Electronic Supplementary Material (ESI) for New Journal of Chemistry.


**Supporting Information**

# Synthesis, antioxidant, and antitumoral activity of new 5'-arylchalcogenyl-3'-N-(E)-feruloyl-3'-amino-3'-deoxythymidine (AFAT) derivatives

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## CHEMISTRY

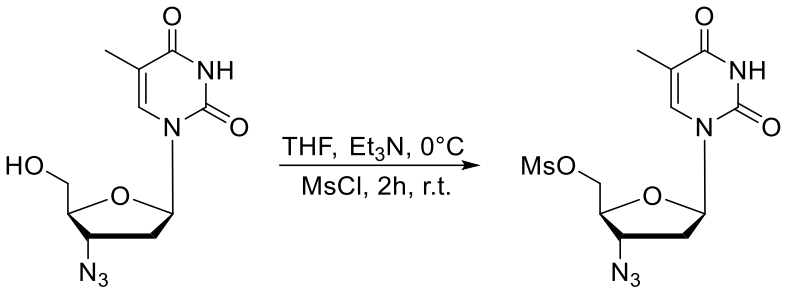
### General Considerations

All Chemicals were of analytical grade and obtained from standard commercial suppliers and some reactions were run under an atmosphere of dry argon. Proton nuclear magnetic resonance spectra ($^{1}$H NMR) were obtained at 400 MHz in a Bruker Avance III HD NMR spectrometer. Spectra were recorded in $CDCl_3$ or DMSO-$d_6$ solutions. Chemical shifts are reported in ppm, referenced to the solvent peak of tetramethylsilane (TMS) as the external reference. Data are reported as follows: chemical shift ($\delta$) expressed in ppm, multiplicity (br = broad, s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, t = triplet, m = multiplet, q = quartet), and coupling constant ($J$) in Hertz and integrated intensity. Carbon-13 nuclear magnetic resonance ($^{13}$C NMR) spectra were obtained either at 100 MHz in an AVANCE III HD NMR spectrometer. Chemical shifts ($\delta$) are reported in ppm, referenced to the solvents peak of $CDCl_3$ or DMSO-$d_6$. High-resolution mass spectra (HRMS) were obtained on a LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). This hybrid system combines an LTQ XL linear ion-trap mass spectrometer and an Orbitrap mass analyzer. The experiments were performed via direct infusion of the sample (flow rate 10 mL/min) in positive-ion mode using electrospray ionization (ESI). Elemental composition calculations were executed using the specific tool included in the Qual Browser module of the Xcalibur (Thermo Fisher Scientific, release 2.0.7) software. Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.25 mm. For visualization, TLC plates were either placed under ultraviolet light, or stained with iodine vapor, or acidic vanillin.

## General Methods

## Synthetic Procedures

### Preparation of 5'-O-(mesyl)zidovudine (**2**)




**1** **2**

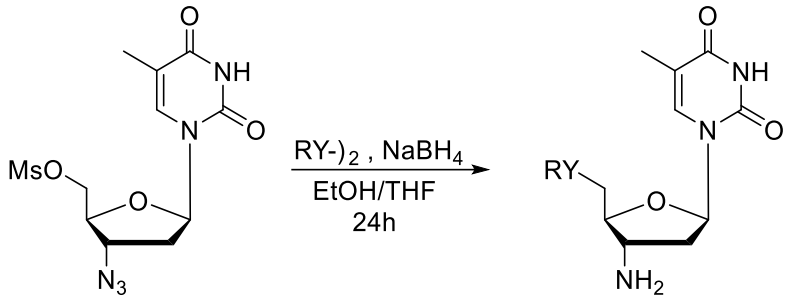
In the two-necked round-bottom flask under an argon atmosphere, 1 mmol zidovudine was added in 7 mL THF. After the dissolution of zidovudine, the system was cooled to 0° C and triethylamine (1.5 mmol) added. After 10 min, while still at 0° C, dropwise mesyl chloride (1.1 mmol) diluted in 3 ml of THF was added. Then, the ice bath was removed and the system allowed to react for 2 h at room temperature. After this period, the reaction was extracted with a saturated solution of $NH_4Cl$ (~ 20 mL) and the organic phase extracted with dichloromethane (3x 20 mL). Organic phases were combined and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the product crystallized in ethyl acetate and dried in high vacuum pump. Mesylate was obtained.

Physical state: White solid; Yield: 92%

RMN $^{1}H$ (DMSO-$d_6$, 400 MHz), $\delta$ (ppm):11.28 (s, 1H), 7.47 (d, $J$ = 1.1 Hz, 1H), 6.15 (t, $J$ = 6.7 Hz, 1H), 4.47 – 4.41 (m, 2H), 4.10 – 4.04 (m, 1H), 3.23 (s, 3H), 2.53 – 2.43 (m, 3H), 1.80 (d, $J$ = 0.8 Hz, 3H).

RMN $^{13}C$ (DMSO-$d_6$, 100 MHz), $\delta$ (ppm): 164.4, 151.2, 136.7, 110.8, 84.9, 81.3, 69.7, 60.9, 37.7, 36.4, 12.7.

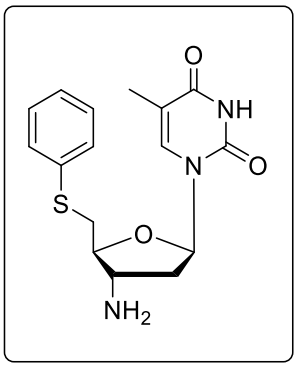
**Preparation of Arylchalcogeno-aminothymidines (4a-4l)**[1]




**2** **4a-l**

In a two-necked round-bottom flask under argon atmosphere was added diaryl dichalcogenide (0.5 mmol), THF (4 mL) and ethanol (3 mL). Afterwards, $NaBH_4$ (5,0 eq., 5 mmol, 0,185 g,) was added and the reaction was stirred until the disappearance of the color. Subsequently, the 5'-O-(mesyl)zidovudine (1 mmol) dissolved in THF (3 mL) was added dropwise to the reaction flask. The system was heated at reflux for 24 h. After completion of the reaction, the mixture was quenched with a saturated solution of $NH_4Cl$ and extracted with ethyl acetate. The solvent was evaporated under reduced pressure, and the crude product was purified by chromatographic column employing a gradient of a mixture of dichloromethane and ethanol (until 70:30) as solvent. Compounds were obtained with yields ranging from 20 to 82%.

**5’-S-(phenyl) -3’-(amino)-thymidine (4a)** [1]

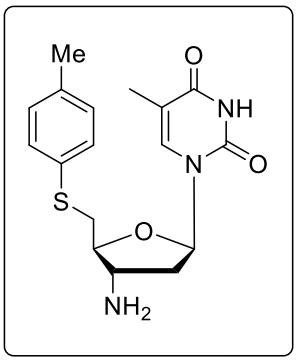



Physical state: light orange solid; Melting Point: 140-143°C; Yield: 82%

RMN $^{1}H$ ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.42 – 7.38 (m, 2H), 7.34 (d, $J$ = 0.8 Hz, 1H), 7.32– 7.28 (m, 2H), 7.23 – 7.17 (m, 1H), 6.20 (dd, $J_1$ = 6.8, $J_2$ = 5.2 Hz, 1H), 3.96 – 3.80 (m,1H), 3.65 – 3.50 (m, 1H), 3.33 (d, $J$ = 5.0 Hz, 2H), 2.33 – 2.16 (m, 2H), 1.82 (d, $J$ = 0.8Hz, 3H).

RMN $^{13}C$ ($CDCl_3$, 100 MHz), $\delta$ (ppm): 163.9, 150.4, 135.6, 135.5, 129.1, 128.9, 126.4, 110.9, 84.9, 84.2, 53.9, 41.2, 36.1, 12.5.

**5’-S-(4-methyl-phenyl)-3’-(amino)-thymidine (4b)** [1]

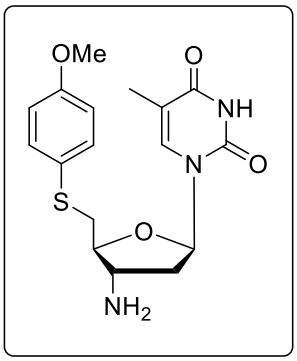



Physical state: Dark orange solid; Melting Point: 150-152°C; Yield: 47%

RMN $^{1}H$ ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.36 (s, 1H), 7.33 – 7.28 (m, 2H), 7.13 – 7.07 (m, 2H), 6.17 (t, $J$ = 6.0 Hz, 1H), 3.91 – 3.82 (m, 1H), 3.63 – 3.50 (m, 1H), 3.33 – 3.21 (m,2H), 2.31 (s, 3H), 2.29 – 2.19 (m, 2H), 1.85 (s, 3H).

RMN $^{13}C$ (DMSO-$d_6$, 100 MHz), $\delta$(ppm): 163.5, 150.2, 135.9, 135.2, 132.4, 129.5, 128.66, 109.4, 84.7, 83.3, 54.2, 35.8, 20.3, 11.9.

**5’-S-(4-methoxy-phenyl)-3’-(amino)-thymidine (4c)** [1]

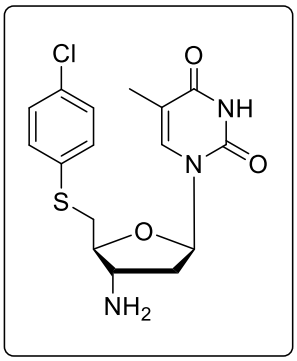



Physical state: light rose solid; Melting Point: 133-136°C; Yield: 60%

RMN $^{1}H$ ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.42 – 7.38 (m, 2H), 7.37 (d, $J$ = 0.8 Hz, 1H), 6.88– 6.82 (m, 2H), 6.16 (dd, $J_1$= 6.4, $J_2$ = 5.2 Hz, 1H), 3.84 – 3.77 (m, 4H), 3.59 – 3.49 (m,1H), 3.23 – 3.15 (m, 2H), 2.30 – 2.12 (m, 2H), 1.88 (d, $J$ = 0.8 Hz, 3H).

RMN $^{13}C$ ($CDCl_3$,100 MHz), $\delta$ (ppm): 164.1, 158.8, 150.4, 135.4, 132.5, 125.7, 114.6, 110.5, 84.2, 55.1,53.9, 40.7, 38.2, 12.2.

**5’-S-(4-chloro-phenyl)-3’-(amino)-thymidine (4d)** [1]

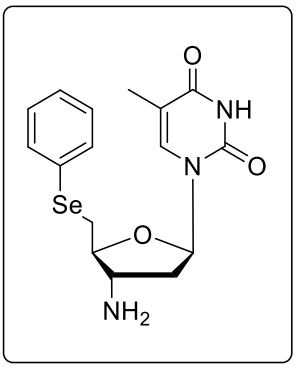



Physical state: light yellow solid; Melting Point: 133-136°C; Yield: 20%

RMN $^{1}H$ ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.35 – 7.30 (m, 2H), 7.27 (m, 1H), 7.26 – 7.22 (m, 2H), 6.17 (dd, $J_1$ = 6.8, $J_2$ = 5.2 Hz, 1H), 4.28 (sl, 2H), 3.95 – 3.79 (m, 1H), 3.63 – 3.44 (m, 1H), 3.39 – 3.17 (m, 2H), 2.37 – 2.06 (m, 2H), 1.83 (d, $J$ = 1.2 Hz, 3H).

RMN $^{13}C$ ($CDCl_3$, 100 MHz), $\delta$ (ppm): 163.9, 150.4, 135.5, 134.3, 132.4, 130.4, 129.1, 110.9, 84.8, 84.4, 54.0, 41.0, 36.5, 12.3.

**5’-Se-(phenyl)-3’-(amino)-thymidine (4e)** [1]

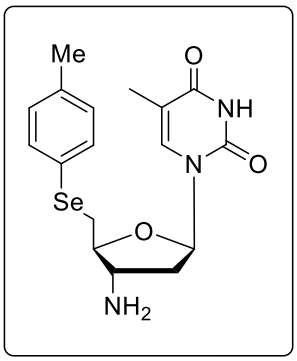



Physical state: light yellow solid; Melting Point: 132-134°C; Yield: 78%

RMN $^{1}$H ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.57 – 7.46 (m, 2H), 7.34 (d, $J$ = 1.2 Hz, 1H), 7.29– 7.18 (m, 3H), 6.16 (dd, $J_1$ = 7.2, $J_2$ = 5.6 Hz, 1H), 3.93 – 3.84 (m, 1H), 3.55 – 3.48 (m,1H), 3.29 – 3.24 (m, 2H), 2.31 – 2.13 (m, 2H), 1.85 (d, $J$ = 1.2 Hz, 3H).

RMN $^{13}$C ($CDCl_3$, 100 MHz), $\delta$ (ppm): 163.7, 150.3, 135.5, 132.3, 129.9, 129.2, 127.2,110.9, 85.7, 84.4, 54.9, 41.4, 30.2, 12.3.

**5’-Se-(4-methyl-phenyl)-3’-(amino)-thymidine (4f)** [1]

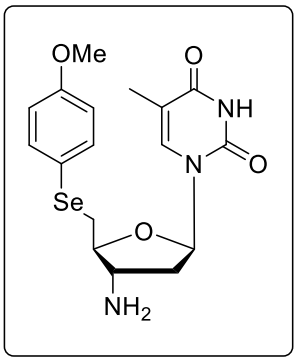



Physical state: light yellow solid; Melting Point: 146-149°C; Yield: 40%

RMN $^{1}H$ ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.46 – 7.41 (m, 2H), 7.37 (d, $J$ = 1.2 Hz, 1H), 7.12– 7.02 (m, 2H), 6.17 (dd, $J_1$ = 6.8, $J_2$ = 5.2 Hz, 1H), 3.90 – 3.79 (m, 1H), 3.56 – 3.45 (m,1H), 3.28 – 3.11 (m, 2H), 2.31 (s, 3H), 2.28 – 2.15 (m, 2H), 1.87 (d, $J$ = 1.2 Hz, 3H).

RMN $^{13}C$ ($CDCl_3$, 100 MHz), $\delta$ (ppm): 163.8, 150.3, 137.4, 135.6, 132.81, 130.1, 125.9,110.9, 85.7, 84.3, 54.8, 41.4, 30.5, 21.0, 12.4.

**5’-Se-(4-methoxy-phenyl)-3’-(amino)-thymidine (4g)** [1]

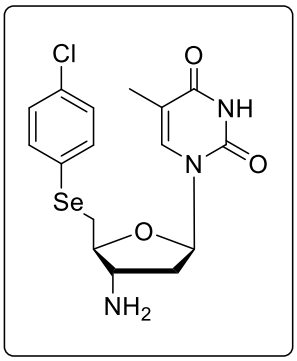



Physical state: beige solid; Melting Point: 124-127°C; Yield: 72%

RMN $^{1}$H ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.52 – 7.44 (m, 2H), 7.37 (d, $J$ = 1.2 Hz, 1H), 6.85– 6.77 (m, 2H), 6.17 (dd, $J_1$ = 6.8, $J_2$ = 5.6 Hz, 1H), 3.90 – 3.82 (m, 1H), 3.78 (s, 3H), 3.55 – 3.44 (m, 1H), 3.17 (d, $J$ = 5.6 Hz, 2H), 2.31 – 2.14 (m, 2H), 1.87 (d, $J$ = 1.2 Hz,3H).

RMN $^{13}$C ($CDCl_3$, 100 MHz), $\delta$ (ppm): 164.1, 159.3, 150.4, 135.5, 135.0, 119.4,114.9, 110.7, 85.6, 84.1, 55.1, 54.6, 41.1. 31.2, 12.4.

**5’-Se-(4-chloro- phenyl)-3’-(amino)-thymidine (4h)** [1]

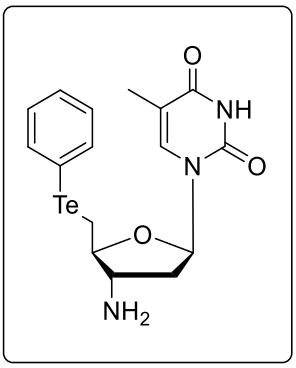



Physical state: beige solid; Melting Point: 144-146°C; Yield: 68%.

RMN $^{1}$H ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.51 – 7.43 (m, 2H), 7.30 (d, $J$ = 1.2 Hz, 1H), 7.26– 7.20 (m, 2H), 6.17 (dd, $J_1$= 6.8, $J_2$ = 5.2 Hz, 1H), 3.90 – 3.79 (m, 1H), 3.56 – 3.42 (m, 1H), 3.33 – 3.11 (m, 2H), 2.33 – 2.12 (m, 2H), 1.87 (d, $J$ = 1.2 Hz, 3H).

RMN $^{13}$C ($CDCl_3$,100 MHz), $\delta$ (ppm): 163.5, 150.3, 135.5, 133.7, 133.6, 129.4, 128.0, 110.9, 85.5, 84.3,54.9, 41.5, 30.5, 12.5.

**5’-Te-(phenyl)-3’-(amino)-thymidine (4i)** [1]

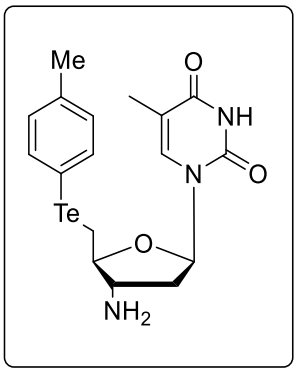



Physical state: beige solid; Melting Point: 199°C; Yield: 48%

RMN $^{1}H$ ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.78 – 7.73 (m, 2H), 7.45 (s, 1H), 7.31 – 7.16 (m,3H), 6.20 – 6.14 (m, 1H), 3.93 – 3.85 (m, 1H), 3.45 – 3.22 (m, 3H), 2.38 – 2.18 (m, 2H),1.87 (d, $J$ = 2.1 Hz, 3H).

RMN $^{13}C$ ($CDCl_3$, 100 MHz), $\delta$ (ppm): 164.3, 150.3, 137.4,135.9, 128.8, 127.3, 111.2, 110.3, 85.5, 83.5, 55.4, 40.0, 11.0, 10.9.

**5’-Te-(4-methyl-phenyl)-3’-(amino)-thymidine (4j)**



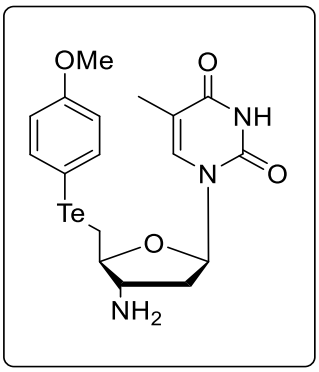

Physical state: White solid; Melting Point: 117-118°C; Yield: 63%

RMN $^{1}$H ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.65 – 7.52 (m, 2H), 7.44 (d, *J* = 1.2 Hz, 1H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.09 (dd, *J* = 7.4, 5.1 Hz, 1H), 3.72 (q, *J* = 6.2 Hz, 1H), 3.36 – 3.24 (m, 2H), 3.16 (dd, *J* = 12.0, 6.5 Hz, 1H), 2.32 (d, *J* = 11.5 Hz, 1H), 2.26 (s, 3H), 2.23 – 2.17 (m, 2H), 2.05 (dt, *J* = 13.4, 7.3 Hz, 1H), 1.77 (d, *J* = 1.2 Hz, 3H), 1.73 (td, *J* = 4.4, 4.0, 1.2 Hz, 1H).

RMN $^{13}$C ($CDCl_3$, 100 MHz), $\delta$ (ppm): 164.45, 151.01, 137.53, 137.19, 136.76, 130.50, 130.47, 110.04, 109.10, 86.41, 83.42, 56.77, 21.14, 12.64, 12.31.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{17}H_{21}N_3O_3Te$ [$(M + H)^+$]: 446.0719; found: 446.0730.

**5’-Te-(4-mehtoxy-phenyl)-3’-(amino)-thymidine (4k)**



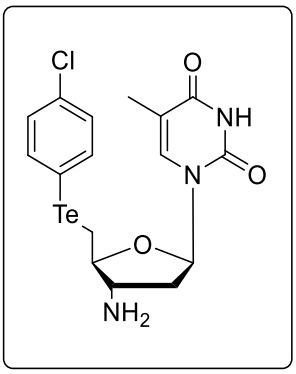

Physical state: White solid; Melting Point: 104-105°C; Yield: 74%

RMN $^{1}$H ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.67 – 7.58 (m, 2H), 7.44 (d, *J* = 1.3 Hz, 1H), 6.85 – 6.76 (m, 2H), 6.10 (dd, *J* = 7.4, 5.2 Hz, 1H), 3.73 (s, 3H), 3.72 – 3.70 (m, 1H), 3.29 – 3.22 (m, 2H), 3.12 (dd, *J* = 11.9, 6.6 Hz, 1H), 2.20 (ddd, *J* = 13.1, 7.6, 5.2 Hz, 1H), 2.04 (dt, *J* = 13.4, 7.2 Hz, 1H), 1.78 (d, *J* = 1.2 Hz, 3H).

RMN $^{13}$C ($CDCl_3$, 100 MHz), $\delta$ (ppm): 164.60, 159.67, 151.14, 139.92, 136.79, 115.77, 110.09, 101.83, 86.55, 83.49, 56.78, 55.52, 40.34, 12.77, 12.70.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{17}H_{21}N_3O_4Te$ [$(M + H)^+$]: 462.0667; found: 462.0688.

**5’-Te-(4-chloro-phenyl)-3’-(amino)-thymidine (4l)**



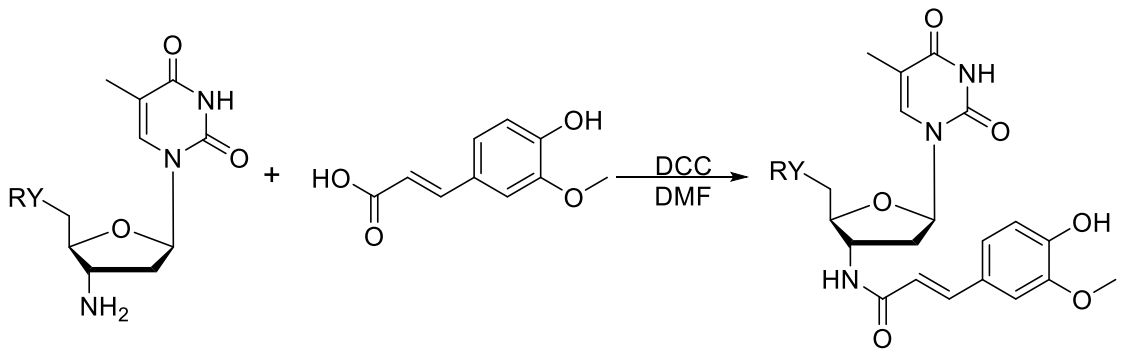

Physical state: White solid; Melting Point: 139-141°C; Yield: 63%

RMN $^{1}H$ ($CDCl_3$, 400 MHz), $\delta$ (ppm): 1H NMR (600 MHz, ) δ 7.66 (d, *J* = 7.2 Hz, 2H), 7.44 (s, 1H), 7.23 (d, *J* = 7.3 Hz, 2H), 6.13 – 6.04 (m, 1H), 3.73 (d, *J* = 12.6 Hz, 1H), 3.39 – 3.33 (m, 1H), 3.26 (d, *J* = 7.4 Hz, 1H), 3.20 (dd, *J* = 12.1, 6.1 Hz, 1H), 2.20 (ddd, *J* = 14.0, 7.1, 3.1 Hz, 1H), 2.10 – 1.75 (m, 1H), 1.76 (s, 3H).

RMN $^{13}C$ ($CDCl_3$, 100 MHz), $\delta$ (ppm): 164.26, 150.88, 138.63, 136.78, 132.72, 129.52, 112.34, 110.06, 86.12, 83.38, 56.79, 12.58, 12.54.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{16}H_{18}ClN_3O_3Te$ $[(M + H)^+]$: 466.0172; found: 466.0184.

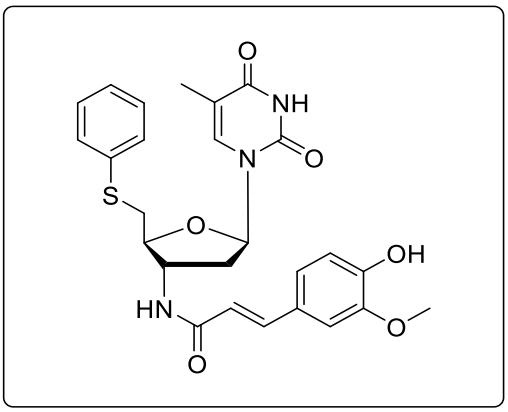
**Preparation of 5'-Arylchalcogenyl-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine [AFAT] (6a-l)**




**4a-l** **5** **6a-l**

In a one round-bottom flask was added Arylchalcogeno-aminothymidine (0.25 mmol), ferulic acid (0.25 mmol), DCC (0.3 mmol 1.2 eq.) and DMF (5mL). Afterwards, the reaction was stirred overnight at 50°C for 18 hours. After completion of the reaction, ethyl acetate was poured in the reaction vessel, and the mixture was extracted with water. The organic phase was dried with $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by chromatographic column employing a gradient of dichloromethane and ethanol (until 95:5) as solvent. Compounds were obtained with yields ranging from 34 to 96%.

**5’-S-(phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6a)**



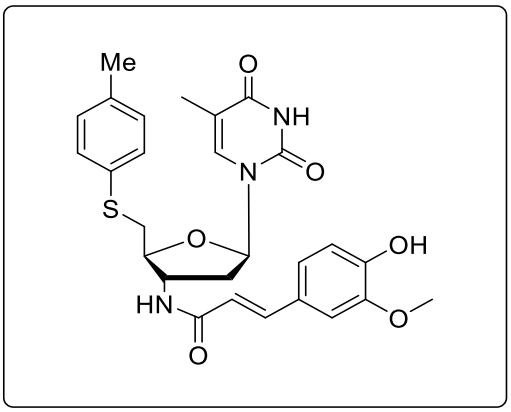

Physical state: White solid; Melting Point: 197-199°C; Yield: 96%

$^{1}$H NMR (398 MHz, DMSO-$d_6$) $\delta$ 11.30 (s, 1H), 9.44 (s, 1H), 8.43 (d, $J$ = 7.3 Hz, 1H), 7.54 (d, $J$ = 1.5 Hz, 1H), 7.42 – 7.35 (m, 3H), 7.29 (t, $J$ = 7.7 Hz, 2H), 7.18 (d, $J$ = 7.3 Hz, 1H), 7.15 (d, $J$ = 2.0 Hz, 1H), 7.02 (dd, $J$ = 8.1, 2.0 Hz, 1H), 6.81 (d, $J$ = 8.1 Hz, 1H), 6.43 (d, $J$ = 15.7 Hz, 1H), 6.22 (t, $J$ = 6.8 Hz, 1H), 4.64 – 4.38 (m, 1H), 4.05 – 3.90 (m, 1H), 3.82 (s, 3H), 3.52 – 3.40 (m, 1H), 2.48 – 2.34 (m, 1H), 2.29 – 2.13 (m, 1H), 1.77 (d, $J$ = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) $\delta$ 165.94, 164.14, 150.87, 148.93, 148.33, 140.20, 136.59, 136.54, 129.45, 128.44, 126.71, 126.19, 122.23, 118.81, 116.15, 111.21, 110.28, 84.02, 82.99, 56.00, 52.55, 36.59, 36.11, 12.56.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{26}H_{27}N_3O_6S$ [$(M + 2H)^{2+}$]: 511.1766; found: 511.1798.

**5’-S-(4-methyl-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6b)**



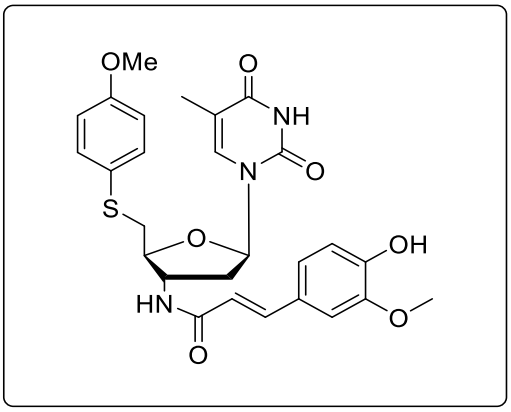

Physical state: white solid; Melting Point: 111-112°C; Yield: 67%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.35 (s, 2H), 9.51 (s, 2H), 8.46 (d, *J* = 7.4 Hz, 2H), 7.54 (d, *J* = 1.6 Hz, 2H), 7.38 (d, *J* = 15.7 Hz, 2H), 7.30 – 7.22 (m, 5H), 7.15 (d, *J* = 2.0 Hz, 2H), 7.10 (d, *J* = 8.1 Hz, 5H), 7.02 (dd, *J* = 8.2, 2.0 Hz, 2H), 6.81 (d, *J* = 8.1 Hz, 2H), 6.43 (d, *J* = 15.7 Hz, 2H), 6.21 (t, *J* = 6.8 Hz, 2H), 4.55 – 4.43 (m, 3H), 3.97 – 3.87 (m, 2H), 3.81 (s, 6H), 3.38 – 3.25 (m, 3H), 2.45 – 2.35 (m, 2H), 2.23 (s, 7H), 2.21 – 2.16 (m, 1H), 1.83 – 1.69 (m, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.92, 164.18, 150.88, 148.91, 148.30, 140.22, 136.56, 135.87, 132.73, 130.12, 129.21, 126.68, 122.25, 118.75, 116.12, 111.12, 110.29, 83.99, 82.98, 55.96, 52.50, 36.76, 36.57, 20.94, 12.56.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{27}H_{29}N_3O_6S$ [$(M + H)^+$]: 524.1850; found: 524.1867.

**5’-S-(4-methoxy-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6c)**



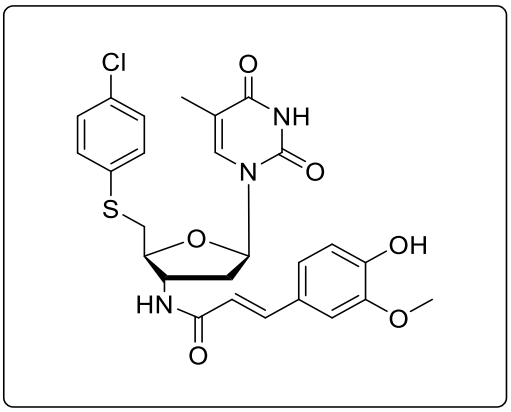

Physical state: white solid; Melting Point: 196-199°C; Yield: 74%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.34 (s, 1H), 9.48 (s, 1H), 8.43 (d, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 1.2 Hz, 1H), 7.44 – 7.32 (m, 3H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.01 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.42 (d, *J* = 15.6 Hz, 1H), 6.21 (t, *J* = 6.9 Hz, 1H), 4.56 – 4.40 (m, 1H), 3.92 – 3.83 (m, 1H), 3.81 (s, 3H), 3.71 (s, 3H), 3.41 – 3.13 (m, 1H), 2.46 – 2.31 (m, 1H), 2.25 – 2.11 (m, 1H), 1.79 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.82, 164.16, 158.82, 150.89, 148.90, 148.30, 140.15, 136.56, 132.44, 126.68, 126.29, 122.22, 118.81, 116.12, 115.17, 111.14, 110.27, 84.00, 83.03, 55.96, 55.62, 52.48, 38.35, 36.56, 12.58.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{27}H_{29}N_3O_7S$ [$(M + H)^+$]: 540.1799; found: 540.1829.

**5’-S-(4-chloro-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6d)**



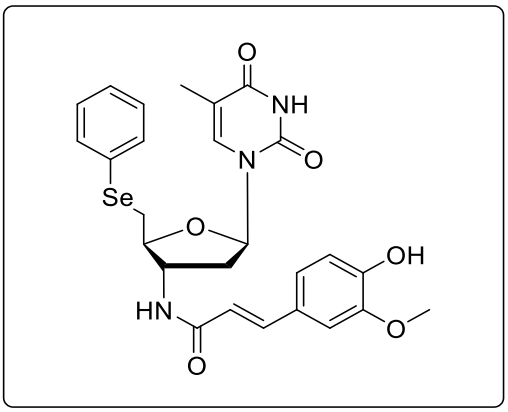

Physical state: light yellowish solid; Melting Point: 202-203°C; Yield: 65%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.36 (s, 1H), 9.52 (s, 1H), 8.47 (d, *J* = 7.3 Hz, 1H), 7.51 (d, *J* = 1.2 Hz, 1H), 7.41 – 7.28 (m, 5H), 7.14 (d, *J* = 1.9 Hz, 1H), 7.02 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 6.42 (d, *J* = 15.7 Hz, 1H), 6.21 (t, *J* = 6.8 Hz, 1H), 4.67 – 4.30 (m, 1H), 4.01 – 3.87 (m, 1H), 3.81 (s, 3H), 3.52 – 3.31 (m, 2H), 2.48 – 2.33 (m, 1H), 2.27 – 2.11 (m, 1H), 1.76 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.92, 164.14, 150.87, 148.90, 148.29, 140.23, 136.52, 135.75, 130.77, 130.08, 129.29, 126.64, 122.25, 118.71, 116.10, 111.08, 110.29, 83.90, 82.85, 55.91, 52.44, 36.47, 36.02, 12.57.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{26}H_{26}ClN_3O_6S$ $[(M + 2H)^{2+}]$: 545.1376; found: 545.1399.

**5’-Se-(phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6e)**



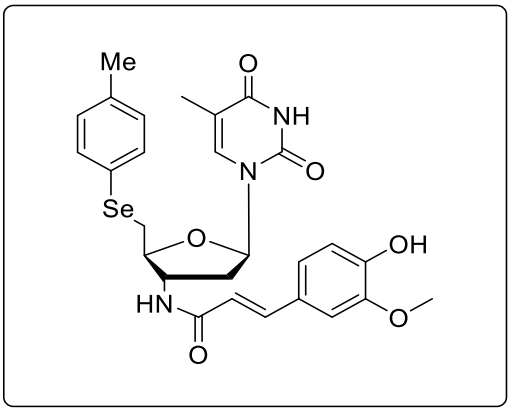

Physical state: White solid; Melting Point: 104-106°C; Yield: 82%

$^{1}$H NMR (398 MHz, ) *δ* 11.34 (s, 1H), 9.50 (s, 1H), 8.45 (d, *J* = 7.5 Hz, 1H), 7.55 (d, *J* = 1.5 Hz, 1H), 7.48 (dd, *J* = 8.2, 1.5 Hz, 2H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.30 – 7.18 (m,46H), 7.14 (d, *J* = 1.7 Hz, 1H), 7.01 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.42 (d, *J* = 15.7 Hz, 1H), 6.20 (t, *J* = 6.7 Hz, 1H), 4.55 – 4.40 (m, 1H), 4.03 – 3.92 (m, 2H), 3.80 (s, 3H), 2.47 – 2.35 (m, 1H), 2.26 – 2.15 (m, 1H), 1.75 (s, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.94, 164.16, 150.87, 148.90, 148.30, 140.22, 136.59, 131.45, 130.84, 129.64, 126.98, 126.66, 122.24, 118.73, 116.11, 111.12, 110.27, 83.80, 83.37, 55.95, 53.01, 36.65, 24.92, 12.56.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{26}H_{27}N_3O_6Se$ $[(M + 2H)^{2+}]$: 559.1210; found: 559.1255.

**5’-Se-(4-methyl-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6f)**



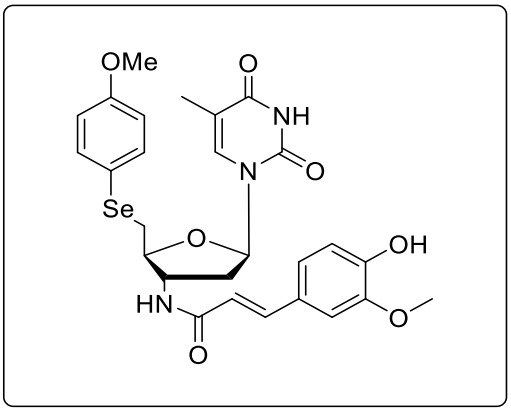

Physical state: white solid; Melting Point: 196-197°C; Yield: 94%

$^{1}$H NMR (398 MHz, DMSO) *δ* 1H NMR (398 MHz, ) *δ* 11.36 (s, 1H), 9.51 (s, 1H), 8.45 (d, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 1.4 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 2H), 7.01 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.42 (d, *J* = 15.7 Hz, 1H), 6.20 (t, *J* = 6.8 Hz, 1H), 4.52 – 4.40 (m, 1H), 4.00 – 3.90 (m, 1H), 3.81 (s, 3H), 3.36 – 3.26 (m, 1H), 2.45 – 2.35 (m, 1H), 2.24 (s, 3H), 2.25 – 2.14 (m, 1H), 1.77 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.86, 164.16, 150.87, 148.89, 148.29, 140.17, 136.61, 132.11, 130.33, 126.81, 126.66, 122.23, 118.76, 116.11, 111.10, 110.26, 83.78, 83.41, 55.94, 52.99, 36.65, 30.80, 21.03, 12.58.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{27}H_{29}N_3O_6Se$ [$(M + H)^+$]: 572.1294; found: 572.1327.

**5’-Se-(4-methoxy-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6g)**



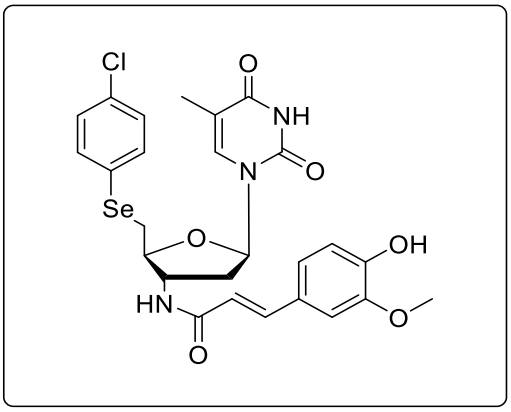

Physical state: white solid; Melting Point: 110-112°C; Yield: 89%

$^{1}$H NMR (398 MHz, DMSO) *δ* 8.45 (d, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 1.2 Hz, 1H), 7.46 (d, *J* = 8.9 Hz, 2H), 7.36 (d, *J* = 15.7 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.01 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.85 (d, *J* = 8.9 Hz, 2H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.41 (d, *J* = 15.7 Hz, 1H), 6.20 (t, *J* = 6.8 Hz, 1H), 4.51 – 4.40 (m, 1H), 3.92 (q, *J* = 5.7 Hz, 1H), 3.81 (s, 3H), 3.71 (s, 3H), 3.24 (d, *J* = 5.9 Hz, 2H), 2.46 – 2.34 (m, 1H), 2.24 – 2.13 (m, 1H), 1.79 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.82, 164.17, 159.18, 150.89, 148.29, 140.17, 136.60, 134.76, 126.62, 122.24, 120.05, 118.74, 116.10, 115.37, 111.07, 110.28, 83.78, 83.44, 55.92, 55.56, 52.96, 36.63, 31.75, 12.61.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{27}H_{29}N_3O_7Se$ $[(M + 2H)^{2+}]$: 589.1316; found: 589.1320.

**5’-Se-(4-chloro-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6h)**



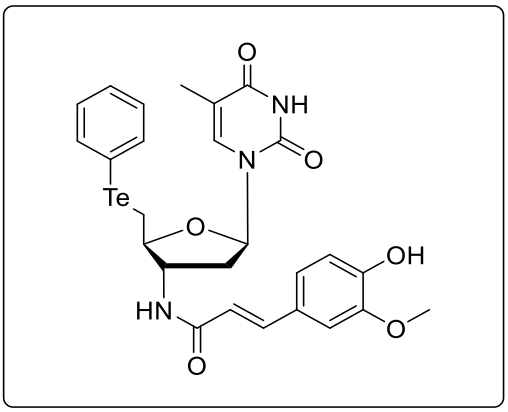

Physical state: white solid; Melting Point: 195-197°C; Yield: 64%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.36 (s, 1H), 9.52 (s, 1H), 8.46 (d, *J* = 7.4 Hz, 1H), 7.56 – 7.49 (m, 3H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.01 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 6.42 (d, *J* = 15.8 Hz, 1H), 6.20 (t, *J* = 6.8 Hz, 1H), 4.54 – 4.38 (m, 1H), 4.01 – 3.92 (m, 1H), 3.81 (s, 3H), 3.37 (d, *J* = 10.5 Hz, 2H), 2.47 – 2.35 (m, 1H), 2.26 – 2.14 (m, 1H), 1.76 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.92, 164.14, 150.86, 148.90, 148.28, 140.22, 136.55, 133.20, 131.80, 129.72, 129.46, 126.64, 122.24, 118.70, 116.10, 111.07, 110.26, 83.73, 83.29, 55.92, 52.94, 36.57, 30.59, 21.24, 12.57.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{26}H_{26}ClN_3O_6Se$ $[(M + 2H)^{2+}]$: 593.0821; found: 593.0818.

**5’-Te-(phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6i)**



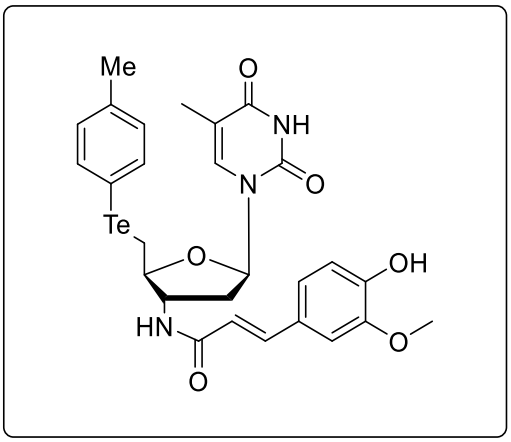

Physical state: light yellowish solid; Melting Point: 161-163°C; Yield: 63%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.36 (s, 1H), 9.53 (s, 1H), 8.46 (d, *J* = 7.4 Hz, 1H), 7.67 (d, *J* = 6.7 Hz, 2H), 7.60 (d, *J* = 1.2 Hz, 1H), 7.38 (d, *J* = 15.7 Hz, 1H), 7.24 (d, *J* = 7.4 Hz, 1H), 7.19 (d, *J* = 7.4 Hz, 2H), 7.15 (d, *J* = 2.0 Hz, 1H), 7.02 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 6.43 (d, *J* = 15.7 Hz, 1H), 6.21 (t, *J* = 6.7 Hz, 1H), 4.44 (p, *J* = 6.7 Hz, 1H), 4.00 (q, *J* = 5.7 Hz, 1H), 3.81 (s, 3H), 3.39 – 3.28 (m, 2H), 2.50 – 2.37 (m, 1H), 2.31 – 2.18 (m, 1H), 1.77 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 166.01, 164.14, 150.85, 148.95, 148.34, 140.20, 137.11, 136.73, 129.68, 127.68, 126.74, 122.22, 118.84, 116.18, 113.21, 111.31, 110.23, 84.06, 83.63, 56.04, 54.21, 36.76, 12.97, 12.54.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{26}H_{27}N_3O_6Te$ [$(M + H)^+$]: 608.1035; found: 608.1046.

**5’-Te-(4-methyl-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6j)**



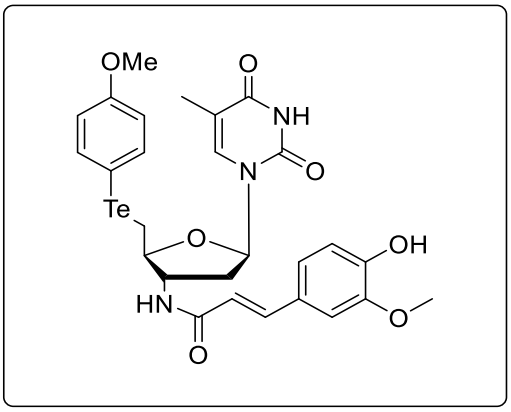

Physical state: white solid; Melting Point: 132-134°C; Yield: 46%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.34 (s, 1H), 9.51 (s, 1H), 8.43 (d, *J* = 7.4 Hz, 1H), 7.63 – 7.52 (m, 3H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.06 – 6.97 (m, 3H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.42 (d, *J* = 15.7 Hz, 1H), 6.20 (t, *J* = 6.7 Hz, 1H), 4.47 – 4.34 (m, 1H), 3.98 (q, *J* = 5.8 Hz, 1H), 3.81 (s, 3H), 3.26 (d, *J* = 5.8 Hz, 2H), 2.49 – 2.36 (m, 1H), 2.25 (s, 3H), 2.32 – 2.15 (m, 1H), 1.77 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.98, 164.19, 150.87, 148.90, 148.30, 140.23, 137.64, 137.29, 136.77, 130.50, 126.67, 122.24, 118.73, 116.12, 111.12, 110.24, 108.78, 84.12, 83.54, 55.98, 54.12, 36.70, 21.11, 13.00, 12.57.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{27}H_{29}N_3O_6Te$ [(M + Na)$^{+}$]: 644.1011; found: 644.1030.

**5’-Te-(4-methoxy-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6k)**



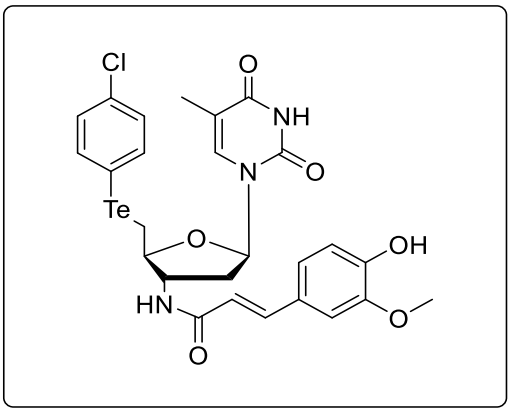

Physical state: white solid; Melting Point: 155-156°C; Yield: 34%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.37 (s, 1H), 9.52 (s, 1H), 8.44 (d, *J* = 7.4 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.58 (d, *J* = 1.4 Hz, 1H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.01 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.86 – 6.69 (m, 3H), 6.42 (d, *J* = 15.7 Hz, 1H), 6.20 (t, *J* = 6.7 Hz, 1H), 4.47 – 4.35 (m, 1H), 4.00 – 3.90 (m, 1H), 3.81 (s, 3H), 3.71 (s, 3H), 3.21 (d, *J* = 6.0 Hz, 2H), 2.48 – 2.36 (m, 1H), 2.28 – 2.16 (m, 1H), 1.79 (d, *J* = 1.4 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 165.91, 164.18, 159.63, 150.88, 148.89, 148.29, 140.19, 139.98, 136.75, 126.66, 122.22, 118.75, 116.10, 115.69, 111.10, 110.25, 101.46, 84.16, 83.54, 60.25, 55.93, 55.43, 21.24, 14.55, 12.60.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{27}H_{29}N_3O_7Te$ [$(M + H)^+$]: 638.1141; found: 638.1141.

**5’-Te-(4-chloro-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6l)**




Physical state: white solid; Melting Point: 142-143°C; Yield: 56%

$^{1}$H NMR (398 MHz, DMSO) *δ* 11.34 (s, 1H), 9.51 (s, 1H), 8.44 (d, *J* = 7.6 Hz, 1H), 7.70 – 7.62 (m, 2H), 7.56 (d, *J* = 1.2 Hz, 1H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.27 – 7.19 (m, 2H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.02 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 6.42 (d, *J* = 15.7 Hz, 1H), 6.20 (t, *J* = 6.6 Hz, 1H), 4.49 – 4.36 (m, 1H), 3.98 (q, *J* = 5.7 Hz, 1H), 3.81 (s, 3H), 3.31 (t, *J* = 5.2 Hz, 1H), 2.49 – 2.36 (m, 1H), 2.32 – 2.17 (m, 1H), 1.76 (d, *J* = 1.2 Hz, 3H).

$^{13}$C NMR (100 MHz, DMSO) *δ* 166.04, 164.15, 150.85, 148.92, 148.31, 140.26, 138.78, 136.74, 132.82, 129.53, 126.67, 122.24, 118.70, 116.12, 111.79, 111.16, 110.22, 83.96, 83.52, 66.82, 55.97, 54.14, 36.62, 13.40, 12.54.

**HRMS** [TOF MS ES+] *m/z* calculated for $C_{26}H_{26}ClN_3O_6Te$ [$(M + Na)^+$]: 664.0465; found: 664.0503.

## NMR and HRMS Charts

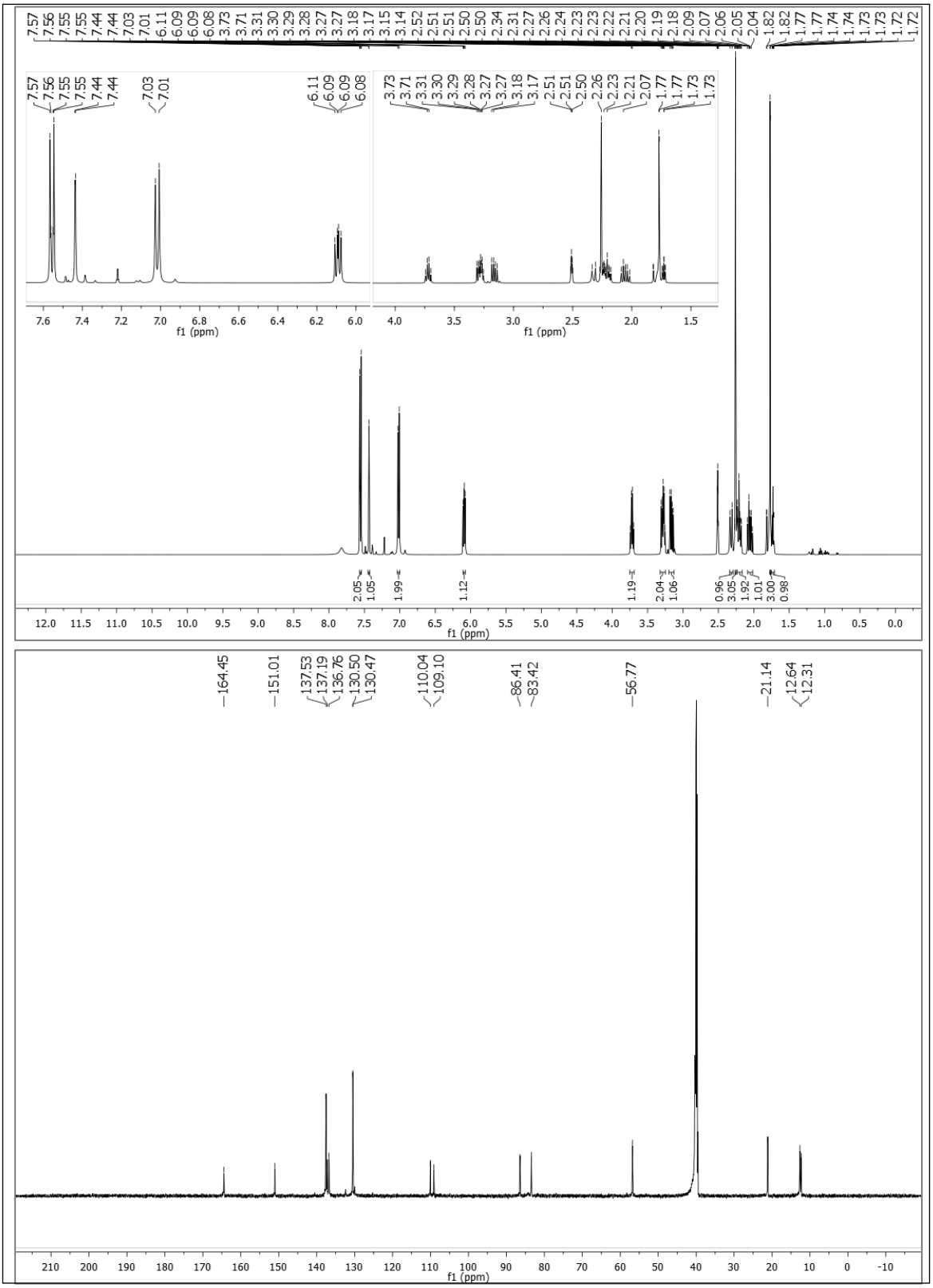


**Figure SI1.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Te-(4-methyl-phenyl)-3'-(amino)-thymidine (4j)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

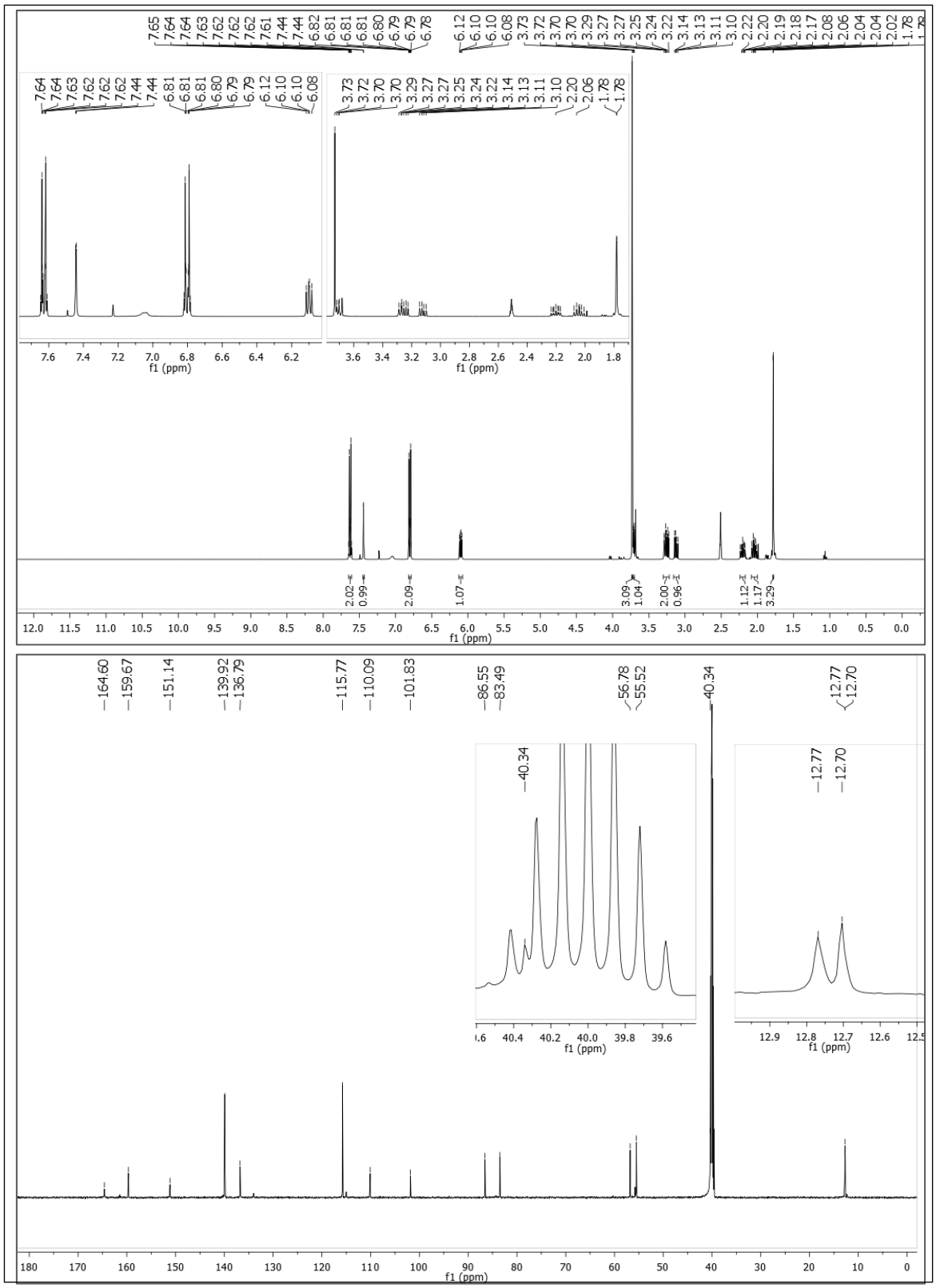


**Figure SI2.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Te-(4-mehtoxy-phenyl)-3'-(amino)-thymidine (4k)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

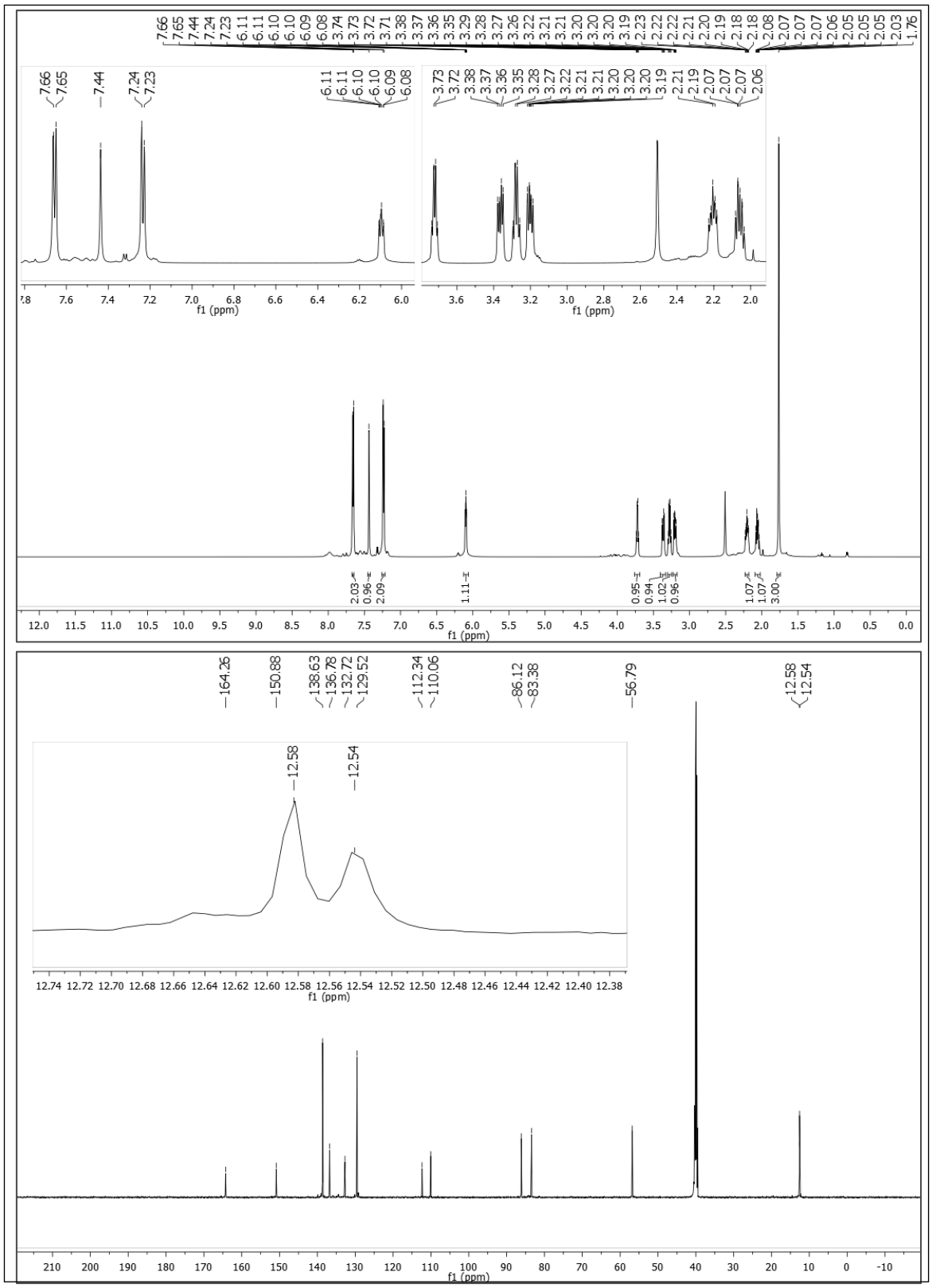


**Figure SI3.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Te-(4-chloro-phenyl)-3'-(amino)-thymidine (4l)** in DMSO-$d^{6}$ at 400 MHz and 100 MHz respectively

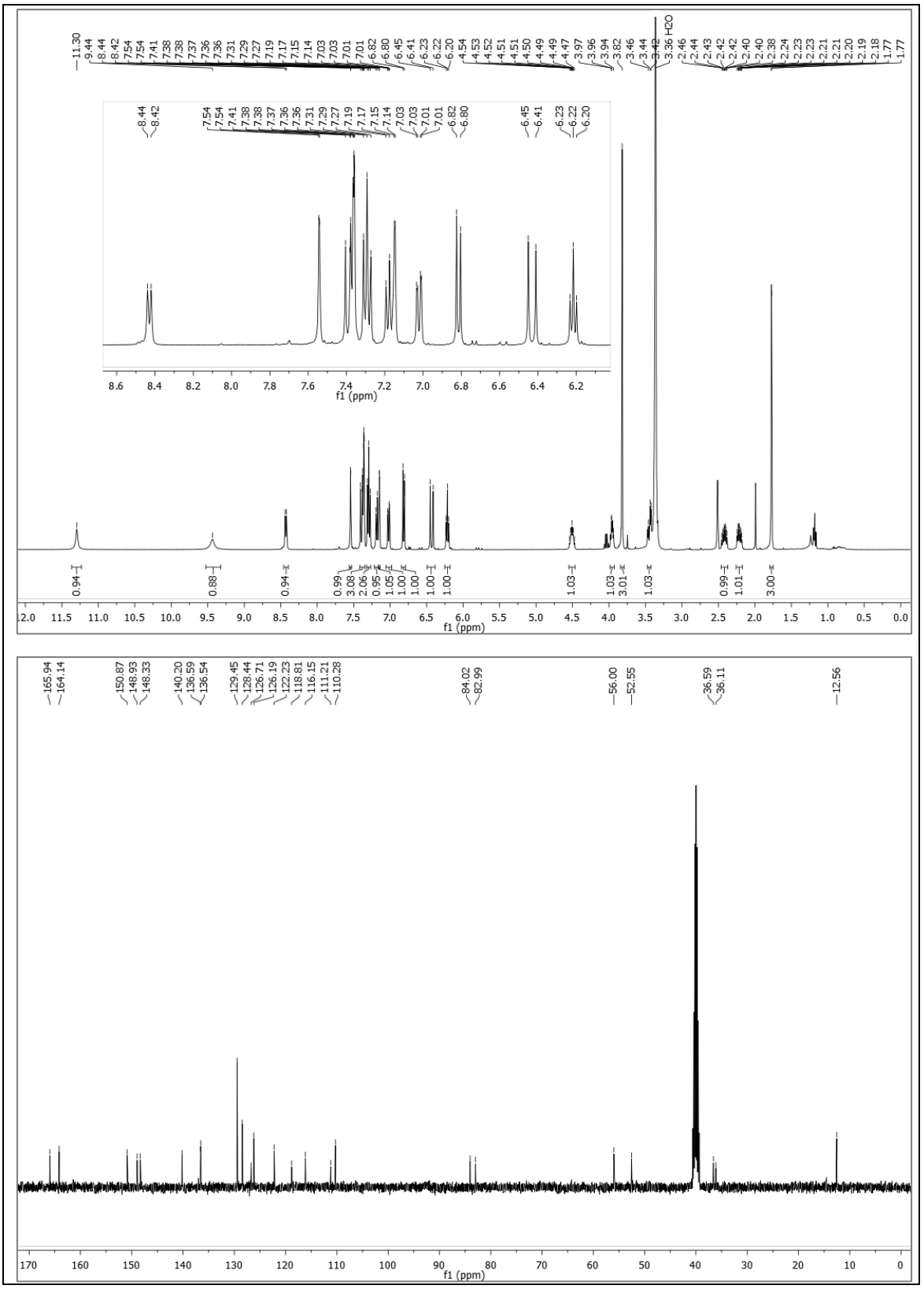


**Figure SI4.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-S-(phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-AminoThymidina (6a)** in DMSO-$d^{6}$ at 400 MHz and 100 MHz respectively

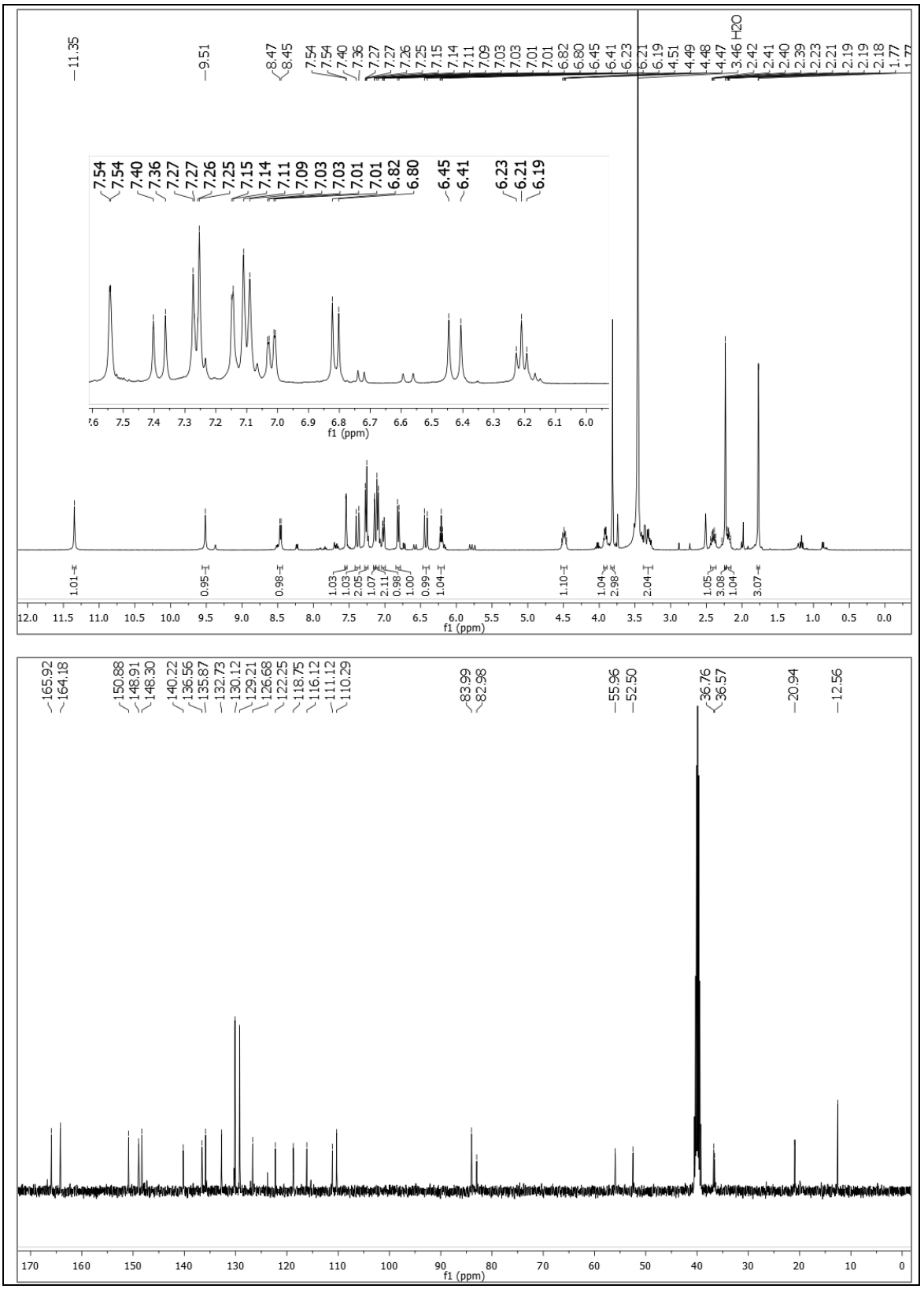



**Figure SI5.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-S-(4-methyl-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6b)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

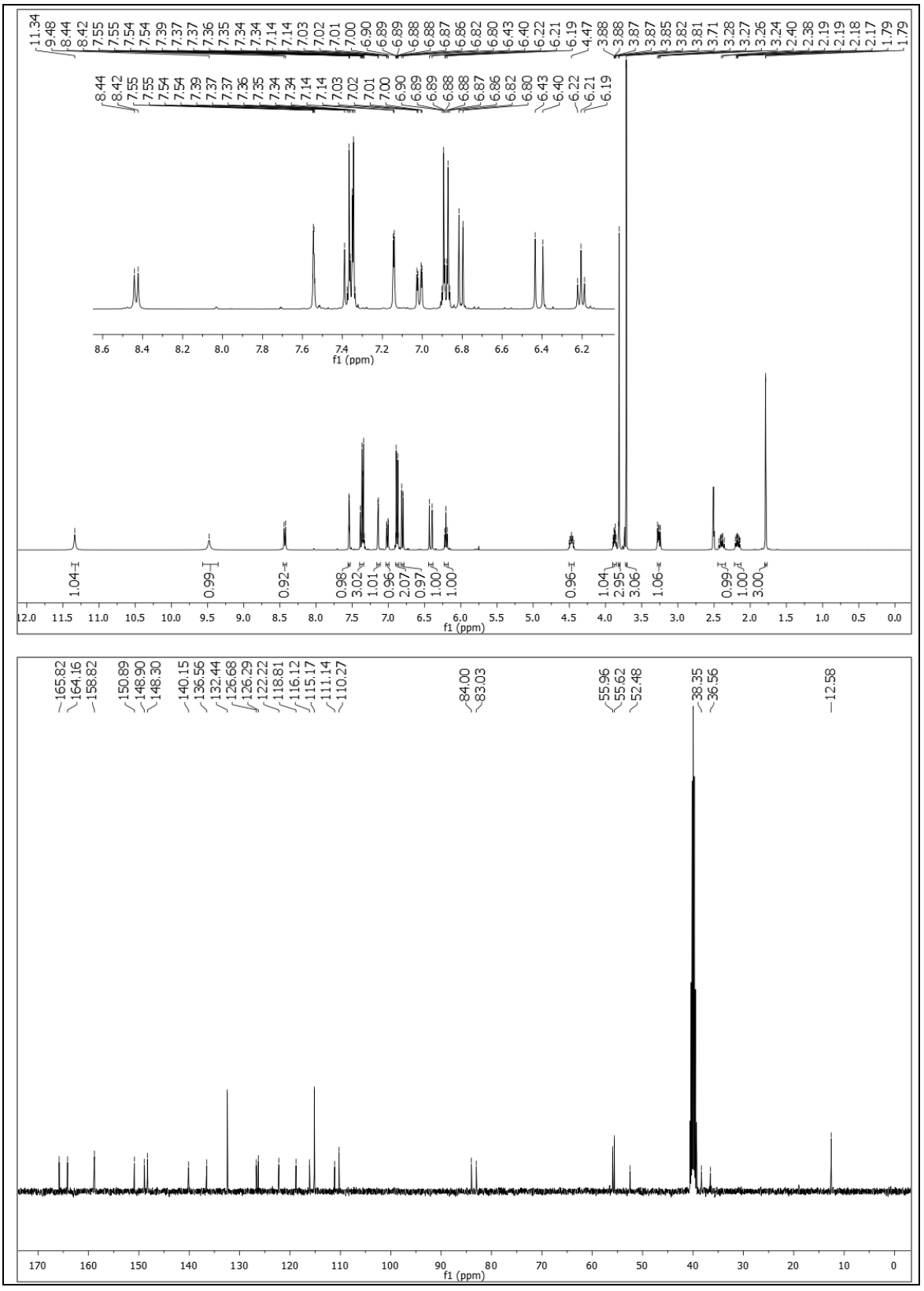


**Figure SI6.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-S-(4-methoxy-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6c)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

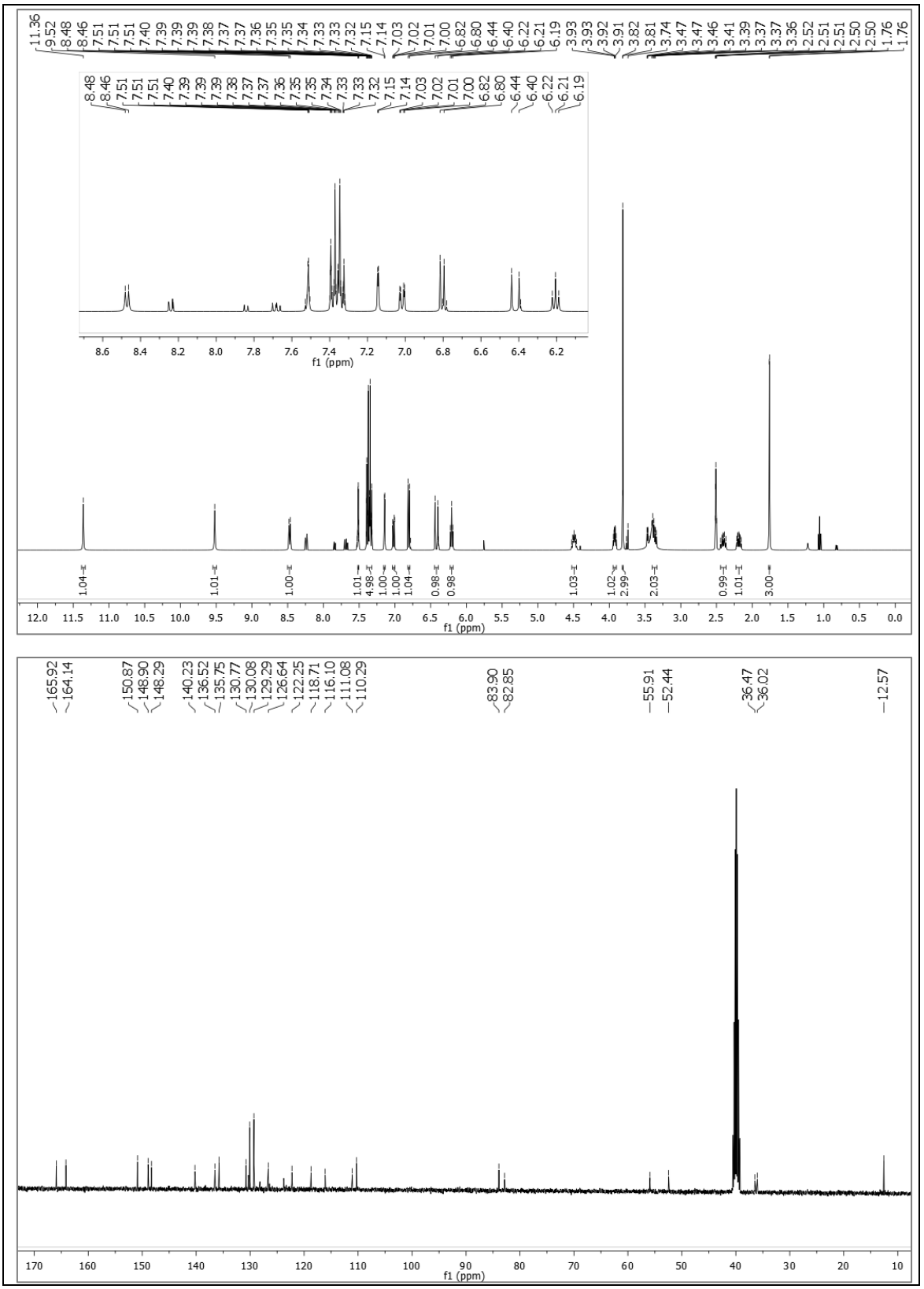


**Figure SI7.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-S-(4-chloro-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6d)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

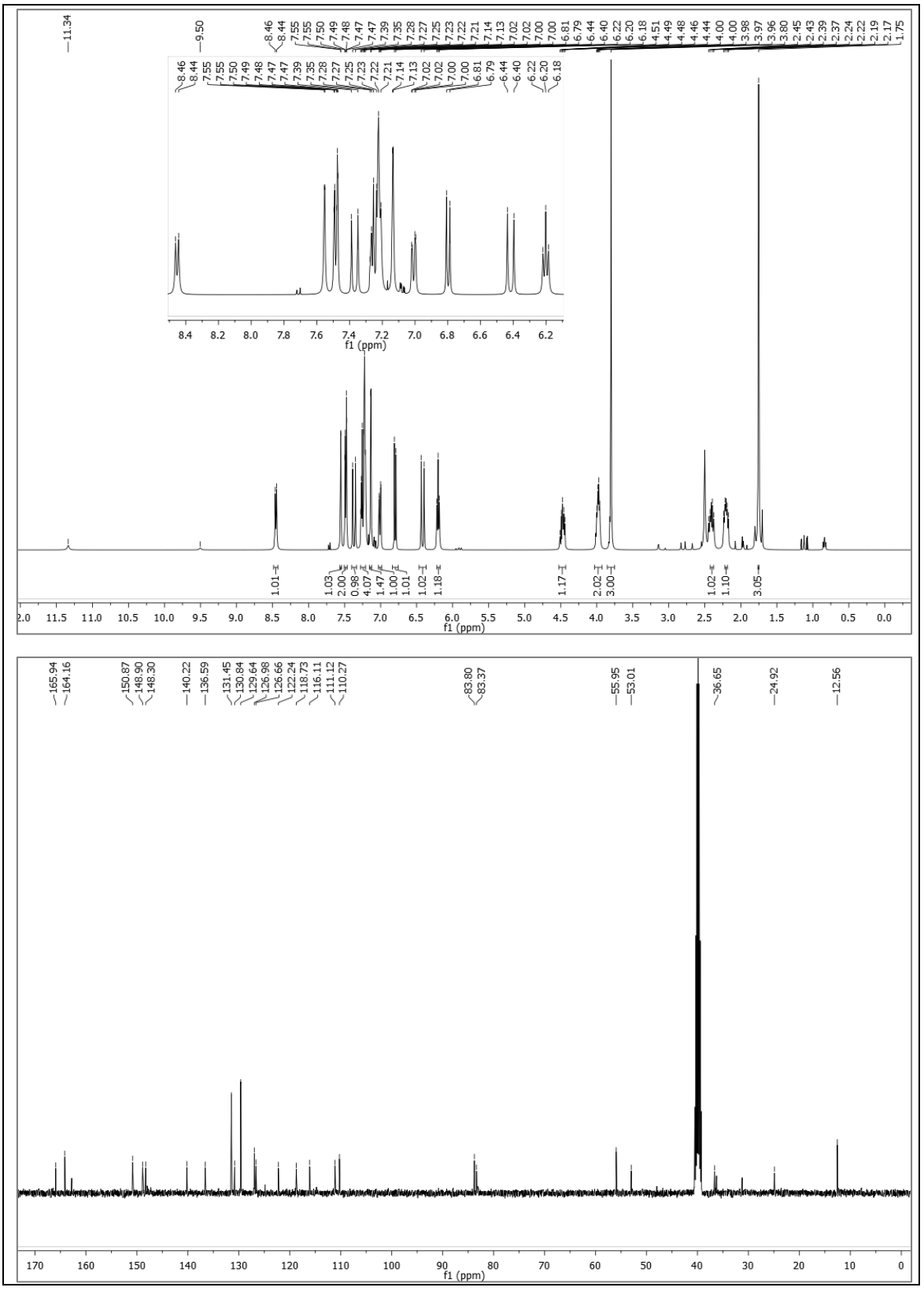


**Figure SI8.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Se-(phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6e)** in DMSO-$d^{6}$ at 400 MHz and 100 MHz respectively

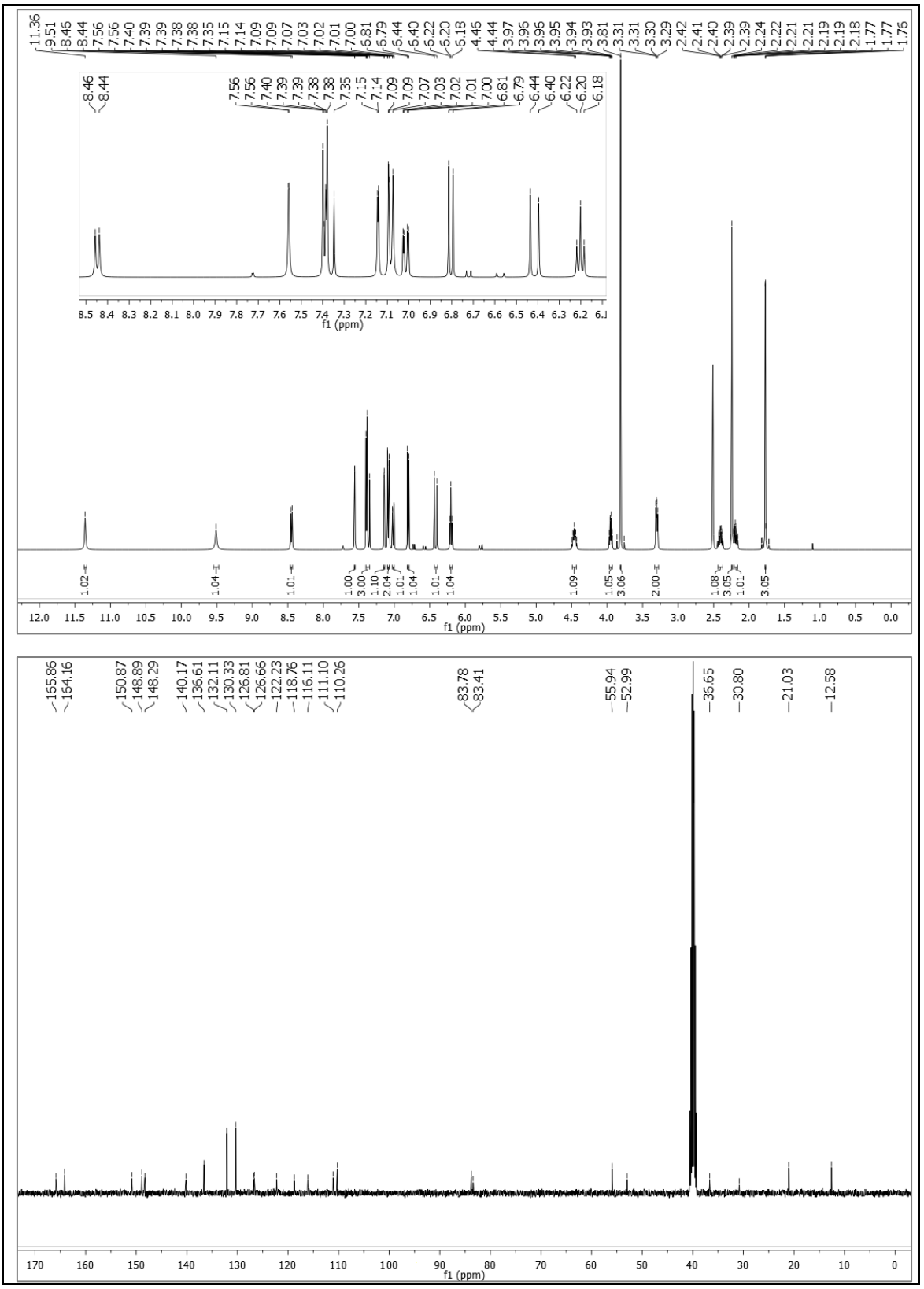


**Figure SI9.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Se-(4-methyl-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6f)** in DMSO-$d^{6}$ at 400 MHz and 100 MHz respectively

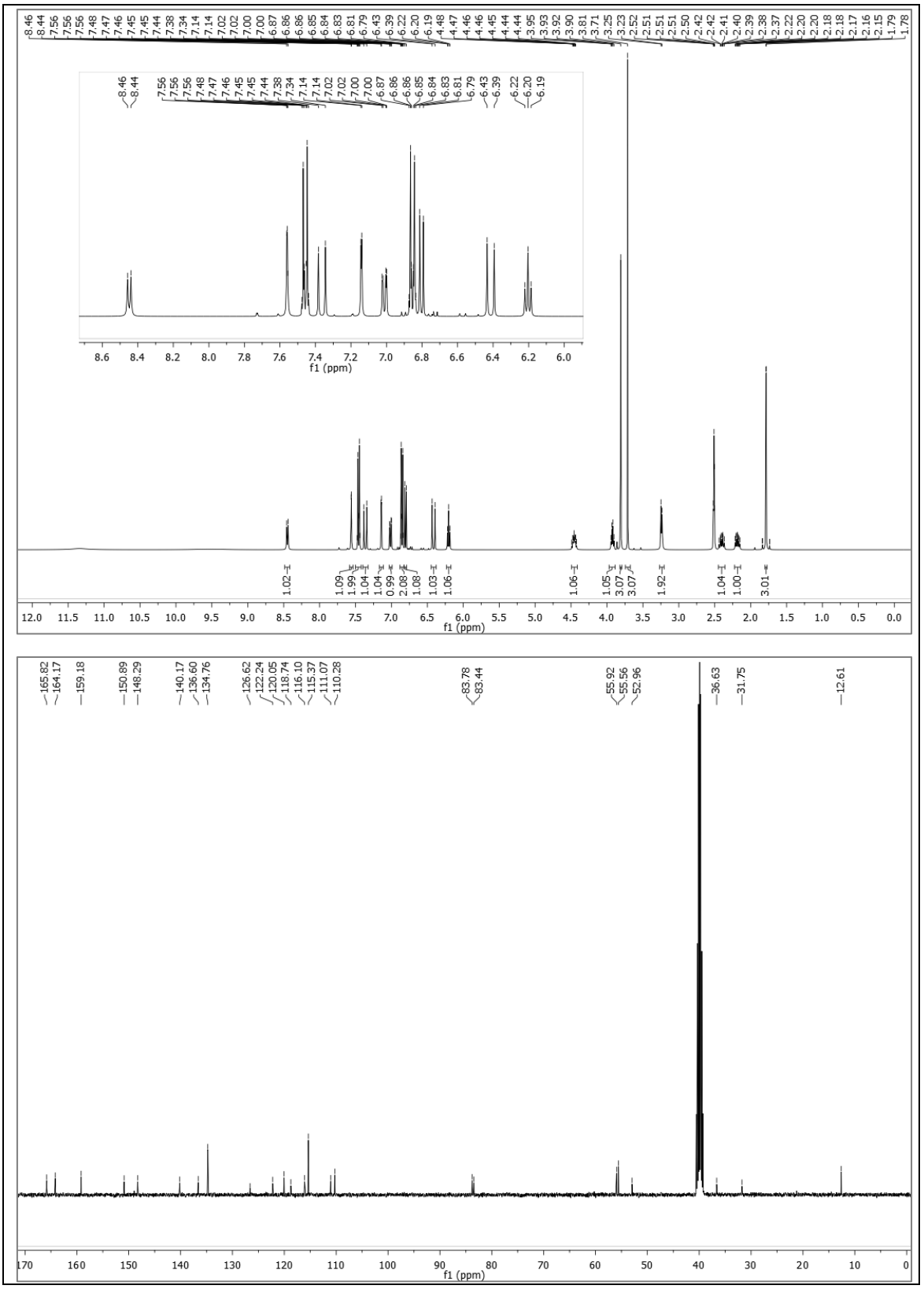



**Figure SI10.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Se-(4-methoxy-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6g)** in DMSO-$d^{6}$ at 400 MHz and 100 MHz respectively

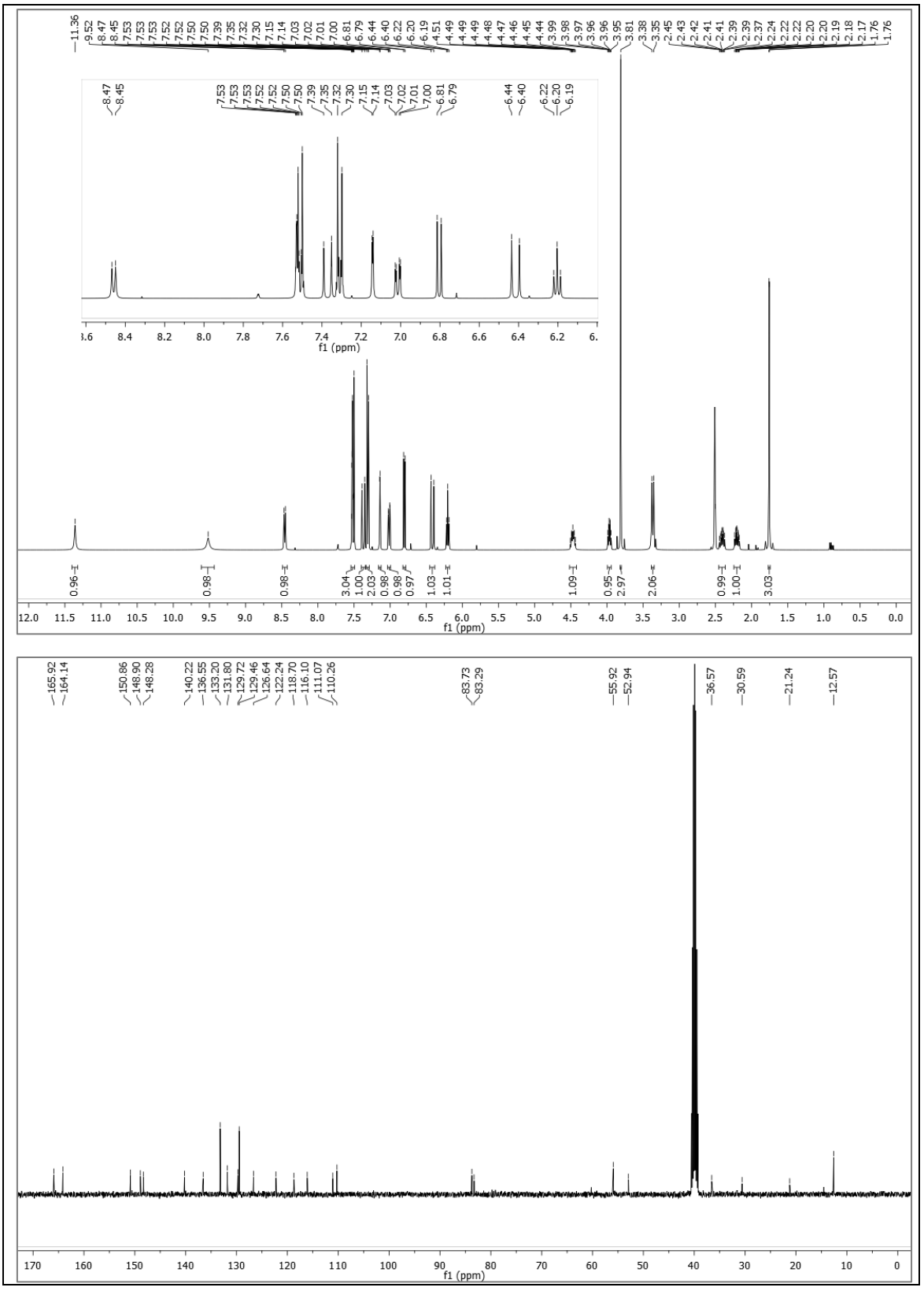


**Figure SI11.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Se-(4-chloro-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6h)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

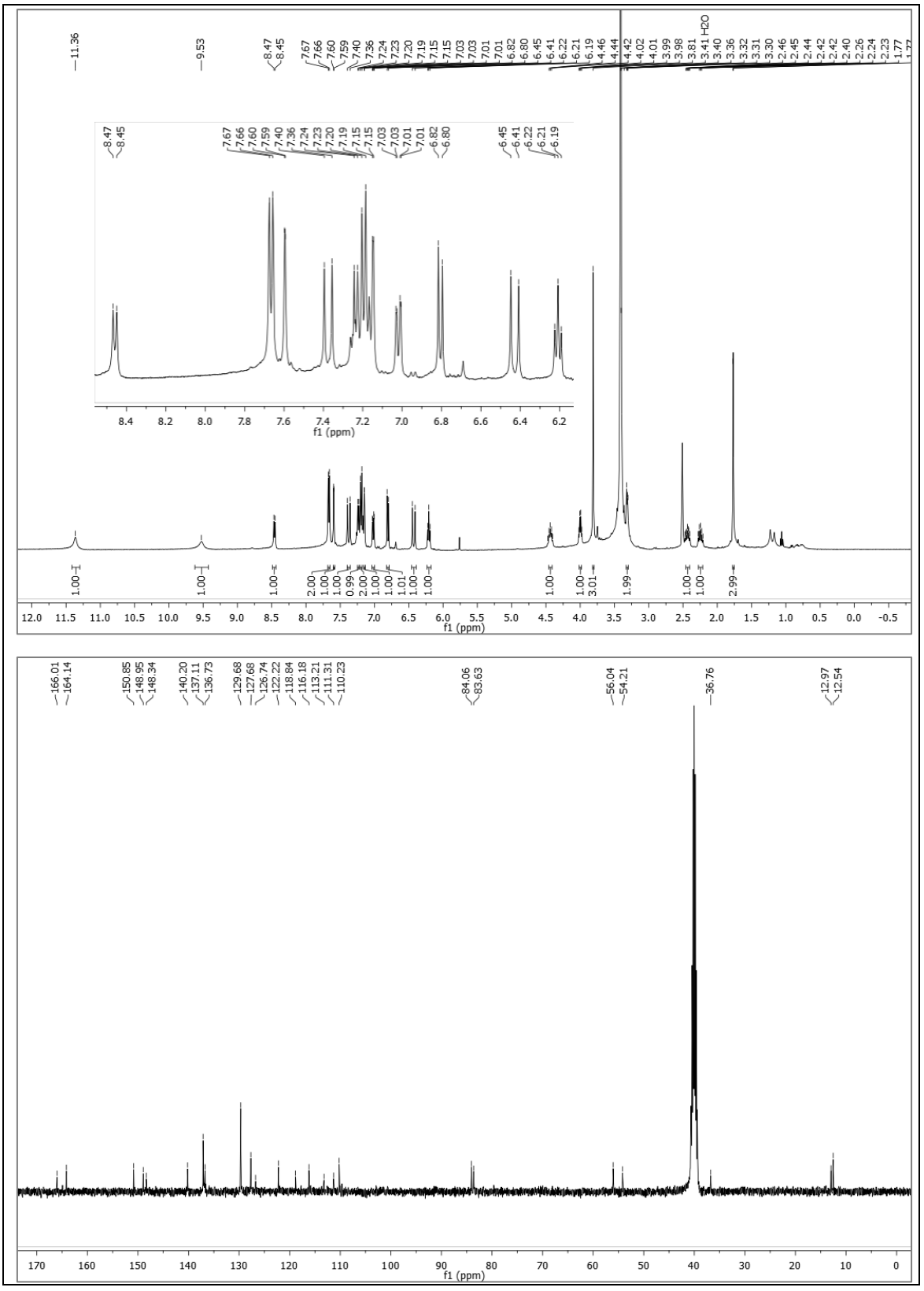


**Figure SI12.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Te-(phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6i)** in DMSO-$d^{6}$ at 400 MHz and 100 MHz respectively

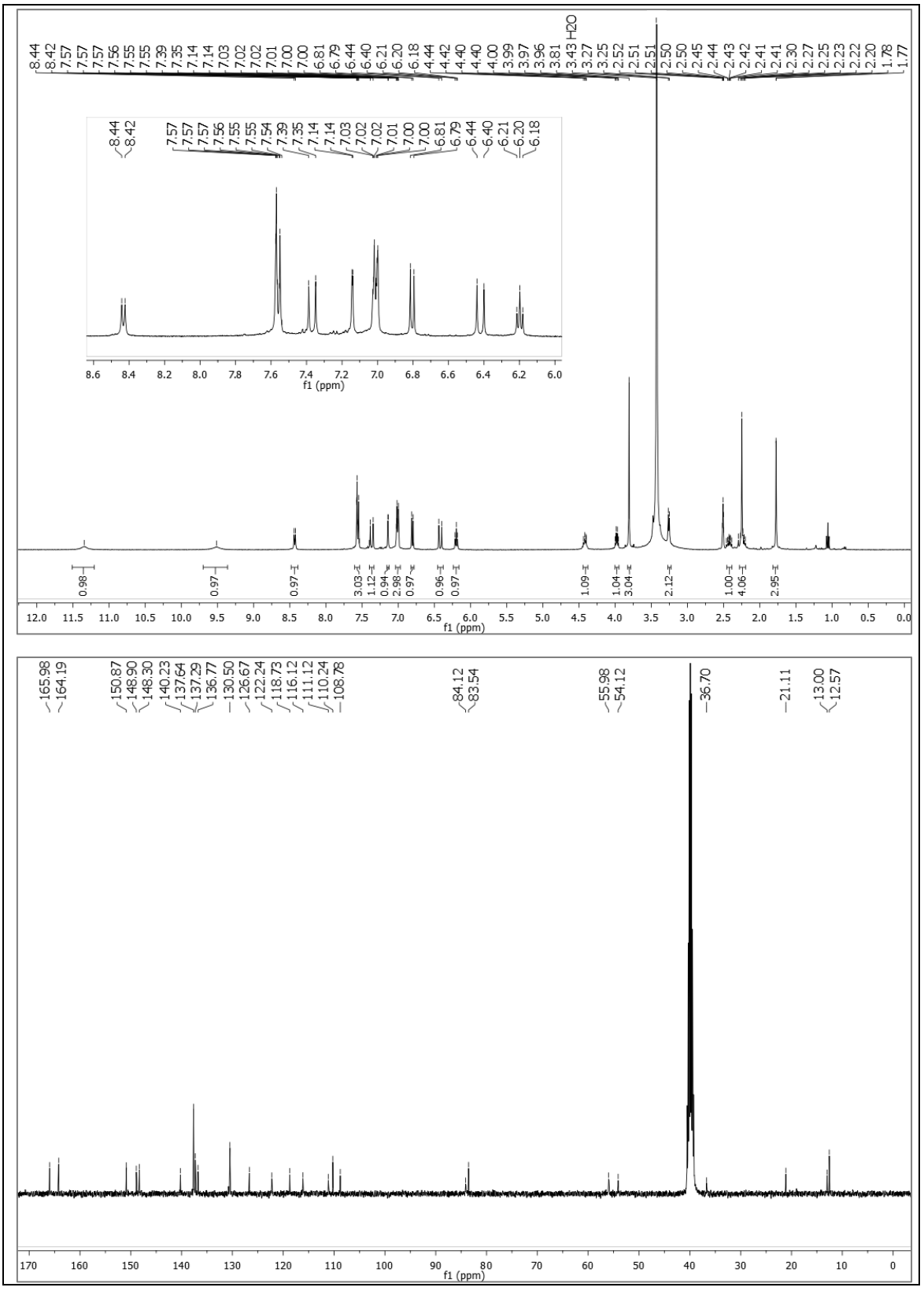


**Figure SI13.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Te-(4-methyl-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6j)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

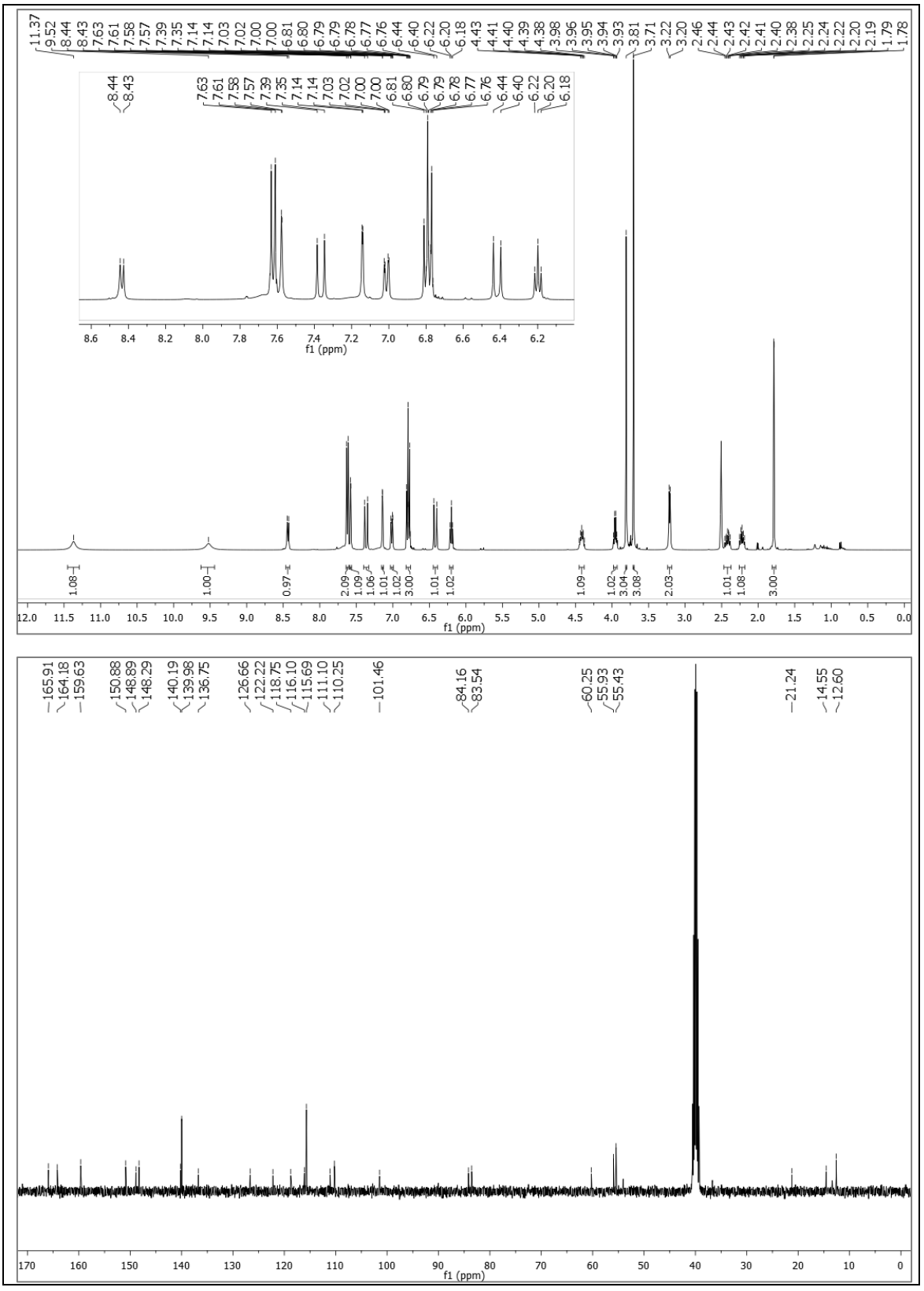


**Figure SI14.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Te-(4-methoxy-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6k)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

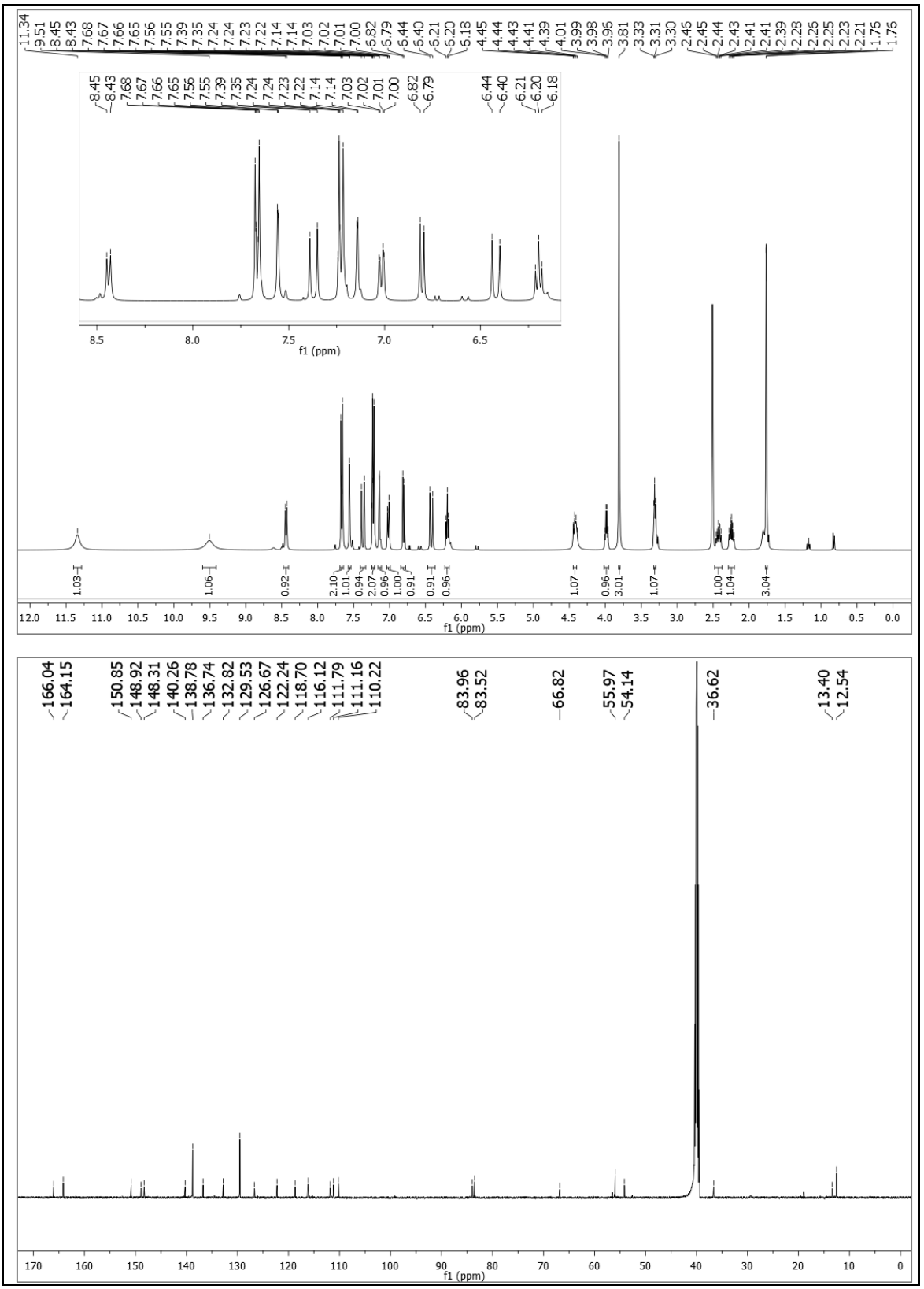


**Figure SI15.** $^{1}H$ and $^{13}C$ NMR of the compound **5'-Te-(4-chloro-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6l)** in DMSO-$d^6$ at 400 MHz and 100 MHz respectively

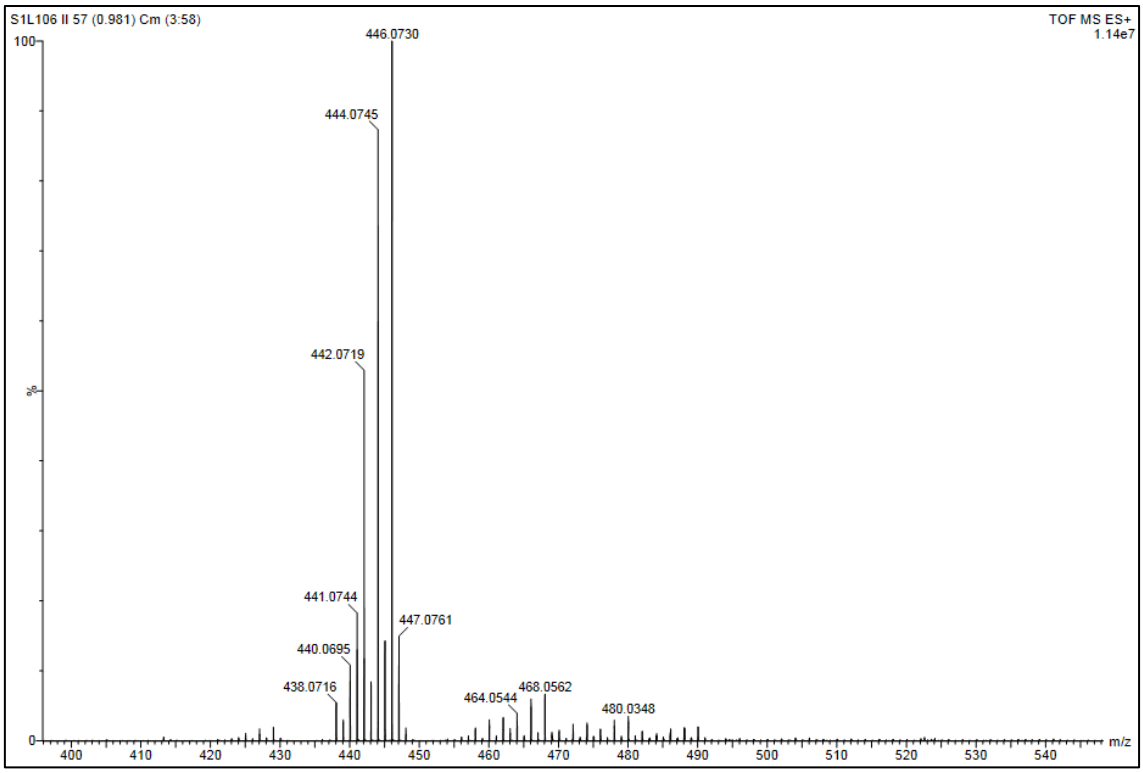



**Figure SI16.** HRMS of compound **5'-Te-(4-methyl-phenyl)-3'-(amino)-thymidine (4j)**.

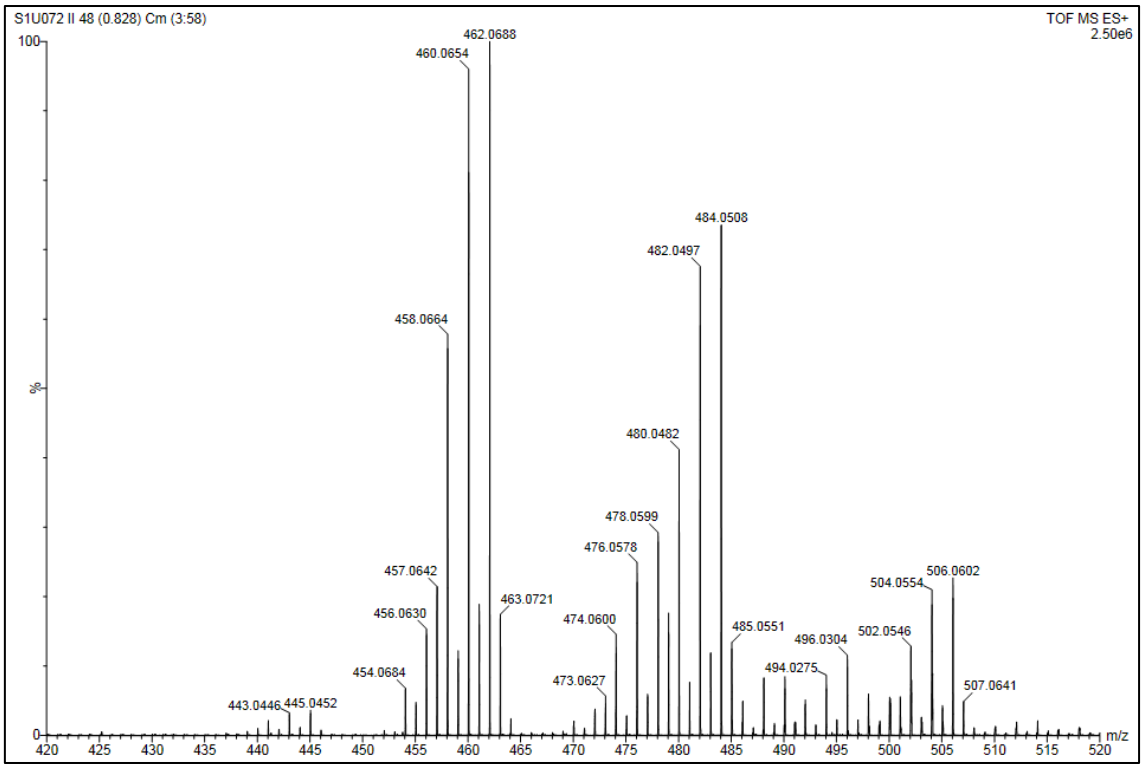



**Figure SI17.** HRMS of compound **5'-Te-(4-mehtoxy-phenyl)-3'-(amino)-thymidine (4k)**.

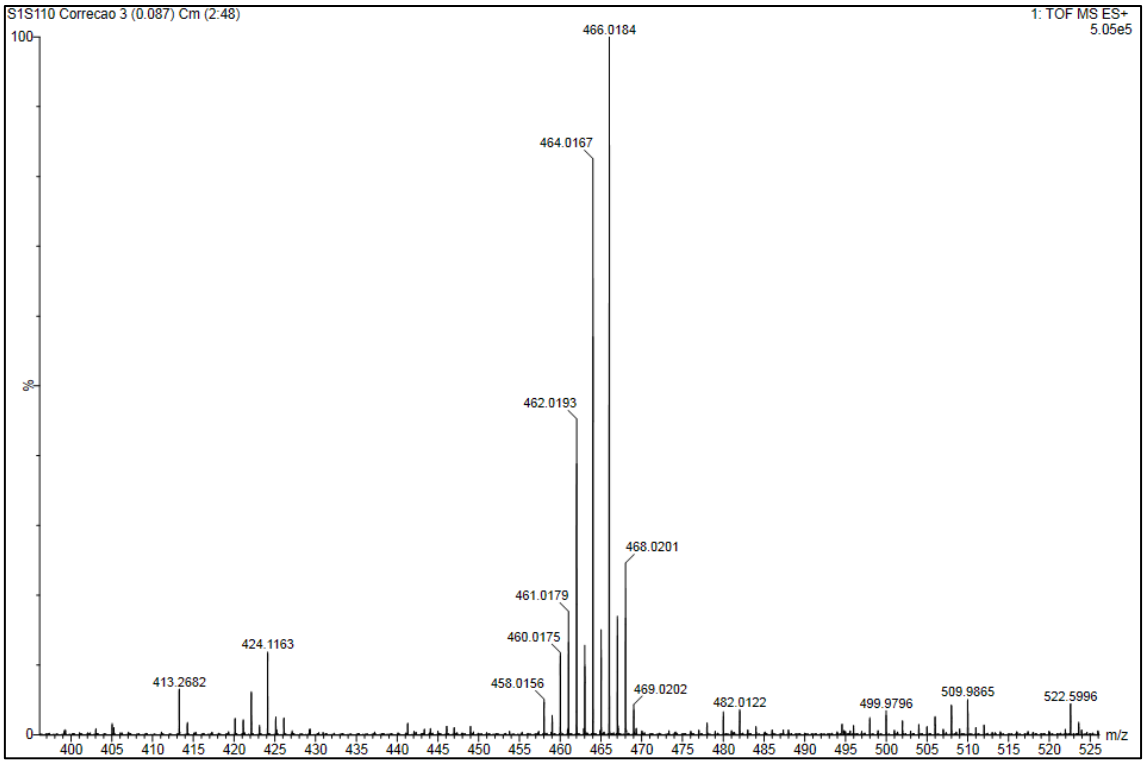



**Figure SI18.** HRMS of compound **5'-Te-(4-chloro-phenyl)-3'-(amino)-thymidine (4l)**.

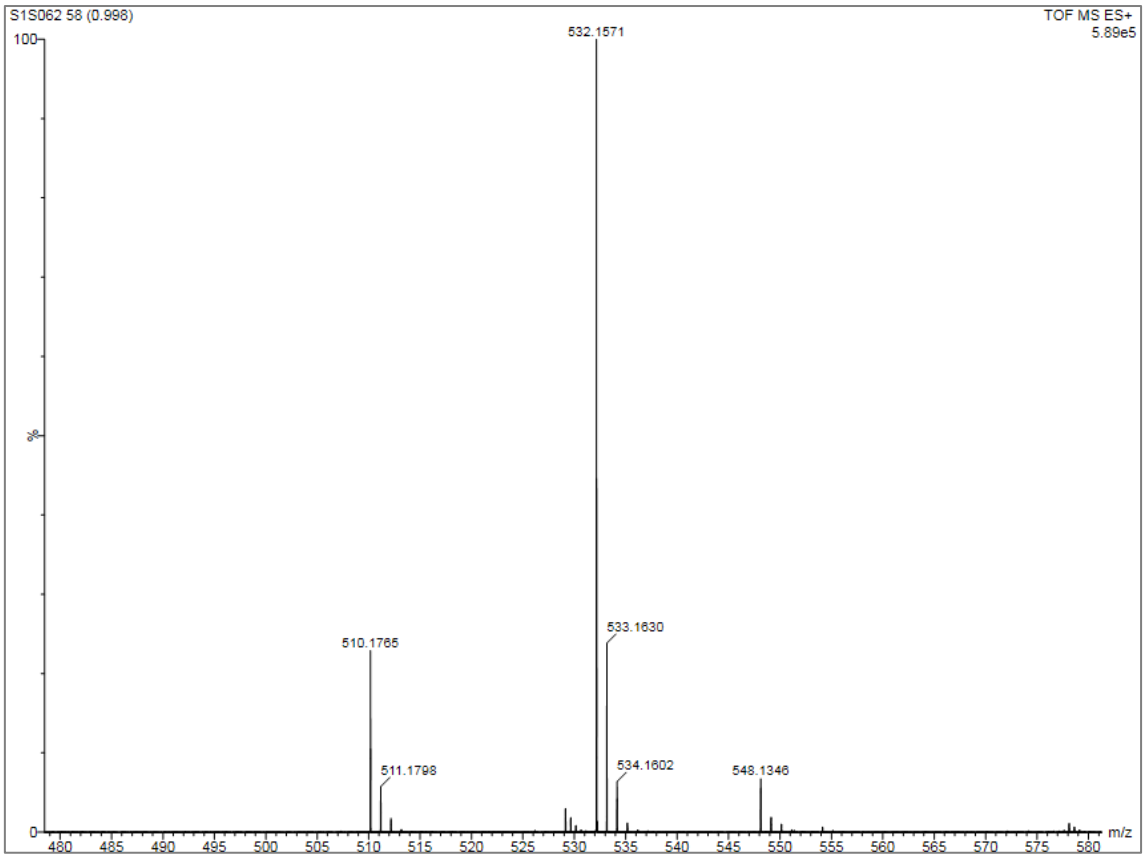



**Figure SI19.** HRMS of compound **5'-S-(phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6a)**.

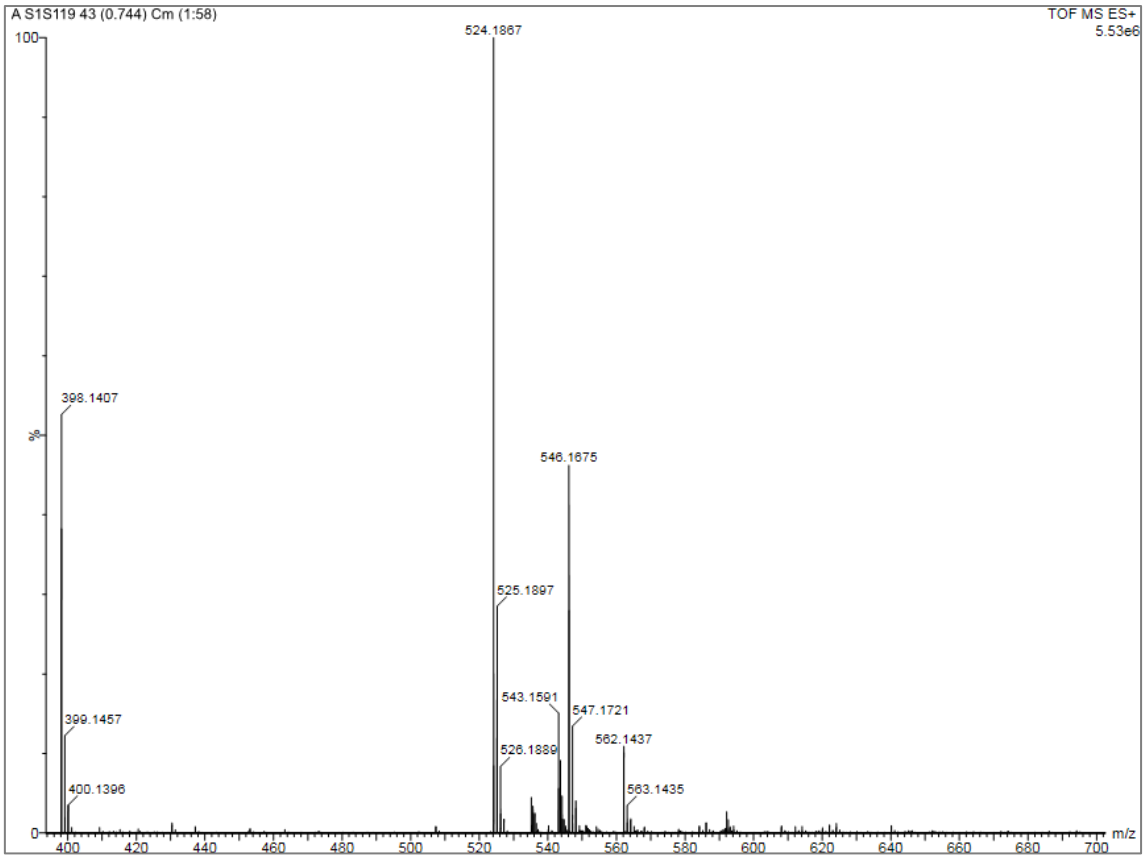



**Figure SI20.** HRMS of compound **5’-S-(4-methyl-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6b)**.

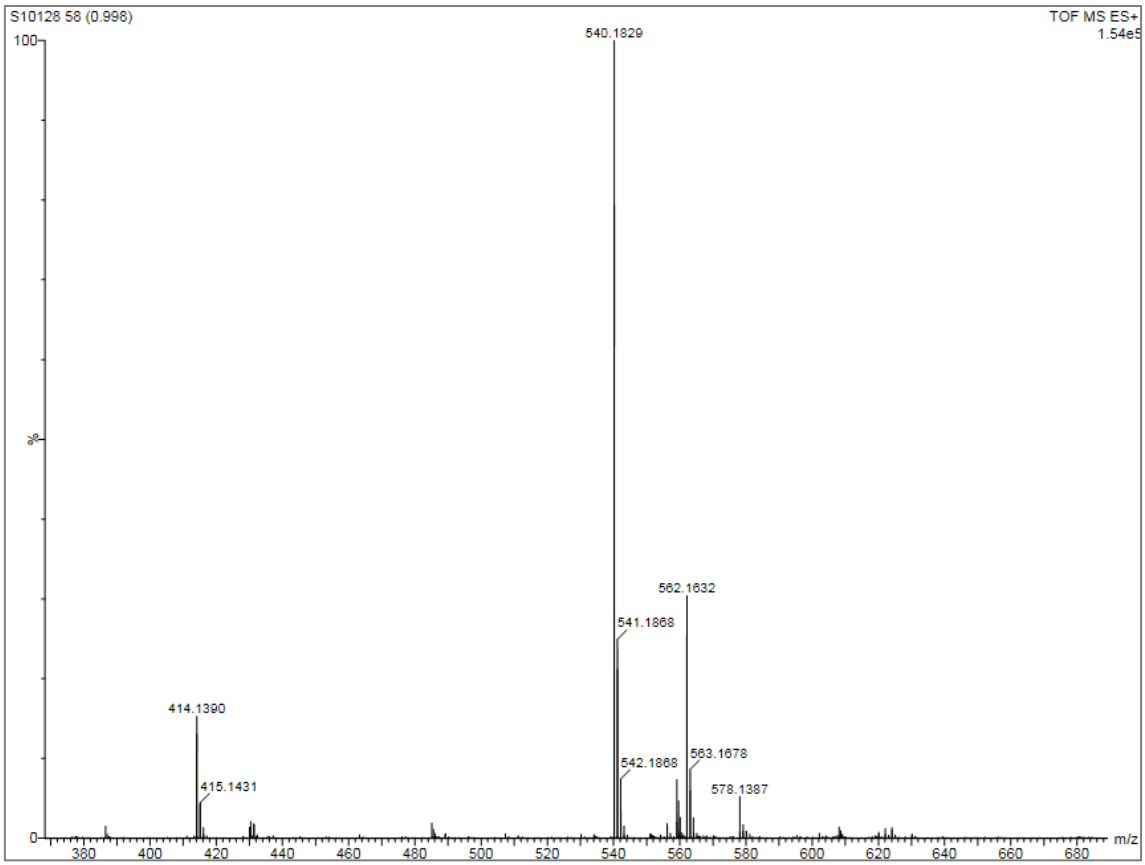



**Figure SI21.** HRMS of compound **5'-S-(4-methoxy-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6c)**.

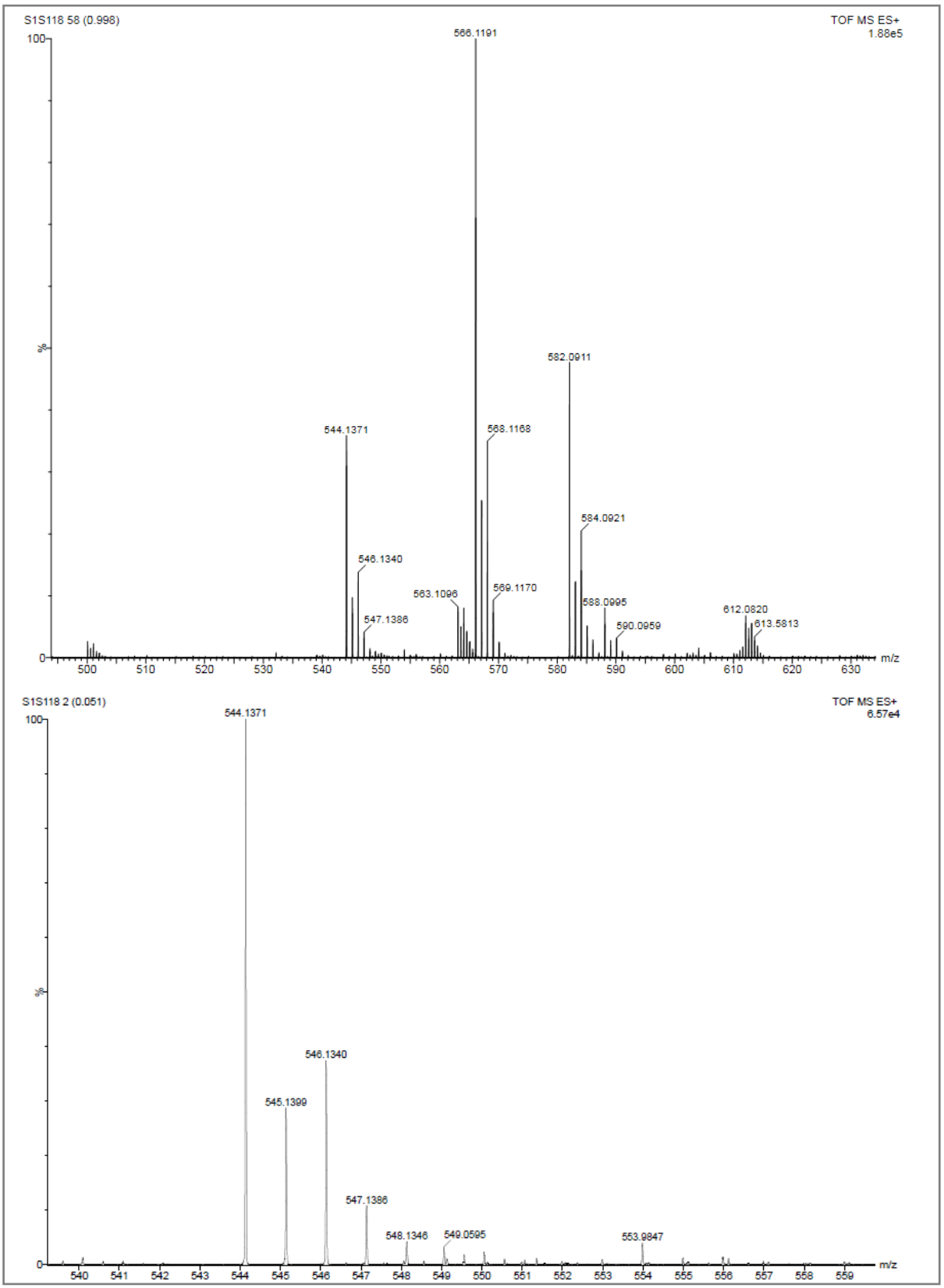



**Figure SI22.** HRMS of compound **5'-S-(4-chloro-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6d)**.

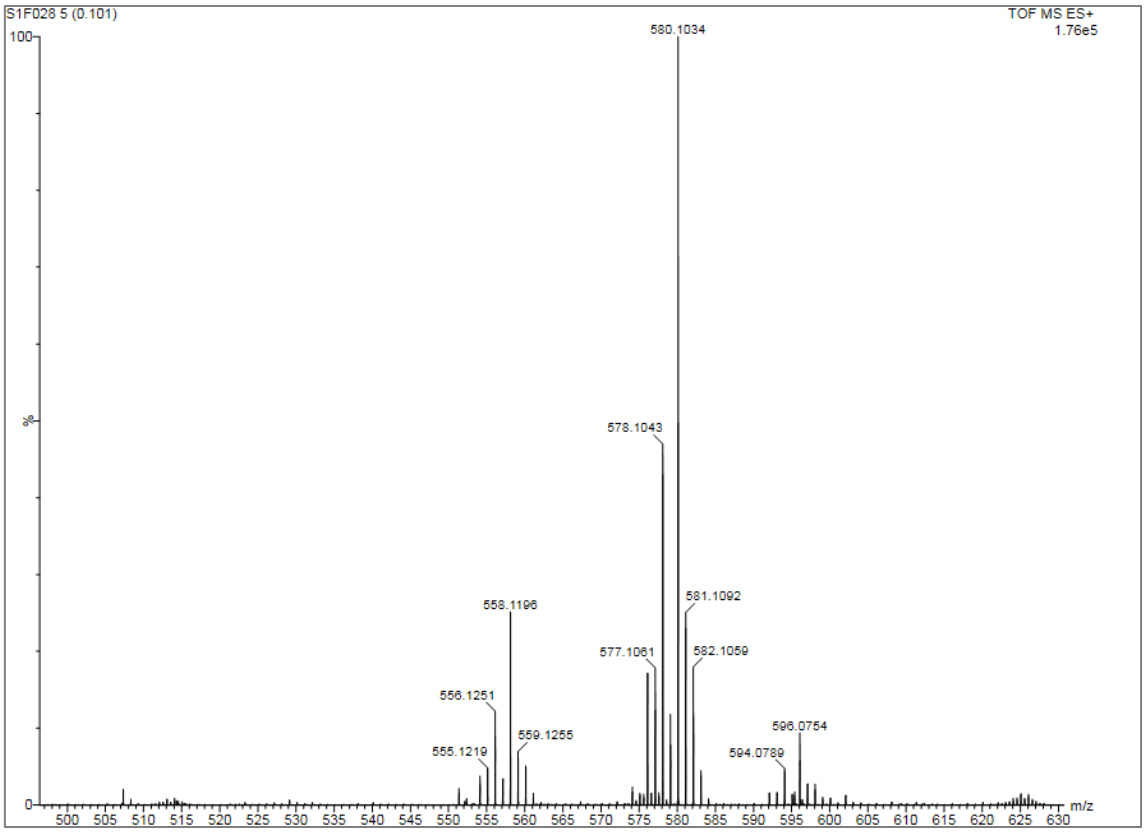



**Figure SI23.** HRMS of compound **5'-Se-(phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6e)**.

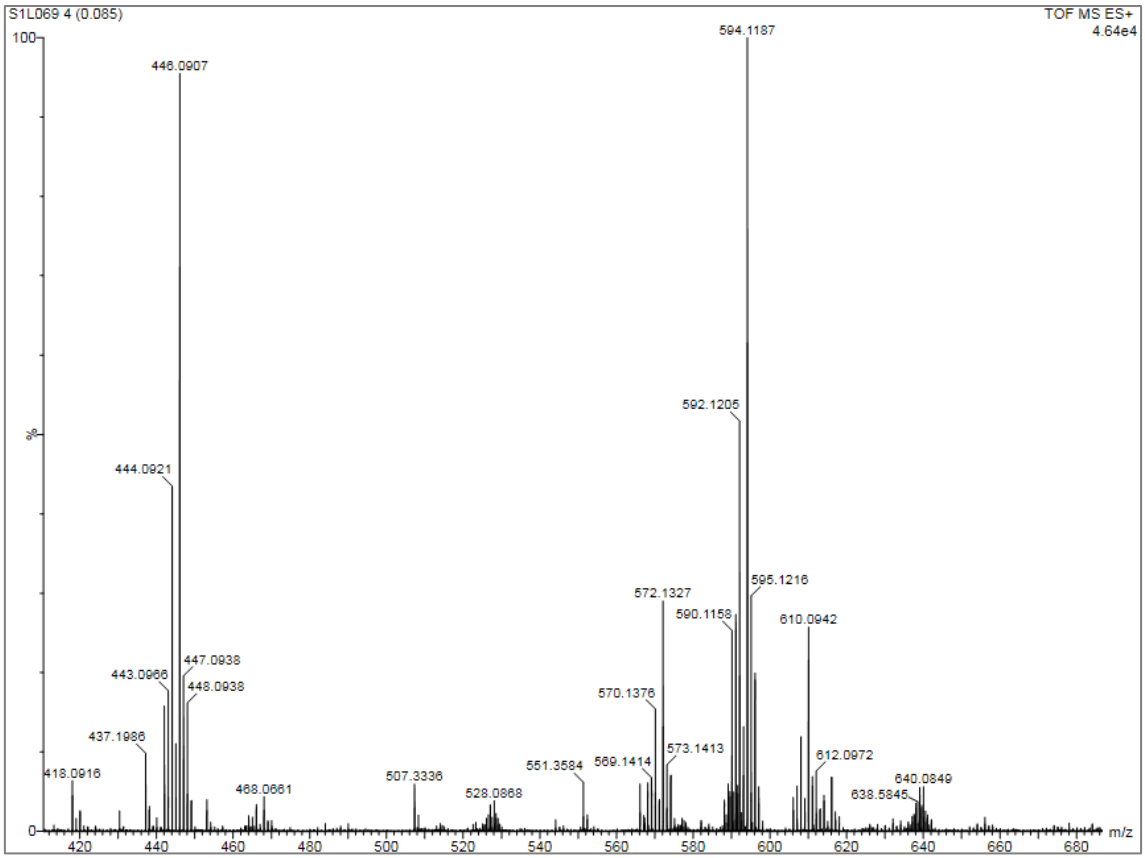



**Figure SI24.** HRMS of compound **5'-Se-(4-methyl-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6f)**.

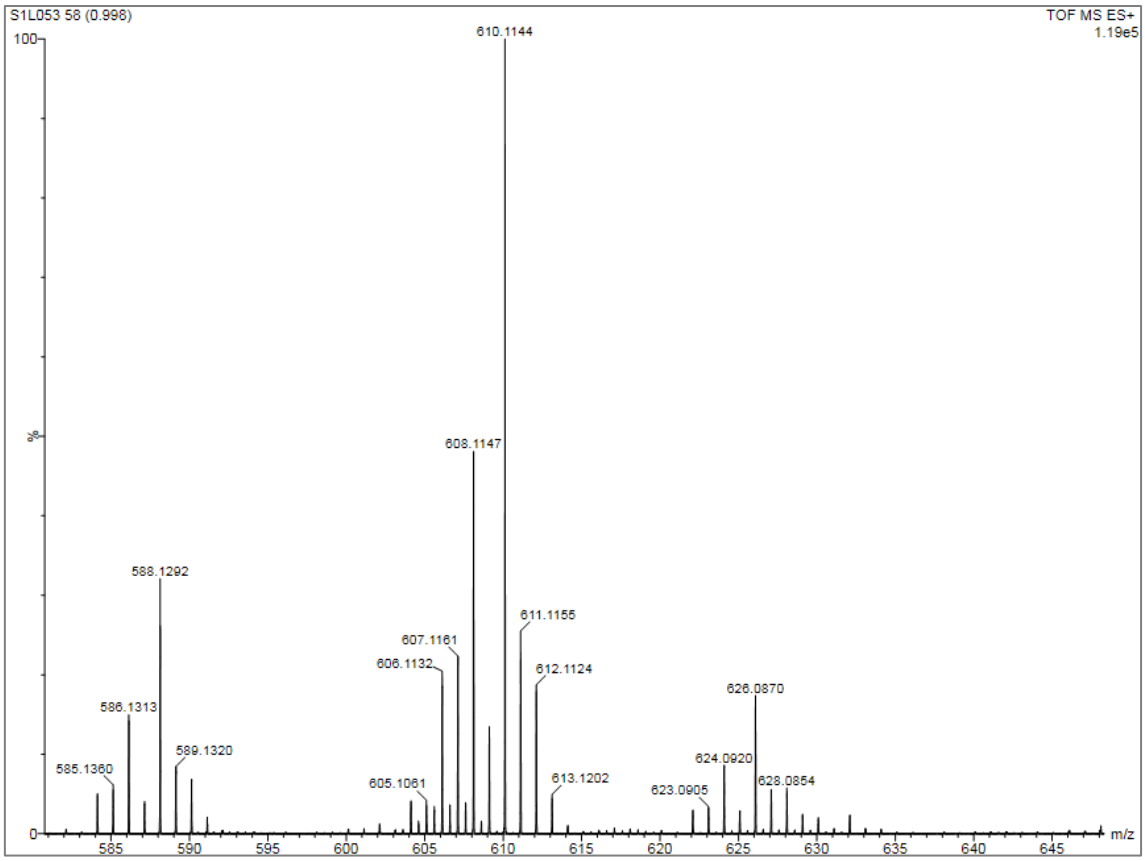



**Figure SI25.** HRMS of compound **5’-Se-(4-methoxy-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6g)**.

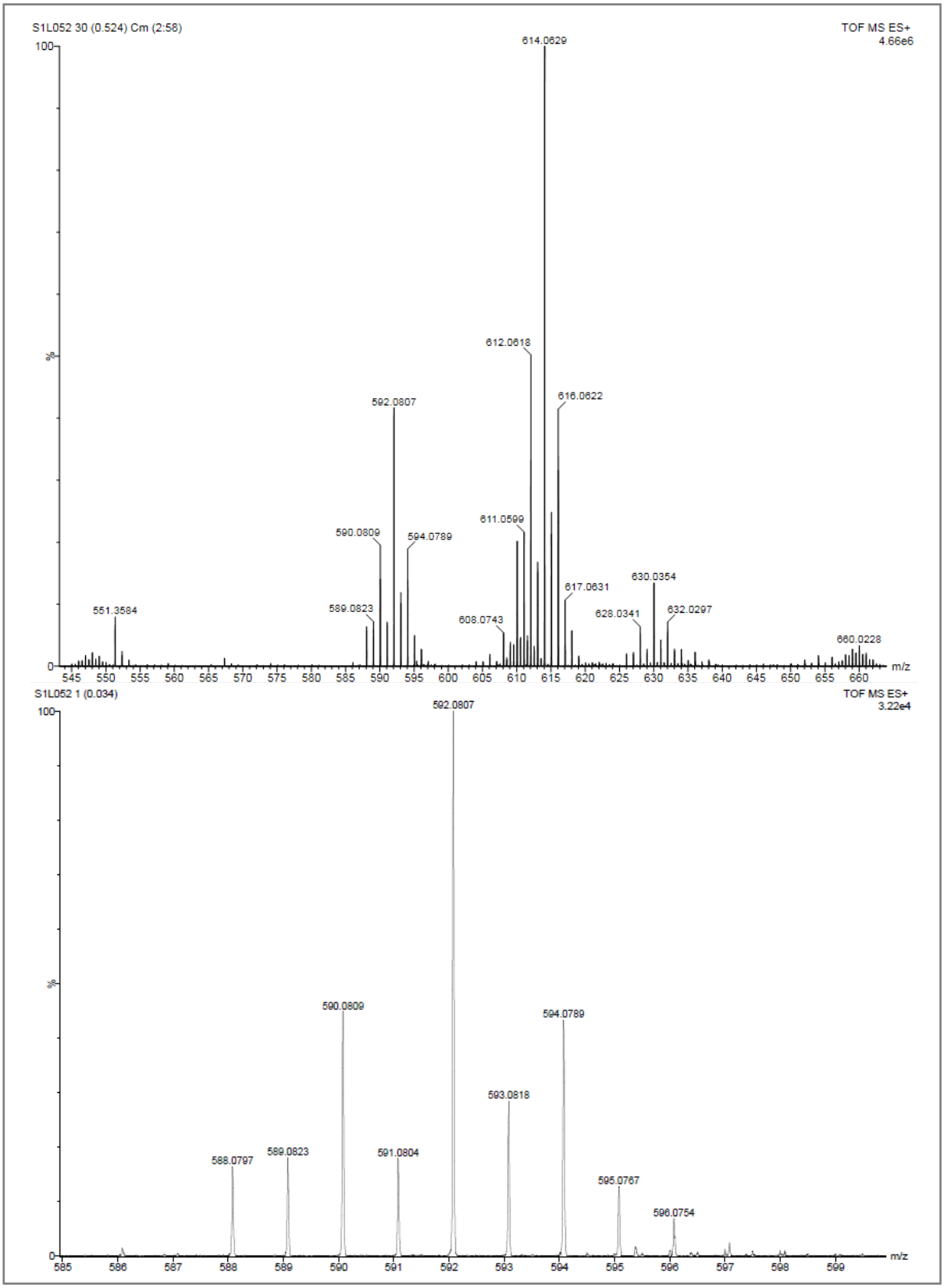



**Figure SI26.** HRMS of compound **5'-Se-(4-chloro-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6h)**.

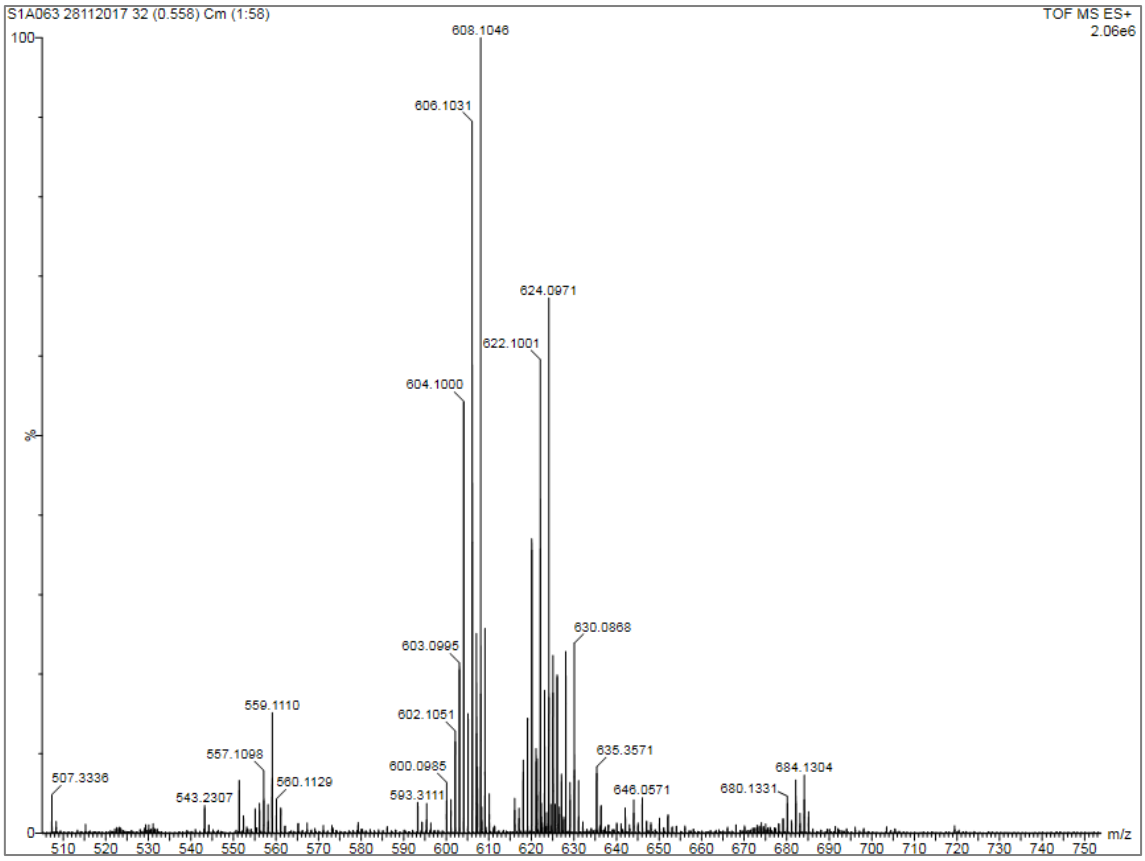



**Figure SI27.** HRMS of compound **5’-Te-(phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6i)**.

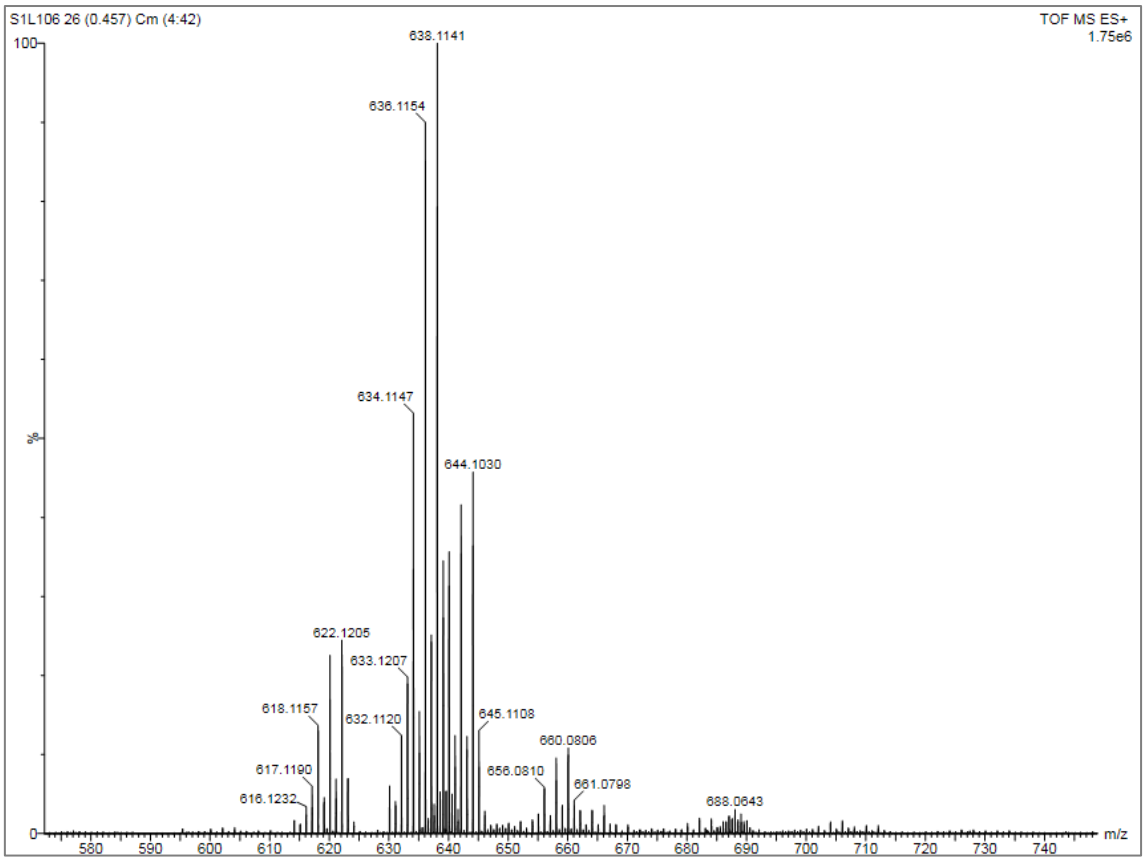



**Figure SI28.** HRMS of compound **5'-Te-(4-methyl-phenyl)-3'-N-(E)-Feruloyl-3',5'-dideoxy-amino-thymidine (6j)**.

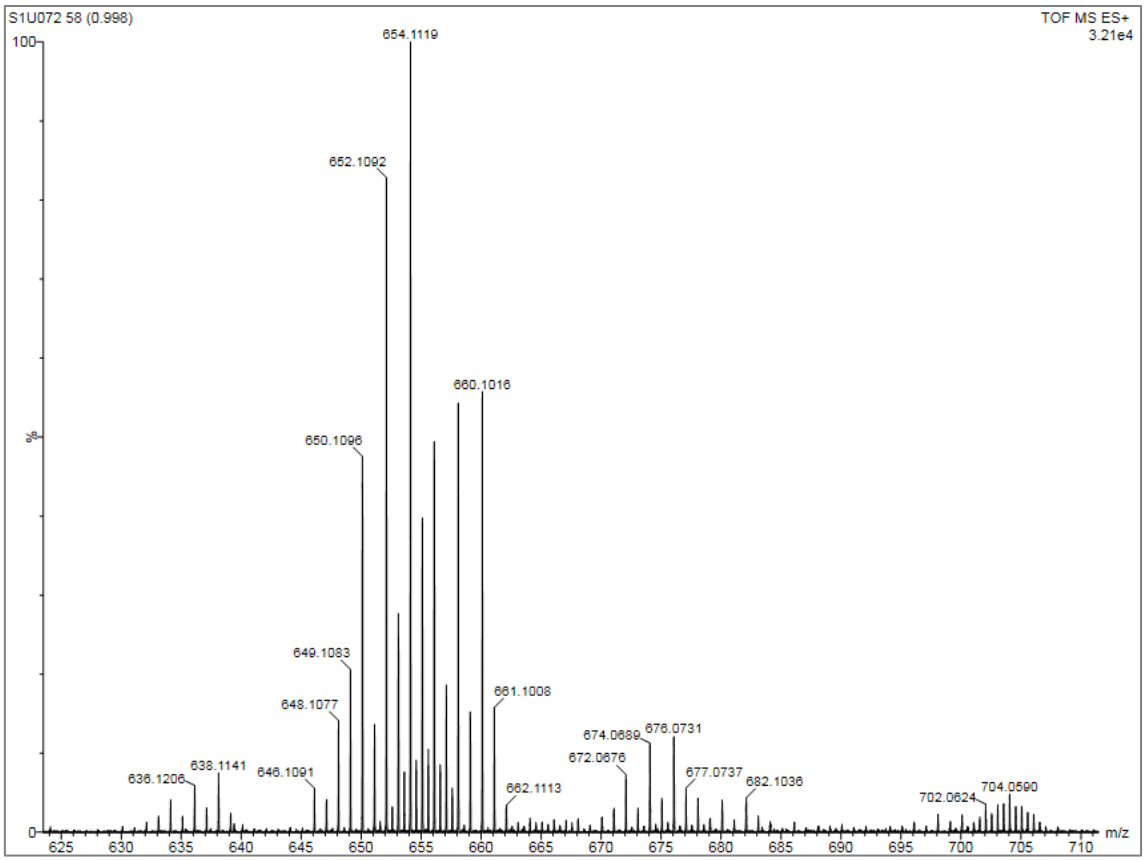



**Figure SI29.** HRMS of compound **5’-Te-(4-methoxy-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6k)**.

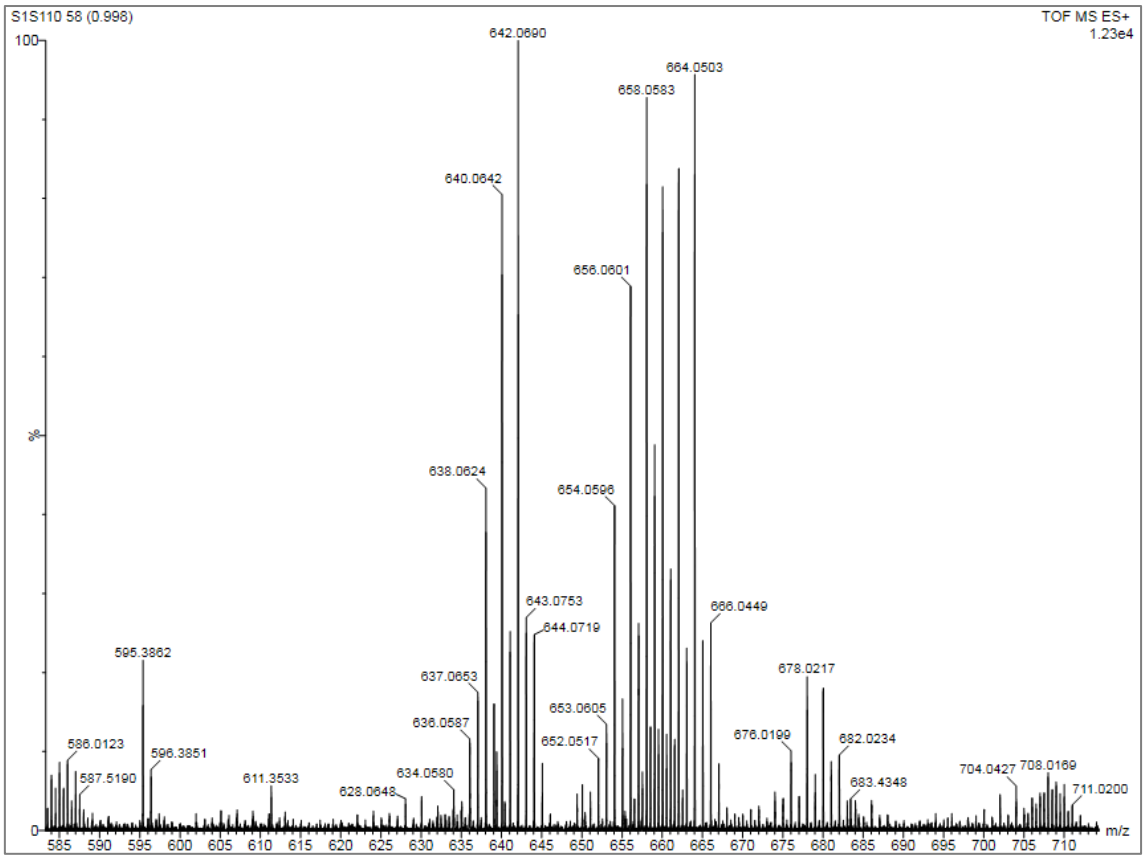



**Figure SI30.** HRMS of compound **5’-Te-(4-chloro-phenyl)-3’-N-(E)-Feruloyl-3’,5’-dideoxy-amino-thymidine (6l)**.

## Materials and methods

### Antioxidant assays

### Scavenger of radical 2,2-diphenylpicrylhydrazyl

The DPPH scavenger method used was proposed by Pereira et al (2014)[2] . For the time-dependent curve, azidothymidine, ferulic acid, and 5'-arylchalcogenyl-3'-N-(E)-feruloyl-3'-amino-3'-deoxythymidine derivatives (1 mM final concentration) were dissolved in DMSO (10 % final concentration) and mixed with 0.3 mM DPPH in ethanol medium. To perform the concentration curve were used 0, 0.01, 0.025, 0.05, 0.1, and 1 mM of the compounds mixed with 0.3 mM DPPH in ethanol. The absorbance was measured in spectrophotometer SpectraMax at 518 nm every 5 min for 180 min. α-tocopherol (0, 0.025, 0.05, 0.1 mM) was used as positive control.

### Lipid peroxidation inhibition

The lipid peroxidation inhibition was evaluated by the TBARS production according to Ohkawa et al (1979)[3]. Phosphatidylcholine (0.4 mg), Fe(II) (55 μM), and Tris-HCl buffer pH 7.4 (1.85 mM) were mixed with azidothymidine, ferulic acid or 5'-arylchalcogenyl-3'-N-(E)-feruloyl-3'-amino-3'-deoxythymidine derivatives (final concentration was ranged between 0 and 200 μM) dissolved in DMSO (final concentration was equal to 3.8 %). The system was incubated for 30 minutes at 37 ° and, subsequently, acetic acid buffer pH 3.4 and thiobarbituric acid (0.22%) were added. The samples were incubated again for one hour at 100 °C. To extract the complex between thiobarbituric acid and lipid peroxidation products (pink coloring) 400 μL of N-butanol was added. Then, the tubes were stirred for 30 s and centrifuged for 10 min at 6000 rpm. The supernatant was read in spectrophotometer SpectraMax at 532 nm. Malondialdehyde (MDA) curve was prepared as a standard. Diphenyl diselenide (200 μM) and α-tocopherol (0 – 200 μM) were used as positive controls.

## Antitumoral activity

### Cell culture and viability assay

Cancer cell line was obtained from the American Type Culture Collection (Rockville, USA) and was grown in Dulbecco Minimal Essential Medium (DMEM) containing 10% fetal bovine serum (FBS). Cell line was maintained at 37°C in a humidified atmosphere containing 5% $CO_2$. Cell viability was determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) test according Mosmann 1983[4]. Cells were seeded in 96-well plates (2 X $10^4$ cells per well) containing 100 µL growth medium. After incubation for 24 h, the cells were treated with medium containing compounds **6a**, **6e**, **6i**, **6j**, **6k** and **6l** at different concentrations (6, 12, 25, 50, 100 µM), dissolved in DMSO (less than 0,25% in each preparation). After incubation, 20 µL/well (5 mg/mL in PBS) was added for 3 h at 37 °C. After incubation for 3 h, after removal of the supernatant, formazan crystals were solubilized with 100 µL of DMSO, and the cell metabolic activity was determined by measuring the formazan absorbance at 492 nm with a automatic plate reader. Inhibition (%) of cell proliferation was determined as follows: inhibitory growth = (1- $Abs_{492 treated\ cells}/Abs_{492 control\ cells}$) x 100 [2]. The $IC_{50}$ value corresponds to the concentration of test compound that caused a decrease of 50% in viability cellular of drug-treated cells compared with untreated cells.

### Cell cycle analysis

T24 cells were plated in 12-well plates and incubated at 37 °C for 24 h. Cells were then treated with different concentrations of compounds **6i**, **6j**, **6k**, **6l** at 40, 40, 40 and 25 µM, respectively, for 48 h. After treatment, cells were harvested (1x$10^5$/mL) and washed with PBS. Subsequently, cells were centrifuged and fixed in 70% ethanol at 4 °C overnight, which were resuspended in PBS containing 100 mL Guava Cell Cycle Reagent, according to the manufacturer's description. Cellular DNA content for cell cycle distribution assay was performed on the flow cytometry Muse Cell Analyser (Millipore Corporation) and results were analyzed using Prism 6.0 software.

### *In vivo* toxicity

Male mice were randomly divided into 4 groups (n = 4 per group) and treated by the subcutaneous route at the dose of 100 µmol/Kg with compounds **6a**, **6e**, and **6i** or DMSO 1 mL/Kg (control group). Clinical signs and symptoms such as death, weight loss, dehydration, piloerection, and bent posture were observed during the treatment period (7 days). After 7 days (168 hours), mice had locomotor and exploratory activities evaluated in the open-field test 57 cm height x 44 cm width x 43 cm length. Subsequently, mice were euthanized and blood was collected by exsanguination to perform toxicity tests. Blood was centrifuged at 3000 rpm for 15 minutes to obtain serum for biochemical dosages.

### Open-field test

Mice were placed individually in the center of the open-field apparatus, which is a box with walls 30 cm high and floor 40 cm long by 40 cm wide, divided into nine quadrants and observed for four minutes to verify the locomotor activity (number of crossing segments with all four legs) and exploratory (expressed by the number of surveys on the hind paws-rearings). This test was performed four minutes before euthanasia.

### Biochemical dosages

The dosage of renal (urea and creatinine levels) and hepatic biomarkers (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities) were performed using commercial kits (Bioclin Quibasa, Belo Horizonte, Minas Gerais).

### Statistical analysis

All results were expressed as mean ± SEM. The results of antioxidant assays and *in vivo* toxicity were analyzed using one or two-way ANOVA followed by Tukey post-hoc test when appropriated. The percent survival was analyzed by

the Log-rank test (Mantel-Cox) Data were considered statistically different when $p \leq 0.05$. The $IC_{50}$ values for antitumoral effect compounds were determined by non-linear regression analysis in the Prism 6.0 software (GraphPad, San Diego,CA, USA).

**Others Toxicity Assays**

Other toxicity parameters analyzed were the open-field test that is used to evaluate the effects of a new environment on the emotional reactions of animals (*i.e.*, new environments generate natural reactions of fear) and to determine the general locomotor activity of mice and rats.[15] The analyses of the number of crossing (Figure SI31) and rearing (Figure SI31) indicated that 5'-arylchalcogenyl-3'-N-(E)-feruloyl-3'-amino-3'-deoxythymidine derivatives **6a**, **6e**, and **6i** did not alter mice locomotion and exploration, respectively.

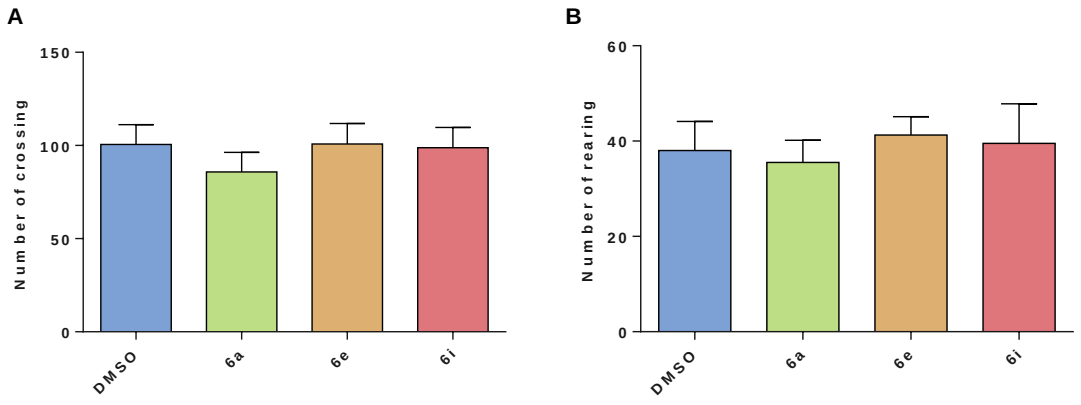



**Figure SI31.** Effect of 5'-arylchalcogenyl-3'-N-(E)-feruloyl-3'-amino-3'-deoxythymidine derivatives **6a**, **6e**, and **6i** (100 µmol/Kg, subcutaneously) treatment on the number of crossing (A) and rearing (B) obtained in the open-field behavioral test. Data were analyzed by one-way ANOVA followed by Tukey *posthoc* test. No significant differences were obtained ($p > 0.05$).

The biomarkers of liver and kidney damage were assessed (Figure SI32). Regarding liver function, serum levels of alanine aminotransferase (ALT) (Figure SI32A) and aspartate aminotransferase (AST) (Figure SI32B) did not change after AFAT's **6a**, **6e**, and **6i** treatments indicating the absence of damage in the

liver of animals. Besides, serum markers of renal damage, urea (Figure SI32C) and creatinine (Figure SI32D) were not altered in the treated mice. Thus, it can be inferred that **6a**, **6e** and **6i** compounds (100 µmol/Kg) did not cause renal damage in the mice.

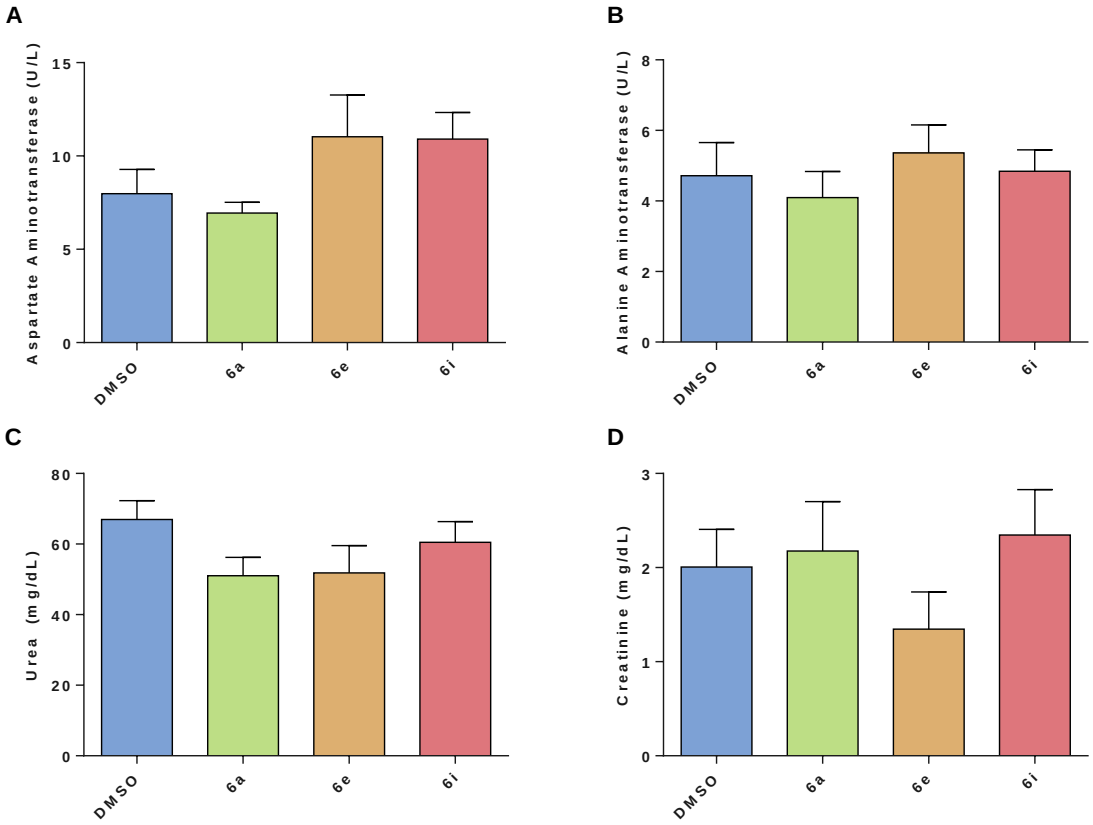



**Figure SI32.** Effect of 5'-arylchalcogenyl-3'-N-(E)-feruloyl-3'-amino-3'-deoxythymidine derivatives **6a**, **6e**, and **6i** treated mice on AST (A), ALT (B), urea (C) and creatinine (D) levels. Data were analyzed by one-way ANOVA followed by Tukey *posthoc* test. No significant differences were obtained ($p > 0.05$).

## References

[1] DA ROSA, R. M. et. al. Synthesis, Antioxidant and Antitumoral Activities of 5'-ArylChalcogeno-3-AminoThymidine (ACAT) derivatives. **Med. Chem. Commun.**, p. 408-414, 2017.

[2] – PEREIRA, R. P.; BOLIGON, A. A.; APPEL, A. S.; FACHINETTO, R.; CERON, C. S.; TANUS-SANTOS, J. E.; ATHAYDE, M. L.; ROCHA, J. B. T. Chemical composition, antioxidant and anticholinesterase activity of Melissa officinalis. Industrial Crops and Products, v. 53, p. 34-45, 2014.

[3] – OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, v. 95, p. 351−358, 1979.

[4] – MOSMANN T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 55-63. https://doi.org/10.1016/0022-1759(83)90303-4.