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Supporting Information

Synthesis of fully protected trinucleotide building blocks on a disulphide-linked soluble support

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Content

S1: Abbrevations

ACN	acetonitrile
Bz	benzoyl
CV	column volume
DCE	dichloroethane
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
DMAc	dimethylacetamide
DMF	dimethylformamide
DMT	dimethoxytrityl
DQF-Cosy	double quantum filtered correlation spectroscopy
EE	ethyl acetate
EtOH	ethanol
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	high performance liquid chromatography
HSQC	heteronuclear single quantum coherence
Ibu	isobutyryl
MeOH	methanol
MS	mass spectrometry
MTM	methylthiomethyl
NMR	nuclear magnetic resonance
PLC	preparative layer chromatography
TCEP	tris(2-carboxyethyl)phosphine
TEA	triethylamine
TEAAc	triethylammonium acetate
THF	tetrahydrofuran
TLC	thin layer chromatography

S2: General information

Mass spectra were recorded on a Bruker microflex MALDI-TOF MS. HPLC spectra were performed on an Äkta Purifier (Amersham Biosciences) with Nucleodur 125/4, CV = 1.571 ml. DNA Oligomers were synthesized using a Gene Assembler Special DNA synthesizer from Amersham Bioscience. NMR spectra were recorded on Bruker Avance 300 MHz (**1, 3**$_{\mathbf{a\text{-}b}}$) or 600 MHz (**13**$_{\mathbf{a\text{-}b}}$) and analyzed via TopSpin 4.0.7. Pyridine was dried overnight over KOH, heated to reflux, distilled off and stored over molecular sieve and argon. TEA was freshly distilled and stored over molecular sieve. DCM used during work-up procedure after coupling reaction was stored over $NaHCO_3$. ACN used for coupling was extra dry (10 ppm H_2O). Solid support (Thymidine 3'-lcaa CPG 500 Å) and Phosphoramidites (5'-*O*-DMT-3'-*O*-methyl phosphoramidites) were purchased from ChemGenes. All other reagents, chemicals and solvents were obtained commercially and used without further purification. All products were visualized via TLC chromatography on aluminium silica gel 60 F254 plates and UV shadowing. Synthesis of 3'-*O*-methylthiomethyl modified nucleosides (**3**$_{\mathbf{a\text{-}b}}$) has been described previously.[1,2] Synthesis of Oligomers (5'-CTACTT-3', 5'-GGTCTT-3') was carried out according to Suchsland *et al*.[2] Synthesis of Tetrakis-*O*-{4-[4-(acetylthiomethyl)-1*H*-1,2,3-triazol-1-ylmethyl]phenyl}-pentaerythritol (**1**) was conducted according to Jabgunde *et al*. by using less amount of propargyl thioacetate and achieving equally high yields.[3] Phosphitylation was done according to McBride and Caruthers.[4]

S3: Experimental procedures

S3.1: Tetrakis-*O*-{4-[4-(acetylthiomethyl)-1*H*-1,2,3-triazol-1-ylmethyl]phenyl}-pentaerythritol (**1**)

0.29 g Tetrakis-{[4-(azidomethyl)phenoxy-]methyl}methane (0.44 mmol), 8.6 mg sodium ascorbate (44 µmol) and 33 mg CuI (0.18 mmol) were dissolved in 1 ml DMAc under dry conditions. Then 250 mg propargylthioacetate (2.2 mmol) were added. After four Pump-Freeze-Thaw cycles, the solution was stirred at 50 °C for 16 h. 2.5 ml H_2O were added and the crude product was extracted three times with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and dried over Na_2SO_4. After evaporation to dryness the crude product was purified via column chromatography (DCM:MeOH / 97:3). Upon lyophilisation the product **1** was obtained with 70% yield (0.36 g, 0.32 mmol).

^{1}H NMR (300 MHz, DMSO): δ [ppm] = 7.92 (s, 4H, H5 of triazole), 7.22 (d, *J* = 8.71 Hz, 8H, H2/H6 of Ph), 6.94 (d, *J* = 8.71 Hz, 8H, H3/H5 of Ph), 5.43 (s, 8H, N-C*H*$_2$-Ph), 4.23 (s, 8H, AcS-C*H*$_2$-), 4.11 (s, 8H, C*H*$_2$-pentaerythritol), 2.33 (s, 12H, -SAc).

^{13}C NMR (300 MHz, DMSO): δ [ppm] = 194.47, 158.32, 143.29, 129.53, 128.34, 122.93, 114.80, 66.00, 52.22, 44.25, 30.20, 23.21.

MS: calculated: 1117.33 m/z, found: 1117.19 m/z.

S3.2: Coupling of start nucleoside to soluble support (**6$_{a-b}$**)

0.22 g Tetrakis-*O*-{4-[4-(acetylthiomethyl)-1*H*-1,2,3-triazol-1-ylmethyl]phenyl}pentaerythritol (**1**, 0.19 mmol) was dissolved in 1.4 ml degassed ACN and 0.7 ml MeOH. Then 1 ml degassed 5 M butylamine solution (in MeOH) was added. The reaction mixture was stirred over night at room temperature. 2.8 ml of a 1:1 solution DCM:ACN were added. After 10 min 50 mg Amberlyst 15 were added. The ion exchange resin was filtered off and the solvents were removed in vacuo to get the deprotected soluble support **2**.

0.58 g (0.80 mmol) 5'-*O*-DMT-3'-*O*-MTM-*N*-Bz-dA (**3$_a$**) were dissolved in 3.2 ml DCM under dry conditions. Then 0.35 ml TEA (3 eq) were added. The solution was stirred in an ice bath. 70 µl (1 eq) freshly distilled SO_2Cl_2 in 0.65 ml dry DCM were added dropwise. The reaction mixture was stirred for another 10 min in an ice bath. 0.23 g (1.5 eq) potassium thiotosylate (in 0.45 ml dry DMF) were added at room temperature and the mixture was stirred for 10 min. The deprotected soluble support with the free thiol (**2**) was completely dissolved in 1.4 ml DCM:ACN / 1:1 and added to the reaction mixture. The solution was stirred for 3.5 h at room temperature. The solution was divided over three 50 ml plastic tubes and each was refilled with MeOH. The tubes were stored in the freezer at -20 °C overnight. Then the tubes were centrifuged at 6000 rpm for 30 min, the solid was isolated and lyophilized. MS: calculated: 3756.37 m/z, 940.09 ($4H^+$/4), found: 940.9 m/z.

Accordingly, 5'-*O*-DMT-3'-*O*-MTM-T (**3$_b$**) was coupled to soluble support using 0.19 mmol soluble support and 1.54 mmol 5'-*O*-DMT-3'-*O*-MTM-T.

Yield over two steps: **6$_a$**= 54% (0.10 mmol, 0.38 g), **6$_b$**= 49% (0.09 mmol, 0.32 g)

S3.3: General procedure for detritylation, coupling, oxidation and cleavage:

Detritylation (7_{a-b}, 10_{a-b})

7.5 ml Dichloroacetic acid solution (4% in DCE) were added to 0.24 g 5'-*O*-DMT-*N*-Bz-dA bound on soluble support (63 µmol, **6_a**) and stirred for 5 min, whereby the solution turned red. 7.5 ml pyridine were added, thereby the solution turned colourless again. Pyridine was evaporated, and the residue was divided over two 50 ml plastic tubes, each of which was filled up with about 40 ml MeOH. After 15 min at -20 °C, the tubes were centrifuged at 6000 rpm for 30 min, the solid was filtered and lyophilized to yield in 5'-OH-3'-*O*-MTM-*N*-Bz-dA bound on soluble support **7_a** to be used for the following phosphoramidite coupling.

Detritylation was carried out in the same manner to get the other 5'-OH-derivatives:

34 µmol **9_a** (5'-*O*-DMT-TdA^{Bz} on soluble support) and 4 ml acid solution to give **10_a**
89 µmol **6_b** (5'-*O*-DMT-T on soluble support) and 11 ml acid solution to give **7_b**
46 µmol **9_b** (5'-*O*-DMT-dG^{Ibu}T on soluble support) and 6 ml acid solution to give **10_b**

Coupling (8_{a-b}, 11_{a-b})

5 ml of a 0.1 M solution of 5'-*O*-DMT protected 3'-*O*-methylphosphoramidite of T in ACN (6 eq) was added under dry conditions to the detritylated nucleoside bound on the soluble support (**7_a**, 63 µmol). Then 2.1 ml 5-(benzylmercapto)-1*H*-tetrazole (0.3 M in ACN) were added, and the reaction mixture was stirred for 3 h at room temperature under argon to form the protected dimer **8_a** which was used immediately for oxidation.

Coupling was carried out in the same manner with the following compounds:

10_a (TdA^{Bz} on soluble support, 34 µmol), 2.2 ml of a 0.1 M solution of the 5'-*O*-DMT-dC^{Bz}-3'-*O*-methylphosphoramidite in ACN and 1.2 ml BMT-solution to give **11_a**
7_b (T on soluble support, 89 µmol), 5.4 ml of a 0.1 M solution of the 5'-*O*-DMT-dG^{Ibu}-3'-*O*-methylphosphoramidite in ACN and 3 ml BMT-solution to give **8_b**
10_b (dG^{Ibu}T on soluble support, 46 µmol), 2.8 ml of a 0.1 M solution of the 5'-*O*-DMT-dG^{Ibu}-3'-*O*-methylphosphoramidite in ACN and 1.6 ml BMT-solution to give **11_b**

Oxidation (9_{a-b}, 12_{a-b})

0.2 M iodine solution (Trimethylpyridine/ACN/H_2O (1:11:5)) were added until a yellow colour remained (about 70 ml). A few drops of a 1 M trimethyl phosphite solution in DMF were added until the solution was colourless again. The solution was divided over four 50 ml plastic tubes, refilled with MeOH and stored in the freezer at -20 °C overnight. The solid was filtered and the product **9_a** was lyophilized (34 µmol, 0.17 g, 53%).

Oxidation was carried out in the same manner for the other oxidation reactions:

11_a (5'-*O*-DMT-dC^{Bz}TdA^{Bz} on soluble support) and about 35 ml iodine solution to give **12_a** (70%, 24 µmol, 0.16 g)
8_b (5'-*O*-DMT-dG^{Ibu}T on soluble support) and about 95 ml iodine solution to give **9_b** (55%, 46 µmol, 0.23 g)
11_b (5'-*O*-DMT-dG^{Ibu}-dG^{Ibu}T on soluble support) and about 40 ml iodine solution to give **12_b** (70%, 33 µmol, 0.22 g)

All yields for oxidized compounds were calculated in relation to DMT-protected starting materials 6_{a-b} and 9_{a-b}.

Cleavage from support (13_{a-b})

0.30 g TCEP were dissolved in a mixture of 2 ml aqueous HEPES buffer (pH 7.5) and 4 ml ACN. This TCEP solution was added to trinucleotide linked soluble support (0.16 g, 27 µmol, **12_a**,) and stirred overnight at room temperature under argon. The reaction mixture was evaporated and lyophilized. The soluble support was washed extensively with ACN, acetone, EtOH, THF and EE, until no colour appeared when the soluble support was treated with acid-solution (dichloroacetic acid, 4% in DCE). The combined organic layers were evaporated and lyophilized. Purification was carried out by PLC (DCM:MeOH / 95:5 + 1% TEA). The product was eluted from silica gel first with DCM + 1% TEA and afterwards with ACN/acetone + 1% TEA. The combined organic layers were evaporated and lyophilized to give **13_a** (44%, 48 µmol, 66 mg).

Cleavage from support was achieved in the same manner for trinucleotide **12_b** (31 µmol) with 0.39 g TCEP to give **13_b** (39%, 48 µmol, 0.07 g).

13_a (5'-*O*-DMT-dC^{Bz}TdA^{Bz}):

^{1}H NMR (600 MHz, DMSO): δ [ppm] = 11.21 (s, 1H, NH), 11.17 (s, 1H, NH), 10.44 (s, 1H, NH), 7.81 (m, 4H, Bz-arom), 7.68-7.65 (m, 2H, A-H6, H8), 7.38 (m, 1H, T-H6), 7.21 (m, 1H, C-H6), 7.20-7.19 (m, 13 H, DMT-arom), 6.88-6.87 (m, 6 H, Bz-arom), 6.48 (m, 1H, H1'), 6.11 (m, 1H, H1'), 5.79 (m, 1H, H1'), 5.39 (m, 1H, C H5), 5.32 (m, 1H, H4'), 4.82 (m, 1H, H4'), 4.68 (m, 1H, H4'), 4.27 (m, 1H, H3'), 3.84 (m, 1H, H3'), 3.76-3.71 (m, 12H, CH_3 (DMT/$POCH_3$)), 3.69 (m, 1H, H3'), 3.59 (m, 2H, H5'/H5''), 3.46 (m, 2H, H5'/H5''), 3.25 (m, 2H, H5'/H5''), 2.85 (m, 2H, H2'/H2''), 2.58 (m, 2H, H2'/H2''), 2.35 (m, 2H, H2'/H2''), 1.34 (s, 3H, T-CH_3).

^{13}C NMR (600 MHz, DMSO): δ [ppm] = 174.25, 166.98, 165.79, 164.76, 158.35, 158.33, 157.71, 156.66, 147.02, 146.98, 144.05, 137.97, 136.23, 131.60, 130.23-128.44, 129.79, 127.18, 127.75-114.88, 118.45, 109.69, 89.54, 89.40, 84.48, 83.74, 78.25, 74.32, 72.90, 69.79, 69.30, 67.80, 65.95, 65.17, 62.62, 55.05, 43.4, 35.11, 31.93, 26.53, 29.8. For more information see table S1 in section NMR spectra (**13a**).

MS: calculated: 1382.41 m/z, found: 1405.63 (**13a** +Na) m/z.

13_b (5'-*O*-DMTdG^{Ibu}dG^{Ibu}T):

^{1}H NMR (600 MHz, DMSO): δ [ppm] = 11.31 (s, 2H, NH), 10.46 (s, 1H, NH), 9.07 (s, 2H, NH Ibu), 7.91 (s, 2H, G-C8), 7.80 (m, 1H, T-H6), 7.16-6.86 (m, 13 H, DMT-arom), 6.65 (m, 2H, H1'), 5.97 (m, 1H, H1'), 5.31 (m, 1H, H3'-OH), 4.46 (m, 1H, H4'), 4.31 (m, 2H, H4'), 4.01 (m, 1H, H3'), 3.87 (m, 2H, H3'), 3.76-3.71 (m, 12H, CH_3 (DMT/$POCH_3$)), 3.51 (m, 2H, H5'/H5''), 3.36 (m, 2H, 2H''), 3.01 (m, 4H, 2H' + 2x Ibu-H), 2.87 (m, 4H, 2x H5'/H5''), 1.97 (m, 2H, H2'/H2''), 1.23 (s, 3H, T- CH_3).

^{13}C NMR (600 MHz, DMSO): δ [ppm] = 180.90, 163.70, 158.41, 158.39, 158.35, 157.71, 143.08, 136.23, 130.23-128.33, 122.95, 122.25, 120.88, 119.24, 113.31, 87.16, 86.37, 84.45, 83.60, 81.42, 77.19, 74.66, 69.78, 65.75, 55.05, 45.69, 39.47, 32.14, 29.96, 18.04. For more information see table S2 in section NMR spectra (**13b**).

MS: calculated: 1370.44 m/z, found: 1370.80 m/z.

S3.4: Synthesis of fully protected phosphoramidite trinucleotides (**14a-b**)

Trinucleotide (**13_a**, 27 µmol, 37 mg) was dried under high vacuum and dissolved in 250 µl DCM under dry conditions. 20 µl TEA were added. 10 µl *N*,*N*-Diisopropylmethyl-phosphonamidic-chloride were

added dropwise. The reaction mixture was stirred for 3.5 h at room temperature under argon. Synthesis was controlled via TLC (EE:DCM:TEA / 45:45:10). Samples were visualized with ethanolic ninhydrin solution. Reaction was stopped by addition of 40 µl MeOH. The organic reaction mixture was washed with saturated $NaHCO_3$-solution containing 2% TEA. The separated organic layer was evaporated at 35 °C, and the remaining oil (32 mg, 21 µmol, **14a**) was lyophilized and stored under argon at -20 °C.

Phosphitylation was carried out in the same manner with trinucleotide **13_b** to give **14b** (66 mg, 48 µmol).

S3.5: Coupling of trinucleotides on DNA synthesizer – 6mer synthesis

The trinucleotide phosphoramidite (5'-CTA-3', **14_a**) was dissolved in DCM (0.1 M) under dry conditions and used for coupling to the sequence 5'-CTT-3' on CPG, assembled directly prior to trinucleotide coupling from standard monomer 3-*O*-methyl-phosphoramidites. Synthesis scale was 1 µmol. Coupling time was 2 x 300 s (double-coupling) for the trinucleotide building block. Synthesis was performed via DMT-on method.

The trinucleotide phosphoramidite (5'-GGT-3', **14_b**) was dissolved in DCM:ACN/3:1 (0.1 M) under dry conditions. Coupling to the sequence 5'-CTT-3' on CPG was carried out as described above for **14_a**.

S3.6: Deprotection of synthesized 6mers

The solid support of each individual synthesis was divided over two screw cap plastic tubes. 1 ml 32% aqueous ammonia solution was added to each vial, and the vials were shaken at room temperature overnight. The oligonucleotide solution was transferred to a new screw cap plastic tube, the support was washed with another 1 ml of ammonia solution, which was then added to the solution in the tube. The tube was sealed and treated at 55°C overnight. The supernatant was removed and the solid support was washed four times with each time 100 µl EtOH:H_2O/1:1. The combined supernatant and washing solutions were evaporated to dryness and the remaining solid was dissolved in buffer A (5% ACN, 0.1 M TEAAc) and analyzed by HPLC.

S3.7: HPLC analysis of 6mers

HPLC conditions: Nucleodur 125/4, CV = 1.571 ml; 1 ml/min; room temperature; buffer A: 5% ACN, 0.1 M TEAAc; buffer B: 30% ACN, 0.1 M TEAAc; Gradient: starting with 0% buffer B for 4 CV, to 40% buffer B over 3 CV, to 60% buffer B over 7 CV, to 100% buffer B over 2 CV, then 100% buffer B for another 2 CV and to 0% buffer B over 3 CV. Detection was by UV-light absorption at 260 nm. Collected fractions corresponding to Peak 2 (see Fig. 4 HPLC Purification of CTACTT (A) and GGTCTT (B) (DMT-on) in main text) were analyzed by MALDI mass spectroscopy.

MS: full length 5'-CTACTT-3' (Peak 2, Fig. 4A) calculated: 1742.18 m/z, found: 1742.15 m/z.
MS: full length 5'-GGTCTT-3' (Peak 2, Fig. 4B) calculated: 1798.21 m/z, found: 1799.21 m/z ($+H^+$).

S3.8: Coupling yields for GGT and CTA building blocks

Coupling yields for trinucleotide phosphoramidites were determined from peak areas of the HPL chromatogram (Figure 4, main text), corrected by the extinction coefficients.

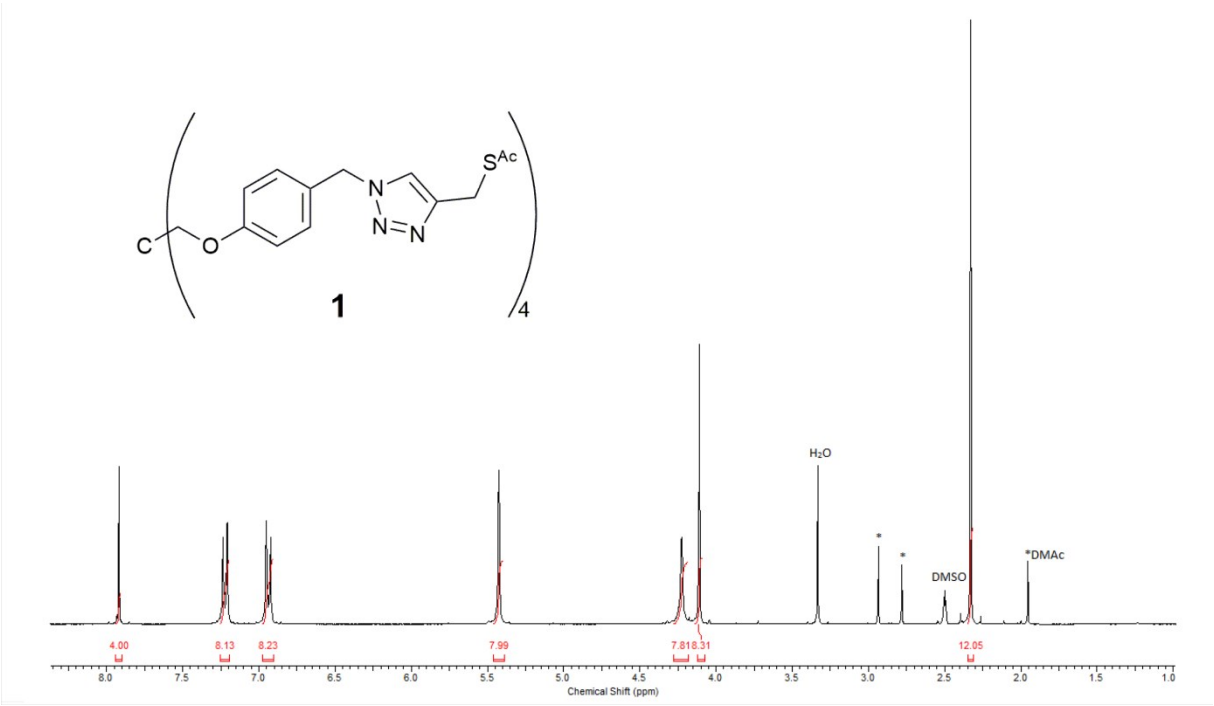
Coupling yield for CTA: 17%.

Coupling yield for GGT: 72%.

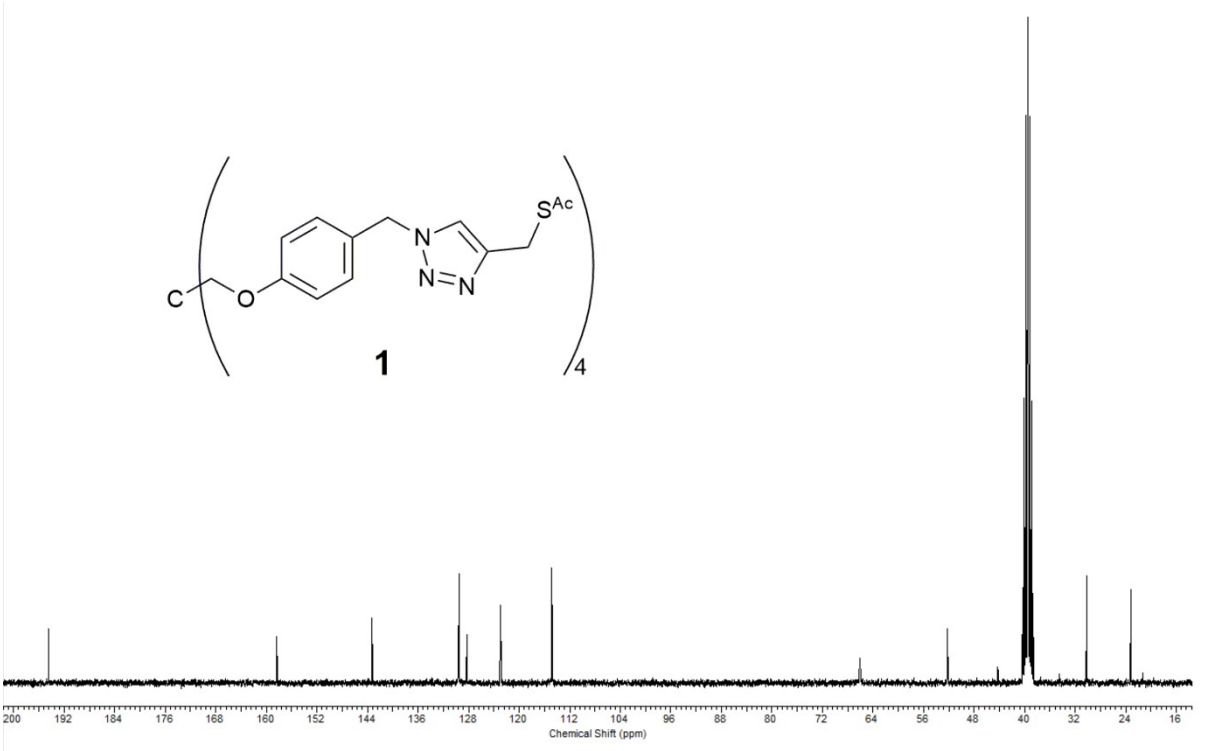
S4: NMR Spectra

Tetrakis-*O*-{4-[4-(acetylthiomethyl)-1*H*-1,2,3-triazol-1-ylmethyl]-phenyl}-pentaerythritol (**1**), ^{1}H,^{13}C spectra

^{1}H spectrum (1):

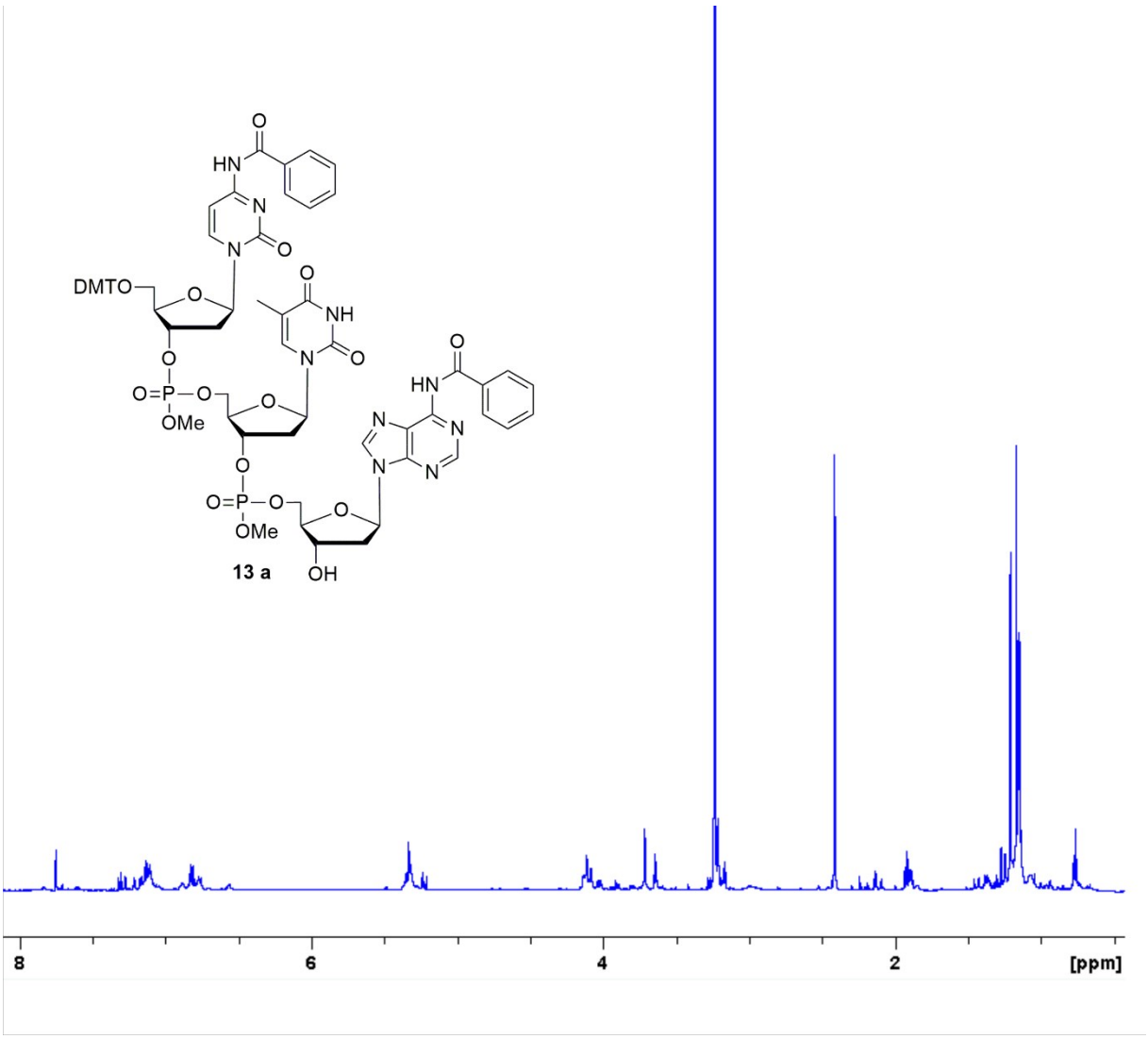


^{13}C spectrum (1):

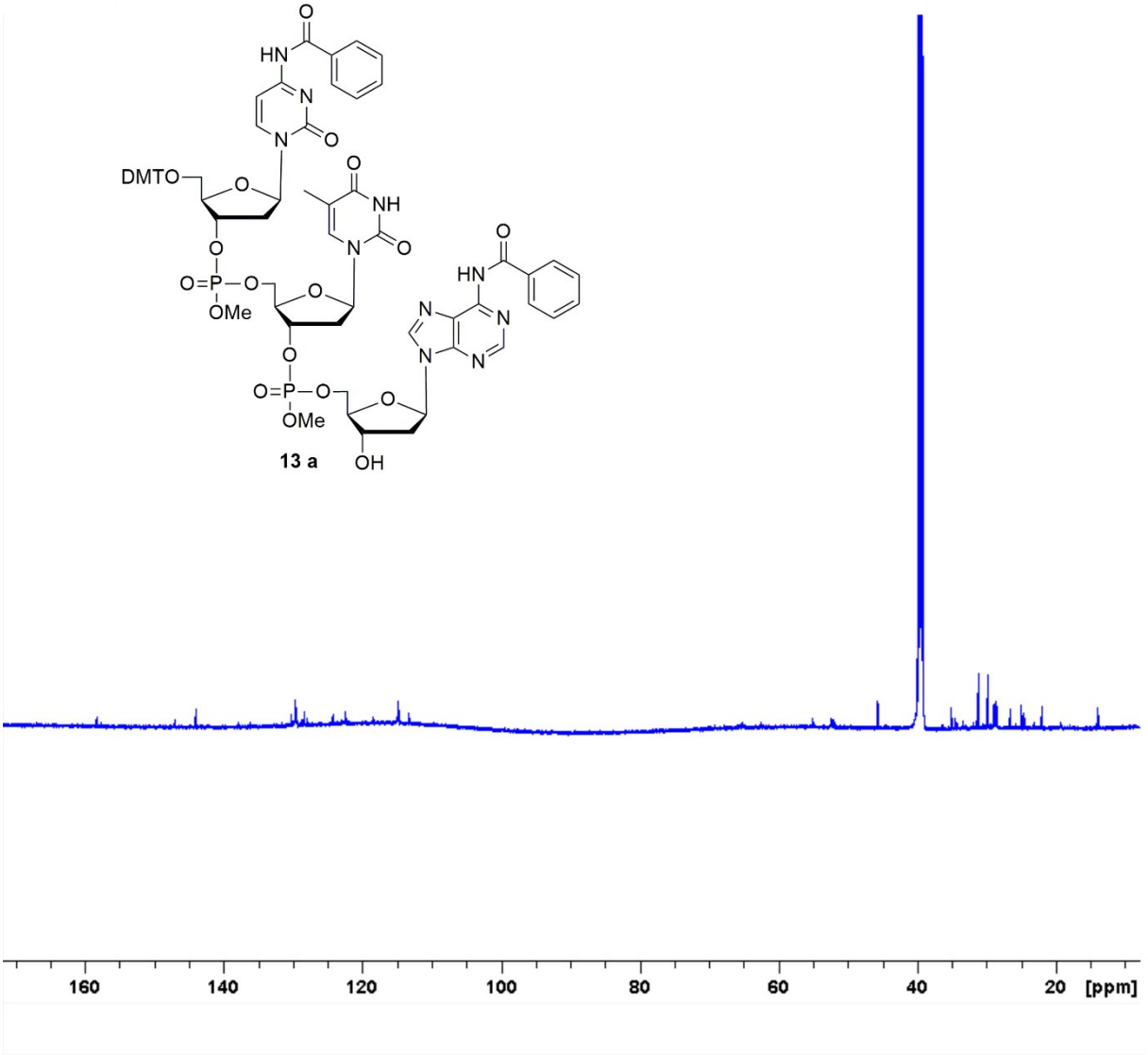


Trinucleotide $C^{Bz}TA^{Bz}$ (**13_a**), ^{1}H, ^{13}C, DEPT, DQF-COSY, HSQC spectra

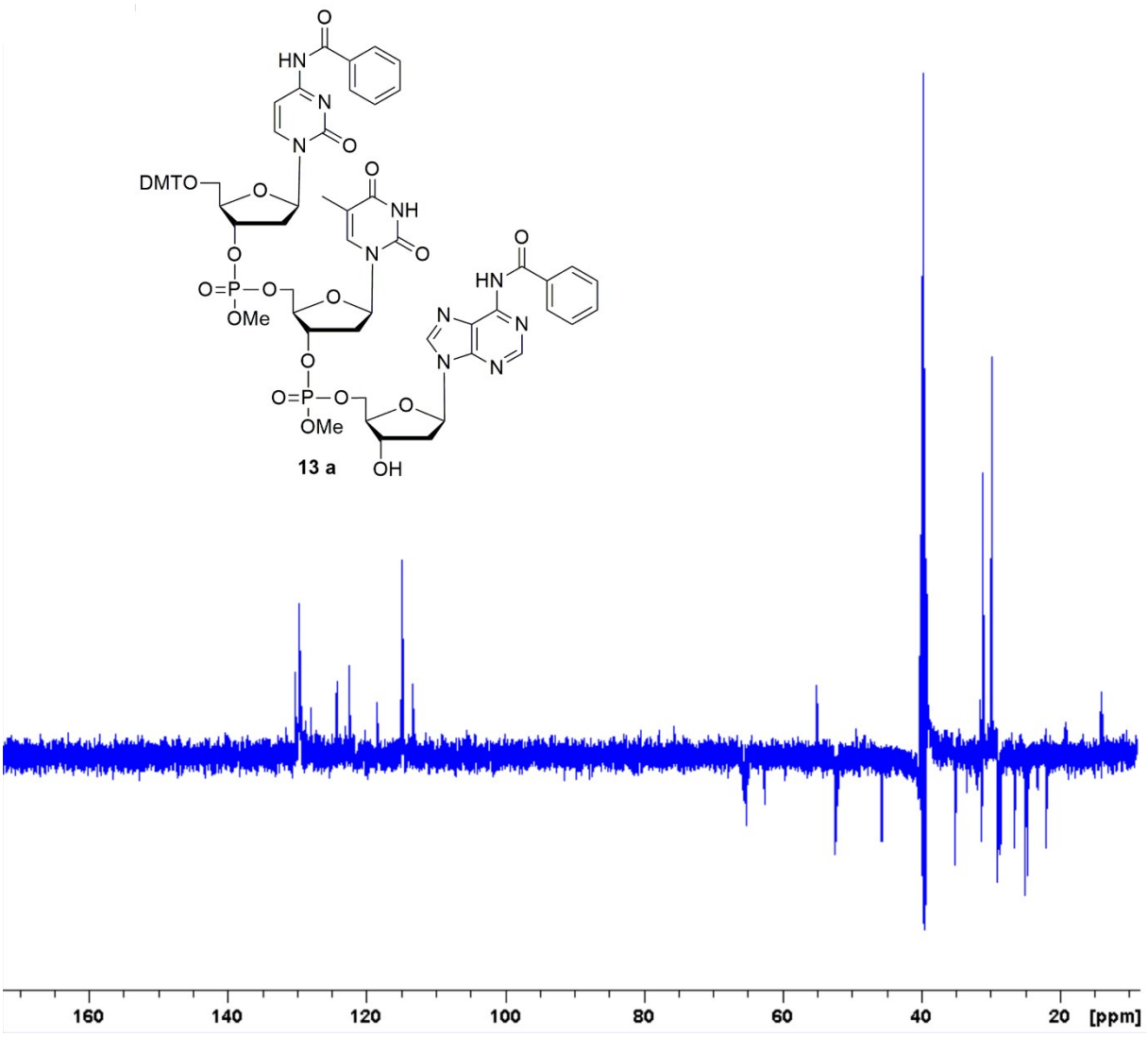
^{1}H spectrum (13a):



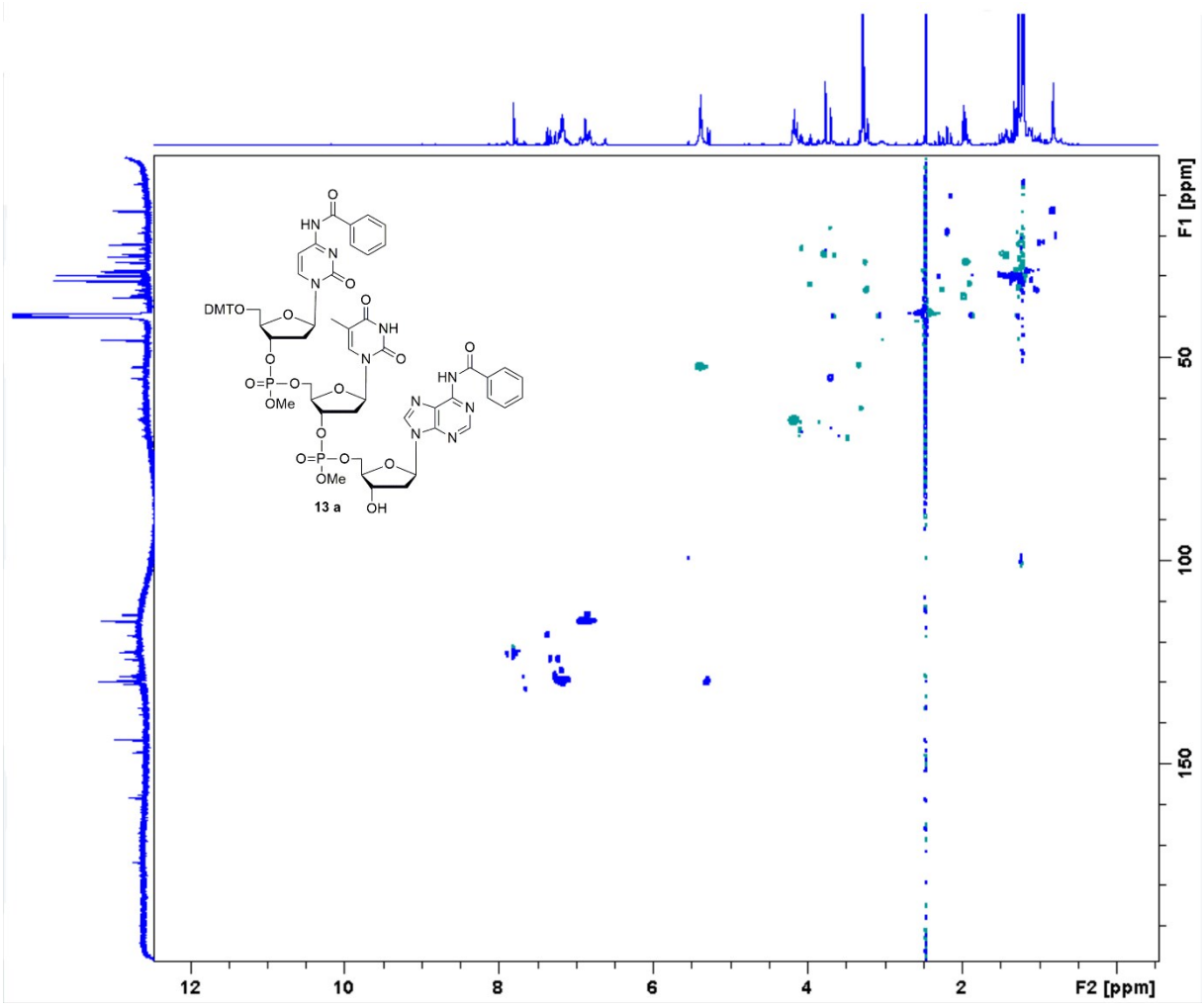
^{13}C spectrum (13a):



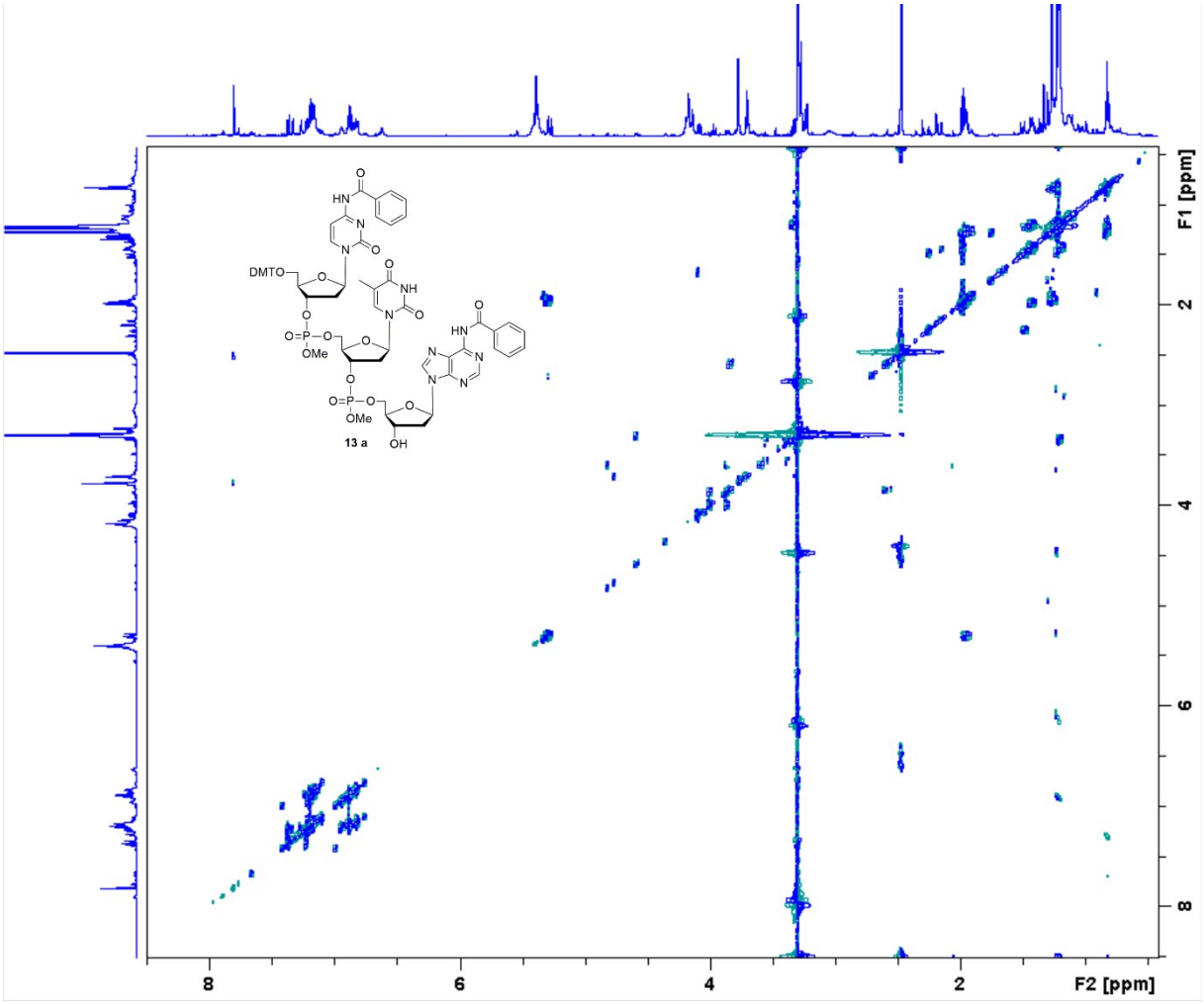
DEPT spectrum (13a):



HSQC spectrum (13a):



DQF-COSY spectrum (13a):



Details of ^{1}H and Cosy spectra (13a):

Object	Integral [abs]	Integral [rel]	Peaks	v(F1) [ppm]	
Integral 1	146872918016.00	1.0874	0	11.2141	NH
Integral 2	128184184832.00	0.9491	0	11.1748	NH
Integral 3	122552209408.00	0.9074	0	10.4424	NH
Integral 4	629390876672.00	4.6600	0	7.8099	BZ arom
Integral 5	187295752192.00	1.3867	0	7.6808	
Integral 6	178513494016.00	1.3217	0	7.6532	Ade H6/H8
Integral 7	189182054400.00	1.4007	0	7.3814	Thy H6
Integral 8	134741446656.00	0.9976	0	7.2162	Cyt H6
Integral 9	692917497856.00	5.1303	0	7.2041	
Integral 10	713390256128.00	5.2819	0	7.1907	DMT arom
Integral 11	421545852928.00	3.1211	0	6.8867	
Integral 12	459441123328.00	3.4017	0	6.8740	Bz arom
Integral 13	106472710144.00	0.7883	0	6.4854	H1'
Integral 14	135063437312.00	1.0000	0	6.1115	H1'
Integral 15	146421608448.00	1.0841	0	5.7955	H1'
Integral 16	199132606464.00	1.4744	0	5.3942	Cyt H5
Integral 17	224739733504.00	1.6640	0	5.3280	H4'
Integral 18	131820859392.00	0.9760	0	4.8246	H4'
Integral 19	122597601280.00	0.9077	0	4.6878	H4'
Integral 20	173989359616.00	1.2882	0	4.2754	H3'
Integral 21	143954042880.00	1.0658	0	3.8494	H3'
Integral 22	959010983936.00	7.1004	0	3.7699	methyl (DMT/POCH3)
Integral 23	798403350528.00	5.9113	0	3.7116	
Integral 24	174303420416.00	1.2905	0	3.6916	H3'
Integral 25	289091543040.00	2.1404	0	3.5970	H5'/"
Integral 26	291325337600.00	2.1570	0	3.4684	H5'/"
Integral 27	328343789568.00	2.4310	0	3.2524	H5'/"
Integral 28	313418035200.00	2.3205	0	2.8574	H2'/"
Integral 29	271217479680.00	2.0081	0	2.5878	H2'/"
Integral 30	268220178432.00	1.9859	0	2.3557	H2'/"
Integral 31	440311259136.00	3.2600	0	1.3349	Thy methyl

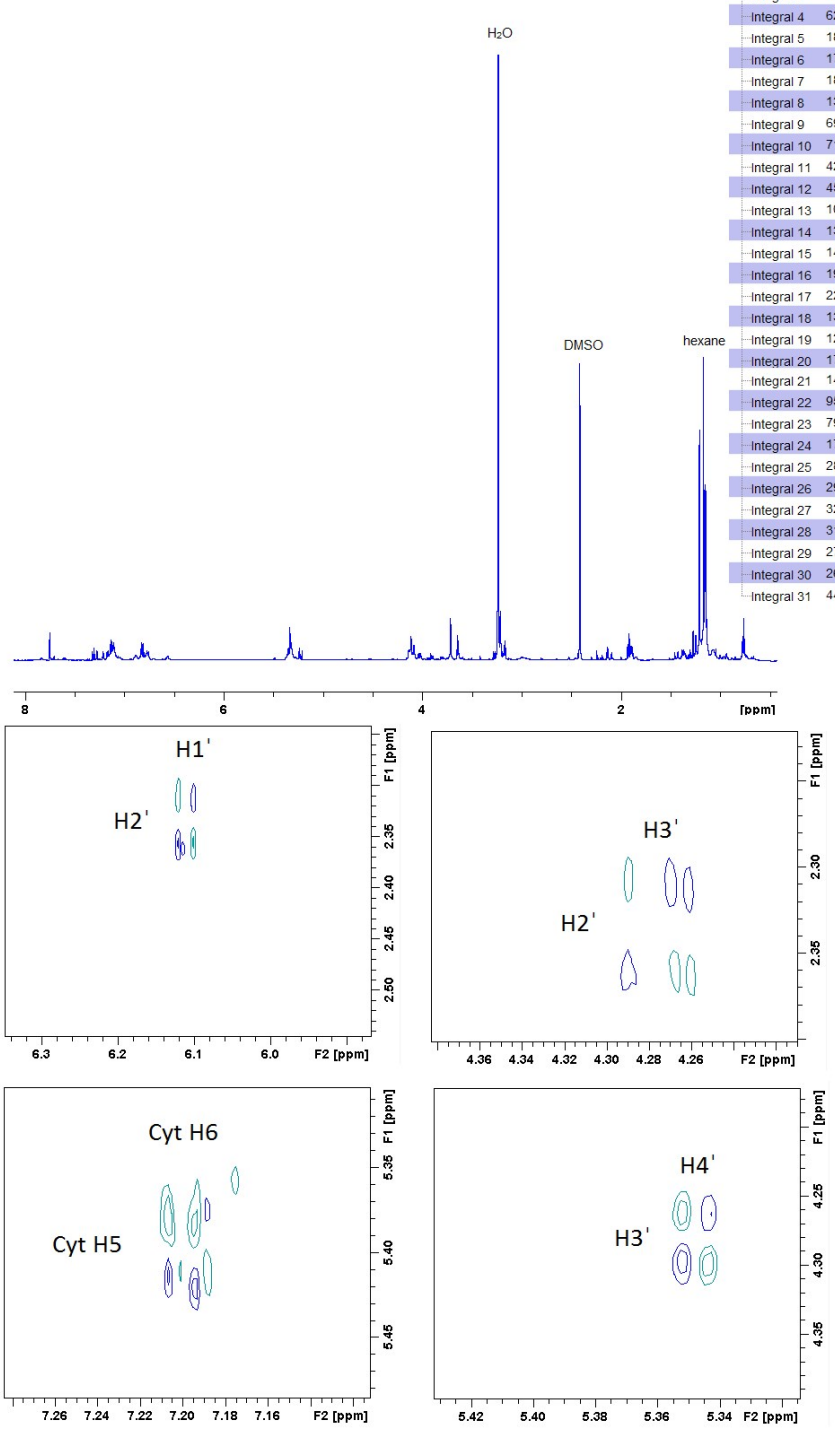
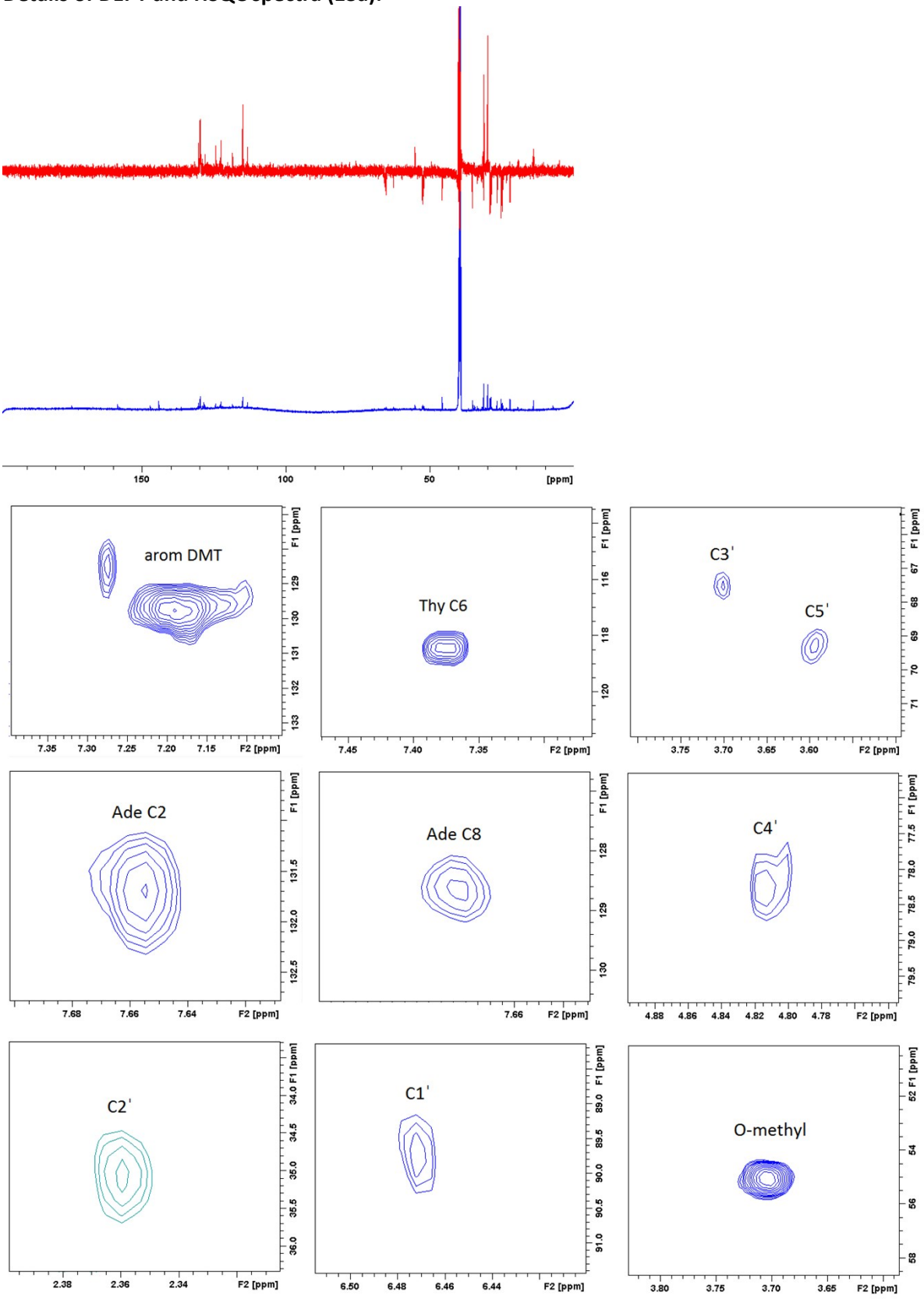


Table S1: Details of ^{13}C and DEPT spectra of **13_a**, DEPT: + ≙ positive signal, - ≙ negative signal, / ≙ no signal.

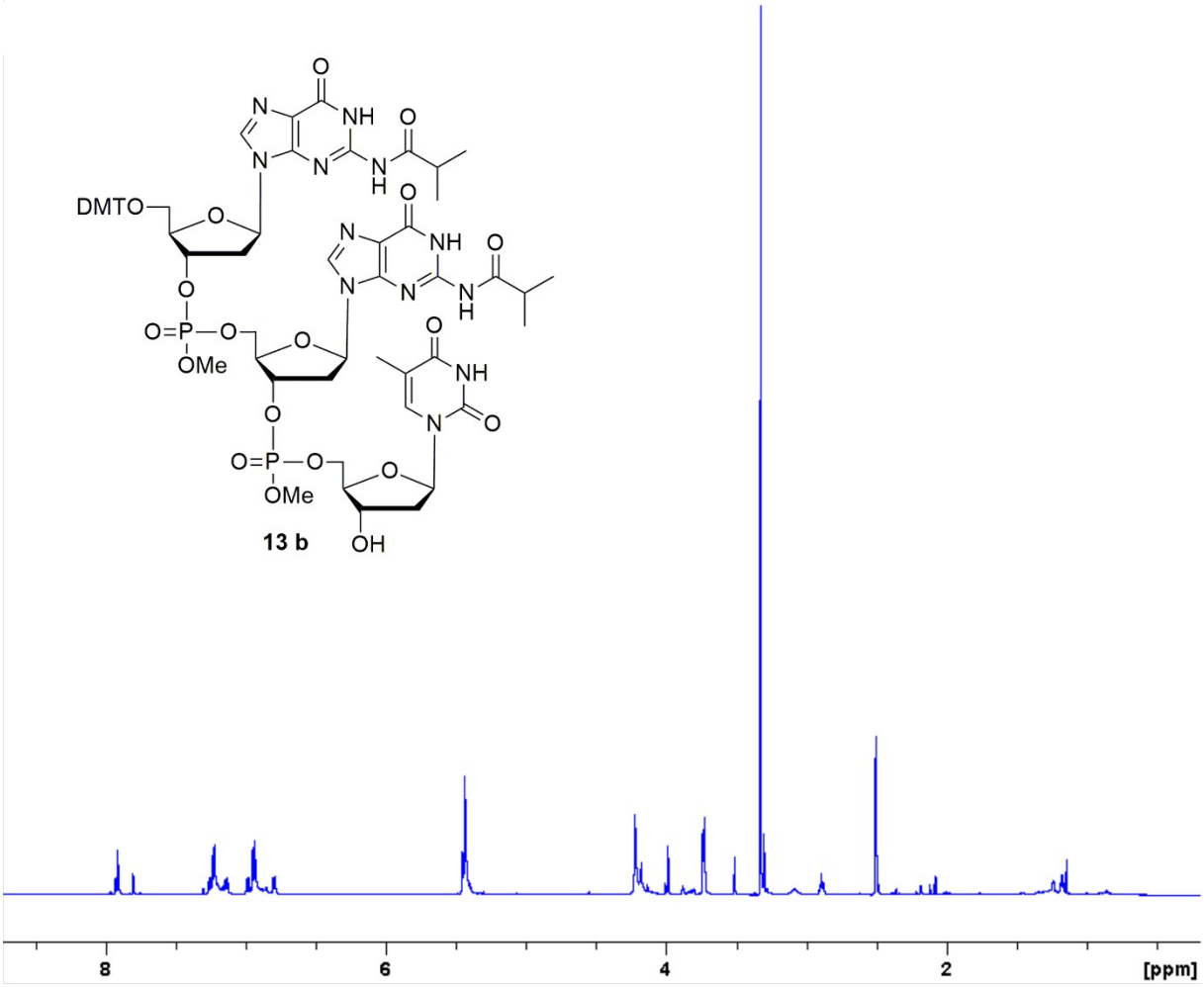
ppm	DEPT	Signal
174.25	/	Cyt C1
166.98, 165.79	/	BZ C=O
164.76	/	Thy C3
158.35, 158.33	/	DMT arom
157.71	/	Ade C5
156.66	/	Cyt C3
147.02, 146.98	/	Ade C3/4
144.05	/	DMT arom
137.97	/	Bz arom
136.23	/	DMT arom
131.60	+	Ade C2
130.23-128.44	+	DMT arom
129.79	+	Cyt C5
127.18	+	Cyt C6
127.75-114.88	+	Bz arom
118.45	+	Thy C6
109.69	/	Thy C5
89.54	+	C1'
89.40	/	DMTq
84.48	+	C1'
83.74	+	C1'
78.25	+	C4'
74.32	+	C4'
72.90	+	C4'
69.79	-	C5'/''
69.30	-	C5'/''
67.80	+	C3'
65.95	+	C3'
65.17	+	C3'
62.62	-	C5'/''
55.05, 43.40	+	O-CH_3 (DMT/$POCH_3$)
35.11	-	C2'/''
31.93	-	C2'/''
26.53	-	2'/''
10.50	-	Thy methyl

Details of DEPT and HSQC spectra (13a):

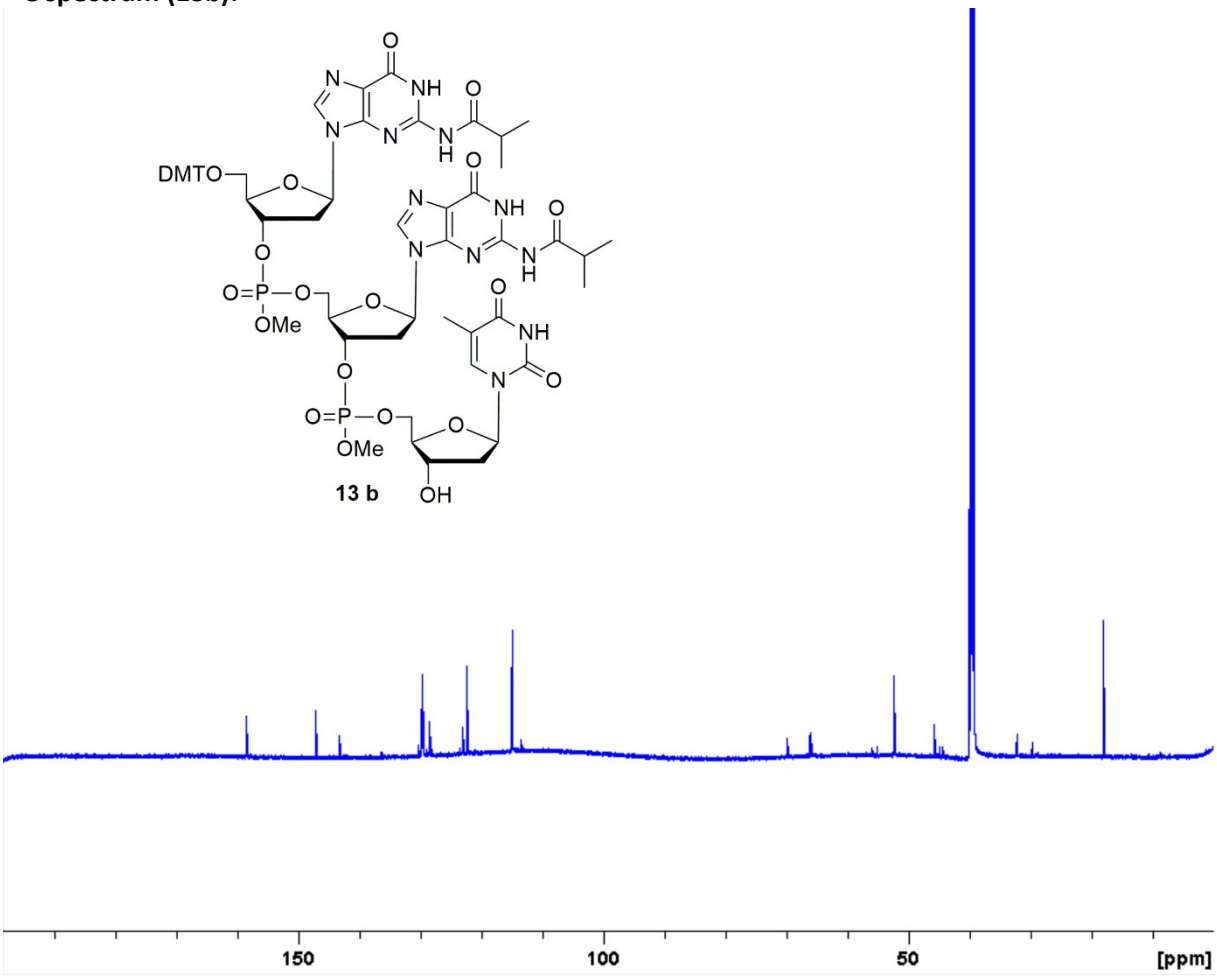


Trinucleotide $G^{Ibu}G^{Ibu}T$, (**13_b**), ^{1}H, ^{13}C, DEPT, DQF-COSY, HSQC spectra

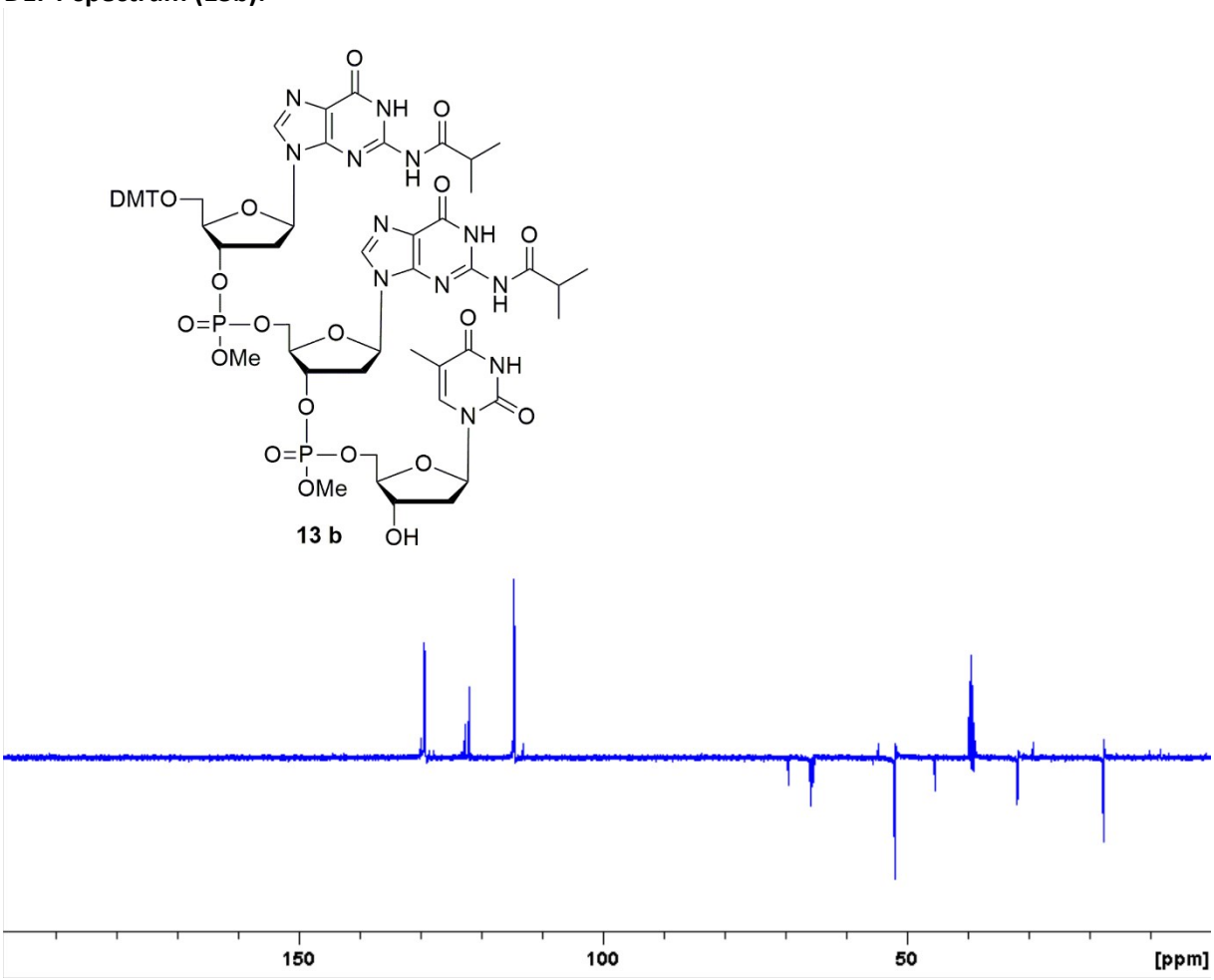
^{1}H spectrum (13b):



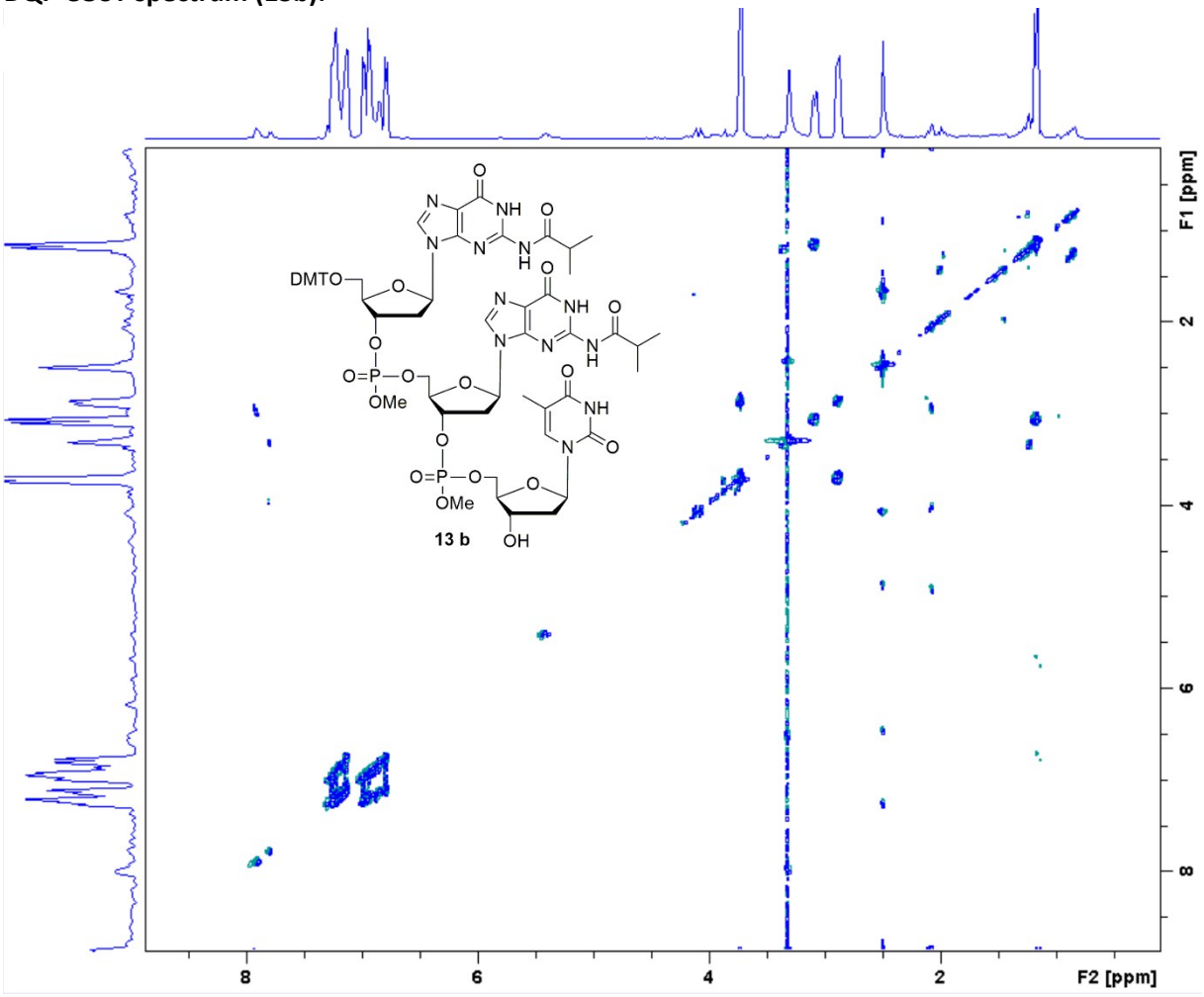
^{13}C spectrum (13b):



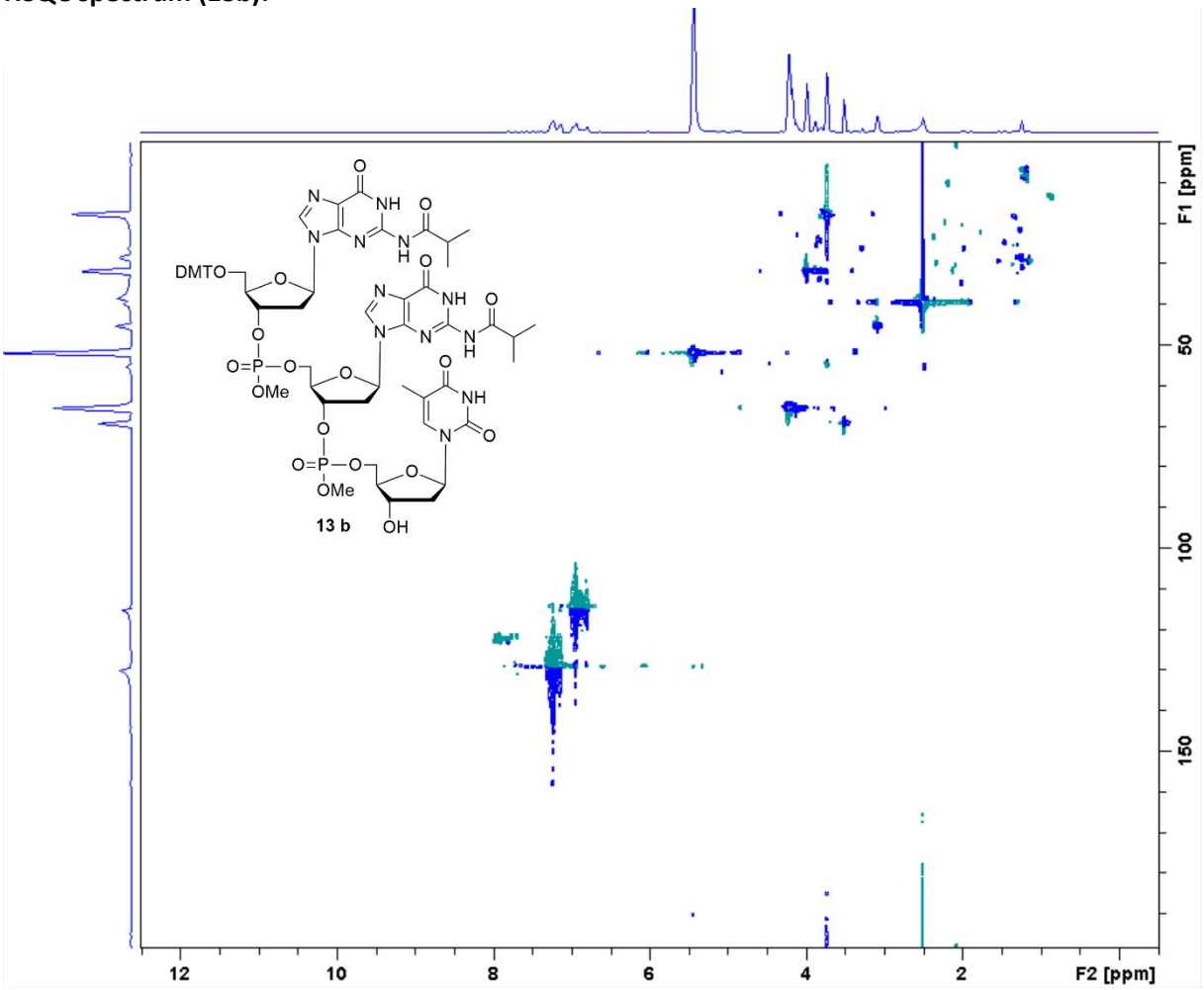
DEPT spectrum (13b):



DQF-COSY spectrum (13b):

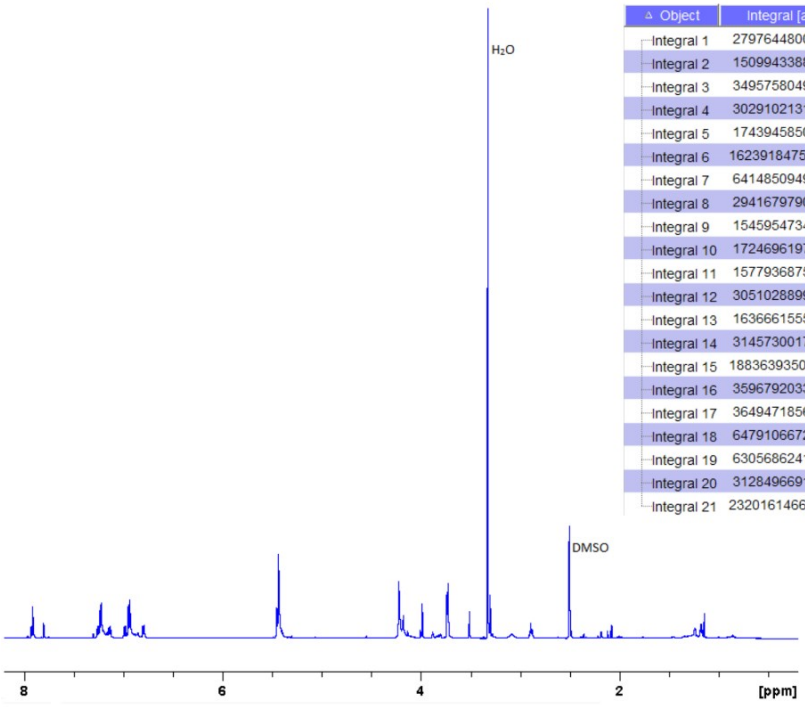


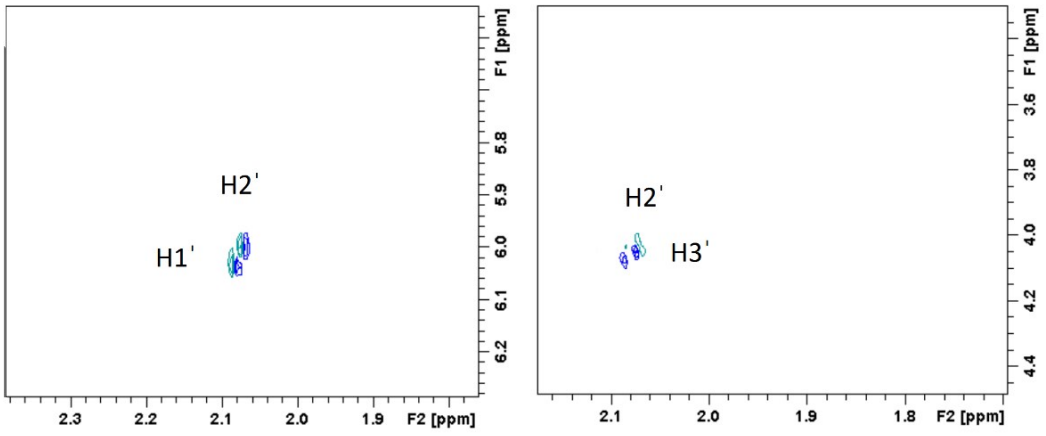
HSQC spectrum (13b):



Details of ^{1}H and COSY spectra (13b):

Object	Integral [abs]	Integral [rel]	Peaks	v(F1) [ppm]	
Integral 1	279764480000.00	1.8097	0	11.3102	2xNH
Integral 2	150994338816.00	0.9767	0	10.4567	NH
Integral 3	349575804928.00	2.2612	0	9.0698	2x NH (Ibu)
Integral 4	302910213120.00	1.9594	0	7.9154	2x Gua C8
Integral 5	174394585088.00	1.1281	0	7.8006	Thy C6
Integral 6	1623918475264....	10.5043	0	7.1645	DMT arom.
Integral 7	641485094912.00	4.1494	0	6.8609	
Integral 8	294167979008.00	1.9028	0	6.6526	2x H1'
Integral 9	154595473408.00	1.0000	0	5.9742	H1'
Integral 10	172469619712.00	1.1156	0	5.3157	H3'-OH
Integral 11	157793687552.00	1.0207	0	4.4658	H4'
Integral 12	305102889984.00	1.9736	0	4.3190	2xH4'
Integral 13	163666155520.00	1.0587	0	4.0044	H3'
Integral 14	314573001728.00	2.0348	0	3.8704	2x H3'
Integral 15	1883639350272....	12.1843	0	3.7262	methyl (DMT/$POCH_3$)
Integral 16	359679203328.00	2.3266	0	3.5068	H5'/''
Integral 17	364947185664.00	2.3607	0	3.3581	2x H''
Integral 18	647910667264.00	4.1910	0	3.0895	2x Ibu-H, 2'
Integral 19	630568624128.00	4.0788	0	2.8754	2x 5'/5''
Integral 20	312849669120.00	2.0237	0	1.9713	H2'/''
Integral 21	2320161466368....	15.0080	0	1.2472	methyl Ibu/Thy





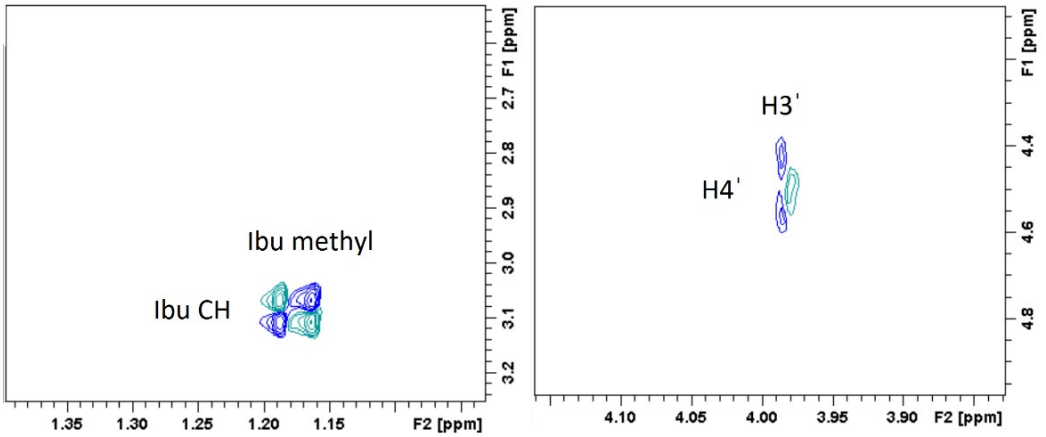
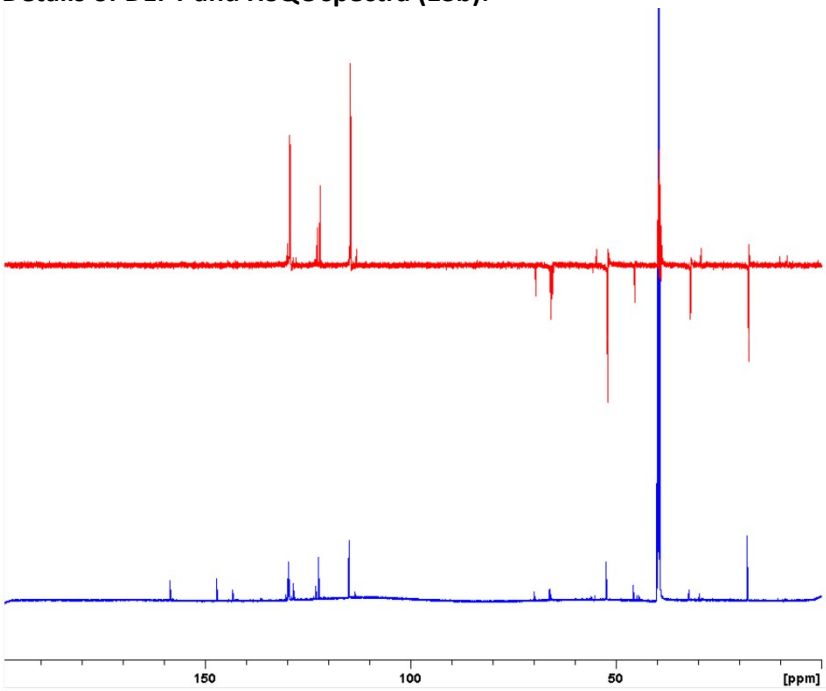
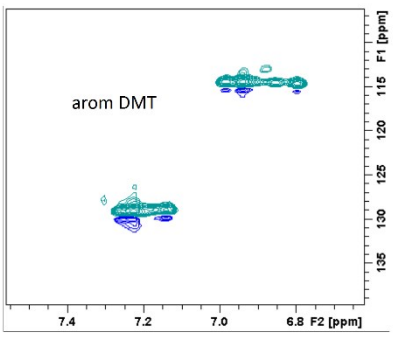


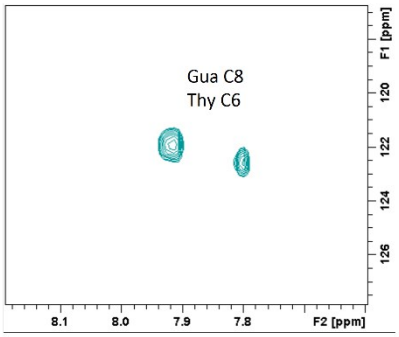
Table S2: Details of ^{13}C and DEPT spectra of **13_b**, DEPT: + ≙ positive signal, - ≙ negative signal / ≙ no signal.

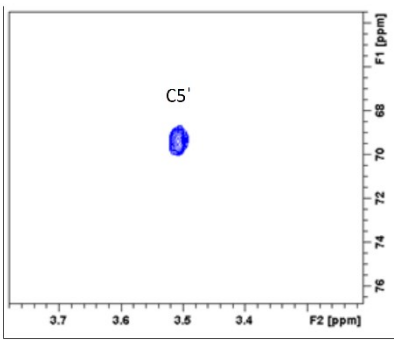
ppm	Dept	Signal
180.90	/	Ibu C=O
163.70	/	Thy C4
158.41, 158.39	/	DMT arom
158.35	/	Gua C6
157.71, 147.02	/	Thy C2; Gua C4
143.08	/	Gua C2
136.23	/	DMT arom
130.22-128.33	+	DMT arom
122.95, 122.25	+	Thy C6, Gua C8
120.88, 119.24	/	Gua C5; Thy C5
113.31	+	DMT arom
87.16	/	DMTq
86.37	+	C4'
84.45	+	C4'
83.60	+	C1'
81.42	+	C1'
77.19	+	C3'
74.66	+	C3'
69.78	-	C5'
65.75	-	C5'
55.05, 39.47	+	O-CH_3 (DMT/$POCH_3$)
45.69	-	C2'
32.14	-	C2'
29.60, 18.04	+	Methyl Thy/Ibu

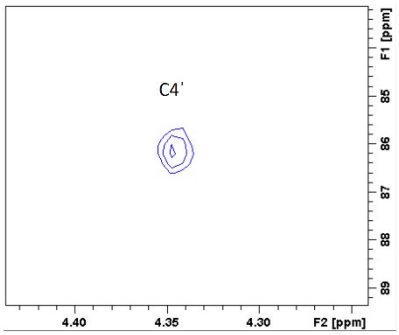
Details of DEPT and HSQC spectra (13b):

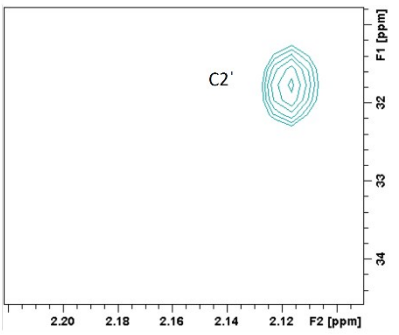


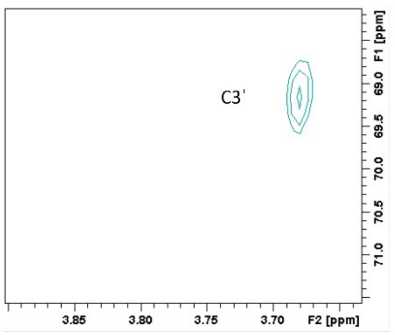


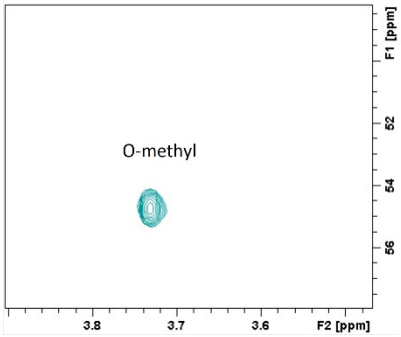


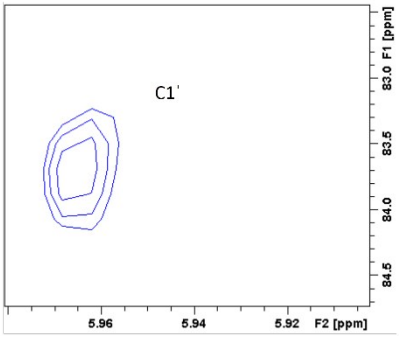












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[2] R. Suchsland, B. Appel and S. Müller, *Curr. Protoc. Nucleic Acid Chem.* **2018**, *75*, e60.
[3] A. M. Jabgunde, A. G. Molina, P. Virta and H. Lönnberg, *Beilstein J. Org. Chem.* **2015**, *11*, 1553.
[4] L. J. McBride and M. H. Caruthers, *Tetrahedron Lett.* **1983**, *24*, 245.