SUPPORTING INFORMATIONS

Applying prodrug strategy to α-phosphonocarboxylate inhibitors of Rab GGTase - synthesis and stability studies

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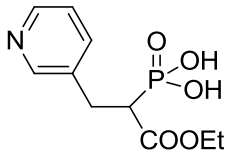
General procedure for the synthesis of monoesters 18 and 21

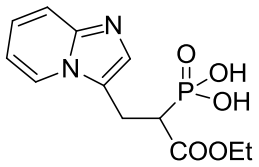
A one-neck flask equipped with a magnetic stir bar was purged with argon and then appropriate triester (1 eq) was placed. After cooling to -5°C, bromotrimethylsilane (0.8 ml/1 mmol of substrate, 6 eq) was slowly added and resulting mixture was stirred for 4 hours (in case of triester bearing imidazo[1,2-a]pyridine ring addition of DCM (1 ml/1 mmol of substrate) was required after 1 hour). Then the volatile material was evaporated under reduced pressure and EtOH (20 ml/1 mmol of substrate), H_2O (2 drops/1 mmol of substrate) were added and stirring was continued for 2 hours. After careful evaporation of the solvent, obtained semi-solid was used in the next step without further purification. In case of synthesis compound **21** reaction could be carried out in presence of TEA (1 eq) used as scavenger of HBr formed in the reaction or present in BTMS.

(1-Ethoxy-1-oxo-3-(pyridin-3-yl)propan-2-yl)phosphonic acid (**18a**). Quantitative yield. **Scale**: 1.05 g of **18a**.



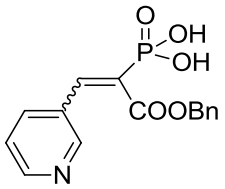
^{31}P NMR (100 MHz, D_2O, pH-7): δ 14.18. **^{1}H NMR** (250 MHz, D_2O): δ 1.04 (t, *J* = 7.1 Hz, $C\underline{H}_3CH_2$, 3H), 3.25-3.42 (m, $C\underline{H}_2CHP$, $CH_2C\underline{H}P$, 3H), 4.07 (dq, *J* = 7.1 and 1.6 Hz, $CH_3C\underline{H}_2$, 2H), 7.98 (dd, *J* = 8.2, 5.8 Hz, $C\underline{H}_{ar\ (Py\text{-}5)}$, 1H), 8.51 (bdt, *J* = 8.2, 1.5 Hz, $C\underline{H}_{ar\ (Py\text{-}4)}$, 1H), 8.62 (dd, *J* = 5.8, 0.8 Hz, $C\underline{H}_{ar\ (Py\text{-}6)}$, 1H), 8.70 (s, $C\underline{H}_{ar\ (Py\text{-}2)}$, 1H). **^{13}C NMR** (63 MHz, D_2O): δ 12.91 (s, $\underline{C}H_3CH_2$, 1C), 29.32 (s, $C\underline{H}_2CHP$, 1C), 47.25 (d, *J* = 119.5, $CH_2\underline{C}HP$, 1C), 62.29 (s, $CH_3\underline{C}H_2$, 1C), 126.88 (s, $\underline{C}H_{ar\ (Py\text{-}5)}$, 1C), 139.13 (s, $\underline{C}H_{ar\ (Py\text{-}4)}$, 1C), 139.36 (s, $\underline{C}_{ar\ (Py\text{-}3)}$, 1C), 140.64 (s, $\underline{C}H_{ar\ (Py\text{-}6)}$, 1C), 147.03 (s, $\underline{C}H_{ar\ (Py\text{-}2)}$, 1C), 170.68 (d, *J* = 4.9 Hz, $\underline{C}O_2Et$, 1C).

(1-Ethoxy-3-(imidazo[1,2-a]pyridin-3-yl)-1-oxopropan-2-yl)phosphonic acid (**18b**). Quantitative yield. **Scale**: 1.3 g of **18b**.



^{31}P NMR (100 MHz, D_2O, pH-7): δ 14.56. **^{1}H NMR** (250 MHz, D_2O): δ 1.11 (t, *J* = 7.1 Hz, $C\underline{H}_3CH_2$, 3H), 3.35-3.69 (m, $C\underline{H}_2CHP$, $CH_2C\underline{H}P$, 3H), 4.10 (q, *J* = 7.1 Hz, $CH_3C\underline{H}_2$, 2H), 7.48 (td, *J* = 6.8, 1.6 Hz, $C\underline{H}_{ar}$, 1H), 8.71 (s, $C\underline{H}_{ar\ (IP\text{-}2)}$, 1H), 7.81-7.95 (m, 2x$C\underline{H}_{ar}$, 2H), 8.60 (bd, *J* = 6.8 Hz, $C\underline{H}_{ar}$, 1H). **^{13}C NMR** (63 MHz, D_2O): δ 11.31 (s, $\underline{C}H_3CH_2$, 1C), 19.05 (s, $C\underline{H}_2CHP$, 1C), 43.15 (d, *J* = 120.8, $CH_2\underline{C}HP$, 1C), 60.81 (s, $CH_3\underline{C}H_2$, 1C), 110.25 (s, $\underline{C}H_{ar}$, 1C), 115.33 (s, $\underline{C}H_{ar}$, 1C), 117.69 (s, $\underline{C}H_{ar}$, 1C), 122.72 (d, *J* = 18.0 Hz, $\underline{C}_{ar}$, 1C), 124.15 (s, $\underline{C}H_{ar}$, 1C), 131.57 (s, $\underline{C}H_{ar}$, 1C), 137.83 (s, $\underline{C}_{ar}$, 1C), 169.45 (d, *J* = 5.3 Hz, $\underline{C}O_2Et$, 1C).

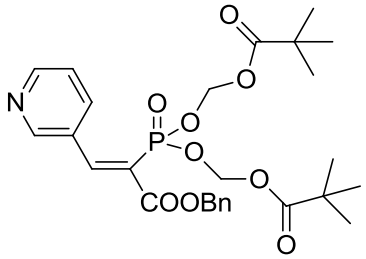
(Z)-(3-(Benzyloxy)-3-oxo-1-(pyridin-3-yl)prop-1-en-2-yl)phosphonic acid (**21**). **Scale**: 0.55 g of **25**. Obtained with quantitative yield as a mixture of isomers E/Z (1:0.07).



^{31}P NMR (100 MHz, D_2O, pH-7): δ 3.54 (isomer Z), 5.90 (isomer E). **^{1}H NMR** (250 MHz, D_2O): δ 5.23 (s, PhC$\underline{H}_2$O, 2H), 7.21-7.28, 7.32-7.40 (2m, $\underline{Ph}CH_2O$, 1/2xPyC$\underline{H}$=CP, C$\underline{H}_{ar\ (Py\text{-}5)}$, 6.5H), 7.45 (s, 1/2xPyC$\underline{H}$=CP, 0.5H), 7.61 (bd, *J* = 8.3 Hz, C$\underline{H}_{ar\ (Py\text{-}4)}$, 1H), 8.34-8.40 (m, C$\underline{H}_{ar\ (Py\text{-}2\ and\ 6)}$, 2H).

Synthesis of compound 22

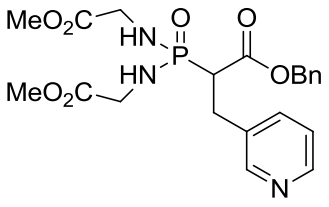
(Z)-(((3-(Benzyloxy)-3-oxo-1-(pyridin-3-yl)prop-1-en-2-yl)phosphoryl)bis(oxy))bis (methylene) bis(2,2-dimethylpropanoate) (**22**). (Prepared according to **procedure A**). Yield 10 %. **Scale**: 0.7 g of **21**. Obtained as a mixture of of isomers E/Z (1:0.07).



^{31}P NMR (100 MHz, $CDCl_3$): δ 10.22 (isomer Z), 13.28 (isomer E). **^{1}H NMR** (250 MHz, $CDCl_3$) δ 5.22 (s, PhC$\underline{H}_2$O, 2H), 5.68, 5.73 (2s, 2xOCH_2O, 4H), 7.13 (dd, *J* = 8.0, 4.9 Hz, C$\underline{H}_{ar\ (Py\text{-}5)}$, 1H), 7.23-7.36 (m-signal overlaping with $CHCl_3$, $\underline{Ph}CH_2O$, 5C), 7.61 (bd, *J* = 7.9 Hz, C$\underline{H}_{ar\ (Py\text{-}4)}$ 1H), 7.77 (d, *J* = 25.6 Hz, PyC$\underline{H}$=CP-isomer E, 1H), 8.56 (bd, *J* = 4.1 Hz, C$\underline{H}_{ar\ (Py\text{-}6)}$, 1H), 8.62 (bs, C$\underline{H}_{ar\ (Py\text{-}2)}$, 1H).

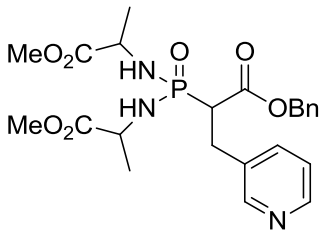
Synthesis of compounds 26-28

Dimethyl 2,2'-(((1-(benzyloxy)-1-oxo-3-(pyridin-3-yl)propan-2-yl)phosphoryl)bis (azanediyl))diacetate (**26**). (Prepared according to **procedure B**). Yield 46 %. **Scale**: 0.4 g of **23a**.



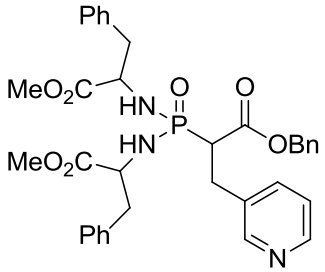
^{31}P NMR (283 MHz, $CDCl_3$): δ 23.30. **^{1}H NMR** (700 MHz, $CDCl_3$): δ 3.16-3.22 (m, $C\underline{H}_aH_bCHP$, 1H), 3.32-3.37 (m, $CH_a\underline{H}_bCHP$, $CH_aH_bC\underline{H}P$, 2H), 3.52-3.58 (m, $2XCH_2N\underline{H}$, 2H), 3.66-3.87 (m, ($2xC\underline{H}_2NH$, 4H), 3.72, 3.75 (2s, $2xCO_2C\underline{H}_3$, 6H), 5.07 (s, $PhC\underline{H}_2O$, 2H), 7.11 (dd, *J* = 7.8, 4.8 Hz, $C\underline{H}_{ar\ (Py\text{-}5)}$, 1H), 7.15-7.18 and 7.27-7.30 (2m, $C\underline{H}_{ar\ (Ph)}$, 5H), 7.47 (dt, *J* = 7.8, 1.9 Hz, $C\underline{H}_{ar\ (Py\text{-}4)}$,1H), 8.44 (dd, *J* = 4.8, 1.4 Hz, $C\underline{H}_{ar\ (Py\text{-}6)}$, 1H), 8.46 (d, *J* = 1.9 Hz, $C\underline{H}_{ar\ (Py\text{-}2)}$, 1H). **^{13}C NMR** (176 MHz, $CDCl_3$): δ 30.52 (d, *J* = 3.2 Hz, $\underline{C}H_2CHP$, 1C), 41.60, 41.80 (2s, $2x\underline{C}H_2NH$, 2C), 49.98 (d, *J* = 101.6 Hz, $CH_2\underline{C}HP$, 1C), 52.47 (s, $2xCO_2\underline{C}H_3$, 2C), 67.75 (s, $Ph\underline{C}H_2O$, 1C), 123.53 (s, $\underline{C}H_{ar\ (Py\text{-}5)}$, 1C), 128.63, 128.72 (2s, $\underline{C}H_{ar\ (Ph)}$, 5C), 134.25 (d, *J* = 15.4 Hz, $\underline{C}_{ar\ (Py\text{-}3)}$, 1C), 135.01 (s, $\underline{C}_{ar\ (Ph)}$, 1C), 136.28 (s, $\underline{C}H_{ar\ (Py\text{-}4)}$, 1C), 148.35 (s, $\underline{C}H_{ar\ (Py\text{-}6)}$, 1C) 150.22 (s, $\underline{C}H_{ar\ (Py\text{-}2)}$, 1C), 170.25 (d, *J* = 2.5 Hz, $\underline{C}O_2Bn$, 1C), 172.02, 172.34 (2d, *J* = 5.5 and 4.5 Hz, $2x\underline{C}O_2Me$, 2C). HRMS: m/z calcd 464.1581 $(M + H)^+$, found 464.1576 $(M + H)^+$.

Dimethyl 2,2'-(((1-(benzyloxy)-1-oxo-3-(pyridin-3-yl)propan-2-yl)phosphoryl)bis(azanediyl))dipropanoate (**27**). (Prepared according to **procedure B**). Yield 37 %. **Scale**: 0.43 g of **23a**. Obtained as a mixture of diastereomers (D1/D2, 1:0.8)



^{31}P NMR (283 MHz, $CDCl_3$): δ 20.74 (D1), 20.89 (D2). **^{1}H NMR** (700 MHz, $CDCl_3$): δ 1.31-1.42 (4d, *J* = 7.1, 7.1, 7.2 and 7.2 Hz, $2xC\underline{H}_3CHNH$ (D1 and D2), 10.8H), 3.11-3.51 (4m, $C\underline{H}_2CHP$ (D1 and D2), $CH_2C\underline{H}P$ (D1 and D2), $2xCH_3CHN\underline{H}$ (D1 and D2), 9H), 3.66-3.75 (4s, $2xCO_2C\underline{H}_3$ (D1 and D2), 10.8H), 4.02-4.16 (m, $2xCH_3C\underline{H}NH$, (D1 and D2), 3.6H), 5.04 (d, *J* = 12.1 Hz, $PhC\underline{H}_aH_bO$ (D1), 1H), 5.11 (d, *J* = 12.1 Hz, $PhCH_a\underline{H}_bO$ (D1), 1H), 5.07 (s, $PhC\underline{H}_2O$ (D2), 1.6H), 7.10-7.13 (m, $C\underline{H}_{ar\ (Py\text{-}5)}$ (D1 and D2), 1.8H), 7.15-7.19 and 7.28-7.31 (2m, $C\underline{H}_{ar\ (Ph)}$ (D2 and D2), 9H), 7.45 (bd, *J* = 7.9 Hz, $C\underline{H}_{ar\ (Py\text{-}4)}$ (D2), 0.8H), 7.47 (bd, *J* = 7.8, Hz, $C\underline{H}_{ar\ (Py\text{-}4)}$ (D1), 1H), 8.43-8.46 (m, $C\underline{H}_{ar\ (Py\text{-}6)}$ (D1 and D2), $C\underline{H}_{ar\ (Py\text{-}2)}$ (D2), 2.6H), 8.47 (d, *J* = 1.9 Hz, $C\underline{H}_{ar\ (Py\text{-}2)}$ (D1), 1H). **^{13}C NMR** (176 MHz, $CDCl_3$): δ 21.10-21.51 (4d, *J* = 4.5, 6.0, 5.2 and 4.7 Hz, $2x\underline{C}H_3CHNH$ (D1 and D2), 3.6C), 30.44, 30.87 (d and bs, *J* = 2.5 Hz, $\underline{C}H_2CHP$ (D1 and D2), 1.8C), 48.46-49.19 (3s, $2xCH_3\underline{C}HNH$ (D1 and D2), 3.6C), 50.00, 50.51 (2d, *J* = 101.2 and 99.3 Hz, $CH_2\underline{C}HP$ (D1 and D2), 1.8C), 52.49, 52.61 (2s, $2xCO_2\underline{C}H_3$ (D1 and D2), 3.6C), 67.68, 67.74 (2s, $Ph\underline{C}H_2O$ (D1 and D2), 1.8C), 123.54 (s, $\underline{C}H_{ar\ (Py\text{-}5)}$ (D1 and D2), 1.8C), 128.62-128.76 (5s, $\underline{C}H_{ar\ (Ph)}$ (D1 and D2), 9C), 134.31, 134.40 (2d, *J* = 12.2 and 13.7 Hz, $\underline{C}_{ar\ (Py\text{-}3)}$ (D1 and D2), 1.8C), 135.07, 135.12 (2s, $\underline{C}_{ar\ (Ph)}$ (D1 and D2), 1.8C), 136.30, 136.31 (2s, $\underline{C}H_{ar\ (Py\text{-}4)}$ (D1 and D2), 1.8C), 148.32, 148.37 (2s, $\underline{C}H_{ar\ (Py\text{-}6)}$ (D1 and D2), 1.8C), 150.23, 150.25 (2s, $\underline{C}H_{ar\ (Py\text{-}2)}$ (D1 and D2), 1.8C), 170.26, 170.36 (2d, *J* = 3.0 and 2.6 Hz, $\underline{C}O_2Bn$ (D1 and D2), 1.8C), 174.49-175.04 (4d, *J* = 5.2, 6.0, 3.8 and 4.9 Hz, $2x\underline{C}O_2Me$ (D1 and D2), 3.6C). HRMS: m/z calcd 492.1894 $(M + H)^+$, found 492.1892 $(M + H)^+$.

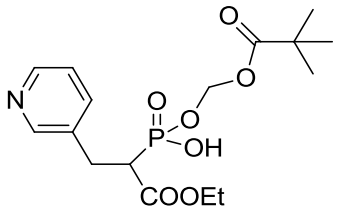
Dimethyl 2,2'-(((1-(benzyloxy)-1-oxo-3-(pyridin-3-yl)propan-2 yl)phosphoryl)bis (azanediyl))bis(3-phenylpropanoate) (**28**). (Prepared according to **procedure B**). Yield 12 %. **Scale**: 0.48 g of **23a**. Obtained as mixture of diastereomers (D1/D2, 1:0.7)**.**



^{31}P NMR (283 MHz, $CDCl_3$): δ 20.51 (D1), 21.01 (D2). **^{1}H NMR** (700 MHz, $CDCl_3$): δ 2.62-3.16 (m, C$\underline{H}_2$CHP (D1 and D2), CH$_2$C$\underline{H}$P (D1 and D2), 2xPhC$\underline{H}_2$CHNH (D1 and D2), PhCH$_2$CHN$\underline{H}$ (D1 and D2), 13.6H), 3.33 (t, *J* = 11.4 Hz, PhCH$_2$CHN$\underline{H}$ (D1), 1H), 3.50 (t, *J* = 10.7 Hz, PhCH$_2$CHN$\underline{H}$ (D2), 0.7H), 3.65-3.73 (2s, 2xCO$_2$C$\underline{H}_3$ (D2), 4.2H), 3.68-3.74 (2s, 2xCO$_2$C$\underline{H}_3$ (D1), 6H), 3.95–3.99 (m, PhCH$_2$C$\underline{H}$NH (D1), 1H), 4.08–4.12 (m, PhCH$_2$C$\underline{H}$NH (D2), 0.7H), 4.40–4.45 (m, PhCH$_2$C$\underline{H}$NH (D1 and D2), 1.7H), 4.89 (d, *J* = 12.2 Hz, PhC$\underline{H}_a$H$_b$O (D2), 0.7H), 4.92 (d, *J* = 12.2 Hz, PhC$\underline{H}_a$H$_b$O (D1), 1H) 4.99 (d, *J* = 12.2 Hz, PhCH$_a$$\underline{H}_b$O (D2), 0.7H), 5.05 (d, *J* = 12.2 Hz, PhCH$_a$$\underline{H}_b$O (D1), 1H), 7.03-7.32 (3m, 3xC$\underline{H}_{ar\ (Ph)}$ (D1 and D2), C$\underline{H}_{ar\ (Py\text{-}5)}$ (D1 and D2), C$\underline{H}_{ar\ (Py\text{-}4)}$ (D2), 27.9H), 7.34 (dt, *J* = 7.8, 1.8 Hz, C$\underline{H}_{ar\ (Py\text{-}4)}$ (D1), 1H), 8.21 (d, *J* = 1.7 Hz, C$\underline{H}_{ar\ (Py\text{-}2)}$ (D2), 0.7H), 8.34 (d, *J* = 1.8 Hz, C$\underline{H}_{ar\ (Py\text{-}4)}$ (D1), 1H), 8.39-8.42 (m, C$\underline{H}_{ar\ (Py\text{-}6)}$ (D1 and D2), 1.7H). **^{13}C NMR** (176 MHz, $CDCl_3$): δ 30.40, 30.56 (2d, *J* = 2.5 and 1.9 Hz, C$\underline{H}_2$CHP (D1 and D2), 1.7C), 40.17-41.11 (4d, *J* = 6.3, 4.6, 5.4 and 5.5 Hz, 2xPhC$\underline{H}_2$CHNH (D1 and D2), 3.4C), 49.78, 50.30 (2d, *J* = 103.2 and 99.9 Hz, CH$_2$C$\underline{H}$P (D1 and D2), 1.7C), 52.10-52.52 (4s, 2xCO$_2$C$\underline{H}_3$ (D1 and D2), 3.4C), 53.80-54.21 (4s, PhCH$_2$C$\underline{H}$NH (D1 and D2), 3.4C), 67.46, 67.55 (2s, PhC$\underline{H}_2$O (D1 and D2), 1.7C), 123.37 (s, C$\underline{H}_{ar\ (Py\text{-}5)}$ (D1 and D2), 1.7C), 126.97-129.97 (18s, 3xC$\underline{H}_{ar\ (Ph)}$ (D1 and D2), 25.5C), 134.44, 134.37 (2d, *J* = 16.1 and 15.6 Hz, C$\underline{}_{ar(Py\text{-}3)}$ (D1 and D2), 1.7C), 135.08, 135.16 (2s, C$\underline{}_{ar\ (PhCH2O)}$ (D1 and D2), 1.7C), 135.82 (s, C$\underline{H}_{ar\ (Py\text{-}4)}$ (D2), 0.7C), 136.03 (s, C$\underline{H}_{ar\ (Py\text{-}4)}$ (D1), 1C), 136.27-136.69 (4s, 2xC$\underline{}_{ar\ (PhCH2CHNH)}$ (D1 and D2), 3.4C), 148.18, 148.20 (2s, C$\underline{H}_{ar\ (Py\text{-}6)}$ (D1 and D2), 1.7C), 150.13, 150.21 (2s, C$\underline{H}_{ar\ (Py\text{-}2)}$ (D1 and D2), 1.7C), 170.01, 170.08 (2d, *J* = 3.6 and 3.2 Hz, C$\underline{}$O$_2$Bn (D1 and D2), 1.7C), 173.23-173.81 (3d and bs**,** *J* = 3.7, 4.6, 2.3 Hz, 2xC$\underline{}$O$_2$Me (D1 and D2), 3.4C). HRMS: m/z calcd 644.2520 $(M + H)^+$, found 644.2507 $(M + H)^+$.

Spectroscopic data for compounds 19 and 29a (byproducts)

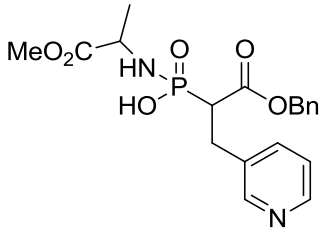
(((1-Ethoxy-1-oxo-3-(pyridin-3-yl)propan-2-yl)(hydroxy)phosphoryl)oxy)methyl pivalate (**19**). Obtained as byproduct in the reaction carried out according to **procedure A**.



^{31}PNMR (100 MHz, D_2O) δ 15.86. **^{1}H NMR** (250MHz, D_2O) δ 1.11 (t, *J* = 7.2, C$\underline{H}_3$CH$_2$, 3H), 1.21 (s, C(C$\underline{H}_3$)$_3$, 9H), 3.16-3.30 (m, C$\underline{H}_2$C$\underline{H}$P, 3H), 4.07 (q, *J* = 7.2, CH$_3$C$\underline{H}_2$, 2H), 5.54-5.61 (m, OC$\underline{H}_2$O, 2H), 7.44 (bt, *J* = 7.2, C$\underline{H}_{ar\ (Py\text{-}5)}$, 1H), 7.89 (d, *J* = 8.0, C$\underline{H}_{ar\ (Py\text{-}4)}$, 1H), 8.42 (s, C$\underline{H}_{ar\ (Py\text{-}2\ and\ 6)}$, 2H). **^{13}C NMR** (63 MHz, D_2O) δ 13.98 (s, C$\underline{}$H$_3$CH$_2$, 1C), 26.86 (s, C(C$\underline{}$H$_3$)$_3$, 3C), 30.80

(s,C$\underline{H}_2$CHP, 1C), 39.25 (s, $\underline{C}(CH_3)_3$, 1C), 51.62 (d, *J* = 118.8, $CH_2\underline{C}HP$, 1C). 62.92 (s, $CH_3\underline{C}H_2$, 1C), 83.56 (d, *J* = 5.6, $O\underline{C}H_2O$, 1C), 125.14 (s, $\underline{C}H_{ar\ (Py\text{-}5)}$, 1C), 136.48 (d, *J* = 15.2, $\underline{C}_{ar\ (Py\text{-}3)}$, 1C), 138.91 (s, $\underline{C}H_{ar\ (Py\text{-}4)}$, 1C), 147.13 (s, $\underline{C}H_{ar\ (Py\text{-}6)}$, 1C), 148.81 (s, $\underline{C}H_{ar\ (Py\text{-}6)}$, 1C), 172.63 (d, *J* = 4.7, $\underline{C}O_2Et$, 1C), 181.07 (s, $\underline{C}C(CH_3)_3$, 1C).

P-(1-(benzyloxy)-1-oxo-3-(pyridin-3-yl)propan-2-yl)-N-(1-methoxy-1-oxopropan-2-yl)phosphonamidic acid (**29a**). Obtained as byproduct in the reaction carried out according to **procedure B**. Mixture of diastereomers (D1/D2, ~1:1). Purified by prepatative HPCL: t_R, 5.3 min, H_2O/ACN, 80:20 (v/v). Ratio of compound **37a** and TEA, 1:0.65.



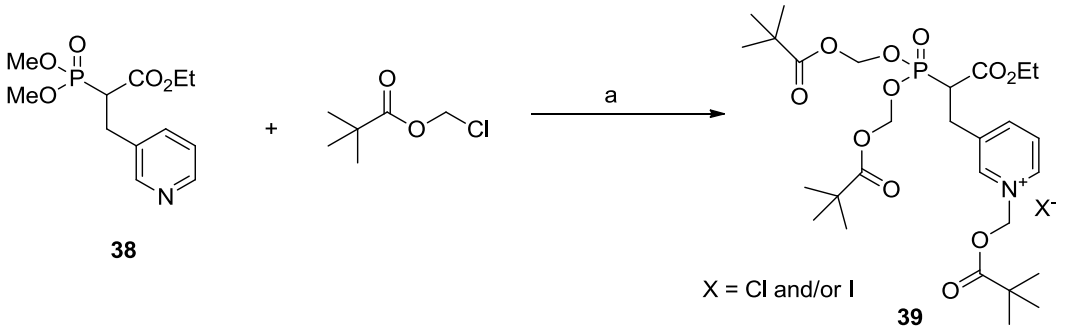
^{31}P NMR (283 MHz, $CDCl_3$): δ 14.65 and 14.79. **^{1}H NMR** (700 MHz, $CDCl_3$): δ 1.32-1.33 (2d, *J* = 7.0 Hz, $C\underline{H}_3CHNH$ (D1 and D2), 6H), 3.08-3.30 (m, $C\underline{H}_2CHP$ (D1 and D2), $CH_2C\underline{H}P$ (D1 and D2), 6H), 3.64-3.66 (4s, $CO_2C\underline{H}_3$ (D1 and D2), 6H), 3.98-4.05 (m, $CH_3C\underline{H}NH$, (D1 and D2), 2H), 4.95-5.07 (m, $PhC\underline{H}_2O$ (D1 and D2), 4H), 7.08 (bs, $C\underline{H}_{ar\ (Py\text{-}5)}$ (D1 and D2), 2H), 7.14-7.17 and 7.22-7.25 (2m, $C\underline{H}_{ar\ (Ph)}$ (D2 and D2), 10H), 7.49 (t, *J* = 9.0 Hz, $C\underline{H}_{ar\ (Py\text{-}4)}$ (D1 and D2), 2H), 8.38 (s, $C\underline{H}_{ar\ (Py\text{-}6)}$ (D1 and D2), 2H), 8.46 (s, $C\underline{H}_{ar\ (Py\text{-}2)}$ (D1 andD2), 2H). **^{13}C NMR** (176 MHz, $CDCl_3$): δ 21.94, 22.36 (2bs, $\underline{C}H_3CHNH$ (D1 and D2), 2C), 31.76 (d, *J* = 9.0 Hz, $\underline{C}H_2CHP$ (D1 and D2), 2C), 50.72 (s, $CH_3\underline{C}HNH$ (D1 and D2), 2C), 51.41, 51.58 (2bd, *J* = 107.4 and 102.3 Hz, $CH_2\underline{C}HP$ (D1 and D2), 2C), 51.99 (2s, $CO_2\underline{C}H_3$ (D1 and D2), 2C), 66.18 (bs, $Ph\underline{C}H_2O$ (D1 and D2), 4C), 123.31 (s, $\underline{C}H_{ar\ (Py\text{-}5)}$ (D1 and D2), 2C), 127.88-128.63 (7s, $\underline{C}H_{ar\ (Ph)}$ (D1 and D2), 10C), 136.40 (bs, $\underline{C}_{ar\ (Py\text{-}3)}$ (D1 and D2), $\underline{C}_{ar\ (Ph)}$ (D1 and D2), $\underline{C}H_{ar\ (Py\text{-}4)}$ (D1 and D2), 6C), 147.47 (2s, $\underline{C}H_{ar\ (Py\text{-}6)}$ (D1 and D2), 2C), 150.28 (bs, $\underline{C}H_{ar\ (Py\text{-}2)}$ (D1 and D2), 2C), 172.18, (s, $\underline{C}O_2Bn$ (D1 and D2), 2C), 176.27, 176.47 (d and bs, *J* = 4.7 Hz, $\underline{C}O_2Me$ (D1 and D2), 2C).

Synthesis of compound 39

To synthesize compounds with acyloxyalkyl moieties (**3**) we tried also an approach utilizing dimethyl ester **38** as starting material. Such approach was successfully applied in synthesis of allylphosphonates prodrugs, where introduction of acyloxyalkyl residue to phoshonic group was achieved on the action of pivaloxyl chloride in the presence of sodium iodide.[*] Applying such conditions have led to formation of product **39** (Scheme 2) characterized by upfield chemical shift in ^{31}P NMR (~19.5 ppm) compared with the expected products **3** (~21.5 ppm). Also, in ^{1}H NMR we observed one additional methylene group, in the area of 6.5 ppm, and in aromatic area, signals of all protons were shifted downfield (0.5-1 ppm). These data suggested formation of compound containing three POM groups **39**, two in the phosphonate residue and one attached to nitrogen in pyridine ring which was confirmed by NMR and MS. Since quaternization of pyridine nitrogen turned out to be faster than trans-esterification of phosphonate group, we

[*] Pradere, U.; Clavier, H.; Roy, V.; Nolan, S. P.; Agrofoglio, L. A., The Shortest Strategy for Generating Phosphonate Prodrugs by Olefin Cross-Metathesis - Application to Acyclonucleoside Phosphonates. *Eur. J. Org. Chem.*, **2011,** (36), 7324-7330.

protected nitrogen atom by forming pyridine oxide. This approach failed and *N*-alkylation reaction could not be suppressed.



Scheme S1. *Formation of quaternized pyridine analog. Reagents and conditions: (a) NaI (2 eq), ACN, 80°C, 3 h.*

Triester **38** (0.1 g, 0.35 mmol) was dissolved in ACN (1 ml). Then NaI (0.15 g, 1.05 mmol, 3 eq) and POM-Cl (0.16 g, 0.15 ml, 1.05 mmol, 3 eq) were added and the resulting mixture was stirred for 3 hours in 80°C. Solvent was evaporated under reduced pressure and residue was dissolved in H_2O (1 ml). After pH adjustment to 9 using Na_2CO_2 (s), mixture was extracted with Et_2O (3x4 ml). Organic layer was dried over anhydrous MgSO4, solvent was evaporated and residue was subjected to column chromatography using DCM:MeOH (25:1) system as eluent to give 96 mg (45 %) of product as yellow oil.

3-(2-(bis((pivaloyloxy)methoxy)phosphoryl)-3-ethoxy-3-oxopropyl)-1-((pivaloyloxy)methyl)pyridin-1-ium chloride and/or iodide (39).

^{31}P NMR (100 MHz, $CDCl_3$): δ 19.74. **^{1}H NMR** (250 MHz, $CDCl_3$): δ 1.22-1.24 (m, $C\underline{H}_3CH_2$, 3xC($C\underline{H}_3$)$_3$, 30H), 3.43-3.52 (m, $C\underline{H}_2CHP$, 2H), 3.67-3.82 (m, $CH_2C\underline{H}P$, 1H), 4.06-4.29 (m, $CH_3C\underline{H}_2$, 2H), 5.61-5.76 (m, 2xOC$\underline{H}_2$O, 4H), 6.63-6.73 (m, $N^+C\underline{H}_2O$, 2H), 8.07 (dd, *J* = 7.8 and 4.8 Hz, $C\underline{H}_{ar\ (Py\text{-}5)}$, 1H), 8.52 (d, *J* = 7.8 Hz, $C\underline{H}_{ar\ (Py\text{-}4)}$, 1H), 9.20-9.24 (m, $C\underline{H}_{ar\ (Py\text{-}2\ and\ 6)}$, 2H). **^{13}C NMR** (63 MHz, $CDCl_3$): δ 14.08 (s, $\underline{C}H_3CH_2$, 1C), 26.86 (s, $N^+CH_2OC(O)C(\underline{C}H_3)_3$, 3C), 26.95 (s, 2xOC(O)C($\underline{C}H_3$)$_3$, 6H), 29.39 (s, $\underline{C}H_2CHP$, 1C), 38.83 (s, 2xOC(O)$\underline{C}$(CH$_3$)$_3$, 2C), 45.74 (d, *J* = 130.4 Hz, $CH_2\underline{C}HP$, 1C), 53.70 (s, $N^+CH_2OC(O)\underline{C}(CH_3)_3$, 1C), 62.66 (s, $CH_3\underline{C}H_2$, 1C), 80.09 (s, $N^+\underline{C}H_2O$, 1C), 82.26, 82.37 (2d, *J* = 6.6 and 6.2 Hz, 2xO$\underline{C}H_2$O, 2C), 128.27 (s, $\underline{C}H_{ar\ (Py\text{-}5)}$, 1C), 139.54 (d, *J* = 13.8 Hz, $\underline{C}_{ar\ (Py\text{-}3)}$, 1C), 143.53 (s, $\underline{C}H_{ar\ (Py\text{-}4)}$, 1C), 145.32 (s, $\underline{C}H_{ar\ (Py\text{-}6)}$, 1C), 148.79 (s, $\underline{C}H_{ar\ (Py\text{-}2)}$, 1C), 166.69 (d, *J* = 5.4 Hz, $\underline{C}O_2Et$, 1C), 177.11 (m, $N^+CH_2O\underline{C}(O)C(CH_3)_3$, 2 x O$\underline{C}$(O)C(CH$_3$)$_3$, 3C).

Table S1. Selected Chemical Shifts (^{31}P and ^{1}H NMR) of obtained prodrugs. Ratios for mixtures of diastereomers.[a]

Compound	^{31}P NMR (Δ)	^{1}H NMR (selected protons)[b]	Ratio of diastereomers[c]
3	21.78	n.a	n.a
4	22.26	n.a	n.a
5	21.44	n.a	n.a
6	22.03	n.a	n.a
7	23.20	n.a	n.a
8	23.38	n.a	n.a
9	21.05, 21.15 (0.1)	7.54, 7.57[d]	1:0.8
10	21.47, 21.99 (0.52)	0.99, 1.02 ($C\underline{H}_3CH_2$) 8.24, 8.37[d] 3.36, 3.54 ($N\underline{H}$)	1:0.9
11	23.42	n.a	n.a
12	20.87, 21.24 (0.37)	8.10, 8.18[d] 1.13, 1.17 ($C\underline{H}_3CH_2$)	1:0.9
13	20.46, 21.11 (0.65)	7.86, 8.06[d]	1:0.9
14	26.66	n.a	n.a
15	22.79, 23.08 (0.34)	7.76, 7.80[d]	1:0.5[e]
16	23.29, 23.57 (0.28)	7.52, 7.65[d]	1:0.8
26	23.30	n.a	n.a
27	20.74, 20.89 (0.15)	5.05, 5.07 ($PhC\underline{H}_2CHNH$)	1:0.8
28	20.51, 21.01 (0.5)	8.21, 8.34[d] 3.33, 3.50 ($N\underline{H}$) 3.97, 4.10 ($PhCH_2C\underline{H}NH$)	1:0.7

[a] ($CDCl_3$, ^{1}H NMR, 700 MHz, ^{31}P NMR, 283 MHz)
[b] determined only for signals distinguished from other;
[c] determined based on ^{31}P NMR for mixtures after flash chromatography.
[d] chemical shifts in ^{1}H NMR are given for the following pairs of protons: H4 in pyridine ring for **9**, **15** and **16**; H2 in pyridine ring for **10** and **28**; H5 in imidazo[1,2-a]pyridine ring for compounds **12** and **13**.
[e] in experimental section data included for one diastereomer
n.a. – not applicable

Table S2. Comparison of Calculated ClogD Values for Differently Modified Compounds.

Compound	ClogD*	
	pH 6.5	pH 7.4
d-RisPC (**1b**)	-5.37	-6.16
diPOM-d-RisPCOEt (**3**)	4.36	4.36
diPOC-d-RisPCOEt (**4**)	3.54	3.55
diPOM-d-RisPCOH (**7**)	1.54	0.81
diGly(OMe)-d-RisPCOEt (**8**)	-1.21	-1.20
diAla(OMe)-d-RisPCOEt (**9**)	-0.16	-0.15
diPhe(OMe)-d-RisPCOEt (**10**)	3.14	3.15
diGly(OMe)-d-RisPCOH (**14**)	-4.18	-4.86
diAla(OMe)-d-RisPCOH (**15**)	-3.08	-3.78
diPhe(OMe)-d-RisPCOH (**16**)	0.28	-0.44
d-MinPC (**2b**)	-4.84	-5.58
diPOM-d-MinPCOEt (**5**)	4.02	4.21
diPOC-d-MinPCOEt (**6**)	3.21	3.39
diGly(OMe)-d-MinPCOEt (**11**)	-1.54	-1.36
diAla(OMe)-d-MinPCOEt (**12**)	-0.49	-0.31
diPhe(OMe)-d-MinPCOEt (**13**)	2.81	2.99

*Calculated with MarvinSketch 15.1.19.0

Table S3. Retention times for compounds 3-16 observed under HPLC conditions used for stability studies

Compound	HPLC conditions*	
	A (min)	**B** (min)
(**3**)	4.02	16.07
(**4**)	3.66	14.20
(**7**)	3.59	14.67
(**5**)	4.08	14.73
(**6**)	3.73	13.23
(**8**)	2.57	7.33
(**9**)	2.94	9.56
(**10**)	3.96, 3.99	13.93
(**11**)	2.68	8.23
(**12**)	3.05	9.82
(**13**)	4.01, 4.03	14.87
(**14**)	1.72	3.20
(**15**)	2.21, 2.27	7.84
(**16**)	3.62, 3.65	15.09

*__A__. Waters Acquity UPLC equipped with autosampler and coupled with mass spectrometer Waters Micromass LCT Premier XE, column Waters UPLC BEH C_{18} (1.7µm, 50mm x 2.1mm). The UV detector was operated at 261 and 280 nm for **1b** and **2b** derivatives respectively. Eluents: A: 10 mM ammonium acetate, pH 7; B: methanol/10 mM ammonium acetate, pH 7 (90/10, v/v). Gradient: from 0 to 0.25 min: 90 % of A, from 3.75 to 4.00 min: 1 % of A at a flow rate of 0.3 mL/min.

B. Dionex Ultimate 3000 – LC system equipped with autosampler, column compartment, diode array detector and column Thermo Hypersil Gold (1.9 µm, 2.1 x 50 mm). The detection was performed at a wavelength of 263 nm or 281 nm for **1b** and **2b** derivatives respectively. Eluents: A: methanol/water/formic acid (5/94.9/0.1, v/v/v); B: methanol/water/formic acid (95/4.9/0.1, v/v/v). Gradient: 0-100% in 15.2 min of B at a flow rate of 0.2 mL/min.

Spectral data of compounds synthesized

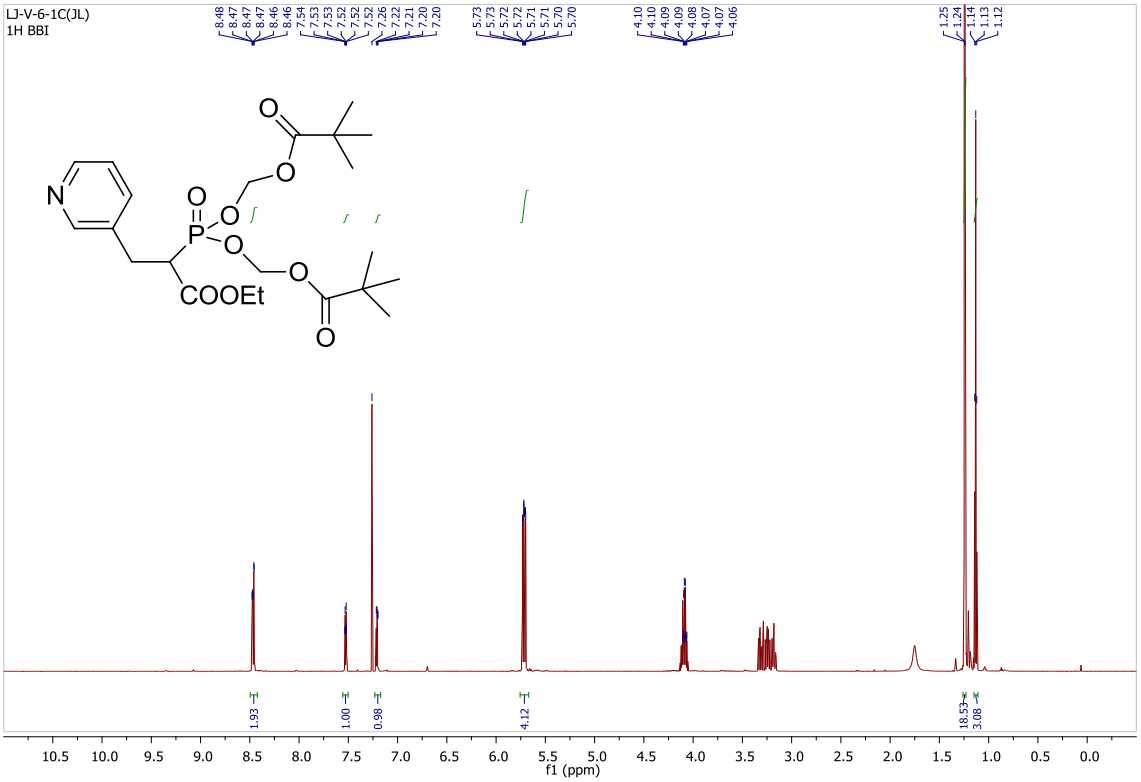


Figure S1. ^{1}H NMR of compound **3** (700 MHz, $CDCl_3$).

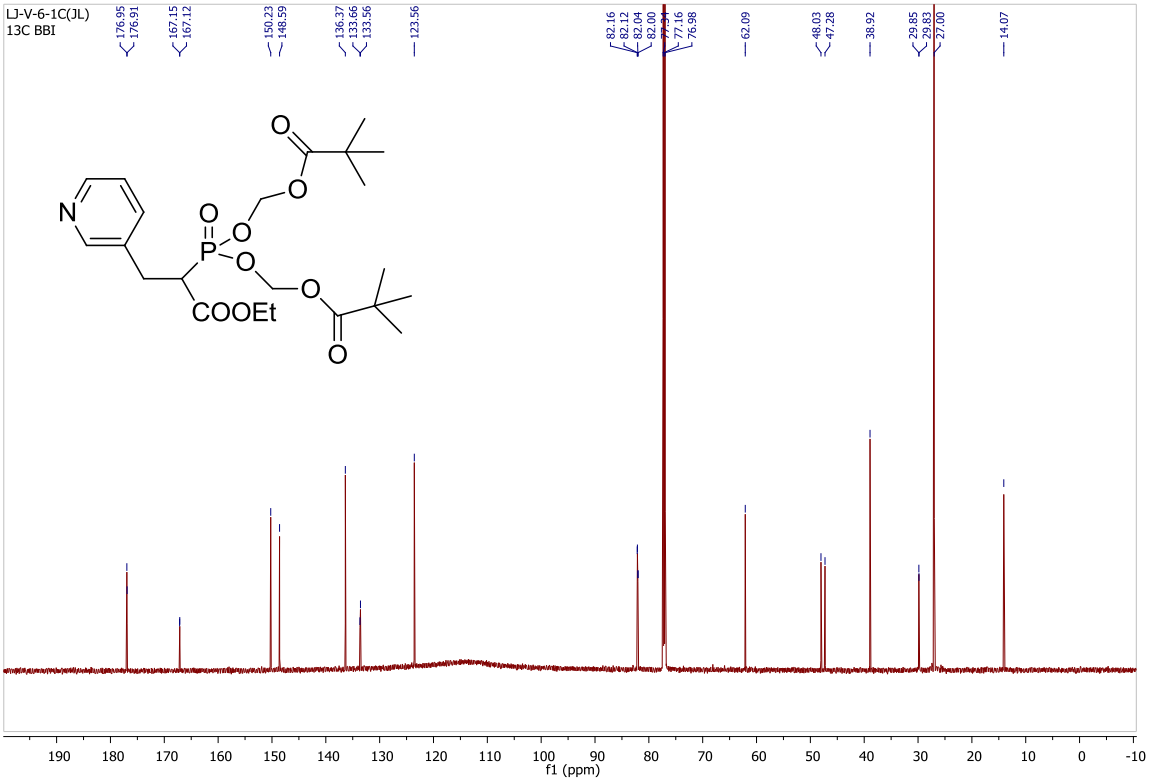


Figure S2. ^{13}C NMR of compound **3** (176 MHz, $CDCl_3$).

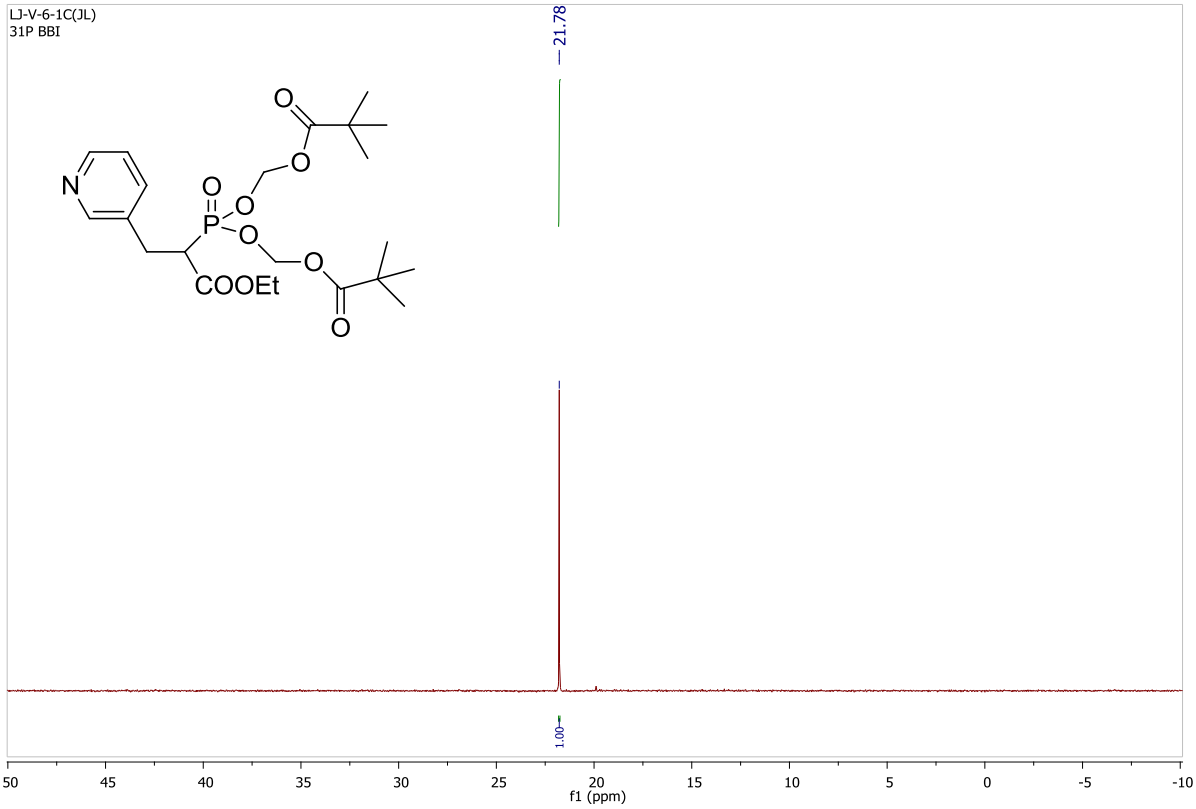


Figure S3. ^{31}P NMR of compound **3** (283 MHz, $CDCl_3$).

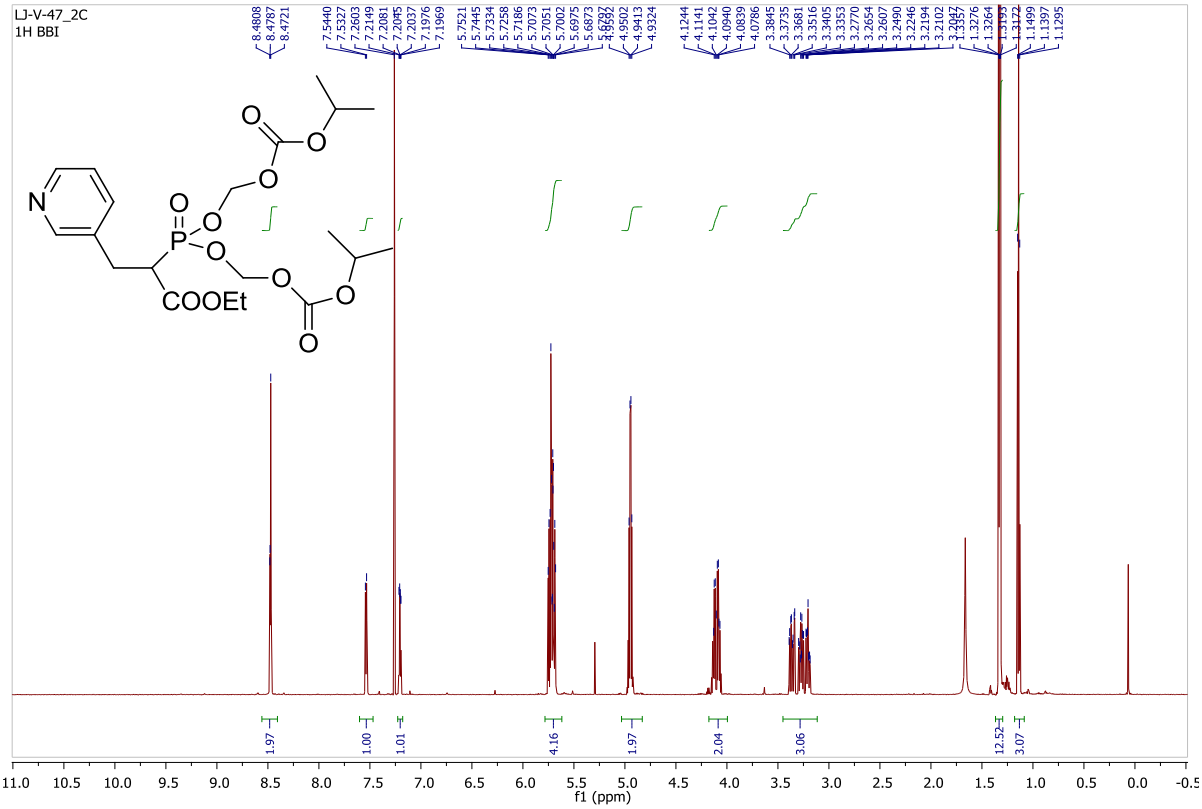


Figure S4. ^{1}H NMR of compound **4** (700 MHz, $CDCl_3$).

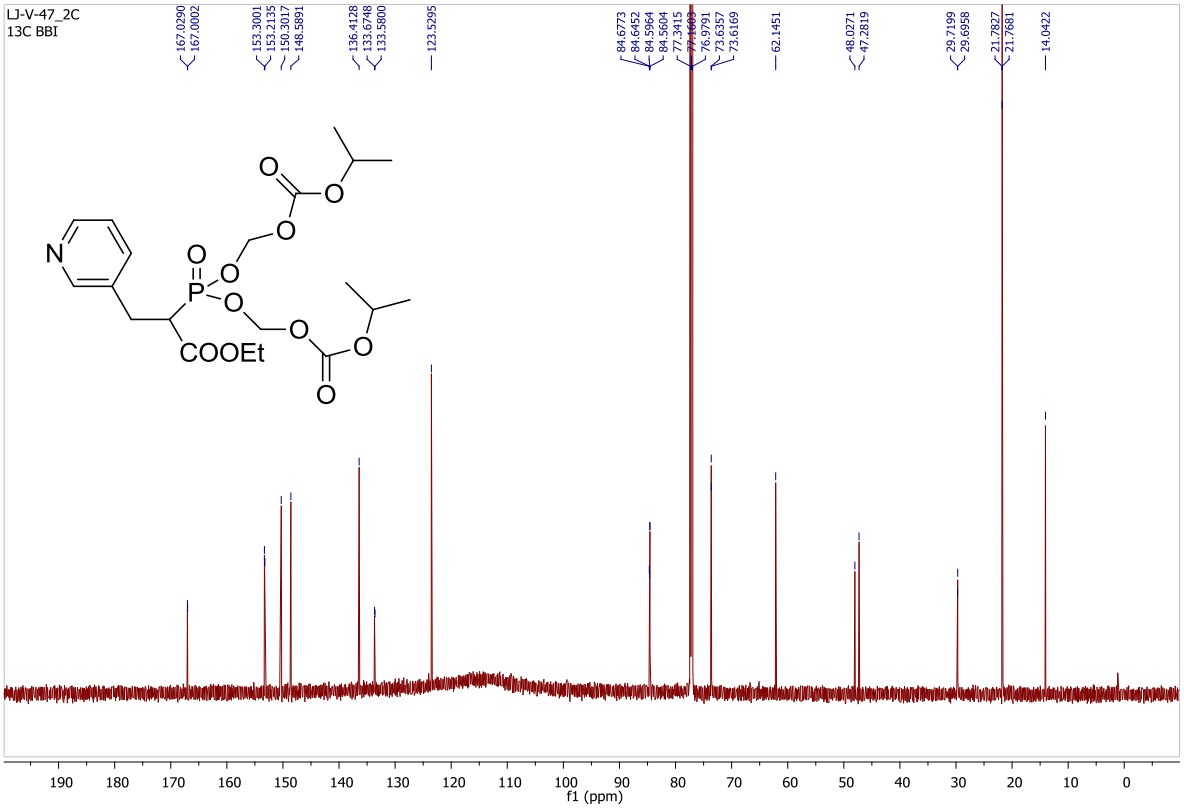


Figure S5. ^{13}C NMR of compound **4** (176 MHz, $CDCl_3$).

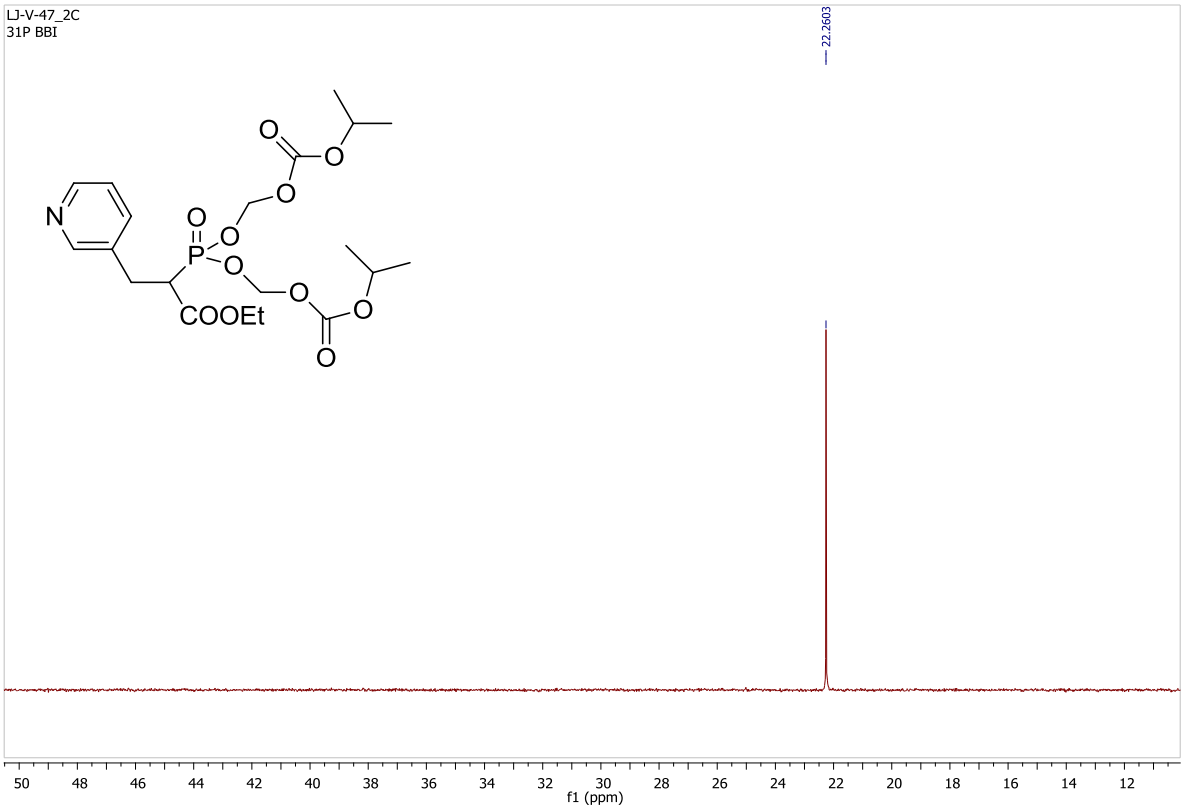


Figure S6. ^{31}P NMR of compound **4** (283 MHz, $CDCl_3$).

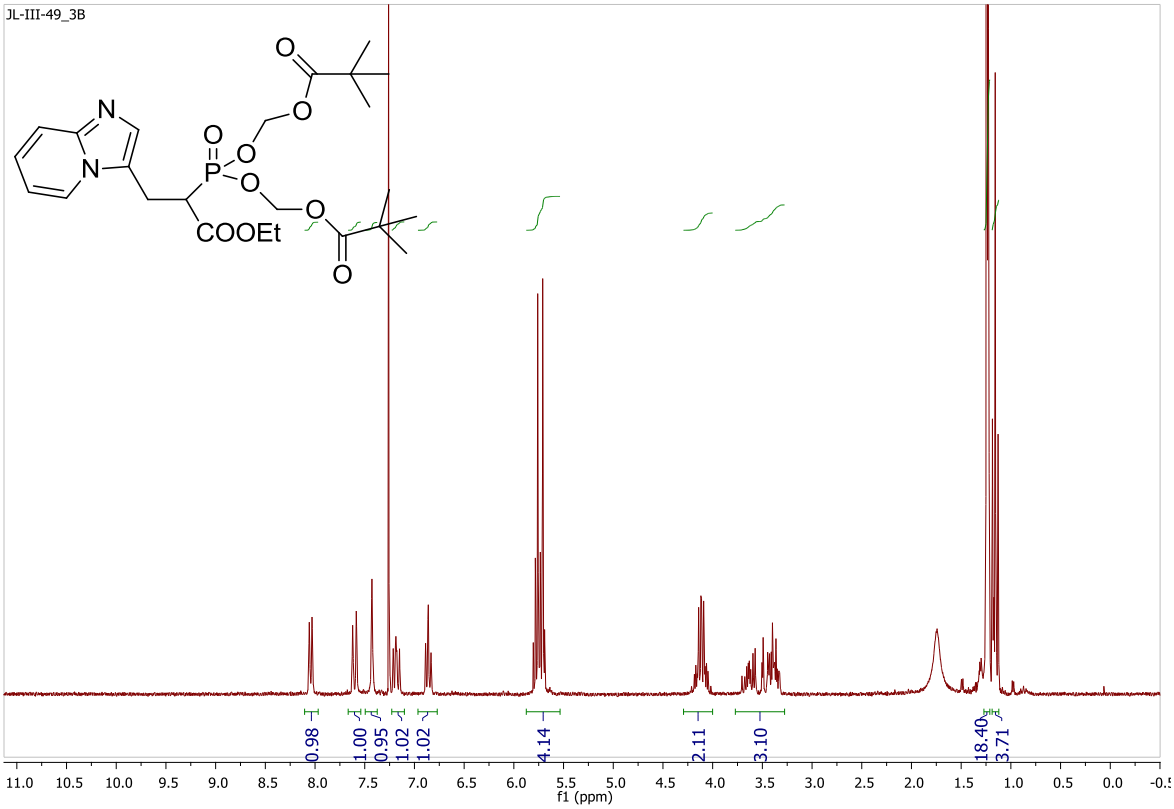


Figure S7. ^{1}H NMR of compound **5** (250 MHz, $CDCl_3$).

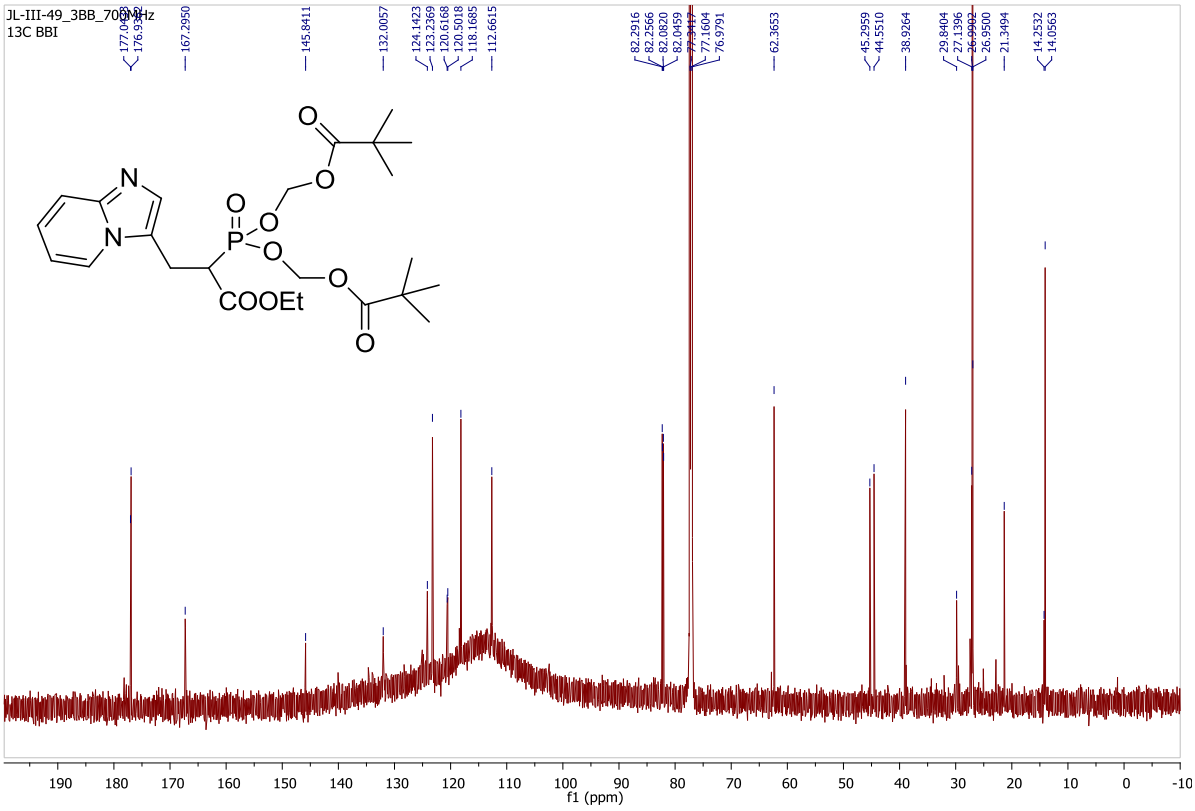


Figure S8. ^{13}C NMR of compound **5** (176MHz, $CDCl_3$).

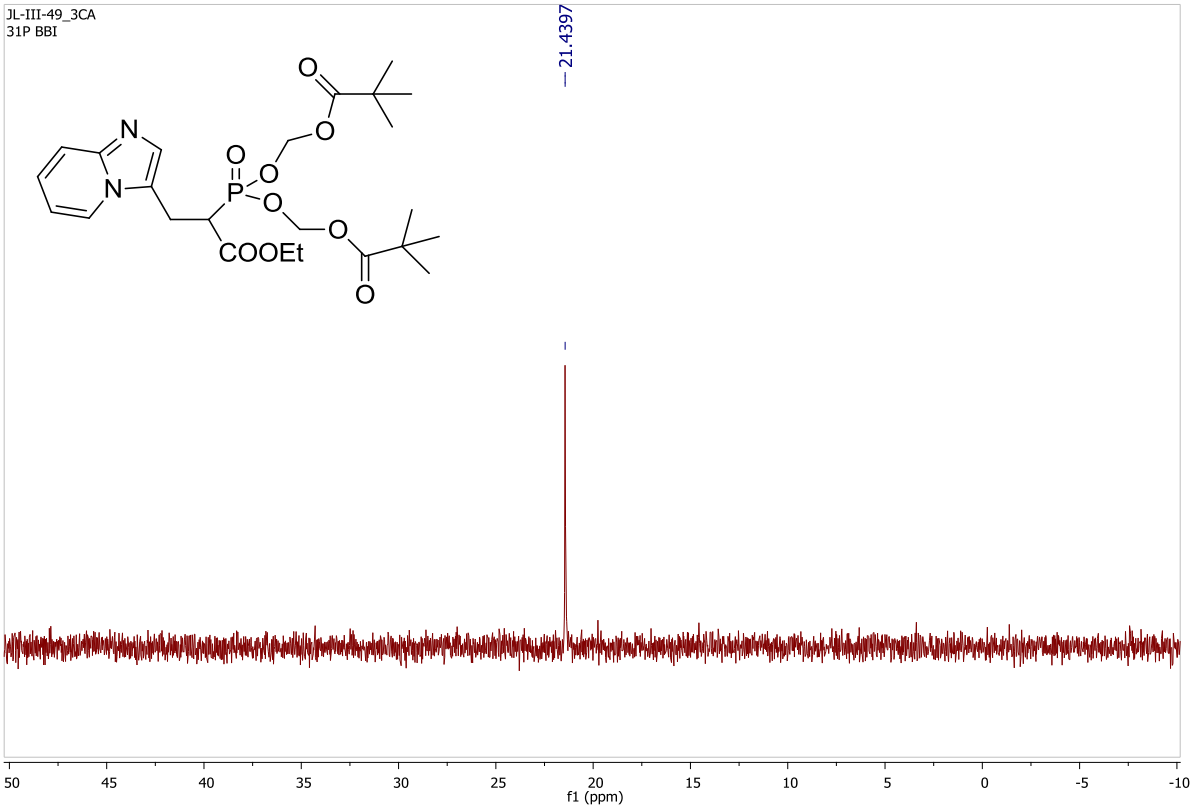


Figure S9. ^{31}P NMR of compound **5** (283 MHz, $CDCl_3$).

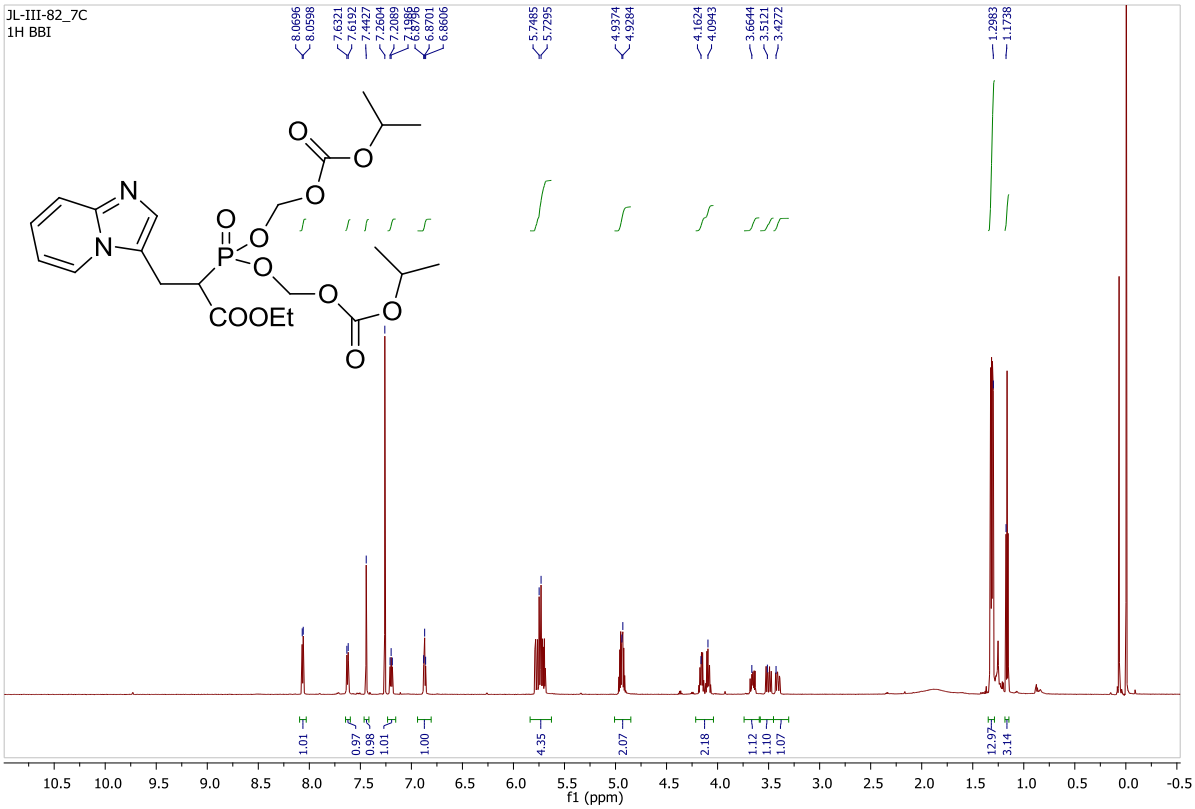


Figure S10. ^{1}H NMR of compound **6** (700 MHz, $CDCl_3$).

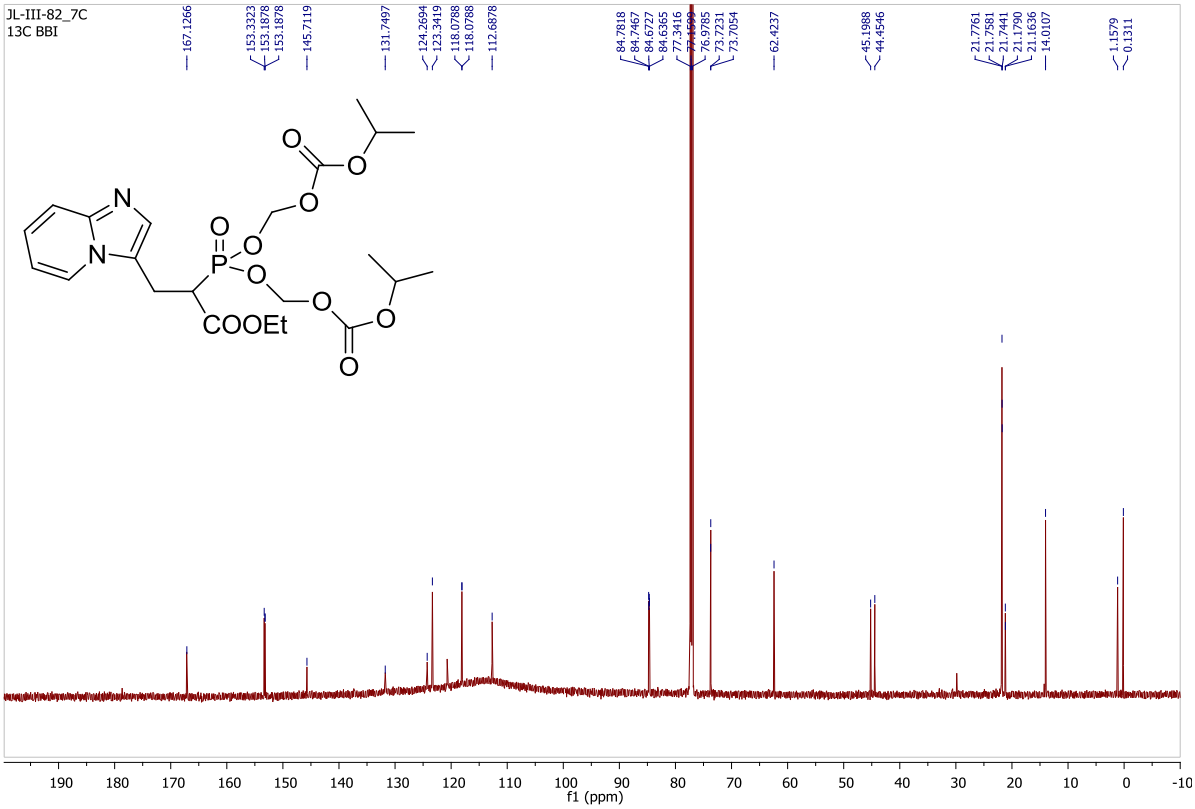


Figure S11. ^{13}C NMR of compound **6** (700 MHz, $CDCl_3$).

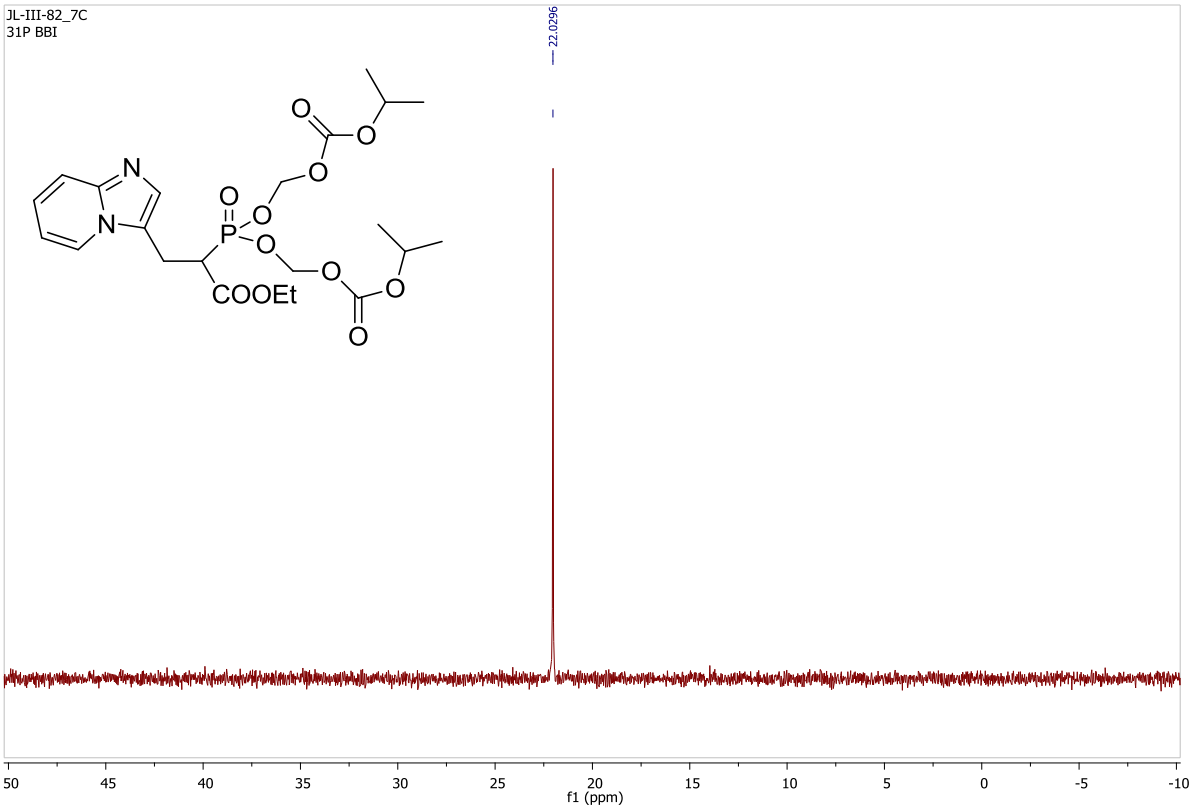


Figure S12. ^{31}P NMR of compound **6** (283 MHz, $CDCl_3$).

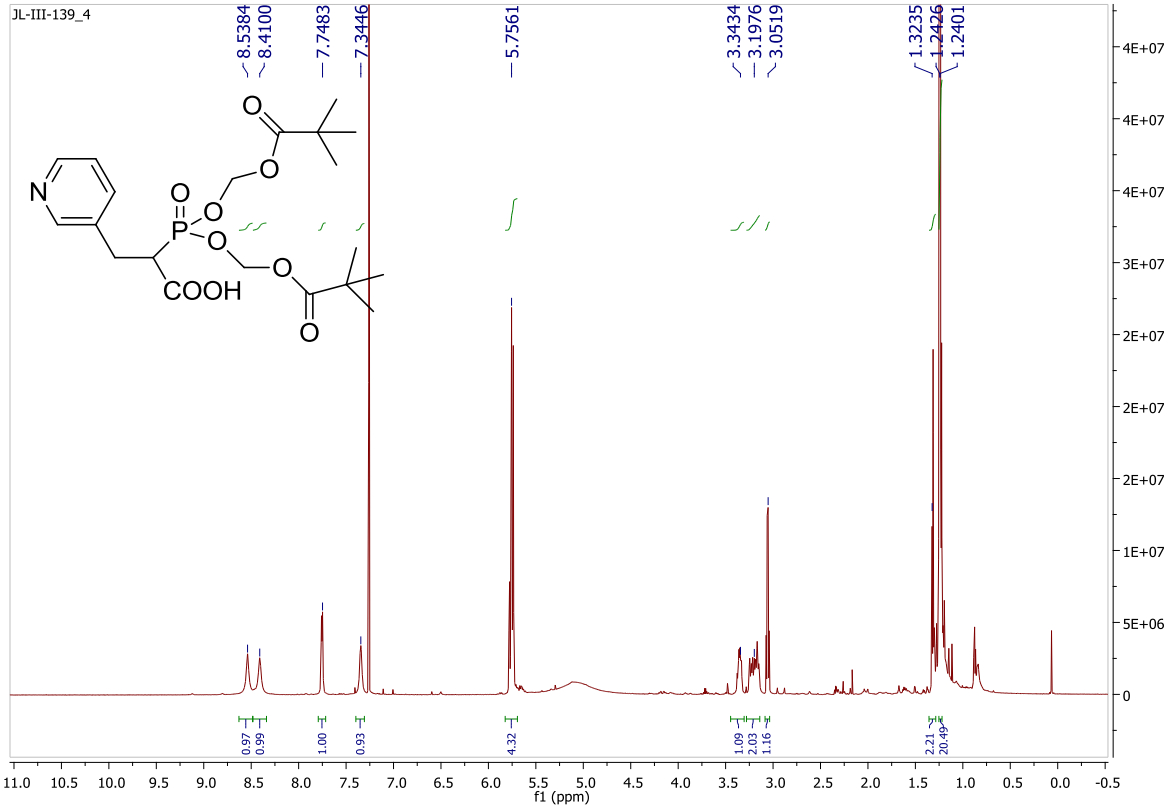


Figure S13. ^{1}H NMR of compound **7** (700 MHz, $CDCl_3$).

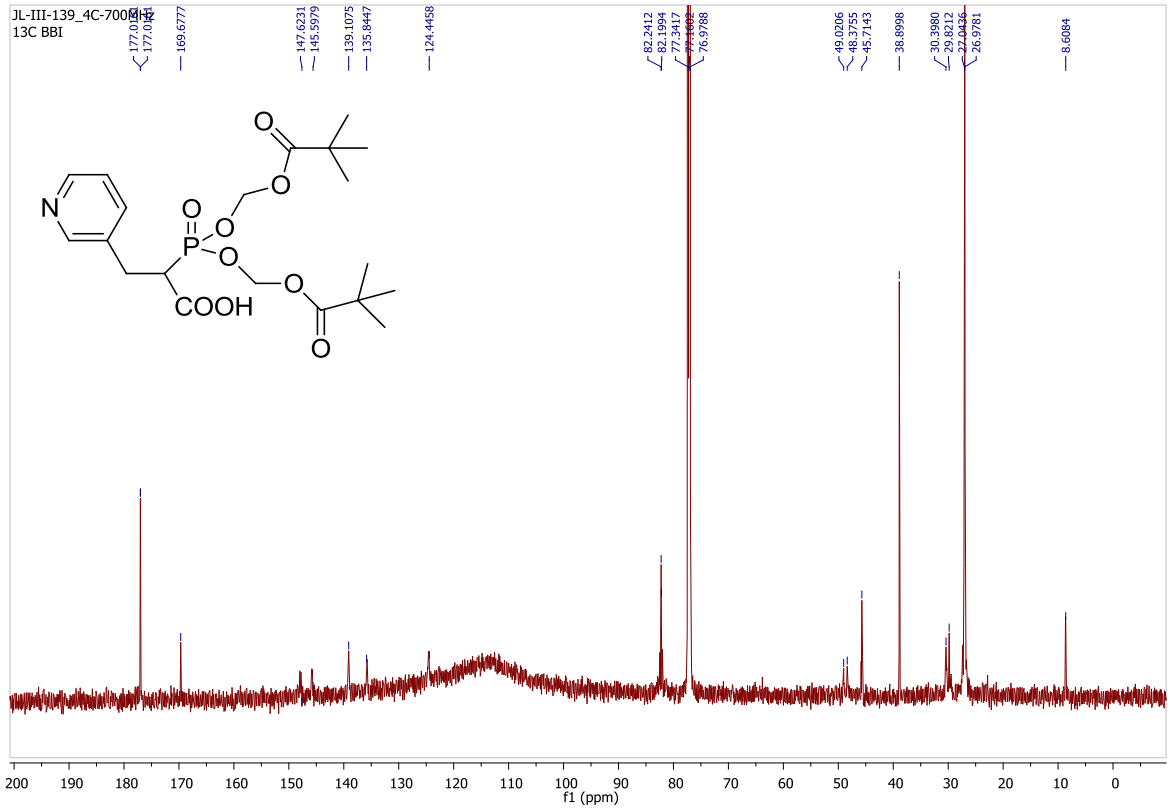


Figure S14. ^{13}C NMR of compound **7** (176 MHz, $CDCl_3$).

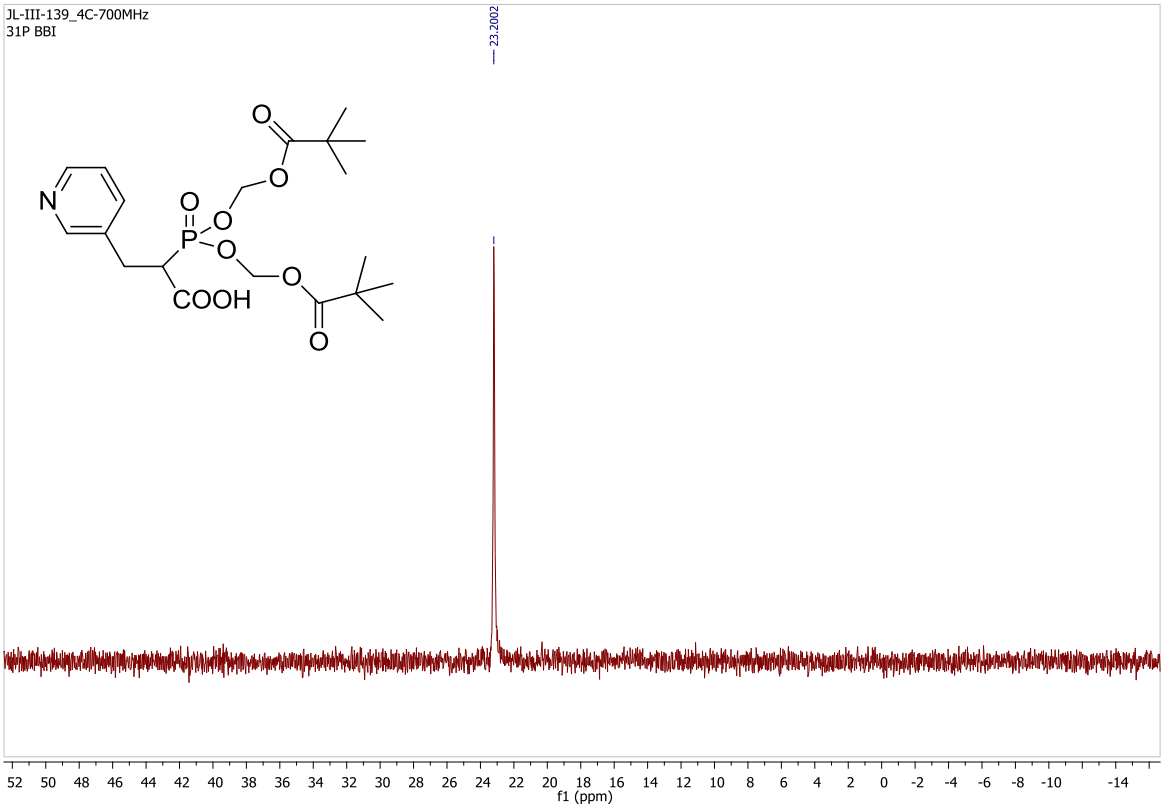


Figure S15. ^{31}P NMR of compound **7** (283 MHz, $CDCl_3$).

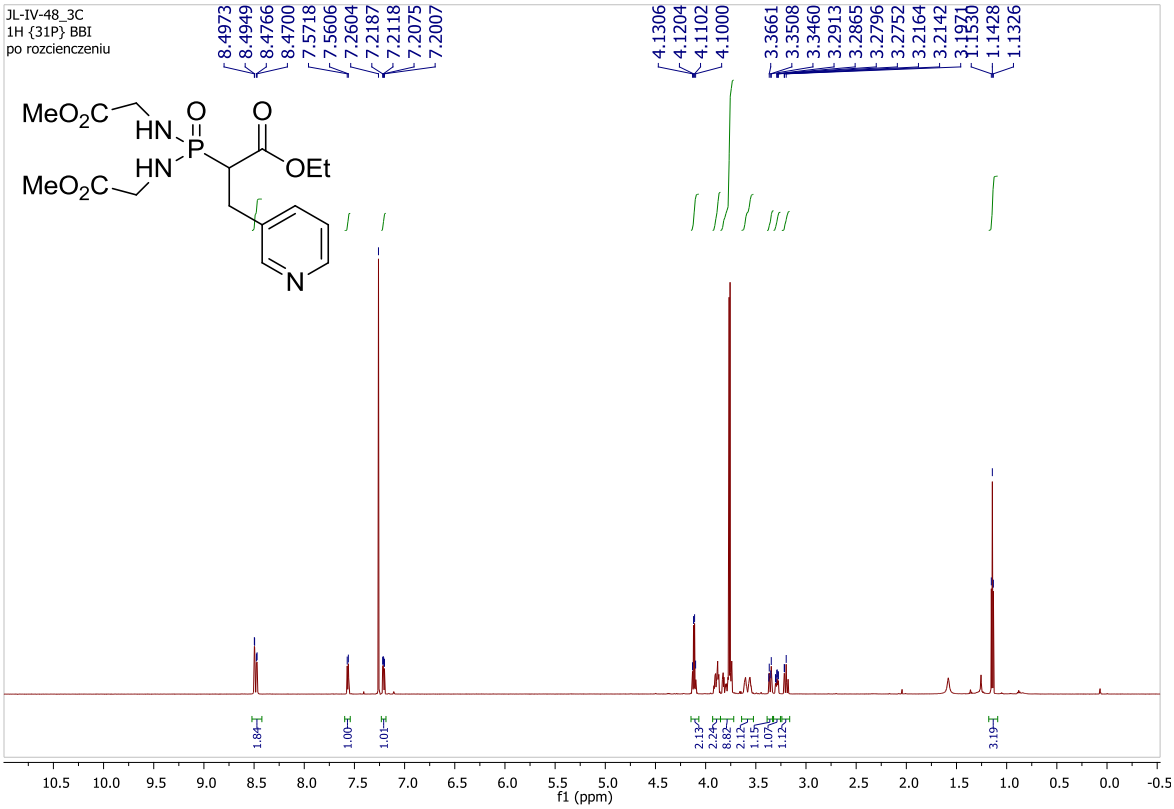


Figure S16. ^{1}H NMR of compound **8** (700 MHz, $CDCl_3$).

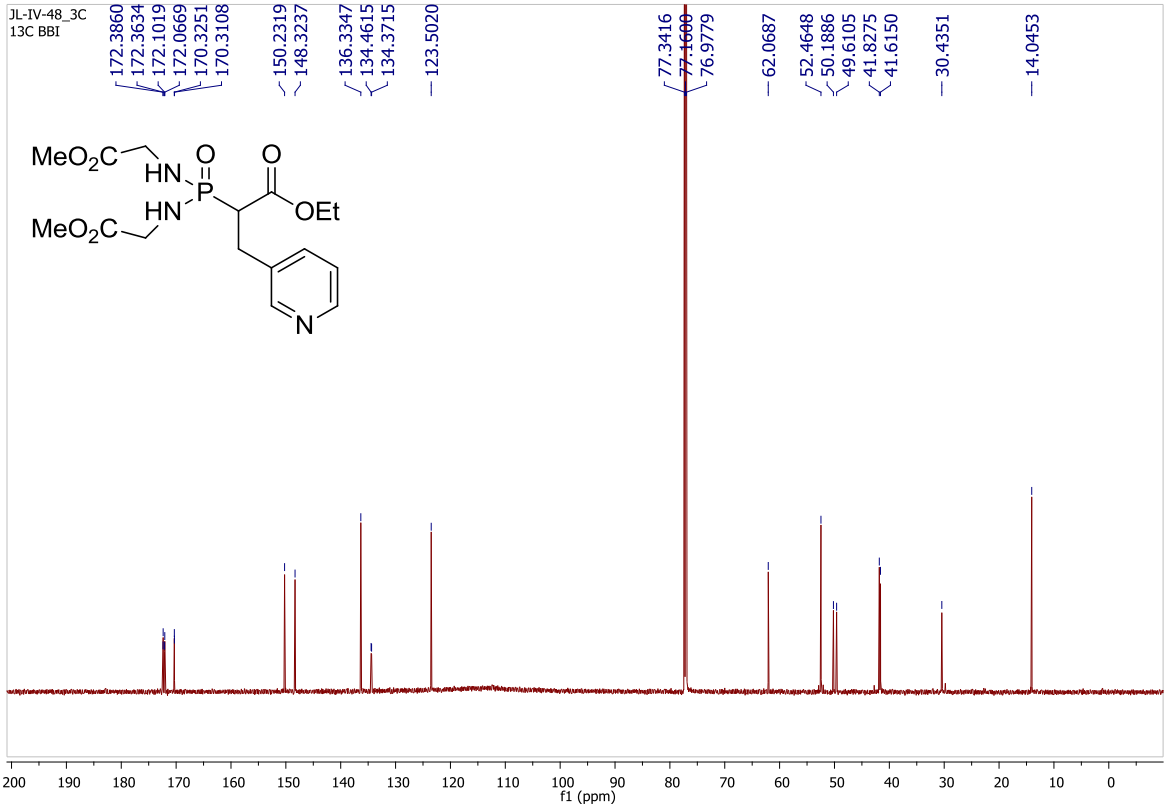


Figure S17. ^{13}C NMR of compound **8** (176 MHz, $CDCl_3$).

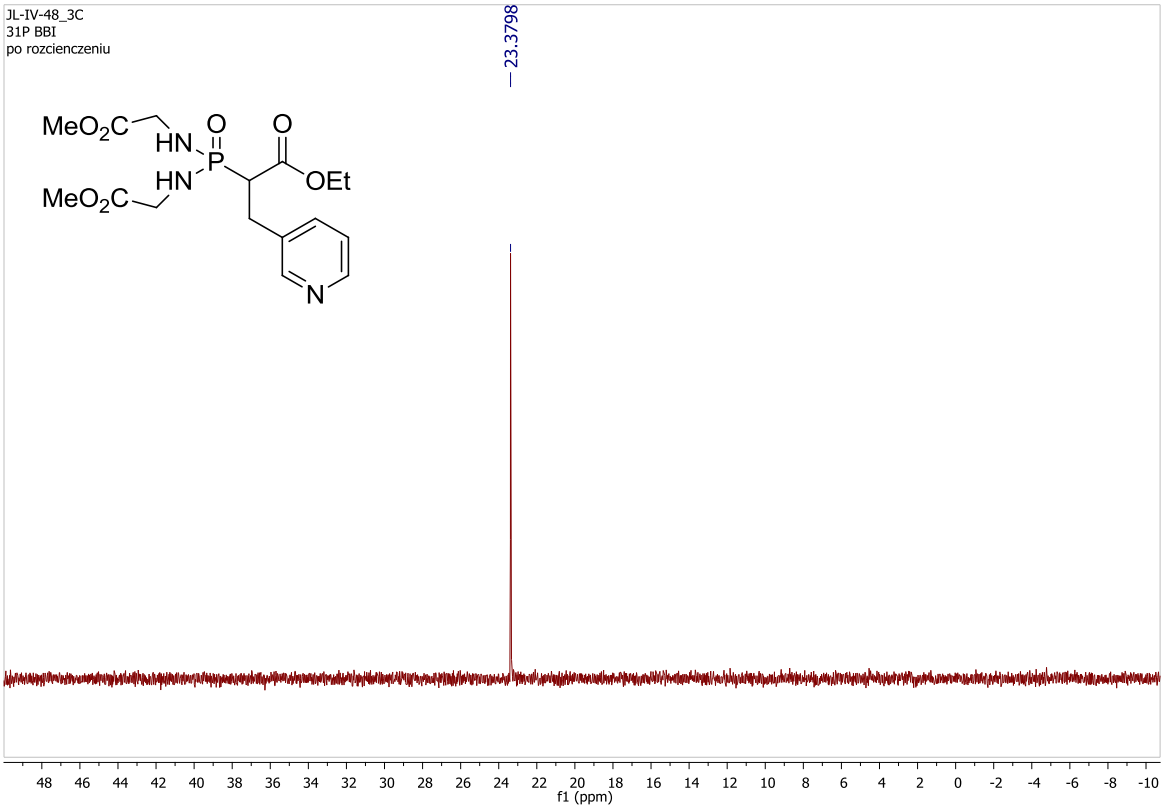


Figure S18. ^{31}P NMR of compound **8** (283 MHz, $CDCl_3$).

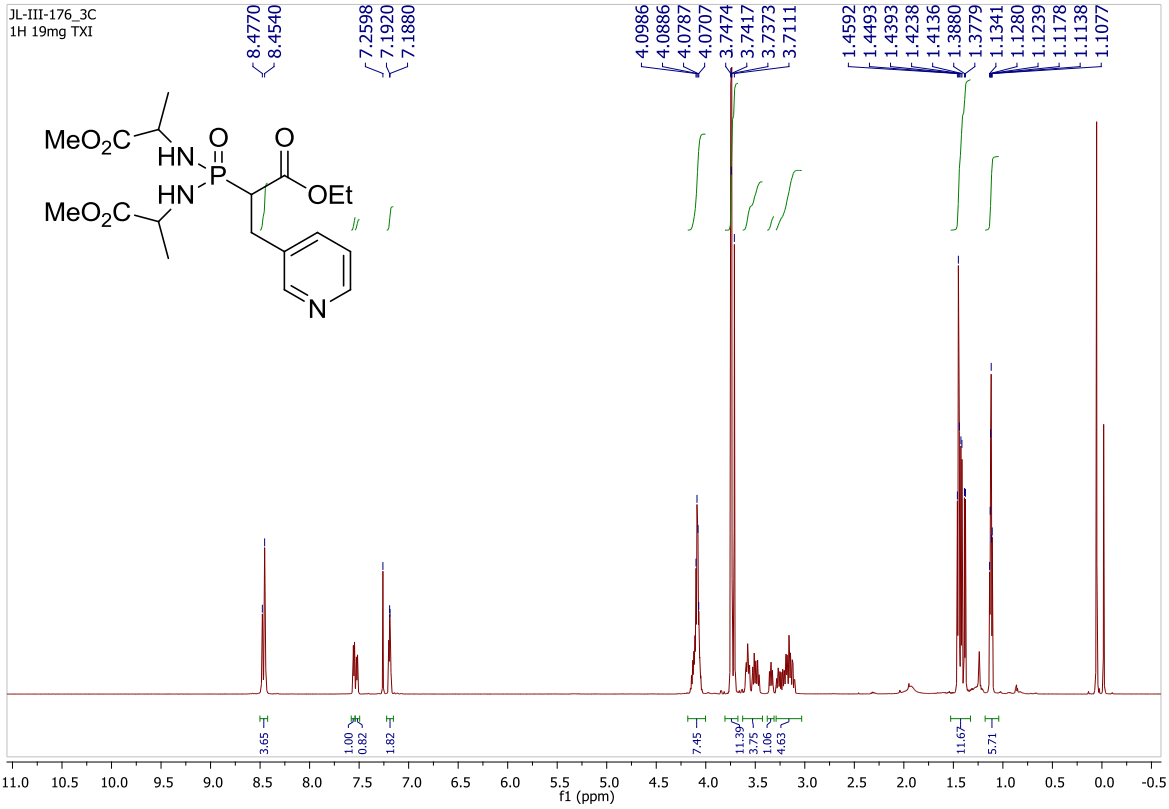


Figure S19. ^{1}H NMR of compound **9** (700 MHz, $CDCl_3$).

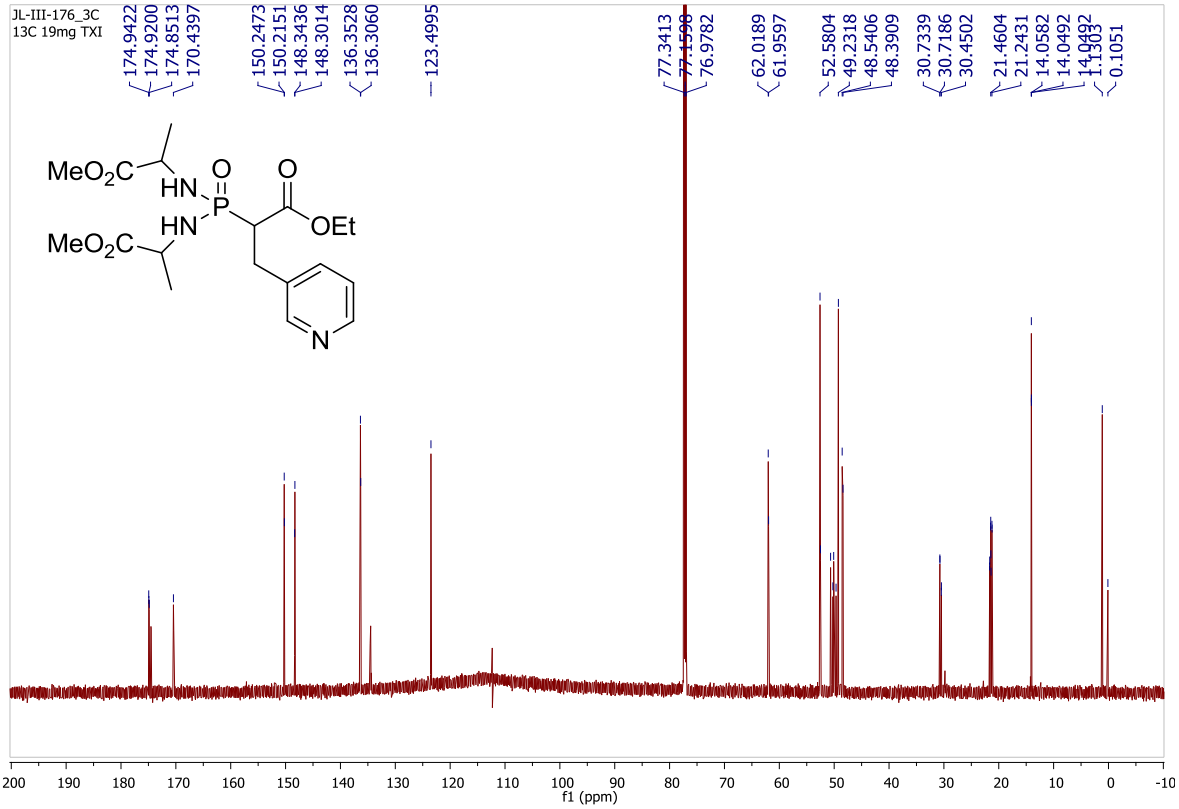


Figure S20. ^{13}C NMR of compound **9** (176 MHz, $CDCl_3$).

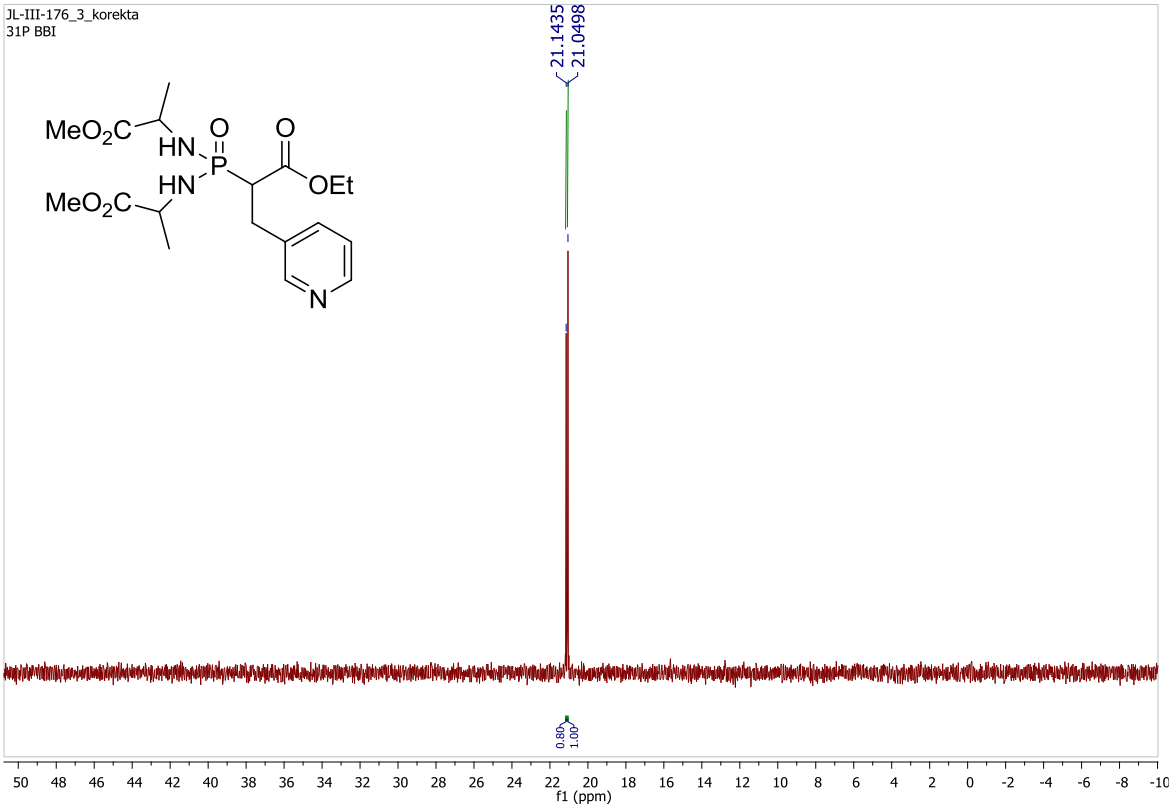


Figure S21. ^{31}P NMR of compound **9** (283 MHz, $CDCl_3$).

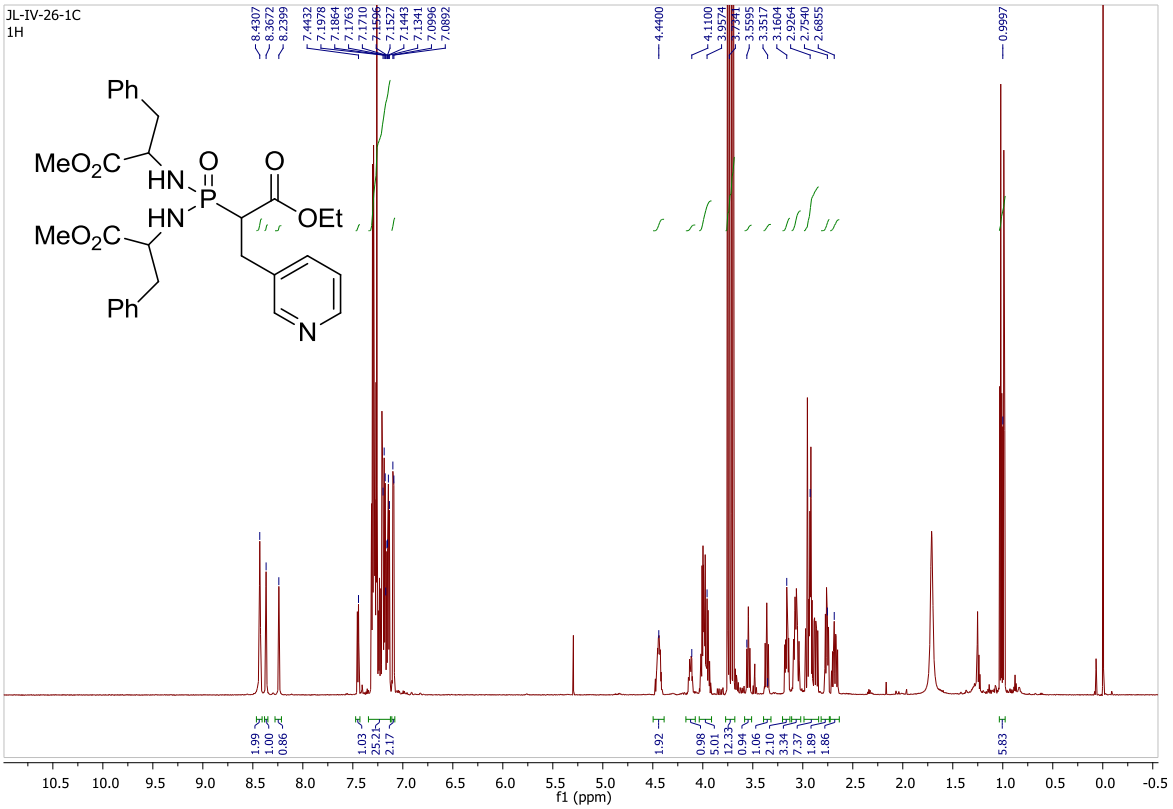


Figure S22. ^{1}H NMR of compound **10** (700 MHz, $CDCl_3$).

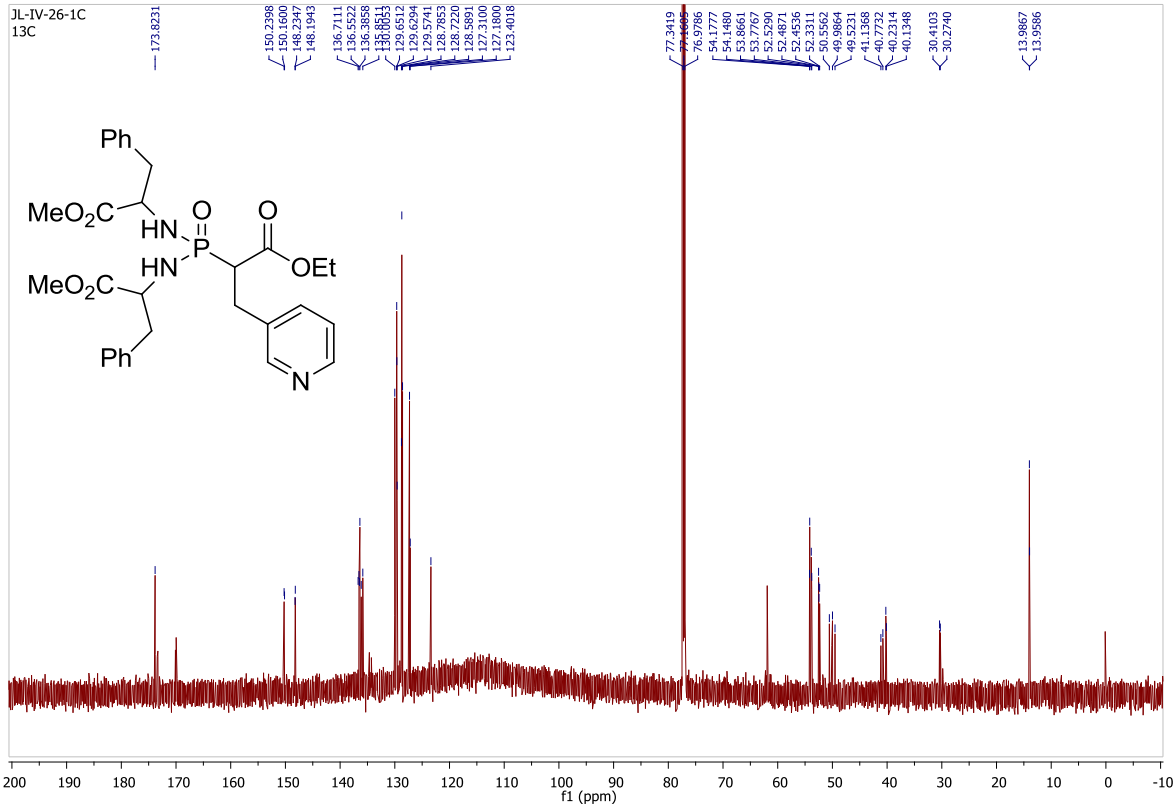


Figure S23. ^{13}C NMR of compound **10** (176 MHz, $CDCl_3$).

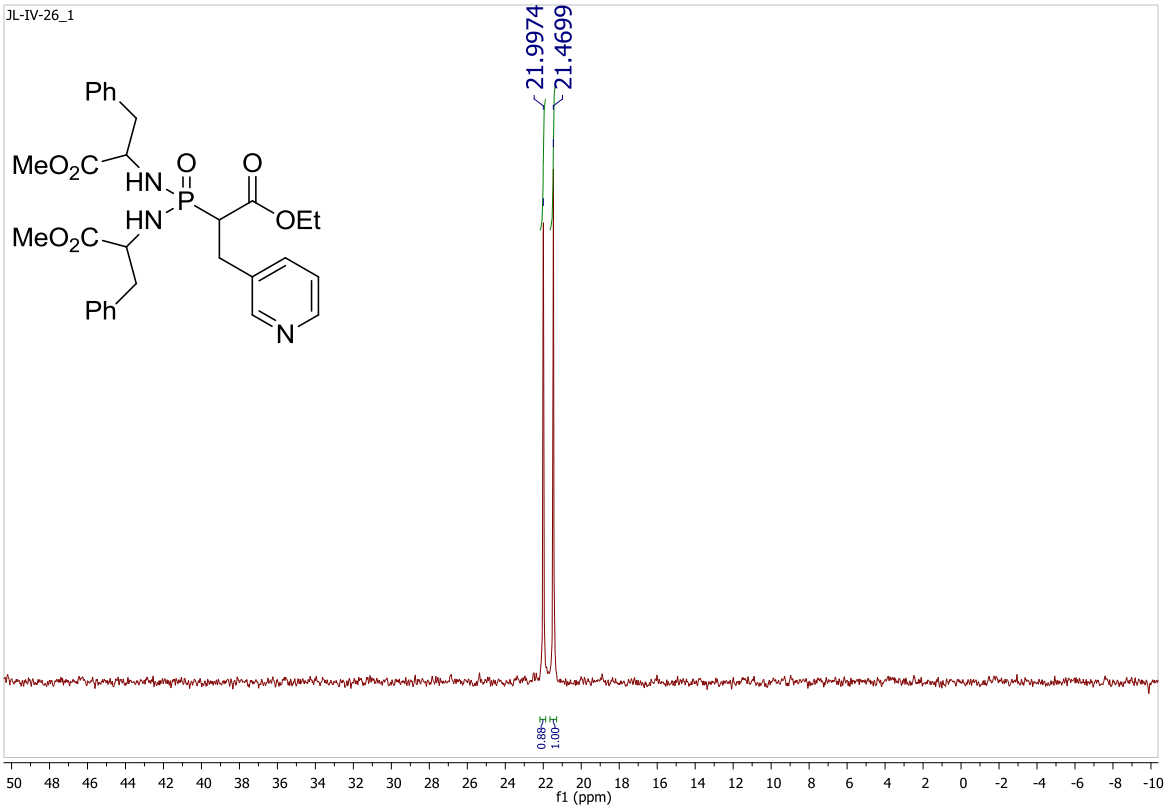


Figure S24. ^{31}P NMR of compound **10** (283 MHz, $CDCl_3$).

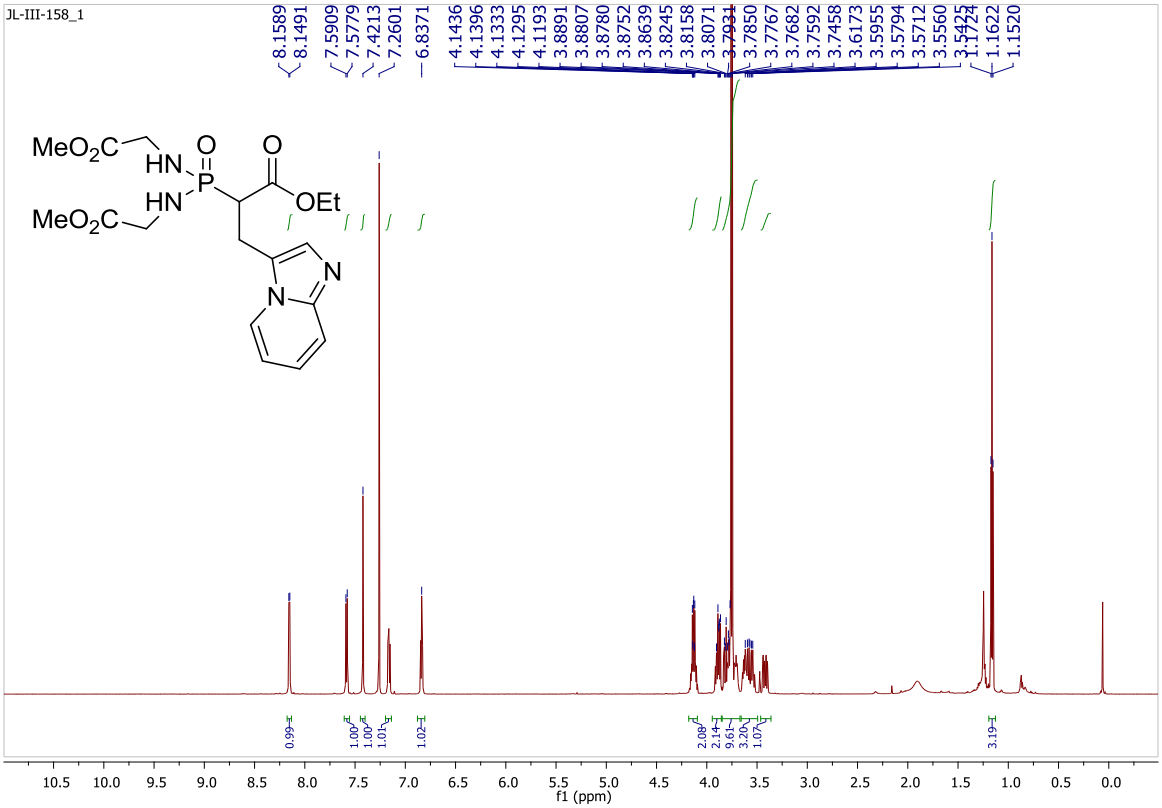


Figure S25. ^{1}H NMR of compound **11** (700 MHz, $CDCl_3$).

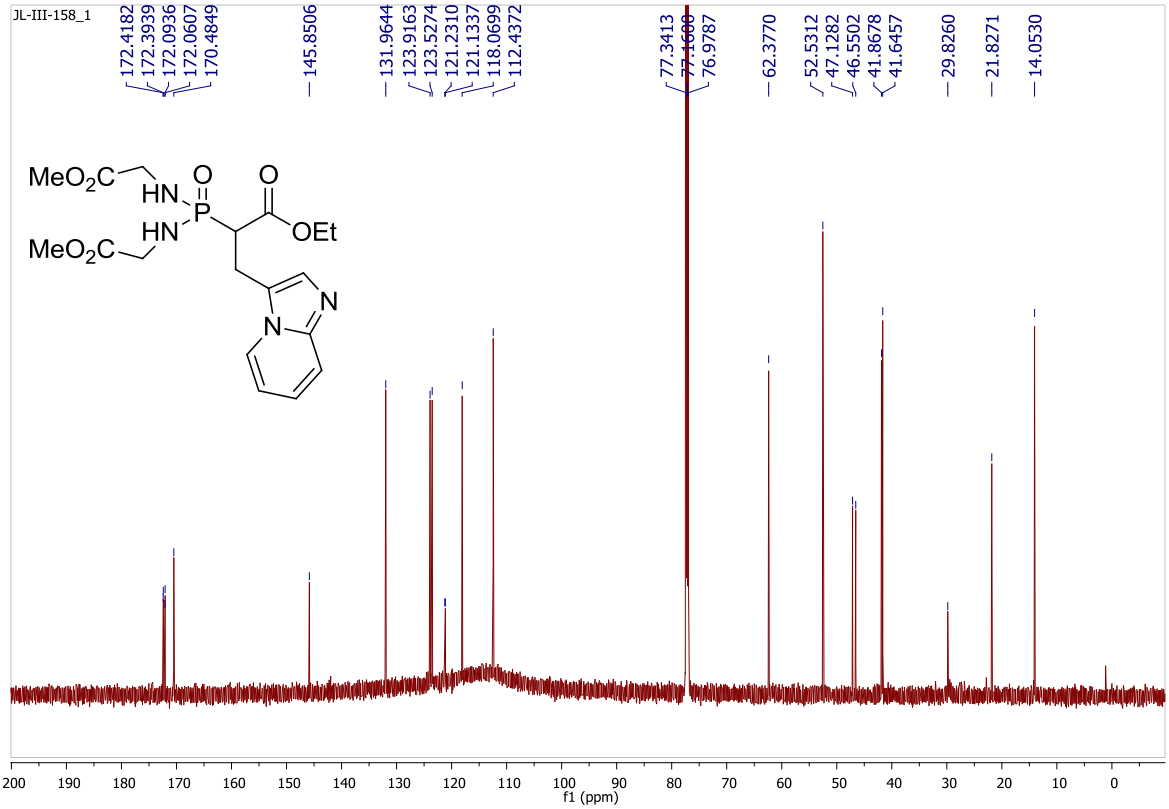


Figure S26. ^{13}C NMR of compound **11** (176 MHz, $CDCl_3$).

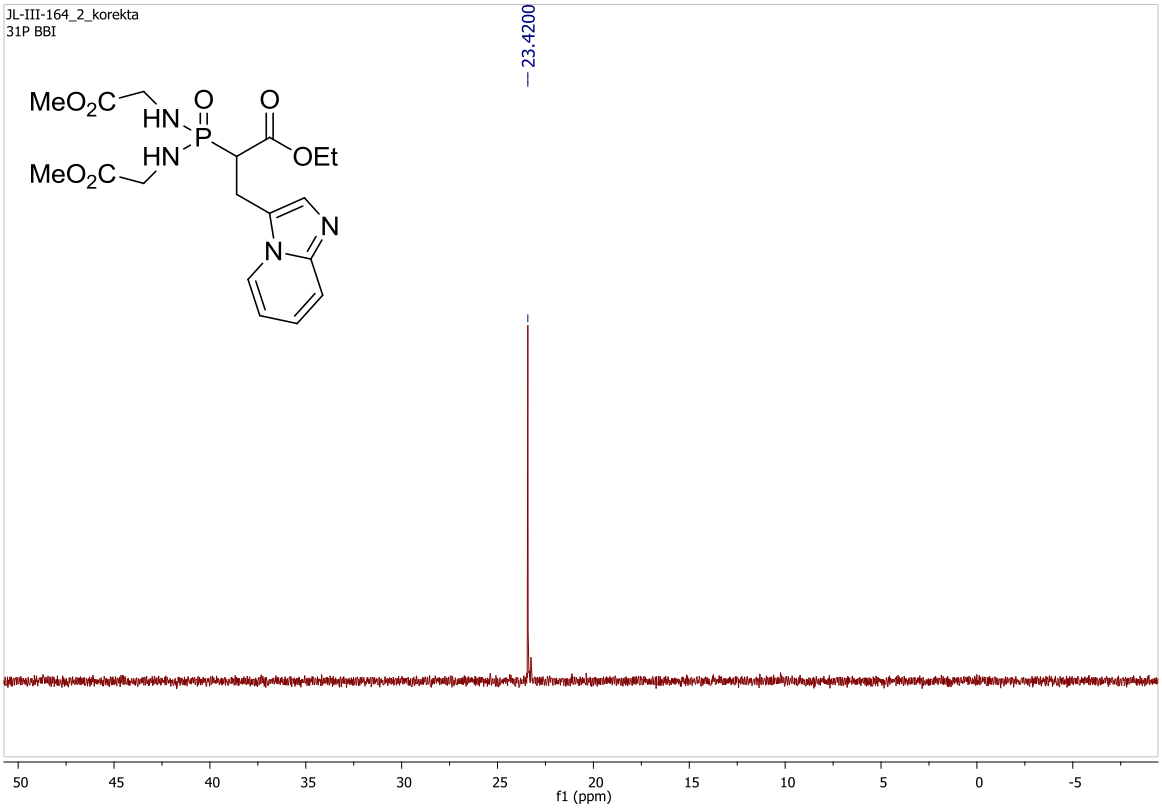


Figure S27. ^{31}P NMR of compound **11** (283 MHz, $CDCl_3$).

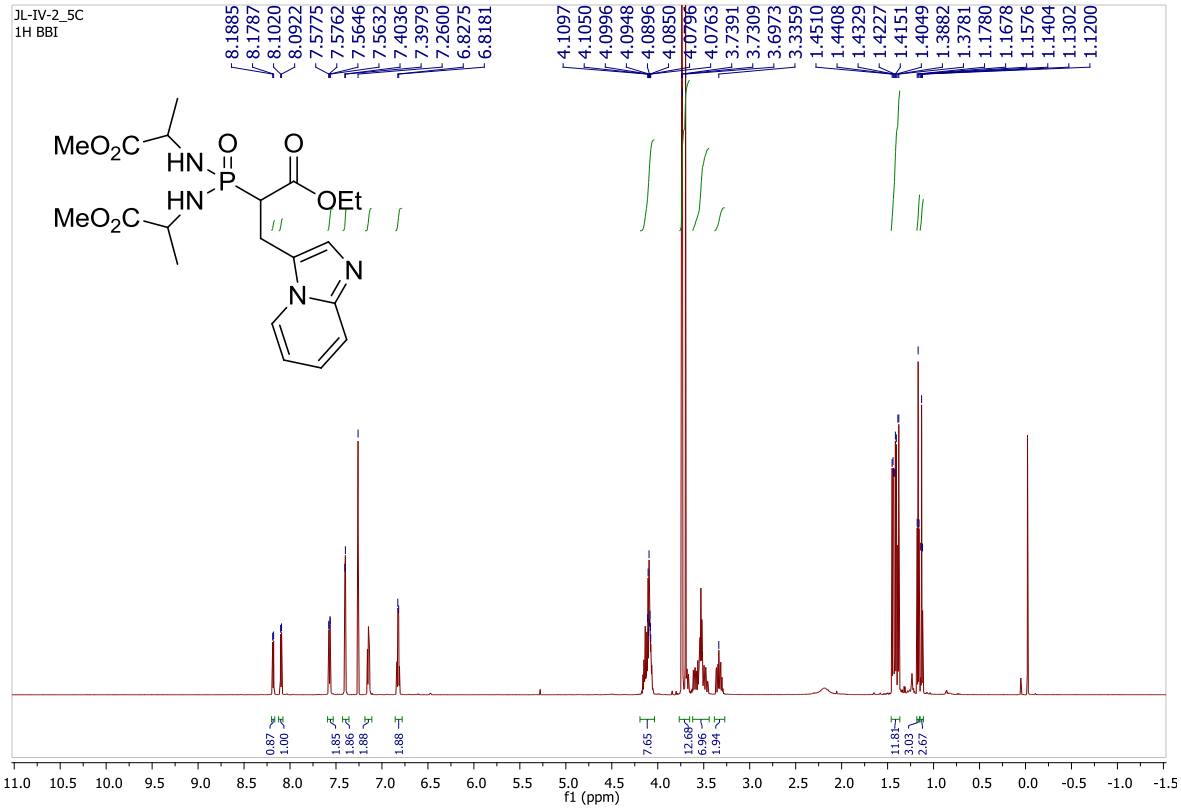


Figure S28. ^{1}H NMR of compound **12** (700 MHz, $CDCl_3$).

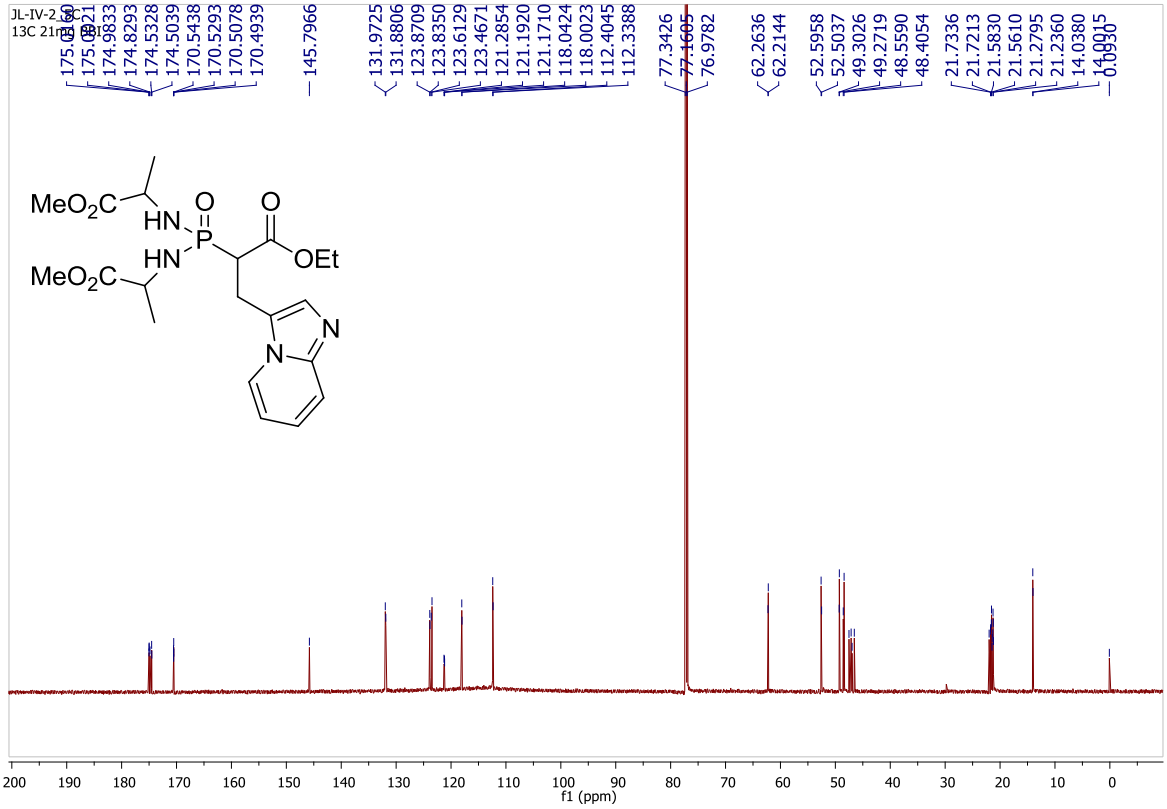


Figure S29. ^{13}C NMR of compound **12** (176 MHz, $CDCl_3$).

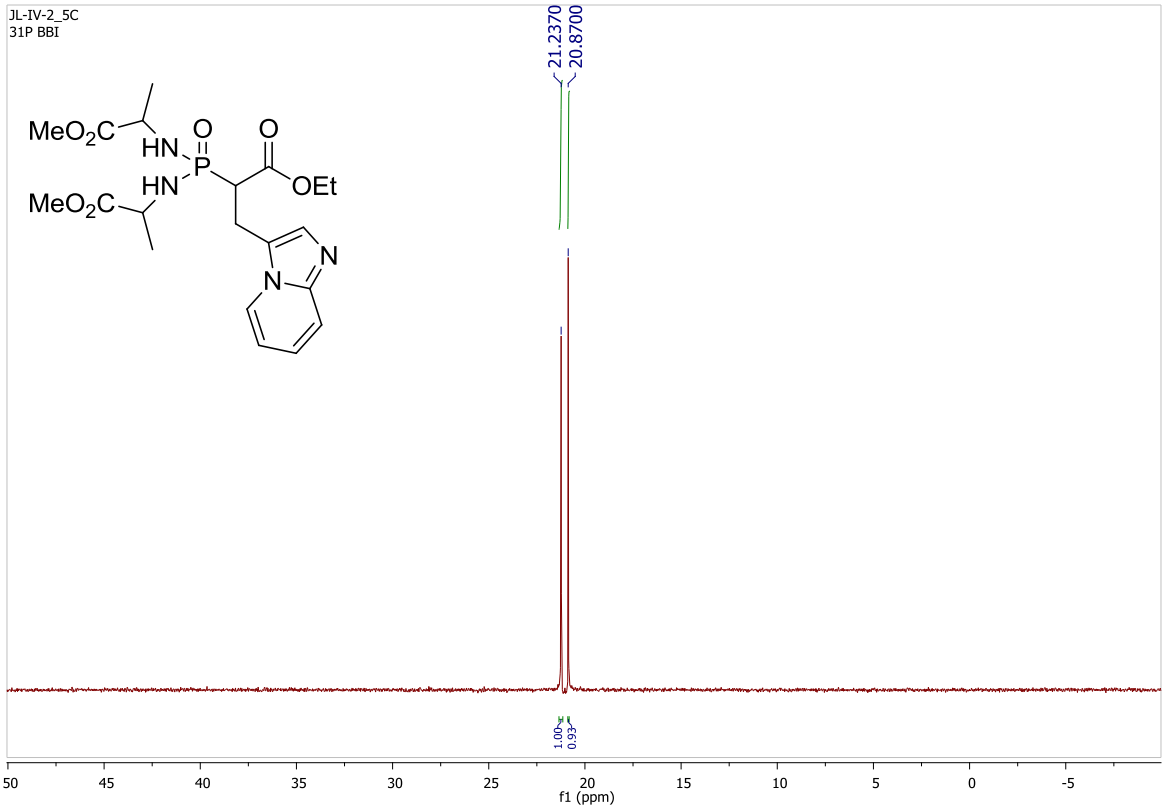


Figure S30. ^{31}P NMR of compound **12** (283 MHz, $CDCl_3$).

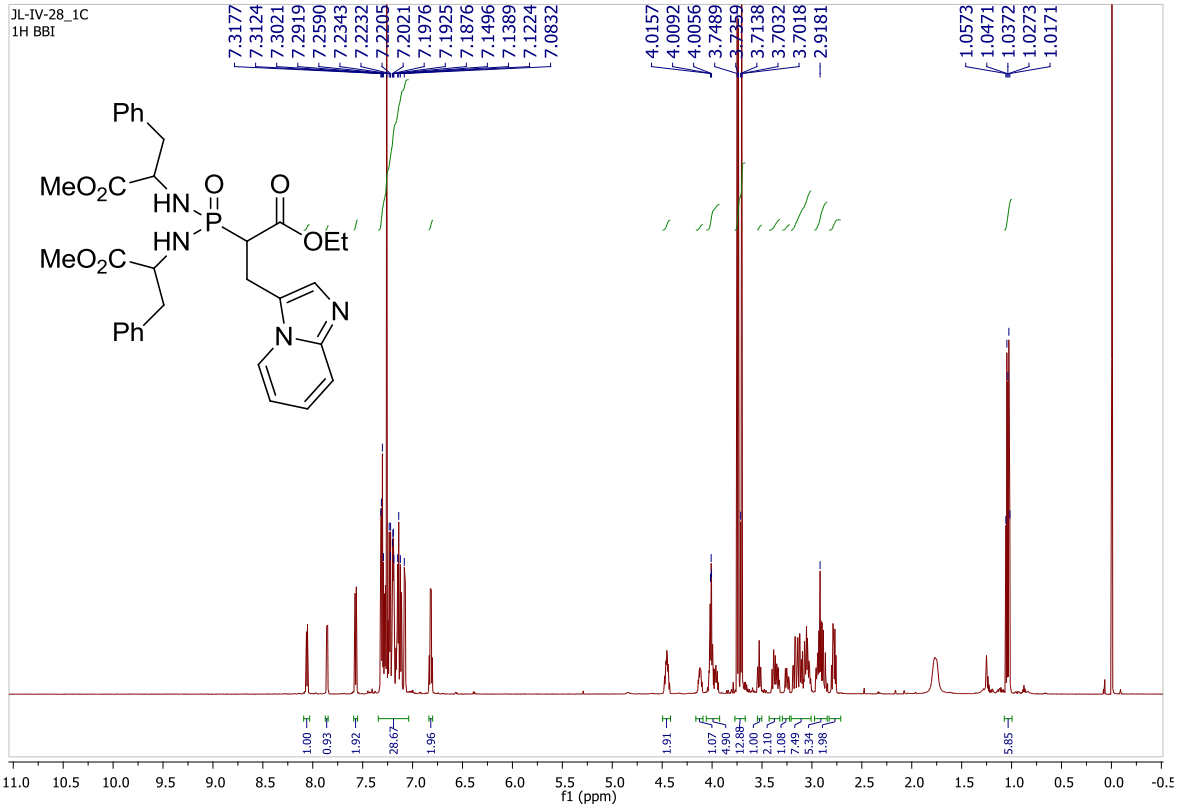


Figure S31. ^{1}H NMR of compound **13** (700 MHz, $CDCl_3$).

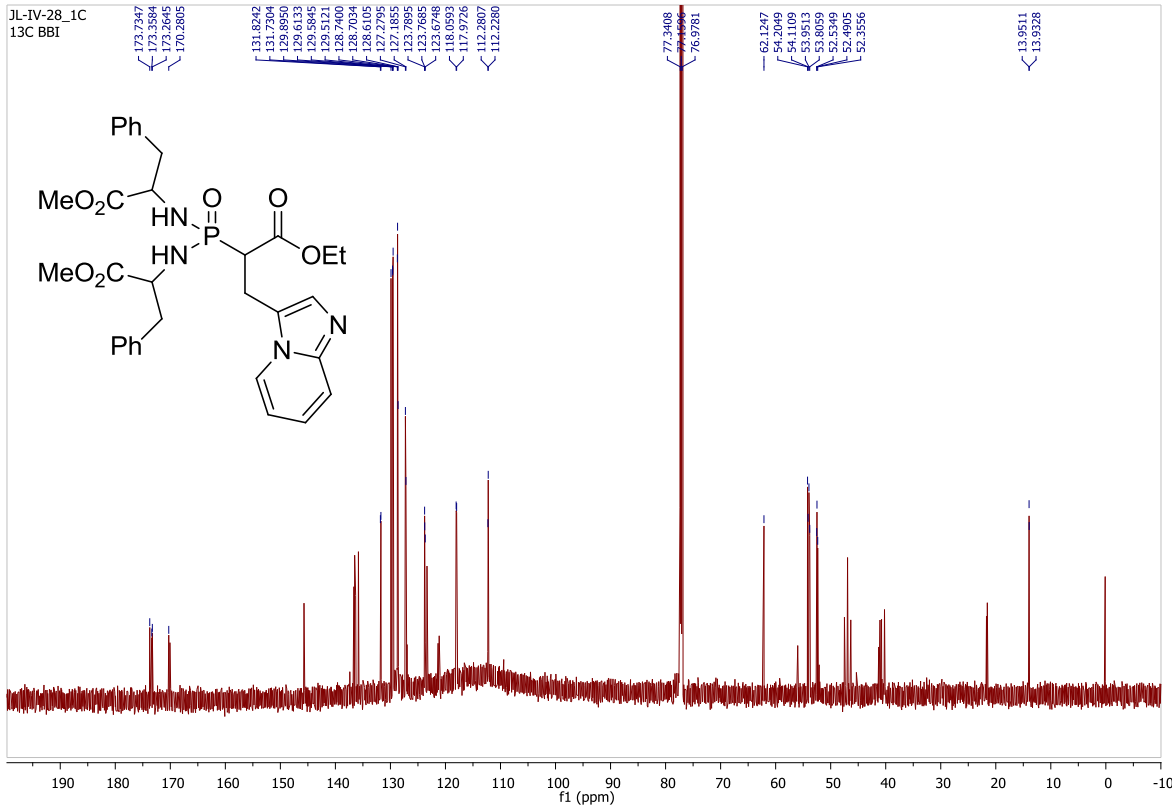


Figure S32. ^{13}C NMR of compound **13** (176 MHz, $CDCl_3$).

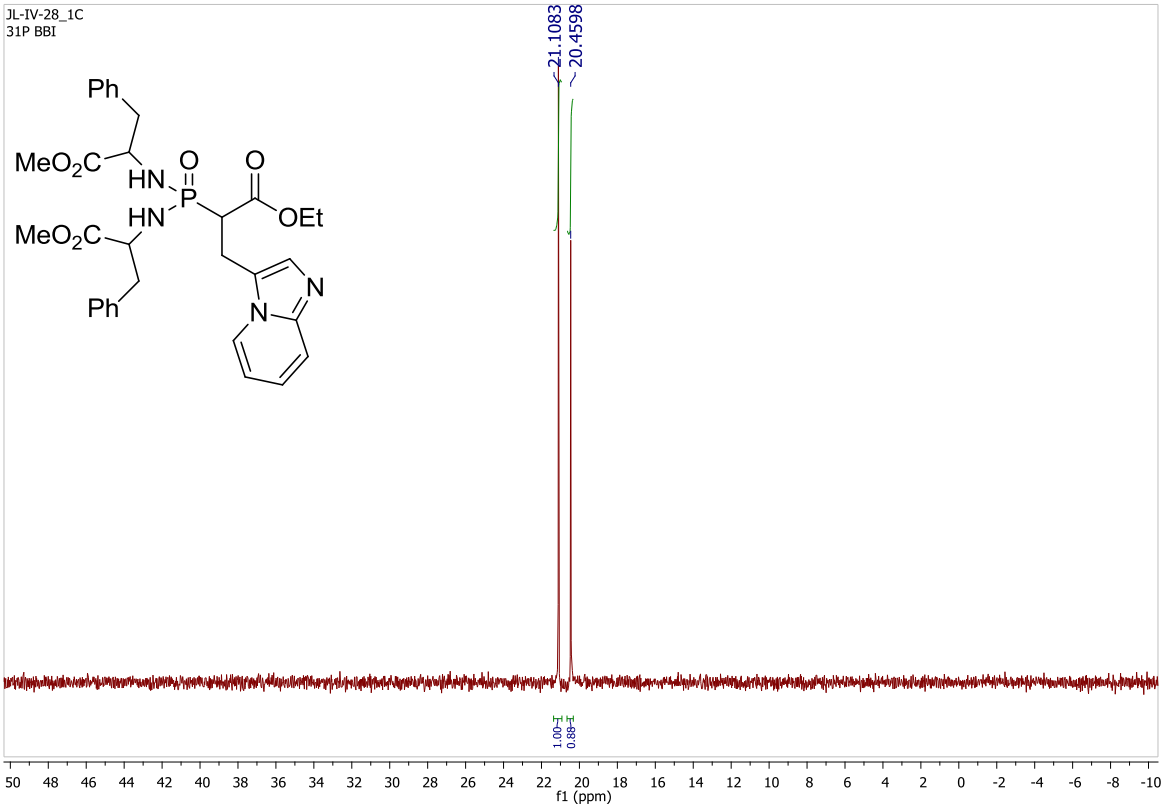


Figure S33. ^{31}P NMR of compound **13** (283 MHz, $CDCl_3$).

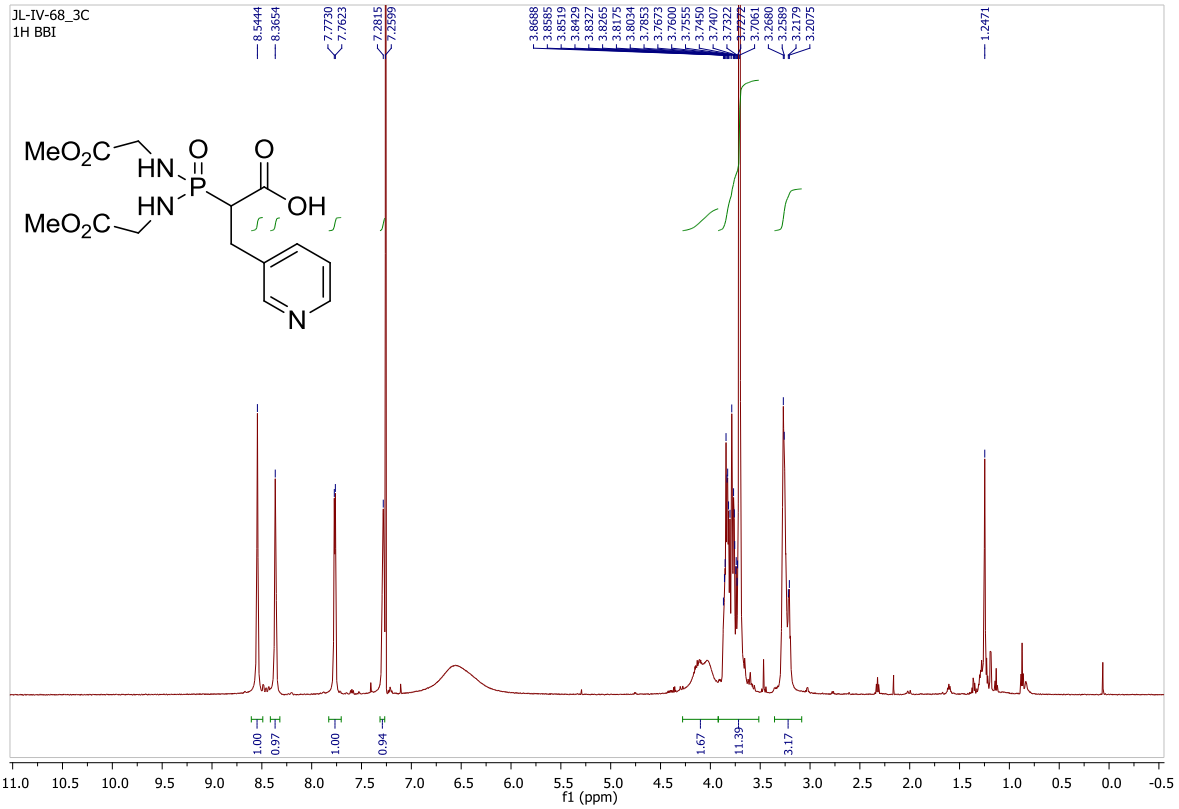


Figure S34. ^{1}H NMR of compound **14** (700 MHz, $CDCl_3$).

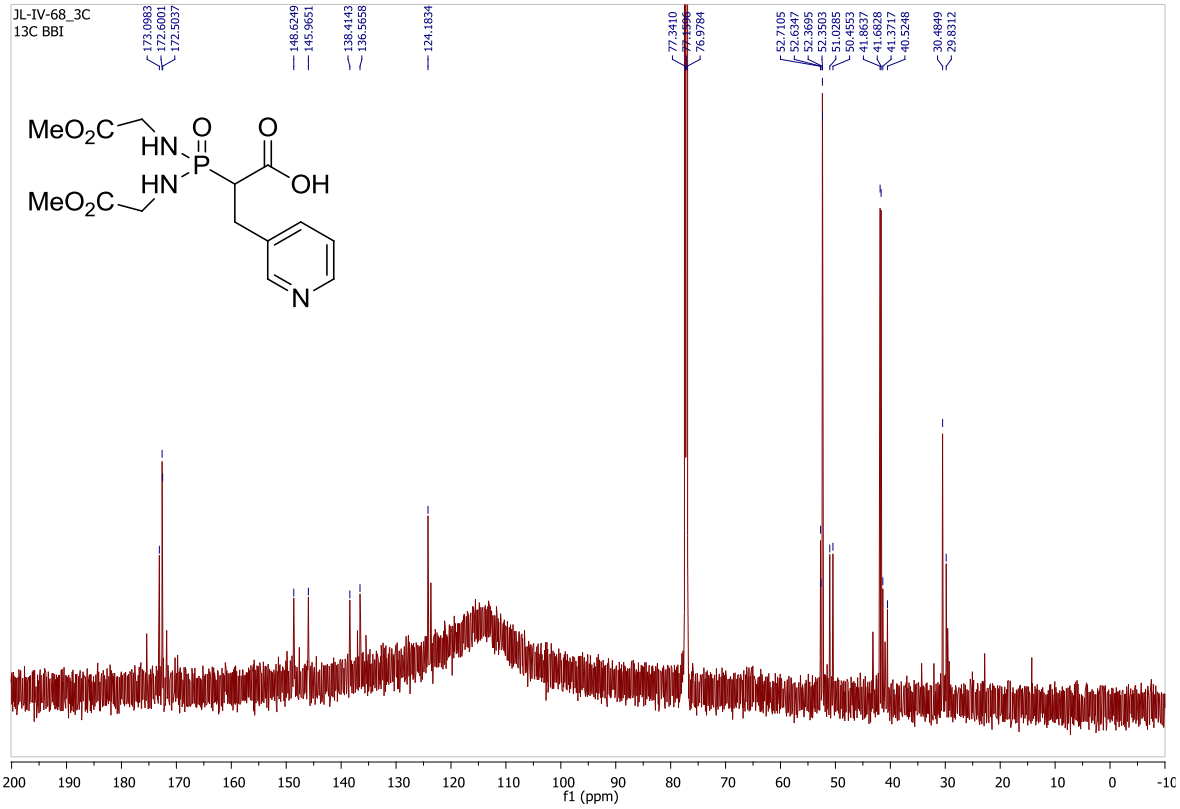


Figure S35. ^{13}C NMR of compound **14** (176 MHz, $CDCl_3$).

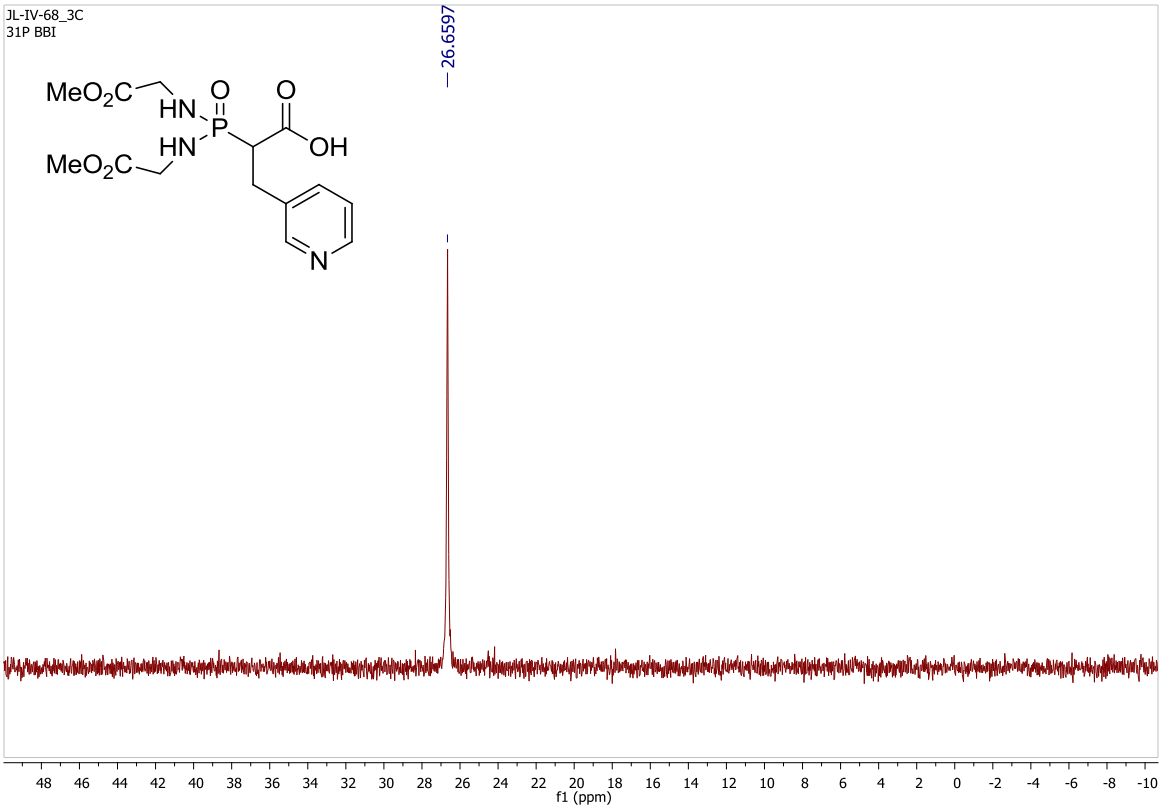


Figure S36. ^{31}P NMR of compound **14** (283 MHz, $CDCl_3$).

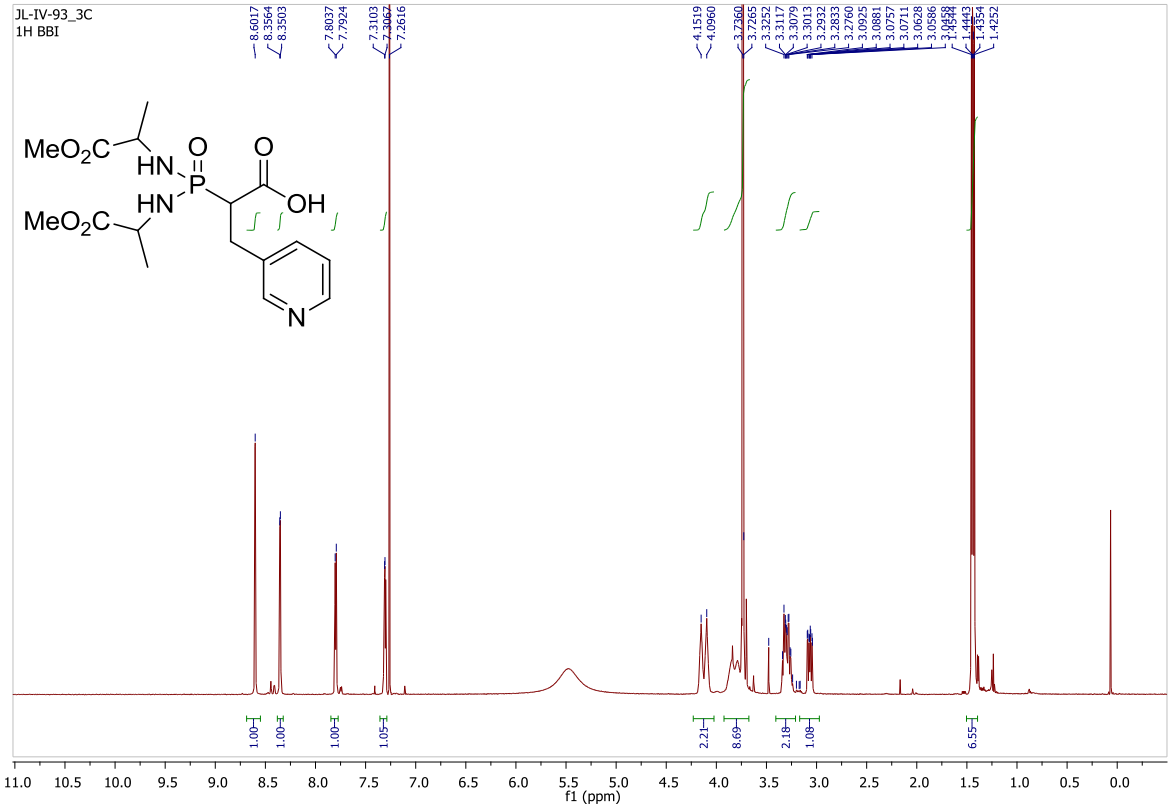


Figure S37. ^{1}H NMR of compound **15** (700 MHz, $CDCl_3$).

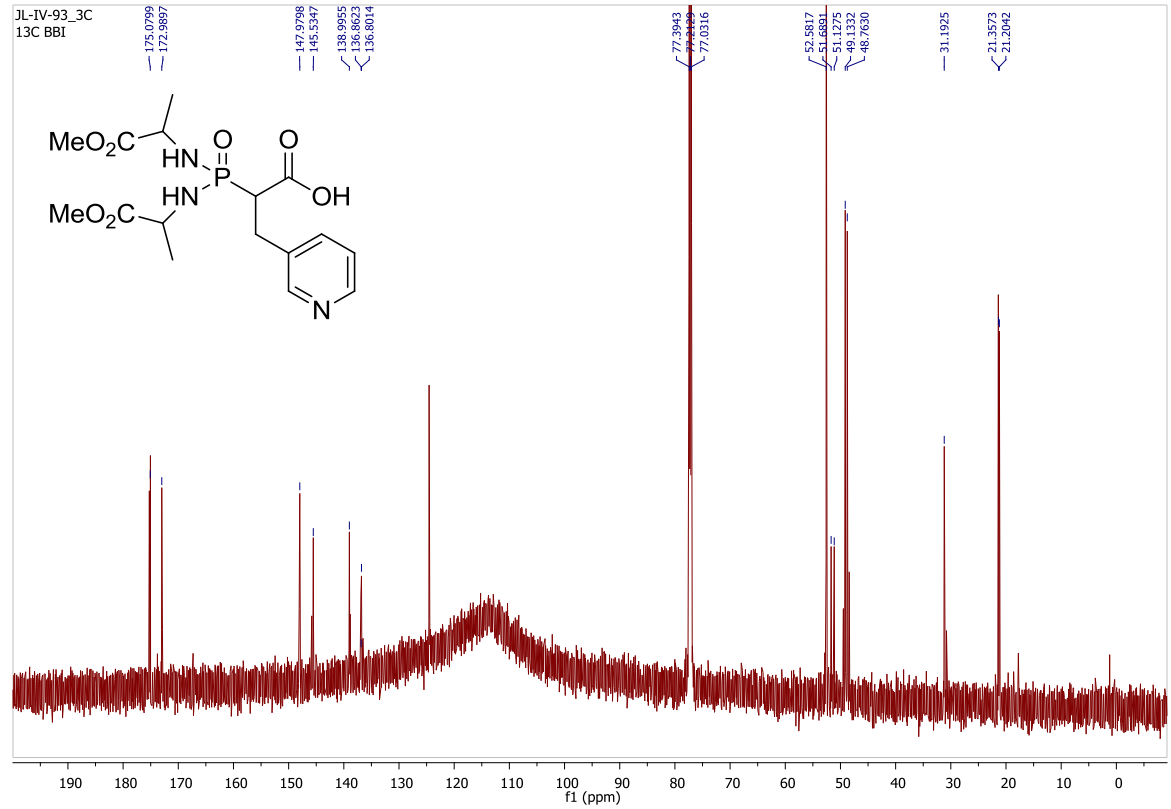


Figure S38. ^{13}C NMR of compound **15** (176 MHz, $CDCl_3$).

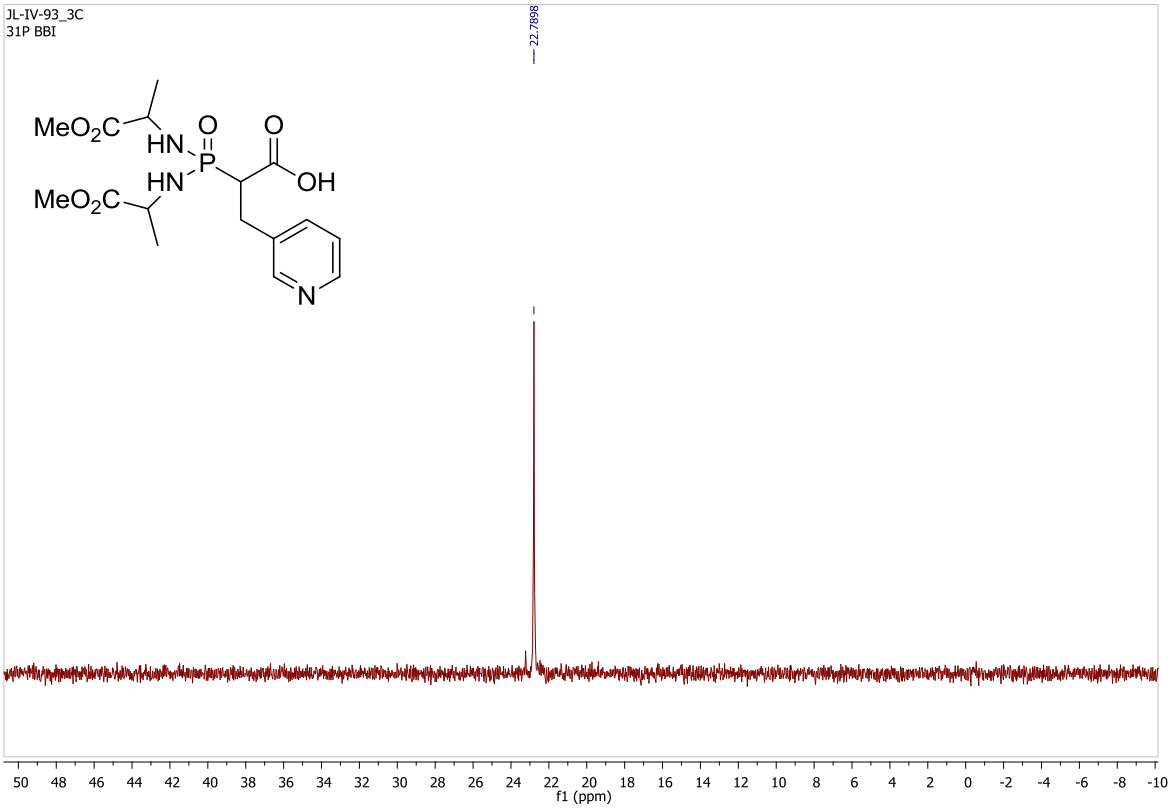


Figure S39. ^{31}P NMR of compound **15** (283 MHz, $CDCl_3$).

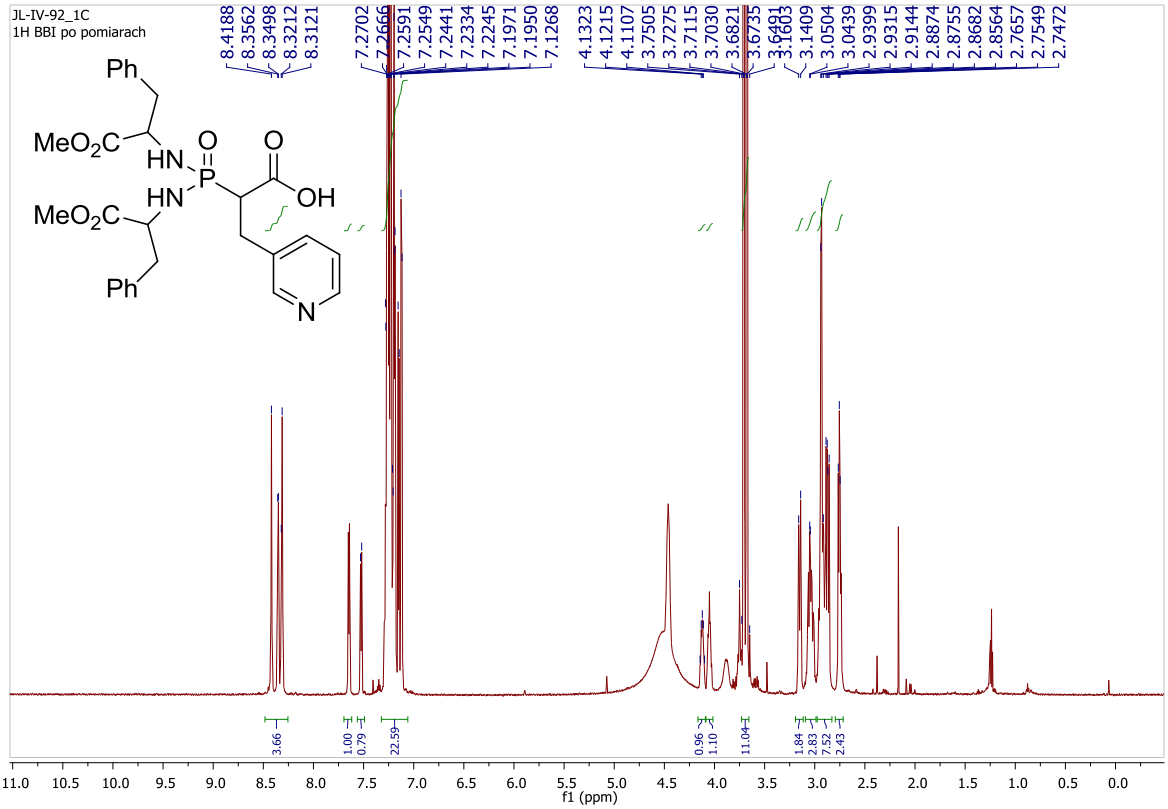


Figure S40. ^{1}H NMR of compound **16** (700 MHz, $CDCl_3$).

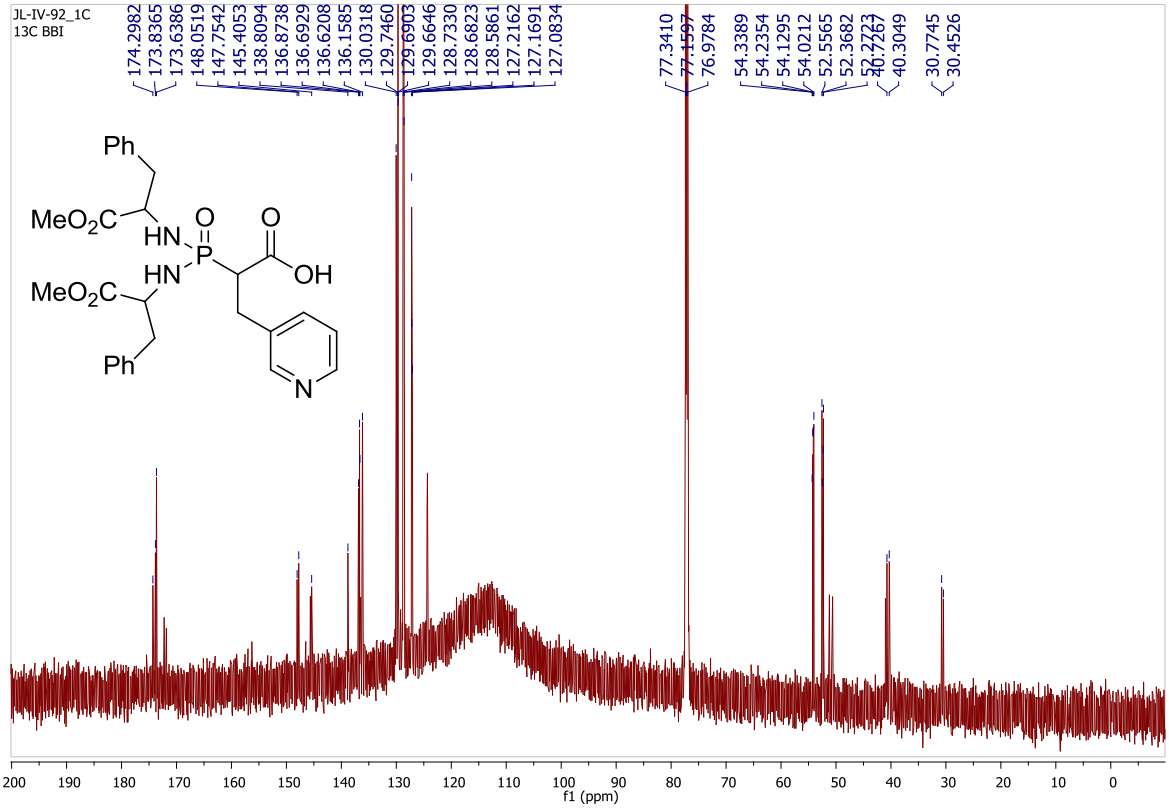


Figure S41. ^{13}C NMR of compound **16** (176 MHz, $CDCl_3$).

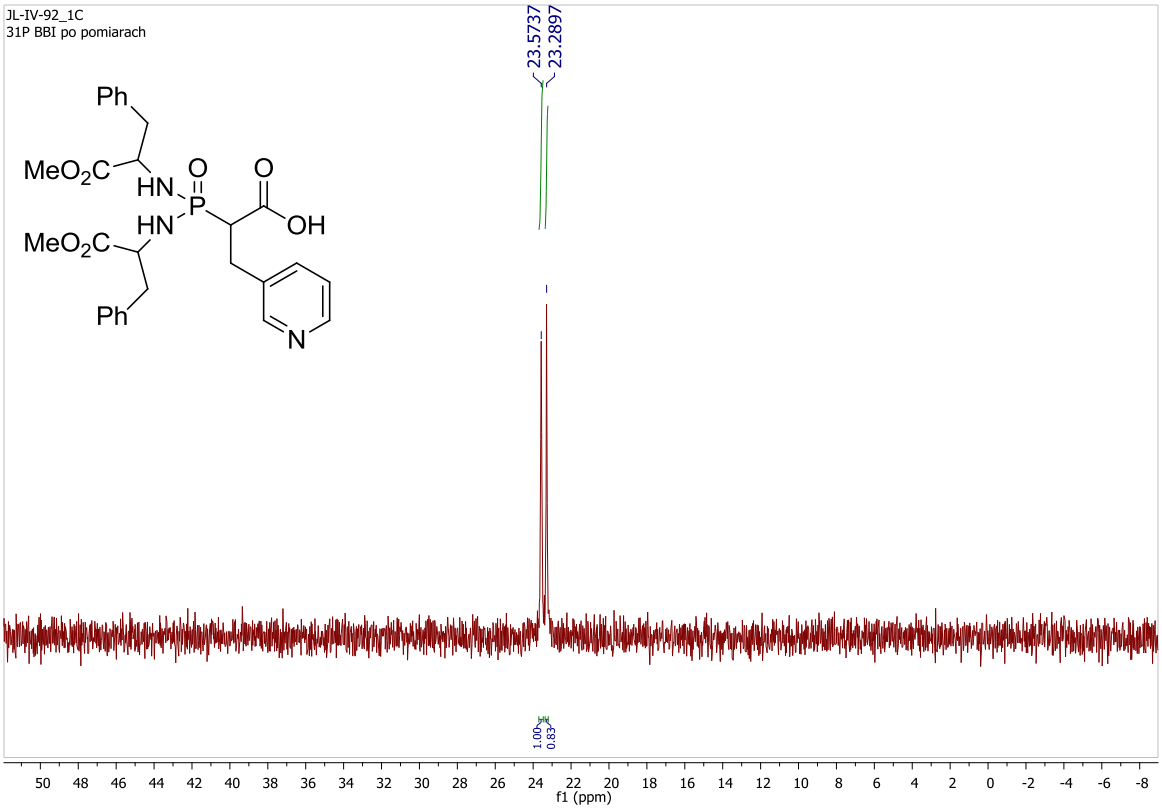


Figure S42. ^{31}P NMR of compound **16** (283 MHz, $CDCl_3$).

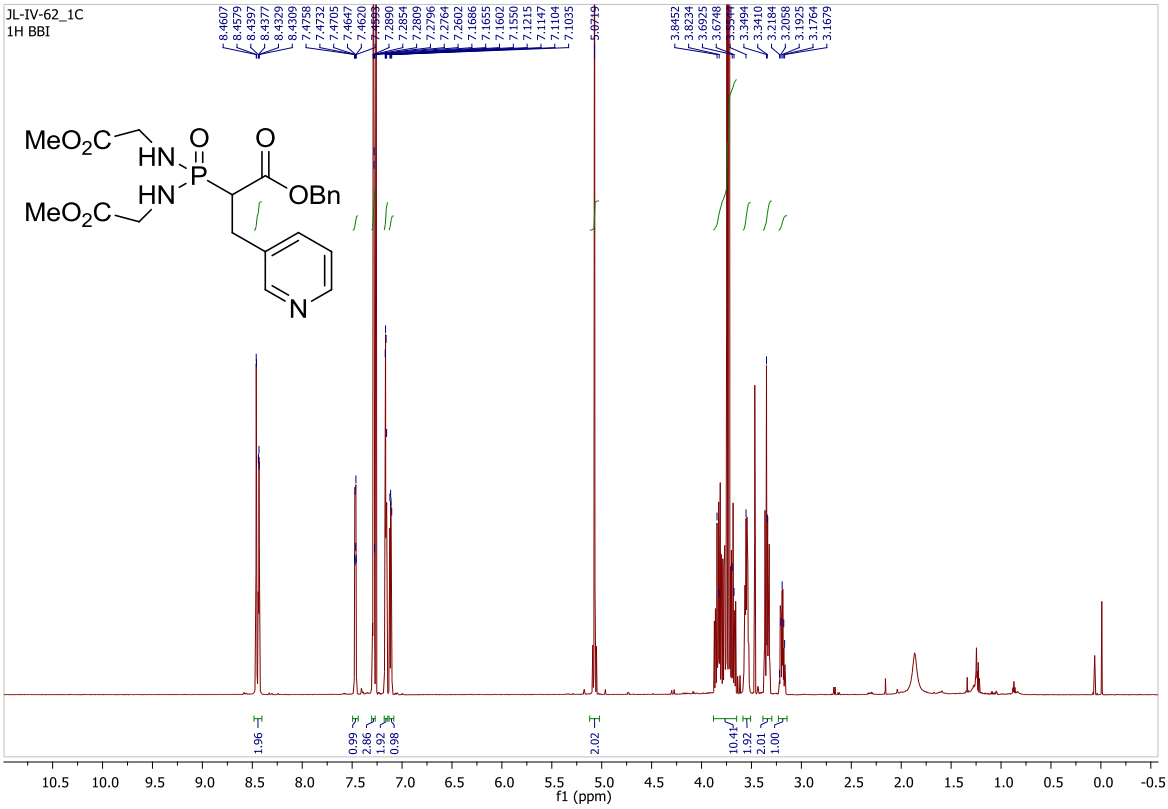


Figure S43. ^{1}H NMR of compound **26** (700 MHz, $CDCl_3$).

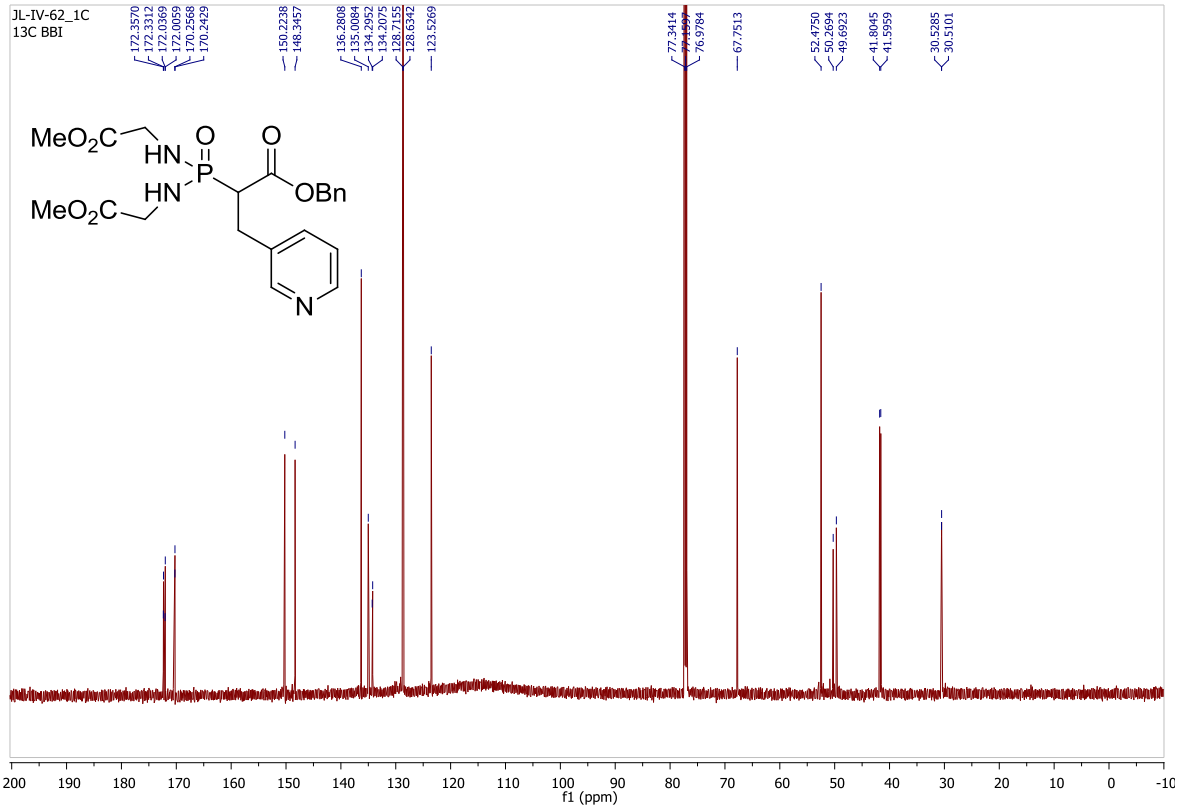


Figure S44. ^{13}C NMR of compound **26** (176 MHz, $CDCl_3$).

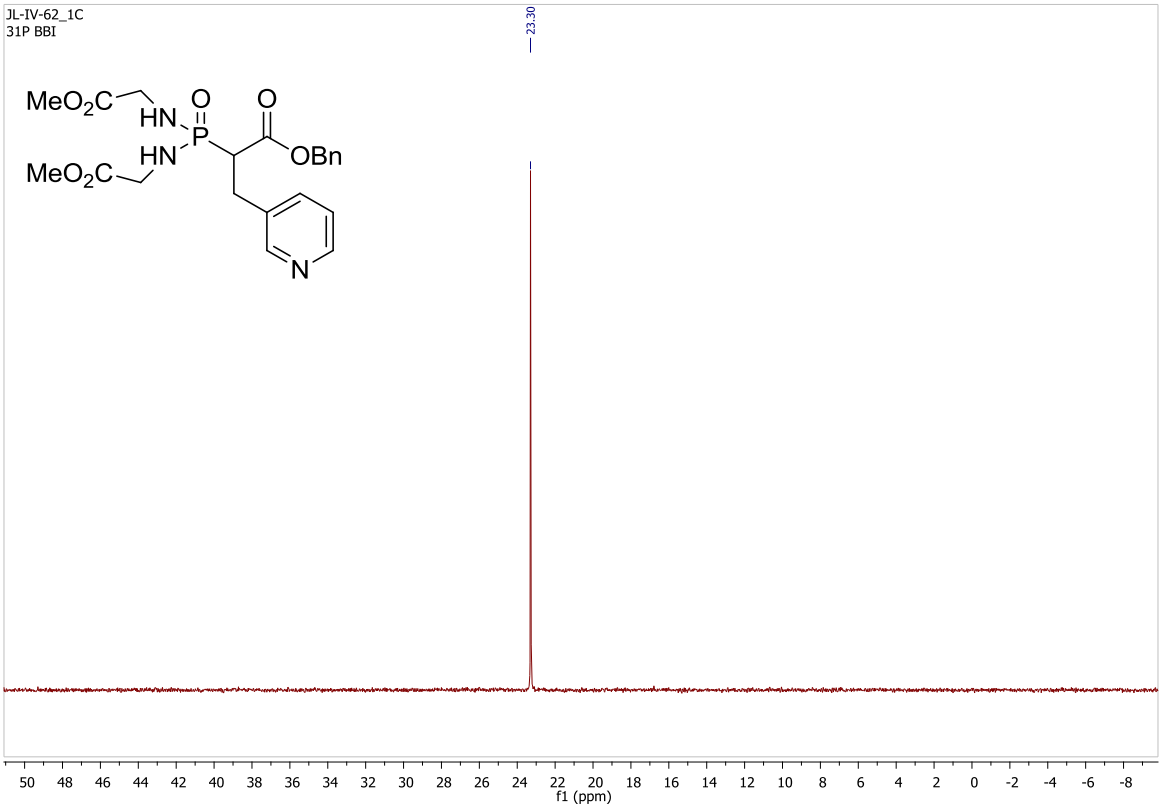


Figure S45. ^{31}P NMR of compound **26** (283 MHz, $CDCl_3$).

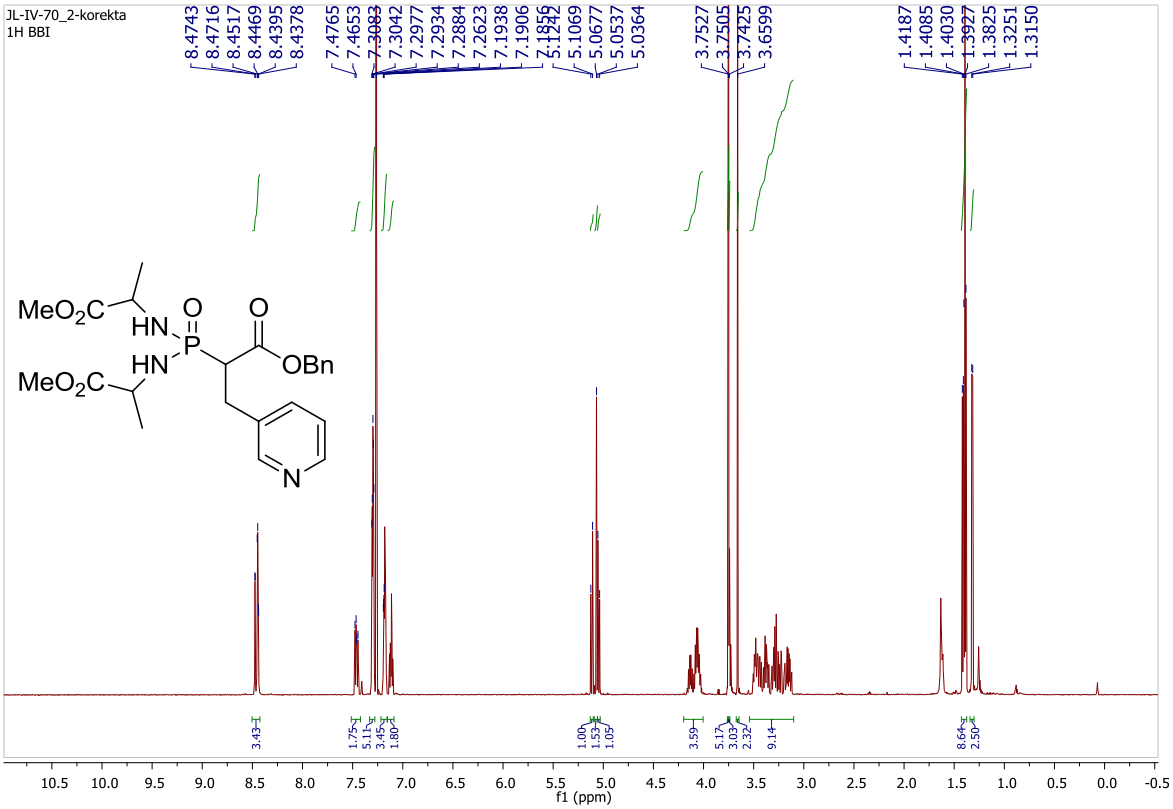


Figure S46. ^{1}H NMR of compound **27** (700 MHz, $CDCl_3$).

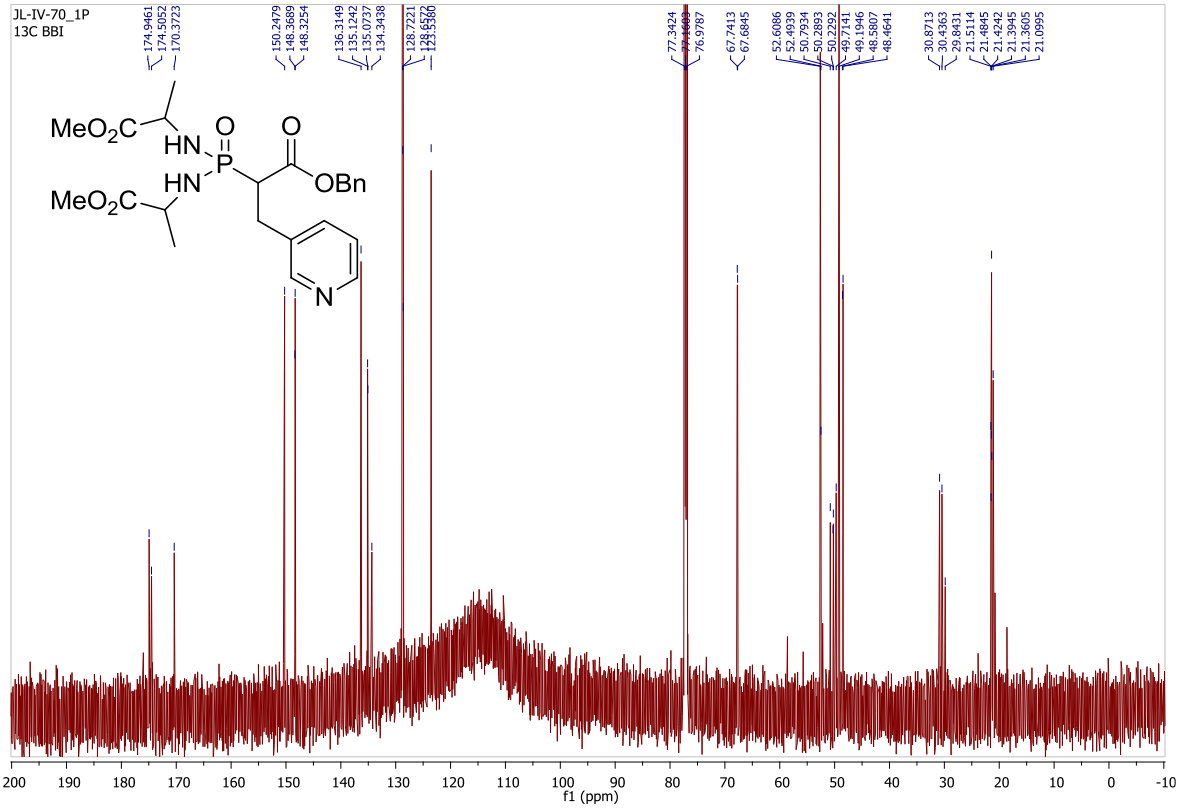


Figure S47. ^{13}C NMR of compound **27** (176 MHz, $CDCl_3$).

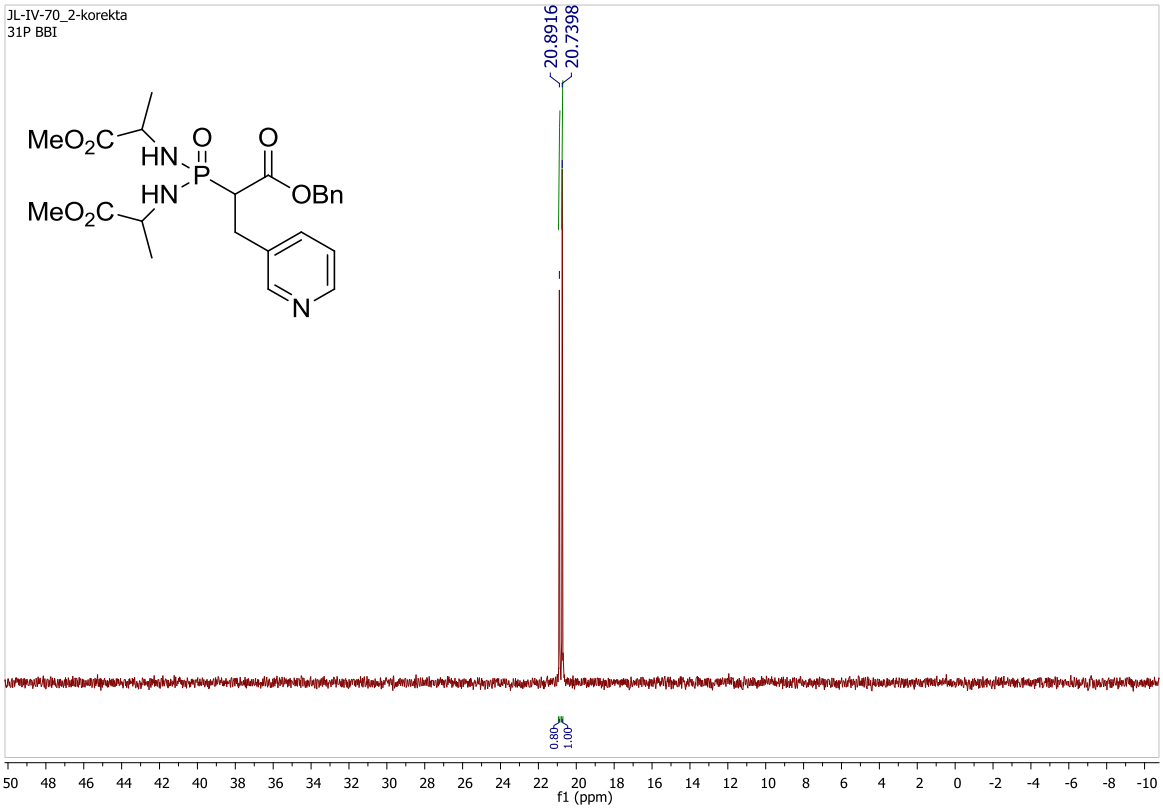


Figure S48. ^{31}P NMR of compound **27** (283 MHz, $CDCl_3$).

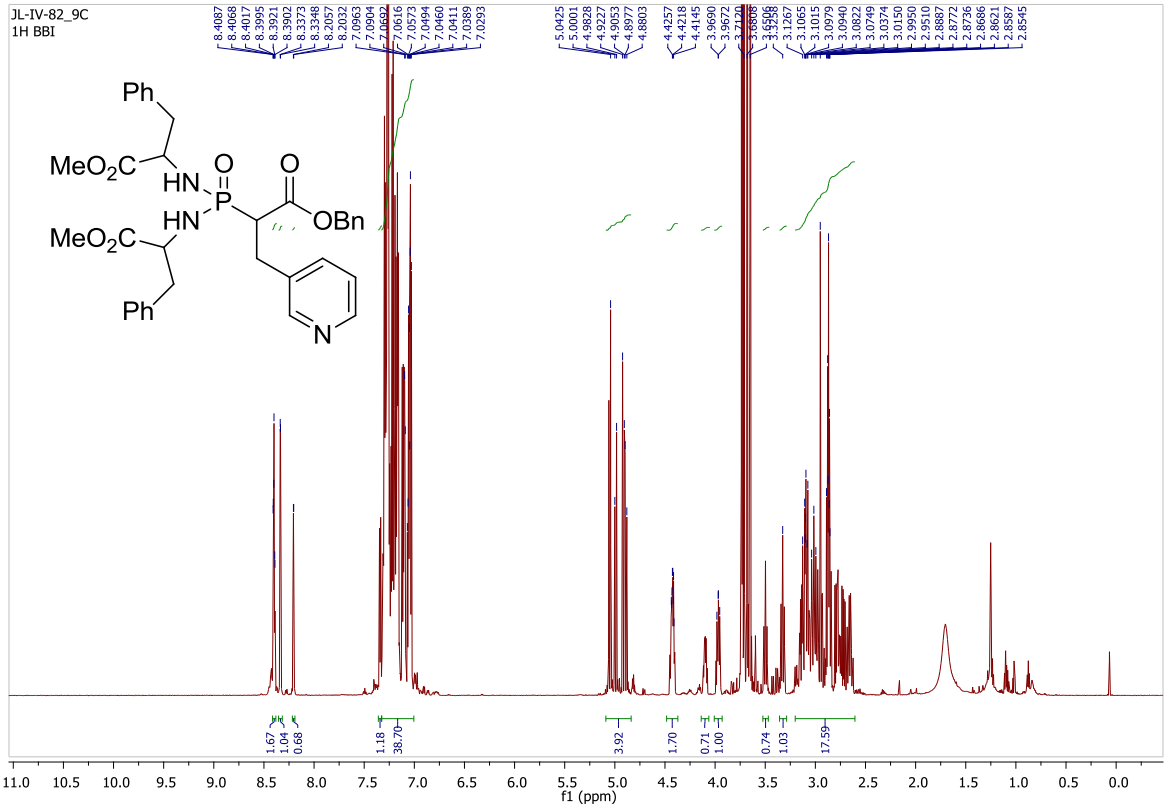


Figure S49. ^{1}H NMR of compound **28** (700 MHz, $CDCl_3$).

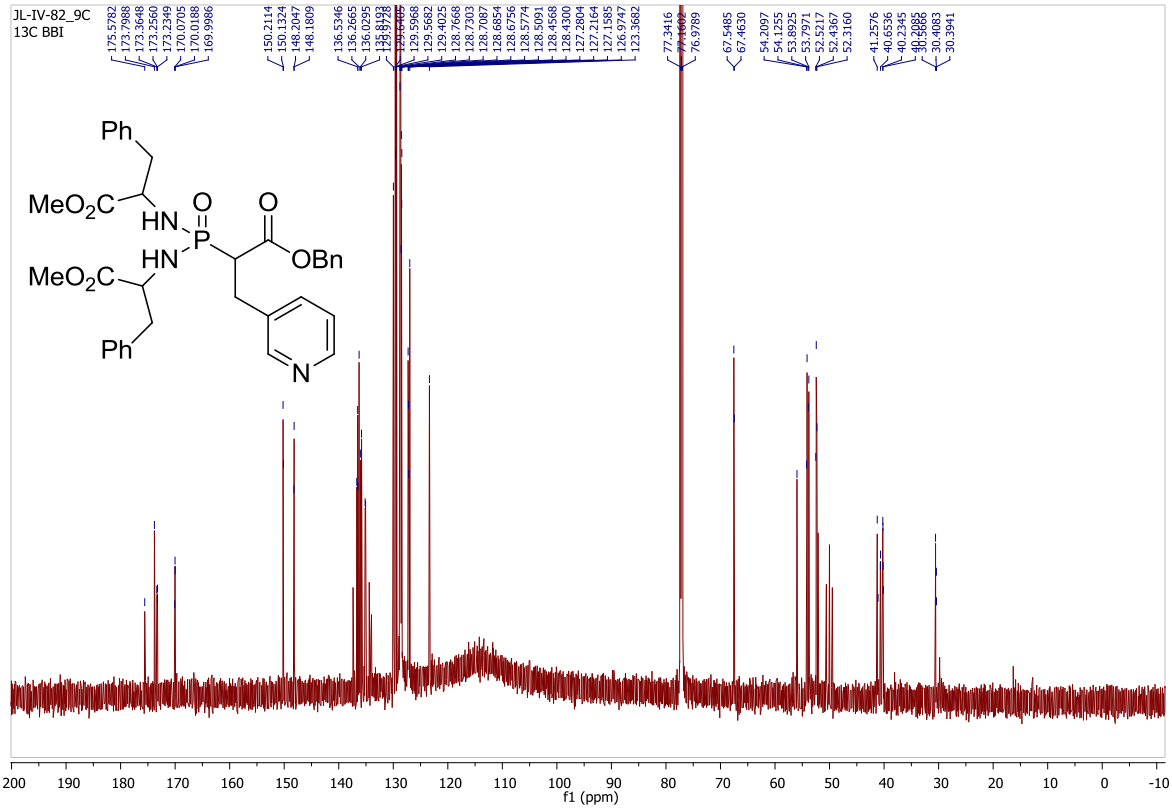


Figure S50. ^{13}C NMR of compound **28** (176 MHz, $CDCl_3$).

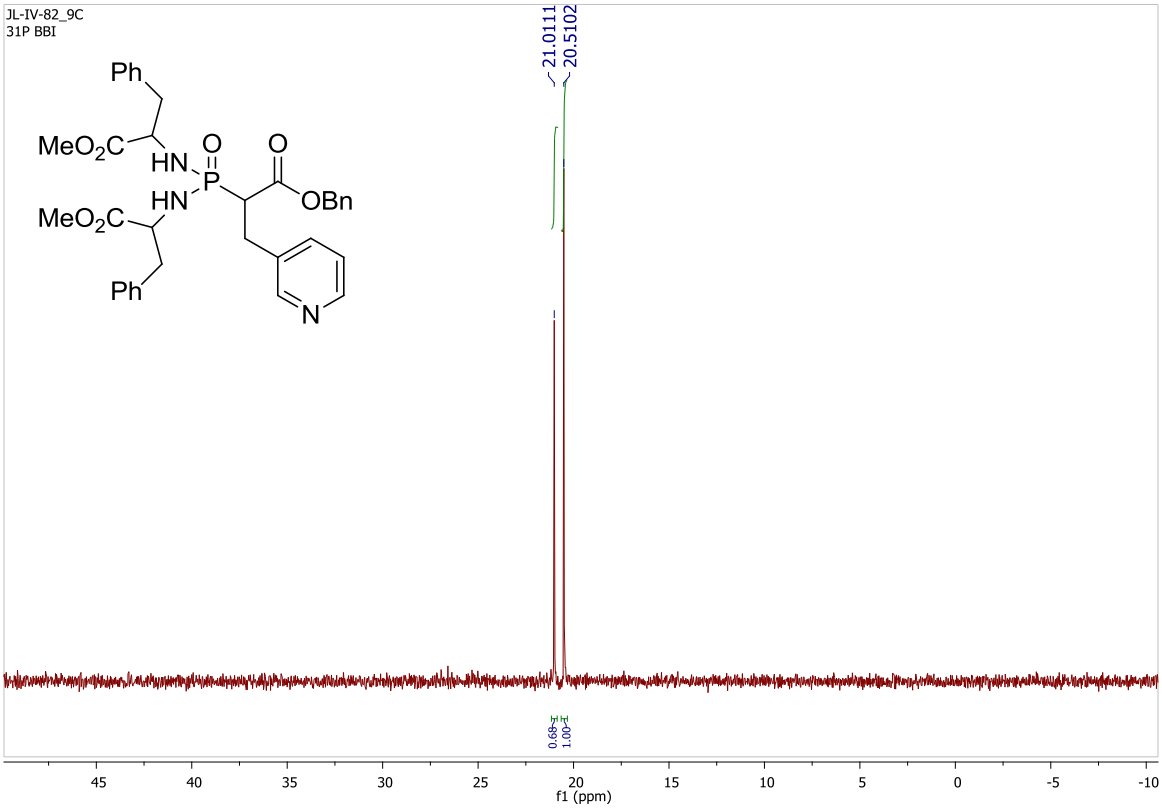


Figure S51. ^{31}P NMR of compound **28** (283 MHz, $CDCl_3$).

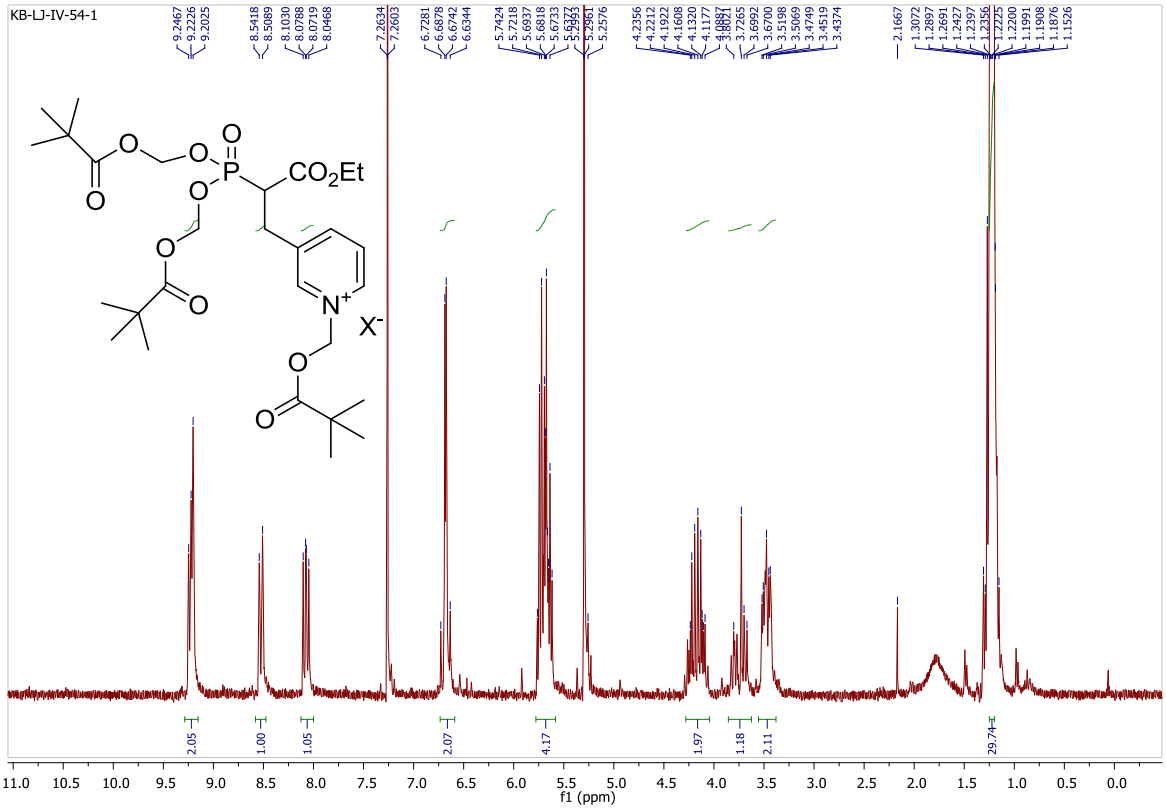


Figure S52. ^{1}H NMR of compound **37** (250 MHz, $CDCl_3$).

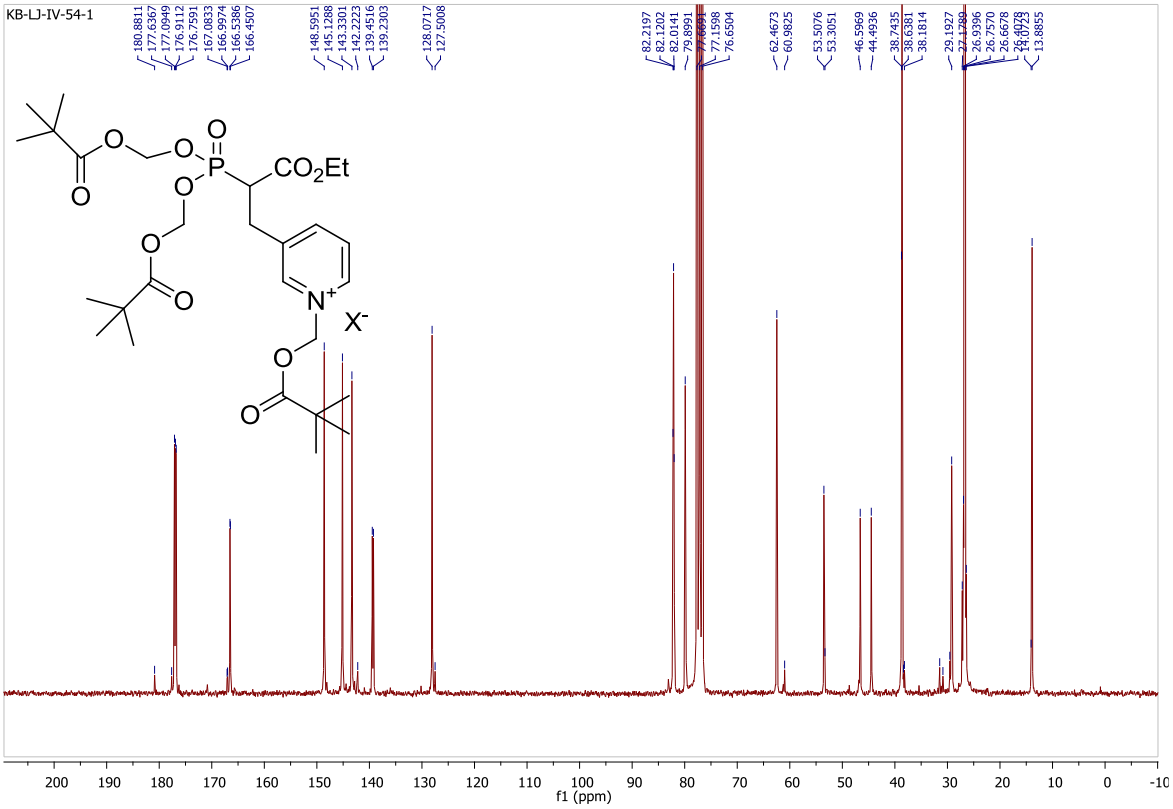


Figure S53. ^{13}C NMR of compound **37** (63 MHz, $CDCl_3$).

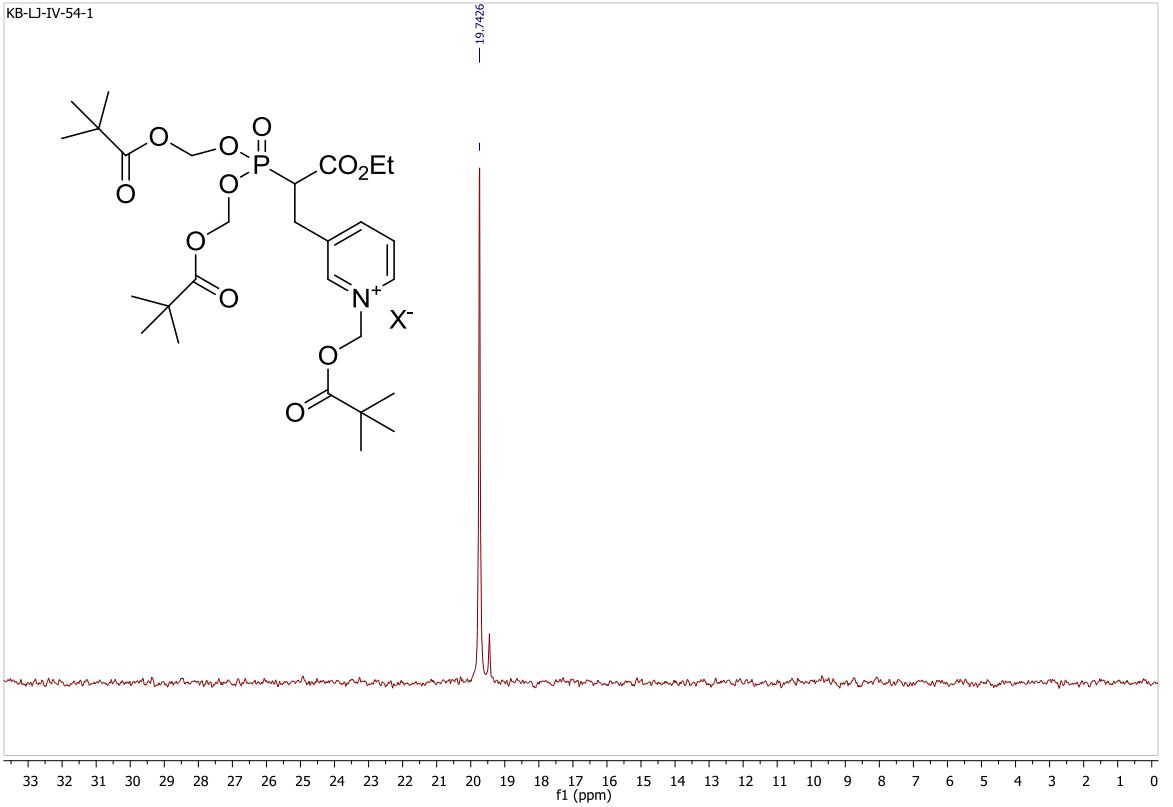


Figure S54. ^{31}P NMR of compound **37** (100 MHz, $CDCl_3$).

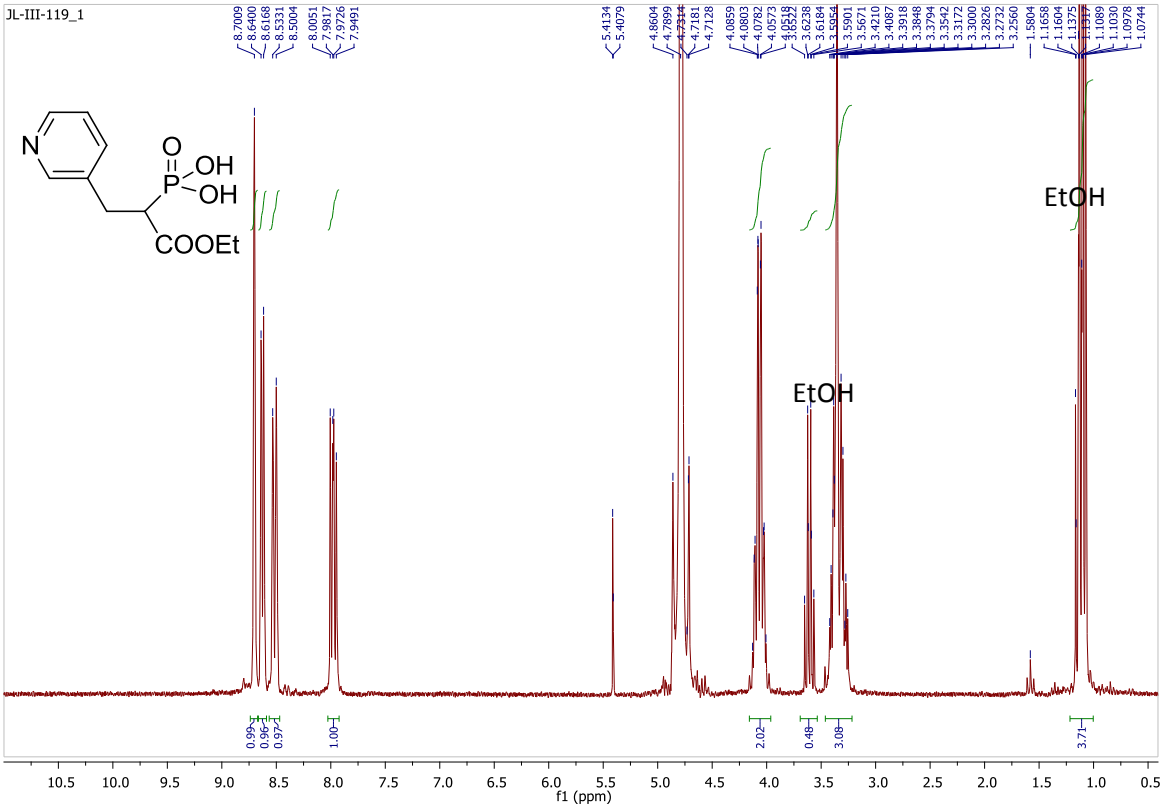


Figure S55. ^{1}H NMR of compound **18a** (250 MHz, D_2O, pH-7).

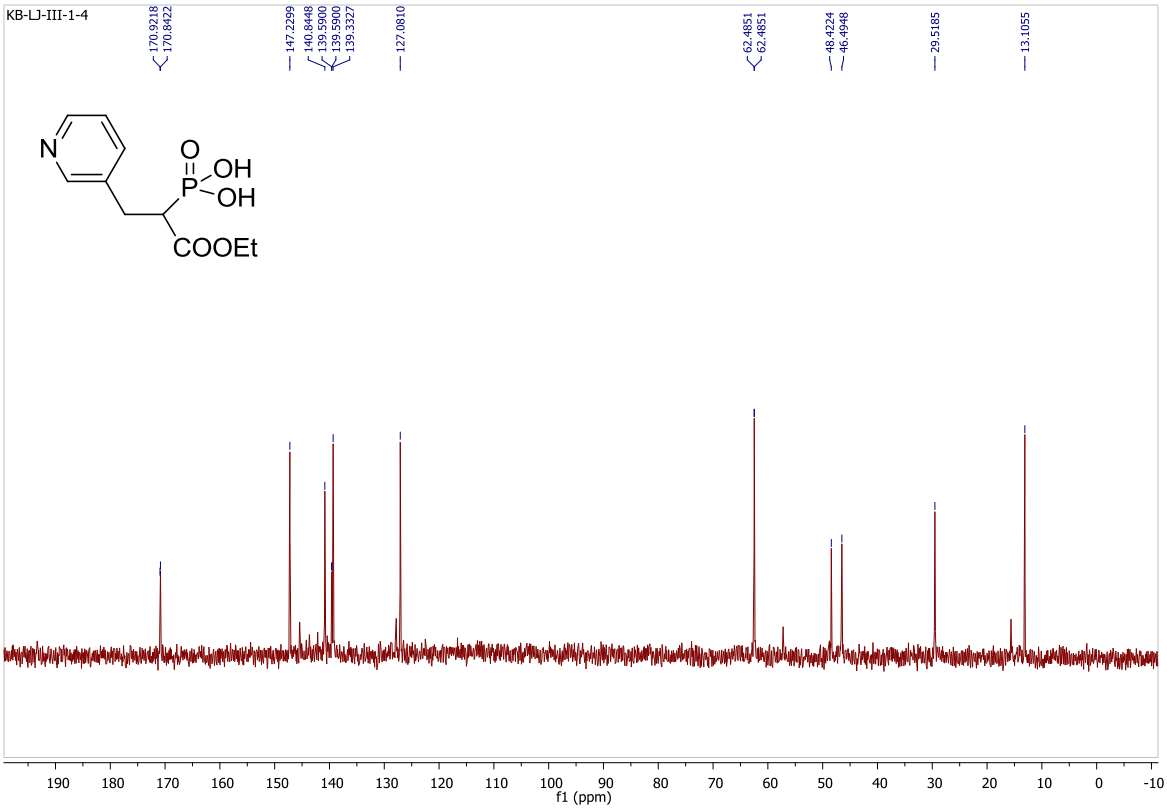


Figure S56. ^{13}C NMR of compound **18a** (63 MHz, D_2O, pH-7).

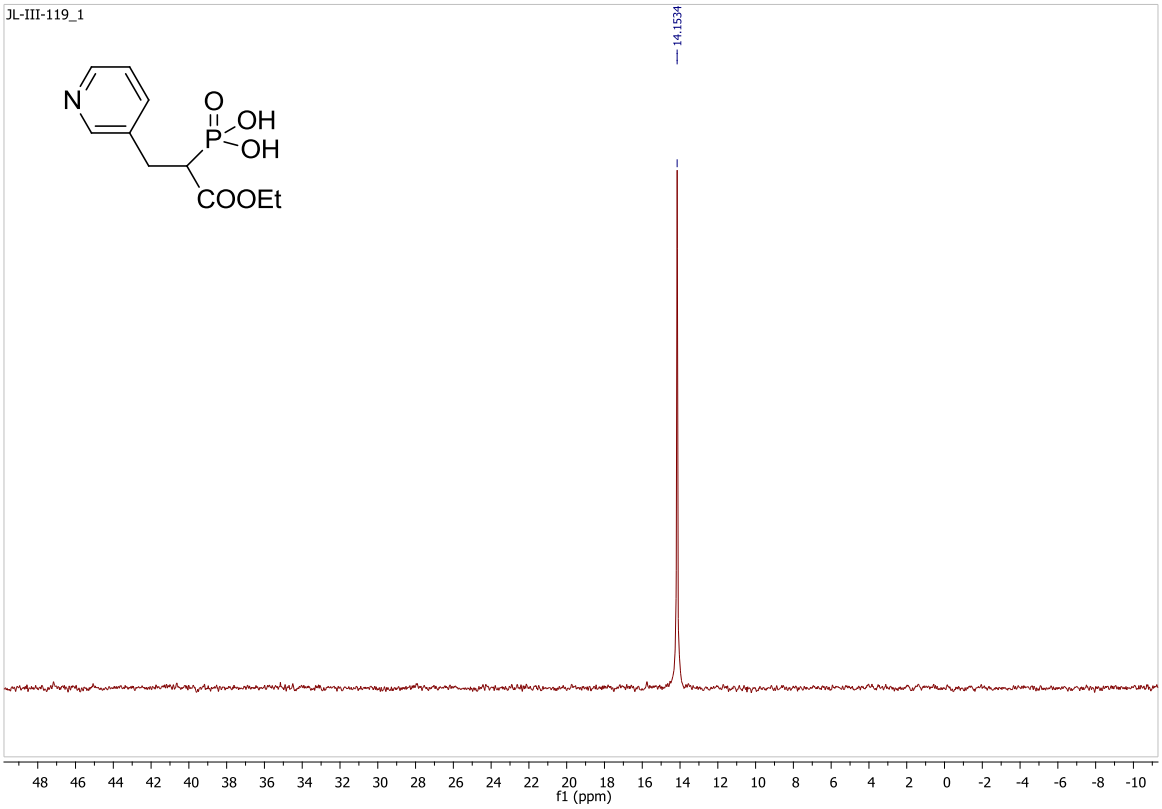


Figure S57. ^{31}P NMR of compound **18a** (100 MHz, D_2O, pH-7).

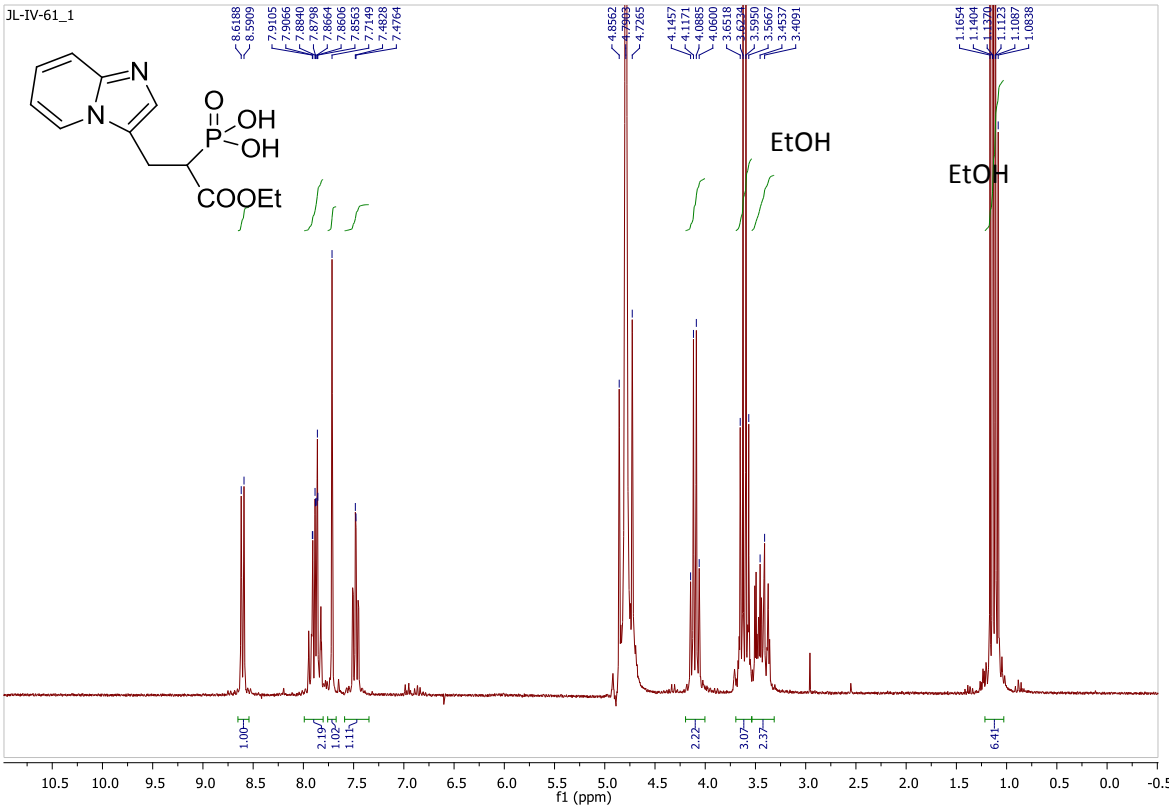


Figure S58. ^{1}H NMR of compound **18b** (250 MHz, D_2O, pH-7).

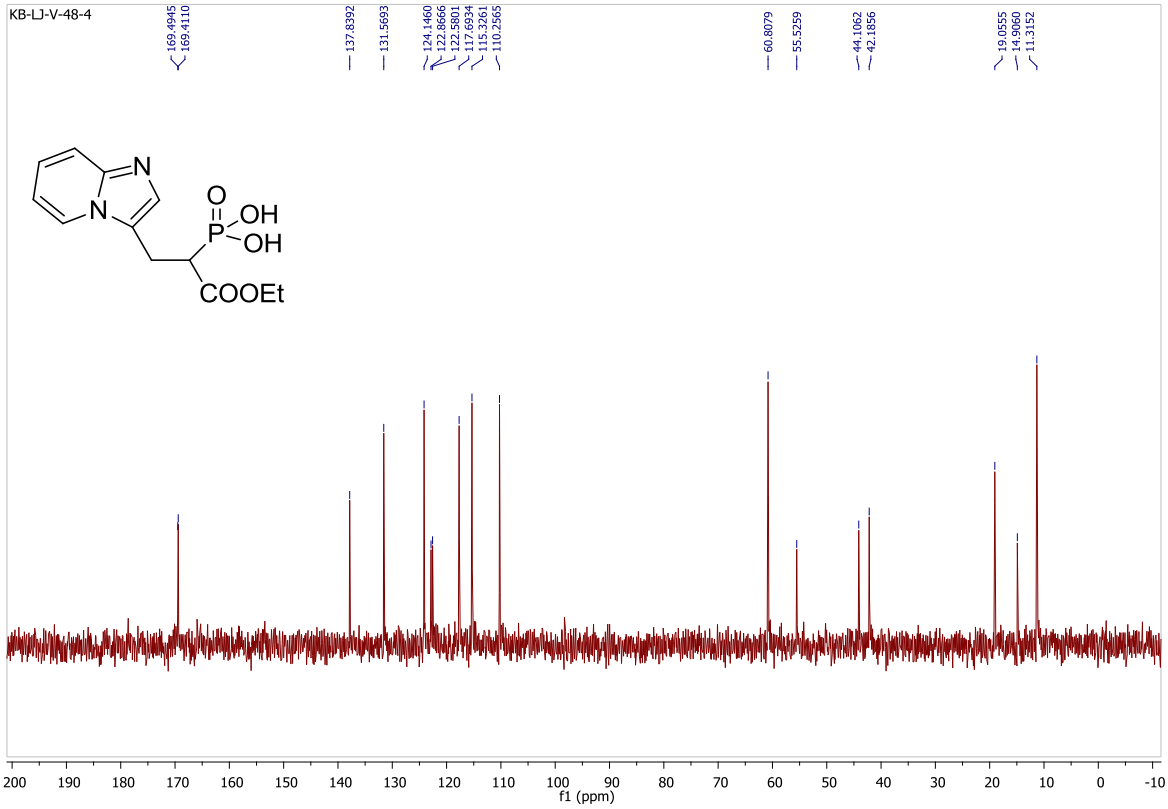


Figure S59. ^{13}C NMR of compound **18b** (63 MHz, D_2O, pH-7).

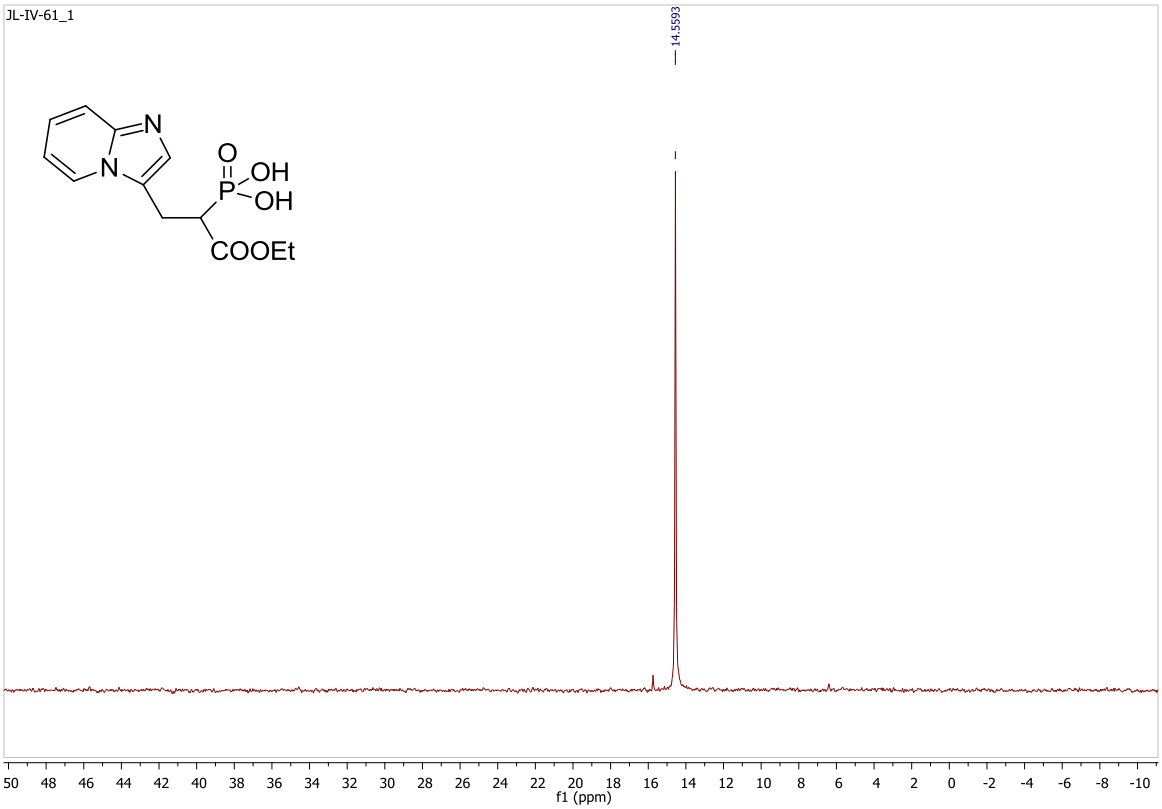


Figure S60. ^{31}P NMR of compound **18b** (100 MHz, D_2O, pH-7).

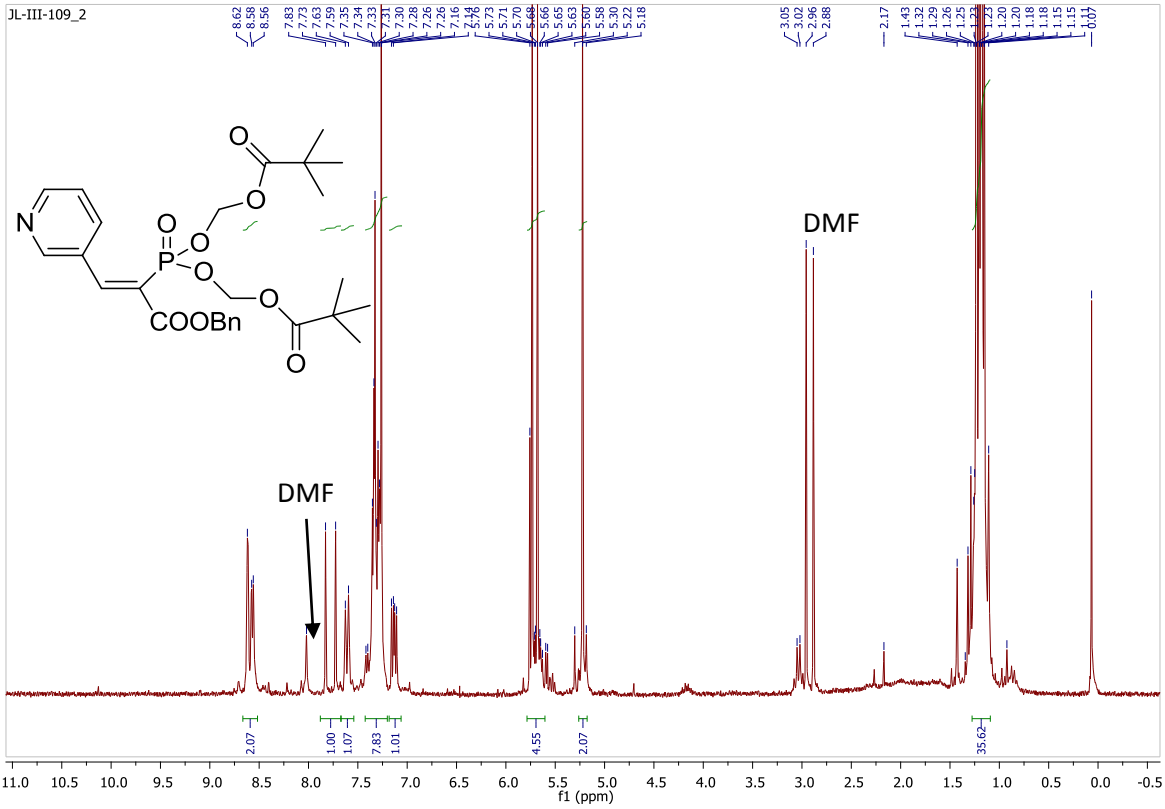


Figure S61. ^{1}H NMR of compound **22** (250 MHz, $CDCl_3$).

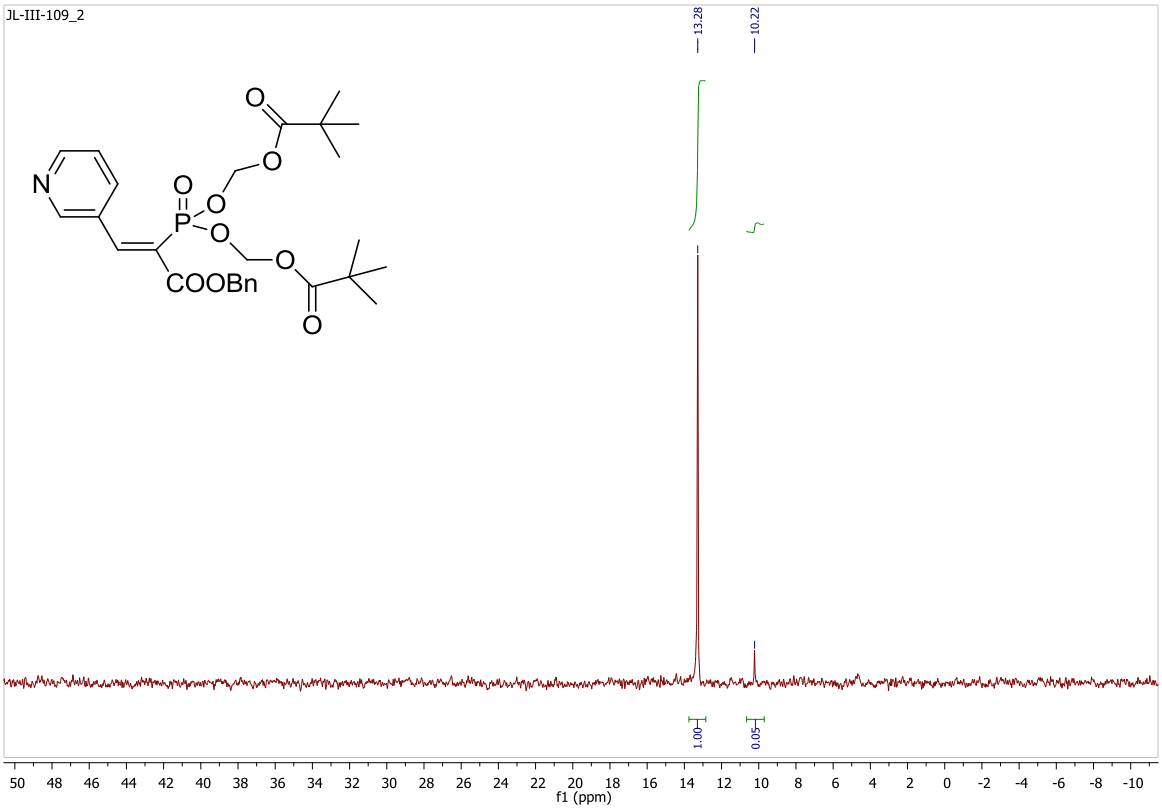


Figure S62. ^{31}P NMR of compound **22** as a mixture of diastereoisomers *Z/E* (100 MHz, $CDCl_3$).

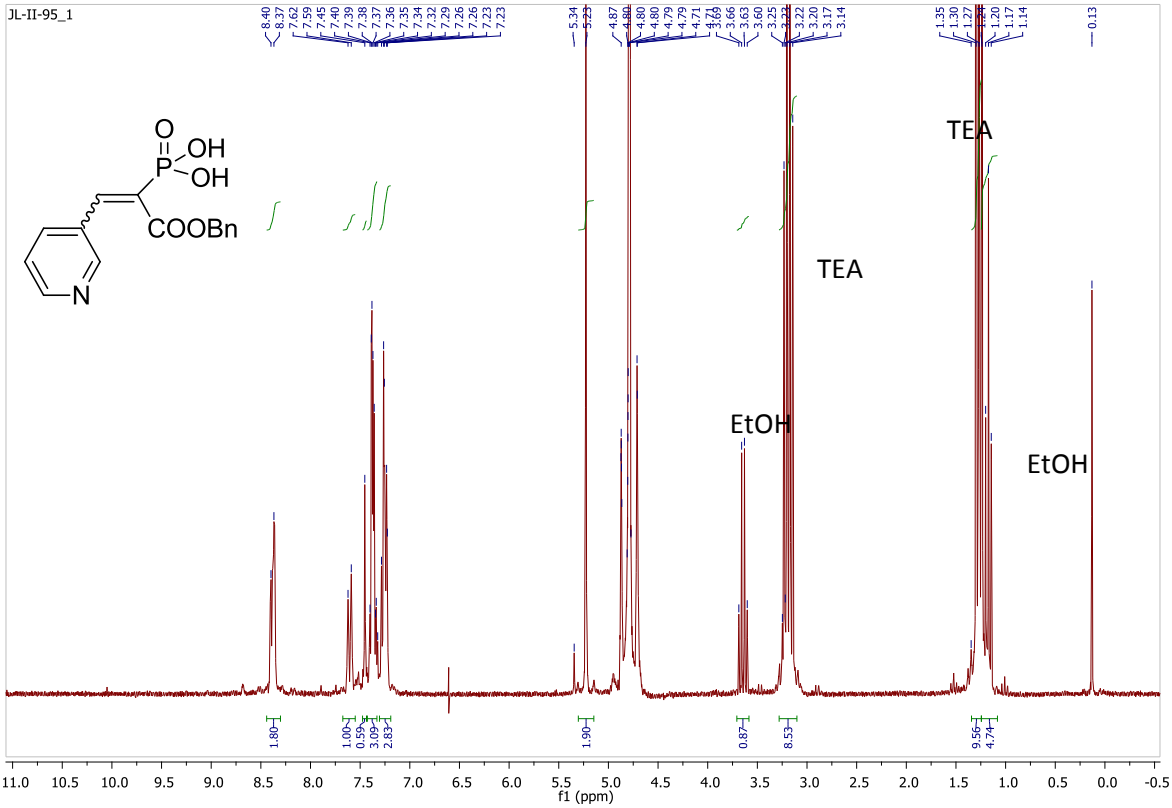


Figure S63. ^{1}H NMR of compound **21** (250 MHz, D_2O, pH-7).

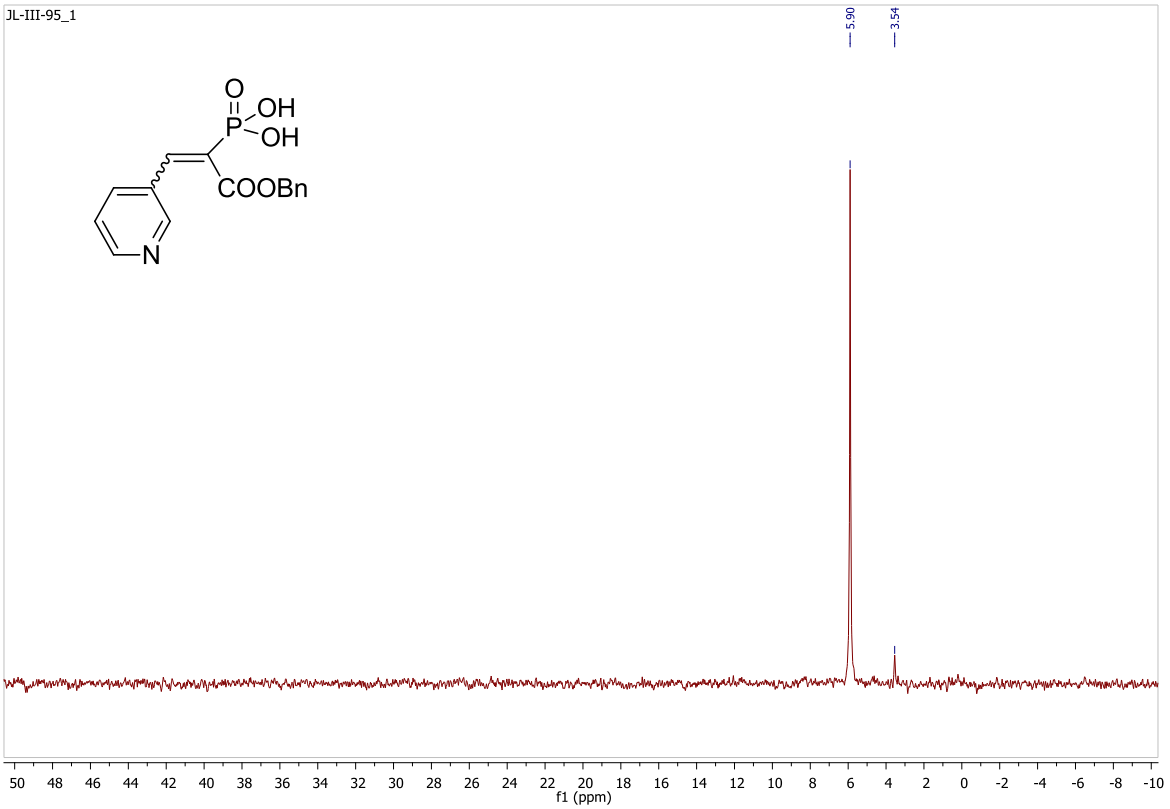


Figure S64. ^{31}P NMR of compound **21** as a mixture of diastereoisomers *Z/E* (100 MHz, D_2O, pH-7).