Supporting Information

Aspartic Acid Based Phosphoramidate Prodrugs as Potent Inhibitors of Hepatitis C Virus Replication

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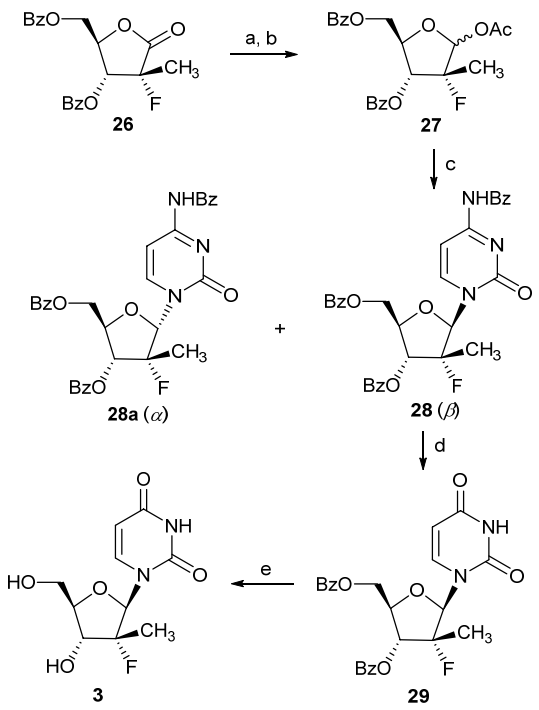


Scheme S1 Synthesis of fluorinated nucleoside **3**. **Reagents and conditions:** a) $Li(O\text{-}tBu)_3AlH$, THF, -20 °C; b) Ac_2O, DMAP, -20 °C; c) N^4-benzoylcytosine, *N*,*O*-bis(trimethylsilyl)acetamide, $SnCl_4$, PhCl, 65 °C, 16 h; d) 75% aqueous acetic acid, 110 °C, 5 h; e) ~7 N NH_3 in MeOH, rt, 30 h.

1-*O*-Acetyl-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methyl-α,β-D-ribofuranose (27). Protected lactone **26** (2 g, 5.4 mmol) was dissolved in dry tetrahydrofuran (45 mL) under nitrogen atmosphere and the solution was cooled to -20 °C. Lithium tri-*tert*-butoxyaluminium hydride (1.0 M in THF, 6.5 mL, 6.5 mmol) was added dropwise over 20 min while maintaining the temperature near -20 °C. Upon completion of the reaction (~ 3 h) based on TLC, that is the formation of lactol (R_f = 0.36, 2:8 EtOAc/Hexane), DMAP (66 mg, 5.4 mmol) and acetic anhydride (4.7 mL, 49.4 mmol) were added to the reaction mixture at -20 °C and stirred for 1.5 h. The reaction mixture was diluted with ethyl acetate and water. The organic layer was collected and the aqueous layer was extracted three times with ethyl acetate. The combined organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to obtain crude acetate **27** which was purified by flash column chromatography using 0-15% EtOAc in hexane to obtain the pure product as clear oil in 95% yield. R_f = 0.5 (2:8 EtOAc/Hexane). ^{1}H NMR (500 MHz, DMSO-d6): δ = 8.03-8.01 (m, 4H, Ar-H), 7.99-7.94 (m, 4H, Ar-H), 7.74-7.70

(m, 2H, Ar-H), 7.68-7.65 (m, 2H, Ar-H), 7.59-7.56 (m, 4H, Ar-H), 7.52-7.49 (m, 4H, Ar-H), 6.19 (d, 1H, J = 4.33 Hz, H-1a), 6.09 (d, 1H, J = 9.65 Hz, H-1b), 5.62 (dd, J = 7.97, 24.50 Hz, 1H, H-3a), 5.62 (dd, J = 6.25, 8.59 Hz, 1H, H-3b), 4.75-4.72 (m, 1H, H-4a), 4.67-4.61 (m, 3H, H-4b & H-5a), 4.57-4.41 (m, 2H, H-5b), 2.14 (s, 3H, OAc-a), 1.92 (s, 3H, OAc-b), 1.62 (d, 3H, J = 22.96 Hz, CH_3-a), 1.50 (d, 3H, J = 23.37 Hz, CH_3-b); ^{13}C NMR (125 MHz, DMSO-d6): δ = 168.9, 168.3 (CO of -OAc), 165.0, 164.8, 164.7, 164.5 (CO of Bz), 133.6, 133.5, 133.2 (Ar-C), 129.2-128.1 (Ar-C), 100.2, 97.4, 95.3, 93.7 (C-1a, C-1b, C-2a & C-2b), 79.0, 77.9 (C-4a, C-4b), 73.3, 73.2, 72.7, 72.6 (C-3a, C-3b), 63.0, 62.6 (C-5a, C-5b), 20.4-20.0 (CH_3), 15.8, 15.6 (-CH_3); HRMS (ESI+) calcd for $C_{22}H_{21}F_1O_7Na$ $[M+Na]^+$ 439.1164, found 439.1160.

3′,5′-di-*O*-benzoyl-2′-deoxy-2′-fluoro-2′-*C*-methyl-*N*^{4}-benzoyl-cytidine (28). To a suspension of *N*^{4}-benzoylcytosine (1.74 g, 8.0 mmol) in anhydrous chlorobenzene (24 mL), *N*,*O*-bis(trimethylsilyl)acetamide (4.5 mL, 18 mmol) was added and the suspension was heated to 80 °C for 2 h. To the clear resultant solution was then cooled to room temperature. A solution of acetate sugar **27** (1.68 g, 4.0 mmol) in chlorobenzene (12 mL) was then added to the silylated base. To this, neat tin (IV) chloride (2.4 mL, 20 mmol) was added dropwise and was heated to 65 °C for 16 h. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. Cold saturated sodium bicarbonate solution was added and the white suspension was then filtered through a celite pad. The organic layer was separated and the aqueous layer was extracted with ethyl acetate several times. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to obtain the crude product (a mixture of *α* (**28a**) and *β* (**28**) isomer) which was purified by flash column chromatography eluting with 20-40% EtOAc in hexane to obtain the pure *β*-isomer (**28**) in 26% yield. R_f = 0.34 for β-isomer (1:1 EtOAc/Hexane) and R_f = 0.2 for α-isomer (1:1 EtOAc/Hexane). *β* isomer (**28**): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.70 (br s, 1H, NH), 8.10-8.06 (m, 5H, Ar-H), 7.89 (d, J = 7.03 Hz, 2H), 7.69-7.61 (m, 3H, Ar-H), 7.55-7.46 (m, 7H, Ar-H), 6.19 (br d, 1H, J = 16.59 Hz, H-1′), 5.55 (br dd, J = 8.6, 20.7 Hz, 1H, H-3′), 4.88 (dd, J = 2.4, 12.7 Hz, 1H, H-5′), 4.72 (m, 1H, H-4′), 4.63 (dd, J = 3.27, 12.7 Hz, 1H, H-5″) , 1.48 (d, 3H, J = 22.4 Hz, -CH_3); HRMS (ESI+) calcd for $C_{31}H_{27}F_1N_3O_7$ $[M+H]^+$ 572.1827, found 572.1832.

3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-fluoro-2′-*C*-methyl-uridine (29). A suspension of compound **28** (0.58 g, 1.0 mmol) in 75% aqueous acetic acid (30 mL) was heated to 110 °C for 5 h. The clear solution was cooled to room temperature and concentrated to dryness under reduced pressure and coevaporated with methanol/water (1:1) for three times to remove traces of acetic acid. The compound **29** was used as such without further purification for the next step. Yield: 90%, R_f = 0.45 (EtOAc/Hexane , 1:1). ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ = 8.05-7.96 (m, 4H, Ar-H), 7.61-7.40 (m, 7H, Ar-H & H-6), 6.22 (d, 1H, *J* = 19.05 Hz, H-1′), 5.51 (dd, *J* = 9.47, 21.2 Hz, 1H, H-3′), 5.42 (d, 1H, *J* = 8.11 Hz, H-5), 4.84 (dd, *J* = 2.65, 12.7 Hz, 1H, H-5′), 4.60 (m, 1H, H-4′), 4.49 (dd, *J* = 3.45, 12.7 Hz, 1H, H-5″) , 1.42 (d, 3H, *J* = 22.4 Hz, -CH_3); HRMS (ESI+) calcd for $C_{24}H_{21}F_1N_2O_7Na$ $[M+Na]^+$ 491.1225, found 491.1229.

2′-deoxy-2′-fluoro-2′-*C*-methyl-uridine (3). NH_3 in methanol (~ 7 N, 30 mL) was added to compound **29** (0.5 g, 1.0 mmol) and was stirred 30 h at room temperature. The reaction mixture was evaporated with silica gel and chromatographed on a flash silica gel column eluting with CH_2Cl_2/MeOH/NH_3 (9.0:1.0:0.2) to obtain compound **3** as white solid (62%). TLC (CH_2Cl_2/MeOH/NH_3, 9.0:1.0:0.2): R_f = 0.23. ^{1}H NMR (600 MHz, CD_3OD): δ = 8.07 (d, *J* = 7.89 Hz, 1H, H-6), 6.12 (d, 1H, *J* = 18.53 Hz, H-1′), 5.71 (d, *J* = 7.89 Hz, 1H, H-5), 4.02-3.79 (m, 4H, H-3′, H-4′, H-5′ & H-5″), 1.35 (d, 3H, *J* = 22.3 Hz, CH_3); ^{13}C NMR (150 MHz, CD_3OD): δ = 165.8 (C-4), 152.3 (C-2), 141.8 (C-6), 102.9 (C-5), 102.0 (d, *J* = 181.0, C-2′), 90.5 (br d, C-1′), 83.3 (C-4′), 72.4 (d, *J* = 18.0, C-3′), 60.0 (C-5′), 16.8 (d, *J* = 25.5, -CH_3). HRMS (ESI+) calcd for $C_{10}H_{13}F_1N_2O_5Na$ $[M+Na]^+$ 283.0701, found 283.0709.

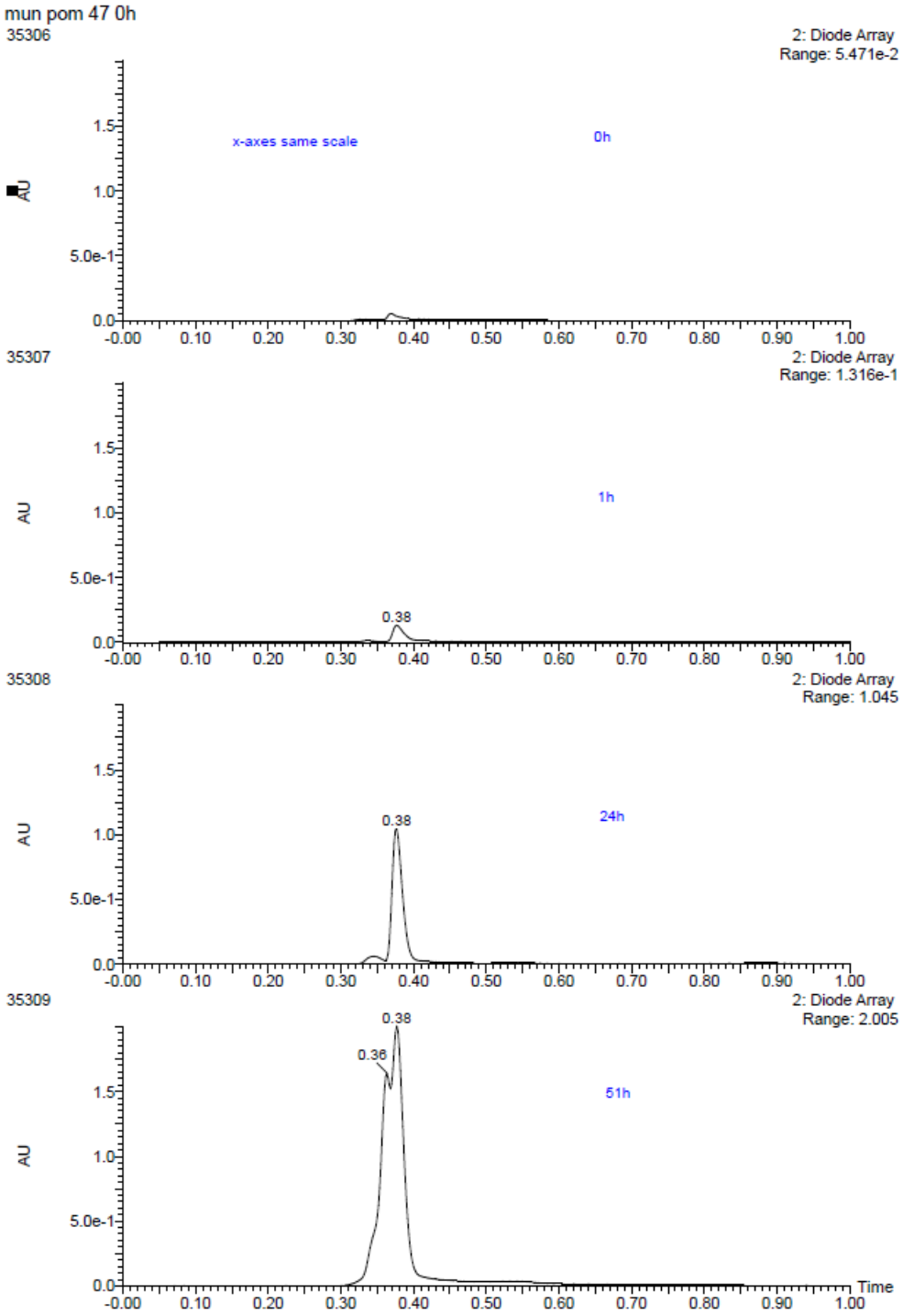


Fig S1 Liquid chromatograms of compound **2c** which is subjected to human liver S9 metabolism study. Retention times 0.38 min and 0.36 min correspond to intermediate and final monophosphate formation, respectively.

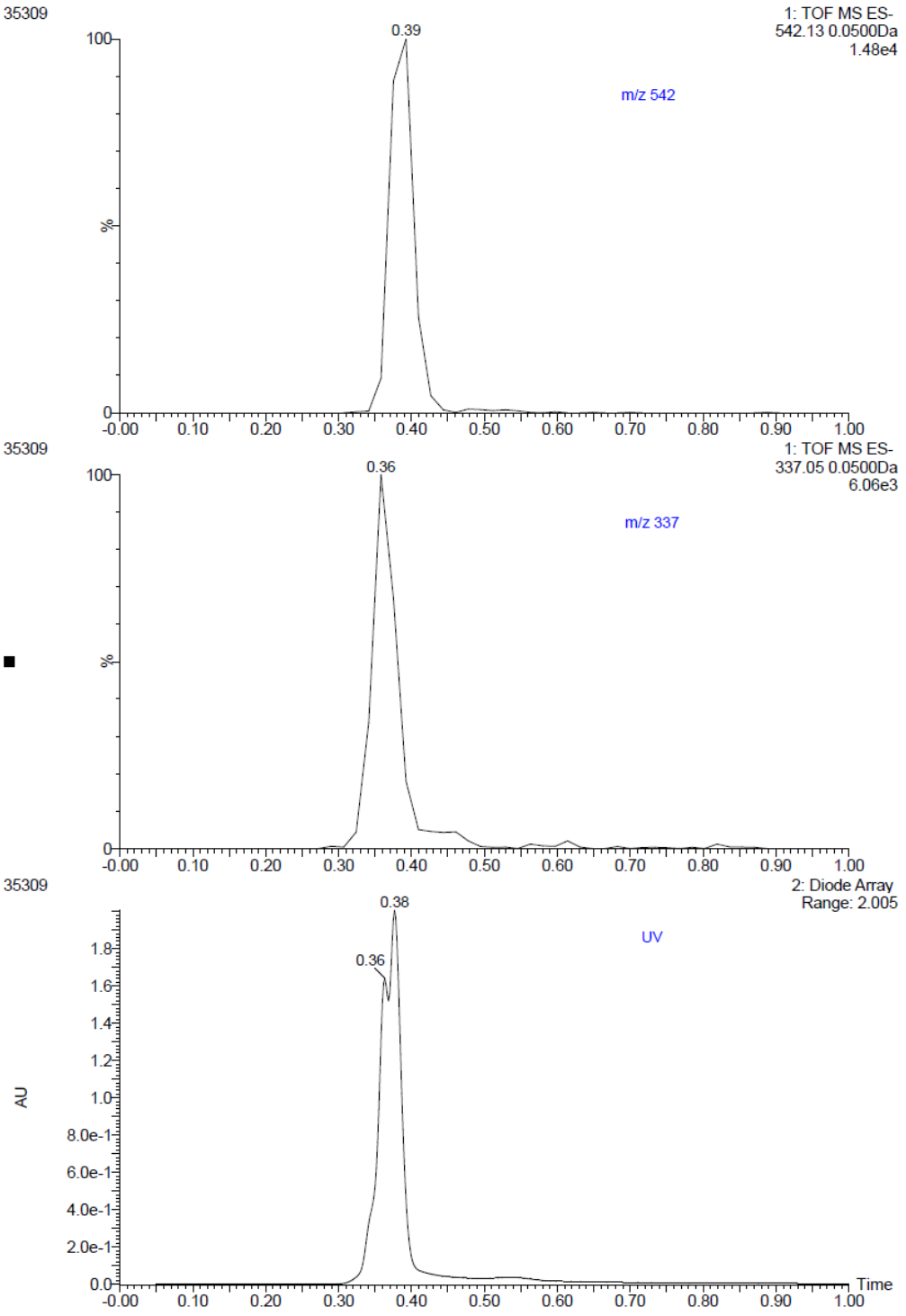
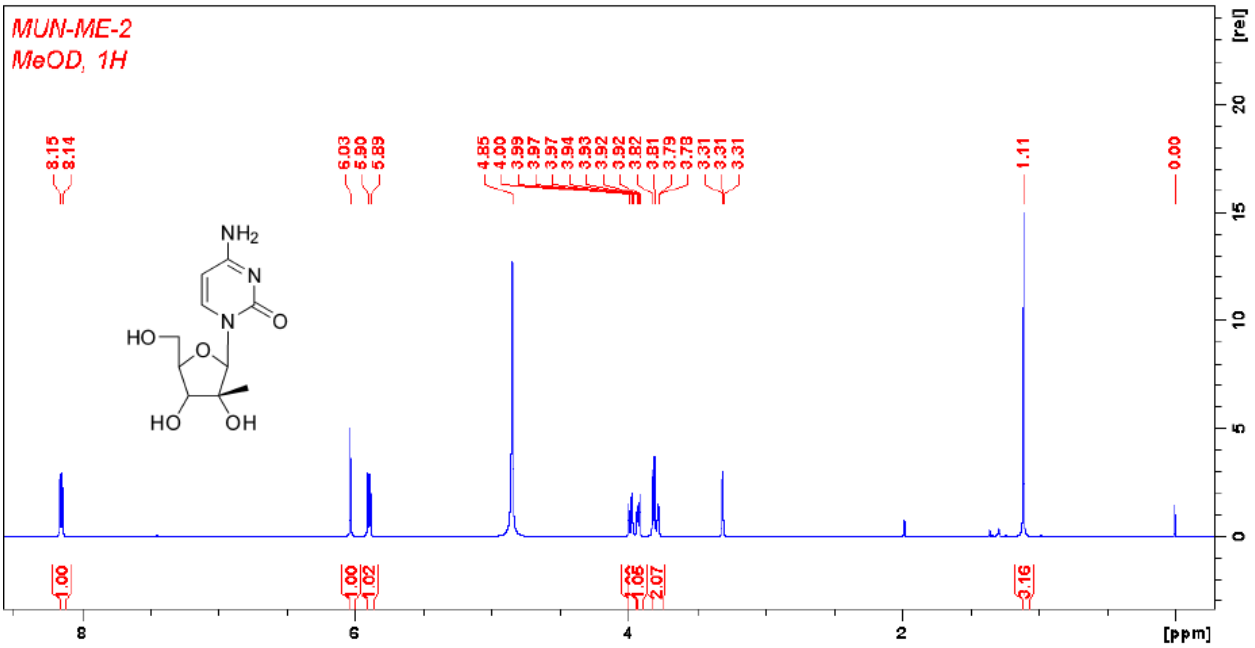


Fig S2 The molecular mass corresponding to the intermediate at retention time 0.38 min (*m*/*z* = 542 in negative mode, top spectrum) and 2′-Me-U-monophosphate at retention time 0.36 min (*m*/*z* = 337 in negative mode, middle spectrum).

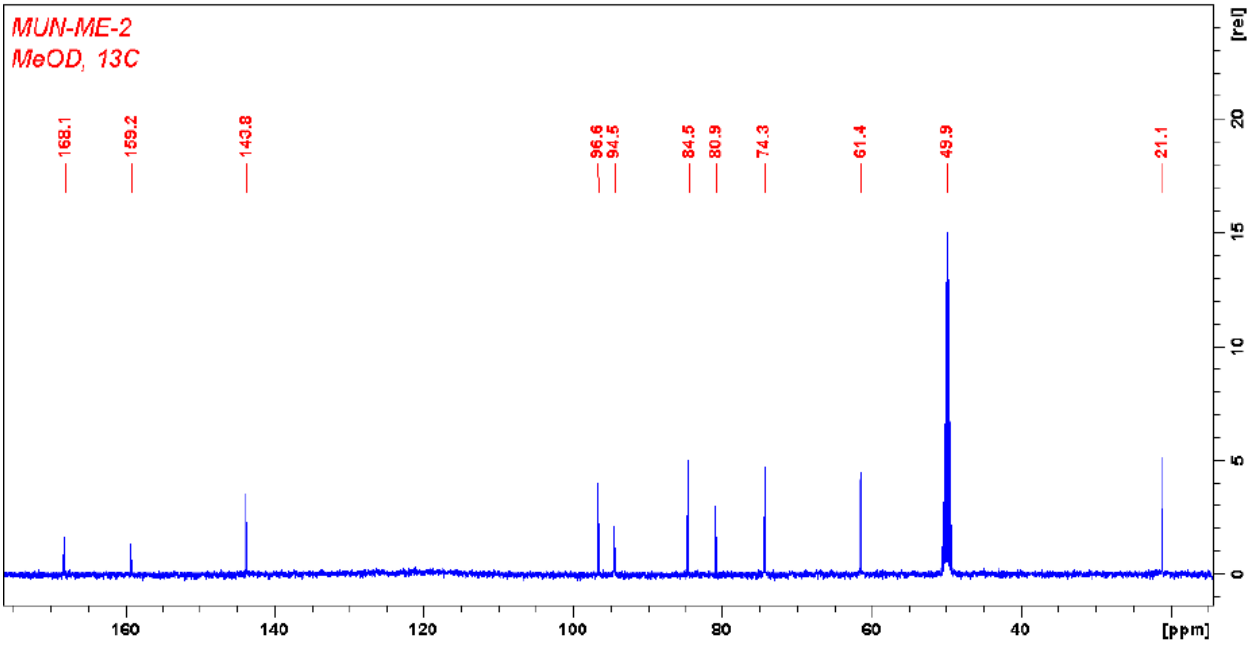
Compound **1**

^{1}H spectrum



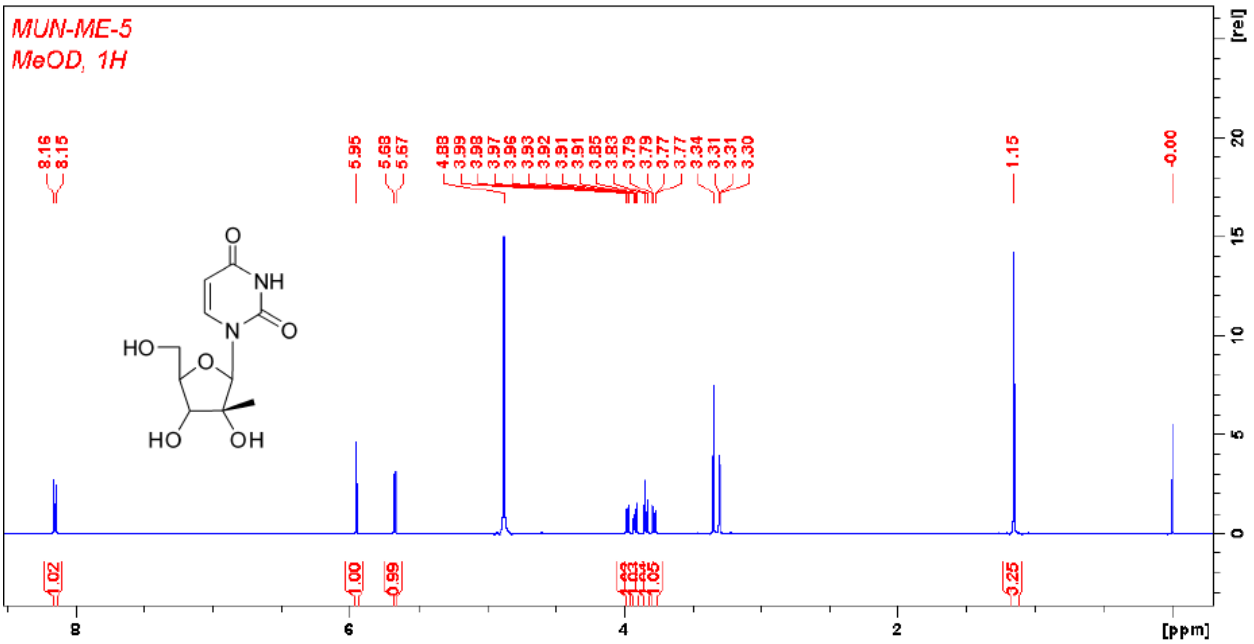
Compound **1**

^{13}C spectrum



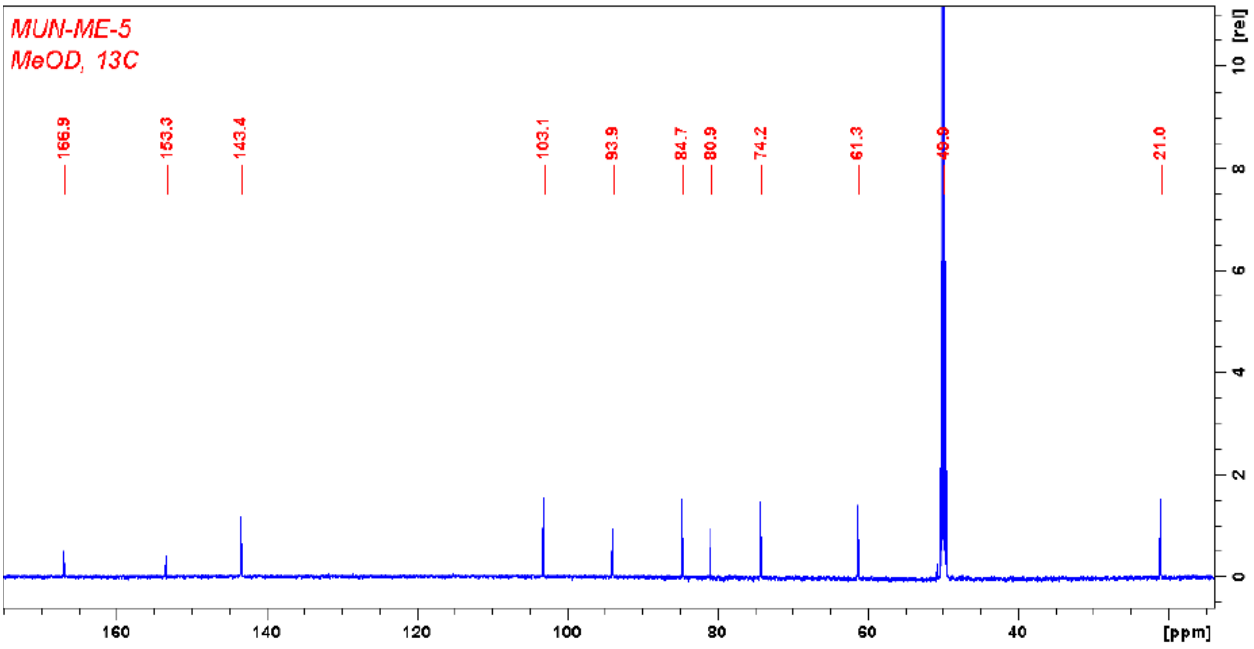
Compound **2**

^{1}H spectrum



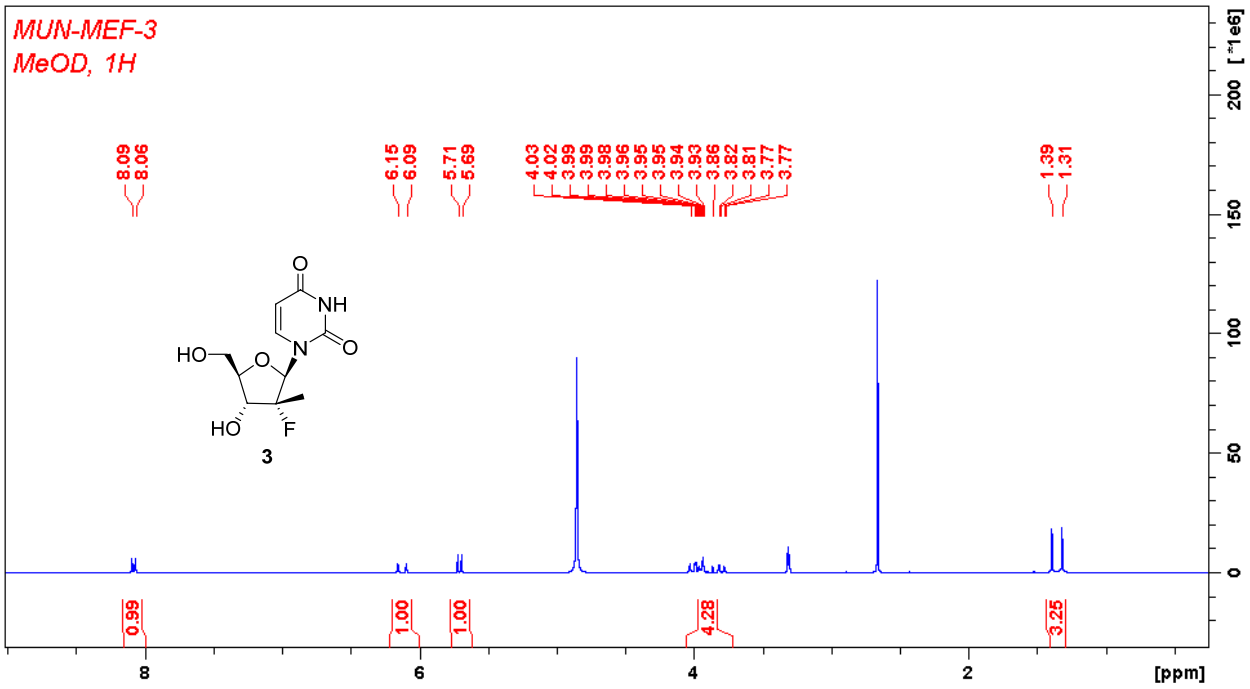
Compound **2**

^{13}C spectrum



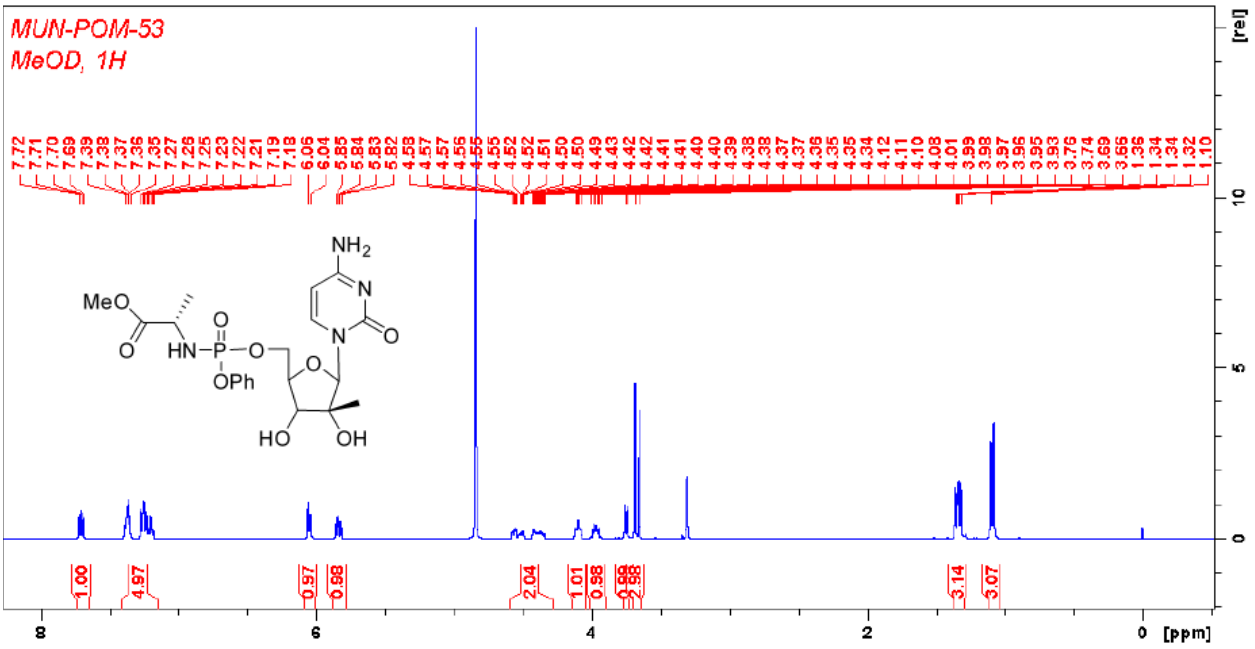
Compound **3**

^{1}H spectrum



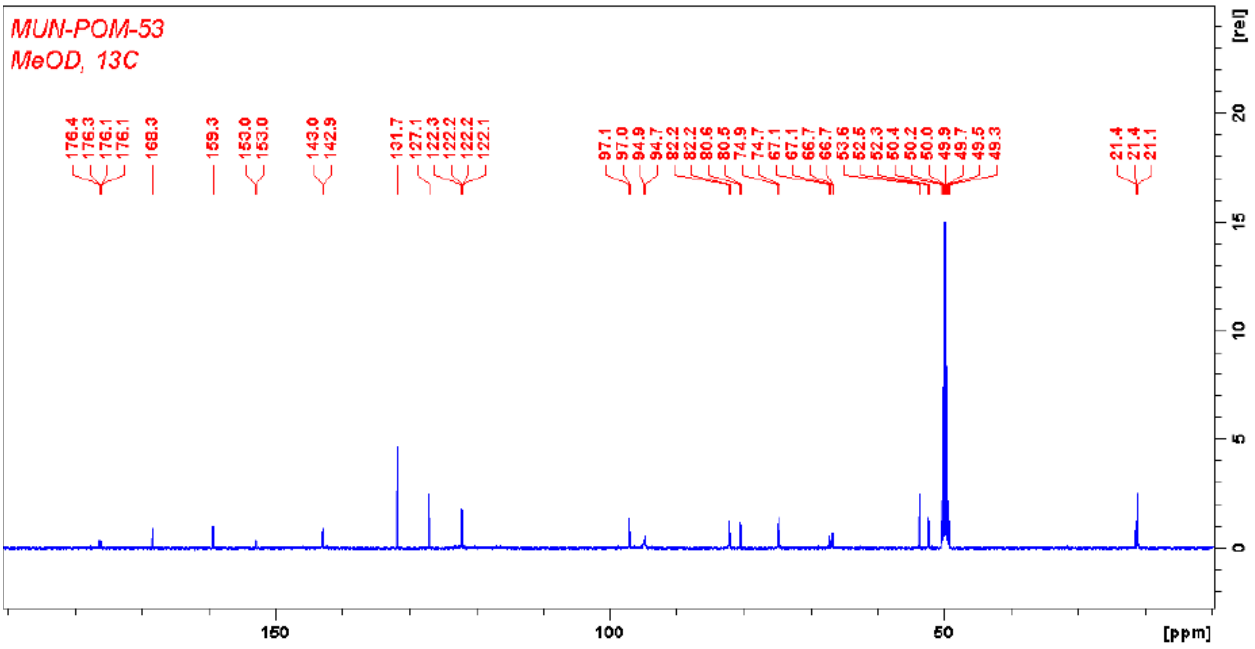
Compound **1a**

^{1}H spectrum



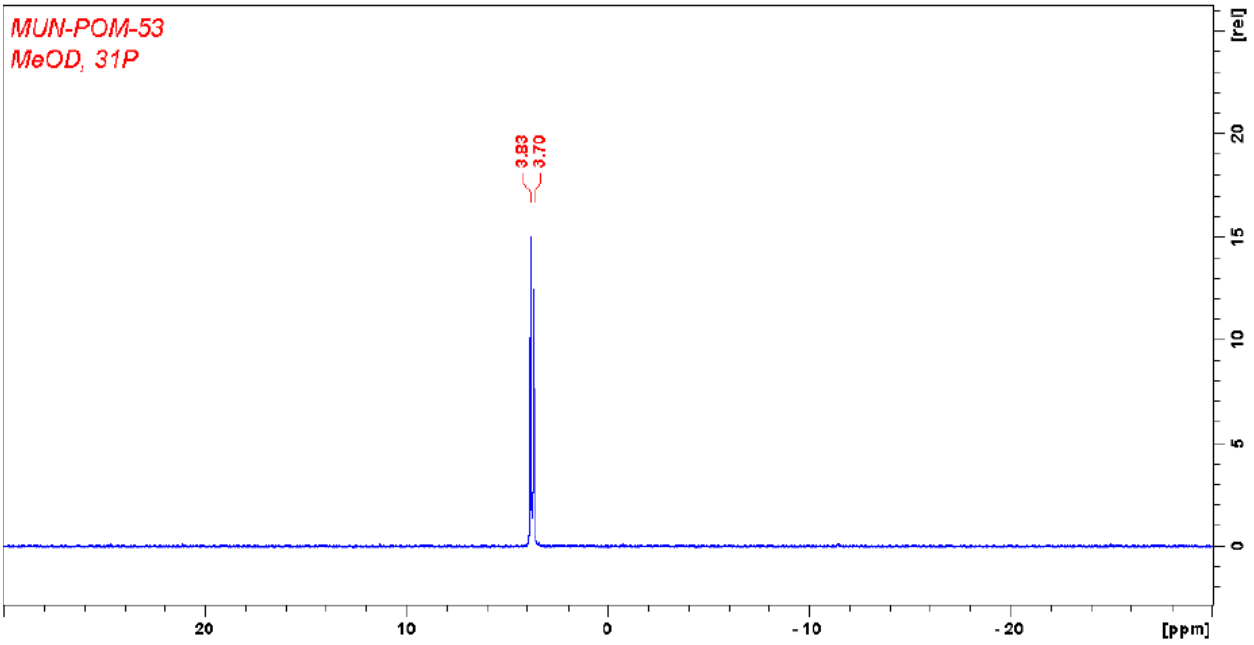
Compound **1a**

^{13}C spectrum



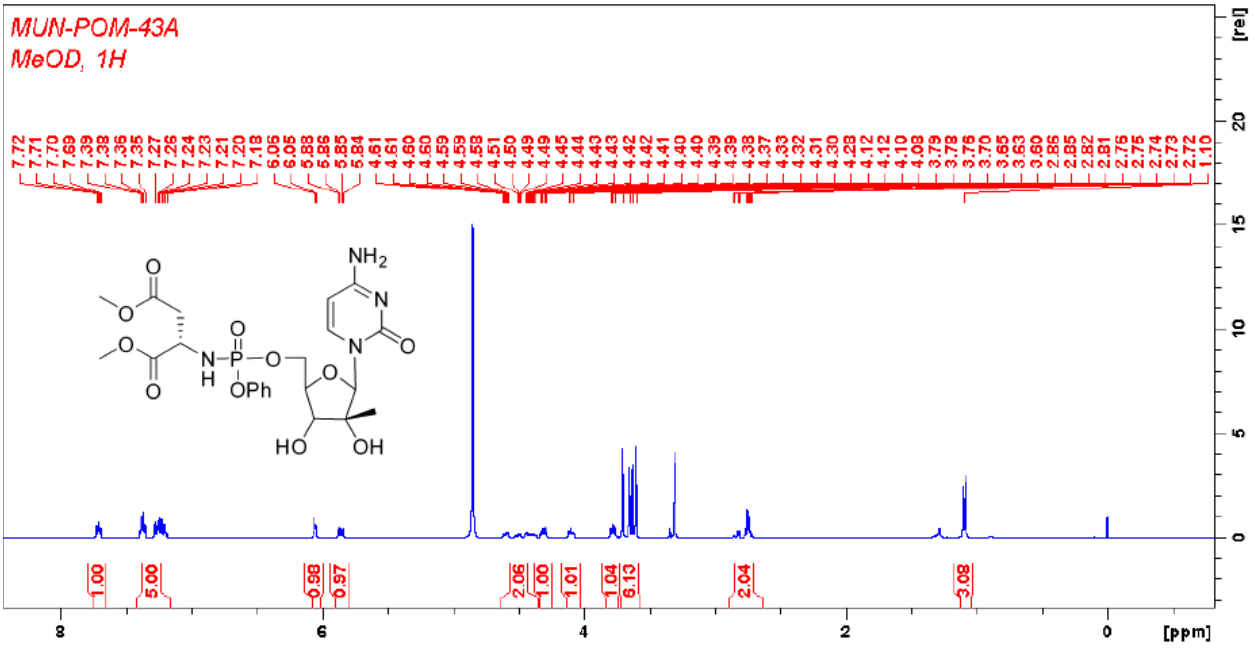
Compound **1a**

^{31}P spectrum



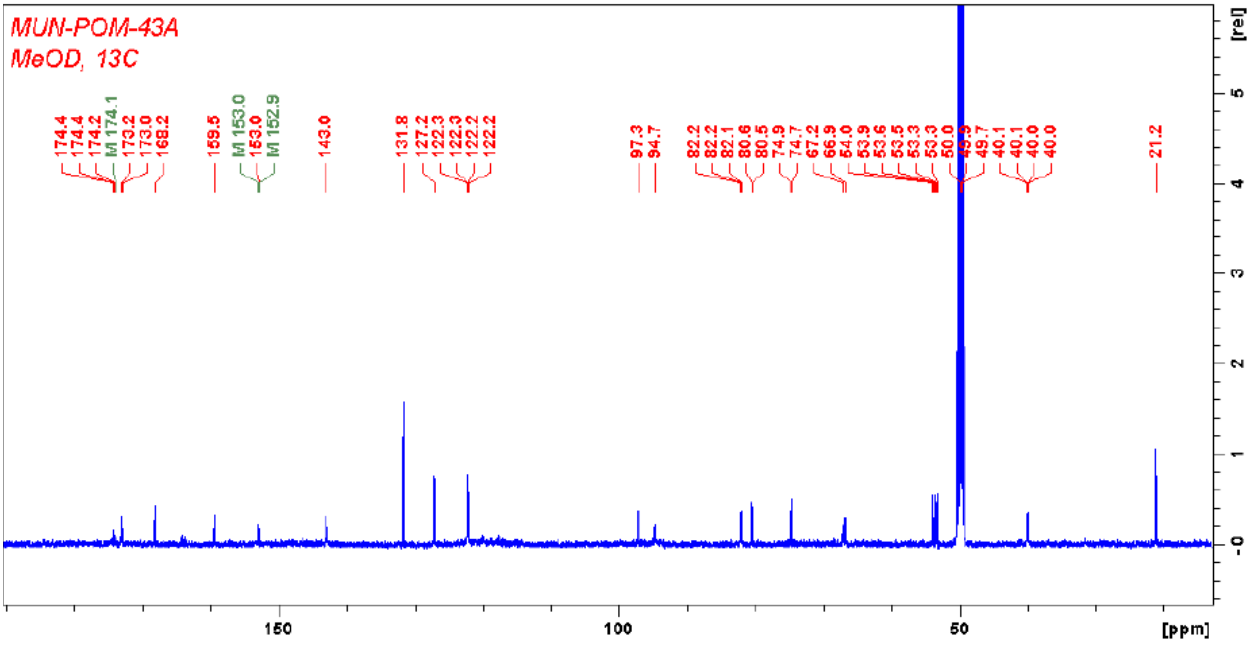
Compound **1b**

^{1}H spectrum



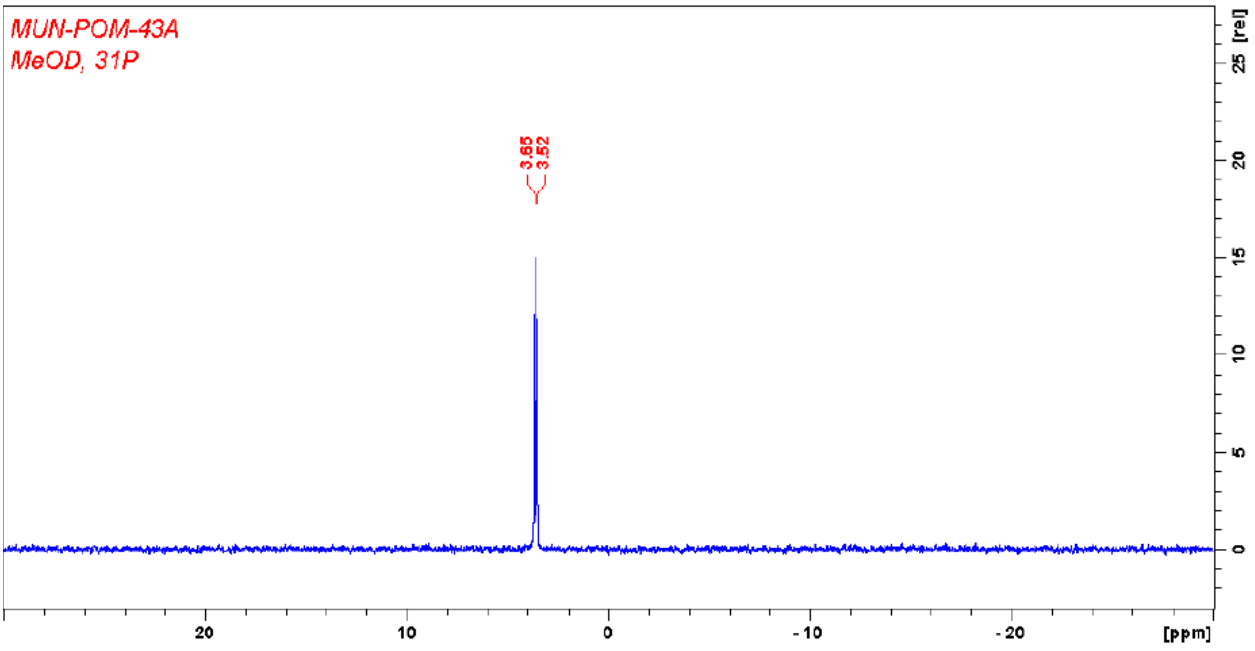
Compound **1b**

^{13}C spectrum



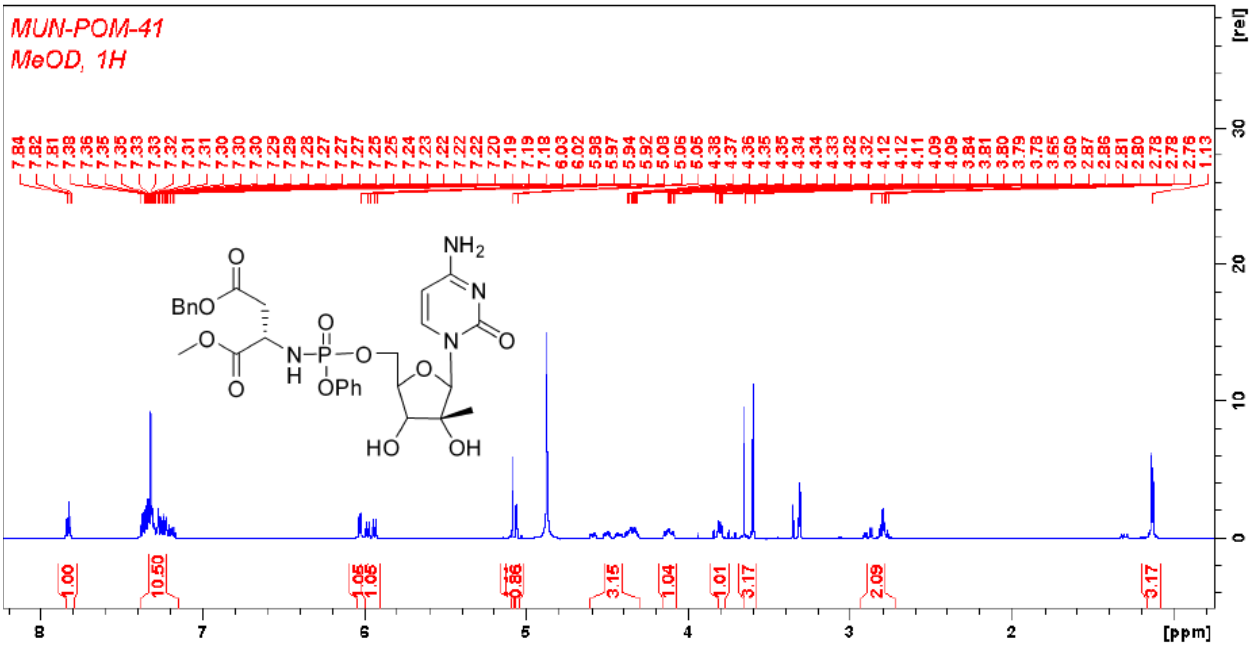
Compound **1b**

^{31}P spectrum



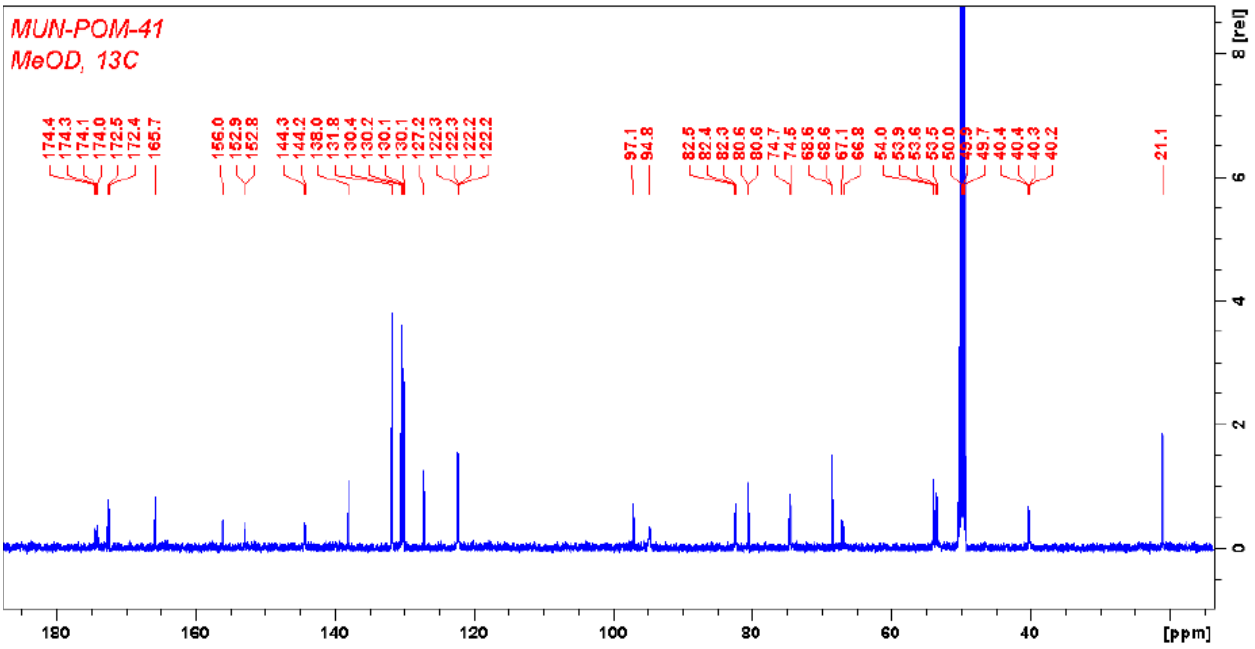
Compound **1c**

^{1}H spectrum



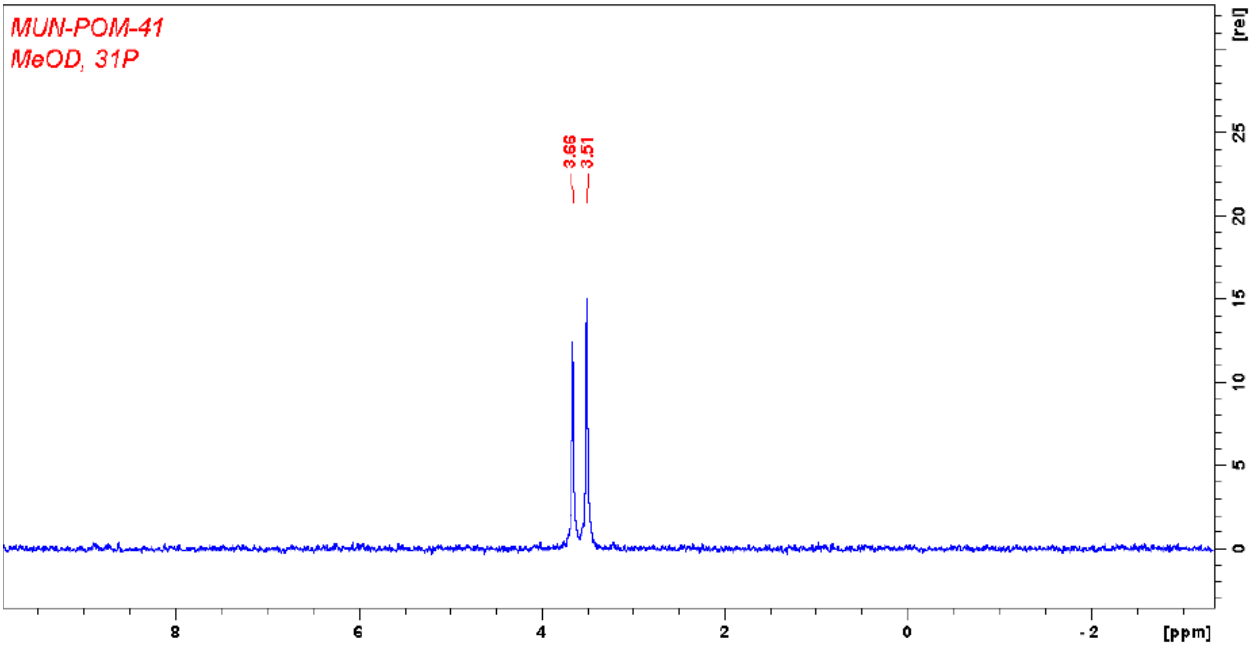
Compound **1c**

^{13}C spectrum



Compound **1c**

^{31}P spectrum



Compound **1d**

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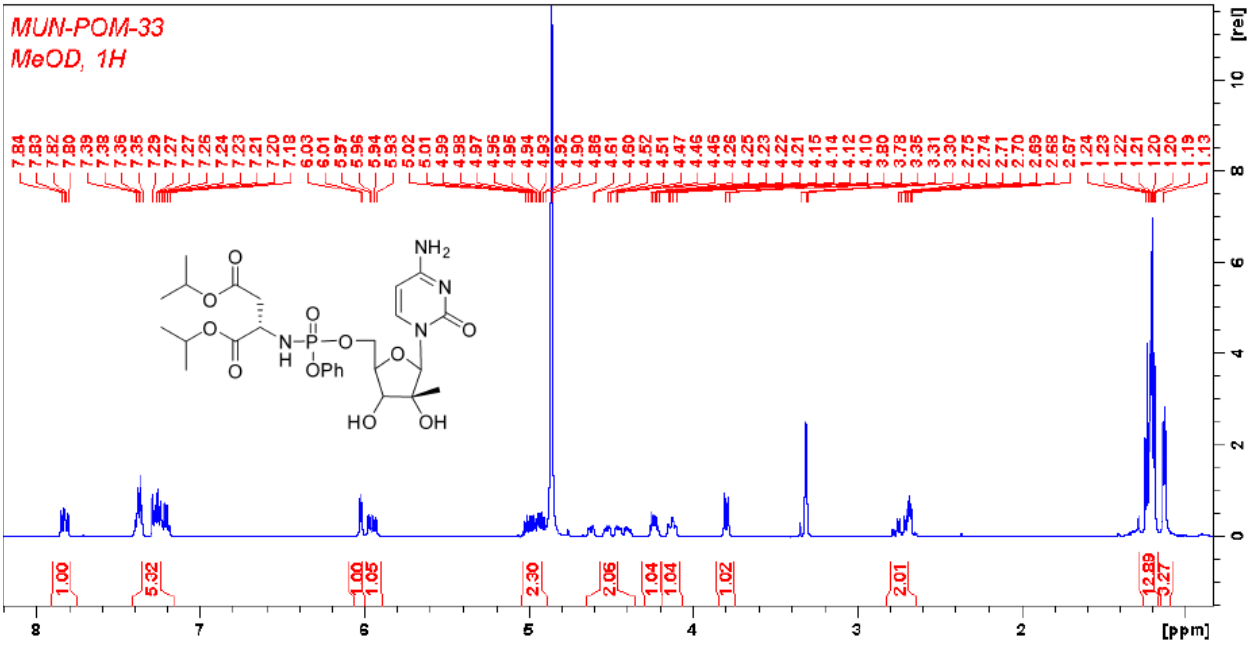
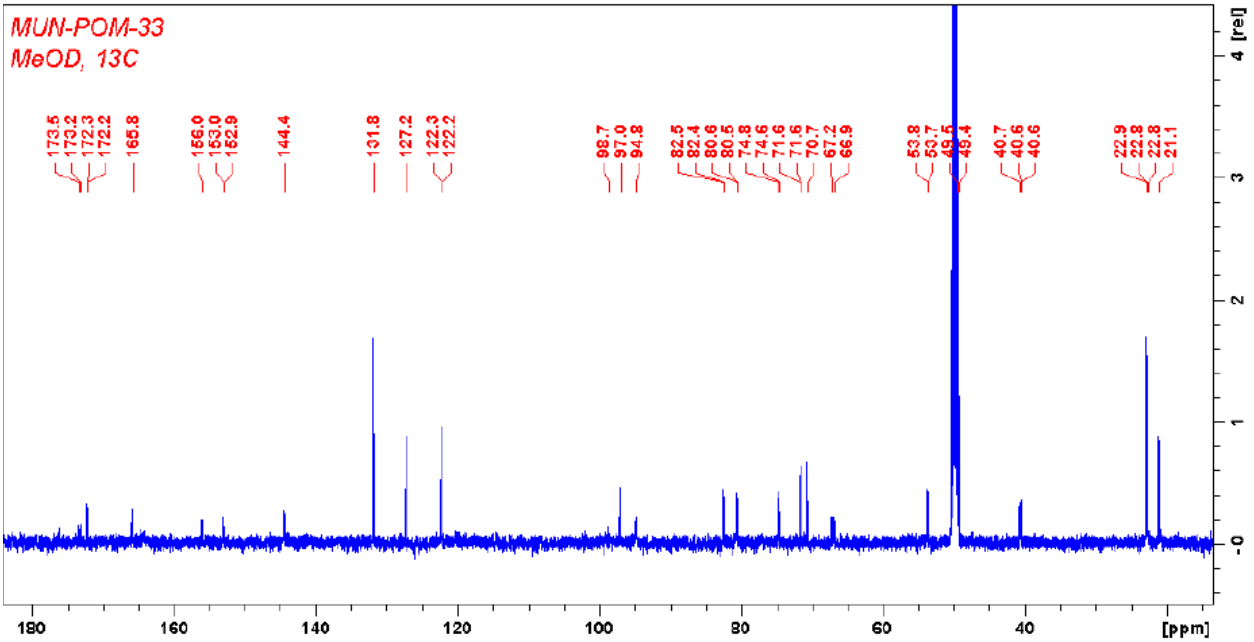


Compound **1d**

^{13}C spectrum



Compound **1d**

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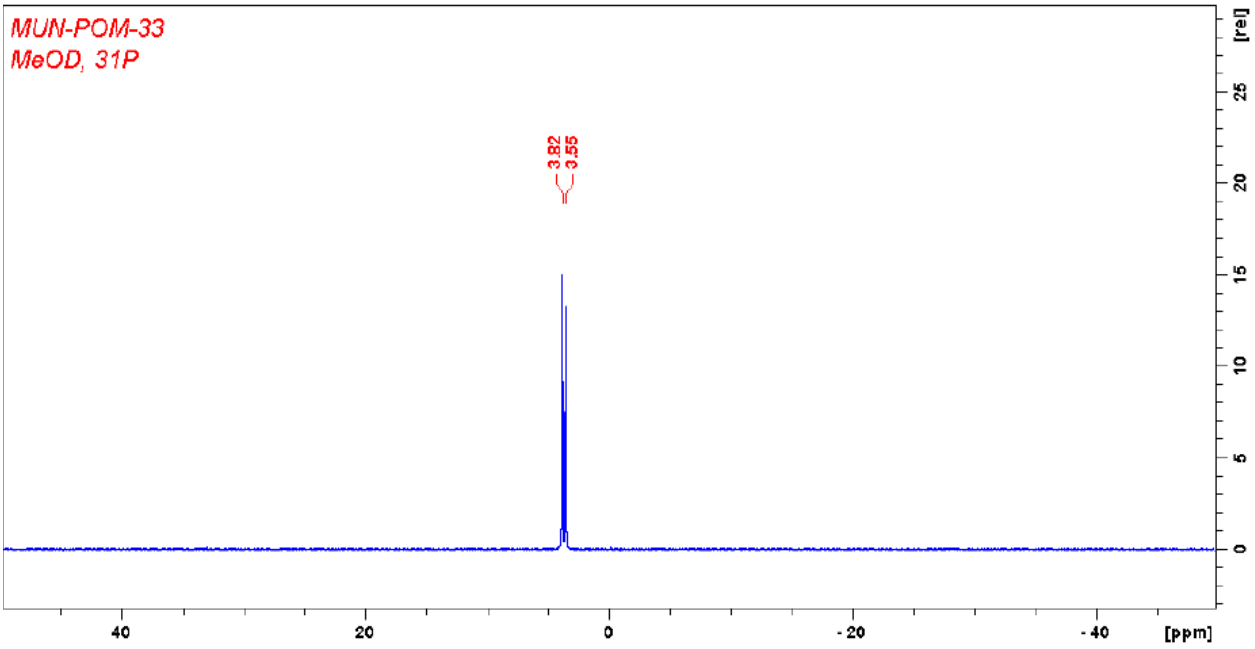
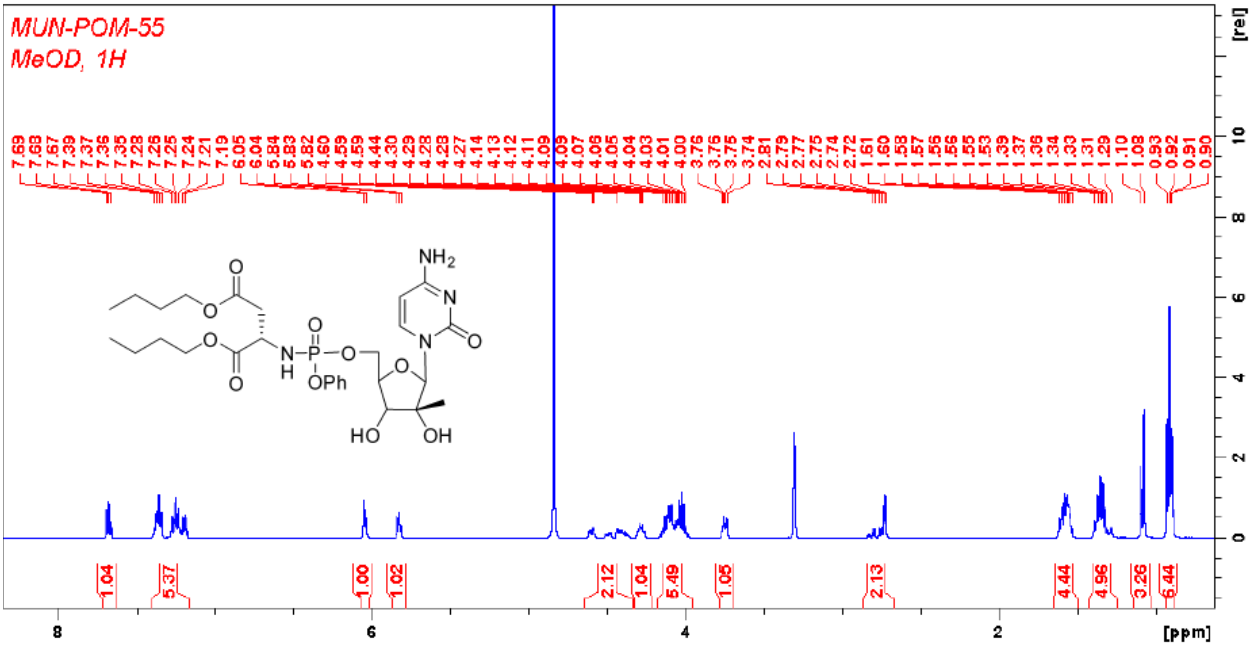


Compound **1e**

^{1}H spectrum



Compound **1e**

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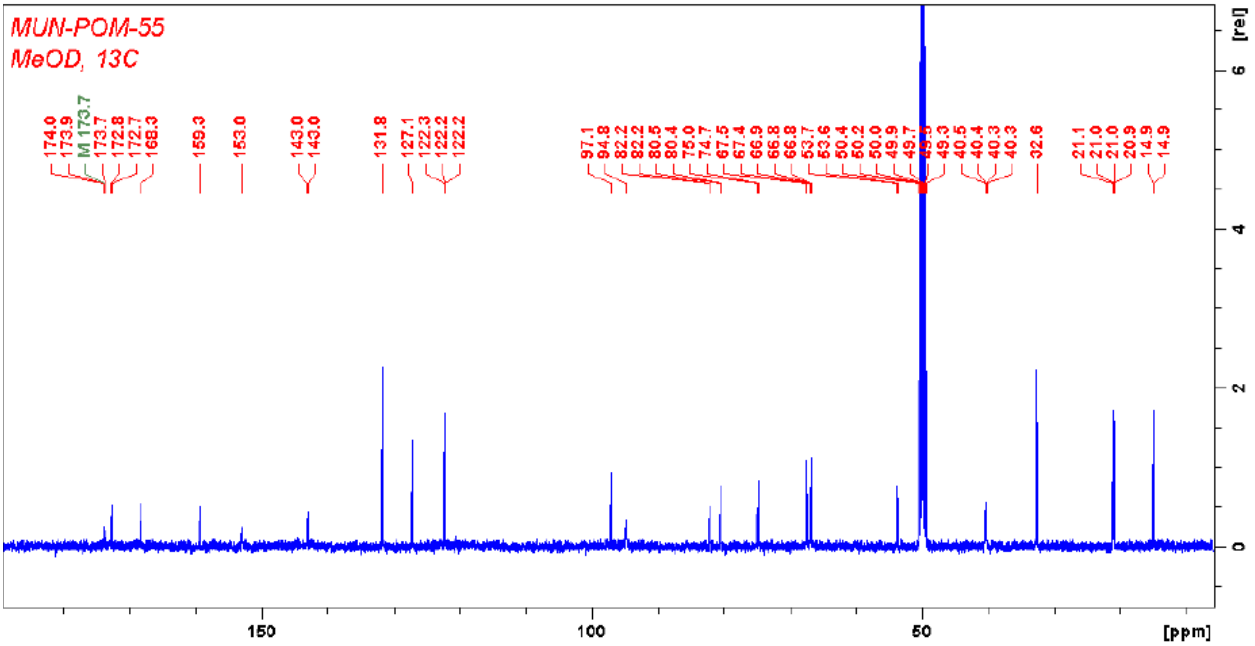

Compound **1e**

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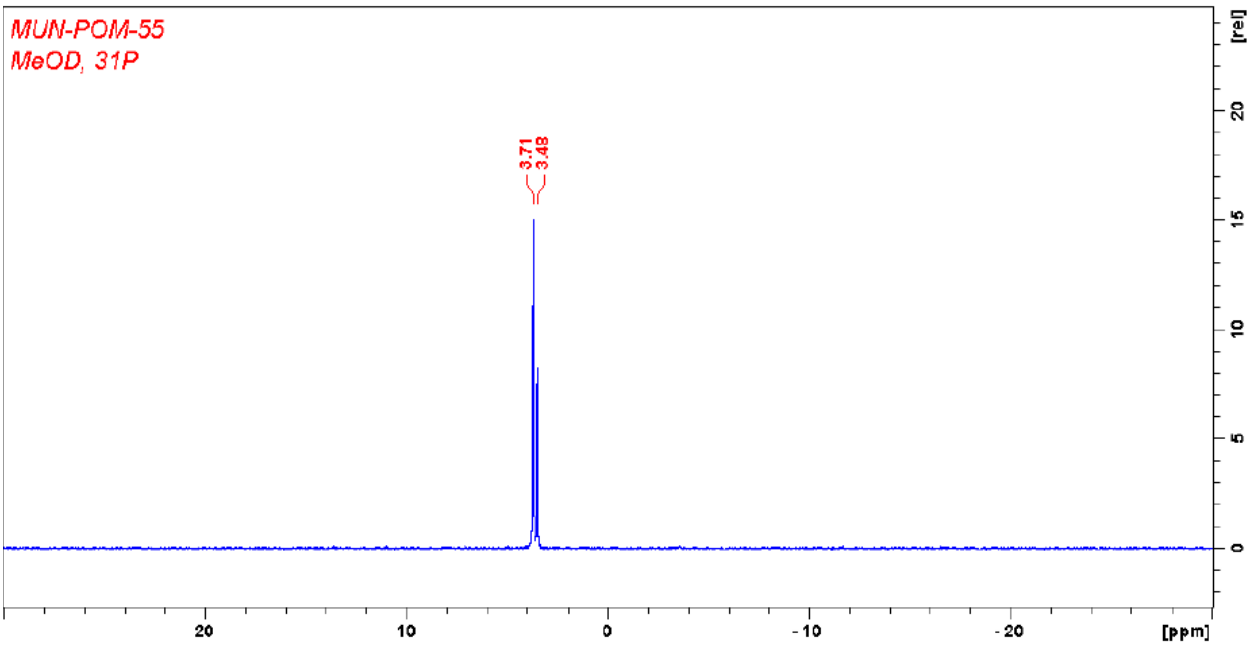
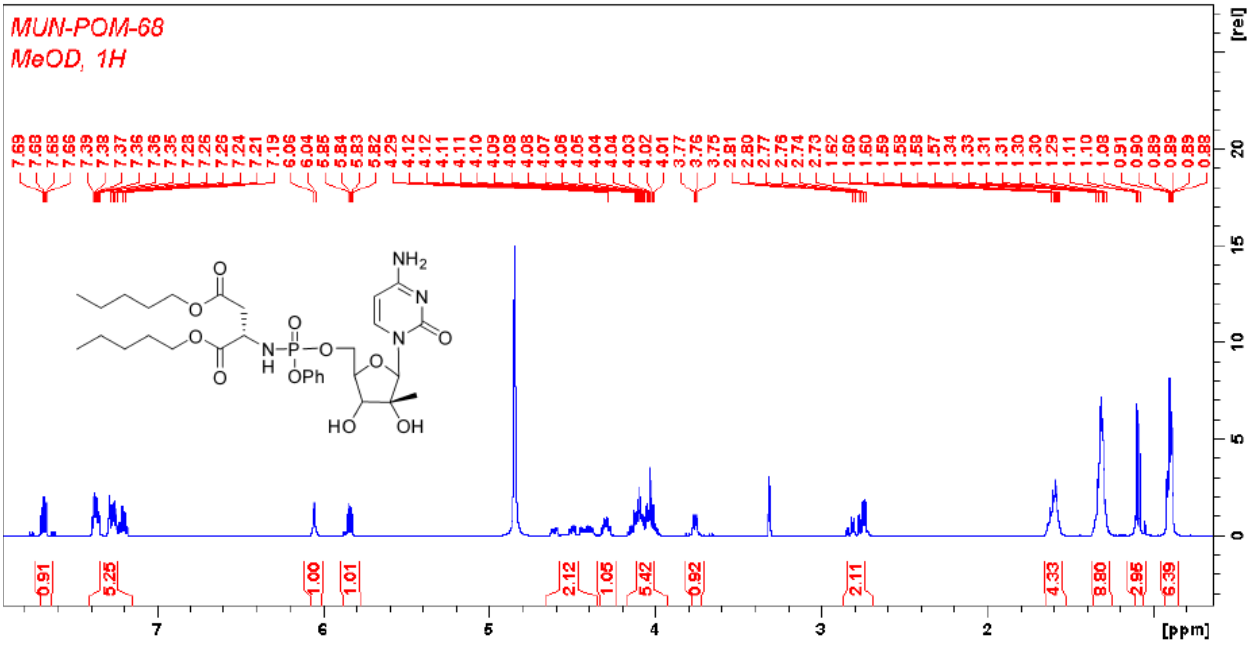


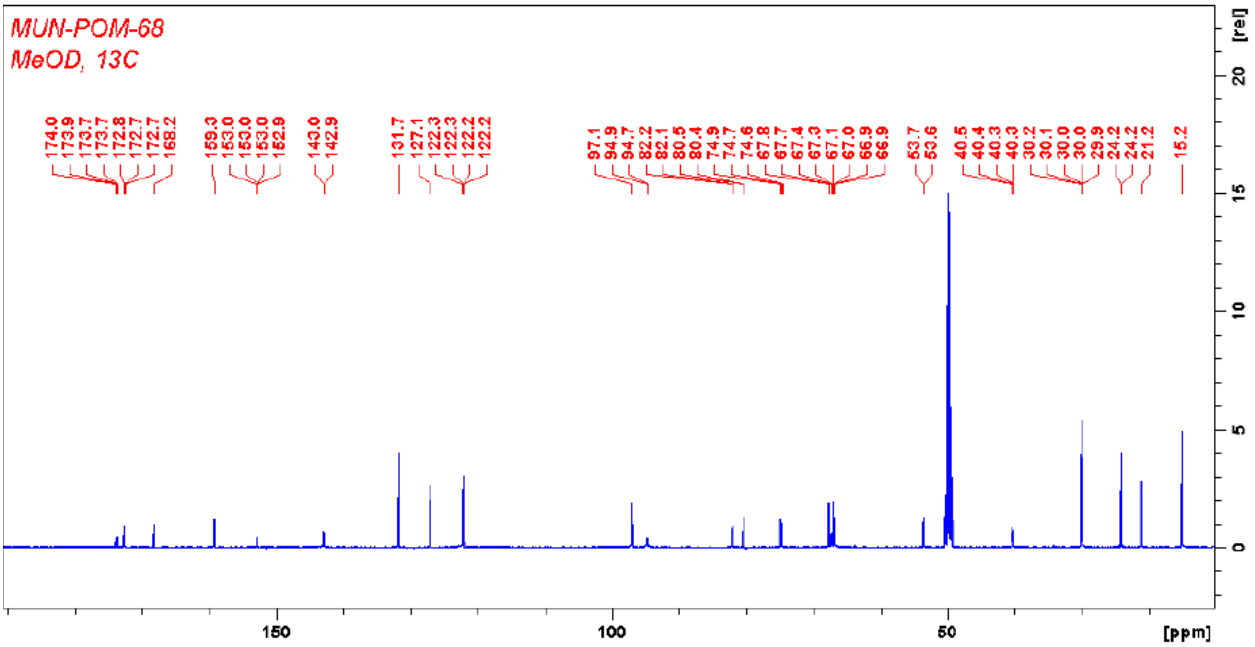
Compound **1f**

^{1}H spectrum



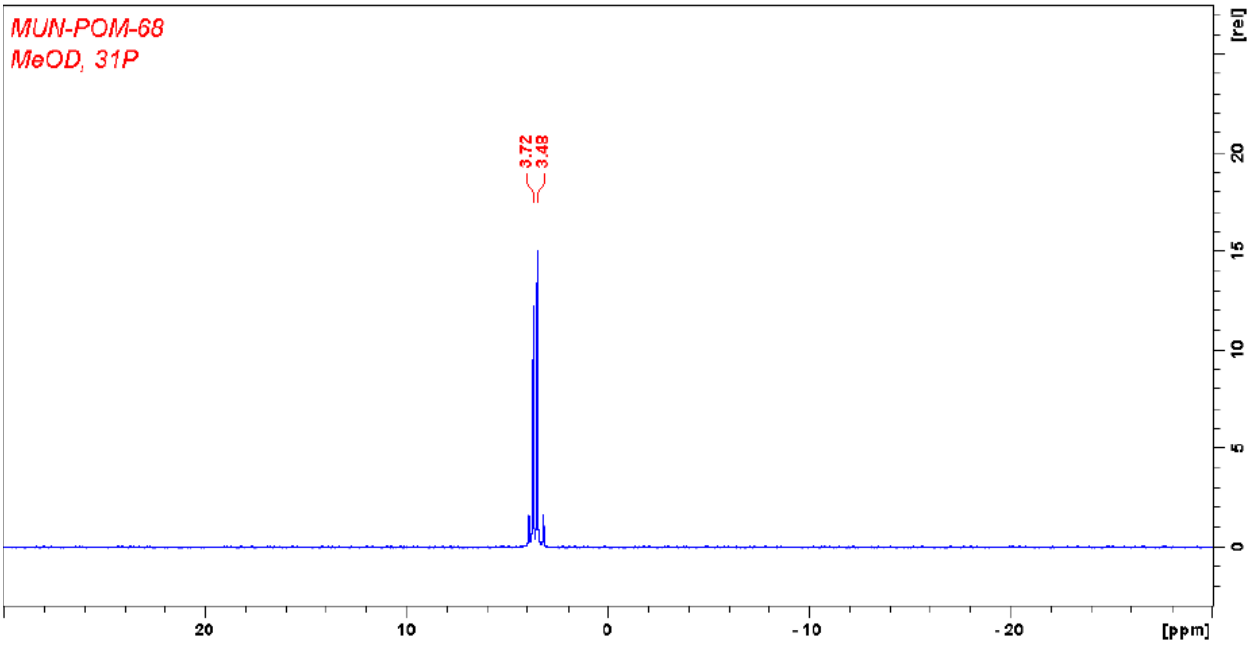
Compound **1f**

^{13}C spectrum



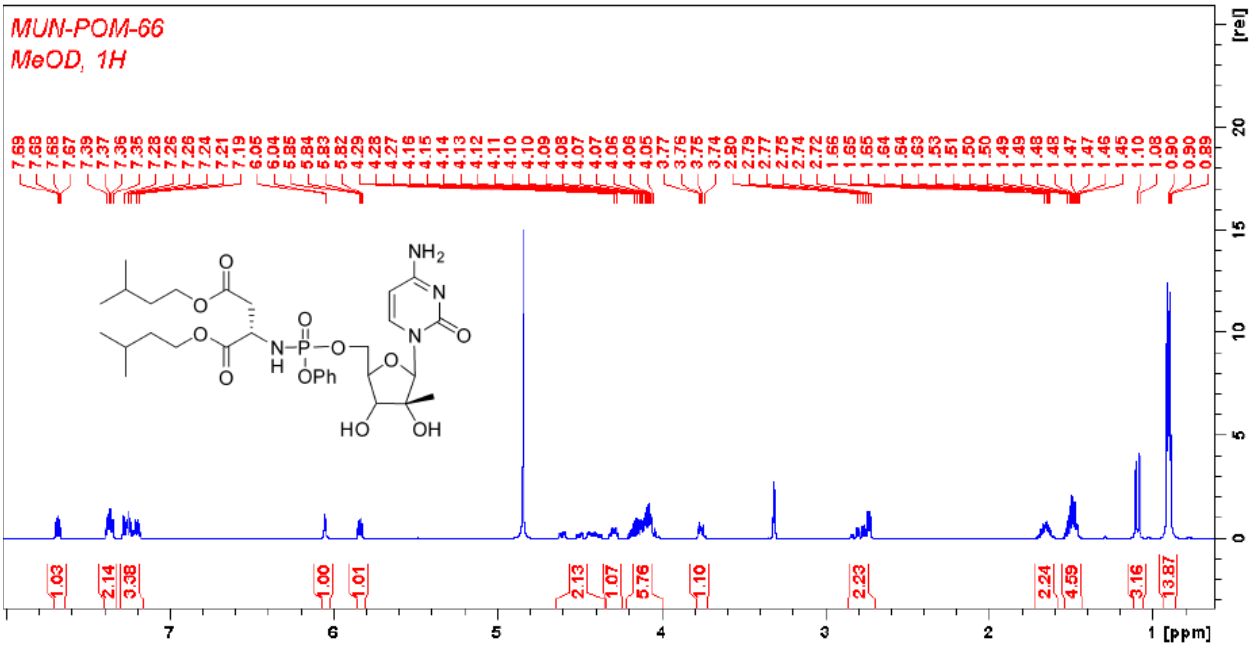
Compound **1f**

^{31}P spectrum



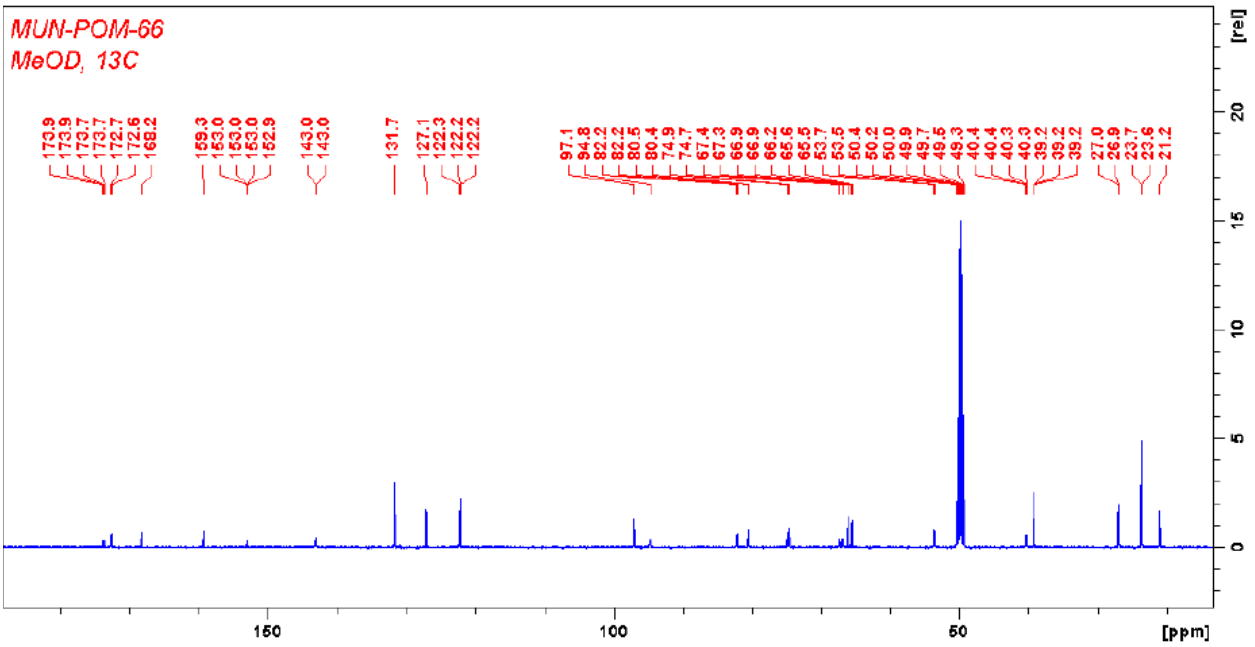
Compound **1g**

^{1}H spectrum



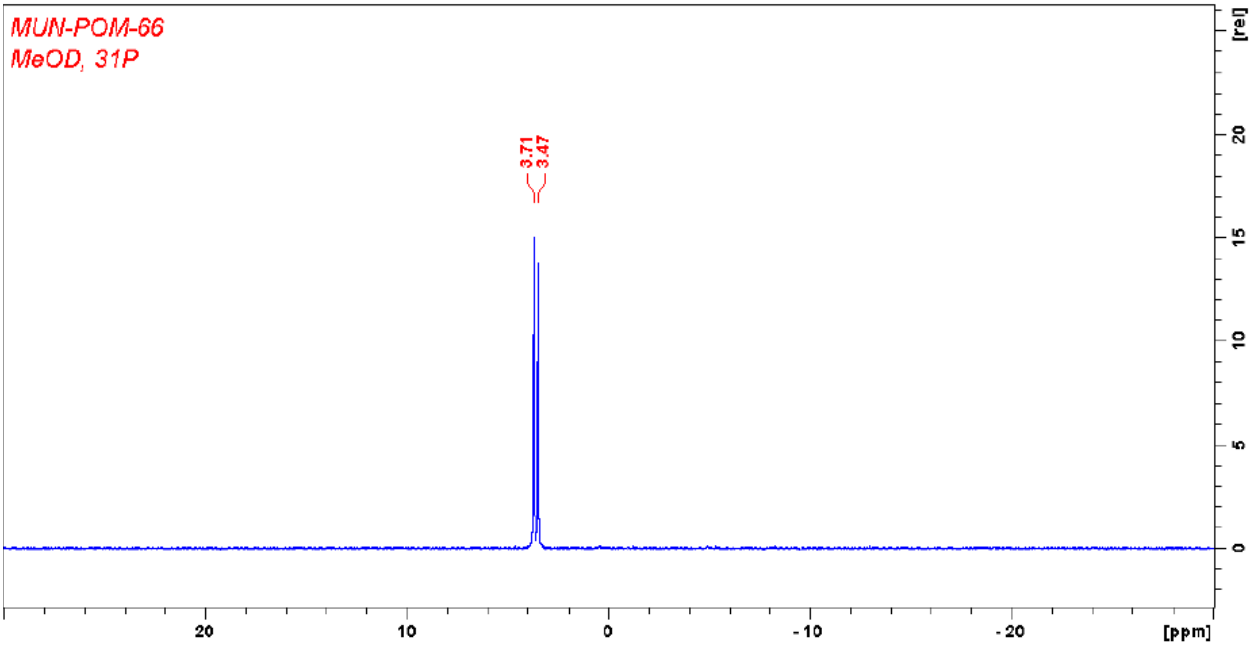
Compound **1g**

^{13}C spectrum



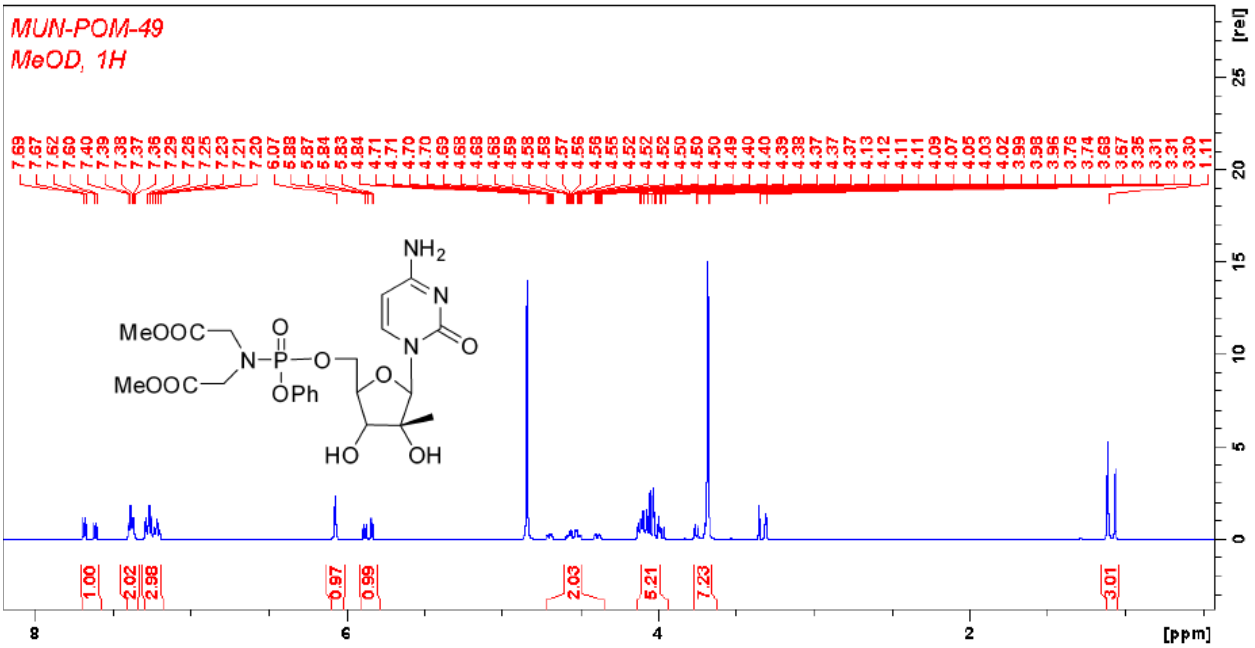
Compound **1g**

^{31}P spectrum



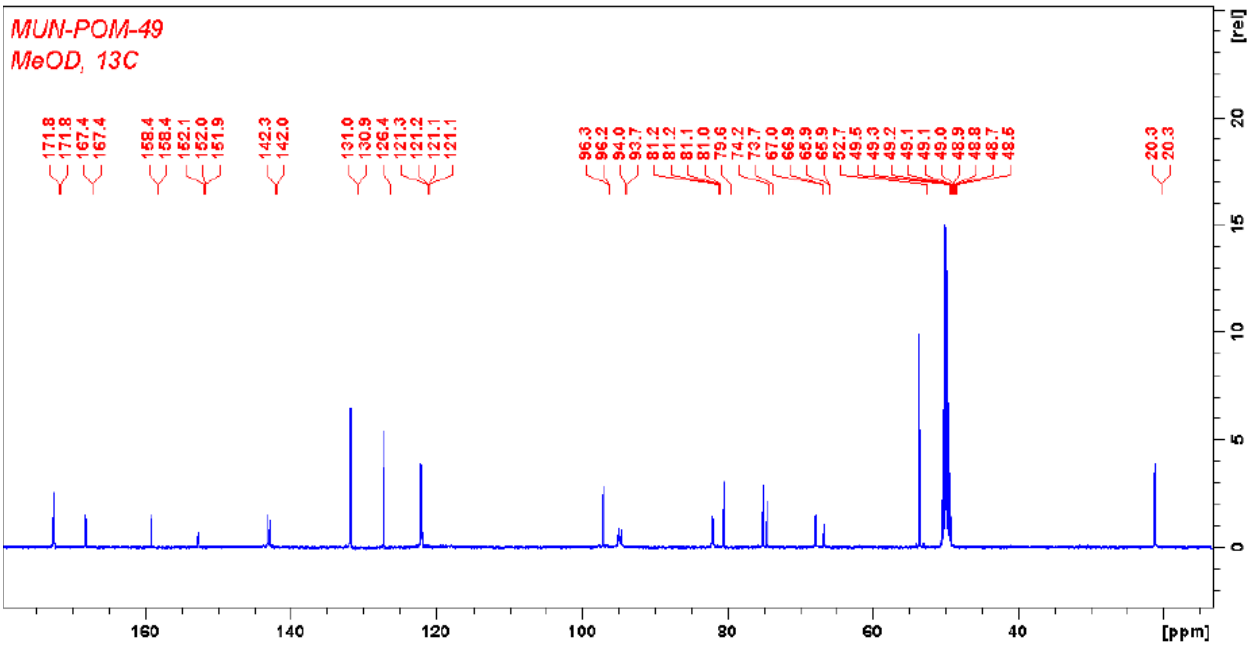
Compound **1h**

^{1}H spectrum



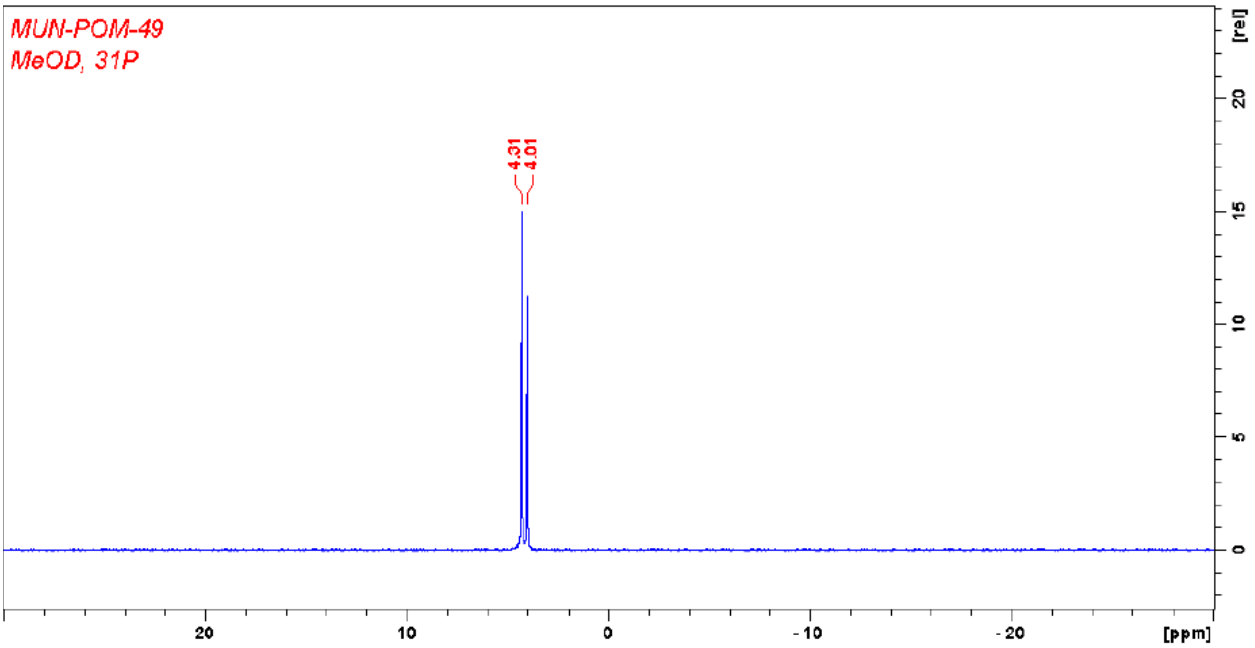
Compound **1h**

^{13}C spectrum



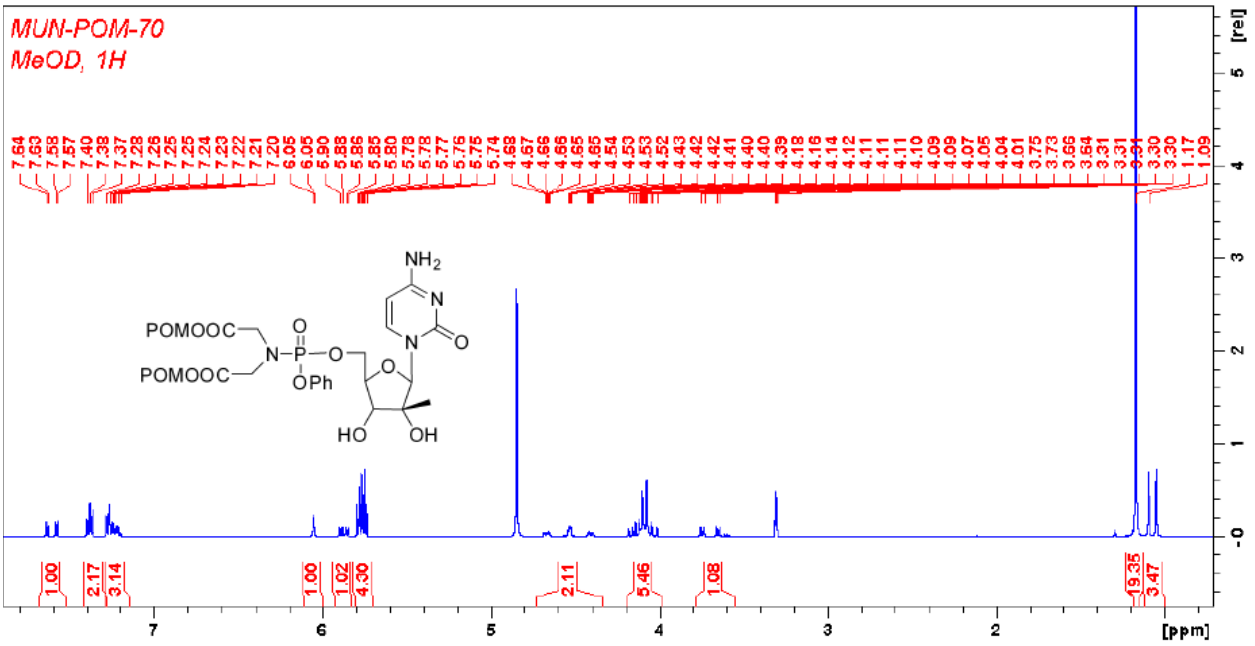
Compound **1h**

^{31}P spectrum



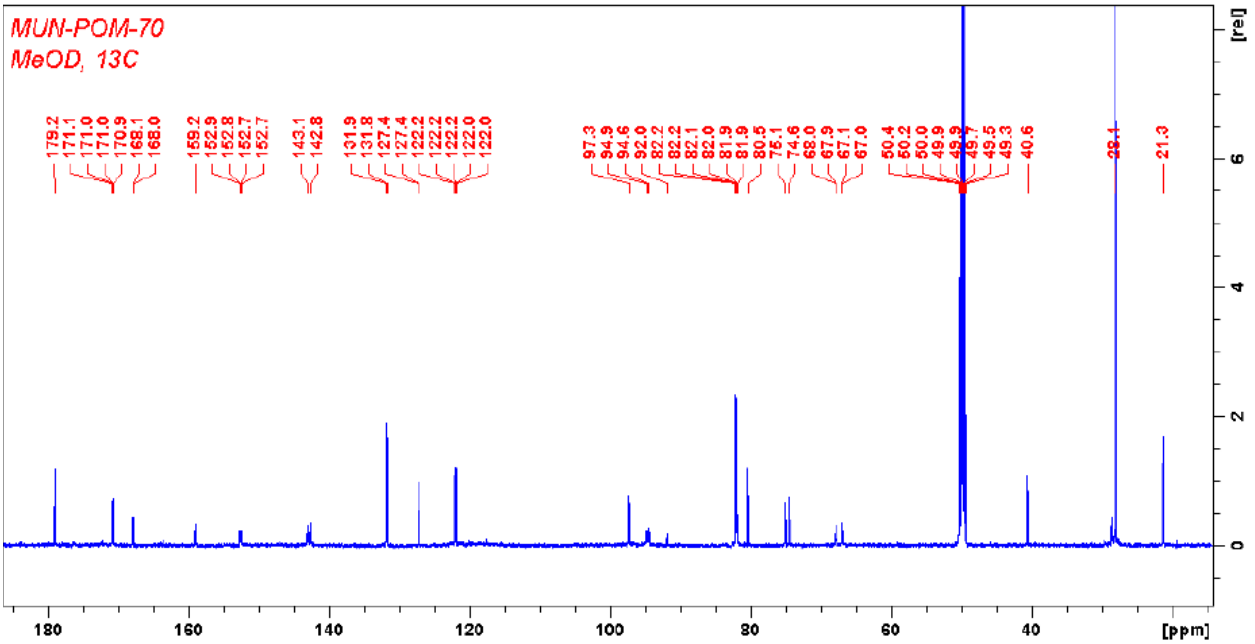
Compound **1i**

^{1}H spectrum



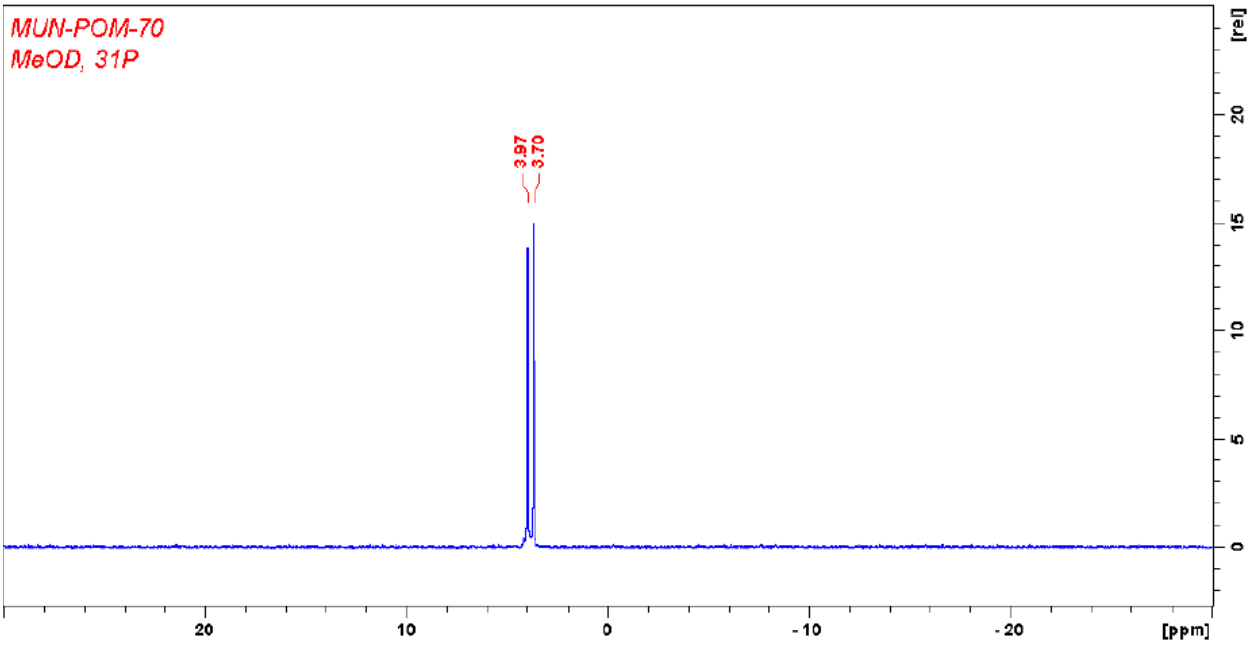
Compound **1i**

^{13}C spectrum



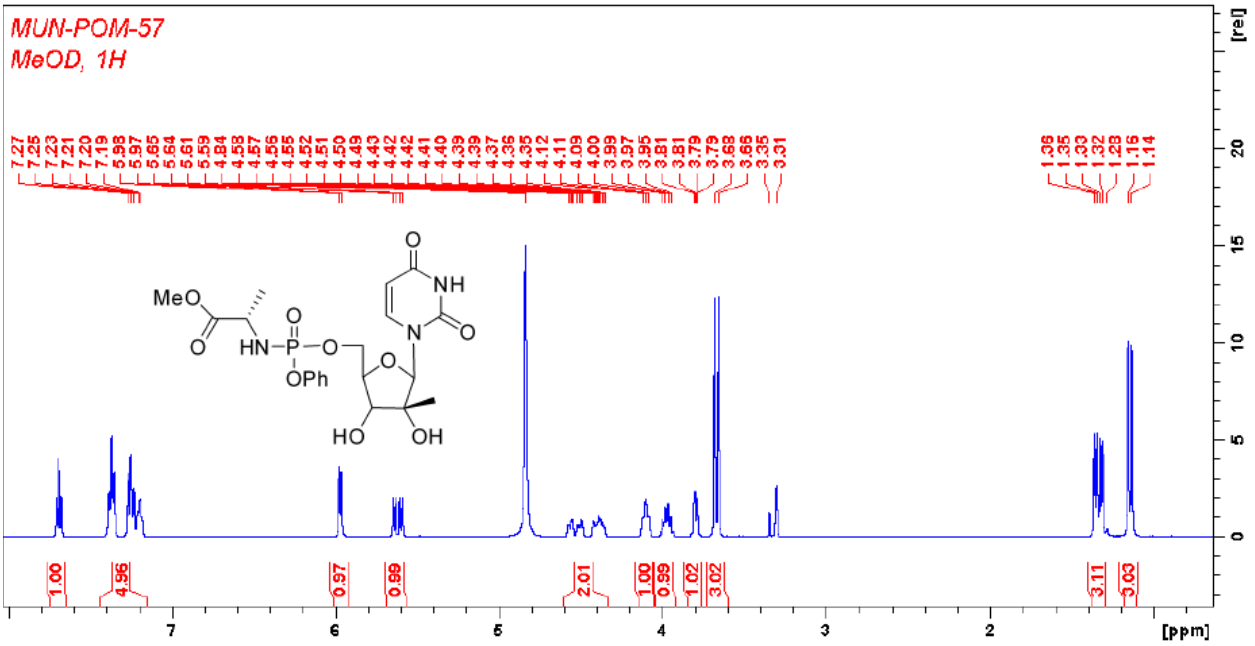
Compound **1i**

^{31}P spectrum



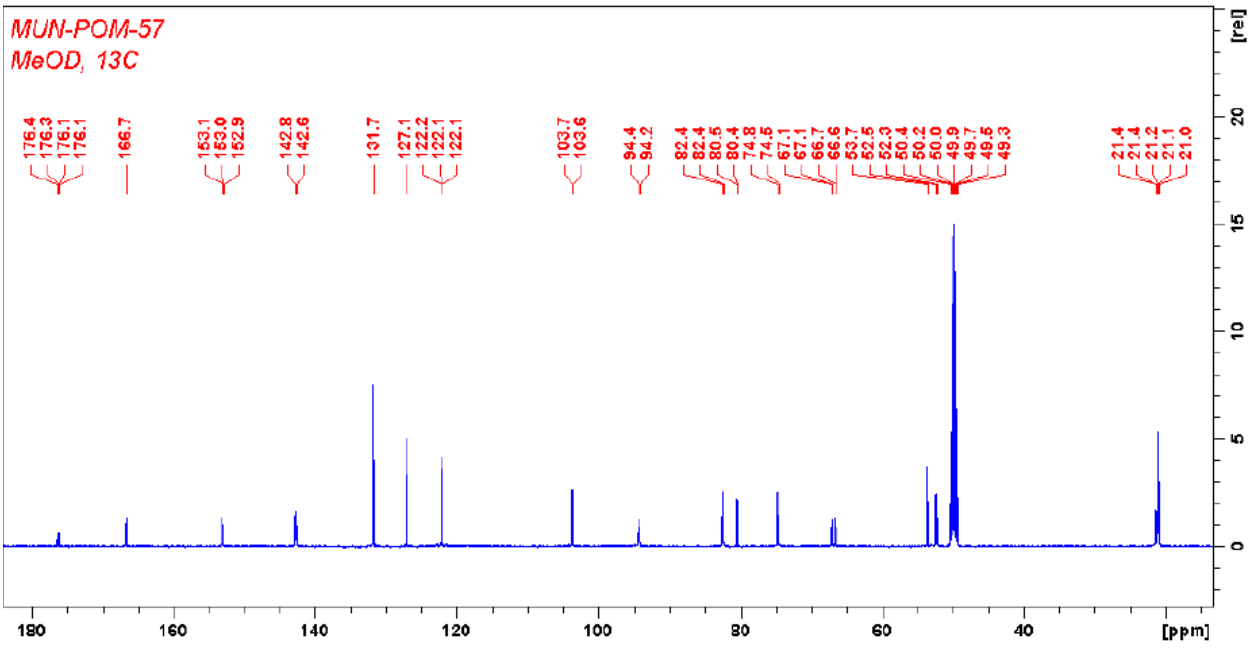
Compound **2a**

^{1}H spectrum



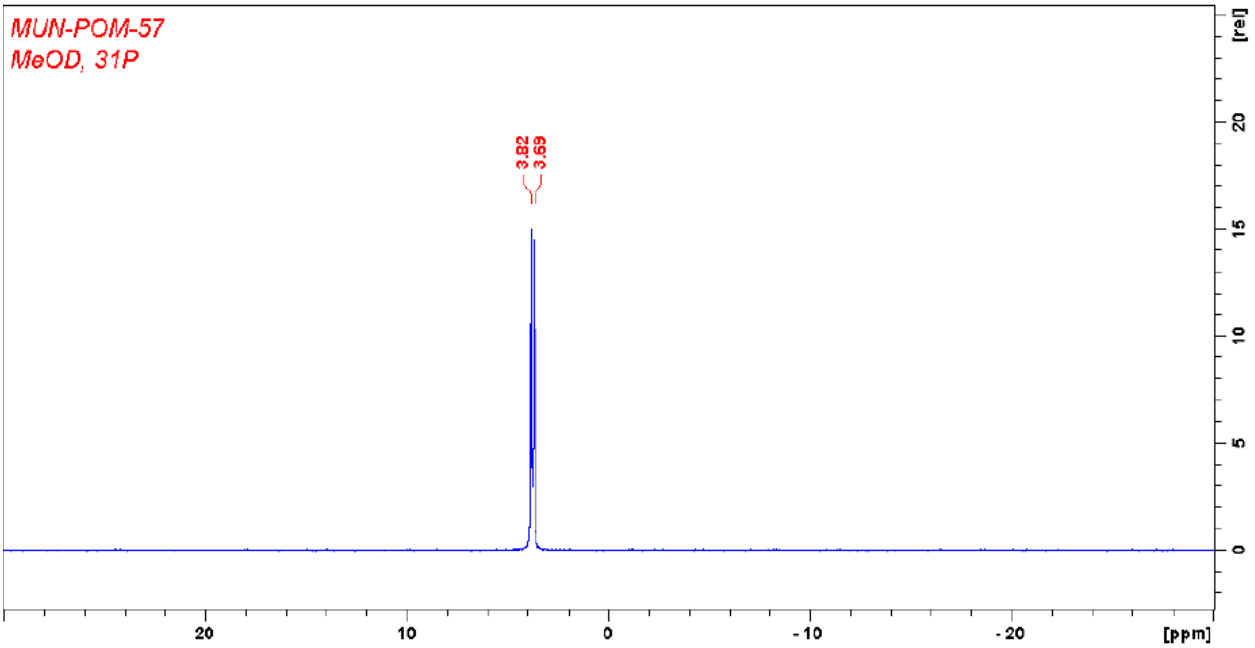
Compound **2a**

^{13}C spectrum



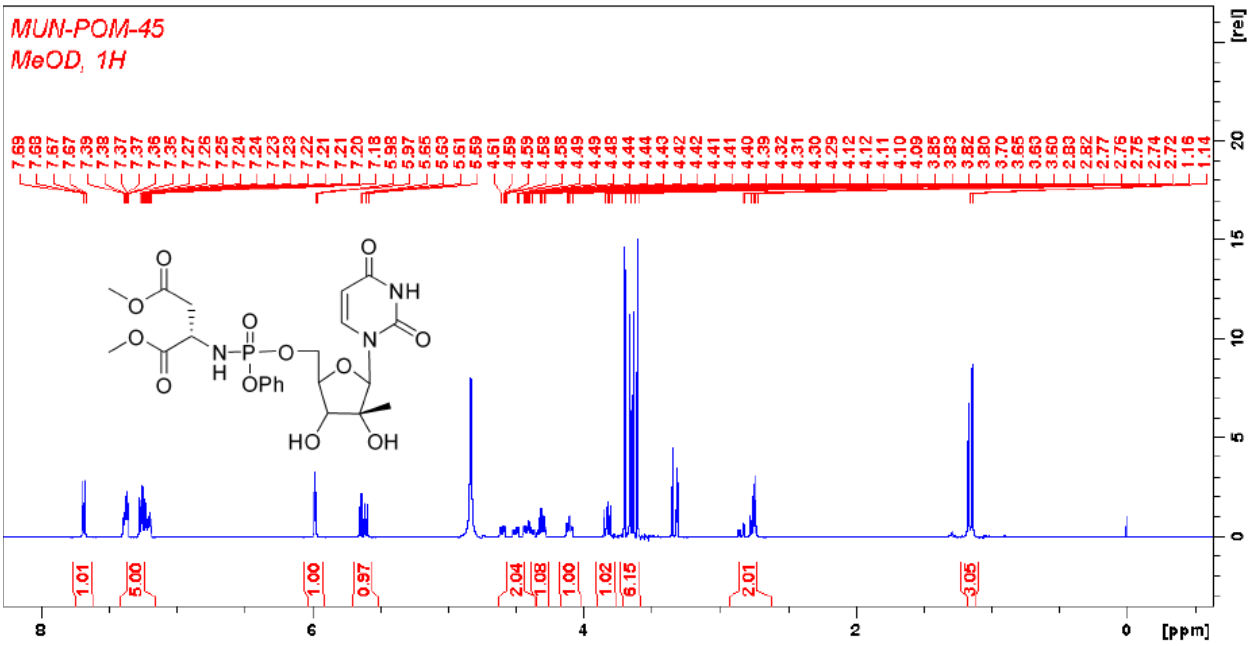
Compound **2a**

^{31}P spectrum



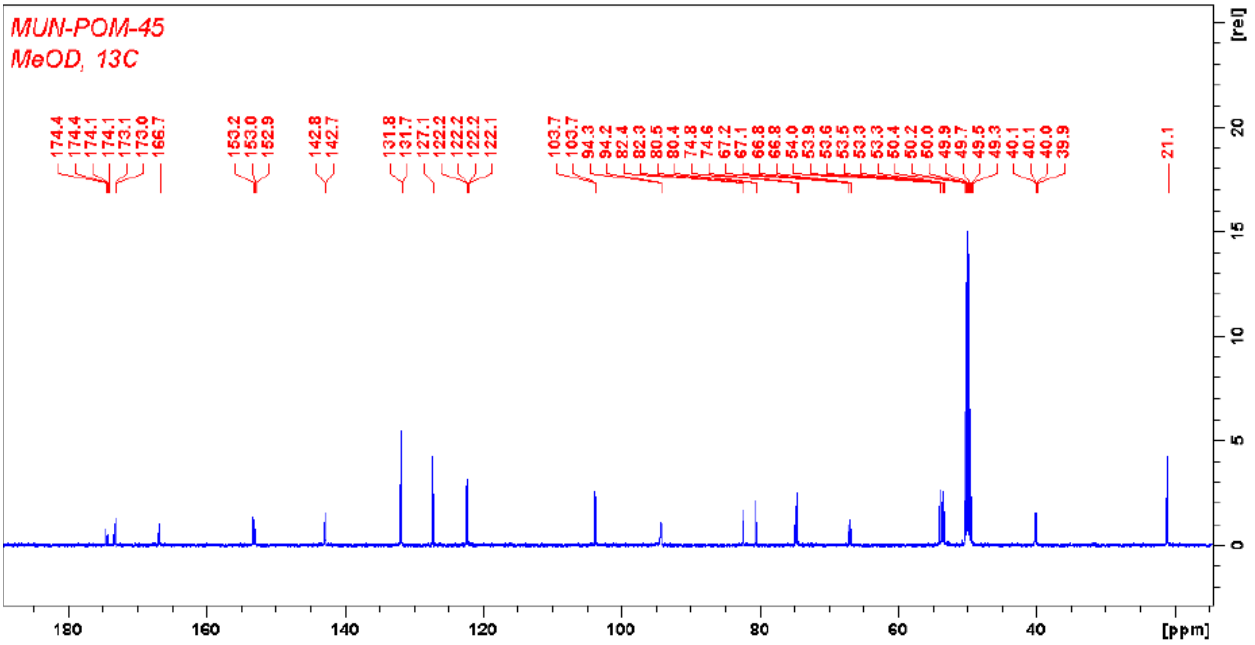
Compound **2b**

^{1}H spectrum



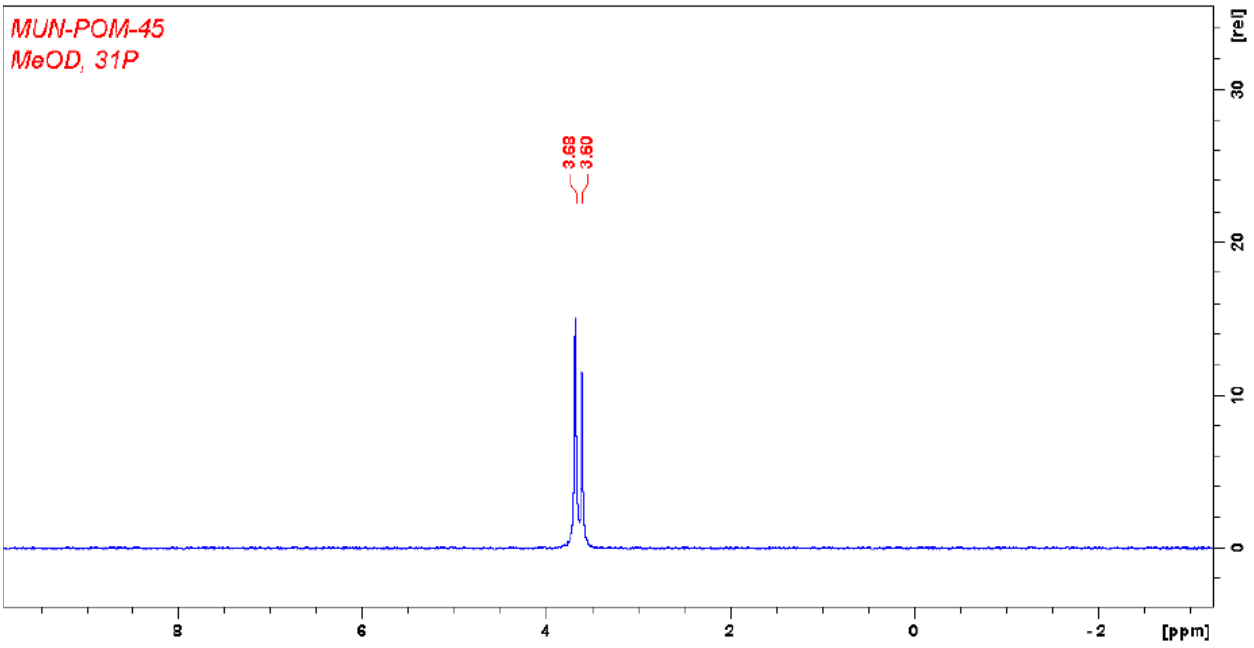
Compound **2b**

^{13}C spectrum



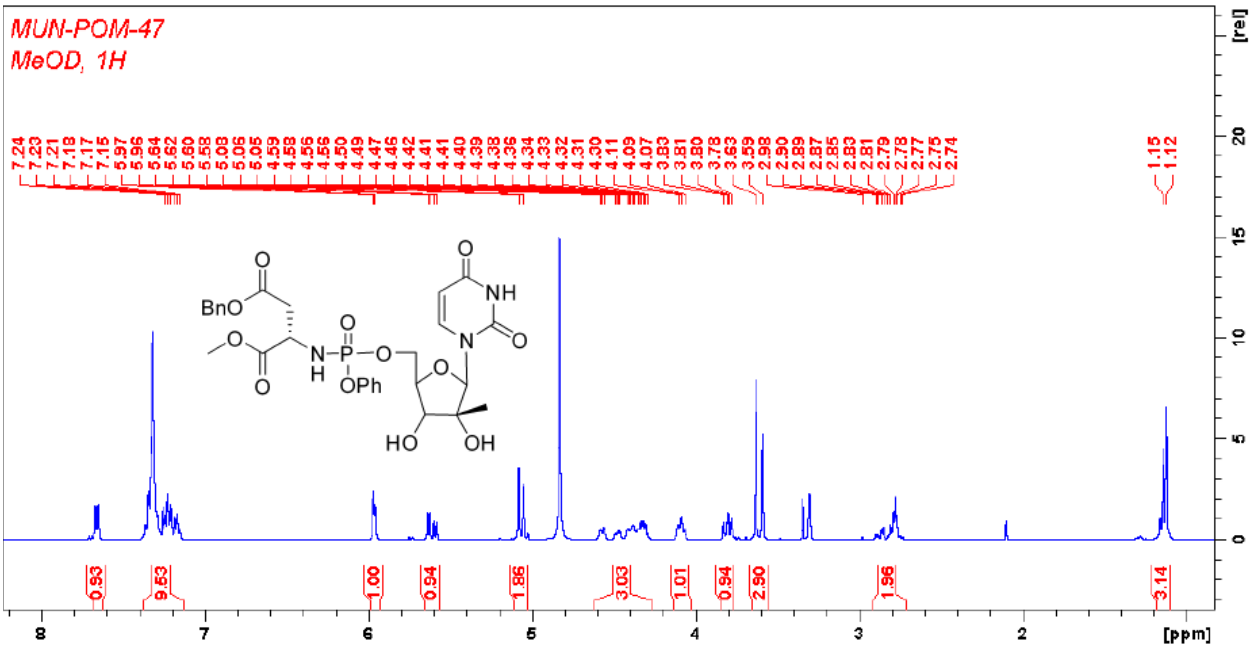
Compound **2b**

^{31}P spectrum



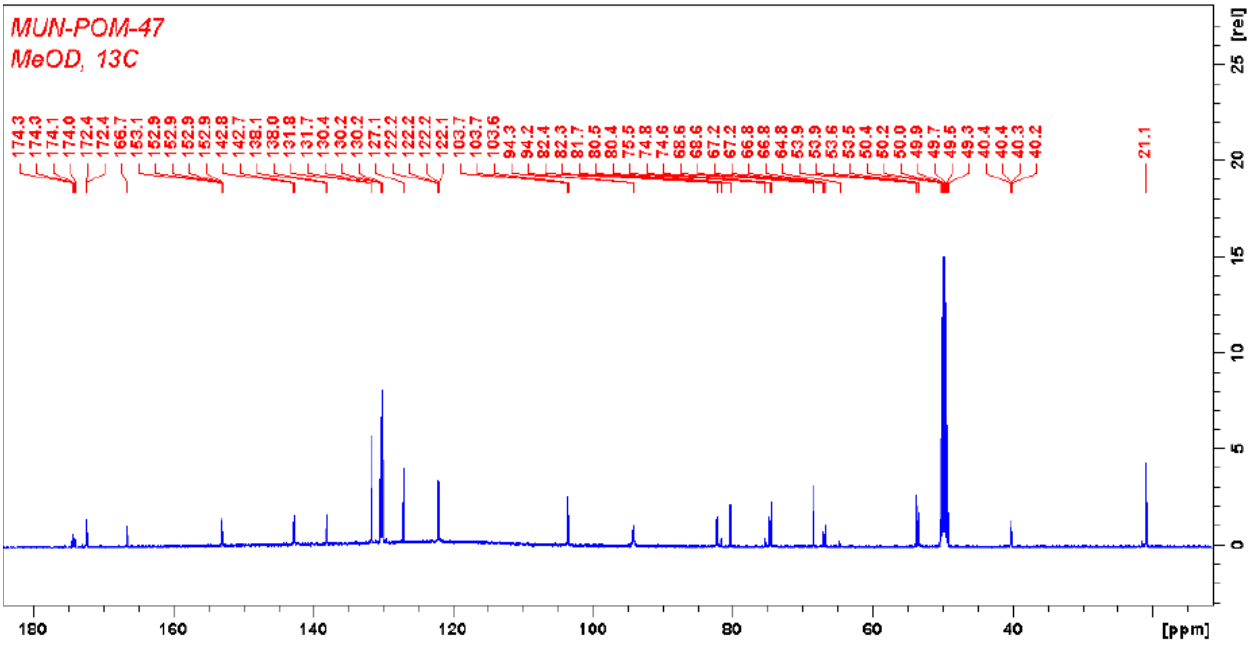
Compound **2c**

^{1}H spectrum



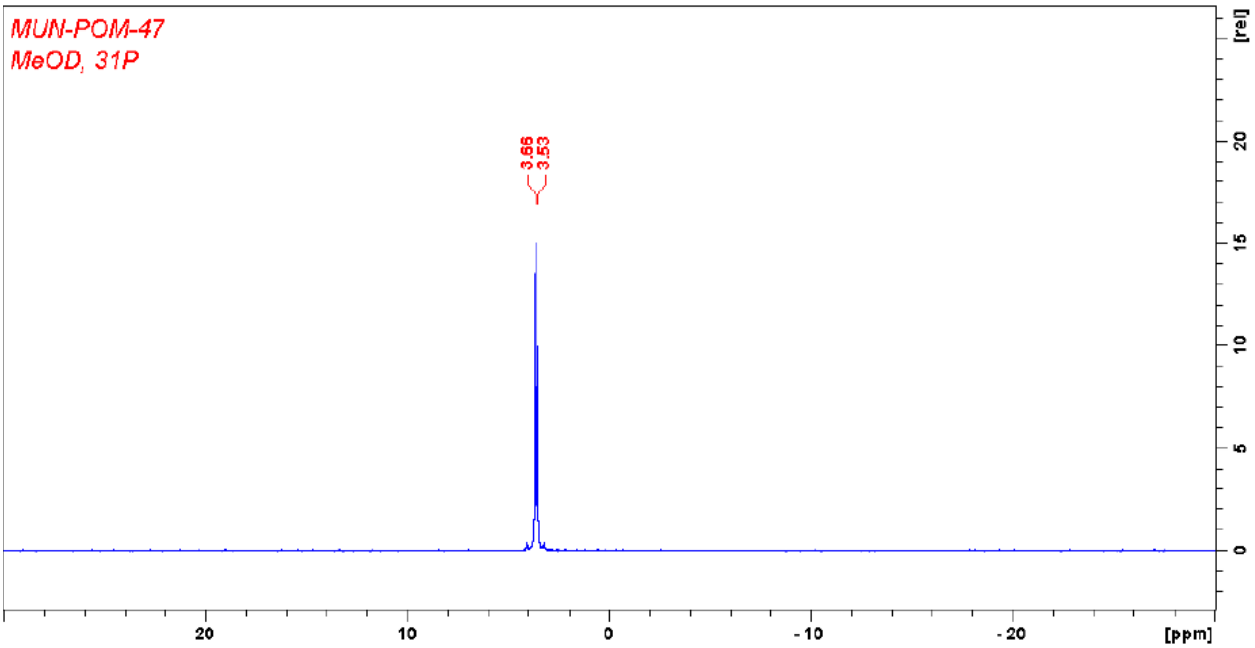
Compound **2c**

^{13}C spectrum



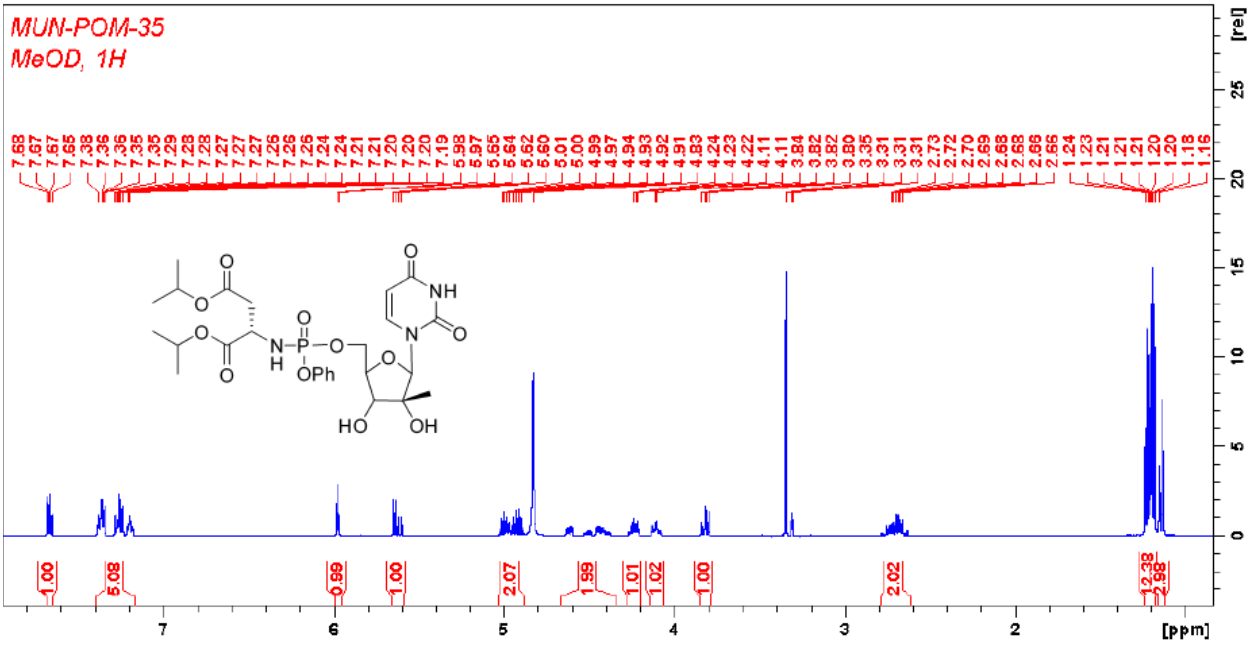
Compound **2c**

^{31}P spectrum



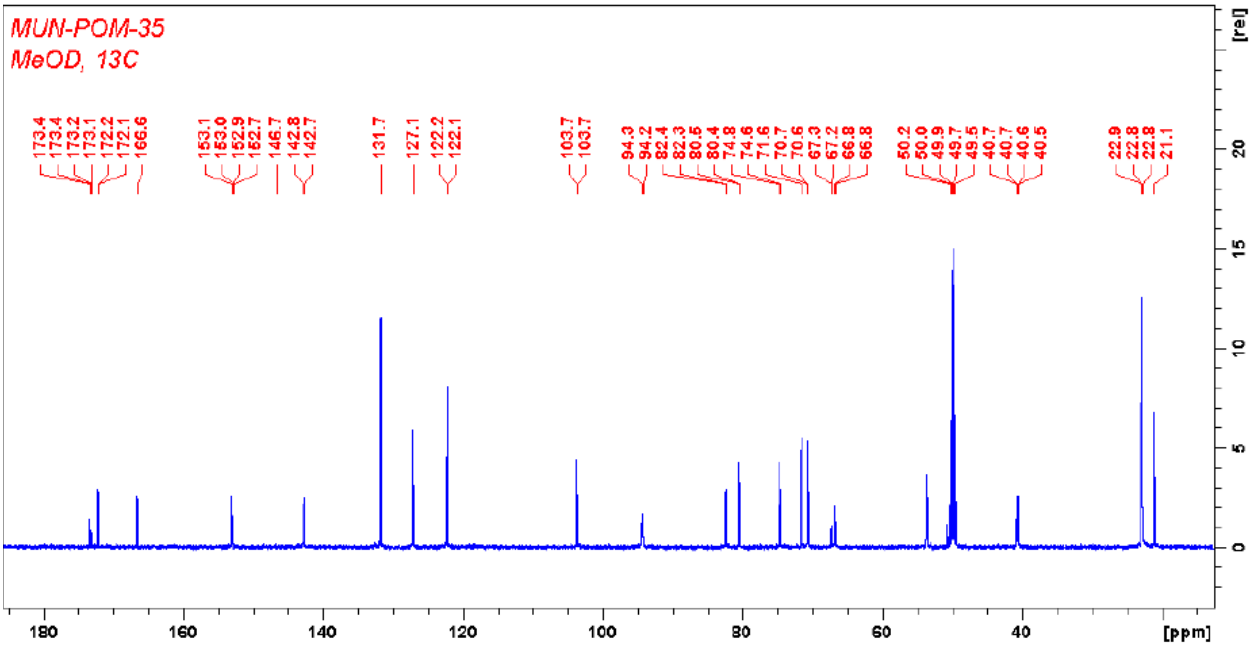
Compound **2d**

^{1}H spectrum



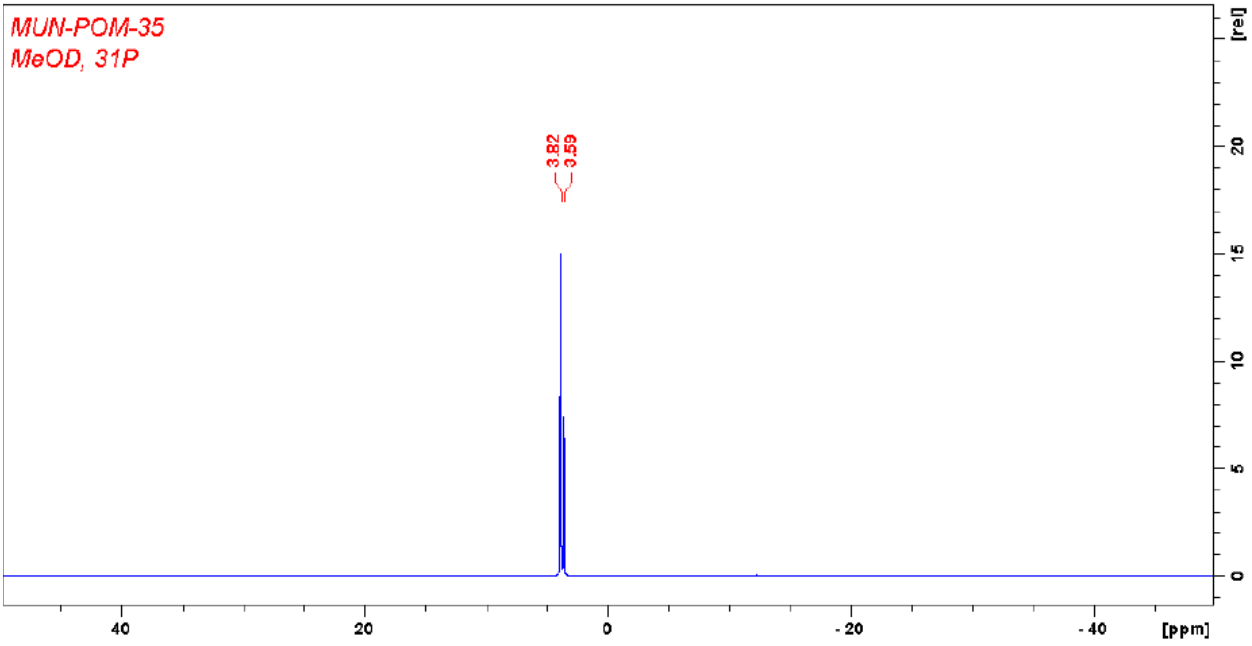
Compound **2d**

^{13}C spectrum



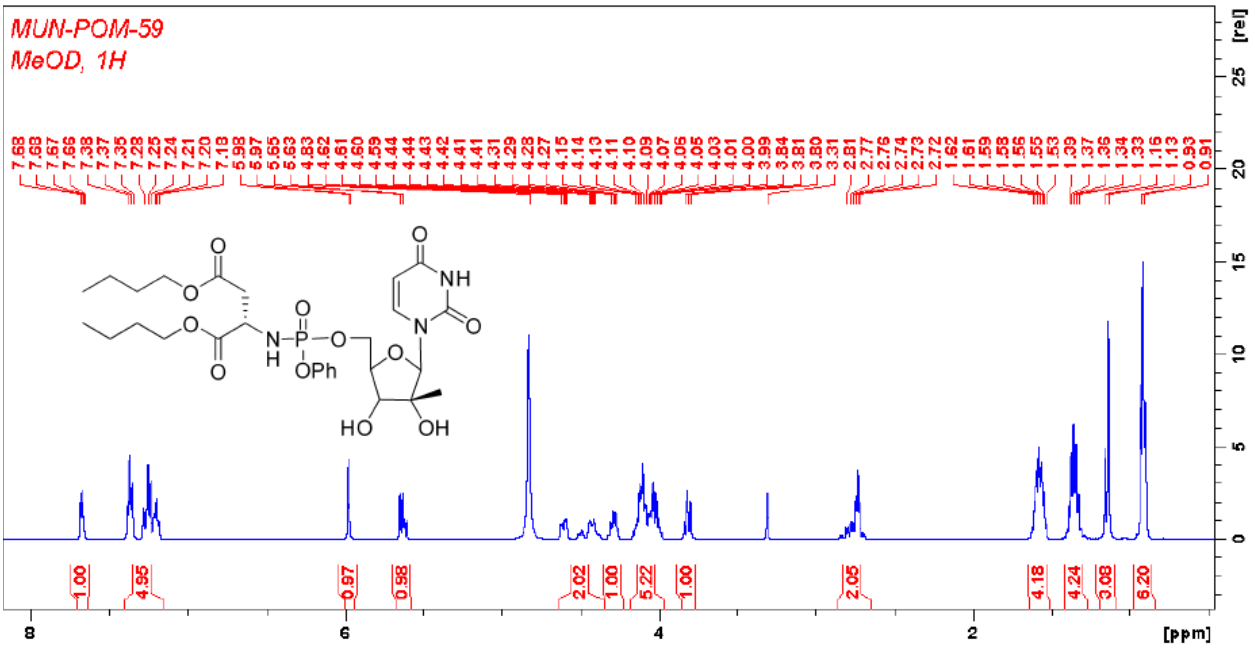
Compound **2d**

^{31}P spectrum



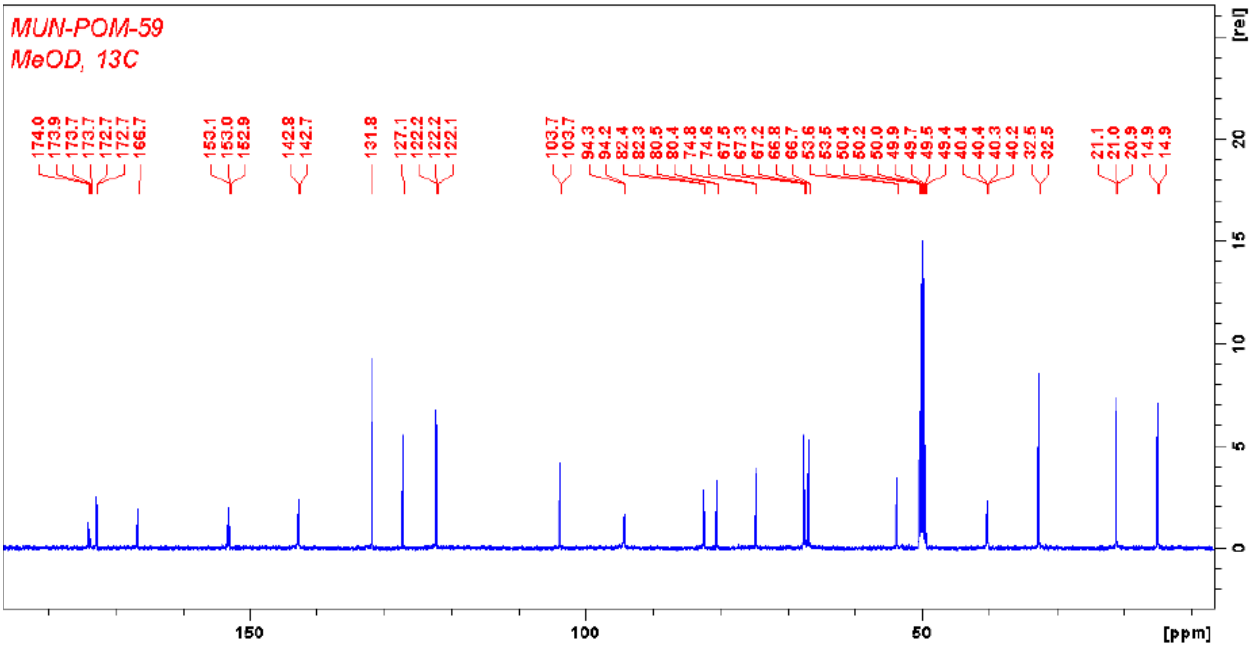
Compound **2e**

^{1}H spectrum



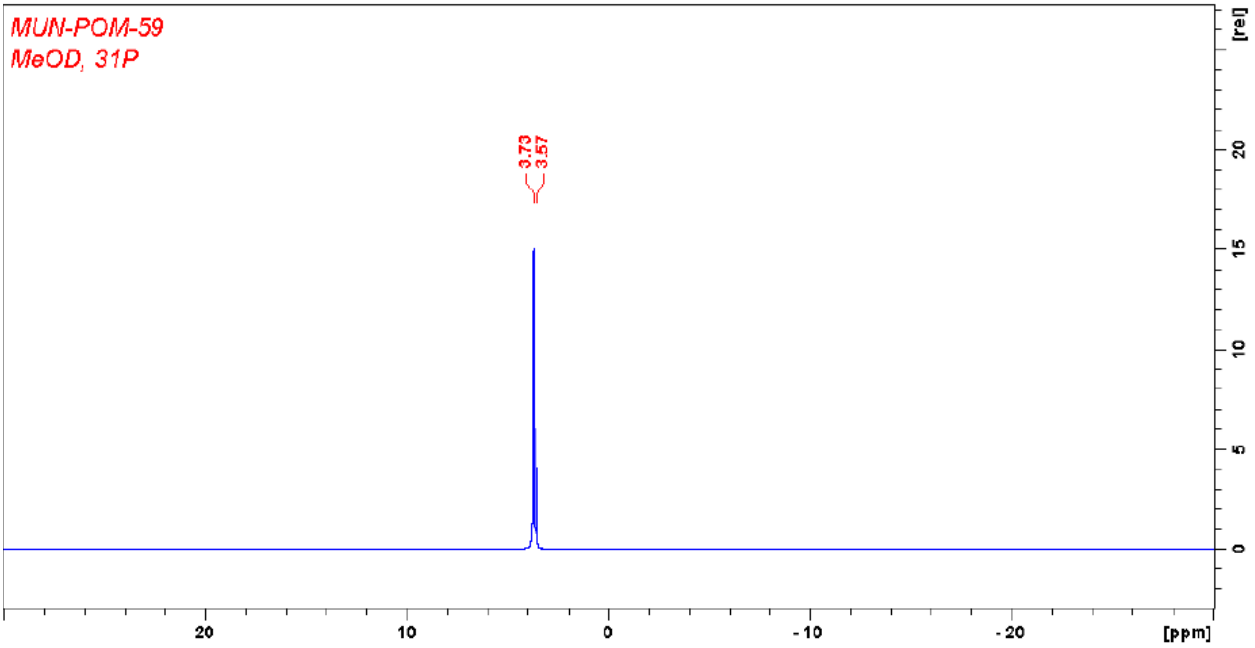
Compound **2e**

^{13}C spectrum



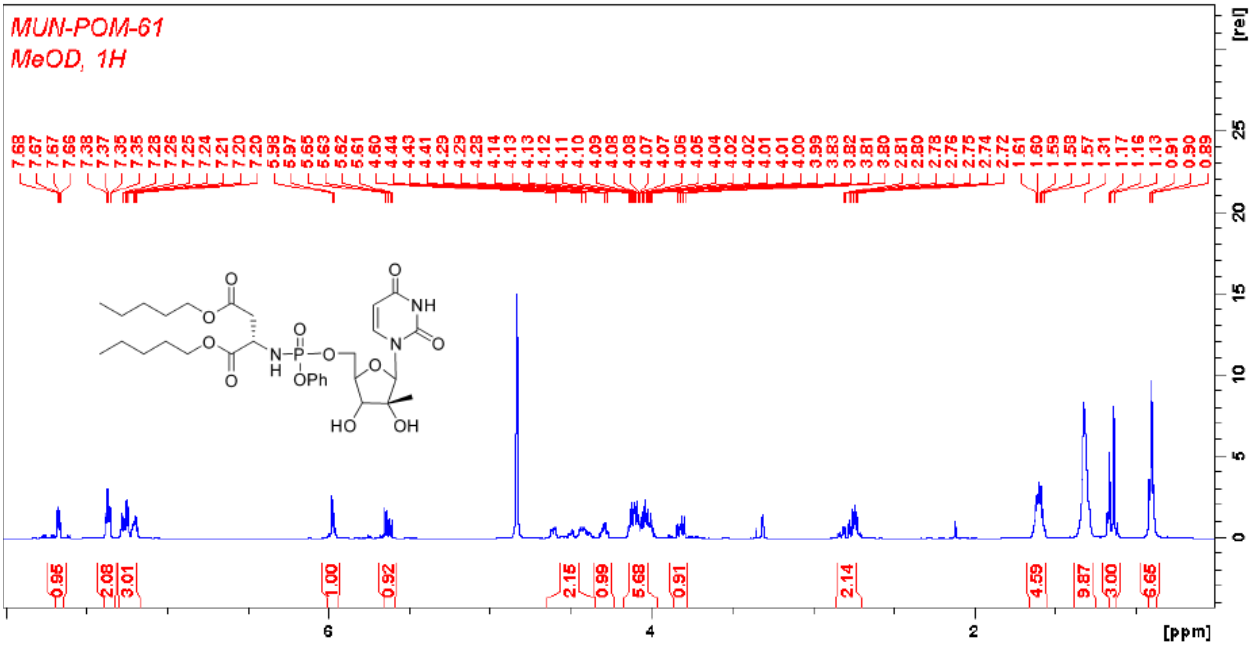
Compound **2e**

^{31}P spectrum



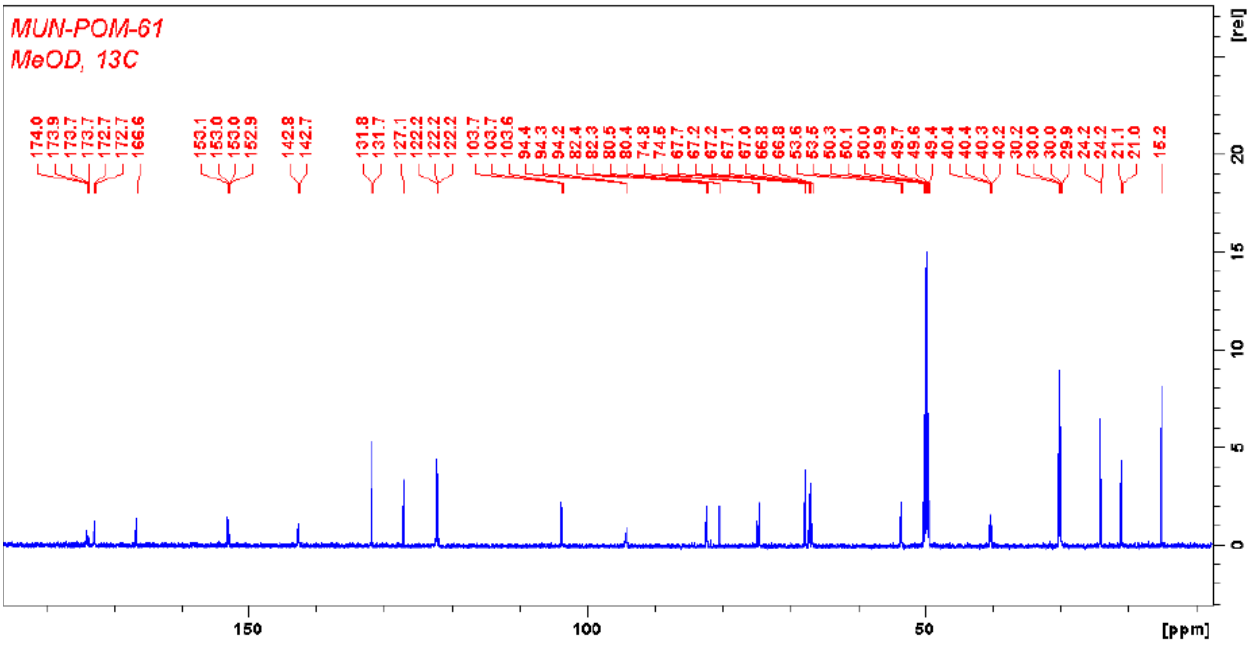
Compound **2f**

^{1}H spectrum



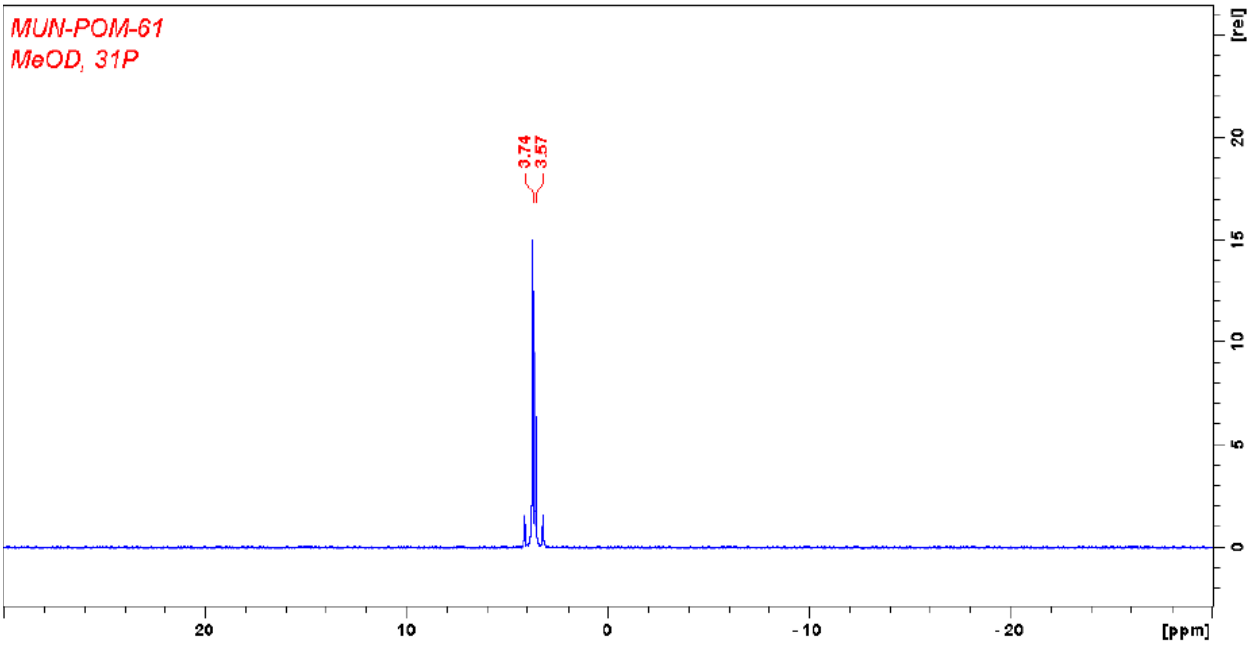
Compound **2f**

^{13}C spectrum



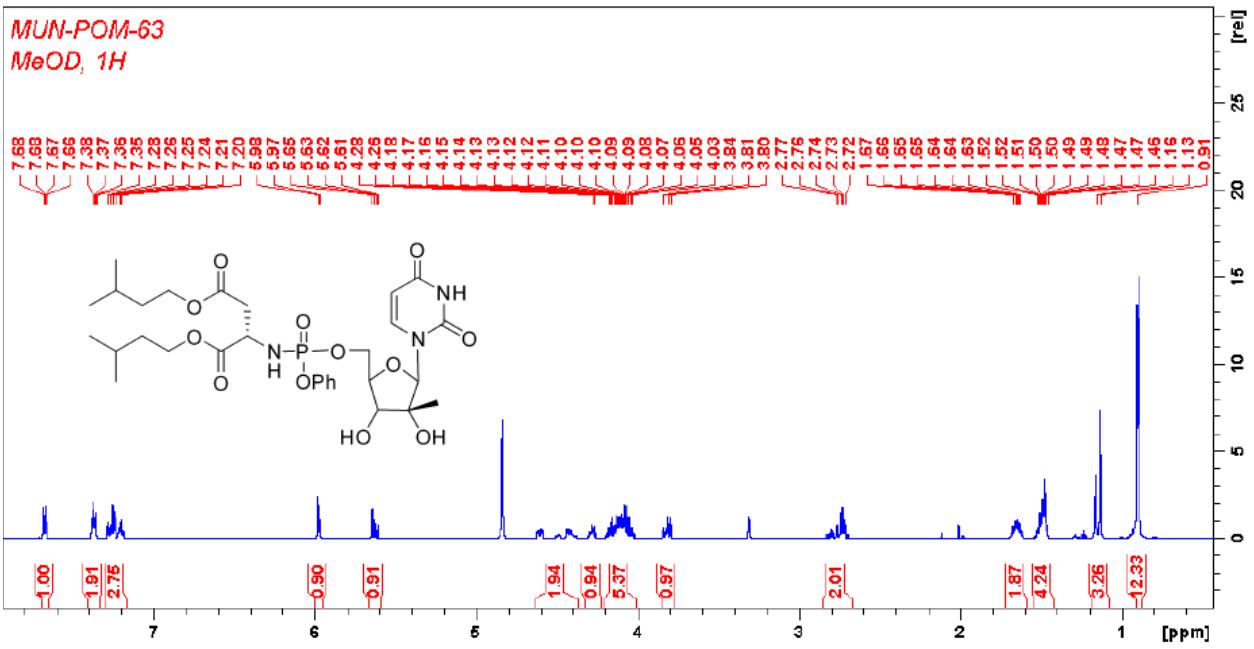
Compound **2f**

^{31}P spectrum



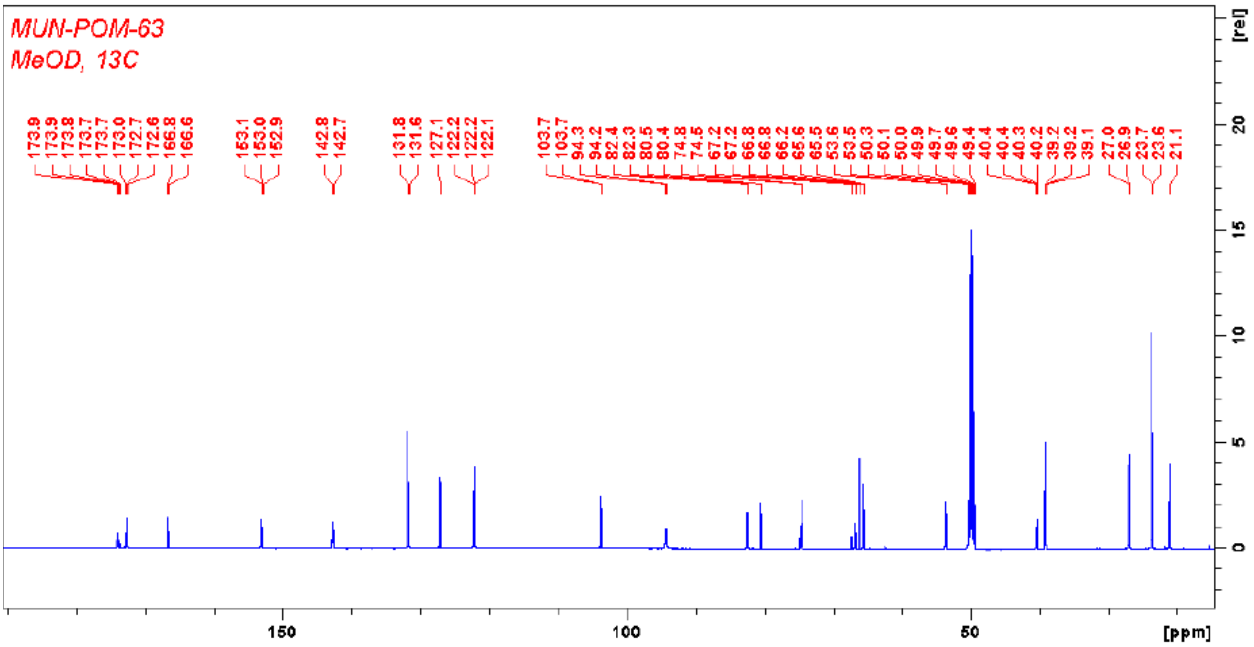
Compound **2g**

^{1}H spectrum



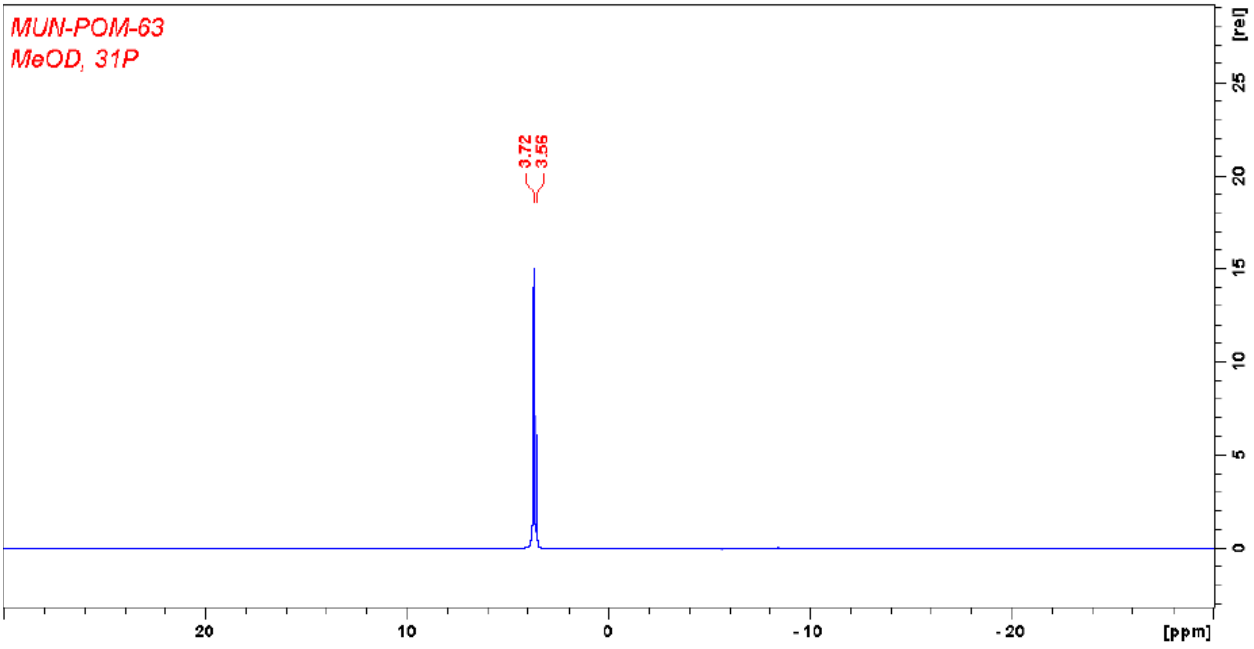
Compound **2g**

^{13}C spectrum



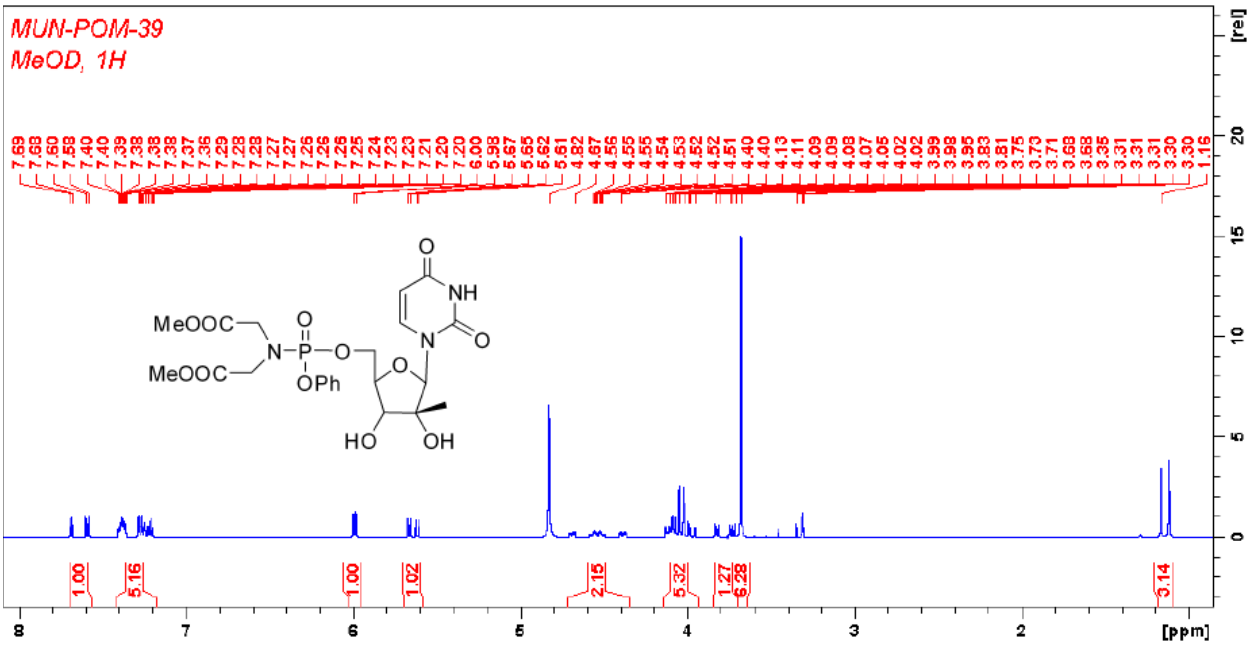
Compound **2g**

^{31}P spectrum



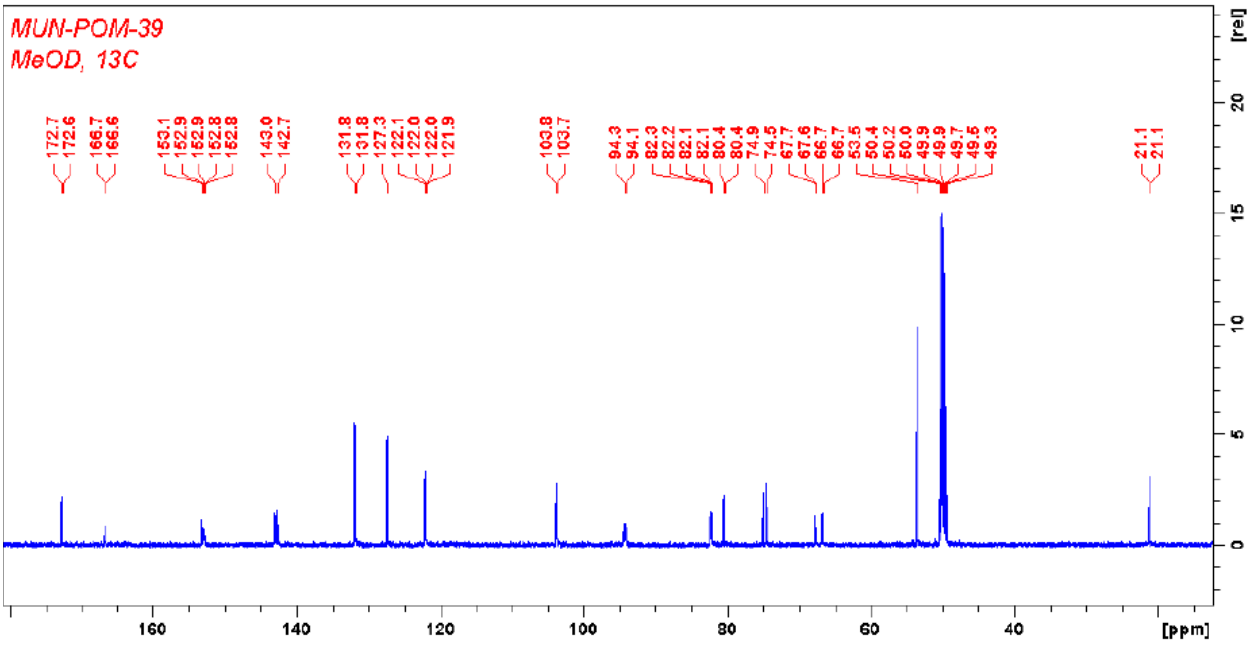
Compound **2h**

^{1}H spectrum



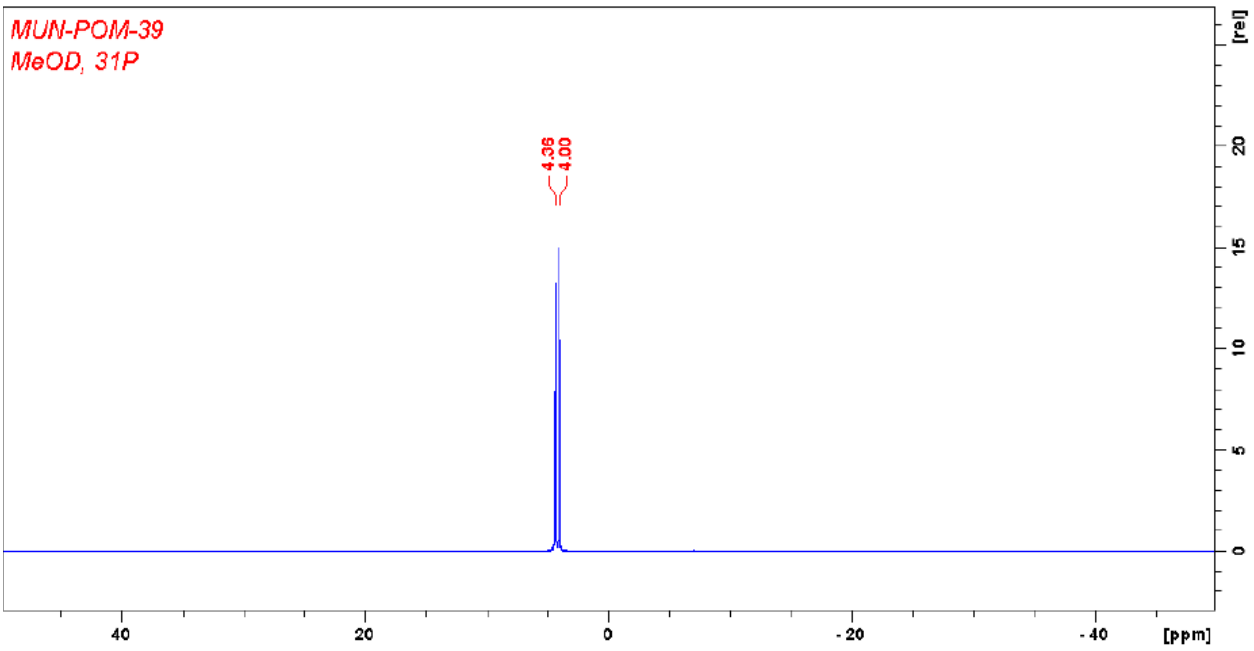
Compound **2h**

^{13}C spectrum



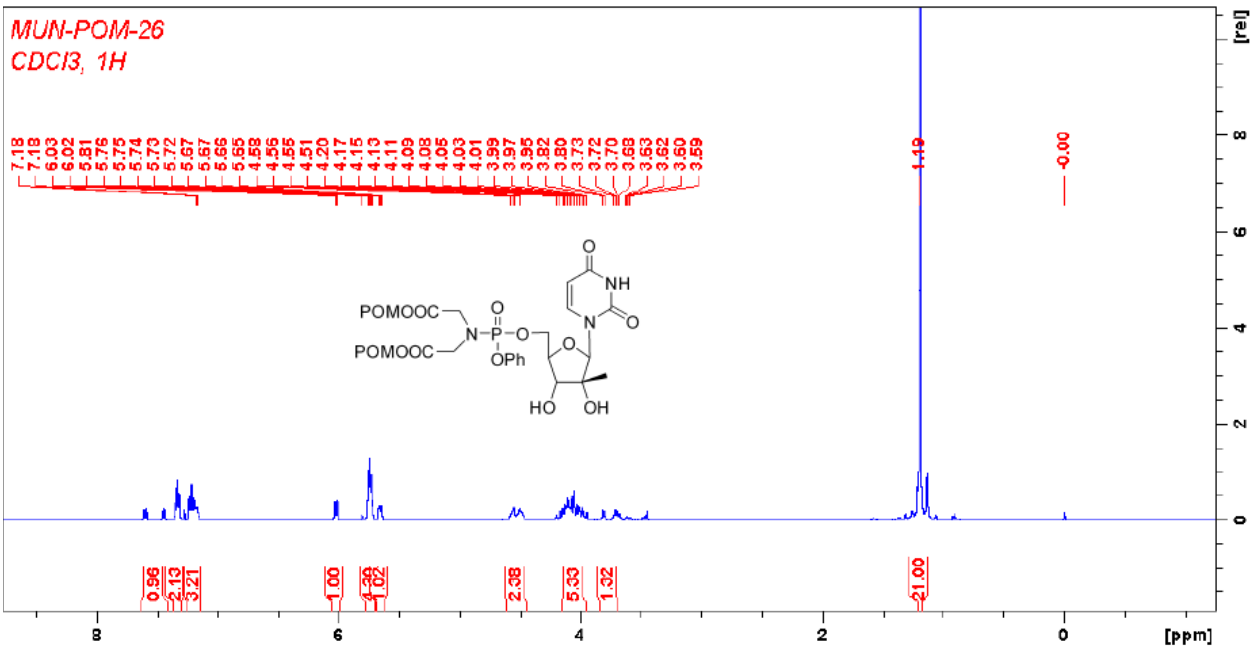
Compound **2h**

^{31}P spectrum



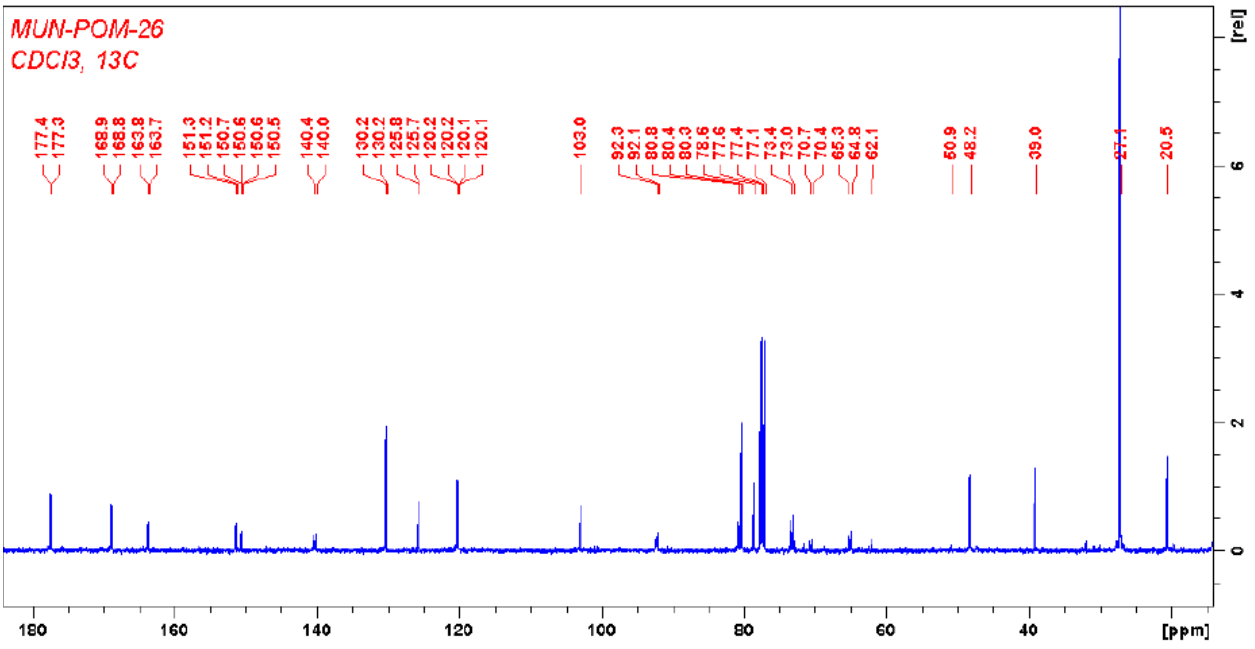
Compound **2i**

^{1}H spectrum



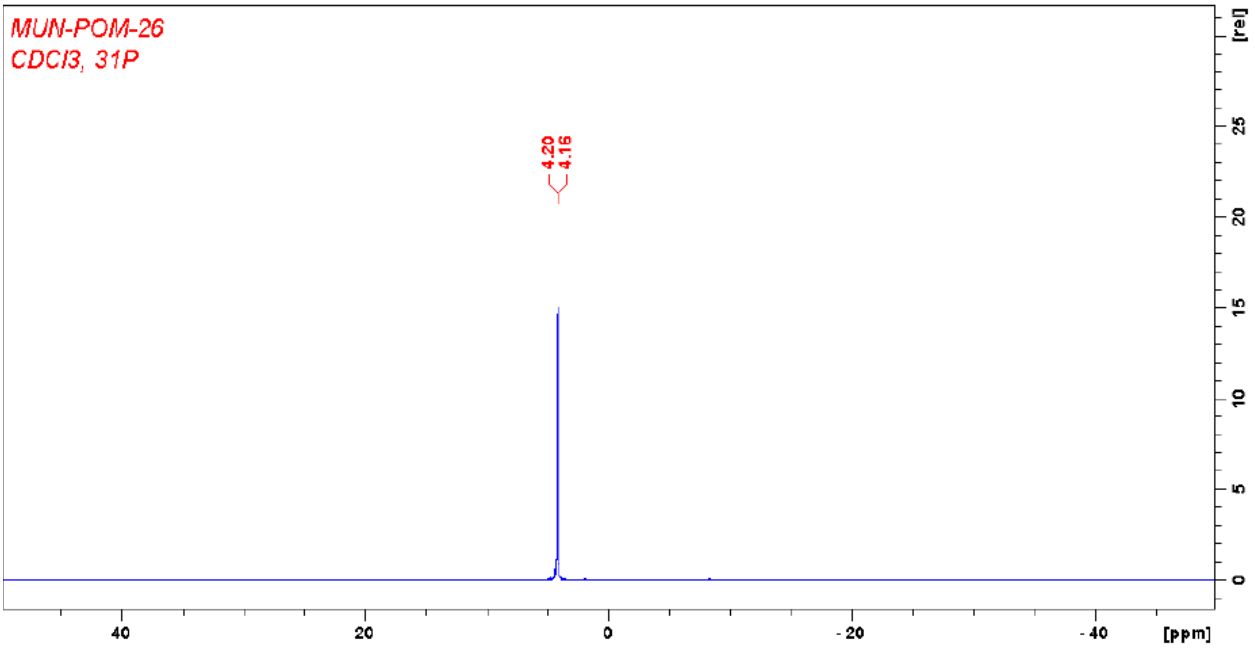
Compound **2i**

^{13}C spectrum



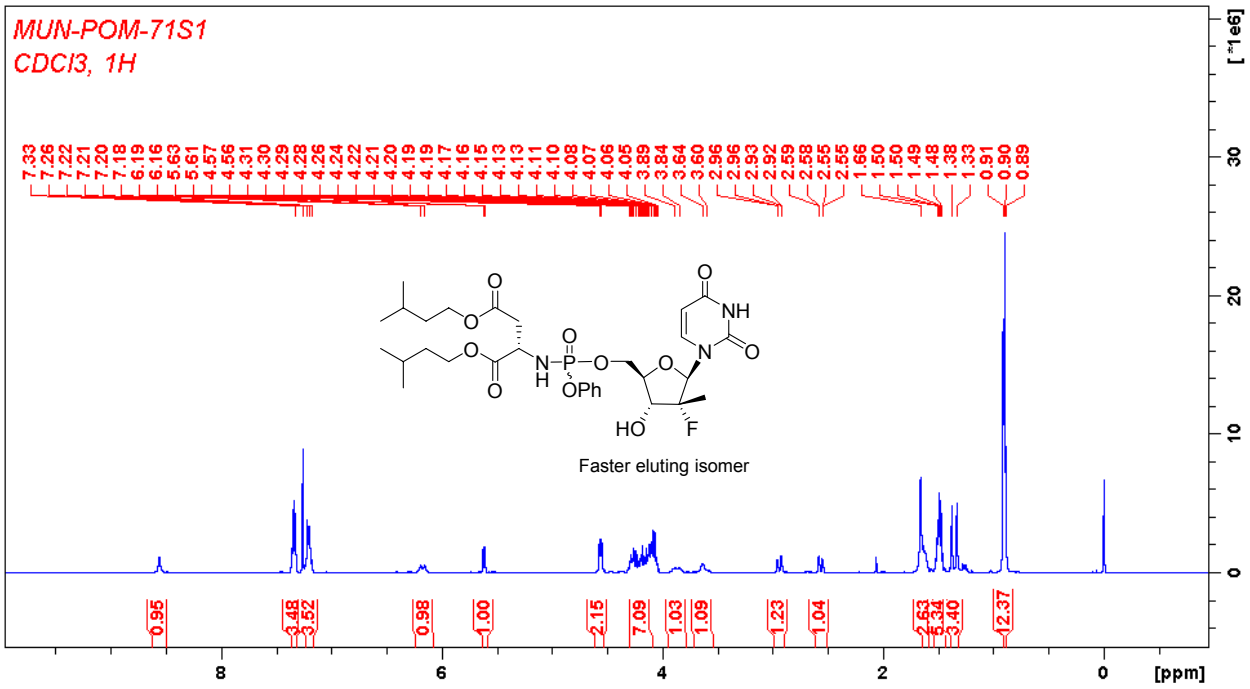
Compound **2i**

^{31}P spectrum



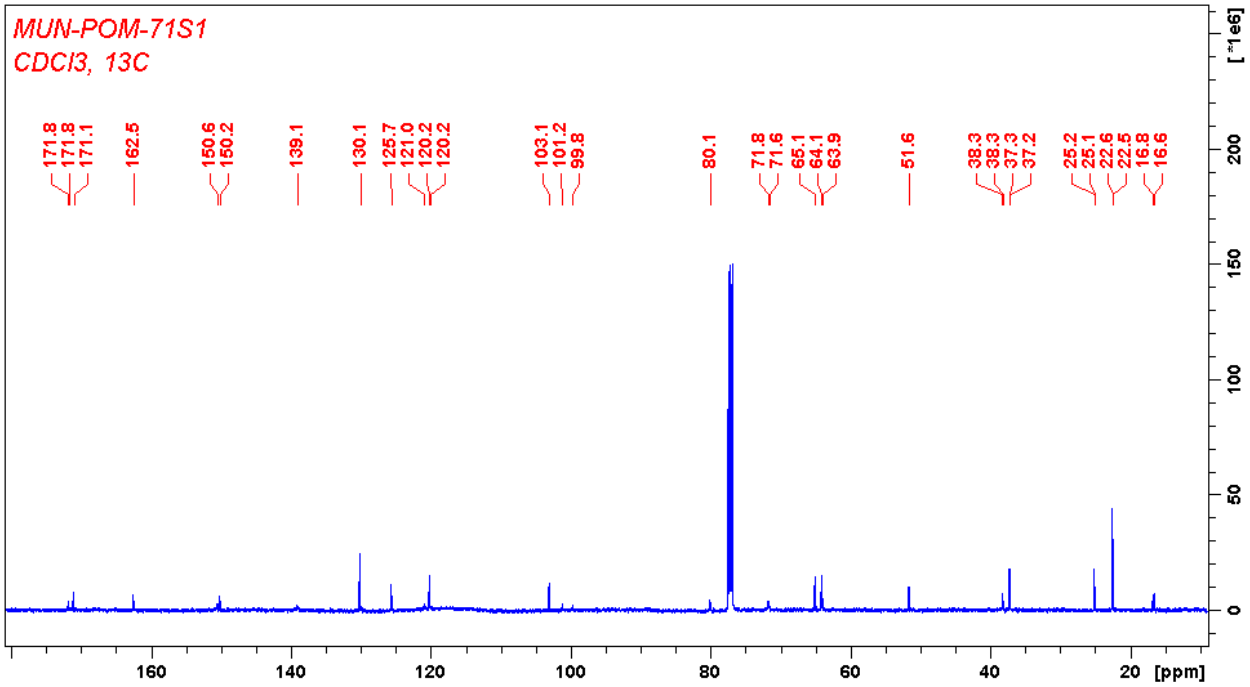
Compound **3a**

^{1}H spectrum



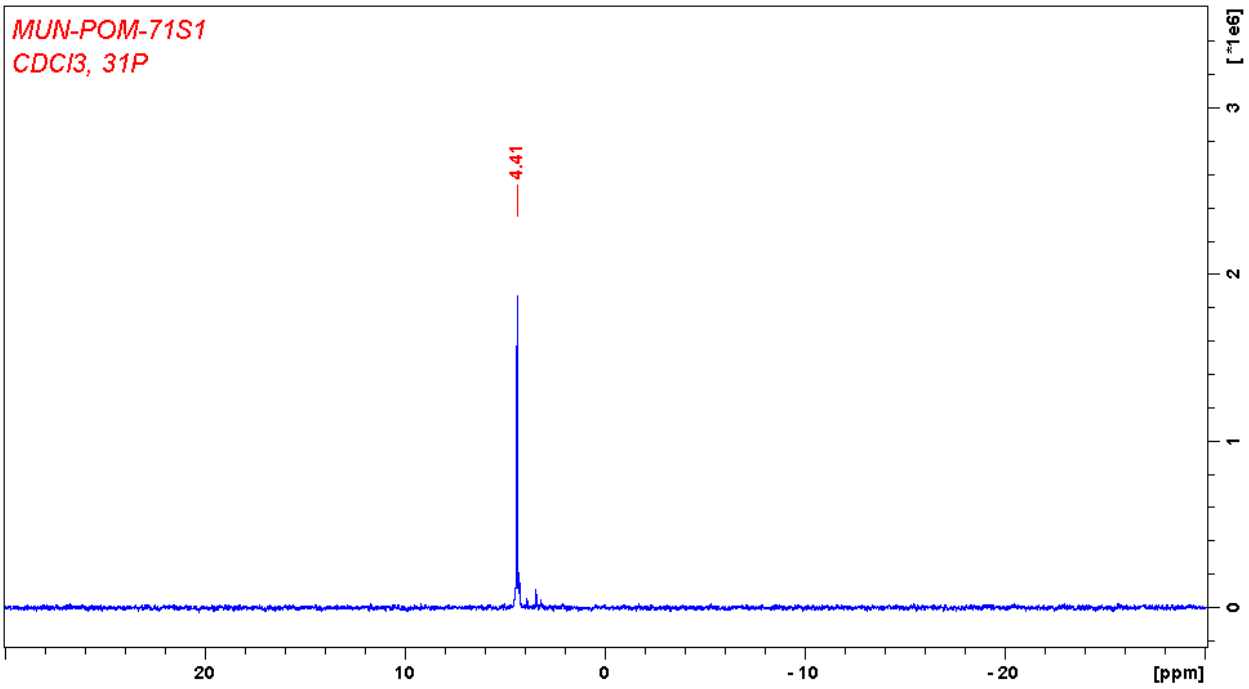
Compound **3a**

^{13}C spectrum



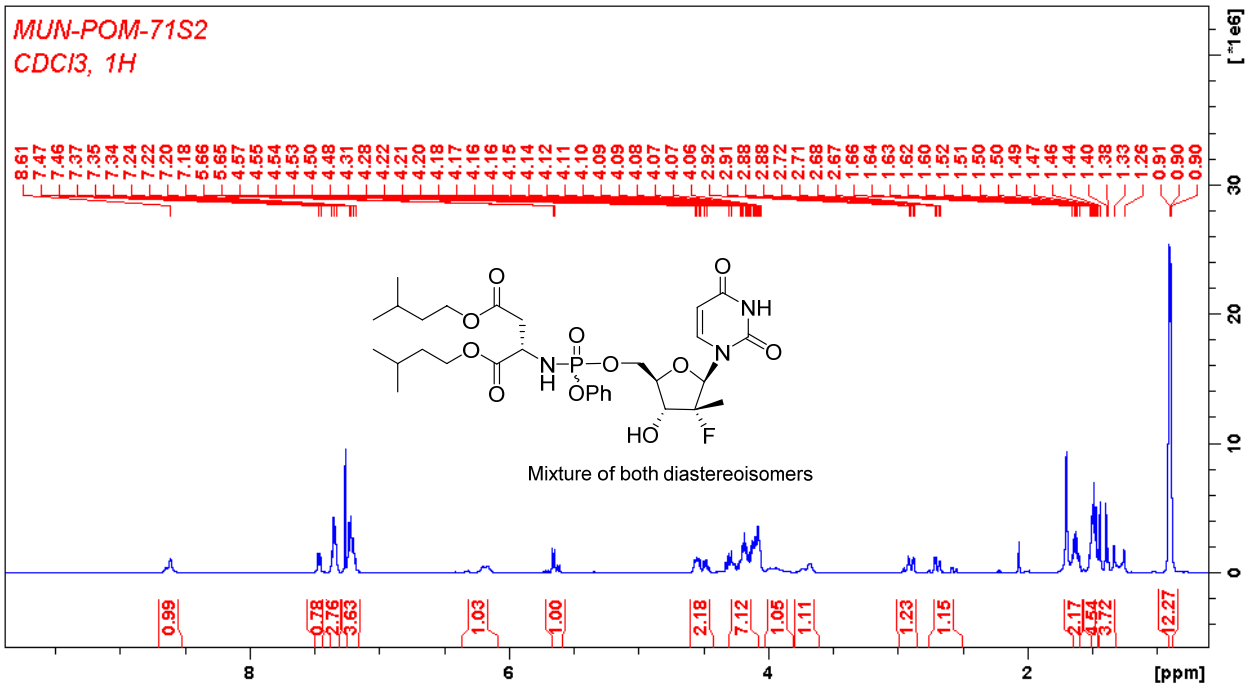
Compound **3a**

^{31}P spectrum



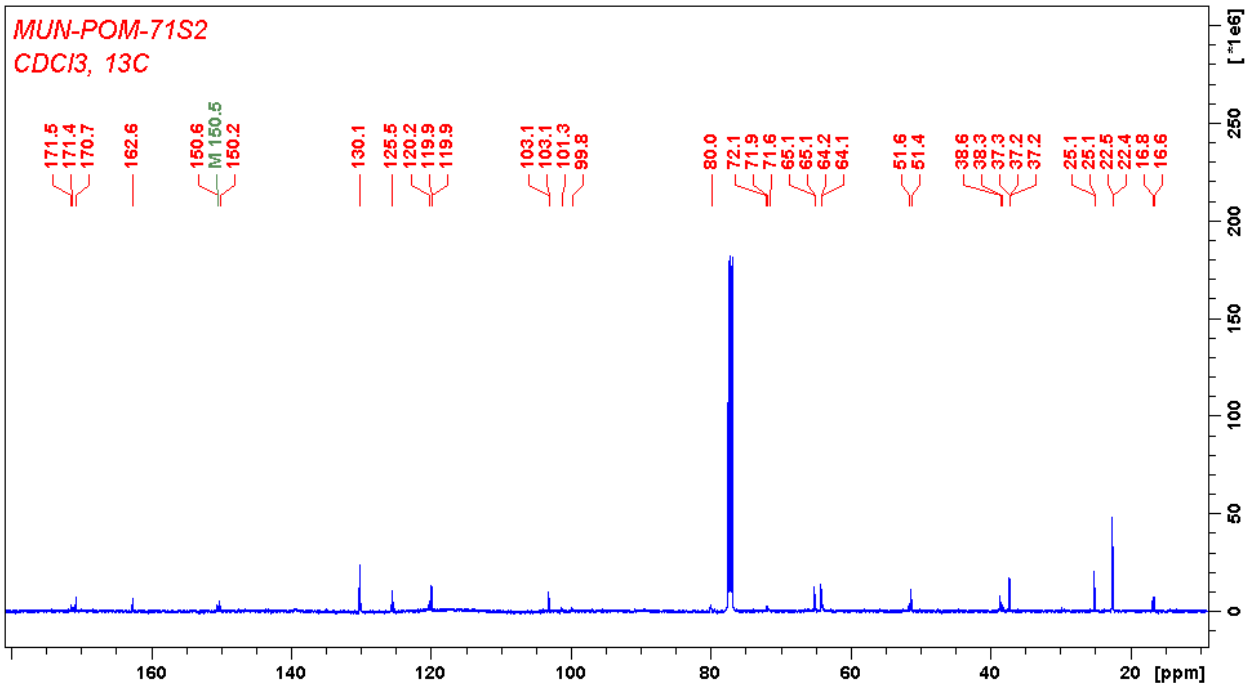
Compound **3b**

^{1}H spectrum



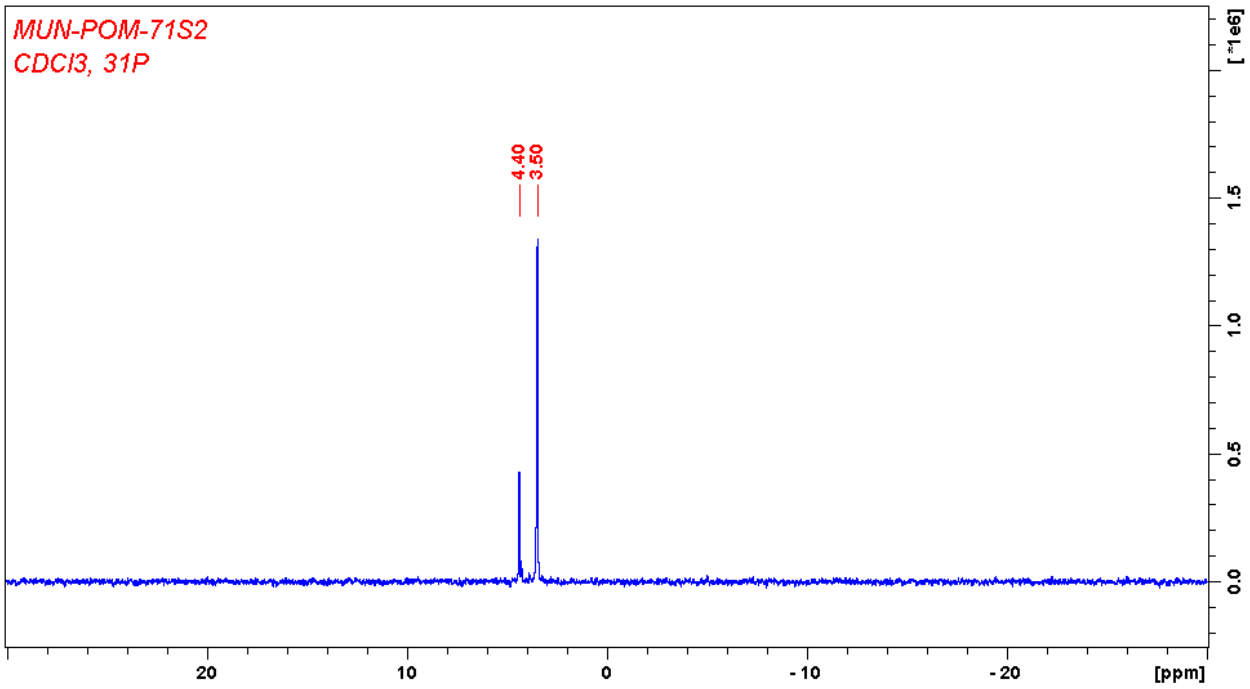
Compound **3b**

^{13}C spectrum



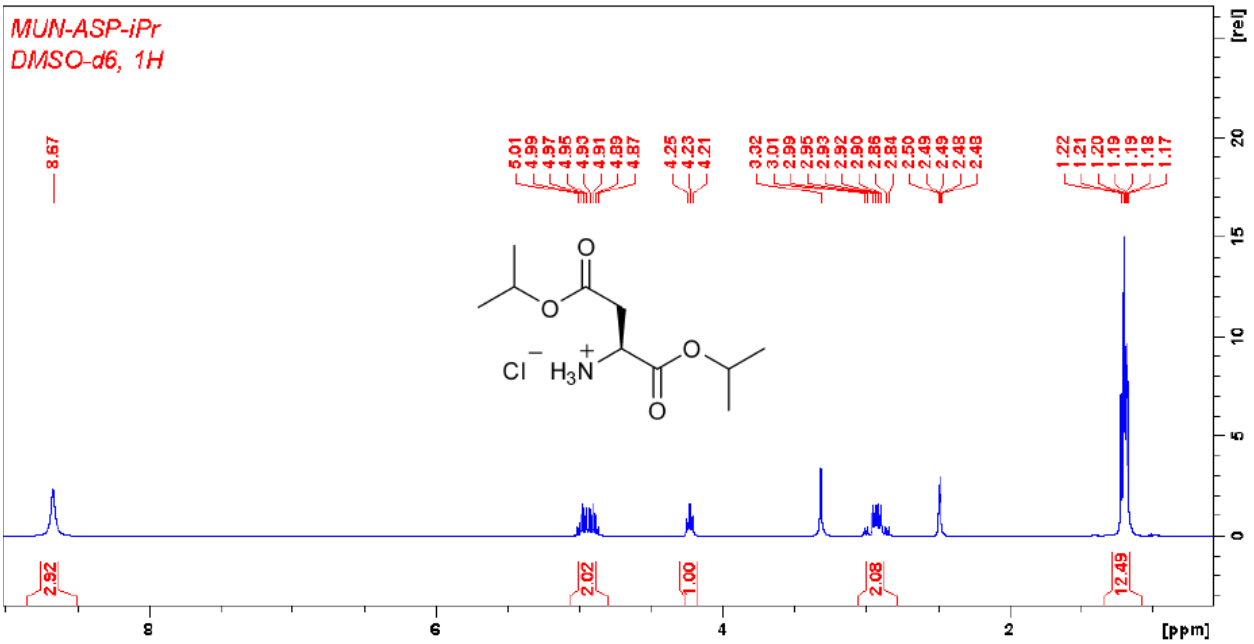
Compound **3b**

^{31}P spectrum



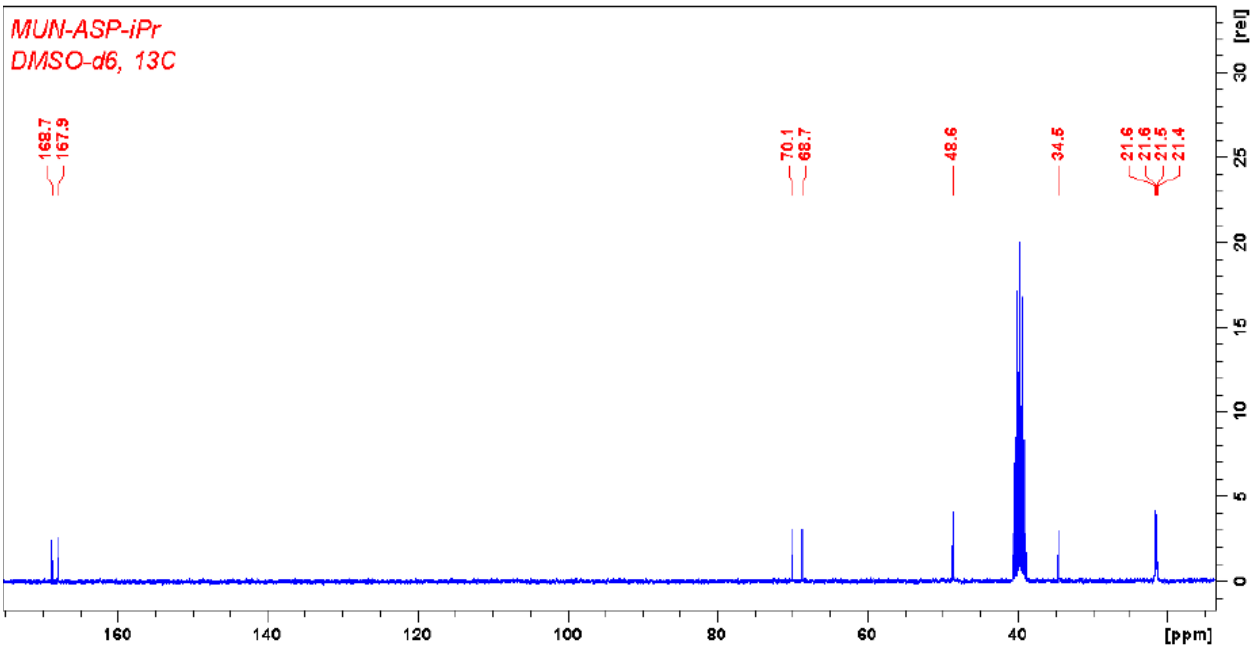
Compound **5**

^{1}H spectrum



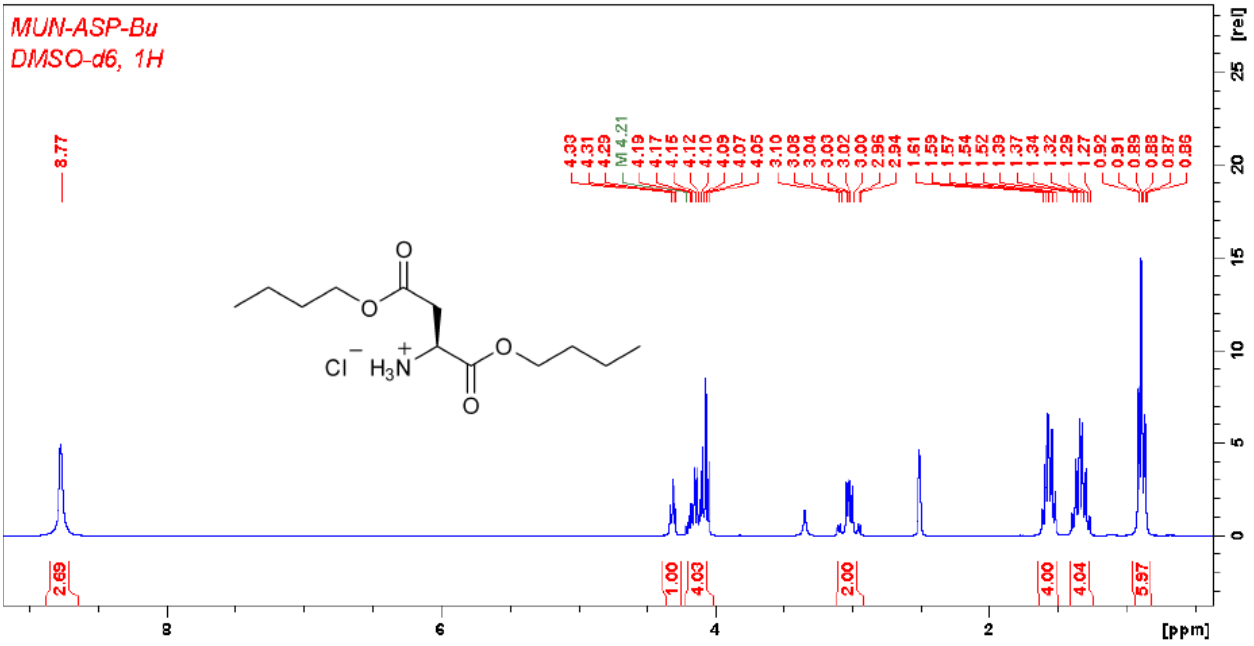
Compound **5**

^{13}C spectrum



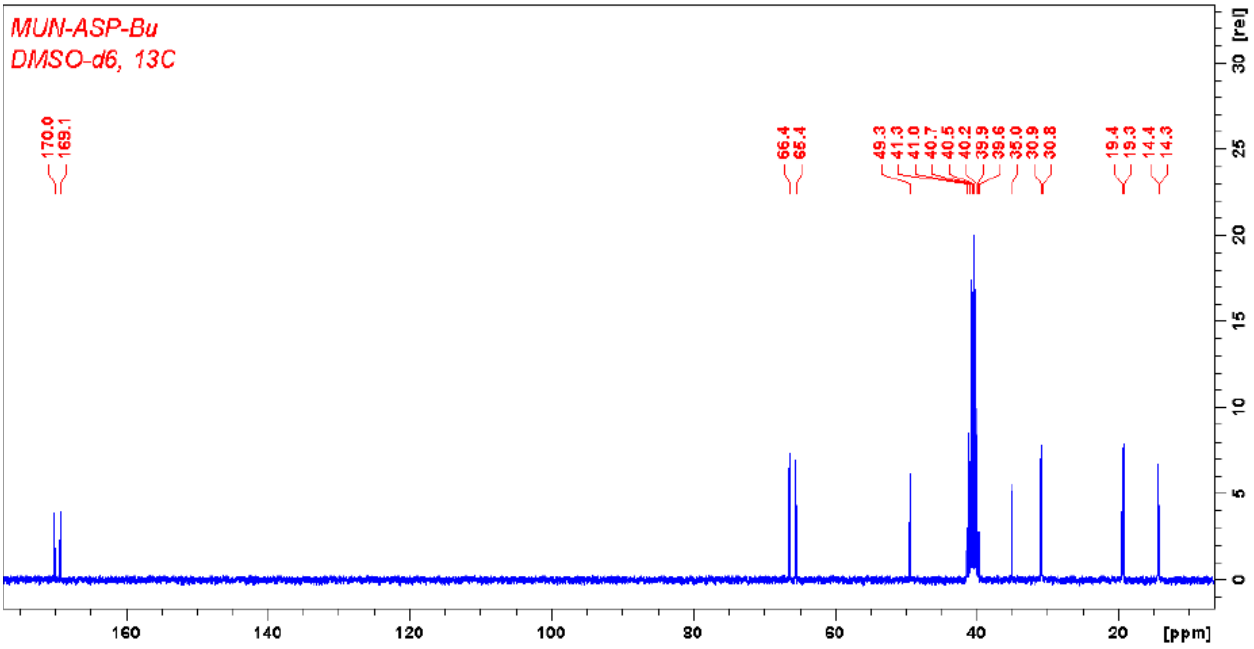
Compound **6**

^{1}H spectrum



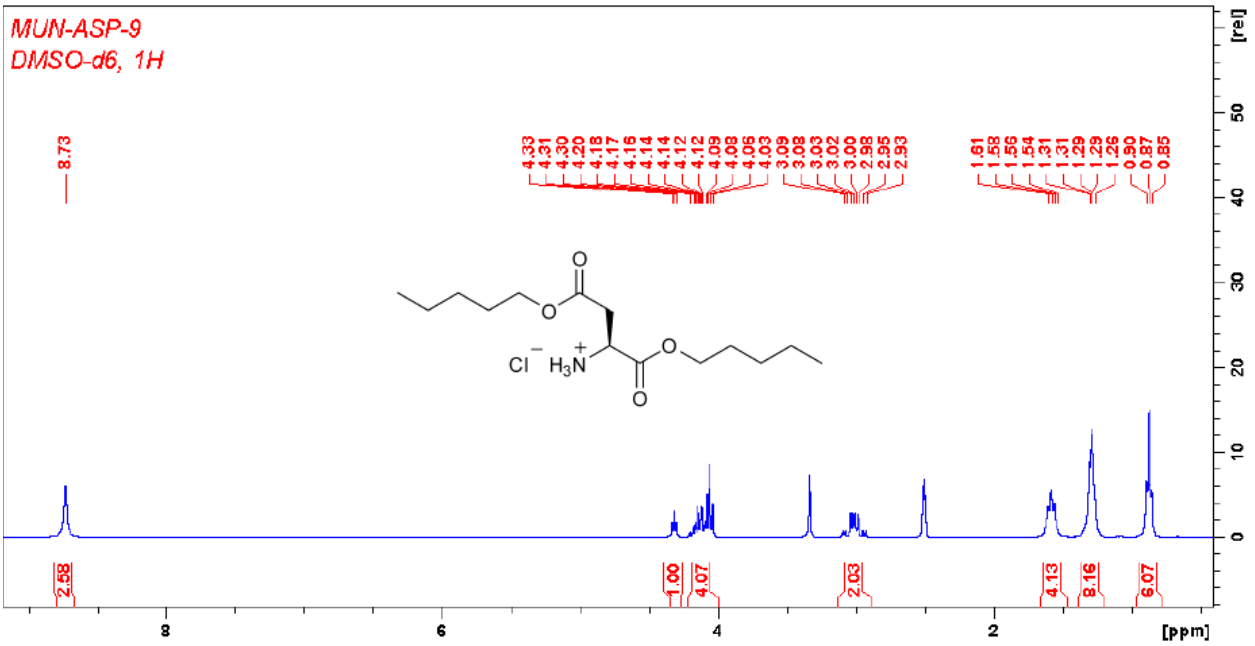
Compound **6**

^{13}C spectrum



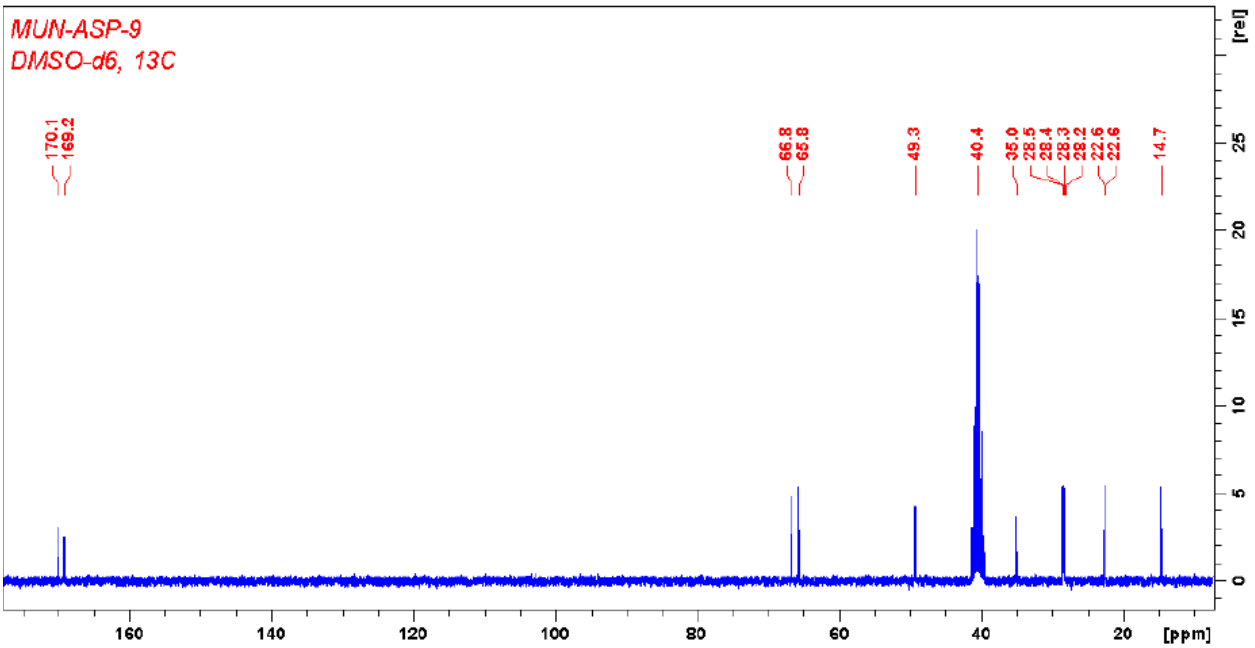
Compound **7**

^{1}H spectrum



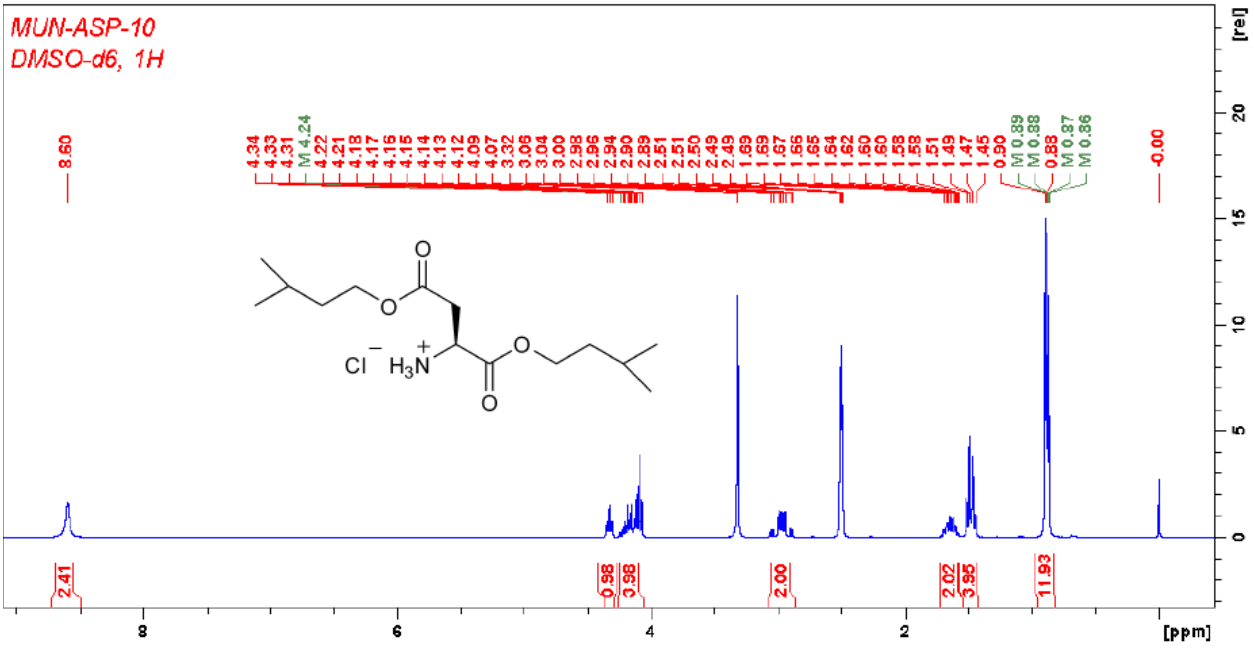
Compound **7**

^{13}C spectrum



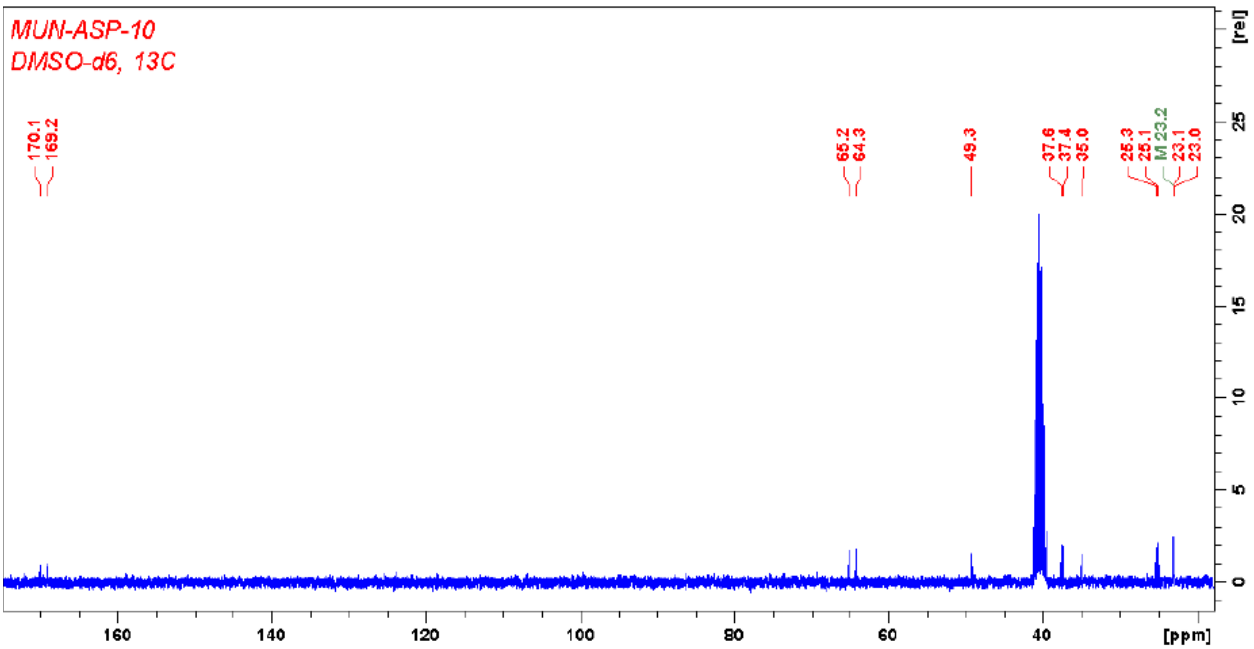
Compound **8**

^{1}H spectrum



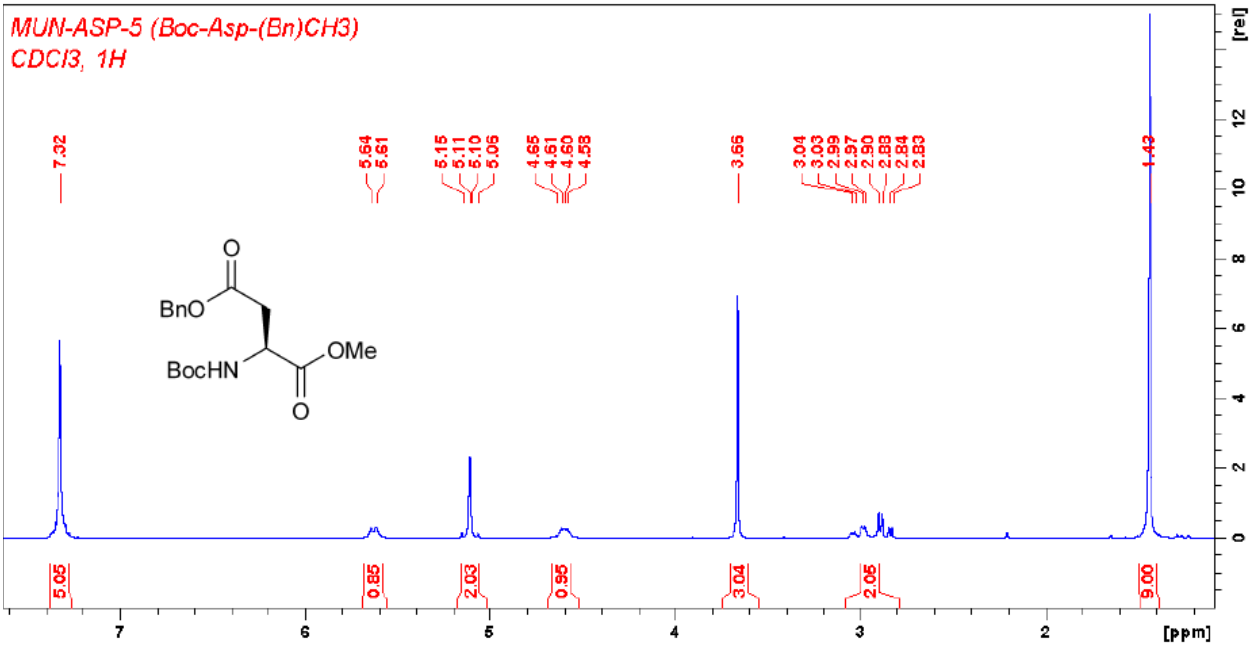
Compound **8**

^{13}C spectrum



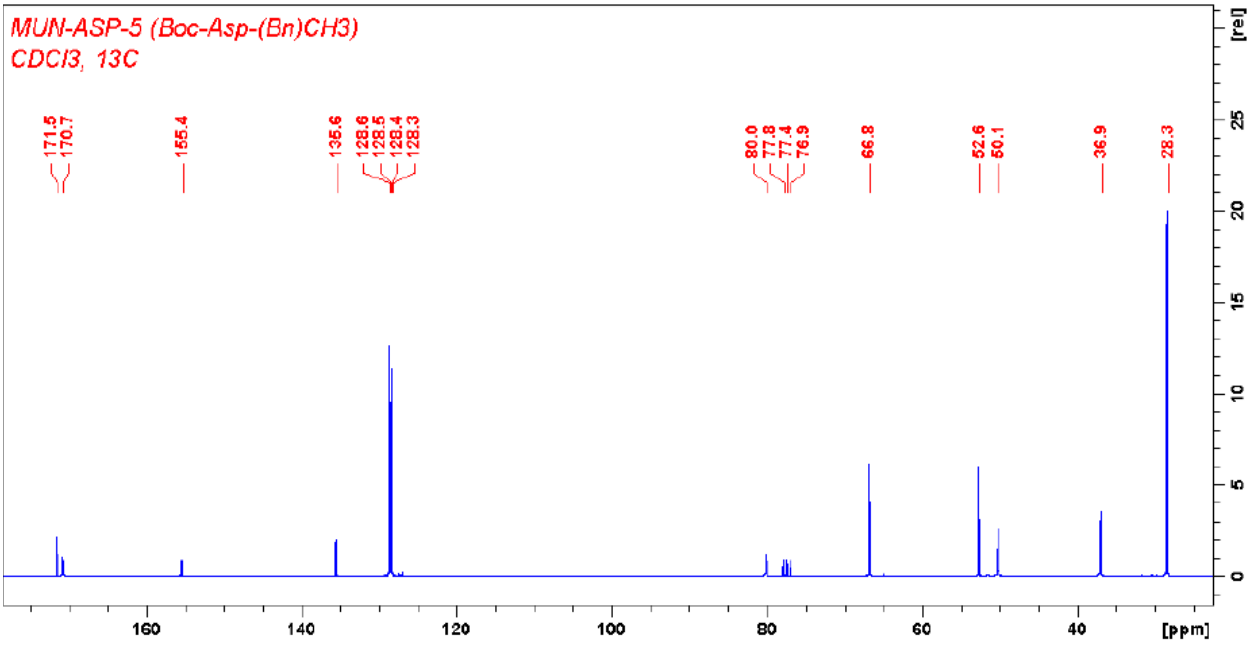
Compound **10**

^{1}H spectrum



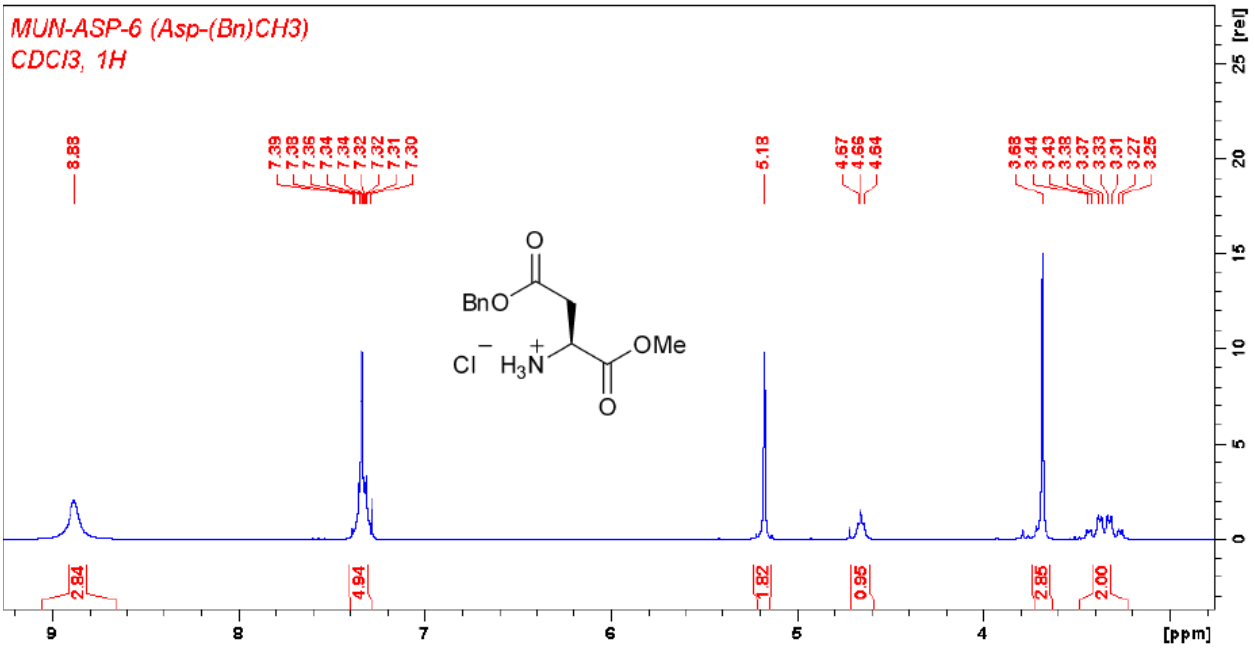
Compound **10**

^{13}C spectrum



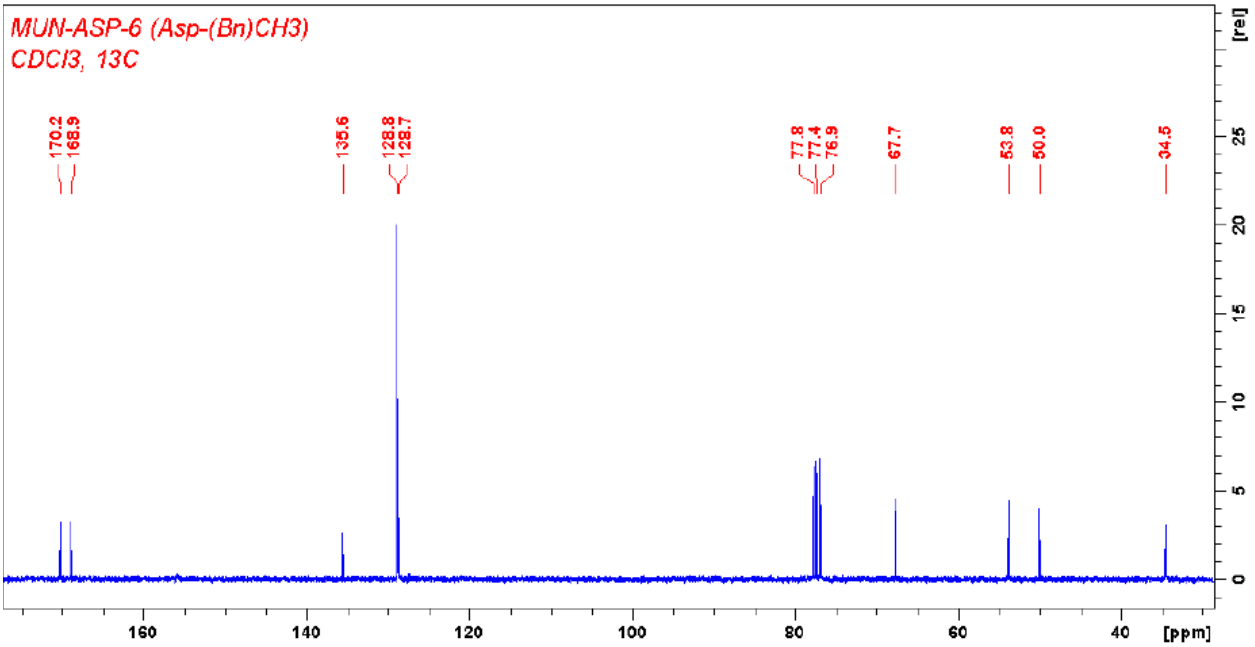
Compound **11**

^{1}H spectrum



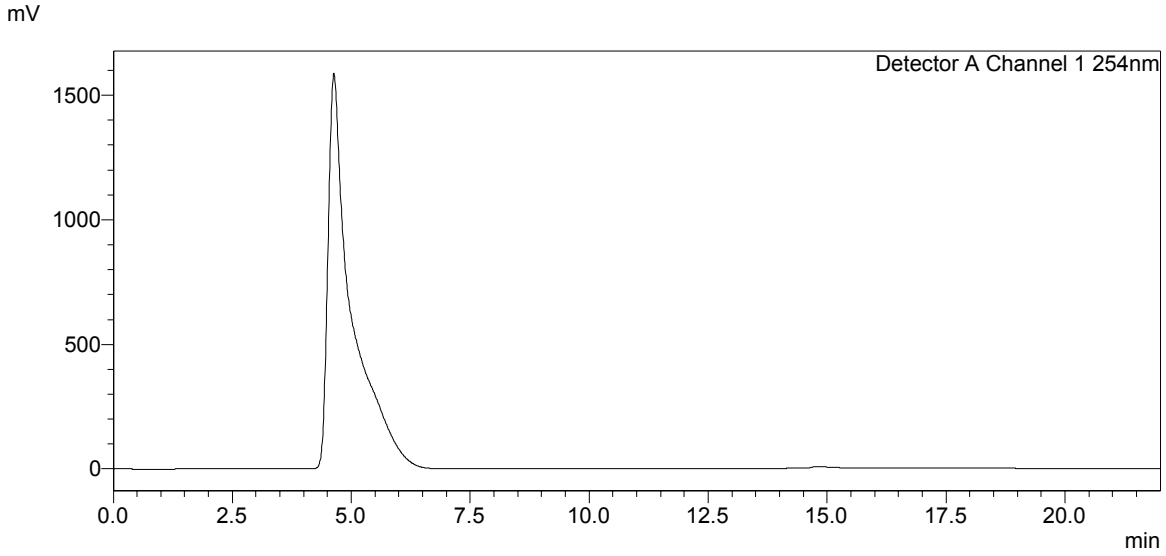
Compound **11**

^{13}C spectrum

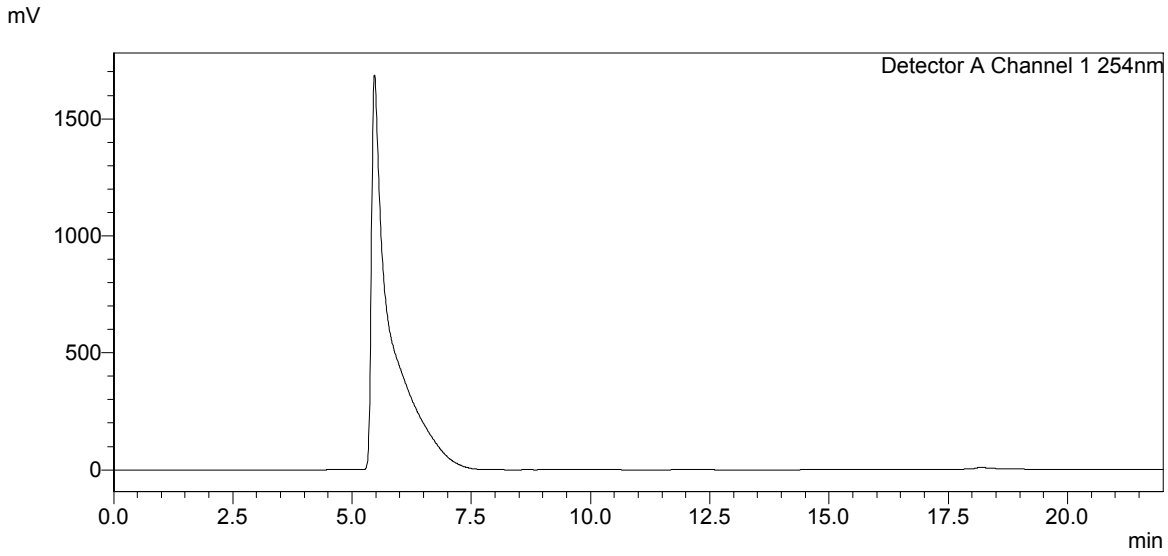


Analytical HPLC for final compounds was performed on a Inertsil ODS-3 (C-18) (4.6 x 100 mm) column, connected to a Shimadzu LC-20AT pump using a Shimadzu SPD-20A UV-detector, using water and acetonitrile as the eluents. The gradient program used contained following steps: 0-15 min 30% CH_3CN in H_2O; 15-25 min 30% to 90% CH_3CN in H_2O; 25-35 min 90% to 30% CH_3CN in H_2O. All recordings were performed at 254 nm.

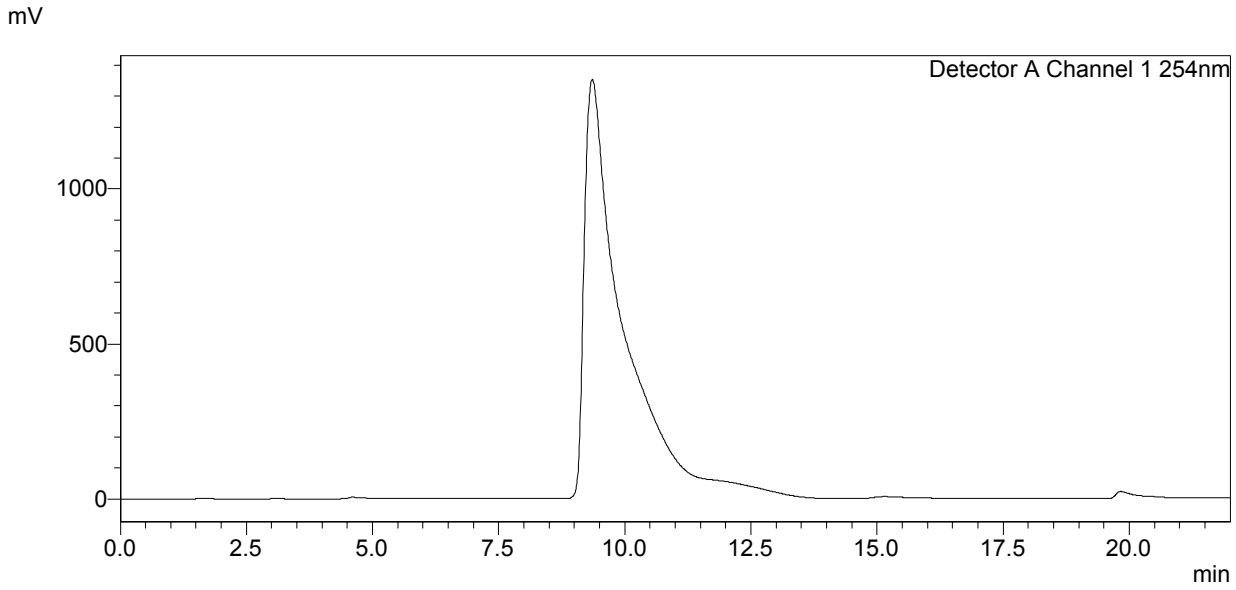
Compound **1**



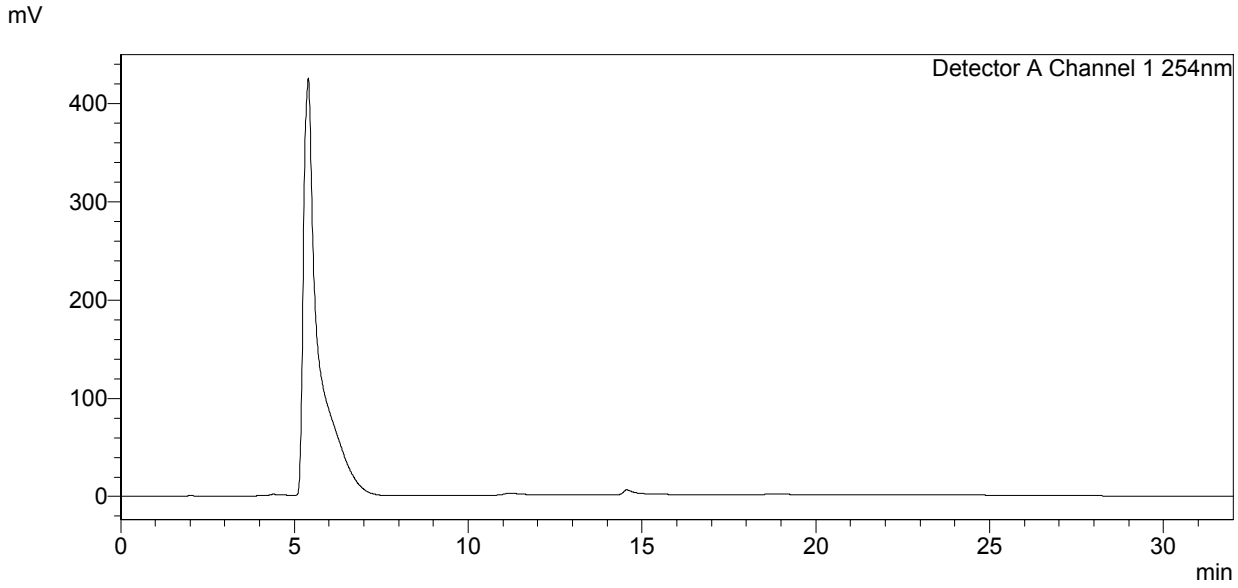
Compound **2**



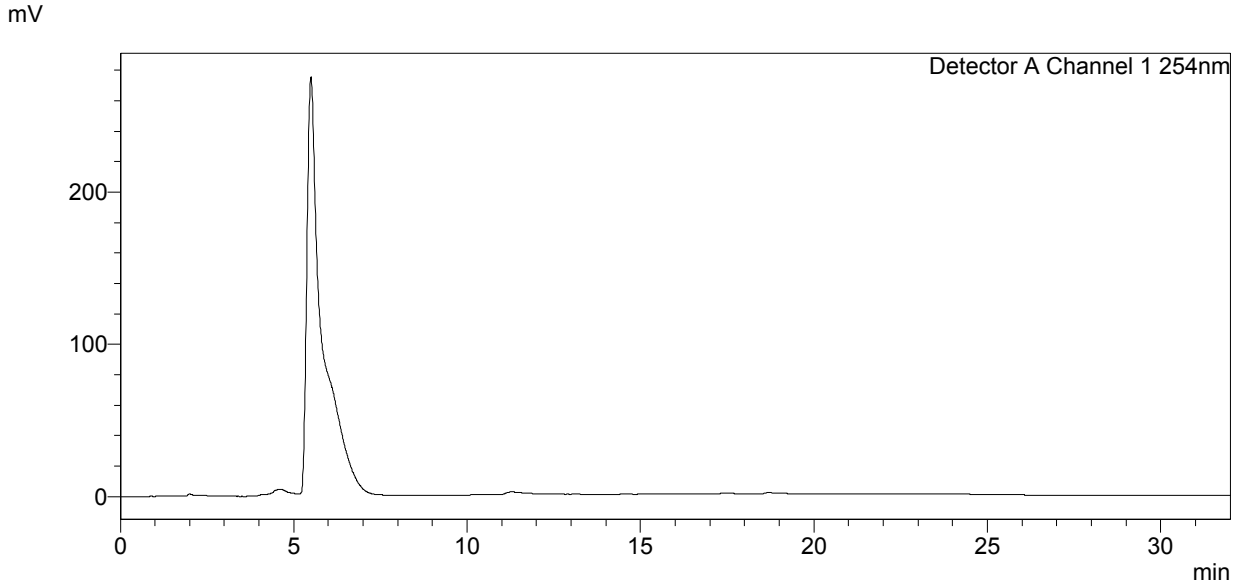
Compound **3**



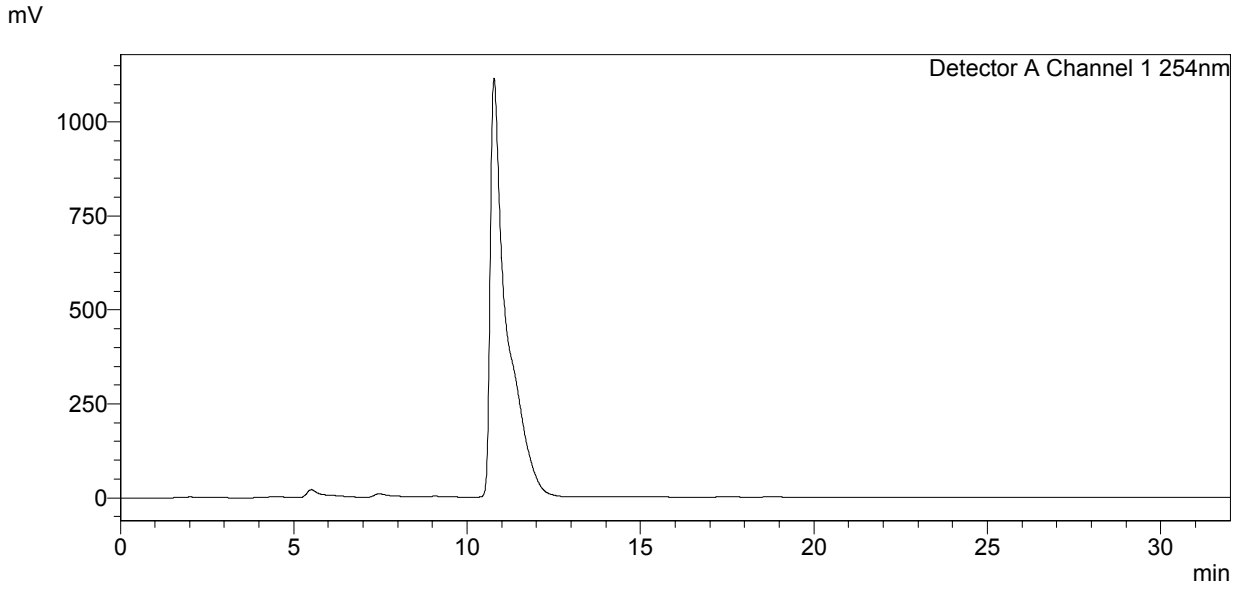
Compound **1a**



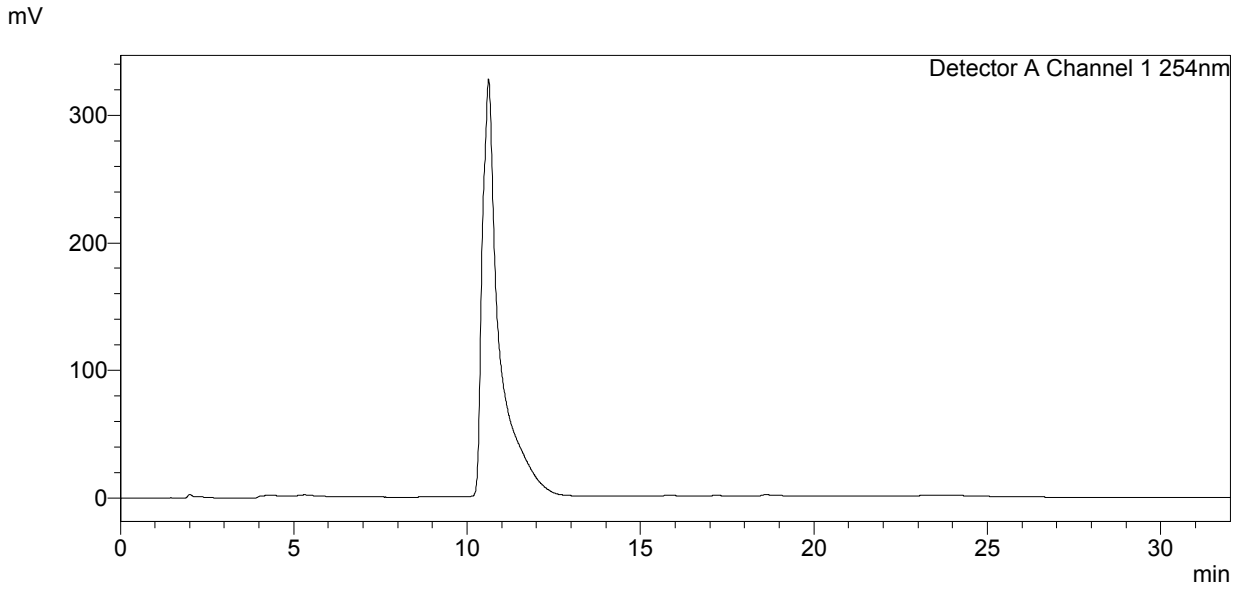
Compound **1b**



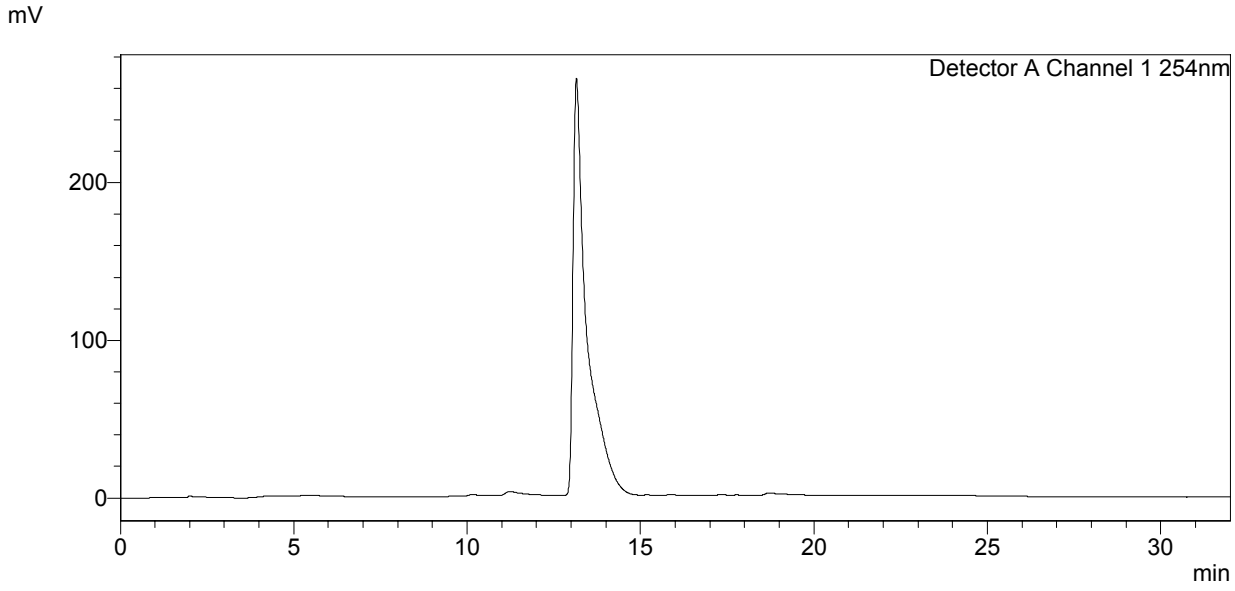
Compound **1c**



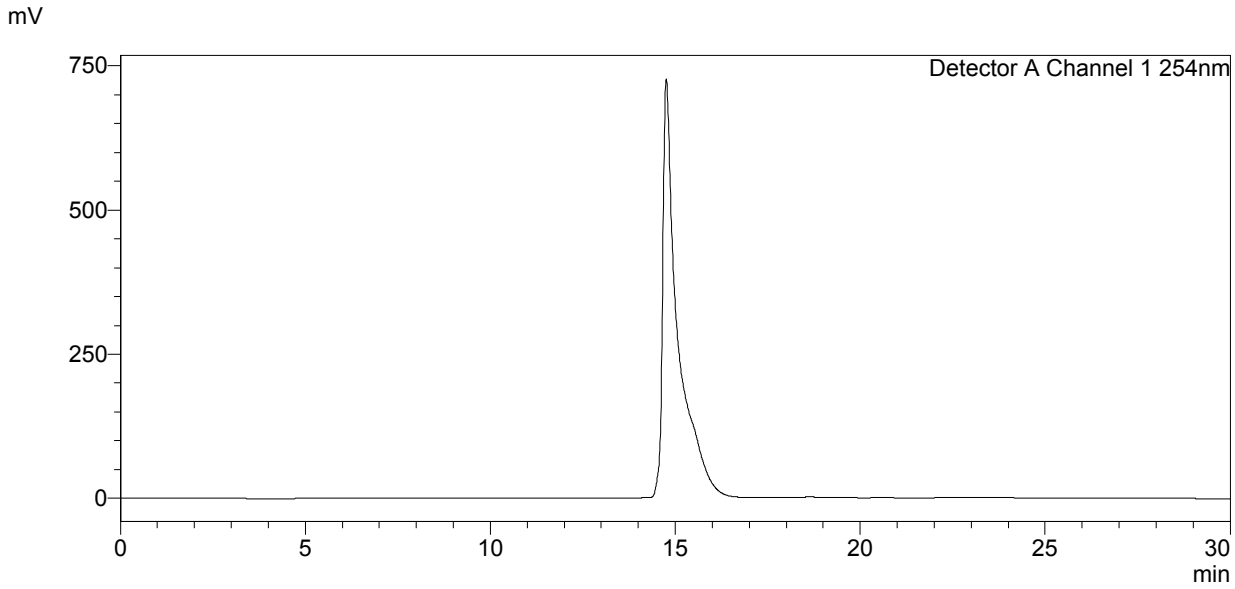
Compound **1d**



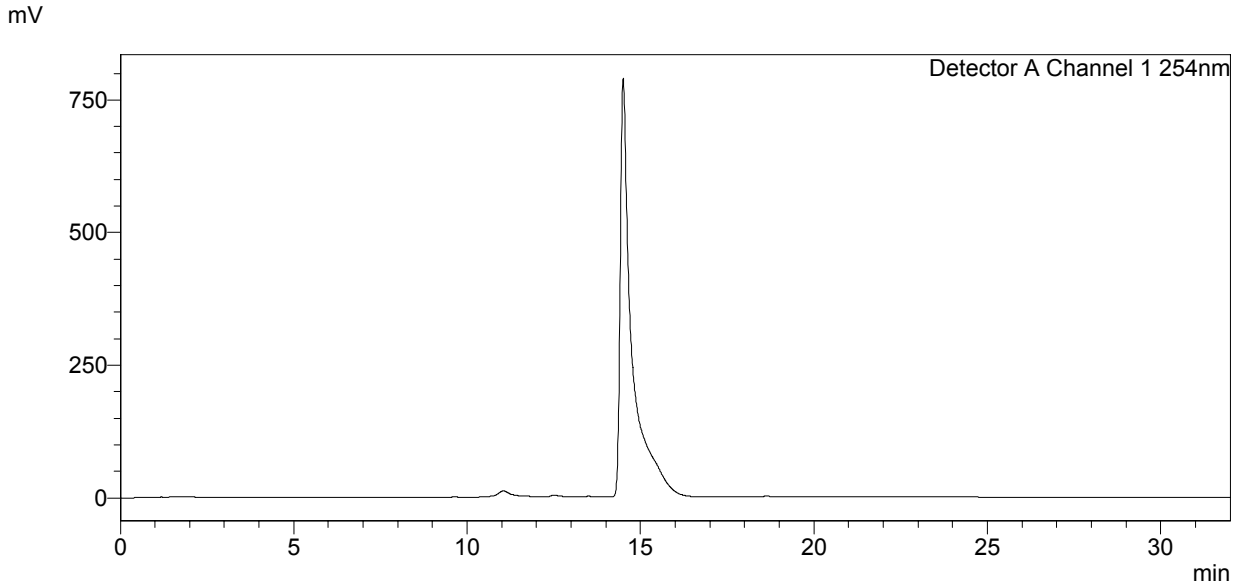
Compound **1e**



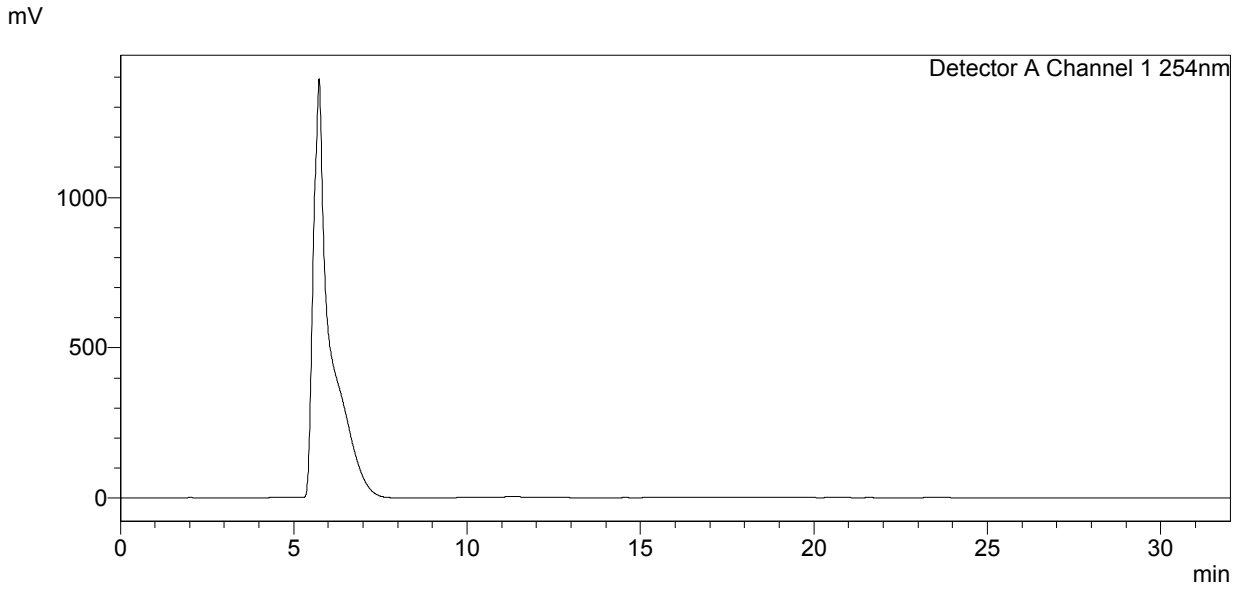
Compound **1f**



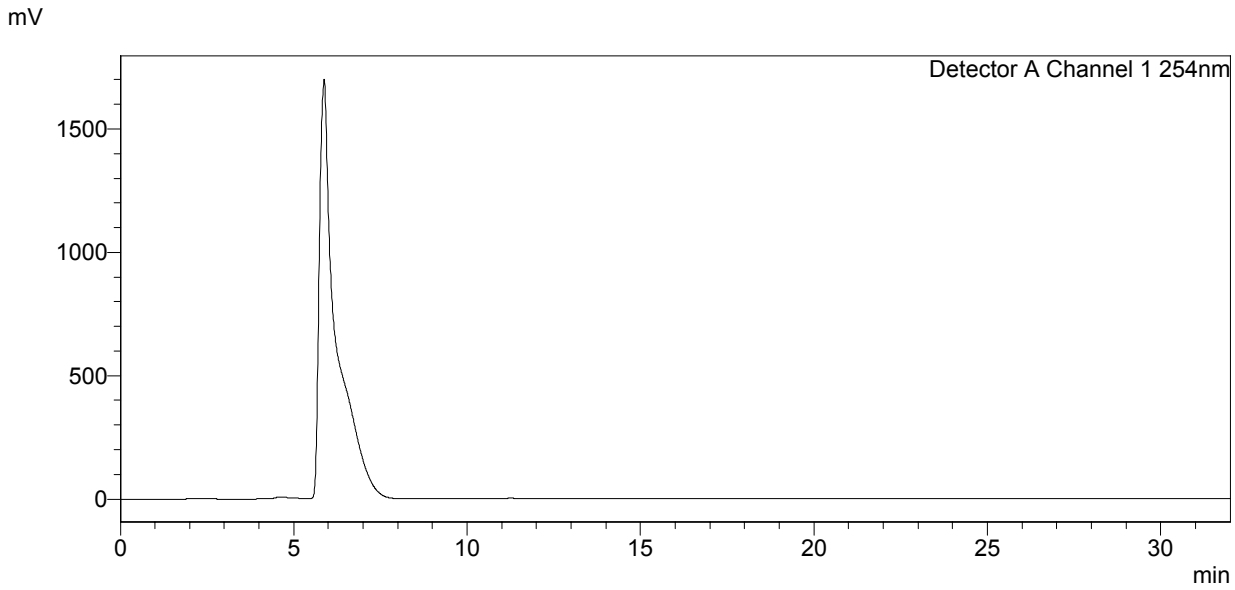
Compound **1g**



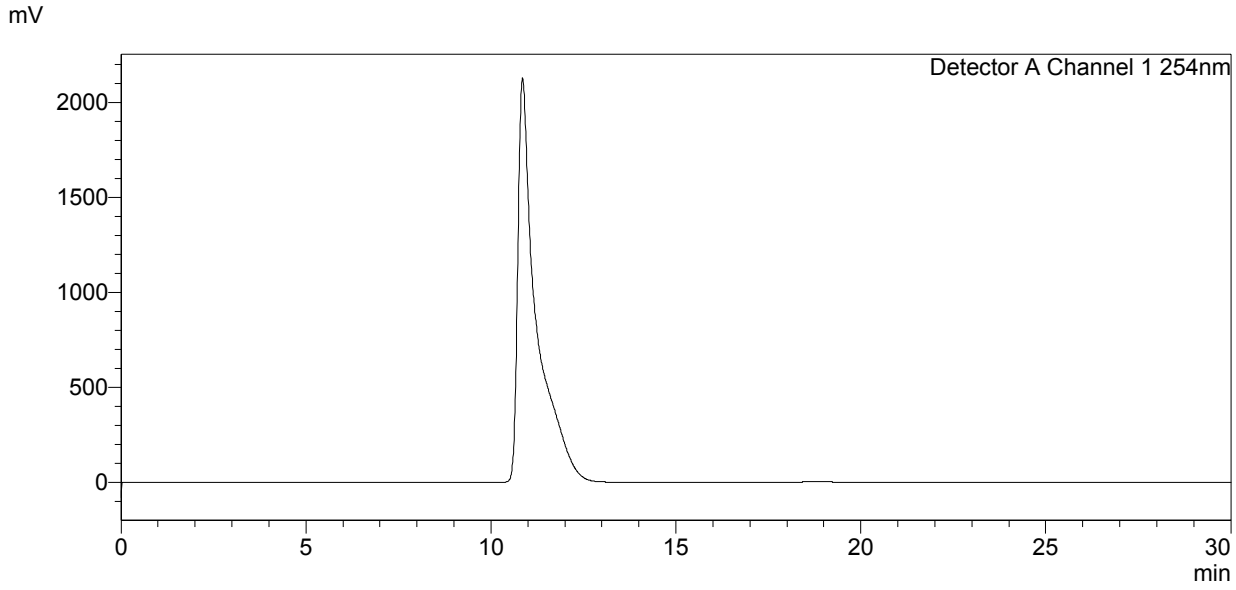
Compound **2a**



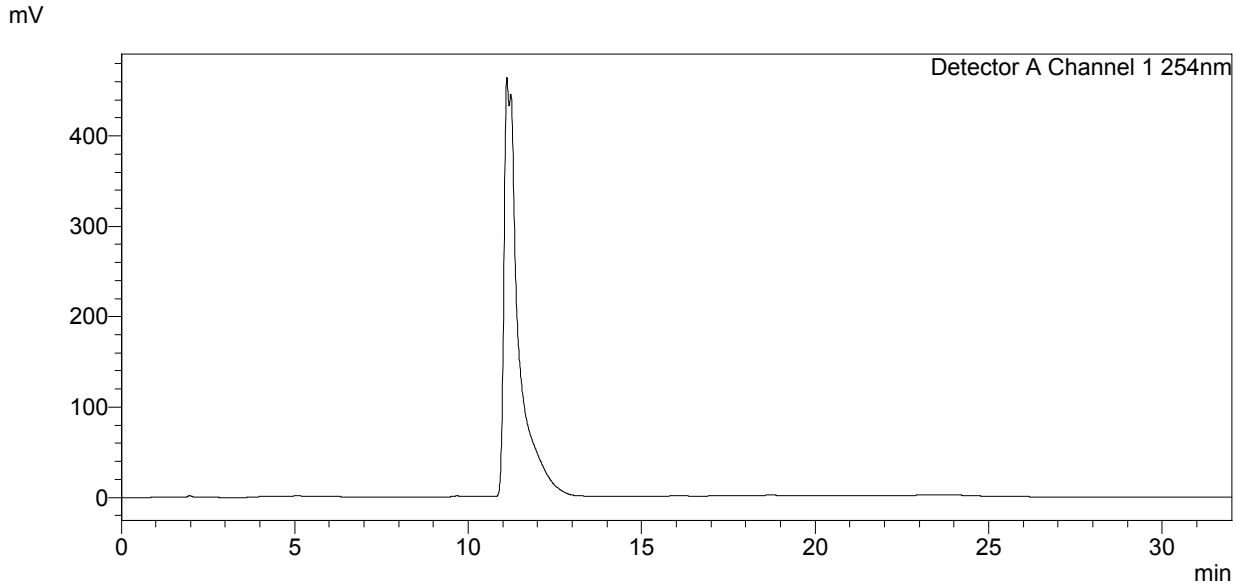
Compound **2b**



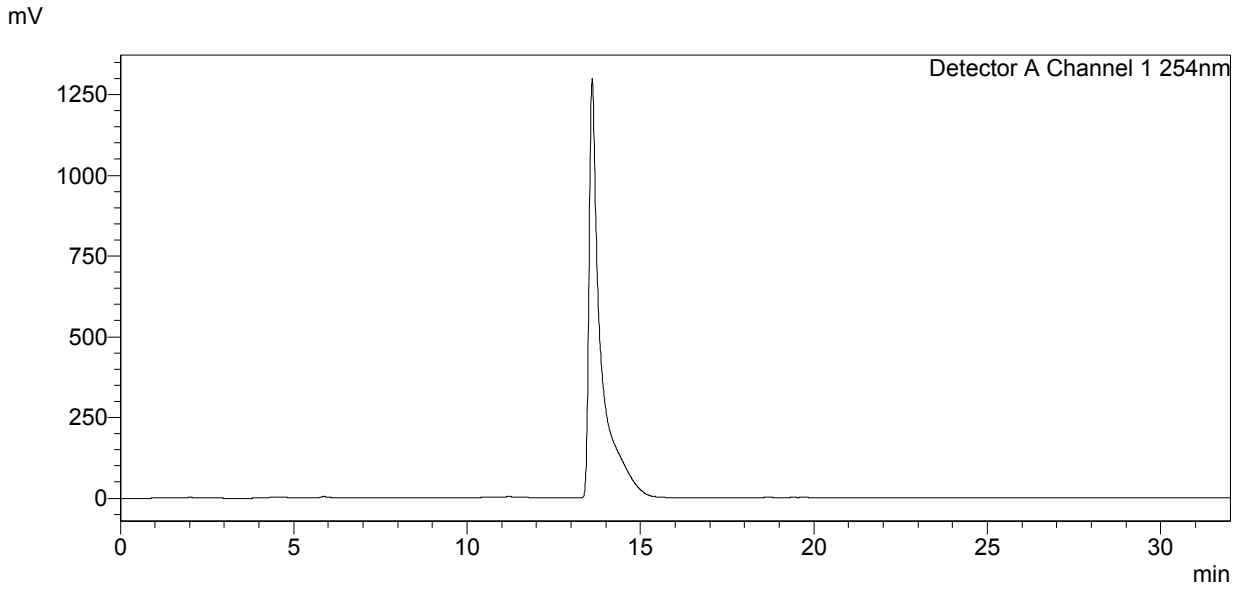
Compound **2c**



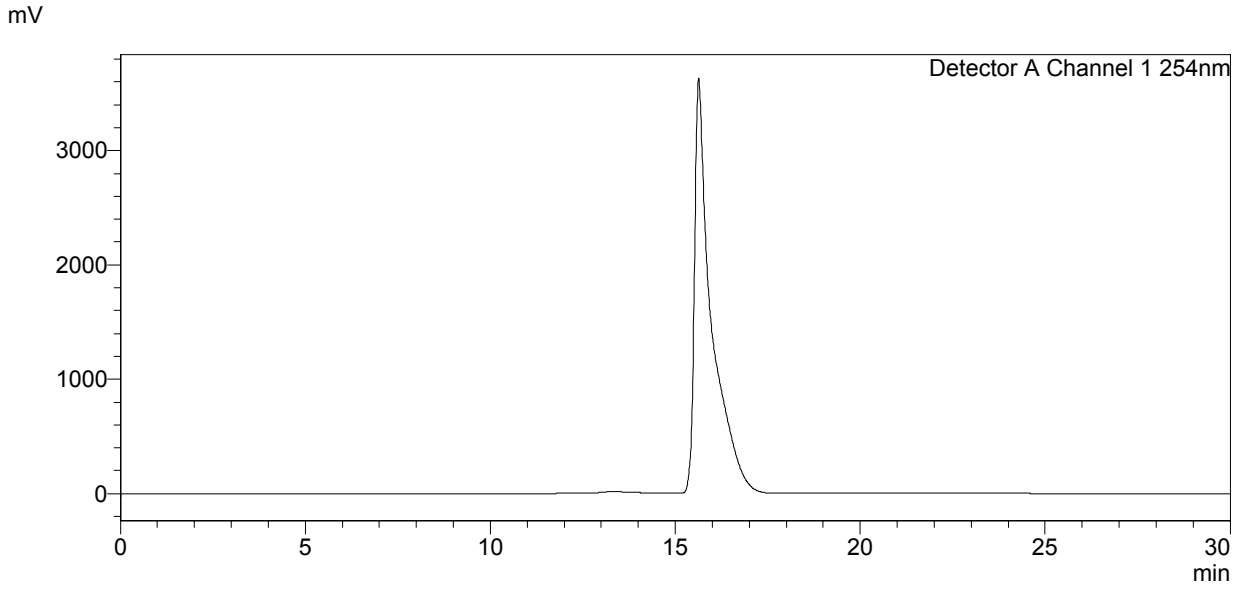
Compound **2d**



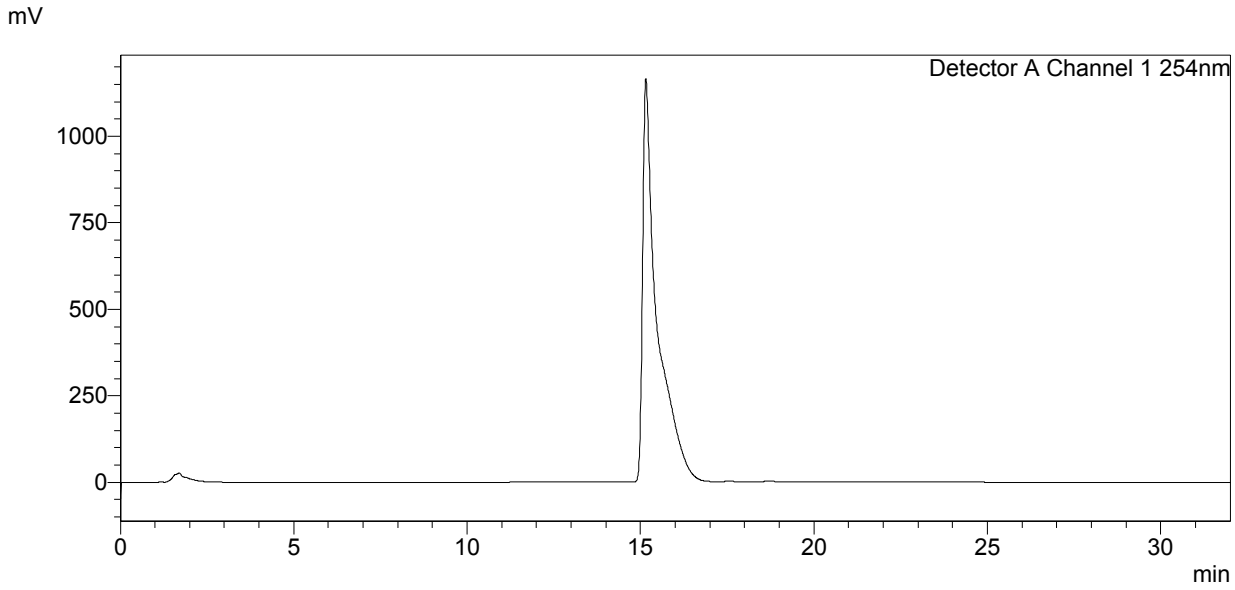
Compound **2e**



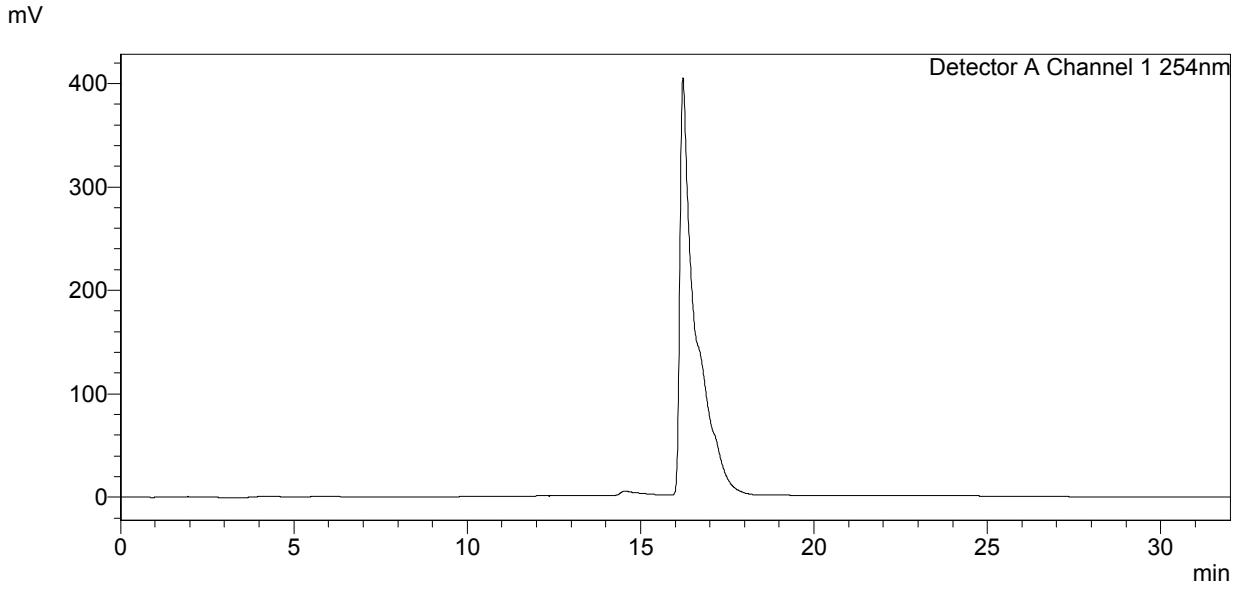
Compound **2f**



Compound **2g**



Compound **3a**



Compound **3b**