

Supporting Information

Deploying solid-phase synthesis to access thymine-containing nucleoside analogs that inhibit DNA repair nuclease SNM1A.

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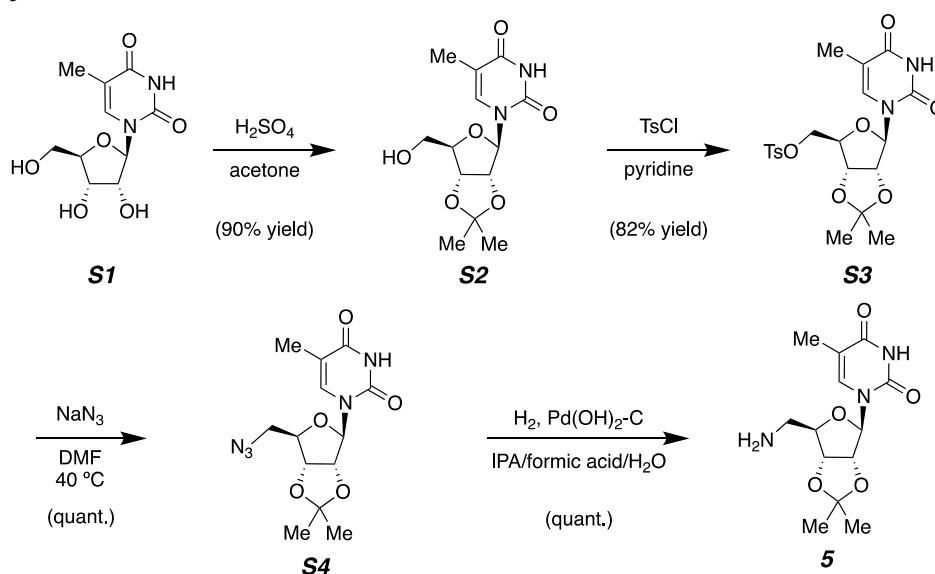
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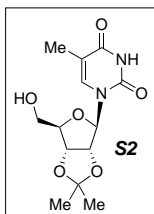
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General Information

Unless otherwise specified, all commercially available reagents were used without further purification. Normal-phase chromatographic purifications were conducted using SiliaFlash Irregular Silica Gel P60, 40 - 63 μm , 60 \AA (Silicycle). Reverse-phase chromatography was performed on a Waters 1525 binary HPLC mounted with a preparative column (Phenomenex P#: 00G-4252-P0-AX, Luna 5 μm C18(2) 100 \AA , LC Column 250 x 21.2 mm), a flow rate of 10 mL/min of MeCN (0.1% TFA) and H₂O (0.1% TFA), and monitored at 260 nm. Thin-layer chromatography (TLC) was performed on Agela Technologies silica gel TLC plates. All compounds were characterized by ¹H, ¹³C NMR, and HRMS. ¹H, ¹³C, and ¹⁹F NMR spectra were recorded on Bruker Avance-III 400 MHz or Bruker 600 MHz spectrometer. All ¹³C and ¹⁹F spectra are recorded with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane (TMS) with the solvent resonance as the internal standard (¹H and ¹³C: CDCl₃, δ = 7.26 and 77.16; DMSO-*d*₆, δ = 2.50 and 39.52; CD₃OD-*d*₄ δ = 3.31 and 49.0; D₂O δ = 4.79). For samples in D₂O, the spectrometer uses the absolute deuterium (D₂O) lock frequency for all multinuclear chemical shift calibrations. ¹⁹F NMR spectra are reported in ppm from residual trifluoroacetic acid (TFA) as an internal standard (CF₃CO₂H δ = -76.55 ppm). Multiplicities are indicated as: s = singlet, d = doublet, t = triplet, m = multiplet or unresolved. NMR assignments are estimated using the ChemNMR feature on ChemDraw 20.0 set to 400 MHz in DMSO. Additionally, yields refer to chromatographically and spectroscopically pure products unless otherwise noted. High-resolution mass spectral analyses were obtained on a Q-TOF high-resolution Agilent 6545 mass spectrometer coupled to an Agilent Infinity 1260 LC system with a Jet Stream ESI source. The AmpC β -lactamase assay was monitored on a SynergyH1 multi-mode plate reader (Biotek).

Scheme S1. Solution-phase synthesis of the C2',3'-O-acetal-protected-C5'-amino-deoxymethyluridine.



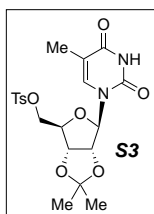


1-((3aR,4R,6R,6aR)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**S2**). Methyl uridine (**S1**) (3 g, 11.617 mmol, 1 equiv) was suspended in acetone (71 mL) and 1.34 mL of H₂SO₄ (96%, 68.4 mmol, 6 equiv) was added dropwise. After the addition of H₂SO₄, the solid dissolved into the solution. The reaction proceeded at ambient temperature overnight (18 h). The reaction was quenched with Et₃N (6 mL) and diluted with H₂O. The pH was adjusted to 7 with 0.5 M HCl. Once the optimal pH was achieved, the desired product was extracted with CH₂Cl₂ (6x 200 mL) and dried over Na₂SO₄. The solution was filtered and concentrated to a solid which was purified by silica gel chromatography (5-15% MeOH/CH₂Cl₂) to isolate the acetal-protected compound in 90% yield (3.09 g). Spectroscopic data matched literature spectrum.¹

¹H NMR (400 MHz, CDCl₃) δ 8.54 (s, 1H), 7.13 (q, *J* = 1.2 Hz, 1H), 5.49 (d, *J* = 3.2 Hz, 1H), 5.09 (dd, *J* = 6.5, 3.2 Hz, 1H), 4.98 (dd, *J* = 6.5, 3.5 Hz, 1H), 4.27 (q, *J* = 3.2 Hz, 1H), 3.92 (dd, *J* = 12.1, 2.6 Hz, 1H), 3.80 (dd, *J* = 12.2, 3.4 Hz, 1H), 1.92 (d, *J* = 1.2 Hz, 3H), 1.58 (s, 3H), 1.36 (s, 3H).

¹³C NMR (101 MHz, CDCl₃) δ 163.4, 150.3, 139.1, 114.5, 111.3, 96.6, 86.7, 83.2, 80.3, 62.8, 27.3, 24.5, 11.6.

R_f(5% MeOH/CH₂Cl₂)= 0.86.



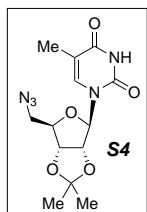
((3aR,4R,6R,6aR)-2,2-dimethyl-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 4-methylbenzenesulfonate (**S3**). Compound **S2** (2 g, 6.7 mmol) and DMAP (81.7 mg, 0.67 mmol, 0.1 equiv) was dissolved in 40 mL CH₂Cl₂ and 11 mL pyridine. Then tosyl chloride (1.52 g, 8 mmol, 1.3 equiv) was dissolved in 16 mL CH₂Cl₂ and added dropwise to the methyl uridine mixture. The mixture was heated to 30 °C under nitrogen for 18 h. The reaction was removed from heat and diluted with CH₂Cl₂ (200 mL) and washed with 0.5 M HCl (3 x 200 mL). The organic layer was dried over Na₂SO₄ and concentrated. The crude product was purified by silica gel chromatography using a mobile phase eluent of 20-50% acetone/hexanes to provide a white solid in 82% yield (3.031 g). Spectroscopic data matched literature spectrum.²

The mixture was heated to 30 °C under nitrogen for 18 h. The reaction was removed from heat and diluted with CH₂Cl₂ (200 mL) and washed with 0.5 M HCl (3 x 200 mL). The organic layer was dried over Na₂SO₄ and concentrated. The crude product was purified by silica gel chromatography using a mobile phase eluent of 20-50% acetone/hexanes to provide a white solid in 82% yield (3.031 g). Spectroscopic data matched literature spectrum.²

¹H NMR (400 MHz, CDCl₃) δ 8.32 (s, 1H), 7.83 – 7.76 (m, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 1.4 Hz, 1H), 5.74 (d, *J* = 2.4 Hz, 1H), 4.93 (dd, *J* = 6.4, 2.4 Hz, 1H), 4.80 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.38 – 4.21 (m, 3H), 2.47 (s, 3H), 1.96 (d, *J* = 1.2 Hz, 3H), 1.57 (s, 3H), 1.35 (s, 3H).

¹³C NMR (101 MHz, CDCl₃) δ 163.3, 149.9, 145.3, 137.6, 132.5, 130.0, 128.0, 114.7, 111.5, 93.9, 84.5, 84.2, 80.3, 69.3, 27.1, 25.2, 21.7, 12.3.

$R_f(1:2 \text{ acetone/hexanes})= 0.32.$

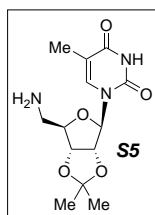


(3aR,4R,6R,6aR)-2,2-dimethyl-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 4-methylbenzenesulfonate (S4). Tosyl-methyl uridine **S3** (1 g, 2.21 mmol) and sodium azide (1.436 g, 22.1 mmol, 10 equiv) were dissolved in 22.1 mL DMF (0.1 M) and heated to 40 °C under nitrogen for 18 h. The reaction was removed from heat and diluted with EtOAc (200 mL) and washed with LiCl/NaCl (saturated in H₂O, 2 x 200 mL), followed by H₂O (200 mL). The

organic layer was dried over Na₂SO₄ and concentrated. The compound was used directly without further purification (98% crude yield, 700.4 mg). Spectroscopic data matched literature spectrum.³

¹H NMR (400 MHz, DMSO) δ 11.45 (s, 1H), 7.58 (d, J = 1.4 Hz, 1H), 5.81 (d, J = 2.4 Hz, 1H), 5.07 (dd, J = 6.5, 2.5 Hz, 1H), 4.77 (dd, J = 6.5, 4.1 Hz, 1H), 4.13 (td, J = 5.5, 4.1 Hz, 1H), 3.59 (d, J = 5.6 Hz, 2H), 1.78 (d, J = 1.2 Hz, 3H), 1.49 (s, 3H), 1.30 (s, 3H).

$R_f(1:2 \text{ acetone/hexanes})= 0.63.$



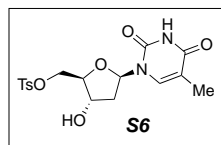
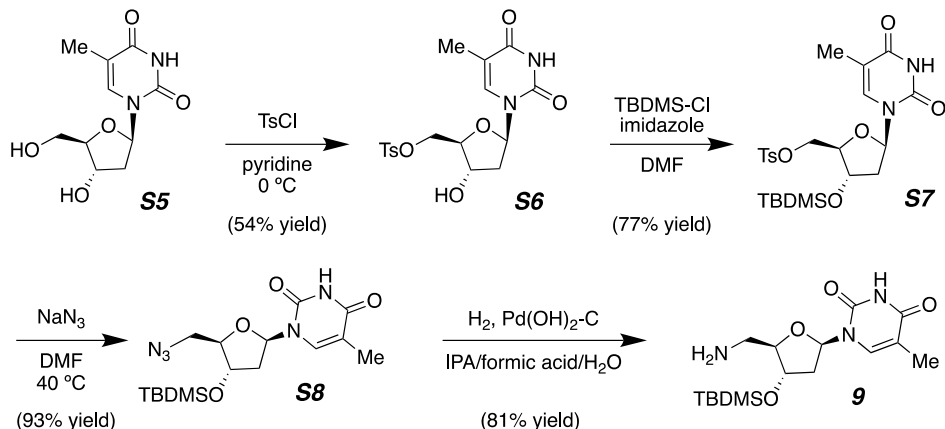
1-((3aR,4R,6R,6aR)-6-(aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (S5). Azido-methyl uridine **S4** (2.21 mmol) and Pd(OH)₂ on carbon (62 mg, 0.442 mmol, 0.2 equiv) was suspended in 16 mL of 2-propanol/formic acid/H₂O (6:1:1). Hydrogen was bubbled through the stirring solution until disappearance of the starting material was

observed by TLC (4 h). The mixture was directly filtered through a bed of celite and concentrated to isolate a yellow foam in quantitative yield (>99% crude yield, 769.6 mg). Spectroscopic data matched literature spectrum.⁴

¹H NMR (400 MHz, MeOD) δ 8.42 (s, 1H), 7.52 (d, J = 1.4 Hz, 1H), 5.68 (d, J = 1.7 Hz, 1H), 5.23 (dd, J = 6.4, 1.6 Hz, 1H), 4.32 (dt, J = 8.4, 3.6 Hz, 1H), 3.38 (dd, J = 13.1, 9.0 Hz, 1H), 3.29 (dd, J = 13.2, 3.4 Hz, 1H), 1.90 (d, J = 1.1 Hz, 2H), 1.56 (s, 2H), 1.38 (s, 2H).

$R_f(1:2 \text{ acetone/hexanes})= 0.04.$

Scheme S2. Solution-phase synthesis of the C3'-O-TBDMS-protected-C5'-amino-deoxythymidine.

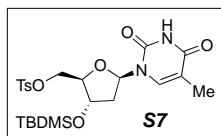


((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate (S6).

Thymidine (**S5**) (1.63 g, 6.73 mmol, 1 equiv) was dissolved in pyridine (11.37 mL, 0.59 M) and cooled to 0 °C. Tosyl chloride (1.625 g, 8.55 mmol, 1.27 equiv) was dissolved in 1.63 mL pyridine and added dropwise to the stirring thymidine solution maintained at 0 °C. The reaction was allowed to warm to ambient temperature and stirred overnight (20 h). The reaction was quenched with the addition of cold H₂O (4 °C, 65 mL, 0.1035 M) resulting in the product crashing out of solution. The solid was collected and washed with Et₂O followed by hexanes. The product was isolated in 54% yield (1.45 g) and used in the next step without further purification. Spectroscopic data matched literature spectrum.⁵

¹H NMR (400 MHz, DMSO) δ 11.32 (s, 1H), 7.82 – 7.74 (m, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 1.4 Hz, 1H), 6.15 (t, *J* = 6.9 Hz, 1H), 4.26 (dd, *J* = 10.9, 3.3 Hz, 1H), 4.19 – 4.13 (m, 2H), 3.87 (dt, *J* = 5.8, 3.6 Hz, 1H), 2.41 (s, 3H), 2.19 – 2.10 (m, 1H), 2.10 – 2.04 (m, 1H), 1.77 (d, *J* = 1.2 Hz, 3H).

R_f(10% MeOH/CH₂Cl₂) = 0.56.



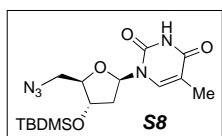
((2R,3S,5R)-3-((tert-butyldimethylsilyloxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate (S7).

Tosyl-appended thymidine **S6** (1 g, 2.52 mmol, 1 equiv), TBDMS-Cl (456 mg, 3.027 mmol, 1.2 equiv), and imidazole (428.4 mg, 6.3 mmol, 2.5 equiv) were combined and dissolved in 3.23 mL DMF (0.78 M) and allowed to stir at ambient temperature under nitrogen for 6 h 30 min. After this time, the reaction was diluted with EtOAc (200 mL) and washed with H₂O (3 x 150 mL) and brine (3 x 150 mL). The organic layer was dried

over Na₂SO₄, filtered, and concentrated. The crude residue was purified by silica gel chromatography using a stepwise mobile phase gradient of 1:2→1:1→100:0 EtOAc/hexanes to provide the TBDMS-protected nucleoside in 77% yield (0.994 g). Spectroscopic data matched literature spectrum.⁶

¹H NMR (400 MHz, CDCl₃) δ 7.85 – 7.66 (m, 2H), 7.41 – 7.34 (m, 3H), 6.30 (t, *J* = 6.7 Hz, 1H), 4.36 (dt, *J* = 6.8, 3.6 Hz, 1H), 4.24 (dd, *J* = 10.9, 2.8 Hz, 1H), 4.16 (dd, *J* = 11.0, 2.5 Hz, 1H), 3.98 (q, *J* = 2.9 Hz, 1H), 2.46 (s, 3H), 2.25 (ddd, *J* = 13.6, 6.2, 3.6 Hz, 1H), 2.11 (dt, *J* = 13.5, 6.7 Hz, 1H), 1.95 (d, *J* = 1.2 Hz, 3H), 0.85 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H).

R_f(1:1 EtOAc/hexanes)= 0.33.

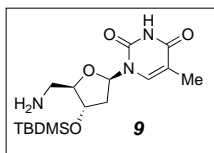


1-((2*R*,4*S*,5*R*)-5-(azidomethyl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (**S8**). TBDMS-protected, tosyl thymidine **S7** (200 mg, 0.392 mmol) and NaN₃ (91 mg, 1.396 mmol, 3.56 equiv) were suspended in 1.86 mL DMF (0.21

M) and heated to 55 °C for 18 h. The reaction was then allowed to cool to ambient temperature and diluted with H₂O (150 mL). The product was extracted with EtOAc (150 mL x 3), and the combined organics were washed with brine (150 mL), dried over Na₂SO₄, filtered, and concentrated. The crude product was purified by silica gel chromatography using a mobile phase eluent of 20-50% EtOAc/hexanes. The desired product was isolated in 93% yield (139.2 mg, off-white sticky solid). Spectroscopic data matched literature spectrum.⁷

¹H NMR (400 MHz, CDCl₃) δ 9.27 (s, 1H), 7.26 (d, *J* = 1.5 Hz, 1H), 6.19 (t, *J* = 6.6 Hz, 1H), 4.29 (dt, *J* = 6.9, 4.4 Hz, 1H), 3.88 (q, *J* = 3.8 Hz, 1H), 3.64 (dd, *J* = 13.3, 3.4 Hz, 1H), 3.44 (dd, *J* = 13.3, 3.7 Hz, 1H), 2.23 (ddd, *J* = 13.6, 6.6, 4.3 Hz, 1H), 2.12 (dt, *J* = 13.6, 6.8 Hz, 1H), 1.89 (d, *J* = 1.3 Hz, 3H), 0.83 (s, 9H), 0.03 (s, 6H).

R_f(1:1 EtOAc/hexanes)= 0.55.



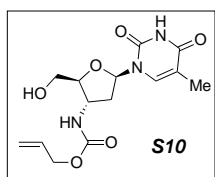
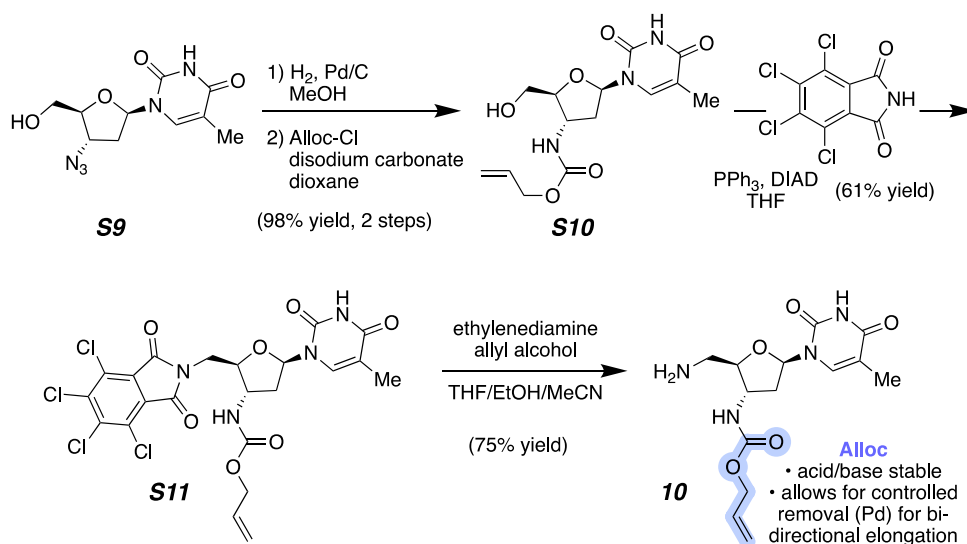
1-((2*R*,4*S*,5*R*)-5-(aminomethyl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (**9**). The azido, TBDMS-protected thymidine **S8** (200 mg, 0.524 mmol, 1 equiv) and palladium hydroxide on carbon (15 mg, 0.1048 mmol, 0.2 equiv)

were dissolved in 5.24 mL of 2-propanol/formic acid/H₂O (6:1:1). Hydrogen gas was bubbled through the stirring solution until disappearance of the starting material was observed by TLC (2 h). The mixture was directly filtered through a bed of celite and concentrated to isolate a yellow-sticky foam in 81% yield (186.3 mg). Spectroscopic data matched literature spectrum.⁸

¹H NMR (400 MHz, CDCl₃) δ 7.32 (s, 1H), 6.04 (t, *J* = 6.8 Hz, 1H), 4.41 (dt, *J* = 7.3, 4.0 Hz, 1H), 3.95 (dq, *J* = 7.5, 4.7, 4.1 Hz, 1H), 3.16 (dd, *J* = 13.4, 3.8 Hz, 1H), 3.05 (dd, *J* = 13.1, 6.4 Hz, 1H), 2.48 – 2.34 (m, 1H), 2.26 – 2.16 (m, 1H), 1.92 – 1.84 (m, 3H), 0.87 (s, 9H), 0.08 (d, *J* = 1.2 Hz, 6H).

R_f(20% MeOH/CH₂Cl₂) = 0.28.

Scheme S3. Solution-phase synthesis of the C3'-N-Alloc-protected-C5'-amino-deoxythymidine.



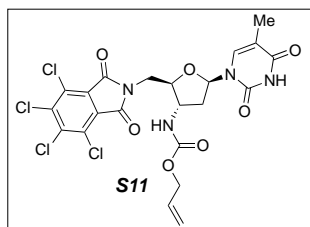
allyl ((2*S*,3*S*,5*R*)-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl)carbamate (**S10**). Compound **S10** was synthesized using a modified literature procedure.⁹ C3'-azido deoxythymidine (**S9**, Zidovudine) (1 g, 3.74 mmol, 1 equiv) and Pd/C (100 mg,

0.935 mmol, 0.25 equiv) were suspended in MeOH (19.94 mL, 0.1875 M). Then hydrogen gas was continually passed into the mixture through a 22 G x 4" hypodermic needle (Air-Tite) submerged into the solution for 1 h and 16 min. The reaction mixture was filtered through a pad of celite to remove the palladium. The elution was collected and concentrated to produce the C3' amine as a white solid and used in the next step without further purification. The white solid was dissolved in 40 mL of H₂O (0.93 M) and 2 equiv of sodium carbonate (792.8 mg in 19.9 mL H₂O) was added to the stirring solution. The mixture was cooled to 0 °C and Alloc-Cl (1.193 mL, 11.22 mmol, 3 equiv) was added in a dropwise manner. The reaction was stirred at 0 °C for 1 h, and then allowed to warm to ambient temperature with one additional hour of stirring. The reaction was acidified to pH 1 with 5 M HCl and the product was extracted with EtOAc (4 x 100 mL). The

combined organic layers were dried over Na₂SO₄, filtered, and concentrated to provide the C3'-Alloc-amine-thymidine **S10** as a white solid in 98% crude yield over 2 steps (1.196 g). Spectroscopic data matched literature spectrum.¹⁰

¹H NMR (400 MHz, CDCl₃) δ 9.49 (s, 1H), 7.66 (s, 1H), 6.25 (t, *J* = 5.8 Hz, 1H), 6.08 (d, *J* = 7.2 Hz, 1H), 5.91 (ddt, *J* = 16.4, 10.9, 5.7 Hz, 1H), 5.30 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.22 (dd, *J* = 10.4, 1.5 Hz, 1H), 4.58 (d, *J* = 5.6 Hz, 2H), 4.33 (p, *J* = 7.0 Hz, 1H), 3.97 – 3.78 (m, 4H), 3.20 (s, 2H), 2.34 (d, *J* = 7.4 Hz, 3H), 1.89 (s, 4H).

R_f(10% MeOH/CH₂Cl₂) = 0.4.



allyl ((2*R*,3*S*,5*R*)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-((4,5,6,7-tetrachloro-1,3-dioxoisindolin-2-yl)methyl)tetrahydrofuran-3-yl)carbamate (**S11**). Alloc-protected, C3'-aminodeoxythymidine **S10** (500 mg, 1.536 mmol, 1 equiv), 4,5,6,7-tetrachloroisindoline-1,3-dione (551.7 mg, 1.937 mmol, 1.26 equiv),

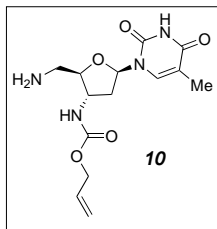
and Ph₃P (508 mg, 1.937 mmol, 1.26 equiv) were suspended in THF (17 mL, 0.09 M). Then diisopropyl azodicarboxylate (DIAD, 380 μL, 1.937 mmol, 1.26 equiv) was added dropwise under nitrogen at ambient temperature. The solids dissolved upon the addition of diisopropyl azodicarboxylate (DIAD) and the reaction was allowed to stir overnight (18 h). Then the reaction was diluted with EtOAc (150 mL) and washed with sat. sodium bicarbonate (100 mL), 6% H₂O₂ in H₂O (100 mL), followed by H₂O (100 mL). The yellow organic layer was dried over Na₂SO₄, filtered, and concentrated. The crude mixture was suspended in 47.9 mL EtOH and the insoluble white solid was collected by filtration; washing with EtOH to remove the remaining yellow color to isolate the phthalimide **S11** in 61% yield (1.104 g).

¹H NMR (400 MHz, DMSO) δ 11.30 (s, 1H), 7.79 (d, *J* = 7.4 Hz, 1H), 6.10 (t, *J* = 6.8 Hz, 1H), 5.86 (ddt, *J* = 16.1, 10.6, 5.4 Hz, 1H), 5.24 (dq, *J* = 17.3, 1.8 Hz, 1H), 5.15 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.42 (d, *J* = 5.4 Hz, 2H), 4.13 (p, *J* = 7.3 Hz, 1H), 4.03 (dt, *J* = 6.9, 5.4 Hz, 1H), 3.97 – 3.82 (m, 2H), 2.40 (dt, *J* = 13.6, 8.0 Hz, 1H), 2.13 (ddd, *J* = 13.7, 6.7, 4.8 Hz, 1H), 1.82 (d, *J* = 1.1 Hz, 3H).

¹³C NMR (101 MHz, DMSO) δ 165.0, 164.2, 163.8, 156.0, 150.8, 138.7, 138.4, 137.1, 133.9, 129.7, 128.7, 128.6, 128.4, 117.5, 110.1, 84.5, 81.0, 65.0, 53.0, 41.4, 36.2, 12.5.

R_f(5% MeOH/CH₂Cl₂) = 0.31.

HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₂₂H₁₉Cl₄N₄O₇Na 612.9822; found 612.9816.



allyl ((2*R*,3*S*,5*R*)-2-(aminomethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl)carbamate (**10**).

Tetrachlorophthalimide-appended thymidine (**S11**) (1 g, 1.688 mmol) was dissolved in MeCN/THF/EtOH/allyl alcohol (2:1:0.72:0.32, 34.5 mL, 0.049 M).

Then ethylene diamine (505 μ L, 7.56 mmol, 4.49 equiv) was added to the stirring solution at ambient temperature. The reaction was concentrated to a yellow oil after 1.5 h and purified by silica gel chromatography. A mobile phase eluent of EtOAc/MeOH (7:3) with 1% Et₃N was used. The desired product was isolated in 75% yield (411.3 mg) as a sticky white solid. Spectroscopic data matched literature spectrum;¹¹ however, due to partial compound solubility in D₂O, the spectrum was obtained in MeOD-*d*₄ as well.

¹H NMR (600 MHz, D₂O) δ 7.39 (s, 1H), 6.11 (dd, *J* = 7.6, 5.4 Hz, 1H), 5.87 (ddt, *J* = 16.2, 10.2, 4.9 Hz, 1H), 5.23 (d, *J* = 17.4 Hz, 1H), 5.16 (d, *J* = 10.6 Hz, 1H), 4.49 (d, *J* = 5.2 Hz, 2H), 4.11 (q, *J* = 7.8 Hz, 1H), 3.82 (td, *J* = 7.2, 3.7 Hz, 1H), 2.97 (dd, *J* = 13.8, 3.7 Hz, 1H), 2.87 (dd, *J* = 13.8, 7.2 Hz, 1H), 2.43 (ddd, *J* = 14.2, 8.8, 5.4 Hz, 1H), 2.30 (dt, *J* = 14.5, 7.4 Hz, 1H), 1.80 (d, *J* = 1.2 Hz, 3H).

¹H NMR (400 MHz, MeOD) δ 7.56 (d, *J* = 1.5 Hz, 1H), 6.15 (dd, *J* = 7.4, 5.5 Hz, 1H), 5.95 (ddt, *J* = 16.3, 10.6, 5.3 Hz, 1H), 5.32 (d, *J* = 17.2 Hz, 1H), 5.21 (d, *J* = 10.5 Hz, 1H), 4.56 (d, *J* = 5.4 Hz, 2H), 4.20 (q, *J* = 7.7 Hz, 1H), 3.77 (td, *J* = 6.9, 3.6 Hz, 1H), 3.02 (dd, *J* = 13.7, 3.7 Hz, 1H), 2.93 (dd, *J* = 13.6, 6.7 Hz, 1H), 2.44 (ddd, *J* = 14.3, 9.0, 5.5 Hz, 1H), 2.32 (dt, *J* = 14.1, 7.3 Hz, 1H), 1.92 (d, *J* = 1.2 Hz, 3H).

R_f(1% Et₃N, 7:3 EtOAc/MeOH) = 0.1.

Nucleoside loading with BAL resin.

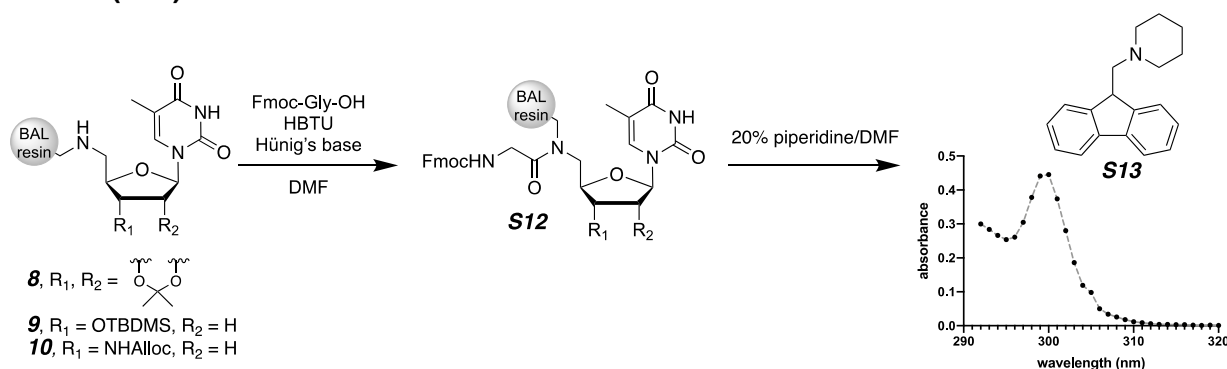
The C5'-amino-nucleosides were attached to BAL resin using a previously established protocol.¹² BAL resin (Advanced Chemtech, 100-200 mesh, 0.6–1.2 mmol/g, 1% DVB) was weighed into a reaction vessel containing a frit (Torviq 10 mL Luer Lock Fritted Syringe from Fisher, Catalog No. NC9299151). The resin was swelled in 1% AcOH/DMF (1 g resin/10 mL) for 1 h. The solvent was removed, and the resin was treated with a 0.2 M mixture of C5'-amino nucleoside (2 equiv) in 1% AcOH/DMF. The reaction was agitated for 1 h at ambient temperature. Then a mixture of NaBH₃CN (2 equiv) in MeOH (0.45 M) was added directly to the slurry of resin and agitated overnight. *Note: A closed reaction vessel is not recommended as production of gas is observed after the addition of NaBH₃CN. After 18 h, the solvent mixture was evacuated and the resin was rinsed with DMF, CH₂Cl₂, MeOH, and H₂O (2 x 5 mL each). The resin, in a slurry of H₂O, was frozen and lyophilized to provide the dried resin.

Fmoc loading determination

An excess of Fmoc-Gly-OH was coupled to the resin using standard solid-phase synthesis procedures. In this case, Fmoc-Gly-OH (5 equiv. relative to resin loading), HATU (5 equiv), and Hünig's base (10 equiv) in DMF was used. Fmoc-containing resin (10 mg, sufficiently dried to allow accurate weighing) was swelled in DMF for 30 min. The solvent was removed and 1 mL of 20% piperidine in DMF was added. The resin was agitated for 15 min and after this time, the solution was collected. To prepare the sample, 10 μ L of the deprotection solution was diluted to 1 mL with 990 μ L of DMF (100 dilution). The absorbance of the fulvene adduct (Extinction coefficient = 7800 $\text{M}^{-1}\text{cm}^{-1}$) at 301 nm was obtained in a 1 cm quartz cuvette compared to a DMF blank using a UV-VIS spectrophotometer. The loading was calculated using the following equation:¹³

$$\text{resin loading} = \frac{(\text{Absorbance}_{301} \times \text{volume} \times \text{dilution})}{(\text{extinction coefficient} \times \text{width of cuvette} \times \text{weight of resin})}$$

Scheme S4. Determining the nucleoside loading using the dibenzofulvene-piperidine adduct (S13).

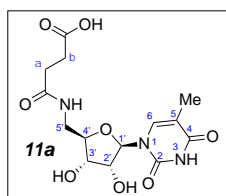


General procedure for Scheme 3A. Resin (50–200 mg, loading: 0.12–0.33 mmol/g, 1 equiv) was weighed into a 10 mL fritted syringe. The resin was swelled for 15 min in CH_2Cl_2 (5 mL). Then a mixture of succinic anhydride (4.75 equiv) in CH_2Cl_2 (5 mL) was added to the resin and the mixture was agitated for 18 h at ambient temperature. After this time, the solvent was removed, and the resin was rinsed with CH_2Cl_2 (2 x).

- *Procedure for methyl uridine and thymidine:* The desired product was cleaved from resin.
- *Procedure for azido thymidine:* The dipeptide was treated with $\text{Pd}(\text{PPh}_3)_4$ (0.8 equiv) with PhSiH_3 (10 equiv) in CH_2Cl_2 (5 mL) and agitated for 1 h. The solvent was removed and a 0.2 M solution of sodium diethyldithiocarbamate in DMF was added and agitated for 30 min to remove residual

Pd adhered to the resin; the solution/resin slurry will turn from dark brown to yellow. Then imidazole sulfonyl azide•HCl (3 equiv), Hünig's base (9 equiv) in DMSO (5 mL) was added to the resin and agitated for 2 h at ambient temperature. The solvent was removed, and the resin was rinsed with DMSO (2x) and DMF (2x). Then a mixture of 20% piperidine/DMF (v/v) was added to the resin and agitated for 20 min. The solvent was removed, and the resin was rinsed with DMF and CH₂Cl₂ (2 x each). Then the desired product was cleaved from resin.

• *Resin cleavage and purification:* The crude product was cleaved from the resin with 2 mL TFA/TIPS/H₂O (95:2.5:2.5) for 2 h (1 mL/h). The TFA cleavage was concentrated in volume under a stream of N₂ and the crude product was precipitated with -20 °C diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellet. The pellet was resuspended in MeCN/H₂O and purified by preparative HPLC (Luna 5 μm C₁₈(2) 100 Å, 250 x 21.2 mm Phenomenex column). The purified product was transferred to 50 mL centrifuge tubes, frozen in LN₂, and lyophilized.



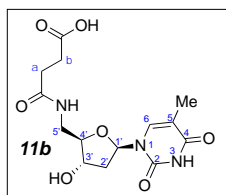
4-((((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-oxobutanoic acid (**11a**). Scale: 50 mg of resin (0.32 mmol/g, 0.016 mmol). Fluffy white solid (75% yield, 4.3 mg).

¹H NMR (600 MHz, D₂O) δ 7.38 (d, *J* = 1.5 Hz, 1H, H-6^T), 5.76 (d, *J* = 4.9 Hz, 1H, H-1'), 4.26 (t, *J* = 5.2 Hz, 1H, H-2'), 4.06 – 3.98 (m, 2H, H-4' and H-3'), 3.53 (dd, *J* = 14.6, 6.1 Hz, 1H, H-5'b), 3.44 (dd, *J* = 14.6, 4.0 Hz, 1H, H-5'a), 2.65 – 2.55 (m, 2H, CH₂^{succinic-β}), 2.52 – 2.43 (m, 2H, CH₂^{succinic-α}), 1.81 (d, *J* = 1.1 Hz, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.8 (CO₂H^{succinic}), 175.2 (C(O)NH^{succinic}), 166.5 (C-4^T), 151.7 (C-2^T), 137.6 (C-6^T), 111.6 (C-5^T), 89.5 (C-1'), 82.1 (C-4'), 72.9 (C-3'), 70.3 (C-2'), 40.3 (C-5'), 30.2 (CH₂^{succinic-β}), 29.1 (CH₂^{succinic-α}), 11.4 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 14.25 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₁₄H₂₀N₃O₈Na 380.1064; found 380.1056.



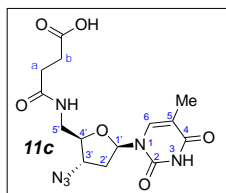
4-((((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-oxobutanoic acid (**11b**). Scale: 50 mg of resin (0.33 mmol/g, 0.0165 mmol). Fluffy white solid (78% yield, 4.4 mg).

¹H NMR (600 MHz, D₂O) δ 7.40 (d, *J* = 1.5 Hz, 1H, H-6^T), 6.15 (t, *J* = 6.8 Hz, 1H, H-1'), 4.28 (q, *J* = 5.0 Hz, 1H, H-4'), 3.93 (dt, *J* = 6.2, 4.2 Hz, 1H, H-3'), 3.47 (dd, *J* = 14.4, 6.4 Hz, 1H, H-5'b), 3.39 (dd, *J* = 14.5, 4.4 Hz, 1H, H-5'a), 2.65 – 2.53 (m, 2H, CH₂^{succinic-β}), 2.47 (td, *J* = 6.6, 3.6 Hz, 2H, CH₂^{succinic-α}), 2.30 – 2.25 (m, 2H, CH₂-2'), 1.81 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.8 (CO₂H^{succinic}), 175.1 (C(O)NH^{succinic}), 166.5 (C-4^T), 151.6 (C-2^T), 136.8 (C-6^T), 111.5 (C-5^T), 85.2 (C-1'), 84.4 (C-4'), 71.1 (C-3'), 40.5 (C-5'), 37.8 (C-2'), 30.2 (CH₂^{succinic-β}), 29.1 (CH₂^{succinic-α}), 11.5 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 15.9 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₄H₂₀N₃O₇⁺ 342.1296; found 342.1299.



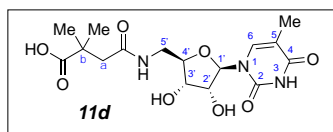
4-((((2R,3S,5R)-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-oxobutanoic acid (**11c**). Scale: 200 mg of resin (0.12 mmol/g, 0.024 mmol). Fluffy white solid (27% yield, 2.4 mg).

¹H NMR (600 MHz, D₂O) δ 7.40 (d, *J* = 1.4 Hz, 1H, H-6^T), 6.07 (dd, *J* = 7.2, 5.6 Hz, 1H, H-1'), 4.16 – 4.10 (m, 1H, H-4'), 3.90 (dt, *J* = 6.2, 4.7 Hz, 1H, H-3'), 3.59 (dd, *J* = 14.6, 5.1 Hz, 1H, H-5'b), 3.38 (dd, *J* = 14.7, 4.3 Hz, 1H, H-5'a), 2.66 – 2.54 (m, 2H, CH₂^{succinic-β}), 2.51 – 2.36 (m, 4H, CH₂-2' & CH₂^{succinic-α}), 1.81 (d, *J* = 1.2 Hz, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.8 (CO₂H^{succinic}), 175.3 (C(O)NH^{succinic}), 166.5 (C-4^T), 151.6 (C-2^T), 137.6 (C-6^T), 111.5 (C-5^T), 84.9 (C-1'), 82.1 (C-4'), 60.2 (C-3'), 39.8 (C-5'), 35.5 (C-2'), 30.1 (CH₂^{succinic-β}), 29.1 (CH₂^{succinic-α}), 11.4 (CH₃^T).

HPLC (20-95% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 15.6 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₄H₁₉N₆O₆⁺ 367.1361; found 367.1352.



4-((((2R,3S,4R,5R)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-2,2-dimethyl-4-oxobutanoic acid (**11d**). Resin (50 mg, loading: 0.32

mmol/g, 0.016 mmol, 1 equiv) was weighed into a 3 mL fritted syringe. The resin was swelled for 15 min in CH₂Cl₂ (2 mL). Then a mixture of anhydride (3 equiv) in CH₂Cl₂ (2 mL) was added to the resin and the mixture was agitated for 18 h at ambient temperature. After this time, the solvent was removed, and the resin was rinsed with CH₂Cl₂ (2 x). The crude product was cleaved from the resin with 2 mL TFA/TIPS/H₂O (95:2.5:2.5) for 2 h (1 mL/h). The TFA cleavage was concentrated in volume under a stream of N₂ and the crude product was precipitated with -20 °C diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellet. The pellet was resuspended in MeCN/H₂O and purified by preparative HPLC (Luna 5 μm C₁₈(2) 100 Å, 250 x 21.2 mm Phenomenex column). The purified product was transferred to 50 mL centrifuge tubes, frozen in LN₂, and lyophilized. Fluffy white solid (98% yield, 6.03 mg).

¹H NMR (600 MHz, D₂O) δ 7.48 (d, *J* = 1.5 Hz, 1H, H-6^T), 5.85 (d, *J* = 4.9 Hz, 1H, H-1'), 4.37 (t, *J* = 5.2 Hz, 1H, H-2'), 4.12 (t, *J* = 5.4 Hz, 1H, H-3'), 4.09 (dt, *J* = 6.6, 4.6 Hz, 1H, H-4'), 3.58 (dd, *J* = 14.5, 6.6 Hz, 1H, H-5'b), 3.52 (dd, *J* = 14.5, 4.2 Hz, 1H, H-5'a), 2.59 (s, 2H, CH₂^{succinic-α}), 1.92 (d, *J* = 1.3 Hz, 3H, CH₃^T), 1.24 (s, 3H, CH₃^{succinic-β}), 1.23 (s, 3H, CH₃^{succinic-β}).

¹³C NMR (151 MHz, D₂O) δ 181.9 (CO₂H^{succinic}), 173.9 (C(O)NH^{succinic}), 166.4 (C-4^T), 151.7 (C-2^T), 137.6 (C-6^T), 111.6 (C-5^T), 89.6 (C-1'), 81.9 (C-4'), 72.9 (C-3'), 70.5 (C-2'), 45.4 (CH₂^{succinic-α}), 40.6 (C^{succinic-β}-Me₂), 40.3 (C-5'), 24.6 (2 x CH₃^{succinic-β}), 11.5 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 15.4 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₆H₂₄N₃O₈⁺ 386.1558; found 386.1563.

Procedure for Scheme 3B, compounds 11e–g.

Preparation of 2,2-difluorosuccinic anhydride (Scheme S5A). To a solution of 2,2-difluorosuccinic acid (500 mg, 3.246 mmol) in *i*-PrOAc (5 mL) was added trifluoroacetic anhydride (541 μL, 3.896 mmol) at ambient temperature. The reaction solution was stirred at 50 °C for 1 h.¹⁴ The anhydride solution was added directly to the resin without purification.

Installation of fluorinated-succinic moiety on resin (Scheme S5B). Resin (50 mg, loading: 0.32 mmol/g, 1 equiv) was weighed into a 3 mL fritted syringe. The resin was swelled for 15 min in CH₂Cl₂ (2 mL) and after this time the solvent was removed. Then a mixture of anhydride (3 equiv, 73.8 μL of 0.65 M *i*-PrOAc solution) was diluted with CH₂Cl₂ to a final volume of 2 mL, added to the swelled resin, and agitated for 18 h at ambient temperature. After this time, the solvent was removed, and the resin was rinsed with CH₂Cl₂ (2 x).

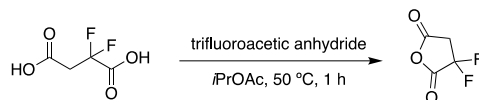
Cleavage procedure for compound 11e: The crude product was cleaved from the resin with 2 mL TFA/TIPS/H₂O (95:2.5:2.5) for 2 h (1 mL/h).

Cleavage procedure for compounds 11f and 11g: The crude product was cleaved from the resin with 2 mL TFA/TIPS/H₂O (95:2.5:2.5) for 40 min (1 mL for 20 min x 2).

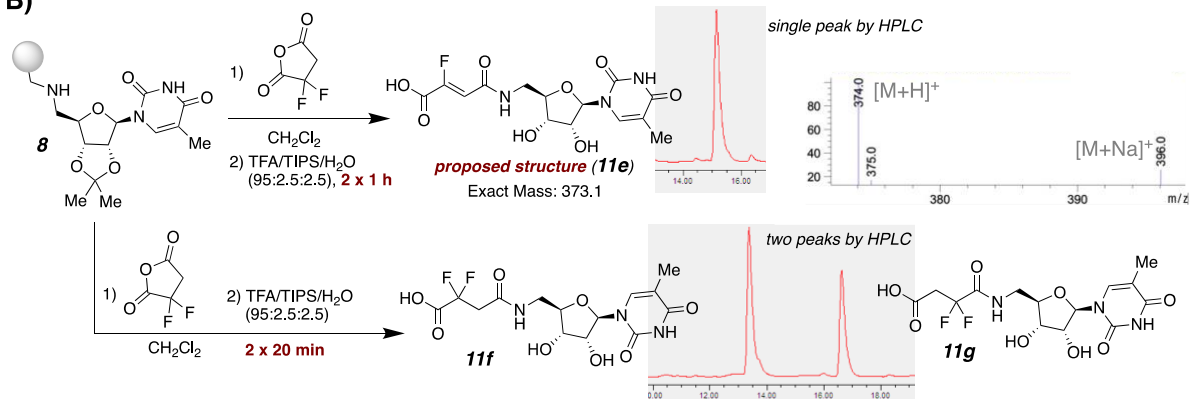
Cleavage work-up and purification for compounds 11e-g. Each TFA cleavage was concentrated in volume under a stream of N₂ and the crude products were precipitated with –20 °C diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellets. The pellets were resuspended in MeCN/H₂O and purified by preparative HPLC (Luna 5 µm C₁₈(2) 100 Å, 250 x 21.2 mm Phenomenex column). The purified products were transferred to 50 mL centrifuge tubes, frozen in LN₂, and lyophilized.

Installation of fluorinated-succinic moiety in solution (11g, Scheme S5C). The amino-modified methyl uridine **5** (15 mg, 0.051 mmol, 1 equiv) was weighed into a 2.0 mL centrifuge tube. Then a mixture of anhydride (0.66 equiv, 51.3 µL of 0.65 M *i*-PrOAc solution) was diluted with CH₂Cl₂ to a final volume of 1 mL, added to the centrifuge tube, and rotated for 2 h at ambient temperature. After this time, the solvent was removed under a stream of N₂, and the crude product was purified by silica gel chromatography (0-20% MeOH/CH₂Cl₂). The TLC R_f of the desired product is 0.5 in 20% MeOH/CH₂Cl₂ (visualized by UV, 254 nm). The isolated product was treated with 1 mL TFA for 20 min. The TFA mixture was concentrated in volume under a stream of N₂ and the crude product was precipitated with –20 °C diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellet. The pellet was resuspended in MeCN/H₂O and purified by preparative HPLC (Luna 5 µm C₁₈(2) 100 Å, 250 x 21.2 mm Phenomenex column). The purified product was transferred to 50 mL centrifuge tubes, frozen in LN₂, and lyophilized. The R_f of the crude product aligns with peak two of the on-resin reaction by LCMS and HPLC.

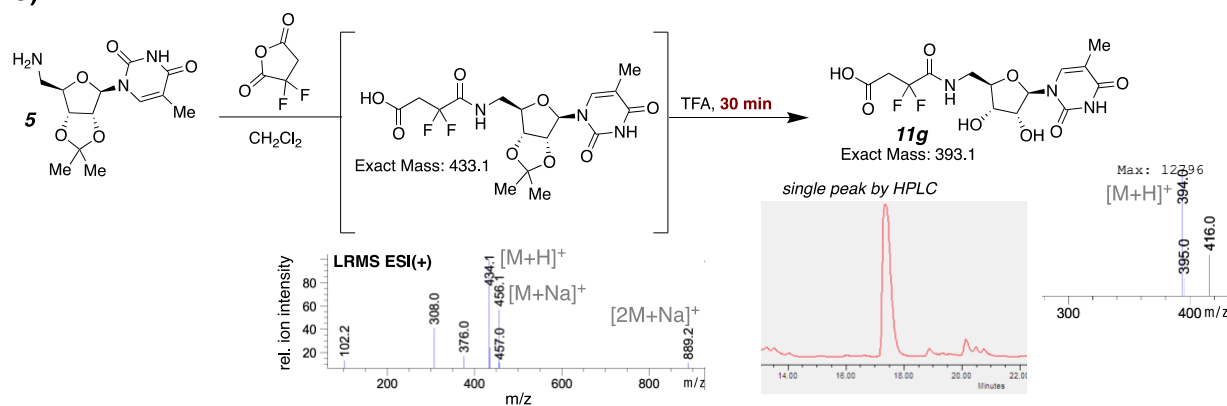
A)



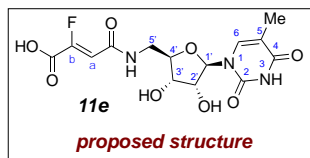
B)



C)



Scheme S5. The synthesis of fluorine-containing succinic derivatives. A) Generation of difluorinated-succinic anhydride. B) Solid-phase and C) solution-phase synthesis of compounds 11e-g.



Proposed structure:[‡] (Z)-4-(((2R,3S,4R,5R)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-3-fluoro-4-oxobut-2-enoic acid (**11e**). Scale: 50 mg of resin (0.32 mmol/g, 0.016 mmol). Fluffy white solid (11% yield, 0.7 mg).

¹H NMR (600 MHz, D₂O) δ 7.45 (d, *J* = 1.5 Hz, 1H, H-6^T), 6.30 (d, *J* = 19.8 Hz, 1H, CF=CH^α), 5.86 (d, *J* = 4.5 Hz, 1H, H-1'), 4.39 (dd, *J* = 5.8, 4.4 Hz, 1H, H-2'), 4.23 (t, *J* = 5.8 Hz, 1H, H-3'), 4.19 – 4.13 (m, 1H, H-4'), 3.70 (qd, *J* = 14.7, 4.8 Hz, 2H, H-5'), 1.89 (d, *J* = 1.4 Hz, 3H, CH₃^T).

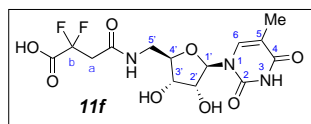
¹H NMR (400 MHz, DMSO) δ 11.33 (s, 1H), 7.57 (s, 1H), 5.76 (d, *J* = 5.8 Hz, 1H), 5.38 (s, 1H), 5.21 (s, 1H), 4.10 (s, 1H), 3.91 (s, 1H), 3.85 (q, *J* = 4.9 Hz, 1H), 3.56 (s, 1H), 3.53 – 3.44 (m, 1H), 1.80 (d, *J* = 1.2 Hz, 3H).[‡]

¹³C NMR (151 MHz, D₂O) δ 166.6 (d, *J* = 18.8 Hz, CO₂H), 166.4 (C(O)NH), 163.6 (d, *J* = 33.2 Hz, C^β-F), 155.4 (C-4^T), 151.7 (C-2^T), 137.6 (C-6^T), 111.5 (C-5^T), 110.7 (d, *J* = 26.9 Hz, CF=C^αH), 89.8 (C-1'), 81.7 (C-4'), 72.9 (C-3'), 70.1 (C-2'), 40.4 (C-5'), 11.5 (CH₃^T).

¹⁹F NMR (565 MHz, D₂O) δ -102.01 (d, *J* = 19.9 Hz).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 15.25 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₄H₁₇FN₃O₈⁺ 374.0994; found 374.0999.



4-((((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-2,2-difluoro-4-oxobutanoic acid (**11f**). Scale: 50 mg of resin (0.32 mmol/g,

0.016 mmol). The fluffy white solid was isolated in 11% yield (0.674 mg) out of the total yield (total: 0.8 mg).

¹H NMR (600 MHz, D₂O) δ 7.46 (s, 1H, H-6^T), 5.86 (d, *J* = 5.1 Hz, 1H, H-1'), 4.36 (t, *J* = 5.4 Hz, 1H, H-2'), 4.15 (t, *J* = 5.5 Hz, 1H, H-3'), 4.12 (q, *J* = 5.1 Hz, 1H, H-4'), 3.65 (dd, *J* = 14.6, 5.7 Hz, 1H, H-5'b), 3.58 (dd, *J* = 14.6, 4.2 Hz, 1H, H-5'a), 3.11 (dd, *J* = 17.5, 15.4 Hz, 2H, CH₂^{succinic-α}), 1.92 (s, 3H, CH₃^T).

¹H NMR (400 MHz, DMSO) δ 11.33 (s, 1H), 8.38 (s, 1H), 7.48 (s, 1H), 5.74 (d, *J* = 5.9 Hz, 1H), 5.35 (s, 1H), 5.15 (s, 1H), 4.06 (s, 1H), 3.85 (t, *J* = 5.0 Hz, 1H), 3.75 (dd, *J* = 7.2, 4.1 Hz, 1H), 3.50 – 3.40 (m, 1H), 3.11 (t, *J* = 15.4 Hz, 2H), 1.80 (s, 3H).

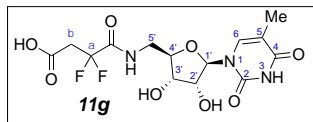
¹³C NMR (151 MHz, D₂O) δ 168.9 (C(O)NH^{succinic}), 168.9 – 168.7 (m, CO₂H^{succinic}), 166.5 (C-4^T), 151.8 (C-2^T), 137.5 (C-6^T), 117.4 – 113.1 (m, CF₂^{succinic-β}), 111.7 (C-5^T), 89.3 (C-1'), 82.0 (C-4'), 72.7 (C-3'), 70.2 (C-2'), 41.5 (t, *J* = 25.1 Hz, CH₂^{succinic-α}), 40.4 (C-5'), 11.4 (CH₃^T).

¹⁹F NMR (565 MHz, D₂O) -103.25 (dt, *J* = 21.4, 16.5 Hz).

HPLC (1-60% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 13.5 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₄H₁₈F₂N₃O₈⁺ 394.1056; found 394.1054.

[‡] We note the absence of the amide N-H and the alkene C-H peaks in the ¹H NMR in DMSO-*d*₆. This suggests there is a possible different structural representation for this compound.



2-(((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)carbamoyl)cyclopentane-1-carboxylic acid (**11g**). Scale: 50

mg of resin (0.32 mmol/g, 0.016 mmol). The fluffy white solid was isolated in 2% yield (0.126 mg) out of the total yield (total: 0.8 mg).

¹H NMR (600 MHz, D₂O) δ 7.45 (s, 1H, H-6^T), 5.87 (d, *J* = 4.4 Hz, 1H, H-1'), 4.36 (t, *J* = 4.6 Hz, 1H, H-2'), 4.17 (t, *J* = 3.6 Hz, 2H, H-3' & H-4'), 3.78 (dd, *J* = 15.0, 4.4 Hz, 1H, H-5'b), 3.65 (dd, *J* = 14.7, 3.0 Hz, 1H, H-5'a), 3.48 – 3.32 (m, 2H, CH₂^{succinic-β}), 1.89 (s, 3H, CH₃^T).

¹H NMR (400 MHz, DMSO) δ 12.87 (s, 1H), 11.32 (s, 1H), 8.90 (t, *J* = 6.1 Hz, 1H), 7.47 (d, *J* = 1.5 Hz, 1H), 5.74 (d, *J* = 6.1 Hz, 1H), 5.33 (s, 1H), 5.13 (s, 1H), 4.05 (t, *J* = 5.9 Hz, 1H), 3.88 (dq, *J* = 15.1, 4.7 Hz, 2H), 3.43 (ddt, *J* = 17.7, 13.1, 6.1 Hz, 2H), 3.23 (t, *J* = 15.5 Hz, 2H), 1.79 (d, *J* = 1.2 Hz, 3H).

¹³C NMR (151 MHz, D₂O) δ 170.2 – 170.1 (m, CO₂H^{succinic}), 166.5 (C-4^T), 165.9 – 165.2 (m, C(O)NH^{succinic}), 151.7 (C-2^T), 137.5 (C-6^T), 117.2 – 113.1 (m, CF₂^{succinic-α}), 111.6 (C-5^T), 89.6 (C-1'), 81.6 (C-4'), 73.0 (C-3'), 70.1 (C-2'), 40.2 (C-5'), 39.1 (t, *J* = 25.9 Hz, CH₂^{succinic-β}), 11.4 (CH₃^T).

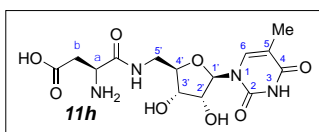
¹⁹F NMR (565 MHz, D₂O) δ -104.28 (ddd, *J* = 265.8, 16.1, 11.7 Hz), -106.26 (ddd, *J* = 265.7, 19.7, 12.6 Hz).

HPLC (1-60% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 16.9 min (260 nm).

HRMS (ESI⁺) m/z: [M+H]⁺ Calcd for C₁₄H₁₈F₂N₃O₈⁺ 394.1056; found 394.1064.

Procedure for Scheme 3B, compounds 11h–i. Resin (50 mg, loading: 0.32 mmol/g, 1 equiv) was weighed into a 3 mL fritted syringe. The resin was swelled for 15 min in DMF (2 mL). Then a mixture of Fmoc-Asp(O*t*Bu)-OH (20 mg, 0.048 mmol, 3 equiv), HBTU (36 mg, 0.048 mmol, 3 equiv), and Hünig's base (9 μL, 0.096 mmol, 6 equiv) in DMF (2 mL) was added to the resin and the mixture was agitated for 2 h at ambient temperature. After this time, the solvent was removed, and the resin was rinsed with DMF (2 x). The resin was then treated with 20% piperidine/DMF (1 mL, 2 x 15 min). The solvent was removed, and the resin was rinsed with DMF (2 x) followed by CH₂Cl₂ (2 x). The crude product was cleaved from the resin with 2 mL TFA/TIPS/H₂O (95:2.5:2.5) for 2 h (1 mL/h). The TFA cleavage was concentrated in volume under a stream of N₂ and the crude product was precipitated with -20 °C diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellet. The pellet was resuspended in MeCN/H₂O and purified by preparative HPLC (Luna 5 μm C₁₈(2) 100 Å, 250 x

21.2 mm Phenomenex column). The purified product was transferred to 50 mL centrifuge tubes, frozen in LN₂, and lyophilized.



(S)-3-amino-4-((((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-oxobutanoic acid (**11h**). Scale: 50 mg of resin

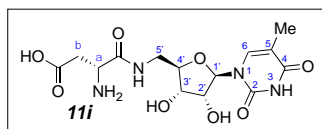
(0.32 mmol/g, 0.016 mmol). Fluffy white solid (28% yield, 1.7 mg).

¹H NMR (600 MHz, D₂O) δ 7.47 (s, 1H, H-6^T), 5.79 (d, *J* = 4.9 Hz, 1H, H-1'), 4.41 (t, *J* = 5.2 Hz, 1H, H-2'), 4.33 (dd, *J* = 7.2, 5.3 Hz, 1H, H-3'), 4.16 – 4.08 (m, 2H, H-4' & CH^{L-Asp(α)}), 3.70 (dd, *J* = 14.5, 7.0 Hz, 1H, H-5'b), 3.58 (dd, *J* = 14.5, 3.9 Hz, 1H, H-5'a), 3.05 (dd, *J* = 17.9, 5.4 Hz, 1H, CH_aH_b^{L-Asp(β)}), 2.99 (dd, *J* = 17.8, 7.3 Hz, 1H, CH_aH_b^{L-Asp(β)}), 1.92 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 173.1 (C(O)NH^{L-Asp}), 169.0 (CO₂H^{L-Asp}), 166.5 (C-4^T), 151.7 (C-2^T), 138.1 (C-6^T), 111.6 (C-5^T), 90.4 (C-1'), 81.5 (C-4'), 72.6 (C-3'), 70.4 (C-2'), 49.8 (CH^{L-Asp(α)}), 40.9 (C-5'), 35.1 (CH₂^{L-Asp(β)}), 11.4 (CH₃^T).

HPLC (1-60% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 12.4 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₄H₂₁N₄O₈⁺ 373.1354; found 373.1362.



(R)-3-amino-4-((((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-oxobutanoic acid (**11i**). Scale: 50 mg of resin (0.32

mmol/g, 0.016 mmol). Fluffy white solid (30% yield, 1.8 mg).

¹H NMR (600 MHz, D₂O) δ 7.48 (d, *J* = 1.6 Hz, 1H, C-6^T), 5.78 (d, *J* = 4.6 Hz, 1H, H-1'), 4.43 (t, *J* = 5.2 Hz, 1H, H-2'), 4.33 (dd, *J* = 7.6, 5.1 Hz, 1H, H-3'), 4.18 (t, *J* = 5.8 Hz, 1H, CH^{D-Asp(α)}), 4.12 – 4.07 (m, 1H, H-4'), 3.68 (dd, *J* = 14.5, 4.3 Hz, 1H, H-5'b), 3.60 (dd, *J* = 14.5, 6.9 Hz, 1H, H-5'a), 3.03 (dd, *J* = 17.8, 5.1 Hz, 1H, CH_aH_b^{D-Asp(β)}), 2.96 (dd, *J* = 17.8, 7.5 Hz, 1H, CH_aH_b^{D-Asp(β)}), 1.92 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 173.3 (CO₂H^{D-Asp}), 169.0 (C(O)NH^{D-Asp}), 166.5 (C-4^T), 151.7 (C-2^T), 138.3 (C-6^T), 111.6 (C-5^T), 90.8 (C-1'), 81.5 (C-4'), 72.6 (C-3'), 70.5 (C-2'), 49.9 (CH^{D-Asp(α)}), 41.0 (CH₂^{D-Asp(β)}), 35.2 (C-5'), 11.4 (CH₃^T).

HPLC (1-60% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 12.5 min (260 nm).

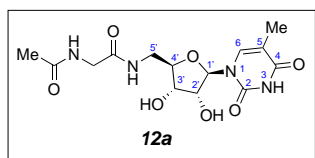
HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₄H₂₁N₄O₈⁺ 373.1354; found 373.1343.

General procedure for Scheme 4. Resin (50-200 mg, loading: 0.28–0.33 mmol/g, 1 equiv) was weighed into a 10 mL fritted syringe. The resin was swelled for 15 min in CH₂Cl₂ (5 mL). Then a mixture of *N*-acetyl-glycine (5 equiv), HBTU (5 equiv), and Hünig's base (10 equiv) in DMF (5 mL) was added to the resin. The mixture was agitated for 2 h at ambient temperature. After this time, the solvent was removed, and the resin was rinsed with DMF (2 x).

• *Procedure for methyl uridine and thymidine:* The desired product was cleaved from resin.

• *Procedure for azido thymidine:* The dipeptide was treated with Pd(PPh₃)₄ (0.8 equiv) with PhSiH₃ (10 equiv) in CH₂Cl₂ (5 mL) and agitated for 1 h. The solvent was removed and a 0.2 M solution of sodium diethyldithiocarbamate in DMF was added and agitated for 30 min to remove residual Pd adhered to the resin; the solution/resin slurry will turn from dark brown to yellow. Then imidazole sulfonyl azide•HCl (3 equiv), Hünig's base (9 equiv) in DMSO (5 mL) was added to the resin and agitated for 2 h at ambient temperature. The solvent was removed, and the resin was rinsed with DMSO (2x) and DMF (2x). Then a mixture of 20% piperidine/DMF (v/v) was added to the resin and agitated for 20 min. The solvent was removed, and the resin was rinsed with DMF and CH₂Cl₂ (2 x each). Then the desired product was cleaved from resin.

• *Resin cleavage and purification:* The crude product was cleaved from the resin with 2 mL TFA/TIPS/H₂O (95:2.5:2.5) for 2 h (1 mL/h). The TFA cleavage was concentrated in volume under a stream of N₂ and the crude product was precipitated with –20 °C diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellet. The pellet was resuspended in MeCN/H₂O and purified by preparative HPLC (Luna 5 μm C₁₈(2) 100 Å, 250 x 21.2 mm Phenomenex column). The purified product was transferred to 50 mL centrifuge tubes, frozen in LN₂, and lyophilized.



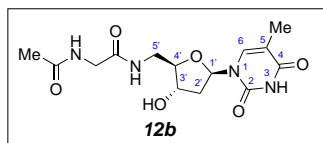
2-acetamido-N-(((2R,3S,4R,5R)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)acetamide (12a). Scale: 50 mg of resin (0.32 mmol/g, 0.016 mmol). Fluffy white solid (87% yield, 5.2 mg).

¹H NMR (600 MHz, D₂O) δ 7.39 (d, *J* = 1.5 Hz, 1H, H-6^T), 5.74 (d, *J* = 5.1 Hz, 1H, H-1'), 4.26 (t, *J* = 5.3 Hz, 1H, H-2'), 4.03 (dt, *J* = 16.1, 5.2 Hz, 2H, H-3' & H-4'), 3.87 – 3.73 (m, 2H, CH₂^{Gly}), 3.53 – 3.44 (m, 2H, CH₂-5'), 1.96 (s, 3H, CH₃^{Ac}), 1.81 (d, *J* = 1.2 Hz, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 174.8 (C(O)^{Ac}), 172.0 (C(O)NH^{Gly}), 166.4 (C-4^T), 151.7 (C-2^T), 137.7 (C-6^T), 111.6 (C-5^T), 89.6 (C-1'), 82.1 (C-4'), 72.8 (C-3'), 70.3 (C-2'), 42.7 (CH₂^{Gly}), 40.4 (C-5'), 21.7 (CH₃^{Ac}), 11.4 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 13.0 min (260 nm).

HRMS (ESI⁺) m/z: [M+Na]⁺ Calcd for C₁₄H₂₁N₄O₇Na 379.1230; found 379.1232.



2-acetamido-N-(((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)acetamide (12b). Scale: 50 mg of resin (0.33 mmol/g, 0.0165 mmol). Fluffy white

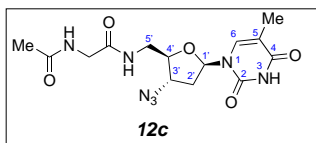
solid (78% yield, 4.4 mg).

¹H NMR (600 MHz, D₂O) δ 7.41 (s, 1H, H-6^T), 6.13 (t, *J* = 6.9 Hz, 1H, H-1'), 4.32 – 4.26 (m, 1H, H-4'), 3.97 – 3.92 (m, 1H, H-3'), 3.84 – 3.75 (m, 2H, CH₂^{Gly}), 3.49 – 3.38 (m, 2H, CH₂-5'), 2.32 – 2.23 (m, 2H, CH₂-2'), 1.96 (s, 3H, CH₃^{Ac}), 1.81 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 174.8 (C(O)^{Ac}), 171.9 (C(O)NH^{Gly}), 166.5 (C-4^T), 151.6 (C-2^T), 137.6 (C-6^T), 111.5 (C-5^T), 85.3 (C-1'), 84.3 (C-4'), 71.2 (C-3'), 42.7 (CH₂^{Gly}), 40.7 (C-5'), 37.7 (C-2'), 21.6 (CH₃^{Ac}), 11.4 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 13.7 min (260 nm).

HRMS (ESI⁺) m/z: [M+Na]⁺ Calcd for C₁₄H₂₁N₄O₆Na 363.1281; found 363.1271.



4-(((2R,3S,5R)-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-oxobutanoic acid (12c). Scale: 150 mg of resin (0.28 mmol/g, 0.042 mmol). Fluffy white solid (53% yield, 8.04 mg).

¹H NMR (600 MHz, D₂O) δ 7.41 (d, *J* = 1.4 Hz, 1H, H-6^T), 6.05 (dd, *J* = 7.1, 6.0 Hz, 1H, H-1'), 4.16 (dt, *J* = 7.7, 6.0 Hz, 1H, H-4'), 3.90 (q, *J* = 5.2 Hz, 1H, H-3'), 3.85 – 3.75 (m, 2H, CH₂^{Gly}), 3.56 (dd, *J* = 14.6, 5.4 Hz, 1H, H-5'b), 3.43 (dd, *J* = 14.6, 4.4 Hz, 1H, H-5'a), 2.47 – 2.36 (m, 2H, CH₂-2'), 1.96 (s, 3H, CH₃^{Ac}), 1.80 (d, *J* = 1.2 Hz, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 174.8 (C(O)^{Ac}), 172.1 (C(O)NH^{Gly}), 166.5 (C-4^T), 151.5 (C-2^T), 137.7 (C-6^T), 111.5 (C-5^T), 85.1 (C-1'), 81.9 (C-4'), 60.4 (C-3'), 42.7 (CH₂^{Gly}), 40.11 (C-5'), 35.5 (C-2'), 21.6 (CH₃^{Ac}), 11.4 (CH₃^T).

HPLC (20-95% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 10.2 min (260 nm).

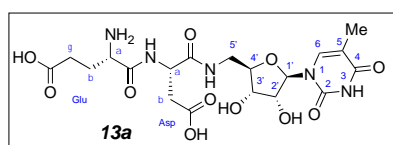
HRMS (ESI⁺) m/z: [M+Na]⁺ Calcd for C₁₄H₂₀N₇O₅Na 388.1345; found 388.1348.

General procedure for Scheme 5. Resin (50-200 mg, loading: 0.12–0.33 mmol/g, 1 equiv) was weighed into a 10 mL fritted syringe. The resin was swelled for 15 min in CH₂Cl₂ (5 mL). Then a mixture of Fmoc-AA(OtBu)-OH (5 equiv), HBTU (5 equiv), and Hünig's base (10 equiv) in DMF (5 mL) was added to the resin. The mixture was agitated for 2 h at ambient temperature. After this time, the solvent was removed, and the resin was rinsed with DMF (2 x). Then a mixture of 20% piperidine/DMF (v/v) was added to the resin and agitated for 20 min. The solvent was removed, and the resin was rinsed with DMF (2 x). Then a mixture of Fmoc-AA(OtBu)-OH (5 equiv), HBTU (5 equiv), and Hünig's base (10 equiv) in DMF (5 mL) was added to the resin. The mixture was agitated for 2 h at ambient temperature.

- *Procedure for methyl uridine and thymidine:* Then a mixture of 20% piperidine/DMF (v/v) was added to the resin and agitated for 20 min. The solvent was removed, and the resin was rinsed with DMF and CH₂Cl₂ (2 x each). Then the desired product was cleaved from resin.

- *Procedure for azido thymidine:* The dipeptide was treated with Pd(PPh₃)₄ (0.8 equiv) with PhSiH₃ (10 equiv) in CH₂Cl₂ (5 mL) and agitated for 1 h. The solvent was removed and a 0.2 M solution of sodium diethyldithiocarbamate in DMF was added and agitated for 30 min to remove residual Pd adhered to the resin; the solution/resin slurry will turn from dark brown to yellow. Then imidazole sulfonyl azide•HCl (3 equiv), Hünig's base (9 equiv) in DMSO (5 mL) was added to the resin and agitated for 2 h at ambient temperature. The solvent was removed, and the resin was rinsed with DMSO (2x) and DMF (2x). Then a mixture of 20% piperidine/DMF (v/v) was added to the resin and agitated for 20 min. The solvent was removed, and the resin was rinsed with DMF and CH₂Cl₂ (2 x each). Then the desired product was cleaved from resin.

- *Resin cleavage and purification:* The crude product was cleaved from the resin with 2 mL TFA/TIPS/H₂O (95:2.5:2.5) for 2 h (1 mL/h). The TFA cleavage was concentrated in volume under a stream of N₂ and the crude product was precipitated with –20 °C diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellet. The pellet was resuspended in MeCN/H₂O and purified by preparative HPLC (Luna 5 μm C₁₈(2) 100 Å, 250 x 21.2 mm Phenomenex column). The purified product was transferred to 50 mL centrifuge tubes, frozen in LN₂, and lyophilized.



(S)-4-amino-5-(((S)-3-carboxy-1-((((2R,3S,4R,5R)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-1-oxopropan-2-

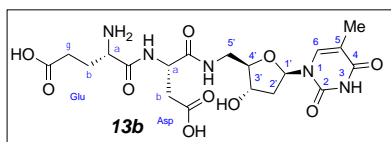
yl)amino)-5-oxopentanoic acid (**13a**). Scale: 100 mg of resin (0.32 mmol/g, 0.032 mmol). Fluffy white solid (95% yield, 15.2 mg).

¹H NMR (600 MHz, D₂O) δ 7.39 – 7.36 (m, 1H, H-6^T), 5.71 (d, *J* = 4.9 Hz, 1H, H-1'), 4.29 (t, *J* = 5.2 Hz, 1H, H-2'), 4.05 – 3.98 (m, 3H, CH^{Asp or Glu-α} & H-3' & H-4'), 3.53 (dd, *J* = 14.4, 7.0 Hz, 1H, H-5'b), 3.47 (dd, *J* = 14.5, 3.9 Hz, 1H, H-5'a), 2.86 (dd, *J* = 17.1, 6.4 Hz, 1H, CH_aH_b^{Asp-β}), 2.76 (dd, *J* = 17.1, 7.5 Hz, 1H, CH_aH_b^{Asp-β}), 2.44 (td, *J* = 7.3, 2.1 Hz, 2H, CH₂^{Glu-γ}), 2.08 (qd, *J* = 7.3, 3.7 Hz, 2H, CH₂^{Glu-β}), 1.81 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.0 (CO₂H^{Glu}), 173.8 (CO₂H^{Asp}), 171.8 (C(O)NH^{Asp}), 169.0 (C(O)NH^{Glu-Asp}), 166.5 (C-4^T), 151.7 (C-2^T), 137.9 (C-6^T), 111.5 (C-5^T), 90.1 (C-1'), 81.7 (C-4'), 72.8 (C-3'), 70.5 (C-2'), 52.2 (CH^{Asp-α}), 50.3 (CH^{Glu-α}), 40.9 (C-5'), 35.3 (CH₂^{Asp-β}), 28.9 (CH₂^{Glu-γ}), 25.8 (CH₂^{Glu-β}), 11.4 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 12.5 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₉H₂₈N₅O₁₁⁺ 502.1780; found 502.1767.



(*S*)-4-amino-5-(((*S*)-3-carboxy-1-((((2*R*,3*S*,5*R*)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-1-oxopropan-2-yl)amino)-5-oxopentanoic acid (**13b**). Scale: 50 mg of resin

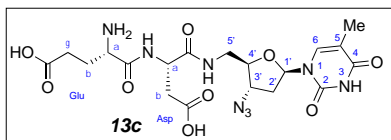
(0.33 mmol/g, 0.0165 mmol). Fluffy white solid (90% yield, 7.2 mg).

¹H NMR (600 MHz, D₂O) δ 7.39 (s, 1H, H-6^T), 6.13 (t, *J* = 6.9 Hz, 1H, H-1'), 4.31 – 4.24 (m, 1H, H-4'), 4.00 (t, *J* = 6.5 Hz, 1H, CH^{Glu or Asp-α}), 3.94 (dt, *J* = 7.9, 4.3 Hz, 1H, H-3'), 3.45 (qt, *J* = 14.4, 7.4 Hz, 2H, CH₂-5'), 2.84 (dd, *J* = 17.0, 6.2 Hz, 1H, CH_aH_b^{Asp-β}), 2.75 (dd, *J* = 17.0, 7.3 Hz, 1H, CH_aH_b^{Asp-β}), 2.44 (td, *J* = 7.4, 2.1 Hz, 2H, CH₂^{Glu-γ}), 2.34 – 2.23 (m, 2H, CH₂-2'), 2.08 (qd, *J* = 7.3, 4.2 Hz, 2H, CH₂^{Glu-β}), 1.81 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.0 (CO₂H^{Glu}), 173.8 (CO₂H^{Asp}), 171.8 (C(O)NH^{Asp}), 169.0 (C(O)NH^{Glu-Asp}), 166.5 (C-4^T), 151.6 (C-2^T), 137.7 (C-6^T), 111.5 (C-5^T), 85.6 (C-1'), 84.0 (C-4'), 71.4 (C-3'), 52.2 (CH^{Asp-α}), 50.3 (CH^{Glu-α}), 41.1 (C-5'), 37.6 (C-2'), 35.4 (CH₂^{Asp-β}), 28.9 (CH₂^{Glu-γ}), 25.8 (CH₂^{Glu-β}), 11.4 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 13.1 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₉H₂₈N₅O₁₀⁺ 486.1831; found 486.1827.



(*S*)-4-amino-5-(((*S*)-1-((((2*R*,3*S*,5*R*)-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-3-carboxy-1-oxopropan-2-yl)amino)-5-oxopentanoic acid (**13c**). Scale: 200 mg of resin (0.12 mmol/g,

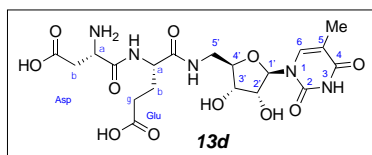
0.024 mmol). Fluffy white solid (32% yield, 3.9 mg).

¹H NMR (600 MHz, D₂O) δ 7.39 (d, *J* = 1.6 Hz, 1H, H-6^T), 6.03 (t, *J* = 6.5 Hz, 1H, H-1'), 4.13 (dt, *J* = 7.6, 5.9 Hz, 1H, H-4'), 4.00 (t, *J* = 6.5 Hz, 1H, CH^{Asp} or Glu-α), 3.90 (td, *J* = 6.0, 4.2 Hz, 1H, H-3'), 3.55 (dd, *J* = 14.5, 6.3 Hz, 1H, H-5'b), 3.44 (dd, *J* = 14.5, 4.3 Hz, 1H, H-5'a), 2.86 (dd, *J* = 17.0, 6.4 Hz, 1H, CH_aH_b^{Asp-β}), 2.77 (dd, *J* = 17.0, 7.2 Hz, 1H, CH_aH_b^{Asp-β}), 2.50 – 2.36 (m, 4H, H-2' & CH₂^{Glu-γ}), 2.08 (dq, *J* = 12.2, 7.3 Hz, 2H, CH₂^{Glu-β}), 1.81 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.0 (CO₂H^{Glu}), 173.8 (CO₂H^{Asp}), 171.9 (C(O)NH^{Asp}), 169.0 (C(O)NH^{Glu-Asp}), 166.5 (C-4^T), 151.5 (C-2^T), 137.9 (C-6^T), 111.5 (C-5^T), 85.5 (C-1'), 81.7 (C-4'), 60.6 (C-3'), 52.2 (CH^{Asp-α}), 50.2 (CH^{Glu-α}), 40.6 (C-5'), 35.4 (C-2'), 35.3 (CH₂^{Asp-β}), 28.9 (CH₂^{Glu-γ}), 25.8 (CH₂^{Glu-β}), 11.4 (CH₃^T).

HPLC (1-85% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 19.0 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₉H₂₇N₈O₉⁺ 511.1896; found 511.1902.



(*S*)-4-((*S*)-2-amino-3-carboxypropanamido)-5-((((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-5-oxopentanoic acid (**13d**). Scale: 100 mg of resin (0.32 mmol/g,

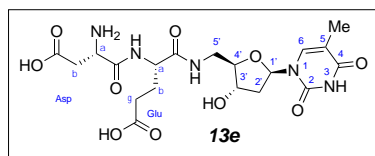
0.032 mmol). Fluffy white solid (43% yield, 6.89 mg).

¹H NMR (600 MHz, D₂O) δ 7.43 – 7.30 (m, 1H, H-6^T), 5.77 (d, *J* = 5.4 Hz, 1H, H-1'), 4.29 (ddt, *J* = 13.5, 10.6, 5.7 Hz, 3H, H-2' & H-3' & CH^{Asp-α}), 4.03 (dq, *J* = 16.8, 4.6 Hz, 2H, H-4' & CH^{Glu-α}), 3.56 (dd, *J* = 14.3, 8.3 Hz, 1H, H-5'b), 3.41 (dd, *J* = 14.3, 4.0 Hz, 1H, H-5'a), 2.98 (dd, *J* = 18.0, 5.2 Hz, 1H, CH_aH_b^{Asp-β}), 2.95 – 2.87 (m, 1H, CH_aH_b^{Asp-β}), 2.36 (t, *J* = 7.6 Hz, 2H, CH₂^{Glu-γ}), 2.04 – 1.95 (m, 1H, CH_aH_b^{Glu-β}), 1.95 – 1.86 (m, 1H, CH_aH_b^{Glu-β}), 1.82 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.7 (CO₂H^{Glu}), 172.7 (CO₂H^{Asp}), 172.7 (C(O)NH^{Asp}), 168.5 (C(O)NH^{Glu-Asp}), 166.4 (C-4^T), 151.8 (C-2^T), 137.6 (C-6^T), 111.7 (C-5^T), 89.4 (C-1'), 81.6 (C-4'), 72.7 (C-3'), 70.7 (C-2'), 53.4 (CH^{Asp-α}), 49.4 (CH^{Glu-α}), 41.0 (C-5'), 34.8 (CH₂^{Asp-β}), 29.5 (CH₂^{Glu-γ}), 26.1 (CH₂^{Glu-β}), 11.4 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 15.25 min (260 nm).

HRMS (ESI⁺) m/z: [M+Na]⁺ Calcd for C₁₉H₂₈N₅O₁₁Na 524.1599; found 524.1603.



(S)-4-(((S)-2-amino-3-carboxypropanamido)-5-((((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-5-oxopentanoic acid (13e).

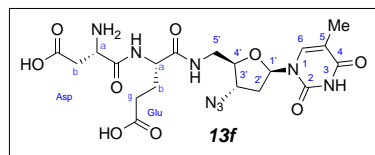
Scale: 50 mg of resin (0.33 mmol/g, 0.0165 mmol). Fluffy white solid (97% yield, 7.8 mg).

¹H NMR (600 MHz, D₂O) δ 7.37 (s, 1H, C-6^T), 6.18 (t, *J* = 7.1 Hz, 1H, H-1'), 4.28 (td, *J* = 7.4, 4.3 Hz, 3H, H-4' & CH^{Glu} and Asp-α), 3.96 (dt, *J* = 8.2, 3.9 Hz, 1H, H-3'), 3.49 (dd, *J* = 14.2, 8.6 Hz, 1H, H-5'b), 3.36 (dd, *J* = 14.2, 4.4 Hz, 1H, H-5'a), 2.97 (dd, *J* = 17.9, 5.1 Hz, 1H, CH_aCH_b^{Asp-β}), 2.89 (dd, *J* = 17.9, 7.6 Hz, 1H, CH_aCH_b^{Asp-β}), 2.36 (t, *J* = 7.6 Hz, 2H, H-2'), 2.32 – 2.22 (m, 2H, CH₂^{Glu-γ}), 1.98 (dq, *J* = 14.5, 7.4 Hz, 1H, CH_aCH_b^{Glu-β}), 1.90 (dq, *J* = 14.9, 7.5 Hz, 1H, CH_aCH_b^{Glu-β}), 1.81 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.7 (CO₂H^{Glu}), 172.9 (CO₂H^{Asp}), 172.7 (C(O)NH^{Asp-Glu}), 168.5 (C(O)NH^{Glu}), 166.4 (C-4^T), 151.7 (C-2^T), 137.5 (C-6^T), 111.6 (C-5^T), 85.4 (C-1'), 83.9 (C-4'), 71.7 (C-3'), 53.4 (CH^{Asp-α}), 49.5 (CH^{Glu-α}), 41.2 (C-5'), 37.5 (C-2'), 34.9 (CH₂^{Asp-β}), 29.6 (CH₂^{Glu-γ}), 26.1 (CH₂^{Glu-β}), 11.5 (CH₃^T).

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t: 13.0 min (260 nm).

HRMS (ESI⁺) m/z: [M+H]⁺ Calcd for C₁₉H₂₈N₅O₁₀⁺ 486.1831; found 486.1842.



(S)-4-(((S)-2-amino-3-carboxypropanamido)-5-((((2R,3S,5R)-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-5-oxopentanoic acid (13f).

Scale: 200 mg of resin (0.12 mmol/g, 0.024 mmol). Fluffy white solid (41% yield, 5.0 mg).

¹H NMR (600 MHz, D₂O) δ 7.37 (d, *J* = 1.5 Hz, 1H, H-6^T), 6.07 (t, *J* = 6.7 Hz, 1H, H-1'), 4.29 (ddd, *J* = 16.6, 7.7, 5.7 Hz, 2H, CH^{Asp} and Glu-α), 4.17 (dt, *J* = 7.3, 5.1 Hz, 1H, H-4'), 3.93 (dt, *J* = 7.3, 4.8 Hz, 1H, H-3'), 3.55 (dd, *J* = 14.3, 7.4 Hz, 1H, H-5'b), 3.40 (dd, *J* = 14.3, 4.6 Hz, 1H, H-5'a), 2.49 – 2.39 (m, 2H, H-2'), 2.37 (t, *J* = 7.4 Hz, 2H, CH₂^{Asp-β}), 2.01 (dq, *J* = 14.4, 7.3 Hz, 1H, CH_aH_b^{Glu-β}), 1.90 (dq, *J* = 14.9, 7.6 Hz, 1H, CH_aH_b^{Glu-β}), 1.81 (s, 3H, CH₃^T).

^{13}C NMR (151 MHz, D_2O) δ 176.8 ($\text{CO}_2\text{H}^{\text{Glu}}$), 172.8 ($\text{CO}_2\text{H}^{\text{Asp}}$), 172.8 ($\text{C}(\text{O})\text{NH}^{\text{Asp-Glu}}$), 168.5 ($\text{C}(\text{O})\text{NH}^{\text{Glu}}$), 166.4 (C-4^{T}), 151.5 (C-2^{T}), 137.7 (C-6^{T}), 111.5 (C-5^{T}), 85.5 ($\text{C-1}'$), 81.6 ($\text{C-4}'$), 61.2 ($\text{C-3}'$), 53.3 ($\text{CH}^{\text{Glu-}\alpha}$), 49.4 ($\text{CH}^{\text{Asp-}\alpha}$), 40.9 ($\text{C-5}'$), 35.2 ($\text{C-2}'$), 34.9 ($\text{CH}_2^{\text{Asp-}\beta}$), 29.6 ($\text{CH}_2^{\text{Glu-}\gamma}$), 26.2 ($\text{CH}_2^{\text{Glu-}\beta}$), 11.5 (CH_3^{T}).

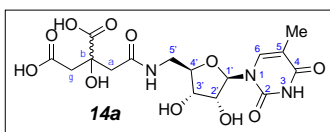
HPLC (1-85% MeCN/ H_2O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t : 19.0 min (260 nm).

HRMS (ESI⁺) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{19}\text{H}_{27}\text{N}_8\text{O}_9^+$ 511.1896; found 511.1896.

General procedure for Scheme 6. Resin (50-200 mg, loading: 0.28–0.33 mmol/g, 1 equiv) was weighed into a 10 mL fritted syringe. The resin was swelled for 15 min in CH_2Cl_2 (5 mL). Then a mixture of citric acid anhydride (2 equiv) in CH_2Cl_2 (5 mL) was added to the resin and the mixture was agitated for 18 h at ambient temperature. After this time, the solvent was removed, and the resin was rinsed with CH_2Cl_2 (2 x).

- *Procedure for methyl uridine and thymidine:* The desired product was cleaved from resin.
- *Procedure for azido thymidine:* The dipeptide was treated with $\text{Pd}(\text{PPh}_3)_4$ (0.8 equiv) with PhSiH_3 (10 equiv) in CH_2Cl_2 (5 mL) and agitated for 1 h. The solvent was removed and a 0.2 M solution of sodium diethyldithiocarbamate in DMF was added and agitated for 30 min to remove residual Pd adhered to the resin; the solution/resin slurry will turn from dark brown to yellow. Then imidazole sulfonyl azide $\cdot\text{HCl}$ (3 equiv), Hünig's base (9 equiv) in DMSO (5 mL) was added to the resin and agitated for 2 h at ambient temperature. The solvent was removed, and the resin was rinsed with DMSO (2x) and DMF (2x). Then a mixture of 20% piperidine/DMF (v/v) was added to the resin and agitated for 20 min. The solvent was removed, and the resin was rinsed with DMF and CH_2Cl_2 (2 x each). Then the desired product was cleaved from resin.

- *Resin cleavage and purification:* The crude product was cleaved from the resin with 2 mL TFA/TIPS/ H_2O (95:2.5:2.5) for 2 h (1 mL/h). The TFA cleavage was concentrated in volume under a stream of N_2 and the crude product was precipitated with $-20\text{ }^\circ\text{C}$ diethyl ether. The resulting slurry was centrifuged, and the TFA/ether supernatant was decanted to yield the crude pellet. The pellet was resuspended in MeCN/ H_2O and purified by preparative HPLC (Luna 5 μm C_{18} (2) 100 Å, 250 x 21.2 mm Phenomenex column). The purified product was transferred to 50 mL centrifuge tubes, frozen in LN_2 , and lyophilized.



2-(2-((((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-2-oxoethyl)-2-hydroxysuccinic acid (**14a**). Scale: 50 mg of resin (0.32

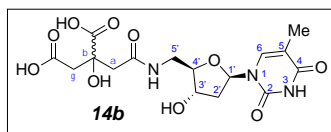
mmol/g, 0.016 mmol). Fluffy white solid (32% yield, 2.2 mg).

¹H NMR (600 MHz, D₂O) δ 7.38 (dd, *J* = 3.3, 1.5 Hz, 1H, H-6^T), 5.75 (d, *J* = 5.0 Hz, 1H, H-1'), 4.29 (dt, *J* = 7.0, 5.4 Hz, 1H, H-2'), 4.04 (dt, *J* = 11.2, 5.4 Hz, 1H, H-3'), 4.00 (dt, *J* = 9.4, 4.4 Hz, 1H, H-4'), 3.52 (td, *J* = 15.1, 6.4 Hz, 1H, H-5'b), 3.43 (td, *J* = 14.2, 4.2 Hz, 1H, H-5'a), 2.95 (dd, *J* = 16.1, 11.6 Hz, 1H, CH_aH_b^{citrate-γ}), 2.79 – 2.62 (m, 3H, CH_aH_b^{citrate-γ} & CH₂^{citrate-α}), 1.82 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 176.9, 176.9, 173.7, 173.6, 171.7, 171.6, 166.5, 151.8, 137.7, 137.7, 111.6, 89.6, 89.5, 81.9, 73.7, 72.8, 72.7, 70.4, 70.3, 44.6, 44.5, 43.2, 43.0, 40.4, 40.4, 11.5.

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 15.6 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₁₆H₂₂N₃O₁₁Na 454.1074; found 454.1064.



2-hydroxy-2-(2-((((2*R*,3*S*,5*R*)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-2-oxoethyl)succinic acid (**14b**). Scale: 63 mg of resin (0.33 mmol/g,

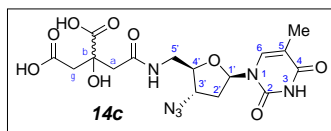
0.021 mmol). Fluffy white solid (13% yield, 1.1 mg).

¹H NMR (600 MHz, D₂O) δ 7.40 (d, *J* = 5.8 Hz, 1H, H-6^T), 6.15 (td, *J* = 6.9, 2.3 Hz, 1H, H-1'), 4.29 (tt, *J* = 7.8, 4.2 Hz, 1H, H-4'), 3.93 (dq, *J* = 8.2, 4.4 Hz, 1H, H-3'), 3.50 – 3.33 (m, 2H, CH₂-5'), 2.93 (dd, *J* = 16.0, 10.7 Hz, 1H, CH_aH_b^{citrate-γ}), 2.76 – 2.68 (m, 2H, CH_aH_b^{citrate-γ} & CH_aH_b^{citrate-α}), 2.63 (dd, *J* = 14.6, 4.5 Hz, 1H, CH_aH_b^{citrate-α}), 2.35 – 2.23 (m, 2H, CH₂-2'), 1.82 (s, 3H, CH₃^T).

¹³C NMR (151 MHz, D₂O) δ 173.8, 171.7, 171.6, 166.5, 151.7, 137.6, 137.5, 111.6, 85.2, 85.2, 84.2, 84.2, 73.7, 71.2, 71.2, 44.6, 44.5, 43.2, 43.0, 40.6, 40.5, 37.6, 11.5.

HPLC (1-75% MeCN/H₂O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t 17.4 min (260 nm).

HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₁₆H₂₂N₃O₁₀Na 438.1125; found 438.1127.



2-(2-((((2*R*,3*S*,5*R*)-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)amino)-2-oxoethyl)-2-hydroxysuccinic acid (**14c**). Scale: 200 mg of resin (0.28

mmol/g, 0.056 mmol). Fluffy white solid (4% yield, 0.89 mg).

¹H NMR (600 MHz, D₂O) δ 7.43 – 7.36 (m, 1H, H-6^T), 6.07 (t, *J* = 6.5 Hz, 1H, H-1'), 4.15 (ddt, *J* = 21.8, 7.6, 5.8 Hz, 1H, H-4'), 3.91 (p, *J* = 5.0 Hz, 1H, H-3'), 3.55 (ddd, *J* = 21.3, 14.7, 5.6 Hz, 1H,

H-5'b), 3.40 (ddd, $J = 24.2, 14.5, 4.4$ Hz, 1H, H-5'a), 2.96 (dd, $J = 16.0, 14.4$ Hz, 1H, $\text{CH}_a\text{H}_b^{\text{citrate-}\gamma}$), 2.80 – 2.71 (m, 2H, $\text{CH}_a\text{H}_b^{\text{citrate-}\gamma}$ & $\text{CH}_a\text{H}_b^{\text{citrate-}\alpha}$), 2.66 (dd, $J = 14.6, 3.5$ Hz, 1H, $\text{CH}_a\text{H}_b^{\text{citrate-}\alpha}$), 2.51 – 2.37 (m, 2H, $\text{CH}_2\text{-}2'$), 1.82 (s, 3H, CH_3^T).

^{13}C NMR (151 MHz, D_2O) δ 174.8, 172.1, 166.5, 151.5, 137.7, 111.5, 85.1, 81.9, 60.4, 42.7, 40.1, 35.5, 21.6, 11.4.

HPLC (1-75% MeCN/ H_2O (0.1% TFA) over 25 min, flow rate: 10 mL/min), R_t : 17.2 min (260 nm).

HRMS (ESI⁺) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{16}\text{H}_{21}\text{N}_6\text{O}_9\text{Na}$ 463.1189; found 463.1191.

Expression and Purification of AmpC β -lactamase

AmpC (pET24a vector) was expressed in BL21-RIL *E. coli* cells by autoinduction. Cultures were grown at 37 °C for 4 h and incubated at 16 °C overnight (16-18 h). The purification was carried out at 4 °C. Cell pellets were resuspended in 50 mL 20 mM HEPES pH 7.5, 50 mM NaCl, with 25 mg lysozyme, 25 μL DNase and 50 μL protease inhibitor cocktail. Cells were sonicated twice for 1.5 min (1 s on/2 s off, 50% amplitude), resting on ice for 5 min between sonication cycles. The lysed cells were centrifuged at 35000 rpm for 65 min using a 45-Ti rotor to pellet the membrane fraction. The supernatant was incubated with 3 x 1 mL Ni-NTA resin columns for 1 hour at 4 °C. The resin was washed with 20 mL Wash 1 (20 mM HEPES pH 7.5, 50 mM NaCl, 20 mM imidazole, 5% glycerol) followed by a wash with 20 mL Wash 2 (20 mM HEPES pH 7.5, 50 mM NaCl, 45 mM imidazole, 5% glycerol). AmpC was eluted in 2 x 1 mL fractions of elution buffer (20 mM HEPES pH 7.5, 50 mM NaCl, 500 mM imidazole, 5% glycerol) and immediately dialyzed to remove imidazole yielding 41.5 mg of protein per 1 L. Presence of protein was verified by SDS-PAGE (Figure S1A).

Sequence of His₆-AmpC [MW: 41.7 kDa. Extinction coefficient: ϵ (280 nm) = 93850 $\text{M}^{-1}\text{cm}^{-1}$]:

MGSSHHHHHSSGLVPRGSHMAPQQINDIVHRTITPLIEQQKIPGMAVAVIYQGKPYFTWGYA
DIAKKQPVTQQTLFELGVSSTFTGVLGGDAIARGEIKLSDPTTKYWPELTAKQWNGITLLHLAY
TAGGLPLQVPDEVKSSDLLRFYQNWQPAWAPGTQRLYANSSIGLFGALAVKPSGLSFEQAMQ
TRVFQPLKLNHTWINVPPAEKKNYAWGYREGKAVHVSPGALDAEAYGVKSTIEDMARWVQSNL
KPLDINEKTLQQGIQLAQSRWQTDGMYQGLGWEMLDWPVNPDSIINGSNDKIALAARPVKAIT
PPTPAVRASWWHKTGATGGFGSYVAFIPEKELGIVML ANKNYPNPARVDA AWQILNALQ

AmpC β -lactamase compound aggregator counter screen

Inhibitor in H₂O (from 2 mM stock, final: 50 μ M) was pipetted into a clear, U-shaped 96-well plate (Corning 3788). The assay buffer (50 mM potassium phosphate (25 mM KH₂PO₄ + 25 mM K₂HPO₄), pH 7 \pm 0.01% Triton X-100 vol/vol) was added to each well. Then the enzyme (AmpC) was added from a 30x, 40 nM stock (final [enzyme]: 1.34 nM) and the mixture was allowed to incubate for 5 minutes. Then nitrocefin from a 5 mM stock in DMSO (final [substrate]: 100 μ M) was added to the wells and mixed to fully distribute the DMSO mixture. The initial rates were measured at 482 nm in the linear portion of the reaction curve over a 5 min period at ambient temperature in the plate reader. Slopes of the reaction in the presence of inhibitor were compared to the control (H₂O only) reaction using the below equation to calculate inhibition. In this equation, v_i and v_c represent the inhibited and uninhibited rates of reaction, respectively.¹⁵

$$\% \text{ Inhibition} = 100 * \left(1 - \frac{v_i}{v_c}\right)$$

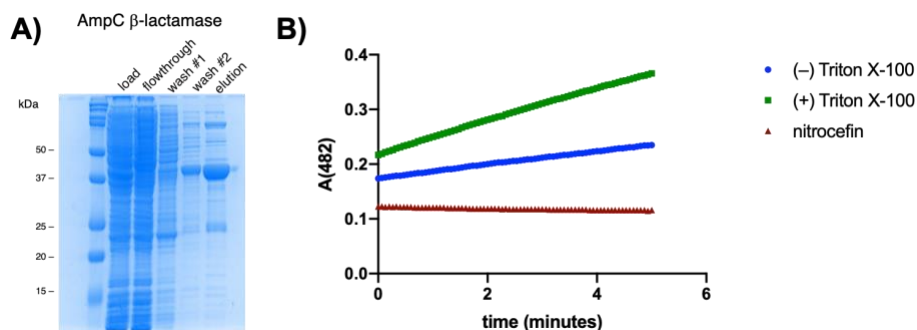


Figure S1. A) SDS-PAGE Gel of the NiNTA purification of AmpC β -lactamase (41.7 kDa) with Coomassie staining and **B)** the uninhibited controls of AmpC in the presence of detergent (+, green), absence of detergent (-, blue), and substrate in the absence of enzyme (Δ , red).

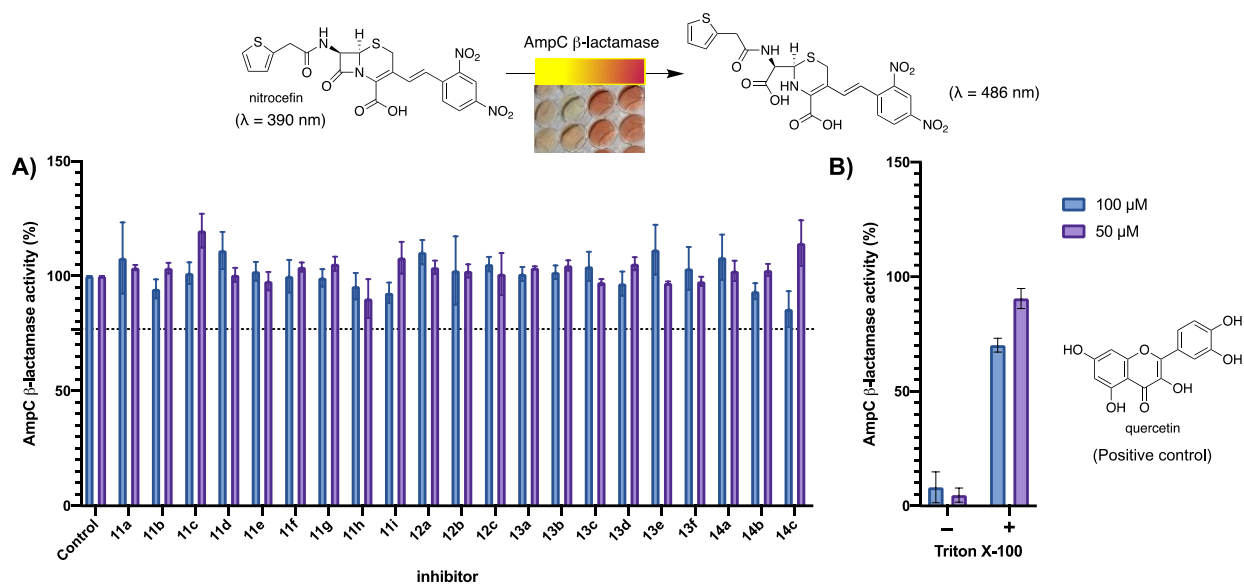


Figure S2. AmpC β -lactamase assay. A) Determination of small molecule, detergent-sensitive aggregates at 50 and 100 μ M. Panel B) is an example of a detergent-sensitive aggregator, quercetin. The compound, at both 50 and 100 μ M, causes significant inhibition of the enzyme in the absence of detergent. However, with the addition of Triton X-100, this inhibition is mitigated. Error bars are given for mean \pm SEM, $n = 3$. The dotted line indicates the threshold at which inhibition is significant (i.e., the activity of AmpC β -lactamase drops below this line).

Gel electrophoresis assay

Gel electrophoresis assays were performed using a modified literature procedure.¹⁶ SNM1A (698-1040) was stored as a 1.0 μ M solution in reaction buffer (20 mM HEPES-KOH, pH 7.5; 50 mM KCl, 10 mM MgCl₂, 0.05% Triton-X, 0.1 mg/mL BSA, 5% glycerol, 0.5 mM DTT). All incubations were carried out with gentle shaking (80 rpm). Modified nucleosides (1 mM or as otherwise specified) were treated with SNM1A (698-1040) (50 fmol) in reaction buffer containing 4% DMSO (10 μ L) on ice and incubated at 37 $^{\circ}$ C for 5 minutes. Thymidine (1 mM) was incubated with SNM1A as a control. A solution of 3'-Cy3-labelled 5'-phosphorylated 21mer oligonucleotide (1 μ L, 0.8 pmol/ μ L) was added and the reaction was incubated at 37 $^{\circ}$ C for 60 minutes. The reaction was stopped by the addition of stop solution (2 μ L, 95% formamide, 10 mM EDTA) followed by heating to 95 $^{\circ}$ C for 3 minutes. Digested oligonucleotides were separated on a 15% acrylamide 6.5 M urea gel (2.9 g urea, 2.7 mL 40% acrylamide-bisacrylamide 25:1, 1.4 mL 5X TBE (0.45 M Tris, 0.45 M boric acid, 0.01 M EDTA pH 8.0), 0.6 mL H₂O) in 1X TBE at 150 V for 75 or 90 min alongside bromophenol blue and xylene cyanol as markers for 8 nt and 28 nt, respectively, and imaged using Typhoon FLA 9500.

Gel electrophoresis assay – supplementary figures

Screening at 1 mM

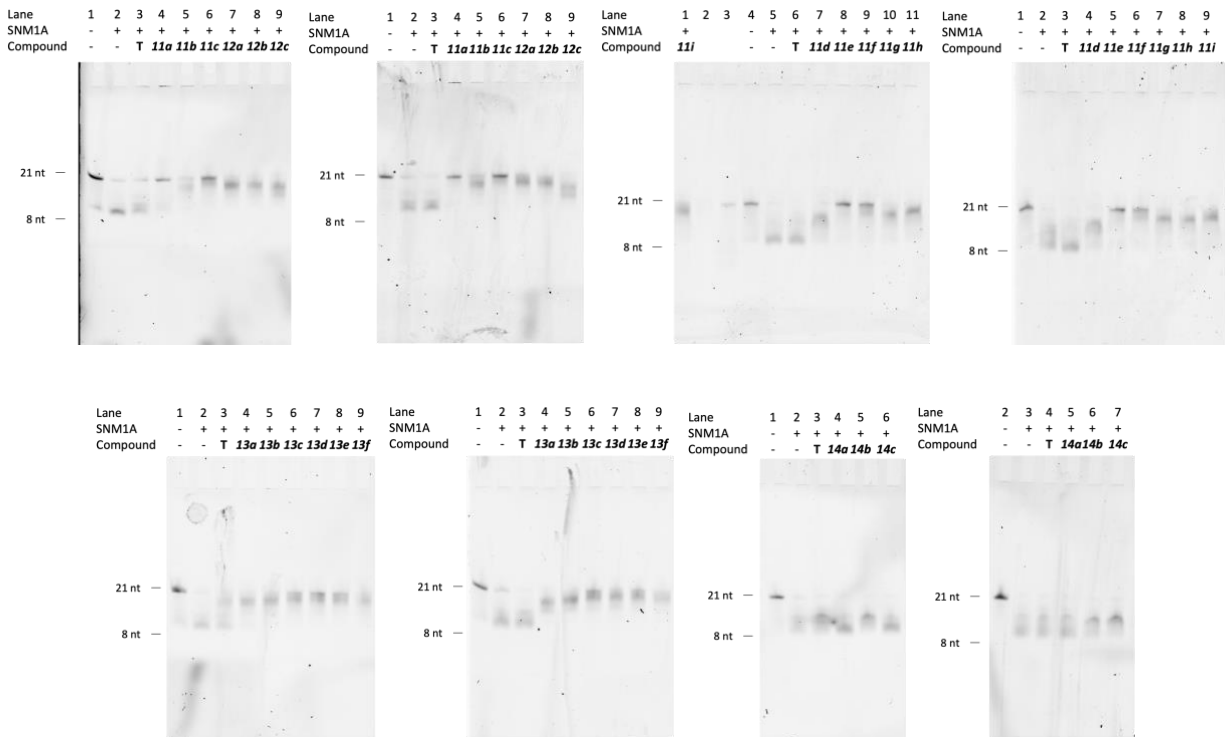


Figure S3. Evaluation of 11-14 as inhibitors of SNM1A at 1 mM. Digestion of fluorescent substrate oligonucleotide (0.8 pmol) after incubation with SNM1A (50 fmol) for 60 minutes at 37 °C following 5 minutes preincubation with 11-14 (1 mM). Experiments run in duplicate. nt = nucleotides, Sequence of fluorescent substrate oligonucleotide: 5'-XTAG CAG TCA GTC AGT CAT CGY-3' X = Thymidine 5'-phosphate, Y = Cy3

Screening at 100 μ M

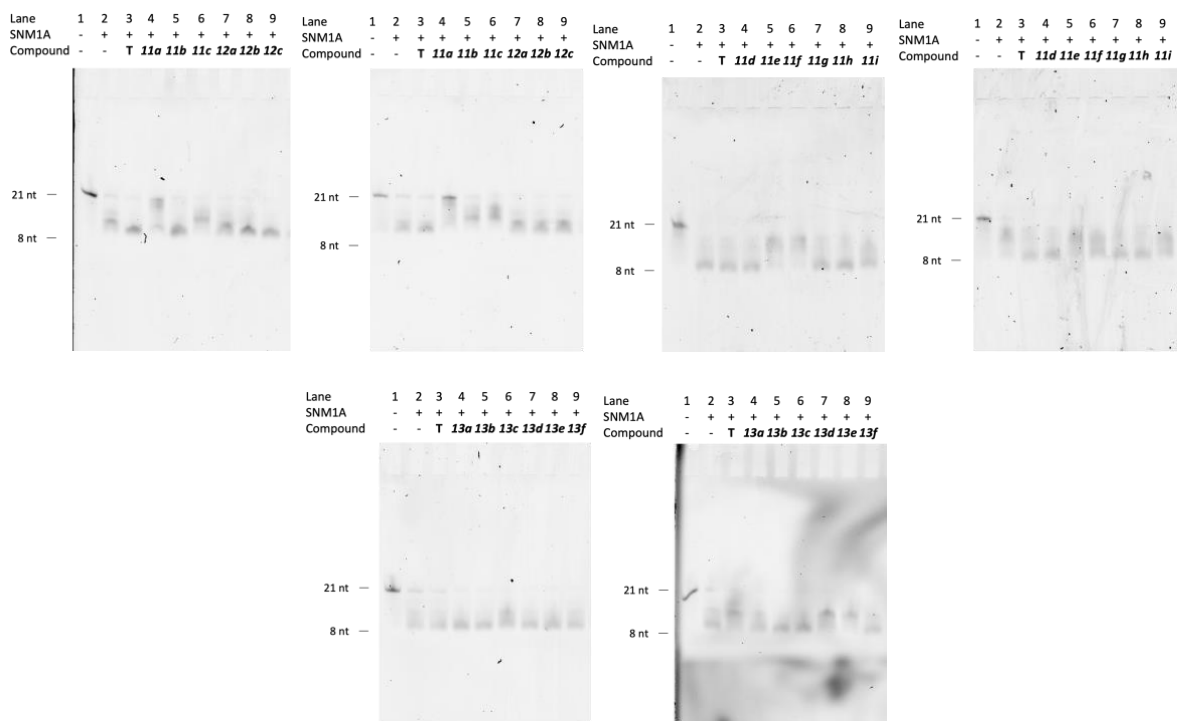


Figure S4. Evaluation of 11-14 as inhibitors of SNM1A at 100 μ M. Digestion of fluorescent substrate oligonucleotide (0.8 pmol) after incubation with SNM1A (50 fmol) for 60 minutes at 37 $^{\circ}$ C following 5 minutes preincubation with 11-14 (100 μ M). Experiments run in duplicate. nt = nucleotides, Sequence of fluorescent substrate oligonucleotide: 5'-XTAG CAG TCA GTC AGT CAT CGY-3' X = Thymidine 5'-phosphate, Y = Cy3

Concentration dependence assays

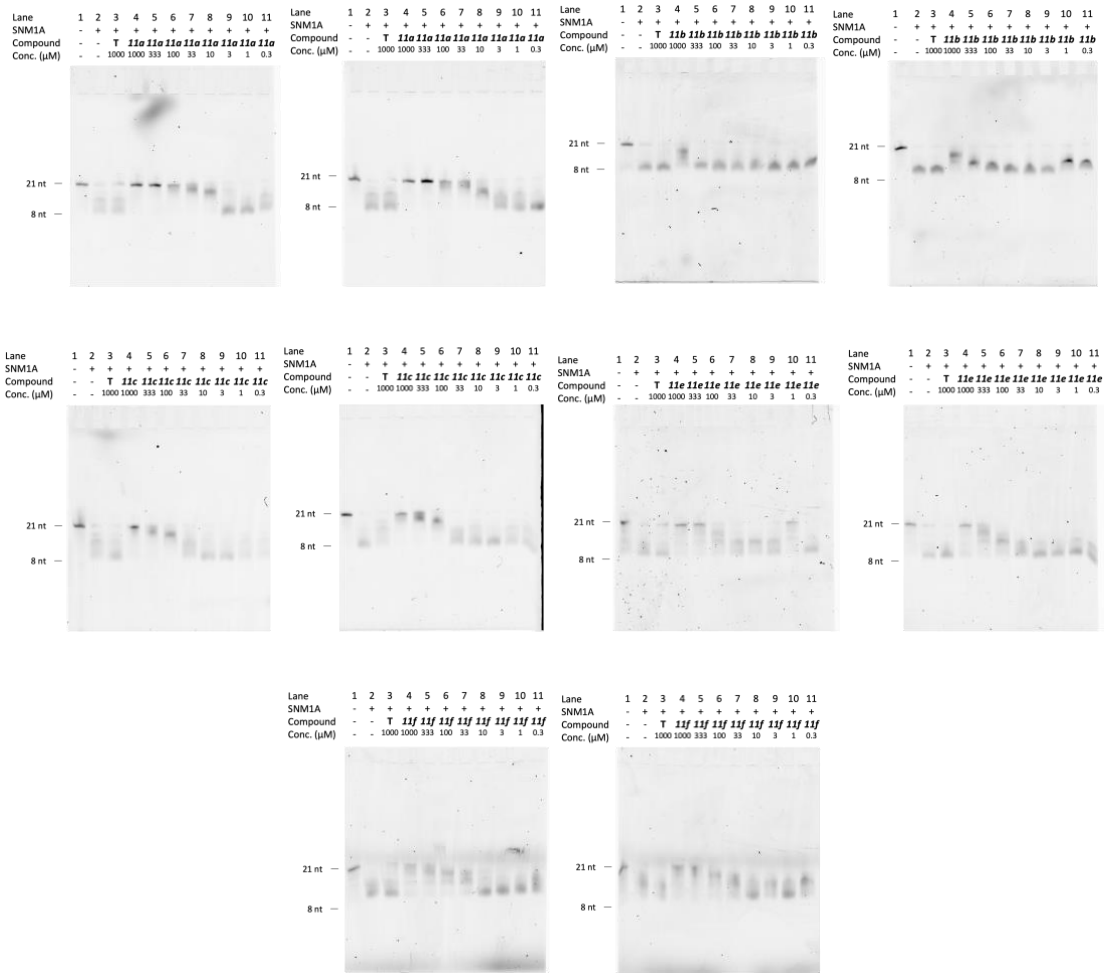


Figure S5. Concentration dependence assays of 11a-c, 11e-f. Digestion of fluorescent substrate oligonucleotide (0.8 pmol) after incubation with SNM1A (50 fmol) for 60 minutes at 37 °C following 5 minutes preincubation with 11a-c, 11e-f (1 mM – 0.3 μM). Experiments run in duplicate. nt = nucleotides, Sequence of fluorescent substrate oligonucleotide: 5'-XTAG CAG TCA GTC AGT CAT CGY-3' X = Thymidine 5'-phosphate, Y = Cy3

Lane	1	2	3	4	5	6	7
SNM1A	-	+	+	+	+	+	+
Compound	-	-	T	T	5-MeU	5-MeU	5-MeU
Conc. (μM)	-	-	1000	1000	333	100	33

Lane	1	2	3	4	5	6	7
SNM1A	-	+	+	+	+	+	+
Compound	-	-	T	T	5-MeU	5-MeU	5-MeU
Conc. (μM)	-	-	1000	1000	333	100	33

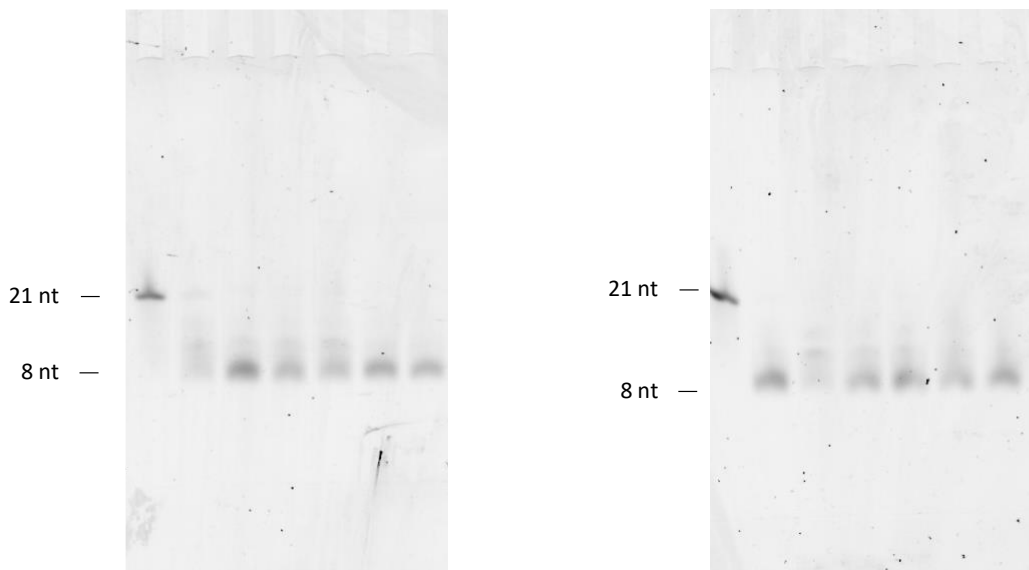


Figure S6. Concentration dependence assays of 5-methyluridine. Digestion of fluorescent substrate oligonucleotide (0.8 pmol) after incubation with SNM1A (50 fmol) for 60 minutes at 37 °C following 5 minutes preincubation with 5-methyluridine (1 mM – 33 μM). Experiments run in duplicate. nt = nucleotides, Sequence of fluorescent substrate oligonucleotide: 5'-XTAG CAG TCA GTC AGT CAT CGY-3' X = Thymidine 5'-phosphate, Y = Cy3

Real-time fluorescence assay

Real-time fluorescence assays were performed using a modified literature procedure¹⁷ utilizing a 20-nucleotide ssDNA substrate of the following sequence: 5'-A[FamT]AATTTGA[BHQT]CATCTATTAT-3' (Eurogentec). This oligonucleotide contained a fluorescein-conjugated thymine (FamT) as the second residue from the 5'-end, and a black-hole quencher moiety conjugated to a thymine residue (BHQT) eight nucleotides away. The oligonucleotide substrate was phosphorylated at the 5'-end using T4 polynucleotide kinase (New England Biolabs) according to the manufacturer's protocol, and made up to 1.25 μM for addition to reactions. Nuclease reactions were carried out in black 384-well microplates in a total volume of 25 μL in reaction buffer (20 mM HEPES-KOH, pH 7.5, 50 mM KCl, 10 mM MgCl_2 , 0.5 mM DTT, 0.05% (v/v) Triton-X100, 5% (v/v) glycerol) containing 4% DMSO, with 125 nM oligonucleotide substrate and 5 nM SNM1A (698-1040). Reactions were carried out in the presence of twelve different concentrations (0-500 μM) of inhibitor **11a**. Five replicates of each reaction were

performed. SNM1A was incubated with the inhibitor in the above nuclease buffer for 6.5 minutes at room temperature, before the reactions were started by the addition of the DNA substrate. The fluorescence spectra were measured at 37 °C using a BioTek Synergy H1 microplate reader (excitation at 495 nm, emission at 525 nm) with readings taken every 46 seconds for 35 minutes. The fluorescence intensity for each reaction was plotted against time, and the rate of increase was determined and normalized to the zero-inhibitor control. This was plotted against inhibitor concentration and the data were fitted using a “log[inhibitor] vs. normalized response” nonlinear regression algorithm on GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) to calculate the IC₅₀ values.

Circular dichroism titrations

Circular dichroism measurements were made using a JASCO J-815 spectrometer. A spectroscopic window of 400-200 nm was used, and data points were taken at an interval of 0.5 nm, scanning at 50 nm min⁻¹. Data are presented as an average of 3 accumulations. Baseline correction from blank solvent was used for all spectra. Spectra were measured in a 1 cm quartz cuvette at 25 °C. Titrations were performed through modification of a literature procedure.¹⁷ A solution of a 21mer ssDNA oligonucleotide in H₂O was made up and diluted to 1.22 μM in the cuvette. A solution of methylene blue, or of compound **11a**, in 4% DMSO in H₂O was aliquoted into the cuvette such that the volume in the cuvette did not increase by more than 10% during the titration. CD spectra of methylene blue and of compound **11a** alone, without the presence of the oligonucleotide, were also recorded at the same concentrations used in the titrations, and these were subtracted from the corresponding spectra measured during the titrations, to allow only changes in the CD spectrum of the oligonucleotide to be visualized (**Figure S6**).

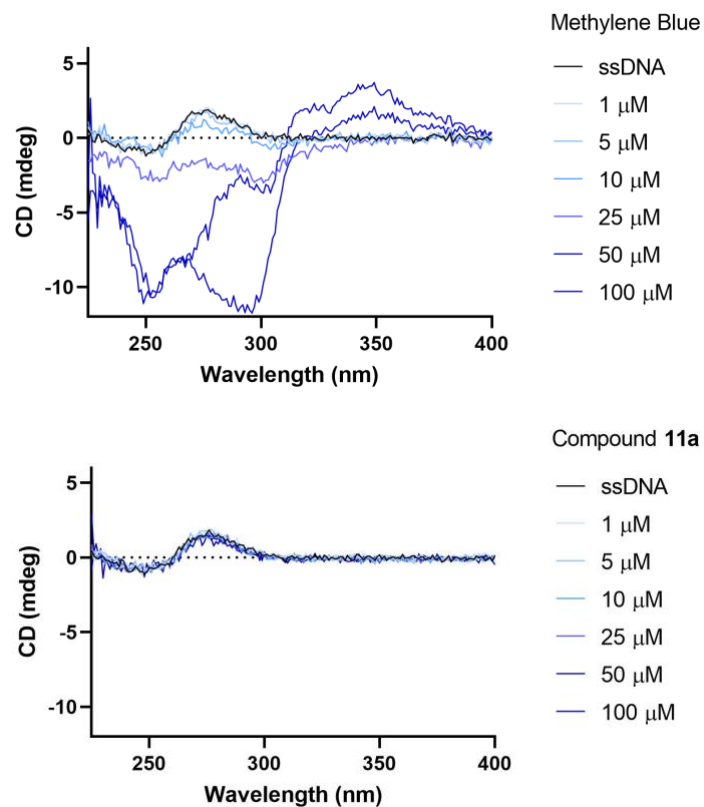
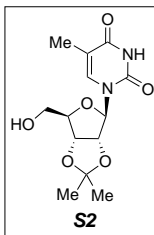
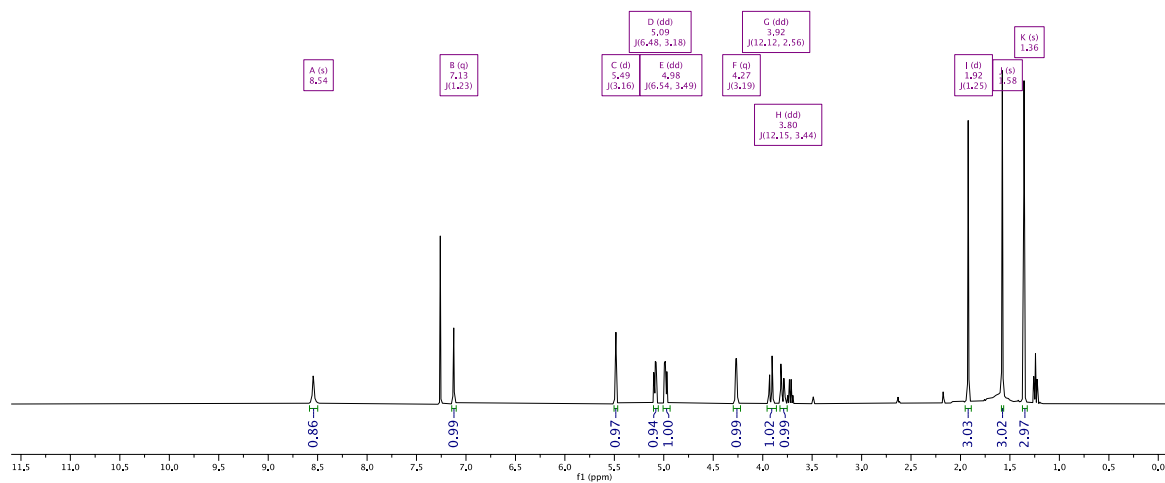


Figure S7. Circular dichroism spectra of a 21mer ssDNA oligonucleotide in the presence of varying concentrations of methylene blue or 11a.

Spectra: ^1H , ^{13}C , ^{19}F NMR, and crude HPLC

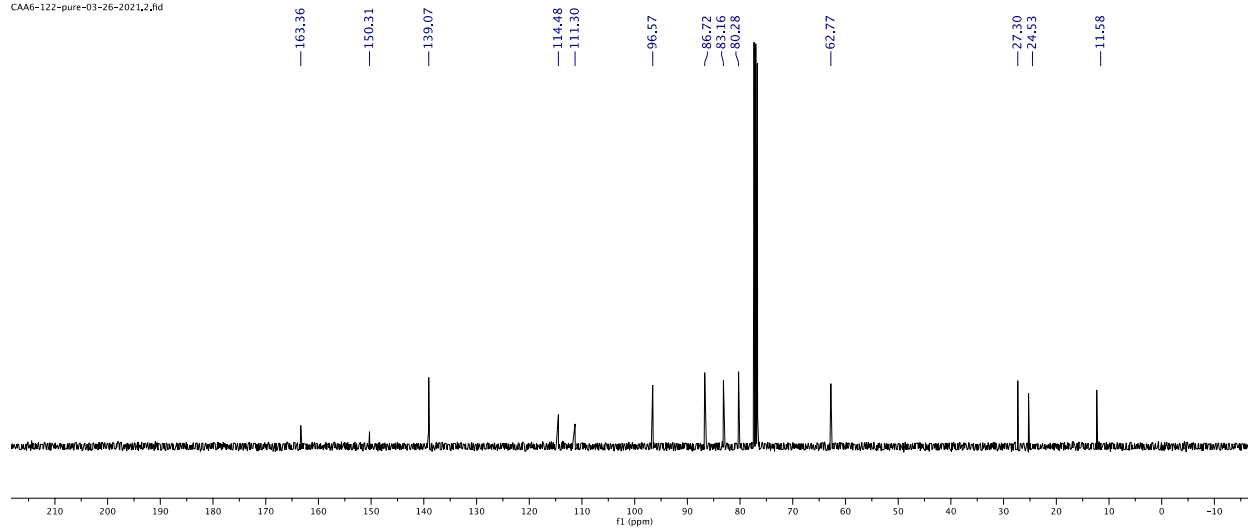


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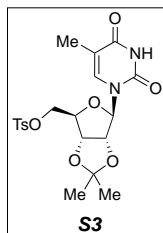


^1H NMR (400 MHz, CDCl_3) spectrum of compound **S2**.

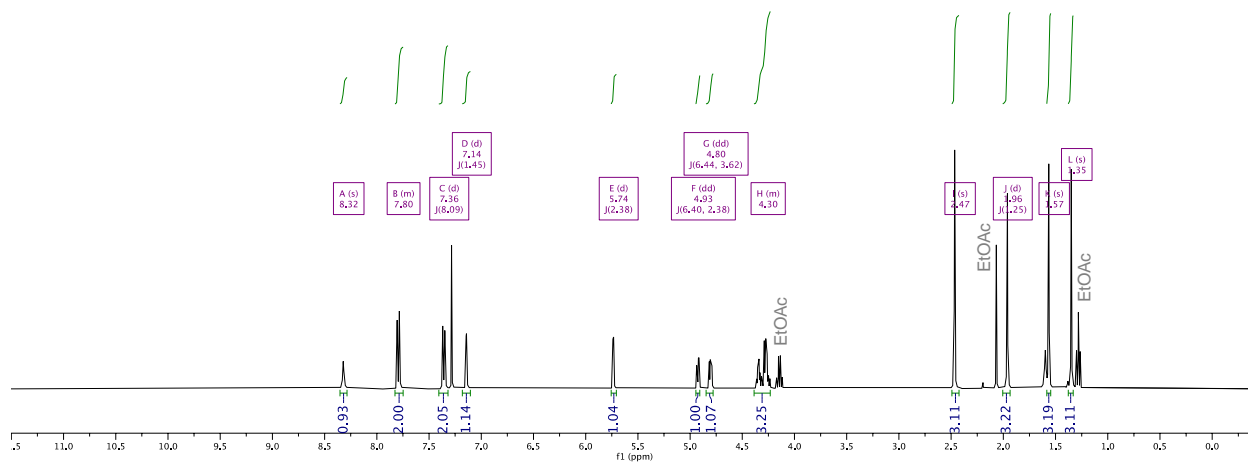
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^{13}C NMR (101 MHz, CDCl_3) spectrum of compound **S2**.

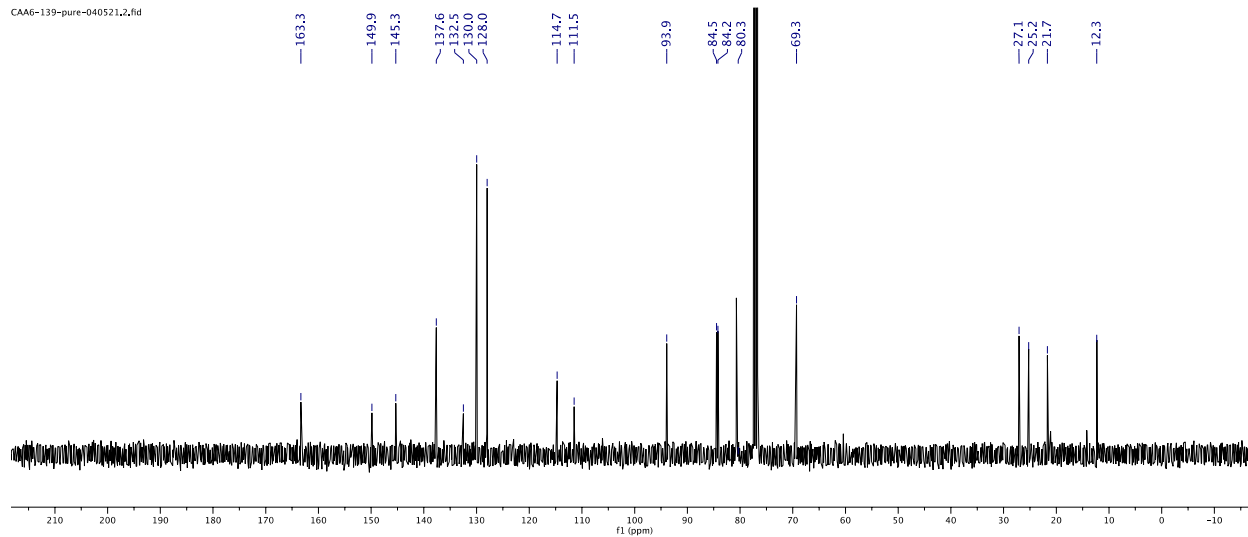


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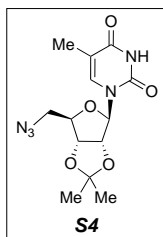


^1H NMR (400 MHz, CDCl_3) spectrum of compound **S3**.

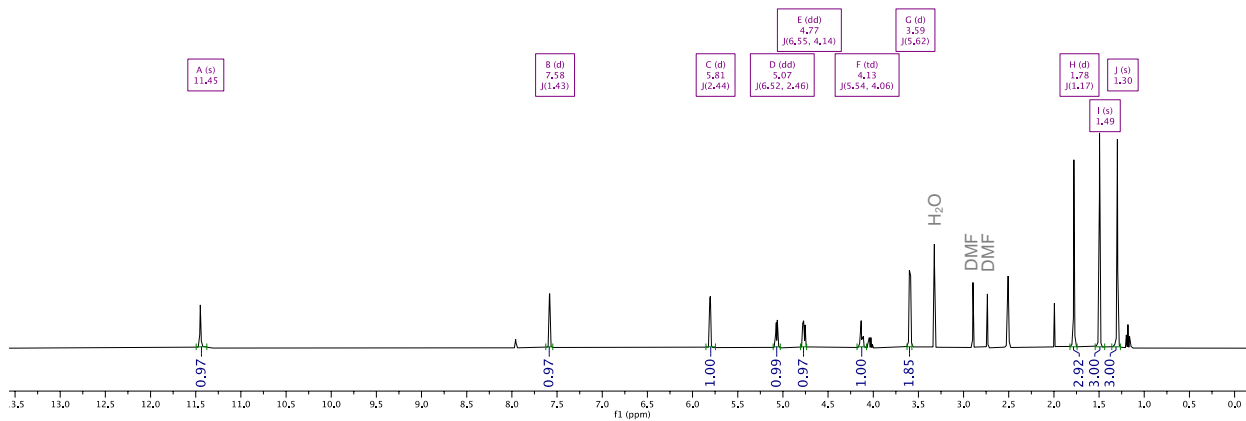
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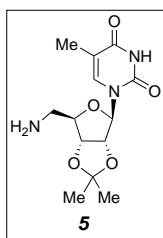
^{13}C NMR (101 MHz, CDCl_3) spectrum of compound **S3**.



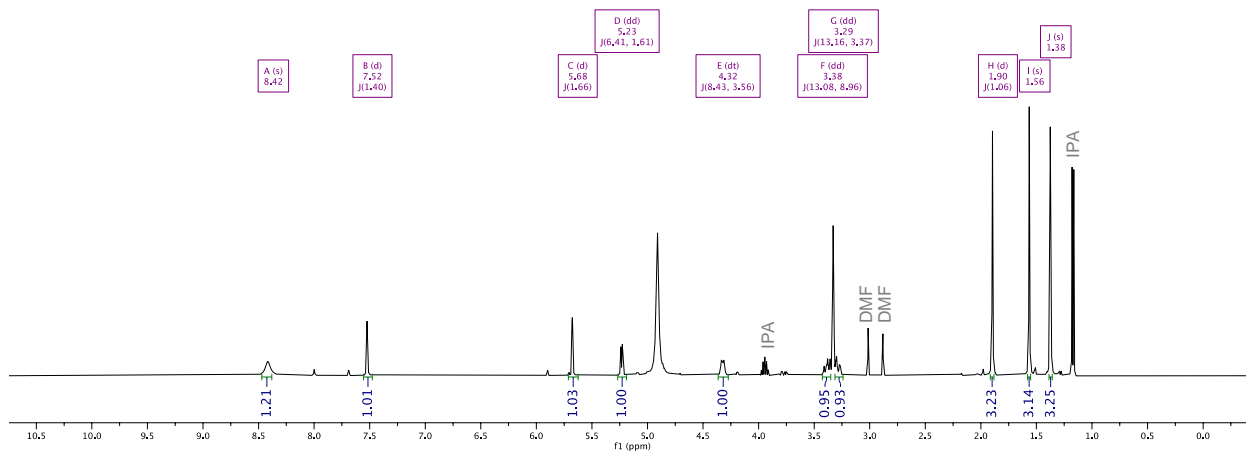
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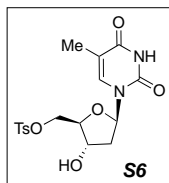
¹H NMR (400 MHz, DMSO-*d*₆) spectrum of compound **S4**.



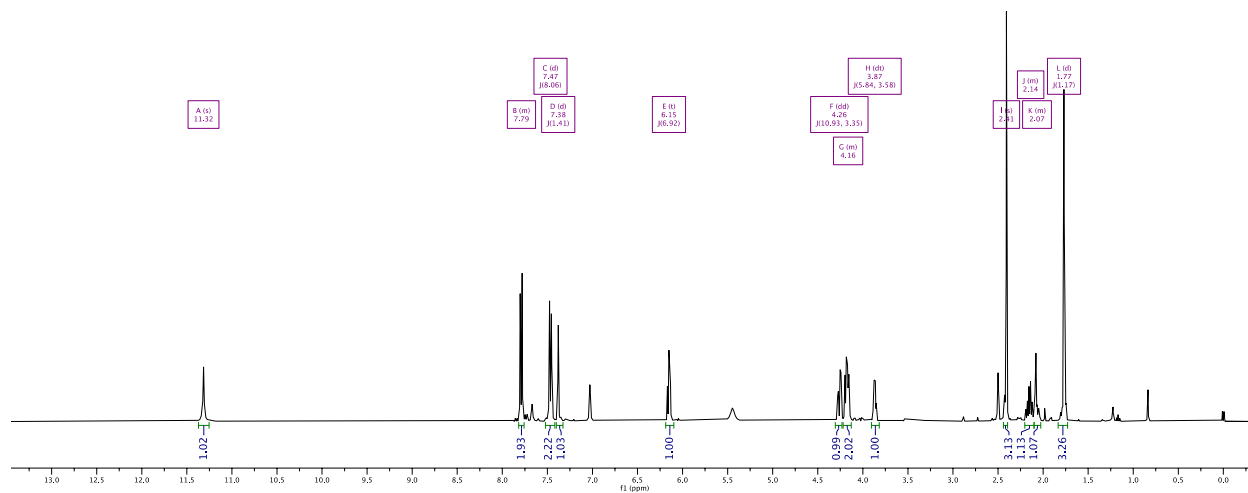
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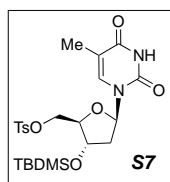
¹H NMR (400 MHz, MeOD-*d*₄) spectrum of compound **5**.



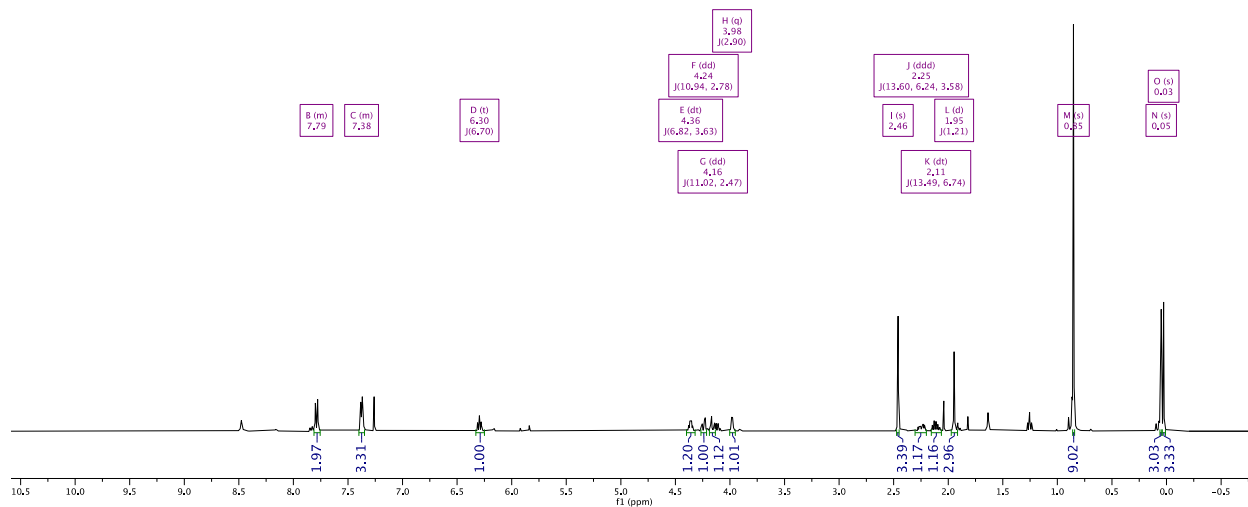
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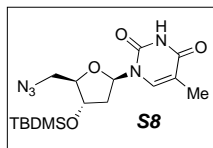
^1H NMR (400 MHz, $\text{DMSO-}d_4$) spectrum of compound **S6**.



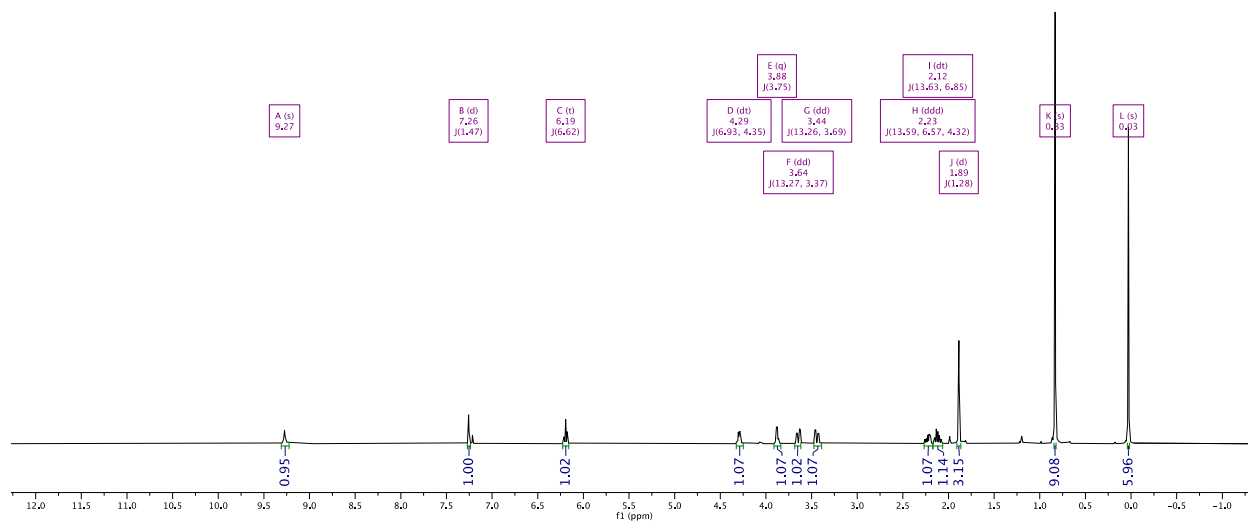
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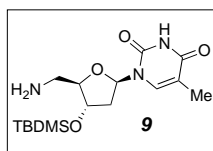
^1H NMR (400 MHz, CDCl_3) spectrum of compound **S7**.



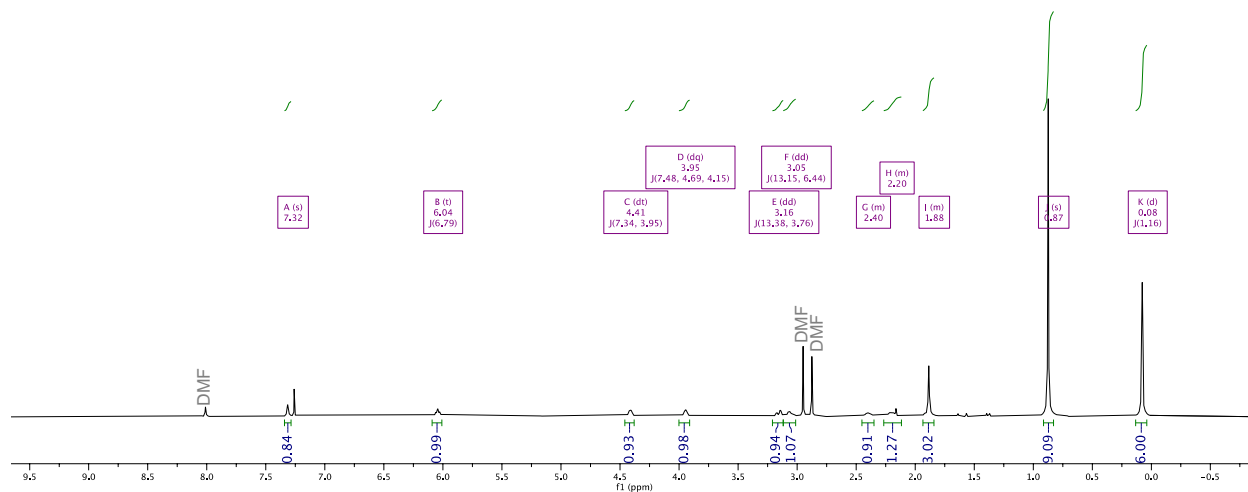
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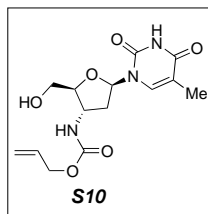
¹H NMR (500 MHz, CDCl₃) spectrum of compound **S8**.



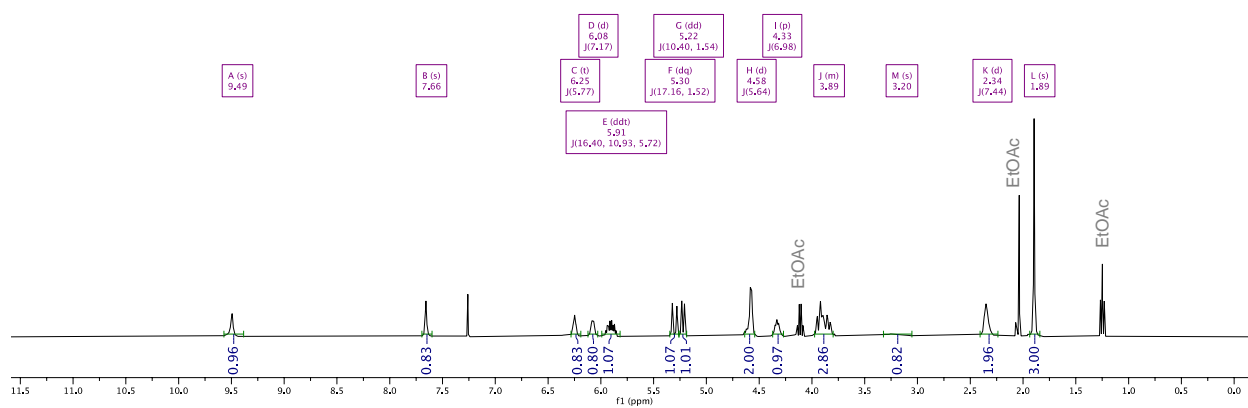
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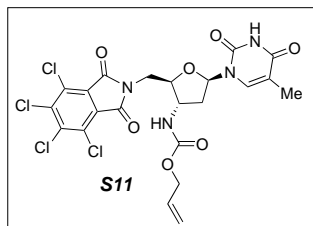
¹H NMR (400 MHz, CDCl₃) spectrum of compound **9**.



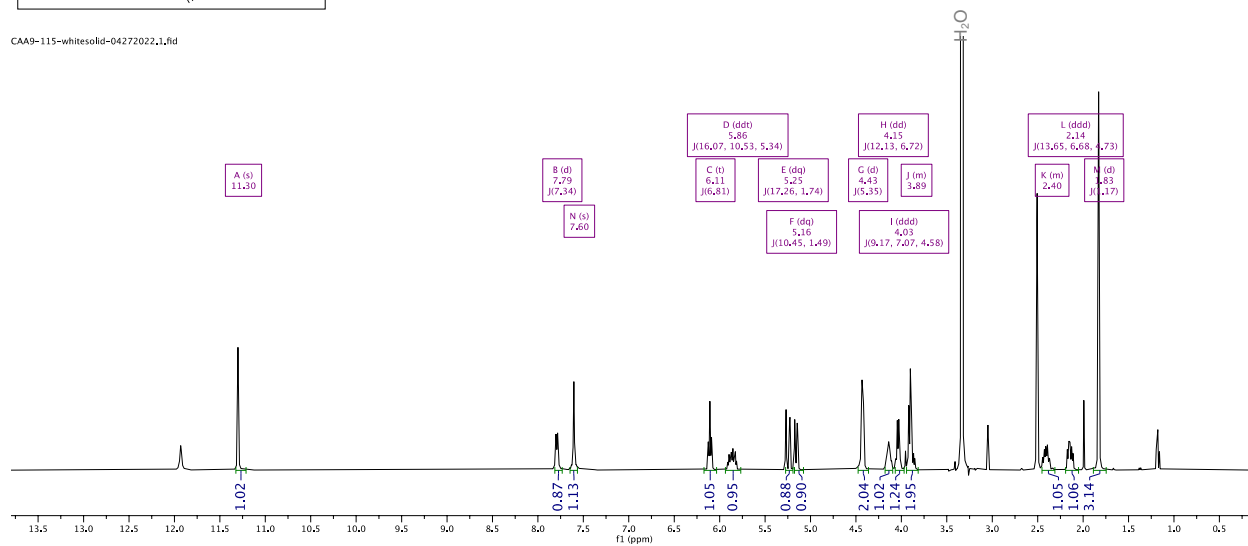
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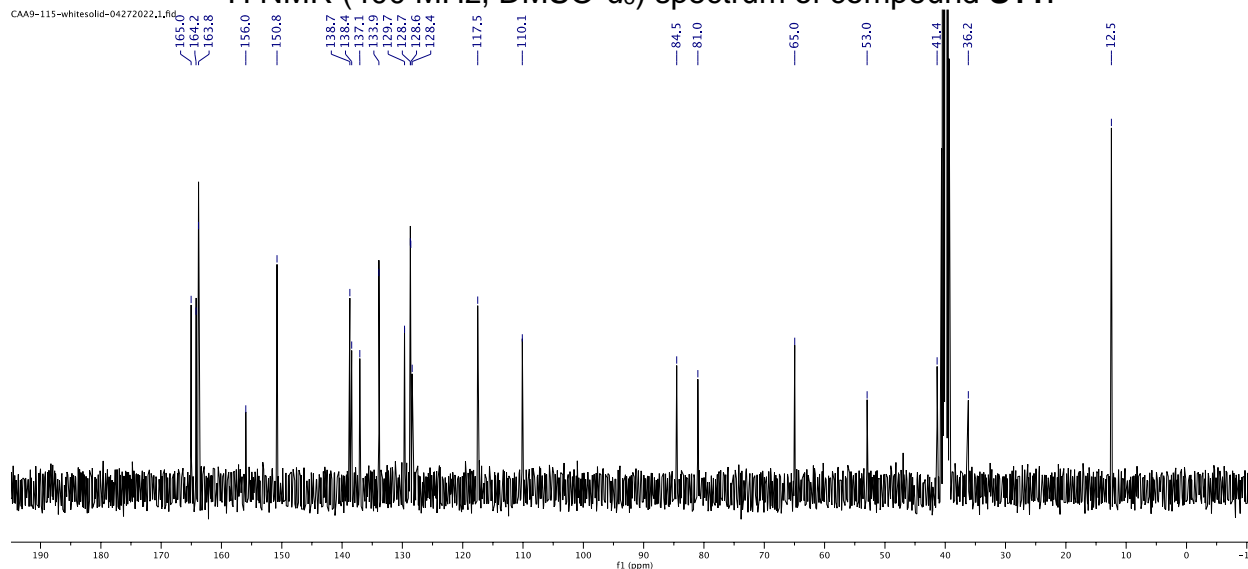
^1H NMR (400 MHz, CDCl_3) spectrum of compound **S10**.



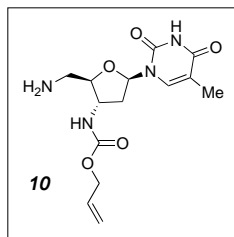
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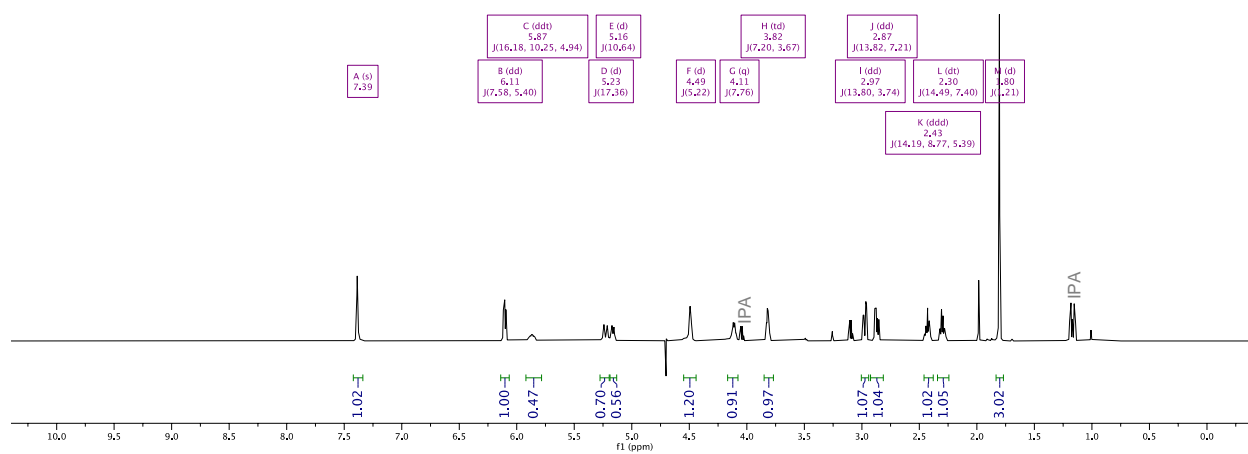
¹H NMR (400 MHz, DMSO-*d*₆) spectrum of compound **S11**.



¹³C NMR (101 MHz, DMSO-*d*₆) spectrum of compound **S11**.

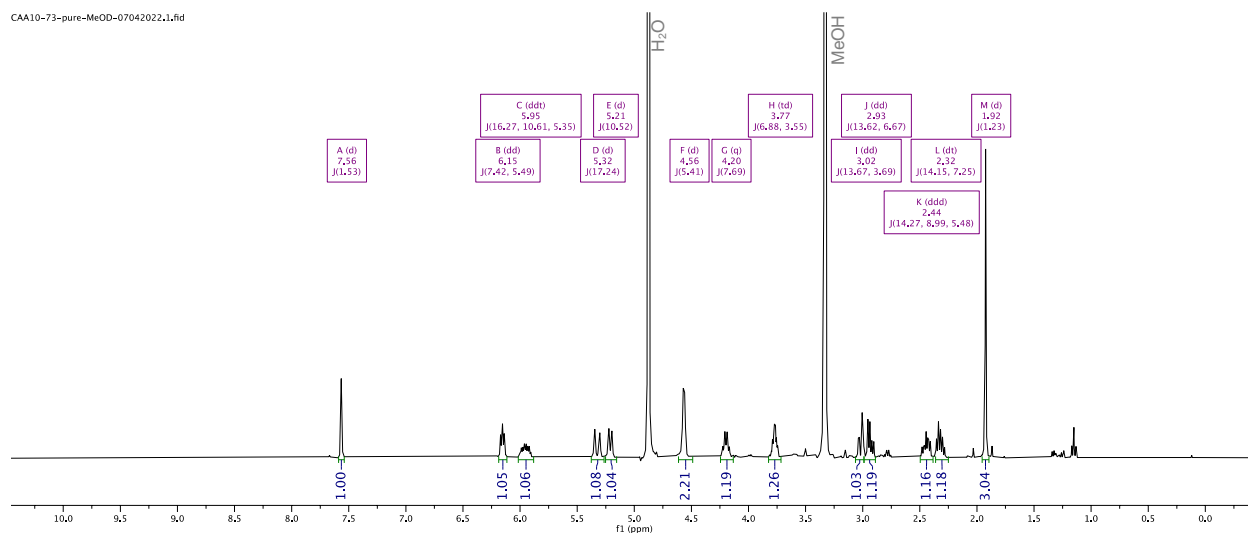


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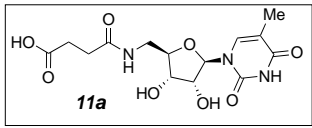


¹H NMR (600 MHz, D₂O) spectrum of compound **10** with water suppression.

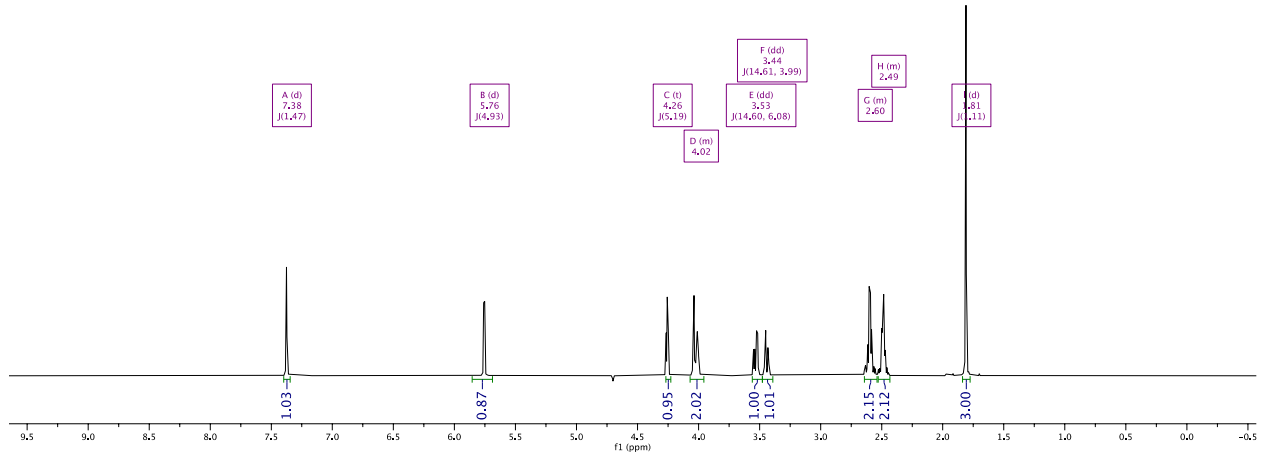
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¹H NMR (600 MHz, MeOD-*d*₄) spectrum of compound **10**.

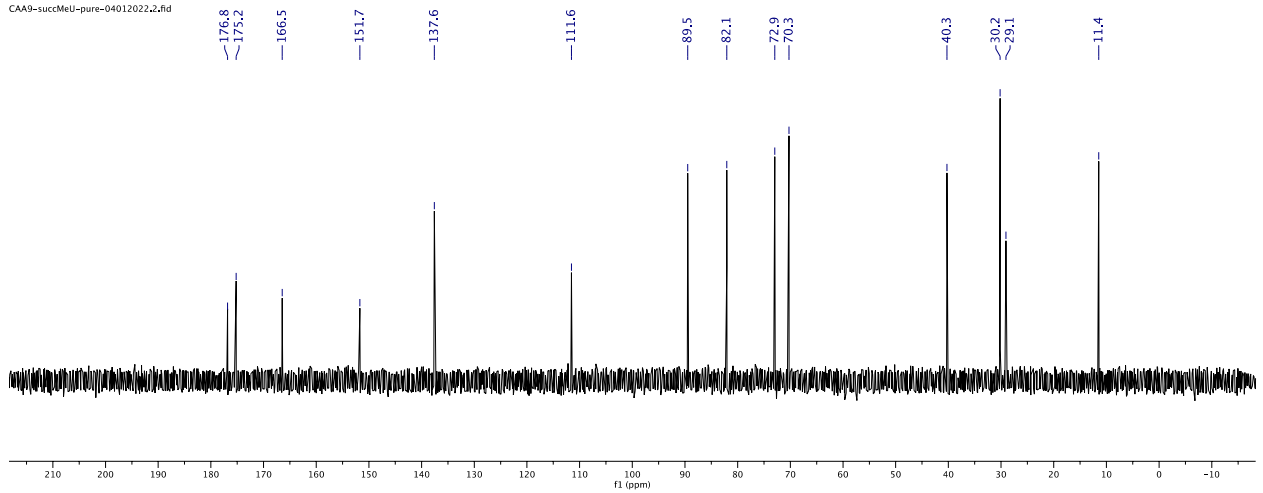


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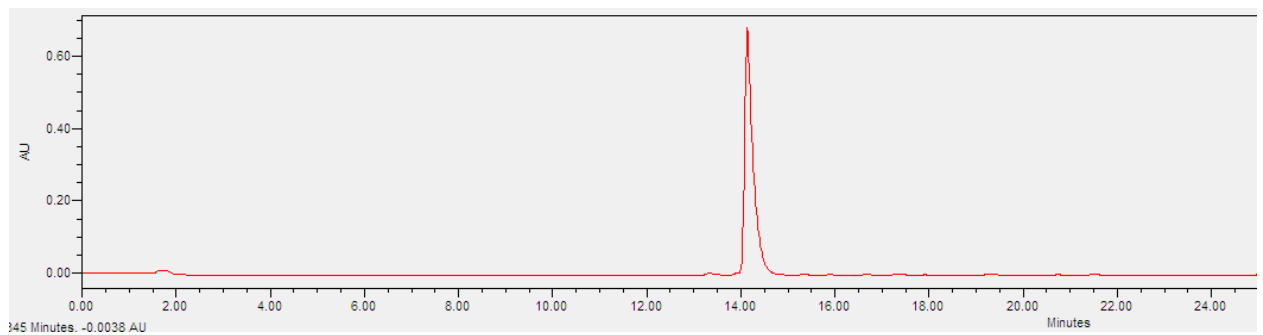


¹H NMR (600 MHz, D₂O) spectrum of compound **11a** with water suppression.

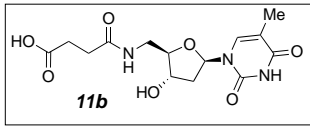
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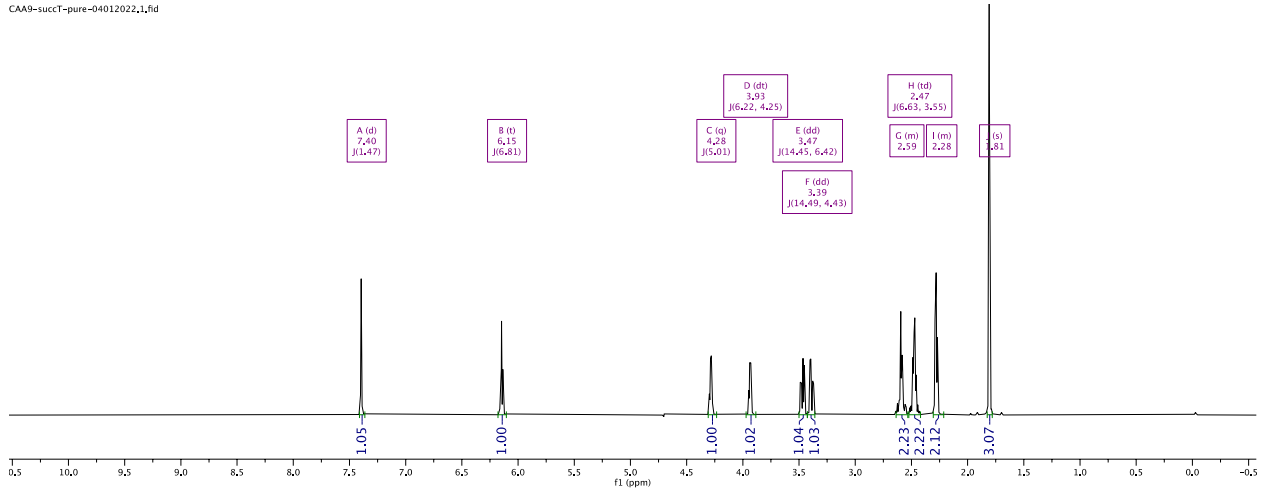
¹³C NMR (151 MHz, D₂O) spectrum of compound **11a**.



Crude HPLC spectrum of compound **11a**.

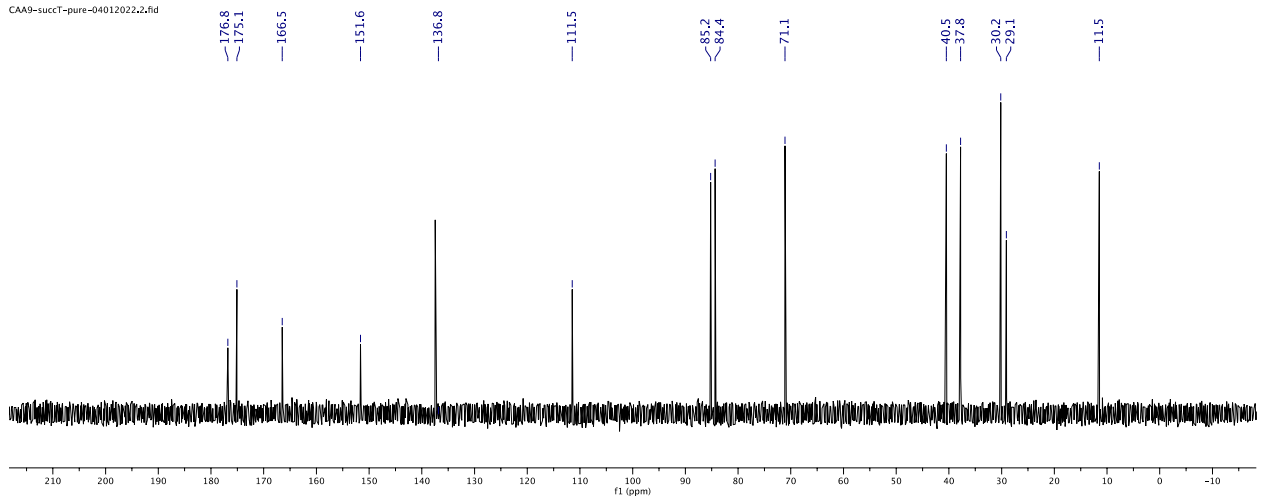


CAA9-sucCT-pure-04012022,1.fid

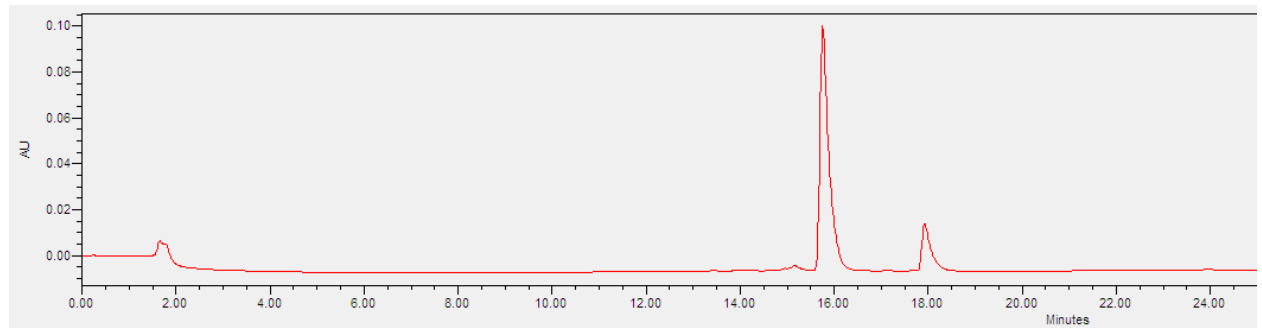


¹H NMR (600 MHz, D₂O) spectrum of compound **11b** with water suppression.

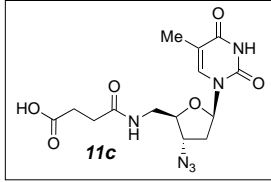
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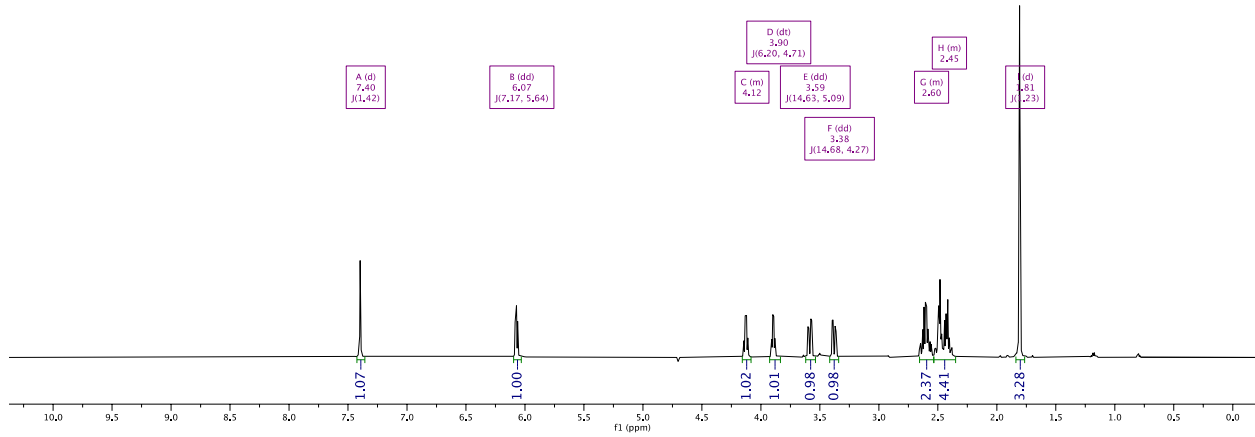
¹³C NMR (151 MHz, D₂O) spectrum of compound **11b**.



Crude HPLC spectrum of compound **11b**.

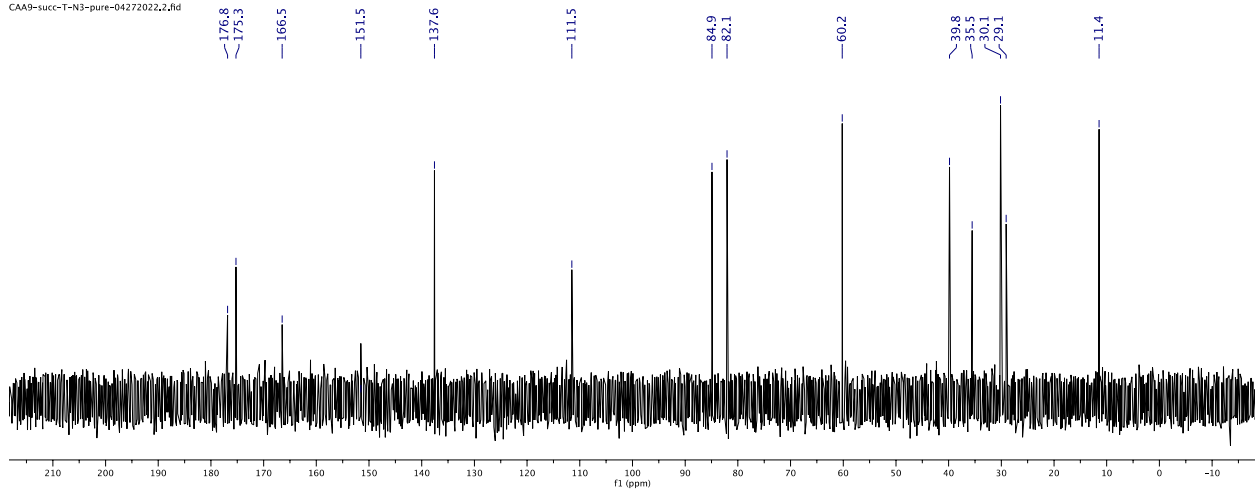


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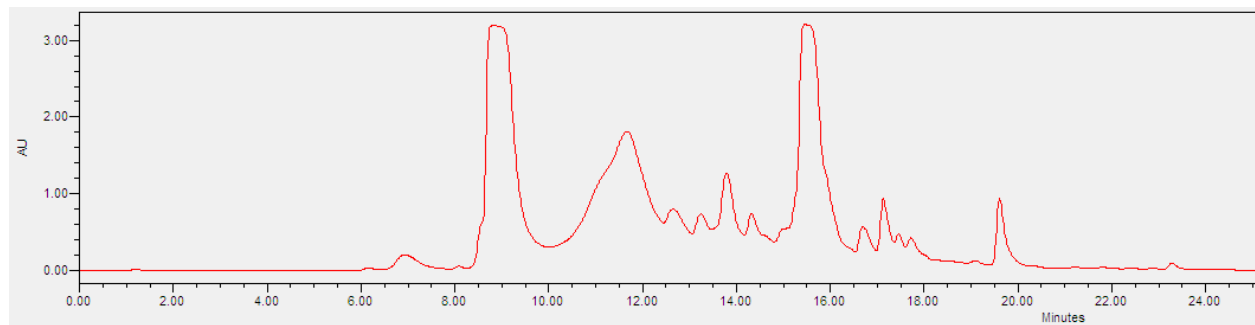


¹H NMR (600 MHz, D₂O) spectrum of compound **11c** with water suppression.

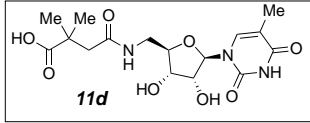
CAA9-succ-T-N3-pure-04272022,2.fid



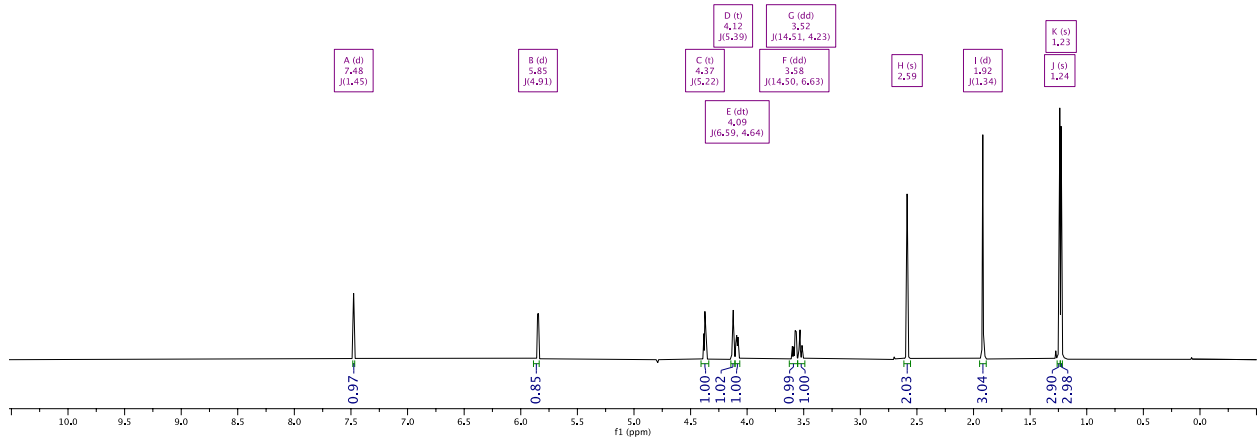
¹³C NMR (151 MHz, D₂O) spectrum of compound **11c**.



Crude HPLC spectrum of compound **11c**.

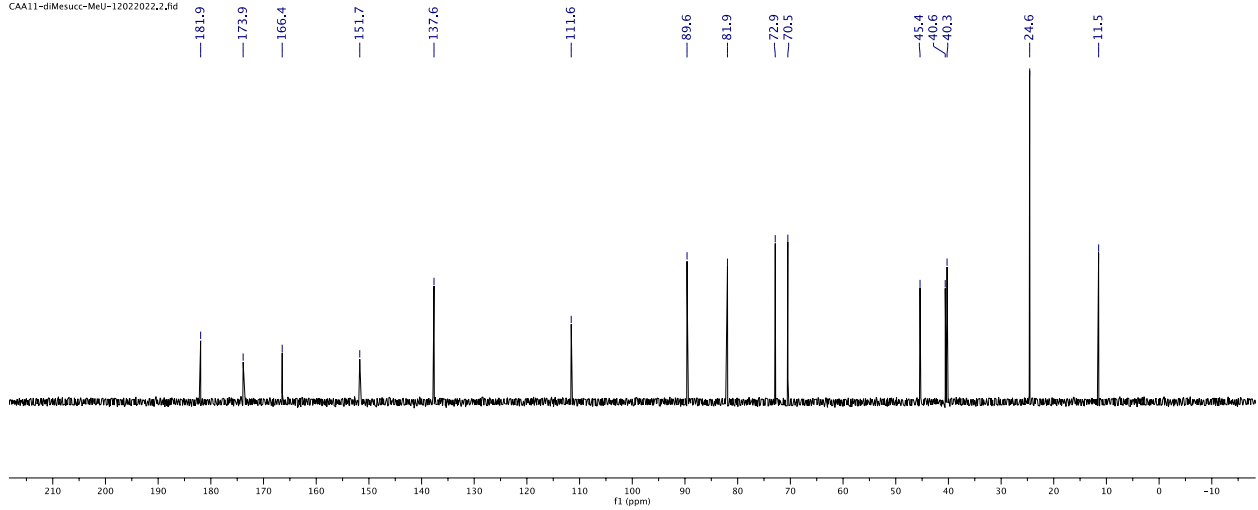


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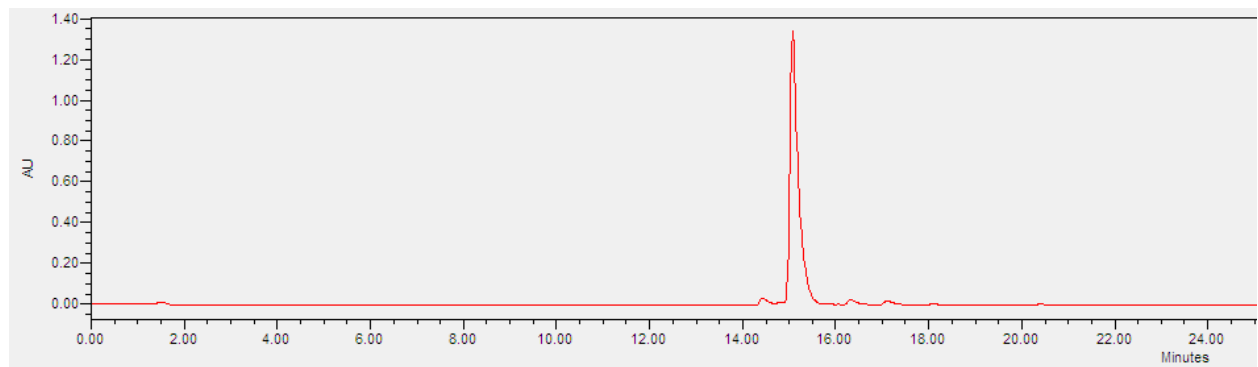


¹H NMR (600 MHz, D₂O) spectrum of compound **11d** with water suppression.

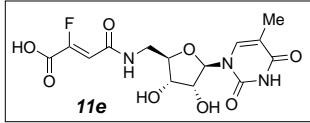
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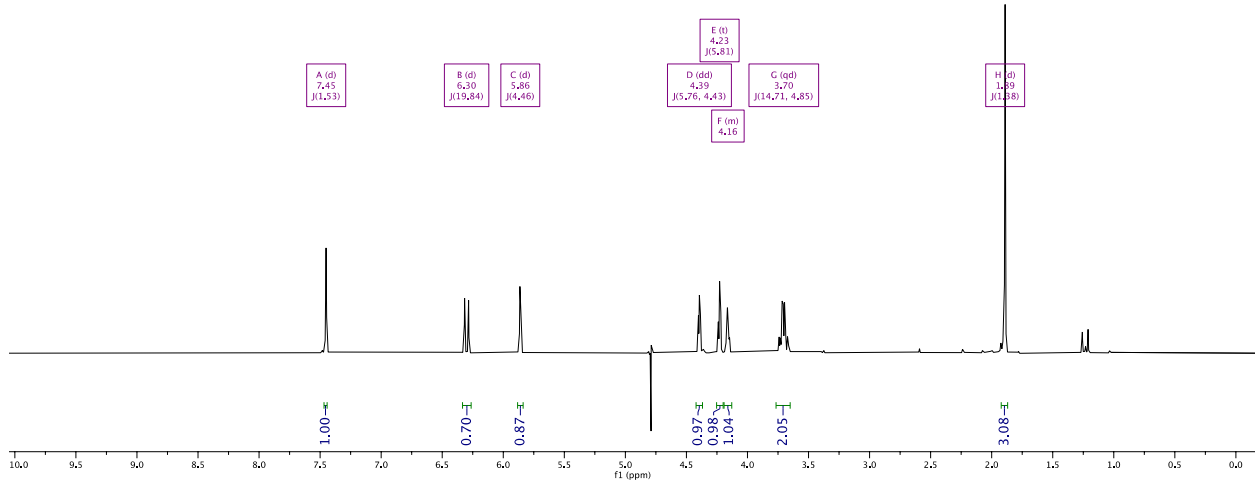
¹³C NMR (151 MHz, D₂O) spectrum of compound **11d**.



Crude HPLC spectrum of compound **11d**.

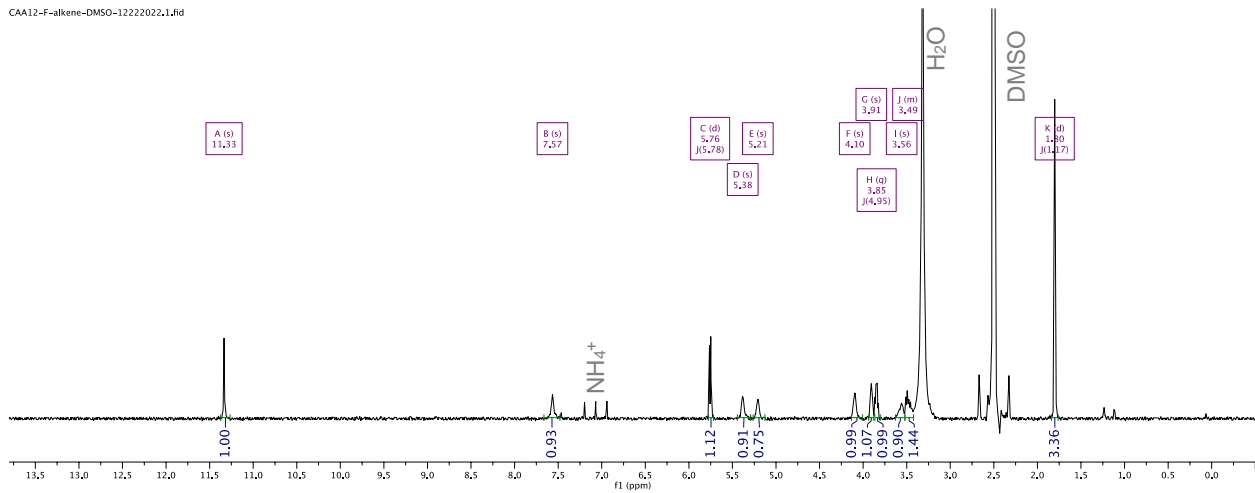


CAA11-dif-succ-Me1-12022022.1.fid

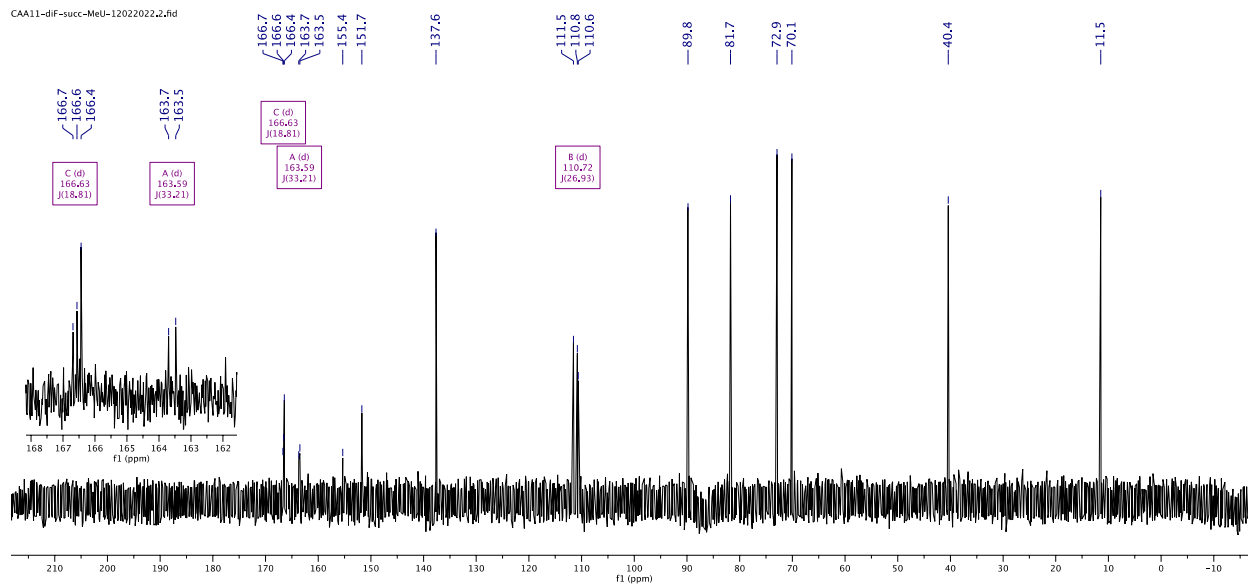


^1H NMR (600 MHz, D_2O) spectrum of compound **11e** with water suppression.

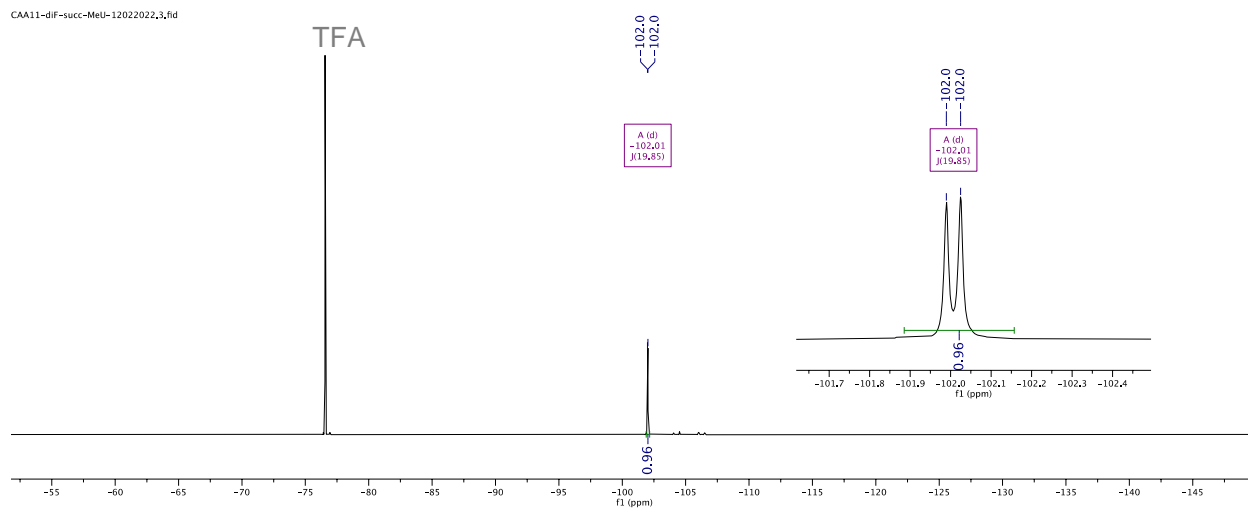
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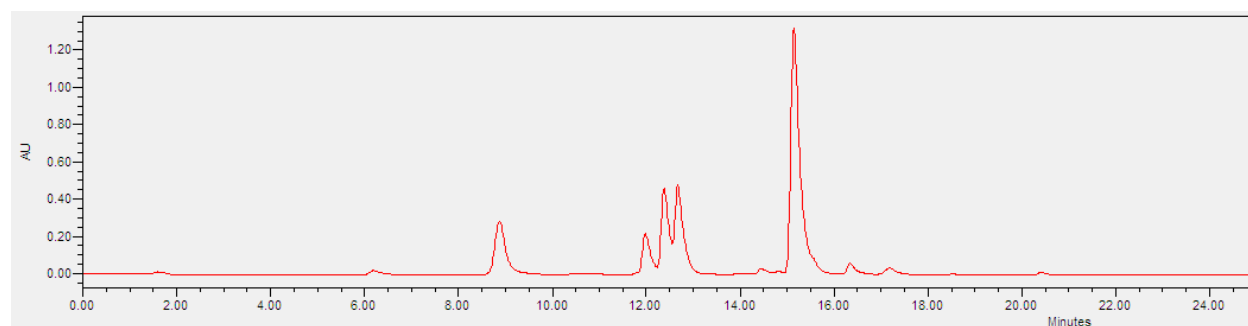
^1H NMR (400 MHz, $\text{DMSO}-d_6$) spectrum of compound **11e**.



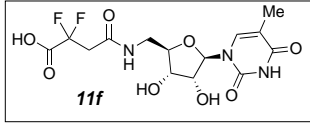
¹³C NMR (151 MHz, D₂O) spectrum of compound **11e**.



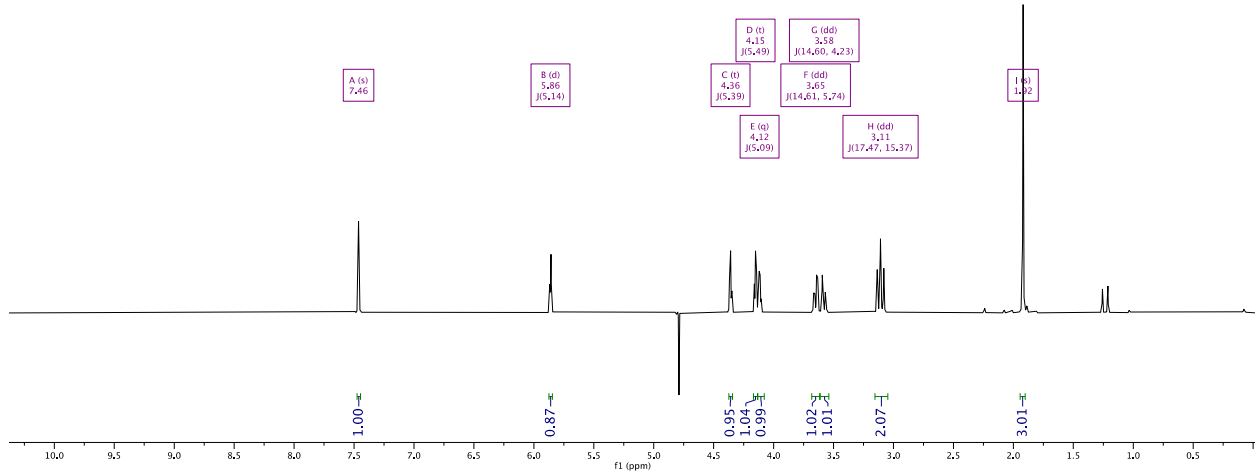
¹⁹F NMR (565 MHz, D₂O) spectrum of compound **11e**.



Crude HPLC spectrum of compound **11e**.

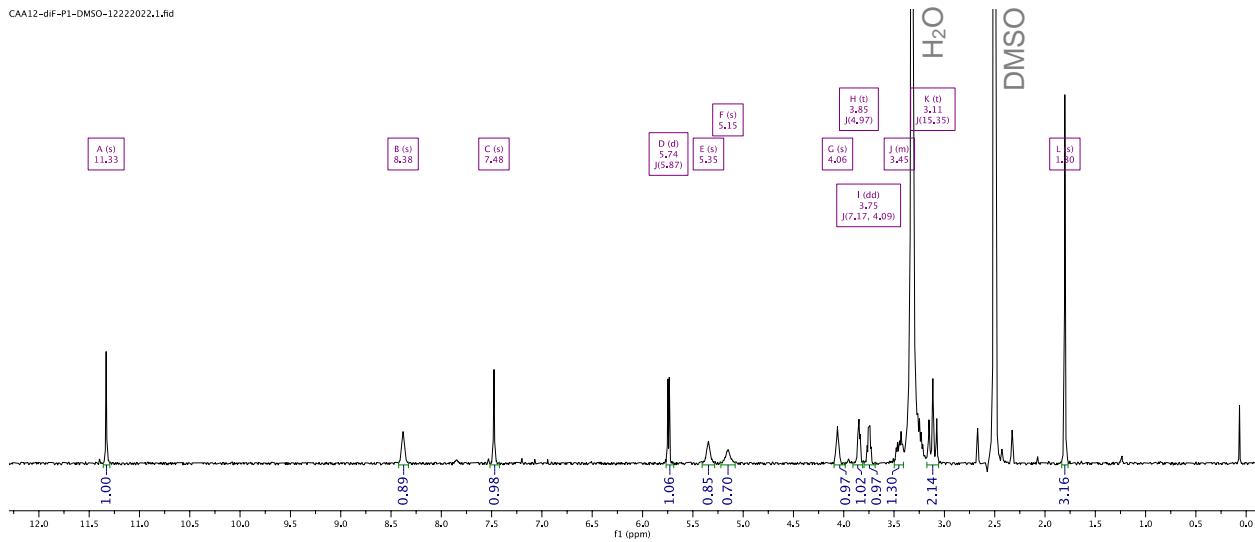


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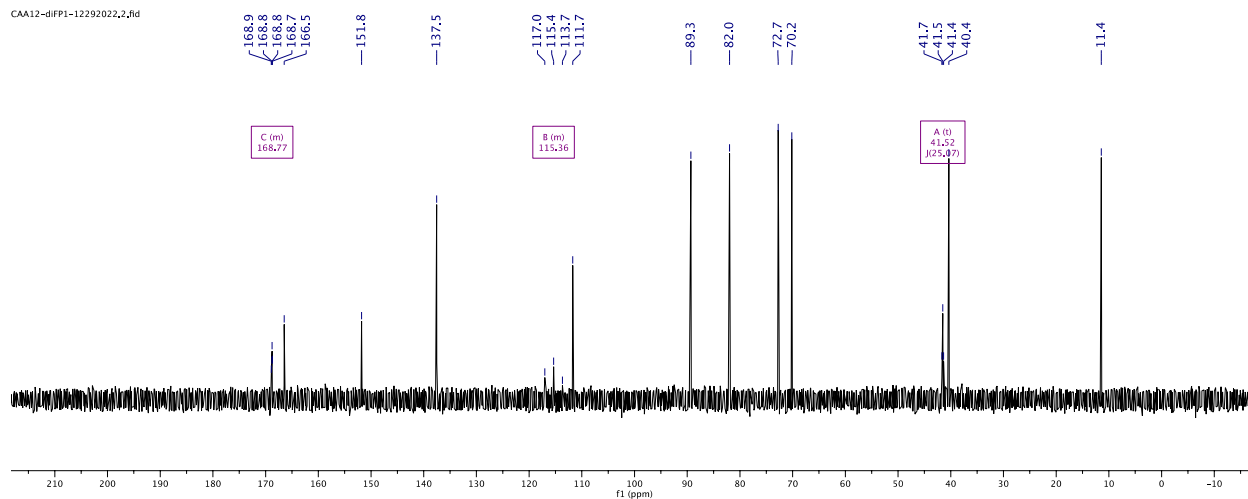


¹H NMR (600 MHz, D₂O) spectrum of compound **11f** with water suppression.

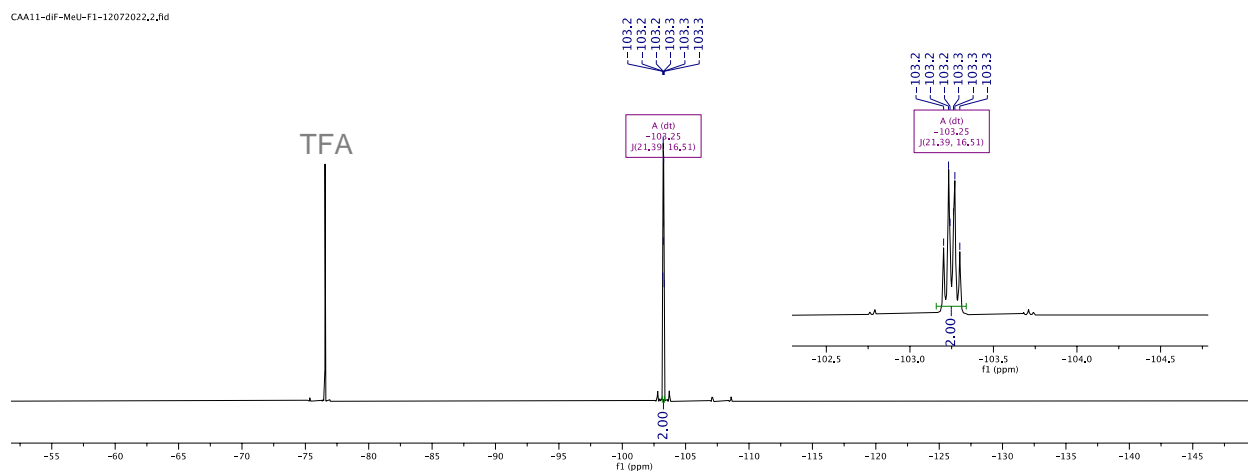
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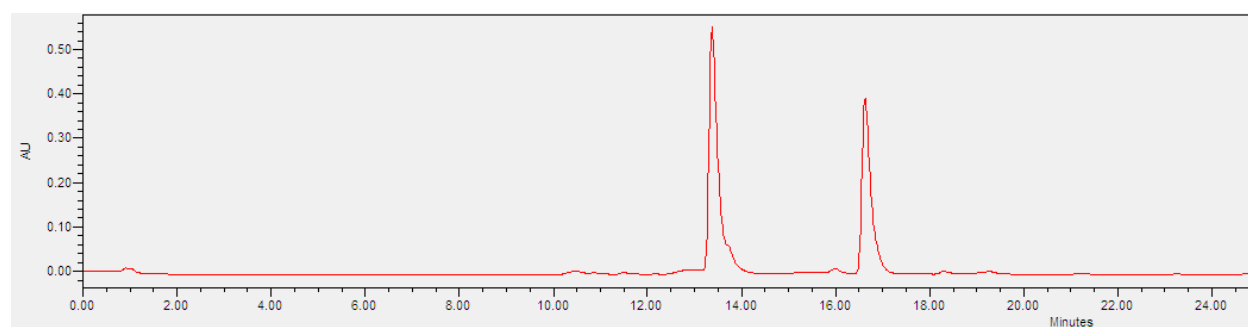
¹H NMR (400 MHz, DMSO-*d*₆) spectrum of compound **11f**.



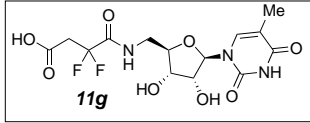
^{13}C NMR (151 MHz, D_2O) spectrum of compound **11f**.



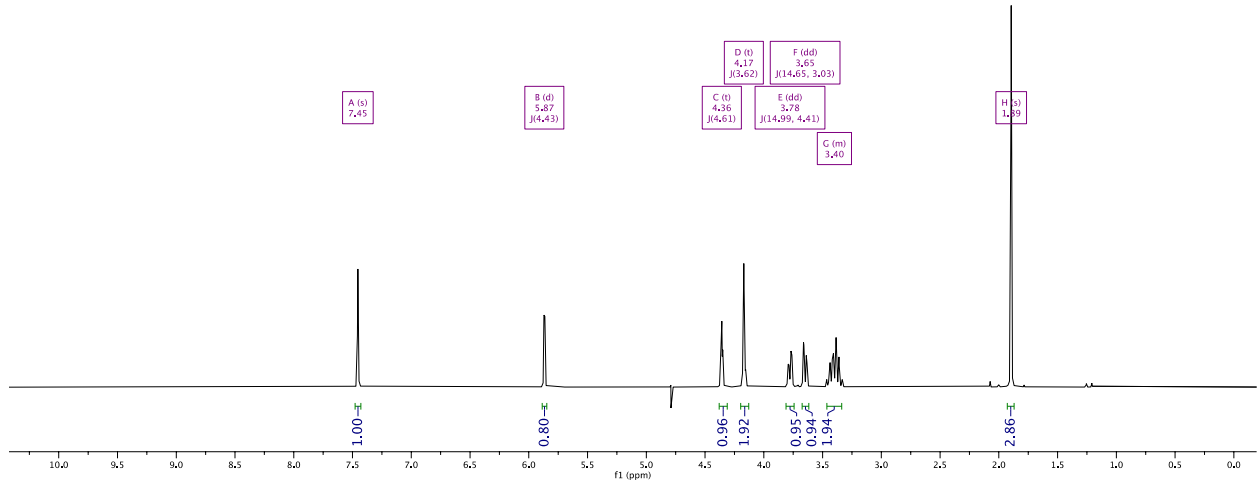
^{19}F NMR (565 MHz, D_2O) spectrum of compound **11f**.



Crude HPLC spectrum of compound **11f**.

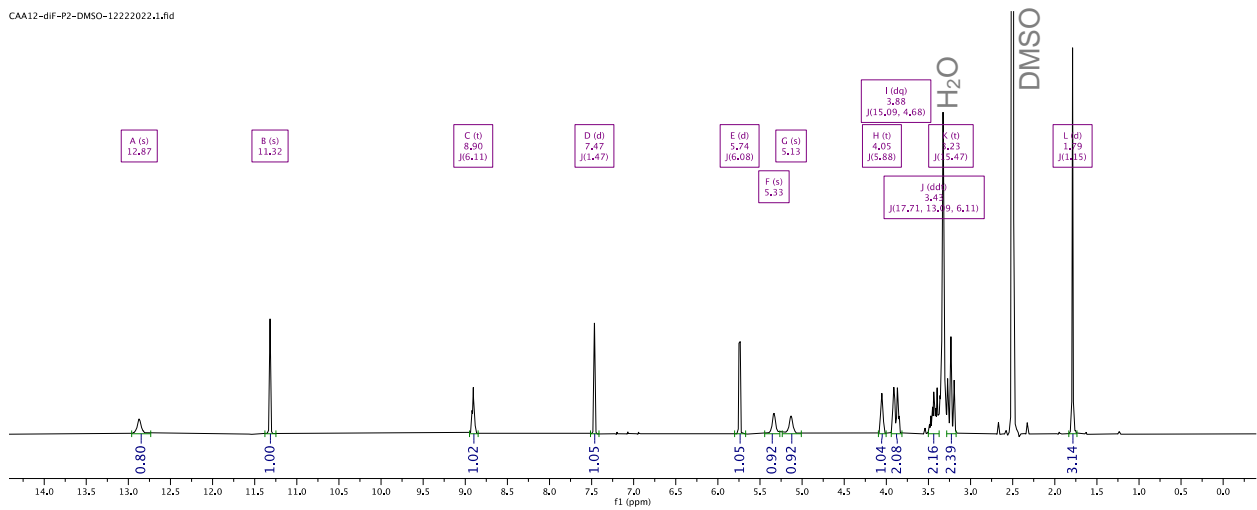


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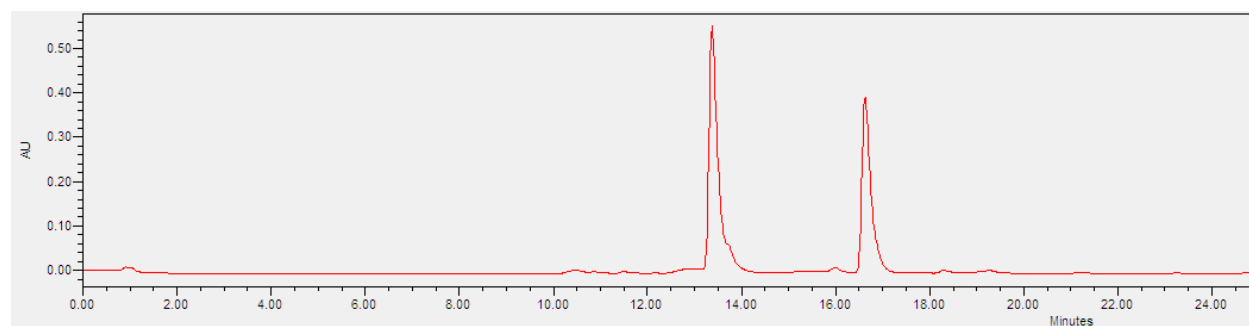
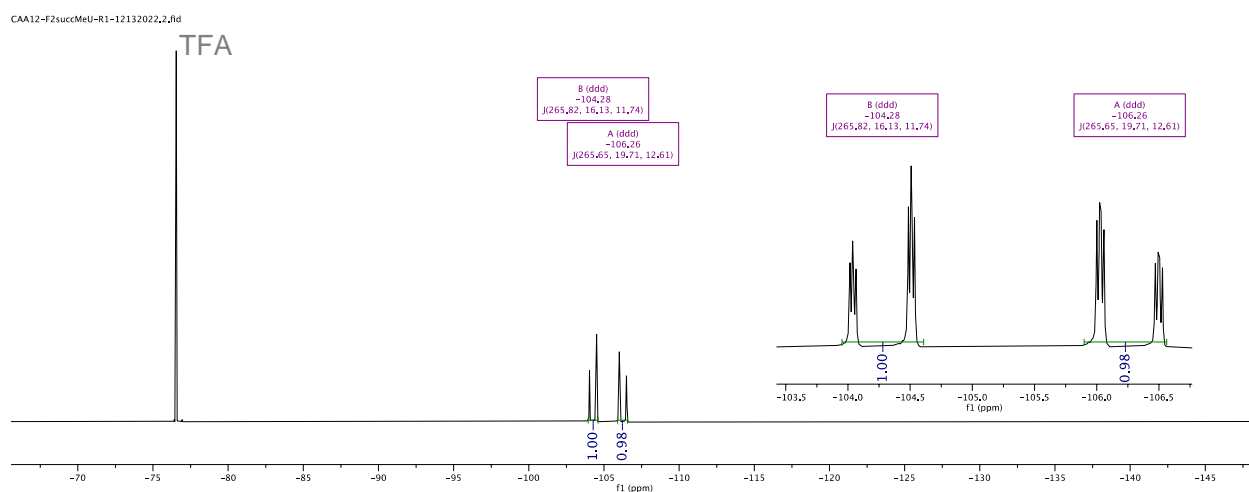
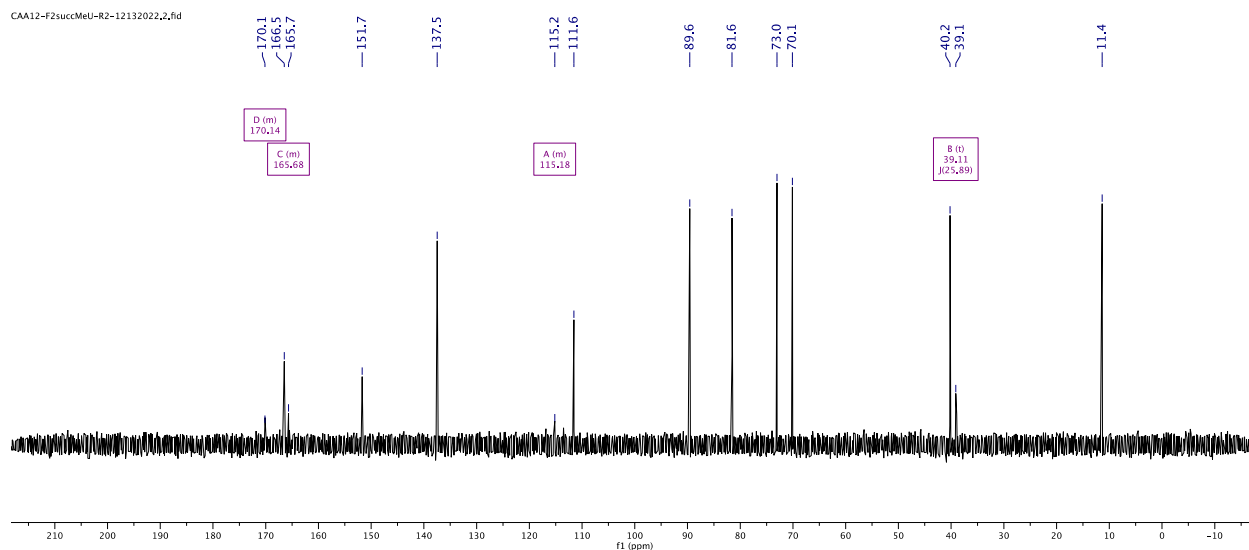


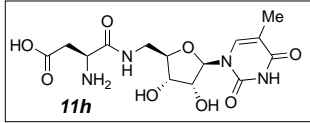
^1H NMR (600 MHz, D_2O) spectrum of compound **11g** with water suppression.

CAA12-dif-P2-DMSO-12222022.1.fid

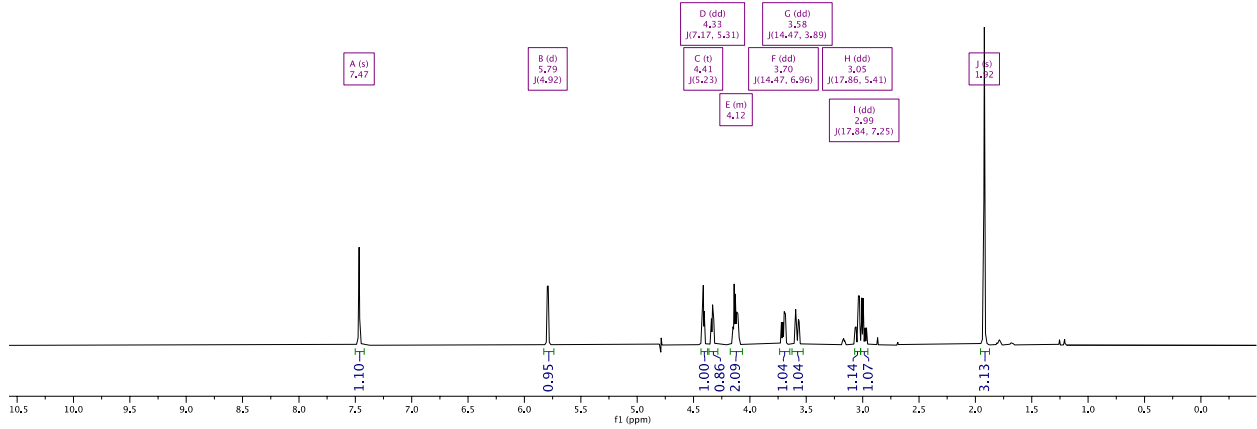


^1H NMR (400 MHz, $\text{DMSO}-d_6$) spectrum of compound **11g**.



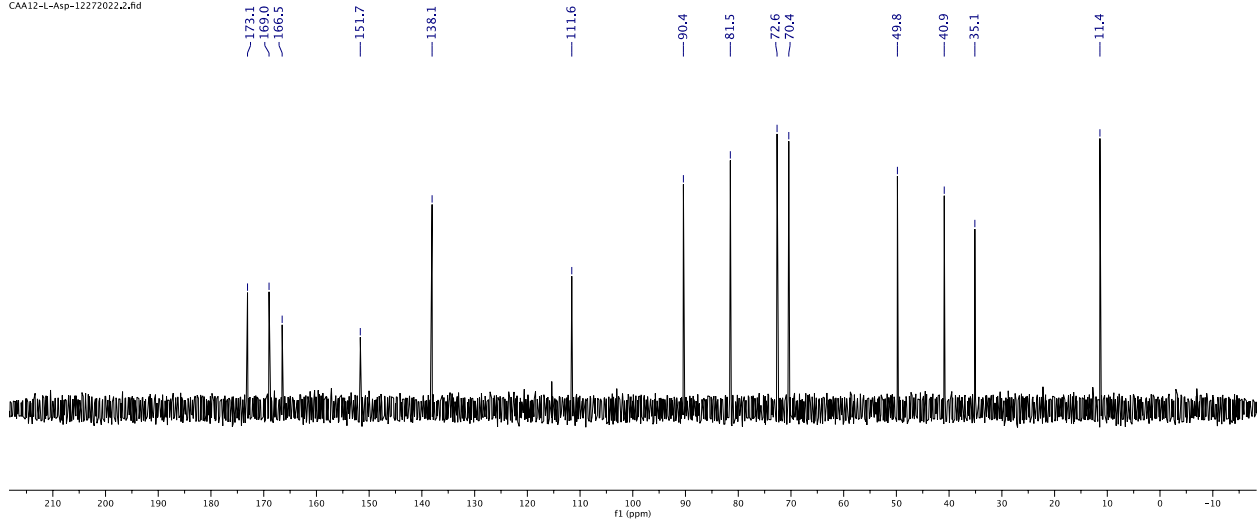


CAA12-L-Asp-12272022.1.fid

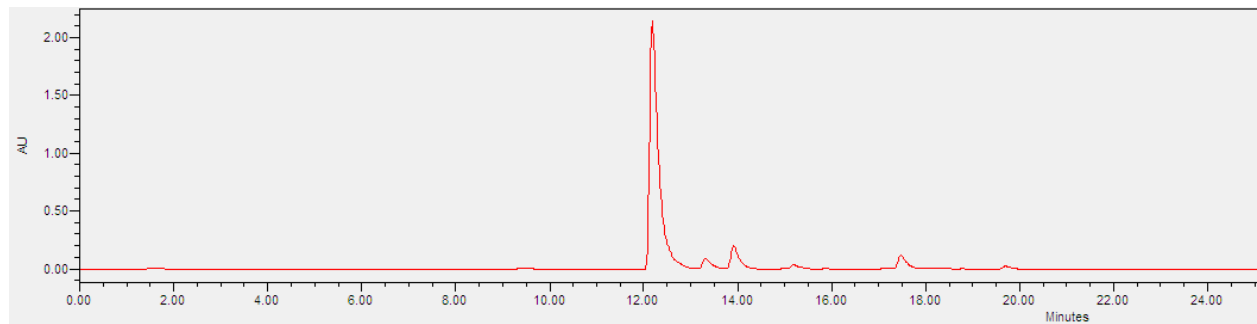


¹H NMR (600 MHz, D₂O) spectrum of compound **11h** with water suppression.

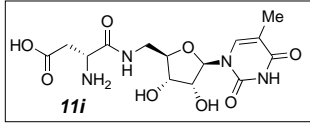
CAA12-L-Asp-12272022.2.fid



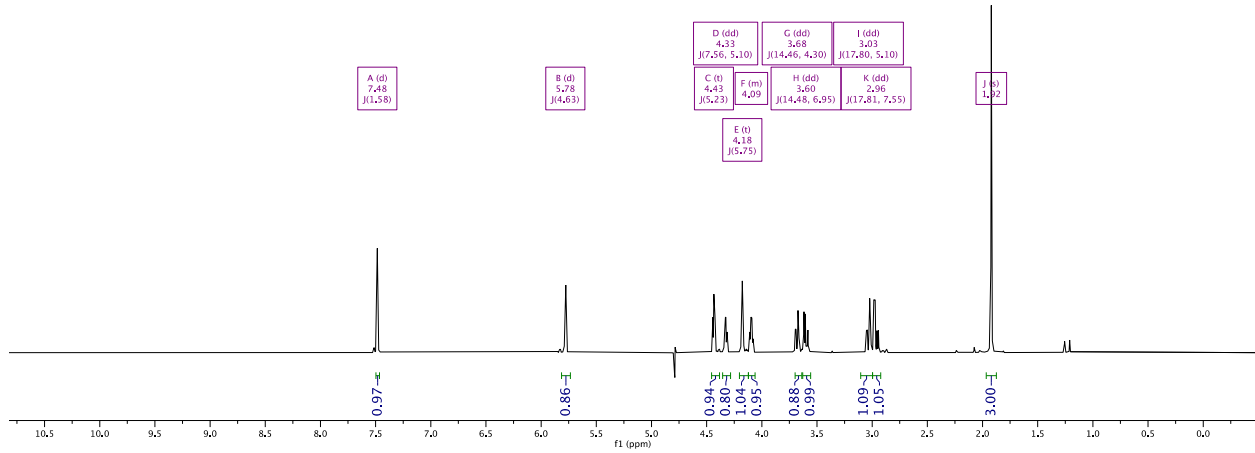
¹³C NMR (151 MHz, D₂O) spectrum of compound **11h**.



Crude HPLC spectrum of compound **11h**.

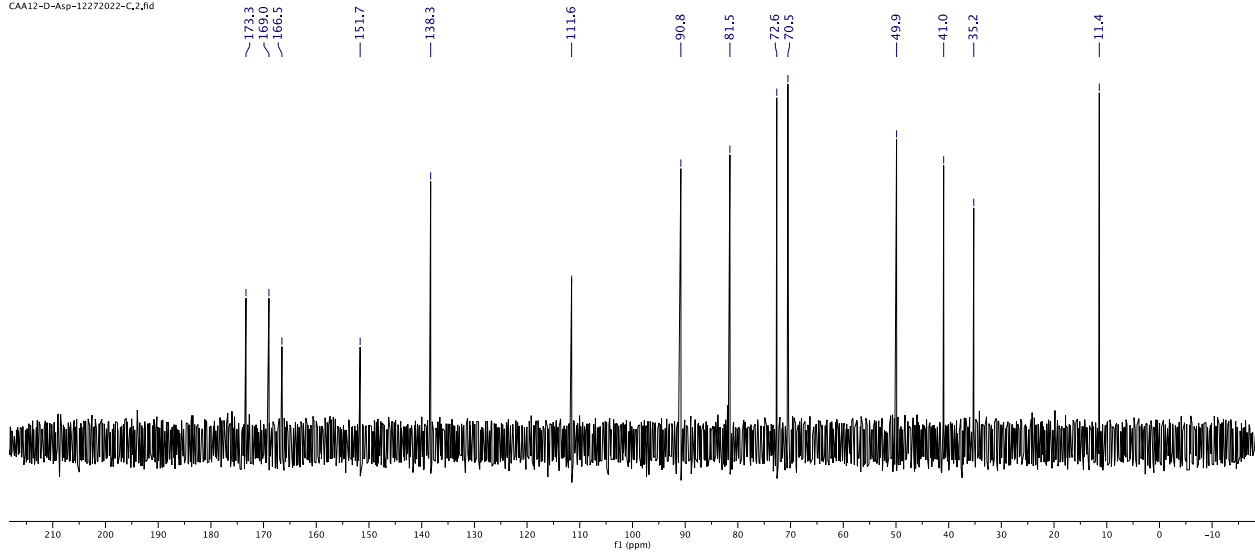


CAA12-D-Asp-12272022-1.fid

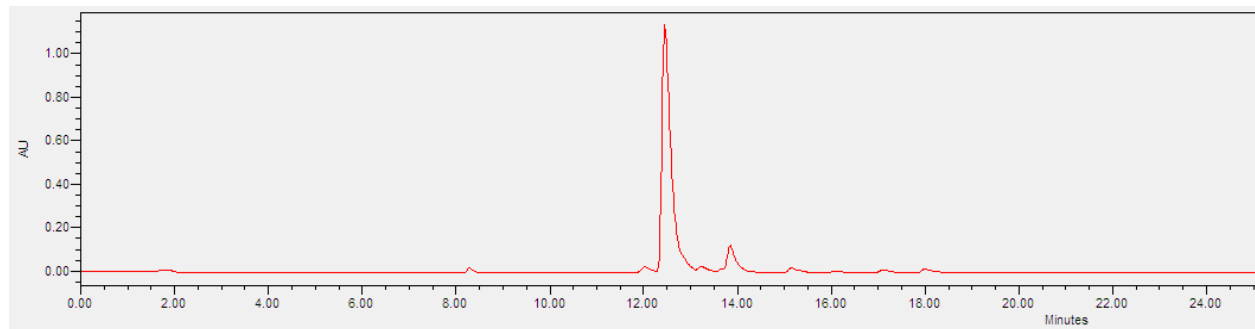


¹H NMR (600 MHz, D₂O) spectrum of compound **11i** with water suppression.

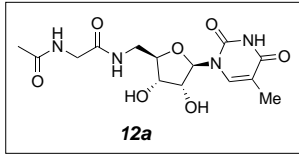
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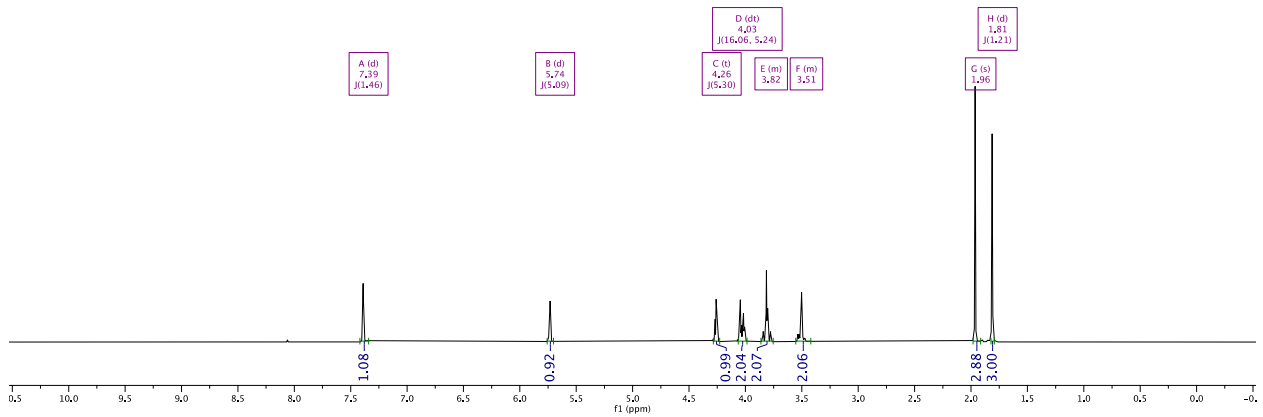
¹³C NMR (151 MHz, D₂O) spectrum of compound **11i**.



Crude HPLC spectrum of compound **11i**.

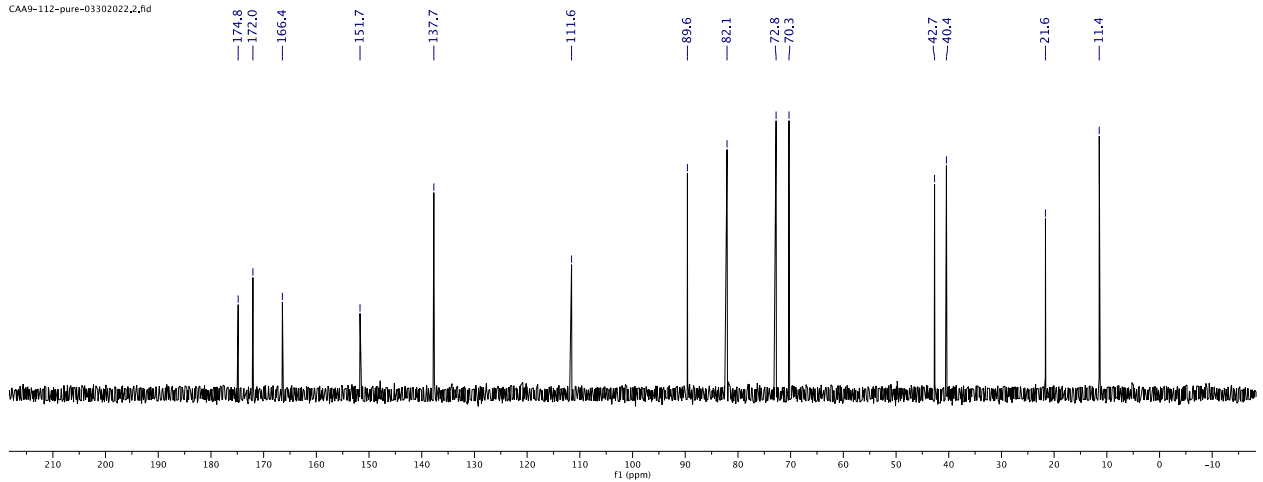


CAA9-112-pure-03302022_1.fid

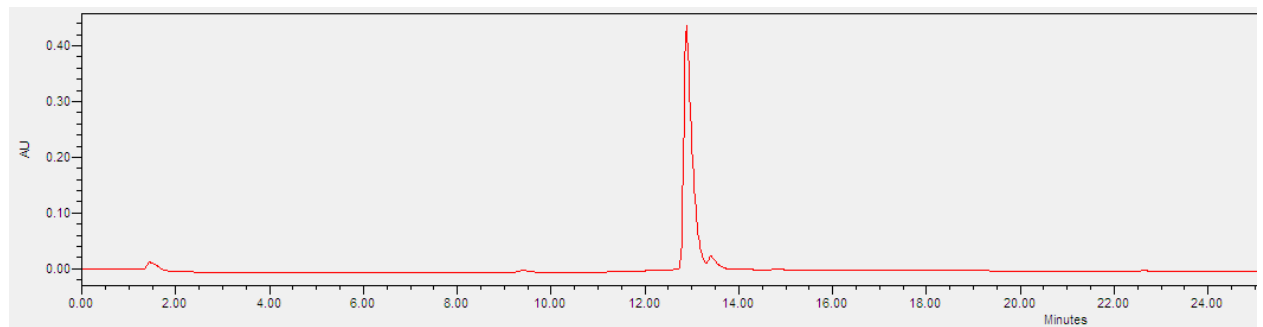


¹H NMR (600 MHz, D₂O) spectrum of compound **12a** with water suppression.

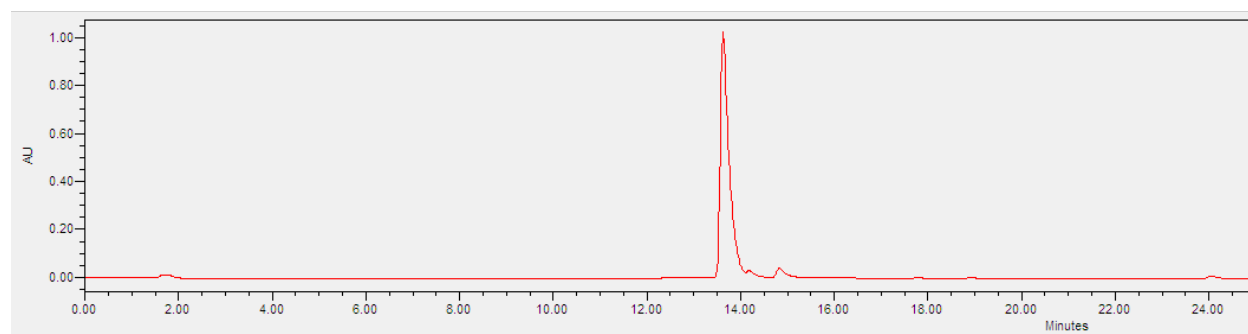
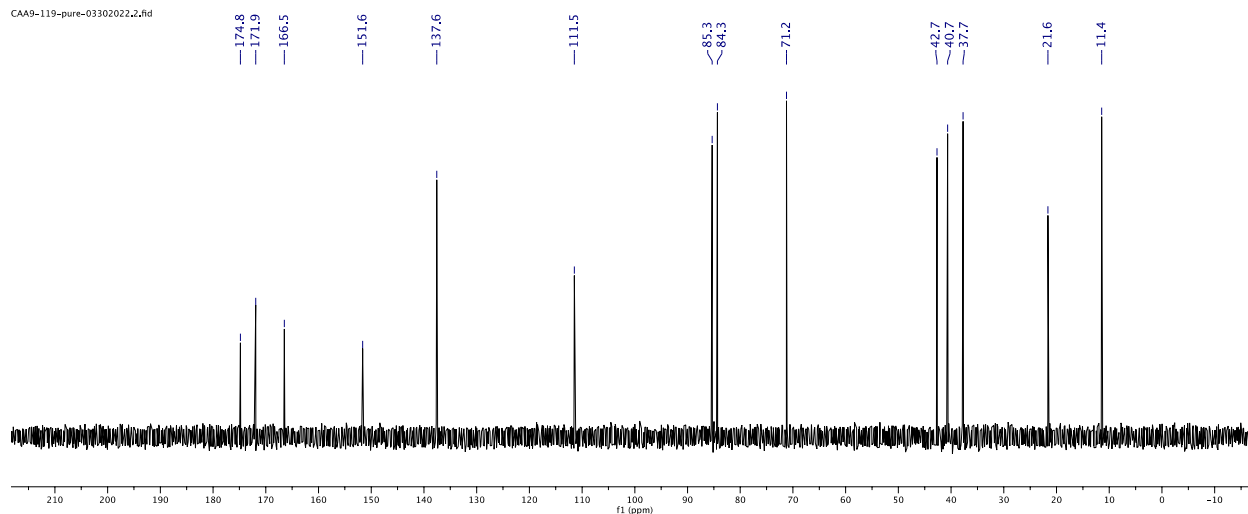
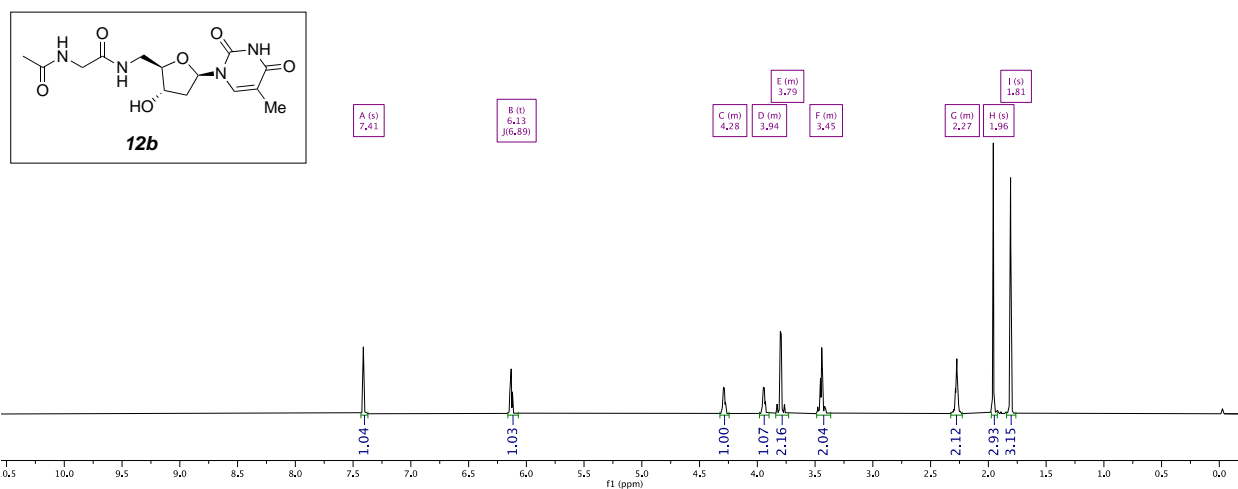
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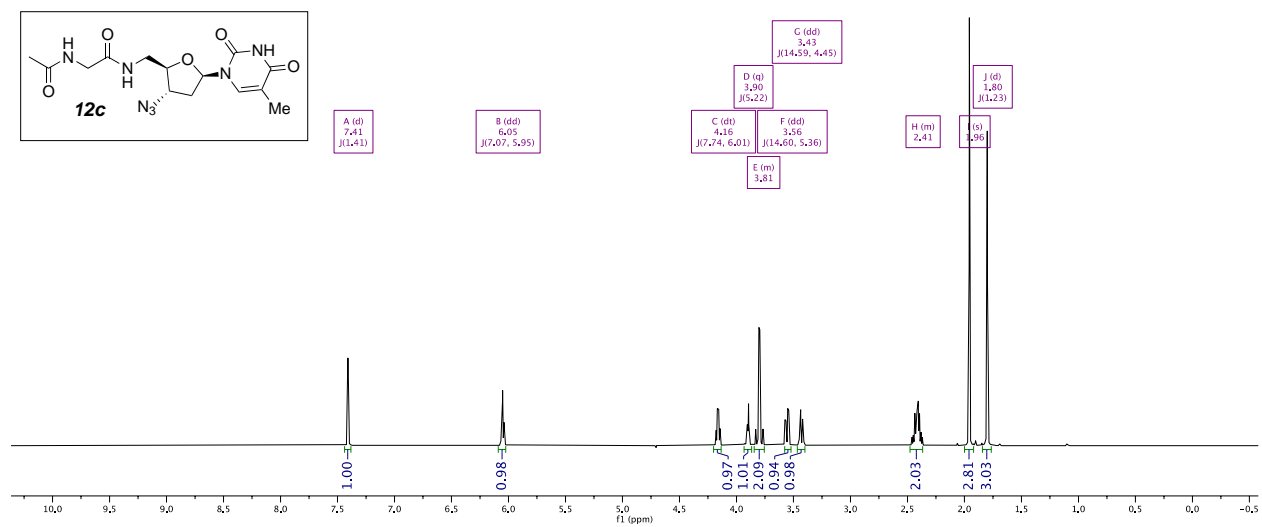


¹³C NMR (151 MHz, D₂O) spectrum of compound **12a**.

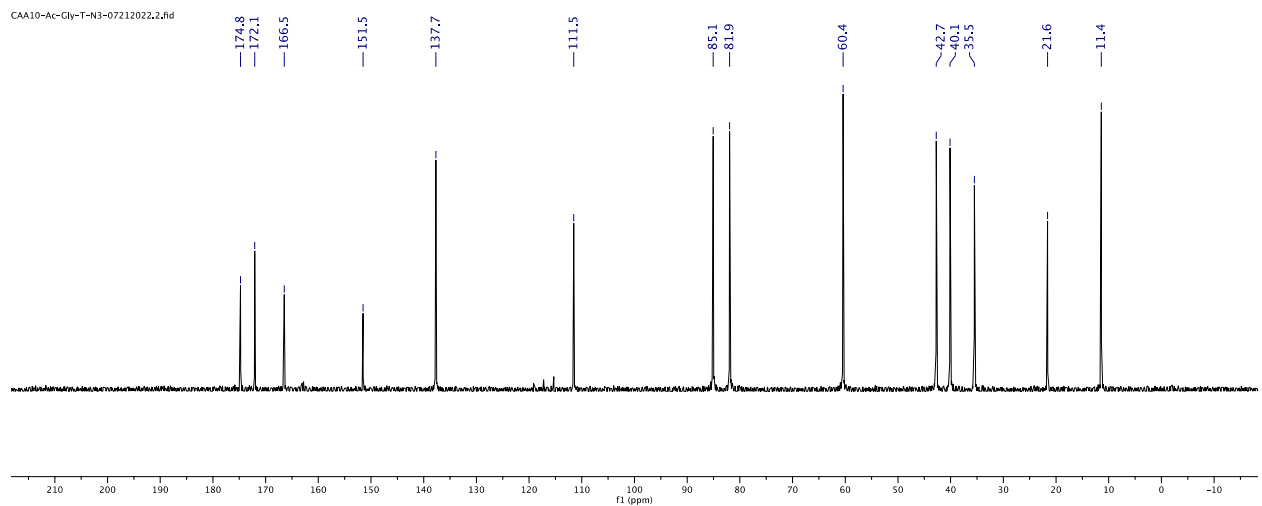


Crude HPLC spectrum of compound **12a**.

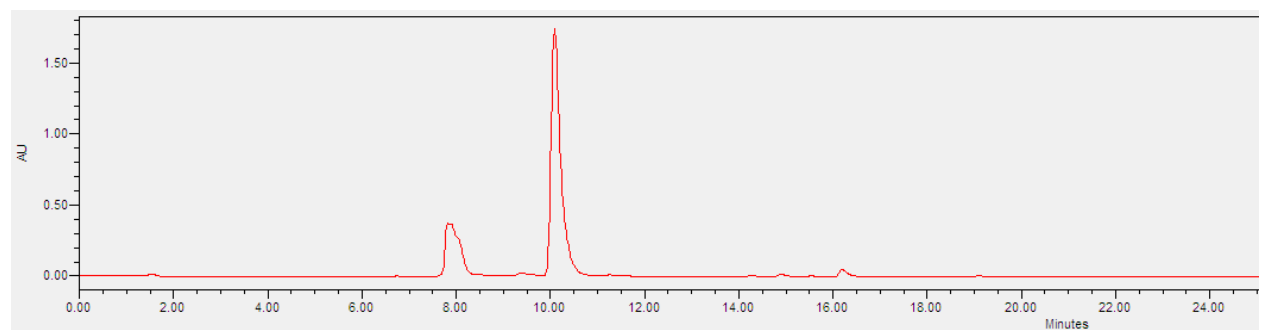




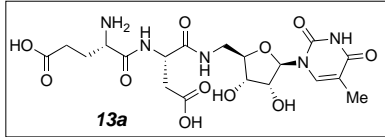
¹H NMR (600 MHz, D₂O) spectrum of compound 12c with water suppression.



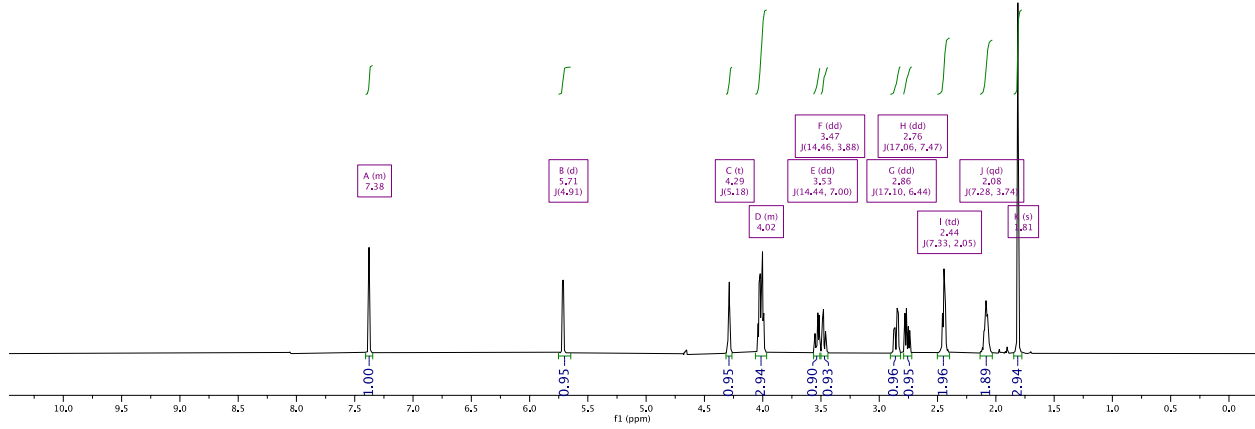
¹³C NMR (151 MHz, D₂O) spectrum of compound 12c.



Crude HPLC spectrum of compound 12c.

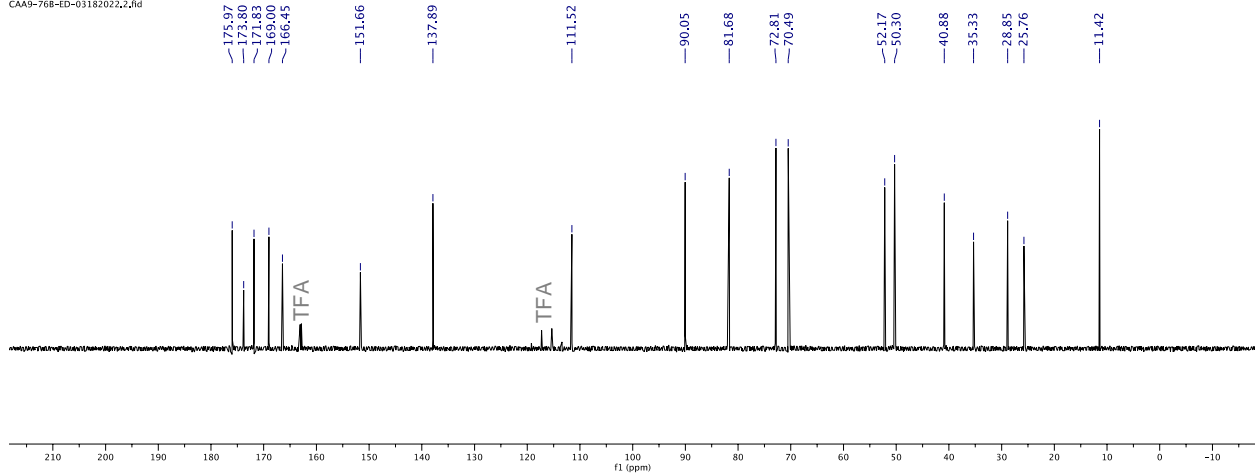


CAA9-76B-ED-03182022,1.fid

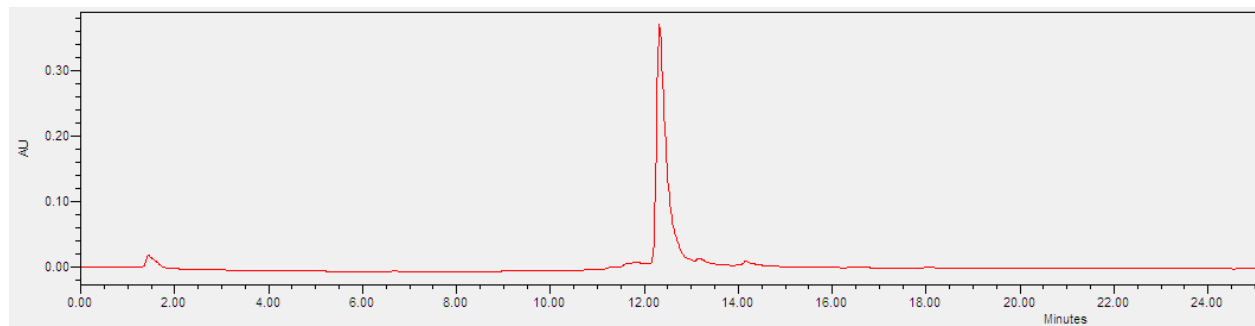


¹H NMR (600 MHz, D₂O) spectrum of compound 13a with water suppression.

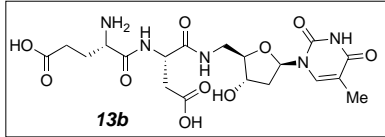
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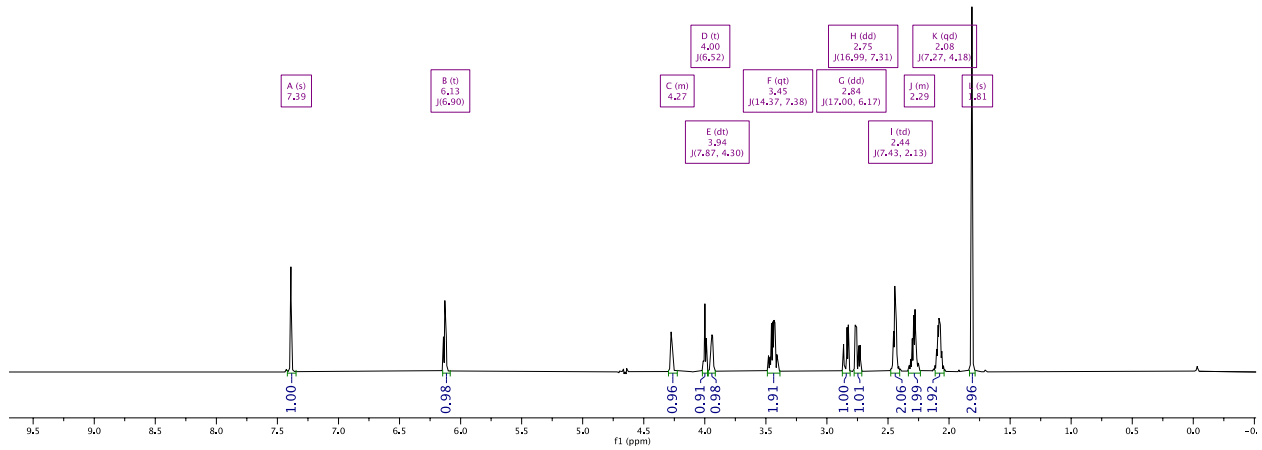
¹³C NMR (151 MHz, D₂O) spectrum of compound 13a.



Crude HPLC spectrum of compound 13a.

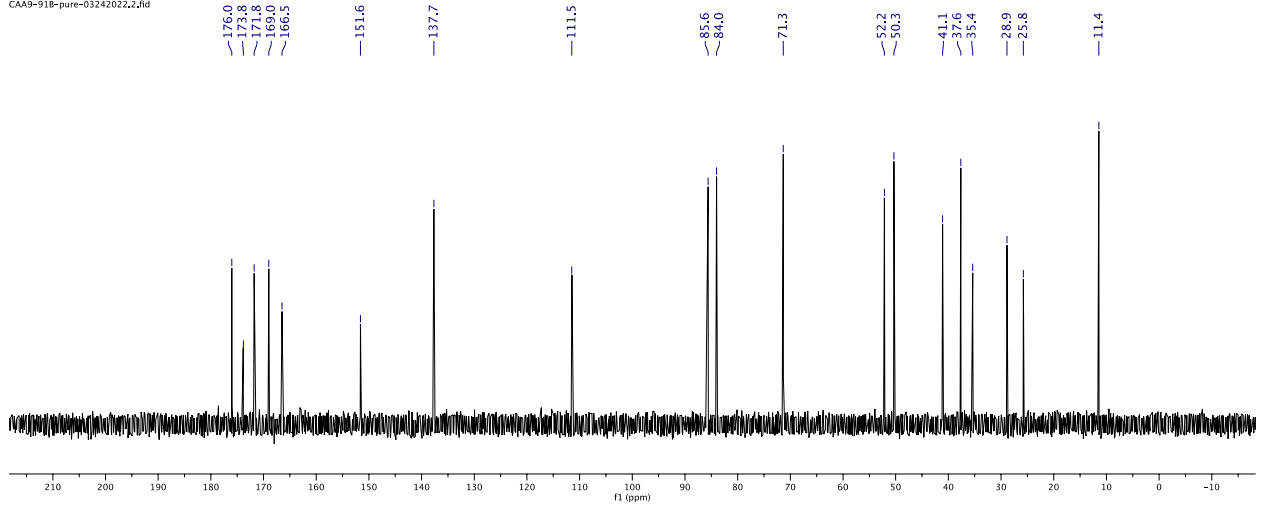


CAA9-91B-pure-03242022.1.fid

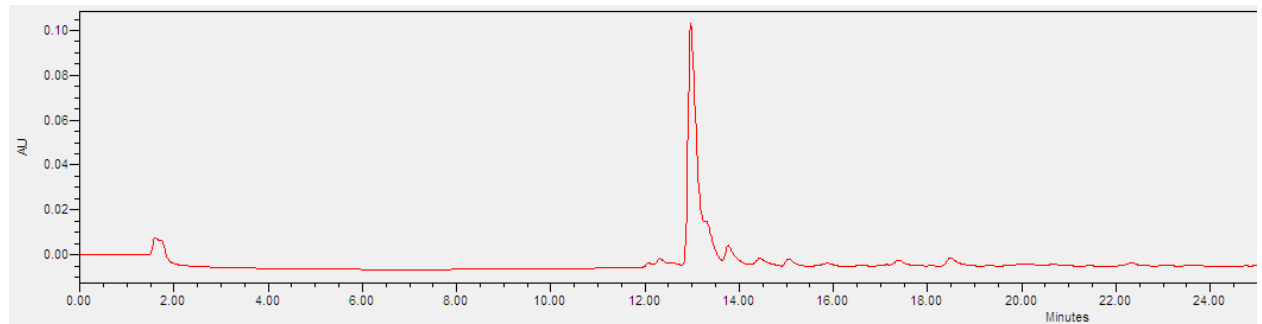


¹H NMR (600 MHz, D₂O) spectrum of compound **13b** with water suppression.

CAA9-91B-pure-03242022.2.fid

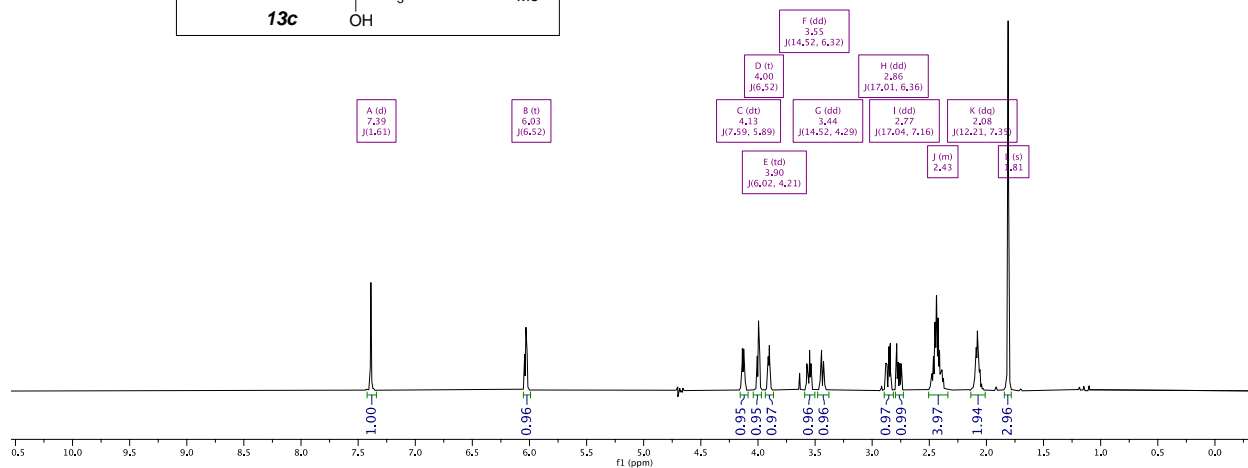
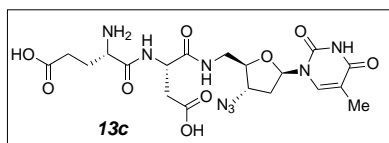


¹³C NMR (151 MHz, D₂O) spectrum of compound **13b**.



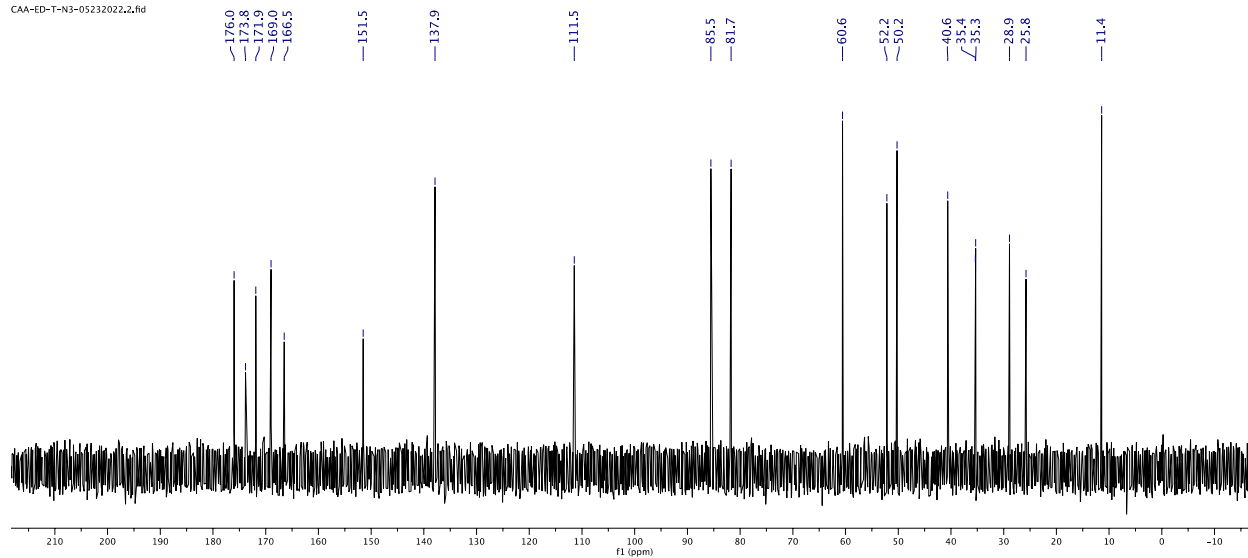
Crude HPLC spectrum of compound **13b**.

CAA-ED-T-N3-05232022,1.fid

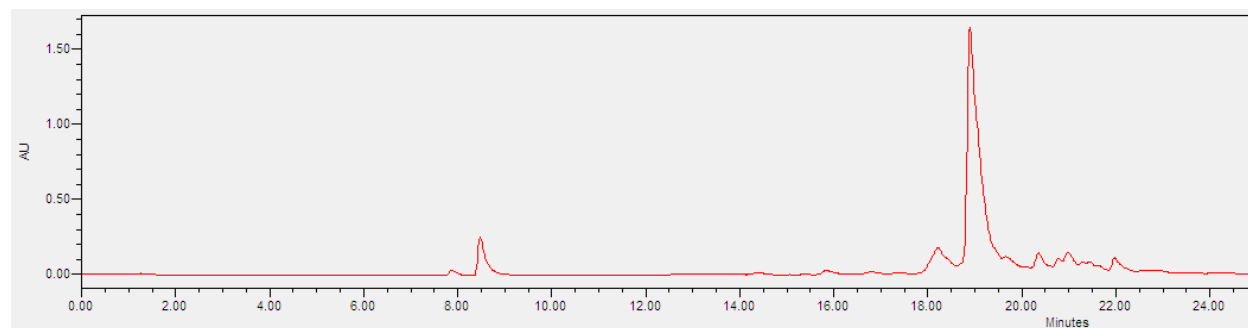


¹H NMR (600 MHz, D₂O) spectrum of compound **13c** with water suppression.

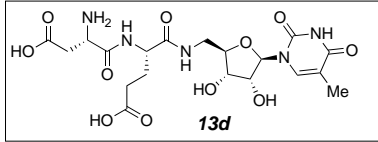
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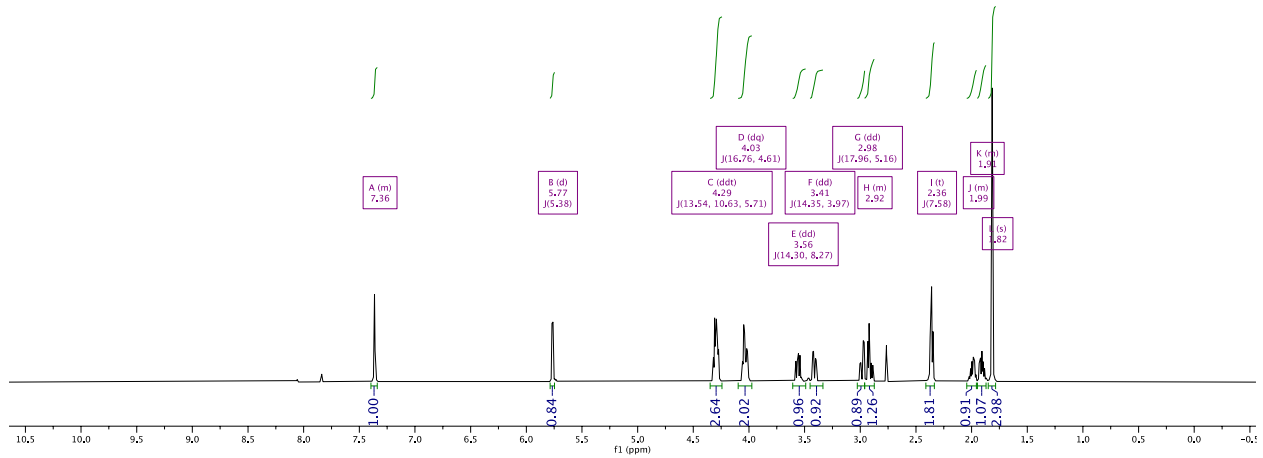
¹³C NMR (151 MHz, D₂O) spectrum of compound **13c**.



Crude HPLC spectrum of compound **13c**.

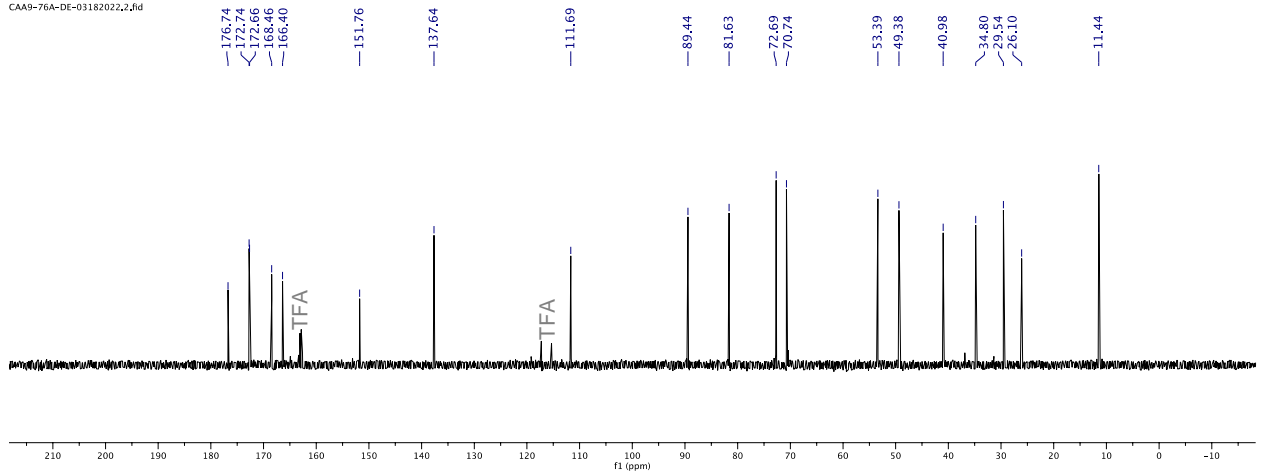


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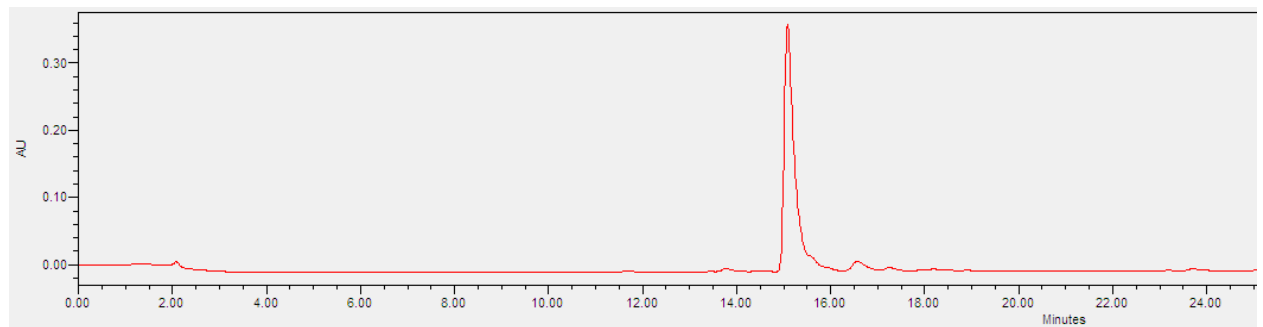


¹H NMR (600 MHz, D₂O) spectrum of compound **13d** with water suppression.

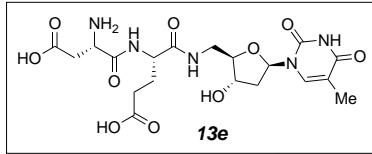
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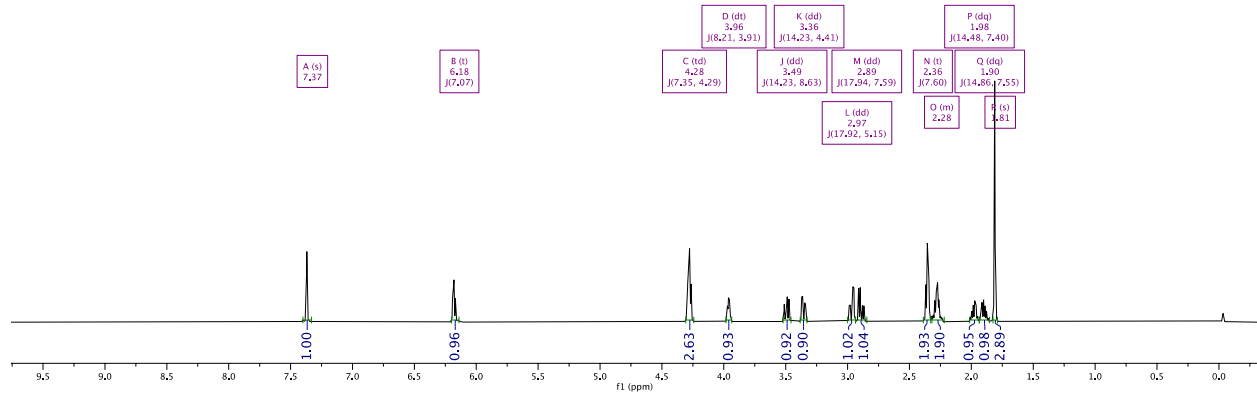
¹³C NMR (151 MHz, D₂O) spectrum of compound **13d**.



Crude HPLC spectrum of compound **13d**.

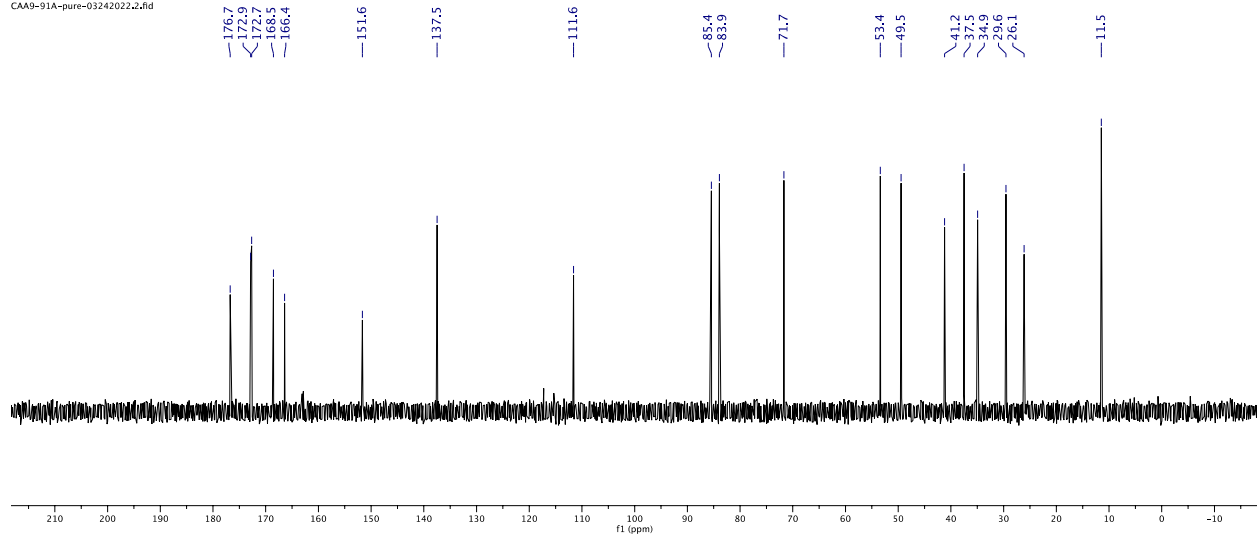


CAA9-91A-pure-03242022_1.fid

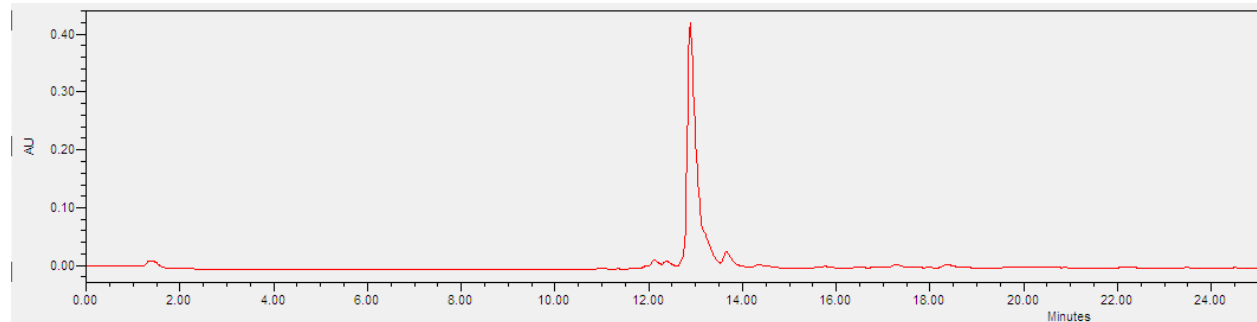


¹H NMR (600 MHz, D₂O) spectrum of compound **13e** with water suppression.

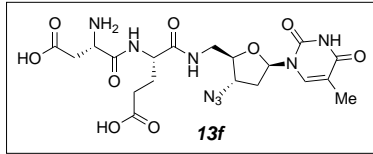
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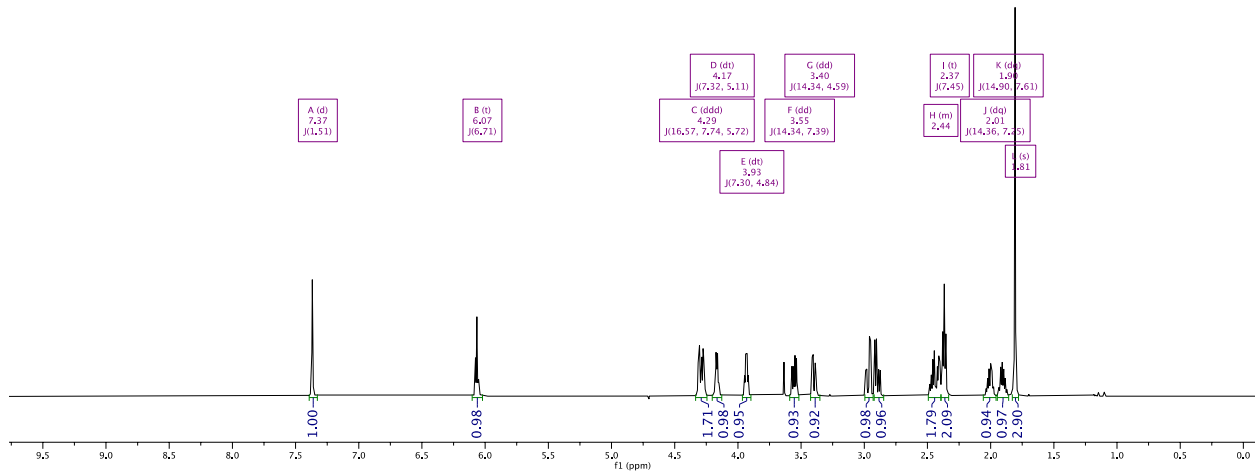
¹³C NMR (151 MHz, D₂O) spectrum of compound **13e**.



Crude HPLC spectrum of compound **13e**.

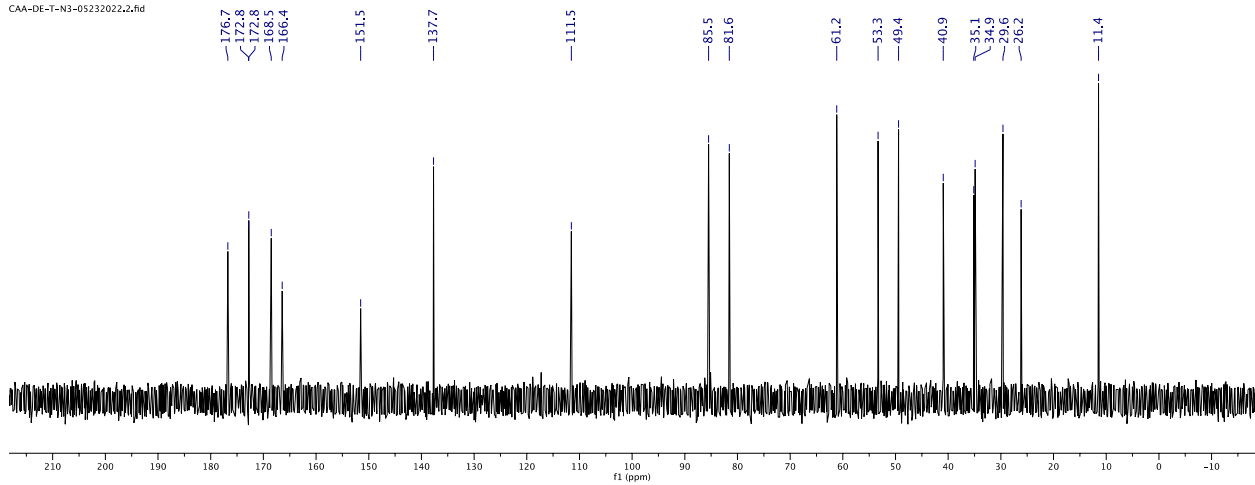


CAA-DE-T-N3-05232022.1.fid

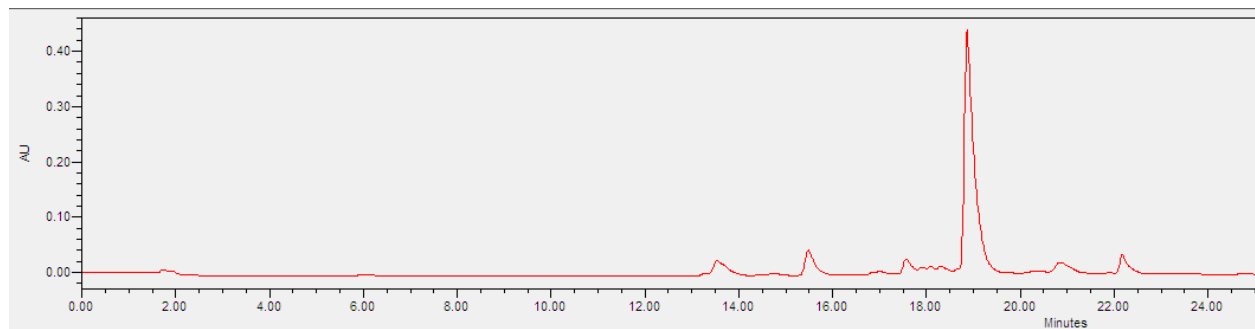


¹H NMR (600 MHz, D₂O) spectrum of compound **13f** with water suppression.

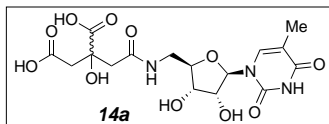
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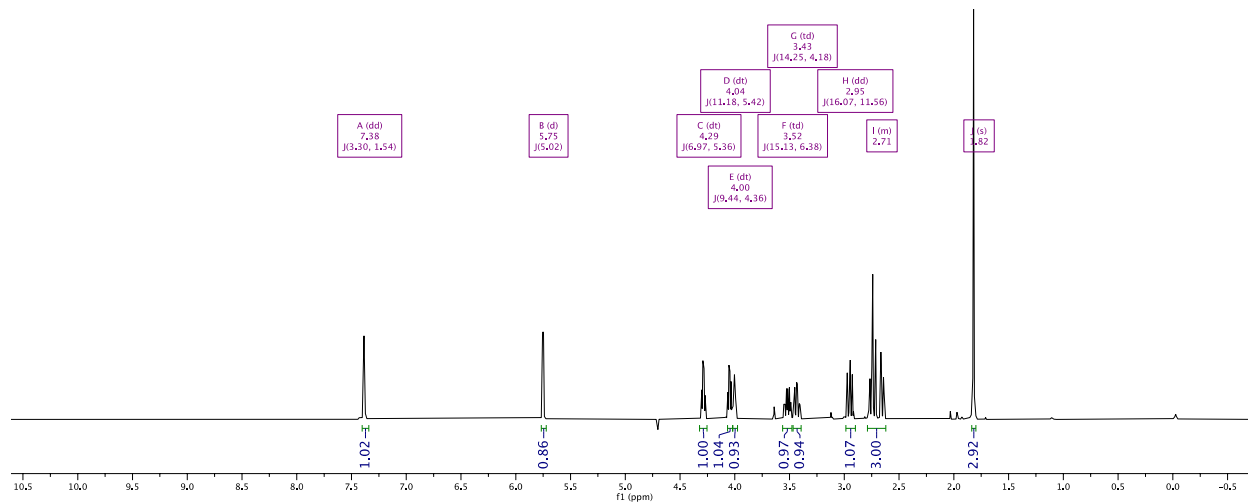
¹³C NMR (151 MHz, D₂O) spectrum of compound **13f**.



Crude HPLC spectrum of compound **13f**.

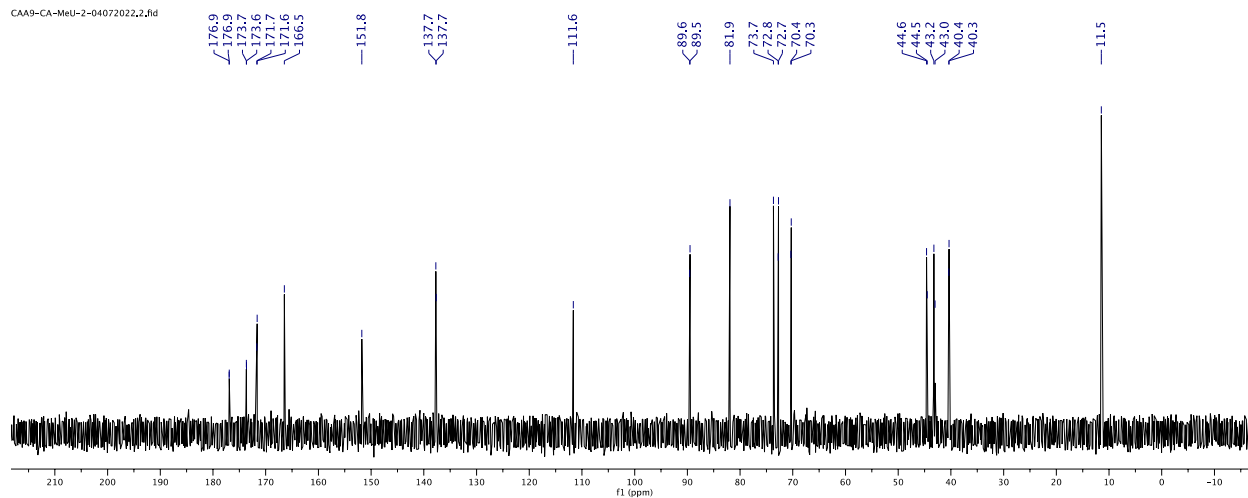


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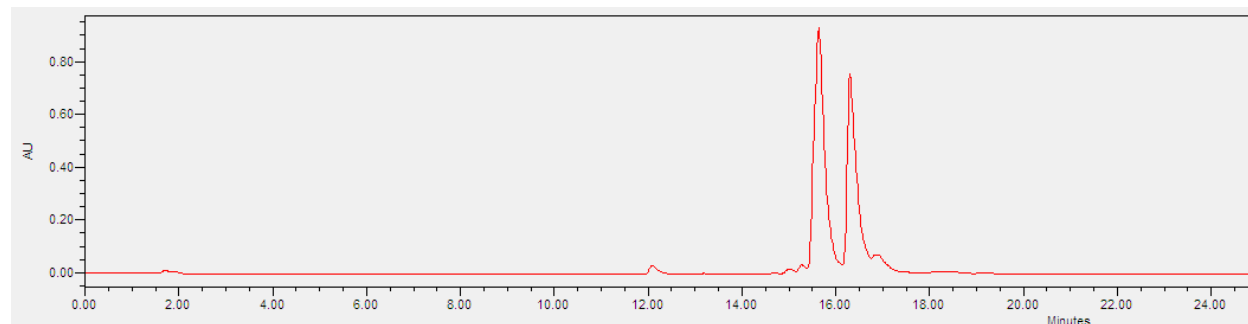


¹H NMR (600 MHz, D₂O) spectrum of compound **14a** with water suppression.

CAA9-CA-MeU-2-04072022.2.fid

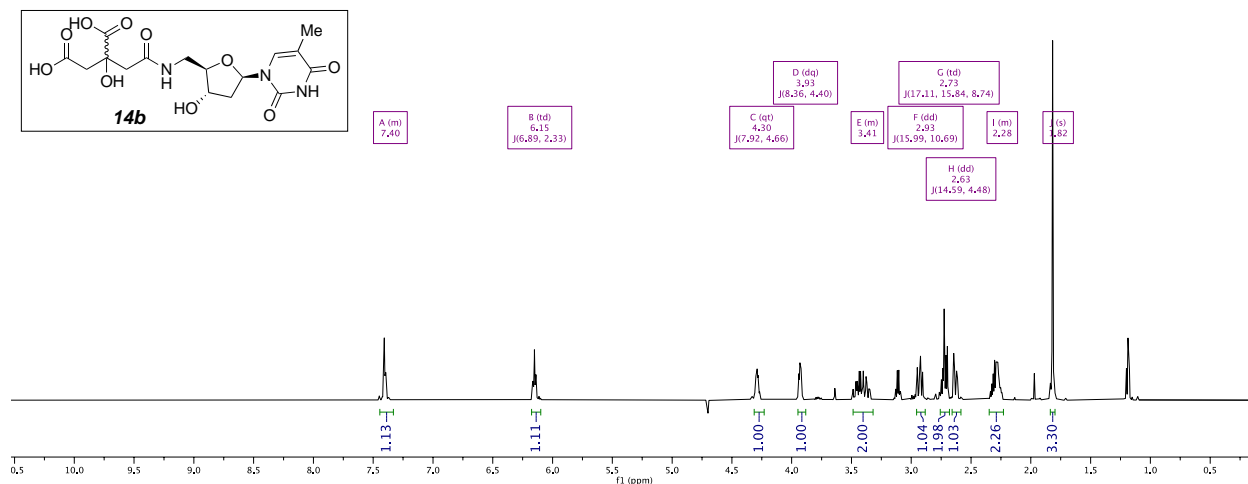


¹³C NMR (151 MHz, D₂O) spectrum of compound **14a**.



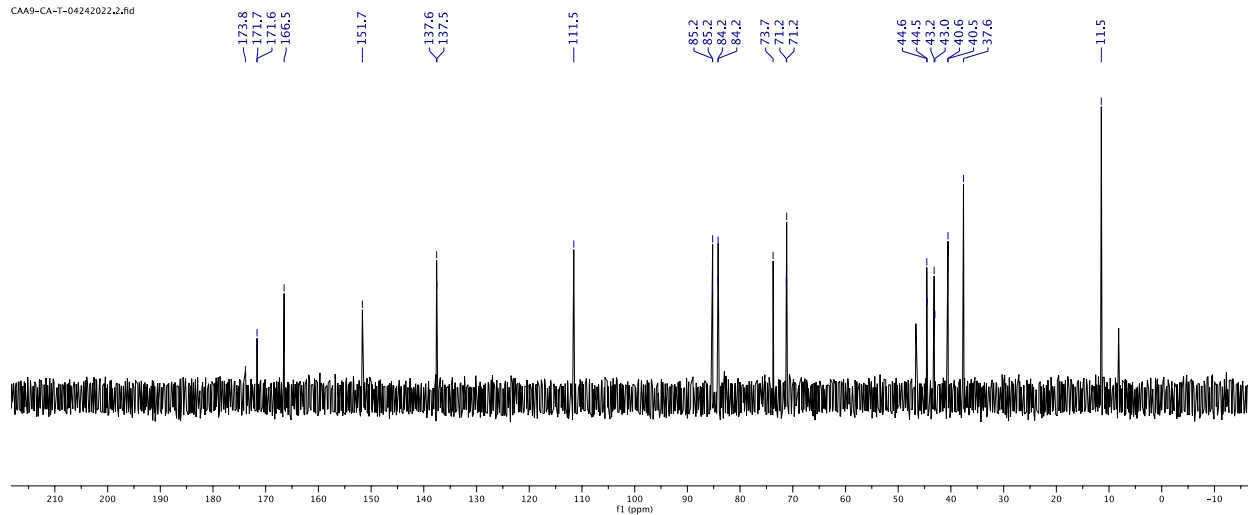
Crude HPLC spectrum of compound **14a**.

CAA9-CA-T-04242022_1.fid

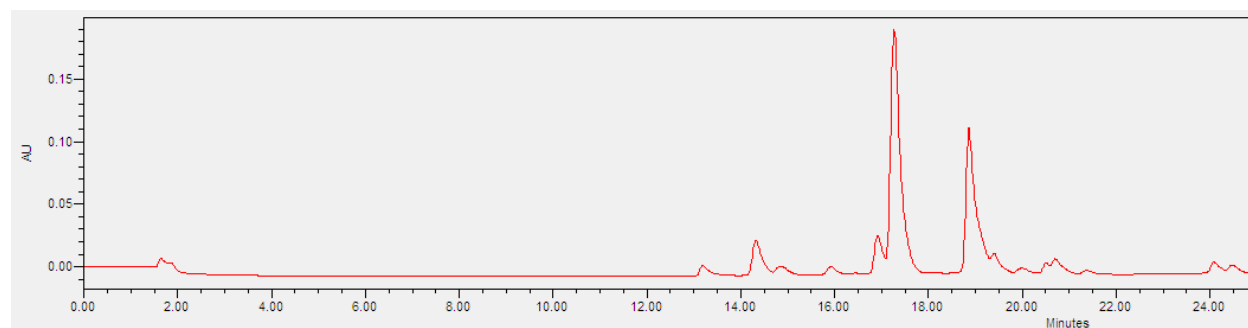


¹H NMR (600 MHz, D₂O) spectrum of compound **14b** with water suppression.

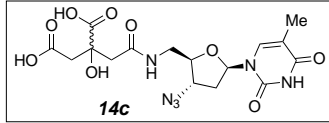
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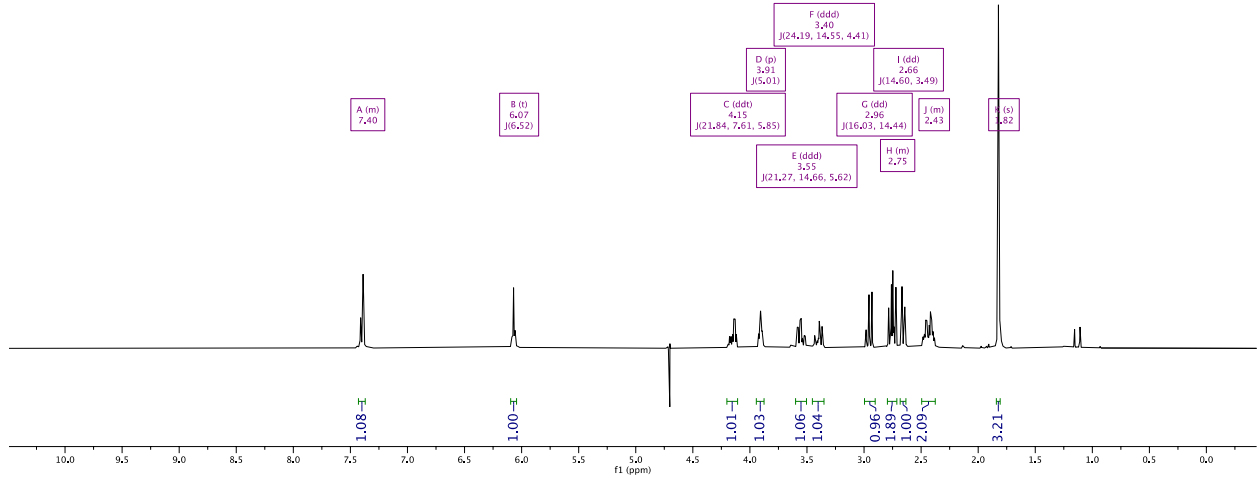
¹³C NMR (151 MHz, D₂O) spectrum of compound **14b**.



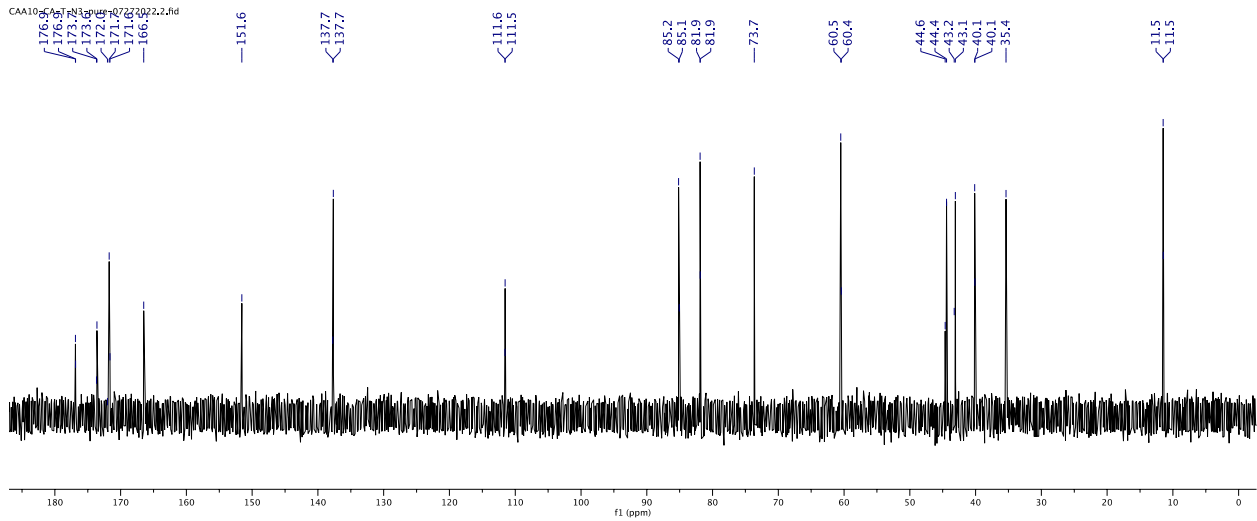
Crude HPLC spectrum of compound **14b**.



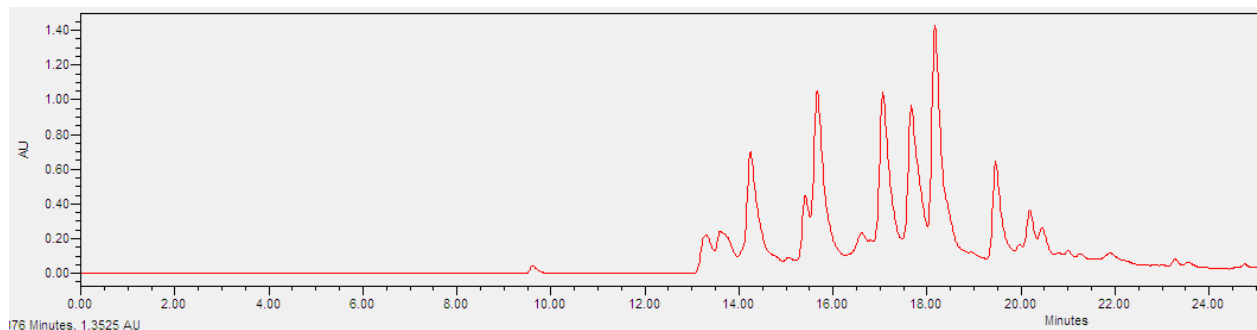
CAA10-CA-T-N3-pure-07272022_1.fid



¹H NMR (600 MHz, D₂O) spectrum of compound 14c with water suppression.



¹³C NMR (151 MHz, D₂O) spectrum of compound 14c.



Crude HPLC spectrum of compound 14c.

References

- (1) Shen, X.-M.; Zheng, B.-Y.; Huang, X.-R.; Wang, L.; Huang, J.-D. The first silicon(iv) phthalocyanine–nucleoside conjugates with high photodynamic activity. *Dalton Transactions* **2013**, 42 (29), 10398-10403, 10.1039/C3DT50910A. DOI: 10.1039/C3DT50910A.
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