Supplementary Information

Synthesis and *in vitro* metabolic stability of sterically shielded antimycobacterial phenylalanine amides

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2. Methods & Materials

General

The purchased starting materials were used as received without further purification. Solvents used for either synthetical or purification purposes were distilled and stored over 4 Å-molecular sieves. Glassware was oven-dried at 110 °C prior to use. For the determination of R_f values and other analytical purposes such as qualitative chromatography Merck TLC silica gel 60 on aluminium sheets with fluorescent indicator F254 were used. Flash chromatography was performed with a puriFlash® 430 instrument (Interchim, Montluçon, France). Columns were packed in either 8 g (v=10 mL/min), 45 g (v=30 mL/min) or 90 g (v=40 mL/min) cartridges with 40 - 63 µm normal phase silica gel produced by Carl Roth. Column loading was performed with the dry load method. NMR spectra were recorded on an Agilent Technologies VNMRS 400 MHz spectrometer. Chemical shifts are reported relative to the residual solvent signal ($CDCl_3$: δ_H = 7.26 ppm; δ_C = 77.36 ppm; CD_3OD δ_H = 3.31 ppm). ^{13}C NMR spectral data were determined as attached-proton-test spectra (APT) and plain ^{13}C spectra. Spectra have been cut, baseline and phase corrected and analyzed utilizing MestreNova 11.0 software (Mestrelab Research, S.L., Spain). APCI-MS (atmospheric pressure chemical ionization) was performed using an expression CMS mass spectrometer (Advion Inc., Ithaca, NY, USA), with both ASAP (atmospheric solids analysis probe) sampling and with the help of the Plate Express TLC-plate extractor. ESI measurements have been conducted on the same expression CMS mass spectrometer with an ESI ionization module and direct injection sampling. HRMS was carried out using a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

X-ray crystallography

Crystals of **2** suitable for single-crystal X-ray diffraction were obtained by slow evaporation from a solution in ethanol. The X-ray intensity data collection was carried out at 100 K on a Bruker AXS D8 Venture diffractometer, using Mo-K_α radiation from an Incoatec IµS Diamond microfocus X-ray source with Montel multilayer optics. A crystal was mounted on a MiTeGen cryo loop using perfluoropolyether PFO-XR75. The programs APEX4 [35] and SAINT [36] were used to control the data collection and to process the raw diffraction data. Scaling and an absorption corrections based on indexed crystal faces was performed with APEX5 [37]. The crystal structure was solved with SHELXT [38] and initially refined with SHELXL-2019/3 [39]. The final structure refinement was carried out by Hirshfeld atom refinement with aspherical scattering factors using NoSpherA2 [30, 31] partitioning in Olex2 1.5 [40] based on electron density from iterative single determinant SCF single point DFT calculations using ORCA v.4.1.1 [41] with a B3LYP functional [42, 43] and a def2-TZVPP basis set. Positions and U_{iso} values of all hydrogen atoms were refined freely. The *R* configuration at the α-carbon atoms was inferred from the known absolute configuration of the starting material and the absolute structure was confirmed by a Flack *x* parameter [44] and a Hooft *y* parameter [45] close to zero (**Table S1**). Structure pictures were drawn with Mercury [46]. Crystal data and refinement details are given in **Table S1**. **Figure S1** shows a displacement ellipsoid plot and **Table S2** lists the corresponding hydrogen bond parameters. CCDC 2293688 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe via www.ccdc.cam.ac.uk/structures.

Table S1 *Crystal data and refinement details for* ***2****.*

Empirical formula	$C_{26}H_{26}FN_3O_4S$
M_r	495.578
T (K)	100(2)
λ (Å)	0.71073
Crystal system, space group	Triclinic, $P1$
a (Å)	9.5510(8)
b (Å)	10.4458(9)
c (Å)	12.6068(10)
α (°)	73.785(4)
β (°)	78.752(4)
γ (°)	85.654(4)
V (Å^3)	1184.22(17)
Z, ρ_{calc} (mg m^{-3})	2,
μ (mm^{-1})	0.184
F(000)	520.570
Crystal size (mm)	0.197 × 0.165 × 0.118
θ range (°)	2.03 - 36.38
Reflections collected / unique	551394
R_{int}	0.062
Completeness to θ_{full} (%)	99.67
Data / restraints / parameters	23006 / 3 / 839
Observed data [$I > 2\sigma(I)$]	21992
Goodness-of-fit on F^2	1.2264
$R1$ [$I > 2\sigma(I)$]	0.0179
$wR2$ (all data)	0.0374
Flack x parameter	−0.002(6)[a]
Hooft y parameter	−0.009(6)[b]
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.24, −0.11
CCDC No.	2293688

[a] Calculated with Olex2.

[b] Calculated with PLATON [47].

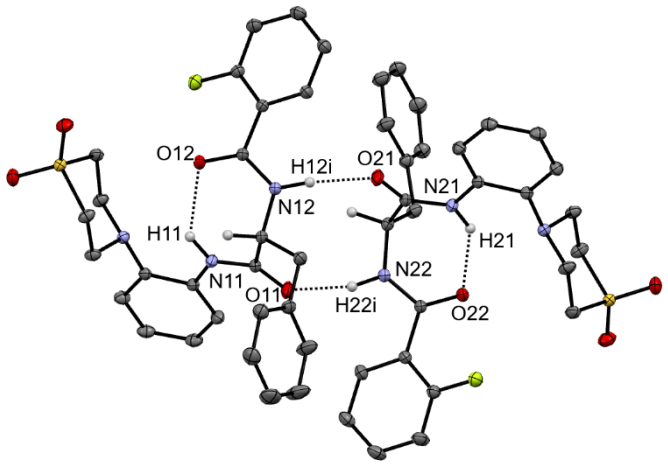

Figure S1 *Asymmetric unit of* ***2****. Displacement ellipsoids are drawn at the 50 % probability level. Nitrogen-bound hydrogen atoms and the carbon-bound hydrogen atoms attached to centres of chirality are represented by small spheres of arbitrary radius, otherwise omitted for clarity. Colour scheme: C, grey; H, white; N, blue; O, red; F, light green; S, yellow.*

Table S2 *Hydrogen bond parameters in* ***2****.*

D*–H···*A	***d*(D–H) (Å)**	***d*(H···*A*) (Å)**	***d*(*D*···*A*) (Å)**	**<(*DHA*) (°)**
N11 H11 O12	1.006(7)	2.287(7)	3.0836(6)	135.2(6)
N12 H12i O21	0.988(7)	1.913(7)	2.8929(5)	170.9(6)
N21 H21 O22	0.991(8)	2.134(8)	2.9709(6)	141.1(6)
N22 H22i O11	0.984(8)	1.843(8)	2.7998(5)	163.3(6)

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Plasma stability

Plasma stability in human and murine plasma was determined at five different time points over 120 min using HPLC-MS/MS analysis. Pooled human plasma, anti-coagulated with trisodium citrate, and non-sterile murine plasma, anti-coagulated with Li-heparin (Bienta/Enamine Ltd., batch #M190406), were used. The human plasma was used by a third-party lab and the authors were not involved in the extraction and preparation of the samples. The source of the samples was the Kyiv city blood center, Kiew, Ukraine.The plasma stability is given as the percentage of substance remaining in plasma over time. All measurements were performed using the Shimadzu Prominence HPLC system including vacuum degasser, gradient pumps, reverse phase column (ZORBAX Extend-C18 column, 2.1x50 mm, 5 µm), column oven and autosampler. The HPLC system was coupled with tandem mass API 3000 (AB Sciex). Both positive and negative ion modes of the TurboIonSpray ion source were used. Acquisition and analysis of the data were performed using Analyst 1.6.3 software (PE Sciex).

Incubations of every compound + references (Verapamil and Propantheline) were carried out in 5 aliquots of 60 µL each (one for each time point), in duplicates. Plasma was spiked with test compounds from a 10 mM DMSO stock solution to yield a test concentration of 1 µM, final DMSO concentration 1%. The aliquots were incubated at 37 °C with shaking at 100 rpm. Five time points over 120 min have been analyzed. The reactions were stopped by adding 300 µL of methanol containing internal standard with subsequent plasma proteins sedimentation by centrifuging at 6000 rpm for 4 minutes. Supernatants were analyzed by the HPLC system that was coupled with a tandem mass spectrometer. The percentage of the test compounds remaining after incubation were calculated.

Microsomal stability

Microsomal stability in human and murine microsomal suspensions was determined at five different time points over 40 min using HPLC-MS/MS analytic. The microsomes were purchased commercially from XenoTech: human liver microsomes (pooled, mixed gender, H0630/lot N#1210097) and mouse liver microsomes (pooled, male BALB/c, M3000/lot #2010026). All measurements were performed using the Shimadzu Prominence HPLC system including vacuum degasser, gradient pumps, reverse phase column (Phenomenex Luna®-C18 column, 2.1x50 mm, 5 µm), column oven and autosampler. The HPLC system was coupled with tandem mass API 3000 (AB Sciex). Both positive and negative ion

modes of the TurboIonSpray ion source were used. Acquisition and analysis of the data were performed using Analyst 1.6.3 software (PE Sciex).

Incubations of every compound + references (Diclofenac and Propranolol for human microsomes, Propranolol and Imipramin for murine microsomes) were carried out in 5 aliquots of 30 µL each (one for each time point), in duplicates. Liver microsomal incubation medium contained 0.42 mg of liver microsomal protein as well as phosphate buffer (100 mM, pH 7.4), $MgCl_2$ (3.3 mM), NADPH (3 mM), glucose-6-phosphate (5.3 mM) and glucose-6-phosphate dehydrogenase (0.67 units/ml). In the control reactions, the NADPH-cofactor system was substituted with phosphate buffer. Microsomal suspensions were spiked with test compounds from a 10 mM DMSO stock solution to yield a test concentration of 2 µM, final DMSO concentration 1.6 %. The aliquots were incubated at 37 °C with shaking at 100 rpm. Five time points over 40 min have been analyzed. The reactions were stopped by adding 5 volumes of 90 % acetonitrile containing internal standard with subsequent protein sedimentation by centrifuging at 5500 rpm for 5 minutes. Supernatants were analyzed by the HPLC system that was coupled with a tandem mass spectrometer. The percentage of the test compounds remaining after incubation were calculated. The elimination constants (k_{el}) and half-lives ($t_{1/2}$) of the compounds were determined in a plot of ln(AUC) versus time, using linear regression analysis:

$$k_{el} = -slope$$

$$t_{1/2} = \frac{0.693}{k_{el}}$$

HPLC

Analytical HPLC

All described final compounds described were confirmed to be of >95 % purity. The used Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consists of a CBM-40 control unit, a DGU-403 degassing unit, two LC-40D chromatography pumps, a SIL-40C autosampler unit, a CTO-40C column oven and an SPD-M40 PDA UV detector. The standard method for purity determination utilized an Agilent Poroshell 120, EC-C18, 3,0x50mm, 2,7 µm analytical column at a flow rate of 1.2 mL/min at room temperature. The 6 min gradient started at 15 % and increased to 85 % acetonitrile in water and was used in all cases. All solvents used are HPLC grade purity. UV absorbance at 254 nm was measured and the purity was derived from the integrated intensity signal.

Preparative HPLC

If necessary, final compounds were purified by preparative HPLC. The Shimadzu HPLC system (Shimadzu, Kyoto, Japan) used consists of a CBM-40 control unit, two LC-20AP chromatography pumps, a SIL-40C autosampler unit, a CTO-40C column oven, an SPD-M40 PDA UV detector and an FRC-10A fraction collector. A preparative column manufactured by Agilent (Polaris C18-A, 5 µ, 250 X 21.2mm) was used at a flow rate of 15 mL/min with a standard gradient of 50 % to 95% acetonitrile in water over 20 min at room temperature.

Microbiological assays

MIC determination against *M. intracellulare* ATCC 35761 pTEC27 and *M. abscessus* ATCC 19977 pTEC27

M. intracellulare ATCC 35761 pTEC27 and *M. abscessus* ATCC 19977 pTEC27 expressing tomato RFP were used for the activity assays. Cryo-stocks of the bacteria grown in 7H9 broth supplemented with 10 % ADS and 0.05 % Tween 80 were stored with approximately 15 % glycerol at -80°C. Using an inoculation loop, bacteria were streaked on 7H10 plates supplemented with 0.5 % glycerol, 10 % ADS containing and 400 µg/mL hygromycin and grown for 5 days in an incubator at 37°C. A single colony was picked from the 7H10 plates and the liquid culture was grown in 7H9 broth supplemented with 10 % ADS, 0.05 % Tween 80 and 400 µg/mL hygromycin. The culture volume was 10 mL in a 50 mL Falcon tube. The tubes were shaken in an incubator at 37°C.

MIC values were determined by the broth microdilution method. 96-well flat bottom tissue culture plates (Sarstedt, 83.3924.500) were used[31]. In the third well of each row two times the desired highest concentration of each compound was added in 7H9 medium supplemented with 10 % ADS and 0.05 % Tween 80. Each compound was diluted twofold in a nine-point serial dilution in 50 µL medium. (All drugs were prepared as 10 mM stocks in dimethyl sulfoxide (DMSO).)

The starting inoculum was diluted from a liquid culture described above at the mid-log phase (OD_{600} 0.3 to 0.7) and an OD_{600} of 0.1 was correlated to 1 x 10^8 CFU/mL. A starting inoculum of 5 x 10^5 cells/mL was used and 50 µl were added to each well. The plates were sealed with parafilm, placed in a container with moist tissue and incubated for three days at 37 °C (*M. abscessus*) or five days (*M. intracellulare*). After incubation the plates were monitored by OD measurement at 550 nm (BMG labtech Fluostar Optima) and by measurement of fluorescence (λ_{ex} = 544 nm λ_{em} = 590 nm). The assay was performed in duplicate, and the results were averaged.

Data analysis:

Every assay plate contained eight wells with dimethyl sulfoxide (1 %) as negative control, which corresponds to 100 % bacterial growth and eight wells with amikacin (100 µM) as positive control in which 100 % inhibition of bacterial growth was reached. Controls were used to monitor the assay quality through determination of the Z' score. The Z' factor was calculated as follows:

$$Z' = 1 - \frac{3(SD_{amikacin} + SD_{DMSO})}{M_{amikacin} - M_{DMSO}}$$

(SD = standard deviation, M = mean)

The percentage of growth inhibition was calculated by the equation:

$$\%\ \text{growth inhibition} = 100\ \% \times \frac{\text{signal(DMSO)} - \text{signal(sample)}}{\text{signal(DMSO)} - \text{signal(amikacin)}}$$

MIC determination against *M. avium ssp. hominissuis strain 109 (MAC109)*

MIC value determination by optical density at 600 nm [OD_{600}] was carried out in 96-well plate format. 96-well plates were initially set up with 100 µl of 7H9 per well. For each compound, a 10-point twofold dilution series starting at twice the desired highest concentration was dispensed onto the 96-well plates using a Tecan D300e Digital Dispenser, with the DMSO concentration normalized to 2 % v/v. A bacteria culture grown to mid-log-phase (OD_{600},0.4 to 0.6) was diluted to OD_{600} = 0.1 ($1*10^7$ CFU/mL). 100 µl of the resulting bacteria suspension was dispensed onto the 96-well plates containing the sample compounds to give a final volume of 200 µl per well with an initial OD_{600} = 0.05 ($5*10^6$ CFU/mL) and a final DMSO concentration of 1 % v/v. Final compound concentration ranges were typically 50 to 0.098 µM or 6.25 to 0.012 µM. Untreated control wells, which contained bacteria suspension and 1 % v/v DMSO, were included on each plate. Plates were sealed with parafilm, stored in boxes with wet paper towels, and incubated at 37°C with shaking (110 rpm) and were incubated for 5 days.

To determine growth, OD_{600} was measured using a Tecan Infinite M200 plate reader on day 0 and day 3. Two biological replicates were performed. Clarithromycin was included in each experiment as a positive control. For each well on the 96-well plate, bacterial growth was calculated by subtracting the day 0 OD_{600} value from the day 3 OD_{600} value. For each compound series, the bacterial growth values for the untreated control wells were averaged to give the average drug-free bacterial growth. For compound-containing wells, percentage growth was calculated by dividing their growth values by the average drug-free bacterial growth for the compound series and multiplying by 100. For each compound series, we plotted percentage growth versus compound concentration. By visual inspection of the dose-response curve, we determined the MIC of a compound as the compound concentrations that would result in 90 % growth inhibition. The MIC determination was performed two times with different starter cultures. The MIC values shown in the script are the averaged results of biological duplicates.

MIC determination against *M. tuberculosis* H37Rv

MICs against Mtb H47Rv were determined as described in [48]: Yang, X.; Wedajo, W.; Yamada, Y.; Dahlroth, S. L.; Neo, J. J. L.; Dick, T.; Chui, W. K. 1,3,5-Triazaspiro[5.5]Undeca-2,4-Dienes as Selective Mycobacterium Tuberculosis Dihydrofolate Reductase Inhibitors with Potent Whole Cell Activity. *Eur J Med Chem* **2018**, *144*, 262–276. https://doi.org/10.1016/J.EJMECH.2017.12.017. Briefly, compounds were serially diluted in flat-bottom 96-well plates, and a mid-log-phase culture was mixed with the compound-containing broth (final OD_{600} = 0.05). Plates were sealed with Breathe-Easy sealing membrane (Sigma), placed in humidified plastic boxes and incubated at 37°C for 7 days, shaking at 80 rpm. Growth was monitored by measuring turbidity at 600 nm using a Tecan Infinite 200 Pro microplate reader (Tecan). MIC_{90} values were deduced from the generated dose–response curves. The MIC values shown in the script are the averaged results of the two biological replicates.

Cytotoxicity against immortalized human kidney epithelial

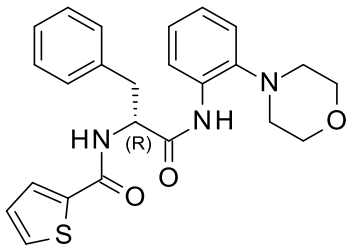
For evaluation of possible cytotoxic effects on mammalian cells, 20.000 HEK293 cells (immortalized human kidney epithelial cells; DSMZ, Braunschweig, Germany) were seeded out per well of a 96-well plate (Sarstedt, Germany) in DMEM supplemented with 10% FCS (both Gibco) and cultured in a humidified incubator at 37°C and 5% CO_2. After 4 hours and attaching cells to the bottom, all compounds in DMSO were added to a final concentration of 50 µM. A sample containing only 0.5%

DMSO was used as control. Cells were incubated for 24 h under the above-described conditions before AlamarBlue reagent (Thermo Fisher Scientific, Germany) was added. Samples were incubated again for 4 h before measurement was performed. The plates were analyzed in a microtiter plate reader (BMG, Germany) and the forming resorufin was quantified in fluorescence channel with Ex:550/Em:590 nm. The data are means of triplicates with standard deviation.

3. Structures and synthetic protocols

MMV

(2R)-N-[2-(morpholin-4-yl)phenyl]-3-phenyl-2-[(thiophen-2-yl)formamido]propenamide



Synthesis & Purification:

Described as compound **2B-(R)** in [18]: Lang, M., Ganapathy, U. S., Mann, L., Abdelaziz, R., Seidel, R. W., Goddard, R., Sequenzia, I., Hoenke, S., Schulze, P., Aragaw, W. W., Csuk, R., Dick, T., & Richter, A. (2023). Synthesis and Characterization of Phenylalanine Amides Active against Mycobacterium abscessus and Other Mycobacteria. *Journal of Medicinal Chemistry*. https://doi.org/10.1021/ACS.JMEDCHEM.3C00009

Analyses:

R_F value: 0.37 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 1**) (400 MHz, Chloroform-*d*) δ 8.83 (s, 1H), 8.40 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.52 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.47 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.32 – 7.25 (m, 4H), 7.23 – 7.17 (m, 1H), 7.16 – 7.05 (m, 3H), 7.03 (dd, *J* = 5.0, 3.7 Hz, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 4.95 (td, *J* = 8.0, 5.6 Hz, 1H), 3.68 – 3.53 (m, 4H), 3.39 (dd, *J* = 13.6, 5.6 Hz, 1H), 3.25 (dd, *J* = 13.6, 8.1 Hz, 1H), 2.69 – 2.50 (m, 4H).

^{13}C NMR APT (**Spectrum 2**) (101 MHz, Chloroform-*d*) δ 168.77, 161.56, 141.19, 137.97, 136.50, 132.91, 130.75, 129.38, 128.86, 128.66, 127.81, 127.12, 125.69, 124.33, 120.80, 119.52, 67.25, 56.19, 52.46, 38.47.

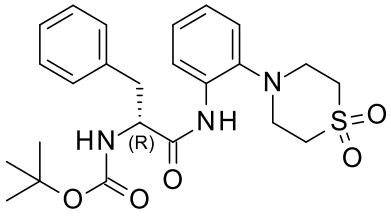
Mass: Calculated m/z for $C_{24}H_{26}N_3O_3S^+$ $[M+H]^+$= 436.1689; $C_{24}H_{25}N_3O_3SNa^+$ $[M+Na]^+$= 458.1509; found APCI: 436.1; found HRMS: $[M+H]^+$ 436.1691; $[M+Na]^+$ 458.1506

HPLC purity: 98.9 % t_R=4.526 min (**HPLC-Trace 1**); (50 mm Eclipse Plus C18 1.8 µm, 4.6 mm, acetonitrile/ water 45:55, v=1.0 mL/min, λ=220 nm

Specific optical rotation: Boc-deprotected intermediate (**2-D**) $[\alpha]^{22.7}_D$ 46.01 (*c* 0.119, MeOH); final compound (**3-D**) $[\alpha]^{22.7}_D$ 56.74 (*c* 0.1375, MeOH)

1

tert-butyl N-[(1R)-1-benzyl-2-[2-(1,1-dioxo-1,4-thiazinan-4-yl)anilino]-2-oxo-ethyl]carbamate



Synthesis & Purification:

Described as compound **23** in: Lang, M., Ganapathy, U. S., Mann, L., Abdelaziz, R., Seidel, R. W., Goddard, R., Sequenzia, I., Hoenke, S., Schulze, P., Aragaw, W. W., Csuk, R., Dick, T., & Richter, A. (2023). Synthesis and Characterization of Phenylalanine Amides Active against Mycobacterium abscessus and Other Mycobacteria. *Journal of Medicinal Chemistry*. https://doi.org/10.1021/ACS.JMEDCHEM.3C00009

Analyses:

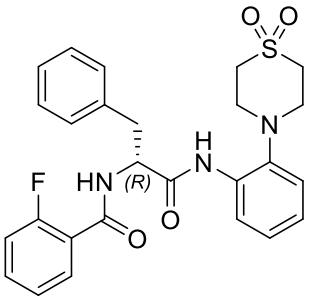
R_F value: 0.31 ethyl acetate/heptane 1:1

^{1}H NMR () (400 MHz, Chloroform-*d*) δ 8.89 (s, 1H), 8.38 (d, *J* = 8.1 Hz, 1H), 7.31 – 7.11 (m, 7H), 7.08 – 7.01 (m, 1H), 5.40 – 5.13 (m, 1H), 4.47 (q, *J* = 7.3 Hz, 1H), 3.33 – 3.00 (m, 10H), 1.37 (s, 9H).

Mass: Calculated m/z for $C_{24}H_{32}N_3O_5S^+$ $[M+H]^+$= 474.2057; found APCI: 474.2

2

(2R)-2-[(2-fluorophenyl)formamido]-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)phenyl]-3-phenylpropanamide



Synthesis & Purification:

Described as compound **24** in: Lang, M., Ganapathy, U. S., Mann, L., Abdelaziz, R., Seidel, R. W., Goddard, R., Sequenzia, I., Hoenke, S., Schulze, P., Aragaw, W. W., Csuk, R., Dick, T., & Richter, A. (2023). Synthesis and Characterization of Phenylalanine Amides Active against Mycobacterium abscessus and Other Mycobacteria. *Journal of Medicinal Chemistry*. https://doi.org/10.1021/ACS.JMEDCHEM.3C00009

Analyses:

R_F value: 0.30 ethyl acetate

^{1}H NMR (**Spectrum 4**) (500 MHz, Chloroform-*d*) δ 9.16 (s, 1H), 8.44 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.08 (td, *J* = 7.9, 1.9 Hz, 1H), 7.52 (dddd, *J* = 8.3, 7.2, 5.3, 1.9 Hz, 1H), 7.36 – 7.22 (m, 7H), 7.20 (ddd, *J* = 8.4, 7.5, 1.5 Hz, 1H), 7.18 – 7.13 (m, 2H), 7.06 (td, *J* = 7.7, 1.5 Hz, 1H), 5.04 (qd, *J* = 6.9, 2.2 Hz, 1H), 3.40 – 3.30 (m, 2H), 3.21 (t, *J* = 5.3 Hz, 4H), 3.12 (t, *J* = 5.1 Hz, 4H).

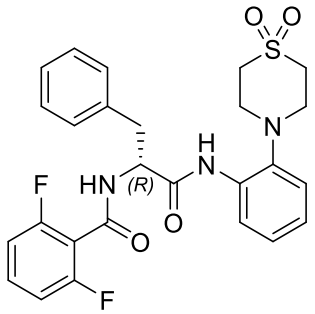
^{13}C NMR (**Spectrum 5**) (126 MHz, Chloroform-*d*) δ 168.70, 163.62 (d, *J* = 3.3 Hz), 160.82 (d, *J* = 248.0 Hz), 139.97, 136.41, 134.36 (d, *J* = 9.5 Hz), 133.12, 131.91 (d, *J* = 1.8 Hz), 129.41, 128.89, 127.19, 126.84, 125.29 (d, *J* = 3.2 Hz), 124.25, 121.51, 120.06, 119.43 (d, *J* = 10.8 Hz), 116.47 (d, *J* = 24.7 Hz), 56.07, 51.92, 51.27, 36.92.

Mass: Calculated m/z for $C_{26}H_{27}FN_3O_4S^+$ $[M+H]^+$= 496.1701; found APCI: 496.6; found HRMS: $[M+H]^+$ 496.1698

HPLC purity: 98.86 %; t_R=3.0 min (**HPLC-Trace 2**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

3

(2R)-2-[(2,6-difluorophenyl)formamido]-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)phenyl]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product from **2**: 156 mg (0.42 mmol, 1 eq.); **THF** (10 ml); **2,6-difluorobenzoic acid** (80 mg, 0.50 mmol, 1.2 eq., BLDpharm); **PyBOP** (328 mg, 0.63 mmol, 1.5 eq., Carbolution); **DIPEA** (214 µL, 0.60 mmol, 3 eq., Aldrich)

Procedure: Boc-deprotected crude product from **2, 2,6-difluorobenzoic acid** and **PyBOP** were dissolved in **THF.** Subsequently, **DIPEA** was added, and the mixture was stirred at room temperature overnight.

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 80 % heptane to 20 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Yield: 194 mg; 90 %

Analyses:

R_F value: 0.30 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 6**) (500 MHz, Chloroform-*d*) δ 9.07 (s, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 7.40 – 7.29 (m, 5H), 7.27 – 7.22 (m, 1H), 7.21 – 7.14 (m, 2H), 7.08 (td, *J* = 7.7, 1.4 Hz, 1H), 6.91 (tt, *J* = 8.5, 2.1 Hz, 3H), 5.03 (q, *J* = 7.4 Hz, 1H), 3.40 – 3.11 (m, 10H).

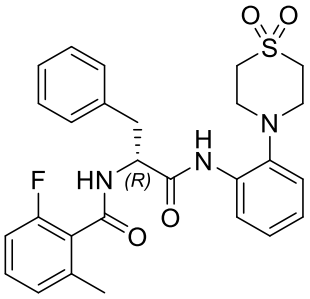
^{13}C NMR (**Spectrum 7**) (126 MHz, Chloroform-*d*) δ 168.61, 160.78, 160.01 (d, *J* = 253.2 Hz), 159.96 (d, *J* = 252.7 Hz), 140.24, 136.32, 132.99, 132.40 (t, *J* = 10.2 Hz), 129.41, 128.79, 127.13, 126.68, 124.44, 121.57, 120.20, 112.93 (d, *J* = 18.7 Hz), 112.25 (d, *J* = 4.3 Hz), 112.09 (d, *J* = 4.1 Hz), 56.10, 51.87, 51.27, 37.08.

Mass: Calculated m/z for $C_{26}H_{26}F_2N_3O_4S^+$ $[M+H]^+$= 514.1607; found APCI: 514.6; found HRMS: $[M+H]^+$ 514.1602

HPLC purity: 98.8 %; t_R=4.7 min (**HPLC-Trace 3**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 10 min, v=1.2 mL/min, λ=254 nm)

4

(2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)phenyl]-2-[(2-fluoro-6-methylphenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product from **2**: 183 mg (0.49 mmol, 1 eq.); **THF** (10 ml); **2-fluoro-6-methylbenzoic acid** (91 mg, 0.59 mmol, 1.2 eq., BLDpharm); **PyBOP** (382 mg, 0.74 mmol, 1.5 eq., Carbolution); **DIPEA** (250 µL, 1.47 mmol, 3 eq., Aldrich)

Procedure: Boc-deprotected crude product from **2, 2-fluoro-6-methylbenzoic acid** and **PyBOP** were dissolved in **THF.** Subsequently, **DIPEA** was added, and the mixture was stirred at room temperature overnight.

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 75 % heptane to 25 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Preparative HPLC afterwards. Yield: 203 mg; 81 %

Analyses:

R_F value: 0.31 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 8**) (400 MHz, Chloroform-*d*) δ 9.15 (s, 1H), 8.35 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.32 – 7.13 (m, 8H), 7.07 (td, *J* = 7.7, 1.5 Hz, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 6.84 (t, *J* = 8.9 Hz, 1H), 6.68 – 6.59 (m, 1H), 5.07 (q, *J* = 7.8 Hz, 1H), 3.35 (dd, *J* = 14.2, 6.9 Hz, 1H), 3.31 – 3.12 (m, 9H), 2.06 (s, 3H).

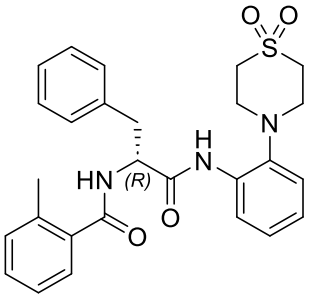
^{13}C NMR (**Spectrum 9**) (101 MHz, Chloroform-*d*) δ 168.88, 165.77, 159.04 (d, *J* = 246.0 Hz), 140.19, 138.37 (d, *J* = 2.8 Hz), 136.52, 133.07, 130.91 (d, *J* = 8.9 Hz), 129.29, 128.82, 127.11, 126.72, 126.34 (d, *J* = 2.9 Hz), 124.40, 123.71 (d, *J* = 17.0 Hz), 121.58, 120.27, 112.90 (d, *J* = 21.8 Hz), 55.66, 51.76, 51.29, 36.82, 18.84.

Mass: Calculated m/z for $C_{27}H_{29}FN_3O_4S^+$ $[M+H]^+$= 510.1858; found APCI: 510.3; found HRMS: $[M+H]^+$ 510.1852

HPLC purity: 98.8 %; t_R=3.0 min (**HPLC-Trace 4**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

5

(2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)phenyl]-2-[(2-methylphenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product from **2**: 42 mg (0.11 mmol, 1 eq.); **THF** (5 ml); **2-methylbenzoic acid** (18 mg, 0.13 mmol, 1.2 eq., TCI); **PyBOP** (88 mg, 0.17 mmol, 1.5 eq., Carbolution); **DIPEA** (44 µL, 0.34 mmol, 3 eq., Aldrich)

Procedure: Boc-deprotected crude product from **2, 2-methylbenzoic acid** and **PyBOP** were dissolved in **THF.** Subsequently, **DIPEA** was added, and the mixture was stirred at room temperature overnight.

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 75 % heptane to 25 % over 10 CV on 8 g normal phase silica gel 0.63 – 0.2 µm particle size. Afterwards preparative HPLC. Yield: 29 mg; 54 %

Analyses:

R_F value: 0.42 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 10**) (400 MHz, Chloroform-*d*) δ 9.18 (s, 1H), 8.39 (d, *J* = 8.6, 1.5 Hz, 1H), 7.32 (d, *J* = 4.3 Hz, 4H), 7.30 (dd, *J* = 7.3, 1.7 Hz, 1H), 7.28 – 7.23 (m, 1H), 7.22 – 7.17 (m, 4H), 7.14 (td, *J* = 7.4,

1.2 Hz, 1H), 7.11 – 7.05 (m, 1H), 6.39 (d, *J* = 7.7 Hz, 1H), 4.96 (q, *J* = 7.5 Hz, 1H), 3.35 (dd, *J* = 14.1, 7.4 Hz, 1H), 3.31 – 3.14 (m, 9H), 2.30 (s, 3H).

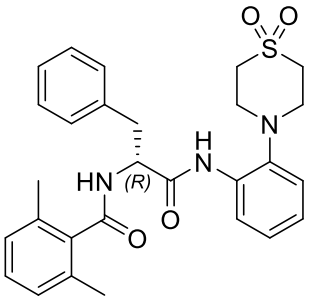
^{13}C NMR (**Spectrum 11**) (101 MHz, Chloroform-*d*) δ 170.23, 169.04, 140.01, 136.52, 136.43, 134.69, 133.09, 131.33, 130.56, 129.27, 128.88, 127.17, 126.80, 126.63, 125.86, 124.36, 121.53, 120.17, 55.92, 51.86, 51.30, 36.97, 19.79.

Mass: Calculated m/z for $C_{27}H_{29}FN_3O_4S^+$ $[M+H]^+$= 492.1952; $[M+Na]^+$= 514.1771; found APCI: 492.2; found HRMS: $[M+H]^+$ 492.1956; $[M+Na]^+$ 514.1775

HPLC purity: 97.2 %; t_R=3.0 min (**HPLC-Trace 5**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

6

(2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)phenyl]-2-[(2,6-dimethylphenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product from **2**: 42 mg (0.11 mmol, 1 eq.); **DMF** (10 ml); **2,6-dimethylbenzoic acid** (21 mg, 0.14 mmol, 1.2 eq., TCI); **PyBOP** (117 mg, 0.23 mmol, 2 eq., Carbolution); **DIPEA** (56 µL, 0.33 mmol, 3 eq., Aldrich)

Procedure: Boc-deprotected crude product from **2, 2,6-dimethylbenzoic acid** and **PyBOP** were dissolved in **THF.** Subsequently, **DIPEA** was added, and the mixture was stirred at room temperature for two days.

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 80 % heptane to 30 % over 10 CV on 8 g normal phase silica gel 0.63 – 0.2 µm particle size. Afterwards preparative HPLC. Yield: 21 mg; 38 %

Analyses:

R_F value: 0.39 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 12**) (400 MHz, Chloroform-*d*) δ 9.30 (s, 1H), 8.40 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.35 – 7.28 (m, 4H), 7.28 – 7.19 (m, 3H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.12 – 7.07 (m, 1H), 6.94 (d, *J* = 7.6 Hz, 2H),

6.16 (d, *J* = 7.8 Hz, 1H), 4.99 (ddd, *J* = 8.8, 7.8, 6.8 Hz, 1H), 3.44 – 3.19 (m, 9H), 3.13 (dd, *J* = 14.2, 8.8 Hz, 1H), 2.00 (s, 6H).

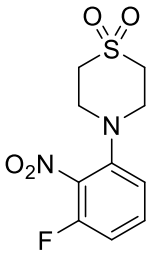
^{13}C NMR (**Spectrum 13**) (101 MHz, Chloroform-*d*) δ 170.84, 168.91, 139.99, 136.42, 136.22, 134.09, 133.11, 129.23, 129.09, 128.92, 127.51, 127.21, 126.86, 124.34, 121.55, 120.14, 55.55, 51.88, 51.39, 36.60, 18.68.

Mass: Calculated m/z for $C_{28}H_{31}N_3O_4S^+$ $[M+H]^+$= 506.2108; found APCI: 506.4; found HRMS: $[M+H]^+$ 506.2101

HPLC purity: 99.0 %; t_R=3.1 min (**HPLC-Trace 6**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

7

4-(3-fluoro-2-nitrophenyl)-1λ^6-thiomorpholine-1,1-dioxide



Synthesis:

Quantities: **1,3-Difluoro-2-nitrobenzene** (1114 mg, 7 mmol, 1 eq., BLDpharm); **Thiomorpholinedioxide** (946 mg, 7 mmol, 1 eq., BLDpharm); **DIPEA** (2382 µL, 14 mmol, 2 eq., Aldrich)

Procedure: **1,3-Difluoro-2-nitrobenzene** and **thiomorpholinedioxide** were dissolved in **DIPEA** and stirred at 50 °C for 3 days.

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/chloroform gradient 40 % heptane to 0 % over 10 CV on 90 g normal phase silica gel 0.63 – 0.2 µm particle size. Yield: 1261 mg; 95 %

Analyses:

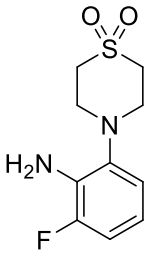
R_F value: 0.76 dichloromethane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 14**) (400 MHz, Chloroform-*d*) δ 7.47 (td, *J* = 8.4, 6.0 Hz, 1H), 7.15 – 7.02 (m, 2H), 3.65 – 3.43 (m, 4H), 3.27 – 3.03 (m, 4H).

Mass: Calculated m/z for $C_{10}H_{12}FN_2O_4S^+$ $[M+H]^+$= 275.0497; found APCI: 275.0

8

4-(2-amino-3-fluorophenyl)-1λ^6-thiomorpholine-1,1-dioxide



Synthesis:

Quantities: **7** (1261 mg, 4.60 mmol, 1 eq.); **H_2** (Balloon); **Pd(OH)$_2$/C 20 %** (252 mg, 20 m %., Aldrich); **EtOH 96 % v/v** (90 mL)

Procedure: **7** was dissolved in **EtOH**, add catalyst; close the flask with a septum and purge the mixture with hydrogen for 30 min under stirring; add a hydrogen balloon; check for full conversion with TLC; after completion remove the catalyst with paper filter and wash the filter thoroughly with ethyl acetate; evaporate the solvents under reduced pressure

Purification:

Product was used without further purification. Yield: 955 mg; 85 %

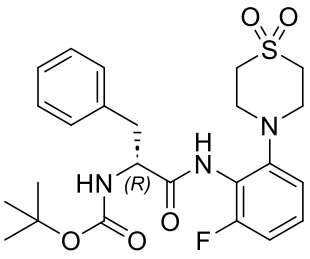
Analyses:

R_F value: 0.25 ethyl acetate/heptane 1:1

Mass: Calculated m/z for $C_{10}H_{14}FN_2O_2S^+$ $[M+H]^+$= 245.0755; found APCI: 245.2

9

tert-butyl N-[(1R)-1-{[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-fluorophenyl]carbamoyl}-2-phenylethyl]carbamate



Synthesis:

Quantities: **Pyridine** (20 mL); **EtOAc** (40 mL); **8** (955 mg, 3.91 mmol, 1 eq.); ***N*-Boc-(*R*)-phenylalanine** (1141 mg, 4.30 mmol, 1.1 eq., TCI); **T3P 50 % m/V in EtOAc** (4655 µL, 7.82 mmol, 2 eq., Aldrich)

Procedure: **8** and ***N*-Boc-(*R*)-phenylalanine** were dissolved in a 1:2 mixture of distilled **pyridine** and **EtOAc**. The mixture was cooled to -20 °C with an isopropanol dry-ice bath before **T3P 50 % m/V in EtOAc** was added. After the addition, the cooling bath was removed, and the mixture was stirred for

20 h at room temperature. Afterwards the mixture was washed with 0.25 M KH_2PO_4 solution three times before the organic phase was evaporated on silica gel under reduced pressure for purification.

Purification:

Flash chromatography heptane/methyl tert-butyl ether gradient 50 % heptane to 0 % over 10 CV on 90 g normal phase silica gel 0.63 – 0.2 µm particle size. Yield: 1769 mg; 92 %

Analyses:

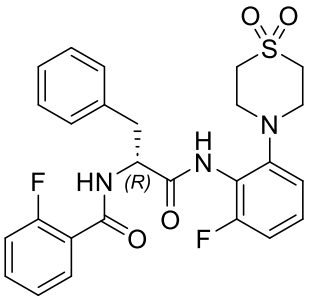
R_F value: 0.42 heptane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 15Spectrum 14**) (400 MHz, cdcl$_3$) δ 7.78 (s, 1H), 7.34 – 7.21 (m, 5H), 7.17 (td, *J* = 8.2, 5.9 Hz, 1H), 6.92 – 6.81 (m, 2H), 5.15 (d, *J* = 7.6 Hz, 1H), 4.54 (q, *J* = 7.2 Hz, 1H), 3.45 – 3.00 (m, 10H), 1.39 (s, 9H).

Mass: Calculated m/z for $C_{10}H_{12}FN_2O_4S^+$ $[M+H]^+$= 492.1963; found APCI: 492.6

10

((2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-fluorophenyl]-2-[(2-fluorophenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: **DCM** (10 mL); **TFA** (10 mL); **9** (1769 mg, 3.60 mmol) Boc-deprotected crude product isolation: 1019 mg; Used for next step: 201 mg (0.51 mmol, 1 eq.); **THF** (10 ml); **2-fluorobenzoic acid** (86 mg, 0.62 mmol, 1.2 eq., TCI); **DEPBT** (169 mg, 0.57 mmol, 1.1 eq., BLDpharm); **DIPEA** (174 µL, 1.02 mmol, 2 eq., Aldrich)

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 70 % heptane to 20 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Yield: 238 mg; 91 %

Analyses:

R_F value: 0.15 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 16**) (400 MHz, Chloroform-*d*) δ 8.08 (s, 1H), 7.97 (t, *J* = 7.8 Hz, 1H), 7.50 – 7.43 (m, 1H), 7.36 – 7.20 (m, 7H), 7.16 (td, *J* = 8.2, 5.8 Hz, 1H), 7.09 (dd, *J* = 12.1, 8.3 Hz, 1H), 6.94 – 6.83 (m, 2H), 5.07 (qd, *J* = 6.9, 1.7 Hz, 1H), 3.42 – 3.23 (m, 6H), 3.23 – 3.02 (m, 4H).

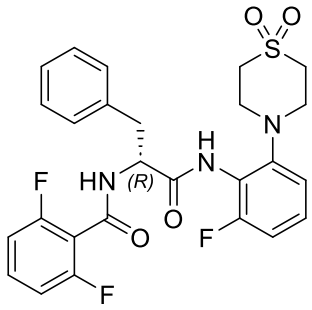
^{13}C NMR (**Spectrum 17**) (101 MHz, Chloroform-*d*) δ 169.65, 163.93 (d, *J* = 3.0 Hz), 160.73 (d, *J* = 248.7 Hz), 157.63 (d, *J* = 252.4 Hz), 147.82, 136.03, 134.12 (d, *J* = 9.3 Hz), 131.77 (d, *J* = 1.8 Hz), 129.44, 128.83, 127.72 (d, *J* = 9.3 Hz), 127.29, 125.06 (d, *J* = 3.2 Hz), 119.81 (d, *J* = 10.7 Hz), 119.47 (d, *J* = 13.5 Hz), 116.36 (d, *J* = 3.4 Hz), 116.26 (d, *J* = 24.4 Hz), 112.90 (d, *J* = 20.7 Hz), 55.62, 51.96, 50.66, 37.36.

Mass: Calculated m/z for $C_{26}H_{26}F_2N_3O_4S^+$ $[M+H]^+$= 514.1607; found APCI: 514.5; found HRMS: $[M+H]^+$ 514.1601

HPLC purity: 97.6 %; t_R=2.7 min (**HPLC-Trace 7**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

11

(2R)-2-[(2,6-difluorophenyl)formamido]-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-fluorophenyl]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product of **10** (184 mg, 0.47 mmol, 1 eq.); **THF** (10 ml); **2,6-difluorobenzoic acid** (89 mg, 0.56 mmol, 1.2 eq., TCI); **DEPBT** (295 mg, 0.99 mmol, 2.1 eq., BLDpharm); **DIPEA** (160 µL, 1.41 mmol, 3 eq., Aldrich)

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 70 % heptane to 25 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Afterwards preparative HPLC. Yield: 170 mg; 68 %

Analyses:

R_F value: 0.75 ethyl acetate

^{1}H NMR (**Spectrum 18**) (400 MHz, Acetone-d_6) δ 8.85 (s, 1H), 8.31 (d, *J* = 8.3 Hz, 1H), 7.52 – 7.37 (m, 3H), 7.36 – 7.21 (m, 4H), 7.09 – 6.91 (m, 4H), 5.19 (q, *J* = 8.3 Hz, 1H), 3.50 – 3.23 (m, 7H), 3.22 – 3.10 (m, 3H).

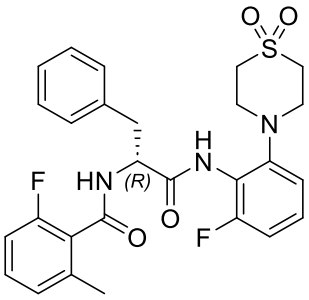
^{13}C NMR (**Spectrum 19**) (101 MHz, Acetone-d_6) δ 169.76 (d, *J* = 8.5 Hz), 160.34 (d, *J* = 8.2 Hz), 159.63 (d, *J* = 250.2 Hz), 159.56 (d, *J* = 250.3 Hz), 158.16 (d, *J* = 250.0 Hz), 149.09, 137.28, 131.76 (t, *J* = 10.0 Hz), 129.35, 128.29, 127.71 (d, *J* = 9.5 Hz), 126.64, 119.99 – 119.35 (m), 116.33 (d, *J* = 3.0 Hz), 115.25 – 114.38 (m), 111.82 – 111.41 (m), 55.35, 52.00, 50.39, 37.74.

Mass: Calculated m/z for $C_{26}H_{25}F_3N_3O_4S^+$ $[M+H]^+$= 532.1513; found APCI: 532.5; found HRMS: $[M+H]^+$ 532.1507

HPLC purity: 98.9 %; t_R=2.6 min (**HPLC-Trace 8**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

12

(2R)-N-[2-(1,1-dioxo--thiomorpholin-4-yl)-6-fluorophenyl]-2-[(2-fluoro-6-methylphenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product of **10** (193 mg, 0.49 mmol, 1 eq.); **THF** (10 ml); **2-fluoro-6-methylbenzoic acid** (91 mg, 0.59 mmol, 1.2 eq., TCI); **DEPBT** (308 mg, 1.03 mmol, 2.1 eq., BLDpharm); **DIPEA** (251 µL, 1.47 mmol, 3 eq., Aldrich)

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 70 % heptane to 25 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Yield: 245 mg; 95 %

Analyses:

R_F value: 0.25 heptane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 20**) (400 MHz, Chloroform-*d*) δ 8.09 (d, *J* = 10.3 Hz, 1H), 7.37 – 7.22 (m, 5H), 7.22 – 7.12 (m, 2H), 6.93 – 6.79 (m, 4H), 6.70 (q, *J* = 7.9 Hz, 1H), 5.16 (tdd, *J* = 8.0, 6.9, 6.4, 2.6 Hz, 1H), 3.45 – 3.23 (m, 5H), 3.20 – 3.03 (m, 5H), 2.08 (s, 3H).

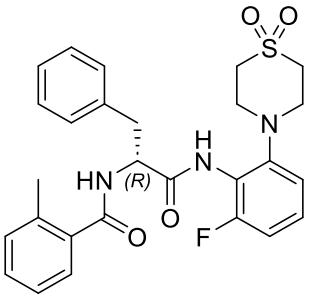
^{13}C NMR (**Spectrum 21**) (101 MHz, Chloroform-*d*) δ 169.61, 165.82, 159.08 (d, *J* = 245.5 Hz), 157.78 (d, *J* = 251.1 Hz), 148.01, 138.83 – 137.77 (m), 136.13, 130.84 (d, *J* = 8.8 Hz), 129.37, 128.81, 127.91 (d, *J* = 9.7 Hz), 127.25, 126.27 (d, *J* = 2.6 Hz), 123.77 (d, *J* = 16.6 Hz), 119.17 (d, *J* = 13.4 Hz), 116.25 (d, *J* = 3.0 Hz), 112.86 (d, *J* = 21.7 Hz), 112.70 (d, *J* = 20.4 Hz), 54.93, 51.84, 50.54, 37.40, 18.79.

Mass: Calculated m/z for $C_{27}H_{28}F_2N_3O_4S^+$ $[M+H]^+$= 528.1763; found APCI: 528.5; found HRMS: $[M+H]^+$ 528.1758

HPLC purity: 99.3 %; t_R=2.7 min (**HPLC-Trace 9**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

13

(2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-fluorophenyl]-2-[(2-methylphenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product of **10** (306 mg, 0.82 mmol, 1 eq.); **DMF** (10 ml); **2-methylbenzoic acid** (134 mg, 0.98 mmol, 1.2 eq., TCI); **PyBOP** (640 mg, 1.23 mmol, 1.5 eq., BLDpharm); **DIPEA** (418 µL, 2.46 mmol, 3 eq., Aldrich)

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 70 % heptane to 30 % over 10 CV on 8 g normal phase silica gel 0.63 – 0.2 µm particle size. Afterwards preparative HPLC. Yield: 311 mg; 74 %

Analyses:

R_F value: 0.20 heptane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 22**) (400 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.41 – 7.24 (m, 6H), 7.22 – 7.09 (m, 4H), 6.93 – 6.81 (m, 2H), 6.48 (d, *J* = 7.6 Hz, 1H), 5.05 (q, *J* = 7.4 Hz, 1H), 3.46 – 3.23 (m, 5H), 3.18 (dd, *J* = 13.8, 7.2 Hz, 1H), 3.13 – 3.02 (m, 4H), 2.29 (s, 3H).

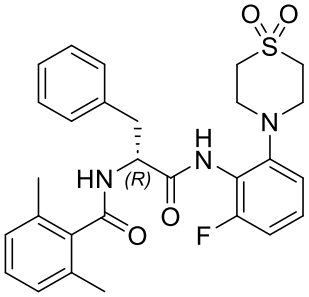
^{13}C NMR (**Spectrum 23**) (101 MHz, Chloroform-*d*) δ 170.66, 169.92, 157.74 (d, *J* = 251.5 Hz), 147.83, 136.37, 136.20, 134.69, 131.21, 130.54, 129.39, 128.89, 127.85 (d, *J* = 9.4 Hz), 127.35, 126.84, 125.86, 119.25 (d, *J* = 13.4 Hz), 116.20 (d, *J* = 2.7 Hz), 112.76 (d, *J* = 20.7 Hz), 55.25, 51.96, 50.58, 37.19, 19.72.

Mass: Calculated m/z for $C_{27}H_{29}FN_3O_4S^+$ $[M+H]^+$= 510.1858$^+$; $[M+Na]^+$= 532.1677; found APCI: 528.5; found HRMS: $[M+H]^+$ 510.1864; $[M+Na]^+$ 532.1679

HPLC purity: 99.5 %; t_R=2.7 min (**HPLC-Trace 10**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

14

(2R)-2-[(2,6-dimethylphenyl)formamido]-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-fluorophenyl]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product from **10**: 195 mg (0.50 mmol, 1 eq.); **THF** (10 ml); **2,6-dimethylbenzoic acid** (90 mg, 0.60 mmol, 1.2 eq., TCI); **PyBOP** (313 mg, 1.05 mmol, 2.1 eq., Carbolution); **DIPEA** (255 µL, 1.5 mmol, 3 eq., Aldrich)

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 60 % heptane to 15 % over 10 CV on 8 g normal phase silica gel 0.63 – 0.2 µm particle size. Afterwards preparative HPLC. Yield: 99 mg; 38 %

Analyses:

R_F value: 0.25 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 24**) (500 MHz, Chloroform-*d*) δ 8.25 – 8.15 (m, 1H), 7.32 (dd, *J* = 23.3, 4.4 Hz, 5H), 7.24 – 7.16 (m, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 7.7 Hz, 2H), 6.89 (dd, *J* = 12.0, 8.3 Hz, 2H), 6.33 (d, *J* = 7.9 Hz, 1H), 5.17 (q, *J* = 7.2, 6.2 Hz, 1H), 3.45 – 3.27 (m, 5H), 3.16 – 3.03 (m, 5H), 2.07 (s, 6H).

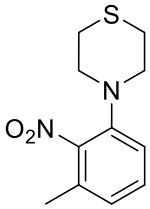
^{13}C NMR (**Spectrum 25**) (126 MHz, Chloroform-*d*) δ 171.19, 169.73, 157.72 (d, *J* = 251.8 Hz), 147.79, 136.19, 136.15, 134.32, 129.31, 129.13, 128.93, 127.83 (d, *J* = 9.9 Hz), 127.53, 127.38, 119.14 (d, *J* = 14.3 Hz), 116.14, 112.83 (d, *J* = 20.6 Hz), 54.80, 52.01, 50.57, 37.37, 18.76.

Mass: Calculated m/z for $C_{28}H_{31}FN_3O_4S^+$ $[M+H]^+$= 524.2014; found APCI: 524.5; found HRMS: $[M+H]^+$ 524.2010

HPLC purity: 98.4 %; t_R=2.9 min (**HPLC-Trace 11**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

15

4-(3-methyl-2-nitrophenyl)thiomorpholine



Synthesis:

Quantities: **1-Fluoro-3-methyl-2-nitrobenzene** (1000 mg, 6.45 mmol, 1 eq., BLDpharm); **thiomorpholine** (731 mg, 7.09 mmol, 1.1 eq., abcr GmbH); **DIPEA** (2194 µL, 12.90 mmol, 2 eq., Aldrich)

Procedure: Mix **1-fluoro-3-methyl-2-nitrobenzene** and **thiomorpholine**; add **DIPEA**; stir for 24 h at 50 °C.

After the reaction time the crude reaction mixture was directly evaporated on silica gel.

Purification:

Flash chromatography heptane/dichloromethane gradient 95 % heptane to 50 % over 10 CV on 90 g normal phase silica gel 0.63 – 0.2 µm particle size. Yield: 313 mg; 18 %

Analyses:

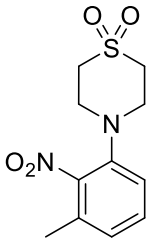
R_F value: 0.30 dichloromethane/heptane 1:1

^{1}H NMR (**Spectrum 26**) (400 MHz, Chloroform-*d*) δ 7.30 (t, *J* = 7.9 Hz, 1H), 7.09 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.03 (dt, *J* = 7.6, 1.0 Hz, 1H), 3.20 – 3.13 (m, 4H), 2.74 – 2.65 (m, 4H), 2.25 (s, 3H).

Mass: Calculated m/z for $C_7H_7FNO_2^+$ $[M+H]^+$= 239.0849; found APCI: 239.1

16

4-(3-methyl-2-nitrophenyl)--thiomorpholine-1,1-dioxide



Synthesis:

Quantities: **15** (331 mg, 1.39 mmol, 1 eq.); **mCPBA 77 % m/m** (747 mg, 3.33 mmol, 2.4 eq., Aldrich); **DCM** (15 + 15 mL)

Procedure: Dissolve **15** in 15 mL **DCM**; cool the solution to -20 °C (iso-propanol bath); dissolve mCPBA in 15 mL **DCM** and add it to the **15** solution over 30 min; stir for 4 h at 0 °C; wash the mixture with saturated $NaHCO_3$ solution; concentrate under reduced pressure

Purification:

Was used without further purification. Crude product yield: 376 mg; 100 %

Analyses:

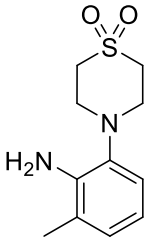
R_F value: 0.7 ethyl acetate

^{1}H NMR (**Spectrum 27**) (400 MHz, Chloroform-*d*) δ 7.36 (t, *J* = 7.9 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 7.7 Hz, 1H), 3.49 – 3.41 (m, 4H), 3.16 – 3.07 (m, 4H), 2.28 (s, 3H).

Mass: Calculated m/z for $C_{11}H_{15}N_2O_4S^+$ $[M+H]^+$= 271.3103; found APCI: 271.2

17

4-(2-amino-3-methylphenyl)-1λ^6-thiomorpholine-1,1-dioxide



Synthesis:

Quantities: **16** (376 mg, 1.39 mmol, 1 eq.); **PdOH/C 20 % m/m** (75 mg (20 % m of **16**), Aldrich); **methanol** (40 mL)

Procedure: Dissolve **15** in 40 mL **methanol**; add catalyst; close the flask with a septum and purge the mixture with hydrogen for 30 min under stirring; add a hydrogen balloon; check for full conversion with TLC; after completion remove the catalyst with paper filter and wash the filter thoroughly with ethyl acetate; evaporate the solvents under reduced pressure

Purification:

Was used without further purification. Crude product yield: 196 mg; 58 %

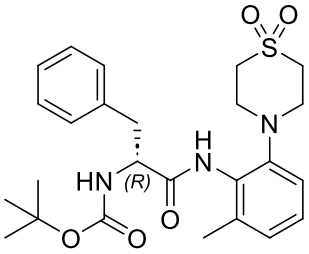
Analyses:

R_F value: 0.25 ethyl acetate/heptane 1:1

Mass: Calculated m/z for $C_{11}H_{15}N_2O_4S^+$ $[M+H]^+$= 241.1005; found APCI: 241.1

18

tert-butyl-N-[(1R)-1-{[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-methylphenyl]carbamoyl}-2-phenylethyl]carbamate



Synthesis:

Quantities: **Pyridine** (15 mL); **EtOAc** (30 mL); **17** (196 mg, 0.82 mmol, 1 eq.); **N-Boc-D-phenylalanine** (238 mg, 0.90 mmol, 1.1 eq., TCI); **T3P 50 % m/V in EtOAc** (976 µL, 1.64 mmol, 2 eq., Aldrich)

Procedure: Dissolve **17** and **(*N*)-Boc-(*R*)-phenylalanine** in **pyridine** and **ethyl acetate**; cool the mixture to -20 °C before adding **T3P**; keep the temperature at 0 °C for 20 h; wash with 0.25 M KH_2PO_4 solution 3x; evaporate the solvents under reduced pressure

Purification:

Flash chromatography heptane/methyl tert-butyl ether gradient 50 % heptane to 10 % over 8 CV on 90 g normal phase silica gel 0.63 – 0.2 µm particle size. Yield: 222 mg; 56 %

Analyses:

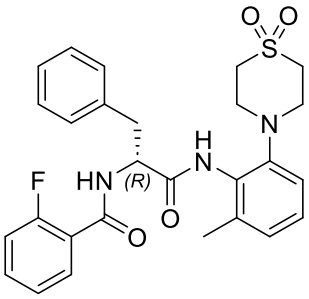
R_F value: 0.35 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 28**) (400 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 7.35 – 7.23 (m, 5H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 7.8 Hz, 1H), 5.23 – 5.10 (m, 1H), 4.58 (q, *J* = 7.5 Hz, 1H), 3.34 – 3.17 (m, 5H), 3.15 – 3.00 (m, 5H), 2.09 (s, 3H), 1.41 (s, 9H).

Mass: Calculated m/z for $C_{25}H_{34}N_3O_5S^+$ $[M+H]^+$= 488.2214; found APCI: 488.2

19

(2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-methylphenyl]-2-[(2-fluorophenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: **DCM** (10 mL); **TFA** (10 mL); **18** (222 mg, 0.46 mmol) Boc-deprotected crude product isolation: 145 mg; Used for next step: 59 mg (0.15 mmol, 1 eq.); **THF** (10 ml); **2-fluorobenzoic acid** (26 mg, 0.18 mmol, 1.2 eq., TCI); **DEPBT** (68 mg, 0.23 mmol, 1.5 eq., BLDpharm); **DIPEA** (78 µL, 0.47 mmol, 3 eq., Aldrich)

Procedure: Dissolve **18** in 10 mL **DCM** and add 10 mL **TFA** under stirring; stir for 1 h and check for full conversion by TLC; after conversion evaporate the solvents under reduced pressure; resuspend the residue in ethyl acetate and wash with saturated $NaHCO_3$ 3x; dry the organic phase with $NaSO_4$ and concentrate under reduced pressure; dissolve the Boc-deprotected reactant, the **2-fluorobenzoic acid** and **DEPBT** in 10 mL **THF**; add **DIPEA**; stir over night

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 80 % heptane to 40 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Preparative HPLC afterwards. Yield: 48 mg; 62 %

Analyses:

R_F value: 0.30 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 29**) (400 MHz, Chloroform-*d*) δ 8.10 (s, 1H), 8.05 (td, *J* = 7.9, 1.9 Hz, 1H), 7.55 – 7.47 (m, 1H), 7.40 – 7.24 (m, 7H), 7.17 – 7.10 (m, 2H), 7.02 (d, *J* = 7.5 Hz, 1H), 6.96 (dd, *J* = 7.9, 1.4 Hz, 1H), 5.14 (qd, *J* = 7.1, 2.0 Hz, 1H), 3.39 (dd, *J* = 13.8, 7.0 Hz, 1H), 3.34 – 3.19 (m, 5H), 3.18 – 3.01 (m, 4H), 2.10 (s, 3H).

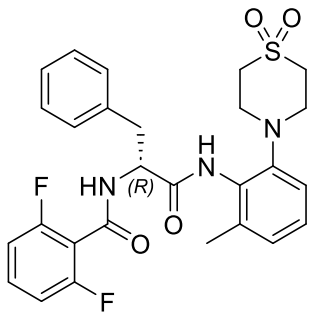
^{13}C NMR (**Spectrum 30**) (101 MHz, Chloroform-*d*) δ 169.15, 163.77 (d, *J* = 3.3 Hz), 160.76 (d, *J* = 248.3 Hz), 146.54, 136.36, 136.20, 134.21 (d, *J* = 9.5 Hz), 131.99, 129.97, 129.50, 128.92, 127.82, 127.37, 127.28, 125.17 (d, *J* = 3.2 Hz), 119.68 (d, *J* = 11.0 Hz), 118.46, 116.27 (d, *J* = 24.7 Hz), 55.67, 52.00, 51.07, 37.30, 18.98.

Mass: Calculated m/z for $C_{27}H_{29}FN_3O_4S^+$ $[M+H]^+$= 510.1858; found APCI: 510.3; found HRMS: $[M+H]^+$ 510.1852

HPLC purity: 98.6 %; t_R=2.8 min (**HPLC-Trace 12**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

20

(2R)-2-[(2,6-difluorophenyl)formamido]-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-methylphenyl]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product of **19** (61 mg, 0.16 mmol, 1 eq.); **THF** (10 ml); **2,6-difluorobenzoic acid** (30 mg, 0.19 mmol, 1.2 eq., TCI); **DEPBT** (71 mg, 0.24 mmol, 1.5 eq., BLDpharm); **DIPEA** (80 µL, 0.47 mmol, 3 eq., Aldrich)

Procedure: Dissolve the Boc-deprotected reactant of **19**, the **2,6-difluorobenzoic acid** and **DEPBT** in 10 mL **THF**; add **DIPEA**; stir over night

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 70 % heptane to 30 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Preparative HPLC afterwards. Yield: 67 mg; 81 %

Analyses:

R_F value: 0.50 heptane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 31**) (400 MHz, Chloroform-*d*) δ 8.01 (s, 1H), 7.40 – 7.23 (m, 6H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 7.9 Hz, 1H), 6.90 (t, *J* = 8.2 Hz, 2H), 6.81 (d, *J* = 8.0 Hz, 1H), 5.15 (q, *J* = 7.4 Hz, 1H), 3.36 (dd, *J* = 13.9, 7.4 Hz, 1H), 3.32 – 3.18 (m, 5H), 3.14 – 3.03 (m, 4H), 2.07 (s, 3H).

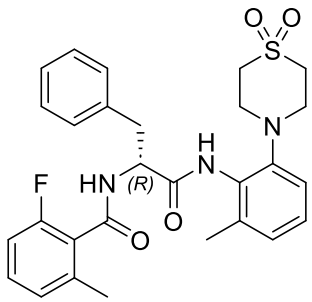
^{13}C NMR (**Spectrum 32**) (101 MHz, Chloroform-*d*) δ 168.88, 160.79, 159.94 (d, *J* = 252.9 Hz), 159.87 (d, *J* = 252.8 Hz), 146.72, 136.52, 135.98, 132.33 (t, *J* = 10.4 Hz), 129.71, 129.50, 128.88, 127.62, 127.44, 127.38, 118.40, 113.12 (t, *J* = 19.6 Hz), 112.35 – 111.90 (m), 55.32, 51.92, 50.95, 37.48, 18.82.

Mass: Calculated m/z for $C_{27}H_{28}F_2N_3O_4S^+$ $[M+H]^+$= 528.1763; found APCI: 528.3; found HRMS: $[M+H]^+$ 528.1760

HPLC purity: 99.0 %; t_R=2.7 min (**HPLC-Trace 13**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

21

(2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-methylphenyl]-2-[(2-fluoro-6-methylphenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product of **19** (64 mg, 0.17 mmol, 1 eq.); **THF** (10 mL); **2-fluoro-6-methylbenzoic acid** (31 mg, 0.20 mmol, 1.2 eq., TCI); **DEPBT** (74 mg, 0.25 mmol, 1.5 eq., BLDpharm); **DIPEA** (84 µL, 0.50 mmol, 3 eq., Aldrich)

Procedure: Dissolve the Boc-deprotected reactant of **19**, the **2-fluoro-6-methylbenzoic acid** and **DEPBT** in 10 mL **THF**; add **DIPEA**; stir over night

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 60 % heptane to 20 % over 10 CV on 45 g normal phase silica gel 0.63 – 0.2 µm particle size. Preparative HPLC afterwards. Yield: 50 mg; 58 %

Analyses:

R_F value: 0.55 heptane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 33**) (400 MHz, Chloroform-*d*) δ 8.10 (s, 1H), 7.36 – 7.12 (m, 7H), 7.03 (d, *J* = 7.7 Hz, 1H), 6.95 (dd, *J* = 16.5, 7.8 Hz, 2H), 6.86 (t, *J* = 9.0 Hz, 1H), 6.57 (t, *J* = 9.4 Hz, 1H), 5.18 (td, *J* = 8.4, 6.3 Hz, 1H), 3.44 (dd, *J* = 14.1, 6.2 Hz, 1H), 3.29 (q, *J* = 6.1, 5.1 Hz, 4H), 3.14 (tdd, *J* = 15.4, 10.6, 5.3 Hz, 5H), 2.14 (s, 3H), 2.09 (s, 3H).

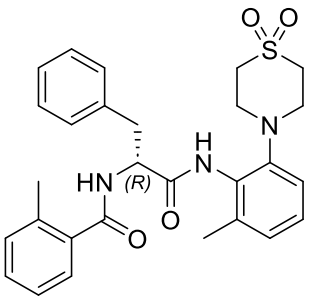
^{13}C NMR (**Spectrum 34**) (101 MHz, Chloroform-*d*) δ 169.24, 165.63, 159.14 (d, *J* = 245.3 Hz), 146.70, 138.56 (d, *J* = 2.8 Hz), 136.54, 136.25, 130.90 (d, *J* = 9.0 Hz), 129.81, 129.31, 128.89, 127.70, 127.43, 127.31, 126.39 (d, *J* = 2.8 Hz), 123.73 (d, *J* = 17.0 Hz), 118.43, 112.90 (d, *J* = 22.0 Hz), 55.01, 51.97, 50.99, 37.55, 18.88, 18.85.

Mass: Calculated m/z for $C_{28}H_{31}FN_3O_4S^+$ $[M+H]^+$= 524.2014; found APCI: 524.4; found HRMS: $[M+H]^+$ 524.2010

HPLC purity: 98.0 %; t_R=2.8 min (**HPLC-Trace 14**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

22

(2R)-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-methylphenyl]-2-[(2-methylphenyl)formamido]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product of **19** (67 mg, 0.17 mmol, 1 eq.); **THF** (10 mL); **2-methylbenzoic acid** (28 mg, 0.21 mmol, 1.2 eq., TCI); **PyBOP** (135 mg, 0.26 mmol, 1.5 eq., Carbolution); **DIPEA** (88 µL, 0.52 mmol, 3 eq., Aldrich)

Procedure: Dissolve the Boc-deprotected reactant of **19**, the **2-methylbenzoic acid** and **PyBOP** in 10 mL **THF**; add **DIPEA**; stir over night

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 75 % heptane to 30 % over 8 CV on 8 g normal phase silica gel 0.63 – 0.2 µm particle size. Preparative HPLC afterwards. Yield: 14 mg; 16 %

Analyses:

R_F value: 0.20 heptane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 35**) (400 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.41 – 7.26 (m, 6H), 7.23 – 7.12 (m, 4H), 7.04 (d, *J* = 7.6 Hz, 1H), 6.98 (dd, *J* = 7.9, 1.4 Hz, 1H), 6.31 (d, *J* = 7.6 Hz, 1H), 5.03 (q, *J* = 7.5 Hz, 1H), 3.42 (dd, *J* = 14.0, 7.1 Hz, 1H), 3.39 – 3.24 (m, 4H), 3.20 (dd, *J* = 14.0, 7.9 Hz, 1H), 3.18 – 3.09 (m, 4H), 2.32 (s, 3H), 2.15 (s, 3H).

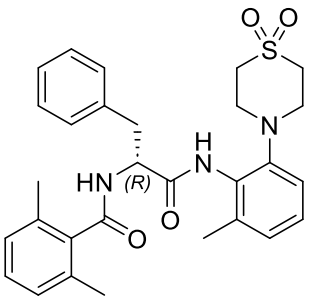
^{13}C NMR (**Spectrum 36**) (101 MHz, Chloroform-*d*) δ 170.40, 169.57, 146.31, 136.49, 136.32, 136.25, 134.64, 131.33, 130.60, 129.81, 129.34, 128.99, 127.83, 127.44, 127.36, 126.72, 125.92, 118.33, 55.38, 52.06, 51.08, 37.37, 19.84, 18.99.

Mass: Calculated m/z for $C_{28}H_{32}N_3O_4S^+$ $[M+H]^+$= 506.2108; $[M+Na]^+$= 528.1927; found APCI: 506.1; found HRMS: $[M+H]^+$ 506.2112; $[M+Na]^+$ 528.1932

HPLC purity: 97.3 %; t_R=2.8 min (**HPLC-Trace 15**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

23

(2R)-2-[(2,6-dimethylphenyl)formamido]-N-[2-(1,1-dioxo-1λ^6-thiomorpholin-4-yl)-6-methylphenyl]-3-phenylpropanamide



Synthesis:

Quantities: Boc-deprotected crude product of **19** (74 mg, 0.19 mmol, 1 eq.); **DMF** (10 mL); **2,6-dimethylbenzoic acid** (35 mg, 0.23 mmol, 1.2 eq., TCI); **PyBOP** (198 mg, 0.38 mmol, 2 eq., Carbolution); **DIPEA** (97 µL, 0.57 mmol, 3 eq., Aldrich)

Procedure: Dissolve the Boc-deprotected reactant of **19**, the **2,6-dimethylbenzoic acid** and **PyBOP** in 10 mL **DMF**; add **DIPEA**; stir over night

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography heptane/ethyl acetate gradient 70 % heptane to 30 % over 8 CV on 8 g normal phase silica gel 0.63 – 0.2 µm particle size. Preparative HPLC afterwards. Yield: 76 mg; 77 %

Analyses:

R_F value: 0.30 heptane/ethyl acetate 1:1

^{1}H NMR (**Spectrum 37**) (400 MHz, Chloroform-*d*) δ 8.26 (s, 1H), 7.38 – 7.31 (m, 4H), 7.31 – 7.26 (m, 1H), 7.17 (t, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 7.8 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 2H), 6.20 (d, *J* = 7.6 Hz, 1H), 5.13 (ddd, *J* = 9.6, 7.6, 5.8 Hz, 1H), 3.44 (dd, *J* = 14.3, 5.8 Hz, 1H), 3.41 – 3.25 (m, 4H), 3.20 (s, 4H), 3.07 (dd, *J* = 14.3, 9.6 Hz, 1H), 2.18 (s, 3H), 2.03 (s, 6H).

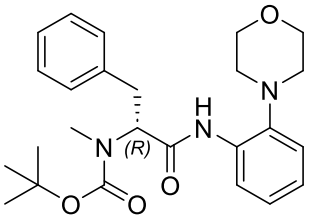
^{13}C NMR (**Spectrum 38**) (126 MHz, Chloroform-*d*) δ 170.97, 169.82, 146.18, 136.33, 136.25, 136.15, 134.27, 129.80, 129.17, 129.07, 129.01, 127.88, 127.52, 127.42, 127.28, 118.29, 54.89, 52.07, 51.08, 37.76, 19.13, 18.81.

Mass: Calculated m/z for $C_{29}H_{34}N_3O_4S^+$ $[M+H]^+$= 520.2265; $[M+Na]^+$= 542.2084; found APCI: 520.5; found HRMS: $[M+H]^+$ 520.2271; $[M+Na]^+$ 542.2088

HPLC purity: 99.3 %; t_R=3.0 min (**HPLC-Trace 16**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

24

tert-butyl (*R*)-methyl(1-((2-morpholinophenyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate



Synthesis:

Quantities: **Pyridine** (5 mL); **EtOAc** (10 mL); **2-morpholinoaniline** (166 mg, 0.94 mmol, 1 eq.); ***N*-Boc-*N*-methyl-*(R)*-phenylalanine** (289 mg, 1.1 mmol, 1.1 eq); **T3P 50 % m/v in EtOAc** (559 µL, 1.88 mmol, 2.0 eq.)

Procedure: **2-Morpholinoaniline** and ***N*-Boc-*N*-methyl-(*R*)-phenylalanine** were dissolved in a 1:2 mixture of distilled **pyridine** and **EtOAc**. The mixture was cooled to -20 °C with an isopropanol dry-ice bath before **T3P 50 % m/V in EtOAc** was added. After the addition, the cooling bath was removed, and the mixture was stirred for 20 h at room temperature. Afterwards the mixture was washed with 0.25 M KH_2PO_4 solution three times before the organic phase was evaporated on silica gel under reduced pressure for purification.

Purification:

Flash chromatography ethyl acetate/heptane gradient 80 % heptane to 40 % over 8 CV on 45 g. Yield: 162 mg; 39 %

Analyses:

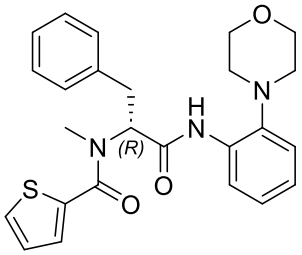
R_F value: 0.25 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 39**) (400 MHz, Chloroform-*d*) δ 9.25 – 9.14 (m, 1H), 8.51 – 8.41 (m, 1H), 7.32 – 7.05 (m, 8H), 5.27 – 5.20 (m, 1H), 4.86 – 4.78 (m, 0H), 3.89 – 3.75 (m, 4H), 3.59 – 3.47 (m, 1H), 3.01 – 2.93 (m, 1H), 2.91 – 2.66 (m, 7H), 1.42 – 1.10 (m, 9H).

Mass: Calculated m/z for $C_{25}H_{34}N_3O_4^+$ $[M+H]^+$= 440.2544; found APCI: $[M+H]^+$ 440.1

25

(*R*)-*N*-methyl-*N*-(1-((2-morpholinophenyl)amino)-1-oxo-3-phenylpropan-2-yl)thiophene-2-carboxamide



Synthesis:

Quantities: **DCM** (2 mL); **TFA** (2 mL); **24** (100 mg, 0.23 mmol, 1.0 eq.); **thiophene-2-carboxylic acid** (30 mg, 0.23 mmol, 1.0 eq.); **PyBOP** (132 mg, 0.25 mmol, 1.1 eq.); **DMF** (5 mL), **DIPEA** (120 µL, 0.69 mmol, 3.0 eq.)

Procedure: Dissolve **24** in **DCM** and add **TFA** under stirring; stir for 1 h and check for full conversion by TLC; after conversion evaporate the solvents under reduced pressure; followed by coevaporation with toluene 3x, DCM 3x; dissolve the Boc-deprotected reactant, the **thiophene-2-carboxylic acid** and **PyBOP** in **DMF**; add **DIPEA**; stir over night at 65 °C under argon and light protection.

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography ethyl acetate/heptane gradient 95 % heptane to 50 % over 10 CV on 45 g. Yield: 38 mg; 37 %

Analyses:

R_F value: 0.25 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 40**) (400 MHz, Chloroform-*d*) δ 9.44 (s, 1H), 8.46 (d, *J* = 8.1 Hz, 1H), 7.46 (d, *J* = 5.0 Hz, 1H), 7.36 – 7.26 (m, 4H), 7.24 – 7.05 (m, 5H), 7.03 – 6.97 (m, 1H), 5.63 (s, 1H), 3.93 (s, 4H), 3.51 (dd, *J* = 14.8, 6.7 Hz, 1H), 3.23 (s, 1H), 3.19 (s, 3H), 2.88 – 2.75 (m, 4H).

^{13}C NMR (**Spectrum 41**) (126 MHz, Chloroform-*d*) δ 168.1, 165.6, 141.4, 137.1, 134.0, 130.3, 129.0, 128.8, 127.1, 126.9, 125.8, 124.2, 121.2, 119.7, 67.4, 59.5, 52.9, 33.9.

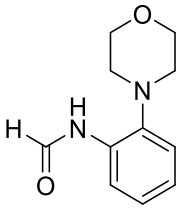
Additional HSQC is provided because of superimposed signals (**Spectrum 42**).

Mass: Calculated m/z for $C_{25}H_{28}N_3O_3S^+$ $[M+H]^+$= 450.1846; $[M+Na]^+$= 472.1665; found APCI: $[M+H]^+$ 450.1; found HRMS: $[M+H]^+$ 450.1850; $[M+Na]^+$ 472.1668

HPLC purity: 98.4 %; t_R=3.3 min (**HPLC-Trace 17**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

26

N-(2-morpholinophenyl)formamide



Synthesis:

Quantities: **Formic acid** (422 µL, 11.2 mmol, 4.0 eq.); **2-morpholinoaniline** (500 mg, 2.8 mmol, 1.0 eq.); **sodium formate** (38 mg, 0.56 mmol, 0.2 eq.)

Procedure: **2-morpholinoaniline** and **sodium formate** were suspended in **formic acid** and stirred at room temperature overnight.

Purification:

The reaction mixture was poured in ethyl acetate and washed with saturated $NaHCO_3$ solution three times. Followed by flash chromatography ethyl acetate/DCM gradient 70 % ethyl acetate to 30 % over 13 CV on 45 g. Yield: 456 mg; 79 %

Analyses:

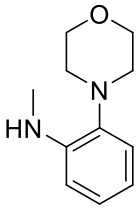
R_F value: 0.15 ethyl acetate/DCM 9:1

^{1}H NMR (**Spectrum 43**) (400 MHz, Chloroform-*d*) δ 8.87 (d, *J* = 11.9 Hz, 0.4H), 8.51 (s, 1H), 8.39 (dd, *J* = 8.0, 1.5 Hz, 0.6H), 8.14 (s, 1H), 7.25 – 7.08 (m, 3H), 3.93 – 3.78 (m, 4H), 2.93 – 2.79 (m, 4H).

Mass: Calculated m/z for $C_{11}H_{15}N_2O_2^+$ $[M+H]^+$= 207.1128; found APCI: $[M+H]^+$ 207.0

27

N-methyl-2-morpholinoaniline



Synthesis:

Quantities: **26** (457 mg, 2.21 mmol, 1.0 eq.); **$LiAlH_4$ 1 M in THF** (4.4 mL, 4.42 mmol, 2.0 eq.); **THF** (10 mL)

Procedure: **26** was dissolved in **THF** and **$LiAlH_4$ 1 M in THF** was added dropwise over 30 minutes at 0 °C under argon atmosphere. The reaction mixture was warmed to room temperature and stirred overnight.

Purification:

The reaction mixture was quenched by addition of water and the aqueous phase was extracted with ethyl acetate 3x. Followed by flash chromatography ethyl acetate/heptane gradient 95 % ethyl acetate to 50 % over 8 CV on 45 g. Yield: 219 mg; 51 %

Analyses:

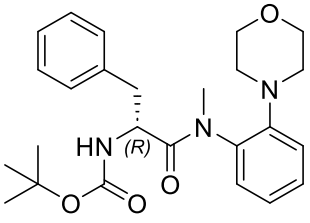
R_F value: 0.45 ethyl acetate/heptane 1:1

^{1}H NMR (**Spectrum 44**) (400 MHz, Chloroform-*d*) δ 7.08 (td, *J* = 7.7, 1.5 Hz, 1H), 7.02 (dd, *J* = 7.8, 1.5 Hz, 1H), 6.71 (td, *J* = 7.6, 1.4 Hz, 1H), 6.64 (dd, *J* = 8.0, 1.4 Hz, 1H), 4.73 (s, 1H), 3.85 (t, *J* = 4.5 Hz, 4H), 2.92 – 2.88 (m, 4H), 2.87 (s, 3H).

Mass: Calculated m/z for $C_{11}H_{17}N_2O^+$ $[M+H]^+$= 193.1335; found APCI: $[M+H]^+$ 193.0

28

tert-butyl (*R*)-(1-(methyl(2-morpholinophenyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate



Synthesis:

Quantities: **Pyridine** (5 mL); **EtOAc** (10 mL); **27** (125 mg, 0.65 mmol, 1.0 eq.); ***N*-Boc-(*R*)-phenylalanine** (189 µg, 0.72 mmol, 1.1 eq.); **T3P 50 % m/v in EtOAc** (774 µL, 1.30 mmol, 2.0 eq.)

Procedure: **27** and ***N*-Boc-(*R*)-phenylalanine** were dissolved in a 1:2 mixture of distilled **pyridine** and **EtOAc**. The mixture was cooled to -20 °C with an isopropanol dry-ice bath before **T3P 50 % m/V in EtOAc** was added. After the addition, the cooling bath was removed, and the mixture was stirred for 20 h at room temperature. Afterwards the mixture was washed with 0.25 M KH_2PO_4 solution three times before the organic phase was evaporated on silica gel under reduced pressure for purification.

Purification:

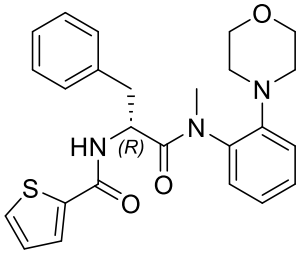
Flash chromatography ethyl acetate/heptane gradient 80 % ethyl acetate to 50 % over 9 CV on 45 g. Yield: 95 mg; 33 %

Analyses:

Mass: Calculated m/z for $C_{25}H_{34}N_3O_4^+$ $[M+H]^+$= 440.2544; found APCI: $[M+H]^+$ 440.2

29

(*R*)-*N*-(1-(methyl(2-morpholinophenyl)amino)-1-oxo-3-phenylpropan-2-yl)thiophene-2-carboxamide



Synthesis:

Quantities: **DCM** (2 mL); **TFA** (2 mL); **28** (95 mg, 0.22 mmol, 1.0 eq.); **thiophene-2-carboxylic acid** (28 mg, 0.22 mmol, 1.0 eq.); **PyBOP** (125 mg, 0.24 mmol, 1.1 eq.); **DCM** (2 mL), **DIPEA** (114 µL, 0.66 mmol, 3.0 eq.)

Procedure: Dissolve **28** in **DCM** and add **TFA** under stirring; stir for 1 h and check for full conversion by TLC; after conversion evaporate the solvents under reduced pressure; followed by coevaporation with toluene 3x, DCM 3x; dissolve the Boc-deprotected reactant, the **thiophene-2-carboxylic acid** and **PyBOP** in **DMF**; add **DIPEA**; stir over night at 65 °C under argon and light protection.

After the reaction time the crude reaction mixture was directly evaporated on silica gel without previous washing.

Purification:

Flash chromatography ethyl acetate/heptane gradient 80 % heptane to 40 % over 7 CV on 45 g. Yield: 76 mg; 77 %

Analyses:

R_F value: 0.45 ethyl acetate/heptane 4:1

^{1}H NMR (**Spectrum 45**) (400 MHz, Chloroform-*d*) δ 7.52 (dd, *J* = 3.7, 1.0 Hz, 1H), 7.47 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.24 – 7.18 (m, 4H), 7.11 – 7.02 (m, 2H), 7.02 – 6.93 (m, 3H), 6.86 (td, *J* = 7.6, 1.4 Hz, 1H), 6.18 (dd, *J* = 7.8, 1.5 Hz, 1H), 4.95 – 4.87 (m, 1H), 3.65 – 3.57 (m, 2H), 3.55 – 3.45 (m, 2H), 3.21 (s, 3H), 2.93 – 2.83 (m, 4H), 2.78 – 2.70 (m, 2H).

^{13}C NMR (**Spectrum 46**) (126 MHz, Chloroform-*d*) δ 172.7, 160.4, 148.4, 139.1, 136.5, 136.5, 130.3, 129.7, 128.9, 128.5, 128.1, 128.1, 127.9, 126.9, 124.2, 120.4, 67.1, 52.5, 51.7, 41.0, 37.1.

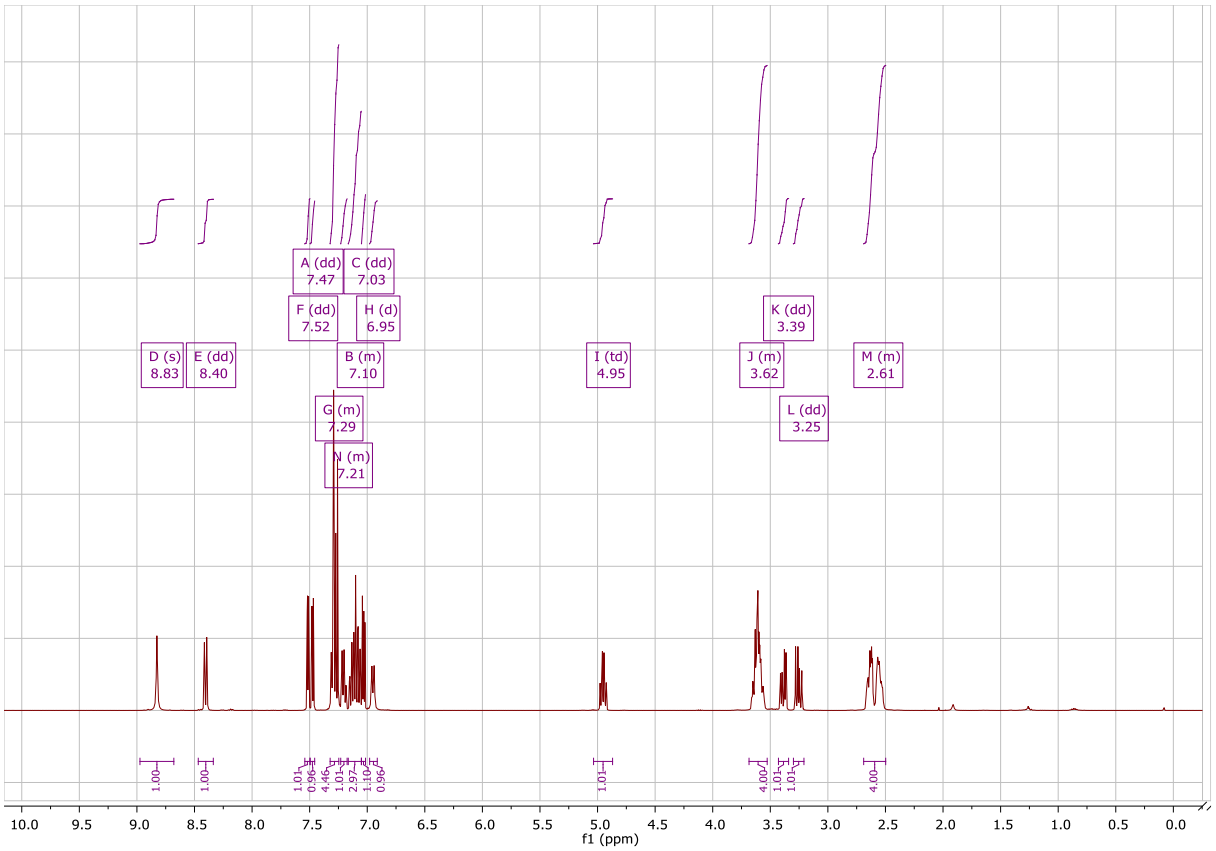
Mass: Calculated m/z for $C_{25}H_{28}N_3O_3S^+$ $[M+H]^+$= 450.1846; $[M+Na]^+$= 472.1665; found APCI: $[M+H]^+$ 450.1; found HRMS: $[M+H]^+$ 450.1850; $[M+Na]^+$ 472.1670

HPLC purity: 96.3 %; t_R=2.9 min (**HPLC-Trace 18**); (Agilent Poroshell 120, EC-C18, 3,0x50 mm, 2,7 µm, acetonitrile/ water 15:85, 6 min, v=1.2 mL/min, λ=254 nm)

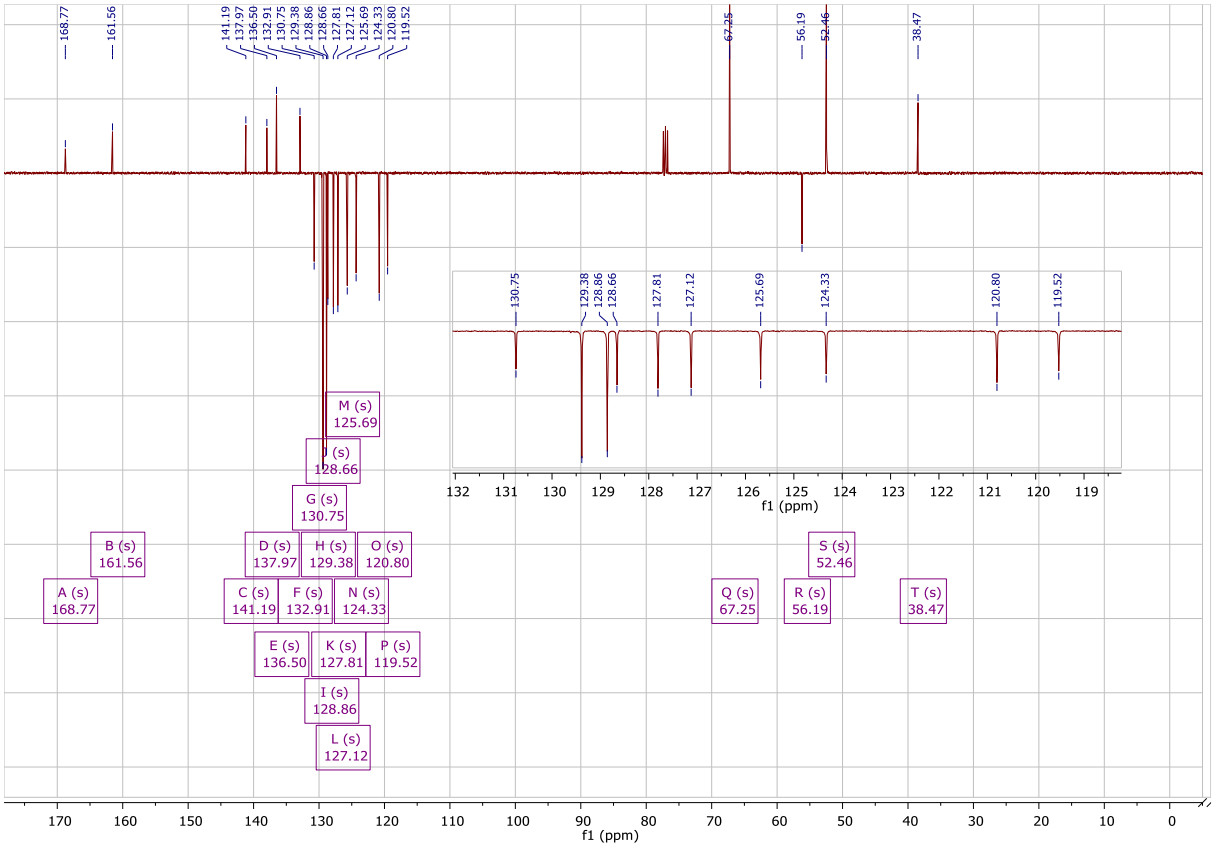
4. NMR spectra and HPLC traces

Table of NMR spectra:

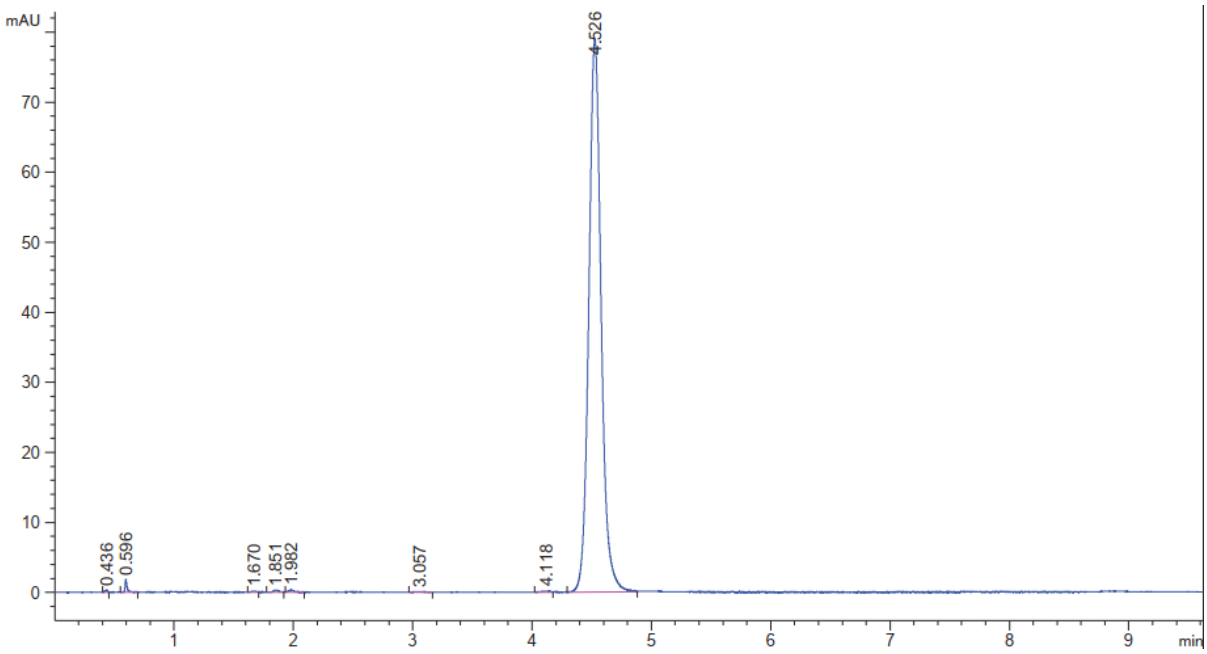
Table of HPLC-Traces



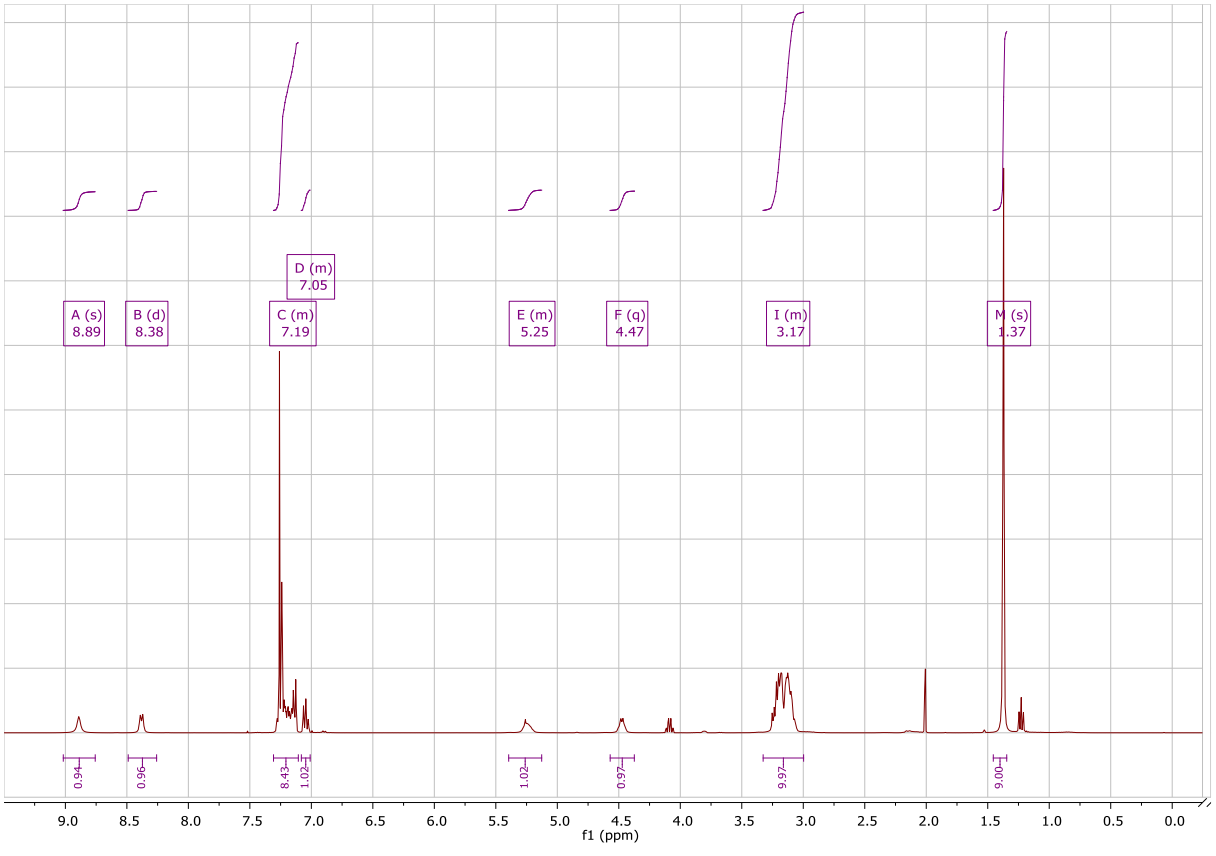
Spectrum 1 *^{1}H-NMR* ***MMV***



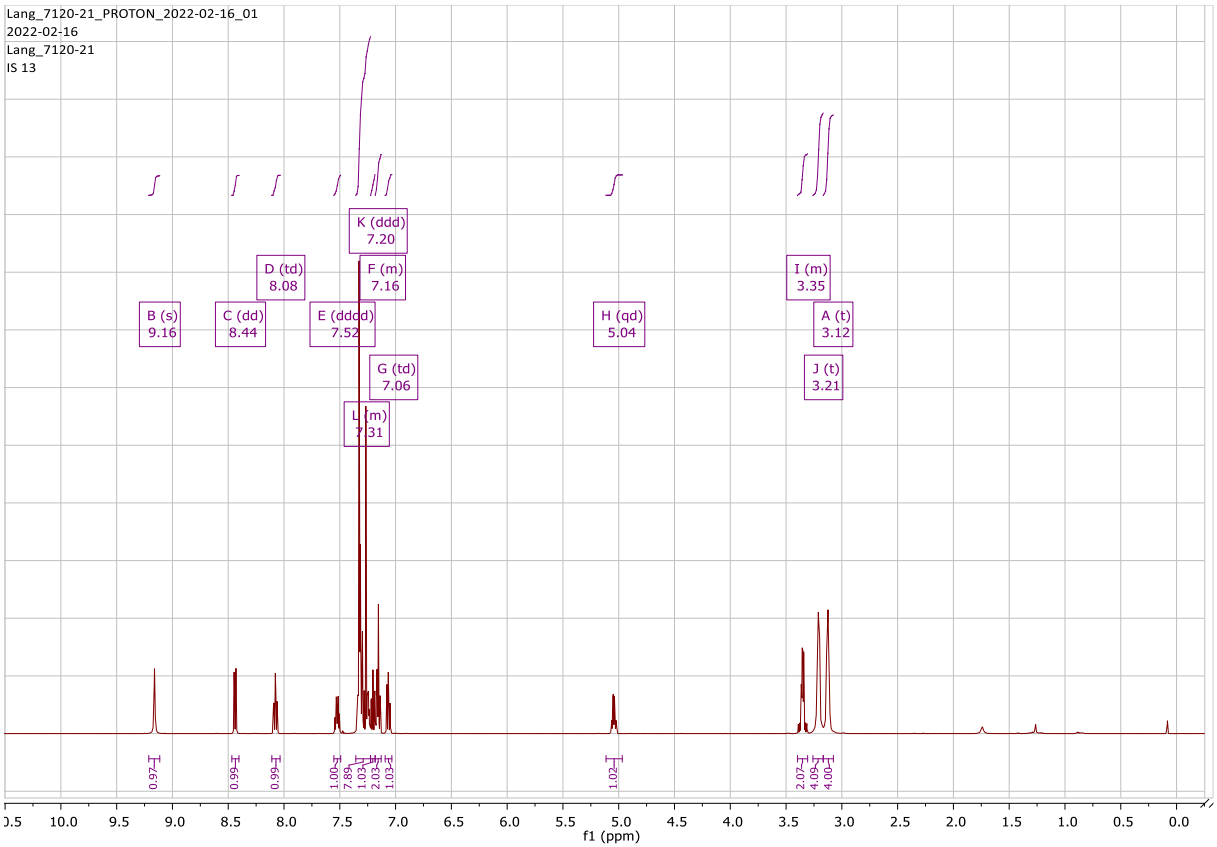
Spectrum 2 *APT ^{13}C-NMR* ***MMV***



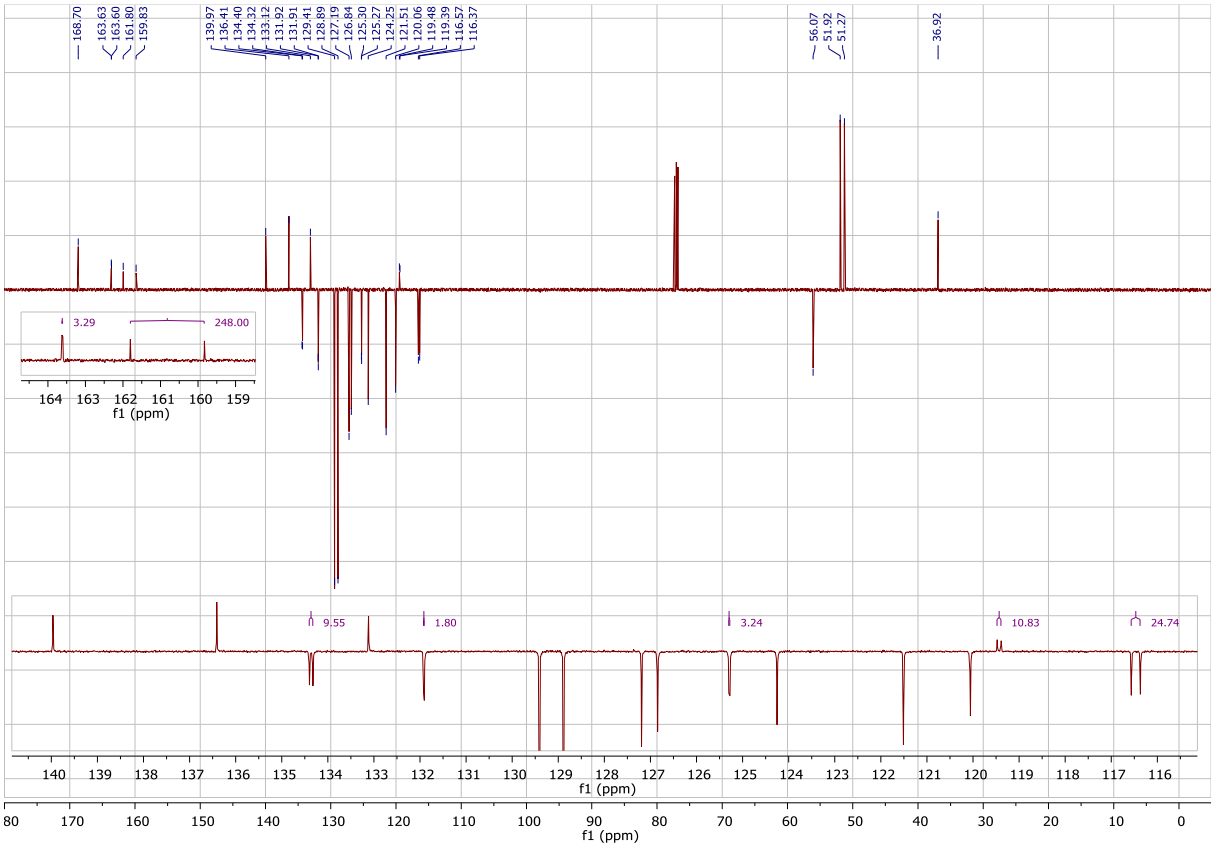
HPLC-Trace 1 *HPLC-purity* ***MMV***



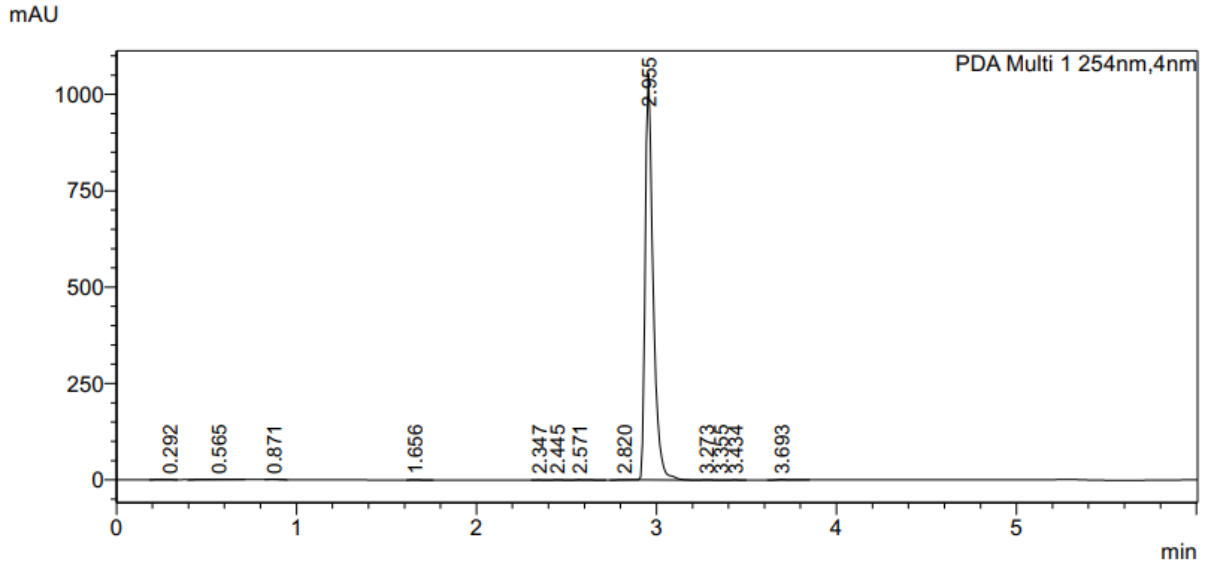
Spectrum 3 *^{1}H-NMR* ***1***



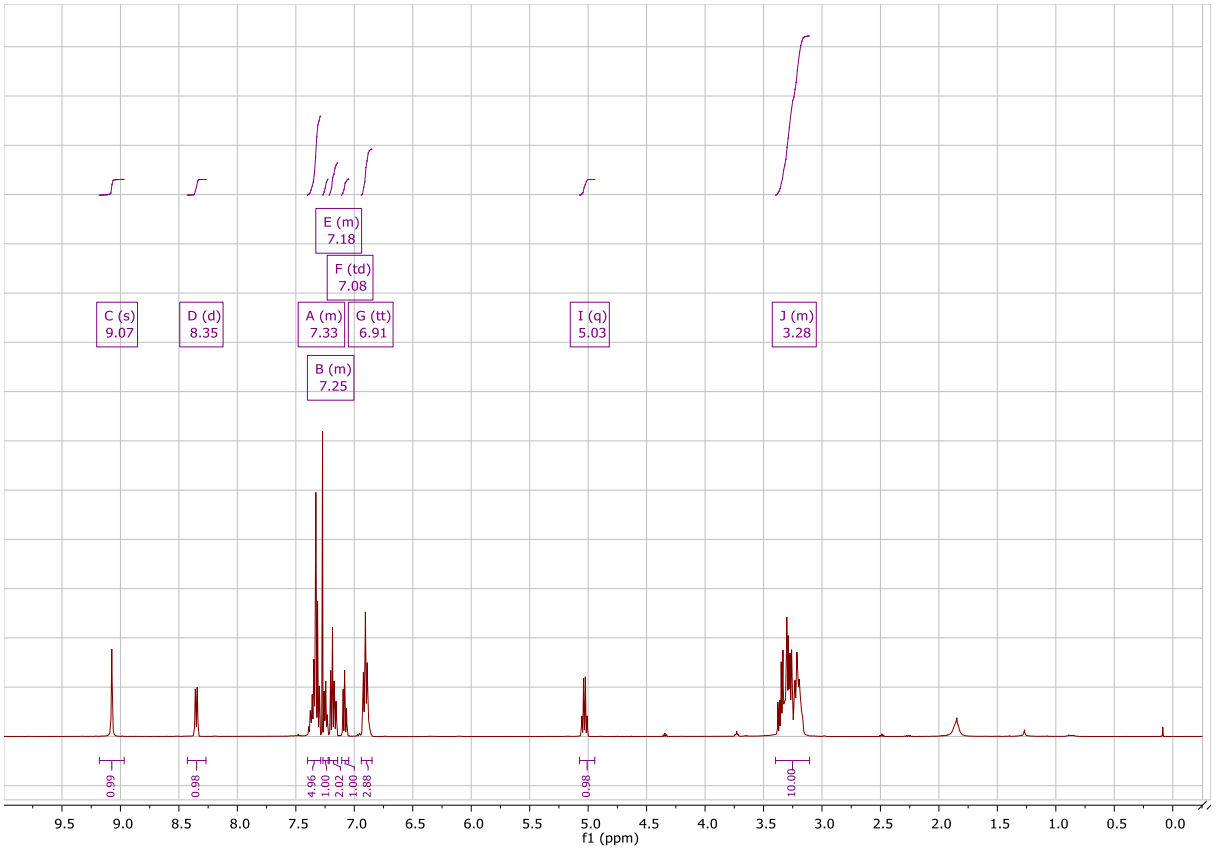
Spectrum 4 *^{1}H-NMR* **2**



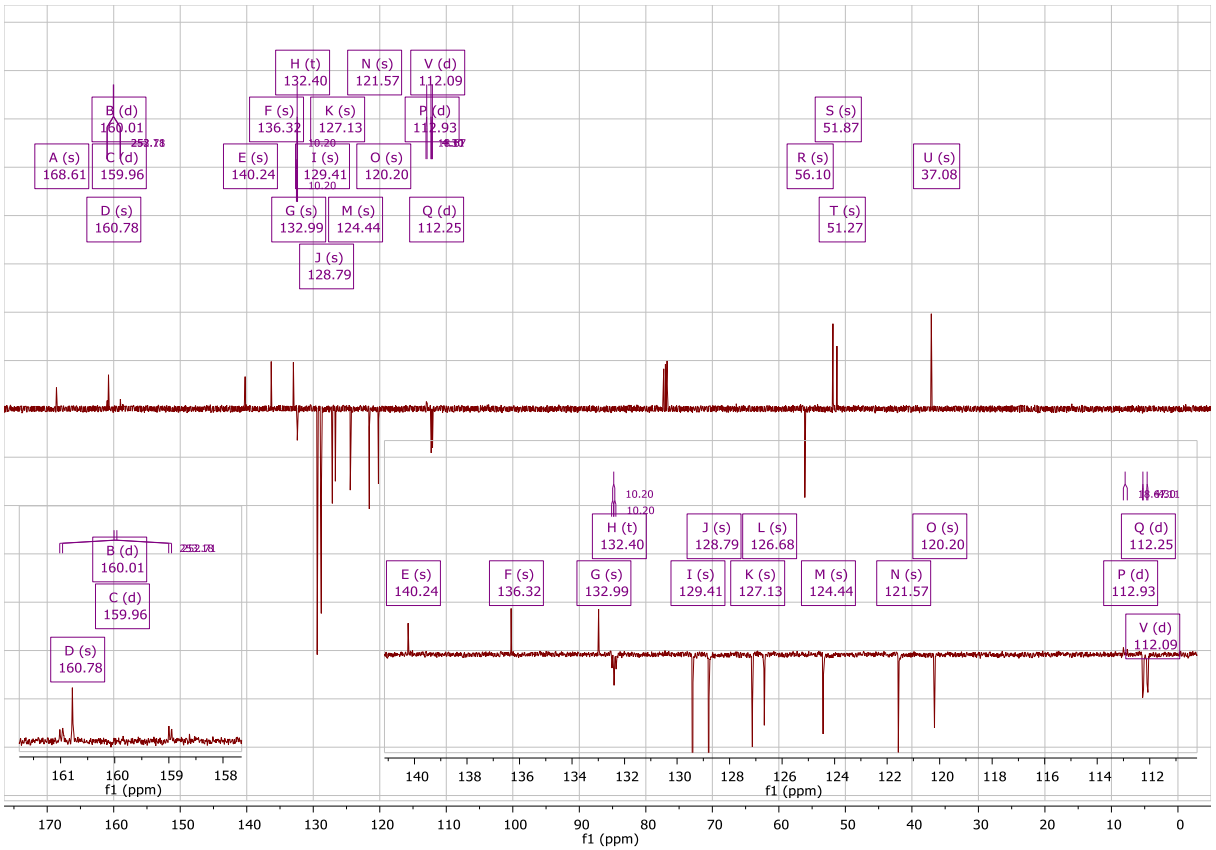
Spectrum 5 *APT ^{13}C-NMR* ***2***



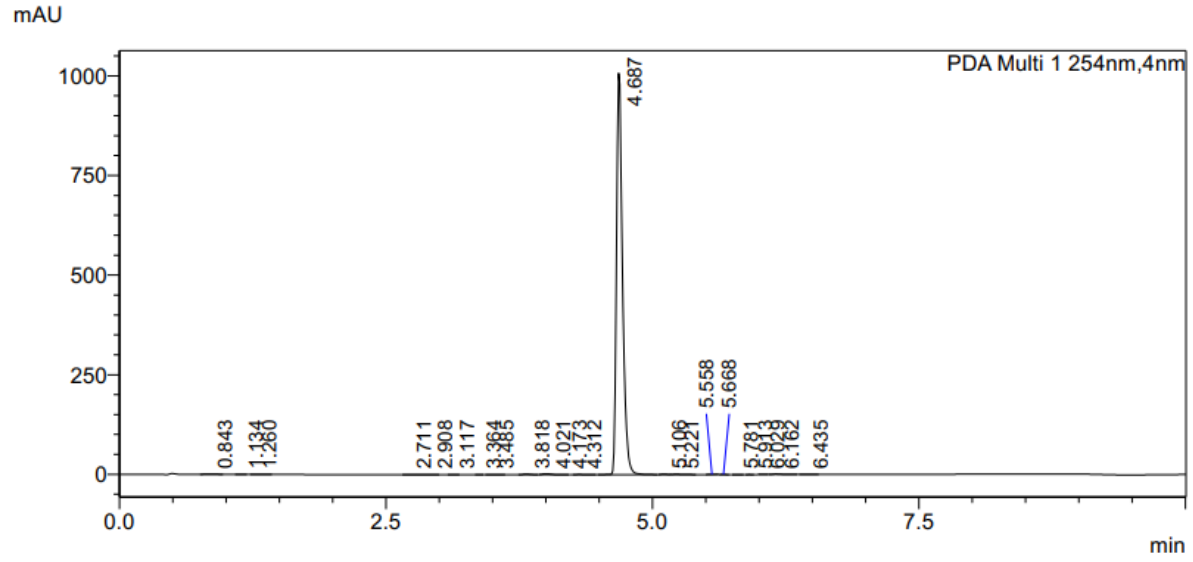
HPLC-Trace 2 *HPLC-purity* ***2***



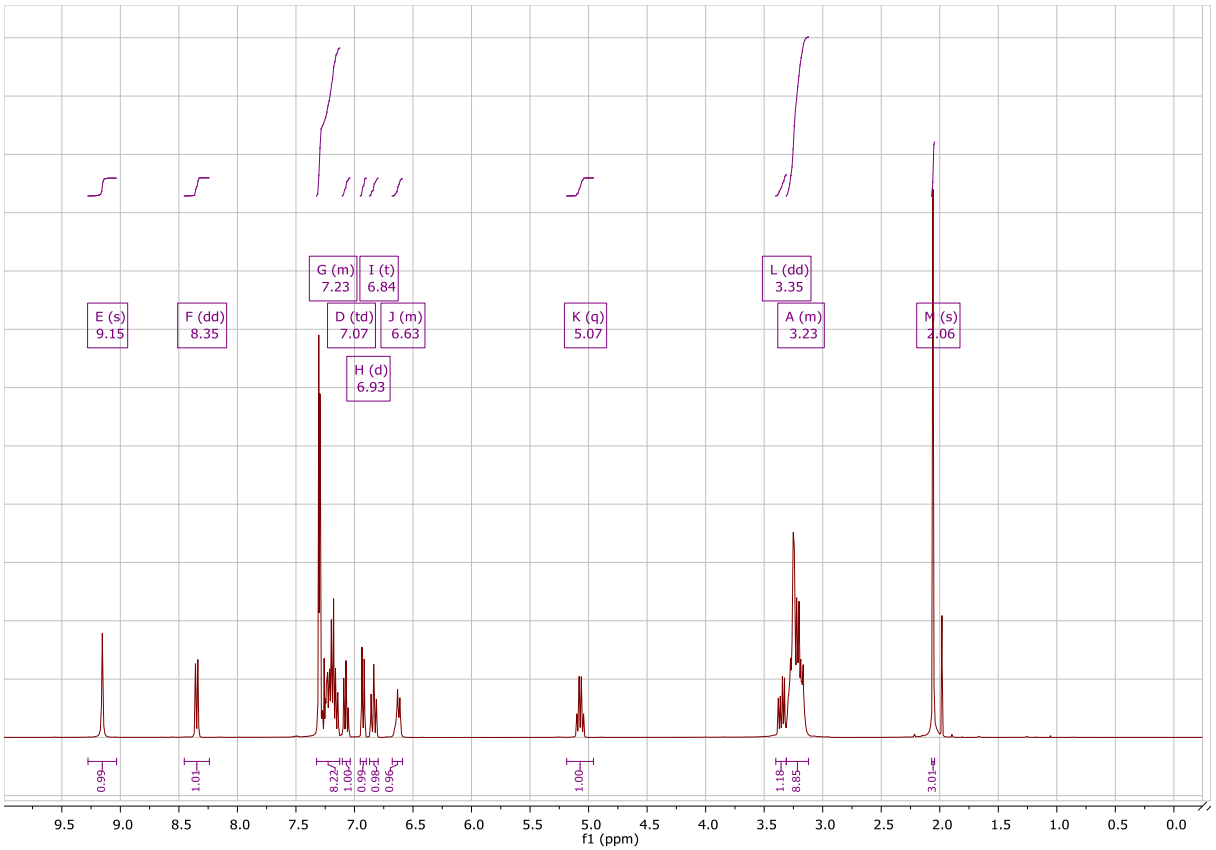
Spectrum 6 *^{1}H-NMR* ***3***



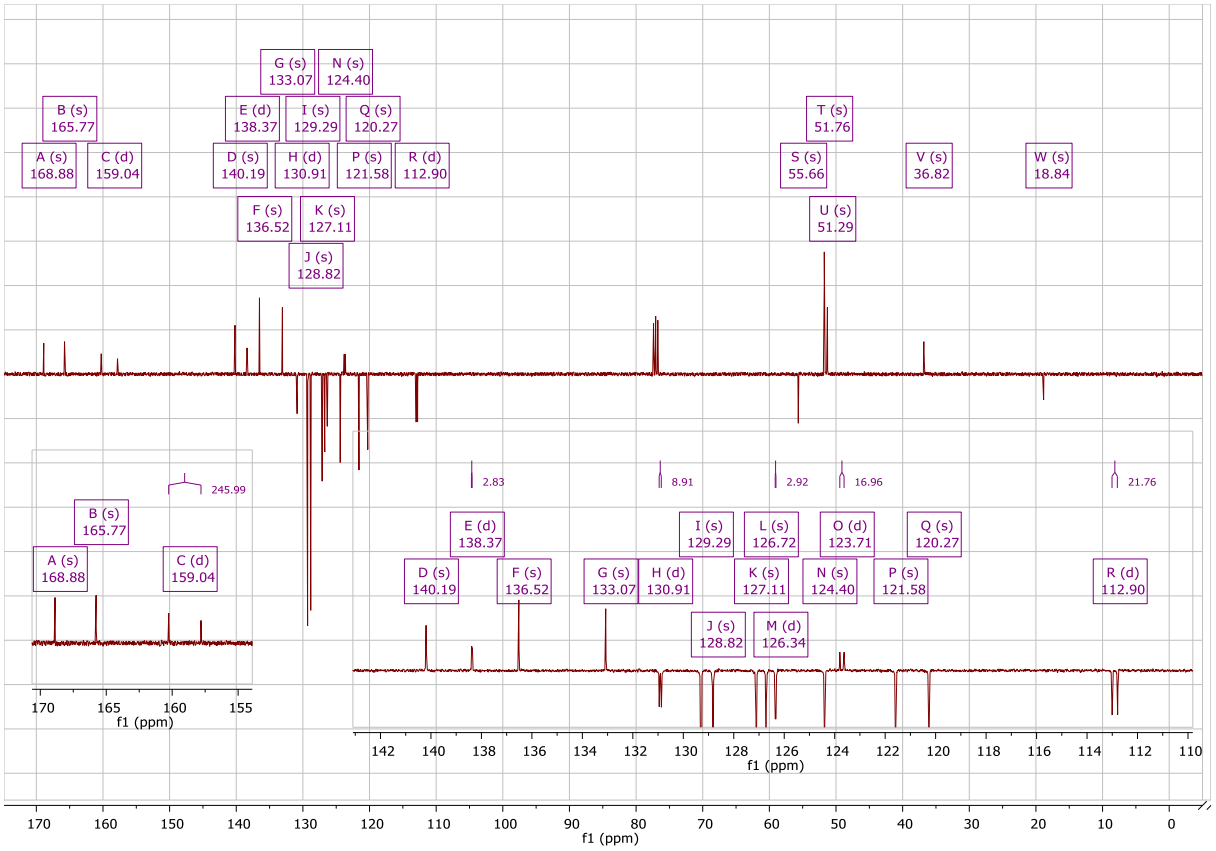
Spectrum 7 *APT ^{13}C-NMR* ***3***



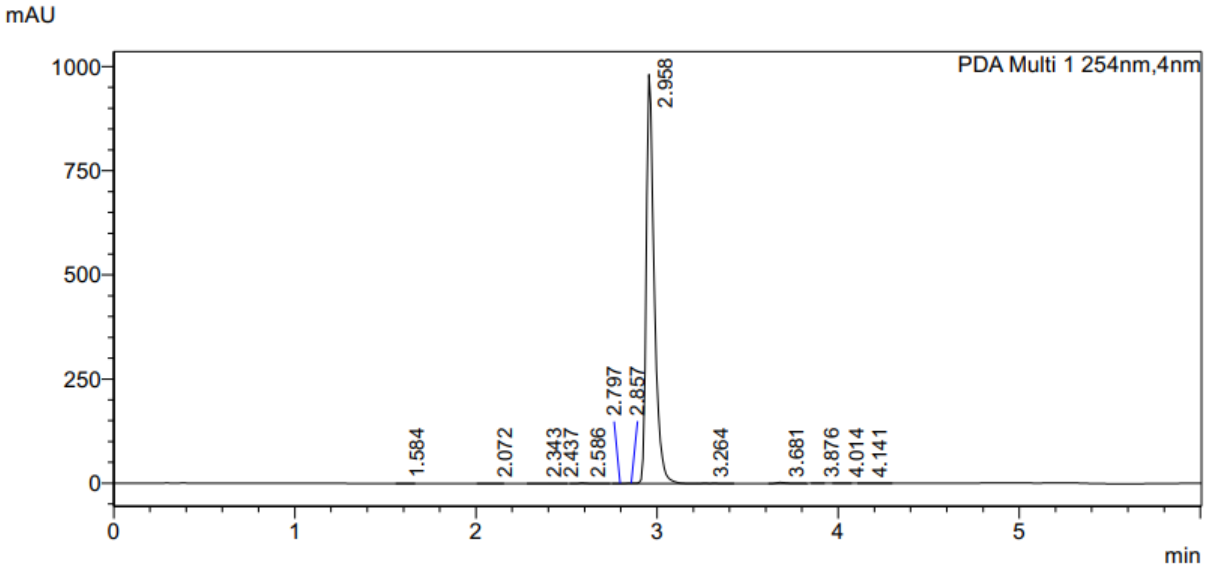
HPLC-Trace 3 *HPLC-purity* ***3***



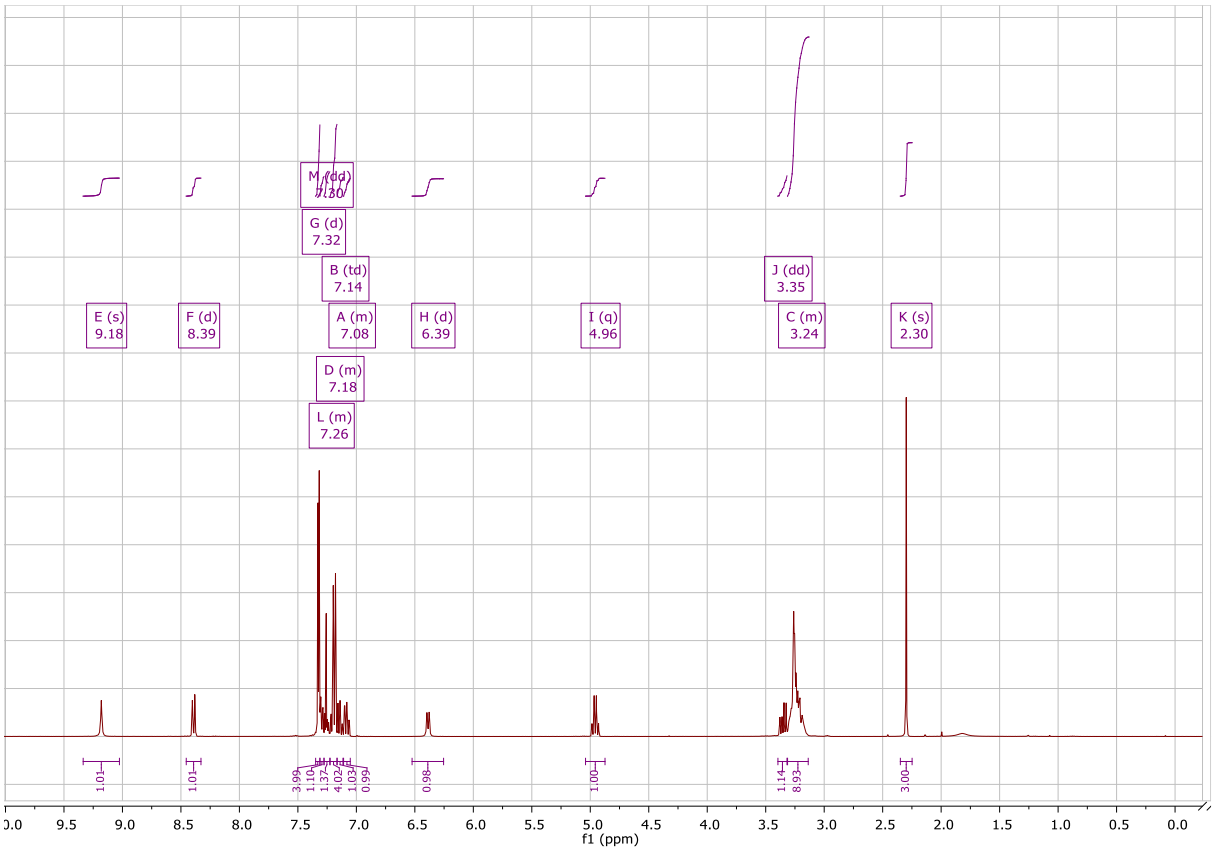
Spectrum 8 *^{1}H-NMR* ***4***



Spectrum 9 *APT ^{13}C-NMR* ***4***

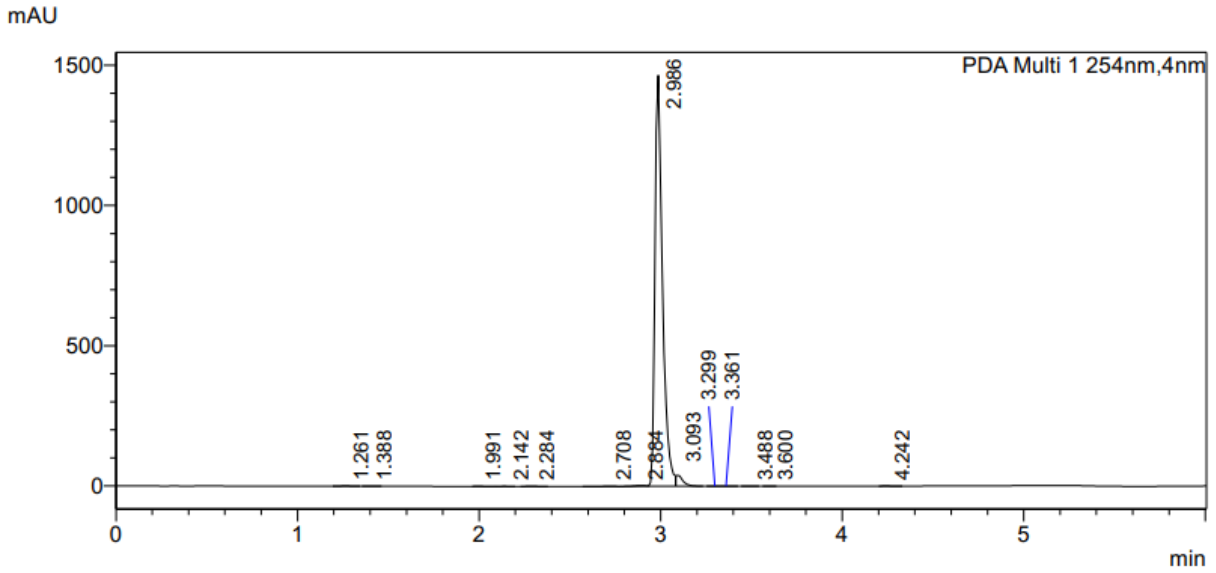


HPLC-Trace 4 *HPLC-purity* ***4***

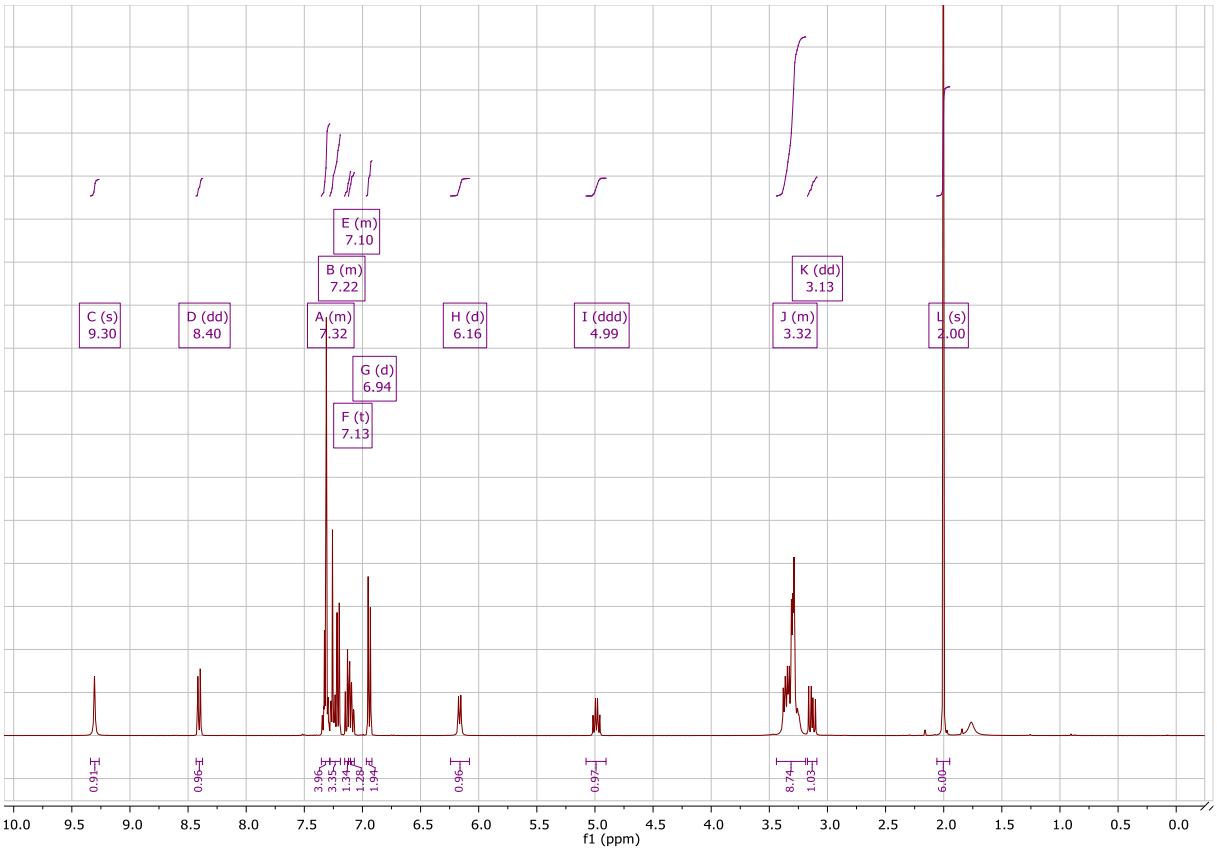


Spectrum 10 *^{1}H-NMR* ***5***

Spectrum 11 *APT ^{13}C-NMR* ***5***

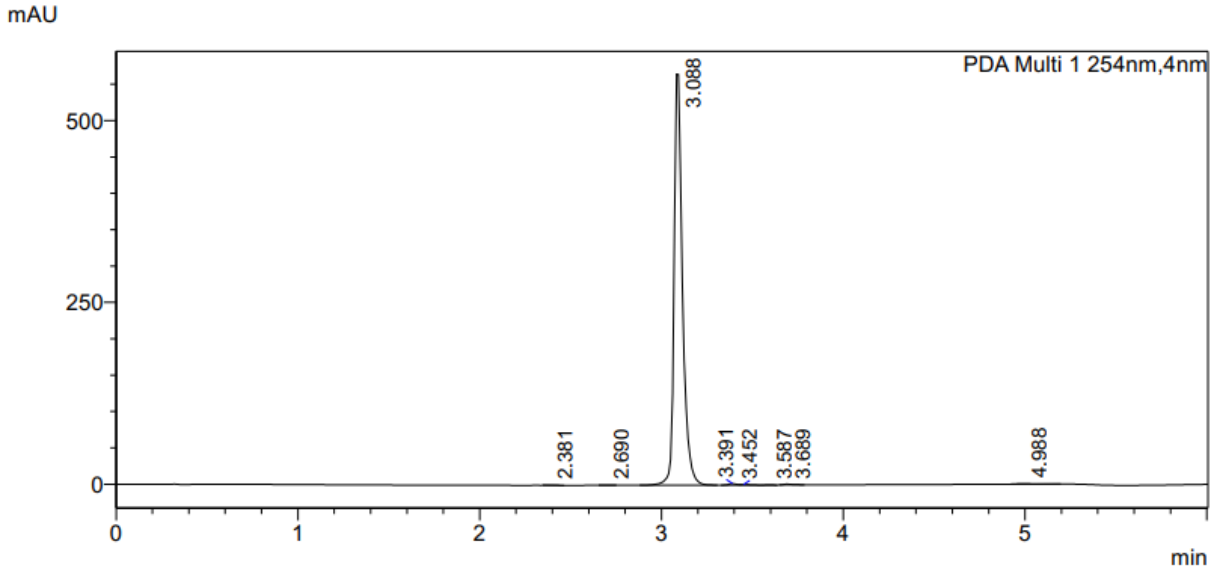


HPLC-Trace 5 *HPLC-purity* ***5***

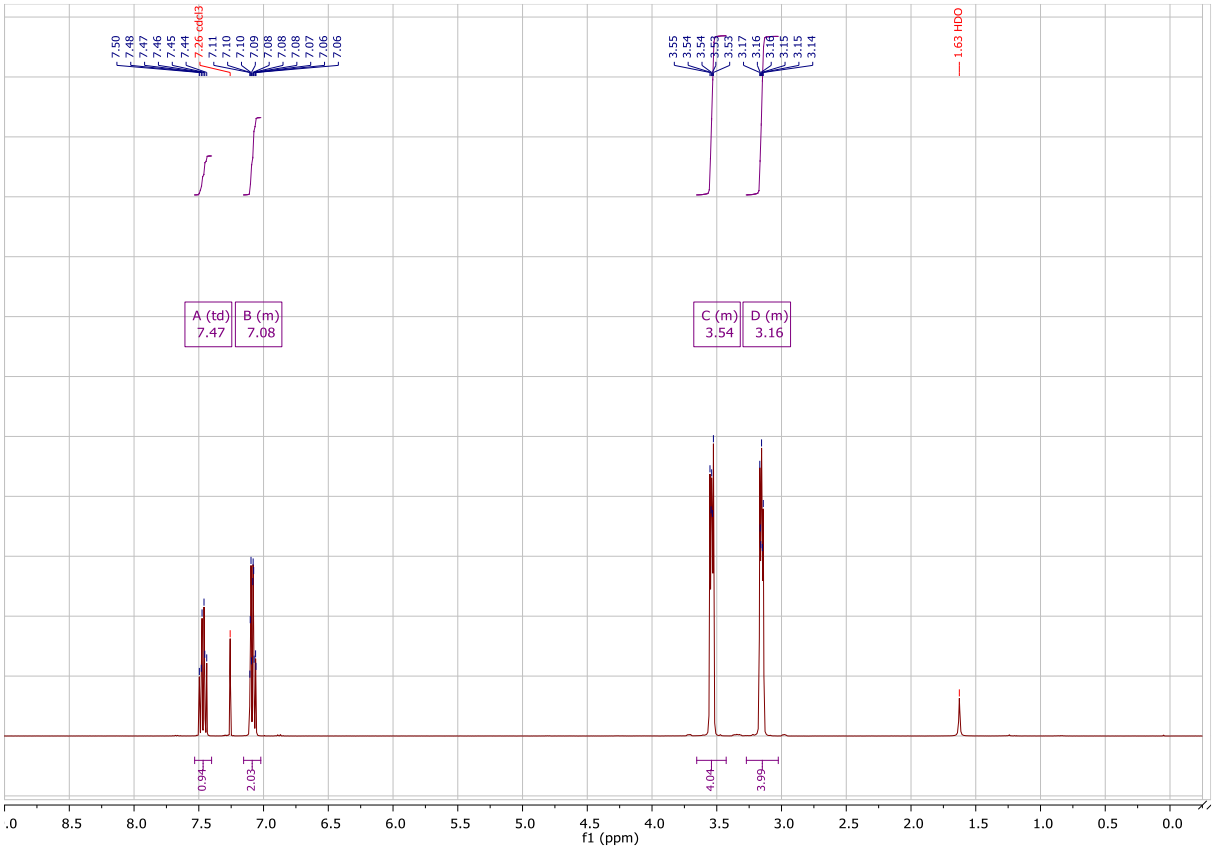


Spectrum 12 *^{1}H-NMR* ***6***

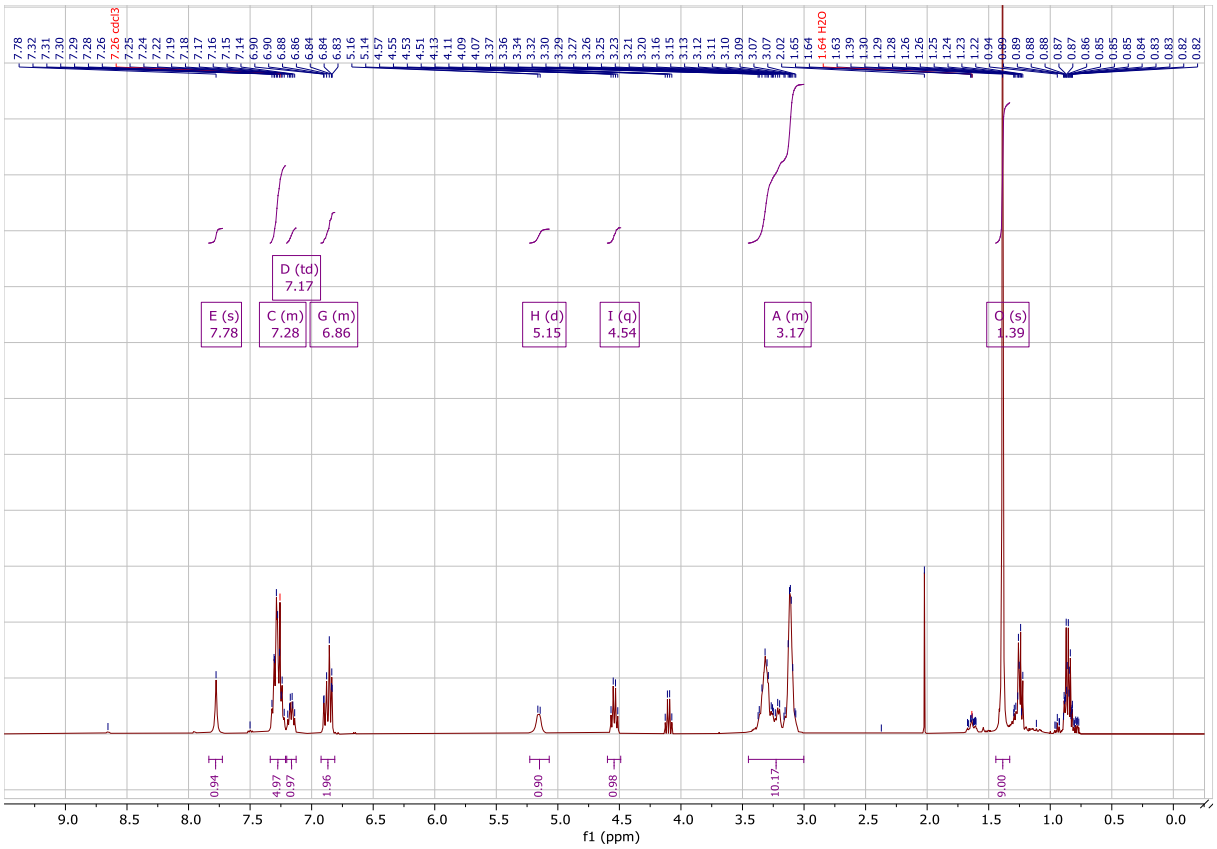
Spectrum 13 *APT ^{13}C-NMR* ***6***



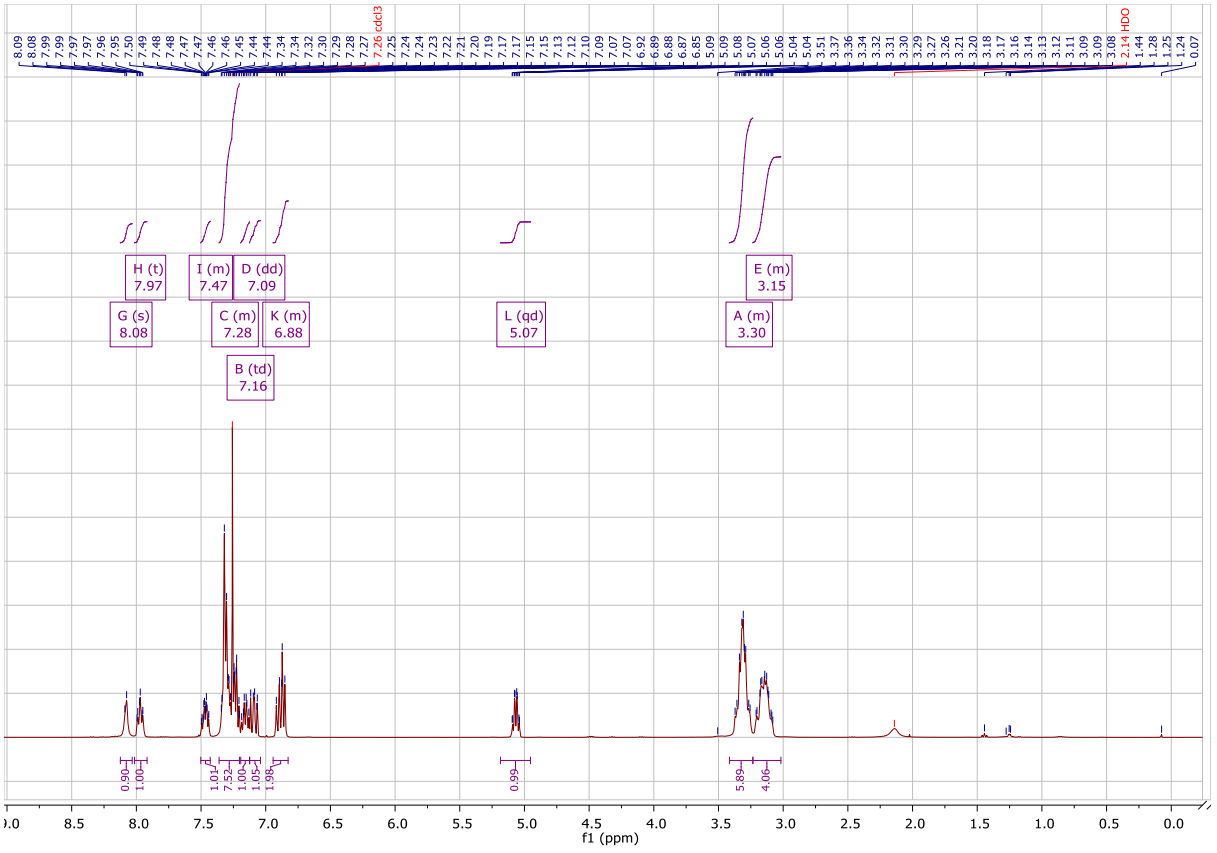
HPLC-Trace 6 *HPLC-purity* ***6***



Spectrum 14 *^{1}H-NMR* ***7***

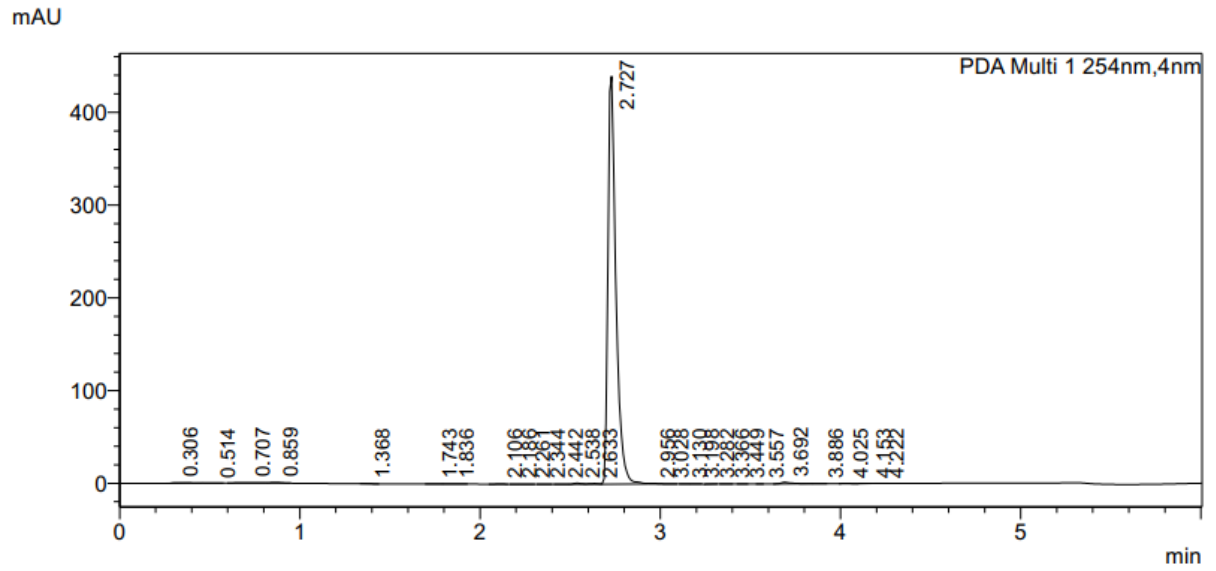


Spectrum 15 *^{1}H-NMR* ***9***

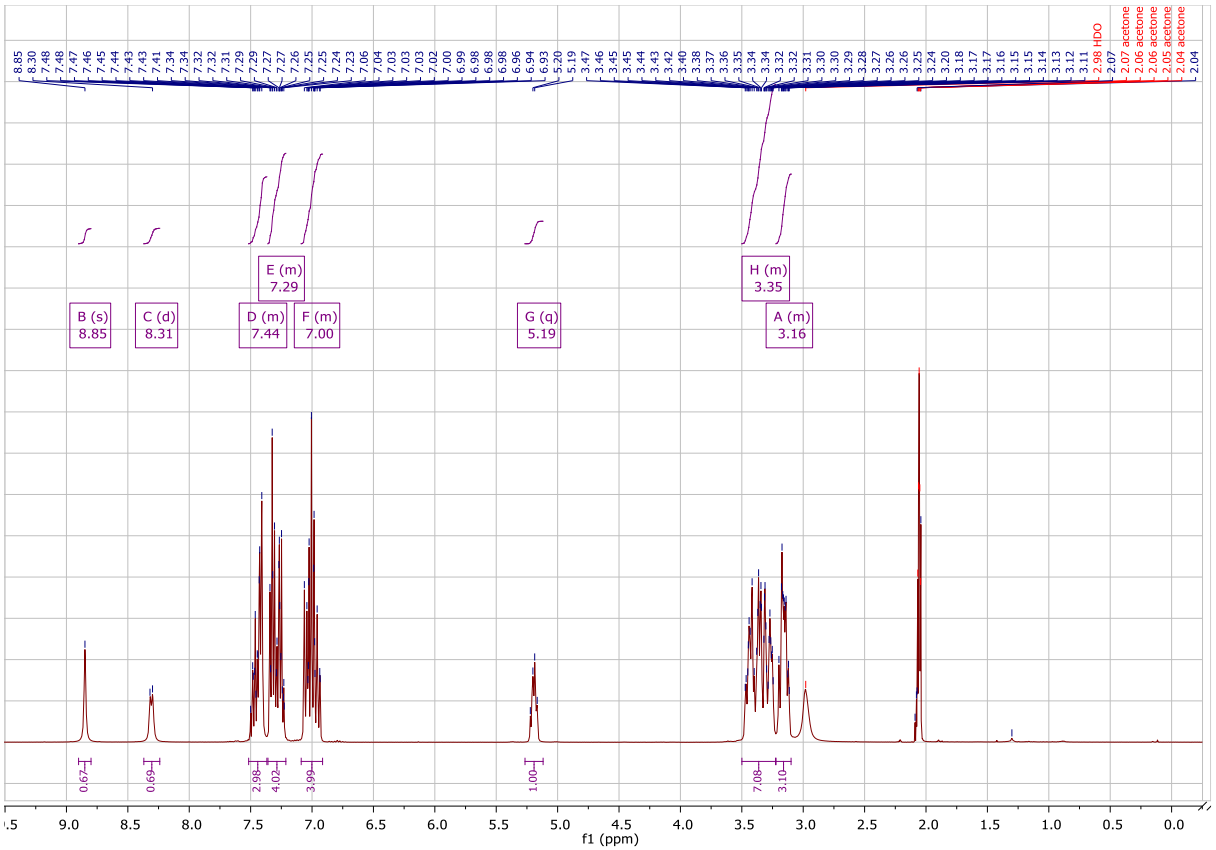


Spectrum 16 *^{1}H-NMR* ***10***

Spectrum 17 *APT* ^{13}*C-NMR* ***10***

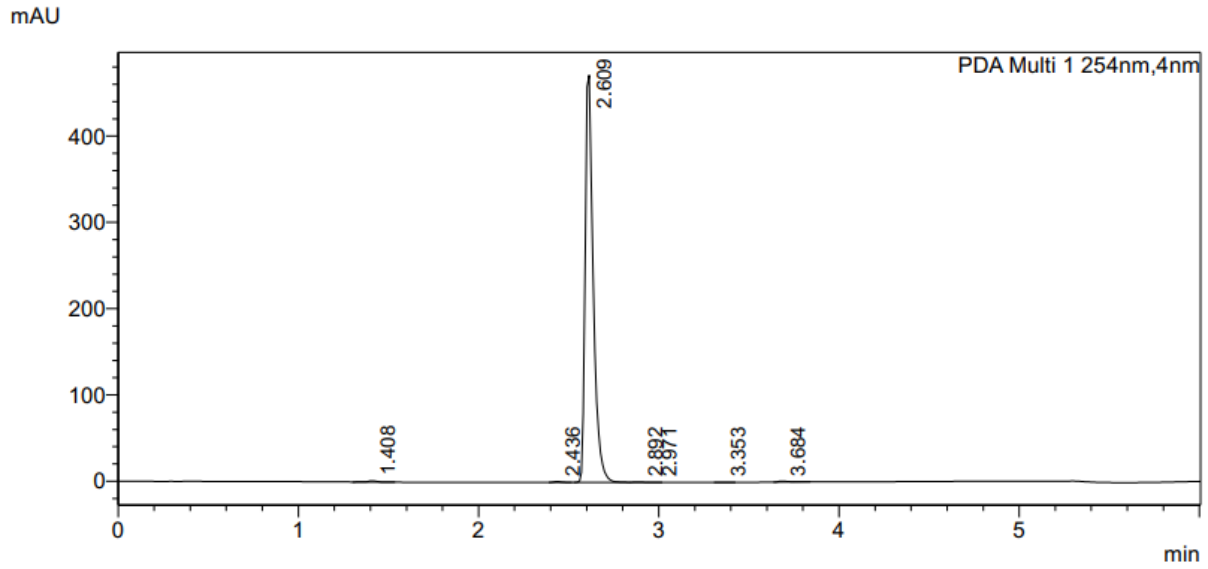


HPLC-Trace 7 *HPLC-purity* ***10***

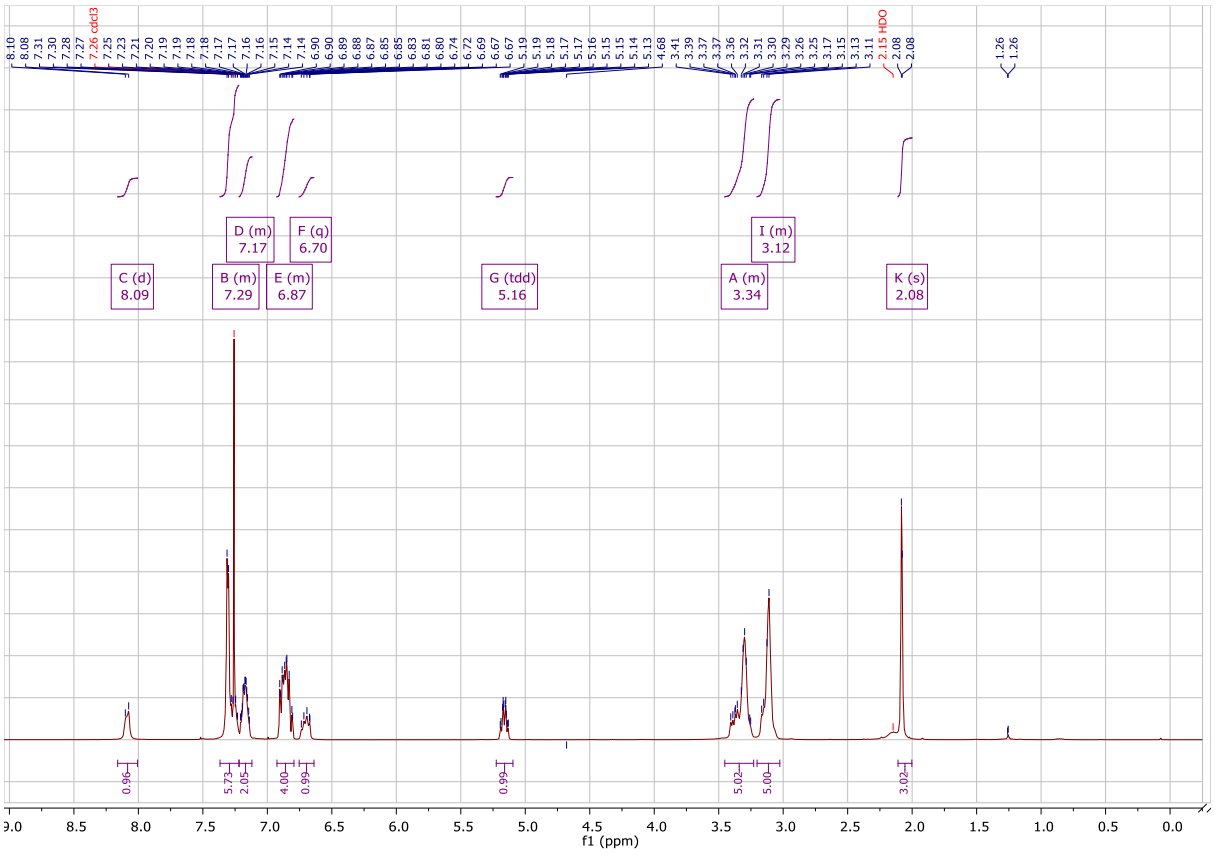


Spectrum 18 *^{1}H-NMR* ***11***

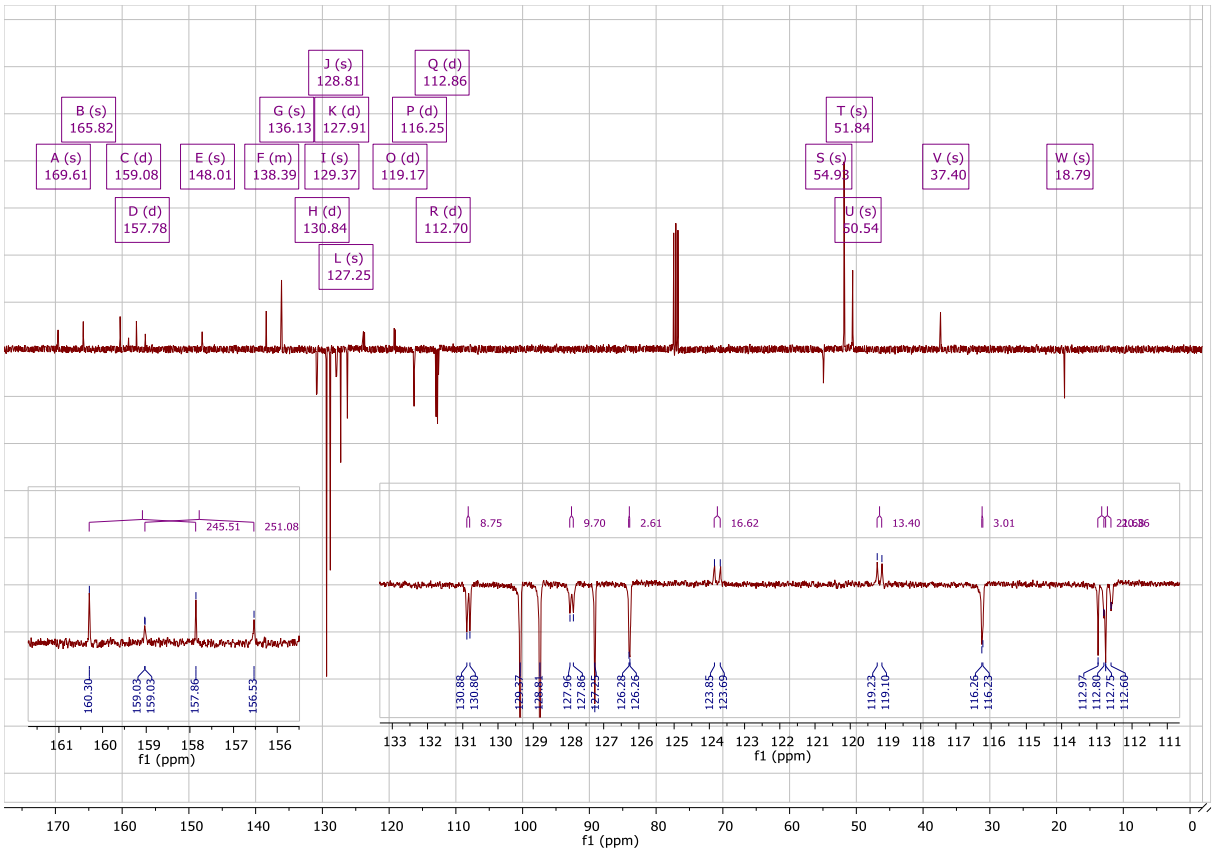
Spectrum 19 *APT ^{13}C-NMR* ***11***



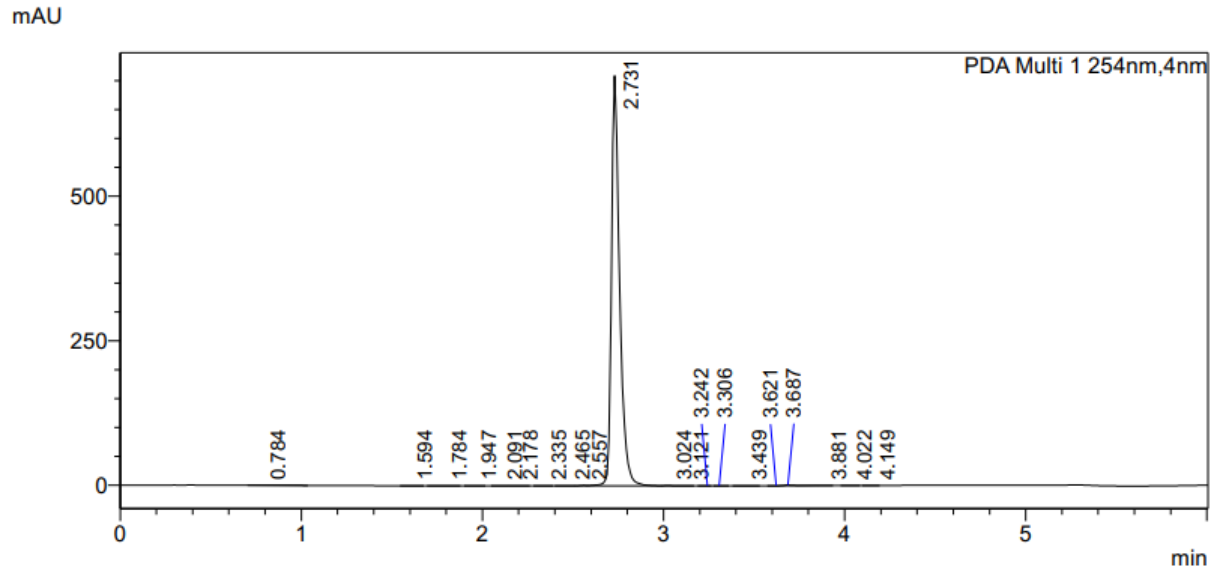
HPLC-Trace 8 *HPLC-purity* ***11***



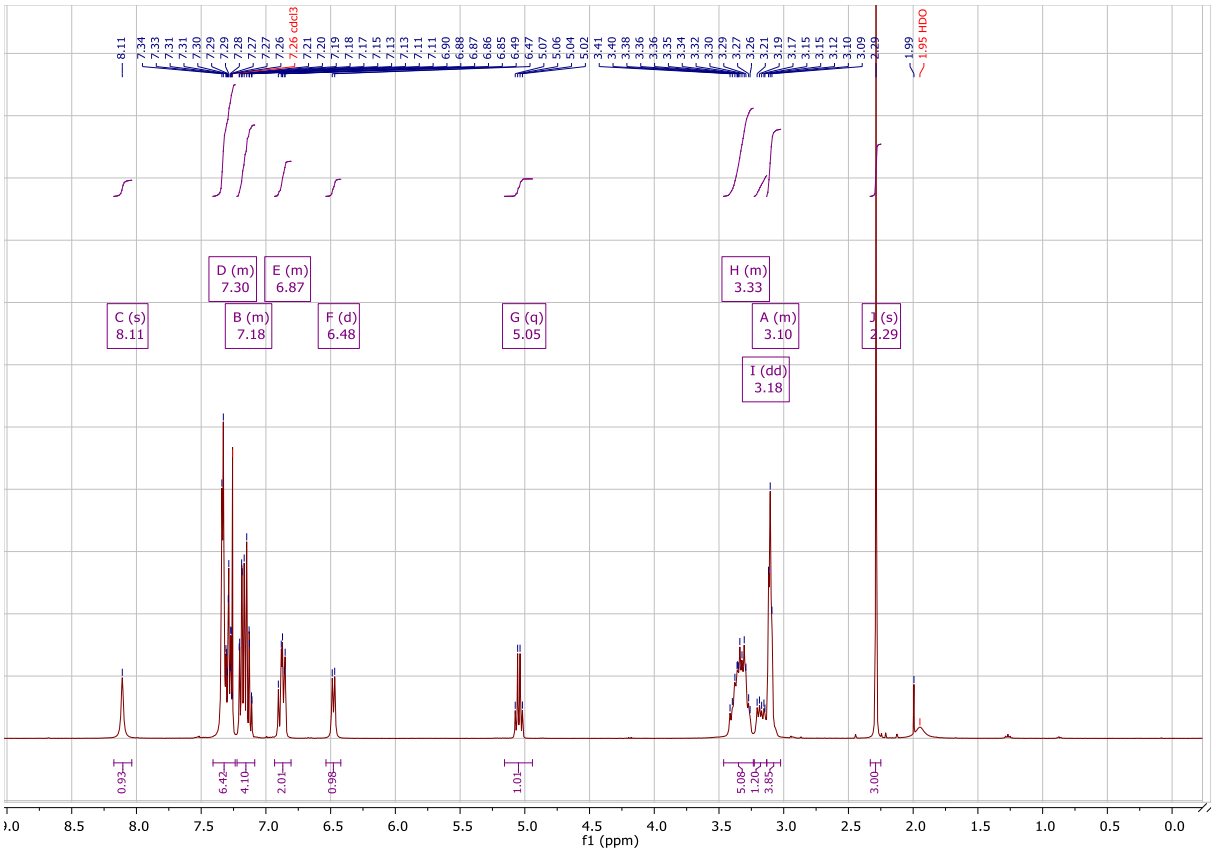
Spectrum 20 *^{1}H-NMR* ***12***



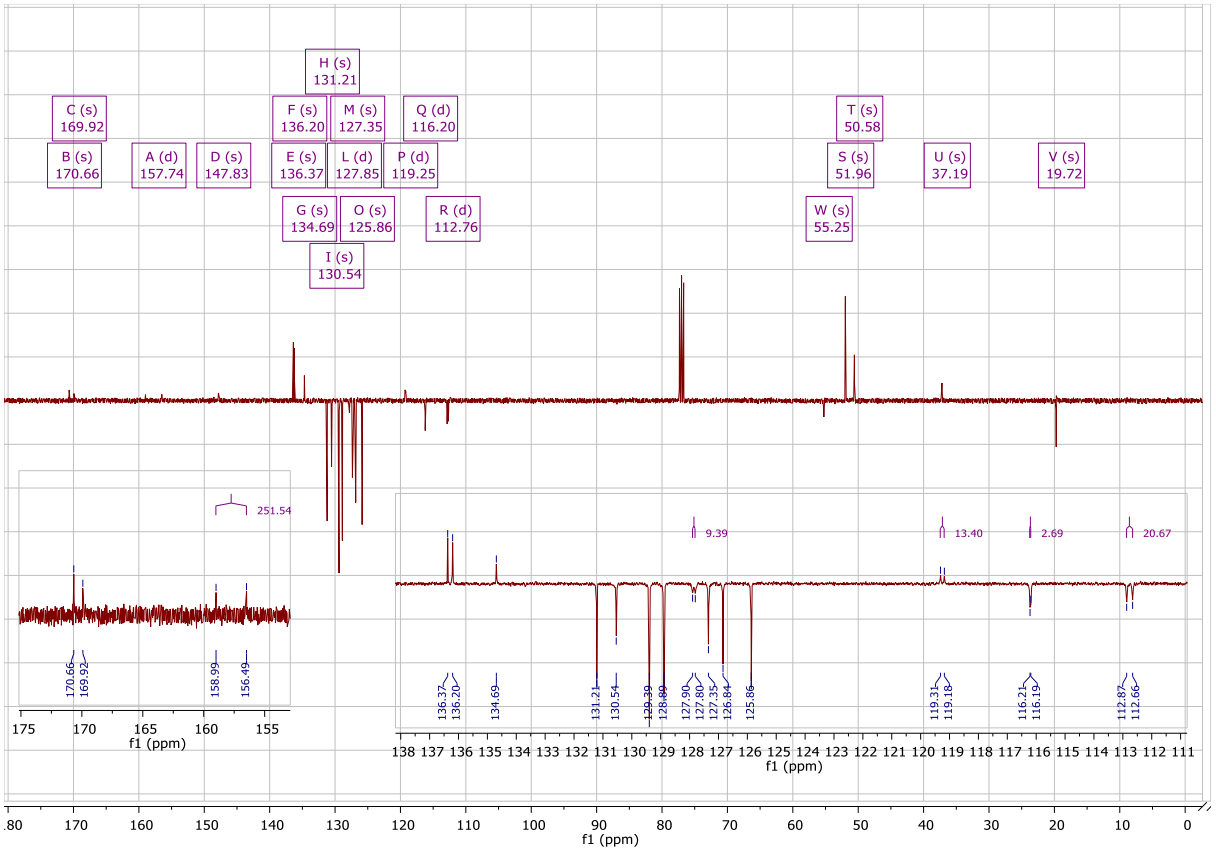
Spectrum 21 *APT* ^{13}*C-NMR* ***12***



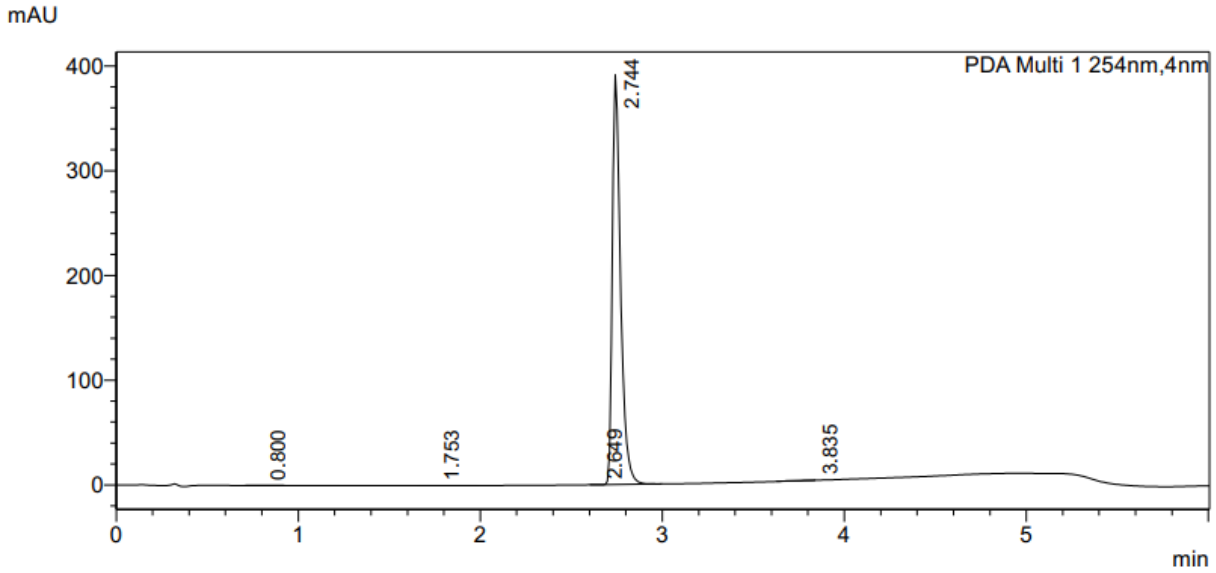
HPLC-Trace 9 *HPLC-purity* ***12***



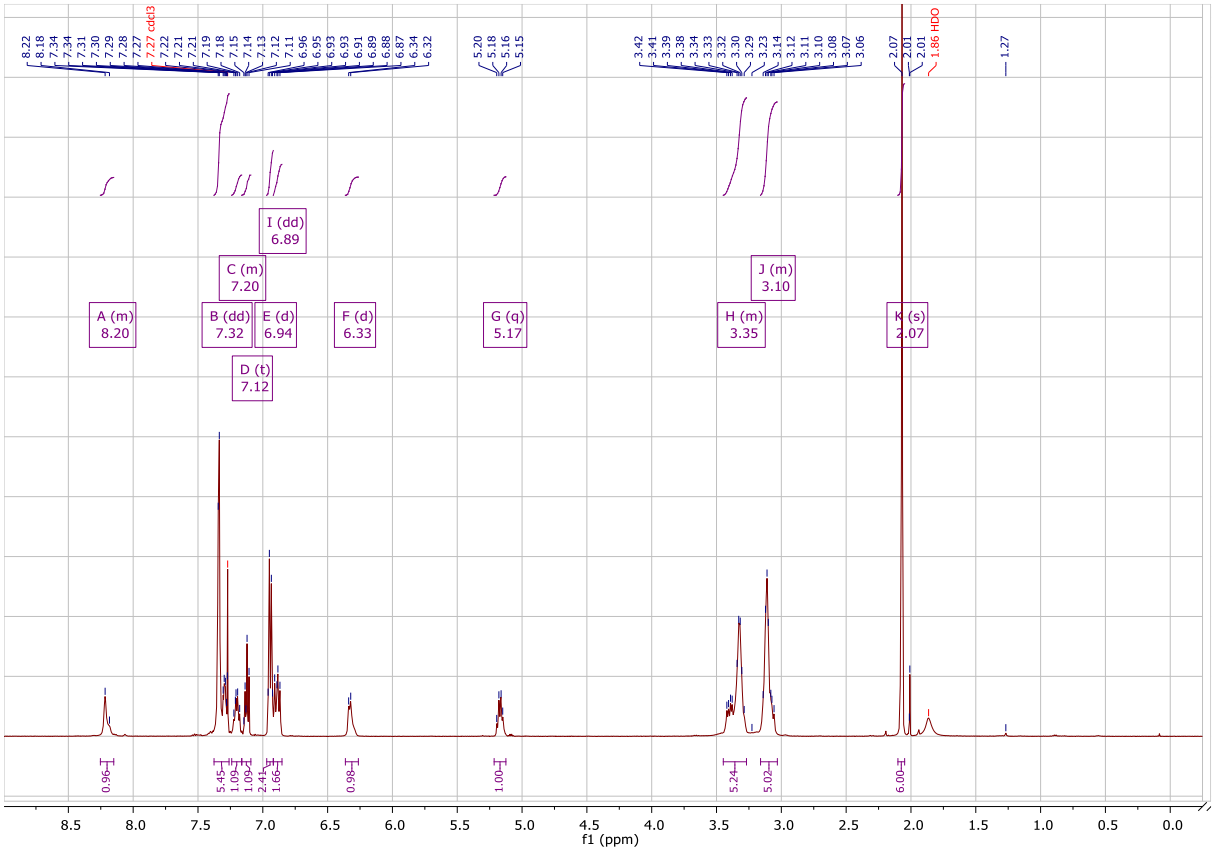
Spectrum 22 *^{1}H-NMR* ***13***



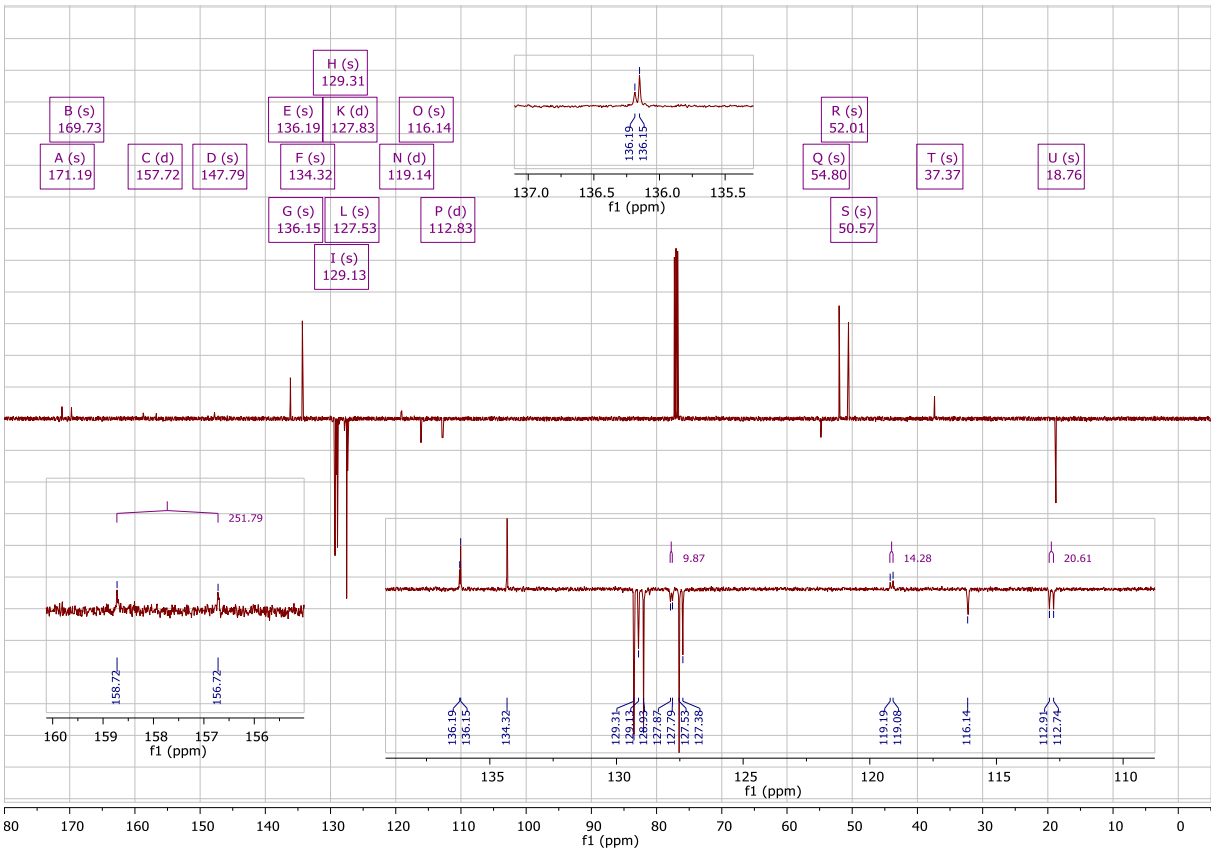
Spectrum 23 *APT ^{13}C-NMR* ***13***



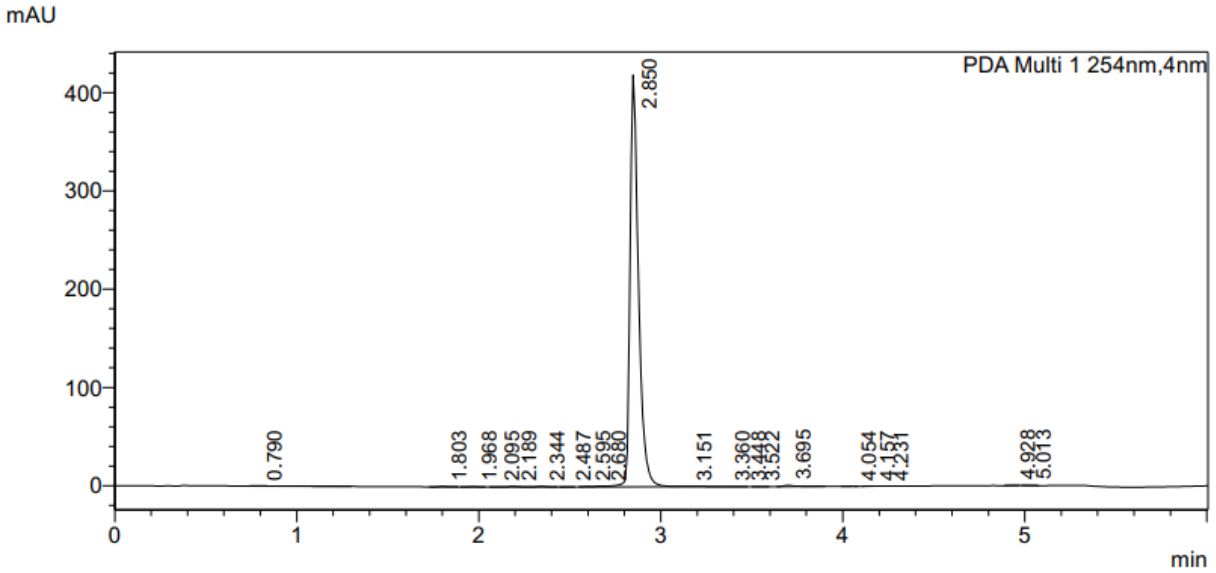
HPLC-Trace 10 *HPLC-purity* ***13***



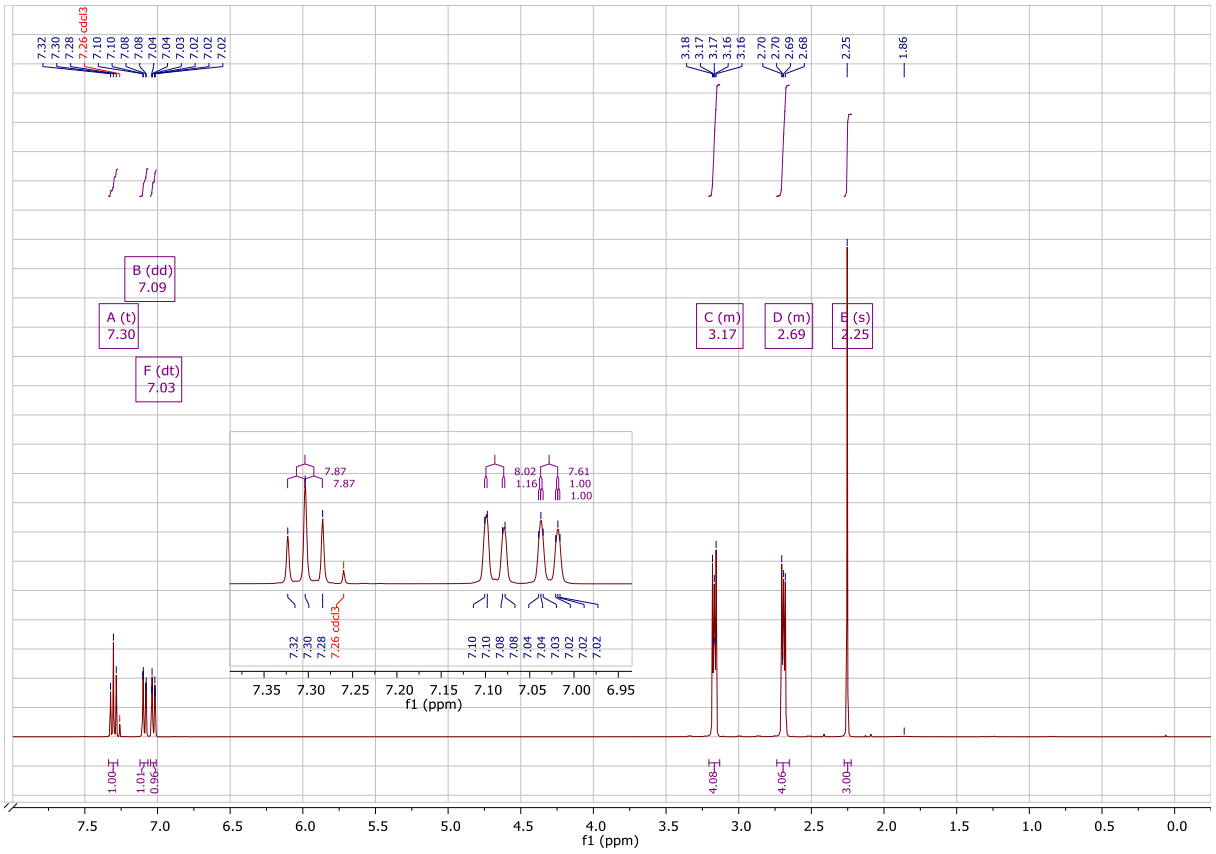
Spectrum 24 *^{1}H-NMR* ***14***



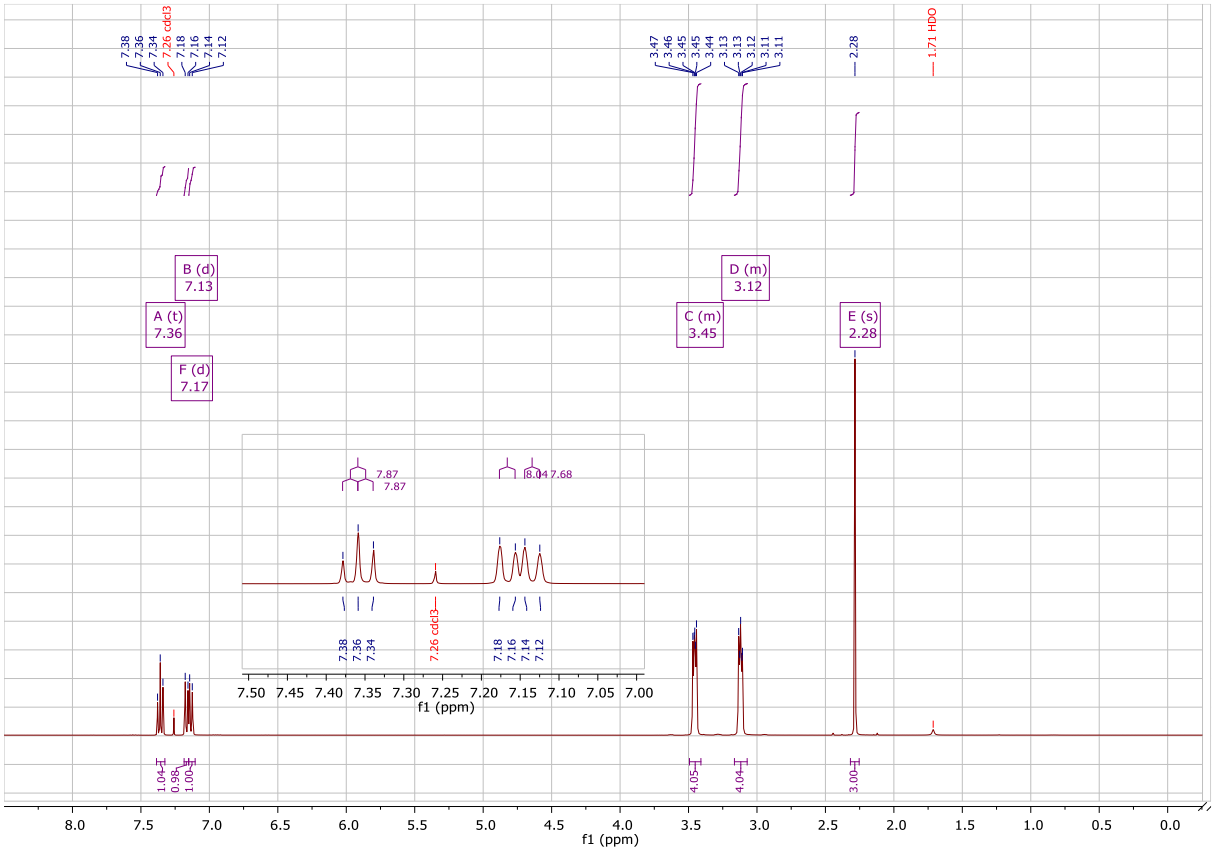
Spectrum 25 *APT ^{13}C-NMR* ***14***



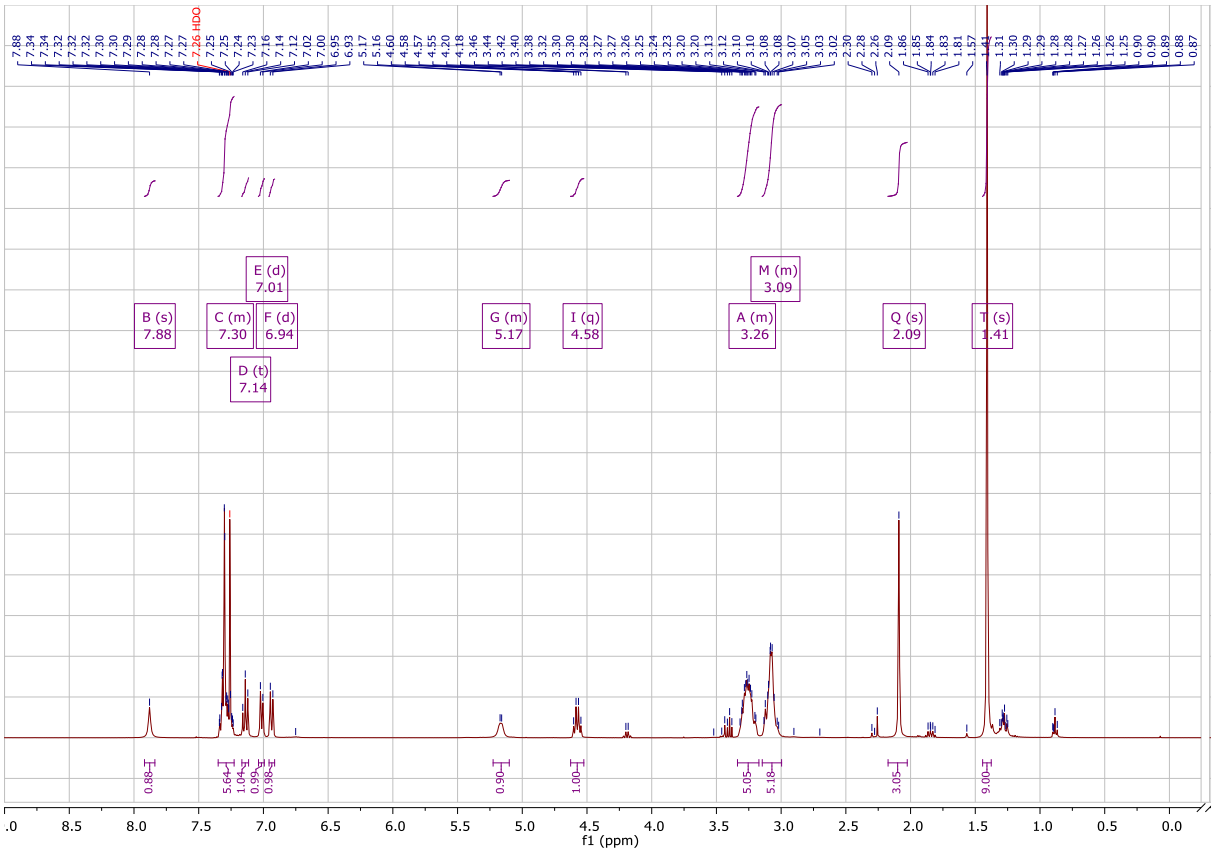
HPLC-Trace 11 *HPLC-purity* ***14***



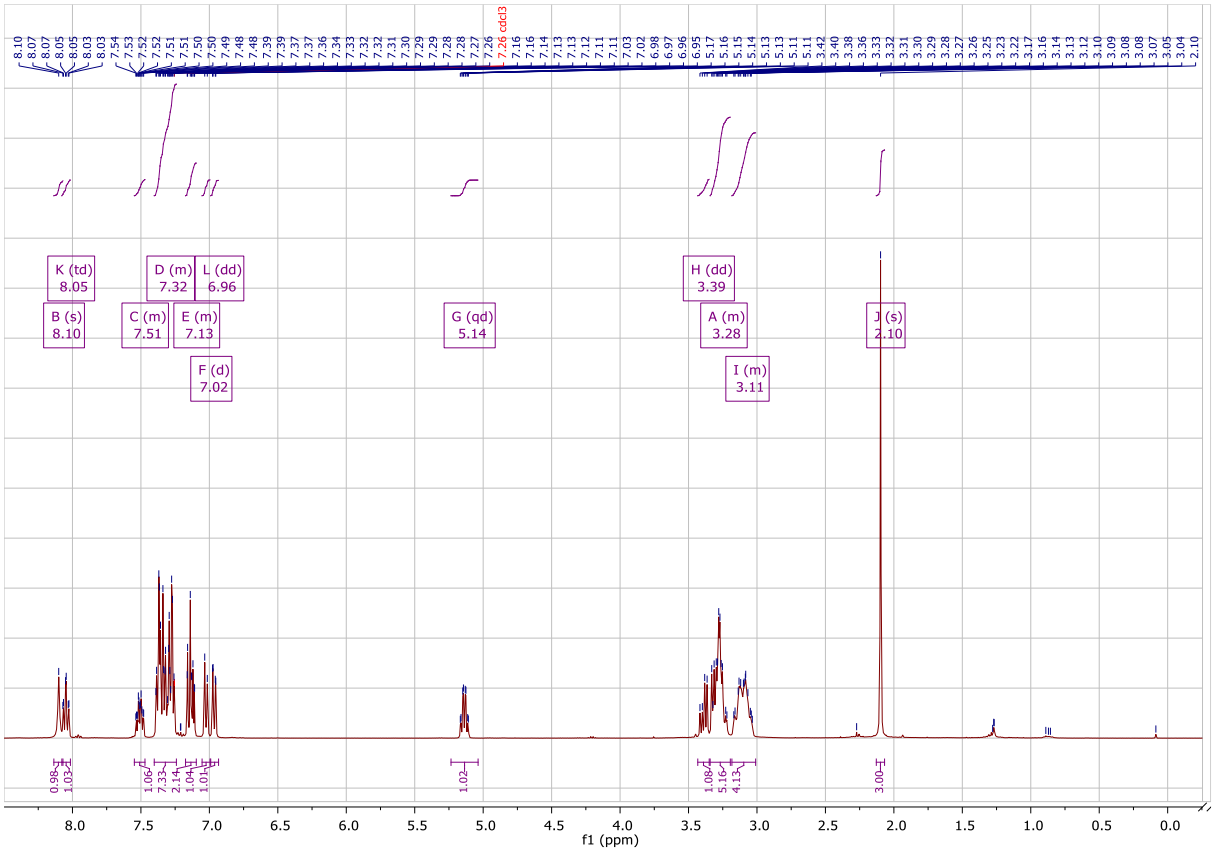
Spectrum 26 *^{1}H-NMR* ***15***



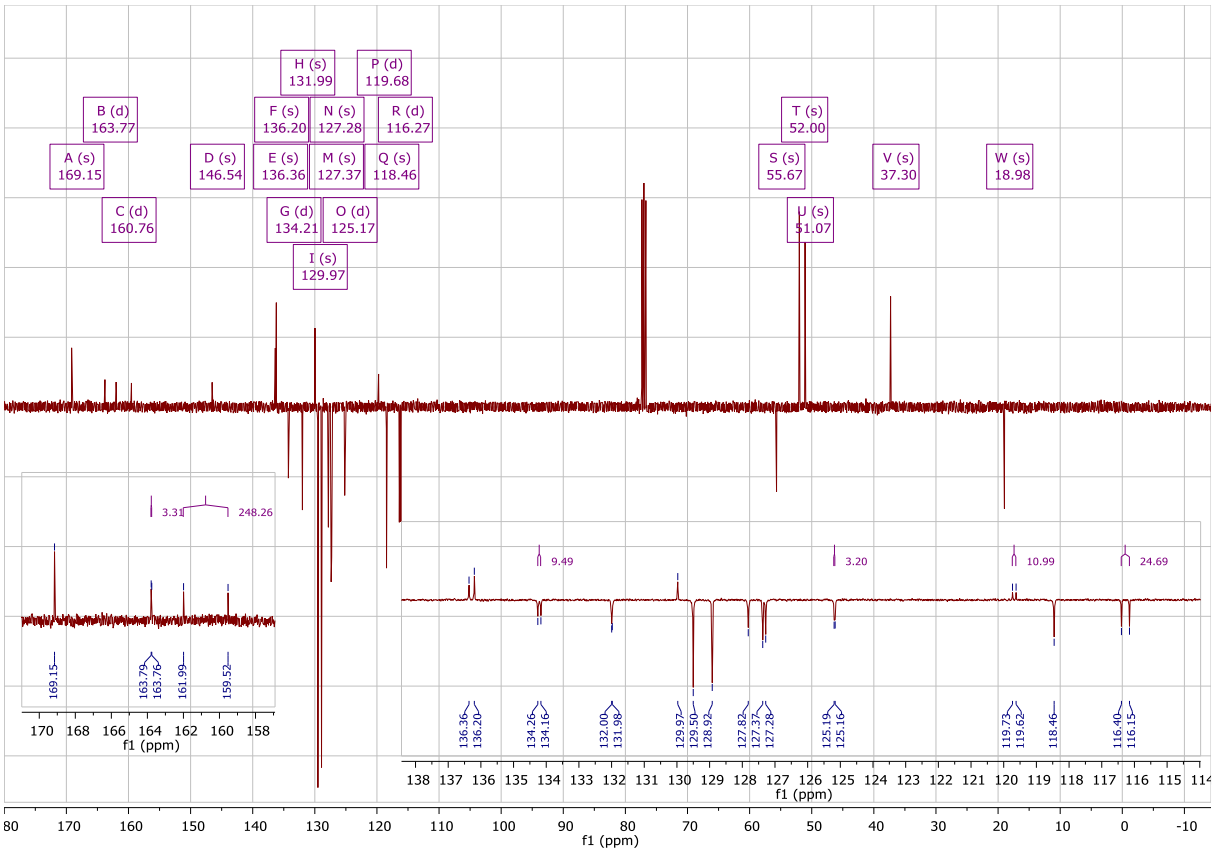
Spectrum 27 *^{1}H-NMR* ***16***



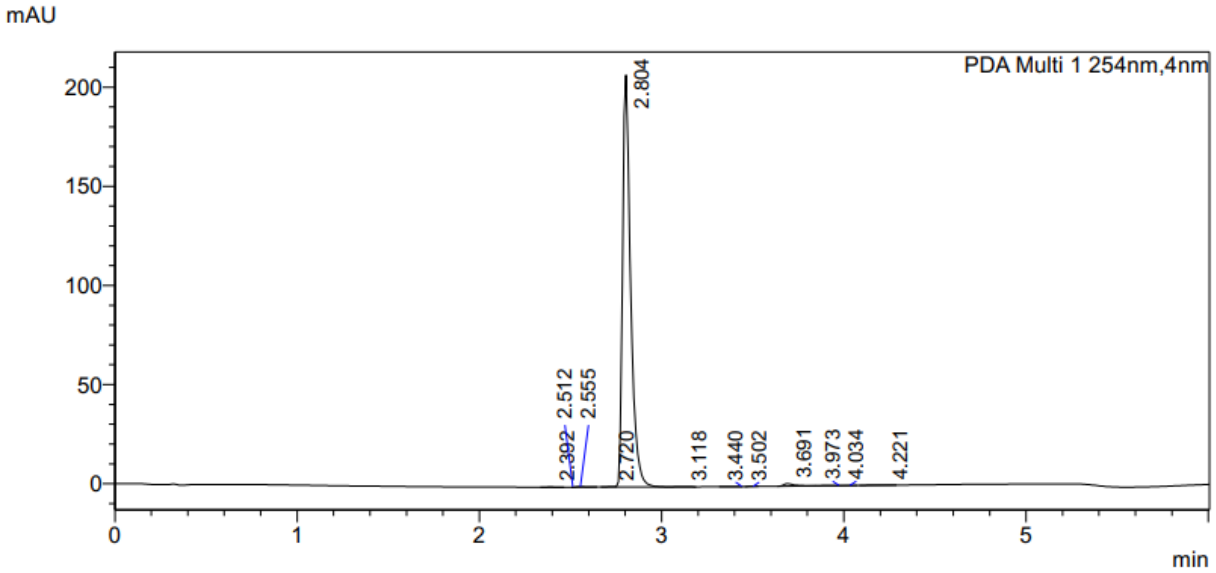
Spectrum 28 *^{1}H-NMR* ***18***



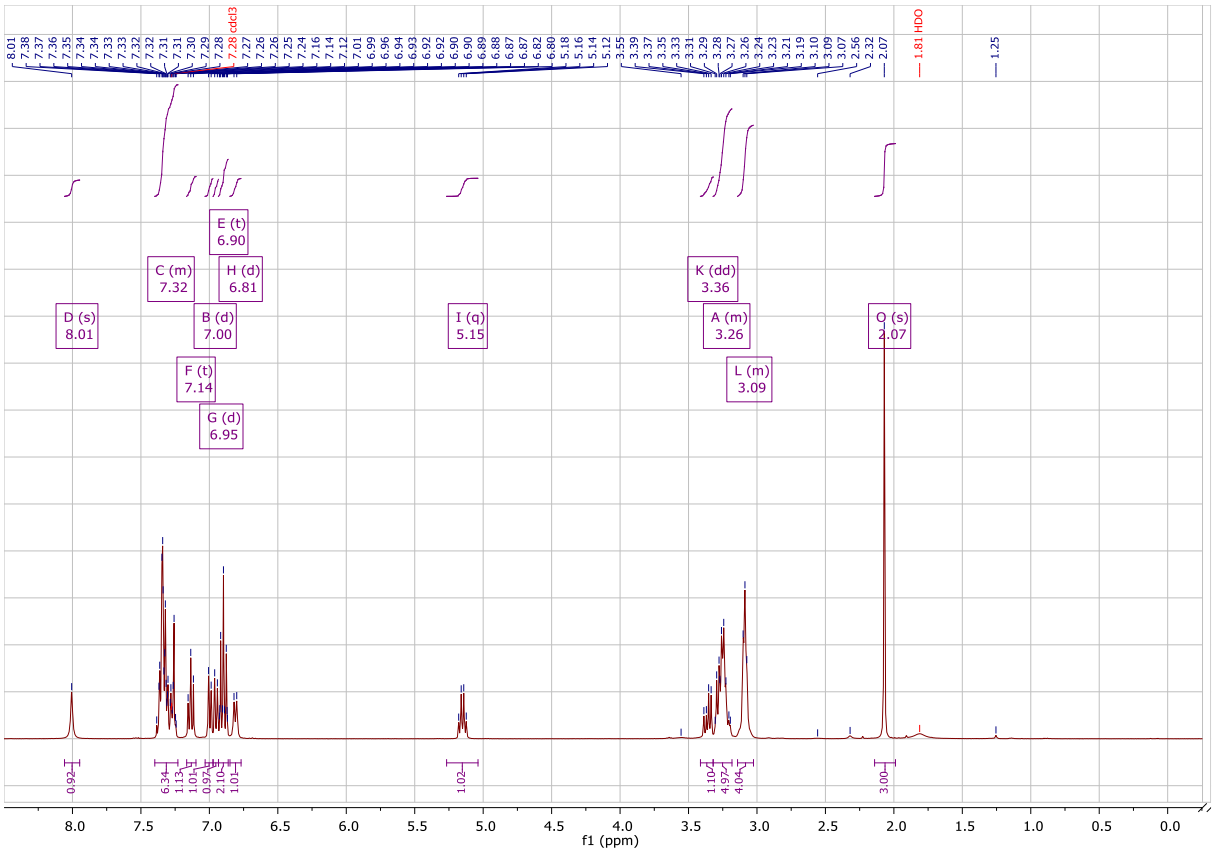
Spectrum 29 *^{1}H-NMR* ***19***



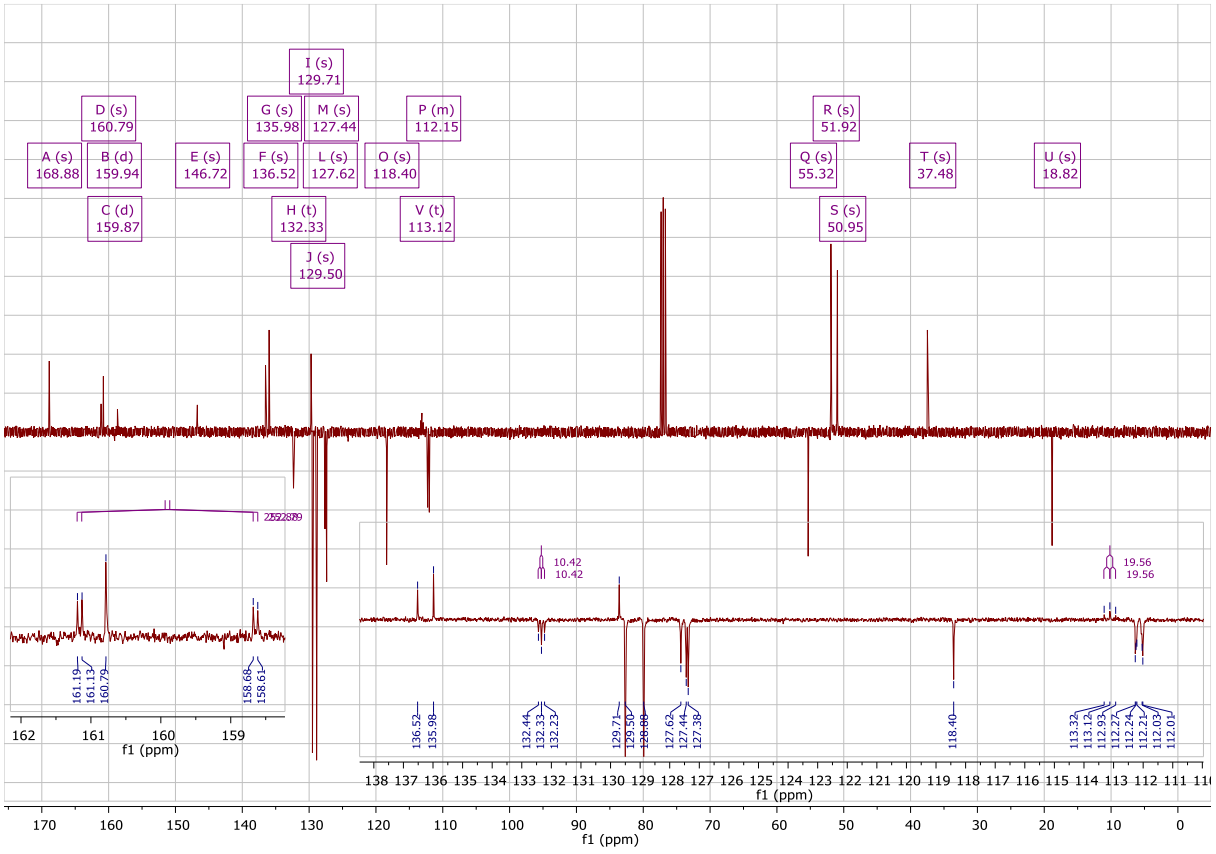
Spectrum 30 *APT ^{13}C-NMR* ***19***



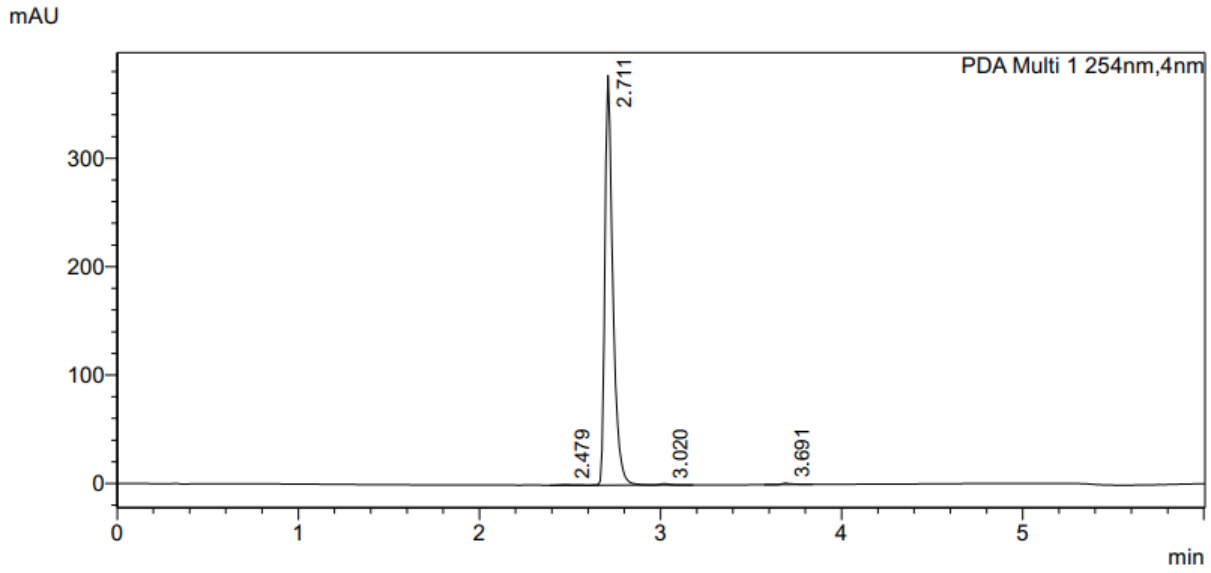
HPLC-Trace 12 *HPLC-purity* ***19***



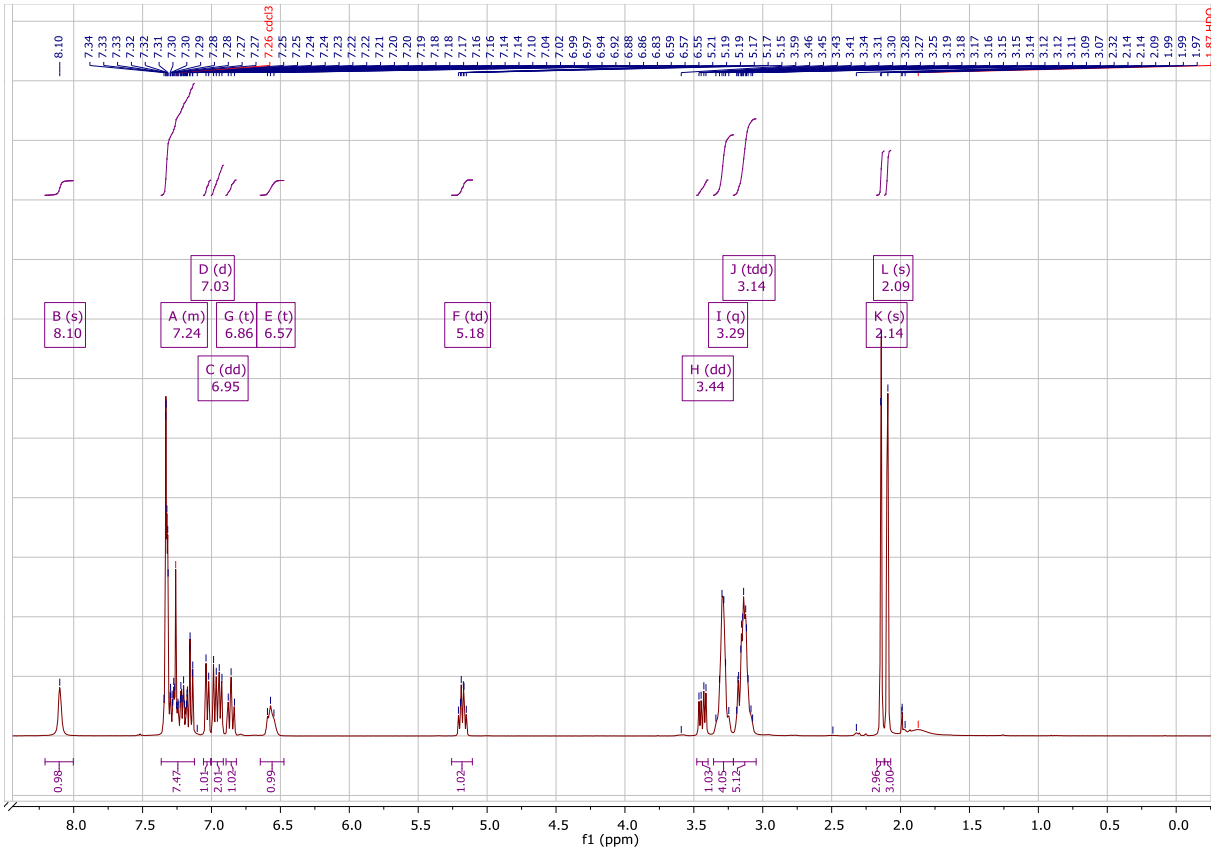
Spectrum 31 *^{1}H-NMR* ***20***



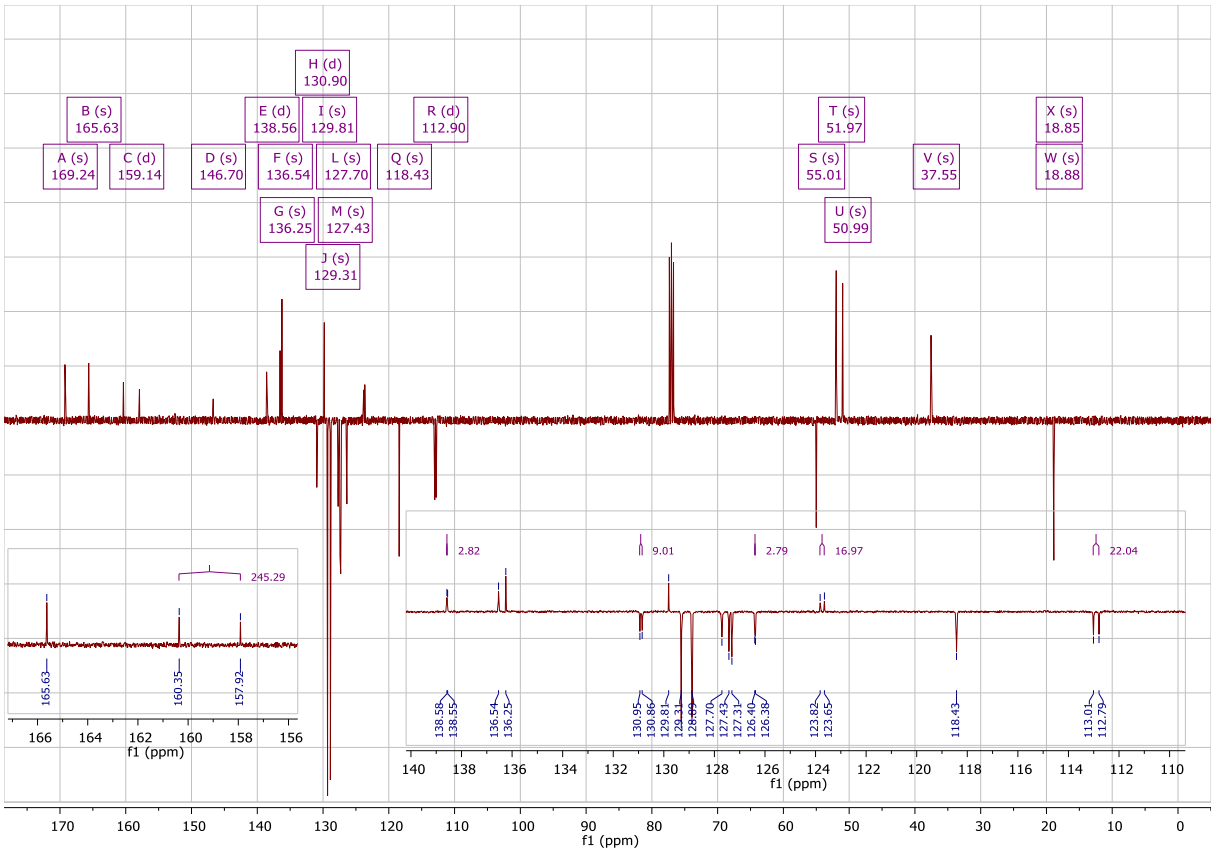
Spectrum 32 *APT ^{13}C-NMR* ***20***



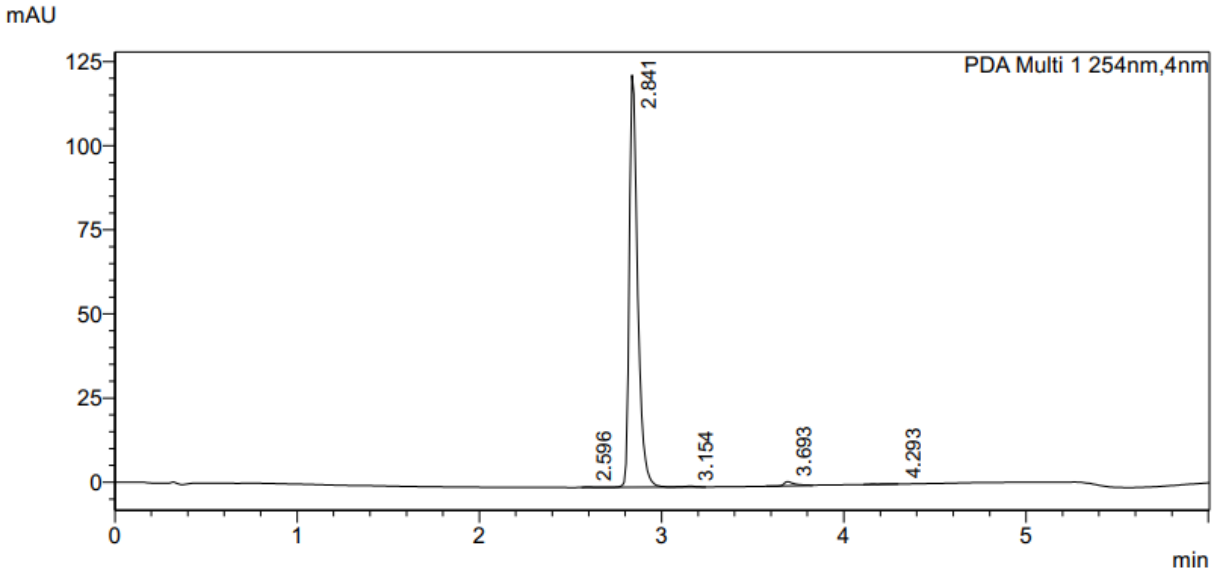
HPLC-Trace 13 *HPLC-purity* ***20***



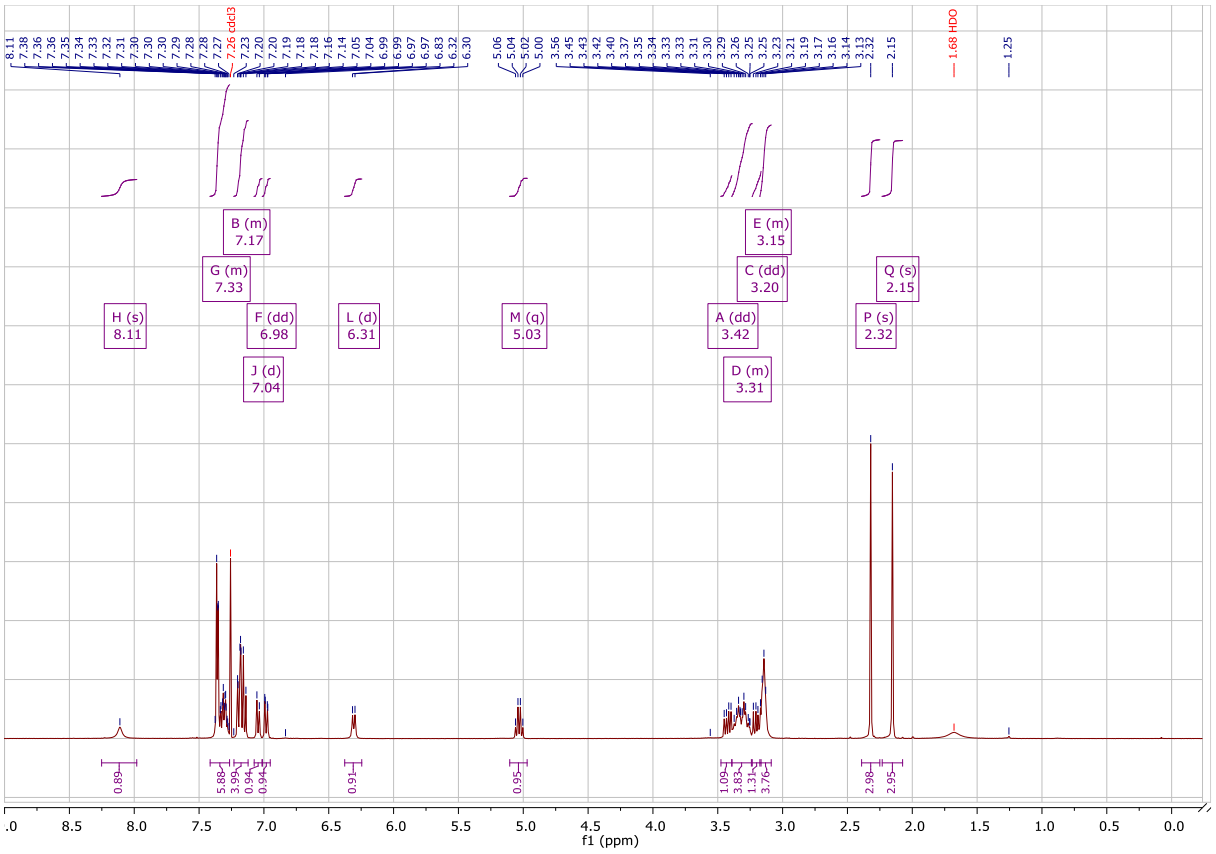
Spectrum 33 *^{1}H-NMR* ***21***



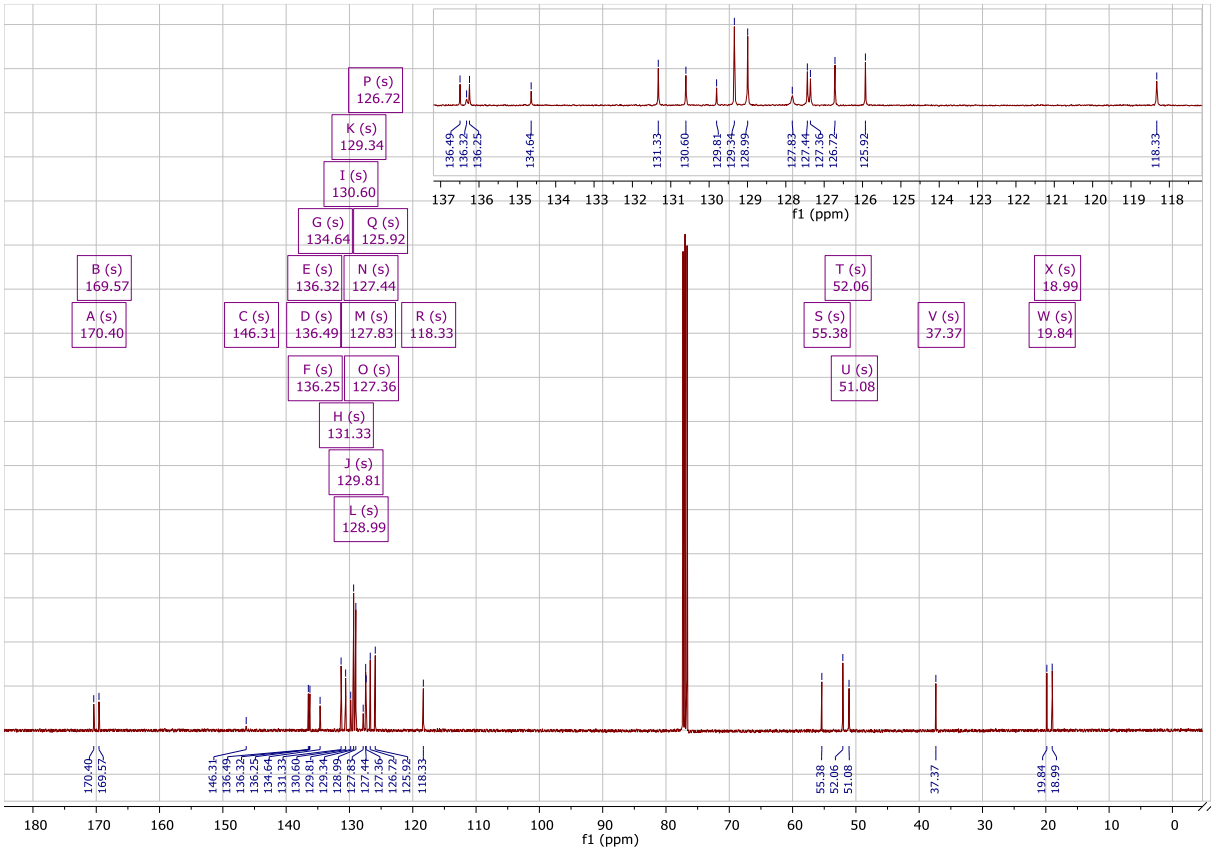
Spectrum 34 *APT* ^{13}*C-NMR* ***21***



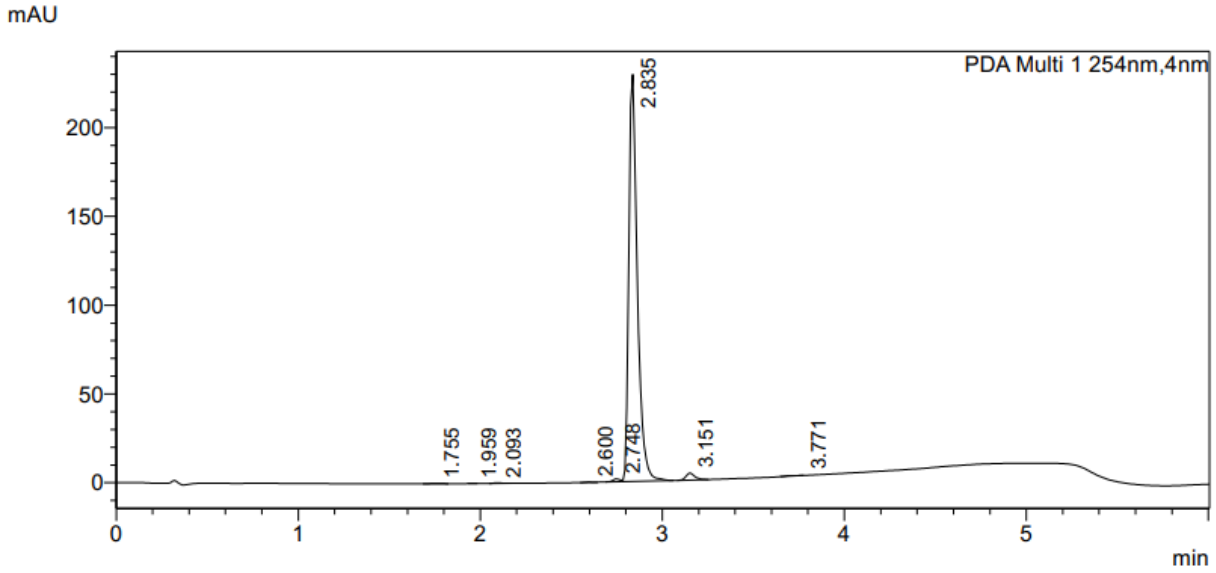
HPLC-Trace 14 *HPLC-purity* ***21***



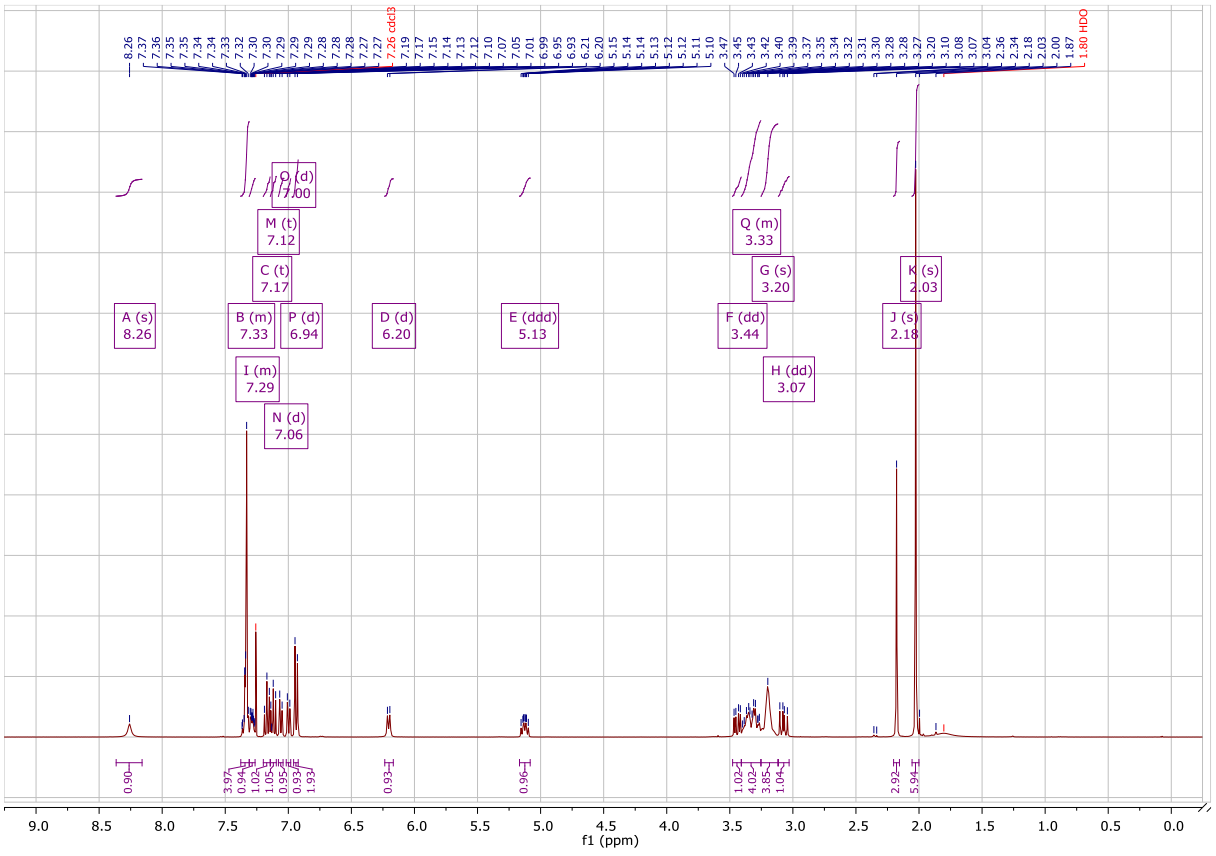
Spectrum 35 *[1]H-NMR* ***22***



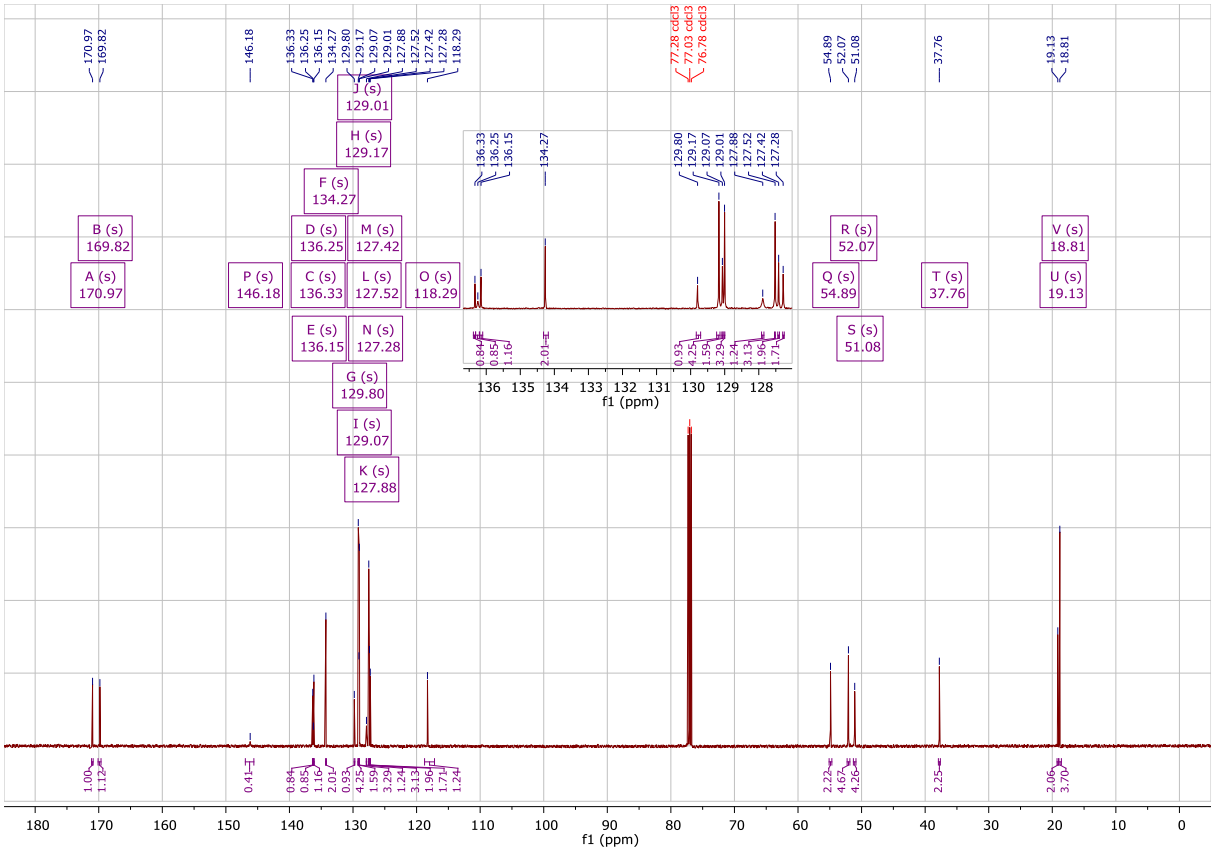
Spectrum 36 *[13]C-NMR* ***22***



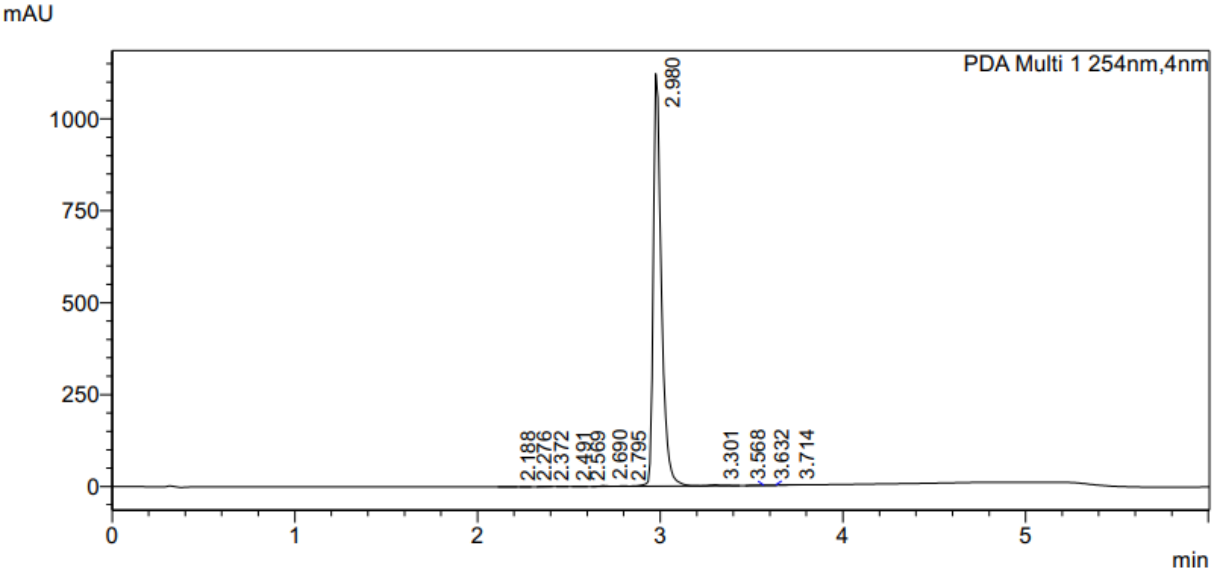
HPLC-Trace 15 *HPLC-purity* ***22***



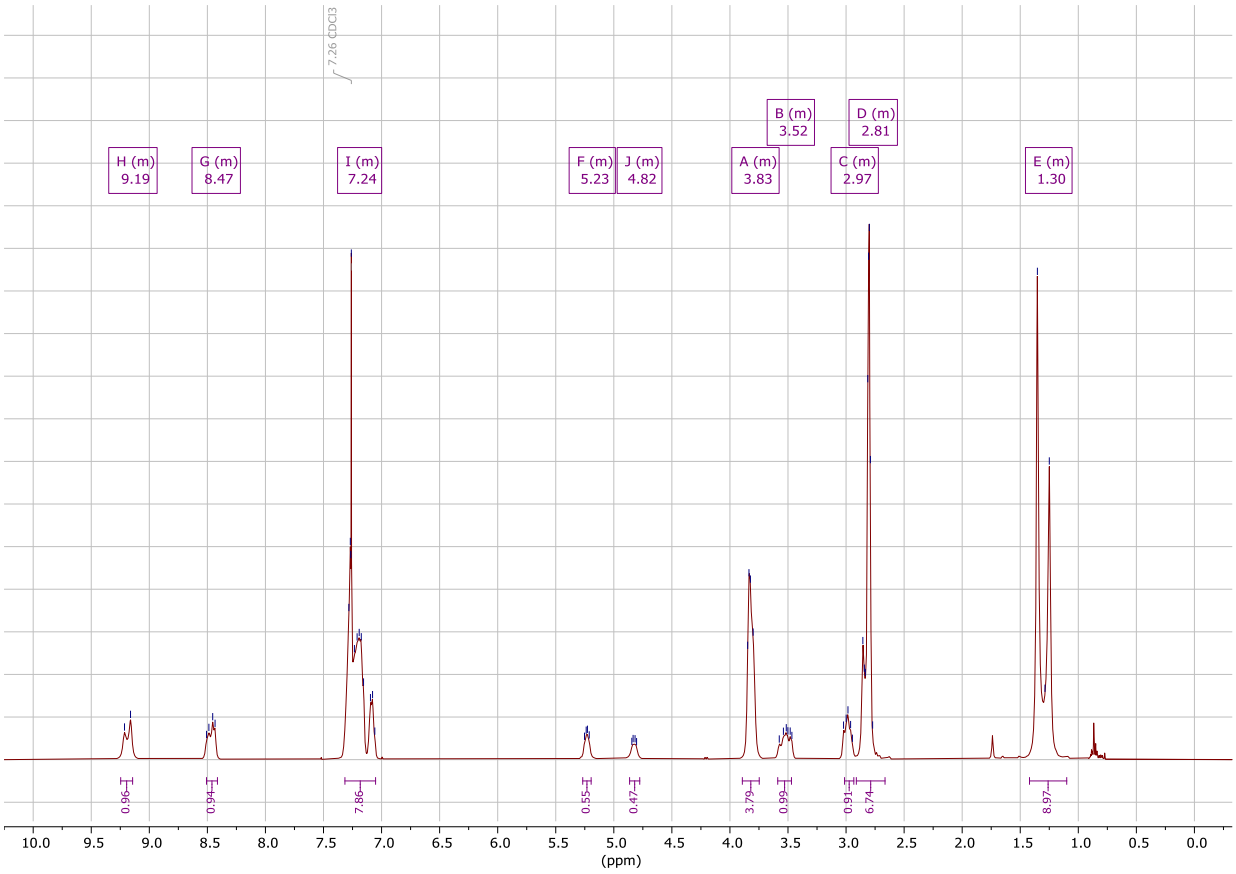
Spectrum 37 *^{1}H-NMR* ***23***



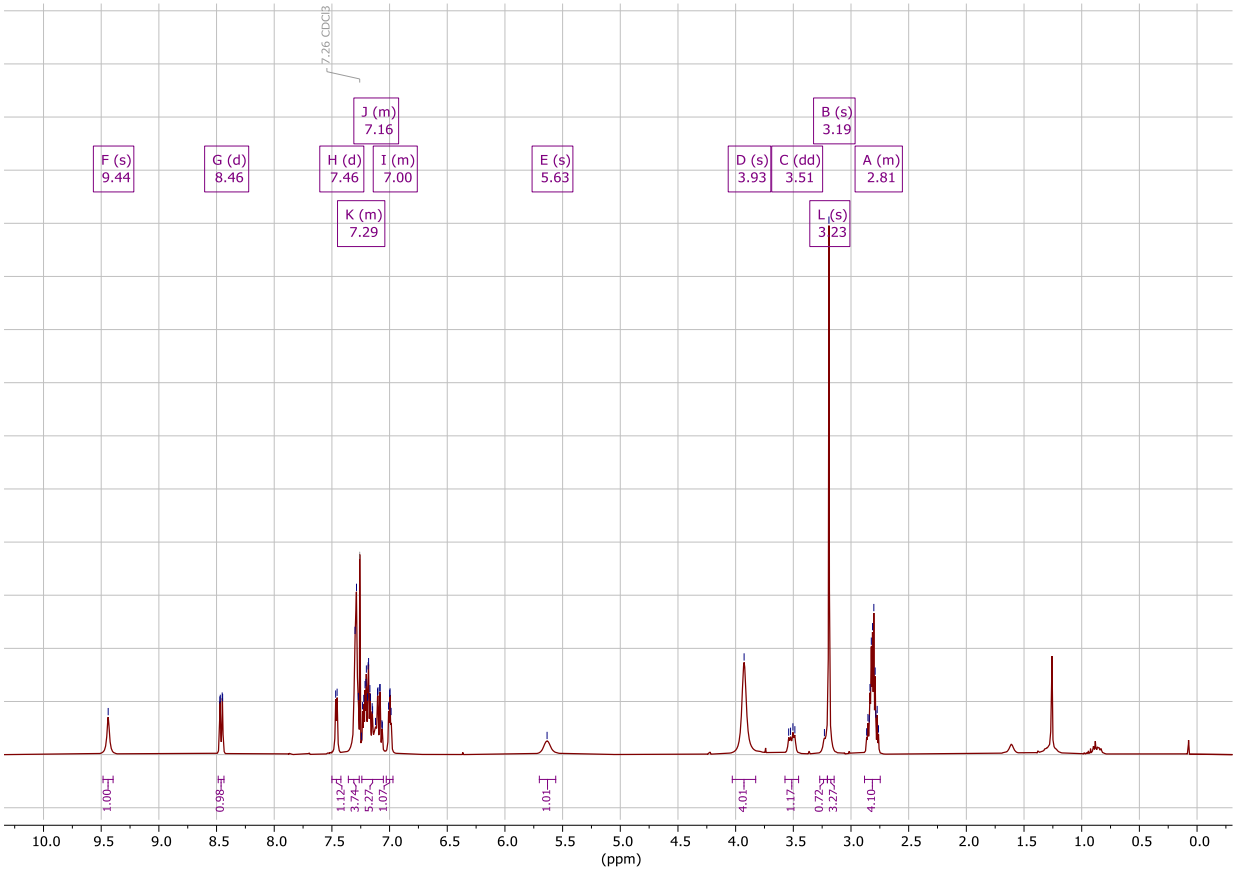
Spectrum 38 *^{13}C-NMR* ***23***



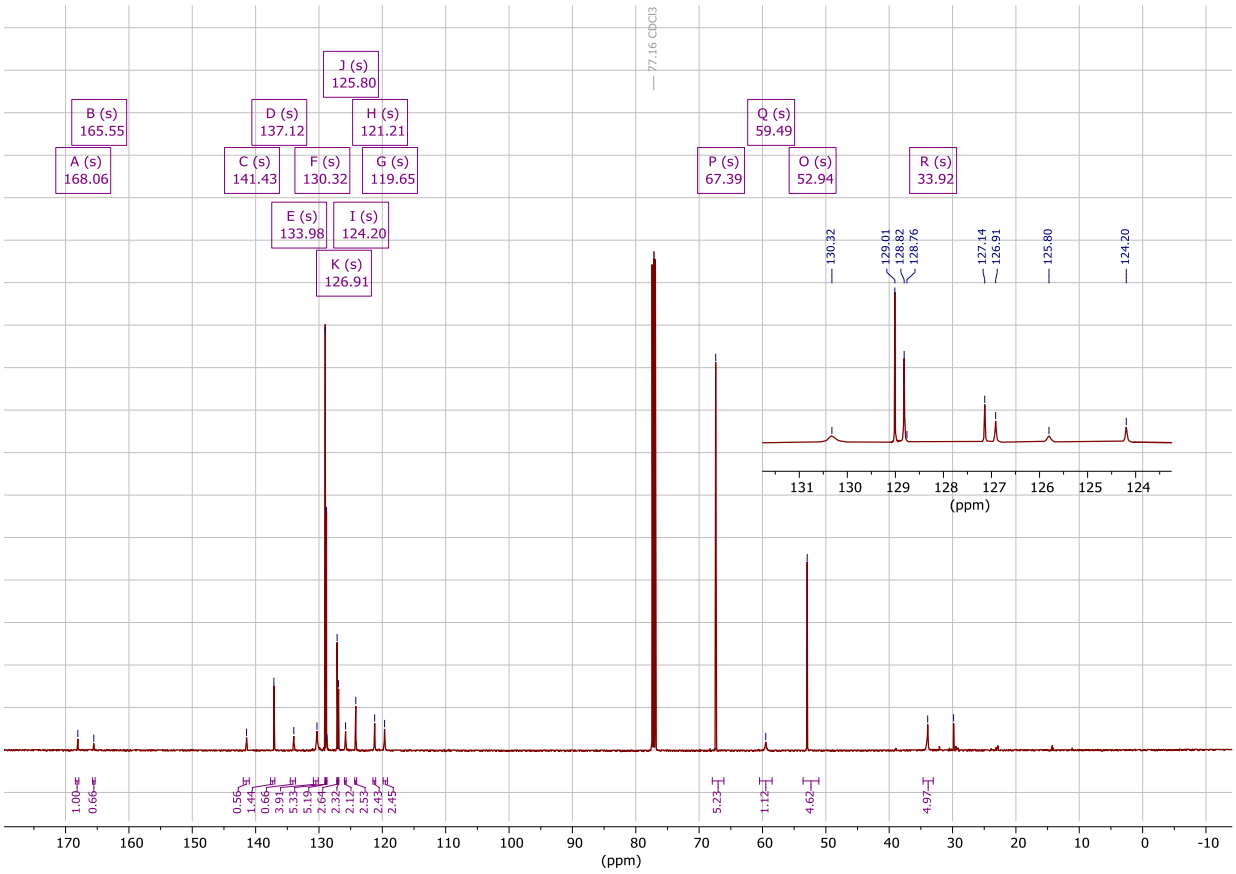
HPLC-Trace 16 *HPLC-purity* ***23***



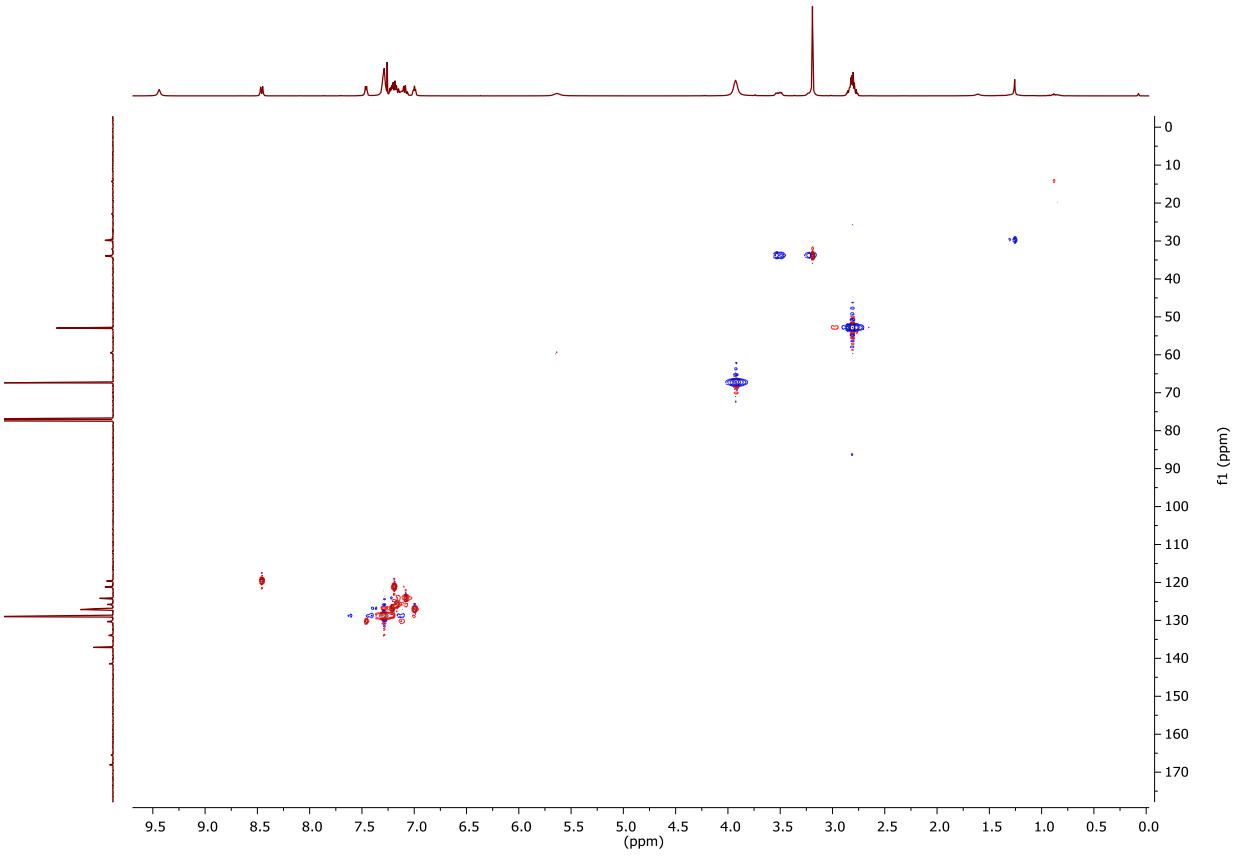
Spectrum 39 *^{1}H-NMR* ***24***



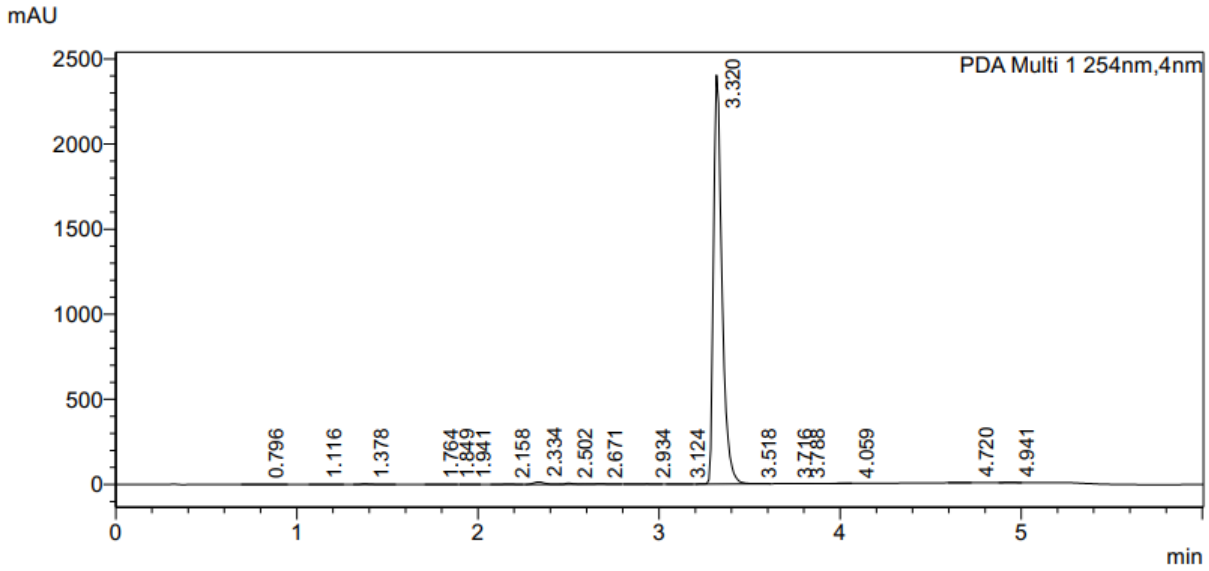
Spectrum 40 *^{1}H-NMR* ***25***



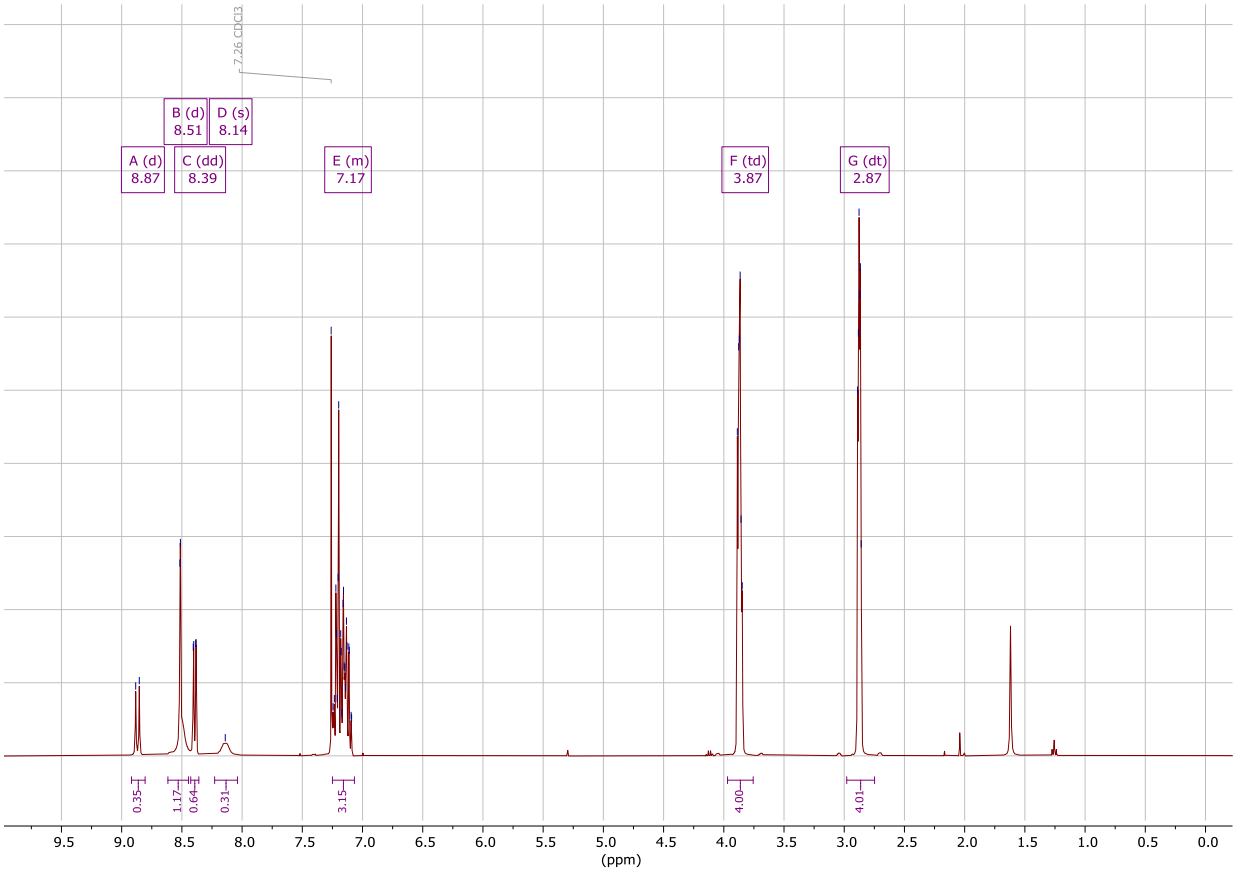
Spectrum 41 *^{13}C-NMR* ***25***



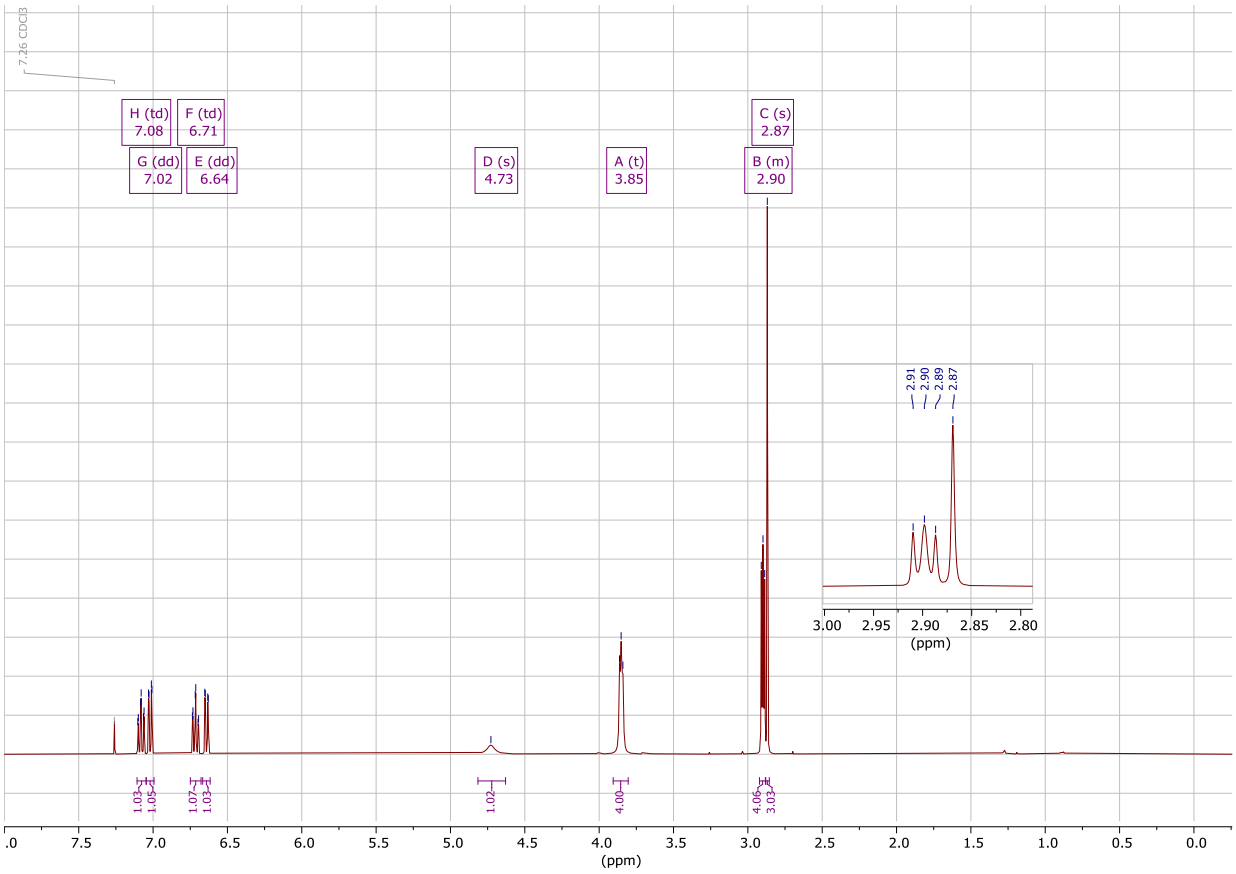
Spectrum 42 *HSQC* ***25***



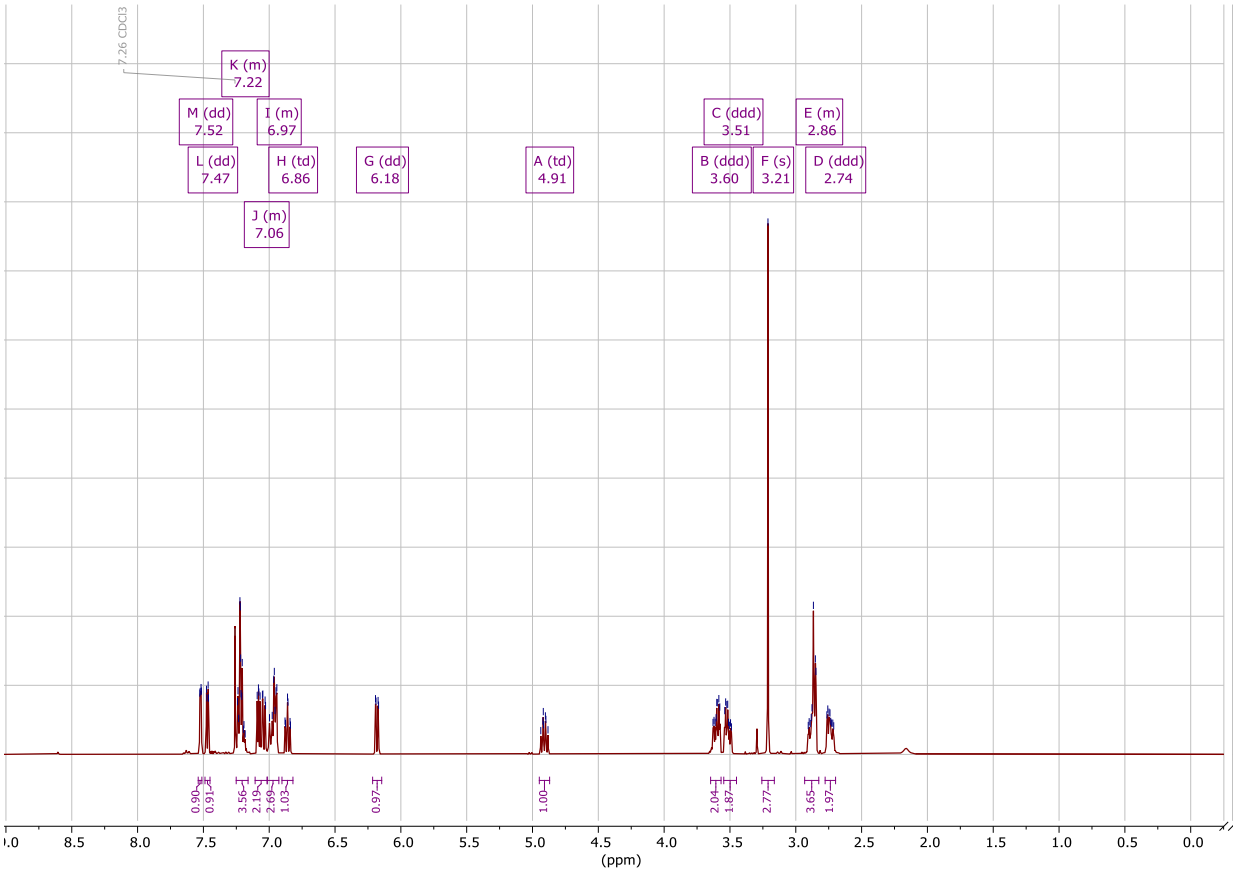
HPLC-Trace 17 *HPLC-purity* ***25***



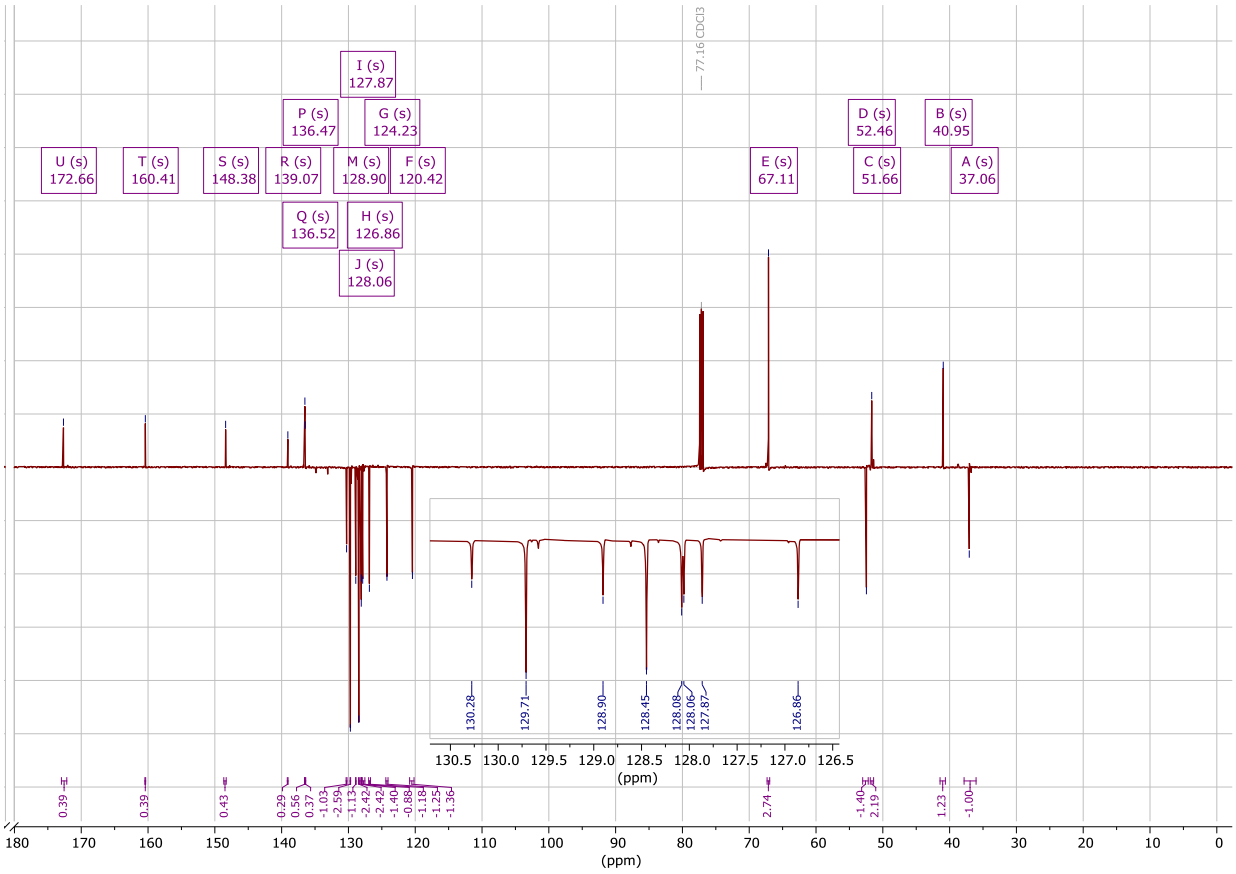
Spectrum 43 *^{1}H-NMR* ***26***



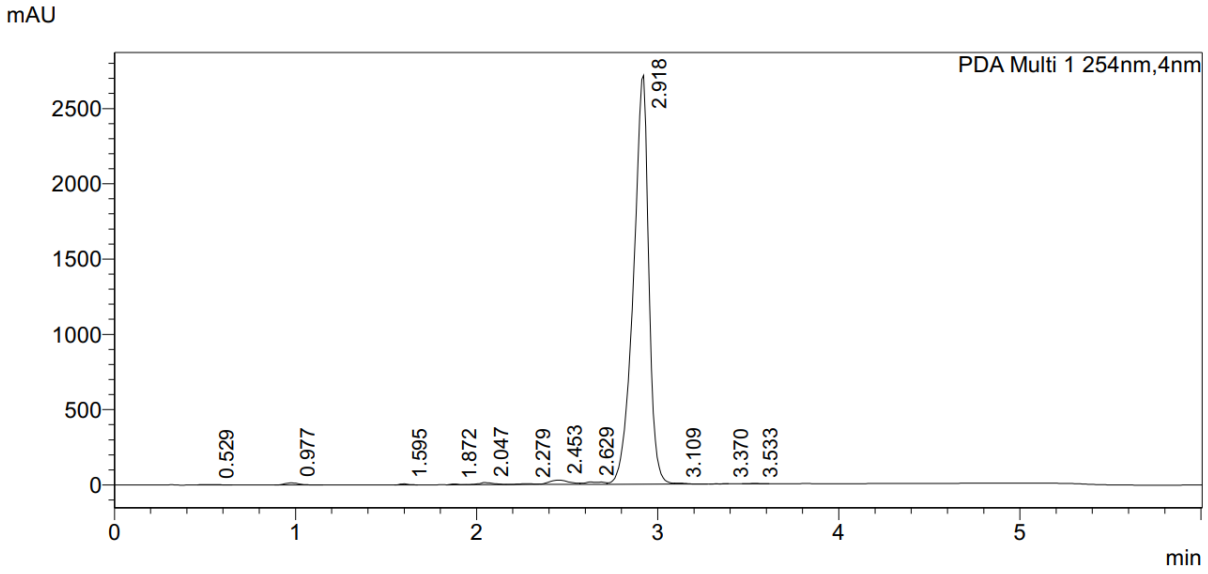
Spectrum 44 *^{1}H-NMR* ***27***



Spectrum 45 *^{1}H-NMR* ***29***



Spectrum 46 *APT ^{13}C-NMR* ***29***



HPLC-Trace 18 *HPLC-purity* ***29***