

Synthesis of Nucleoside Triphosphates from 2'-3'-Protected Nucleosides using Trimetaphosphate

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Experimental

General. All reagents and starting nucleosides were obtained from commercial sources unless stated otherwise. DABCO and phthalimide were used without further purification. Strictly anhydrous conditions are essential for obtaining the reported yields. Acetonitrile was distilled from calcium hydride under Ar. Pyridine was distilled from potassium hydroxide and stored in a Schlenk tube under Ar. 2',3'-di-O-acetyl-uridine, *N*-acetyl-2',3'-di-O-acetyl-guanosine and *N*-acetyl-2',3'-di-O-acetyl-cytidine were prepared according using literature procedures.^{1a-d} All reactions were conducted in flame-dried flasks under an inert atmosphere of Ar. All NMR spectra were recorded using D₂O as solvent. All triphosphates were treated with Chelex resin before obtaining NMR spectra. For ¹H-NMR spectra, chemical shifts are reported in ppm relative to the solvent residual peak (δ 4.79). For proton-decoupled ¹³C-NMR spectra, chemical shifts are reported in ppm relative to CH₃OH in D₂O (δ 49.5, external standard). For proton-decoupled ³¹P-NMR, chemical shifts are reported in ppm relative to aqueous 85% H₃PO₄ (δ 0 ppm, external standard). Preparative reversed phase chromatography was performed using a Biotage Isolera One Flash purification system equipped with two C-18 reverse phase preparative Biotage 30 g columns (30g) connected in series. High resolution negative ion mass spectra (ESI HRMS) were obtained using a Thermo Scientific Q-exactive Orbitrap mass spectrometer.

Preparation of tris(tetrabutylammonium) trimetaphosphate (1). The trisodium salt of TriMP (about 1.5 g) was dissolved in 30 mL water and was converted into its pyridinium salt using a Dowex-50W (25-30 g) ion exchange column in its pyridinium form (the pyridinium form of the Dowex H⁺ resin is prepared by passing 125 mL of a 25% aqueous pyridine solution through it, then washing twice with water). Fractions containing the desired pyridinium salt were pooled and then a dilute solution of tetrabutylammonium hydroxide was added with stirring

until pH 7.0 was reached. The solution was concentrated by high vacuum rotary evaporation to approximately one-seventh the original volume then lyophilized to give compound **1** a white powder in near quantitative yield. Compound **1** can be stored for months at -20 °C without any detectable decomposition. ³¹P-NMR (D₂O, 121 MHz): δ -19.16. Before use, an appropriate quantity of **1** was dissolved in acetonitrile (approximately 2 mL for 500 mg of compound **1**) followed by the addition of a small quantity (approximately 0.8 mL for 500 mg compound **1**) of toluene and the solution was concentrated by rotary evaporation to dryness. This was repeated. The residue was dissolved in dry acetonitrile and concentrated by rotary evaporation to dryness. This was repeated. The residue was subjected to high vacuum overnight. The flask was removed from the vacuum line under Ar then dissolved in the appropriate solvent (6 mL of dry acetonitrile or dry pyridine per 500 mg **1**). Properly activated 4Å molecular sieves (approximately one third of the volume of the solution) were added and the solution was allowed to stand for 24h before use.² This solution can be stored at room temperature and used for at least 5 days.

4-methyl-coumarin-7-triphosphate (4). To a mixture of **1** (0.54 mmol, 520 mg) and DABCO (1.62 mmol, 180 mg) in dry acetonitrile (6 mL) was added mesitylenesulfonyl chloride (0.48 mmol, 106 mg). The mixture was stirred at room temperature for one minute then 7-hydroxy-4-methylcoumarin (HMC, 0.27 mmol, 48 mg) was added and the reaction mixture was allowed to stir at room temperature for 3 h. A white turbidity appears after about 45 min. The reaction mixture was then cooled in an ice bath and quenched by adding triethylammonium acetate buffer (100 mM, pH 7.0, 8 mL). The solution was washed with chloroform (3 x 10 mL). The solution was then purified by reversed-phase chromatography using a linear gradient of 80% buffer A (10 mM tributylamine-30 mM acetic acid in water), 20% buffer B (15 mM tributylamine in MeOH)

to 45% buffer A, 65% buffer B over 40 min ($t_r = 29$ min). The flow rate was 25 mL/min and eluate was monitored at 254 and 280 nm. Fractions containing the desired product were pooled, concentrated by high vacuum rotary evaporation, and the residue was dissolved in water and repeatedly freeze-dried until the $^1\text{H-NMR}$ spectrum indicated that no residual buffer was present (four times). The resulting white powder was converted to its ammonium salt using a Dowex-50-W ion-exchange resin in NH_4^+ form to yield 114 mg (90%) of compound **4** as triammonium salt after lyophilization. $^1\text{H-NMR}$ (D_2O , 300 MHz): δ 2.27 (s, 3H, CH_3), 6.10 (s, 1H, H_3), 7.11 (brd, $J = 12.2$ Hz, 2H, $\text{H}_{6,8}$), 7.58 (d, $J = 8.4$ Hz, 1H, H_5). $^{13}\text{C-NMR}$ (D_2O , 75 MHz): δ 17.8, 108.3 (d, $J = 5.1$ Hz), 111.8, 116.4, 117.5 (d, $J = 4.6$ Hz), 126.4, 153.3, 154.5 (d, $J = 6.8$ Hz), 156.1, 164.4. $^{31}\text{P-NMR}$ (D_2O , 121 MHz): δ -8.25 (d, $J = 18.2$ Hz), -14.56 (d, $J = 19.5$ Hz), -20.79 (overlapping dd, $J = 19.4, 20.0$ Hz). HRMS (ESI-): $m/z = 414.93900$, $\text{C}_{10}\text{H}_{10}\text{O}_{12}\text{P}_3$ [$\text{M} - \text{H}$] $^-$, requires 414.93906.

4-biphenylmethyl triphosphate (5). To a mixture of **1** (0.54 mmol, 520 mg) and DABCO (0.61 mmol, 69 mg) in dry pyridine (6 mL) was added mesitylenesulfonyl chloride (0.50 mmol, 109 mg). The mixture was stirred at room temperature for one minute then 4-biphenylmethanol (0.135 mmol, 25 mg) was added and the reaction mixture was allowed to stir at room temperature for 4 min then phthalimide (0.61 mmol, 89 mg) was added and the reaction mixture was stirred at room temperature for 24h. The reaction mixture was then cooled in an ice bath and quenched by adding triethylammonium acetate buffer (100 mM, pH 7.0, 10 mL). The solution was washed with chloroform (3 x 10 mL). The solution was then lyophilized. The residue was dissolved in 6 mL water and purified by reversed-phase chromatography using a linear gradient of 75% buffer A (10 mM tributylamine-30 mM acetic acid in water), 25% Buffer B (15 mM tributylamine in MeOH) to 25% buffer A, 75% buffer B over 45 min. The flow rate was 25

mL/min and eluate was monitored at 254 and 280 nm. Fractions containing the desired product ($t_r = 35$ min) were pooled, concentrated by high vacuum rotary evaporation, and the residue was dissolved in water and repeatedly freeze-dried until the $^1\text{H-NMR}$ spectrum indicated that no residual buffer was present (four times). The resulting white powder was converted to its ammonium salt using a Dowex-50-W ion-exchange resin in NH_4^+ form to yield 54 mg (85%) of **5** as its triammonium salt after lyophilisation. $^1\text{H-NMR}$ (D_2O , 300 MHz): δ 4.92 (d, $J = 6.8$ Hz, 2H, CH_2), 7.27 - 7.45 (m, 5H, $\text{H}_{2',3',4',5',6'}$), 7.55 (d, $J = 8.1$ Hz, 4H, $\text{H}_{2,3,5,6}$). $^{13}\text{C-NMR}$ (D_2O , 75 MHz): δ 67.6 (d, $J = 5.4$ Hz), 126.8, 126.9, 127.6, 128.3, 129.0, 136.5, 136.6, 140.0 (d, $J = 7.8$ Hz). $^{31}\text{P-NMR}$ (D_2O , 121 MHz): δ -8.33 (d, $J = 20.9$ Hz), -8.69 (d, $J = 19.1$ Hz), -20.68 (overlapping dd, $J = 19.1, 20.3$ Hz). HRMS (ESI-): $m/z = 422.97850$, $\text{C}_{13}\text{H}_{14}\text{O}_{10}\text{P}_3$ [$\text{M} - \text{H}$], requires 422.97943.

Synthesis of ATP (12), GTP (13), CTP (14), UTP (15), dATP (16) AZTTP (17). To a mixture of tetrabutylammonium trimetaphosphate (0.54 mmol, 520 mg in case of CTP, GTP and 2'-deoxyATP, and 0.675 mmol, 650 mg in case of ATP, AZTTP and UTP) and DABCO (0.61 mmol, 69 mg for CTP, GTP, and 2'-deoxyATP or 0.74 mmol, 83 mg for ATP, AZTTP and UTP) in dry pyridine (6 mL for CTP, GTP, 2'-deoxyATP, 7.5 mL for ATP, AZTTP, and UTP) was added mesitylenesulfonyl chloride (0.50 mmol, 109 mg for CTP, GTP, 2'-deoxyATP 0.63 mmol, 139 mg for ATP, AZTTP, and UTP). The mixture was stirred at room temperature for one minute then **6**, **7**, **8**, or **9** (0.135 mmol) was added and the reaction mixture was allowed to stir at room temperature for 4 min then phthalimide (0.61 mmol, 89 mg for CTP, GTP, and 2'-deoxyATP, 0.742 mmol, 109 mg for ATP, AZTTP, and UTP) was added and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then cooled in an ice bath and quenched by adding 100mM triethylammonium acetate buffer (pH 7.0, 10 mL) The

solution was washed with chloroform (3 x 10 mL).³ The solution was then lyophilized. The residue after lyophilisation was dissolved in 4.5 mL water and then 10 mL methanol followed by 1.5 mL triethylamine were added, and the solution was stirred at room temperature for 24h. The solution was then concentrated to about 5 mL on rotary evaporator (at room temperature) and lyophilized. The lyophilized powder was dissolved in 6 mL water and purified by reversed-phase chromatography using the Biotage system. Fractions containing the desired product were pooled, concentrated by high vacuum rotary evaporation, and the residue was dissolved in water and repeatedly freeze-dried until the ¹H-NMR spectrum indicated that no residual buffer was present (four times). The resulting white powder was converted to its ammonium salt using a Dowex-50-W ion-exchange resin in NH₄⁺ form followed by lyophilisation.

Adenosine 5'-triphosphate, triammonium salt (12). Obtained in 74% yield as its triammonium salt (56 mg) after purification by RP-chromatography followed by passage through a Dowex-50-W-NH₄⁺ ion exchange column. For RP-chromatography a linear gradient of 100% buffer A (10 mM tributylamine-30 mM acetic acid in water), 0% buffer B (15 mM tributylamine in MeOH) to 50% buffer A, 50% buffer B over 30 min then 50% buffer A, 50% buffer B to 0% buffer A, 100% buffer B over 20 min was used ($t_r = 35$ min). The flow rate was 25 mL/min and eluate was monitored at 265 and 280 nm. ¹H-NMR (D₂O, 300 MHz): δ 4.14-4.26 (m, 3H, H_{4',5'}), 4.36 (t, $J = 4.1$ Hz, 1H, H_{3'}), 4.49 (t, $J = 4.8$ Hz, 1H, H_{2'}) 5.92 (d, $J = 4.8$ Hz, 1H, H_{1'}), 8.24 (s, 1H, H₂), 8.41 (s, 1H, H₈). ¹³C-NMR (D₂O, 75 MHz): δ 64.9 (d, $J = 5.2$ Hz), 69.9, 74.7, 83.8 (d, $J = 9.0$ Hz), 87.5, 117.9, 141.9, 145.2, 147.8, 149.8. ³¹P-NMR (D₂O, 121 MHz): δ -8.07 (d, $J = 17.6$ Hz), -8.62 (d, $J = 18.0$ Hz), -20.17 (overlapping dd, $J = 18.2, 20.7$ Hz). HRMS (ESI-): $m/z = 505.98828$. C₁₀H₁₅O₁₃N₅P₃ [M - H]⁻, requires 505.98737.

Guanosine 5'-triphosphate, triammonium salt (13). Obtained in 70% yield as its triammonium salt (54 mg) after purification by RP-chromatography followed by passage through a Dowex-50-W-NH₄⁺ ion exchange column. For RP-chromatography a linear gradient of 100% buffer A (10 mM tributylamine-30 mM acetic acid in water), 0% buffer B (15 mM tributylamine in MeOH) to 55% buffer A, 45% buffer B over 40 min was used (*t_r* = 34 min). The flow rate was 25 mL/min and eluate was monitored at 265 and 280 nm. ¹H-NMR (D₂O, 300 MHz): δ 3.83 (brs, 2H, H_{5'}), 4.15 (brs, 1 H, H_{4'}), 4.30 (t, *J* = 4.4 Hz, 1H, H_{3'}), 4.56 (t, *J* = 5.4 Hz, 1H, H_{2'}), 5.72 (d, *J* = 5.7 Hz, 2H, H_{1'}), 8.01 (s, 1H, H₈). ¹³C-NMR (D₂O, 75 MHz): δ 63.3 (d, *J* = 4.2 Hz), 70.5, 73.9, 84.3 (d, *J* = 8.4 Hz), 86.5, 115.9, 137.4, 151.4, 153.8, 158.8. ³¹P-NMR (D₂O, 121 MHz): δ -3.15 (d, *J* = 18.3 Hz), -8.37 (d, *J* = 20.8 Hz), -18.85 (overlapping dd, *J* = 20.8, 18.3 Hz). HRMS (ESI-): *m/z* = 521.98372. C₁₀H₁₅O₁₄N₅P₃ [M - H]⁻, requires 521.98338.

Cytidine 5'-triphosphate, triammonium salt (14). Obtained in 70% yield as its triammonium salt (51 mg) after purification by RP-chromatography followed by passage through a Dowex-50-W-NH₄⁺ ion exchange column. For RP-chromatography, a linear gradient of 100% buffer A (10 mM tributylamine-30 mM acetic acid in water), 0% Buffer B (15 mM tributylamine in MeOH) to 60% buffer A, 40% buffer B over 40 min was used (*t_r* = 29 min). The flow rate was 25 mL/min and eluate was monitored at 265 and 280 nm. ¹H-NMR (D₂O, 300 MHz): δ 4.10 (brs, 3H, H_{4'}, 5'), 4.15 (t, *J* = 4.9Hz, 1H, H_{3'}), 4.20 (t, *J* = 4.8Hz, 1H, H_{2'}) 5.81 (d, *J* = 4.2 Hz, 1H, H_{1'}), 5.97 (d, *J* = 7.6 Hz, 1H, H₅), 7.81 (d, *J* = 7.6 Hz, 1H, H₆). ¹³C-NMR (D₂O, 75 MHz): δ 64.5 (d, *J* = 5.4 Hz), 69.0, 74.0, 82.5 (d, *J* = 9.0 Hz), 89.0, 96.3, 141.6, 156.5, 165.2. ³¹P-NMR (D₂O, 121 MHz): δ -4.74 (d, *J* = 19.3 Hz), -8.55 (d, *J* = 20.4 Hz), -21.05 (overlapping dd, *J* = 18.6, 19.3 Hz). HRMS (ESI-): *m/z* = 481.97708. C₉H₁₅O₁₄N₃P₃ [M - H]⁻, requires 481.97723.

Uridine 5'-triphosphate, triammonium salt (15). Obtained in 77% yield as its triammonium salt (56 mg) after purification by RP-Chromatography followed by passage through a Dowex-50-W-NH₄⁺ ion exchange column. For RP-chromatography, a linear gradient of 100% buffer A (10 mM tributylamine-30 mM acetic acid in water), 0% Buffer B (15 mM tributylamine in MeOH) to 60% buffer A, 40% buffer B over 40 min was used ($t_r = 33$ min). The flow rate was 25 mL/min and eluate was monitored at 265 and 280 nm. ¹H-NMR (D₂O, 300 MHz): δ 3.79 (br, 2H, H_{5'}), 4.11 (brs, 1H, H_{4'}), 4.21 (brs, 1H, H_{3'}), 4.26 (t, 1H, $J = 5.1$ Hz), 5.82 (brd, 2H, $J = 8.4$ Hz, H_{1',5'}), 7.96 (d, 1H, $J = 8.0$ Hz, H₆). ¹³C-NMR (D₂O, 75 MHz): δ 62.9 (d, $J = 4.3$ Hz), 69.9, 73.8, 84.0 (d, $J = 8.7$ Hz), 88.1, 102.5, 141.9, 151.9, 166.3. ³¹P-NMR (D₂O, 121 MHz): δ -5.46 (d, $J = 18.7$ Hz), -9.07 (d, $J = 19.7$ Hz), -20.20 (overlapping dd, $J = 18.6, 18.0$ Hz). HRMS (ESI-): $m/z = 482.96059$. C₉H₁₄O₁₅N₂P₃ [M - H]⁻, requires 482.96015.

dATP (16). Obtained in 71% yield as its triammonium salt (52 mg) after purification by RP-chromatography followed by passage through a Dowex-50-W-NH₄⁺ ion exchange column. For RP-chromatography a linear gradient of 100% buffer A (10 mM tributylamine-30 mM acetic acid in water), 0% Buffer B (15 mM tributylamine in MeOH) to 40% buffer A, 60% buffer B over 45 min was used ($t_r = 38$ min). The flow rate was 25 mL/min and eluate was monitored at 265 and 280 nm. ¹H-NMR (D₂O, 300 MHz): δ 2.45-2.50 (m, 1H, H_{2'}), 2.67-2.71 (m, 1H, H_{2'}), 4.07-4.17 (m, 3H, H_{3',5'}), 6.34 (br, 1H, H_{1'}), 8.06 (s, 1H, H₂), 8.33 (s, 1H, H₈). ¹³C-NMR (D₂O, 75 MHz): δ 38.85, 65.0 (d, $J = 5.5$ Hz), 70.5, 83.4, 85.5 (d, $J = 8.7$ Hz), 118.4, 139.7, 148.48, 152.5, 155.3, 160.2 ³¹P-NMR (D₂O, 121 MHz): δ -10.09 (d, $J = 18.5$ Hz), -10.60 (d, $J = 21.4$ Hz), -22.29 (overlapping dd, $J = 18.6, 18.6$ Hz). HRMS (ESI-): $m/z = 489.99307$. C₁₀H₁₅O₁₂N₅P₃ [M - H]⁻, requires 489.99355.

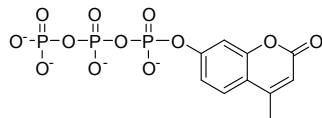
AZT-TP (17). Obtained in 79% yield as its triammonium salt (57 mg) after purification by RP-Chromatography followed by passage through a Dowex-50-W-NH₄⁺ ion exchange column. For RP-chromatography, a linear gradient of 100% buffer A (10 mM tributylamine-30 mM acetic acid in water), 0% Buffer B (15 mM tributylamine in MeOH) to 50% buffer A, 50% buffer B over 45 min was used ($t_r = 37$ min). The flow rate was 25 mL/min and eluate was monitored at 265 and 280 nm. ¹H-NMR (D₂O, 300 MHz): δ 1.27 (s, 3H, CH₃), 2.30 (s, 2H, H₂), 3.79 (s, 3H, H_{5,4}), 4.38 (s, 1H, H₃), 6.44 (t, 1H, $J = 6.0$ Hz, H₁), 7.53 (s, 1H, H₆). ¹³C-NMR (D₂O, 75 MHz): δ 11.4, 36.0, 60.6, 65.4 (d, $J = 5.4$ Hz), 82.6 (d, $J = 8.9$ Hz), 84.7, 111.5, 137.0, 151.4, 166.2. ³¹P-NMR (D₂O, 121 MHz): δ -6.37 (d, $J = 19.4$ Hz), -10.45 (d, $J = 19.8$ Hz), -21.24 (overlapping dd, $J = 19.9, 18.8$ Hz). HRMS (ESI-): $m/z = 505.98906$. C₁₀H₁₅O₁₃N₅P₃ [M - H]⁻, requires 505.98847.

References and Notes

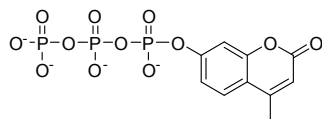
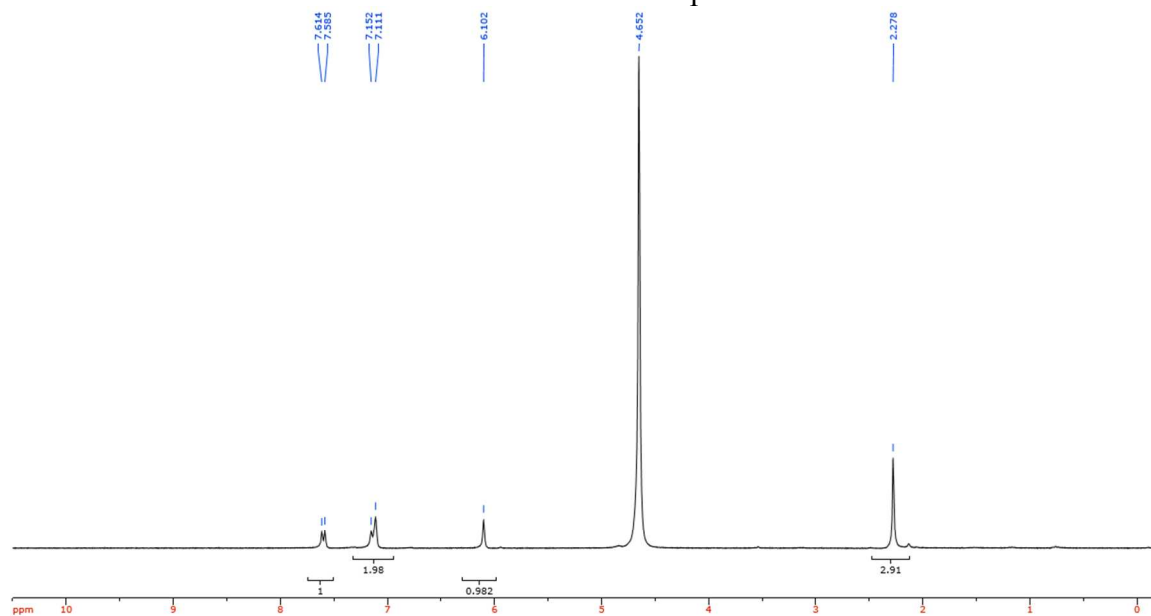
1. (a) Wolf, S.; Zismann, T.; Lunau, N.; Meier, C. *Chem. Eur. J.* **2009**, *15*, 7656. (b) Ogilvie, K. K.; Iwacha, D. *Tetrahedron Lett.* **1973**, *4*, 317. (c) Ogilvie, K. K.; Sadana, K. L.; Thompson, E. A.; Quilliam, M. A.; Westmore, J. B. *Tetrahedron Lett.* **1974**, *33*, 2861. (d) Ogilvie, K. K.; Schifman, A. L.; Penny, C. *Can. J. Chem.* **1979**, *57*, 2230.

2. It is strongly recommended to check that the stock solution of TriMP in pyridine is sufficiently dry before attempting a triphosphorylation. This is done by adding MstSO_2Cl (0.93 equiv), DABCO (1.15 equiv) and phthalimide (1.15 equiv) to a solution of TriMP (1.00 equiv) in dry pyridine (approximately 1 mL dry pyridine/0.1 mmol TriMP) and then immediately obtaining the ^{31}P -NMR spectrum of the mixture before the accumulation of a significant quantity of precipitate. There should be no peak corresponding to TriMP at -19 ppm. If a peak due to TriMP is present then more sieves should be added to the stock solution of TriMP and the solution allowed to sit for 24 h. This solution should be checked again as described above.

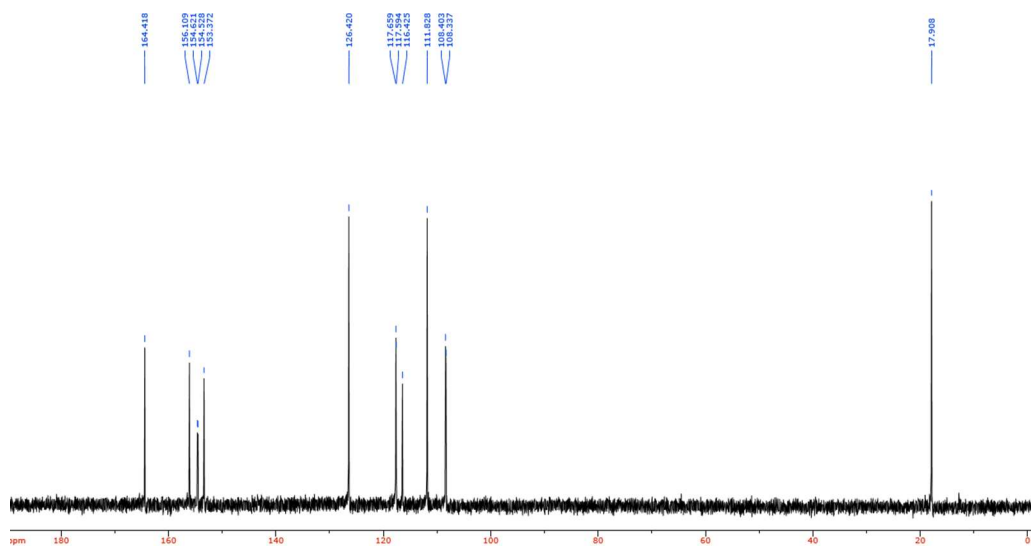
3. The use of benzoyl OH protecting groups is not recommended as these increase the hydrophobicity of the OH-protected product which results in their partial extraction into the chloroform layer.

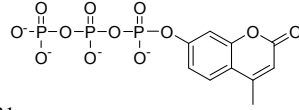


¹H-NMR of compound 4

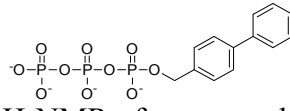
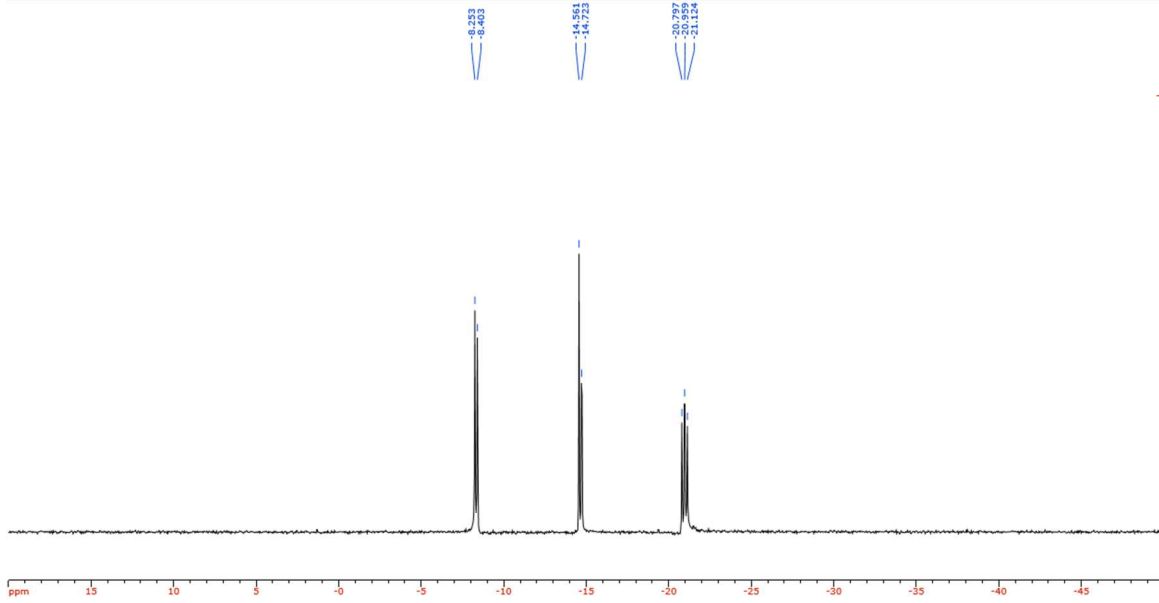


¹³C-NMR of compound 4

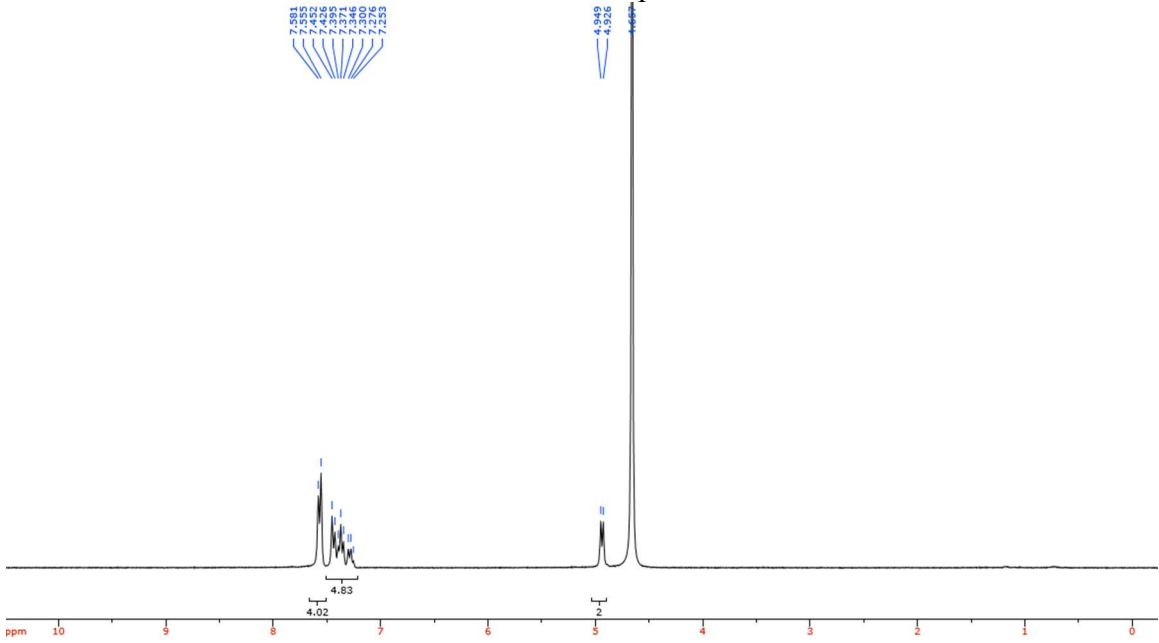


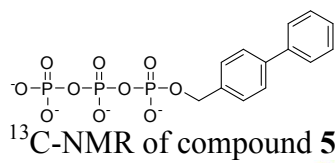


³¹P-NMR of compound 4



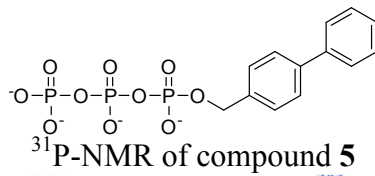
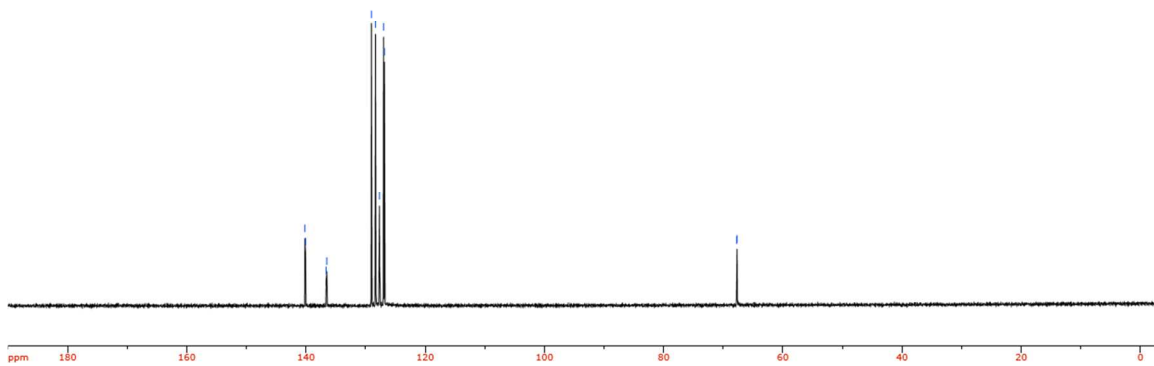
¹H-NMR of compound 5





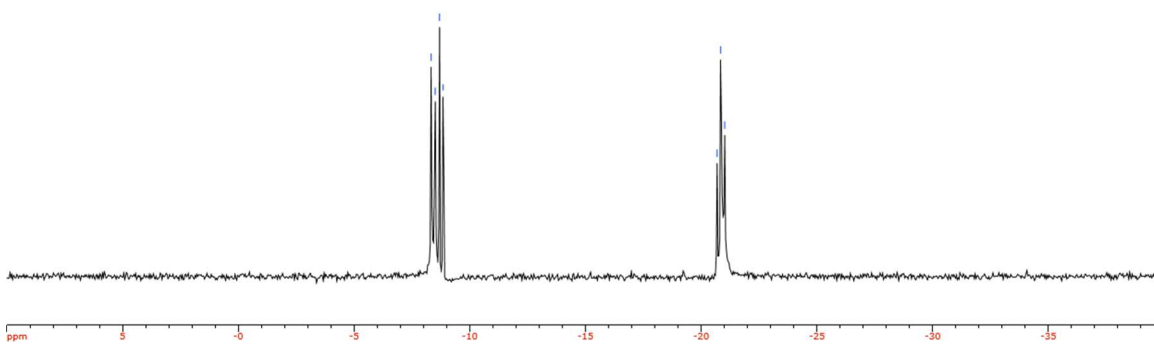
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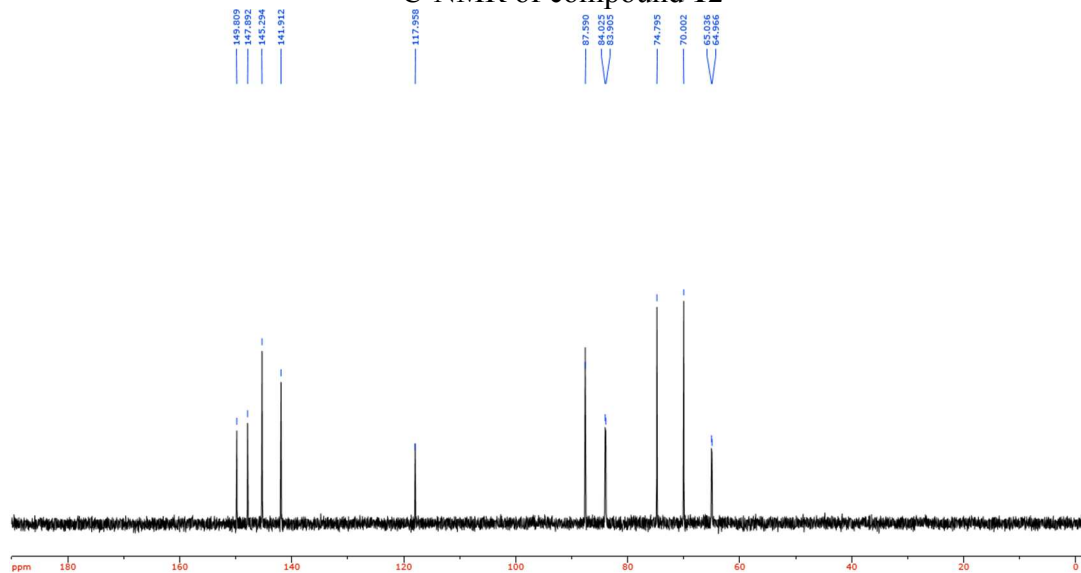
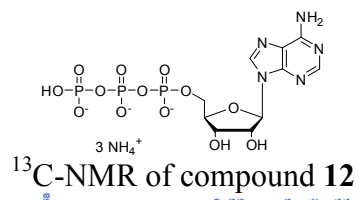
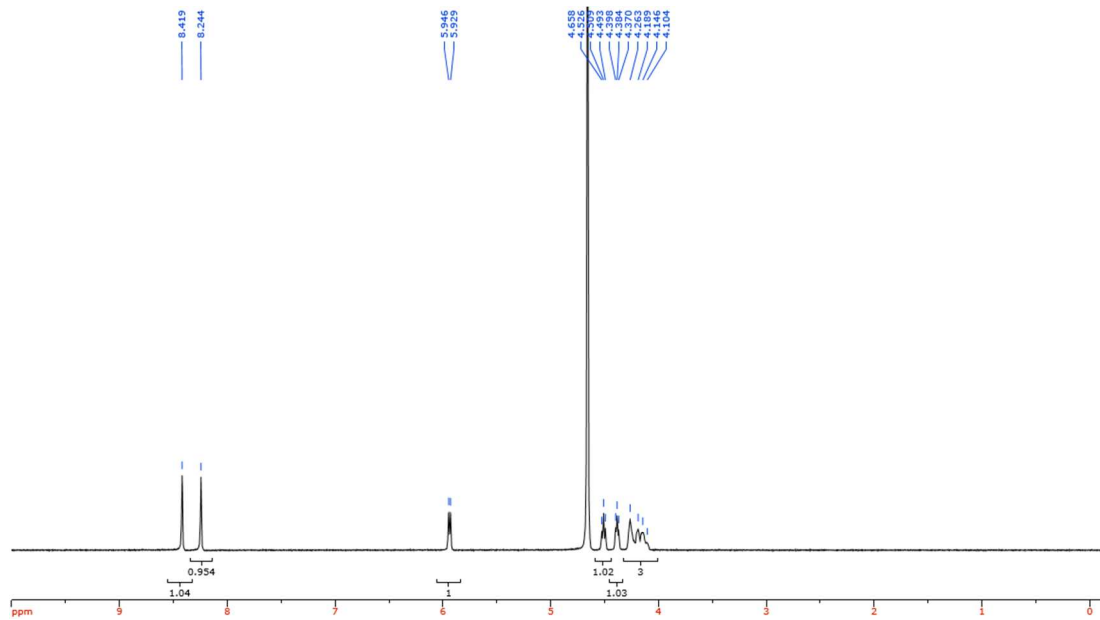
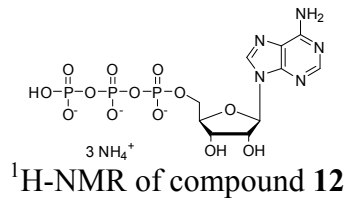
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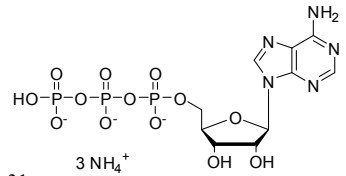


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20.372

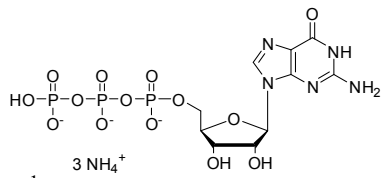
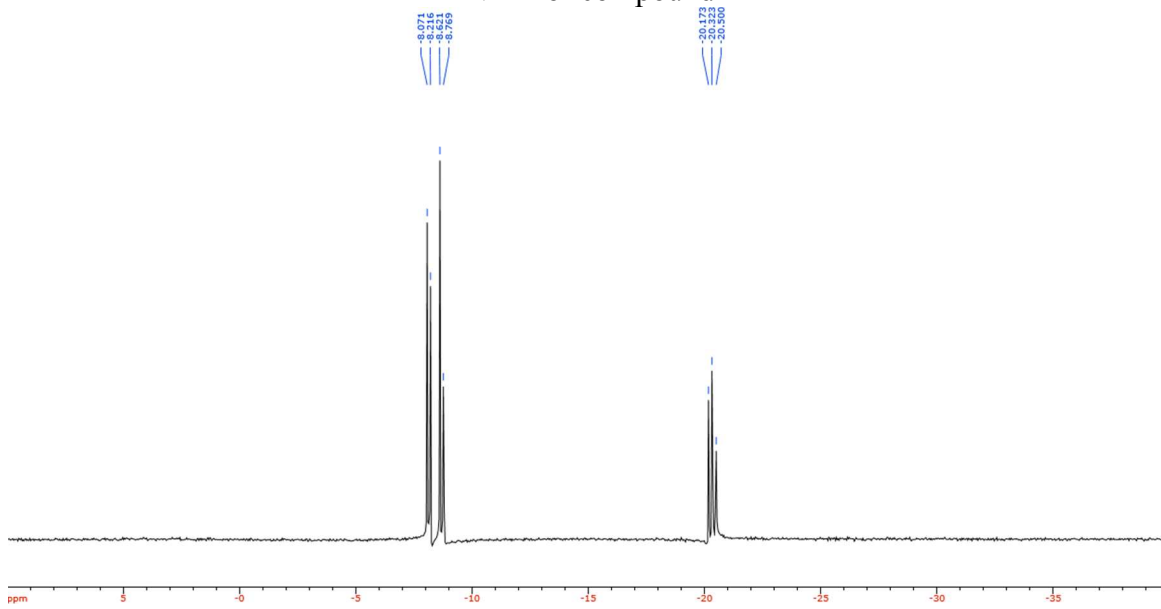
20.686
20.843
21.014



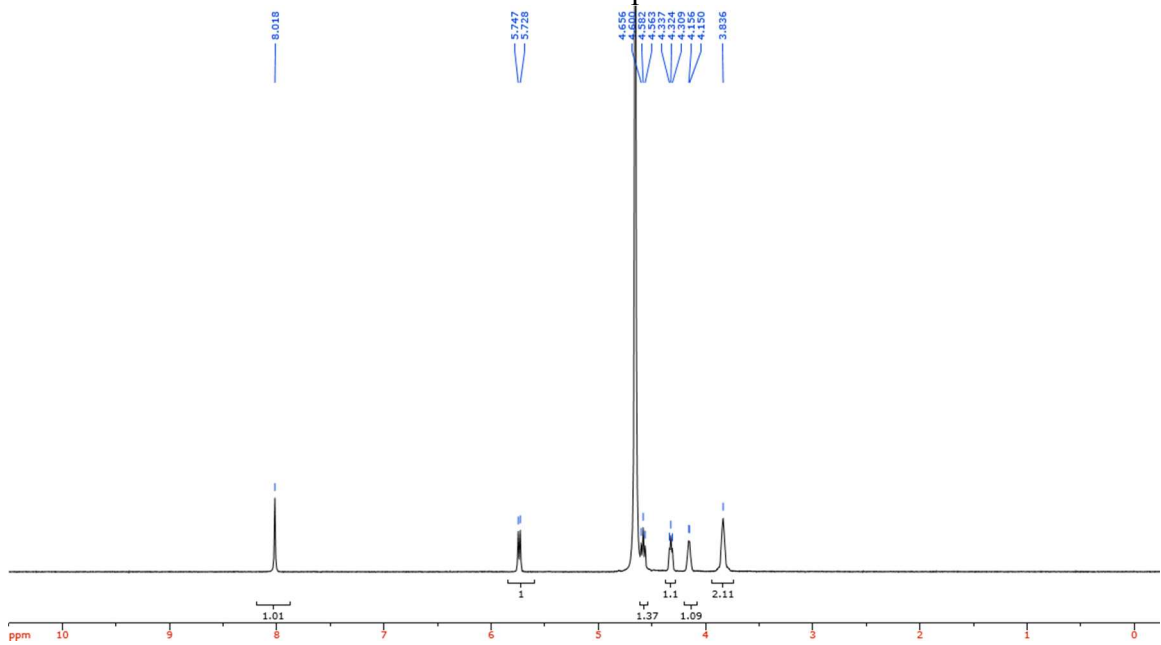


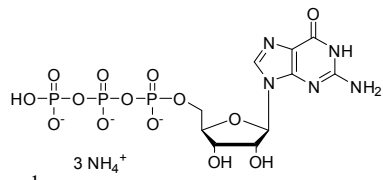


³¹P-NMR of compound 12

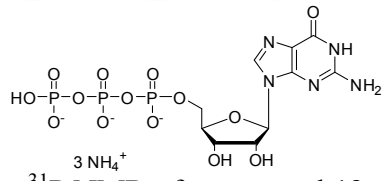
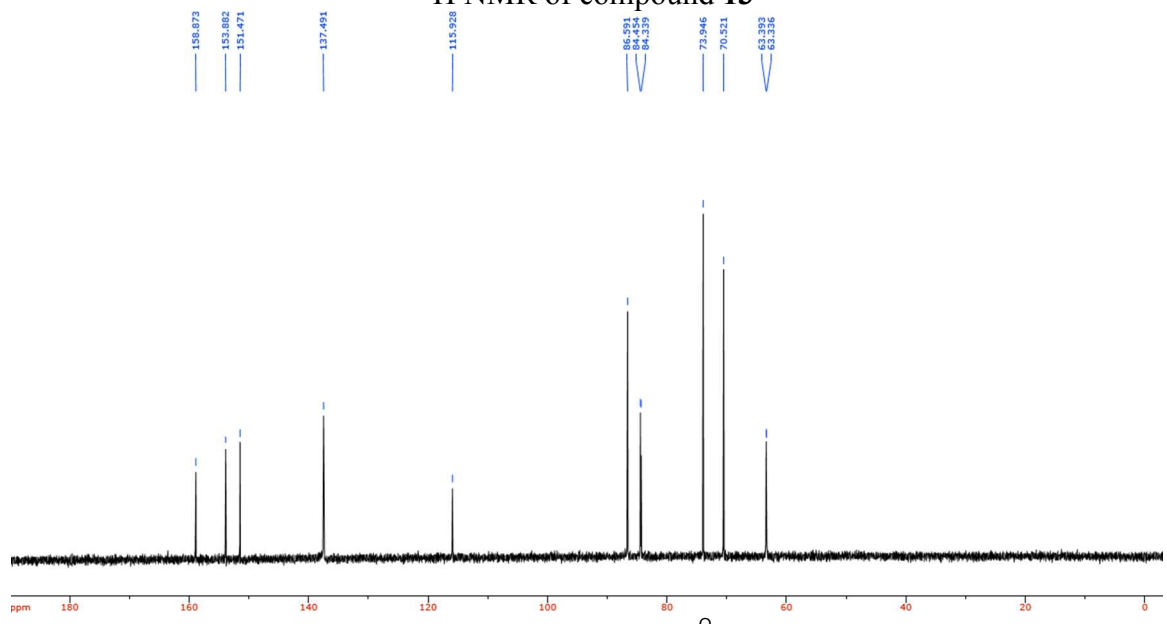


¹H NMR of compound 13

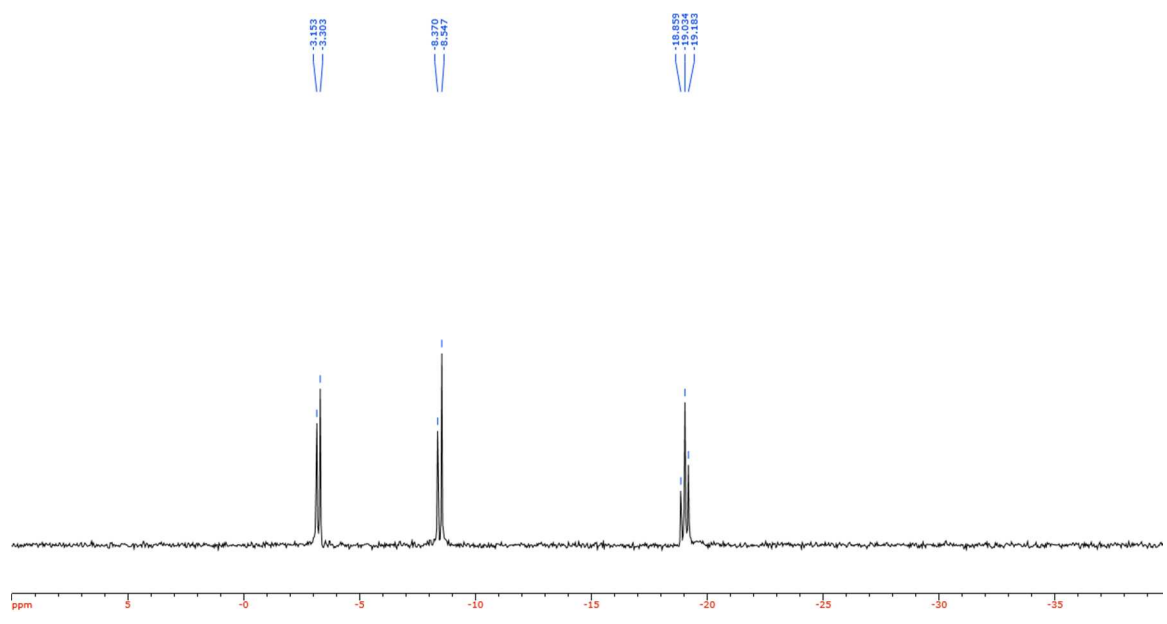


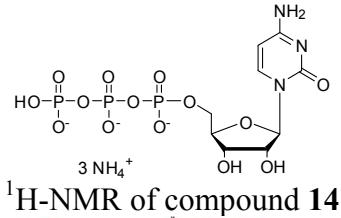


¹H NMR of compound 13

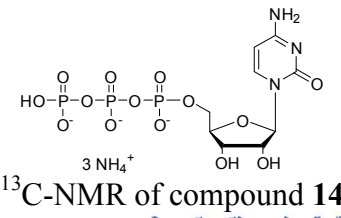
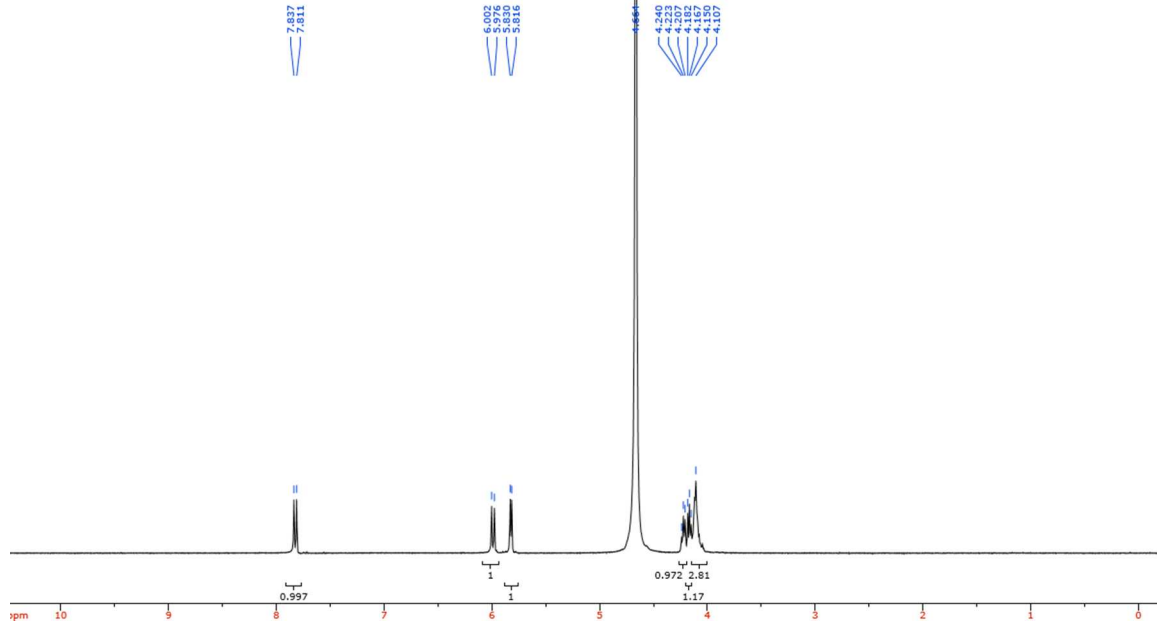


³¹P NMR of compound 13

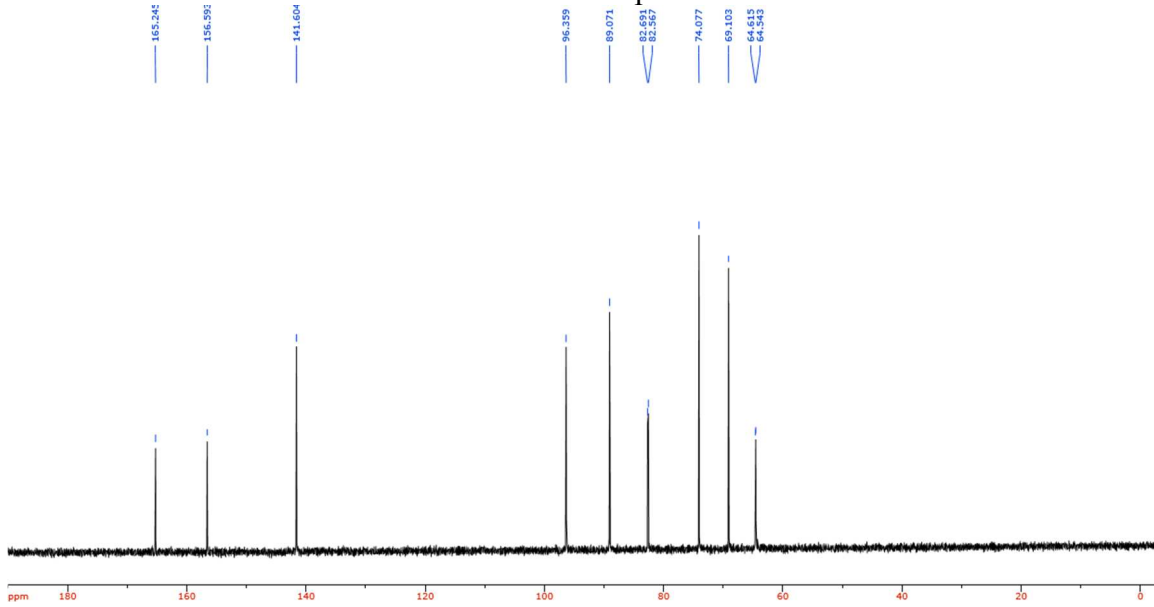


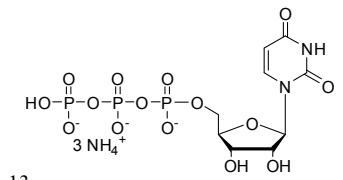


$^1\text{H-NMR}$ of compound 14

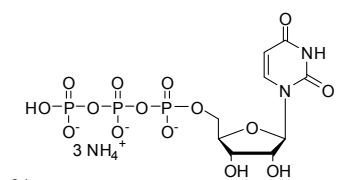
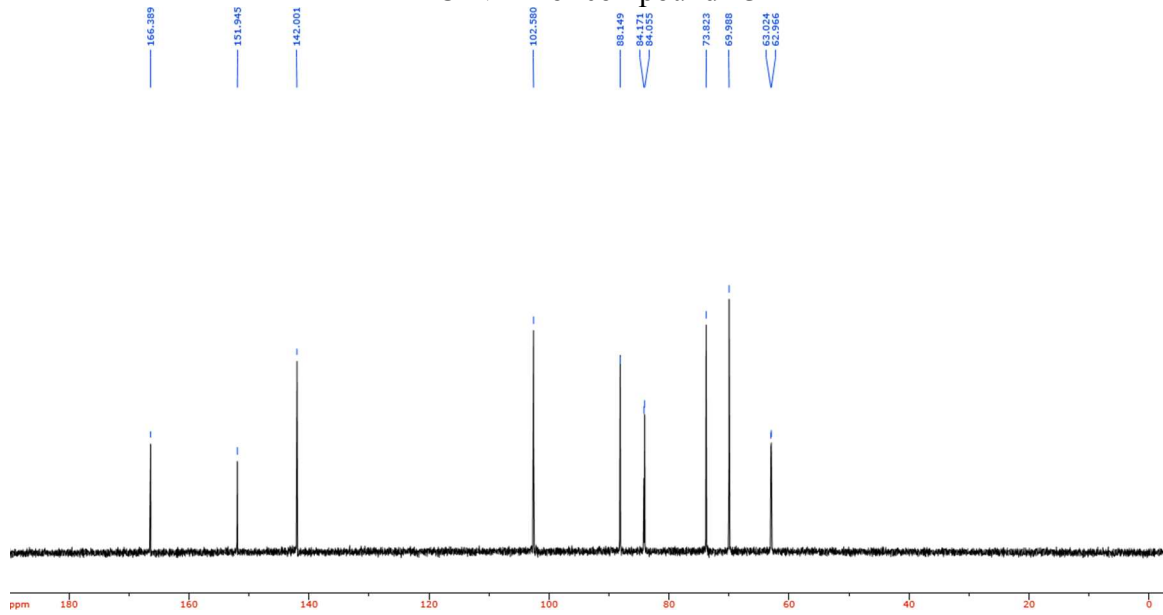


$^{13}\text{C-NMR}$ of compound 14

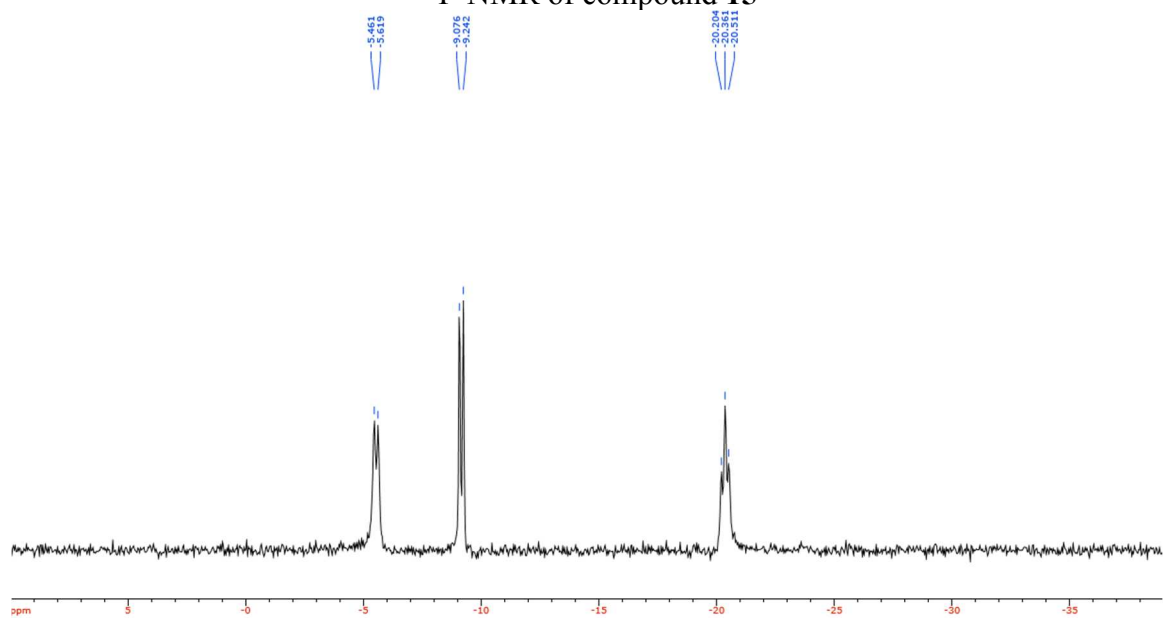


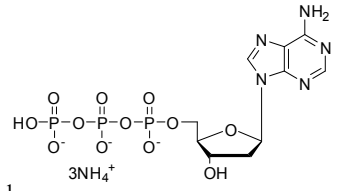


^{13}C -NMR of compound 15

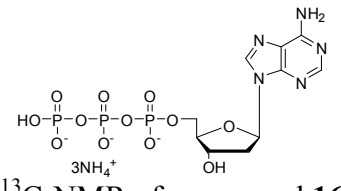
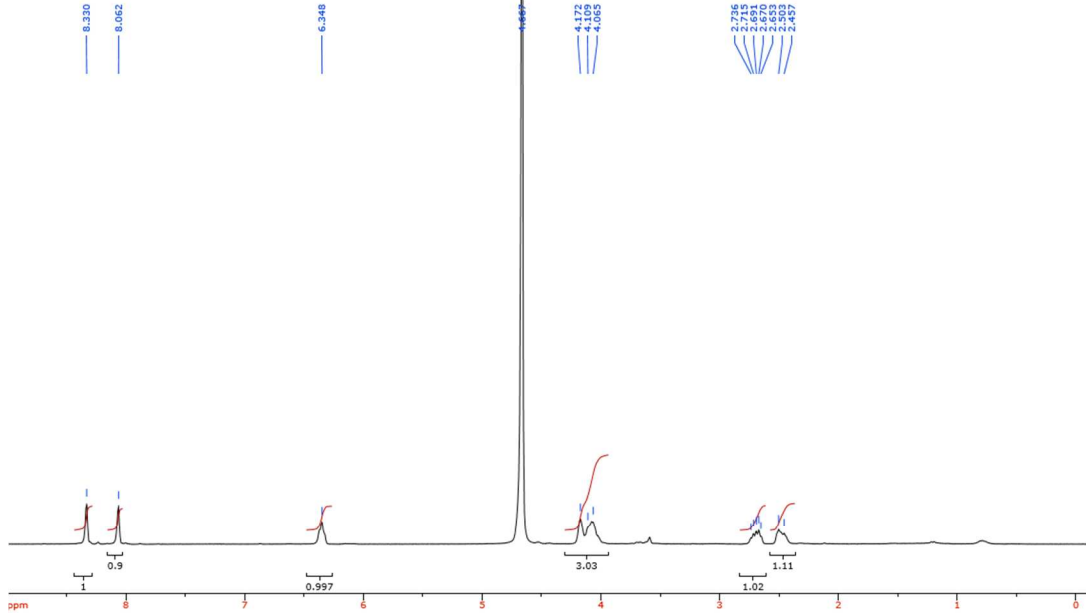


^{31}P -NMR of compound 15

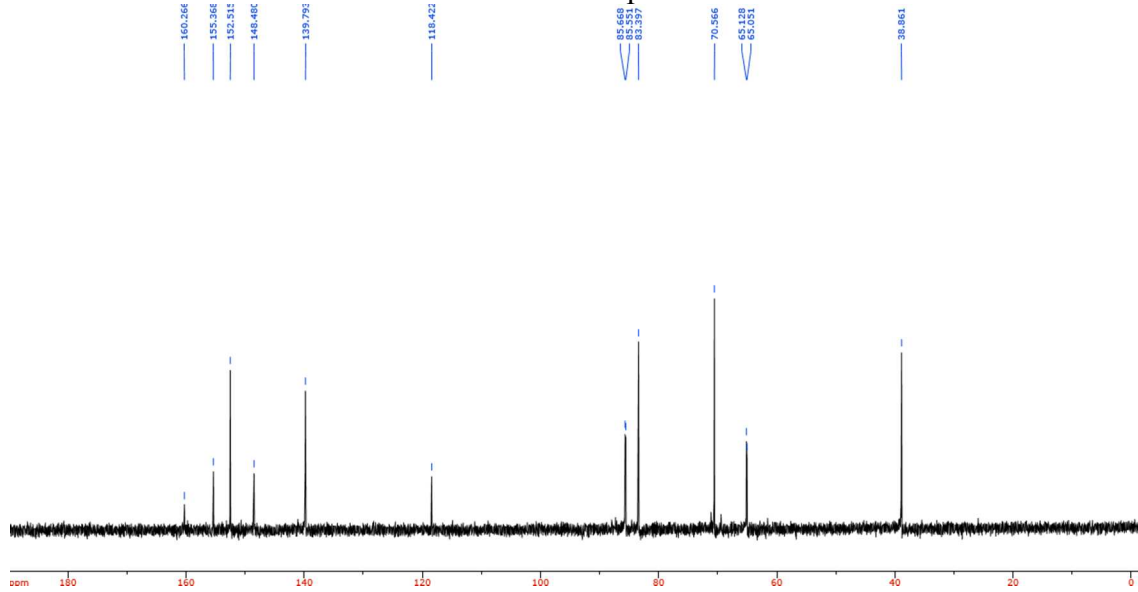


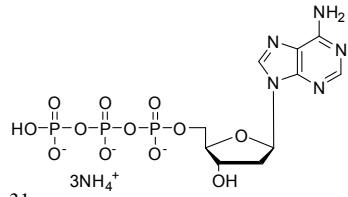


$^1\text{H-NMR}$ of compound 16

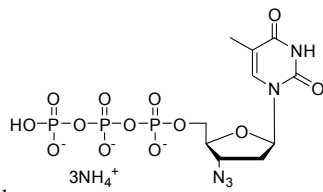
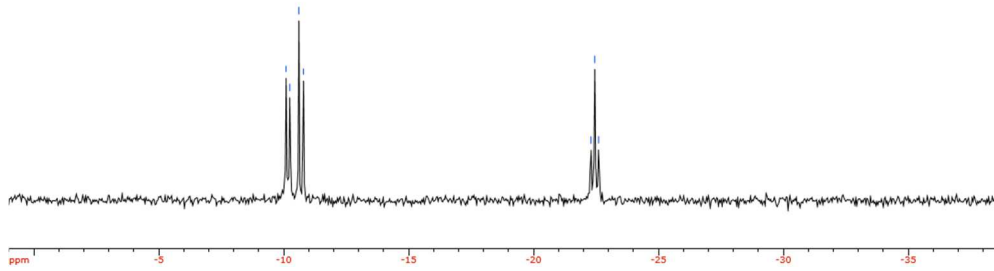


$^{13}\text{C-NMR}$ of compound 16

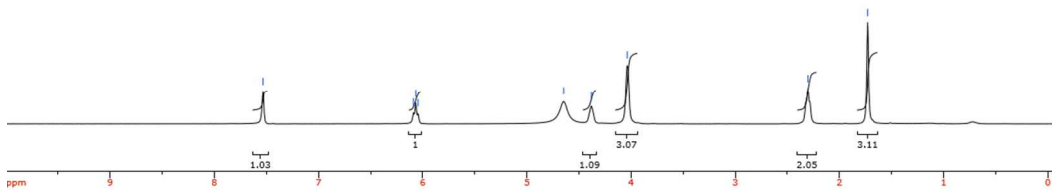


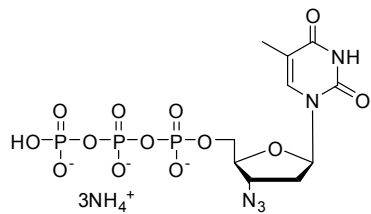


^{31}P -NMR of compound 16

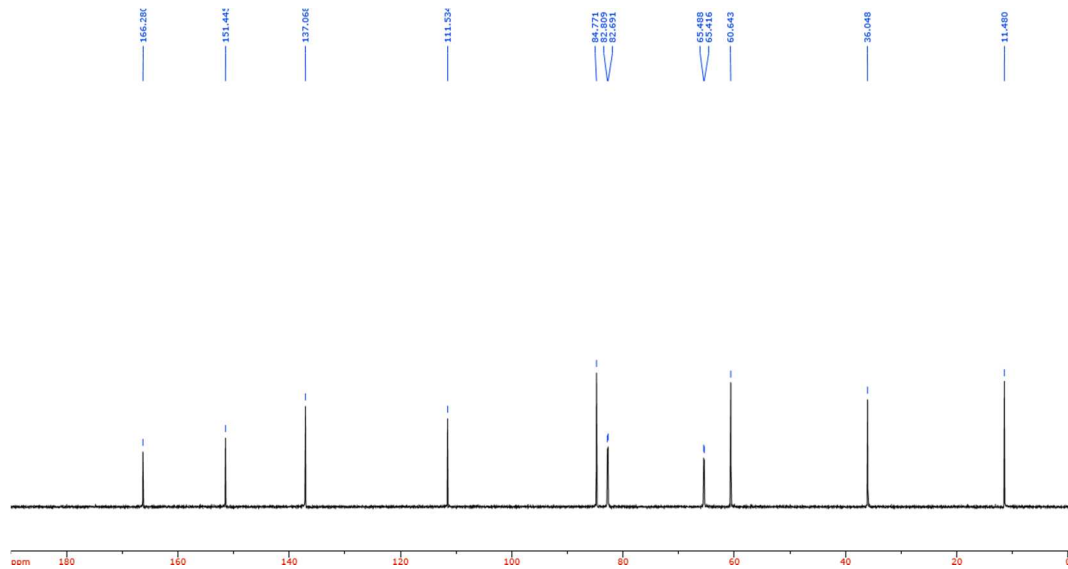


^1H -NMR of compound 17

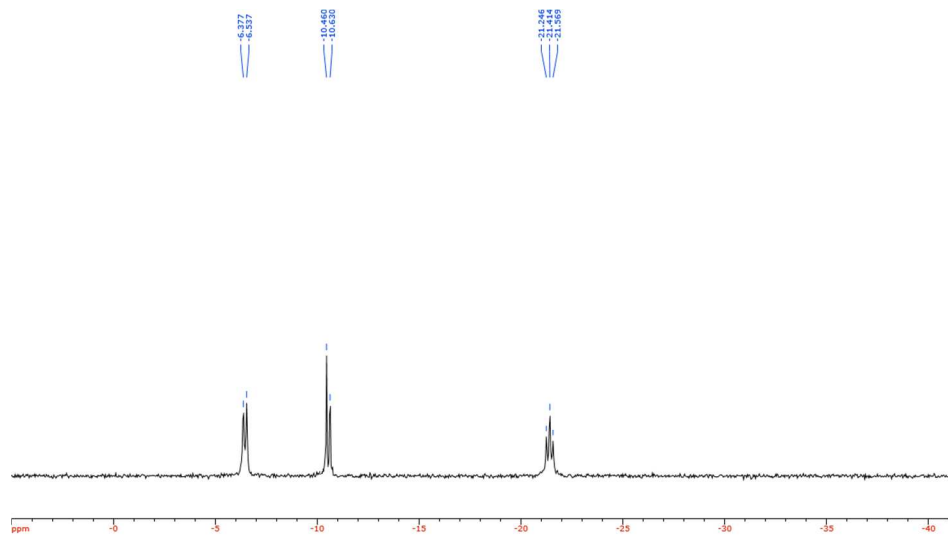




¹³C-NMR of compound 17



³¹P-NMR of compound 17



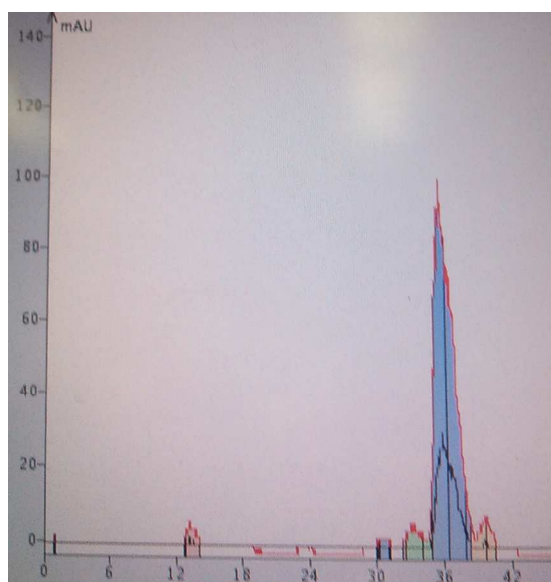


Figure S.1 Preparative RP chromatogram of crude compound **5** using a Biotage Isolera purification system. The peak corresponding to compound **5** is shaded in blue. A linear gradient of 75% buffer A (10 mM tributylamine-30 mM acetic acid in water), 25% buffer B (15 mM tributylamine in MeOH) to 25% buffer A, 75% buffer B over 45 min was used. The flow rate was 25 mL/min and eluate was monitored at 254 (red trace) and 280 nm (black trace).

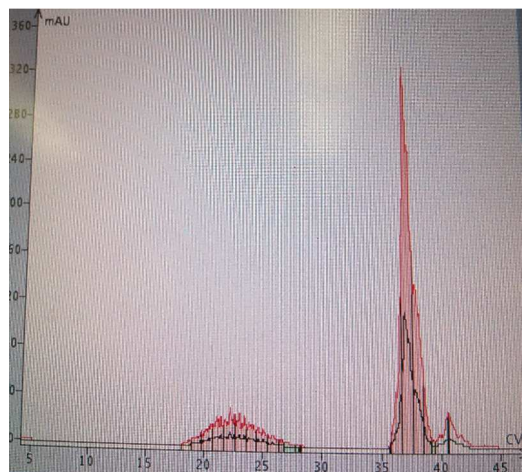


Figure S.2. Preparative RP chromatogram of crude compound **12** using a Biotage Isolera purification system. The peak corresponding to compound **12** is the large relatively sharp peak appearing at 35.5-39.5 min. A linear gradient of 75% buffer A (10 mM tributylamine-30 mM acetic acid in water), 25% buffer B (15 mM tributylamine in MeOH) to 25% buffer A, 75%

buffer B over 45 min was used. The flow rate was 25 mL/min and eluate was monitored at 254 (red trace) and 280 nm (black trace).