

Supporting Information to

Enzymatic synthesis of 2'-methylseleno-modified RNA

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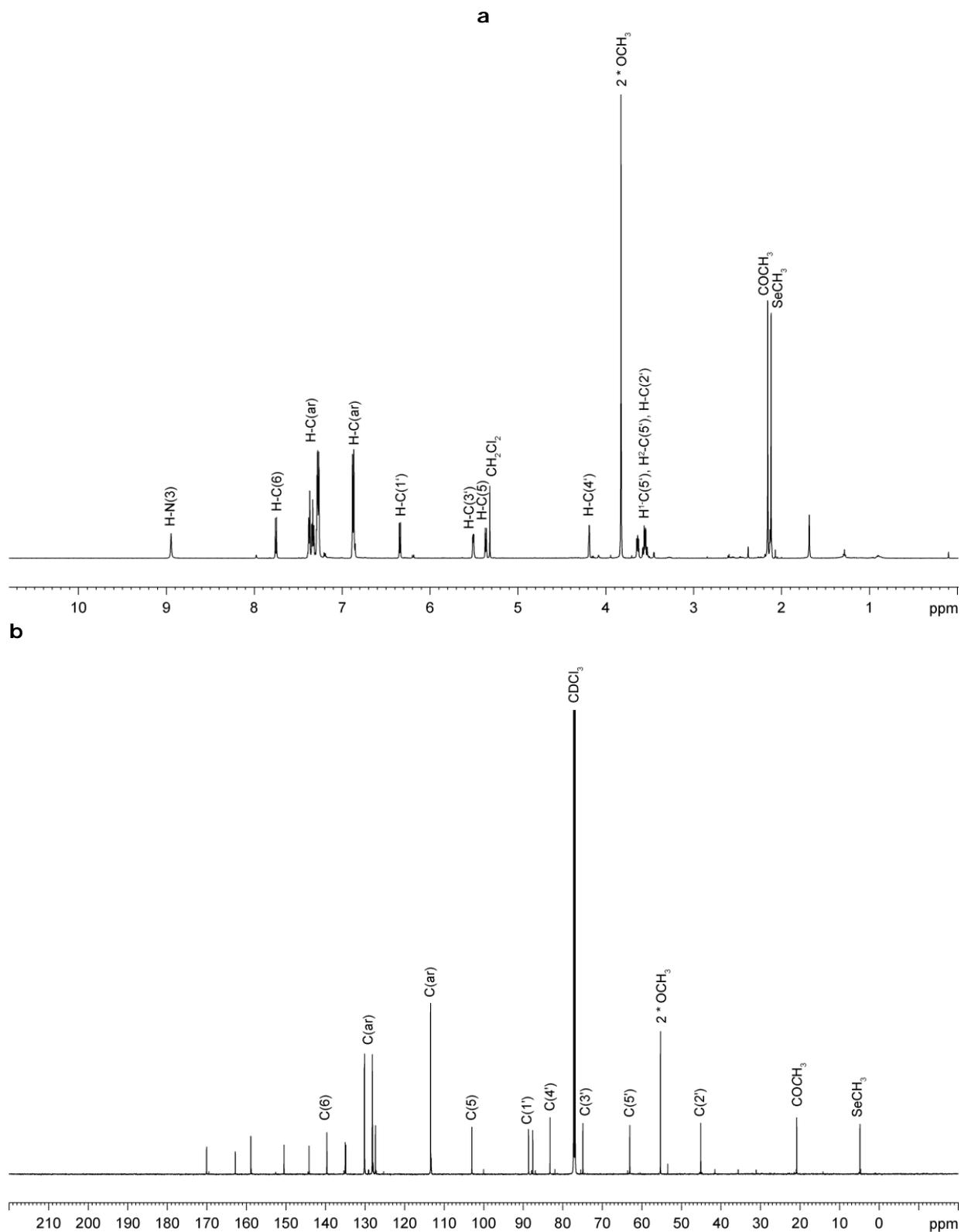
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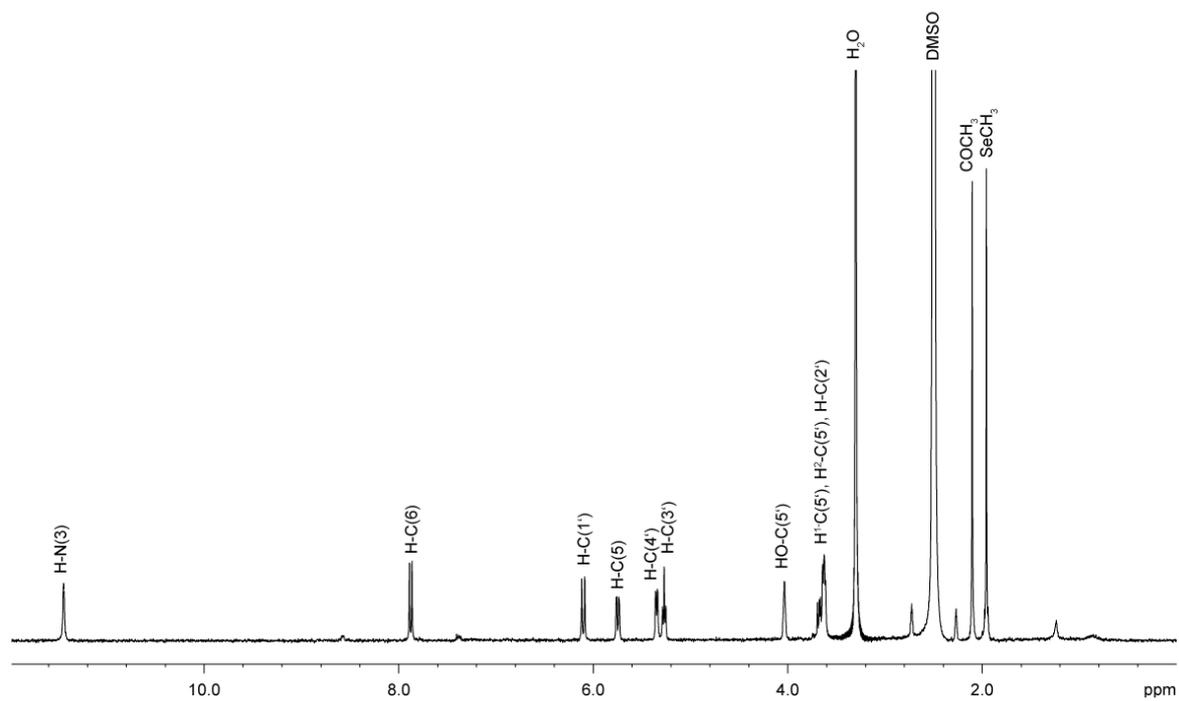
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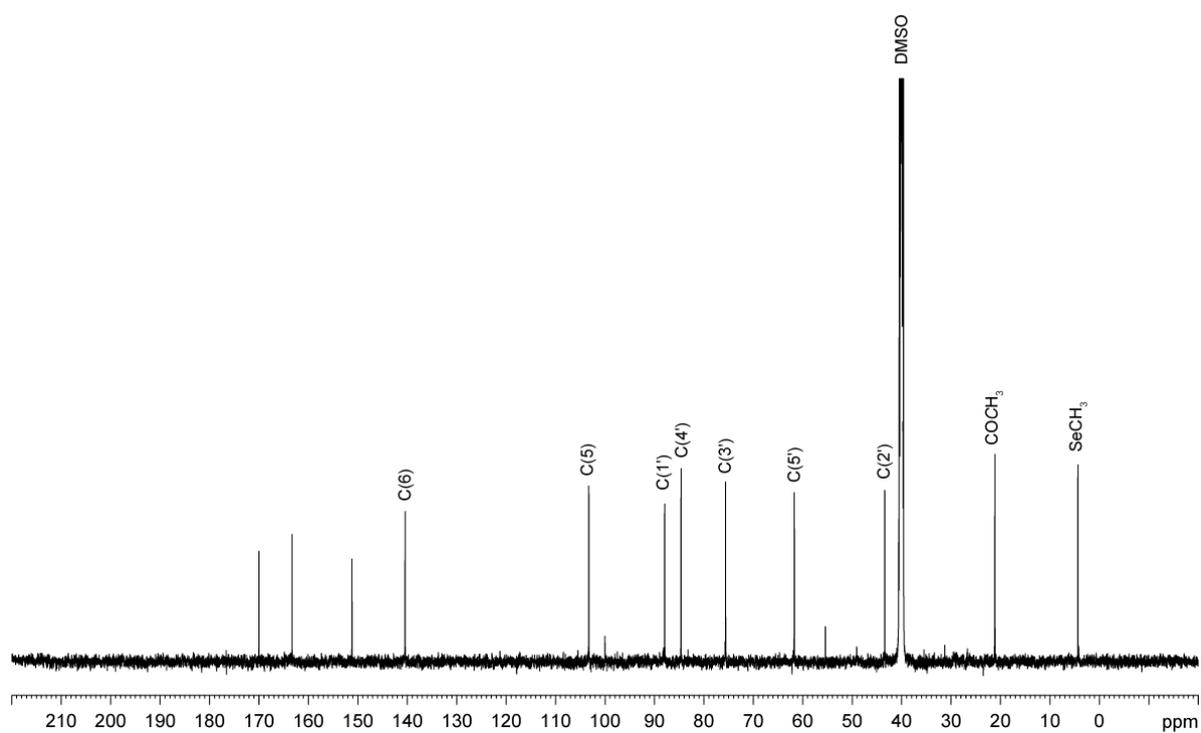


Supporting Figure 1. NMR spectra of 3'-acetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-methylseleno-2'-deoxyuridine (**2**). (a) $^1\text{H-NMR}$ (300 MHz, CDCl_3). (b) $^{13}\text{C-NMR}$ (150 MHz, CDCl_3).

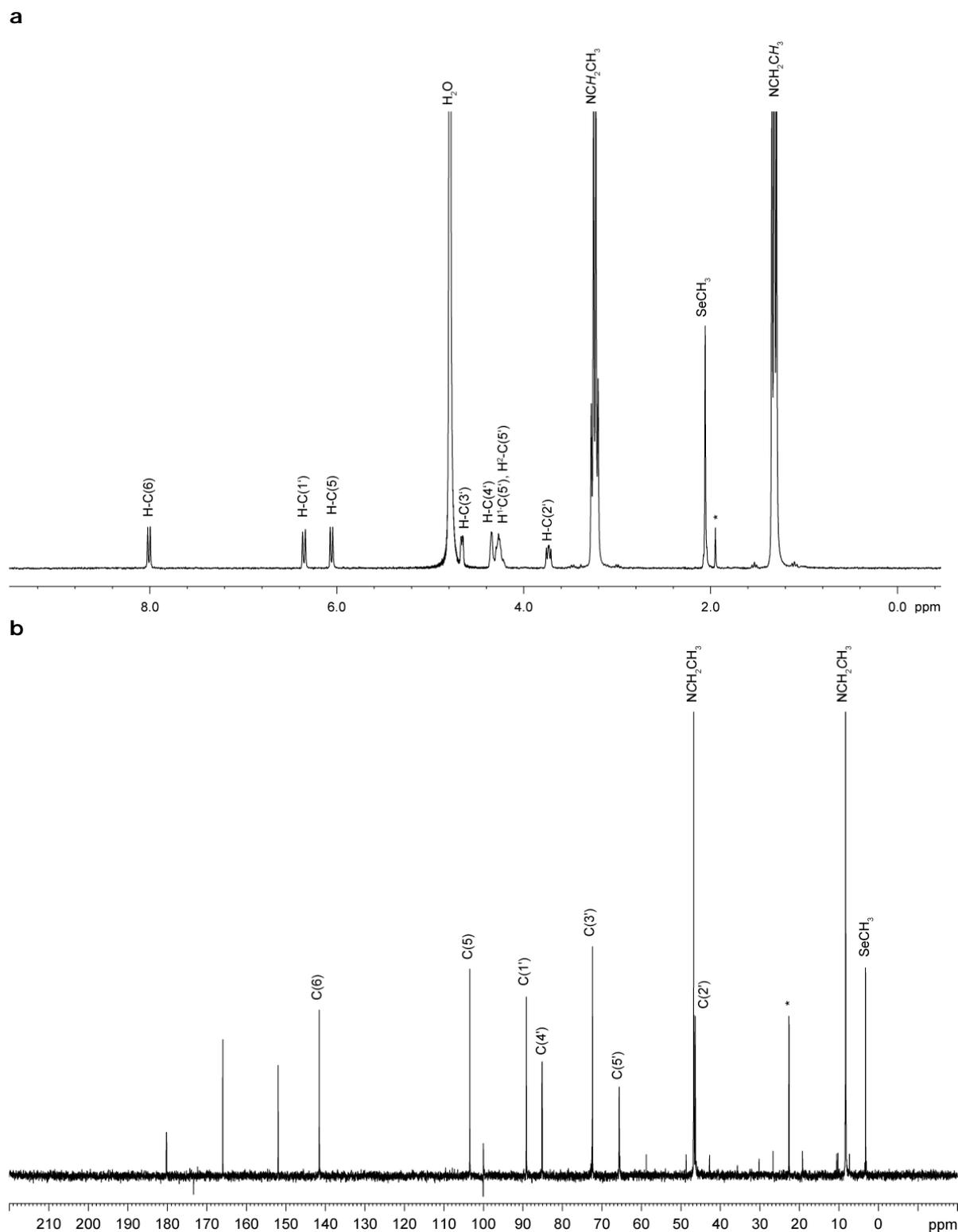
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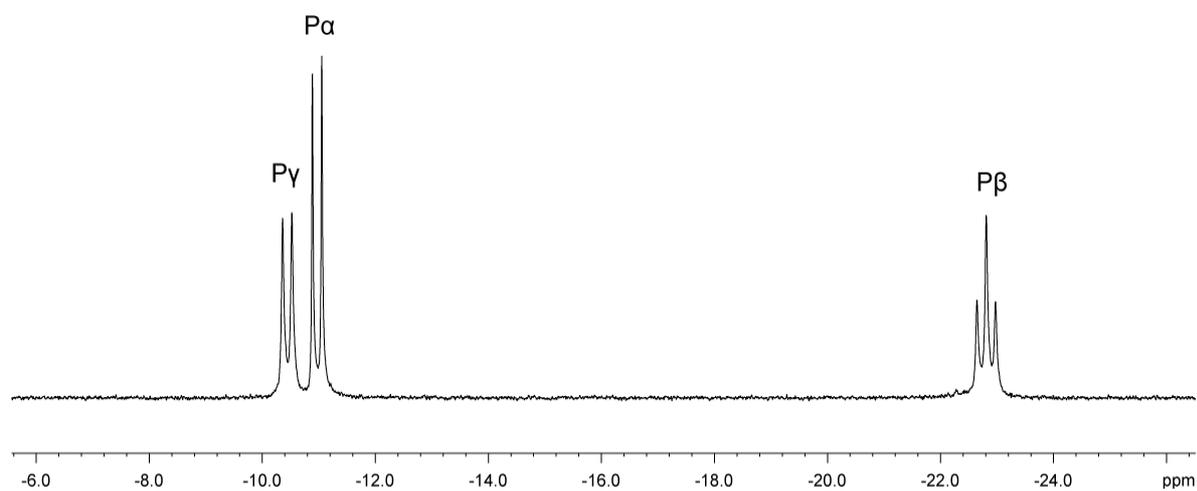
b



Supporting Figure 2. NMR spectra of 3'-acetyl-2'-methylseleno-2'-deoxyuridine (**3**).
(a) ^1H -NMR (300 MHz, $\text{DMSO}-d_6$). (b) ^{13}C -NMR (150 MHz, $\text{DMSO}-d_6$).

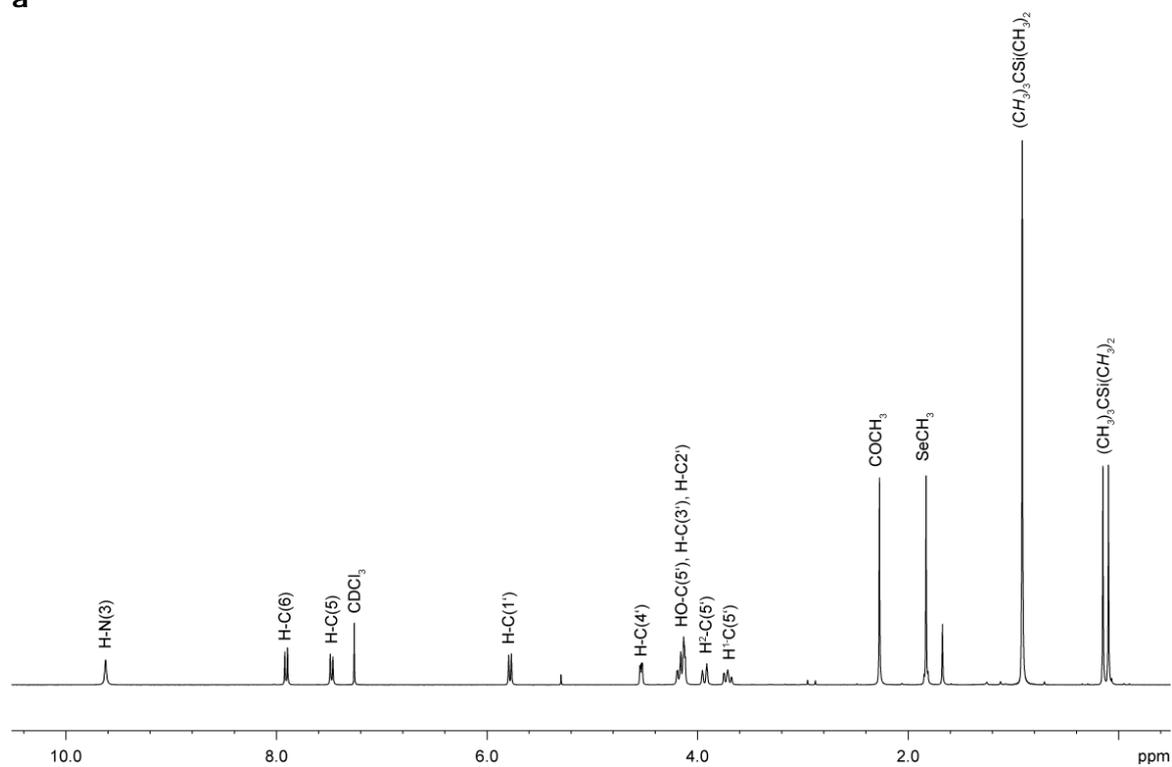


Supporting Figure 3. NMR spectra of 2'-methylseleno-2'-deoxyuridine 5'-triphosphate (**4**). (a) $^1\text{H-NMR}$ (300 MHz, D_2O). (b) $^{13}\text{C-NMR}$ (150 MHz, D_2O). * Resonances flagged with an asterisk indicate residual amounts of acetate anion from TEAA buffer.

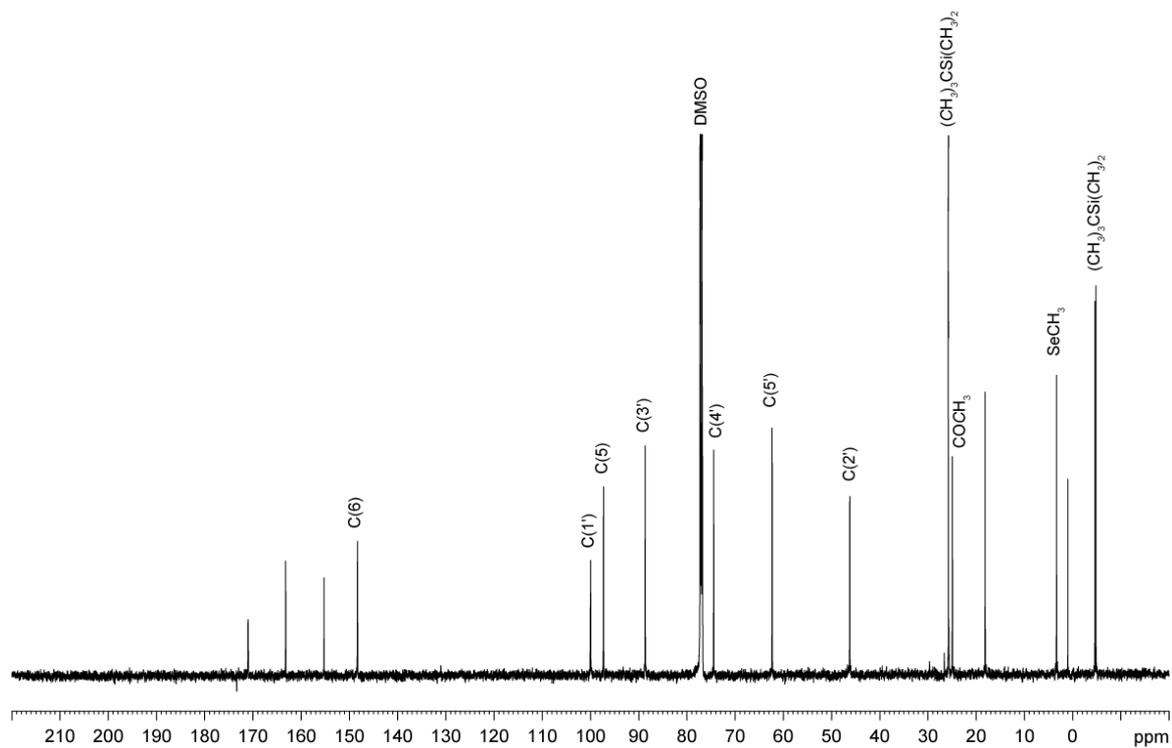


Supporting Figure 4. ^{31}P -NMR spectrum (121 MHz, D_2O) of 2'-methylseleno-2'-deoxyuridine 5'-triphosphate (**4**).

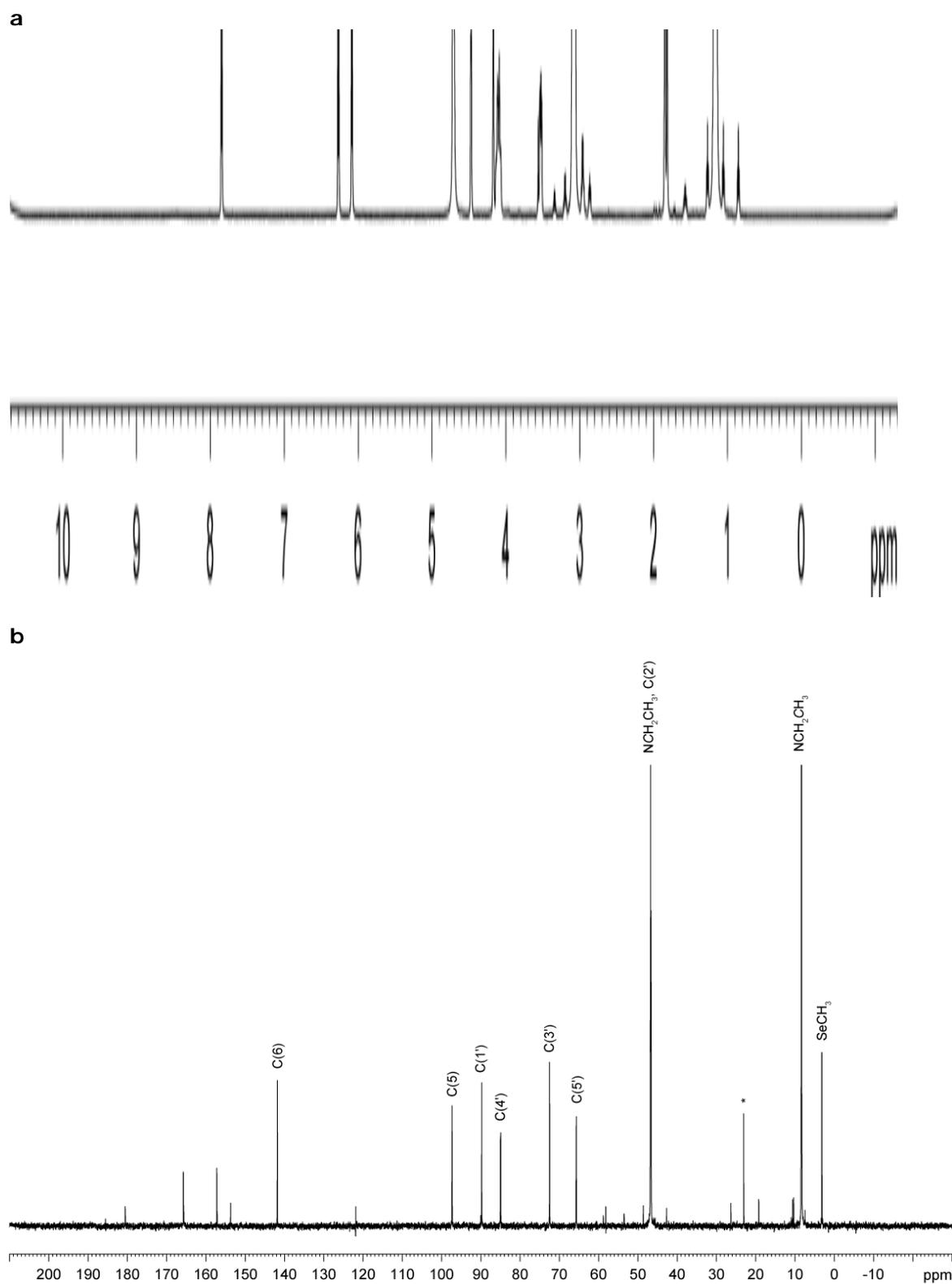
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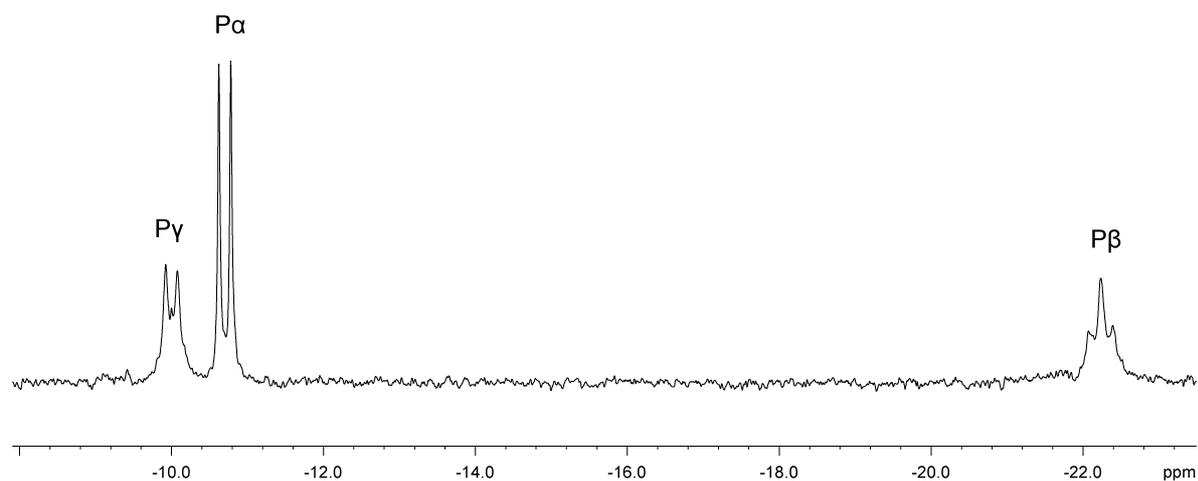
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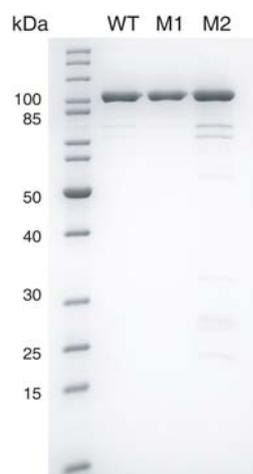
Supporting Figure 5. NMR spectra *M*⁴-acetyl-3'-tert.-butyldimethylsilyl-2'-methylseleno-2'-deoxycytidine (**6**). (a) ¹H-NMR (300 MHz, CDCl₃). (b) ¹³C-NMR (150 MHz, DMSO-*d*₆).



Supporting Figure 6. NMR spectra of 2'-methylseleno-2'-deoxycytidine 5'-triphosphate (**7**). (a) ¹H-NMR (500 MHz, D₂O). (b) ¹³C-NMR (150 MHz, D₂O). * Resonances flagged with an asterisk indicate residual amounts of acetate anion from HPLC buffer.



Supporting Figure 7. ^{31}P -NMR spectrum (121 MHz, D_2O) of 2'-methylseleno-2'-deoxycytidine 5'-triphosphate (**7**).



Supporting Figure 8. SDS-PAGE gel (12%) of expressed and purified T7 RNA polymerases (WT: wildtype; M1: Mutant 1 Y639F, H784A; M2: Mutant 2 Y639V, H784G, E593G, V685A).