
Supplementary information

Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis

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Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis

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Supplementary Table 1: RNA substrates

RNA oligonucleotide	Sequence
Product	/56-FAM/rUrGrA rGrCrC rUrArC rGrCrA rGrU
Template 1	5' rUrUrU rArArC rUrGrC rGrUrA ddC 3'
Template 2	5 rCrCrC rUrArC rUrGrC rGrUrA ddC 3'
Template 3	5' rUrUrU rCrArC rUrGrC rGrUrA ddC 3'
Template 4	5' rUrUrU rGrArC rUrGrC rGrUrA ddC 3'
Template 5	5' rUrUrU MrArC rUrGrC rGrUrA ddC 3'
Template 6 (structure)	5' rGrGrG MrArC rUrGrC rGrUrA-3'
RNA-hairpin duplex (structure)	5' rGrCrU rCrArU rArCrC rGrUrA rUrUrG rArGrA rCrCrU rUrUrU rGrGrU rCrUrC rArArU rArCrG rGrUrA rUrGrA rGrCrC rUrArC rGrCrA rGrUrA 3'
RNA-hairpin duplex (structure)	5' rGrCrU rCrArU rArCrC rGrUrA rUrUrG rArGrA rCrCrU rUrUrU rGrGrU rCrUrC rArArU rArCrG rGrUrA rUrGrA rGrCrC rUrArC rGrCrA rGrUrG 3'
RNA-hairpin duplex	/56-FAM/rUrUrU rUrCrA rUrGrC rArCrC rGrCrG rUrArG rUrUrU rUrCrU rArCrG rCrG-3'

Supplementary Table 2. Thermal melting analysis

sequence R – Y	5'CACUGCGUAG R ^a 3'GUGACGCAUC Y GAGU5'	5'CACUGCGUAG R CUCA3' 3'GUGACGCAUC Y GAGU5'	5'CA Y UGCGUAGGCUCA3' 3'GU R ACGCAUCCGAGU5'
G – C	64.7 °C	77.6 °C	77.6 °C
G – M	61.2 °C	71.2 °C	73.2 °C
A – M	60.6 °C	68.2 °C	73.8 °C

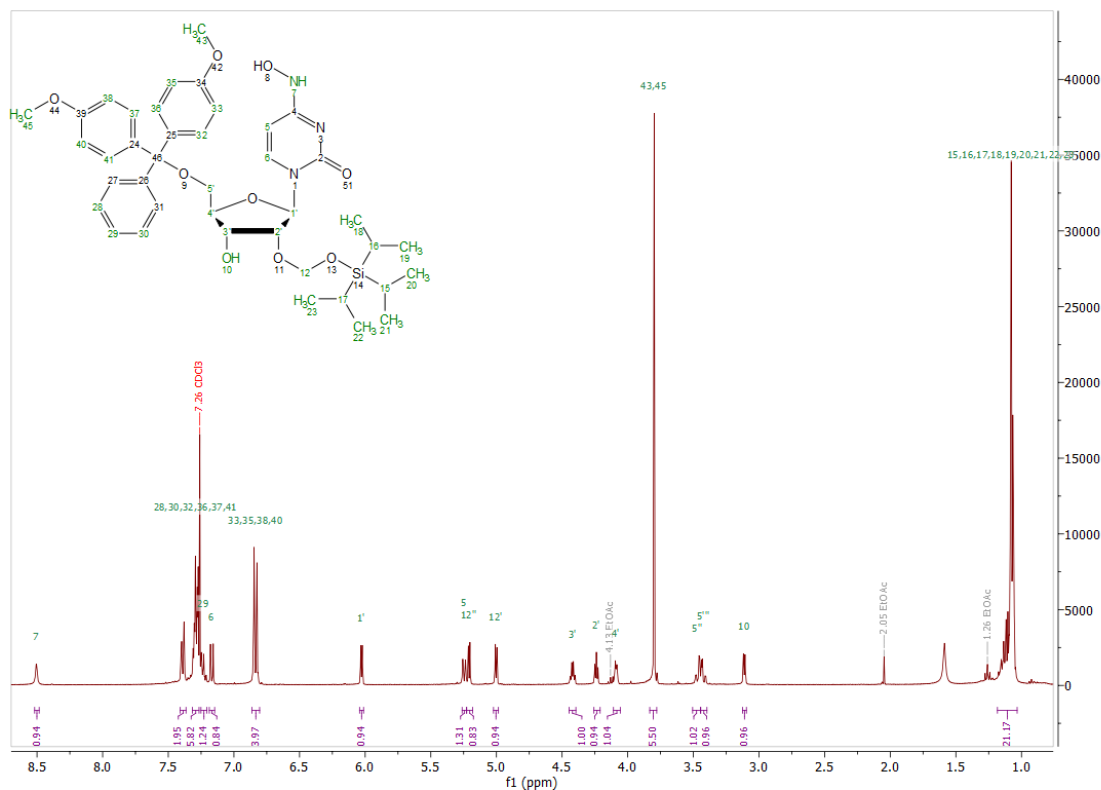
^a R = G or A, Y = C or M

Supplementary Table 3. Sequences and high-resolution ESI-MS data of NHC/M**-modified RNA oligonucleotides**

Name	5'-sequence-3'	nt	formula	Mass calc.	Mass found
M_T	GGG M ACUGCGUAp	12	C ₁₁₅ H ₁₄₄ N ₄₈ O ₈₆ P ₁₂	3944.5221	3944.5297
M_1	M ACUGCGUAGGCUCA	15	C ₁₄₂ H ₁₇₈ N ₅₆ O ₁₀₄ P ₁₄	4764.6688	4764.6760
M_3	C M UGCGUAGGCUCA	15	C ₁₄₂ H ₁₇₈ N ₅₆ O ₁₀₄ P ₁₄	4764.6688	4764.6797
M_5	UGAG M CUACGCAGUG	15	C ₁₄₃ H ₁₇₈ N ₅₈ O ₁₀₄ P ₁₄	4804.6749	4804.6684

Supplementary Data Set 1: NMR spectra of synthetic compounds

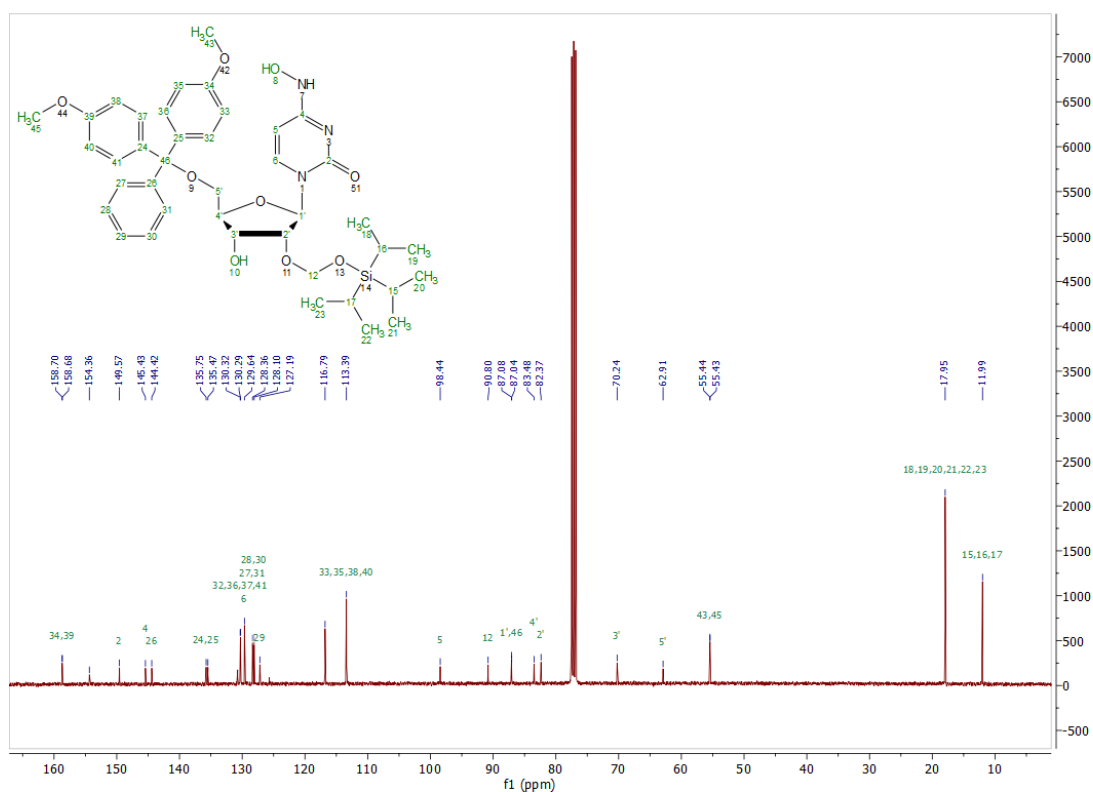
5'-*O*-(4,4'-Dimethoxytrityl)-*N*⁴-hydroxy-2'-*O*-(triisopropylsilyloxy)methylcytidine (compound 1)



¹H-NMR (400 MHz, CDCl₃) of compound 1.

¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.04 – 1.15 (m, 21H, Si(CH(CH₃)₂)₃), 3.13 (d, J = 4.7 Hz, 1H, C3'-OH), 3.40 – 3.45 (dd, J = 2.8, 10.8 Hz, 1H, H-5'b), 3.45 – 3.49 (dd, J = 2.4, 10.8 Hz, 1H, H-5'a), 3.80 (s, 6H, OCH₃), 4.07 – 4.11 (dt, J = 2.6, 5.1 Hz, 1H, H-4'), 4.24 (t, J = 4.8 Hz, 1H, H-2'), 4.38 – 4.46 (q, J = 4.8 Hz, 1H, H-3'), 5.00 (d, J = 4.8 Hz, 1H, OCH₂O), 5.20 (d, J = 4.8 Hz, 1H, OCH₂O), 5.25 (d, J = 8.3 Hz, 1H, H-5), 6.03 (d, J = 4.4 Hz, 1H, H-1'), 6.79 – 6.88 (m, 4H, DMT), 7.17 (d, J = 8.3 Hz, 1H, H-6), 7.18 – 7.27 (m, 1H, DMT), 7.24 – 7.33 (m, 6H, DMT), 7.35 – 7.42 (m, 2H, DMT), 8.53 (s, NH).

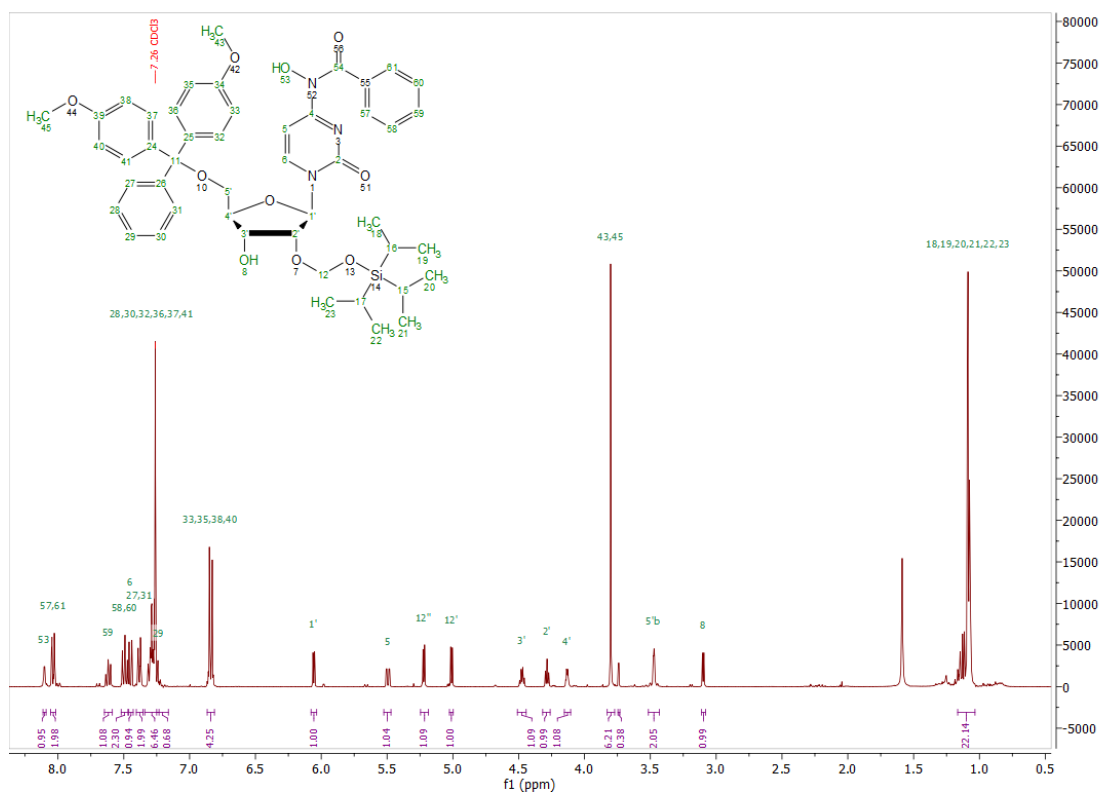
HR-MS (ESI⁺): Exact mass calculated for C₄₀H₅₃NaN₃O₉Si [M+Na]⁺: 770.3443, found: 770.3459.



^{13}C -NMR (100 MHz, CDCl_3) of compound 1.

^{13}C -NMR (100 MHz, CDCl_3): δ (ppm) = 11.99, 17.95 ($\text{Si}(\text{CH}(\text{CH}_3)_2)_3$), 55.43, 55.44 (OCH_3), 62.91 ($\text{C}5'$), 70.24 ($\text{C}3'$), 82.37 ($\text{C}2'$), 83.48 ($\text{C}4'$), 87.04, 87.08 ($\text{C}1'$, $\text{C}_q\text{-DMT}$), 90.80 (OCH_2O), 98.44 ($\text{C}5$), 113.39 (DMT), 127.19 (DMT), 128.10 (DMT), 128.36 (DMT), 129.64 ($\text{C}6$), 130.29, 130.32 (DMT), 135.47 135.75 ($\text{C}_q\text{-DMT}$), 144.42 ($\text{C}_q\text{-DMT}$), 145.43 ($\text{C}4$), 149.57 ($\text{C}2$), 158.68, 158.70 ($\text{C}_q\text{-OCH}_3$).

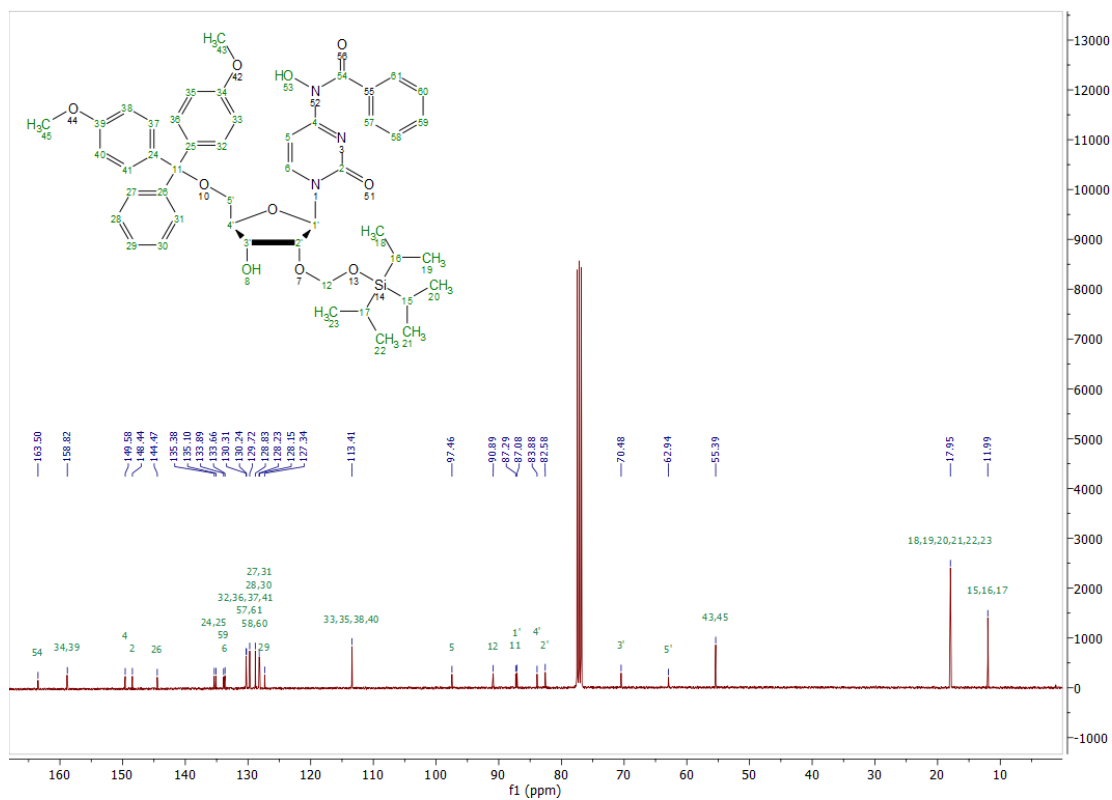
***N*⁴-Benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-*N*⁴-hydroxy-2'-*O*-(triisopropylsilyloxy)methyl cytidine (compound 2)**



¹H-NMR (400 MHz, CDCl₃) of compound 2.

¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.05 – 1.10 (m, 21H, Si(CH(CH₃)₂)₃), 3.10 (d, J = 4.4 Hz, 1H, C3'-OH), 3.44 – 3.50 (m, 2H, H-5'), 3.80 (2s, 6H, OCH₃), 4.11 – 4.16 (m, 1H, H-4'), 4.28 (t, J = 4.9 Hz, 1H, H-2'), 4.48 (q, J = 4.6 Hz, 1H, H-3''), 5.01 (d, J = 4.8 Hz, 1H, OCH₂O), 5.22 (d, J = 4.8 Hz, 1H, OCH₂O), 5.49 (dd, J = 2.3, 8.3 Hz, 1H, H-5), 6.06 (d, J = 4.7 Hz, 1H, H-1'), 6.81 – 6.86 (m, 4H, DMT), 7.22 – 7.34 (m, 6H, DMT), 7.36 – 7.40 (m, 2H, DMT), 7.45 (d, J = 8.3 Hz, 1H, H-6), 7.48 – 7.53 (m, 2H, bz), 7.60 – 7.64 (m, 1H, bz), 8.02 – 8.05 (m, 2H, bz), 8.10 (d, J = 2.2 Hz, 1H, OH).

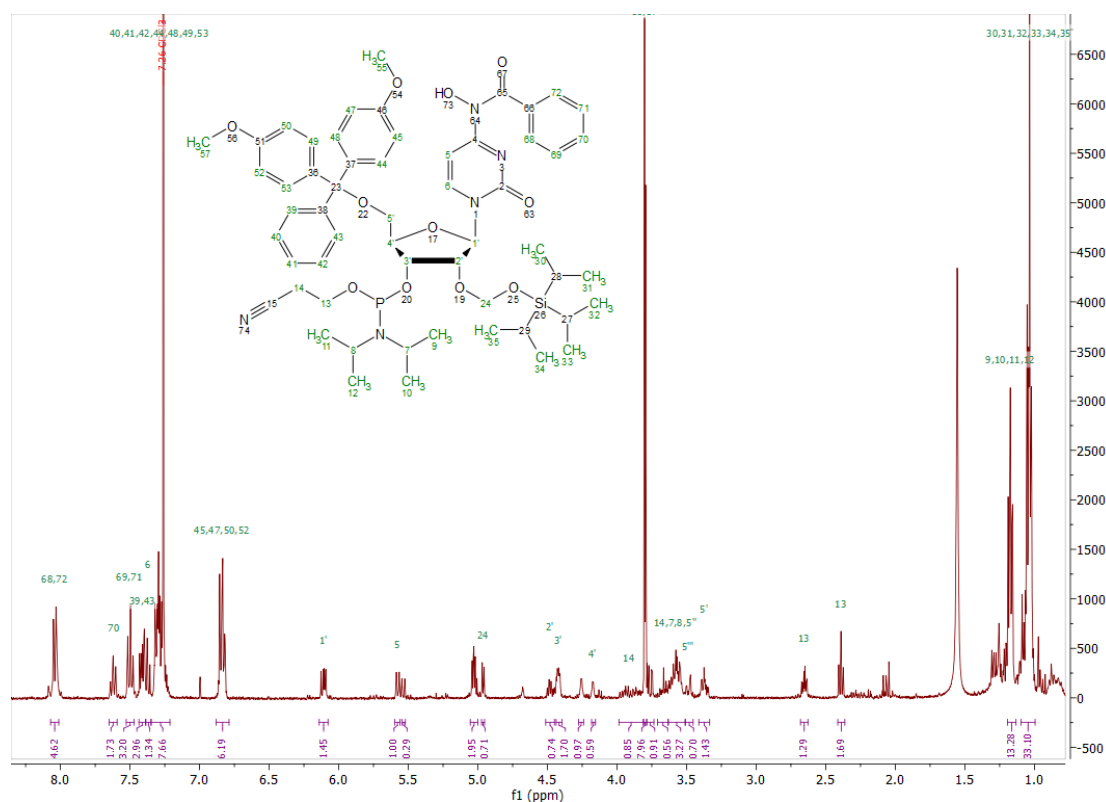
HR-MS (ESI⁺): Exact mass calculated for C₄₇H₅₇N₃NaO₁₀Si [M+Na]⁺: 874.3705, found: 874.3727.



^{13}C -NMR (100 MHz, CDCl_3) of compound 2.

^{13}C - NMR (100 MHz, CDCl_3): δ (ppm) = 11.99, 17.95 ($\text{Si}(\text{CH}(\text{CH}_3)_2)_3$), 55.39 (OCH_3), 62.94 ($\text{C}5'$), 70.48 ($\text{C}3'$), 82.58 ($2'$), 83.88 ($4'$), 87.08 ($1'$), 87.29 ($\text{C}_q\text{-DMT}$), 90.89 (OCH_2O), 97.46 ($\text{C}5$), 113.41 (DMT), 127.34 (DMT), 128.15, 128.23 (DMT), 128.83 (bz), 129.72 (bz), 130.24, 130.31 (DMT), 130.66 ($\text{C}6$), 133.89 (bz), 135.10, 135.38 ($\text{C}_q\text{-DMT}$), 144.47 ($\text{C}_q\text{-DMT}$), 1448.44 ($\text{C}2$), 149.58 ($\text{C}4$), 158.82 ($\text{C}_q\text{-OCH}_3$), 163.50 ($\text{C}=\text{O}$).

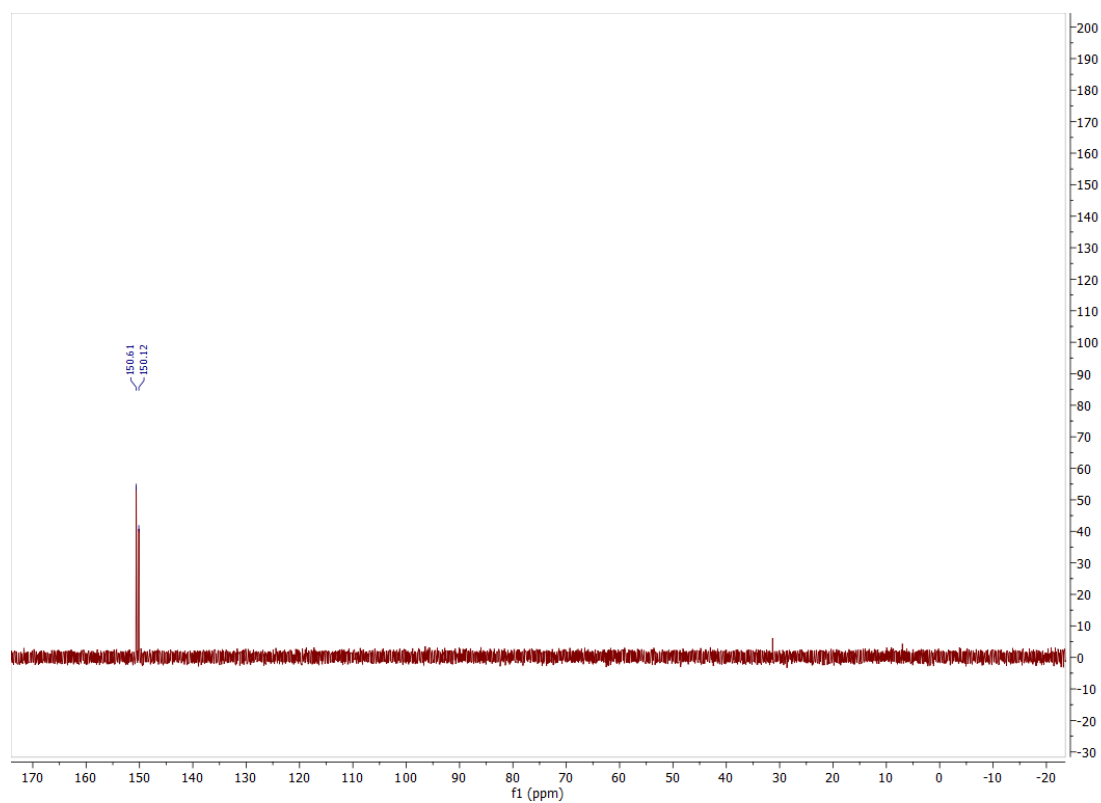
***N*⁴-Benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-*N*⁴-hydroxy-2'-*O*-(triisopropylsilyloxy)methyl cytidine 3'-cyanoethyl-*N,N*-diisopropylphosphoramidite (compound 3, M-PA)**



¹H-NMR (400 MHz, CDCl₃) of compound 3, M-PA.

¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.00 – 1.10 (m, 33H, Si(CH(CH₃)₂)₃, diast.), 1.13 – 1.19 (m, 13H, NCH(CH₃)₂), 2.36 – 2.41 (t, *J* = 6.4 Hz, 2H, POCH₂), 2.63 – 2.68 (td, *J* = 2.9, 6.4 Hz, 1H, POCH₂, diast.), 3.33 – 3.41 (m, 1H, H-5', diast.), 3.45 – 3.51 (m, 1H, H-5', diast.), 3.51 – 3.63 (m, 3H, H-5', NCH(CH₃)₂, CH₂-CN), 3.63 – 3.71 (m, 1H, CH₂-CN, diast.), 3.79 – 3.81 (s, 6H, OCH₃), 3.81 – 3.99 (m, 1H, CH₂-CN), 4.15 – 4.18 (m, 1H, H-4', diast.), 4.24 – 4.28 (m, 1H, H-4'), 4.39 – 4.44 (m, 2H, H-2', H-3'), 4.45 – 4.51 (q, *J* = 5.6 Hz, 1H, H-2'), 4.96 (d, *J* = 5.0 Hz, 1H, OCH₂O, diast.), 5.00 – 5.05 (m, 2H, OCH₂O), 5.54 (d, *J* = 8.2 Hz, 1H, C-5, diast.), 5.58 (d, *J* = 8.2 Hz, 1H, C-5, diast.), 6.08 – 6.14 (2d, *J* = 8.2 Hz, 1H, H-1'), 6.79 – 6.88 (m, 6H, DMT), 7.21 – 7.35 (m, 8H, DMT), 7.35 – 7.39 (m, 1H, H-6), 7.39 – 7.44 (m, 3H, DMT), 7.47 – 7.53 (m, 3H, bz), 7.59 – 7.65 (m, 2H, bz), 8.03 (m, 5H, bz).

HR-MS (ESI⁺): Exact mass calculated for C₅₆H₇₅N₅O₁₁PSi [M+H]⁺: 1052.4964, found: 1052.4933 and for C₅₆H₇₄N₅NaO₁₁PSi [M+Na]⁺: 1074.4784, found: 1074.4749



^{31}P -NMR (162 MHz, CDCl_3) of compound 3, M-PA.

^{31}P NMR (162 MHz, CDCl_3): δ (ppm) = 150.12, 150.61.