

SUPPORTING INFORMATION

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1. NMR and ESI-MS spectra of new compounds

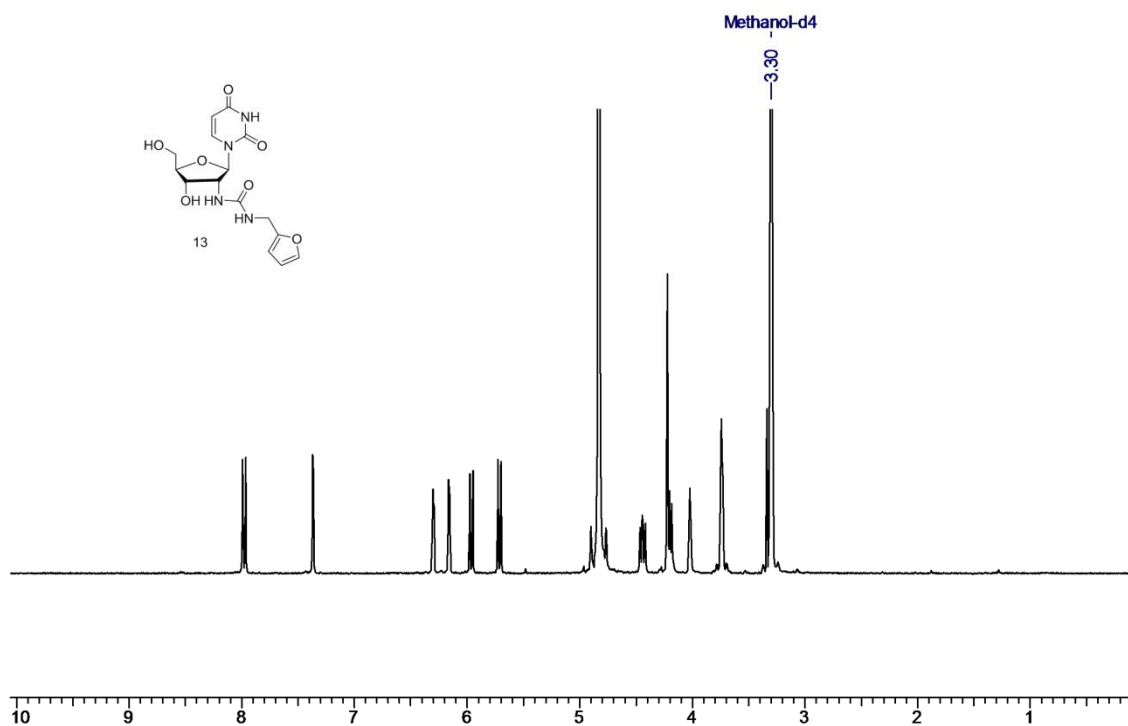


Figure S1: ^1H -NMR (MeOD, 300 MHz) of nucleoside **13**

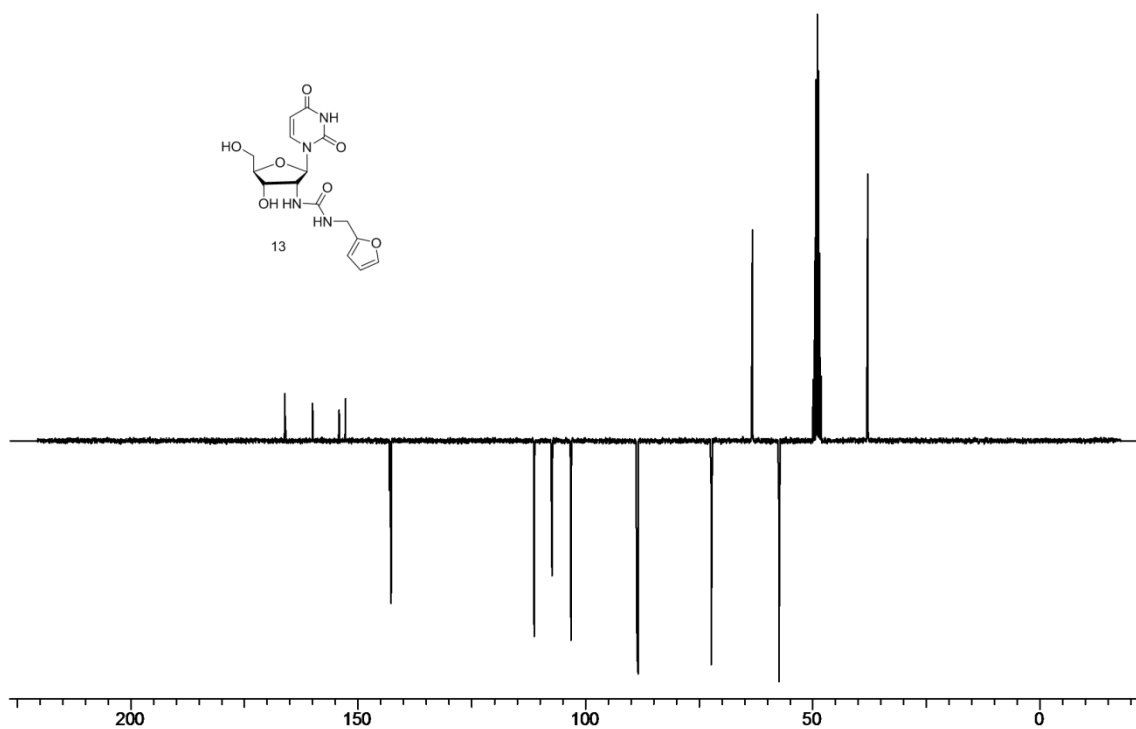


Figure S2: ^{13}C -NMR (MeOD, 75 MHz) of nucleoside **13**

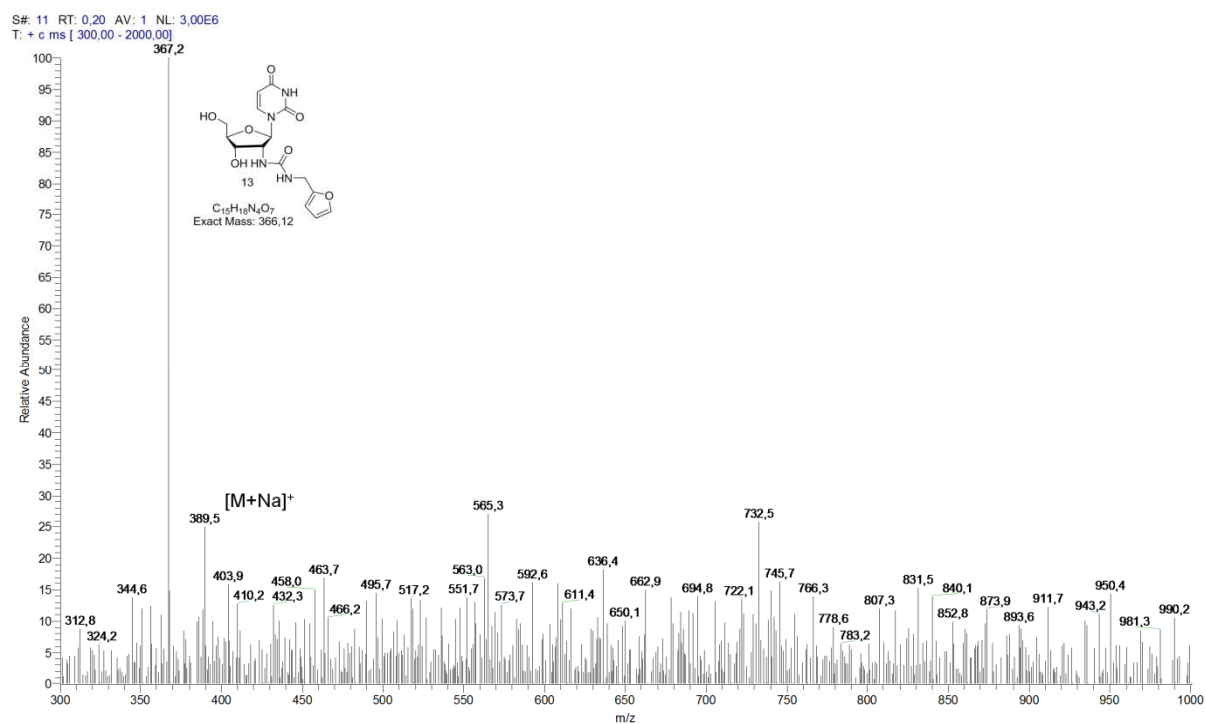


Figure S3: ESI-MS of nucleoside **13**

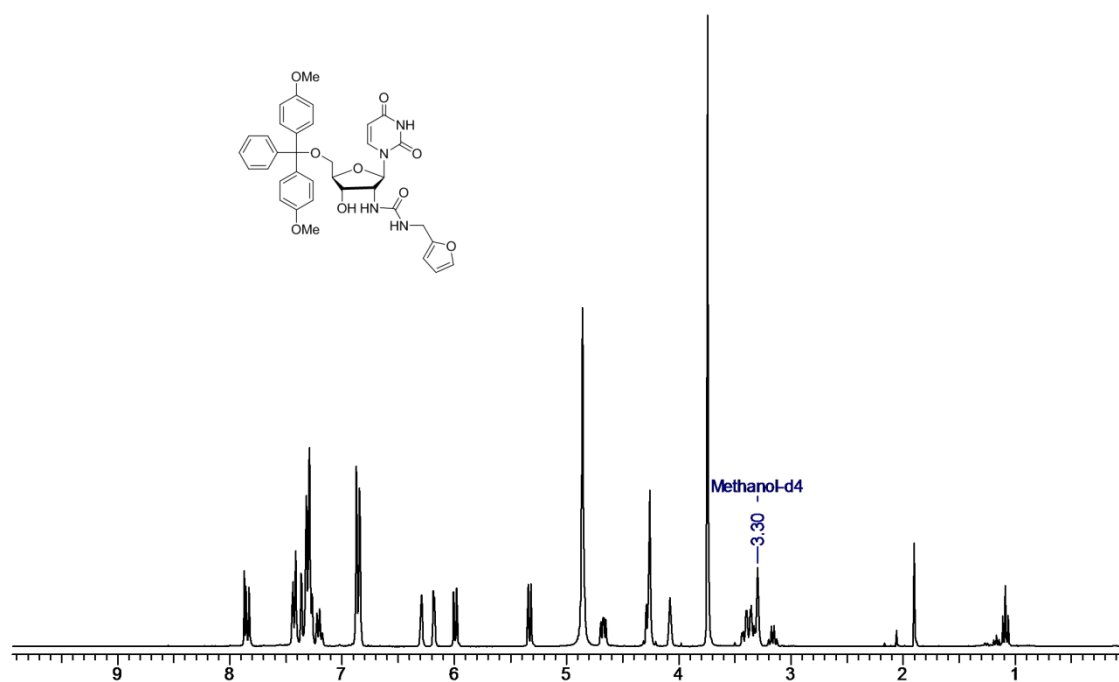


Figure S4: $^1\text{H-NMR}$ (MeOD, 300 MHz) of the DMTr-protected form of nucleoside **13**

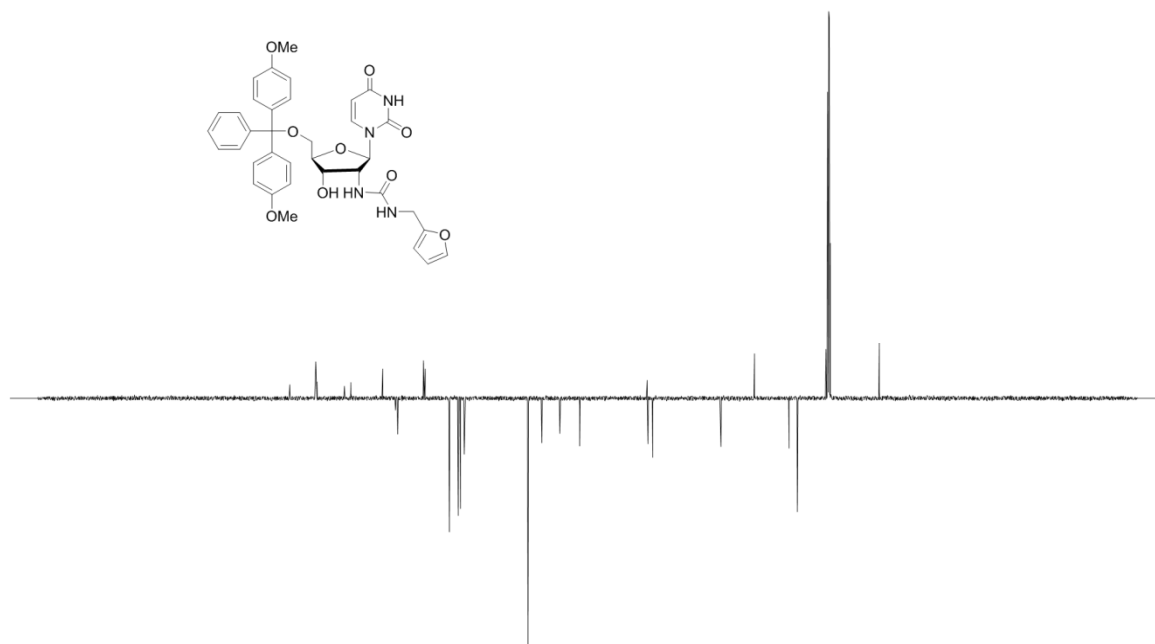


Figure S5: ^{13}C -NMR (MeOD, 125 MHz) of the DMTr-protected form of nucleoside **13**

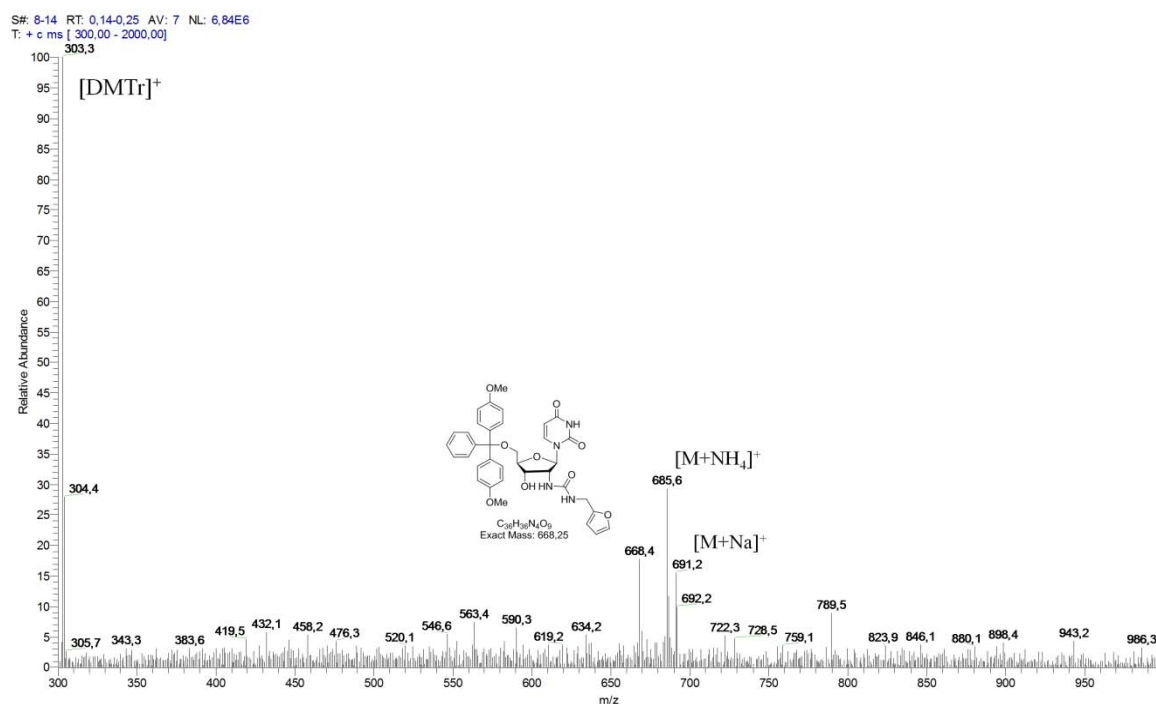


Figure S6: ESI-MS of the DMTr-protected form of nucleoside **13**

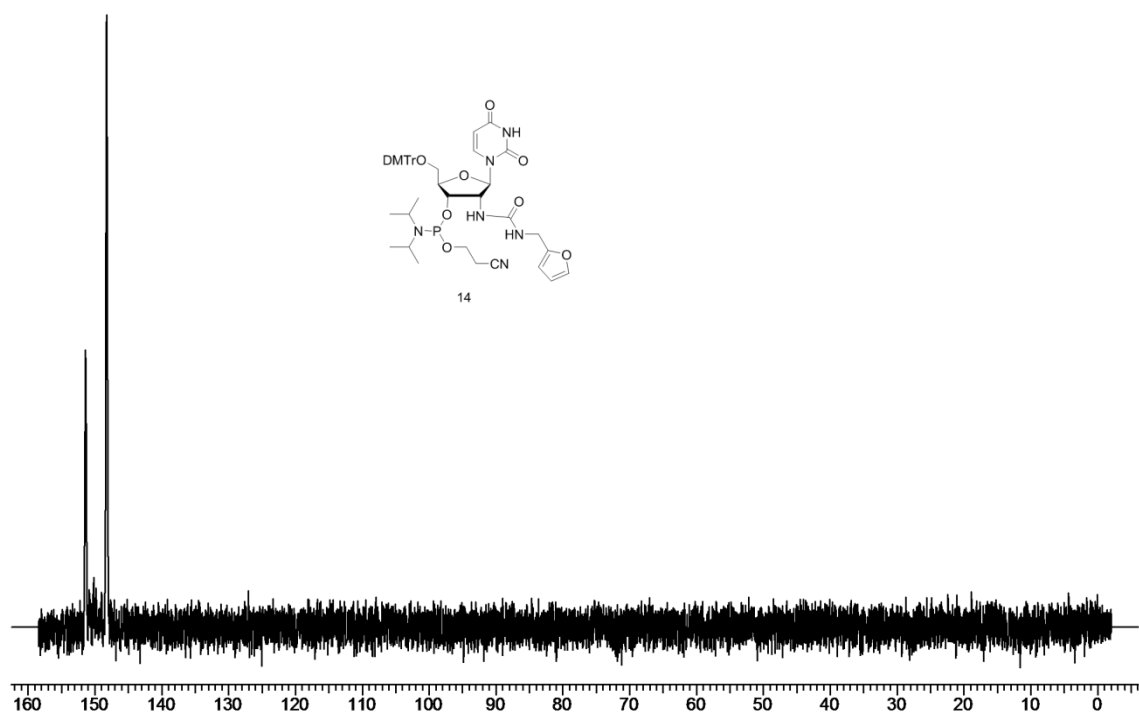


Figure S7: ^{31}P -NMR (CDCl_3 , 121 MHz) of nucleoside **14**

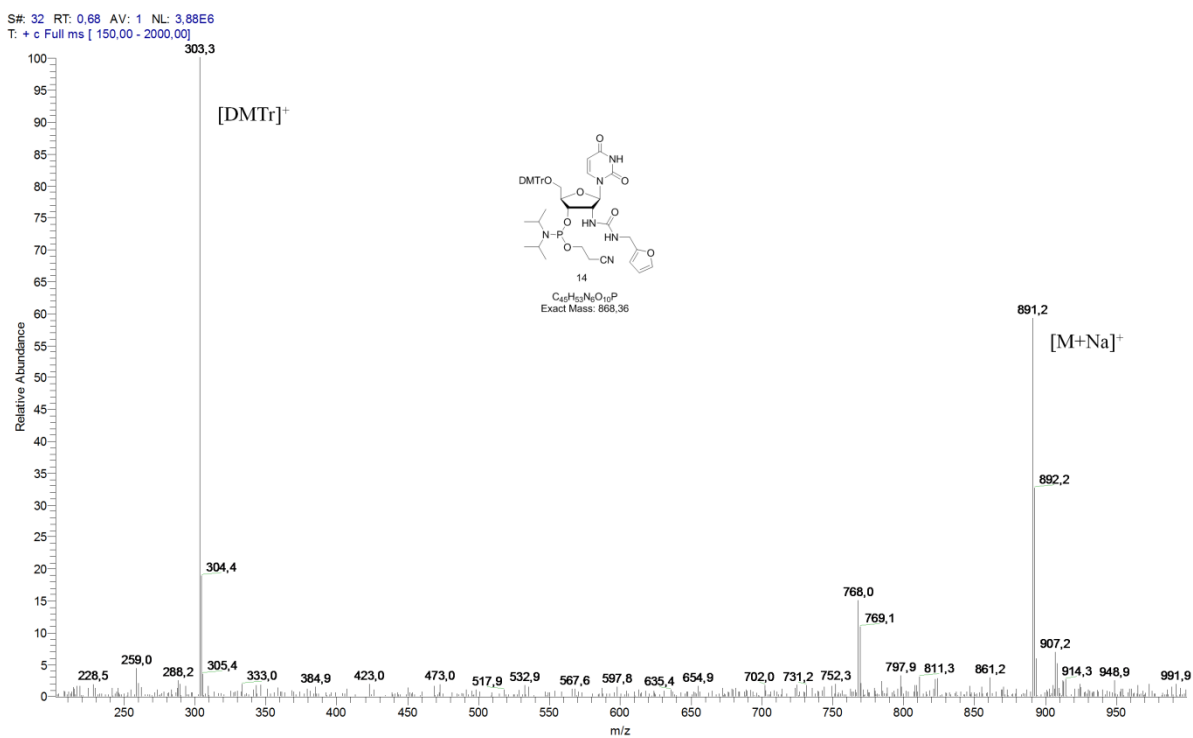


Figure S8: ESI-MS of nucleoside **14**

2. *Synthesis of 2,2'-O-anhydro-1-(β -D-arabinofuranosyl)uracil 7*

Uridine (4.99 g, 25.6 mmol) was added to a solution of diphenylcarbonate (5.49 g, 25.6 mmol) in dry DMF. The mixture was heated to 80°C until a white turbid solution was obtained. Then NaHCO₃ (0.046 g, 0.55 mmol) was added and the reaction mixture was stirred at 115°C. Shortly after, the solution became clear yellow and gas evolution could be observed. After about 4 h, gas evolution subsided and a white precipitate was formed. The reaction mixture was allowed to cool to room temperature and the white precipitate was filtered and washed with methanol. Partial evaporation of the filtrate resulted in further precipitation, the product could again be filtrated and the same protocol was repeated twice more. Ultimately, the product was obtained as a white powder in 74% yield. See ref 44 for analytical data.

3. *Synthesis of 2'-azido-2'-deoxyuridine 8*

A solution of LiF (0.620 g, 23.9 mmol) in dry DMF (13.3 ml) was heated until 105°C, after which Me₃SiN₃ (3.2 ml, 38.0 mmol) and TMEDA (16.6 ml) were added. The solution was allowed to stir for 30' after which nucleoside **7** (3.00 g, 13.3 mmol) was added and the mixture was stirred for another 64 h. After allowing the reaction mixture to cool to room temperature, it was concentrated by rotary evaporation and the residue was redissolved in MeOH (10 ml). The majority of the salts and starting material were then precipitated with ethylacetate (40 ml). The residue was filtered off and washed with MeOH:EtOAc (1:4). The filtrate was evaporated on silica (50 ml) and a first rudimentary purification is performed by flash chromatography (EtOAc:MeOH 4:1). The resulting brown foam is again purified by flash chromatography using DCM:MeOH 9:1 after which the product is obtained as an off-white foam (2.59 g, 9.62 mmol) in 72 % yield. See ref 45 for analytical data.

4. *Synthesis of crosslinked dinucleoside 24*

3',5'-O-bisacetyl-2'-deoxy-2'-[3-(2-furyl)propanamido]uridine 19

Acetic anhydride (80 μ l, 0.846 mmol) was added to a solution of nucleoside **9** (49.5 mg, 0.136 mmol), DMAP (2.6 mg, 0.021 mmol) and TEA (130 μ l, 0.925 mmol) in acetonitrile (1.5 ml) upon which the turbid solution rapidly turned clear. The reaction was allowed to stir for 2 h at room temperature before it was taken up in EtOAc (20 ml) and washed twice with saturated $\text{NaHCO}_{3,\text{aq}}$ (2 x 20 ml) and once with saturated NaCl_{aq} (20 ml). The organic phase was dried over Na_2SO_4 , filtered and concentrated under vacuum. The residue was purified by flash chromatography with DCM:MeOH 98:2 after which the product (40.7 mg, 0.091 mmol) was obtained in 67% yield. Rf: 0.53 (DCM:MeOH 9:1); $^1\text{H-NMR}$ (MeOD, 300 Hz): 6.16 (d, J = 8.3 Hz, 1H), 5.78 (m, 1H), 4.72 (m, 1H), 4.57 (d, J = 8.9 Hz, 1H), 4.46 (m, 1H), 4.28 (d, J = 8.1 Hz, 1H), 3.68 (m, 1H), 2.93 (dd, J = 11.3 and 3.6 Hz, 1H), 2.78 (m, 2H), 1.79 (m, 1H), 1.38 (m, 2H), 1.03 (m, 2H), 0.63 (m, 3H); $^{13}\text{C-NMR}$ (MeOD, 75 MHz): 175.3, 172.3, 166.0, 155.6, 152.6, 141.9, 111.2, 106.2, 103.5, 88.1, 85.2, 82.8, 73.9, 71.6, 65.3, 56.1, 54.2, 35.0, 24.8, 20.7; HRMS (+ESI): Calcd for $\text{C}_{20}\text{H}_{24}\text{N}_3\text{O}_9$ ($\text{M}+\text{H}^+$): 450.1507; found: 450.1506.

3',5'-O-bisacetyl-2'-deoxycytidine 21

Acetic anhydride (250 μ l, 2.64 mmol) was added to a solution of 2'-deoxycytidine **20** (0.301 g, 1.32 mmol), DMAP (14.8 mg, 0.121 mmol) and TEA (410 μ l, 2.92 mmol) in acetonitrile (15 ml). The reaction mixture was stirred overnight at room temperature, then quenched with MeOH (100 μ l) and concentrated by rotary evaporation. The residue was purified by flash chromatography with DCM:MeOH 95:5. The product (0.278 mg, 0.894 mmol) was obtained in 68% yield. Rf: 0.31 (DCM:MeOH 9:1), $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): 7.48 (d, J = 7.5 Hz, 1H), 6.18 (dd, J = 7.6 and 6.0 Hz, 1H), 5.89 (d, J = 7.6 Hz, 1H), 5.16 (m, 1H), 4.26 (m, 2H), 4.21 (m, 1H), 2.55 (m, 1H), 2.04 (m, 7H); $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz):

176.5, 170.3, 165.8, 155.5, 139.8, 95.3, 86.4, 82.1, 74.2, 63.8, 38.2, 20.7; HRMS (+ESI): Calcd for $C_{13}H_{18}N_3O_6$ ($M+H^+$): 312.1190; found: 312.1191.

Crosslinked dinucleoside **24**

Protected nucleoside **19** (20.1 mg, 0.045 mmol) was dissolved in THF:acetone: H_2O 5:4:2 (1 ml) after which pyridine (11 μ l, 0.136 mmol) and NBS (8.5 mg, 0.048 mmol) were added. The reaction was followed by TLC, after 1 h the starting nucleoside was completely consumed and protected cytidine **21** (13.8 mg, 0.045 mmol) was added. The reaction was allowed to stir overnight at room temperature, then quenched by the addition of acetone (2 ml) and evaporated to dryness. The resulting mixture was purified by flash chromatography using a gradual elution from DCM:MeOH 99:1 to 9:1. The desired product (26.0 mg, 0.0334 mmol) was obtained together with the partially deprotected product. LC-MS (negative mode): t_r : 10.92 min. (nucleoside **22** – Ac, calculated for $C_{31}H_{38}N_6O_{15}$: 734.2, found: 733.2 $[M]^-$), t_r : 11.61 min. (nucleoside **22**, calculated for $C_{33}H_{40}N_6O_{16}$: 776.3, found: 775.1 $[M]^-$)

Crosslinked dinucleoside **22** (26.0 mg; 0.034 mmol) was then dissolved in methanolic ammonia (1.25 ml, 1.4 N) and was allowed to react overnight at room temperature. After evaporation to dryness, the product (22.0 mg, 0.036 mmol) was obtained in high enough purity to proceed with the next reaction. The overall yield of the crosslinking and deprotection reactions was 80%. The purity of the material was checked by HPLC and the identity checked by ESI-MS.

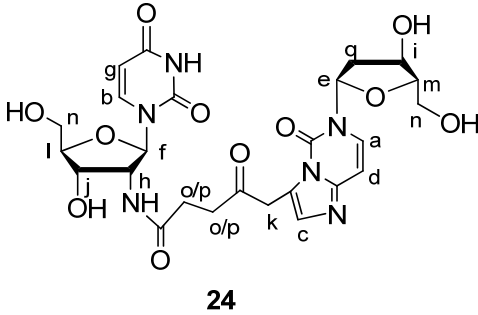
RP-HPLC showed the presence of two diastereomers with t_r : 10.1 min. and t_r : 10.5 min. However under the conditions used for LC-MS both diastereomers eluted together. HRMS (+ESI): Calcd for $C_{25}H_{33}N_6O_{12}$ ($M+H^+$): 609.2151; found: 609.2144.

Subsequently, dinucleoside **23** (10 mg, 0.016 mmol) was dissolved in 0.1M HCl_{aq} (10 ml). The reaction was stirred at room temperature and followed by RP-HPLC. After 9 days the

reaction mixture was diluted with 40 ml H₂O and lyophilized to dryness. The product was purified by RP-HPLC, allowing unambiguous characterization by NMR.

HRMS (+ESI): Calcd for C₂₅H₃₁N₆O₁₁ (M+H⁺): 591.2046; found: 591.2040; Calcd for C₂₅H₃₀N₆O₁₁Na (M+Na⁺): 613.1895; found: 613.1863; Calcd for C₂₅H₃₀N₆O₁₁K (M+K⁺): 629.1605; found: 629.1584

¹H-NMR (D₂O, 700 MHz):

structuur	atomen	δ (ppm)	integratie	multipliciteit	J (Hz)
 <p style="text-align: center;">24</p>	a	7.88	1	d	7.9
	b	7.56	1	d	7.9
	c	7.22	1	s	
	d	6.73	1	d	7.9
	e	6.44	1	t	6.8
	f	6.04	1	d	8.4
	g	5.91	1	d	8.3
	h	4.63	1	dd	8.1 and 5.9
	i	4.53	1	m	
	j	4.37	1	dd	5.7 and 2.2
	k	4.28	2	d	4.0
	l	4.22	1	m	
	m	4.11	1	dd	8.8 and 4.0
	n	3.86	4	m	
	o	3.00	2	m	
	p	2.62	2	m	
	q	2.43	2	m	

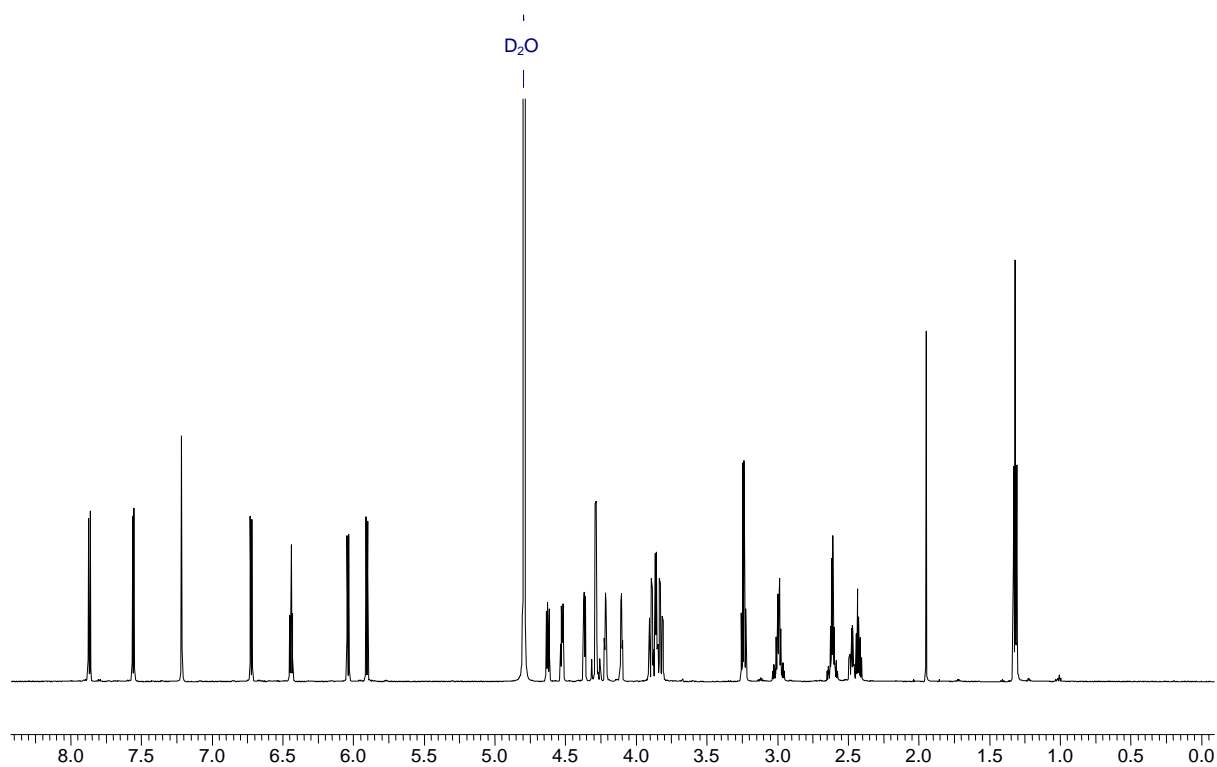
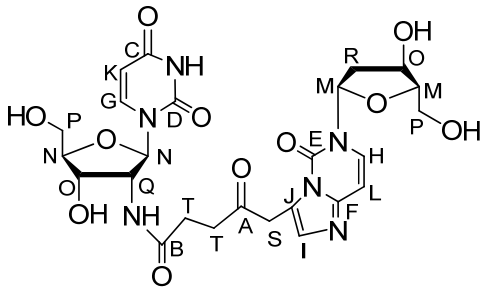


Figure S9: ^1H -NMR (D_2O , 700 MHz) of crosslinked dinucleoside **24**

¹³C-NMR (D₂O, 176 MHz):

structuur	atomen	δ (ppm)	atomen	δ (ppm)
 <p style="text-align: center;">24</p>	A	210.3	K	102.8
	B	175.6	L	99.2
	C	166.0	M	86.9 + 86.8
	D	151.9	N	86.4 + 85.8
	E	148.0	O	70.6 + 70.1
	F	146.8	P	61.5 + 61.4
	G	141.8	Q	55.0
	H	132.3	R	39.2
	I	128.7	S	39.1
	J	122.6	T	36.9 + 29.2

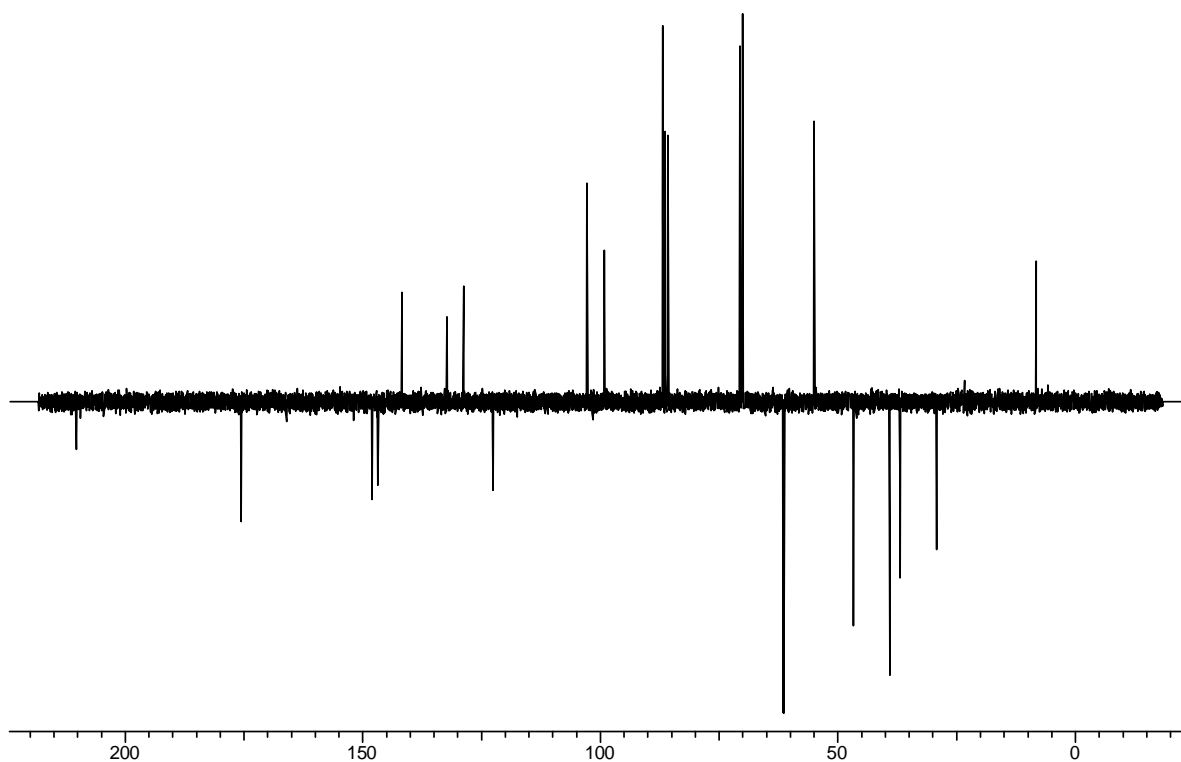


Figure S10: ¹³C-NMR (D₂O, 176 MHz) of crosslinked dinucleoside **24**

ESI-MS:

S#: 6 RT: 0,09 AV: 1 NL: 1,18E7
T: + c Full ms [100,00 - 1500,00]

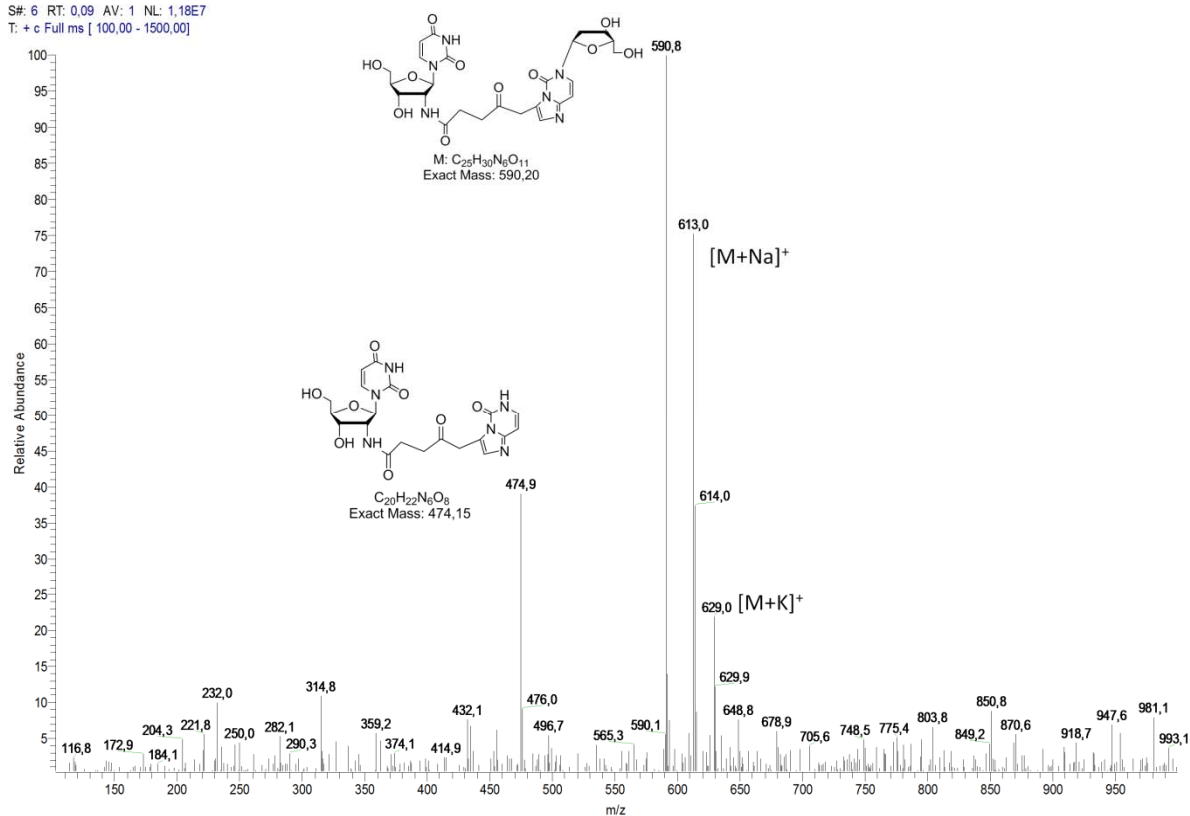
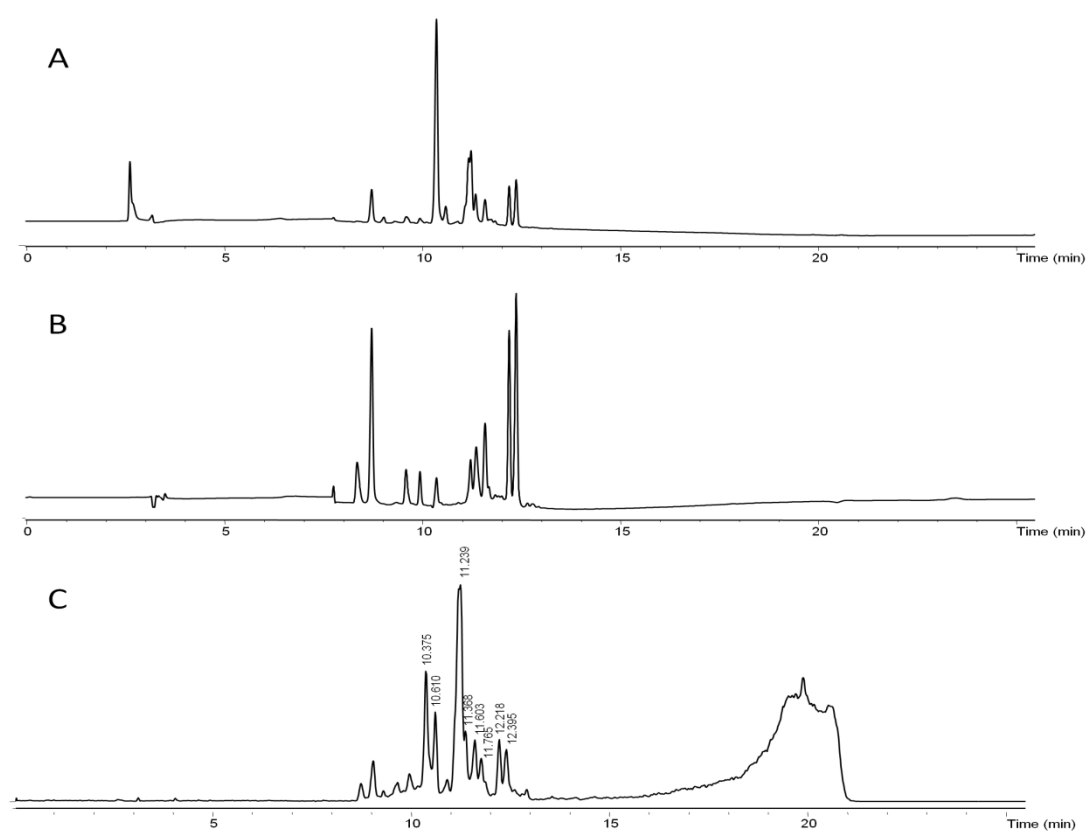
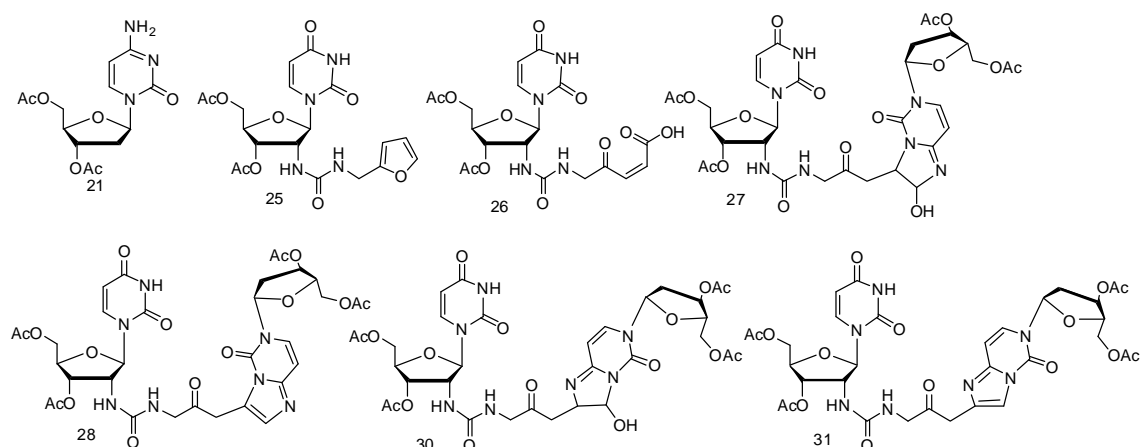


Figure S11: ESI-MS spectrum for crosslinked dinucleoside **24**

5. LC-MS spectrum of the reaction mixture of dinucleoside 27



Tr (min.)	compound	Chemical formula	Calculated mass	Mass found
10.38	nucleoside 21	$C_{13}H_{17}N_3O_6$	311.1	310.0
10.61	nucleoside 26 – 2Ac	$C_{15}H_{18}N_4O_9$	398.1	397.0
11.24	nucleoside 26	$C_{19}H_{22}N_4O_{11}$	482.1	480.0
11.37, 11.60	nucleosides 27 & 30	$C_{32}H_{39}N_7O_{16}$	777.3	776.1
11.77	nucleoside 25	$C_{19}H_{22}N_4O_9$	450.1	449.0
12.22, 12.40	nucleosides 28 & 31	$C_{32}H_{37}N_7O_{15}$	759.2	758.1

Figure S12: A. LC-chromatogram at 260 nm, B. LC-chromatogram at 310 nm, C. total ion abundance chromatogram in negative mode

6. HPLC-spectra of the synthesized modified oligonucleotides

ODN-synthesis was carried out as described in the experimental section. To save precious material, the modified phosphoramidites were introduced manually. If desired for convenience they can also be included in the automated synthesis cycles.

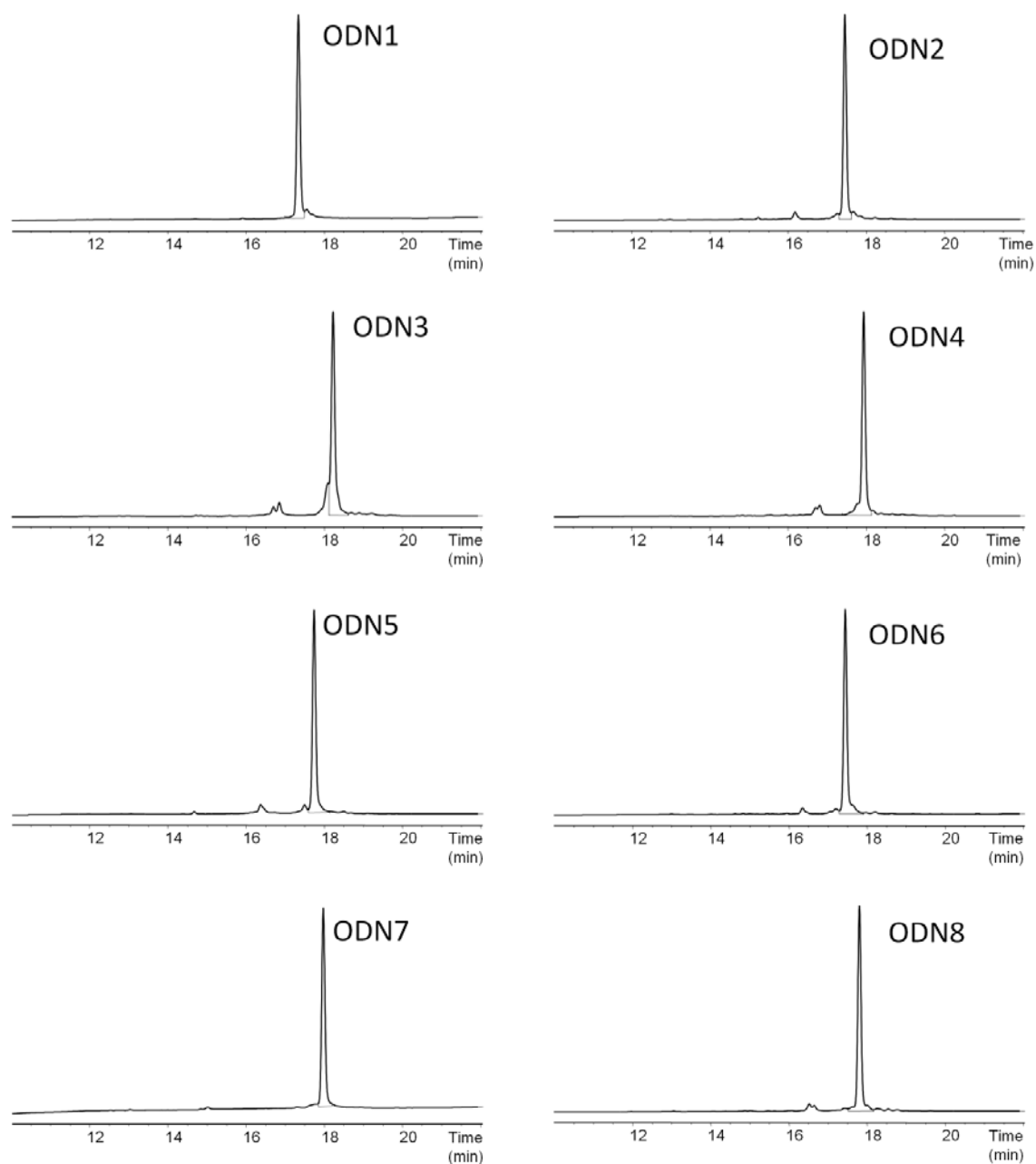


Figure S13: RP-HPLC chromatograms of furan-modified **ODN1-8**

7. *ESI-MS data of the synthesized oligonucleotides*

Modified sequence	Calculated mass	Mass found (Da)
ODN1: 5'-CTG ACG G1G TGC-3'	3800.5	3799.7 (M-H) ⁻
ODN2: 5'-CTG ACG G2G TGC-3'	3801.5	3800.4 (M-H) ⁻ , 3838.0 (M-2H+K) ⁻
ODN3: 5'-CTG ACG T1T TGC-3'	3750.5	3749.6 (M-H) ⁻
ODN4: 5'-CTG ACG T2T TGC-3'	3751.5	3750.6 (M-H) ⁻ , 3788.6 (M-2H+K) ⁻
ODN5: 5'-CTG ACG C1C TGC-3'	3720.5	3719.8 (M-H) ⁻
ODN6: 5'-CTG ACG C2C TGC-3'	3721.5	3720.4 (M-H) ⁻ , 3758.7 (M-2H+K) ⁻
ODN7: 5'-CTG ACG A1A TGC-3'	3768.5	3768.0 (M-H) ⁻
ODN8: 5'-CTG ACG A2A TGC-3'	3769.5	3768.6 (M-H) ⁻ , 3806.4 (M-2H+K) ⁻

Table S1: ESI-MS data of **ODN1-8**

8. RP-HPLC spectra of the crosslinking and aromatization reactions

Crosslinking reactions and aromatization reactions are carried out as described in the Experimental Section.

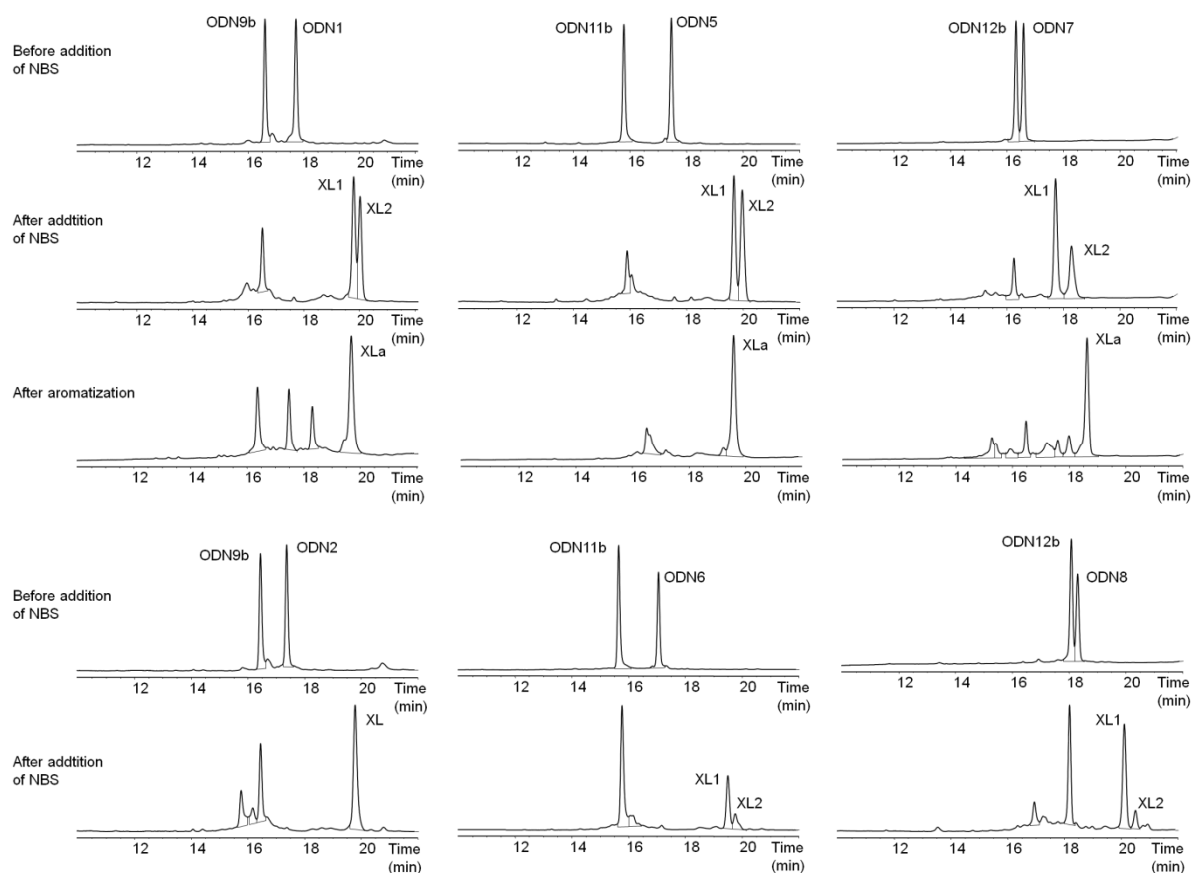
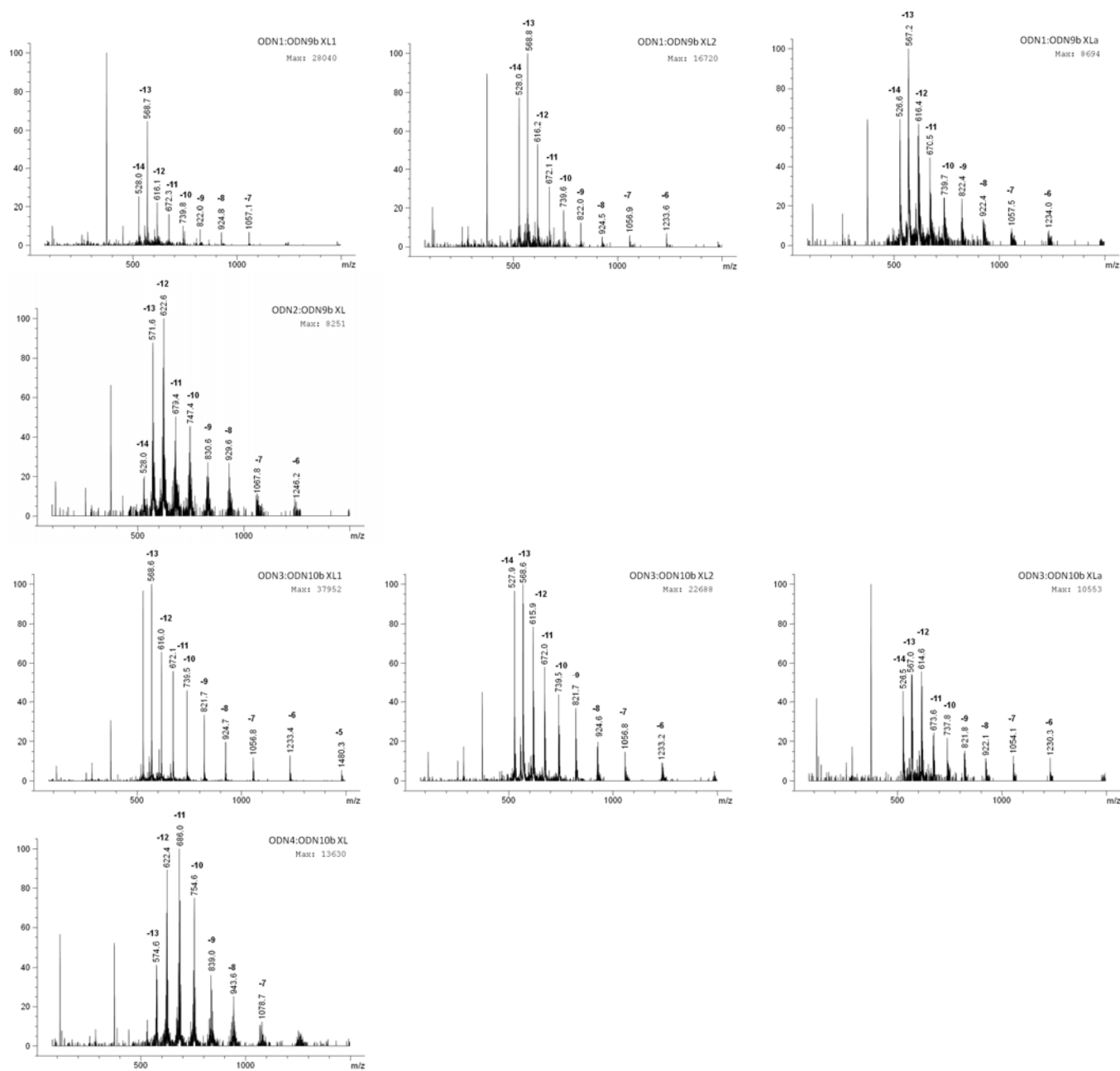


Figure S14: RP-HPLC of the crosslinking and aromatization reactions

Modified sequence	Complementary sequence
ODN1: 5'-CTG ACG G1G TGC-3'	ODN9a-d: 3'-GAC TGC CNC ACG-5'
ODN2: 5'-CTG ACG G2G TGC-3'	
ODN3: 5'-CTG ACG T1T TGC-3'	ODN10a-d: 3'-GAC TGC ANA ACG-5'
ODN4: 5'-CTG ACG T2T TGC-3'	
ODN5: 5'-CTG ACG C1C TGC-3'	ODN11a-d: 3'-GAC TGC GNG ACG-5'
ODN6: 5'-CTG ACG C2C TGC-3'	
ODN7: 5'-CTG ACG A1A TGC-3'	ODN12a-d: 3'-GAC TGC TNT ACG-5'
ODN8: 5'-CTG ACG A2A TGC-3'	

Table S1: Modified ODN sequences and their complements
(a:N= A, b:N= C, c: N=G, d: N=T)

9. *ESI-MS-data of crosslinked duplexes*



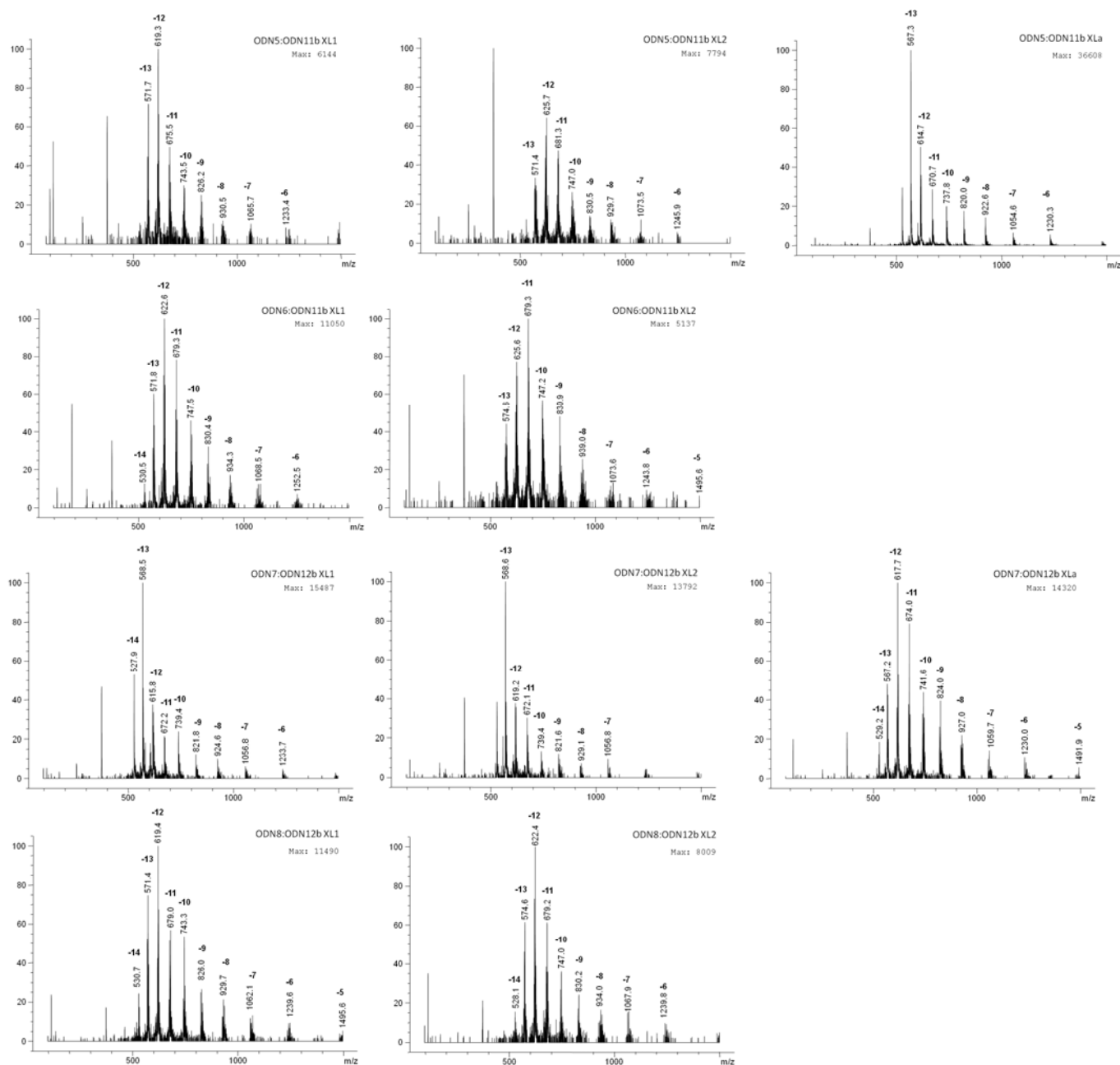


Figure S15: ESI-MS spectra of the crosslinked duplexes. Because of cation adduction (Na^+ and K^+), the molecular ions are dispersed among several different species of different m/z values. For each cluster the z -value is shown. For clarity reasons only the mass of the most abundant ion of the cluster has been indicated on the spectrum. From the different m/z values, the mass of the parent molecular adduct can be calculated by computational deconvolution using the Agilent LC/MSD ChemStation software. The obtained values are shown in Table S2.

Crosslinked duplex	Calculated mass (Da)	Observed Mass (Da)
ODN1:ODN9b XL1	7407.9	7406.3 (M-H) ⁻
ODN1:ODN9b XL2	7407.9	7406.4 (M-H) ⁻ , 7484.1 (M-3H+2K) ⁻
ODN1:ODN9b XLa	7389.8	7387.3 (M-H) ⁻ , 7408.6 (M-2H+Na) ⁻ , 7427.6 (M-2H+K) ⁻
ODN2:ODN9b XL	7408.8	7483.5 (M-3H+2K) ⁻ , 7523.4 (M-4H+3K) ⁻ , 7503.5 (M-4H+Na+2K) ⁻ , 7469.4 (M-3H+Na+K) ⁻ , 7445.4 (M-2H+K) ⁻ , 7406.0 (M-H) ⁻ , 7563.8 (M-5H+4K) ⁻
ODN3:ODN10b XL1	7405.9	7404.6 (M-H) ⁻ , 7442.2 (M-2H+K) ⁻ , 7480.0 (M-3H+2K) ⁻ , 7425.1 (M-2H+Na) ⁻
ODN3:ODN10b XL2	7405.9	7480.5 (M-3H+2K) ⁻ , 7404.5 (M-H) ⁻ , 7442.7 (M-2H+K) ⁻
ODN3:ODN10b XLa	7387.9	7386.5 (M-H) ⁻ , 7423.1 (M-2H+K) ⁻ , 7405.4 (M-2H+Na) ⁻ , 7465.3 (M-3H+2K) ⁻ , 7443.7 (M-3H+Na+K) ⁻
ODN4:ODN10b XL	7406.9	7557.2 (M-5H+4K) ⁻ , 7481.2 (M-3H+2K) ⁻ , 7443.1 (M-2H+K) ⁻ , 7596 (M-6H+5K) ⁻ , 7404.8 (M-H) ⁻
ODN5:ODN11b XL1	7407.9	7444.3 (M-2H+K) ⁻ , 7407.5 (M-H) ⁻ , 7483.5 (M-3H+2K) ⁻ , 7465.4 (M-3H+Na+K) ⁻
ODN5:ODN11b XL2	7407.9	7481.4 (M-3H+2K) ⁻ , 7520.4 (M-4H+3K) ⁻ , 7444.3 (M-2H+K) ⁻ , 7467.0 (M-3H+Na+K) ⁻ , 7504.8 (M-4H+Na+2K) ⁻ , 7425.9 (M-2H+Na) ⁻ , 7559.4 (M-5H+4K) ⁻ , 7544.0 (M-5H+Na+3K) ⁻
ODN5:ODN11b XLa	7389.8	7388.2 (M-H) ⁻ , 7425.7 (M-2H+K) ⁻ , 7407.8 (M-2H+Na) ⁻
ODN6:ODN11b XL1	7407.9	7483.3 (M-3H+2K) ⁻ , 7445.3 (M-2H+K) ⁻ , 7521.0 (M-4H+3K) ⁻ , 7467.3 (M-3H+Na+K) ⁻ , 7504.6 (M-4H+Na+2K) ⁻ , 7428.6 (M-2H+Na) ⁻
ODN6:ODN11b XL2	7407.9	7482.3 (M-3H+2K) ⁻ , 7519.2 (M-4H+3K) ⁻ , 7468.6 (M-3H+Na+K) ⁻ , 7448.6 (M-2H+K) ⁻
ODN7:ODN12b XL1	7405,9	7404.2 (M-H) ⁻ , 7443.2 (M-2H+K) ⁻ , 7425.8 (M-2H+Na) ⁻
ODN7:ODN12b XL2	7405,9	7404.4 (M-H) ⁻ , 7441.8 (M-2H+K) ⁻ , 7425.6 (M-2H+Na) ⁻ , 7484.6 (M-3H+2K) ⁻ , 7461,4 (M-3H+Na+K) ⁻
ODN7:ODN12b XLa	7387,9	7424.9 (M-2H+K) ⁻ , 7387.0 (M-H) ⁻ , 7445.0 (M-3H+Na+K) ⁻ , 7461.8 (M-3+2K) ⁻ , 7409.6 (M-2+Na) ⁻
ODN8:ODN12b XL1	7406.9	7443.2 (M-2H+K) ⁻ , 7480.7 (M-3H+2K) ⁻ , 7465.9 (M-3H+Na+K) ⁻ , 7405.9 (M-H) ⁻ , 7518.6 (M-4H+3K) ⁻ , 7501.3 (M-3H+Na+K) ⁻ , 7428.1 (M-2H+Na) ⁻
ODN8:ODN12b XL2	7406.9	7481.2 (M-3H+2K) ⁻ , 7442.6 (M-2H+K) ⁻ , 7519.7 (M-4H+3K) ⁻ , 7465.7 (M-3H+Na+K) ⁻ , 7503.8 (M-4H+Na+2K) ⁻ , 7405.8 (M-H) ⁻ , 7428.1 (M-2H+Na) ⁻

Table S2: ESI-MS analysis of crosslinked duplexes

10. Melting temperatures of the crosslinked duplexes

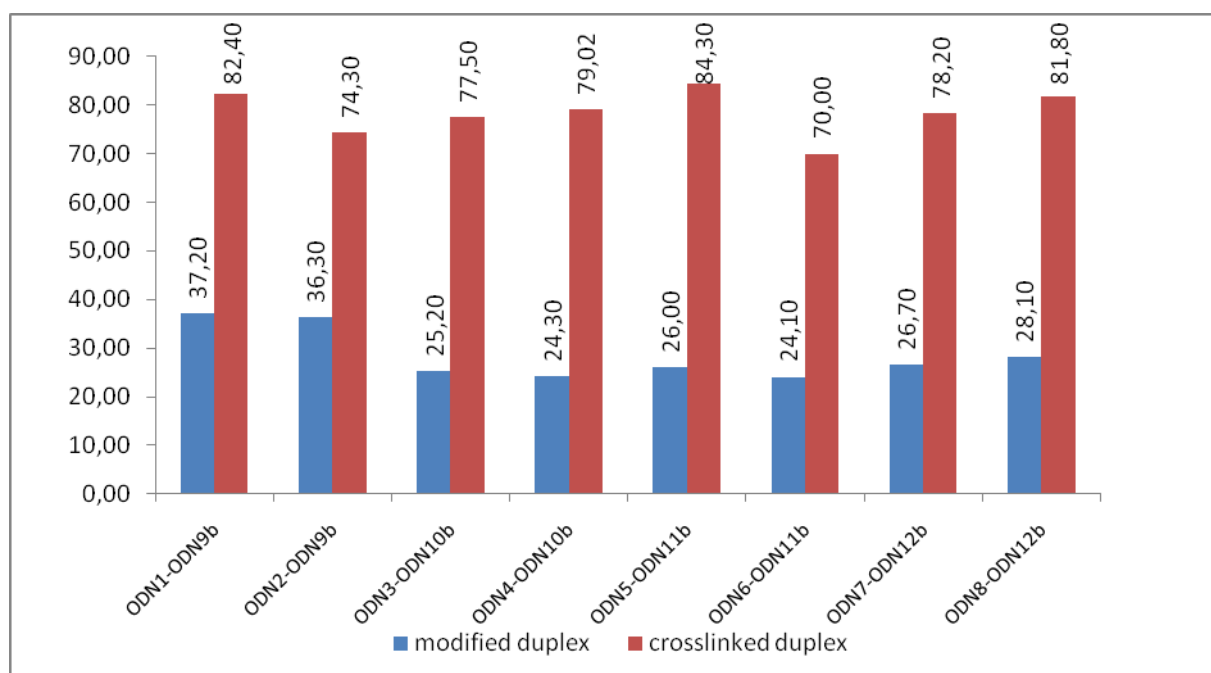


Figure S16: Comparison of duplex stability for modified versus crosslinked duplexes as observed through T_m determination