Supporting Information

Development of 2-desoxystreptamine-nucleobase conjugates for the inhibition of oncogenic miRNAs production

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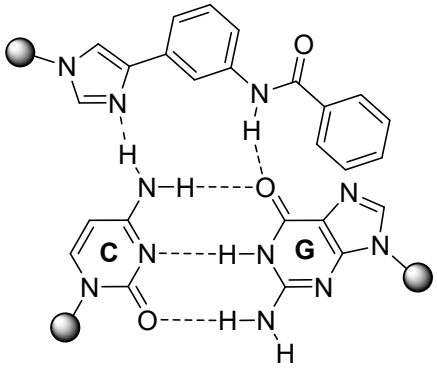
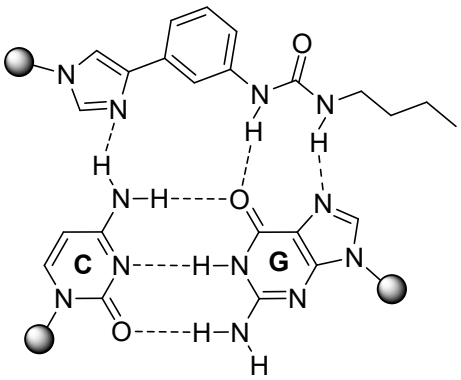
Figure S1. Artificial nucleobases $\mathbf{D_3}$ and $\mathbf{D_4}$ and their interactions with DNA (or RNA) base pairs.[1, 2]



D_3.CG



D_4.CG

Figure S2. Chemical structures of alkynes **10a-h** employed in this study.

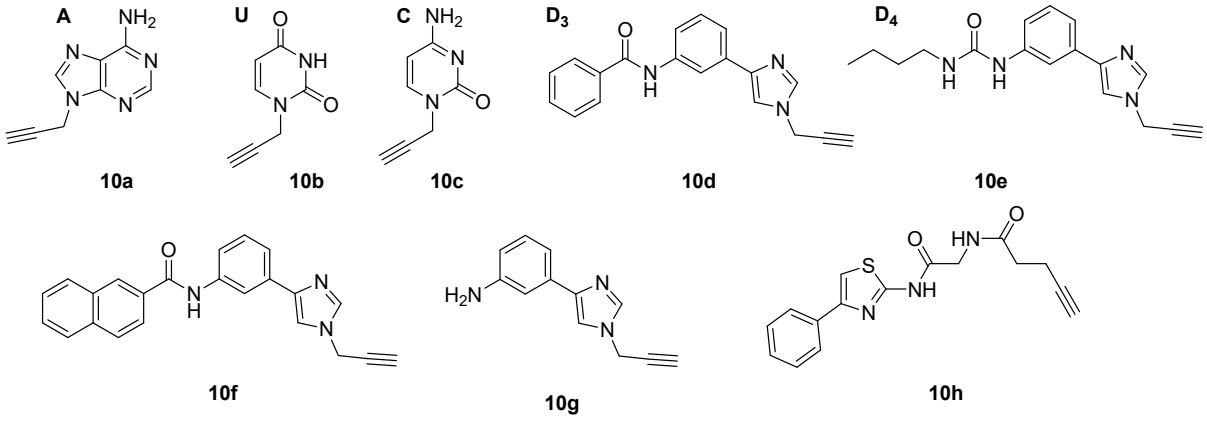


Figure S3. Dissociation constants (K_D) curves for compounds **5a-h** binding to pre-miR-372.

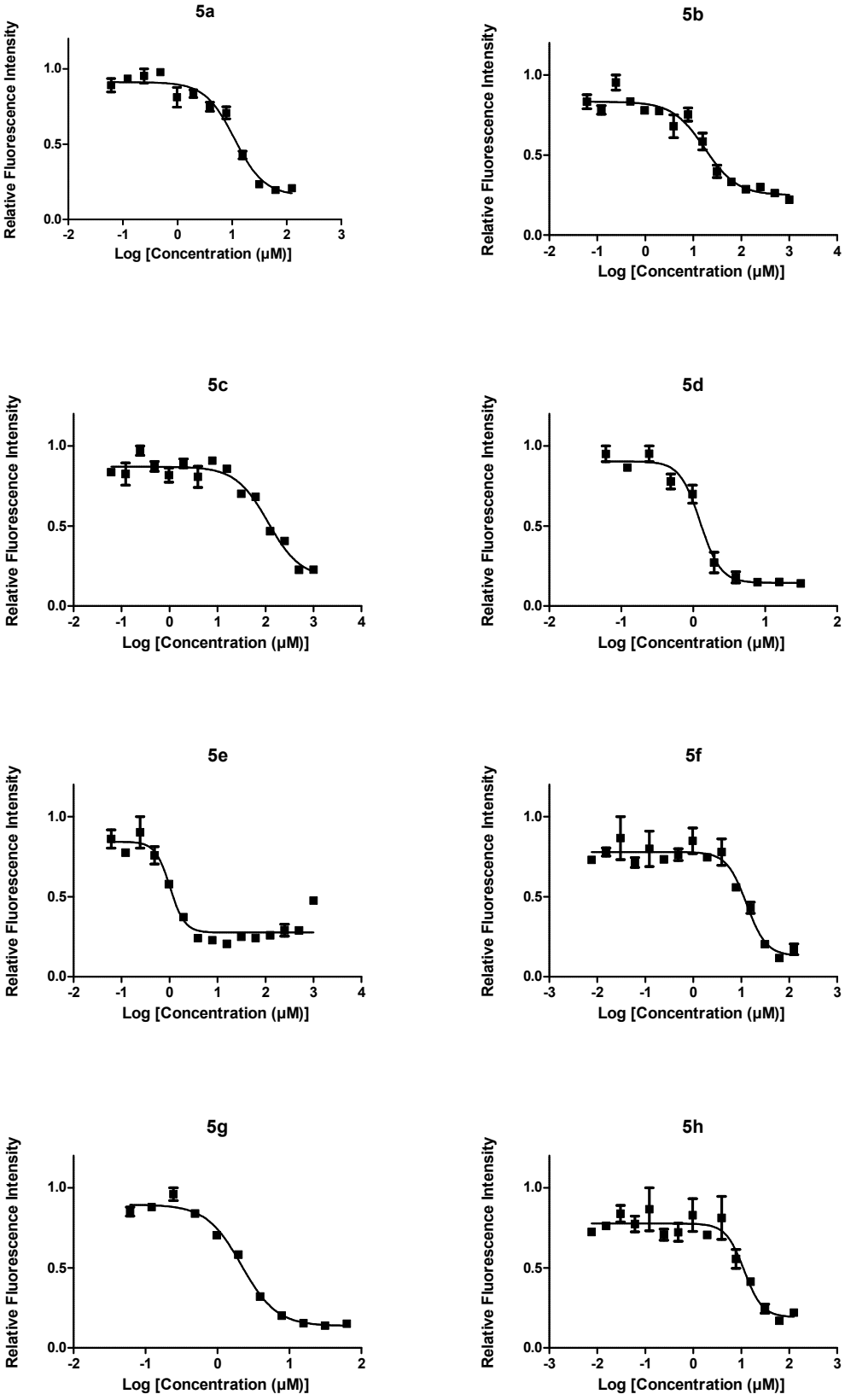
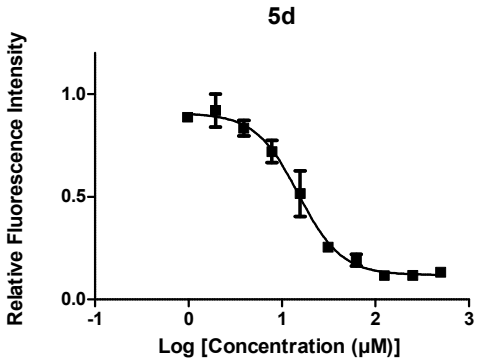
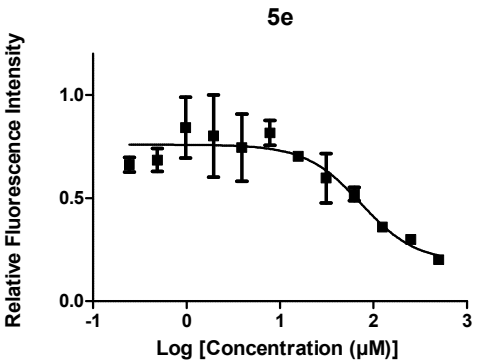
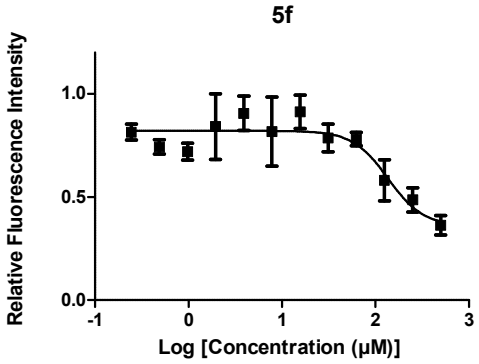
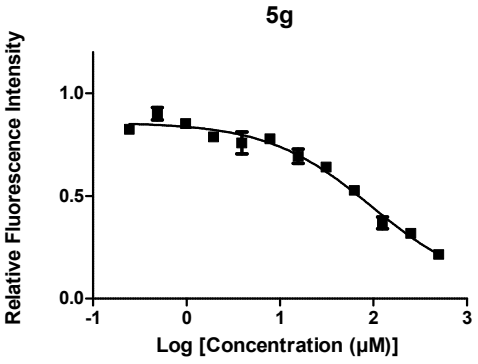


Figure S4. Inhibition activity (IC_{50}) curves for compounds **5d-h** against pre-miR-372 maturation.









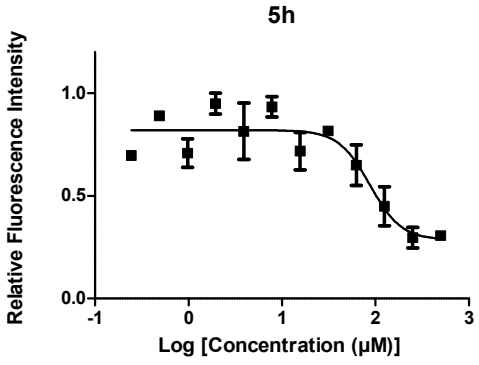


Table S1. Dissociation constants (K_D) and inhibition activities (IC_{50}) for compounds **5a-h** and **9** against pre-miR-21, pre-miR-18a and pre-miR-148a.

ID	K_D[a] pre-miR-21	IC_{50}[b] pre-miR-21	K_D[a] pre-miR-18a	IC_{50}[b] pre-miR-18a	K_D[a] pre-miR148a	IC_{50}[b] pre-miR-148a
5a	15.9	no inhibition	no binding	no inhibition	50.8	no inhibition
5b	61.4	no inhibition	no binding	no inhibition	no binding	no inhibition
5c	144	no inhibition	no binding	no inhibition	no binding	no inhibition
5d	0.628	53.8	0.899	> 500	1.70	21.7
5e	2.59	> 500	not determined	not determined	not determined	not determined
5f	18.0	> 500	6.28	177	7.90	> 500
5g	2.50	no inhibition	3.31	20.3	3.12	18.2
5h	2.23	62.6	6.72	89.5	6.35	85.3
9	1.2	79.8	1.99	76.0	1.99	28.0

[a] Binding studies were performed on 5'-FAM-pre-miR-372 in buffer A (20 mM Tris–HCl (pH 7.4), 12 mM NaCl, 2.5 mM $MgCl_2$ and 1 mM DTT). The K_D values are given with an uncertainty of ±10%. [b] The IC_{50} experiments were performed in the presence of 50 nM premiR-372 beacon 5'-labeled with FAM and 3'-labeled with dabcyl (DAB) and 0.5 U of recombinant Dicer in buffer A (20 mM Tris–HCl (pH 7.4), 12 mM NaCl, 2.5 mM $MgCl_2$ and 1 mM DTT). The IC_{50} values are given with an uncertainty of ±10%.

Synthetic procedures

General methods have been described in the main manuscript. Compounds **10a-e** have been prepared following a previously reported procedure.[3]

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-2-deoxytreptamine (1). A solution of neamine tetrahydrochloride (2.0 g, 4.27 mmol) in aqueous 48% HBr (12 mL) was heated under reflux for 2 days. The reaction mixture became colored rather quickly. The solution was evaporated *in vacuo* to dryness, 10 mL of water were added and again evaporated to dryness. This process was repeated twice to insure complete removal of the excess of HBr. TLC indicated that the major component of the crude product was 2-deoxystreptamine, R_f = 0.16 (*i*PrOH/EtOH/$CHCl_3$/NH_4OH 4/5/2/5). The crude product was then converted into compound **1** without further purification by the following procedure: sodium hydroxide (2.56 g, 64.0 mmol), Boc_2O (9.30 g, 42.6 mmol) in a mixture of dioxane and water (60 mL, 3:1) were added to the crude product. This solution was heated at 60^0C for 1h, then concentrated *in vacuo* and the residue was partitioned between water (30 mL) and EtOAc (60 mL). The aqueous layer was separated and extracted with EtOAc (2×25mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. A flash column chromatography using a mixture CH_2Cl_2/MeOH 95:5 as the eluent afforded the desired product **1** as a white solid in 50% yield (773 mg). R_f = 0.26 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (200 MHz, CD_3OD) δ (ppm): 3.40-3.30 (m, 3H), 3.20-3.10 (m, 2H), 2.10-2.00 (m, 1H), 1.45-1.44 (br, 18H), 1.30-1.15 (m, 1H); ^{13}C NMR 50 MHz (CD_3OD) δ (ppm): 158.2, 80.1, 78.3, 76.3, 52.7, 35.9, 28.8; MS (ESI) *m/z* = 363.4 $[M+H]^+$ (theoretical *m/z* 363.4).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine (2). Compound **1** (100 mg, 0.28 mmol) was treated with 1,1-dimethoxycyclohexane (273 µL, 1.82 mmol) in dry DMF (2 mL), in the presence of *p*-TsOH monohydrate (2.6 mg, 0.015 mmol) at 55°C and 50 mbar for 1 h. The reaction was then quenched by the addition of Et_3N (1 mL). After evaporation, the crude product was purified by silica gel chromatography using a miwture CHX/EtOAc 6:4 as the eluent to afford desired compound **2** as a white solid in 98% yield (122 mg). R_f= 0.39 (CHX/EtOAc 1:1); ^{1}H NMR (200 MHz, CD_3OD) δ (ppm): 3.75-3.35 (m, 5H), 2.20-2.10 (m, 1H), 1.65 (br, 8H), 1.45 (br, 20H), 1.35-1.25 (m, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ (ppm): 157.2, 156.7, 111.9, 81.1, 79.3, 78.8, 73.0, 36.5, 36.3, 27.7, 25.1, 23.7; MS (ESI): *m/z* 465.6 $[M+Na]^+$ (theoretical *m/z* 465.3).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[2'-azidoethyl]ether (3). To a solution of compound **2** (750 mg, 1.70 mmol) in dry THF (15 mL), NaH (60% dispersion in mineral oil, 340 mg, 8.5 mmol) and 2-azidoethyl-p-toluensulfonate[4] (820 mg, 3.4 mmol) were added under argon atmosphere. The reaction mixture was stirred at 30°C under argon for 3h, then concentrated under reduced pressure. The residue was dissolved in Et_2O (50 mL) and washed with a saturated solution of $NaHCO_3$ (10 mL) and then with water (30 mL). The organic layer was separated and dried over $MgSO_4$. Flash chromatography (CHX/EtOAc 85:15) afforded **3** as a white solid in 33% yield (287 mg). R_f = 0.44 (CHX/EtOAc 7:3); ^{1}H NMR (200 MHz, CD_3OD) δ (ppm): 4.20-4.00 (m, 2H), 3.80-3.60 (m, 2H), 3.50-3.35 (m, 5H), 2.20-2.05 (m, 1H), 1.75-1.60 (m, 8H); 1.40-1.50 (m,

21H); ^{13}C NMR (50 MHz, CD_3OD) δ (ppm): 157.0, 129.1, 112.0, 81.1, 79.3, 78.8, 68.4, 51.7, 51.1, 36.5, 36.2, 27.7, 27.0, 25.1, 23.9; MS (ESI) m/z = 534.0 $[M+Na]^+$ (theoretical *m/z* 534.3).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-(adenyl-*N*-methyl)]ethyltriazole (4a). General procedure **A** was applied to compound **3** and *N*-9-propargyl adenine **10a** (37.4 mg, 0.216 mmol) and the reaction mixture was stirred at room temperature for 4h, then heated at 50°C overnight. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 98:2 as the eluent affording desired compound **4a** as a yellow solid in 87% yield (117 mg). R_f = 0.55 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (200 MHz, CD_3OD) δ (ppm): 8.25 (s, 1H), 8.22 (s, 1H), 8.16 (s, 1H), 5.56 (s, 2H), 4.70-4.50 (m, 2H), 4.20-4.10 (m, 1H), 4.00-3.90 (m, 1H), 3.70-3.60 (m, 1H), 3.55-3.35 (m, 4H), 2.20-2.00 (m, 1H), 1.60-1.20 (m, 29H); ^{13}C NMR (50 MHz, CD_3OD) δ (ppm): 161.4, 157.7, 157.3, 154.0, 150.5, 142.5, 126.2, 113.9, 113.0, 82.0, 81.8, 80.3, 70.1, 56.4, 55.8, 54.8, 52.0, 39.6, 37.4, 28.8, 26.0, 24.8; MS (ESI) m/z = 685.3 $[M+H]^+$ (theoretical *m/z* 685.4).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-(uracil-*N*-methyl)]ethyltriazole (4b). General procedure **A** was applied to compound **3** and *N*-1-propargyluracil **10b** (32.4 mg, 0.216 mmol) and the reaction mixture was stirred at room temperature for 3h. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 98:2 as the eluent affording desired compound **4b** as a yellow solid in 74% yield (95.9 mg). R_f = 0.53 (CH_2Cl_2/MeOH 95/5); ^{1}H NMR (200 MHz, CD_3OD) δ (ppm): 8.10 (s, 1H), 7.74 (d, *J* = 8.1 Hz, 1H), 5.67 (d, *J* = 8.1 Hz, 1H), 5.00 (d, *J* = 4.2 Hz, 2H), 4.60-4.50 (m, 2H), 4.25-4.15 (m, 1H), 4.00-3.95 (m, 1H), 3.75-3.60 (m, 5H), 2.20-2.00 (m, 1H), 1.60-1.30 (m, 29H); ^{13}C NMR (50 MHz, CD_3OD) δ (ppm): 179.8, 166.7, 157.9, 157.8, 152.5, 146.9, 126.5, 113.0, 102.6, 81.9, 80.4, 79.7, 55.8, 52.8, 52.0, 44.2, 37.4, 37.2, 28.8, 26.1, 24.9; MS (ESI) *m/z* = 662.1 $[M+H]^+$ (theoretical *m/z* 662.3).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-(cytosyl-*N*-methyl)]ethyltriazole (4c). General procedure **A** was applied to compound **3** and *N*-1-propargylcytosine **10c** (32.6 mg, 0.216 mmol) and the reaction mixture was stirred at room temperature for 3h, then heated at 50°C overnight. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 95:5 as the eluent affording desired compound **4c** as a yellow solid in 46% yield (59.7 mg). R_f = 0.23 (CH_2Cl_2/MeoH 98/2); ^{1}H NMR (200 MHz, CD_3OD) δ (ppm): 8.06 (s, 1H), 7.72 (d, 1H, *J* = 8.1 Hz), 5.87 (d, 1H, *J* = 8.1 Hz), 5.02 (s, 2H), 4.60-4.50 (m, 3H), 4.25-4.15 (m, 1H), 4.00-3.95 (m, 1H), 3.75-3.65 (m, 1H), 3.50-3.35 (m, 3H), 2.10-2.00 (m, 1H), 1.70-1.50 (m, 10H), 1.50-1.30 (m, 19H); ^{13}C NMR (50 MHz, CD_3OD) δ (ppm): 169.5, 157.8, 157.7, 151.0, 141.5, 1325., 126.7, 126.0, 113.1, 94.5, 91.9, 81.9, 80.3, 79.7, 52.8, 51.9, 51.2, 37.5, 37.2, 30.7, 28.8, 26.1, 24.9; MS (ESI) m/z = 661.4 $[M+H]^+$ (theoretical *m/z* = 661.4).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-((3-benzamidophenyl)imidazol-*N*-methyl)]ethyltriazole (4d). General procedure **A** was applied to compound **3** and to compound **10d** (63.6 mg, 0.216 mmol) and the reaction mixture was stirred at 50°C

for 1h. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 99:1 as the eluent affording desired compound **4d** as a yellow oil in 87% yield (138 mg). R_f = 0.35 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (200 MHz, Acetone-d_6) δ ppm: 9.57 (br s, 1H), 8.27 (br s, 1H), 8.18 (s, 1H), 8.03 (d, J = 8.0 Hz, 3H), 7.76 (d, J = 8.0 Hz, 2H), 7.55-7.45 (m, 5H), 7.30-7.25 (m, 1H), 5.38 (s, 2H), 4.58 (d, J = 4.2 Hz, 2H), 4.15-4.00 (m, 2H), 3.75-3.50 (m, 5H), 2.30-2.20 (m, 1H), 1.70-1.50 (m, 8H), 1.50-1.30 (m, 21H); ^{13}C NMR (50 MHz, Acetone-d_6) δ ppm: 166.3, 157.6, 156.3, 156.0, 140.2, 136.1, 132.3, 129.5, 129.2, 128.4, 125.2, 125.1, 121.5, 119.6, 112.1, 81.5, 79.0, 78.9, 69.8, 66.1, 57.7, 51.3, 49.3, 40.3, 37.4, 37.0, 36.9, 29.4, 25.6, 24.5; MS (ESI): m/z = 813.3 $[M+H]^+$ (theoretical *m/z* 813.3).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-(phenyl-(3-butylurea)-imidazol-*N*-methyl)]ethyltriazole (4e). General procedure **A** was applied to compound **3** and to compound **10g** (64.0 mg, 0.216 mmol). The reaction mixture was stirred at 50°C for 1h. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 98:2 as the eluent affording desired compound **4g** as a yellow solid in 61% yield (96.5 mg); R_f = 0.35 (CH_2Cl_2/MeOH 98:2); ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 8.16 (s, 1H), 8.00-7.90 (m, 1H), 7.75-7.65 (m, 2H), 7.45-7.35 (m, 2H), 7.24 (s, 1H), 5.37 (s, 2H), 4.70-4.50 (m, 2H), 4.25-4.15 (m, 1H), 4.00-3.90 (m, 1H), 3.70-3.55 (m, 2H), 3.50-3.40 (m, 3H), 3.22 (t, J = 8.0 Hz, 2H), 2.20-2.10 (m, 1H), 1.70-1.50 (m, 8H), 1.50-1.25 (m, 25H), 0.97 (t, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm: 157.0, 156.4, 156.3, 149.7, 142.8, 140.0, 128.8, 124.7, 121.1, 118.8, 117.7, 115.5, 113.1, 111.7, 80.8, 80.4, 78.9, 68.9, 53.4, 51.3, 39.2, 36.0, 35.8, 32.0, 27.4, 27.3, 24.7, 23.4, 19.7, 12.8; MS (ESI): m/z = 807.3 $[M+H]^+$ (theoretical *m/z* 807.6).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-((3-(2-naphthamido)phenyl)-imidazol-*N*-methyl)]ethyltriazole (4f). General procedure **A** was applied to compound **3** and to compound **10f** (75.8 mg, 0.216 mmol). The reaction mixture was stirred at 50°C overnight. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 95:5 as the eluent affording desired compound **4f** as a yellow solid in 34% yield (54.5 mg); R_f = 0.35 (CH_2Cl_2/MeOH 95/5); ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 8.54 (s, 1H), 8.20-8.10 (m, 2H), 8.10-7.90 (m, 5H), 7.80-7.70 –m, 2H), 7.65-7.55 (m, 3H), 7.43 (s, 1H), 5.36 (s, 2H), 4.70-4.50 (m, 5H), 4.20-4.10 (m, 1H), 4.00-3.90 (m, 1H), 3.60-3.50 (m, 2H), 2.10-2.00 (m, 1H), 1.70-1.30 (m, 29H); ^{13}C NMR (50 MHz, CD_3OD) δ ppm: 169.0, 157.7, 136.4, 134.0, 133.5, 130.1, 129.4, 129.2, 129.0, 128.8, 127.9, 125.2, 121.3, 113.1, 82.3, 81.7, 80.3, 97.2, 70.2, 52.7, 52.0, 37.4, 37.2, 28.8, 28.7, 26.0, 24.8; MS (ESI): m/z = 863.1 $[M+H]^+$ (theoretical *m/z* 863.2).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-((3-aminophenyl)-imidazol-*N*-methyl)]ethyltriazole (4g). General procedure **A** was applied to compound **3** and to compound **10g** (42.5 mg, 0.216 mmol). The reaction mixture was stirred at 50°C for 1h. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 99:1 as the eluent affording desired compound **4g** as a yellow oil in 71% yield (98.6

mg); R_f = 0.59 (CH_2Cl_2/MeOH 90:10); ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 8.13 (s, 1H), 7.81 (s, 1H), 7.45 (s, 1H), 7.20-7.00 (m, 3H), 6.70-6.60 (m, 1H), 5.33 (s, 2H), 4.65-4.50 (m, 2H), 4.50-4.40 (m, 1H), 4.00-3.90 (m, 1H), 3.75-3.60 (m, 2H), 3.50-3.40 (m, 3H), 2.10-2.00 (m, 1H), 1.75-1.45 (m, 29H); ^{13}C NMR (50 MHz, CD_3OD) δ ppm: 167.4, 158.0, 157.9, 157.7, 154.5, 150.1, 147.7, 138.8, 130.4, 126.0, 119.4, 116.2, 113.1, 90.2, 82.2, 81.8, 76.5, 70.3, 59.7, 52.7, 52.0, 50.3, 42.9, 37.4, 28.8, 26.0, 24.8; MS (ESI): m/z = 709.4 $[M+H]^+$ (theoretical *m/z* 709.5).

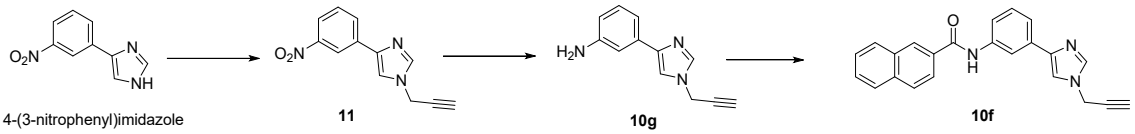
1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-(2-oxo-2-((4-phenylthiazol-2-yl)amino)-*N*-ethylpropanamide)]ethyltriazole (4h). To a solution of **3** (20.0 mg, 0.04 mmol) in DMF (3.0 mL), compound **10h** (9.0 mg, 0.044 mmol, 1.1 eq.), CuI (152.4 mg, 0.8 mmol, 2.0 eq.) and DiPEA (0.3 mL, 2.0 mmol, 5.0 eq.) were added. The reaction mixture was stirred at room temperature during 5 h. The solvent was evaporated under reduced pressure and the reaction mixture was then solubilized in CH_2Cl_2 and washed with water. The organic phases were dried with Na_2SO_4, filtered and evaporated under reduced pressure. The mixture was purified by flash chromatography on a silica gel column using a mixture of CH_2Cl_2/ MeOH (9:1) to give **4h** as a white solid in 53% yield (20.0 mg). R_f = 0.4 (CH_2Cl_2/MeOH 9:1); ^{1}H RMN (400MHz, CD_3OD) δ (ppm): 7.90-7.80 (m, 3H), 7.40-7.30 (m, 3H), 7.30-7.25 (m, 1H), 4.60-4.50 (m, 2H), 4.20-4.10 (m, 3H), 4.00-3.90 (m, 1H), 3.75-3.65 (m, 1H), 3.60-3.40 (m, 4H), 3.10-3.00 (m, 2H), 2.75-2.65 (m, 2H), 2.15-2.05 (m, 1H), 1.70-1.60 (m, 8H), 1.50-1.40 (m, 21H); ^{13}C (100MHz, CD_3OD) δ (ppm): 167.1, 157.7, 135.9, 129.6, 128.9, 127.1, 113.1, 108.6, 81.9, 80.3, 74.0, 70.2, 55.5, 52.7, 51.8, 43.4, 37.4, 37.3, 36.0, 30.8, 28.8, 28.7, 26.1, 24.9, 24.8, 22.4; MS (ESI) *m/z* = 768.2 $[M+Na]^+$ (theoretical *m/z* 768.9).

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-(1S,3R,4S,5R,6R)-deoxystreptamine-4-*O*-[4-((3-benzamidophenyl)imidazol-*N*-methyl)]ethyltriazole (8). To a solution of compound **7** (50 mg, 0.0987 mmol) in CH_3CN (4 mL) were added compound **7** (37.4 mg, 0.124 mmol, 1.3 eq.), CuI (44.6 mg, 0.234 mmol, 2eq.) and DIPEA (125 µL, 0.705 mmol, 6 eq.) and the reaction mixture was stirred at 50°C for 1h. After removal of the solvent under reduced pressure, flash chromatography was performed using a mixture CH_2Cl_2/MeOH 99:1 as the eluent affording desired compound **8** as a yellow oil in 55% yield (44 mg). R_f = 0.35 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 8.14 (s, 1H), 7.95 (d, *J* = 8.0 Hz, 3H), 7.90 (br s, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.60-7.50 (m, 5H), 7.40-7.30 (m, 1H), 5.36 (s, 2H), 4.65-4.60 (m, 2H), 4.20-4.10 (m, 21), 4.00-3.95 (m, 1H), 3.70-3.50 (m, 2H), 3.50-3.35 (m, 3H), 2.10-2.00 (m, 1H), 1.70-1.50 (m, 8H), 1.50-1.30 (m, 21H); ^{13}C NMR (50 MHz, CD_3OD) δ ppm: 167.8, 158.8, 145.5, 139.8, 138.4, 136.2, 135.0, 134.9, 131.8, 129.4, 129.1, 128.6, 128.3, 125.3, 120.2, 118.8, 113.6, 80.6, 80.3, 77.4, 76.7, 66.6, 51.9, 51.7, 47.1, 41.6, 35.1, 28.4, 25.5, 22.3; MS (ESI): m/z = 813.3 $[M+H]^+$ (theoretical *m/z* 813.3).

2-(1S,3R,4S,5R,6R)-deoxystreptamine-4-*O*-[4-((3-benzamidophenyl)imidazol-*N*-methyl)]ethyltriazole (9). Compound **8** (44 mg, 0.0542 mmol) was dissolved in CH_2Cl_2 (1 mL) and H_2O (0.5 mL) in the presence of TFA (208 µL, 2.71 mmol, 50 eq.). Pure compound **9** was obtained after precipitation in CH_2Cl_2 and Chelex resin treatment as a white solid in 97% yield (40.0 mg). Retention

time 2.78 min; ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 8.28 (s, 1H), 8.00-7.95 (m, 3H), 7.70-7.50 (m, 8H), 5.60 (s, 2H), 4.72 (t, J = 2.0 Hz, 2H), 4.50-4.45 (m, 1H), 4.20-4.15 (m, 1H), 3.45-3.35 (m, 4H), 3.15-3.10 (m, 1H), 2.45-2.40 (m, 1H), 1.80-1.75 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ ppm: 169.5, 141.4, 136.4, 133.6, 131.4, 130.9, 130.1, 129.1, 123.5, 83.0, 77.7, 75.1, 73.5, 72.2, 49.9, 49.7, 49.6, 30.2; MS (ESI) *m/z* 533.3 (theoretical 533.3).

Synthesis of *N*-(3-(1-(prop-2-yn-1-yl)-1*H*-imidazol-4-yl)phenyl)-2-naphthamide (10f).

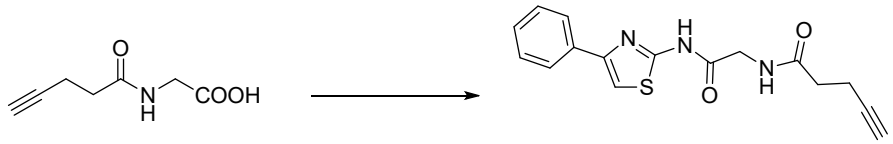


4-(3-Nitrophenyl)-*N*-1-propargylimidazole (11). To a solution of 4-(3-nitrophenyl)imidazole[3] (1 g, 5.3 mmol) in acetone (50 mL) was added K_2CO_3 (2.2 g, 15.9 mmol). After stirring at 60°C for 30min, TBAI (98 mg) and 80% weight solution of propargyl bromide in toluene (0.95 mL, 10.6 mmol) were added respectively. The reaction mixture was stirred at 60°C under reflux overnight. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography using a mixture CH_2Cl_2/MeOH 95:5 as the eluent to afford compound **11** as a yellow solid in 56% yield (67 mg). R_f = 0.42 (CH_2Cl_2/MeOH 98:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.56 (br s, 1H), 8.20-8.10 (m, 2H), 7.66 (s, 1H), 7.60-7.50 (m, 2H), 4.79 (d, J = 2.6 Hz, 2H), 2.56 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 146.5, 140.7, 137.5, 135.9, 130.7, 129.7, 121.6, 119.7, 115.9, 76.1, 75.7, 36.9; MS (ESI) *m/z* = 228.0 $[M+H]^+$ (theoretical *m/z* 228.0).

4-(3-Aminophenyl)-*N*-1-propargylimidazole (10g). A mixture of **11** (100 mg, 0.53 mmol), iron powder (148 mg, 2.65 mmol) and NH_4Cl (113 mg, 2.12 mmol) was refluxed in aqueous ethanol (4.5 mL of alcohol and 1.25 mL of water) at 80°C for 1h. The reaction mixture was treated with saturated aqueous $NaHCO_3$ and filtered through filter paper, then concentrated under reduced pressure. The orange solution was concentrated *in vacuo* and the residue was extracted with EtOAc (5 mL×2). The organic phase was dried over $MgSO_4$ and then concentrated to afford **10g** in 94% yield (98.2 mg). R_f = 0.33 (CH_2Cl_2/MeOH 98:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.59 (s, 1H), 7.27 (s, 1H), 7.20-7.10 (m, 3H), 6.60-6.50 (m, 1H), 4.74 (br s, 2H), 2.51 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 146.7, 142.7, 136.7, 134.8, 129.5, 115.4, 114.5, 113.8, 111.6, 74.9, 36.5; MS (ESI) *m/z* = 198.0 $[M+H]^+$ (theoretical *m/z* 198.1).

***N*-(3-(1-(prop-2-yn-1-yl)-1*H*-imidazol-4-yl)phenyl)-2-naphthamide (10f).** To a solution of **10g** (77 mg, 0.34 mmol) in CH_2Cl_2 (1.6 mL) were added naphthoyl chloride (77.8 mg, 0.4 mmol), pyridine (41 μL, 0.51 mmol). The reaction mixture was stirred at room temperature for 3h. The solvent was then concentrated under reduced pressure and the obtained residue purified on a silica gel column using a mixture CH_2Cl_2/acetone 98:2 as the eluent to afford compound **10g** as a yellow solid in 67% yield (80 mg). R_f = 0.51 (30% CHX/EtOAc 7/3); ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 8.80 (s, 1H), 8.54 (s, 1H), 8.22 (s. 1H), 8.05-7.95 (m, 5H), 7.75-7.70 (m, 1H), 7.65-7.60 (m, 2H), 7.55-7.50 (m, 1H), 5.16 (d, J =

2.6 Hz, 2H), 3.26 (t, J = 2.6 Hz, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ ppm: 169.0, 149.2, 148.2, 147.6, 141.7, 140.9, 136.4, 134.0, 133.1, 130.9, 130.1, 129.5, 129.2, 129.1, 128.8, 128.0, 125.1, 122.9, 122.6, 119.2, 78.1, 76.4, 39.3; MS (ESI): *m/z* = 352.3 $[M+H]^+$ (theoretical *m/z* 352.2).



N-(1-oxo-4-pentyn-1-yl)-glycine **10h**

***N*-(2-oxo-2-(4-phenylthiazol-2-ylamino)ethyl)pent-4-ynamide (10h).** Compound **10h** was synthesized starting from *N*-(1-oxo-4-pentyn-1-yl)-glycine (250 mg, 1.61 mmol) that was dissolved in DMF (10 mL). Then, HOSu (204 mg, 1.1 eq) and EDC (339 mg, 1.1 eq) were added. The mixture was stirred at room temperature during 30 min. Then, 2-amino-4-phenylthiazole (312 mg, 1.1 eq) was added. The mixture was stirred overnight at room temperature. The solvent was then removed under reduced pressure and the crude product washed with DCM and water (3 x 50 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The product was purified by flash chromatography on a silica gel column using a mixture of DCM/MeOH 99 :1 and desired compound **10h** was obtained as a yellow solid in 53% yield (266 mg). R_f = 0.24 (DCM/MeOH 99:1); ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 7.80-7.70 (m, 2H), 7.40-7.30 (m, 3H), 7.30-7.25 (m, 1H), 4.13 (s, 2H), 2.60-2.50 (m, 4H), 2.30 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm: 174.7, 169.4, 159.1, 151.3, 135.9, 129.6, 128.9, 127.0, 108.7, 83.5, 70.3, 43.4, 35.8, 15.5; MS (ESI): *m/z* = 352.3 $[M+H]^+$ (theoretical *m/z* 352.2).

Biochemical procedures

RNA and biochemicals. The buffers and solutions used in the fluorescence experiments were filtered through Millipore filters (0.22 mm; GP ExpressPLUS membrane). Human recombinant Dicer enzyme (Genlantis) had a concentration of 0.5 U/µL. 1. Tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl) 20 mM (pH 7.4), containing 12 mM NaCl, 3 mM $MgCl_2$, and 1 mM DTT) was used for the FRET assays and K_D determination.

RNA oligonucleotides. Oligonucleotides were purchased from IBA GmbH (Goettingen, Germany). RNA folding was performed in TRIS buffer upon incubation at 90°C during 2 minutes, 4°C during 10 minutes and slowly returning to room temperature during 15 minutes.

For pre-miR-372:

5′-FAM-

GUGGGCCUCAAAUGUGGAGCACUAUUCUGAUGUCCAAGUGGAAAGUGCUGCGACAUU

UGAGCGUCAC-

3′-DABCYL (ODN1)

5′-FAM-
GUGGGCCUCAAAUGUGGAGCACUAUUCUGAUGUCCAAGUGGAAAGUGCUGCGACAUU
UGAGCGUCAC-
3′ (ODN2)
For pre-miR-21
5′-FAM-
UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGA
UGGGCUGUCUGACA-3′-DABCYL (ODN3)
5′-FAM-
UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGA
UGGGCUGUCUGACA-3′ (ODN4)
For pre-miR-18a
5′-FAM-
UGUUCUAAGGUGCAUCUAGUGCAGAUAGUGAAGUAGAUUAGCAUCUACUGCCCUAAG
UGCUCCUUCUGGCA-3′-DABCYL (ODN5)
5′-FAM-
UGUUCUAAGGUGCAUCUAGUGCAGAUAGUGAAGUAGAUUAGCAUCUACUGCCCUAAG
UGCUCCUUCUGGCA-3′ (ODN6)
For pre-miR-148a
5′-FAM-
GAGGCAAAGUUCUGAGACACUCCGACUCUGAGUAUGAUAGAAGUCAGUGCACUACAGA
ACUUUGUCUC-3′-DABCYL (ODN7)
5′-FAM-
GAGGCAAAGUUCUGAGACACUCCGACUCUGAGUAUGAUAGAAGUCAGUGCACUACAGA
ACUUUGUCUC-3′ (ODN8)
DNA duplex sequence
5′-CGTTTTAAATTTTGC-3′ (ODN9) and 5′-GCTTTTAAATTTTGC-3′

FRET Dicer assay.

The Dicer assay was performed in 384-well plates (Greiner bio-one) in a final volume of 40 µL by using a 5070 EpMotion automated pipetting system (Eppendorf). Each experiment was performed in duplicate and repeated three times. A beacon of ODN1, ODN3, ODN5 or ODN7 at 50 nM was used in each well and the reaction mixtures containing inhibitors were preincubated at room temperature for 30 min. Human recombinant Dicer (0.25 U; Genlantis) or *Escherichia coli* RNase III (0.25U, Ambion) were added to the reaction mixtures. When cell lysates were used instead of recombinant enzymes, HEK293 whole cell lysates were purchased from Tebu-Bio. For the IC_{50} experiments, each ligand was added in 12 dilutions (0.244 pM–500 mM). The fluorescence increase was measured after 4 h incubation at 37°C

on a GeniosPro (Tecan) with excitation and emission filters of l=485±10 and 535±15 nm, respectively. Each point was measured (10x) with an integration time 500 ms and was averaged. The inhibition data were analyzed by using Prism 5 (GraphPad Software) by using nonlinear regression that followed the equation: Y = bottom + (top - bottom)/1+ $^{10[(\text{Log IC50-X}) \text{ x hills slope}]}$).

Binding experiments and K_D determination.

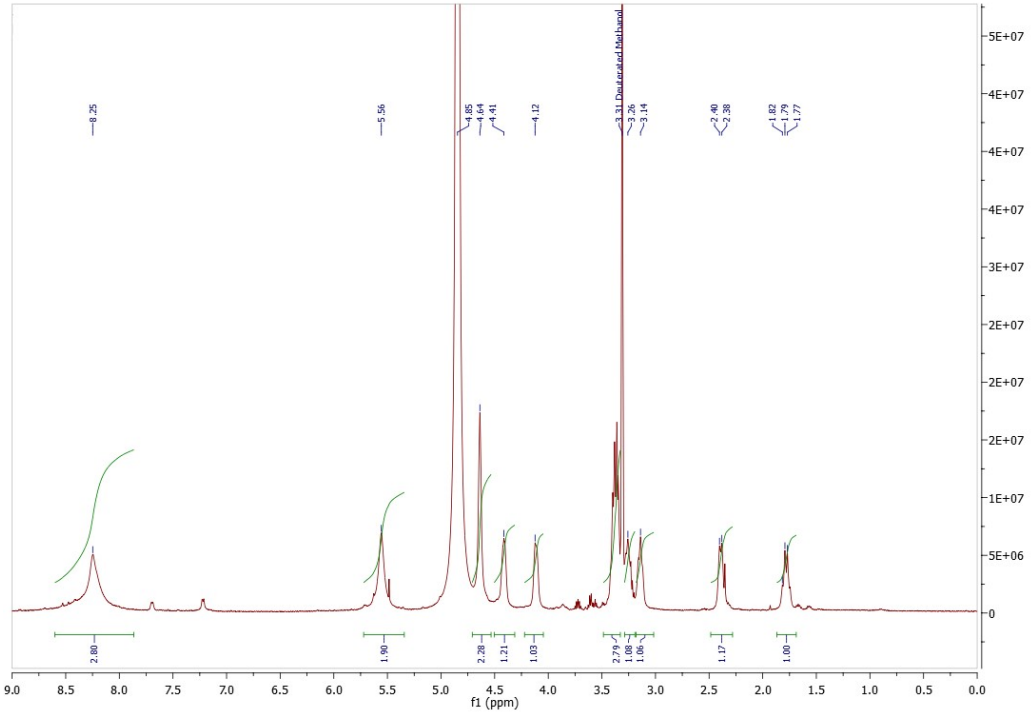
Binding experiments were performed in 384-well plates (Greiner bio-one) in a final volume of 60 mL by using a 5070 EpMotion automated pipetting system (Eppendorf). Each experiment was performed in duplicate and repeated at least three times. A beacon (ODN2, ODN4, ODN6 and ODN8) of 10 nM was used in each well. Each ligand was added in 15 dilutions (0.030 nM–0.5 mM). The fluorescence increase measured after 4 h on a GeniosPro (Tecan) with excitation and emission filters of l=485±10 and 535±15 nm, respectively. Each point was measured (10x) with an integration time of 500 ms and was averaged. The binding data were analyzed by using Graphpad Prism 5 software. Unless otherwise stated, the binding profiles were well modeled by using a simple model that assumed 1:1 stoichiometry.

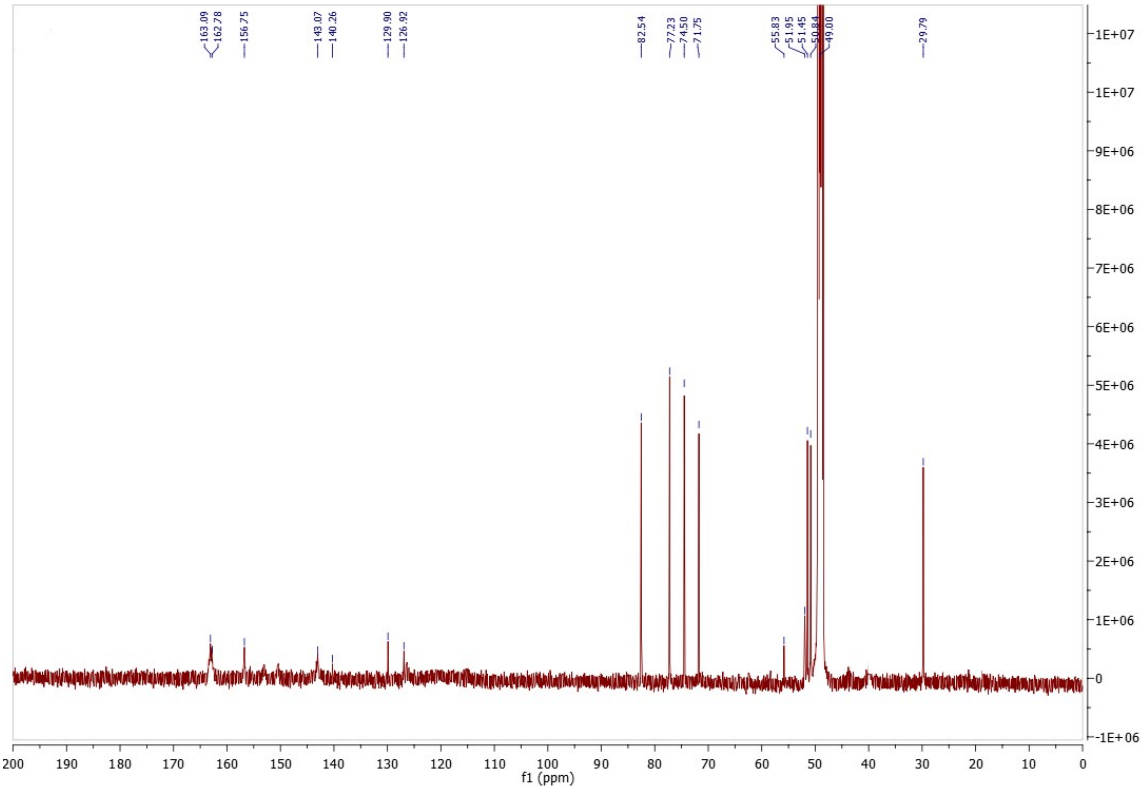
In the competition experiments, 100 eq. of tRNA structured as pre-miR-372 beacon were added to the RNA solution and 100 eq. of duplex DNA (mixture of ODN9 and ODN10 incubated at 90°C during 5 minutes then slowly returned at room temperature) were added to the RNA solution.

Molecular modeling and docking.

The MC-Fold/MC-Sym pipeline (http://www.major.iric.ca/MC-Pipeline/) is a web-hosted service for RNA secondary and tertiary structure prediction. The pipeline consists in uploading RNA sequence to MC-Fold, which output secondary structures that are directly input to MC-Sym, which outputs tertiary structures. Pre-miRNA sequences were obtained from the miRBase database (http://www.mirbase.org/). The hairpin loop of pre-miR-372 was chosen to predict the 3D structure using the MC-Fold/MC-Sym pipeline. Energy optimization was further conducted on the 3D model using the TINKER Molecular Modeling Package (http://dasher.wustl.edu/tinker/). For docking with AutoDock,20 polar hydrogen atoms, Kollman united charges and solvent parameters were applied to the RNA using pmol2q script (http://www.sourcefiles.org/Scientific/Biology/Proteins/pmol2q_2.3.0.tar.gz). This script converts the .pdb file format of the RNA template to the .pdbqt file format that is compatible with AutoDock program version 4 (http://autodock.scripps.edu/). Pre-miR-372/**9** (or **Neo-D_3**) molecular docking was conducted using AutoDock program version 4. The rotational bonds of the ligand were treated as flexible, whereas the receptor was kept rigid. Grid box was fixed in order to include the entire RNA sequence. RNA-ligand interactions were analyzed and visualized using Discovery Studio Visualizer version 4.1 (http://accelrys.com/products/discoverystudio/).

Figure S5. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **5a**.





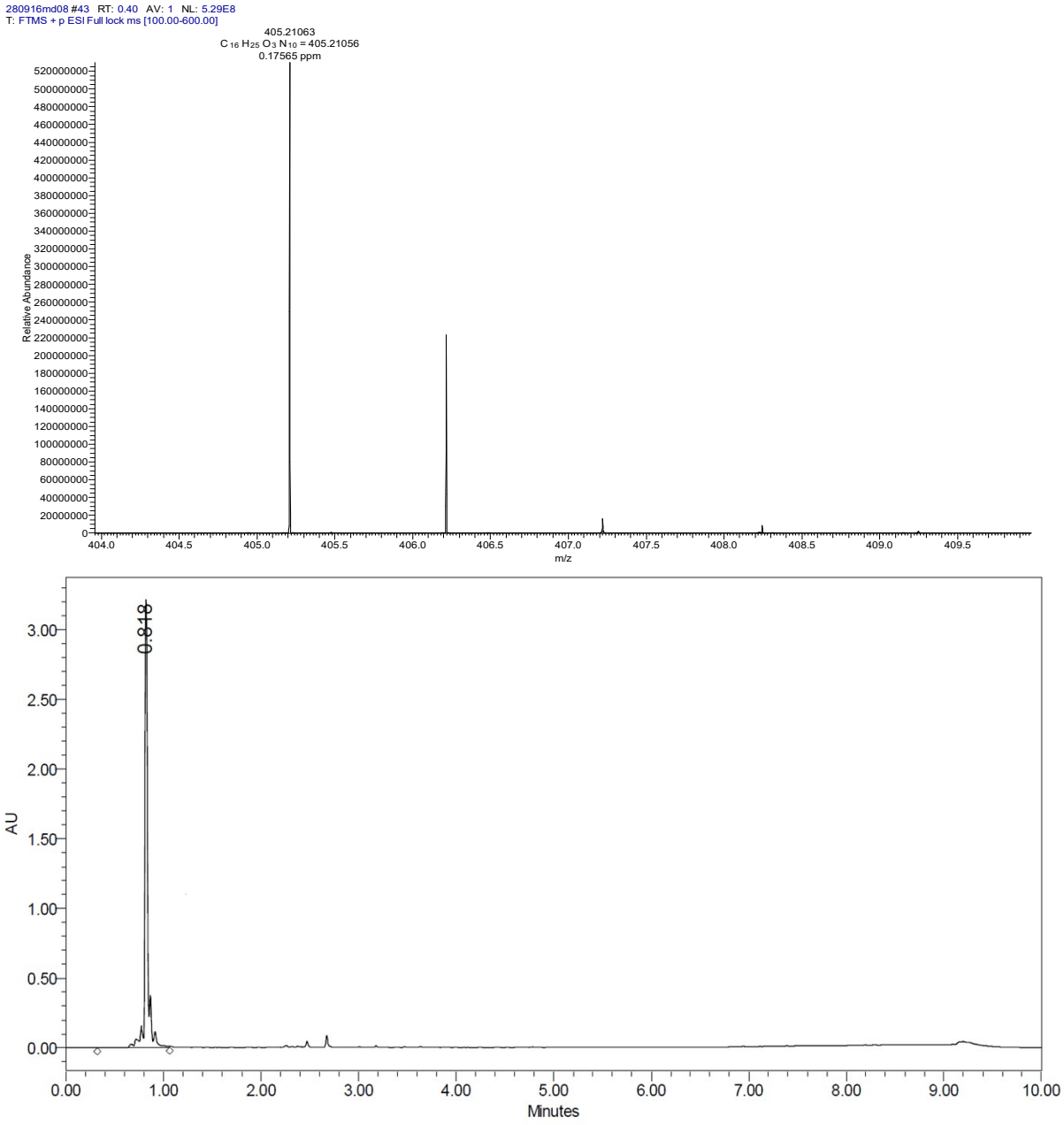
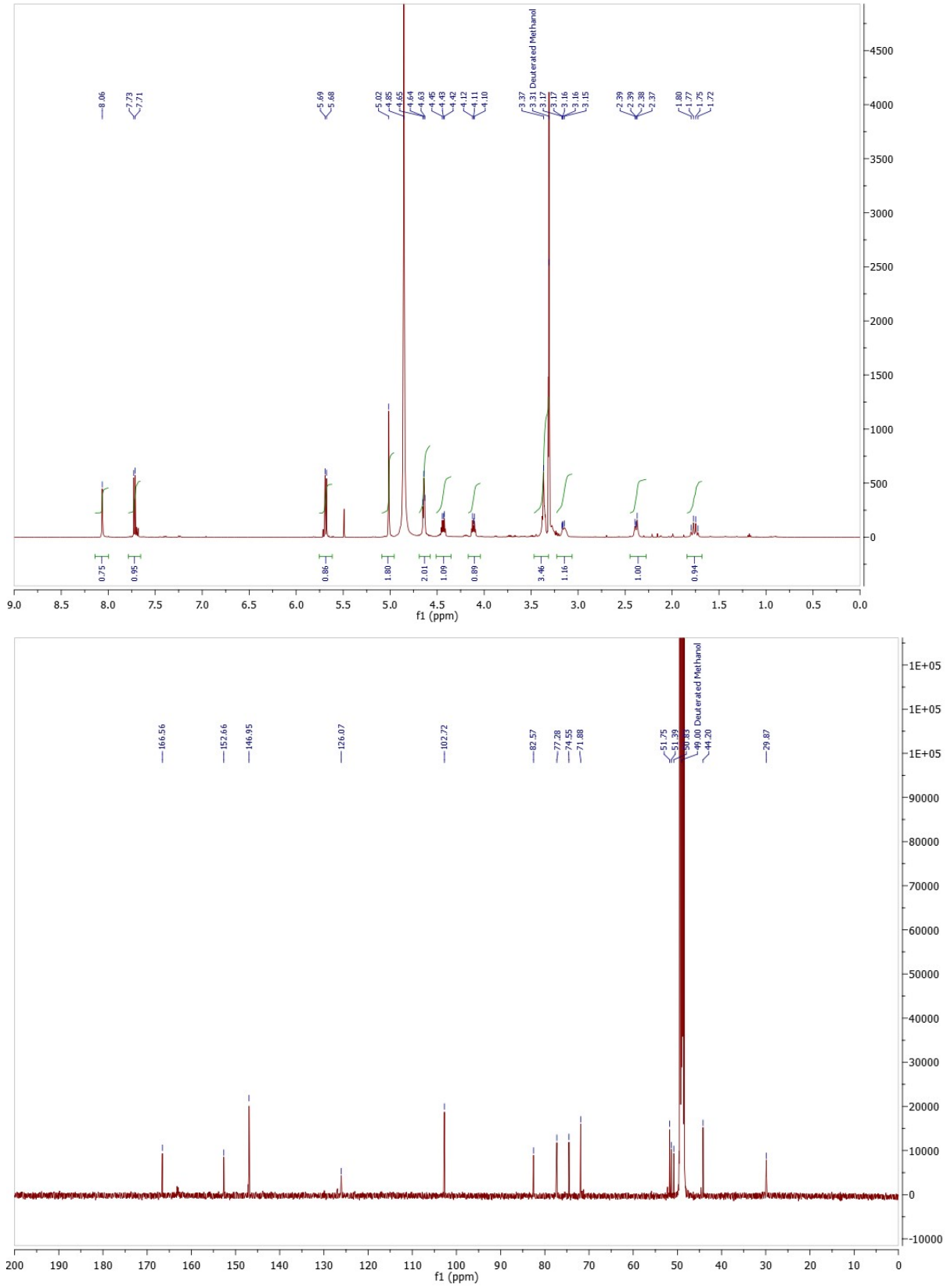

280916md08 #43 RT: 0.40 AV: 1 NL: 5.29E8
T: FTMS + p ESI Full lock ms [100.00-600.00]
405.21063
C 16 H25 O3 N10 = 405.21056
0.17565 ppm
Relative Abundance
m/z
0.818
AU
Minutes

Figure S6. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **5b**.



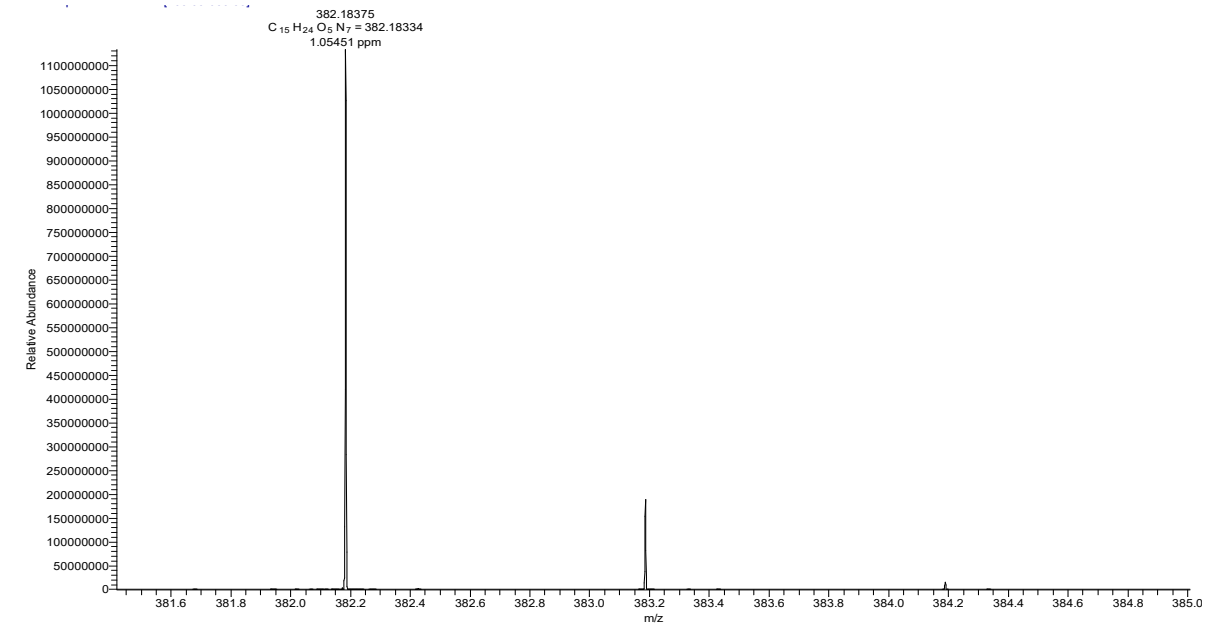
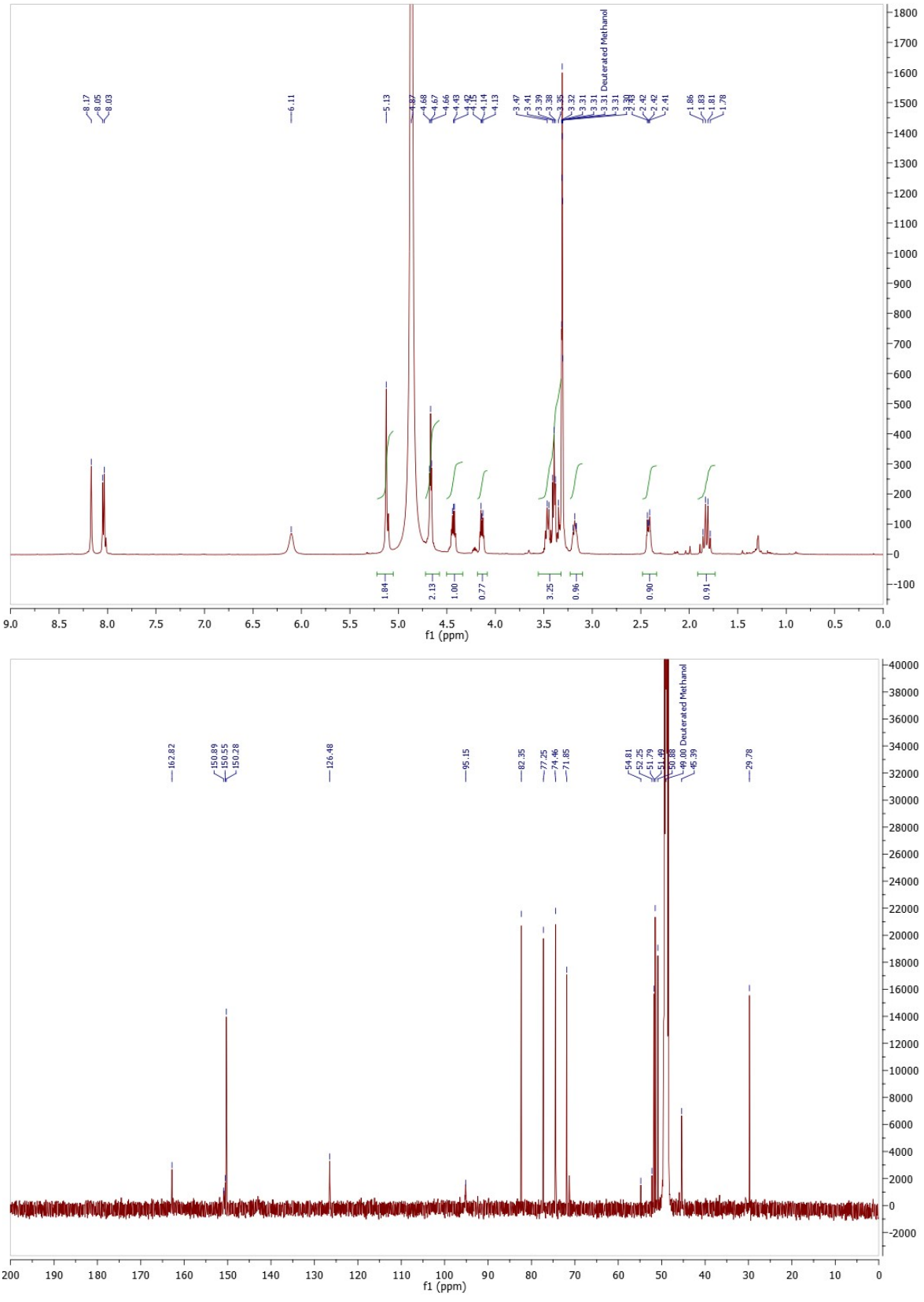
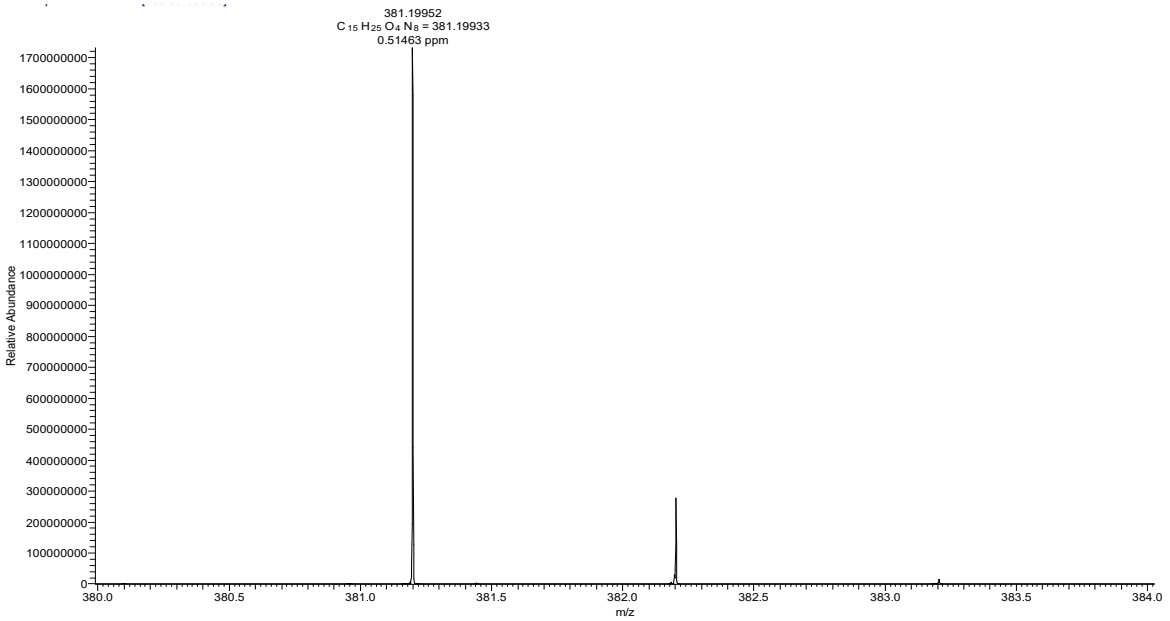

382.18375
C 15 H 24 O 5 N 7 = 382.18334
1.05451 ppm
Relative Abundance
m/z

Figure S7. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **5c**.




381.19952
C 15 H25 O4 N8 = 381.19933
0.51463 ppm
Relative Abundance
m/z

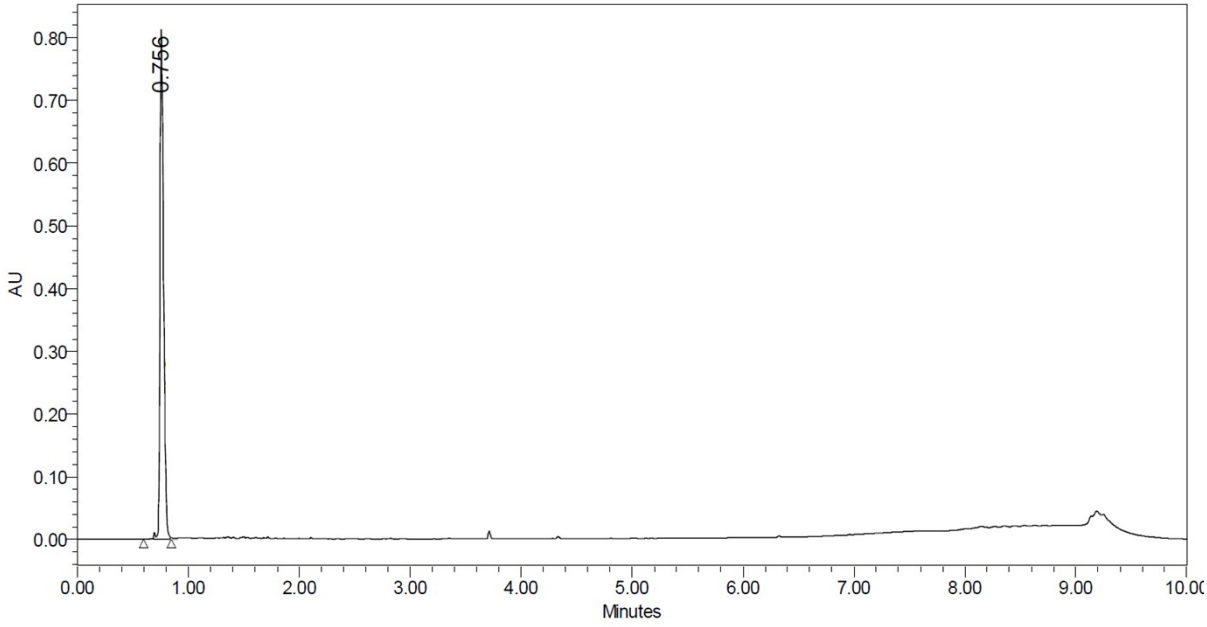
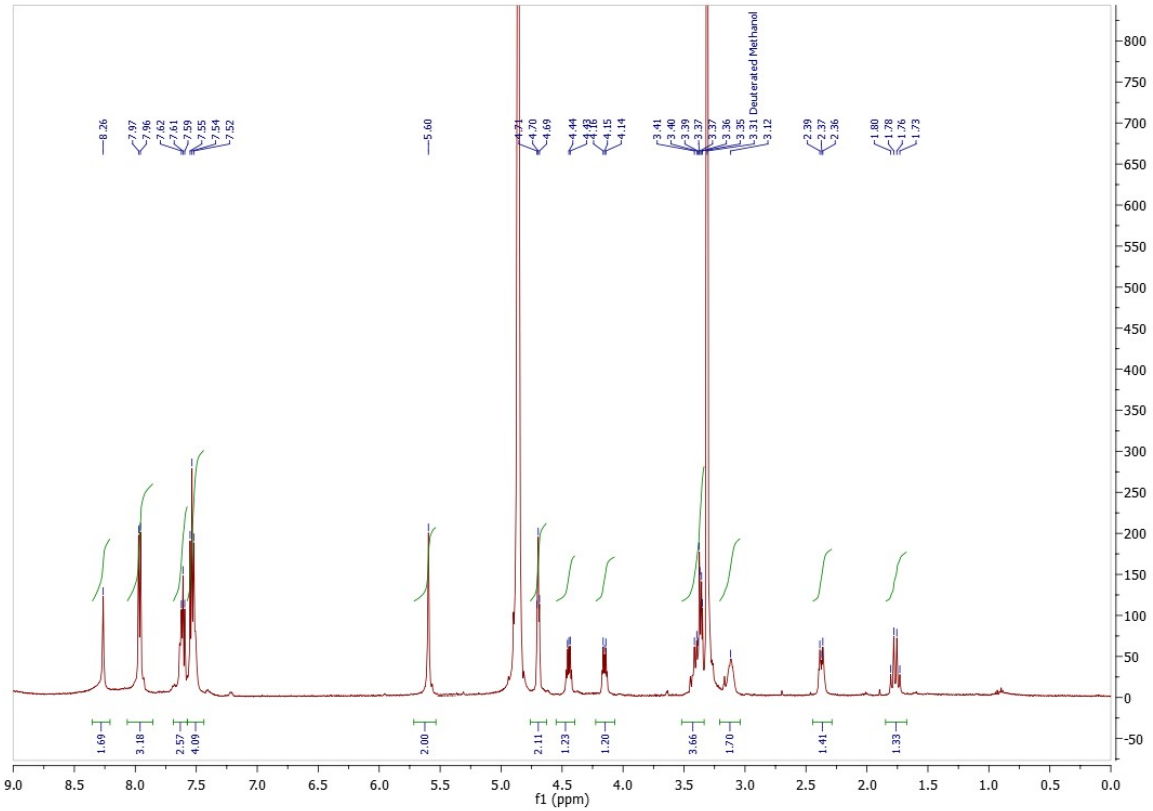
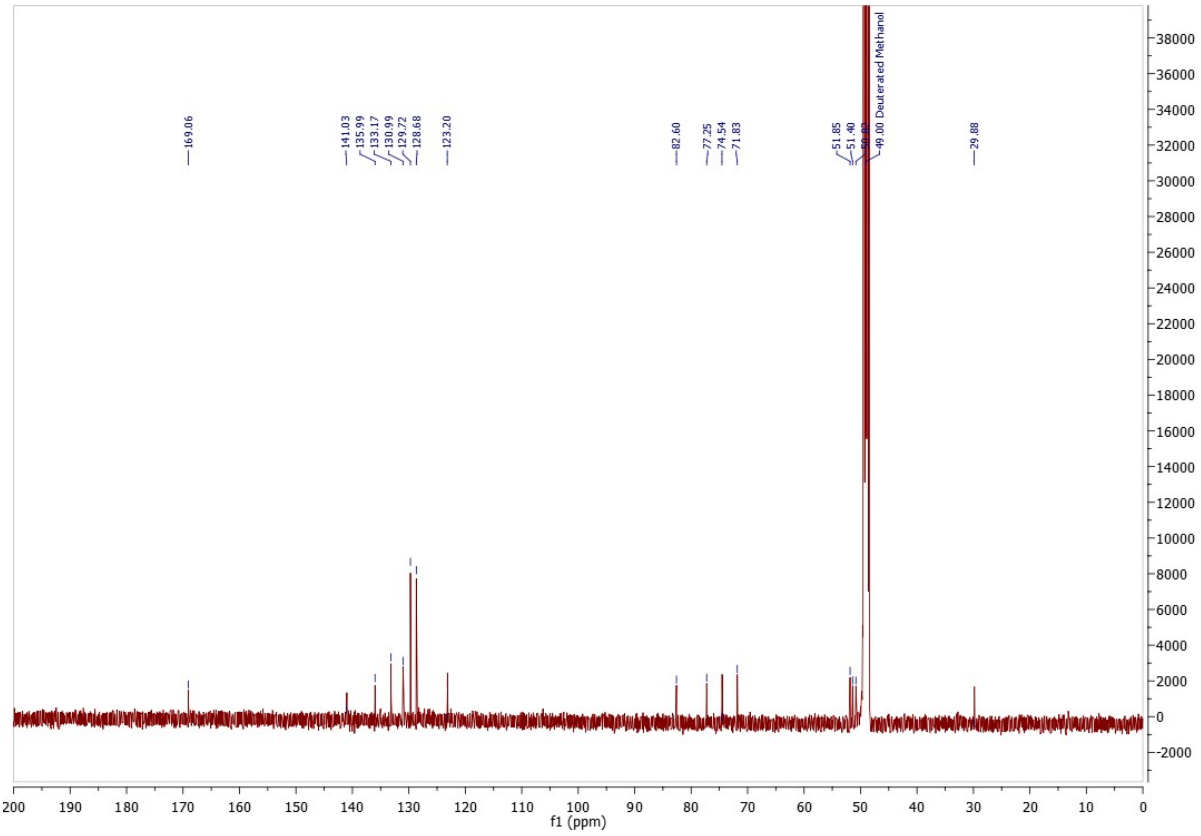
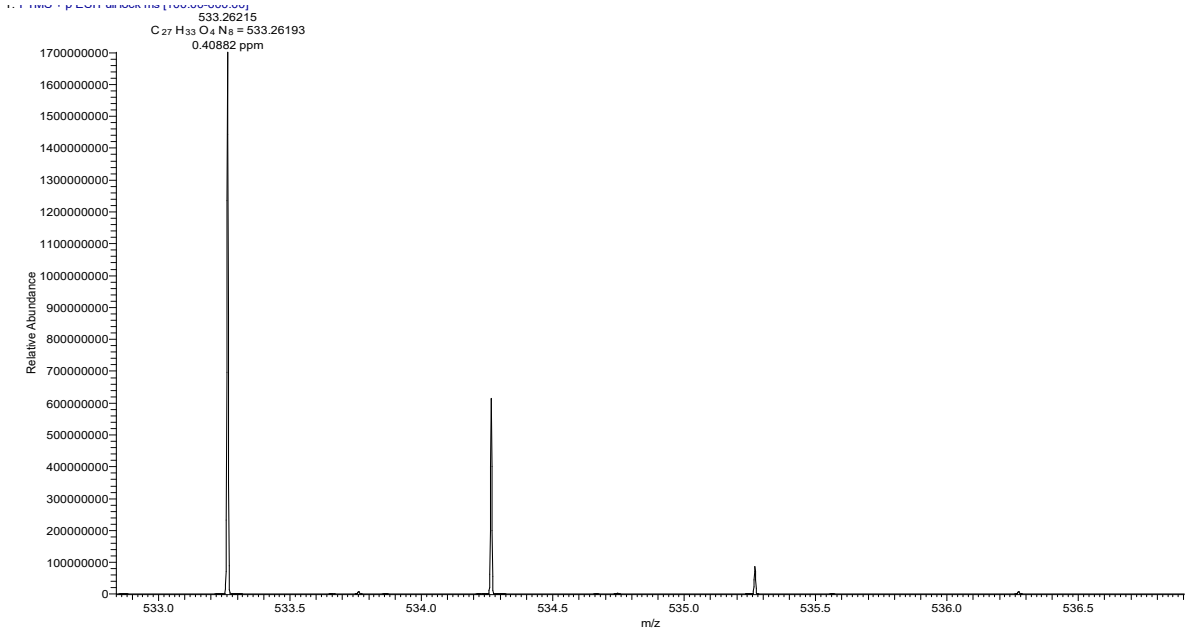

0.766
AU
Minutes

Figure S8. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **5d**.






533.26215
C 27 H 33 O 4 N 8 = 533.26193
0.40882 ppm
Relative Abundance
m/z

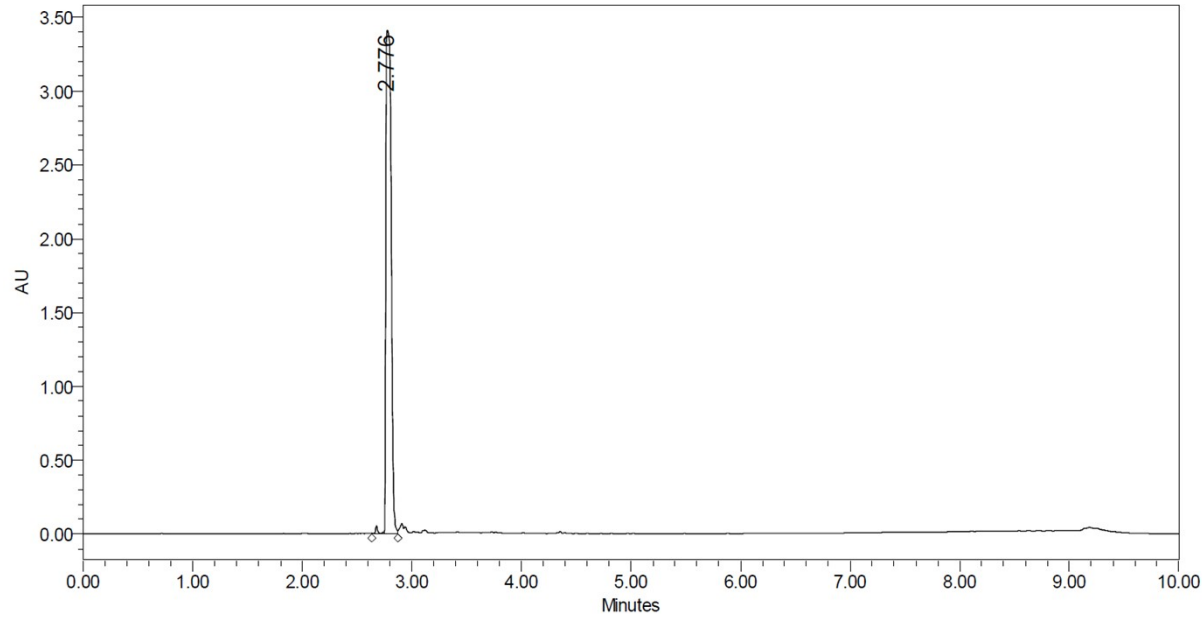
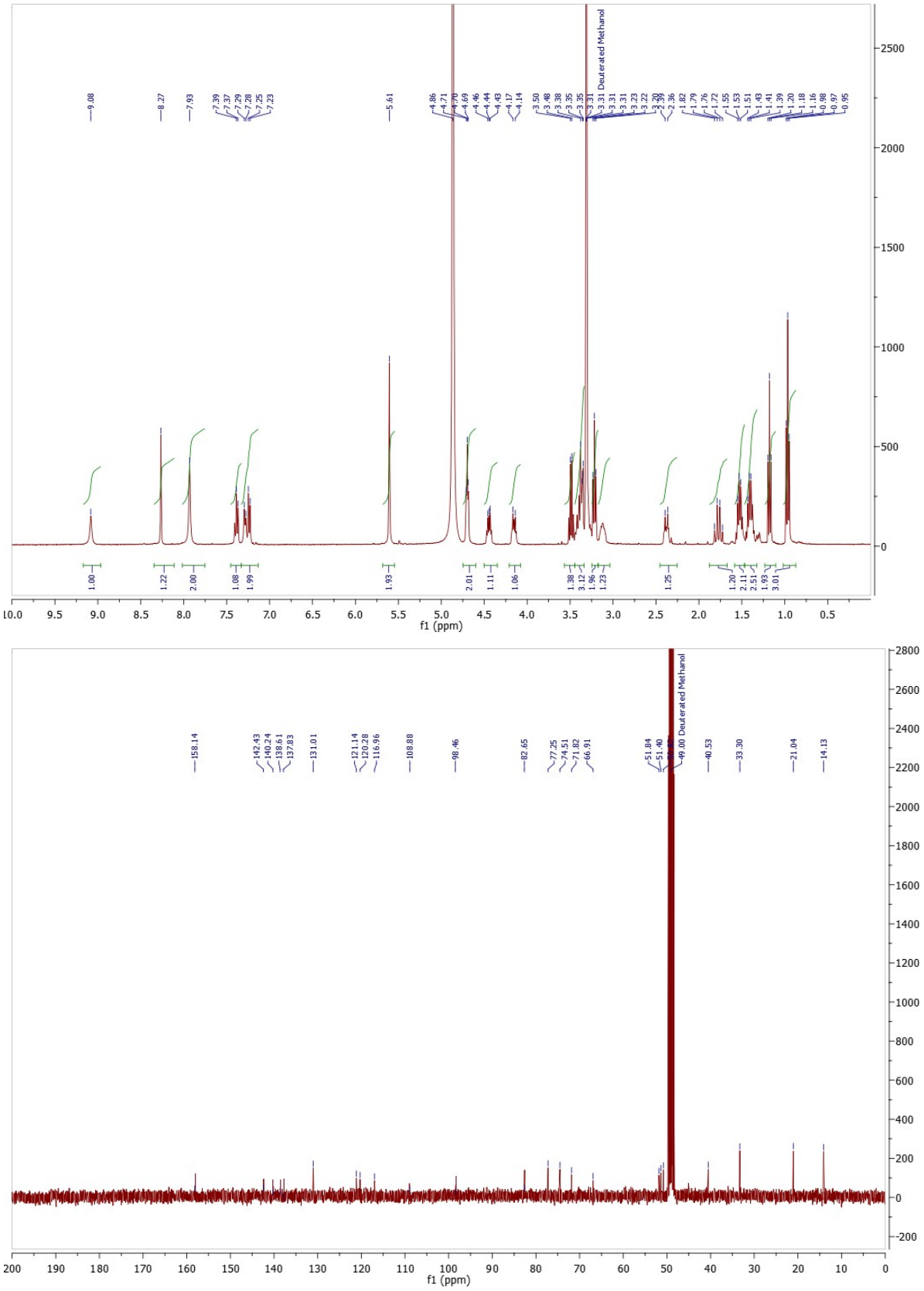
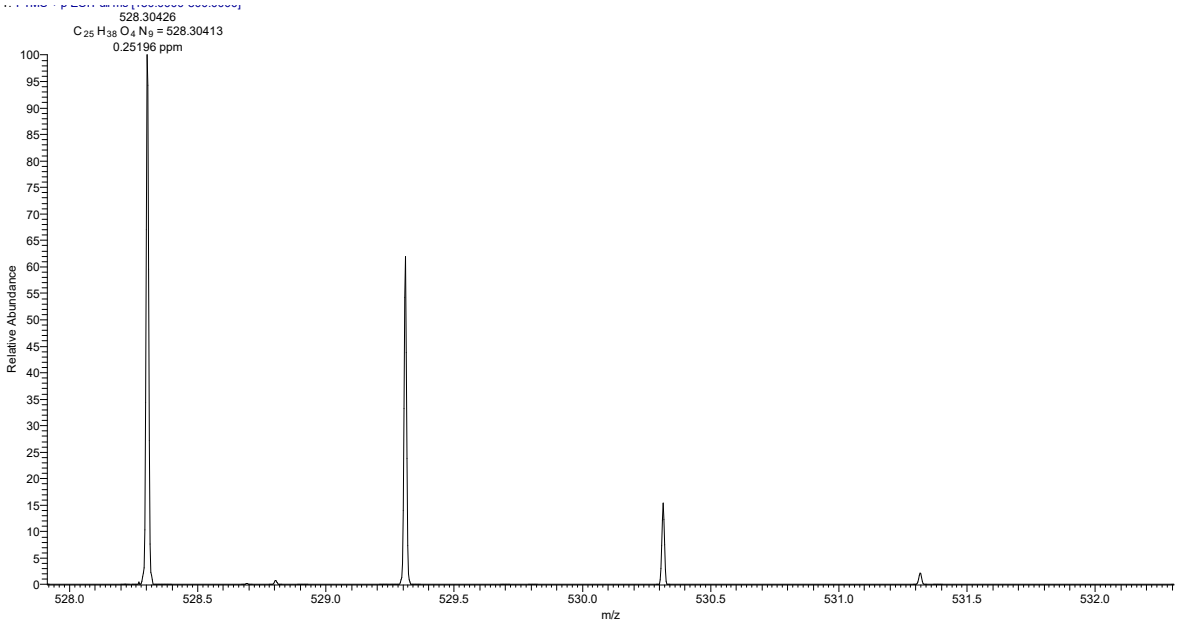

2.776
AU
Minutes

Figure S9. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **5e**.




528.30426
C25 H38 O4 N9 = 528.30413
0.25196 ppm
Relative Abundance
m/z

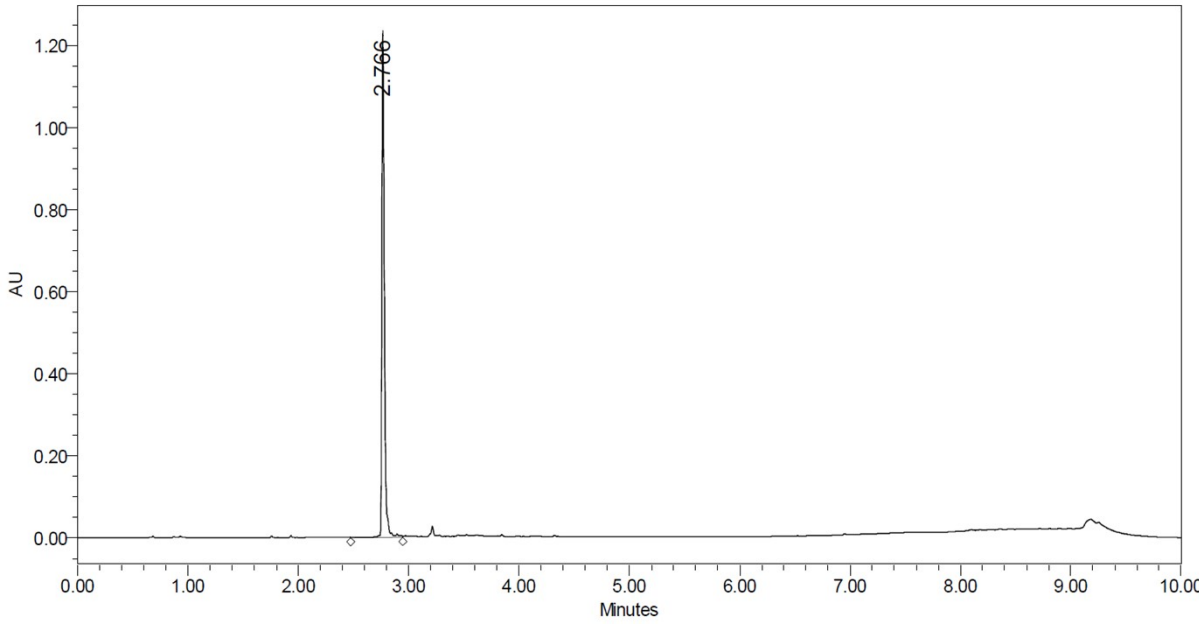
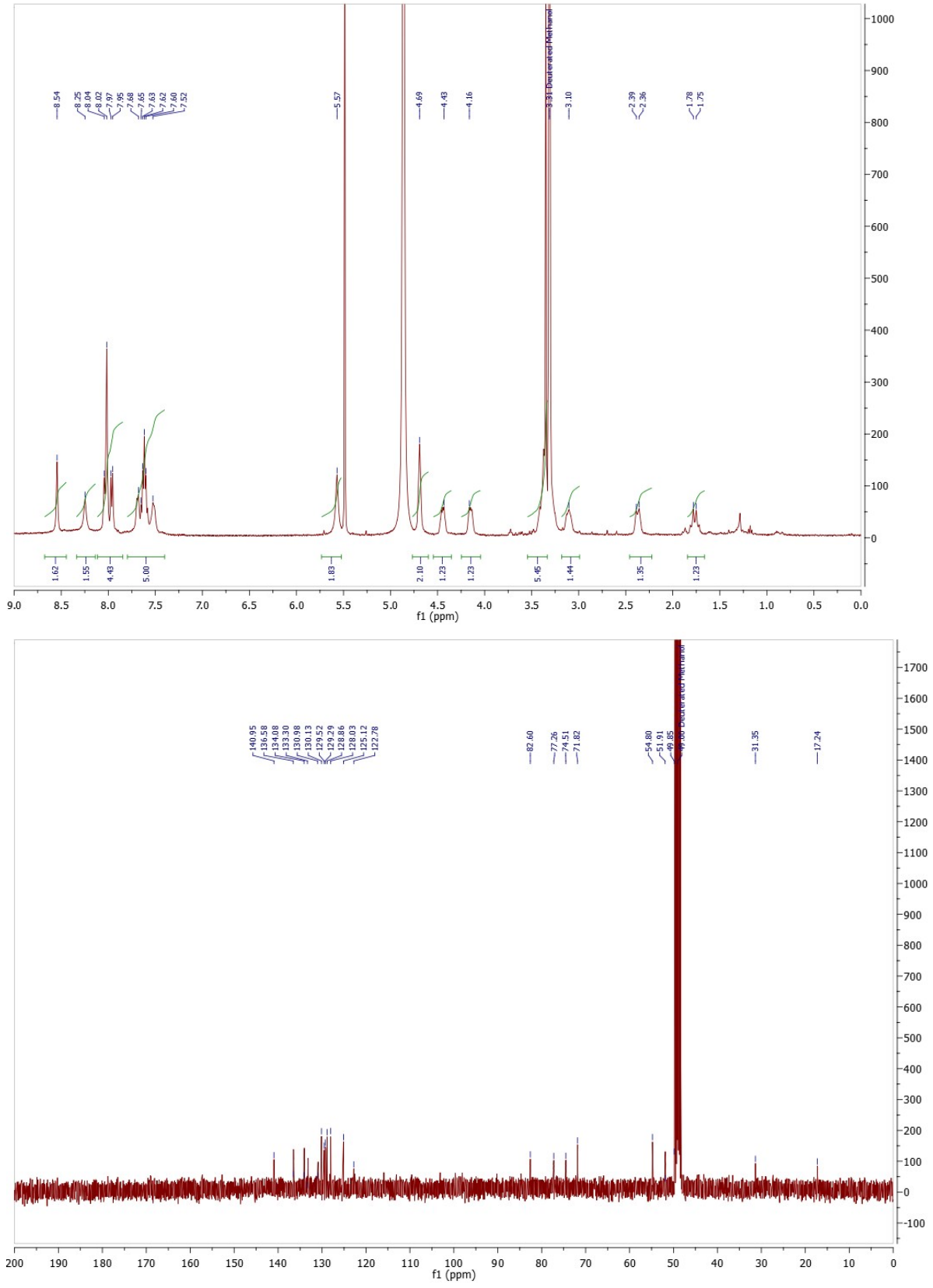

2.766
AU
Minutes

Figure S10. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **5f**.



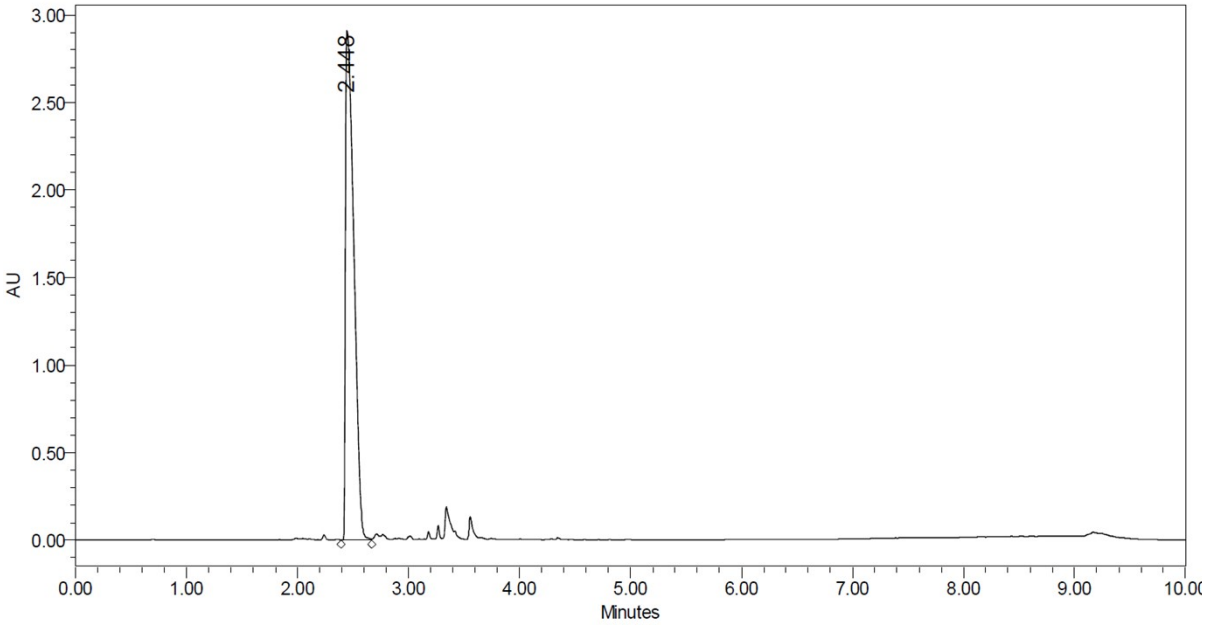
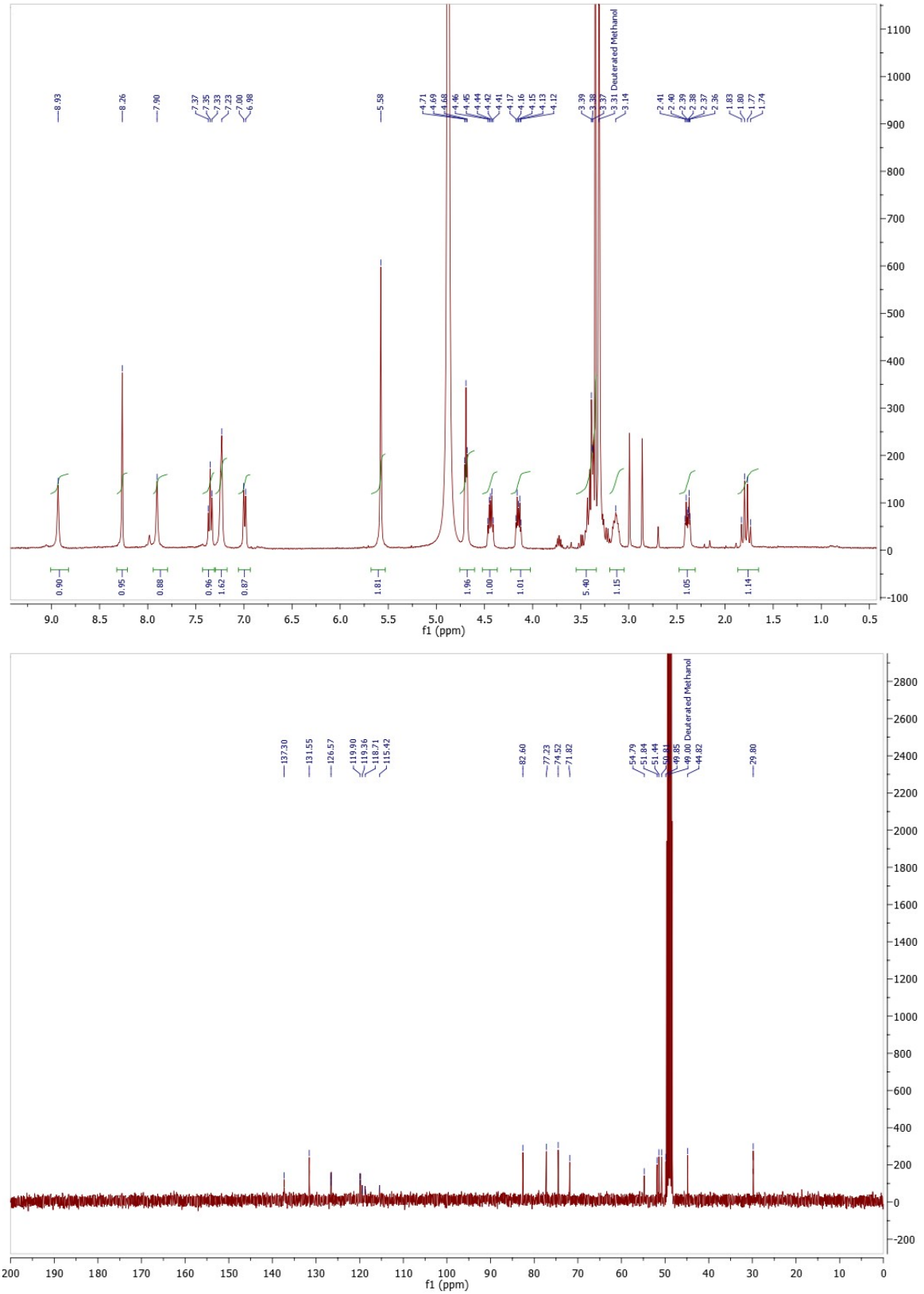
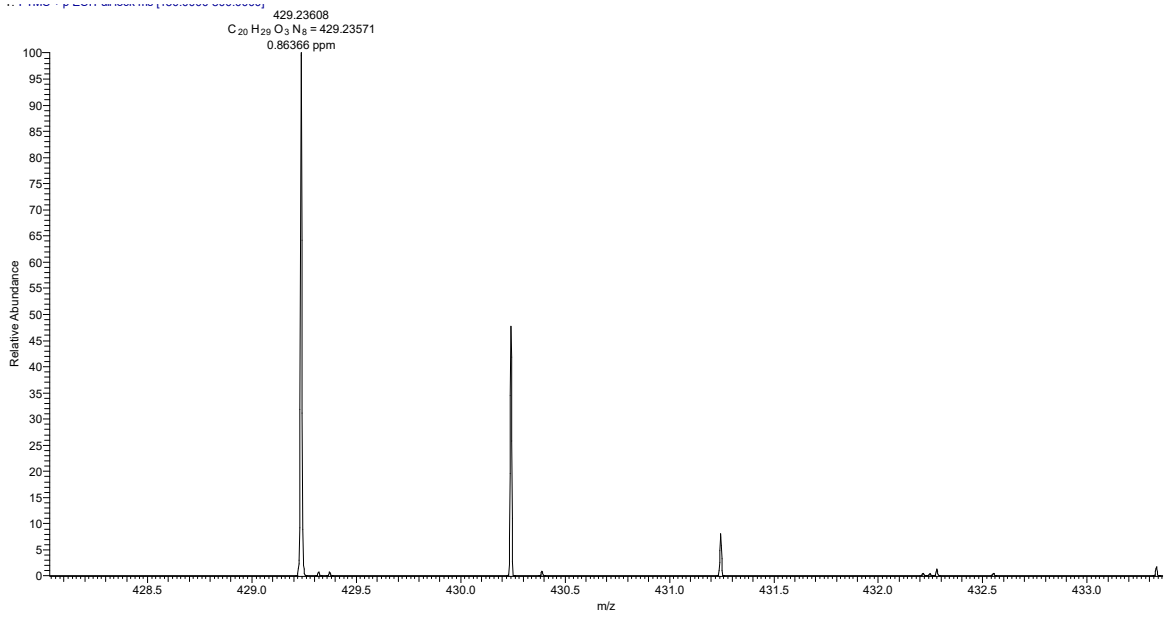

3.00
2.50
2.00
1.50
1.00
0.50
0.00
AU
2.448
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
Minutes

Figure S11. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compound **5g**.




429.23608
C 20 H 29 O 3 N 8 = 429.23571
0.86366 ppm
Relative Abundance
m/z

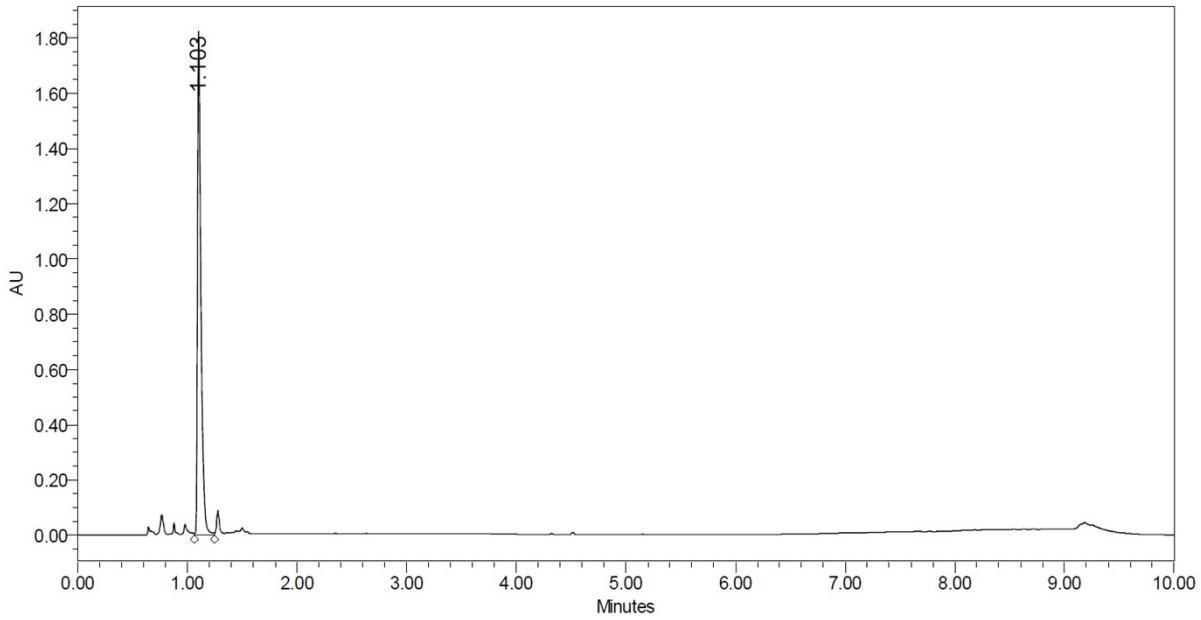
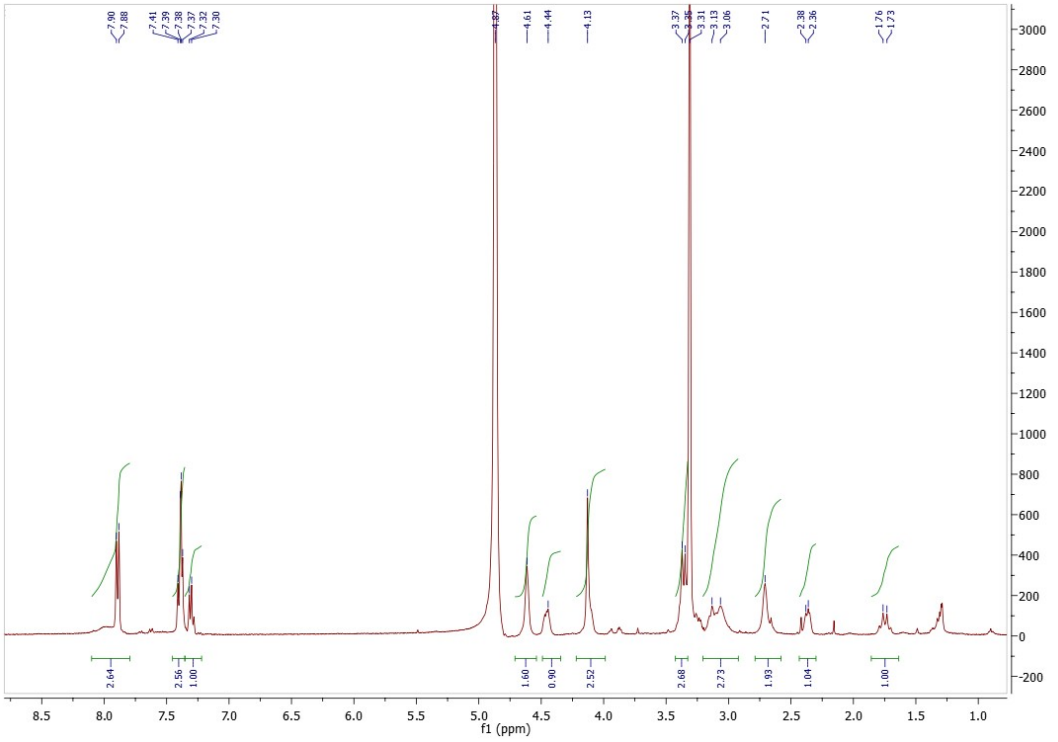
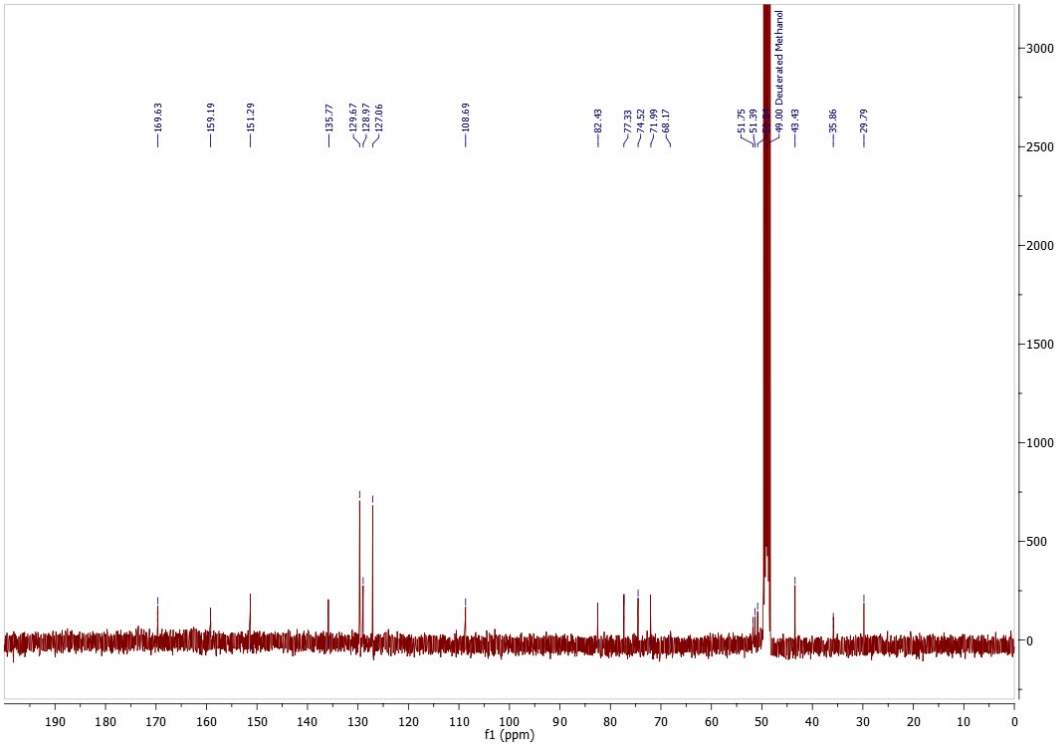
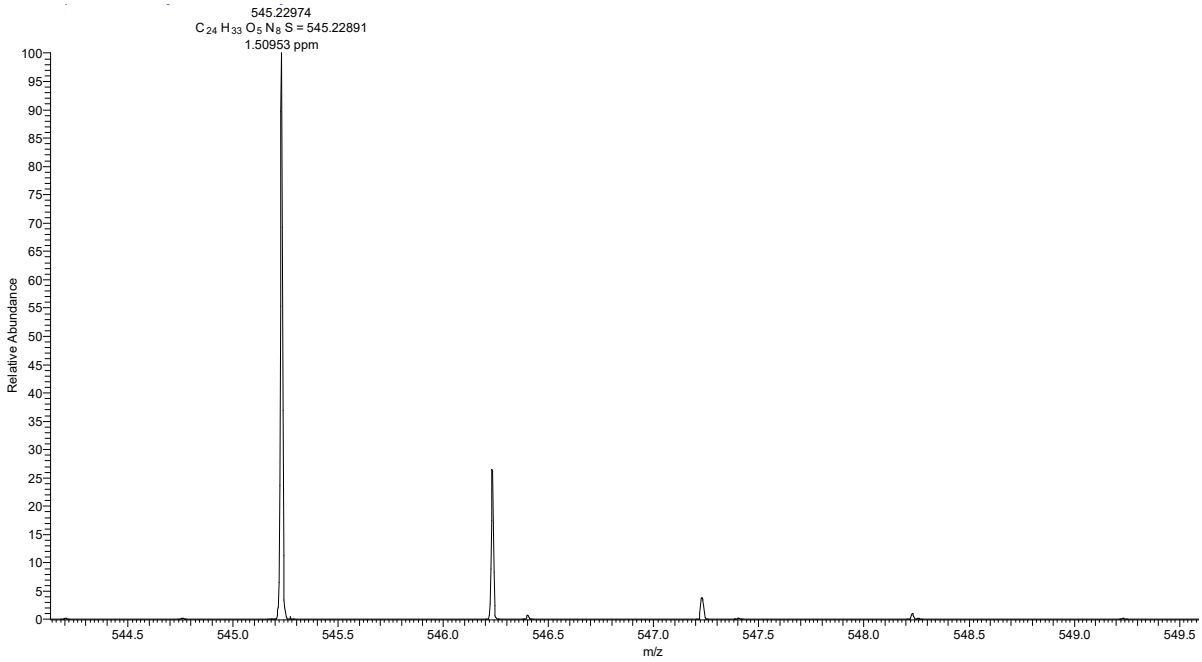
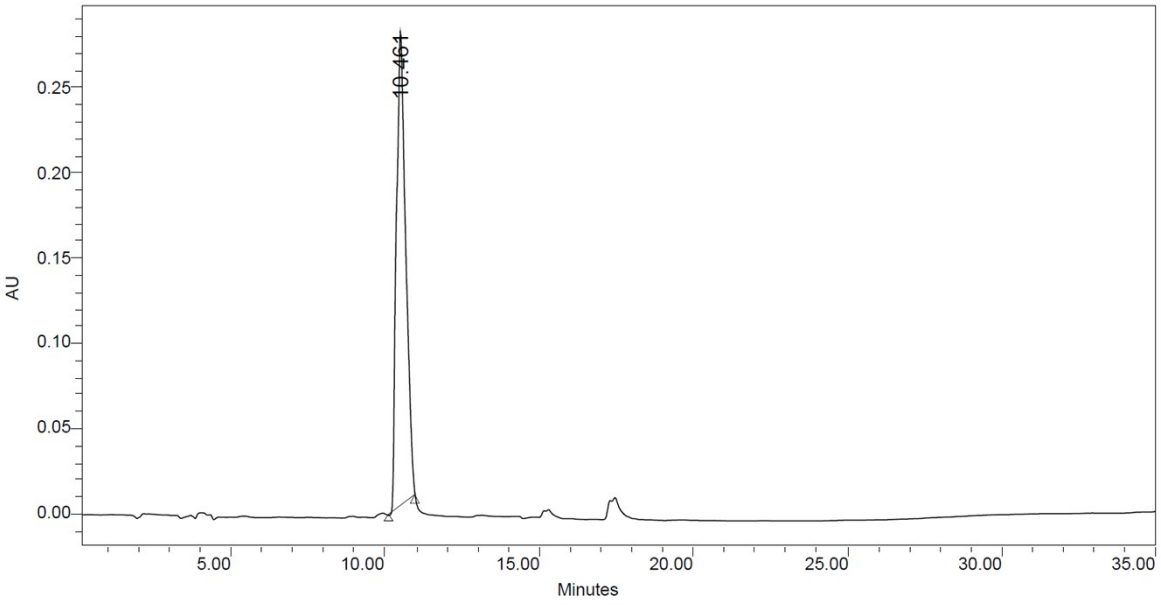

1.103
AU
Minutes

Figure S12. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **5h**.






545.22974
C 24 H 33 O 5 N 8 S = 545.22891
1.50953 ppm
Relative Abundance
m/z


10.461
AU
Minutes

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2. Wang, W.; Purwanto, M. G.; Weisz, K., CG base pair recognition by substituted phenylimidazole nucleosides. *Org Biomol Chem* **2004,** *2* (8), 1194-8.
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