

Supplemental Information for
Synthesis and RNA Transcription of Colored 4-Selenouridine Triphosphate
with Atom Mutagenesis

Authors

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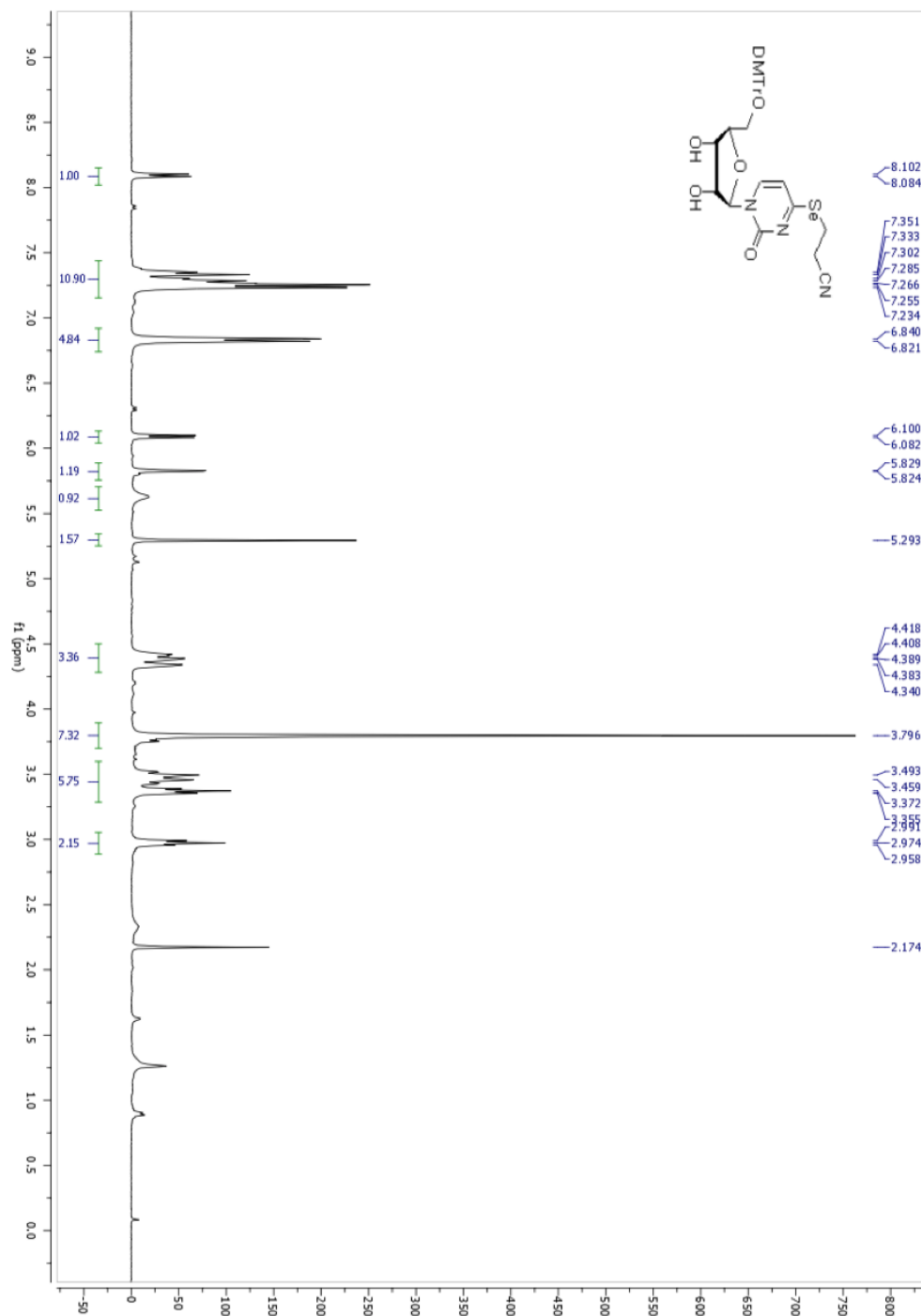


Figure S1. ^1H -NMR spectra of 5'-O-4,4'-dimethoxytrityl-4-cyanoethylselanyluridine.

^1H -NMR (400 MHz, CDCl_3) δ : 8.09 (d, $J = 7.0$ Hz, 1H, H-6), 7.42 – 7.17 (m, 10H, aromatic and N-H), 6.83 (m, 4H, aromatic), 6.09 (d, $J = 7.0$ Hz, 1H, H-5), 5.83 (d, $J = 2.2$ Hz, 1H, H-1'), 5.71 – 5.51 (br, 1H, 2'-OH), 4.53 – 4.25 (m, 3H, H-3', H-4', 3'-OH), 3.80 (s, 6H, OMe), 3.58 – 3.41 (m, 3H, H-5', H-2'), 3.37 (t, $J = 6.7$ Hz, 2H, $\text{CH}_2\text{-CN}$), 2.97 (t, $J = 6.6$ Hz, 2H, $\text{CH}_2\text{-Se}$).

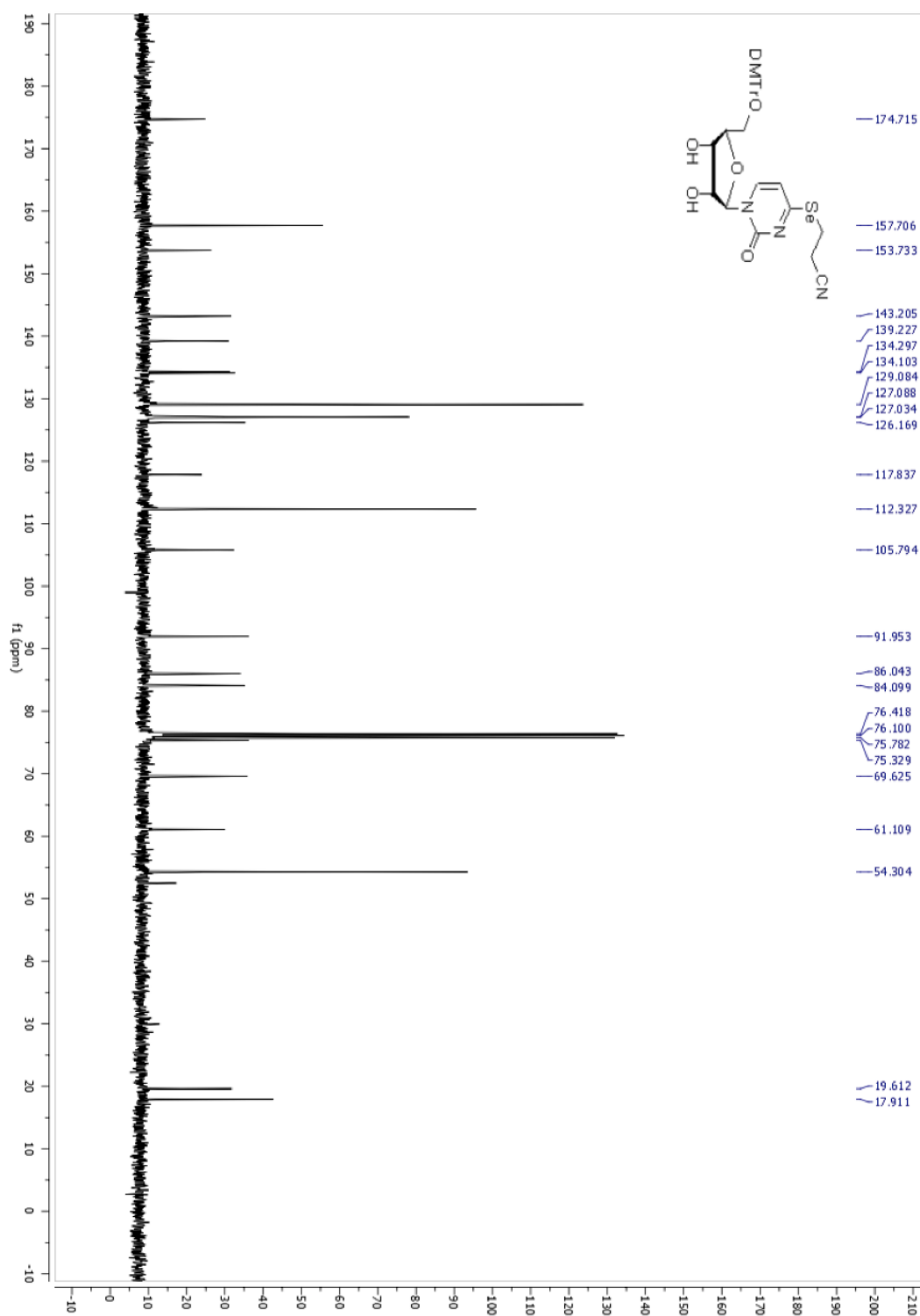


Figure S2. ¹³C-NMR spectra of 5'-O-4,4'-dimethoxytrityl-4-cyanoethylselanyluridine. ¹³C-NMR (101 MHz, CDCl₃) δ: 174.71 (C-4), 153.73 (C-2), 139.23 (C-6), 157.70, 143.20, 139.23, 134.30, 134.10, 60 129.08, 127.09, 127.03, 126.17, 112.33 (Ar), 117.84 (CN), 105.79 (C-5), 91.95 (C-1'), 86.04 (C-Ar), 84.10 (C-4'), 75.33 (C-2'), 69.62 (C-3'), 61.11 (C-5'), 54.30 (OCH₃), 19.61 (CH₂CH₂CN), 17.91 (CH₂-CN).

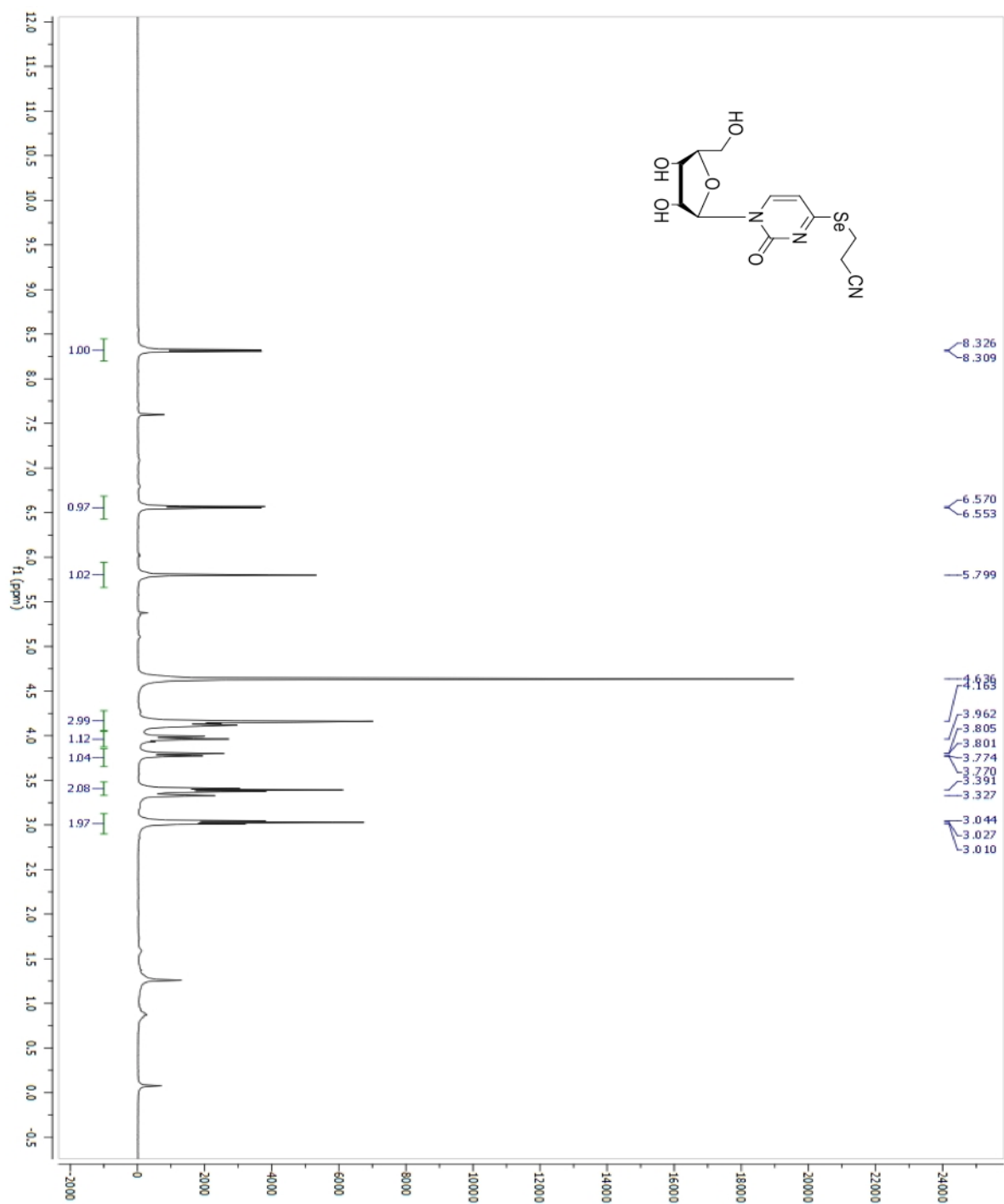


Figure S3. ¹H-NMR spectra of 4-cyanoethylselanyluridine.

¹H-NMR (400 MHz, MeOD) δ: 8.32 (d, *J* = 7.0 Hz, 1H, H-5), 6.56 (d, *J* = 7.0 Hz, 1H, H-6), 5.80 (s, 1H, H-1'), 4.14 (m, 3H, H-2', H-3', H-4'), 3.98 (dd, 1H, H5'), 3.79 (dd, 1H, H5''), 3.38 (m, 2H, CH₂-CN), 3.03 (m, 2H, CH₂-Se).

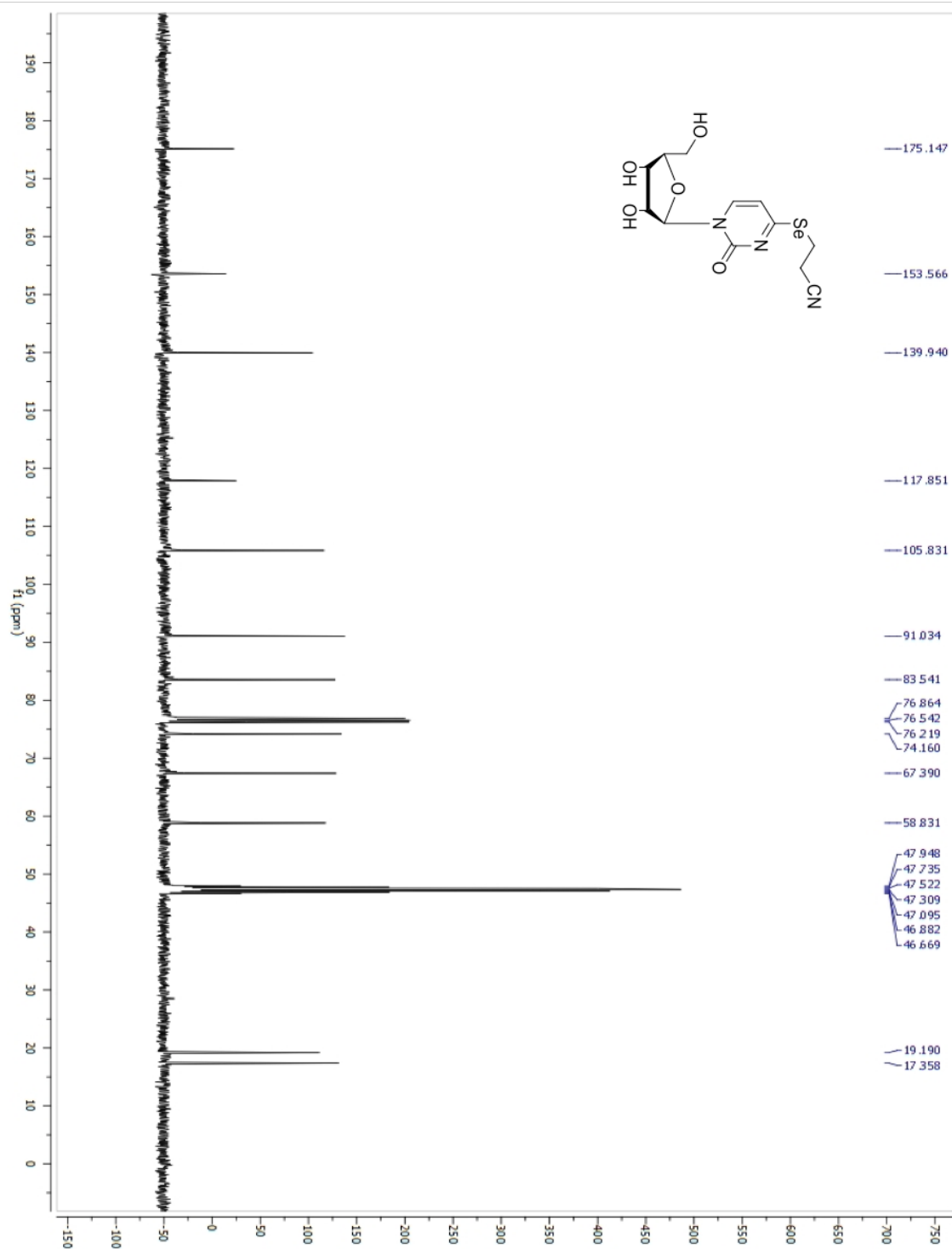


Figure S4. ¹³C-NMR spectra of 4-cyanoethylselanyluridine.

¹³C-NMR (101 MHz, MeOD) δ : 175.15 (C-4), 153.57 (C-2), 139.94 (C-6), 117.85 (CN), 105.83 (C-5), 91.03 (C-1'), 83.54 (C-4'), 74.16 (C-2'), 67.39 (C-3'), 58.83 (C-5'), 19.19 (CH₂CH₂CN), 17.36 (CH₂-CN).

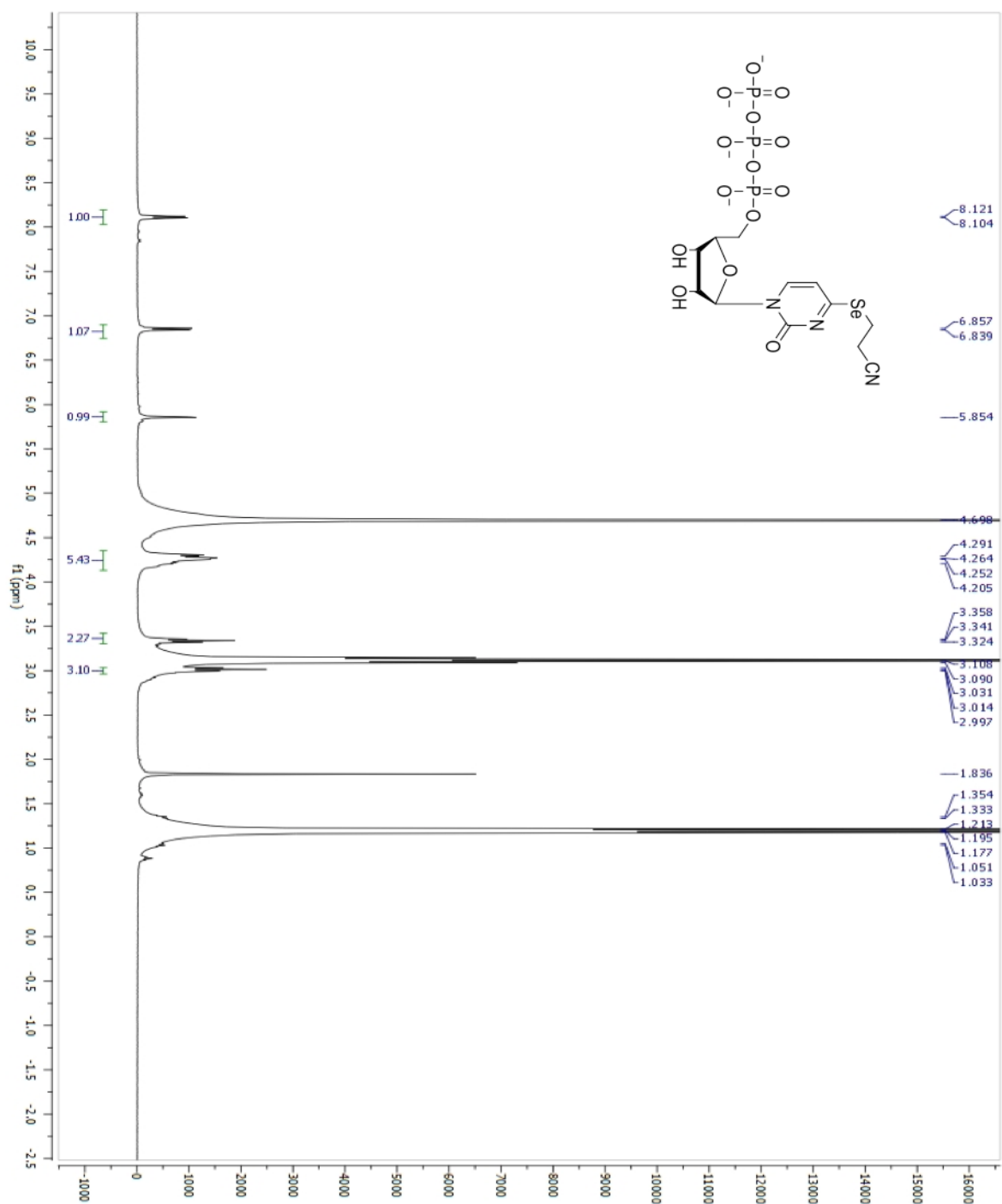


Figure S5. ^1H -NMR of $^4\text{Cy-SeUTP} \cdot 4 \times [\text{NH}(\text{Et})_3]^+$.

^1H -NMR (400 MHz, D_2O) δ 8.11 (d, $J = 7.1$ Hz, 1H, H-5), 6.85 (d, $J = 7.1$ Hz, 1H, H-6), 5.85 (s, 1H, H-1'), 4.45 – 3.97 (m, 5H, H-2', H-3', H-4', H-5'), 3.34 (t, $J = 6.8$ Hz, 2H, $\text{CH}_2\text{-CN}$), 3.12 – 2.92 (m, 2H, $\text{CH}_2\text{-Se}$).

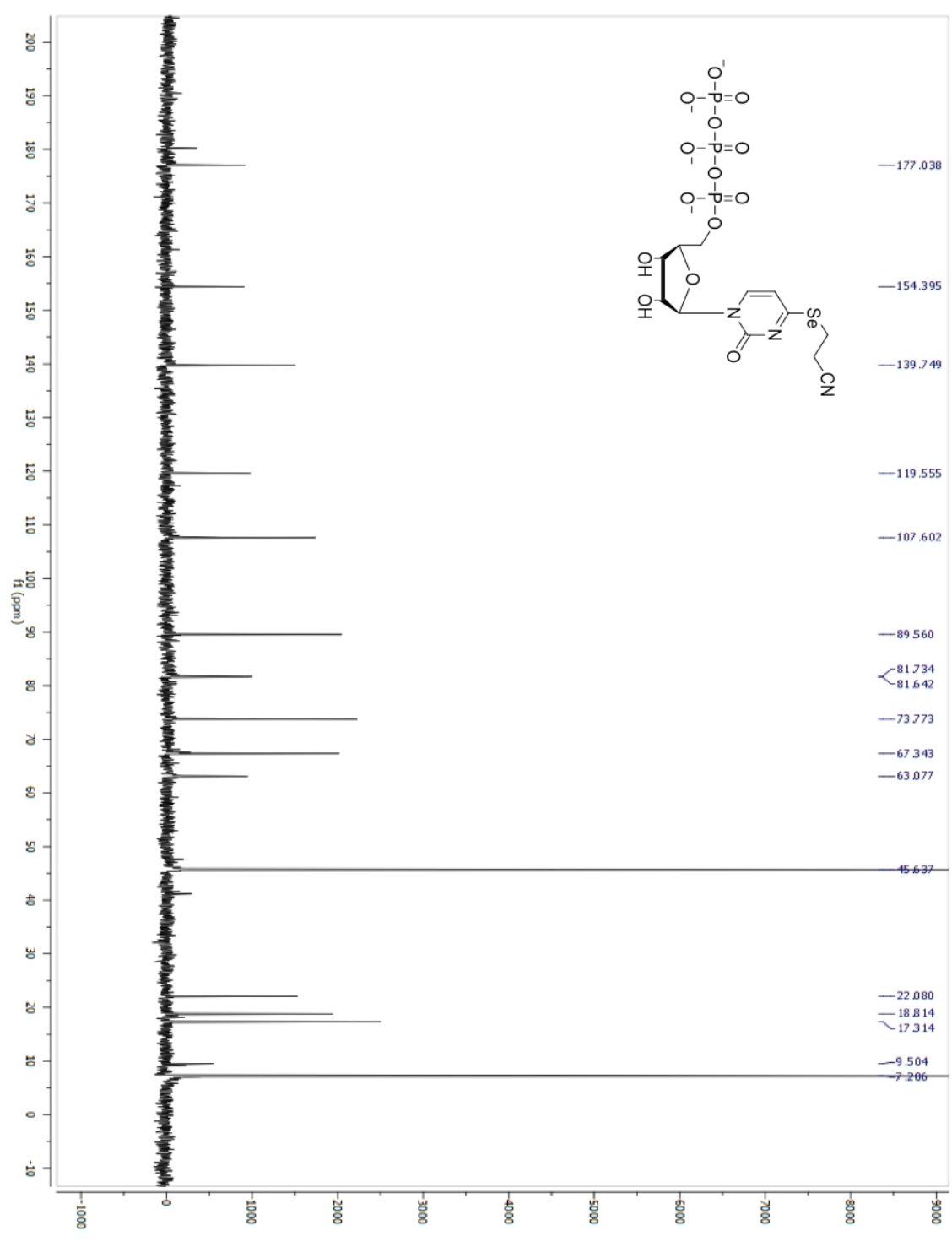


Figure S6. ^{13}C -NMR of $4\text{Cy-SeUTP} \cdot 4 \times [\text{NH}(\text{Et})_3]^+$.

^{13}C -NMR (101 MHz, MeOD) δ : 177.04 (C-4), 154.40 (C-2), 139.75 (C-6), 119.56 (CN), 107.60 (C-5), 89.56 (C-1'), 81.73 (C-4'), 73.77 (C-2'), 67.34 (C-3'), 63.08 (C-5'), 18.81 (CH₂CH₂CN), 17.31 (CH₂-CN).

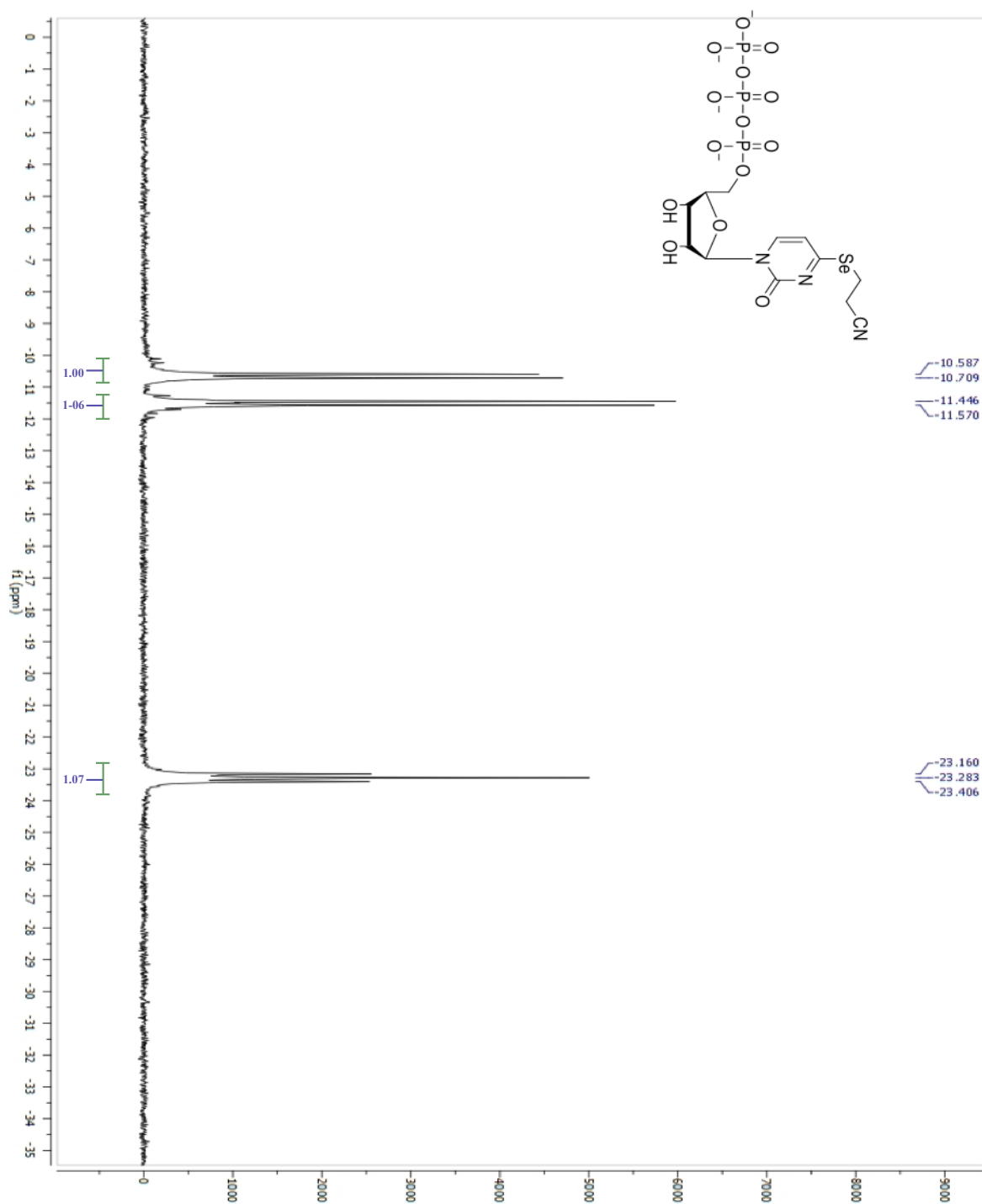


Figure S7. ³¹P-NMR of ⁴Cy-SeUTP·4×[NH(Et)₃]⁺.

³¹P-NMR (162 MHz, D₂O) δ -10.65 (d, J = 19.8 Hz, α-P), -11.51 (d, J = 20.1 Hz, γ-P), -23.28 (t, J = 20.0 Hz, β-P).

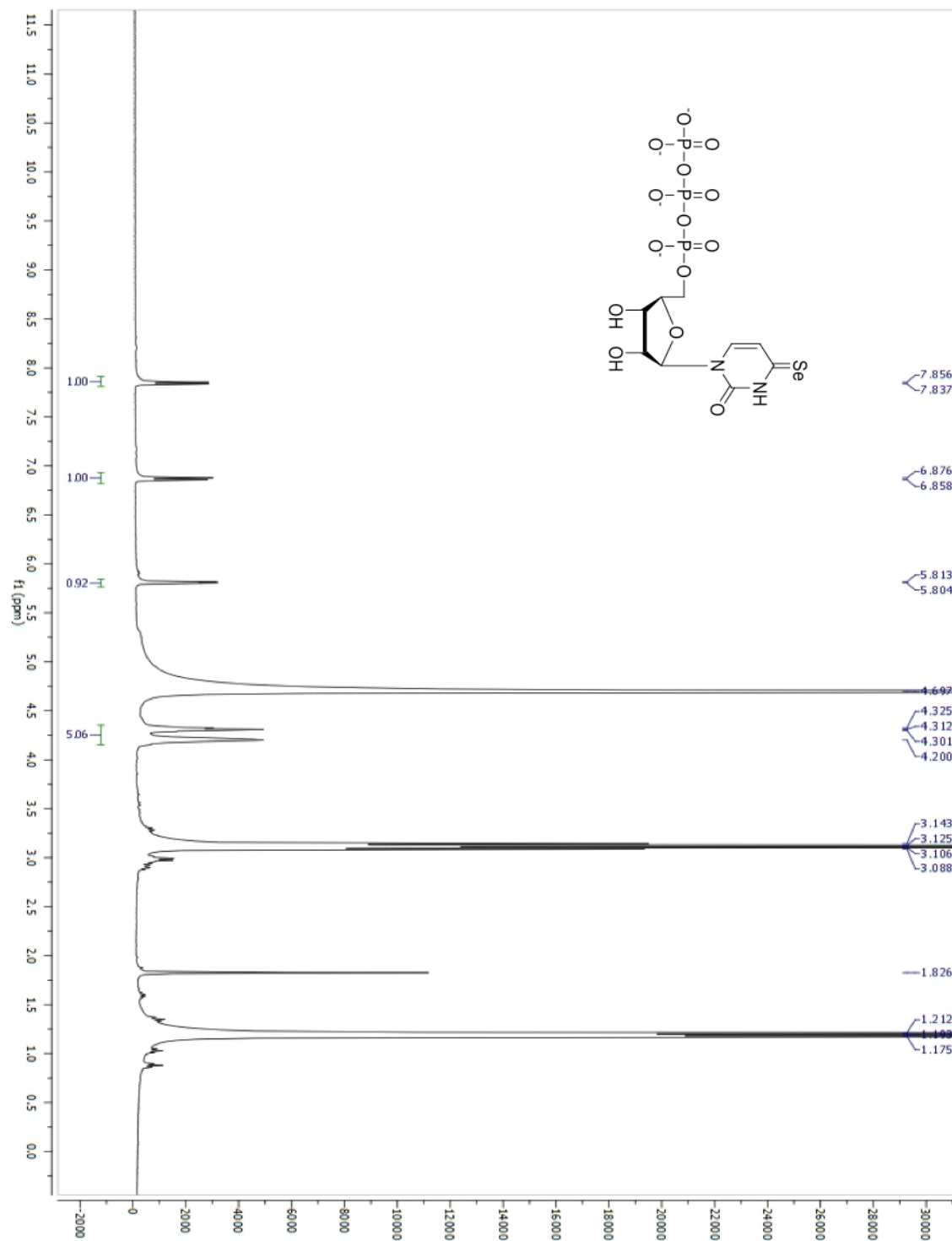


Figure S8. ¹H-NMR of ⁴SeUTP·4×[NH(Et)₃]⁺.

¹H-NMR (400 MHz, D₂O) δ: 7.85 (d, J = 7.4 Hz, 1H, H-5), 6.87 (d, J = 7.4 Hz, 1H, H-6), 5.81 (d, J = 3.7 Hz, 1H, H-1'), 4.31 – 4.20 (m, 5H, H-2', H-3', H-4', H-5').

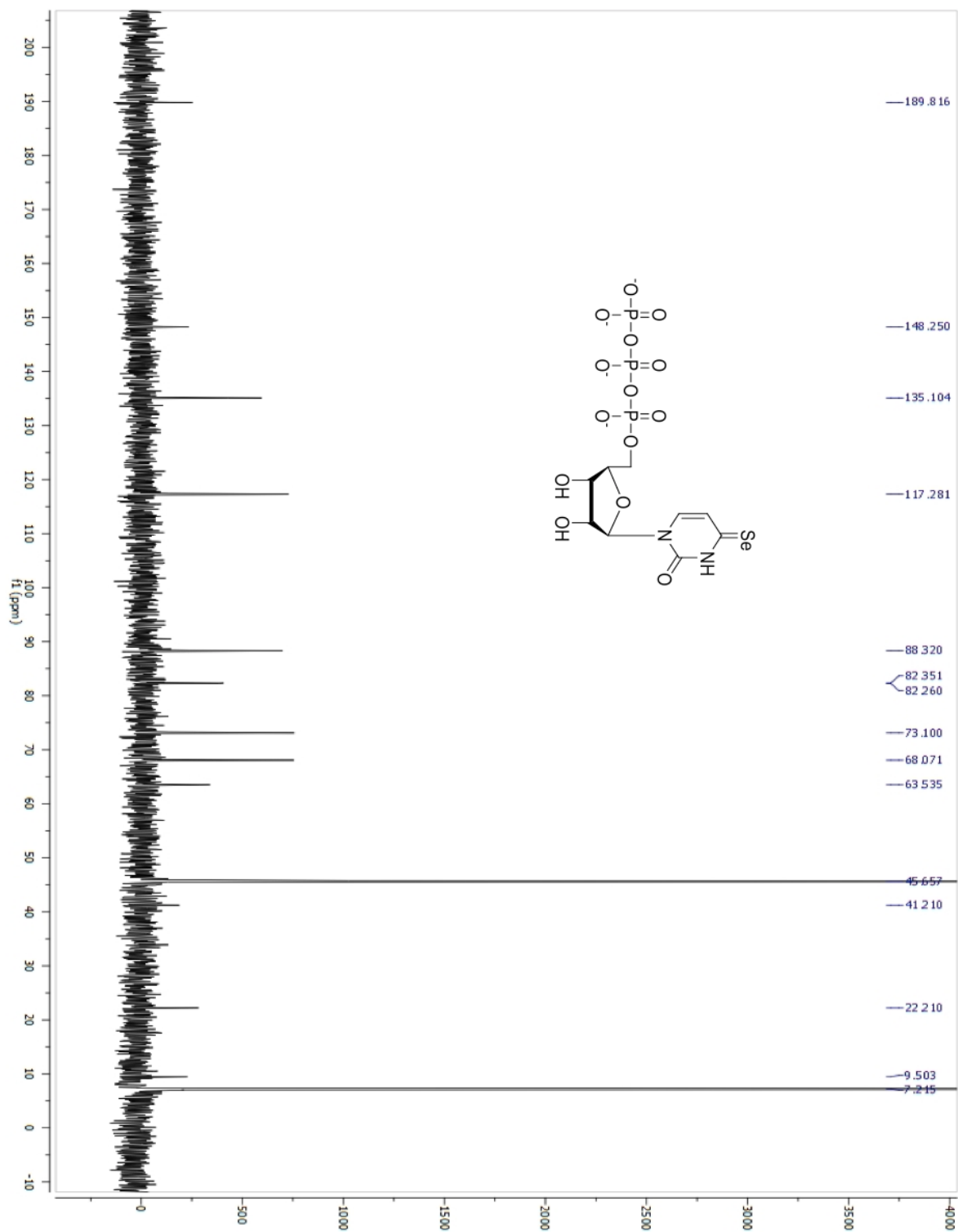


Figure S9. ^{13}C -NMR of $^4\text{SeUTP} \cdot 4 \times [\text{NH}(\text{Et})_3]^+$.

^{13}C -NMR (101 MHz, D_2O) δ 189.82 (C-4), 148.25 (C-2), 135.10 (C-6), 117.28 (C-5), 88.32 (C-1'), 82.31 (C-4'), 73.10 (C-2'), 68.07 (C-3'), 63.53 (C-5').

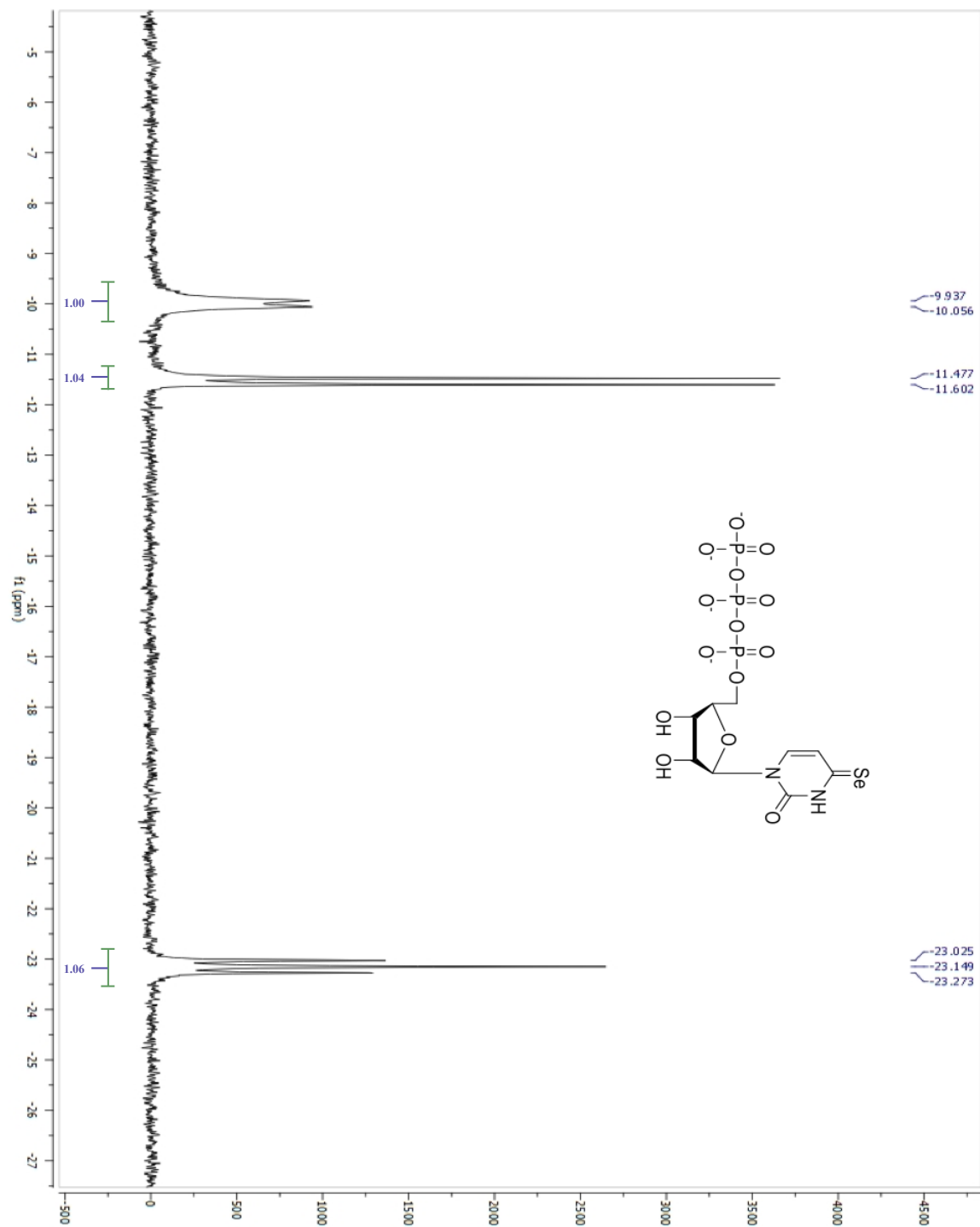


Figure S10. ^{31}P -NMR of $4\text{SeUTP} \cdot 4 \times [\text{NH}(\text{Et})_3]^+$.

^{31}P -NMR (162 MHz, D_2O) δ : -9.99 (d, $J = 19.3$ Hz, $\alpha\text{-P}$), -11.54 (d, $J = 20.2$ Hz, $\gamma\text{-P}$), -23.15 (t, $J = 20.1$ Hz, $\beta\text{-P}$).

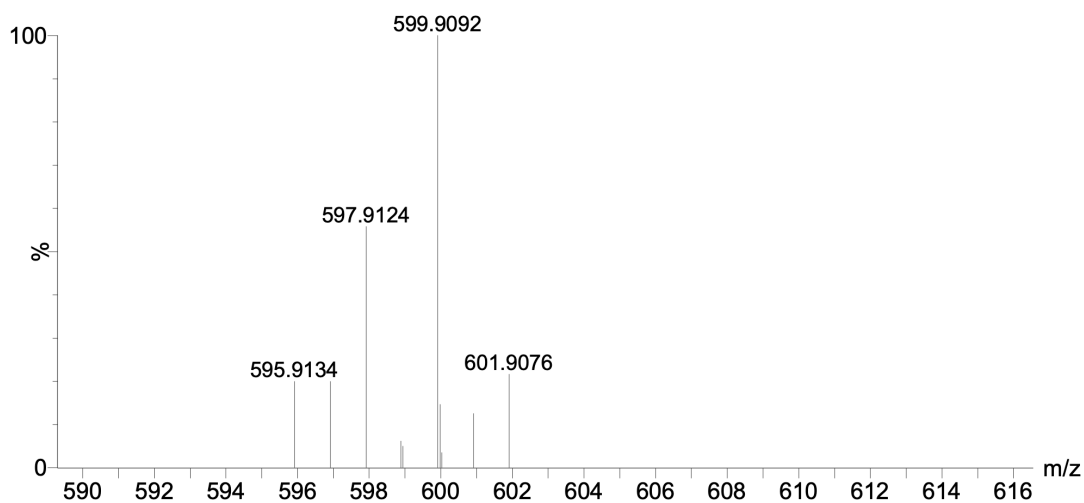


Figure S11. Mass spectrum of $^4\text{Cy-SeUTP}$. HR-MS (ESI-TOF): $[\text{M-H}^+]^- = 599.9092$ (calc. 599.9094), in 50% MeOH.

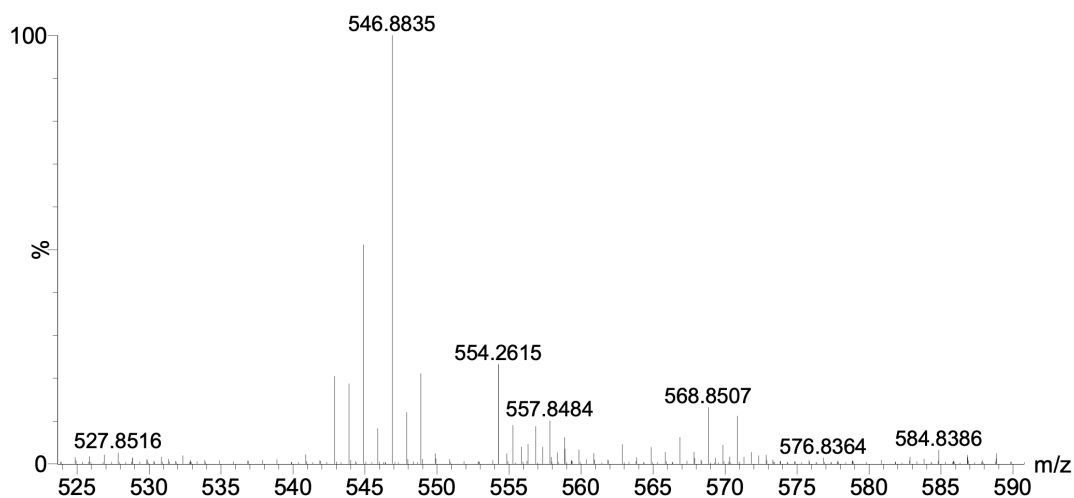


Figure S12. Mass spectrum of $^4\text{SeUTP}$. HR-MS (ESI-TOF): $[\text{M-H}^+]^- = 546.8835$ (calc. 546.8829), in 50% MeOH+0.5% NH_4OH

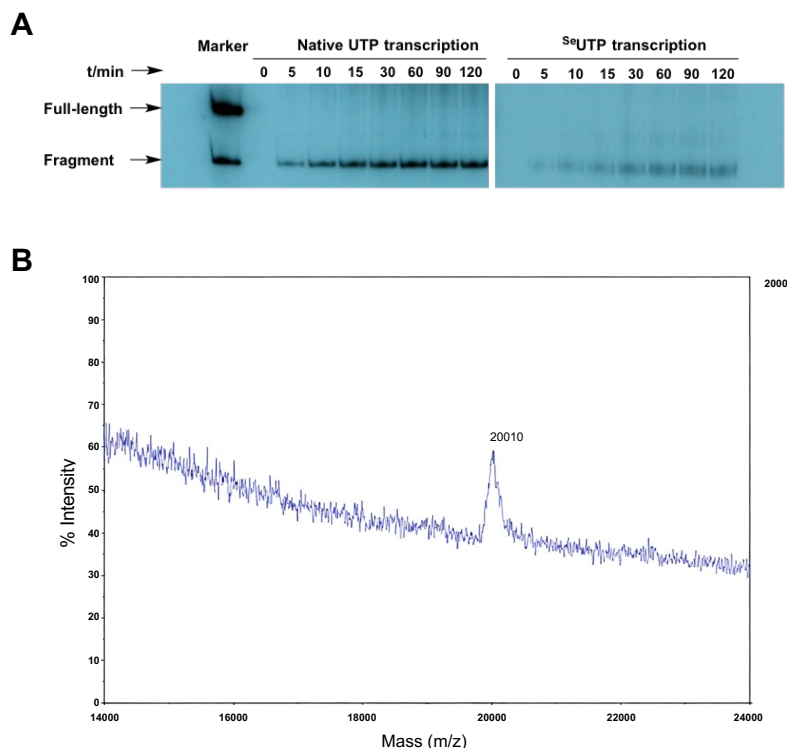


Figure S13. Transcription of the wild-type native and 4-Se-modified ribozymes. (A) The transcription and catalysis of the ribozymes with the self-cleaving activity. (B) MALDI-TOF MS analysis of the cleaved 4-Se-U-WHR fragment (molecular formula: $C_{543}H_{672}N_{218}O_{385}P_{56}Se_{13}$); the matrix molecule: 3-hydroxypicolinic acid (3HPA, molecular formula: $C_6H_5NO_3$). The complex mass of the 4-Se-U-WHR fragment accompanied by six matrix molecules was observed: 20010 (calc. 20011).

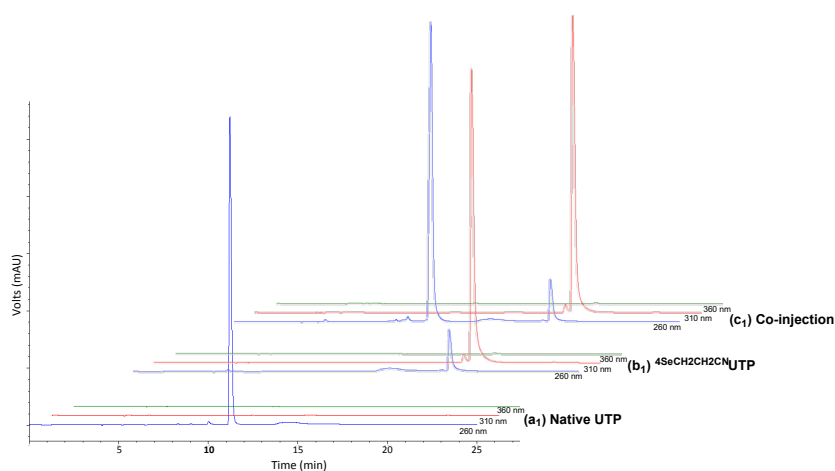


Figure S14. HPLC analyses of 4SeCH_2CH_2CNUTP ($^4Cy-SeUTP$) at multiwavelength (260 nm, blue; 310 nm, red; 360 nm, green). a1: Native UTP (retention time: 11.3 min); b1: 4SeCH_2CH_2CNUTP (retention time: 17.8 min); c1: co-injection of both native UTP and 4SeCH_2CH_2CNUTP (retention time: 11.3 min and 17.8 min).