Synthesis and RNA binding selectivity of oligonucleotides modified with five-atom TANA backbone structures

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Experimental Procedure:

Melting points of samples were determined in open capillary tubes using Buchi Melting point B-540 apparatus and are uncorrected. IR spectra were recorded on an infrared Fourier Transform spectrometer .Column chromatographic separations were performed using silica gel 60-120 and 230-400 mesh, Ethyl acetate, Petroleum ether, Dichloromethane and Methanol as the solvent system. ^{1}H and ^{13}C spectra were obtained using Bruker AC 200(200MHz), 400(400MHz) and 500(500MHz) NMR spectrometers. The optical rotation values were measured on Bellingham-Stanley Ltd, ADP220 polarimeter.

Synthesis of 5'-*O*-tosyl thymidine (2a): A solution of thymidine **1**, 2.42g (10 mmol) in 30 ml anhydrous pyridine was cooled to 0 ˚C. Tosyl chloride (2.3g, 12 mmol) dissolved in 10 ml of pyridine was then added drop wise during 4 hours. The reaction temperature was maintained at 0 ˚C during the addition. The reaction mixture was stirred for additional 4 h at room temperature. Pyridine was removed the under vacuum. The residue was dissolved in 100 ml ethyl acetate and the organic layer was washed with 10% $NaHCO_3$ solution followed by water .The tosyl thymidine **2a** slowly crystallized out. The product was filtered and was dried under vacuum. Yield 3.2g (80%).

M.p. 160-164ºC. ^{1}HNMR: ($CDCl_3$ +1-drop DMSO-d6), (200MHz) δ1.89(s, 3H), 2.1-2.25(m, 2H), 2.4(s, 3H), 3.98-4.1 (m, 3H), 4.24(m, 1H), 6.2(t, 1H), 7.33(d, 3H), 7.78(d, 2H), 10.14(s, 1H).

Mass calculated 396.39 observed 396.46.

Synthesis of 5'*O*-tosyl, 3'*O*-*tert* butyl dimethyl silyl thymidine (2b):

A solution of 5'*O*- tosyl thymidine **2a** (3.1g, 7.8 mmol), *tert*-butyl dimethyl silyl chloride (1.4g, 9.4 mmol), imidazole (1.33g, 19.6mmol) in 10 ml anhydrous DMF was stirred for

6 h. DMF was removed the in *vacuo* and the residue was dissolved in 200 ml ethyl acetate. The organic layer was washed with water (3 x 50 ml) followed by brine (2 x 30 ml). The organic layer was dried over anhydrous Na_2SO_4 and solvent was then removed in *vacuo*. The residue was redissolved in 20 ml DCM and subjected to filtration column purification (50% Pet Ether: Ethyl acetate). Pure compound **2b** was obtained in 95% yield, 3.8 g.

^{1}HNMR: ($CDCl_3$), (200MHz) δ 0.06(s, 6H), 0.86(s, 9H) 1.89(s, 3H), 2.1 (m, 1H), 2.29(m,1H), 3.98-4.1 (m, 3H), 4.24(m,1H), 6.2(t, 1H), 7.33(d, 3H), 7.8(d, 2H), 9.7(s, 1H).

Mass calculated 511.2 observed 511.87

Synthesis of ethyl-[*S*- 5'-(3'-*O*-TBDMS- Thymidinyl)-mercapto]-acetate 3:

Solution of NaH (0.35g, 60% solution in hexane, 8.7 mmol.) and ethyl mercapto acetate (1 ml, 8.7 mmol) in 5 ml anhydrous DMF was stirred for 30 minutes. 5'*O*-tosyl, 3'*O*-*tert* butyl dimethyl silyl thymidine **2b** (3.7g, 7.23 mmol) dissolved in 5 ml DMF was then added slowly. The reaction mixture was stirred for 1 h at RT. DMF was removed under reduced pressure, and the residue was dissolved in 200 ml ethyl acetate. The organic layer was washed with water (3 x 50 ml) followed by washing with brine (2 x 30 ml). The organic layer was dried over anhydrous Na_2SO_4 and solvent removed in *vacuo*. The product was subjected to column chromatography purification (50% Pet Ether: Ethyl acetate) to give pure **3**, 2.75g, 80%.

M.p. 122-124 ºC $[\propto]_{25}^{D}$= +19.4˚ (c 0.5, $CHCl_3$).

^{1}HNMR: ($CDCl_3$), (400MHz) δ 0.05(s, 6H), 0.86(s,9H) 1.9(s, 3H), 2.09 (m,1H), 2.26(m,1H), 2.9-3 (m,2H), 3.22-3.31(q, 2H), 4(q,1H), 4.16(q, 2H), 4.26(m,1H) 6.2(t, 1H), 7.33(d,1H), 9.4(b, 1H)

^{13}C NMR δ -4.8, -4.7, 12.5, 14.1, 17.9, 25.7, 34.2, 34.3, 40.5, 61.5, 73.5, 84.7, 85.7, 111.2, 135.4, 150.1, 163.7, 170.0.

Mass calculated 458.64 observed 497.4($+K^+$).

Synthesis of *S*- 5'-(3'-*O*-TBDMS-thymidinyl)-mercapto-acetic acid 4:

5 ml 2M NaOH solution was added to the solution of **3** (2.6g, 5.47 mmol) in 10 ml methanol. The reaction mixture was stirred for 30 minutes. The sodium salt of the acid and excess NaOH present in the solution was neutralized by DOWEX 50 H^+ resin. The resin filtered and washed with 2:1 mixture of methanol:water. The filtrate was concentrated under vacuum and the dried by vacuum desiccation to give **5,** 2.3 g, (95%)

M.p. 134-136ºC. ^{1}H NMR (D_2O) δ 0.06(s, 6H), 0.85(s, 9H), 1.66(s, 3H), 2.16(m, 2H), 2.71-2.76(m, 2H), 3.22(q, 2H), 3.88(dd, 1H), 4.2(m, 1H), 5.99(t, 1H), 7.3(s, 1H).
Mass calculated 430.6 observed 453.11(+ Na^+).

Synthesis of 3'-deoxy-3'-azido- 5'-*O*-dimethoxytrityl- thymidine 6:

A mixture of AZT **5** (2.68g, 10 mmol), dimethoxytrityl chloride (4g, 12 mmol), DMAP (1mmol), triethylamine (7ml,50mmol) in 50 ml anhydrous pyridine was stirred at RT for 6 h. Pyridine was removed in *vacuo* and residue redissolved in 200 ml DCM. The organic layer was washed with 10% $NaHCO_3$ solution (2 x 30 ml) followed by water (2 x 50 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. Column purification using 1% methanol-dichloromethane gave pure **6,** 5.1 g, 90%.

IR, ν(cm^{-1}) (CHCl3) ; 3128, 3019, 2110, 1724, 1670 cm^{-1} ^{1}HNMR: ($CDCl_3$, 200MHz) δ 1.51 (s, 3H), 2.4-2.5(m, 2H), 3.3-3.5(m, 2H), 3.8(s, 6H), 4.0- (d, 1H), 4.4(q, 1H), 6.25(t, 1H), 6.8-8.6(m,14H) Mass calculated 569.6 observed 568.3.

Synthesis of 3'-deoxy-3'-amino 5'-*O*-dimethoxytrityl- thymidine 7:

Compound **6** was dissolved (1.5 g, 2.6 mmol) in 10 ml methanol and to it was added 0.15g (10%) Pd-C catalyst. Then mixture was subjected to catalytic hydrogenation at 40 Psi of hydrogen pressure for 3.5 h. The catalyst was removed by filtration over celite and concentration of the filtrate in *vacuo* give **7,** 1.3g, 90%.

IR, ν(cm^{-1}) (CHCl3) 3398, 3018, 2924, 2853, 1701, and 1686. ^{1}HNMR: ($CDCl_3$, 200MHz) δ 1.52 (s,3H), 2.17-2.3 (m,2H), 3.38(dd,1H), 3.48(dd,1H), 3.7-3.8(m,2H) 3.8(s,6H), 6.29 (t,1H), 6.86(d,4H), 7.2-7.5 (m,9H), 8.64 (s,1H). Mass calculated 543.6 observed 543.7.

Synthesis of 3'-deoxy-3'-azido-5'-*O*-dimethoxytrityl-C^4-(-1, 2, 4-triazol-1-yl)-pyrimidine 2'-deoxyribonucleoside 8:

Triethylamine (12.7ml, 91mmol) was added drop wise to a stirred mixture of 1,2,4-triazole (7g, 100 mmol) and phosphoryl chloride (2ml, 22 mmol) in 50ml CH_3CN at 0ºC. The solution of **6** (3.5g, 9.1 mmol) in 15ml dry CH_3CN was then added dropwise. The reaction mixture was stirred at room temperature for 2.5 h. Triethylamine (4.4 ml) and water (1.1ml) were added at 0ºC and the mixture was stirred for another 10 minutes. The solvent was evaporated and the residue was re-dissolved in 50 ml ethyl acetate. The organic layer washed with water (2x50ml) and saturated aqueous NaCl (2x 20ml), dried over Na_2SO_4 and evaporated to dryness to give 3.6g (92%) **8** as yellow foam, which was used immediately for the next reaction.

Synthesis of 2',3'-dideoxy-3'-azido-5'-dimethoxytrityl-5-methylcytidine 9:

Concentrated aqueous ammonia (10 ml) was added to the solution of **8** (3.5g, 8mmol) in 50 ml dioxane. The reaction mixture was stirred at room temperature for 2.5 hours. The solvents were evaporated *in vacuo* and the residue redissolved in CH_2Cl_2 and purified by silica-gel column chromatography (eluted with 0-5%methanol in CH_2Cl_2) to afford **9** (3g, 96%) as white foam.

IR, $\nu(cm^{-1})$ 3192, 2984, 2827, 2114, 1687.6 ^{1}HNMR: ($CDCl_3$, 200MHz) δ 1.52 (d, 3H), 2.44 (m,1H), 2.6(m, 1H) 3.33(dd, 1H), 3.56(dd, 1H), 3.8(s, 6H) 4.0 (m,1H),4.3(m,1H), 6.25(t,1H), 6.84(d, 4H), 7.3-7.5(m, 9H), 7.75(s, 1H).

Mass Calculated 568.6 observed 568.4.

5'-*O*-dimethoxy-trityl-N^4-benzoyl-2',3'-dideoxy-3'-azido -5- methylcytidine 10:

Benzoyl chloride (2.6ml, 22.7 mmol) was added drop wise slowly to a solution of **9** (2.9g, 7.56 mmol) in 40 ml pyridine at 0ºC. The mixture was then stirred at room temperature overnight. Then 8 ml water were added and the reaction mixture was stirred for 5 min at 0ºC, followed by addition of concentrated ammonia (8 ml) at 0ºC and stirring for another 30 minute. The solvent was evaporated *in vacuo* and the residue redissolved in 50ml ethyl acetate. Washed the organic layer with 10% $NaHCO_3$ solution (3 x 30 ml), water (2 x 50ml) and saturated aqueous NaCl (2 x 20 ml). The organic layer was then

dried over Na_2SO_4 and evaporated to dryness. The residue was redissolved in 10 ml DCM and purified by silica gel (60-120 mesh) column filtration (10% ethyl acetate in petroleum ether) to afford (2.9g,76%) **10** as a white foam.

IR, ν(cm^{-1}) (CHCl3) 3189, 3024, 2110, 1715, 1705, 1696, 1685 ^{1}HNMR: ($CDCl_3$, 200MHz) δ1.7(d, 3H), 2.5 (m, 2H), 3.36(dd,1H), 3.58(dd,1H), 3.8(s, 6H) 4.0 (m,1H),4.3(m,1H), 6.28(t,1H), 6.87(m, 4H), 7.3-8.3(m,15H). Mass calculated 673.33 observed 673.31, 695.27(+ Na^+).

5'-*O*-dimethoxy-trityl-*N*^{4}-benzoyl-2',3'-dideoxy-3'-amino-5-methylcytidine 11:

Compound **10 (**2.8g, 4.1mmol) was dissolved in 15% triethylamine in pyridine (5 ml) and H_2S gas was bubbled into the solution at 0º C for 15 minutes. The solution was then stirred at room temperature for an additional 30 minutes and solvent removed *in vacuo*. The residue was purified by flash silica gel (200-400 mesh) column chromatography (2-5 % methanol in DCM) to afford **11**, 2.2g (80%).

IR, ν(cm^{-1}) (CHCl3) 3189, 3024, 2810, 1718, 1709, 1692, 1683.

^{1}HNMR: ($CDCl_3$, 200MHz) δ1.71(d, 3H), 2.26 (m, 1H), 2.4(m, 1H), 3.38(dd, 1H), 3.56(dd, 1H), 3.76(m, 2H), 3.8(s, 6H), 6.24(t, 1H), 6.87(m, 4H), 7.32-8.3(m, 15H).

Mass calculated 647.33, observed 647.32, 669.29(+ Na^+).

Synthesis of 5'-*O*-DMT -tst-3'-*O*-TBDMS Dimer 12:

To compound **4** (1g, 2.32 mmol) in dry acetonitrile (5 ml), HBTU (1g, 2.8 mmol), DIPEA (1.2 ml, 7 mmol) and HOBt (0.16g, 1.2 mmol) were added and stirred for 15min. compound **7** (1.25g, 2.3 mmol) was dissolved in 3 ml acetonitrile and was then added into the reaction mixture and further stirred at room temperature for 1h. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate (30ml) and washed with 5% $NaHCO_3$ solution (2 x10 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to get the crude product. This was purified by column chromatography using CH_2Cl_2/MeOH to get pure product **12,** 1.7g, 78%.

^{1}HNMR: ($CDCl_3$, 200MHz) δ 0.07(s, 6H), 0.87(s, 9H), 1.82 (s, 3H),1.84(s, 3H), 2.3-2.4 (m, 4H), 2.8-2.96 (m, 2H), 3.3 (q, 2H), 3.66-3.82 (m, 2H), 3.94 (m, 1H), 3.99(m, 1H),

4.36(m, 1H), 4.41 (m,1H), 6.13-6.2 (m, 2H), 6.8-6.84 (d, 4H), 7.17-7.69 (m, 11H), 8.63 (d, 2H).

Mass calculated 955.96 observed 979.0 ($+Na^+$), 994.23 ($+K^+$).

Synthesis of 5'-*O*-DMT- cst-3'-*O*-TBDMS Dimer 15:

To compound **4** (1g, 2.32 mmol) in dry acetonitrile (5ml), HBTU (1g, 2.8 mmol), DIPEA (1.2ml, 7mmol) and HOBt (0.16g, 1.2 mmol) were added and stirred for 15min. **11** (1.5, 2.3 mmol.) dissolved in 4 ml acetonitrile was then added into the reaction mixture and further stirred at room temperature for 1h. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate (30 ml) and washed with 5% $NaHCO_3$ solution (2 x 10 ml). The organic layer was dried over anhydrous. Na_2SO_4 and concentrated to get the crude product. This was purified by column chromatography using CH_2Cl_2/MeOH to get pure product **15,** 2g, 80%.

^{1}HNMR: ($CDCl_3$, 200MHz) δ 0.08 (s, 6H), 0.88 (s, 9H), 1.58 (s, 3H), 1.9 (s, 3H), 2.15-2.34 (m, 2H), 2.46-2.53 (m, 2H), 2.85 (m, 2H), 3.28 (q, 2H), 3.47 (m, 2H), 3.96 (m, 1H), 4.1(d, 1H), 4.29 (m,1H), 4.76 (m,1H), 6.06 (t, 1H), 6.4 (t, 1H), 6.82-6.87 (d, 4H), 7.24-7.72 (m, 15H), 7.82 (s,1H),8.25 (s,1H), 8.29 (s,1H).

Mass calculated 1059.18 observed 1059.47, 1081.44 (+ Na^+).

Synthesis of 5'-*O*-DMT tst-3'-OH Dimer 13:

A solution of **12** (1.6g, 1.7 mmol) and TBAF (0.65g, 2.5 mmol) in 15 ml anhydrous THF was stirred at RT for 1h. THF was evaporated in *vacuo* and residue re-dissolved in DCM (50 ml). The solution was washed with water (2 x 20 ml) followed by brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Silica gel column chromatography gave the pure product **13**, 1.25g, 90 %.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.4 (s, 3H), 1.9 (s, 3H), 2.27 (m, 1H), 2.4(m, 1H), 2.46(m, 2H), 2.92 (m, 2H), 3.32 (q, 2H), 3.46(m, 2H), 3.79 (s, 6H), 4.08-4.14 (m, 2H), 4.38(m, 1H), 4.75 (m, 1H), 6.2 (t, 1H), 6.4 (t, 1H), 6.84 (d, 4H), 7.2-7.8 (m, 9H), 7.64 (s, 1H), 8.0 (b, NH).

Mass calculated 841.93 observed 841.4.

Synthesis of 5'-*O*-DMT-cst -3'-OH Dimer 16:

A solution of **13** (1.8g, 1.67 mmol) and TBAF (0.67g, 2.54 mmol) in 15 ml anhydrous THF was stirred at RT for 1h. The THF was evaporated in *vacuo* and residue was re-dissolved in DCM (50 ml). The solution was washed with water (2 x 20 ml) followed by brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Silica gel column chromatography gave the pure product **15**, 1.48g, 92%.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.6 (s, 3H), 1.9 (s, 3H), 2.35 (t, 2H), 2.5 (m, 2H), 2.8-2.9 (m, 4H), 3.5 (m, 2H), 3.8 (s, 6H), 4.02 (m, 1H), 4.1 (m, 1H), 4.4 (m, 1H), 4.73 (m, 1H), 6.1 (t, 1H), 6.4 (t, 1H), 6.85 (d, 4H), 6.8 (d, 4H), 7.2-7.53 (m, 13H), 7.8 (s, 1H), 8.25 (d, 2H).

Mass calculated 945.05 observed 968.2(+Na^+).

5'-*O*-(4, 4'-dimethoxy) trityl -tst- 3'-*O*-(2-cyanoethyl-N, N-diisopropylphosphoramidite)-dimer 14:

Compound **13** (0.9g, 1.07 mmol) was dissolved in dry DCM (10ml) followed by the addition of tetrazole (0.12g,1.7 mmol) and 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphorodiamidite (0.55 ml,1.7 mmol) and the reaction mixture was stirred at room temperature for 12 h. The contents were then diluted with dry DCM and washed with 5% $NaHCO_3$ solution. The organic phase was dried over anhydrous. Na_2SO_4 and concentrated to a foam. The residue was dissolved in DCM and precipitated with hexane to obtain **14** (0.73g, 65%). The phosphoramidite **14** was dried overnight over P_2O_5 and KOH in a desiccator before applying on DNA synthesizer. TLC shows two close moving spots for two diasteromers (R_f =0.5, 2% methanol-dichlromethane)

^{31}P NMR ($CDCl_3$) δ 149.0, 149.08. ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.2(d, 12H), 1.41(s, 3H), 1.92(s, 3H), 2.47(m, 4H), 2.47(s, 2H), 2.78(m, 2H), 2.96(m, 2H), 3.29(m, 2H), 3.5-3.61(m, 6H), 3.73(m, 1H), 3.8(s, 6H), 3.85(m, 1H), 4.13-4.24(m, 4H), 4.5(m, 1H), 4.7(m, 1H), 6.1(t, 1H), 6.4(t, 1H), 8.85(d, 4H), 7.2-7.4(m, 10H), 7.6(s, 1H).

Mass calculated 1042.1 observed 1041.5.

5'-*O*-(4, 4'-dimethoxy) trityl-cst-3'-*O*-(2-cyanoethyl-N, N-diisopropylphosphoramidite) dimer 17:

Compound **16** (1g, 1.05 mmol) was dissolved in dry DCM (10 ml) followed by the addition of tetrazole (0.12 g,1.7 mmol) and 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphorodiamidite (0.54 ml,1.7 mmol) and the reaction mixture was stirred at room temperature for 16h. The contents were then diluted with dry dichloromethane and washed with 5% $NaHCO_3$ solution. The organic phase was dried over anhydrous. Na_2SO_4 and concentrated to a foam. The residue was dissolved in DCM and precipitated with hexane to obtain **17** (0.72g, 60%). The phosphoramidite **17** was dried overnight over P_2O_5 and KOH in a desiccator before using on DNA synthesizer. TLC shows two close moving spots for two diastreomers (Rf =0.6, 2% methanol-dichlromethane)

^{31}P NMR ($CDCl_3$) δ 148.91, 147.03. ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.2(d, 12H), 1.6(s, 3H), 1.9(d, 3H), 2.4-2.5(m, 4H), 2.7(m, 2H), 2.8(m, 1H), 2.9-3.0(m, 2H), 3.2-3.35(m, 2H), 3.45-3.65(m, 5H), 3.73(m, 1H), 3.8(s, 6H), 3.9(m, 1H), 4.1-4.3(m, 3H), 4.5(m, 1H), 4.8(m, 1H), 6.1(q, 1H), 6.4(t, 1H), 6.85(d, 4H), 7.2-7.5(m, 13H), 7.8(s, 1H), 8.3(d, 2H). Mass calculated 1145.3 Observed 1145.06.

Reference

1. Divakar K.J. ; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1*, **1982**, 1171-1176.

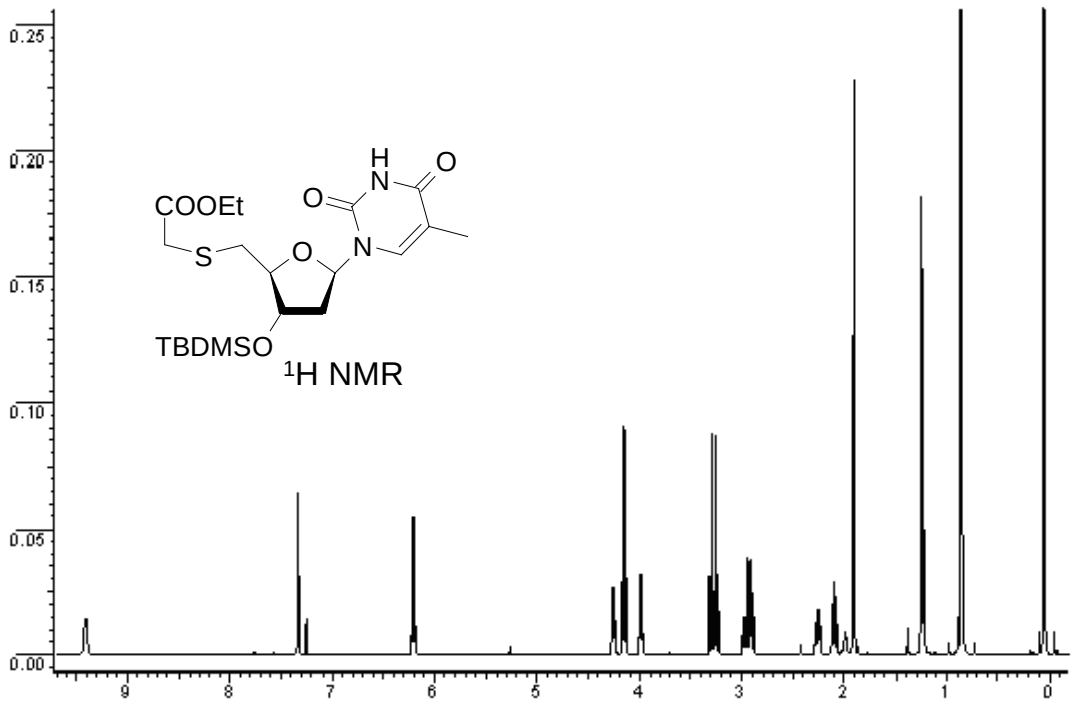


Figure S1 ^{1}H NMR of **3**

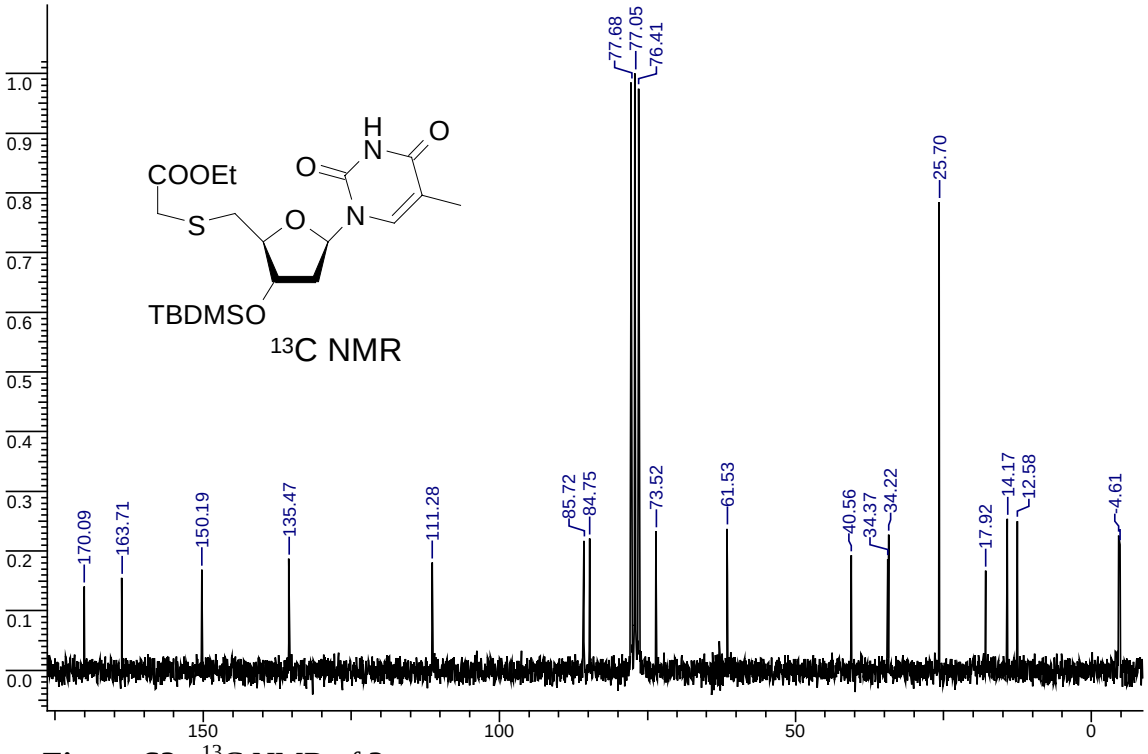


Figure S2 ^{13}C NMR of **3**

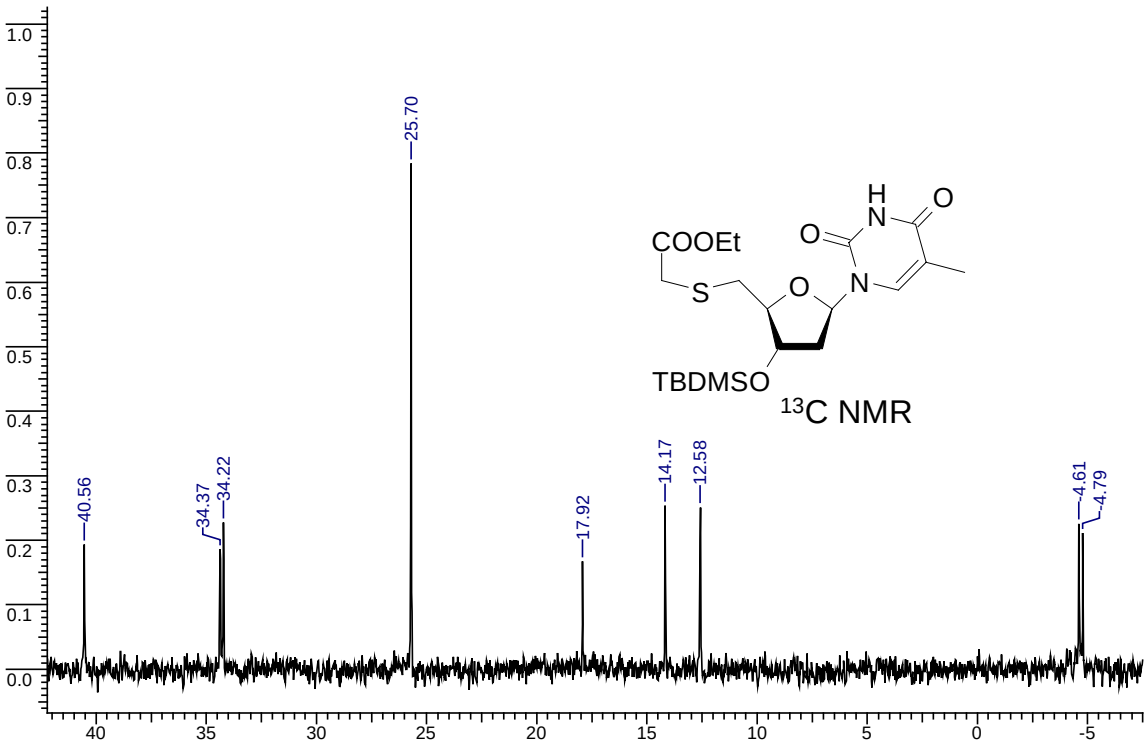


Figure S3: Expanded ^{13}C of **3**

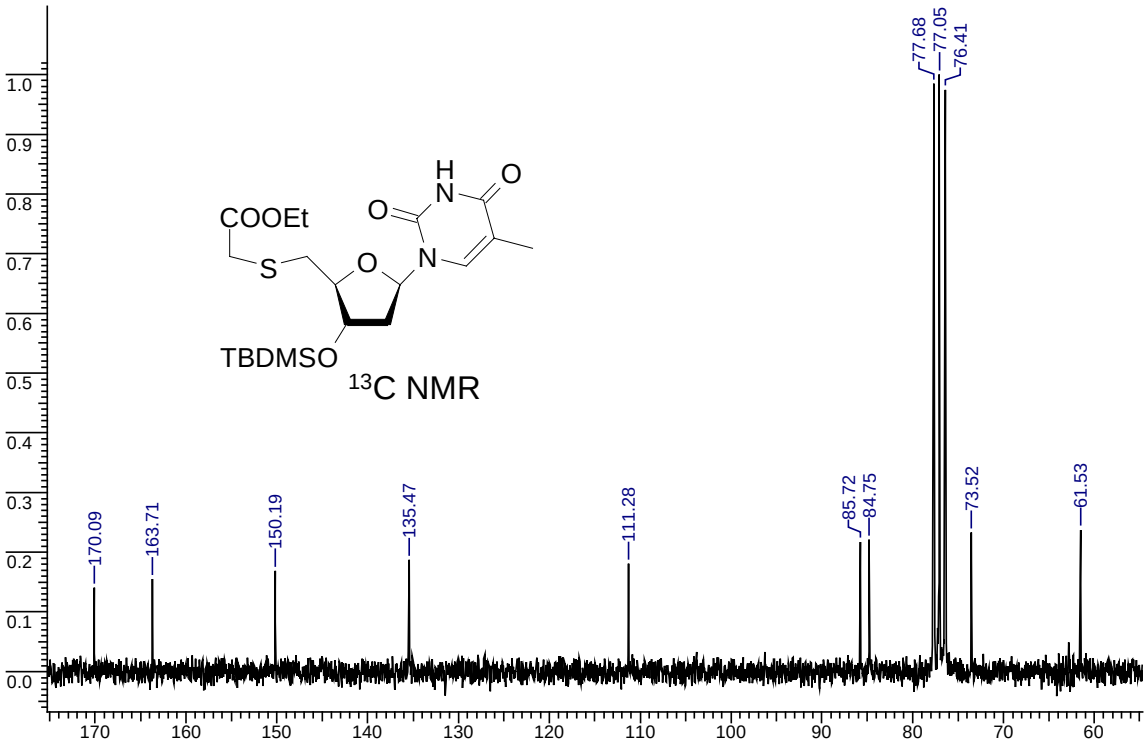


Figure S4: Expanded ^{13}C of **3**

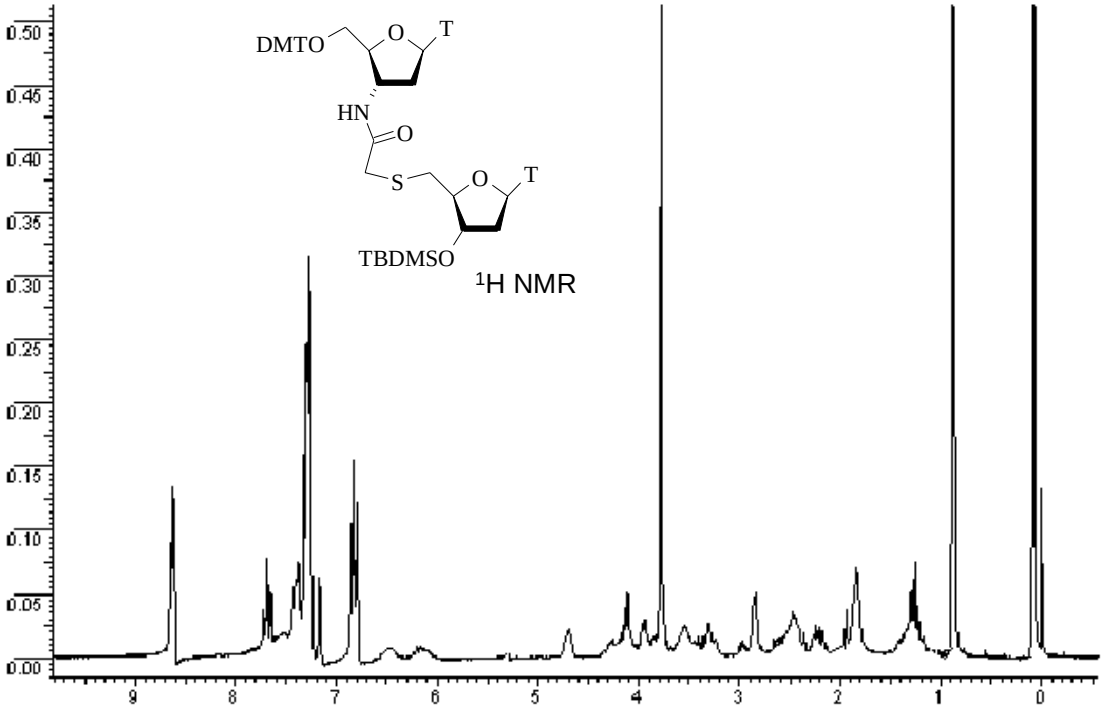


Figure S5: ^{1}H NMR **12**

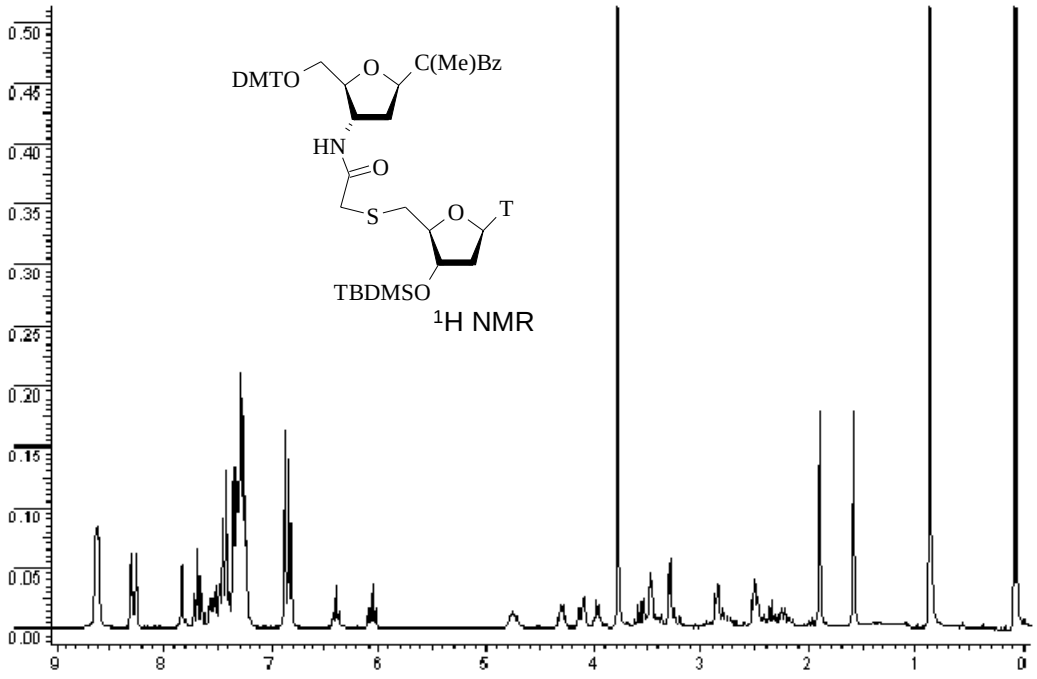


Figure S6: ^{1}H NMR **15**

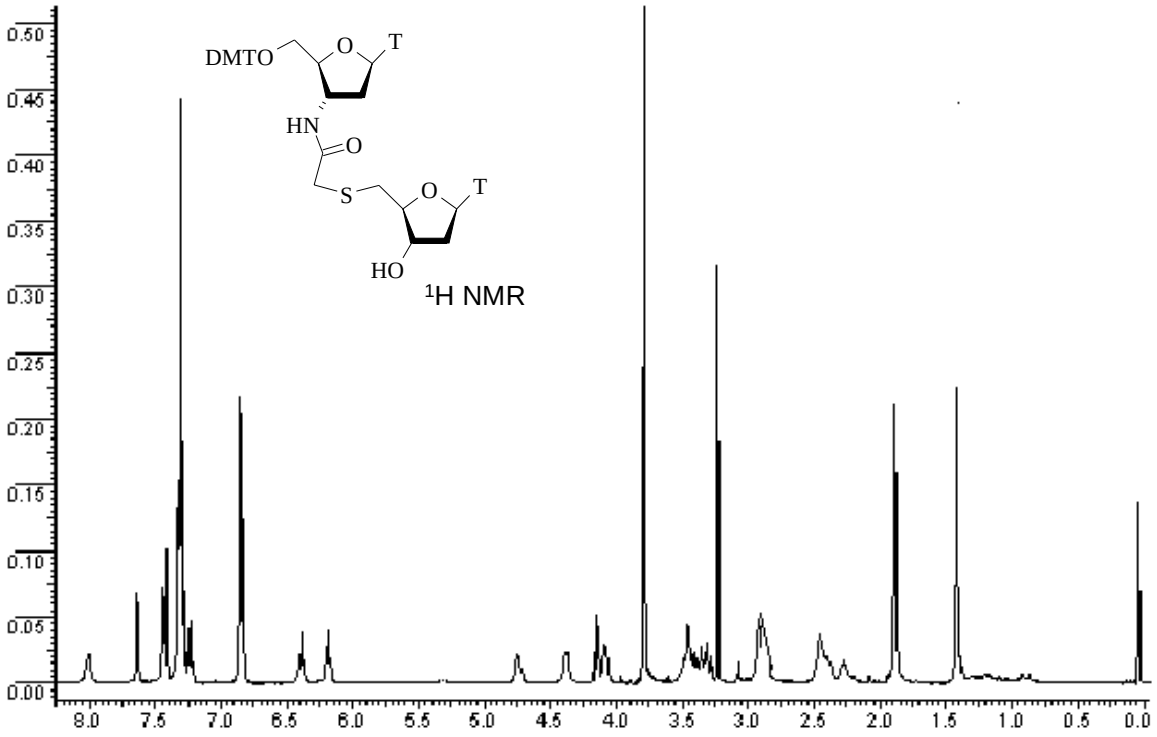


Figure S7: ^{1}H NMR of **13**

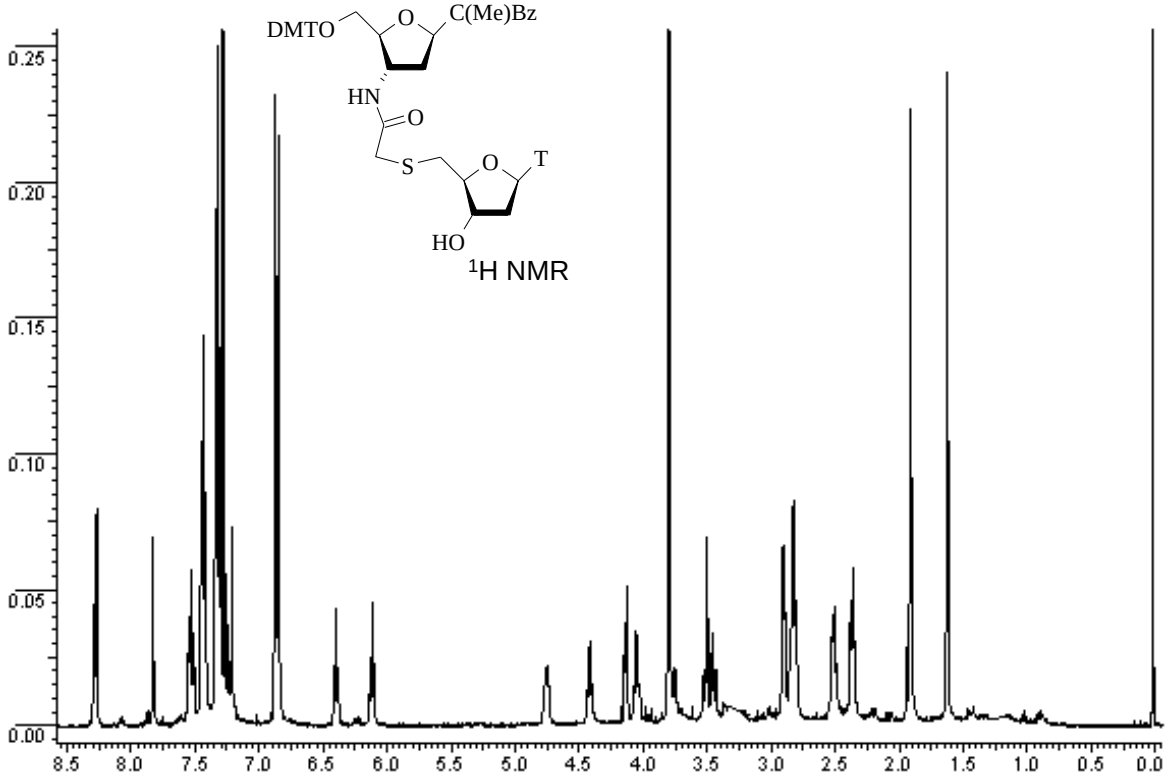


Figure S8: ^{1}H NMR of **16**

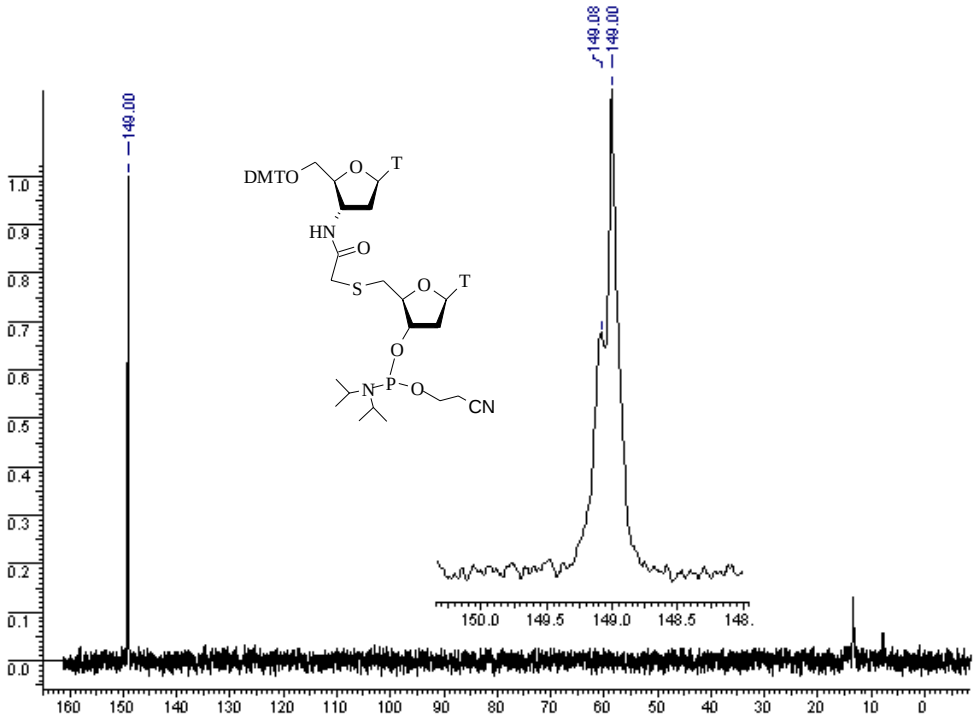


Figure S9: ^{31}P NMR of **14**

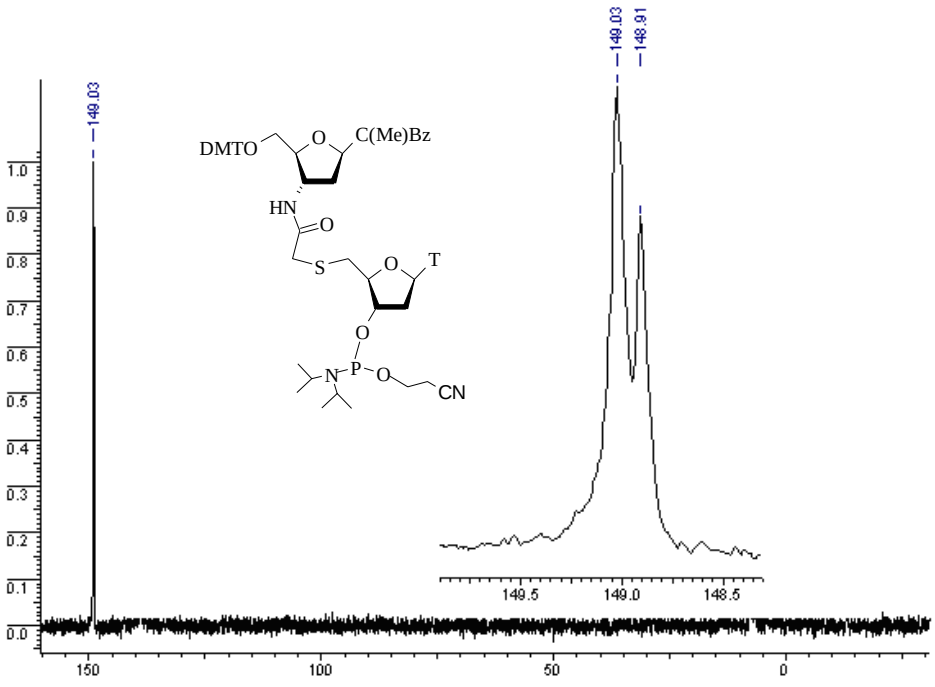


Figure S10: ^{31}P NMR of **17**

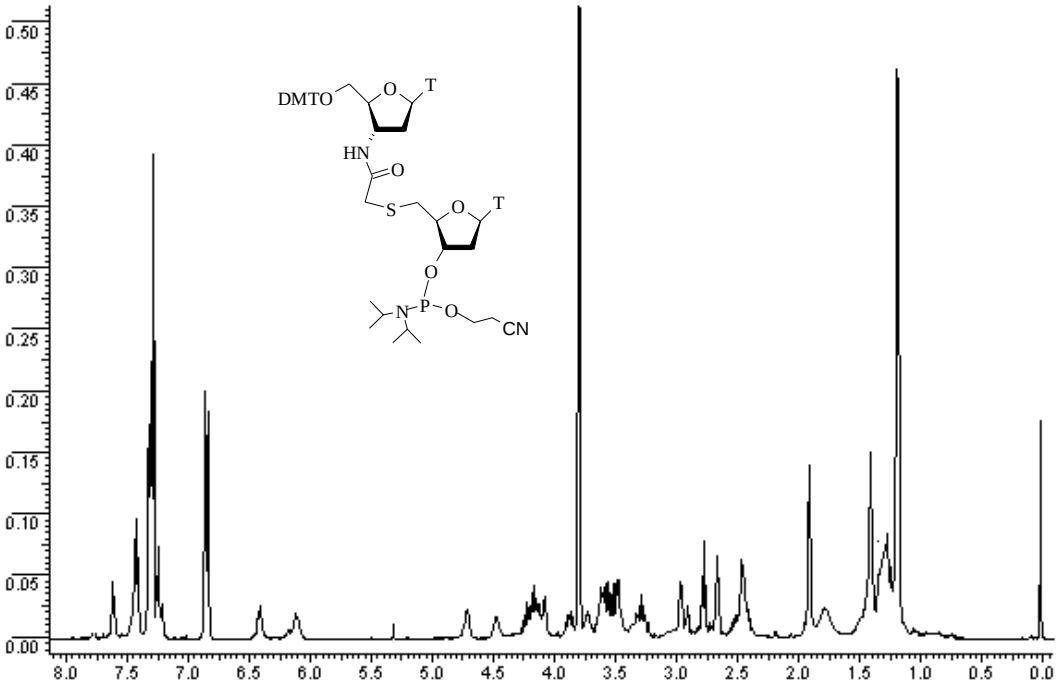


Figure S11: ^{1}H NMR of **14**

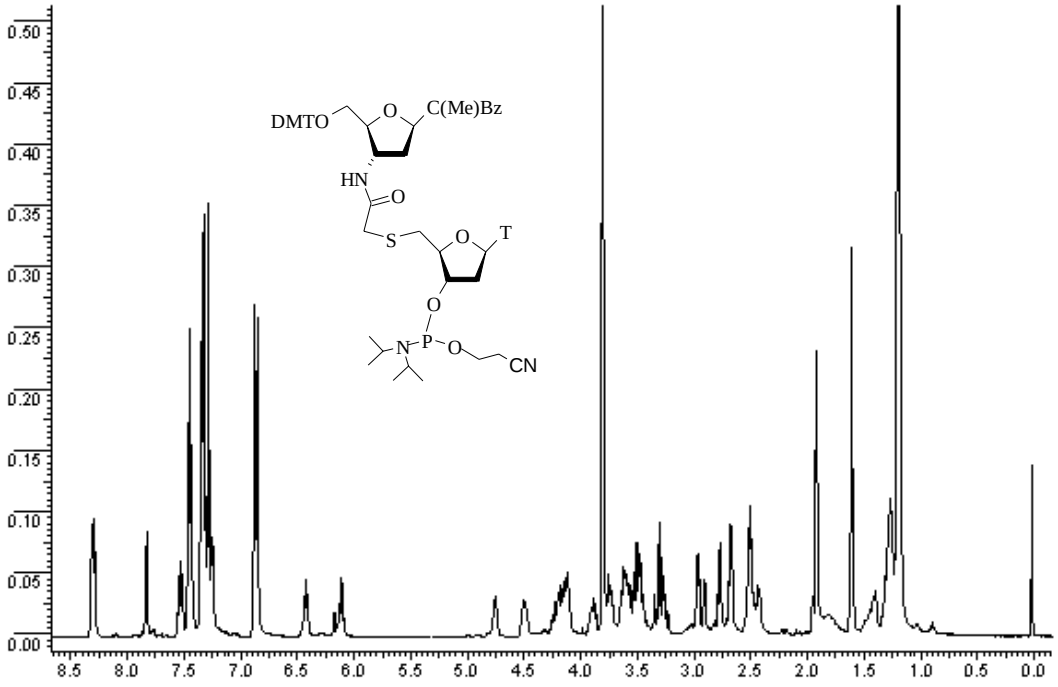


Figure S12: ^{1}H NMR of **17**

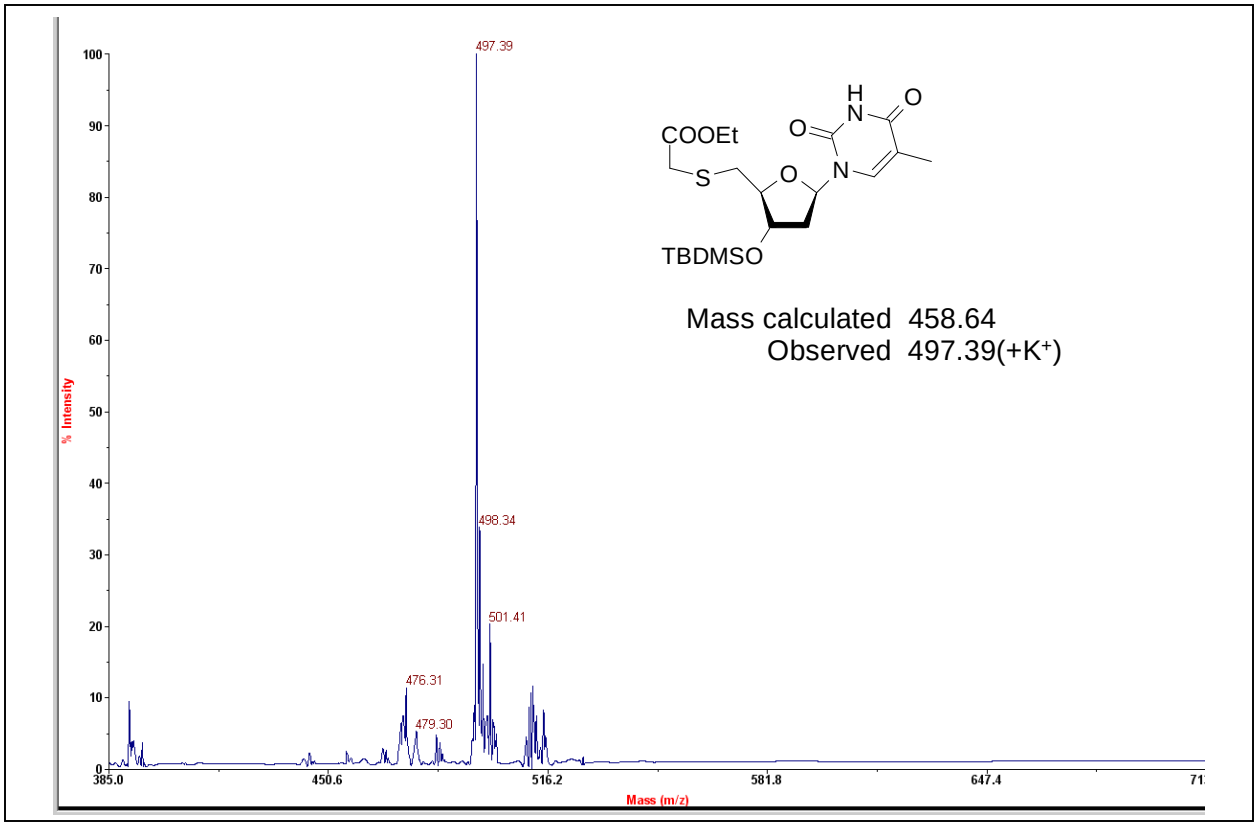


Figure S13: Mass of **3**

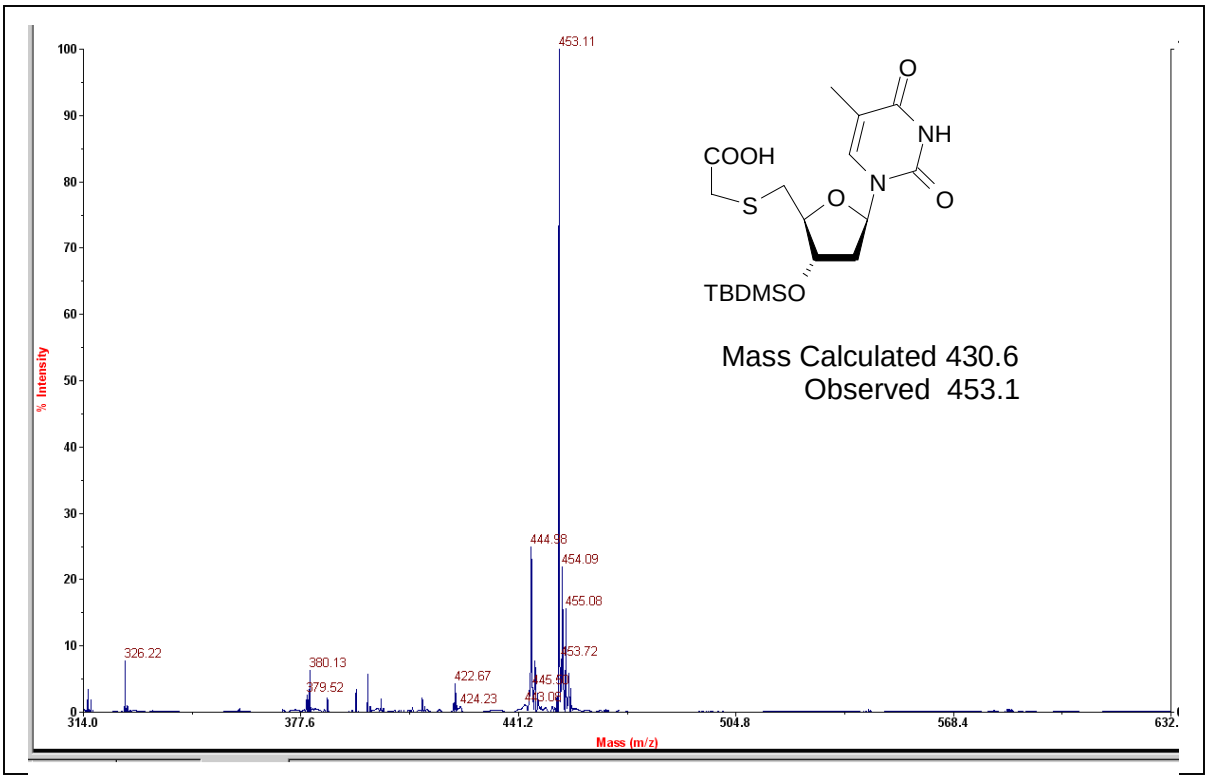


Figure S14: Mass of **4**

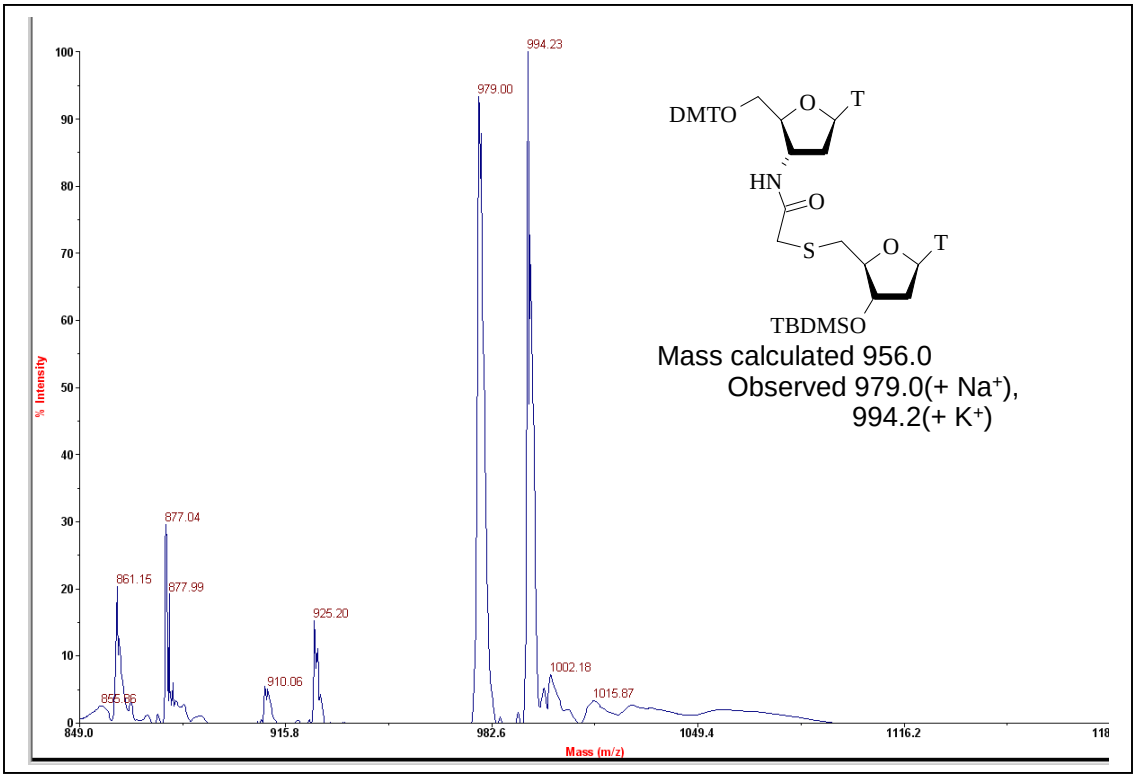


Figure S15: Mass of **12**

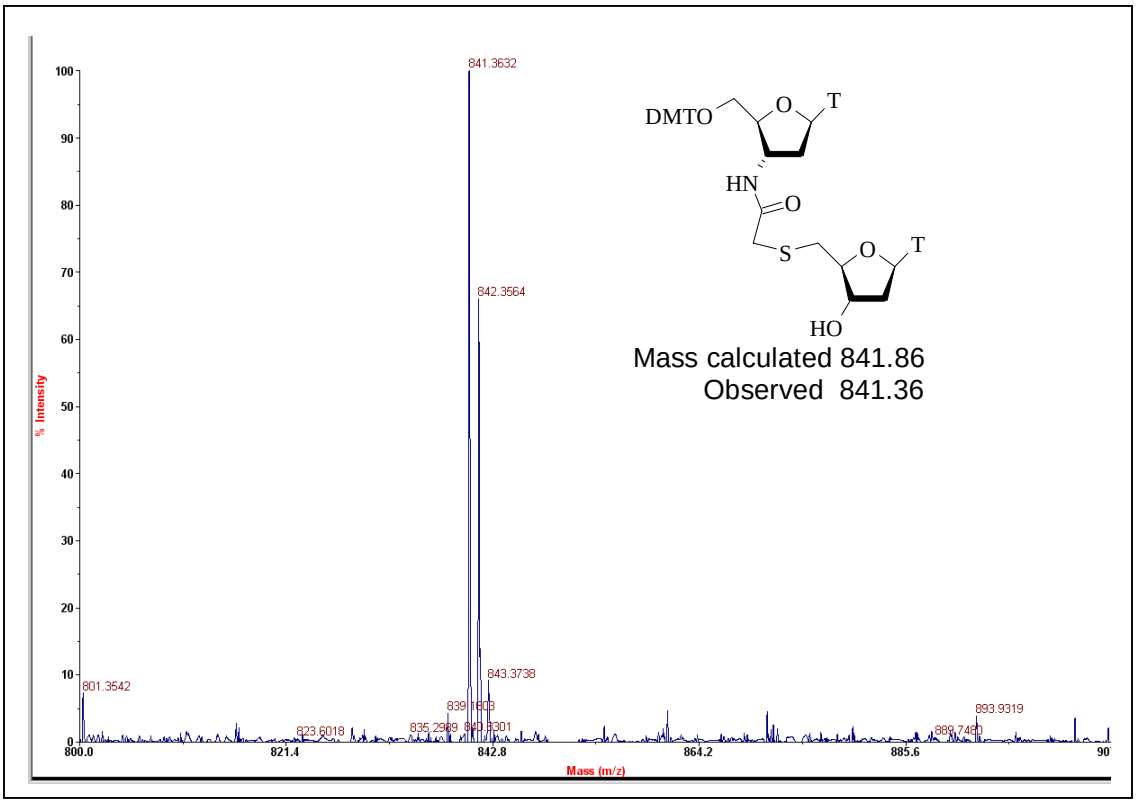


Figure S16: Mass of **13**

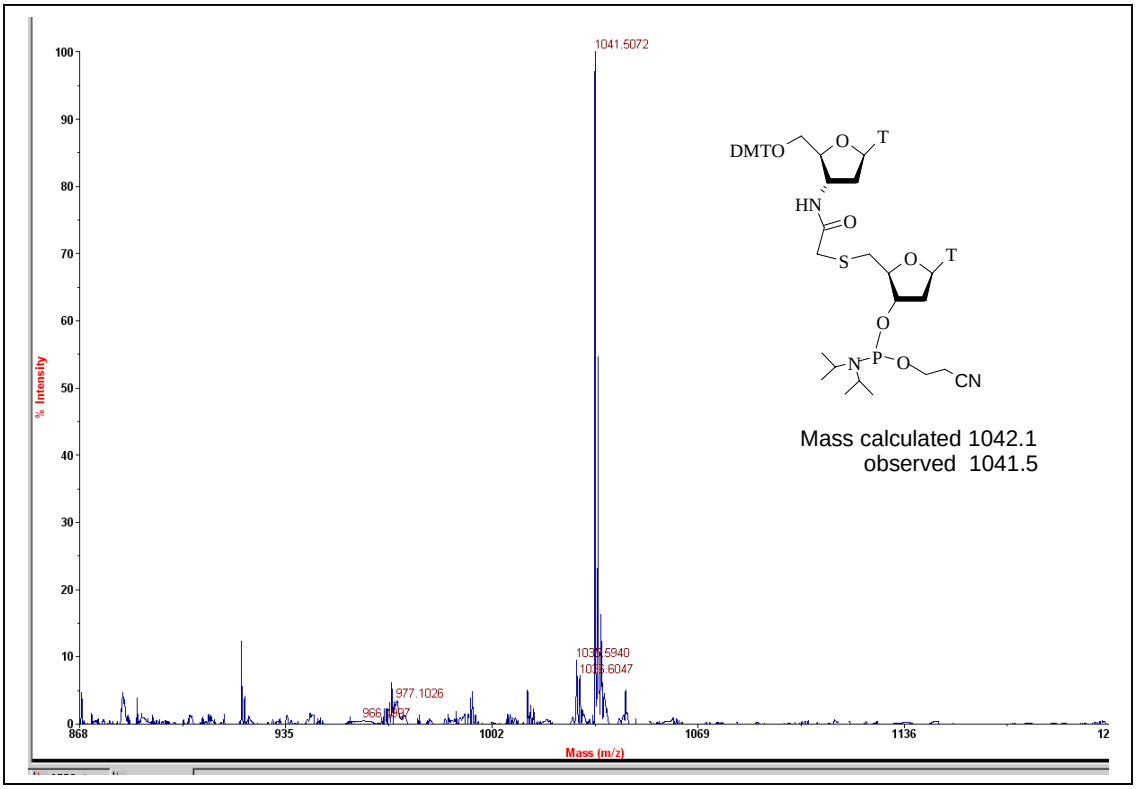


Figure S17: Mass of **14**

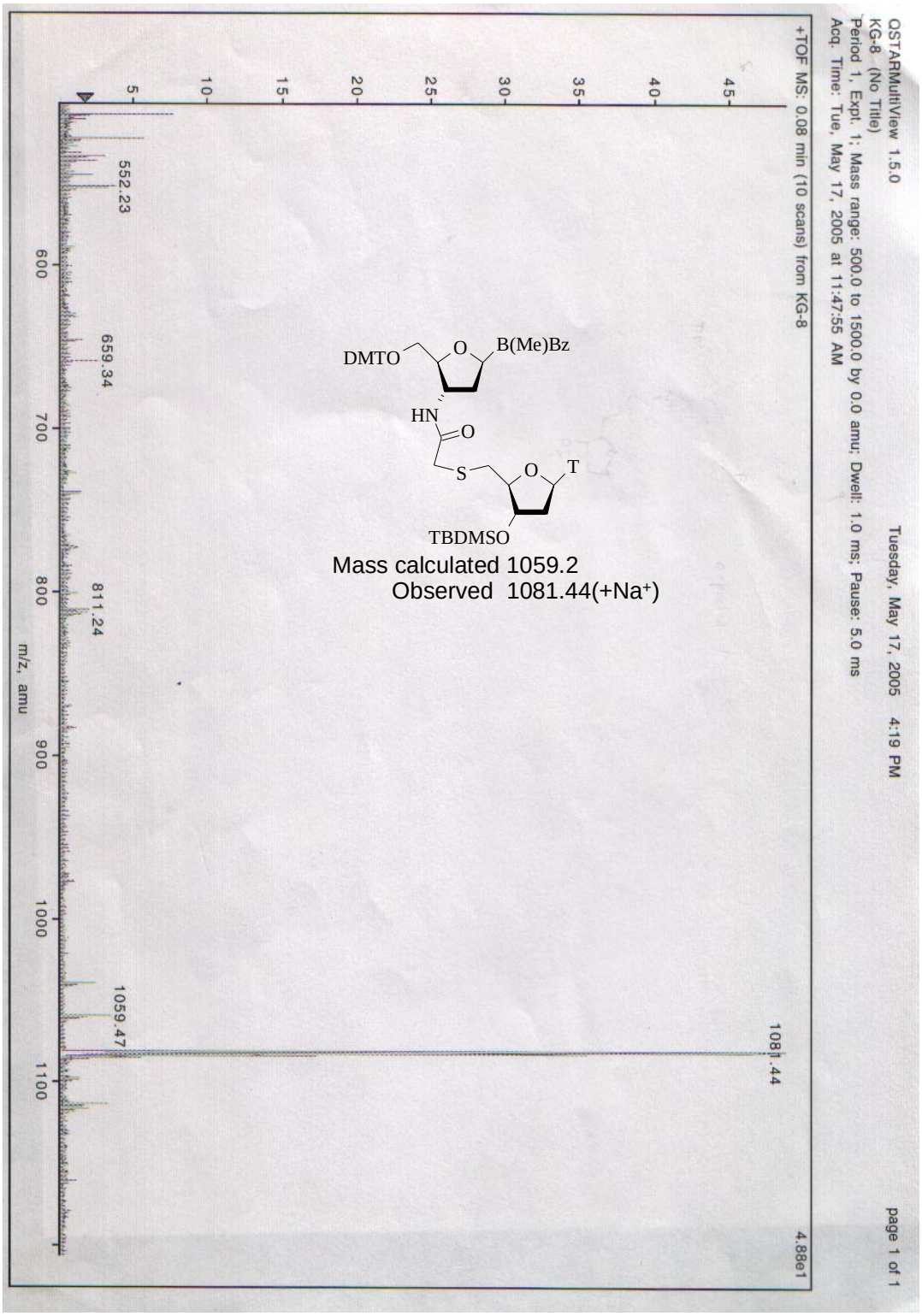


Figure S18: Mass of **15**

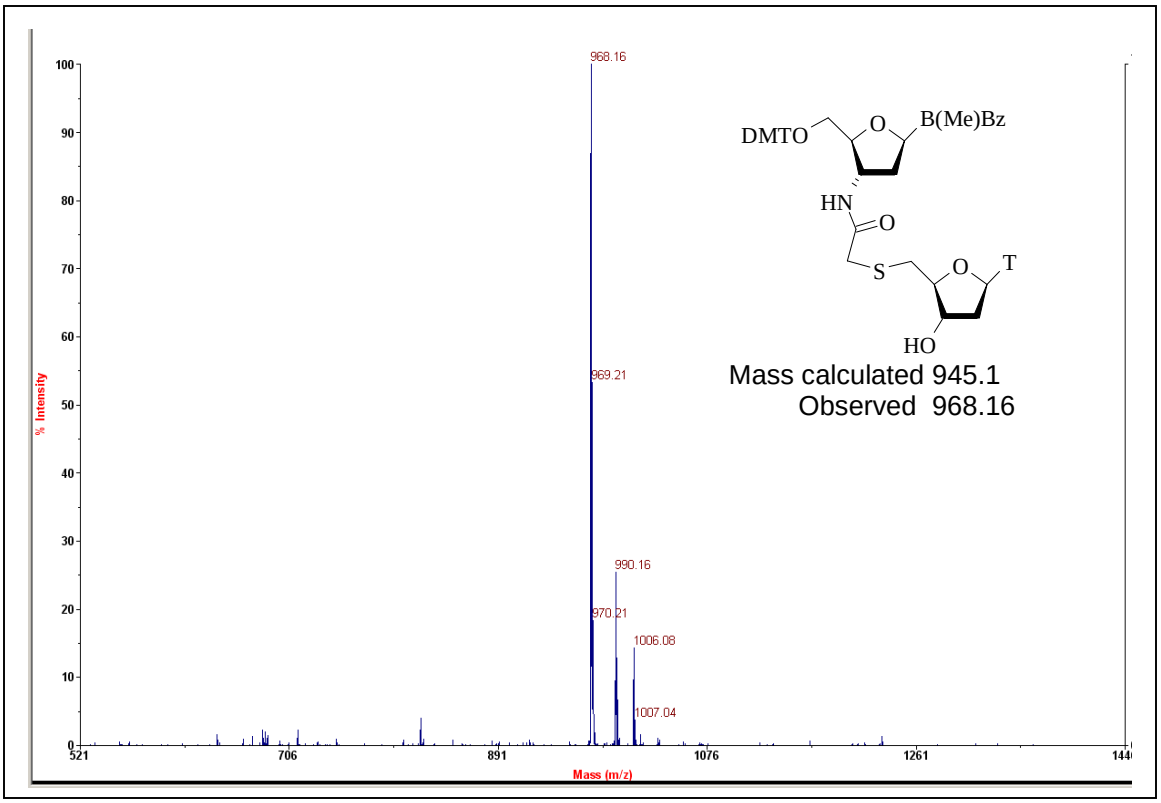
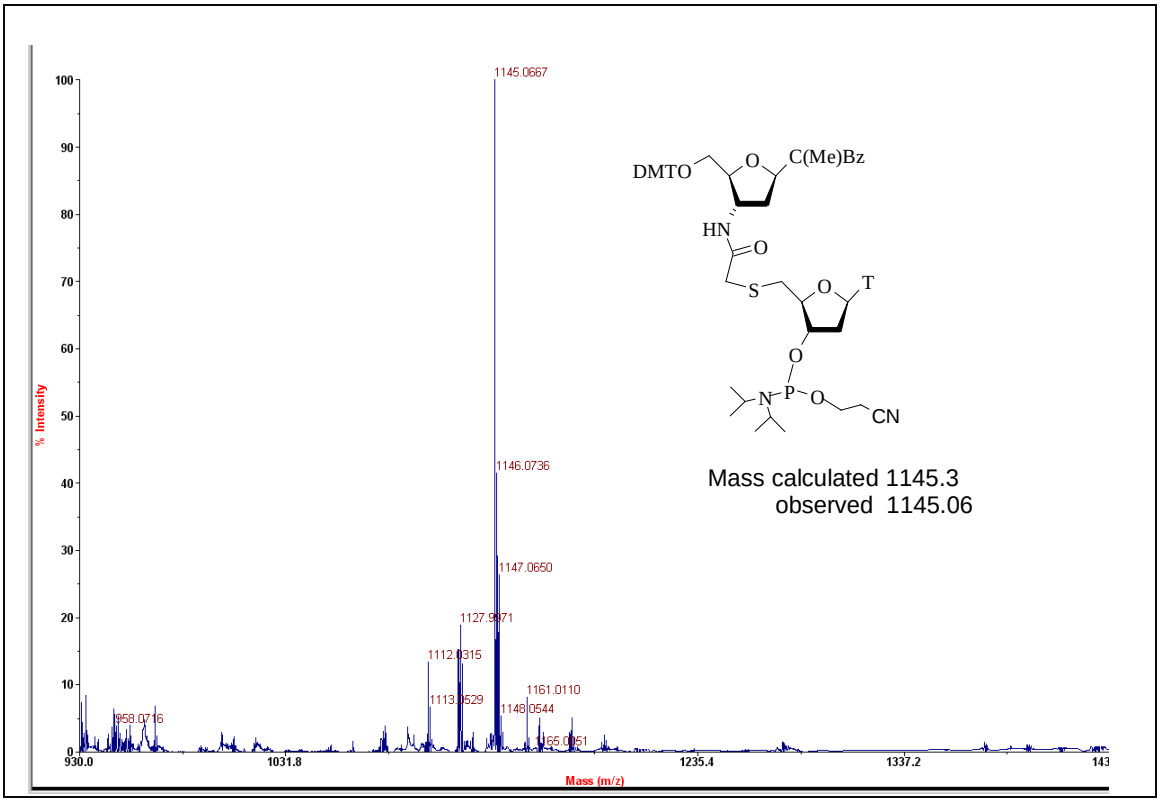


Figure S19: Mass of **16**



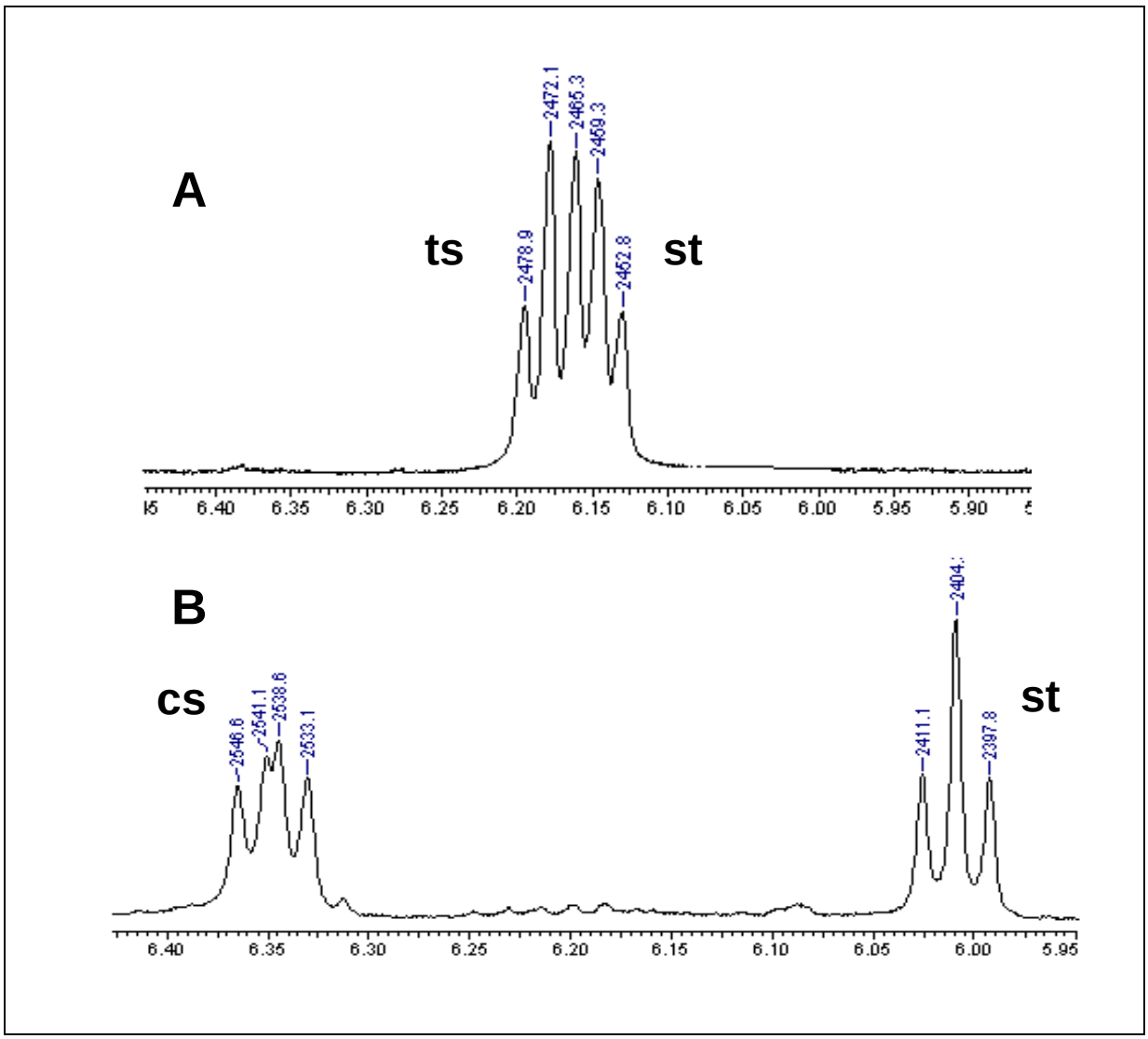
FigureS20: Mass of **17**

TableS1 Conformational study of sugar residues in dinucleoside blocks **18** and **19**. p- indicates phosphodiester linkage, s- indicates mercaptoacetamide linkage. The 2'-endo conformer population (%S) is determined from the sum of the coupling constants of H1' and H2' and H2" ($^3J_{1'-2'}$ and $^3J_{1'-2"}$). % S = (∑ H1' - 9.8) / 5.9 where ∑ H1'= $^3J_{1'-2'}$ + $^3J_{1'-2"}$, according to Rinkel and Altona.[1]

Dinucleotide		∑ H1' (Hz)	%S	Predominant Sugar Ring pucker
TpT [2]	Tp	13.8	68	2'-endo
	pT	13.4	61	2'-endo
18 (tst)	ts	13.6	64.4	2'-endo
	st	12.5	45.8	3'-endo/2'-endo
19(cst)	cs	13.2	57.6	2'-endo
	st	13.3	59.3	2'-endo

1. Rinkel, L. J.; Altona, C. J. *Biomol.Struct. Dyn*. **1987**, *4*, 621-649

2. Nawrot, B.; Boczkowska, M.; Wojcik, M.; Sochaki, M.; Kazmierski, S.; Stec, W.J. *Nucleic Acids Res*. **1998**, *26*, 2650-2658



FigureS21: H1' region of ^{1}H NMR spectra of **A**. **18(tst)** and **B. 19(cst)** at 25ºC. 5'-terminal ribose H1' signals are downfield of 3'-terminal ribose H1' signals.

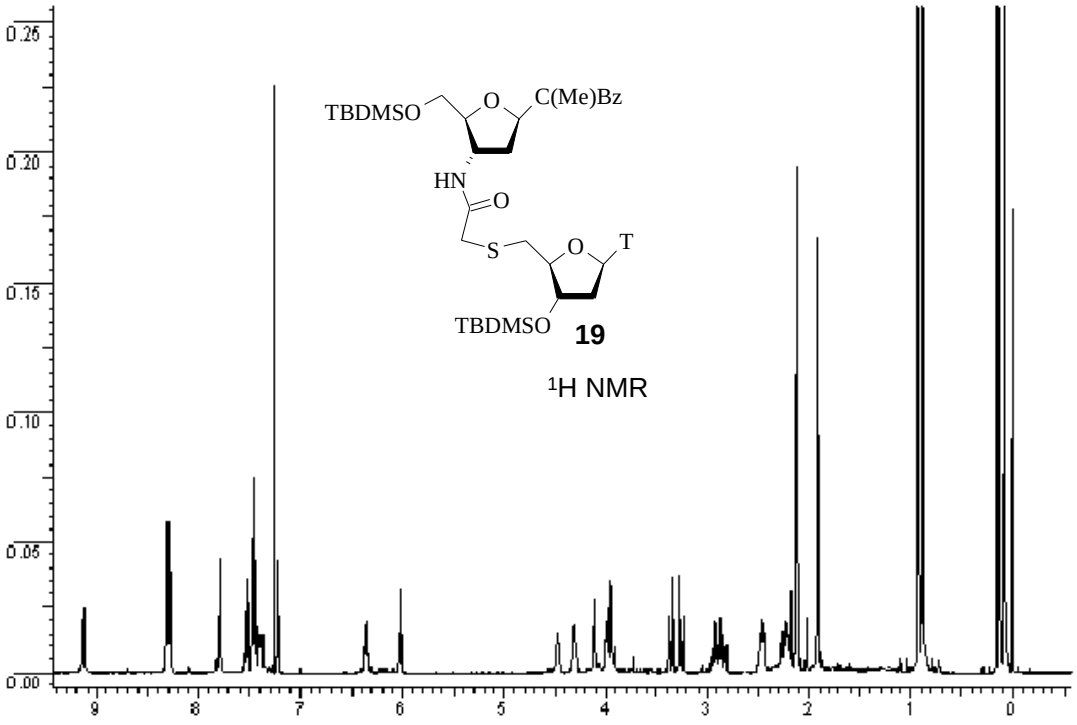


Figure S22: ^{1}H NMR of **19**

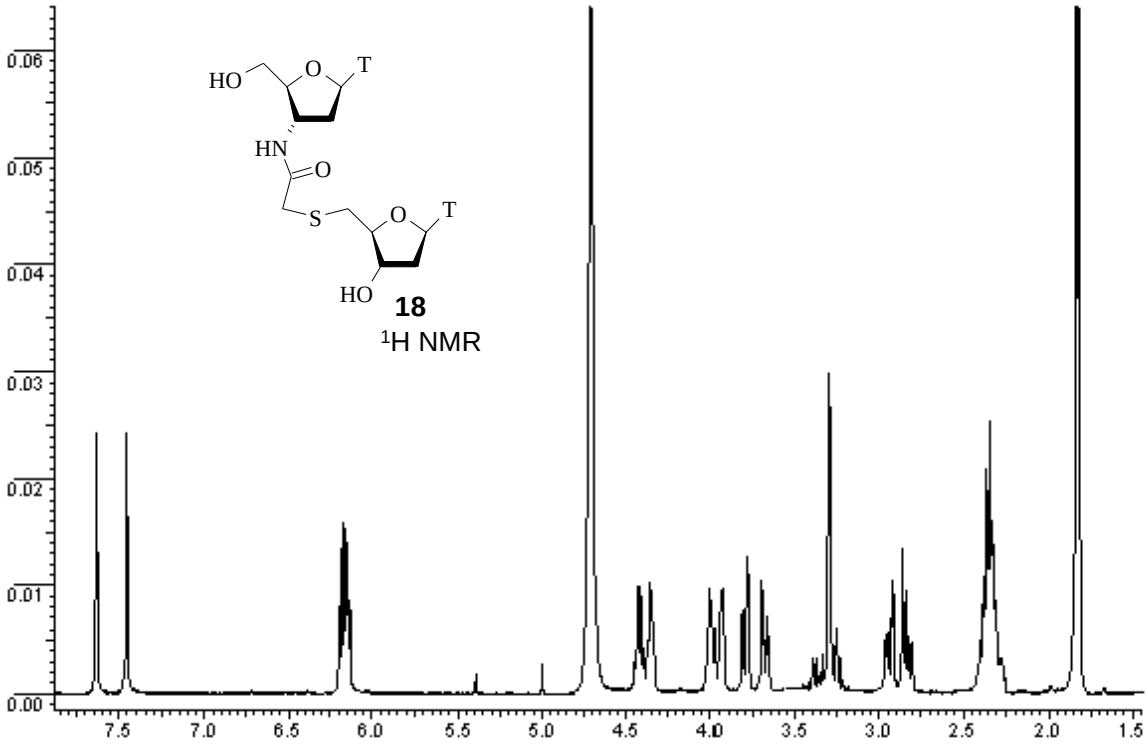


Figure S23: ^{1}H NMR of **18**

Oligomer Synthesis: The oligomers were synthesized on an Applied Biosystems 3900 DNA Synthesizer at 40 nmol scale. Dinucleotide phosphoramidites **14** and **17** were used as solution in acetonitrile at concentration of 0.1-0.15 M. Purification of all the oligonucleotides was carried out by RP-HPLC C18 colunm .

MALDI-TOF Mass spectral analysis was performed on a Voyager-De-STR (Applied Biosystems) MALDI-TOF. A nitrogen laser (337 nm) was used for desorption and ionization. Spectra were acquired in linear mode. The matrix used for analysis was THAP (Trihydroxy acetophenone) and diammonium citrate as additive.

Table S2. Oligomers synthesized, their HPLC t_R and MALDI-TOF mass characterization

Entry	Sequence	HPLCt_R(min)	Mass $_{calc/observed}$
1	5’ CG TT**tst**TTT TGC 3’ **20**	7.9	3606.54/3606.64
2	5’ CGTT **tst** TT **tst** GC 3’ **21**	8.3	3596.64/5597.01
3	5’ CG **tst tst tst tst** GC 3’ **22**	9.2	3576.86/3576.65
4	5’ TCT C **tst** TCT T 3’ **23**	8.6	2933.16/2933.16
5	5’ TCT C **tst** TC **tst** 3’ **24**	9.2	2923.26/2924.06
6	5’ CCT C **tst** ACC TCA G TT ACA 3’ **26**	8.9	5366.78/ 5366.88
7	5’ CCT C **tst** ACC TCA G **tst** ACA 3’ **27**	9.3	5356.9/5356.92
8	5’ T**cst** CTT TCTT 3’ **29**	8.0	2948.2/2948.1
9	5’ T**cst** CTT T**cst** T 3’ **30**	8.8	2938.3/2938.77
10	5’ T**cst cst**T T**cst** T 3’ **31**	9.0	2928.4/2928.7
11	5’ TCA **cst** A GAT G 3’ **32**	8.3	3036.26/ 3037.0

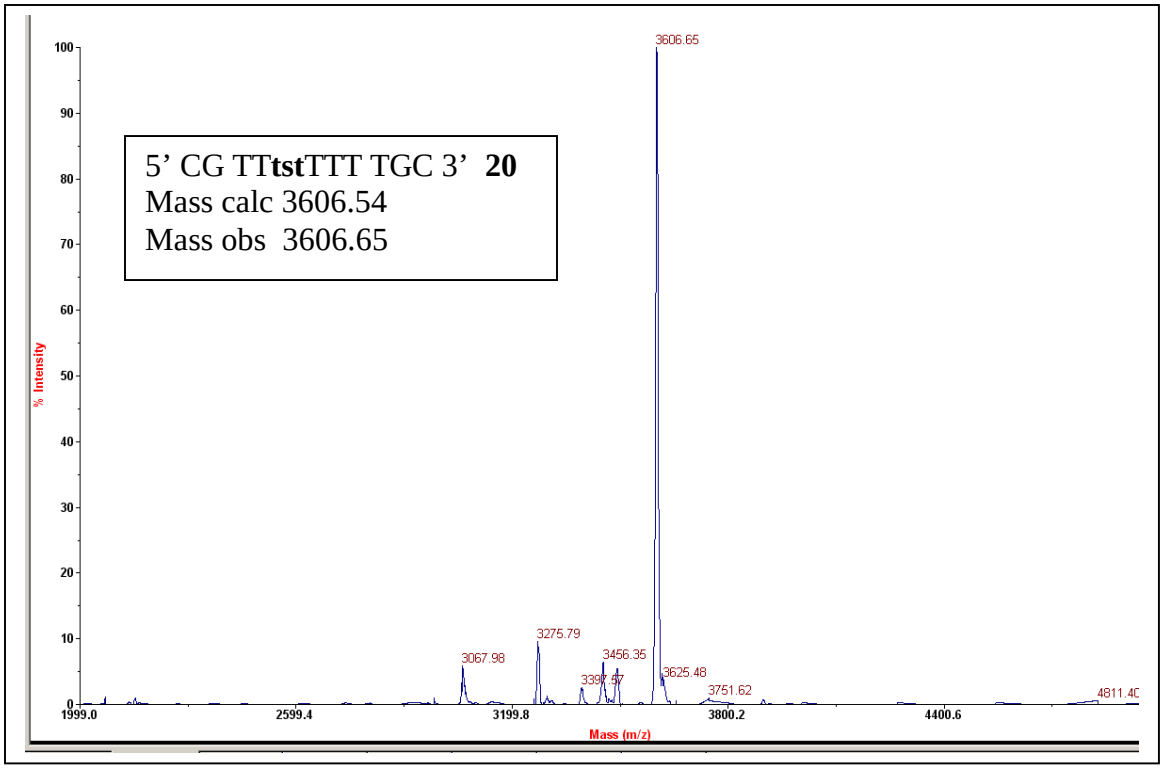


Figure S24: MALDI-TOF mass of **20**

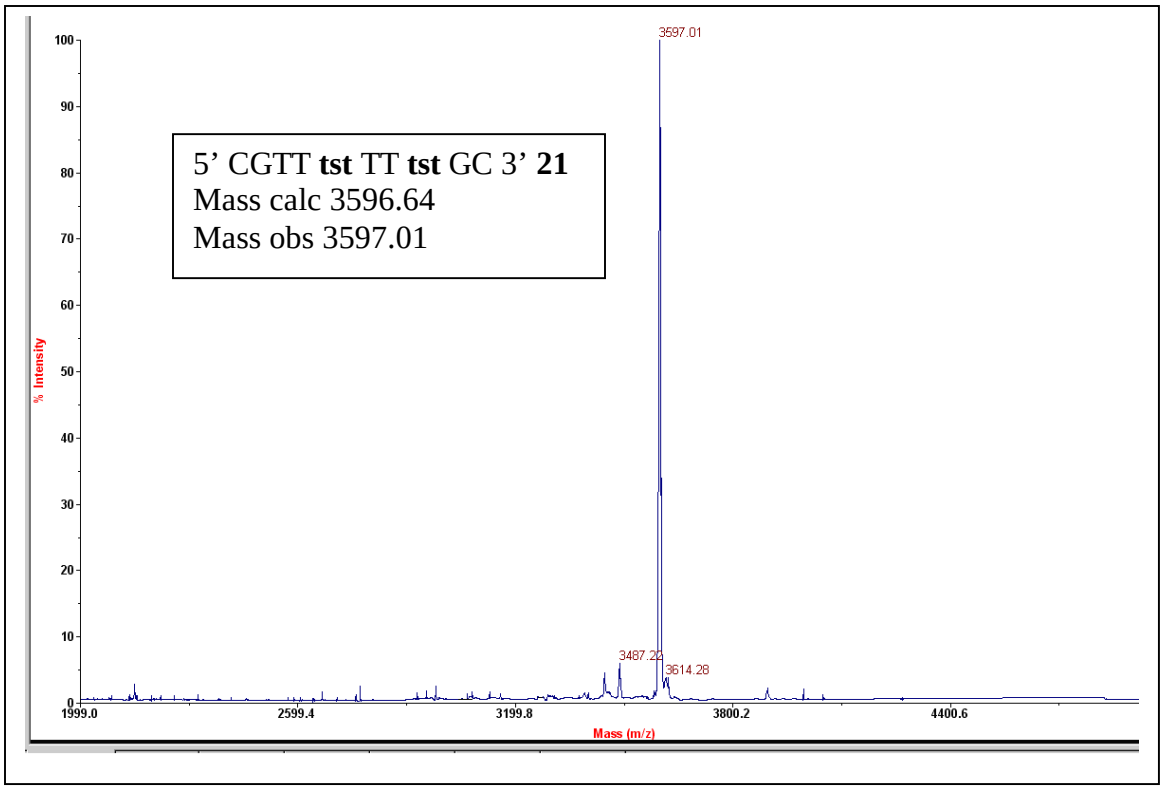


Figure S25: MALDI-TOF mass of **21**

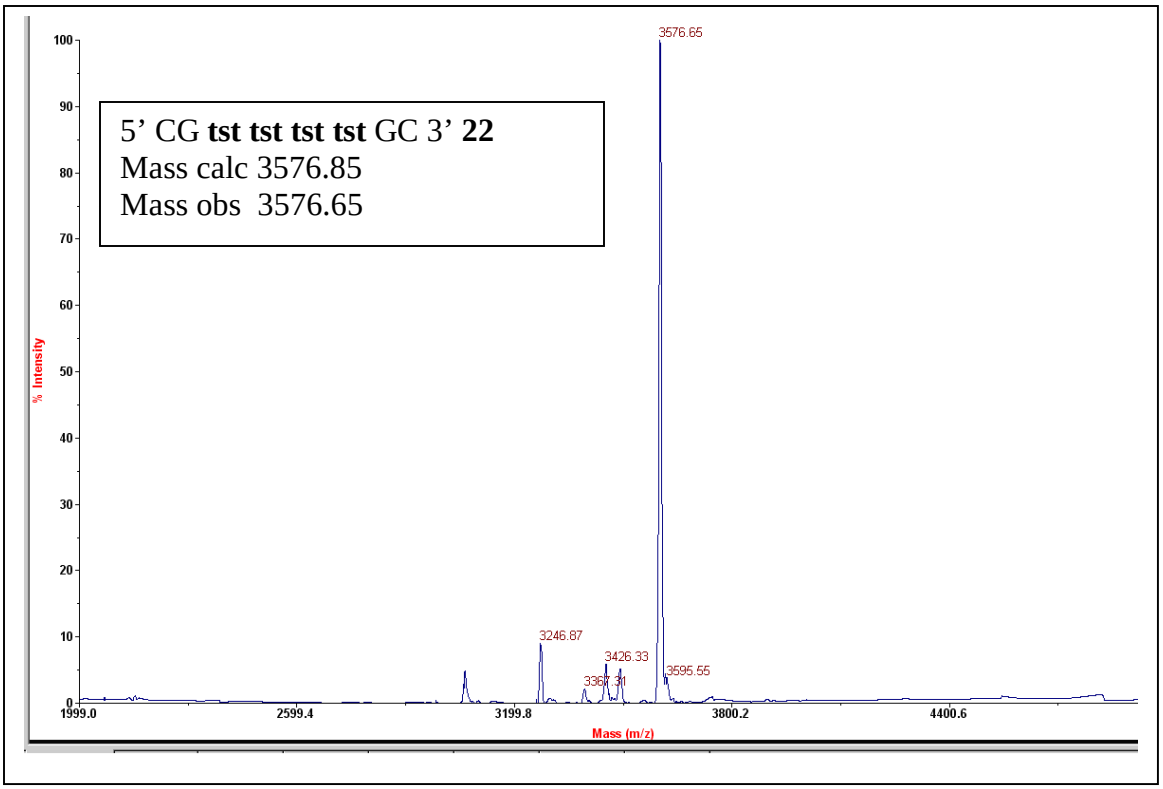


Figure S26: MALDI-TOF mass of **22**

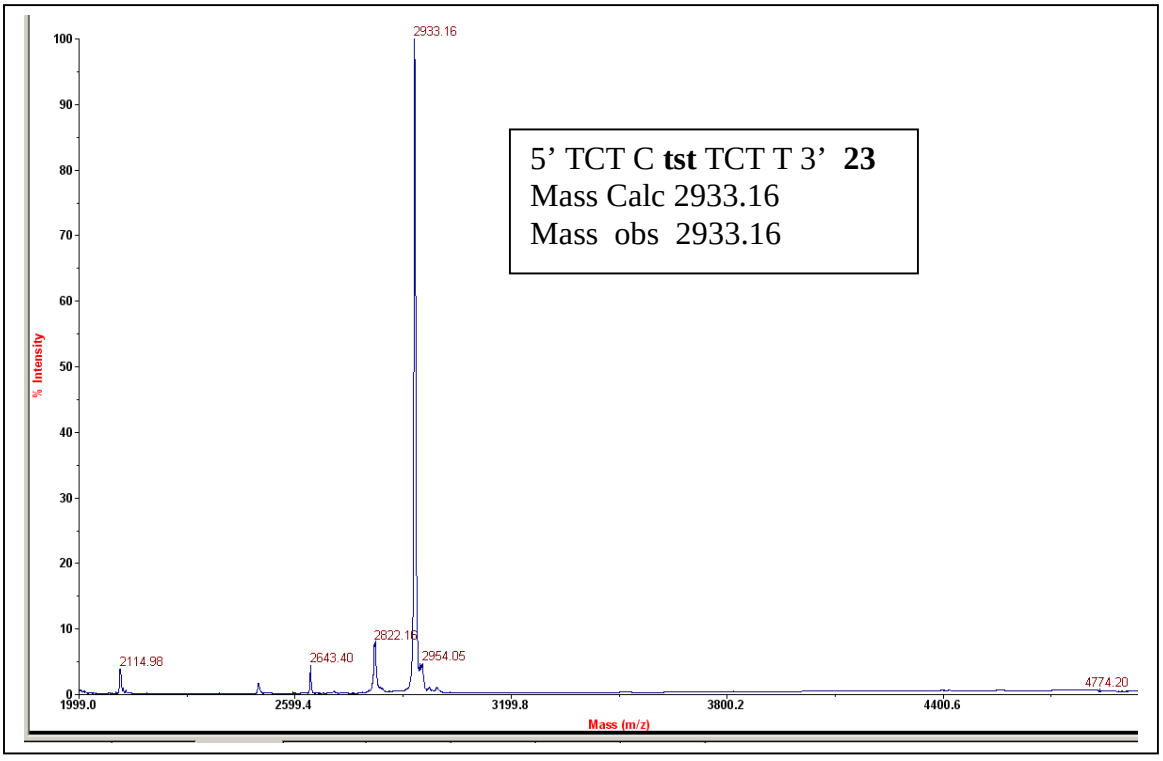


Figure S27: MALDI-TOF mass of **23**

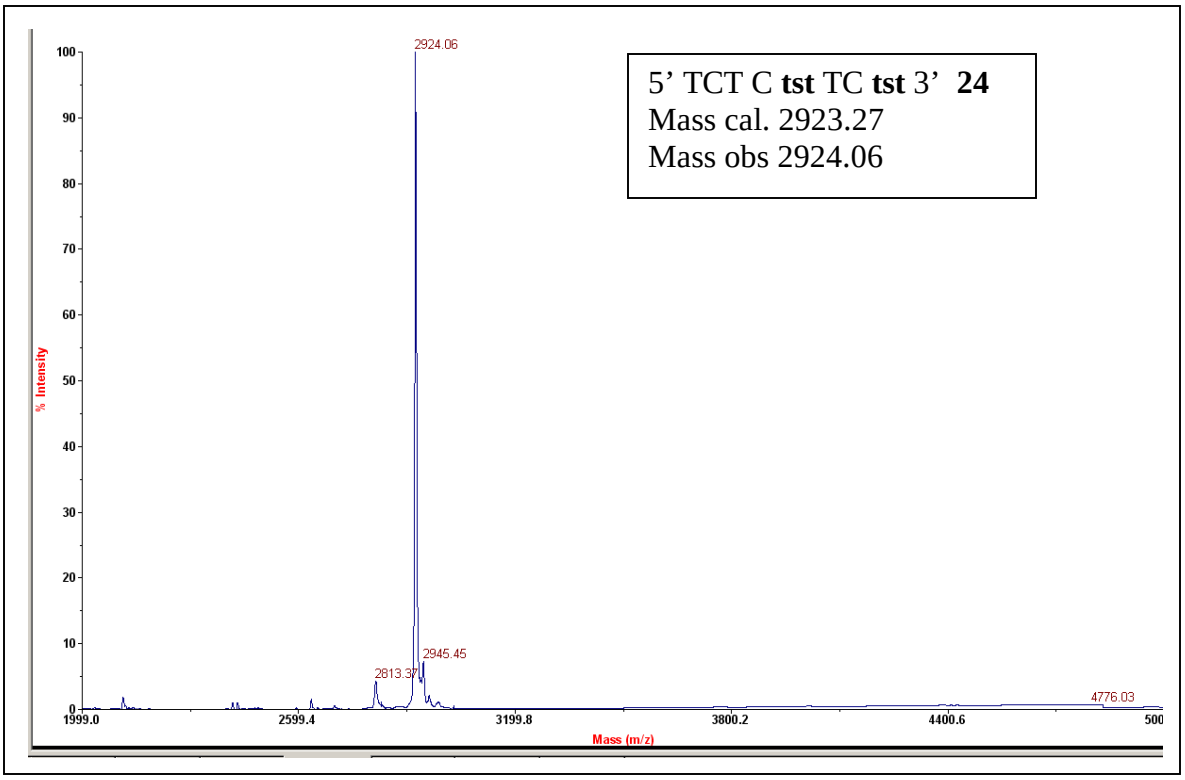


Figure S28: MALDI-TOF mass of **24**

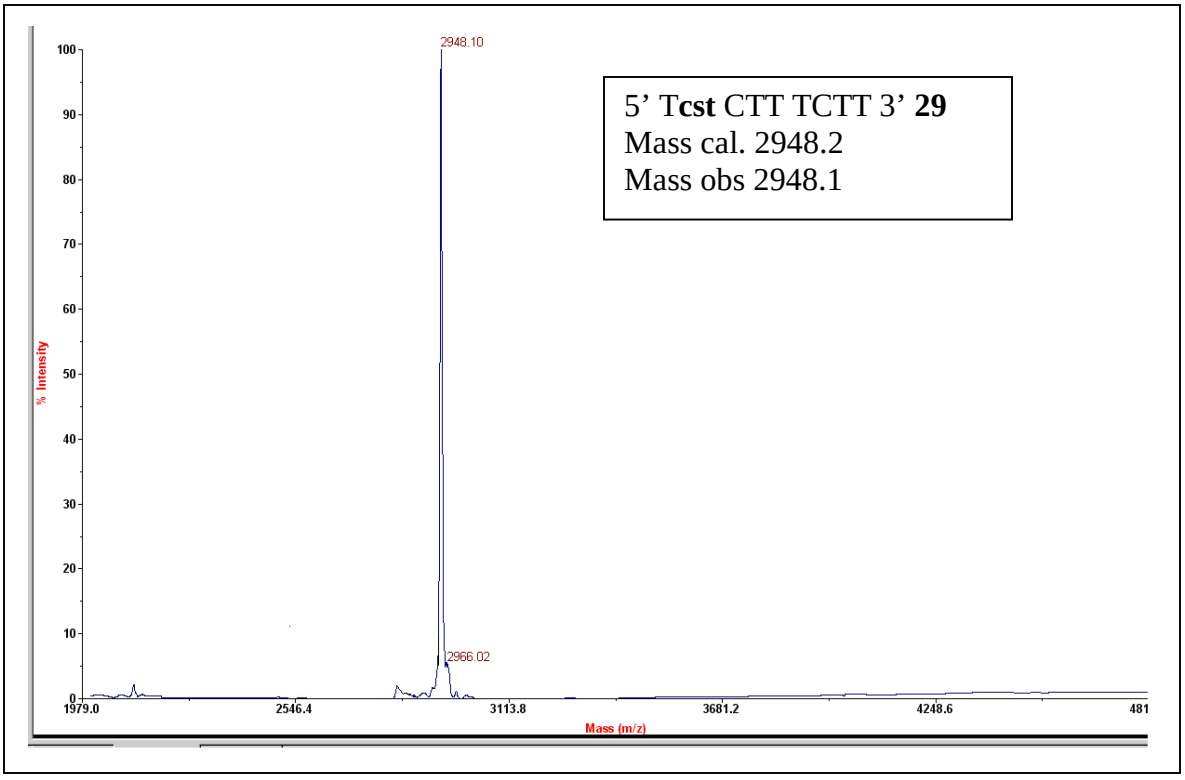


Figure S29: MALDI-TOF mass of **29**

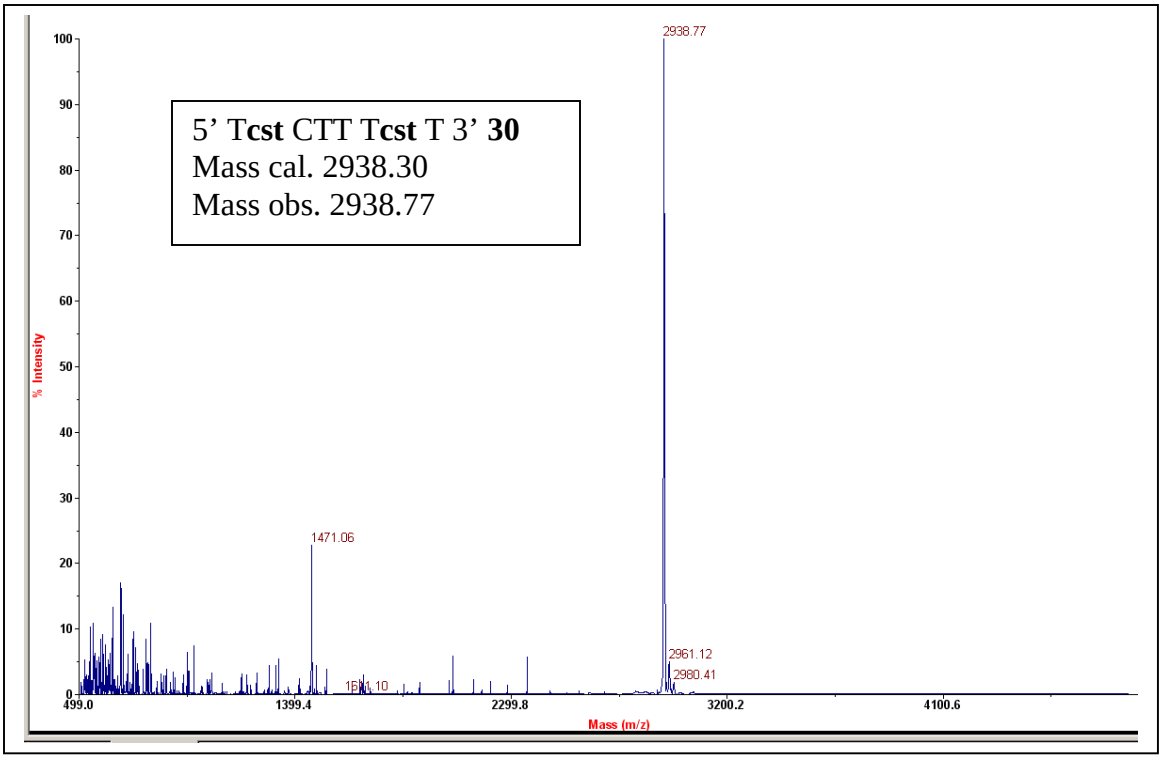


Figure S30: MALDI-TOF mass of **30**

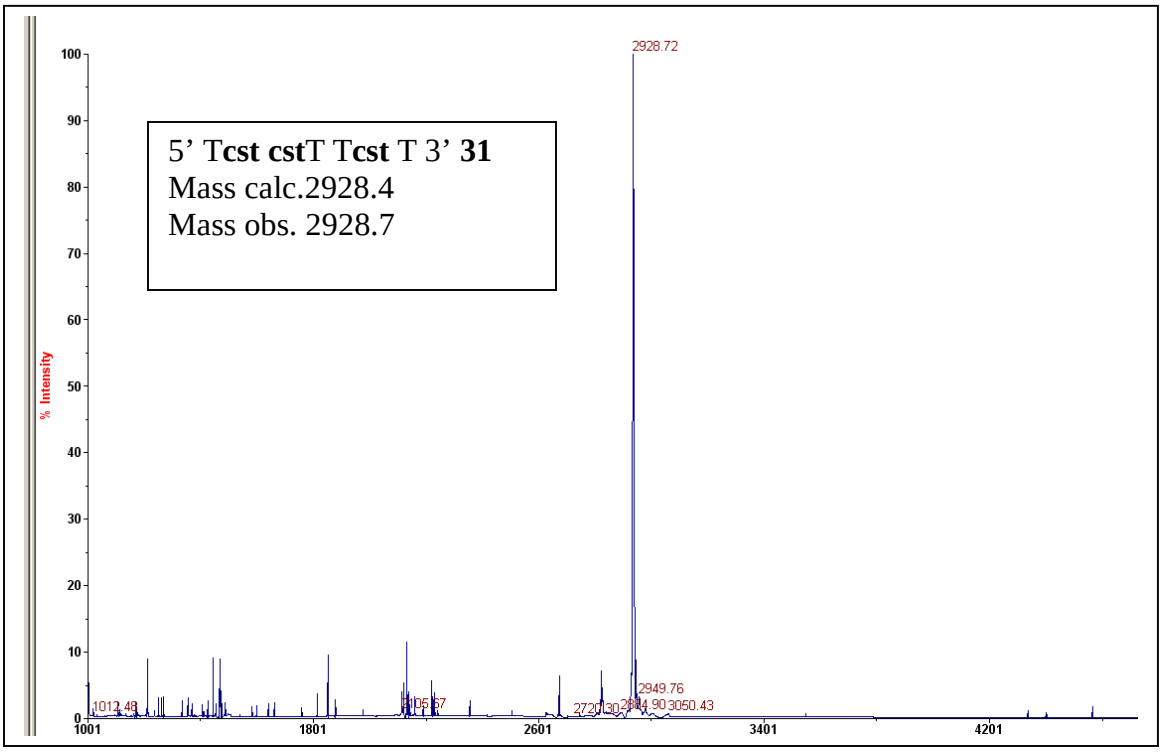


Figure S31: MALDI-TOF mass of **31**

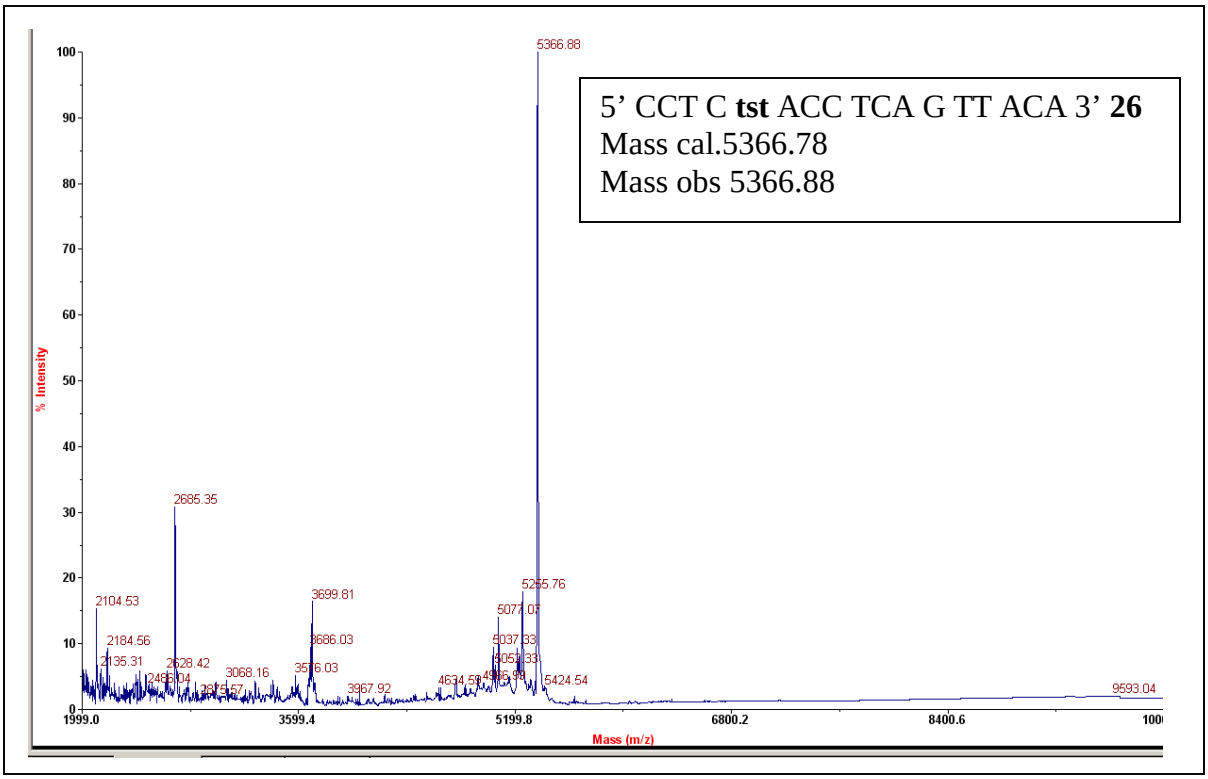


Figure S32: MALDI-TOF mass of **26**

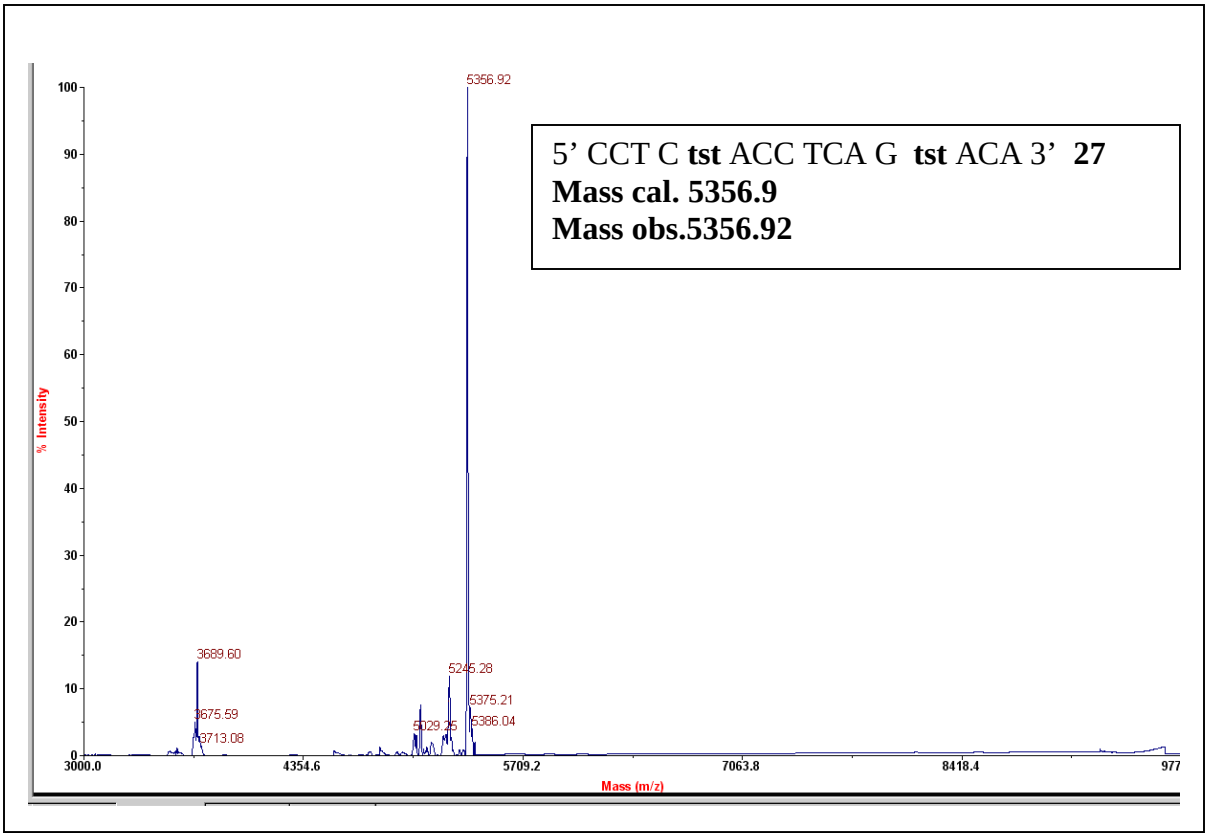


Figure S33: MALDI-TOF mass of **27**

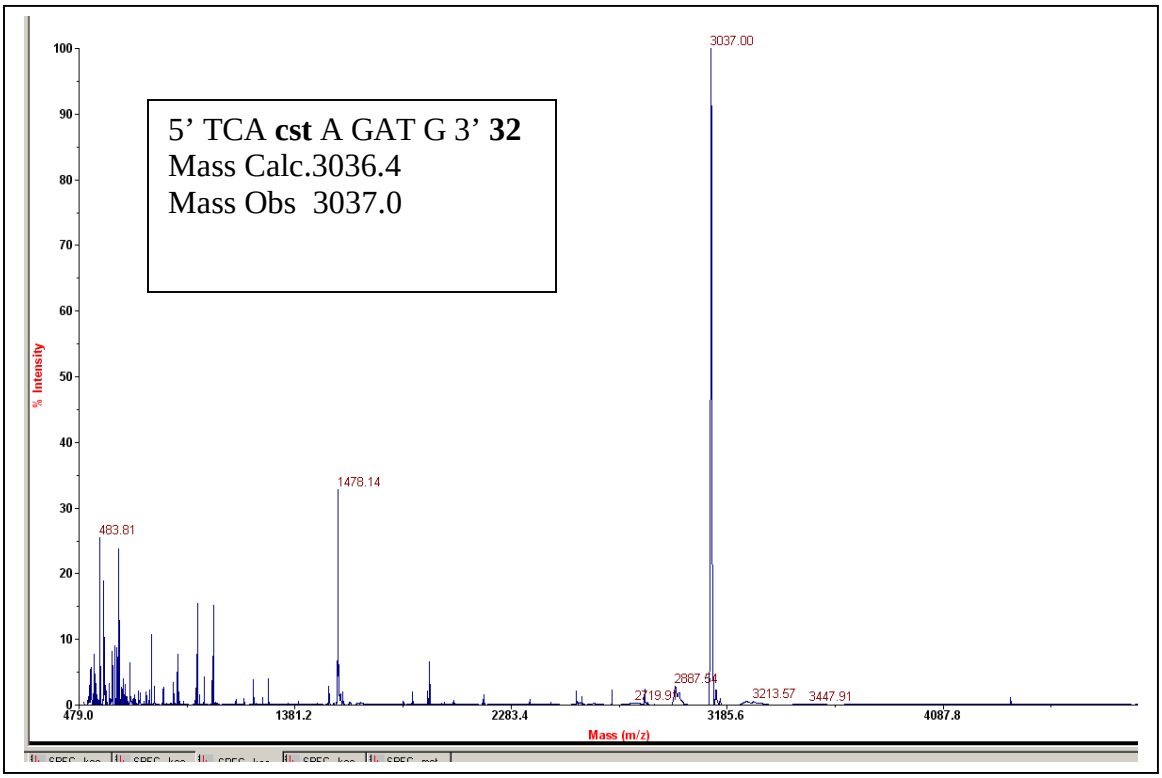


Figure S34: MALDI-TOF mass of **32**

UV-*Tm* measurements: The complementary DNA and RNA oligomers were synthesized on an Applied Biosystems 3900 DNA Synthesizer. The concentration was calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases. The experiments were performed in 1-2 µM concentrations. The complexes were prepared in 10 mM sodium phosphate buffer, pH 7.0 containing NaCl (100 mM) and EDTA (0.1 mM) and were annealed by keeping the samples at 90°C for 5 minutes followed by slow cooling to room temperature. Absorbance versus temperature profiles were obtained by monitoring at 260 nm with Perkin-Elmer Lambda 35 spectrophotometer scanning from 5 to 85°C at a ramp rate of 0.5°C per minute. The data were processed using Microcal Origin 6.1 and T_m values derived from the derivative curves. All values are an average of at least 3 experiments and accurate to within ± 0.5ºC.

Table S3 *T*m(ºC) values of ON: DNA / RNA duplex

Entry	DNA Sequence	DNA**34**	RNA**35**	Δ*T*m (RNA-DNA)
1	5’ CGTTTTTTTGC 3’ **33**	40	32.0	- 8.0
2	5’ CG TT**tst**TTT TGC 3’ **20**	23.7	32.3	8.6
3	5’ CGTT **tst** TT **tst** GC 3’ **21**	nd	50.0	-
4	5’ CG **tst tst tst tst** GC 3’ **22**	nd	47.81	-

DNA **34** 5’ GCAAAAAAAACG 3’ RNA **35** r (5’ GCAAAAAAAACG 3’)

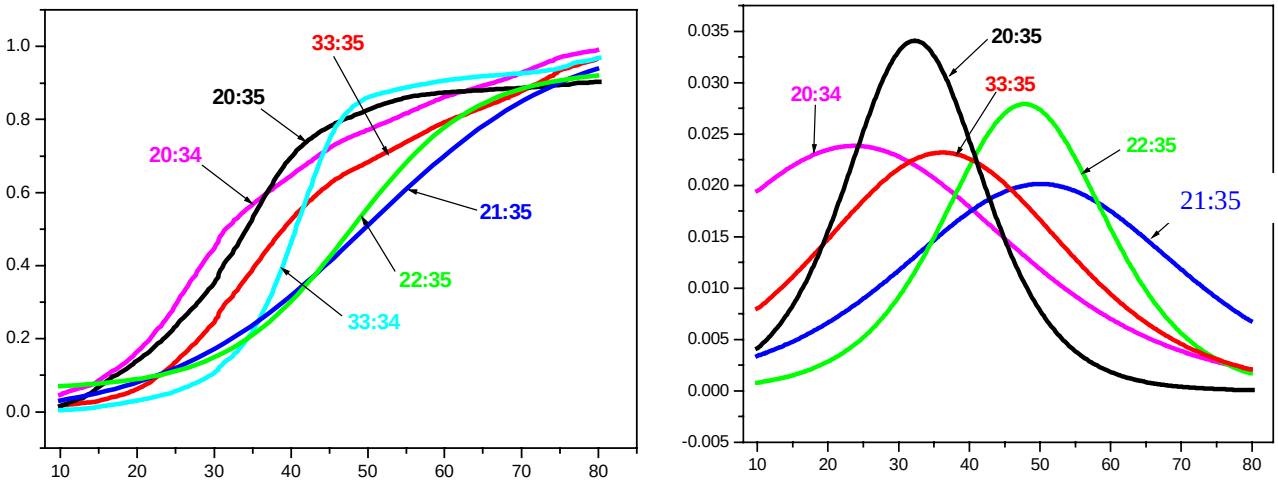


Figure S35: A. UV-melting Curves of **20**, **21**, **22** and **33** with cDNA **(34)** and cRNA **(35) B.** Corresponding first derivative Curves**.**

Table S4: *T*m(ºC) values of ON:DNA/RNA duplex

Entry	Sequence	DNA **37**	RNA **38**	$\Delta Tm_{(RNA-DNA)}$
1	5’ CCT CTT ACC TCA GTT ACA 3’ **36**	54.6	54.7	0.1
2	5’ CCT C **tst** ACC TCA G TT ACA 3’ **26**	39.6	47.5	7.9
3	5’ CCT C **tst** ACC TCA G **tst** ACA 3’ **27**	43.5	52.8	9.3

DNA **37** 5’ TGT AAC TGA GGT AAG AGG 3’
RNA **38** r (5’ TGT AAC TGA GGT AAG AGG 3’)

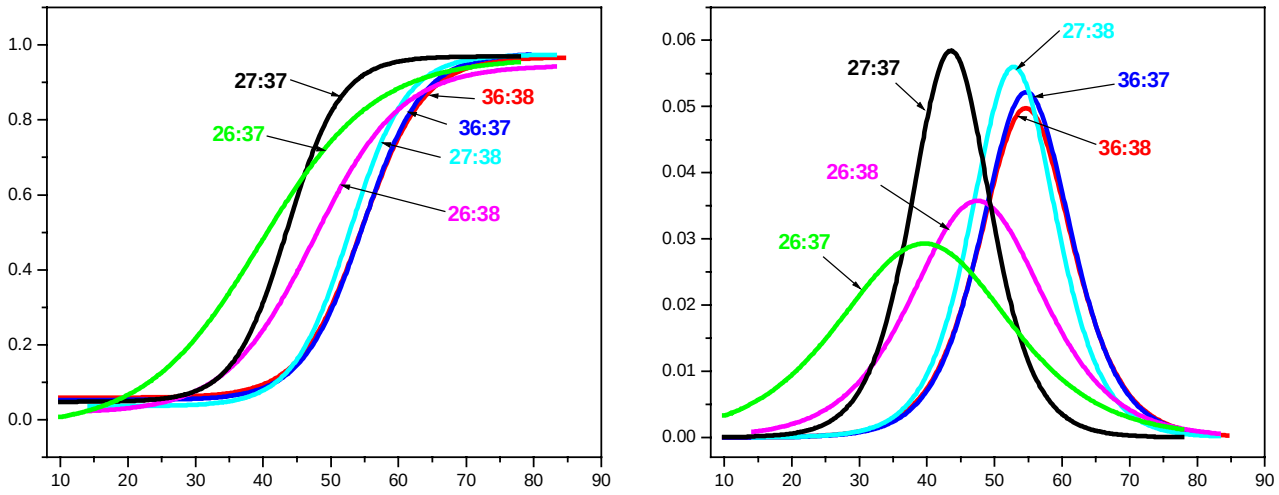


Figure S36: C. UV-melting Curves of **26**, **27** and **36** with cDNA **(37)** and cRNA **(38) D.** Corresponding first derivative Curves**.**

Table S5: *T*m(ºC) values of ON:DNA /RNA duplex

Entry	Sequence	DNA **40**	RNA **41**	$\Delta Tm_{(RNA-DNA)}$
1	5’ TCT CTT TCT T 3’ **39**	23.6	27.3	3.7
2	5’ TCT C **tst** TCT T 3’ **23**	nd	31	-
3	5’ TCT C **tst** TC **tst** 3’ **24**	nd	33.8	-
4	5’ T**cst** CTT TCTT 3’ **29**	18.5	27.5	9
5	5’ T**cst** CTT T**cst** T 3’ **30**	nd	32.6	-
6	5’ T**cst cst**T T**cst** T 3’ **31**	nd	39.7	-

DNA **40** 5’AAG AAA GAG A3’ RNA **41** r (5’ AAG AAA GAG A 3’)

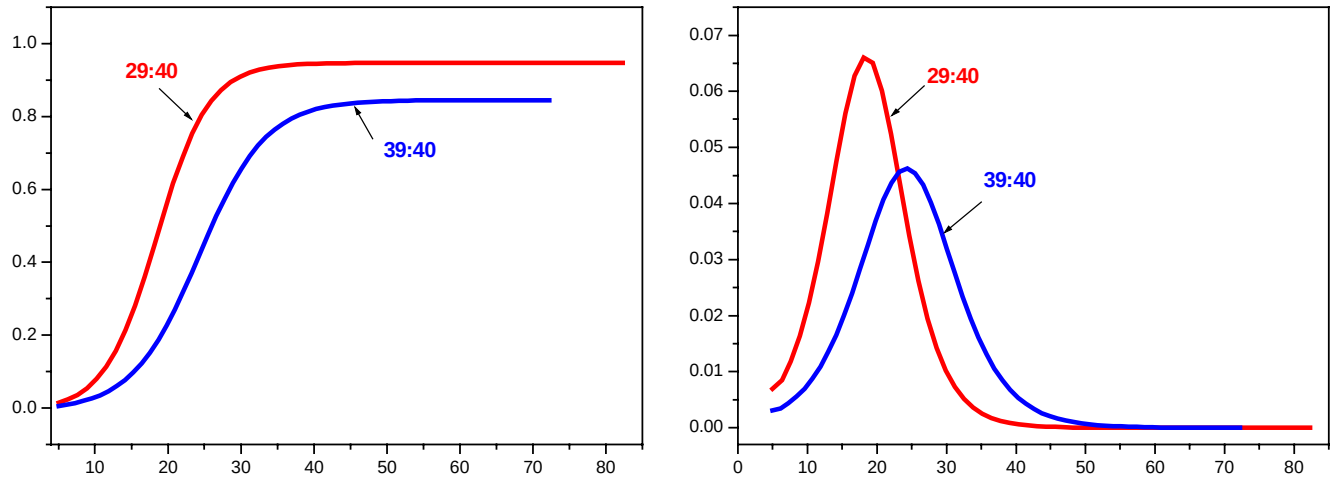


Figure S37: E. UV-melting Curves of **29** and **39** with cDNA **(40) D.** Corresponding first derivative Curves**.**

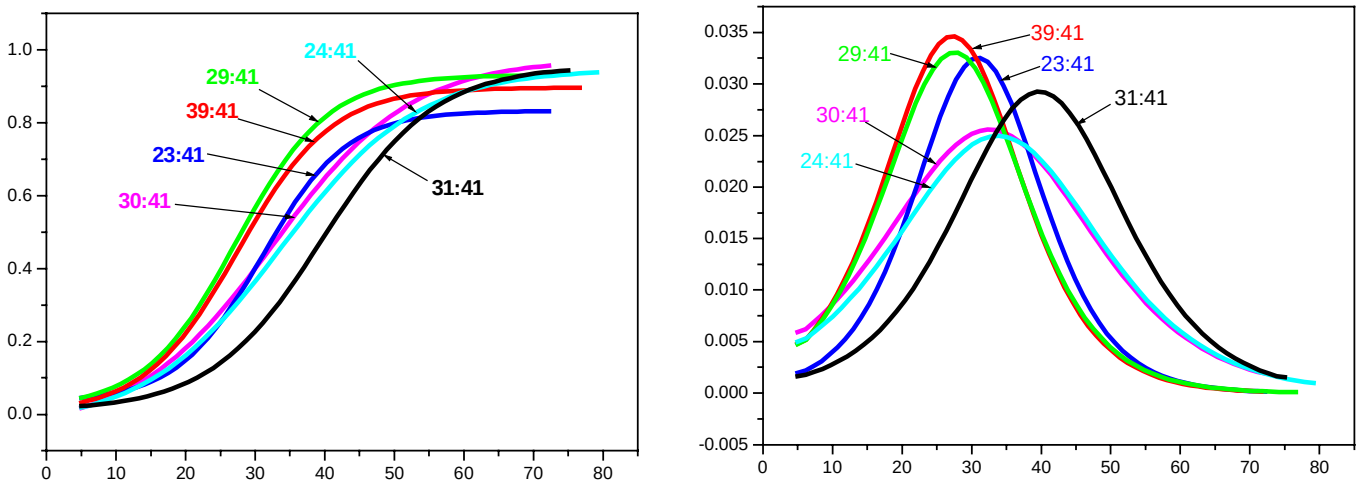


Figure S38: G. UV-melting Curves of **23**, **24, 29, 30, 31** and **39** with cRNA **(41) H.** Corresponding first derivative Curves**.**

Table S6: *T*m(ºC) values of ON:DNA /RNA duplex

Entry	Sequence	DNA **43**	RNA **44**	$\Delta Tm_{(RNA-DNA)}$
1	5’ TCA CTA GAT G 3’ **42**	24.3	24.7	0.4
2	5’ TCA **cst** A GAT G 3’ **32**	16.3	29.6	13.3

DNA **43** 5’CAT CTA GAG A3’ RNA **44** r (5’CAT CTA GAG A3’)

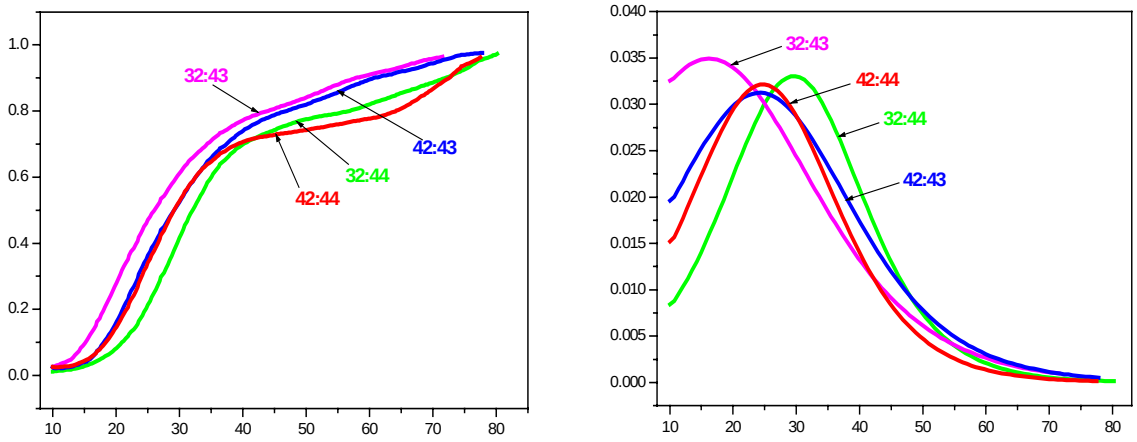


Figure S39: I. UV-melting Curves of **32** and **42** with cDNA (**43**) and cRNA **(44) J.** Corresponding first derivative Curves**.**

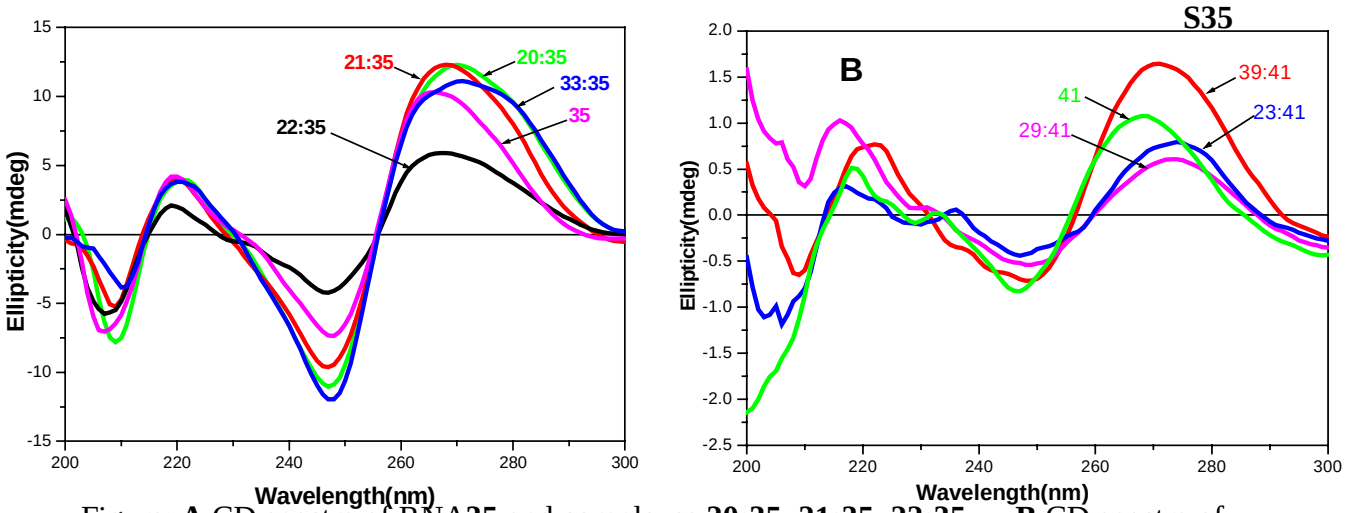


Figure: **A.**CD spectra of RNA**35** and complexes **20:35, 21:35, 22:35** **B**.CD spectra of RNA **41** and complexes **39:41, 23:41, 29:41.**

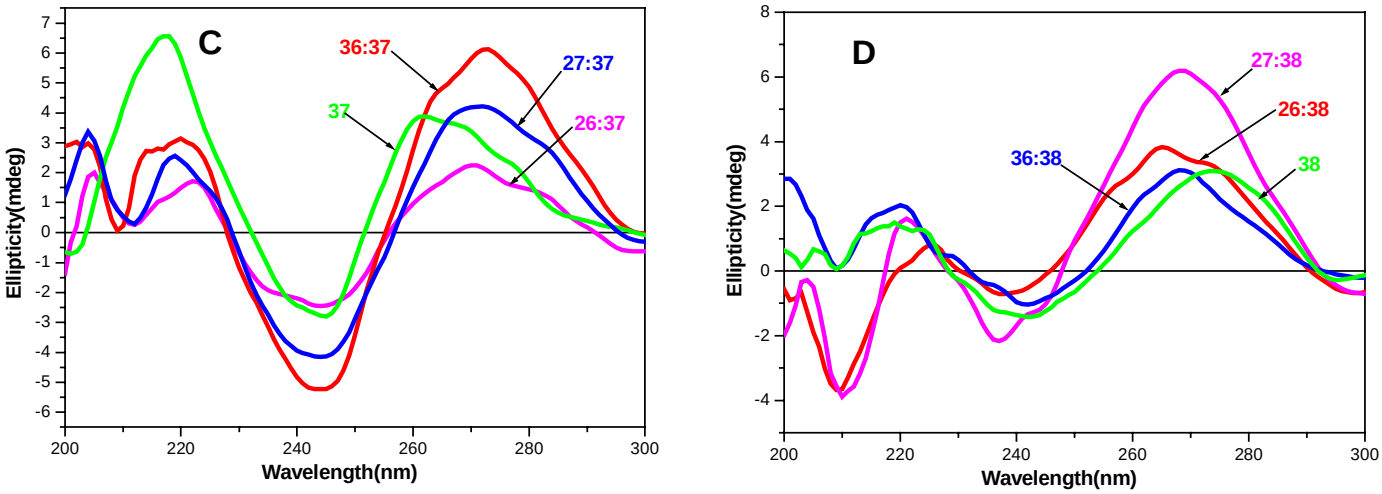


Figure: **C**. CD spectra of DNA**37** and complexes **36:37, 26:37** and **27:37** **D**. CD spectra of RNA**38** and complexes **36:38, 26:38** and **27:38**

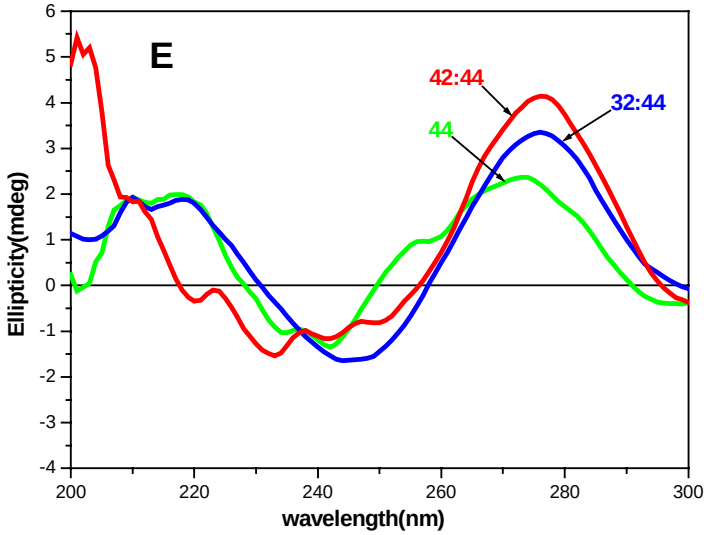


Figure: **E.** CD spectra of RNA 44 and complexes 42:44, 32:44.

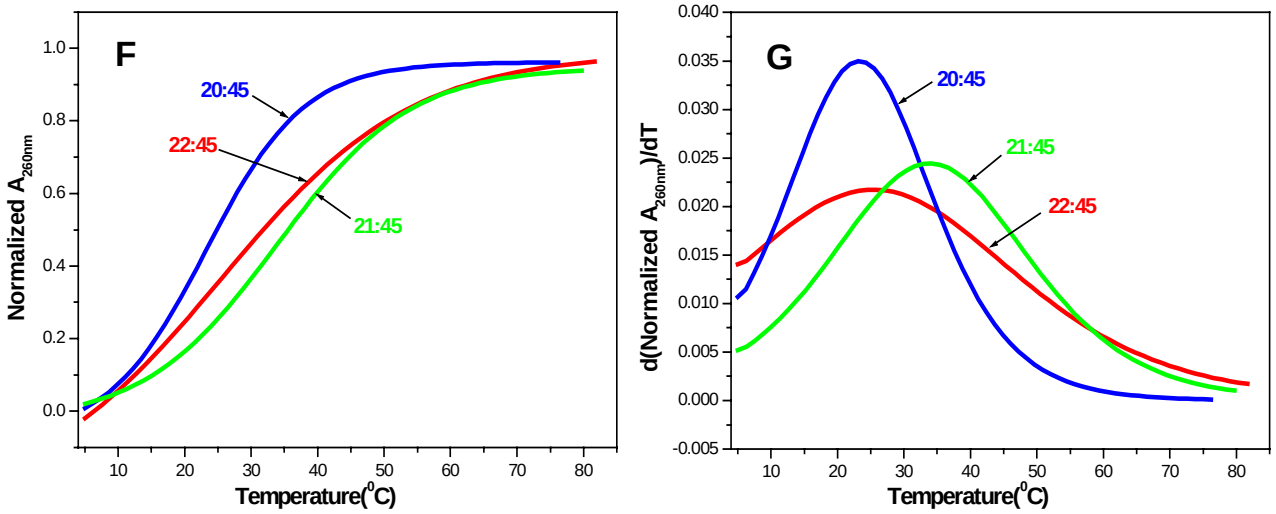


Figure: **F.** Mismatch melting curves of ONs **20**, **21** & **22** with mismatch RNA **45** r (5' GCAAA**U**AAAACG 3')
G.Corresponding derivative curves.

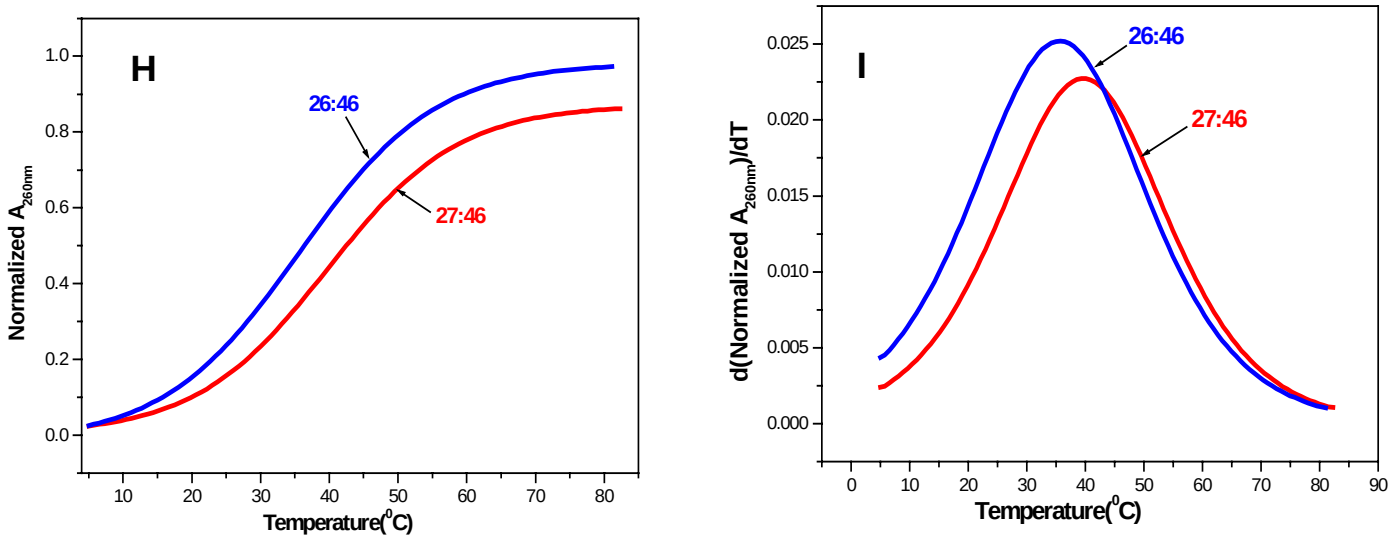


Figure: **H.** Mismatch melting curves of ONs **26** & **27** with mismatch RNA **46** r (5' UGU AAC UGA **C**GU AAG AGG 3') **I.**Corresponding derivative curves

Table S7: Mismatch UV-melting studies of modified ONs:RNA

Entry	ON Sequences	ON: cRNA T_m ^oC	ON:Mismatch RNA T_m ^oC
1	5’ CG TT**tst**TTT TGC **20**	**20:35**	**20:45**
		32.3	23.3 (-9)
2	5’ CGTT **tst** TT **tst** GC **21**	**21:35**	**21:45**
		50.0	33.9 (-16.1)
3	5’ CG **tst tst tst tst** GC **22**	**22:35**	**22:45**
		47.8	25.4 (-22.4)
4	5’ CCT C **tst** ACC TCA G TT ACA **26**	**26:38**	**26:46**
		47.5	35.7 (-11.8)
5	5’ CCT C **tst** ACC TCA G **tst** ACA **27**	**27:38**	**27:46**
		52.8	39.7 (-13.1)

RNA **35** r (5’ GCAAAAAAAACG 3’) , RNA **45** r (5’ GCAAA**U**AAAACG 3’)
RNA **38** r (5’ UGU AAC UGA GGU AAG AGG 3’)
RNA **46** r (5’ UGU AAC UGA **C**GU AAG AGG 3’)
Values in the parenthesis indicate the decreased melting temperature when hybridized with mismatch RNA