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Synthesis of a pteroic-based conjugate with ibuprofen moiety and macrocyclic chelator DOTA-GA

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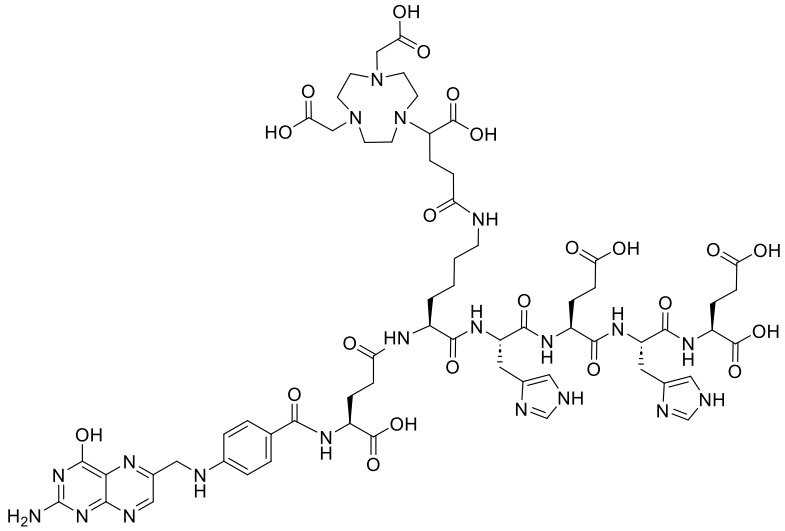
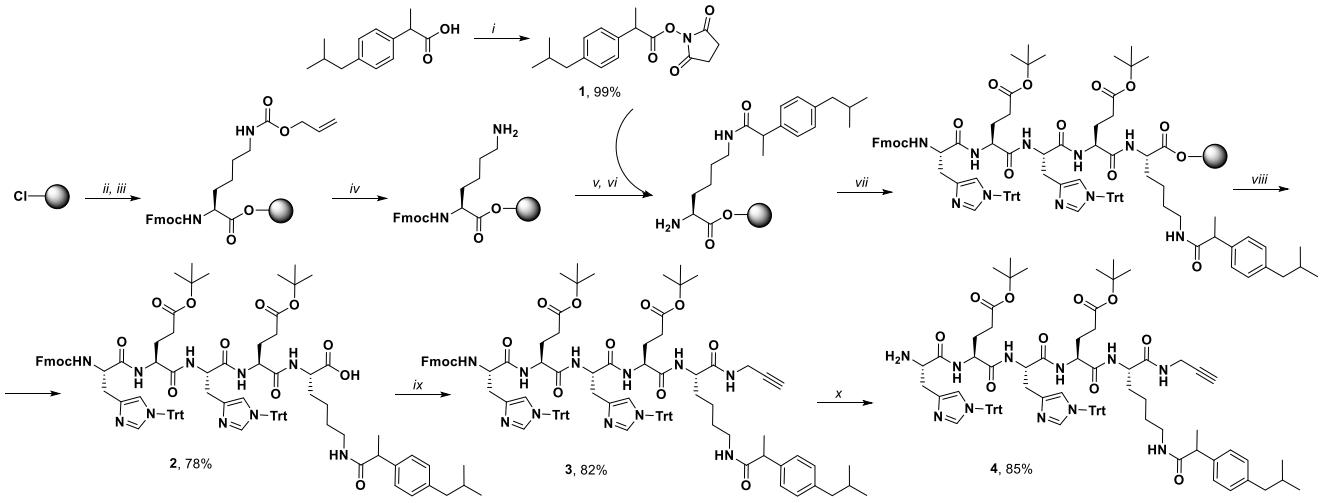
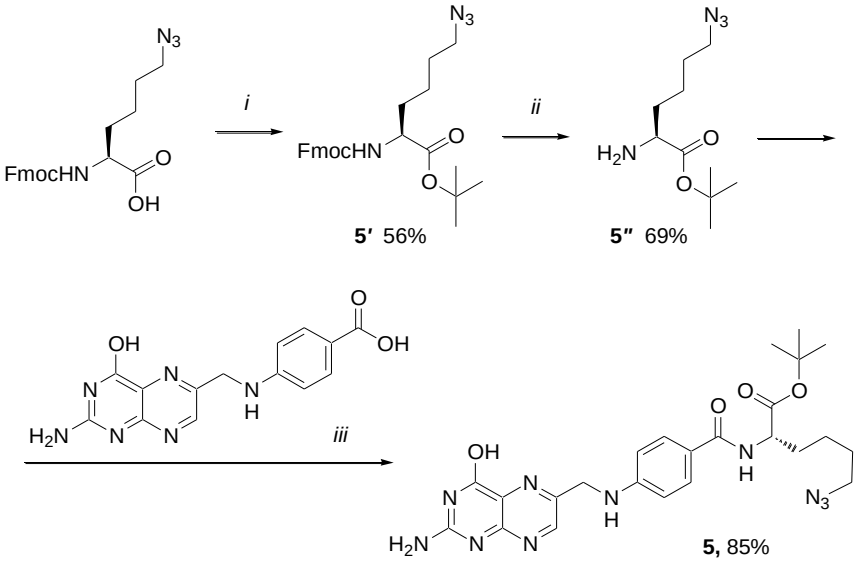


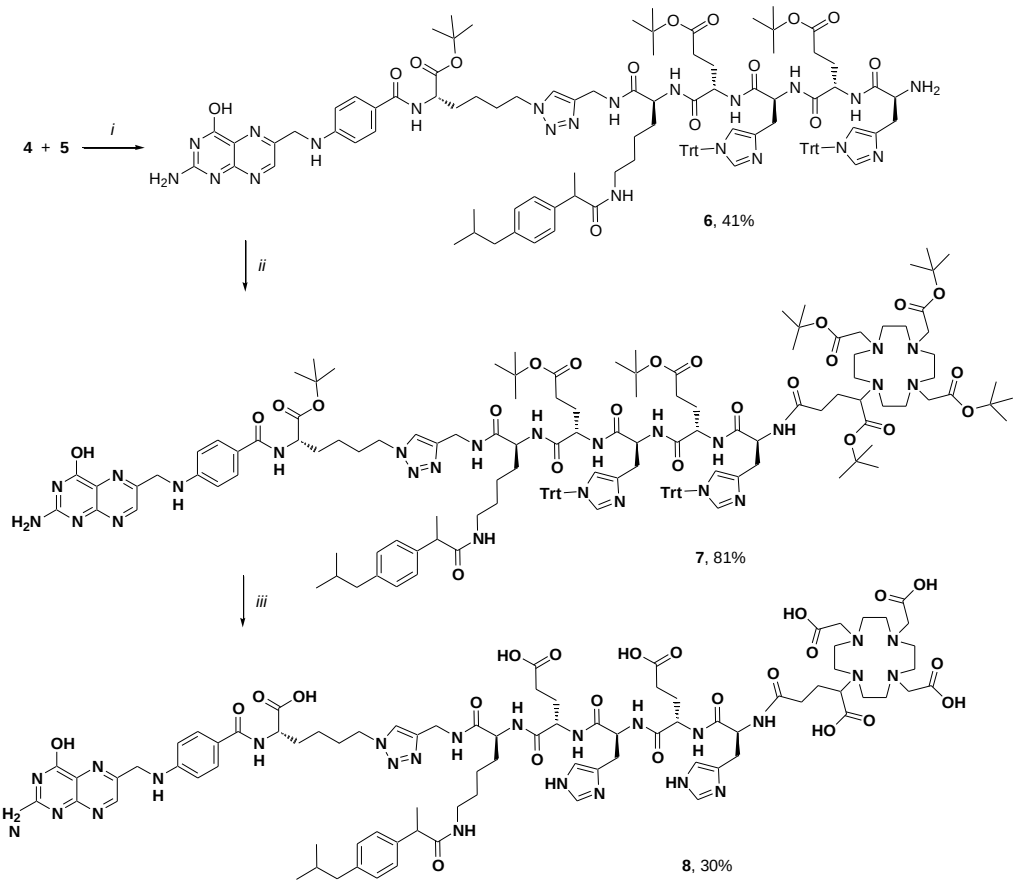
Scheme S1 Structure of previously obtained and studied conjugate of folic acid with peptide continuity $(HE)_2$.



Scheme S2 *Reagents and conditions*: i, NHS, EDC·HCl, DCM; ii, $SOCl_2$ (3 equiv), DMF (0.2 equiv), DCM; iii, Fmoc-Lys(Alloc)-OH(2 eq), DIPEA(10 eq), DMF, 2 h; iv, $Pd(PPh_3)_4$ (0.2 eq), $PhSiH_3$ (12 eq), CH_2Cl_2, 1h, Ar; v, DIPEA (1.25 eq), CH_2Cl_2; vi, 20% 4-MePip, DMF; vii, multistep peptide synthesis; viii, 0.75% TFA, DCM; ix, propargylamine, DIPEA, DMF; x, Et_2NH, MeCN.



Scheme S3 *Reagents and conditions*: i, $(Boc)_2O$, DMAP, Bu^tOH; ii, Et_2NH, MeCN; iii, HBTU (hexafluorophosphate benzotriazole tetramethyl uronium), HOBt (1-hydroxy-benzotriazole), DIPEA (*N*,*N*-diisopropylethylamine), DMF.



Scheme S4 *Reagents and conditions*: i, $CuSO_4 \cdot 5H_2O$, AscNa, DMF; ii, DOTA-GA(OBu^t)$_4$, HOBt, HBTU, DIPEA, DMF; iii, TFA, TIPS, H_2O.

Acid-labile groups deprotection

When removing the protective groups at the last stage of the synthesis (Scheme S3), LCMS results revealed that in addition to the target compound, the mixture contained mono-, di-, and tri-tert-butylated derivatives of compound **8**, which, due to the insignificant difference in molecular weight, could not be separated by column chromatography. This led us to select the conditions presented in **Table S1**.

Table S1 Optimization of conditions for obtaining the target conjugate **8**.

Entry	Conditions	Amount of conjugate **8** by LCMS, %
1	TFA (90%), TIPS (5%), H_2O (5%), 2 h, rt	48
2	TFA (90%), TIPS (5%), H_2O (5%), 5 h, rt	65
3	CH_2Cl_2 (40%), TFA (50%), TIPS (5%), H_2O (5%), 5 h, rt	55
4	10 H HCl/H_2O, dioxane, 18 h, rt	0

Based on the results of the optimization of the conditions, we selected *method 2*. After conducting preparative chromatography, we were able to obtain the target compound **8** with a yield of 30%.

1. Synthesis

1.1. General information

All used solvents were purified according to procedures described in [S1]. All starting compounds are commercially available reagents (SigmaAldrich, Fluka®Analytical, abcr, Carbosynth) and were used without further purification.

Characterization

^{1}H and ^{13}C NMR spectra were registered on Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 101 MHz for ^{13}C) in $CDCl_3$ or DMSO-d_6. Preparative column chromatography was performed on INTERCHIM puriFlash 430. For purification and analysis of samples we used Shimadzu Prominence LC-20 system with Phenomenex Luna 3µm C18 100A (150 x 4.6 mm) column in column oven at 40ºC and fraction collector coupled to single quadrupole mass-

spectrometer Shimadzu LCMS-2020 with dual DUIS-ESI-APCI ionization source. Mobile phases: A - 0.1% formic acid in water, B - 10 mM ammonium formate in water, D - acetonitrile. LC parameters for analyses were: gradient flow of 1 ml/min (0-0.5 min - 5% D, 0.5 -10.5 min - 5% to 100% D, 10.5-12 min - 100% D, 12-14.5 min - 100% to 5% D) with optional UV detection for some compounds. MS parameters: drying gas 15.0 L/min, nebulizing gas 1.5 L/min, desolvation line temperature 250ºC, heat block temperature 400ºC, interface voltage -3.5 kV, corona needle voltage -3.5 kV. Positive (mass range 250-2000 Da, in some cases 155-2000 Da) and negative ions (mass range 100-2000 Da) were registered simultaneously. For purification we used identical LC parameters except gradient which was tailored for each compound (in some cases we used mobile phase B instead of A). Fractionation was based on UV detection only; fractions were collected based on UV signal level and slope. High resolution mass spectra were registered on Orbitrap Elite mass spectrometer (Thermo Scientific) with ESI ionization source. Compounds with concentration of 0.1-10 μg/ml (in 1% formic acid in acetonitrile) were directly infused into the ion source with syringe pump (5 μl/min). We didn't use auxiliary and sheath gases, spray voltage was +-3.5 kV, capillary temperature was set to 275ºC. MS spectra were registered by Orbitrap analyzer with 480000 resolution (1 microscan, AGC target value of 1×106, max inject time 1000 ms, averaged on 10 spectra, MS range 100-2000 Da, in some cases 200-4000 Da). We used DMSO and di-iso-octyl phthalate as internal calibration signals (m/z 157.03515 and 413.26623) in positive mode and dodecylsulfate (m/z 265.14790) in negative mode. HPLC MS study of conjugate 10 was performed using an ONYX MONOLITHIC, C18 50x3mm column; 1.8ml/min; Columns Reg Valve. Gradient: From 100% Phase A to 100% Phase B in 2 min followed by 100% Phase B in 0.6 min. Phase A: 0.1% TFA, 2.5% acetonitrile in water. Phase B: 0.1% TFA in acetonitrile [S2].

The synthesis of ibuprofen NHS ester **1** was carried out from racemic ibuprofen according to procedures presented in the literature [S3].[3]

Synthesis of (5*S*,8*S*,11*S*,14*S*,17*S*)-8,14-bis(3-(*tert*-butoxy)-3-oxopropyl)-1-(9*H*-fluoren-9-yl)-17-(4-(2-(4-isobutylphenyl)propanamido)butyl)-3,6,9,12,15-pentaoxo-5,11-bis((1-trityl-1*H*-imidazol-4-yl)methyl)-2-oxa-4,7,10,13,16-pentaazaoctadecan-18-oic acid (2) (see Scheme S2)

1) Activation of 2-chlorotrityl chloride resin (2-CTC): resin (410 mg, 1 equiv., capacity 1.0-1.5 mmol/g) was stirred in DCM (10 ml) for 10 min. Then $SOCl_2$ (131 μl, 3 eq.) was added dropwise, followed by DMF (7 μl, 5% v/v, relative to $SOCl_2$). The resulting mixture was stirred

at 40 °C for 4 h. Next, the resin was filtered off, transferred to a polypropylene reactor and washed with DMF (7 ml, 3 times for 1 min) and DCM (7 ml, 3 times for 1 min).

2) Immobilization of Fmoc-Lys-(*L*)-(NHAlloc)-OH on 2-CTC resin: Fmoc-Lys-(*L*)-(NHAlloc)-OH (545 mg, 2 eq.) and DIPEA (1049 µl, 10 eq.) were added to a mixture of activated 2-CTC resin in DMF (7 ml). The resulting mixture was stirred for 2 h, after which the resin was filtered and successively washed with methanol (7 ml, 3 times for 5 min), DCM (7 ml, 3 times for 1 min), DMF (7 ml, 3 times for 1 min) and DCM (7 ml, 3 times for 1 min).

3) Removal of the Alloc-protective group: Fmoc-Lys-(*L*)-(NHAlloc)-OH on 2-CTC resin was washed with DCM (7 ml, 1 times for 1 min), then was added DCM (10 ml), $PhSiH_3$ (902 µl, 12 eq.) and stirred for 2 minutes. $Pd(PPh_3)_4$ (139 mg, 0,2 eq.) in DCM (5 ml) was added to the resulting mixture and the mixture was srirred for 30 min under atmosphere Ar. The resin was filtered, washed with DCM (7 ml, 3 times for 1 min), DMF (7 ml, 3 times for 1 min), DCM (7 ml, 3 times for 1 min) and the process was repeated once. Then the resin was filtered off, washed with DMF (7 ml, 3 times for 1 min) and DCM (7 ml, 3 times for 1 min).

4) Acylation reaction of ibuprofen NHS-ester (1): to the amino acid immobilized on the resin was added DCM (15 ml). NHS-ester of ibuprofen (228 mg, 1,25 eq.) and DIPEA (131 µl, 1,25 eq.) were added to the resulting mixture and the mixture was stirred overnight. The resin was then filtered off and washed with DMF (7 ml, 3 times for 1 min) and DCM (7 ml, 3 times for 1 min).

5) Removal of the Fmoc-protective group: Fmoc-protected amino acid immobilized on 2-CTC resin was washed with DMF (7 ml, 2 times for 1 min), then a solution of 4-methylpiperidine in DMF (7 ml, 20% v/v) was added and stirred for 15 min. The resin was filtered, washed with DMF (7 ml, 3 times for 1 min), a solution of 4-methylpiperidine in DMF (7 ml, 20% v/v) was added and stirred for 15 min. Then the resin was filtered off, washed with DMF (7 ml, 3 times for 1 min) and dichloromethane (7 ml, 3 times for 1 min).

6) Immobilization of Fmoc-Glu(OBu^t)-OH on 2-CTC resin: to the amino acid immobilized on the resin (1 eq.) was added DMF (7 ml). Fmoc-Glu(OBu^t)-OH (513 mg, 2 eq.), HOBt (46 mg, 0.5 eq.), HBTU (457 mg, 2 eq.) and DIPEA (210 µl , 3 eq.) were added to the resulting mixture and the mixture was stirred for 2 h. The resin was then filtered off and washed with DMF (7 ml, 3 times for 1 min) and dichloromethane (7 ml, 3 times for 1 min).

7) Removal of the Fmoc-protective group: Fmoc-protected amino acid immobilized on 2-CTC resin was washed with DMF (7 ml, 2 times for 1 min), then a solution of 4-methylpiperidine in DMF (7 ml, 20% v/v) was added and stirred for 15 min. The resin was filtered, washed with DMF (7 ml, 3 times for 1 min), a solution of 4-methylpiperidine in DMF (7 ml, 20% v/v) was

added and stirred for 15 min. Then the resin was filtered off, washed with DMF (7 ml, 3 times for 1 min) and dichloromethane (7 ml, 3 times for 1 min).

8) Immobilization of Fmoc-His(Trt)-OH on 2-CTC resin: to the amino acid immobilized on the resin (1 eq.) was added DMF (7 ml). Fmoc-His(Trt)-OH (747 mg, 2 eq.), HOBt (46 mg, 0.5 eq.), HBTU (457 mg, 2 eq.) and DIPEA (210 μl , 3 eq.) were added to the resulting mixture and the mixture was stirred for 2 h. The resin was then filtered off and washed with DMF (7 ml, 3 times for 1 min) and dichloromethane (7 ml, 3 times for 1 min).

9) Removal of the Fmoc-protective group: Fmoc-protected amino acid immobilized on 2-CTC resin was washed with DMF (7 ml, 2 times for 1 min), then a solution of 4-methylpiperidine in DMF (7 ml, 20% v/v) was added and stirred for 15 min. The resin was filtered, washed with DMF (7 ml, 3 times for 1 min), a solution of 4-methylpiperidine in DMF (7 ml, 20% v/v) was added and stirred for 15 min. Then the resin was filtered off, washed with DMF (7 ml, 3 times for 1 min) and dichloromethane (7 ml, 3 times for 1 min).

10) Immobilization of Fmoc-Glu(OtBu)-OH on 2-CTC resin: to the amino acid immobilized on the resin (1 eq.) was added DMF (10 ml). Fmoc-Glu(OBu^t)-OH (513 mg, 2 eq.), HOBt (46 mg, 0.5 eq.), HBTU (457 mg, 2 eq.) and DIPEA (210 μl , 3 eq.) were added to the resulting mixture and the mixture was stirred for 2 h. The resin was then filtered off and washed with DMF (10 ml, 3 times for 1 min) and dichloromethane (10 ml, 3 times for 1 min).

11) Removal of the Fmoc-protective group: Fmoc-protected amino acid immobilized on 2-CTC resin was washed with DMF (10 ml, 2 times for 1 min), then a solution of 4-methylpiperidine in DMF (10 ml, 20% v/v) was added and stirred for 15 min. The resin was filtered, washed with DMF (10 ml, 3 times for 1 min), a solution of 4-methylpiperidine in DMF (10 ml, 20% v/v) was added and stirred for 15 min. Then the resin was filtered off, washed with DMF (7 ml, 3 times for 1 min) and dichloromethane (10 ml, 3 times for 1 min).

12) Immobilization of Fmoc-His(Trt)-OH on 2-CTC resin: to the amino acid immobilized on the resin (1 eq.) was added DMF (10 ml). Fmoc-His(Trt)-OH (747 mg, 2 eq.), HOBt (46 mg, 0.5 eq.), HBTU (457 mg, 2 eq.) and DIPEA (210 μl , 3 eq.) were added to the resulting mixture and the mixture was stirred for 2 h. The resin was then filtered off and washed with DMF (10 ml, 3 times for 1 min) and dichloromethane (10 ml, 3 times for 1 min).

13) Removal of immobilized pentapeptide with ibuprofen fragment from the resin: 0.75% v/v solution of trifluoroacetic acid in dichloromethane (15 ml) was added to the pentapeptide on CTC-resin, stirred for 15 min, then filtered, the resin washed with DCM (3 times for 1 min, 15 ml) and the process was repeated twice. From the obtained filtrate the solvent was removed under reduced pressure. After purification by column chromatography (InterchimPuriflash 40 g, 15μ,

gradient from 0% methanol to 5% methanol in 20 min, then from 5% methanol to 10% in 5 min, then from 10% methanol to 15% in 4 min, then from 15% methanol to 30% in 5 min flow rate - 30 ml/min) 396 mg (78% yield) of compound **2** as a white powder was obtained.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.55 (s, 1H, COOH), 8.25 (m, 2H, Ar), 8.08 (d, J = 7.2 Hz, 1H, NH), 7.98 (d, J = 7.7 Hz, 1H, NH), 7.89 (d, J = 7.8 Hz, 3H, Ar+NH), 7.66 – 7.47 (m, 3H, Ar+NH), 7.44 – 7.21 (m, 21H, Ar+NH), 7.18 (d, J = 7.8 Hz, 2H, Ar), 7.14 – 6.95 (m, 15H, Ar), 6.92 (s, 1H, Ar), 4.53 (m, 1H, CH), 4.34 (m, 1H, CH), 4.31 – 4.06 (m, 5H, CH_2+3CH), 3.92 (m, 1H, CH), 3.50 (q, J = 6.6 Hz, 2H, CH_2), 3.05 – 2.87 (m, 4H, CH_2), 2.78 (m, 2H, CH_2), 2.37 (td, J = 8.7, 7.1, 4.7 Hz, 2H, CH_2), 2.22 (m, 3H, CH_2+CH), 1.88 (m, 2H, CH_2), 1.82 – 1.65 (m, 3H, CH_2+CH), 1.58 (m, 1H, CH_2), 1.48 (m, 1H, CH_2), 1.40 – 1.13 (m, 25H, CH_2+CH_3), 0.82 (dd, J = 6.6, 1.7 Hz, 6H, CH_3).

^{13}C NMR (101 MHz, DMSO-d_6) δ 173.24, 171.92, 171.81, 170.99, 170.84, 170.27, 155.79, 143.75, 143.70, 142.29, 142.25, 140.72, 140.70, 139.69, 139.09, 129.27, 128.71, 128.12, 128.08, 127.91, 127.67, 127.08, 126.96, 125.32, 125.30, 121.41, 120.10, 120.05, 79.57, 79.49, 74.49, 44.76, 44.28, 29.64, 27.71, 22.19, 18.70.

HPLC-MS: positive ions, target compound content – 100%, t_R=11.075 min.

HRMS (m/z, ESI): calculated for $C_{102}H_{112}N_{10}O_{13}$ – $[M+H]^+$ 1685.84831, found: 1685.8423

Synthesis of *tert*-butyl (5*S*,8*S*,11*S*,14*S*)-8-(3-(*tert*-butoxy)-3-oxopropyl)-1-(9*H*-fluoren-9-yl)-14-(((2*S*)-6-(2-(4-isobutylphenyl)propanamido)-1-oxo-1-(prop-2-yn-1-ylamino)hexan-2-yl)carbamoyl)-3,6,9,12-tetraoxo-5,11-bis((1-trityl-1*H*-imidazol-4-yl)methyl)-2-oxa-4,7,10,13-tetraazaheptadecan-17-oate (3)

To a solution of compound **2** (156 mg, 0.0925 mmol, 1 eq) in DMF at 0 ºC was successively added propargylamine (6 μl, 0.0935 μmol, 1.01 eq), HBTU (52 mg, 0.1379 mmol, 1.5 eq), HOBT (14 mg, 0.0925 mmol, 1 eq) and DIPEA (37 μl, 0.213 mmol, 2.3 eq). The reaction mixture was stirred overnight at room temperature. The solvent was then removed under reduced pressure. The resulting residue was dissolved in DCM (10 mL) and extracted with H_2O (2*10 mL), 0.1M HCl (1*10 mL), NaCl (1*10 mL). The organic fraction was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. of compound **3** was obtained as a white powder (164 mg, yield 84%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (m, 1H, NH), 8.17 (m, 2H, NH), 8.02 (m, 1H, NH), 7.85 (m, 3H, Ar+NH), 7.62 (t, *J* = 6.7 Hz, 2H, Ar), 7.46 (d, *J* = 8.3 Hz, 1H, NH), 7.41 – 7.12 (m, 27H, Ar+NH), 7.01 (m, 14H, Ar), 6.73 – 6.65 (d, *J* = 12.1 Hz, 2H, Ar), 4.40 (q, *J* = 7.0 Hz, 1H, CH), 4.33 – 4.25 (m, 1H, CH), 4.12 (m, 5H, CH_2+CH), 3.77 (d, *J* = 5.4 Hz, 2H, CH_2), 3.48 (q, *J* = 6.9 Hz, 1H, CH), 3.03 (t, *J* = 2.5 Hz, 1H, CH), 2.94 – 2.81 (m, 4H, CH_2), 2.75 – 2.66 (m, 2H, CH_2), 2.35 (d, *J* = 7.1 Hz, 2H, CH_2), 2.18 (m, 3H, CH_2+CH), 1.96 – 1.80 (m, 2H, CH_2), 1.75 (m, 3H, CH_2+CH), 1.55 (m, 1H, CH_2), 1.42 – 1.11 (m, 28H, CH_3+CH_2), 0.81 (d, *J* = 6.7 Hz, 6H, CH_3).

HPLC-MS: positive ions, target compound content – 100%, t_R=12.678 min.

HRMS (m/z, ESI): calculated for $C_{105}H_{115}N_{11}O_{12}$ – $[M+Na]^+$ 1745,8621, found: 1745,8619

Synthesis of *tert*-butyl (9*S*,12*S*,15*S*,18*S*)-18-((*S*)-2-amino-3-(1-trityl-1*H*-imidazol-4-yl)propanamido)-12-(3-(*tert*-butoxy)-3-oxopropyl)-2-(4-isobutylphenyl)-3,11,14,17-tetraoxo-9-(prop-2-yn-1-ylcarbamoyl)-15-((1-trityl-1*H*-imidazol-4-yl)methyl)-4,10,13,16-tetraazahenicosan-21-oate (4) (see Scheme S2)

Et_2NH (196 µl, 1.9 mmol, 20 eq) was added to a solution of compound **3** (164 mg, 0.0952 mmol, 1 eq) in CH_3CN under stirring at room temperature. The reaction was monitored by TLC (eluent was 5% MeOH-95% DCM). After the reaction time, the solvent was removed under reduced pressure. After purification by column chromatography (InterchimPuriflash 20 g, 15µ, gradient from 0% methanol to 10% methanol in 20 min, then from 10% methanol to 15% in 5 min, then from 15% methanol to 20% in 4 min, then from 20% methanol to 30% in 5 min flow rate - 20 ml/min) 116 mg (82% yield) of compound **4** as a white powder was obtained.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (m, 1H, NH), 8.18 (m, 1H, NH), 8.11 (m, 1H, NH), 8.01 (m, 1H, NH), 7.85 (m, 1H, NH), 7.37 – 7.29 (m, 20H, Ar+NH_2), 7.18 – 7.14 (m, 3H, Ar+NH), 7.07 – 6.99 (m, 15H, Ar), 6.67 (d, *J* = 6.0 Hz, 2H, Ar), 4.40 (m, 1H, CH), 4.15 (m, 3H, CH), 3.77 (m, 2H, CH_2), 3.49 (q, *J* = 7.1 Hz, 1H, CH), 3.03 (t, *J* = 2.5 Hz, 1H, CH), 2.98 – 2.81 (m, 4H, CH_2), 2.73 (m, 2H, CH_2), 2.36 (d, *J* = 7.0 Hz, 2H, CH_2), 2.17 (m, 3H, CH_2+CH), 1.95 – 1.63 (m, 5H, CH_2+CH), 1.55 (m, 1H, CH_2), 1.43 – 1.19 (m, 26H, CH_3+CH_2), 1.14 (m, 2H, CH_2), 0.81 (dd, *J* = 6.7, 1.3 Hz, 6H, CH_3).

HPLC-MS: positive ions, target compound content – 98,7%, t_R=8,766 min.

HRMS (m/z, ESI): calculated for $C_{90}H_{105}N_{11}O_{10}$ – $[M+H]^+$ 1500,8119, found: 1500,8119; $[M+Na]^+$ 1522,7938, found: 1522,7938

Synthesis of *tert*-butyl (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-azidohexanoate (5′) (see Scheme S3)

To a solution of Fmoc-azidolysine (616 mg, 1.56 mmol, 1 eq.) in anhydrous tBuOH (9.24 mL) was added Boc_2O (850 mg, 3.9 mmol, 2.5 eq.), DMAP (57 mg, 0.47 mmol, 0.3 eq.) and stirred for 30 min at 35 °C, followed by 15 h at room temperature. The reaction was monitored by TLC (EtOAc/hexane 2:1). The solvent was then removed under reduced pressure, the resulting solid residue was dissolved in EtOAc (15 mL)/H_2O (1 mL) mixture and the resulting mixture was washed with a saturated solution of $NaHCO_3$ (3*10mL) and H_2O (3*10mL). The organic fraction was dried over Na_2SO_4 and the solvent was removed under reduced pressure. After purification by column chromatography (InterchimPuriflash 40 g, 15μ, gradient from 0% EtOAc to 10% EtOAc in 22 min, then from 10% EtOAc to 25% in 8 min, then from 25% methanol to 80% in 8 min flow rate - 30 ml/min) 224 mg (61% yield) of compound **5′** as a yellow oil was obtained.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.78 (d, *J* = 7.5 Hz, 2H, Ar), 7.61 (d, *J* = 7.5 Hz, 2H, Ar), 7.41 (t, *J* = 7.5 Hz, 2H, Ar), 7.33 (td, *J* = 7.5, 1.2 Hz, 2H, Ar), 5.35 (d, *J* = 8.1 Hz, 1H, NH), 4.40 (d, *J* = 7.1 Hz, 2H, CH_2), 4.32 – 4.20 (m, 2H, CH), 3.29 (t, *J* = 6.8 Hz, 2H, CH_2), 1.86 (m, 1H, CH_2), 1.75 – 1.54 (m, 4H, CH_2), 1.49 (m, 12H, CH_3+CH_2).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.59, 156.14, 143.87, 143.82, 140.77, 127.67, 127.08, 125.29, 120.16, 80.50, 65.61, 54.37, 50.53, 46.69, 30.35, 27.86, 27.66, 22.83.

Synthesis of tert-butyl (*S*)-2-amino-6-azidohexanoate (5″) (see Scheme S3)

To a solution of compound **5′** (224 mg, 0.497 mmol, 1 eq) in CH_3CN (10 mL) was added Et_2NH (1.026 mL, 9.95 mmol, 20 eq) under stirring and the reaction was followed by TLC (EtOAc/hexane 2:1). The solvent was then removed under reduced pressure, the resulting solid residue was purified by flash chromatography on silica gel (first 100% DCM followed by 100% MeOH). 131 mg (59% yield) of compound **5″** was obtained as a colorless oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.30 (t, *J* = 6.8 Hz, 2H, CH_2), 3.15 (dd, *J* = 6.9, 5.6 Hz, 1H, CH), 2.05 (br s, 2H, NH_2), 1.56 – 1.47 (m, 3H, CH_2), 1.43 – 1.32 (m, 12H, CH_3+CH_2).

Synthesis of *tert*-butyl (*S*)-2-(4-(((2-amino-4-hydroxypteridin-6-yl)methyl)amino)benzamido)-6-azidohexanoate (5) (see Scheme S3)

To a solution of pteroic acid (149 mg, 0.47 mmol, 1 eq) in DMSO (15 mL) was successively added compound **5″** (131 mg, 0.57 mmol, 1.2 eq), HBTU (217 mg, 0.57 mmol, 1.2 eq), HOBT (37 mg, 0.24 mmol, 0.5 eq), DIPEA (250 μL, 1.44 mmol, 3 eq) and stirred overnight

at room temperature. The solvent was then removed under reduced pressure, the product was precipitated with MeOH from the resulting dry residue and the precipitate was centrifuged. 159 mg (65% yield) of compound **5** was obtained as an orange-red powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H, OH), 8.63 (s, 1H, Ar), 8.09 (d, J = 7.5 Hz, 1H, NH), 7.64 (d, J = 8.5 Hz, 2H, Ar), 6.93 (t, J = 5.9 Hz, 1H, NH), 6.62 (d, J = 8.4 Hz, 2H, Ar), 4.47 (d, J = 5.9 Hz, 2H, NH_2), 4.22 (q, J = 7.3 Hz, 1H, CH), 3.29 (m, 2H, CH_2) 3.15 (d, J = 4.1 Hz, 2H, CH_2), 1.74 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.41 (m, 1H, CH_2), 1.38 (s, 10H, CH_3+CH_2).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.83, 166.46, 150.78, 148.73, 129.07, 129.00, 127.97, 127.95, 121.36, 111.19, 80.26, 64.96, 52.99, 50.54, 48.63, 45.93, 30.31, 27.93, 27.71, 23.11, 15.20.

HRMS (m/z, ESI): calculated for $C_{24}H_{30}N_{10}O_4$ – $[M+Na]^+$ 545,2344, found: 545,2348

Synthesis of *tert*-butyl (9*S*,12*S*,15*S*,18*S*)-18-((*S*)-2-amino-3-(1-trityl-1*H*-imidazol-4-yl)propanamido)-9-(((1-((*S*)-5-(4-(((2-amino-4-hydroxypteridin-6-yl)methyl)amino)benzamido)-6-(*tert*-butoxy)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)methyl)carbamoyl)-12-(3-(*tert*-butoxy)-3-oxopropyl)-2-(4-isobutylphenyl)-3,11,14,17-tetraoxo-15-((1-trityl-1*H*-imidazol-4-yl)methyl)-4,10,13,16-tetraazahenicosan-21-oate (6) (see Scheme S4)

Compound **5** (37 mg, 0.0703 mmol, 1 eq.) and compound **4** (116 mg, 0.0773 mmol, 1,1 eq.) were dissolved in a mixture of DMF/H_2O = 3/1. The flask was filled with argon, then aqueous solutions of sodium ascorbate (17 mg, 0.0843 mmol, 1.2 eq.) and $CuSO_4$*$5H_2O$ (7 mg, 0.0281 mmol, 0,4 eq.) were added to the system. The reaction mixture was stirred for 18 hours. Afterwards EDTA (19 mg, 0.0562 mmol, 0.8 eq.) was added and stirred for another 2 hours with access of air oxygen. The solvent was removed under reduced pressure. Then the product was isolated individually by reverse phase column chromatography (InterchimPuriflash C18 20 g, 15µ, gradient from 10% acetonitrile to 20% acetonitrile in 4 min, from 20% acetonitrile to 35% in 5 min, then from 35% acetonitrile to 55% in 4 min, then from 55% acetonitrile to 70% in 3 min, then from 70% acetonitrile to 100% in 3 min, flow rate 20 ml/min). 55 mg (35% yield) of compound **6** was obtained as a yellow powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H, Ar), 8.31 – 7.99 (m, 5H, Ar+NH), 7.84 (s, 1H, NH), 7.79 (s, 1H, CH), 7.63 (d, J = 8.6 Hz, 2H, Ar), 7.32 (s, 20H, Ar+NH), 7.16 (d, J = 7.3 Hz, 2H, Ar), 7.11 – 6.87 (m, 17H, Ar+NH+NH_2), 6.62 (d, J = 8.6 Hz, 2H, Ar), 4.46 (d, J = 5.8 Hz, 3H, CH_2+CH), 4.28 – 4.11 (m, 8H, CH_2+CH), 3.81 (m, 1H, CH), 3.49 (d, J = 7.1 Hz, 2H,

CH), 3.07 – 2.64 (m, 4H, CH_2), 2.33 (d, *J* = 7.1 Hz, 2H, CH_2), 2.17 (m, 4H, CH_2), 1.88 (m, 1H, CH), 1.73 (m, 6H, CH_2), 1.57 (m, 1H, CH_2), 1.40 – 1.10 (m, 38H, CH_3+CH_2), 0.80 (d, *J* = 6.6 Hz, 6H, CH_3).

HPLC-MS: positive ions, target compound content – 90,3%, t_R=8,246 min.

HRMS (m/z, ESI): calculated for $C_{114}H_{135}N_{21}O_{14}$ – $[M+2H]^{2+}$ 1012,0322, found: 1012,0283

Synthesis of di-*tert*-butyl (4*S*,7*S*,10*S*,13*S*)-4-(((2*S*)-1-(((1-((*S*)-5-(4-(((2-amino-4-hydroxypteridin-6-yl)methyl)amino)benzamido)-6-(*tert*-butoxy)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-(2-(4-isobutylphenyl)propanamido)-1-oxohexan-2-yl)carbamoyl)-10-(3-(*tert*-butoxy)-3-oxopropyl)-6,9,12,15-tetraoxo-18-(4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-7,13-bis((1-trityl-1*H*-imidazol-4-yl)methyl)-5,8,11,14-tetraazanonadecane-1,19-dioate (7)

To a solution of compound **6** (36 mg, 0.0178 mmol, 1 eq) in DMF (5 mL) was added successively DOTA-GA(OBu^t)$_4$ (14 mg, 0.0196 mmol, 1.1 eq), HBTU (10 mg, 0.027 mmol, 1.5 eq), HOBt (4 mg, 0.0267 mmol, 1.5 eq), DIPEA (12 μL, 0.0712 mmol, 4 eq) and stirred overnight at room temperature, after which the solvent was removed under reduced pressure. After purification by column chromatography (InterchimPuriflash 25 g, 15μ, gradient from 0% methanol to 10% methanol in 11 min, then from 10% methanol to 15% in 3 min, then from 15% methanol to 25% in 3 min, then from 25% methanol to 55% in 3 min, flow rate - 20 ml/min) 39 mg (81% yield) of compound **7** as a yellow solid was obtained.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (br s, 1H, OH), 8.63 (s, 1H, Ar), 8.18 (m, 2H, NH), 8.08 (d, *J* = 7.2 Hz, 1H, NH), 7.95 (d, *J* = 8.3 Hz, 2H, Ar), 7.84 (m, 1H, NH), 7.79 (s, 1H, CH), 7.68 (d, *J* = 8.3 Hz, 2H, NH_2), 7.63 (d, *J* = 8.5 Hz, 2H, Ar), 7.49 (t, *J* = 7.5 Hz, 2H, NH), 7.41 – 7.27 (m, 19H, Ar+NH), 7.20 – 7.13 (m, 3H, Ar+NH), 7.11 – 6.96 (m, 14H, Ar), 6.93 (m, 1H, NH), 6.62 (t, *J* = 7.6 Hz, 3H, Ar), 4.46 (d, *J* = 6.0 Hz, 2H, CH_2), 4.40 (m, 1H, CH), 4.29 – 4.07 (m, 8H, CH_2+CH), 3.48 (m, 2H, CH), 3.20 – 2.56 (m, 17H, CH_2+CH), 2.34 (d, *J* = 7.0 Hz, 3H, CH_2+CH), 2.16 (m, 6H, CH_2), 1.94 (d, *J* = 23.0 Hz, 5H, CH_2), 1.82 – 1.47 (m, 10H, CH_2), 1.46 – 1.04 (m, 84H, CH_3+CH_2), 0.80 (d, *J* = 6.6 Hz, 6H, CH_3).

HRMS (m/z, ESI): calculated for $C_{149}H_{197}N_{25}O_{23}$ – $[M+3Na]^{3+}$ 909,8351, found: 909,8357

Synthesis of (4*S*,7*S*,10*S*,13*S*)-7,13-bis((1*H*-imidazol-4-yl)methyl)-4-(((2*S*)-1-(((1-((*S*)-5-(4-(((2-amino-4-hydroxypteridin-6-yl)methyl)amino)benzamido)-5-carboxypentyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-(2-(4-isobutylphenyl)propanamido)-1-oxohexan-2-yl)carbamoyl)-10-(2-carboxyethyl)-6,9,12,15-tetraoxo-18-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-5,8,11,14-tetraazanonadecane-1,19-dioic acid (8) (see Scheme S4)

Method 1

The protected compound **7** (40 mg, 0.0144 mmol) was dissolved in a mixture of trifluoroacetic acid (3.6 ml, 90% v/v), triisopropylsilane (0.2 ml, 5% v/v) and water (0.2 ml, 5% v/v). The reaction mixture was stirred for 2 h. Then the solvent was removed under reduced pressure, after which the dry residue was precipitated with diethyl ether, decanted and the precipitate was washed three times with Et_2O. Then the solvent was removed under reduced pressure.

HPLC-MS: negative ions, target compound content – 48,4%, t_R=10,001 min.

Method 2

The protected compound **7** (40 mg, 0.0144 mmol) was dissolved in a mixture of trifluoroacetic acid (5.4 ml, 90% v/v), triisopropylsilane (0.3 ml, 5% v/v) and water (0.3 ml, 5% v/v). The reaction mixture was stirred for 5 h. Then the solvent was removed under reduced pressure, after which the dry residue was precipitated with diethyl ether, decanted and the precipitate was washed three times with Et_2O. Then the solvent was removed under reduced pressure. After preparative chromatography (mobile phases: A - 0.1% formic acid in water, B - 9 mM ammonium formate in water, D - acetonitrile, 0 - 0.5 min - 5% D, 0.5 - 9.5 min - from 5% to 90% D, 9.5 - 12 min - 90% D, 12 - 14.5 min - from 90% to 5% D, flow rate - 1 ml/min) 8 mg (30% yield) of compound **8** were obtained as a yellow powder.

HPLC-MS: negative ions, target compound content – 64.9%, t_R=10.321 min.

HPLC-MS after preparative separation: negative ions, target compound content – 100%, t_R=10.563 min.

Method 3

The protected compound **7** (5 mg) was dissolved in a mixture of trifluoroacetic acid (2.5 ml, 50% v/v), DCM (2 ml, 40% v/v), triisopropylsilane (0.25 ml, 5% v/v) and water (0.25 ml, 5% v/v). The reaction mixture was stirred for 5 h. Then the solvent was removed under reduced

pressure, after which the dry residue was precipitated with diethyl ether, decanted and the precipitate was washed three times with Et_2O. Then the solvent was removed under reduced pressure.

HPLC-MS: negative ions, target compound content – 54.6%, t_R=10.084 min.

Method 4

The protected compound **7** (5 mg) was dissolved in a mixture of 10N HCl in water (2 ml, 50% v/v) and dioxane (2 ml, 50% v/v). The reaction mixture was stirred for 18 h at room temperature. Then the solvent was removed under reduced pressure, after which the dry residue was precipitated with diethyl ether, decanted and the precipitate was washed three times with Et_2O. Then the solvent was removed under reduced pressure.

HPLC-MS: positive and negative ions, target compound content – 0%.

Radiochemical synthesis and analysis

The radiolabeling with lutetium-177 and further radiochemical purity determination were carried out in accordance with previously described protocols [S4,S5]. No-carrier-added lutetium-177 was obtained from Research Institute of Atomic Reactors (Dimitrovgrad, Russia) as solution in 0.04 M HCl with an activity of 286 GBq/mL and a specific activity of 92.9 Ci/mg. A 15μL aliquot of **8** in PBS (1 mg/mL) was mixed with 200 μL of 1.0 M sodium acetate, 5 mg of gentisic acid, 175 μL of 0.5 M $HCl_{aq.}$, 400 μL of ethanol abs. (pharm-grade), 5 μL of [^{177}Lu]$LuCl_3$ solution (1.2 GBq, [Lu]:[**8**] molar ratio was 1:5) and water (q.s. to 1 mL). The final concentration of sodium acetate was 0.2 M and pH was 4.7. The activity was monitored with an ISOMED 2010 dose calibrator. The reaction mixtur was incubated for 15 min at 60 °C. No further purification of sample or any additional manipulations for the reformulation of sample after synthesis were carried out. Radio-TLC and radio-HPLC methods were used to analyze the radiochemical conversion and radiochemical purity of [^{177}Lu]Lu-**8** preparation.

For TLC analisys a silica gel coated aluminum TLC plates (5553, Merck) with acetonitrile-water mixture (1:1) as solvent were used. Radiography of the TLC-strips was performed using a miniGita* radio-TLC scanner (Raytest). HPLC analysis of preparation was carried out using LicArt-62 chromatograph (Labconcept LLC, Russia), equipped with a diode array detector and flow radioactivity detector GABI Nova (2 × 2” NaI-PMT detector, Elysia-Raytest, Germany) with a 5 μL measuring cell. The reversed phase C_{18}-column Phenomenex Luna® 150 × ⌀4 mm (5 μm, 100 Å) was used. Elution was performed in gradient mode with 1 mL/min flow rate: 0-15 min =

5-80% B (solvent A was 0.1% (*v*/*v*) TFA in water and solvent B was 0.1% (*v*/*v*) TFA in acetonitrile). The columns were thermostated at 30 °C.

According to the analysis results, the radiochemical conversion was >99%, and the radiochemical purity was 97.6%. In the reaction mixture, about 1.7% of unidentifiable related impurities were determined, most likely associated with the purity of the starting precursor **10**. Thus, under the implemented synthesis conditions, it was possible to achieve a specific activity of [^{177}Lu]Lu-**10** preparation of 142 MBq/nmol.

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S1. L. Tietze, T. Eicher, U. Diederichsen, A. Speicher and N. Schützenmeister, *Reactions and Syntheses: In the Organic Chemistry Laboratory, 2nd edn.,* Wiley, 2015; https://books.google.ru/books?id=OwnVBQAAQBAJ&hl=ru&source=gbs_navlinks_s.

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S5. A. Larenkov, I. Mitrofanov, E. Pavlenko and M. Rakhimov, *Molecules*, 2023, **28,** 1884; https://doi.org/10.3390/molecules28041884.

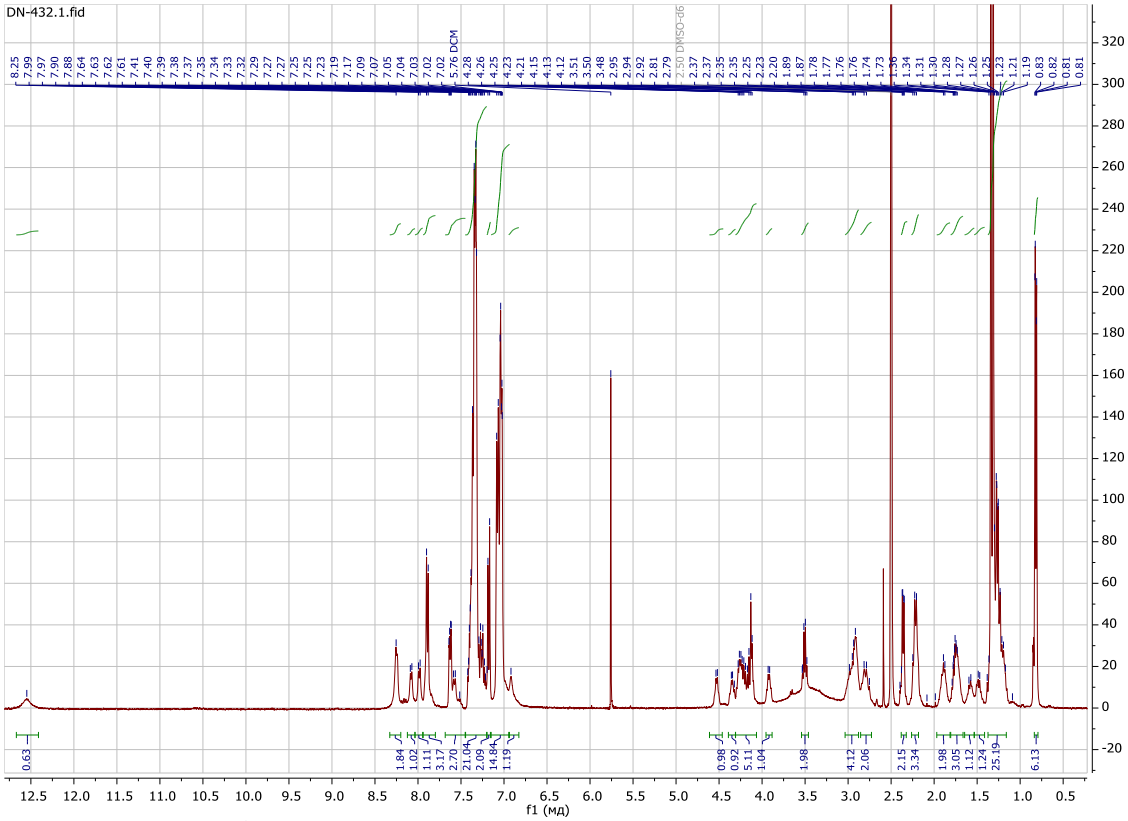

Figure S1. ^{1}H NMR spectra for compound **2**

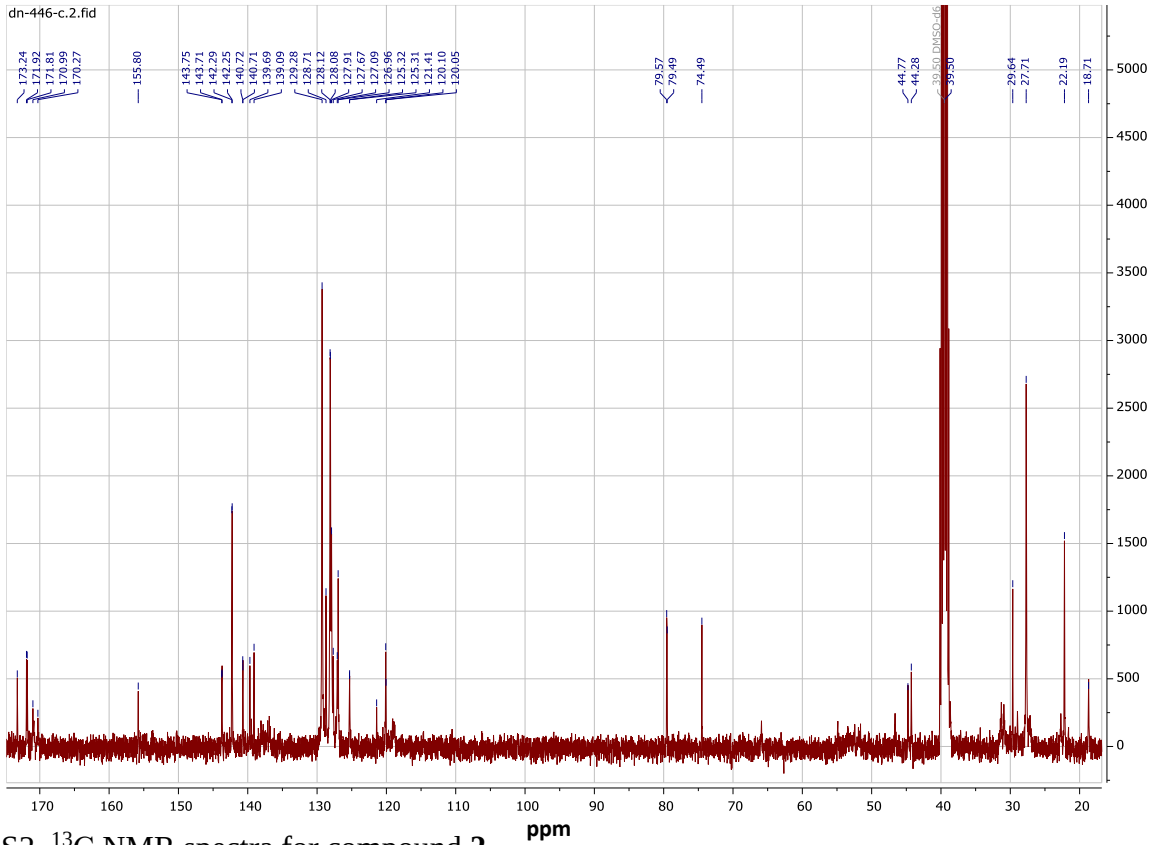

Figure S2. ^{13}C NMR spectra for compound **2**

HPLC-MS spectra for compound **2:**

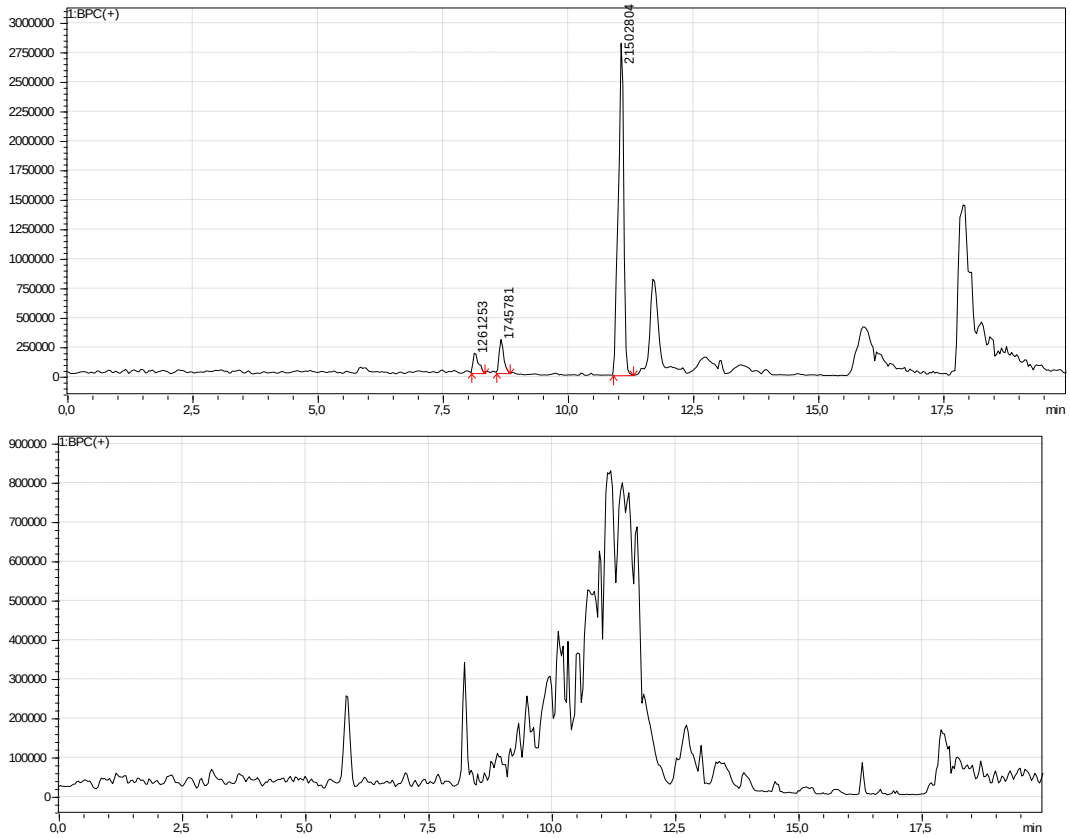

Figure S3. Positive ions (chromatogram and blank)

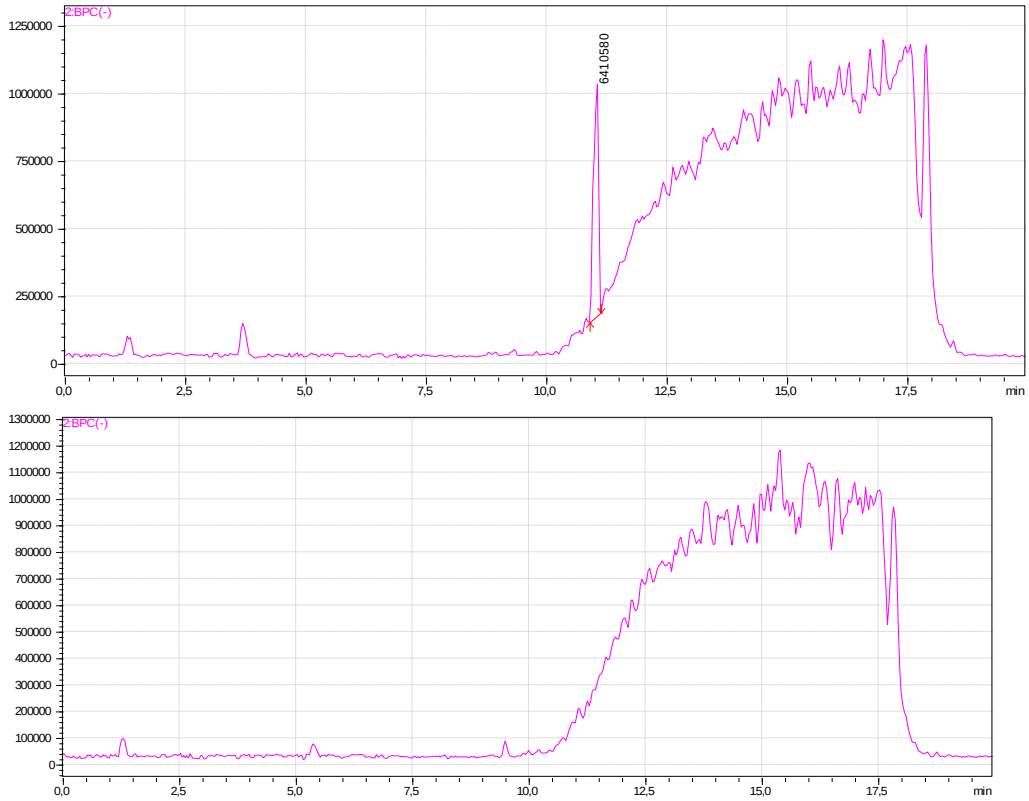

Figure S4. Negative ions (chromatogram and blank)

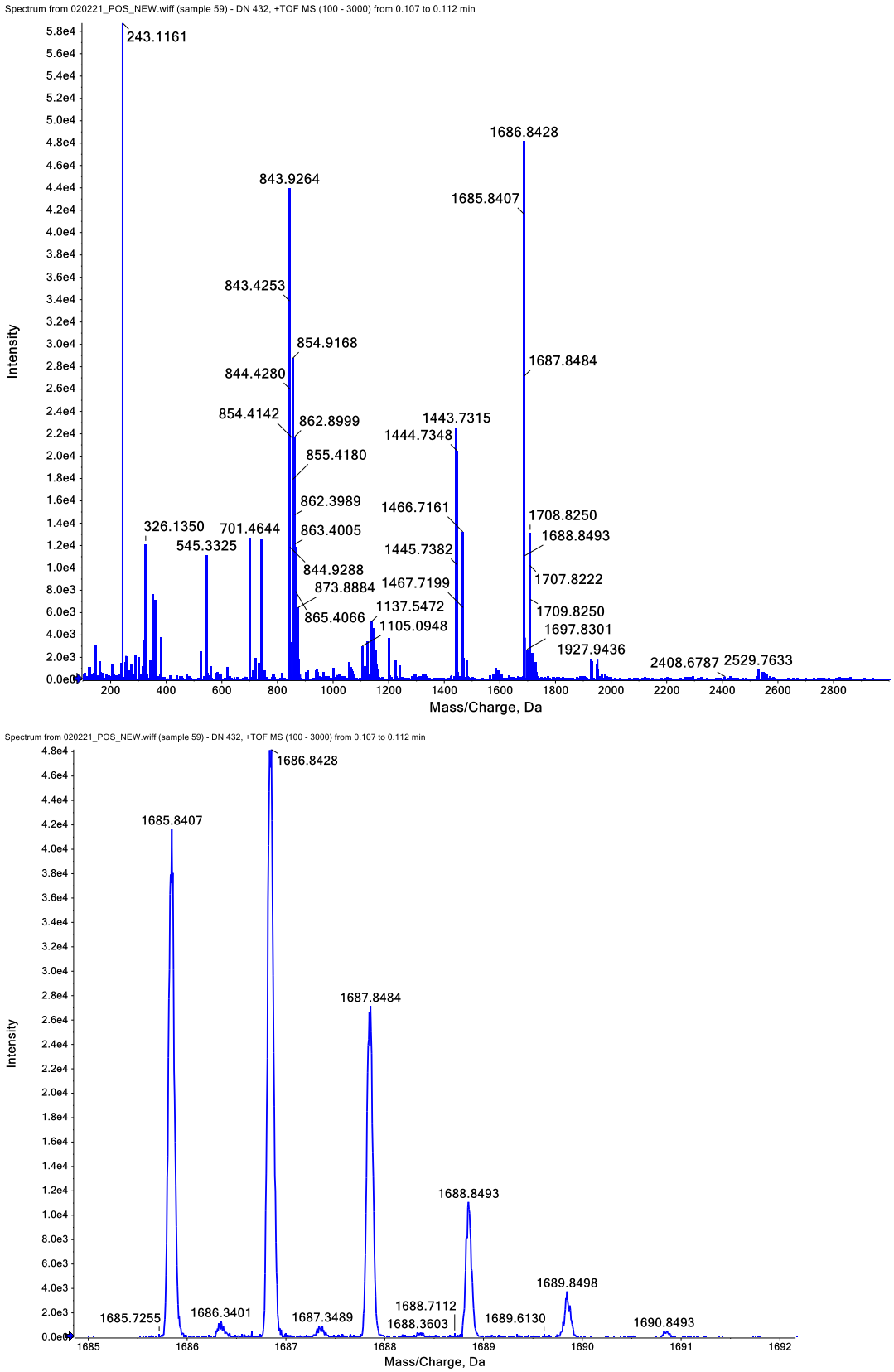

Figure S5. HRMS spectra for compound **2**

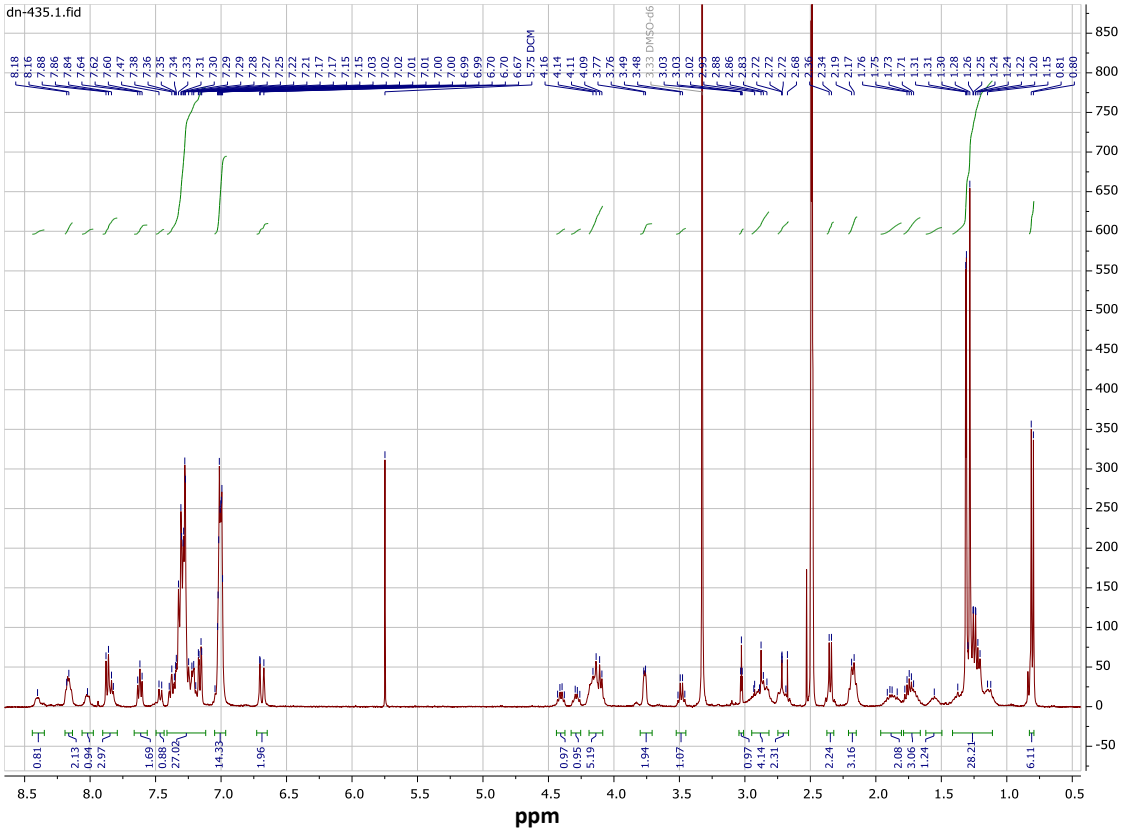

Figure S6. ^{1}H NMR spectra for compound **3**

HPLC-MS spectra for compound **3:**

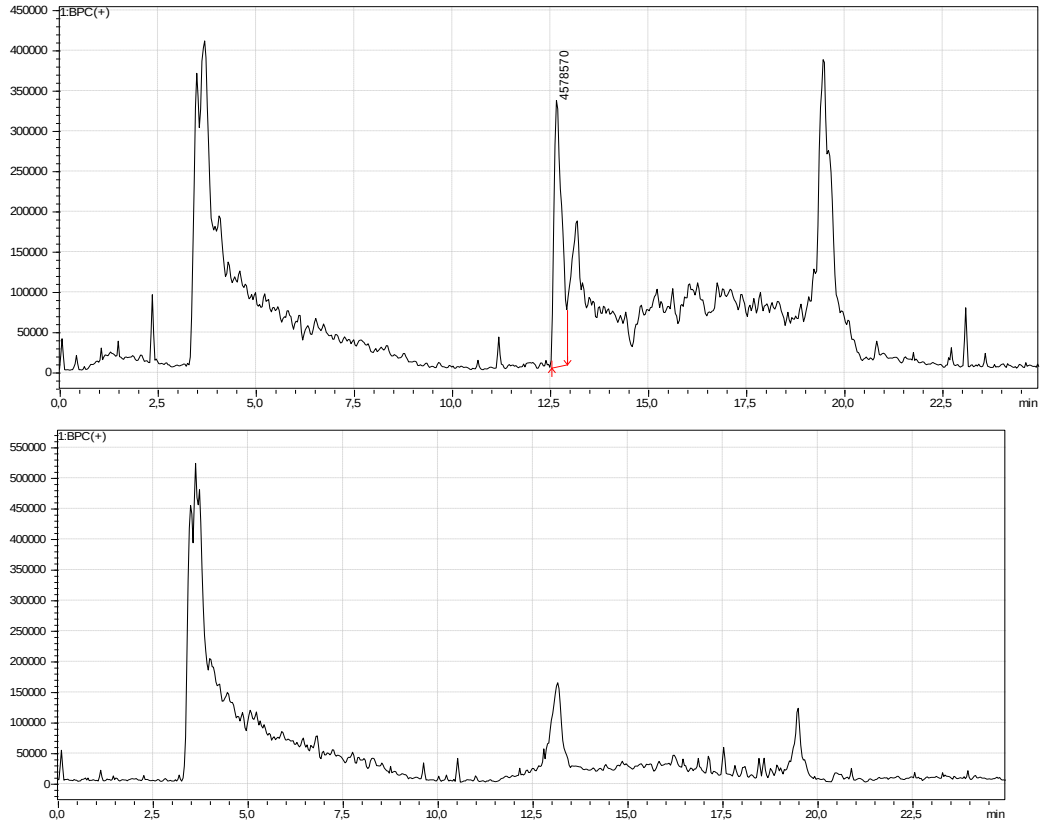

Figure S7. Positive ions (chromatogram and blank)

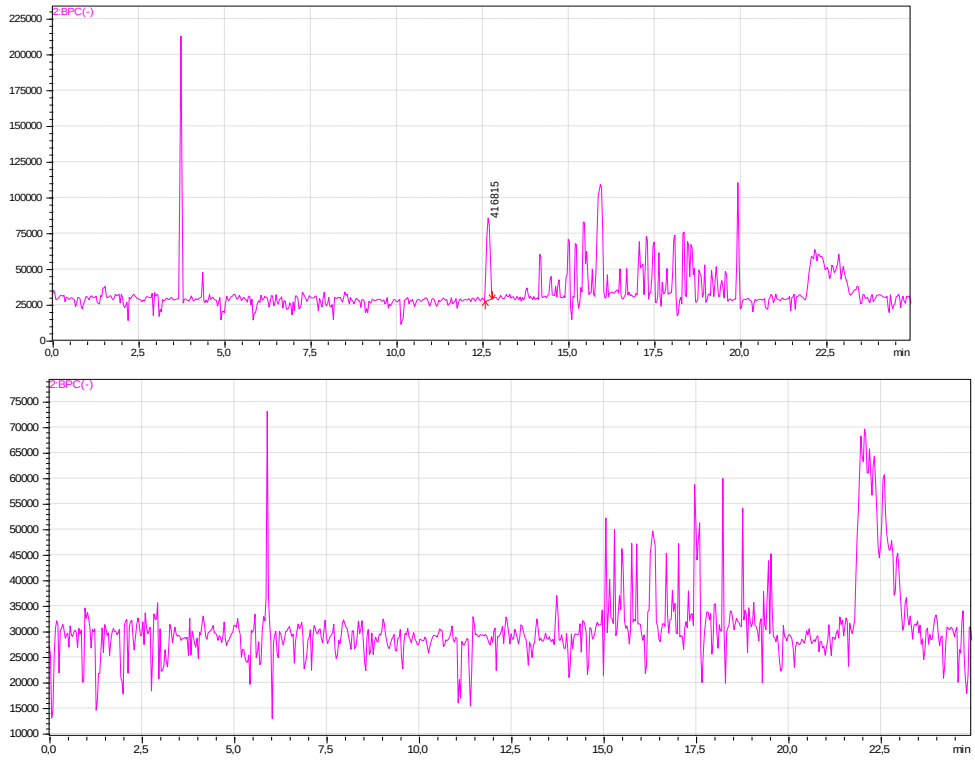

Figure S8. Negative ions (chromatogram and blank)

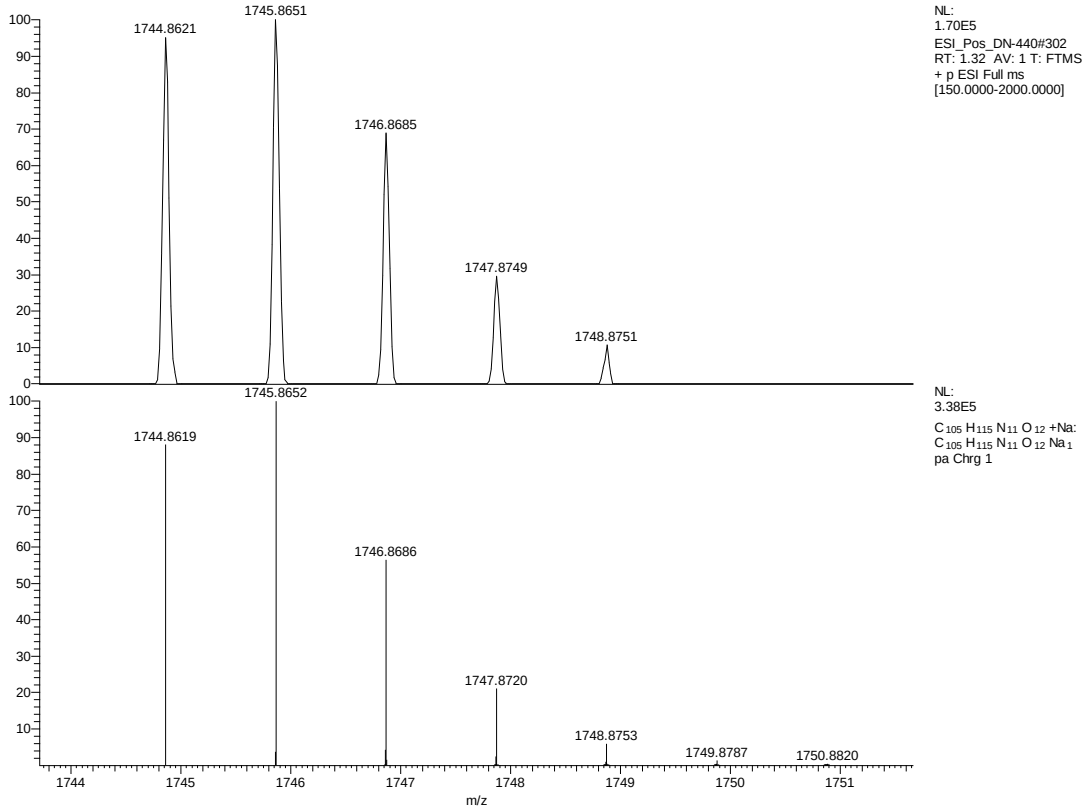

Figure S9. HRMS spectra for compound **3**

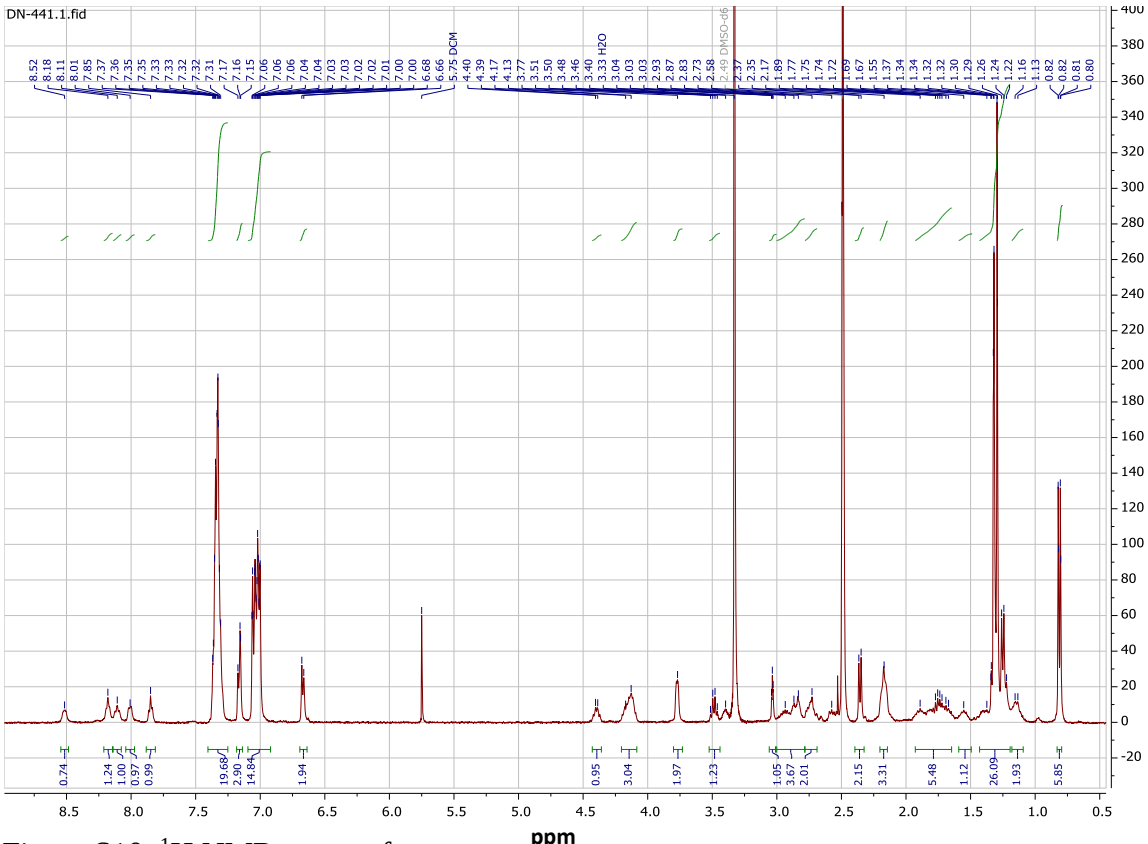

Figure S10. ^{1}H NMR spectra for compound **3**

HPLC-MS spectra for compound **4:**

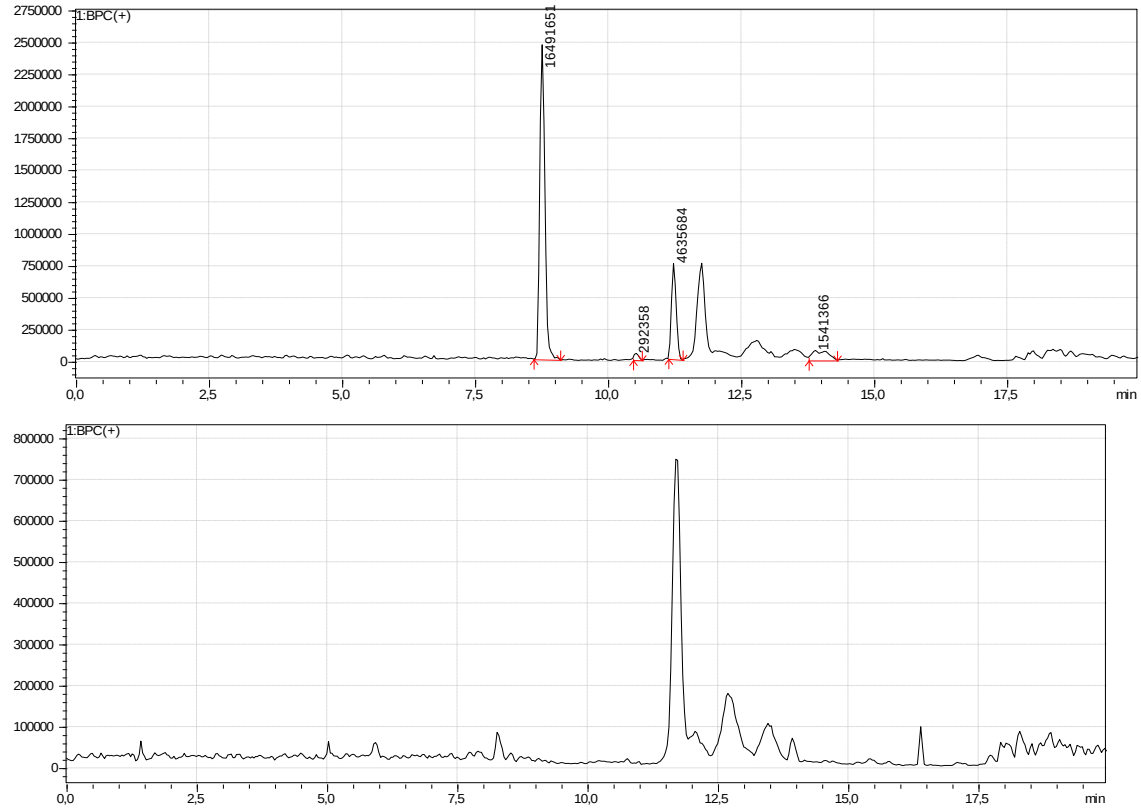

Figure S11. Positive ions (chromatogram and blank)

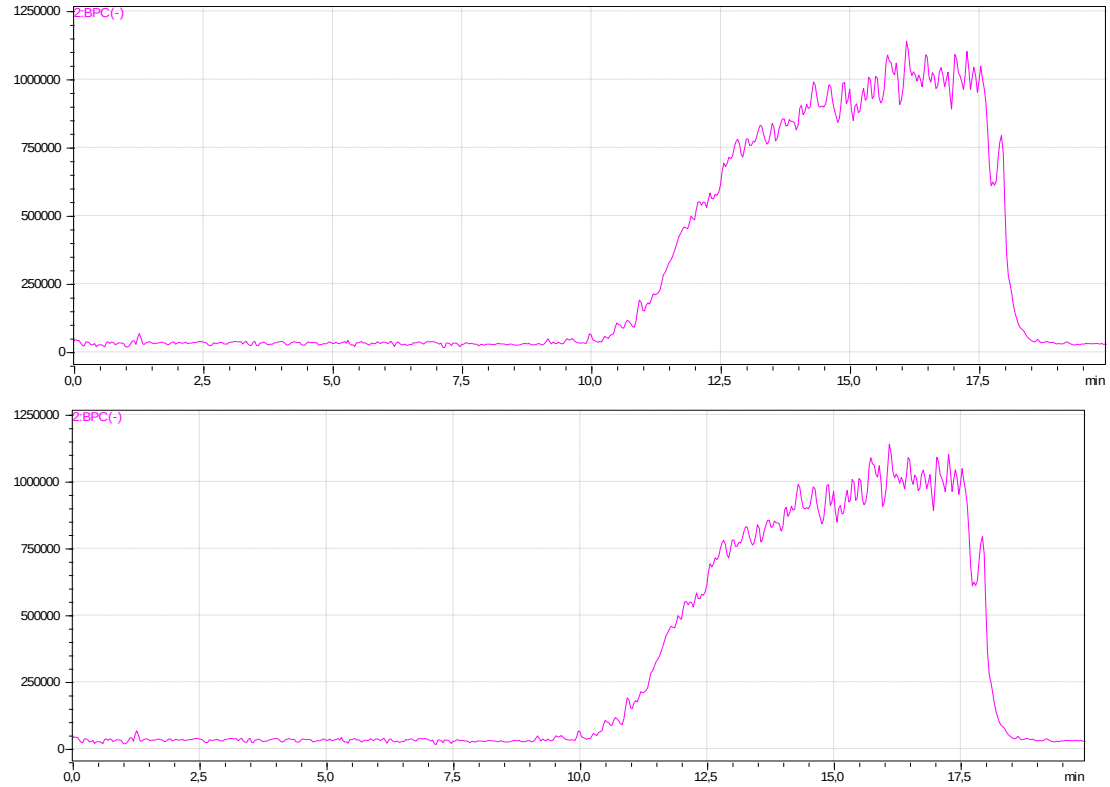

Figure S12. Negative ions (chromatogram and blank)

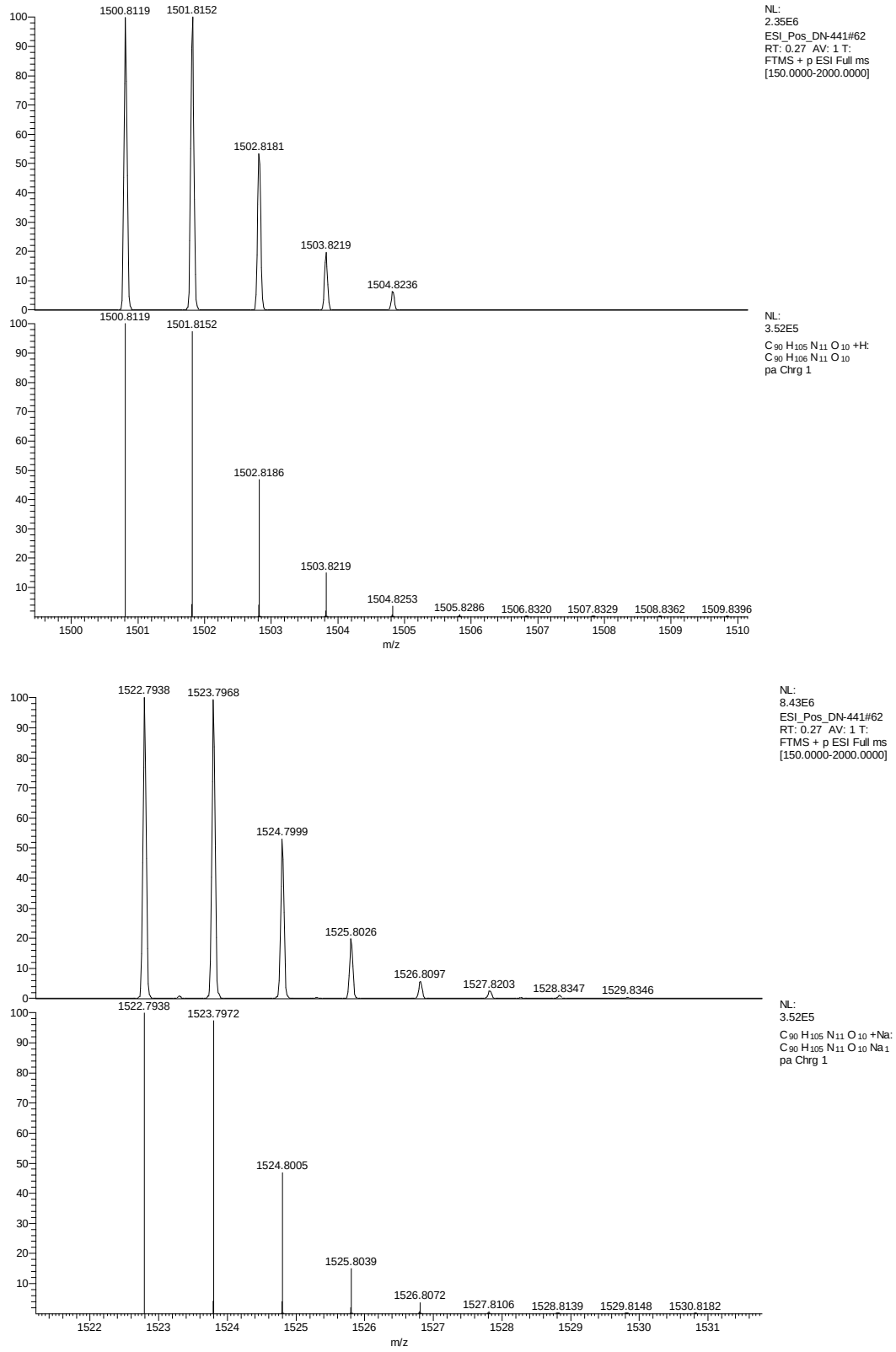

Figure S13. HRMS spectra for compound **4**

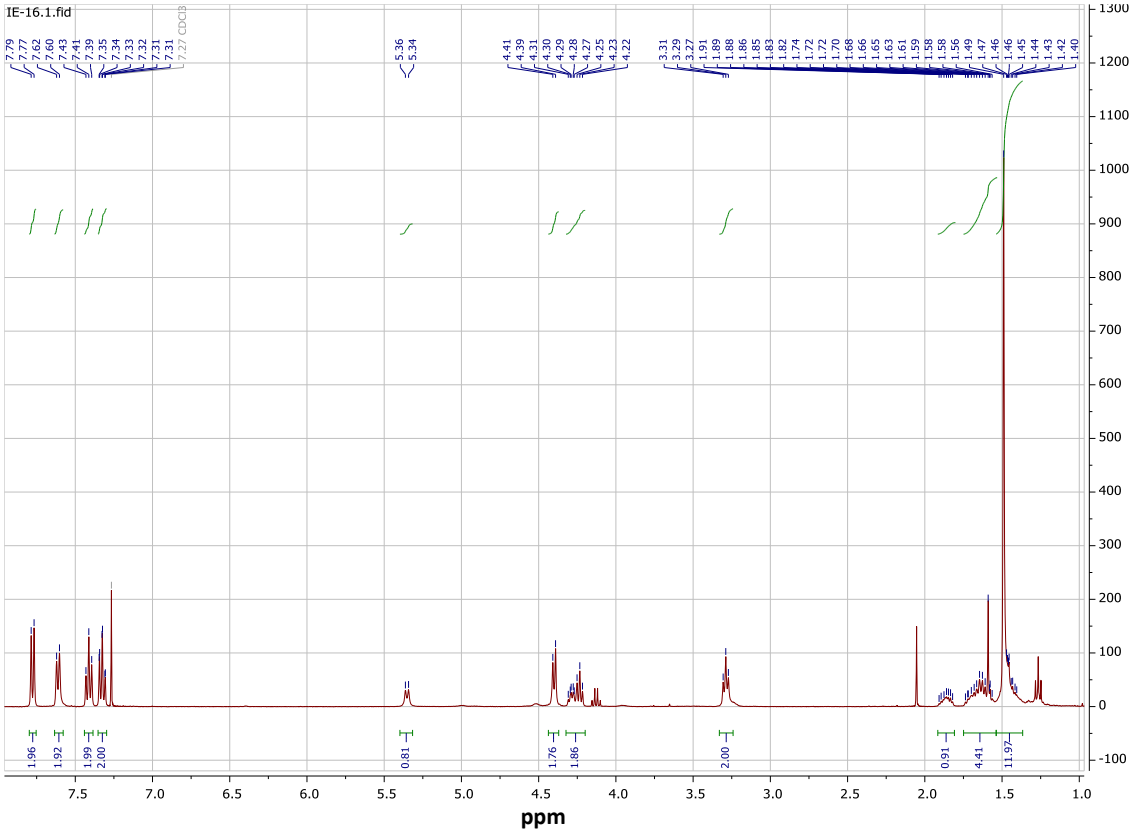

Figure S14. ^{1}H NMR spectra for compound **5′**

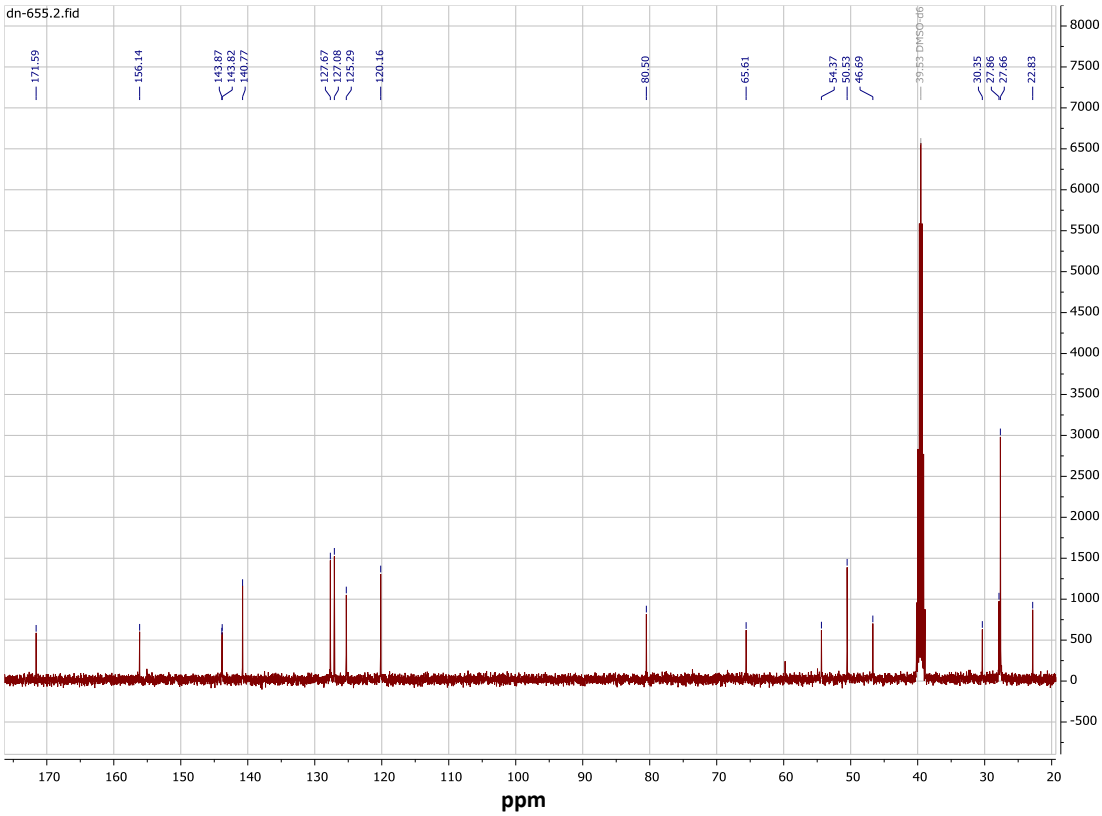

Figure S15. ^{13}C NMR spectra for compound **5′**

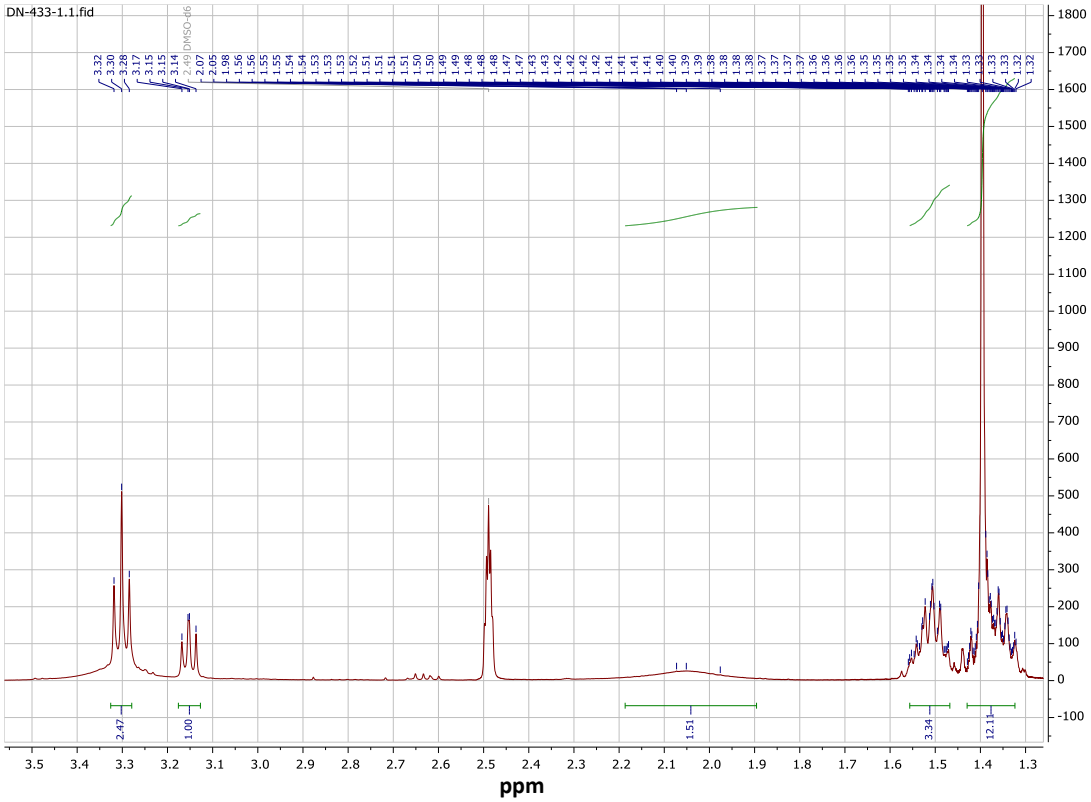

Figure S16. ^{1}H NMR spectra for compound **5″**

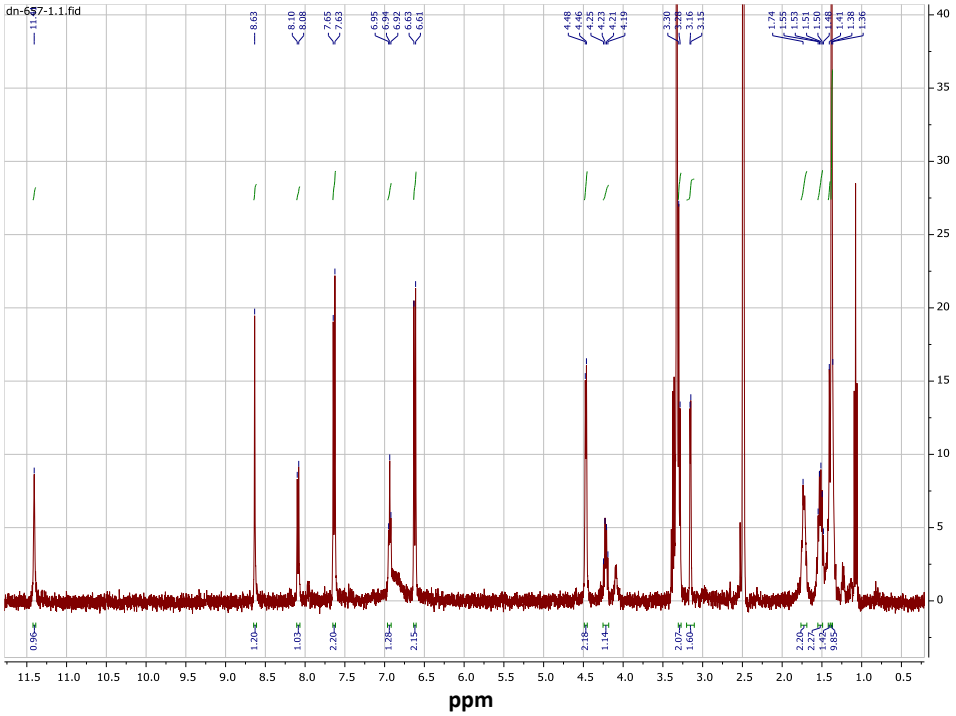

Figure S17. ^{1}H NMR spectra for compound **5**

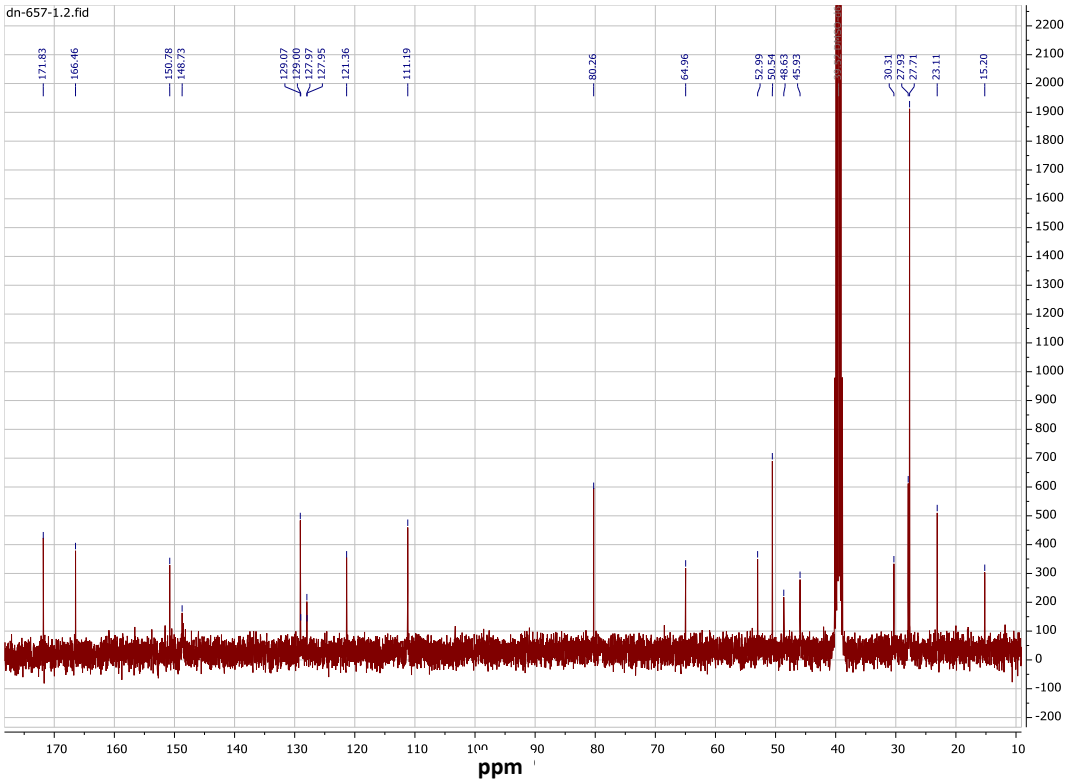

Figure S18. ^{13}C NMR spectra for compound **5**

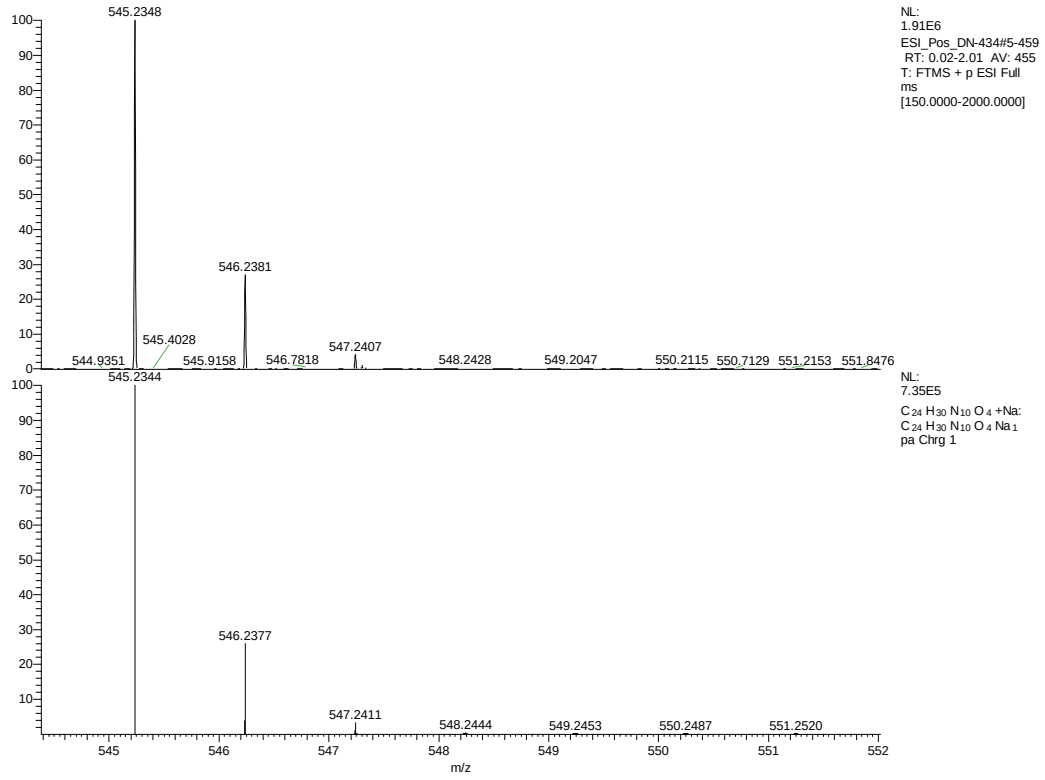

Figure S19. HRMS spectra for compound **5**

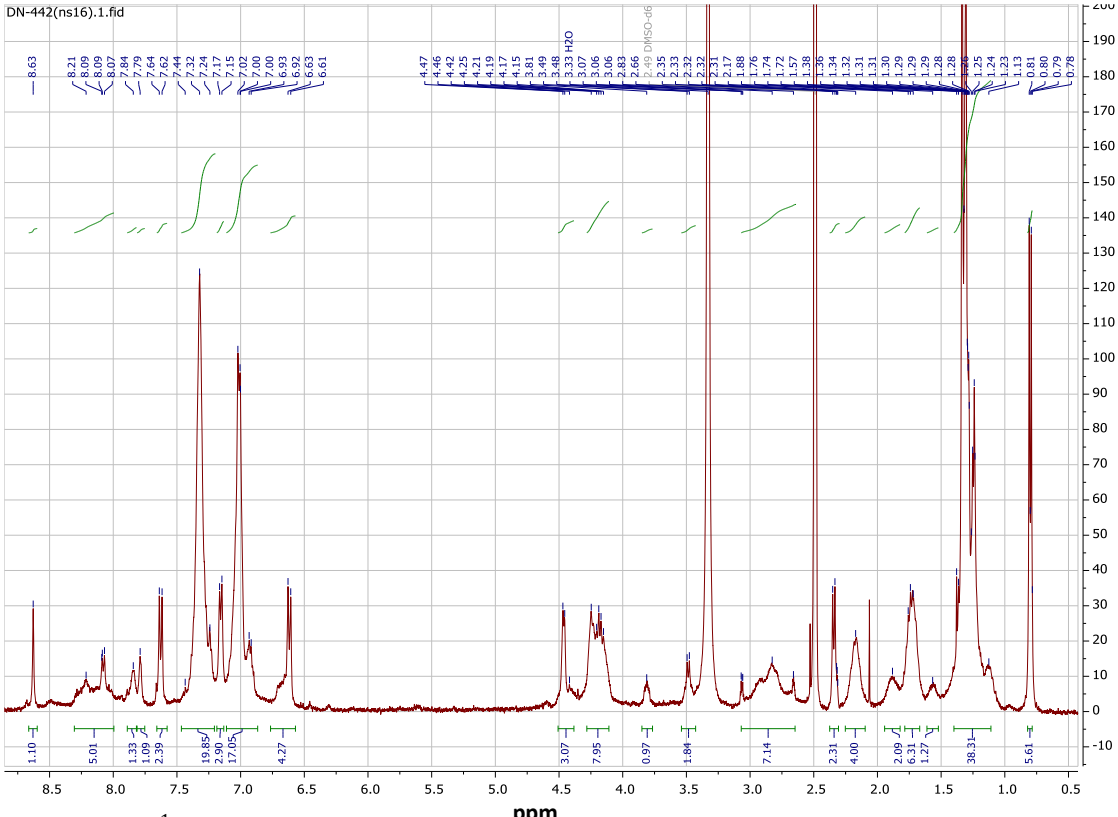

Figure S20. ^{1}H NMR spectra for compound **6**

HPLC-MS spectra for compound **6:**

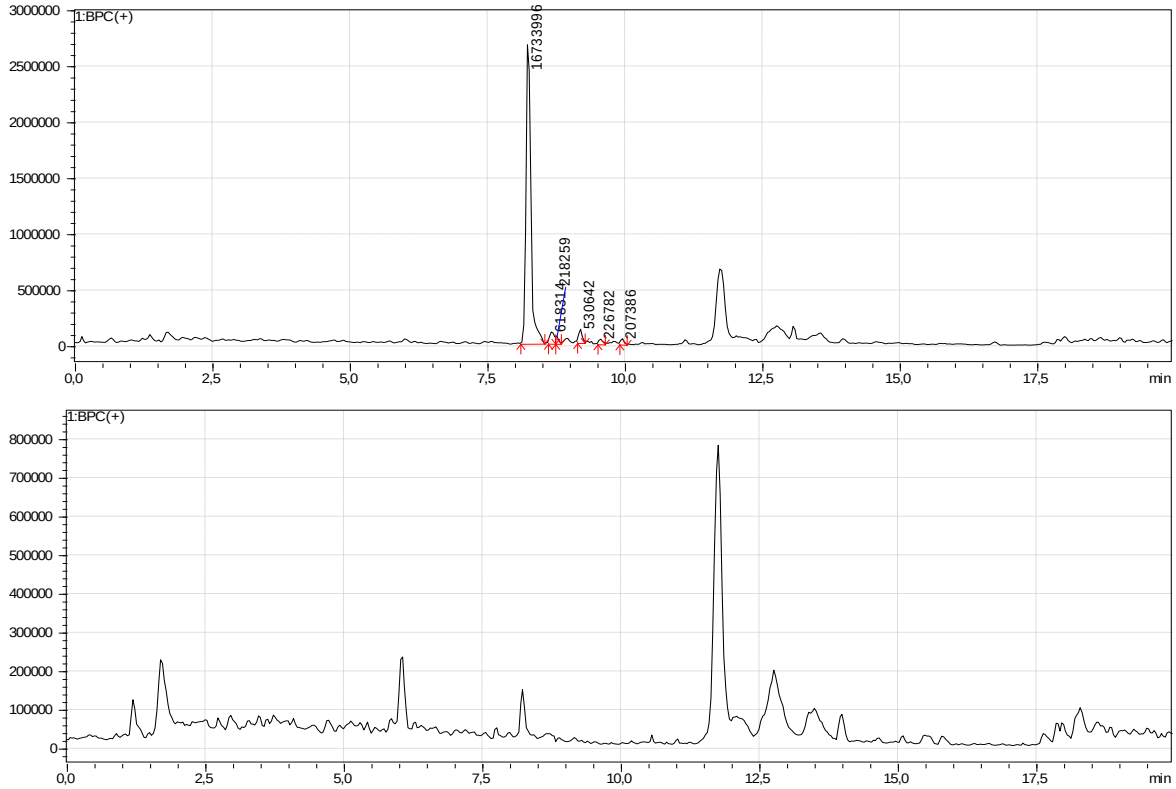

Figure S21. Positive ions (chromatogram and blank)

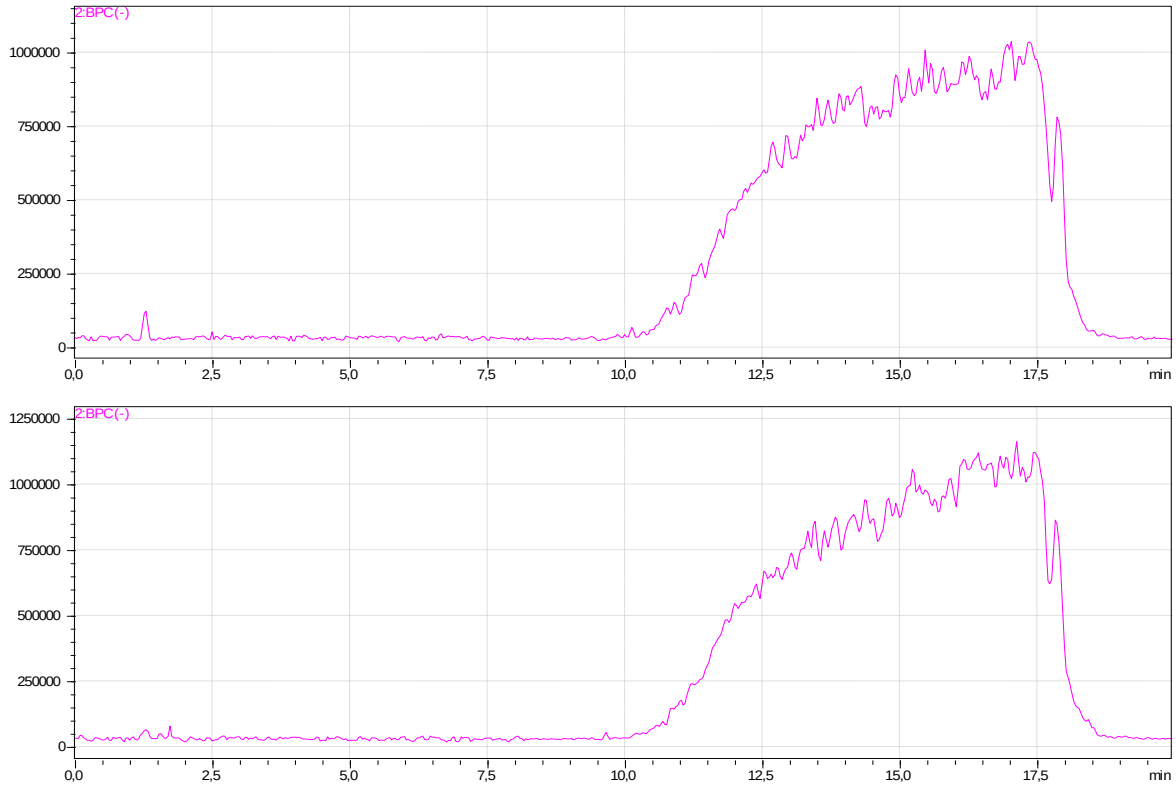

Figure S22. Negative ions (chromatogram and blank)

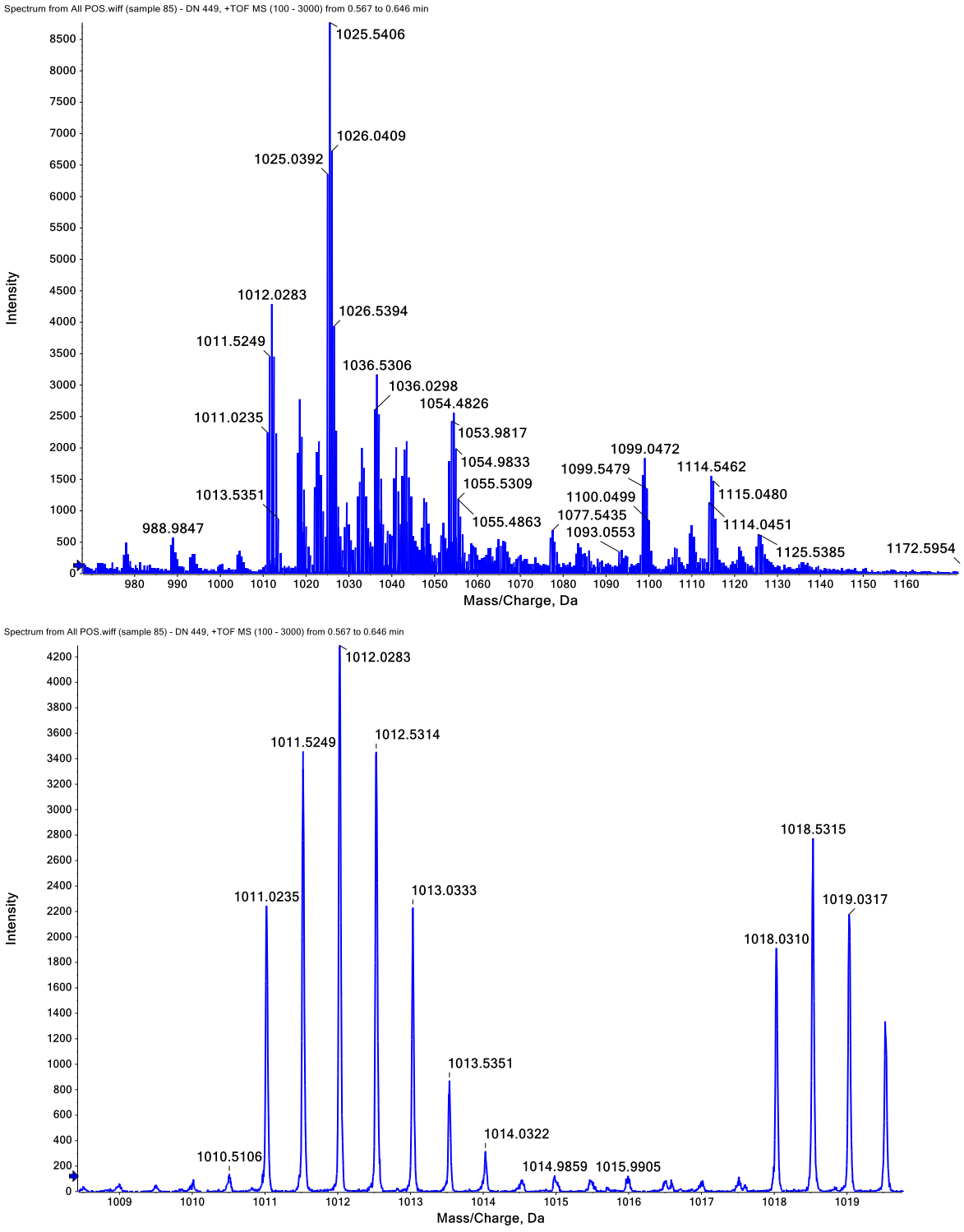

Figure S23. HRMS spectra for compound **6**

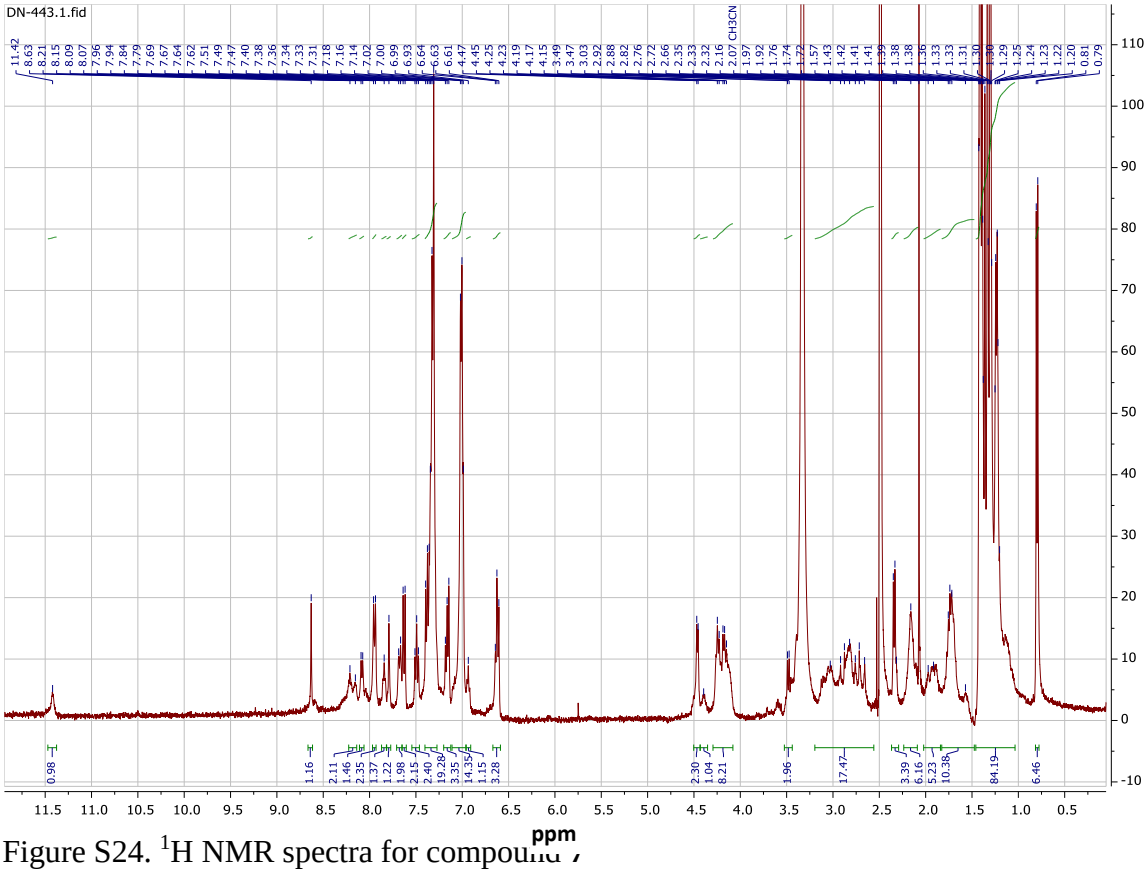

Figure S24. ^{1}H NMR spectra for compound **7**

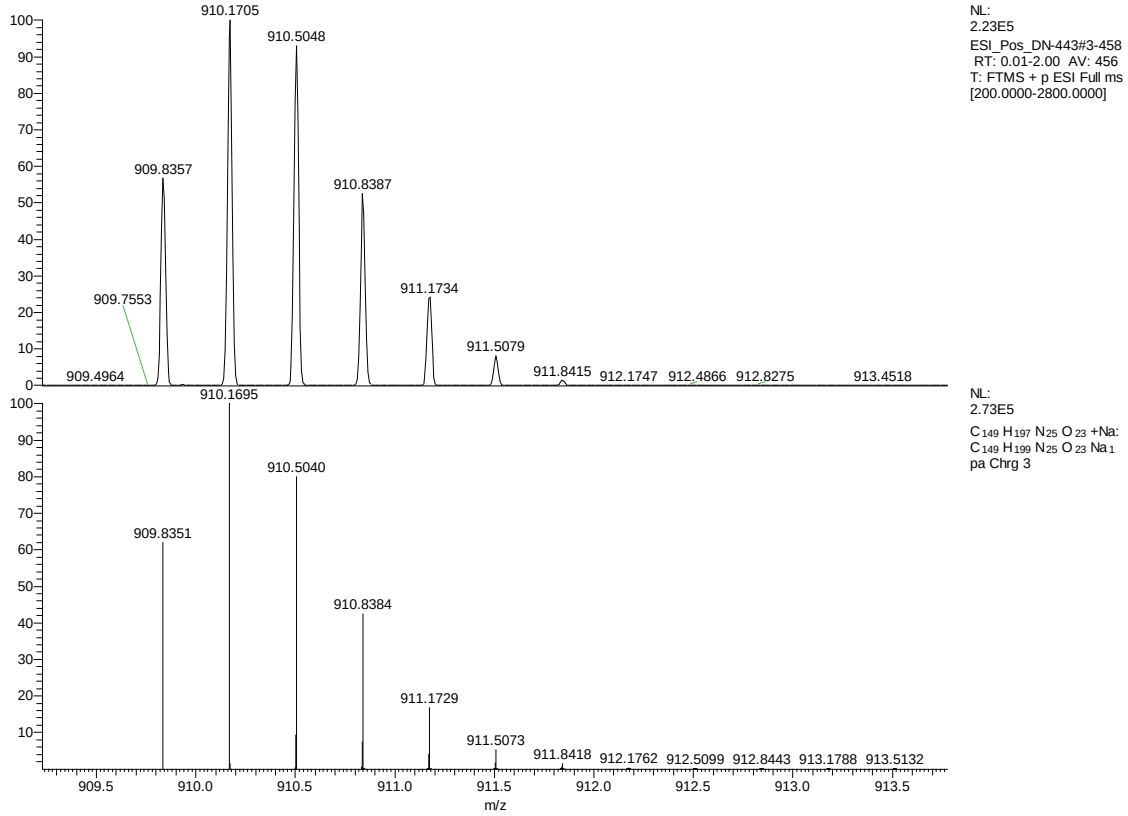

Figure S25. HRMS spectra for compound **7**

HPLC-MS spectra for compound **8:**

Method 1

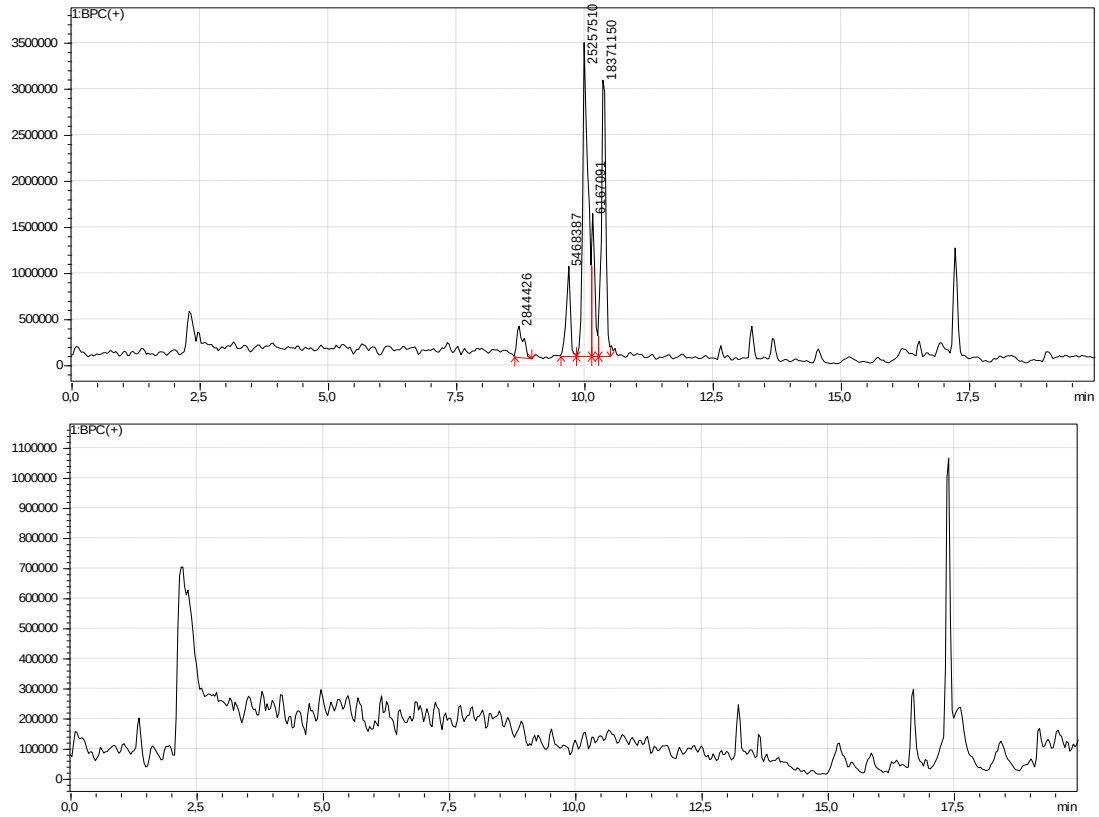

Figure S26. Positive ions (chromatogram and blank)

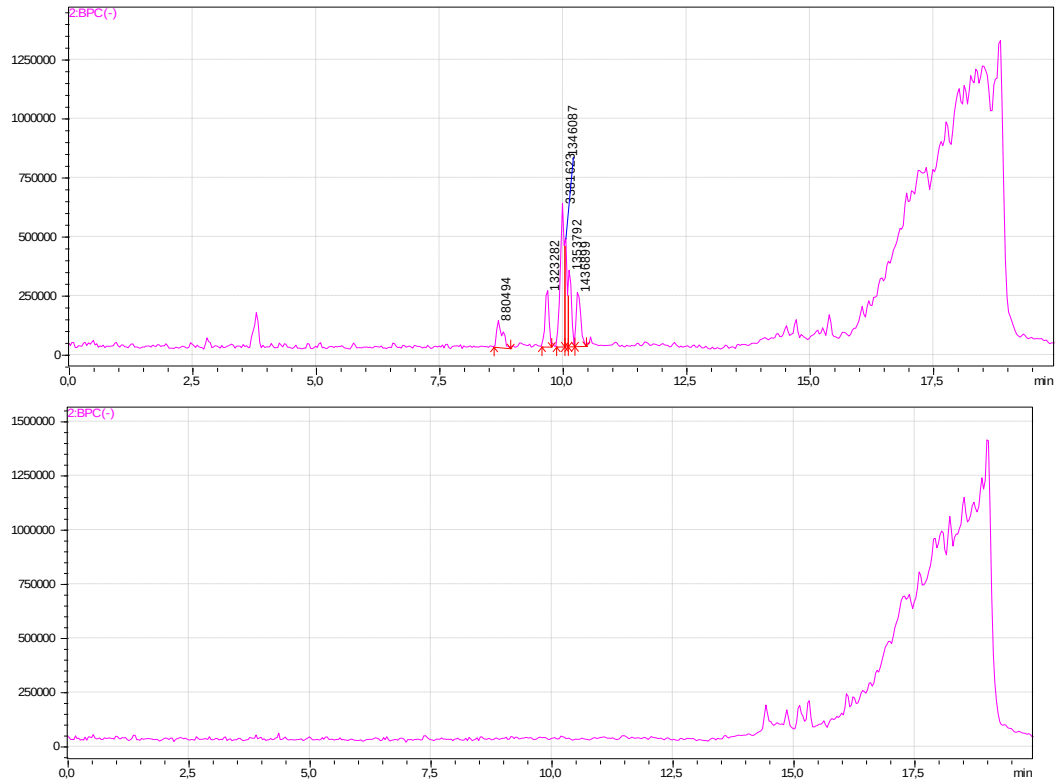

Figure S27. Negative ions (chromatogram and blank)

Method 2

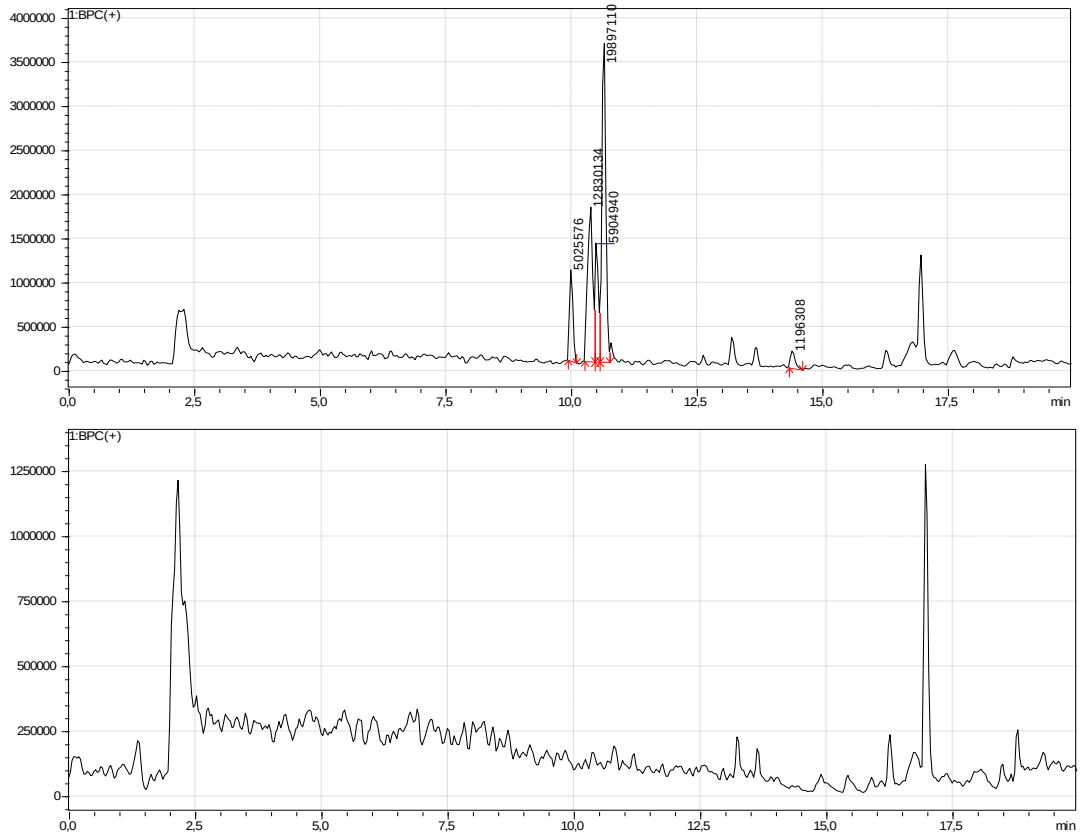

Figure S28. Positive ions (chromatogram and blank)

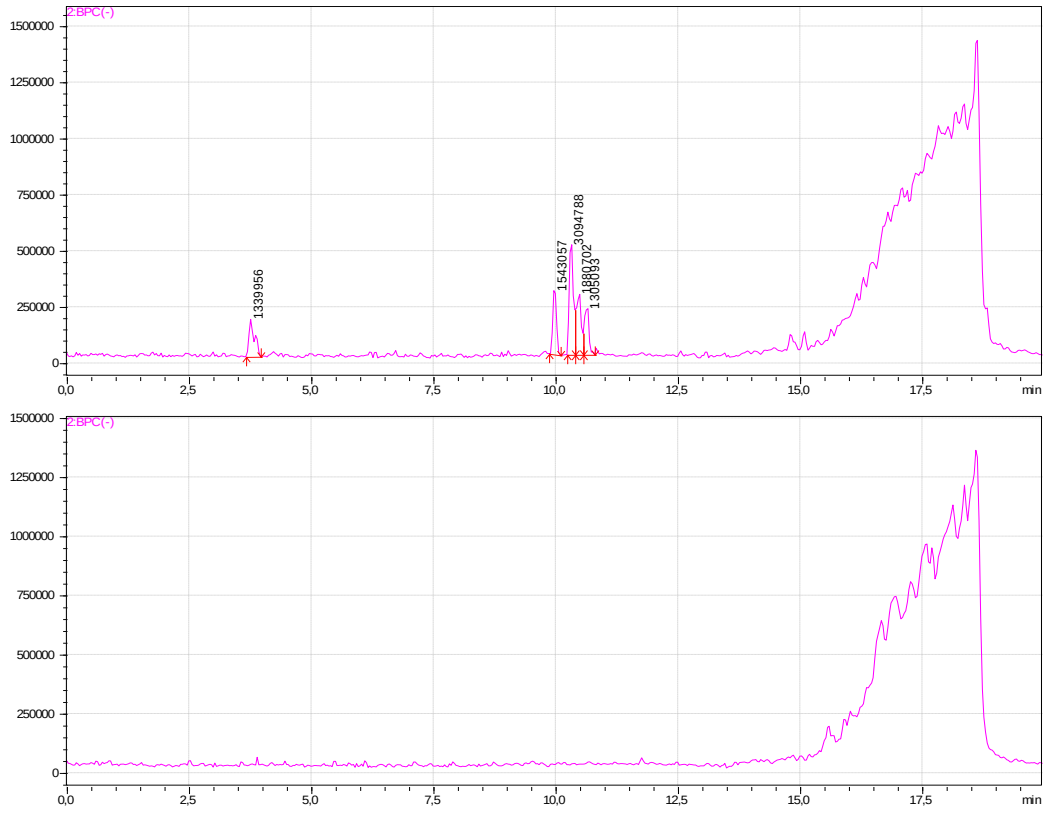

Figure S29. Negative ions (chromatogram and blank)

Method 3

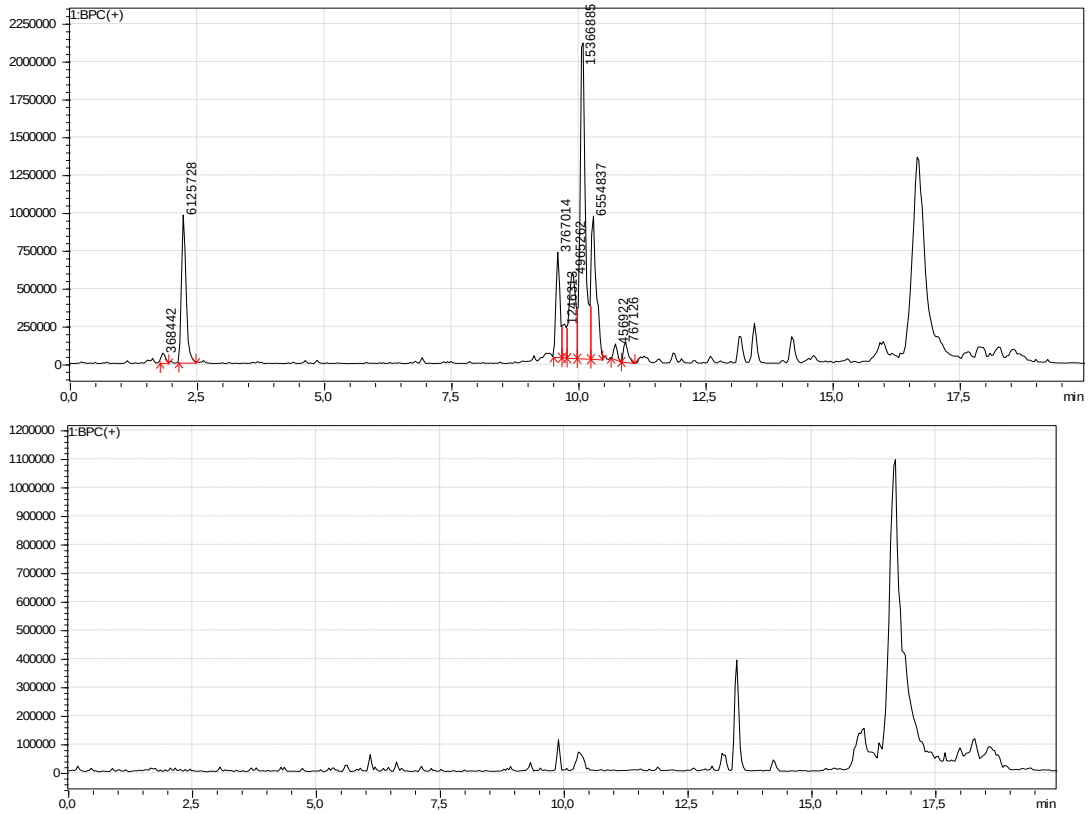

Figure S30. Positive ions (chromatogram and blank)

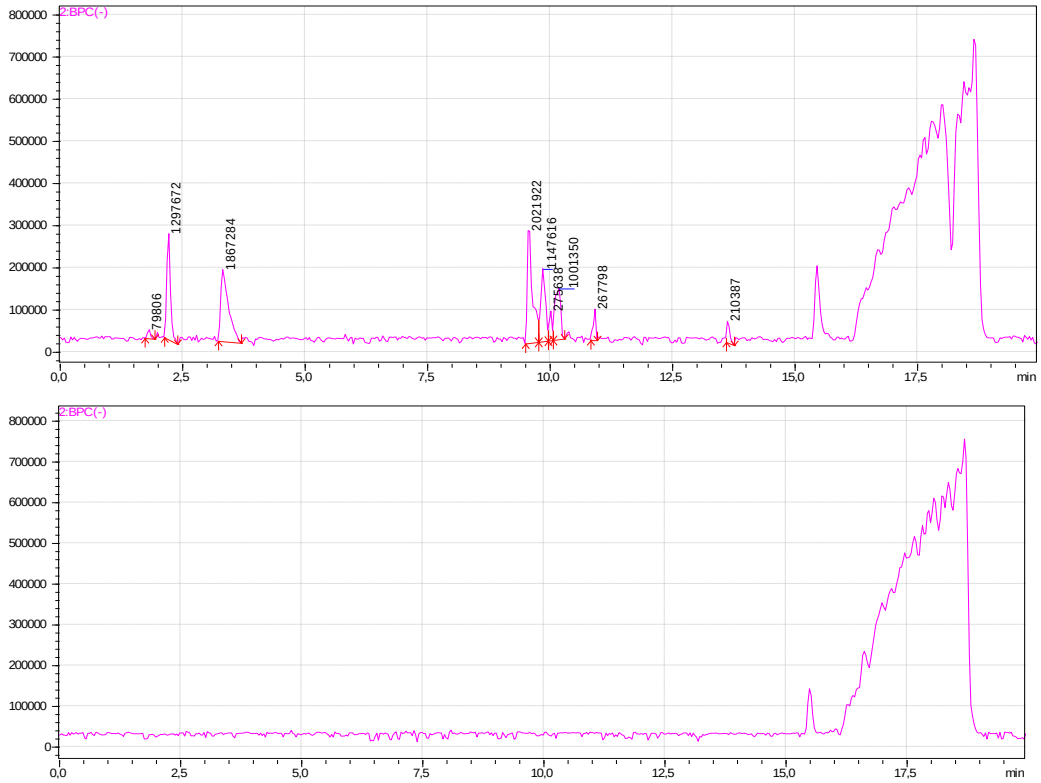

Figure S31. Negative ions (chromatogram and blank)

Method 4

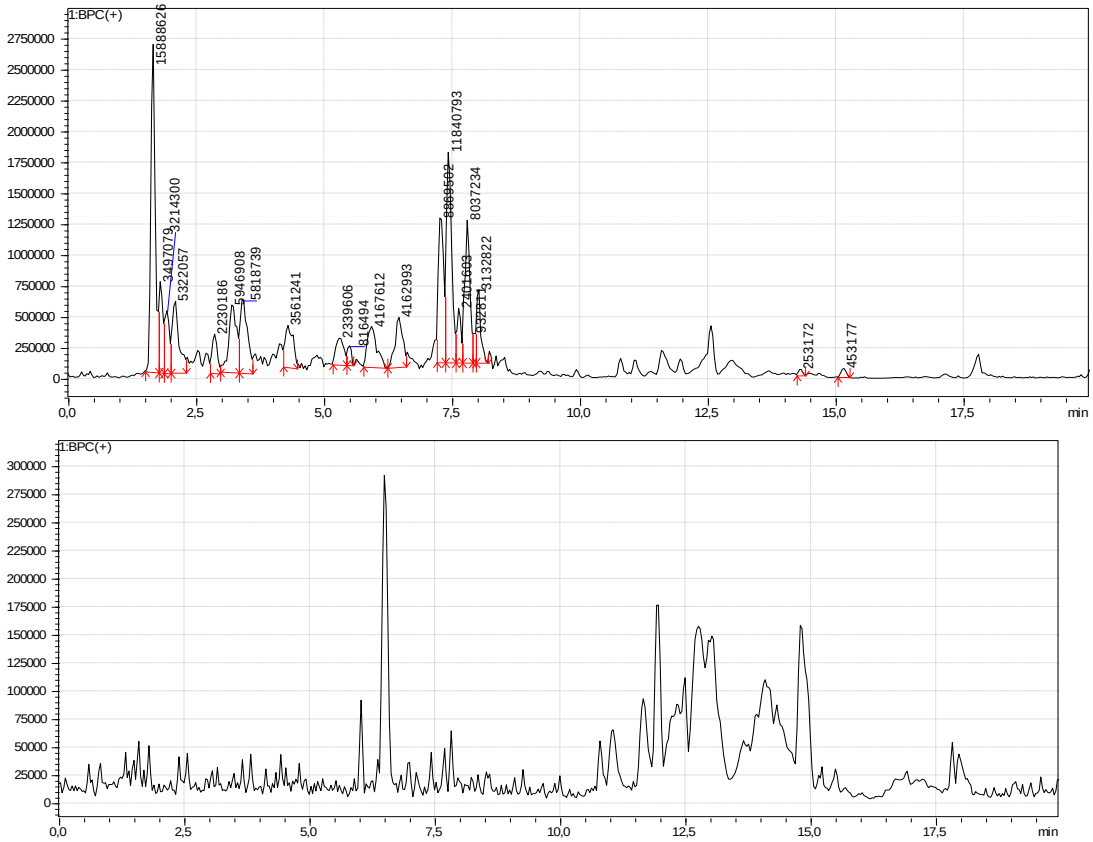

Figure S32. Positive ions (chromatogram and blank)

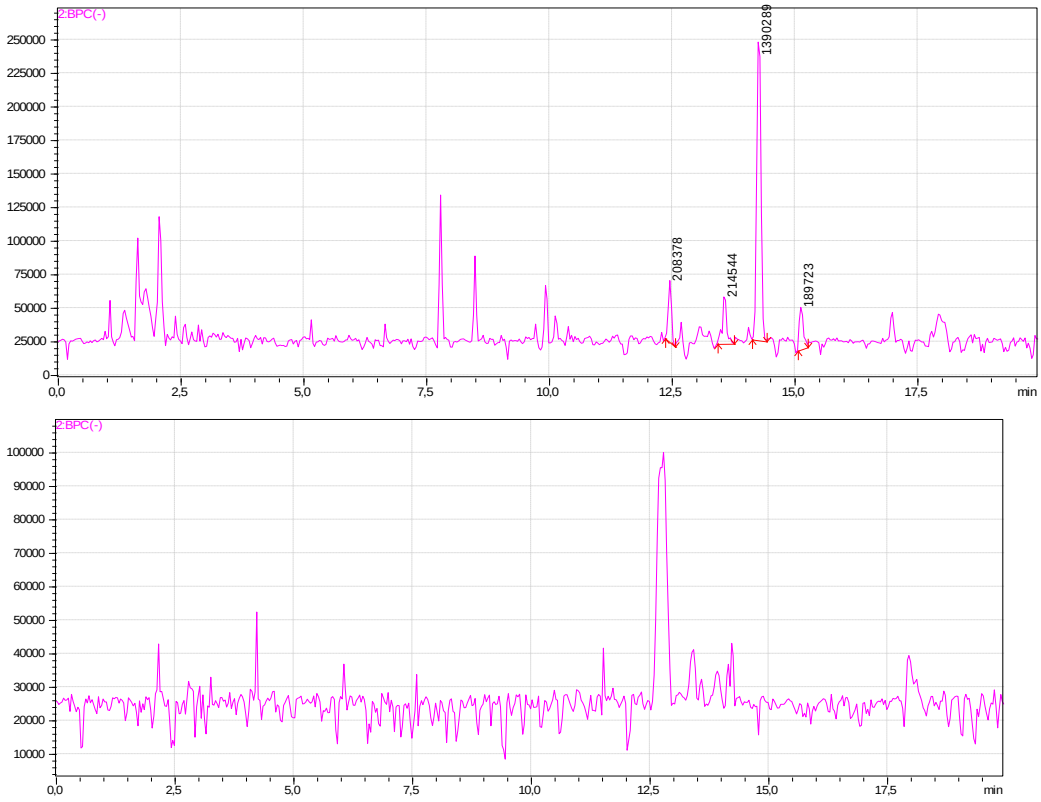

Figure S33. Negative ions (chromatogram and blank)

HPLC-MS spectra for compound **8** after preparative separation:

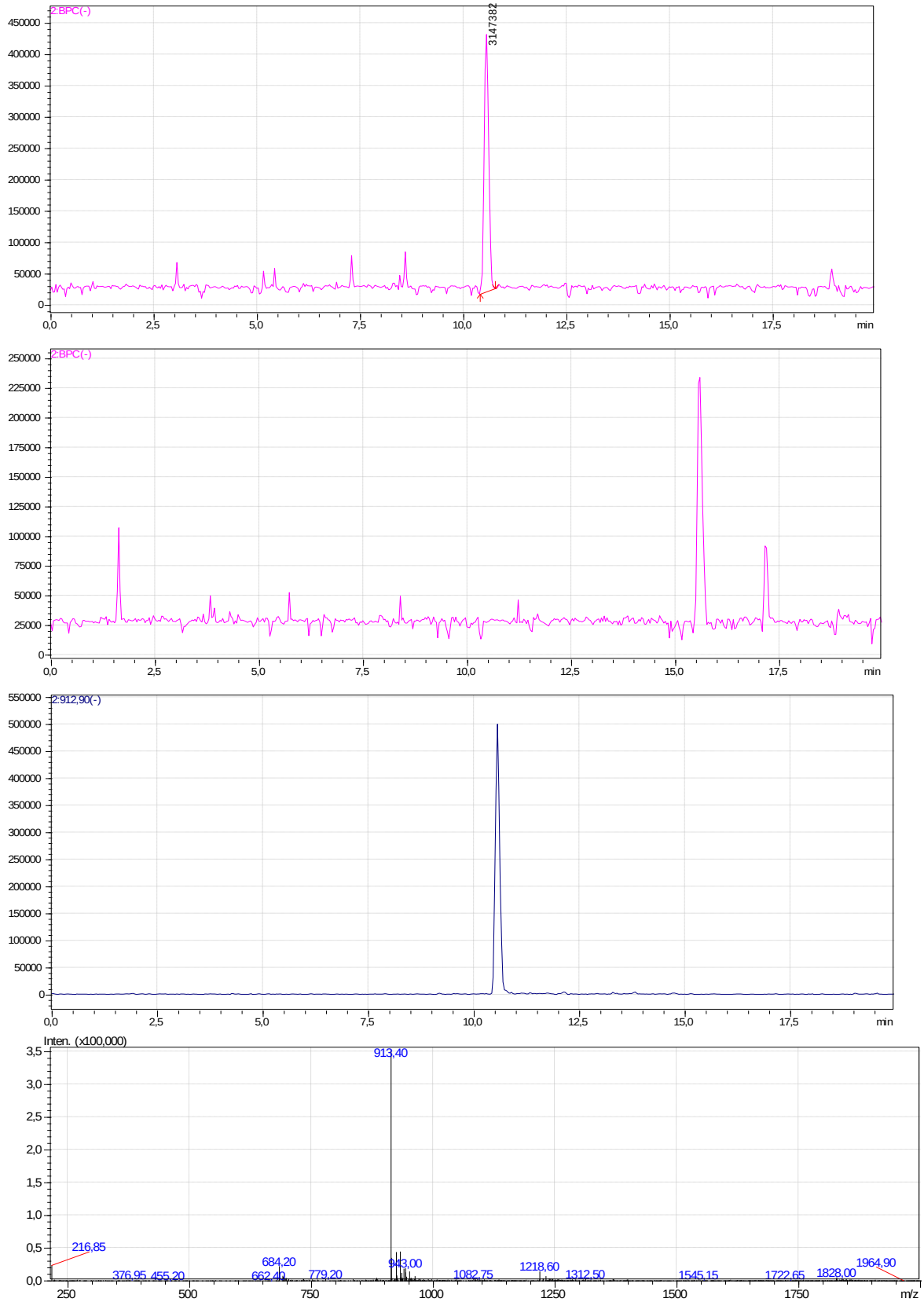

Figure S34. Negative ions (chromatogram, blank, extracted ion and mass spectra)

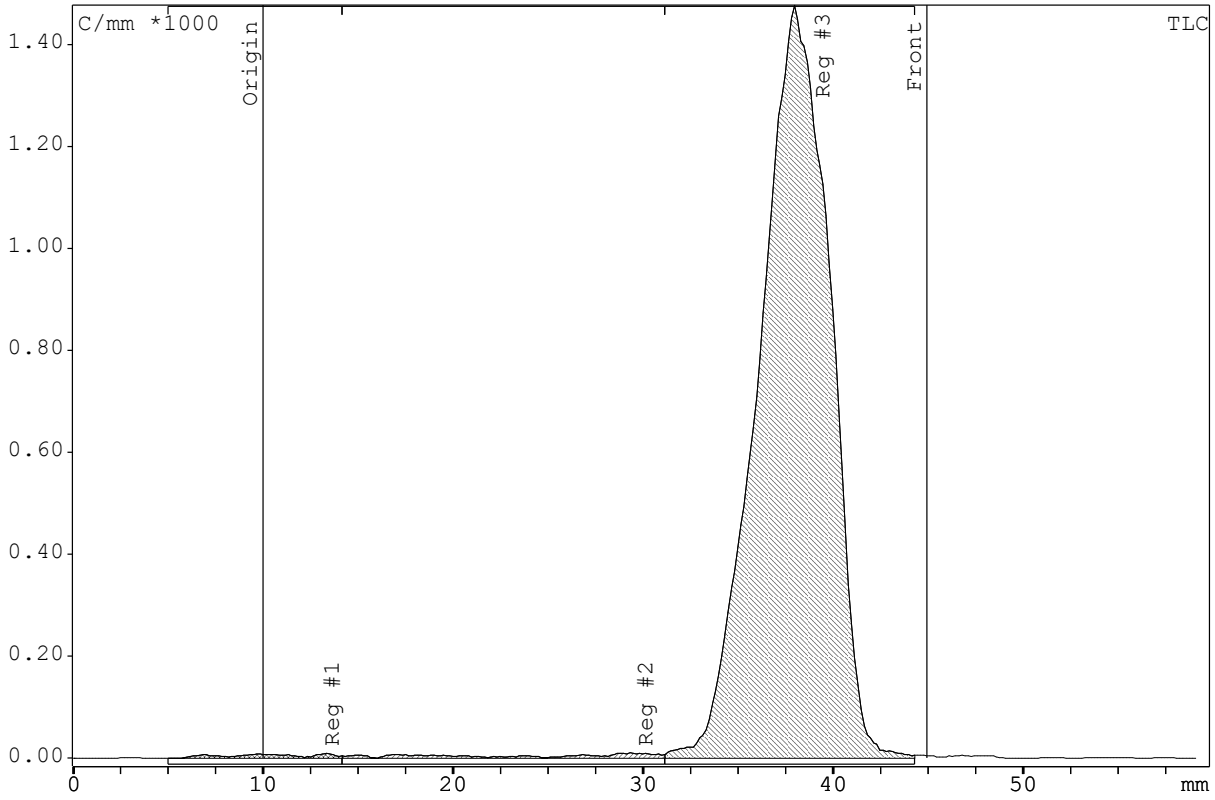

Figure S35. Radio-TLC chromatogram of radiolabeled conjugate [^{177}Lu]Lu-**8**, radiochemical conversion > 99% (reaction mixture analysis; *Rf* for [^{177}Lu]Lu-**8** is 0.8 ± 0.05, *Rf* for [^{177}Lu]Lu^{3+} is 0.0-0.1).

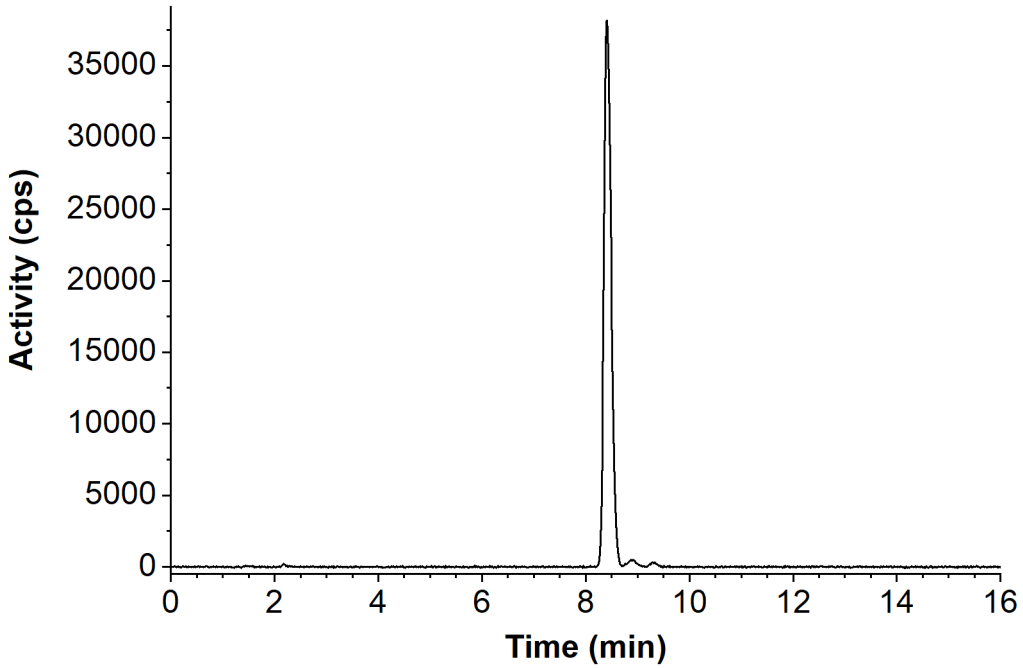

Figure S36. Radio-HPLC chromatogram of radiolabeled conjugate [^{177}Lu]Lu-**8**, radiochemical purity – 97.6% (reaction mixture analysis; *Rt* for [^{177}Lu]Lu-**8** is 8.35 min).