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Biotin-Conjugated *N*-Methylisatoic Anhydride a Chemical Tool for Nucleic Acid Separation by Selective 2'-Hydroxyl Acylation of RNA.

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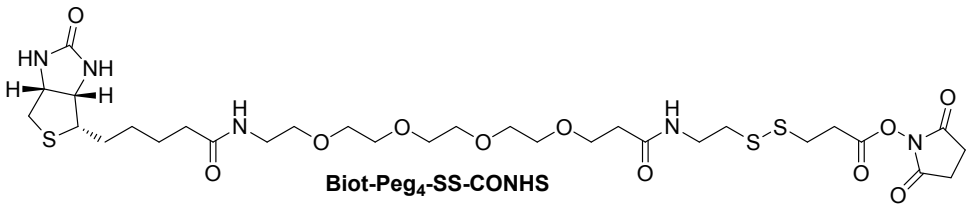
1) Table of Contents

2) General

Chemistry

NMR spectra were recorded in chloroform-*d* or DMSO-d_6 at 400 or 500 MHz for ^{1}H NMR spectra and 100 MHz or 125 MHz for ^{13}C NMR spectra. Chemical shifts were reported in ppm and multiplicities were described as follows: bs, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Coupling constants '*J*' were reported in Hz. IR spectra were recorded on KBr discs. Melting points were determined on a Kofler melting point apparatus. High resolution mass spectra were performed by positive or negative electrospray (HRMS/ESI). Melting points were determined on Kofler melting point apparatus. 2,5-dioxopyrrolidin-1-yl 3-{[2-(1-{5-[(3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}propanoate (**Biot-Peg$_4$-SS-CONHS**) was bought from quanta biodesign (USA). All commercially available compounds were used as received without further purification except THF which was distilled from sodium/benzophenone. Silica gel 0.06-0.2 mm-60 Å was used for all column chromatography except for compounds **6** and **7** where RP-18-40-63μm silica gel was used.



Nucleic acids tagging, extraction and amplification

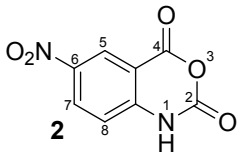
For synthetic RNA/DNA, tagging LC/MS analyses were performed on an Alliance HT Waters 2795 apparatus equipped with a 2996 UV diode-array detector, a ZQ2000 mass spectrometer and an Xterra C18 4.6*30 2.5μm analytical column. The following gradient was used A (98%)/B (0%)/C (2%) to A (24%)/B(74%)/C (2%) in 18 min (A: H_2O, B: MeCN, C: 500 mM aqueous ammonium formate solution) before returning to initial conditions in 2 min. 27-nt synthetic RNA from Eurogentec with the following sequence was used: 5'-AAC-CGC-AGU-GAC-ACC-CUC-AUC-AUU-ACA-3'. 27-nt synthetic DNA from Eurogentec with the following sequence was used: 5'- AAC-CGC-AGT-GAC-ACC-CTC-ATC-ATT-ACA -3'. For enzymatic digestion Nuclease P1 (NP1, 1U.μL^{-1}) and Phosphatase alkaline (AKP, 7U.μL^{-1}) were purchased from Sigma-Aldrich (ref N8630 and P7923 respectively).

HIV-transcript RNA (1083 nucleotides) was a purchased from bioMérieux (France, Nuclisens EasyQ VIH-1 v2.0, Ref 285036). Calf genomic DNA was purchased from Sigma-Aldrich (ref D4522). Buffer solutions and magnetic silica particles were purchased from bioMérieux. MagPrep® Streptavidin magnetic beads were purchased from Merck (72190). DynaMag stands were used as magnetic stands. Detection and quantification of biological nucleic acids was performed using a Qubit fluorometer (Q32857, Invitrogen) and Quant-iT kits (RNA assay kit 5-100ng, Q32855 ; dsDNA HS assay kit 0.2-100ng, Q32854).

RT-PCR experiments were performed on a Roche LightCycler 2.0 using a Roche LightCycler RNA Master HybProbe (03018954001). On-chip electrophoresis were performed using a 2100 Bioanalyzer® instrument (Agilent) and an Agilent DNA 1000 kit (5067-1504).

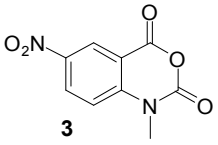
3) Synthesis of Compounds 2-7

6-Nitro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione 2[1]



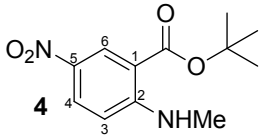
In a round bottom flask at 0°C were introduced 2-amino-5-nitrobenzoïc acid **1** (1.20 g, 6.59 mmol), THF (25 mL) and $COCl_2$ (4.16 mL, 7.91 mmol, 20% in PhMe). The mixture was allowed to stir at room temperature for 0.5 h and evaporated under reduced pressure. 30mL of PhMe were added and the solution was evaporated under reduced pressure. The resulting solid was then recristallized from MeCN (40 mL) to give 1.26 g of **2** (92%) as a yellow powder. mp > 260 °C; IR (KBr) *v* 3195, 3073, 1778, 1761, 1697, 1629, 1541, 1354, 1334, 1034, 855, 753, 658, 530 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.36 (bs, 1H, N*H*), 8.57 (m, 1H, *H5*), 8.52 (m, 1H, *H7*), 7.30 (d, ^3J = 8.9 Hz, *H8*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.9, 146.7, 146.3, 142.6, 131.4, 124.7, 116.9, 111.2. HRMS/ESI: calcd for $C_8H_5N_2O_5$ $[M+H]^+$ 209.0198, found 209.0188.

1-Methyl-6-nitro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione 3



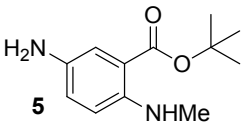
In a round bottom flask at room temperature were introduced **2** (2.00 g, 9.61 mmol), dry DMF (20 mL), DIEA (3.17 mL, 19.18 mmol) and MeI (1.80 mL, 28.91 mmol). The mixture was allowed to stir at room temperature for 1 h, cooled to 0°C and Et_2O (30 mL) was added. The precipitate was filtered and washed with Et_2O (3x30 mL). The filtrate was then dried with $MgSO_4$, concentrated and recristallized from MeCN (30 mL) to afford **3** (1.60 g, 75%) as a yellow powder. mp = 189-191 °C; IR (KBr) *v* 3075, 1792, 1732, 1614, 1599, 1526, 1497, 1469, 1338, 1309, 1135, 1037, 835, 750, 742, 670 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.62 (m, 1H, *H5*), 8.58 (m, 1H, *H7*), 7.64 (dd, ^3J = 9.2 Hz, ^5J = 1.5 Hz, 1H, *H8*), 3.53 (s, 3H, -NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.8, 147.3, 146.7, 142.5, 131.3, 124.5, 116.4, 112.4, 32.4; HRMS/ESI: calcd for $C_9H_7N_2O_5$ $[M+H]^+$ 223.0355, found 223.0363.

***tert*-Butyl 2-(methylamino)-5-nitrobenzoate 4**



In a round bottom flask at room temperature were introduced **3** (1.00 g, 4.5 mmol), DCM (40 mL) and *t*-BuONa (1.08 g, 11.25 mmol). The mixture was stirred at room temperature for 0.5 h and a 1M NaH_2PO_4 solution (50 mL) was added. The solution was then extracted with DCM (3x50 mL), dried with $MgSO_4$ and evaporated. The crude was purified by column chromatography using PE/Et_2O (9/1) as the eluant to afford **4** (1.02 g, 90%) as a yellow solid. mp = 137-138 °C; IR (KBr) *v* 3320, 2977, 2963, 2925, 1688, 1608, 1591, 1309, 1267, 1129, 1118, 1076, 864, 751, 709, 641 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, ^4J = 2.7 Hz, 1H, *H6*), 8.65 (bs, 1H, -N*H*Me), 8.19 (dd, ^3J = 9.4 Hz, ^4J = 2.7 Hz, 1H, *H4*), 6.63 (d, ^3J = 9.4 Hz, 1H, *H3*), 2.99 (d, ^3J = 5.1 Hz, 3H, -NHCH_3), 1.59 (s, 9H, -C(CH_3)$_3$; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 155.8, 135.8, 129.7, 129.3, 110.6, 110.4, 82.5, 29.7, 28.3 (3C); HRMS/ESI: calcd for $C_{12}H_{17}N_2O_4$ $[M+H]^+$ 253.1188, found 2253.1190.

***tert*-Butyl 5-amino-2-(methylamino)benzoate 5**

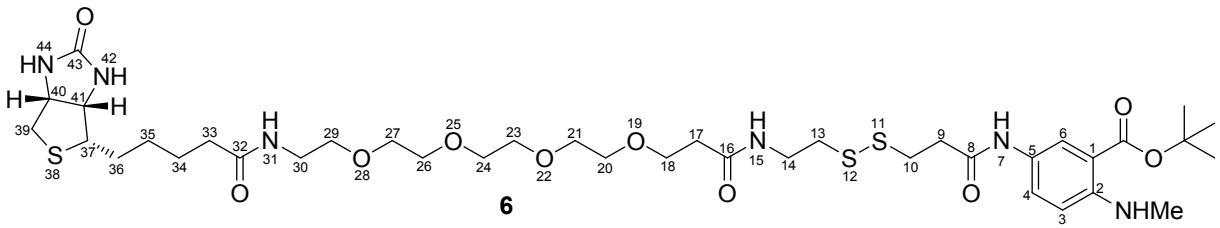


In a round bottom flask at room temperature under nitrogen were introduced **4** (1.75 g, 6.94 mmol), AcOEt (50 mL) and Pd/C (175 mg, 2.5 mol %). The flask was filled with hydrogen and stirred at room temperature for 15 h. The solution was filtered on celite and evaporated. The crude was purified by

[1] Jagattaran Das, Sitaram Kumar M., Subrahmanyam D., Sastry T. V. R. S., Prasad Narasimhulu C., Laxman Rao C. V., Kannan M., Roshaiah M., Riti Awasthi, Santosh N. Patil, Sarnaik H. M., Rao Mamidi N. V. S., Selvakumar N., Javed Iqbal, Substituent activity relationship studies on new azolo benzoxazepinyl oxazolidinones, Bioorganic & *Medicinal Chemistry*, **2006**, 14, 8032.

column chromatography using PE/Et_2O (7/3) as the eluant to afford **5** (1.54 g, 100%) as an orange oil. IR (KBr) *v* 3391, 2976, 1676, 1520, 1245, 1229, 1162, 813, 545 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 7.28 (s, 1H, *H6*), 6.87 (dd, ^3J = 8.7 Hz, ^4J = 2.4 Hz, 1H, *H4*), 6.55 (d, ^3J = 8.7 Hz, 1H, *H3*), 2.85 (s, 3H, -NHCH_3), 1.56 (s, 9H, C(CH_3)$_3$), NH signals are missing; ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 168.1, 147.4, 132.1, 124.5, 119.4, 112.1 (2C), 80.7, 30.0, 28.4 (3C); HRMS/ESI: calcd for $C_{12}H_{19}N_2O_2$ $[M+H]^+$ 223.1447, found 223.1447.

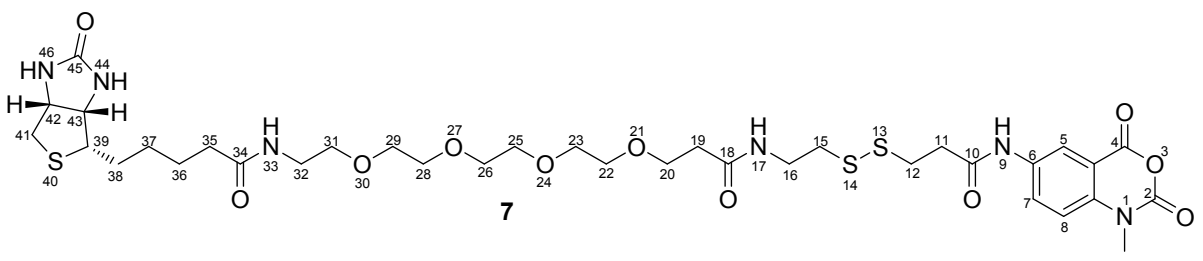
***tert*-Butyl 5-(3-{[2-(1-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}propanamido)-2-(methylamino)benzoate 6**



6

In a round bottom flask at room temperature were introduced **4** (0.30 g, 1.35 mmol), DCM (6 mL), NEt_3 (0.19 mL, 1.35 mmol) and Biot-Peg_4-SS-CONHS (1.01 g, 1.35 mmol). The mixture was stirred at room temperature for 2 h and evaporated. The crude was purified by reverse phase column chromatography using H_2O/MeCN (100/0 to 40/60 in 25 min) to afford **6** (0.96 g, 83%) as a pale yellow solid. mp was impossible to determine due to the extreme hygroscopic nature of **6**; IR (KBr) *v* 3377, 2923, 1698, 1676, 1645, 1552, 1519, 1246, 1159, 1087, 815 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 8,77 (bs, 1H, -N*H7*), 7.96 (d, ^4J = 1,8 Hz, 1H, *H6*), 7.62 (dd, ^3J = 7.2 Hz, ^4J = 1.8 Hz, 1H, *H4*), 7.51 (bs, 1H, -N*H*Me), 7.32 (t, ^3J = 4.5 Hz, 1H, -N*H15*), 6.98 (t, ^3J = 4.3 Hz, 1H, -N*H31*), 6.58 (d, ^3J = 7.2 Hz, 1H, *H3*), 6.48 (bs, 1H, -N*H42 or* –N*H44*), 5.58 (bs, 1H, -N*H42 or* –N*H44*), 4.46 (dd, ^3J = 6.0 Hz, ^3J = 4.0 Hz, 1H, *H40*), 4.27 (dd, ^3J = 5.9 Hz, ^3J = 4.0 Hz, 1H, *H41*), 3.71 (t, ^3J = 4.6 Hz, 2H, *H18*), 3.62-3.59 (m, 12H, *H20-27*), 3.56 (m, 2H, *H14*), 3.52 (t, ^3J = 4.1 Hz, 2H, *H29*), 3.39 (m, 2H, *H30*), 3.10 (m, 1H, *H37*), 3.07 (t, ^3J = 5.9 Hz, 2H, *H9*), 2.88-2.84 (m, 6H, -NHCH_3-*H13-H39a*), 2.75 (t, ^3J = 5.9 Hz, 2H, *H10*), 2.70 (d, ^2J = 10.0 Hz, 1H, *H39b*), 2.47 (t, ^3J = 4.6 Hz, 2H, *H17*), 2.20 (t, ^3J = 6.0 Hz, 2H, *H33*), 1.73-1.59 (m, 4H, *H34-H36*), 1.54 (s, 9H, -C(CH_3)$_3$), 1.43-1.37 (m, 2H, *H35*); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 173.7 (*C32*), 172.2 (*C16*), 169.6 (*C8*), 168.0 (*C*O_2tBu), 164.1 (*C43*), 149.4 (*C2*), 128.1 (*C4*), 126.0 (*C5*), 123.9 (*C6*), 111.2 (2C, *C1-C3*), 111.0 (*C*$(CH_3)_3$), 80.9 (*C20-27*), 70.5 (2C, *C20-27*), 70.4 (*C20-27*), 70.3 (*C20-27*), 70.1 (*C20-27*), 70.1 (*C29*), 67.3 (*C18*), 61.9 (*C41*), 60.3 (*C40*), 55.7 (*C37*), 40.6 (*C39*), 39.3 (*C30*), 38.5 (2C, *C13-C14*), 37.4 (*C10*), 36.9 (*C17*), 35.9 (*C33*), 34.8 (*C9*), 29.8 (-NH*C*H_3), 28.4 (3C, C(*C*H_3)$_3$), 28.3 (*C35*), 28.2 (*C36*), 25.7 (*C34*); HRMS/ESI: calcd for $C_{38}H_{63}N_6O_{10}S_3$ $[M+H]^+$ 859.3768, found 859.3785.

1-{5-[(3a*S*,4*S*,6a*R*)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-*N*-[2-({2-[(1-methyl-2,4-dioxo-1*H*-benzo[*d*][1,3]oxazin-6-yl)carbamoyl]ethyl}disulfanyl)ethyl]-3,6,9,12-tetraoxapentadecan-15-amide 7



7

In a round bottom flask at room temperature were introduced **6** (0.55 g, 0.64 mmol), DCM (10 mL) and $COCl_2$ (0.34 mL, 0.64 mmol). The mixture was stirred at room temperature for 0.25 h and evaporated. The crude was purified by reverse phase column chromatography using H_2O/MeCN (100/0 to 50/50 in 20 min) to afford **7** (0.37 g, 70%) as a pale yellow solid. mp was impossible to determine due to the extreme hygroscopic nature of **7**; IR (KBr) *v* 3401, 2923, 1778, 1723, 1688, 1650, 1554, 1507, 1468, 1309, 1081, 1039, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 10.03 (bs, 1H, -N*H9*), 8.35 (dd, ^3J = 7.2 Hz, ^4J = 1.9 Hz, 1H, *H7*), 8.15 (d, ^4J = 1.9 Hz, 1H, *H5*), 7.43 (t, ^3J = 4.6 Hz, 1H, -N*H17*), 7.27 (d, ^3J = 7.2 Hz, 1H, *H8*), 7.04 (t, ^3J = 4.2 Hz, 1H, -N*H33*), 6.50 (bs, 1H, -N*H44* or -N*H46*), 5.70 (bs, 1H, -N*H44* or -N*H46*), 4.50 (m, 1H, *H42*), 4.31 (m, 1H, *H43*), 3.74 (t, ^3J = 4.6 Hz, 2H, *H20*), 3.62-3.60 (m, 12H, *H22-29*), 3.57-3.53 (m, 7H, -NC*H*$_3$-*H16-H31*), 3.40 (m, 2H, *H32*), 3.12 (m, 1H, *H39*), 3.07 (t, ^3J = 5.7 Hz, 2H, *H11*), 2.89-2.85 (m, 3H, *H15-H41a*), 2.83 (t, ^3J = 5.7 Hz, 2H, *H12*), 2.72 (d, ^2J = 10.2 Hz, 1H, *H41b*), 2.51 (m, 2H, *H19*), 2.21 (t, ^3J = 5.9 Hz, 2H, *H35*), 1.73-1.57 (m, 4H, *H36-H38*), 1.43-1.37 (m, 2H, *H37*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8 (*C34*), 172.5 (*C18*), 170.5 (*C10*), 164.2 (*C45*), 158.6 (*C4*), 148.0 (*C2*), 137.7 (*C8a*), 135.9 (*C6*), 129.2 (*C7*), 120.1 (*C5*), 114.7 (*C8*), 111.7 (*C4a*), 70.5 (3C, *C22-29*), 70.4 (*C22-29*), 70.3 (*C22-29*), 70.1 (*C22-29*), 70.0 (*C31*), 67.3 (*C20*), 61.9 (*C43*), 60.3 (*C42*), 55.7 (*C39*), 40.7 (*C41*), 39.4 (*C32*), 38.8 (*C16*), 38.5 (*C15*), 37.6 (*C12*), 37.0 (*C19*), 36.0 (*C35*), 35.1 (*C11*), 32.0 (-N*C*H$_3$), 28.3 (*C37*), 28.2 (*C38*), 25.8 (*C36*); HRMS/ESI: calcd for $C_{35}H_{53}N_6O_{11}S_3$ $[M+H]^+$ 829.2934, found 829.2946.

4) Synthetic RNA/DNA Tagging Experiments

For the tagging of synthetic RNA or DNA, the following two-step procedure (tagging/enzymatic hydrolysis) was achieved in triplicate and results are given as mean of these three experiments:

RNA or DNA tagging. In a 2mL eppendorf tube were introduced RNA or DNA (27 nucleotides-8nmol), 40µL of water, 20µL of triethylammonium acetate buffer (1M in water, pH = 7) and 20µL of **7** (120mM in DMSO). The eppendorf tube was incubated at 65 °C for 1h. The crude was precipitated in 212µL of water, 18µL of $LiClO_4$ (3M solution in water), 900µL of acetone and the supernatant was removed (This operation was repeated twice). The resulting pellet was diluted with acetone (900 µL) and dried with a speedvac concentrator.

Enzymatic hydrolysis. The reaction mixture was diluted in 33µL of H_2O/DMSO (85/15). 2.5µL of the solution were diluted in 17.5µL of H_2O/DMSO (1/1) and the obtained solution (20µL) was injected in the LC/MS. To the remaining reaction mixture was then added 2µL of NP1 (1U/µL) and 1µL of AKP (7U/µL), the mixture was incubated at room temperature for 3 hours. Finally, 20µL of the crude were directly injected in the LC/MS (Figure 2).

LC/MS ratio determination. For each experiments after DNA or RNA tagging and enzymatic hydrolysis, different tagged nucleosides (Figure 1) and free nucleosides were detected by LC/MS (Figure 2). For each experiments, the ratio was calculated at 260nm (UV absorbance) by dividing the area of each peak (tagged or free nucleosides) by the total area of tagged and free nucleosides (Formula 1.).

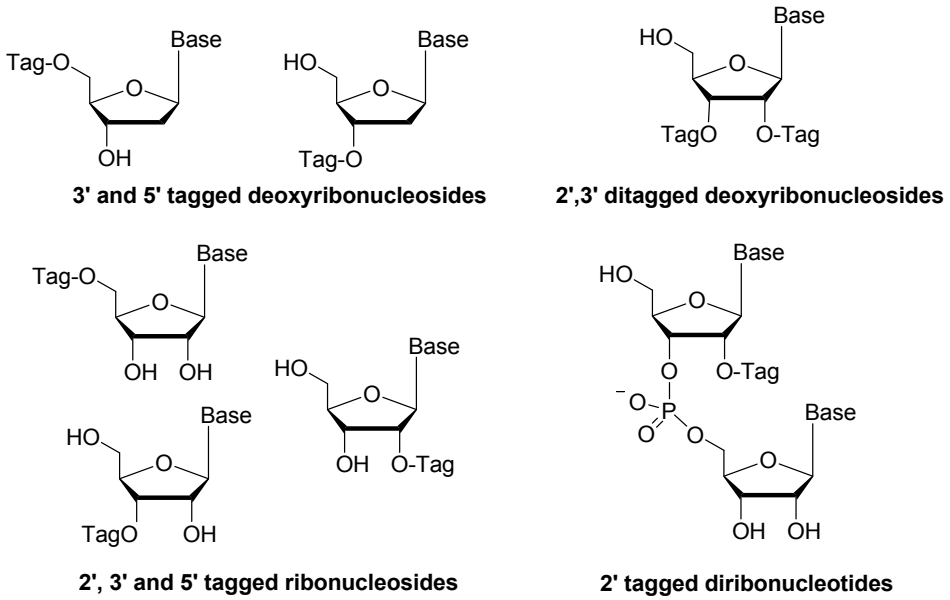


Figure 1. Tagged nucleosides observed by LC/MS.

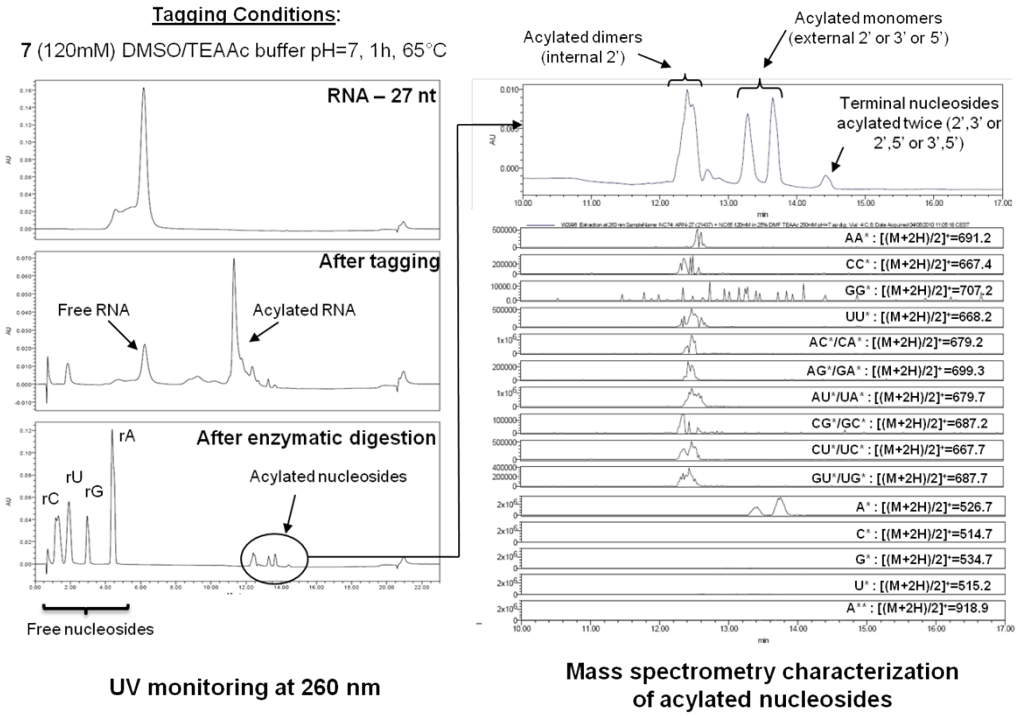


Figure 2. Example of LC/MS analysis after tagging and enzymatic hydrolysis of RNA with **7** (120 mM).

Formula 1.

$$\text{Free nucleosides Ratio} = \frac{Free\ nucleosides\ peaks\ Area}{(Free\ nucleosides\ peak\ area + tagged\ nucleosides\ peak\ area)} \times 100$$

$$\text{Tagged nucleosides Ratio} = \frac{Tagged\ nucleosides\ peak\ area}{(Free\ nucleosides\ peak\ area + tagged\ nucleosides\ peak\ area)} \times 100$$

5) Extraction of Biological RNA

RNA or DNA tagging. In a 250µL eppendorf tube were introduced nucleic acids, triethylammonium acetate buffer (1M in water, pH = 7) and **7** (in DMSO), quantities for each experiments are reported in Table 1. The eppendorf tube was incubated at 65 °C for 1h and tag excess was removed using the following procedure.

The samples were transferred to a 1.5mL eppendorf tube. To respect the binding capacity of magnetic silica particles used during the purification step (1mg of particle for 2µg of nucleic acids), experiments 3 and 4 were divided in four 2.1µL samples and experiment 5 was divided in five 2.1µL samples. 900µL of buffer I (EasyMAG buffer 280134, Biomérieux) and 50µL of magnetic silica particles (1mg, EasyMAG silica 280133, Biomérieux) were added. The solution was vortexed immediately, incubated for 10 minutes at room temperature, magnetized using a magnetic stand and the supernatant was removed. 500µL of buffer II (EasyMAG buffer 280130, Biomérieux) were added. The solution was vortexed, magnetized using a magnetic stand and the supernatant was removed. 900µL of wash buffer III (EasyMAG buffer 280131, Biomérieux) were added. The solution was vortexed, magnetized using a magnetic stand and the supernatant was removed. 500µL of wash buffer III were added. The solution was vortexed, magnetized using a magnetic stand and the supernatant was removed. 500µL of buffer IV (EasyMAG buffer 280132, Biomérieux) were added. The solution was vortexed, magnetized using a magnetic stand and the supernatant was removed. 20µL of buffer IV were added and the mixture was stirred at 70°C and 1400rpm for 5 minutes. The solution was magnetized using a magnetic stand and the supernatant collected.

Table 1. Concentrations and volumes of reagent for nucleic acids tagging.

Experiment	RNA (HIV transcript)		DNA (calf genomic DNA)		RNA/DNA (9/1)		TEAAc Buffer		**7** in DMSO		
	C ($\mu g.\ \mu L^{-1}$)	V (µL)	C ($\mu g.\ \mu L^{-1}$)	V (µL)	C ($\mu g.\ \mu L^{-1}$)	V (µL)	C (M)	V (µL)	C (mM)	V (µL)	Cf* (mM)
1	0.5	4	-	-	-	-	1	2	6	2	1.5
2	0.5	4	-	-	-	-	1	2	120	2	30
3	-	-	1.9	5	-	-	1	2.5	6	2.5	1.5
4	-	-	1.9	5	-	-	1	2.5	120	2.5	30
5	-	-	-	-	1.9	6	1	3	30	3	1.5

***** Cf = Tag final concentration

Streptavidin capture. 40µg of MagPrep-25 particles were introduced in a 0.2mL tube and washed twice with 80µL of PBS (1x)+SDS (0.1%). 5µL of PBS (4x)+SDS (0.4%) solution and 15µL of the previously obtained eluates were added, the tube was gently stirred during 10 minutes at room temperature, magnetized using a magnetic stand and the supernatant was removed.

DTT cleavage. The previously obtained pellet was diluted with 80µL of a solution of PBS (1x)+SDS (0.1%) for experiments 1-4 or a solution of PBS (1x)+Triton X-100 (0.05%) for experiment 5, heated at 65 °C for 5 minutes, magnetized using a magnetic stand and the supernatant was removed (this operation was repeated twice). For experiments 1-2, the obtained pellets were diluted with 80µL of PBS (1x) solution, magnetized using a magnetic stand and the supernatants were removed. For each experiments divided on the purification step (experiments 3-5), the obtained pellets were diluted with 20µL of PBS (1x) solution, gathered, magnetized using a magnetic stand and the supernatants were removed. 8µL of DTT solution (100 mM in PBS (1x)) were added and the mixtures were stirred

at 40°C for 1h. The mixtures were magnetized using a magnetic stand and the supernatants were collected and analyzed by fluorometry to determine nucleic acids quantities.

6) Amplification of Tagged Biological RNA

HIV-transcript RNA was tagged with 15 mM of **7**, captured with streptavidin and cleaved with DTT using the procedure described in §5). 1000, 100 and 50 tagged RNA solutions were prepared by dilution and RT-PCR was performed for all the samples (Figure 3).

Reverse transcription was performed at 45 °C for 20 min followed by 0.5 min of incubation at 95 °C for the denaturation. The following condition of thermal cycling was used for amplification: PCR amplification, 60 cycles at 95 °C for 5 sec, at 55 °C for 15 sec and at 65 °C for 15 sec and cooling at 40 °C for 30 sec.

RT-PCR amplicons were checked by on-chip electrophoresis using a 2100 Bioanalyzer® instrument (Agilent) with an Agilent DNA 1000 kit (Figure 4).

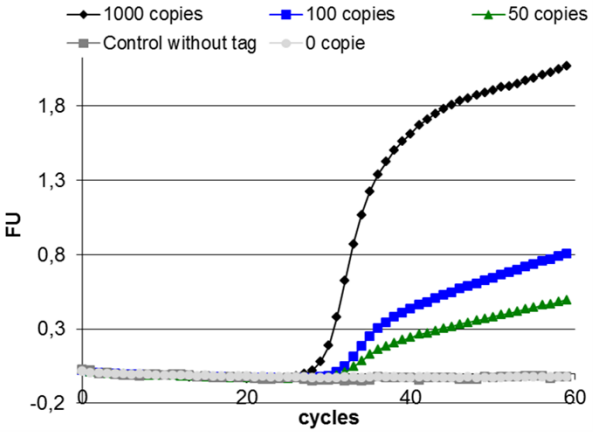


Fig. 3a: Control experiments with non-acylated HIV transcripts

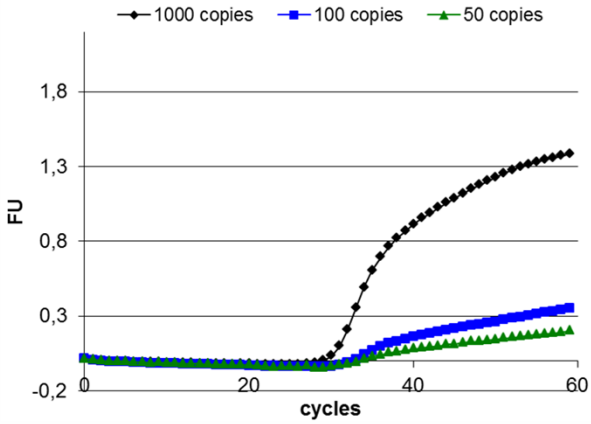


Fig. 3b: RT-PCR with tagged HIV transcripts

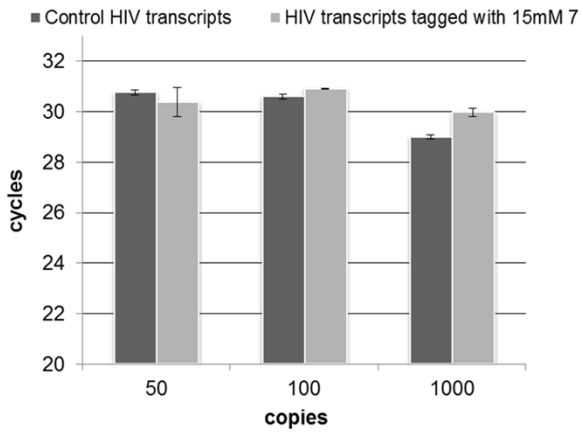


Fig. 3c: Cycle Treshold (CT)

Figure 3. RT-PCR amplification of extracted HIV RNA

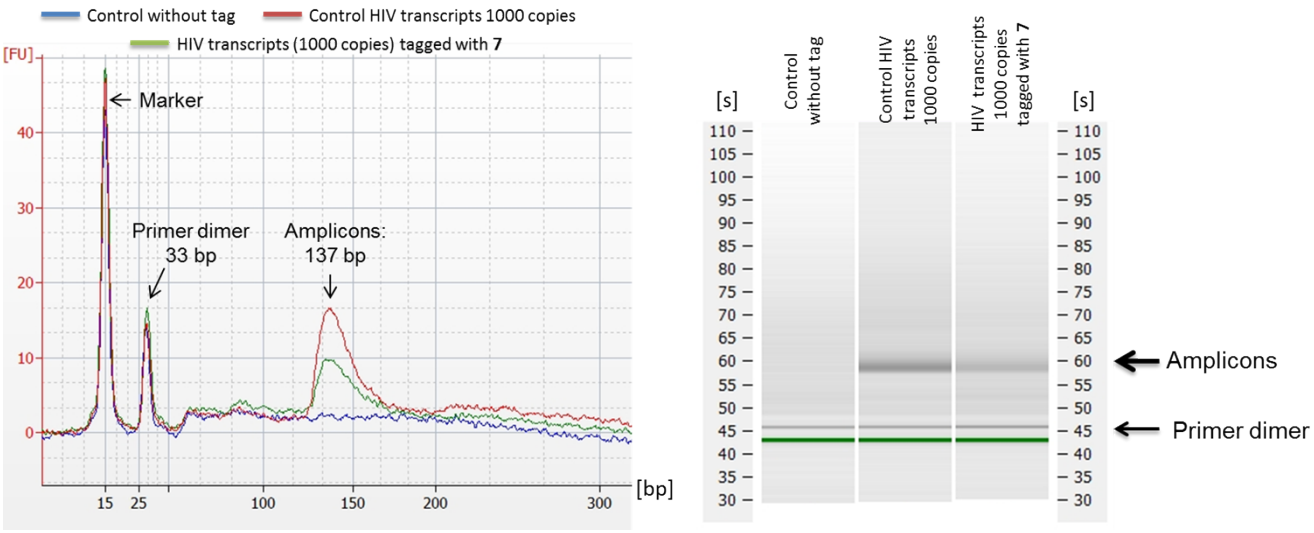
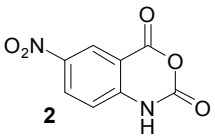


Figure 4. RT-PCR amplicons – On-chip electrophoresis

7) ^{1}H NMR of Compounds 2-7

^{1}H NMR of 6-nitro-2,4-dihydro-*1H*-3,1-benzoxazine-2,4-dione 2



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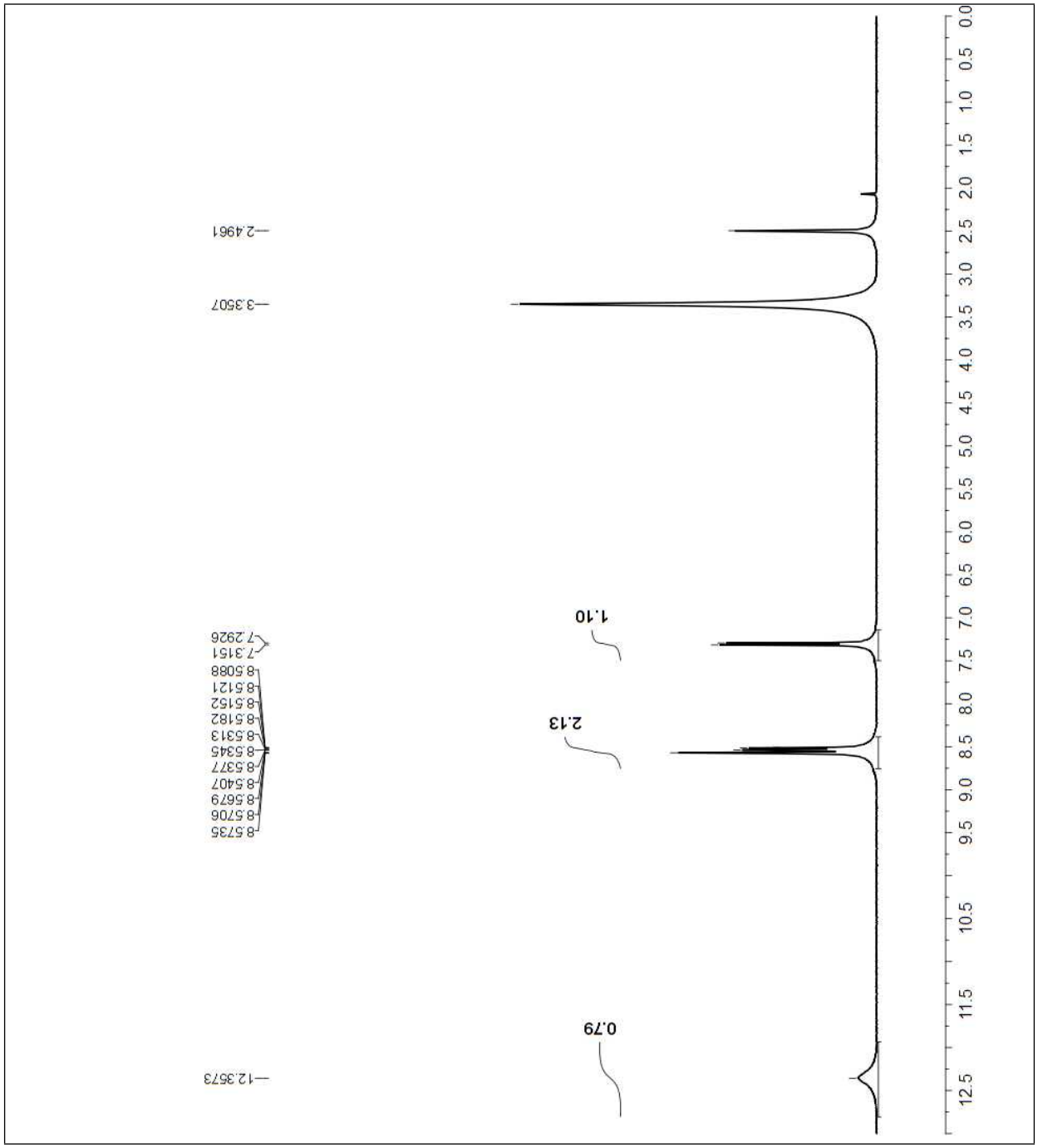
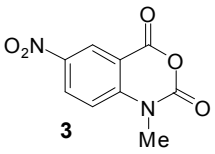


^{1}H NMR of 1-methyl-6-nitro-2,4-dihydro-*1H*-3,1-benzoxazine-2,4-dione 3



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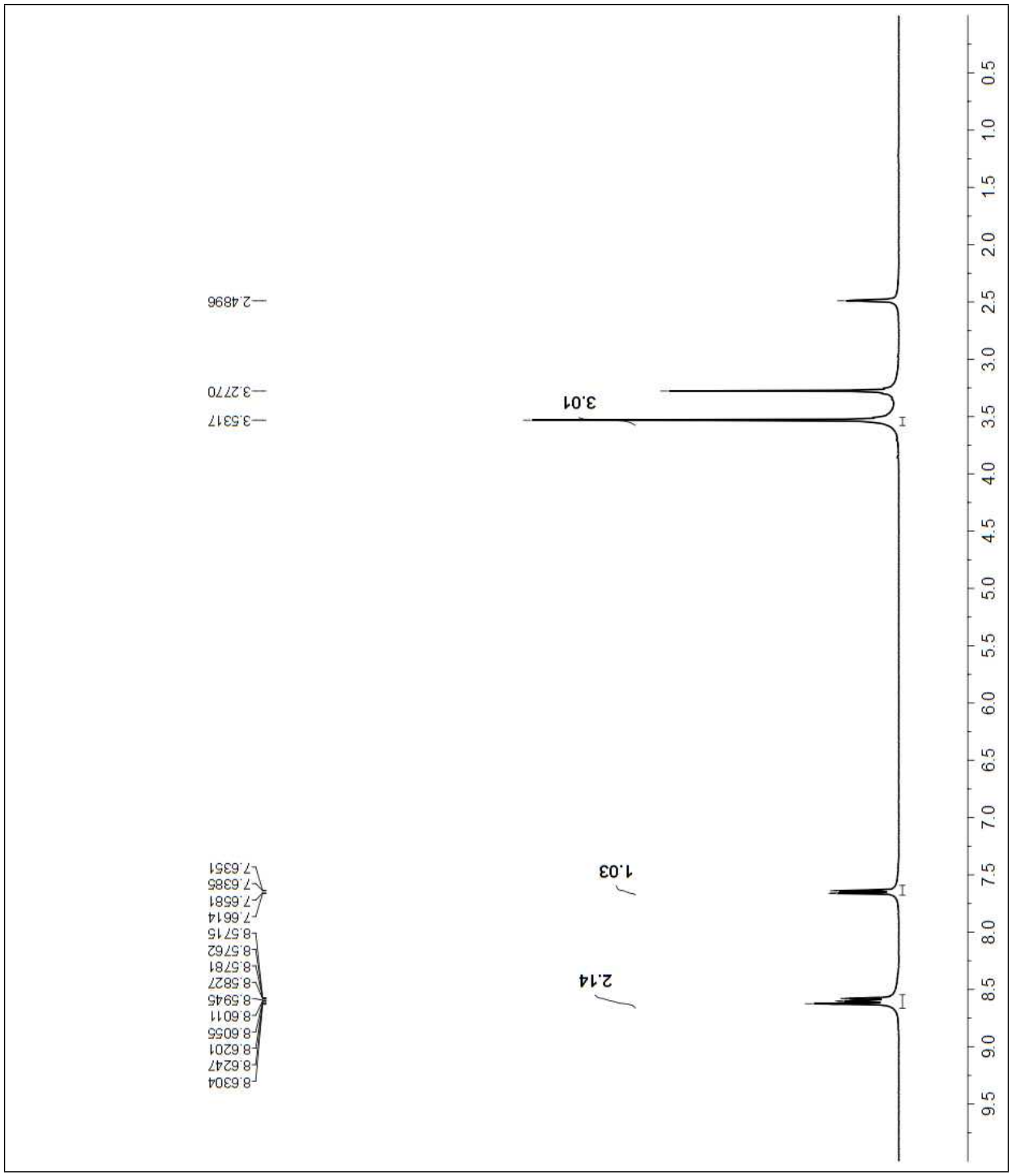
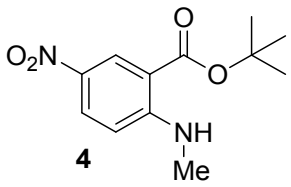


^{1}H NMR of *tert*-butyl 2-(methylamino)-5-nitrobenzoate 4



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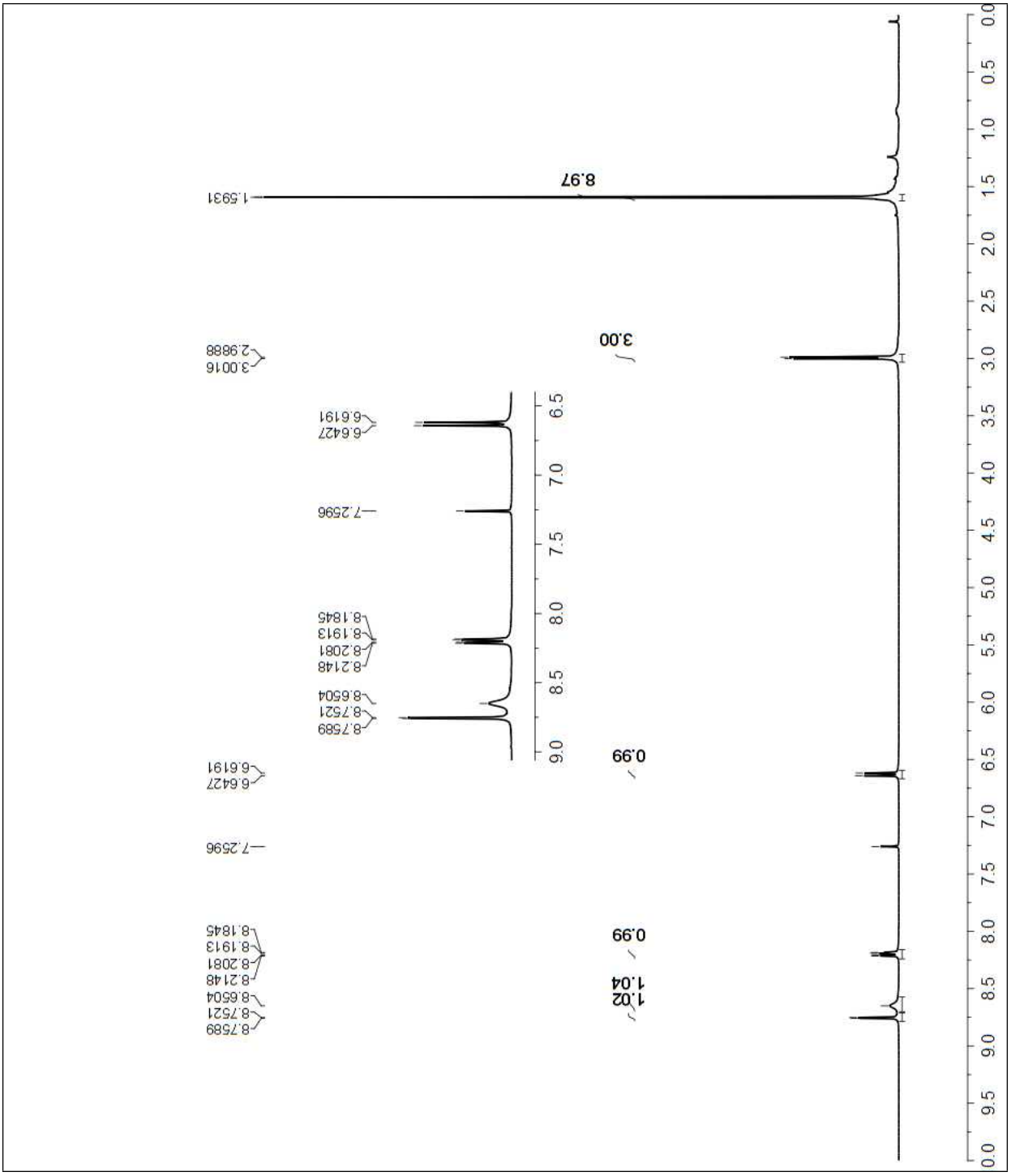
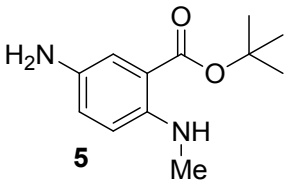


^{1}H NMR of *tert*-Butyl 5-amino-2-(methylamino)benzoate 5



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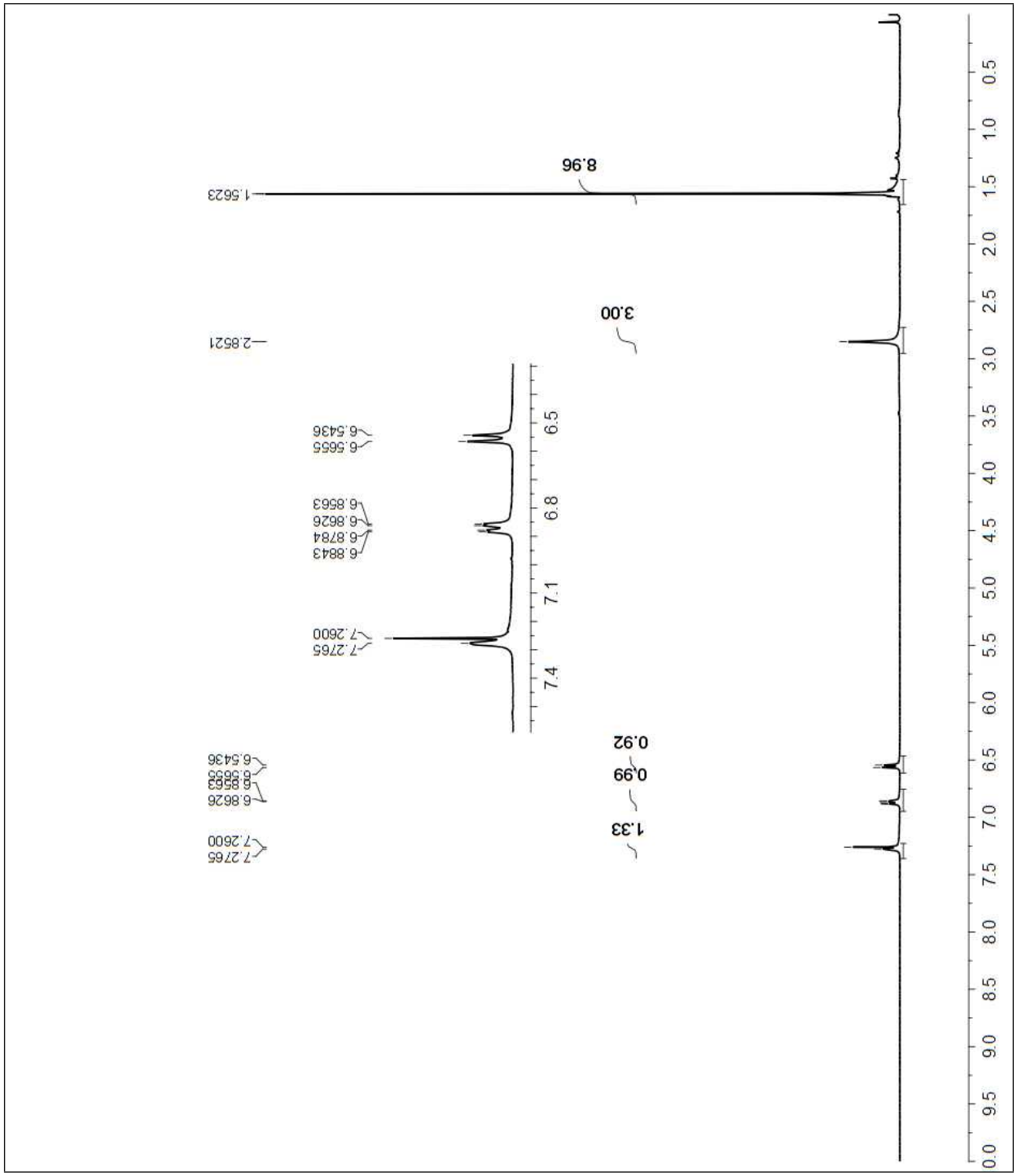
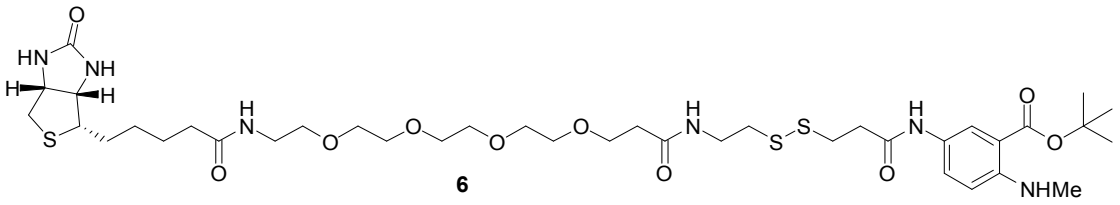


^{1}H NMR of *tert*-butyl 5-(3-{[2-(1-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}propanamido)-2-(methylamino)benzoate 6



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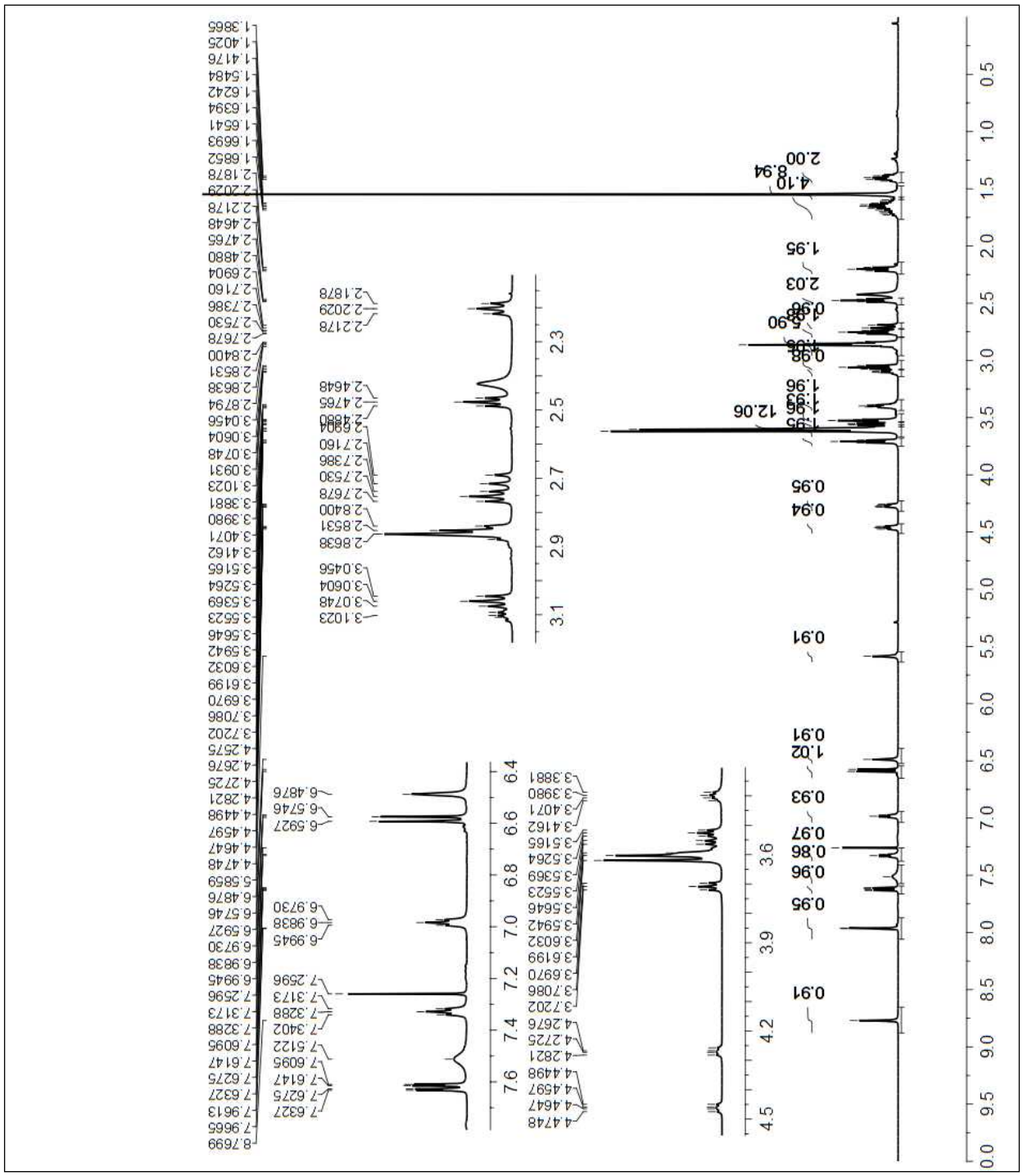
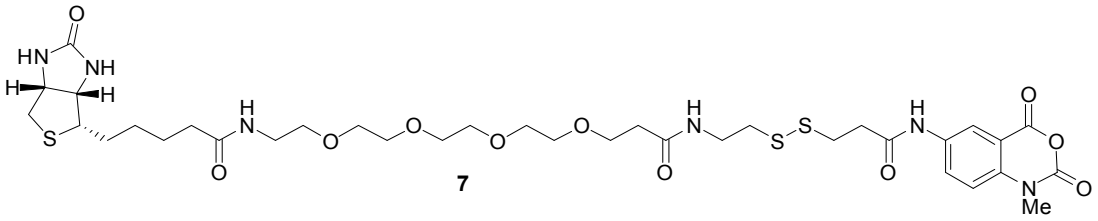


^{1}H NMR of 1-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-N-[2-({2-[(1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazin-6-yl)carbamoyl]ethyl}disulfanyl)ethyl]-3,6,9,12-tetraoxapentadecan-15-amide 7



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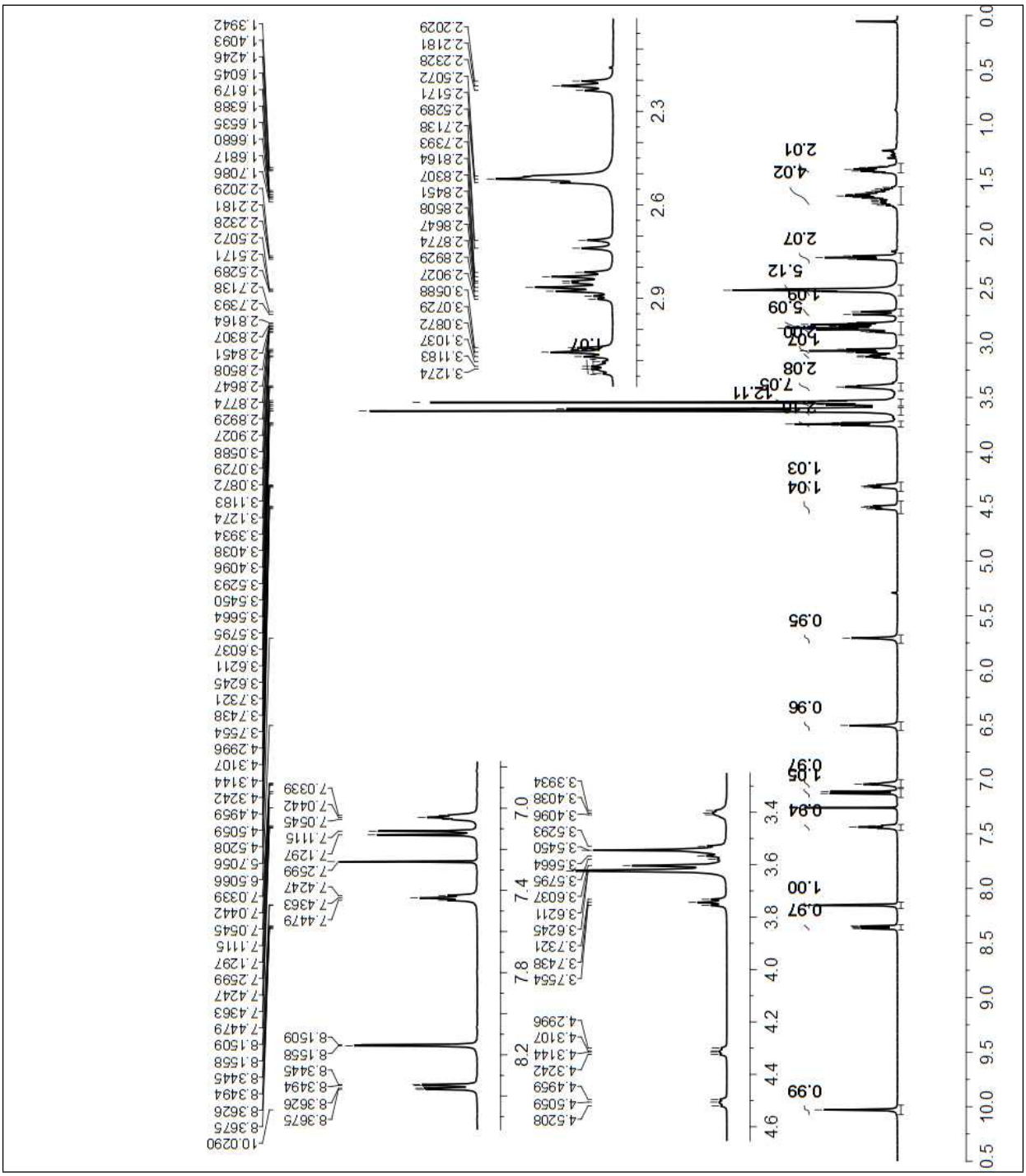
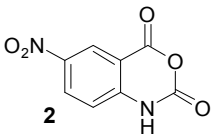


8) ^{13}C NMR of Compounds 2-7

^{13}C NMR of 6-nitro-2,4-dihydro-*1H*-3,1-benzoxazine-2,4-dione 2



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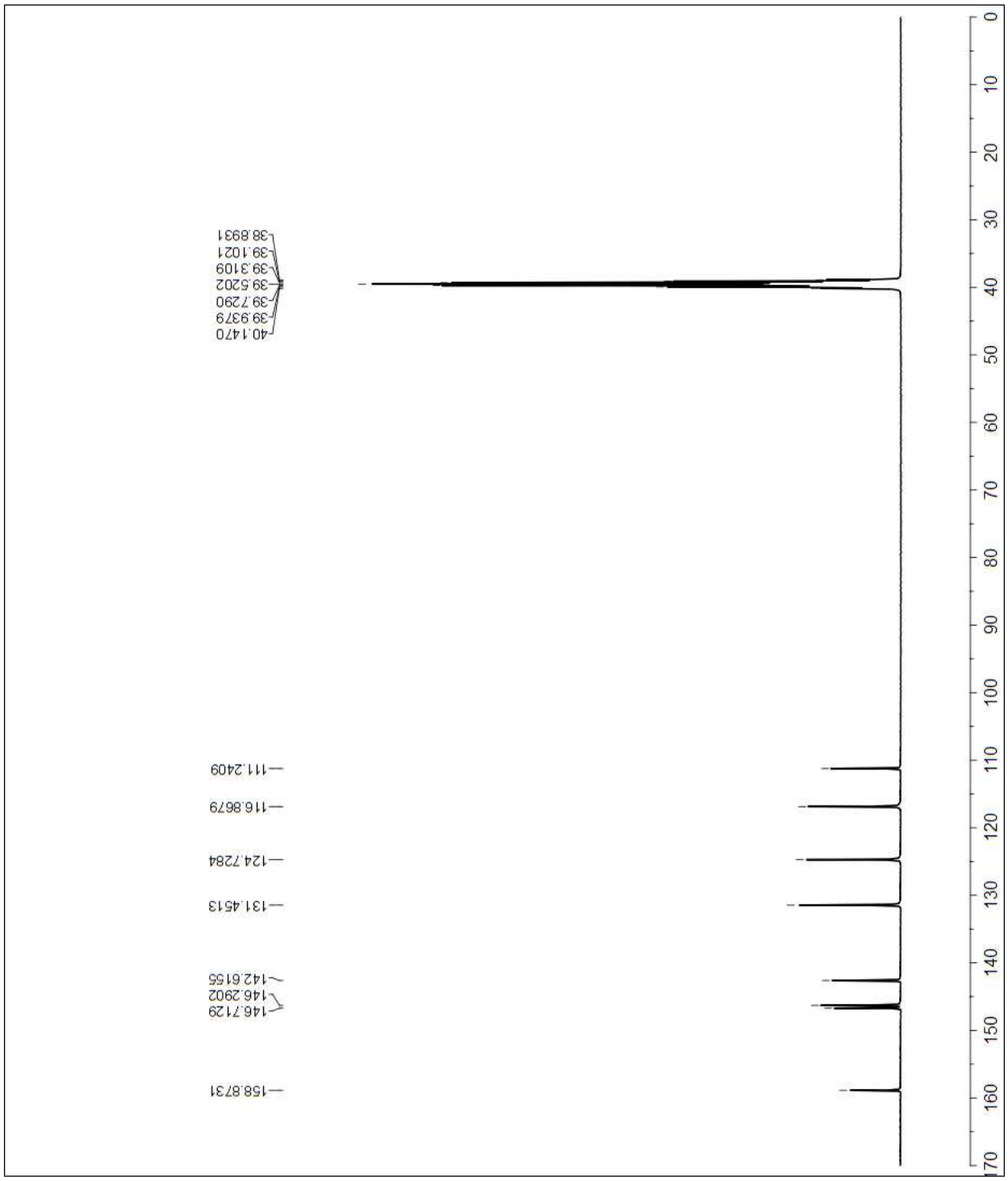
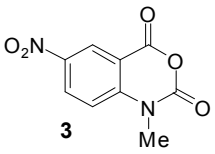


^{13}C NMR of 1-methyl-6-nitro-2,4-dihydro-*1H*-3,1-benzoxazine-2,4-dione 3



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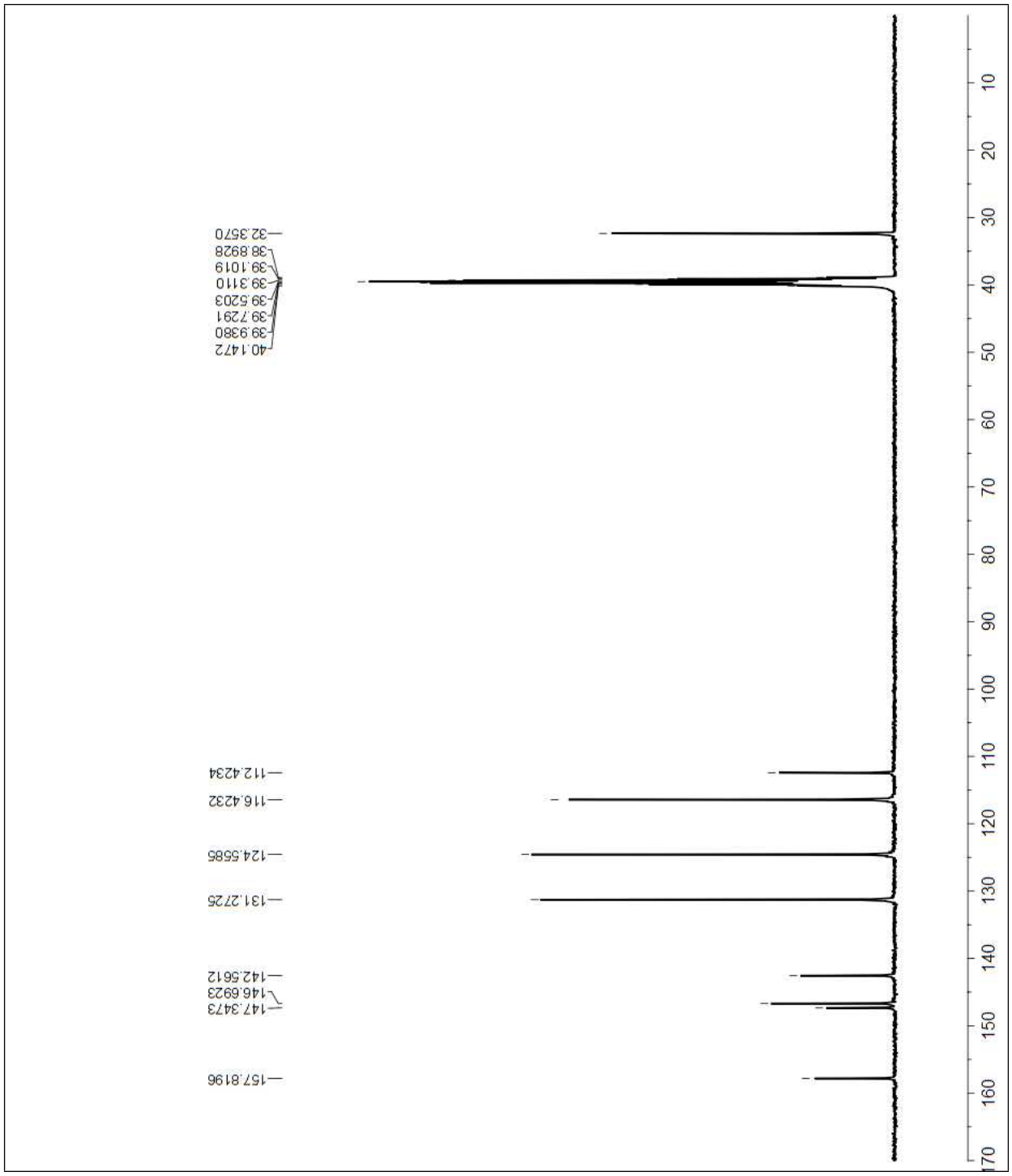
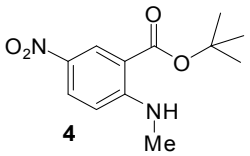


^{13}C NMR of *tert*-butyl 2-(methylamino)-5-nitrobenzoate 4



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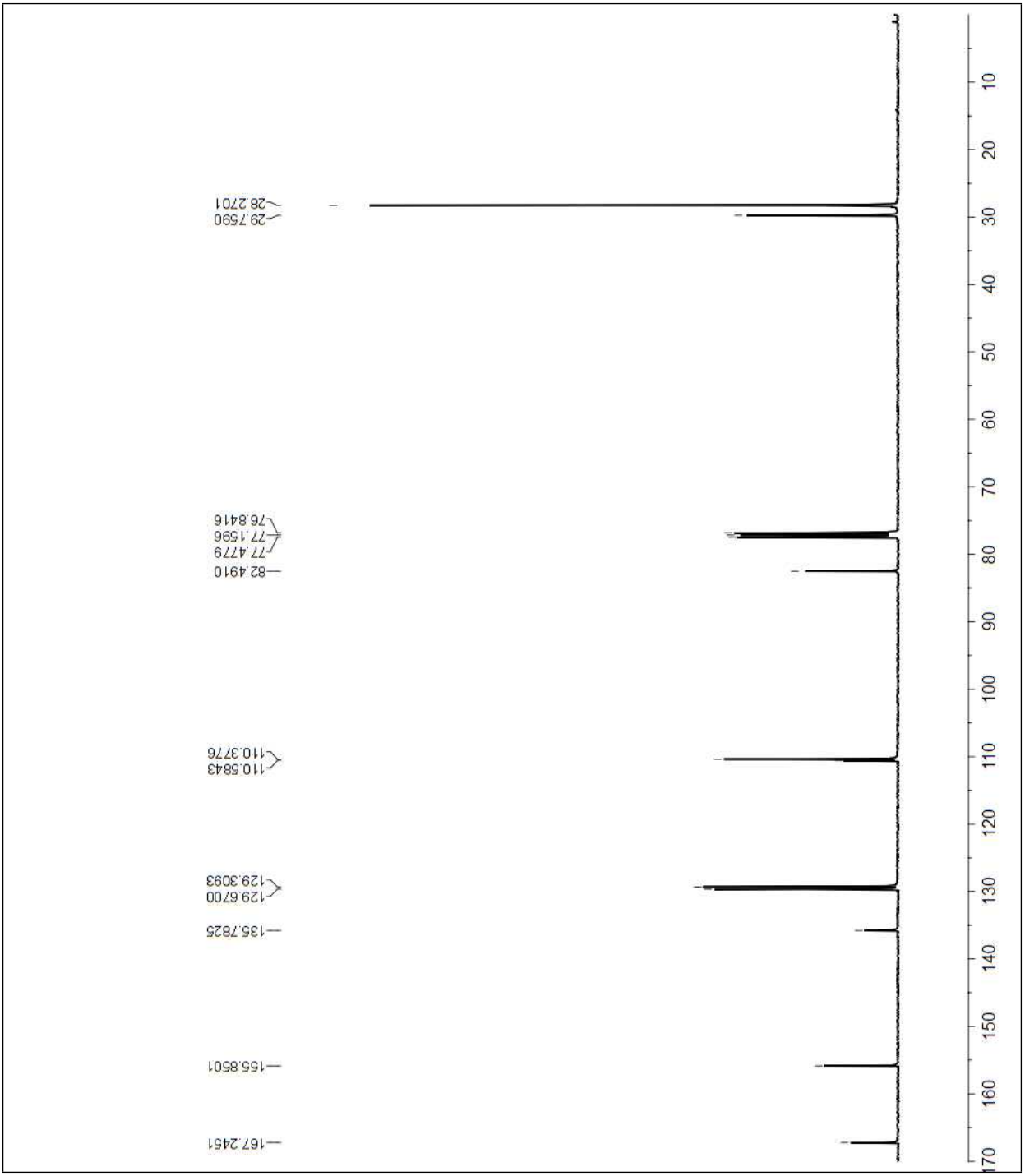
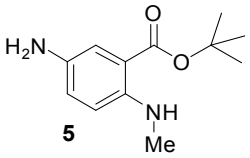


^{13}C NMR of *tert*-Butyl 5-amino-2-(methylamino)benzoate 5



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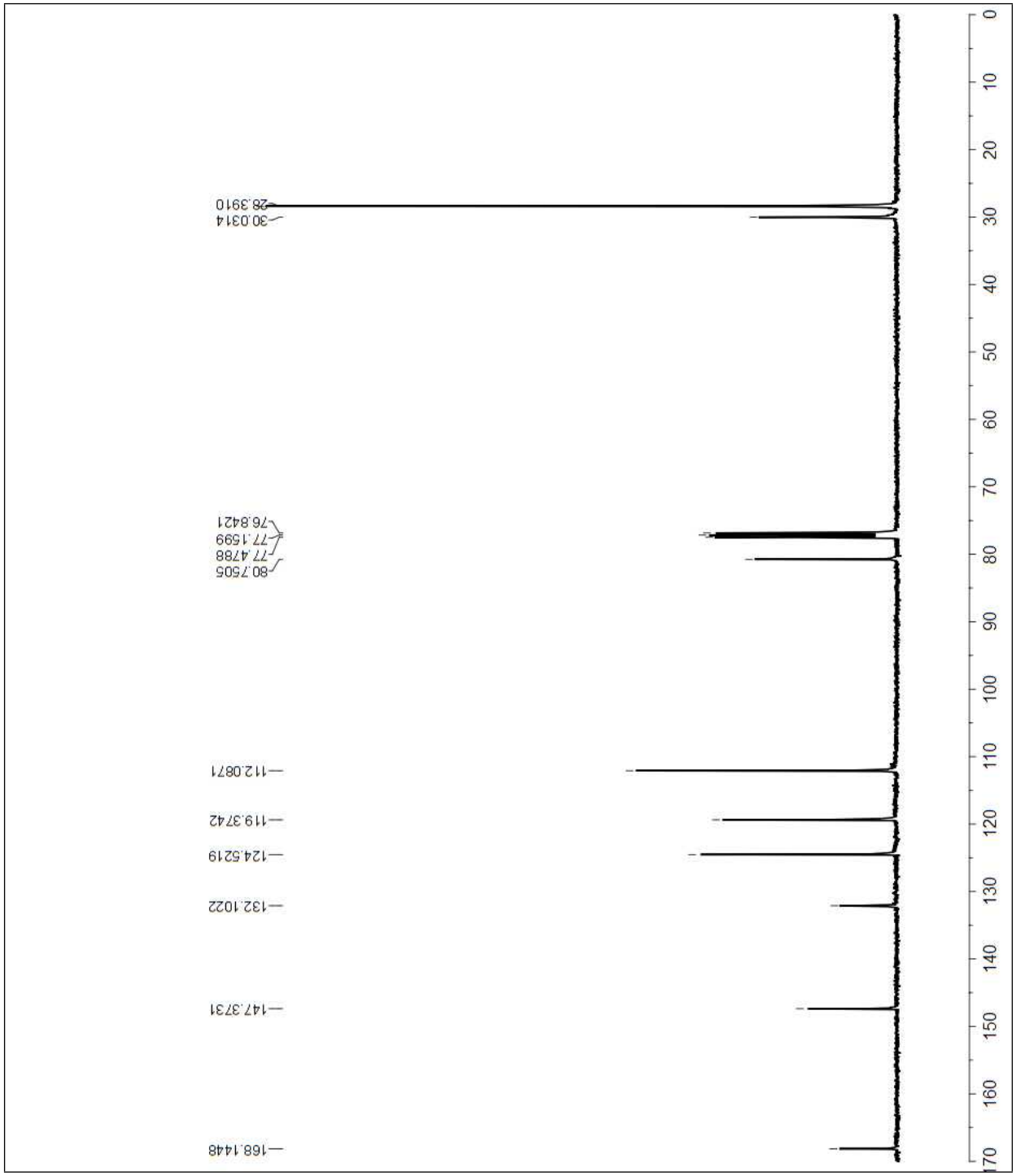
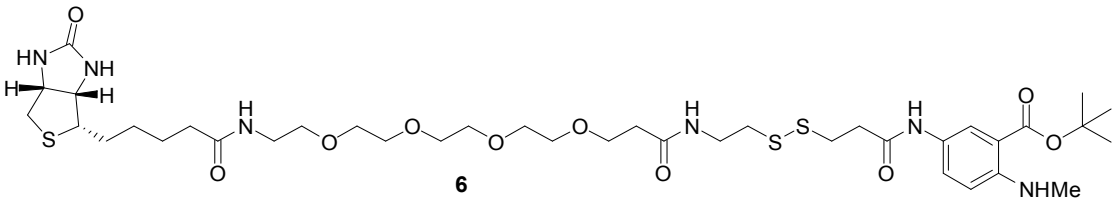


^{13}C NMR of *tert*-butyl 5-(3-{[2-(1-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}propanamido)-2-(methylamino)benzoate 6



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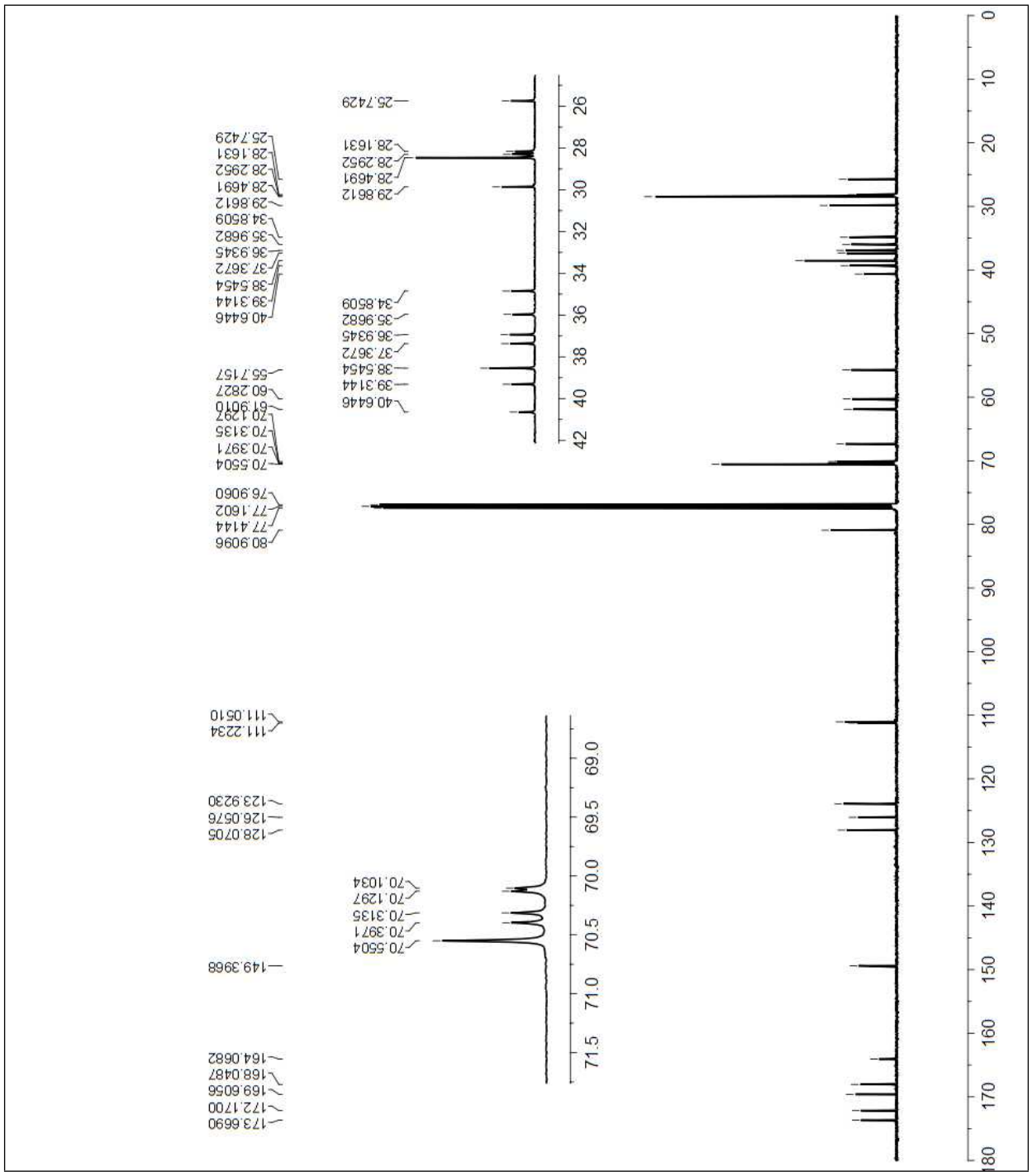
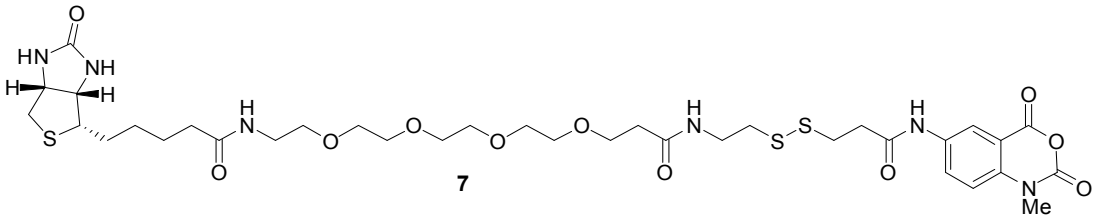


^{13}C NMR of 1-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-N-[2-({2-[(1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazin-6-yl)carbamoyl]ethyl}disulfanyl)ethyl]-3,6,9,12-tetraoxapentadecan-15-amide 7



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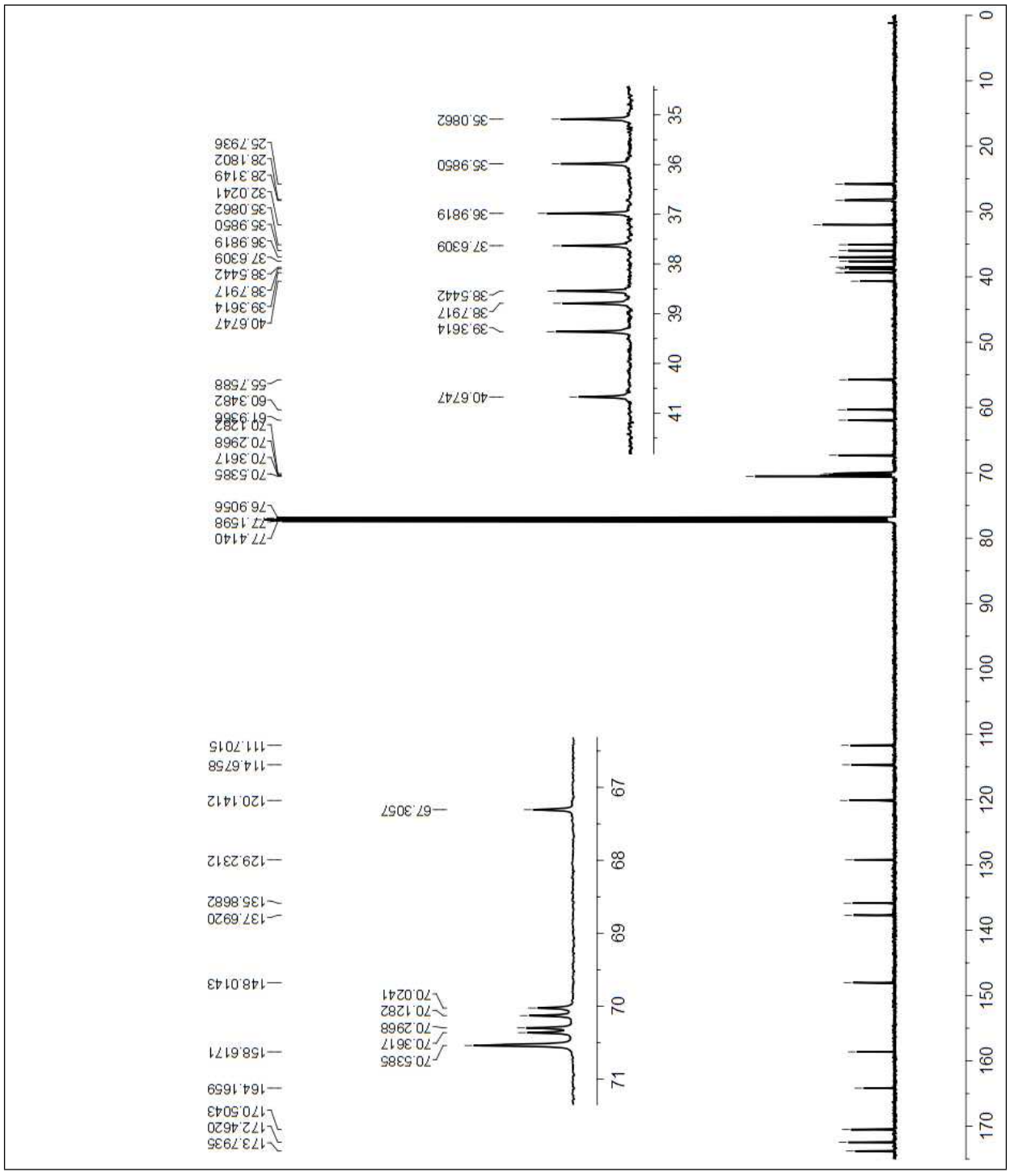
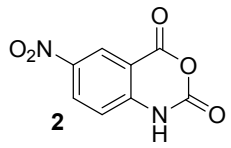


9) HRMS of Compounds 2-7

HRMS of 6-nitro-2,4-dihydro-*1H*-3,1-benzoxazine-2,4-dione 2



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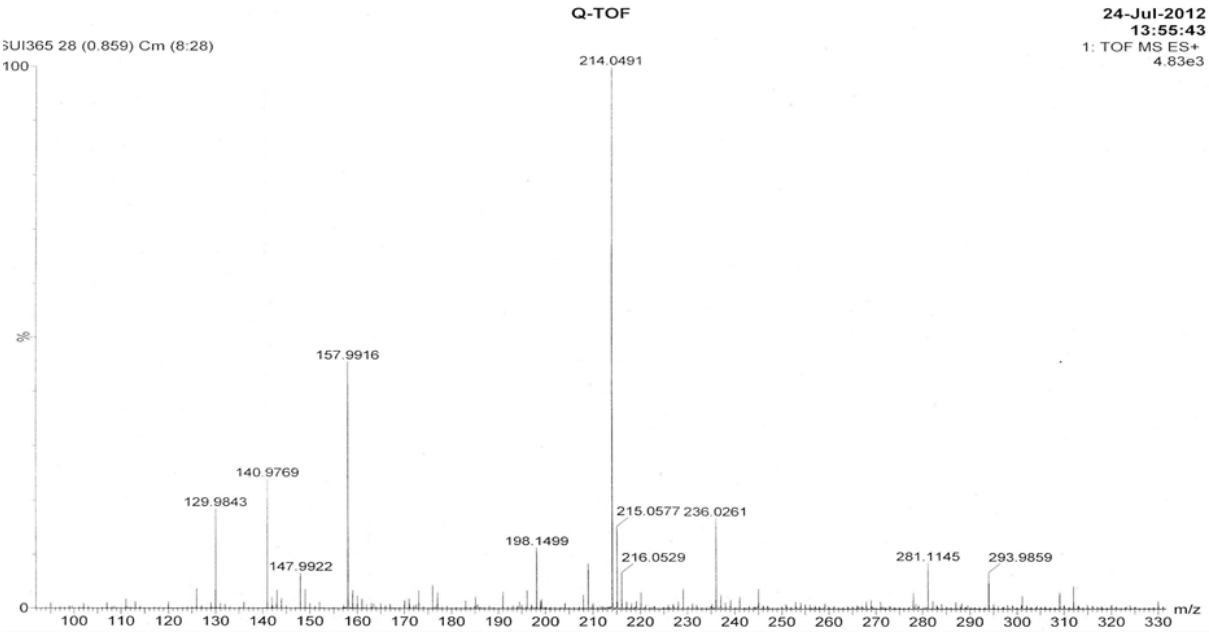
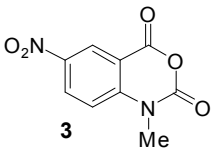


HRMS of 1-methyl-6-nitro-2,4-dihydro-*1H*-3,1-benzoxazine-2,4-dione 3



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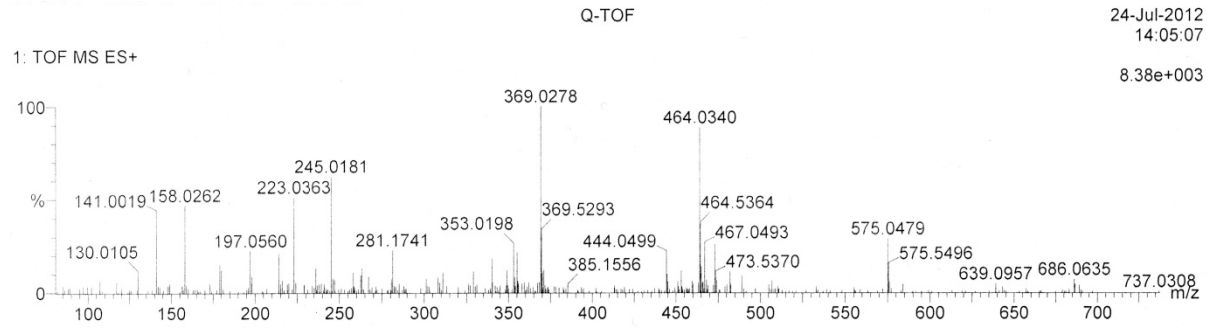

Minimum:				-1000.0		
Maximum:		10.0	10.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
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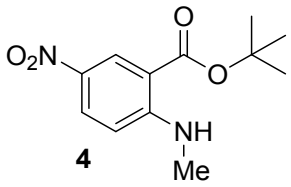
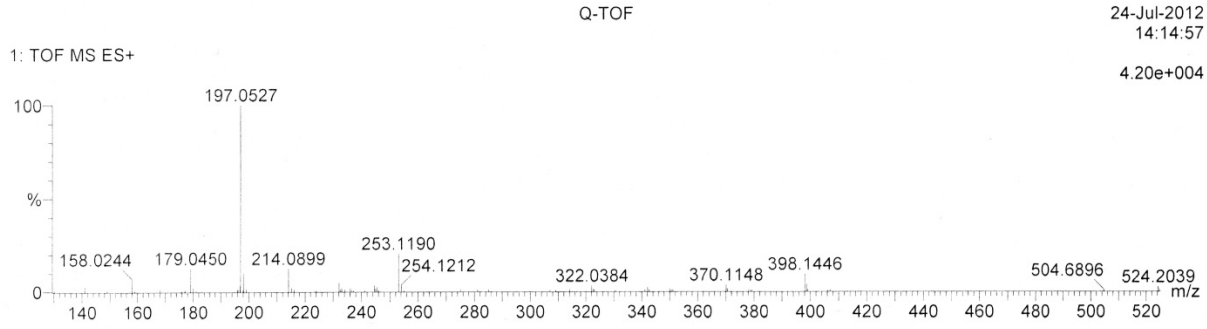
HRMS of *tert*-butyl 2-(methylamino)-5-nitrobenzoate 4





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Maximum:		10.0	10.0	1000.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
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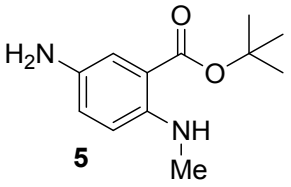
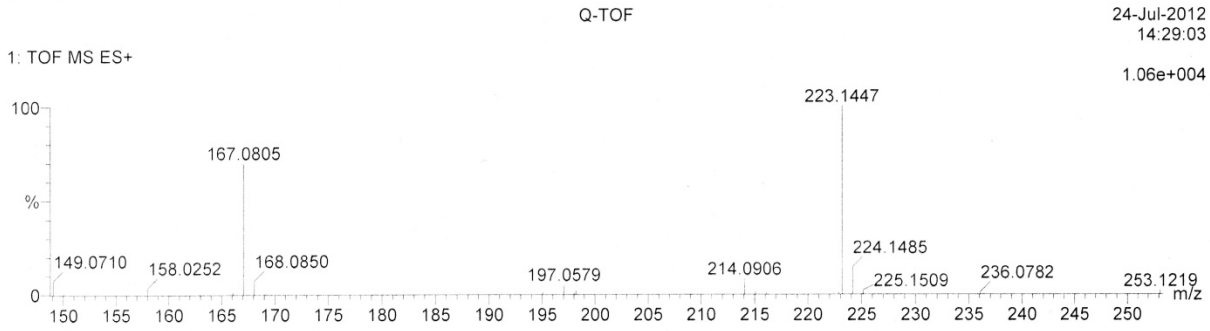
HRMS of *tert*-Butyl 5-amino-2-(methylamino)benzoate 5





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Maximum:		10.0	10.0	1000.0		
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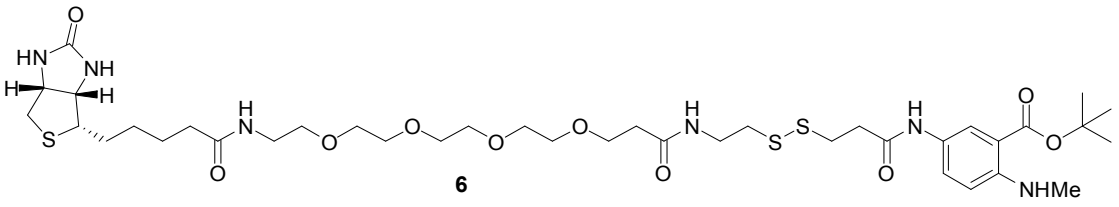
HRMS of *tert*-butyl 5-(3-{[2-(1-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}propanamido)-2-(methylamino)benzoate 6



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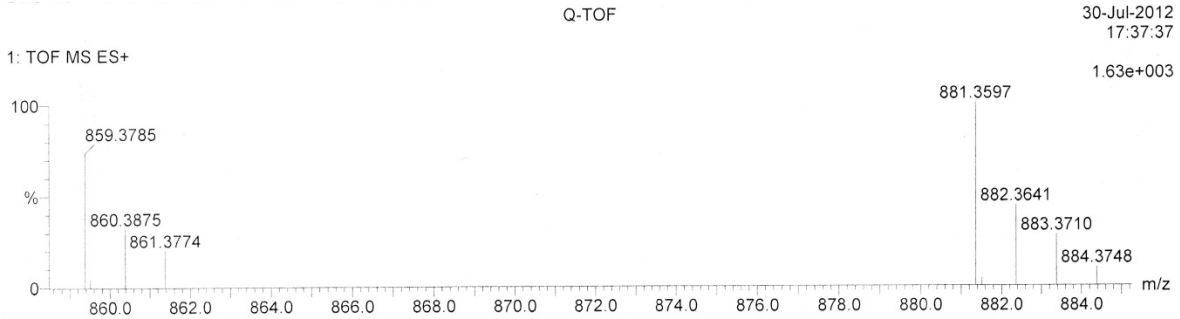

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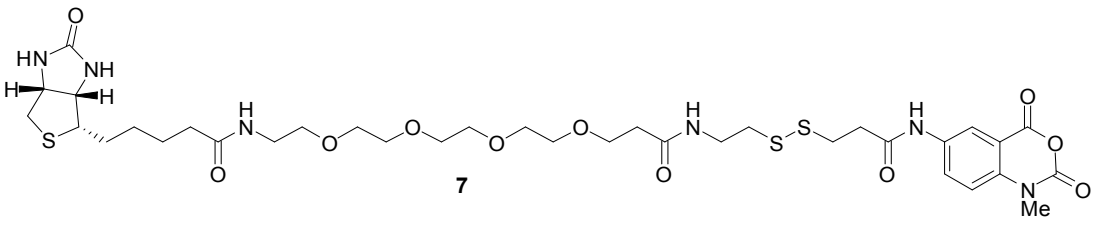
HRMS of 1-{5-[(3aS,4S,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-N-[2-({2-[(1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazin-6-yl)carbamoyl]ethyl}disulfanyl)ethyl]-3,6,9,12-tetraoxapentadecan-15-amide 7



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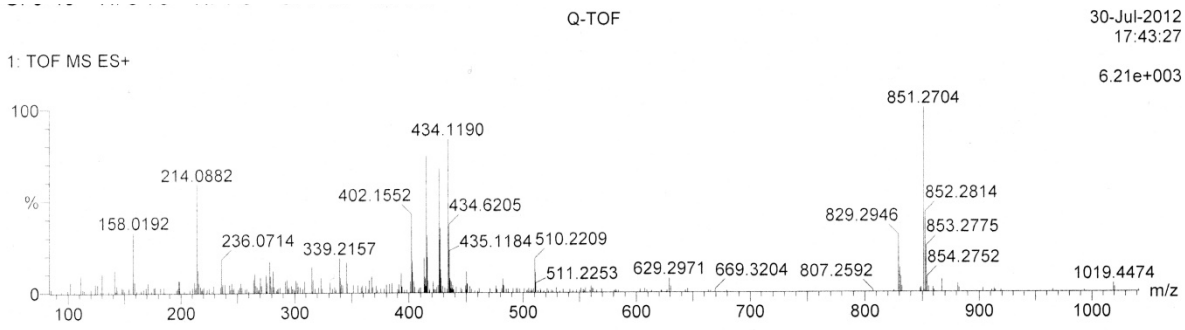

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