

# Supporting Information

## Reduction Sensitive Lipid Conjugates of Tenofovir: Synthesis, Stability, and Antiviral

### Activity

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## Biological Assays

### Anti-HIV Assay

All HIV assays were performed by ImQuest BioSciences (Frederick, MD). All compounds were solubilized at 40 mM in DMSO and stored at -20°C. Test materials were evaluated up to 100  $\mu$ M, and five serial logarithmic dilutions. AZT and 3TC were obtained from the NIH AIDS Research and Reference Reagent Program and used as controls in the anti-HIV and anti-HBV assay, respectively. Fresh human PBMCs were obtained from a commercial source and determined to be HIV and HBV negative. The leukophoresed blood cells were washed repeatedly with PBS, then diluted 1:1 with Dulbecco's phosphate buffered saline (PBS) and layered over 15 ml of Ficoll-Hypaque density gradient in a 50 ml conical centrifuge tube. The tubes were centrifuged for 30 min at 600 g. Banded PBMCs were gently aspirated from the resulting interface and washed three times with PBS. After the final wash, cell number was determined by Trypan Blue dye exclusion and cells re-suspended at  $1 \times 10^6$  cells/mL in RPMI 1640 with 15% Fetal Bovine Serum (FBS), 2 mmol/L L-glutamine, 2  $\mu$ g/mL PHA-P, 100 U/ml penicillin and 100  $\mu$ g/mL streptomycin and allowed to incubate for 48-72 hr at 37°C. After incubation, PBMCs were centrifuged and re-suspended in tissue culture medium (RPMI 1640 with 15% Fetal Bovine Serum (FBS), 2 mmol/L L-glutamine, 2  $\mu$ g/mL PHA-P, 100 U/ml penicillin and 100  $\mu$ g/mL streptomycin, 3.6 ng/mL recombinant human IL-2). The cultures were maintained until use by half-volume culture changes with fresh IL-2 containing tissue culture medium every 3 days. Assays were initiated with PBMCs at 72 hr post PHA-P stimulation. Immediately prior to use, target cells were re-suspended in fresh tissue culture medium at  $1 \times 10^6$  cells/ml and plated in the interior wells of a 96-well round bottom microliter plate at 50  $\mu$ L/well. Then, 100  $\mu$ L of 2X concentrations of compound-containing medium was transferred to the 96-well plate containing the cells in 50  $\mu$ L of the medium. Following addition of test compound to the wells, 50  $\mu$ L of a predetermined dilution of HIV virus (prepared at 4x of final desired in-well concentration) was added, and mixed well. For infection, 50-150 TCID<sub>50</sub> of each virus was added per well (final MOI approximately 0.002). PBMCs were exposed in triplicate to virus and cultured in the presence or absence of the test compound at varying concentrations as described for the 96-well microliter plates. After 7 days, HIV-1 replication was quantified in the tissue culture supernatant by measurement of reverse transcriptase activity. Wells with cells and virus only served as virus controls. Separate plates were identically prepared without virus for cytotoxicity studies. Reverse transcriptase activity was measured in cell-free supernatants using a standard radioactive incorporation polymerization assay.

### Anti-HBV Assay

One hundred microliters (100  $\mu$ L) of wells of a 96-well flat-bottom plate at a density of  $1 \times 10^4$  cells per well were incubated at 37°C in 5% CO<sub>2</sub> for 24 hours. Following incubation, six ten-fold serial dilutions of test compound prepared in RPMI1640 medium with 10% fetal bovine serum were added to individual wells of the plate in triplicate. Six wells in the plate received medium alone as a virus control only. The plate was incubated for 6 days at 37°C at 5% CO<sub>2</sub>. The culture medium was changed on day 3 with medium containing the indicated concentration of each compound. One hundred microliters (100  $\mu$ L) of supernatant was collected from each well for analysis of viral DNA by qPCR and cytotoxicity was evaluated by XTT staining of the cell culture monolayer on the sixth day (see below). Ten microliters (10  $\mu$ L) of cell culture supernatant collected on the sixth

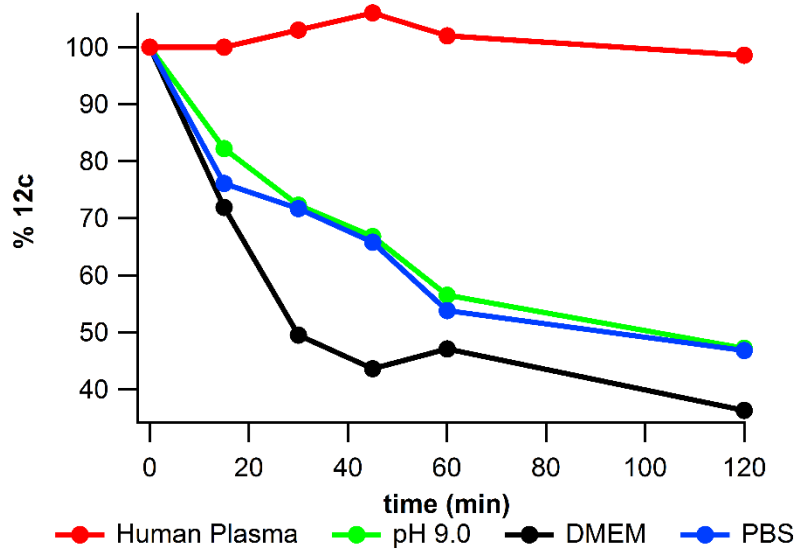
day was diluted in qPCR dilution buffer (40  $\mu\text{g}/\text{ml}$  sheared salmon sperm DNA) and boiled for 15 minutes. Quantitative real time PCR was performed in 386 well plates using an Applied Biosystems 7900HT Sequence Detection System and the supporting SDS 2.4 software. Five microliters (5  $\mu\text{L}$ ) of boiled DNA for each sample and serial 10-fold dilutions of a quantitative DNA standard were subjected to real time Q-PCR using Platinum Quantitative PCR SuperMix-UDG (Invitrogen) and specific DNA oligonucleotide primers (IDT, Coralville, ID) HBV-AD38-qF1 (5'-CCG TCT GTG CCT TCT CAT CTG-3'), HBV-AD38-qR1 (5'-AGT CCA AGA GTY CTC TTA TRY AAG ACC TT-3') and HBV-AD38-qP1 (5'-FAM-CCG TGT GCA /ZEN/CTT GCG TTC ACC TCT GC-3'BHQ1) at a final concentration of 0.2  $\mu\text{M}$  for each primer in a total reaction volume of 15  $\mu\text{l}$ . The final HBV DNA copy number in each sample was interpolated from the standard curve by the SDS2.4 software and the data were analyzed by Excel.

## Cytotoxicity Studies

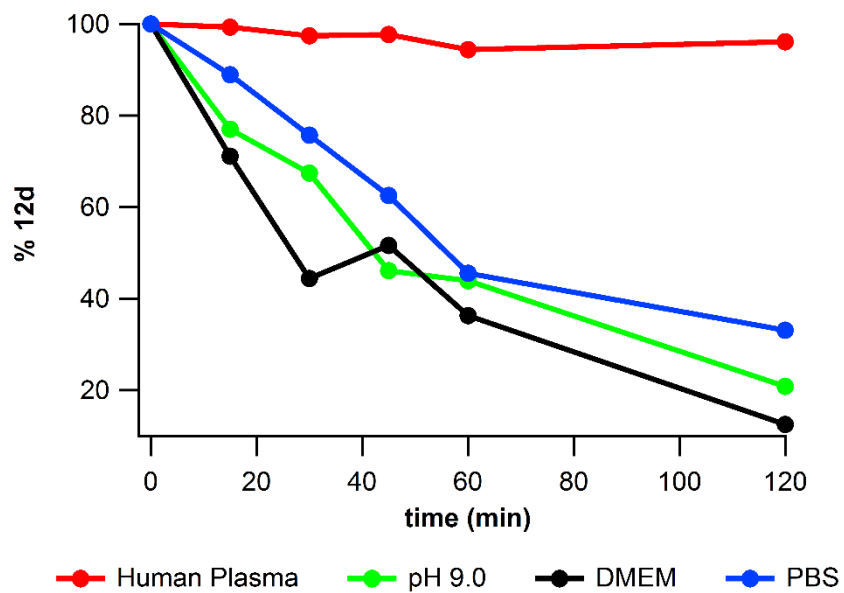
Cytotoxicity was evaluated by staining uninfected cells with tetrazolium dye XXT with spectrophotometric readings at 450/650 nm with a Molecular Devices Vmax plate reader. These experiments were run in triplicate for each compound tested.

## Stability Studies

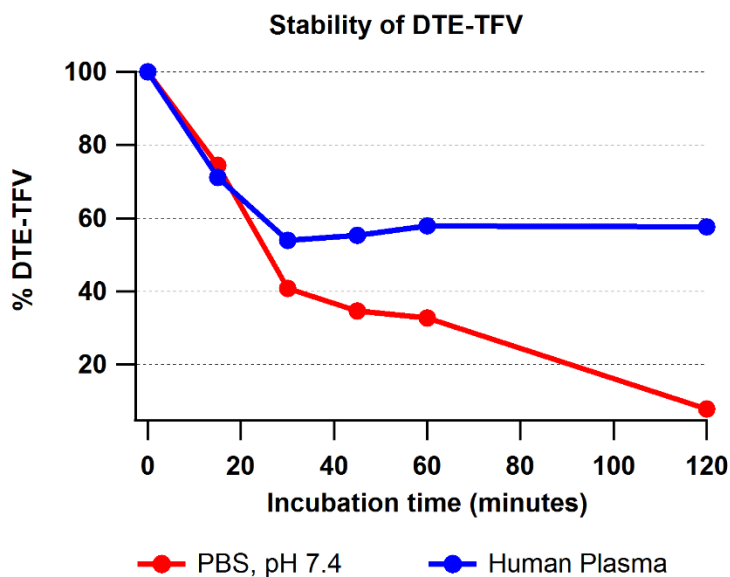
Stock solutions of the target compound (2.0 mg) were prepared in methanol at a concentration 100-fold that of the desired concentration. 10  $\mu\text{L}$  of this solution was added to either 990  $\mu\text{L}$  of human plasma, PBS, DMEM, or sodium carbonate buffer at pH = 9 in Kimax glass tubes that were pre-warmed in a reciprocal water bath shaker at 37  $^{\circ}\text{C}$  prior to addition of compound. The mixture was then vortexed and compound (**12c**, **12d**, or DTE-TFV) was added to the solution. Vortexing continued at 150 rpm at 37 $^{\circ}\text{C}$ . At each time point (0, 15, 30, 45, 120, 1440 min) a 100 $\mu\text{L}$  aliquot was taken from the solution and mixed with 400  $\mu\text{L}$  of an internal standard (0.5  $\mu\text{L}$  hexadecyloxypropyl 2'-deoxy-2'-fluorodine in methanol with 0.1% formic acid) in 1.5 mL polypropylene micro-centrifuge tubes. The aliquots were vortexed for 10 seconds at maximum speed, centrifuged at 13,000 rpm, and decanted into LC vials. The supernatant was analyzed by LC-MS by noting the relative UV intensity of the title compound at 254 nm.



SI Figure 1: Stability of compound 12c in various media.



SI Figure 2: Stability of 12d in various media.



**SI Figure 3:** Stability of DTE-TFV in PBS and Human Plasma.

**SI Table 1:** Half-Life Data for **12c**, **12d**, and DTE-TFV in Various Media.

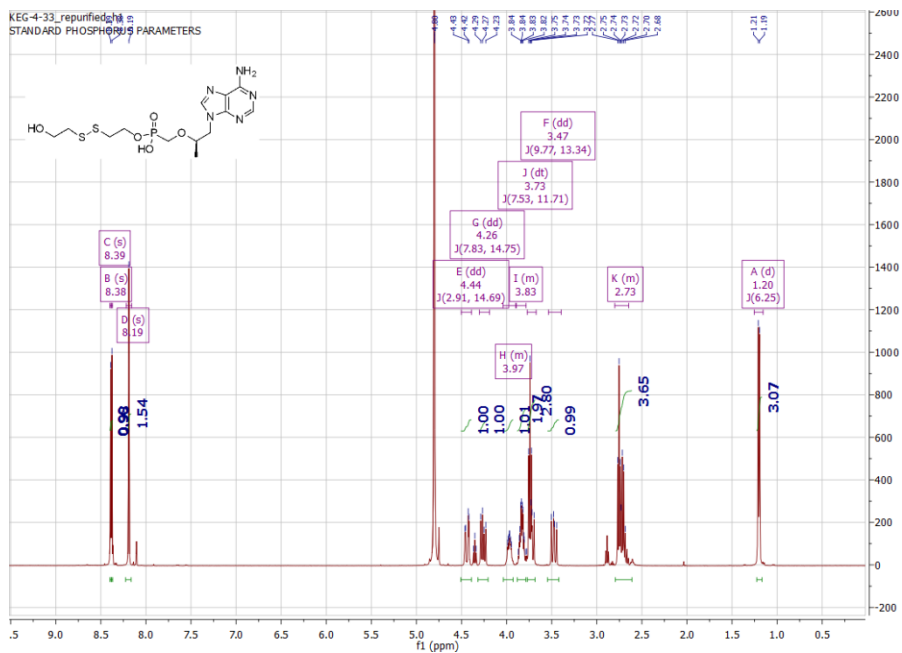
	Human Plasma <sup>b</sup>	pH 9.0 <sup>c</sup>	DMEM <sup>d</sup>	PBS <sup>e</sup>
12c	> 24 h	2 h	1.5 h	2 h
12d	> 24 h	1 h	45 min	1.2 h
DTE-TFV	34 min	-	-	3.5 h

<sup>a</sup>All solutions incubated at 37°C prior to analysis. <sup>b</sup>pH 7.4 with K<sub>2</sub>EDTA. <sup>c</sup>Sodium carbonate/bicarbonate buffer. <sup>d</sup>Dulbecco's Modified Eagle Medium without fetal bovine serum. <sup>e</sup>Phosphate-buffered saline, pH 7.4

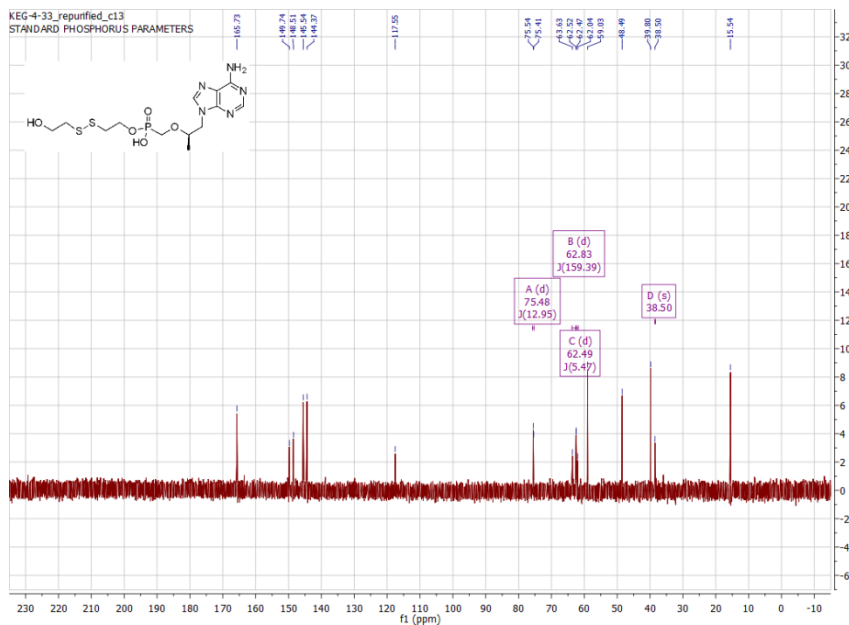
## Associated Spectra

### 2-((2-hydroxyethyl)disulfanyl)ethyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (DTE-TV)

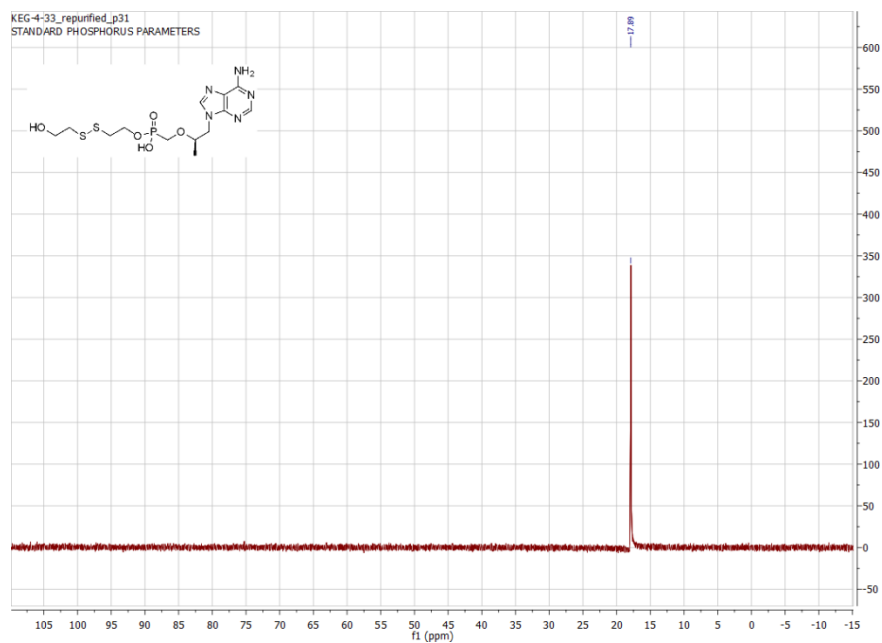
$^1\text{H}$  NMR (400 MHz in  $\text{D}_2\text{O}$ )



$^{13}\text{C}$  NMR (101 MHz in  $\text{D}_2\text{O}$ )

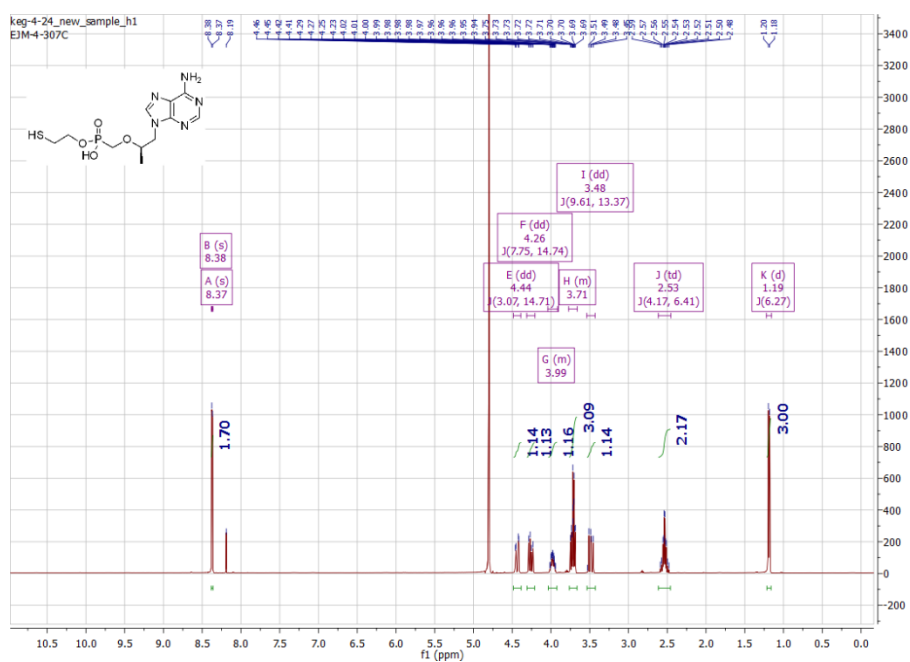


$^{31}\text{P}$  NMR (162 MHz in  $\text{D}_2\text{O}$ )



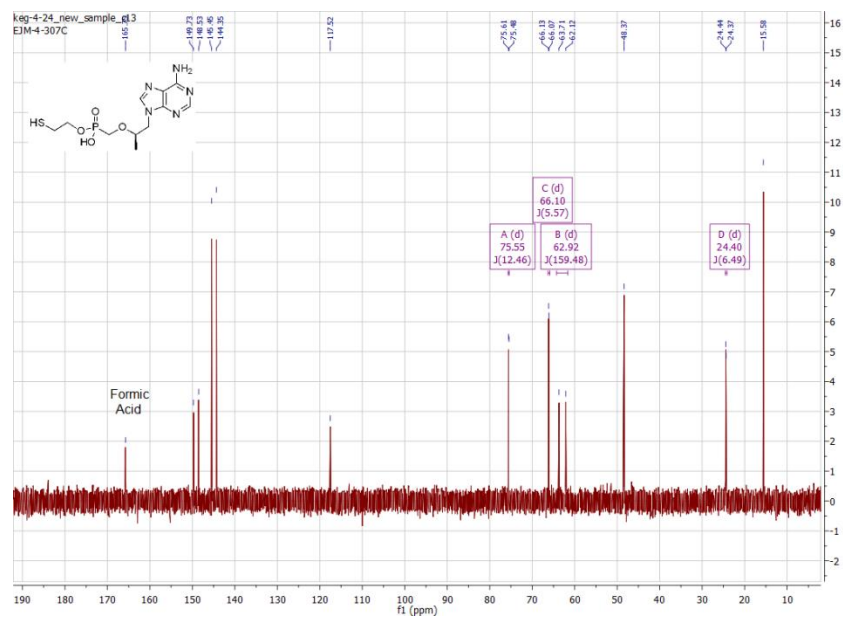
**2-mercaptoethyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (9)**

$^1\text{H}$  NMR (400 MHz in  $\text{D}_2\text{O}$ )

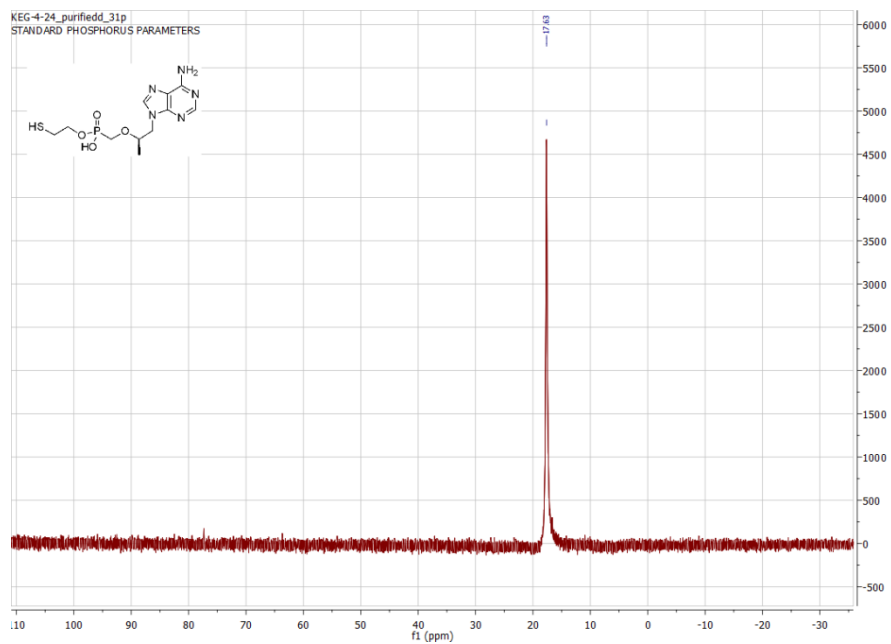




$^{13}\text{C}$  NMR (101 MHz in  $\text{D}_2\text{O}$ )

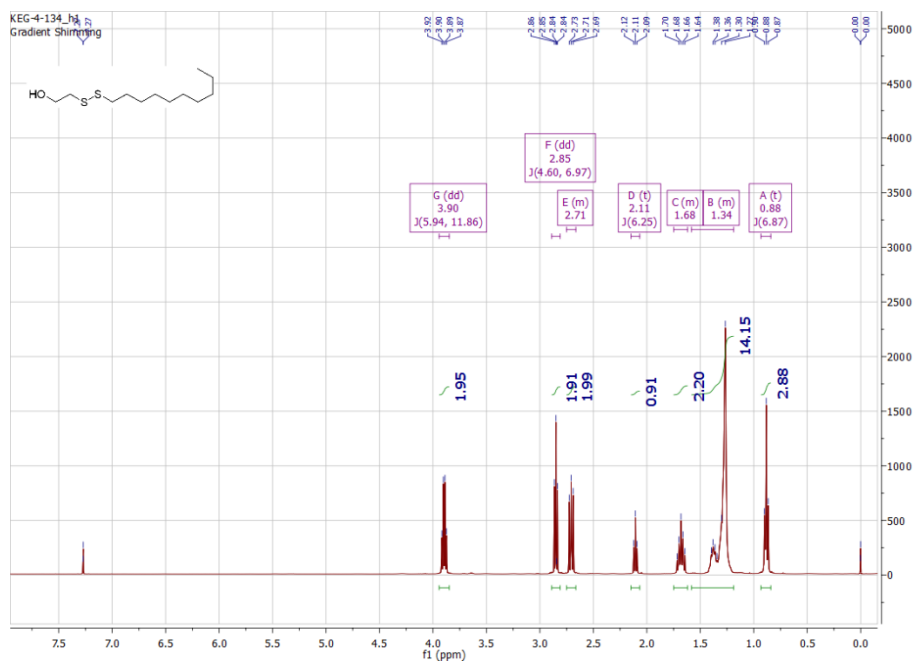


$^{31}\text{P}$  NMR (162 MHz in  $\text{D}_2\text{O}$ )

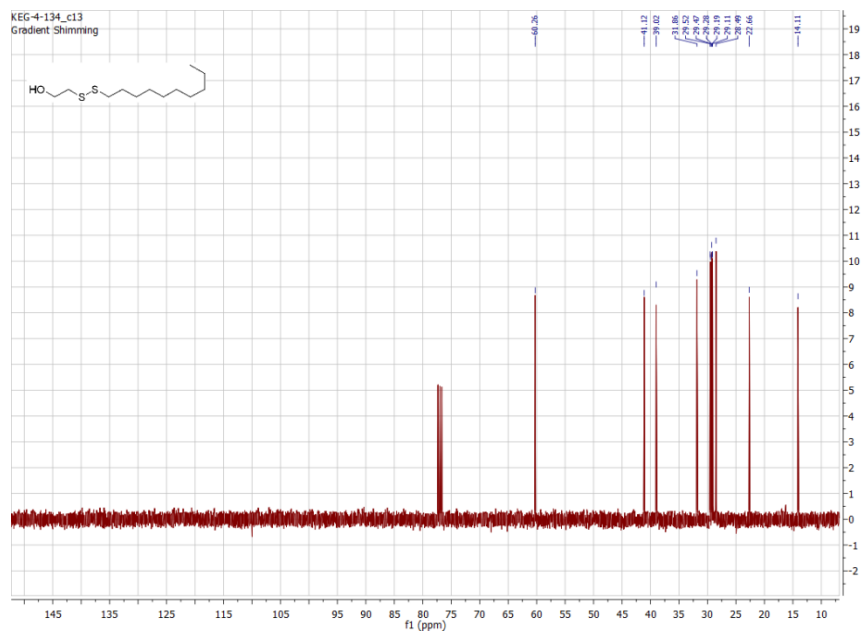


## 2-(decyldisulfanyl)ethanol (11a)

$^1\text{H}$  NMR (400 MHz in  $\text{CDCl}_3$ )

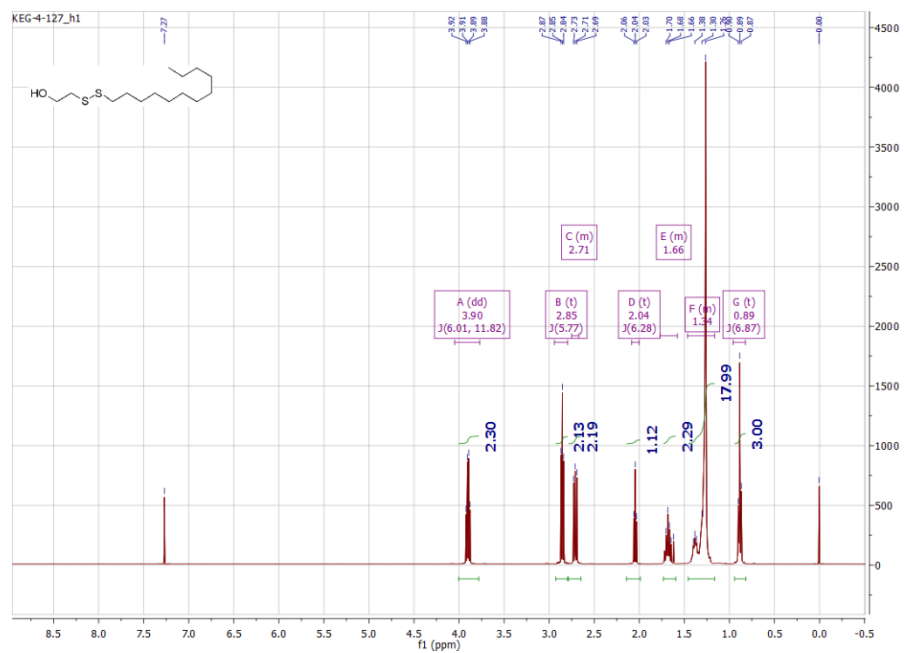


$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

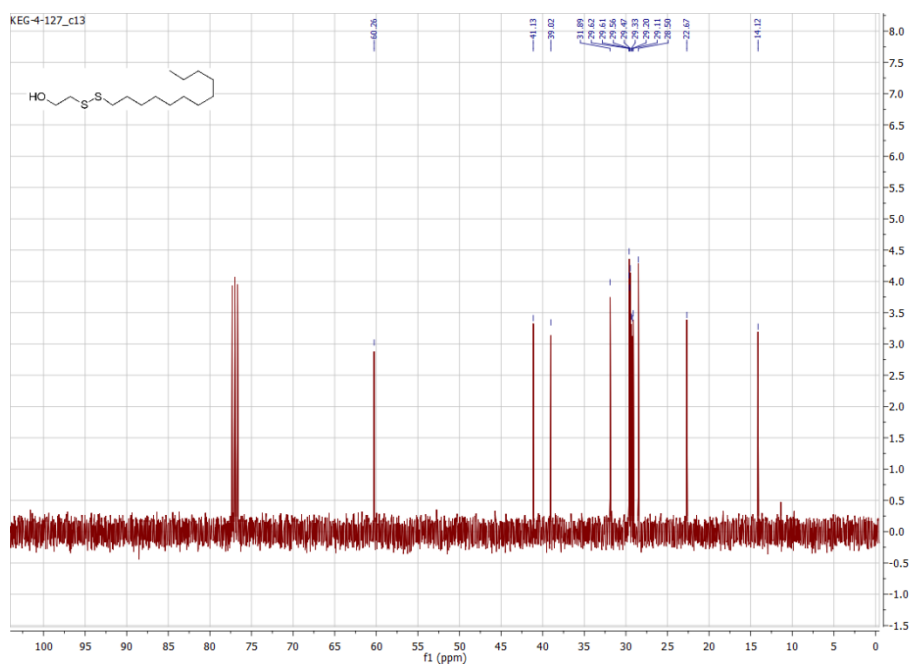


## 2-(dodecyldisulfanyl)ethanol (11b)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

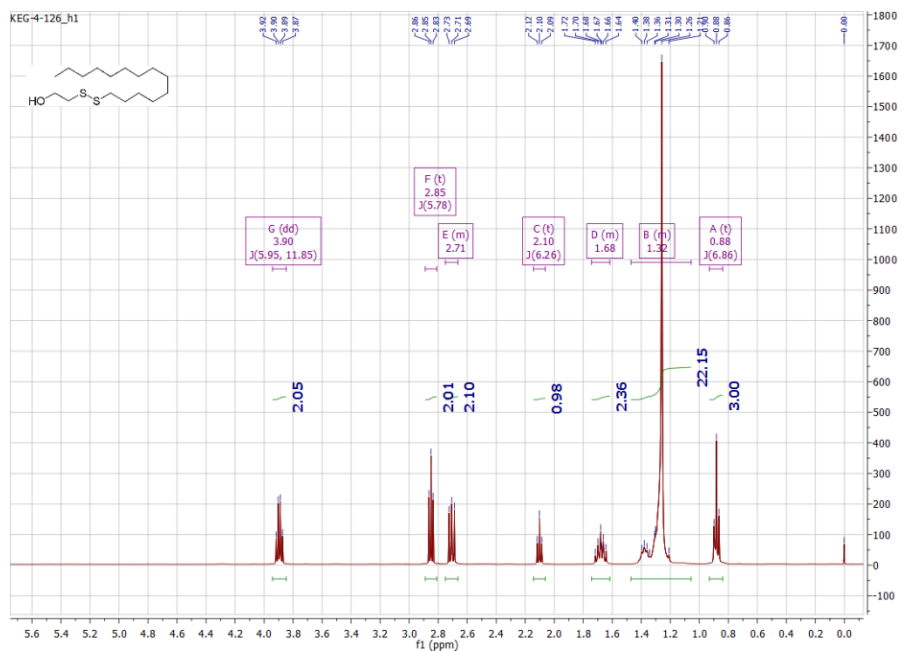


$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

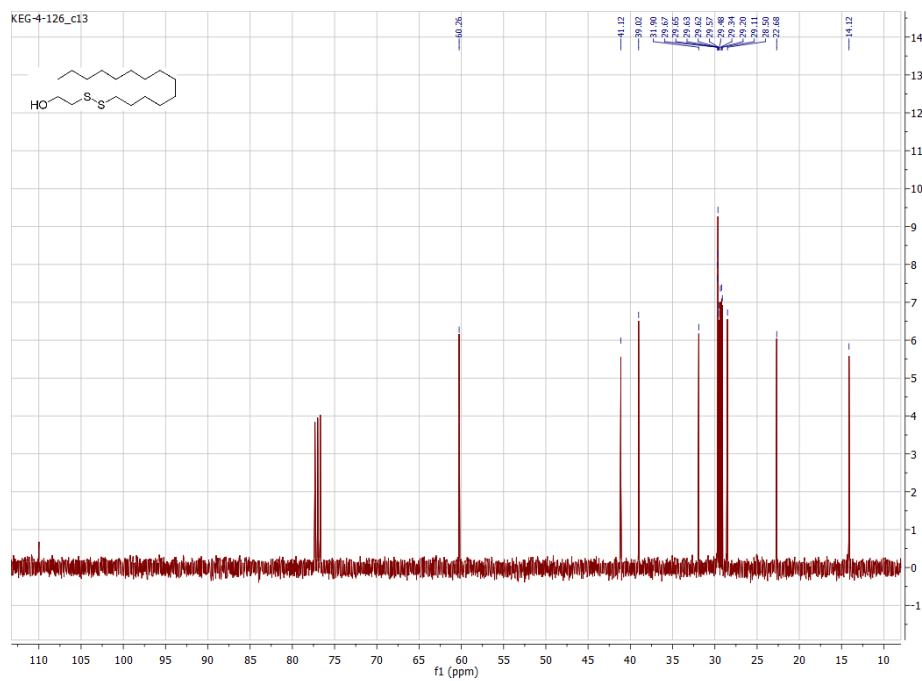


## 2-(tetradecyldisulfanyl)ethanol (11c)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

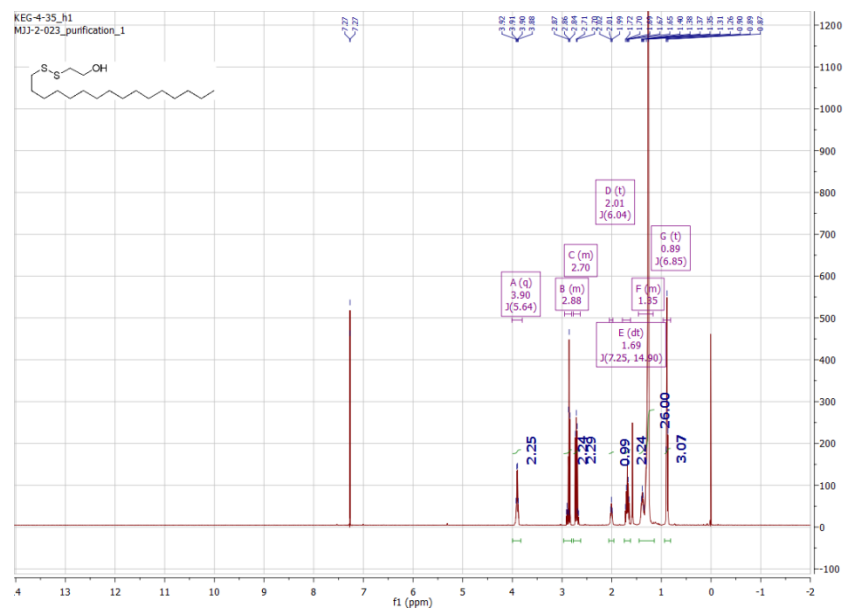


$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

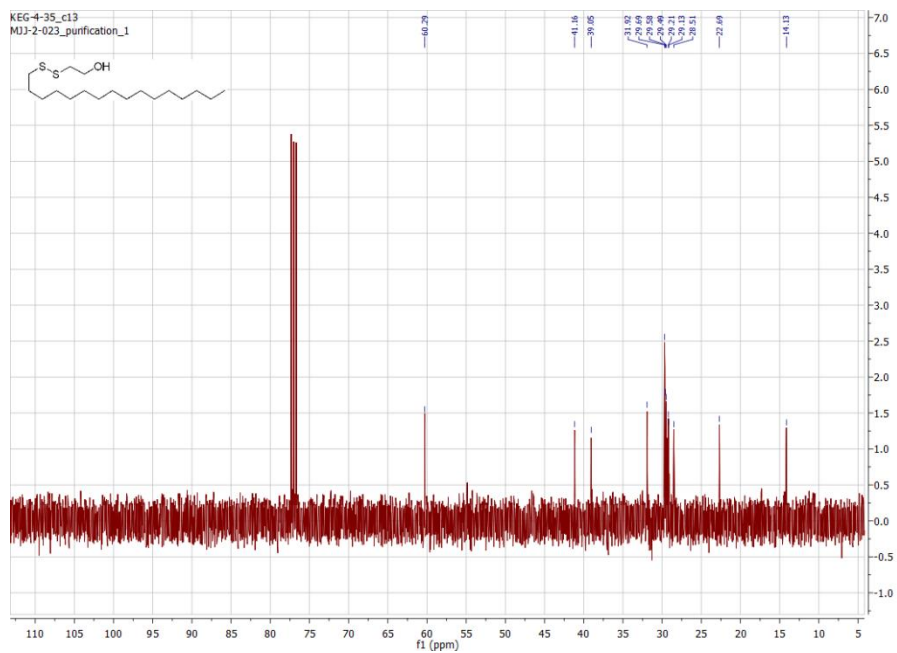


## 2-(hexadecyldisulfanyl)ethanol (11d)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

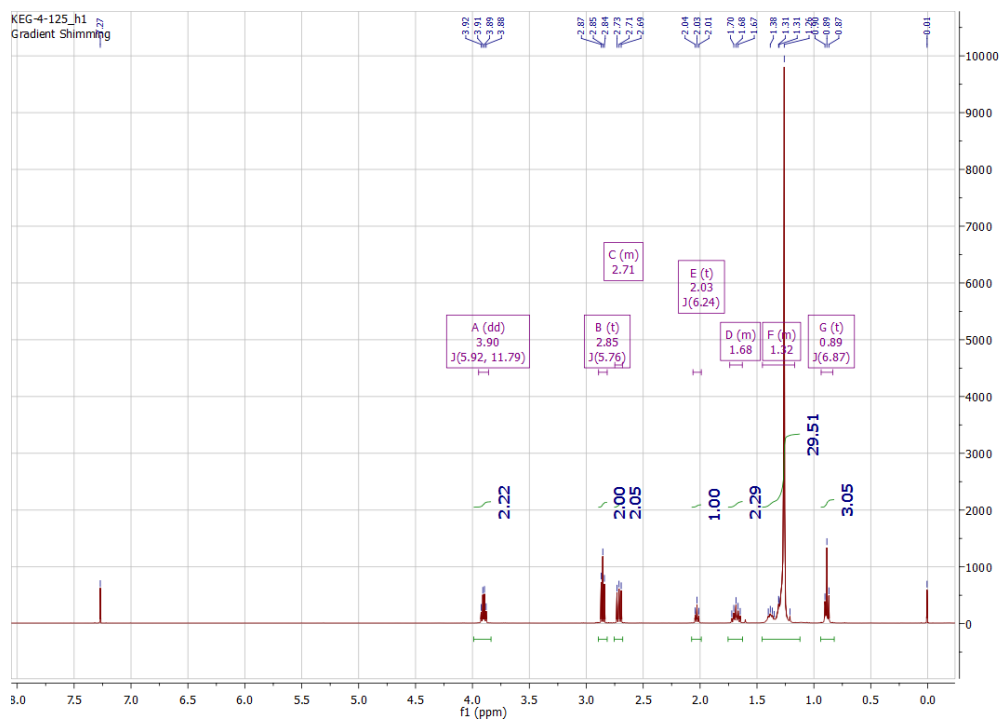


$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

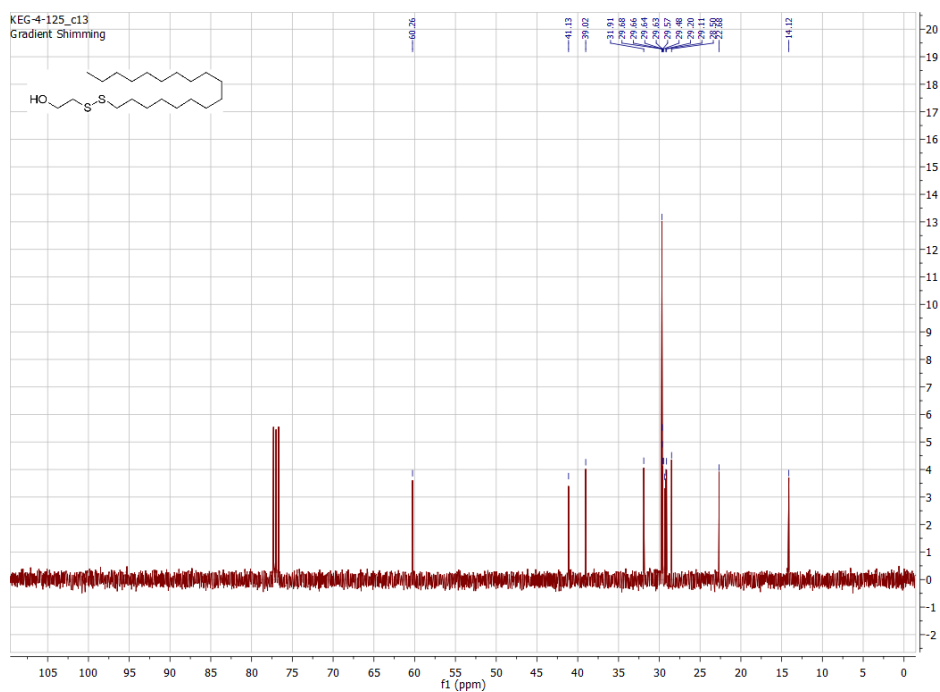


## 2-(octadecyldisulfanyl)ethanol (11e)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

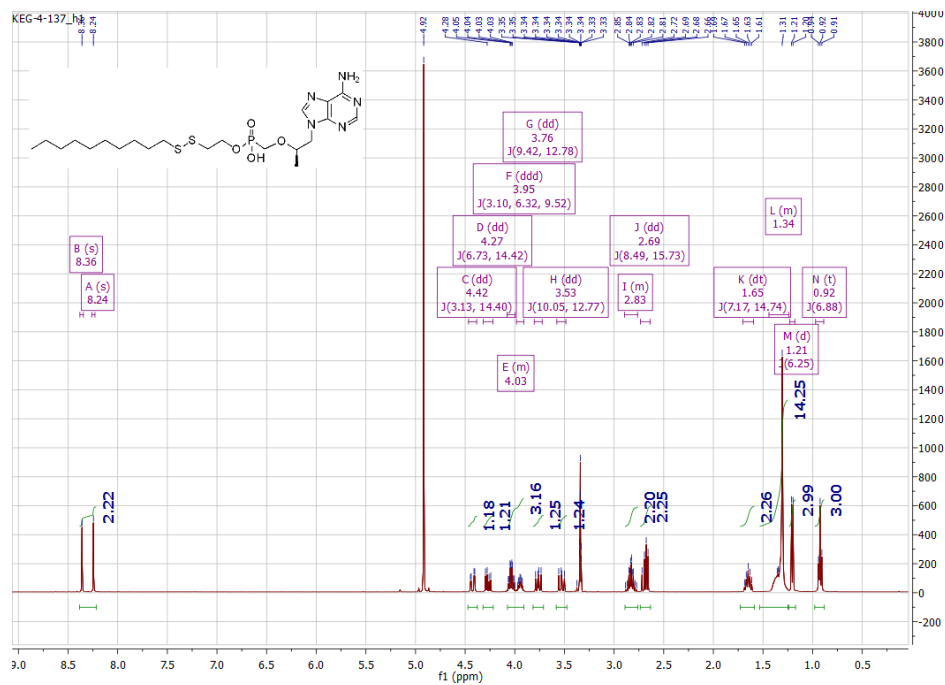


$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

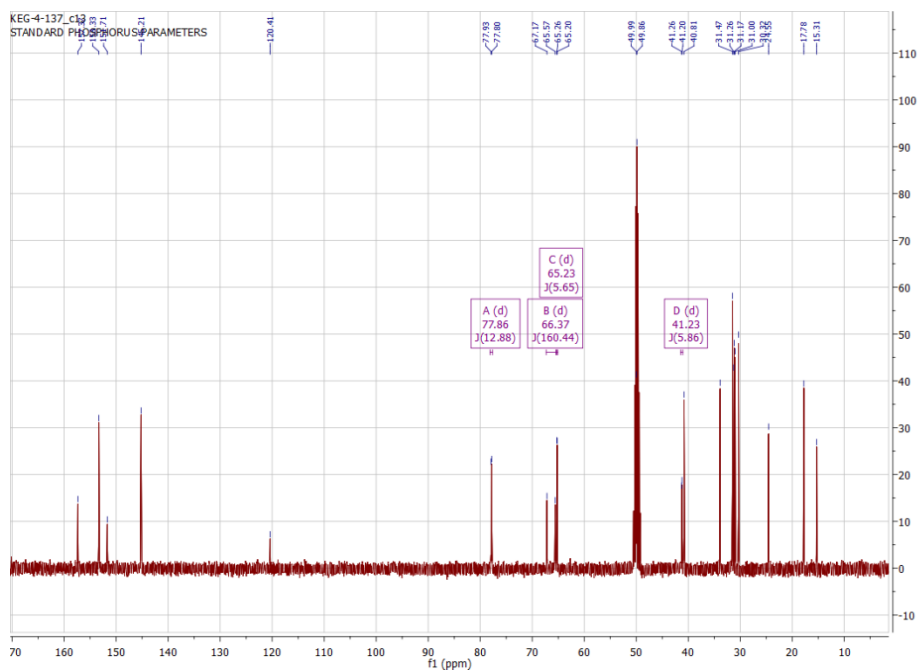


**2-(decyldisfany)ethyl hydrogen (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (12a)**

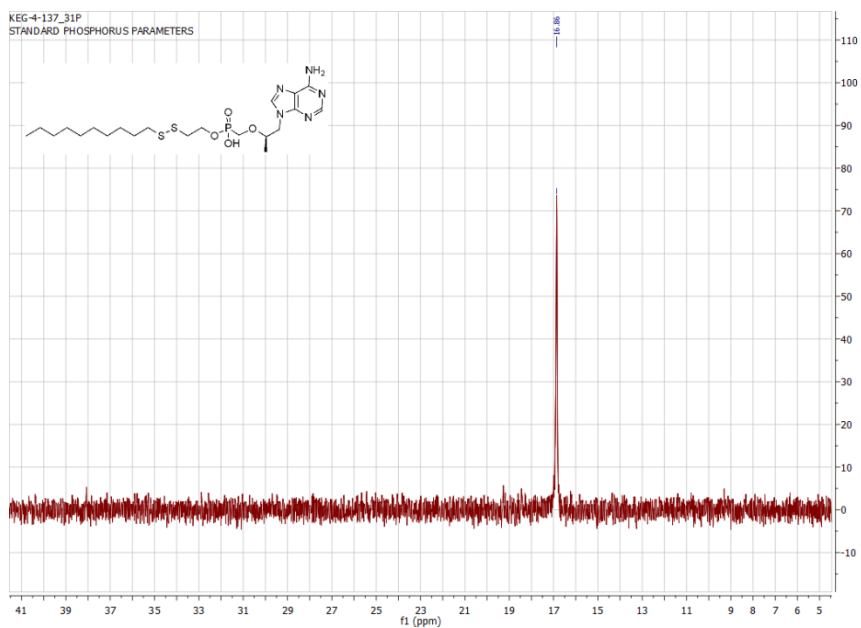
<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)



<sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD)

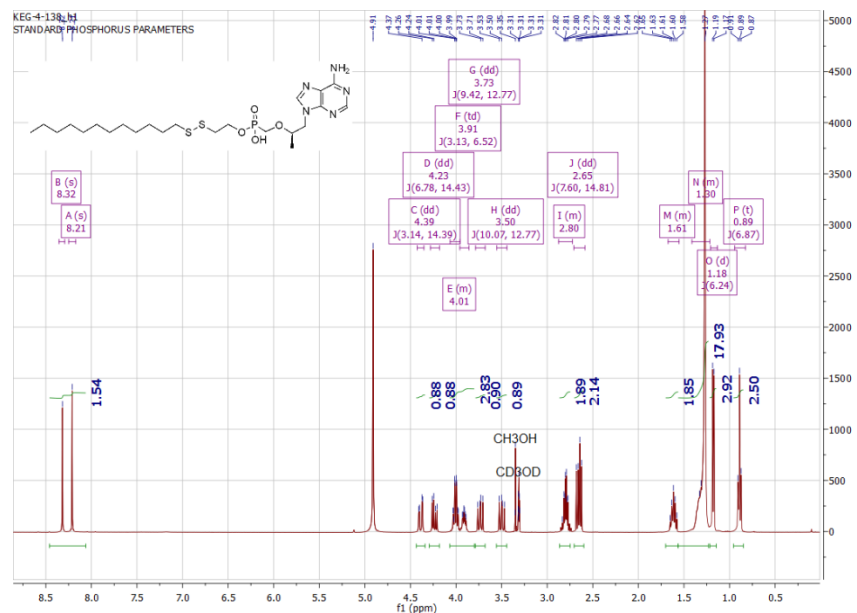


$^{31}\text{P}$  NMR (162 MHz,  $\text{CD}_3\text{OD}$ )



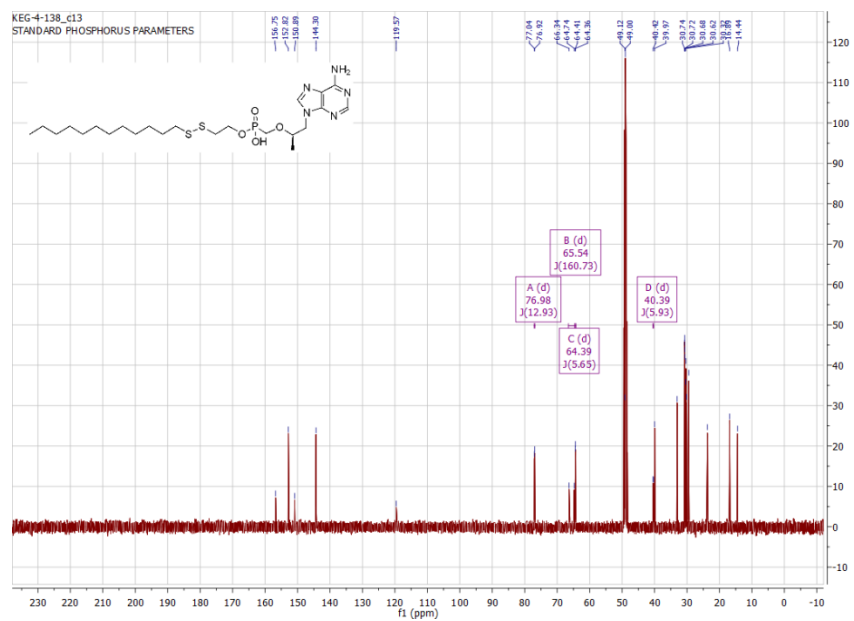
**2-(dodecyldisulfanyl)ethyl hydrogen (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (12b)**

$^1\text{H}$  NMR (399 MHz,  $\text{CD}_3\text{OD}$ )

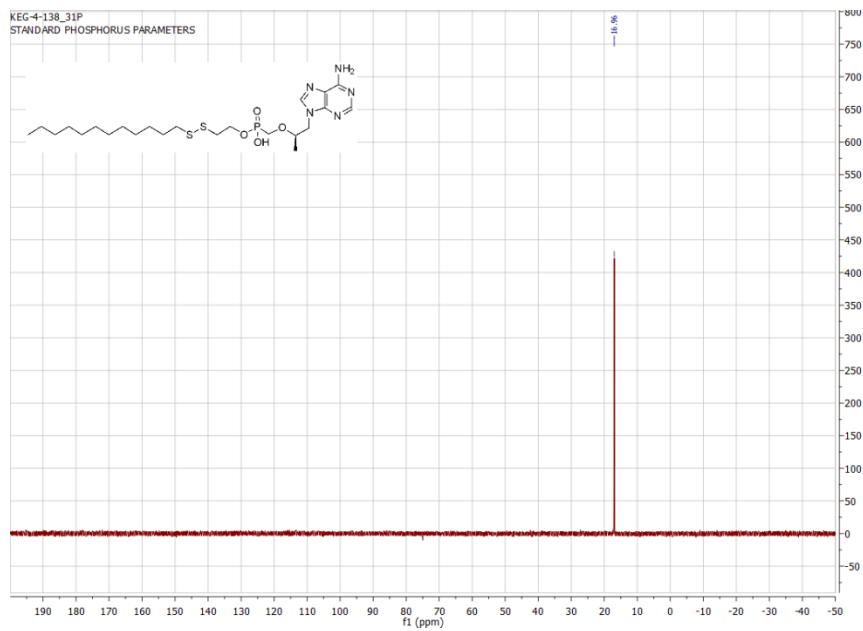




$^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )

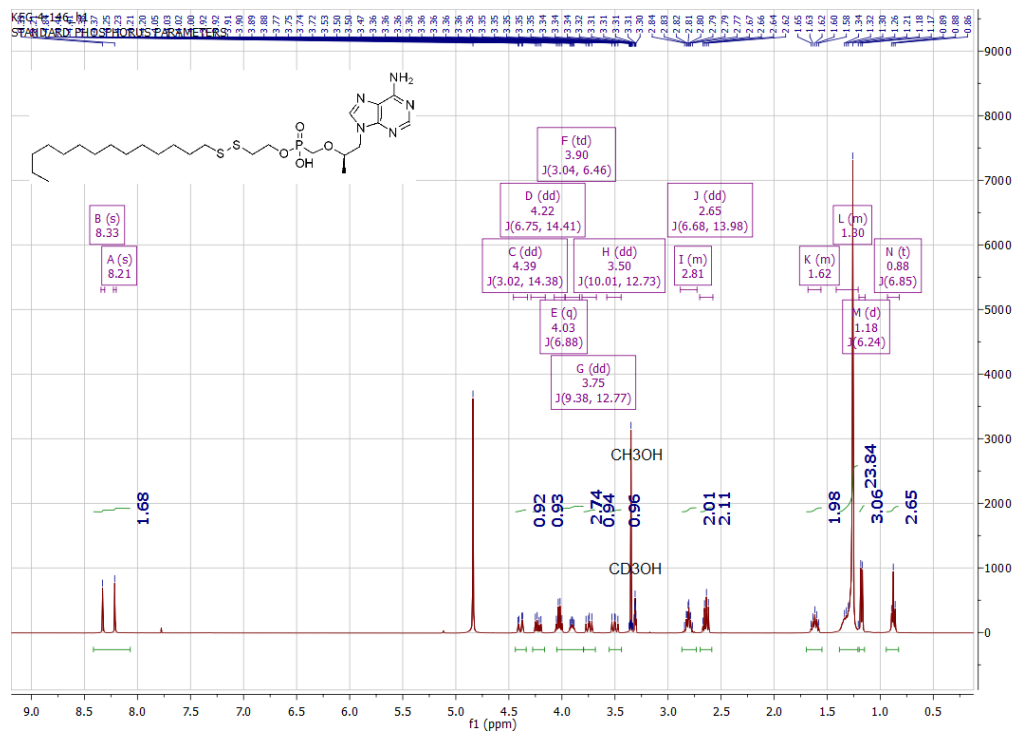


$^{31}\text{P}$  NMR (162 MHz,  $\text{CD}_3\text{OD}$ )

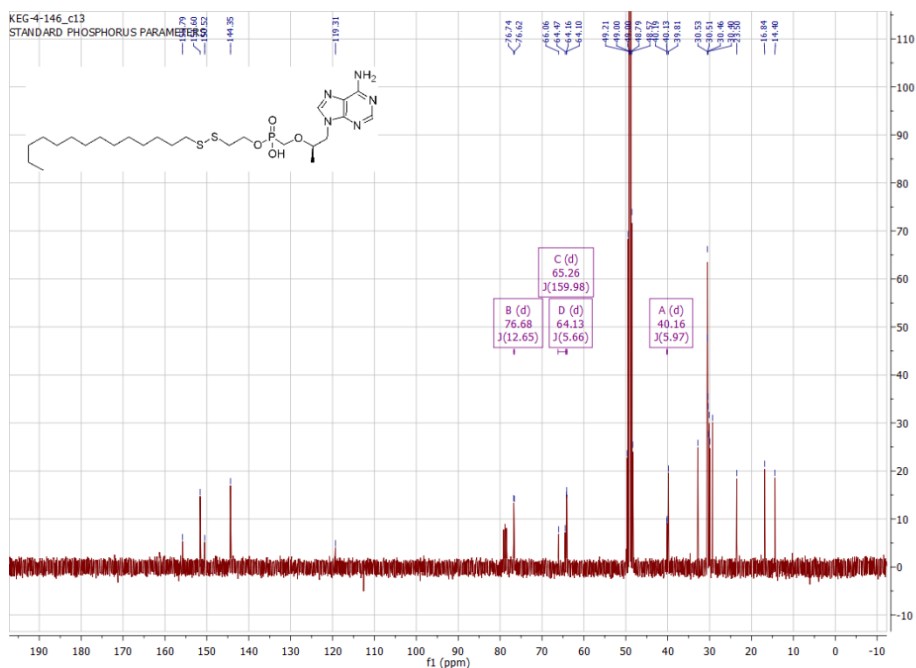


## 2-((tetradecyldisulfanyl)ethyl hydrogen (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (12c)

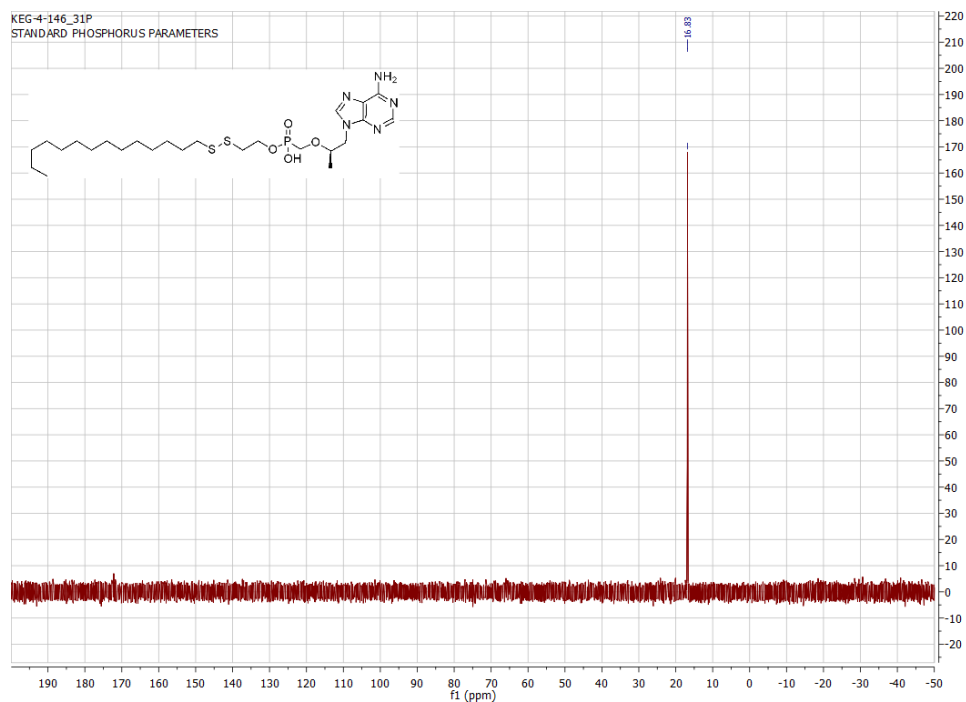
$^1\text{H}$  NMR (399 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ )



$^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ )

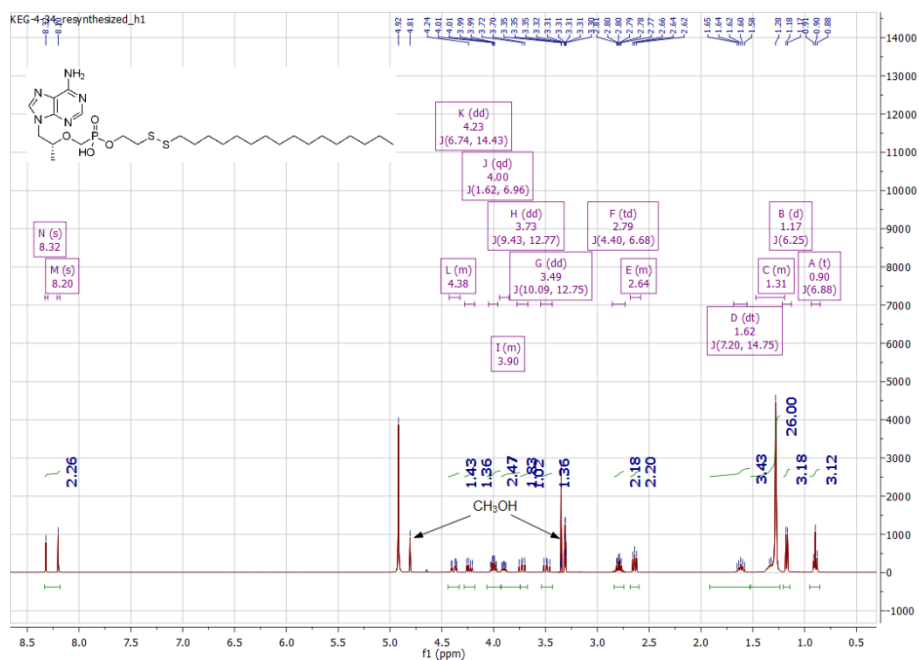


$^{31}\text{P}$  NMR (162 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ )

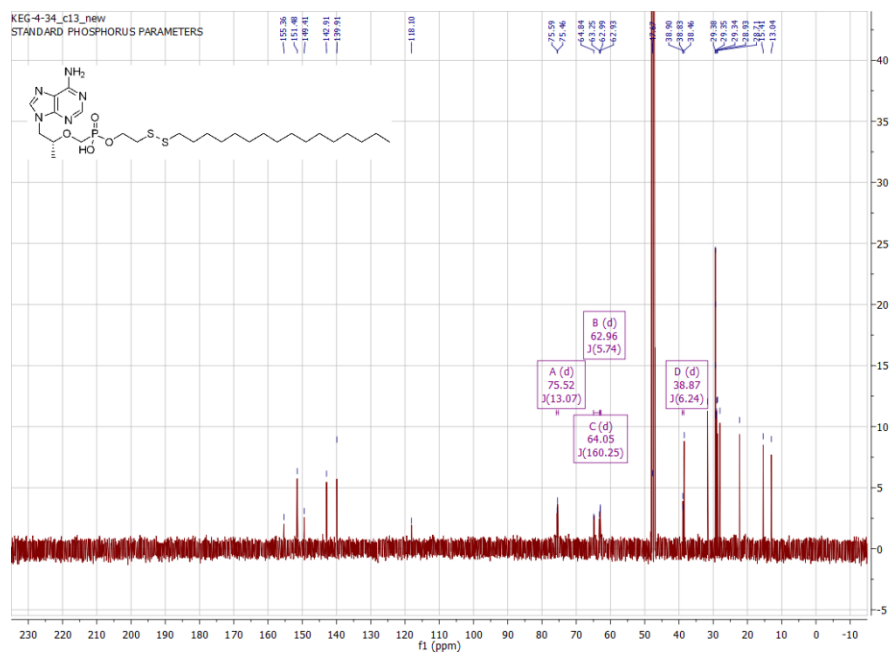


## 2-(hexadecyldisulfanyl)ethyl hydrogen (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl phosphonate (12d)

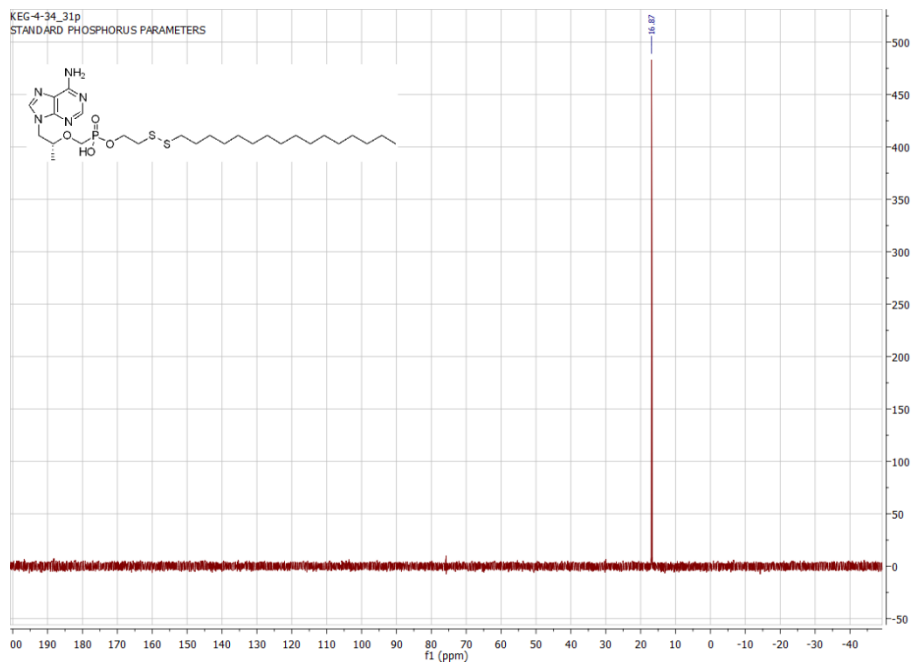
$^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



$^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ )

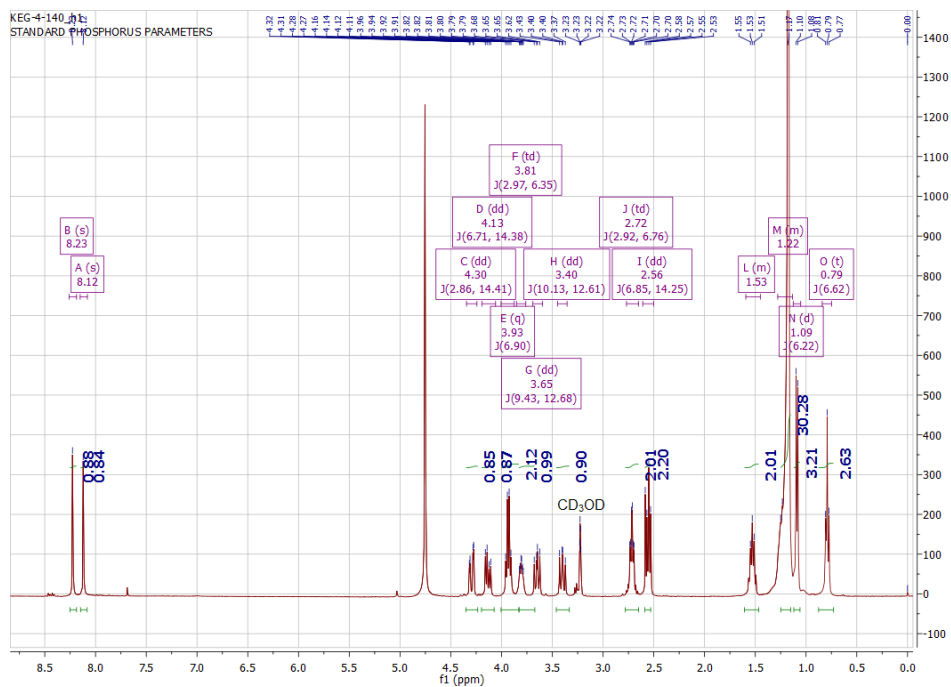


$^{31}\text{P}$  NMR (162 MHz,  $\text{CD}_3\text{OD}$ )

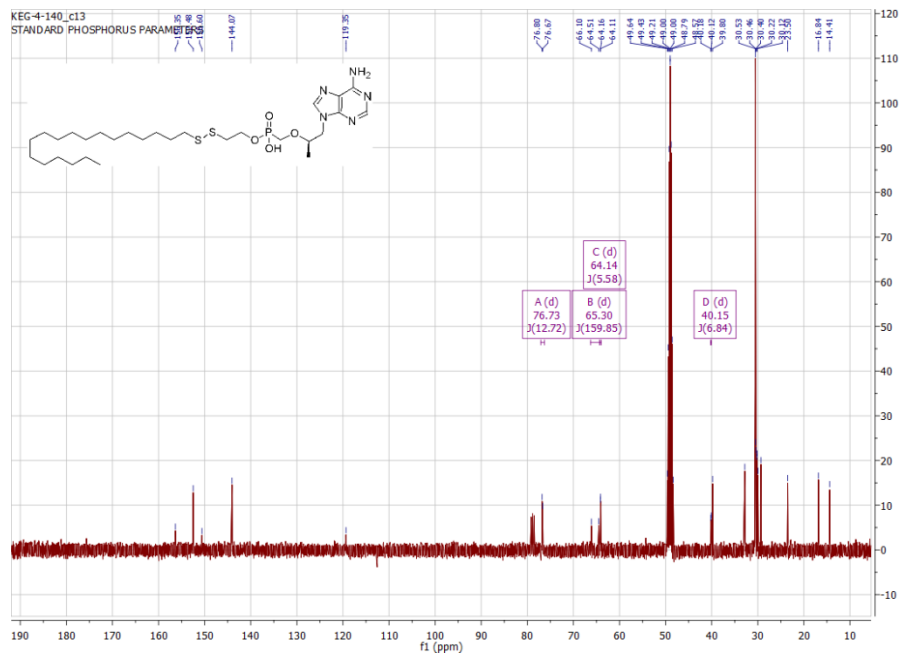


# 2-(octadecylsulfanyl)ethyl hydrogen (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (12e)

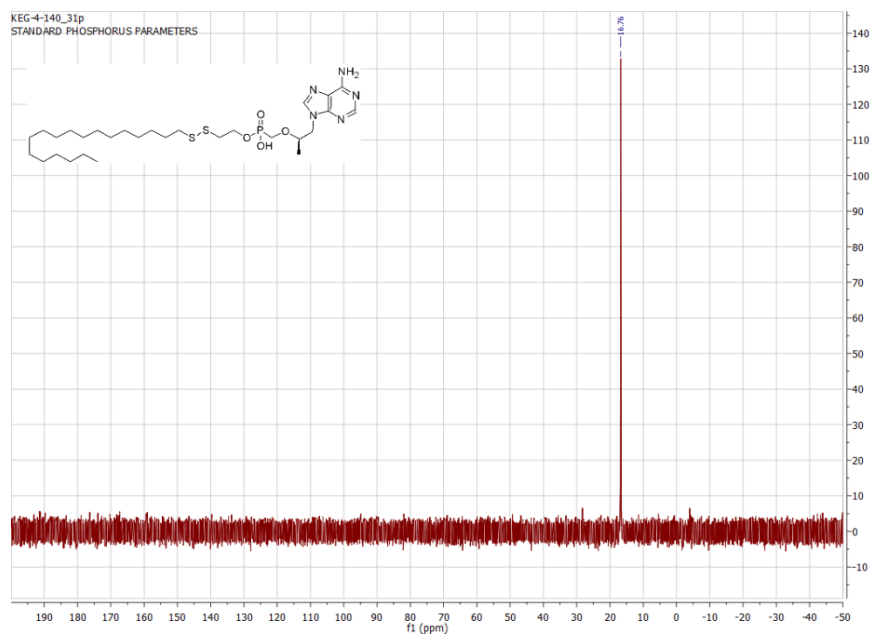
$^1\text{H}$  NMR (399 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ )



$^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ )

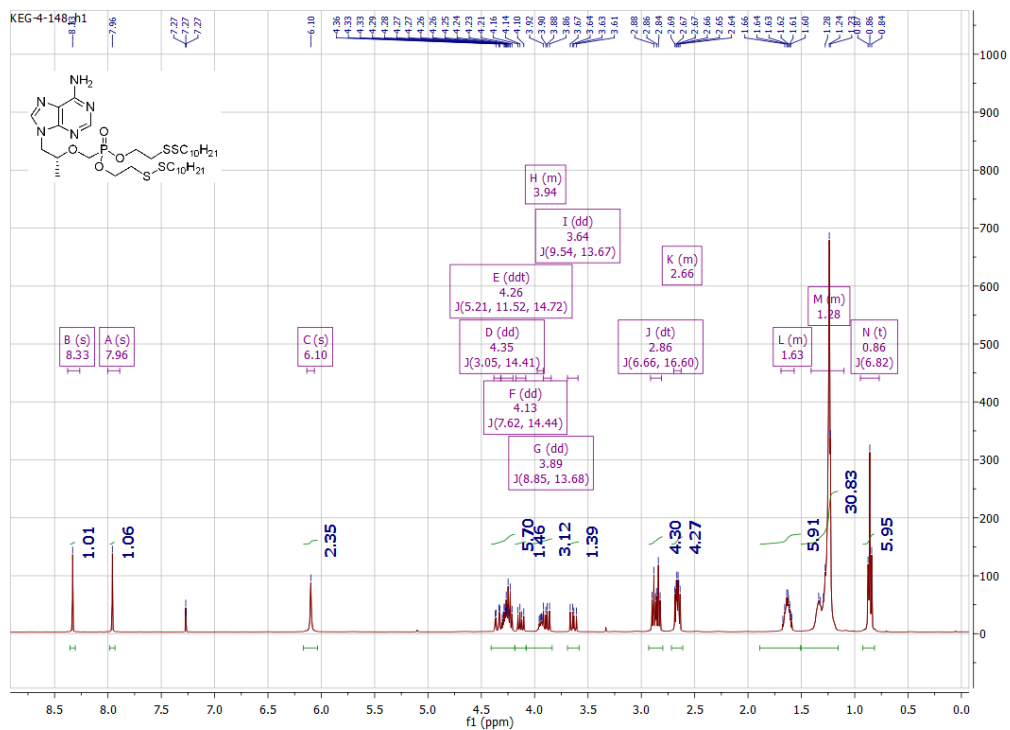


$^{31}\text{P}$ NMR (162 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ )



**2-(decyldisulfanyl)ethyl (2-(decylsulfinothioyl)ethyl) (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl phosphonate (13a)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

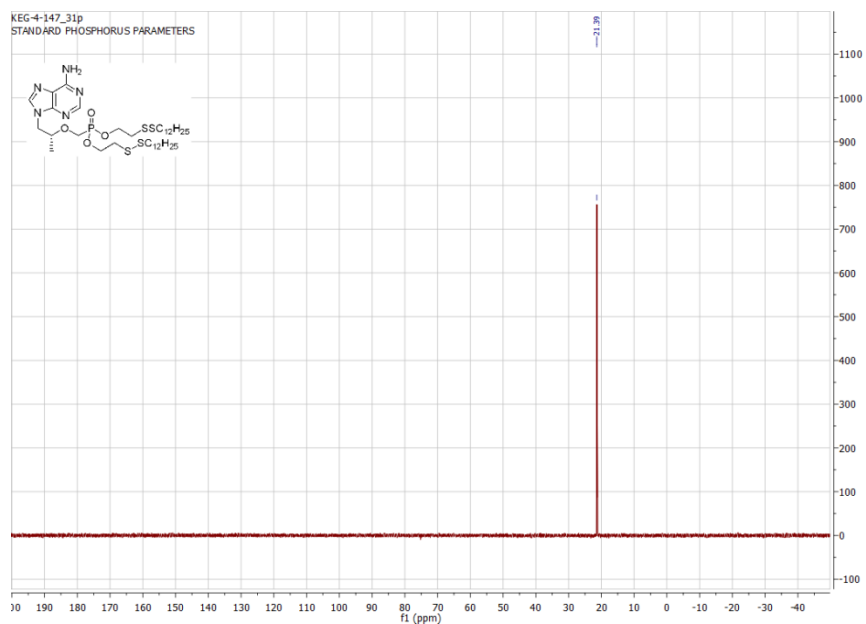






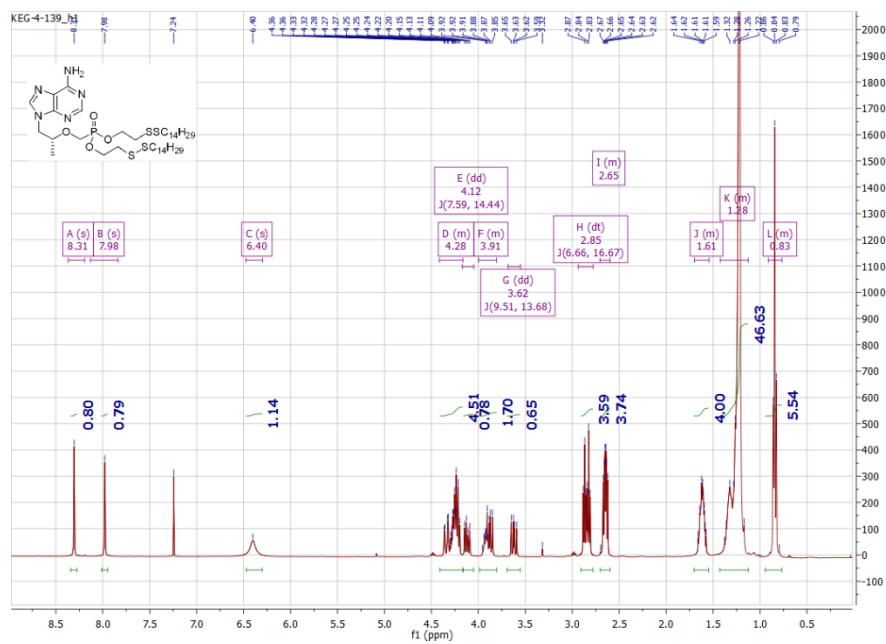


$^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )  $\delta$  21.39

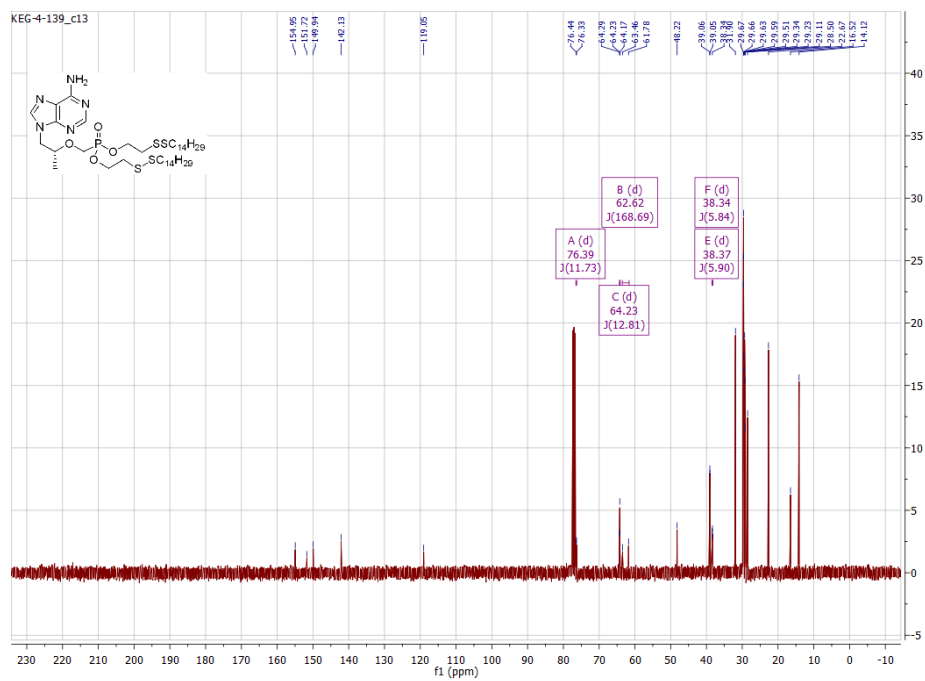


**2-(tetradecyldisulfanyl)ethyl (2-(tetradecylsulfinothioyl)ethyl) (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (13c)**

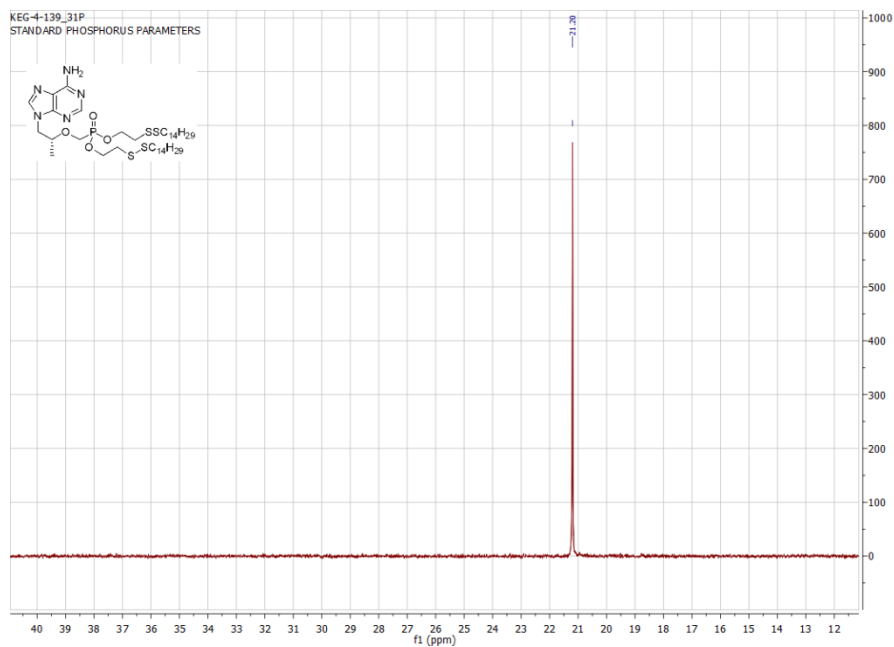
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

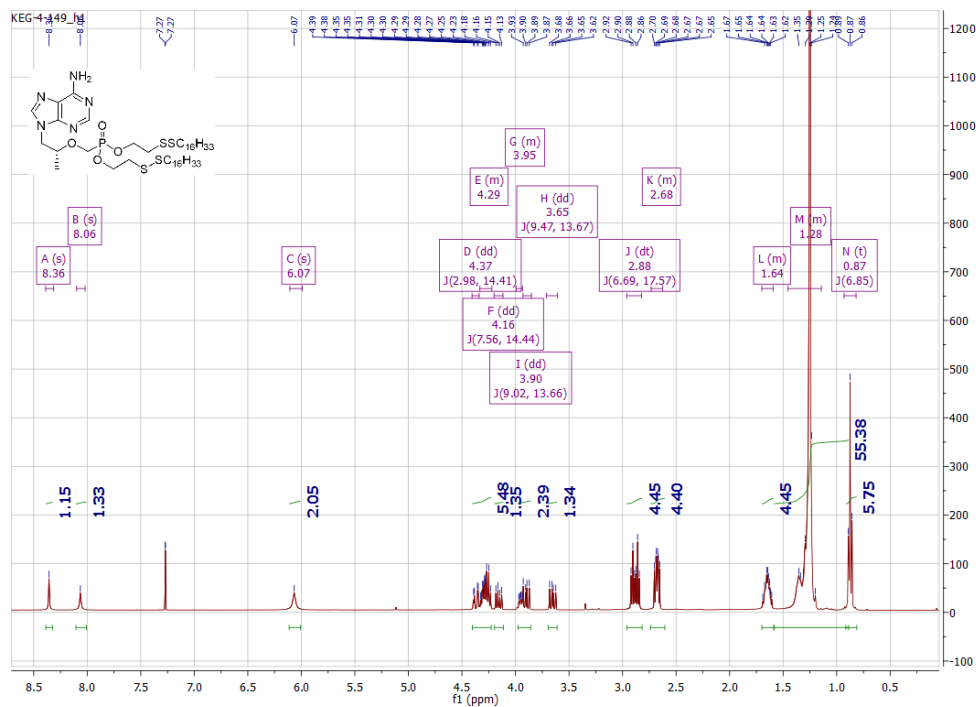


$^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )

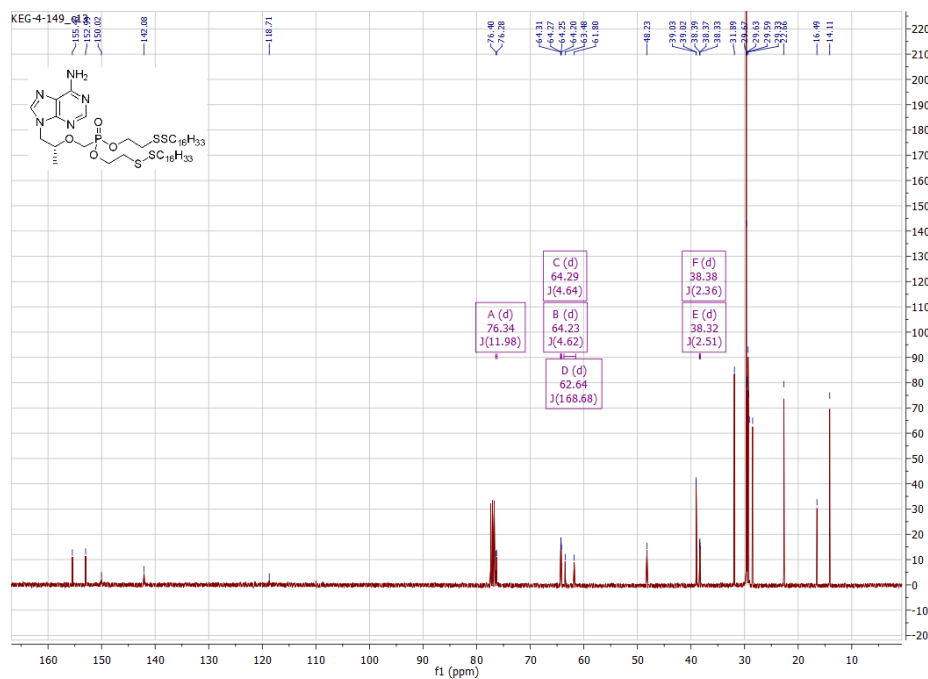


**2-(hexadecyldisulfanyl)ethyl 2-(hexadecylsulfinothioyl)ethyl (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (13d)**

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)

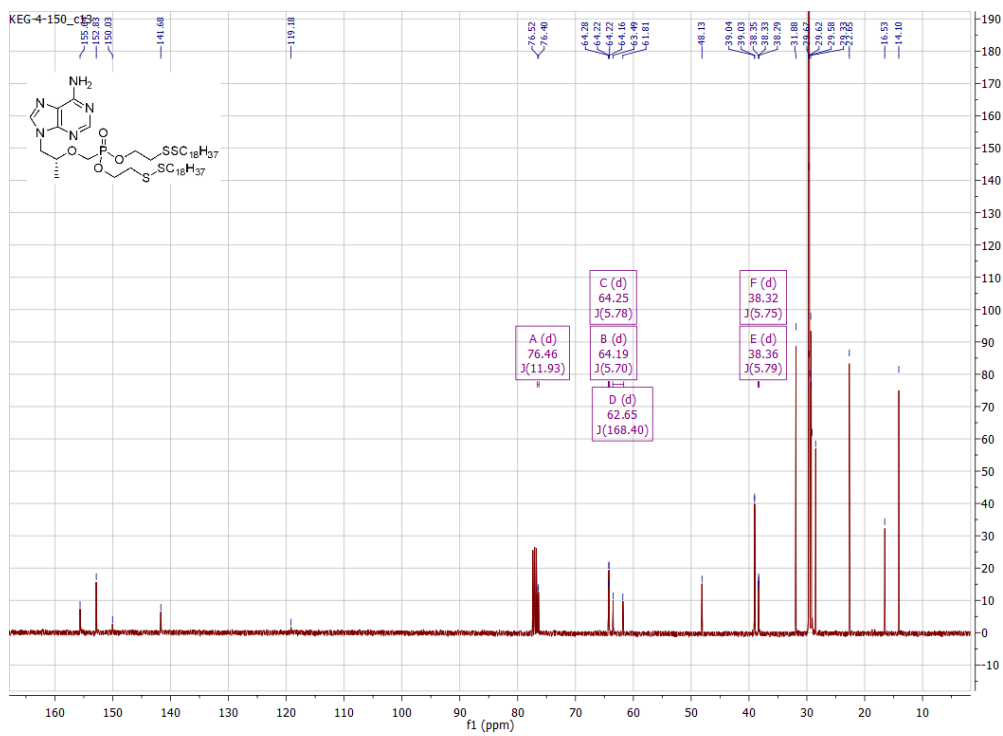


<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)

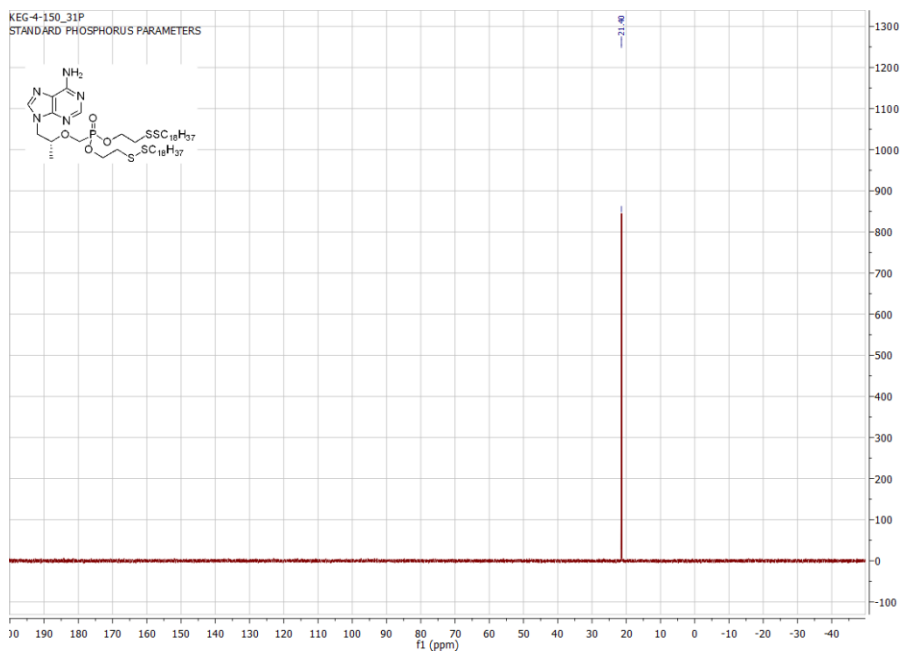




<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)

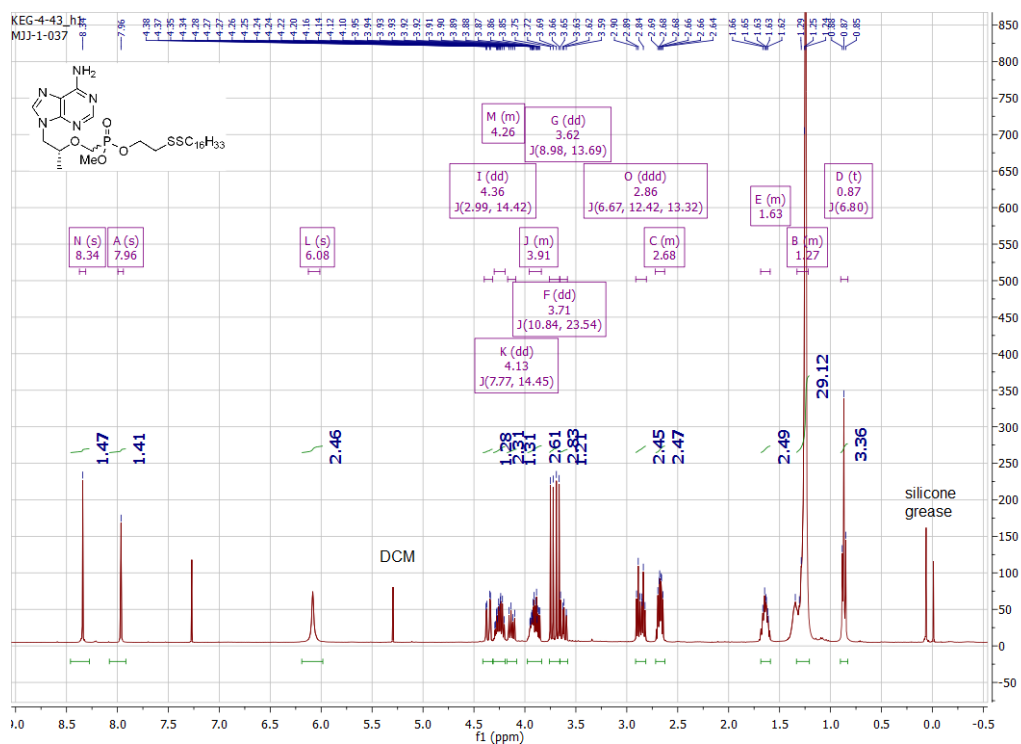


<sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>)

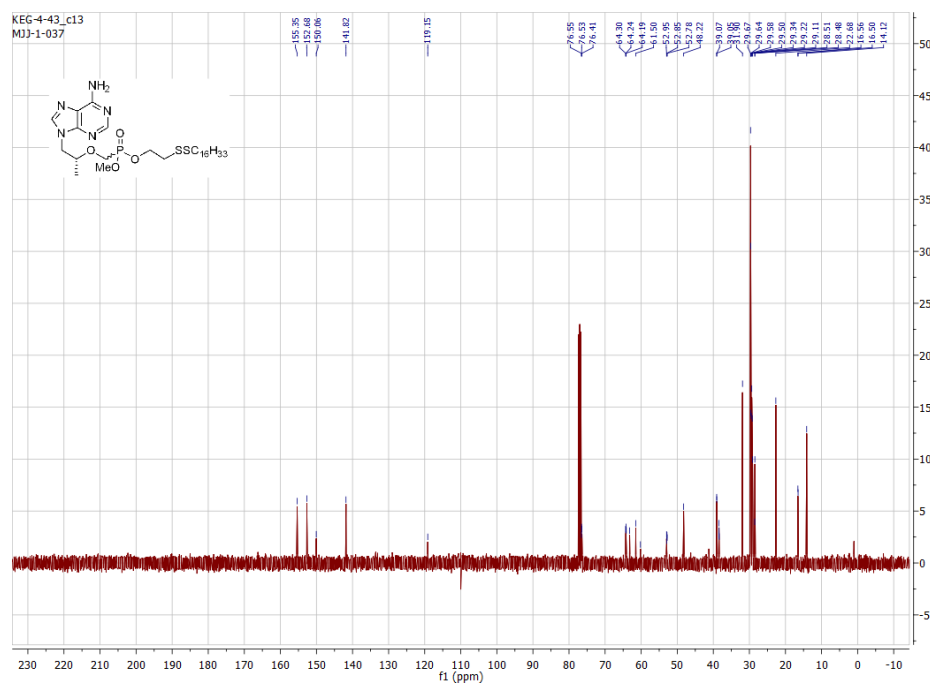


# 2-(hexadecyldisulfanyl)ethyl methyl (((1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (14)

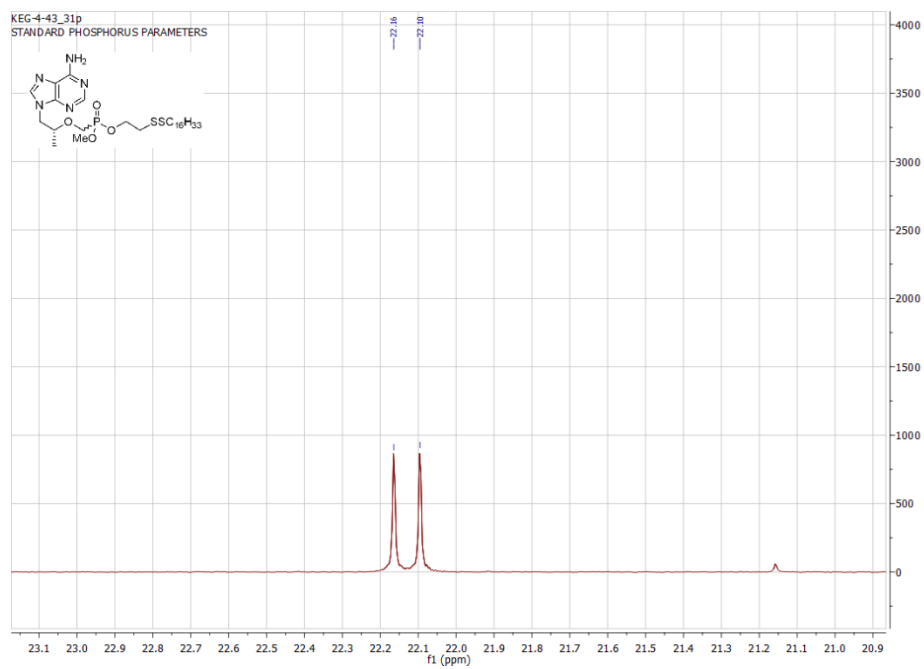
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

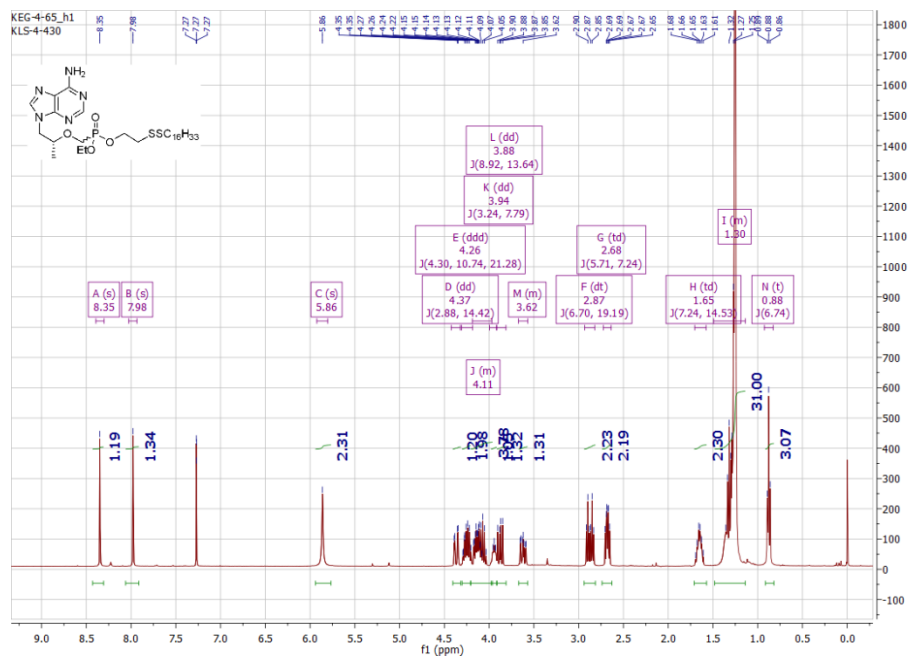


$^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )

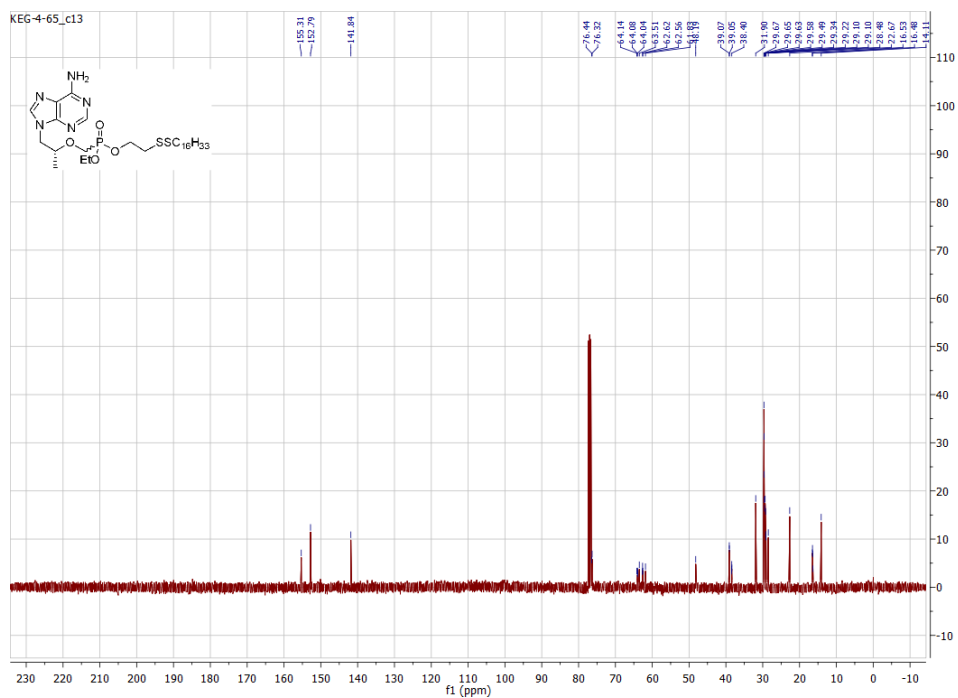


**(2-(hexadecyldisulfanyl)ethyl) (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (15)**

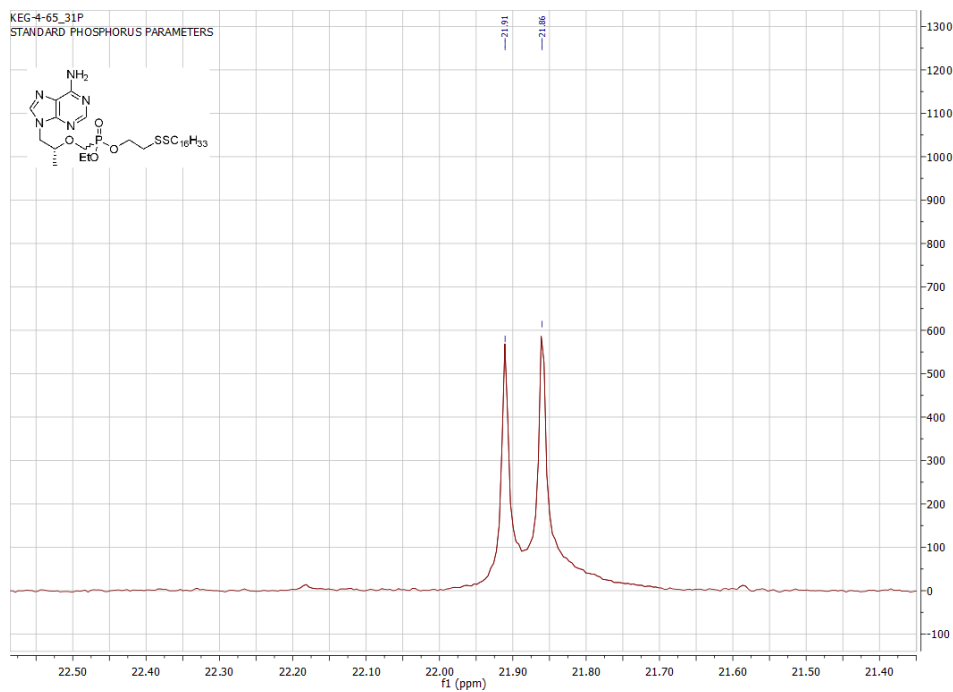
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )



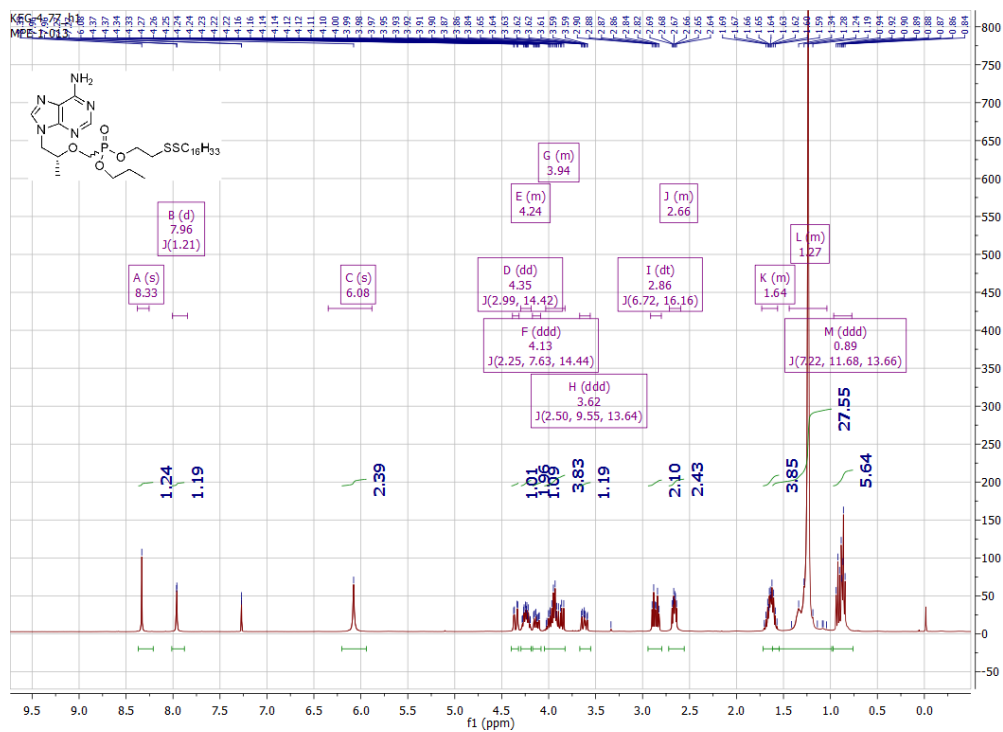
$^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )



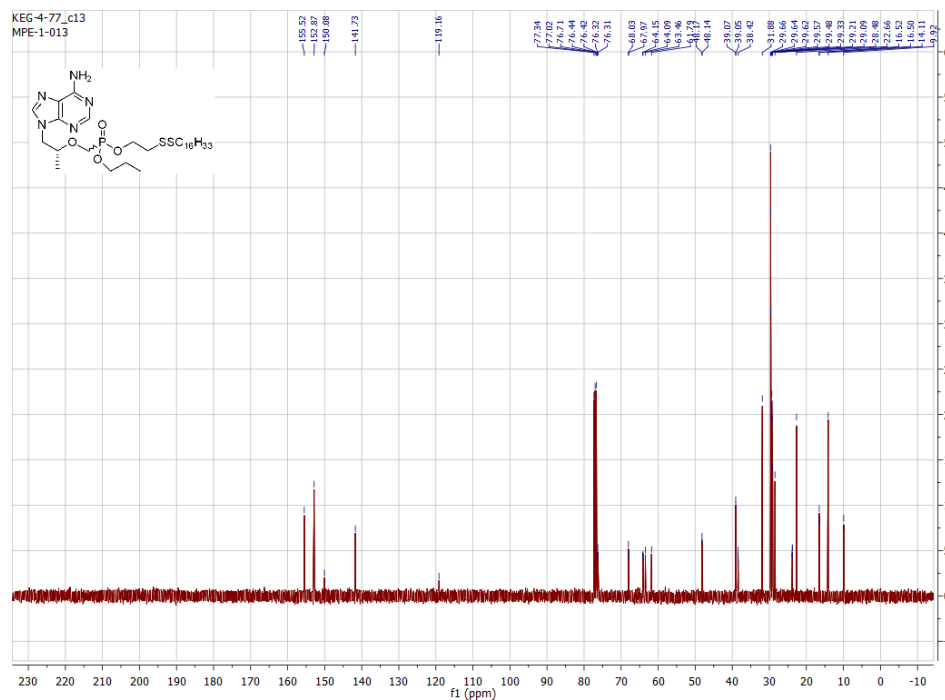


# 2-(hexadecyldisulfanyl)ethyl propyl (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (16)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

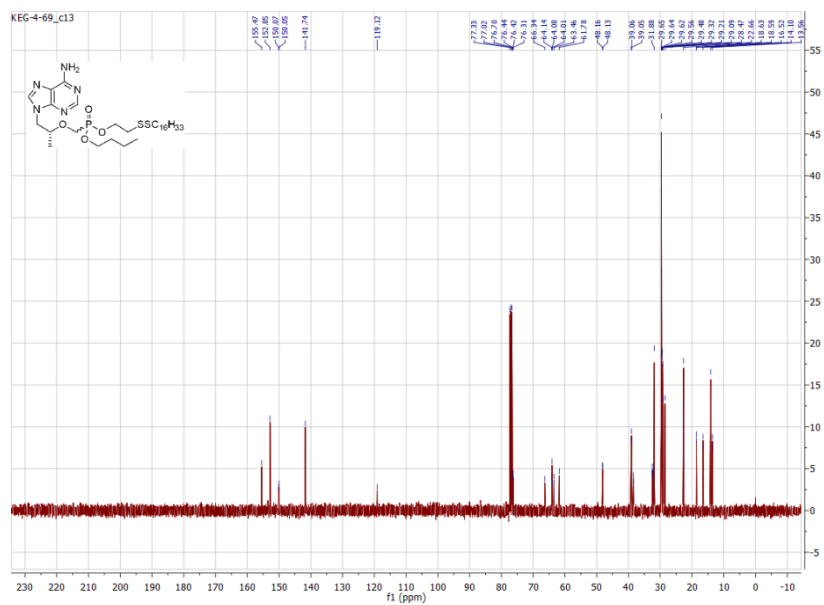


$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

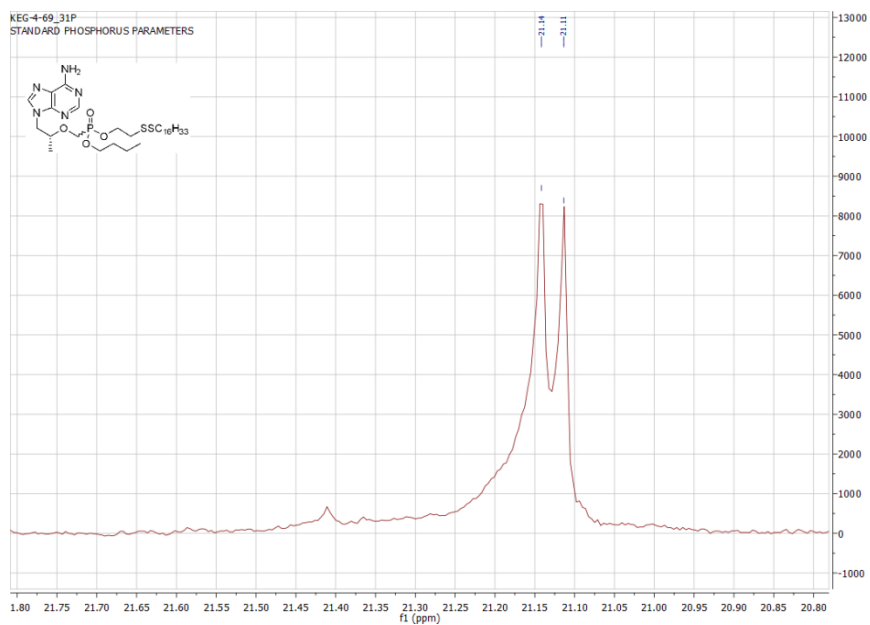




$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

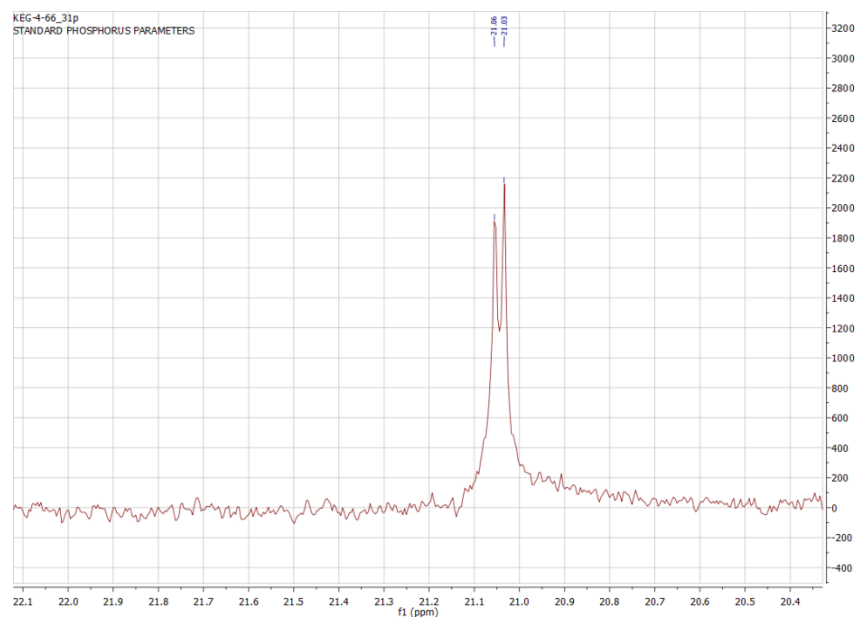


$^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ )



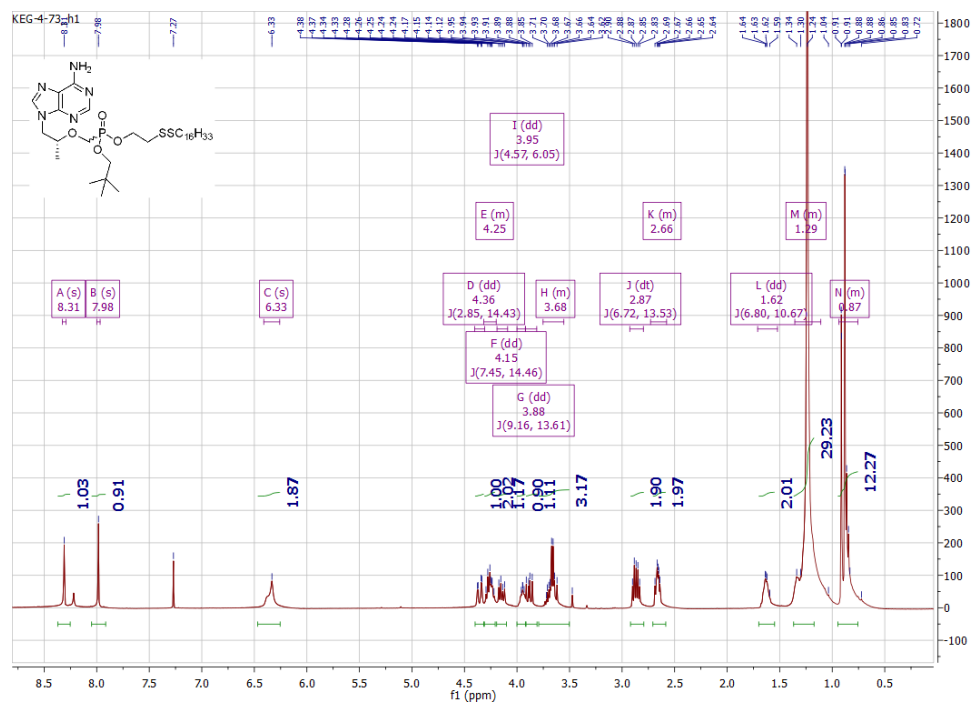


$^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ )

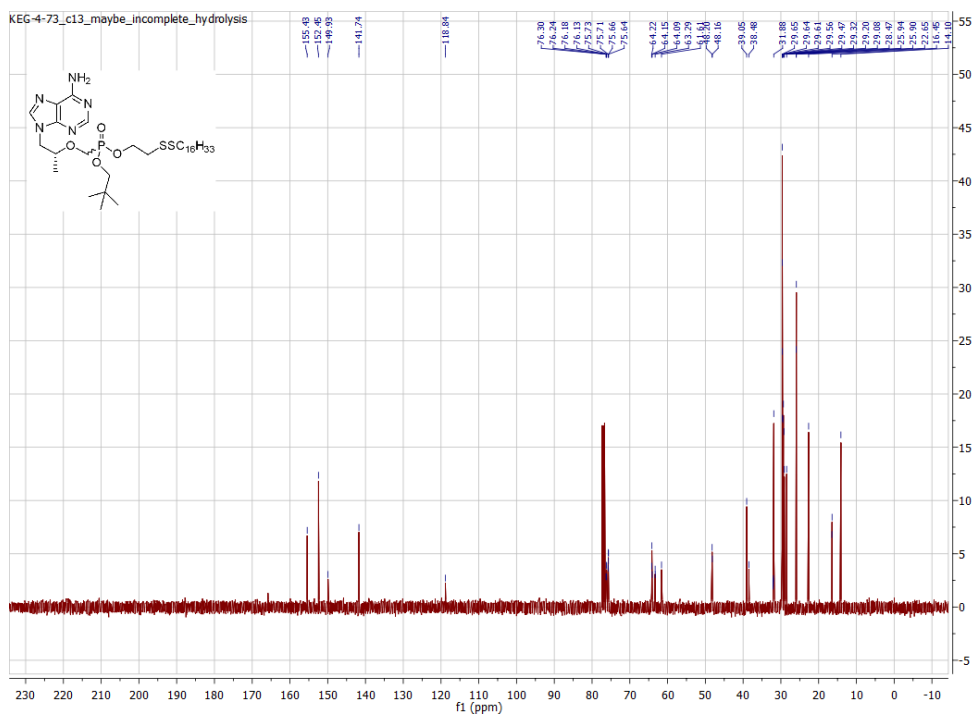


**2-(hexadecylsulfanyl)ethyl neopentyl (((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonate (19)**

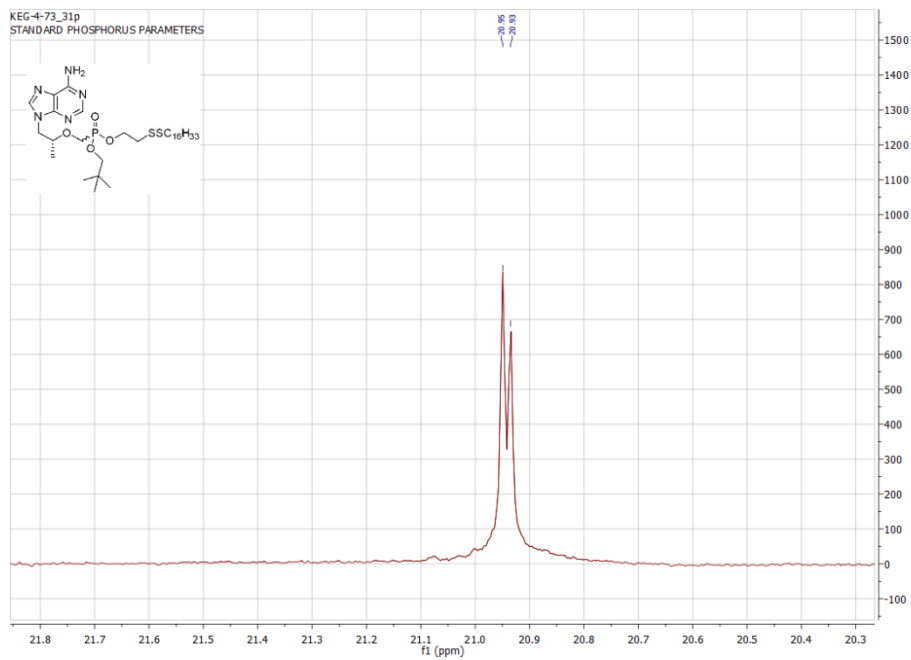
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )

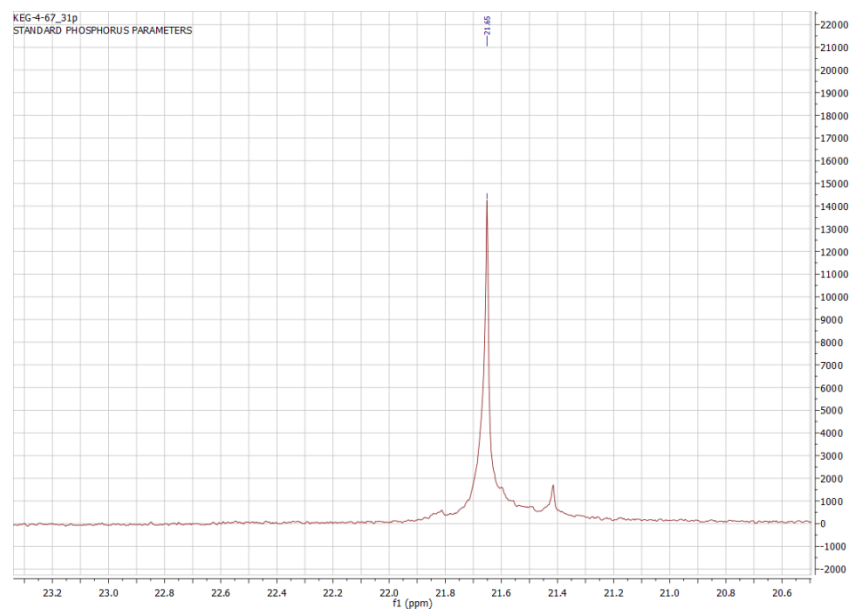


$^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )



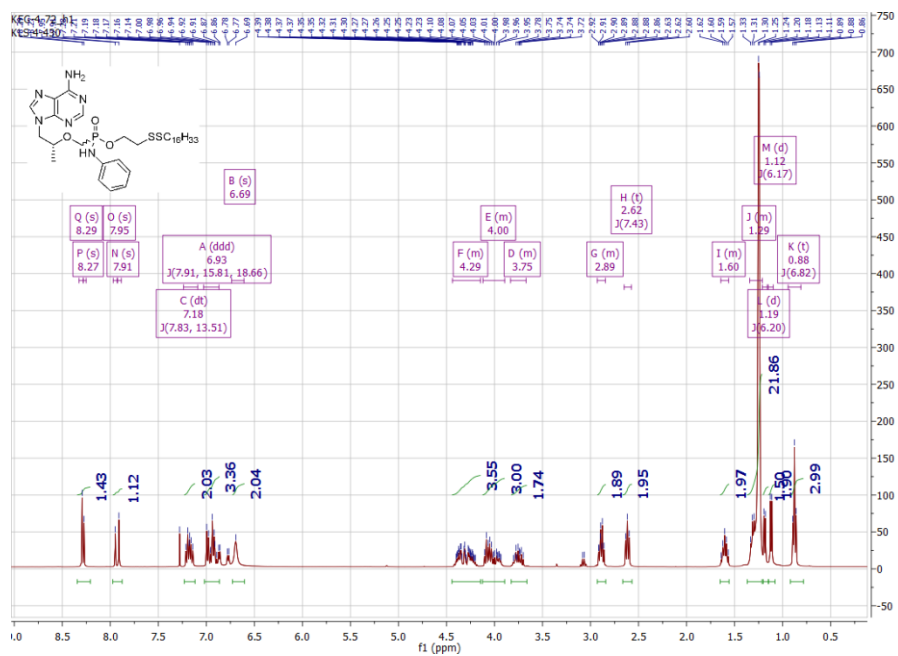


$^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ )



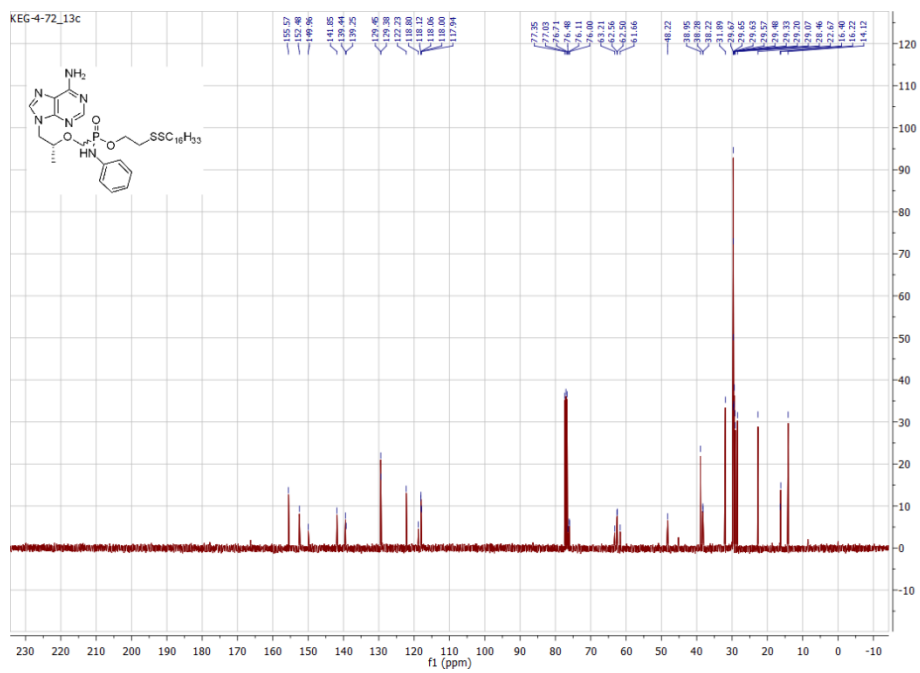
**2-(hexadecyldisulfanyl)ethyl P-(((*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)-*N*-phenylphosphoramidate (21)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

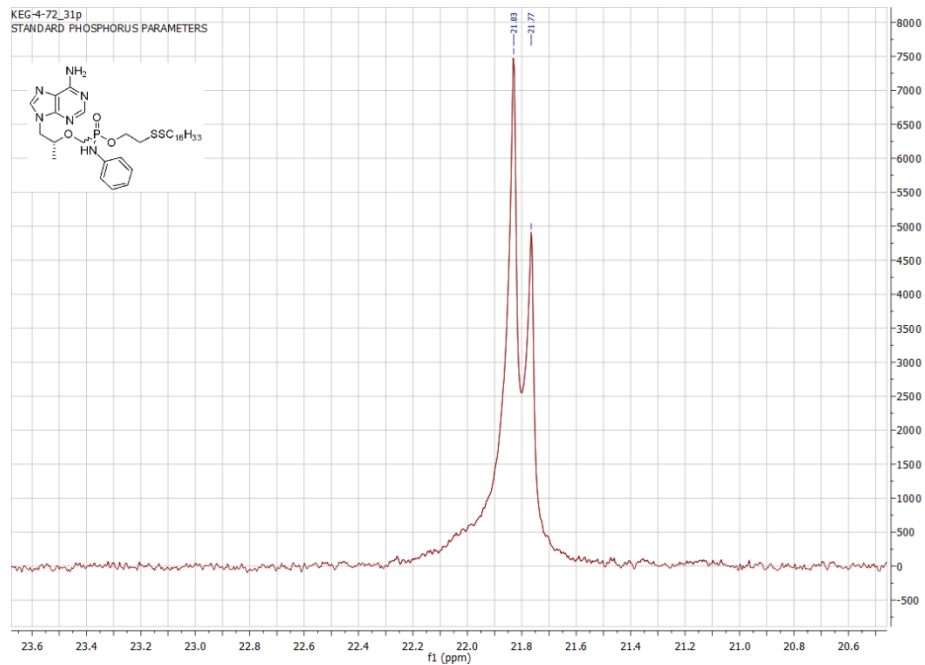




$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )



$^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ )





$^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )

