Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Biotin-Conjugated Pyridine-Based Isatoic Anhydride, a Selective Room Temperature RNA-Acylating Agent for Nucleic Acid Separation.

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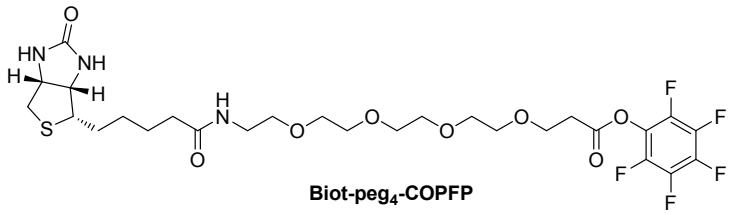
2) General

Chemistry

^{1}H and ^{13}C NMR spectra were recorded, respectively, at 400 MHz and 100 MHz with a Jeol Lambda 400 NMR spectrometer and at 500 MHz and 125 MHz with a Bruker Avance 500 spectrometer. 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).
Reactant and Reagents: 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. 2,3,4,5,6-pentafluorophenyl 1-{5-[(3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-oate (**Biot-peg$_4$-COPFP**) was bought from quanta biodesign (USA).



Biot-peg$_4$-COPFP

Flash Chromatographies were performed on a VWR SPOT II Essential instrument with RP-18-40-63 µm silica. Column's size and flow rate were used according to manufacturer's recommendation. HPLC grade water and acetonitrile were used for all flash chromatographies. After collection and pooling, the fractions containing the desired product were immediately extracted with dichloromethane, dried over $MgSO_4$ and evaporated under *vacuum*.

Nucleic acids tagging:
For synthetic RNA/DNA tagging, LCMS (ESI) analyses were performed on a Alliance HT Waters 2795 apparatus equipped with a 2996 UV diode-array detector, a ZQ 2000 mass spectrometer and an Xterra C18 4.6*30 2.5 µm analytical column. The following linear 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 with a 1 mL/min flow (AF Method). 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).

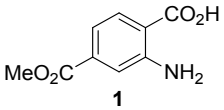
Extraction of biological nucleic acids:
HIV-transcript RNA (1083 nucleotides) was a purchased from bioMérieux (France, Nuclisens EasyQ VIH-1 v2.0, Ref 285036). Calf genomic DNA was purchased from Sigma-Aldrich (ref D4522). Buffer solutions and magnetic silica particles were also purchased from bioMérieux. MagPrep® Streptavidin magnetic beads were purchased from Merck (72190). DynaMag stands were used as magnetic stands. Detection and quantification of biological nucleic acids were 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).

Amplification of HIV transcripts:

RT-PCR experiments were performed on a Roche LightCycler 2.0 using a Roche LightCycler RNA Master HybProbe (03018954001).

3) Synthesis of Compounds 1-15

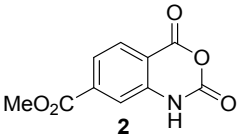
2-Amino-4-(methoxycarbonyl)benzoic acid 1



1

In a round bottom flask, under nitrogen, were introduced 2-aminoterephtalic acid (10.00 g, 55.20 mmol), methanol (200 mL) and chlorotrimethylsilane (10.50 mL, 82.80 mmol). The resulting mixture was stirred and refluxed (65-70°C) for 14 h. After cooling at room temperature, the reaction was concentrated and 200 mL of a saturated aqueous solution of K_2CO_3 were added. The solution was extracted with EtOAc (3x200 mL) and the combined organic layers were washed with a saturated aqueous solution of K_2CO_3 (3x50 mL). The combined aqueous layers were acidified at pH 5 with AcOH (25 mL) and extracted with EtOAc (3x200 mL). The organic layer was dried over $MgSO_4$ and evaporated to afford **2** (9.00 g, 84%) as a yellow powder. **mp =** 223-225°C; **IR (KBr):** ν, 3484 (NH_2), 3376 (NH_2), 3055-2796 (OH acid), 1727 (C=O), 1678 (C=O), 1598, 1551, 1423, 1319, 1245, 748 cm^{-1}; **^{1}H NMR (400 MHz, DMSO-*d6*):** δ 3.81 (s, 3H), 7.01 (d, 1H, ^3J = 8.3 Hz), 7.40 (s. 1H), 7.78 (dd, 1H, ^4J = 1.4 Hz, ^3J = 8.3 Hz); **^{13}C NMR (100 MHz, DMSO-*d6*):** δ 52.3, 113.0, 114.4, 117.4, 131.7, 133.9, 151.3, 166.1, 169.1; **HRMS/ESI:** *m/z* calcd for $C_9H_{10}NO_4$ $[M+H]^+$ 196.0610, found 196.0614.

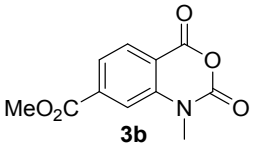
Methyl 2,4-dioxo-1*H*-benzo[*d*][1,3]oxazine-7-carboxylate 2



2

In a round bottom flask, under nitrogen, were introduced **1** (4.00 g, 20.51 mmol), dioxane (80 mL) and a solution of phosgene at 20% in toluene (12.95 mL, 24.62 mmol). The mixture was stirred at room temperature for 1 h. After concentration, 50 mL of Et_2O was added and the resulting solid was filtered, washed with Et_2O (3x50 mL), evaporated and dried under *vacuum* to afford **2** (4.25 g, 94%) as a yellow powder. **mp =** 211-213°C**; IR (KBr):** ν, 3514 (N-H), 3424, 1787 (C=O), 1742 (C=O), 1720 (C=O), 1635, 1599, 1436, 1295, 1239, 1003, 752 cm^{-1}; **^{1}H NMR (400 MHz, DMSO-*d6*):** δ 3.88 (s, 3H), 7.67 (s, 1H), 7.69 (d, 1H, ^3J = 7.5 Hz), 8.00 (d, 1H, ^3J = 7.5 Hz), 11,93 (s, 1H); **^{13}C NMR (100 MHz, DMSO-*d6*):** δ 53.0, 114.0, 116.0, 123.2, 129.6, 136.4, 141.5, 146.9, 159.4, 164.9; **HRMS/ESI:** *m/z* calcd for $C_{10}H_8NO_5$ $[M+H]^+$ 222.0402, found 222.0408.

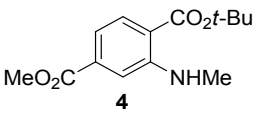
Methyl 1-methyl-2,4-dioxobenzo[*d*][1,3]oxazine-7-carboxylate 3b



3b

In a round bottom flask were introduced **2** (2.00 g, 9.04 mmol), DMF (25 mL), iodomethane (0.68 mL, 10.85 mmol) and K_2CO_3 (1.25 g, 9.04 mmol). The reaction was stirred at room temperature for 45 min. The mixture was then cooled at 0°C and 12.5 mL of water were added. The resulting precipitate was filtered, washed with Et_2O (3x30 mL), evaporated and dried under *vacuum* in presence of P_2O_5 to afford **3b** (1.56 g, 74%) as a yellow powder. **mp =** 193-195°C; **IR (KBr):** ν, 1778 (C=O), 1741 (C=O), 1717 (C=O), 1614, 1432, 1334, 1282, 1260, 1238, 1031 cm^{-1}; **^{1}H NMR (400 MHz, DMSO-*d6*):** δ 3.50 (s, 3H), 3.92 (s, 3H), 7.80 (m, 2H), 8.11 (d, 1H, ^3J = 8.1 Hz); **^{13}C NMR (100 MHz, DMSO-*d6*):** δ 31.8, 53.0, 115.1, 115.2, 123.4, 130.0, 136.7, 142.3, 147.6, 158.5, 165.1; **HRMS/ESI:** *m/z* calcd for $C_{11}H_{10}NO_5$ $[M+H]^+$ 236.0559, found 236.0557.

1-*tert*-Butyl 4-methyl 2-(methylamino)benzene-1,4-dicarboxylate 4

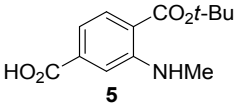


4

In a round bottom flask at room temperature were introduced **3b** (2.00 g, 8.51 mmol) and THF (80 mL). *t*-BuONa (1.03 g, 9.36 mmol) was then added portionwise and the resulting mixture was stirred at room temperature for 20 min. After removal of the solvent, 75 mL of an aqueous solution of K_2CO_3 5% were added and the solution was extracted with EtOAc (5x75 mL). The combined organic layers were dried over MgSO4 and evaporated to afford **4** (1.74 g, 77%) as a yellow powder. **mp =** 101-103°C; IR (KBr): ν, 3366 (N-H), 2970, 1722 (C=O), 1677 (C=O), 1242, 1161, 1133, 1107, 760 cm^{-1}; **^{1}H NMR**

(400 MHz, $CDCl_3$): δ 1.58 (s, 9H), 2.95 (d, 3H, ^3J = 3.7 Hz), 3.92 (s, 3H), 7.18 (dd, 1H, ^4J = 1.6 Hz, ^3J = 8.3 Hz), 7.31 (d, 1H, ^4J = 1.6 Hz), 7.74 (bs, 1H), 7.88 (d, 1H, ^3J = 8.3 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.2 (3C); 29.6; 52.2; 81.2; 111.8; 114.5; 114.7; 131.8; 134.6; 151.6; 167.1; 167.7; **HRMS/ESI:** *m/z* calcd for $C_{14}H_{20}NO_4$ $[M+H]^+$ 266.1392, found 266.1389.

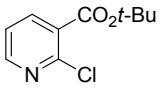
4-[(*tert*-Butoxy)carbonyl]-3-(methylamino)benzoic acid 5



5

In a round bottom flask at room temperature were added **4** (0.85 g, 3.22 mmol), THF (16 mL) and an aqueous solution of LiOH 1M (16.10 mL, 16.10 mmol). The mixture was stirred at room temperature for 4 h. THF was then evaporated and 30 mL of water were added. The resulting aqueous layer was acidified at pH 5 with AcOH and extracted with EtOAc (4x50 mL). The combined organic layers were dried over $MgSO_4$ and co-evaporated with toluene to afford **5** (0.75 g, 92%) as a yellow powder. **mp =** 163-165°C; **IR (KBr):** ν, 3379 (N-H), 3031-2814 (OH acid), 1712 (C=O), 1683 (C=O), 1582, 1246, 1172, 1140, 1122, 750 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.59 (s, 9H), 2.96 (s, 3H), 7.26 (dd, 1H, ^4J = 1.6 Hz, ^3J = 8.3 Hz), 7.39 (d, 1H, ^4J = 1.6 Hz), 7.92 (d, 1H, ^3J = 8.3 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.2 (3C), 29.6, 81.4, 112.4, 115.0, 115.4, 131.9, 133.7, 151.6, 167.6, 172.1; **HRMS/ESI:** *m/z* calcd for $C_{13}H_{18}NO_4$ $[M+H]^+$ 252.1236, found 252.1233.

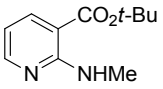
***tert*-Butyl 2-chloropyridine-3-carboxylate 6**



6

In a round bottom flask at 0°C under nitrogen, were introduced 2-chloronicotinic acid (5.00 g, 31.73 mmol), THF (100 mL), some drops of DMF and $(COCl)_2$ (5.37 mL, 63.47 mmol). After stirring the reaction mixture for 2 h at room temperature, the solvent was evaporated to afford a yellow oil. 100 mL of THF was added and the mixture was cooled at -10°C. *t*-BuOK (4.27 g, 38.08 mmol) was introduced portionwise and the reaction was stirred at room temperature for 2 h. After concentration, 200 mL of an aqueous solution of K_2CO_3 5% were added and the solution was then extracted with DCM (3x150 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to afford **6** as an oil (5.97 g, 88%). **IR (KBr):** ν, 2981, 1732 (C=O), 1579, 1403, 1370, 1315, 1288, 1173, 1144, 1065, 1056 cm^{-1}; **^{1}H NMR (400MHz, $CDCl_3$)**: δ 1.62 (s, 9H), 7.31 (dd, 1H, ^3J = 7.8 Hz, ^3J = 4.4 Hz), 8.07 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.9 Hz), 7.31 (dd, 1H, ^3J = 4.4 Hz, ^4J = 1.9 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.0 (3C), 83.3, 122.0, 128.8, 139.8, 149.4, 151.2, 163.9; **HRMS/ESI:** *m/z* calcd for $C_{10}H_{13}NO_2Cl$ $[M+H]^+$ 214.0635, found 214.0638.

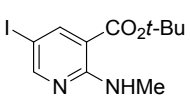
***tert*-Butyl 2-(methylamino)pyridine-3-carboxylate 7**



7

In a sealed tube, were introduced **6** (2.82 g, 13.20 mmol), MeOH (4.60 mL) and an aqueous solution of $MeNH_2$ 40% (4.60 mL, 53.26 mmol) and the reaction mixture was stirred at 100°C for 2 h. After concentration, 75 mL of water was added and the solution was extracted with DCM (3×75 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The crude was purified by silica gel chromatography (gradient: PE to PE/Et_2O 9.5/0.5) to afford **7** (2.35 g, 85%) as an oil. **IR (KBr):** ν, 3380 (NH), 2978, 1684 (C=O), 1595, 1583, 1520, 1392, 1305, 1262, 1250, 1172, 1126 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.57 (s, 9H), 3.05 (d, 3H, ^3J = 4.9 Hz), 6.49 (dd, 1H, ^3J = 7.5 Hz, ^3J = 4.4 Hz), 7.98 (bs, 1H), 8.04 (dd, 1H, ^3J = 7.5 Hz, ^4J = 2.0 Hz), 8.28 (dd, 1H, ^3J = 4.4 Hz, ^4J = 2.0 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 27.8, 28.2 (3C), 81.2, 107.5, 110.4, 139.9, 153.0, 159.3, 167.1; **HRMS/EI:** *m/z* calcd for $C_{11}H_{16}N_2O_2$ $[M]^{+\bullet}$ 208.1212, found 208.1213.

***tert*-Butyl 5-iodo-2-(methylamino)pyridine-3-carboxylate 8**

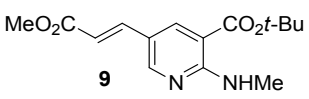


8

In a round bottom flask were introduced **7** (4.50 g, 21.61 mmol), DCM (25 mL), AcOH (5 mL) and NIS (5.83 g, 25.93 mmol) and the reaction mixture was stirred at room temperature. After 30 min, the reaction was quenched with 10 mL of an aqueous solution of sodium thiosulfate. 100 mL of an aqueous solution of K_2CO_3

5% were added and the solution was extracted with DCM (3×100 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The crude was purified by silica gel chromatography (gradient: PE to PE/Et_2O 9/1) to afford **8** (6.92 g, 96%) as a yellow powder. **mp =** 101-103°C; **IR (KBr):** ν, 3371 (N-H), 2980, 1679 (C=O), 1588, 1569, 1505, 1367, 1307, 1243, 1167, 1140, 1107, 796 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.57 (s, 9H), 3.01 (d, 3H, ^3J = 4.9 Hz), 7.97 (bs, 1H), 8.21 (d, 1H, ^4J = 2.0 Hz), 8.40 (d, 1H, ^4J = 2.0 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 27.8; 28.2 (3C); 73.1; 82.0; 109.8; 146.8; 157.9; 158.3; 166.0; **HRMS/EI:** *m/z* calcd for $C_{11}H_{15}N_2O_2I$ $[M]^{+\bullet}$ 334.0179, found 334.0167.

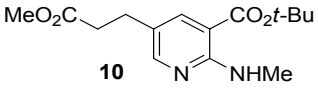
tert-Butyl 5-[(_1E_)-3-methoxy-3-oxoprop-1-en-1-yl]-2-(methylamino)pyridine-3-carboxylate 9



9

In a round bottom flask, under nitrogen, were added PPh_3 (0.39 g, 1.50 mmol), $Pd(OAc)_2$ (0.17 g, 0.75 mmol), TEA (2.08 mL, 14.96 mmol) in dioxane (50 mL, prealably degassed under nitrogen during 15 min) and the mixture was stirred at 100°C. After 5 min, **8** (5.00 g, 14.96 mmol) and methyl acrylate (6.74 mL, 74.82 mmol) were added and the reaction was strirred at 100°C for 4 h. After concentration, the mixture was poured in DCM and filtered through a pad of celite. 200 mL of water were added and the solution was extracted with DCM (3×150 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The crude was purified by silica gel chromatography (gradient: PE to PE/Et_2O 7/3) to afford **9** (3.33 g, 76%) as a yellow powder. **mp =** 113-115°C; **IR (KBr):** ν, 3373 (N-H), 2975, 1720 (C=O), 1693 (C=O), 1634, 1604, 1585, 1526, 1317, 1266, 1253, 1203, 1188, 1161, 1134 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.59 (s, 9H), 3.09 (d, 3H, ^3J = 4.9 Hz), 3.80 (s, 3H), 6.27 (d, 1H, ^3J = 15.8 Hz), 7.59 (d, 1H, ^3J = 15.8 Hz), 8.22 (d, 1H, ^4J = 2.4 Hz), 8.30 (bs, 1H), 8.41 (d, 1H, ^4J = 2.4 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.0, 28.2 (3C), 51.5, 82.1, 107.7, 113.7, 117.6, 138.2, 141.2, 153.9, 159.6, 166.5, 167.6; **HRMS/EI:** *m/z* calcd for $C_{15}H_{20}N_2O_4$ $[M]^{+\bullet}$ 292.1423, found 292.1413.

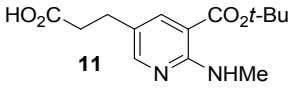
tert-Butyl 5-(3-methoxy-3-oxopropyl)-2-(methylamino)pyridine-3-carboxylate 10



10

In a round bottom flask at room temperature under nitrogen were introduced **9** (6.00 g, 20.52 mmol), EtOAc (250 mL) and Pd/C (2.18 g, 10 mol %). The flask was filled with hydrogen and stirred at room temperature for 24 h. The solution was filtered on celite and evaporated. The crude was purified by silica gel chromatography (gradient: PE to PE/Et_2O 7/3) to afford **10** (4.88 g, 81%). **mp =** 69-71°C; **IR (KBr):** ν, 3392 (NH), 2953, 1736 (C=O), 1690 (C=O), 1574, 1520, 1371, 1227, 1194, 1173, 1156, 1127, 1090, 802 cm^{-1}; **^{1}H NMR (400MHz, $CDCl_3$):** δ 1.57 (s, 9H), 2.58 (t, 2H, ^3J = 7.8 Hz), 2.82 (t, 2H, ^3J = 7.8 Hz), 3.03 (d, 3H, ^3J = 4.9Hz), 3.68 (s, 3H), 7.85 (bs, 1H), 7.87 (s, 1H), 8.14 (s, 1H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 27.1, 27.9, 28.2 (3C), 35.8, 51.7, 81.4, 107.3, 122.0, 139.7, 152.7, 158.2, 167.0, 173.1; **HRMS/ESI:** *m/z* calcd for $C_{15}H_{23}N_2O_4$ $[M+H]^+$ 295.1658, found 295.1655.

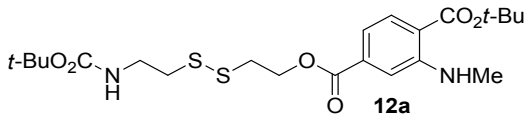
3-{5-[(_tert_-Butoxy)carbonyl]-6-(methylamino)pyridin-3-yl}propanoic acid 11



11

In a round bottom flask were introduced **10** (4.87 g, 16.54 mmol), THF (50 mL) and an aqueous solution of LiOH 1M (50 mL, 50.00 mmol). After stirring the reaction at room temperature for 45 min, THF was evaporated. The aqueous layer was then acidified at pH 6 with acetic acid and extracted with EtOAc (4×50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to afford **11** as a yellow powder (4.25 g, 92%). **mp =** 105-107°C; **IR (KBr):** ν, 3374 (N-H), 2967, 1713 (C=O), 1683 (C=O), 1584, 1538, 1367, 1342, 1304, 1282, 1245, 1192, 1169, 1139, 1101, 801 cm^{-1}; **^{1}H NMR (400MHz, $CDCl_3$):** δ 1.57 (s, 9H), 2.62 (d, 2H, ^3J = 7.8 Hz), 2.85 (t, 2H, ^3J = 7.8 Hz), 3.02 (t, 3H, ^3J = 4.9 Hz), 7.92 (d, 1H, ^4J = 2.0 Hz), 7.95 (bs, 1H), 8.20 (s, 1H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 27.0, 28.1, 28.2 (3C), 35.9, 81.6, 107.8, 122.2, 140.5, 152.6, 157.9, 166.8, 177.0; **HRMS/ESI:** *m/z* calcd for $C_{14}H_{21}N_2O_4$ $[M+H]^+$ 281.1501, found 281.1500.

4-{2-[(2-{[(*tert*-Butoxy)carbonyl]amino}ethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 12a

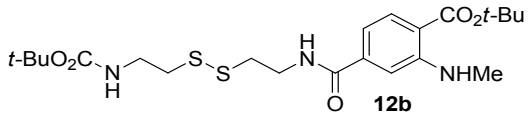


12a

In a round bottom flask were introduced **21** (0.75 g, 2.98 mmol), DCM (7.50 mL), EDCI (0.86, 4.48 mmol), HOBt (0.61 g, 4.48 mmol) and TEA (0.83 mL, 5.97 mmol). After stirring the mixture 10 min at room temperature, **5** (1.51 g, 5.97 mmol) was added. The reaction was stirred at room temperature for 4 h. After concentration, 50 mL of water were added and the solution was extracted with EtOAc (4x50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The crude was purified by silica gel chromatography (gradient: cyclohexane/EtOAc 9.75/0.25 to Cyclohexane/EtOAc 9/1) to afford **12a** (1.05 g, 72%) as a yellow oil.

IR (KBr): ν, 3377 (N-H), 2977, 2930, 1720 (C=O), 1683 (C=O), 1579, 1517, 1367, 1248, 1169, 1138, 1113 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.44 (s, 9H), 1.58 (s, 9H), 2.83 (t, 2H, ^3J = 6.1 Hz), 2.95 (d, 3H, ^3J = 5,0 Hz), 3.05 (t, 2H, ^3J = 6,7 Hz), 3.46 (m, 2H), 4.58 (t, 2H, ^3J = 6,7 Hz), 4.93 (bs, 1H), 7.18 (dd, 1H, ^4J = 1,6 Hz, ^3J = 8,3 Hz), 7.32 (d, 1H, ^3J = 1,6 Hz), 7.75 (bd, 1H, ^3J = 5.0Hz), 7.89 (d, 1H, ^3J = 8.3 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.2 (3C), 28.3 (3C), 29.6, 36.9, 38.6, 39.1, 63.1, 79.5, 81.3, 111.9, 114.6, 114.9, 131.9, 134.3, 151.6, 155.7, 166.4, 167.7; **HRMS/ESI:** *m/z* calcd for $C_{22}H_{35}N_2O_6S_2$ $[M+H]^+$ 487.1937, found 487.1937.

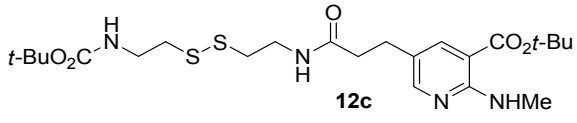
***tert*-Butyl 4-({2-[(2-{[(*tert*-butoxy)carbonyl]amino}ethyl)disulfanyl]ethylcarbamoyl)-2-(methylamino)benzoate 12b**



12b

In a round bottom flask were introduced **5** (0.65 g, 2.61 mmol), DCM (7.50 mL), EDCI (0.75, 3.91 mmol) and HOBt (0.53 g, 3.91 mmol). After stirring the mixture 10 min at room temperature, *N*-Boc cystamine **12b** (0.72 g, 2.87 mmol) was added. The reaction was stirred at room temperature for 45 min. After removal of the solvent, 30 mL of water were added and the solution was extracted with EtOAc (4x30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The crude was purified by silica gel chromatography (gradient: Cyclohexane/EtOAc 9/1 to Cyclohexane/EtOAc 7/3) to afford **23** (0.95 g, 75%) as a white powder. **mp =** 223-225°C; **IR (KBr):** ν, 3369 (N-H), 3256 (N-H), 2977, 1680 (C=O), 1638 (C=O), 1575, 1550, 1513, 1267, 1250, 1170, 1135 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.43 (s, 9H), 1.58 (s, 9H), 2.81 (t, 2H, ^3J = 6.6Hz), 2.95 (m, 5H), 3.46 (q, 2H, ^3J = 6.1Hz), 3.77 (q, 2H, ^3J = 6.1Hz), 5.01 (bs, 1H), 6.88 (d, 1H, ^3J = 8.3Hz), 6.98 (bs, 1H), 7.13 (s, 1H), 7.76 (bs, 1H), 7.87 (d, 1H, ^3J = 8.3Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.2 (3C), 28.3 (3C), 29.5, 38.0 (2C), 38.9, 39.3, 79.6, 81.1, 109.9, 111.6, 113.6, 132.1 (2C), 139.1, 151.9, 155.8, 167.7; **HRMS/ESI:** *m/z* calcd for $C_{22}H_{36}N_3O_5S_2$ $[M+H]^+$ 486.2096, found 486.2086.

***tert*-Butyl 5-[2-({2-[(2-{[(*tert*-butoxy)carbonyl]amino}ethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 12c**

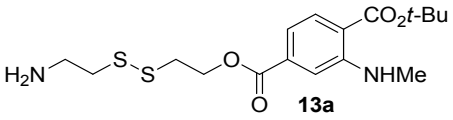


12c

In a round bottom flask were introduced **11** (3.00 g, 10.70 mmol), DCM (50 mL), EDCI (3.08 g, 16.06 mmol) and HOBt (2.17 g, 16.06 mmol) and the mixture was stirred at room temperature. After 10 min, Boc-cystamine (2.97 g, 11.77 mmol) was added and the reaction was stirred at room temperature for 2 h. After concentration, 75 mL of a satured aqueous solution of sodium bicarbonate was added and the solution was extracted with EtOAc (4×75 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The crude was purified by silica gel chromatography (gradient: DCM/EtOAc 8/2 to DCM/EtOAc 2/8) to afford **12c** (5.20 g, 94%) as a white powder. **mp =** 106-108°C; **IR (KBr):** ν, 3385 (N-H), 3338 (N-H), 3275 (N-H), 2980, 1682 (C=O), 1654 (C=O), 1569, 1547, 1538, 1511, 1389, 1366, 1303, 1289, 1253, 1229, 1165, 1126 cm^{-1}; **^{1}H NMR (400MHz, $CDCl_3$):** δ 1.43 (s, 9H),

1.57 (s, 9H), 2.47 (t, 2H, ^3J = 7.5 Hz), 2.74 (t, 2H, ^3J = 6.8 Hz), 2.83 (m, 4H), 3.03 (d, 3H, ^3J = 4.9Hz), 3.42 (q, 2H, ^3J = 6.4 Hz), 3.55 (q, 2H, ^3J = 5.8 Hz), 5.03 (bs, 1H), 6.51 (bs, 1H), 7.84 (bs, 1H), 7.87 (d, 1H, ^4J = 1.3 Hz), 8.14 (d, 1H, ^4J = 1.3 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 27.9 (2C), 28.2 (3C), 28.3 (3C), 37.4, 38.0, 38.3, 38.5, 39.5, 79.7, 81.3, 107.2, 122.4, 139.8, 152.8, 155.9, 158.2, 167.0, 172.2; **HRMS/ESI:** *m/z* calcd for $C_{23}H_{39}N_4O_5S_2$ $[M+H]^+$ 515.2362, found 515.2342.

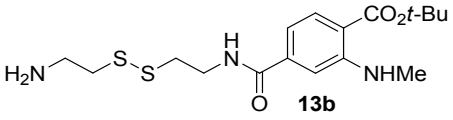
4-{2-[(2-Aminoethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 13a



13a

In a round bottom flask were introduced **12a** (0.80 g, 1.64 mmol), DCM (12 mL) and TFA (4 mL). The reaction was stirred at room temperature for 30 min. After concentration, 50 mL of a saturated aqueous solution of sodium bicarbonate were added and the solution was extracted with Et_2O (3×50 mL). The combined organic layers were washed with water (2×50 mL), dried over $MgSO_4$ and evaporated to afford **13a** (0.63 g, 100%) as a yellow oil. **IR (KBr):** ν, 3378 (N-H), 2925, 2930, 1683 (C=O), 1579, 1517, 1368, 1248, 1170, 1138, 1115; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.58 (s, 9H), 2.85 (t, 2H, ^3J = 6,1 Hz), 2.94 (d, 3H, ^3J = 5.1 Hz), 2.98 (bs, 2H), 3.05-3.10 (m, 4H), 4.58 (t, 2H, ^3J = 6.5Hz), 7.17 (dd, 1H, ^4J = 1.5 Hz, ^3J = 8.3 Hz), 7.31 (d, 1H, ^3J = 1,5 Hz), 7.74 (bd, 1H, ^3J = 5.0 Hz), 7.88 (d, 1H, ^3J = 8.3 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.2 (3C), 29.6, 36.9, 39.8, 40.5, 63.0, 81.3, 111.9, 114.5, 114.9, 131.9, 134.3, 151.6, 166.4, 167.6; **HRMS/ESI:** *m/z* calcd for $C_{17}H_{27}N_2O_4S_2$ $[M+H]^+$ 387.1412, found 387.1394.

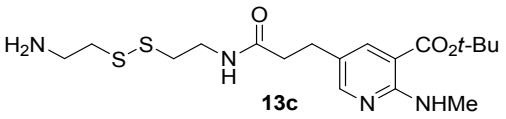
***tert*-Butyl 5-[2-({2-[(2-aminoethyl)disulfanyl]ethyl}carbamoyl)]-2-(methylamino)benzoate 13b**



13b

In a round bottom flask were introduced **11** (0.50 g, 1.03 mmol), DCM (8 mL) and TFA (2 mL). The reaction was stirred at room temperature for 30 min. After concentration, 50 mL of a saturated aqueous solution of $NaHCO_3$ were added and the solution was extracted with EtOAc (4×50 mL). The combined organic layers were washed with water (2×50 mL), dried over $MgSO_4$ and evaporated to afford **13b** (0.39 g, 100%) as an oil. **IR (KBr):** ν, 3375 (NH_2), 3253 (NH_2), 1685 (C=O), 1638 (C=O), 1576, 1551, 1265, 1249, 1200, 1170, 1133 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 1.58 (s, 9H), 2.07 (bs, 2H), 2.81 (t, 2H, ^3J = 5.7 Hz), 2.92 (m, 5H), 3.04 (bs, 2H), 3.79 (q, 2H, ^3J = 5.7 Hz), 6.79 (bs, 1H), 6.82 (d, 1H, ^3J = 8.0 Hz), 7.10 (s, 1H), 7.78 (bs, 1H), 7.87 (d, 1H, ^3J = 8.0 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 28.2 (3C), 29.5, 37.5, 38.9, 40.2, 41.3, 81.1, 109.7, 111.5, 113.7, 132.1, 139.1, 151.9, 167.6, 167.7; **HRMS/ESI:** *m/z* calcd for $C_{17}H_{28}N_3O_3S_2$ $[M+H]^+$ 386.1572, found 386.1558.

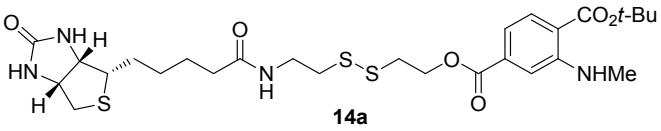
***tert*-Butyl 5-[2-({2-[(2-aminoethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 13c**



13c

In a round bottom flask were introduced **12c** (2.00 g, 3.89 mmol), DCM (15 mL) and TFA (5 mL). The reaction was stirred at room temperature for 30 min. After concentration, 50 mL of a saturated aqueous solution of sodium bicarbonate were added and the solution was extracted with EtOAc (4×50 mL). The combined organic layers were washed with water (2×50 mL), dried over $MgSO_4$ and evaporated to afford **13c** (1.60 g, 100%) as an oil. **IR (KBr):** ν, 3378 (N-H), 2977, 2931, 1682 (C=O), 1612, 1578, 1520, 1368, 1304, 1228, 1160, 1131, 1095, 910, 732 cm^{-1}; **^{1}H NMR (400MHz, $CDCl_3$):** δ 1.58 (s, 9H), 1.64 (bs, 2H), 2.42 (t, 2H, ^3J = 7.6 Hz), 2.76 (t, 4H, ^3J = 6.1 Hz), 2.84 (t, 2H, ^3J = 7.6 Hz), 3.03 (d, 3H, ^3J = 4.9 Hz), 3.03 (m, 2H), 3.58 (q, 2H, ^3J = 6.1 Hz), 6.00 (bs, 1H), 7.84 (bs, 1H), 7.87 (d, 1H, ^4J = 2.4 Hz), 8.14 (d, 1H, ^4J = 2.4 Hz); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 27.7, 27.8, 28.1 (3C), 37.4, 37.9, 38.3, 40.3, 41.9, 81.3, 107.1, 122.2, 139.7, 152.5, 158.0, 166.8, 171.9; **HRMS/ESI:** *m/z* calcd for $C_{18}H_{31}N_4O_3S_2$ $[M+H]^+$ 415.1838, found 415.1821.

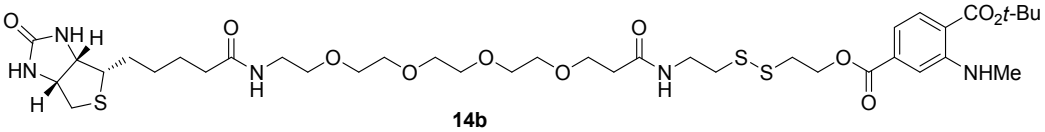
***tert*-Butyl 4-({2-[(2-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl}carbamoyl)-2-(methylamino)benzoate 14a**



14a

In a round bottom flask were introduced **13a** (0.37 g, 0.96 mmol), DMF (5 mL), Biot-CONHS (0.49 g, 1.43 mmol) and TEA (0.20 mL, 1.43 mmol). The reaction was stirred at room temperature for 1 h. After concentration the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 3/7 in 30 min) to afford **14a** (0.45 g, 75%) as a yellow powder. **IR (KBr):** ν, 3374 (N-H), 3294 (N-H), 2928, 1706 (C=O), 1682 (C=O), 1644 (C=O), 1578, 1245, 1138, 1167, 1113, 751; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.40-1.46 (m, 2H), 1.58 (s, 9H), 1.62-1.70 (m, 4H), 2.23 (t, 2H, ^3J = 6.0 Hz), 2.72 (d, 1H, ^3J = 10,2 Hz), 2.88 (m, 3H), 2,95 (d, 3H, ^3J = 4,0 Hz), 3.05 (t, 2H, ^3J = 5,4 Hz), 3.12 (m, 1H), 3.56 (q, 2H, ^3J = 4.8 Hz), 4.30 (dd, 1H, ^3J = 4.0 Hz, ^3J = 6.0 Hz), 4.50 (dd, 1H, ^3J = 4.0 Hz, ^3J = 6.0 Hz), 4.58 (m, 2H, ^3J = 5,4 Hz), 5.57 (bs, 1H), 6.57 (bs, 1H), 6.68 (bt, 1H, ^3J = 4.4 Hz), 7.17 (dd, 1H, ^4J = 1.3 Hz, ^3J = 6,6 Hz), 7.30 (d, 1H, ^4J = 1.3 Hz), 7.75 (bq, 1H, ^3J = 4.0 Hz), 7.89 (d, 1H, ^3J = 6.6 Hz); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 25.6, 28.1, 28.2, 28.3 (3C), 29.6, 35.9, 36.7, 38.0, 38.2, 40.6, 55.7, 60.2, 61.7, 63.2, 81.4, 111.9, 114.6, 115.0, 131.9, 134.2, 151.6, 164.0, 166.5, 167.6, 173.5; **HRMS/ESI:** *m/z* calcd for $C_{27}H_{41}N_4O_6S_3$ $[M+H]^+$ 613.21827, found 613.21768.

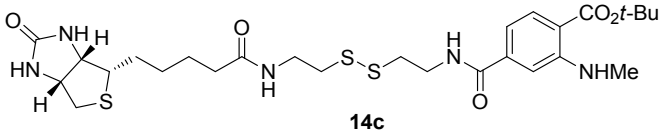
4-(2-{[2-(1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl) 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 14b



14b

In a round bottom flask were introduced **13a** (0.40 g, 1.04 mmol), DCM (15 mL), Biot-peg$_4$-COPFP (0.68 g, 1.04 mmol) and TEA (0.14 mL, 1.04 mmol). The reaction was stirred at room temperature for 30 min. After concentration the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 4/6 in 30 min) to afford **14b** (0.73 g, 82%) as a yellow powder. **IR (KBr):** ν, 3379 (N-H), 2926, 1682 (C=O), 1649 (C=O), 1577, 1517, 1458, 1306, 1247, 1114 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.41-1.47 (m, 2H), 1.58 (s, 9H), 1.63-1.78 (m, 4H), 2.23 (t, 2H, ^3J = 6.0 Hz), 2.49 (t, 2H, ^3J = 4,6 Hz), 2.74 (d, 1H, ^3J = 10.2 Hz), 2.87 (m, 3H), 2.95 (d, 3H, ^3J = 4.0 Hz), 3.06 (t, 2H, ^3J = 5.4 Hz), 3.13 (m, 1H), 3.42 (m, 2H), 3.57 (m, 4H), 3.63 (m, 12H), 3.73 (t, 2H, ^3J = 4,6 Hz), 4.31 (dd, 1H, ^3J = 4,0 Hz, ^3J = 5,8 Hz), 4.50 (dd, 1H, ^3J = 4,0 Hz, ^3J = 5.8 Hz), 4.58 (t, 2H, ^3J = 5.4 Hz), 5.57 (bs, 1H), 6.53 (bs, 1H), 6.86 (bs, 1H), 7.05 (t, 1H, ^3J = 4.5 Hz), 7.17 (dd, 1H, ^4J = 1.2 Hz, ^3J = 6.6 Hz), 7.31 (d, 1H, ^3J = 1,2 Hz), 7.75 (m, 1H), 7.88 (d, 1H, ^3J = 6.6 Hz); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 25.6, 28.1, 28.2, 28.3 (3C), 29.6, 35.9, 36.8, 36.8, 38.0, 38.1, 39.2, 40.5, 55.6, 60.2, 61.8, 63.1, 67.3, 70.0, 70.1, 70.2, 70.3, 70.4, 70.4, 70.5, 81.3, 111.9, 114.6, 115.0, 131.9, 134.3, 151.6, 163.9, 166.4, 167.6, 171.8, 173.4; **HRMS/ESI:** *m/z* calcd for $C_{38}H_{62}N_5O_{11}S_3$ $[M+H]^+$ 860.36025, found 859.36030.

4-{2-[(2-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 14c

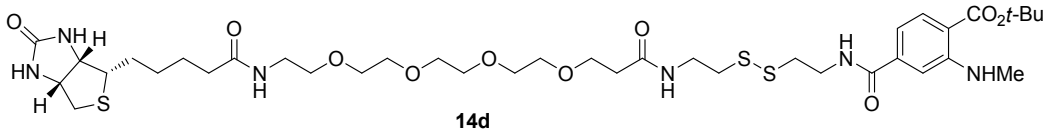


14c

In a round bottom flask were introduced **13b** (0.30 g, 0.78 mmol), DMF (3 mL), Biot-CONHS (0.27 g, 0.78 mmol) and TEA (0.11 mL, 0.78 mmol). The reaction was stirred at room temperature for 1 h. After concentration the crude was purified by reverse phase flash

chromatography (gradient: H_2O to H_2O/ACN 6/4 in 30 min) to afford **14c** (0.33 g, 70%) as a white powder. **mp =** 119-121°C; **IR (KBr):** ν, 3369 (N-H), 3296 (N-H), 2928, 1702 (C=O), 1681 (C=O), 1643 (C=O), 1574, 1540, 1513, 1266, 1249, 1166, 1134 cm^{-1}; **^{1}H NMR (500 MHz, DMSO-*d6*):** δ 1.28-1.34 (m, 2H); 1.42-1.63 (m, 4H); 1.55 (s, 9H); 2.08 (m, 2H); 2.58 (d, 1H, ^3J = 9.9 Hz); 2.81 (m, 3H); 2.89 (d, 3H, ^3J = 4.0 Hz); 2.91 (m, 2H); 3.09 (m, 1H); 3.33 (m, 2H); 3.55 (q, 2H, ^3J = 5.1 Hz); 4.12 (m, 1H); 4.30 (m, 1H); 6.37 (s, 1H); 6.44 (s, 1H); 6.98 (d, 1H, ^3J = 6.6 Hz); 7.11 (s, 1H); 7.65 (q, 1H, ^3J = 4.0 Hz); 7.78 (d, 1H, ^3J = 6.6 Hz); 8.01 (t, 1H, ^3J = 4.4 Hz); 8.73 (t, 1H, ^3J = 4.4 Hz); **^{13}C NMR (125 MHz, DMSO-*d6*):** δ 25.2, 27.8 (3C), 28.0, 28.1, 29.3, 35.1, 36.9, 37.2, 37.8, 38.9, 39.8, 55.4, 59.1, 61.0, 80.7, 109.7, 112.3, 112.4, 131.3, 139.3, 151.2, 162.7, 166.1, 166.9, 172.2; **HRMS/ESI:** *m/z* calcd for $C_{27}H_{42}N_5O_5S_3$ $[M+H]^+$ 612.23426, found 612,23374.

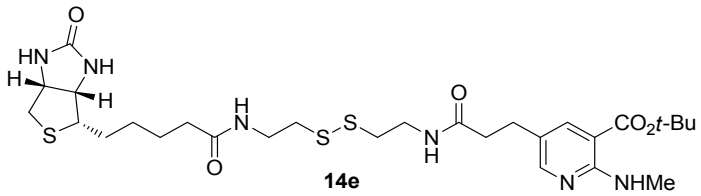
***tert*-Butyl 4-[(2-{[2-(1-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl)carbamoyl]-2-(methylamino)benzoate 14d**



14d

In a round bottom flask were introduced **13b** (0.40 g, 1.04 mmol), DCM (15 mL), Biot-peg$_4$-COPFP (0.68 g, 1.04 mmol) and TEA (0.14 mL, 1.04 mmol). The reaction was stirred at room temperature for 1 h. After concentration the crude was purified by silica gel chromatography (gradient: EtOAc to EtOAc/MeOH 8/2) to afford **14d** (0.81 g, 91%) as a white powder. **IR (KBr):** ν, 3377 (N-H), 2925, 1683 (C=O), 1649 (C=O), 1572, 1543, 1513, 1250, 1166, 1134, 1096 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.40-1.45 (m, 2H), 1.61-1.76 (m, 4H), 1.58 (s, 9H), 2.22 (t, 2H, ^3J = 6.0 Hz), 2.49 (t, 2H, ^3J = 4.7 Hz), 2.72 (d, 1H, ^3J = 10.1 Hz), 2.88 (m, 3H), 2.94 (d, 3H, ^3J = 4.0 Hz), 2.97 (t, 2H, ^3J = 5.2 Hz), 3.12 (m, 1H), 3.41 (m, 2H), 3.56 (m, 4H), 3.62 (m, 12H), 3.74 (m, 4H), 4.29 (dd, 1H, ^3J = 4.0 Hz; ^3J = 6.1 Hz), 4.48 (dd, 1H, ^3J = 4,0 Hz, ^3J = 6,1 Hz), 5.57 (bs, 1H), 6.51 (bs, 1H), 6.94 (m, 2H), 7.16 (d, 1H, ^4J = 1,2 Hz), 7.31 (t, 1H, ^3J = 4,6 Hz), 7.50 (t, 1H, ^3J = 4,6 Hz), 7.74 (q, 1H, ^3J = 3,9 Hz), 7.86 (d, 1H, ^3J = 6.6 Hz); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 25.6, 28.0, 28.2, 28.3 (3C), 29.6, 35.9, 36.8, 37.8, 37.9, 38.3, 39.2, 39.3, 40.5, 55.6, 60.2, 61.8, 67.2, 69.9, 70.0, 70.2, 70.3, 70.4 (3C), 81.1, 110.0, 112.0, 113.6, 132.1, 139.2, 151.9, 164.0, 167.7, 167.8, 172.0, 173.4; **HRMS/ESI:** *m/z* calcd for $C_{38}H_{63}N_6O_{10}S_3$ $[M+H]^+$ 859.37623, found 859.37604.

***tert*-Butyl 5-[2-({2-[(2-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 14e**

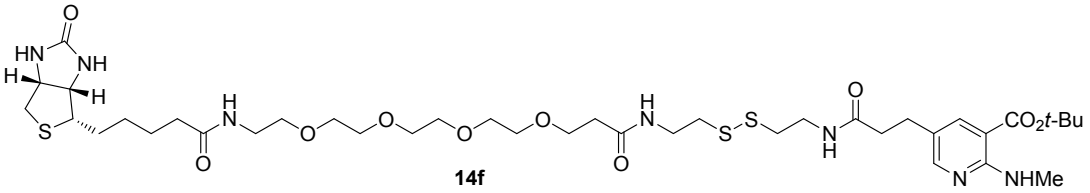


14e

In a round bottom flask were introduced **13c** (0.50 g, 1.21 mmol), DMF (20 mL), Biot-CONHS (0.41 g, 1.21 mmol) and TEA (0.17 mL, 1.21 mmol). The reaction was stirred at room temperature for 1 h. After concentration, the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 3/7 in 30 min) to afford **14e** (0.55 g, 70%) as a white powder. **mp =** 108-110°C; **IR (KBr):** ν, 3297 (N-H), 3074, 2928, 2853, 1704 (C=O), 1683 (C=O), 1642 (C=O), 1613, 1578, 1520, 1226, 1159, 1094, 803, 727, 597 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.42 (m, 2H), 1.57 (s, 9H), 1.60-1.73 (m, 4H), 2.23 (td, ^3J = 7.2 Hz, ^4J = 2.9 Hz, 2H), 2.47 (t, ^3J = 7.5 Hz, 2H), 2.71 (d, ^2J = 12.8 Hz, 1H), 2.78-2.88 (m, 6H), 2.89 (dd, ^2J = 12.8 Hz, ^3J = 4.9 Hz, 1H), 3.01 (d, ^3J = 4.9 Hz, 3H), 3.10-3.15 (m, 1H), 3.50-3.54 (m, 4H), 4.30 (dd, ^3J = 7.5 Hz, ^3J = 4.5 Hz, 1H), 4.50 (dd, ^3J = 7.5 Hz, ^3J = 4.9 Hz, 1H), 5.71 (bs, 1H), 6.58 (bs, 1H), 6.85 (t, ^3J = 5.8 Hz, 1H), 7.09 (t, ^3J = 5.8 Hz, 1H), 7.83 (q, ^3J =

4.9 Hz, 1H), 7.88 (d, ^4J = 2.4 Hz, 1H), 8.14 (d, ^4J = 2.4 Hz, 1H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 25.6, 27.9, 27.9, 28.0, 28.1, 28.3 (3C), 35.7, 37.6, 38.0, 38.2, 38.4, 38.5, 40.6, 55.7, 60.2, 61.7, 81.5, 107.4, 122.6, 140.0, 152.6, 158.2, 164.1, 167.0, 172.6, 173.8; **HRMS/ESI:** *m/z* calcd for $C_{28}H_{45}N_6O_5S_3$ $[M+H]^+$ 641.2614, found 641.2626.

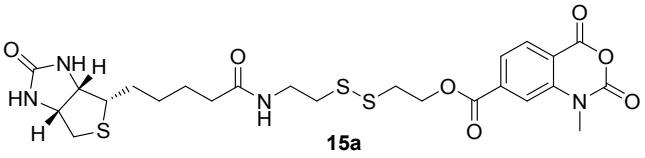
***tert*-Butyl 5-{2-[(2-{[2-(1-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl)carbamoyl]ethyl}-2-(methylamino)pyridine-3-carboxylate 14f.**



14f

In a round bottom flask were introduced **13c** (0.40 g, 0.96 mmol), DCM (15 mL), Biot-peg$_4$-COPFP (0.64 g, 0.97 mmol) and TEA (0.13 mL, 0.96 mmol). The reaction was stirred at room temperature for 45 min. After concentration, the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 4/6 in 30 min) to afford **14f** (0.73 g, 85%) as a white powder. **IR (KBr):** ν, 3400 (N-H), 2925, 1681 (C=O), 1644 (C=O), 1579, 1525, 1157, 1124, 804, 618 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.40-1.46 (m, 2H), 1.57 (s, 9H), 1.61-1.78 (m, 4H), 2.22 (t, ^3J = 7.1 Hz, 2H), 2.46-2.49 (m, 4H), 2.73 (d, ^2J = 12.7 Hz, 1H), 2.77-2.83 (m, 6H), 2.90 (dd, ^2J = 12.7 Hz, ^3J = 5.0 Hz, 1H), 3.02 (d, ^3J = 4.9 Hz, 3H), 3.13 (m, 1H), 3.39-3.44 (m, 2H), 3.52-3.55 (m, 6H), 3.62-3.63 (m, 12H), 3.72 (t, ^3J = 5.9 Hz, 2H), 4.31 (m, 1H), 4.50 (m, 1H), 5.41 (bs, 1H), 6.33 (bs, 1H), 6.85 (t, ^3J = 5.5 Hz, 1H), 6.95 (t, ^3J = 5.6 Hz, 1H), 7.28 (t, ^3J = 5.8 Hz, 1H), 7.82 (q, ^3J = 4.9 Hz, 1H), 7.88 (d, ^4J = 2.5 Hz, 1H), 8.14 (d, ^4J = 2.5 Hz, 1H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 24.6, 26.9, 26.9, 27.1, 27.1, 27.3 (3C), 34.8, 35.8, 36.2, 37.2, 37.3, 37.4, 37.5, 38.2, 39.5, 54.5, 59.1, 60.8, 66.2, 68.9, 69.0, 69.2, 69.3, 69.5 (3C), 80.4, 106.3, 121.7, 138.9, 151.8, 157.2, 162.7, 166.1, 171.0, 171.5, 172.3; **HRMS/ESI:** *m/z* calcd for $C_{39}H_{66}N_7O_{10}S_3$ $[M+H]^+$ 888.4033, found 888.4020.

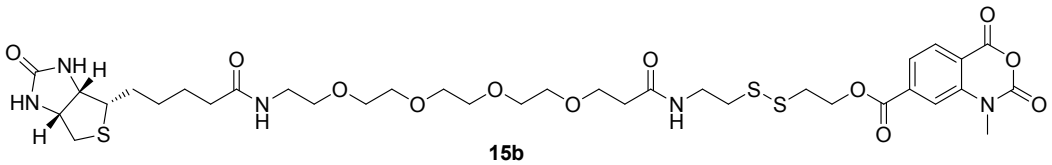
2-[(2-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl 1-methyl-2,4-dioxobenzo[*d*][1,3]oxazine-7-carboxylate 15a



15a

In a round bottom flask at 0°C were introduced **14a** (0.35 g, 0.57 mmol), DCM (20 mL) and a solution of phosgene at 20% in toluene (0.45 mL, 0.86 mmol). The reaction was stirred at room temperature for 30 min. After concentration the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 5/5 in 30 min) to afford **15a** (0.23 g, 67%) as a yellow powder. **IR (KBr):** ν, 3288 (N-H), 2928, 1786 (C=O), 1728 (C=O), 1703 (C=O), 1640, 1620, 1470, 1446, 1333, 1269, 1242, 1118, 1035, 743, 671 cm^{-1}; **^{1}H NMR (500 MHz, DMSO-*d6*):** 1.27-1.33 (m, 2H), 1.42-1.63 (m, 4H), 2.06 (t, ^3J = 7.2 Hz, 2H), 2.57 (d, ^2J = 12.4 Hz, 1H), 2.79-2.83 (m, 3H), 3.08 (m, 1H), 3.16 (t, ^3J = 5.9 Hz, 2H), 3.32 (m, 2H), 3.52 (s, 3H), 4.12 (m, 1H), 4.30 (m, 1H), 4.59 (t, ^3J = 5.9 Hz, 2H), 6.37 (bs, 1H), 6.43 (bs, 1H), 7.84 (m, 2H), 8.00 (t, ^3J = 5.2 Hz, 1H), 8.16 (d, ^3J = 8.0 Hz, 1H); **^{13}C NMR (125 MHz, DMSO-*d6*):** 25.9, 27.9, 28.1, 31.8, 35.1, 36.1, 37.4, 37.6, 39.8, 55.4, 59.1, 61.0, 63.5, 115.1, 115.3, 123.4, 130.0, 136.6, 142.3, 147.5, 158.5, 162.7, 164.3, 172.2; **HRMS/ESI:** *m/z* calcd for $C_{24}H_{31}N_4O_7S_3$ $[M+H]^+$ 583.13494, found 583.13475.

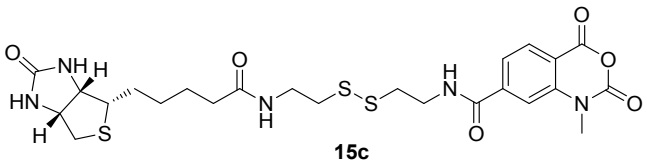
2-{[2-(1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl 1-methyl-2,4-dioxobenzo[*d*][1,3]oxazine-7-carboxylate 15b.



15b

In a round bottom flask at 0°C were introduced **14b** (0.50 g, 0.58 mmol), DCM (20 mL) and a solution of phosgene at 20% in toluene (0.46 mL, 0.87 mmol). The reaction was stirred at room temperature for 30 min. After concentration the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 6/4 in 30 min) to afford **15b** (0.31 g, 64%) as a yellow powder. **IR (KBr):** ν, 3413 (N-H), 2924, 2855, 1784 (C=O), 1729 (C=O), 1694 (C=O), 1652, 1548, 1450, 1334, 1270, 1249, 1117, 1036, 745 cm^{-1}; **^{1}H NMR(500 MHz, $CDCl_3$):** δ 1.38-1.46 (m, 2H), 1.61-1.78 (m, 4H), 2.23 (t, ^3J = 7.3 Hz, 2H), 2.49 (t, ^3J = 5.8 Hz, 2H), 2.74 (d, ^2J = 12.8 Hz, 1H), 2.85-2.91 (m, 3H), 3.08 (t, ^3J = 6.5 Hz, 2H), 3.14 (m, 1H), 3.42 (t, ^3J = 4.7 Hz, 2H), 3.55-3.57 (m, 4H), 3.59-3.65 (m, 15H), 3.73 (t, ^3J = 5.8 Hz, 2H), 4.31 (m, 1H), 4.49 (m, 1H), 4.66 (t, ^3J = 6.5 Hz, 2H), 5.55 (m, 1H), 6.47 (m, 1H), 6.95 (m, 1H), 7.12 (t, ^3J = 5.7 Hz, 1H), 7.87 (s, 1H), 7.94 (dd, ^3J = 8.1 Hz, ^4J = 1.2 Hz, 1H), 8.24 (d, ^3J = 8.1 Hz, 1H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 25.6, 28.1, 28.2, 32.2, 35.9, 36.7, 36.8, 37.9, 38.2, 39.2, 40.5, 55.6, 60.2, 61.2, 64.0, 67.3, 70.0 (2C), 70.2, 70.3, 70.4 (3C), 114.8, 115.3, 124.5, 131.2, 137.7, 142.1, 147.6, 157.8, 163.9, 164.5, 171.8, 173.5; **HRMS/ESI:** *m/z* calcd for $C_{35}H_{52}N_6O_{11}S_3$ $[M+H]^+$ 830.27691, found 830.27712.

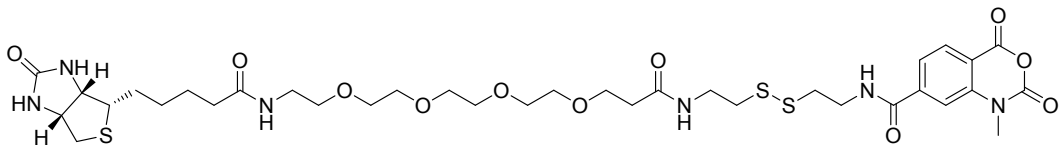
5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]-*N*-[2-({2-[(1-methyl-2,4-dioxobenzo[*d*][1,3]oxazin-7-yl)formamido]ethyl}disulfanyl)ethyl]pentanamide 15c



15c

In a round bottom flask, at 0°C, were introduced **14c** (0.25 g, 0,41 mmol), DCM (20 mL) and a solution of phosgene at 20% in toluene (0.32 mL, 0.61 mmol). The reaction was stirred at room temperature for 1 h. After concentration, the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 6/4 in 30 min) to afford **15c** (0.12 g, 50%) as a white powder. **IR (KBr):** ν, 3287 (N-H), 2926, 1783 (C=O), 1726 (C=O), 1704 (C=O), 1643, 1619, 1585, 1543, 1468, 1442, 1342, 1293, 1244, 1032, 742, 681 cm^{-1}; **^{1}H NMR (500 MHz, DMSO-*d6*):** 1.25-1.31 (m, 2H), 1.39-1.62 (m, 4H), 2.05 (t, ^3J = 7.3 Hz, 2H), 2.55 (d, ^2J = 12.4 Hz, 1H), 2.76-2.81 (m, 3H), 2.92 (t, ^3J = 6.7 Hz, 2H), 3.07 (m, 1H), 3.31 (m, 2H), 3.50 (s, 3H), 3.58 (q, ^3J = 6.3 Hz, 2H), 4.10 (m, 1H), 4.28 (m, 1H), 6.35 (bs, 1H), 6.41 (bs, 1H), 7.72 (m, 2H), 7.99 (t, ^3J = 5.4 Hz, 1H), 8.08 (d, ^3J = 8.0 Hz, 1H), 9.04 (t, ^3J = 5.3 Hz, 1H). **^{13}C NMR (125 MHz, DMSO-*d6*):** 25.2, 28.0, 28.1, 31.8, 35.1, 36.8, 37.2, 37.8, 38.9, 39.8, 55.4, 59.1, 61.0, 113.4, 113.6, 122.0, 129.6, 141.6, 142.2, 147.7, 158.6, 162.7, 164.8, 172.2; **HRMS/ESI:** *m/z* calcd for $C_{24}H_{32}N_5O_6S_3$ $[M+H]^+$ 582.15092, found 582.15104.

1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-d]imidazolidin-4-yl]pentanamido}-*N*-[2-({2-[(1-methyl-2,4-dioxobenzo[*d*][1,3]oxazin-7-yl)formamido]ethyl}disulfanyl)ethyl]-3,6,9,12-tetraoxapentadecan-15-amide 15d.

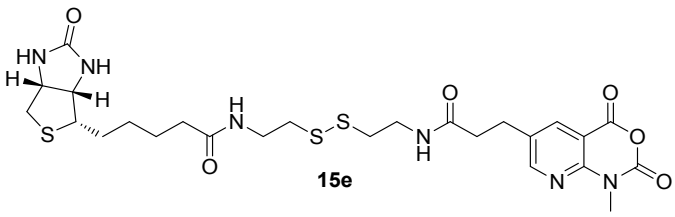


15d

In a round bottom flask, at 0°C, were introduced **14d** (0.40 g, 0.47 mmol), DCM (20 mL) and a solution of phosgene at 20% in toluene (0.37 mL, 0.70 mmol). The reaction was stirred at room temperature for 1 h. After concentration the crude was purified by reverse phase flash

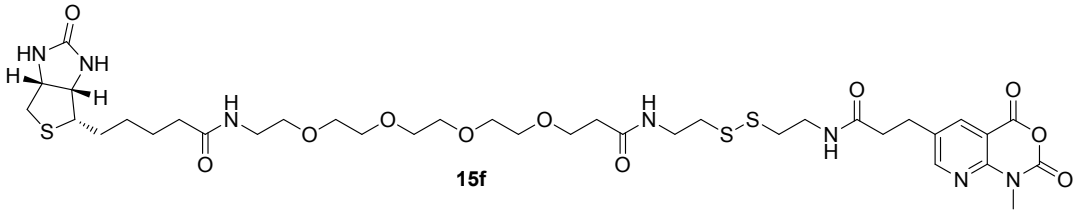
chromatography (gradient: H_2O to H_2O/ACN 6/4 in 30 min) to afford **15d** (0.31 g, 80%) as a white powder. **IR (KBr):** ν, 3443 (N-H), 2923, 1782 (C=O), 1727 (C=O), 1645, 1545, 1346, 1293, 1247, 1100, 744, 683 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.34-1.43 (m, 2H), 1.57-1.74 (m, 4H), 2.21 (t, ^3J = 7.5 Hz, 2H), 2.48 (t, ^3J = 5.8 Hz, 2H), 2.72 (d, ^2J = 12.7 Hz, 1H), 2.85 (t, ^3J = 6.9 Hz, 2H), 2.89 (dd, ^2J = 12.7 Hz, ^3J = 4.9 Hz, 1H), 3.01 (t, ^3J = 6.3 Hz, 2H), 3.10-3.14 (m, 1H), 3.35-3.42 (m, 2H), 3.53-3.56 (m, 4H), 3.62-3.64 (m, 15H), 3.71 (t, ^3J = 5.8 Hz, 2H), 3.75 (q, ^3J = 6.3 Hz, 2H), 4.29 (dd, ^3J = 7.7 Hz, ^3J = 5.5 Hz, 1H), 4.50 (dd, ^3J = 7.7 Hz, ^3J = 4.9 Hz, 1H), 5.69 (bs, 1H), 6.54 (bs, 1H), 6.94 (t, ^3J = 5.5 Hz, 1H), 7.43 (t, ^3J = 5.9 Hz, 1H), 7.84-7.86 (m, 2H), 8.17 (d, ^3J = 8.4 Hz, 1H), 8.56 (t, ^3J = 5.6 Hz, 1H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 25.6, 28.0, 28.1, 32.1, 35.8, 36.8, 37.3, 38.4, 38.6, 39.2, 39.7, 40.5, 55.6, 60.2, 61.8, 67.1, 69.9, 70.0, 70.2, 70.2, 70.4 (3C), 113.3, 113.9, 122.6, 130.8, 142.1, 142.5, 147.8, 158.2, 164.0, 165.7, 172.2, 173.5; **HRMS/ESI:** *m/z* calcd for $C_{35}H_{53}N_6O_{11}S_3$ $[M+H]^+$ 829.29290, found 829,29177.

5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]-*N*-(2-{[2-(3-{1-methyl-2,4-dioxopyrido[2,3-*d*][1,3]oxazin-6-yl}propanamido)ethyl]disulfanyl}ethyl)pentanamide 15e



In a sealed tube were introduced **14e** (0.50 g, 0,78 mmol), Et_2O (20 mL), TEA (0.33 mL, 2.34 mmol) and a solution of phosgene at 20% in toluene (1.23 mL, 2.34 mmol). The reaction was stirred at room temperature for 30 min. After concentration the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 4/6 in 30 min) to afford **15e** (0.20 g, 42%) as a white powder. **mp =** 125-127°C; **IR (KBr):** ν, 3299 (N-H), 2927, 1785 (C=O), 1735 (C=O), 1704 (C=O), 1641 (C=O), 1612, 1488, 1326, 1232, 1179, 1069, 1045, 979, 787, 745, 674 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.24-1.34 (m, 2H), 1.41-1.59 (m, 4H), 2.05 (t, ^3J = 7.4 Hz, 2H), 2.44 (t, ^3J = 7.4 Hz, 2H), 2.56 (d, ^2J = 12.4 Hz, 1H), 2.68-2.73 (m, 4H), 2.80 (dd, ^2J = 12.4 Hz, ^3J = 5.0 Hz, 1H), 2.90 (t, ^3J = 7.4 Hz, 2H), 3.08 (m, 1H), 3.26-3.29 (m, 4H), 3.47 (s, 3H), 4.11 (m, 1H), 4.28 (m, 1H), 6.35 (bs, 1H), 6.41 (bs, 1H), 7.97 (t, ^3J = 5.5 Hz, 1H), 8.06 (t, ^3J = 5.5 Hz, 1H), 8.21 (d, ^4J = 2.2 Hz, 1H), 8.62 (d, ^4J = 2.2 Hz, 1H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 25.2, 26.9, 27.9, 28.1, 29.9, 35.1, 36.0, 37.2 (2C), 37.8, 37.8, 39.8, 55.4, 59.2, 61.0, 107.2, 132.7, 137.8, 147.7, 150.9, 155.4, 158.4, 162.7, 170.9, 172.2; **HRMS/ESI:** *m/z* calcd for $C_{25}H_{35}N_6O_6S_3$ $[M+H]^+$ 611.1780, found 611.1777.

1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-*N*-(2-{[2-(3-{1-methyl-2,4-dioxopyrido[2,3-*d*][1,3]oxazin-6-yl}propanamido)ethyl]disulfanyl}ethyl)-3,6,9,12-tetraoxapentadecan-15-amide 15f



In a sealed tube were introduced **14f** (0.20 g, 0,22 mmol) prealably adsorbed on 1.25 g of C18 silica, Et_2O (20 mL), TEA (0.09 mL, 0.67 mmol) and a solution of phosgene at 20% in toluene (0.35 mL, 0.67 mmol). The reaction was stirred at room temperature for 30 min. After concentration the crude was purified by reverse phase flash chromatography (gradient: H_2O to H_2O/ACN 4/6 in 30 min) to afford **15f** (0.05 g, 26%) as a white powder. **IR (KBr):** ν, 3426 (N-H), 2926, 2875, 1782 (C=O), 1729 (C=O), 1641 (C=O), 1550, 1490, 1369, 1330, 1093, 788, 746, 677 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 1.41-1.47 (m, 2H), 1.58-1.78 (m, 4H), 2.23 (t, ^3J = 7.4 Hz, 2H), 2.49 (t, ^3J = 5.7 Hz, 2H), 2.64 (t, ^3J = 7.2 Hz, 2H), 2.75 (m, 3H), 2.78 (t, ^3J = 5.9 Hz, 2H), 2.91 (dd, ^2J = 12.5 Hz, ^3J = 4.9 Hz, 1H), 3.05 (t, ^3J = 7.2 Hz, 2H),

3.15 (m, 1H), 3.39-3.45 (m, 2H), 3.46-3.51 (m, 4H), 3.56 (t, ^{3}J = 5.0 Hz, 2H), 3.63-3.64 (m, 12H), 3.66 (s, 3H), 3.74 (t, ^{3}J = 5.7 Hz, 2H), 4.33 (m, 1H), 4.52 (m, 1H), 5.51 (bs, 1H), 6.37 (bs, 1H), 6.90 (t, ^{3}J = 5.4 Hz, 1H), 7.38 (t, ^{3}J = 5.7 Hz, 1H), 7.49 (t, ^{3}J = 5.7 Hz, 1H), 8.29 (d, ^{4}J = 2.2 Hz, 1H), 8.60 (d, ^{4}J = 2.2 Hz, 1H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 24.6, 26.7, 27.0, 27.1, 29.4, 34.8, 35.6, 35.7, 35.8, 37.1, 37.6, 37.7, 38.2, 39.5, 54.5, 59.2, 60.8, 64.8, 66.2, 68.9, 69.0, 69.1, 69.2, 69.4 (2C), 105.8, 132.3, 137.7, 146.8, 150.1, 155.5, 157.1, 162.8, 170.8, 171.2, 172.4; **HRMS/ESI:** *m/z* calcd for $C_{35}H_{56}N_7O_{11}S_3$ $[M+H]^+$ 858.3200, found 858.3185.

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 were introduced RNA or DNA (27 nucleotides-8nmol), 40µL of water, 20µL of triethylammonium acetate buffer (1M in water, pH = 7) and 20µL of isatoic anhydride derivative (120mM in DMSO). The eppendorf was incubated at 65 °C or room temperature 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 (900µL) 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 and 1µL of AKP, the mixture was incubated at room temperature for 3 hours. Finally, 20µL of the crude were directly injected in the LC/MS.

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).

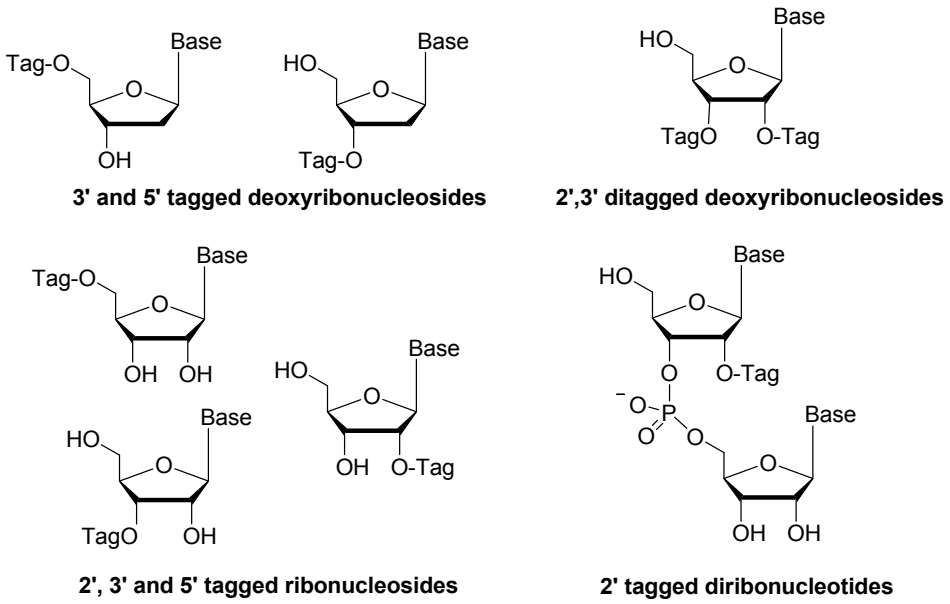


Figure 1. Tagged nucleosides observed by LC/MS.

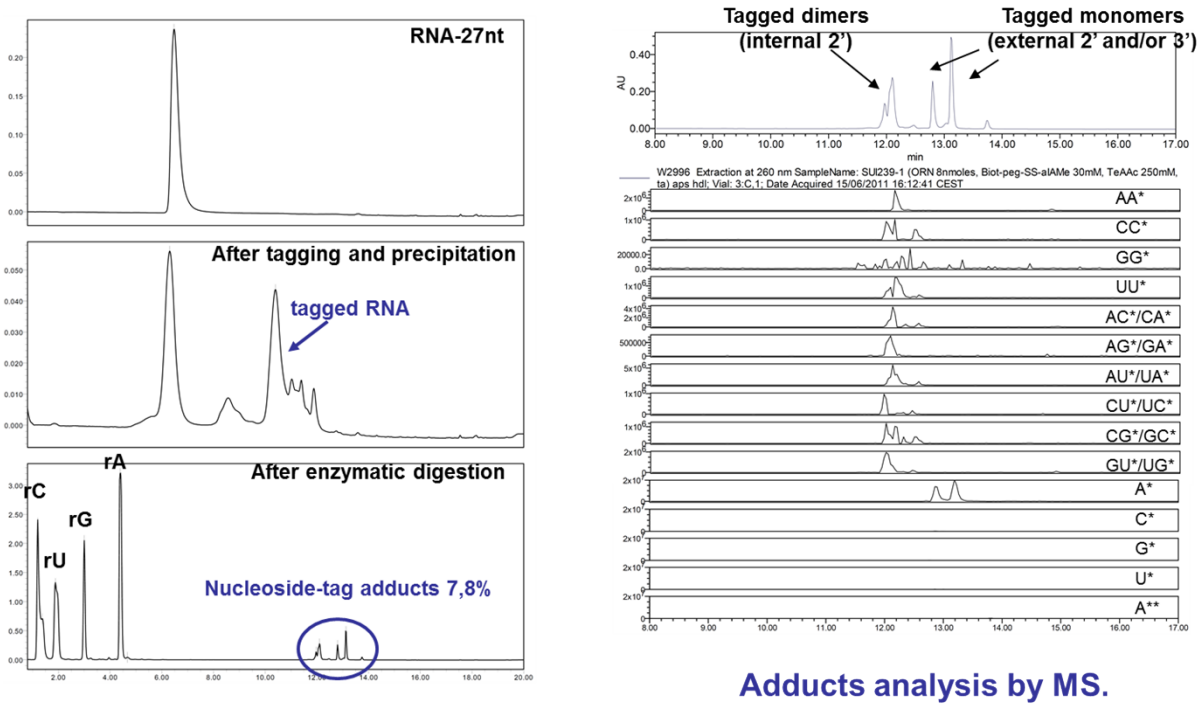


Figure 1. Example of LC/MS analysis after tagging and enzymatic hydrolysis of RNA with **15f** (30 mM at room temperature).

Formula 1.

$$\text{Free nucleosides Ratio} = \frac{Free\ nucleosides\ peaks\ Area}{(Free\ nucleosides\ peak\ area + tagged\ nucleosides\ peak\ area)} \text{ x100}$$

$$\text{Tagged nucleosides Ratio} = \frac{Tagged\ nucleosides\ peak\ area}{(Free\ nucleosides\ peak\ area + tagged\ nucleosides\ peak\ area)} \text{ x100}$$

5) Extraction of Biological RNA

Three kind of extraction experiments were performed according to the nucleic acids used: RNA alone (1), DNA alone (2) and a mixture of DNA/RNA 9/1 (3).

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

Experiment	RNA: HIV transcript		DNA: calf genomic DNA		RNA/DNA (9/1)		TEAAc Buffer		IA* 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) RNA alone	0.5	4	-	-	-	-	1	2	4x	2	1x
(2) DNA alone	-	-	1.9	5	-	-	1	2.5	4x	2.5	1x
(3) RNA/DNA 9/1	-	-	-	-	2.0	5	1	2.5	4x	2.5	1x

IA* = isatoic anhydride derivative **Cf = Tag final concentration

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

Purification. The samples were transferred to a 1.5 mL eppendorf tube. Regarding the binding capacity of magnetic silica particles used during the purification step (1 mg of particle for 2 µg of nucleic acids), experiment 2 (DNA alone) were divided in four 2.1 µL samples and experiment 3 (DNA/RNA mixture 9/1) was divided in five 2.1 µL samples. 900 µL of extraction buffer (EasyMAG® buffer 280134, Biomérieux) and 50 µL of magnetic silica particles (1 mg, 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. 500 µL of buffer I (EasyMAG® buffer 280130, Biomérieux) were added. The solution was vortexed, magnetized using a magnetic stand and the supernatant was removed. 900 µL of wash buffer II (EasyMAG® buffer 280131, Biomérieux), 500 µL of wash buffer II and 500 µL of buffer III (EasyMAG® buffer 280132, Biomérieux) were added with systematic vortexing, centrifugation, magnetization and supernatant elimination after each step. Finally, 20 µL of buffer III were added and the mixture was stirred at 70°C and 1400 rpm for 5 minutes. The solution was magnetized using a magnetic stand and the supernatant collected.

Streptavidin capture. 40 µg of MagPrep-25 particles (8 µL) were introduced in a 0.2 mL tube and washed twice with 80 µL of PBS (1x)+SDS (0.1%). 5 µL of PBS (4x) + SDS (0.4%) solution and 15 µL 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 80 µL of a solution of PBS (1x) + SDS (0.1%) for experiment 1-2 or a solution of PBS (1x) + Triton X-100 (0.05%) for experiment 3, heated at 65 °C for 5 minutes, magnetized using a magnetic stand and the supernatant was removed (this operation was repeated twice). For experiment 1, the obtained pellets were diluted with 80 µL of PBS (1x) solution, magnetized using a magnetic stand and the supernatant was removed. For each

experiments divided on the purification step (experiments 2 and 3), the obtained pellets were diluted with 20 µL of PBS (1x) solution, gathered, magnetized using a magnetic stand and the supernatants were removed. 8 µL 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 or 180 mM of **15f**, captured with streptavidin and cleaved with DTT using the procedure described in §5. 1000, 100, 50 and 10 copies 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, 45 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 0.5 min.

RT-PCR amplicons were checked by on-chip electrophoresis using a 2100 Bioanalyzer® instrument (Agilent) with an Agilent DNA 1000 kit (Figure 3).

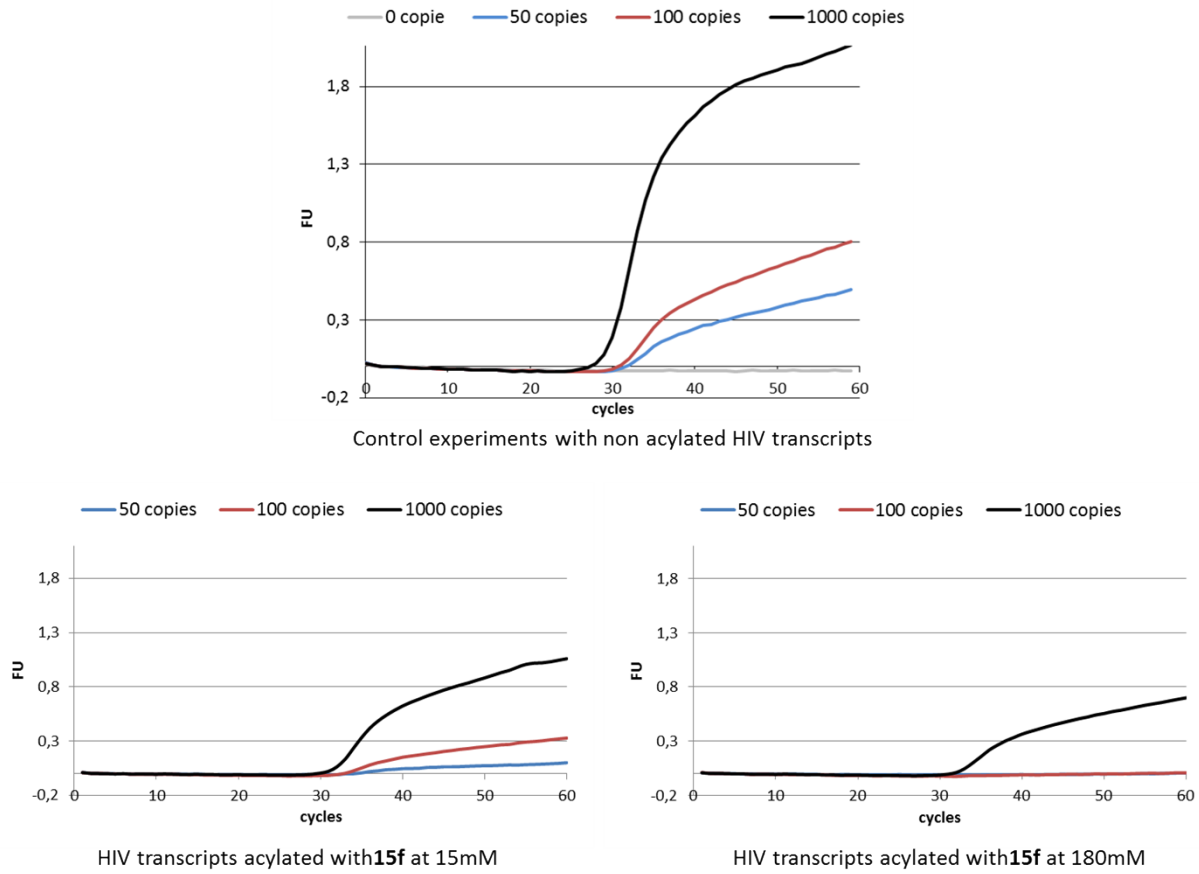


Figure 2. RT-PCR amplification of extracted HIV RNA

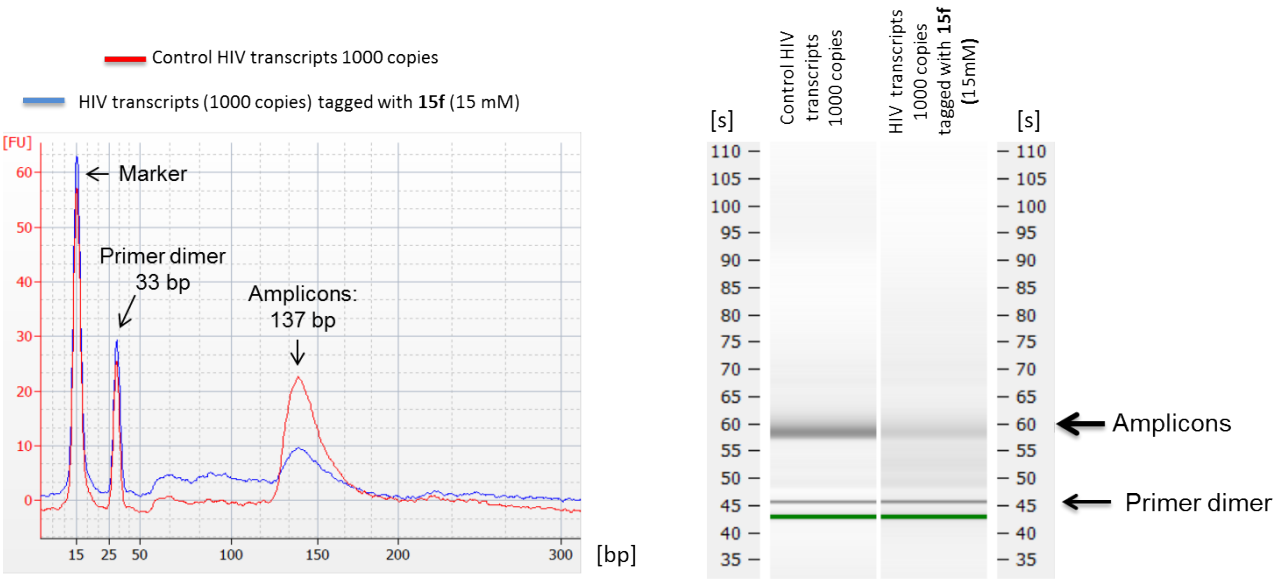
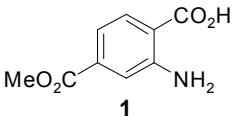


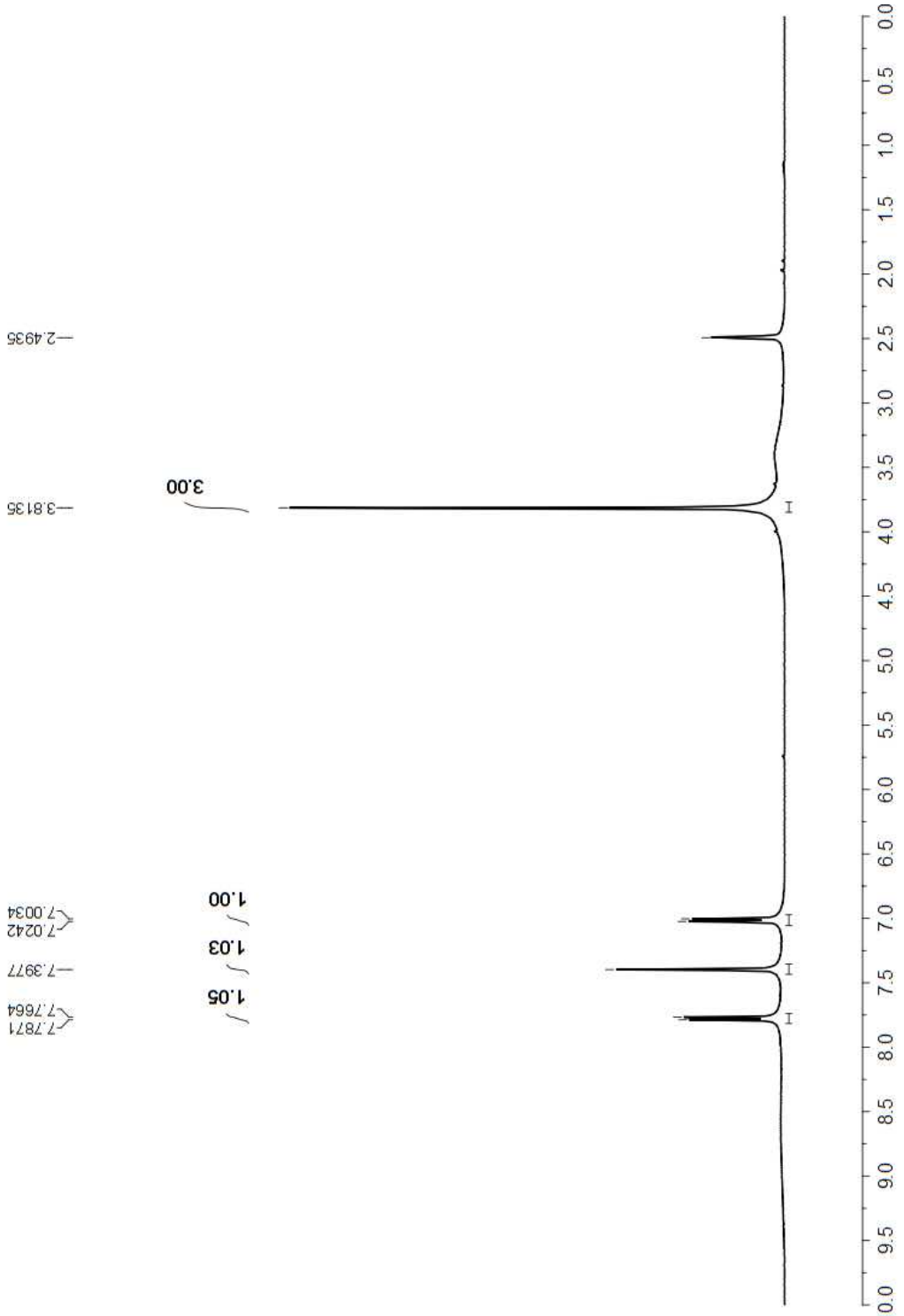
Figure 3: RT-PCR amplicons – On-chip electrophoresis

7) ^{1}H NMR of Compounds 1-15

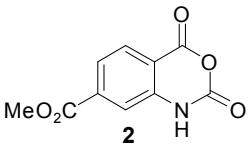
2-Amino-4-(methoxycarbonyl)benzoic acid 1



1



Methyl 2,4-dioxo-*1H*-3,1-benzoxazine-7-carboxylate 2



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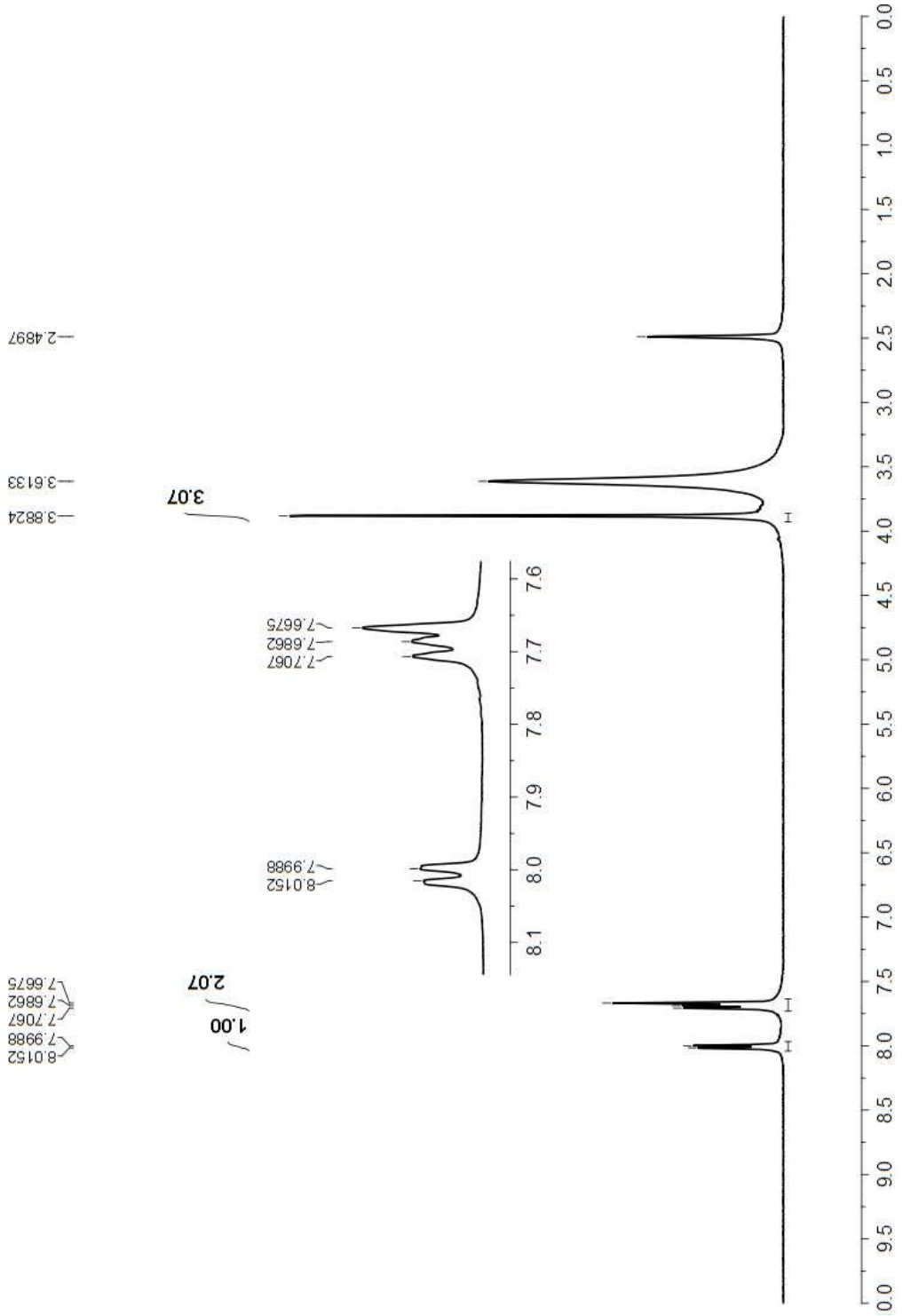
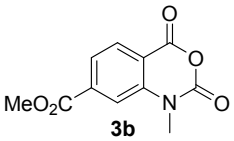
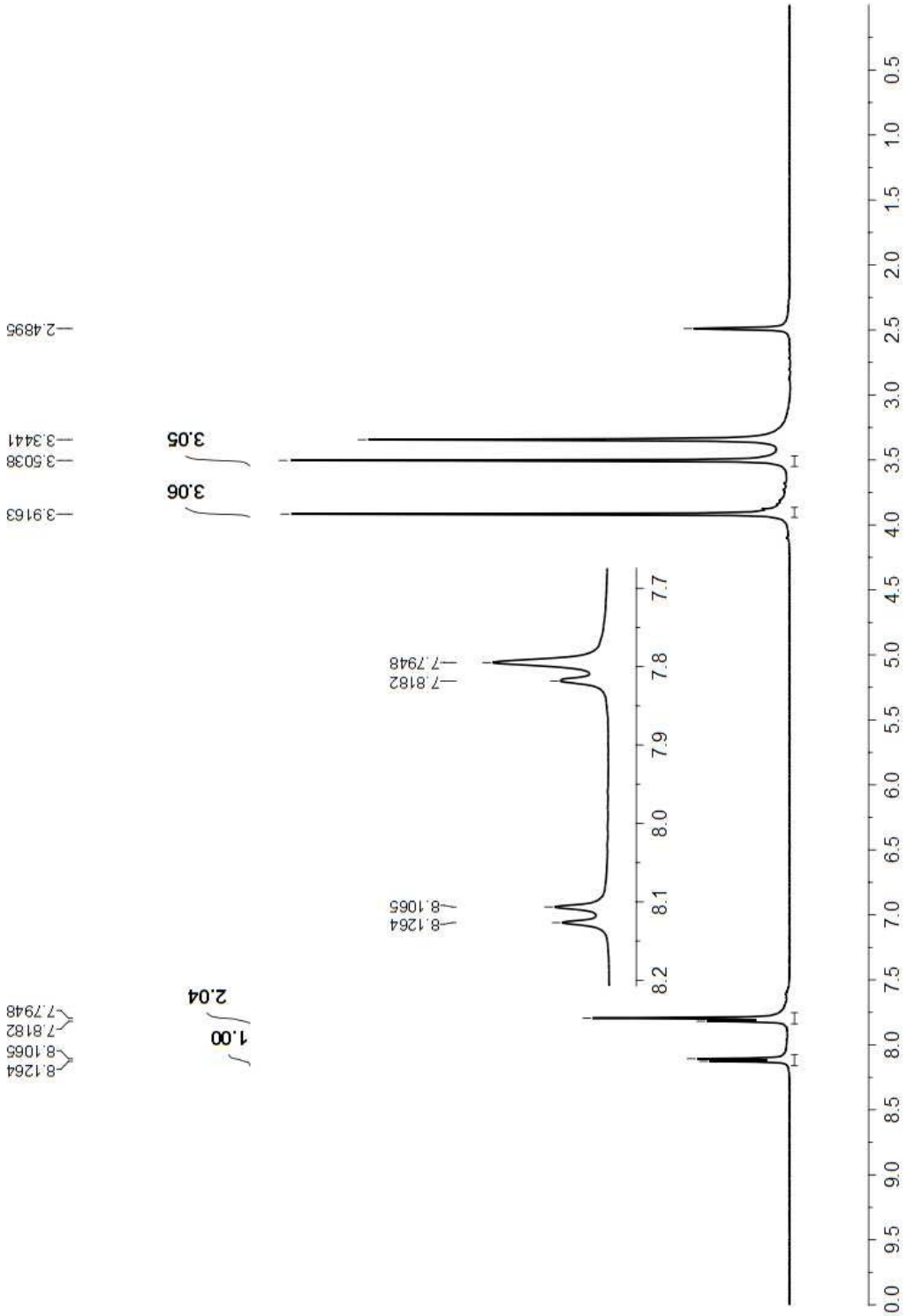
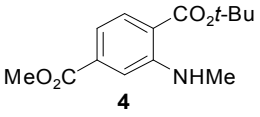


Methyl 1-methyl-2,4-dioxo-3,1-benzoxazine-7-carboxylate 3a





1-*tert*-Butyl 4-methyl 2-(methylamino)benzene-1,4-dicarboxylate 4



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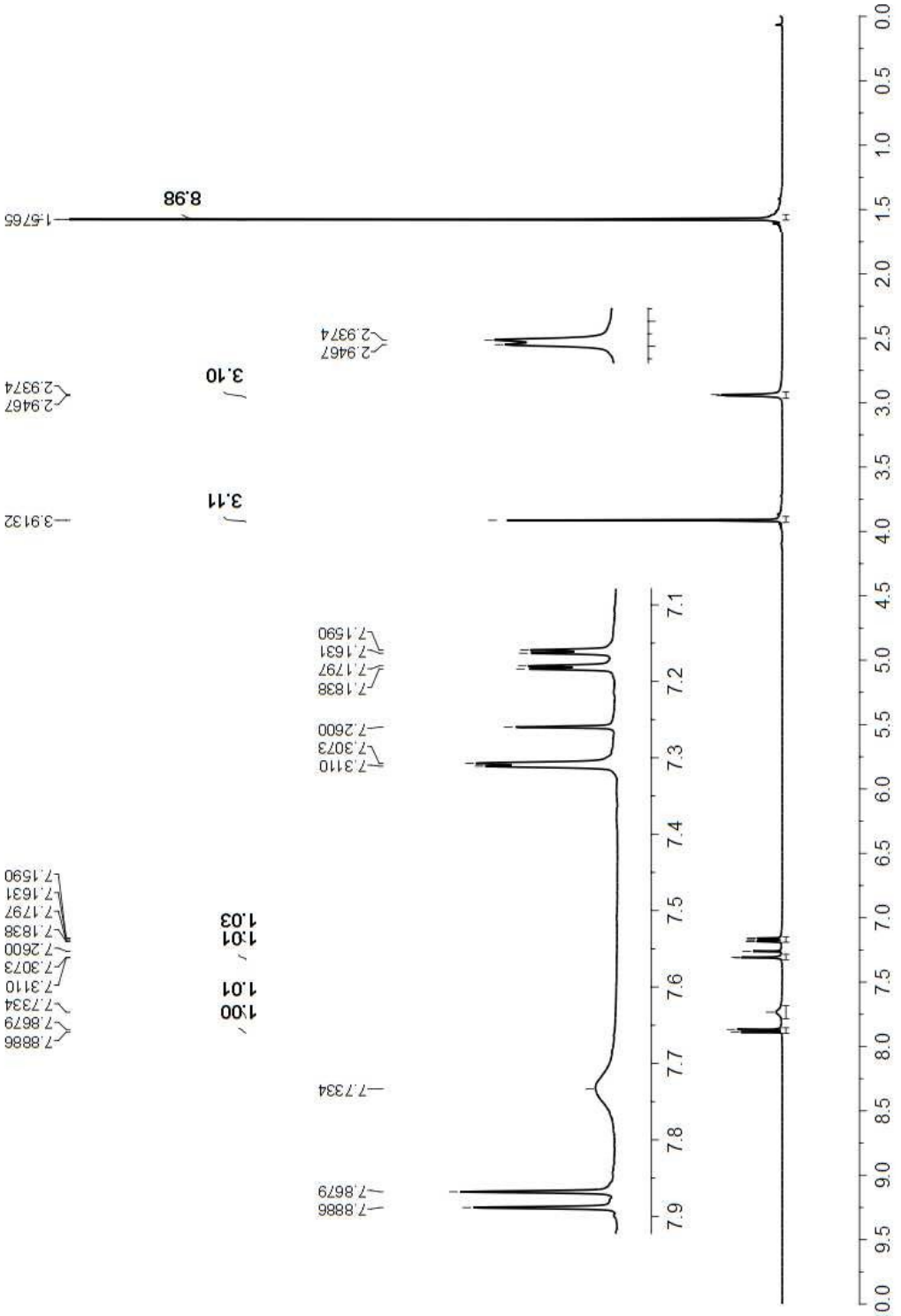
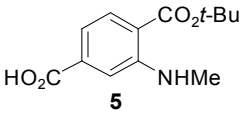


4-[(*tert*-Butoxy)carbonyl]-3-(methylamino)benzoic acid 5



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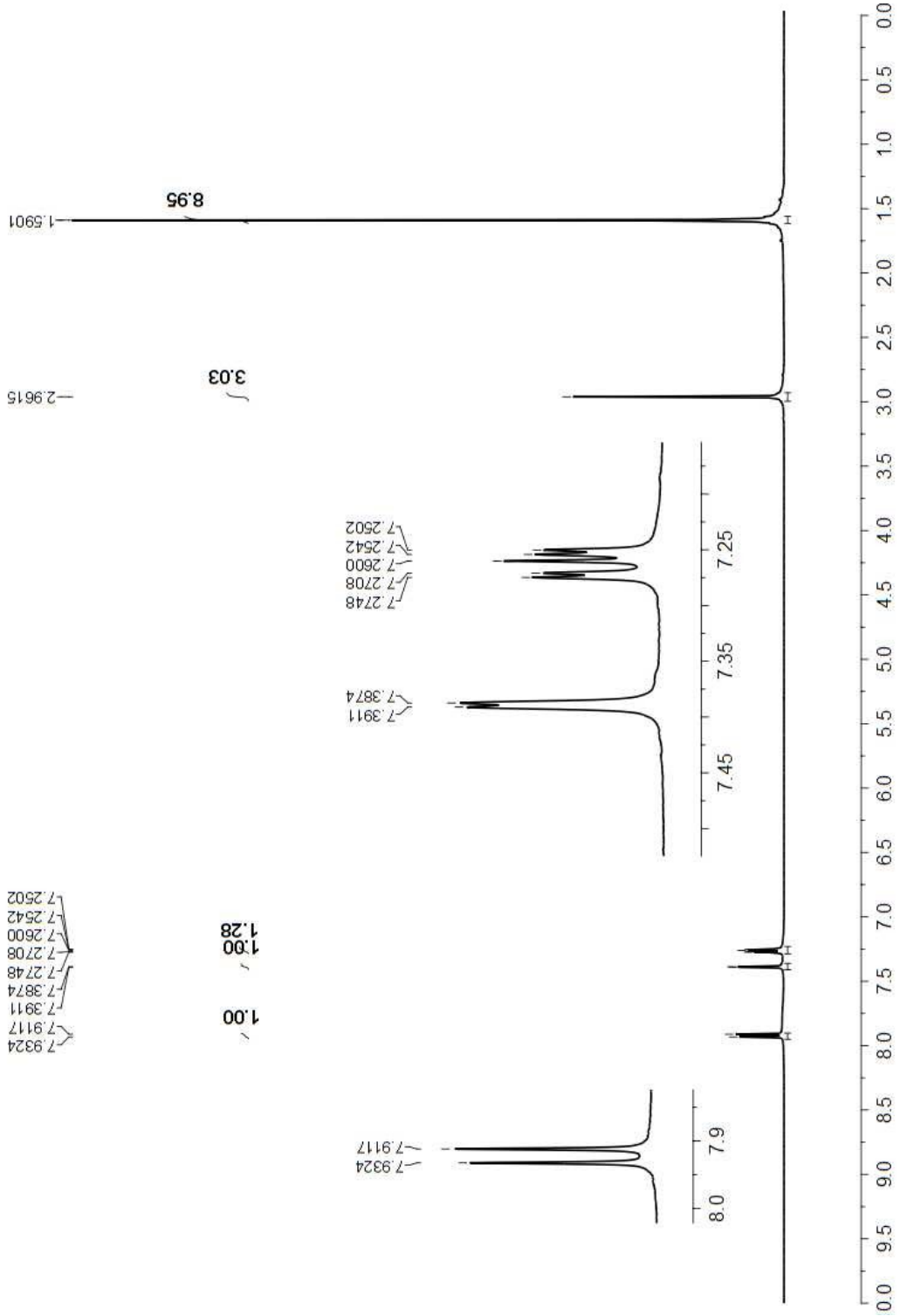
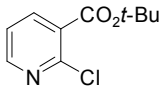


***tert*-Butyl 2-chloropyridine-3-carboxylate 6**



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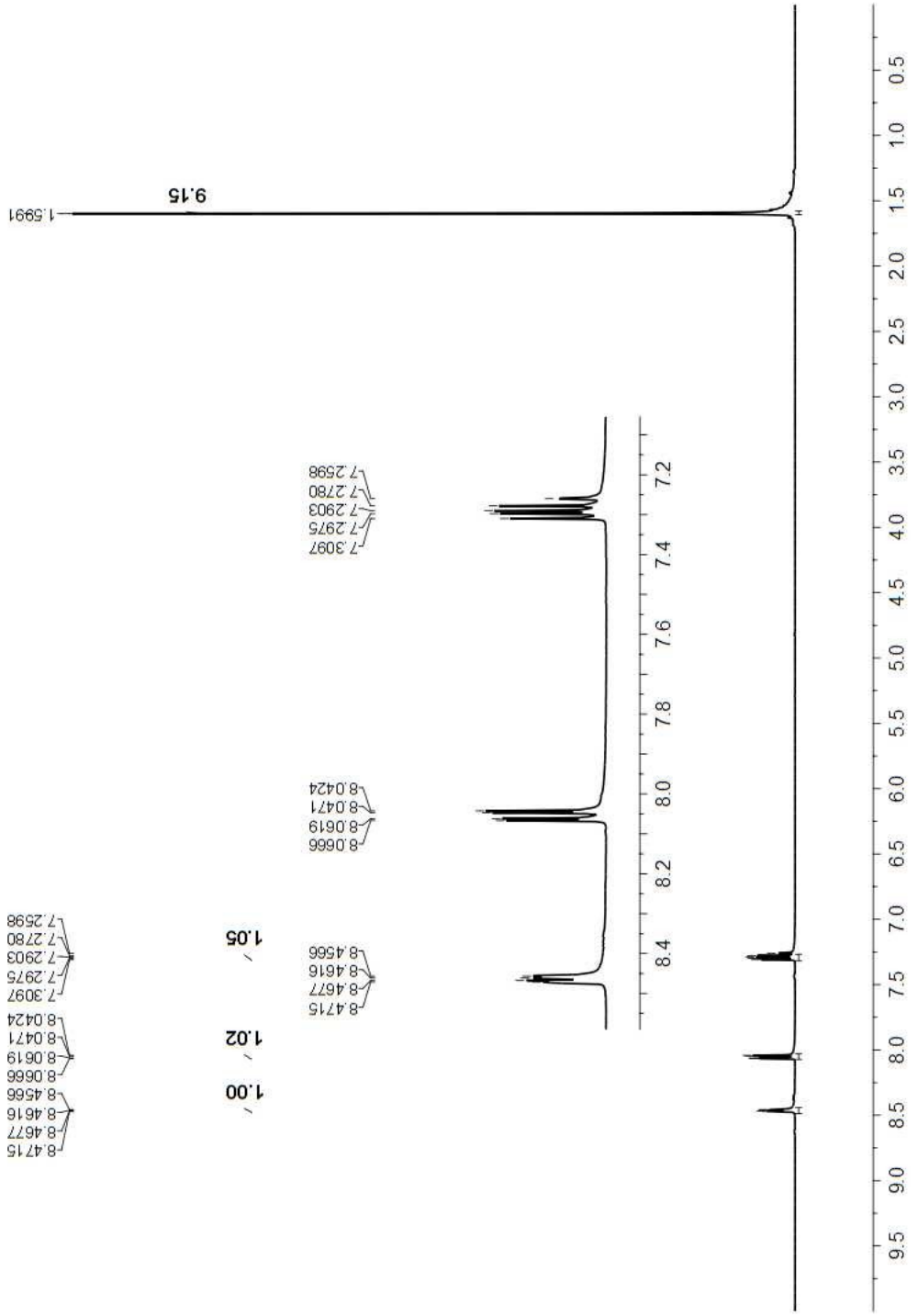

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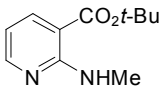

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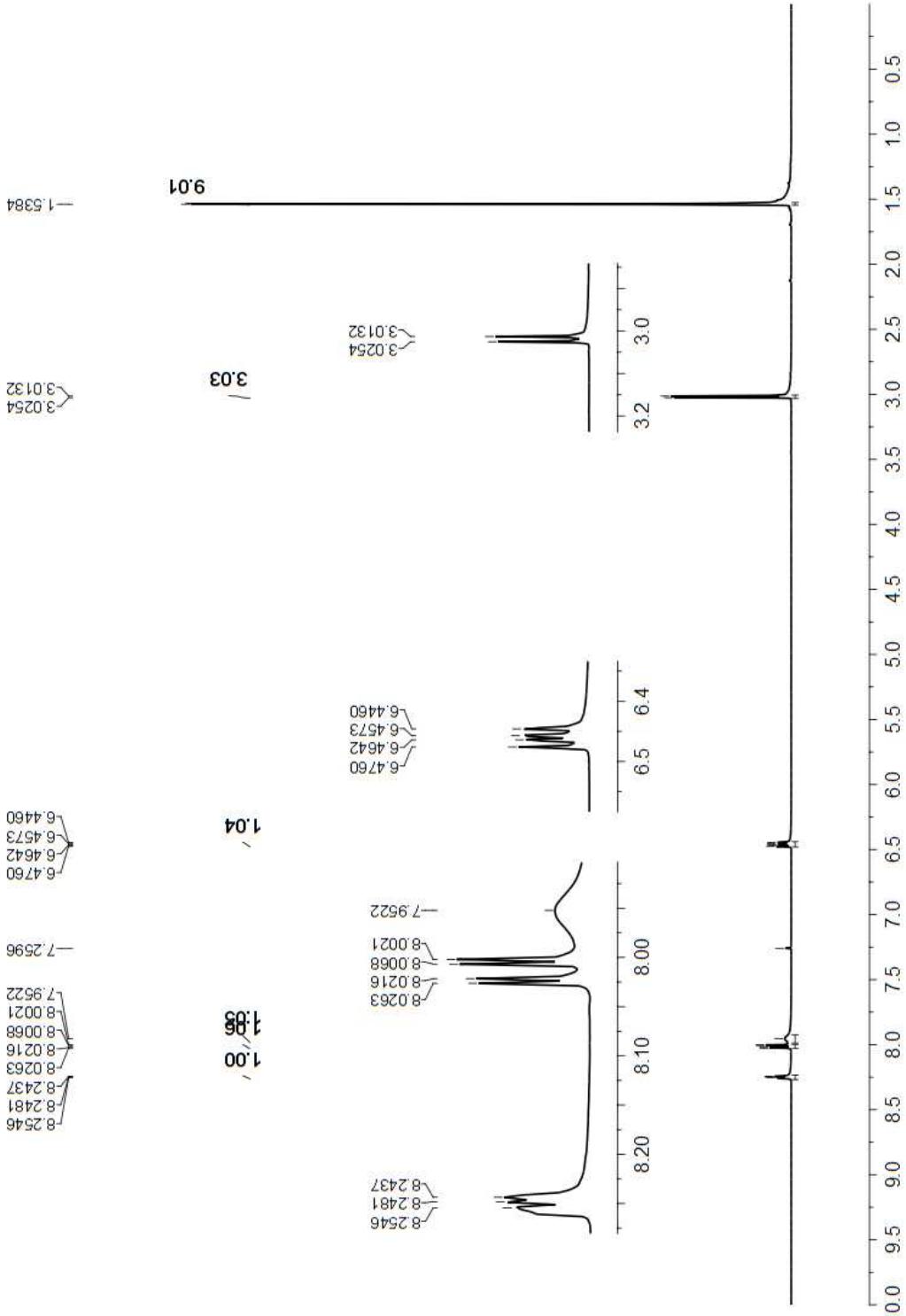

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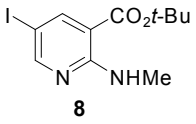

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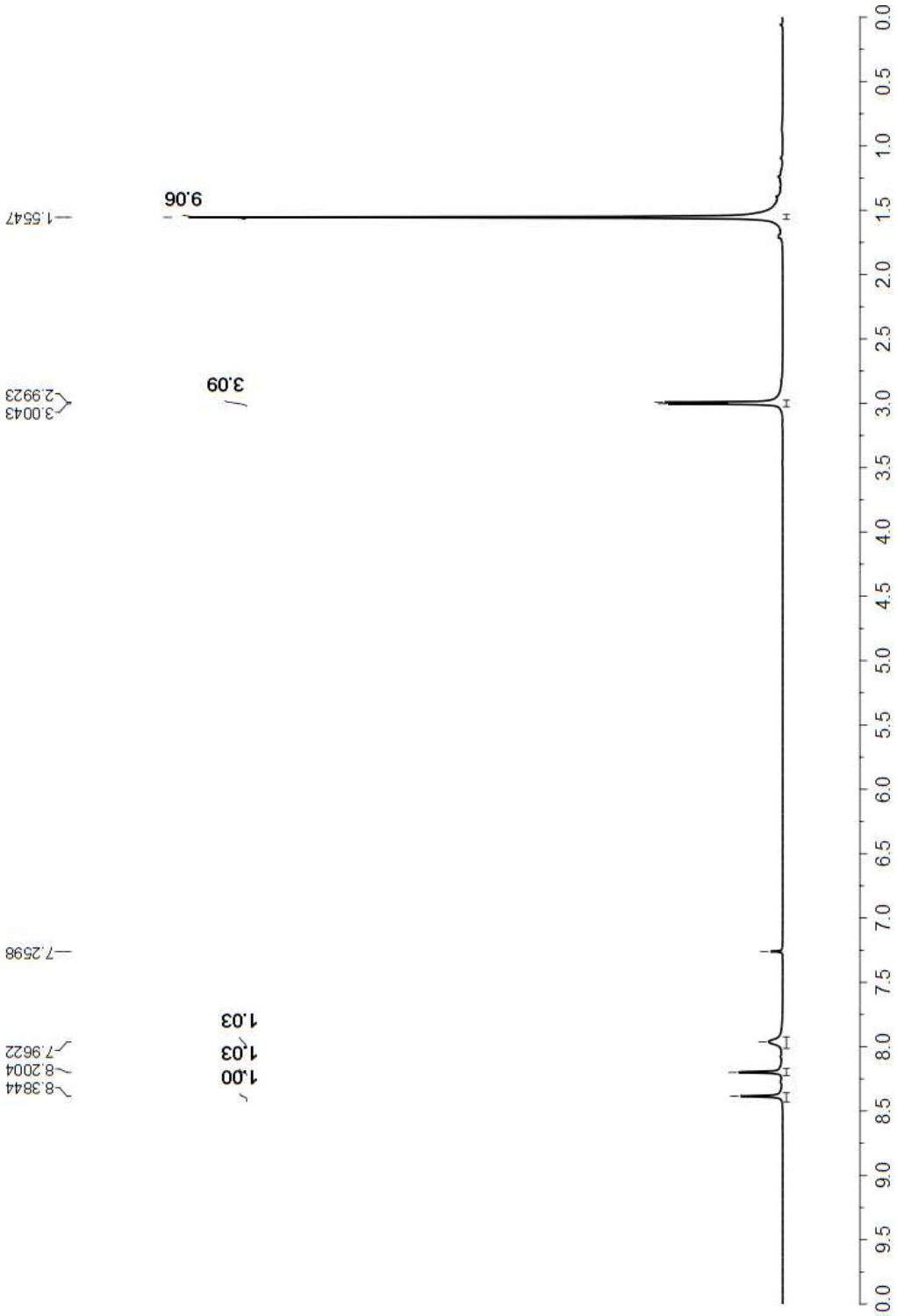

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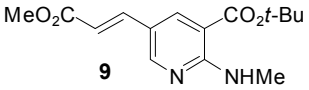

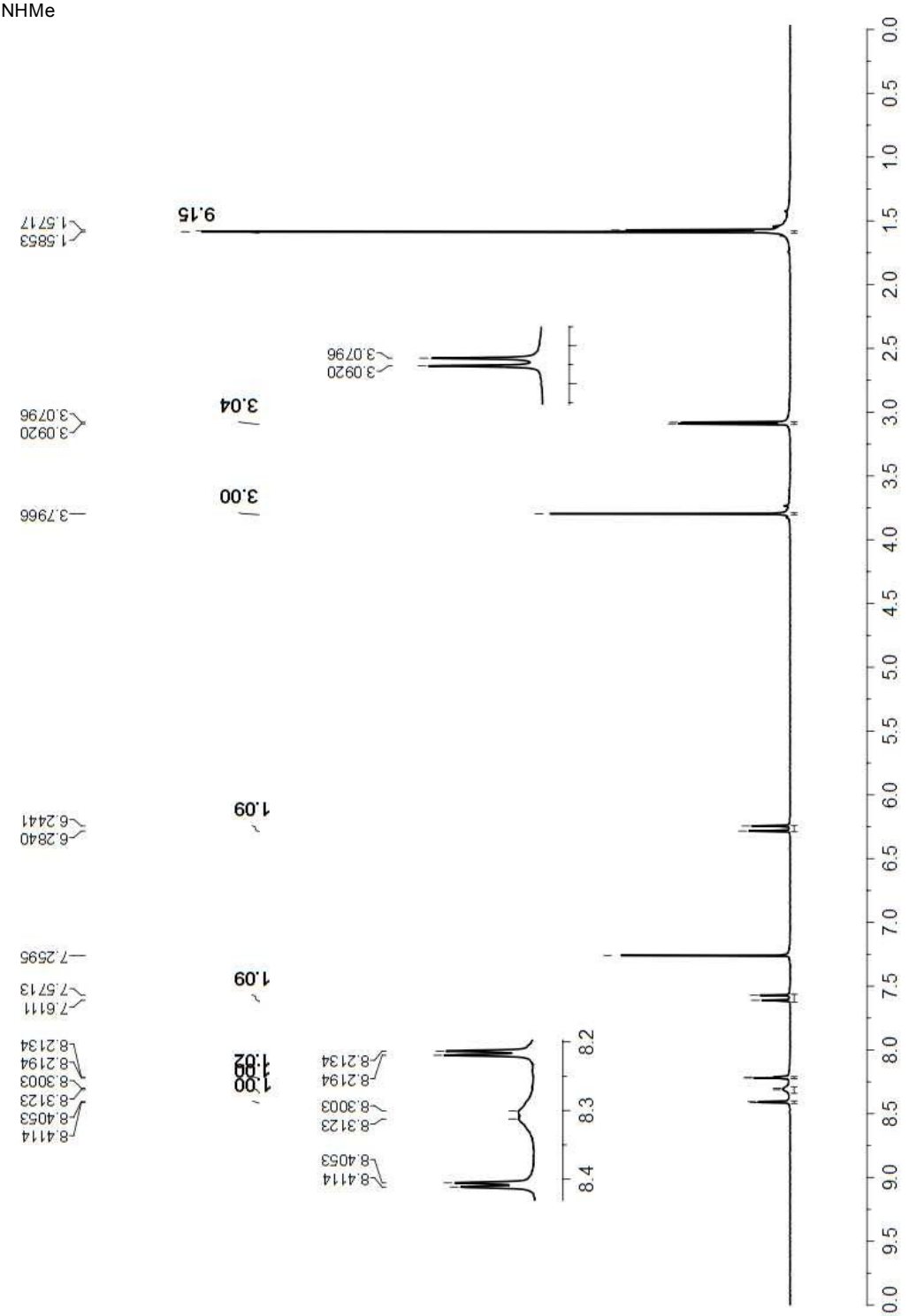

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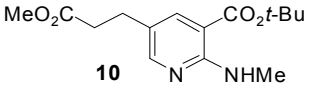
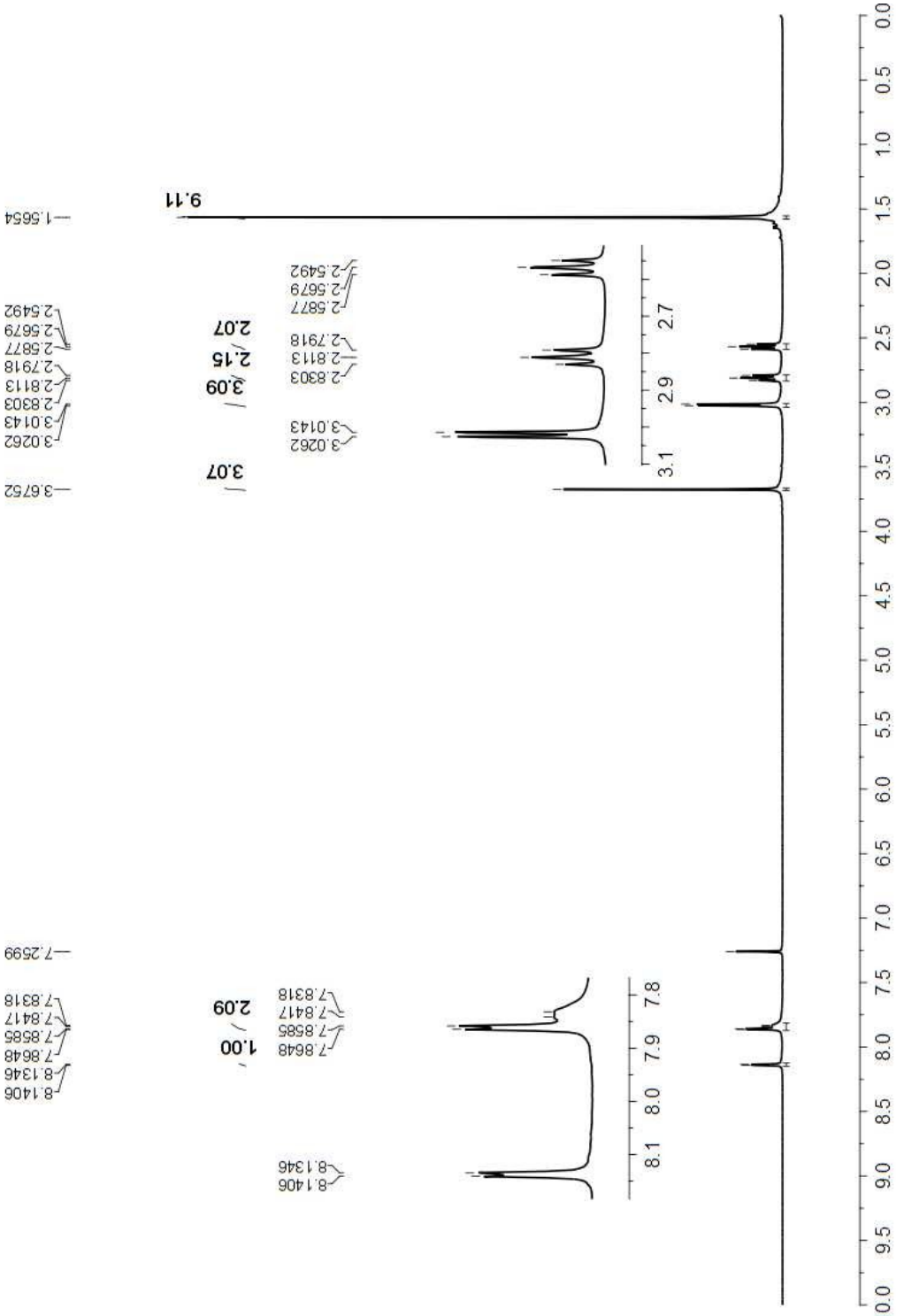
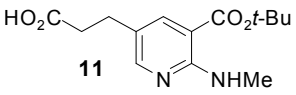
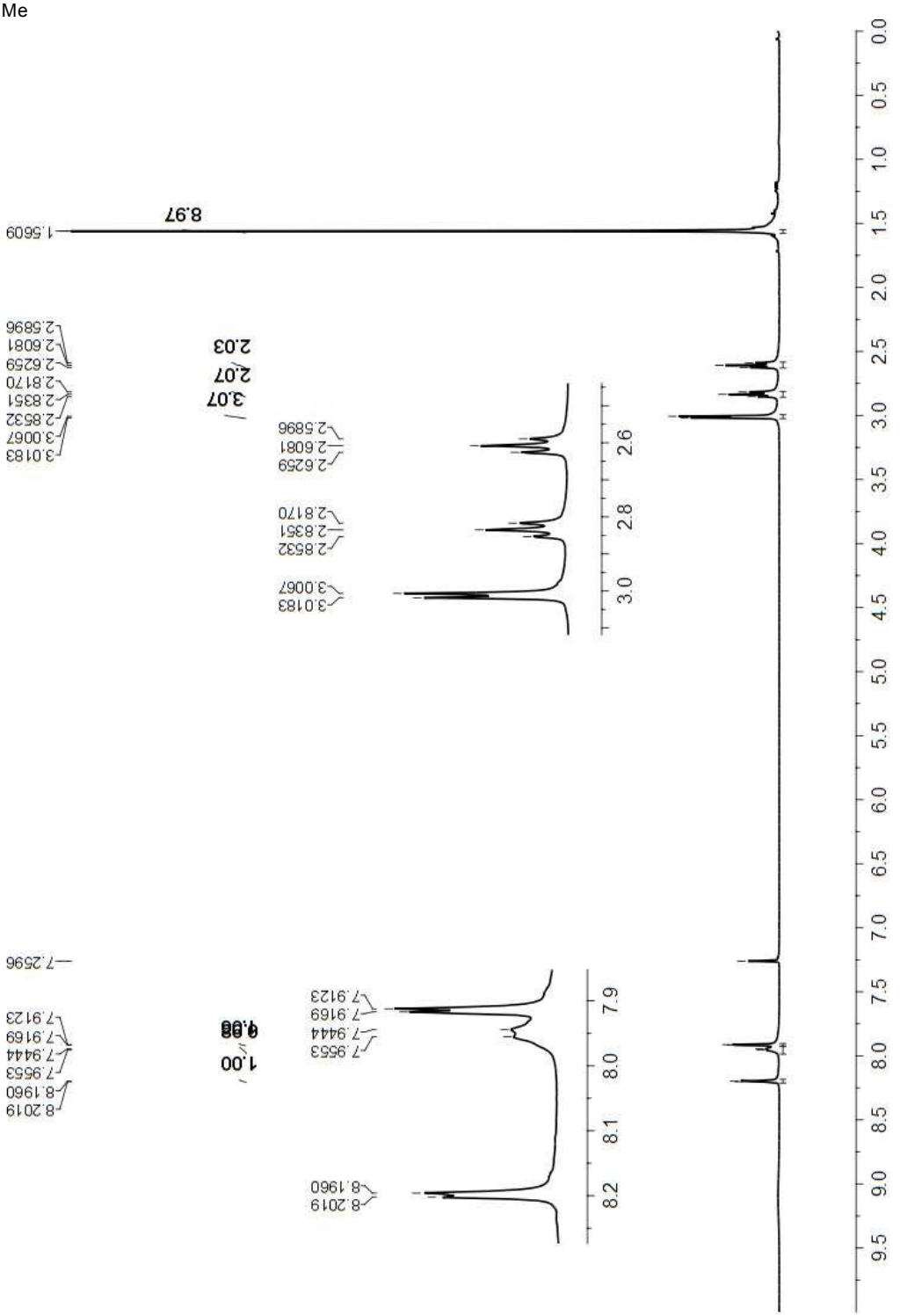


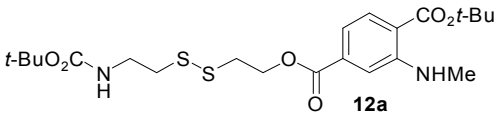


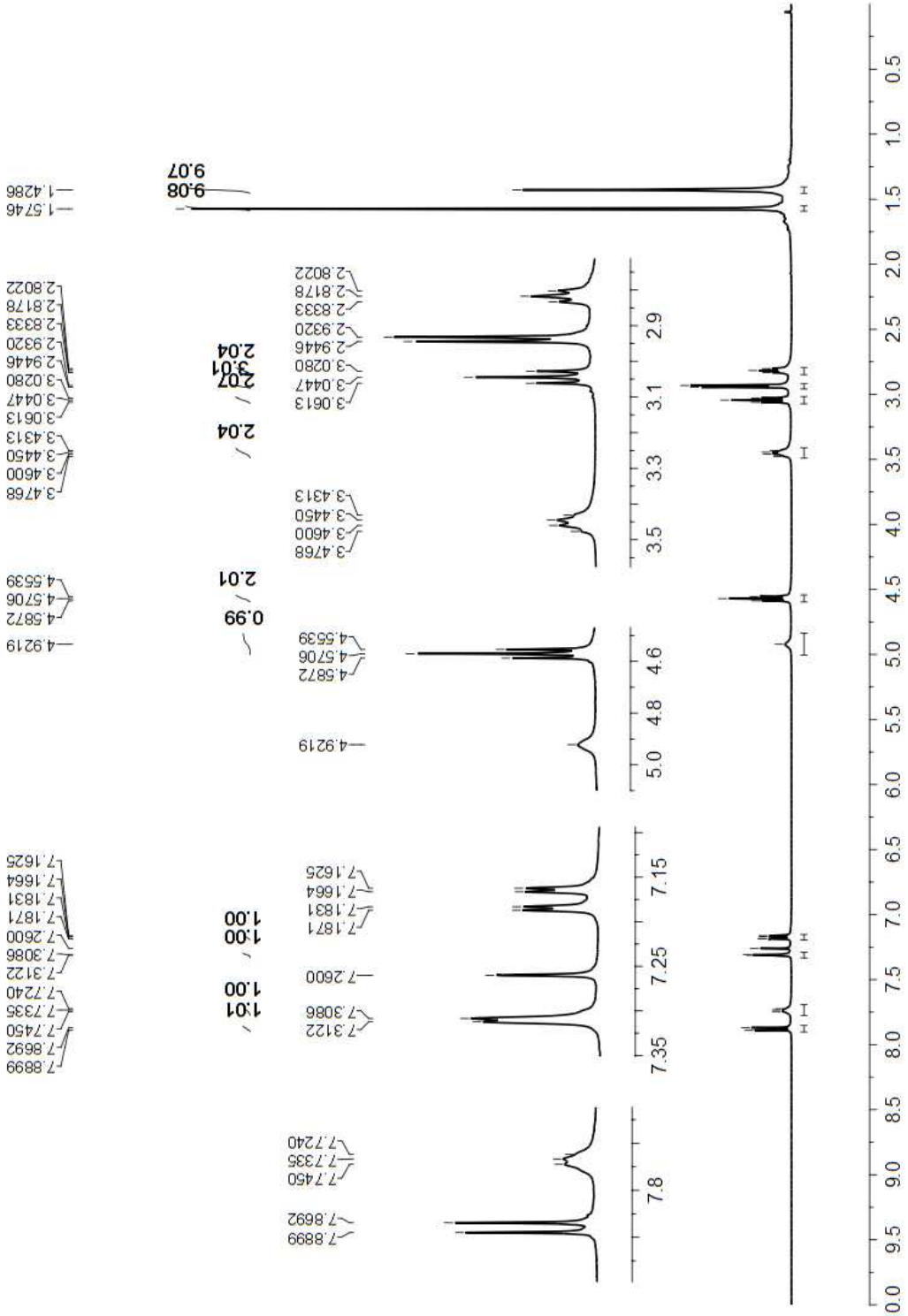
3-{5-[(*tert*-Butoxy)carbonyl]-6-(methylamino)pyridin-3-yl}propanoic acid 11





4-{2-[(2-{[(*tert*-Butoxy)carbonyl]amino}ethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 12a





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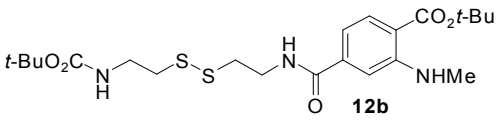

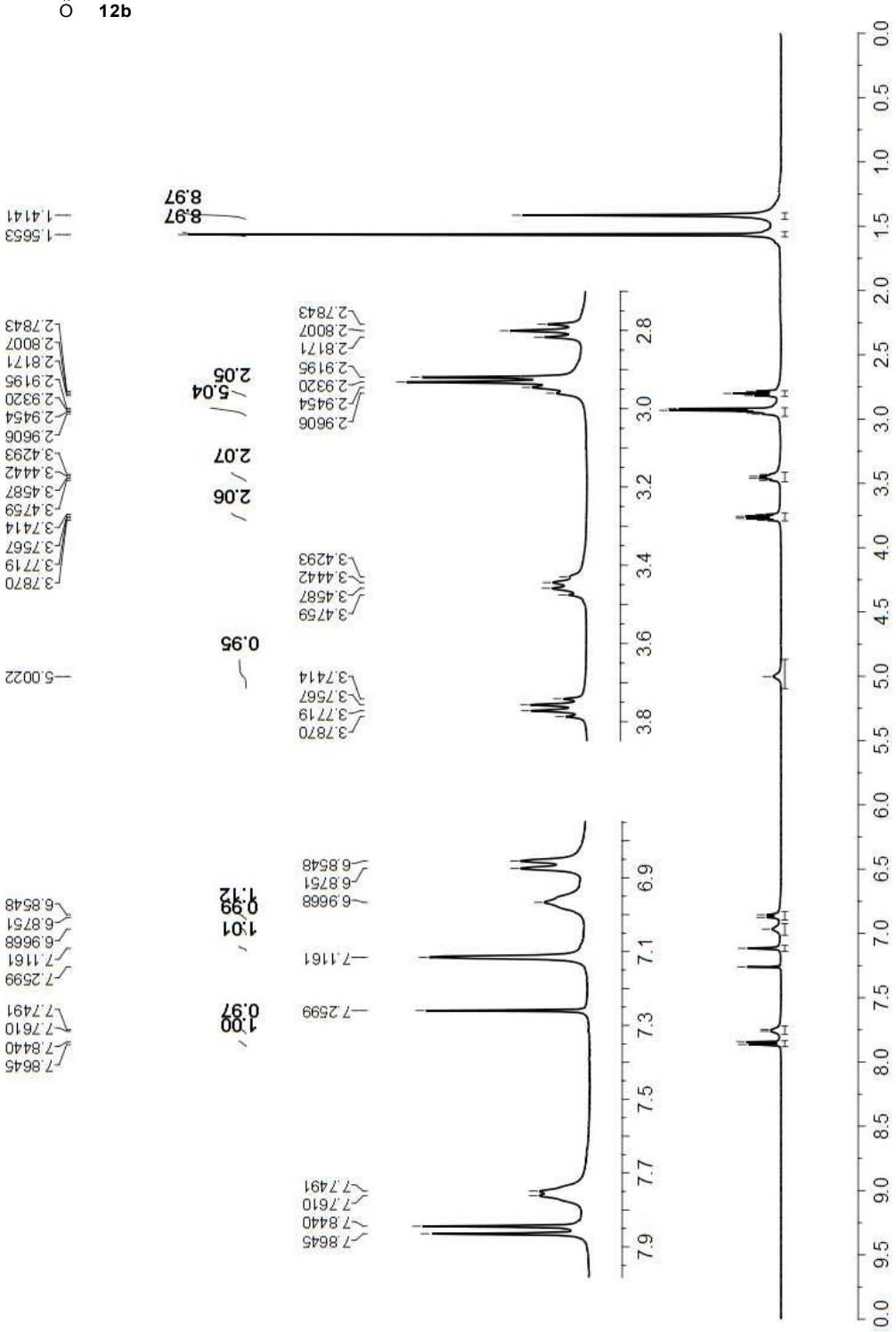

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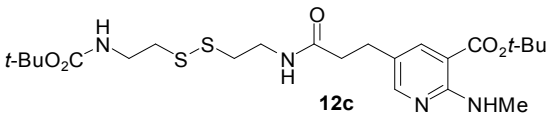
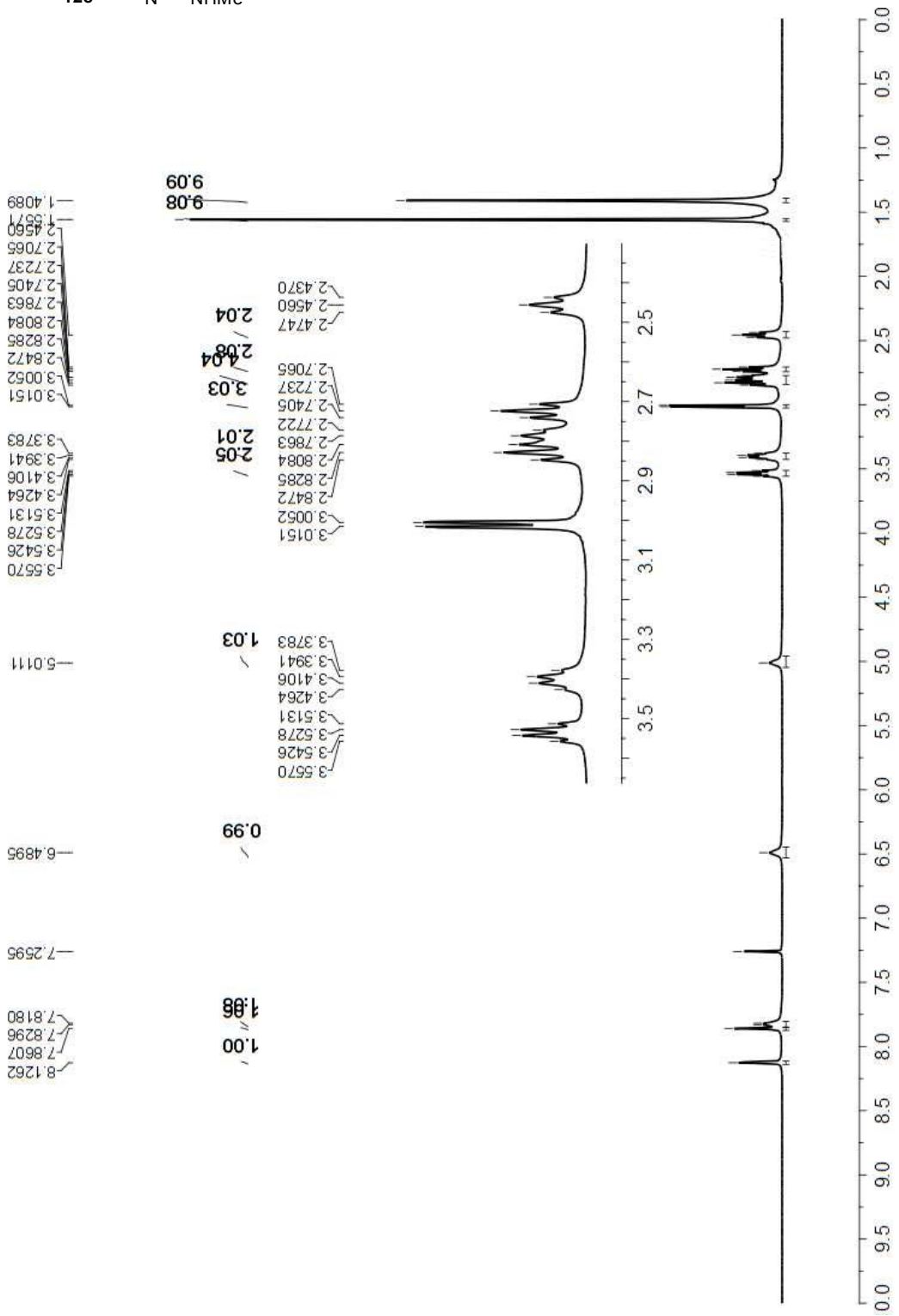
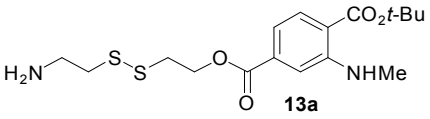
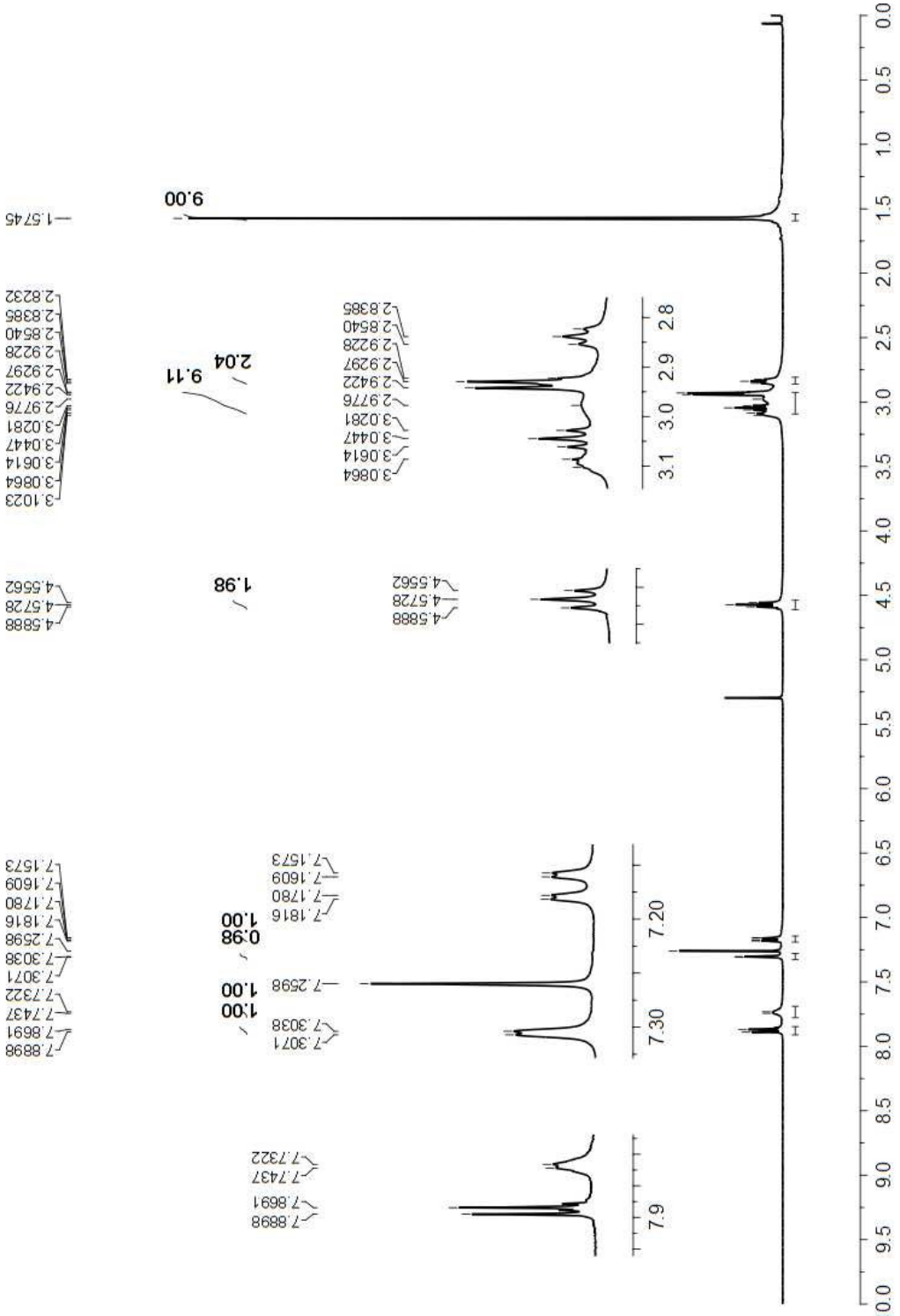




4-{2-[(2-Aminoethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 13a





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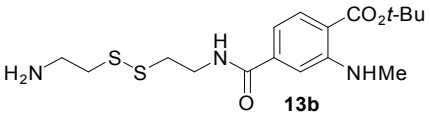
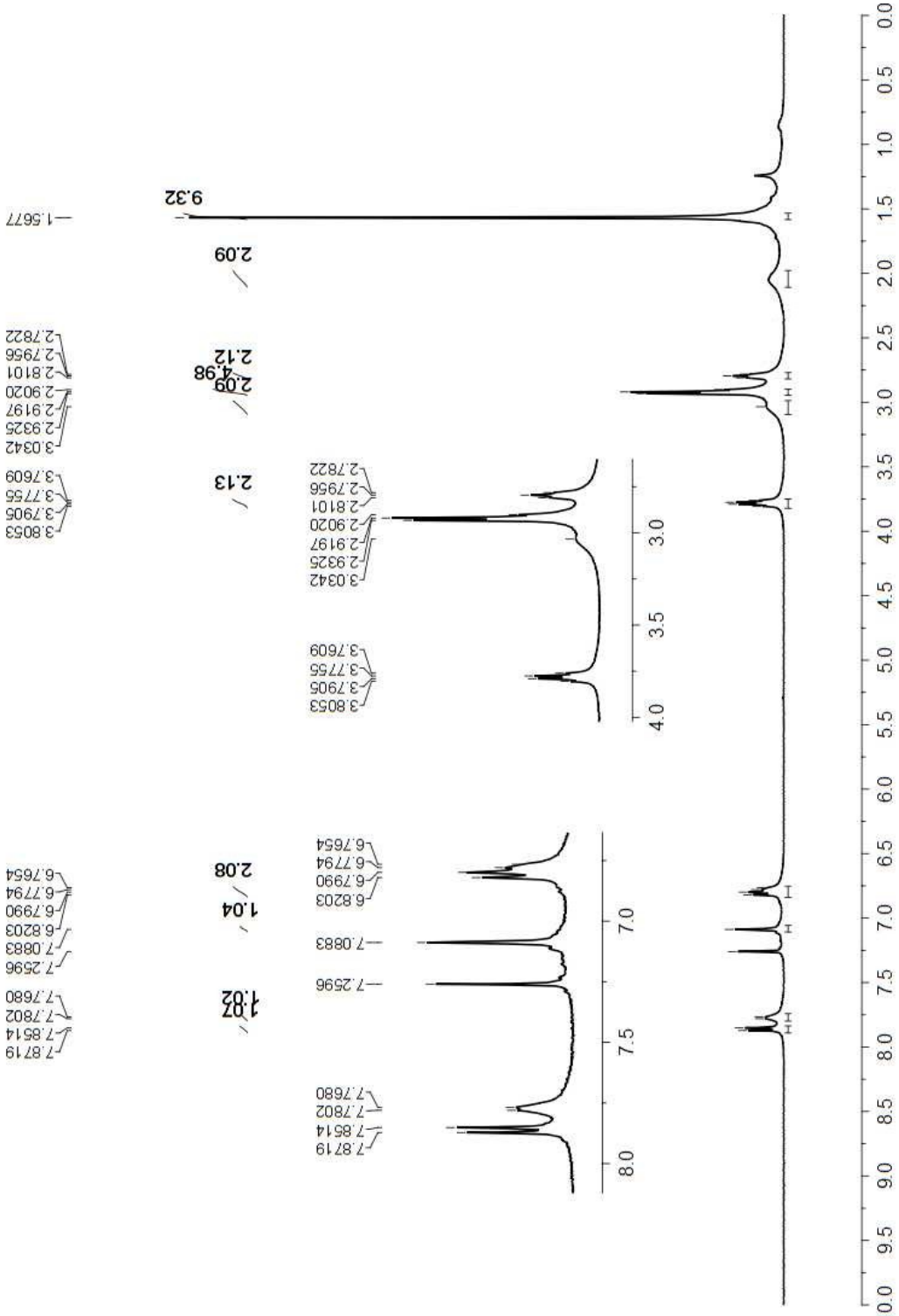
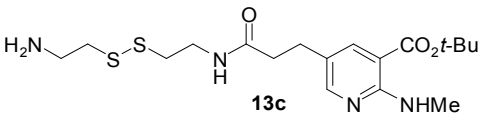


13b



***tert*-Butyl 5-[2-({2-[(2-aminoethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 13c**



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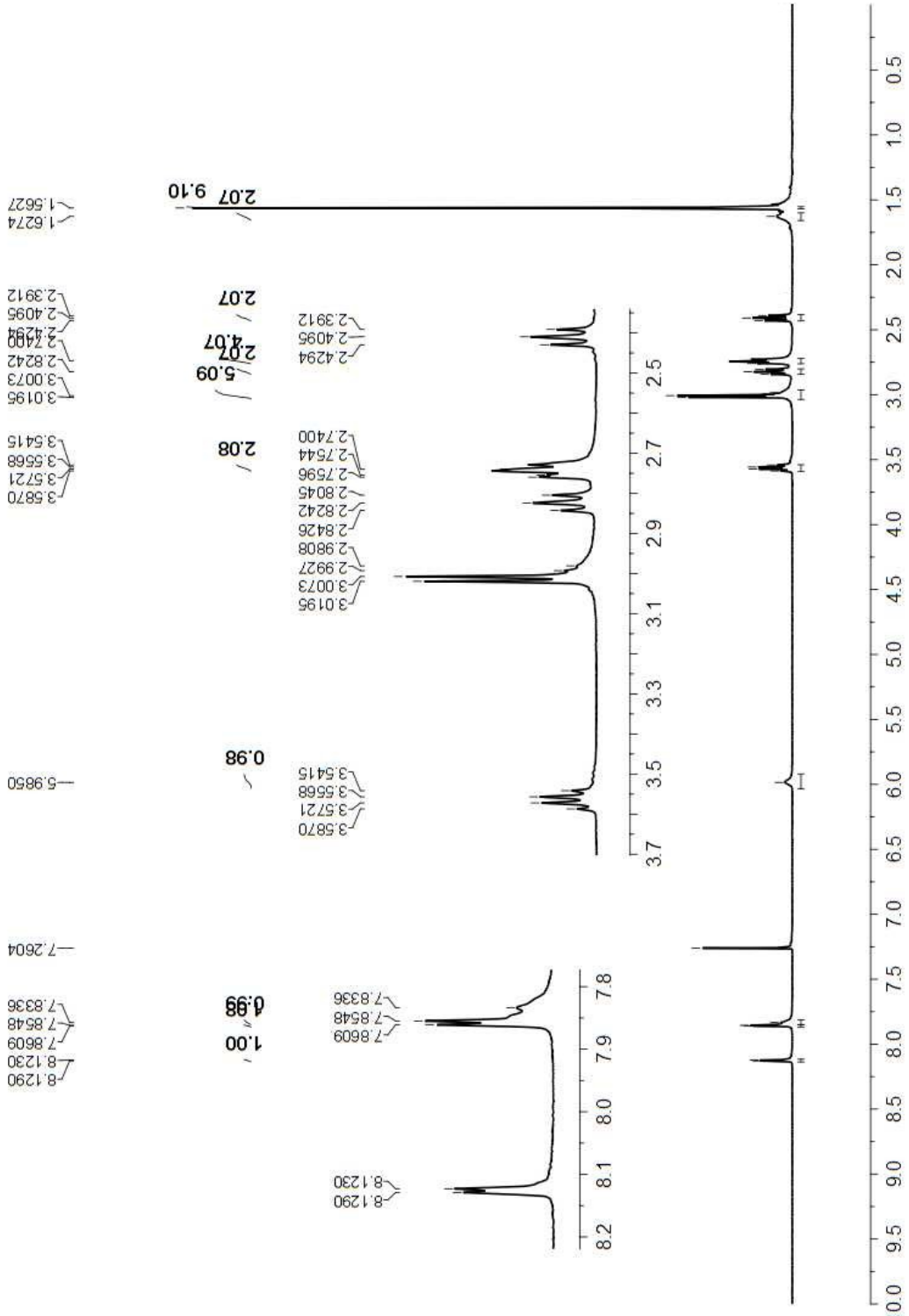
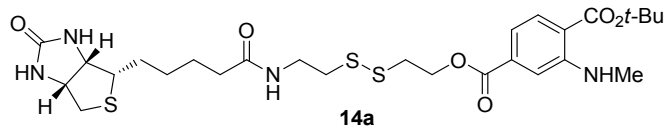
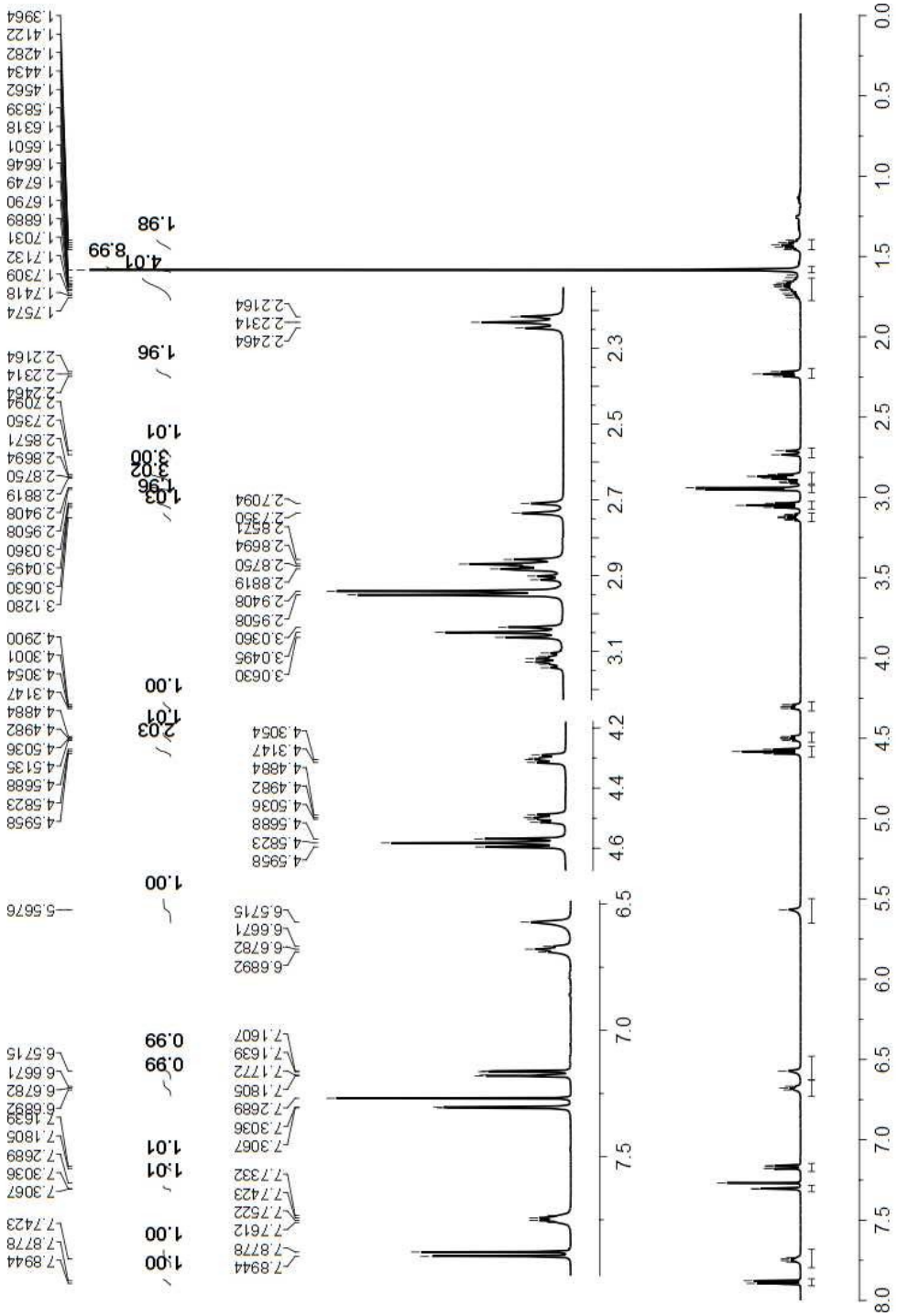


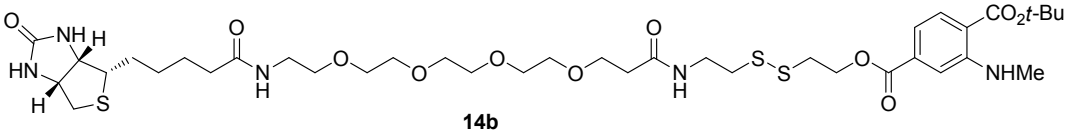
***tert*-Butyl 4-({2-[(2-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl}carbamoyl)-2-(methylamino)benzoate 14a**



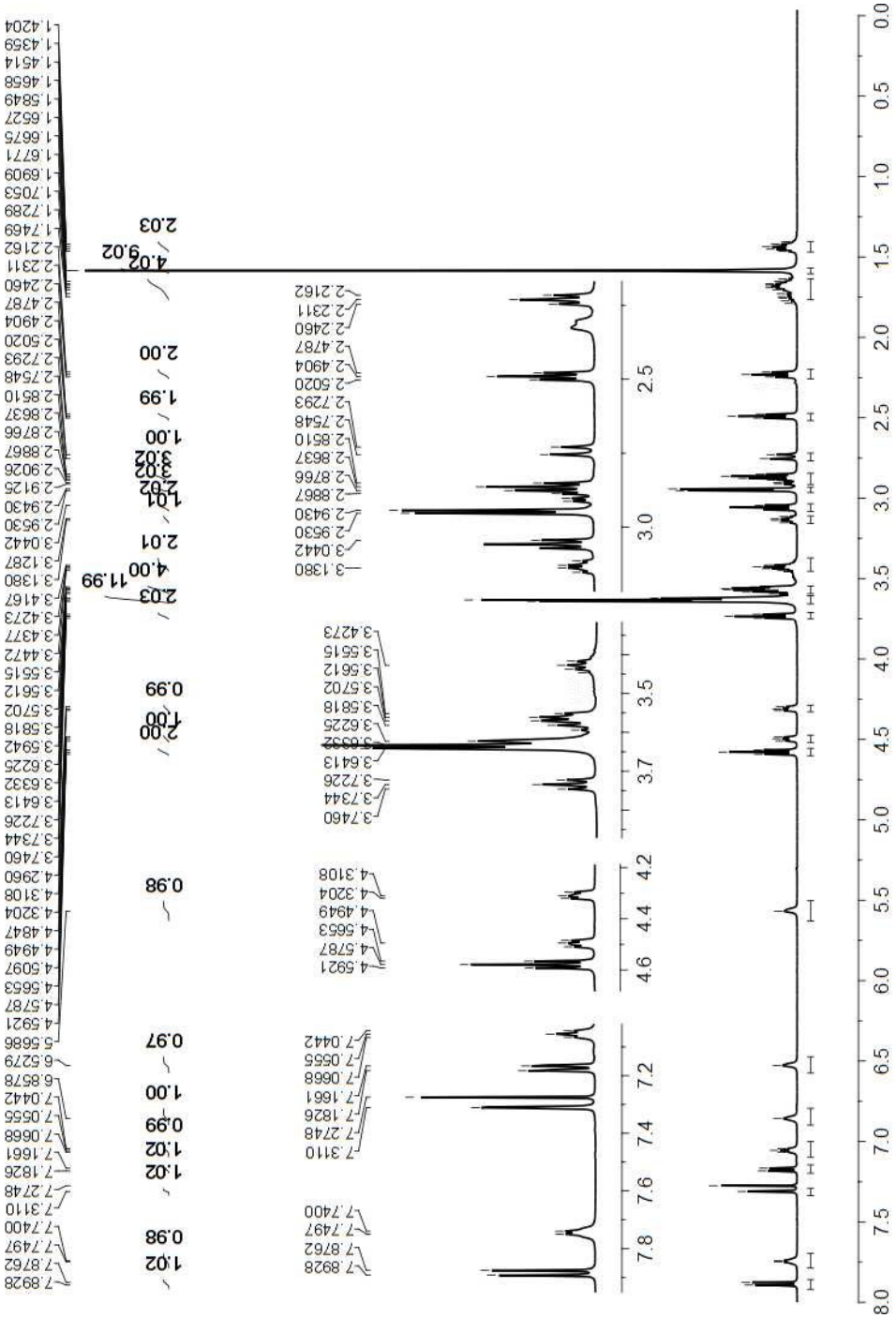
14a



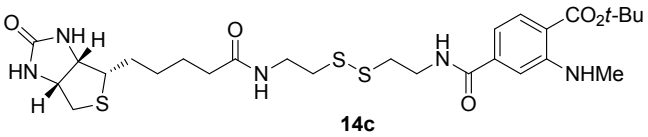
4-(2-{[2-(1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl) 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 14b



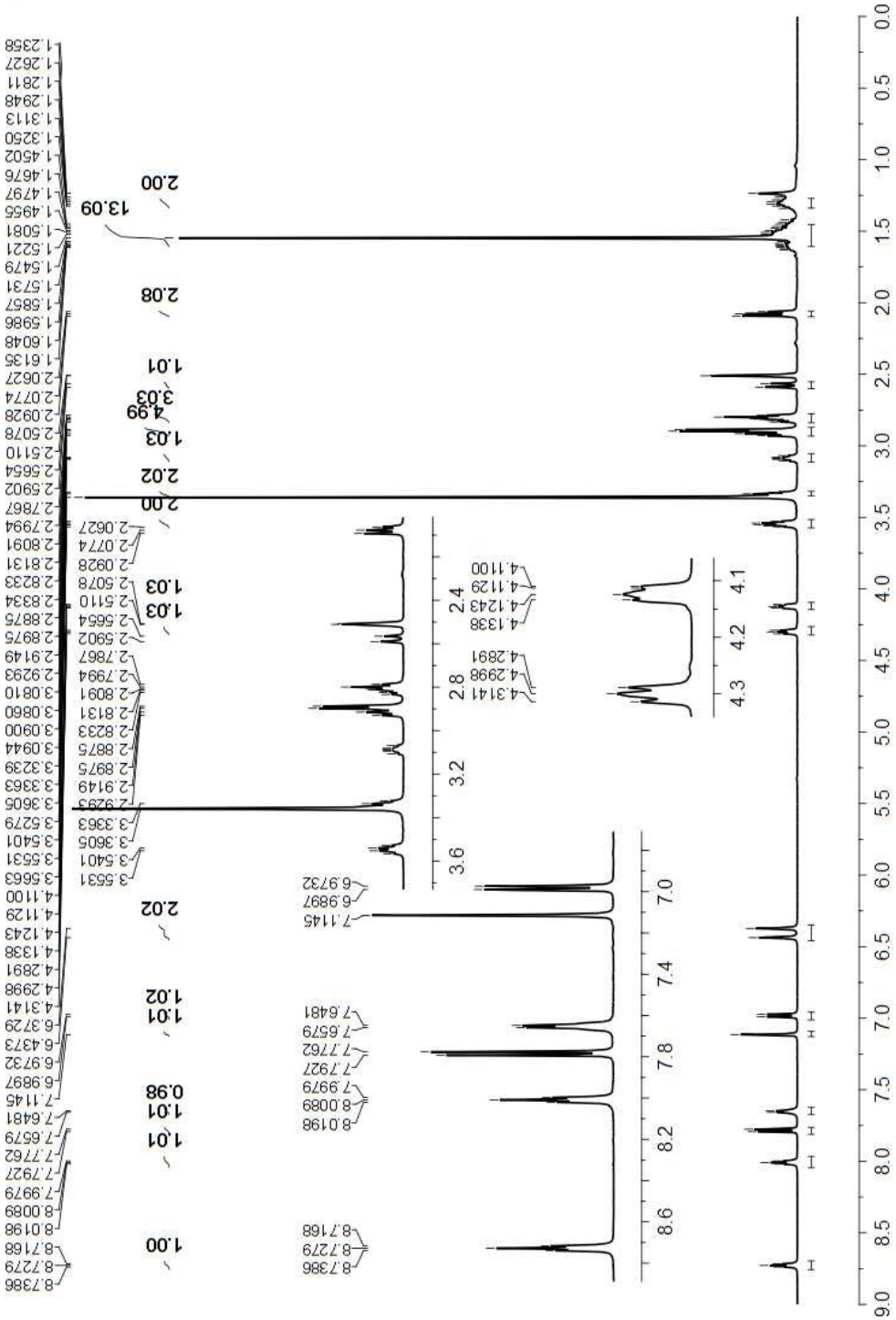
14b



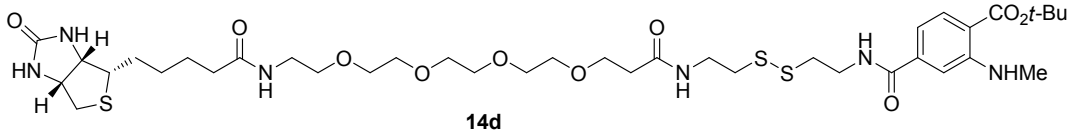
4-{2-[(2-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 14c



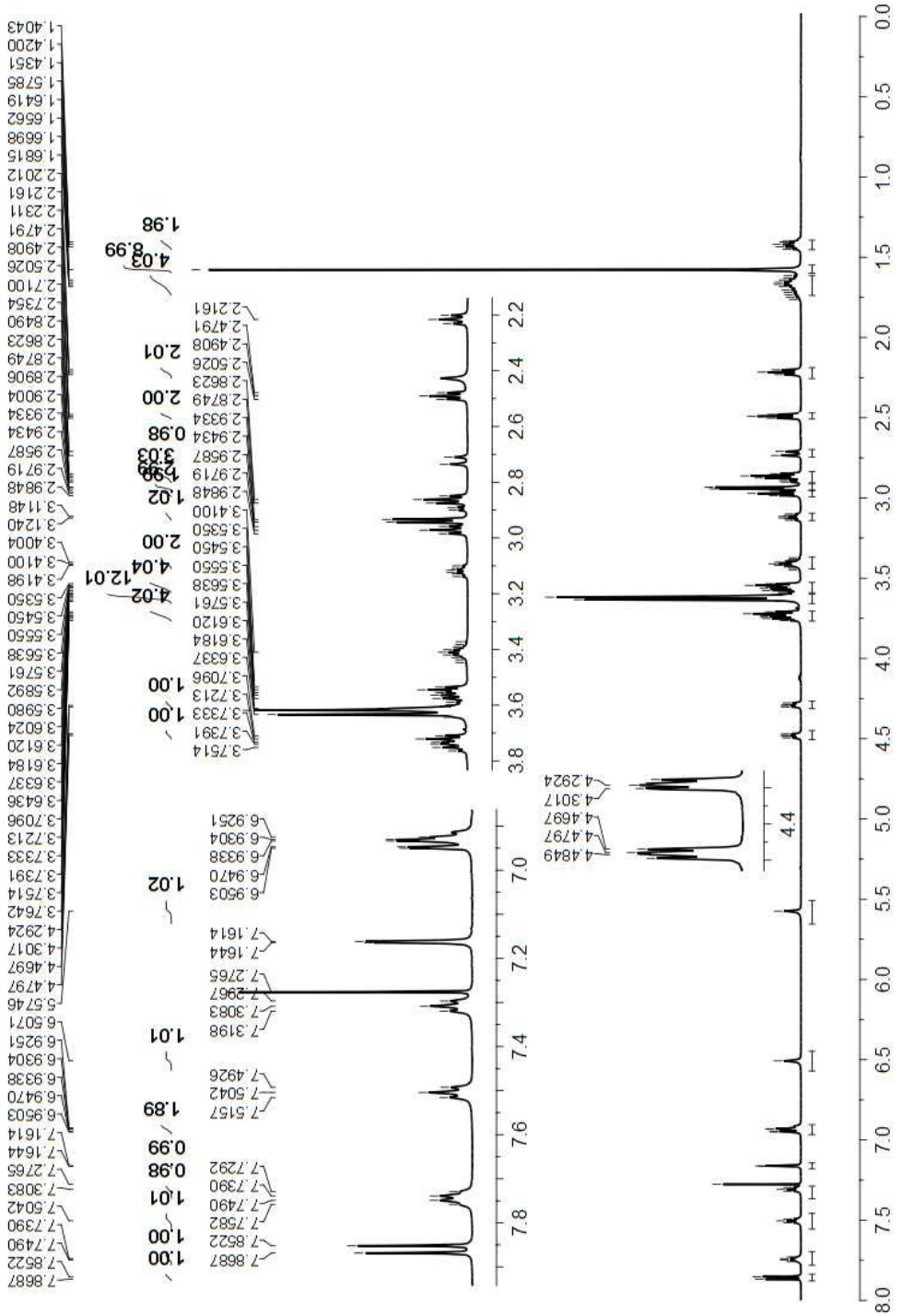
14c



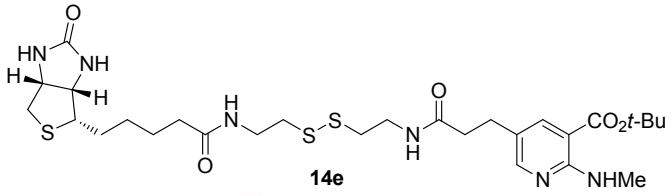
***tert*-Butyl 4-[(2-{[2-(1-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl)carbamoyl]-2-(methylamino)benzoate 14d**



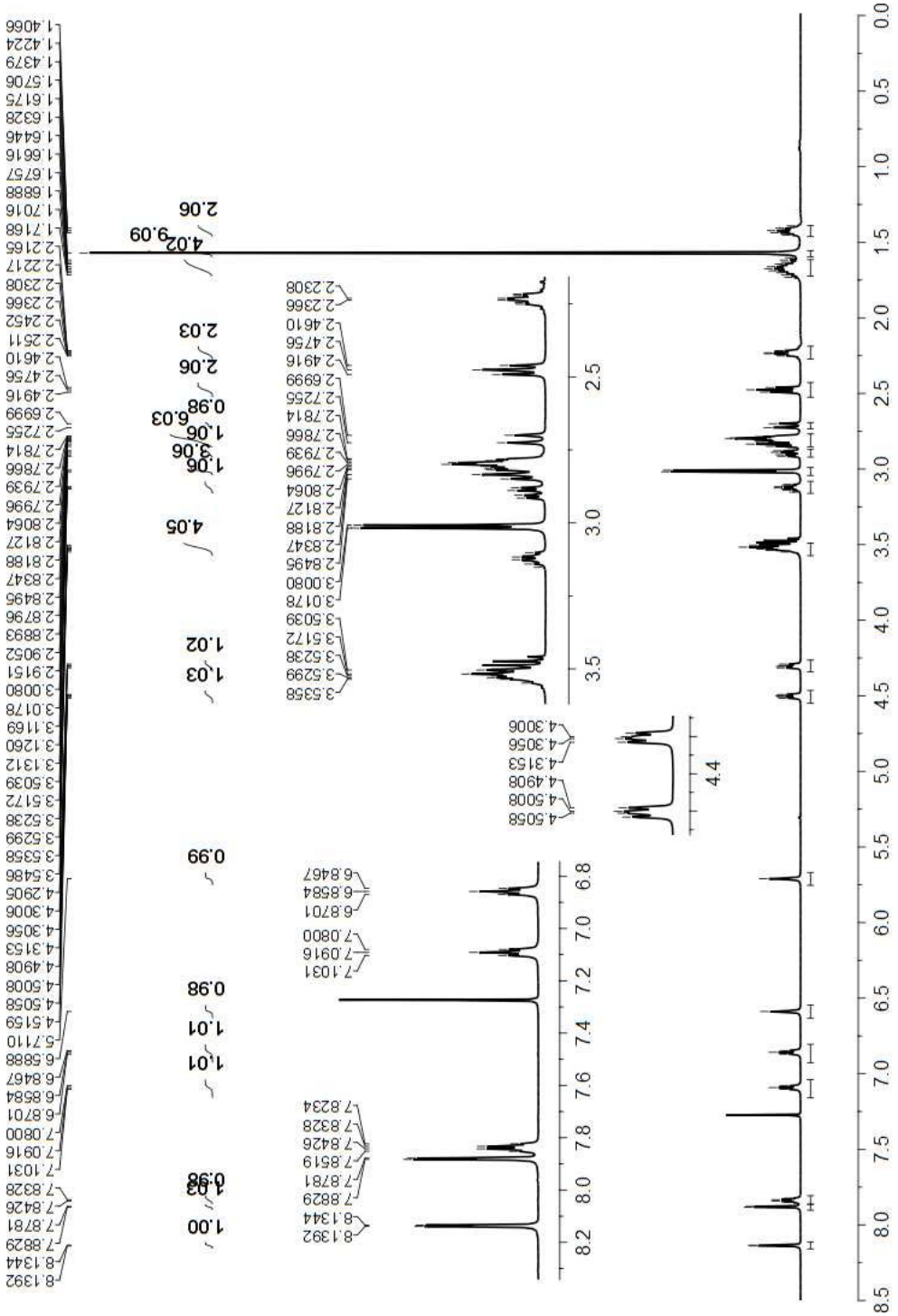
14d



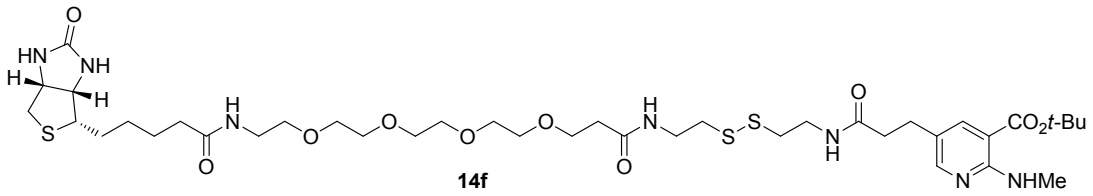
***tert*-Butyl 5-[2-({2-[(2-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 14e**



14e



***tert*-Butyl 5-{2-[(2-{[2-(1-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl)carbamoyl]ethyl}-2-(methylamino)pyridine-3-carboxylate 14f**



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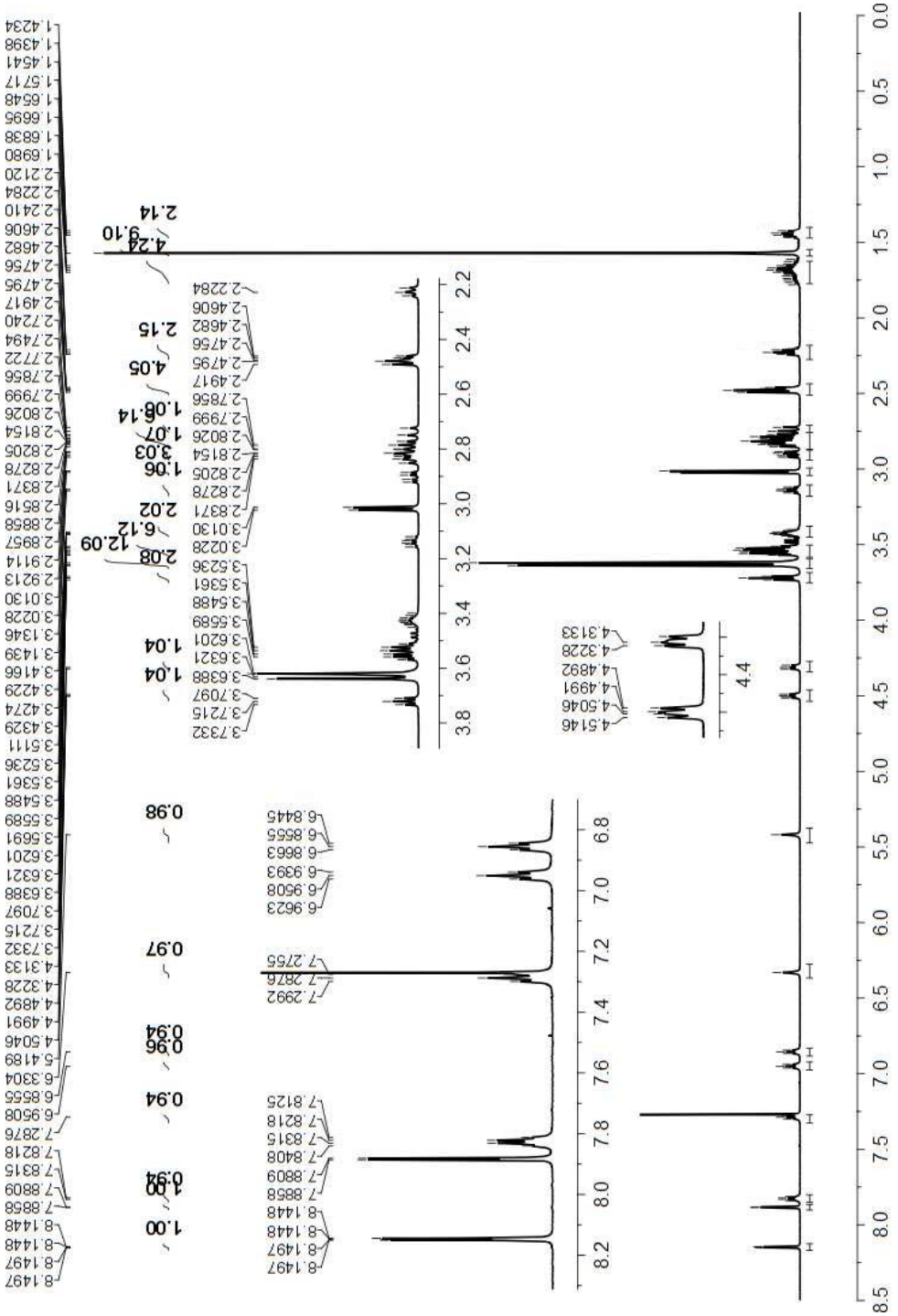
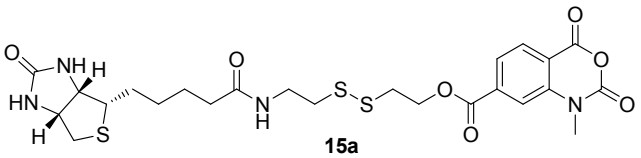
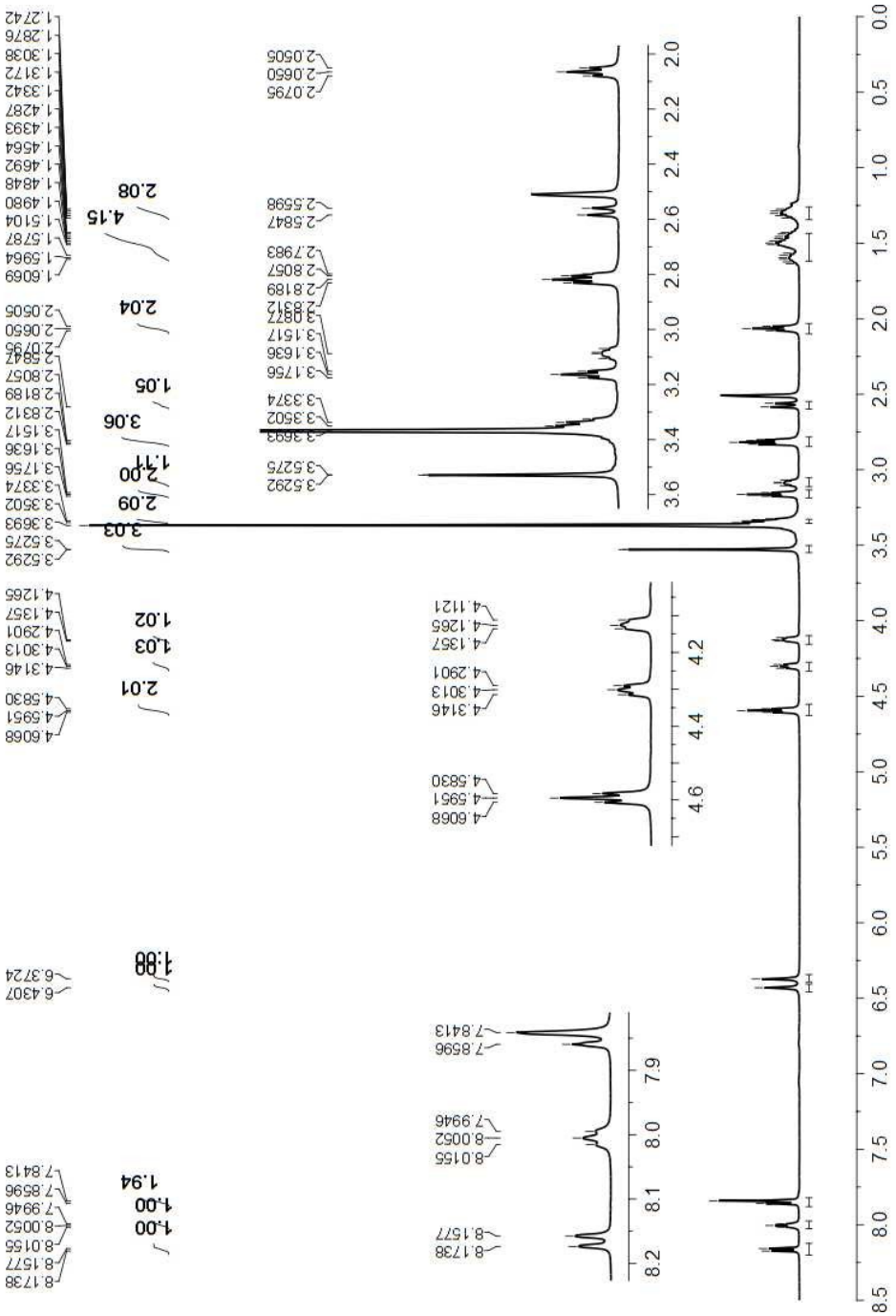


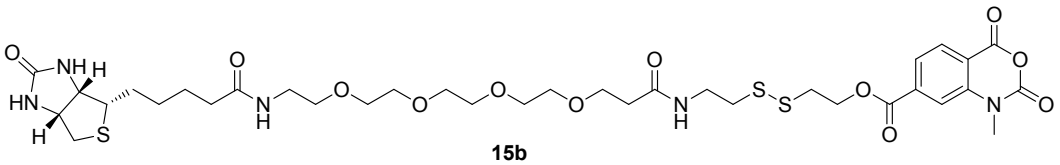
2-[(2-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl 1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazine-7-carboxylate 15a



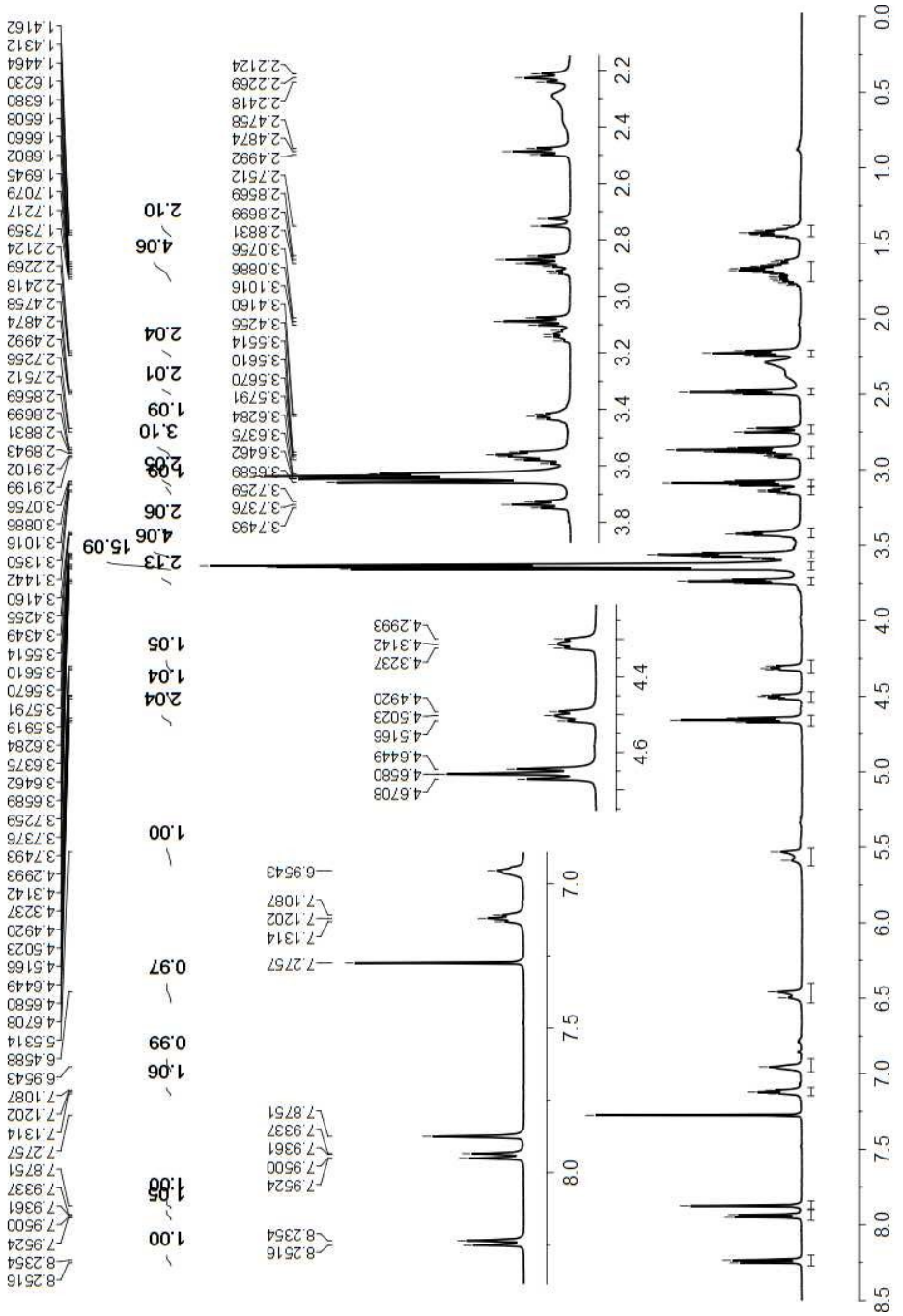
15a



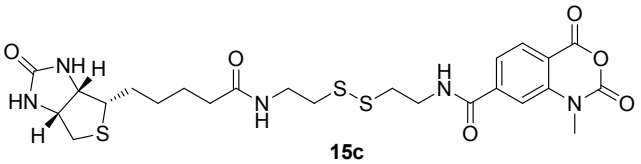
2-{[2-(1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl 1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazine-7-carboxylate 15b



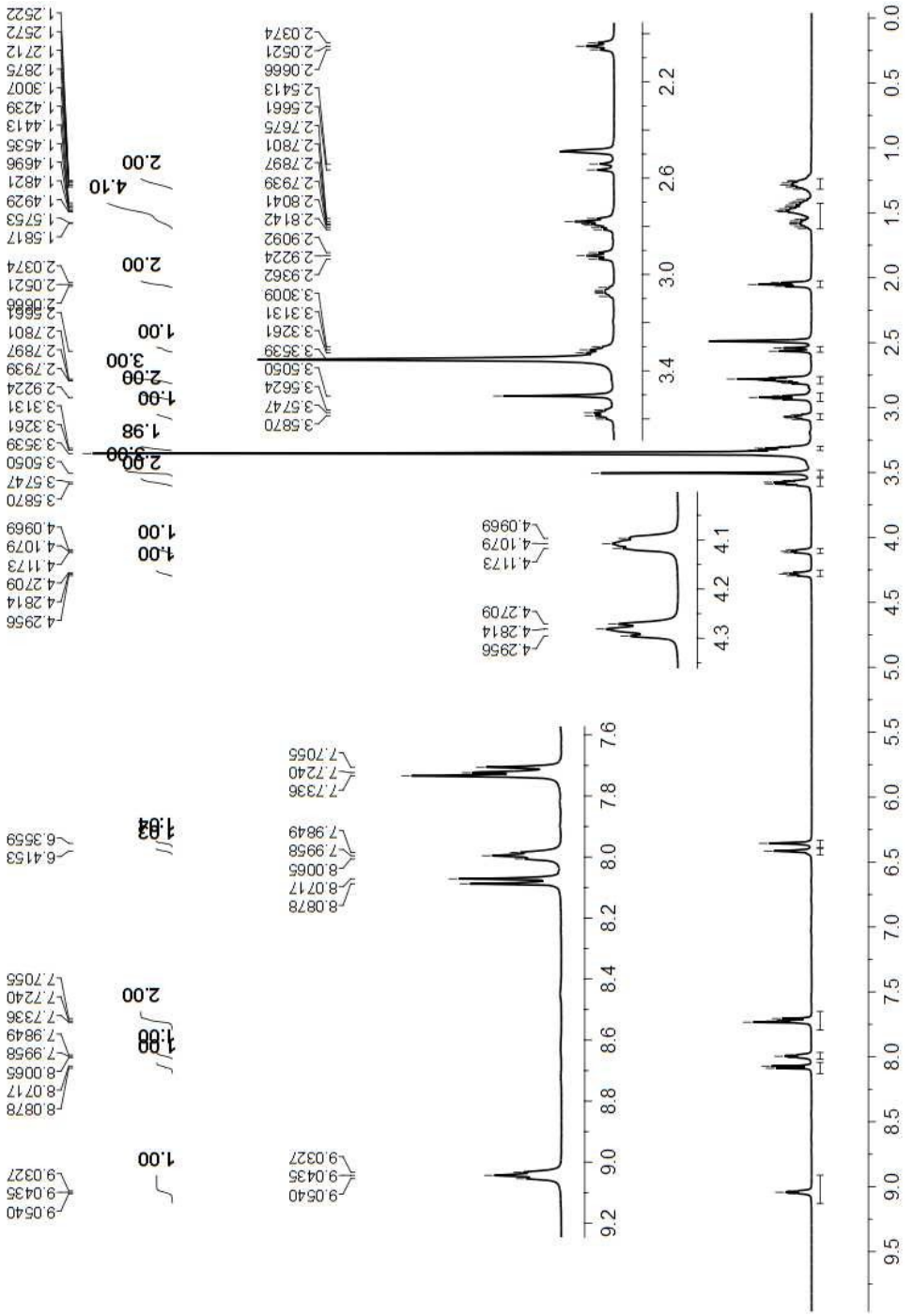
15b



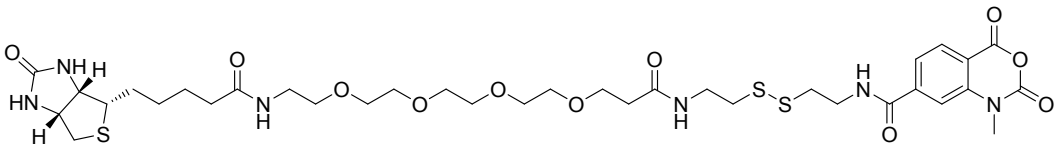
5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]-*N*-[2-({2-[(1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazin-7-yl)formamido]ethyl}disulfanyl)ethyl]pentanamide 15c



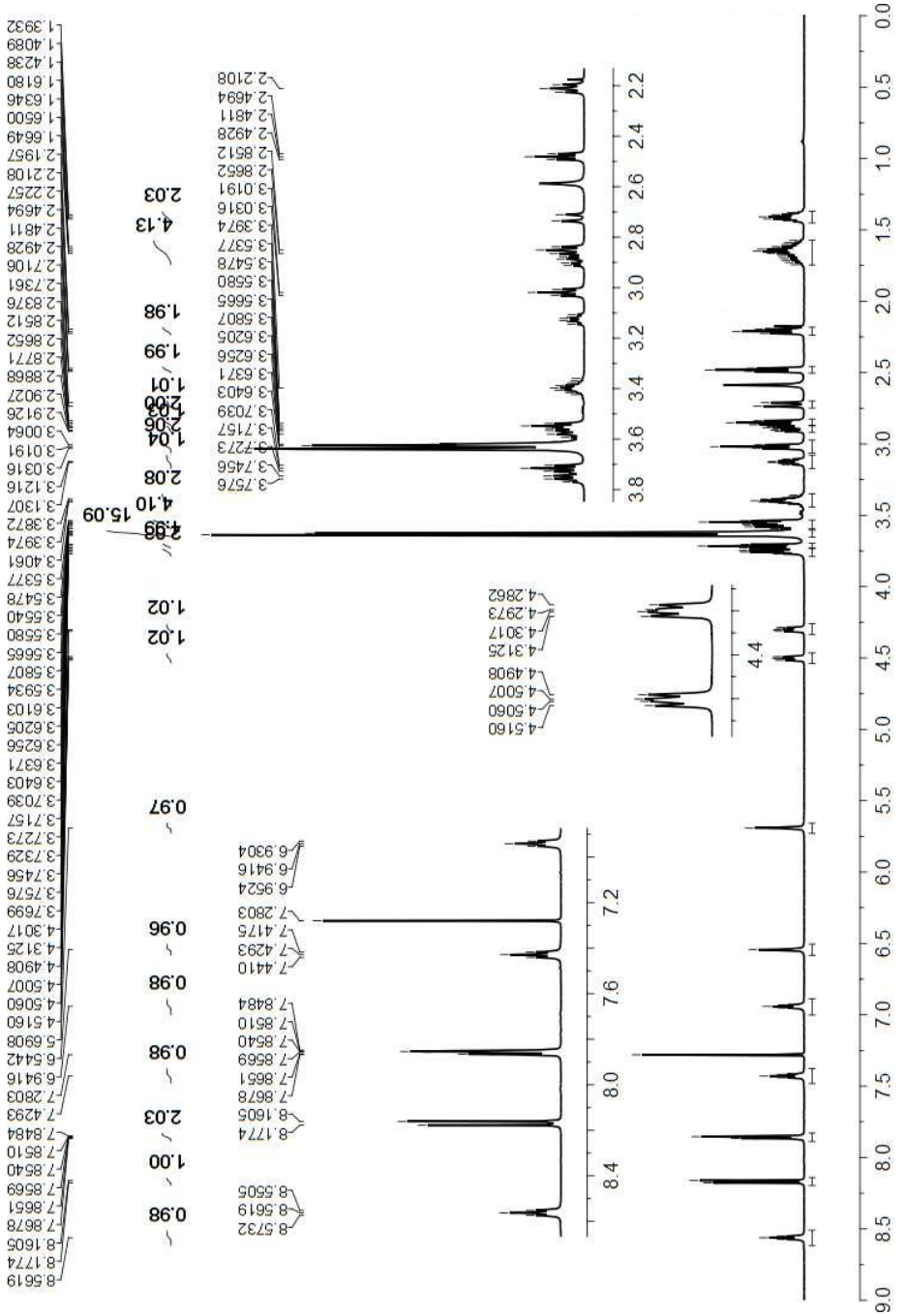
15c



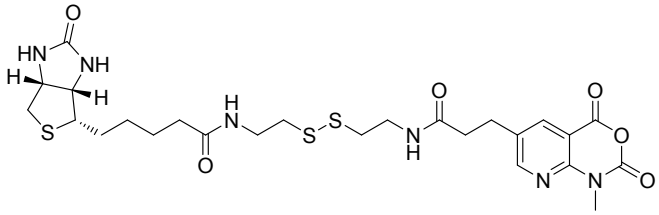
1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-d]imidazolidin-4-yl]pentanamido}-*N*-[2-({2-[(1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazin-7-yl)formamido]ethyl}disulfanyl)ethyl]-3,6,9,12-tetraoxapentadecan-15-amide 15d

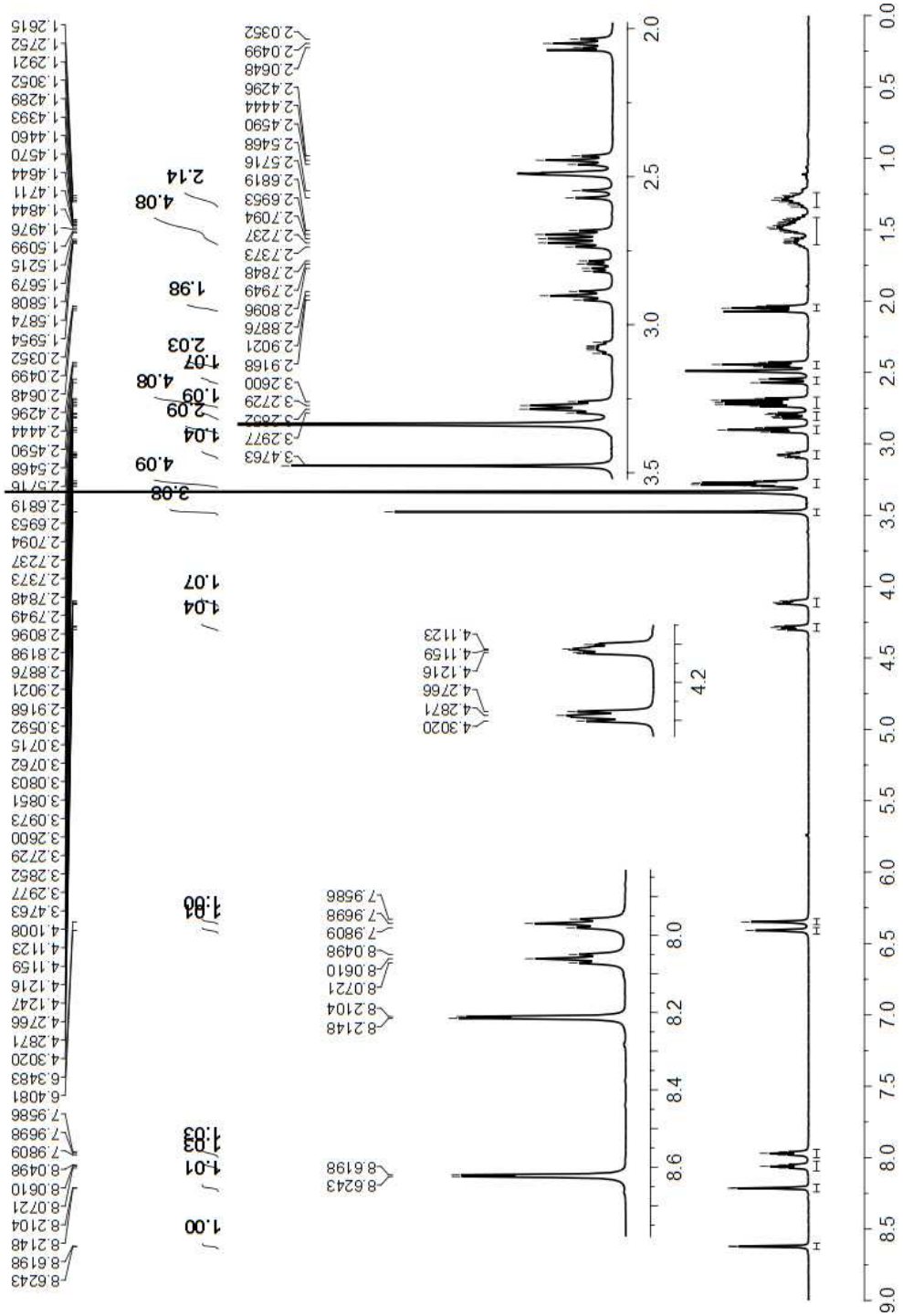


15d

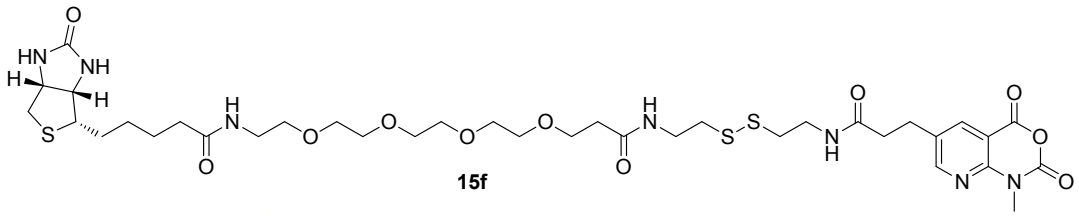


5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]-*N*-(2-{[2-(3-{1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-*d*][1,3]oxazin-6-yl}propanamido)ethyl]disulfanyl}ethyl)pentanamide 15e





1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-*N*-(2-{[2-(3-{1-methyl-2,4-dioxo-*1H*,*2H*,*4H*-pyrido[2,3-*d*][1,3]oxazin-6-yl}propanamido)ethyl]disulfanyl}ethyl)-3,6,9,12-tetraoxapentadecan-15-amide 15f



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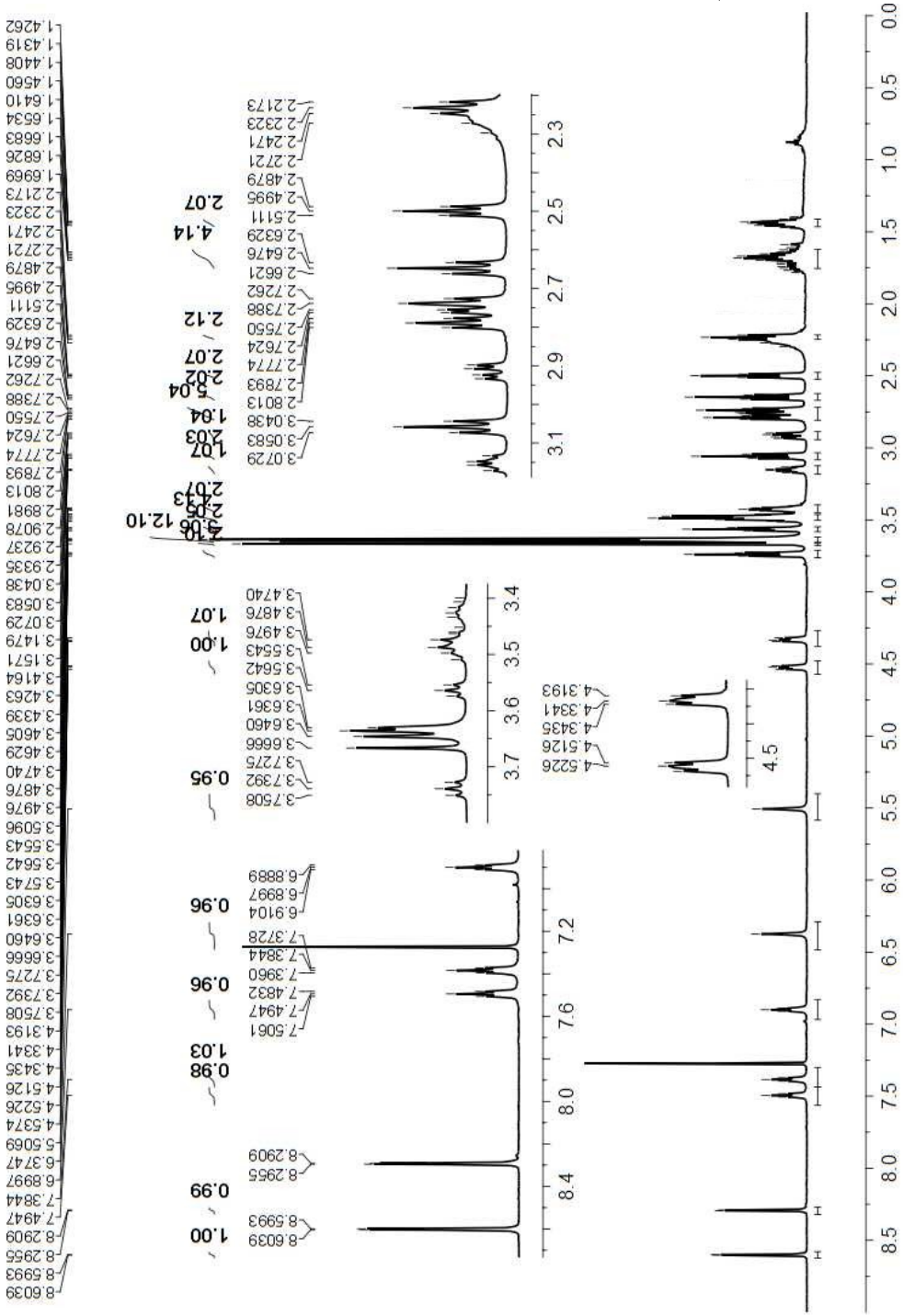
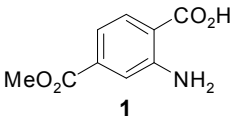


8) ^{13}C NMR of Compounds 1-15

2-Amino-4-(methoxycarbonyl)benzoic acid 1



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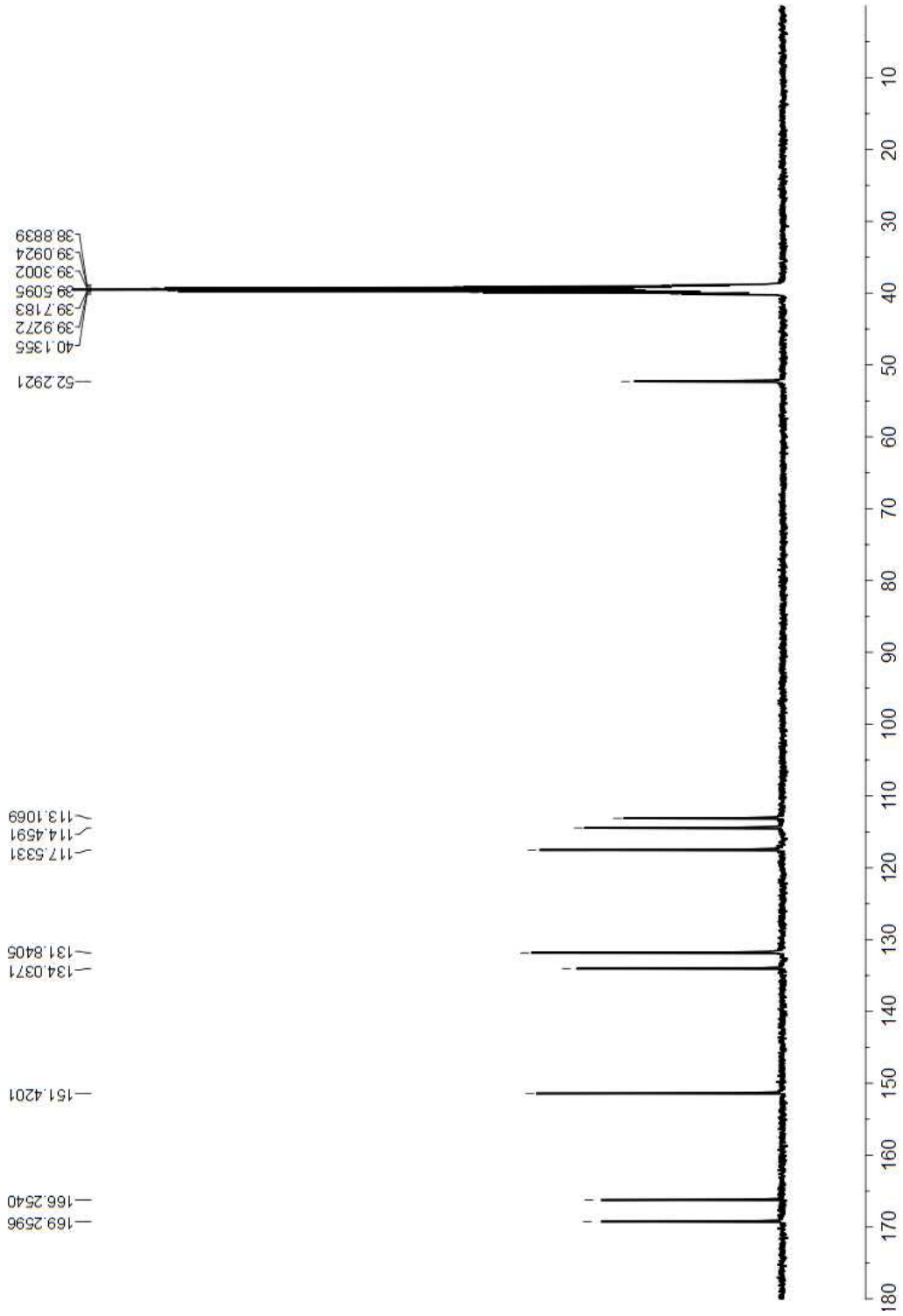
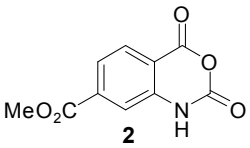
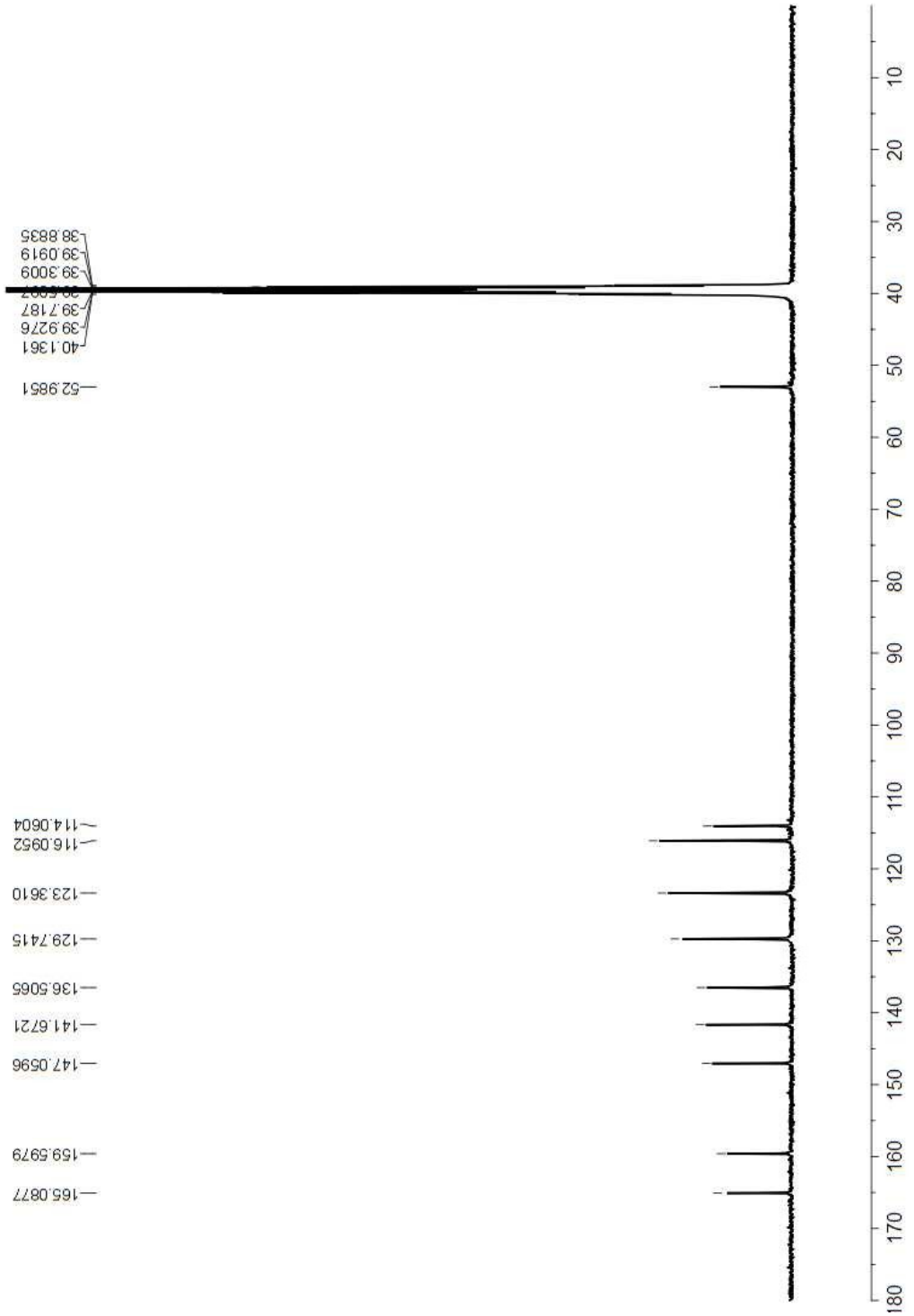


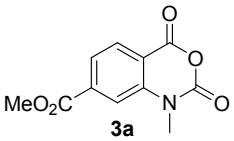
Methyl 2,4-dioxo-*1H*-3,1-benzoxazine-7-carboxylate 2

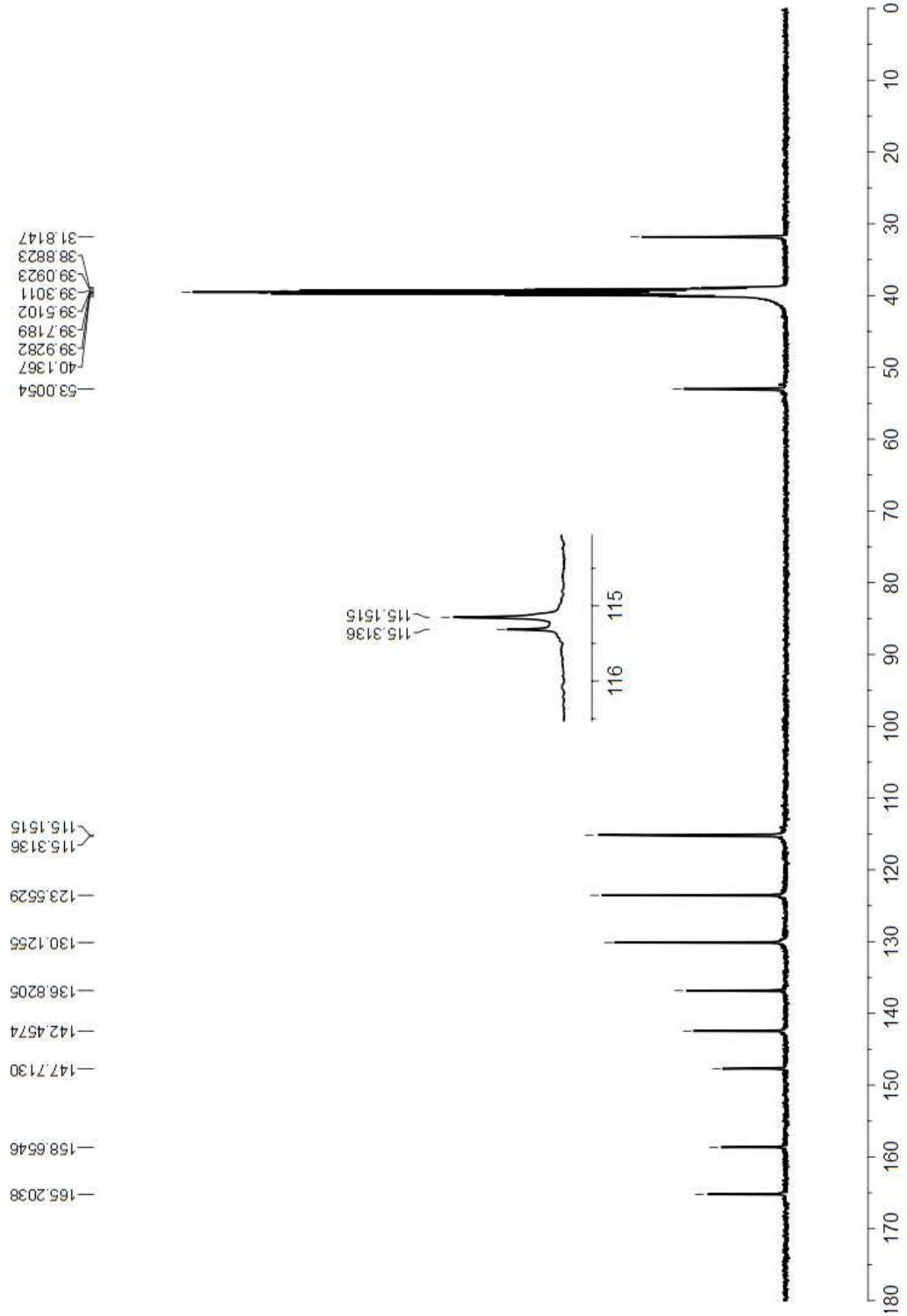


2

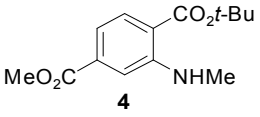


Methyl 1-methyl-2,4-dioxo-3,1-benzoxazine-7-carboxylate 3a





1-*tert*-Butyl 4-methyl 2-(methylamino)benzene-1,4-dicarboxylate 4



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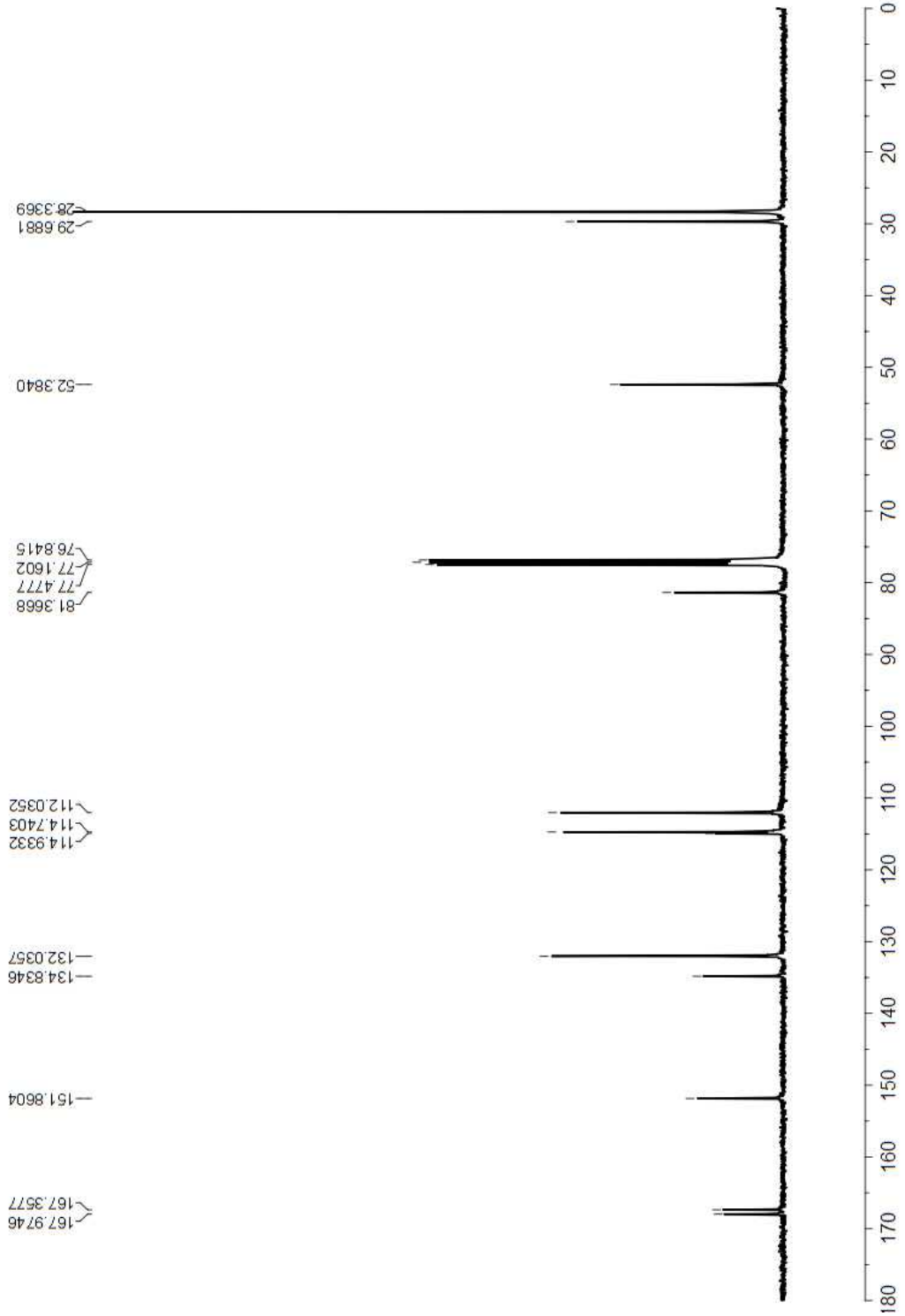
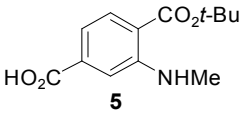


4-[(*tert*-Butoxy)carbonyl]-3-(methylamino)benzoic acid 5



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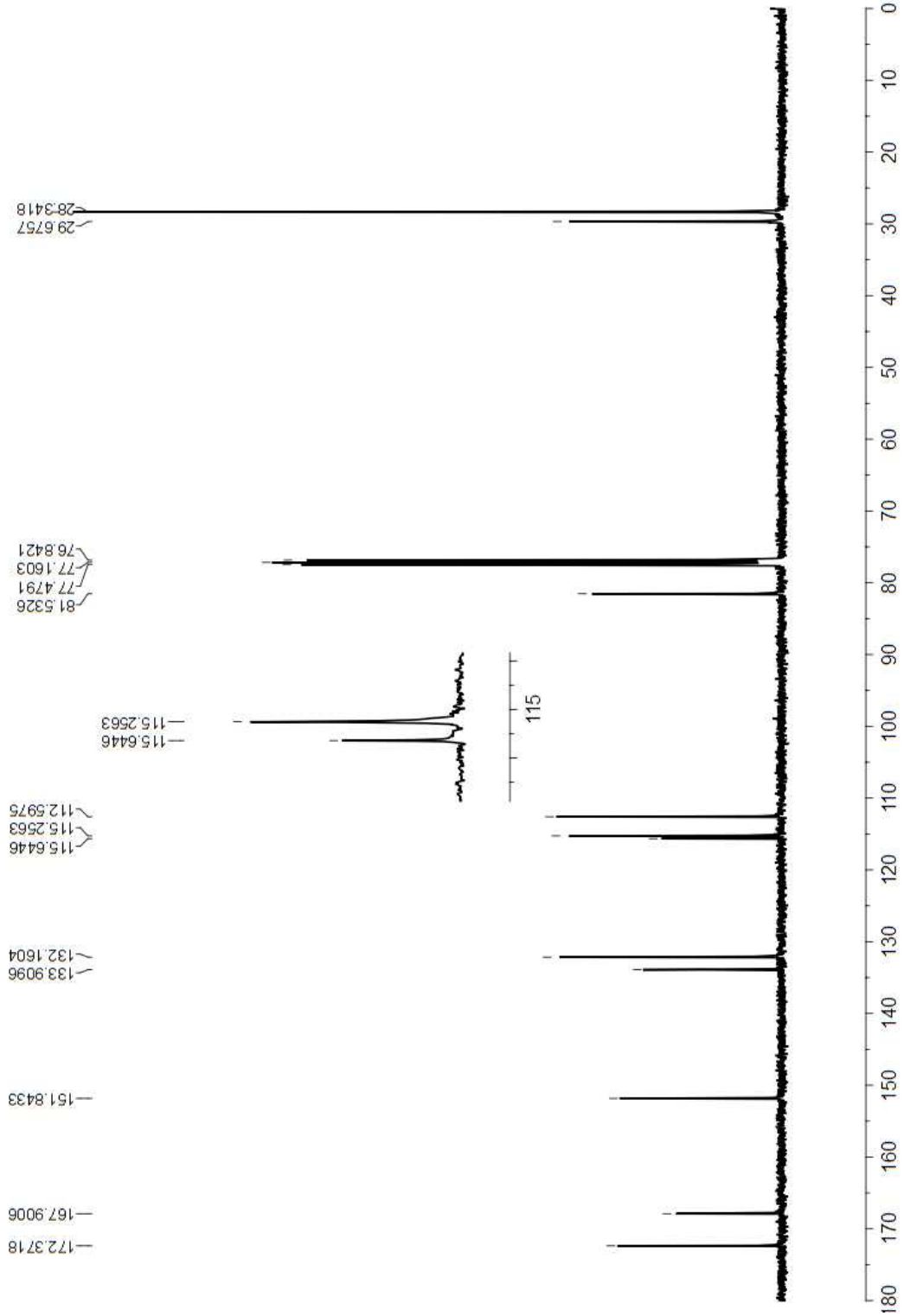
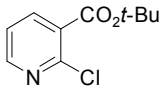


***tert*-Butyl 2-chloropyridine-3-carboxylate 6**



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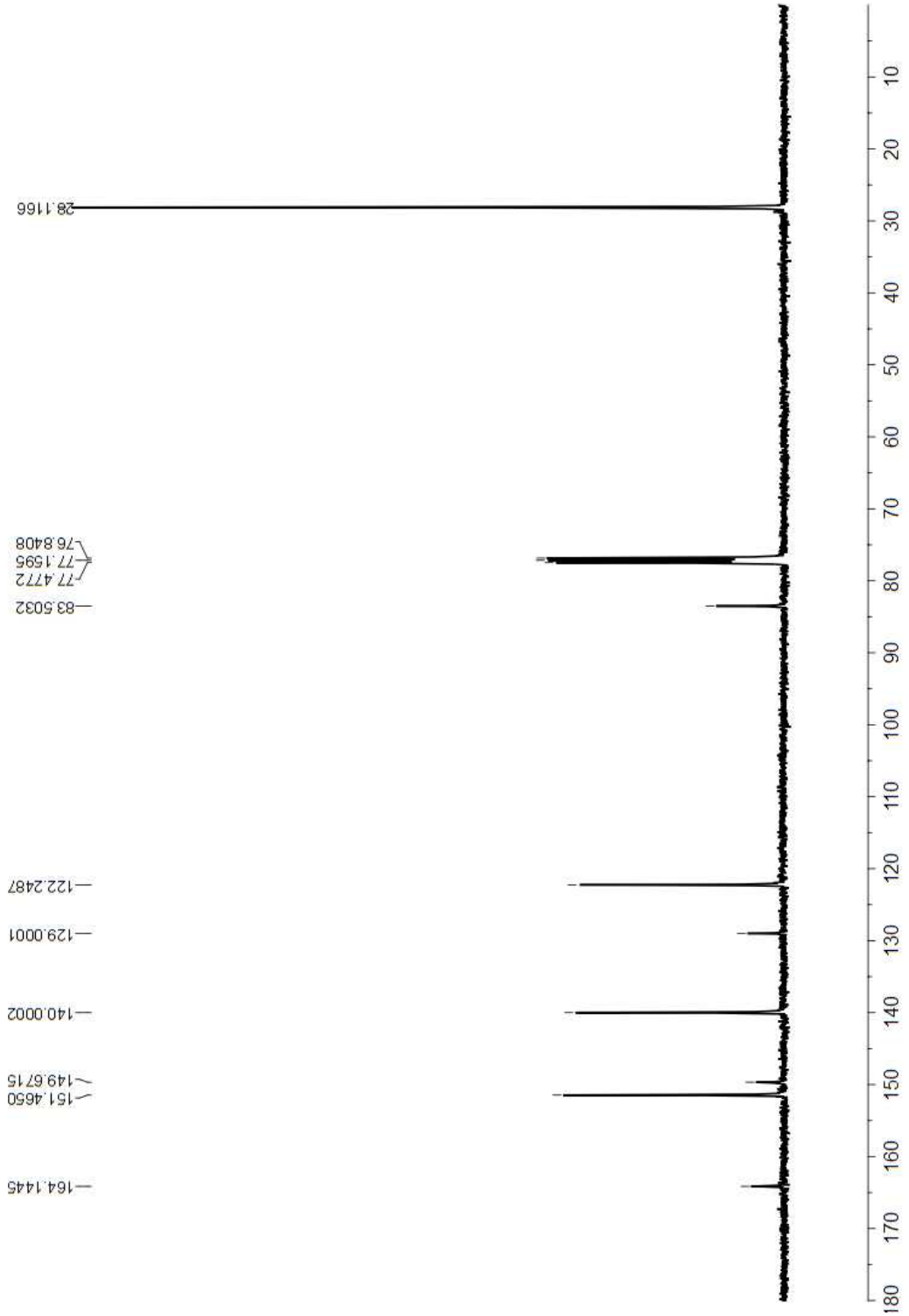
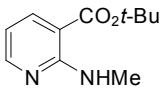


***tert*-Butyl 2-(methylamino)pyridine-3-carboxylate 7**



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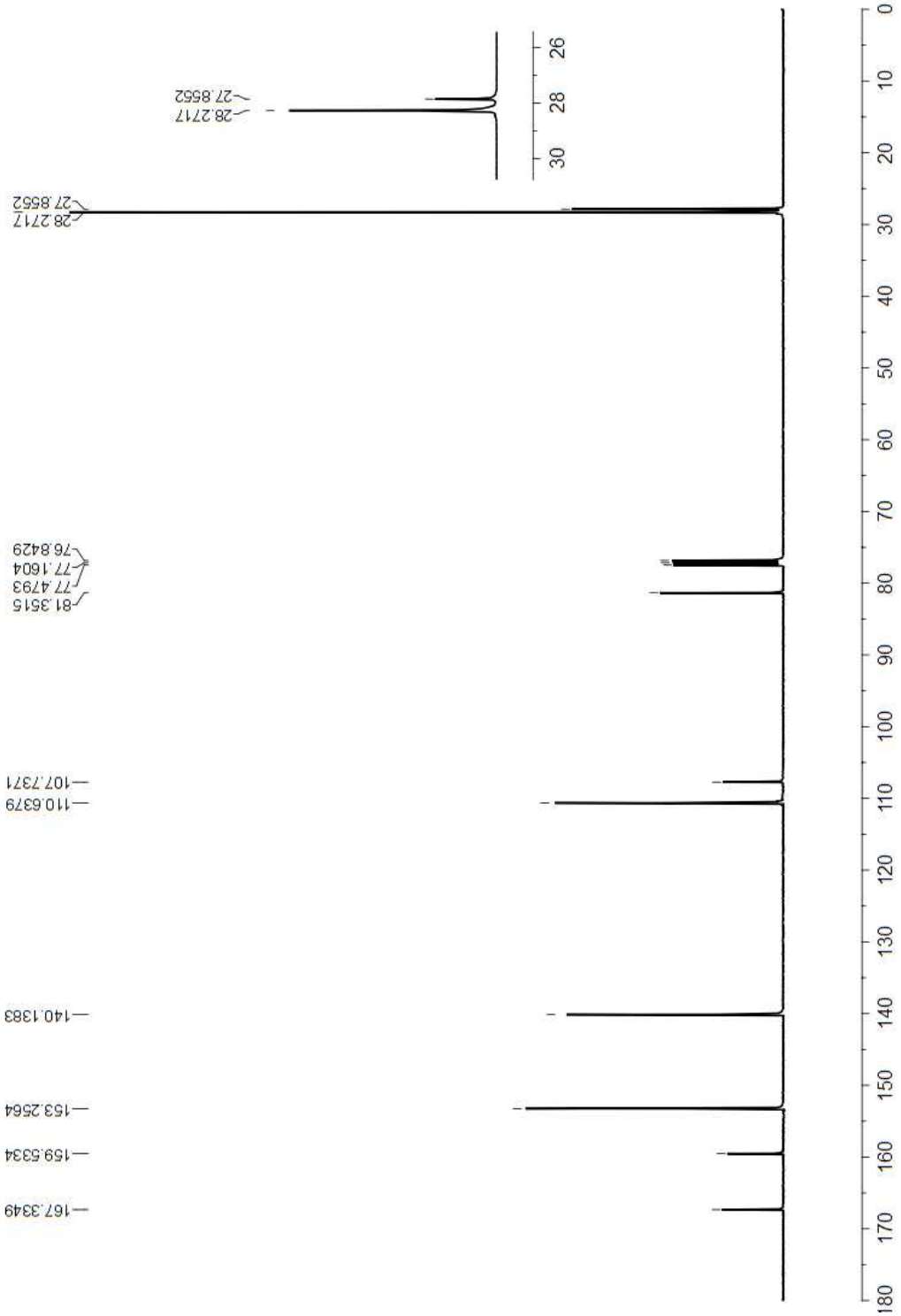
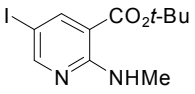


***tert*-Butyl 5-iodo-2-(methylamino)pyridine-3-carboxylate 8**



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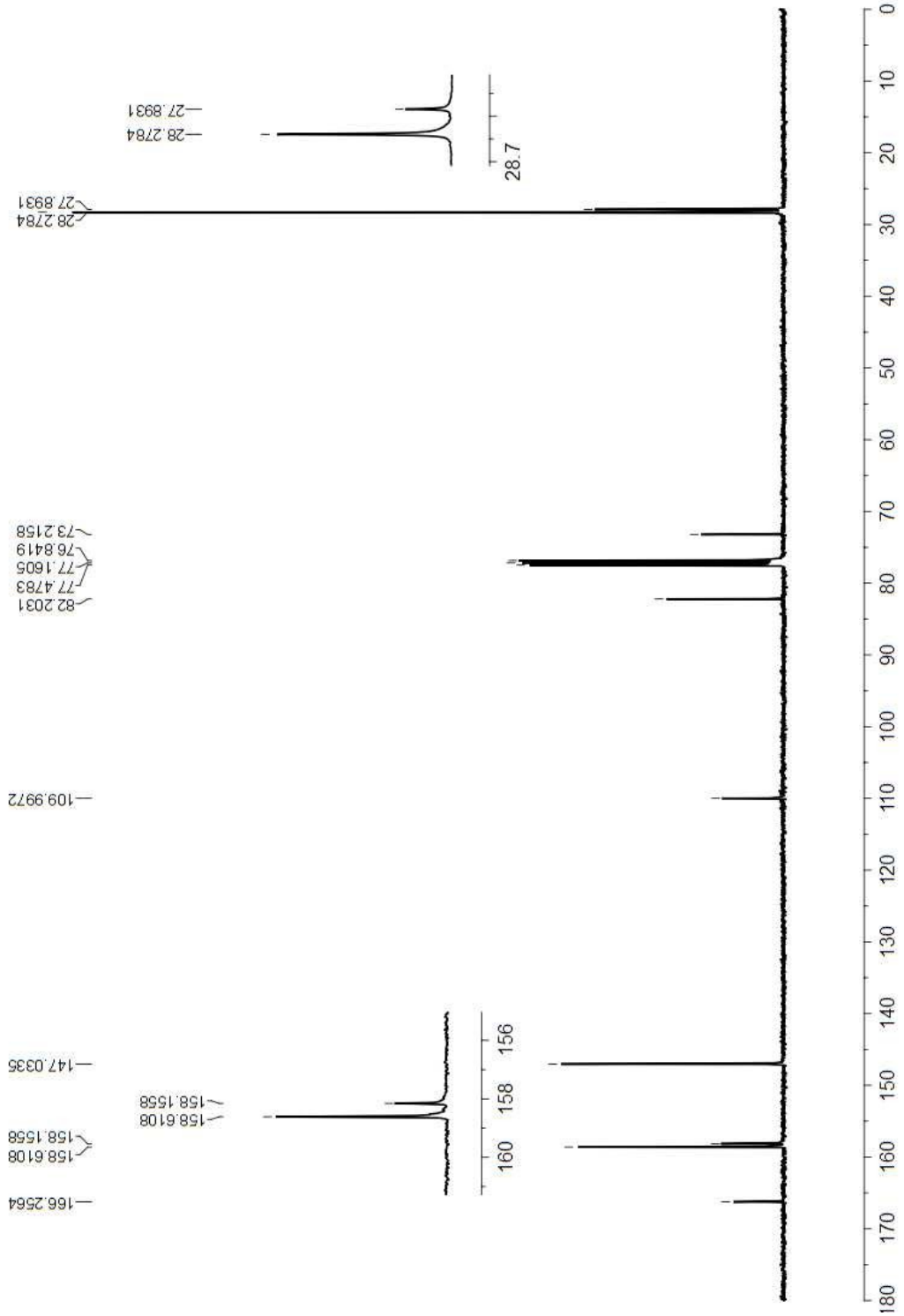
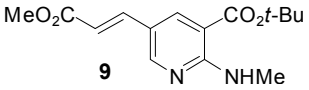


***tert*-Butyl 5-[(*1E*)-3-methoxy-3-oxoprop-1-en-1-yl]-2-(methylamino)pyridine-3-carboxylate 9**



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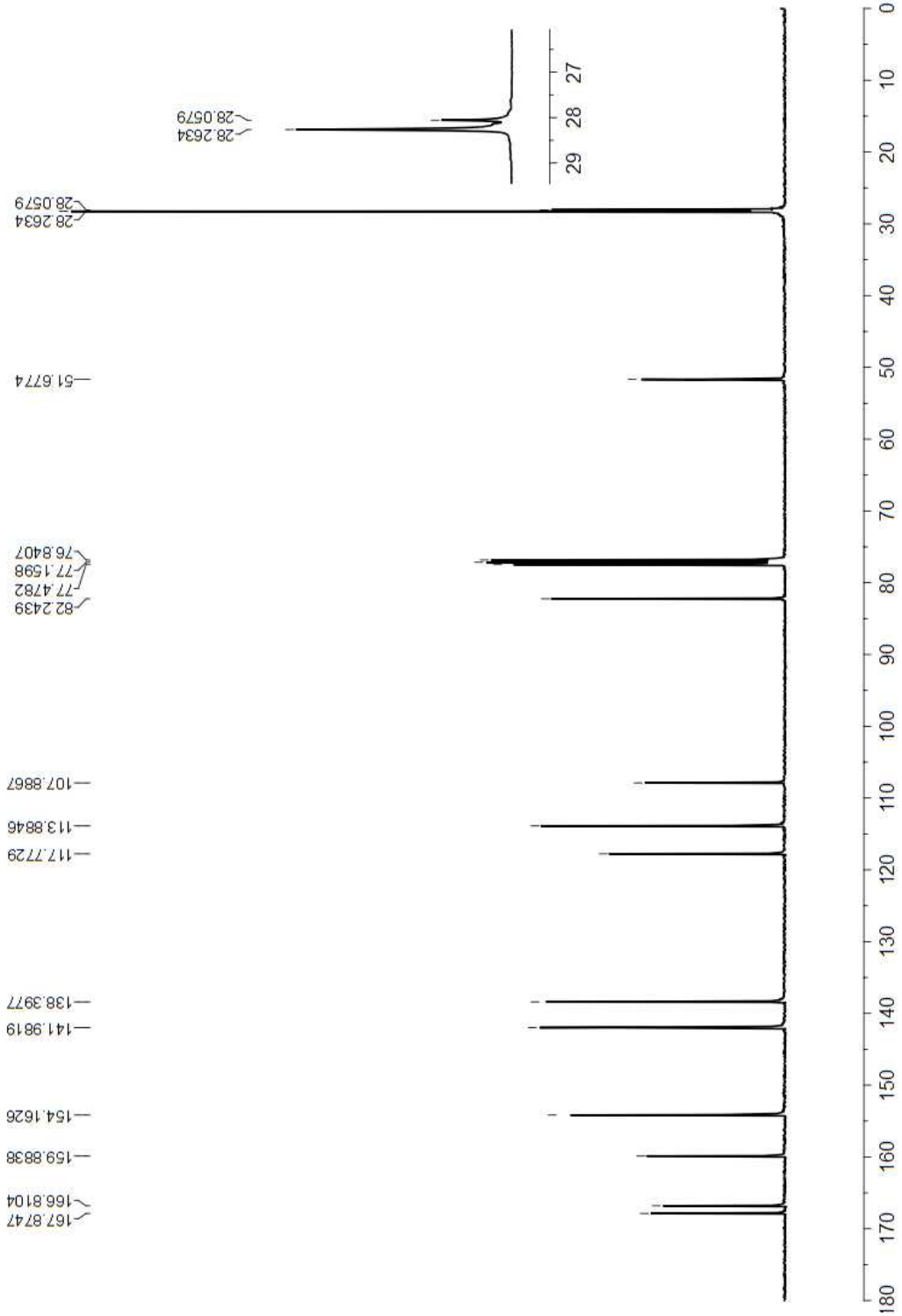
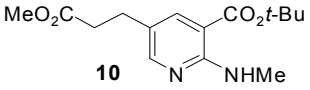
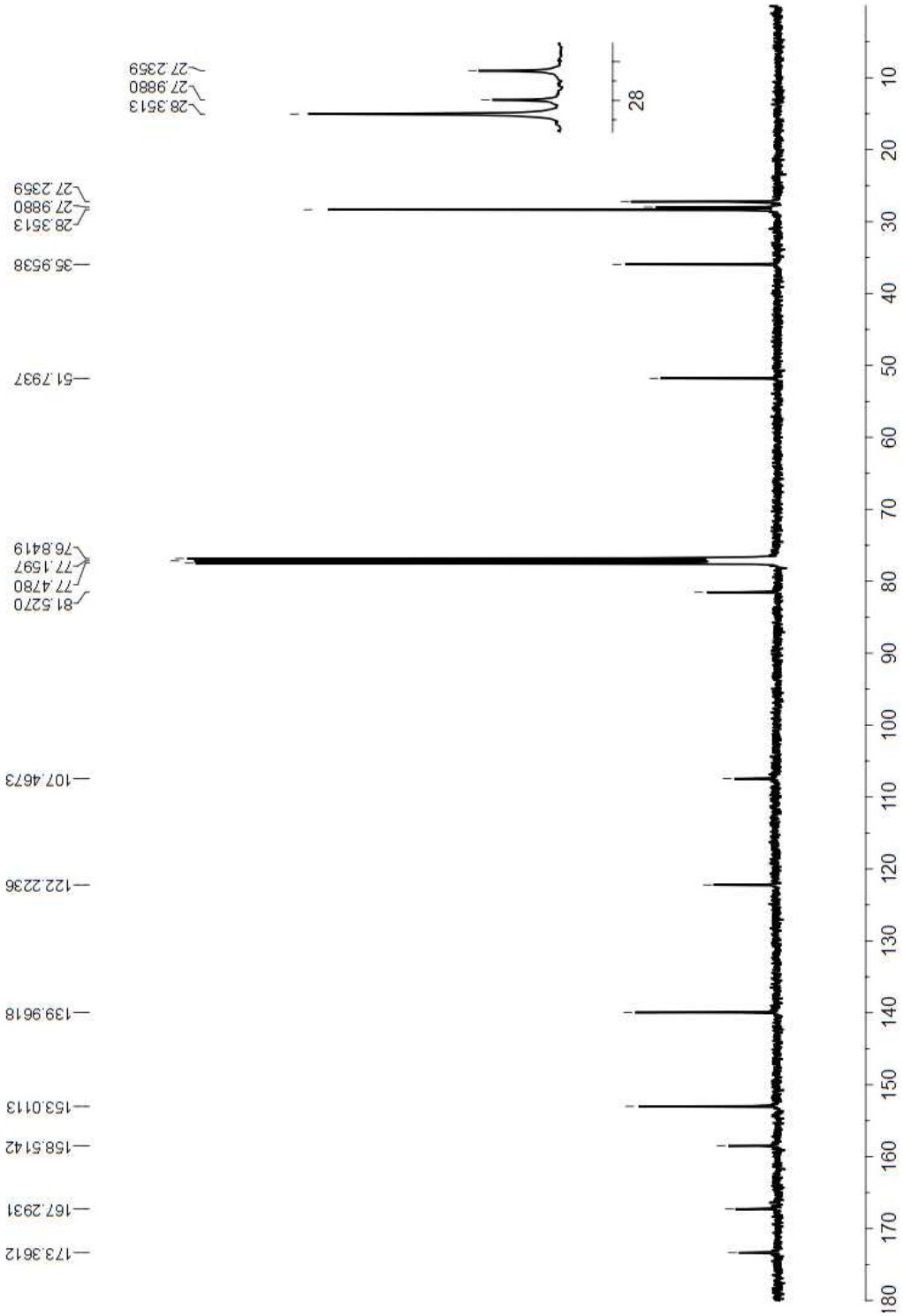
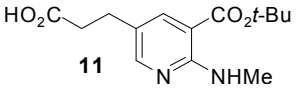


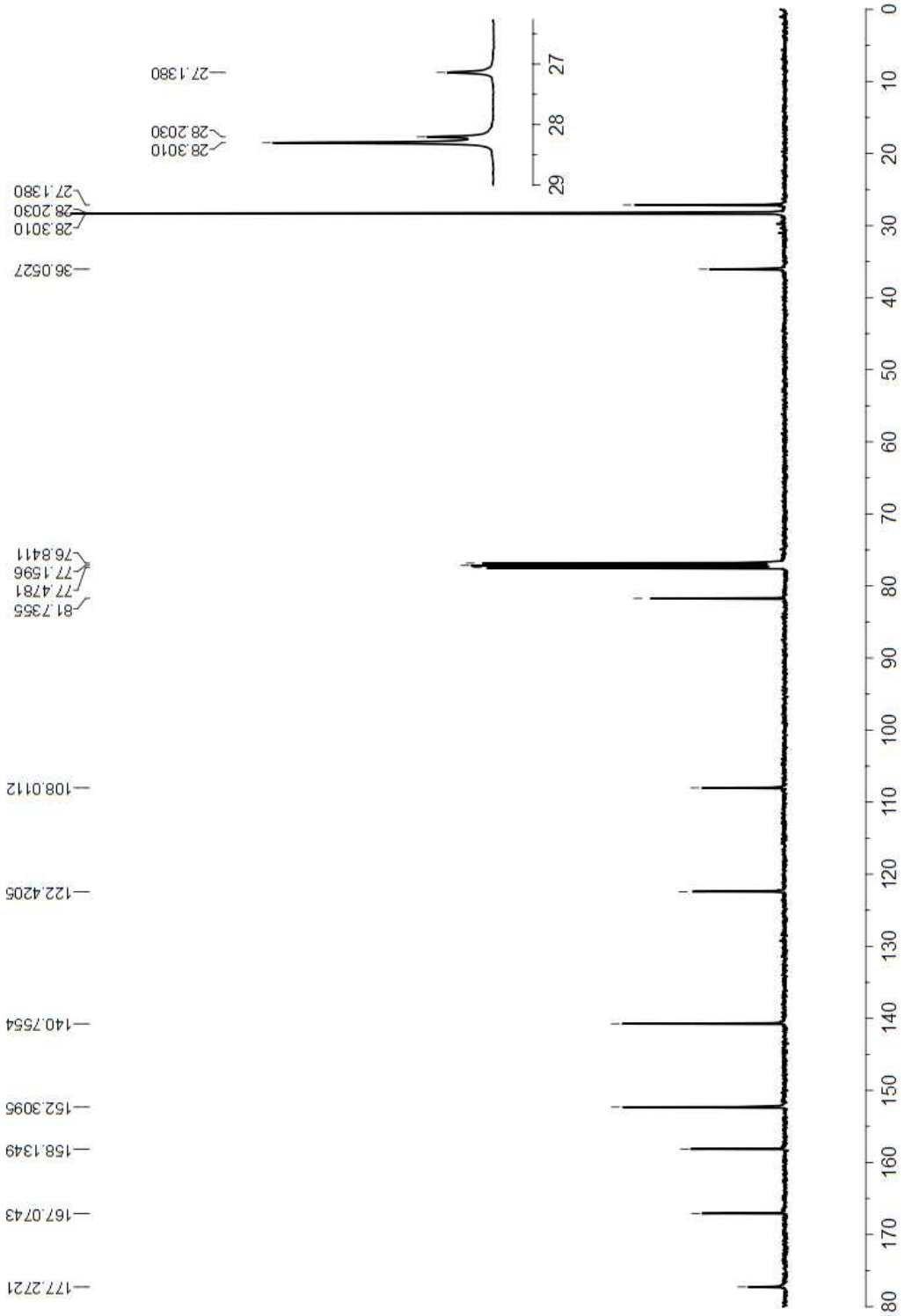
***tert*-Butyl 5-(3-methoxy-3-oxopropyl)-2-(methylamino)pyridine-3-carboxylate 10**



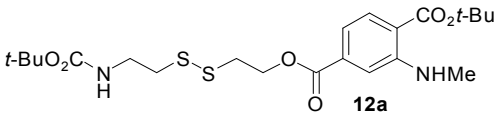


3-{5-[(*tert*-Butoxy)carbonyl]-6-(methylamino)pyridin-3-yl}propanoic acid 11

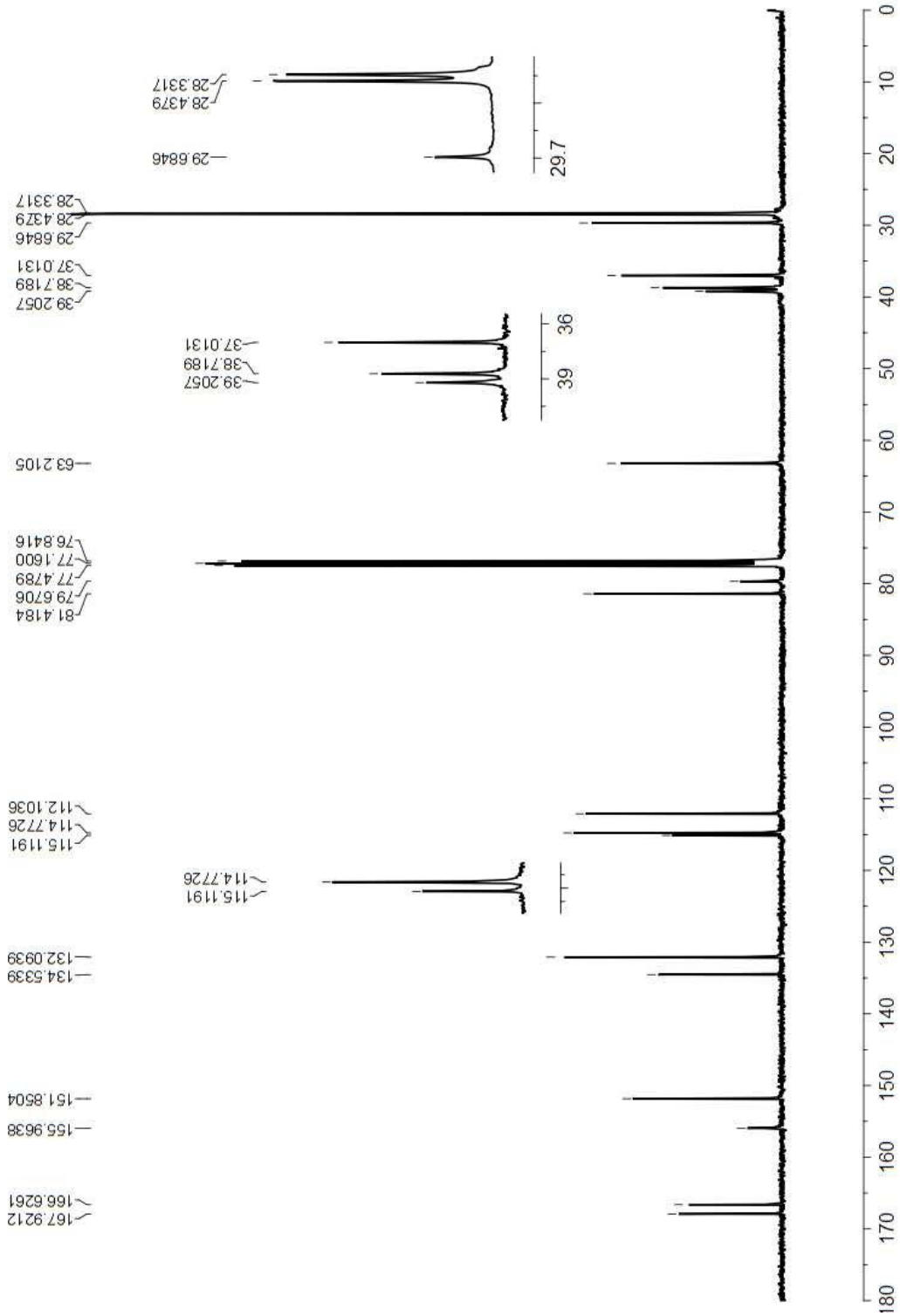




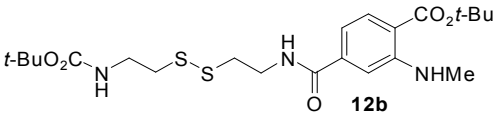
4-{2-[(2-{[(*tert*-Butoxy)carbonyl]amino}ethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 12a



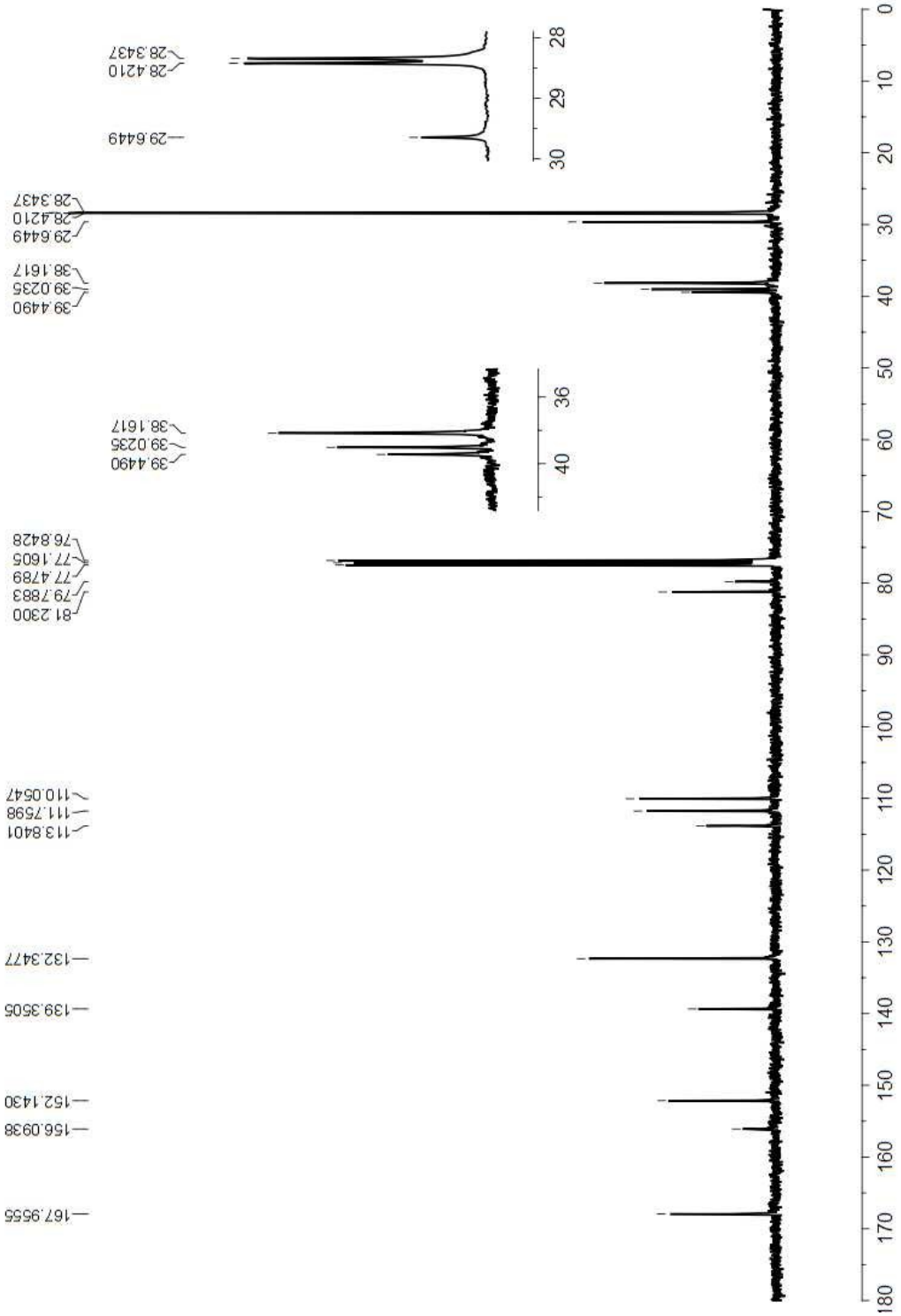
12a



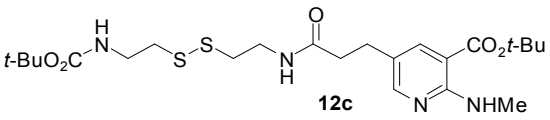
***tert*-Butyl 4-({2-[(2-{[(*tert*-butoxy)carbonyl]amino}ethyl)disulfanyl]ethylcarbamoyl)-2-(methylamino)benzoate 12b**



12b



***tert*-Butyl 5-[2-({2-[(2-{[(*tert*-butoxy)carbonyl]amino}ethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 12c**



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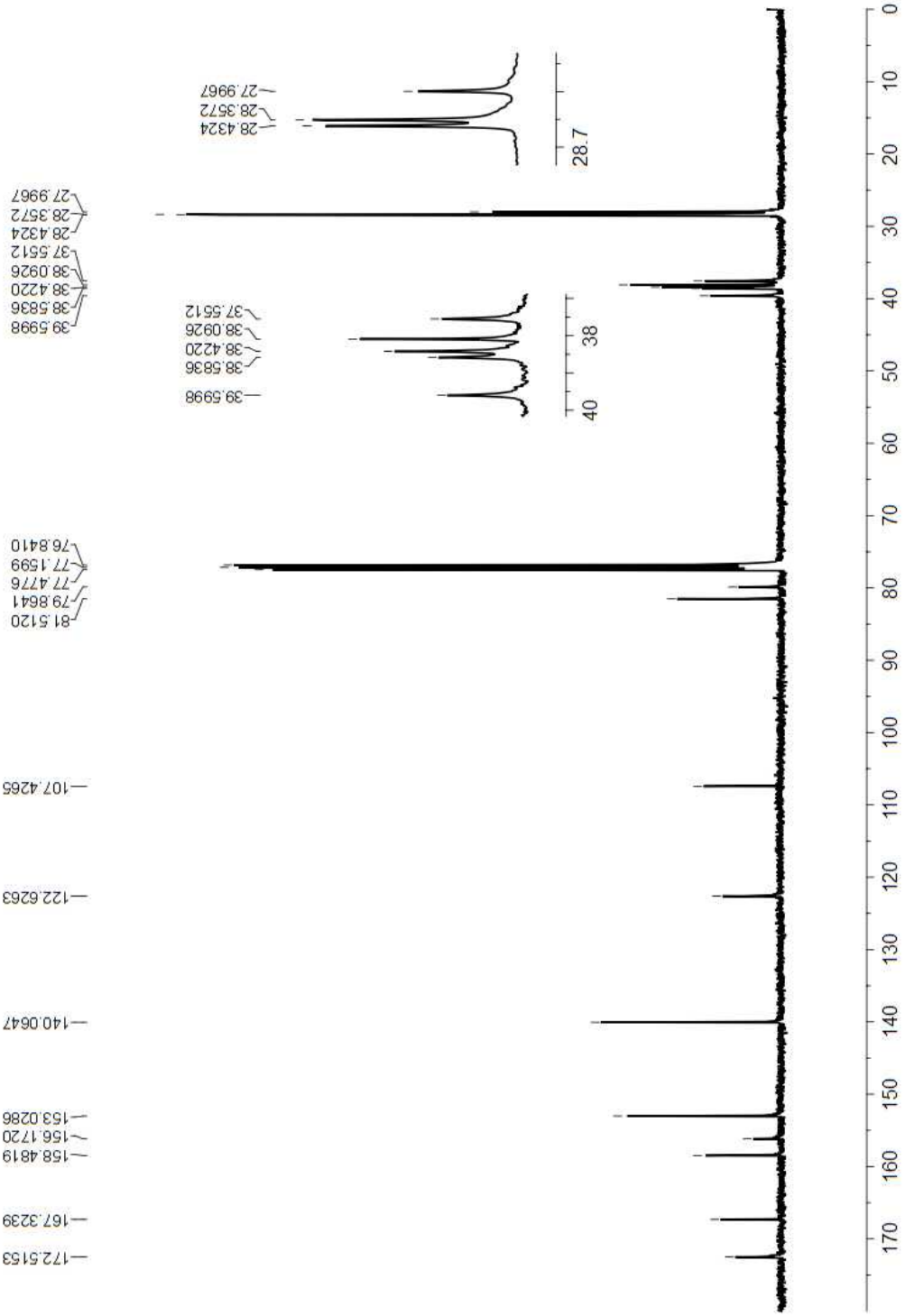
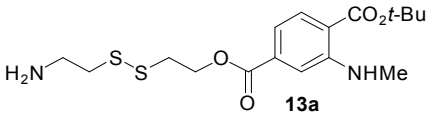
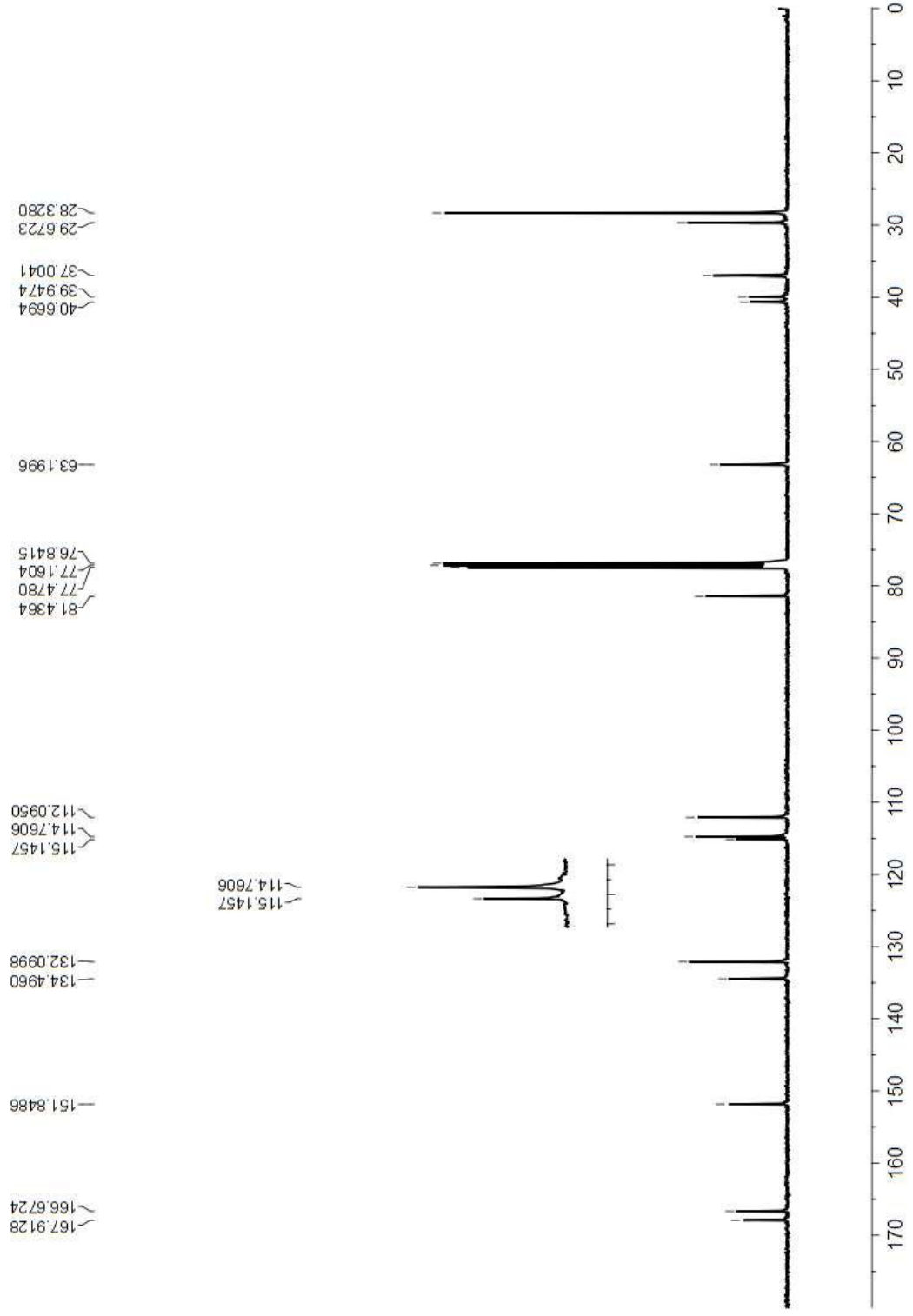


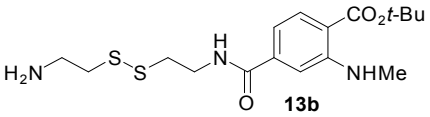
4-{2-[(2-Aminoethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 13a

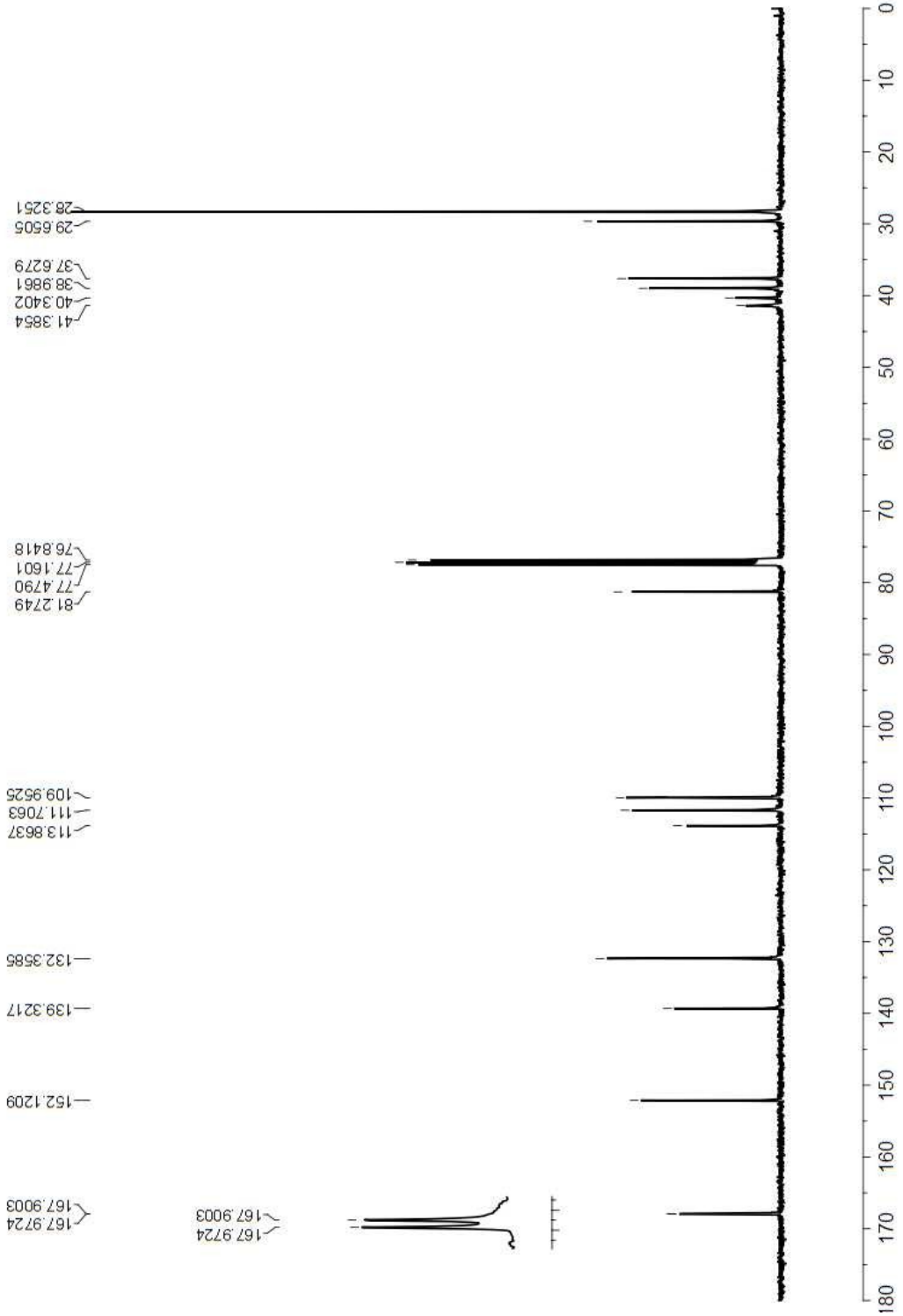


13a

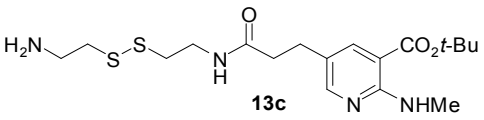


***tert*-Butyl 5-[2-({2-[(2-aminoethyl)disulfanyl]ethyl}carbamoyl)]-2-(methylamino)benzoate 13b**

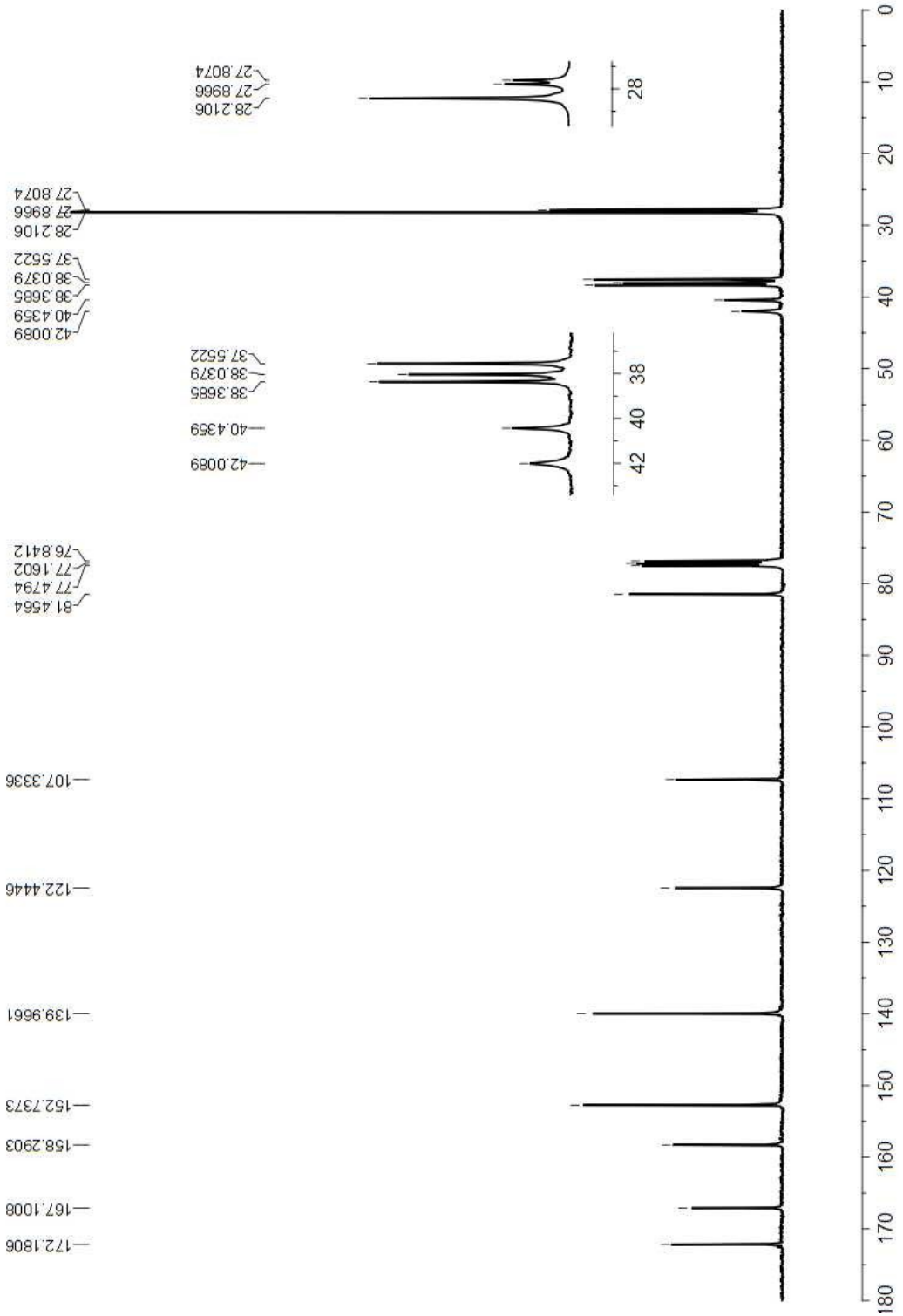




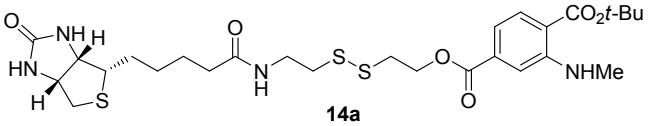
***tert*-Butyl 5-[2-({2-[(2-aminoethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 13c**



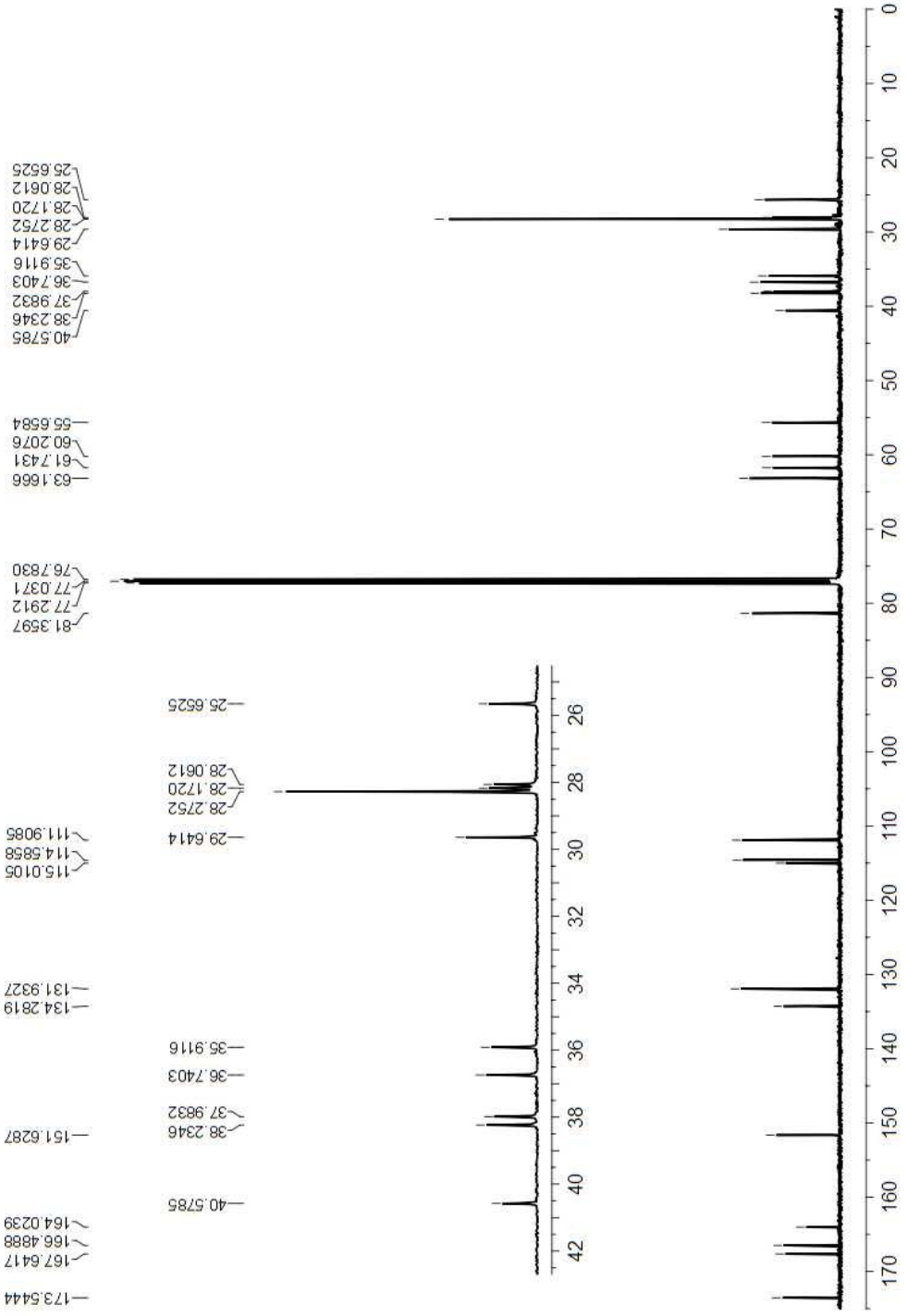
13c



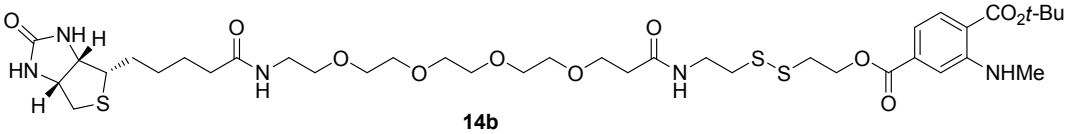
***tert*-Butyl 4-({2-[(2-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl}carbamoyl)-2-(methylamino)benzoate 14a**



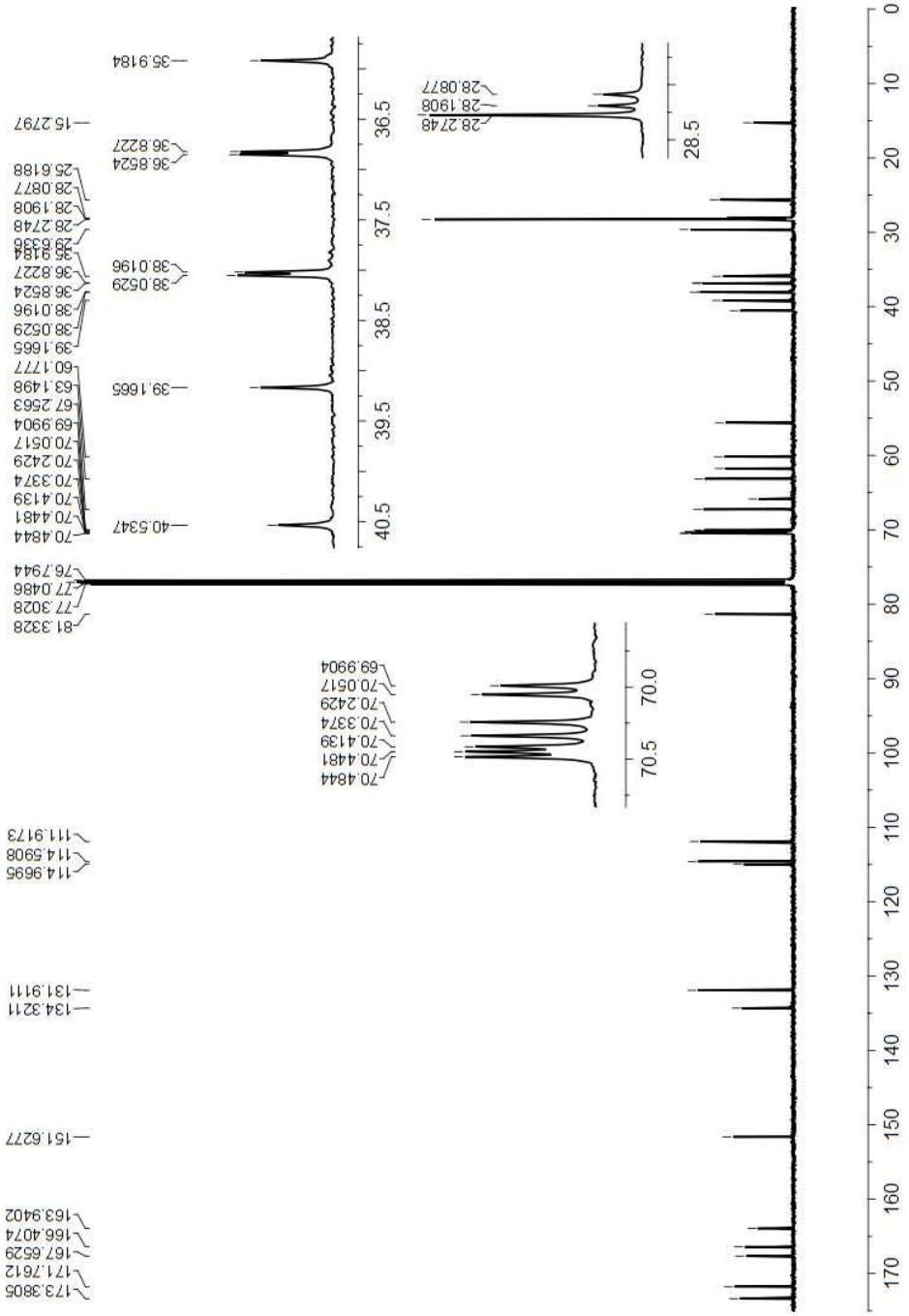
14a



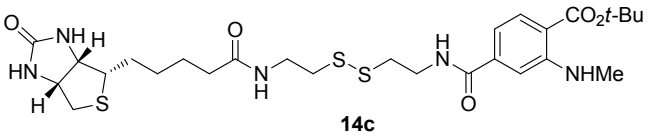
4-(2-{[2-(1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl) 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 14b



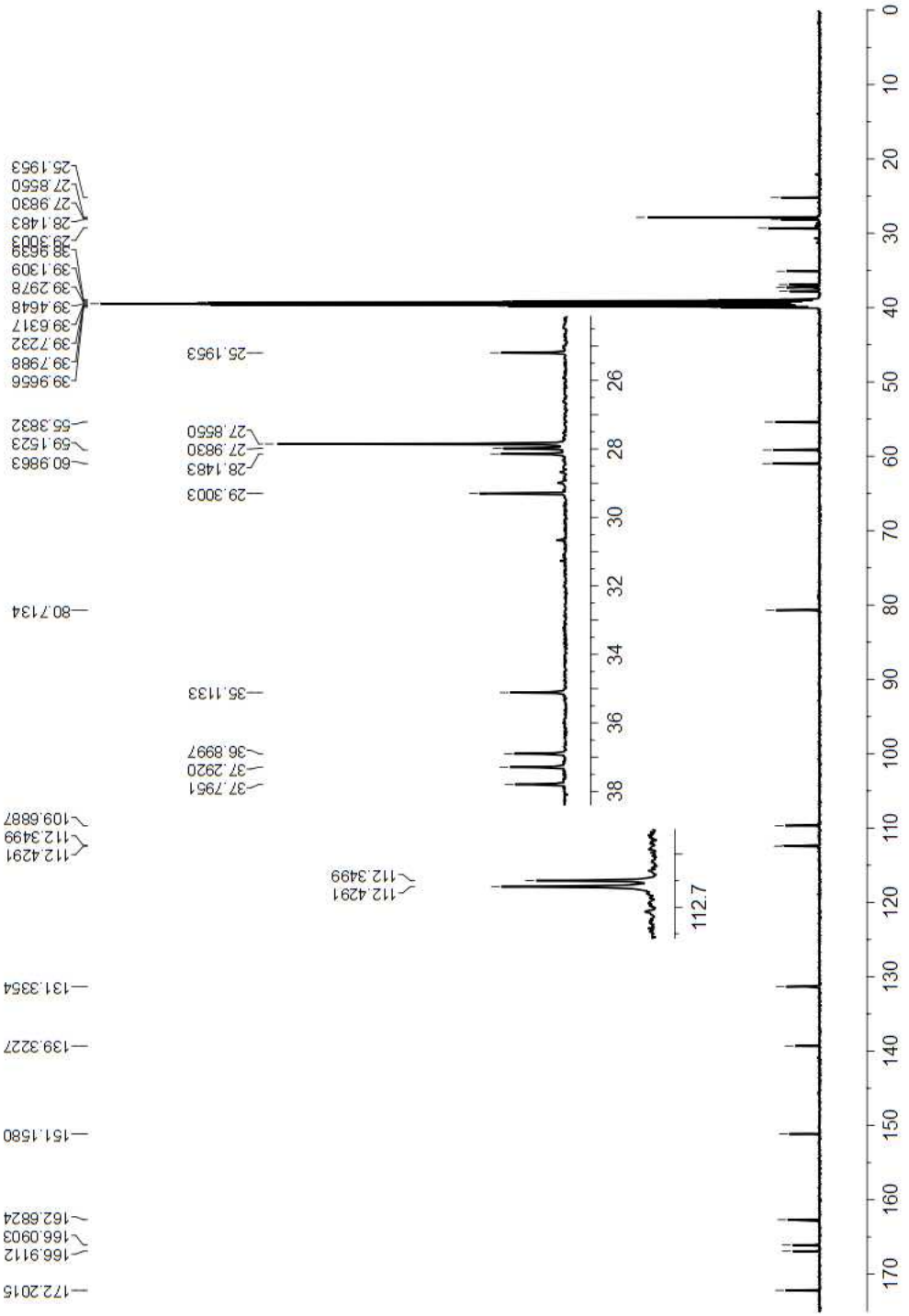
14b



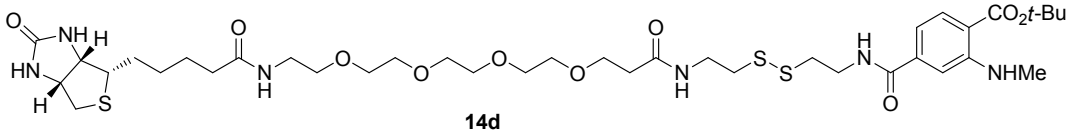
4-{2-[(2-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl} 1-*tert*-butyl 2-(methylamino)benzene-1,4-dicarboxylate 14c



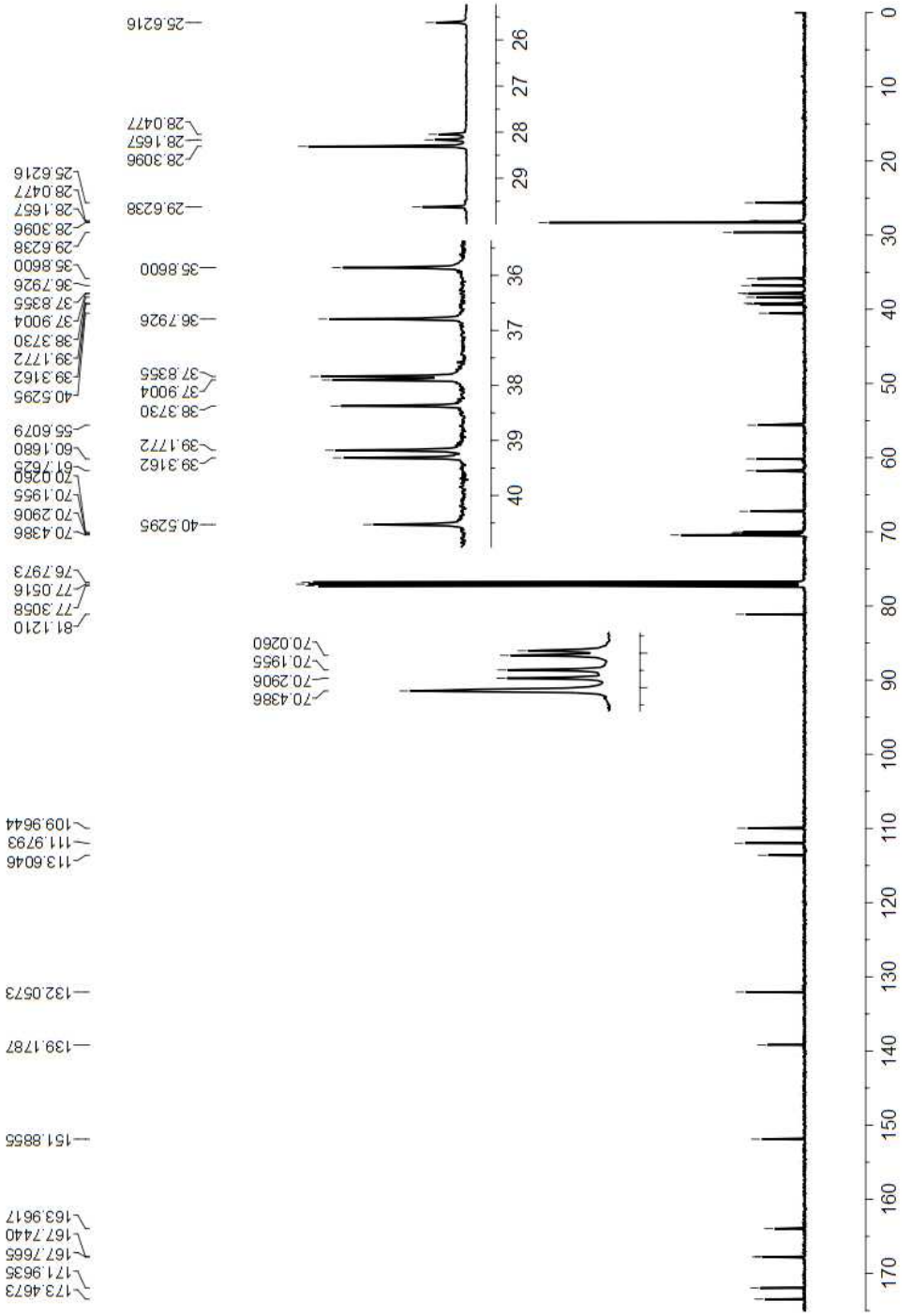
14c



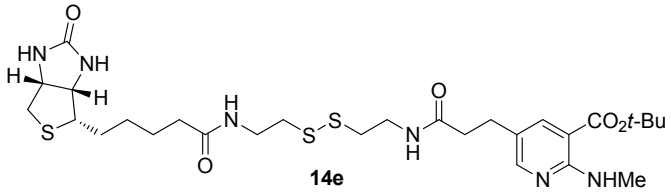
***tert*-Butyl 4-[(2-{[2-(1-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl)carbamoyl]-2-(methylamino)benzoate 14d**



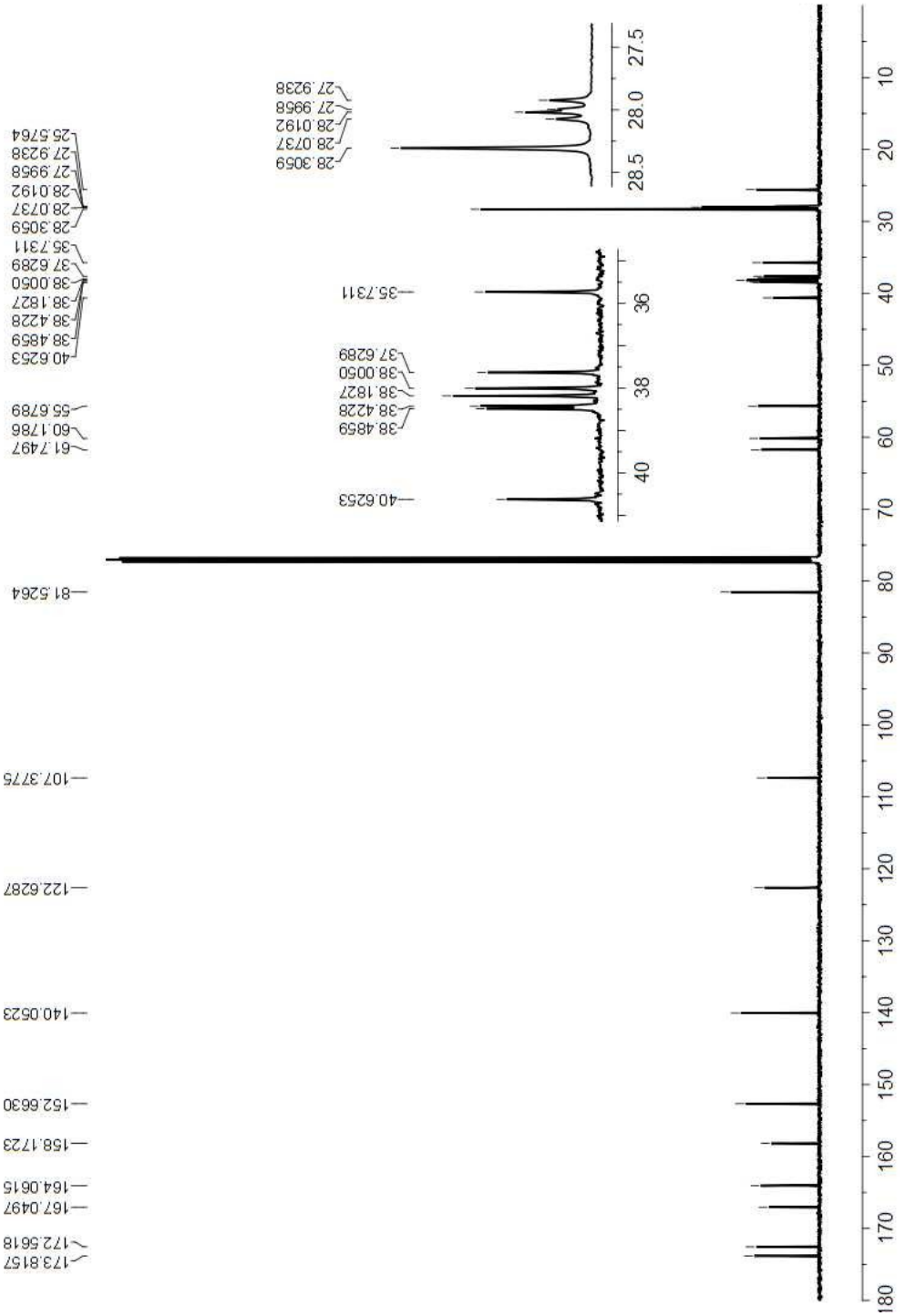
14d



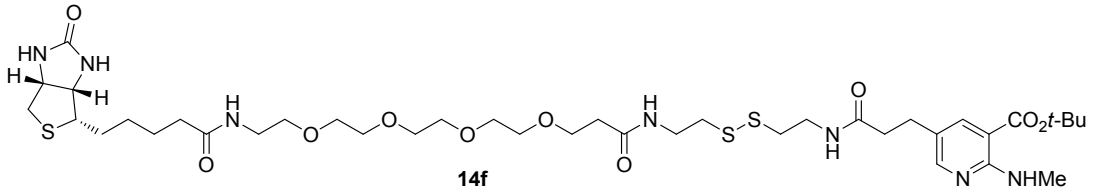
***tert*-Butyl 5-[2-({2-[(2-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl}carbamoyl)ethyl]-2-(methylamino)pyridine-3-carboxylate 14e**

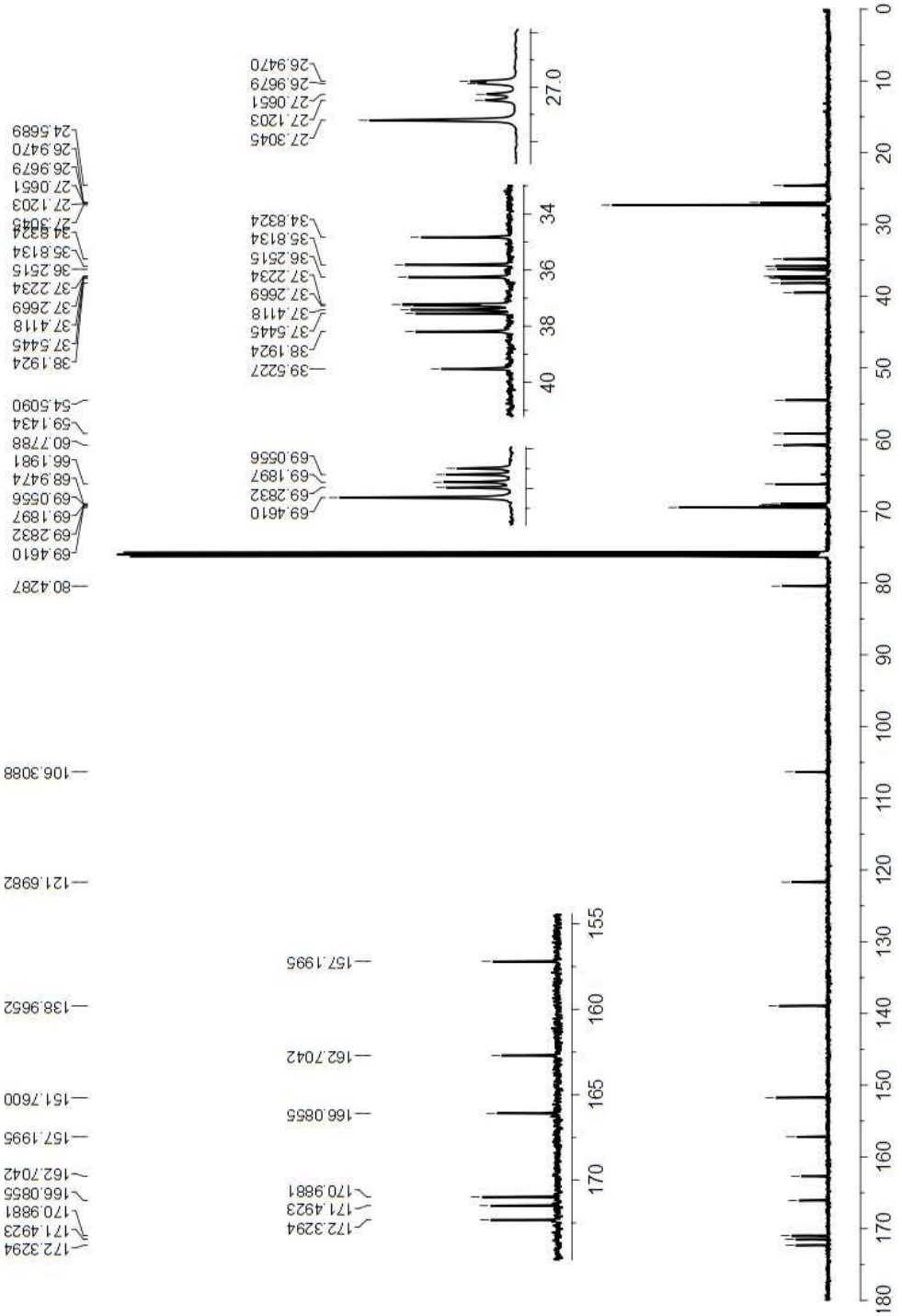


14e

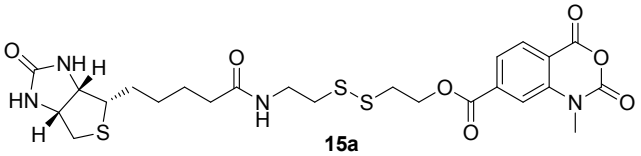


***tert*-Butyl 5-{2-[(2-{[2-(1-{5-[(3aS,6aR)-2-oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl)carbamoyl]ethyl}-2-(methylamino)pyridine-3-carboxylate 14f**

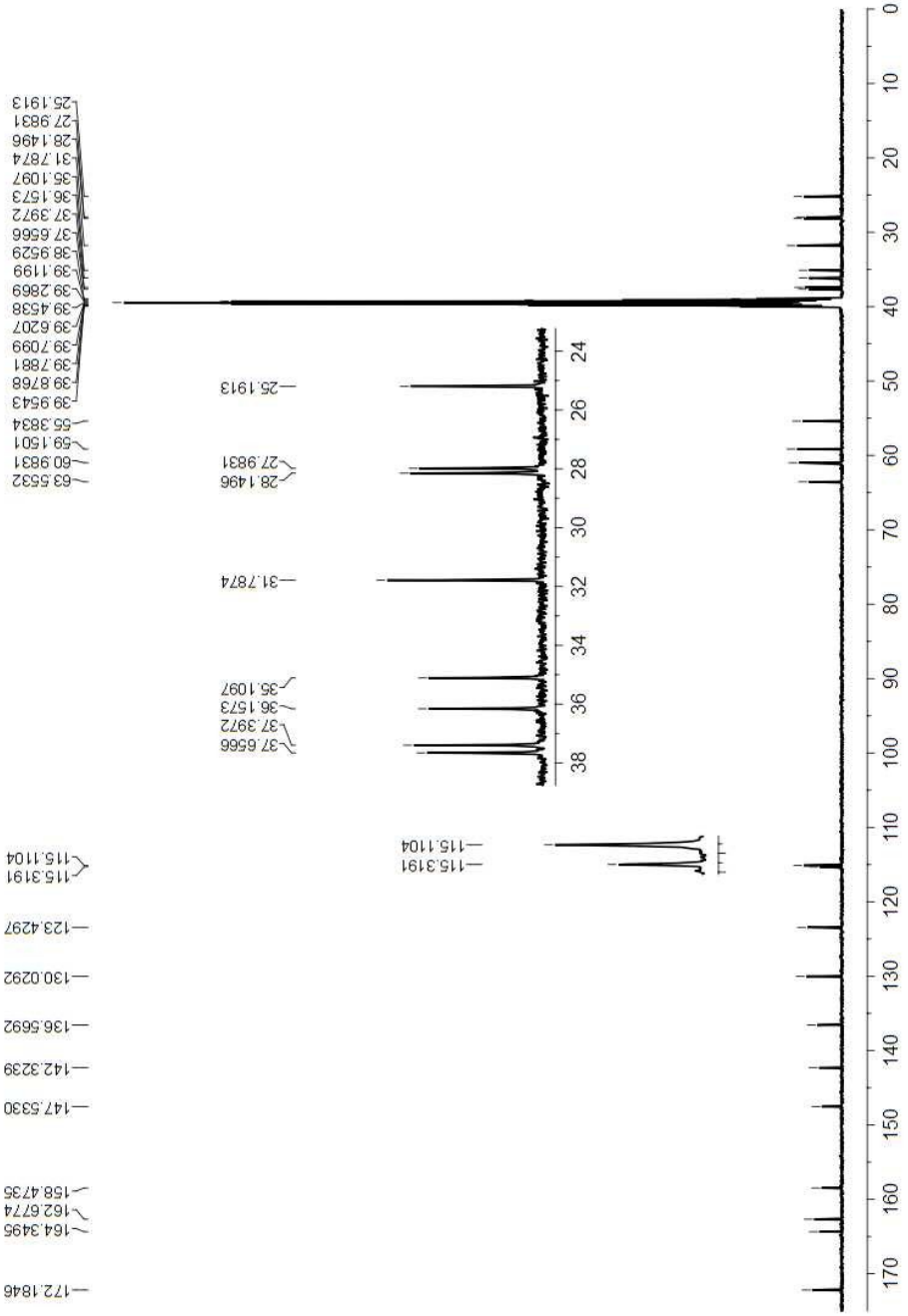




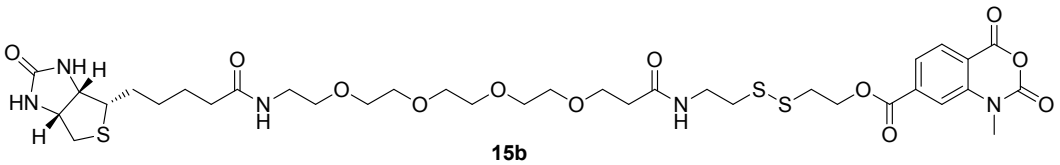
2-[(2-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}ethyl)disulfanyl]ethyl 1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazine-7-carboxylate 15a



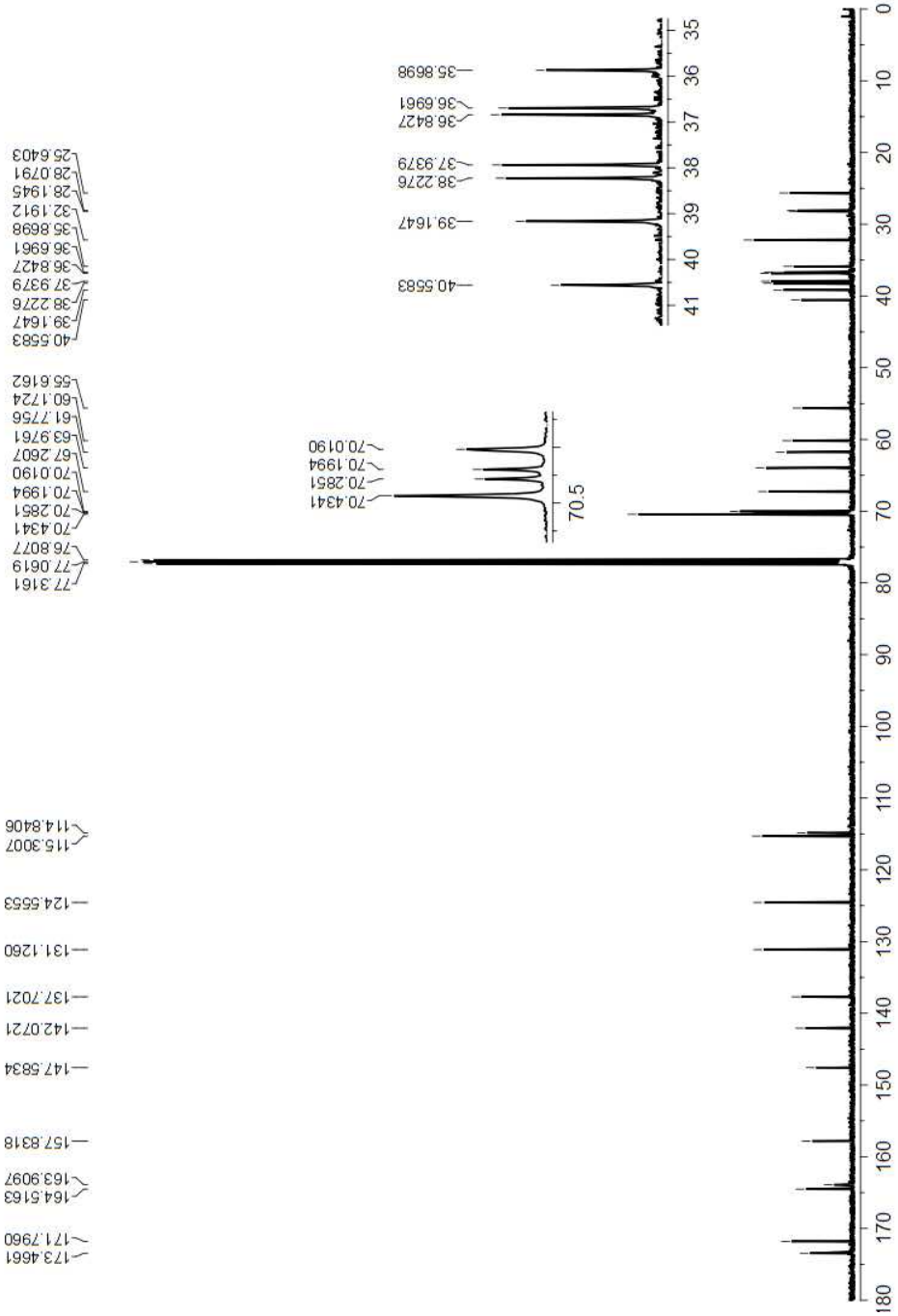
15a



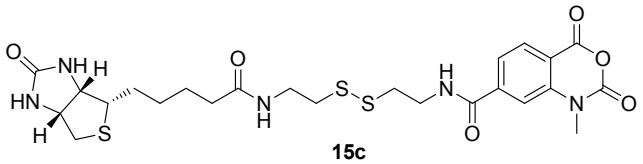
2-{[2-(1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]disulfanyl}ethyl 1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazine-7-carboxylate 15b



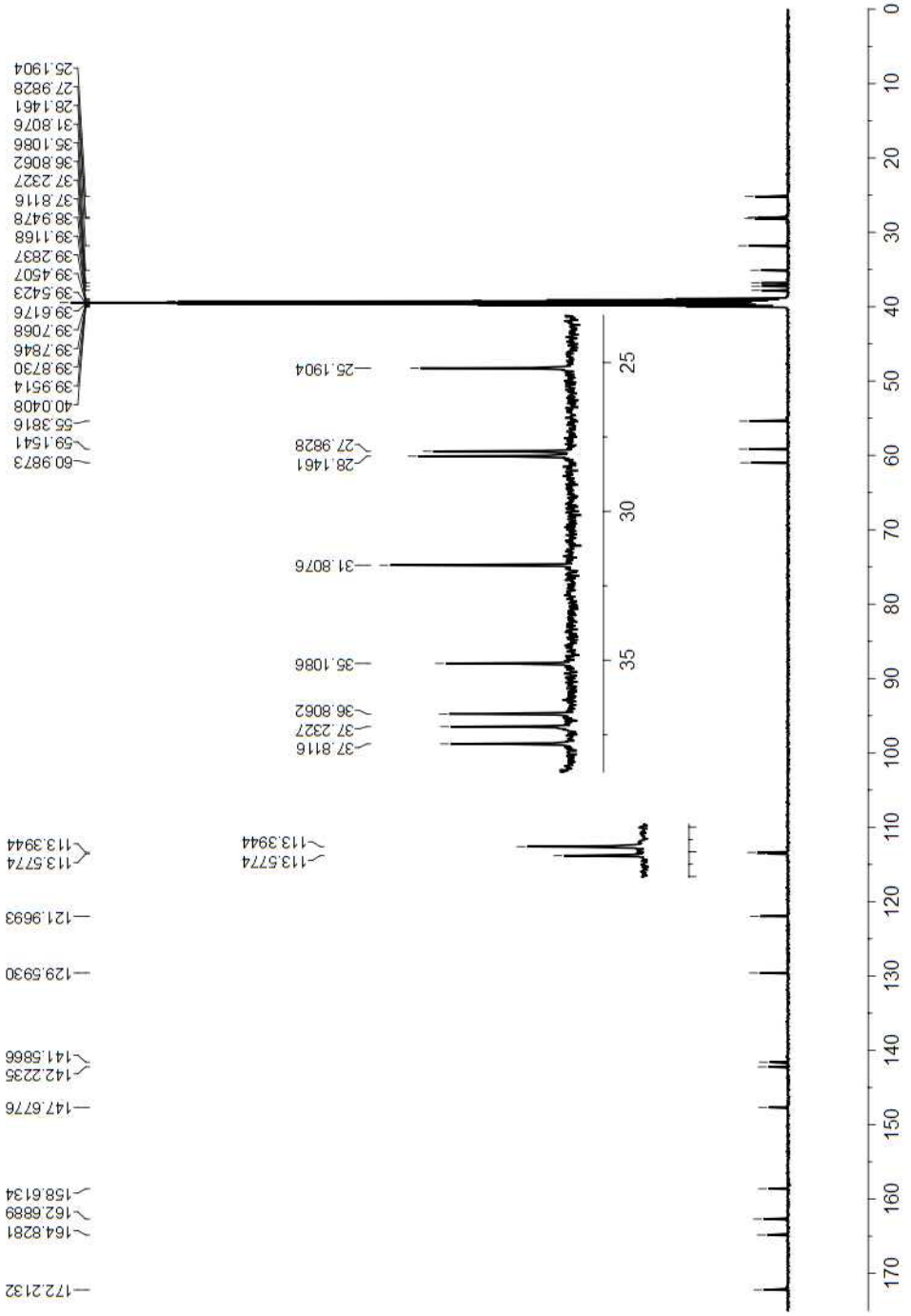
15b



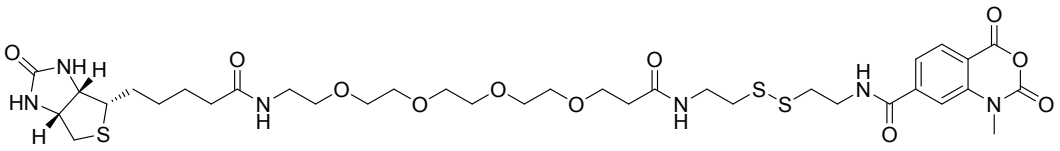
5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]-*N*-[2-({2-[(1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazin-7-yl)formamido]ethyl}disulfanyl)ethyl]pentanamide 15c



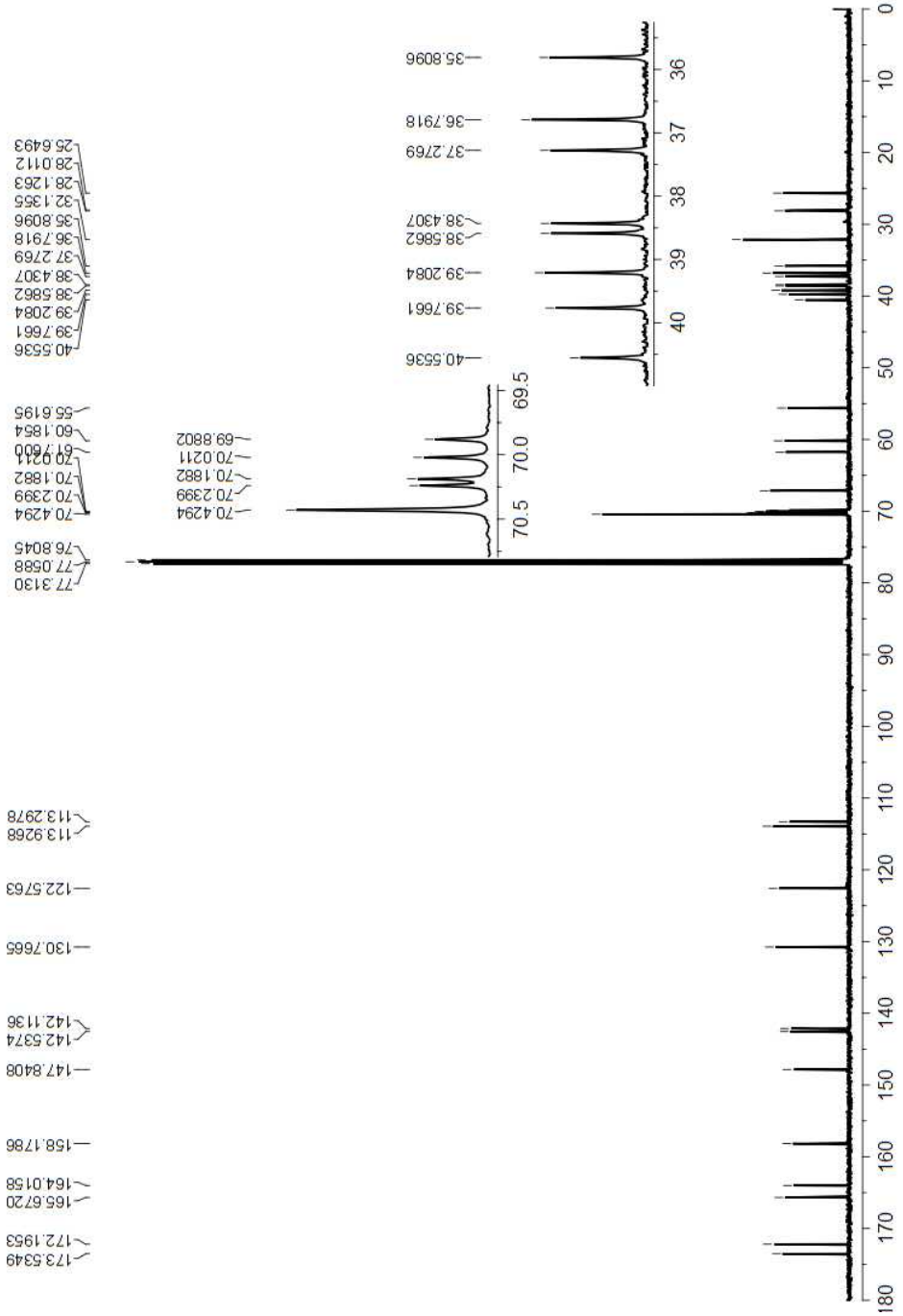
15c



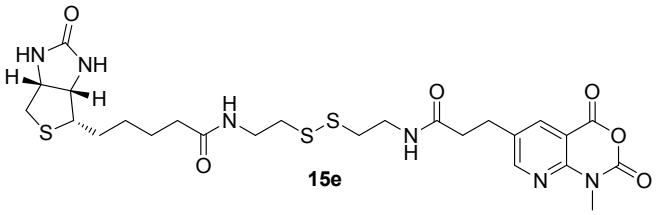
1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-d]imidazolidin-4-yl]pentanamido}-*N*-[2-({2-[(1-methyl-2,4-dioxo-2,4-dihydro-*1H*-3,1-benzoxazin-7-yl)formamido]ethyl}disulfanyl)ethyl]-3,6,9,12-tetraoxapentadecan-15-amide 15d



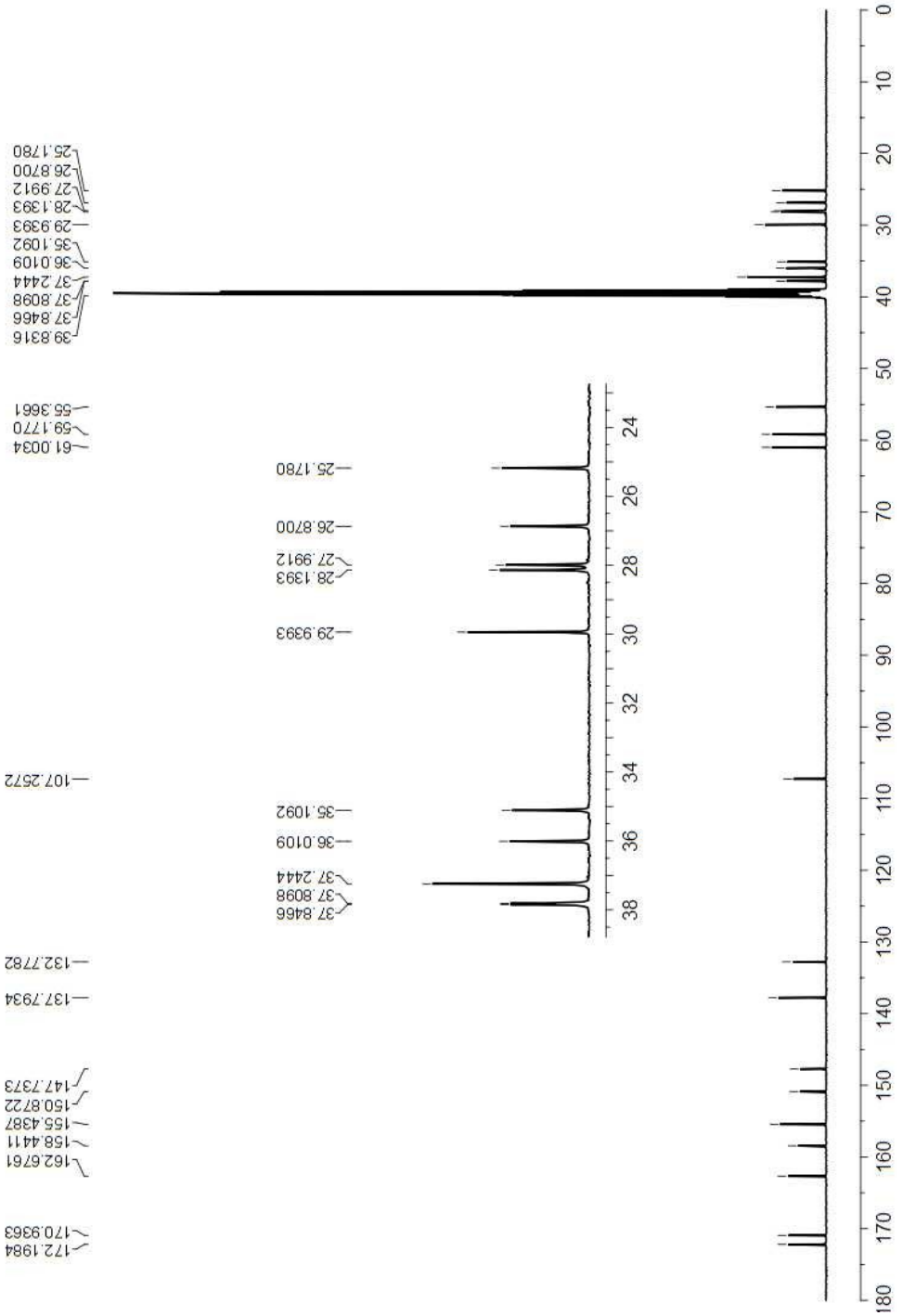
15d



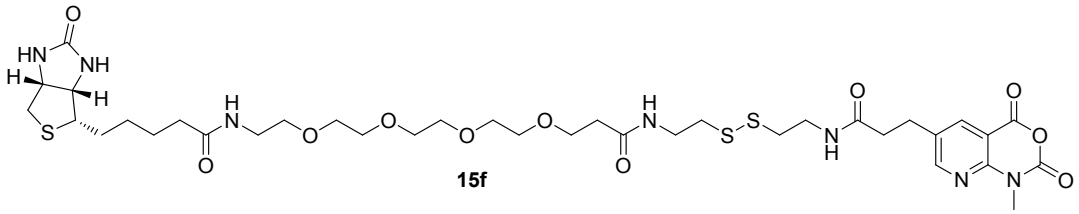
5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]-*N*-(2-{[2-(3-{1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-*d*][1,3]oxazin-6-yl}propanamido)ethyl]disulfanyl}ethyl)pentanamide 15e



15e



1-{5-[(3aS,6aR)-2-Oxo-hexahydro-*1H*-thieno[3,4-*d*]imidazolidin-4-yl]pentanamido}-*N*-(2-{[2-(3-{1-methyl-2,4-dioxo-*1H*,*2H*,*4H*-pyrido[2,3-*d*][1,3]oxazin-6-yl}propanamido)ethyl]disulfanyl}ethyl)-3,6,9,12-tetraoxapentadecan-15-amide 15f



15f