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Table of Contents

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Experimental Procedures

General Methods

Silica gel 60 F254 aluminum backed thin layer chromatography plates (EMD) were used. Flash column chromatography was performed with a Mini-Flash Pump (Scientific Systems, Inc, State College, PA) using EZ-Flash cartridges (Sorbent Technologies, Norcross, GA). NMR spectra were recorded on a 500 MHz Bruker Avance II in the Solution NMR Core Facility in the Penn State College of Medicine. Chemical shifts for the ^{1}H and ^{13}C spectra are reported in parts per million (ppm, δ) relative to TMS, while those for the ^{31}P spectra are reported relative to H_3PO_4. Mass spectra were recorded on a MDS-Sciex 4000 QTrap with electrospray ionization in the Mass Spectrometry and Proteomics Core at the Penn State College of Medicine in Hershey. . High resolution mass spectra were recorded on a Waters Q-TOF Premier tandem mass spectrometer at the Proteomics and Mass Spectrometry Core Facility in University Park.

Reagents. [^{32}P]ATP (6000 Ci/mmol) was purchased from Perkin Elmer, and T4 polynucleotide kinase from USB/Affymetrix. The dNTPs (ultrapure grade) were purchased from GE Healthcare, and the concentrations were determined by UV absorbance.[1] 5(6)-Carboxyfluorescein azide (FAM-N_3) was purchased from Tenova Pharmaceuticals (San Diego, CA). dGTP was purchased from (ultrapure grade) were purchased from GE Healthcare, and N^2-methyl-dGTP was purchased from TriLink Biotechnologies (San Diego, CA). The concentrations of all dGTPs was determined spectrophotometrically.

Oligodeoxynucleotides. Oligodeoxynucleotides were purchased from Integrative DNA technologies. The ssDNA concentration was determined at 260 nm on a Beckman DU640 UV-vis spectrophotometer. The theoretical molar extinction coefficients of the DNA sequences were determined by the method of Borer.[2] The primer strand was 5′- GCA CCG CAG ACG CAG -3' and the template strand was 5'-CTG CGA CGX CTG CGT CTG CGG TGC-3', in which X = dC, except in the fidelity studies, in which dA, dG, and dT were also employed. Primer strands were radioactively labeled at their 5'end using T4 polynucleotide kinase and [γ-^{32}P]ATP following the manufacturer's protocol. Primers and templates were annealed by incubation of a 20% excess of template at 95°C for 5min followed by cooling to RT over 4 h. End concentrations were 1 μM for primer and 1.2 μM for template.

Polymerase purification and concentration. Pol δ(exo-) and PCNA were purified as described.[3] The polymerase consists of four subunits in which the exonuclease activity of the catalytic subunit was inactivated. Pol κ, η and ι were purified from Sf9 insect cells as described.[4] The polymerases are full length proteins with an N-terminal His-tag. His-tagged human pol β was purified as described.[5] Pol ν was purified by a modification of the method described by Takata et al[6] as we previously described.[7] The protein has a deletion of a C-terminal poly proline segment, and an N-terminal His-tag, and C-terminal FLAG-tag. The concentrations of pol β, η, ι, κ, and ν were determined kinetically by reaction with 300 nM DNA and 100 μM dCTP and fit to equation 2. The amplitude was set as the active polymerase concentration.

Biochemical Methods

Polymerase kinetics. Enzyme reactions were initiated by mixing equal volumes of the DNA / polymerase solution in buffer with dNTP and $MgCl_2$ in H_2O at 37°C. The final concentrations for pol β η, ι, κ, and ν were 100 nM DNA, 10 nM polymerase, 40 mM Tris-HCl (pH 8.0), 3 mM DTT, 10 μg/mL BSA, 2.5% glycerol. The pol δ reactions contained 1 nM DNA, 0.1 nM pol δ (exo-), 10 nM PCNA, 20 mM Tris-HCl (pH 7.5), 8 mM $MgCl_2$, 40 μg/mL BSA, 5% glycerol. The reactions were quenched with equal volumes of STOP solution containing 10% 0.5 M Na_2EDTA, 90% formamide and 0.025% (w/v) xylene cyanol and 0.025% (w/v) bromophenol blue. Rapid reactions were performed on a RQF-3 (Kin-Tek Corporation) and quenched with 300 mM EDTA (pH 8). The reaction mixtures were analyzed by 15% polyacrylamide/7M urea denaturing gels and the radioactivity on the gels were visualized with a Typhoon 9200. The progress of the reaction was quantitated by dividing the total radioactivity in the product band(s) by the radioactivity in the product and reactant bands. Multiple product bands appeared when the incorrect dNTP was added to the reaction.

Data analysis. Data were fitted by nonlinear regression using the program Prism version 5 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). The V_{max} and K_m values for the pol δ catalyzed reactions were determined by fitting the data to hyperbolic equation 1, v_0 is the initial rate, V_{max} the maximum velocity, and N_0 the dNTP concentrations.

$$v_o = \frac{V_{max} N_0}{N_0 + K_m} \quad (1)$$

The time courses for the enzyme in excess were fitted to equation 2 in which *P* is the product concentration, *A* the burst amplitude, *k* the burst rate constant, k_{ss} the steady-state rate constant and *t* is time. If burst and slow phases were not evident, the data were fit to a first-order equation in which k_{ss} was set to 0. The dNTP concentration dependence was

quantified by fitting the burst equation parameters to the hyperbolic equation (1) in which v_0 is the value of the parameter at a specific [dNTP] and V_{max} is the maximum value of that parameter.

$$P = A(1 - e^{-kt}) + k_{ss}t \quad (2)$$

Cell Biology Methods

Cell lines. The human pancreatic ductal adenocarcinoma MiaPaCa-2 cell line was purchased from American Tissue Cell Culture (ATCC, Manassas, VA, USA) and Mouse embryo fibroblast (MEF) Polk$^{+/+}$, Polk$^{-/-}$ cell lines were a gift from Cyrus Vaziri, University of North Carolina. The cells were maintained in culture with Dulbecco's Modified Eagle's Medium (DMEM, ThermoFisher), supplemented with penicillin (100 IU/mL), streptomycin (100 µg/mL) and heat-inactivated fetal bovine serum (FBS) to a final concentration of 10% (v/v) at 37 °C in a water saturated 5% CO_2 atmosphere. Every 2 - 3 days cells were passaged using trypsinization for cells to detach in order to maintain them in culture.

EBdG Treatment. Cells were grown on sterile glass cover slips in a 6 well culture plate in DMEM media. After 40-50 % confluency was reached, EBdG (dissolved in DMSO) was added and the cells incubated for 1 to 24 h. The final concentration of DMSO were 0.2%. At the appropriate time, the coverslips were washed three times with PBS. The cells were fixed by incubation with freshly prepared 4% paraformaldehyde in PBS for 20 minutes at room temperature. The coverslip was washed with PBS.

EBdG Staining click chemistry. The fixed cells on the cover slips were incubated with 1 mL of Click Reaction Solution containing 0.5 mM CuTBTA, 25 µM 5(6)-carboxyfluorescein azide (5(6)-FAM azide), 100 mM sodium ascorbate in 55% DMSO/PBS for 2 h at room temperature. After reaction, the cells were washed several times with PBS containing 0.5% Triton X-100. The cells were DAPI-stained to reveal cellular DNA.

Fluorescence Microscopy The cells were mounted in standard mounting media and images were collected 50-ms exposure time with identical illumination and camera settings by using a Nikon Eclipse TE 2000-U fluorescent microscope.**.**

Synthetic Protocols.

The syntheses of N^2-butyl-dGTP (**5a**) and N^2-benzy-dGTP (**5b**) were carried out as described in Scheme 1. 3′,5′O-Bis(tert-butyldimethylsilyl)-O^6-(*p*-nitrophenylethyl)-2′-deoxy-2-fluoroguanosine (**2**) was synthesized as described by Adib et al. [8] The fluoride was displaced by treatment of 2 equivalents of amine in DMSO at room temperature. The reaction was followed by TLC. During the reaction, the *p*-nitrophenyl group was partially deprotected. Therefore, the crude product (**3**) was fully deprotected by treatment with TBAF prior to purification. The nucleosides purified by silica gel chromatography were contaminated with the tetra butylammonium cation. This material was further purified by reverse-phase HPLC. The triphosphates were made via the Ludwig procedure.[9] A similar protocol was employed, in the synthesis of N^2-4-ethynylbenzyl- 2'-deoxyguanosine (**4c**). except the O^6-position was protected with the trimethylsilylethyl group.[10] This group was stable during the amination with 4-ethynylbenzylamine.

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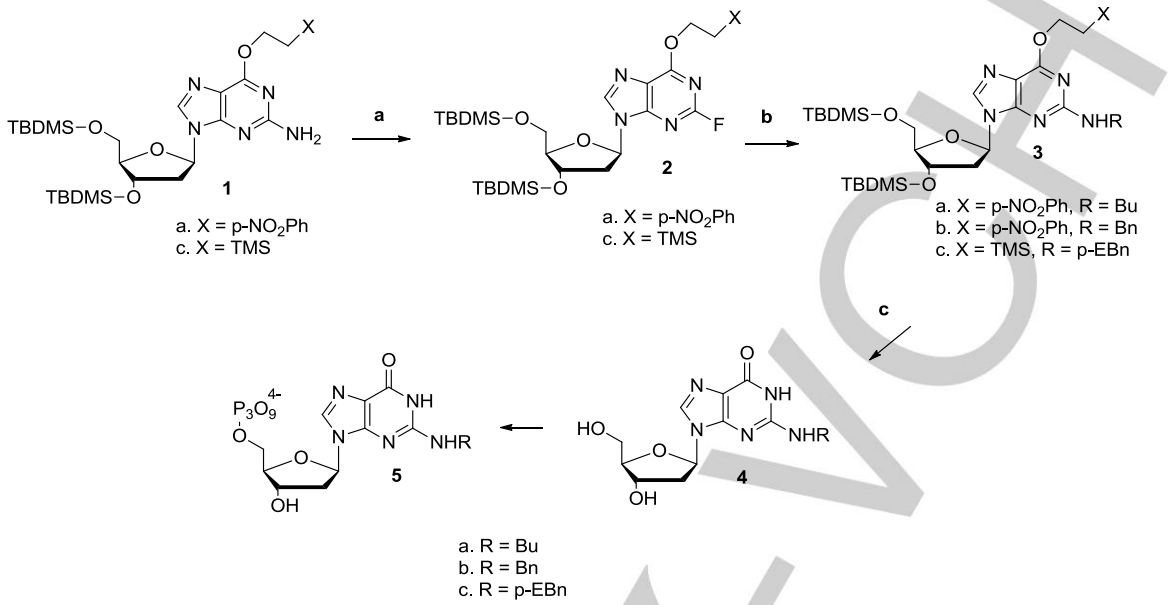
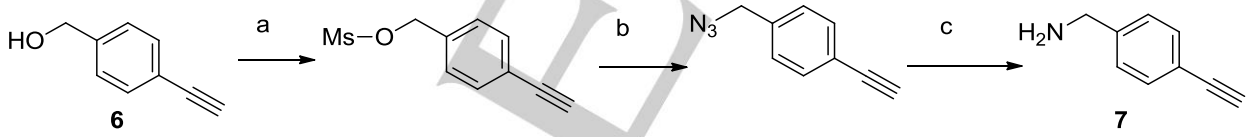
Scheme S1. Synthesis of N^2-alkyl-dGTP



a, PVPHF; b, R-NH_2/DMSO; c, TBAF; d, $POCl_3$ followed by tributylammonium pyrophosphate.

4-Ethynylbenzylamine (**7**) was prepared from 4-ethynylbenzyl alcohol (**6**) according to Scheme S2. The alcohol was mesylated, displaced with azide and reduced with LiAlH4 in a single pot.

Scheme S2. Synthesis of 4-ethynylbenzylamine



a. Ms-Cl; b, NaN_3; c, $LiAlH_4$.

3′,5′-O-Bis(tert-butyldimethylsilyl)-O^6-trimethylsilylethyl-2′-deoxyguanosine (1c)**.** 3′,5′-O-Bis(*tert*-butyldimethylsilyl)-2′-deoxy-guanosine (2.0 g, 4 mmol) was reacted with 2.1 g PPh_3 (8 mmol), 1.15 mL trimethylsilylethanol (8 mmol) and 1.25 mL diethyl azodicarboxylate (8 mmol) in 50 mL anhydrous THF for 8 h at room temperature. The reaction mixture was poured into 100 mL saturated $NaHCO_3$ and the product extracted with 100 mL ethyl acetate (3×). The combined organic layers were dried with brine and then $MgSO_4$. After filtration, the solution was evaporated and the product dissolved in 10 mL methylene chloride, which was loaded onto a 120 g flash column equilibrated with 10% EtOAc/hexane. The product was eluted with 20% EtOAc/hexane in a 50% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (s, 1H), 6.29 (t, J = 6.6 Hz, 1H), 4.92 (s, 2H), 4.55 (m, 3H), 3.95 (m, 1H), 3.77 (d/d, J = 10.8, 4.3, 1H), 3.73 (d/d, J = 10.9, 3.3 ,1H) , 2.54 (m, 1H), 2.32 (m, 1H), 1.20 (m, 2H), 0.89 (s, 9H), 0.88 (s, 9H), 0.077 (s, 6H), 0.055 (s, 9H), 0.049 (s, 3H), 0.043 (s, 3H) , 8.18 (s, 1H), 6.38 (t, J = 6.3 Hz, 1H), , 4.68 (m, 2H), 4.61 (m, 1H) , 4.00 (m, 1H), 3.87 11.6, 4.3, 3.77 11.1, 3.3, 2.60 (m, 1H), 2.42 (m, 1H), 1.27 (m, 2H), 0.91 (s, 9H), 0.90 (s, 9H), 0.10 (s, 6H), 0.09 (s, 9H), 0.085 (s, 3H), 0.081 (s, 3H). **^{13}C NMR** (500 MHz, $CDCl_3$) δ 161.37, 159.34, 153.46, 137.26, 116.02, 87.63, 83.55, 72.01, 64.78, 62.87, 40.83, 25.96, 25.76, 18.39, 17.99, 17.59, -1.42, -4.69, -4.80, -5.40, -5.52.

3′,5′-O-Bis(tert-butyldimethylsilyl)-O^6-trimethylsilylethyl-2-fluoro-2′-deoxy-guanosine (2c). 3′,5′-O-Bis(*tert*-butyldimethylsilyl)-*O*^{6}-trimethylsilylethyl-2′-deoxy-guanosine (**1c**) (1.12 g, 1.85 mmol) was dissolved in 50 mL toluene and cooled in NaCl/ice water. Polyvinylpyridine-HF (6.7 g) and *tert*-butyl nitrite (1.6 mL, 13.5 mmol) were added. After 1h, the

reactions was poured into 300 mL cold saturated $NaHCO_3$. The solid was filtered, and the product extracted with EtOAc. The product was purified by silica gel chromatography by elution with 10% EtOAc/hexane in a 45% yield.

^{1}H NMR 8.18 (s, 1H), 6.38 (t, J = 6.3 Hz, 1H), 4.68 (m, 2H), 4.61 (m, 1H) , 4.00 (m, 1H), 3.87 11.6, 4.3, 3.77 11.1, 3.3, 2.60 (m, 1H), 2.42 (m, 1H), 1.27 (m, 2H), 0.91 (s, 9H), 0.90 (s, 9H), 0.10 (s, 6H), 0.09 (s, 9H), 0.085 (s, 3H), 0.081 (s, 3H). **^{13}C NMR** (500 MHz, CDCl3) 162.69, 162.55, 158.88, 157.18, 152.61, 152.46, 140.89, 140.86, 120.22, 120.17, 88.04, 84.58, 71.86, 66.69, 62.74, 41.27, 25.96, 25.75, 18.41, 18.00, 17.59, -1.46, -4.68, -4.82, -5.42, -5.51,

3',5'-O-Bis(tert-butyldimethylsilyl)-O^6-trimethylsilylethyl-N^2-p-ethynylbenzyl-2′-deoxy-guanosine.(3c). 3′,5′-O-Bis(tert-butyldimethylsilyl)-*O*^{6}-trimethylsilylethyl-2′-deoxy-2-fluoroguanosine (**2c**) (0.5 mmol) was reacted with alkylamine (1.0 mmol) for 5 h in 3 mL anhydrous DMSO. The reaction was poured into sat. $NaHCO_3$ and extracted with ethyl acetate (3 ×). The combined organic phase was dried by washing with brine and then $MgSO_4$. The filtered solution was evaporated and the product was purified by flash chromatograph, eluting with 10% EtOAc/hexane in a 60% yield

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.43(d, J = 7.9 Hz, 2H), 7.31(d, J = 7.9 Hz, 2H), 6.28 (t, J = 6.5 Hz, 1H) 5.32 (t, J = 6.0 Hz, 1H), 4.62 (d, J = 6.0 Hz, 2H), 4.5 (m, 3H), 3.96 (m, 1H), 3.79 (d/d, J= 11.2, 4.6 Hz, 1H), 3.75 (d/d, J= 11.2, 3.6 Hz, 1H), 3.04 (s, 1H), 2.57(m, 1H), 2.28 (m, 1H), 1.19 (m, 2H), 0.905 (s, 9H) 0.90 (s, 9H), 0.090 (s, 9H), 0.0635 (s, 3H), 0.061 (s, 3H), 0.045 (s, 9H). **^{13}C NMR** (500 MHz, $CDCl_3$) δ 161.14, 158.79, 153.45, 140.78, 138.25, 132.22, 127.31, 120.78, 115.87, 87.56, 83.80, 83.55, 72.16, 64.63, 62.98, 45.78, 40.61, 25.97, 25.76, 18.40, 17.99, 17.60, -1.44, -4.67, -4.77, -5.37, -5.46.

N^2-butyl-2′-deoxyguanosine (4a). 3′,5′-O-Bis(tert-butyldimethylsilyl)-*O*^{6}-(*p*-nitrophenylethyl)-2′-deoxy-2-fluoroguanosine[8] (**2a**) (350 mg, 0.54 mmol) was reacted with 106 μL butylamine (1.08 mmol) for 6 h in 3 mL anhydrous DMSO. The fluoride was displaced by treatment of 2 equivalents of amine in DMSO at room temperature. The reaction was poured into sat. $NaHCO_3$ and extracted with EtOAc (3 ×). The combined organic phase was dried by washing with brine and then $MgSO_4$. During the reaction, the *p*-nitrophenyl group was partially deprotected, thus the crude product was completely deprotected. The filtered solution was evaporated and the oil was dissolved in 2 mL 1 M tetra-*n*-butylammonium fluoride. After 2h the solution was loaded onto a silica column (120 g) which was eluted with EtOAc, 10% MeOH/EtOAc, 5% H_2O/10% MeOH/EtOAc. The yield was 30%.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.45 (s, 1H), 7.88 (s, 1H), 6.44 (t, J = 5.35 Hz,, 1H), 6.11 (d/d, J = 7.53, 6.43 Hz, 1H) , 5.22 (d, J =4.05 Hz, 1H), 4.80 (t, J = 5.35 Hz, 1H), 4.33 (m, 1H) , 3.8 (m, 1H), 3.55 (m, 1H), 3.48 (m, 1H), 3.25 (m, 2H), 2.61 (m, 1H), 2.20 (m, 1H), 1.5 (m,2H), 1.3 (m,2H), 0.90(t, J = 7.35 Hz, 3H). **^{13}C NMR** (500 MHz, DMSO-d_6): 157.16, 153.04, 151.01, 136.28, 117.33, 88.11, 83.37, 71.40, 62.37, 58.0, 31.33, 19.94, 14.12. **MS-ESI** *m/z* 324.3 ([M+H], 208.3 [M – deoxyribose +2H] +. **HRMS-ESI+,** Calculated for $C_{14}H_{22}N_5O_4$, 324.1679 , found 324.1679.

N^2-Benzyl-2′-deoxyguanosine (4b). 3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-O^6-(*p*-nitrophenylethyl)-2′-deoxy-2-fluoroguanosine (**2a**) (235 mg, 0.36 mmol) was reacted with 81 μL benzylamine (0.74 mmol) for 5 h in 2 mL anhydrous DMSO. The reaction was poured into sat. $NaHCO_3$ and extracted with ethyl acetate (3 ×). The combined organic phase was dried by washing with brine and then $MgSO_4$. The filtered solution was evaporated and the oil was dissolved in 2 mL 1 M tetra-n-butylammonium fluoride. After 2h the solution was loaded onto a 40 g silica column which was eluted with EtOAc, 10% MeOH/EtOAc, 5% H_2O/10% MeOH/EtOAc. The yield was 33 %.

^{1}H NMR (500 MHz, DMSO-d_6) 10.45 (s, 1H), 7.88 (s, 1H), 7.3 (m, 4H). 7.0 (m, 1H), 6.15 (d/d, J= 7.48, 6.48, 1H), 5.23 (d, J = 4.05 Hz, 1H), 4.82 (t, J = 5.4 Hz, 1H), 4.32 (m, 1H), 3.79 (m, 1H), 3.545 (m, 1H), 3.45 (m, 1H), 2.56 (m, 1H), 2.16 (m, 1H). **^{13}C NMR** (500 MHz, DMSO-d_6):157.33, 153.05, 150.81, 139.52, 136.34, 128.85, 127.92, 127.50, 117.59, 85.82, 83.40, 79.65, 71.34, 62.30, 48.23, 44.53. MS-ESI m/z 358.2 $[M+H]^+$, 241.1 [M-deoxyribose+H]$^+$ **HRMS-ESI+:** Calculated for $C_{17}H_{20}N_5O_4$ 358.1515, found 358.1512.

***N^2-p*-ethynylbenzyl-2′-deoxyguanosine.** (4c) .3′,5′-O-Bis(tert-butyldimethylsilyl)-*O*^{6}-trimethylsilylethyl-*N*^{2}-*p*-ethynylbenzyl-2′-deoxy-2-fluoroguanosine was stirred with 10 equivalents of 1 M tetra-n-butylammonium fluoride for 1 h at room temperature. The solution was loaded onto a 40 g silica column which was eluted with EtOAc and then 10% MeOH/EtOAc. The yield was 66%.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.65 (s, 1H), 7.96 (s, 1H), 7.45(d,, 2H), 7.35 (d, 2H), 6.9 (m, 1H), , 6.10 (t, 1H) , 5.41 (d, 1H), 4.80 (t, 1H), 4.33 (m, 1H) , 3.8 (m, 1H), 3.55 (m, 1H), 3.48 (m, 1H), 3.25 (t, 2H), 2.61 (m, 1H), 2.20 (m, 1H), 1.5 (m,2H), 1.3 (m,2H), 0.90(t, 3H). **MS-ESI.** 382.2 [M+1], 266.3[M+1-dR]. **HRMS-ESI+:** Calculated for $C_{19}H_{20}N_5O_4$, 382.1515, found 382.1510.

***N^2*-butyl-2′-deoxyguanosine 5'-O-triphosphate (5a) and *N^2*-benzyl-2′-deoxyguanosine 5'-O-triphosphate** (5b). *N*^{2}-Alkyl-2′-deoxyguanosine (4) (170 mmol) and tributylamine (100 μL, 430 mmol) were dissolved in 1 mL trimethyl phosphate

that was dried over molecular sieves. The solution was cooled in a NaCl/ice water bath. Freshly distilled $POCl_3$ (24 μL, 255 mmol) was added and the reaction was stirred for 5 min. An additional 16 μL POCl3 (170 mmol) was added and the reaction was stirred for an additional 5 min. To this solution was added an ice-cold solution of anhydrous acetonitrile (1 mL) containing 75 mg tributylammomnium pyrophosphate (200 mmol) and 245 μL tributylamine (1050 mmol). After stirring for 10 min, the reaction was quenched by addition of 10 mL cold H_2O. The aqueous solution was washed with CH_2Cl_2 and evaporated. The solid was redissolved in H_2O and loaded onto a 1 × 10 cm DEAE-cellulose that was equilibrated with 50 mM triethylamine-carbonate (pH 7.5). The column was eluted with a 500 mL gradient from 50 mM to 1 M triethylamine-carbonate (pH 7.5). The triphosphate eluted at approximately 500 mM buffer. The total conversion was 10-15 % for the various syntheses.

***N*^{2}-butyl-2′-deoxyguanosine 5'-triphosphate.** **^{1}H NMR** (500 MHz, D_2O) δ 10.45 (s, 1H), 7.88 (s, 1H), 6.44 (t, J = 5.35 Hz,, 1H), 6.11 (d/d, J = 7.53, 6.43 Hz, 1H) , 5.22 (d, J =4.05 Hz, 1H), 4.80 (t, J = 5.35 Hz, 1H), 4.33 (m, 1H) , 3.8 (m, 1H), 3.55 (m, 1H), 3.48 (m, 1H), 3.25 (t, J = , 2H), 2.61 (m, 1H), 2.20 (m, 1H), 1.5 (m,2H), 1.3 (m,2H), 0.90(t, J = 7.35 Hz, 3H). **^{31}P NMR** (500 MHz, D_2O) -10.4, -11.1, -23.1. **MS-ESI** 564.4 [M+1], 208.2 [M-dRTP+1]. **HRMS-ESI-**: calculated for $C_{14}H_{23}N_5O_{13}P_3$: 562.0505; found: 562.0491.

***N*^{2}-benzyl-2'-deoxyguanosine 5'-triphosphate.** **^{1}H NMR** (500 MHz, D_2O) 10.45 (s, 1H), 7.88 (s, 1H), 7.3 (m, 4H). 7.0 (m, 1H), 6.15 (d/d, J= 7.48, 6.48, 1H), 5.23 (d, J = 4.05 Hz, 1H), 4.82 (t, J = 5.4 Hz, 1H), 4.32 (m, 1H), 3.79 (m, 1H), 3.545 (m, 1H), 3.45 (m, 1H), 2.56 (m, 1H), 2.16 (m, 1H). **^{31}P NMR** (500 MHz, D_2O) -9.6, -11.1, -22.9. **MS-ESI** 598.2 [M+1], 242.1 [M-dRTP+1]. **HRMS-ESI-** calculated for $C_{14}H_{23}N_5O_{13}P_3$: 596.0349; found: 596.0339.

4-Ethynylbenzylamine (7). (4-Ethynylphenyl)methanol (**6**) (2.7 g, 20.4 mmol) was dissolved in 50 mL anhydrous THF and cooled in an ice bath under nitrogen atmosphere. Triethylamine (4.3 mL, 25.5 mmol) and 1.9 mL methanesulfonyl chloride (25 mmol) were added and the reaction was stirred for 45 min. The solvent was reduced to 10 mL and the reaction quenched by the addition of 20 mL cold $NaHCO_3$. The product was extracted with CH_2Cl_2 (3x). The combined extracts were dried with brine and $MgSO_4$. The filtered CH_2Cl_2 was evaporated to an oil which was dissolved in anhydrous DMF (30 mL). Sodium azide (2.7 g, 42 mmol) was added and the reaction was stirred overnight. The DMF was evaporated, the oil mixed with 50 mL H_2O and the product was extracted with 50 mL CH_2Cl_2 (3x). The combined organic extracts were dried and evaporated. The crude azide was dissolved in 30 mL anhydrous THF and cooled in an ice H_2O bath. 2.4 g $LiALH_4$ was added and stirred for 1 h.

^{1}H NMR (500 MHz, $CDCl_3$) 7.74 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 3.87 (s, 3H), 3.05 (s, 1H), 1.47 (s, 2H). **HRMS-ESI+**: calculated for $C_9H_{10}N$, 132.0813; found: 132.0805.

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Results

List of Tables

List of Figures

SUPPORTING INFORMATION

Table S1. Kinetic parameters for the incorporation of N^2-alkyl-dGTPs opposite dC.[a]

	dGTP		N^2-Me-dGTP		N^2-Bu-dGTP		N^2-Bn-dGTP	
pol	k_{pol} (s^{-1})	K_d (μM)	k_{pol} (s^{-1})	K_d (μM)	k_{pol} (s^{-1})	K_d(μM)	k_{pol} (s^{-1})	K_d (μM)
β	4.3 ± 0.2	12 ± 2	0.0088 ± 0.0001	84 ± 4	$k_{pol}/K_d^{dNTP} < 10^{-6}$		$k_{pol}/K_d^{dNTP} < 10^{-6}$	
η	14 ± 3	23 ± 10	15± 7	84 ± 58	(20 ± 10) × 10^{-4}	50 ± 5	$k_{pol}/K_d^{dNTP} < 10^{-6}$	
ι	12 ± 1	21 ± 6	6.5 ± 1.3	18 ± 9	(9 ± 2) × 10^{-4}	180 ± 10	(11 ± 1) × 10^{-4}	29 ± 1
κ	12 ± 1	11 ± 2	11 ± 1	20 ± 5	0.56 ± 0.02	14 ± 2	9.3 ± 2.7	23 ± 6
ν	17 ± 1	26 ± 5	(6.0 ± 0.2) × 10^{-4}	80 ± 4	(3.7 ± 0.2) × 10^{-4}	74 ± 14	(8.2 ± 0.3) × 10^{-4}	210 ± 20
	k_{cat} (s^{-1})	**K_m** (μM)	k_{cat} (s^{-1})	**K_m** (μM)	k_{cat} (s^{-1})	Km (μM)	k_{cat} (s^{-1})	**K_m** (μM)
δ	0.066 ± 0.003	54 ± 6	(3.1 ± 0.2) × 10^{-4}	88 ± 12	(1.9 ± 0. 2)× 10^{-5}	81 ± 24	(1.5 ± 0.4) × 10^{-6}	81 ± 76

a. Reactions contained 100 nM DNA, 10 nM polymerase, 40 mM Tris-HCl (pH 8.0), 3 mM DTT, 10 μg/mL BSA, 2.5% glycerol. The pol δ reactions contained 1 nM DNA, 0.1 nM pol δ (exo-), 10 nM PCNA, 20 mM Tris-HCl (pH 7.5), 8 mM $MgCl_2$, 40 μg/mL BSA, 5% glycerol. The values are the mean of three experiments ± s.e.m.. The DNA substrate was:

5′- G C A C C G C A G A C G C A G -3′
3′- C G T G G C G T C T G C G T C **C** G C A G C G T C -5'

Table S2. Fidelity of pol κ incorporation of dGTP and N^2-Bn-dGTP[a]

dGTP				
template	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	f_{inc}
dA	0.071 ± 0.0060	226 ± 43	5.22 ± 0.59	0.072
dC	0.51 ± 0.02	117 ± 12	72 ±5	1
dG	0.092 ± 0.008	304 ± 55	5.013 ± 0.49	0.069
dT	0.089 ± 0.006	275 ± 42	5.37 ± 0.45	0.074

N^2-Bn-dGTP				
template	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	f_{inc}
dA	0.021 ± 0.003	599 ± 186	0.59 ± 0.09	0.014
dC	0.170 ± 0.006	71 ± 7	40 ± 3	1
dG	0.0073 ± 0.0003	174 ± 22	0.70 ± 0.07	0.017
dT	0.0064 ± 0.0004	169 ± 38	0.63 ± 0.11	0.015

a. Reactions were conducted with 10 nM DNA, 0.1 nM polymerase, 40 mM Tris-HCl (pH 8.0), 3 mM DTT, 10 μg/mL BSA, 2.5% glycerol, at 37 °C. The DNA substrate was:

5'- G C A C C G C A G A C G C A G -3'
3'- C G T G G C G T C T G C G T C **X** G C A G C G T C -5'

In which the template base (X) is listed. The incubation times varied from 5 - 10 min for dC as template to 0.5 - 2 h for mispair formation. The values are the mean of three experiments ± the standard error.

b. $f_{inc} = (k_{cat}/K_m)^{dN} / (k_{cat}/K_m)^{dC}$

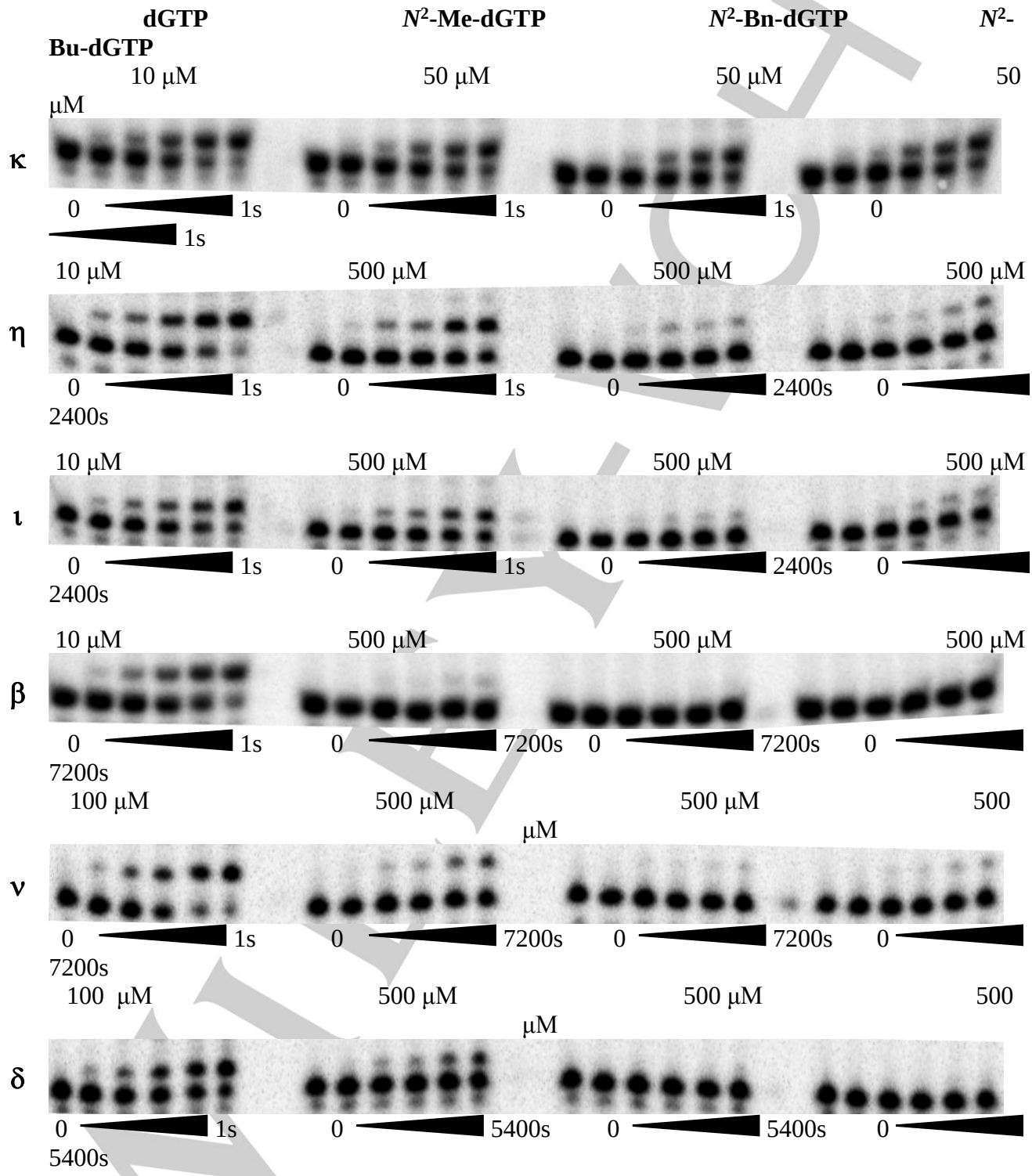


Figure S1. PAGE analysis of the incorporation of dGTP and N^2-alkyl-dGTPs opposite dC by pol κ, η, ι, β, ν, and δ/PCNA. The DNA concentration was 10 nM, the dNTP concentration is as listed. The polymerase

concentration varied from 10 to 100 nM to obtain similar reaction profiles for the dGTP reactions. In each panel, the lower band is the 15-mer starting material and the upper band is the 16-mer product.

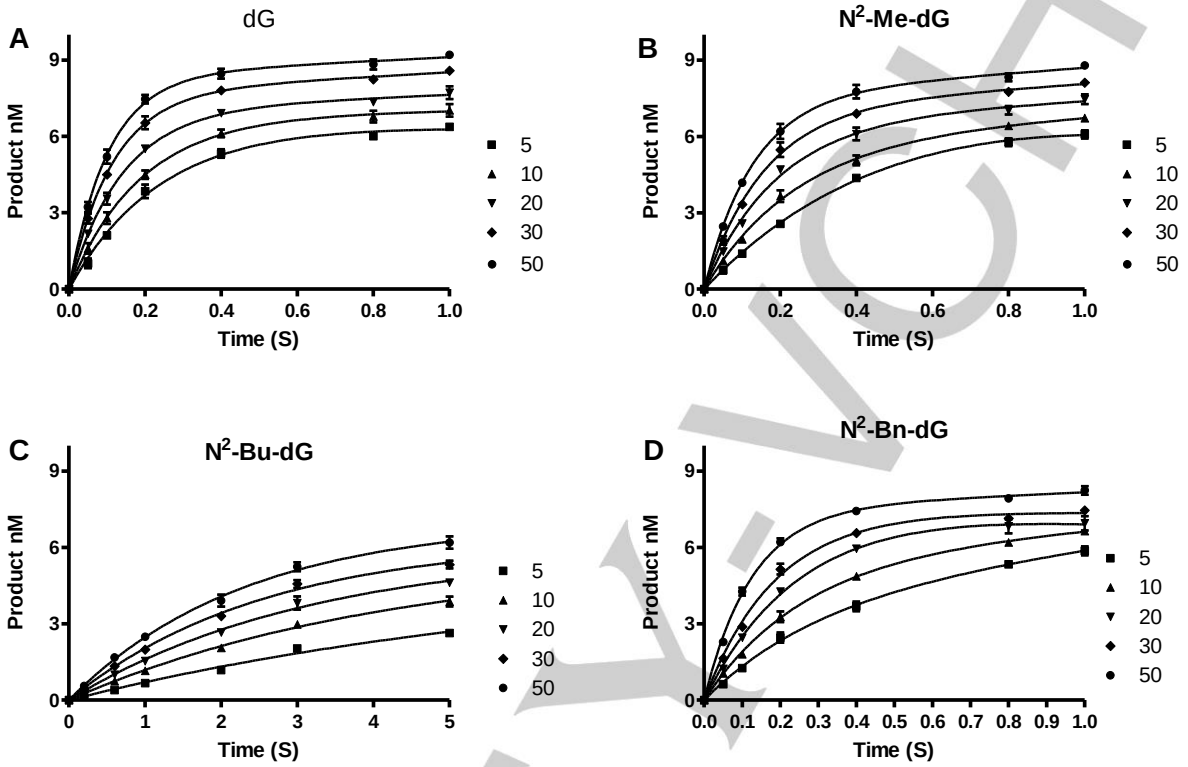


Figure S2. Pol κ catalyzed incorporation of *N*^{2}-alkyl-dGTP opposite dC time course. The reaction was carried out with 100 nM pol κ, 10 nM DNA, with various concentrations of (A) dGTP, (B) *N*^{2}-Me-dGTP, (C) *N*^{2}-Bu-dBGTP, and (D) *N*^{2}-Bn-dGTP. The lines are the best fit to the burst equation.

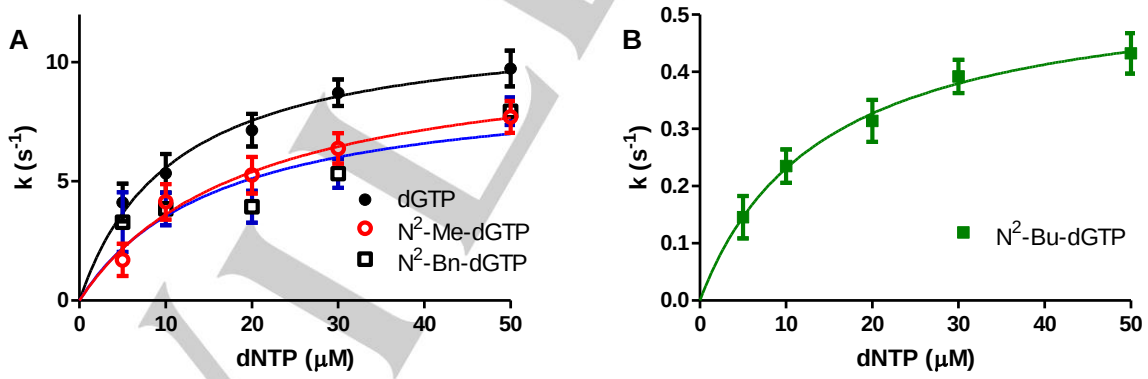


Figure S3. Pol κ catalyzed incorporation of *N*^{2}-alkyl-dGTP opposite dC. dNTP-concentration dependence on the burst rate constant. The lines are the best fit to the hyperbolic equation.

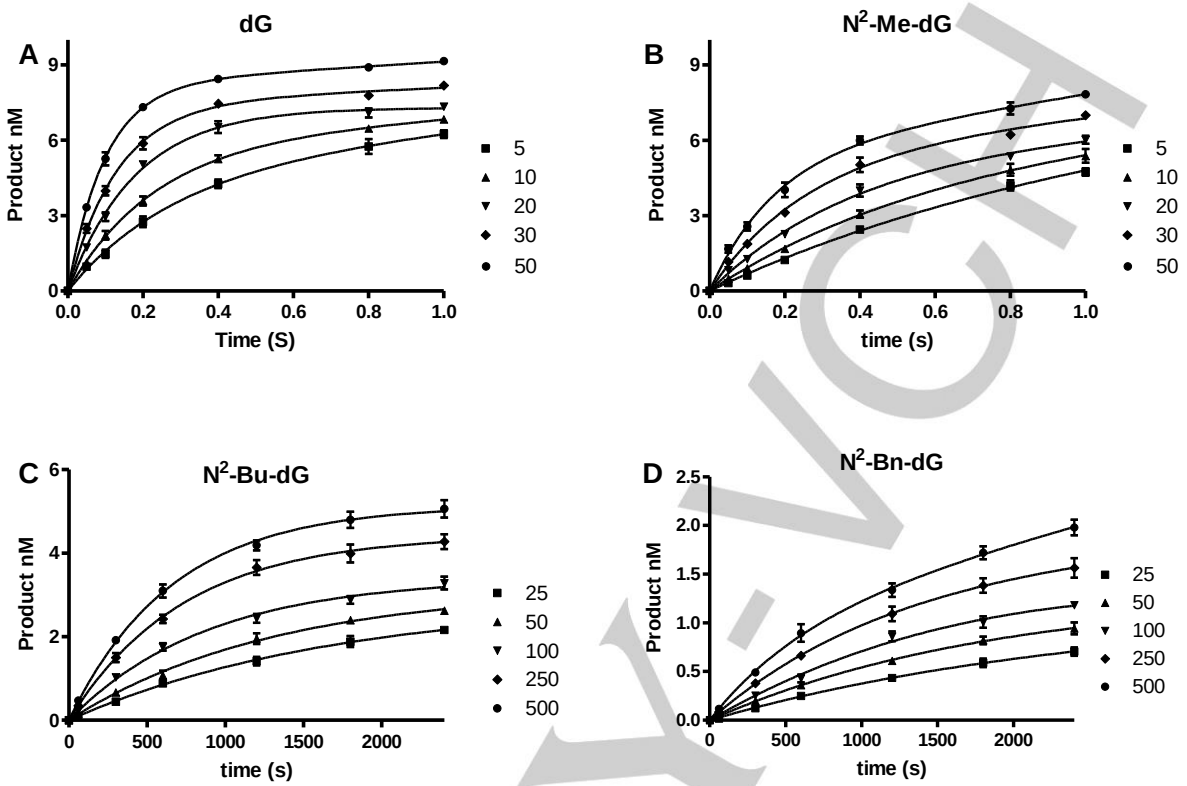


Figure S4. Pol η catalyzed incorporation of *N*^{2}-alkyl-dGTP opposite dC time course. The reaction was carried out with 100 nM pol κ, 10 nM DNA, with various concentrations of (A) dGTP, (B) *N*^{2}-Me-dGTP, (C) *N*^{2}-Bu-dBGTP, and (D) *N*^{2}-Bn-dGTP.

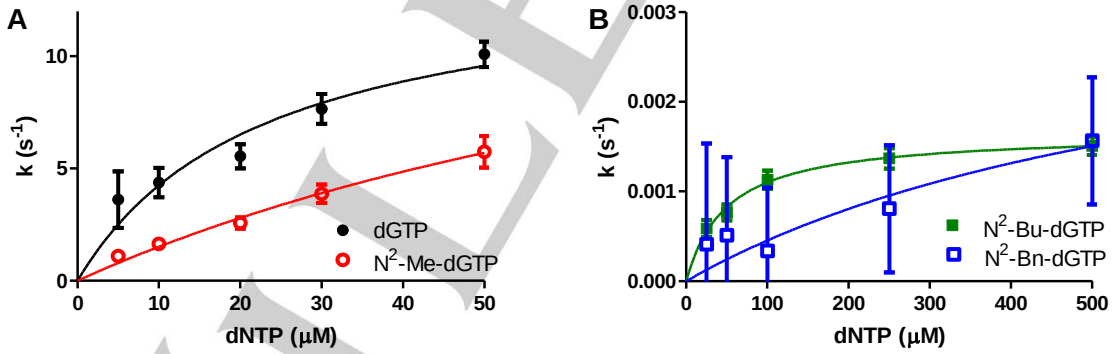


Figure S5. Pol η catalyzed incorporation of *N*^{2}-alkyl-dGTP opposite dC. dNTP-concentration dependence on the burst rate constant. The lines are the best fit to the hyperbolic equation.

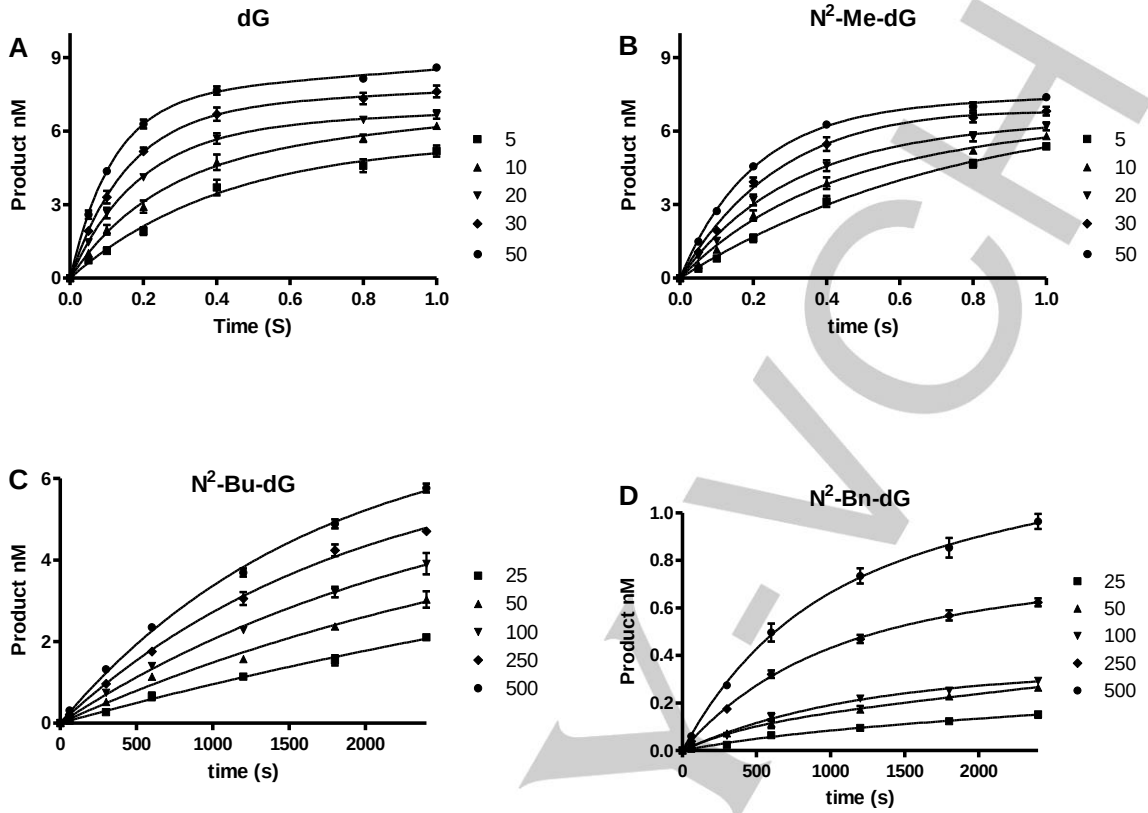


Figure S6. Pol ι catalyzed incorporation of N^2-alkyl-dGTP opposite dC time course. The reaction was carried out with 100 nM pol κ, 10 nM DNA, with various concentrations of (A) dGTP, (B) N^2-Me-dGTP, (C) N^2-Bu-dBGTP, and (D) N^2-Bn-dGTP.

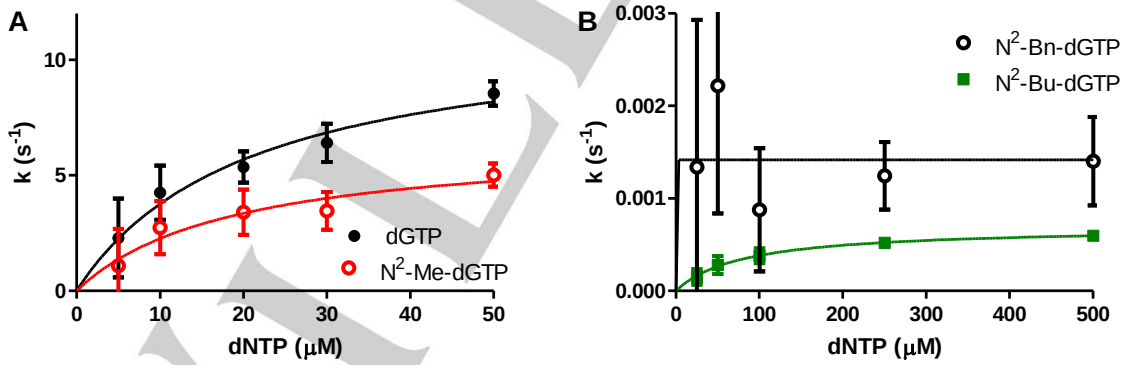


Figure S7. Pol ι catalyzed incorporation of N^2-alkyl-dGTP opposite dC. dNTP-concentration dependence on the burst rate constant. The lines are the best fit to the hyperbolic equation. The N^2-Bn-dGTP reaction does not give k_{pol} or K_d values but gives an upper estimate of k_{pol}/K_d.

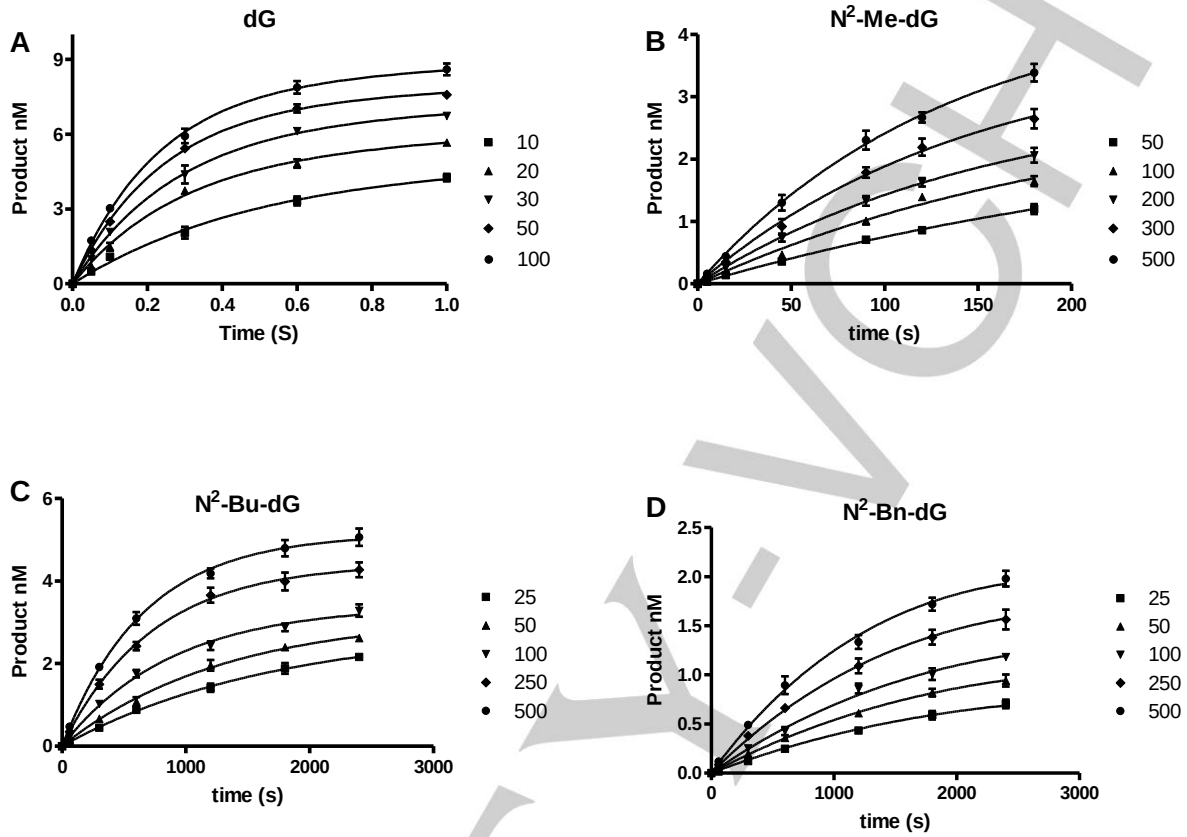


Figure S8. Pol β catalyzed incorporation of N^2-alkyl-dGTP opposite dC. The reaction was carried out with 100 nM pol κ, 10 nM DNA, with various concentrations of (A) dGTP, (B) N^2-Me-dGTP, (C) N^2-Bu-dBGTP, and (D) N^2-Bn-dGTP.

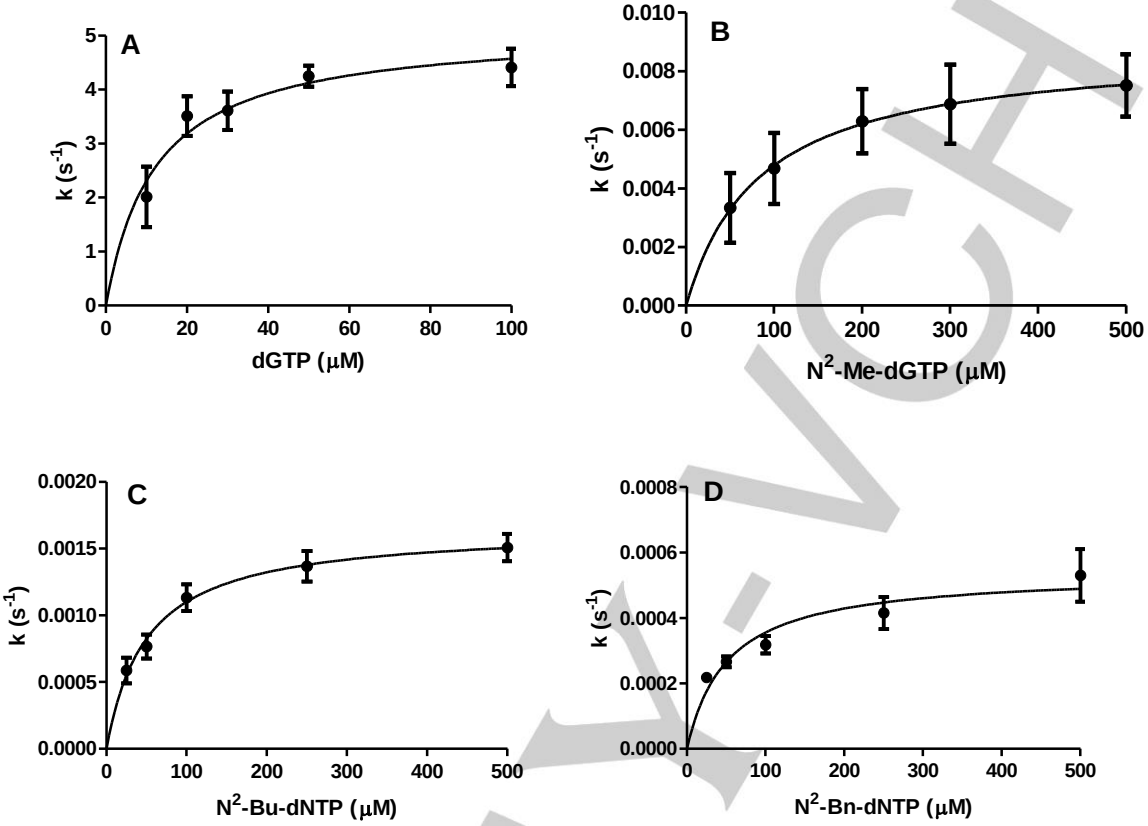


Figure S9. Pol β catalyzed incorporation of *N*^{2}-alkyl-dGTP opposite dC. dNTP-concentration dependence on the burst rate constant. The lines are the best fit to the hyperbolic equation

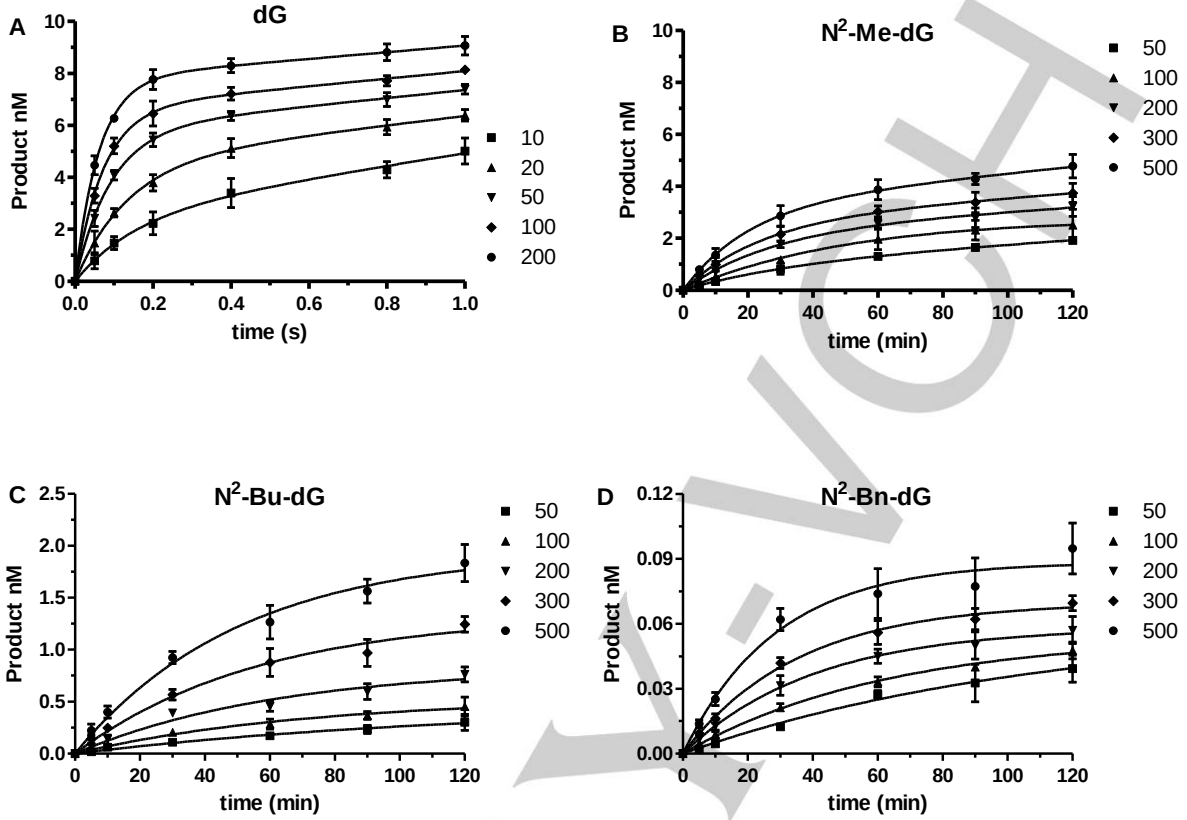


Figure S10. Pol ν catalyzed incorporation of N^2-alkyl-dGTP opposite dC. The reaction was carried out with 100 nM pol κ, 10 nM DNA, with various concentrations of (A) dGTP, (B) N^2-Me-dGTP, (C) N^2-Bu-dBGTP, and (D) N^2-Bn-dGTP. The lines are the best fit to the burst equation (A and B) and the first-order equation (C and D).

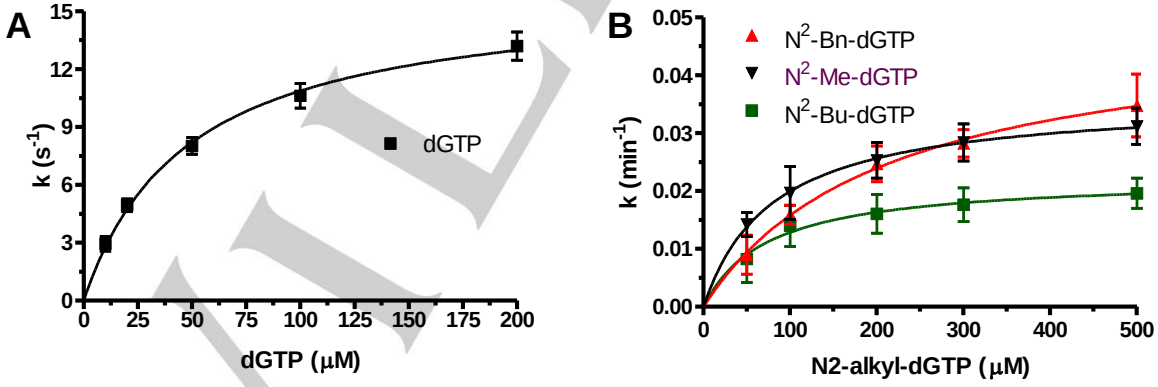


Figure S11. Pol ν catalyzed incorporation of N^2-alkyl-dGTP opposite dC. dNTP-concentration dependence on the burst rate constant. The lines are the best fit to the hyperbolic equation

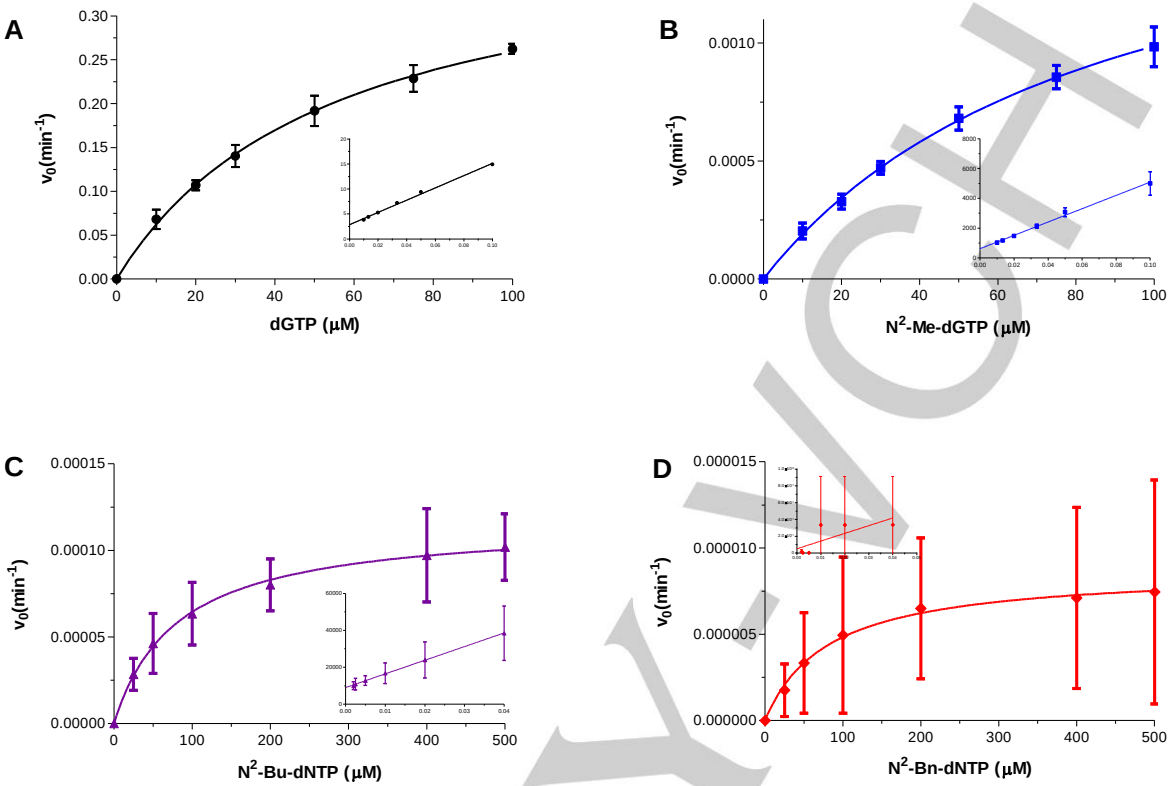


Figure S12. Pol δ-catalyzed incorporation of N^2-alkyl-dGTP opposite dC. The reaction was carried out with 0.1 nM pol δ, 10 nM PCNA, 1 nM DNA, with various concentrations of (A) dGTP, (B) N^2-Me-dGTP, (C) N^2-Bu-dBGTP, and (D) N^2-Bn-dGTP. The lines are the best fit to the hyperbolic equation. The inserts are the Lineweaver-Burk plots.

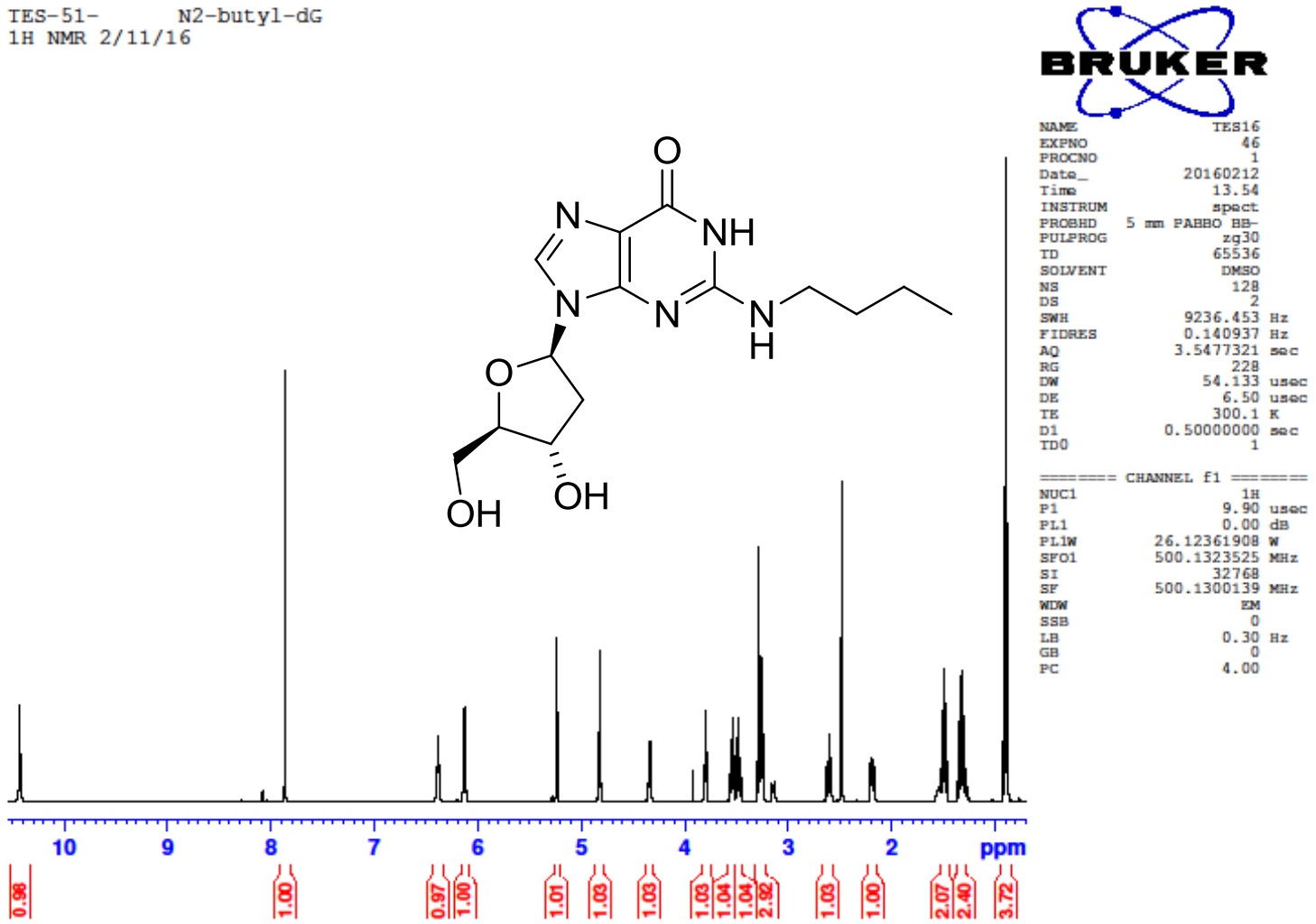


Figure S13. 1H NMR (DMSO-d6) N2-butyl-2'-deoxyguanosine.

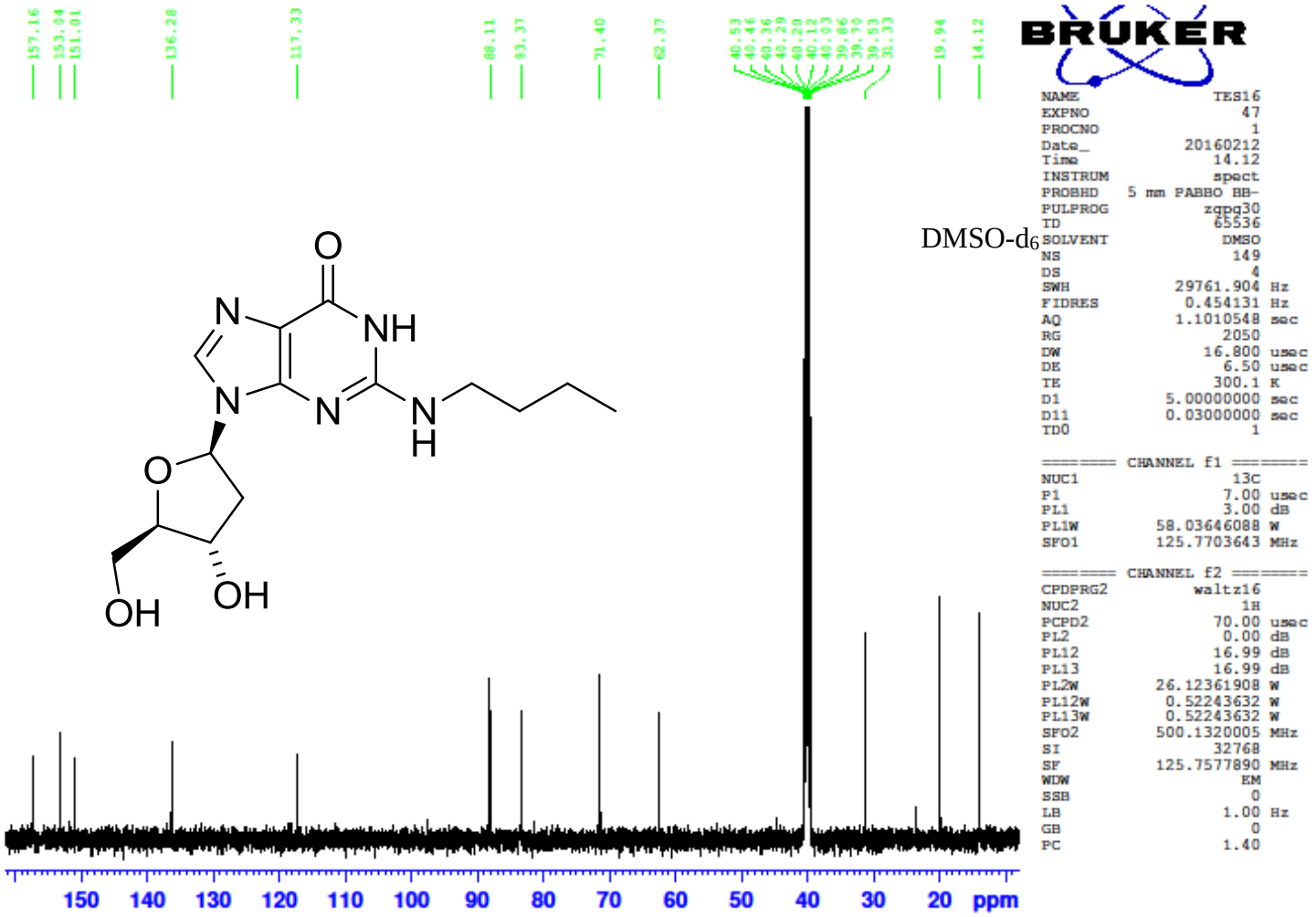


Figure S14. ^{13}C NMR (DMSO-d$_6$) *N*^{2}-butyl-2'-deoxyguanosine.

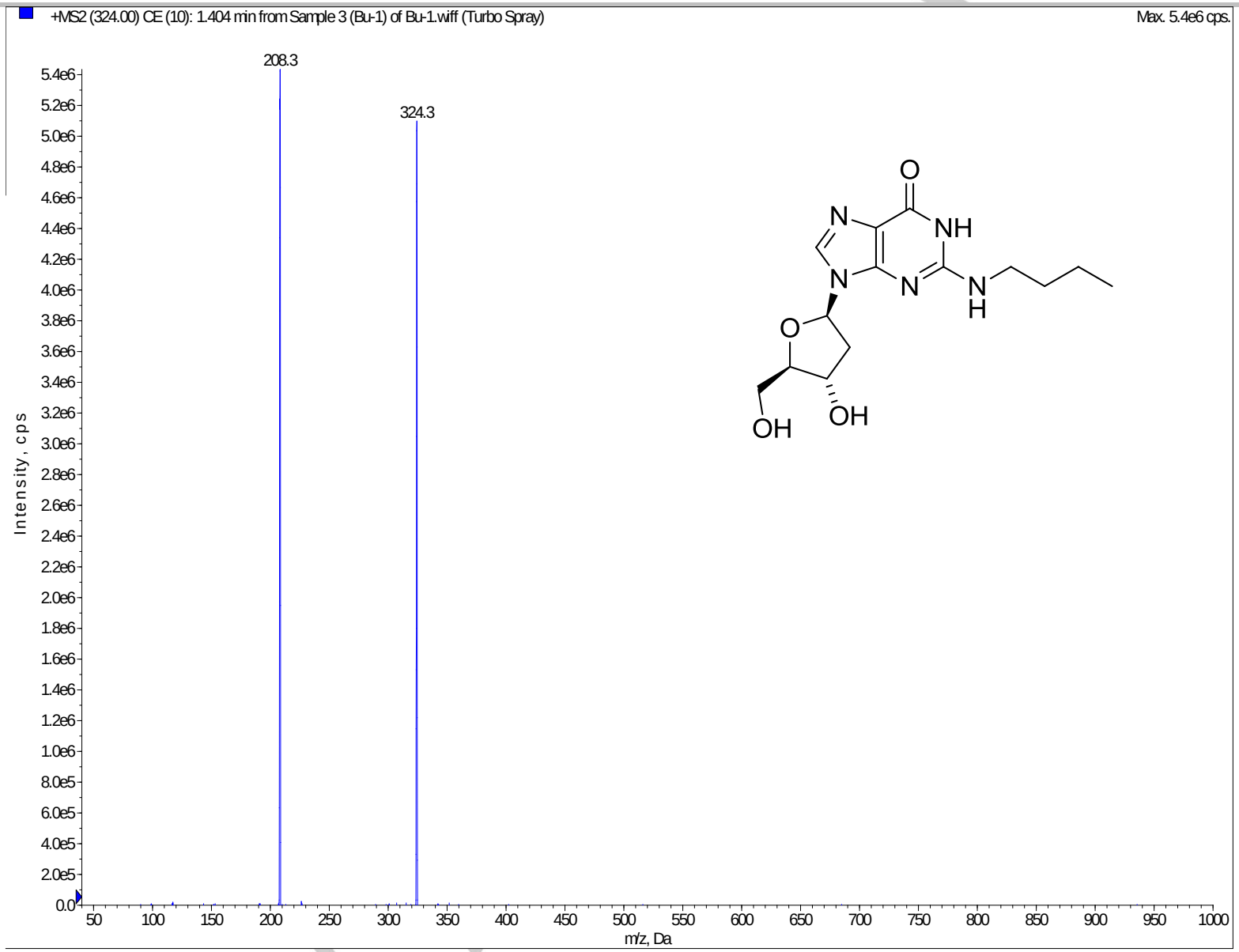


Figure S15. ESI MS N^2-butyl-dG

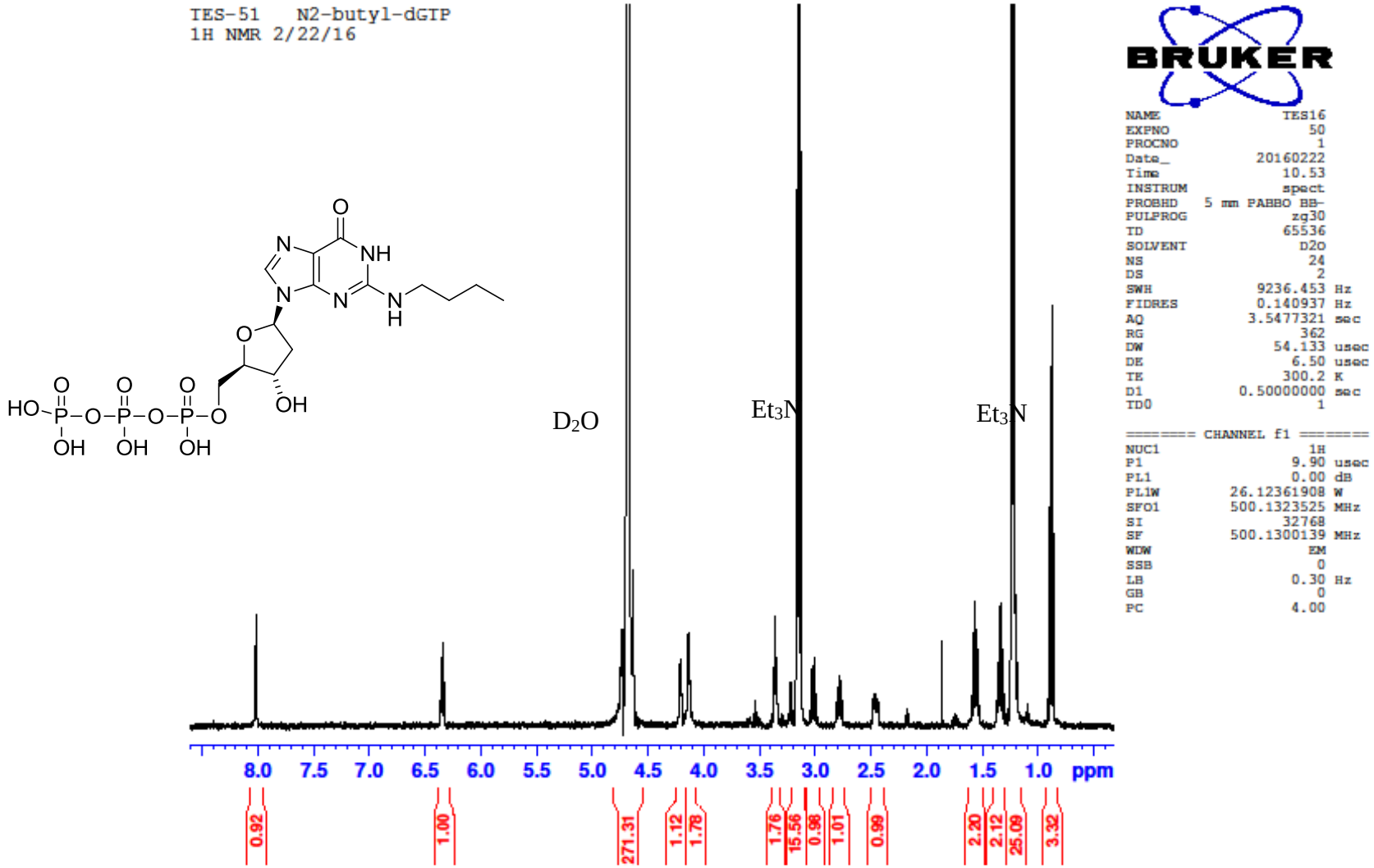


Figure S16. ^{1}H NMR (D_2O) N^2-butyl-dGTP

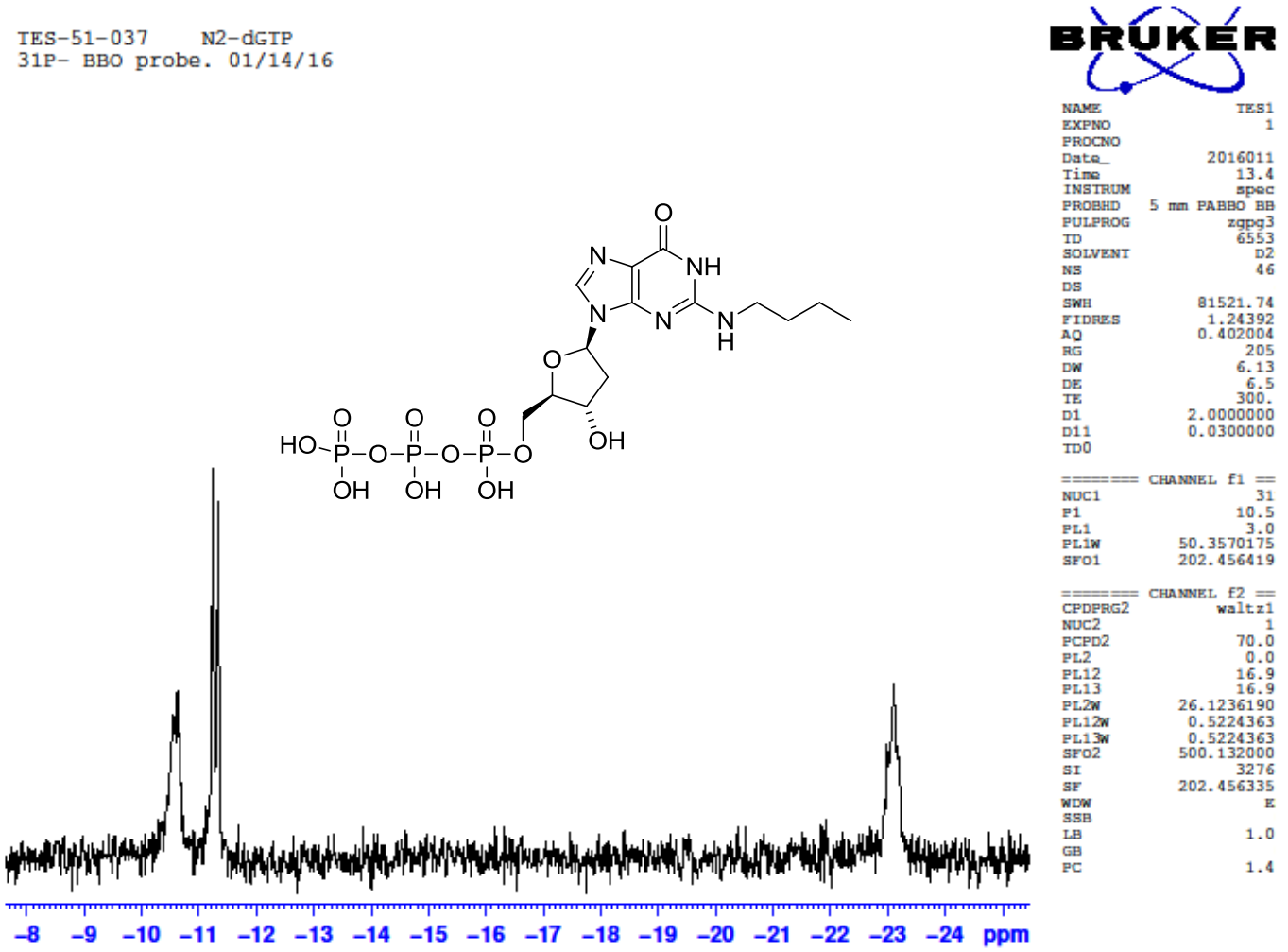


Figure S17. ^{31}P NMR (D_2O) N^2-butyl-dGTP

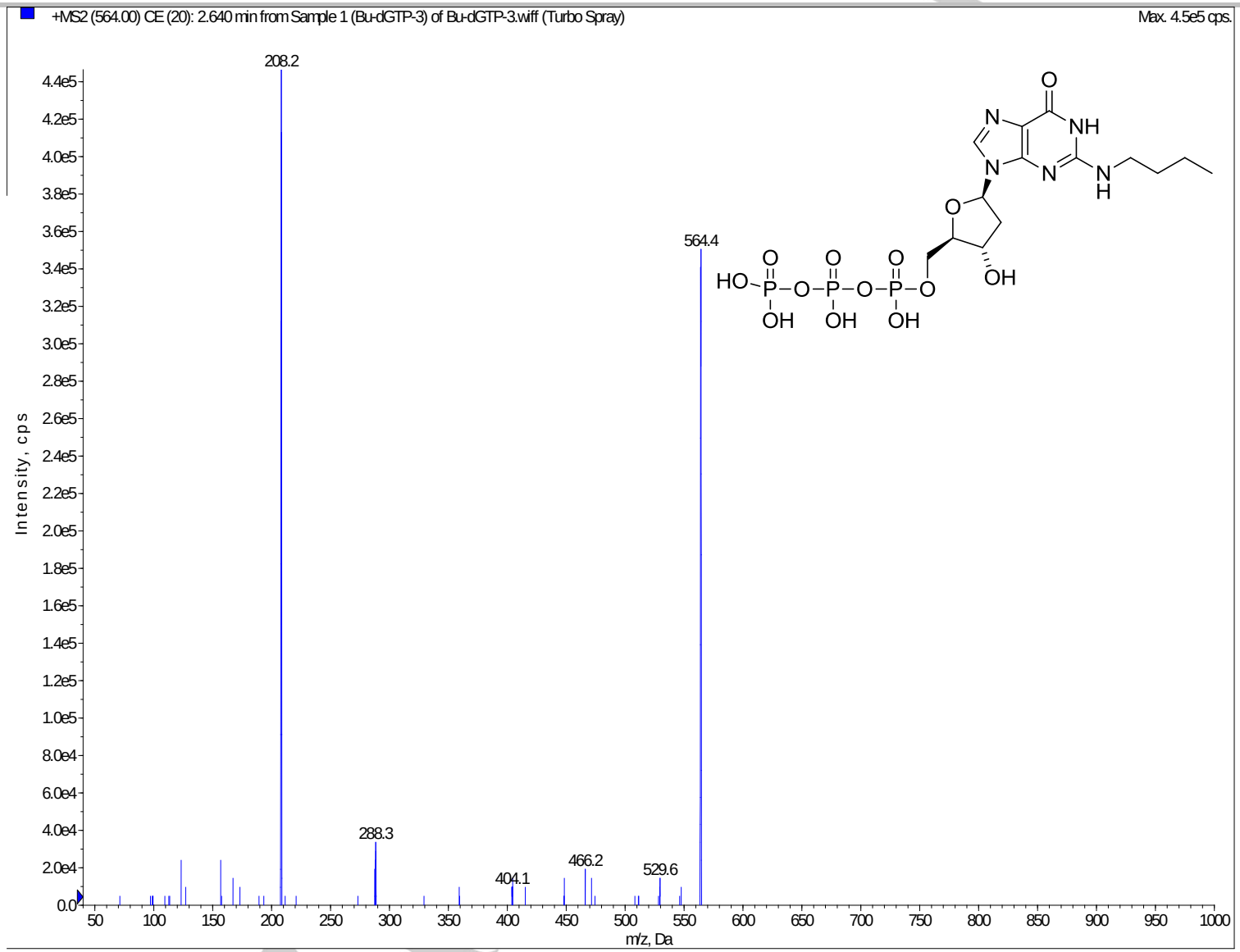


Figure S18. ESI MS N^2-butyl-dGTP

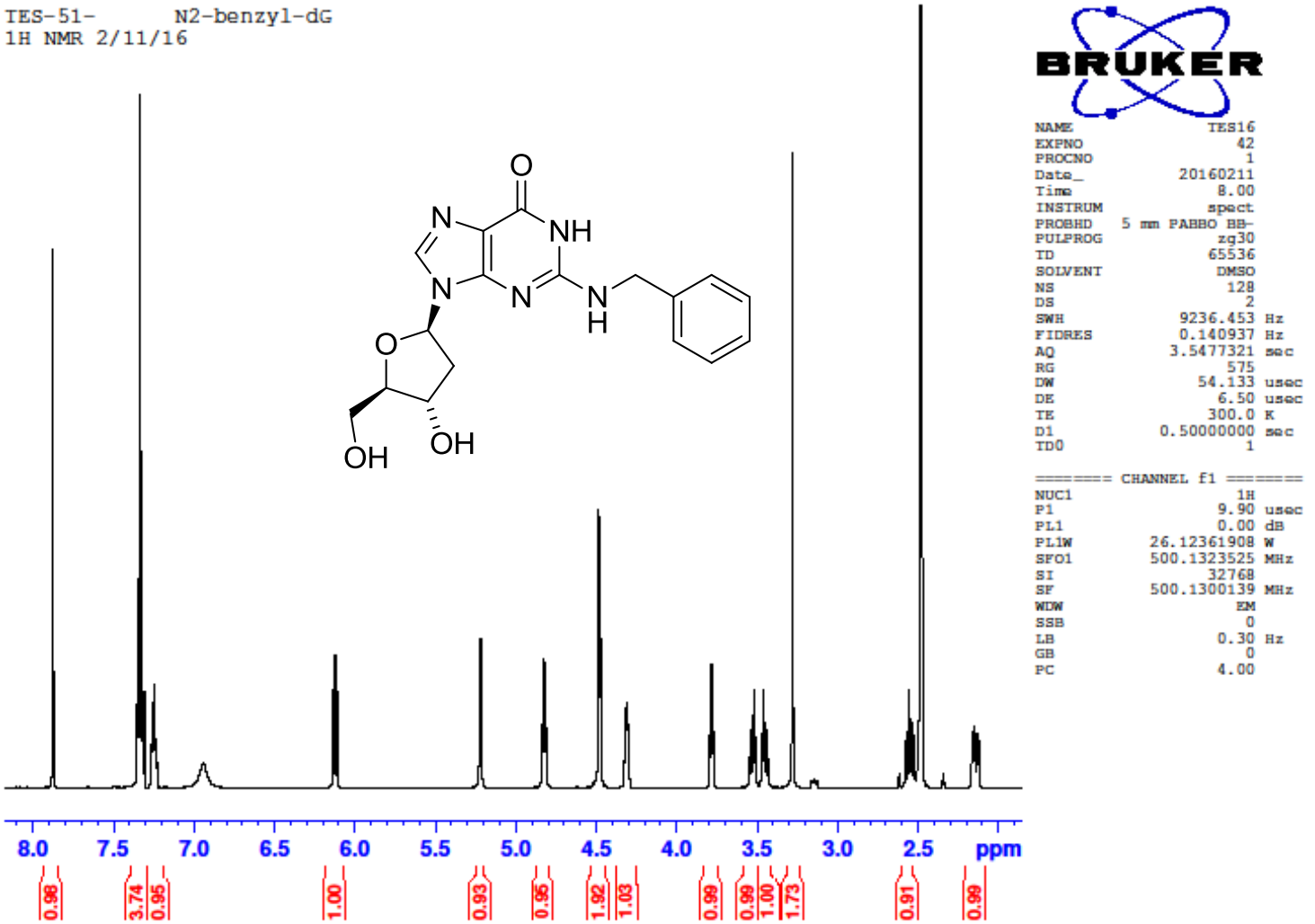


Figure S19. ^{1}H NMR (DMSO-d_6) of N^2-benzyl-2'-deoxyguanosine.

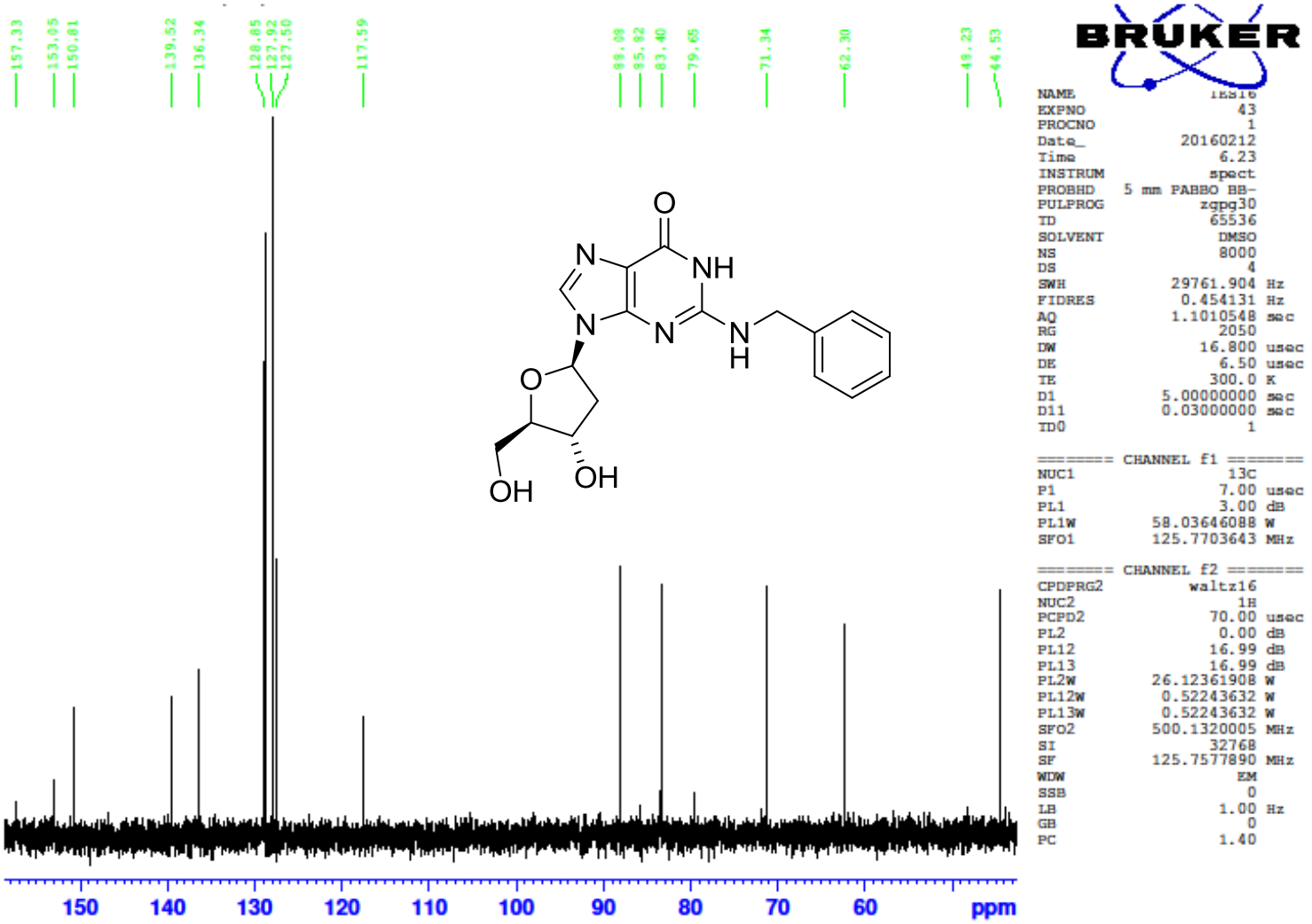


Figure S20. ^{13}C NMR (DMSO-d_6) N^2-benzyl-2'-deoxyguanosine.

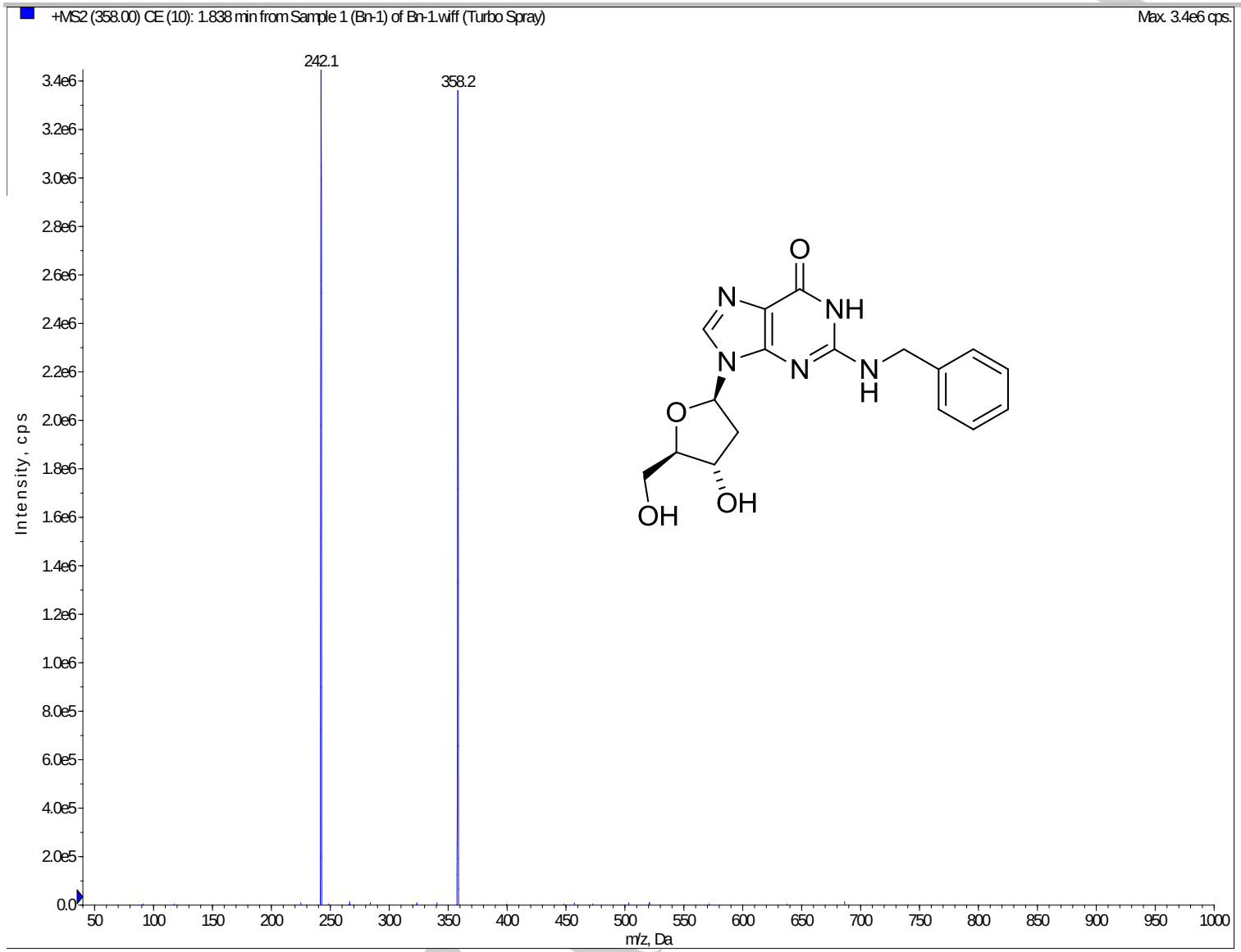


Figure S21. ESI MS N^2-Bn-dG

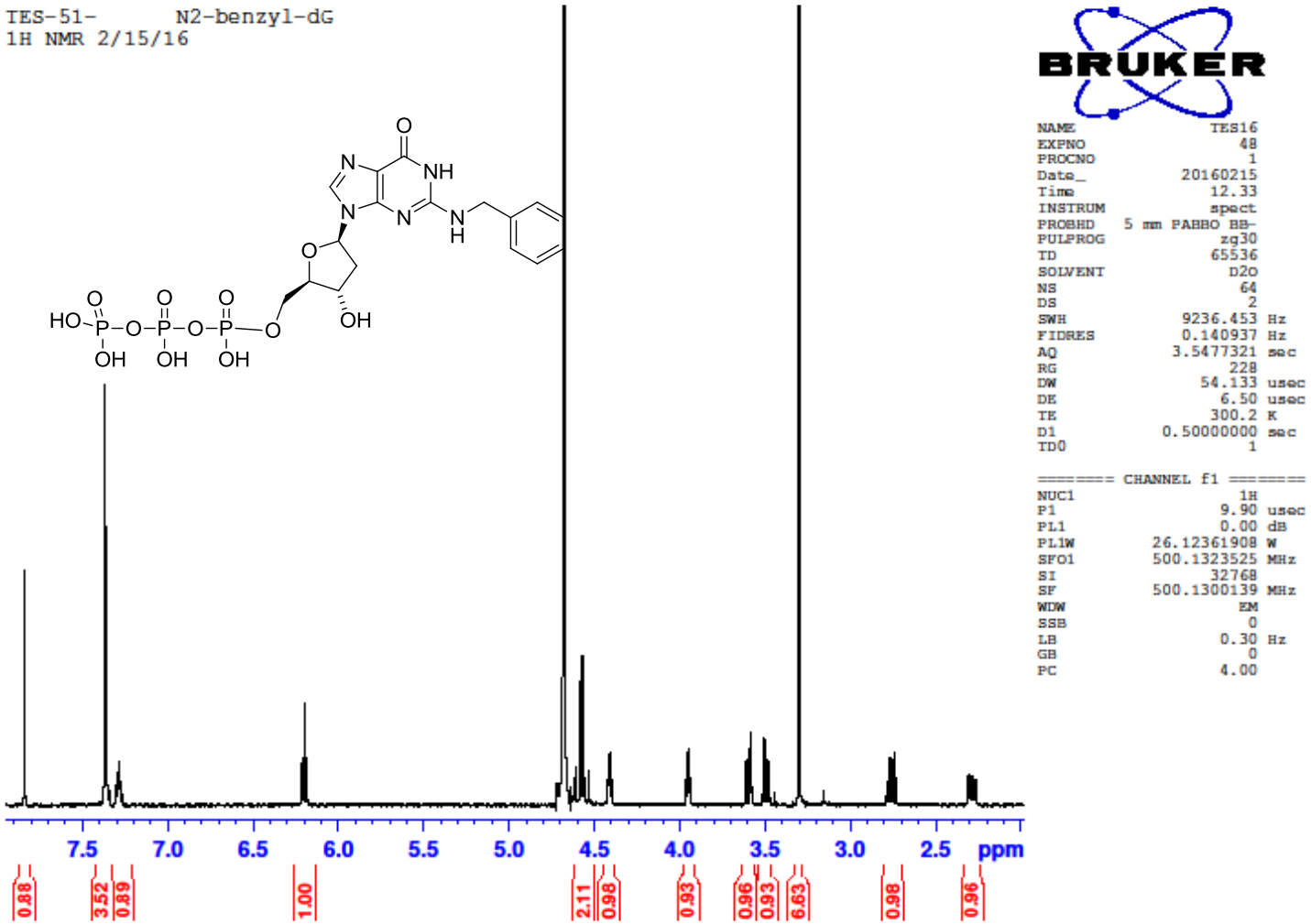


Figure S22. ^{1}H NMR (D_2O) *N*^{2}-benzyl-dGTP

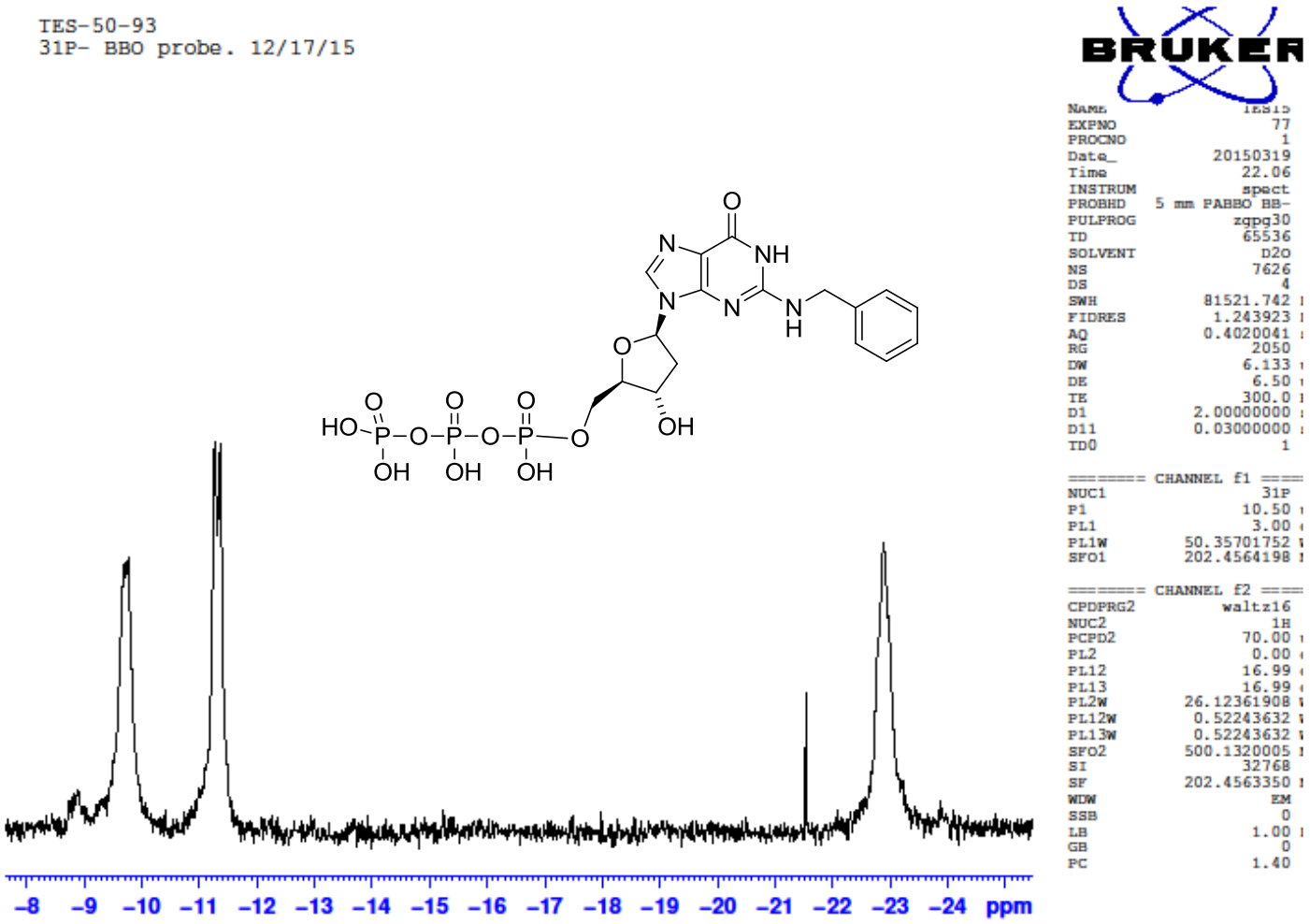


Figure S23. ^{31}P NMR (D_2O) N^2-benzyl-dGTP

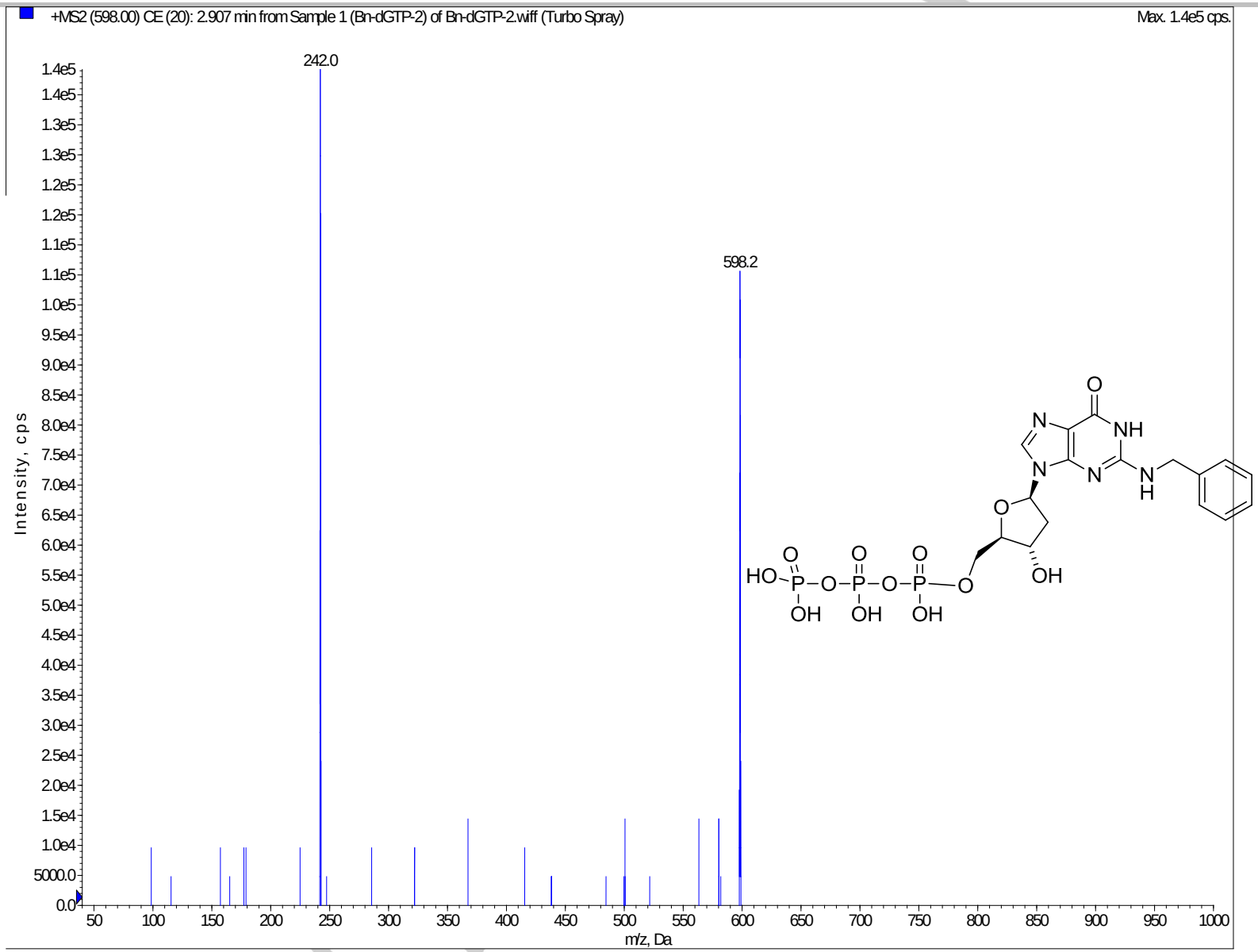
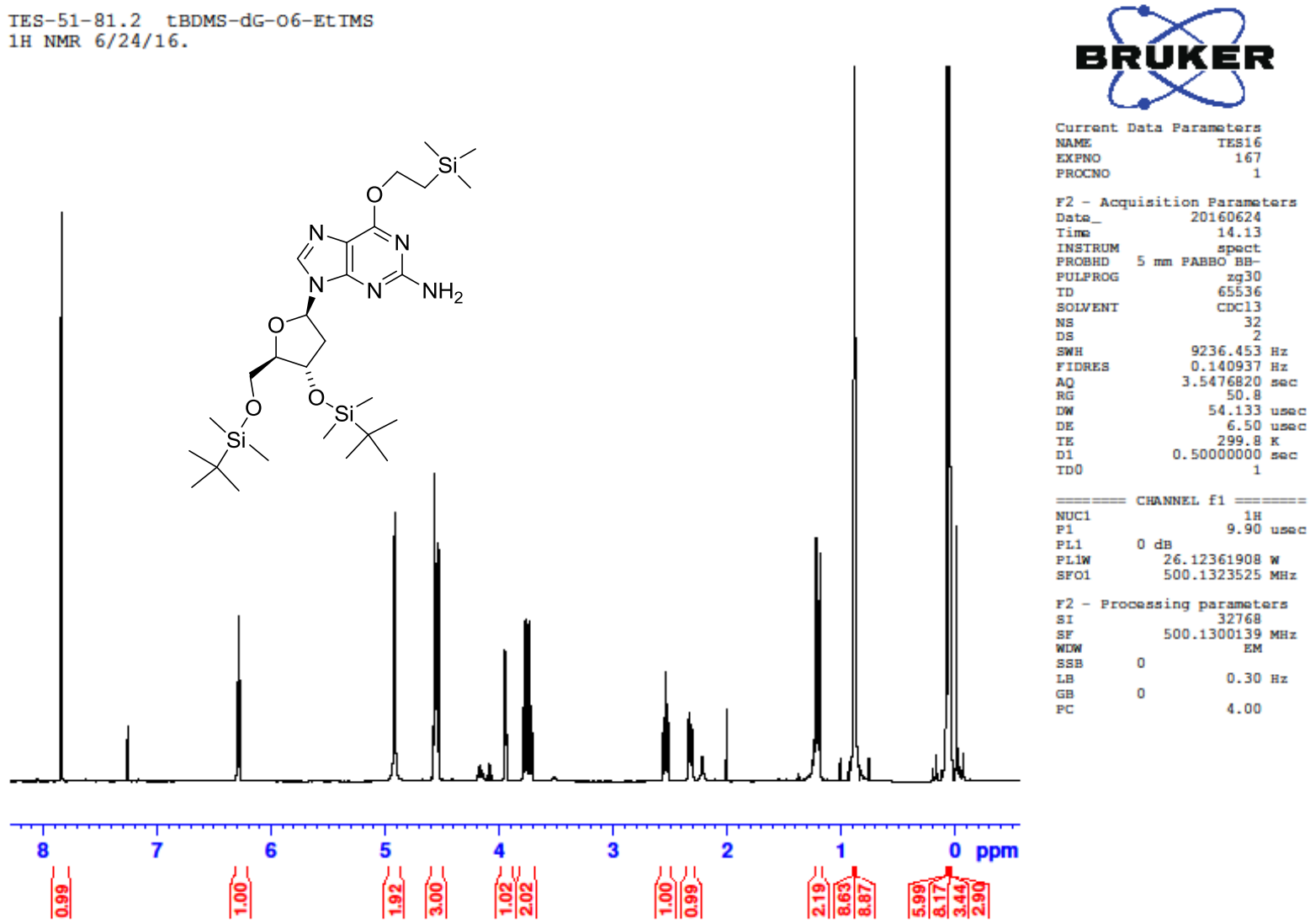


Figure S24. ESI MS N^2-benzyl-dGTP



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DW                   54.133 usec
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TE                   299.8 K
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TD0                       1

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Figure S25. ^{1}H NMR ($CDCl_3$) 3′,5′-O-Bis(*tert*-butyldimethylsilyl)-O^6-trimethylsilylethyl-2′-deoxy-guanosine.

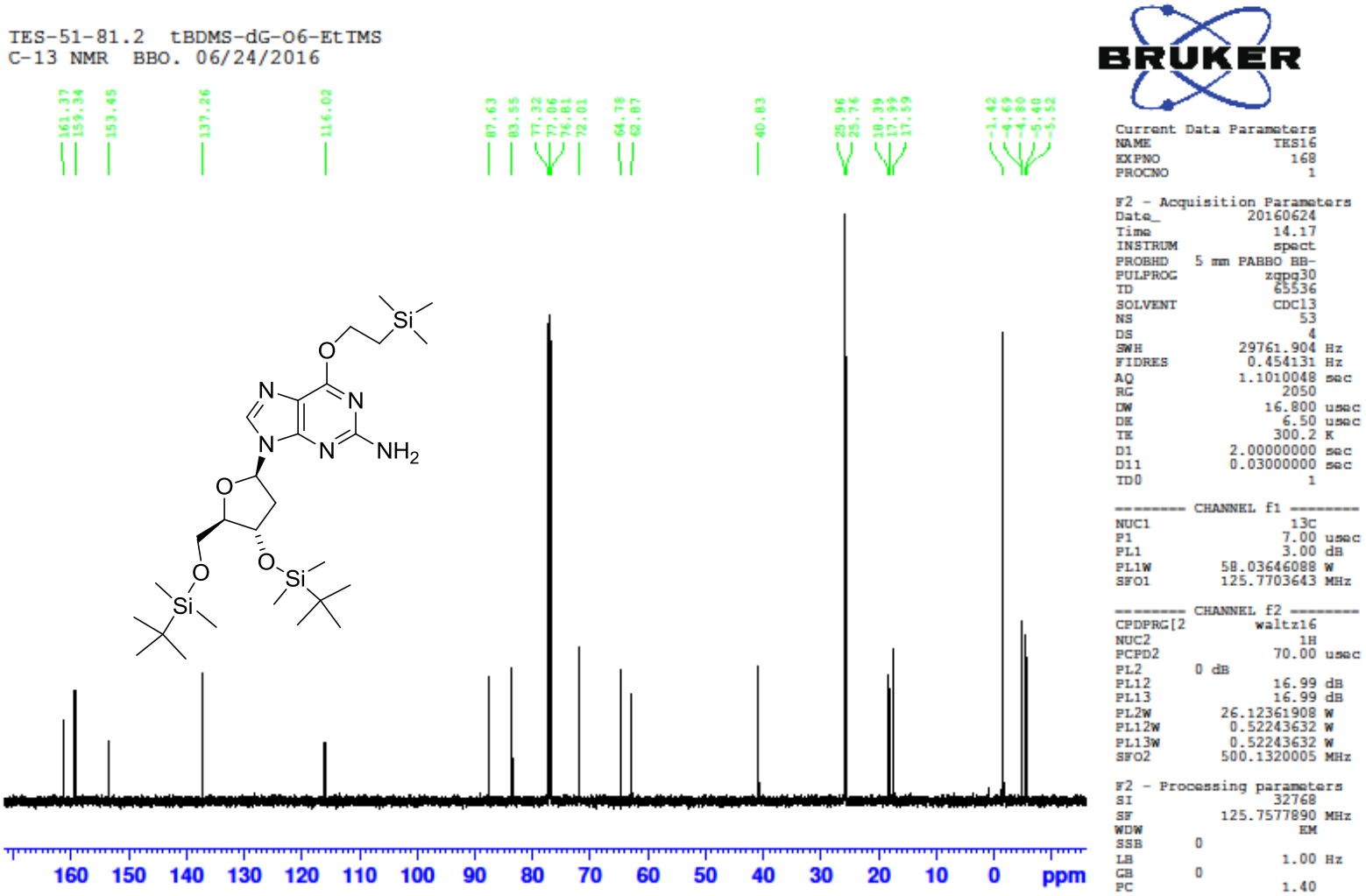


Figure S26. ^{13}C NMR ($CDCl_3$) 3′,5′-O-Bis(*tert*-butyldimethylsilyl)-O^6-trimethylsilylethyl-2′-deoxy-guanosine.

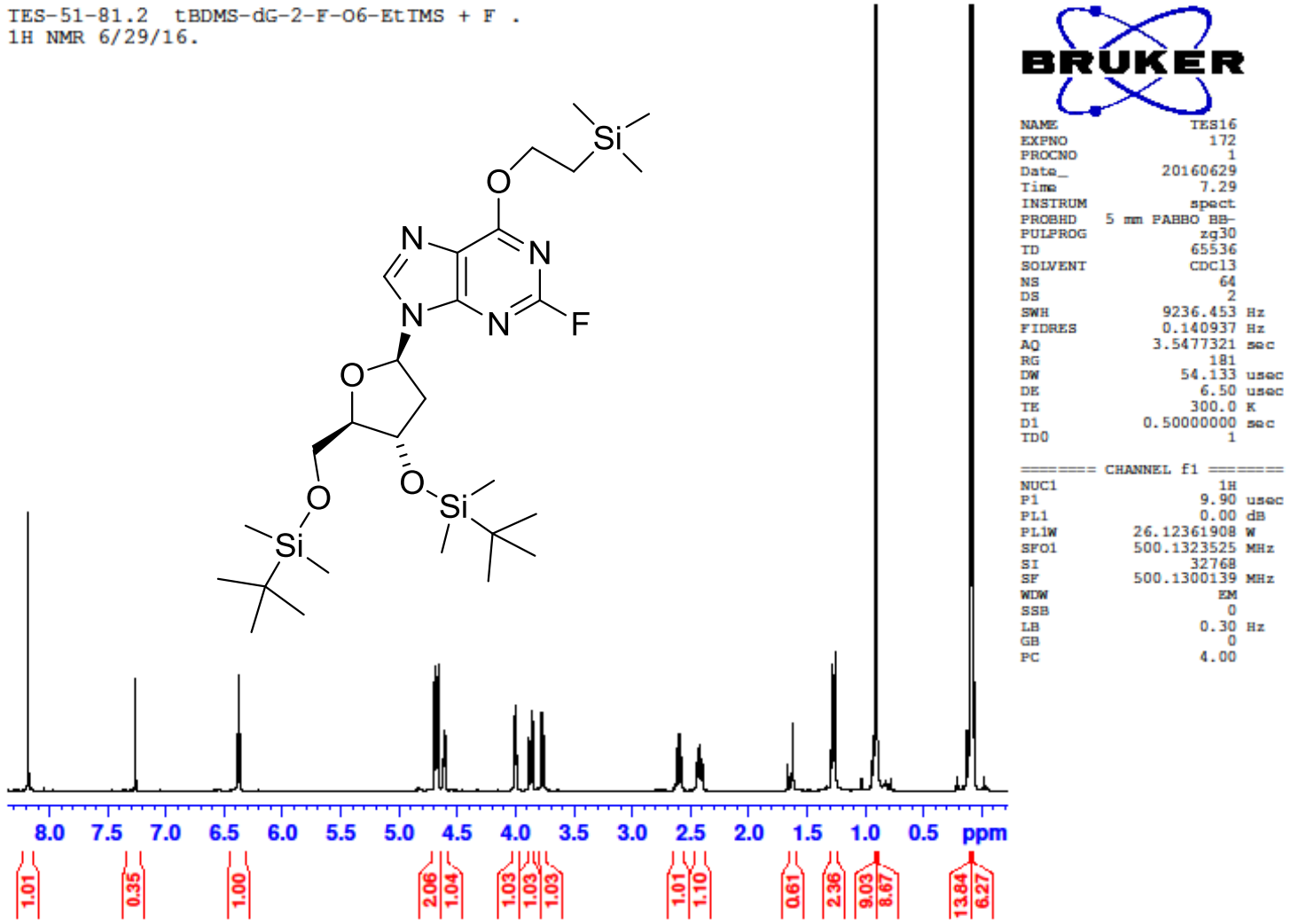


Figure S27. ^{1}H NMR ($CDCl_3$) 3′,5′-O-Bis(*tert*-butyldimethylsilyl)-O^6-trimethylsilylethyl-2′-deoxy-2-fluoroguanosine.

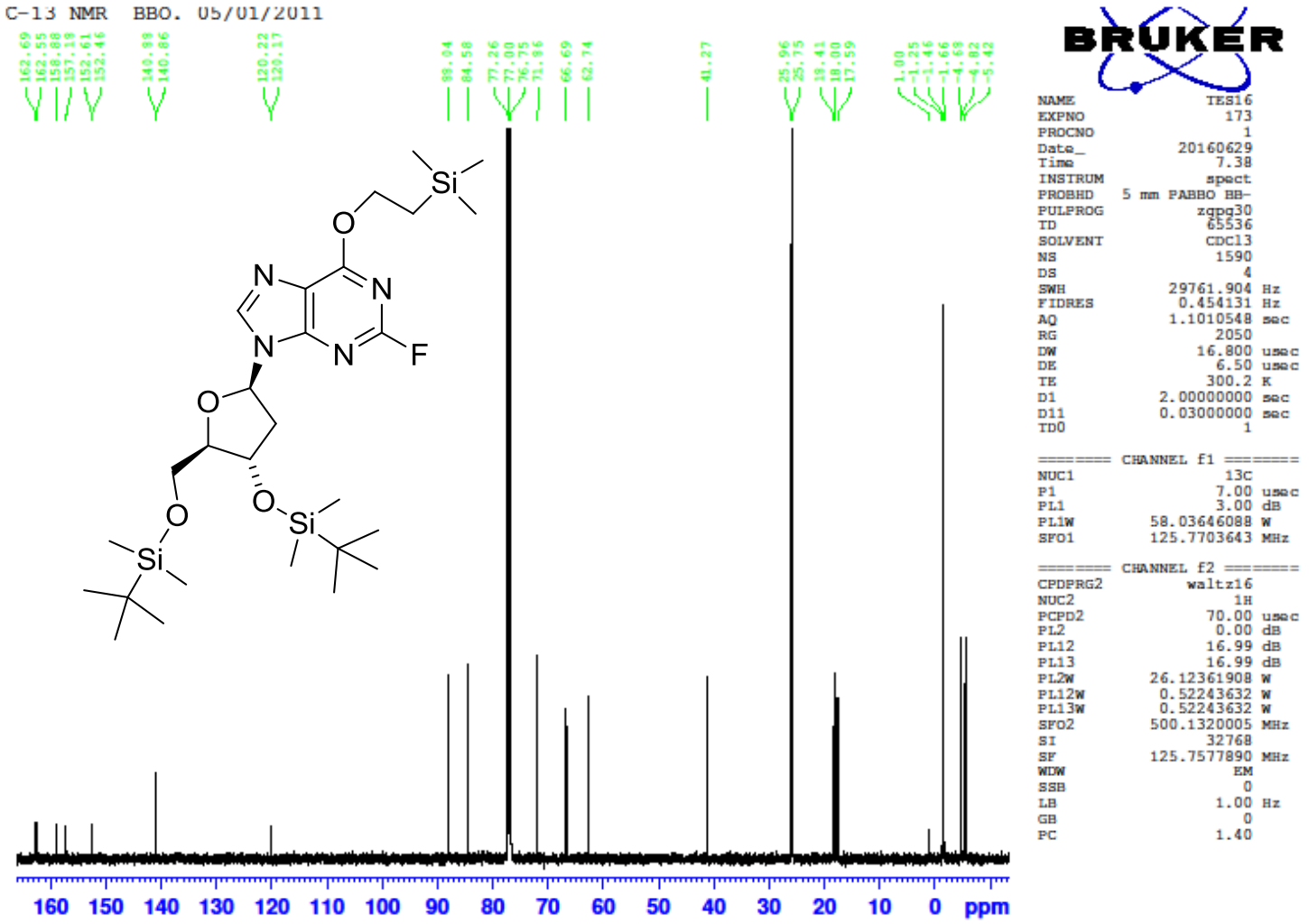


Figure S28. ^{13}C NMR ($CDCl_3$) 3′,5′-O-Bis(*tert*-butyldimethylsilyl)-O^6-trimethylsilylethyl-2′-deoxy-2-fluoroguanosine

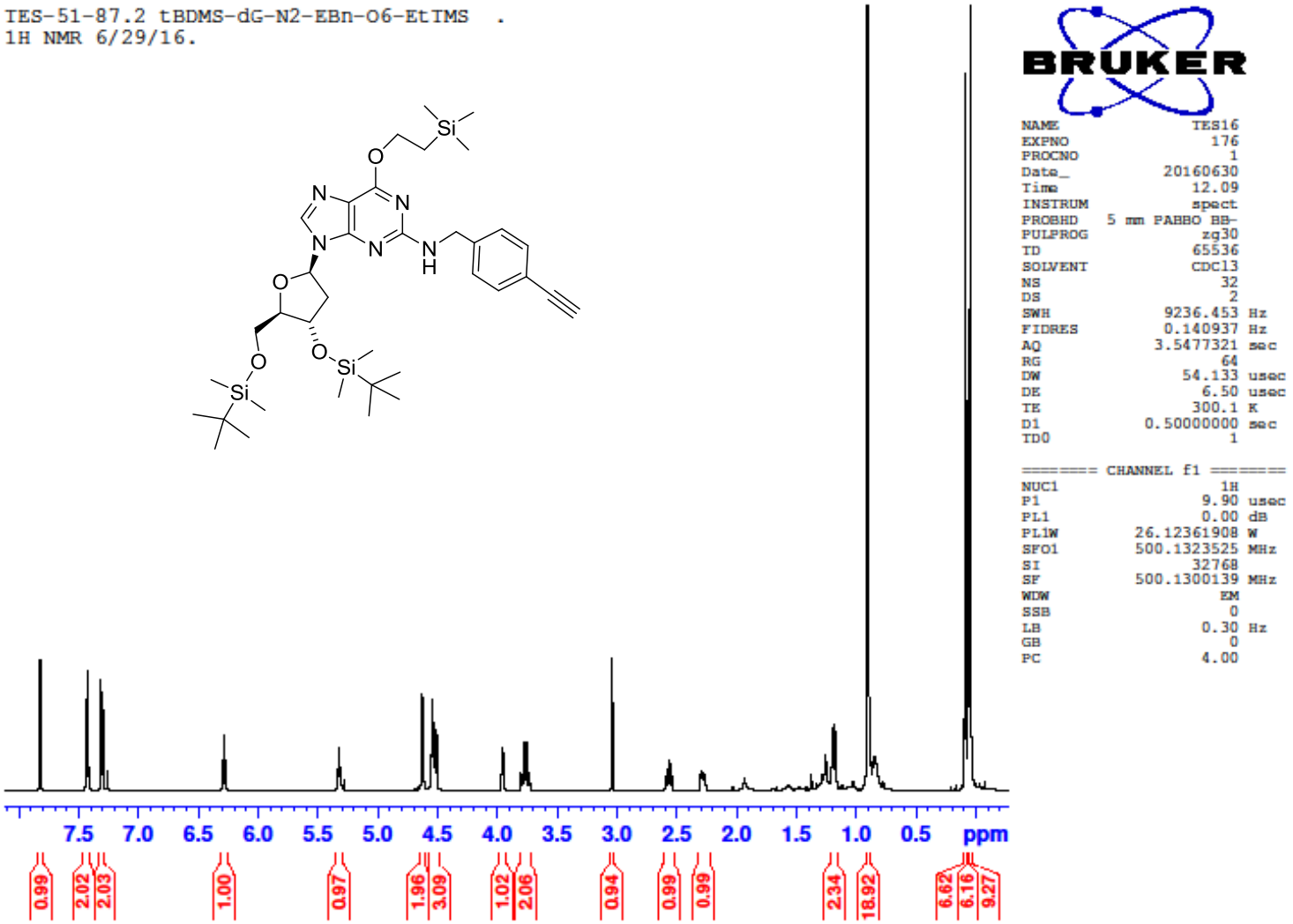


Figure S29. ^{1}H NMR ($CDCl_3$) Bis(*tert*-butyldimethylsilyl)-O^6-trimethylsilylethyl-N^2-*p*-ethynylbenzyl-2′-deoxy-guanosine

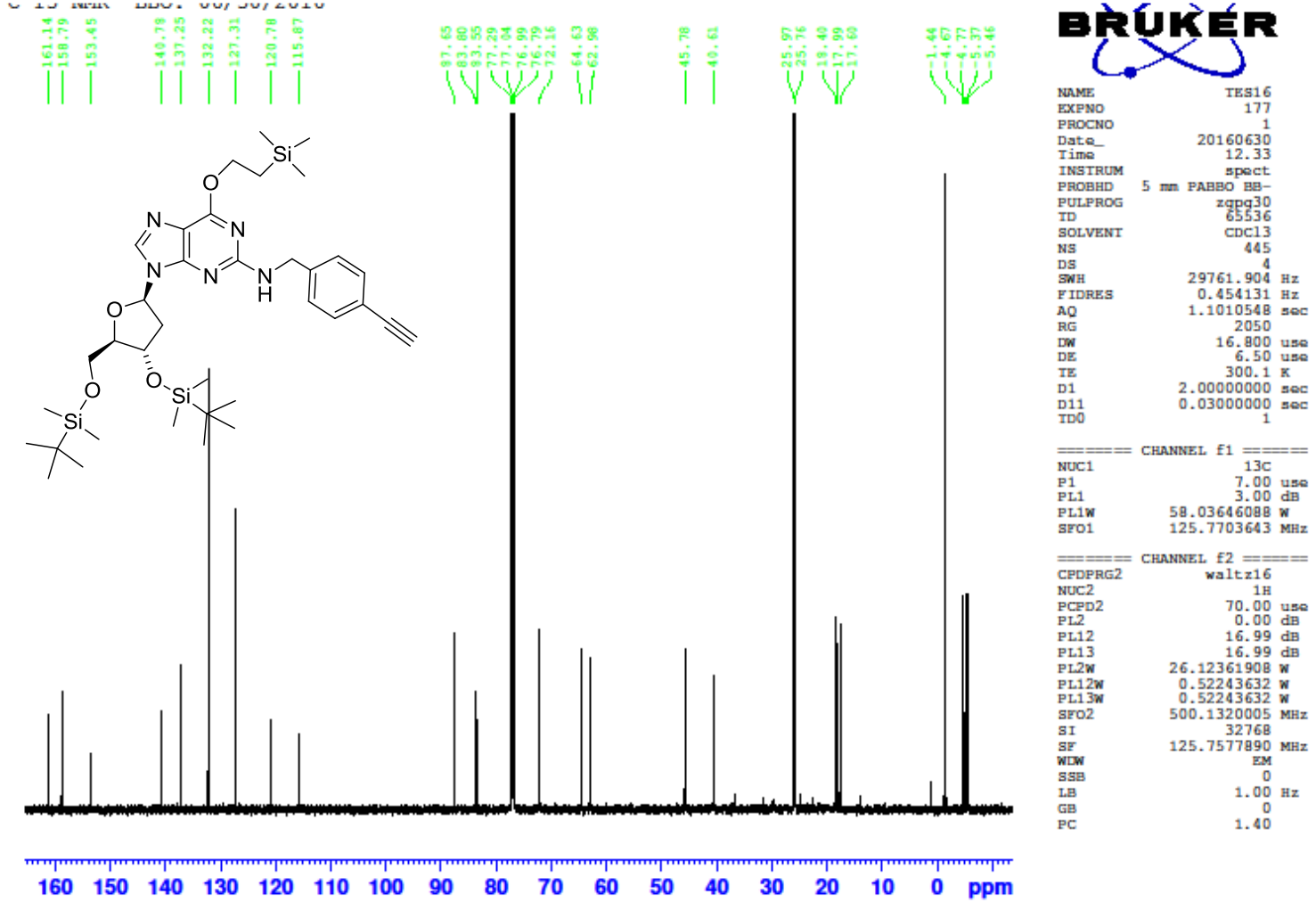
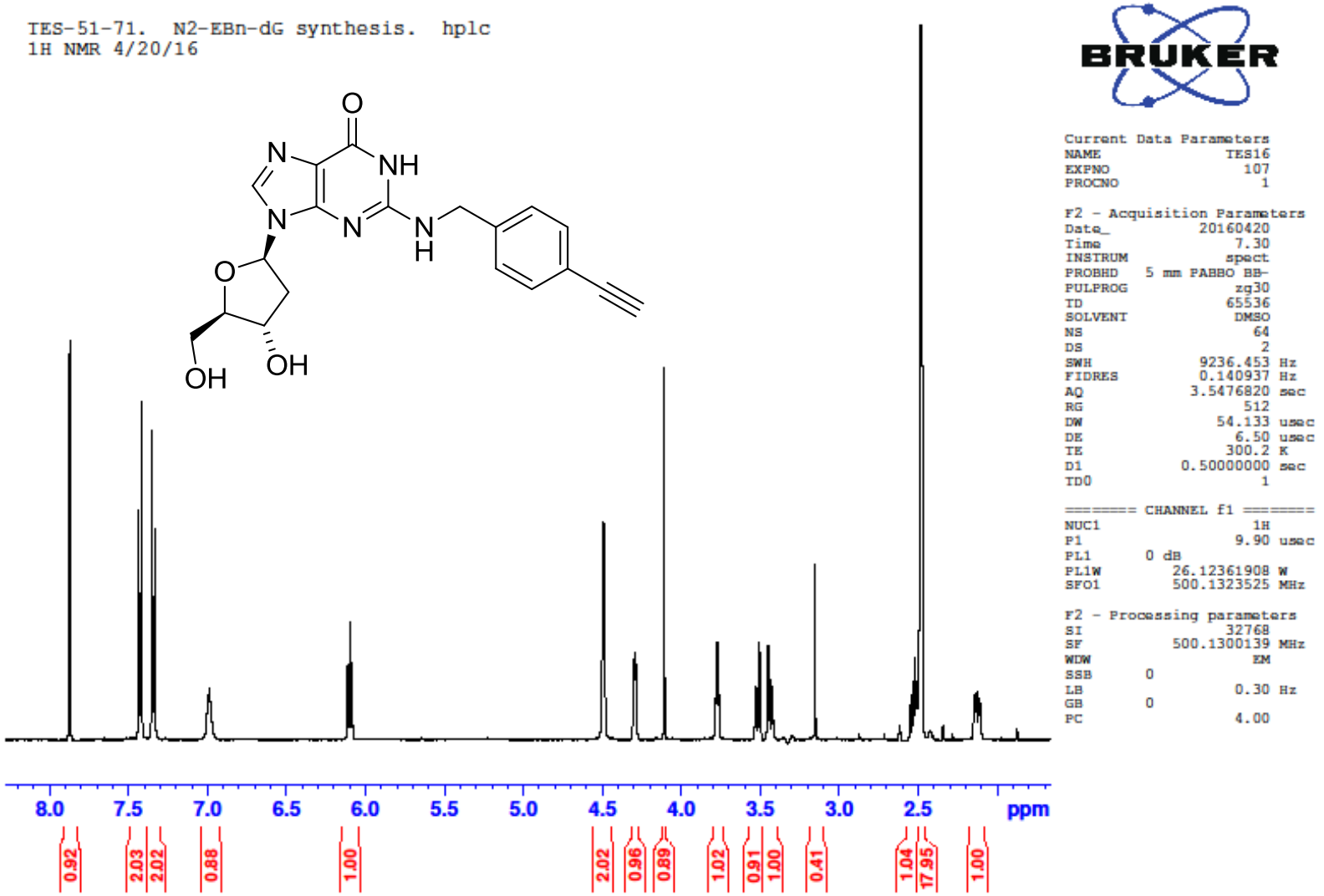


Figure S30. ^{13}C NMR ($CDCl_3$). Bis(*tert*-butyldimethylsilyl)-O^6-trimethylsilylethyl-N^2-*p*-ethynylbenzyl-2'-deoxy-guanosine



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RG                       512
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DE                     6.50 usec
TE                    300.2 K
D1                 0.50000000 sec
TD0                       1

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PL1          0 dB
PL1W          26.12361908 W
SFO1          500.1323525 MHz

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WDW                      EM
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Figure S31. ^{1}H NMR (DMSO-d_6). *N*^{2}-p-ethynylbenzyl-2'-deoxy-guanosine

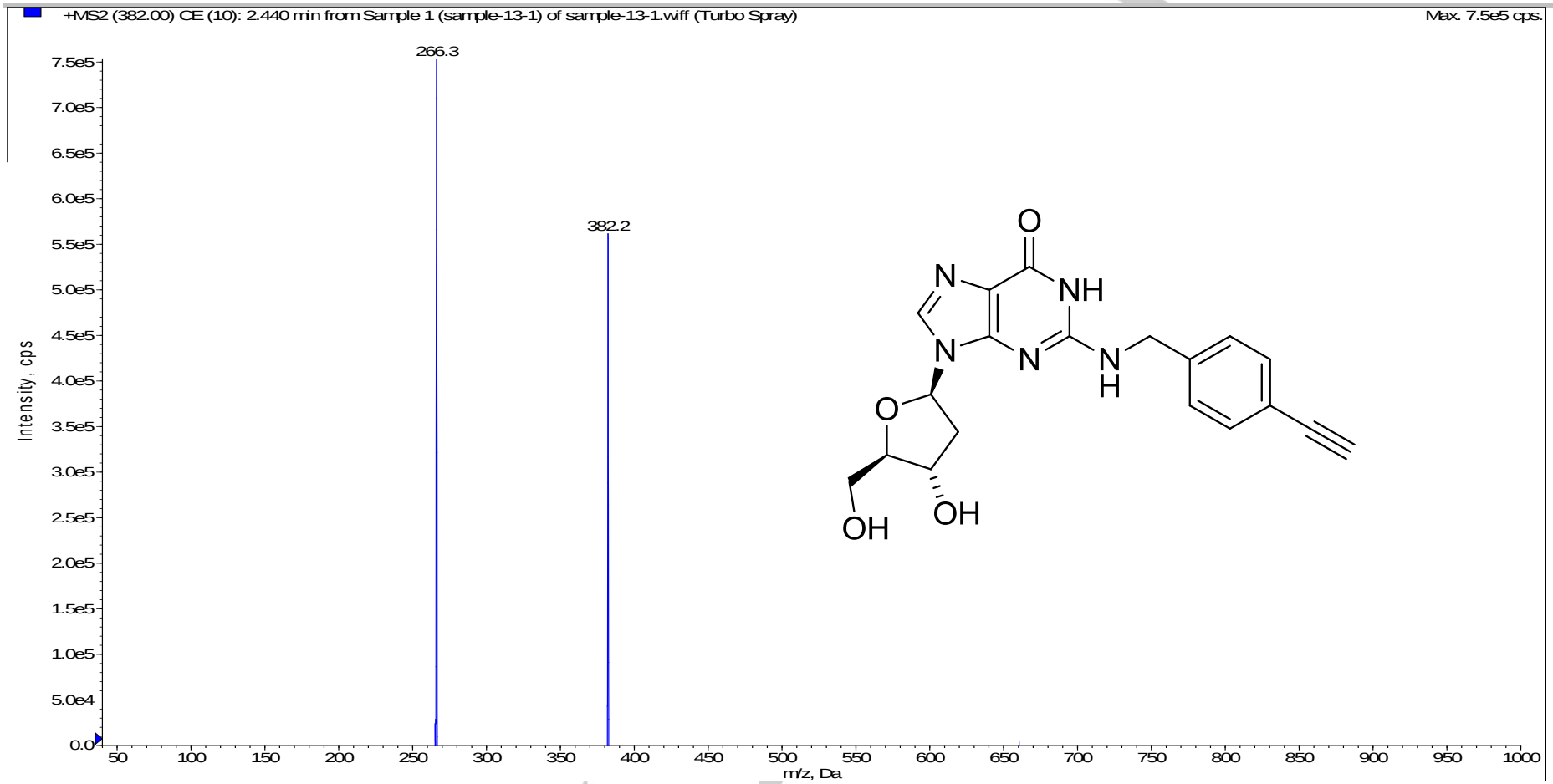

Figure S32. ESI-MS N^2-p-ethynylbenzyl-2′-deoxy-guanosine

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Author Contributions

A.S. Prakasha Gowda performed the biochemical and cell biology experiments.

Marietta Y. Lee provide the DNApolymerase δ

Thomas E. Spratt synthesized the compounds, wrotethe manuscript, and supervised the project.