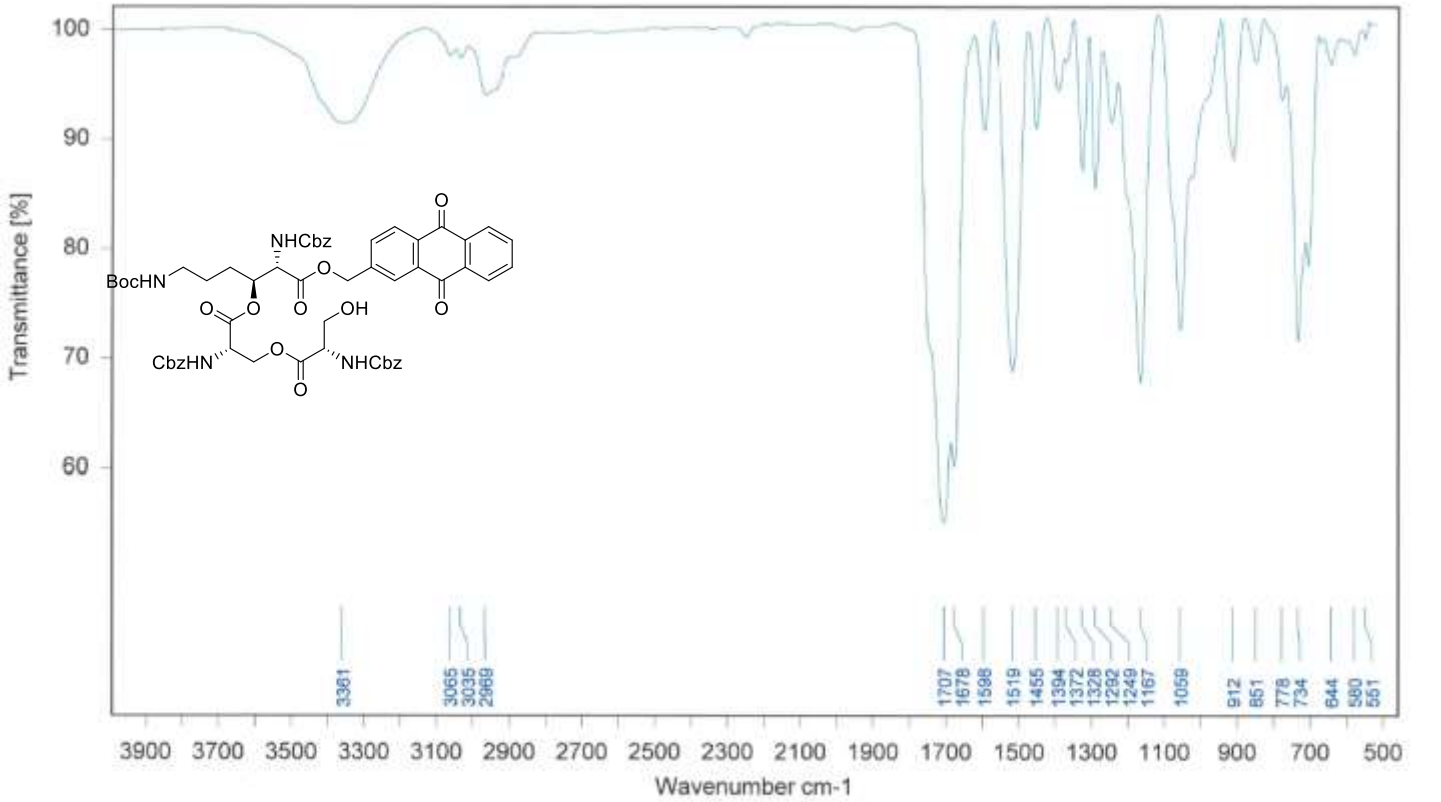




(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(((benzyl-oxy)-carbonyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)-amino)hexanoate (16) (HRMS)



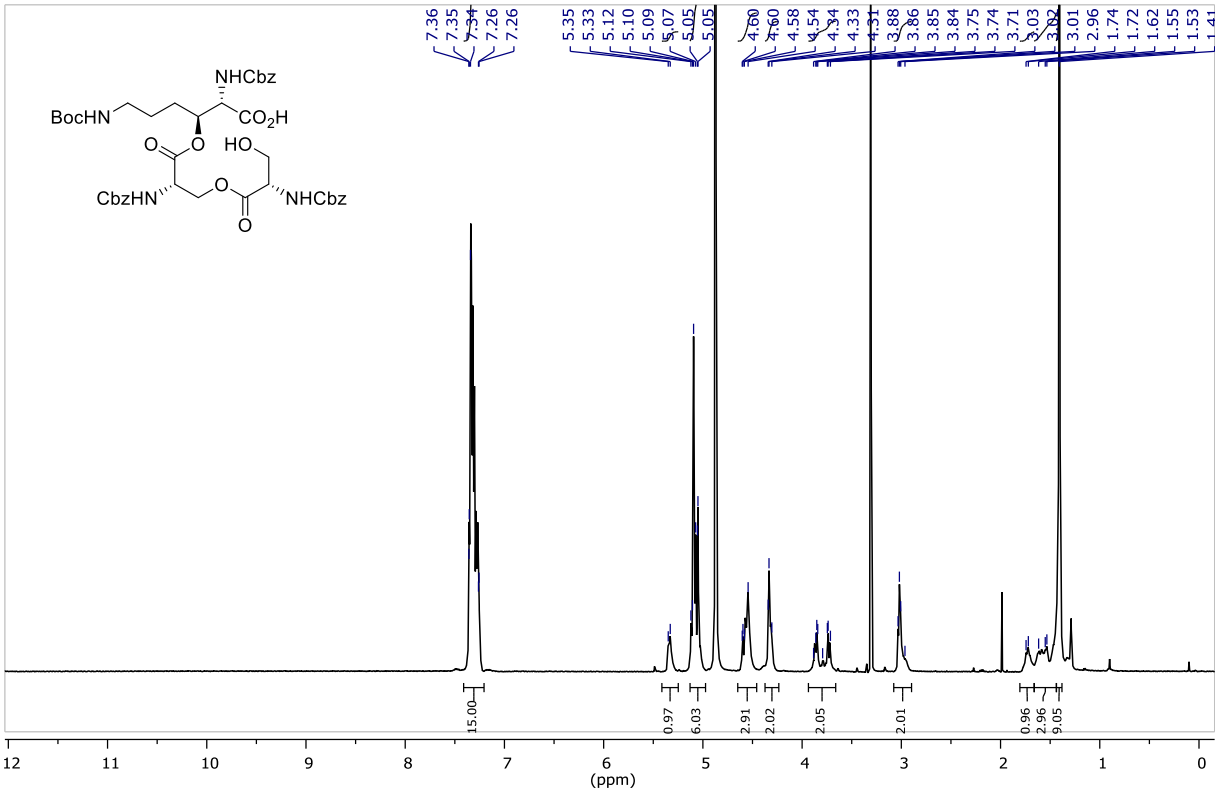
(9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl (2S,3S)-3-((N-((benzyloxy)carbonyl)-O-(((benzyl-oxy)-carbonyl)-L-seryl)-L-seryl)oxy)-2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)-amino)hexanoate (16) (ATR-IR)

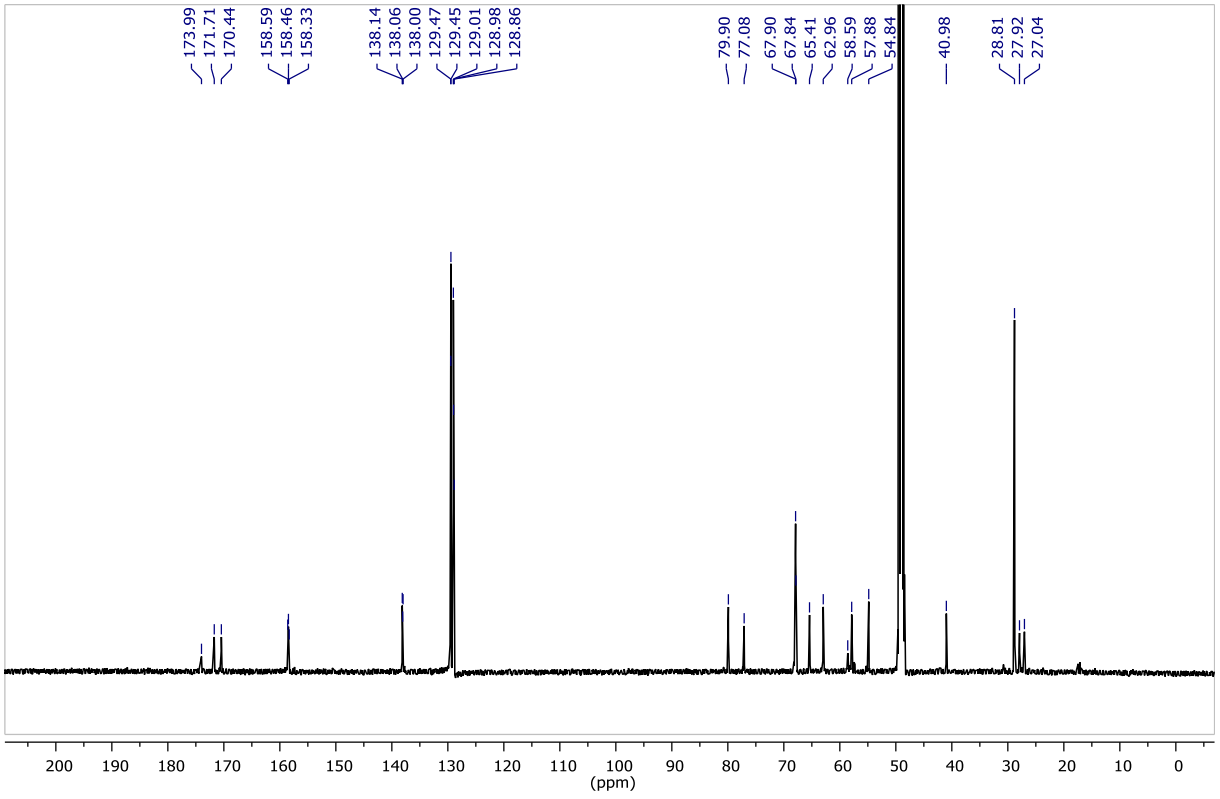


Instrument: Bruker Tensor 27	
Filename: zsr28693.1	Number of Scans: 32
Sample Name: KL-RZ4-073	Operator Name: Default
Technique: Diami. -ATR	ate & Time of Measurement: 20/08/2019 09:39:16

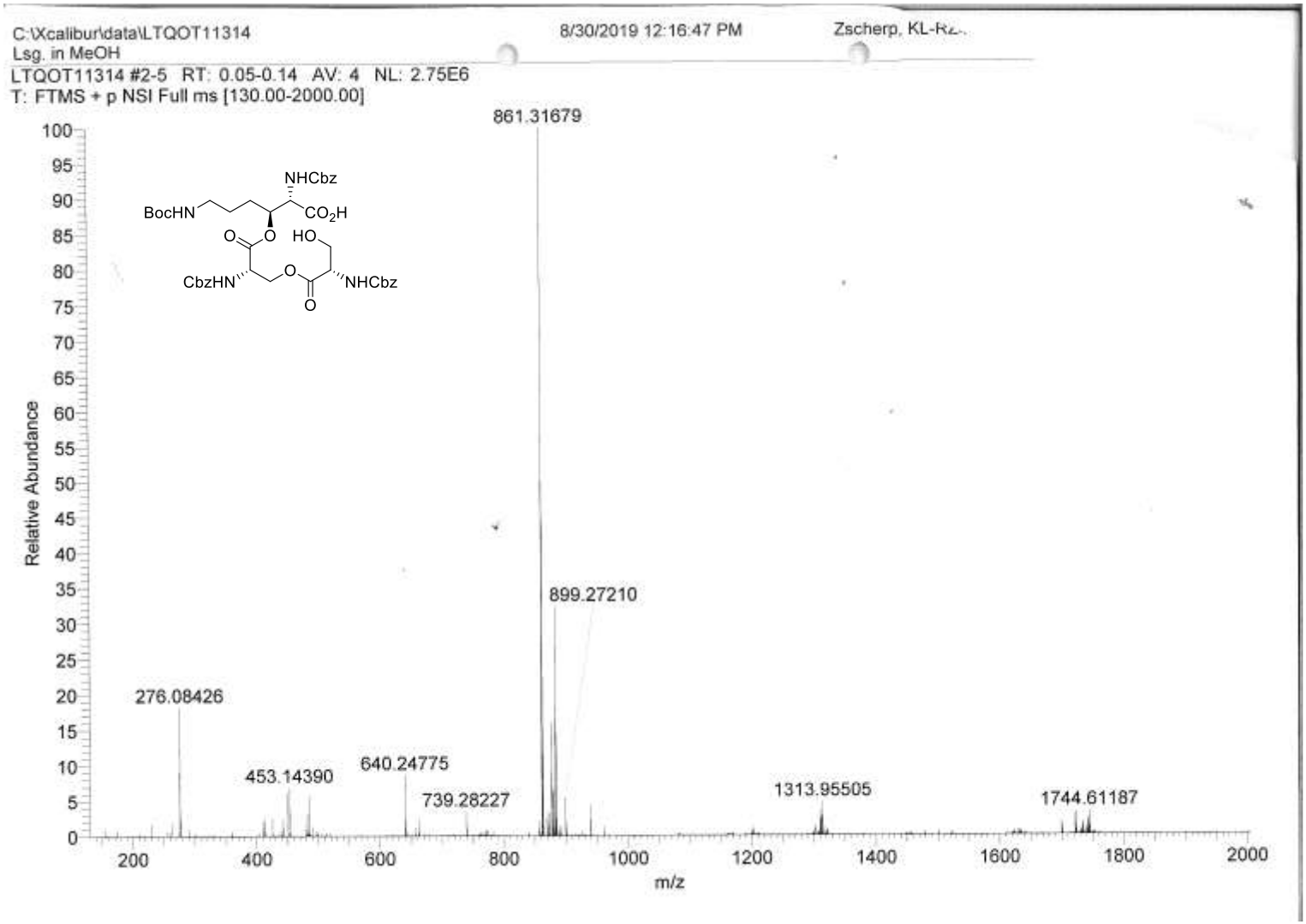
05.01.2020

(5S,9S,12S,13S)-9,13-bis(((Benzyloxy)carbonyl)amino)-12-(3-((tert-butoxycarbonyl)amino)propyl)-5-(hydroxymethyl)-3,6,10-trioxo-1-phenyl-2,7,11-trioxa-4-azatetradecan-14-oic acid (17) (^{1}H and ^{13}C NMR)

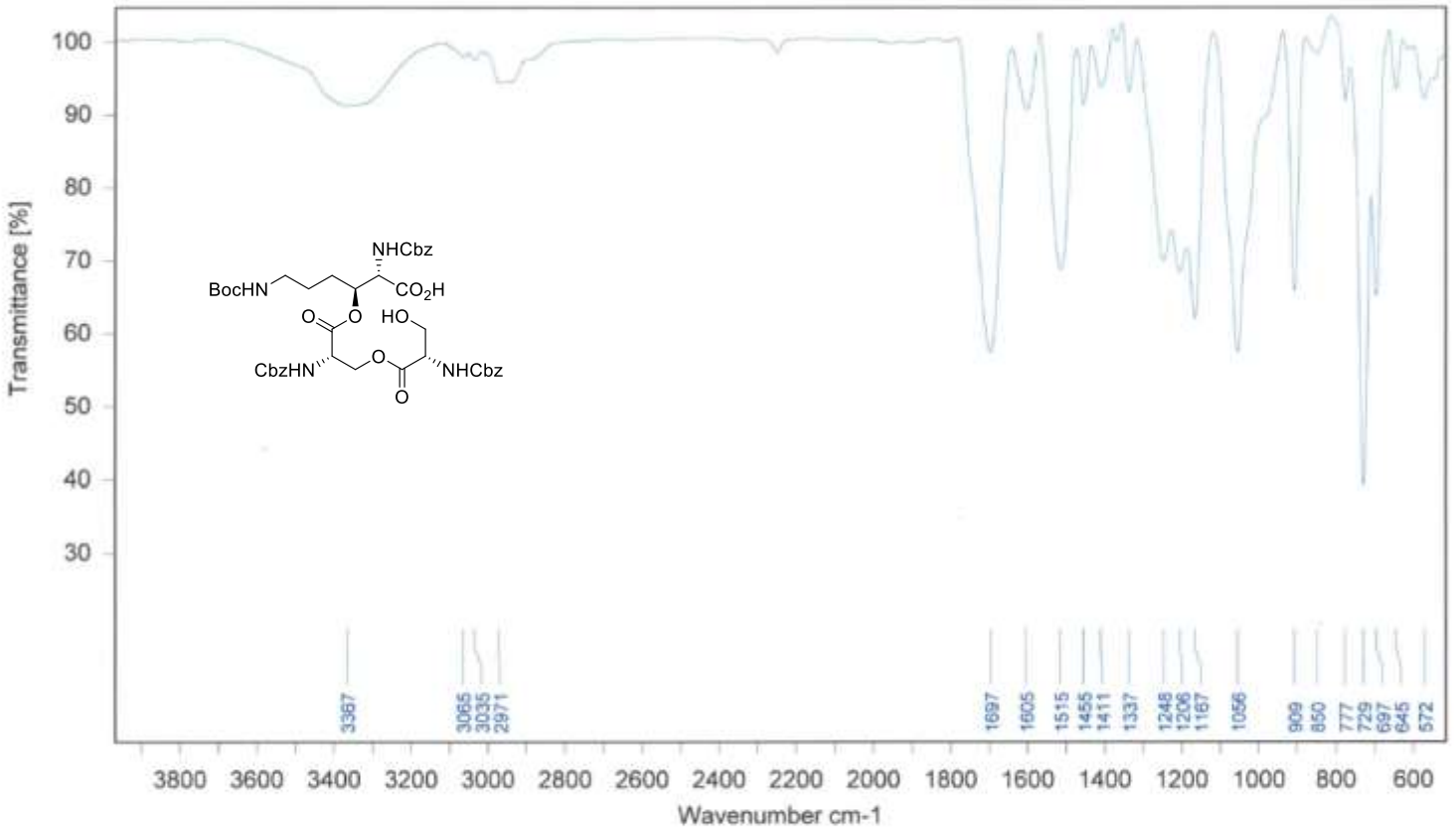




(5S,9S,12S,13S)-9,13-bis(((Benzyloxy)carbonyl)amino)-12-(3-((tert-butoxycarbonyl)amino)propyl)-5-(hydroxymethyl)-3,6,10-trioxo-1-phenyl-2,7,11-trioxa-4-azatetradecan-14-oic acid (17) (HRMS)



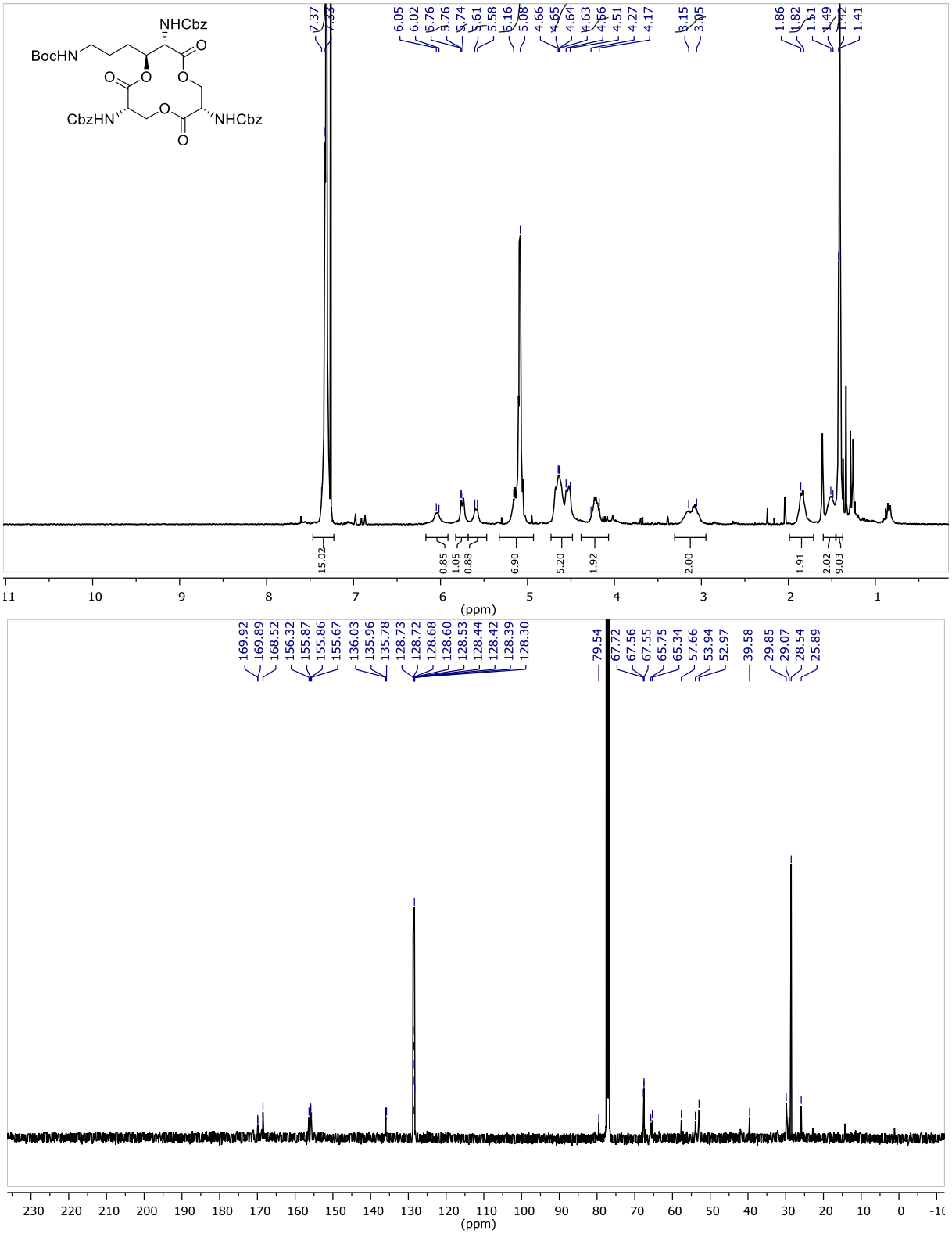
(5S,9S,12S,13S)-9,13-bis(((Benzyloxy)carbonyl)amino)-12-(3-((tert-butoxycarbonyl)amino)propyl)-5-(hydroxymethyl)-3,6,10-trioxo-1-phenyl-2,7,11-trioxa-4-azatetradecan-14-oic acid (17) (ATR-IR)



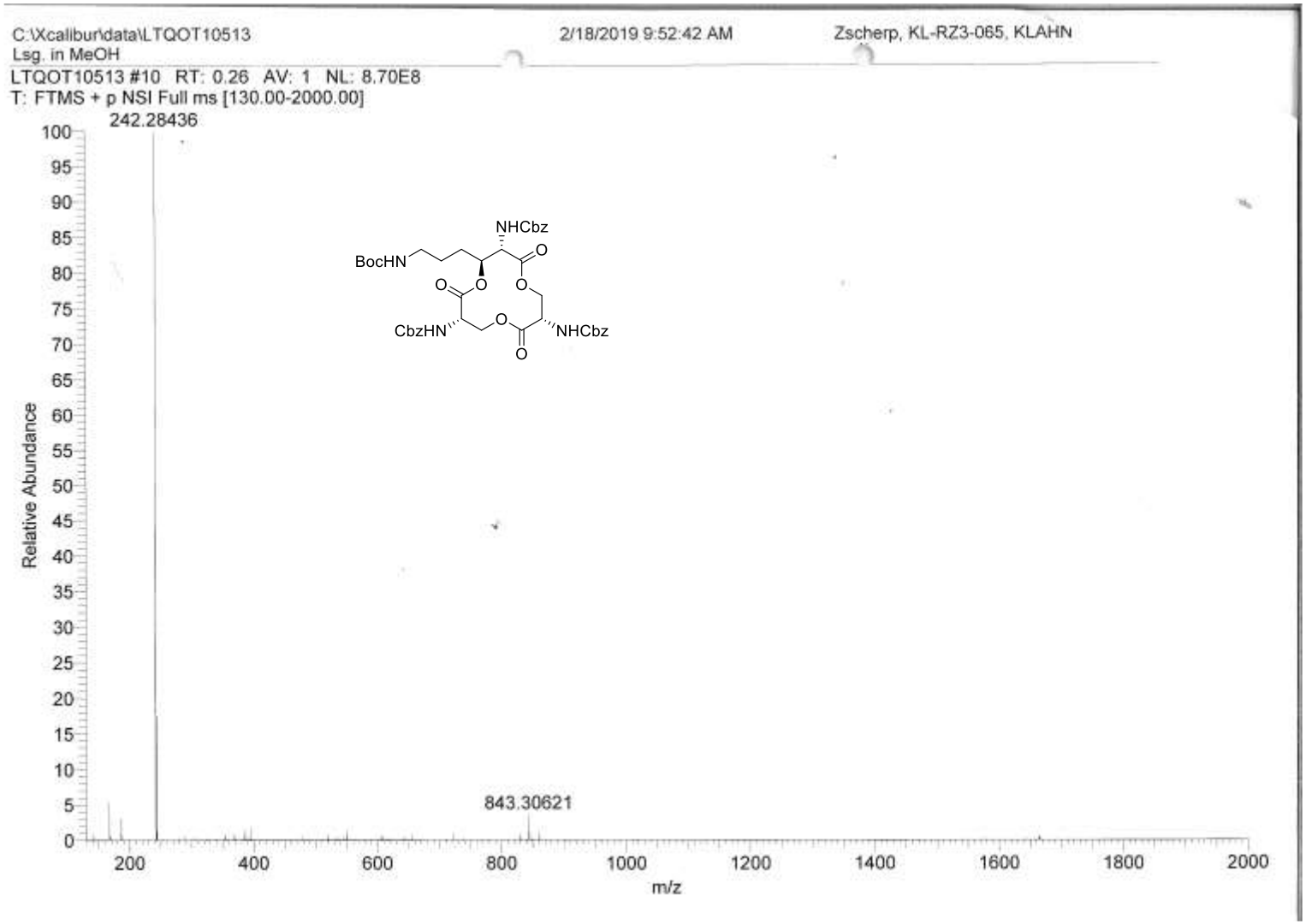
Instrument: Bruker Tensor 27	
Filename: zsr28766.1	Number of Scans: 32
Sample Name: KL-RZ4-064	Operator Name: Default
Technique: Diama... ATR	...te & Time of Measurement: 28/08/2019 13:08:40

27.03.2020

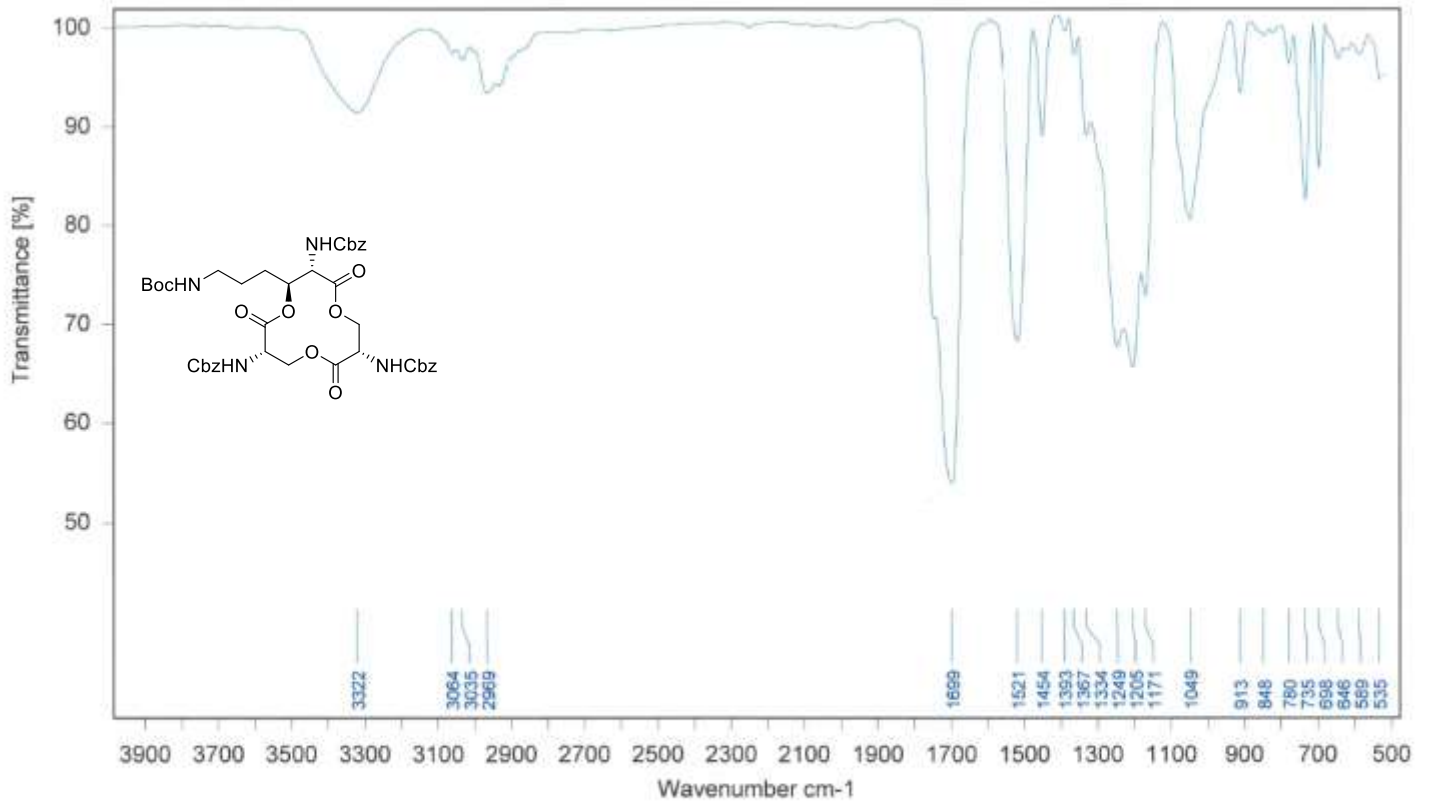
Tribenzyl ((3S,4S,7S,11S)-4-(3-((tert-butoxycarbonyl)amino)propyl)-2,6,10-trioxo-1,5,9-trioxacyclodo-decane-3,7,11-triyl)tricarbamate (18) (^{1}H and ^{13}C NMR)



Tribenzyl ((3S,4S,7S,11S)-4-(3-((tert-butoxycarbonyl)amino)propyl)-2,6,10-trioxo-1,5,9-trioxacyclodo-decane-3,7,11-triyl)tricarbamate (18) (HRMS)



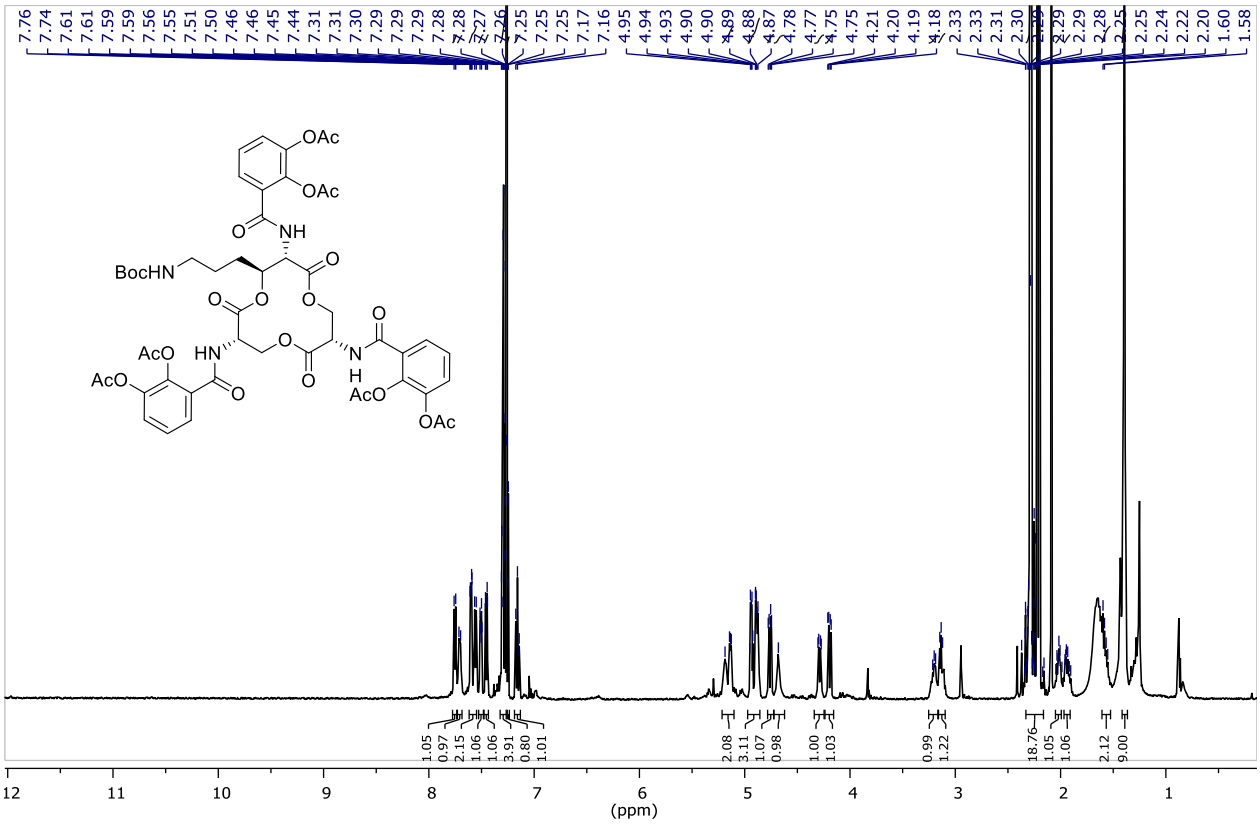
Tribenzyl ((3S,4S,7S,11S)-4-(3-((tert-butoxycarbonyl)amino)propyl)-2,6,10-trioxo-1,5,9-trioxacyclodo-decane-3,7,11-triyl)tricarbamate (18) (ATR-IR)

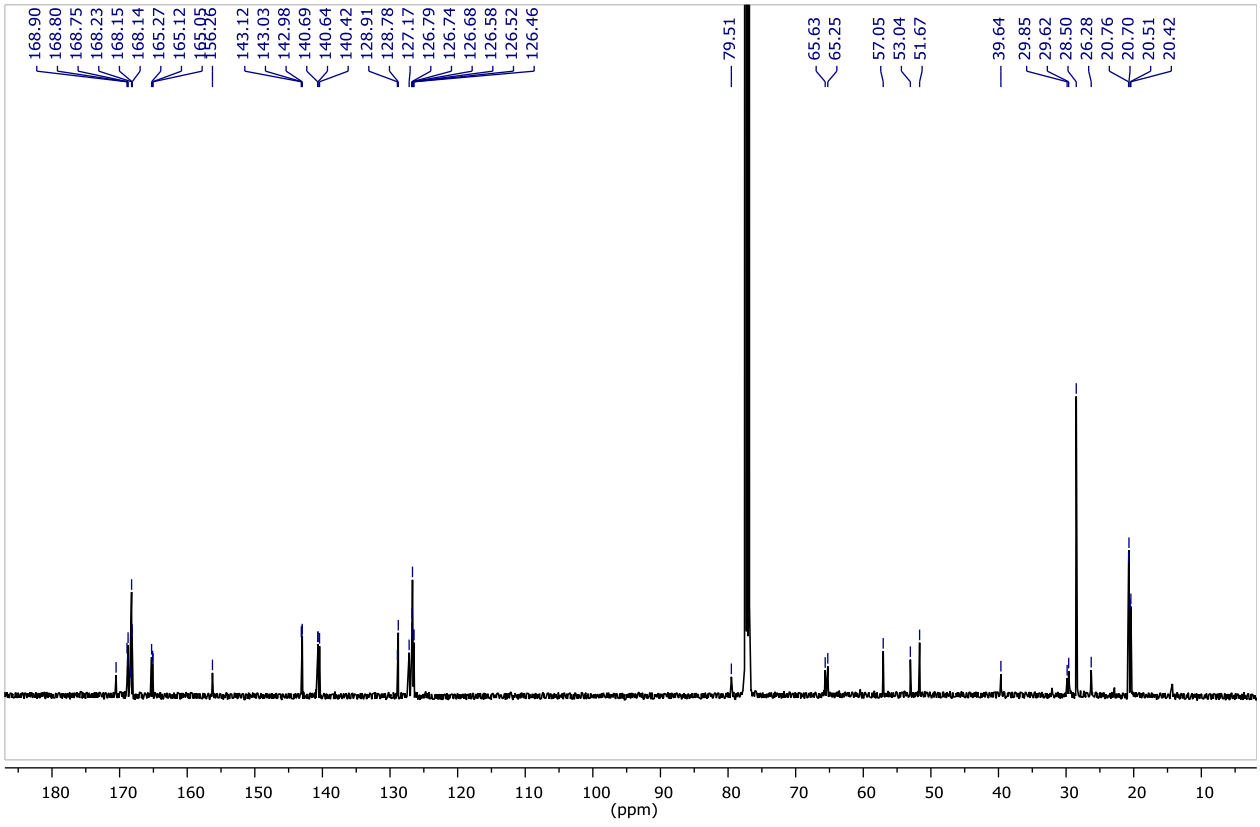


Instrument: Bruker Tensor 27	
Filename: zsr28954.1	Number of Scans: 32
Sample Name: KL-RZ4-141	Operator Name: Default
Technique: Diama...-ATR	...ate & Time of Measurement: 23/10/2019 07:11:35

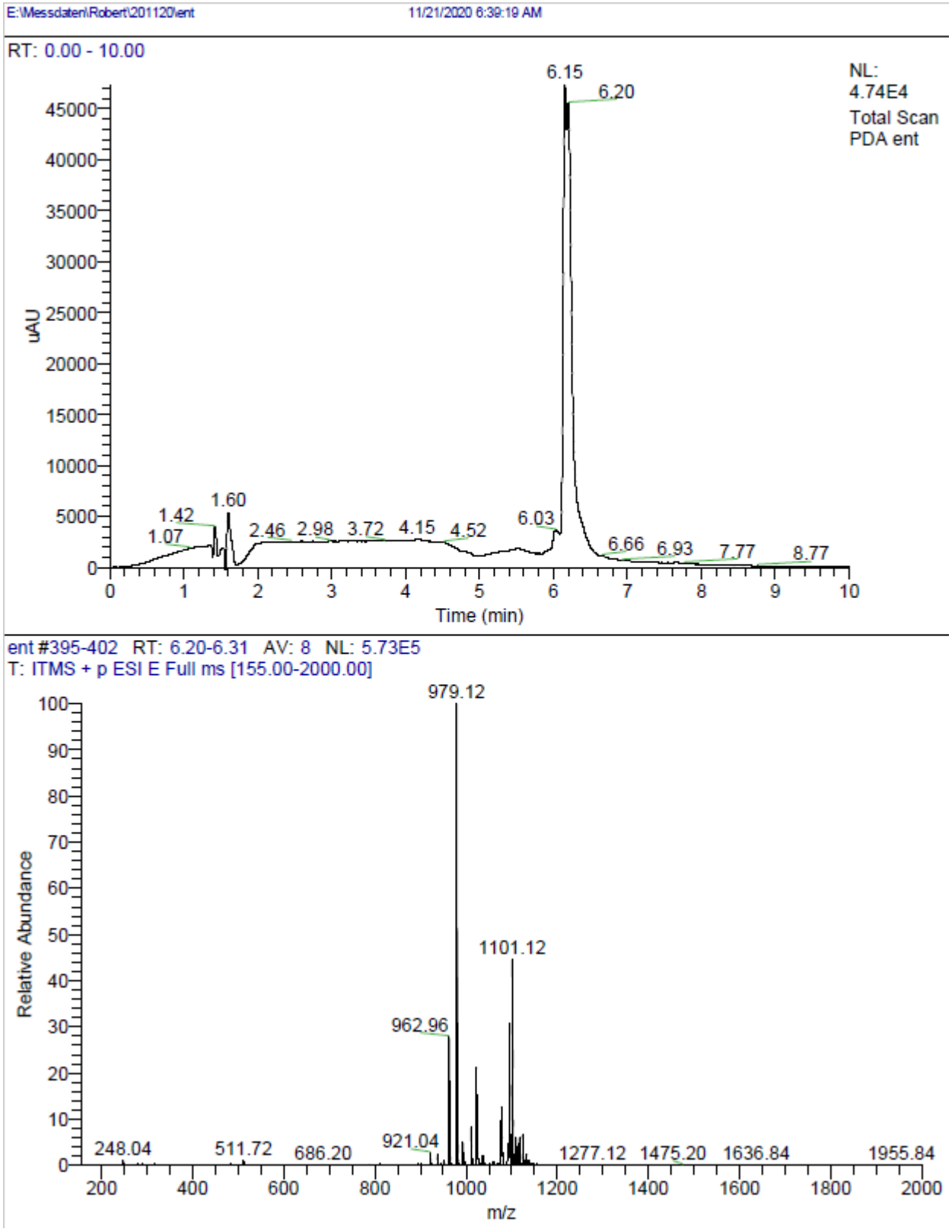
05.01.2020

(AcO)Ent$_{KL}$ (^{1}H and ^{13}C NMR)

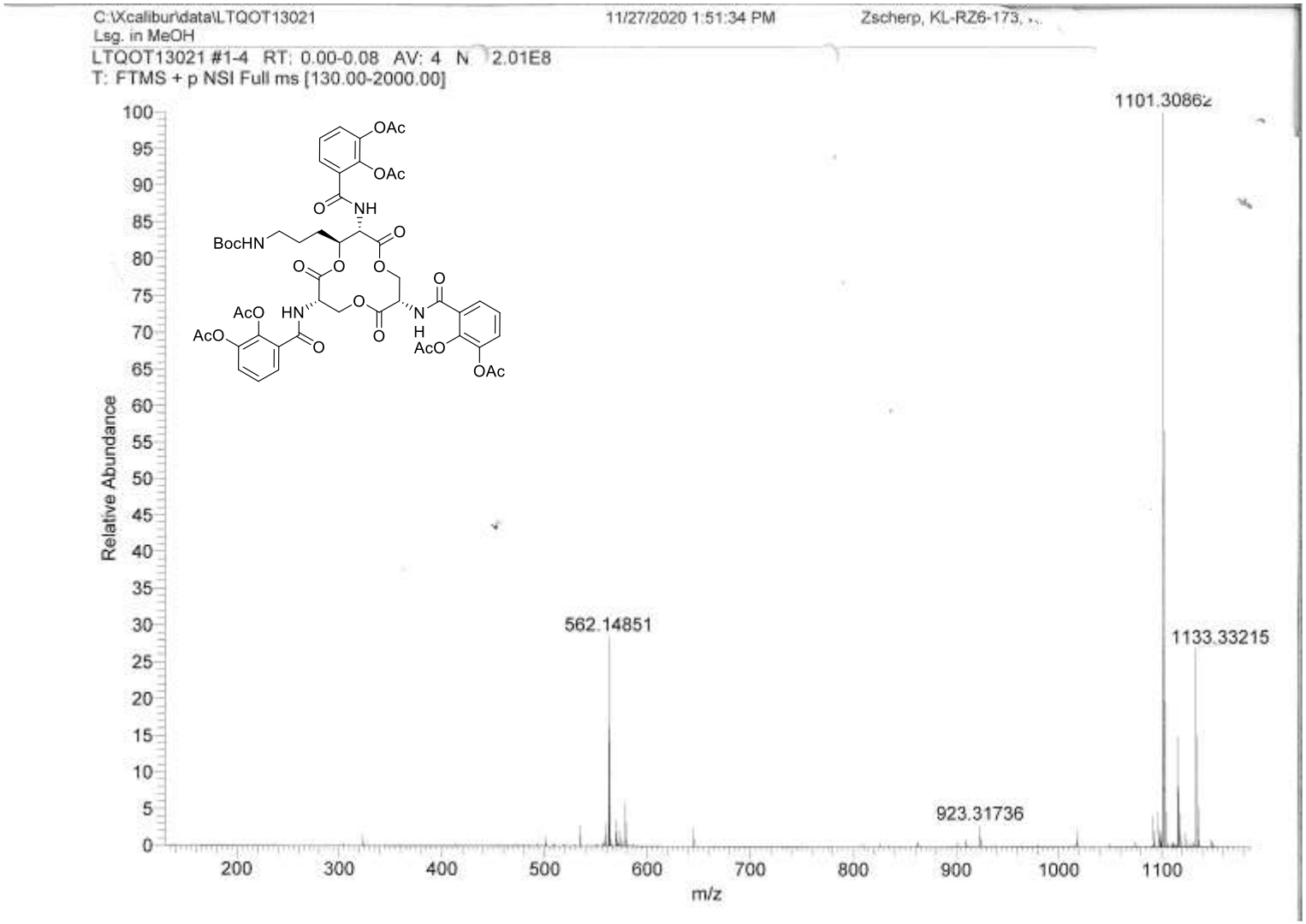




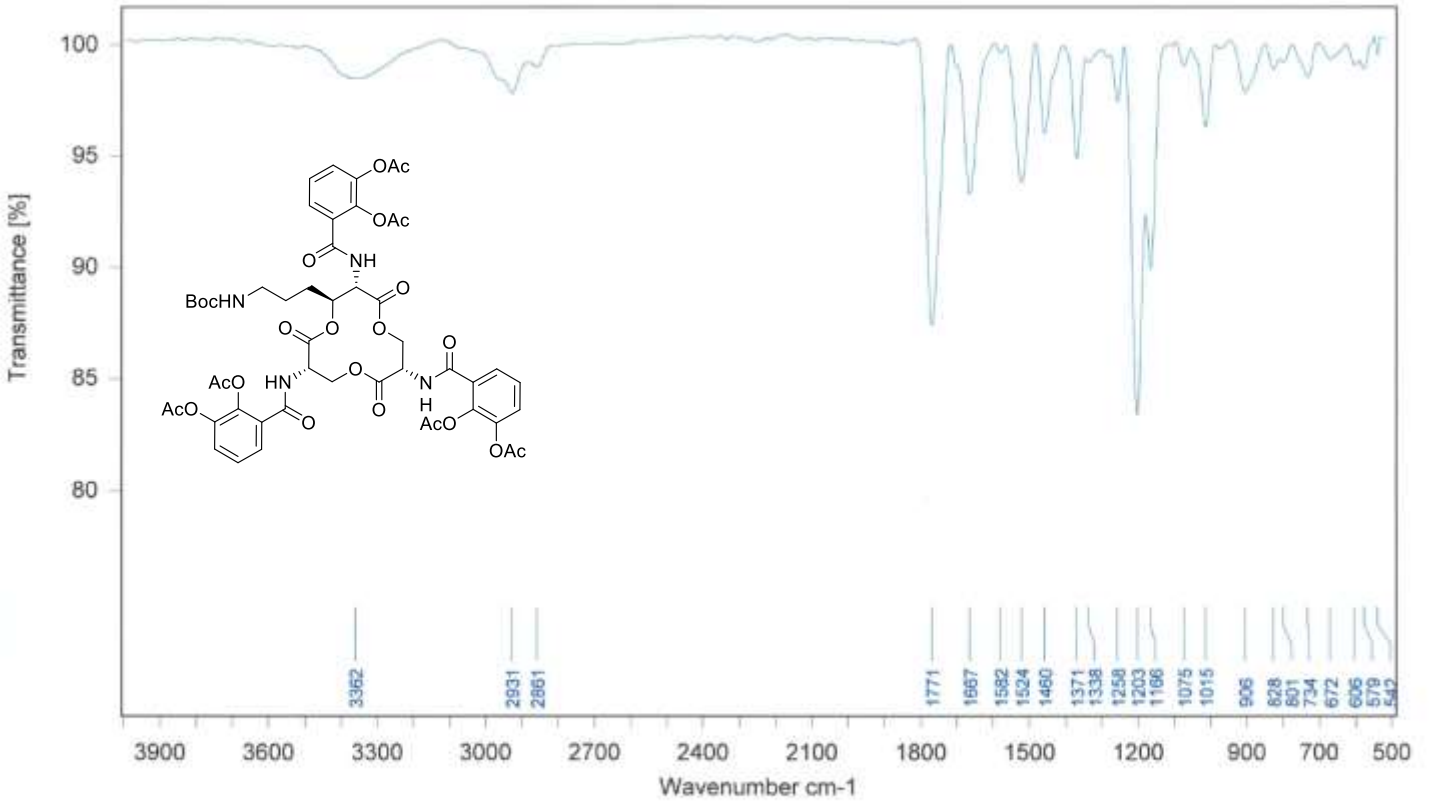
(AcO)Ent$_{KL}$ (HPLC-LRMS)



(AcO)Ent$_{KL}$ (HRMS)



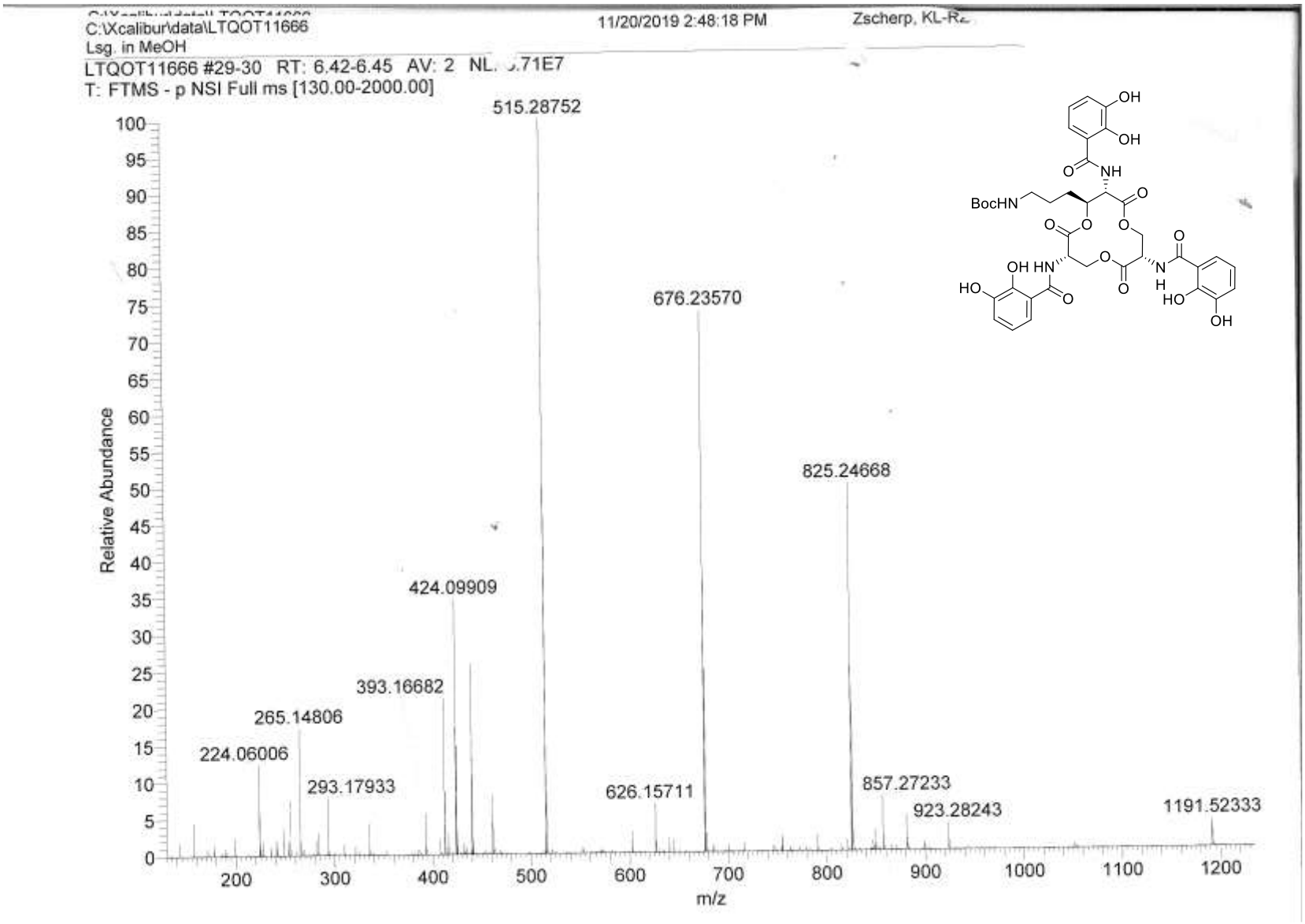
(AcO)Ent$_{KL}$ (ATR-IR)



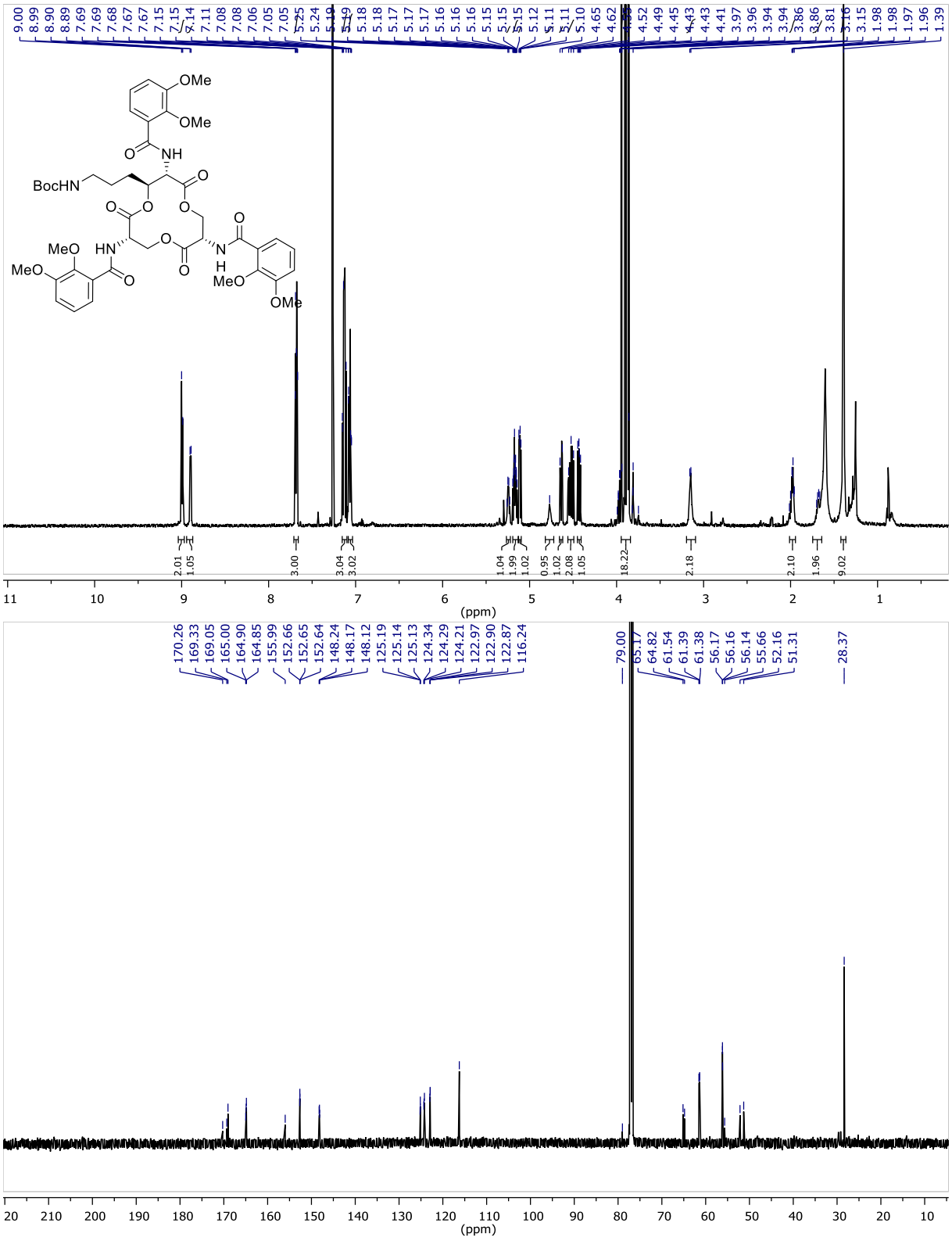
Instrument: Bruker Tensor 27	
Filename: zsr28961.1	Number of Scans: 32
Sample Name: KL-RZ4-148	Operator Name: Default
Technique: Diam. ..-ATR	..te & Time of Measurement: 28/10/2019 13:14:44

05.01.2020

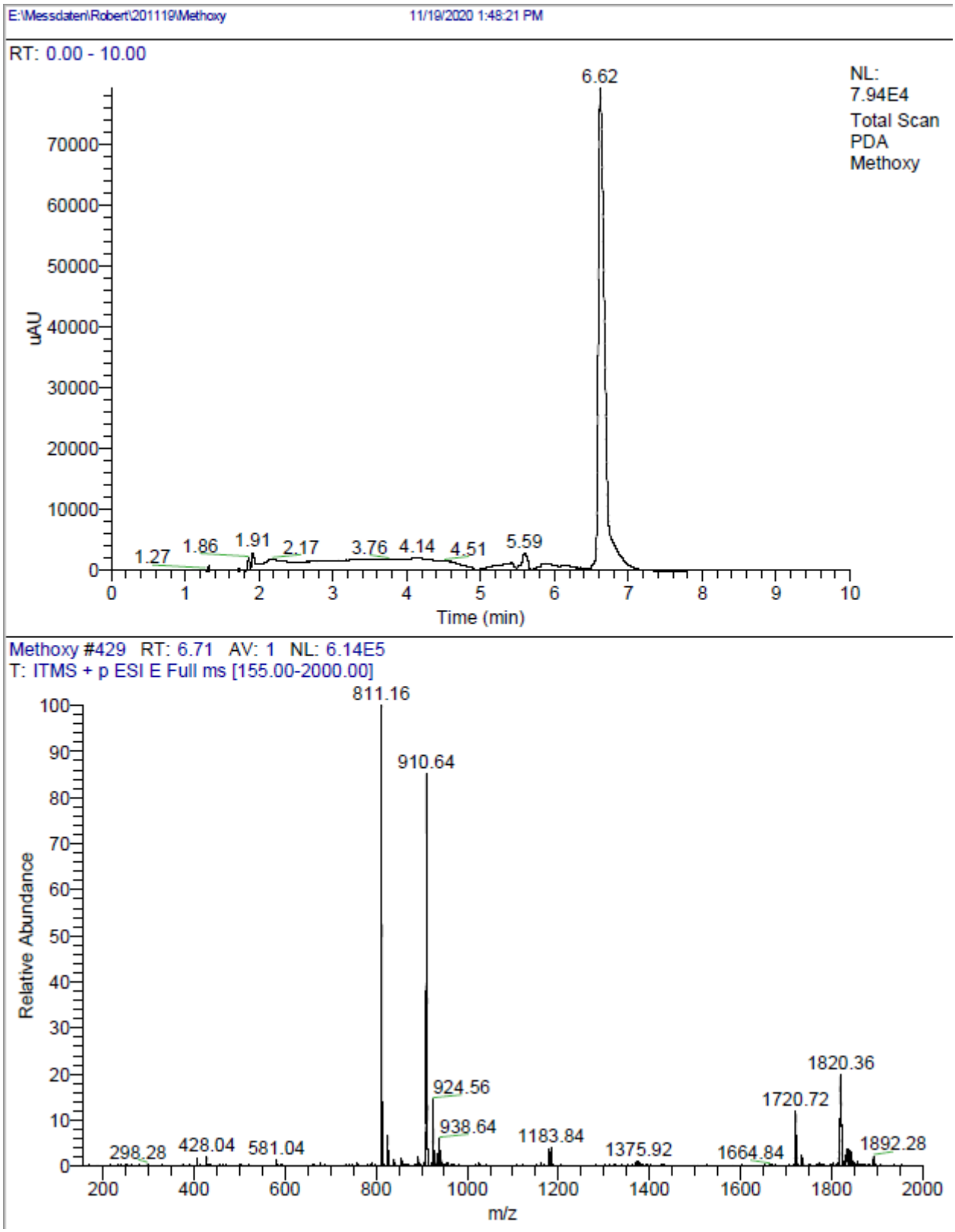
Ent_{KL} (HRMS)


C:\Xcalibur\data\LTQOT11666
11/20/2019 2:48:18 PM
Lsg. in MeOH
LTQOT11666 #29-30 RT: 6.42-6.45 AV: 2
T: FTMS - p NSI Full ms [130.00-2000.00]
515.28752
676.23570
825.24668
424.09909
393.16682
265.14806
224.06006
293.17933
626.15711
857.27233
923.28243
1191.52333
Relative Abundance
m/z
BocHN
OH
NH
HN
HO
O

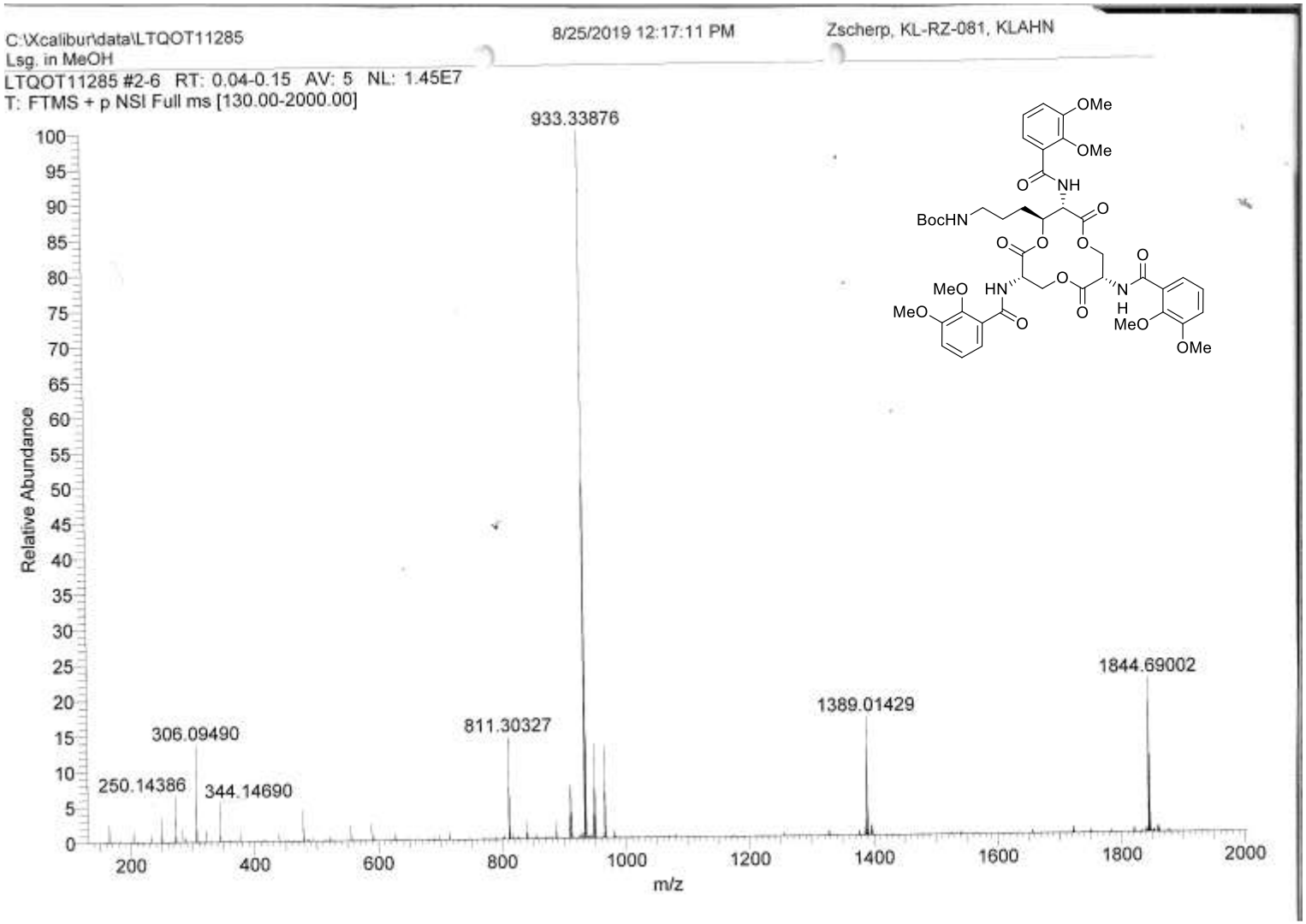
(MeO)Ent$_{KL}$ (^{1}H and ^{13}C NMR)



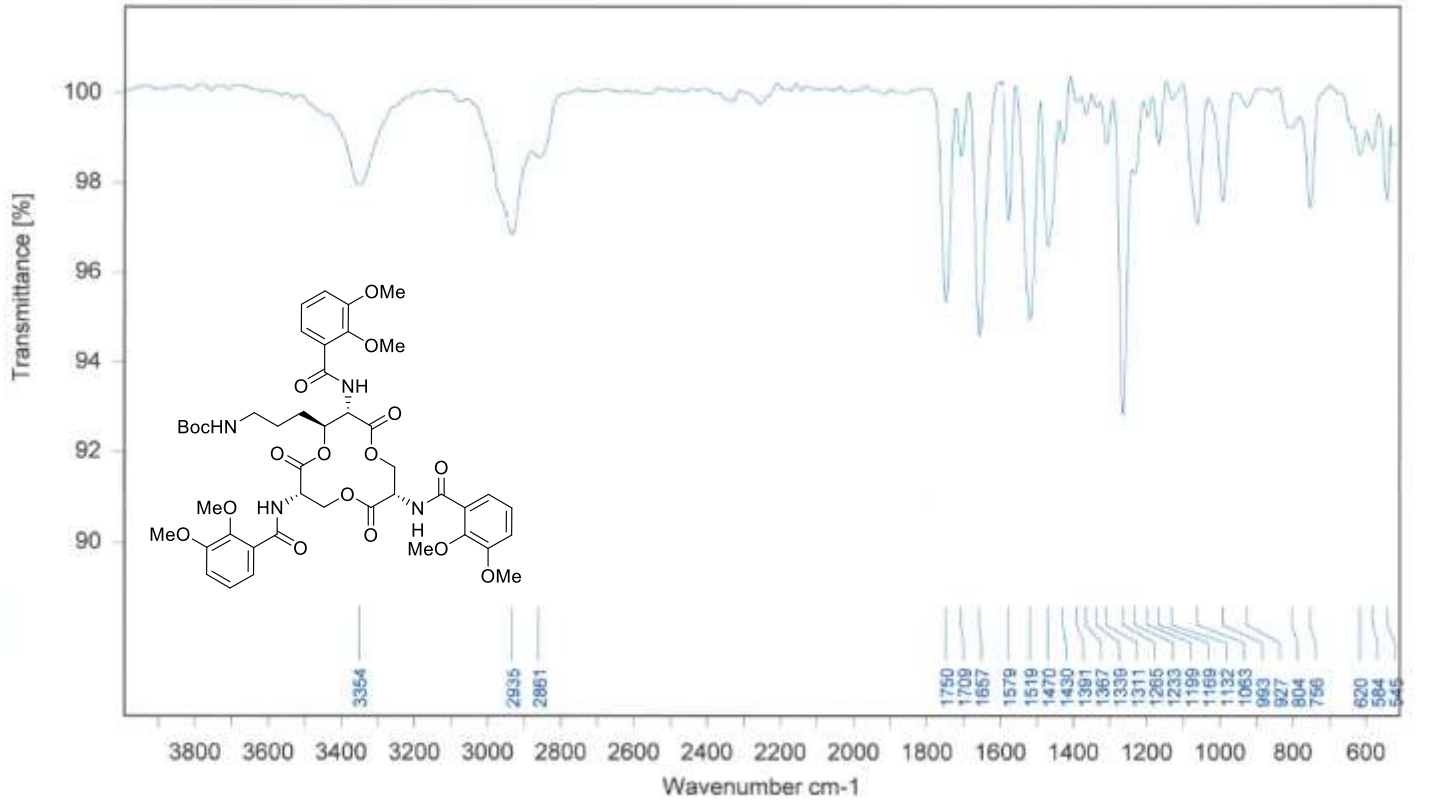
(MeO)Ent$_{KL}$ (HPLC-LRMS)



(MeO)Ent$_{KL}$ (HRMS)



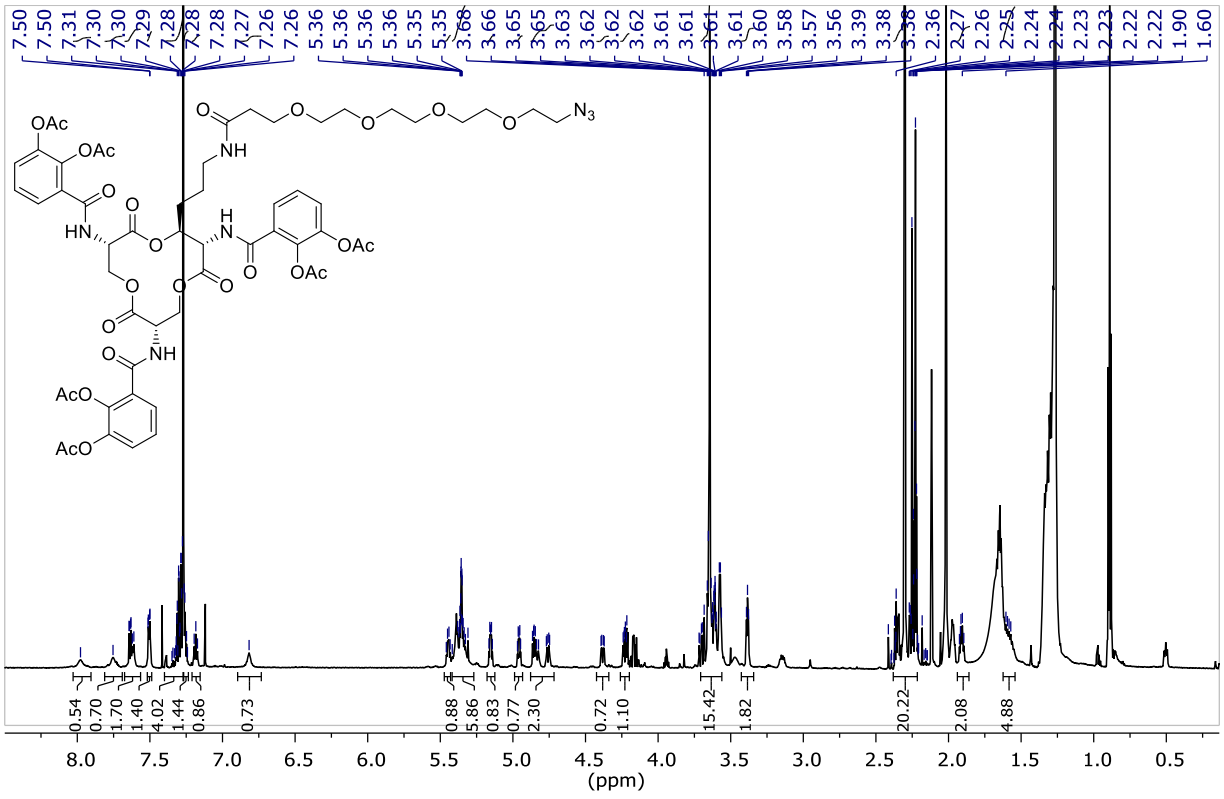
(MeO)Ent$_{KL}$ (ATR-IR)

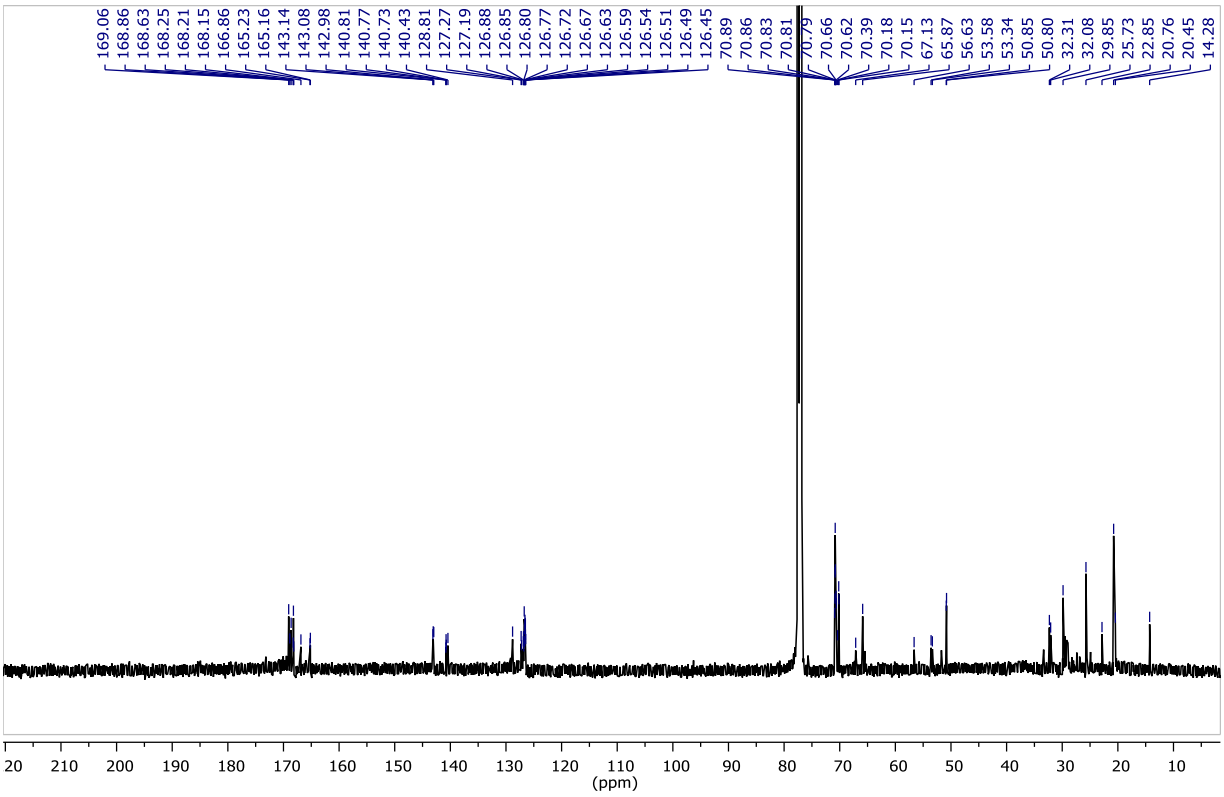


Instrument: Bruker Tensor 27	
Filename: zsr29147.1	Number of Scans: 32
Sample Name: KL-RZ5-013	Operator Name: Default
Technique: Diam. -ATR	ate & Time of Measurement: 17/12/2019 07:42:45

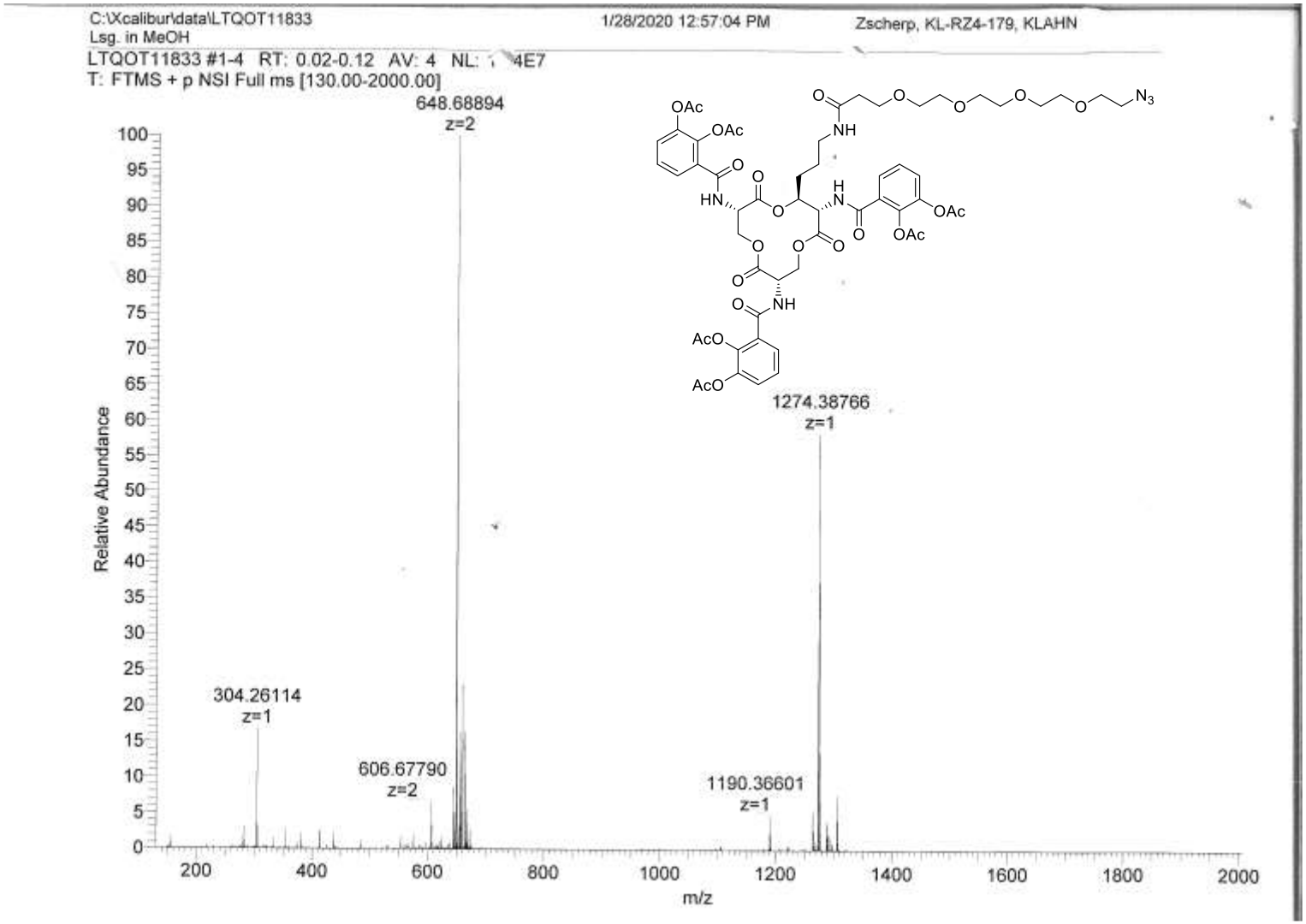
05.01.2020

(AcO)Ent$_{KL}$-PEG$_4$-N$_3$ (^{1}H and ^{13}C NMR)

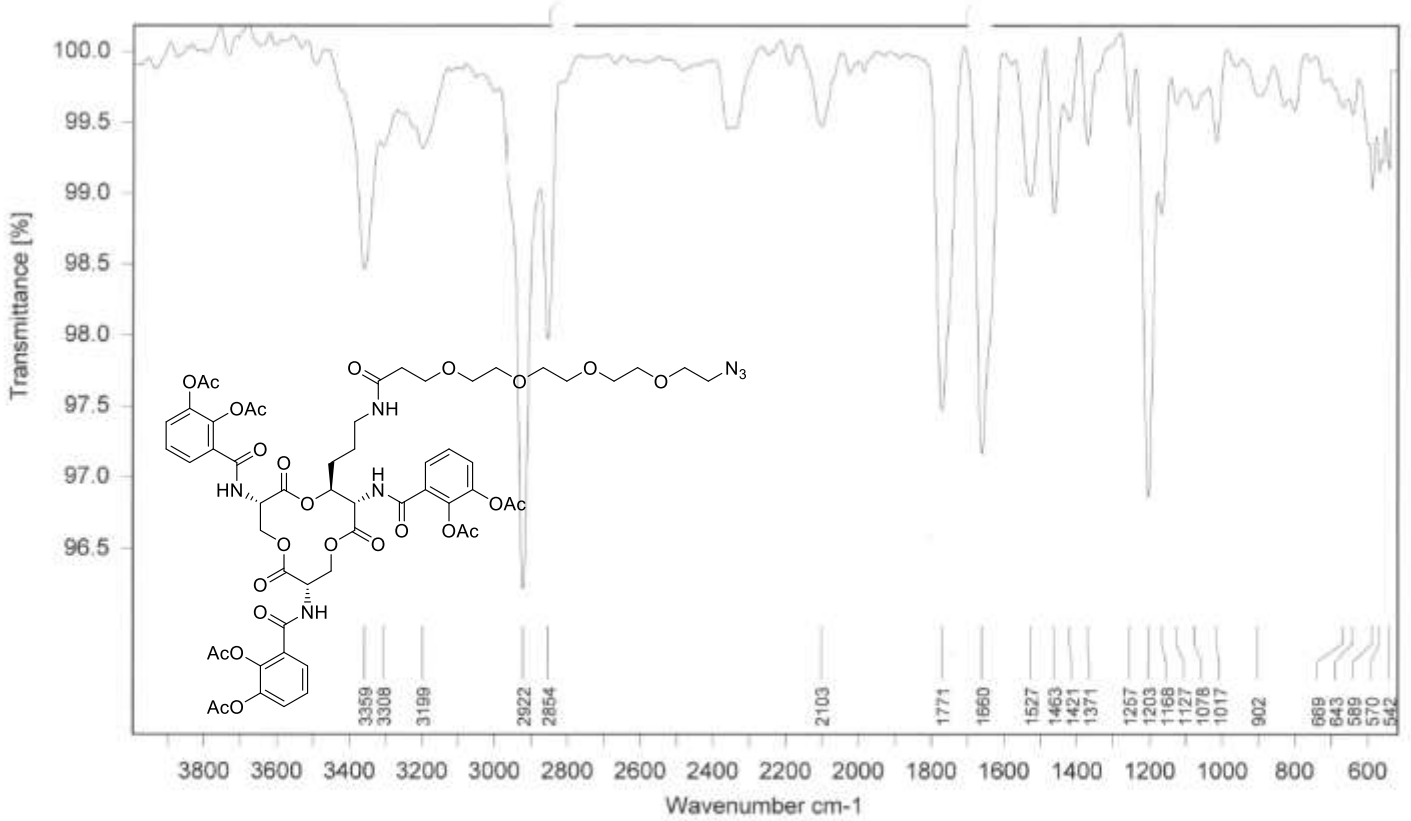




(AcO)Ent$_{KL}$-PEG$_4$-N$_3$ (HRMS)



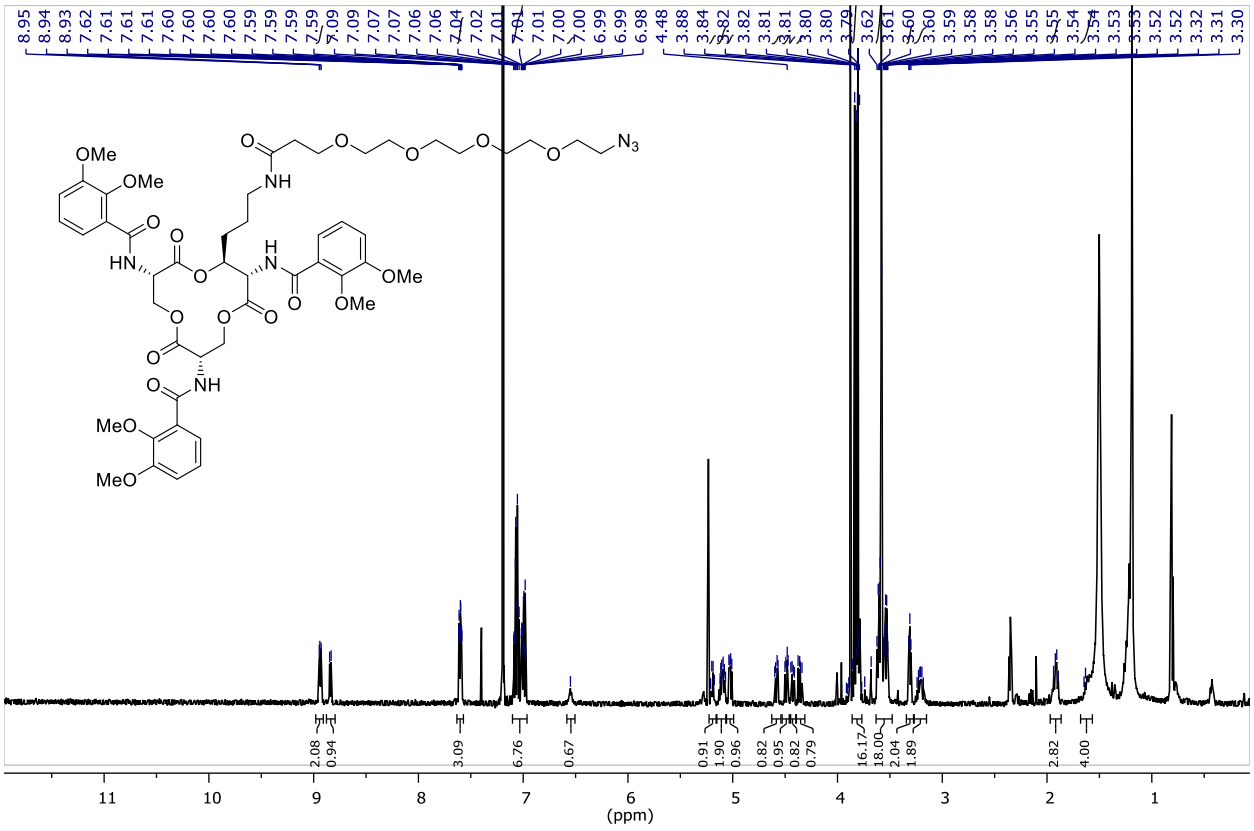
(AcO)Ent$_{KL}$-PEG$_4$-N$_3$ (ATR-IR)

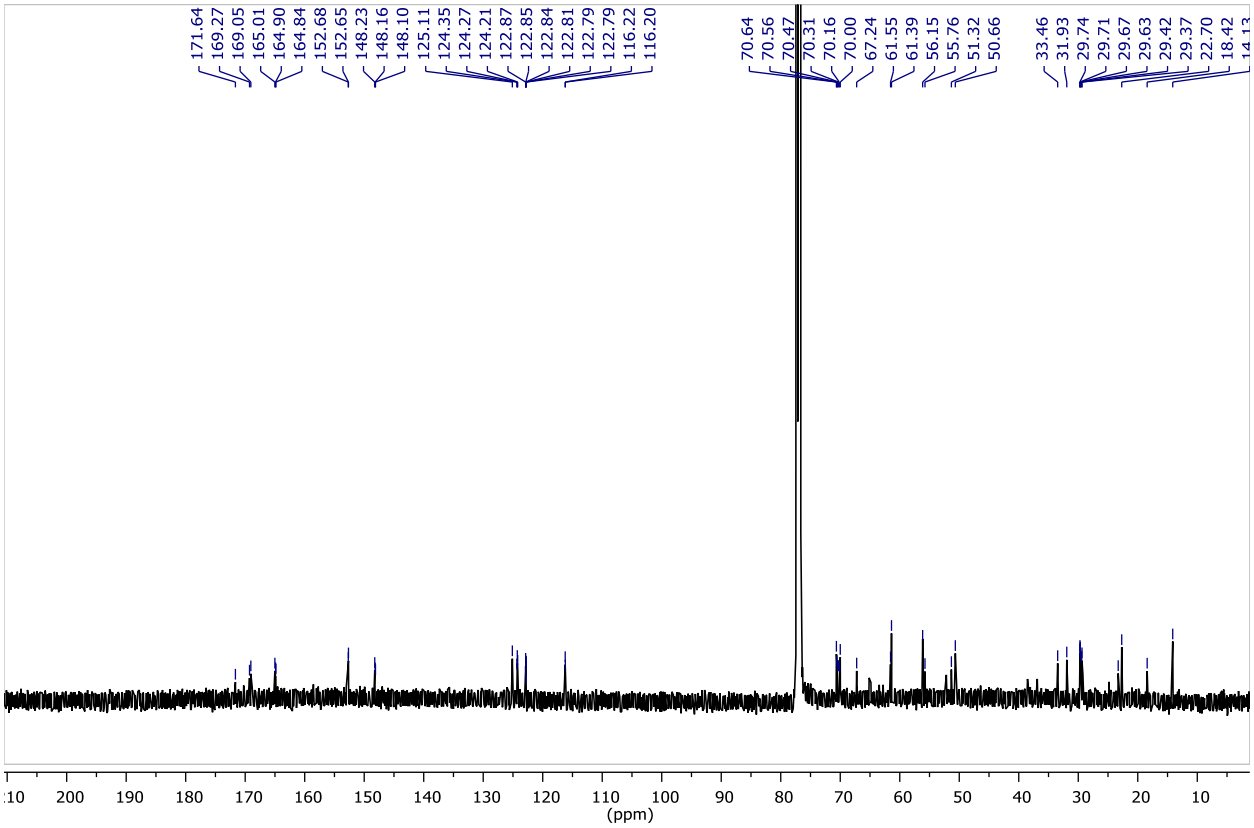


Instrument: Bruker Tensor 27	
Filename: zsr29213.1	Number of Scans: 32
Sample Name: KL-RZ4-179	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 27.01.2020 10:30:49

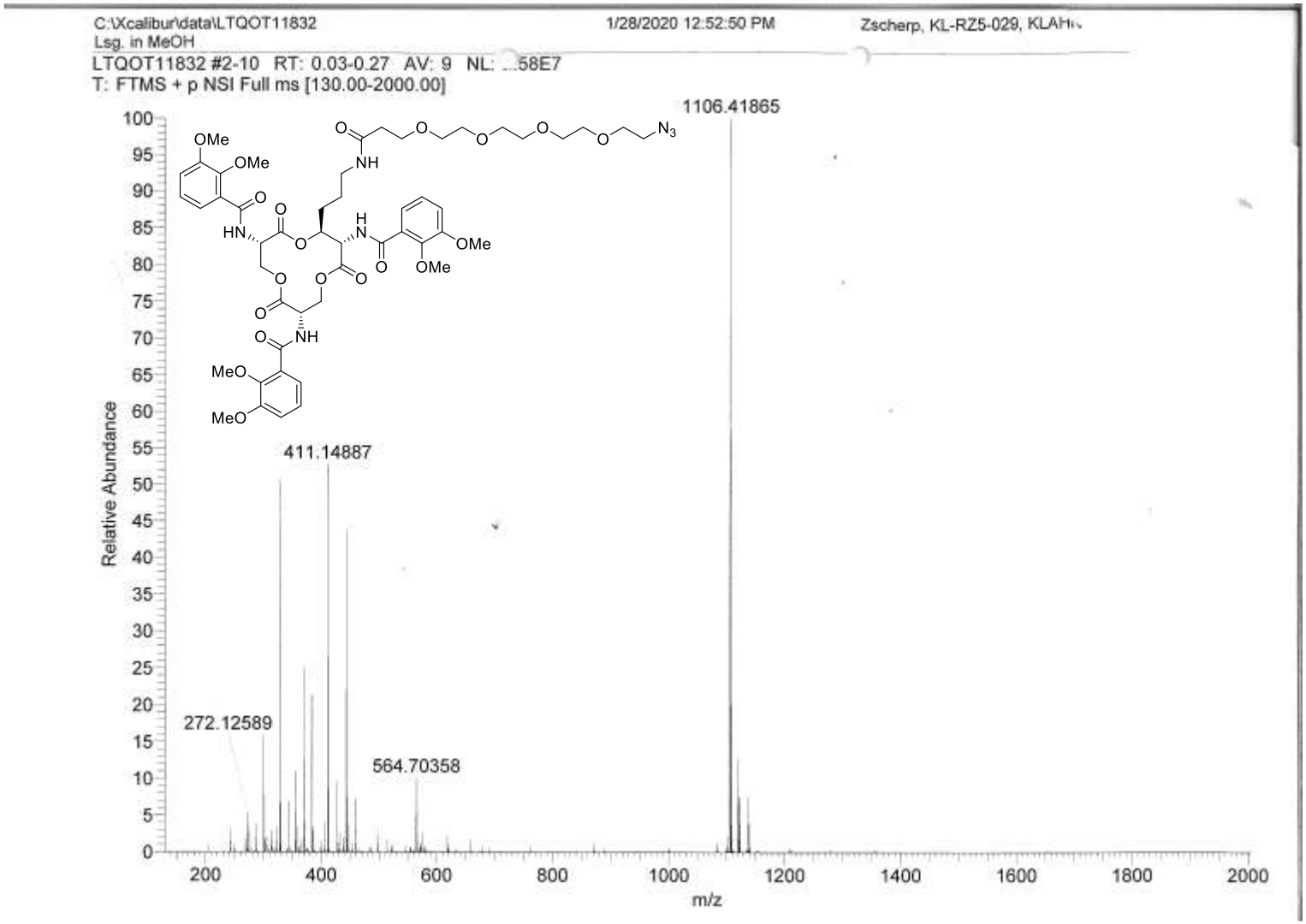
27.01.2020

(MeO)Ent$_{KL}$-PEG$_4$-N$_3$ (^{1}H and ^{13}C NMR)

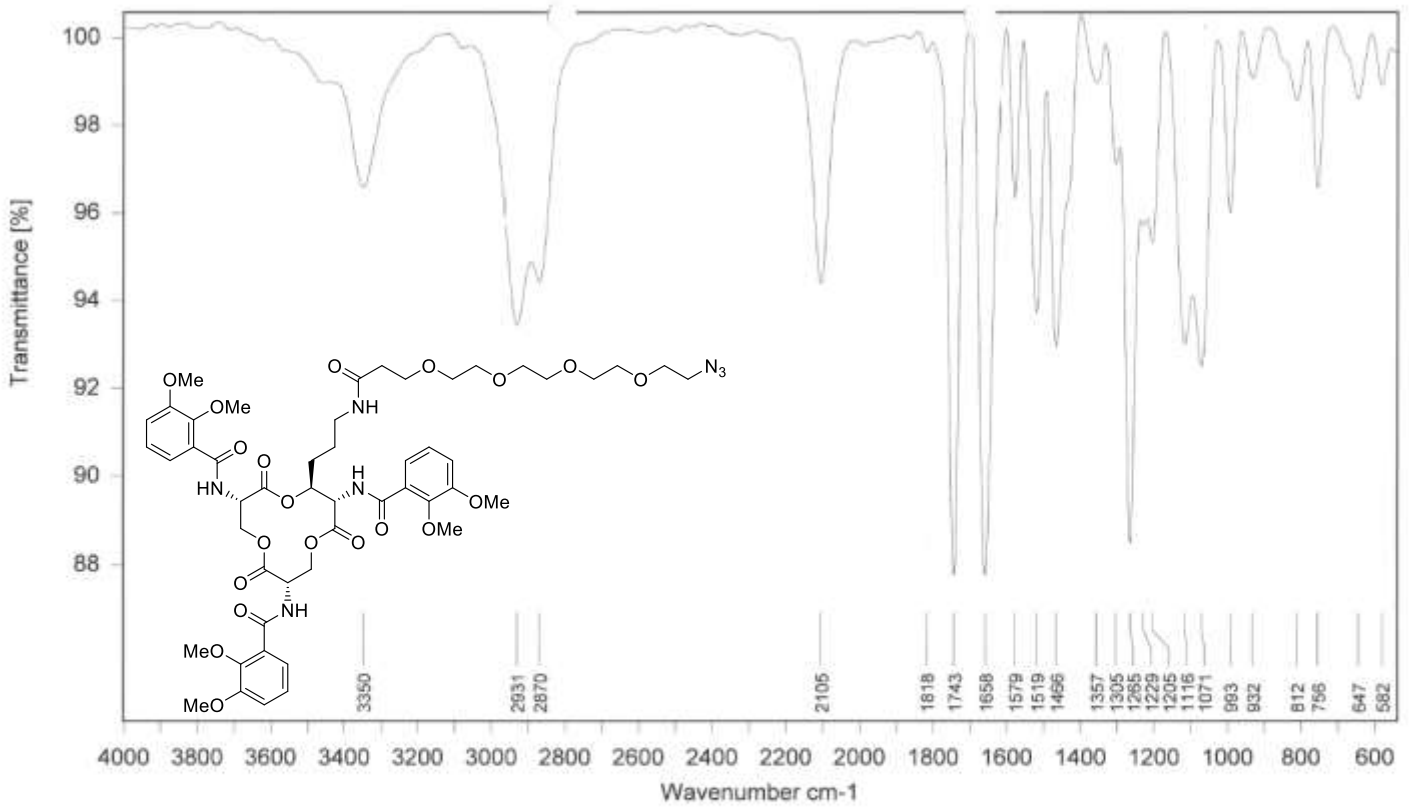




(MeO)Ent$_{KL}$-PEG$_4$-N$_3$ (HRMS)



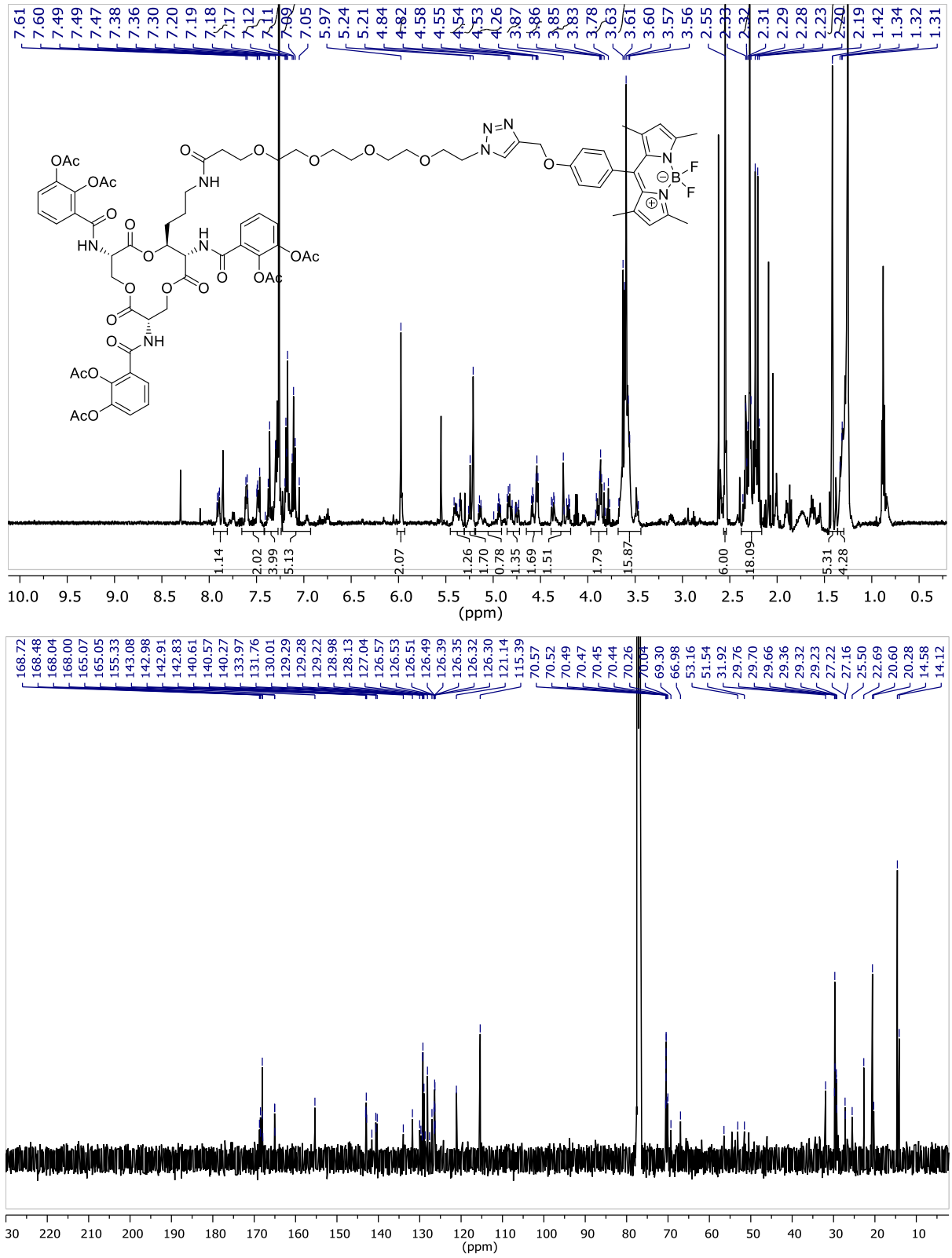
(MeO)Ent$_{KL}$-PEG$_4$-N$_3$ (ATR-IR)



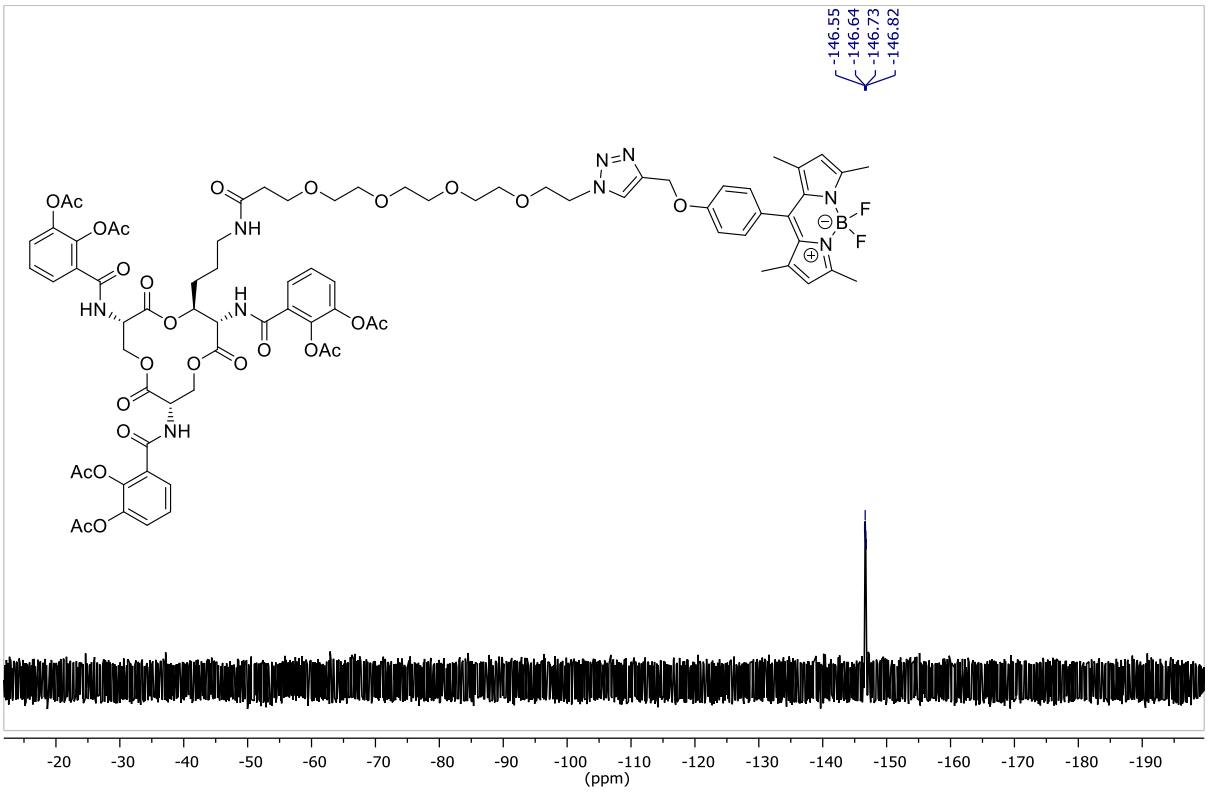
Instrument: Bruker Tensor 27	
Filename: zsr292112.0	Number of Scans: 32
Sample Name: KL-RZ5-029	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 27.01.2020 10:18:40

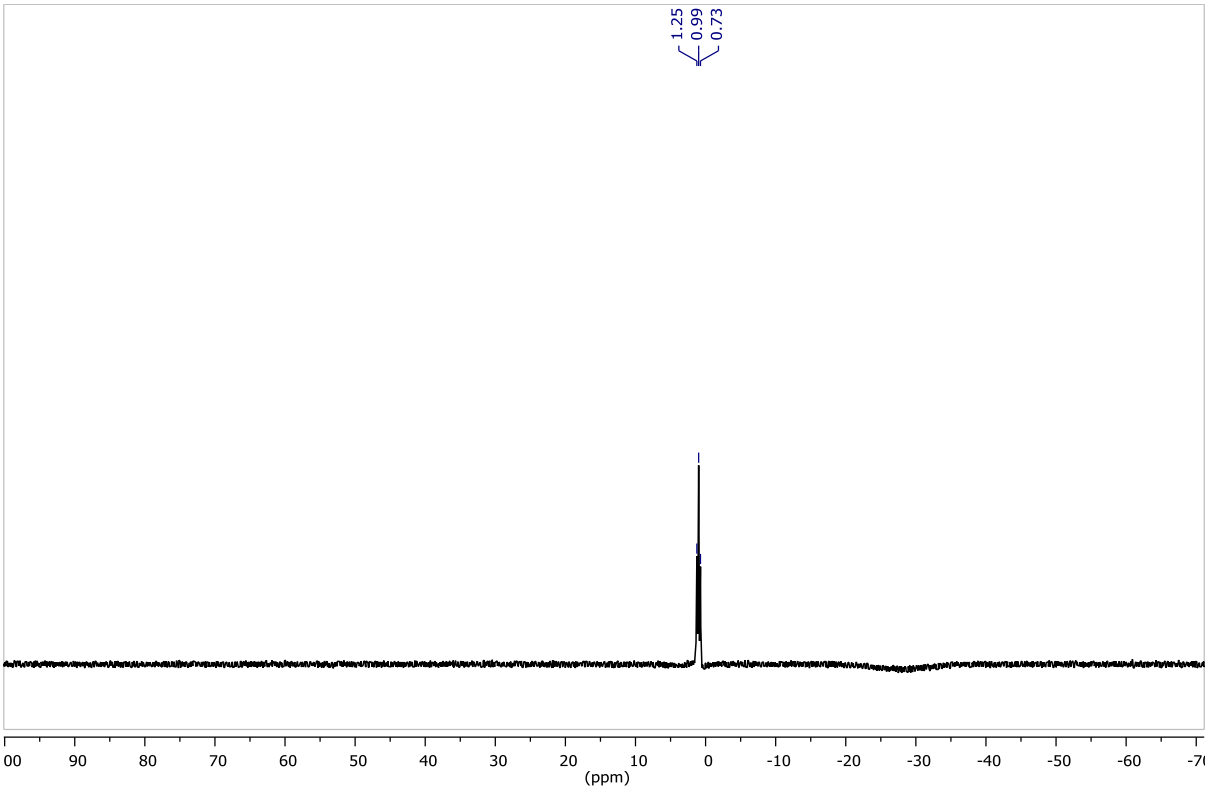
27.01.2020

(AcO)Ent$_{KL}$-PEG$_4$-BODIPY (^{1}H and ^{13}C NMR)

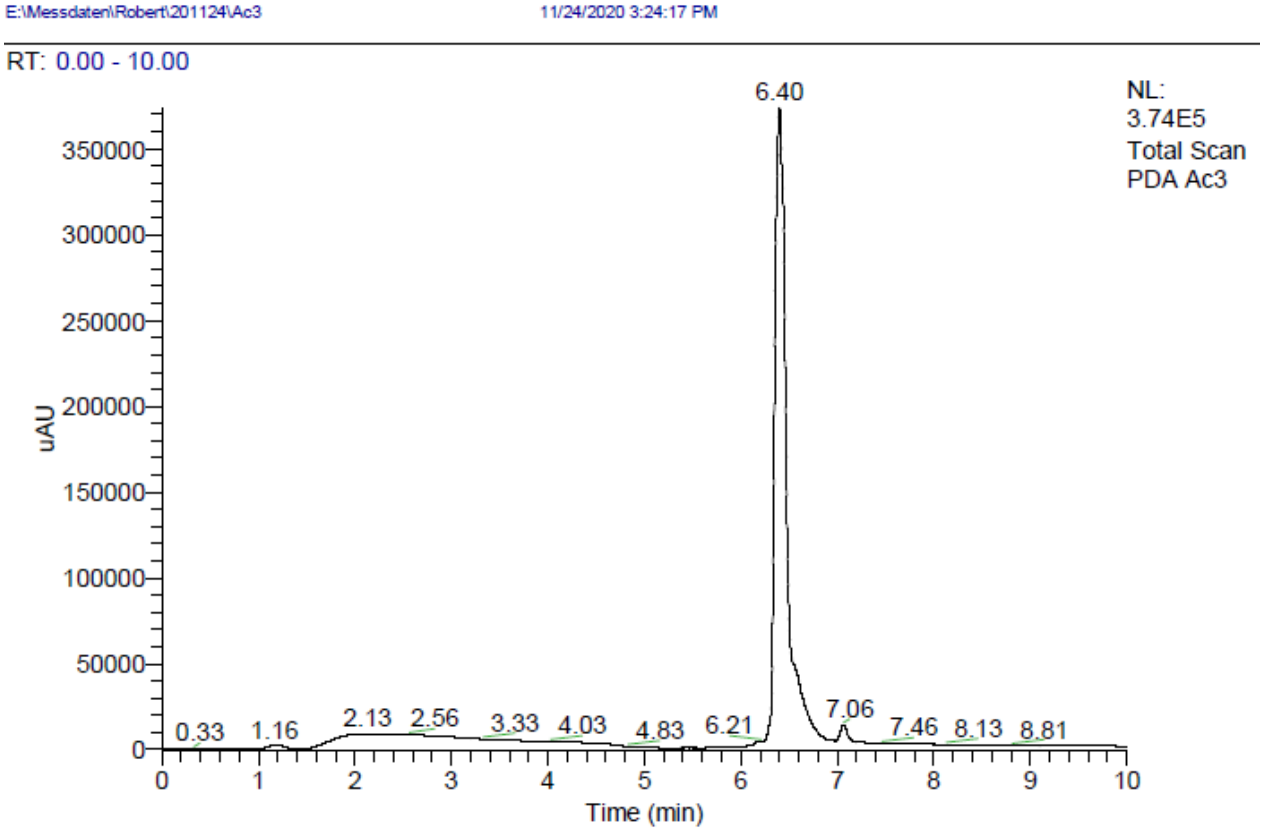


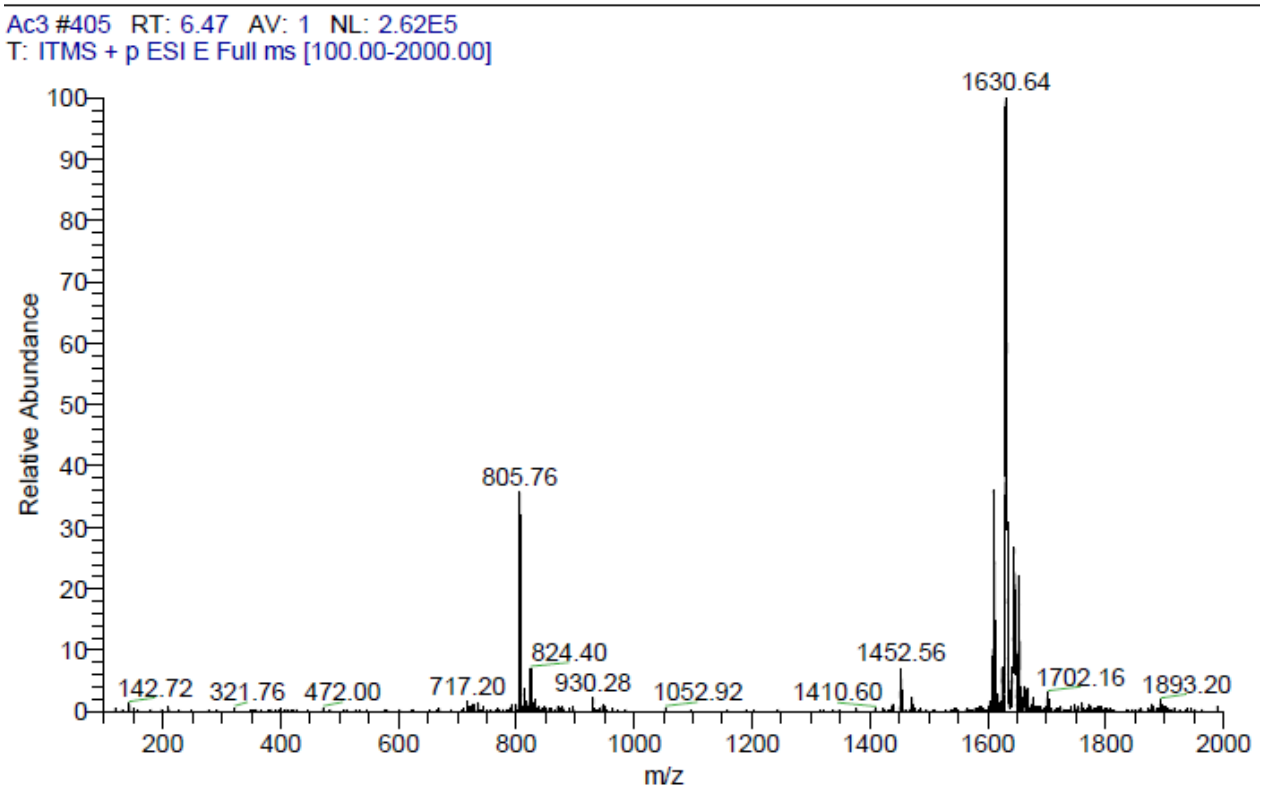
(AcO)Ent$_{KL}$-PEG$_4$-BODIPY (^{19}F and ^{11}B NMR)



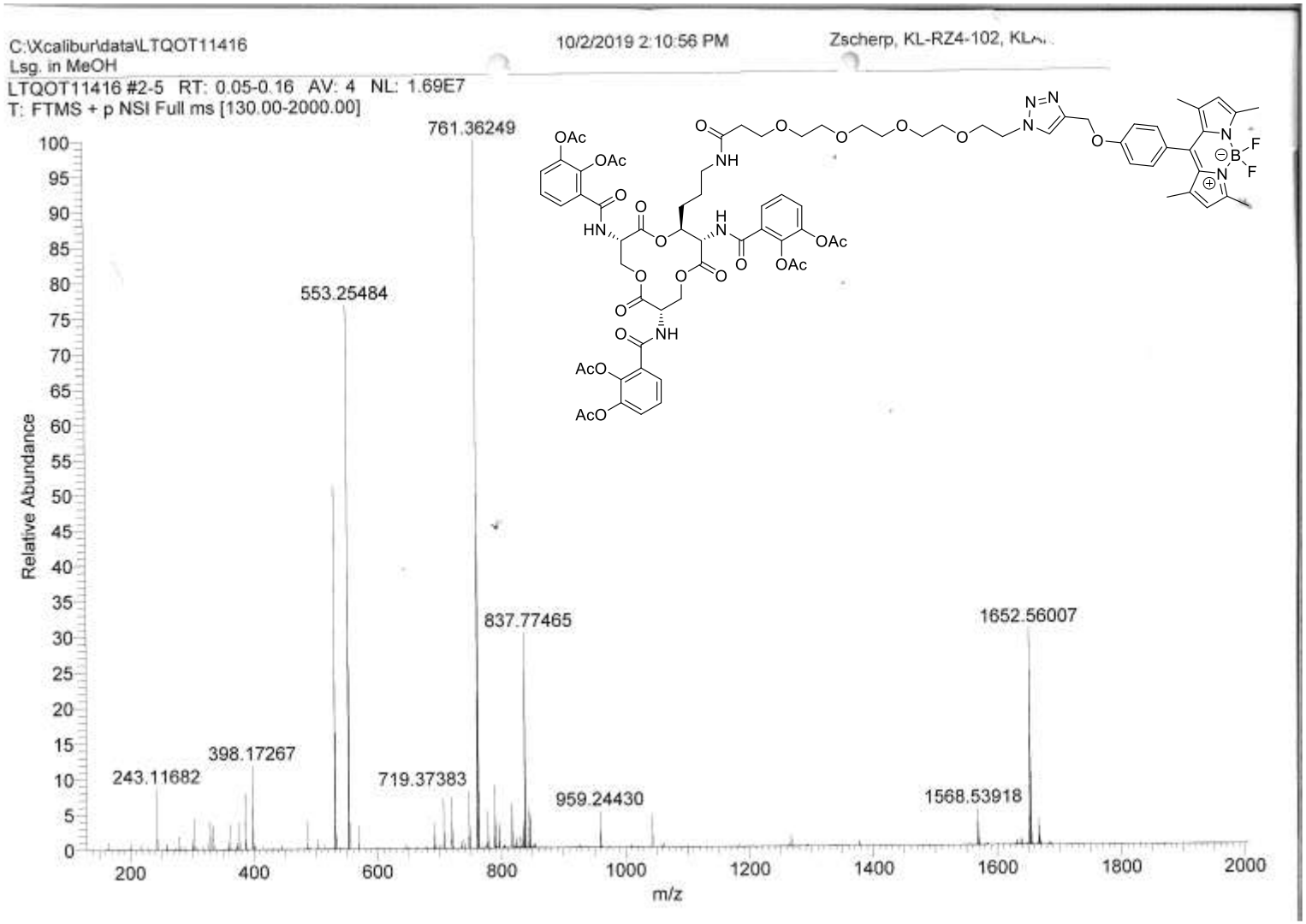


(AcO)Ent$_{KL}$-PEG$_4$-BODIPY (HPLC-LRMS)



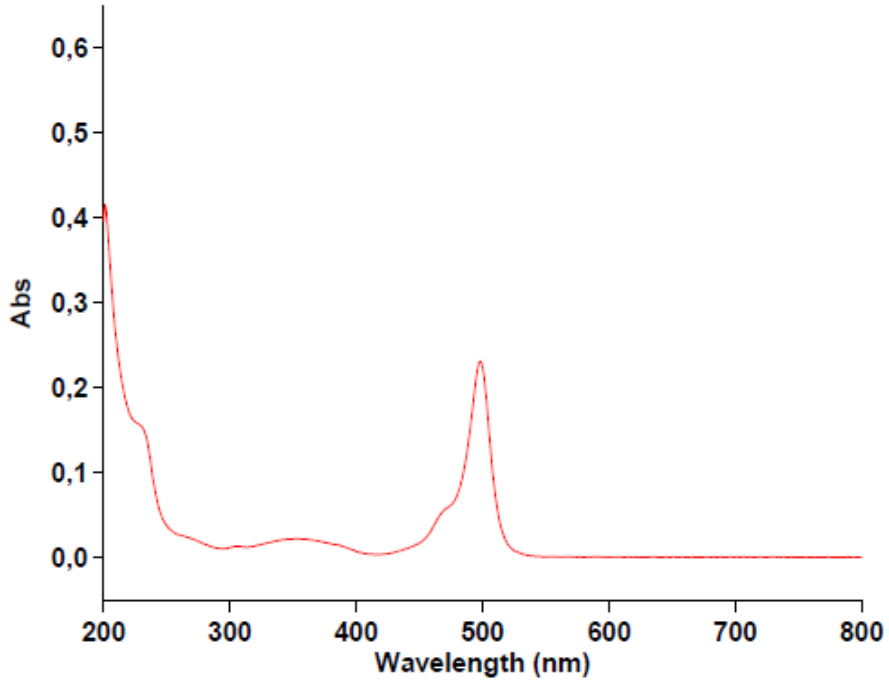


(AcO)Ent$_{KL}$-PEG$_4$-BODIPY (HRMS)



(AcO)Ent$_{KL}$-PEG$_4$-BODIPY (UV/Vis)

04.12.2020 17:06:58 Page 1 of 1



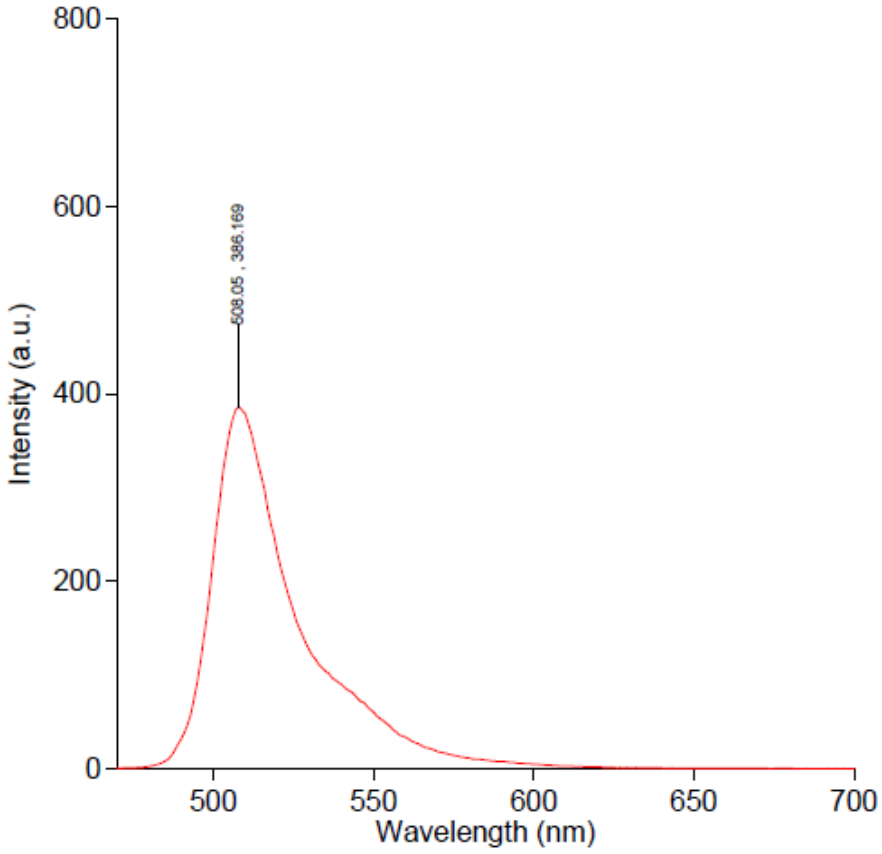
Sample Name: KL-RZ4-102

Collection Time	24.04.2020 16:18:51
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
498,00	0,232
353,00	0,023
201,00	0,416

102 µg in 10 mL in MeOH

(AcO)Ent$_{KL}$-PEG$_4$-BODIPY (Fluorescence Emission)



Sample name: KL-RZ4-102

Peak table

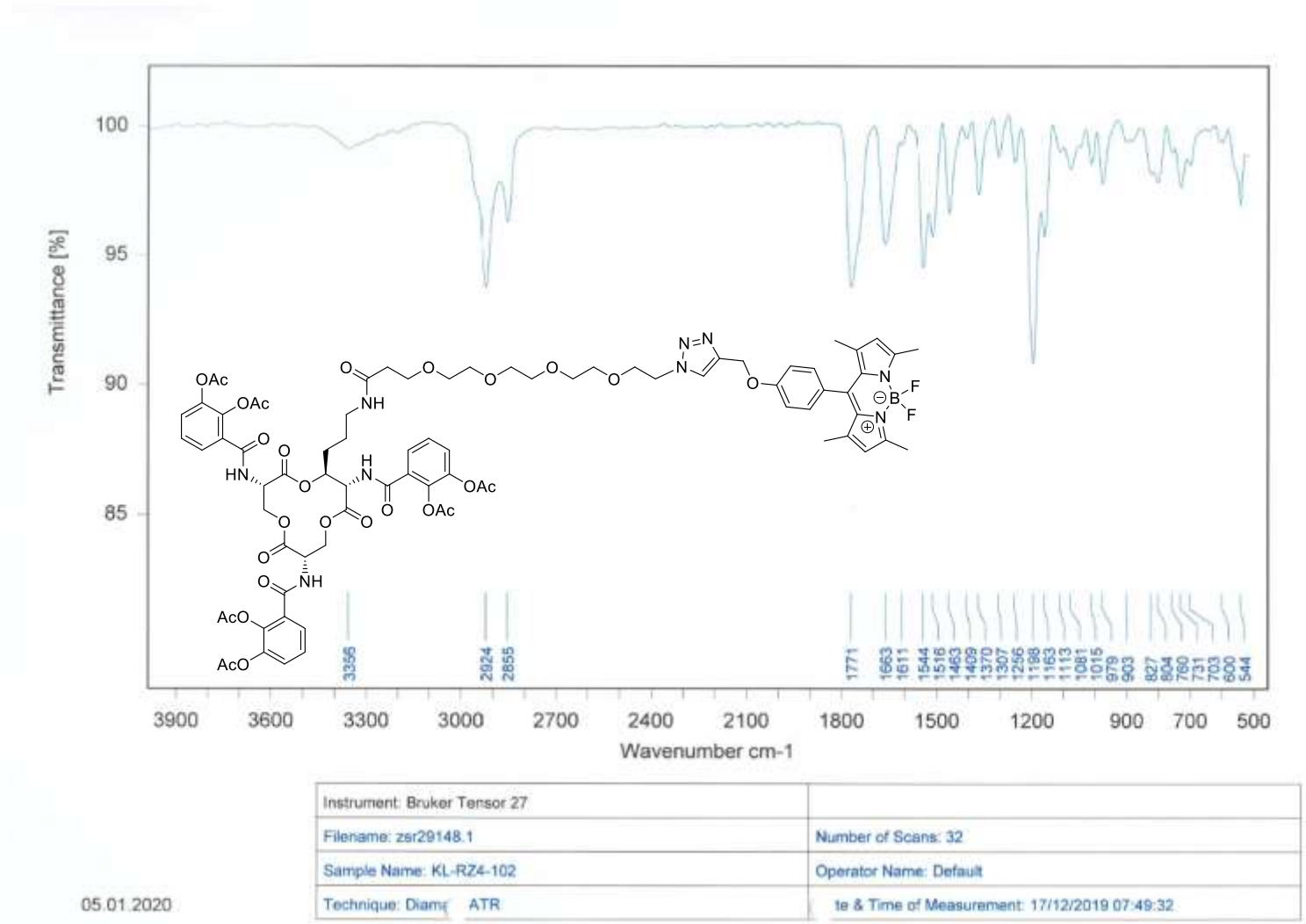
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
508.05	386.169

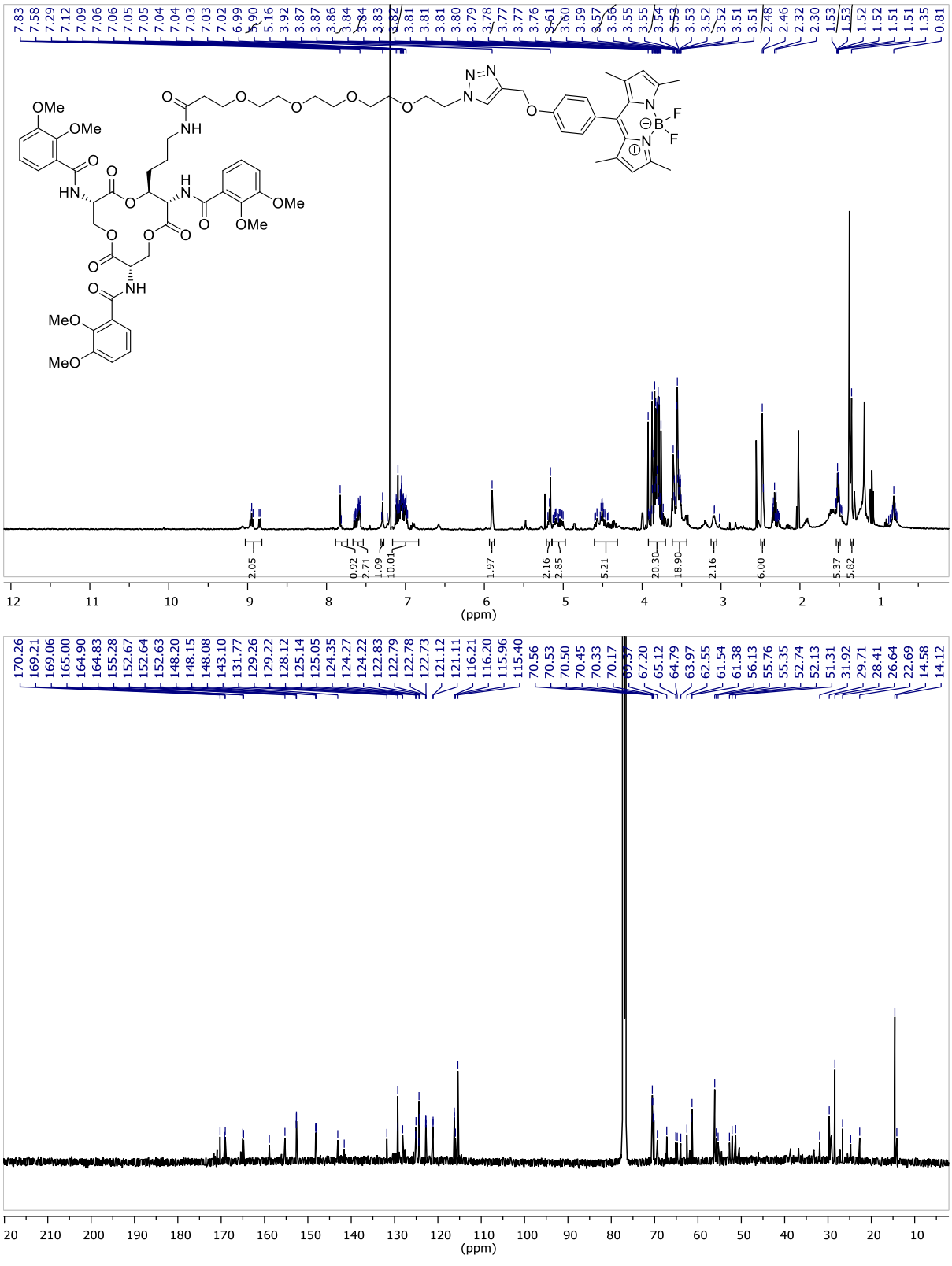
102 µg in 10 mL MeOH

Excitation wavelength: 498 nm

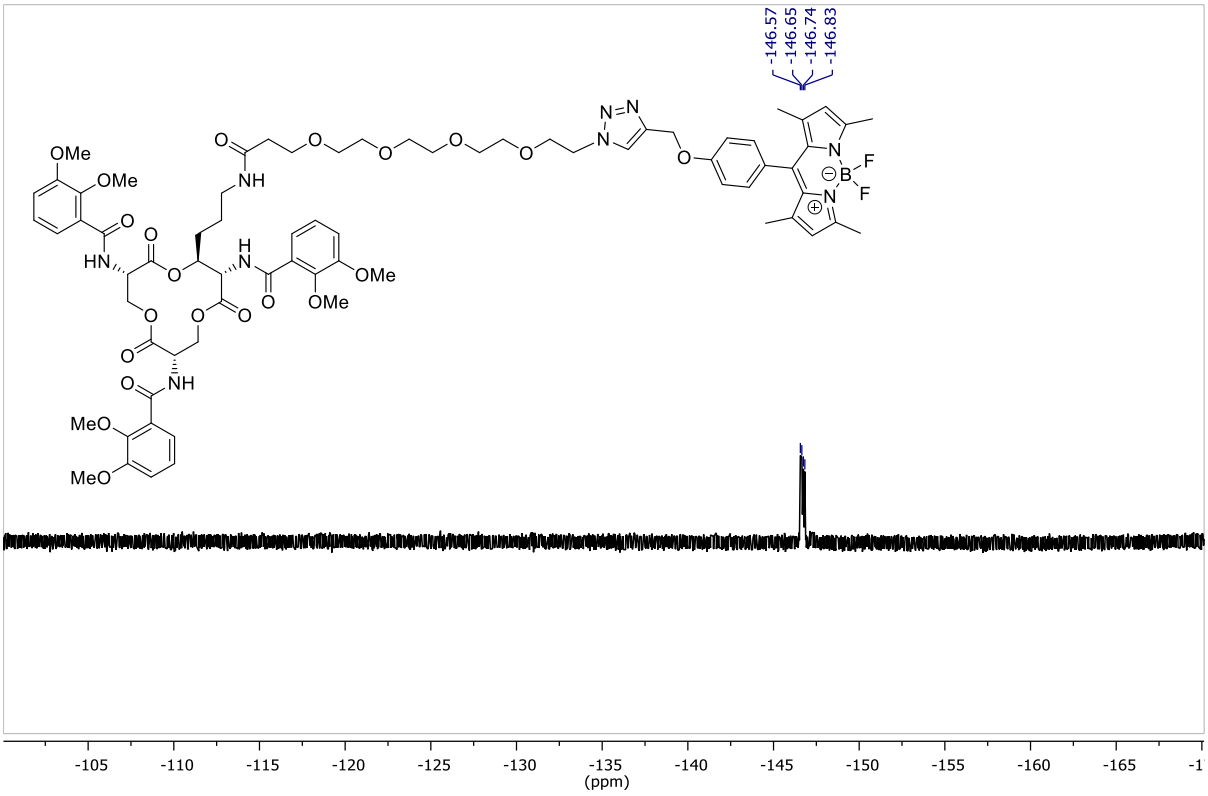
(AcO)Ent$_{KL}$-PEG$_4$-BODIPY (ATR-IR)

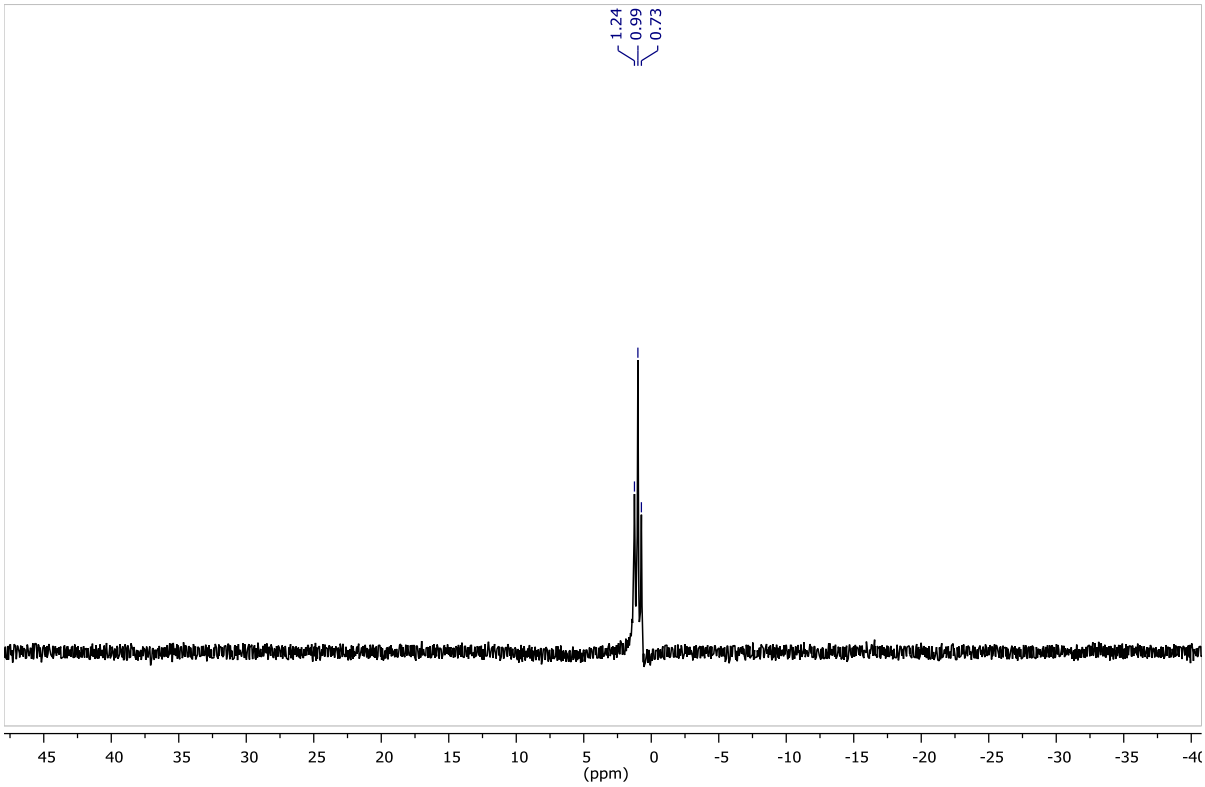


(MeO)Ent$_{KL}$-PEG$_4$-BODIPY (^{1}H and ^{13}C NMR)

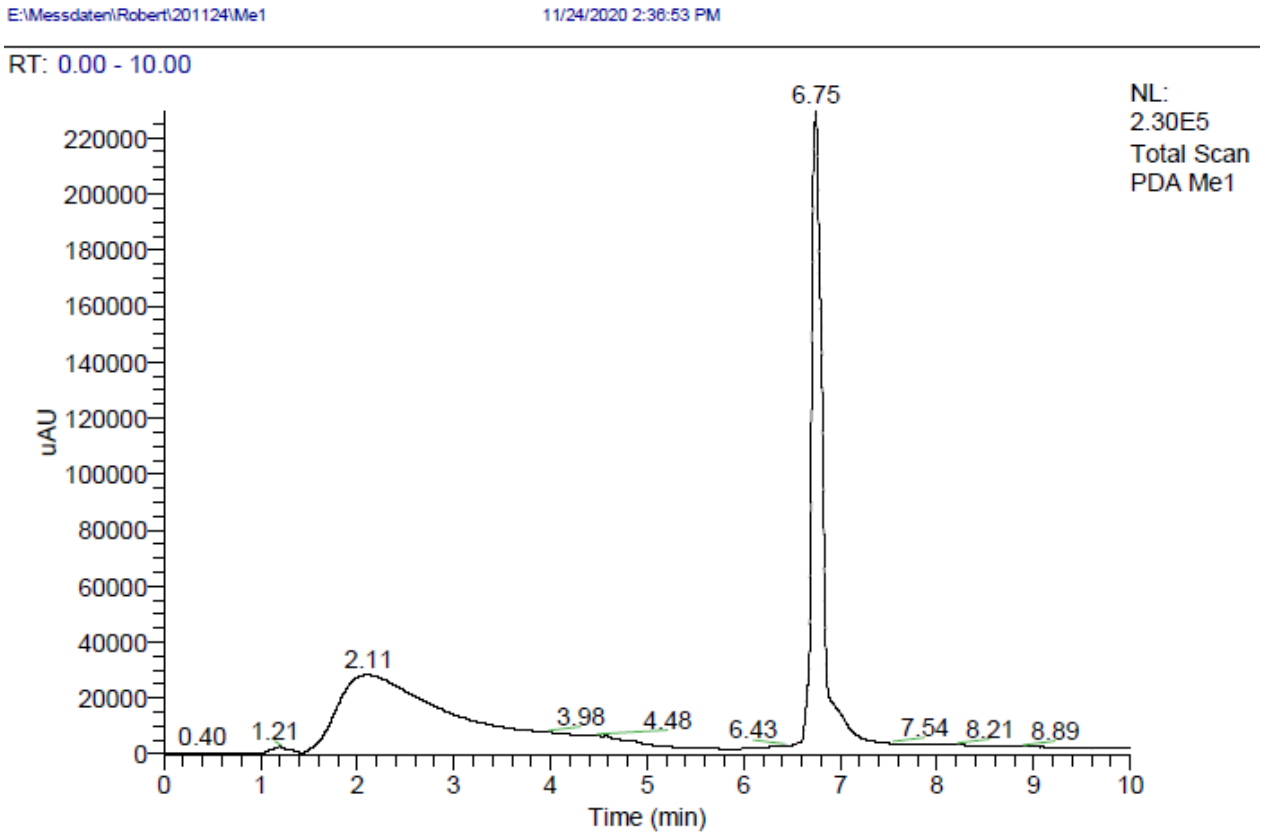


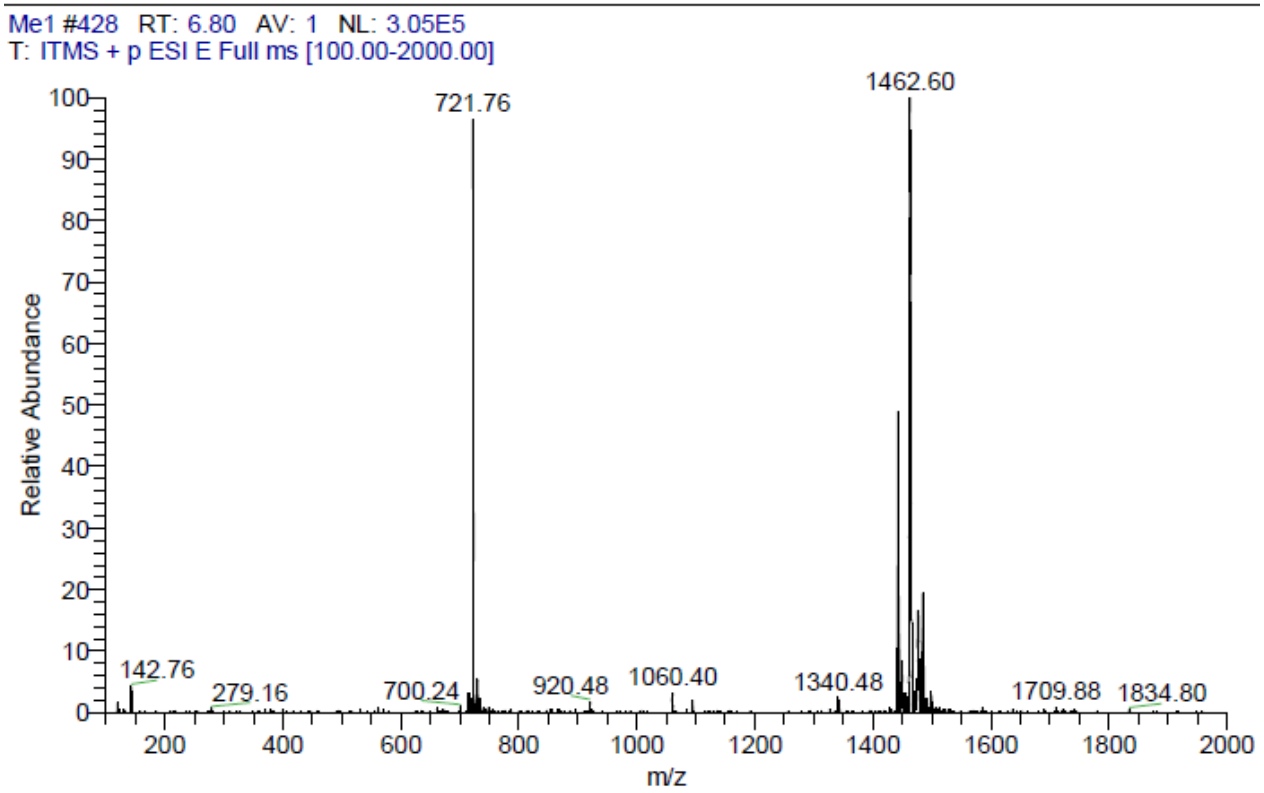
(MeO)Ent$_{KL}$-PEG$_4$-BODIPY (^{19}F and ^{11}B NMR)



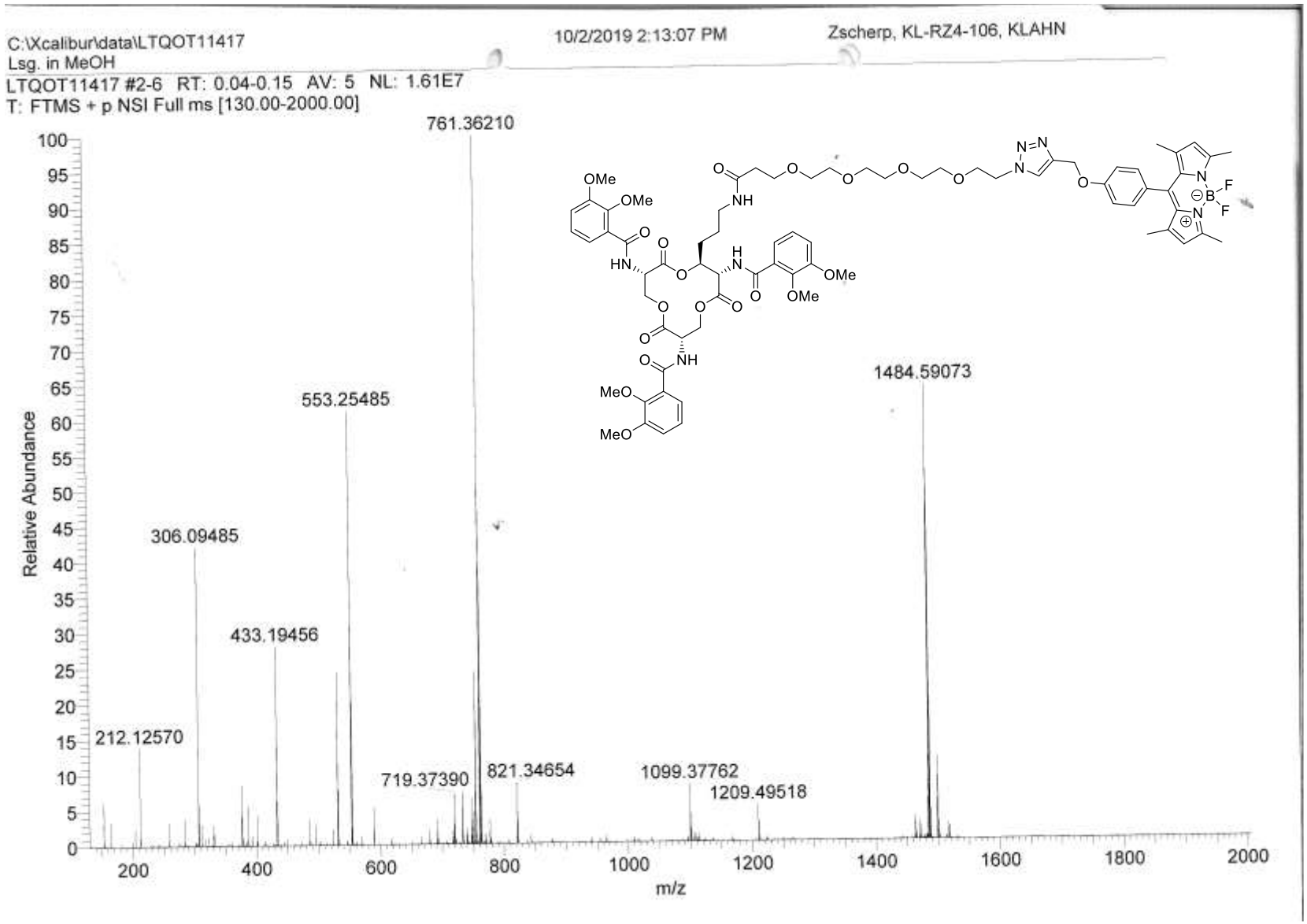


(MeO)Ent$_{KL}$-PEG$_4$-BODIPY (HPLC-LRMS)





(MeO)Ent$_{KL}$-PEG$_4$-BODIPY (HRMS)



(MeO)Ent$_{KL}$-PEG$_4$-BODIPY (UV/Vis)

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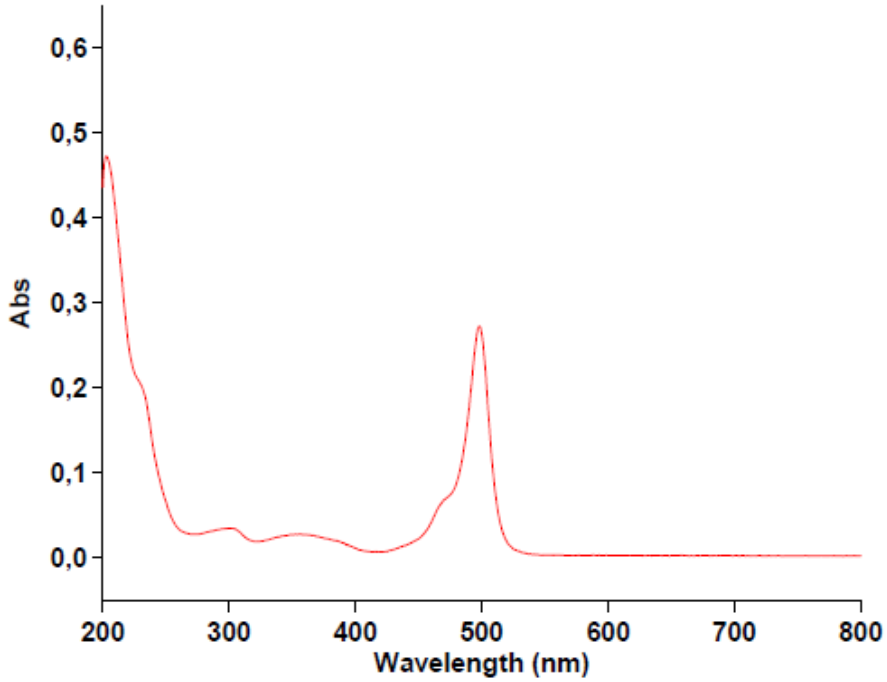


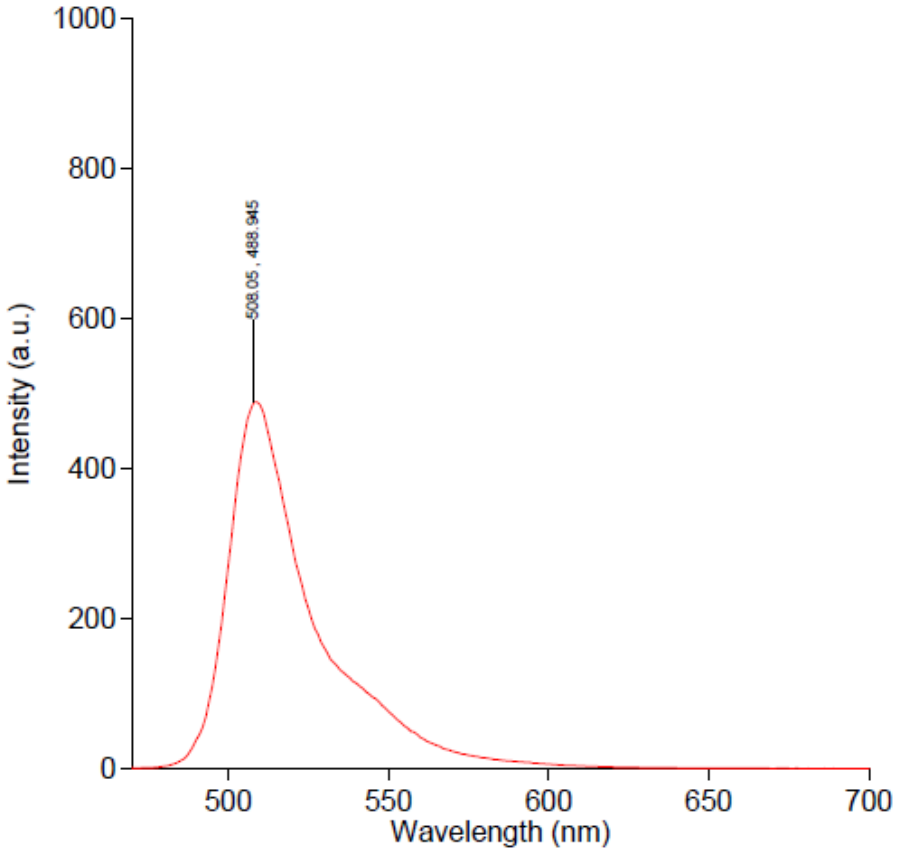
Sample Name: KL-RZ4-106

Collection Time	24.04.2020 16:58:40
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
498,00	0,273
203,00	0,473

120 µg in 10 mL in MeOH

(MeO)Ent$_{KL}$-PEG$_4$-BODIPY (Fluorescence Emission)



Sample name: KL-RZ4-106

Peak table

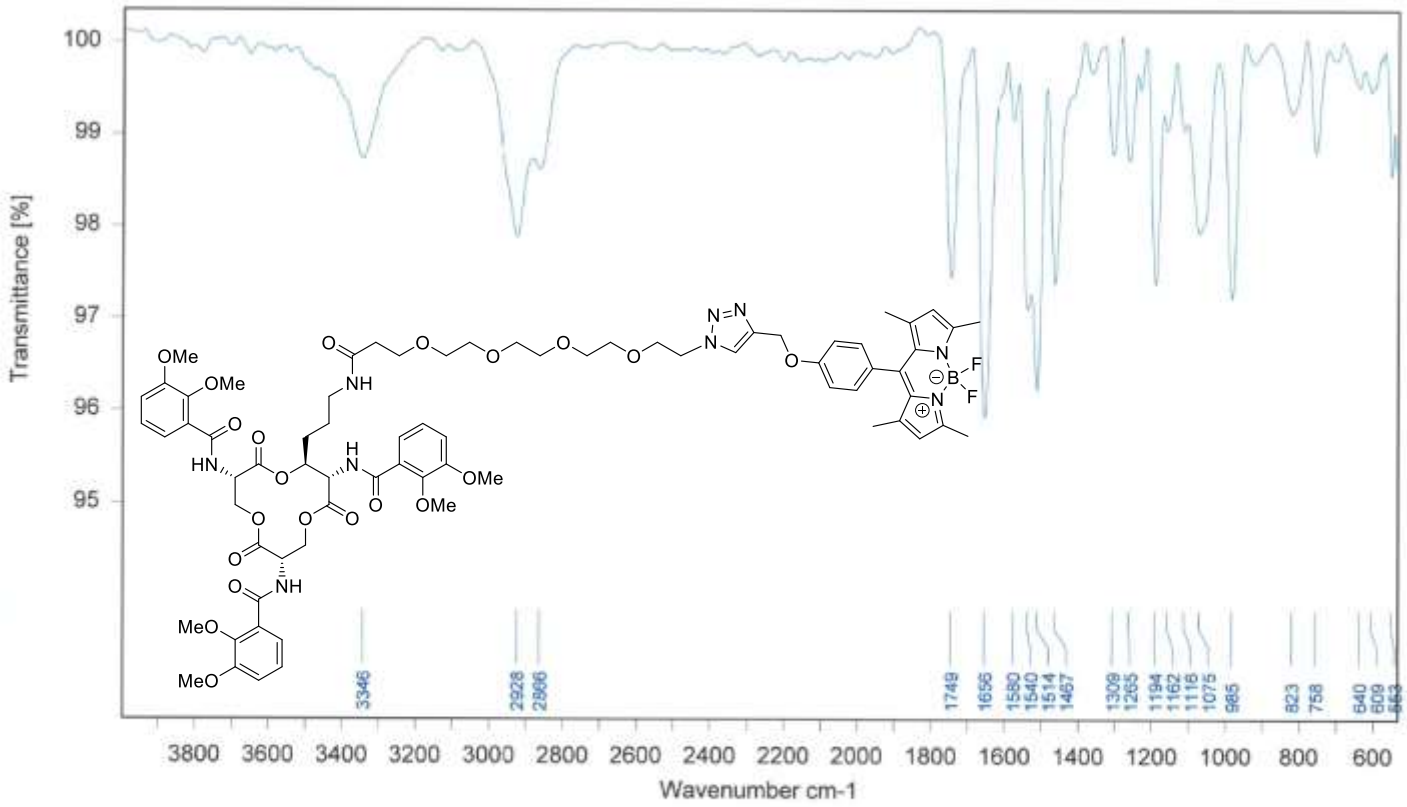
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
508.05	488.945

96 µg in 10 mL MeOH

Excitation wavelength: 498 nm

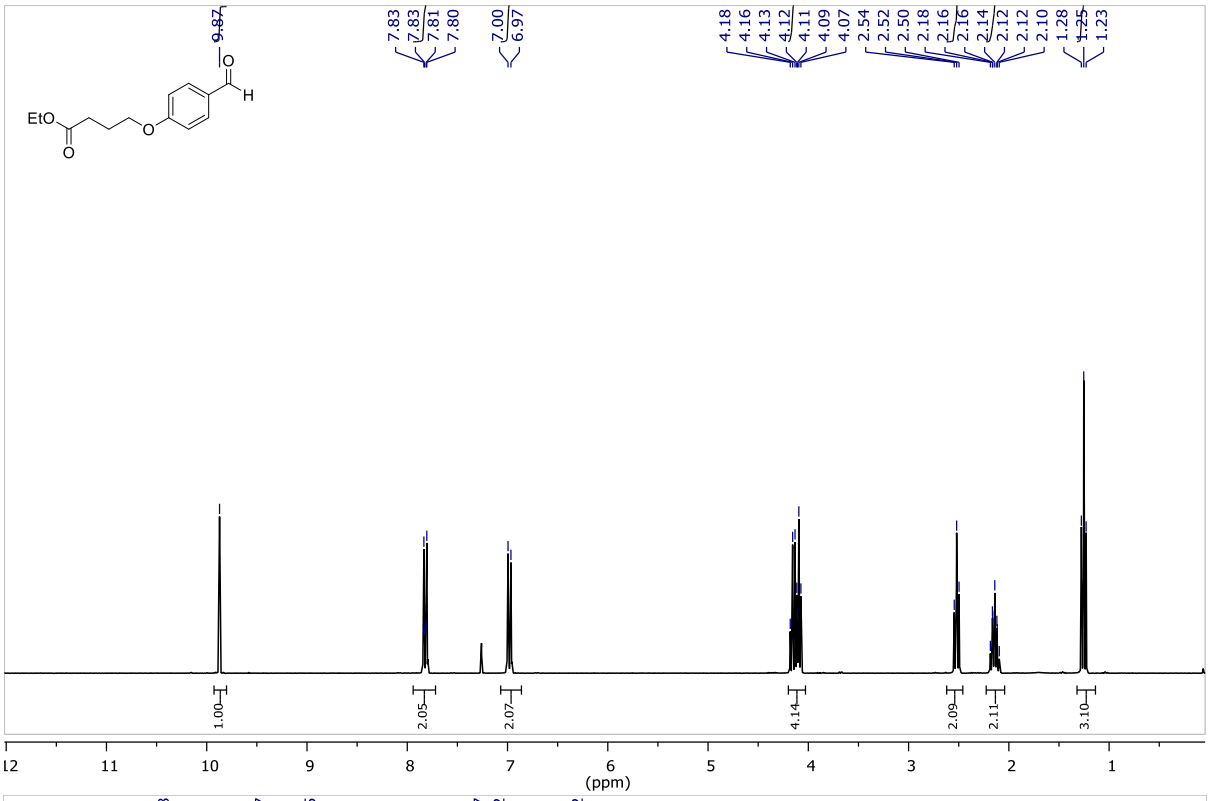
(MeO)Ent$_{KL}$-PEG$_4$-BODIPY (ATR-IR)

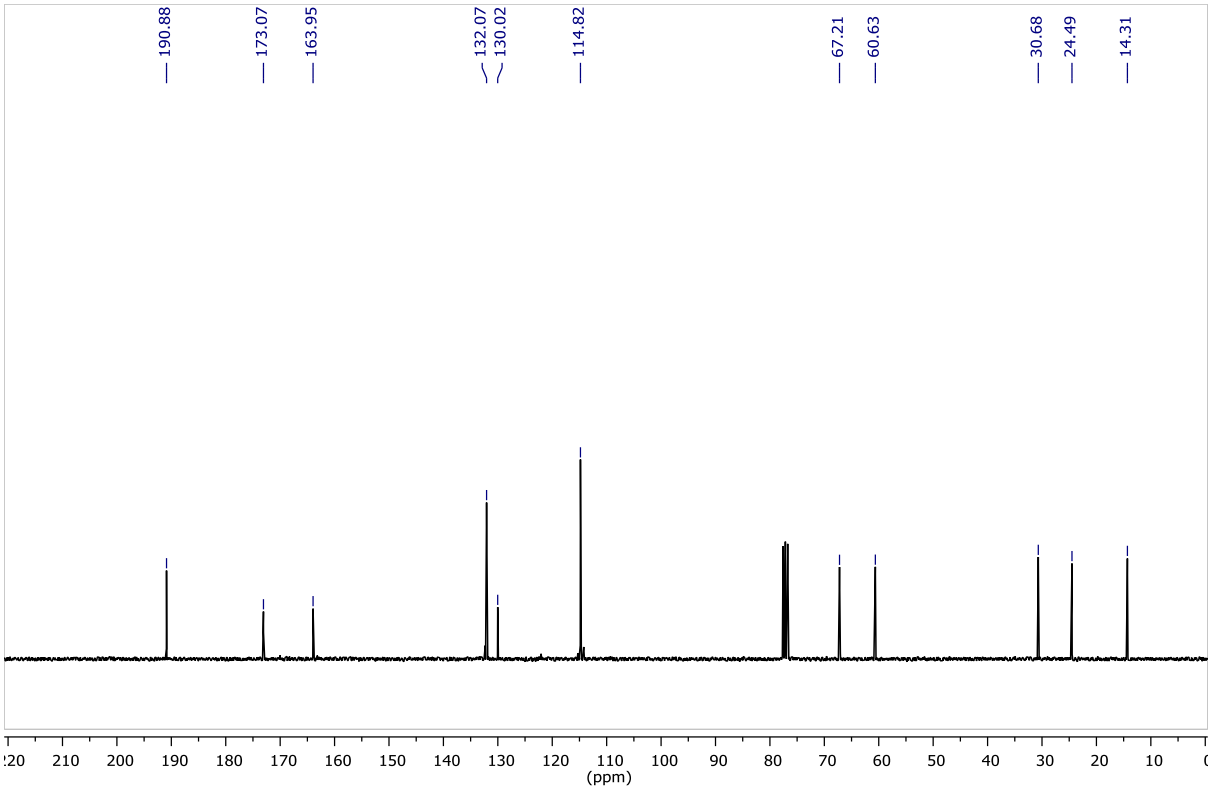


Instrument: Bruker Tensor 27	
Filename: zsr29867.2	Number of Scans: 32
Sample Name: KL-RZ4-106	Operator Name: Default
Technique: Diam[illegible]-ATR	[illegible]ate & Time of Measurement: 04/11/2019 08:49:17

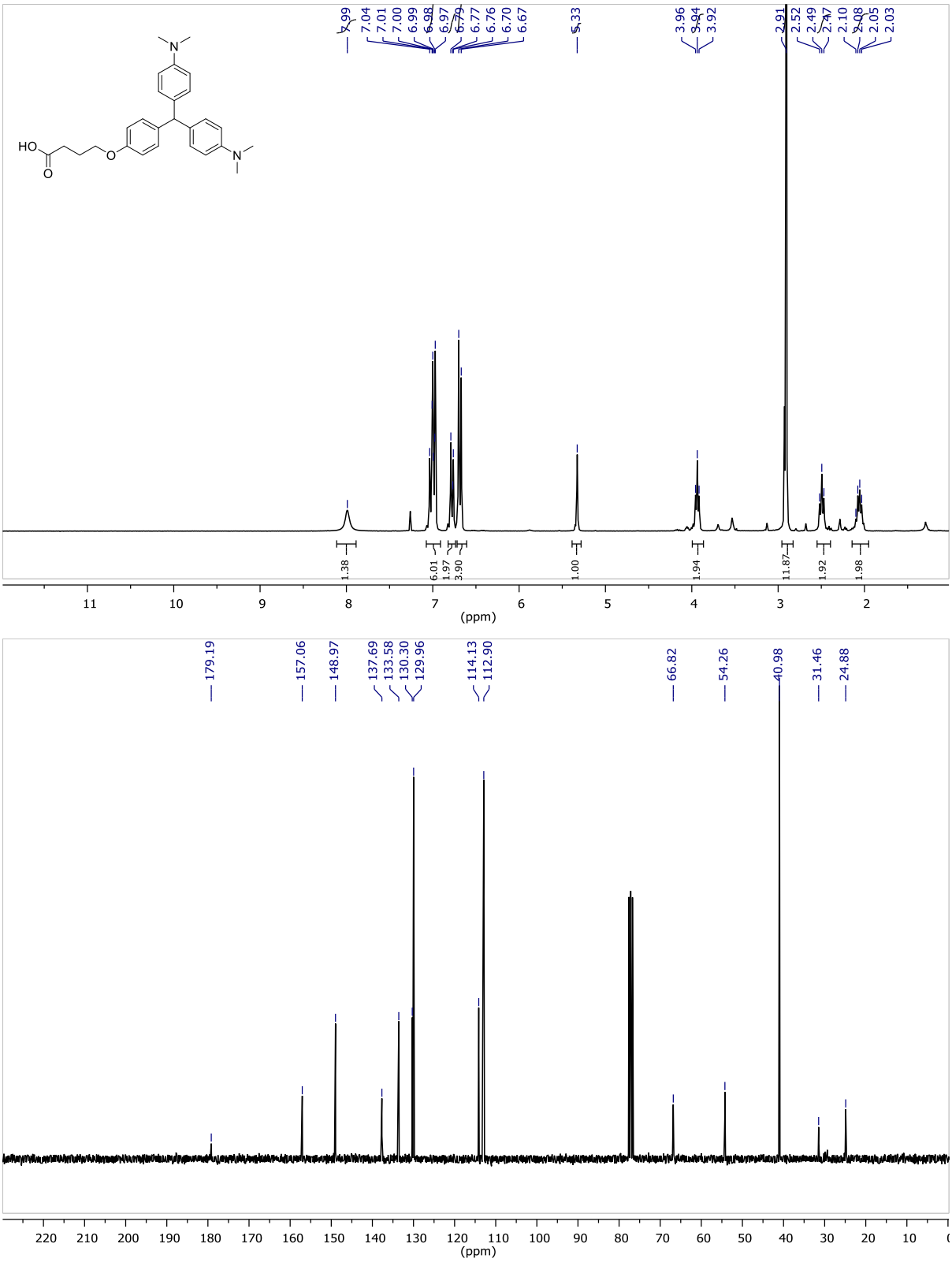
05.01.2020

Ethyl 4-(4-formylphenoxy) butanoate (^{1}H and ^{13}C NMR)

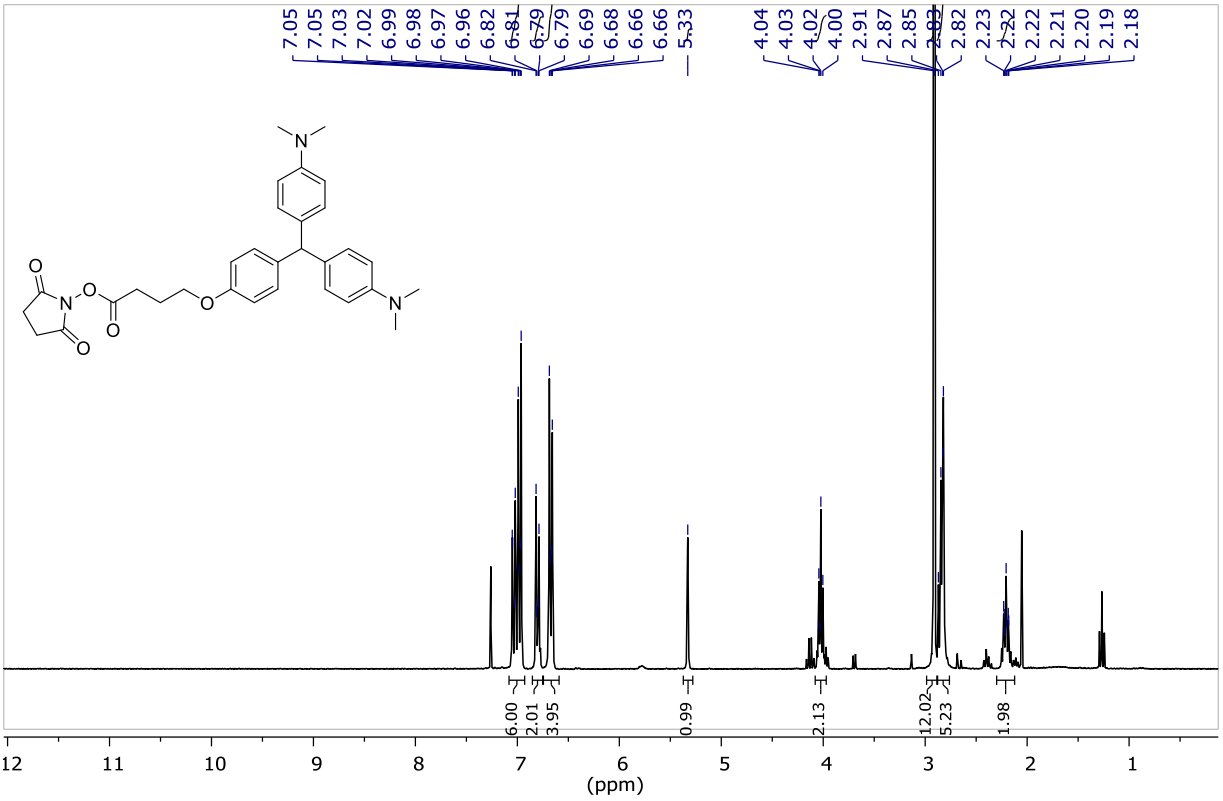


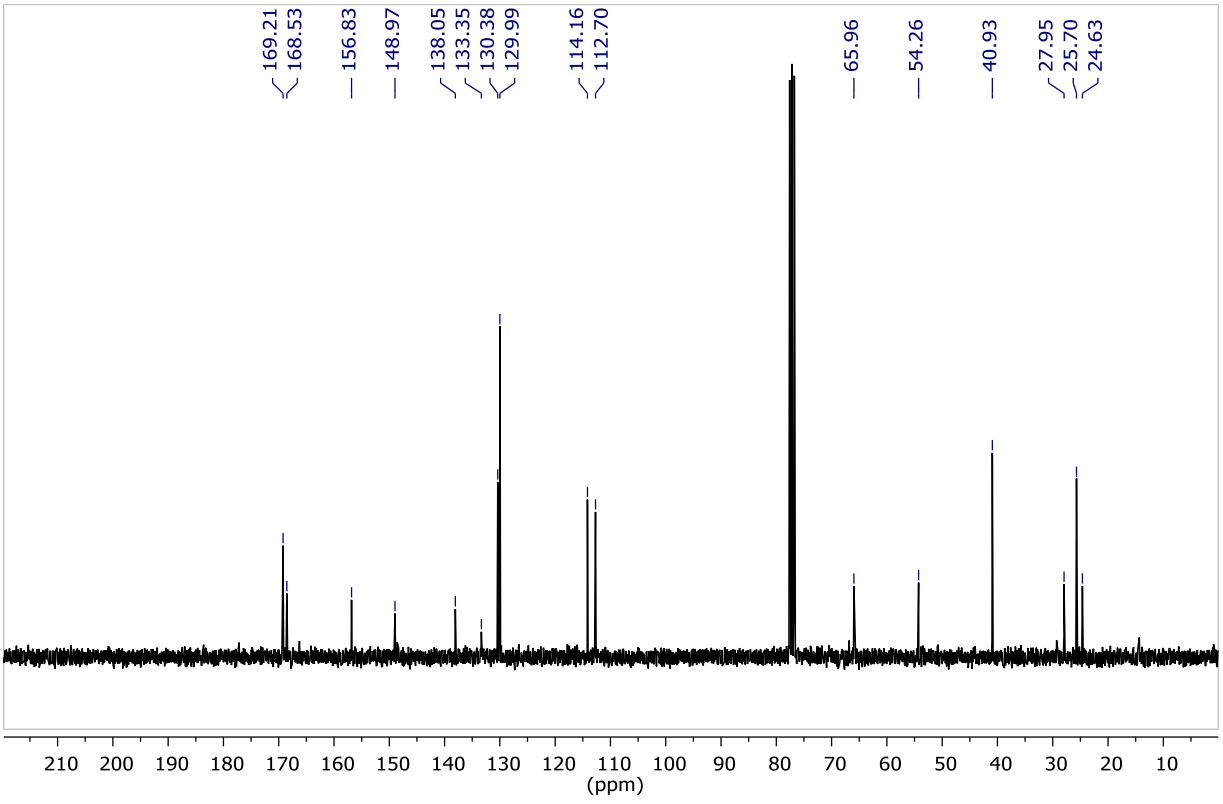


4-(4-(Bis(4-(dimethylamino)phenyl)methyl)phenoxy)butanoic acid (^{1}H and ^{13}C NMR)

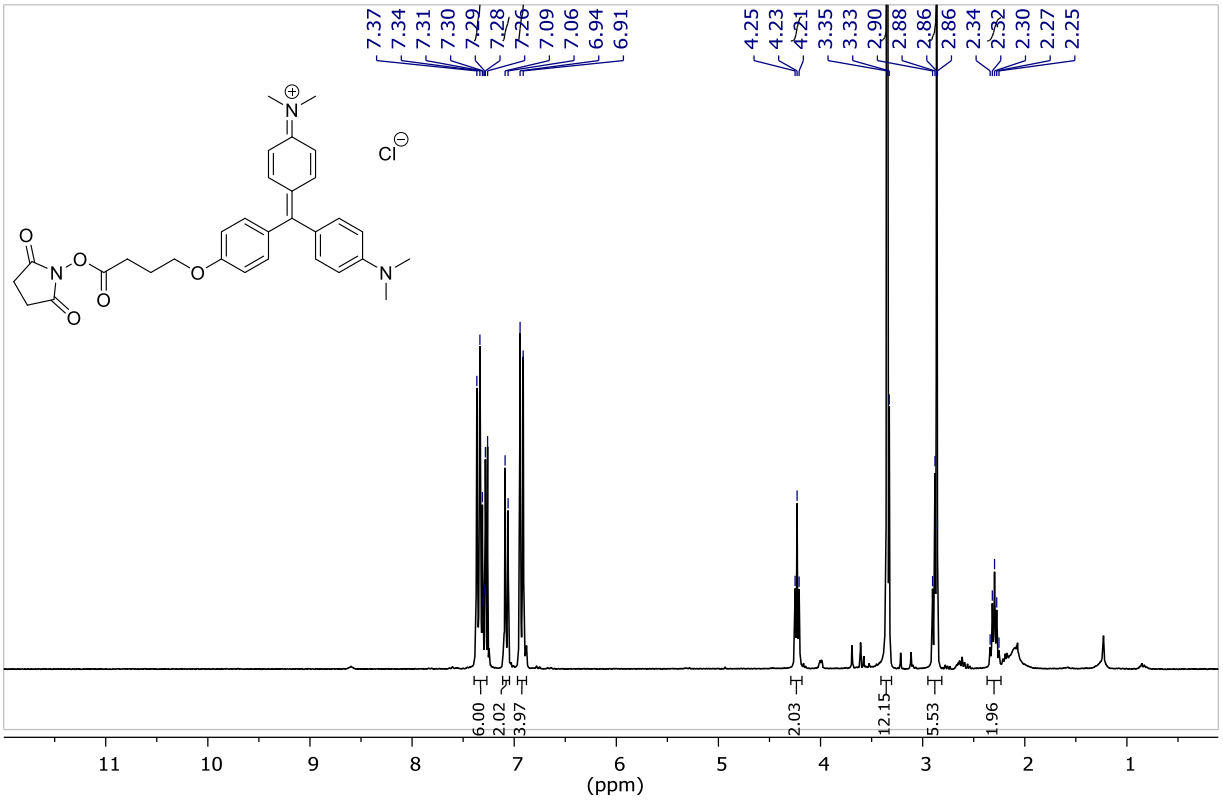


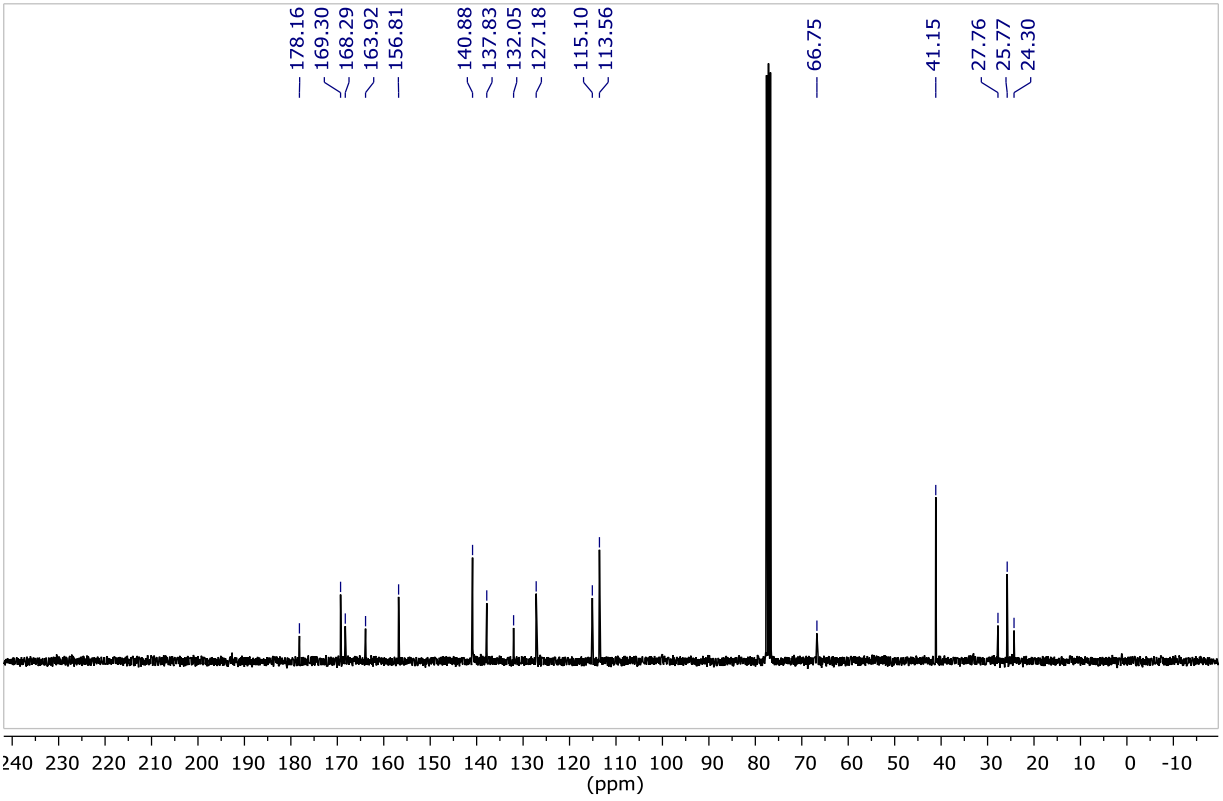
2,5-Dioxopyrrolidin-1-yl 4-(4-(bis(4-(dimethylamino)phenyl)methyl)phenoxy)butanoate (^{1}H and ^{13}C NMR)



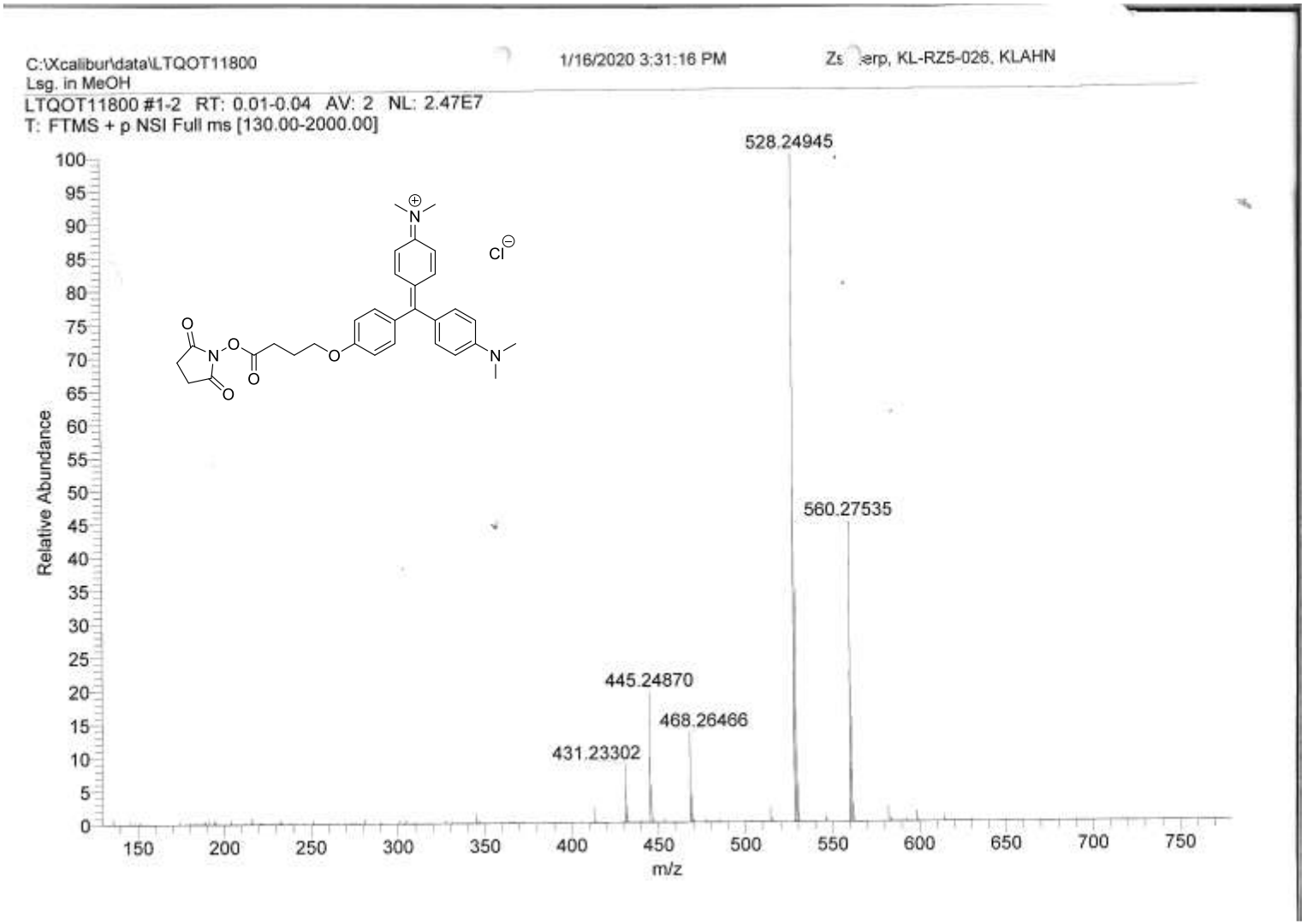


N-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)-methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium chloride (MG-NHS) (^{1}H and ^{13}C NMR)

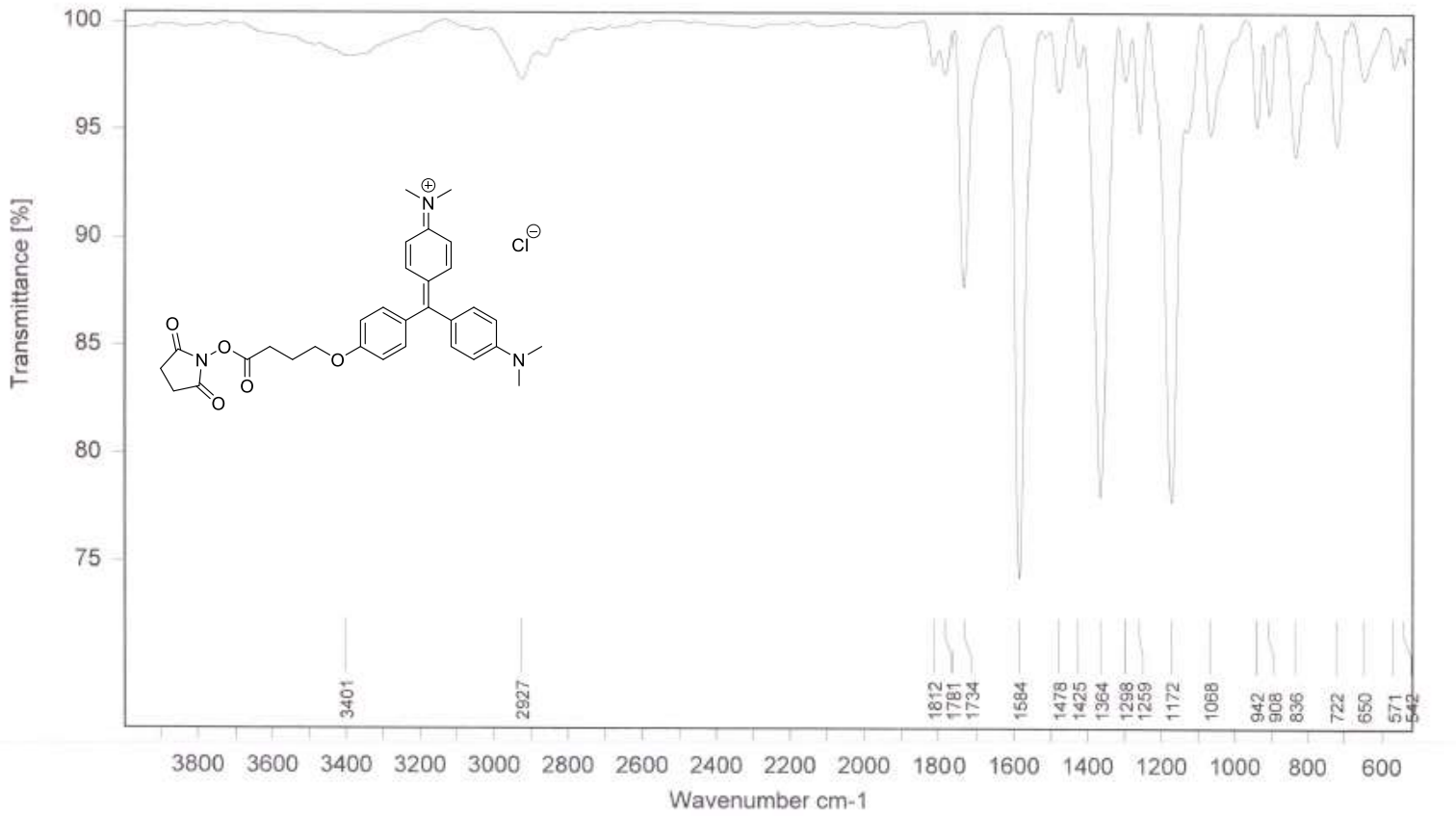




N-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)-methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium chloride (MG-NHS) (HRMS)



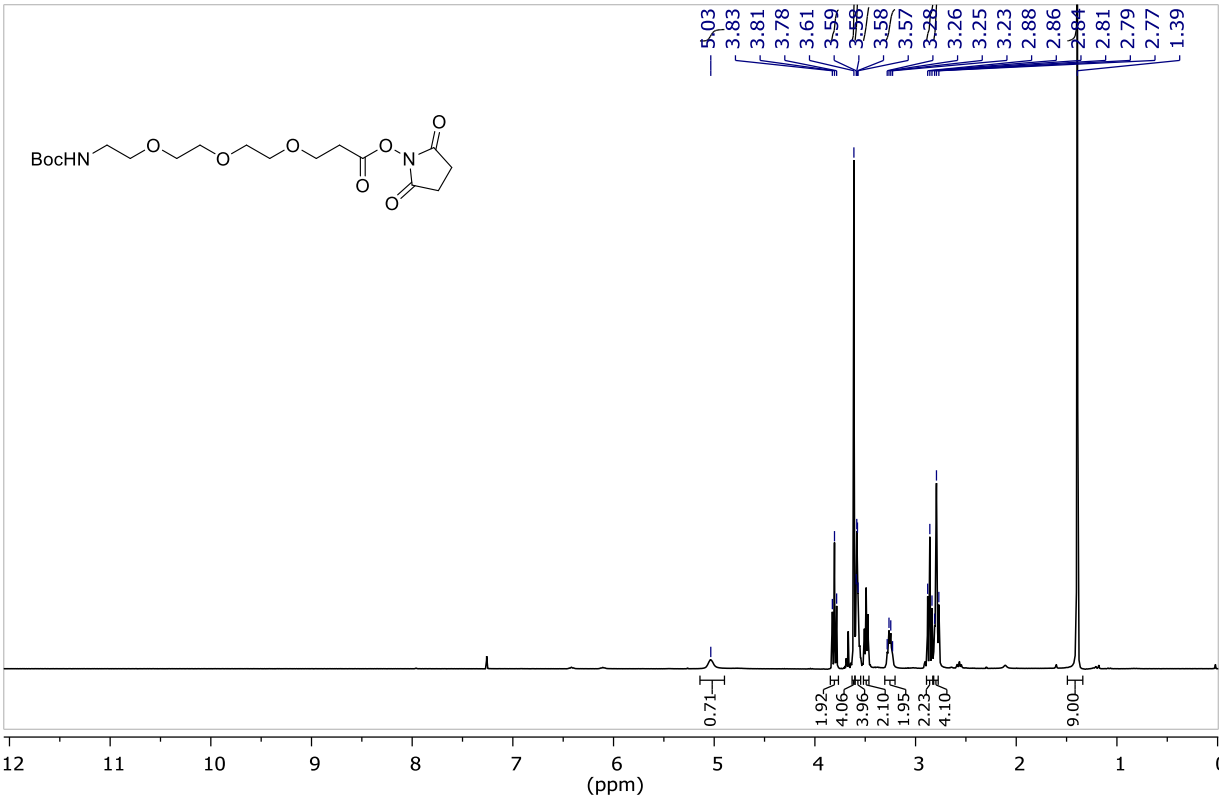
N-(4-((4-(dimethylamino)phenyl)(4-(4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutoxy)phenyl)-methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium chloride (MG-NHS) (ATR-IR)

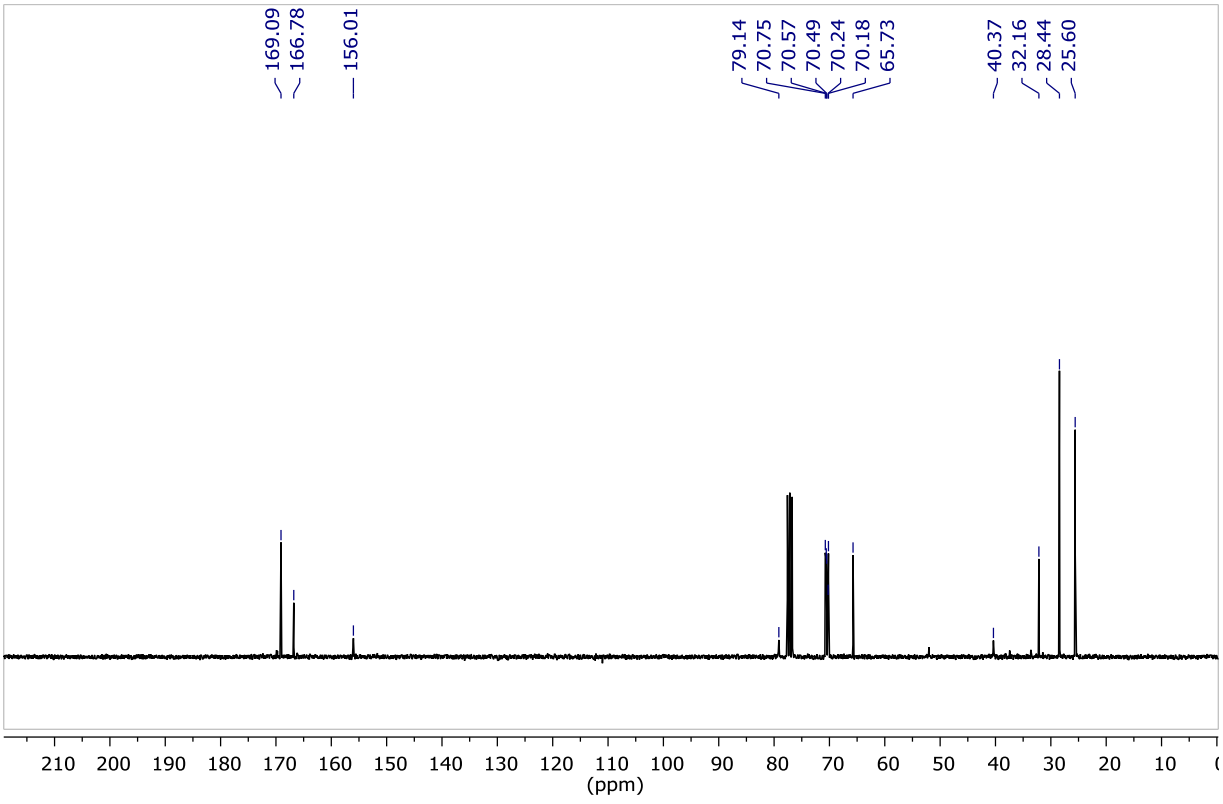


Instrument: Bruker Tensor 27	
Filename: zsr29880.0	Number of Scans: 32
Sample Name: RZ6-NHS-MG	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 26.11.2020 08:25:56

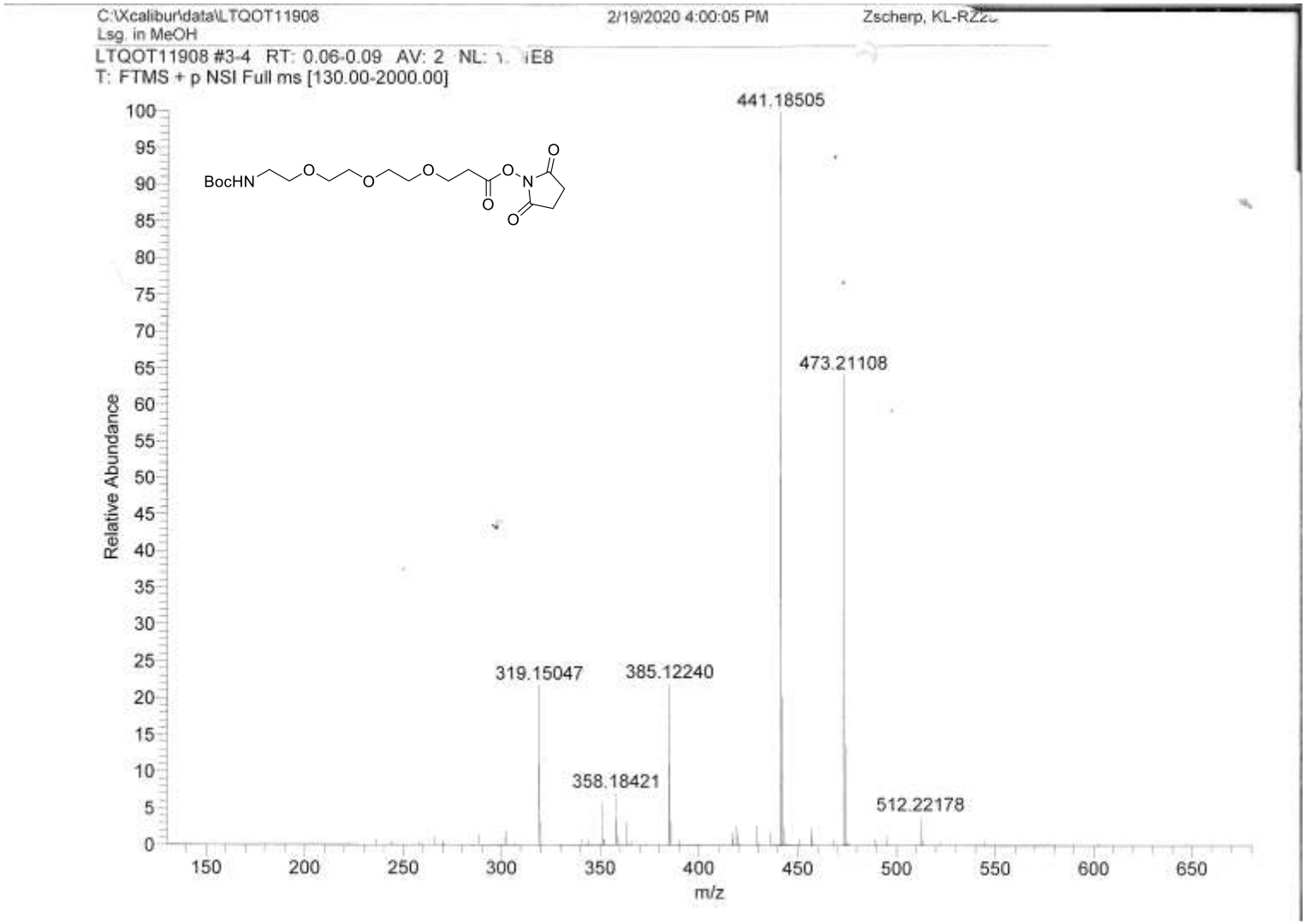
26.11.2020

2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (NHS-PEG$_3$-NHBoc) (^{1}H and ^{13}C NMR)

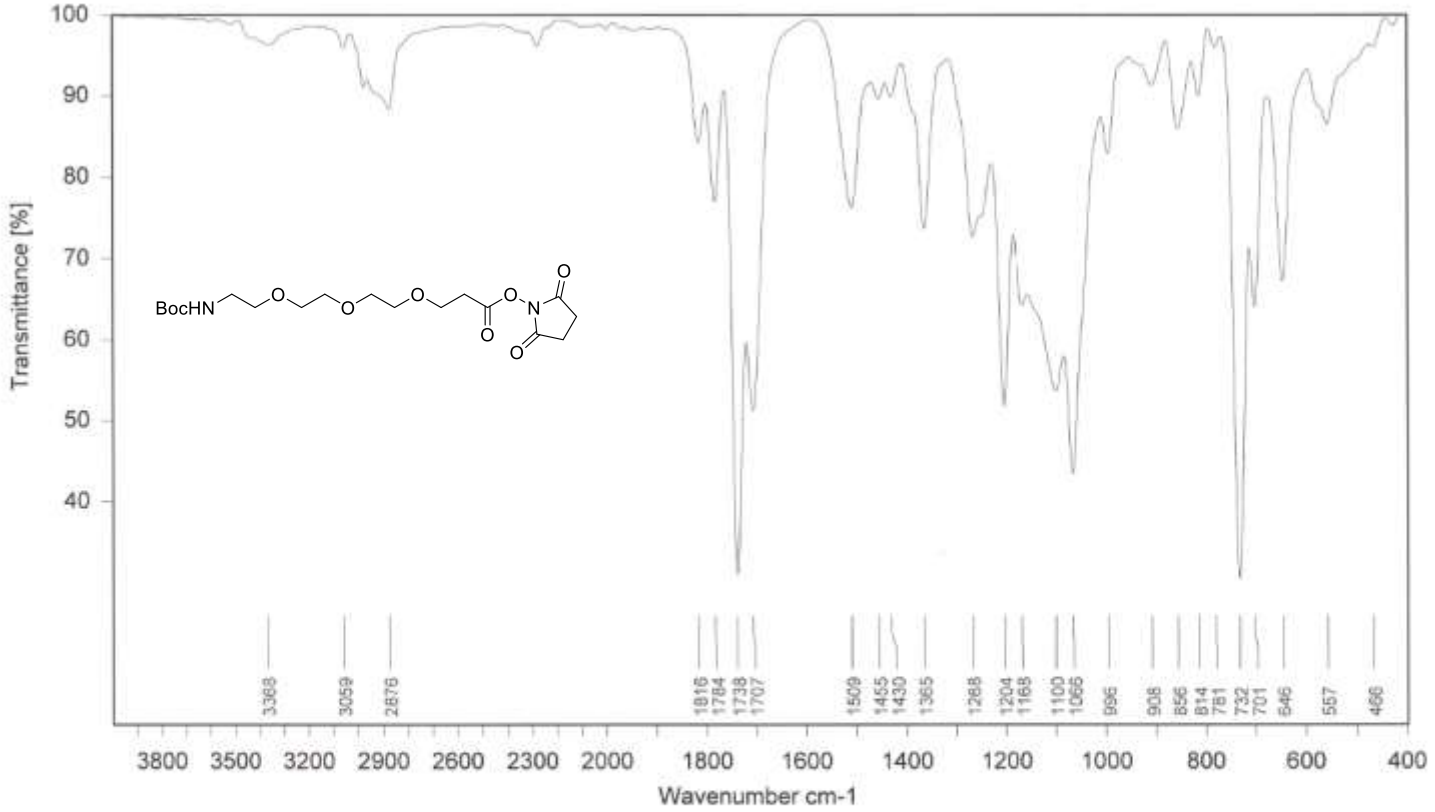




2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (NHS-PEG$_3$-NHBoc) (HRMS)



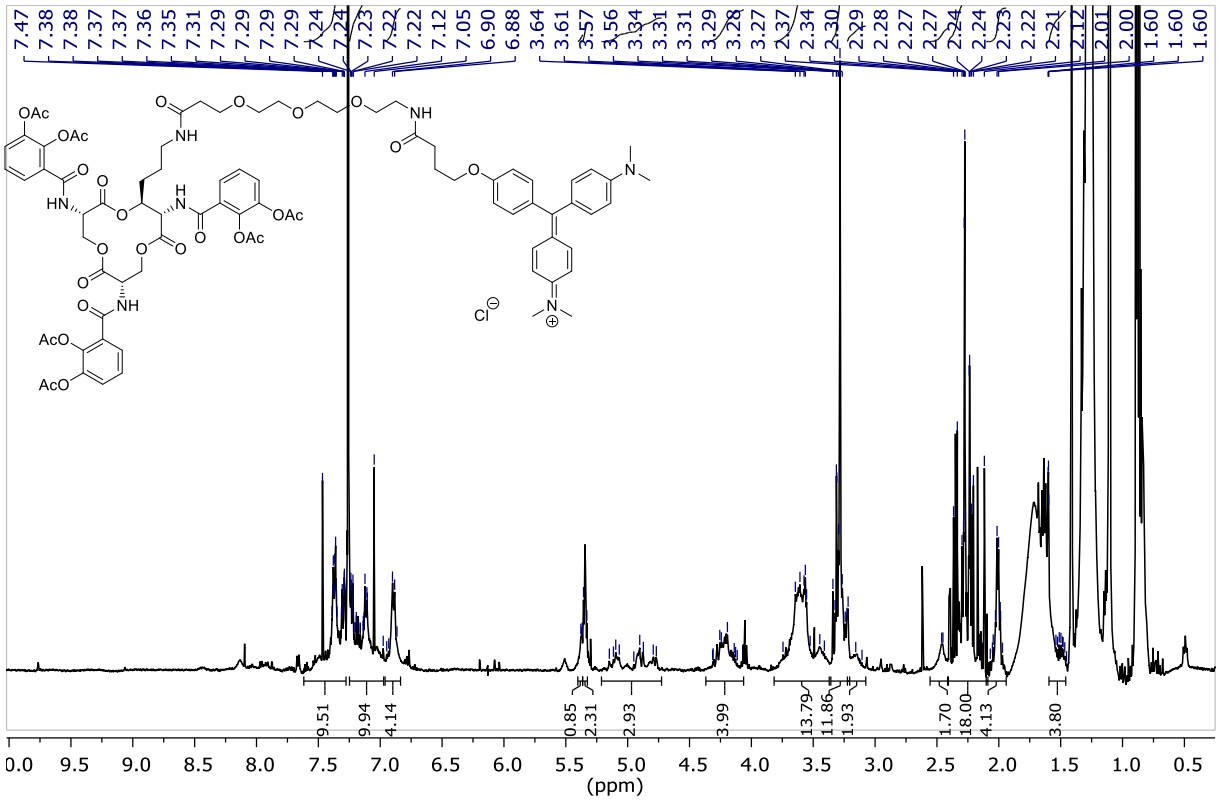
2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (NHS-PEG$_3$-NHBoc) (ATR-IR)



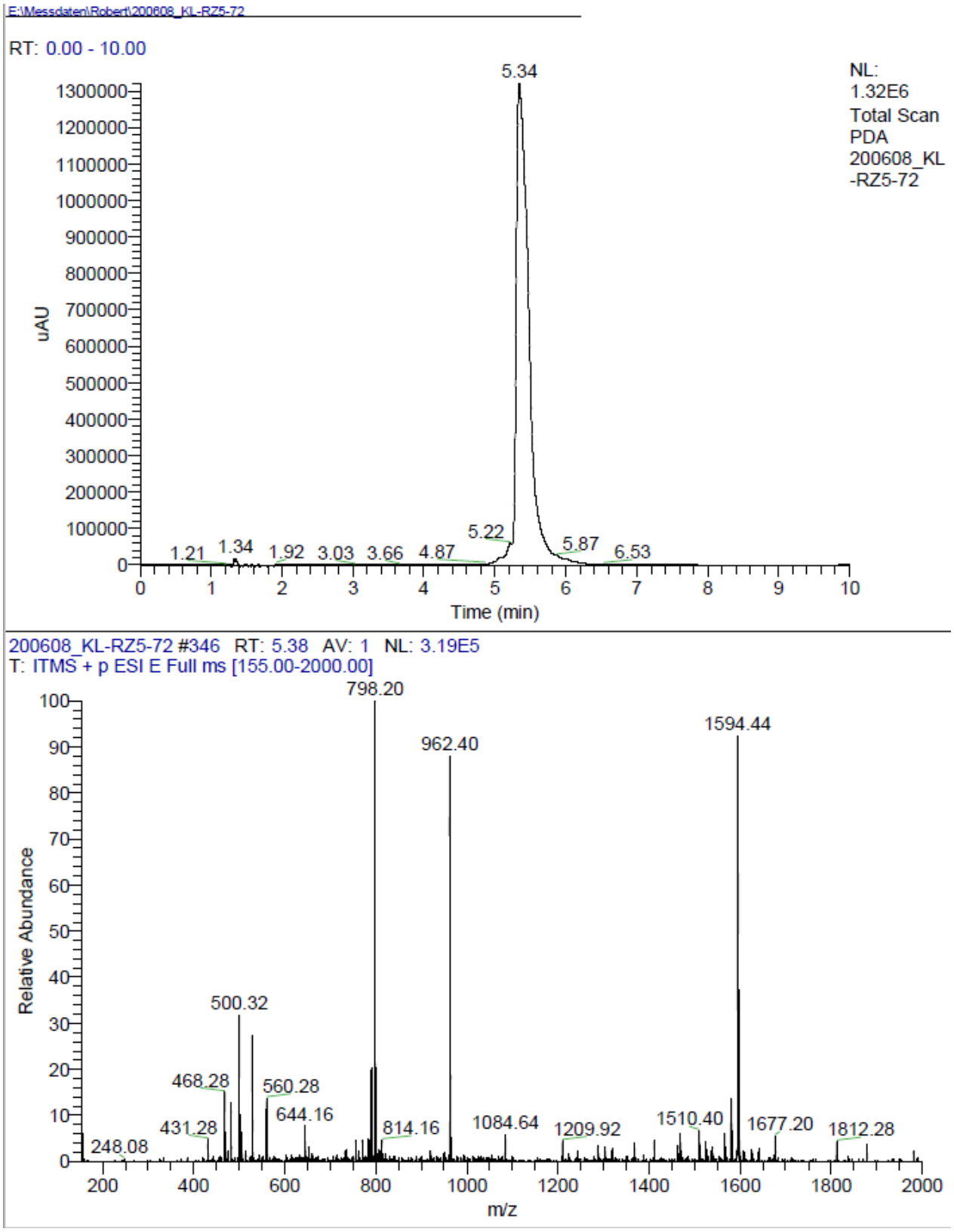
Instrument: Bruker Alpha	
Filename: 200325_0944.0	Number of Scans: 16
Sample Name: KL-RZ5-017	Operator Name: ir-user
Technique: ATR Platinum Diamond 1 Reflection	Date & Time of Measurement: 25/03/2020 09:44:27

25.03.2020

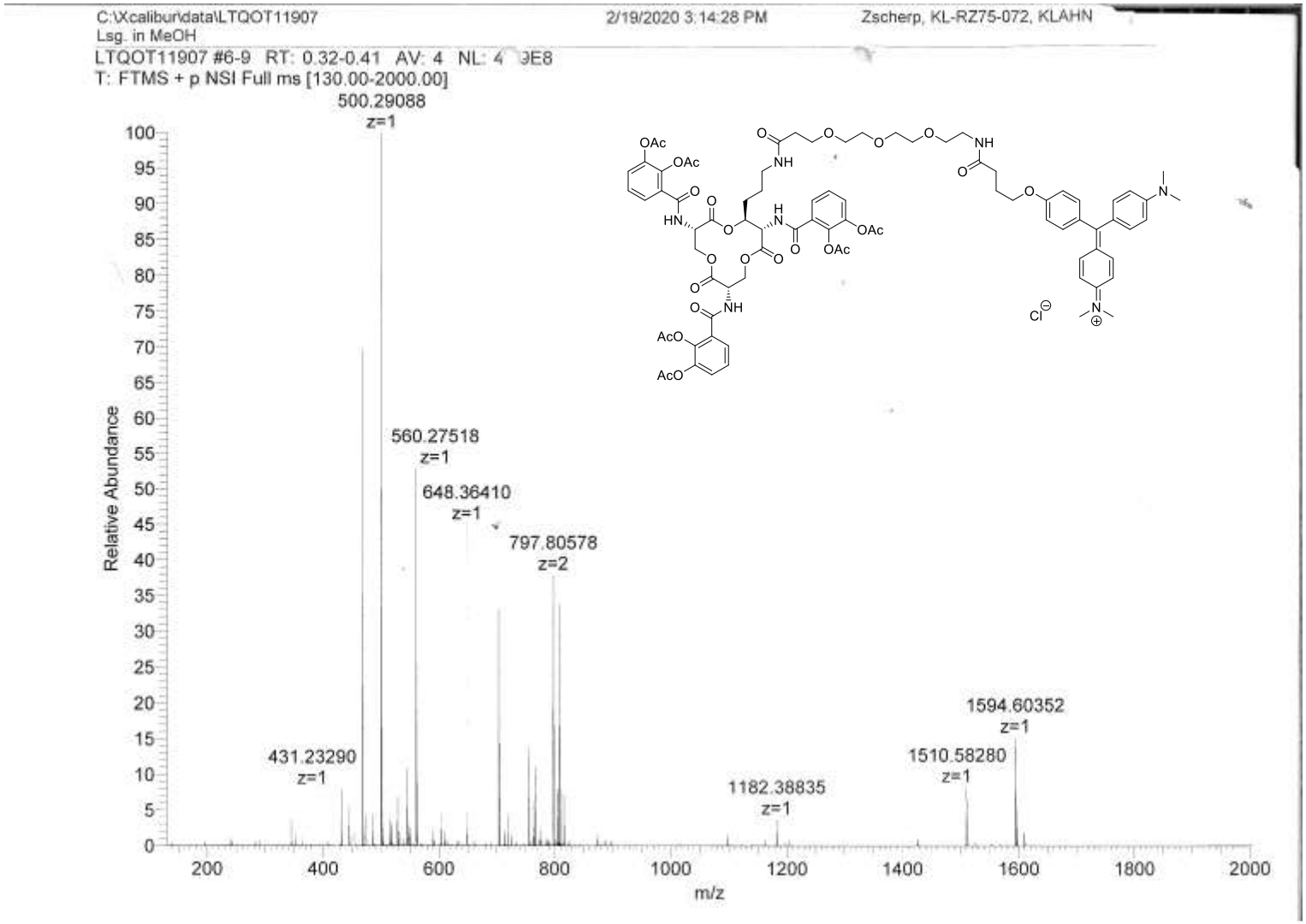
(AcO)Ent$_{KL}$-PEG$_3$-MG (^{1}H NMR)



(AcO)Ent$_{KL}$-PEG$_3$-MG (HPLC-LRMS)



(AcO)Ent$_{KL}$-PEG$_3$-MG (HRMS)



(AcO)Ent$_{KL}$-PEG$_3$-MG (UV/Vis)

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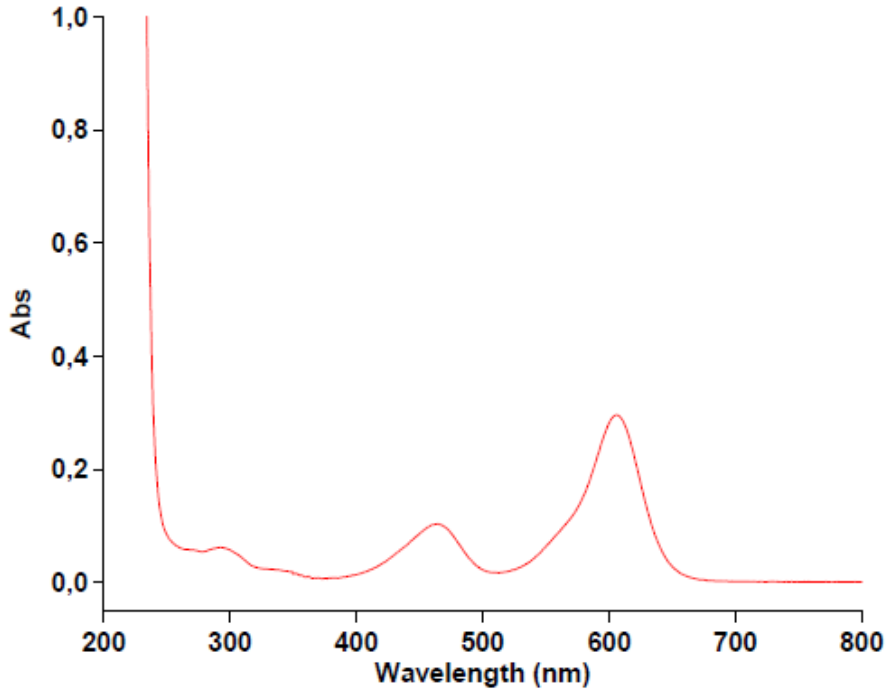


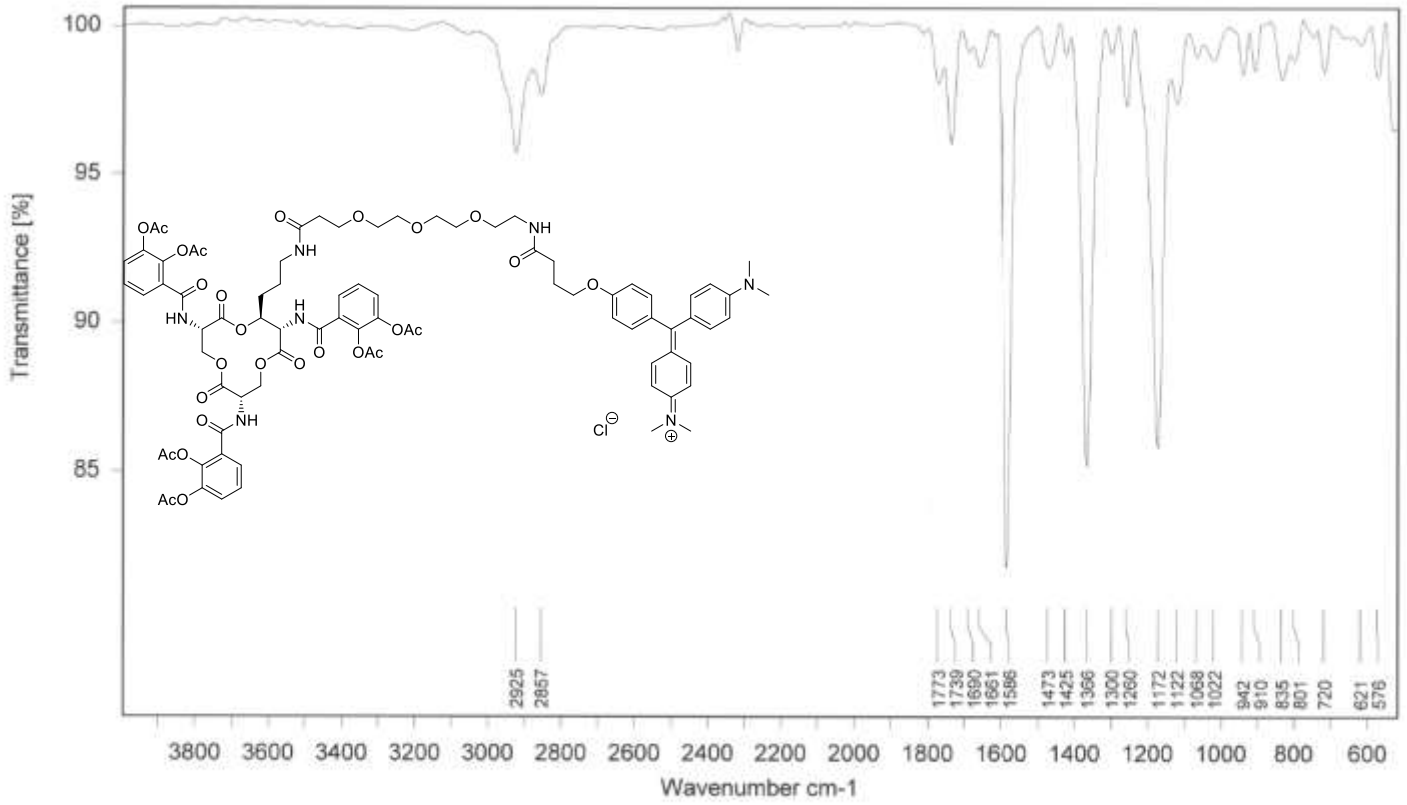
Sample Name: KL-RZ5-072

Collection Time	11.06.2020 14:24:08
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
606,00	0,296
465,00	0,104
215,00	3,128

61 µg in 10 mL MeOH

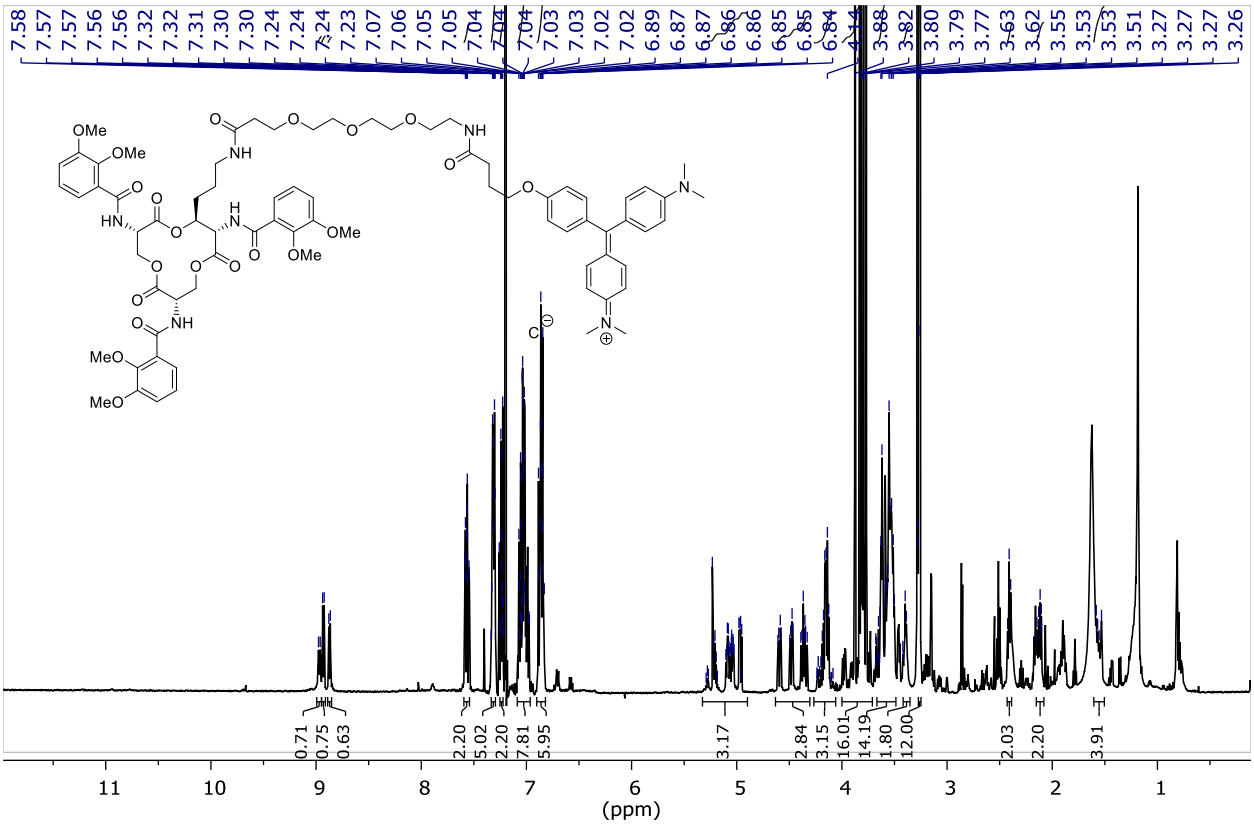
(AcO)Ent$_{KL}$-PEG$_3$-MG (ATR-IR)



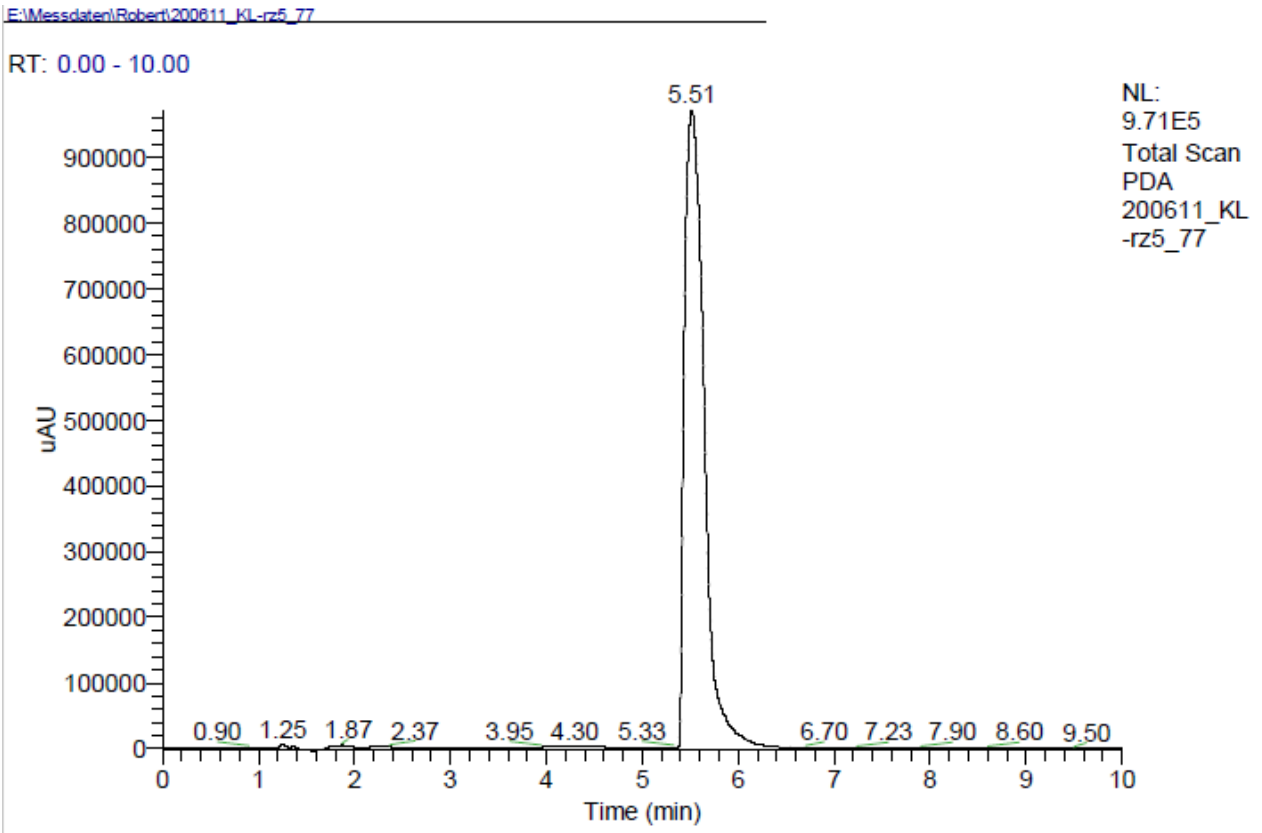
Instrument: Bruker Tensor 27	
Filename: zsr29281.0	Number of Scans: 32
Sample Name: KL-RZ5-072	Operator Name: Default
Technique: Diamond ATR	Date & Time of Measurement: 13.03.2020 11:43:24

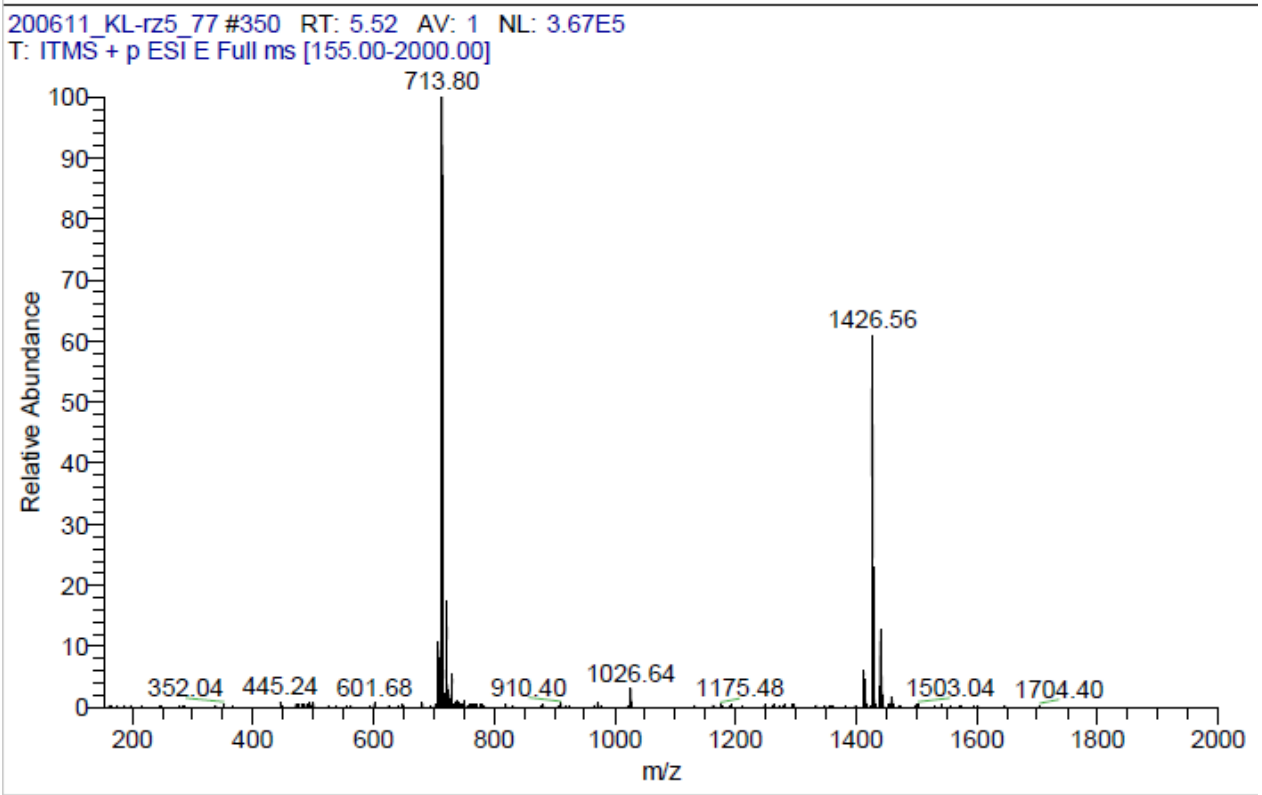
13.03.2020

(MeO)Ent$_{KL}$-PEG$_3$-MG (^{1}H NMR)

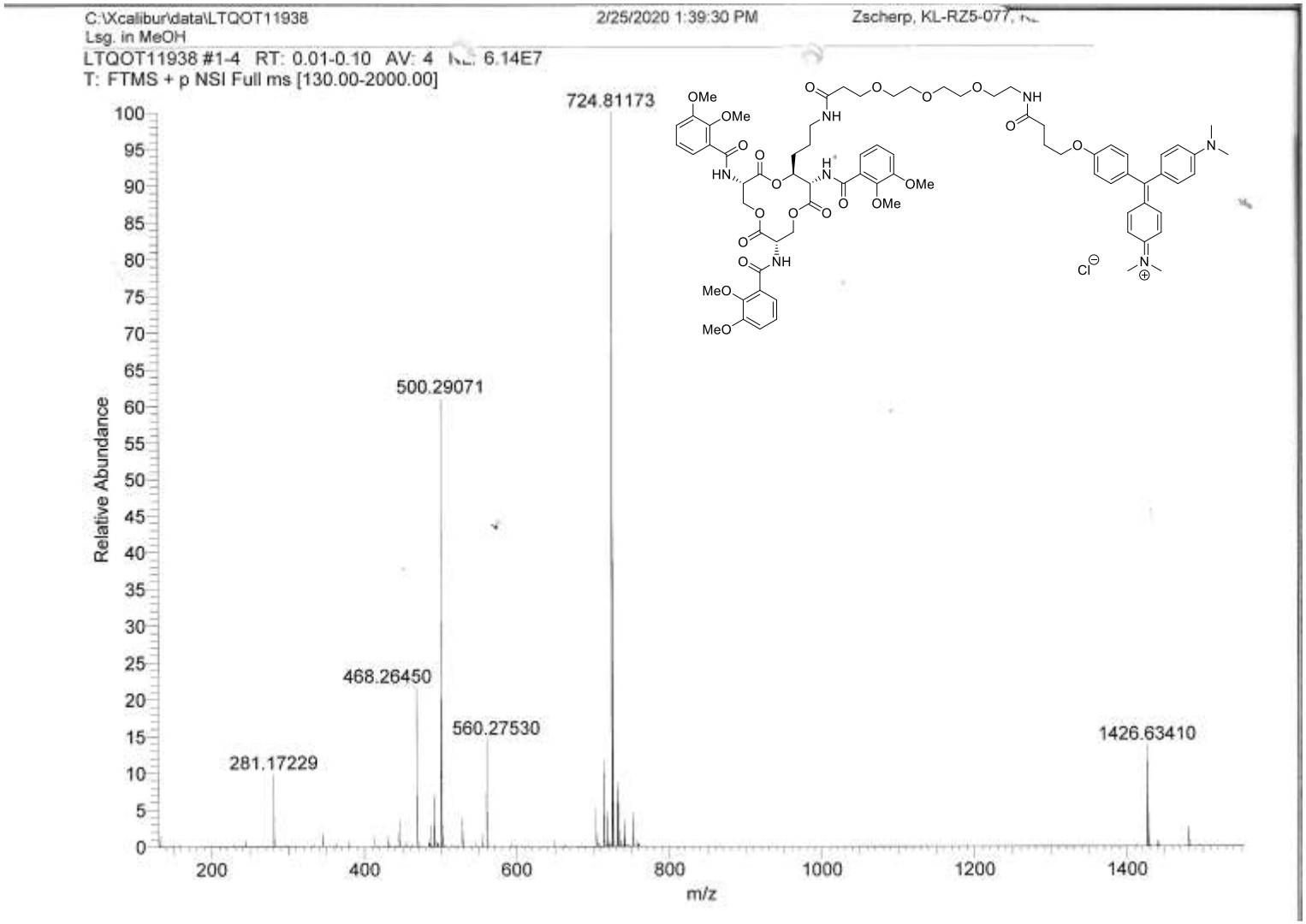


(MeO)Ent$_{KL}$-PEG$_3$-MG (HPLC-LRMS)



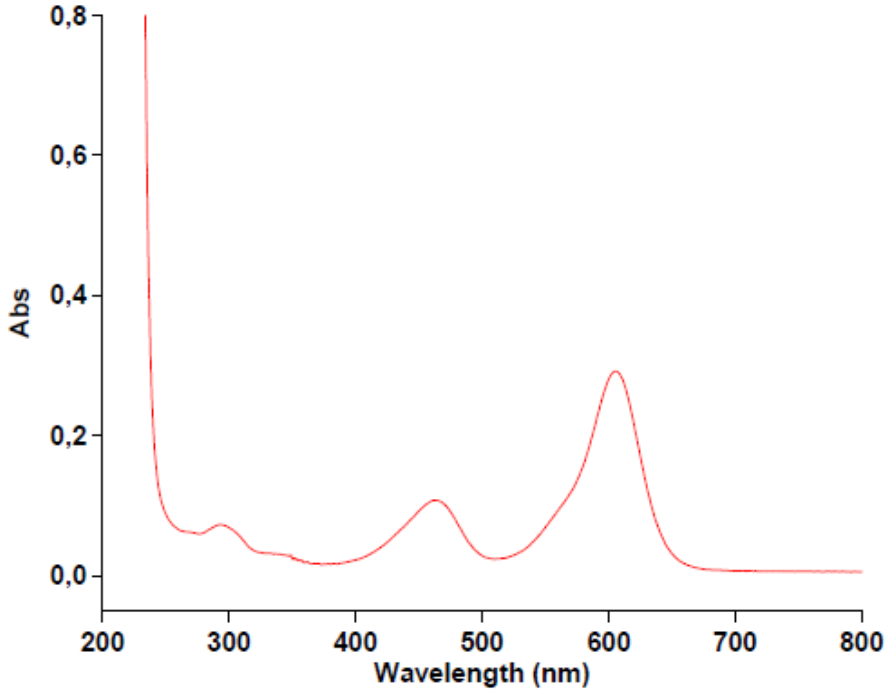


(MeO)Ent$_{KL}$-PEG$_3$-MG (HRMS)



(MeO)Ent$_{KL}$-PEG$_3$-MG (UV/Vis)

04.12.2020 16:59:48 Page 1 of 1



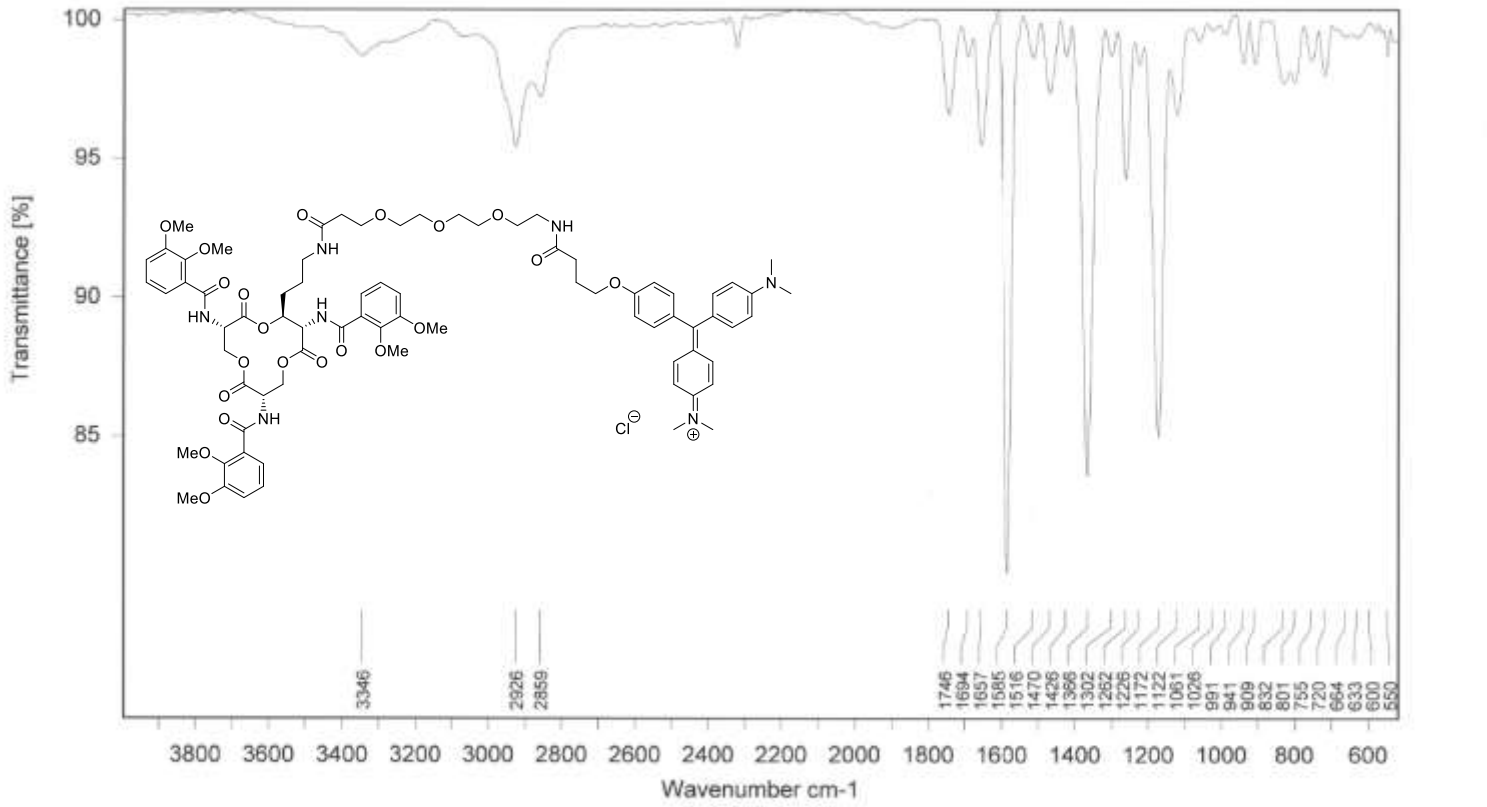
Sample Name: KL-RZ5-077

Collection Time	11.06.2020 14:34:37
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
605,00	0,292
464,00	0,108
293,00	0,073
217,00	3,085

54 µg in 10 mL MeOH

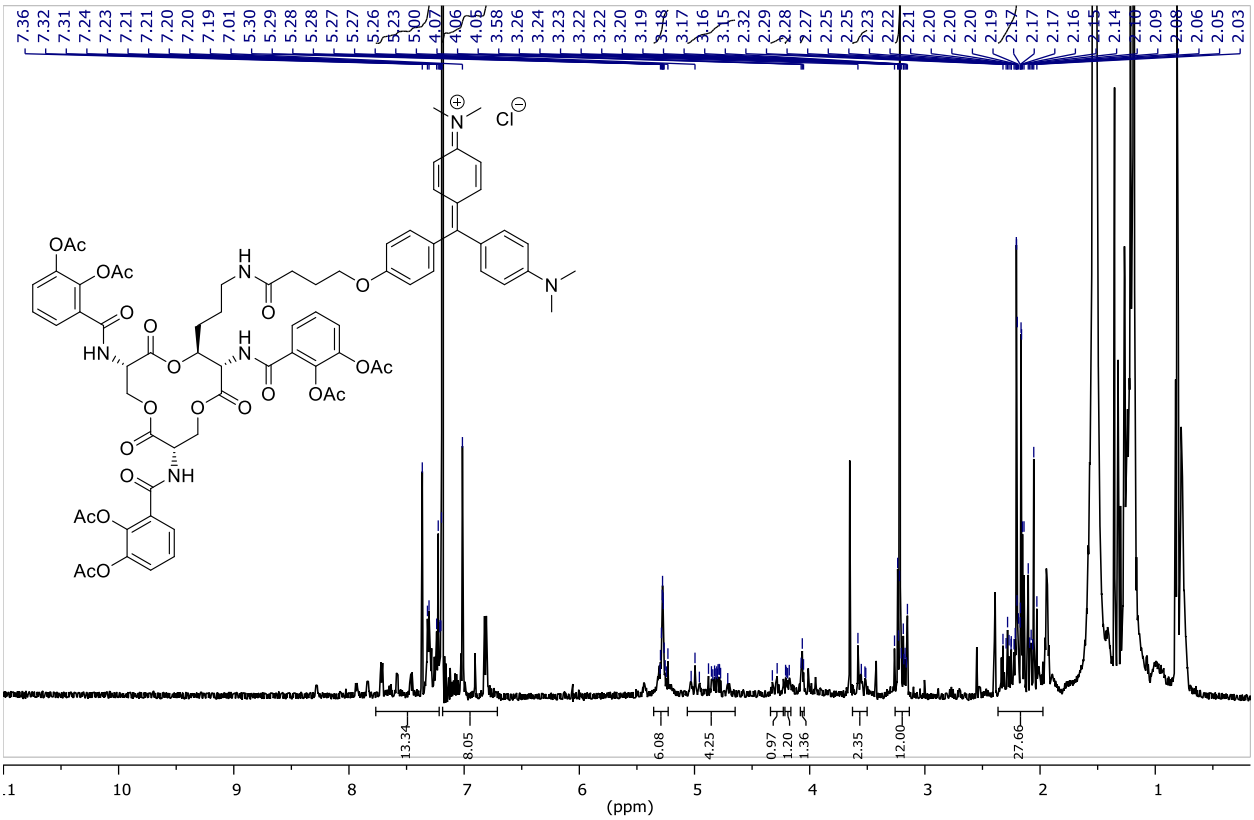
(MeO)Ent$_{KL}$-PEG$_3$-MG (ATR-IR)



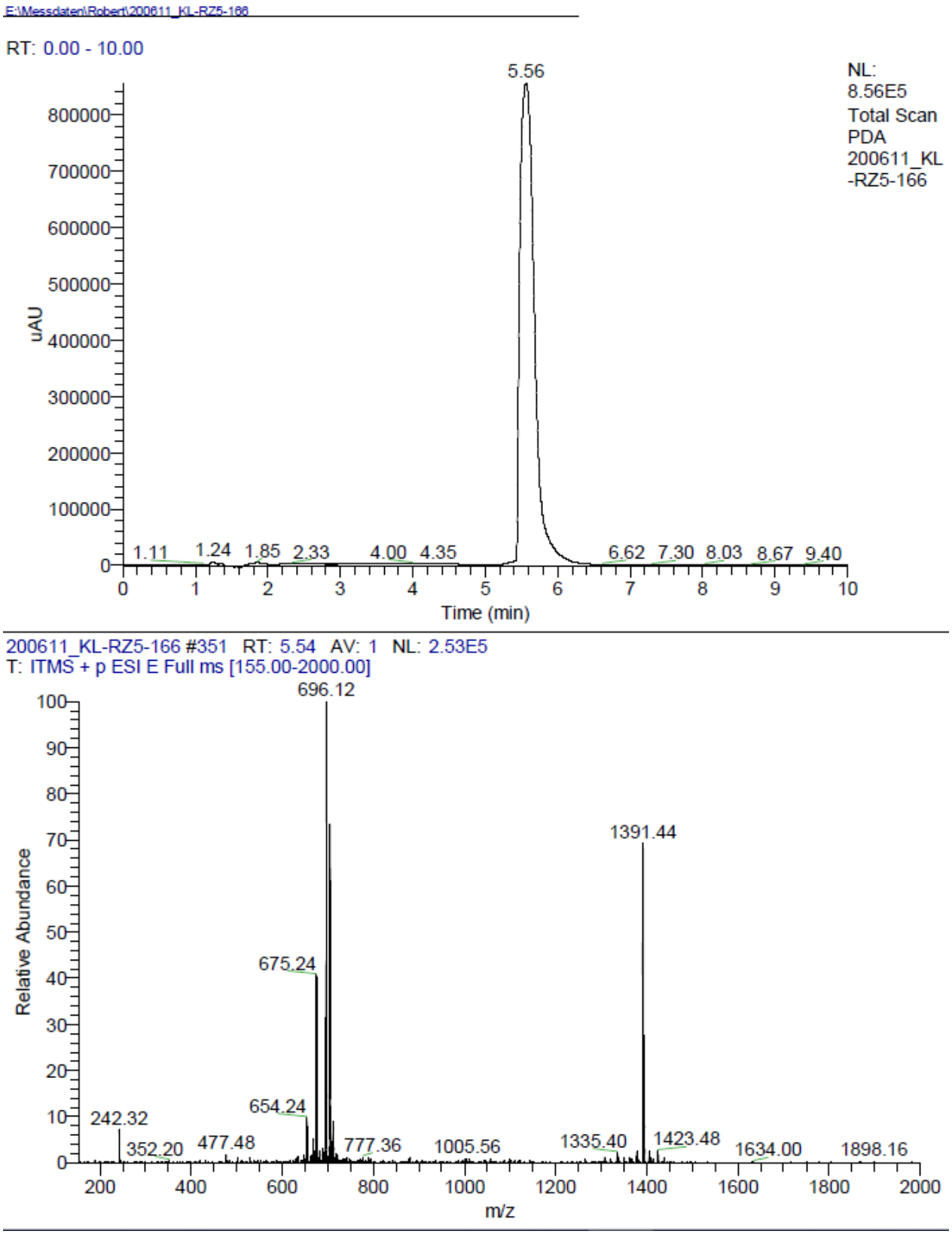
Instrument: Bruker Tensor 27	
Filename: zsr29282.0	Number of Scans: 32
Sample Name: KL-RZ5-077	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 13.03.2020 11:49:25

13.03.2020

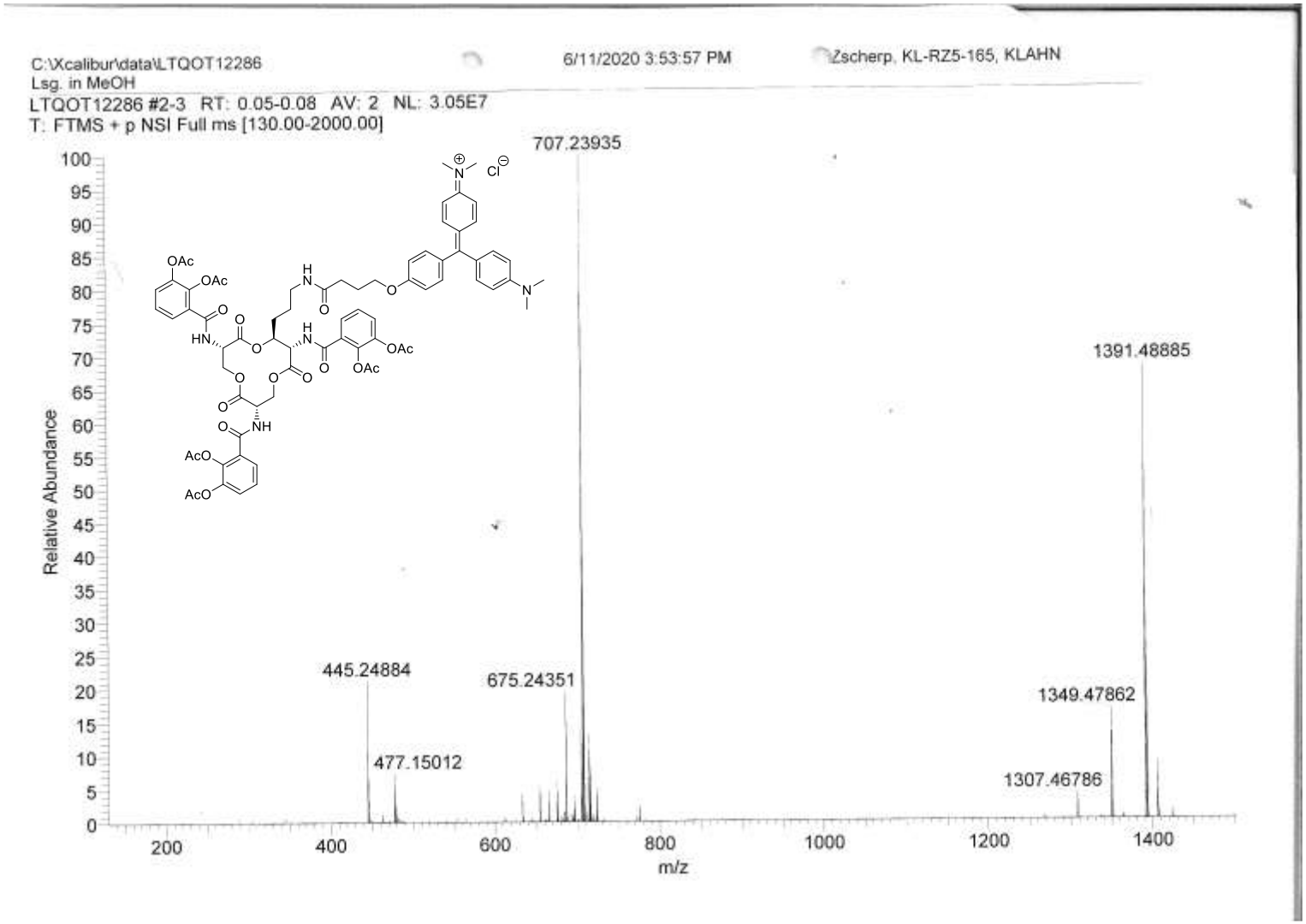
(AcO)Ent$_{KL}$-MG (^{1}H NMR)



(AcO)Ent$_{KL}$-MG (HPLC-LRMS)



(AcO)Ent$_{KL}$-MG (HRMS)



(AcO)Ent$_{KL}$-MG (UV/Vis)

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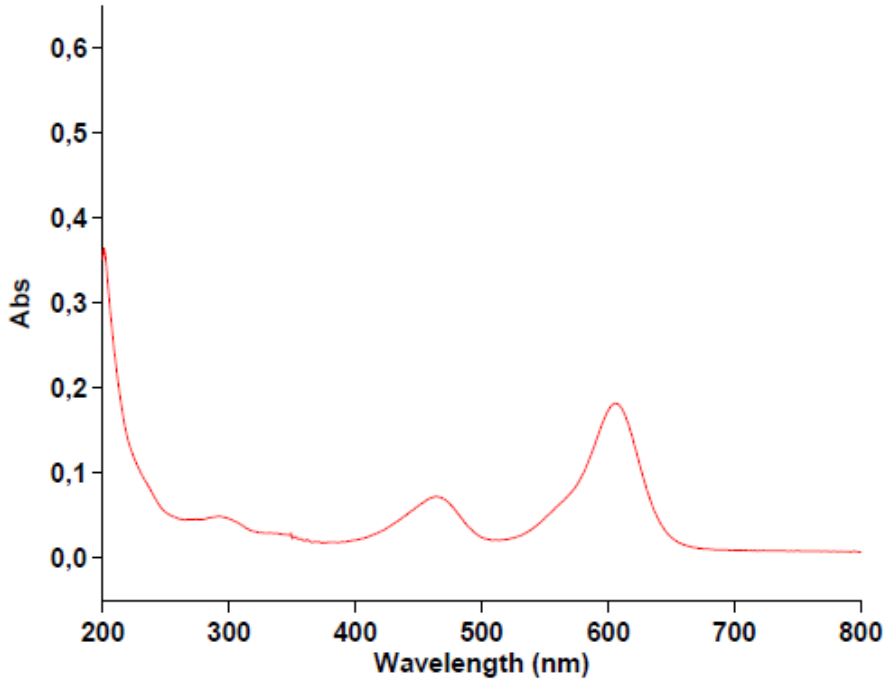


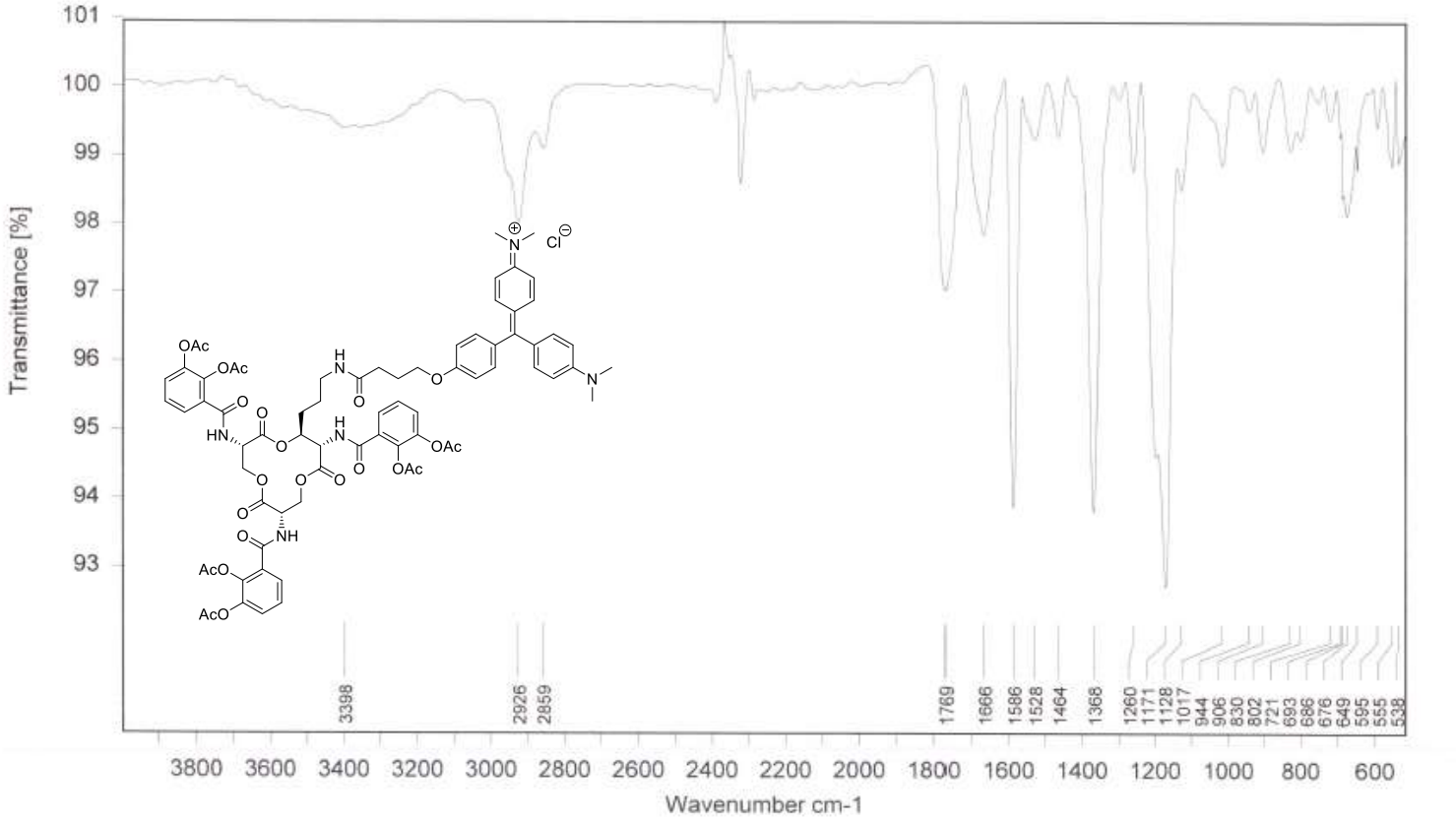
Sample Name: KL-RZ5-166

Collection Time	11.06.2020 14:48:52
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
606,00	0,182
463,00	0,073
201,00	0,365

41 µg in 10 mL MeOH

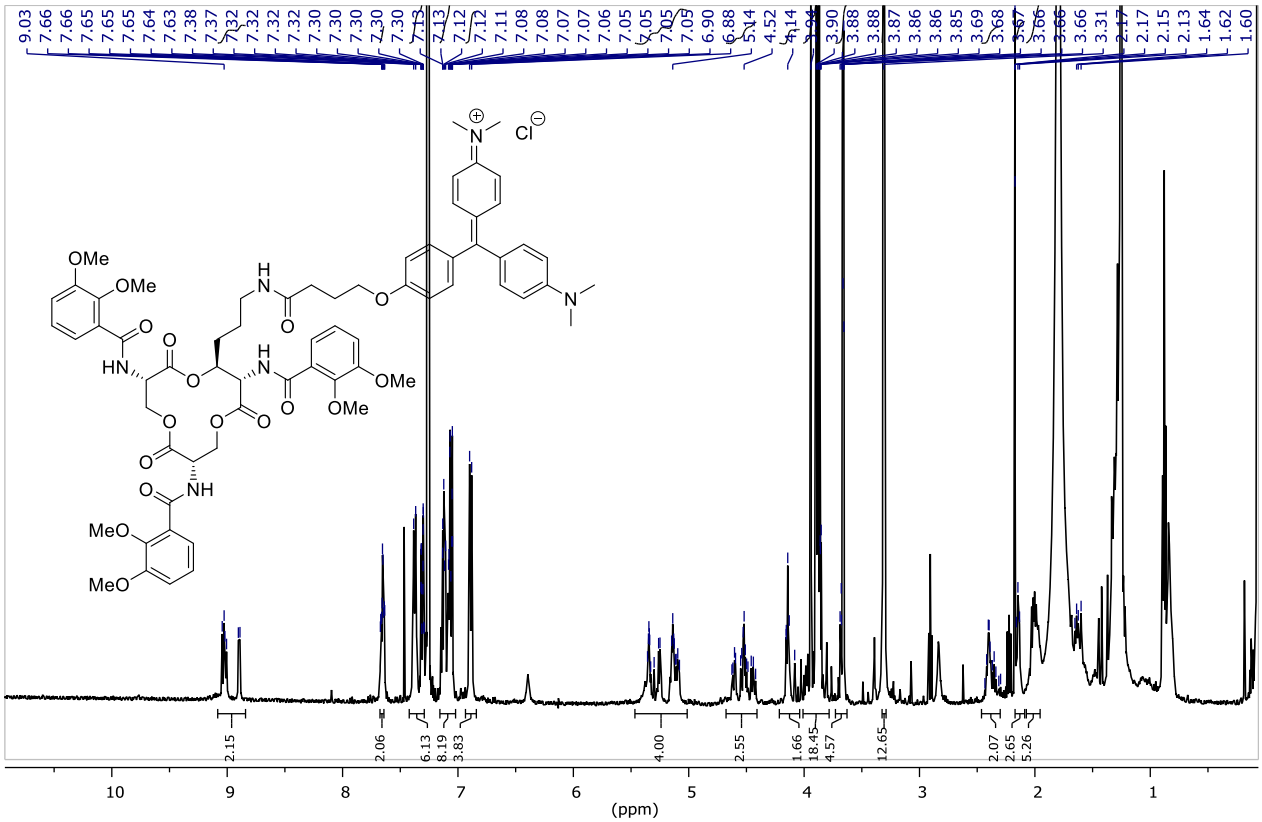
(AcO)Ent$_{KL}$-MG (ATR-IR)



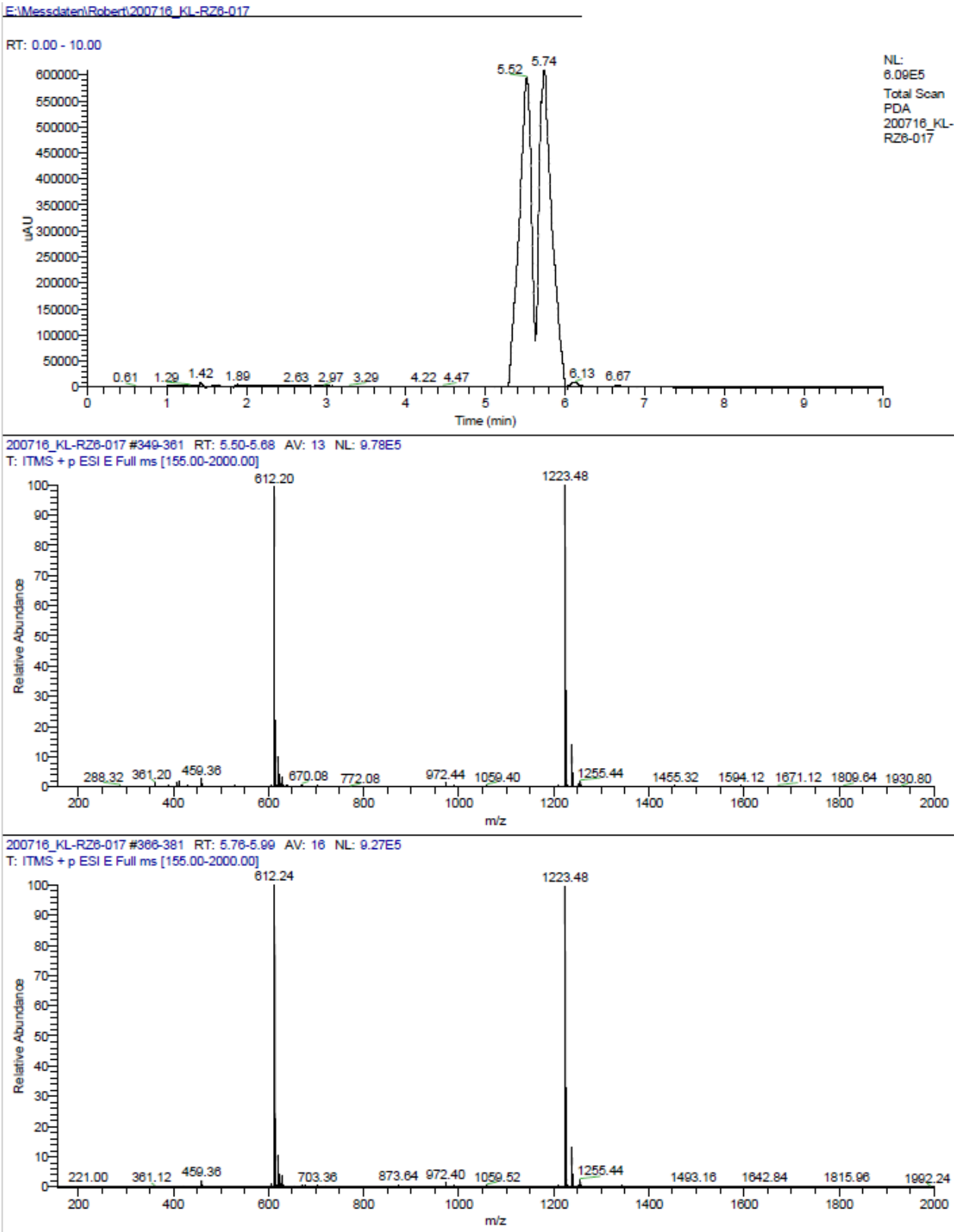
Instrument: Bruker Tensor 27	
Filename: zsr29607.4	Number of Scans: 32
Sample Name: KL-RZ5-166	Operator Name: Default
Technique: Diamant-ATR	Date * Time of Measurement: 12.06.2020 11:53:07

12.06.2020

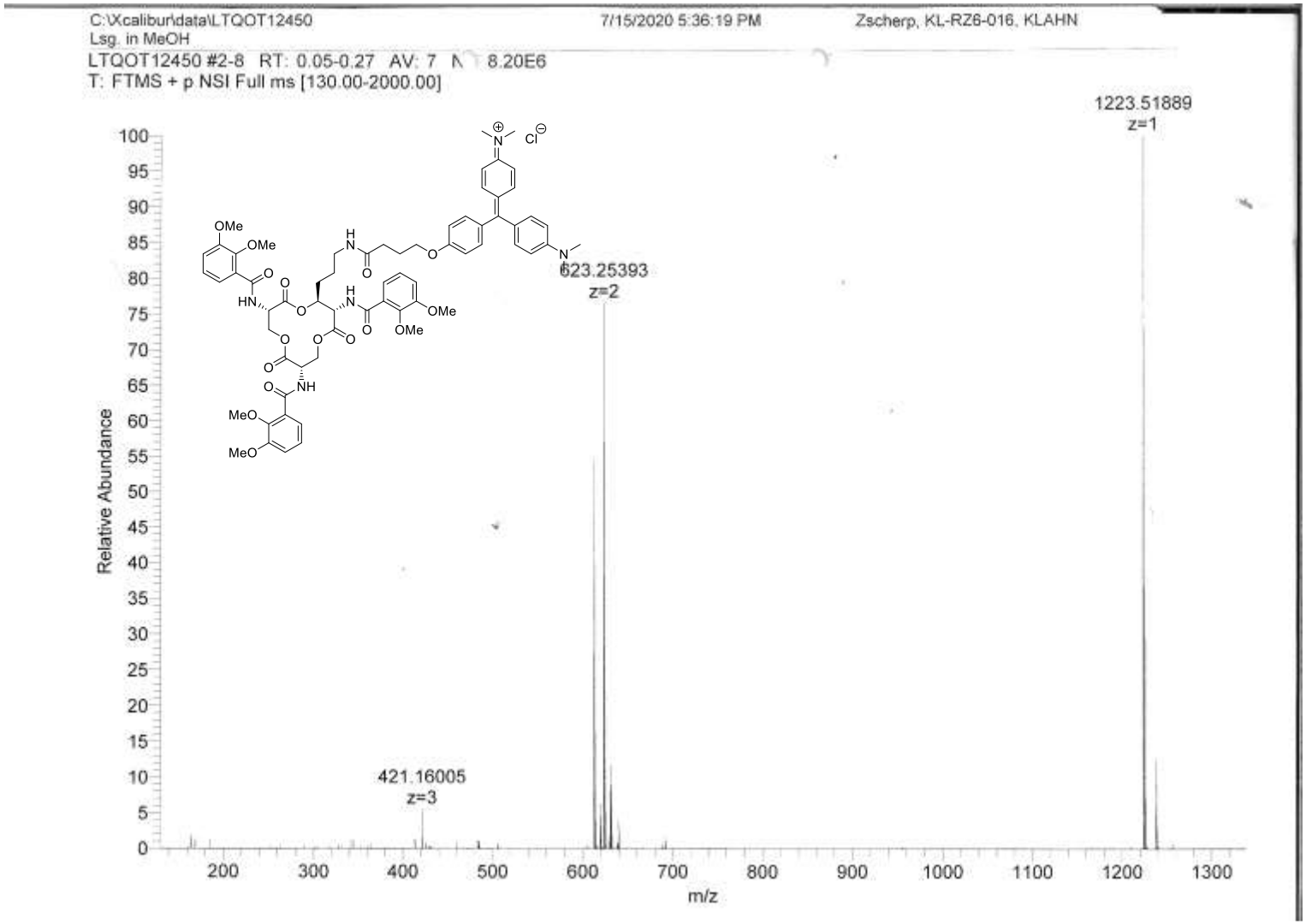
(MeO)Ent$_{KL}$-MG (^{1}H NMR)



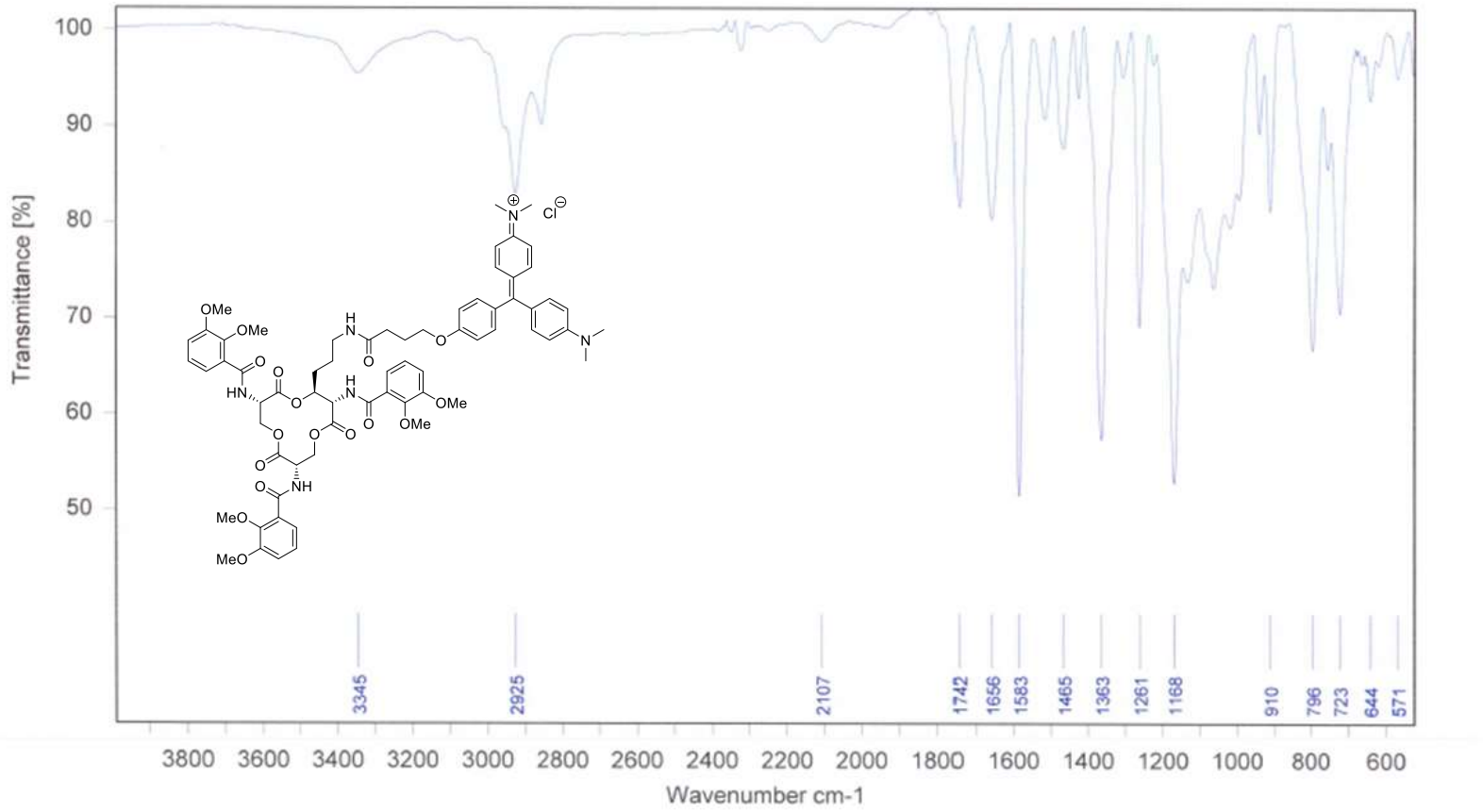
(MeO)Ent$_{KL}$-MG (HPLC-LRMS)



(MeO)Ent$_{KL}$-MG (HRMS)



(MeO)Ent$_{KL}$-MG (ATR-IR)



Instrument: Bruker Tensor 27	
Filename: zsr29683.1	Number of Scans: 46
Sample Name: KL-RZ6-017	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 16/07/2020 11:21:25

16.07.2020

(MeO)Ent$_{KL}$-MG (UV/Vis)

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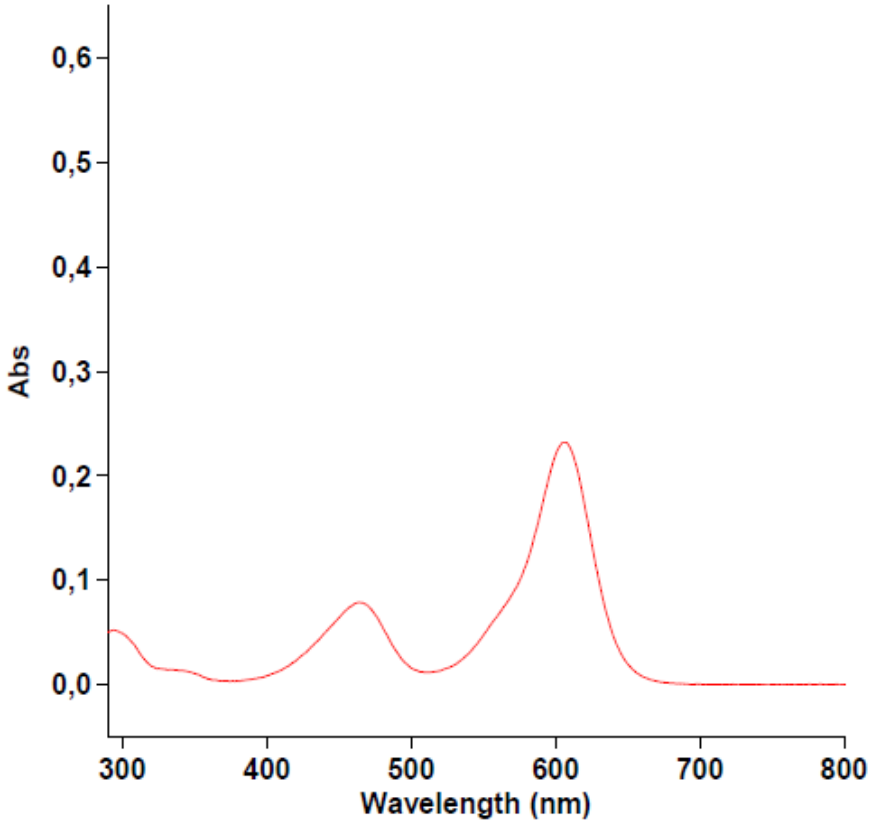


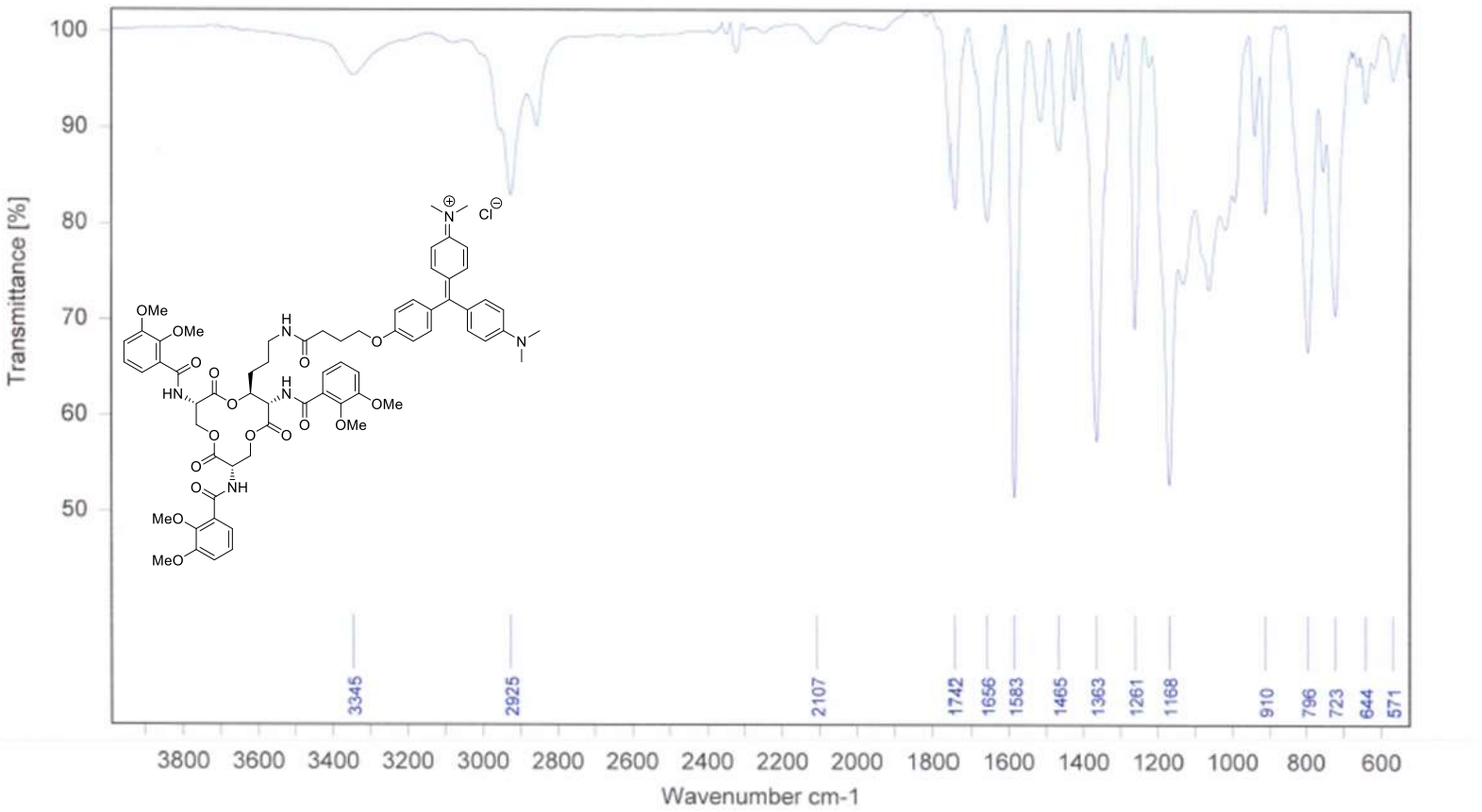
Sample Name: KL-RZ6-017

Collection Time	16.07.2020 13:37:29
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	900,00nm to 290,00nm

Wavelength (nm)	Abs
606,00	0,232
465,00	0,079

74µg in 10 mL MeOH

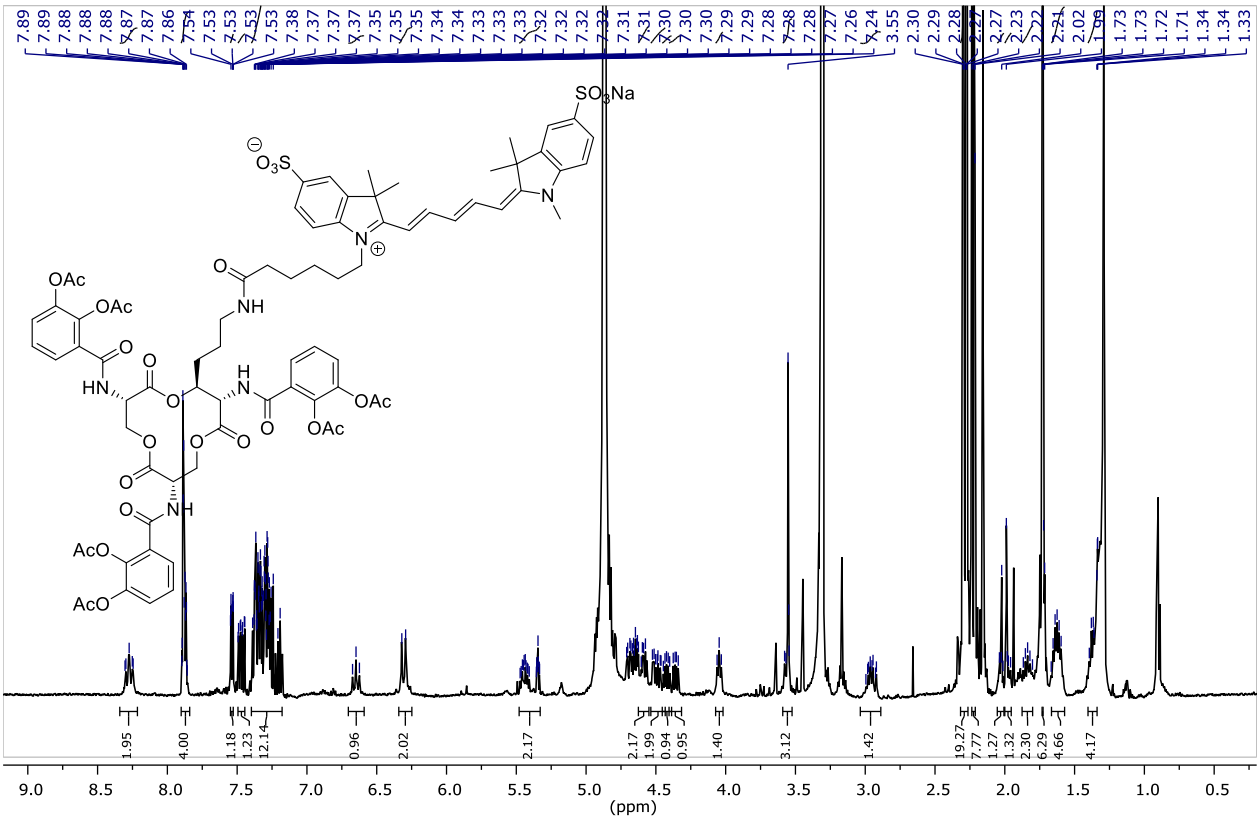
(MeO)Ent$_{KL}$-MG (ATR-IR)



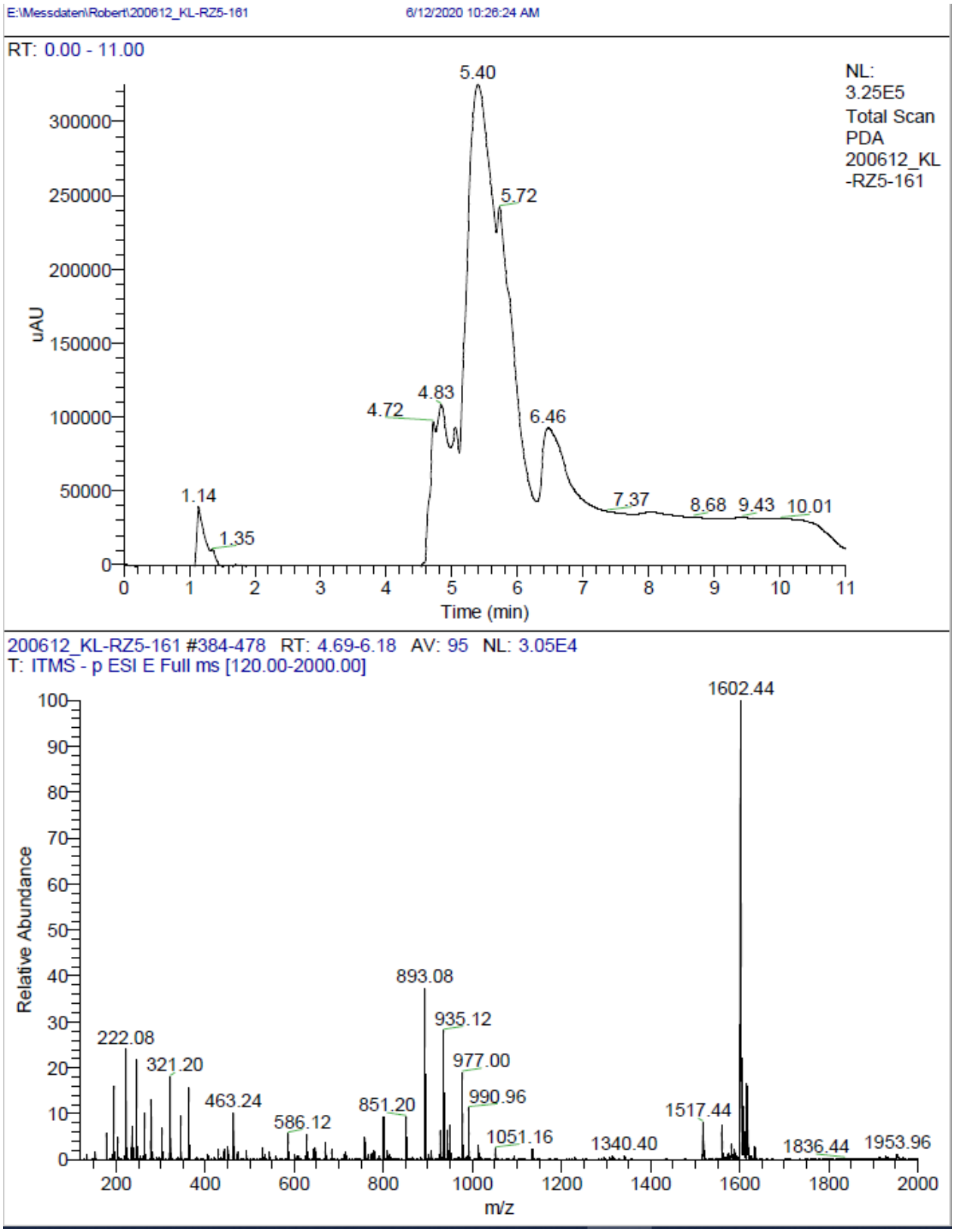
Instrument: Bruker Tensor 27	
Filename: zsr29683.1	Number of Scans: 46
Sample Name: KL-RZ6-017	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 16/07/2020 11:21:25

16.07.2020

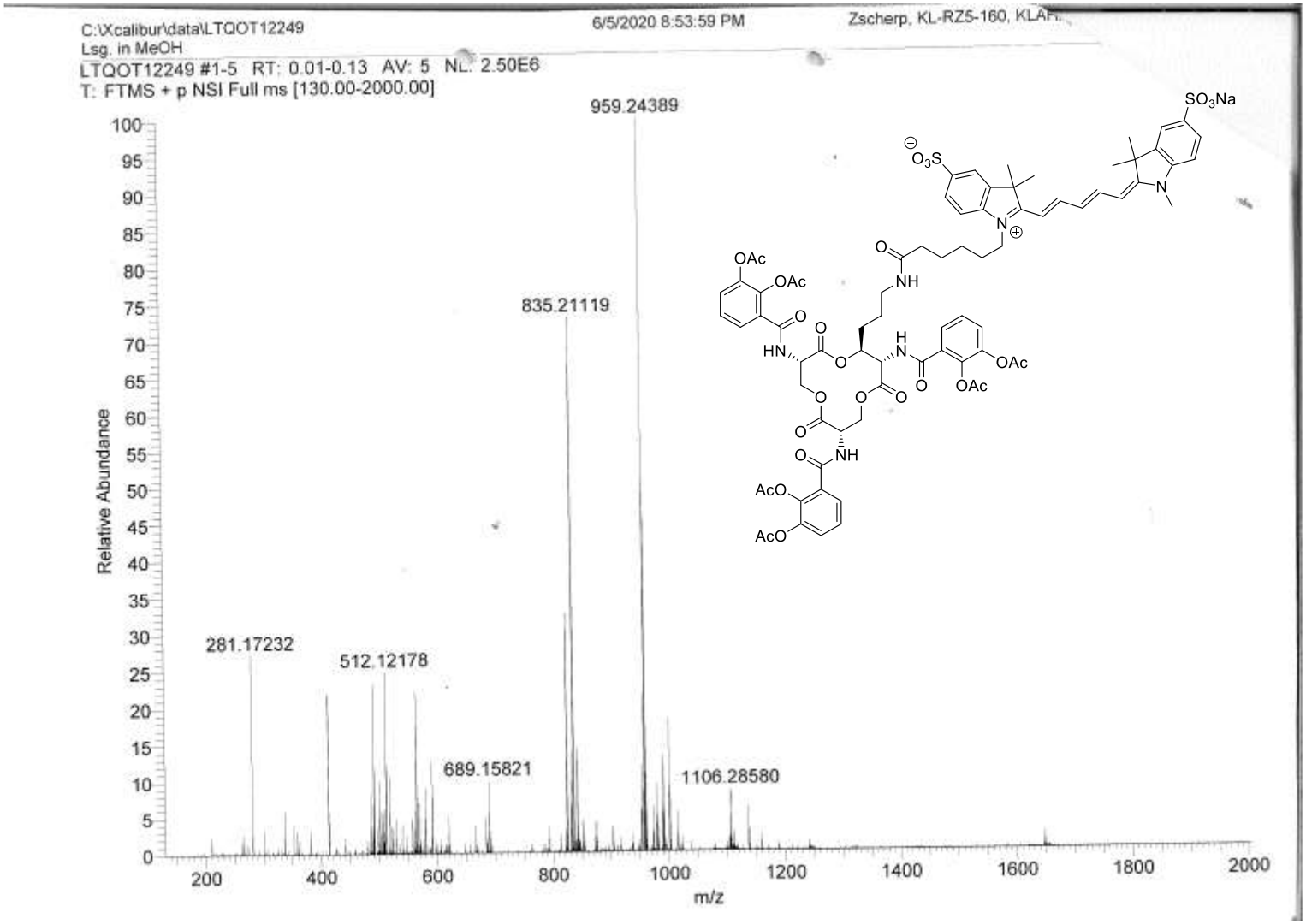
(AcO)Ent$_{KL}$-SulfoCy5 (^{1}H NMR)

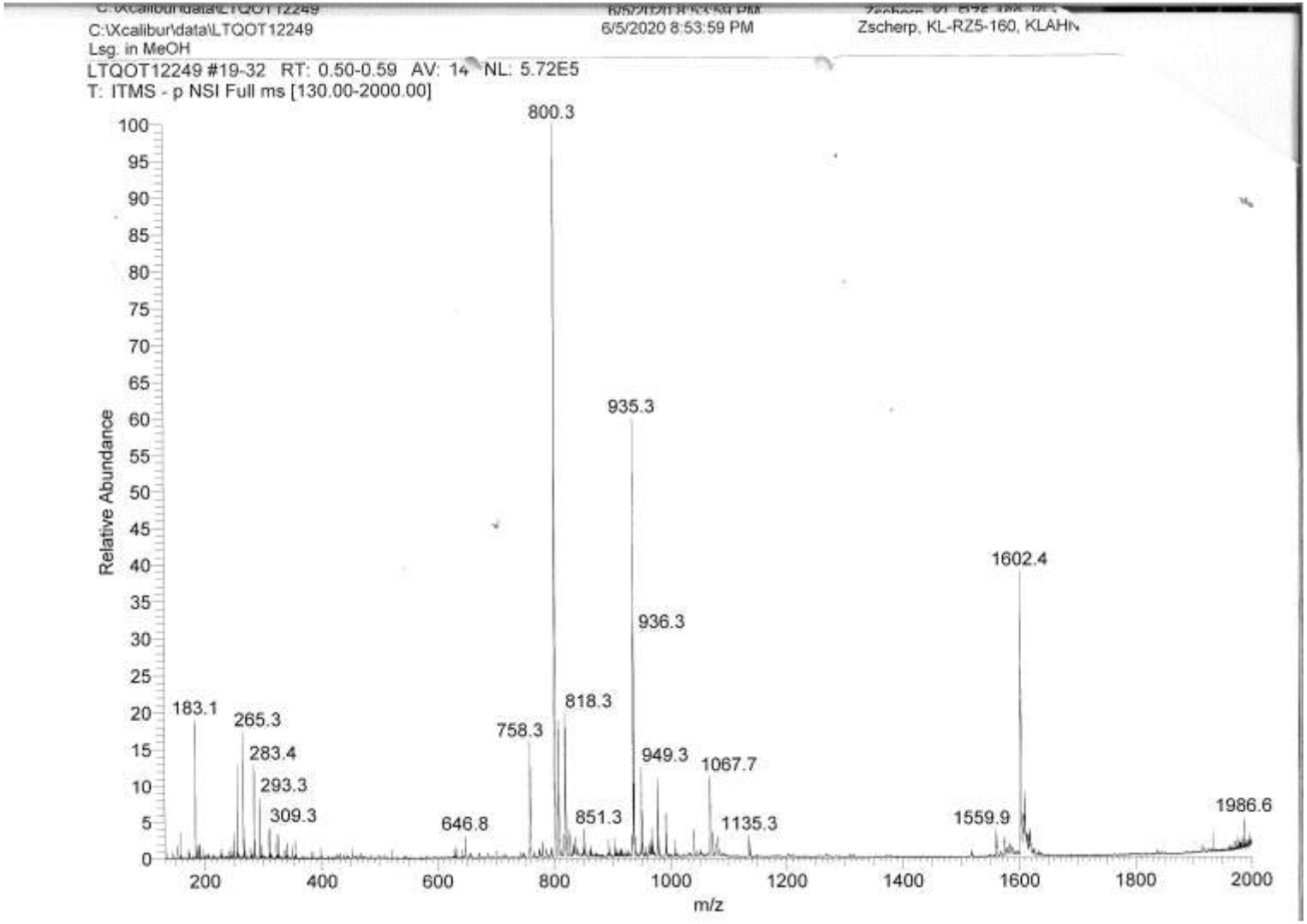


(AcO)Ent$_{KL}$-SulfoCy5 (HPLC-LRMS)

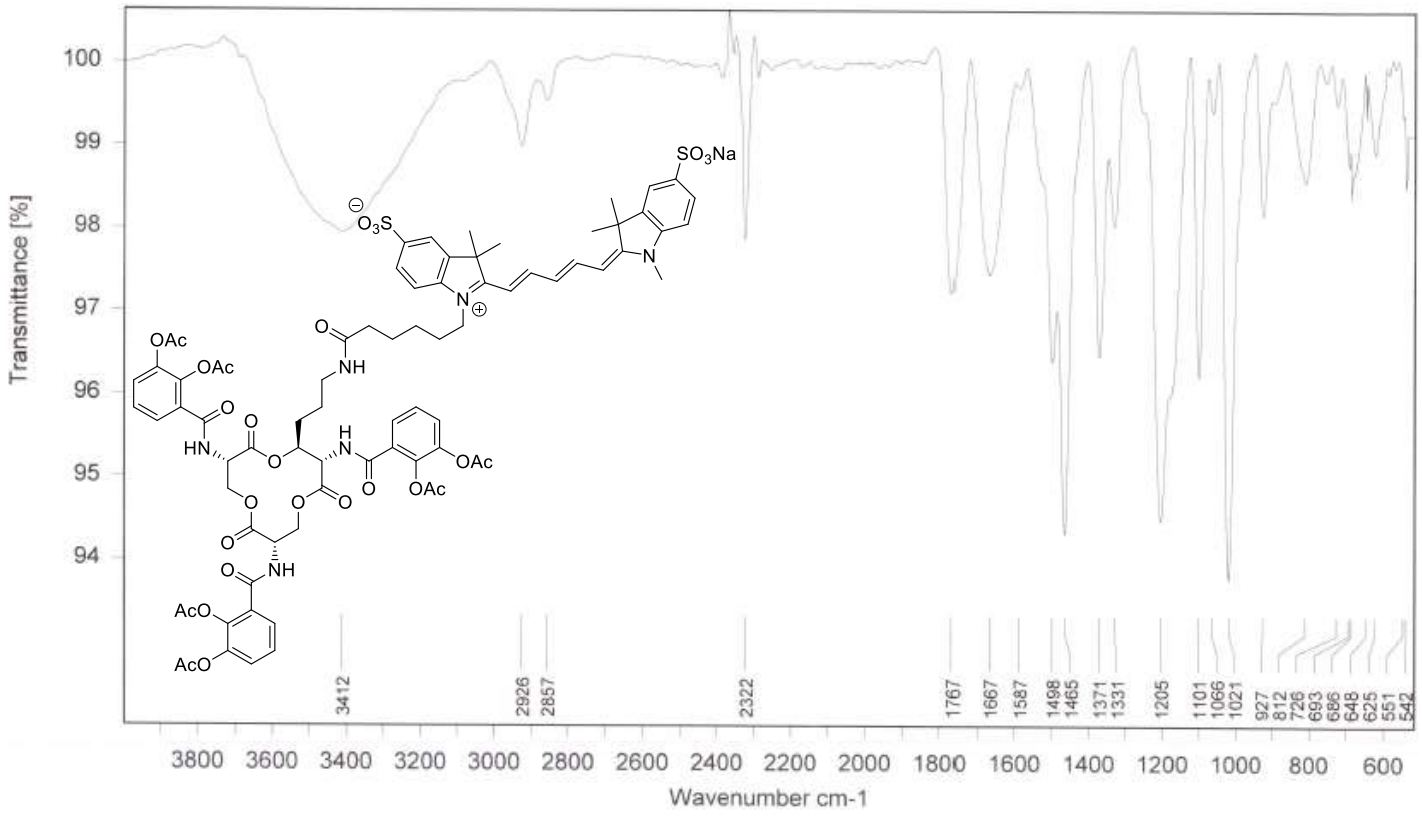


(AcO)Ent$_{KL}$-SulfoCy5 (HRMS)




C:\Xcalibur\data\LTQOT12249
6/5/2020 8:53:59 PM
Lsg. in MeOH
LTQOT12249 #19-32 RT: 0.50-0.59 AV: 14 NL: 5.72E5
T: ITMS - p NSI Full ms [130.00-2000.00]
Relative Abundance
m/z
800.3
935.3
1602.4
936.3
818.3
183.1
265.3
758.3
283.4
949.3
1067.7
293.3
309.3
646.8
851.3
1135.3
1559.9
1986.6

(AcO)Ent$_{KL}$-SulfoCy5 (ATR-IR)

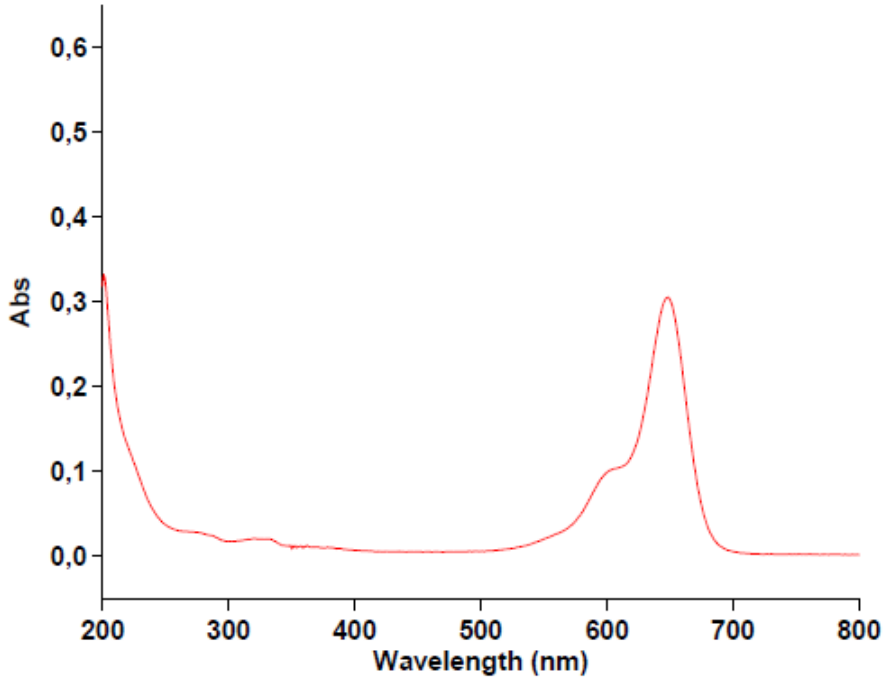


Instrument: Bruker Tensor 27	
Filename: zsr29605.0	Number of Scans: 32
Sample Name: KL-RZ5-160	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 12.06.2020 11:24:28

12.06.2020

(AcO)Ent$_{KL}$-SulfoCy5 (UV/Vis)

04.12.2020 17:02:40 Page 1 of 1



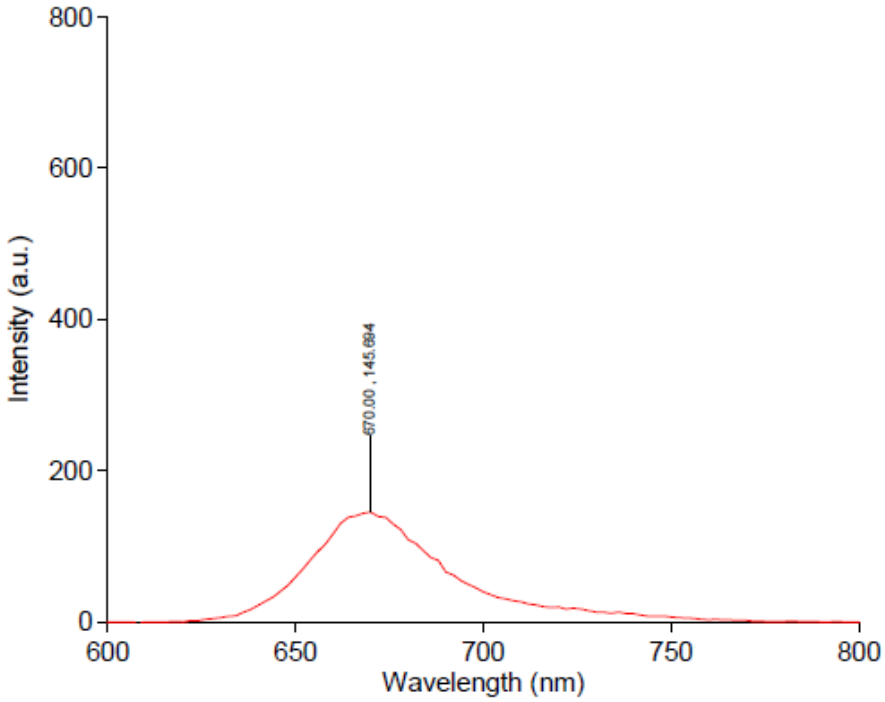
Sample Name: KL-RZ5-160

Collection Time	11.06.2020 14:01:27
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
649,00	0,306
201,00	0,333

44 µg in 10 mL MeOH

(AcO)Ent$_{KL}$-SulfoCy5 (Fluorescence Emission)



Sample name: KL-RZ5-160

Peak table

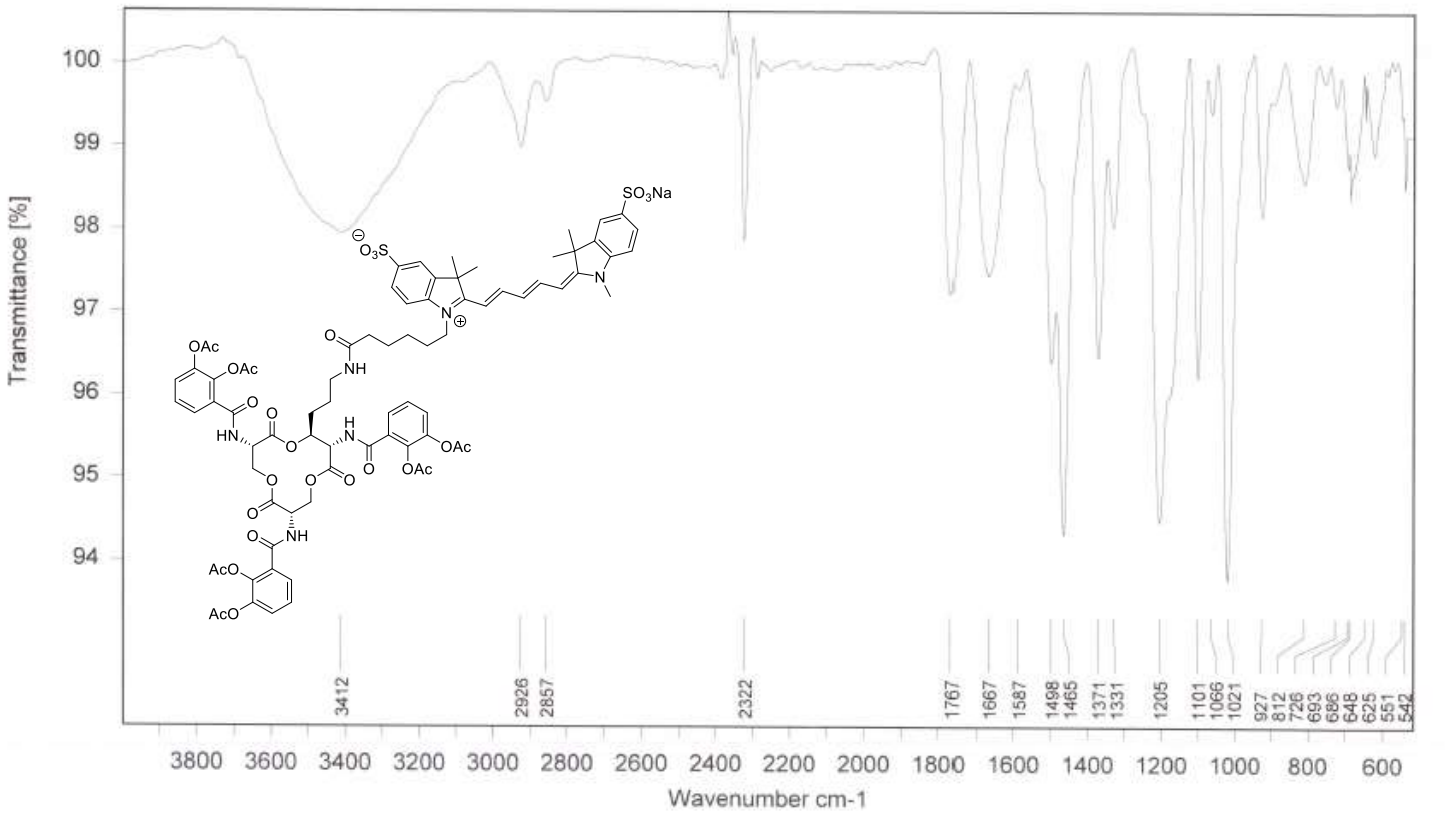
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
670.00	145.694

44 µg in 10 mL MeOH

Excitation wavelength: 640 nm

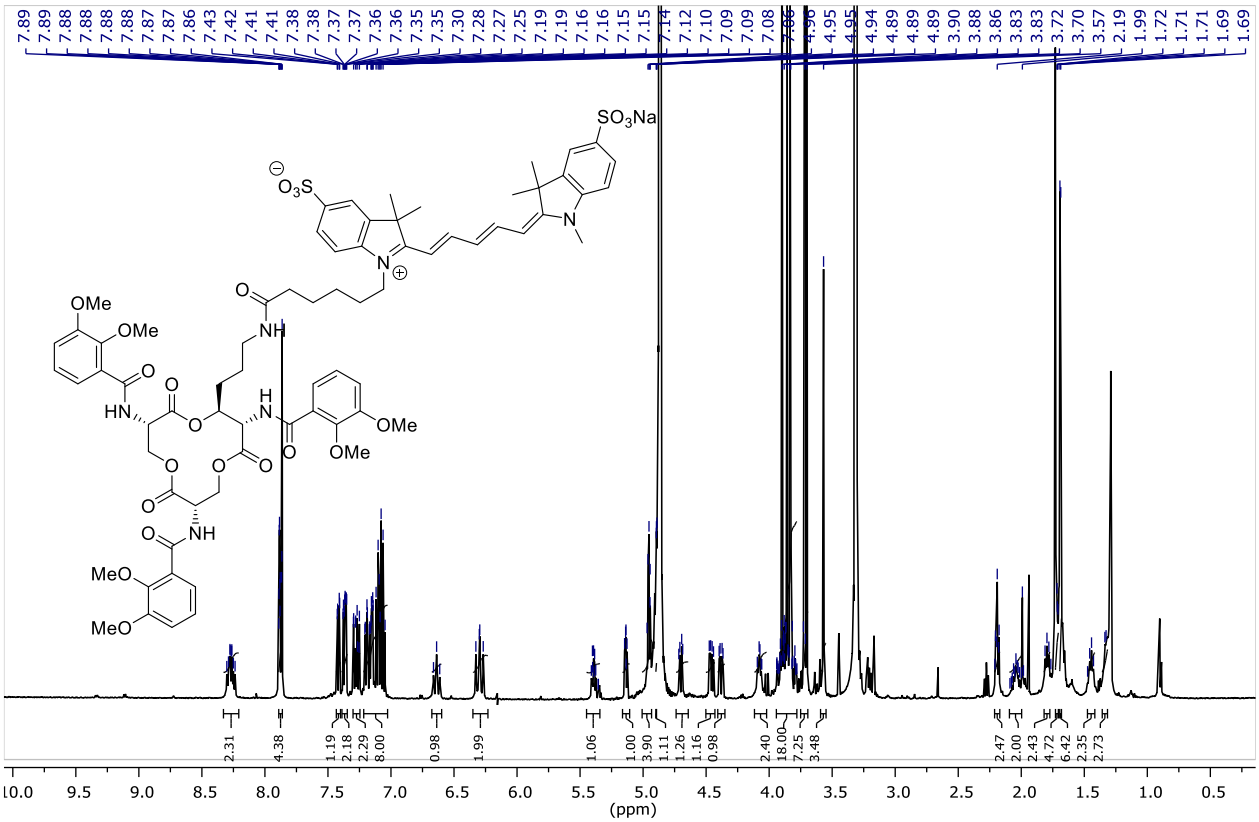
(AcO)Ent$_{KL}$-SulfoCy5 (ATR-IR)



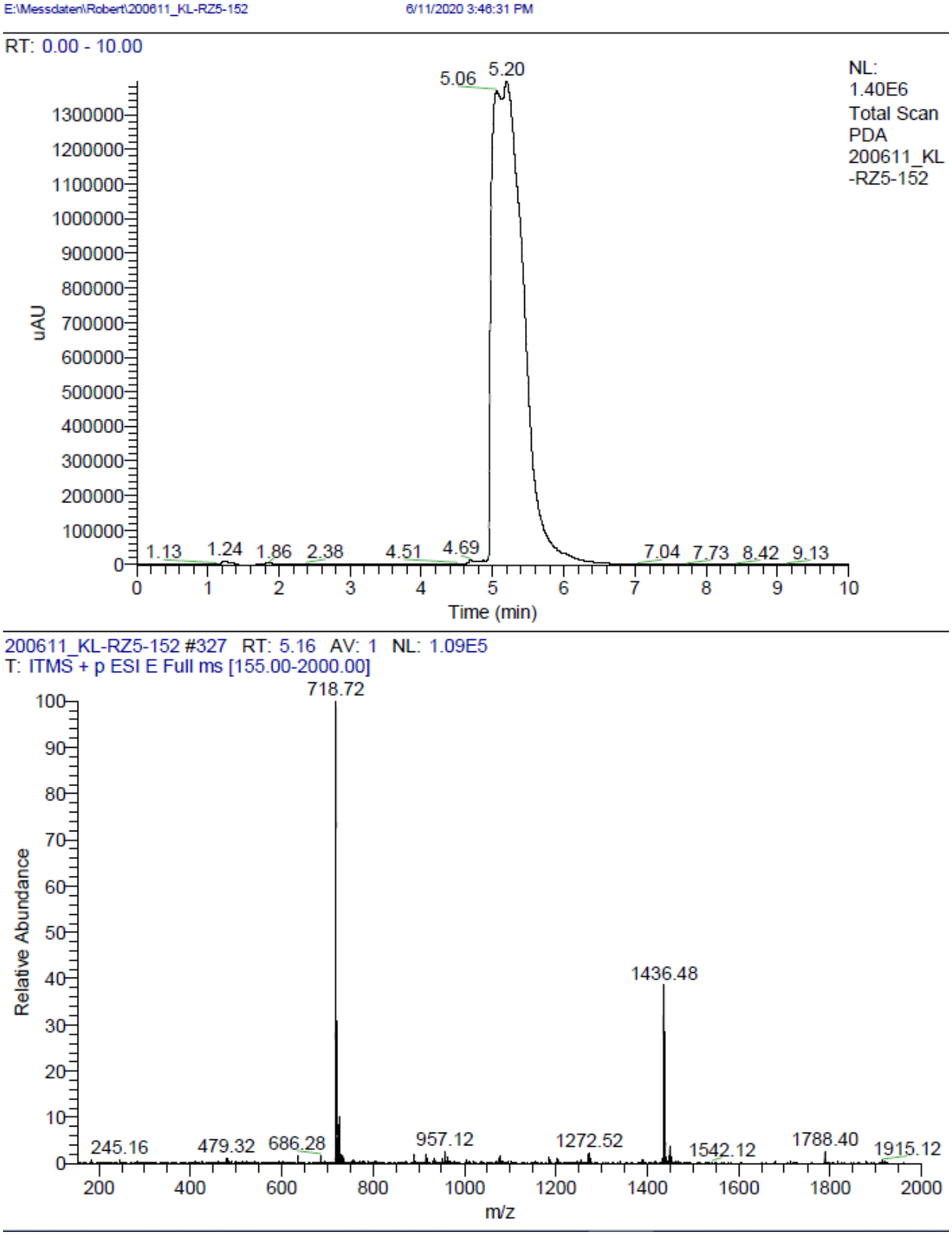
Instrument: Bruker Tensor 27	
Filename: zsr29605.0	Number of Scans: 32
Sample Name: KL-RZ5-160	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 12.06.2020 11:24:28

12.06.2020

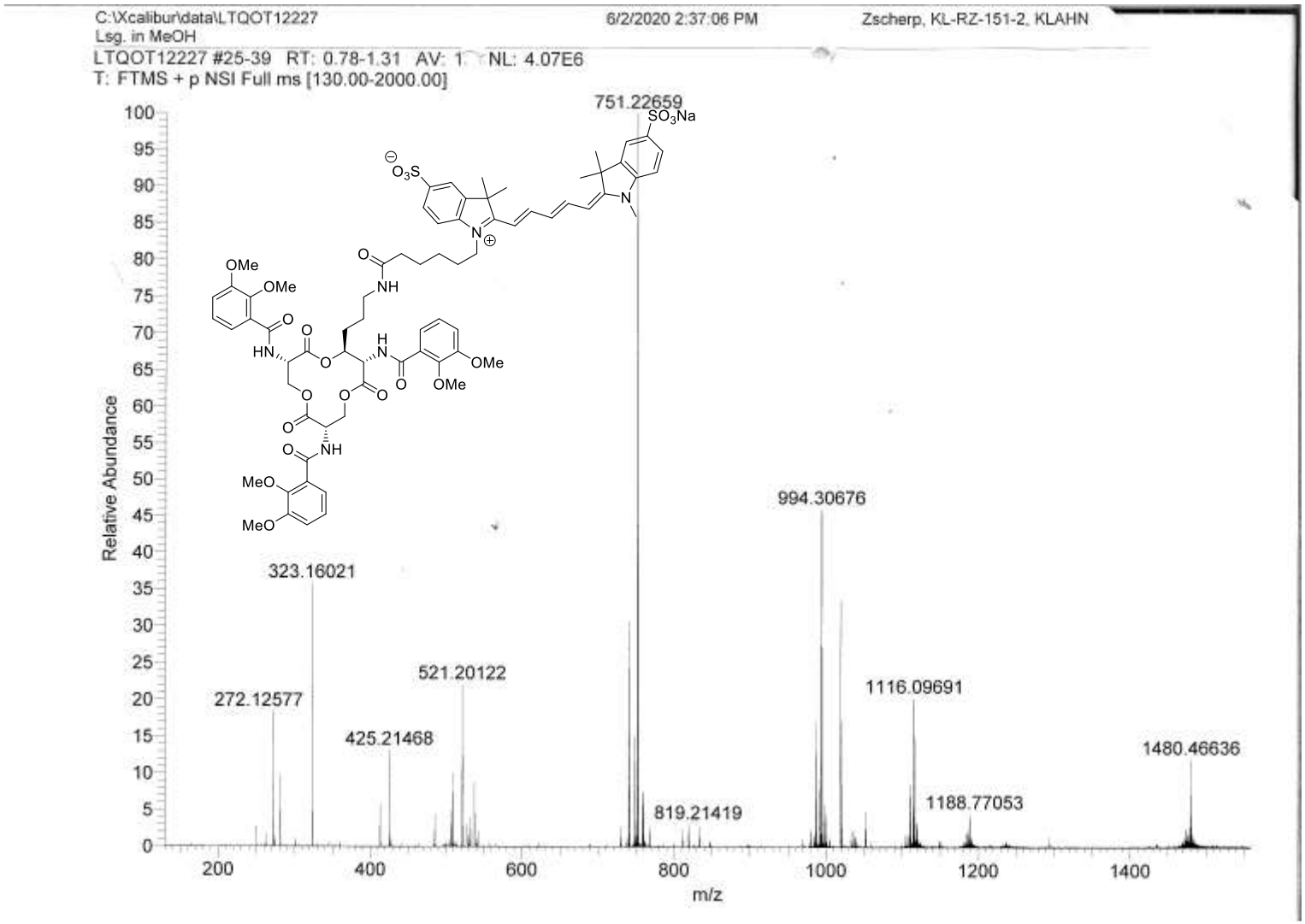
(MeO)Ent$_{KL}$-SulfoCy5 (^{1}H NMR)

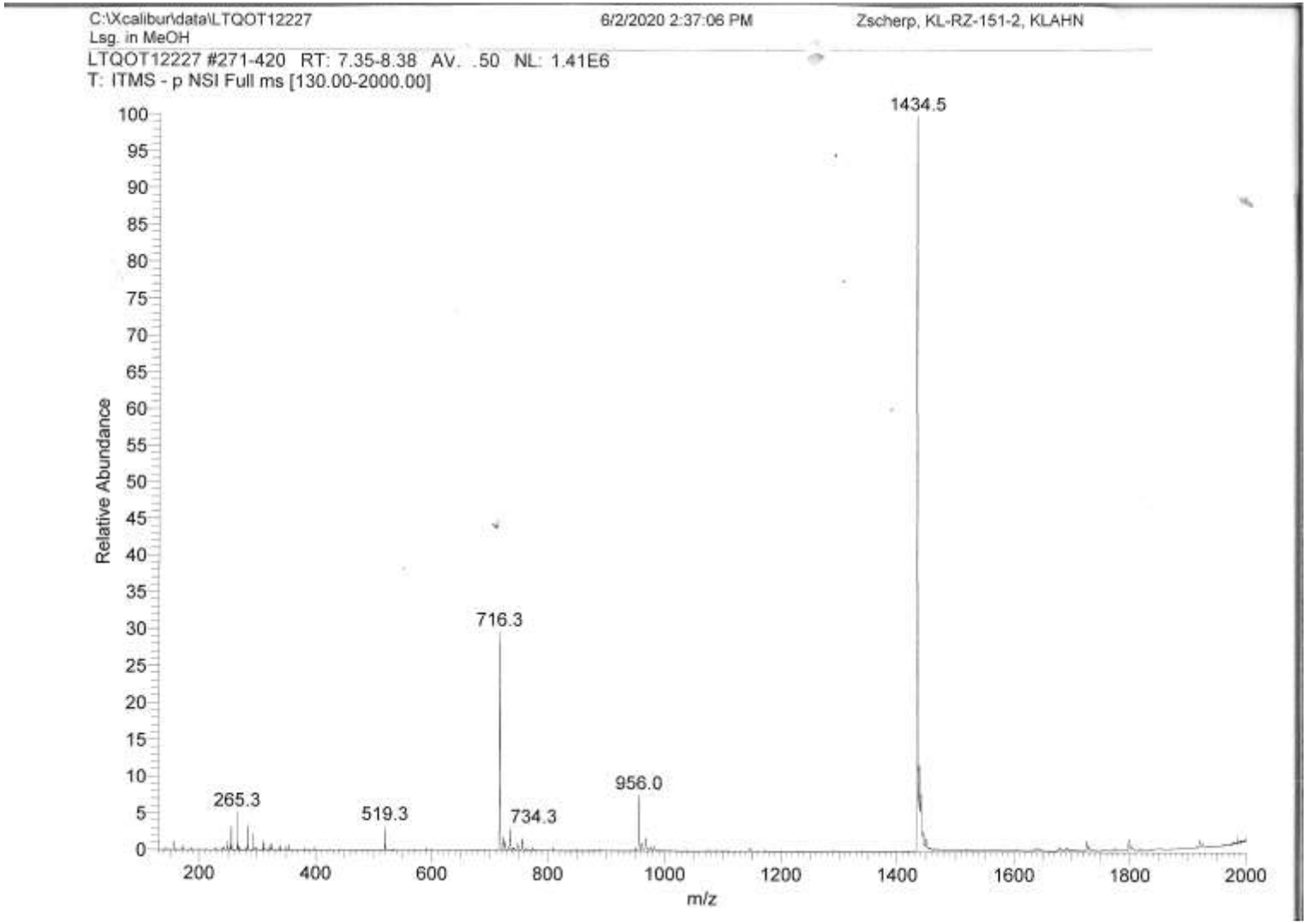


(MeO)Ent$_{KL}$-SulfoCy5 (HPLC-LRMS)



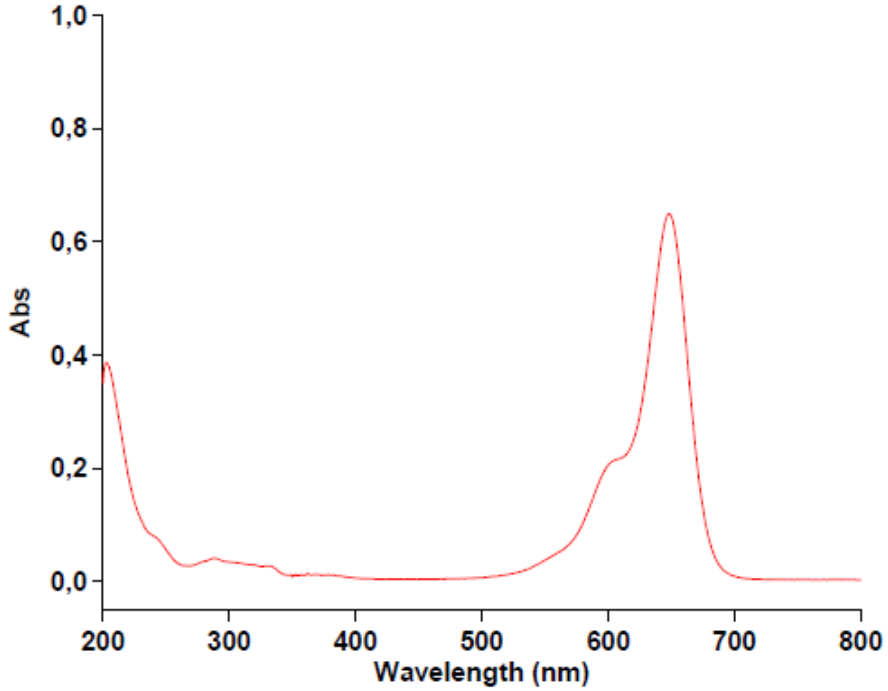
(MeO)Ent$_{KL}$-SulfoCy5 (HRMS)




C:\Xcalibur\data\LTQOT12227
6/2/2020 2:37:06 PM
Zscherp, KL-RZ-151-2, KLAHN
Lsg. in MeOH
LTQOT12227 #271-420 RT: 7.35-8.38 AV: 50 NL: 1.41E6
T: ITMS - p NSI Full ms [130.00-2000.00]
1434.5
716.3
956.0
265.3
519.3
734.3
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
200
400
600
800
1000
1200
1400
1600
1800
2000

(MeO)Ent$_{KL}$-SulfoCy5 (UV/Vis)

04.12.2020 17:04:07 Page 1 of 1



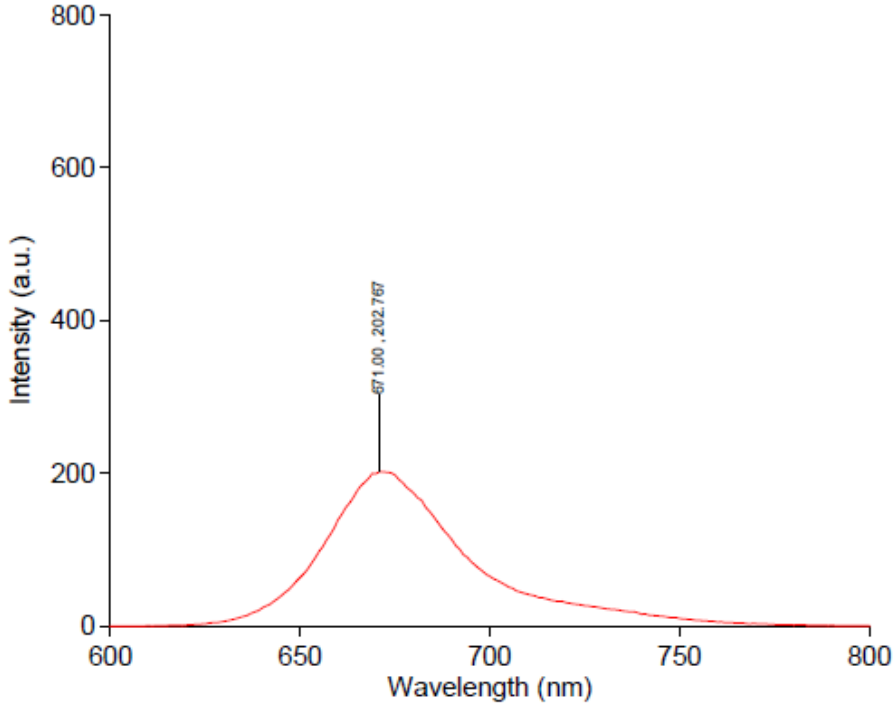
Sample Name: KL-RZ5-151

Collection Time	11.06.2020 13:38:02
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0100
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
648,00	0,649
288,00	0,041
203,00	0,387

56 µg in 10 mL MeOH

(MeO)Ent$_{KL}$-SulfoCy5 (Fluorescence Emission)



Sample name: KL-RZ5-151

Peak table

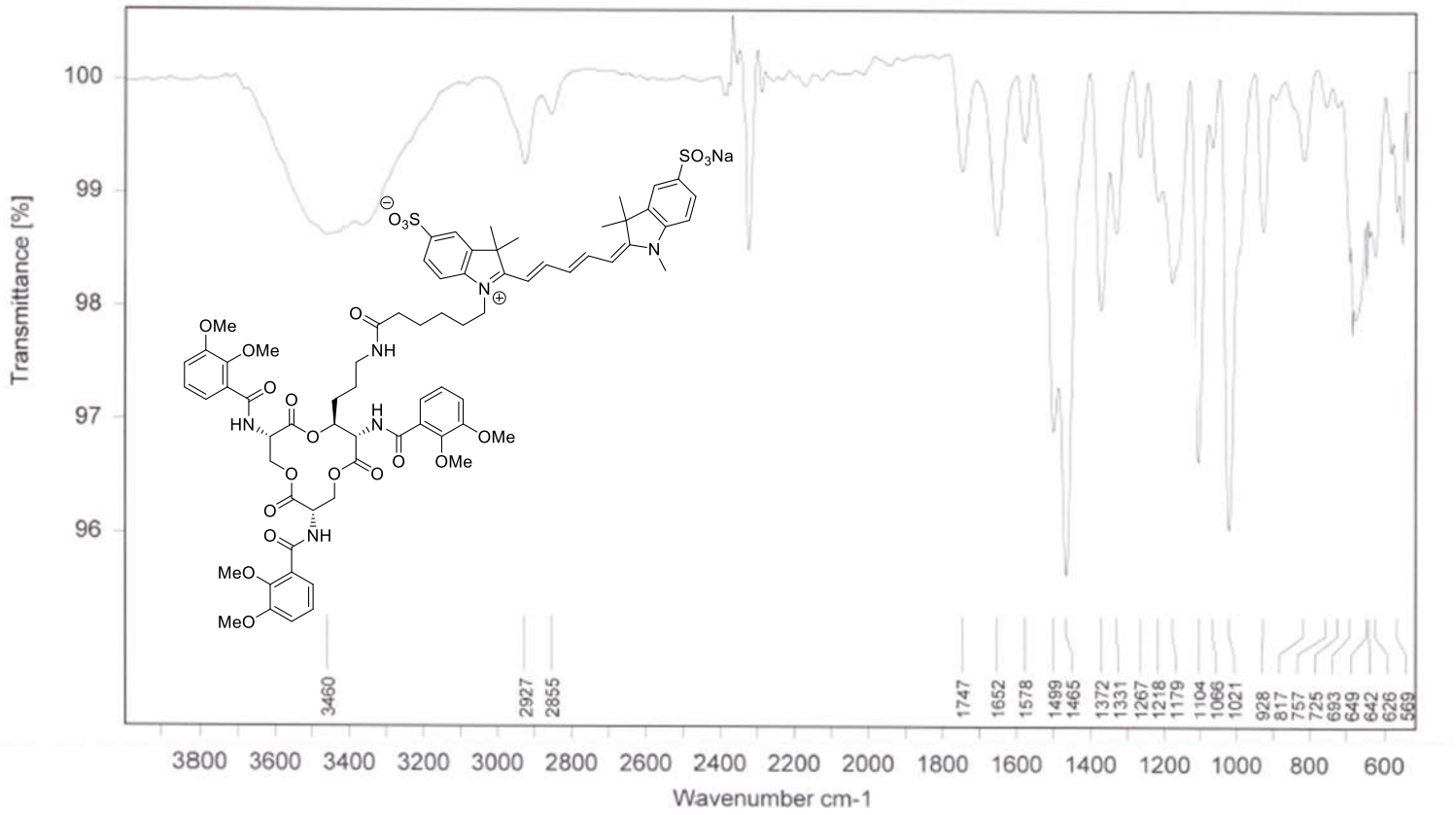
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
671.00	202.767

56 µg in 10 mL MeOH

Excitation wavelength: 640 nm

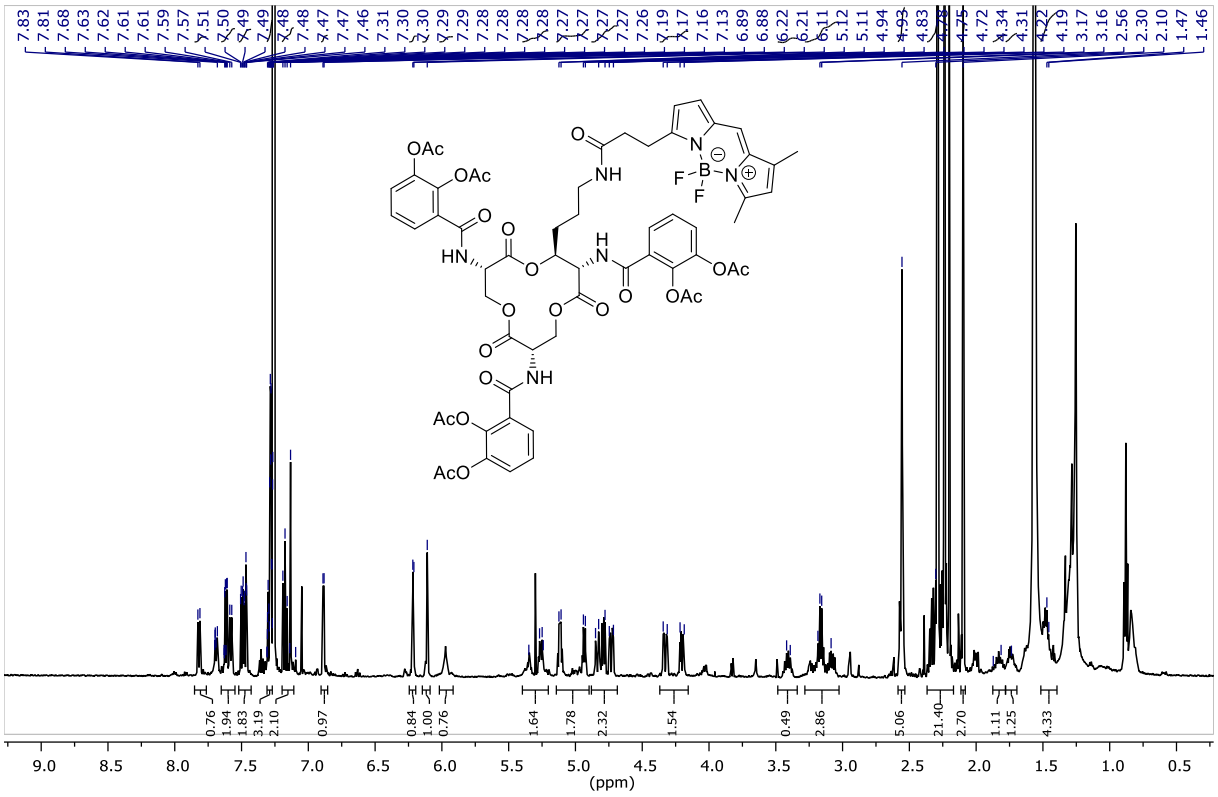
(MeO)Ent$_{KL}$-SulfoCy5 (ATR-IR)



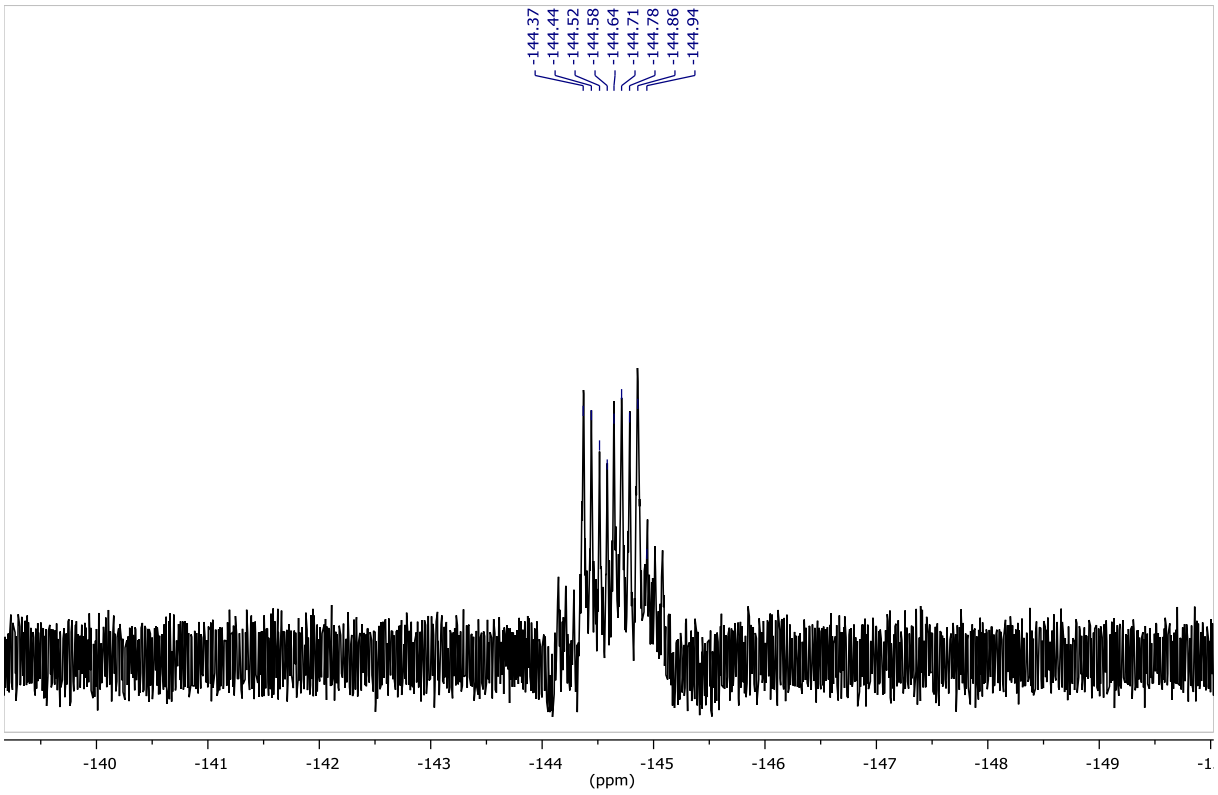
Instrument: Bruker Tensor 27	
Filename: zsr29606.0	Number of Scans: 32
Sample Name: KL-RZ5-154	Operator Name: Default
Technique: Diamant-ATR	Date / Time of Measurement: 12.06.2020 11:32:26

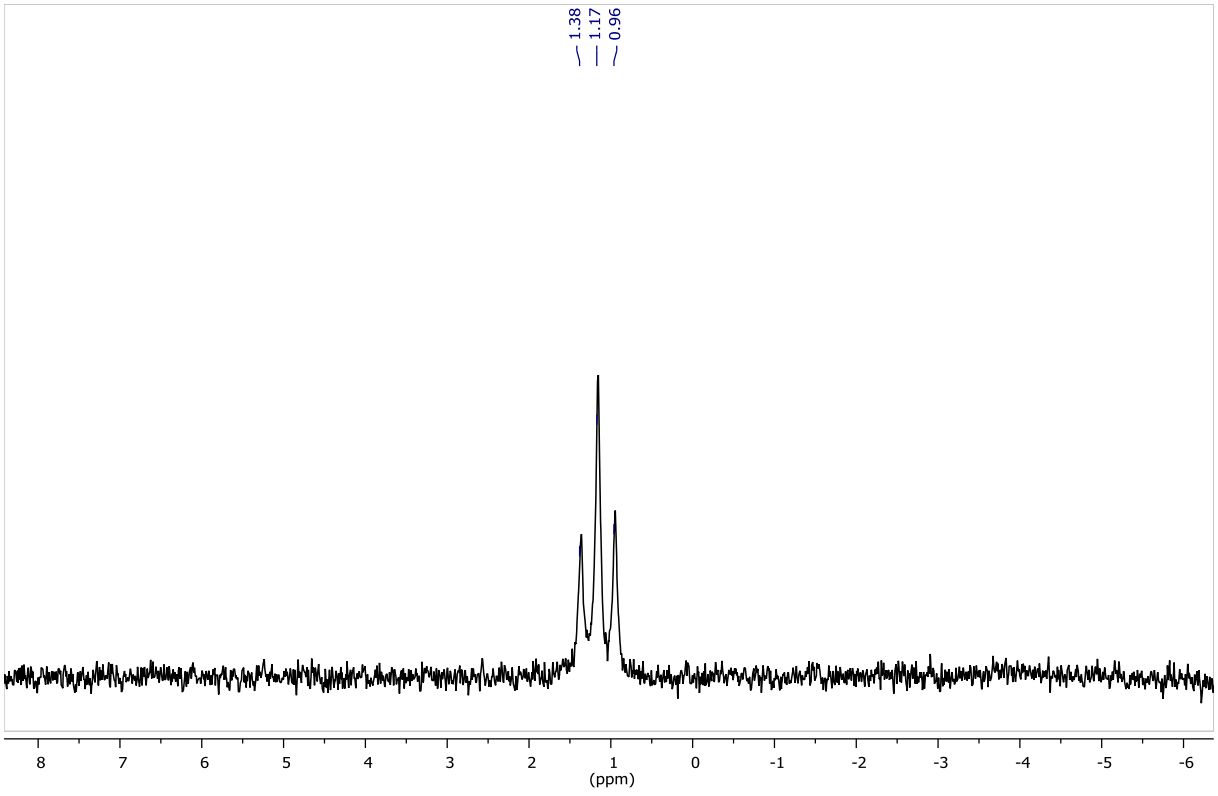
12.06.2020

(AcO)Ent$_{KL}$-BODIPY$_{FL}$ (^{1}H NMR)

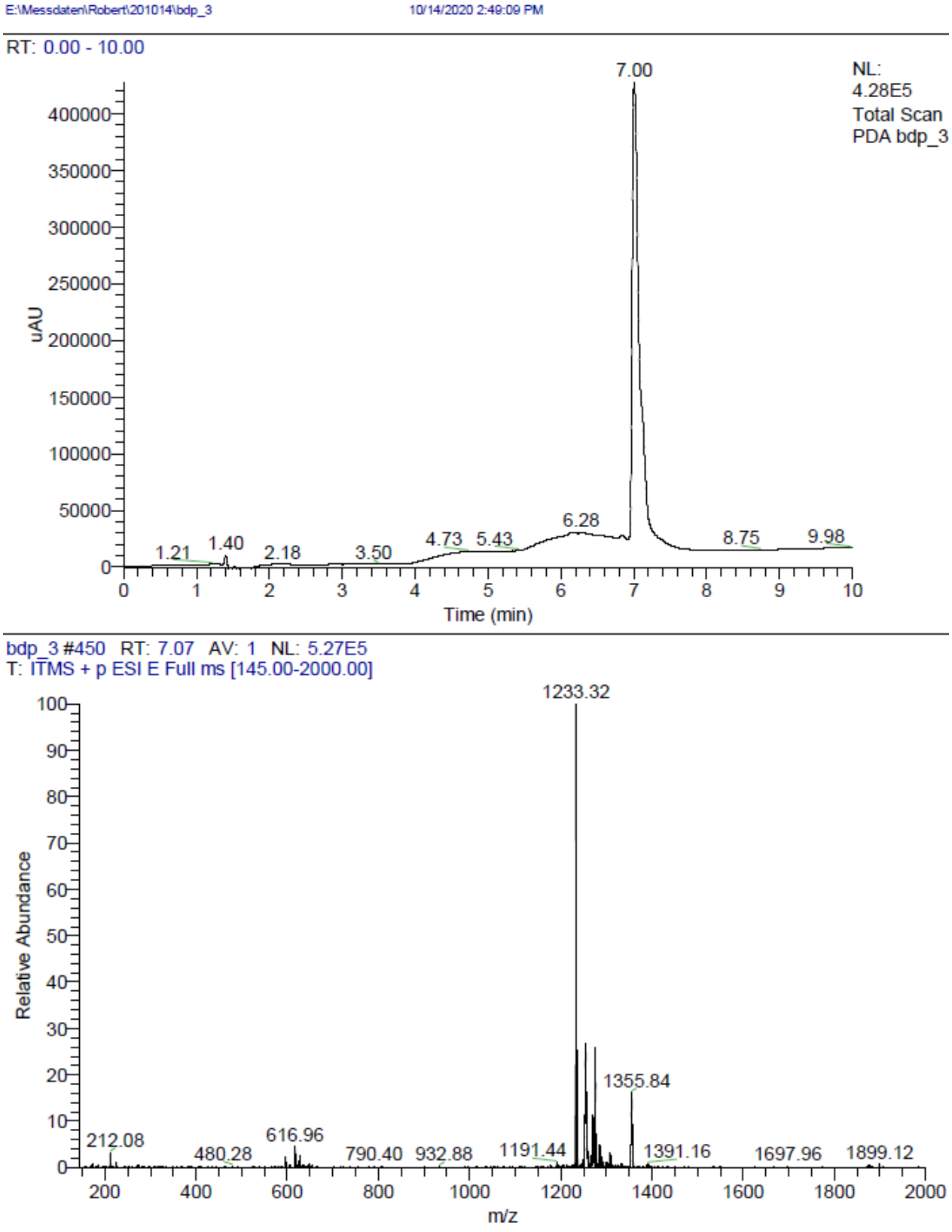


(AcO)Ent$_{KL}$-BODIPY$_{FL}$ (^{19}F and ^{11}B NMR)

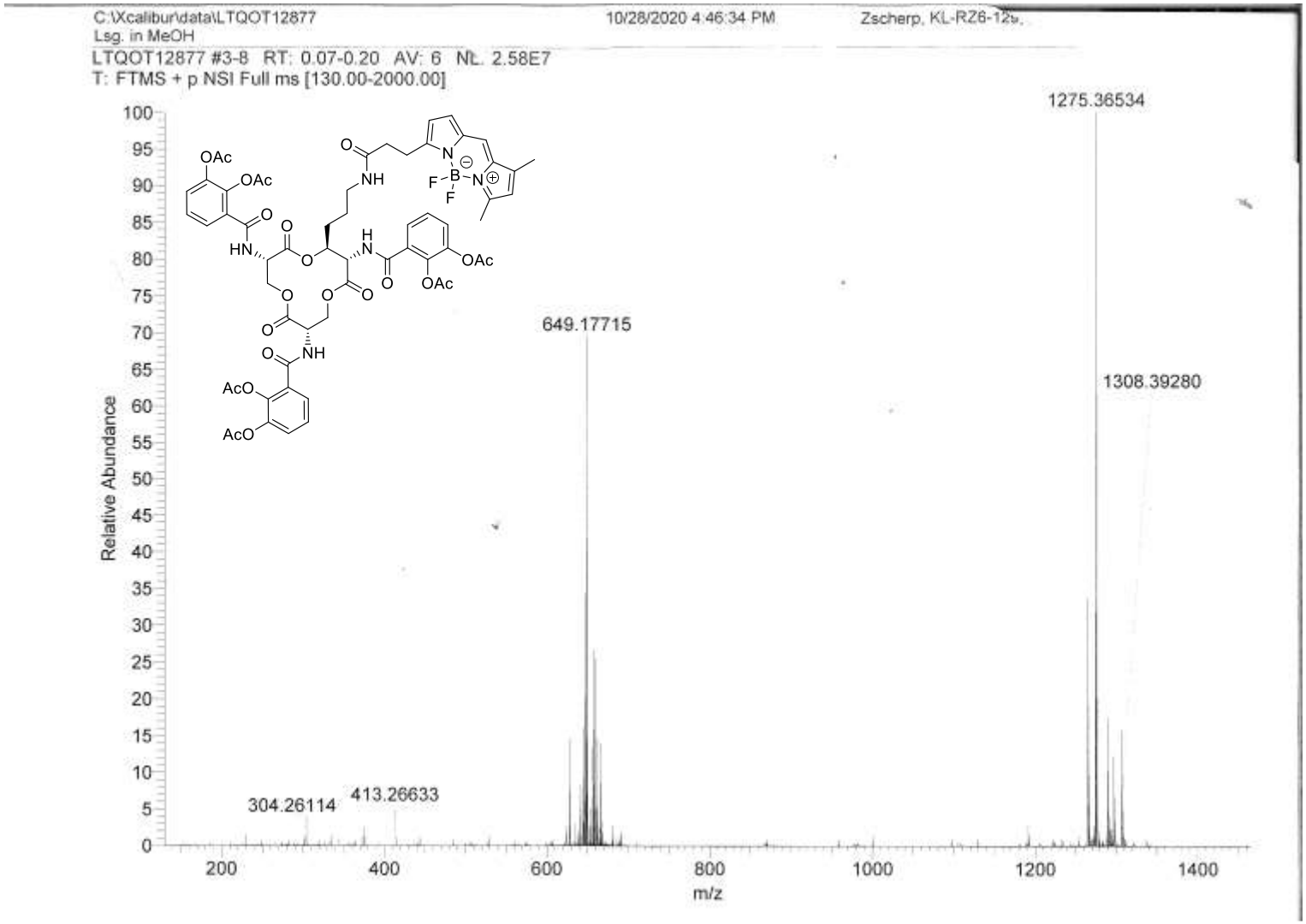




(AcO)Ent$_{KL}$-BODIPY$_{FL}$ (HPLC-LRMS)

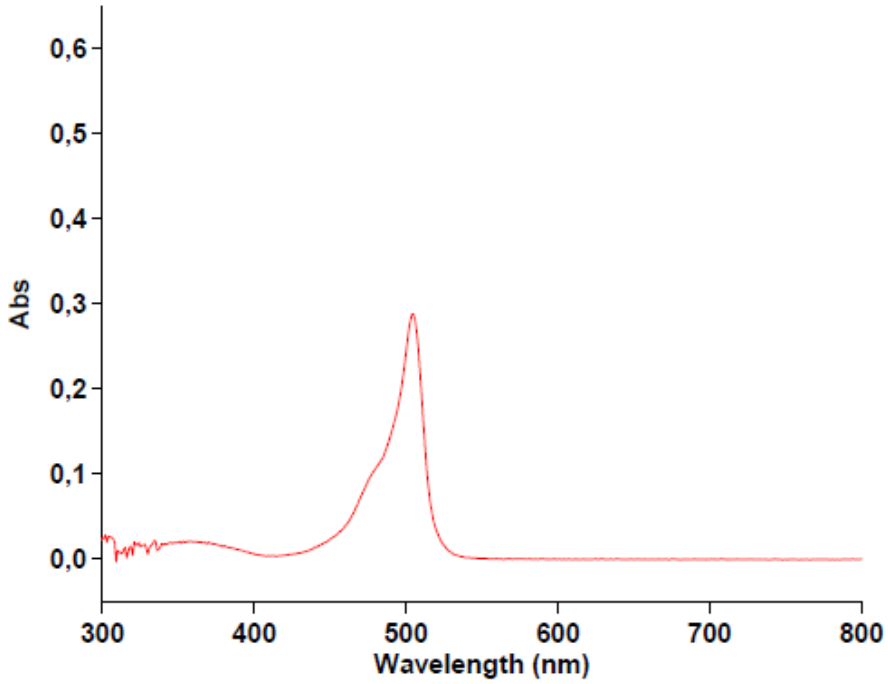


(AcO)Ent$_{KL}$-BODIPY$_{FL}$ (HRMS)



(AcO)Ent$_{KL}$-BODIPY$_{FL}$ (UV/Vis)

04.12.2020 16:45:26 Page 1 of 1



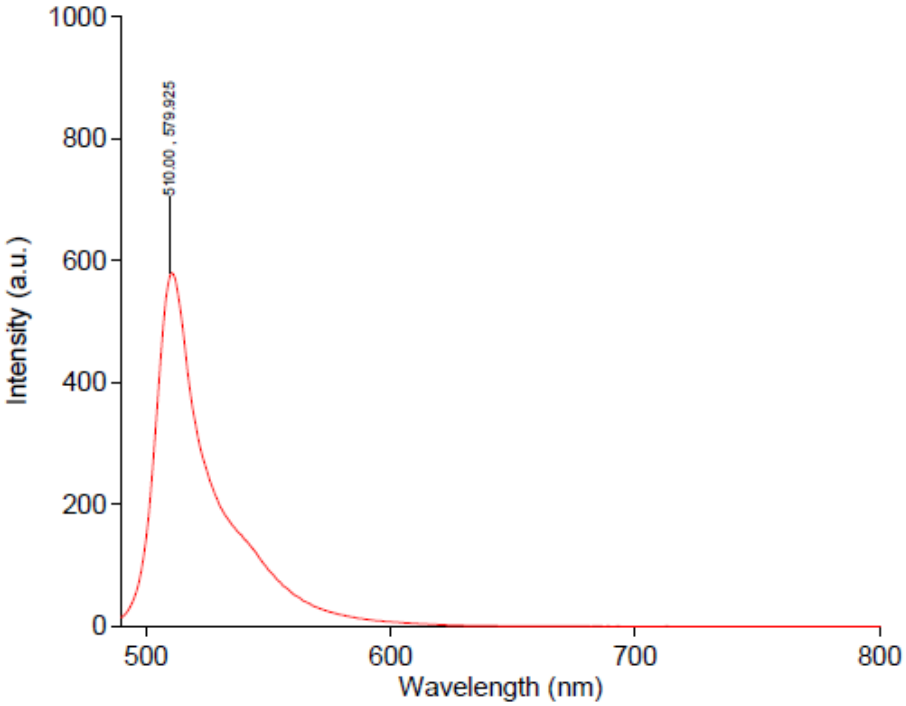
Sample Name: KL-RZ6-128

Collection Time	30.10.2020 16:51:31
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0010
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
504,00	0,288

73 µg in 10 mL MeOH

(AcO)Ent_{KL}-$BODIPY_{FL}$ (Fluorescence Emission)



Sample name: KL-RZ6-128

Peak table

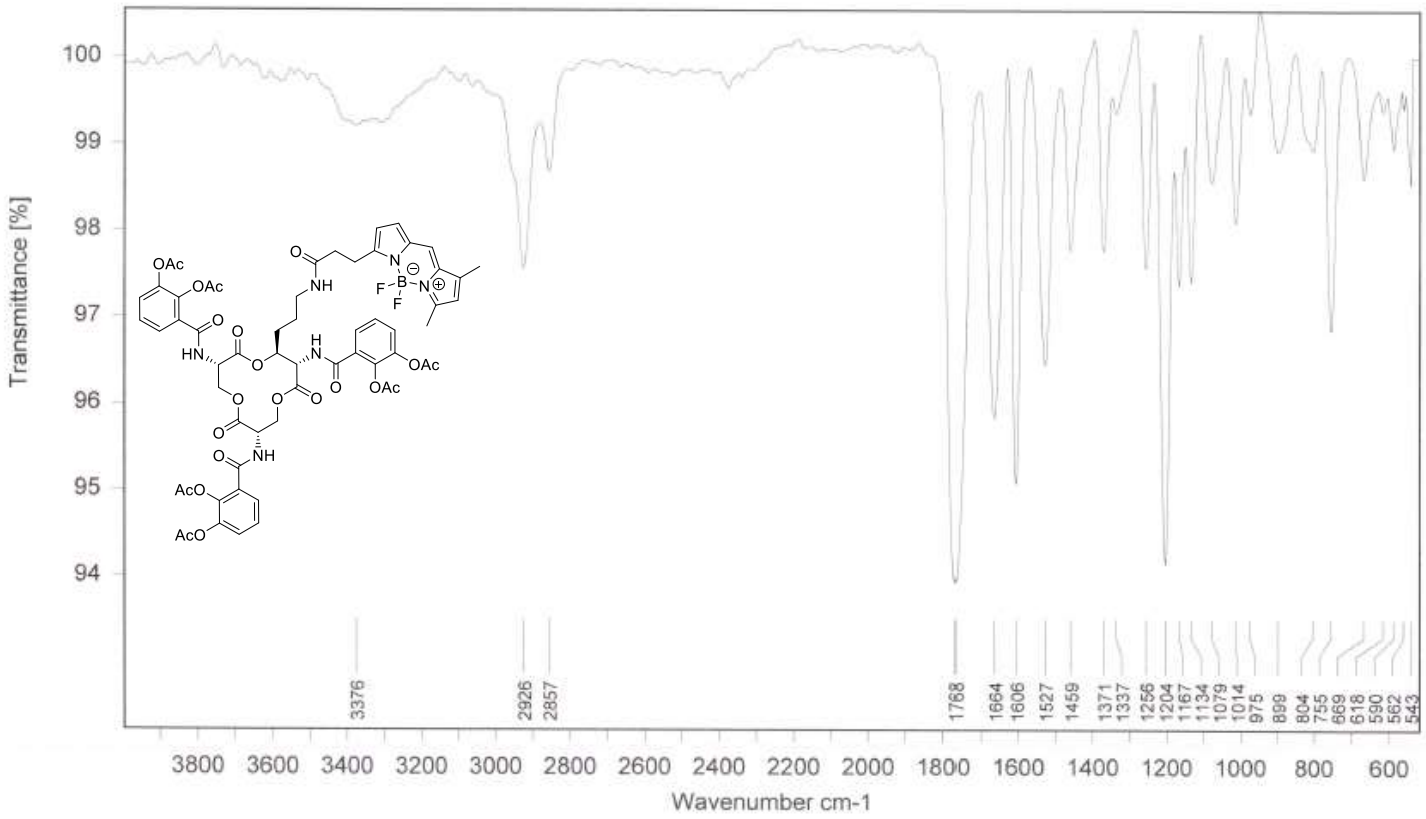
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
510.00	579.925

22 µg in 10 mL MeOH

Excitation wavelength: 504 nm

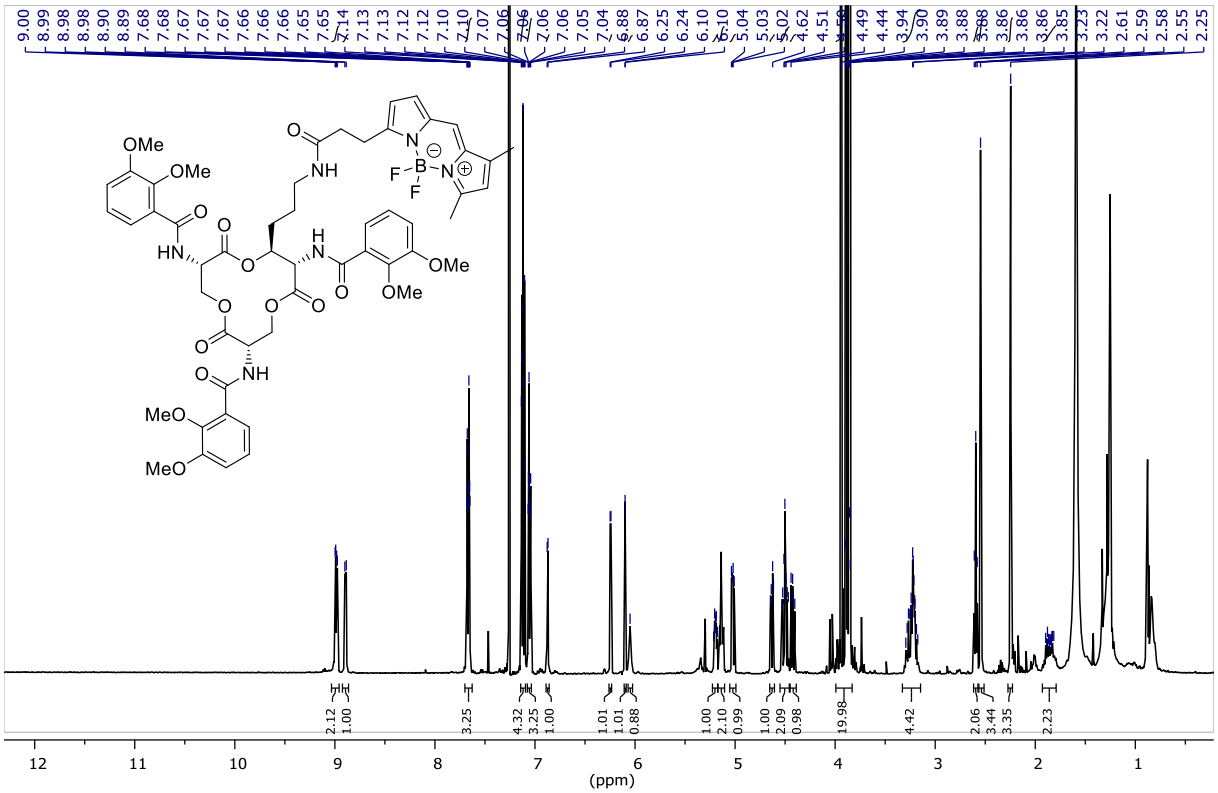
(AcO)Ent$_{KL}$-BODIPY$_{FL}$ (ATR-IR)



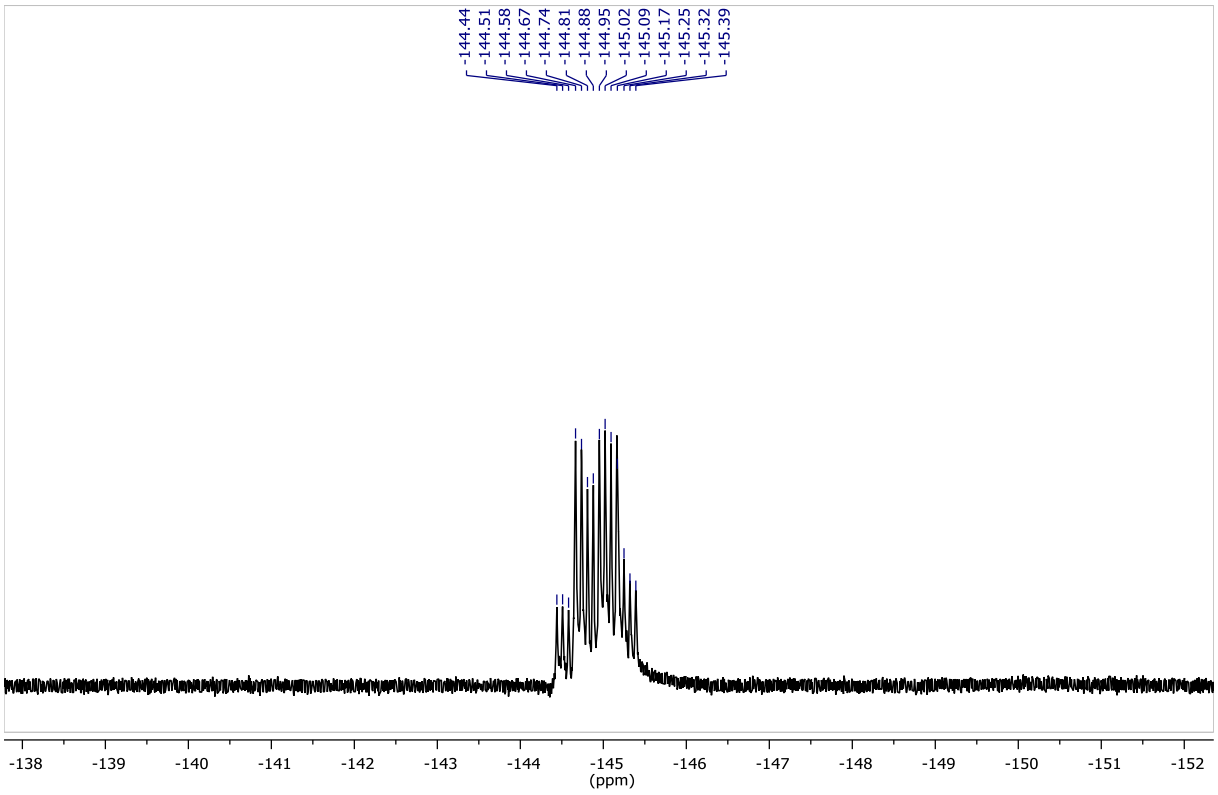
Instrument: Bruker Tensor 27	
Filename: zsr29848.1	Number of Scans: 32
Sample Name: OAc-EntKL-BDP	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 30.10.2020 15:03:22

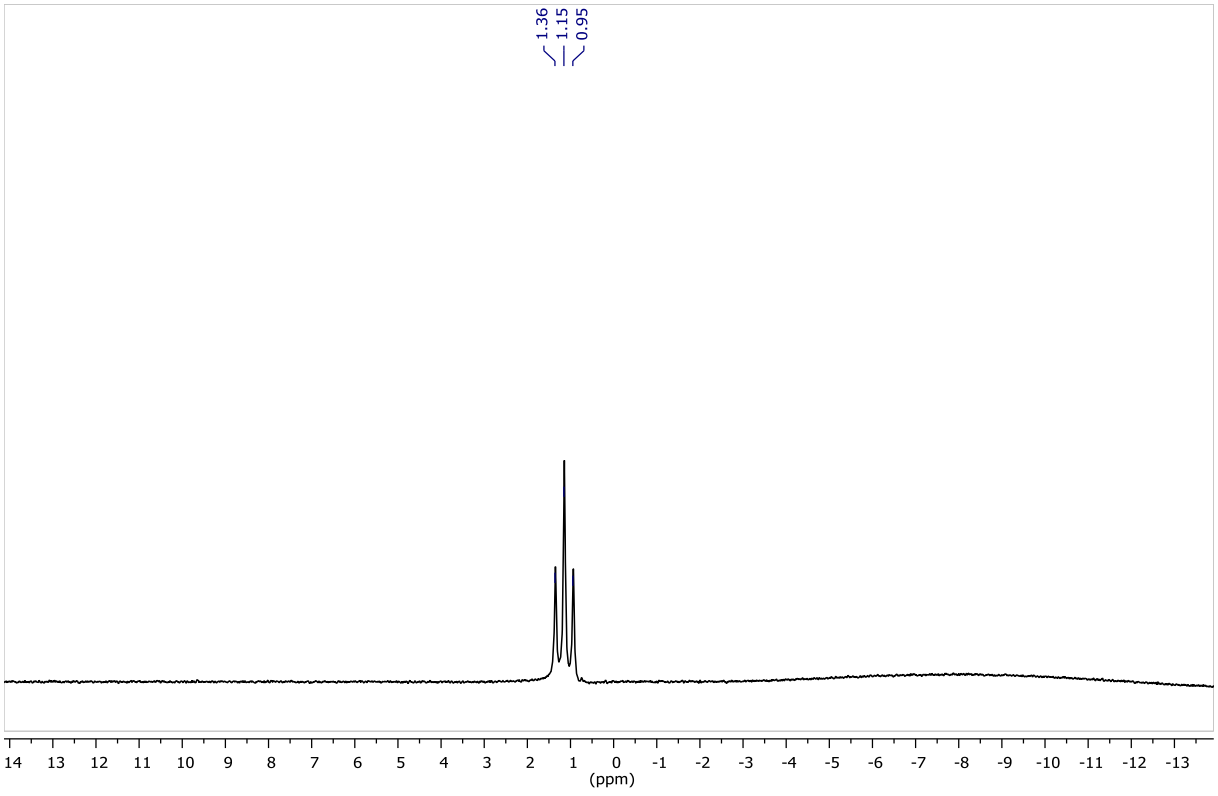
30.10.2020

(MeO)Ent$_{KL}$-**BODIPY$_{FL}$** (^{1}H NMR)

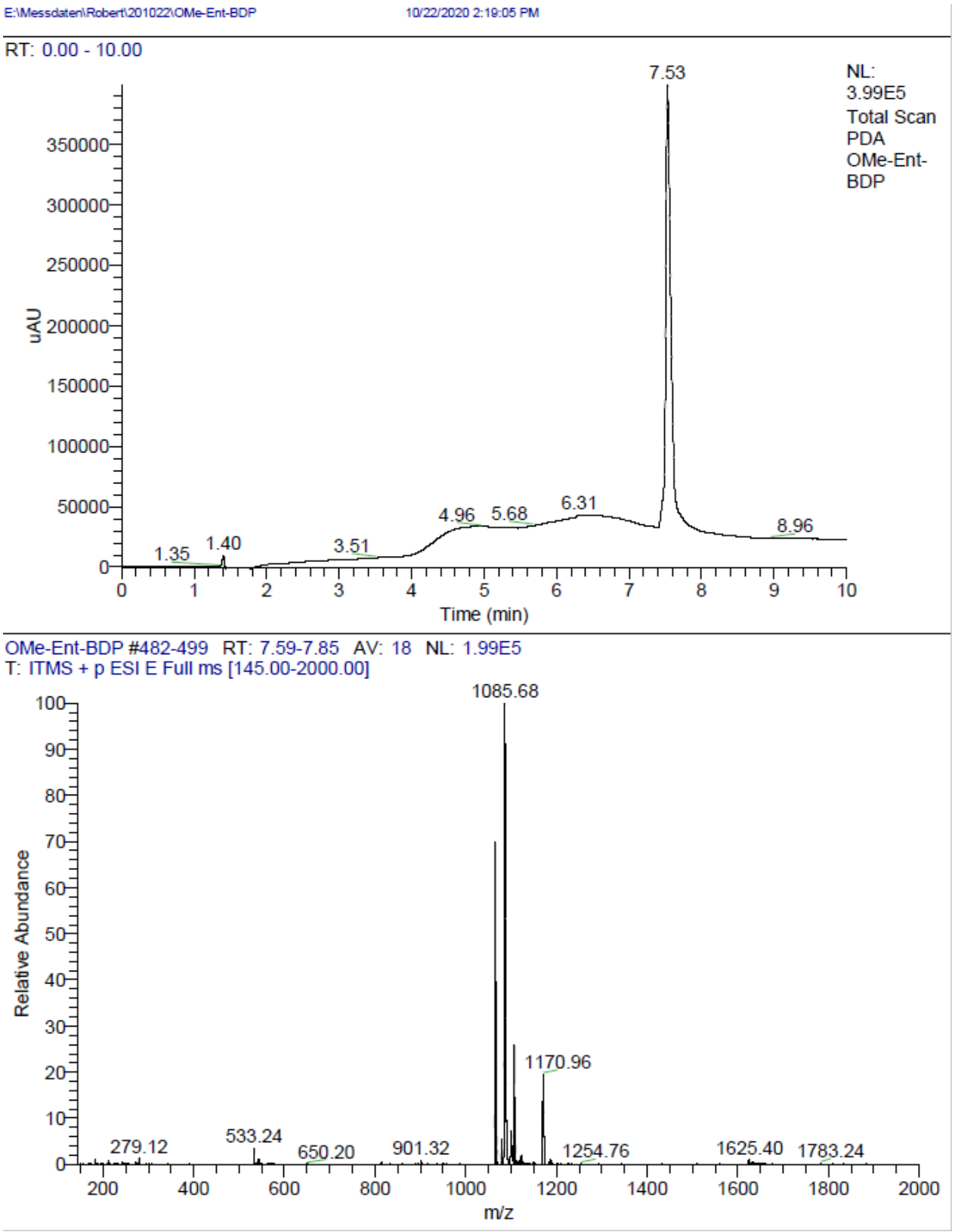


(MeO)Ent$_{KL}$-**BODIPY$_{FL}$** (^{19}F and ^{11}B NMR)

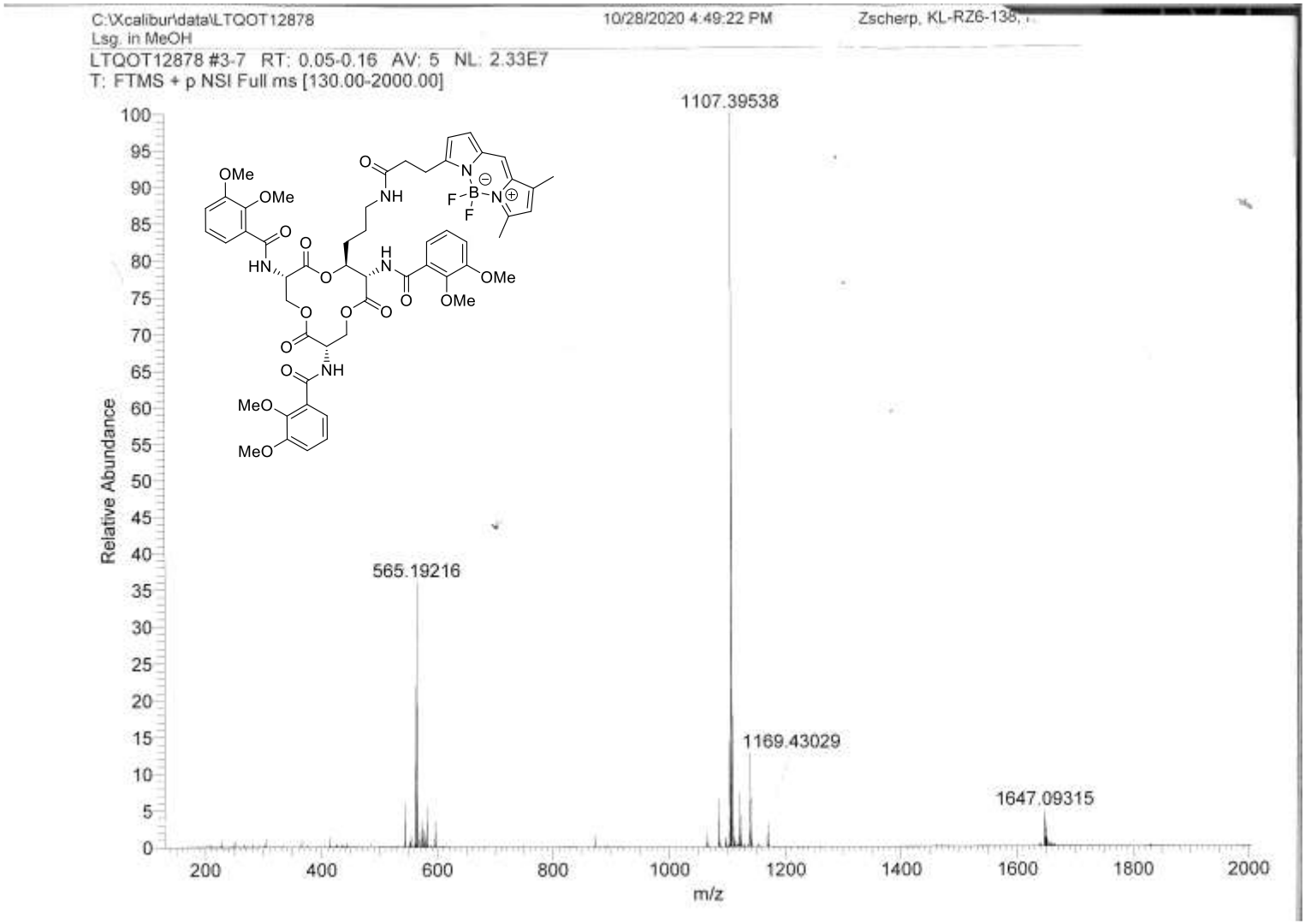




(MeO)Ent$_{KL}$-**BODIPY$_{FL}$** (HPLC-LRMS)



(MeO)Ent$_{KL}$-**BODIPY$_{FL}$** (HRMS)



(MeO)Ent$_{KL}$-**BODIPY$_{FL}$** (UV/Vis)

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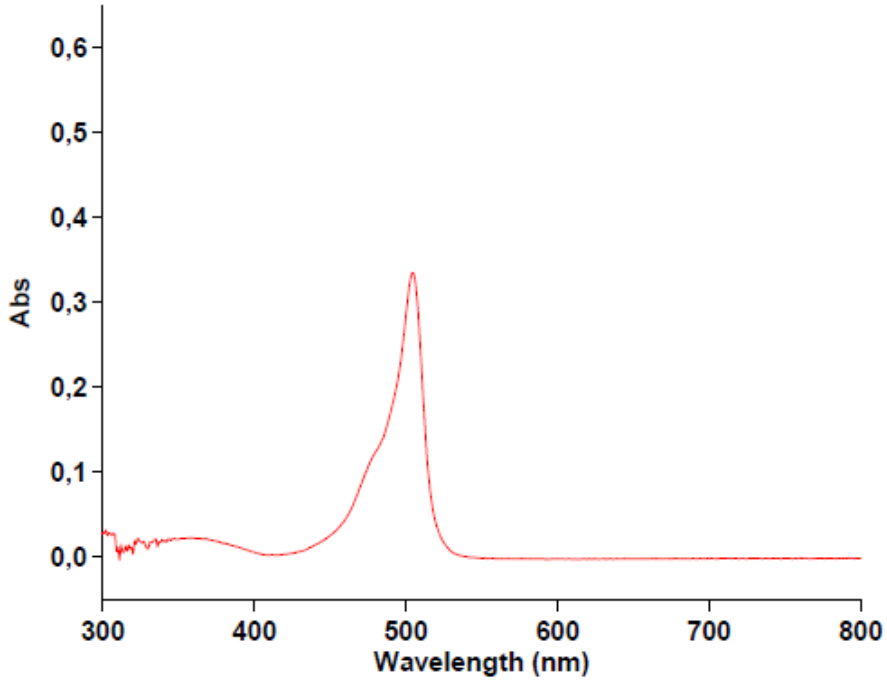


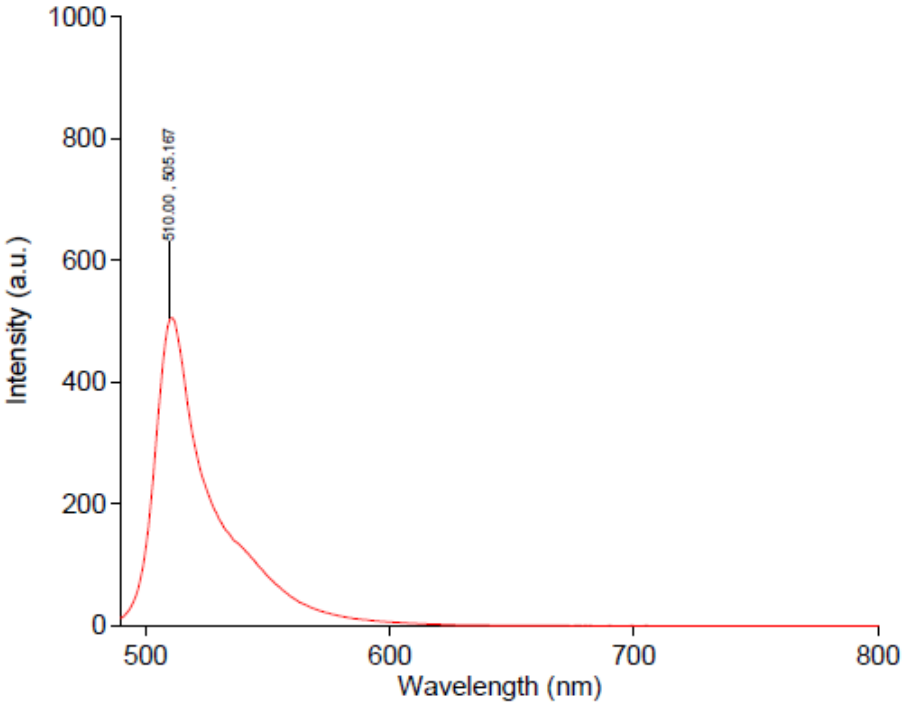
Sample Name: KL-RZ6-138

Collection Time	30.10.2020 16:46:17
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0010
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
504,00	0,336

96 µg in 10 mL MeOH

(MeO)Ent$_{KL}$-**BODIPY$_{FL}$** (Fluorescence Emission)



Sample name: KL-RZ6-138

Peak table

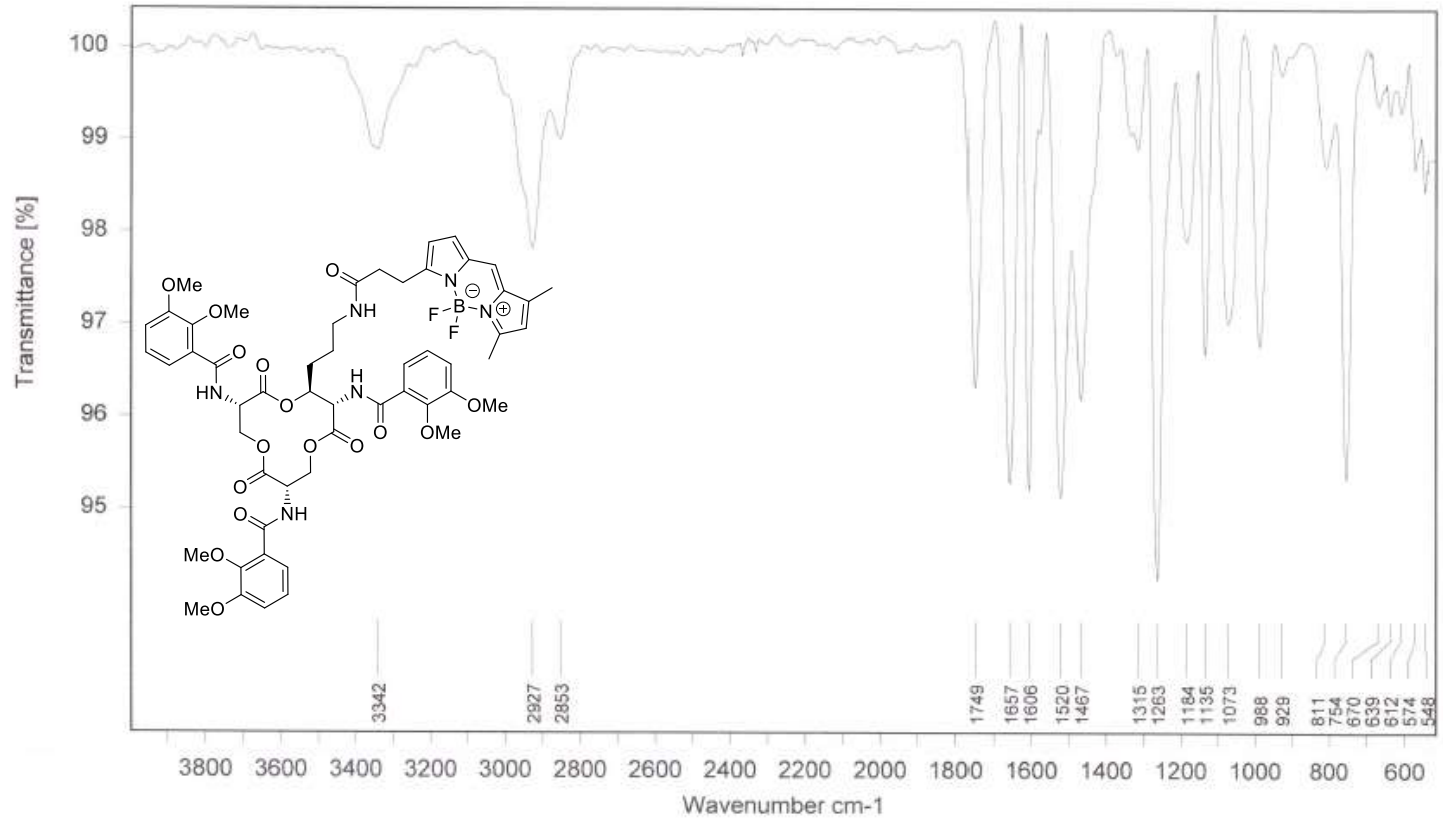
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
510.00	505.167

25 µg in 10 mL MeOH

Excitation wavelength: 504 nm

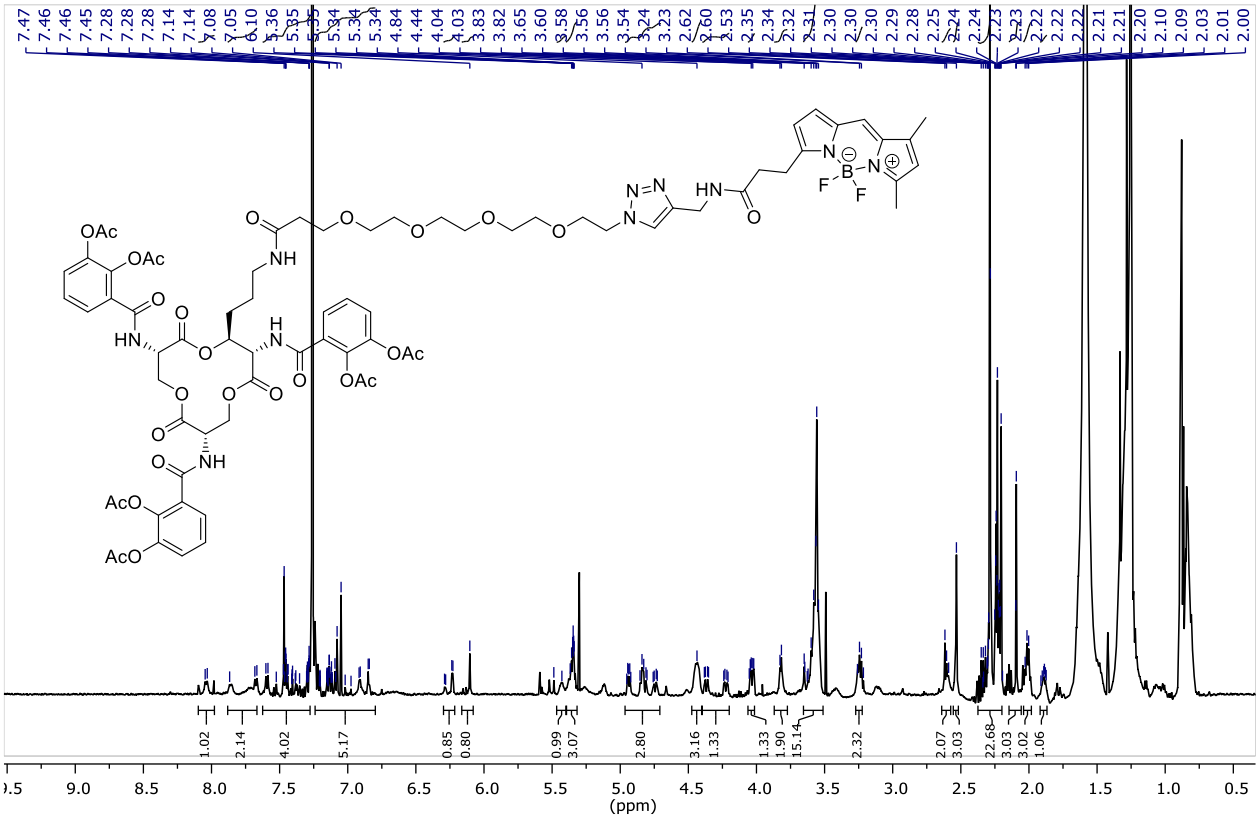
(MeO)Ent$_{KL}$-**BODIPY$_{FL}$** (ATR-IR)



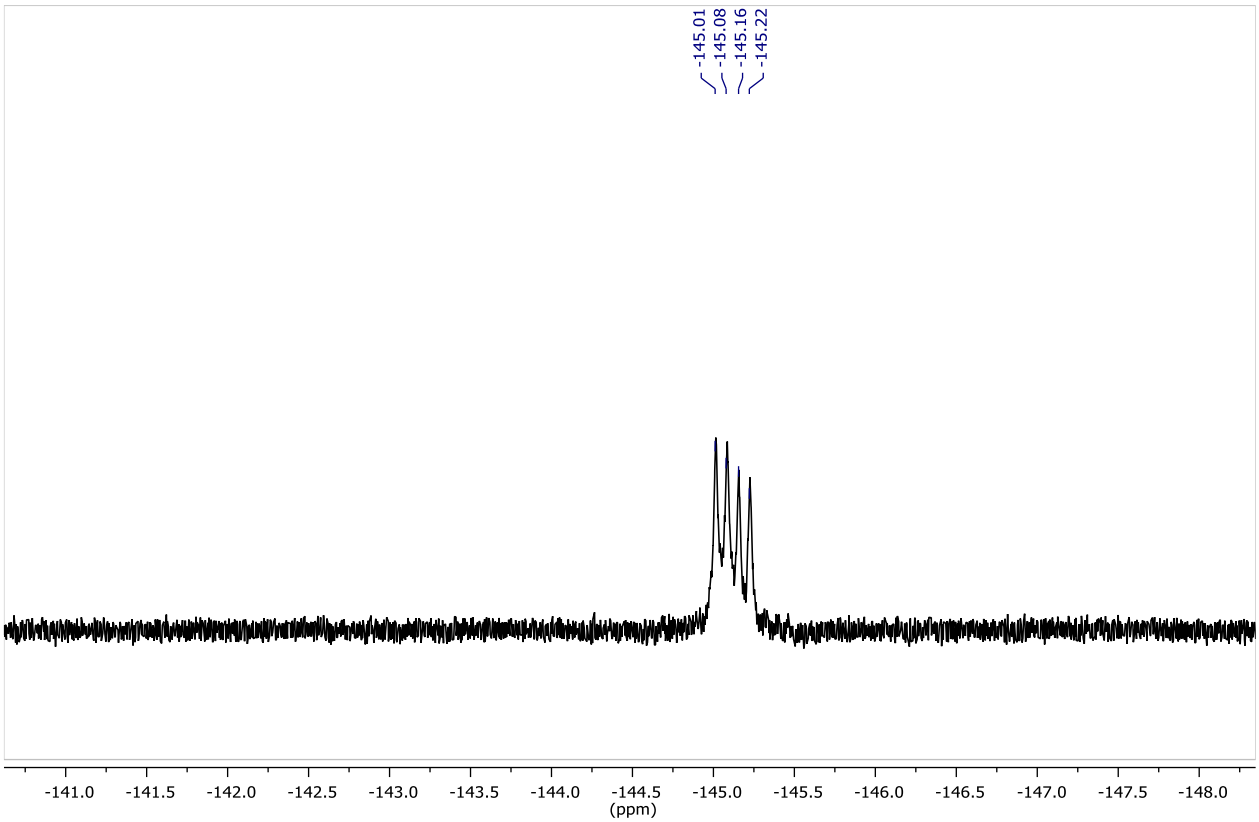
Instrument: Bruker Tensor 27	
Filename: zsr29846.0	Number of Scans: 32
Sample Name: OMe-EntKL-BDP	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 30.10.2020 14:03:00

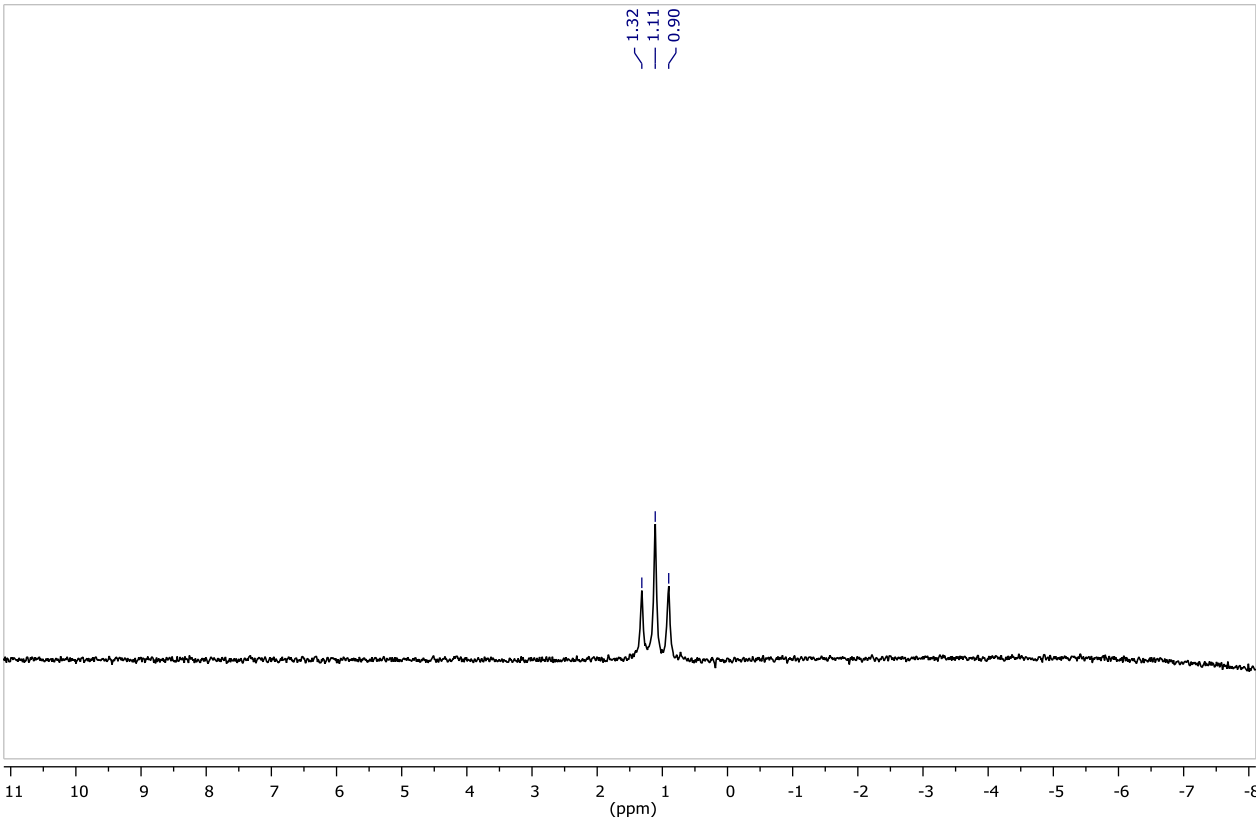
30.10.2020

(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (^{1}H NMR)

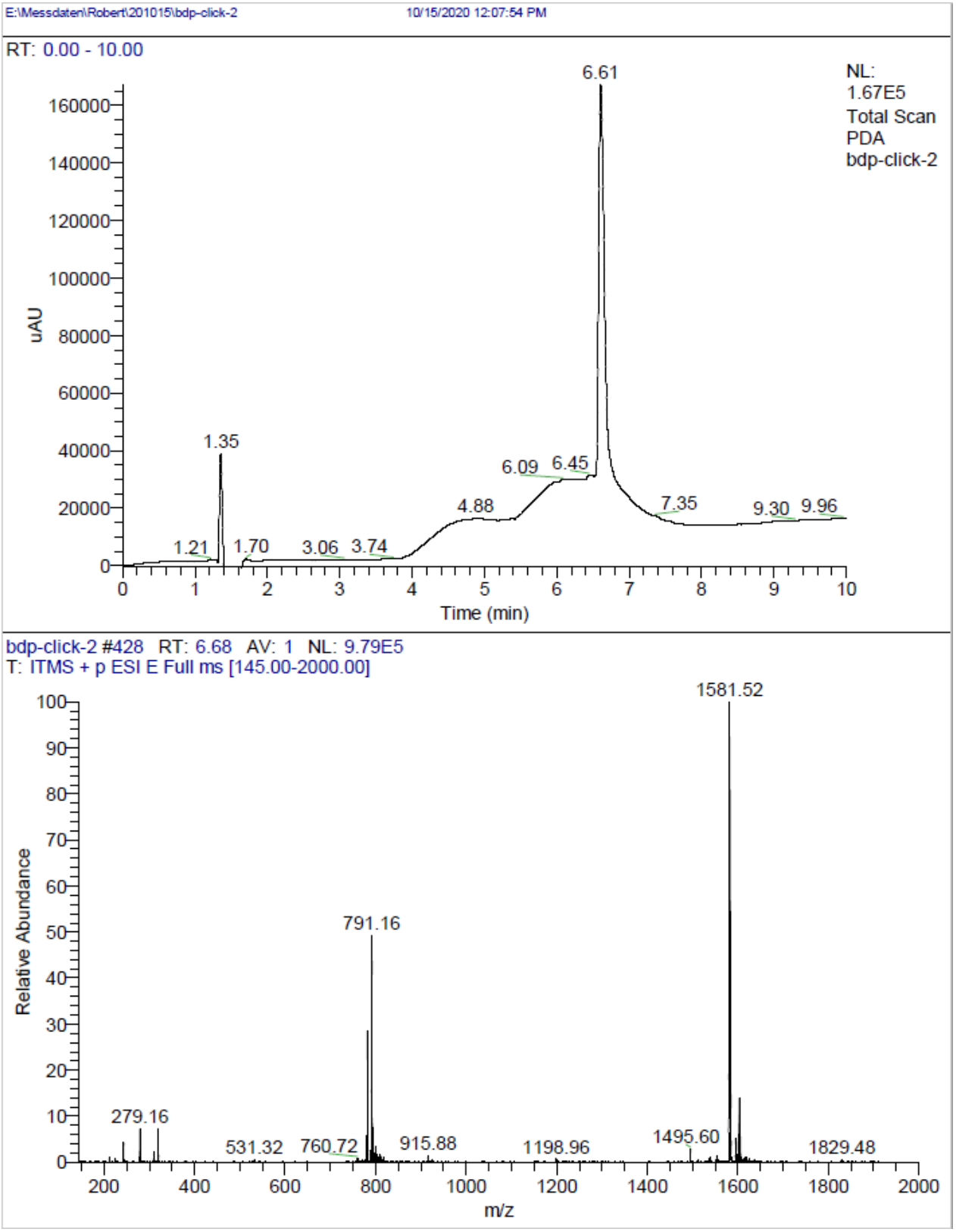


(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (^{19}F and ^{11}B NMR)

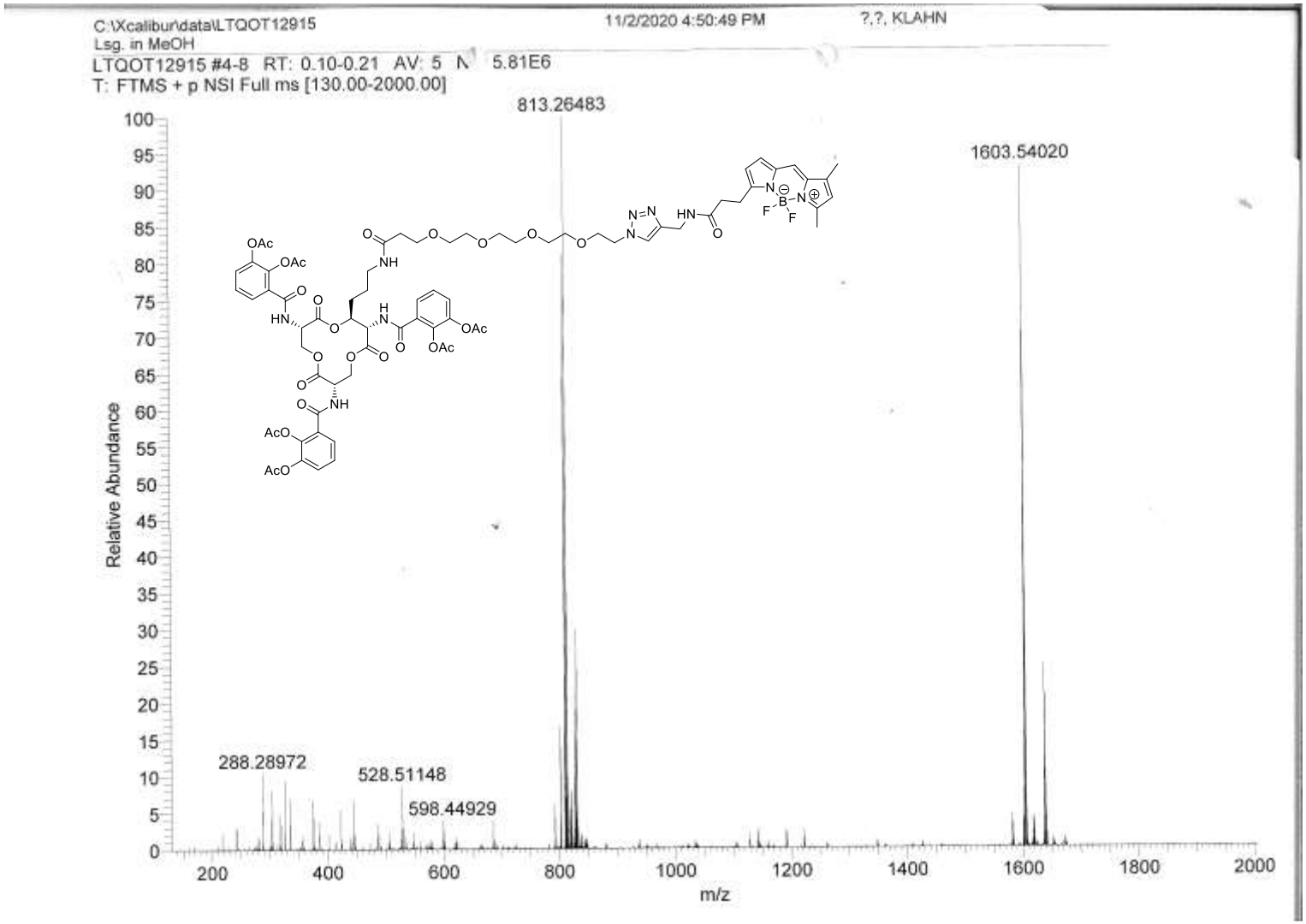




(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (HPLC-LRMS)



(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (HRMS)



(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (UV/Vis)

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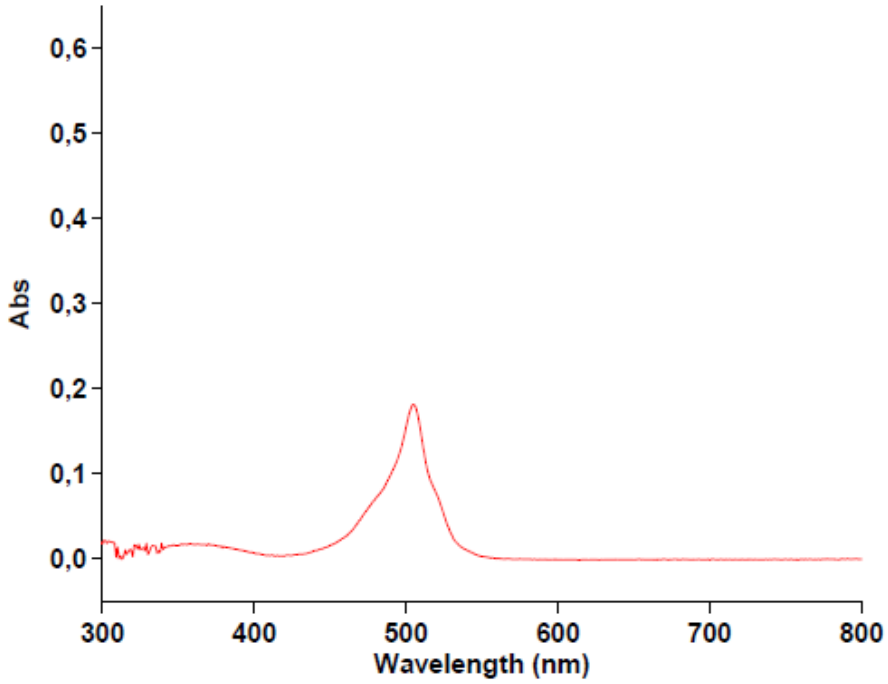


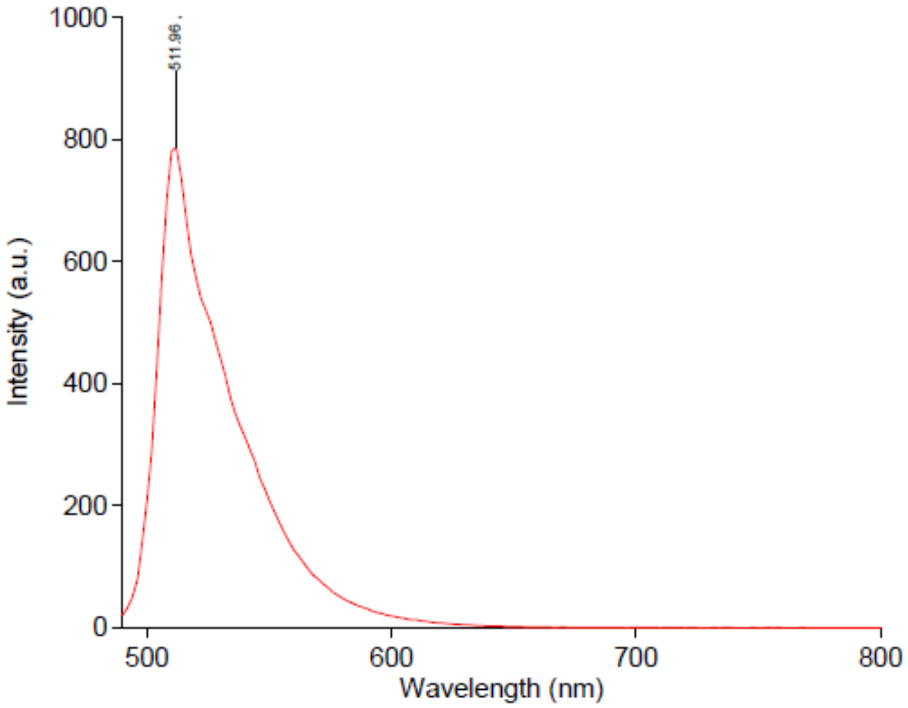
Sample Name: KL-RZ6-130

Collection Time	30.10.2020 17:01:19
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0010
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
505,00	0,182

102 µg in 10 mL MeOH

(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (Fluorescence Emission)



Sample name: KL-RZ6-130

Peak table

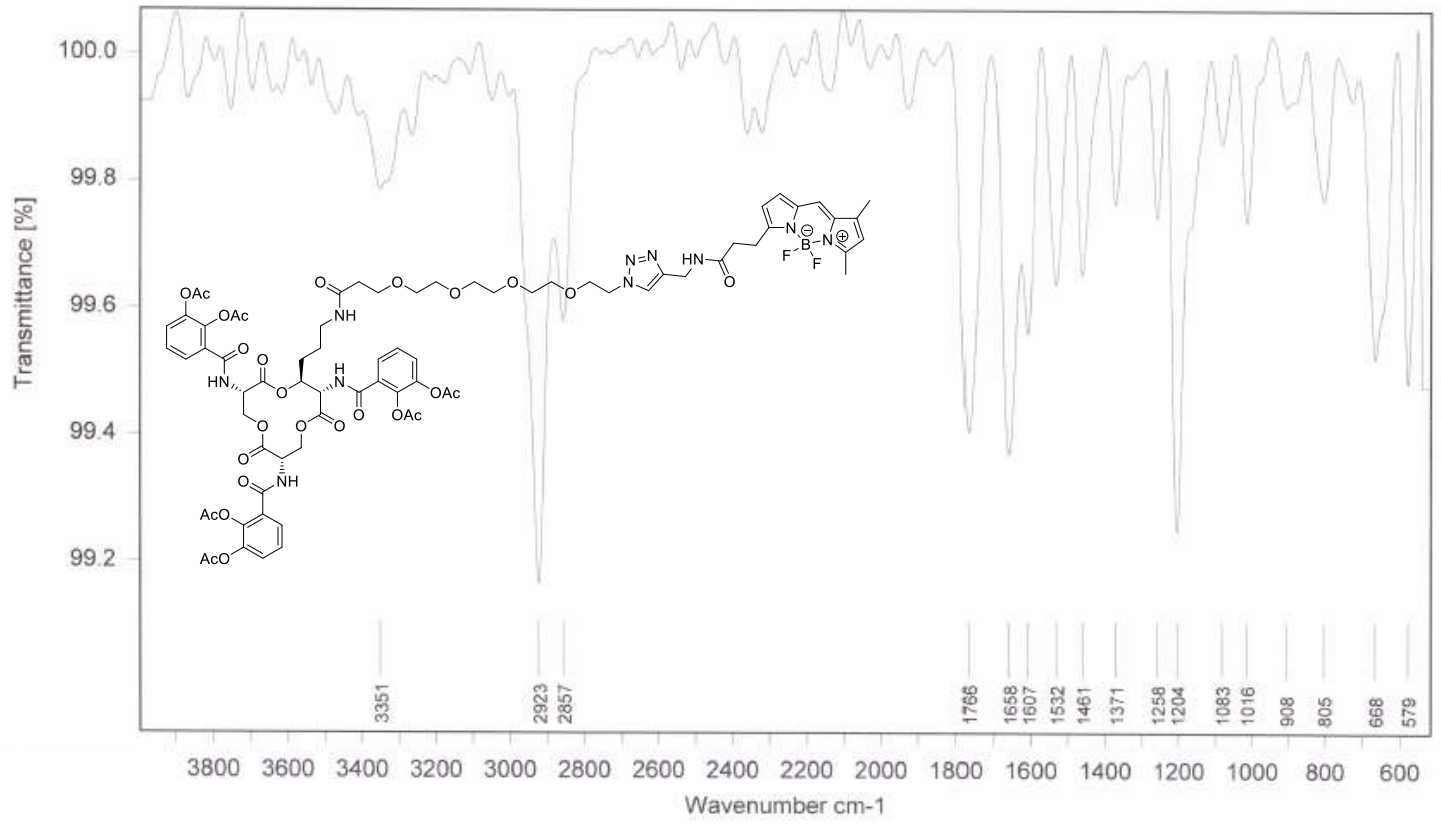
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
511.96	786.816

102 µg in 10 mL MeOH

Excitation wavelength: 505 nm

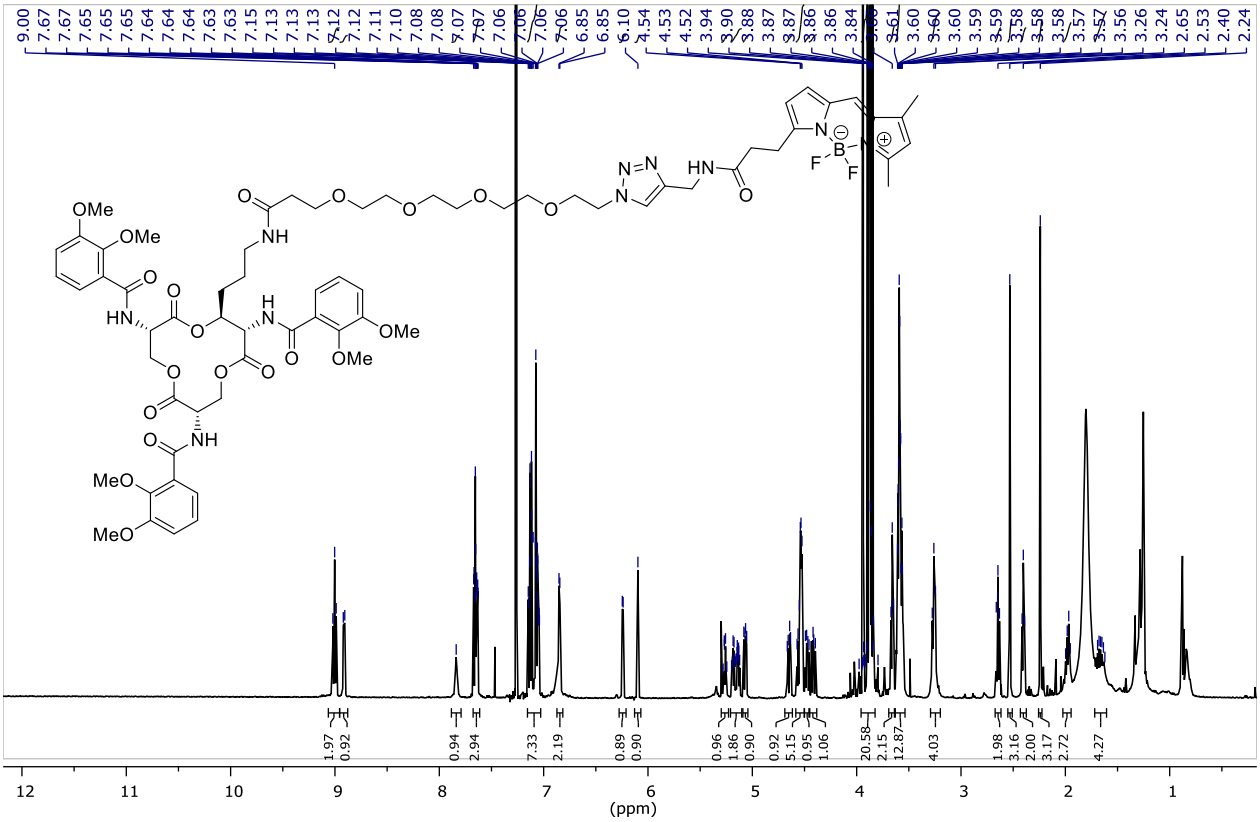
(AcO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (ATR-IR)



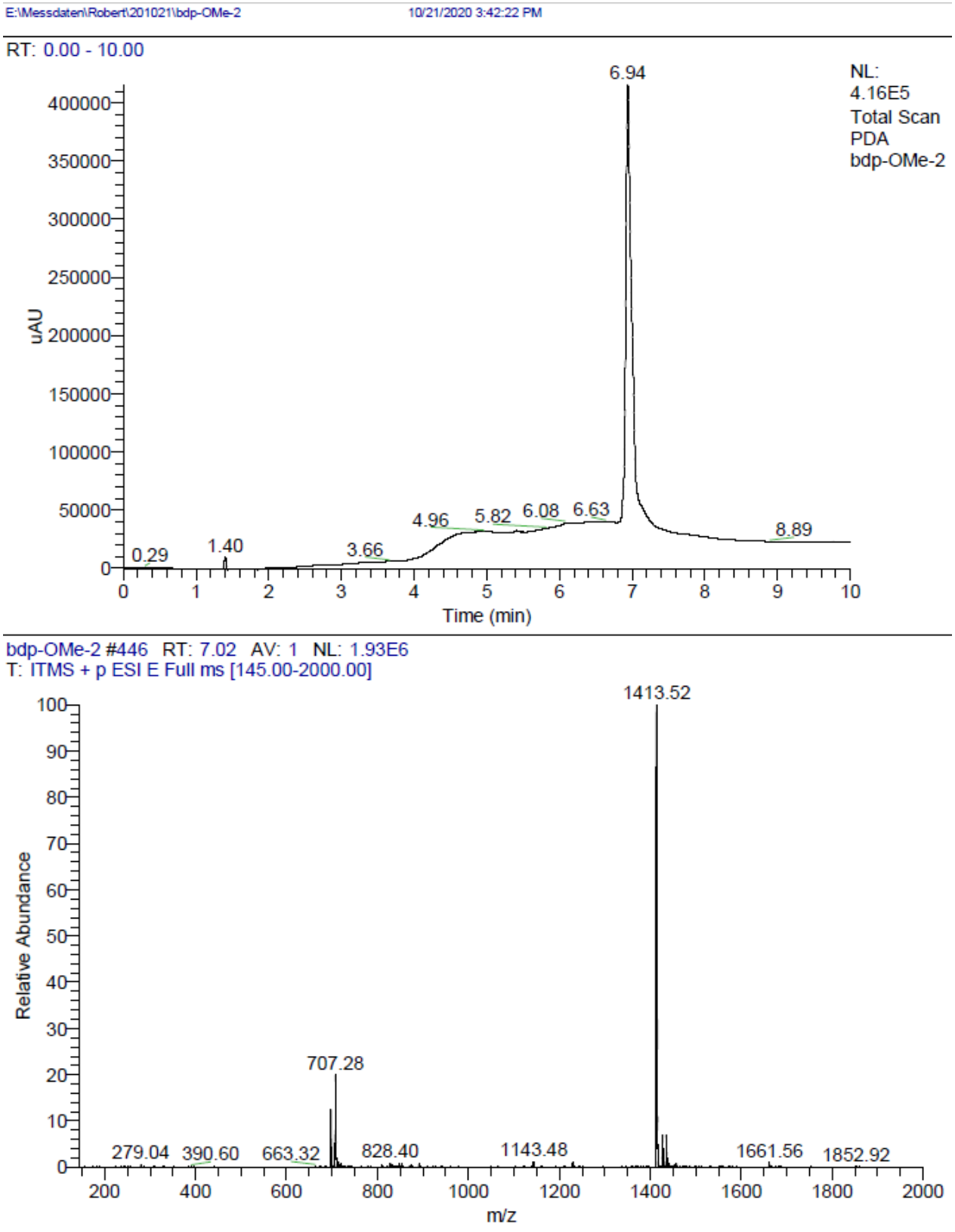
Instrument: Bruker Tensor 27	
Filename: zsr29849.3	Number of Scans: 32
Sample Name: OAc-EntKL-PEG-BDP	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 30.10.2020 15:25:27

30.10.2020

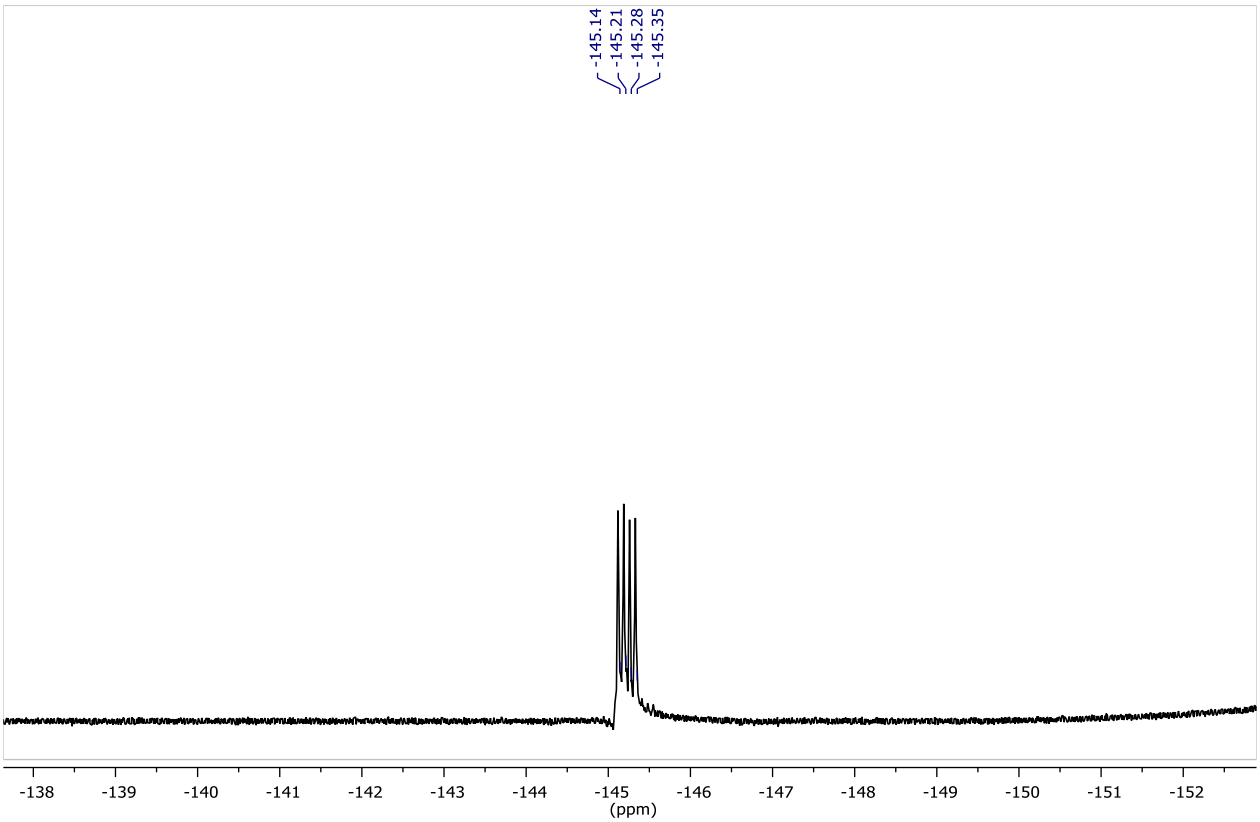
(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (^{1}H NMR)

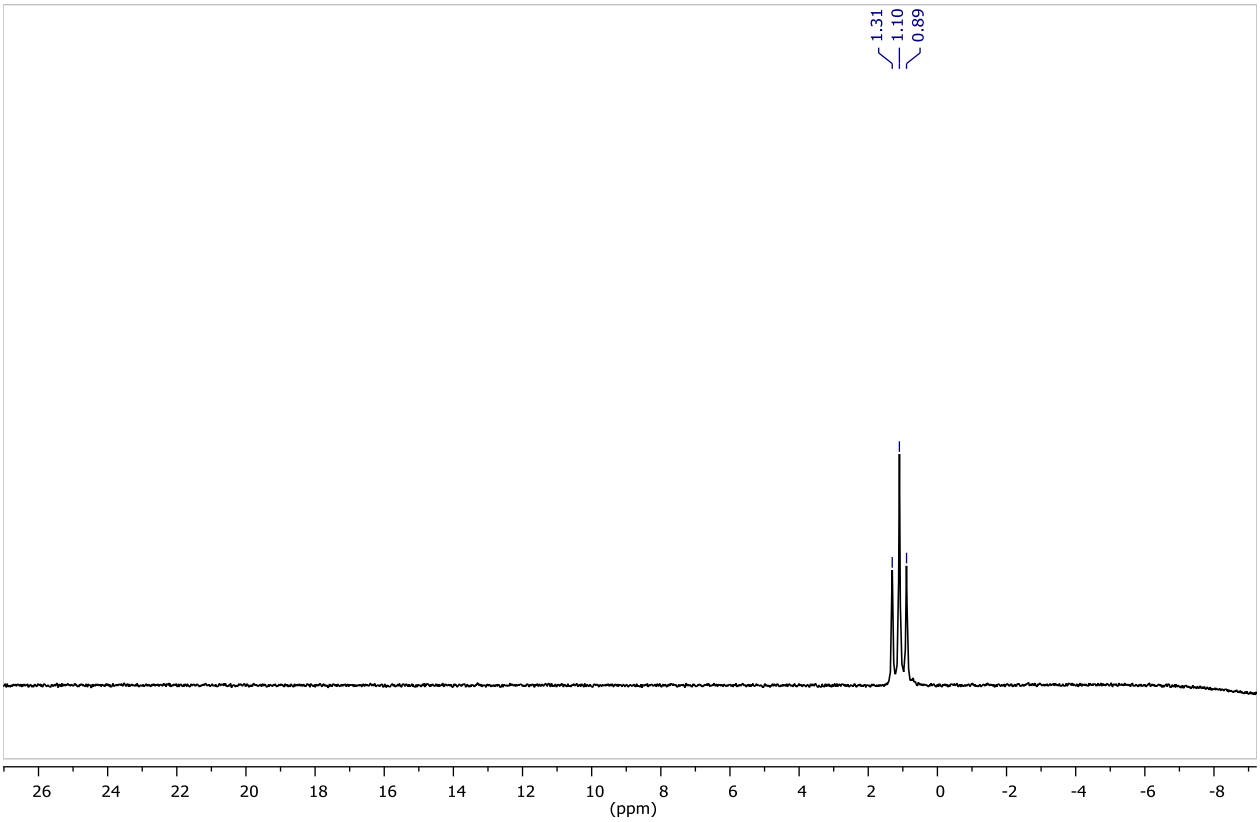


(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (HPLC-LRMS)



(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (^{19}F and ^{11}B NMR)





(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (UV/Vis)

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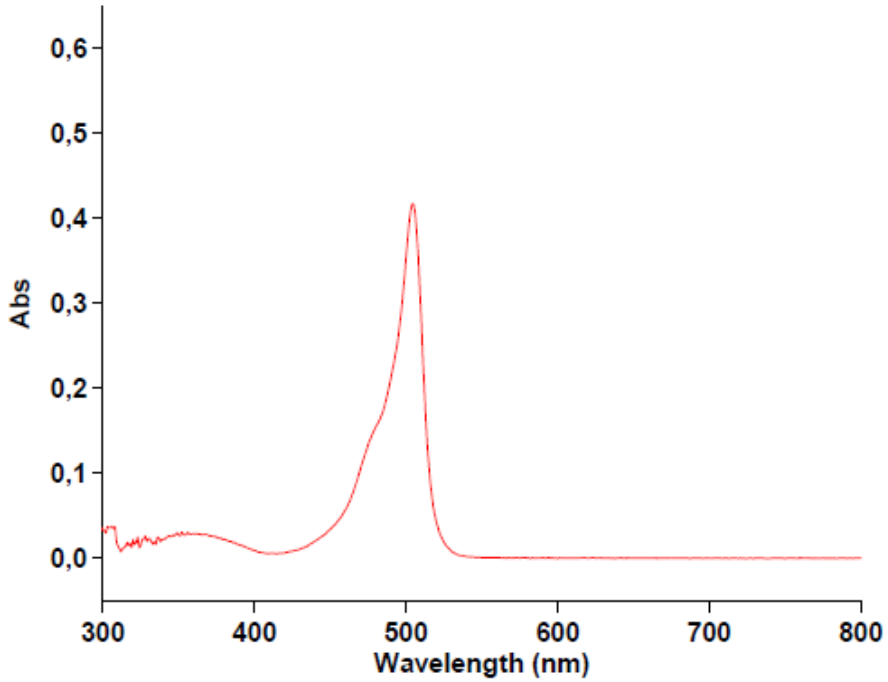


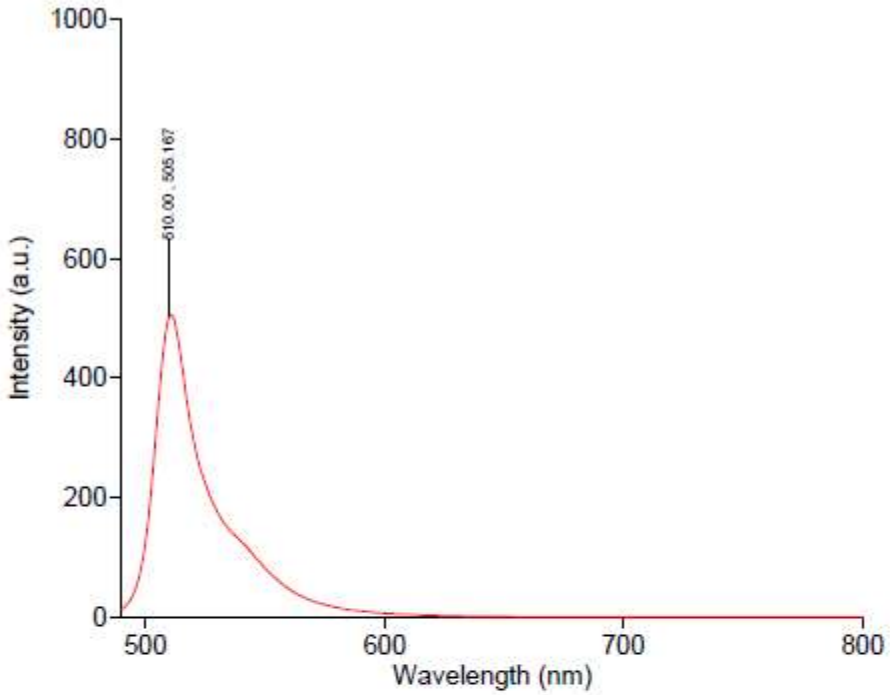
Sample Name: KL-RZ6-136

Collection Time	30.10.2020 15:20:38
Peak Table	
Peak Style	Peaks
Peak Threshold	0,0010
Range	800,00nm to 200,00nm

Wavelength (nm)	Abs
504,00	0,418

123 µg in 10 mL MeOH

(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (Fluorescence Emission)



Sample name: KL-RZ6-136

Peak table

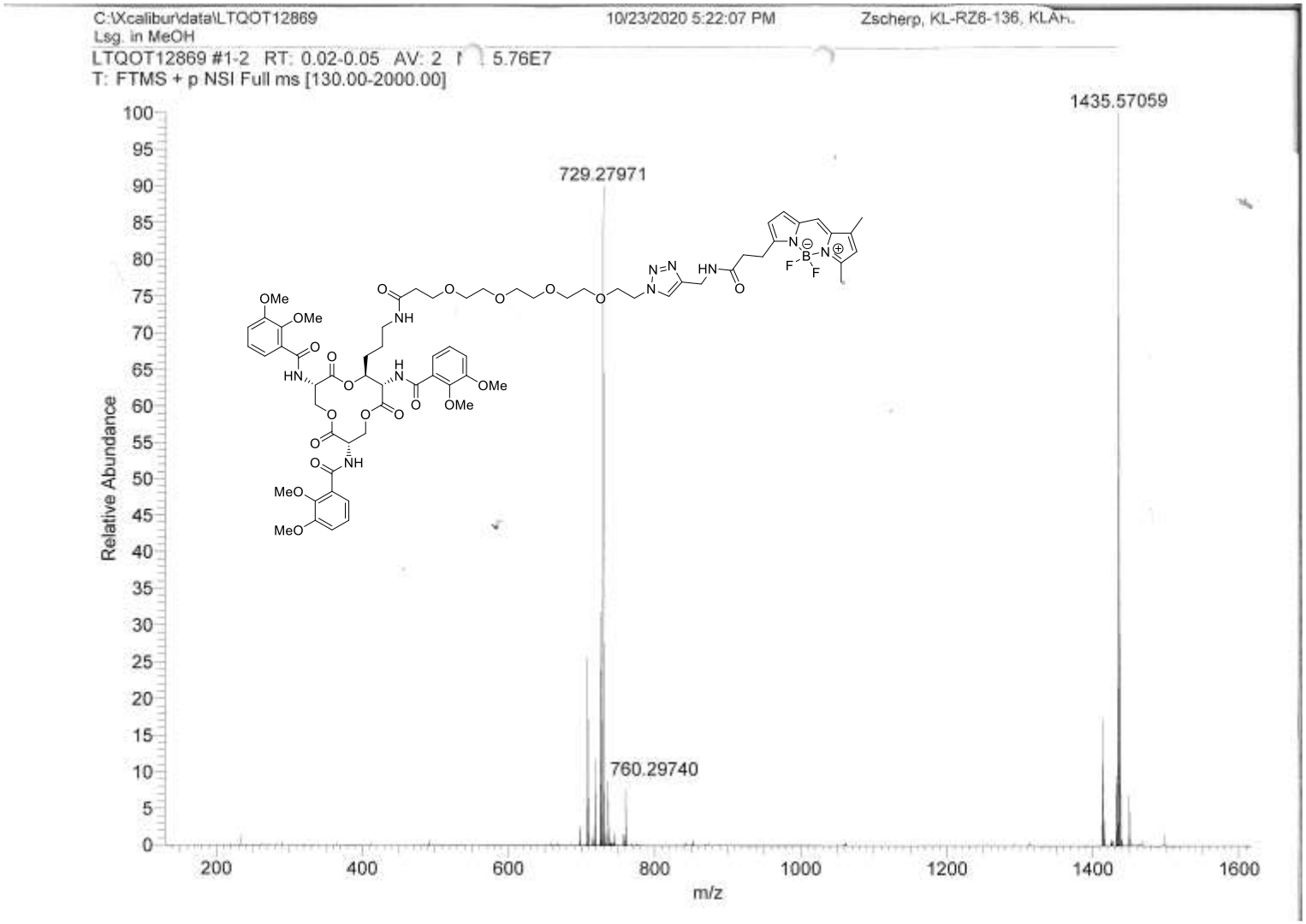
Peak Style	Peaks
Peak Threshold	50.000

Wavelength (nm)	Int. (a.u.)
510.00	505.167

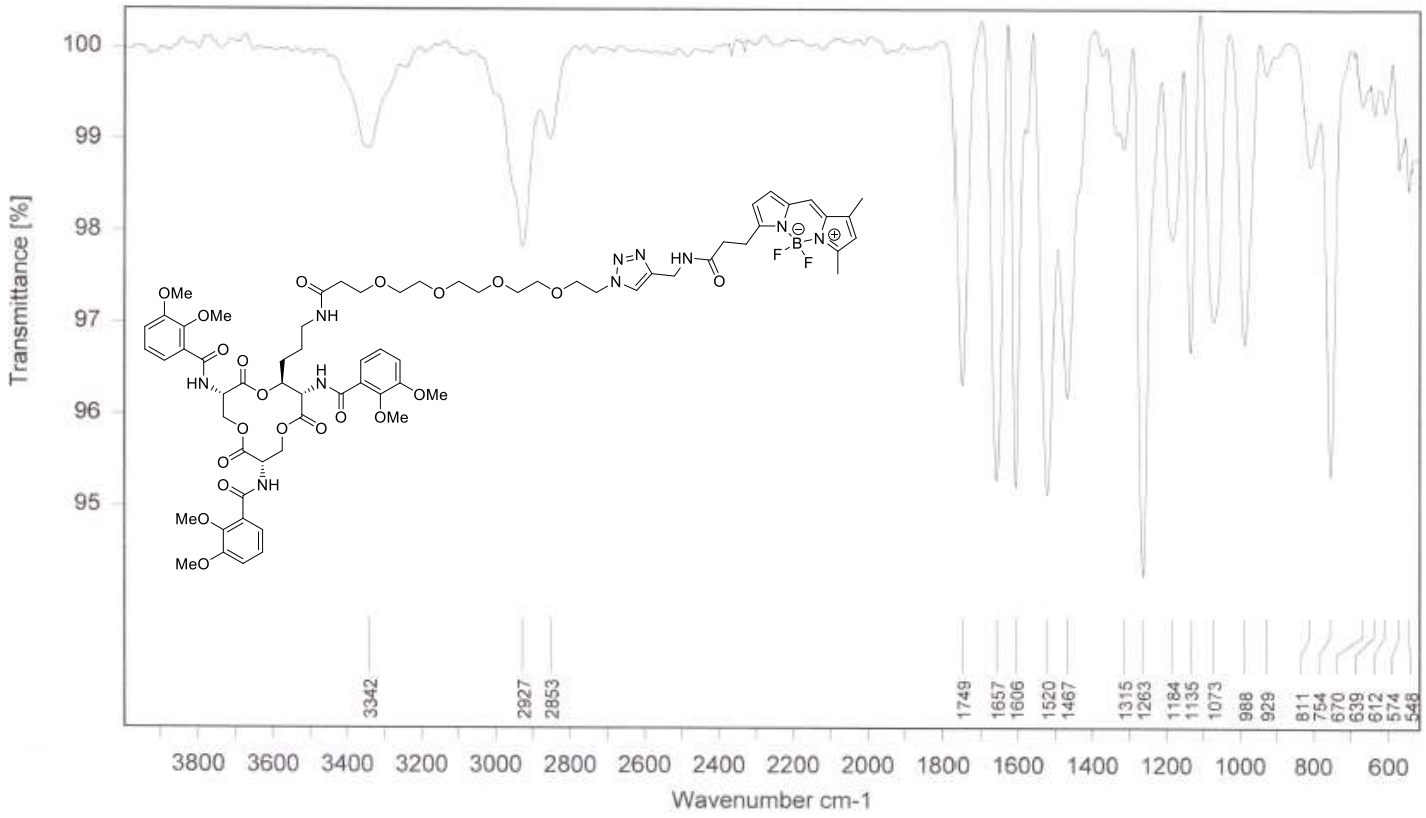
25 µg in 10 mL MeOH

Excitation wavelength: 504 nm

(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (HRMS)



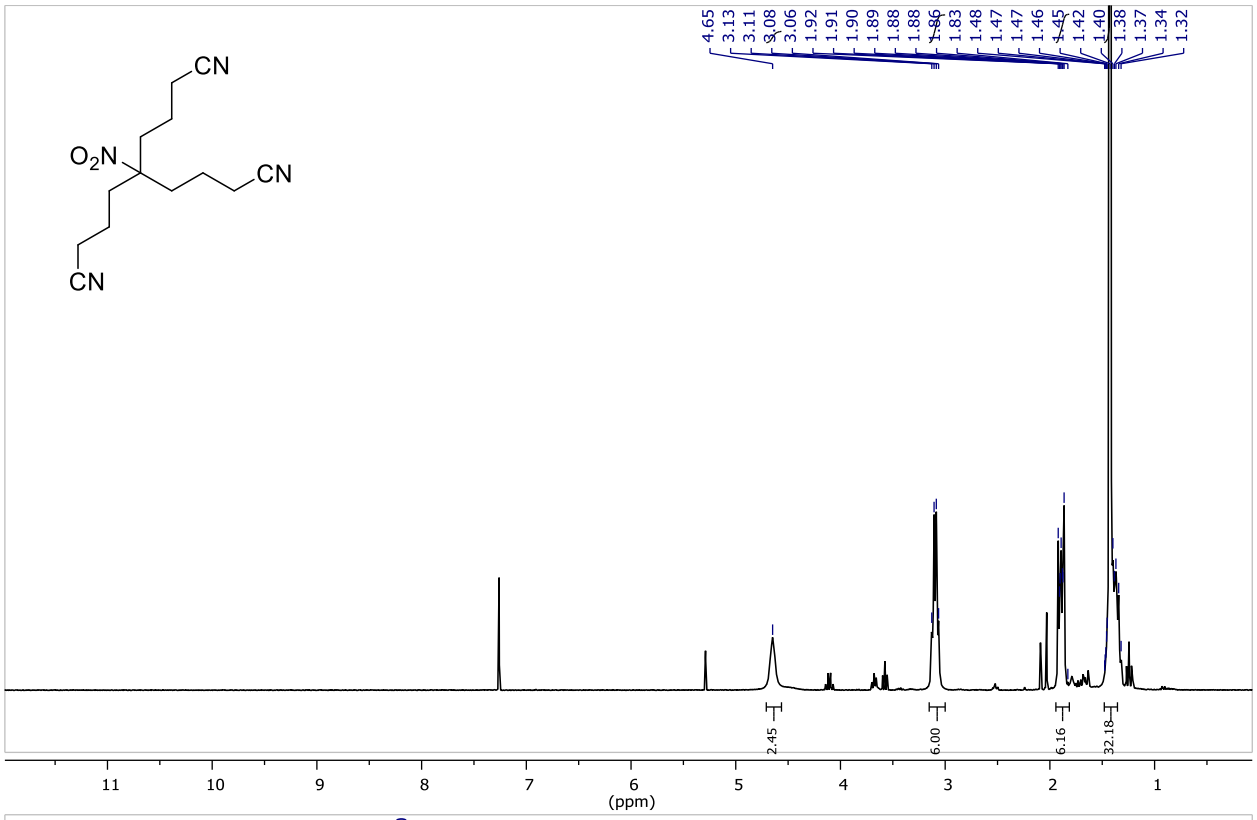
(MeO)Ent$_{KL}$-PEG$_4$-BODIPY$_{FL}$ (ATR-IR)

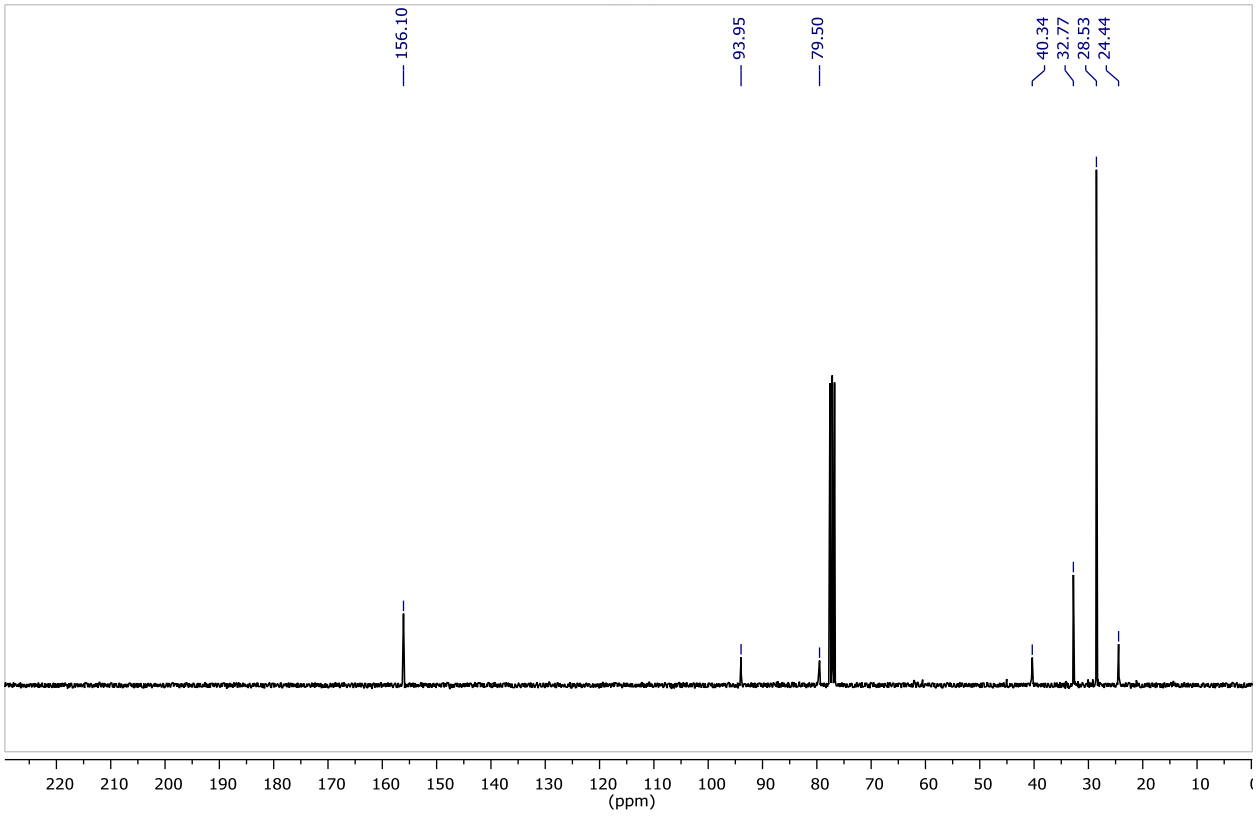


Instrument: Bruker Tensor 27	
Filename: zsr29846.0	Number of Scans: 32
Sample Name: OMe-EntKL-BDP	Operator Name: Default
Technique: Diamant-ATR	Date & Time of Measurement: 30.10.2020 14:03:00

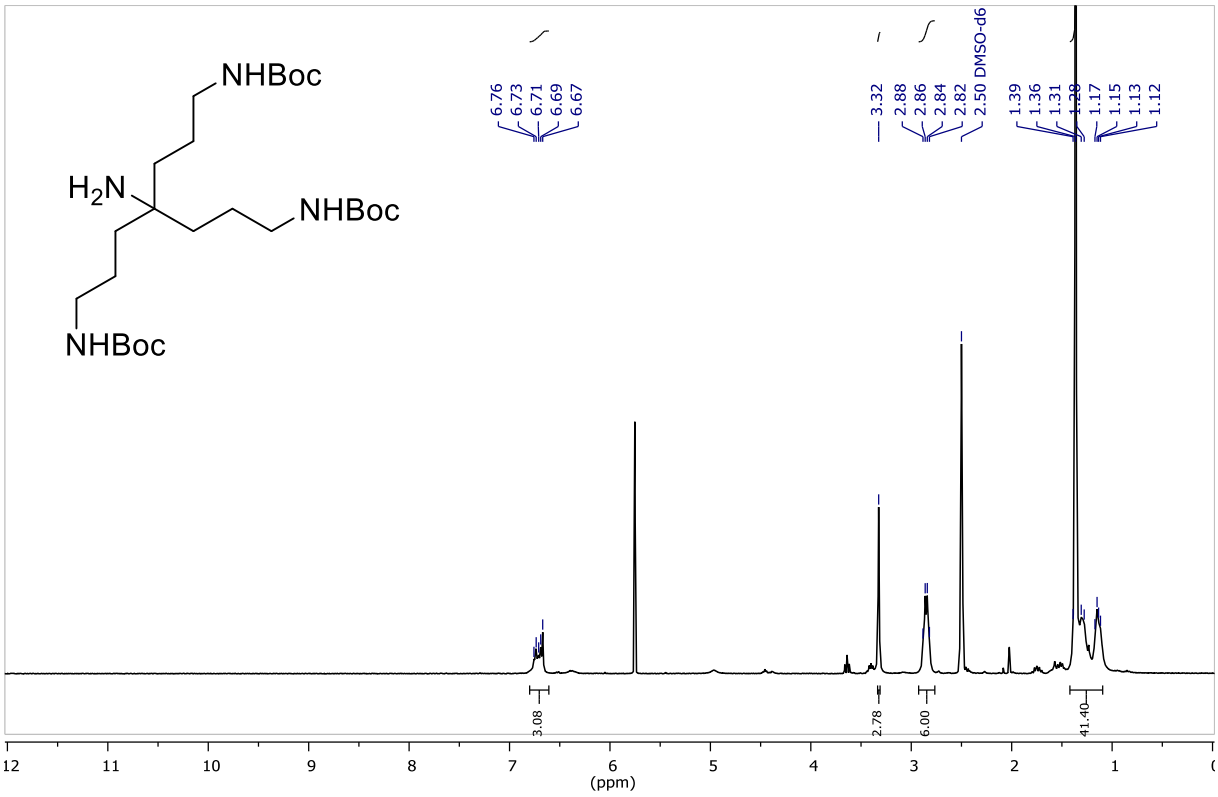
30.10.2020

Di-tert-butyl (4-(3-((tert-butoxycarbonyl)amino)propyl)-4-nitroheptane-1,7-diyl)dicarbamate

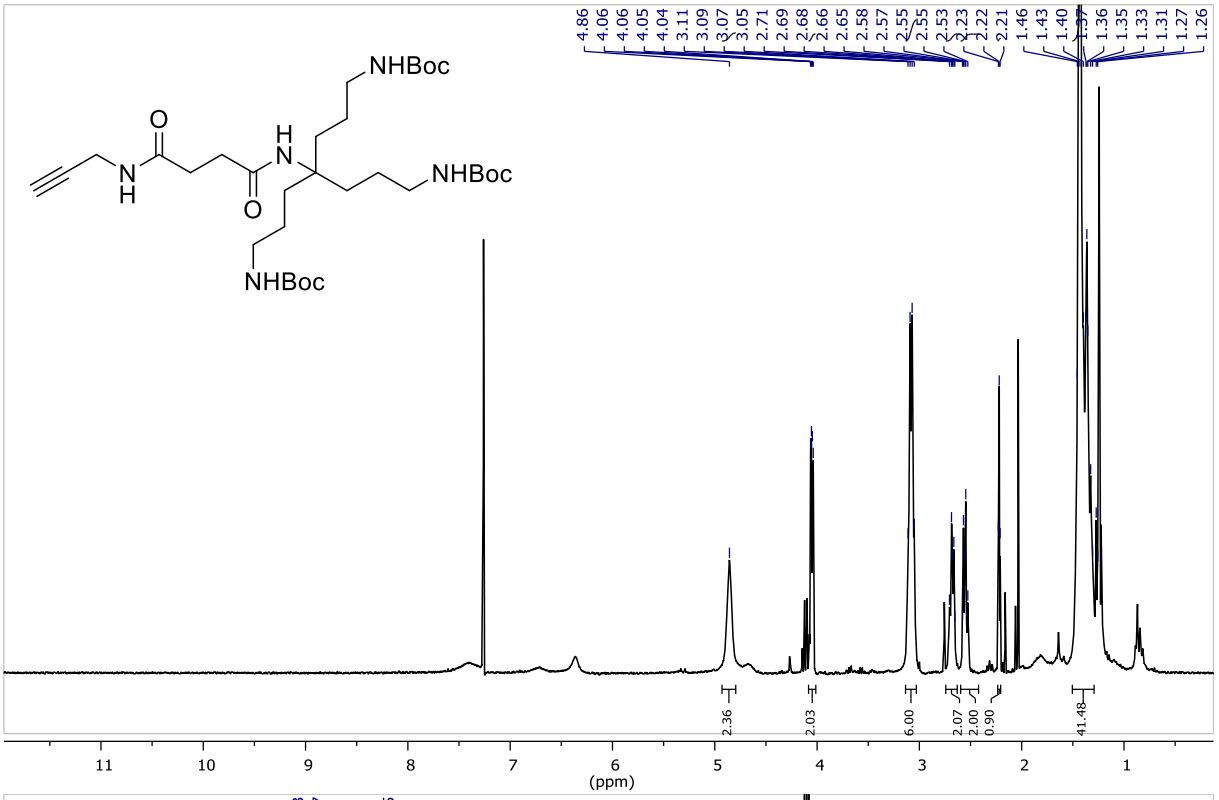


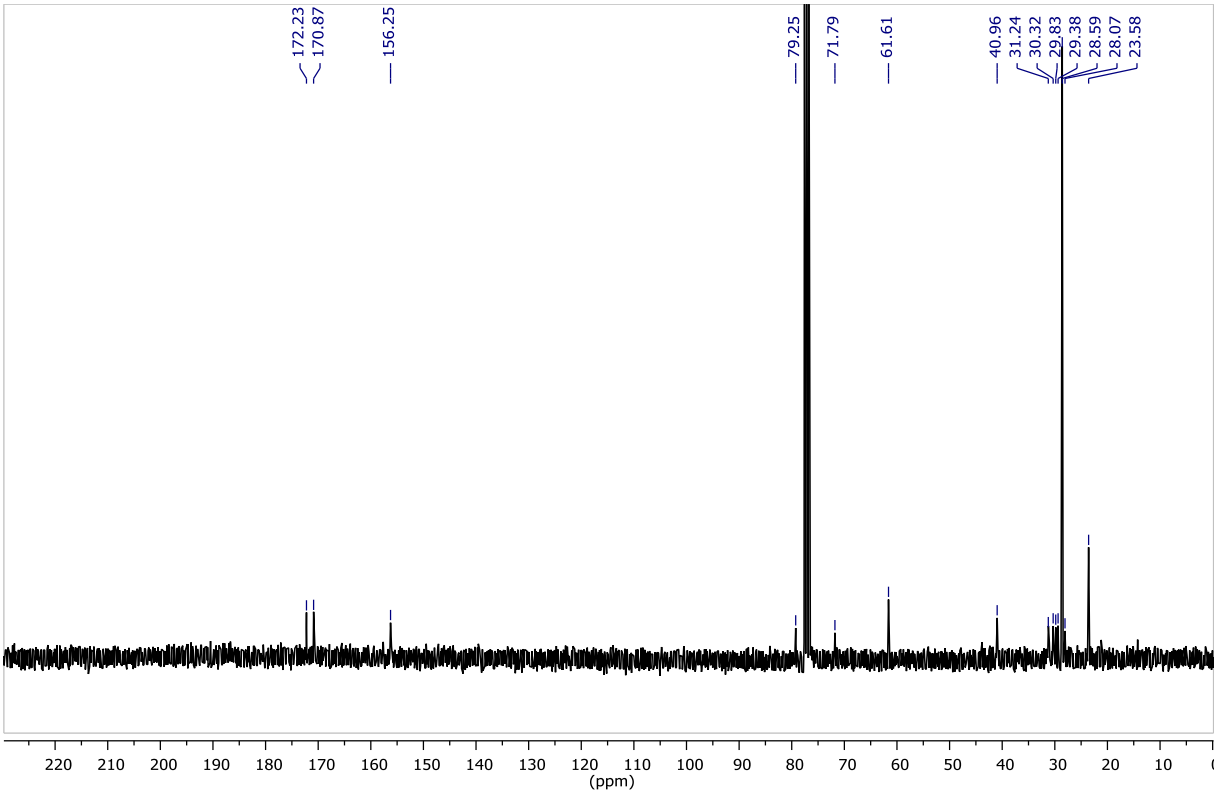


Di-tert-butyl (4-amino-4-(3-((tert-butoxycarbonyl)amino)propyl)heptane-1,7-diyl)dicarbamate

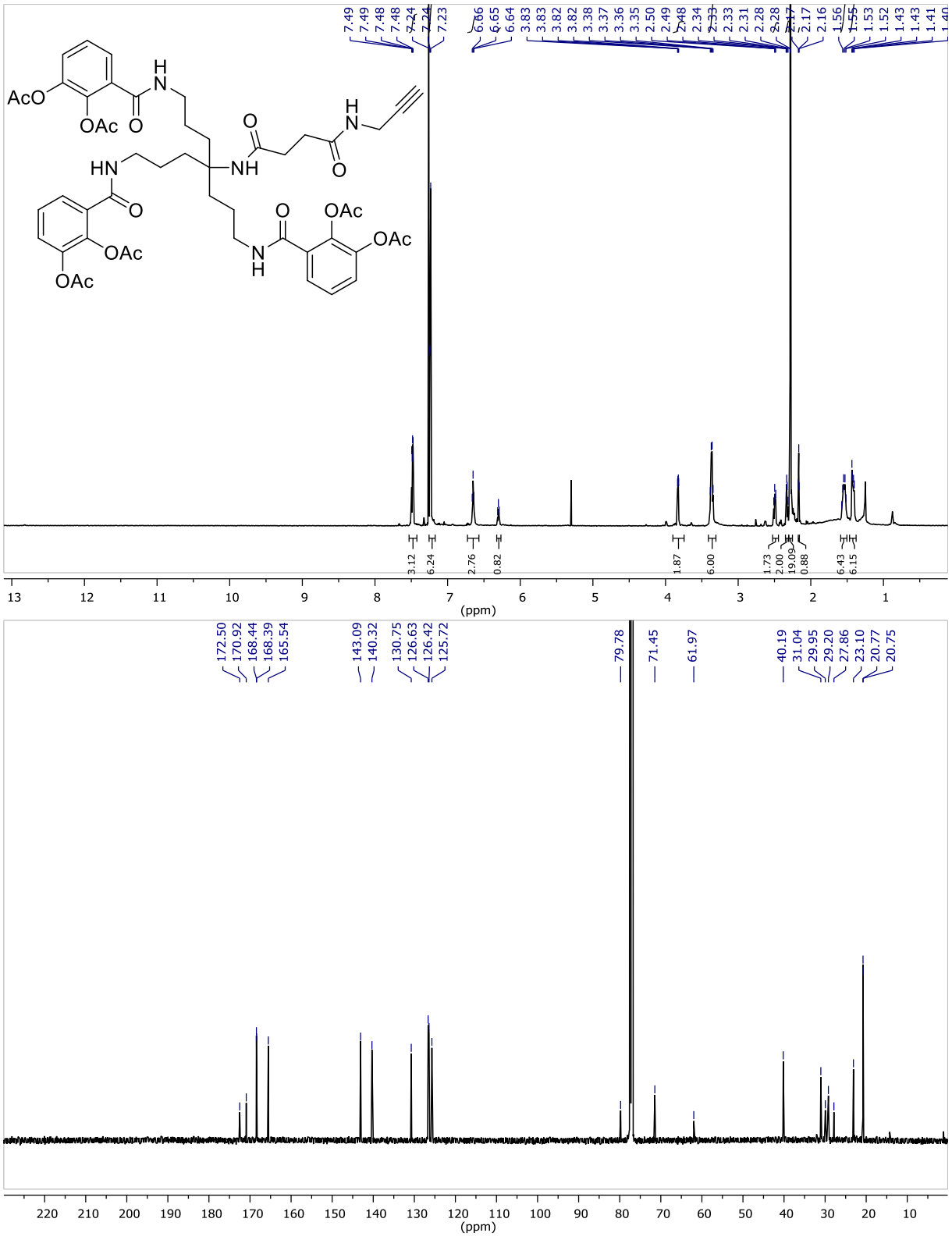


Di-tert-butyl (4-(3-((tert-butoxycarbonyl)amino)propyl)-4-(4-oxo-4-(prop-2-yn-1-ylamino)butan-amido)heptane-1,7-diyl)dicarbamate

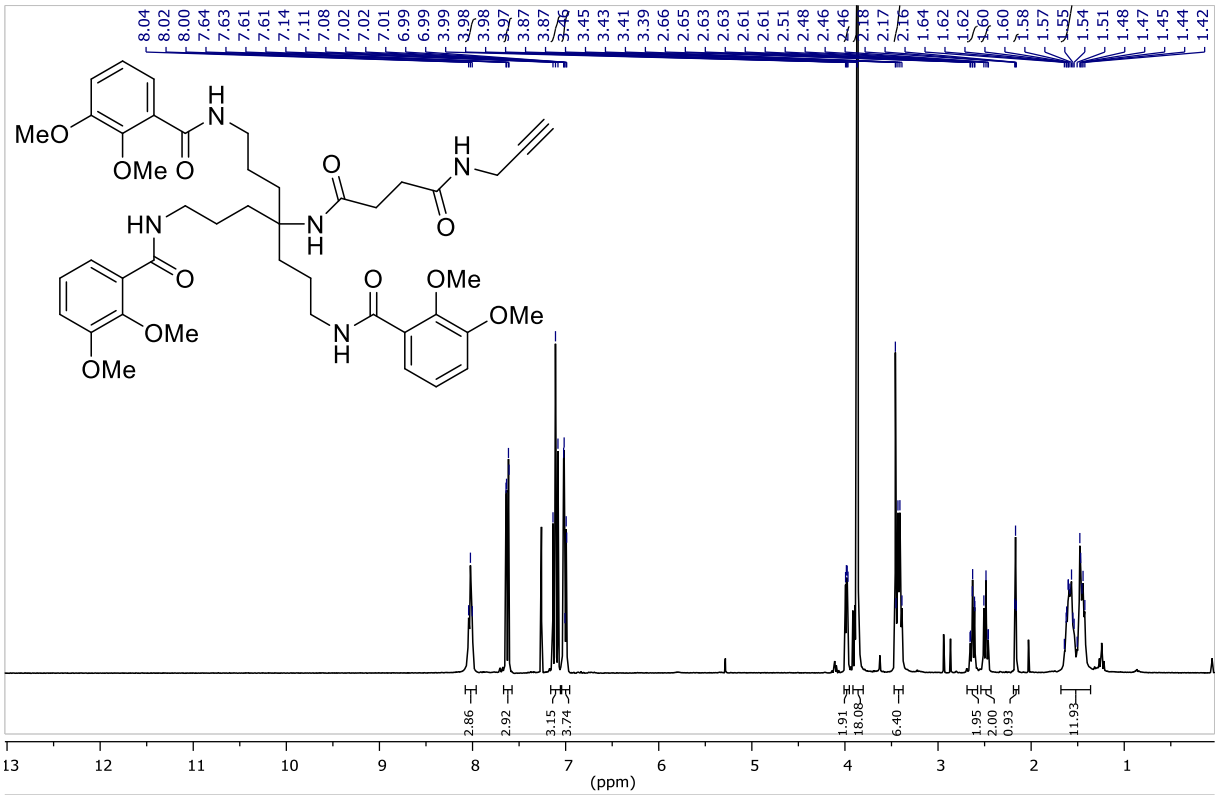




(AcO)Ent$_M$


7.49
7.49
7.48
7.48
7.24
7.24
7.23
6.66
6.65
6.64
3.83
3.83
3.82
3.82
3.38
3.37
3.36
3.35
2.50
2.49
2.48
2.34
2.33
2.33
2.31
2.28
2.28
2.17
2.16
1.56
1.55
1.53
1.52
1.43
1.43
1.41
1.40
AcO
OAc
OAc
HN
NH
OAc
OAc
OAc
OAc
3.12
6.24
2.76
0.82
1.87
6.00
1.73
2.00
19.09
0.88
6.43
6.15
(ppm)
172.50
170.92
168.44
168.39
165.54
143.09
140.32
130.75
126.63
126.42
125.72
79.78
71.45
61.97
40.19
31.04
29.95
29.20
27.86
23.10
20.77
20.75
(ppm)

(MeO)Ent$_M$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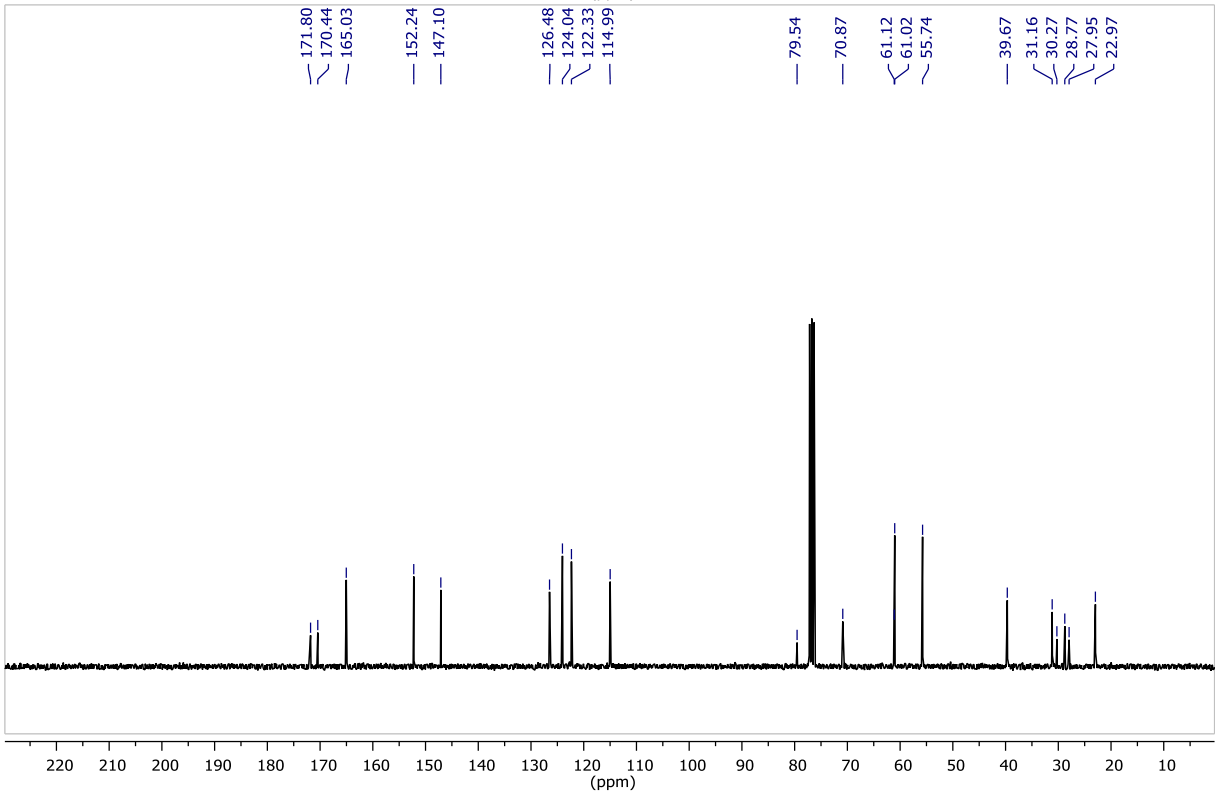
MeO
OMe
OMe
OMe
OMe
OMe
2.86 2.92 3.15 3.74 1.91 18.08 6.40 1.95 2.00 0.93 11.93
(ppm)


171.80 170.44 165.03 152.24 147.10 126.48 124.04 122.33 114.99 79.54 70.87 61.12 61.02 55.74 39.67 31.16 30.27 28.77 27.95 22.97
(ppm)