Supplementary Material (ESI) for Organic & Biomolecular Chemistry

The synthesis of double-headed nucleosides by the CuAAC reaction and their effect in secondary nucleic acid structures

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Supporting information

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Preparation of *N*6-Benzoyl-*N*9-propargyladenine (6)

*N*9-propargyladenine, **10**, (123 mg, 0.71 mmol) was coevaporated twice with anhydrous pyridine and dissolved in the same solvent (4 mL). Benzoyl chloride (0.10 mL, 0.86 mmol) was added and the reaction mixture was stirred at room temperature for 17 h and then at 120 °C for 6 h. Methanol (0.2 mL) was added and the mixture was concentrated under reduced pressure. The residue was coevaporated twice with toluene and purified by silica gel column chromatography (0 – 10% MeOH in CH_2Cl_2) to give a crude product **11** as a white solid (101 mg, ~85% pure, estimated yield 43%), which was used without further purification; R_f 0.58 (10% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (br s, 1H, NH), 8.78 (s, 1H, H2), 8.55 (s, 1H, H8), 8.09 – 8.03 (m, 2H, Ph), 7.68 – 7.48 (m, 3H, Ph), 5.19 (d, 2H, J = 2.5 Hz, CH_2), 3.54 (t, 1H, J = 2.5 Hz, C≡CH); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.7 (C=O), 151.9, 151.7 (C4, C6), 150.3 (C2), 143.9 (C8), 133.4, 132.8, 132.4, 130.8, 129.2, 128.5, 128.4 (Ph), 125.2 (C5), 77.9 (C≡CH), 76.2(C≡CH), 32.7 (CH_2); HRMS-ESI: m/z: 300.0842 $[MNa]^+$; calcd ($C_{15}H_{11}N_5O$-Na^+): 300.0856.

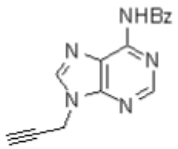
MALDI MS-data of oligonucleotides

Name	Sequence	MW calcd	MW exp
K6	5'-d(GCT CAC **K**CT CCC A)-3'	4021.7	4022.1
L6	5'-d(GCT CAC **L**CT CCC A)-3'	4030.7	4026.6
M6	5'-d(GCT CAC **M**CT CCC A)-3'	4049.7	4050.6
K1	3'-d(GCG **K**AT AAG CG)-5'	3572.4	3576.0
K2	3'-d(GCG TA**K** AAG CG)-5'	3572.4	3573.2
K3	5'-d(CGC ATA T**K**C GC)-3'	3483.4	3483.8
K4	5'-d(CGC ATA **K**TC GC)-3'	3483.4	3480.0
K5	5'-d(CGC A**K**A TTC GC)-3'	3483.4	3479.2
L1	3'-d(GCG **L**AT AAG CG)-5'	3581.4	3577.7
L2	3'-d(GCG TA**L** AAG CG)-5'	3581.4	3578.6
L3	5'-d(CGC ATA T**L**C GC)-3'	3492.4	3488.2
L4	5'-d(CGC ATA **L**TC GC)-3'	3492.4	3491.6
L5	5'-d(CGC A**L**A TTC GC)-3'	3492.4	3490.8
M1	3-d(GCG **M**AT AAG CG)-5'	3600.5	3596.0
M2	3-d(GCG TA**M** AAG CG)-5'	3600.5	3597.4
M3	5'-d(CGC ATA T**M**C GC)-3'	3511.4	3508.8
M4	5'-d(CGC ATA **M**TC GC)-3'	3511.4	3514.3
N1	3-d(GCG **N**AT AAG CG)-5'	3628	3626
N2	3-d(GCG TA**N** AAG CG)-5'	3628	3626
N3	5'-d(CGC ATA T**N**C GC)-3'	3538	3539
N4	5'-d(CGC ATA **N**TC GC)-3'	3538	3539
M7	3-d(GCG **M**A**M** AAG CG)-5'	3819.7	3815.1
M8	5'-d(CGC A**M**A **M**TC GC)-3'	3730.6	3732.8

Multiple incorporations of **M** in zipper motifs

				ΔT_m /°C[a] [$\Delta\Delta T_m$] /°C[b]		
ON	**Duplex**		**Zipper**	**ON**	**Duplex**	
ref.	5′-d(CGC ATA TTC GC)		−1	**M4**	5′-d(CGC ATA **M**TC GC)	−14.0
ref.	3′-d(GCG TAT AAG CG)			**M2**	3′-d(GCG TA**M** AAG CG)	[−1.5]
	5′-d(CGC ATA TTC GC)	−6.0	−2	**M3**	5′-d(CGC ATA T**M**C GC)	−14.0
M1	3′-d(GCG **M**AT AAG CG)			**M2**	3′-d(GCG TA**M** AAG CG)	[−1.5]
	5′-d(CGC ATA TTC GC)	−6.0	−3	**M4**	5′-d(CGC ATA **M**TC GC)	−12.0
M2	3′-d(GCG TA**M** AAG CG)			**M1**	3′-d(GCG **M**AT AAG CG)	[+0.5]
	5′-d(CGC ATA TTC GC)	−12.0	−4	**M3**	5′-d(CGC ATA T**M**C GC)	−12.0
M7	3′-d(GCG **M**A**M** AAG CG)			**M1**	3′-d(GCG **M**AT AAG CG)	[+0.5]
M3	5′-d(CGC ATA T**M**C GC)	−6.5	−1/−3	**M4**	5′-d(CGC ATA **M**TC GC)	−19.0
	3′-d(GCG TAT AAG CG)			**M7**	3′-d(GCG **M**A**M** AAG CG)	[−0.5]
M4	5′-d(CGC ATA **M**TC GC)	−6.5	−1/−3	**M8**	5′-d(CGC A**M**A **M**TC GC)	−19.0
	3′-d(GCG TAT AAG CG)			**M1**	3′-d(GCG **M**AT AAG CG)	[+1.0]
M8	5′-d(CGC A**M**A **M**TC GC)	−14.0	−2/−4	**M3**	5′-d(CGC ATA T**M**C GC)	−21.0
	3′-d(GCG TAT AAG CG)			**M7**	3′-d(GCG **M**A**M** AAG CG)	[−3.0]
			+1/−1	**M8**	5′-d(CGC A**M**A **M**TC GC)	−22.5
				M2	3′-d(GCG TA**M** AAG CG)	[−2.5]
			+1/−1	**M8**	5′-d(CGC A**M**A **M**TC GC)	−30.5
				M7	3′-d(GCG **M**A**M** AAG CG)	[−4.5]

[a] Differences in melting temperatures as compared to the unmodified duplex; $\Delta T_m = T_{m(duplex)} - T_{m(ref)}$. Melting temperatures ($T_m$ values/°C) were obtained from the maxima of the first derivatives of the melting curves (A_{260} *vs.* temperature) recorded in a medium salt buffer (Na_2HPO_4 (2.5 mM), NaH_2PO_4 (5 mM), NaCl (100 mM), EDTA (0.1 mM), pH 7.0) using 1.0 μM concentrations of each strand. **M** corresponds to the incorporation of the amidite **15**. [b] Differences in melting temperatures as compared to singly modified duplexes; $\Delta\Delta T_m = \Delta T_{m(x:y)} - (\Delta T_{m(x:ref)} + \Delta T_{m(ref:y)})$.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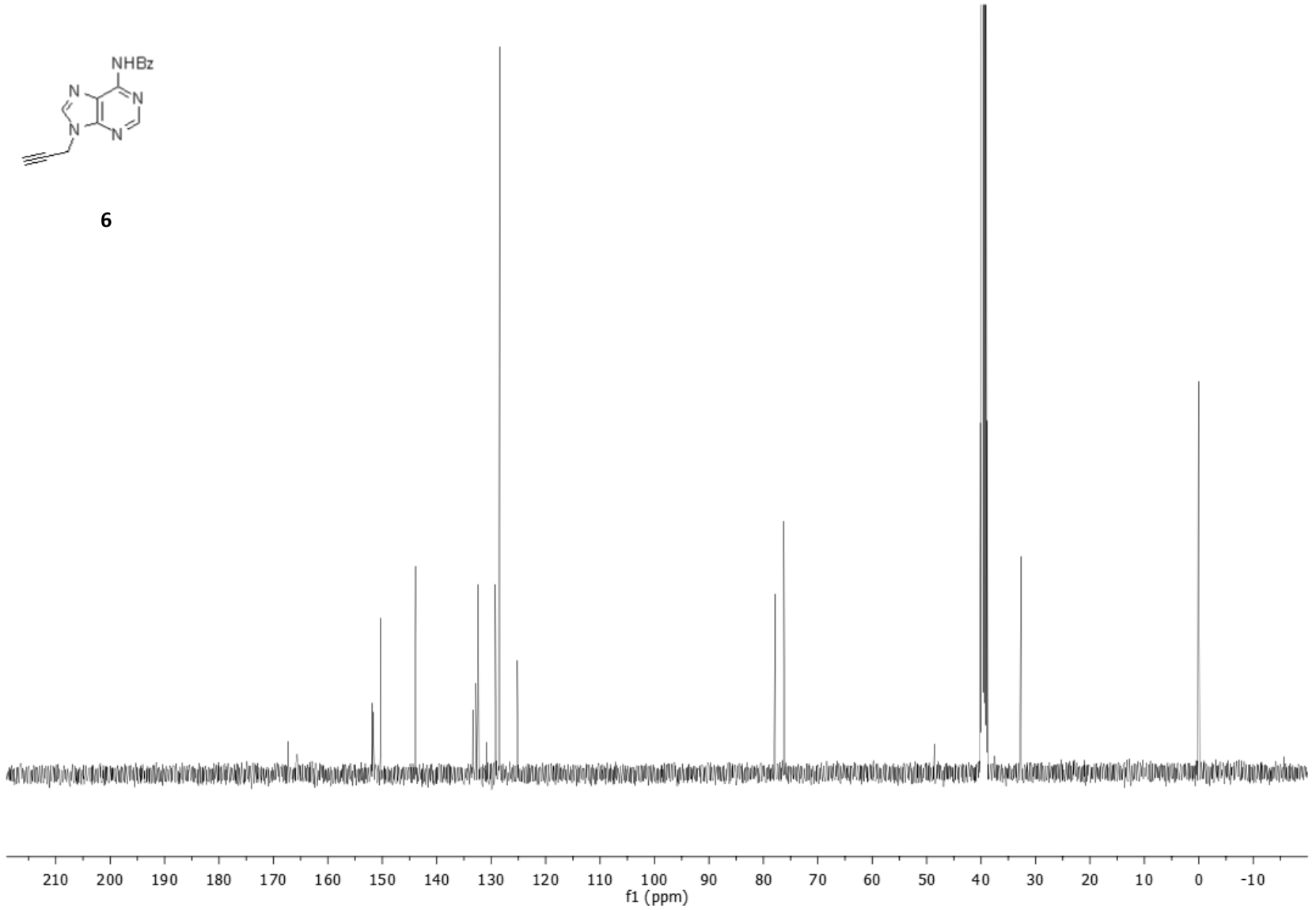
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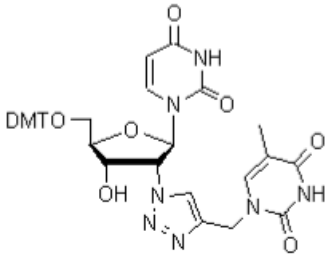
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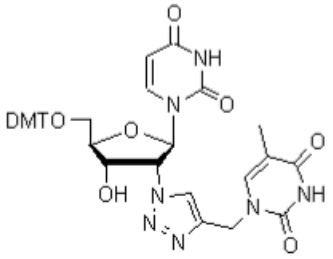
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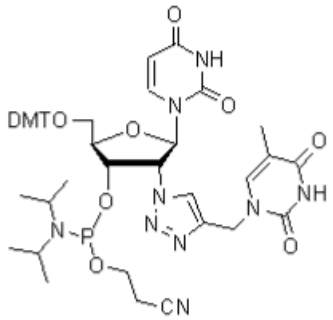
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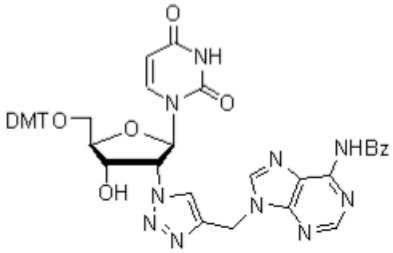
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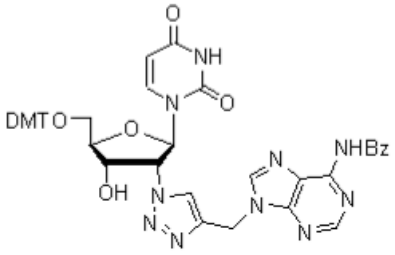
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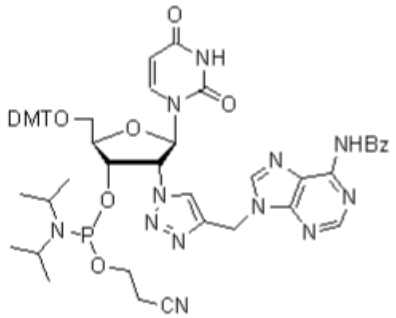
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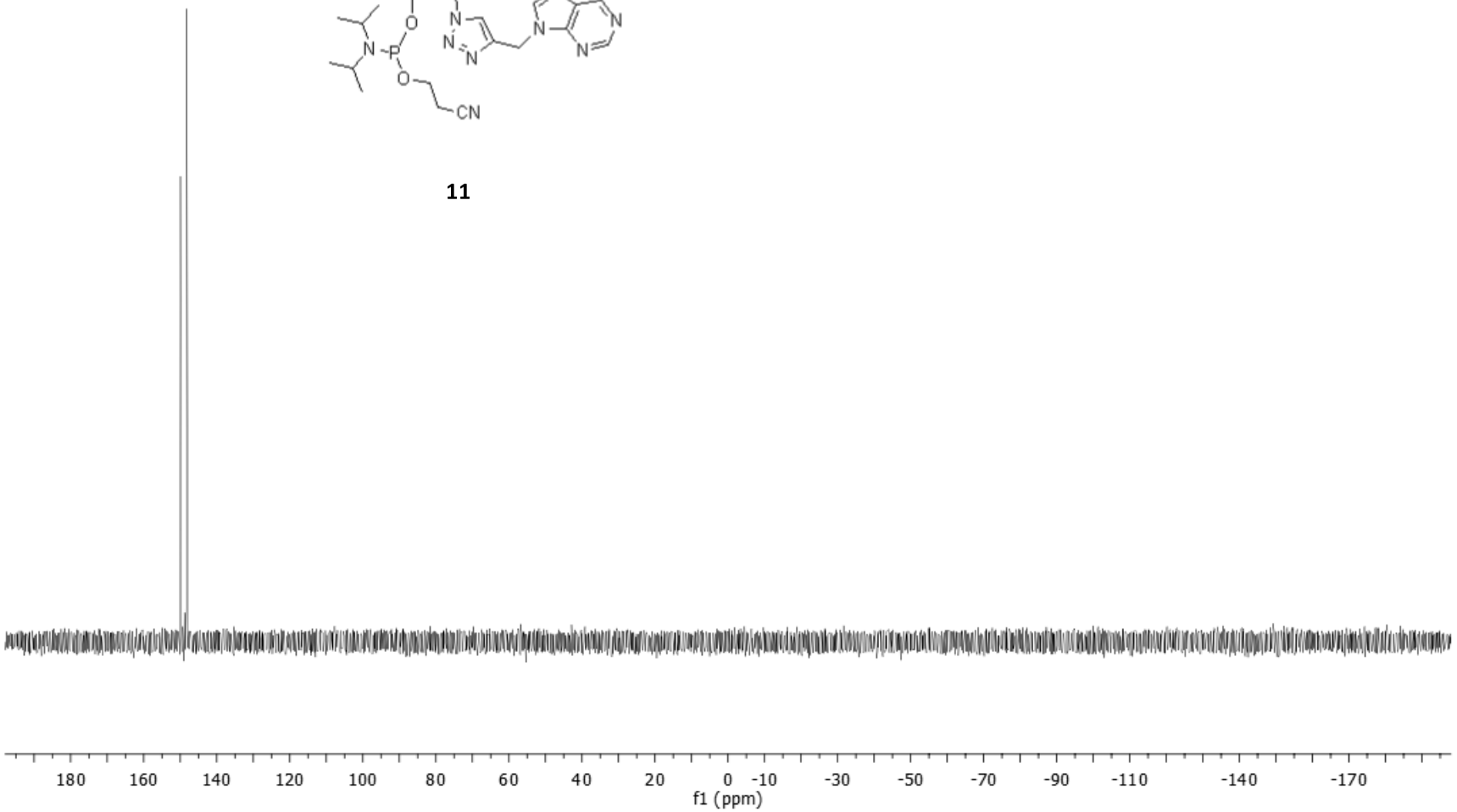
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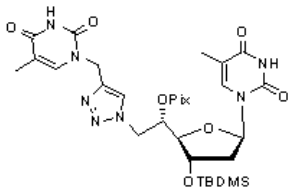

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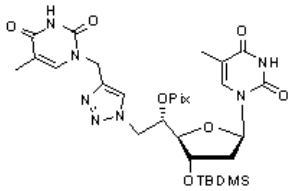

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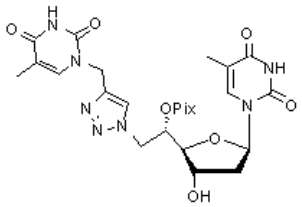

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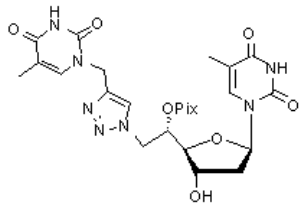

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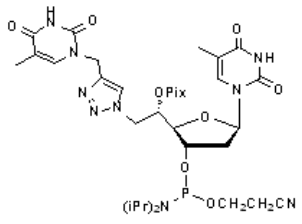

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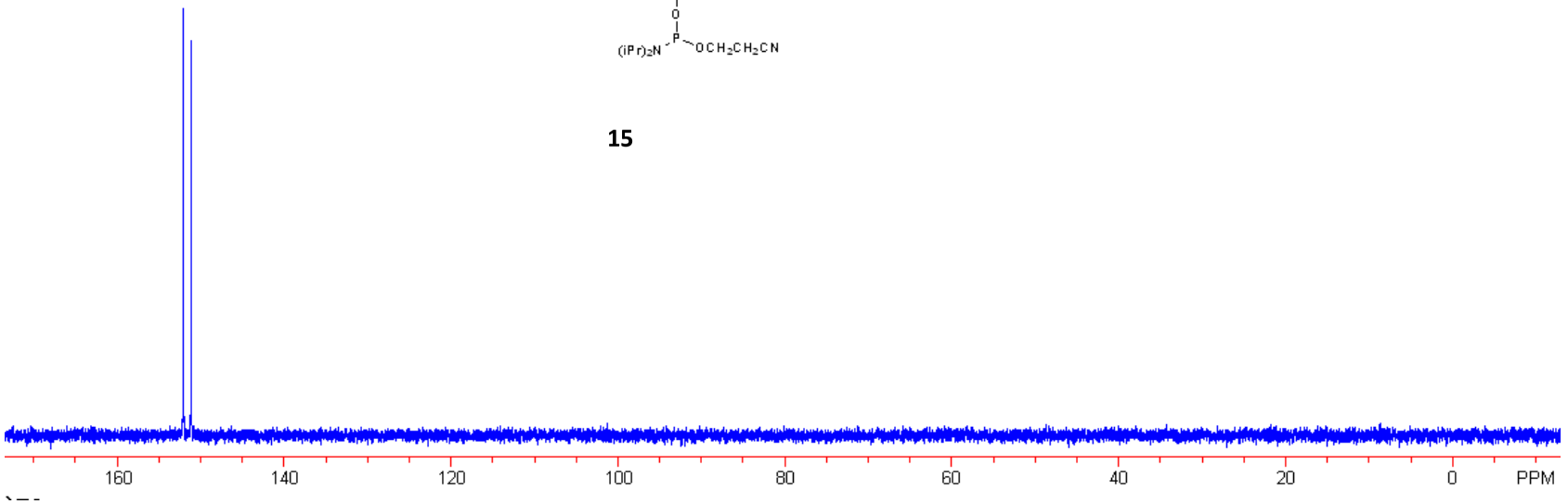

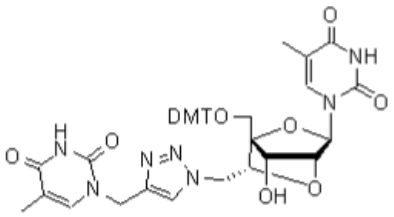
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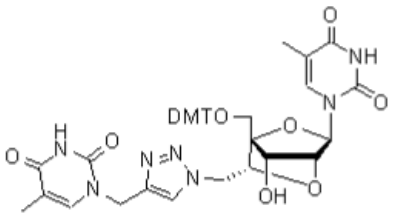
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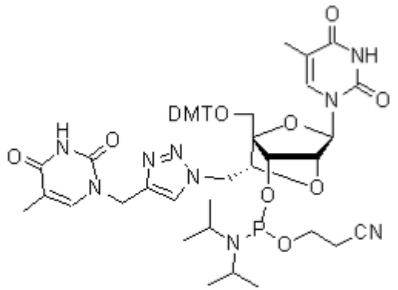
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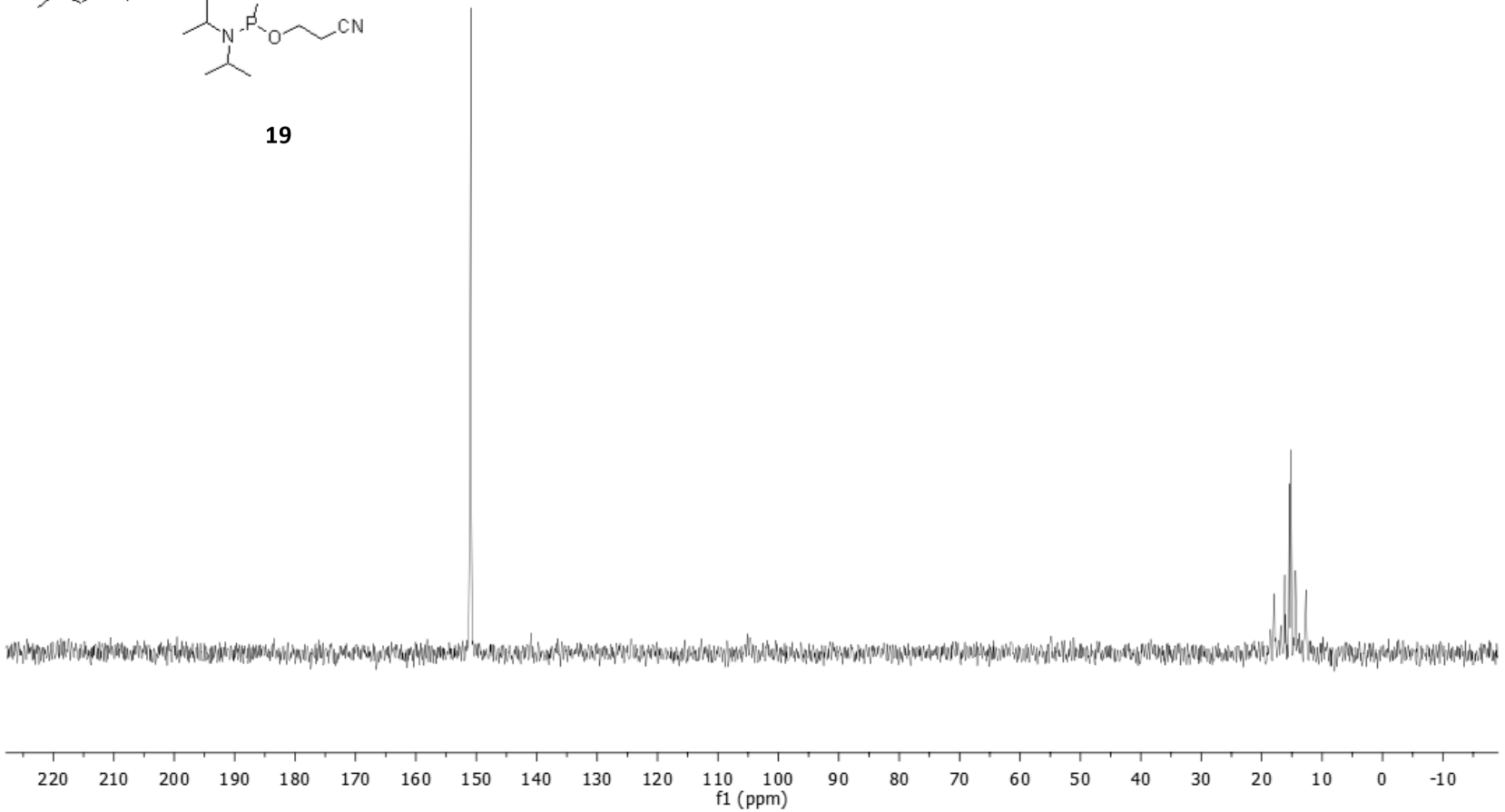
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