Supplementary Information

A 2', 2'-disulfide-bridged dinucleotide conformationally locks RNA hairpins

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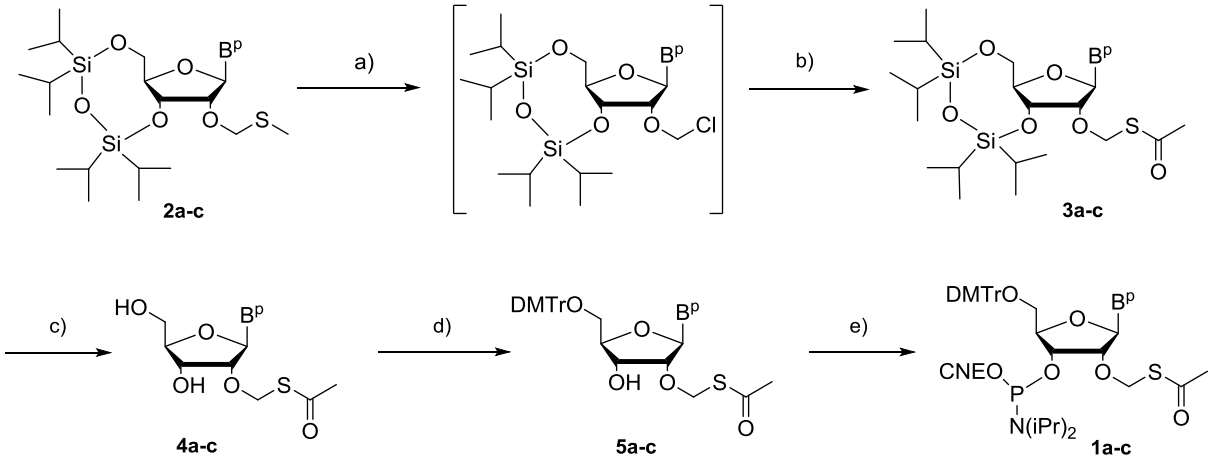
Contents

General procedures

CH_3CN, pyridine and DIEA were distilled over calcium hydride. All reactions were performed in anhydrous conditions under argon. NMR experiments were accomplished on Bruker DRX 400 and AM 300 spectrometers at 20°C. HRMS analyses were obtained with electrospray ionization (ESI) in positive or negative mode on a Q-TOF Micromass spectrometer.

Synthesis of 2'-*O*-AcSM ribonucleosides phosphoramidites 1a-c



B^p: N^4-acetyl cytosine (a); N^6-Pac adenine (b); N^2-Pac guanine (c)

Scheme S1: Synthesis of 2'-O-AcSM ribonucleoside phosphoramidites 1a-c. Reagent and conditions : a) SO_2Cl_2, CH_2Cl_2, RT, 1.5h, for **2a** and **2b**; SO_2Cl_2, CH_2Cl_2, 4-Cl-styrene, 0°C then RT, 1h, for **2c**; b) CH_3COSK, 18-crown-6, CH_2Cl_2, RT, 3h for **3a** and **3b**, CH_3COSK, 18-crown-6, CH_2Cl_2, RT, 2h for **3c**; c) $Et_3N.3HF$, THF, RT, 2h; d) DMTrCl, DIEA, CH_2Cl_2, RT, 1.5h ; e) $iPr_2NPCl(OCNE)$, DIEA, CH_2Cl_2, RT, 2h

Synthesis conditions

2'-*O*-acetylthiomethyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*N4*-acetyl cytidine 3a. To a solution of 2'-*O*-methylthiomethyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl) cytidine **2a** (3.00 g, 5.11 mmol, 1.0 equiv) in dry CH_2Cl_2 (34 mL) was added dropwise under argon a 1.0 M sulfuryl chloride solution in CH_2Cl_2 (8.00 mL, 7.67 mmol, 1.5 equiv). The mixture was stirred for 1.5h at room temperature. After completion of the reaction, the chloromethylether derivative was obtained as brown foam after evaporation of the solvent and was directly used in the next step. 18-crown-6 (1.01 g, 3.83 mmol, 0.75 equiv) was added to potassium thioacetate (1.00 g, 8.79 mmol, 1.72 equiv) solution in CH_2Cl_2 (13 mL). The solution was added dropwise to the chloromethyl ether derivative in dry CH_2Cl_2 (13 mL). After stirring at room temperature for 3h, the mixture was evaporated and diluted in ethyl acetate. The solution was filtered and

washed with water. The aqueous layer was then extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography with cyclohexane/ethyl acetate (70/30). The desired compound **3a** was obtained as white foam (1.89 g, 3.07 mmol, 60%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H, NH) ; 8.26 (d, J = 7.6 Hz, 1H, H_6) ; 7.41 (d, J = 7.2 Hz, 1H, H_5) ; 5.79 (s, 1H, $H_{1'}$) ; 5.46, 5.35 ($2d_{AB}$, J_{AB} = 11.2 Hz, 2H, OCH_2S) ; 4.25 (d, J = 13.6 Hz, 1H, $H_{5'}$) ; 4.16-4.07 (m, 3H, $H_{2'}$, $H_{3'}$, $H_{4'}$) ; 3.96 (dd, J = 13.6 Hz, J = 2.0 Hz, 1H, $H_{5''}$) ; 2.36 (s, 3H, $SCOCH_3$) ; 2.28 (s, 3H, CH_3 Ac) ; 1.09-0.98 (m, 28H, iPr). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.2 (SC=O) ; 171.0 (C=O) ; 162.9 (C=O), 144.3 (C_6) ; 96.3 (C_5) ; 89.5 ($C_{1'}$) ; 81.9 ($C_{4'}$) ; 80.7 ($C_{2'}$) ; 69.9 (OCH_2S) ; 67.3 ($C_{3'}$) ; 59.3 ($C_{5'}$) ; 30.9 ($SCOCH_3$) ; 24.9 (CH_3) ; 17.4-16.8 (CH_3, iPr) ; 13.4-12.5 (CH, iPr). HRMS (ESI^-) m/z calcd for $C_{26}H_{45}N_3O_8Si_2S$ $(M+H)^-$ 616.2544, Found 616.2550.

2'-*O*-acetylthiomethyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*N6*-phenoxyacetyl adenosine 3b. Using the same procedure as for the synthesis of **3a**, starting from **2b** (7.52 g, 10.70 mmol), compound **3b** was obtained as a white foam (5.69 g, 7.78 mmol, 73%). Purification conditions: silica gel column chromatography with cyclohexane/ethyl acetate (60/40). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.48 (s, 1H, NH) ; 8.75 (s, 1H, H_2) ; 8.26 (s, 1H, H_8) ; 7.36-7.03 (m, 5H, HAr) ; 6.02 (s, 1H, $H_{1'}$) ; 5.37 (s, 2H, O*CH*$_2$S) ; 4.87 (s, 2H, CH_2 PAC) ; 4.83 (dd, J = 9.6 Hz, J = 5.4 Hz, 1H, $H_{3'}$) ; 4.54 (d, J = 4.8 Hz, 1H, $H_{2'}$) ; 4.18 (dd, J = 13.2 Hz, J = 1.2 Hz, 1H, $H_{5'}$) ; 4.11 (dt, J = 9.2 Hz, J = 2.0 Hz, 1H, $H_{4'}$); 4.01 (dd, J = 13.2 Hz, J = 2.4 Hz, 1H, $H_{5''}$) ; 2.32 (s, 3H, CH_3) ; 1.10-1.06 (m, 28H, iPr). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.2 (SC=O) ; 166.6 (C=O) ; 157.0 (Cq PAC) ; 152.5 (C_2) ; 150.9 (C_4) ; 148.3 (C_6) ; 141.8 (C_8) ; 129.8, 122.4, 115.0 (Car) ; 122.9 (C_5) ; 88.6 ($C_{1'}$) ; 81.6 ($C_{4'}$) ; 80.5 ($C_{2'}$) ; 70.2 (O*CH*$_2$S) ; 69.3 ($C_{3'}$) ; 68.1 (CH_2 PAC) ; 59.8 ($C_{5'}$) ; 30.9 (*CH*$_3$) ; 17.4-16.9 (*CH*$_3$ iPr) ; 13.4-12.6 (*CH* iPr). HRMS (ESI^+) m/z calcd for $C_{33}H_{49}N_5O_8Si_2S$ $(M+H)^+$ 732.2919, Found 732.2924.

2'-*O*-acetylthiomethyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*N2*-phenoxyacetyl guanosine 3c. To a solution of 2'-*O*-methylthiomethyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl) guanosine **2c** (6.67 g, 9.26 mmol, 1.0 equiv) in dry CH_2Cl_2 (100 mL) was added dropwise under argon a 1.0 M sulfuryl chloride solution in CH_2Cl_2 (11.10 mL, 11.10 mmol, 1.2 equiv) and 4-chloro-styrene (1.23 mL, 10.19 nmol, 1.1 eq). The mixture was stirred for 1h at room temperature. After completion of the reaction, the chloromethylether derivative was obtained as brown foam after evaporation of the solvent and was directly used in the next step. 18-crown-6 (1.84 g, 6.95 mmol, 0.75 equiv) was added to a CH_2Cl_2 (25 mL) potassium thioacetate solution

(1.82 g, 15.93 mmol, 1.72 equiv). The solution was added dropwise to the chloromethyl ether derivative in dry CH_2Cl_2 (50 mL). After stirring at room temperature for 2h, the mixture was evaporated and diluted in ethyl acetate. The solution was filtered and washed with water. The aqueous layer was then extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography with CH_2Cl_2/Methanol (99/1). The desired compound **3c** was obtained as white foam (4.52 g, 6.04 mmol, 65%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.84 (s, 1H, NH) ; 9.60 (s, 1H, NH) ; 8.01 (s, 1H, H_8) ; 7.36-6.97 (m, 5H, Ar) ; 5.88 (s, 1H, $H_{1'}$) ; 5.48, 5.39 ($2d_{AB}$, J_{AB} = 11.2 Hz, 1H+1H, O*CH*$_2$S) ; 4.72 (s, 2H, CH_2 PAC) ; 4.44 (dd, J = 9.2 Hz, J = 4.4 Hz, 1H, $H_{3'}$) ; 4.33 (d, J = 4.4 Hz, 1H, $H_{2'}$) ; 4.19 (d, J = 13.2 Hz, 1H, $H_{5'}$) ; 4.11 (dt, J = 9.3 Hz, J = 2.0 Hz, 1H, $H_{4'}$); 3.98 (dd, J = 13.2 Hz, J = 2.4 Hz, 1H, $H_{5''}$) ; 2.26 (s, 3H, CH_3) ; 1.09-0.99 (m, 28H, iPr). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.7 (SC=O) ; 169.8 (C=O) ; 156.5 (Cq PAC) ; 155.2 (C_6) ; 146.6 (C_4) ; 146.4 (C_8) ; 136.7 (C_2) ; 129.9, 122.9, 114.9 (Car) ; 122.2 (C_5) ; 87.8 ($C_{1'}$) ; 81.7 ($C_{4'}$) ; 80.9 ($C_{2'}$) ; 70.2 (O*CH*$_2$O) ; 68.6 ($C_{3'}$) ; 67.0 (CH_2 PAC) ; 59.6 ($C_{5'}$) ; 30.8 (*CH*$_3$) ; 17.4-16.8 (*CH*$_3$iPr) ; 13.4-12.5 (*CH*iPr). HRMS (ESI$^+$) m/z calcd for $C_{33}H_{49}N_5O_9Si_2S$ $(M+H)^+$ 748.2868, Found 748.2869.

2'-*O*-acetylthiomethyl-*N4*-acetyl cytidine 4a. To a solution of **3a** (3.58 g, 5.82 mmol, 1.0 equiv) in anhydrous THF (83 mL) was added Et_3N-3HF solution (1.90 mL, 11.63 mmol, 2.0 equiv). After stirring for 2h at room temperature, the reaction mixture was treated with triethylammoniumacetate buffer (2M, pH 7). The solvent was concentrated under reduced pressure. The crude material was coevaporated with water and ACN. The residue was purified by silica gel column chromatography with a step gradient of CH_2Cl_2 and methanol (0-5%). The desired compound **4a** was obtained as white foam (1.61 g, 4.31 mmol, 74%).^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H, NH) ; 8.39 (d, J = 7.6 Hz, 1H, H_6) ; 7.19 (d, J = 7.6 Hz, 1H, H_5) ; 5.86 (d, J = 2.8 Hz, 1H, $H_{1'}$) ; 5.25 (s, 2H, OCH_2S) ; 5.19 (m, 1H, OH) ; 4.09 (dd, J = 6.4 Hz, J = 5.2 Hz, 1H, $H_{3'}$) ; 4.01 (dd, J = 5.2 Hz, J = 3.2 Hz, 1H, $H_{2'}$) ; 3.88 (m, 1H, $H_{4'}$) ; 3.73 (dd, J = 12.4 Hz, J = 2.8 Hz, 1H, $H_{5'}$) ; 3.57 (dd, J = 12.4 Hz, J = 3.2 Hz, 1H, $H_{5''}$) ; 3.38 (d, J = 9.2 Hz, 1H, OH) ; 2.35 (s, 3H, $SCOCH_3$) ; 2.10 (s, 3H, CH_3 Ac). ^{13}C-NMR (100 MHz, DMSO-d_6) δ 194.4 (SC=O) ; 171.0 (C=O) ; 162.4 (C=O), 154.6 (C_6) ; 145.2 (Cq); 95.4 (C_5) ; 87.9 ($C_{1'}$) ; 84.4 ($C_{4'}$) ; 81.1 ($C_{2'}$) ; 69.1 (OCH_2S) ; 67.4 ($C_{3'}$) ; 59.5 ($C_{5'}$) ; 30.8 ($SCOCH_3$) ; 24.3 (CH_3). HRMS (ESI$^+$) m/z calcd for $C_{14}H_{19}N_3O_7S$ $(M+H)^+$ 374.1022, Found 374.1021.

2'-*O*-acetylthiomethyl-*N6*-phenoxyacetyl adenosine 4b. Using the same procedure as for synthesis of **4a**, starting from **3b** (5.59 g, 7.65 mmol), compound **4b** was obtained as white

foam (3.31 g, 6.77 mmol, 88%). Purification conditions: silica gel column chromatography with a step gradient of CH_2Cl_2 and methanol (0-5%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.63 (s, 1H, NH) ; 8.78 (s, 1H, H_2) ; 8.10 (s, 1H, H_8) ; 7.36-7.03 (m, 5H, HAr) ; 5.95 (d, J = 7.6 Hz, 1H, $H_{1'}$) ; 4.98, 4.83 ($2d_{AB}$, J_{AB} = 11.2 Hz, 2H, O*CH*$_2$S) ; 4.88 (s, 2H, CH_2 PAC) ; 4.85 (dd, J = 4.8 Hz, J = 2.8 Hz, 1H, $H_{2'}$) ; 4.64 (d, J = 4.8 Hz, 1H, $H_{3'}$) ; 4.37 (s, 1H, $H_{4'}$) ; 3.96 (dd, J = 12.8 Hz, J = 1.6 Hz, 1H, $H_{5'}$); 3.77 (dd, J = 13.6 Hz, J = 1.2 Hz, 1H, $H_{5''}$) ; 2.22 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.4 (SC=O) ; 166.8 (C=O) ; 156.9 (Cq PAC) ; 152.1 (C_2) ; 150.6 (C_4) ; 149.1 (C_6) ; 143.6 (C_8) ; 129.8, 122.5, 114.9 (Car) ; 124.0 (C_5) ; 89.1 ($C_{1'}$) ; 88.0 ($C_{4'}$) ; 80.7 ($C_{2'}$) ; 70.8 ($C_{3'}$) ; 69.4 (O*CH*$_2$S) ; 68.1 (CH_2 PAC) ; 63.0 ($C_{5'}$) ; 30.7 (*CH*$_3$). HRMS (ESI^+) m/z calcd for $C_{21}H_{23}N_5O_7S$ $(M+H)^+$ 490.1396, Found 490.1396.

2'-*O*-acetylthiomethyl-*N2*-phenoxyacetyl guanosine 4c. Using the same procedure as for synthesis of **4a**, starting from **3c** (4.52 g, 6.04 mmol), compound **4c** was obtained as white foam (2.12 g, 4.20 mmol, 70 %). Purification conditions: silica gel column chromatography with a step gradient of CH_2Cl_2 and methanol (0-5%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H, NH) ; 9.25 (s, 1H, NH) ; 7.88 (s, 1H, H_8) ; 7.43-6.80 (m, 19H, Ar) ; 6.99 (d, J = 4.8 Hz, 1H, $H_{1'}$) ; 5.21, 5.16 ($2d_{AB}$, J_{AB} = 11.2 Hz, 1H+1H, O*CH*$_2$S) ; 4.64-4.62 (m, 2H+1H, CH_2 PAC + $H_{2'}$) ; 4.47 (q, J = 4.8 Hz, 1H, $H_{3'}$) ; 4.22 (q, J = 4.0 Hz, 1H, $H_{4'}$) ; 3.77 (s, 6H, O*CH*$_3$) ; 3.44 (dd, J = 10.4 Hz, J = 2.8 Hz, 1H, $H_{5'}$) ; 3.37 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H, $H_{5''}$) ; 2.27 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ 194.4 (SC=O) ; 169.6 (C=O) ; 158.6, 144.4, 137.4, 135.5, 135.4 (Cq DMTr) ; 156.4 (Cq PAC) ; 155.3 (C_6) ; 147.8 (C_4) ; 146.3 (C_8) ; 137.8 (C_2) ; 130.1, 130.0, 129.9, 129.0, 128.2, 128.1, 127.9, 127.0, 125.3, 122.9, 114.8, 113.2 (Car) ; 122.1 (C_5) ; 86.7 ($C_{1'}$) ; 85.8 ($C_{4'}$) ; 84.2 (OCq, DMTr) ; 81.2 ($C_{2'}$) ; 70.0 (O*CH*$_2$S) ; 69.8 ($C_{3'}$) ; 66.9 (CH_2 PAC) ; 63.3 ($C_{5'}$) ; 55.2 (O*CH*$_3$, DMTr) ; 30.9 (*CH*$_3$). HRMS (ESI^+) m/z calcd for $C_{42}H_{41}N_5O_{10}S$ $(M+H)^+$ 808.2652, Found 808.2652.

2'-*O*-acetylthiomethyl-5'-*O*-(4,4'-dimethoxytrityl)-*N4*-acetyl cytidine 5a A solution of **4a** (1.61 g, 4.31 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (22 mL) was treated under argon with DIEA (1.21 mL, 6.89 mmol, 1.6 equiv) and dimethoxytrityl chloride (2.48 g, 7.32 mmol, 1.7 equiv) was added in small portions over 15 min. The mixture was stirred for 1.5h at room temperature. A saturated aqueous $NaHCO_3$ solution was added. The aqueous layer was then extracted with CH_2Cl_2. The organic layer was washed with water then brine and dried over Na_2SO_4. The solvent was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography with a step gradient of CH_2Cl_2 and methanol (0-2%) containing 1% pyridine. The desired compound **5a** was obtained as yellow foam (2.51 g, 3.72

mmol, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H, NH) ; 8.46 (d, J = 7.2 Hz, 1H, H_6) ; 7.42-7.16 (m, 9H, Har DMTr) ; 7.13 (d, J = 7.2 Hz, 1H, H_5) ; 6.88-6.84 (m, 4H, Har DMTr) ; 5.96 (s, 1H, $H_{1'}$) ; 5.50, 5.37 ($2d_{AB}$, J_{AB} = 11.2 Hz, 1H+1H, OCH_2S) ; 4.44 (m, 1H, $H_{3'}$) ; 4.15 (d, J = 5.2 Hz, 1H, $H_{2'}$) ; 4.04 (dt, J = 9.2 Hz, J = 2.4 Hz, 1H, $H_{4'}$) ; 3.81 (d, J = 1.6 Hz, 6H, 2*OCH_3) ; 3.54 (qd, J = 11.2 Hz, J = 2.0 Hz, 2H, $H_{5'}$ + $H_{5''}$) ; 2.38 (s, 3H, $SCOCH_3$) ; 2.26 (s, 3H, CH_3 Ac). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.9 (SC=O) ; 170.4 (C=O) ; 162.9 (C=O) ; 158.7, 155.0, 144.3, 135.5, 135.2 (Cq, Car) ; 144.6 (C_6) ; 130.1, 129.0, 128.2, 128.1, 128.0, 127.1, 125.3, 113.3 (CH, Car); 96.7 (C_5) ; 88.9 ($C_{1'}$); 87.0 (OCq, DMTr) ; 83.1 ($C_{4'}$); 81.3 ($C_{2'}$) ; 69.7 (OCH_2S) ; 67.6 ($C_{3'}$) ; 60.7 ($C_{5'}$) ; 55.2 (OCH_3, DMTr) ; 31.0 ($SCOCH_3$) ; 24.9 (CH_3 Ac).HRMS (ESI^+) m/z calcd for $C_{35}H_{37}N_3O_9S$ $(M+H)^+$ 676.2329, Found 676.2335.

2'-*O*-acetylthiomethyl -5'-*O*-(4,4'-dimethoxytrityl)-*N6*-phenoxyacetyl adenosine 5b. Using the same procedure as for synthesis of **5a**, starting from **4b** (3.10 g, 6.33 mmol), compound **5b** was obtained as white foam (3.67 g, 4.63 mmol, 73 %). Purification conditions: silica gel column chromatography with a step gradient of CH_2Cl_2 and methanol (0-2%) containing 1% pyridine. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.48 (s, 1H, NH) ; 8.72 (s, 1H, H_2) ; 8.24 (s, 1H, H_8) ; 7.44-6.81 (m, 18H, HAr) ; 6.19 (d, J = 4.8 Hz, 1H, $H_{1'}$) ; 5.18 (s, 2H, O*CH*$_2$S) ; 4.87 (s, 2H, CH_2 PAC) ; 4.85 (t, J = 4.8 Hz, 1H, $H_{2'}$) ; 4.59 (q, J = 5.2 Hz, 1H, $H_{3'}$) ; 4.25 (q, J = 4.0 Hz, 1H, $H_{4'}$) ; 3.78, 3.77 (s+s, 3H+3H, 3*O*CH*$_3$) ; 3.52 (dd, J = 10.4 Hz, J = 3.2 Hz, 1H, $H_{5'}$); 3.40 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H, $H_{5''}$) ; 2.28 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.4 (SC=O) ; 166.6 (C=O) ; 158.5, 144.5, 137.8, 135.6, 135.5 (Cq DMTr) ; 157.0 (Cq PAC) ; 152.5 (C_2) ; 151.5 (C_4) ; 148.3 (C_6) ; 142.2 (C_8) ; 130.0, 129.8, 129.0, 128.2, 128.1, 127.9, 126.9, 125.2, 122.4, 114.9, 113.2 (Car) ; 123.1 (C_5) ; 86.9 ($C_{1'}$) ; 86.6 (OCq, DMTr) ; 84.2 ($C_{4'}$) ; 80.7 ($C_{2'}$) ; 70.0 (O*CH*$_2$S) ; 69.7 ($C_{3'}$) ; 68.1 (CH_2 PAC) ; 62.9 ($C_{5'}$) ; 55.2 (O*CH*$_3$, DMTr) ; 30.8 (*CH*$_3$). HRMS (ESI^+) m/z calcd for $C_{42}H_{41}N_5O_9S$ $(M+H)^+$ 792.2703, Found 792.2711.

2'-*O*-acetylthiomethyl -5'-*O*-(4,4'-dimethoxytrityl)-*N2*-phenoxyacetyl guanosine 5c. Using the same procedure as for synthesis of **5a**, starting from **4c** (0.85 g, 1.69 mmol), compound **5c** was obtained as yellow foam (1.21 g, 1.50 mmol, 87 %). Purification conditions: silica gel column chromatography with a step gradient of CH_2Cl_2 and methanol (0-1%) containing 1% pyridine. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.82 (s, 1H, NH) ; 9.25 (s, 1H, NH) ; 7.88 (s, 1H, H_8) ; 7.43-6.80 (m, 19H, Ar) ; 6.99 (d, J = 4.8 Hz, 1H, $H_{1'}$) ; 5.21, 5.16 ($2d_{AB}$, J_{AB} = 11.2 Hz, 1H+1H, O*CH*$_2$S) ; 4.64-4.62 (m, 2H+1H, CH_2 PAC + $H_{2'}$) ; 4.47 (q, J = 4.8 Hz, 1H, $H_{3'}$) ; 4.22 (q, J = 4.0 Hz, 1H, $H_{4'}$) ; 3.77 (s, 6H, O*CH*$_3$) ; 3.44 (dd, J = 10.4 Hz, J = 2.8 Hz, 1H, $H_{5'}$) ; 3.37 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H, $H_{5''}$) ; 2.27 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.4

(SC=O) ; 169.6 (C=O) ; 158.6, 144.4, 137.4, 135.5, 135.4 (Cq DMTr) ; 156.4 (Cq PAC) ; 155.3 (C_6) ; 147.8 (C_4) ; 146.3 (C_8) ; 137.8 (C_2) ; 130.1, 130.0, 129.9, 129.0, 128.2, 128.1, 127.9, 127.0, 125.3, 122.9, 114.8, 113.2 (Car) ; 122.1 (C_5) ; 86.7 ($C_{1'}$) ; 85.8 ($C_{4'}$) ; 84.2 (OCq, DMTr) ; 81.2 ($C_{2'}$) ; 70.0 (O*CH*$_2$S) ; 69.8 ($C_{3'}$) ; 66.9 (CH_2 PAC) ; 63.3 ($C_{5'}$) ; 55.2 (O*CH*$_3$, DMTr) ; 30.9 (*CH*$_3$). HRMS (ESI^+) m/z calcd for $C_{42}H_{41}N_5O_{10}S$ $(M+H)^+$ 808.2652, Found 808.2652.

2'-*O*-acetylthiomethyl-3'-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-5'-*O*-(4,4'-dimethoxytrityl)-*N4*-acetyl cytidine 1a. To a solution of **5a** (1.83 g, 2.71 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (20 mL) previously passed through an alumina column was added dropwise a mixture of *N,N*-diisopropylethylamine (0.94 mL, 5.42 mmol, 2.0 equiv) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (10.03 mL, 4.61 mmol, 1.7 equiv) in CH_2Cl_2 (4 mL). The mixture was stirred for 2h at room temperature under argon. After reaction completion, ethyl acetate previously washed with a saturated aqueous $NaHCO_3$ solution was added and the reaction mixture was poured into saturated NaCl/$NaHCO_3$ solution (1/1 v/v). The aqueous layer was extracted with ethyl acetate and organic layers were dried over Na_2SO_4. The solvent was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography with an isocratic elution of Ethyl acetate/Cyclohexane (9/1) containing 1% pyridine. The desired phosphoramidite **1a** was obtained as white foam (1.62 g, 1.85 mmol, 68%). ^{31}P-NMR (121 MHz, CD_3CN): δ 150.1, 148.7; HRMS (ESI^+) m/z calcd for $C_{42}H_{54}N_5O_{10}PS$ $(M+H)^+$ 876.3407, Found 876.3406.

2'-*O*-acetylthiomethyl-3'-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-5'-*O*-(4,4'-dimethoxytrityl)-*N6*-phenoxyacetyl adenosine 1b. Using the same procedure as for synthesis of **1a**, starting from **5b** (3.08 g, 3.88 mmol), compound **1b** was obtained as white foam (3.20 g, 3.23 mmol, 83 %). Purification conditions: silica gel column chromatography with an isocratic elution of CH_2Cl_2/ethyl acetate (70/30) containing 1% pyridine. ^{31}P-NMR (121 MHz, CD_3CN): δ 149.9, 149.8; HRMS (ESI^+) m/z calcd for $C_{51}H_{58}N_7O_{10}PS$ $(M+H)^+$ 992.3791, Found 992.3782.

2'-*O*-acetylthiomethyl-3'-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-5'-*O*-(4,4'-dimethoxytrityl)-*N2*-phenoxyacetyl guanosine Using the same procedure as for synthesis of **1a**, starting from **5c** (0.86 g, 1.07 mmol), compound **1c** was obtained as white foam (0.90 g, 0.89 mmol, 84 %). Purification conditions: silica gel column chromatography with an isocratic elution of CH_2Cl_2/ethyl acetate (50/50). ^{31}P-NMR (121 MHz, CD_3CN): δ 150.1, 149.8 ; HRMS (ESI^+) m/z calcd for $C_{51}H_{59}N_7O_{11}PS$ $(M+H)^+$ 1008.3730, Found 1008.3731.

^{1}H, ^{13}C, and ^{31}P NMR spectra of nucleosides

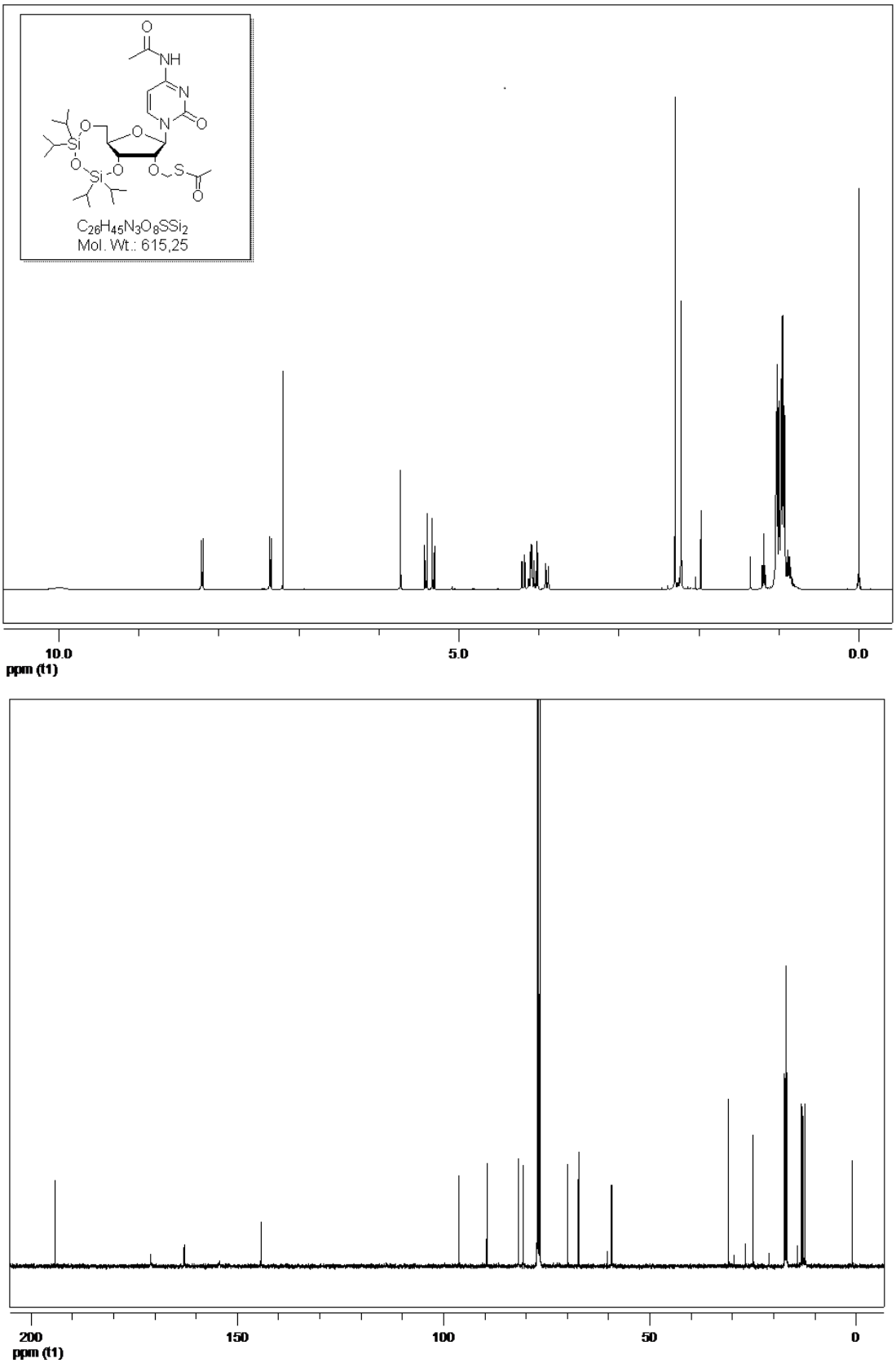


Figure S1: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **3a**

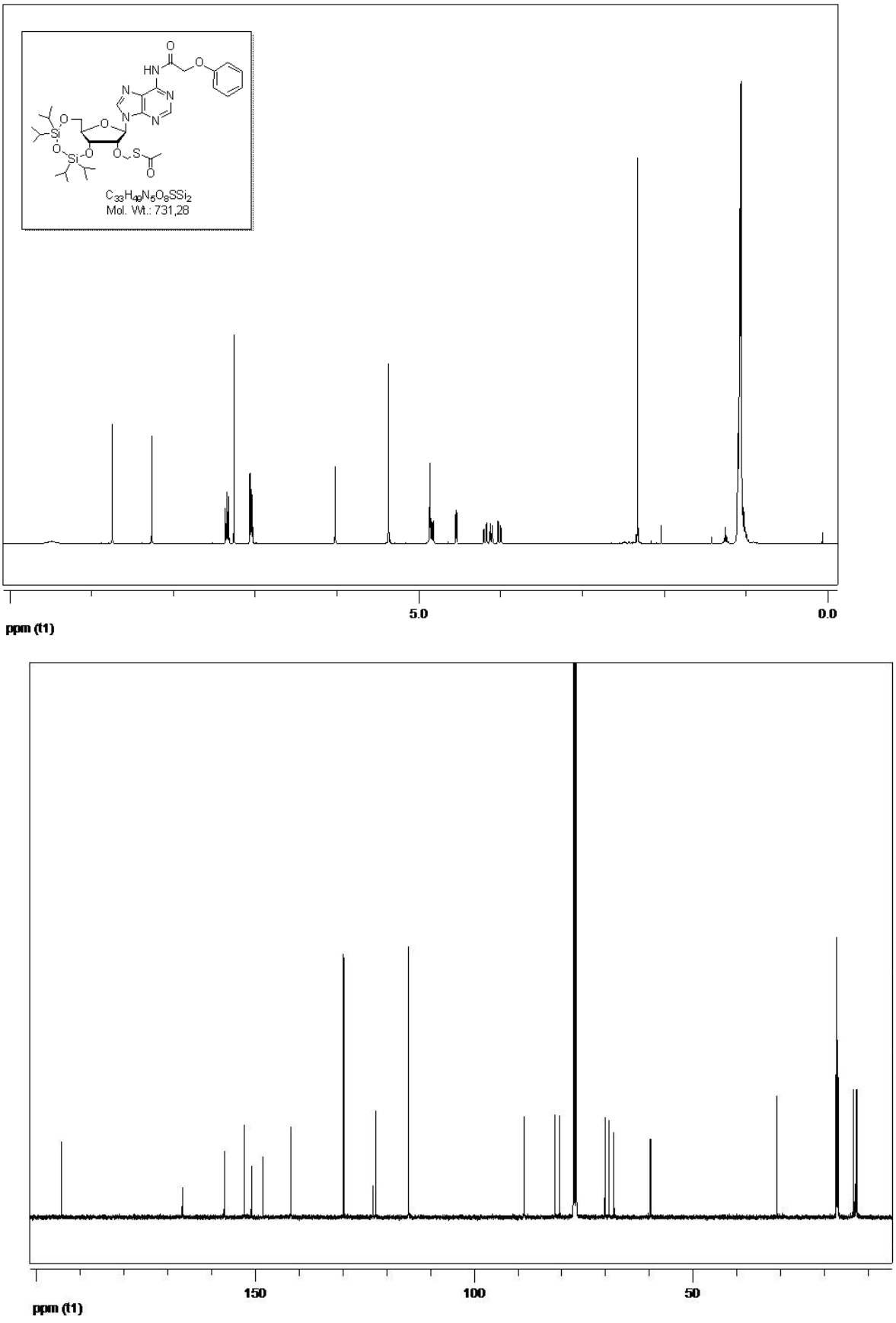
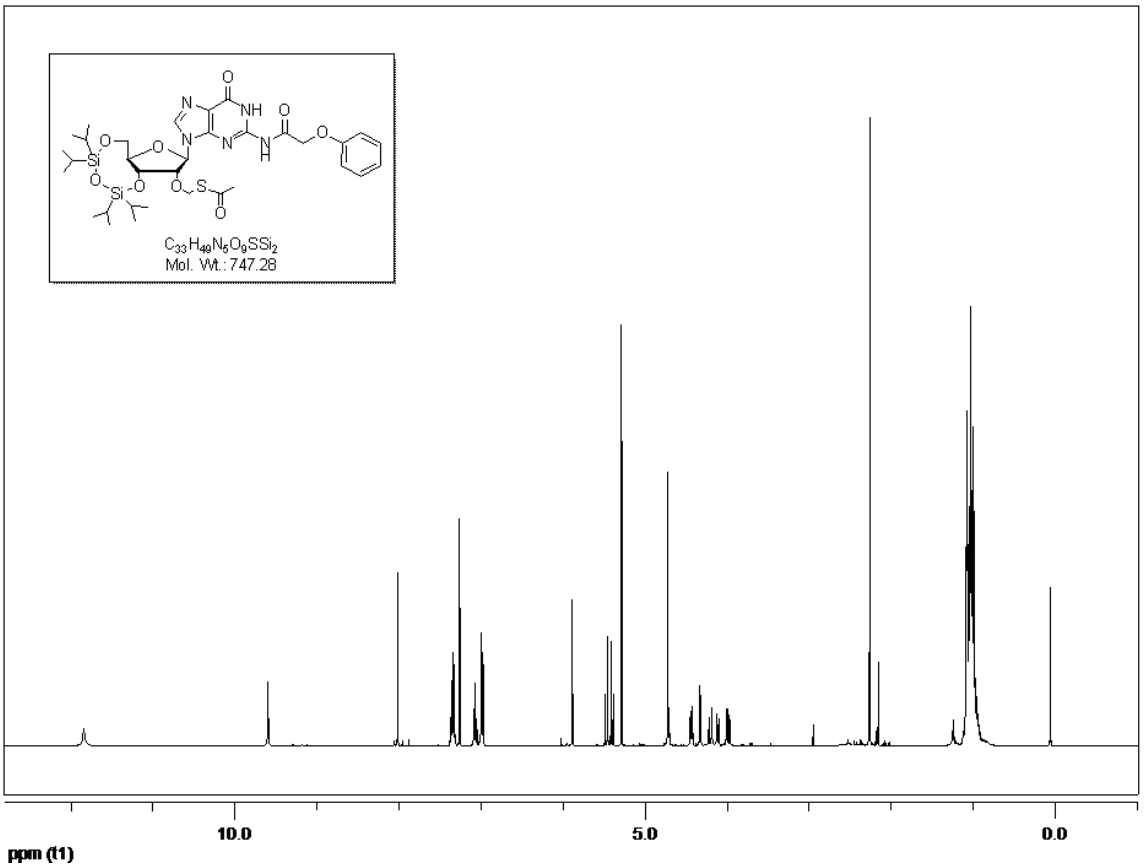


Figure S2: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **3b**



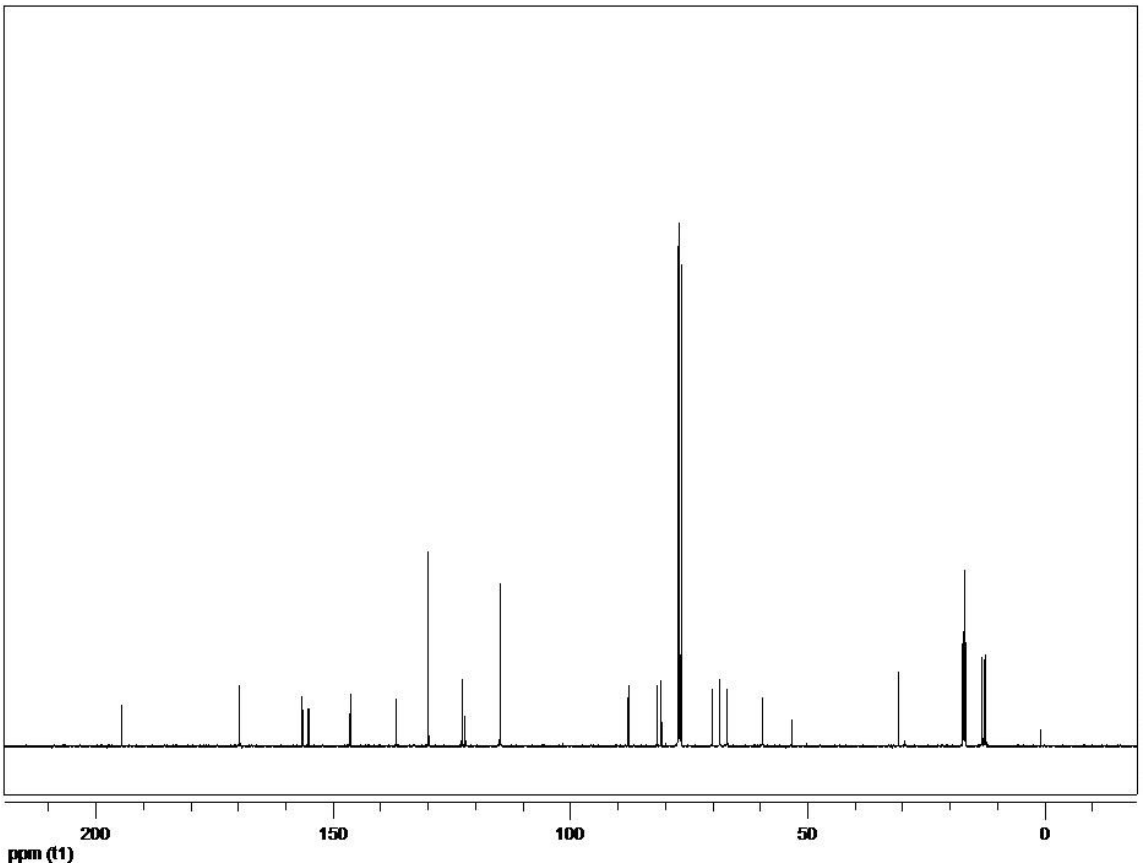
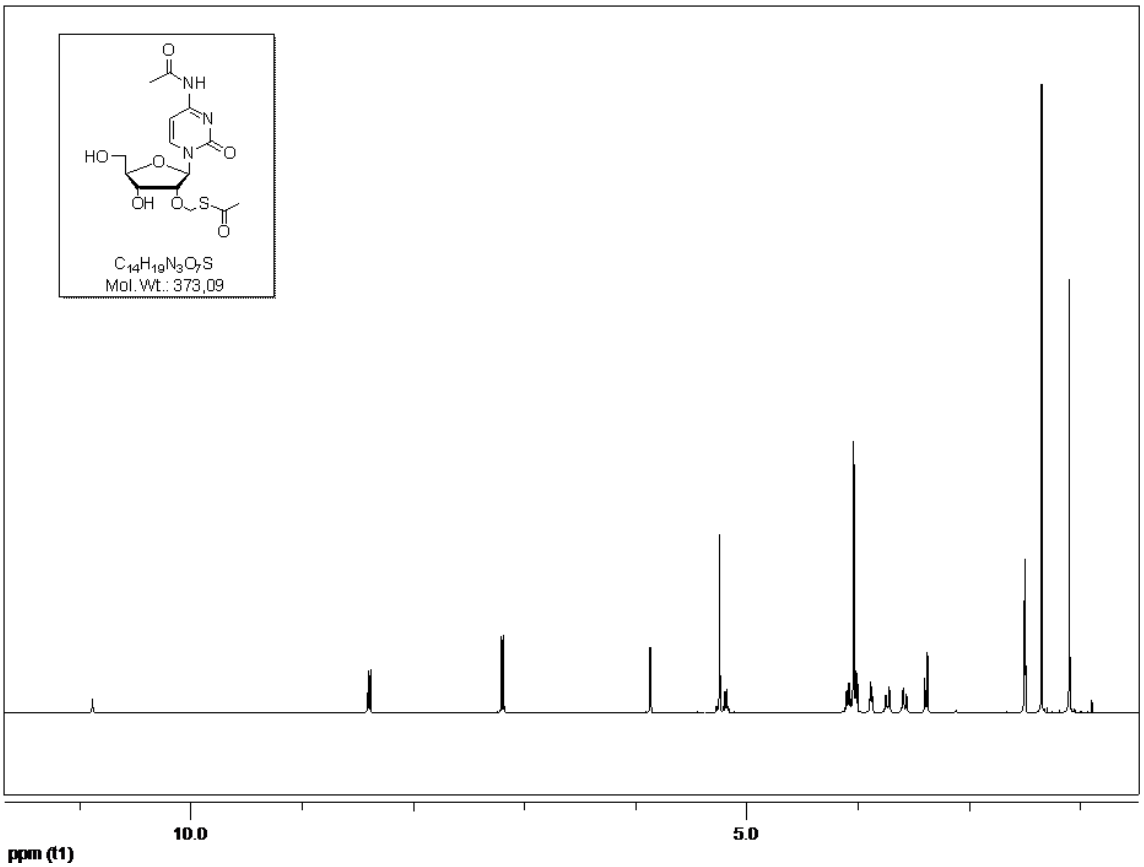


Figure S3: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **3c**



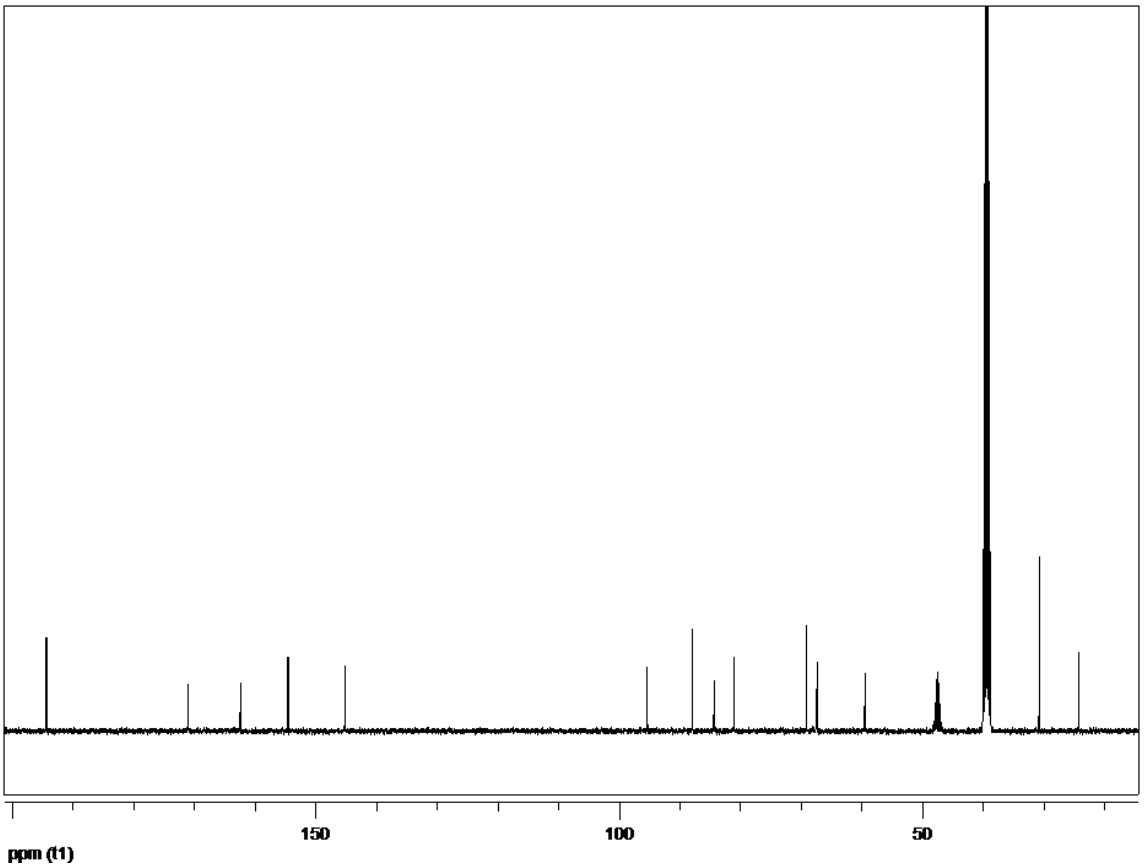


Figure S4: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra (DMSO-d_6) of **4a**

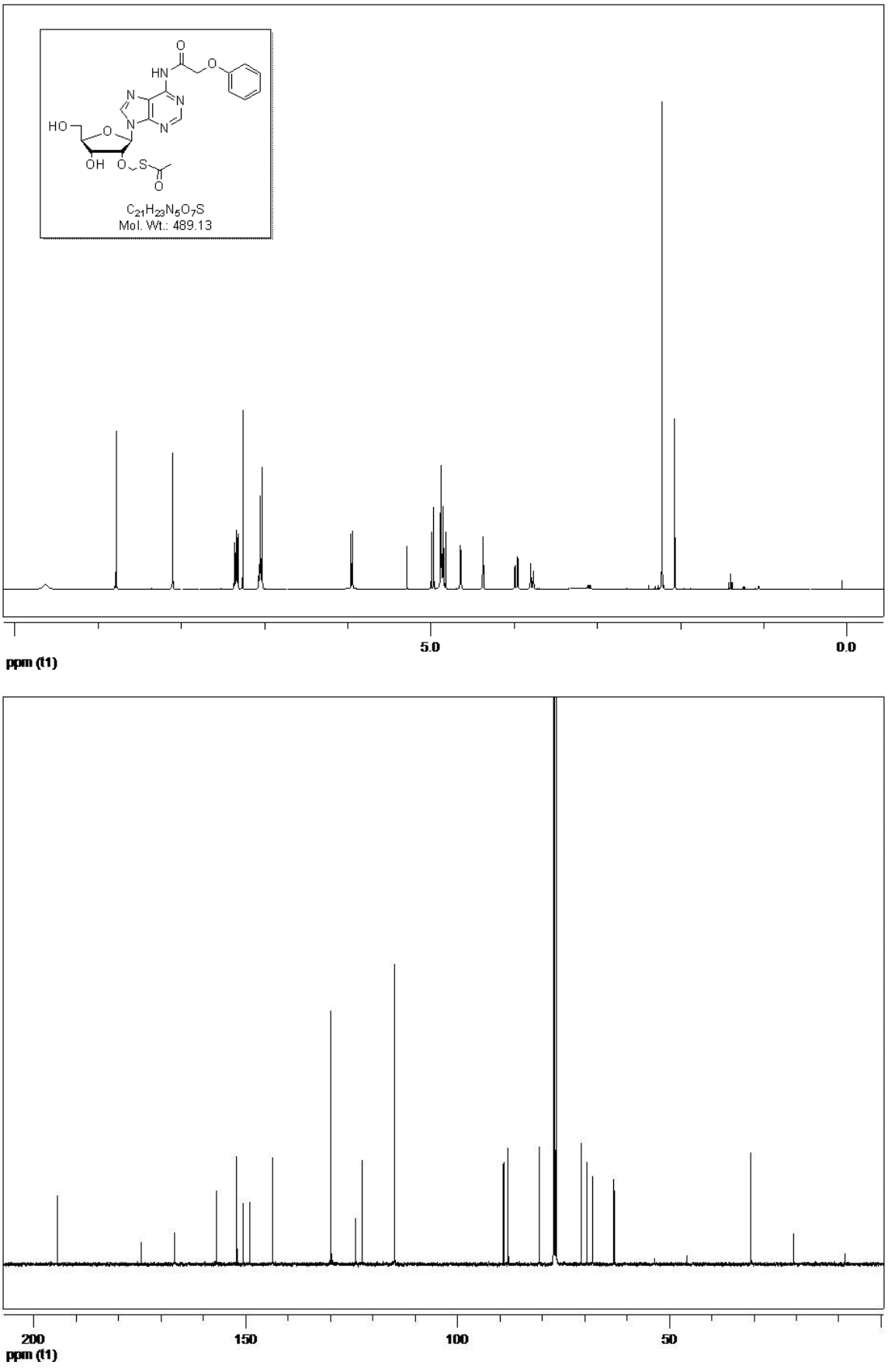
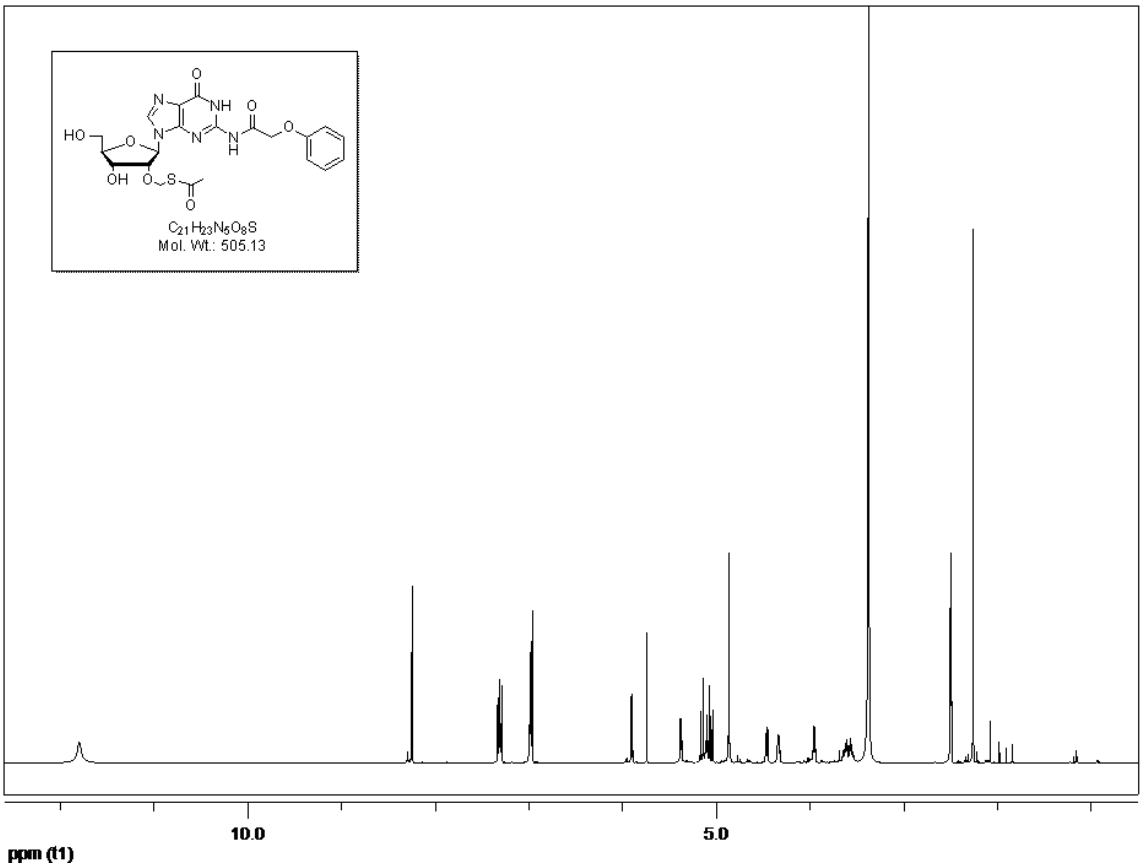


Figure S5: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **<u>4b</u>**



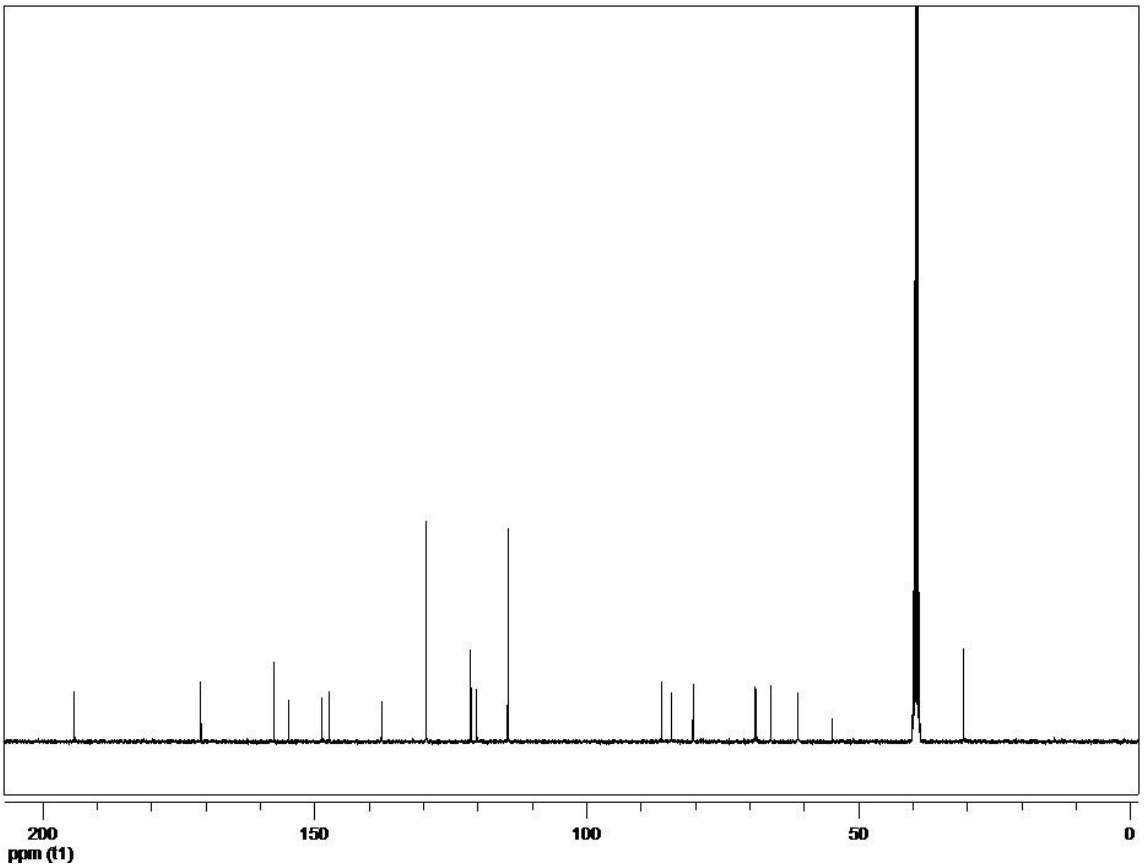


Figure S6: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra (DMSO-d_6) of **4c**

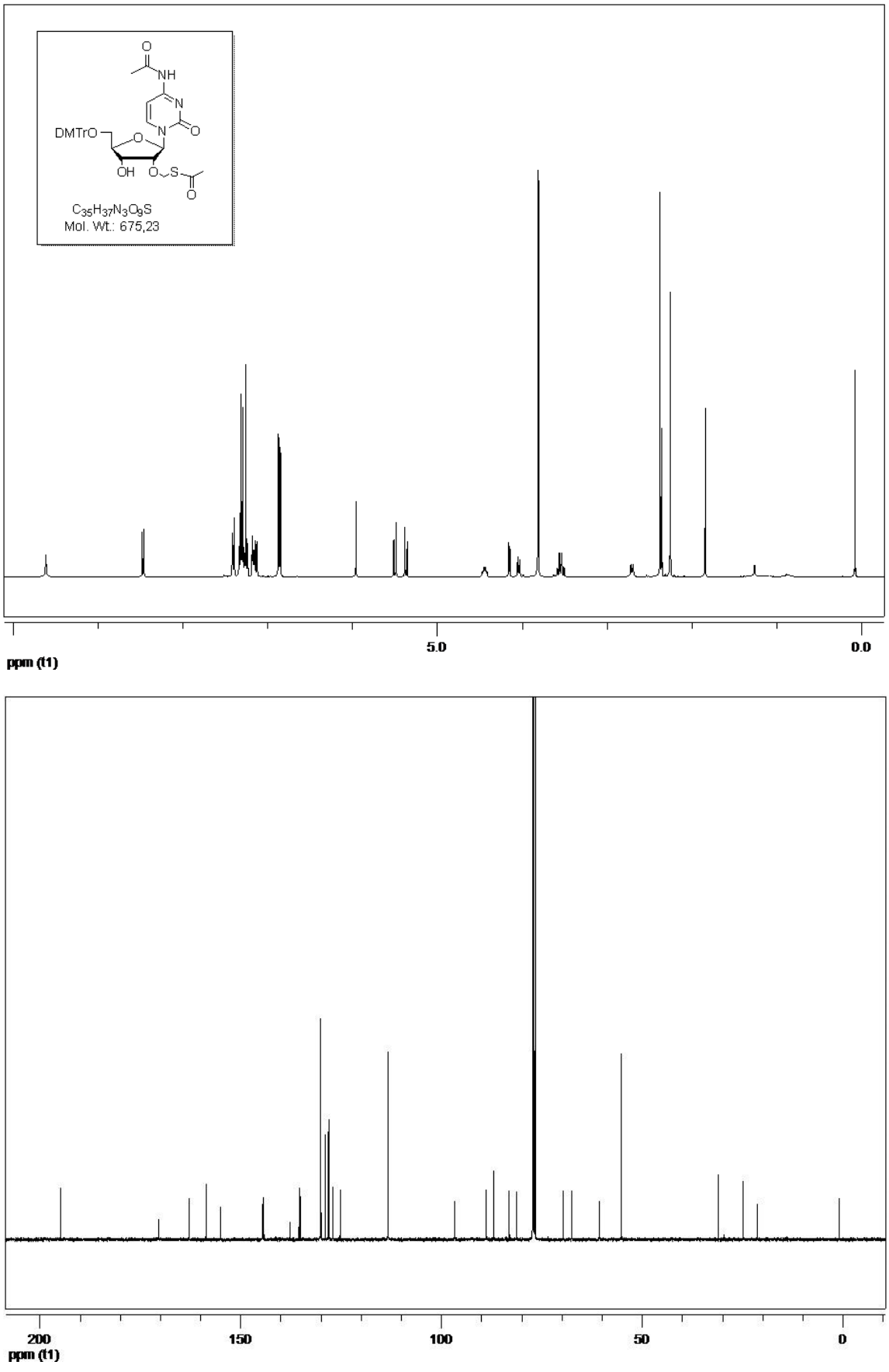


Figure S7: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **5a**

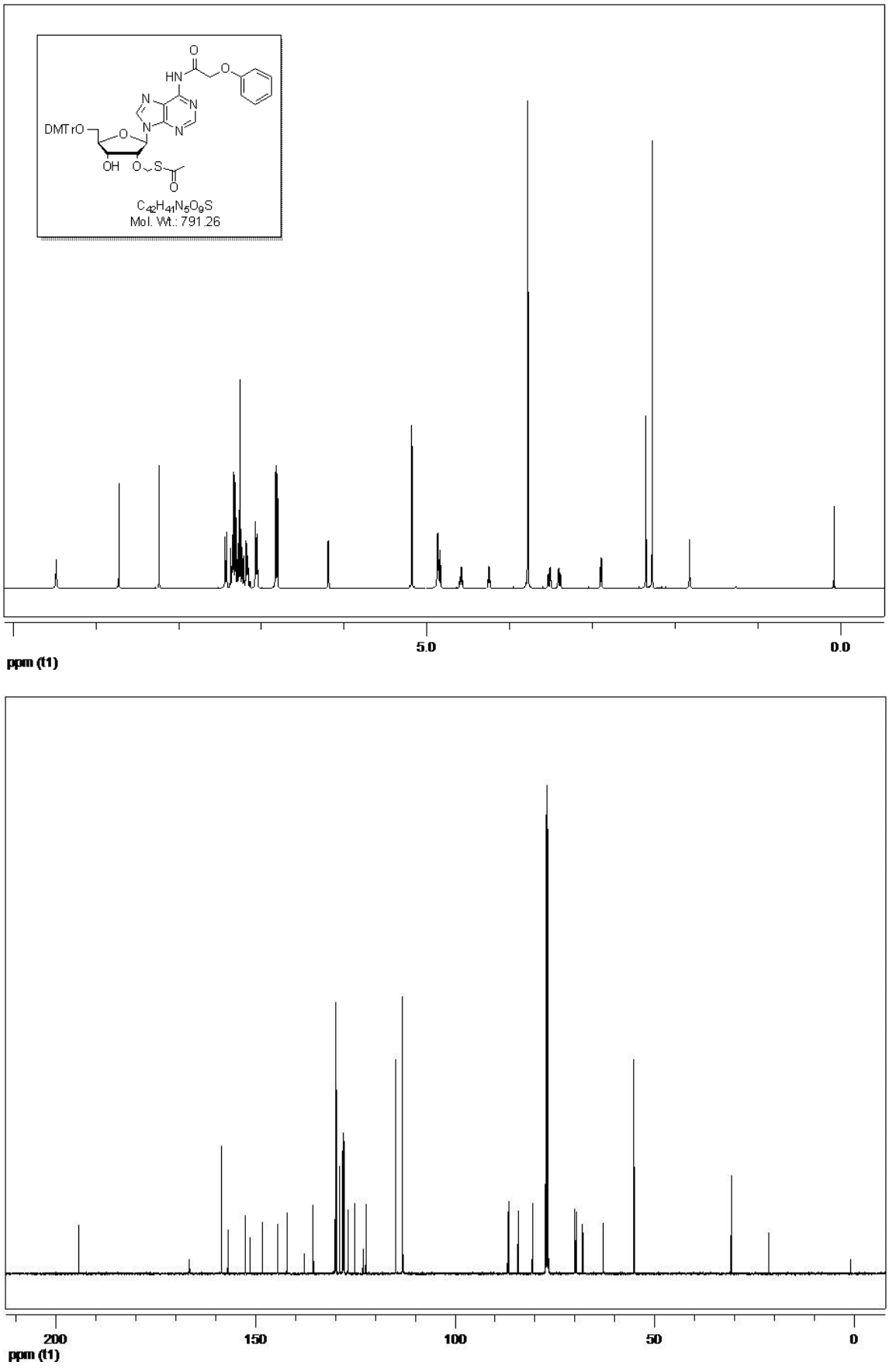


Figure S8: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **5b**

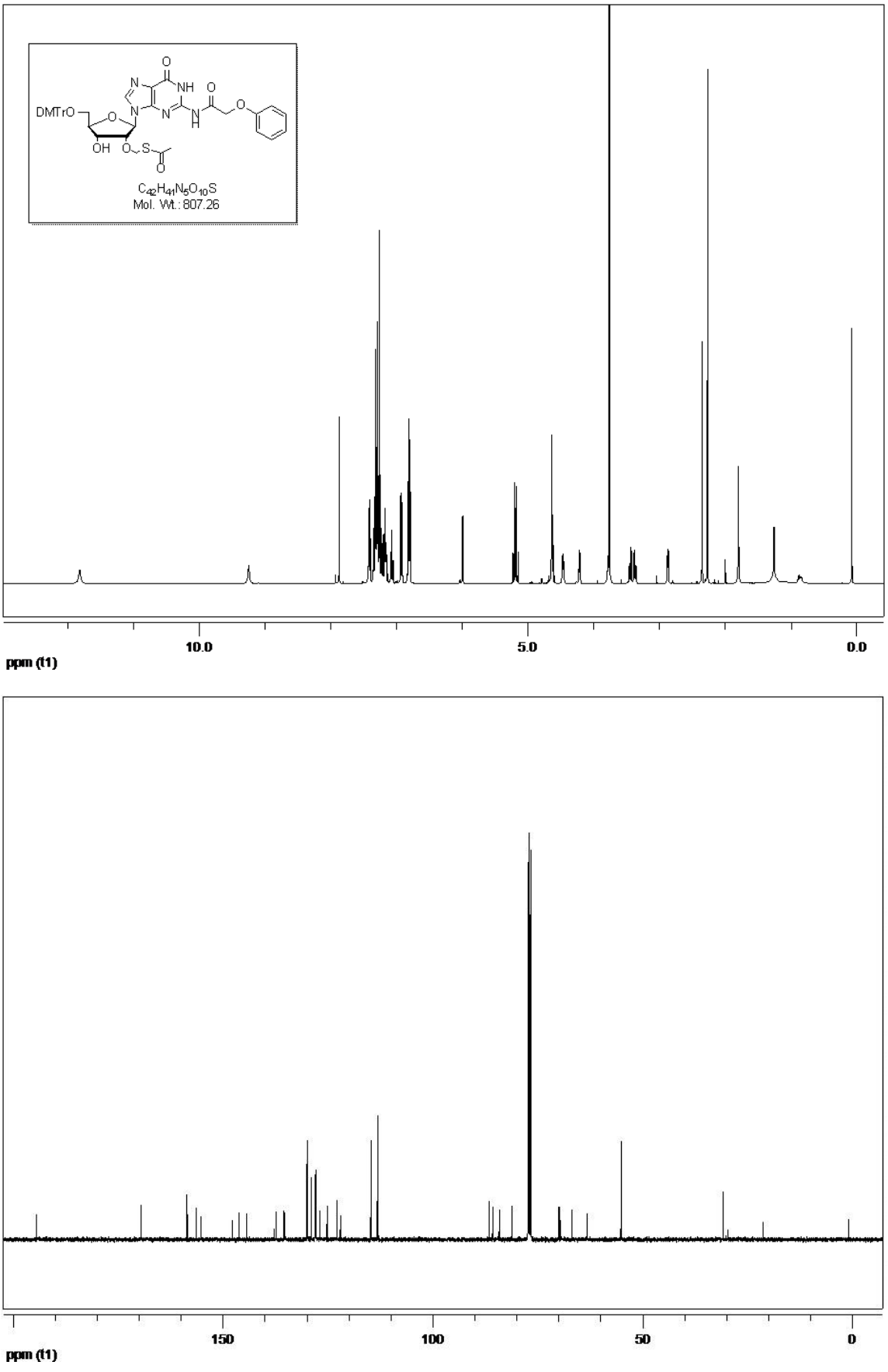


Figure S9: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **5c**

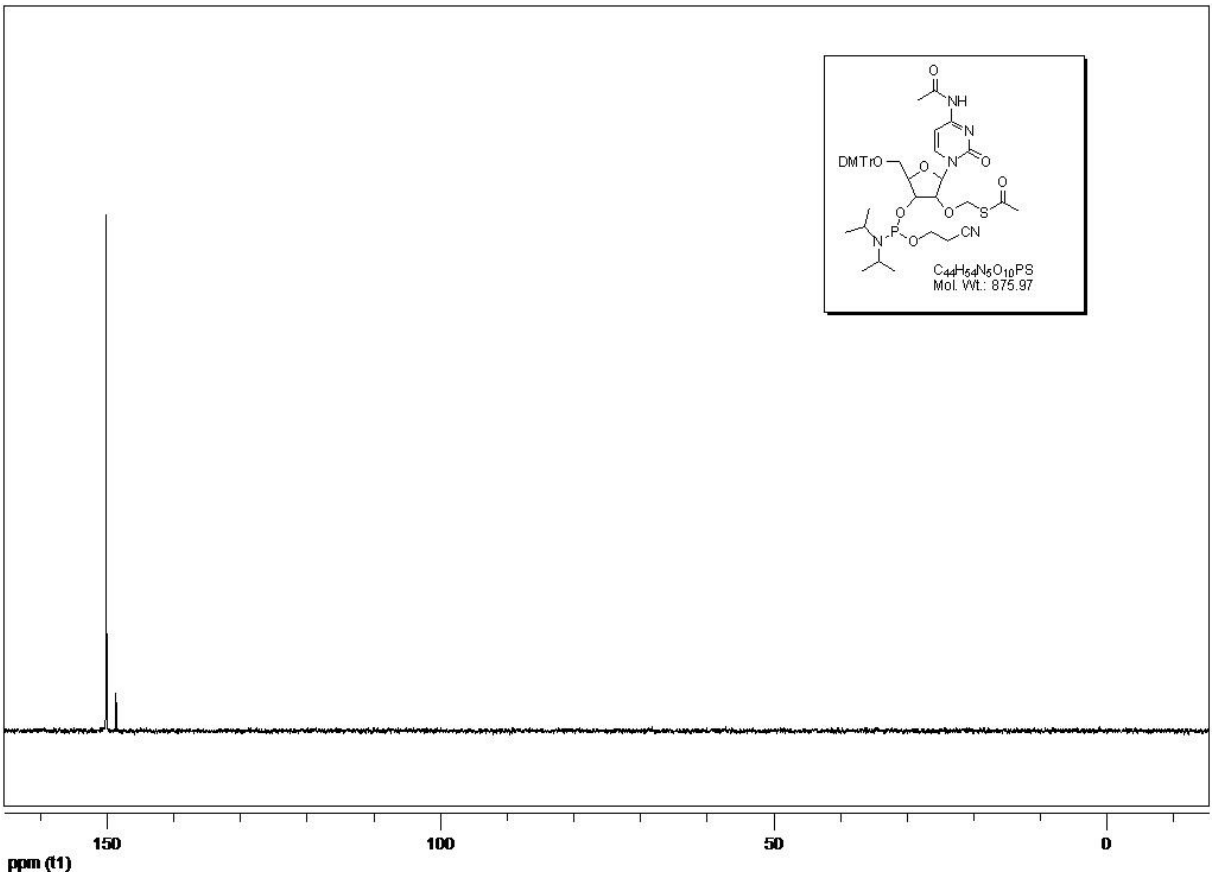


Figure S10: 121 MHz ^{31}P-NMR spectrum (CD_3CN) of **1a**

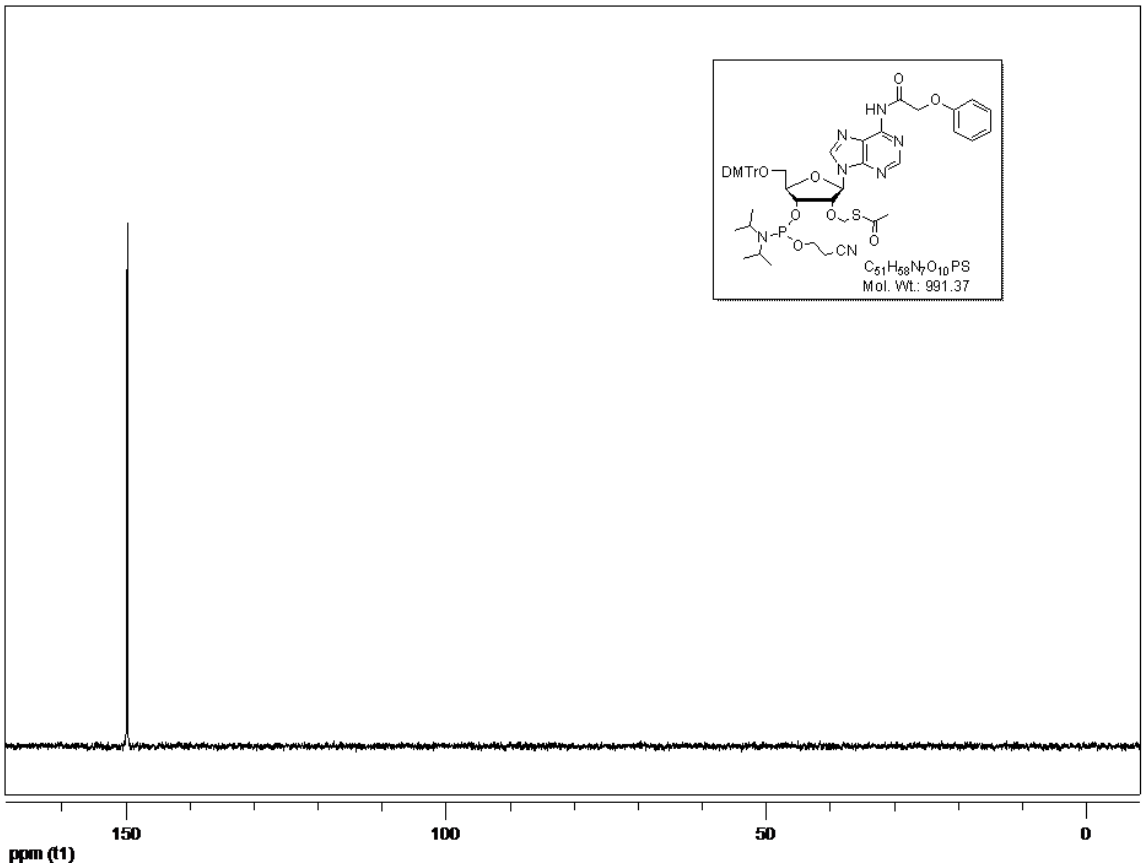


Figure S11: 121 MHz ^{31}P-NMR spectrum (CD_3CN) of **1b**

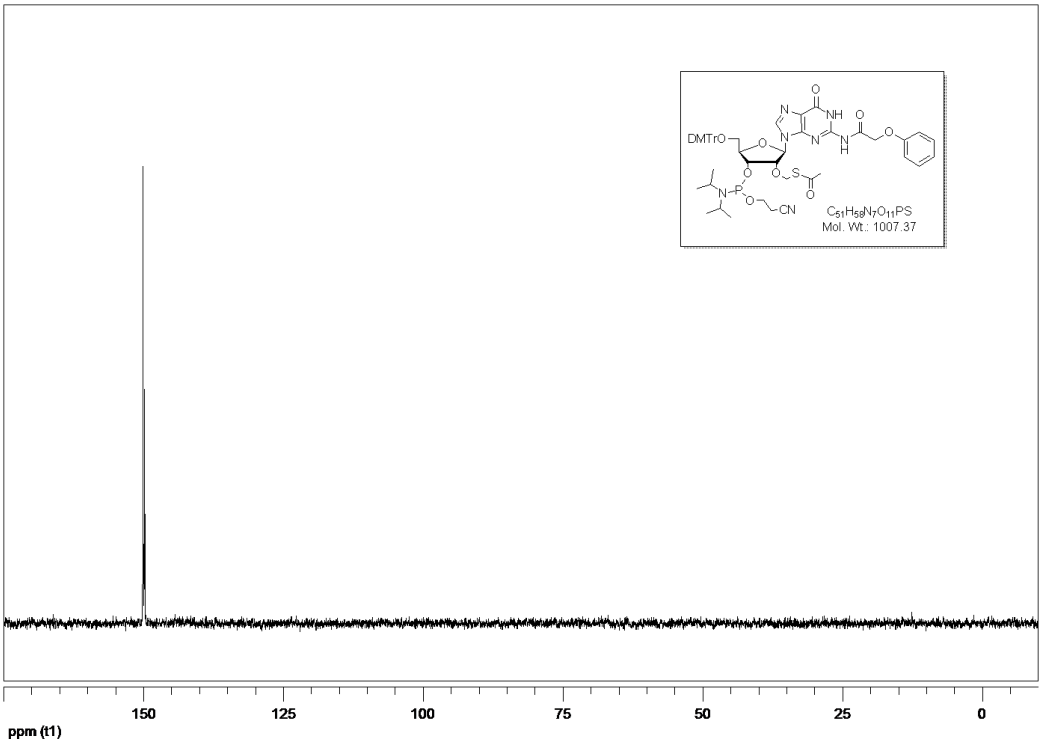


Figure S12: 121 MHz ^{31}P-NMR spectrum (CD_3CN) of **1c**

HPLC chromatograms & MALDI-TOF spectra of purified oligonucleotides

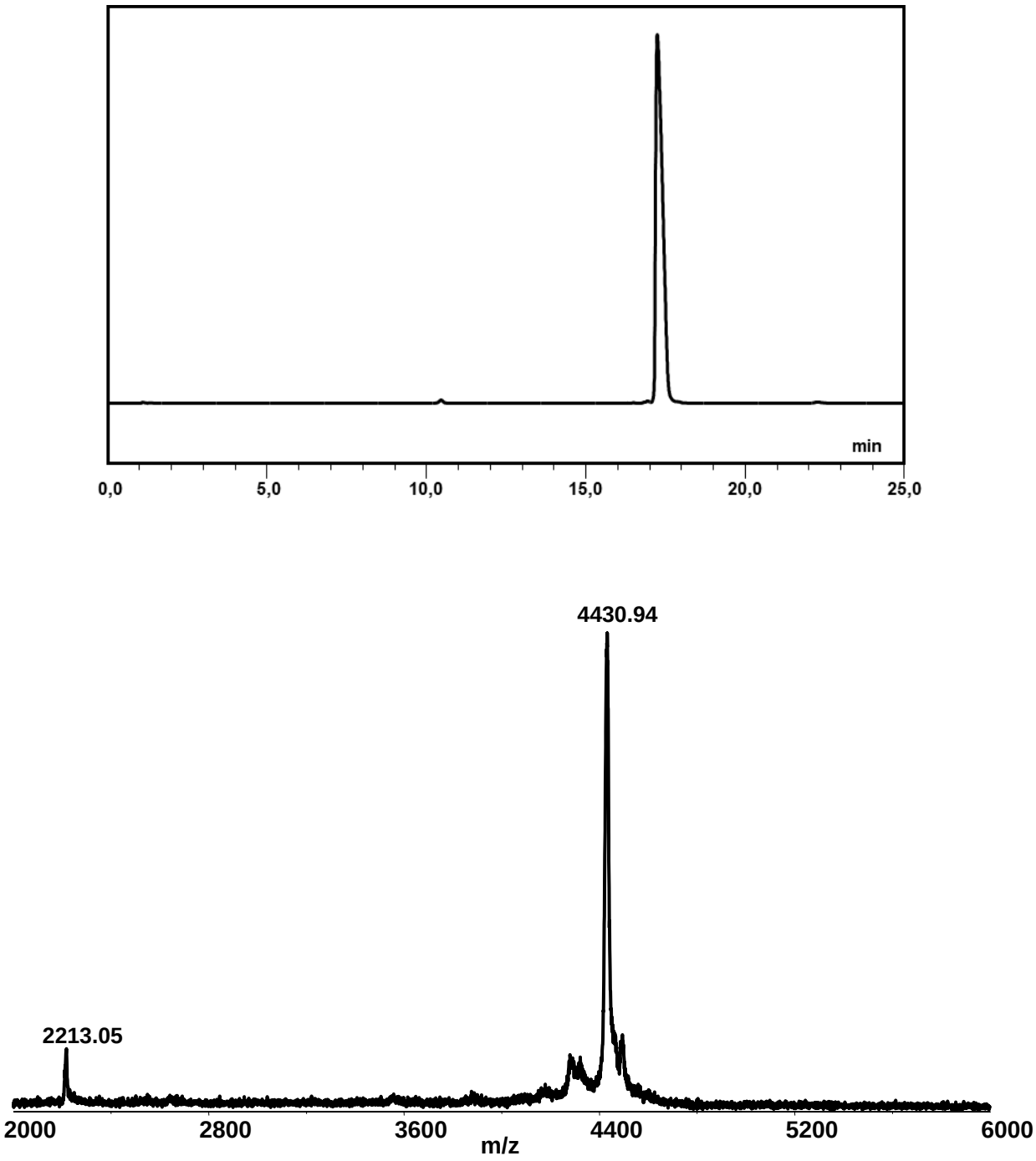


Figure S13: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **S1**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 20 to 100% of B in eluent A. Column temperature 75°C. Flow rate 1.5 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 20% ACN, pH 8

Eluant B : 25 mM Tris HCl, 200 mM $NaClO_4$, 20% ACN, pH 8

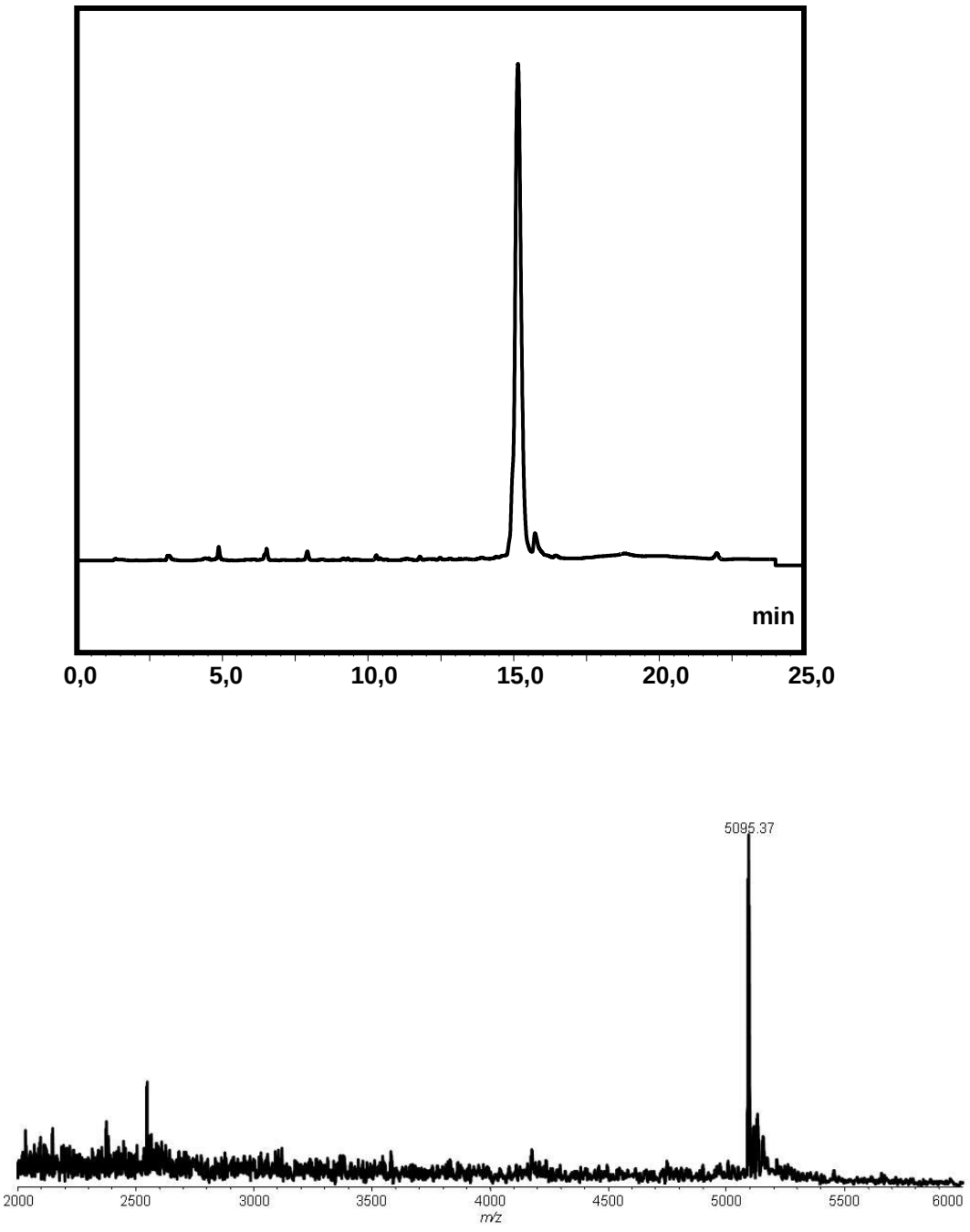


Figure S14: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **H4-1**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 40% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

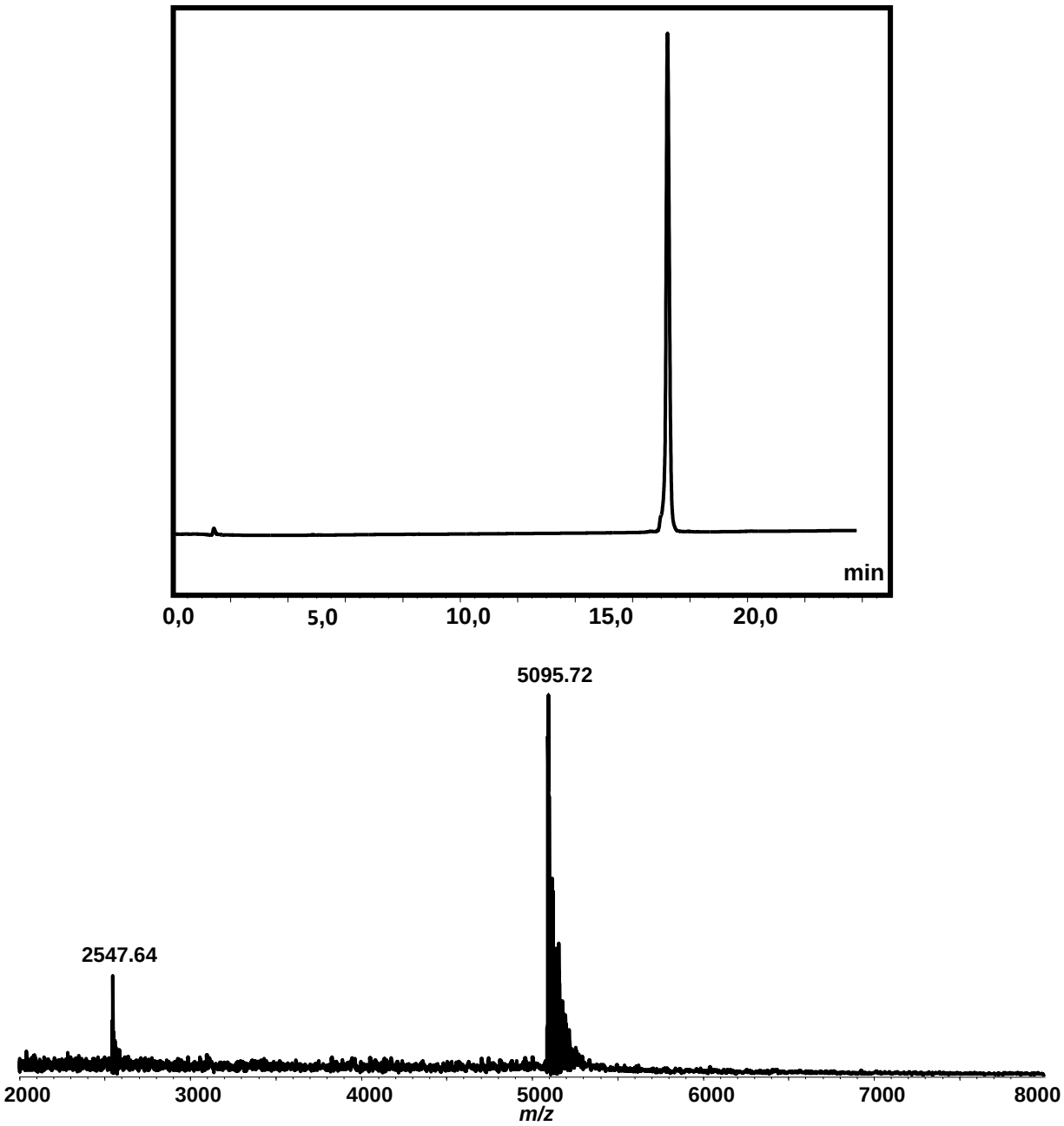


Figure S15: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **H4-2**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 100% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 20% ACN, pH 8

Eluant B : 25 mM Tris HCl, 200 mM $NaClO_4$, 20% ACN, pH 8

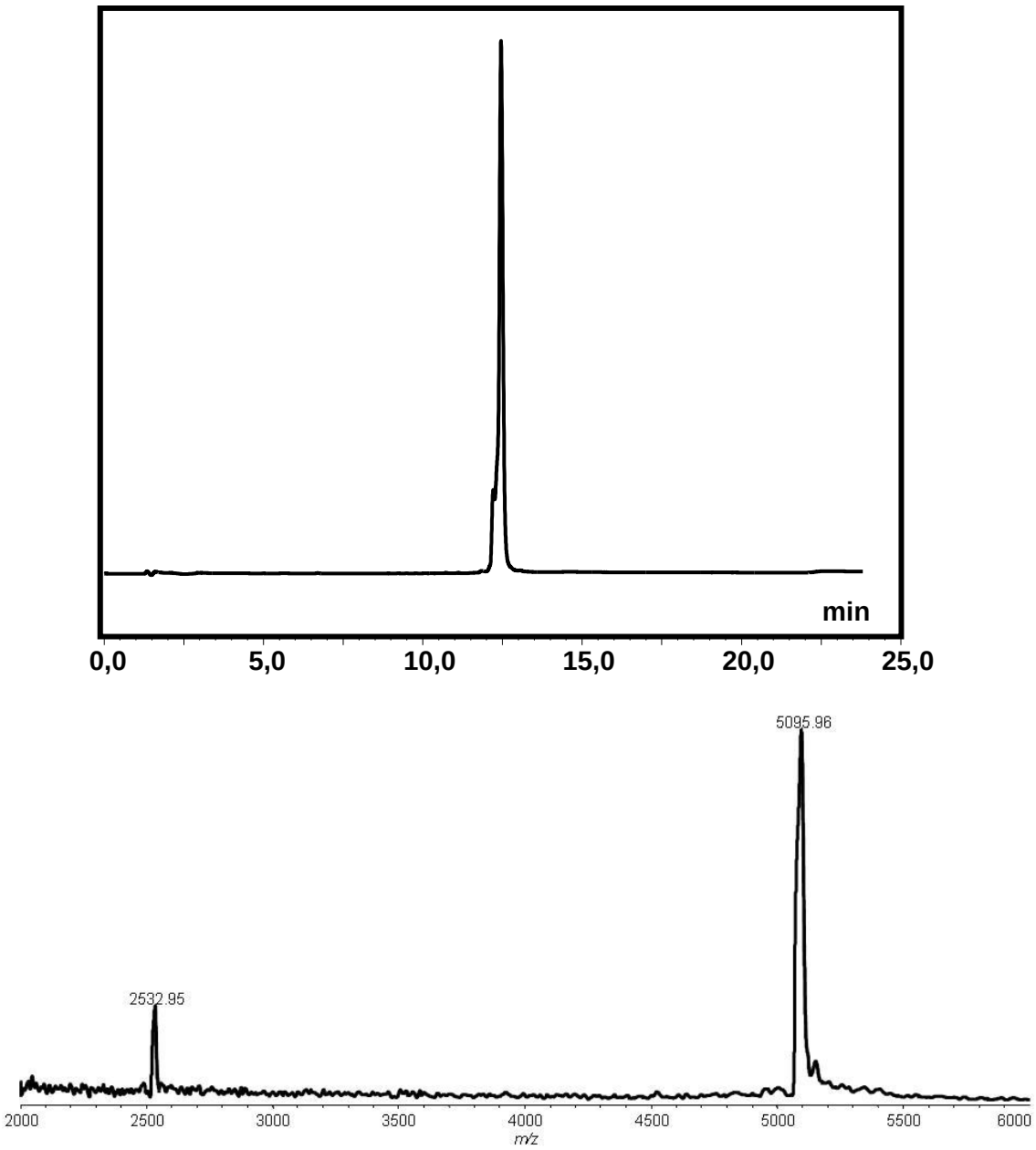


Figure S16: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **H4-3**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 60% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

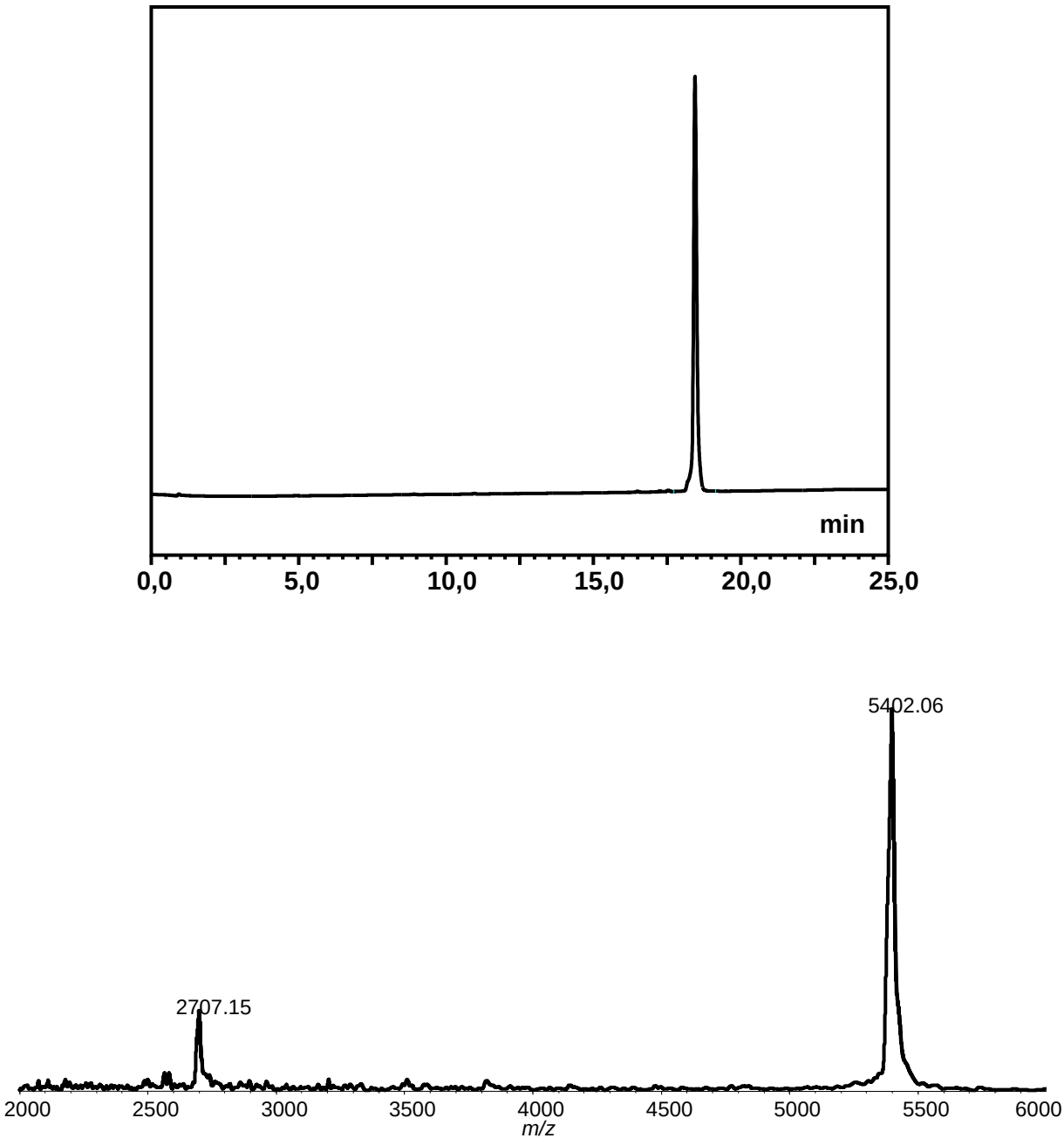


Figure S17: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **H5-1**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 45% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

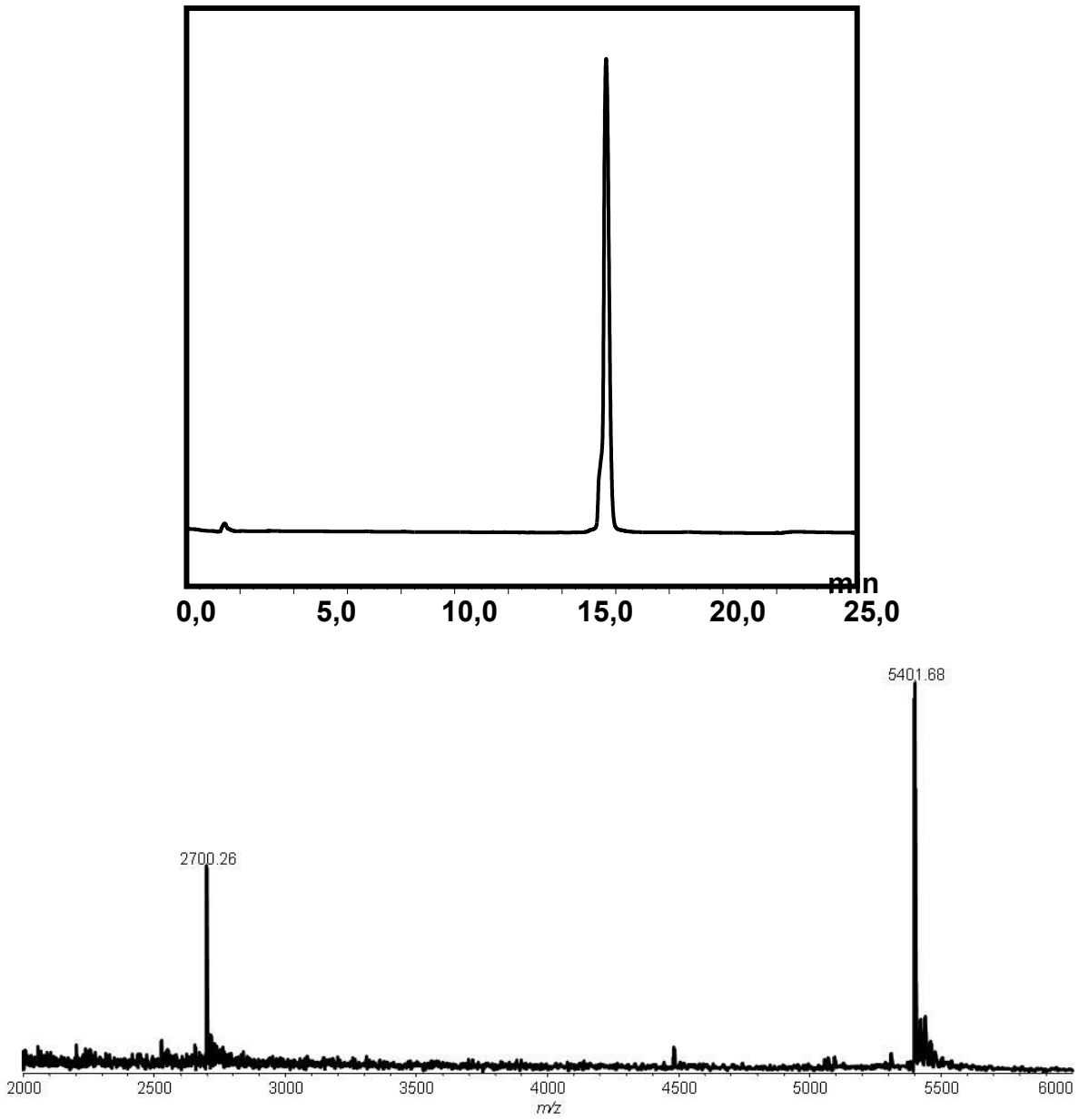


Figure S18: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **H5-2**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 100% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 20% ACN, pH 8

Eluant B : 25 mM Tris HCl, 200 mM $NaClO_4$, 20% ACN, pH 8

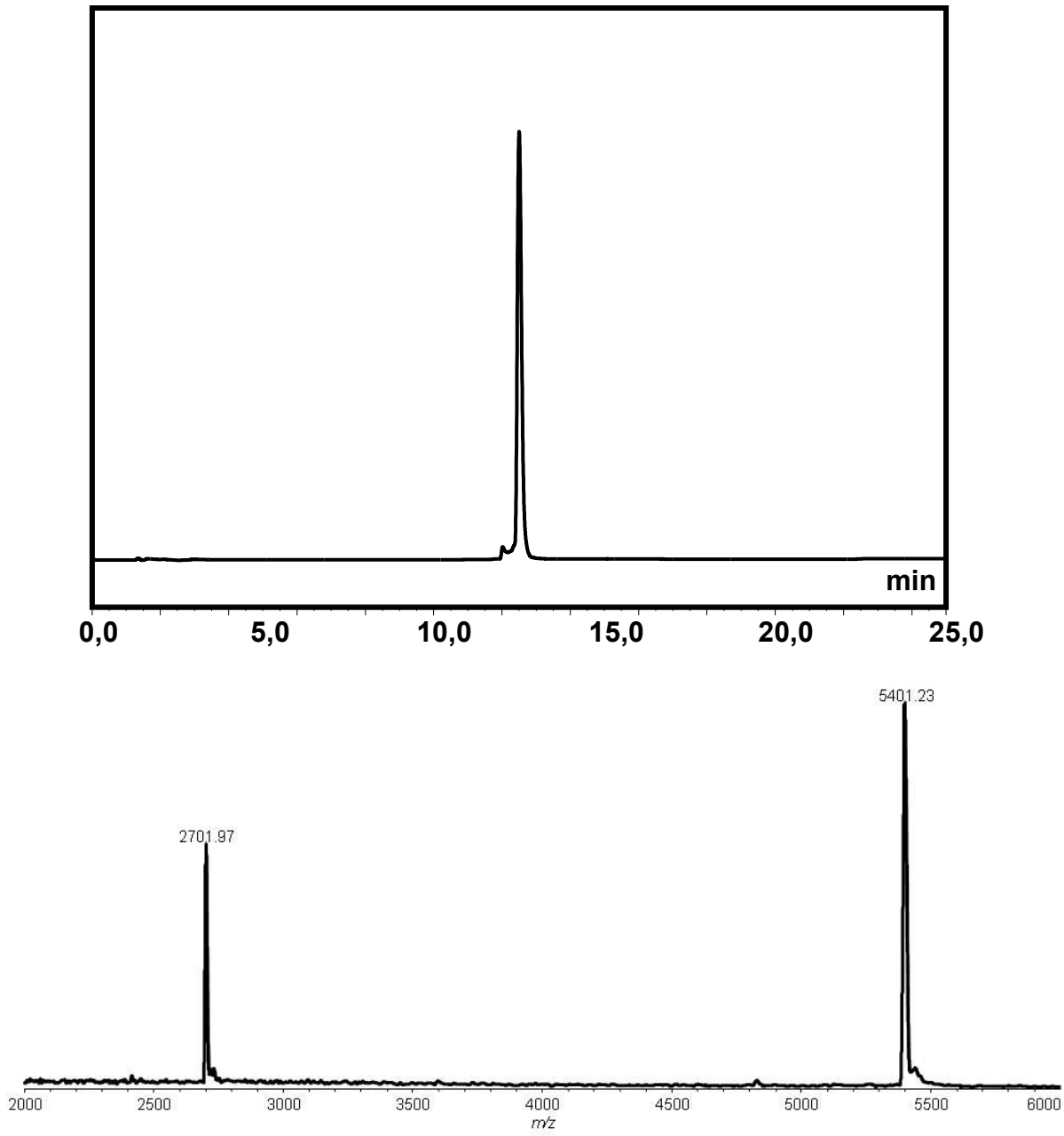


Figure S19: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **H5-3**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 60% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

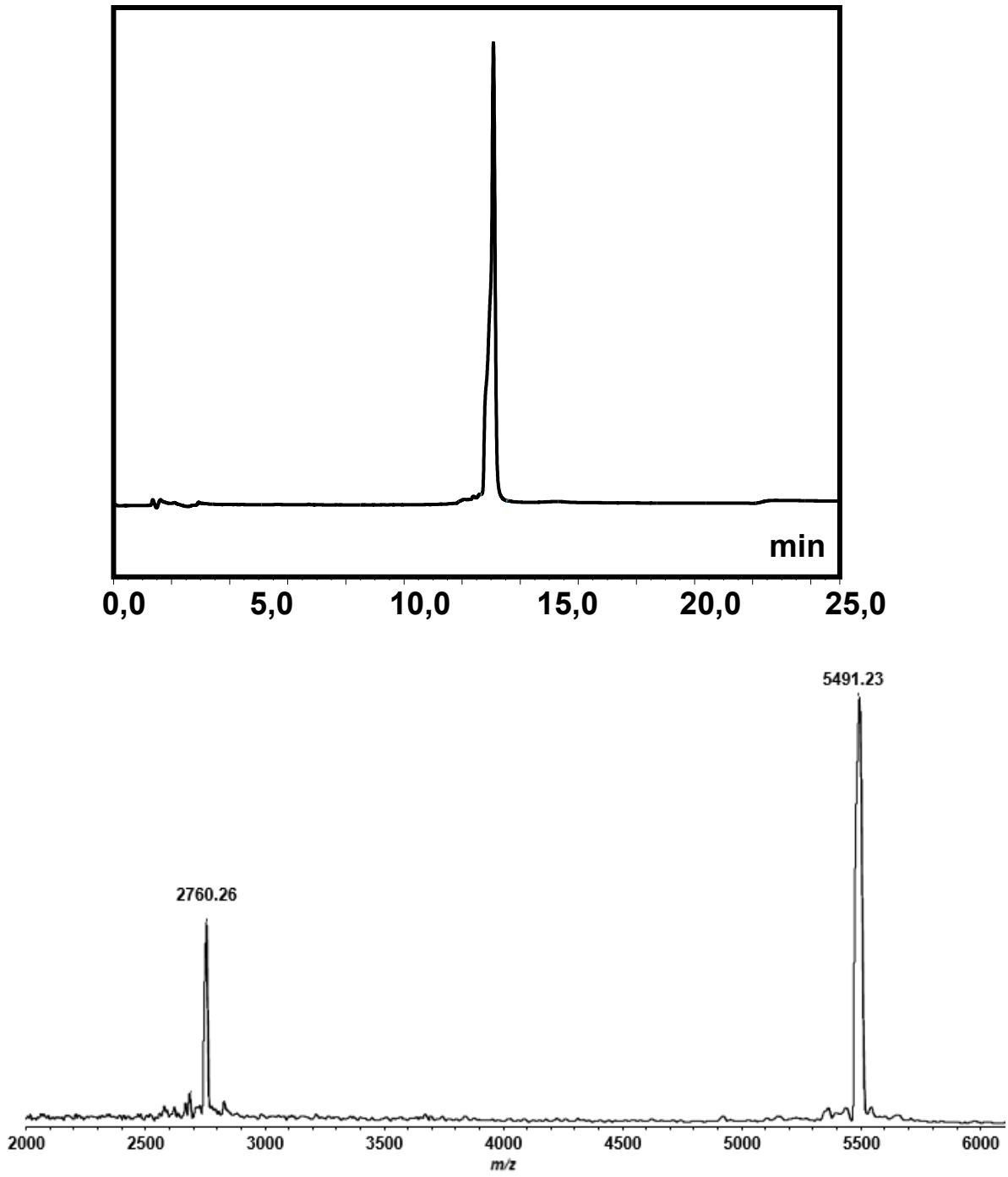


Figure S20: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **H5-4**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 60% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

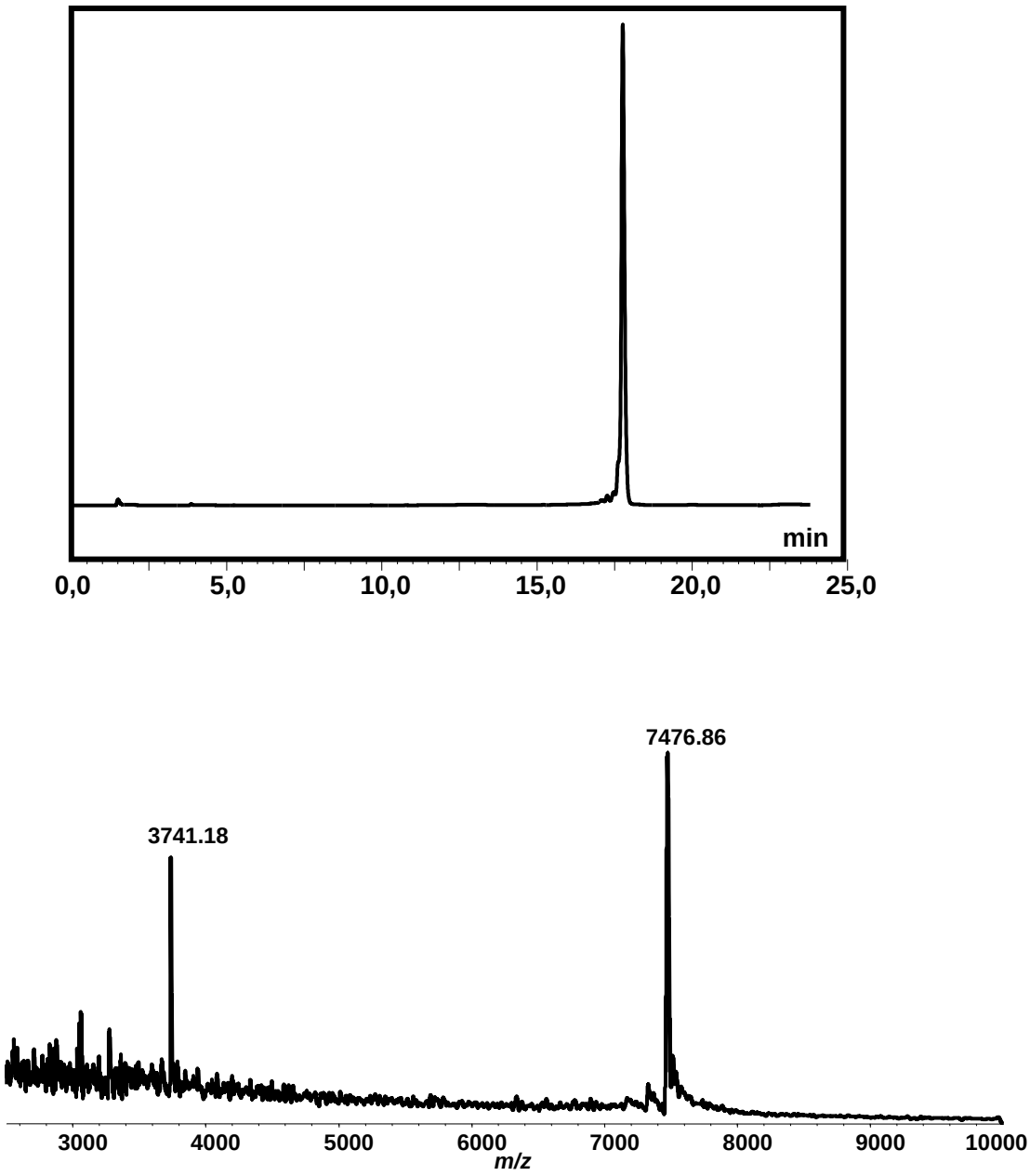


Figure S21: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **DIS-1**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 60% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

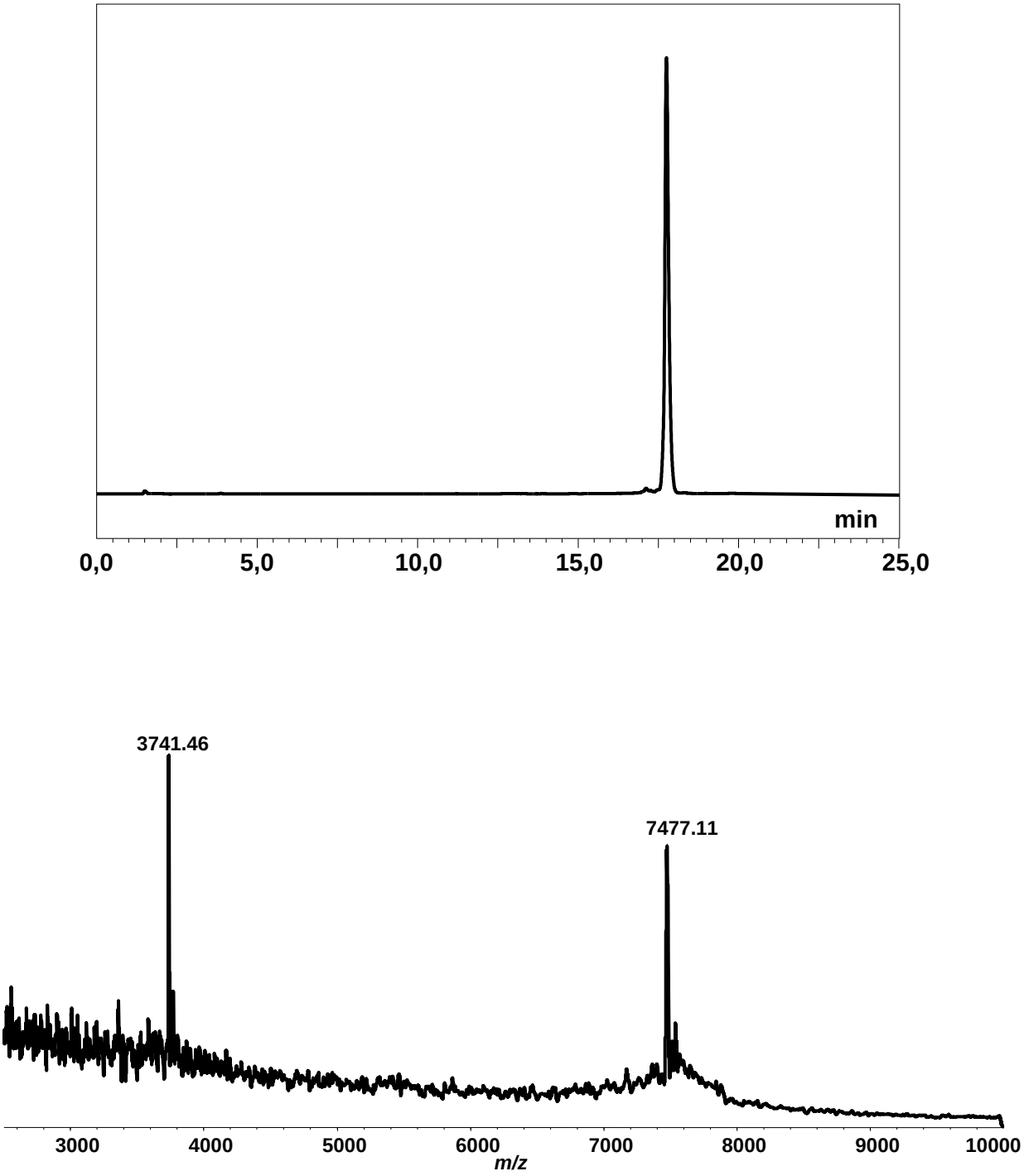


Figure S22: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **DIS-2**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 60% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

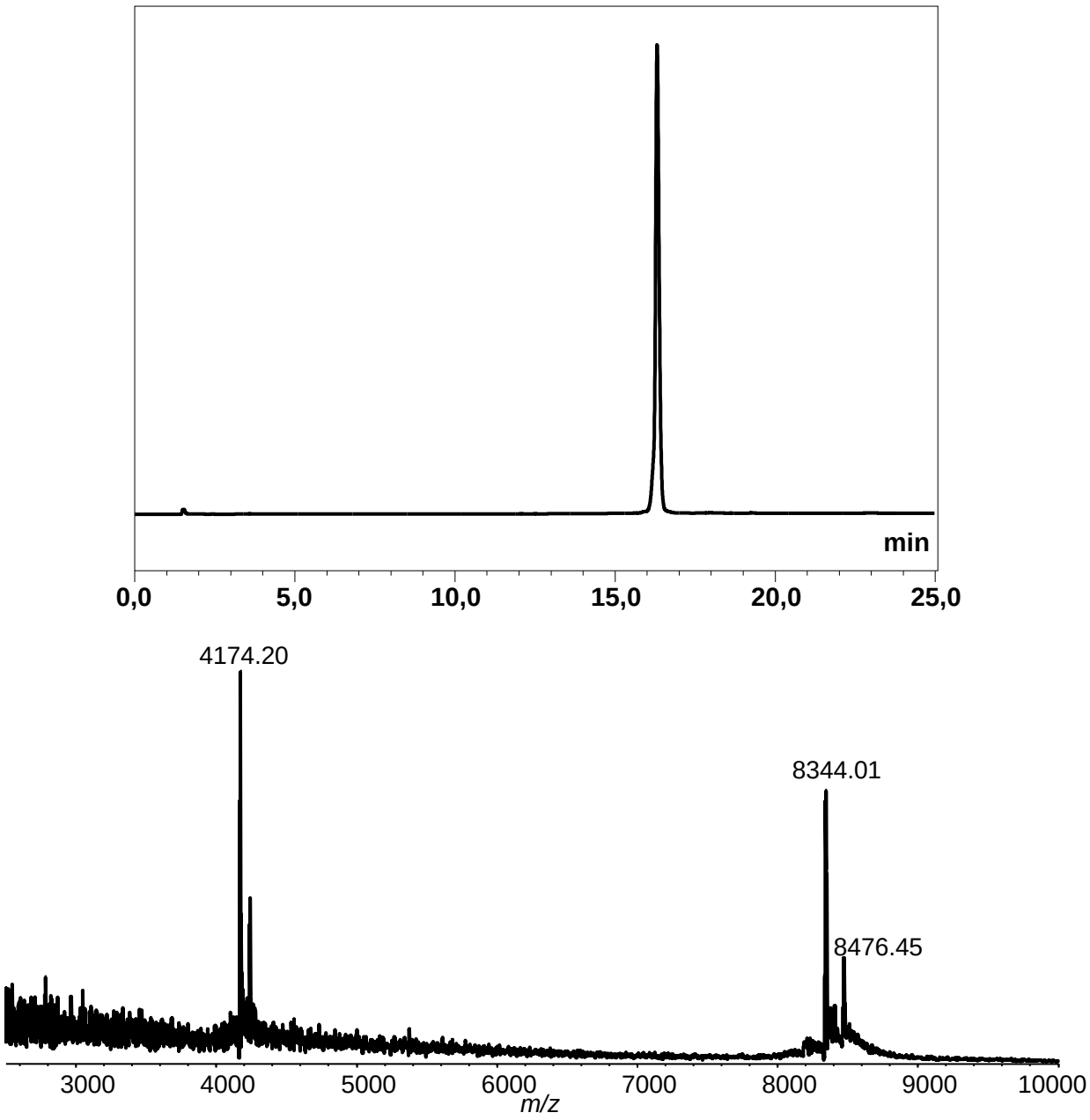


Figure S23: IEX-HPLC and MALDI-TOF MS analysis of purified RNA **DIS-2F**

IEX-HPLC analysis conditions: DNAPac® PA100, 4X250 mm, elution with a 20 min linear gradient of 0 to 80% of B in eluent A. Column temperature 75°C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Eluant A : 25 mM Tris HCl, 5% ACN, pH 8

Eluant B : 25 mM Tris HCl, 400 mM $NaClO_4$, 5% ACN, pH 8

UV Melting curves

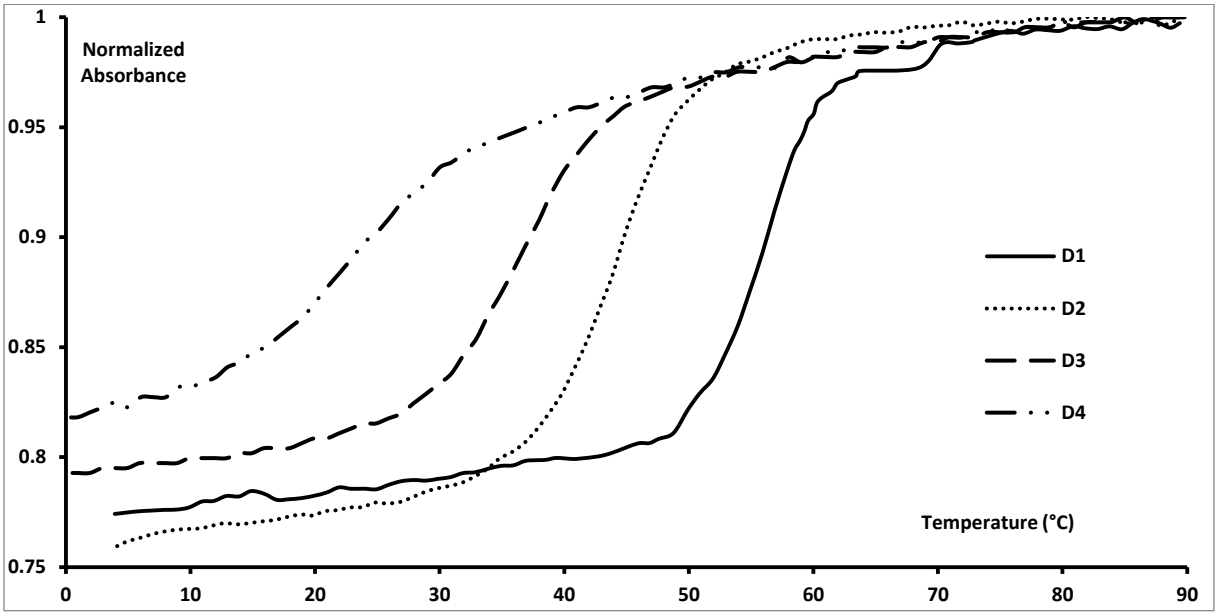


Figure S24: Melting curves of **D1**, **D2**, **D3** and **D4** duplexes
Conditions: Buffer: 10 mM Na cacodylate, 100 mM NaCl, pH 7
1.5µM in 1mL of buffer in a 1cm path length quartz cell,
Heating-cooling-heating cycle in the 0-90°C temperature range with a 0.5°C.min^{-1} gradient
Tm values from two independent experiments were accurate within ± 0.5°C.

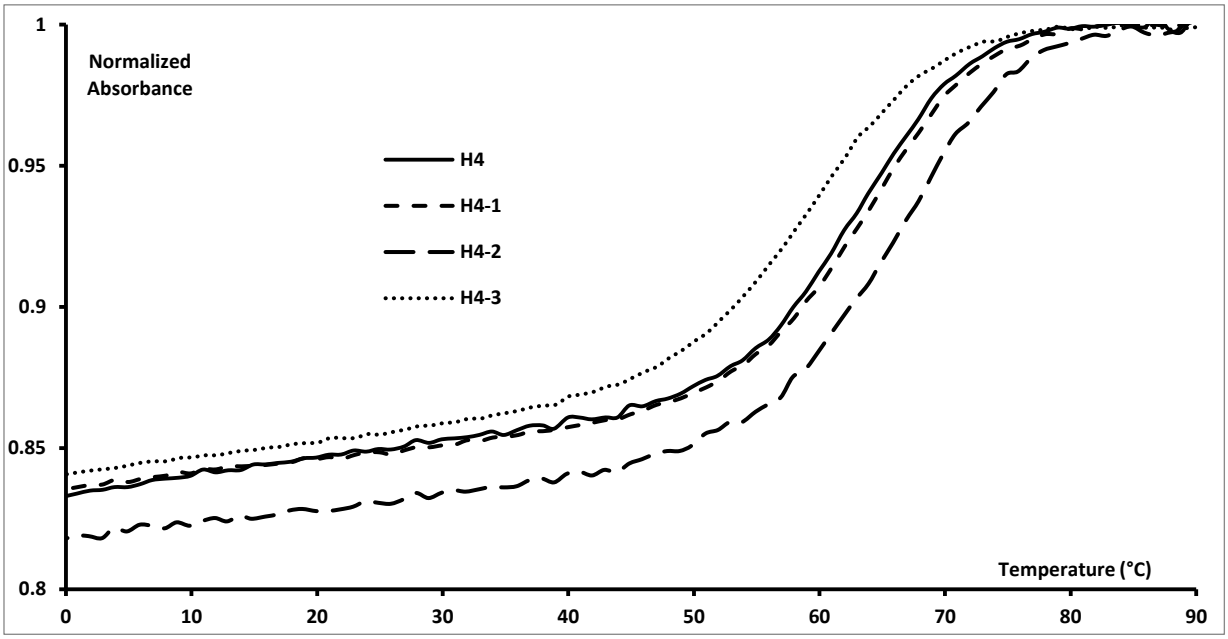
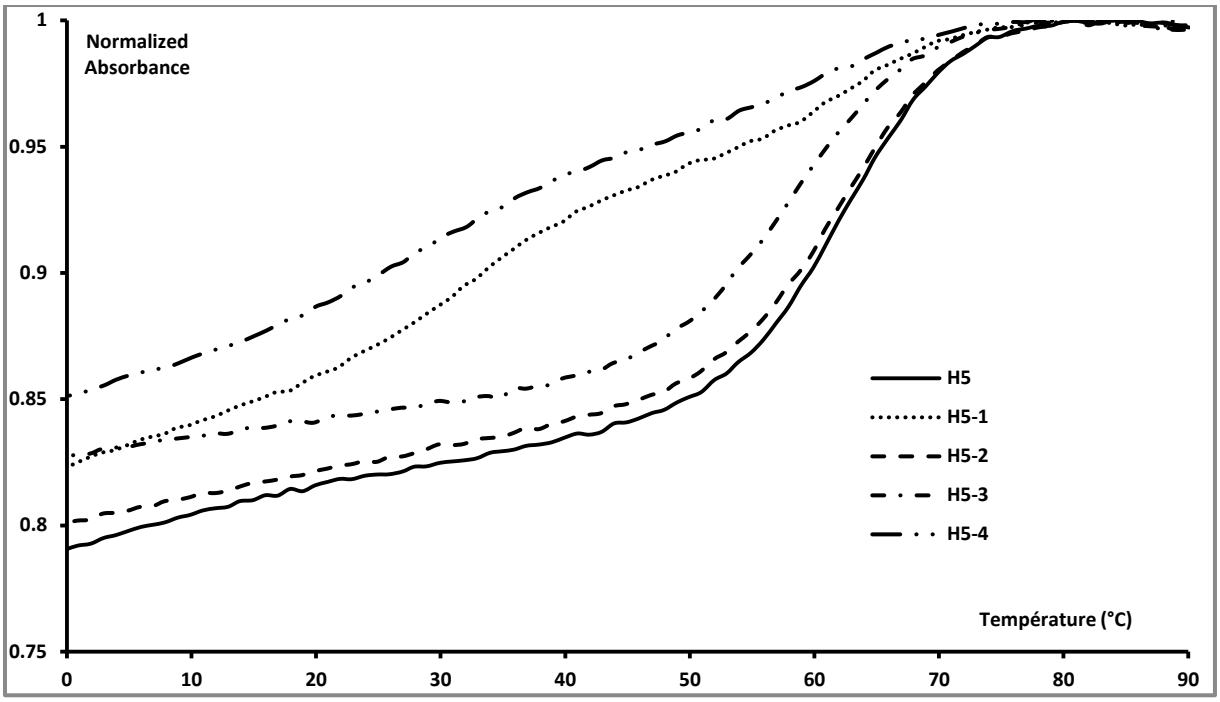


Figure S25: Melting curves of **H4** hairpins
Conditions: Buffer: 10 mM Na cacodylate, 100 mM NaCl, pH 7
1.5µM in 1mL of buffer in a 1cm path length quartz cell,
Heating-cooling-heating cycle in the 0-90°C temperature range with a 0.5°C.min^{-1} gradient
Tm values from two independent experiments were accurate within ± 0.5°C.



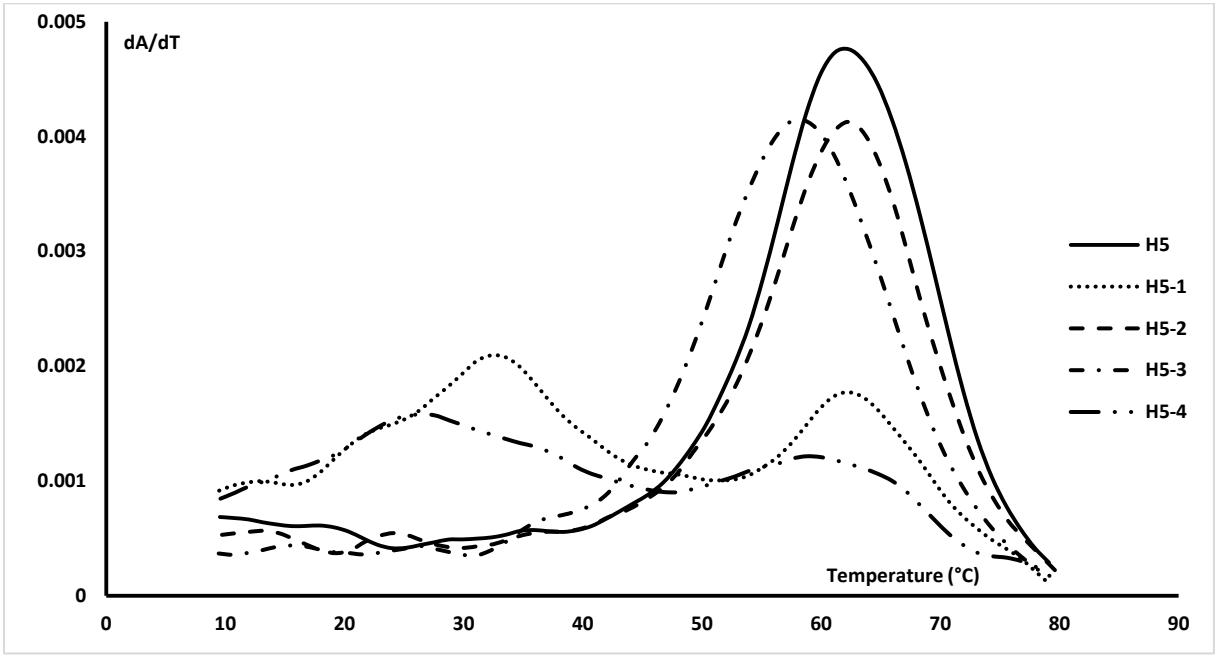


Figure S26: Melting curves and first derivative of **H5** hairpins
Conditions: Buffer: 10 mM Na cacodylate, 100 mM NaCl, pH 7
1.5µM in 1mL of buffer in a 1cm path length quartz cell,
Heating-cooling-heating cycle in the 0-90°C temperature range with a 0.5°C.min^{-1} gradient
Tm values from two independent experiments were accurate within ± 0.5°C.

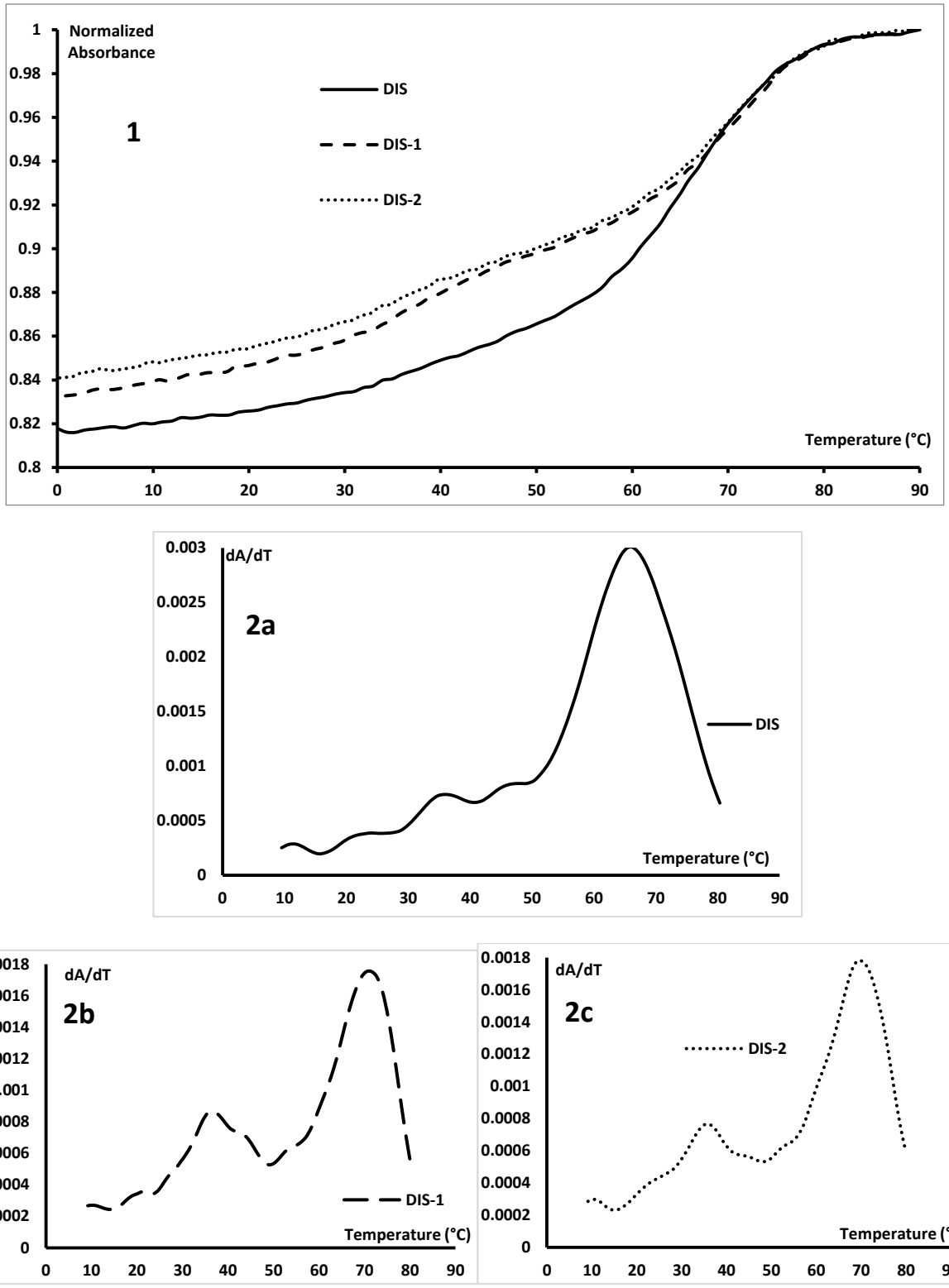


Figure S27: Melting curves **(1)** and first derivatives **(2a, 2b, 2c)** of **DIS** ONs
Conditions: Buffer: 20 mM sodium cacodylate, 25 mM KCl, 5 mM $MgCl_2$, pH 7
1.5µM in 1mL of buffer in a 1cm path length quartz cell,
Heating-cooling-heating cycle in the 0-90°C temperature range with a 0.5°C.min^{-1} gradient
Tm values from two independent experiments were accurate within ± 0.5°C.

Circular Dichroism spectra

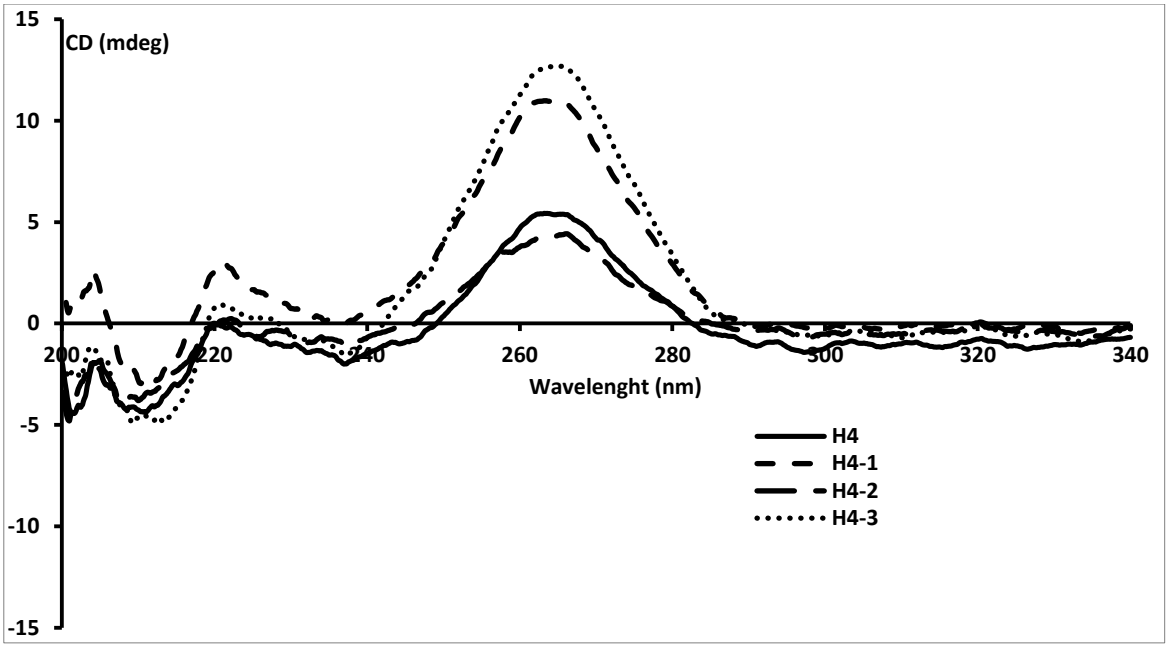


Figure S28: Comparative CD spectra of **H4, H4-1, H4-2** and **H4-3**
Solutions used for Tm experiments conserved at -80°C before CD analyses.
Recorded on a Jasco J-815 spectropolarimeter. Measurements performed at 1°C, wavelength range 340-200 nm, scanning speed of 100 $nm.min^{-1}$.
Raw data were acquired over 2 scans.

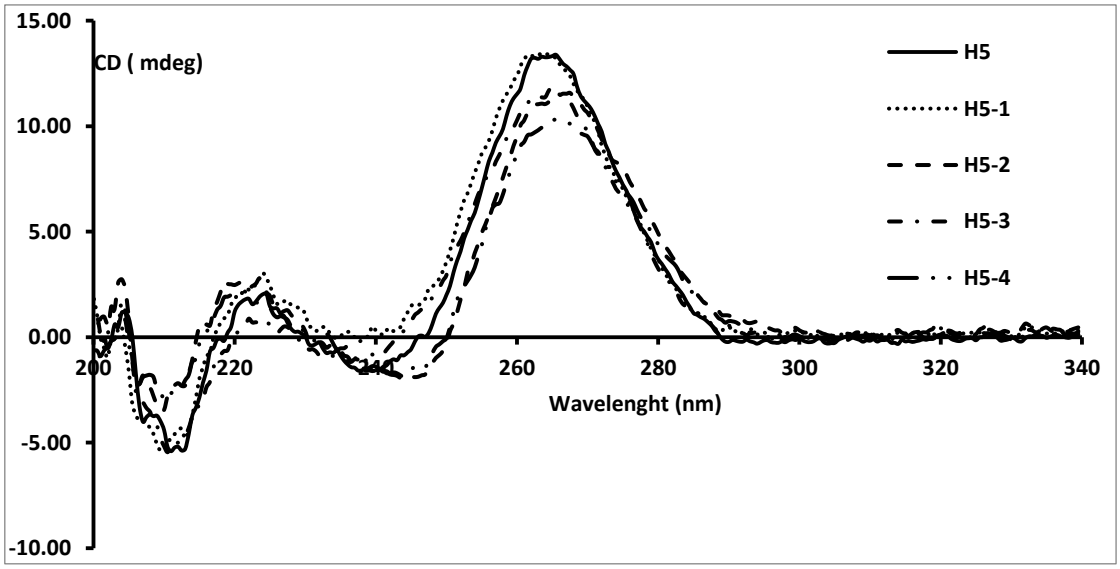


Figure S29: Comparative CD spectra of **H5, H5-1, H5-2, H5-3** and **H5-4** hairpins**.**
Solutions used for Tm experiments conserved at -80°C before CD analyses.
Recorded on a Jasco J-815 spectropolarimeter. Measurements performed at 1°C, wavelength range 340-200 nm, scanning speed of 100 $nm.min^{-1}$.
Raw data were acquired over 2 scans.

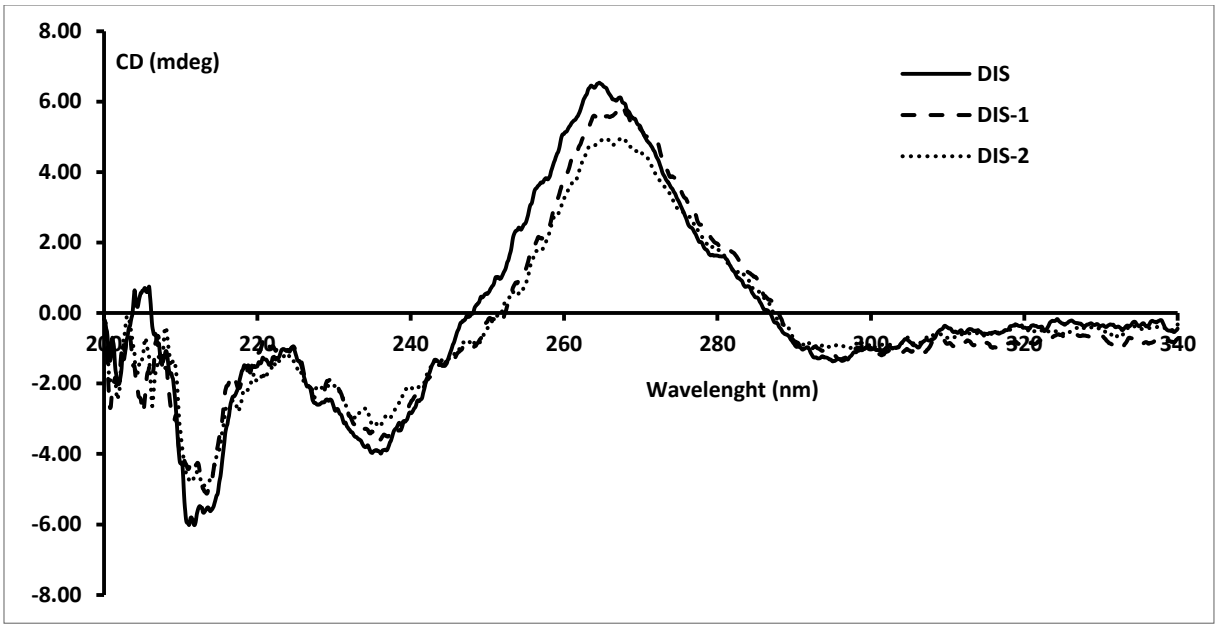


Figure S30: Comparative CD spectra of **DIS, DIS-1** and **DIS-2** ONs**.**
Solutions used for Tm experiments conserved at -80°C before CD analyses.
Recorded on a Jasco J-815 spectropolarimeter. Measurements performed at 1°C, wavelength range 340-200 nm, scanning speed of 100 $nm.min^{-1}$.
Raw data were acquired over 2 scans.

Analyses of H4 & H5 hairpins by gel electrophoresis

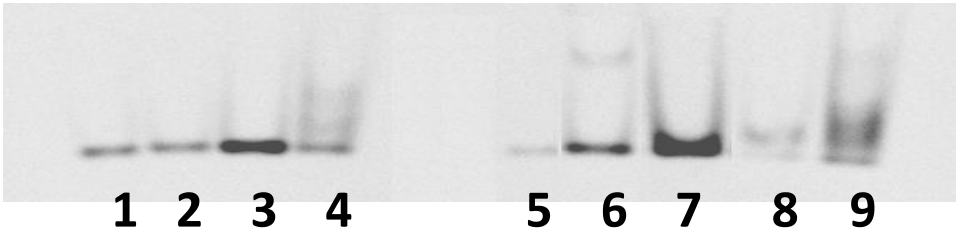


Figure S31: Gel electrophoresis (15% polyacrylamide) of **H4** and **H5** 5µM in Na-cacodylate 10 mM, NaCl 100 mM, pH 7.
Migration in TBE buffer at 4°C. Staining with with GelRed 3X (VWR) in a 0.1M NaCl solution for 1h, revelation with a UV transilluminator.
(1: **H4,** 2: **H4-1,** 3: **H4-2,** 4: **H4-3,** 5: **H5,** 6: **H5-1,** 7: **H5-2,** 8: **H5-3,** 9: **H5-4**)

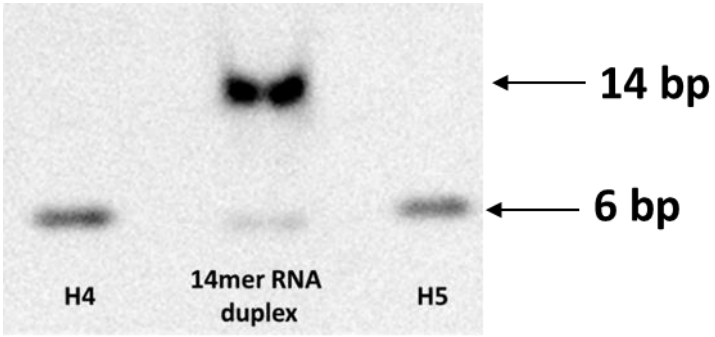


Figure S32: Comparative gel electrophoresis (15% polyacrylamide) of **H4** and **H5** 5µM with a 14-mer RNA duplex in Na-cacodylate 10 mM, NaCl 100 mM, pH 7.
Migration in TBE buffer at 4°C. Staining with with GelRed 3X (VWR) in a 0.1M NaCl solution for 1h, revelation with a UV transilluminator.

Enzymatic stability of RNA hairpins

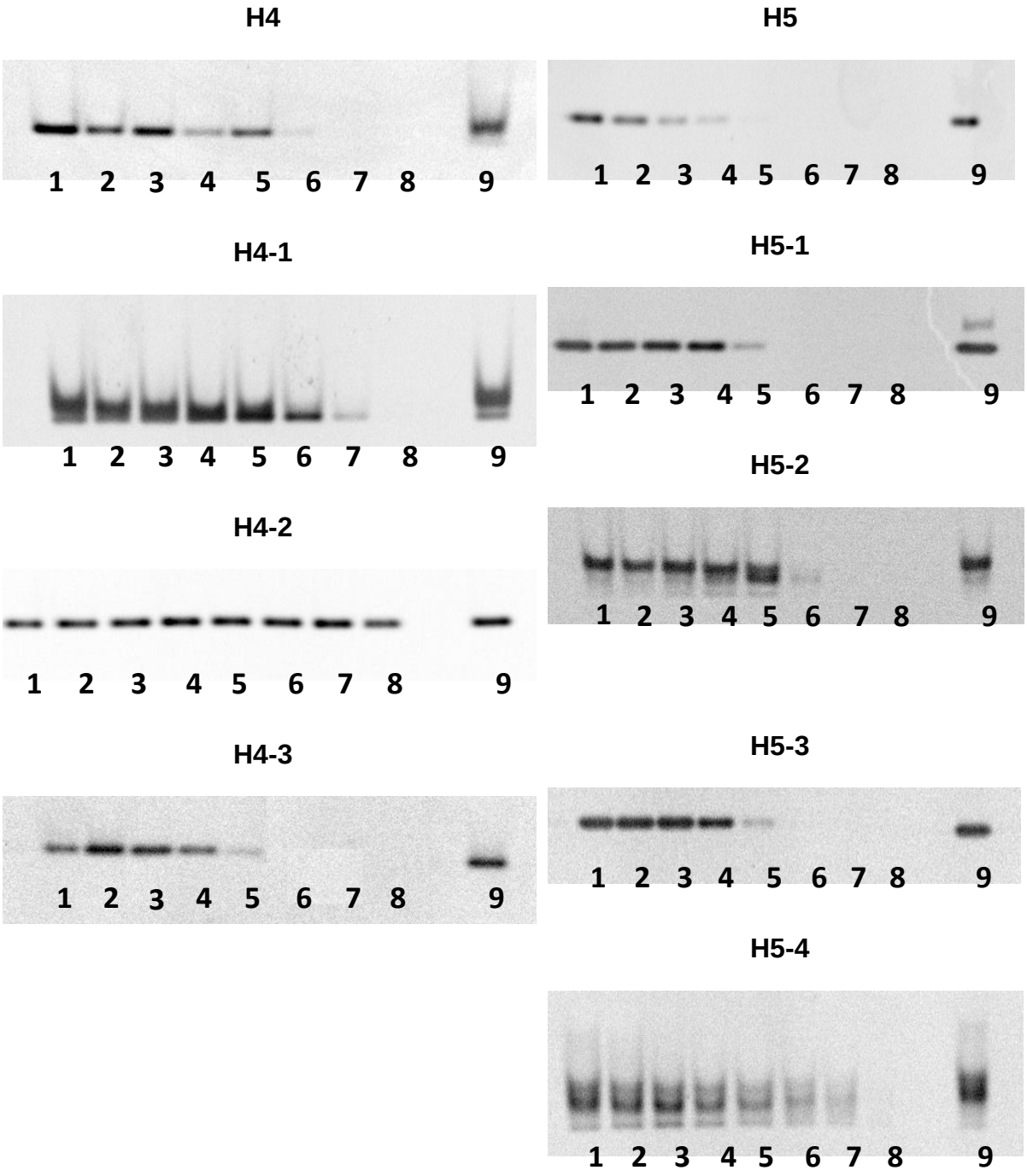


Figure S33: Gel electrophoresis (15% polyacrylamide) of RNAs (48 µM) incubated with SVPDE (2 mU) in a 450 mM ammonium citrate buffer at 37°C. Migration in TBE buffer 1X at 4°C. Staining with with GelRed 3X (VWR) in a 0.1 M NaCl solution for 1h, revelation with a UV transilluminator. **1**: 1 min, **2**: 5 min, **3**: 15 min, **4:** 30 min, **5**: 1h, **6**: 3h, **7**: 6h, **8**: 24h, **9**: reference without enzyme 24h

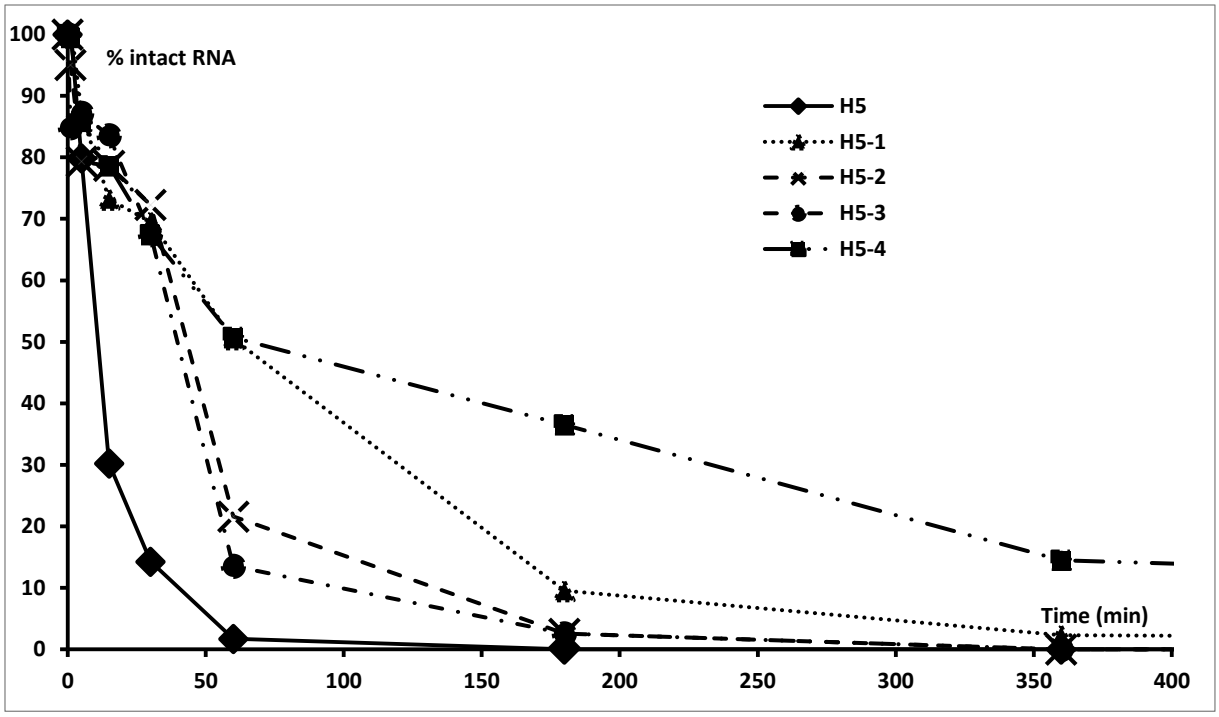


Figure S34: Enzymatic stability of **H5** against SVPDE evaluated by gel electrophoresis. Band analysis was performed using ImageJ Software (Broken Symmetry Software V:1.4.3.67). Percentages of intact RNA were calculated on Microsoft Office Excel by comparison with the reference incubated without enzyme (lane 9 of the gels).

Fluorescence experiments

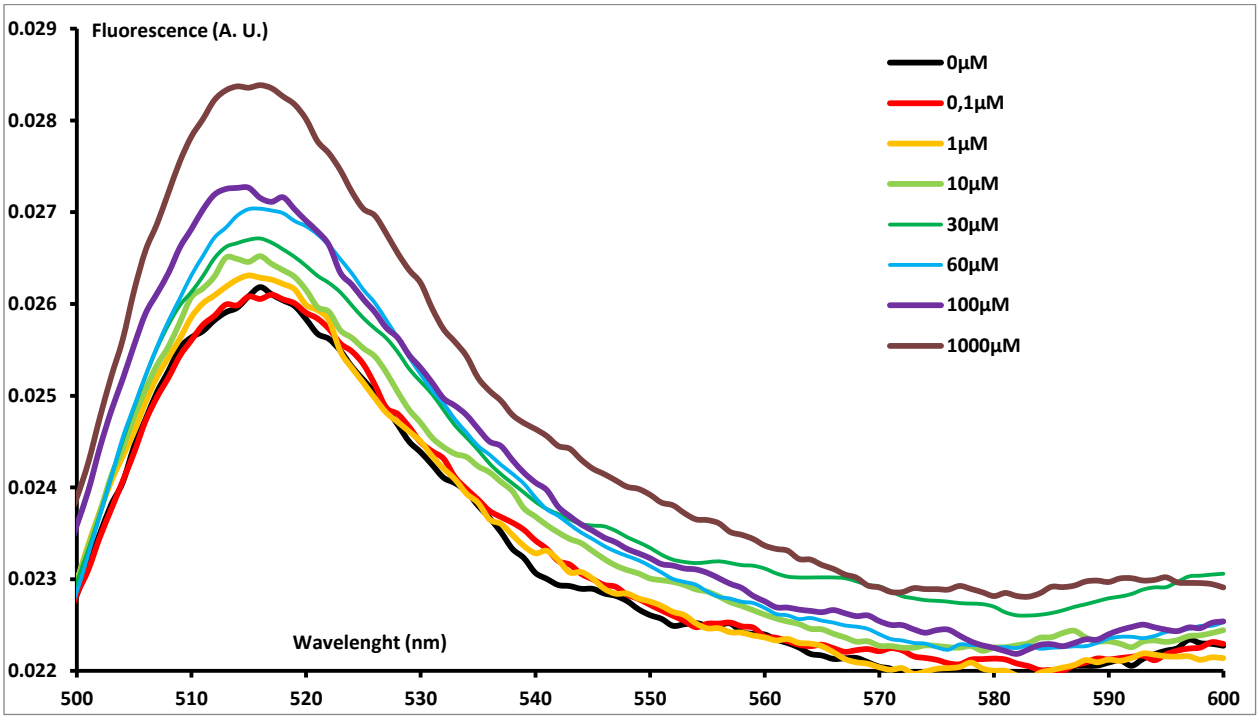


Figure S35: Fluorescence emission spectra of **DIS-2F**, 2 µM in 100 nM to 1 mM glutathione, 20 mM sodium cacodylate, 25 mM KCl, 5 mM $MgCl_2$, pH 7 with a 20 µM concentration of **DIS** (ratio 10:1). Fluorescence emission spectra at 25°C from 400 to 600 nm, excitation wavelength of 494 nm in a 100 µL quartz cell with a 1cm light path. Raw data were acquired in triplicates over 2 scans.

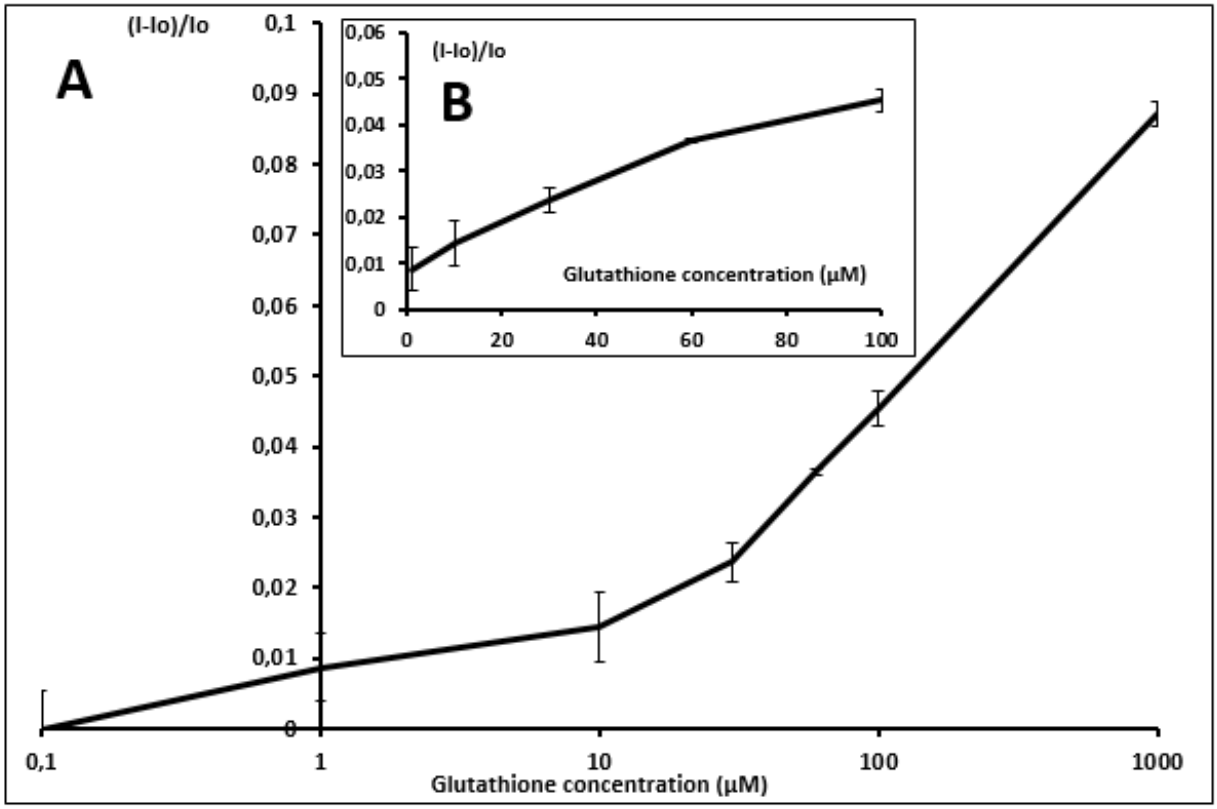


Figure S36: Glutathione concentration dependence of relative fluorescence intensity ((I-Io)/Io) of **DIS-2F**, 2 µM at the fluorescence emission maximum (λ=515nm) (**A**: complete curve with GSH concentration ranging from 0.1 µM to 1000 µM, Log-10 scale was used for the concentration axis, **B**: linear domain from 1 µM to 100 µM
Error bars are the standard deviation of the three repetitive independent experiments.
Buffer: 20 mM sodium cacodylate, 25 mM KCl, 5 mM $MgCl_2$, pH 7 with a 20 µM concentration of **DIS** (ratio 10:1).