# Alpha-heteroatom derivatised analogues of FR900098 as antimalarials

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SUPPORTING INFORMATION - CONTENTS

**Docking results**

**Docking of α-azido derivative 9.**

Both R and S enantiomers have been constructed and docked into PfDXR. All docked poses place the inhibitor in the binding site with positions of the hydroxamate (chelating the metal cation) and phosphonate similar to those observed in the co-crystallized structure of FR. Most of the docked poses, also corresponding to the best scored solutions (top solutions and 10/20 for both R and S) place the N3 group close to Trp_296 in the binding site. In this position, the $N_3$ group is also close to Ser_232 and Glu_233 (Fig.1). The vicinity of an azido group and an indole has further been analyzed by search in the Cambridge structure database and resulted in 15 structures, among which entry JASGOA underlines the possibility of favorable contacts between the indole ring and the $N_3$ function. This experimental structure confirms the possibility of a stabilizing interaction between the azido function of the inhibitor and the indole lateral chain of Trp_296 in the structures obtained by docking simulation

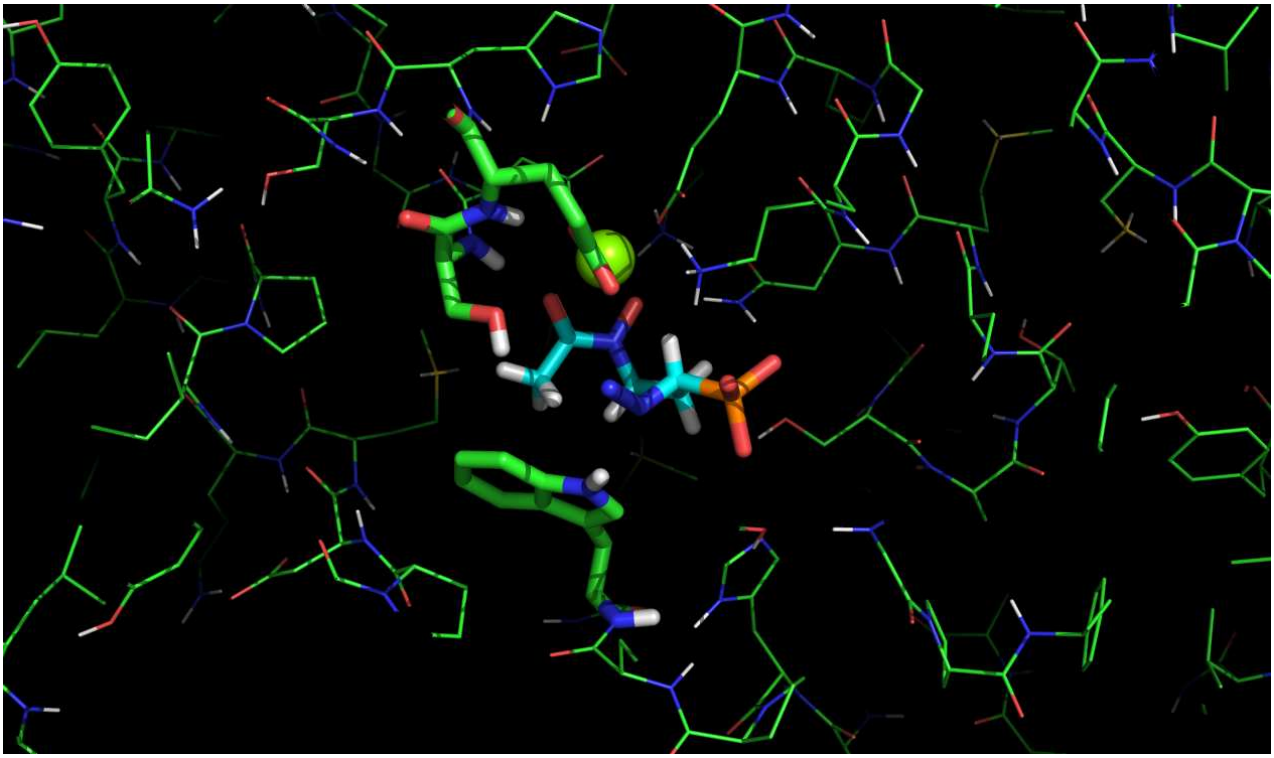


Fig.1 Docking solution (top solution obtained with GOLD program) of (*S*)-**9** and PfDXR.

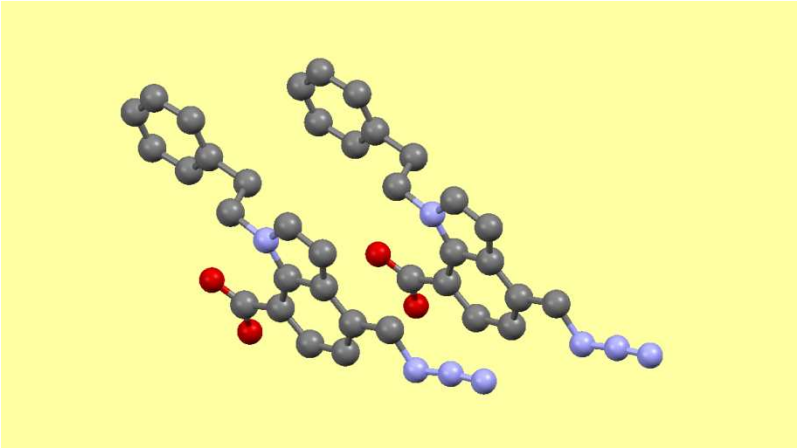


Fig 2. Stabilizing interaction between an azido function and an indole as observerd in entry JASGOA (Cambridge structure database).

**Docking of α-OH-FR900098 (10a)**

Both R and S enantiomers have been constructed and docked into PfDXR. All docked poses place the inhibitor in the binding site with positions of the hydroxamate (chelating the metal cation) and phosphonate similar to those observed in the co-crystallized structure of FR.

For the *R*-enantiomer, most of the poses place the OH group in H-Bond distance to Glu233 (Fig 3.). For the *S*-enantiomer, most of the poses place the OH group in H-Bond distance to Ser_306 (Fig 4 and 5).

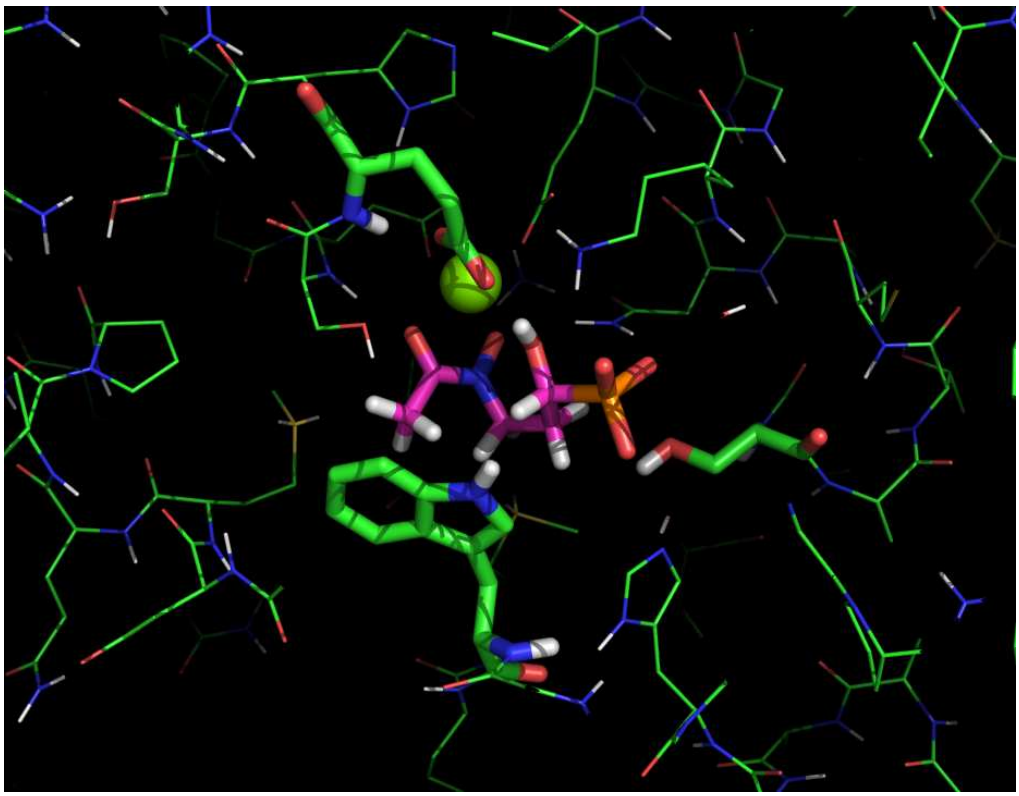


Fig. 3. Docking solution (GOLD program) of (*R*)-**10a** and PfDXR.

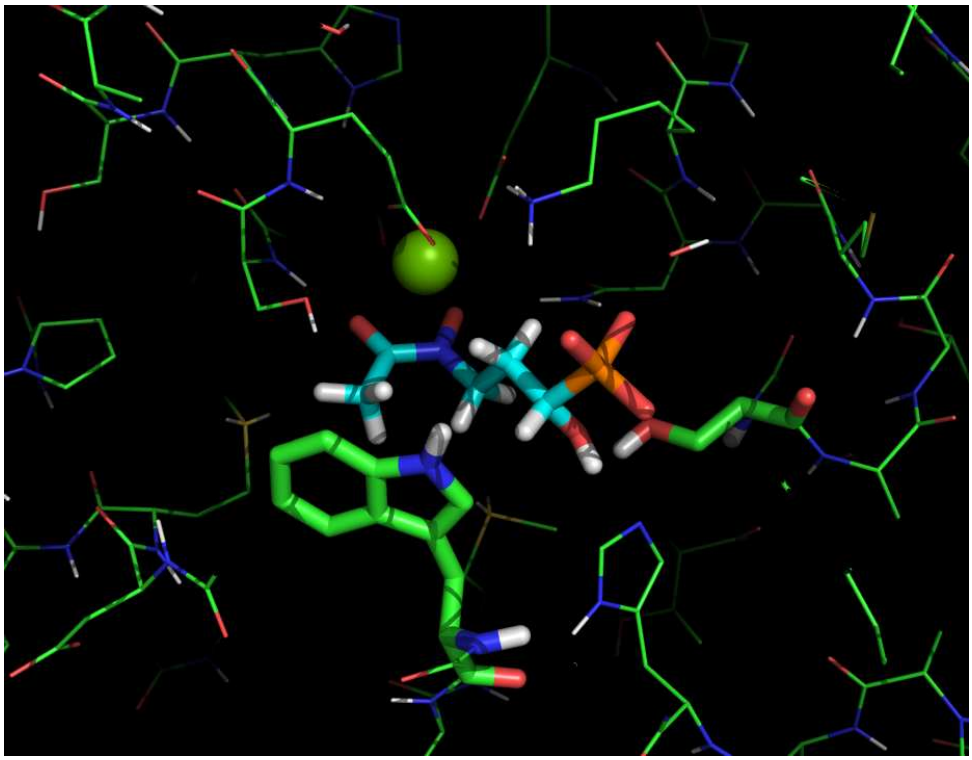


Fig. 4. Docking solution (GOLD program) of (S)-**10a** and PfDXR.

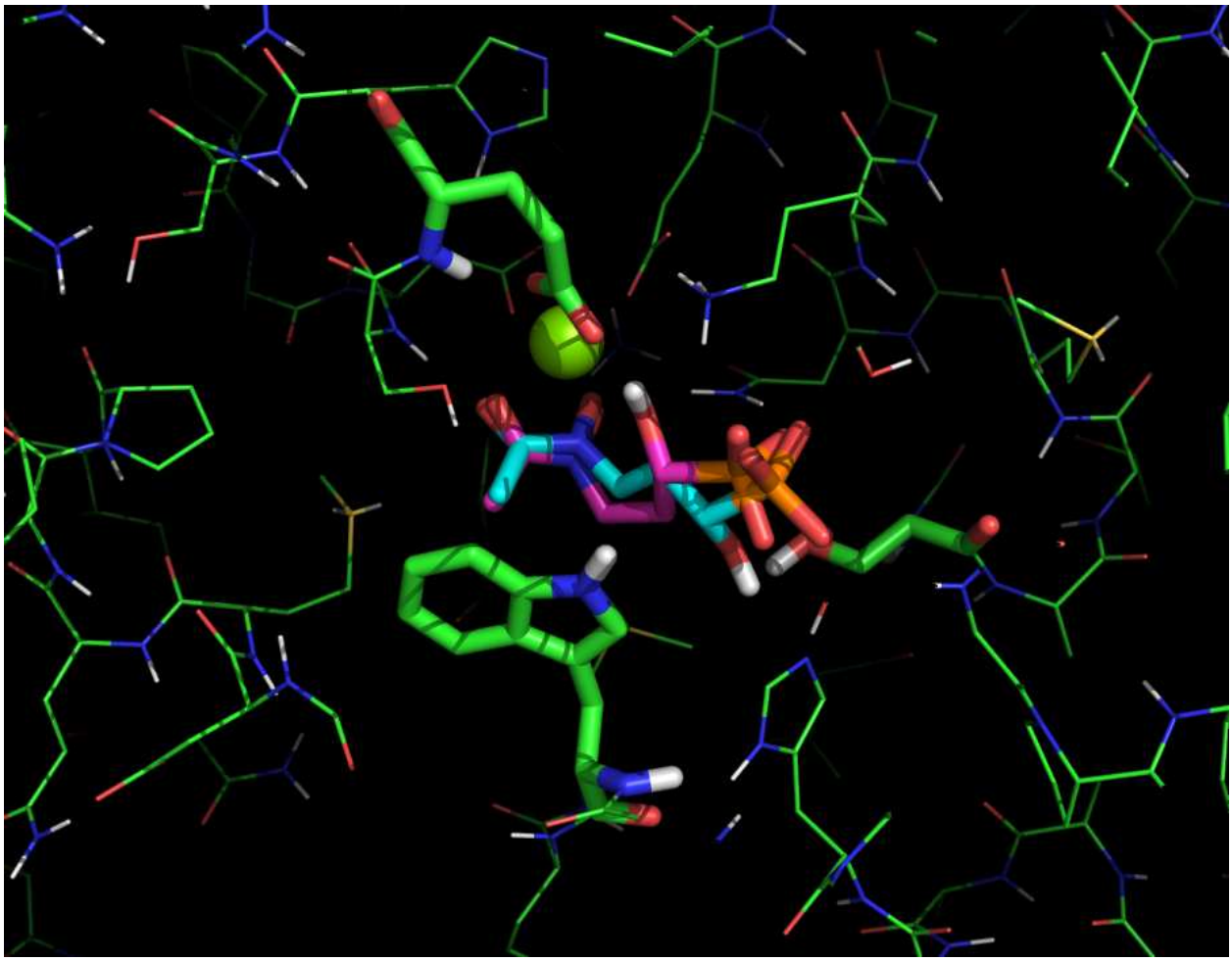


Fig. 5. Comparison of docking solution (GOLD program) of (*R*)-**10a** and (*S*)-**10a** in PfDXR.

***Setup & method***

GOLD program, default parameters for docking

Protein : 3AUA (*Plasmodium falsiparum* tertiary complex with FR)

Ligands have been constructed and 20 conformations are generated by docking.

Docking procedure has been validated by docking of FR compound: the co-crystallized structure is well predicted and reproduced

***tert*-Butyl *N*-(benzyloxy)-*N*-(but-3-enyl)carbamate (17)**. To a solution of *t*-butyl *N*-benzyloxycarbamate (6300 mg, 28.22 mmol) in dry DMF (60mL) was added sodium hydride (1242 mg of a 60% dispersion in mineral oil, 31.04 mmol) in small portions under vigorous stirring at room temperature. After 30 minutes, neat 4-bromobut-1-ene (3.15 mL, 4190 mg, 31.04 mmol) was added dropwise via syringe to the clear solution. The reaction mixture was stirred for 90 minutes, quenched by the addition of saturated aqueous $NH_4Cl$ and partitioned between aqueous 0.1N HCl and diethyl ether. The extraction with diethyl ether was repeated two times, the combined organic phases were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The resulting crude mixture was purified by dry column vacuum chromatography (DCVC) with a gradient of ethyl acetate in hexanes to yield 6.81g (87 %) of **17** as a colourless oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.50 (s, 9 H) 2.20 - 2.50 (m, 2 H) 3.32 - 3.61 (m, 2 H) 4.83 (s, 2 H) 5.05 (ddt, J=3.84, 2.60, 1.32, 1.32 Hz, 1H) 5.11 (q, J=1.46 Hz, 1 H) 5.77 (ddt, J=17.06, 10.25, 6.85, 6.85 Hz, 1 H) 7.27 - 7.51 (m, 5 H) $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ ppm 28.30, 31.44, 49.23, 76.91, 81.19, 116.68, 128.39, 128.44, 129.31, 135.27, 135.69, 156.54; HRMS (ESI) m/z 278.1745 [$(M+H)^+$, calcd for $C_{16}H_{24}NO_3^+$ 278.1751].

***N*-(benzyloxy)-*N*-(but-3-enyl)acetamide (18).** *t*-Butyl *N*-(benzyloxy)-*N*-(but-3-enyl)carbamate **17** (2774 mg, 10 mmol), NaI (2998 mg, 20 mmol) and methanol (dry, 0.81 mL, 20 mmol) were dissolved in 40 mL of dry acetonitrile, resulting in a clear yellow solution. Acetyl chloride (2.85 mL, 40 mmol) was added dropwise while stirring the reaction mixture at room temperature. After 60 minutes the reaction mixture was cooled to 0° C in an icebath and $Et_3N$ (5.6 mL, 40 mmol) was added as well as DMAP (122 mg, 1 mmol). The icebath was removed after 5 minutes and the reaction mixture stirred at room temperature for another 60 minutes. The reaction mixture was then poured into a separating funnel containing 1M aqueous HCl and extracted 3 times with diethyl ether. The combined organic fractions were washed with aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$ and brine and dried over anhydrous $Na_2SO_4$. Purification of the crude material by DCVC (hexanes/ethyl acetate) yielded 2.07g (94%) of **18** as a slightly yellow oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 2.07-2.08 (2s (rotamers), 3 H) 2.28 -

2.53 (m, 2 H) 3.69 (t, J=7.03 Hz, 2 H) 4.81 (s, 2 H) 4.93 - 5.22 (m, 2 H) 5.59 - 5.94 (m, 1 H) 7.28 - 7.51 (m, 5 H) $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ ppm 20.57, 31.26, 45.04, 76.28, 116.97, 128.68, 128.90, 129.11, 134.57, 134.93, 172.48; HRMS (ESI) m/z 220.1320 [$(M+H)^+$, calcd for $C_{13}H_{18}NO_2^+$ 220.1332].

***N*-(benzyloxy)-*N*-(2-formylethyl)acetamide (14).** To a solution of **18** (2200 mg, 10 mmol) in THF (50 mL) was added 40 mg $K_2OsO_4.2H_2O$ and a hot (50° C) solution of $NaIO_4$ (10700 mg, 50 mmol) in 50 mL water. The resulting off-white thick suspension was shielded from light and stirred for 4 hours, after which TLC (90:10 dichloromethane/methanol) confirmed the complete conversion of the starting material. The reaction mixture was then filtered over a glassfibre pad, the filter was rinsed with diethyl ether, the resulting biphasic filtrate was transferred to a separating funnel and aqueous $Na_2SO_4$ was added followed by threefold extraction with diethyl ether. The combined ether phases were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo, yielding 1.85g (84%) of a brown oil that was used without further purification in the next reaction. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 2.05 (s, 3 H) 2.68 (td, J=6.59, 1.46 Hz, 2 H) 3.95 (t, J=6.59 Hz, 2 H) 4.80 (s, 2 H) 7.22 - 7.50 (m, 5 H) 9.73 (t, J=1.46 Hz, 1 H) $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ ppm 20.50, 39.54, 41.32, 76.37, 128.70, 129.01, 129.36, 134.20, 172.80, 200.19; HRMS (ESI) m/z 222.1136 [$(M+H)^+$, calcd for $C_{12}H_{16}NO_3^+$ 222.1125].

**Dibenzyl 3-(*N*-(benzyloxy)acetamido)-1-methoxy-propylphosphonate (19a).** Compound **13** (488 mg, 1 mmol) was dissolved in DMF (4 mL) and Ag2O (348 mg, 1.5 mmol) was added under vigorous stirring. Neat iodomethane (0.62 mL, 1419 mg, 10 mmol) was added via syringe, the reaction mixture was shielded from light and stirred overnight at rt. The reaction mixture was then filtered over a glassfibre pad, the filtrate was concentrated and filtered again, resulting in a clear solution that was evaporated in vacuo. The resulting crude material was purified by dry column vacuum chromatography with a gradient of ethyl acetate in hexanes to yield 304 mg (61%) of **19a** as a colorless oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.85 - 2.02 (m, 1 H), 2.02 - 2.09 (m, 3 H), 2.09 - 2.29 (m, 1 H), 3.41 - 3.57 (m, 4

H), 3.59 - 3.76 (m, 1 H), 3.80 - 4.07 (m, 1 H), 4.63 - 4.86 (m, 2 H), 4.91 - 5.21 (m, 4 H), 7.14 - 7.45 (m, 15 H). HRMS (ESI): calcd for $C_{27}H_{32}NO_6P + H^+$, 498.2040; found [$(M+H)^+$], 498.2035.

**3-(*N*-Dibenzyl 3-(*N*-(benzyloxy)acetamido)-1-phenoxypropylphosphonate (19b).** Dibenzyl 3-(*N*-(benzyloxy)acetamido)-1-hydroxypropylphosphonate (**13**) (725 mg, 1.5 mmol), triphenylphosphine (393 mg, 1.5 mmol) and phenol (141 mg, 1.5 mmol) were dissolved in 0.5 mL THF under sonication at 30° C to give a clear viscous jelly. To this mixture was added diisopropyl azodicarboxylate (0.30 mL, 1.5 mmol) and sonication at 30°C was continued for one hour. Then another half equivalent of phenol (47 mg, 0.5 mmol), triphenylphosphine (131 mg, 0.5 mmol) and diisopropyl azodicarboxylate (0.10 mL, 0.5 mmol) dissolved in 0.250 mL THF were added and the mixture was sonicated at room temperature overnight. Subsequently, all volatiles were removed in vacuo and the crude material was fractionated by DCVC (hexanes/ethyl acetate). All fractions containing product were pooled and evaporated and the resulting material was purified by preparative HPLC (50/50 to 100/0 acetonitrile – water containing 0.2% formic acid) to give 224 mg (27%) of **19b** as a colourless oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.99 (s, 3 H) 2.10 - 2.45 (m, 2 H) 3.62 - 4.01 (m, 2 H) 4.46 - 4.81 (m, 3 H) 4.82 - 5.15 (m, 4 H) 6.82 - 7.10 (m, 3 H) 7.12 - 7.50 (m, 17 H); HRMS (ESI) m/z 560.2203 [$(M+H)^+$, calcd for $C_{32}H_{35}NO_6P^+$ 560.2197].

**3-(*N*-hydroxyacetamido)-1-methoxypropyl-phosphonic acid (10b).** Was prepared according to the same procedure as **10a** yielding an off-white hygroscopic solid (quant.). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 1.83 (dquintet, $J$ = 14.3, 7.5, 7.5, 7.5, 7.5 Hz, 1 H), 2.05 - 2.29 (m, 4 H), 3.16 - 3.28 (m, 1 H), 3.43 - 3.64 (m, 4 H), 3.88 - 4.08 (m, 1 H). $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 20.56, 29.50, 29.52 (d, $J$ = 3.6 Hz), 46.02 (d, J = 13.0 Hz), 60.24, 78.15 (d, J = 158.4 Hz) 173.70. $^{31}$P NMR (121 MHz, $CD_3OD$) $\delta$ 18.83. HRMS (ESI): calcd for $C_6H_{14}NO_6P$ - $H^+$, 226.0486; found [$(M-H)^+$], 226.0520.

**3-(*N*-hydroxyacetamido)-1-phenoxypropylphosphonic acid (10c).** Was prepared according to the same procedure as **10a** yielding a white hygroscopic solid (quant.). $^1$H NMR (300 MHz, METHANOL-d4) δ ppm 1.92 - 2.22 (m, 4 H) 2.22 - 2.54 (m, 1 H) 3.58 - 3.97 (m, 2 H) 4.29 - 4.59 (m, 1 H) 6.89 (t,

J=7.18 Hz, 1 H) 7.05 (d, J=7.91 Hz, 2 H) 7.23 (t, J=7.91 Hz, 2 H) 8.52 (s, 1 H); $^{13}$C NMR (75 MHz, METHANOL-d4) δ ppm 20.36, 29.64, 46.27 (d, J=8.85 Hz), 75.05 (d, J= 158.67Hz), 117.31, 121.93, 130.331, 160.70 (d, J=6.63 Hz) 173.46; $^{31}$P NMR (121 MHz, METHANOL-d4) δ ppm 16.52; HRMS (ESI) m/z 288.0633 [(M-H$^+$), calcd for $C_{11}H_{15}NO_6P^-$ 288.0642].

**Dibenzyl 3-(*N*-(benzyloxy)acetamido)-1-azidopropylphosphonate (20).** Hydrazoic acid solution **(Caution: $HN_3$ is volatile, highly toxic and explosive!):** Sodium azide (4000 mg, 61.5 mmol) was dissolved in water (10 mL). Toluene (50 mL) was added and the resulting biphasic system was cooled on ice to 0°C. Under vigorous stirring, concentrated sulfuric acid (8 mL) was added dropwise. After 30 min of stirring, the organic layer was separated and stored on anhydrous $Na_2SO_4$. Triphenylphosphine (5250 mg, 20 mmol) was dissolved in toluene (60 mL), cooled to 0°C and diisopropylazodicarboxylate (3.96 mL, 20 mmol) was added, followed by 10 mL of the freshly prepared hydrazoic acid stock solution. Then a solution of compound 6 (4880 mg, 10 mmol) in toluene (25 mL) was added and the icebath was removed. Stirring was continued for 2 hours during which the appearance of the reaction mixture shifted from turbid and yellow to clear and almost colourless. The reaction mixture was concentrated in vacuo, the residue was taken up in diethyl ether and triphenylphosphine oxide was crystallized out by addition of heptane and seeding with triphenylphosphine oxide. The resulting crude product was then purified by DCVC (0 to 100% ethyl acetate in hexanes) to yield 3865 mg (76 %) of **20** as an oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.69 - 1.93 (m, 1 H) 2.00 - 2.09 (m, 3 H) 2.15 (ddt, J=14.50, 7.18, 3.81, 3.81 Hz, 1 H) 3.54 (td, J=11.42, 3.22 Hz, 1 H) 3.63 - 3.91 (m, 2 H) 4.77 (s, 2 H) 4.92 - 5.19 (m, 4 H) 7.17 - 7.54 (m, 15 H); HRMS (ESI) m/z 509.1952 [(M+H)$^+$, calcd for $C_{26}H_{30}N_4O_5P^+$ 509.1948].

**Dibenzyl (3-(*N*-(benzyloxy)acetamido)-1-(1*H*-1,2,3-triazol-1-yl)propyl)phosphonate (21a).** Azide **20** (508 mg, 1.01 mmol) was dissolved in vinyl acetate (10 mL, 108 mmol) and the solution was heated in a microwave at 120° C for 6.5 hours, followed by removal of all volatiles in vacuo. The resulting crude was purified by DCVC (0 to 100% ethyl acetate in hexanes followed by 2% ethanol in ethyl

acetate) to yield 370 mg (69 %) of 2**1a** as an oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 2.01 (s, 3 H) 2.36 - 2.68 (m, 2 H) 3.27 - 3.67 (m, 2 H) 4.55 - 4.72 (m, 2 H) 4.72 - 5.14 (m, 5 H) 7.09 - 7.46 (m, 15 H) 7.58 - 7.80 (m, 2 H); HRMS (ESI) m/z 535.2098 [$(M+H)^+$, calcd for $C_{28}H_{32}N_4O_5P^+$ 535.2105].

**Dibenzyl (3-(*N*-(benzyloxy)acetamido)-1-(4-(tert-butyl)-1*H*-1,2,3-triazol-1-yl)propyl)phosphonate (21b).** To a solution of **20** (530 mg, 1.04 mmol) in DMF (10 mL) was added $CuSO_4$ (0.1 mL of a 0.1M aqueous stock solution, 0.01 mmol), sodium ascorbate (0.5 mL of a freshly prepared 0.1M aqueous stock solution, 0.05 mmol) and 3,3-dimethylbut-1-yne (2 mL, 1340 mg, 16.3 mmol). The reaction mixture was heated in a microwave oven for 30 minutes at 60° C. Assessment of the reaction mixture by TLC (25:75 toluene – ethyl acetate) showed the presence of unreacted azide, so another 0.5 mL of sodium ascorbate stock solution and 0.1 mL of $CuSO_4$ stock solution were added and the reaction mixture was irradiated for another 45 minutes at 70° C. At that point all azide was converted and the reaction mixture was concentrated, followed by purification of the resulting crude by DCVC (0 to 100% ethyl acetate in hexanes) to yield 594 mg (97 %) of 2**1b** as a colourless oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.16 - 1.45 (m, 9 H) 2.01 (s, 3 H) 2.37 - 2.68 (m, 2 H) 3.35 - 3.67 (m, 2 H) 4.57 - 4.74 (m, 2 H) 4.74 - 4.91 (m, 2 H) 4.91 - 5.11 (m, 3 H) 7.07 - 7.40 (m, 15 H) 7.44 (d, J=0.88 Hz, 1 H); HRMS (ESI) m/z 591.2731 [$(M+H)^+$, calcd for $C_{32}H_{40}N_4O_5P^+$ 591.2731].

**Dibenzyl (3-(*N*-(benzyloxy)acetamido)-1-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)phosphonate (21c).** To a solution of **20** (536 mg, 1.1 mmol) in DMF (10 mL) was added $CuSO_4$ (0.5 mL of a 0.1M aqueous stock solution, 0.05 mmol), sodium ascorbate (1 mL of a freshly prepared 0.1M aqueous stock solution, 0.1 mmol) and phenylacetylene (3 mL, 2790 mg, 27.3 mmol). The reaction mixture was heated in a microwave oven for 1 hour at 80° C, followed by removal of all volatiles in vacuo. The resulting crude was purified by DCVC (0 to 95% ethyl acetate in hexanes) to yield 610 mg (95 %) of **21c** as an oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.89 - 2.14 (m, 3 H) 2.41 - 2.73 (m, 2 H) 3.34 - 3.74 (m, 2 H) 4.51 - 4.74 (m, 2 H) 4.76 - 4.93 (m, 2 H) 4.93 - 5.16 (m, 3 H) 7.08 - 7.45 (m, 18 H) 7.69 -

7.83 (m, 2 H) 7.83 - 7.91 (m, 1 H); HRMS (ESI) m/z 611.2417 [$(M+H)^+$, calcd for $C_{34}H_{36}N_4O_5P^+$ 611.2418].

**3-(*N*-hydroxyacetamido)-1-(1*H*-1,2,3-triazol-1-yl)propylphosphonic acid (11a).** Prepared according to the same procedure as **10a** yielding a white solid (quant.). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 2.07 (s, 3 H), 2.22 - 2.49 (m, 1 H), 2.51 - 2.76 (m, 1 H), 3.18 - 3.43 (m, 2 H), 3.64 (dt, $J$ = 14.3, 7.4 Hz, 1 H), 4.55 - 4.91 (m, 1 H), 7.72 (s, 1 H), 8.08 (s, 1 H). $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 20.57, 29.98, 46.28 (d, $J$ = 12.7 Hz), 59.92 (d, $J$ = 136.6 Hz), 125.93, 134.15, 173.89. $^{31}$P NMR (121 MHz, $CD_3OD$) δ 11.88. HRMS (ESI): calcd for $C_7H_{13}N_4O_5P$ - $H^+$, 263.0551; found [$(M-H)^+$], 263.0589.

**3-(*N*-hydroxyacetamido)-1-(4-tert-butyl-1*H*-1,2,3-triazol-1-yl)propylphosphonic acid (11b).** To a solution of **21b** (84 mg, 0.14 mmol) in a mixture of methanol – water – tert-butanol (10 mL) was added 10% Pd/C. Hydrogen gas was bubbled through via a glass capillary at atmospheric pressure for 4 hours after which the reaction mixture was filtered and concentrated in vacuo. The residue was taken up in *tert*-butanol, frozen and lyophilized to give the product as a white foam in quantitative yield. $^1$H NMR (300 MHz, METHANOL-d4) d ppm 1.37 (s, 9 H) 2.06 (s, 3 H) 2.41 - 2.79 (m, 2 H) 3.35 - 3.51 (m, 1 H) 3.61 (dd, J=14.35, 7.32 Hz, 1 H) 4.76 - 4.99 (m, 1 H) 7.91 (s, 1 H); $^{13}$C NMR (75 MHz, METHANOL-d4) δ ppm 20.20, 28.34, 30.49, 31.73, 44.49 (d, J=12.72 Hz), 57.82 (d, J=148.17 Hz), 122.30, 157.58, 174.002; $^{31}$P NMR (121 MHz, METHANOL-d4) δ ppm 14.83; HRMS (ESI) m/z 319.1200 [$(M-H^+)$, calcd for $C_{11}H_{20}N_4O_5P^-$ 319.1177].

**3-(*N*-(benzyloxy)acetamido)-1-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propylphosphonic acid (11c).** Was prepared according to the same procedure as **10a** yielding a white solid (quant.). $^1$H NMR (300 MHz, METHANOL-d4) δ ppm 2.07 (s, 3 H) 2.32 - 2.56 (m, 1 H) 2.56 - 2.81 (m, 1 H) 3.33 - 3.52 (m, 1 H) 3.72 (dt, J=14.06, 7.32 Hz, 1 H) 4.59 - 4.86 (m, 1 H) 7.23 - 7.51 (m, 3 H) 7.73 - 7.95 (m, 2 H) 8.39 (s, 1 H); $^{31}$P NMR (121 MHz, METHANOL-d4) δ ppm 12.27; HRMS (ESI) m/z 339.0839 [$(M-H^+)$, calcd for $C_{13}H_{16}N_4O_5P^-$ 339.0864].

**Diethyl 3-(*N*-(benzyloxy)acetamido)-1-hydroxypropylphosphonate (22).** Diethyl phosphite (684 mg, 4.95 mmol) was dissolved in THF (5 mL), the solution was cooled to -78°C and LiHMDS (4.5 mL of a 1M solution in THF) was slowly added. After 30 minutes a solution of aldehyde **14** (996 mg, 4.5 mmol) in 10 mL dry THF was added via syringe. After 20 minutes of stirring at -78°C the reaction was quenched by the addition of saturated aqueous $NH_4Cl$ and extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The resulting crude mixture was purified by DCVC (0 to 100 % acetone in toluene continued by 0 to 20 % methanol in acetone) to yield 1200 mg (74 %) of 16 as an oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.26 - 1.41 (m, 6 H) 1.81 - 2.04 (m, 1 H) 2.09 (s, 3 H) 2.10 - 2.25 (m, 1 H) 3.70 (dt, J=14.64, 4.83 Hz, 1 H) 3.86 (dddd, J=11.35, 8.57, 5.86, 2.93 Hz, 1 H) 3.94 - 4.28 (m, 5 H) 4.48 - 4.73 (m, 1 H) 4.74 - 4.96 (m, 2 H) 7.38 (s, 5 H); HRMS (ESI) m/z 360.1578 [$(M+H)^+$, calcd for $C_{16}H_{27}NO_6P^+$ 360.1571].

**Diethyl 3-(*N*-(benzyloxy)acetamido)-1-azidopropylphosphonate (23).** Hydrazoic acid solution **(Caution: HN3 is volatile, highly toxic and explosive!):** Sodium azide (4000 mg, 61.5 mmol) was dissolved in water (10 mL). Toluene (50 mL) was added and the resulting biphasic system was cooled on ice to 0°C. Under vigorous stirring, concentrated sulfuric acid (8 mL) was added dropwise. After 30 min of stiring, the organic layer was separated and stored on anhydrous $Na_2SO_4$. Triphenylphosphine (525 mg, 2.0 mmol) was dissolved in toluene (5 mL), cooled to 0°C and diisopropylazodicarboxylate (0.40 mL, 2.0 mmol) was added, followed by 5 mL of the freshly prepared hydrazoic acid stock solution, resulting in the formation of copious yellow precipitate. Then a solution of compound **22** (360 mg, 1.0 mmol) in toluene (5 mL) was added and the icebath was removed. Stirring was continued for 4 hours during which the appearance of the reaction mixture shifted to clear and almost colourless. The reaction mixture was concentrated in vacuo, the residue was taken up in diethyl ether and triphenylphosphine oxide was crystallized out by addition of heptane and seeding with triphenylphosphine oxide. The resulting crude product was then purified by DCVC (25 to 100 % ethyl

acetate in toluene continued by 0 to 10 % methanol in ethyl acetate) to yield 336 mg (88 %) of **23** as an oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.34 (td, J=7.10, 0.73 Hz, 6 H) 1.71 - 1.95 (m, 1 H) 2.10 (s, 3 H) 2.12 - 2.32 (m, 1 H) 3.56 (td, J=11.50, 3.37 Hz, 1 H) 3.68 - 3.95 (m, 2 H) 4.08 - 4.29 (m, 4 H) 4.84 (s, 2 H) 7.38 (s, 5 H); HRMS (ESI) m/z 385.1628 [$(M+H)^+$, calcd for $C_{16}H_{26}N_4O_5P^+$ 385.1635].

**Diethyl 3-(*N*-hydroxyacetamido)-1-azidopropylphosphonate (24).** A solution of **23** (334 mg, 0.87 mmol) in dichloromethane (8 mL) was cooled to -75°C and $BCl_3$ (2.6 mL of a 1M solution in dichloromethane, 2.6 mmol) was added dropwise. After 45 minutes of stirring at -75°C the reaction mixture was poured into aqueous $NaHCO_3$ and extracted 4 times with dichloromethane. The combined organic fractions were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The resulting crude mixture was purified by DCVC on a silica column that was previously 'deactivated' by rinsing with 5% triethylamine in hexanes (0 to 20 % methanol in ethyl acetate containing 1% triethylamine) to yield 201 mg (79 %) of **24** as an oil. $^1$H NMR (300 MHz, METHANOL-d4) δ ppm 1.22 - 1.49 (m, 6 H) 1.62 - 1.88 (m, 1 H) 1.98 - 2.25 (m, 4 H) 3.60 - 3.99 (m, 3 H) 4.08 - 4.32 (m, 5 H); HRMS (ESI) m/z 295.1169 [$(M+H)^+$, calcd for $C_9H_{20}N_4O_5P^+$ 295.1166].

***tert*-Butyl 3-(*N*-(benzyloxy)acetamido)-1-tosylpropylcarbamate (25).** A mixture of aldehyde **14** (1106 mg, 5 mmol), tert-butyl carbamate (650 mg, 5.5 mmol), anhydrous sodium *p*-toluenesulfinate (980 mg, 5.5 mmol), water (5 ml), THF (2 ml), acetonitrile (5 mL) and formic acid (540 μL, 14 mmol) was stirred overnight at room temperature, forming a clear solution. As the reaction was not finished at this point according to TLC (25:75 toluene – ethyl acetate), the reaction mixture was stirred for another 6 hours at 50°C. After cooling to room temperature, aqueous $NaHCO_3$ was added and the mixture was extracted three times with diethyl ether, the combined organic phases were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The resulting crude mixture was purified by DCVC (0 to 50 % ethyl acetate in hexanes) to yield 1920 mg (81 %) of **25**. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.10 - 1.32 (m, 9 H) 1.88 - 2.22 (m, 4 H) 2.29 - 2.46 (m, 3 H) 2.46 - 2.69 (m, 1 H) 3.66 - 3.96 (m, 2 H) 4.83 (s, 2 H) 4.90 (td, J=10.54, 4.10 Hz, 1 H) 5.41 (d, J=10.54 Hz, 1 H) 7.18 - 7.35 (m, 2 H)

7.39 (s, 5 H) 7.61 - 7.88 (m, 2 H); HRMS (ESI) m/z 477.2058 [$(M+H)^+$, calcd for $C_{24}H_{33}N_2O_6S^+$ 477.2054].

***tert*-Butyl 1-((benzyloxy)phosphono)-3-(*N*-(benzyloxy)acetamido)propylcarbamate (26).** To a solution of **25** (1920 mg, 4.03 mmol) in THF (15 mL) was added NaH (360 mg of a 60% dispersion in mineral oil, 8.06 mmol) in one batch while stirring at room temperature, resulting in a grey suspension. After 15 minutes, a solution of dibenzyl phosphite (1160 mg, 4.42 mmol) in THF (5 mL) was added dropwise and the reaction mixture was stirred for 1 hour at room temperature. The reaction mixture was then cooled to 0°C, quenched with saturated aqueous $NH_4Cl$ and extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The resulting crude mixture was purified by DCVC (50 to 100 % ethyl acetate in hexanes) to yield 2020 mg (86 %) of **26** as an oil. 1H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.24 - 1.52 (m, 9 H) 1.84 (dt, J=9.15, 4.65 Hz, 1 H) 2.04 (s, 3 H) 2.24 (d, J=8.20 Hz, 1 H) 3.54 - 3.89 (m, 2 H) 4.05 - 4.35 (m, 1 H) 4.68 - 4.82 (m, 2 H) 4.87 (d, J=10.25 Hz, 1 H) 4.92 - 5.12 (m, 4 H) 7.11 - 7.51 (m, 15 H); HRMS (ESI) m/z 583.2568 [$(M+H)^+$, calcd for $C_{31}H_{40}N_2O_7P^+$ 583.2568].

**Dibenzyl 3-(*N*-(benzyloxy)acetamido)-1-aminopropylphosphonate (12).** A solution of **26** (1660 mg, 2.85 mmol) in dichloromethane (30 mL) was cooled to 0°C and TFA (11 mL, 142 mmol) was added dropwise. After 20 minutes of stirring at 0°C the reaction mixture was poured into aqueous $K_2CO_3$ and extracted 4 times with chloroform. The combined organic fractions were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo which resulted in 1350 mg (98 %) of crude **12** that was used as such in the following reactions. HRMS (ESI) m/z 483.2050 [$(M+H)^+$, calcd for $C_{26}H_{32}N_2O_5P^+$ 483.2043]

**Dibenzyl 3-(*N*-(benzyloxy)acetamido)-1-(benzamido)propylphosphonate (27a).** To a solution of **12** (241 mg, 0.5 mmol) in dichloromethane (2.5 mL) was added triethylamine (140 µL, 1 mmol), DMAP (6 mg, 0.05 mmol) and benzoylchloride (87µL, 0.75 mmol). The reaction mixture was stirred at room temperature for 3.5 hours, then poured into aqueous 0.2N HCl and extracted three times with ethyl

acetate. The combined organic phases were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The resulting crude mixture was purified by DCVC (50 to 100 % ethyl acetate in hexanes continued by 0 to 10 % ethanol in ethyl acetate) to yield 261 mg (89 %) of **27a** as an oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.87 (s, 3 H) 1.96 - 2.19 (m, 1 H) 2.21 - 2.44 (m, 1 H) 3.83 (t, J=6.88 Hz, 2 H) 4.43 - 4.62 (m, 2 H) 4.62 - 4.80 (m, 1 H) 4.86 - 5.10 (m, 4 H) 6.98 (d, J=6.74 Hz, 2 H) 7.07 - 7.47 (m, 16 H) 7.50 - 7.65 (m, 2 H); HRMS (ESI) m/z 587.2311 [$(M+H)^+$, calcd for $C_{33}H_{36}N_2O_6P^+$ 587.2305]

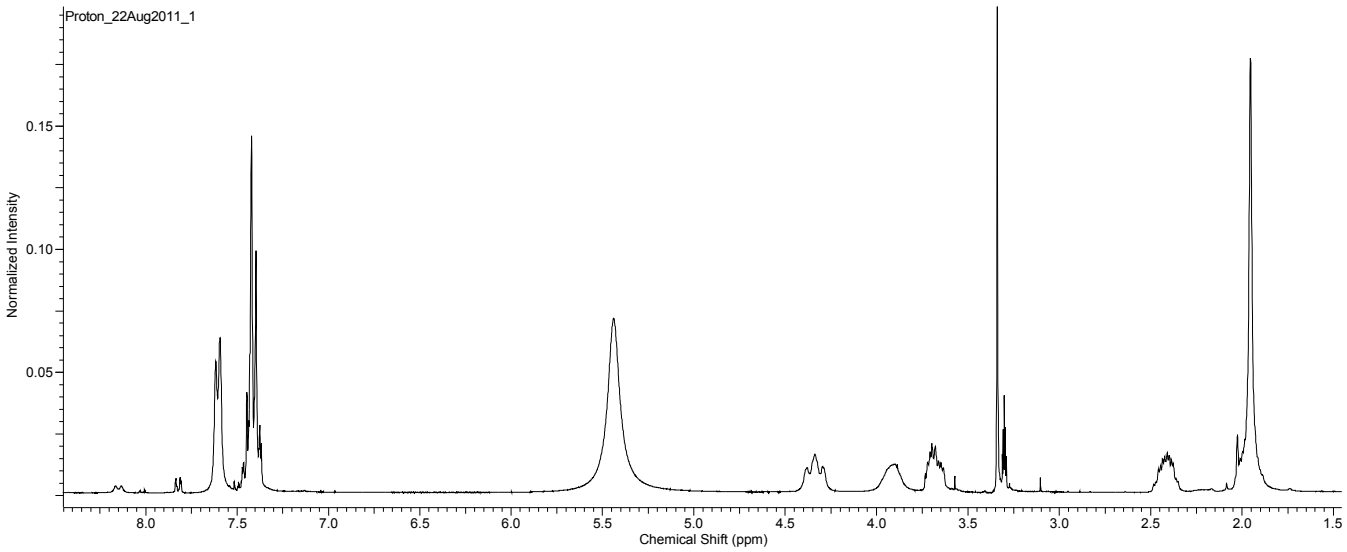
**Dibenzyl 3-(*N*-(benzyloxy)acetamido)-1-(3-phenylureido)propylphosphonate (27b).** To a solution of **12** (241 mg, 0.5 mmol) in dichloromethane (2.5 mL) was added triethylamine (70 μL, 0.5 mmol), DMAP (6 mg, 0.05 mmol) and phenylisocyanate (65μL, 0.6 mmol). The reaction mixture was stirred at room temperature for 4 hours, then poured into aqueous 0.2N HCl and extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The resulting crude mixture was purified by DCVC (50 to 100 % ethyl acetate in hexanes continued by 0 to 10 % ethanol in ethyl acetate) to yield 291 mg (97 %) of **27b** as an oil. $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 1.80 - 2.01 (m, 4 H) 2.27 (br. s., 1 H) 3.46 - 3.85 (m, 2 H) 4.51 - 4.72 (m, 1 H) 4.72 - 4.87 (m, 2 H) 4.89 - 5.12 (m, 4 H) 6.73 (d, J=9.67 Hz, 1 H) 6.90 - 7.09 (m, 1 H) 7.13 - 7.43 (m, 19 H) 7.49 (s, 1 H); HRMS (ESI) m/z 602.2410 [$(M+H)^+$, calcd for $C_{33}H_{37}N_3O_6P^+$ 602.414]

**3-(*N*-hydroxyacetamido)-1-(3-phenylureido)propyl-phosphonic acid (8b).** To a solution of **27b** (321 mg, 0.53 mmol) in a mixture of MeOH–$H_2O$–*t*-BuOH (10 mL) was added 10% Pd/C. Hydrogen gas was bubbled through via a glass capillary at atmospheric pressure for 3 h after which the reaction mixture was filtered and concentrated in vacuo. The residue was taken up in a mixture of water and *t*-BuOH, frozen and lyophilized to give the product as a white foam in quantitative yield. $^1$H NMR (300 MHz, DMSO-d6) $\delta$ 1.58 - 1.82 (m, 1 H), 1.82 - 1.91 (m, 3 H), 1.96 - 2.17 (m, 1 H), 3.29 - 3.58 (m, 2 H), 4.04 (ddd, $J$ = 16.7, 10.8, 3.8 Hz, 1 H), 6.82 - 7.06 (m, 1 H), 7.14 - 7.34 (m, 2 H), 7.50 - 7.72 (m, 2

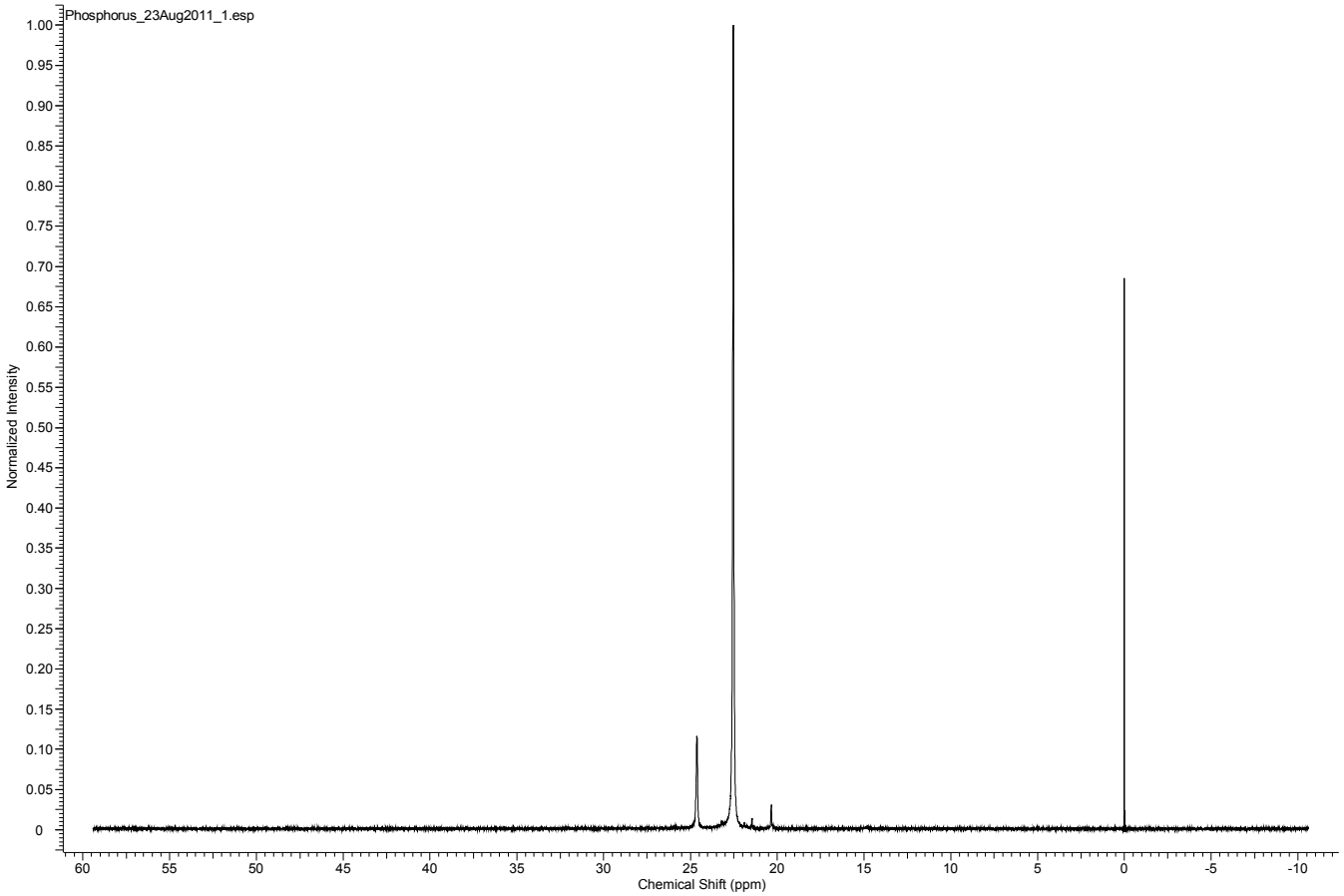
H). $^{13}$C NMR (75 MHz, DMSO-d6) $\delta$ 22.51, 22.56, 27.10, 44.25 (d, $J$ = 153.70 Hz), 47.52 (d, $J$ = 14.9 Hz), 119.19, 119.30, 122.16, 128.39, 139.37, 157.39, 169.03. $^{31}$P NMR (121 MHz, DMSO-d6) $\delta$ 21.12. HRMS (ESI): calcd for $C_{12}H_{18}N_3O_6P$ - $H^+$, 330.0860; found [$(M-H)^+$], 330.0901.

## $^{1}$H NMR and $^{31}$P spectra of final products

$^{1}$H NMR spectrum of structure **8a**




$^{31}$P NMR spectrum of structure **8a**



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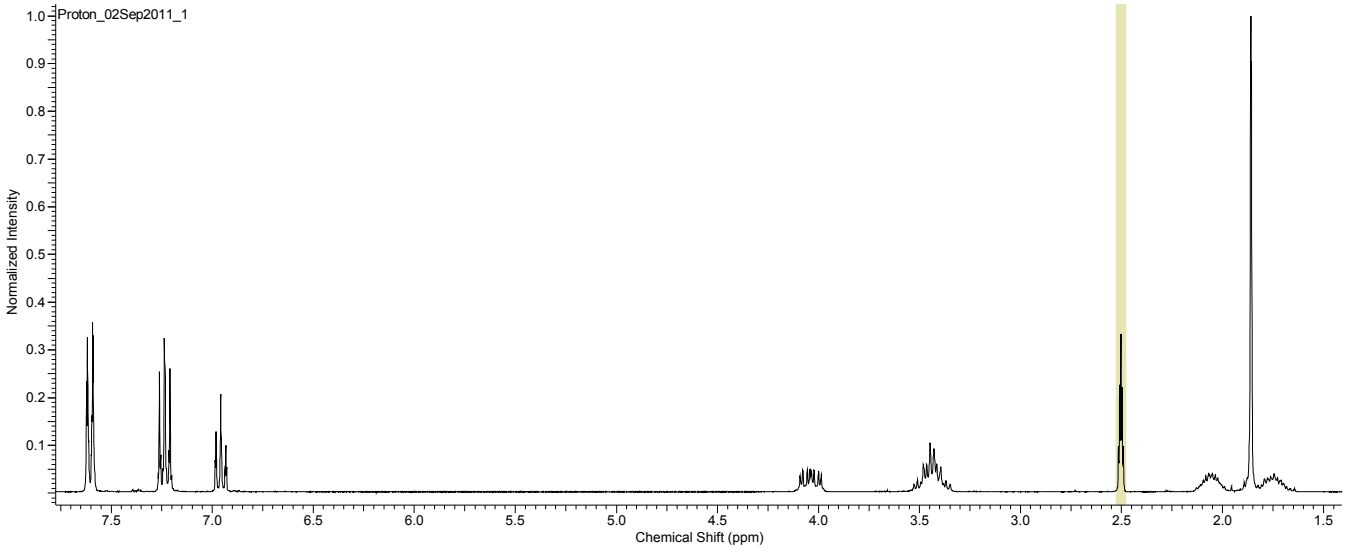


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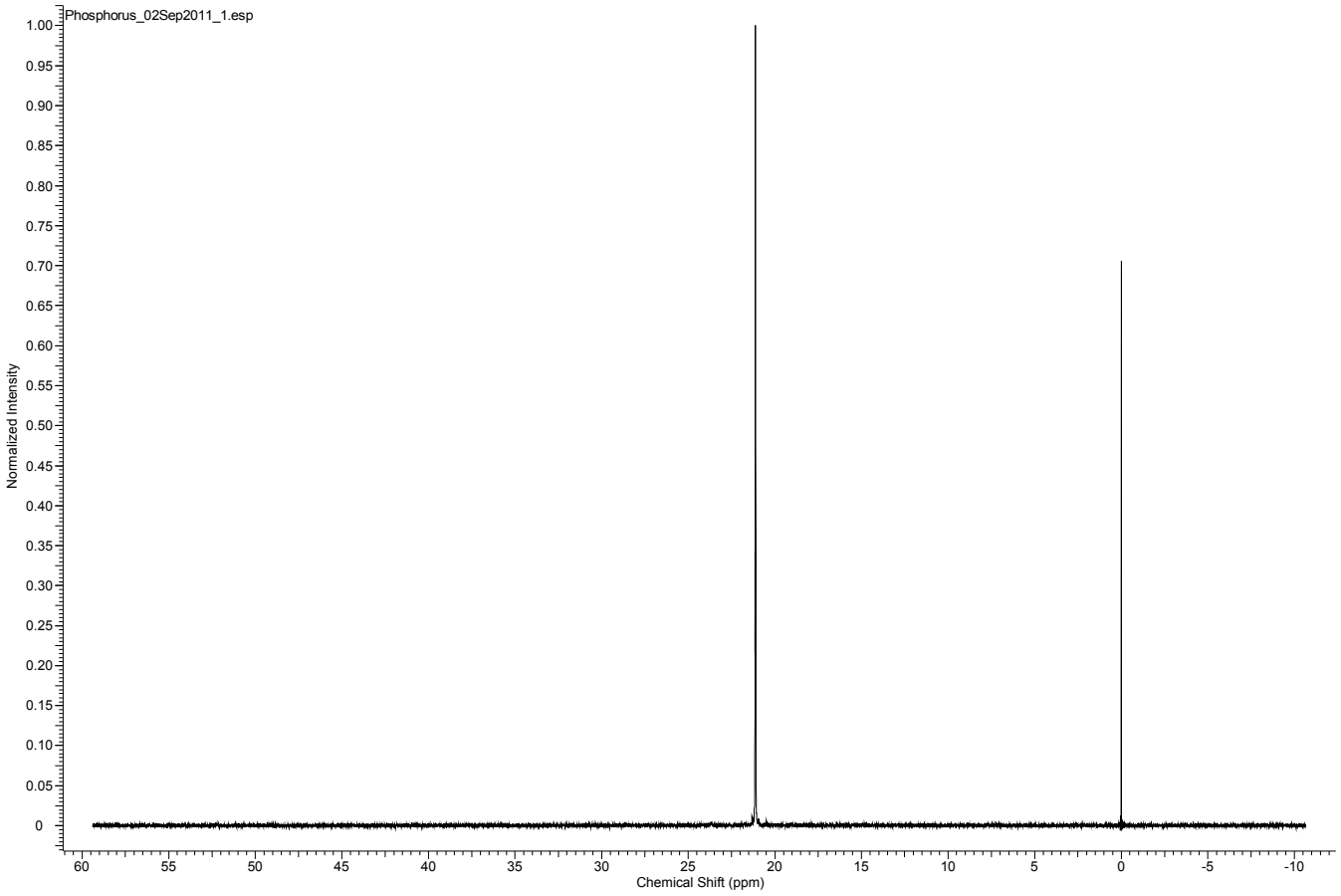
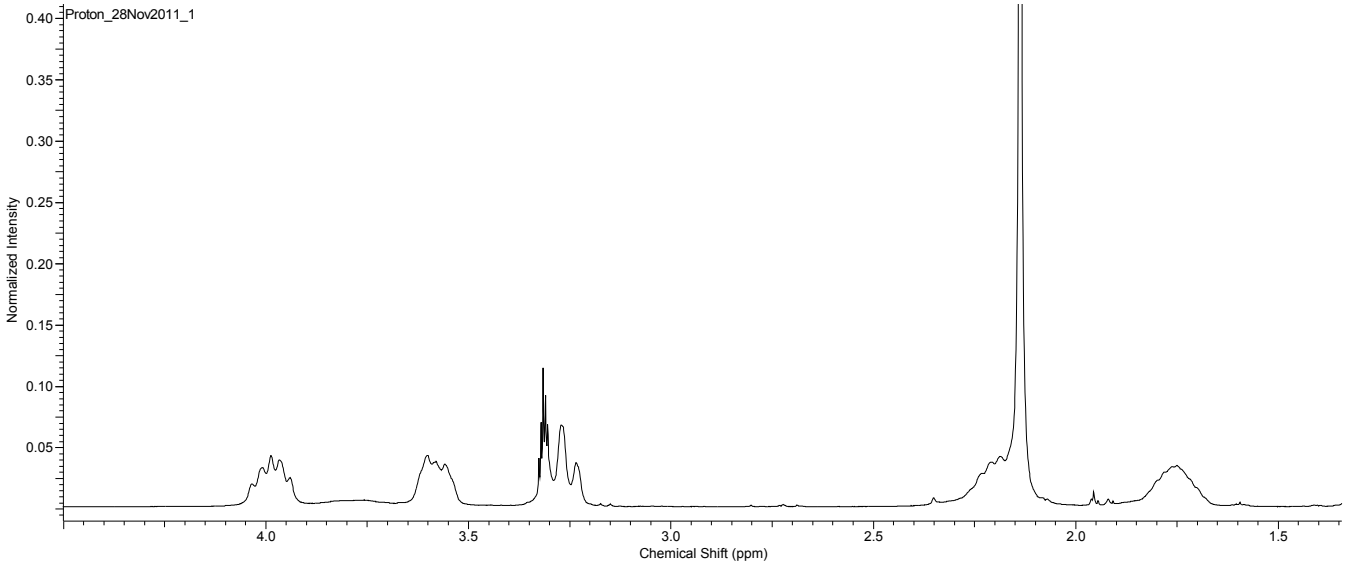
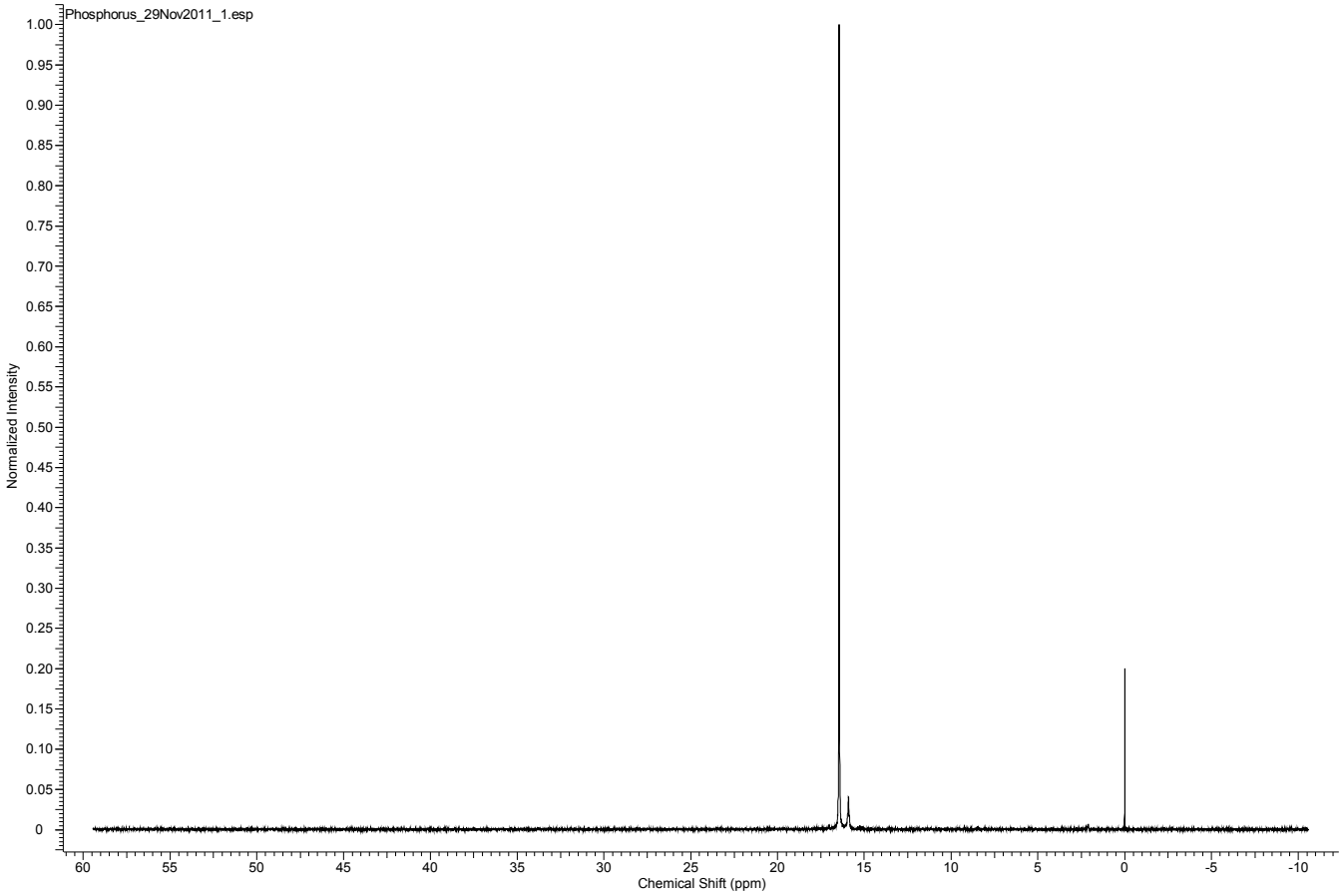


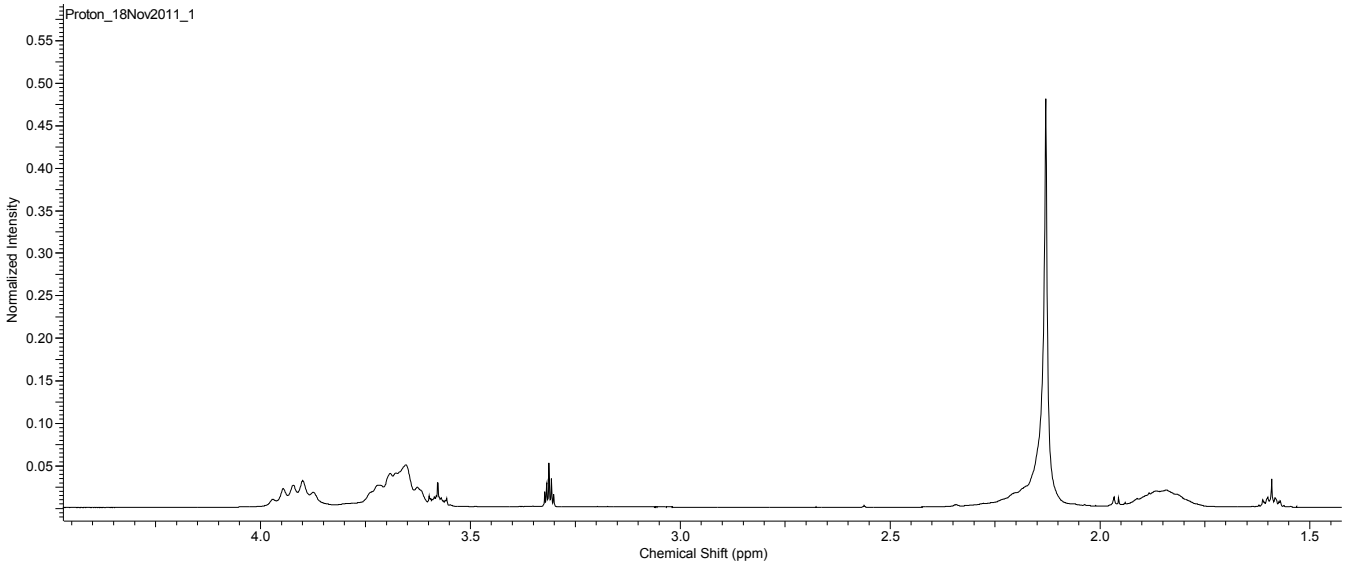
$^{1}$H NMR spectrum of structure **9**



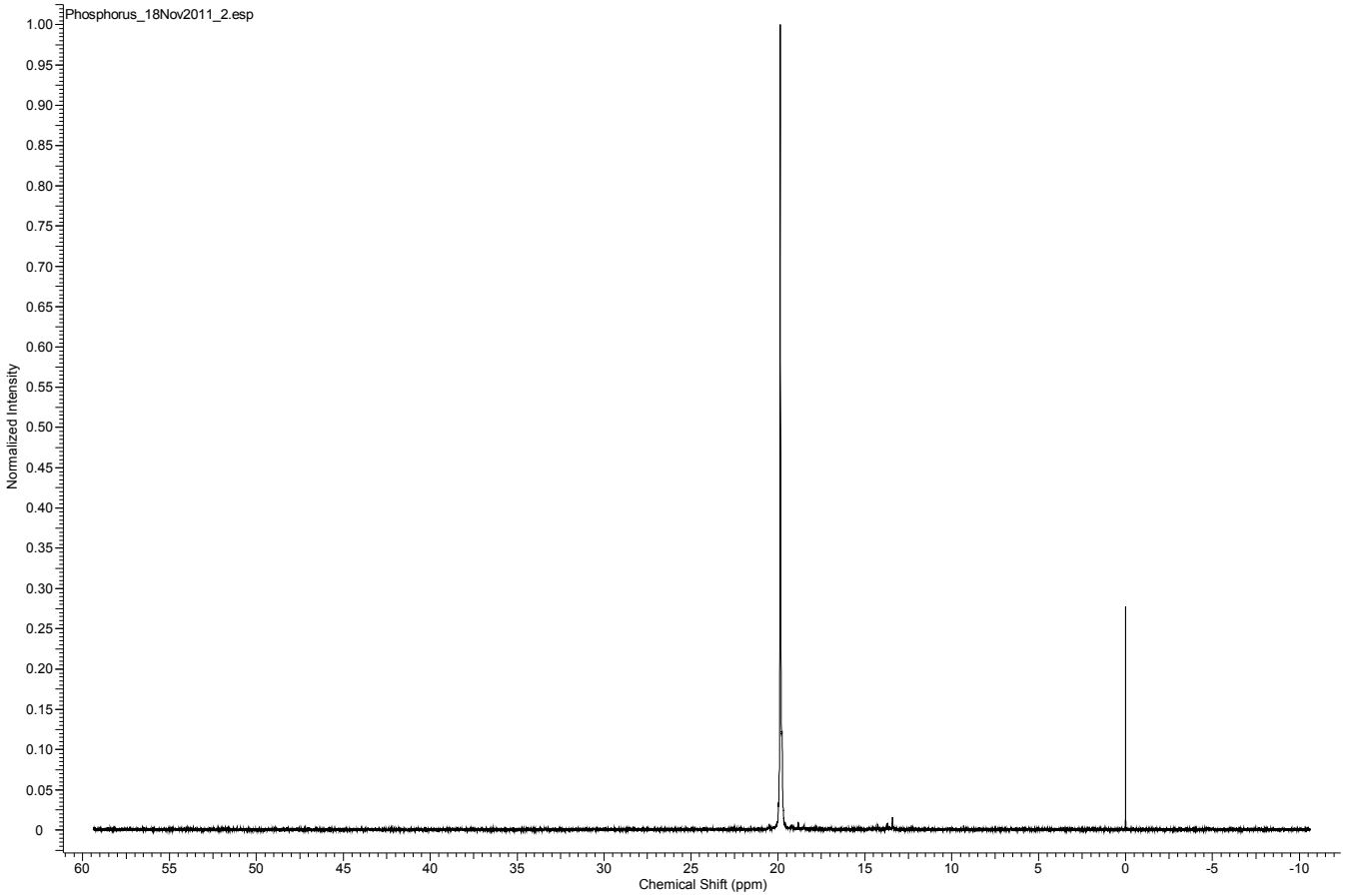

$^{31}$P NMR spectrum of structure **9**



$^{1}$H NMR spectrum of structure **10a**




$^{31}$P NMR spectrum of structure **10a**



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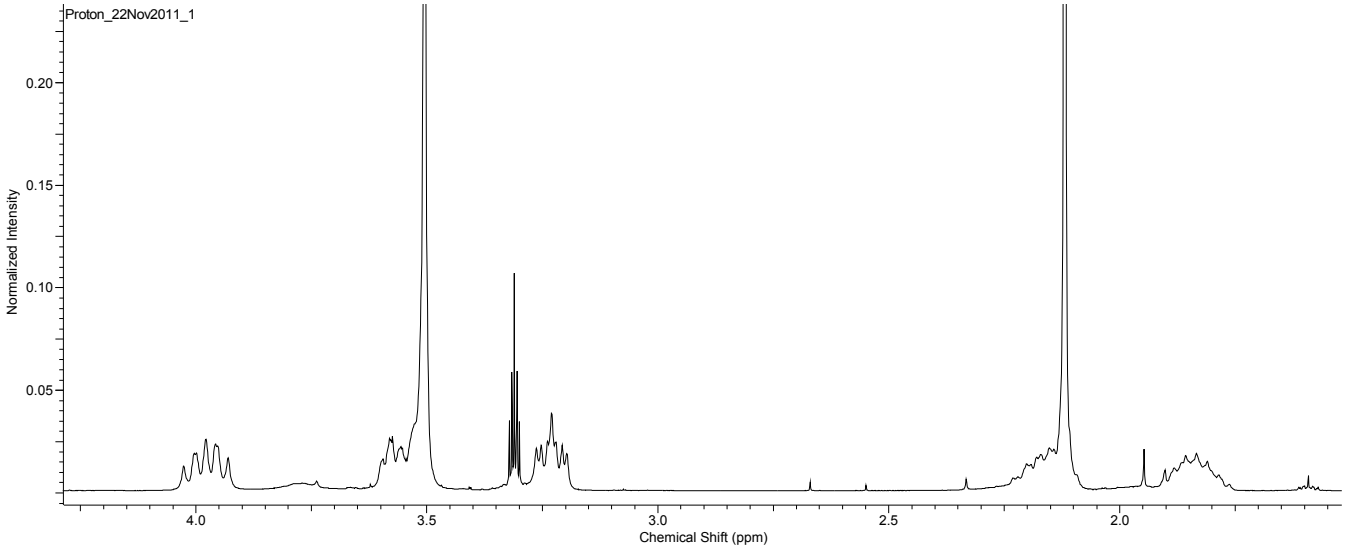


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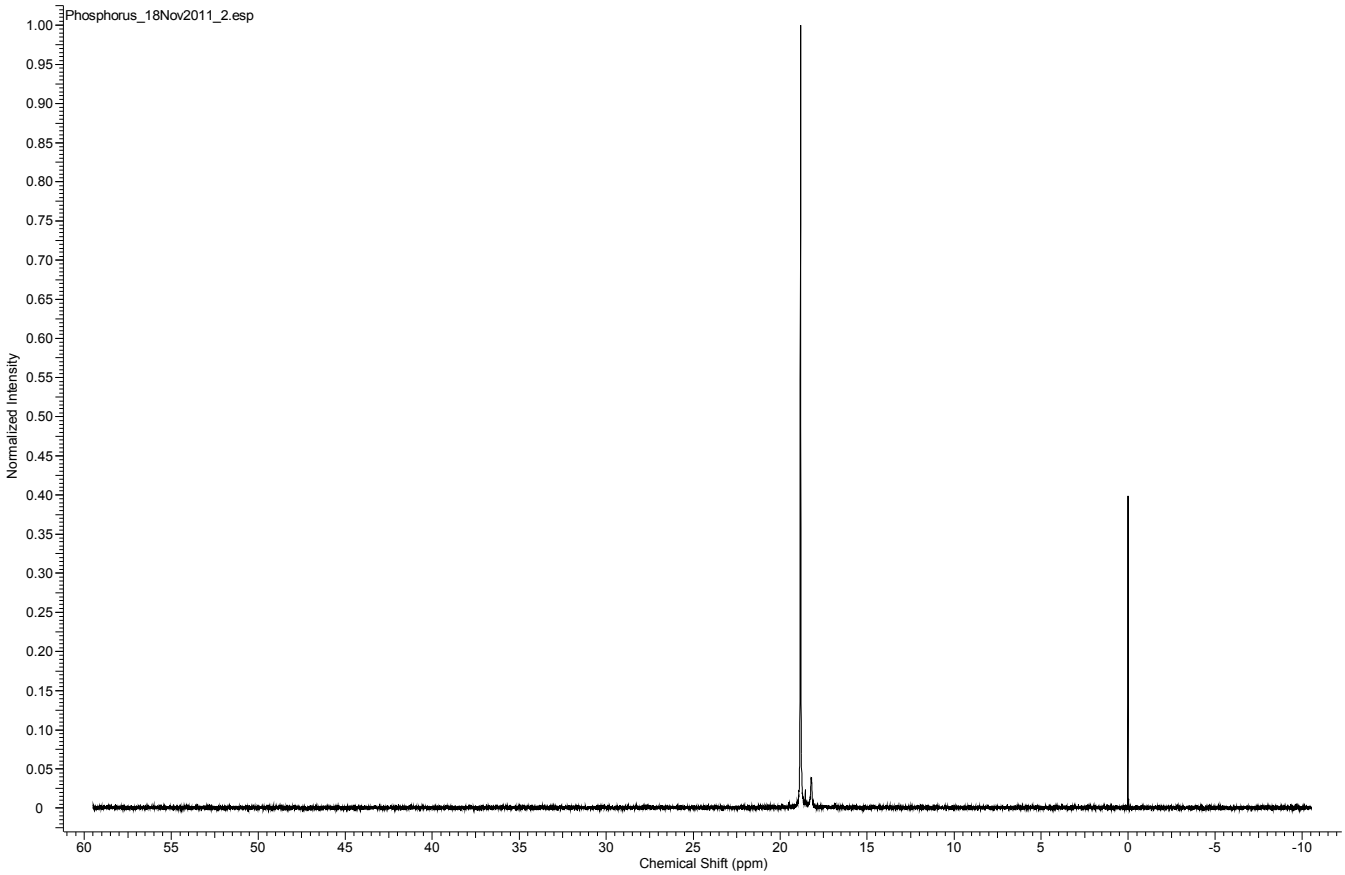
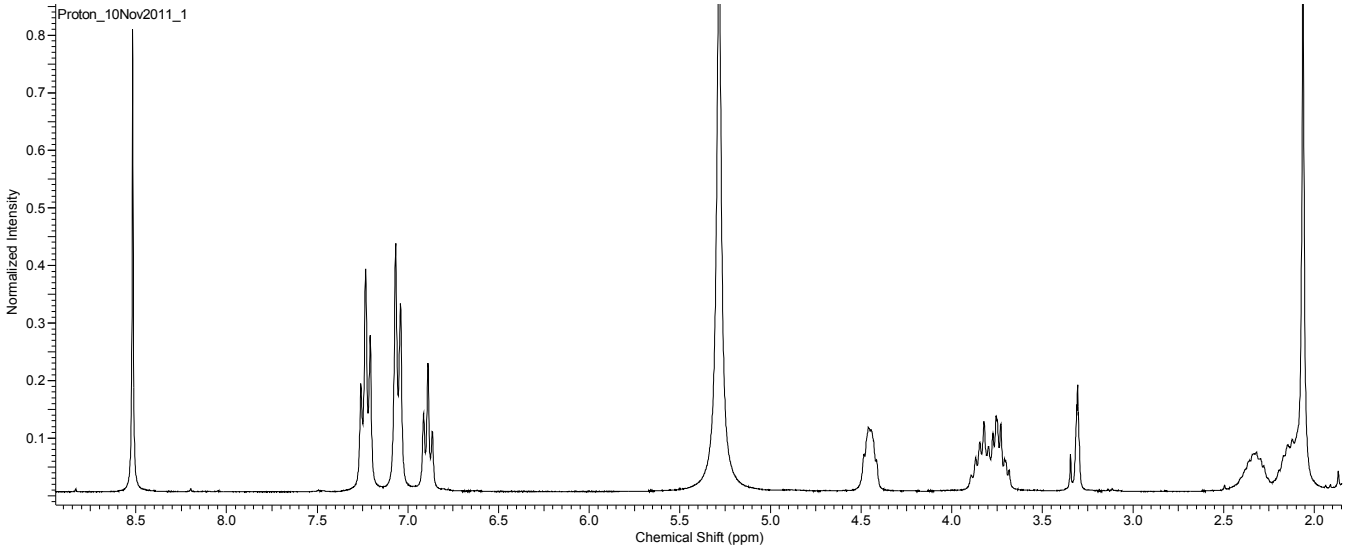


$^{1}$H NMR spectrum of structure **10c**

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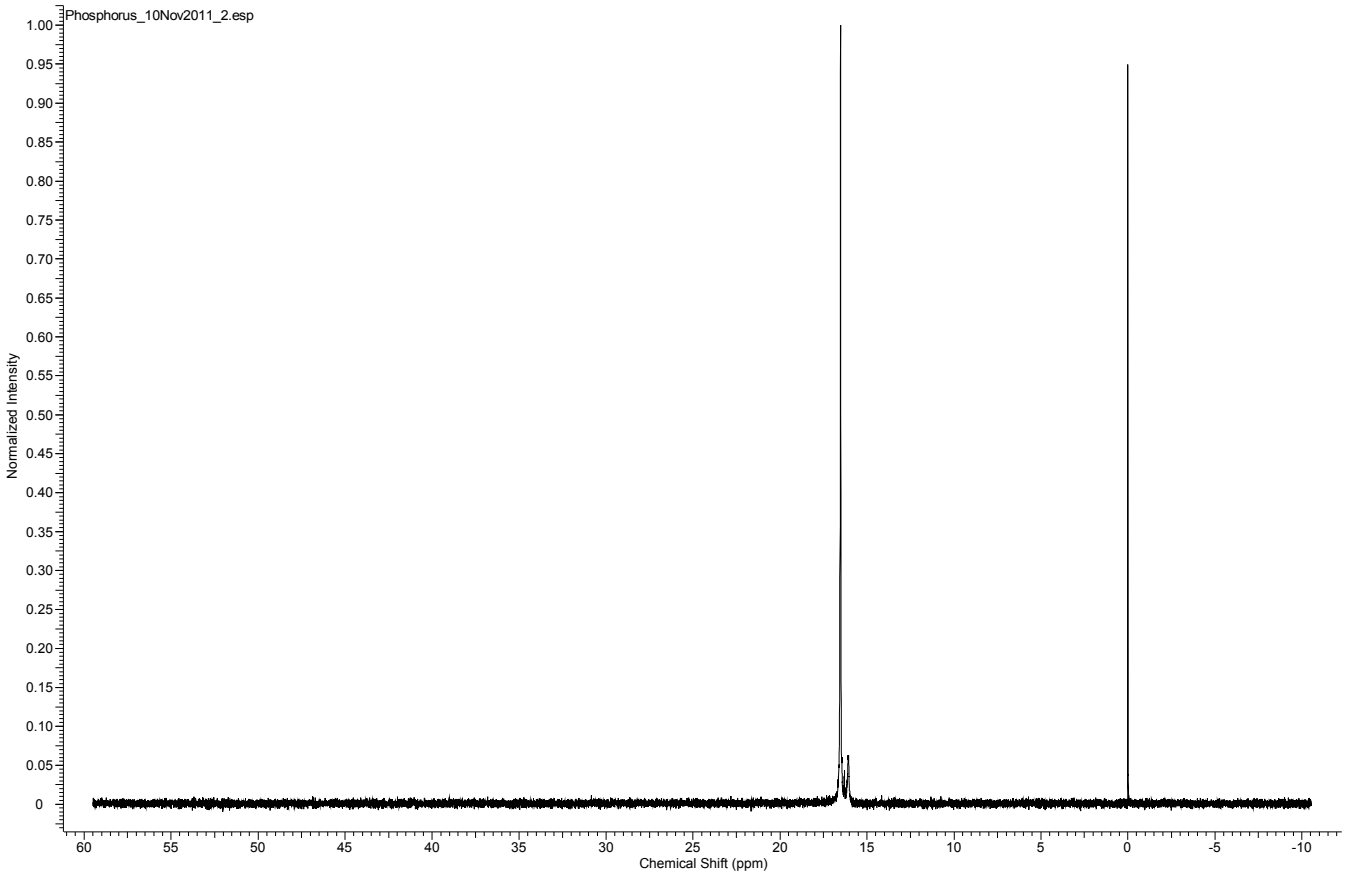
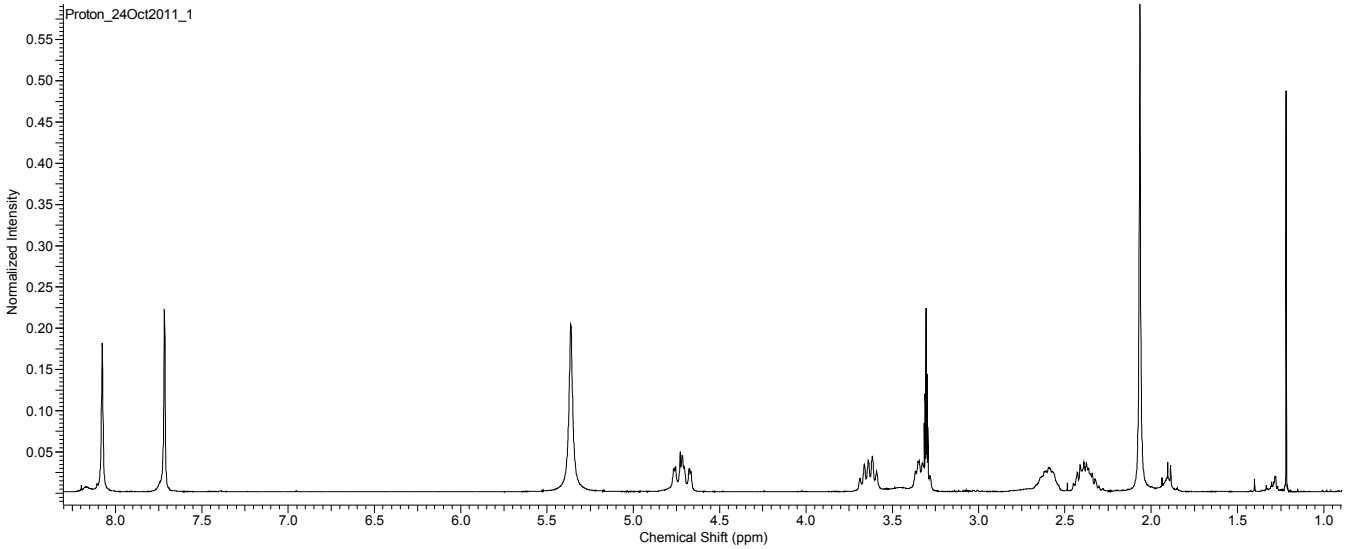
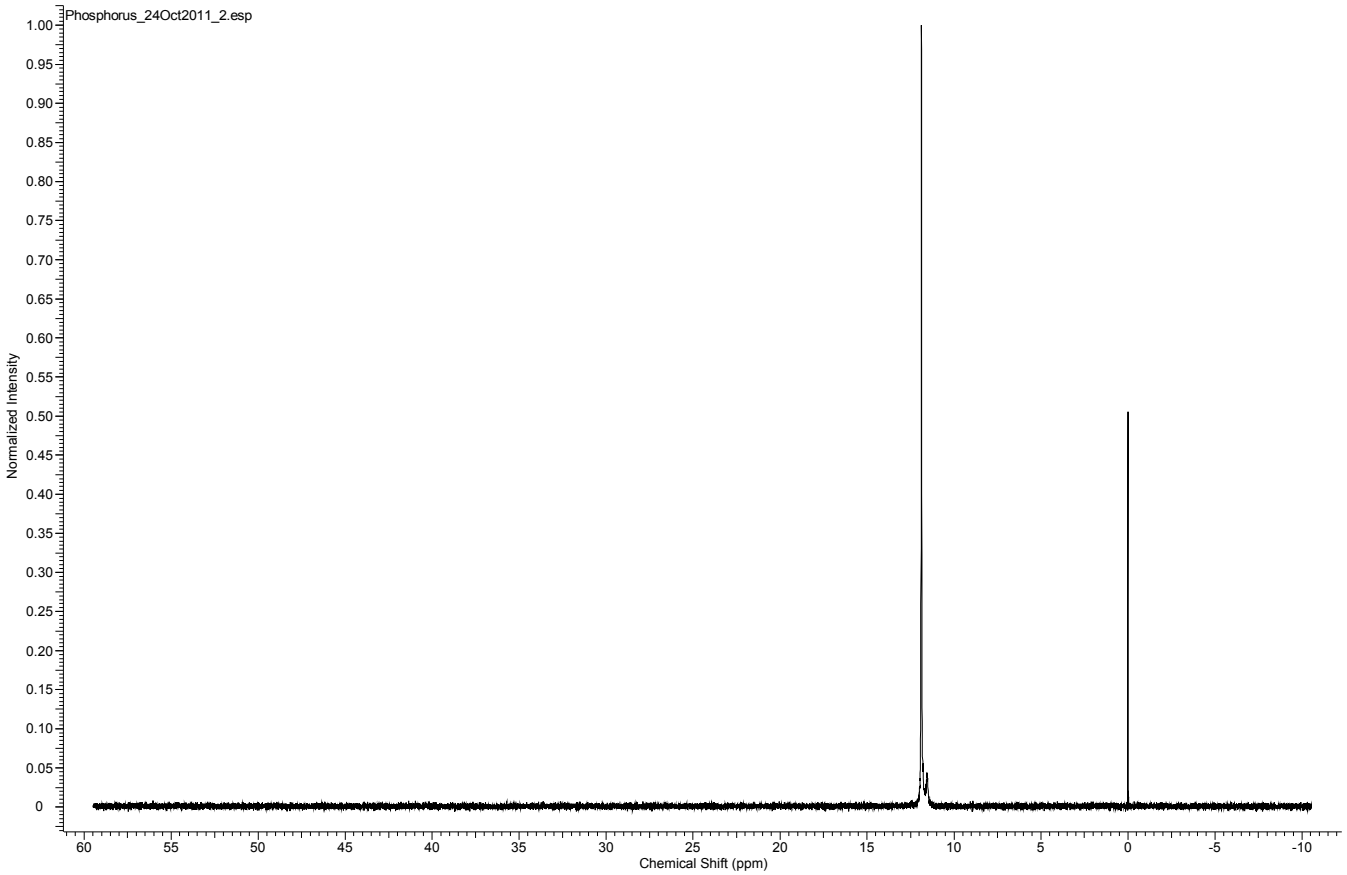


$^{1}$H NMR spectrum of structure **11a**




$^{31}$P NMR spectrum of structure **11a**



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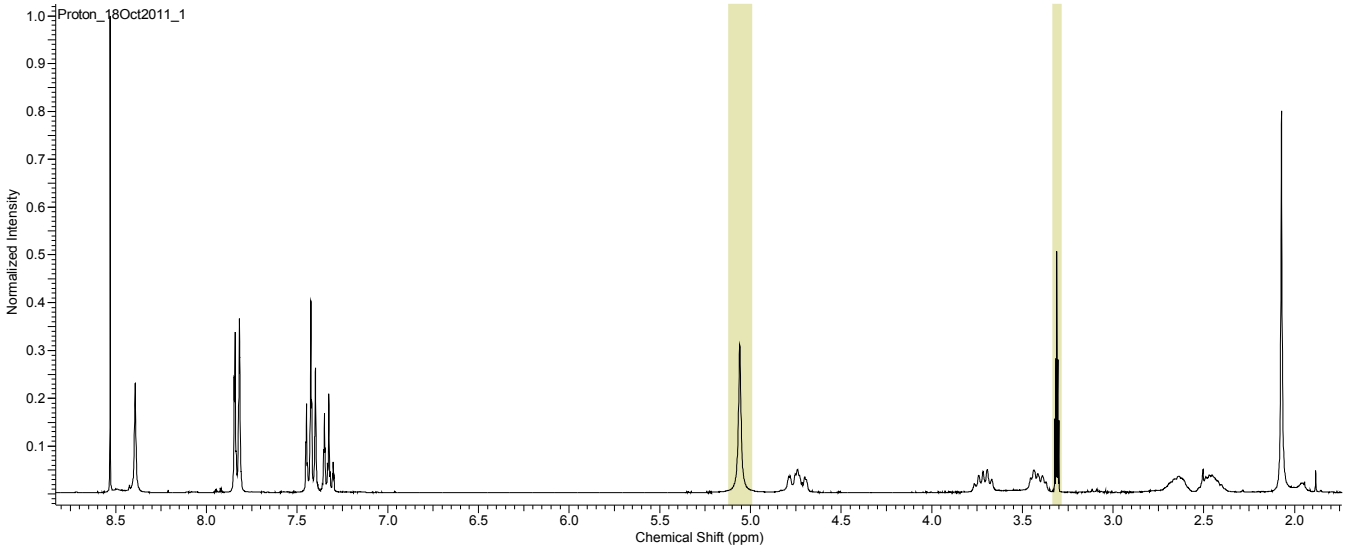


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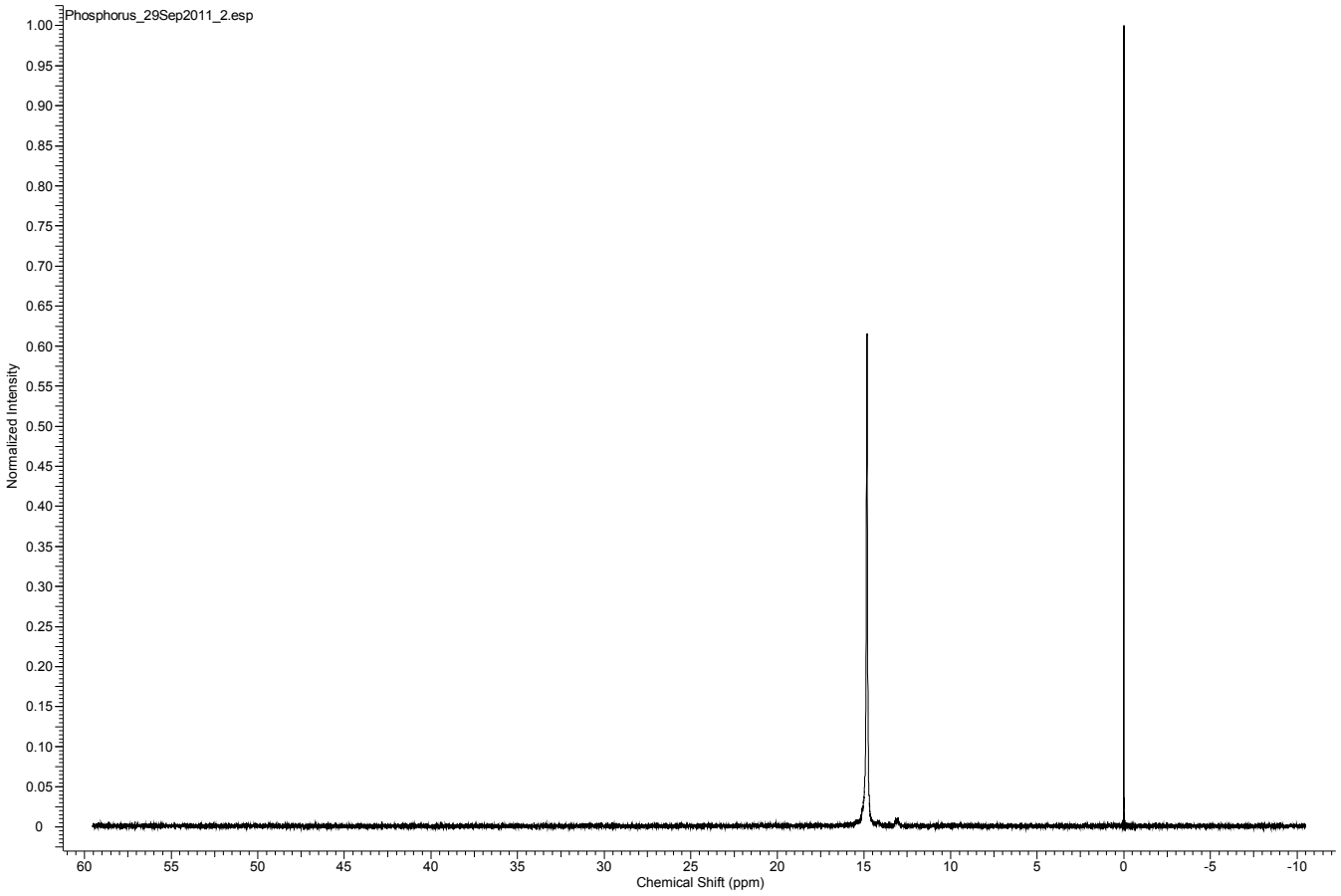
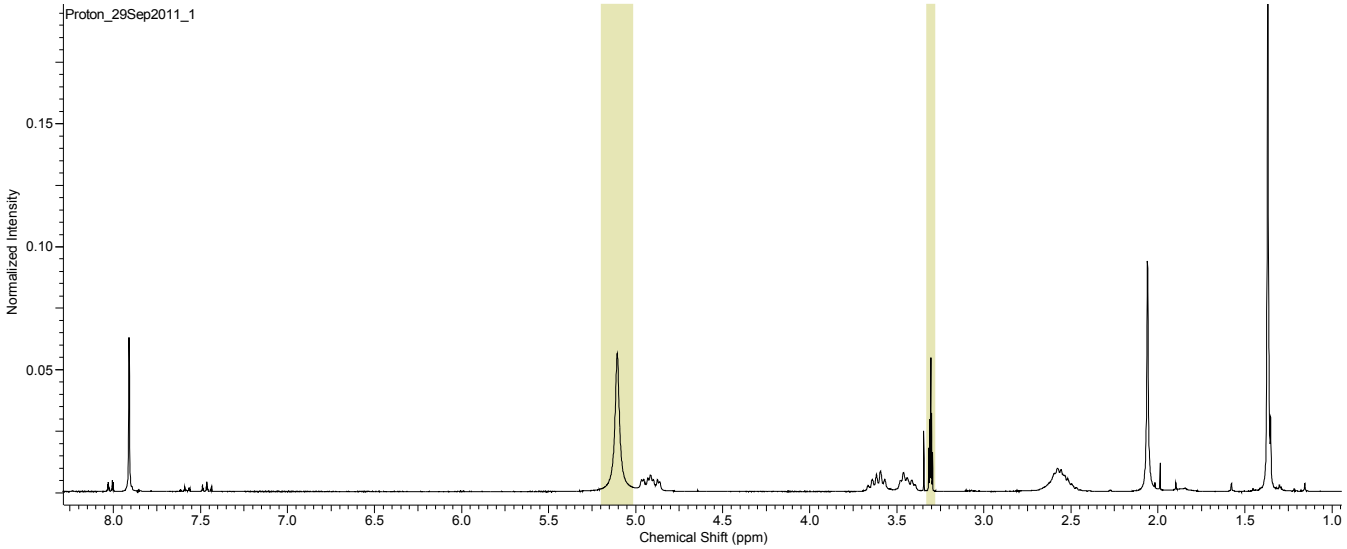


$^{1}$H NMR spectrum of structure **11c**




$^{31}$P NMR spectrum of structure **11c**