Supporting Information

Building of neomycin-nucleobase-amino acid conjugates for the inhibition of oncogenic miRNAs biogenesis.

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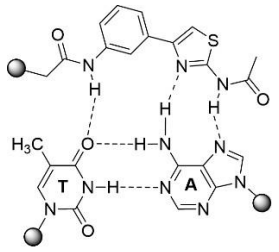
Figure S1. Schematic representation of possible Hoogsteen interactions formed between nucleobase S and T·A base pair.[1]



S.TA[1]

Figure S2. Inhibition curves of compounds **4a-h** (concentrations from 15 nM to 125 µM) in the presence of pre-miR-372 and recombinant Dicer enzyme.

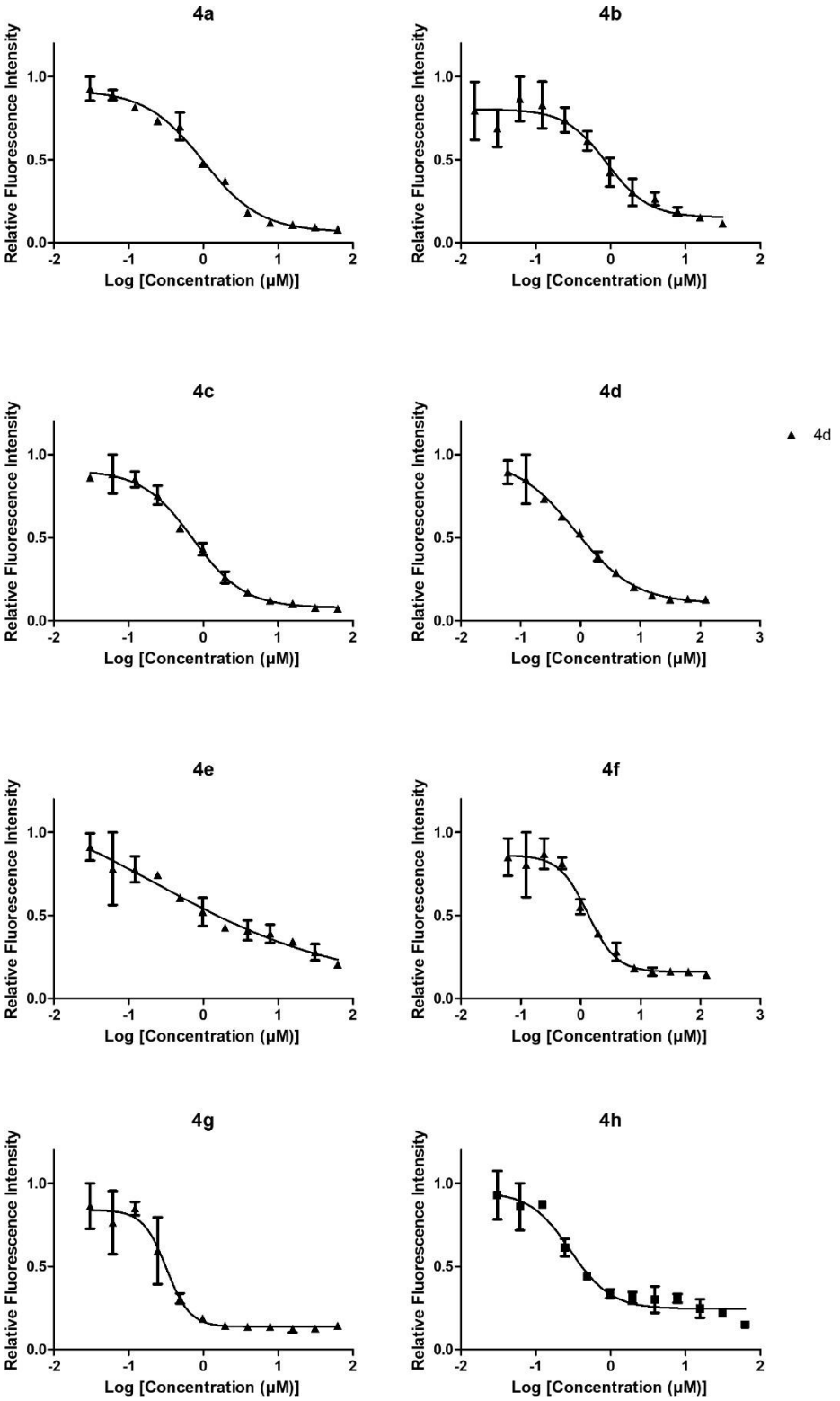


Figure S3. Binding curves of compounds **4a-h** (concentrations from 61 nM to 1 μM) in the presence of pre-miR-372.

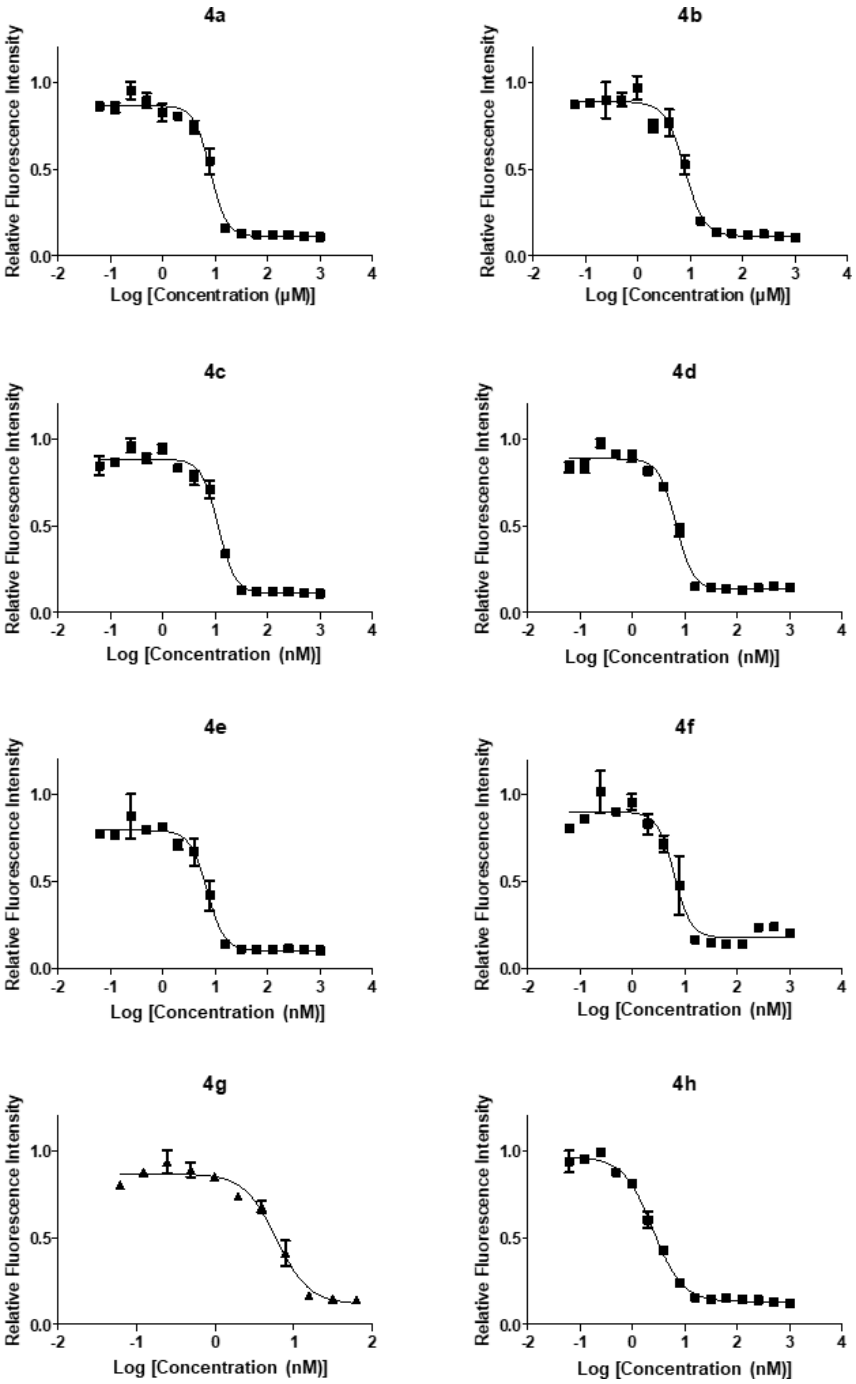


Figure S4. Microscopic observation of gastric adenocarcinoma (AGS) cells without treatment (A), after 48h treatment with 50 µM of compound ,4a (B), 4b (C), 4c (D), 4g (E), 4h (F) or with 25 nM of a scrambled oligonucleotide (G) or of a antimiR-372/373 oligonucleotide (H). Cell layers have been observed in phase contrast on an inverted light microscope (Zeiss) equipped with a 20X objective.

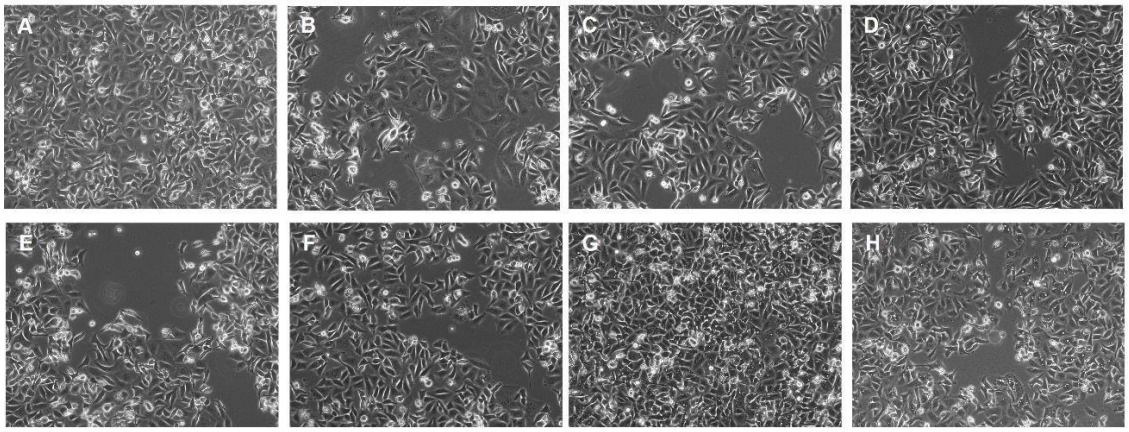


Figure S5. Relative viability of AGS cells that express high miR-372 levels in the presence of increasing concentrations of Neo-S, Neo-S-Ar and 4b. Cell growth was measured using the Cell titer reagent (Promega). Measurements were performed after a treatment of 4 days. Bars represent the mean ± standard deviation (SD) of cell-viability data relative to untreated cells.

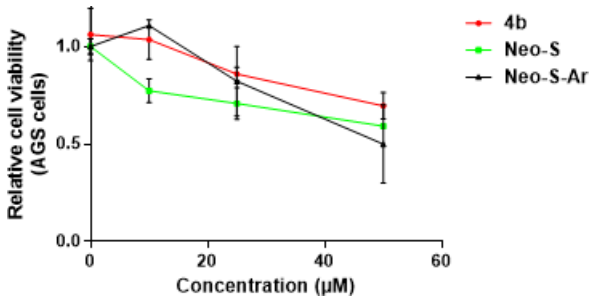


Figure S6. RT-PCR quantification of LATS2 mRNA and pri-miR-371-372-373 after a 4-day treatment by **4b** at the indicated concentrations. Bars represent the mean ± SD of LATS2 or pri-miR-371-373 expressions normalized to the housekeeping genes and compared to untreated cells (n = 4).

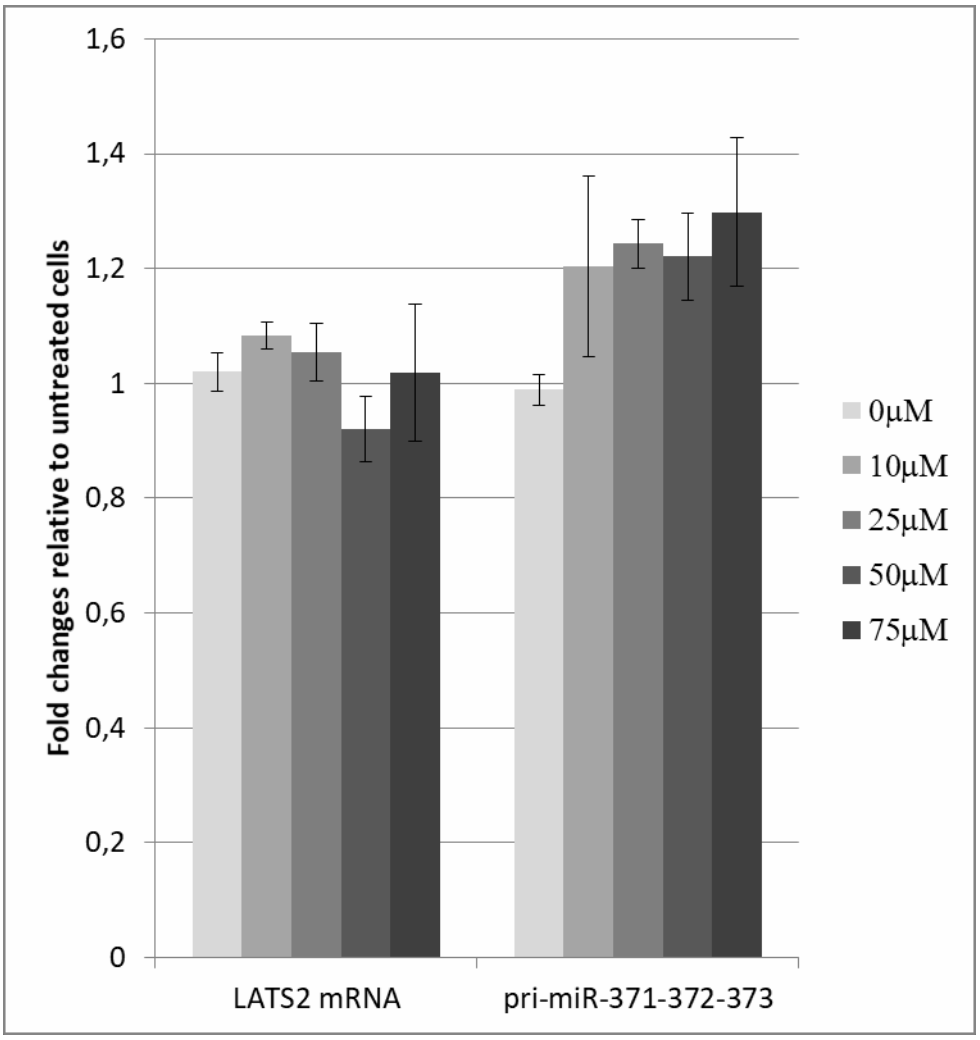


Figure S7. RT-qPCR quantification of miR-371, miR-372, miR-373, miR-17-5p, miR-200b and let-7i after a 4-day treatment of AGS cells in the presence of 50 µM of compound **4b**. Bars represent the mean ± SD of miRNA expression normalized to the small nucleolar RNA RNU49 and compared to untreated cells (n = 4). *** $p < 0.001$, ** $p < 0.01$, *$p < 0.05$ (Student's t test).

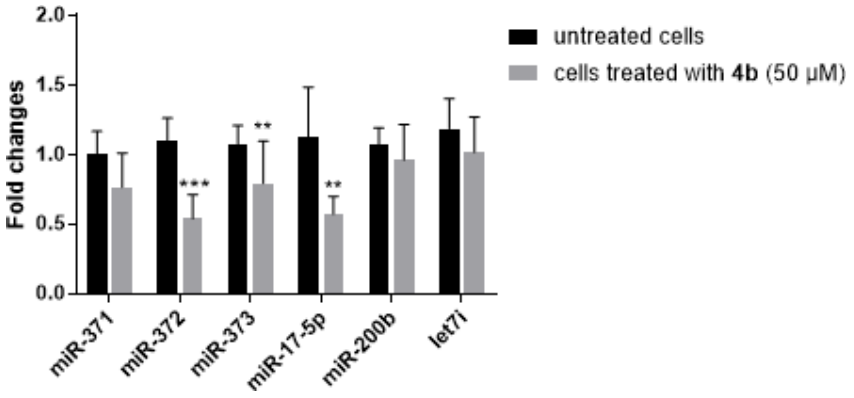


Figure S8. Footprinting analyses. A) Probing of the interaction of pre-miR-372 and **4b** with RNase S1. Lane 1 represents intact RNA; lanes 2 and 3 represent the alkaline-hydrolysis ladder and T1-digestion ladder, respectively; lane 4 represents the cleavage pattern of uncomplexed pre-miR-372 in the presence of 2U of enzyme; lane 5 represents the cleavage patter of uncomplexed pre-miR-372 in the presence of 1U of enzyme; lanes 6-11 represent the cleavage pattern of pre-miR-372 complexed with 0.05, 0.1, 0.5, 1, 5 and 10 mM of **4b** in the presence of 1U of S1 enzyme.

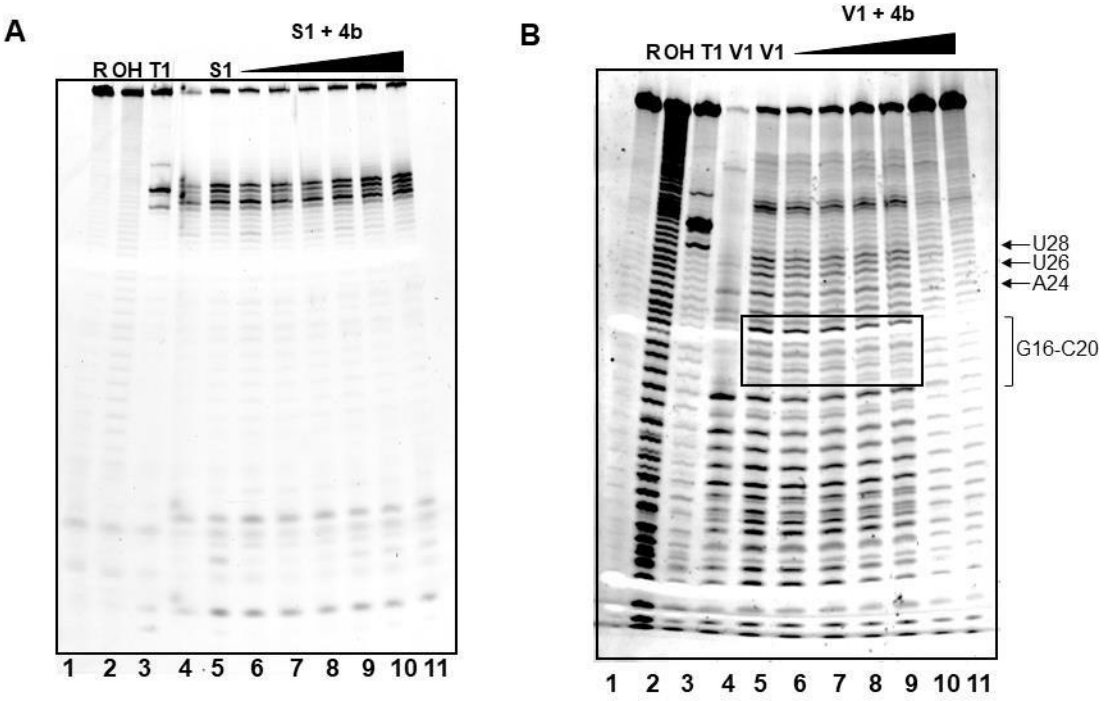


Figure S9. Footprinting analyses. A) Sequence and secondary structure of pre-miR-372. B) Probing of the interaction of pre-miR-372 and **4a** with Dicer enzyme. Lane 1 represents intact RNA; lanes 2 and 3 represent the alkaline-hydrolysis ladder and T1-digestion ladder, respectively; lane 4 represents the cleavage pattern of uncomplexed pre-miR372; lanes 5–9 represent the cleavage pattern of pre-miR-372 complexed with 0.05, 0.1, 0.5, 1, and 5 mM of **4a**.

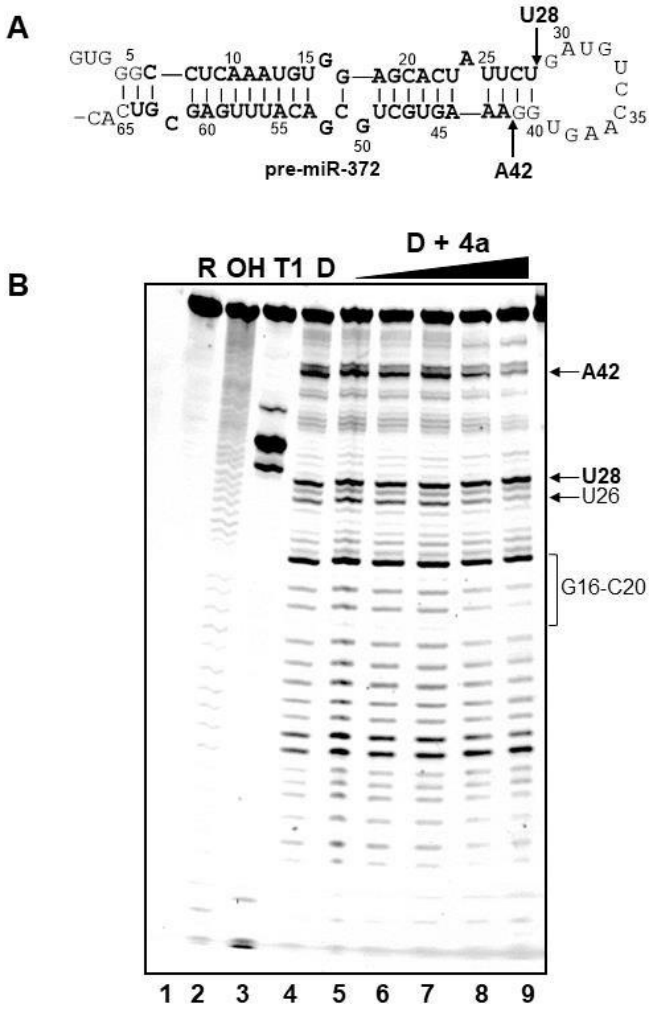


Figure S10. Quantification of Dicer footprinting analyses relative to gels showed in Figure 4 and Figure S7.

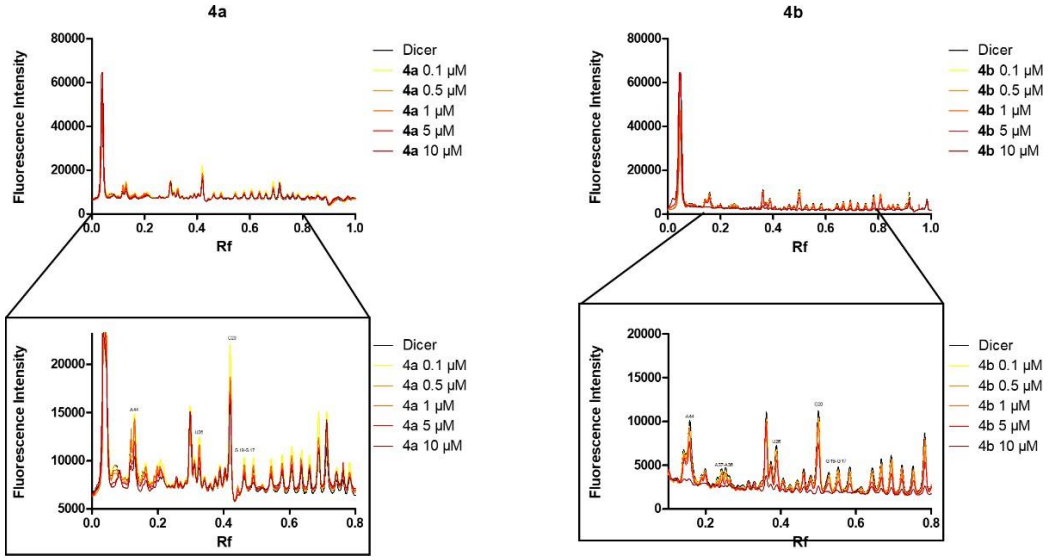
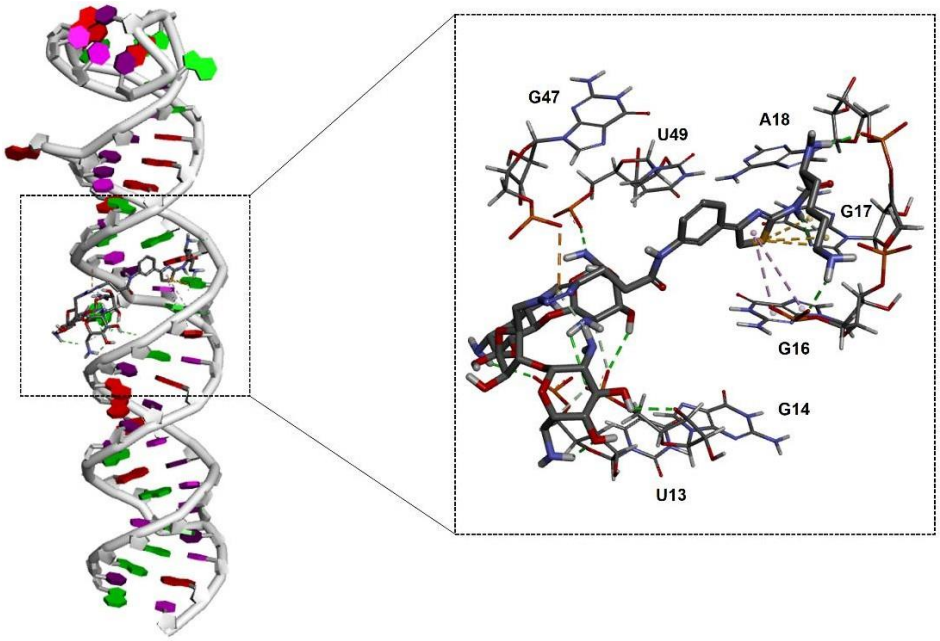
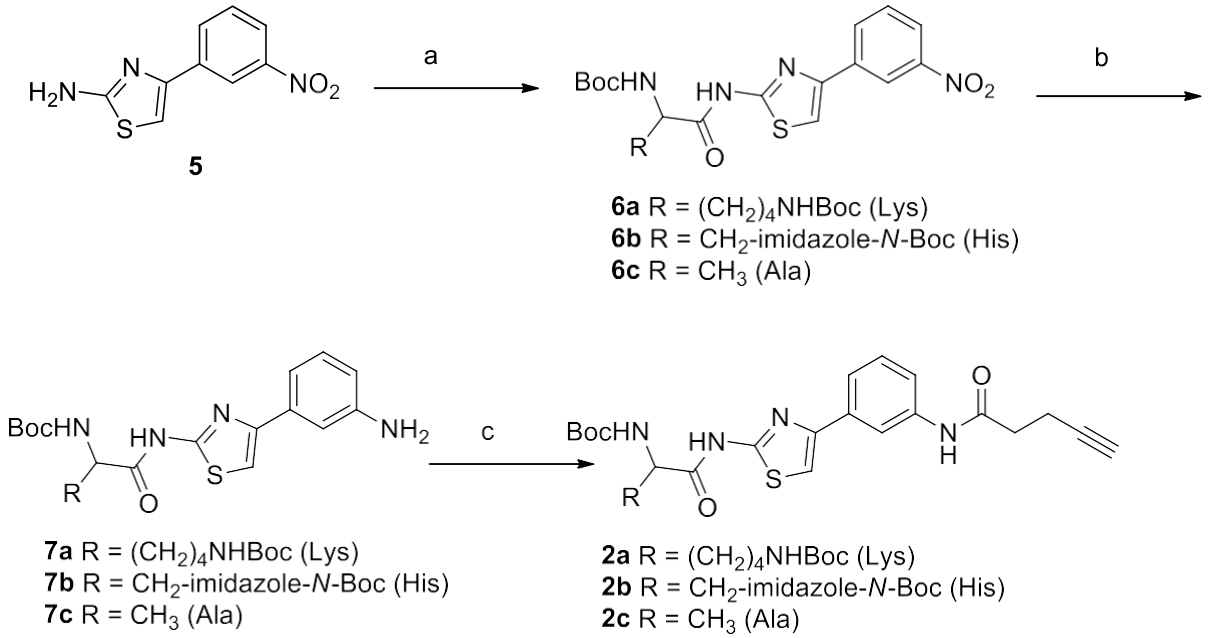


Figure S11. Docking of **4a** with the pre-miR-372 hairpin loop performed by using autodock4, in which the grid boxes were fixed on the entire RNA sequence.



Synthetic Procedures.

Synthesis of amino acids-S nucleobase alkynes.



Scheme S1. Synthesis of alkynes **2a-c**. Reagents: a) Boc-Lys(Boc), Boc-His(Boc) or Boc-Ala, HBTU, DIPEA, DMF, 80°C overnight; b) H_2 Pd/C, DCM, MeOH, r.t., 1h; c) 4-pentynoic acid, Et_3N, chloromethylpyridinium iodide, DCM, r.t., 1h.

4-(3-nitrophenyl)thiazole-2-*N*-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-lysinamide (6a). A mixture of 4-(3-nitrophenyl)thiazol-2-amine[1] (**5**, 500 mg, 2.26 mmol), Boc-Lys(Boc)-OH·DCHA (1.79 g, 3.39 mmol, 1.5 eq.), HBTU (1.29 g, 3.39 mmol, 1.5 eq.), DIPEA (1.18 mL, 6.78 mmol, 3 eq.) in DMF (20 mL) was stirred at 80°C overnight. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on a silica gel column using a mixture DCM/acetone 95:5 as the eluent leading to desired compound **6a** as a yellow solid: 443.5 mg (36%); R_f = 0.25 (DCM/Acetone 95:5); ^{1}H NMR (200MHz, Acetone-d_6) δ (ppm): 11.3 (br, 1H), 8.74 (t, J = 1.9 Hz, 1H), 8.32 (dt, J = 7.8, 1.2 Hz, 1H), 8.16 (ddd, J = 8.2, 2.3, 0.9 Hz, 1H), 7.78 (s, 1H), 7.72 (t, J = 8.0 Hz, 1H), 6.45 (d, J = 7.2 Hz, 1H), 6.00 (t, J = 7.2 Hz, 1H), 4.47 (q, J = 7.2 Hz, 1H), 3.09 (q, J = 6.0 Hz, 2H), 1.99-1.77 (m, 2H), 1.64-1.52 (m, 4H), 1.42 (s, 9H), 1.39 (s, (9H); ^{13}C NMR (50MHz, Acetone-d_6) δ (ppm): 173.5, 160.2, 157.8, 150.7, 149.0, 138.2, 133.4, 131.9, 124.0, 122.3, 112.0, 80.6, 79.4, 56.7, 41.5, 33.3, 29.6, 24.7; mass spectrum (ESI), *m/z* 550.8 $(M+H)^+$ (theoretical 550.2).

4-(3-nitrophenyl)thiazole-2-*N*-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-histidinamide (6b). A solution of 4-(3-nitrophenyl)thiazol-2-amine[1] (5, 500 mg, 2.26 mmol), Boc-His(Boc)-

OH·DCHA (1.82 g, 3.39 mmol, 1.5 eq.), HBTU (1.29 g, 3.39 mmol, 1.5 eq.) and DIPEA (1.18 mL, 6.78 mmol, 3 eq.) in DMF (20 mL) was stirred at 80°C overnight. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on a silica gel column using a mixture DCM/MeOH 9:1 as the eluent leading to compound **6b** as a yellow solid: 204 mg (20%); R_f = 0.73 (Cyclohexane/EtOAc 1:1 + 5% MeOH); ^{1}H NMR (200 MHz, Acetone-d_6) δ 11.60 (s, 1H), 8.69 (s, 1H), 8.28 (d, J = 7.8 Hz, 1H), 8.19-8.06 (m, 2H), 7.73 (s, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.36 (s, 1H), 6.70 (d, J = 7.6 Hz, 1H), 4.82 (m, 1H), 3.34-3.08 (m, 2H), 1.58 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (50 MHz, Acetone-d_6) δ 172.6, 160.1, 157.5, 150.6, 149.0, 148.7, 140.8, 138.8, 138.1, 133.4, 131.8, 123.9, 122.2, 116.7, 112.0, 87.0, 80.8, 56.4, 31.9, 29.5, 28.9; mass spectrum (ESI), *m/z* 459.5 $(M+H\text{-}Boc)^+$ (theoretical 459.2).

4-(3-nitrophenyl)thiazole-2-*N*-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-alaninamide (6c). A mixture of 4-(3-nitrophenyl)thiazol-2-amine[1] (**5**, 500 mg, 2.26 mmol), Boc-Ala-OH (641.5 mg, 3.39 mmol, 1.5 eq.), HBTU (1.29 g, 3.39 mmol, 1.5 eq.) and DIPEA (1.18 mL, 6.78 mmol, 3 eq.) in DMF (20 mL) was stirred at 80°C overnight. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 7:3) as the eluent leading to compound **6c** as a yellow solid: 299 mg (33%); R_f = 0.37 (Cyclohexane/EtOAc 7:3); ^{1}H NMR (200 MHz, Acetone-d_6) δ 11.34 (s, 1H), 8.65 (t, J = 1.9 Hz, 1), 8.30 (dt, J = 7.8, 1.2 Hz, 1H), 8.10 (ddd, J = 8.2, 2.3, 0.9 Hz, 1H), 7.71 (s, 1H), 7.69 (t, J = 8.0 Hz, 1H), 6.46 (d, J = 7.2 Hz, 1H), 4.50 (m, 1H), 1.55 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (50 MHz, Acetone-d_6) δ 160.3, 150.7, 149.0, 138.2, 133.5, 131.9, 124.0, 122.3, 112.0, 80.7, 52.3, 29.5, 18.9; mass spectrum (ESI), *m/z* 415.6 $(M+Na)^+$ (theoretical 415.1).

4-(3-aminophenyl)thiazole-2-*N*-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-lysinamide (7a). Compound **6a** (243.5 mg, 0.44 mmol) was dissolved in a mixture of CH_2Cl_2 and MeOH (1:1, v/v, 20 mL) and was stirred under an hydrogen atmosphere in the presence of 10% palladium on activated carbon (54.2 mg) for 1 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure leading the desired compound **7a** as a yellow solid: 109.4 mg (47%). R_f = 0.5 (1:1 Cyclohexane/EtOAc); ^{1}H NMR (200MHz, Acetone-d_6) δ (ppm): 7.31 (s, 1H), 7.20-7.00 (m, 3H), 6.60-6.55 (m, 1H), 4.46-4.39 (m, 1H), 3.10-3.00 (m, 2H), 1.60-1.50 (m, 4H), 1.41 (s, 9H), 1.39 (s, 9H), 1.25-1.30 (m, 2H); ^{13}C NMR (50MHz, Acetone-d_6) δ (ppm): 158.1, 156.8, 153.2, 151.3, 150.6, 149.4, 136.2, 130.0, 121.2, 119.6, 117.7, 114.8, 107.8, 79.6, 78.4, 55.6, 40.5, 32.3, 27.5, 23.7; mass spectrum (ESI), *m/z* 520.8 $(M+H)^+$ (theoretical 520.2).

4-(3-aminophenyl)thiazole-2-*N*-[*Nα*,*N*ε-(di-tert-butoxycarbonyl)]-histidinamide (7b). A solution of compound **6b** (79 mg, 0.14 mmol) in a mixture of CH_2Cl_2 and methanol (1:1, v/v, 20 mL) was stirred under an hydrogen atmosphere in the presence of 10% palladium on activated carbon (34.5 mg) for 1 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure to give compound **7b** as a yellow solid: 73.6 mg (98%); R_f = 0.1 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (500MHz, CD_3OD) δ (ppm): 8.14 (s, 1H), 7.37-7.18 (m, 4H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.68 (d, *J* = 7.3 Hz, 1H), 4.65-4.39 (m, 1H), 3.16-2.92 (m, 2H), 1.59 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (125MHz, CD_3OD) δ (ppm): 172.1, 158.9, 157.8, 151.8, 149.0, 148.1, 139.6, 138.5, 136.6, 117.3, 116.4, 114.2, 108.5, 87.2, 81.0, 55.6, 45.1, 28.7, 28.0, 27.3, 20.9. mass spectrum (ESI), *m/z* 529.1 $(M+H)^+$ (theoretical 529.2).

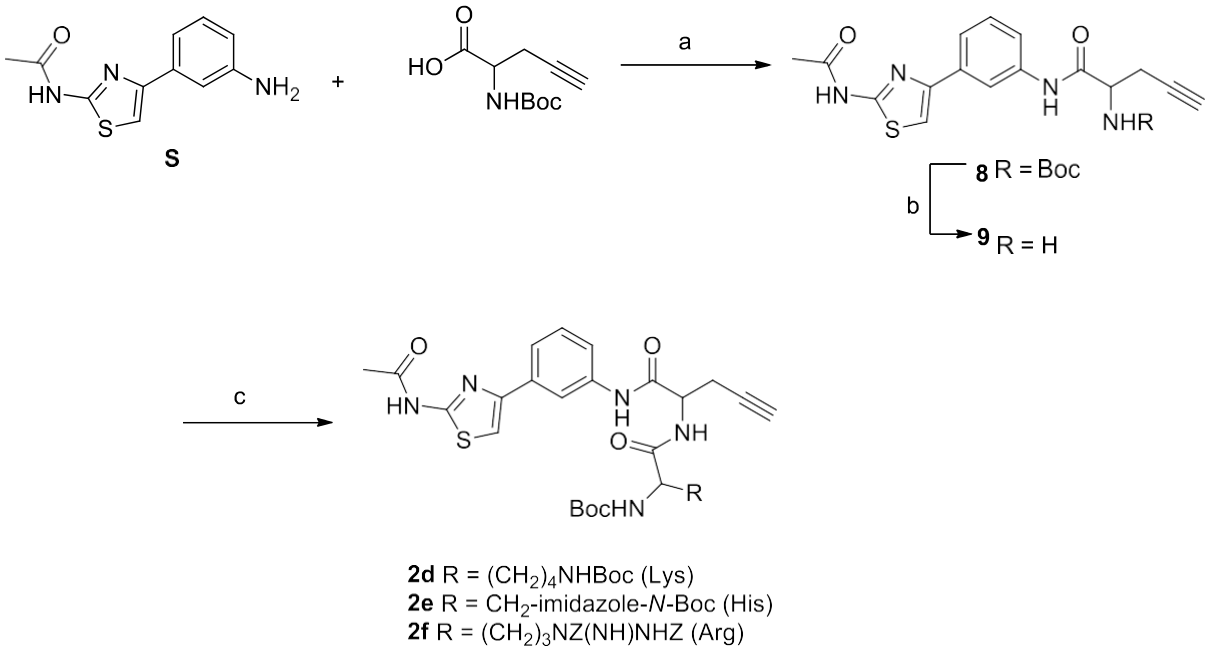
4-(3-aminophenyl)thiazole-2-*N*-[*Nα*,*N*ε-(di-tert-butoxycarbonyl)]-alaninamide (7c). A solution of compound **6c** (151 mg, 0.385 mmol) in a mixture of DCM and MeOH (1:1, v/v, 20 mL) was stirred under a hydrogen atmosphere in the presence of 10% palladium on activated carbon (47 mg) for 1 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure to give the product as a yellow solid: 130 mg (93%); R_f = 0.5 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (200MHz, Acetone-d_6) δ (ppm): 7.55-7.50 (m, 1H), 7.25-7.00 (m, 3H), 6.70-6.60 (m, 1H), 4.55-4.50 (m, 1H), 1.47 (d, J = 6.0 Hz, 3H), 1.41 (s, 9H); ^{13}C NMR (50MHz, Acetone-d_6) δ (ppm): 172.5, 158.1, 156.4, 149.4, 145.3, 135.4, 130.0, 121.7, 115.6, 114.9, 107.8, 79.7, 51.0, 25.7, 18.0; mass spectrum (ESI), *m/z* 385.5 $(M+Na)^+$ (theoretical 385.1).

4-[(3-pentynoylamidophenyl)thiazole-2-*N*-[*Nα*,*N*ε-(di-tert-butoxycarbonyl)]-lysinamide (2a). 4-Pentynoic acid (20.8 mg, 0.21 mmol, 1.1 eq.) was dissolved in dry dichloromethane (20 mL) and then triethylamine (80 µL, 0.38 mmol, 2 eq.), chloromethylpyridinium iodide (98.6 mg, 0.38 mmol, 2 eq.) and compound **7a** (100 mg, 0.19 mmol) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture Cyclohexane/EtOAc 1:1 as the eluent leading to desired compound **2a** as a slightly yellow solid: yield 57 mg (50%); R_f = 0.29 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (200 MHz, Acetone-d_6) δ 11.22 (br, 1H), 9.26 (br, 1H), 8.38 (s, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.42 (s, 1H), 7.31 (t, *J* = 7.9 Hz, 1H), 6.40 (d, *J* = 7.4 Hz, 1H), 5.99 (br, 1H), 4.44 (m, 1H), 3.09 (m, 2H), 2.71-2.46 (m, 4H), 2.39 (t, *J* = 2.3 Hz, 1H), 1.98-1.70 (m, 2H), 1.60-1.38 (m, 22H); ^{13}C NMR (50 MHz, Acetone-d_6) δ 173.3, 171.1, 170.2, 159.5, 157.8, 151.5,

141.6, 137.2, 130.8, 122.8, 120.4, 118.9, 109.6, 85.0, 80.6, 79.4, 71.3, 56.7, 41.6, 37.6, 33.4, 29.6, 28.5, 24.7, 16.0; mass spectrum (ESI), *m/z* 601.0 $(M+H)^+$ (theoretical 601.3).

4-(3-pentynoylamidophenyl)thiazole-2-*N*-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-histidinamide 2b. 4-Pentynoic acid (10.7 mg, 0.11 mmol, 1.1 eq.) was dissolved in dry DCM (15 mL) and then triethylamine (46 µL, 0.20 mmol, 2 eq.), chloromethylpyridinium iodide (56.2 mg, 0.20 mmol, 2 eq.) and compound **7b** (63.6 mg, 0.10 mmol) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 1:1 as the eluent leading to desired compound **2b** as a slightly yellow solid: yield 31.6 mg (47%); R_f = 0.24 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (500 MHz, CD_3OD) δ 8.24-8.09 (m, 2H), 7.63 (d, J = 7.8 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.39-7.31 (m, 3H), 4.64-4.52 (m, 1H), 3.13-2.89 (m, 2H), 2.64-2.42 (m, 4H), 2.29 (t, J = 2.5 Hz, 1H), 1.59 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.4, 172.8, 172.4, 159.2, 157.8, 151.8, 148.6, 140.2, 136.6, 130.1, 123.0, 120.8, 119.0, 109.2, 87.2, 83.6, 70.0, 36.9, 28.7, 28.0, 27.3, 15.6; mass spectrum (ESI), *m/z* 610.0 $(M+H)^+$ (theoretical 610.2).

4-(3-pentynoylamidophenyl)thiazole-2-*N*-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-alaninamide (2c). 4-Pentynoic acid (29.4 mg, 0.36 mmol, 1.1 eq.) was dissolved in dry DCM (30 mL) and then triethylamine (0.12 mL, 0.66 mmol, 2 eq.), chloromethylpyridinium iodide (153.5 mg, 0.66 mmol, 2 eq.) and compound **7c** (120 mg, 0.33 mmol) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 5:5 as the eluent leading to desired compound **2c** as a slightly yellow solid: yield 85 mg (58%); R_f = 0.6 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (500 MHz, Acetone-d_6) δ 11.14 (br, 1H), 9.27 (br, 1H), 8.35 (s, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 7.6 Hz, 1H), 7.40 (s, 1H), 7.31 (d, J = 7.9 Hz, 1H), 6.45 (d, J = 5.5 Hz, 1H), 4.52 (t, J = 6.4 Hz, 1H), 2.67-2.56 (m, 4H), 2.37 (t, J = 2.6 Hz, 1H), 1.48 (d, J = 7.1 Hz, 3H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 173.6, 171.2, 159.5, 157.4, 151.4, 141.5, 137.1, 130.7, 122.8, 120.5, 118.9, 109.6, 84.5, 80.7, 71.3, 52.2, 37.6, 28.4, 19.0, 16.0; mass spectrum (ESI), *m/z* 443.6 $(M+H)^+$ (theoretical 443.2).



Scheme S2. Synthesis of alkynes **2d-f**. Reagents: a) Chloromethylpyridinium iodide, Et_3N, DCM Δ, 1h; b) TFA, DCM, r.t., overnight; c) Boc-Lys(Boc), Boc-His(Boc) or , HBTU, DIPEA, DCM, r.t., overnight.

***Nα*-(*tert*-butoxycarbonyl)-[*N*-(2-*N*-acetylamino-4-(3-aminophenyl)-thiazole)]-propargylglycinamide (8).** Boc-L-propargylglycine (220 mg, 1.03 mmol) was dissolved in dry dichloromethane (55 mL) and then triethylamine (0.43 mL, 2.06 mmol, 2 eq.), chloromethylpyridinium iodide (528 mg, 2.06 mmol, 2 eq.) and compound **S**[1] (265 mg, 1.13 mmol, 1.1 eq.) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 1:1 leading to desired compound **8** as a slightly yellow solid: yield 353.5 mg (80%); R_f = 0.5 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (200 MHz, Acetone-d_6) δ 11.16 (br, 1H), 9.39 (br, 1H), 8.36 (s, 1H), 7.62 (dt, J = 7.8, 1.2 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.39 (s, 1H), 7.33 (t, J = 7.9 Hz, 1H), 6.38 (d, J = 7.9 Hz, 1H), 4.46 (q, J = 7.0 Hz, 1H), 2.77 (td, J = 6.1, 2.6 Hz, 2H), 2.45 (t, J = 2.6 Hz, 1H), 2.29 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (50 MHz, Acetone-d_6) δ 170.8, 170.1, 159.8, 151.1, 141.0, 137.3, 130.8, 123.2, 120.8, 119.3, 109.4, 81.7, 80.9, 73.4, 56.0, 29.5, 25.5, 23.8; mass spectrum (ESI), *m/z* 451.6 $(M+Na)^+$ (theoretical 451.2).

***N*-[2-*N*-acetylamino-4-(3-aminophenyl)-thiazole]-propargylglycinamide (9).** To a solution of **8** (313.5 mg, 0.73 mmol) in DCM (5 mL) was added TFA (0.56 mL, 7.3 mmol, 10 eq.). The reaction was stirred at room temperature overnight. The solvent was then removed under

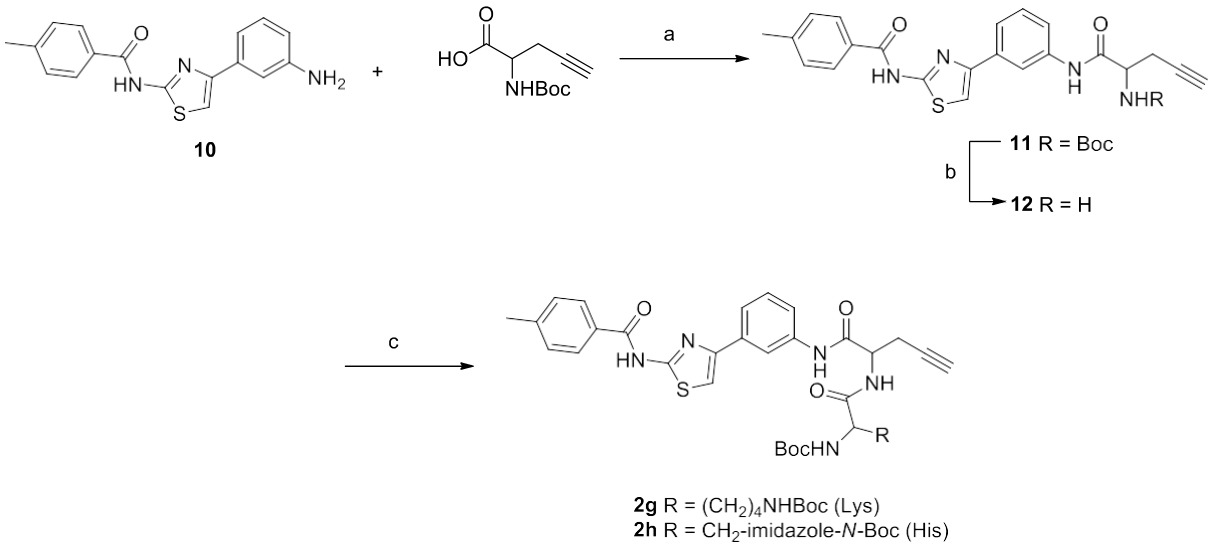
reduced pressure and the crude product was employed in the next step without further purification; mass spectrum (ESI) *m/z* 329.3 $(M+H)^+$ (theoretical 329.1).

***N*-[2-*N*-acetylamino-4-(3-aminophenyl)-thiazole]-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-lysyl-glycinamide (2d).** A mixture of **9** (50 mg, 0.113 mmol), Boc-Lys(Boc)-OH·DCHA (59.6 mg, 0.113 mmol, 1 eq.), HBTU (42.9 mg, 0.113 mmol, 1 eq.) and DIPEA (59 µL, 0.339 mmol, 3 eq.) in DCM (13 mL) was stirred at room temperature overnight. Solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to desired compound **2d** as a yellow solid: 64 mg (86%); R_f = 0.15 (Cyclohexane/EA 1/1); ^{1}H NMR (500 MHz, Acetone-d_6) δ 11.14 (br, 1H), 9.24 (br, 1H), 8.38 (s, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.37 (s, 1H), 7.33 (t, J = 7.9 Hz, 1H), 6.58 (br, 1H), 5.99 (br, 1H), 4.67 (q, J = 6.4 Hz, 1H), 4.11-4.02 (m, 1H), 3.04-2.97 (m, 2H), 2.90-2.80 (m, 2H), 2.53 (t, J = 2.6 Hz, 1H), 2.28 (s, 3H), 1.90-1.72 (m, 2H), 1.57-1.33 (m, 22H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 174.6, 170.4, 170.2, 159.8, 158.1, 151.2, 140.9, 137.2, 130.7, 123.3, 121.0, 119.3, 109.3, 81.6, 81.3, 79.6, 73.4, 57.9, 54.5, 41.4, 39.7, 29.7, 29.6, 28.5, 24.5, 23.8, 22.9; mass spectrum (ESI), *m/z* 658.1 $(M+H)^+$ (theoretical 658.3).

***N*-[2-*N*-acetylamino-4-(3-aminophenyl)-thiazole]-[*Nα*,*N*$_{\text{imidazole}}$-(di-tert-butoxycarbonyl)]-histidyl-glycinamide (2e).** A mixture of **9** (50 mg, 0.113 mmol), Boc-His(Boc)-OH·DCHA (60.6 mg, 0.113 mmol, 1 eq.), HBTU (42.9 mg, 0.113 mmol, 1 eq.) and DIPEA (59 µL, 0.339 mmol, 3 eq.) in DCM (13 mL) was stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound **2e** as a yellow solid: 71 mg (94%); R_f = 0.18 (Cyclohexane/EtOAc 4:6); ^{1}H NMR (500 MHz, Acetone-d_6) δ 11.22; 11.17 (br, 1H), 9.83; 9.57 (br, 1H), 8.36; 8.32 (s, 1H), 8.13 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.40-7.06 (m, 3H), 6.65; 6.49 (br, 1H), 4.70 (m, 1H), 4.50-4.34 (m, 1H), 3.18-3.01 (m, 2H), 2.81 (m, 2H), 2.47; 2.44 (t, J = 2.6 Hz, 1H), 2.28 (s, 3H), 1.59; 1.40-1.33 (s, 18H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 172.9, 169.9, 169.8, 169.6, 159.3, 157.2, 150.6, 150.5, 148.3, 140.4, 140.2, 130.2, 130.1, 122.7, 120.8, 119.2, 108.7, 86.7, 80.9, 80.6, 72.9, 56.2, 53.9, 39.2, 28.9, 28.3, 23.3, 22.5; mass spectrum (ESI), *m/z* 667.1 $(M+H)^+$ (theoretical 667.3).

***N*-[2-*N*-acetylamino-4-(3-aminophenyl)-thiazole]-[*Nα*-(tert-butoxycarbonyl)-*N*,*N*-(di-benzyloxycarbonylguanidino]-arginyl-glycinamide (2f).** A mixture of **9** (50 mg, 0.113 mmol), di-Z-Arg(Boc)-OH (61.3 mg, 0.113 mmol, 1 eq.), HBTU (42.9 mg, 0.113 mmol, 1 eq.), DIPEA (59 µL, 0.339 mmol, 3 eq.) in DCM (13 mL) was stirred at room temperature overnight.

Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound **2f** as a yellow solid: 87.5 mg (91%); R_f = 0.29 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (500 MHz, Acetone-d_6) δ 11.15 (br, 1H), 9.46 (br, 2H), 9.26 (br, 1H), 8.37 (s, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.65-7.57 (m, 2H), 7.50-7.22 (m, 13H), 6.50 (d, J = 5.1 Hz, 1H), 5.28 (s, 2H), 5.12 (s, 2H), 4.66 (q, J = 6.6 Hz, 1H), 4.21-3.97 (m, 3H), 2.81-2.77 (m, 2H), 2.45 (t, J = 2.6 Hz, 1H), 2.28 (s, 3H), 1.86-1.68 (m, 4H), 1.38 (m, 9H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 174.1, 170.2, 170.1, 165.5, 162.5, 159.8, 158.2, 157.6, 151.2, 140.9, 139.4, 130.7, 130.5, 130.3, 130.2, 130.1, 129.7, 129.5, 123.2, 120.9, 119.3, 109.3, 81.6, 81.0, 73.6, 70.4, 68.2, 57.3, 54.6, 46.0, 39.7, 29.6, 27.1, 23.8, 23.0; mass spectrum (ESI), m/z 854.6 $(M+H)^+$ (theoretical 854.3).



Scheme S3. Synthesis of alkynes **2g-h**. Reagents: a) Chloromethylpyridinium iodide, Et_3N, DCM, Δ, 1h; b) TFA, DCM, r.t., overnight; c) Boc-Lys(Boc) or Boc-His(Boc), HBTU, DIPEA, DCM, r.t., overnight.

***Nα*-(*tert*-butoxycarbonyl)-[*N*-(2-*N*-toluoylamino-4-(3-aminophenyl)-thiazole)]-propargylglycinamide (11).** Boc-L-propargylglycine (100 mg, 0.47 mmol) was dissolved in dry DCM (25 mL) and triethylamine (0.2 mL, 0.94 mmol, 2 eq.), chloromethylpyridinium iodide (240 mg, 0.94 mmol, 2 eq.) and compound **10**[2] (160 mg, 0.52 mmol, 1.1 eq.) were added successively. The reaction mixture was stirred under reflux 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 7:3 as the eluent leading to desired compound **11** as a slightly yellow solid: yield 177.6 mg (75%); R_f = 0.34 (cyclohexane/ethyl acetate 7/3); ^{1}H

NMR (200 MHz, Acetone-d_6) δ 11.54 (br, 1H), 9.41 (br, 1H), 8.42 (s, 1H), 8.12 (d, J = 8.3 Hz, 2H), 7.66 (dt, J = 7.7, 1.2 Hz, 1H), 7.54-7.48 (m, 1H), 7.48 (s, 1H), 7.41-7.31 (m, 3H), 6.40 (d, J = 7.9 Hz, 1H), 4.46 (q, J = 7.0 Hz, 1H), 2.77 (td, J = 6.1, 2.6 Hz, 2H), 2.51 (t, J = 2.6 Hz, 1H), 2.44 (s, 3H), 1.44 (s, 9H); ^{13}C NMR (50 MHz, Acetone-d_6) δ 170.9, 166.8, 160.3, 157.3, 151.5, 145.1, 141.0, 137.4, 131.6, 131.2, 130.8, 129.9, 123.2, 120.8, 119.4, 109.9, 81.7, 73.4, 73.3, 56.1, 29.5, 23.8, 22.5; mass spectrum (ESI), *m/z* 505.7 $(M+H)^+$ (theoretical 505.3).

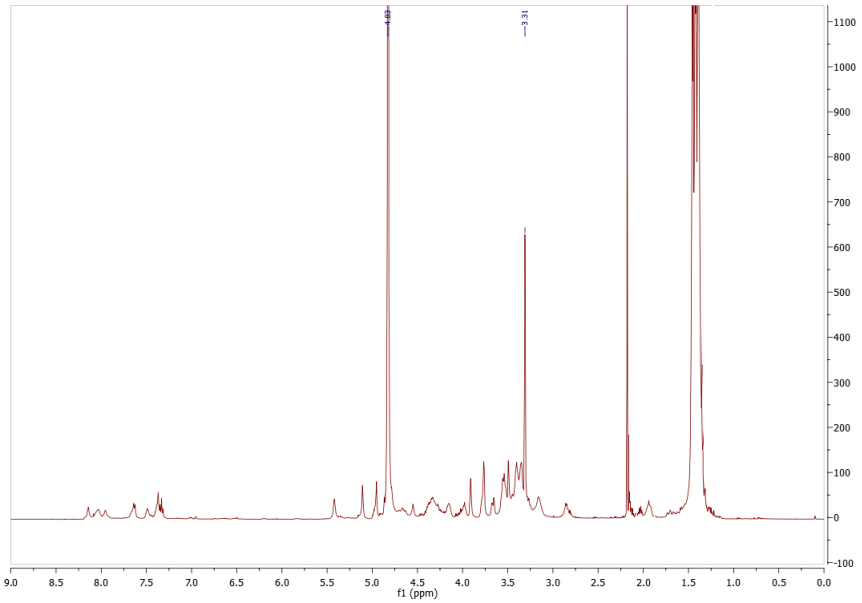
[*N*-(2-*N*-toluoylamino-4-(3-aminophenyl)-thiazole)]-propargylglycinamide (12). To a solution of **11** (167.6 mg, 0.33 mmol) in DCM (3 mL) was added TFA (0.26 mL, 3.3 mmol, 10 eq.) and stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product was used in the next step without further purification: 118 mg (88%); ^{1}H NMR (200 MHz, CD_3OD) δ 8.24 (t, J = 1.5 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.75 (dt, J = 7.6, 1.5 Hz, 1H), 7.53-7.34 (m, 5H), 4.20 (t, J = 6.6 Hz, 1H), 3.02-2.90 (m, 2H), 2.72 (t, J = 2.6 Hz, 1H), 2.43 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD) δ 167.6, 166.9, 160.2, 150.9, 144.9, 139.3, 137.0, 130.9, 130.5, 130.4, 129.1, 123.8, 120.7, 118.9, 109.6, 77.2, 75.2, 53.6, 22.5, 21.6; mass spectrum (ESI), *m/z* 405.4 $(M+H)^+$ (theoretical 405.1).

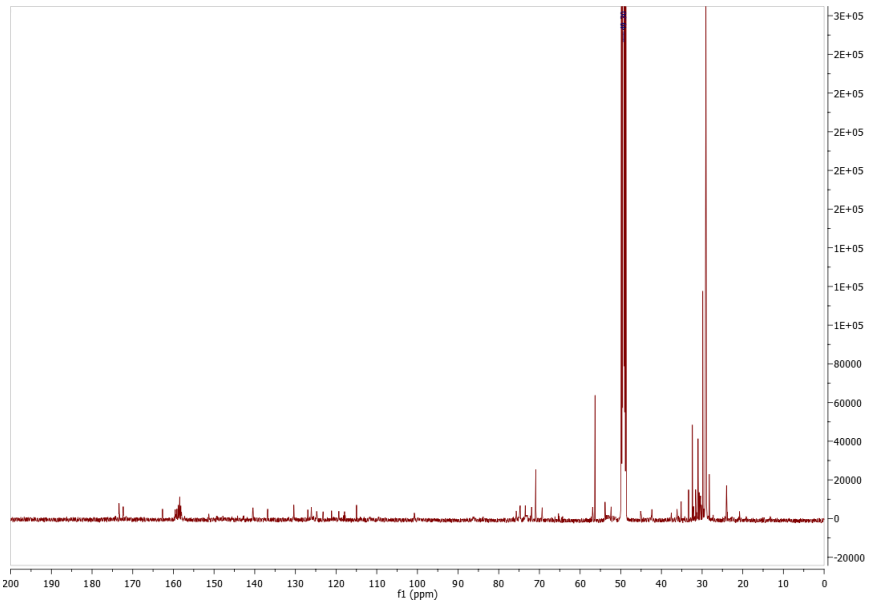
***N*-[2-*N*-toluoylamino-4-(3-aminophenyl)-thiazole]-[*Nα*,*Nε*-(di-tert-butoxycarbonyl)]-lysyl-glycinamide (2g).** A mixture of **12** (50 mg, 0.096 mmol), Boc-Lys(Boc)-OH·DCHA (50.9 mg, 0.096 mmol, 1 eq.), HBTU (36.6 mg, 0.096 mmol, 1 eq.), DIPEA (50 µL, 0.29 mmol, 3 eq.) in DCM (10 mL) was stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound **2g** as a yellow solid that was employed in the following step without further purification: 55.5 mg (78%); R_f = 0.39 (Cyclohexane/EA 1/1); mass spectrum (ESI), *m/z* 734.3 $(M+H)^+$ (theoretical 734.3).

***N*-[2-*N*-toluoylamino-4-(3-aminophenyl)-thiazole]-[*Nα*,$N_{\text{imidazole}}$-(di-tert-butoxycarbonyl)]-histidyl-glycinamide (2h).** A mixture of **12** (50 mg, 0.096 mmol), Boc-His(Boc)-OH·DCHA (51.7 mg, 0.096 mmol, 1 eq.), HBTU (36.6 mg, 0.096 mmol, 1 eq.), DIPEA (50 µL, 0.29 mmol, 3 eq.) in DCM (10 mL) was stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound **2h** as a yellow solid: 59.6 mg (83%); R_f = 0.24 (Cyclohexane/EtOAc 1:1); ^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (s, 1H), 8.07 (s, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.36 (m, 1H), 7.34-7.31 (m, 4H), 4.67 (t, J = 6.2 Hz, 1H), 4.42 (t, J = 6.2 Hz, 1H), 3.07 (dd, J = 14.7, 4.7 Hz, 1H), 2.94 (dd, J = 14.7, 4.7 Hz, 1H),

2.83-2.74 (m, 3H), 2.40 (s, 3H), 1.54 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.0, 170.3, 167.5, 160.0, 151.0, 148.1, 144.8, 139.6, 138.5, 136.6, 130.8, 130.5, 130.1, 129.0, 123.4, 121.2, 119.6, 116.5, 109.4, 87.1, 81.1, 80.2, 72.7, 56.0, 54.2, 38.9, 28.7, 28.0, 22.5, 21.6; mass spectrum (ESI), *m/z* 743.2 $(M+H)^+$ (theoretical 743.3).

Figure S12. ^{1}H, ^{13}C and HRMS spectra for compound **3a**.





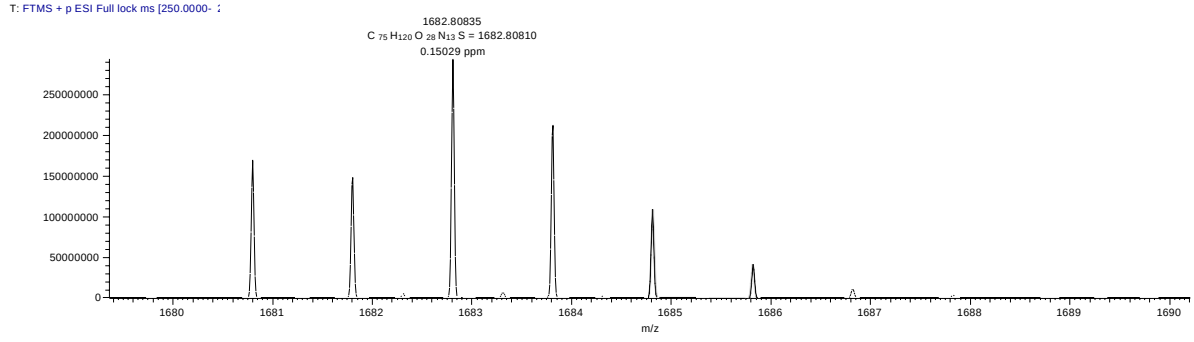
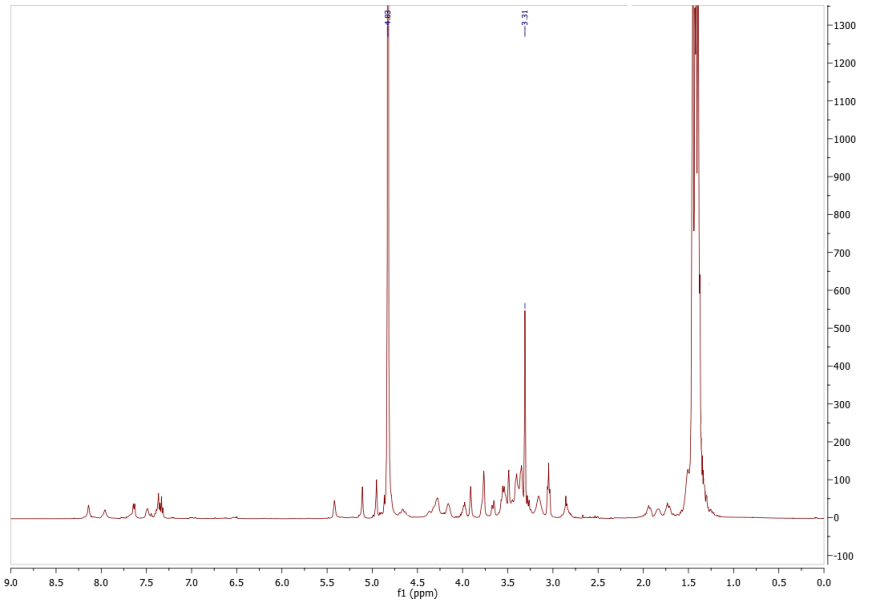
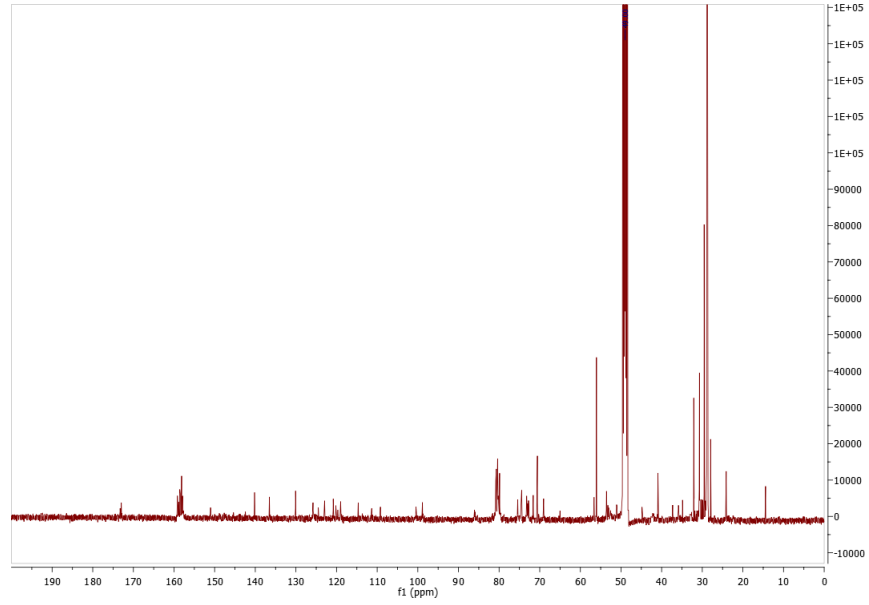


Figure S13. ^{1}H, ^{13}C and HRMS spectra for compound **3b**.





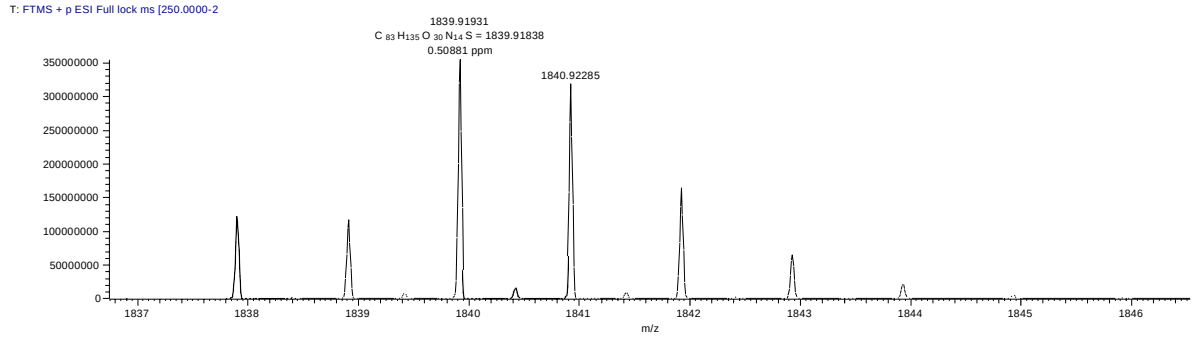


Figure S14. ^{1}H, ^{13}C and HRMS spectra for compound **3c**.

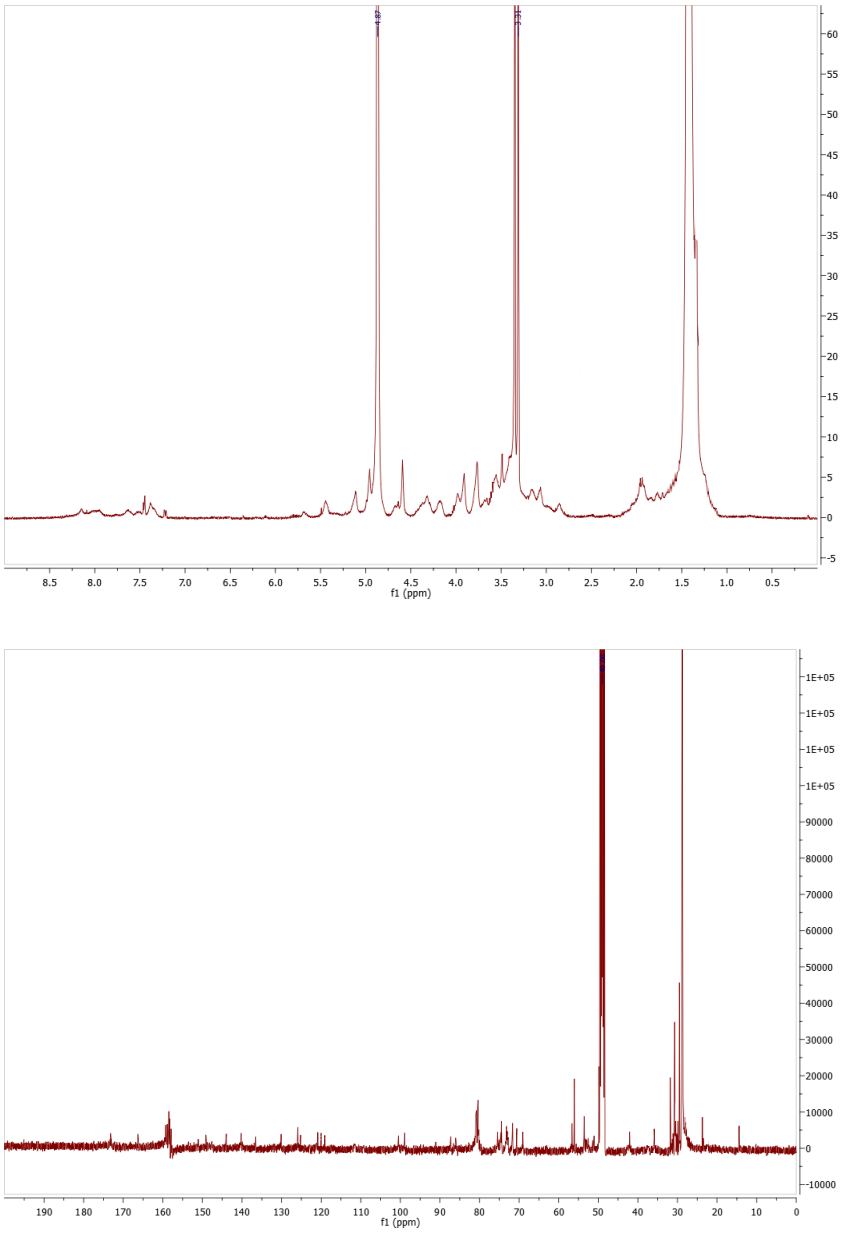
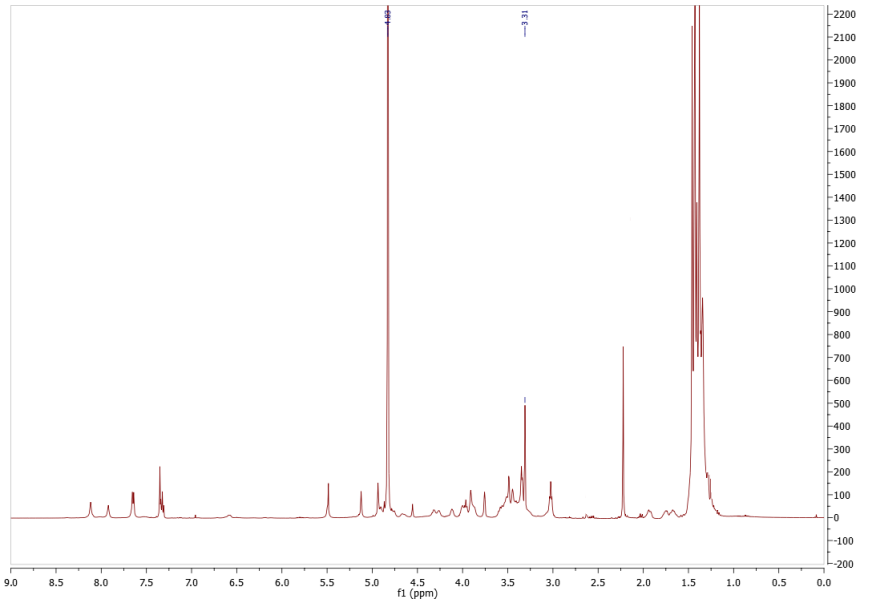
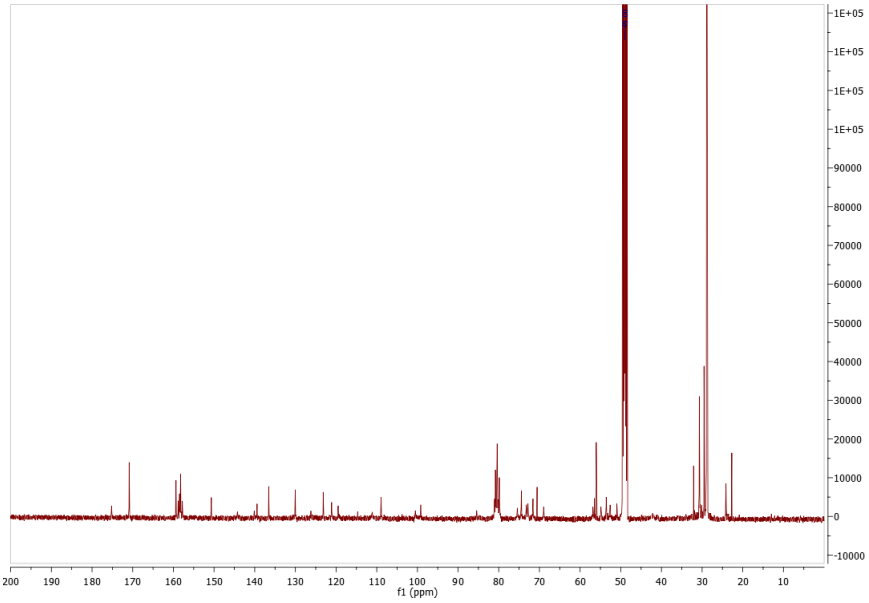


Figure S15. ^{1}H, ^{13}C and HRMS spectra for compound **3d**.





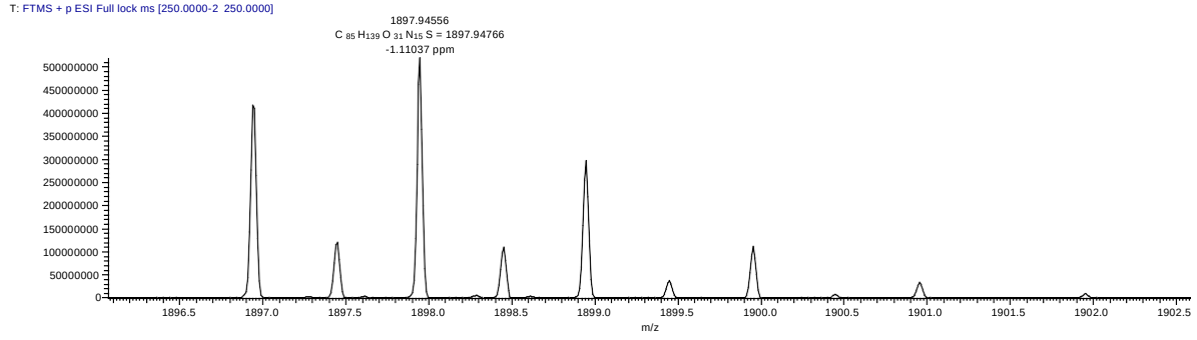
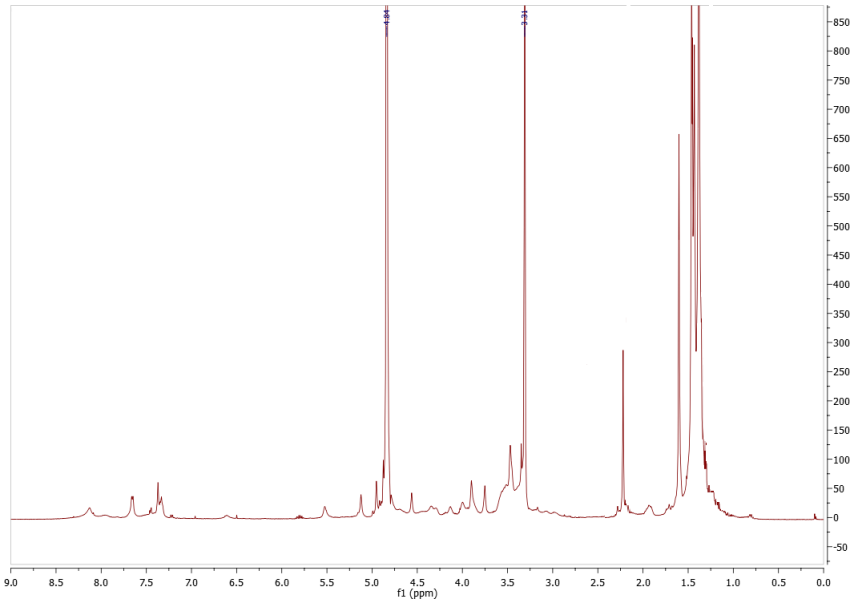
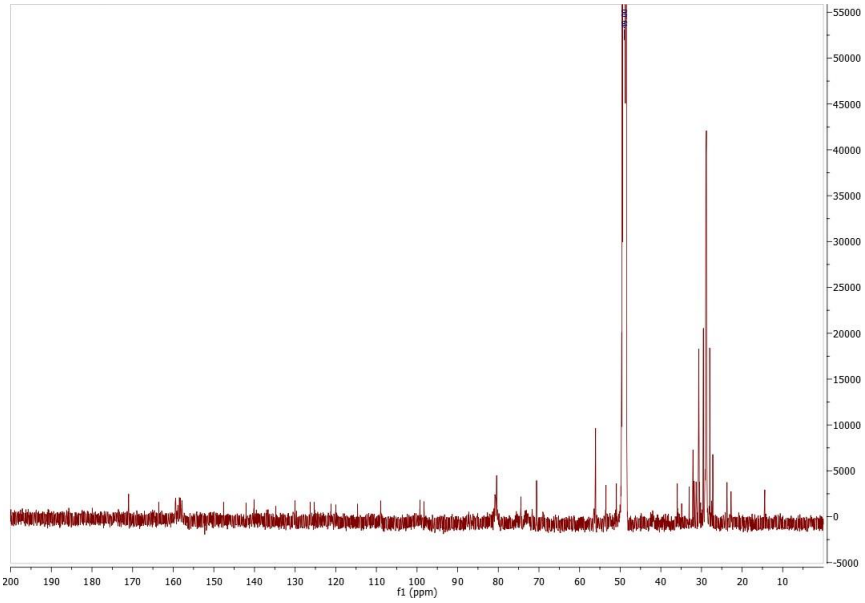


Figure S16. ^{1}H, ^{13}C and HRMS spectra for compound **3e**.





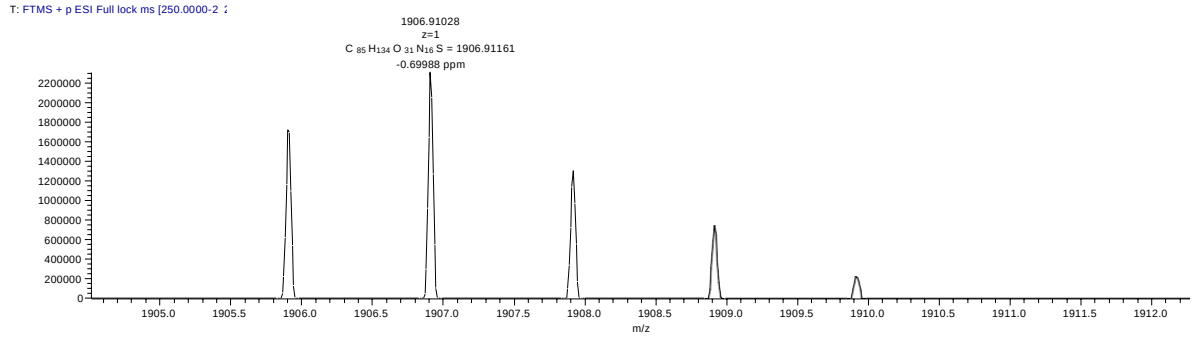
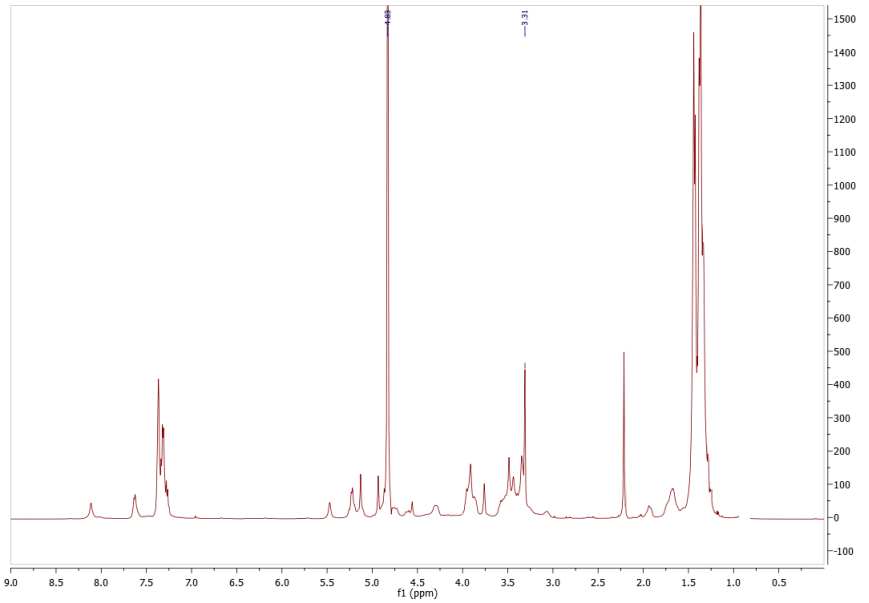
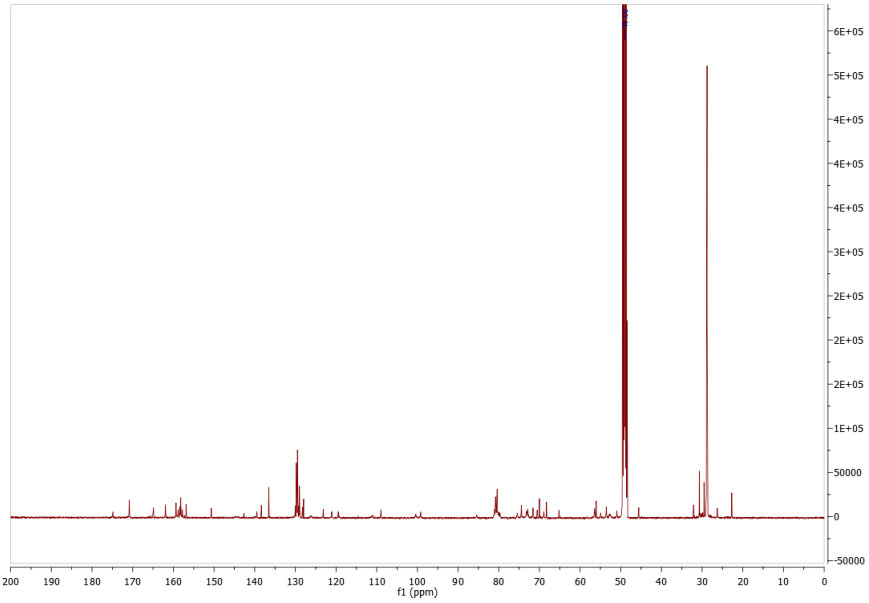


Figure S17. ^{1}H, ^{13}C and HRMS spectra for compound **3f**.





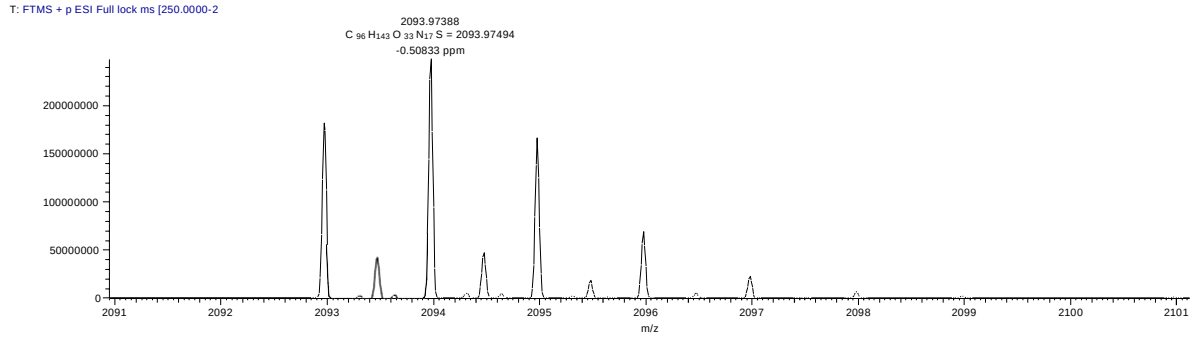


Figure S18. ^{1}H, ^{13}C and HRMS spectra for compound **3g**.

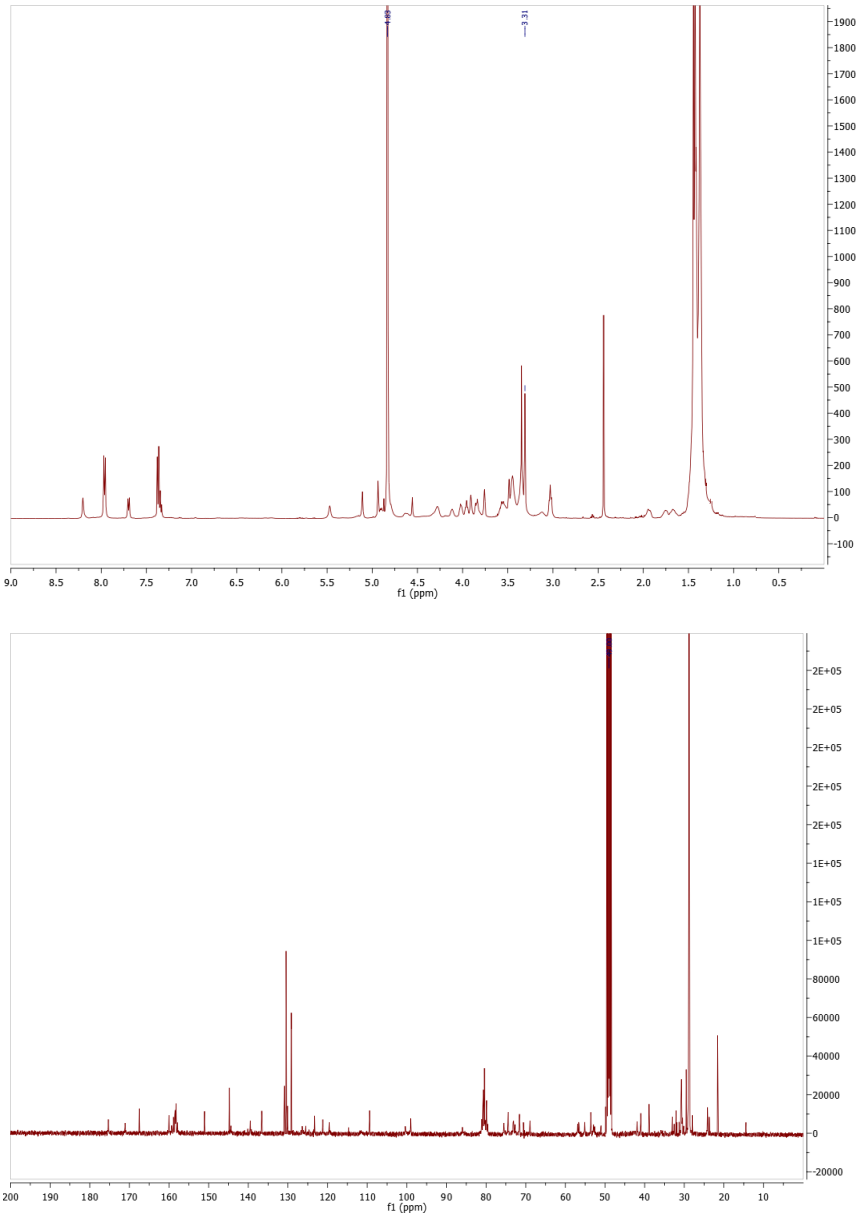
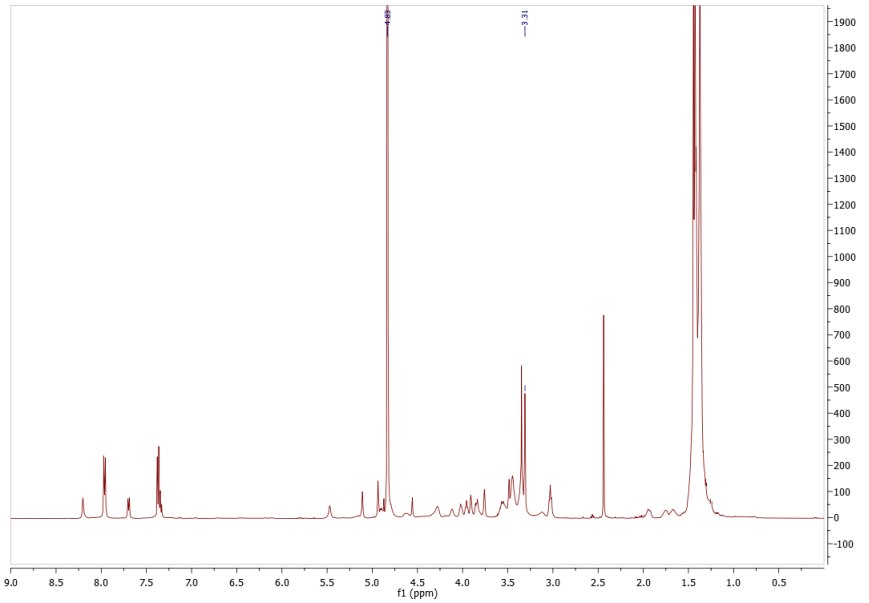
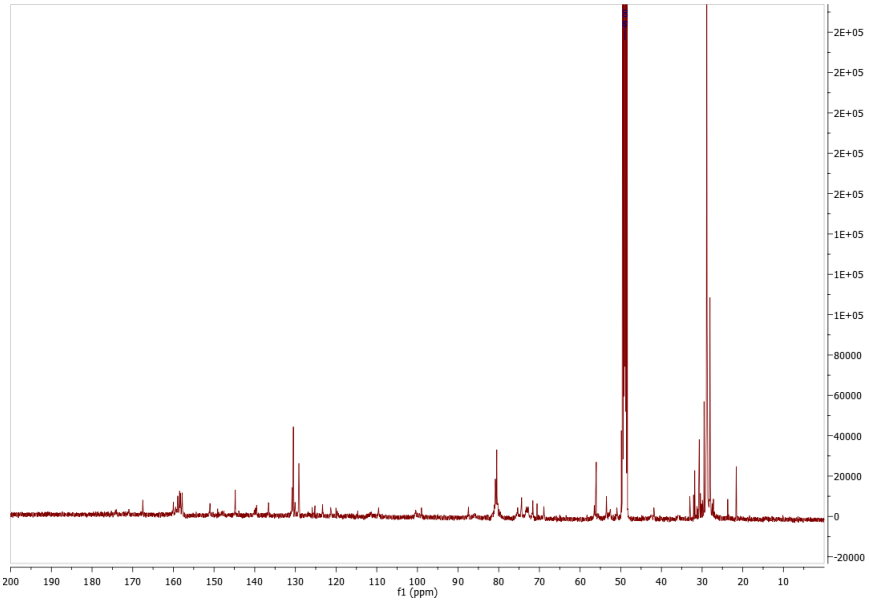


Figure S19. ^{1}H, ^{13}C and HRMS spectra for compound **3h**.





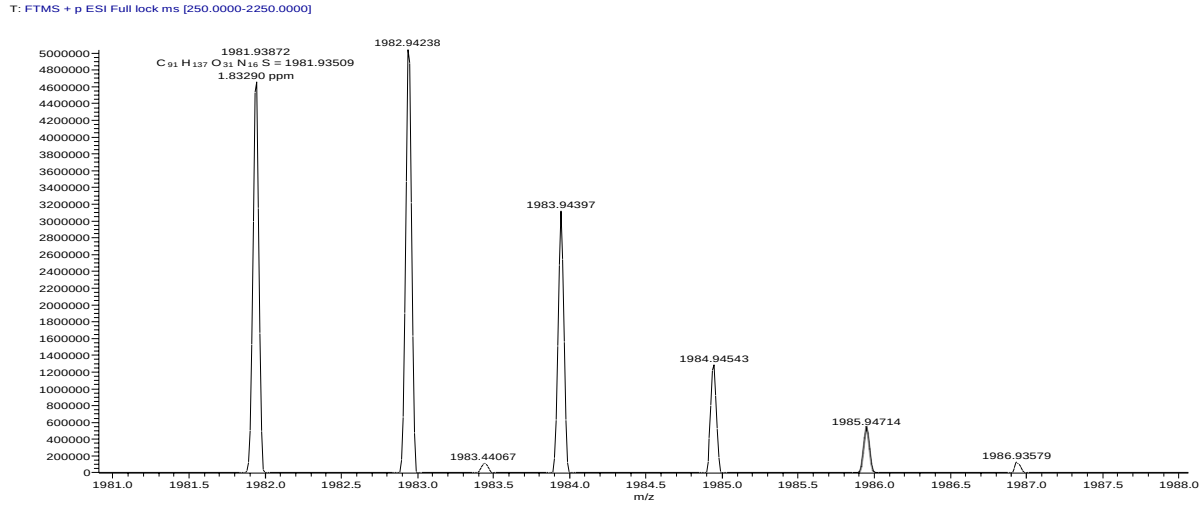
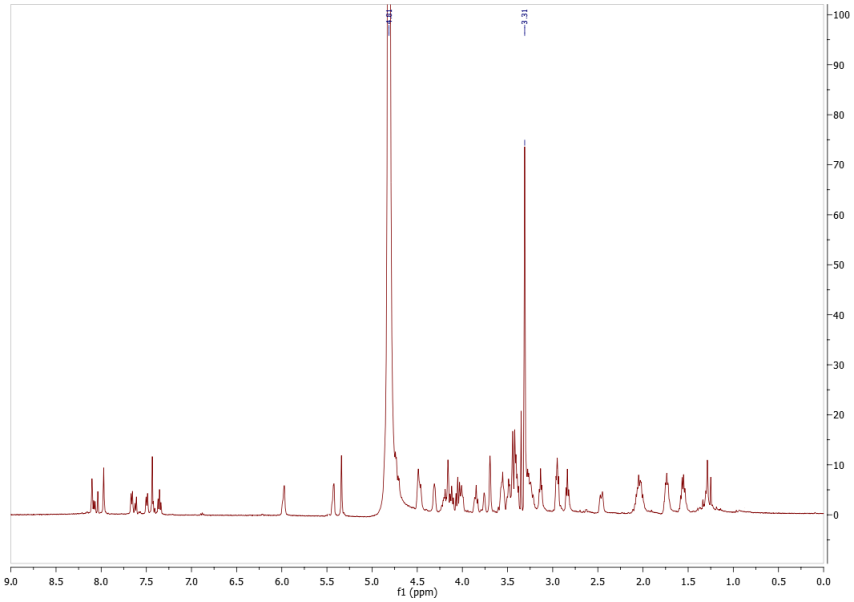
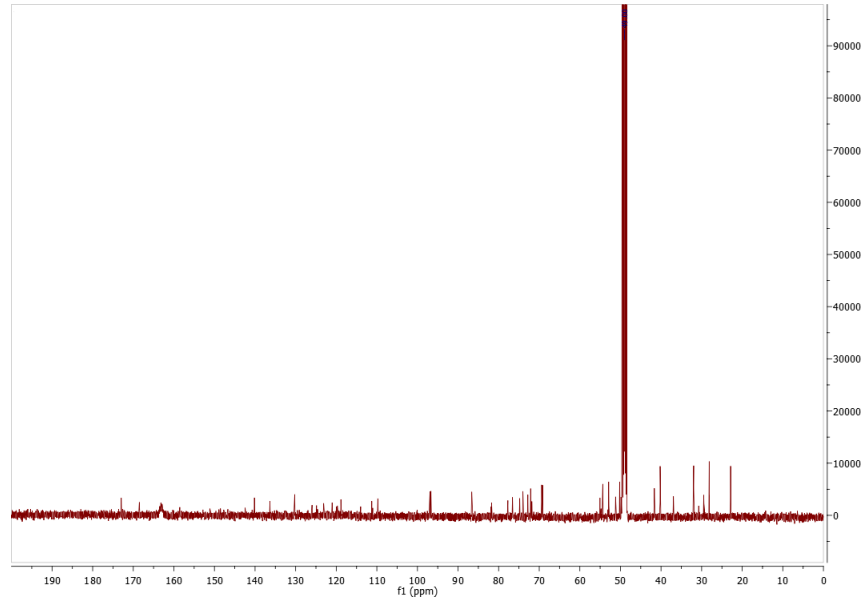


Figure S20. ^{1}H, ^{13}C and HRMS spectra for compound **4a**.





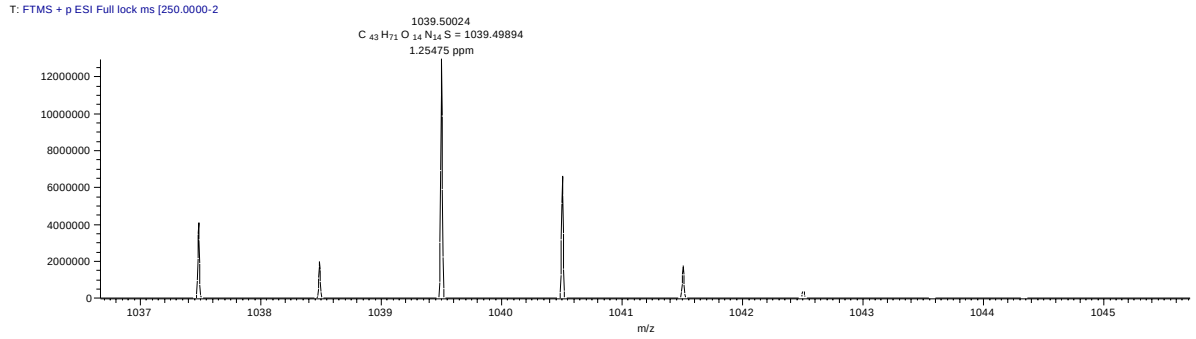
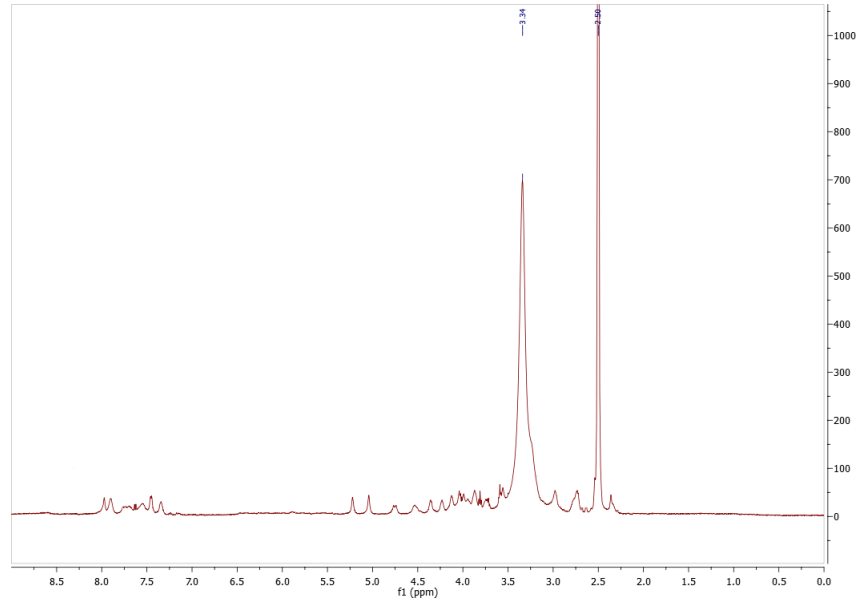
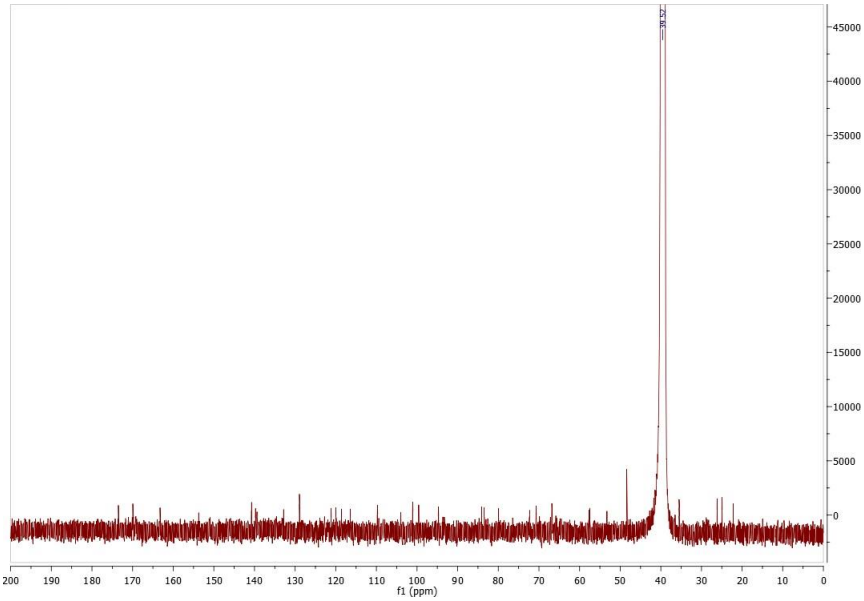


Figure S21. ^{1}H, ^{13}C and HRMS spectra for compound **4b**.





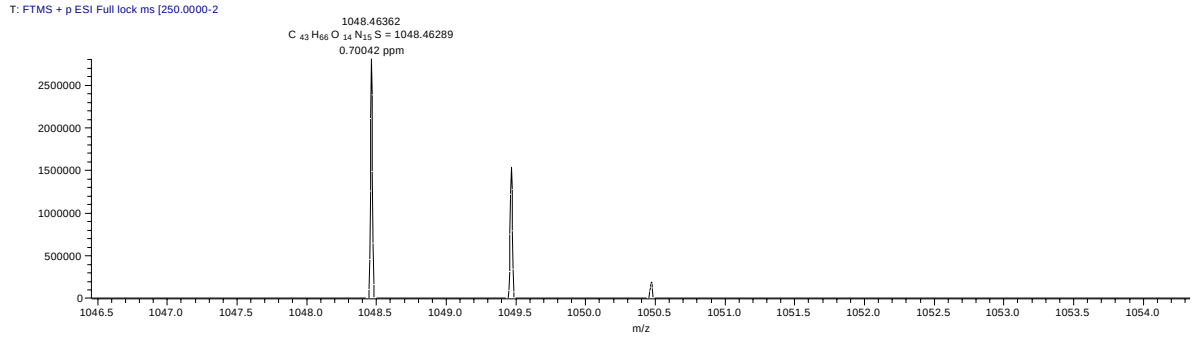
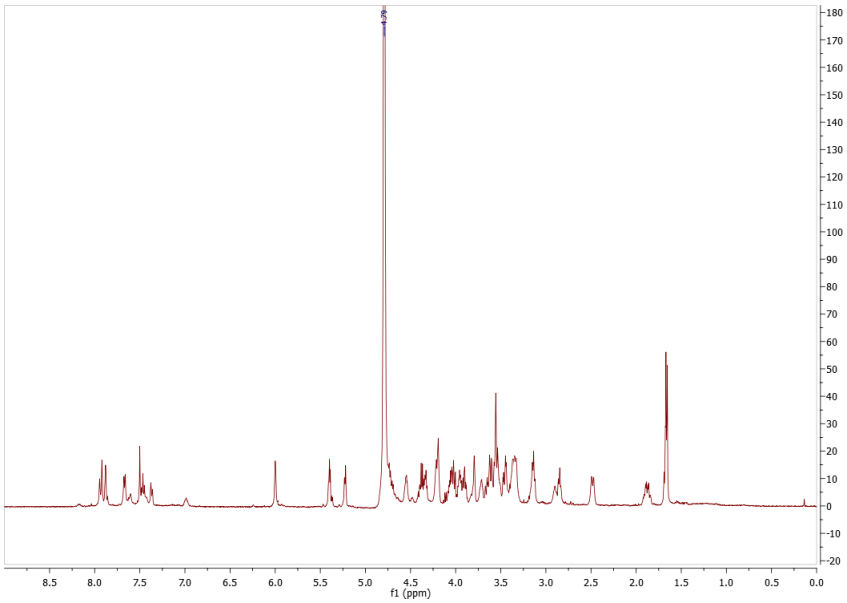
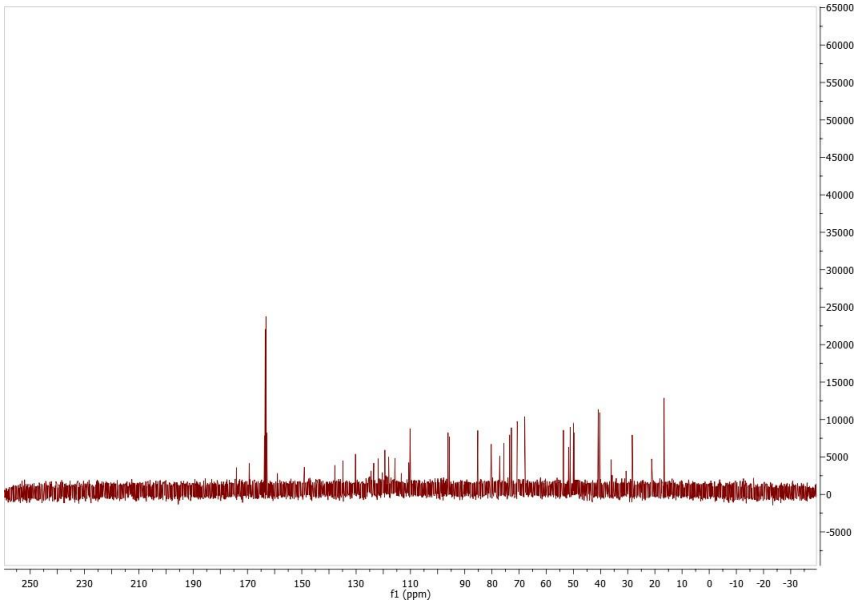


Figure S22. ^{1}H, ^{13}C and HRMS spectra for compound **4c**.





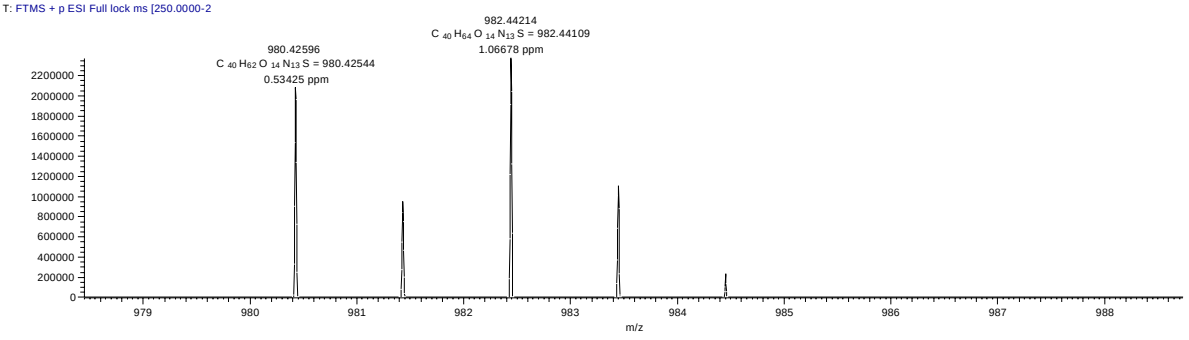
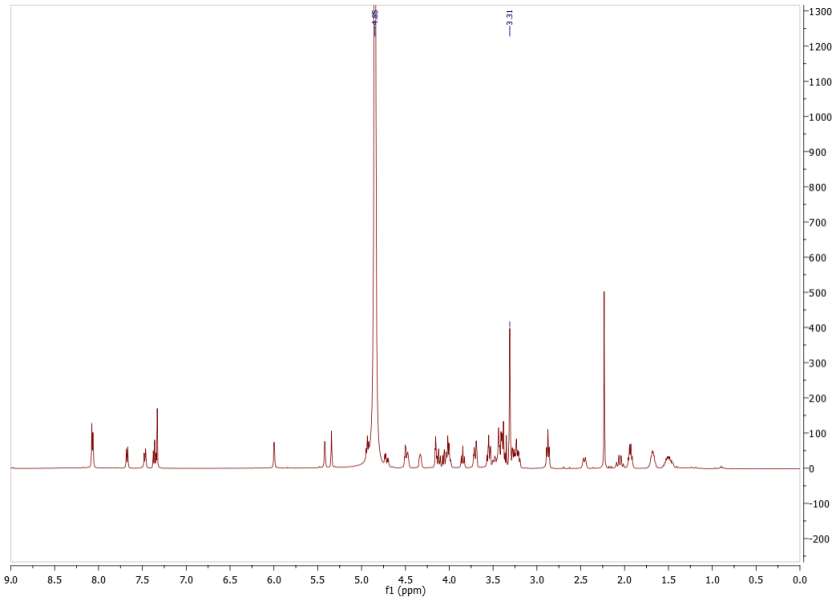
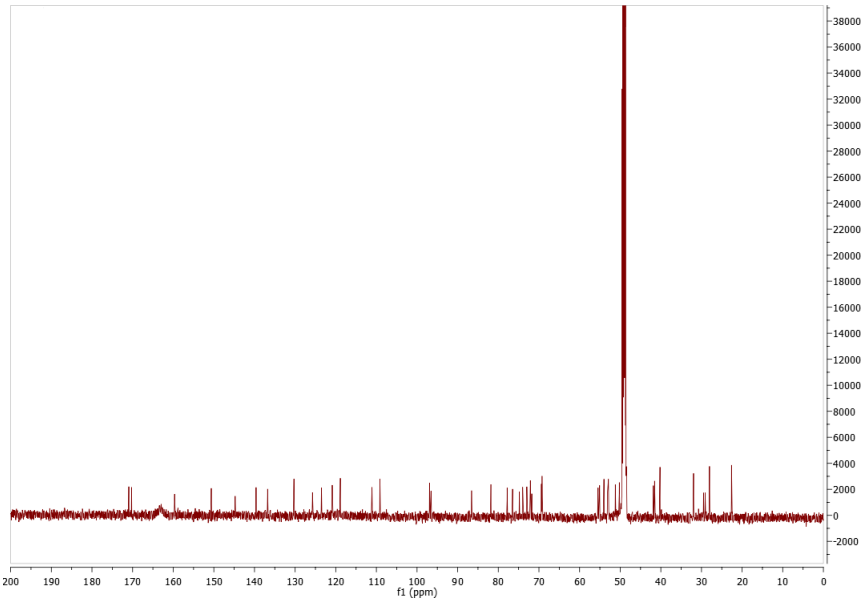


Figure S23. ^{1}H, ^{13}C and HRMS spectra for compound **4d**.





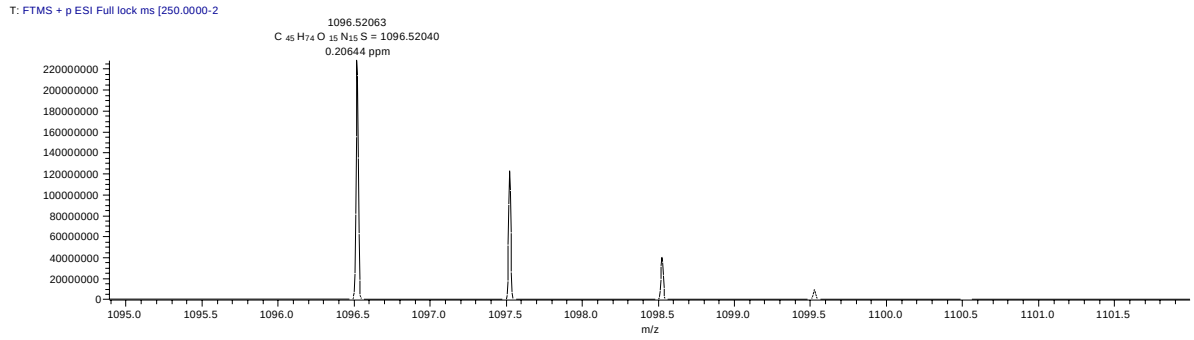
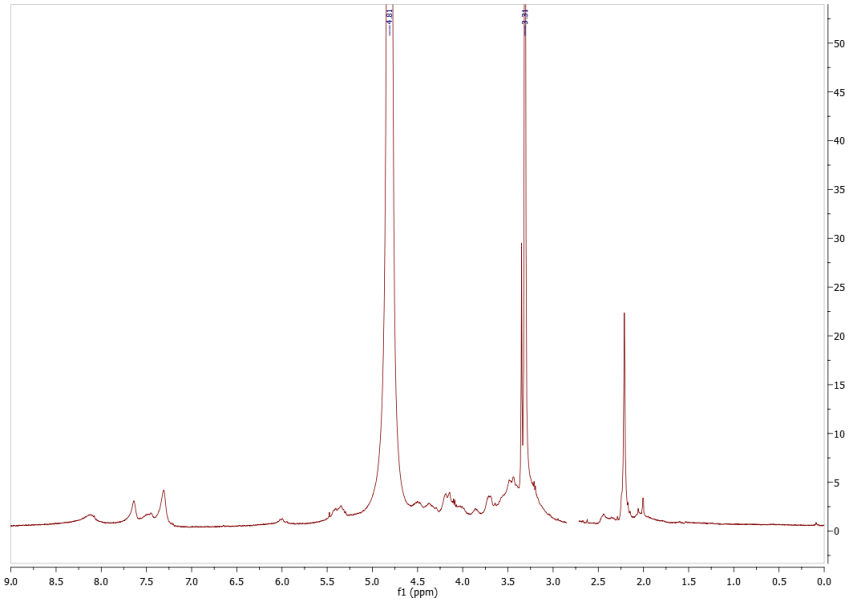
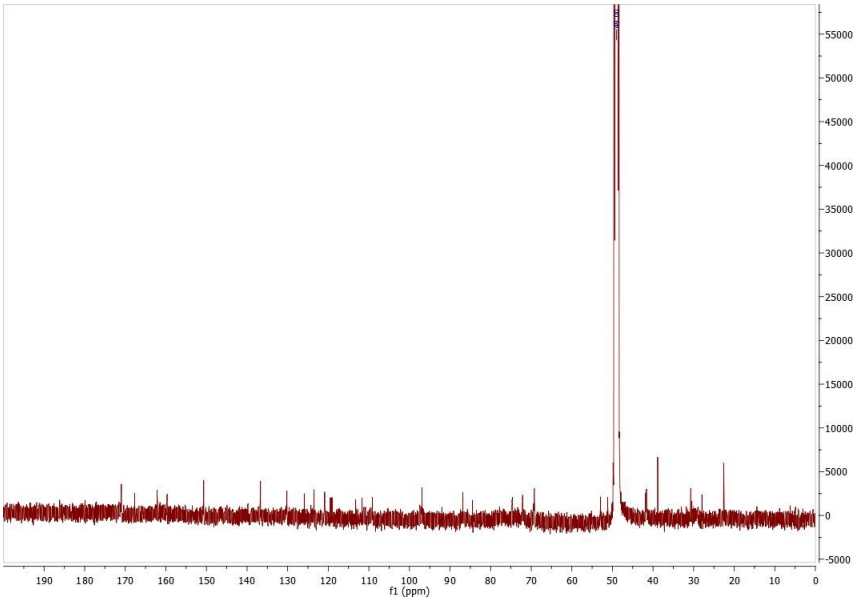


Figure S24. ^{1}H, ^{13}C and HRMS spectra for compound **4e**.





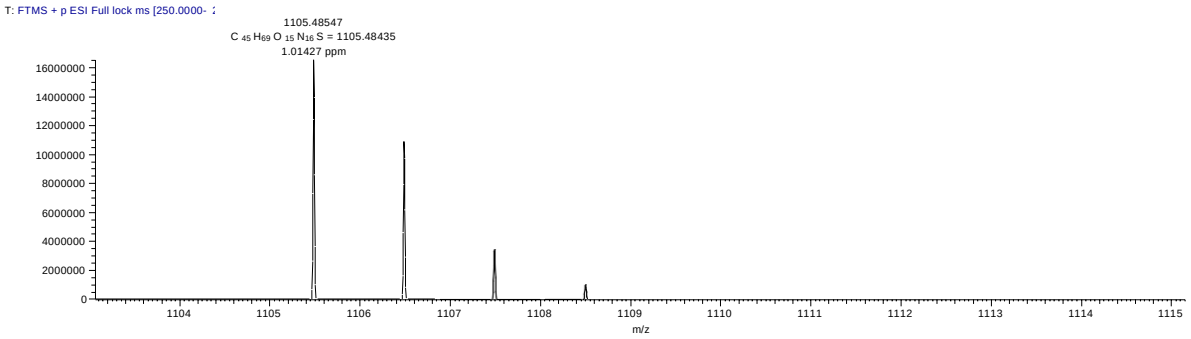
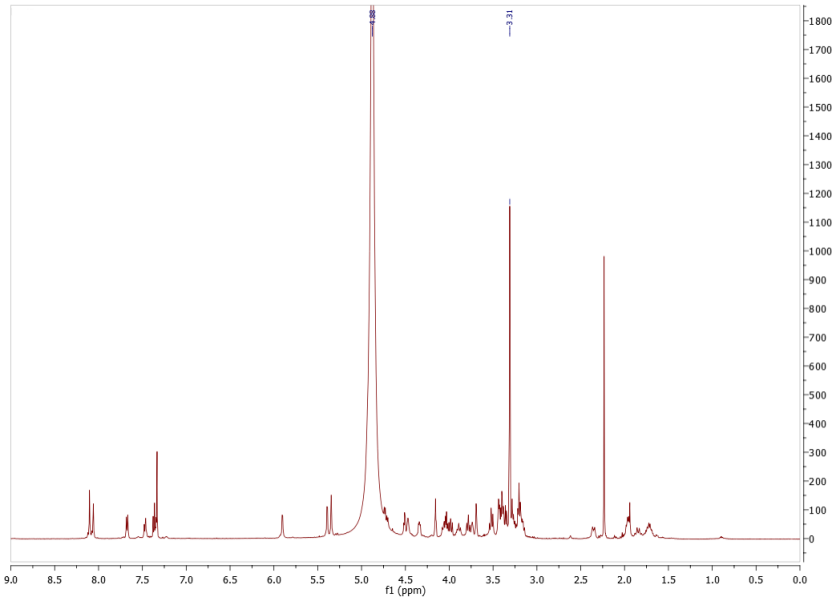
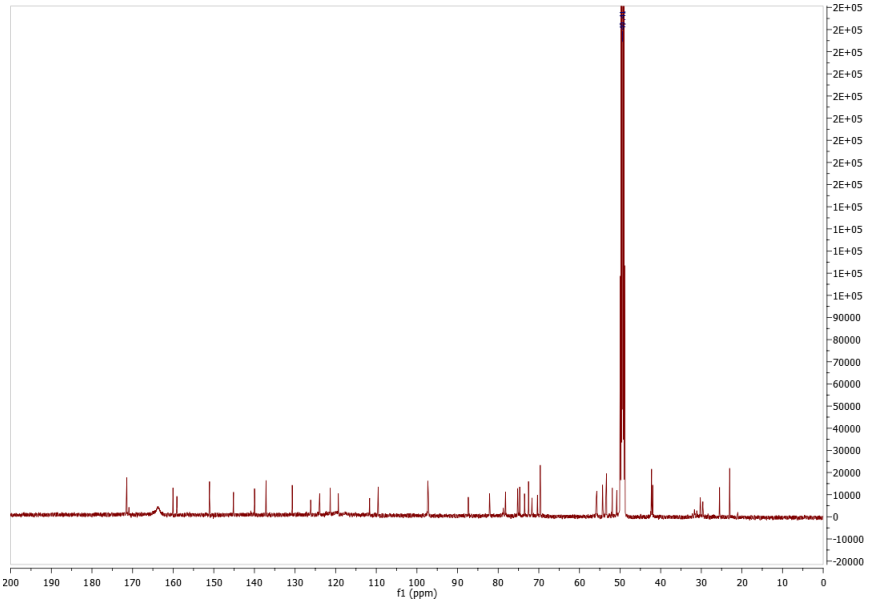


Figure S25. ^{1}H, ^{13}C and HRMS spectra for compound **4f**.





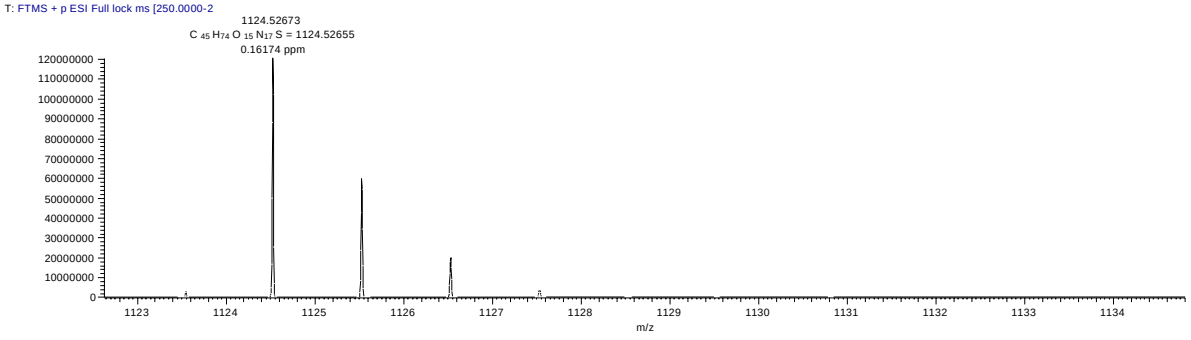
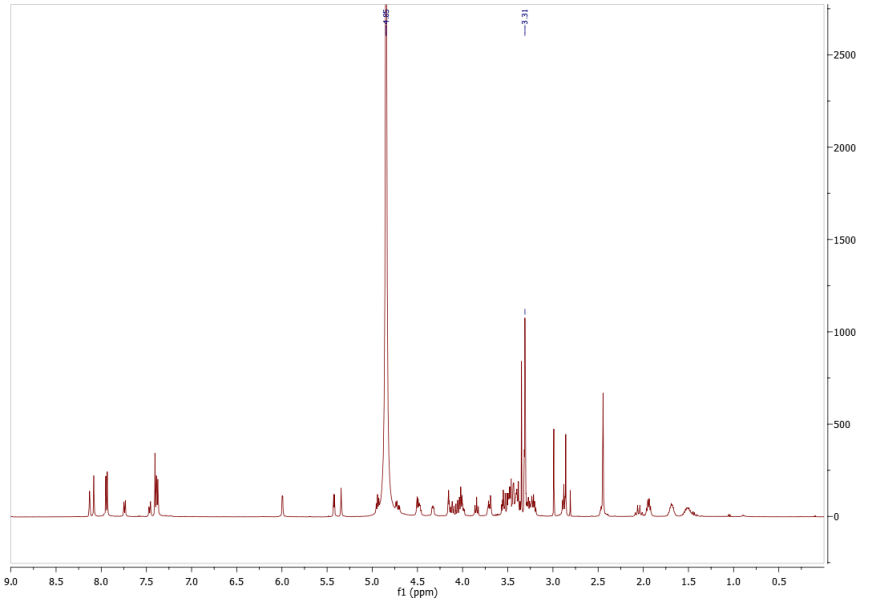
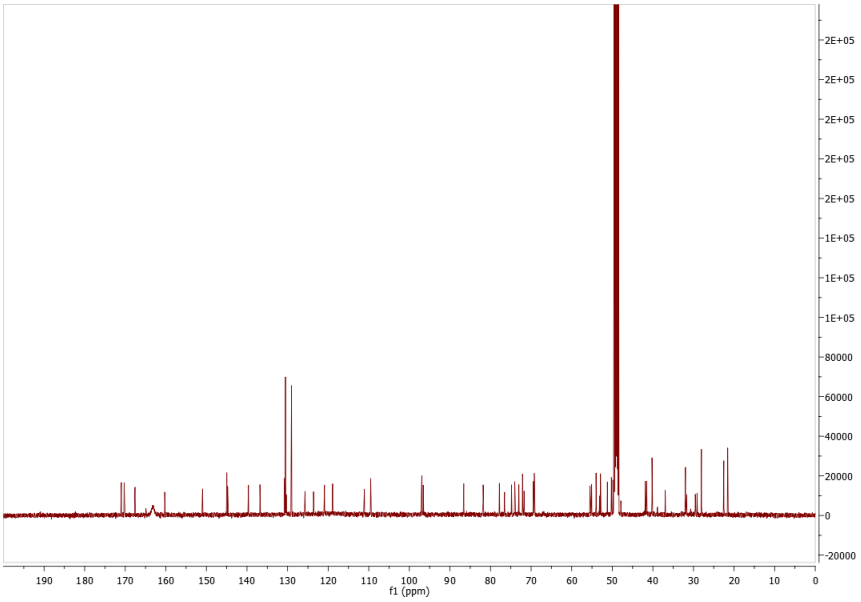


Figure S26. ^{1}H, ^{13}C and HRMS spectra for compound **4g**.





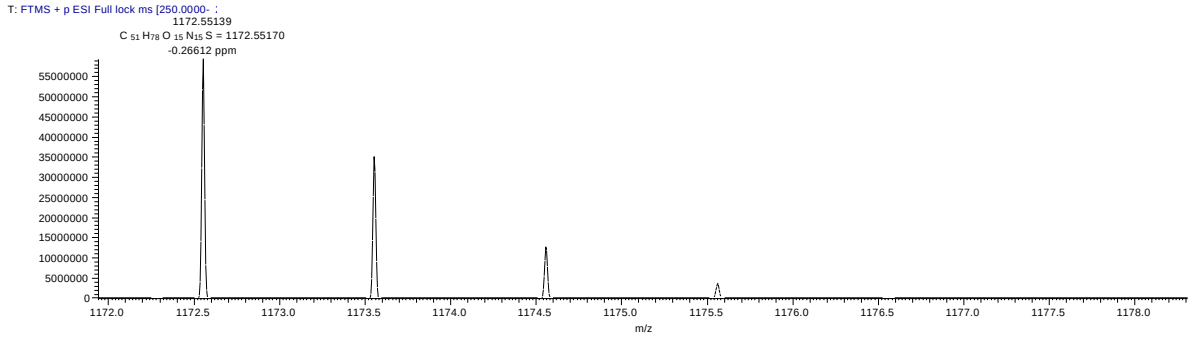
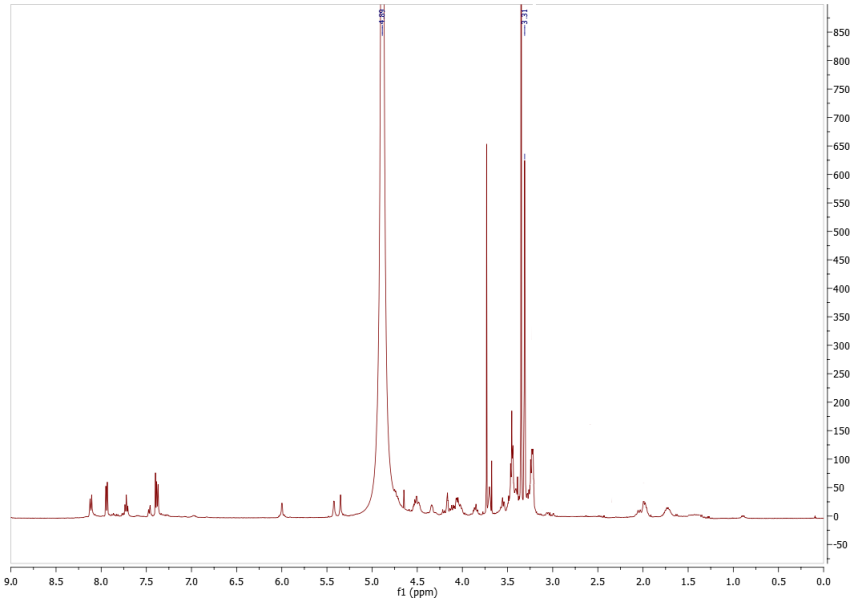
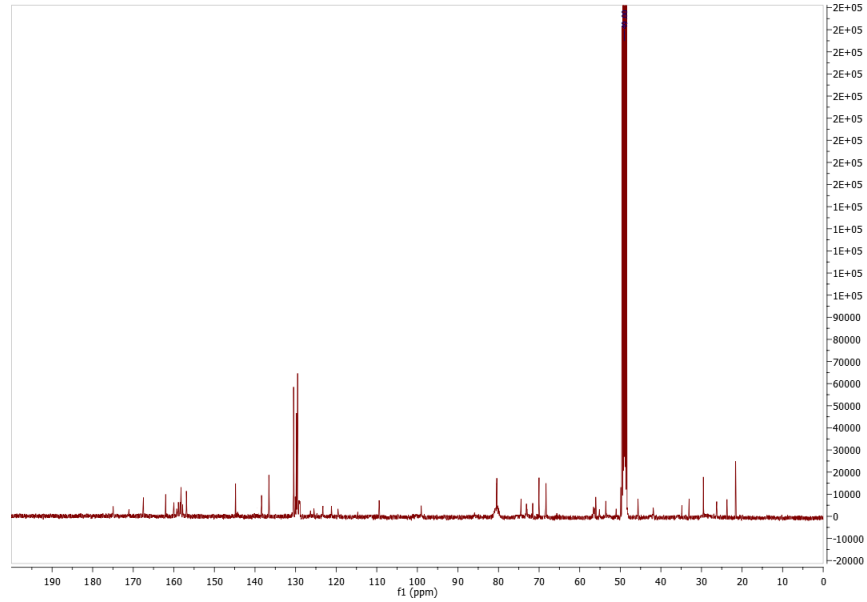
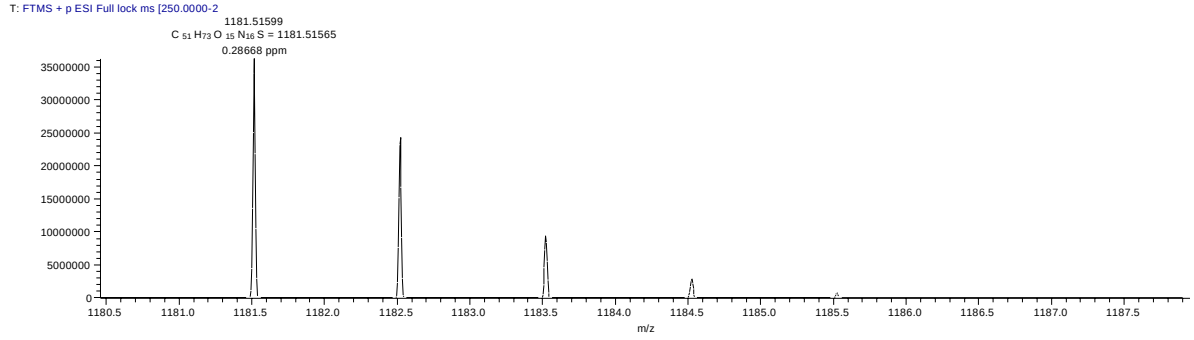


Figure S27. ^{1}H, ^{13}C and HRMS spectra for compound **4h**.







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