Supplementary Information (SI) for RSC Medicinal Chemistry.
This journal is © The Royal Society of Chemistry 2025

Supporting Information

2D and 3D Anticancer Activity of Diiron Bis-Cyclopentadienyl Complexes Incorporating Flurbiprofen and Chlorambucil

Lorenzo Biancalana,[a] Michele De Franco,[b] Valentina Gandin,[b,*] Fabio Marchetti [a,*]

[a] University of Pisa, Department of Chemistry and Industrial Chemistry, Via G. Moruzzi 13, I-56124 Pisa, Italy

[b] University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Via F. Marzolo 5, I-35131 Padova, Italy

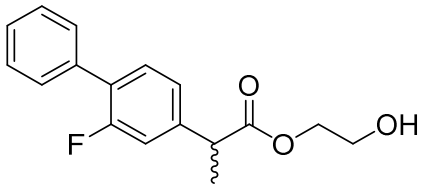
Table of contents **Page(s)**

Synthesis of Flurbiprofen and Chlorambucil 2-hydroxyethyl esters

General procedure for 2-hydroxyethyl esters. A solution of the carboxylic acid (130-160 mg), EDCI·HCl (1.25 equivalents) and DMAP (7 mg, 0.06 mmol) in anhydrous THF (2 mL) under N_2 was treated with ethylene glycol (0.9 mL, 16 mmol) and stirred at room temperature overnight. Next, volatiles were removed under vacuum. The residue was suspended in water (10 mL) and extracted with CH_2Cl_2 (2 x 15 mL). The combined organic fractions were extracted with water (3 x 10 mL) then taken to dryness under vacuum. The residue was dissolved in CH_2Cl_2 and moved on top of a silica gel column (h 6, d 2.3 cm). Impurities were eluted with CH_2Cl_2 then the title compound was eluted with Et_2O. Volatiles were removed under vacuum from the eluate solution (40 °C).

Flurbiprofen, 2-hydroxyethyl ester, FLU-$CO_2(CH_2)_2OH$ (Chart S1).

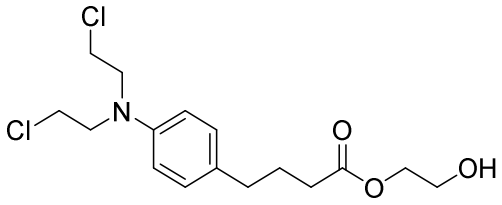
Chart S1. Structure of **FLU**-$CO_2(CH_2)_2OH$.



The title compound was previously prepared by a similar method.[1] Synthesized according to the general procedure using flurbiprofen (**FLU**-CO_2H; 164 mg, 0.671 mmol). The colorless oil was re-dissolved in Et_2O, cooled at − 20 °C and then dried under vacuum without heating. The resulting colorless solid was stored at 4 °C under N_2. Yield: 151 mg, 78%. ^1H NMR ($CDCl_3$): δ/ppm = 7.56–7.51 (m, 2H), 7.47–7.34 (m, 4H), 7.19–7.10 (m, 2H) (Ph + C_6H_3F); 4.31–4.19 (m, 2H, OCH_2); 3.86–3.77 (m, 3H, OCH_2 + CH–C=O); 1.56 (d, $^3J_{HH}$ = 7.2 Hz, 3H, CH_3).

Chlorambucil, 2-hydroxyethyl ester, CMB-$CO_2(CH_2)_2OH$ (Chart S2).

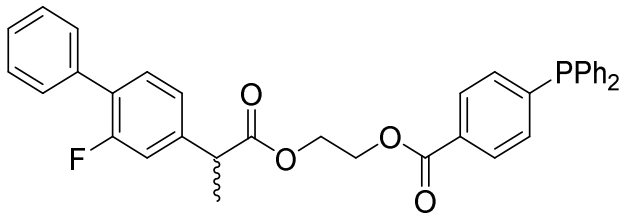
Chart S2. Structure of **CMB**-$CO_2(CH_2)_2OH$.



The title compound was previously prepared by a similar method.[2] Synthesized according to the general procedure, using chlorambucil (**CMB**-CO_2H; 137 mg, 0.449 mmol). Colorless oil, stored at 4 °C under N_2. Yield: 150 mg, 96%. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.07 (d, $^3J_{HH}$ = 8.5 Hz, 2H, C_6H_4); 6.63 (d, $^3J_{HH}$ = 8.6 Hz, 2H, C_6H_4); 4.24–4.16 (m, 2H, C(=O)O−CH_2); 3.87–3.77 (m, 2H, CH_2OH); 3.75–3.67 (m, 4H, CH_2N); 3.65–3.59 (m, 4H, CH_2Cl); 2.56 (t, $^3J_{HH}$ = 7.5 Hz, 2H, CH_2−C=O); 2.36 (t, $^3J_{HH}$ = 7.5 Hz); 1.99–1.85 (m) (4H, CH_2CH_2); 1.75 (br, 1H, OH). Resonances for the CH_2N and CH_2Cl groups were unambiguously assigned by the ^{1}H-^{13}C HMBC experiment.

Flurbiprofen, 2-hydroxyethyl 4-(diphenylphosphanyl)benzoate, P^{FLU} (Chart S3).

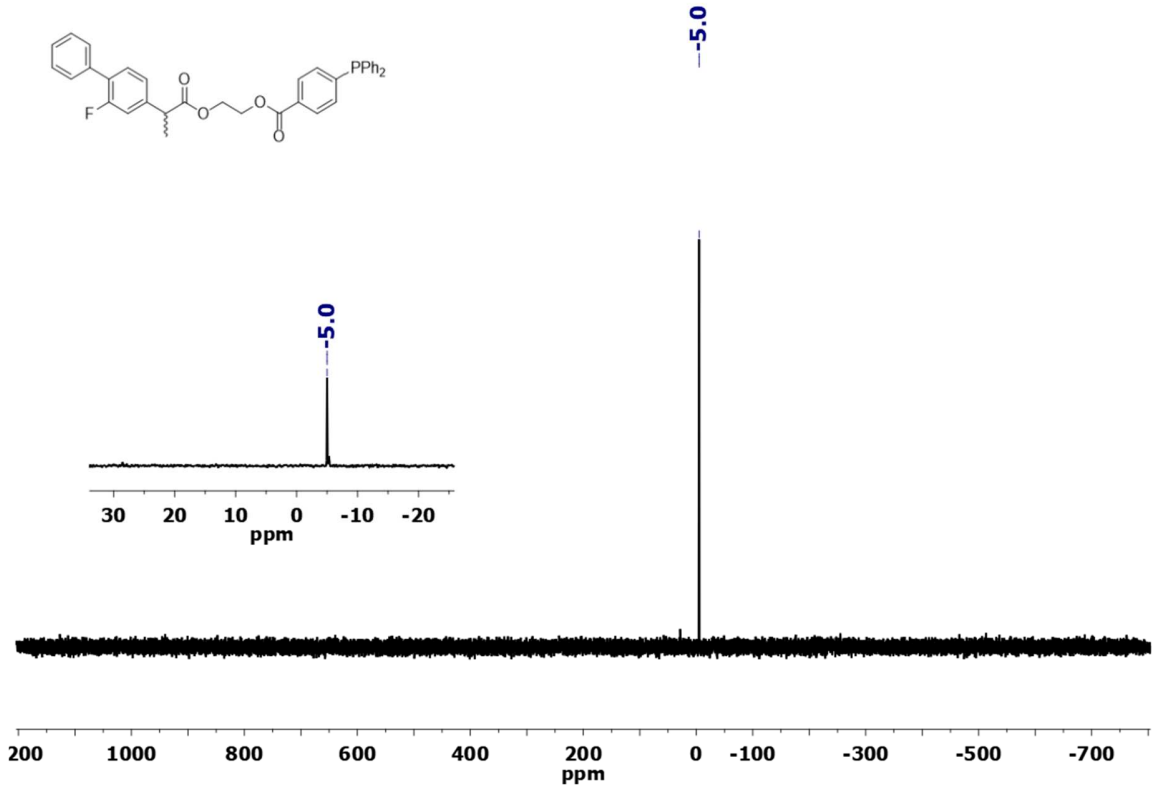
Chart S3. Structure of Flurbiprofen, 2-hydroxyethyl 4-(diphenylphosphanyl)benzoate, P^{FLU}.



In a 15 mL Schlenk tube under N_2, 4-(diphenylphosphanyl)benzoic acid (38 mg, 0.12 mmol), **FLU**-$CO_2(CH_2)_2OH$ (35 mg, 0.12 mmol), DMAP (4 mg, 0.03 mmol), EDCI·HCl (30 mg, 0.16 mmol) and anhydrous CH_2Cl_2 (4 mL) were introduced in this order. The colorless solution was stirred overnight at room temperature then diluted with CH_2Cl_2 and extracted with water (3 x 10 mL). Volatiles were removed under vacuum from the organic solution, affording a pale-yellow solid. Yield: 70 mg, > 90%. The purity was considered satisfactory for the subsequent reaction with [**1-MeCN**]CF_3SO_3 therefore silica gel chromatography was avoided. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.89 (d, $^3J_{HH}$ = 7.6 Hz, 2H, C_6H_4P); 7.48–7.27

(m, 18H, Ph + C_6H_3F); 7.11 (app. t, $^3J_{HH} \approx {}^3J_{HP} \approx 8$ Hz, 2H, C_6H_4P); 4.52–4.41 (m, 4H, OCH_2); 3.78 (q, $^3J_{HH} = 7.3$ Hz, 1H, CH–C=O), 1.53 (d, $^3J_{HH} = 7.2$ Hz, 3H, CH_3). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = – 5.0.

Figure S1. $^{31}P\{^1H\}$ NMR spectrum (162 MHz, $CDCl_3$) of $\mathbf{P_{FLU}}$.



IR, NMR and MS spectra of diiron compounds

Figure S2. Solid-state IR spectrum (650-4000 cm^{-1}) of $[Fe_2Cp_2(CO)\{Ph_2P(4\text{-}C_6H_4CO_2H)\}(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, [**2**]$CF_3SO_3$.

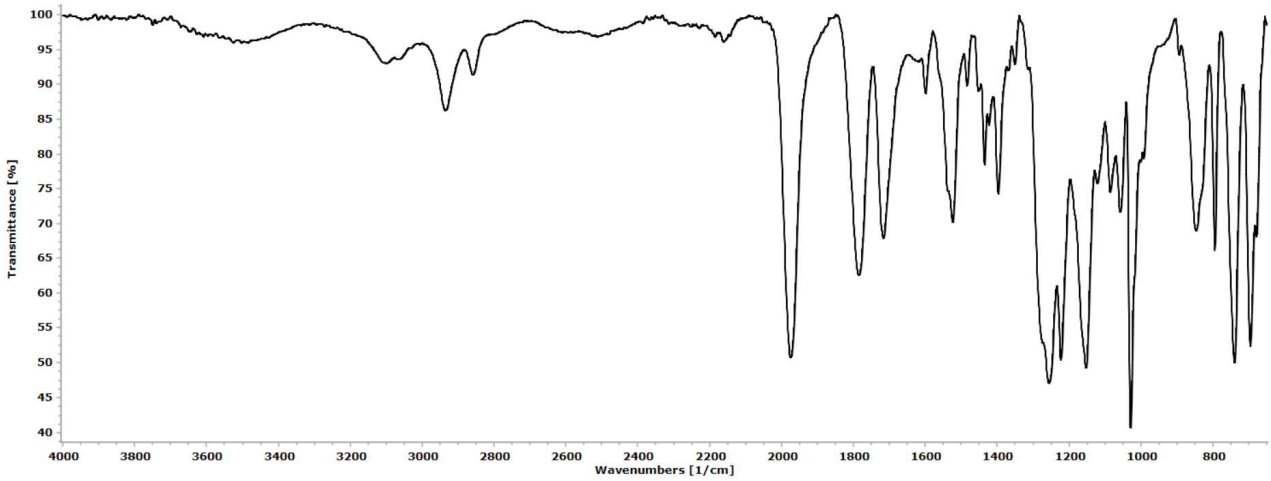


Figure S3. Solid-state IR spectra (650-4000 cm^{-1}) of $[Fe_2Cp_2(CO)\{Ph_2P(4\text{-}C_6H_4CO_2)\}(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]$, **2**$^{-H}$ (dark blue line) and [**2**]CF_3SO_3 (red line).

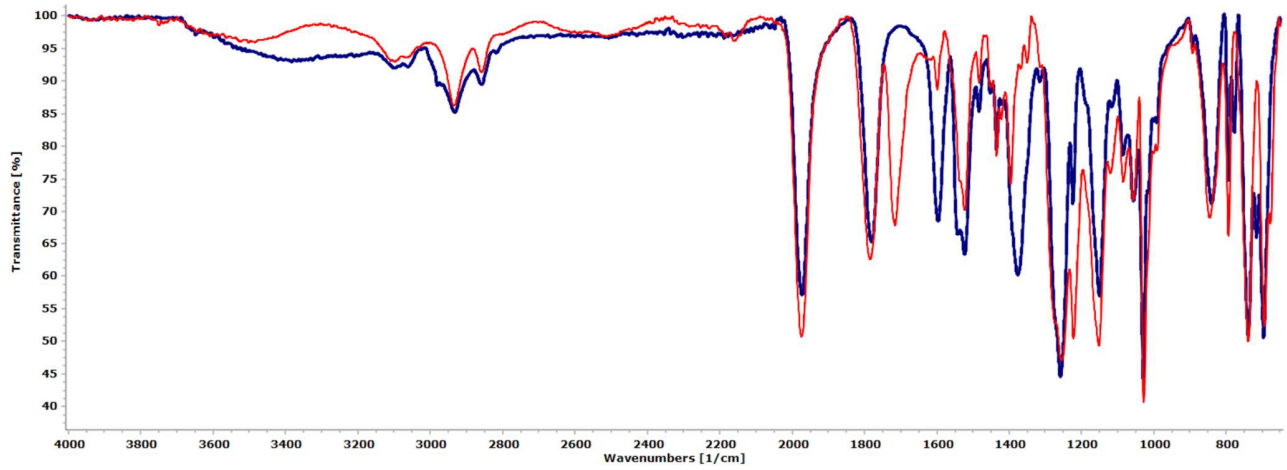


Figure S4. Solid-state IR spectrum (650-4000 cm^{-1}) of $[Fe_2Cp_2(CO)\{Ph_2P(4\text{-}C_6H_4CO_2(CH_2)_2OC(O)\text{-}\mathbf{FLU})\}(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, [**3**]$CF_3SO_3$.

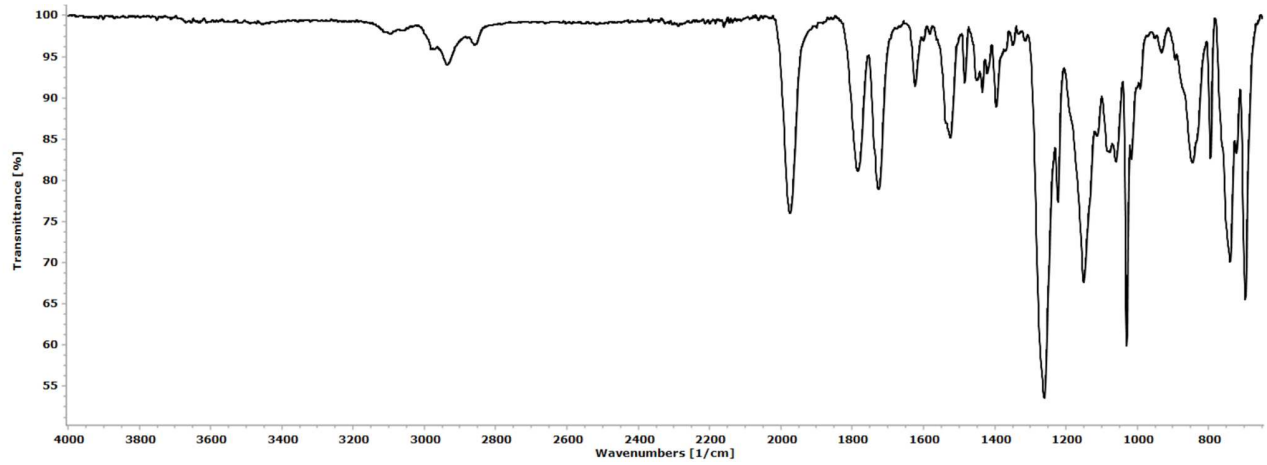


Figure S5. Solid-state IR spectrum (650-4000 cm^{-1}) of $[Fe_2Cp_2(CO)\{Ph_2P(4\text{-}C_6H_4CO_2(CH_2)_2OC(O)\text{-}\mathbf{CMB})\}(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, [**4**]$CF_3SO_3$.

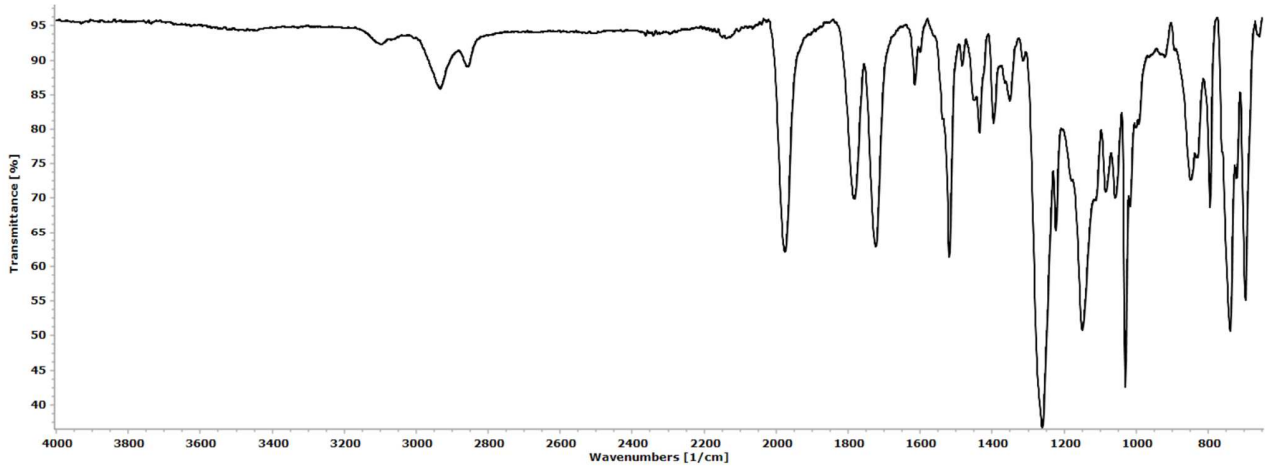


Figure S6. Comparison of solid-state IR spectra (1300-2100 cm^{-1}) of [**2**]CF_3SO_3 (top, blue line), **2**$^{-H}$ (yellow line), [**3**]CF_3SO_3 (red line), [**4**]CF_3SO_3 (bottom, cyan line), normalized with respect to the intensity of the μ–CO stretching absorption (≈ 1800 cm^{-1}).

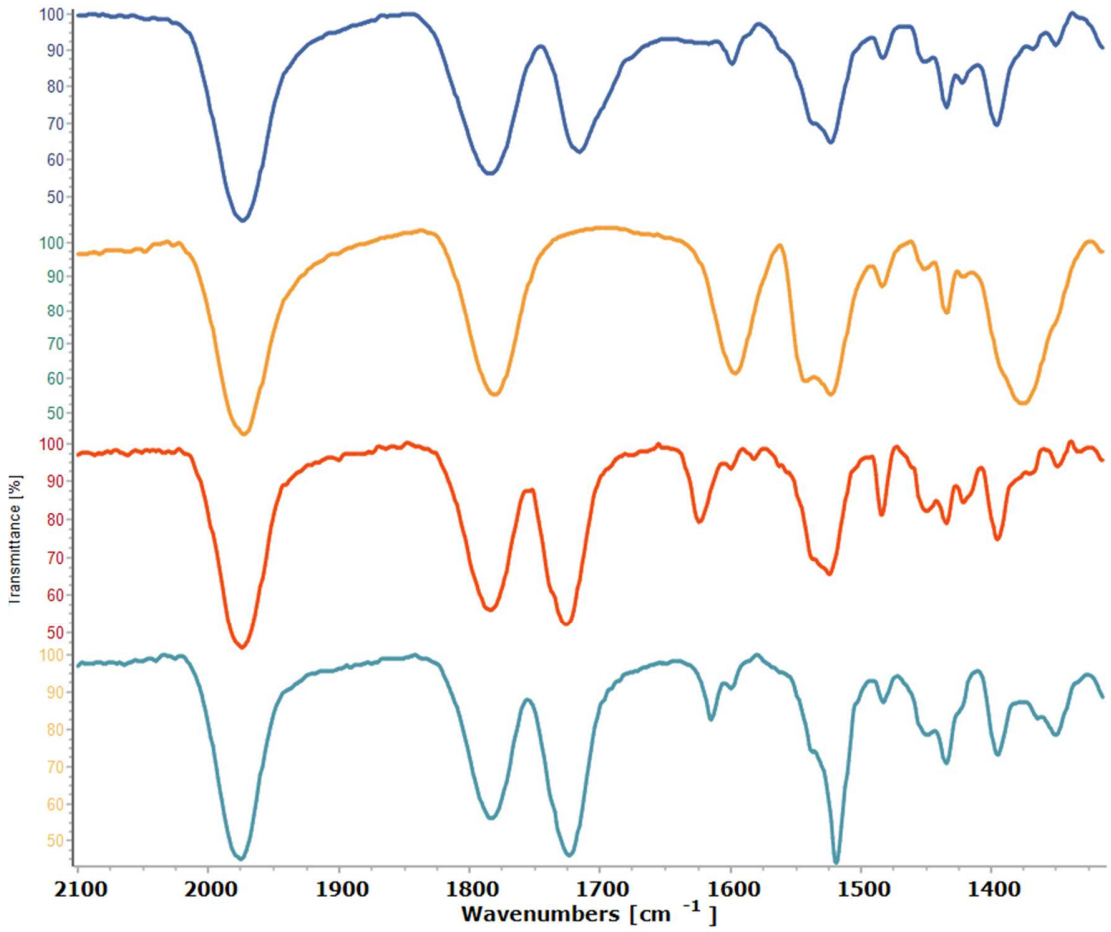


Figure S7. Comparison of IR spectra (1500-2100 cm^{-1}) of CH_2Cl_2 solutions of [**2**]CF_3SO_3 (top, blue line), $\mathbf{2}^{-H}$ (yellow line), [**3**]CF_3SO_3 (red line), [**4**]CF_3SO_3 (bottom, cyan line), normalized with respect to the intensity of the μ–CO stretching absorption (≈ 1800 cm^{-1}). Compound [**2**]CF_3SO_3 displays two stretching bands for the carboxylic C=O, probably related to dimer formation in solution.

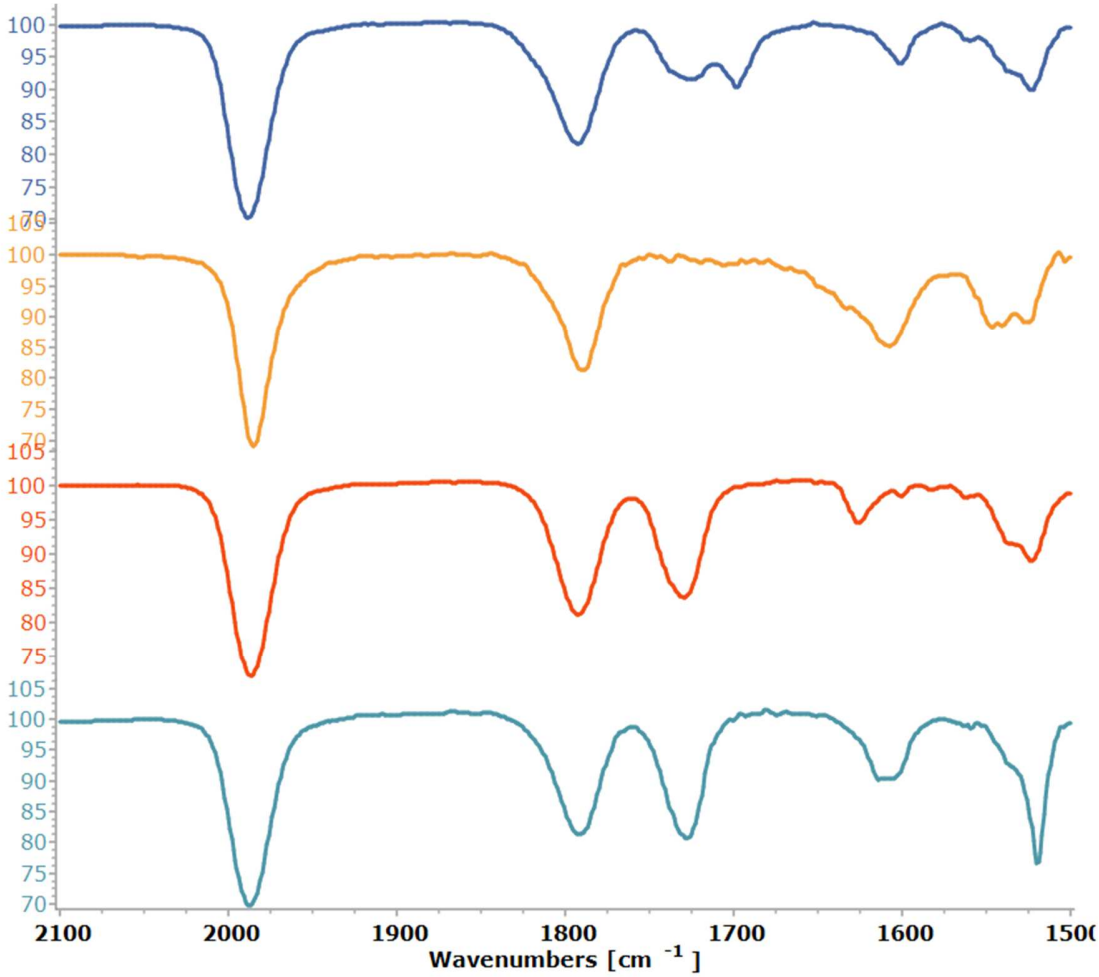


Figure S8. ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [**2**]CF_3SO_3. Inset shows the 8-16 ppm region.

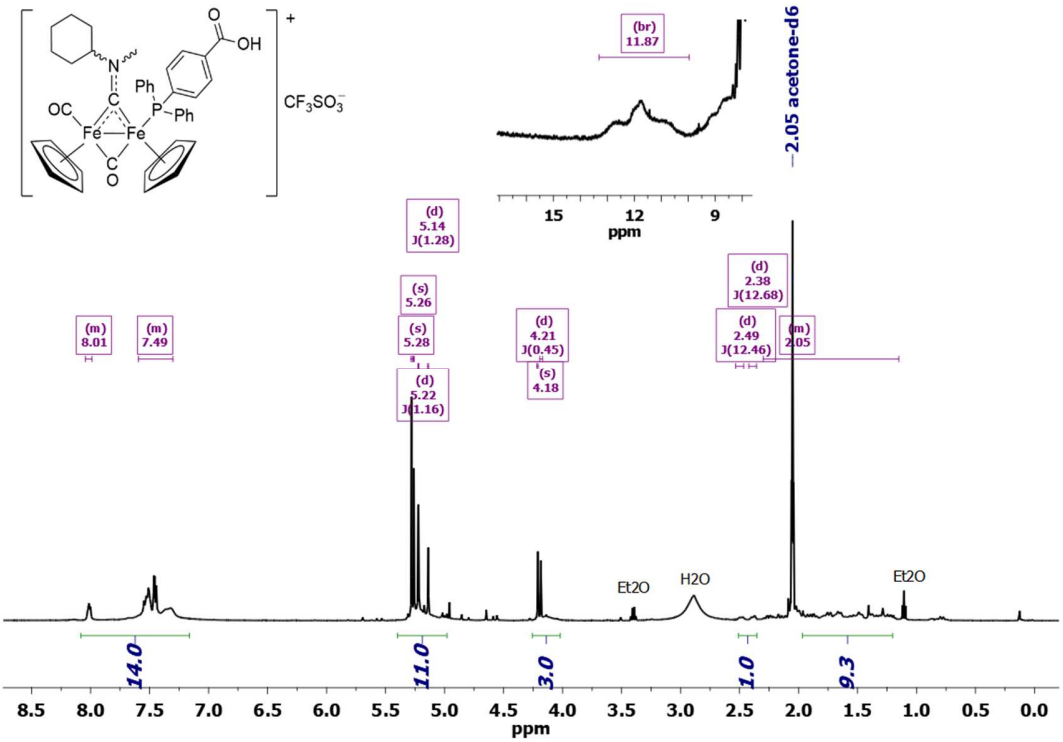


Figure S9. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, acetone-d_6) of [**2**]CF_3SO_3. Doublets in the 140-120 ppm region are not highlighted.

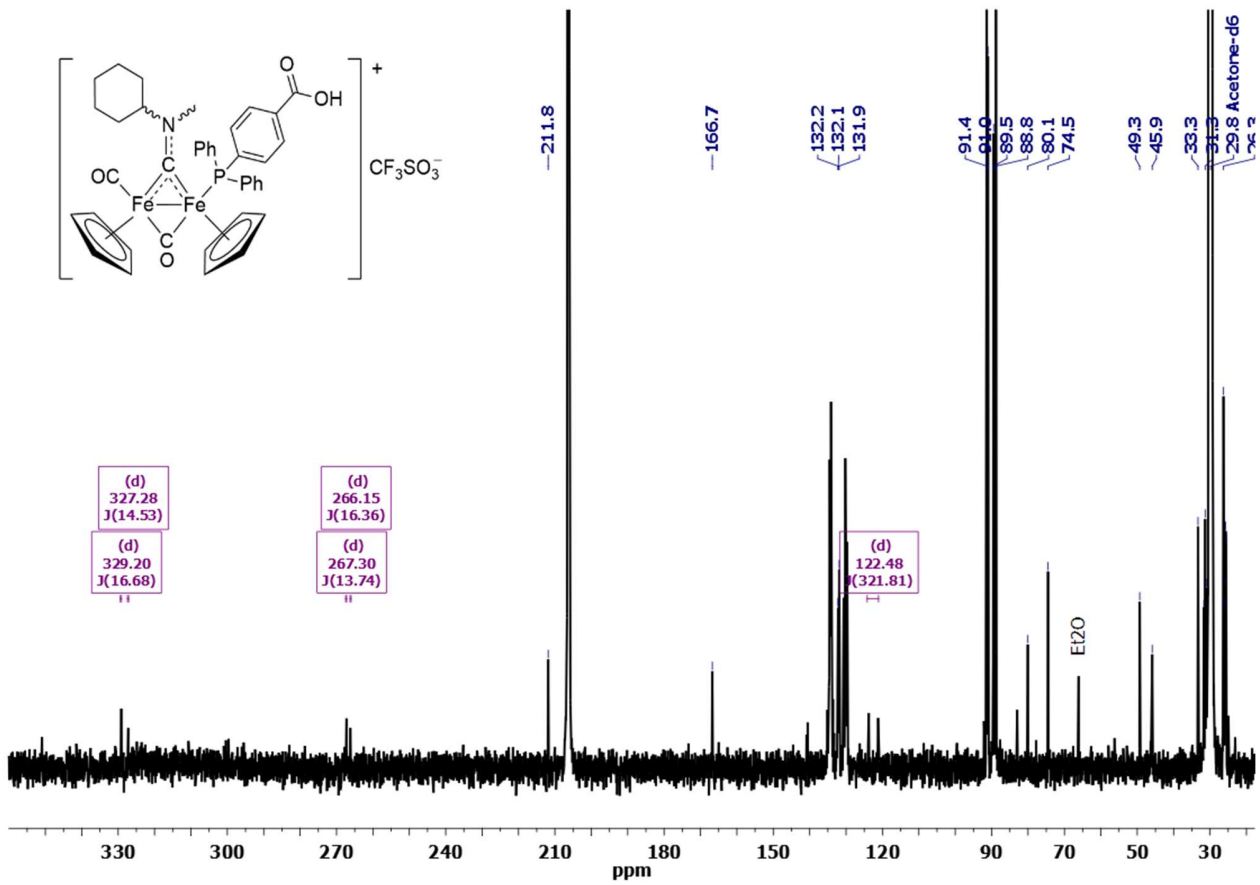


Figure S10. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, acetone-d_6) of [**2**]CF_3SO_3.

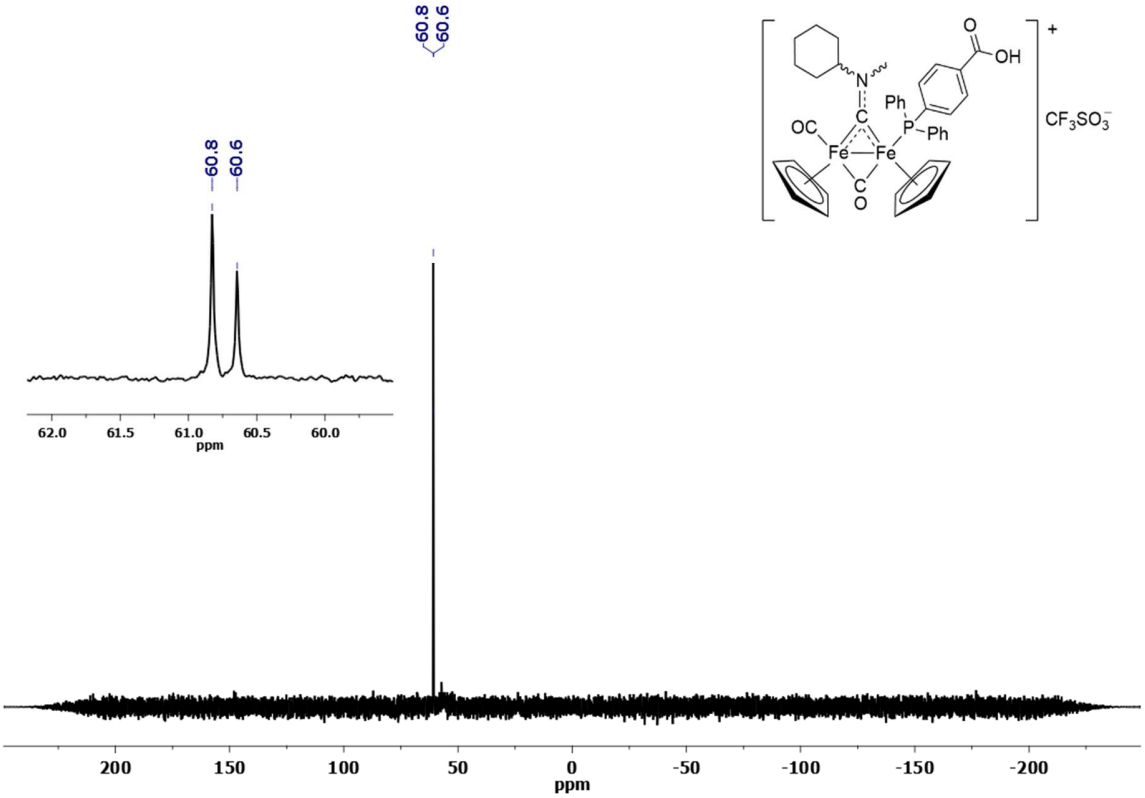


Figure S11. Black line: ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [**2**]CF_3SO_3. Red line: ^{1}H NOESY with irradiation at 5.22 ppm (Cp^P of the *Z* isomer). Blue line: ^{1}H NOESY with irradiation at 5.14 ppm (Cp^P of the *E* isomer). Observed NOEs are indicated by the arrows.

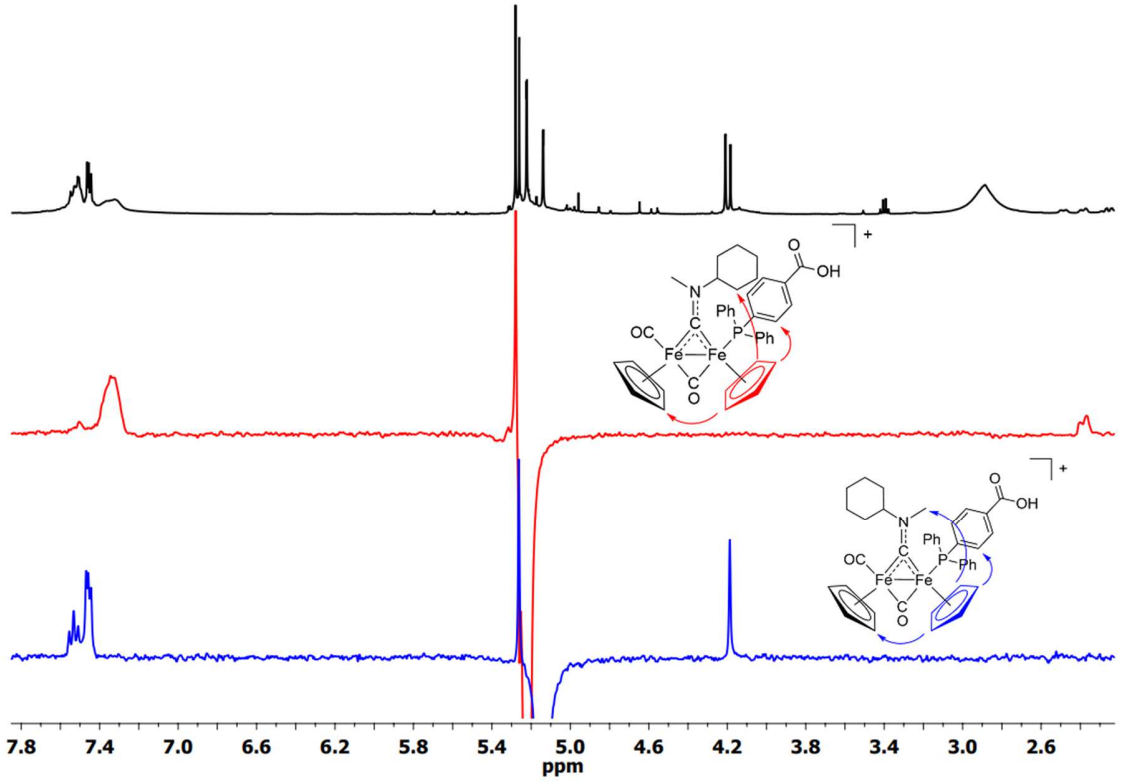


Figure S12. ^{1}H NMR spectrum (401 MHz, CD_3OD) of **2**$^{-H}$. Signals due to the potassium 4-(diphenylphosphanyl oxide)benzoate impurity are marked with (*).

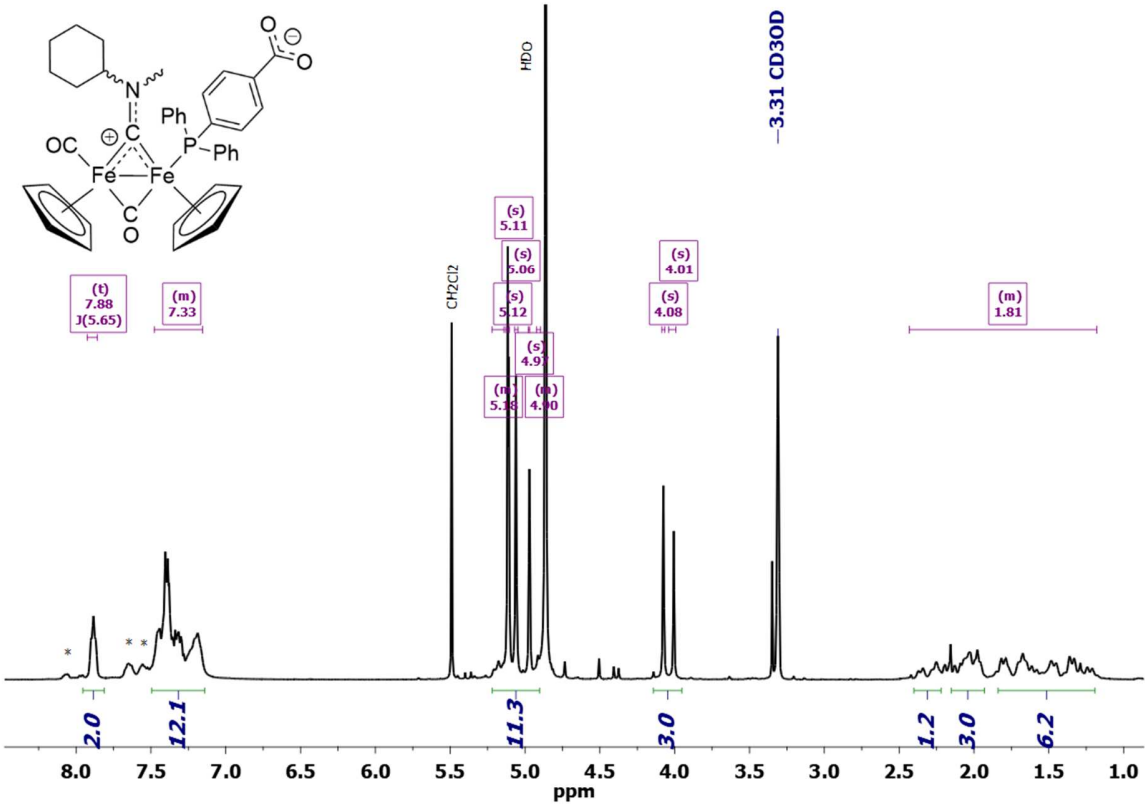


Figure S13. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, CD_3OD) of **2**$^{-H}$. Doublets in the 140-120 ppm region are not highlighted.

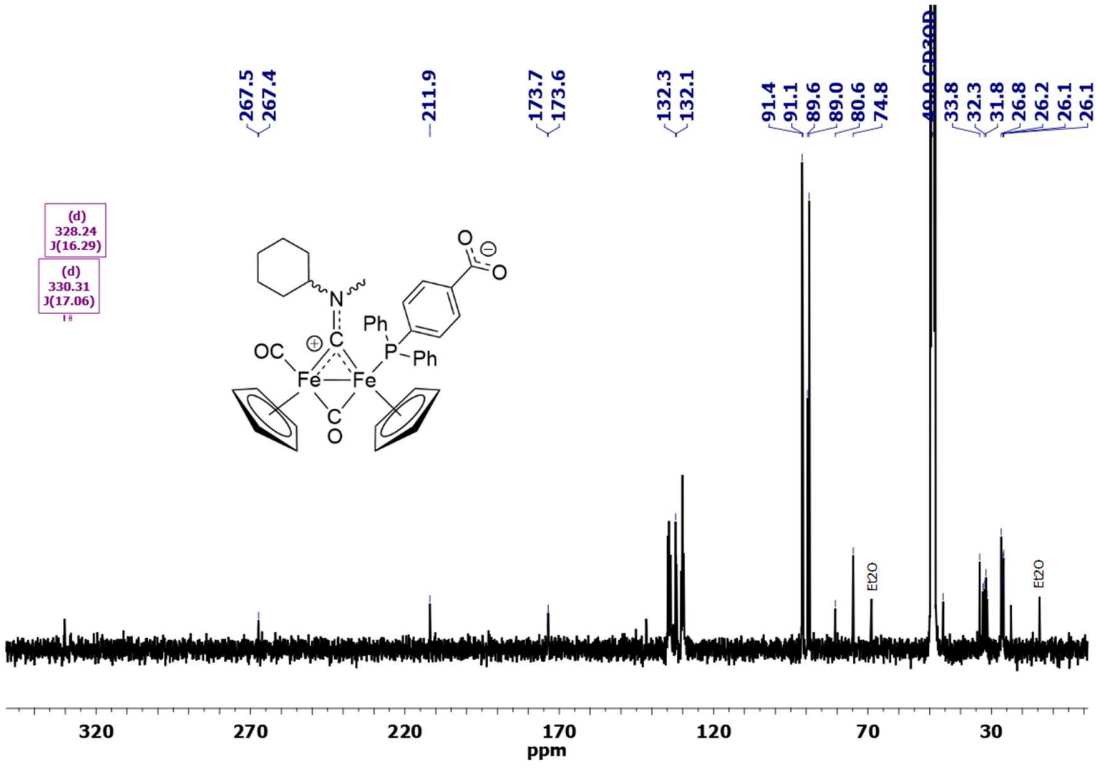


Figure S14. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, CD_3OD) of **2**$^{-H}$. The signal due to the potassium 4-(diphenylphosphanyl oxide)benzoate impurity is marked with (*).

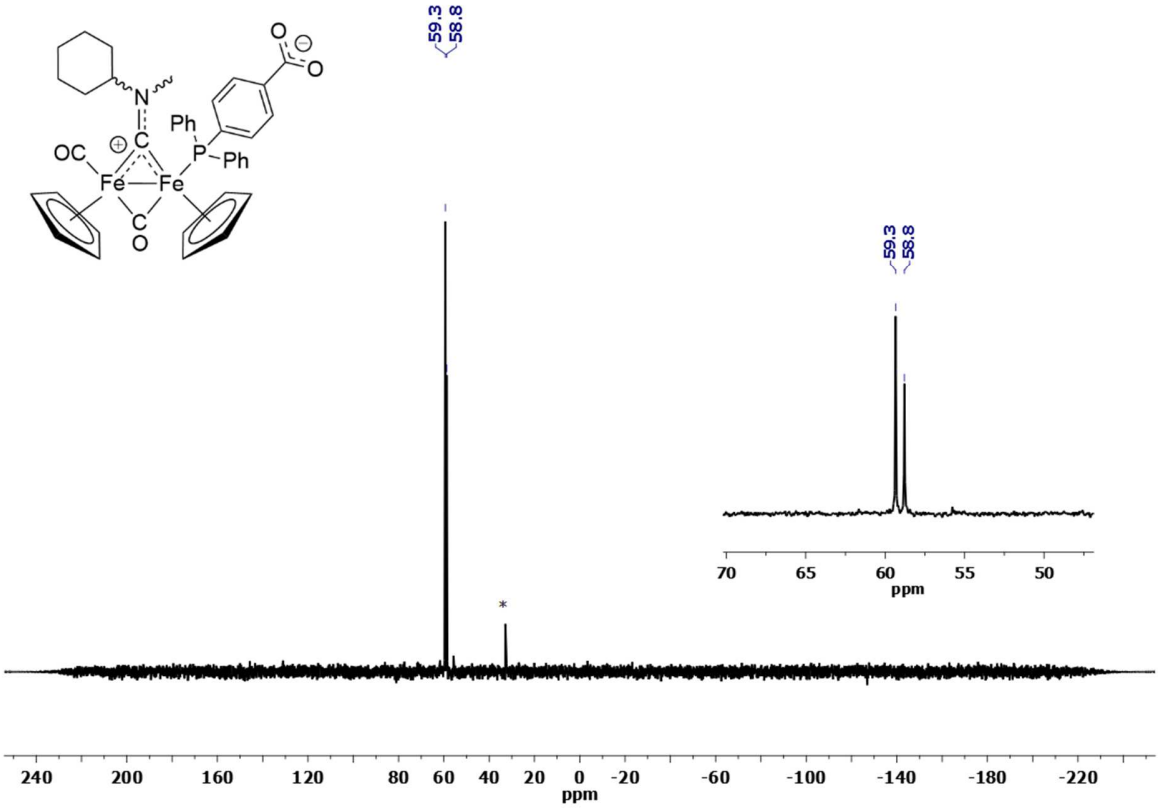


Figure S15. Black line: ^{1}H NMR spectrum (401 MHz, CD_3OD) of **2**$^{-H}$. Red line: ^{1}H NOESY with irradiation at 5.06 ppm (Cp^P of the *Z* isomer). Blue line: ^{1}H NOESY with irradiation at 4.97 ppm (Cp^P of the *E* isomer). Observed NOEs are indicated by the arrows.

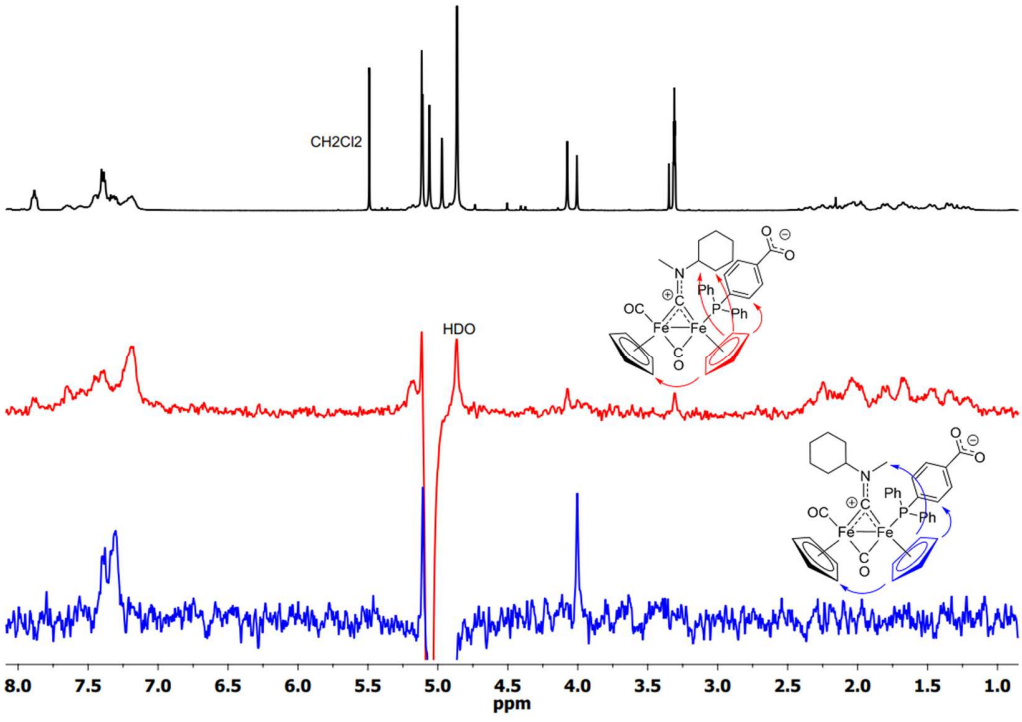


Figure S16. ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [**3**]CF_3SO_3.

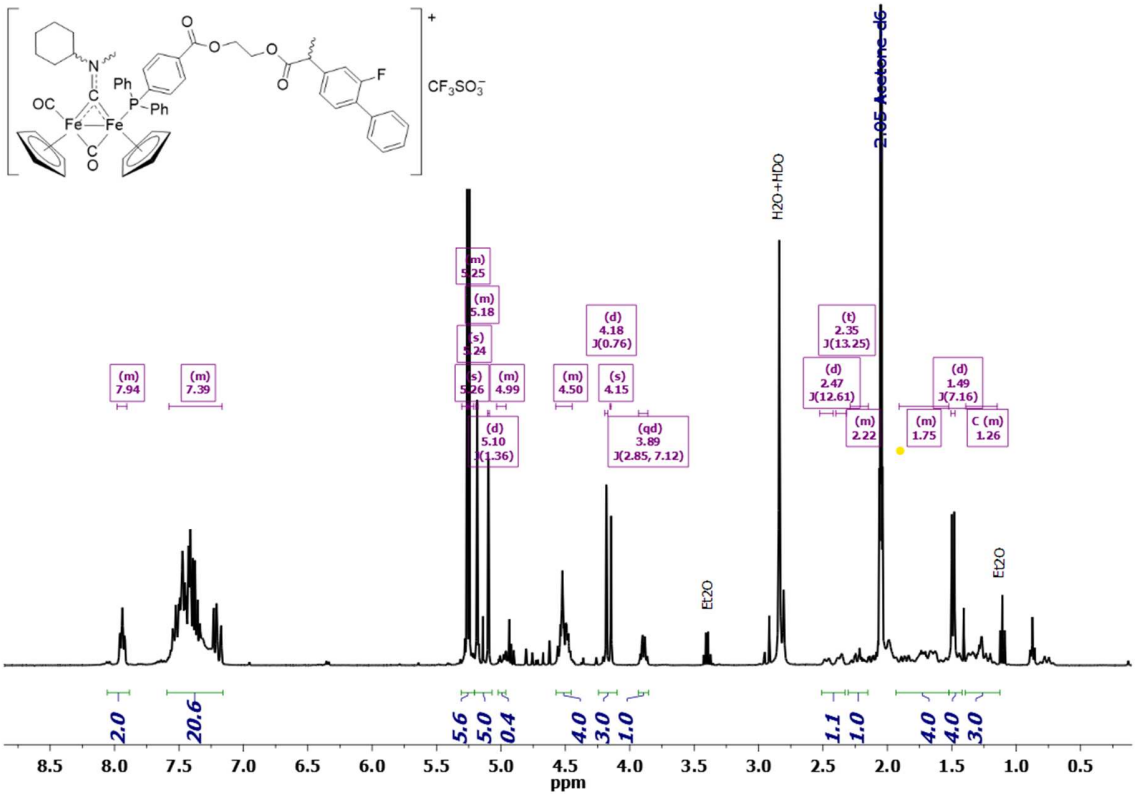


Figure S17. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, acetone-d_6) of [**3**]CF_3SO_3. Doublets in the 140-120 ppm region are not highlighted.

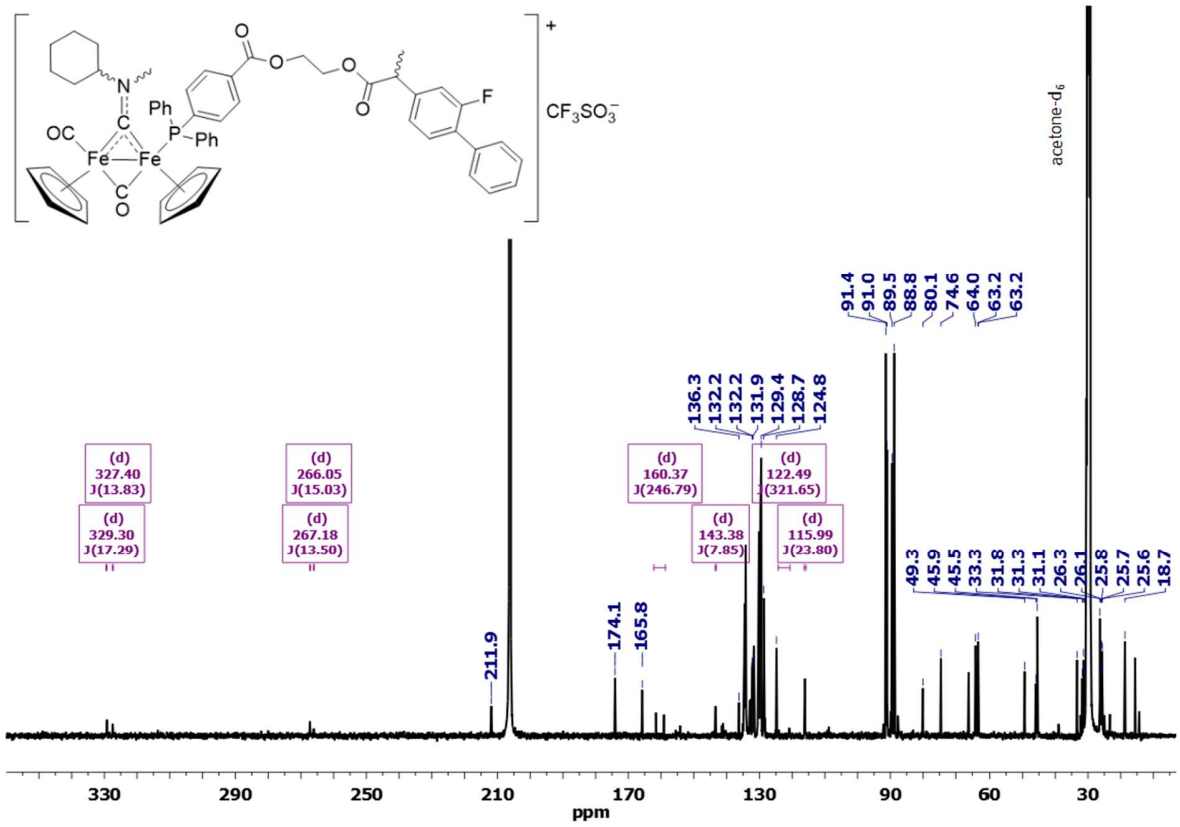


Figure S18. $^{19}F\{^{1}H\}$ NMR spectrum (376 MHz, acetone-d_6) of [**3**]CF_3SO_3.

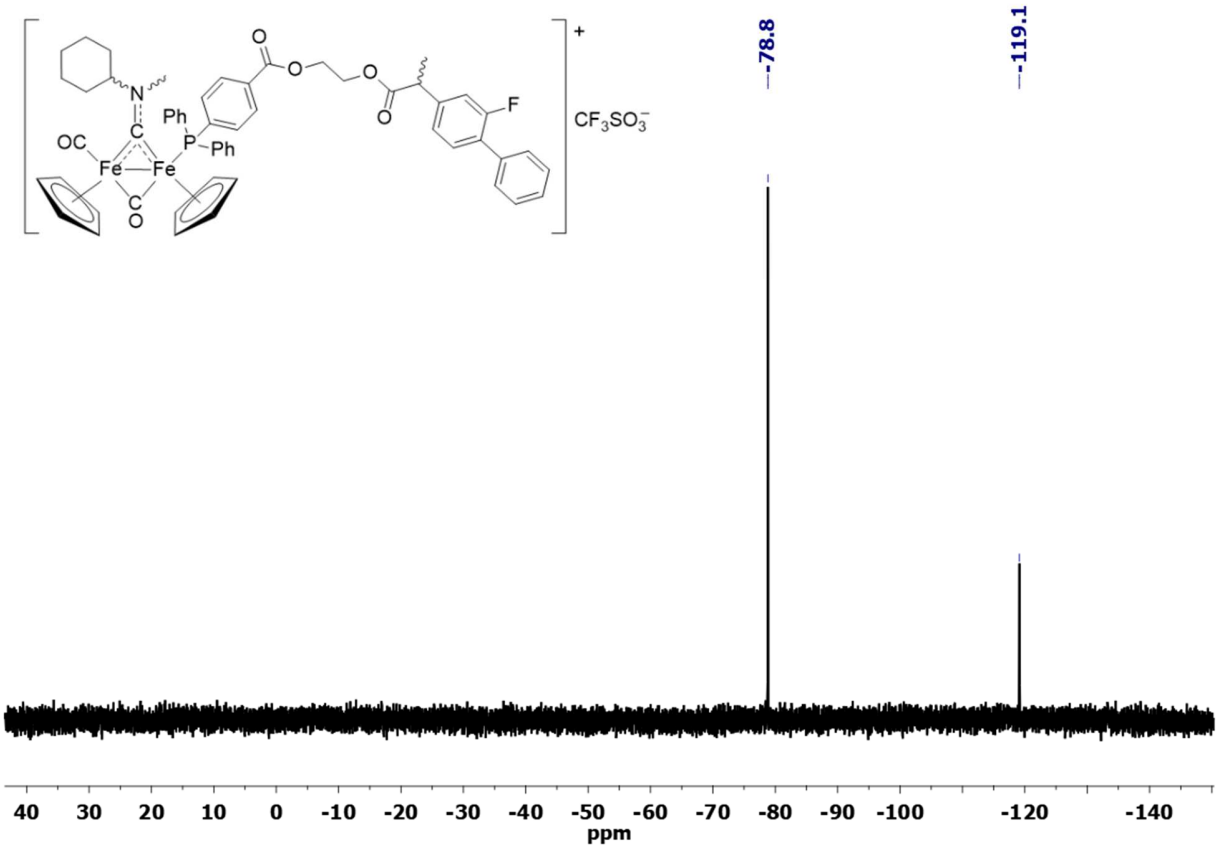


Figure S19. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, acetone-d_6) of [**3**]CF_3SO_3.

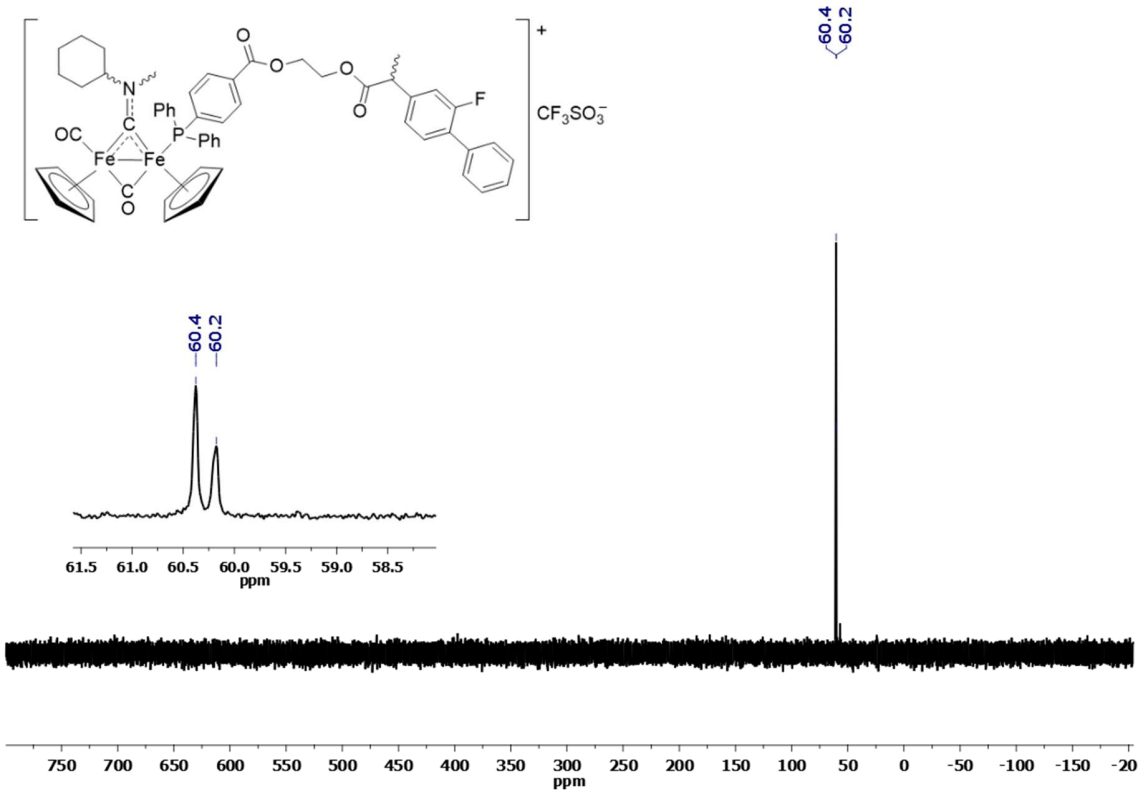


Figure S20. Black line: ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [**3**]CF_3SO_3. Red line: ^{1}H NOESY with irradiation at 5.18 ppm (Cp^P of the *Z* isomer). Blue line: ^{1}H NOESY with irradiation at 5.10 ppm (Cp^P of the *E* isomer). R = $(CH_2)_2OC(O)$-**FLU**. Observed NOEs are indicated by the arrows.

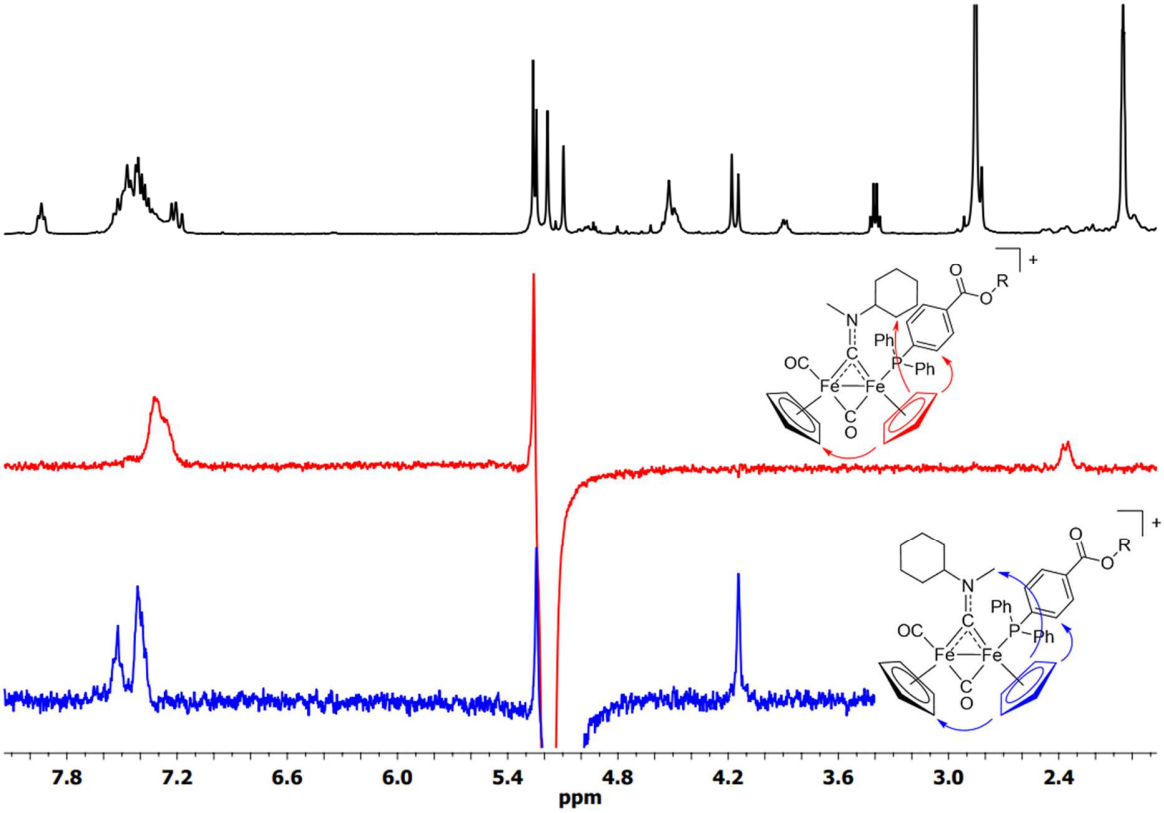


Figure S21. ^{1}H-^{13}C HSQC NMR spectrum of (acetone-d_6) of [**3**]CF_3SO_3.

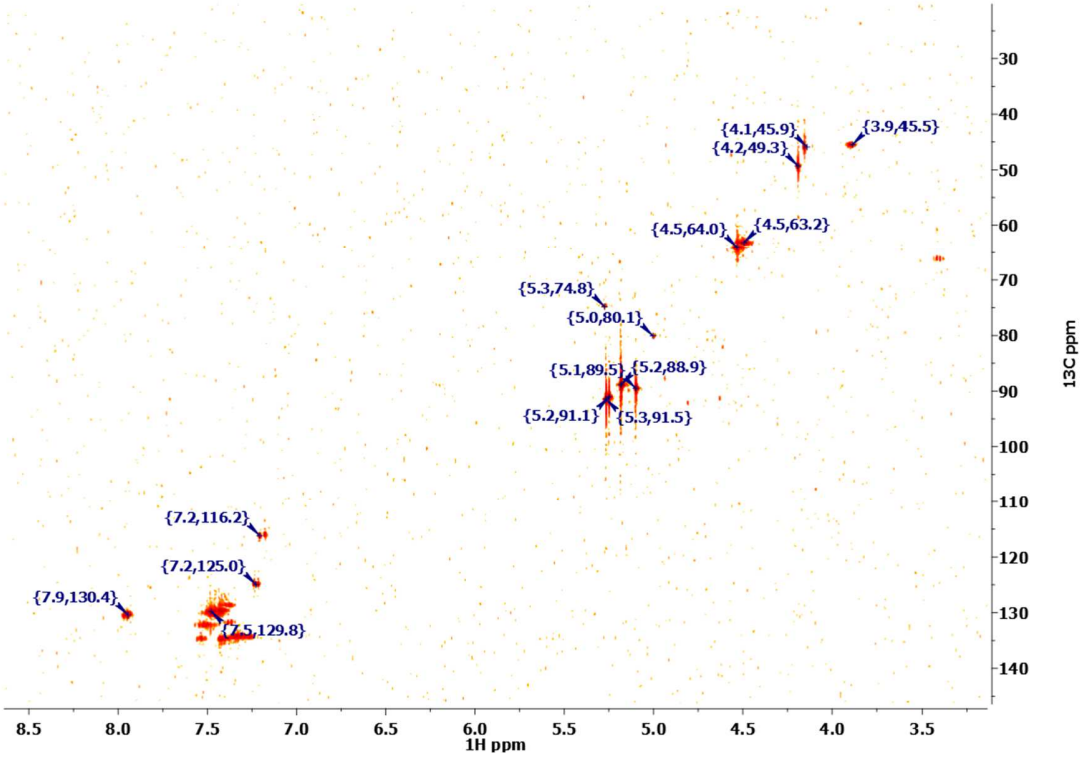


Figure S22. ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [**4**]CF_3SO_3.

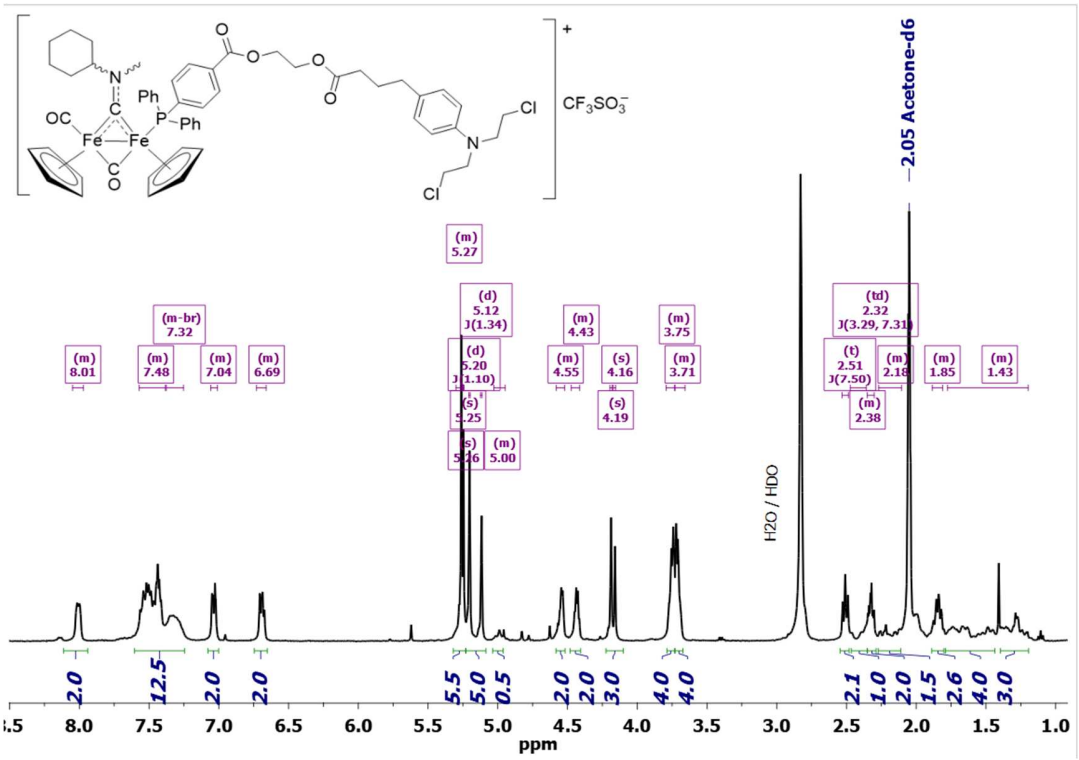


Figure S23. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, acetone-d_6) of [**4**]CF_3SO_3. Doublets in the 140-120 ppm region are not highlighted.

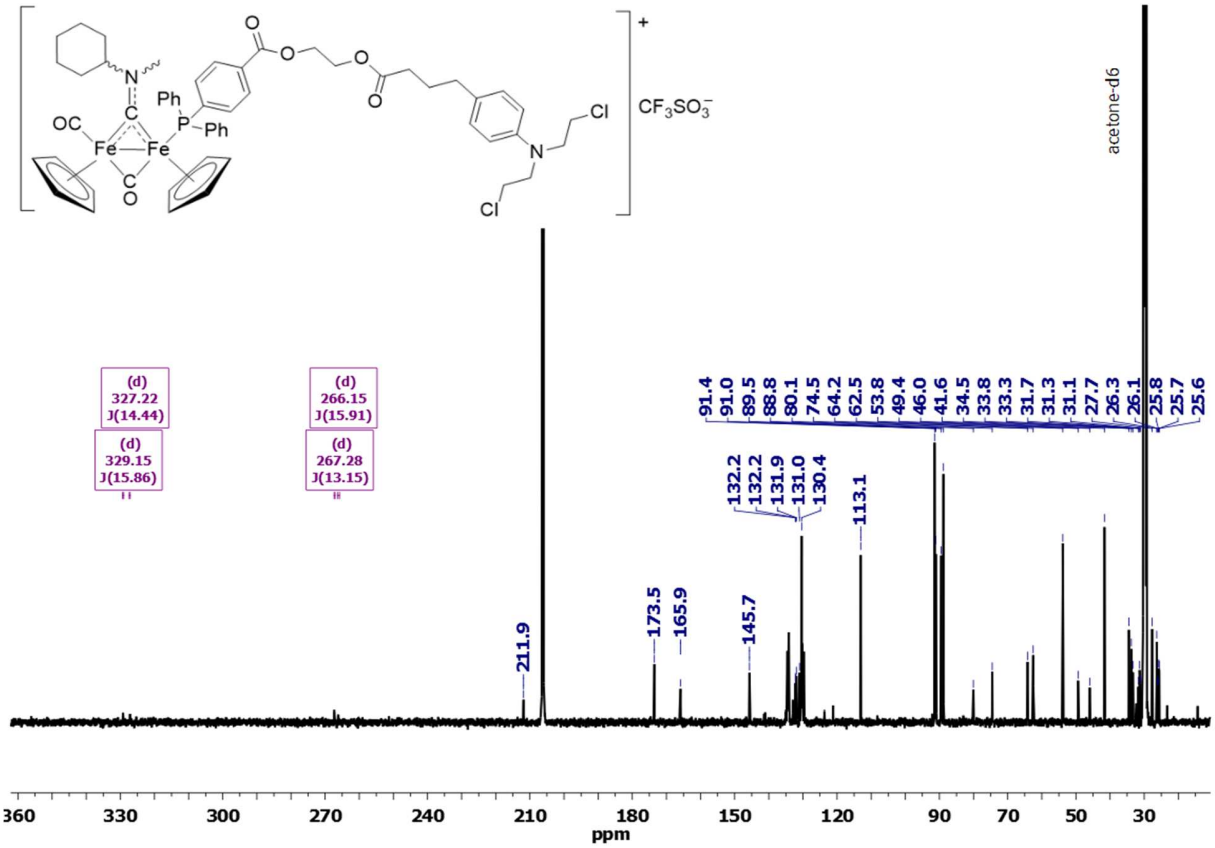


Figure S24. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, acetone-d_6) of [**4**]CF_3SO_3.

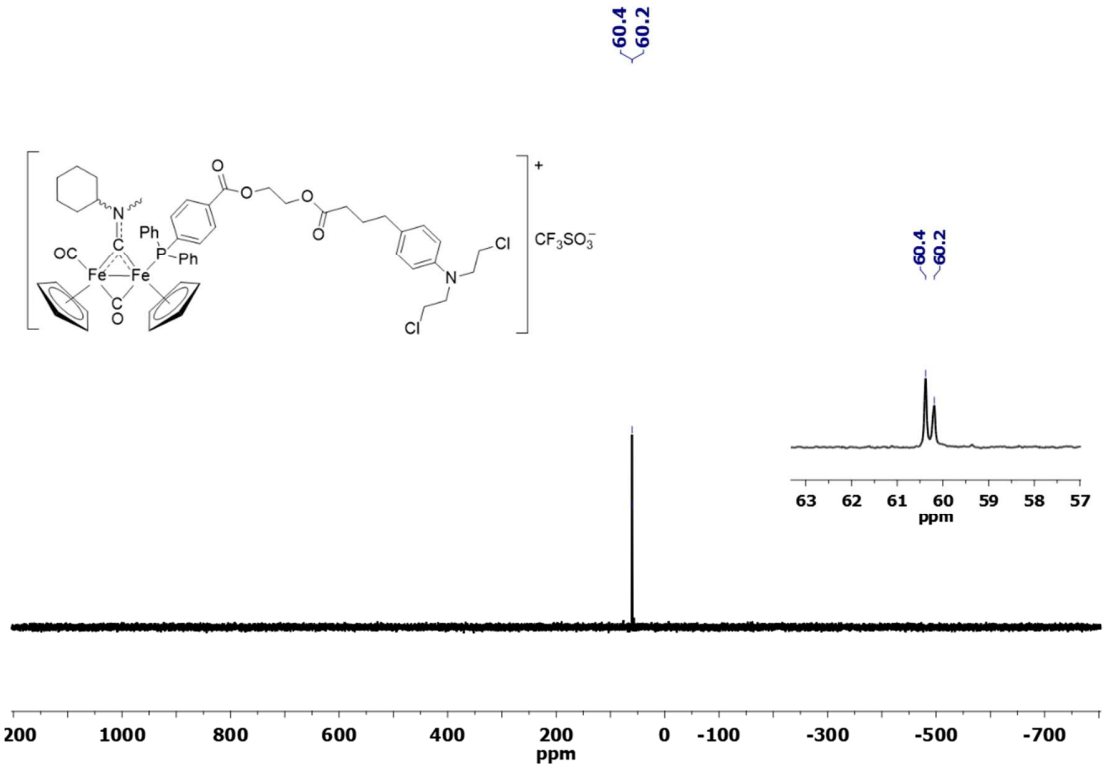


Figure S25. Black line: ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [**4**]CF_3SO_3. Red line: ^{1}H NOESY with irradiation at 5.20 ppm (Cp^P of the *Z* isomer). Blue line: ^{1}H NOESY with irradiation at 5.12 ppm (Cp^P of the *E* isomer). R = $(CH_2)_2OC(O)$-**CMB**. Observed NOEs are indicated by the arrows.

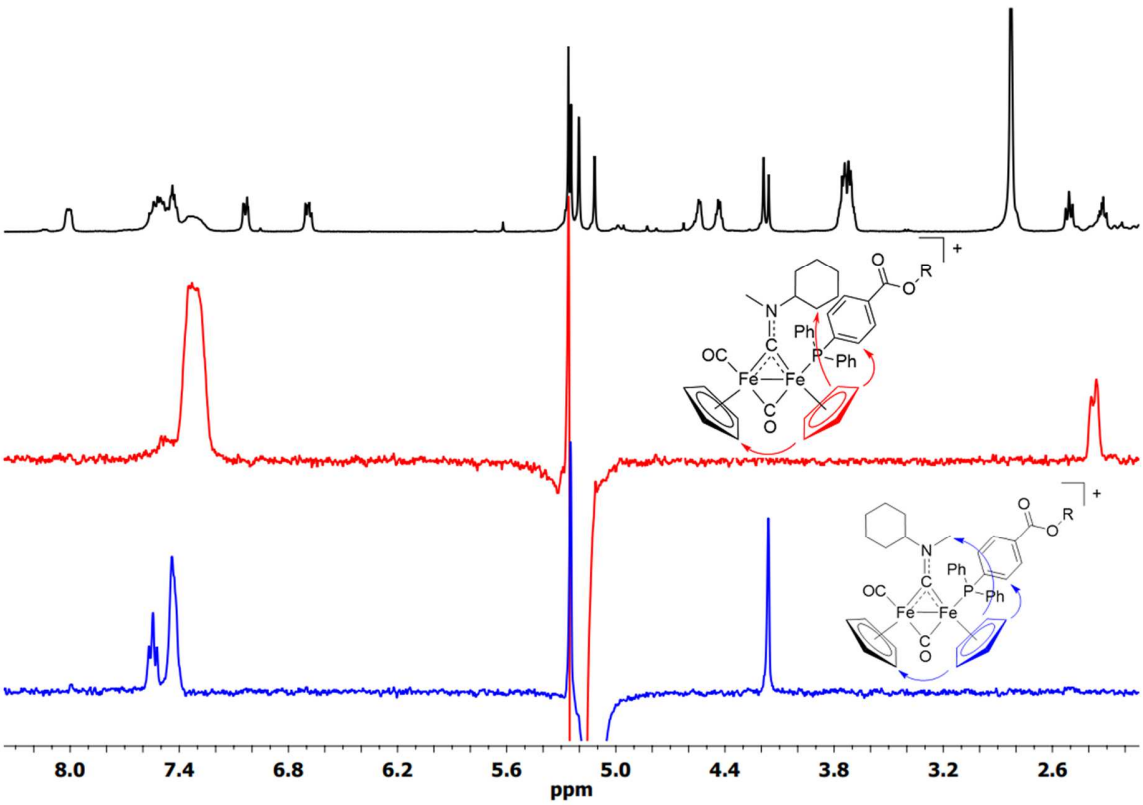


Figure S26. ^{1}H-^{13}C HSQC NMR spectrum of (acetone-d_6) of [**4**]CF_3SO_3.

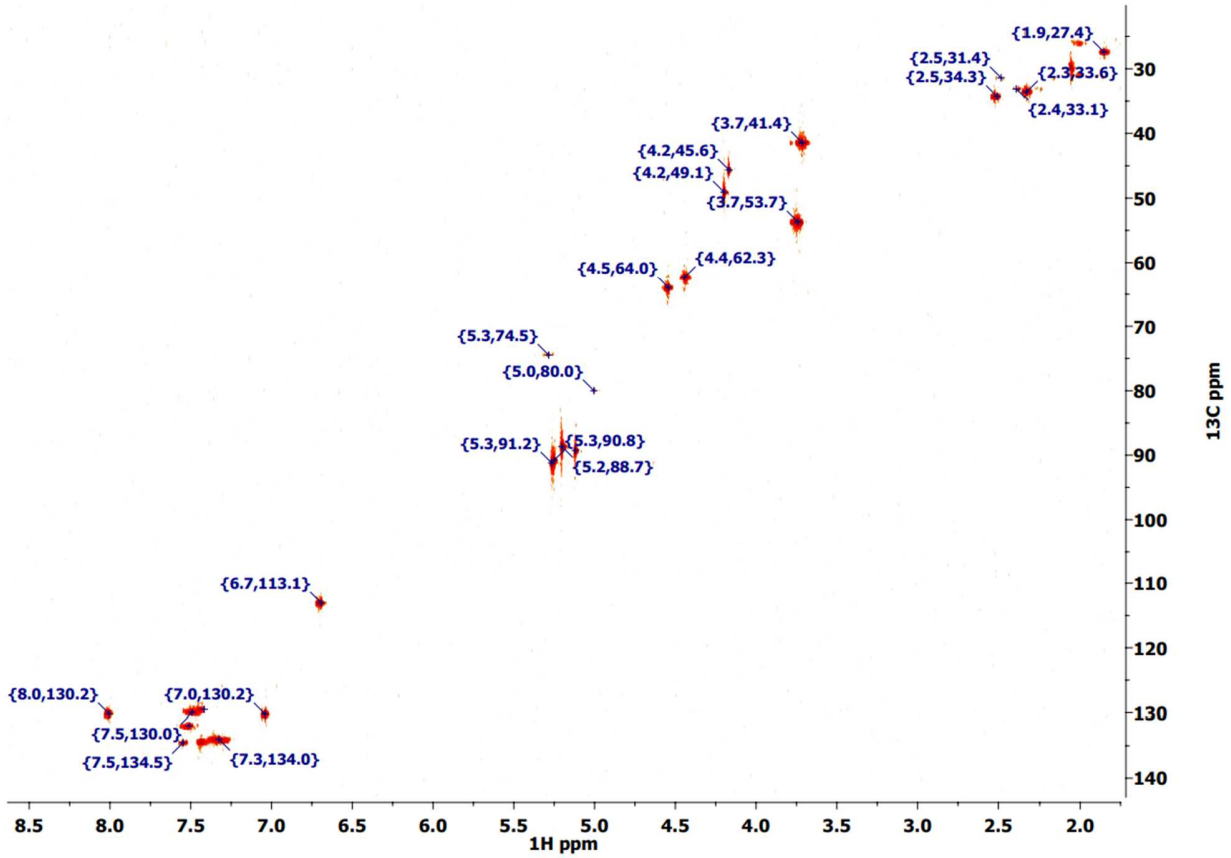


Figure S27. FIA-ESI(+)-MS spectrum of [**3**]CF_3SO_3 in MeOH (black line) and calculated isotopic pattern (red boxes). Calcd. base peak for [**3**]$^{+}$ ($C_{56}H_{54}FFe_2NO_6P$): 998.2465 Da.

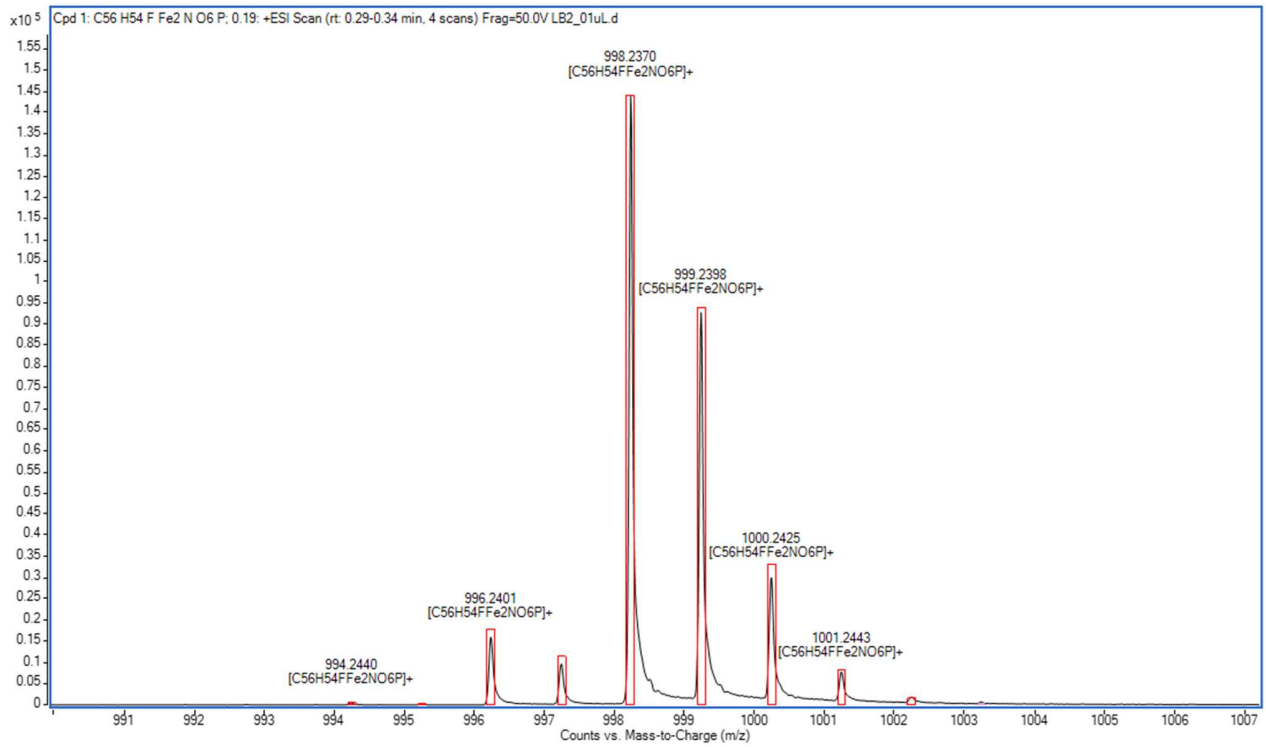
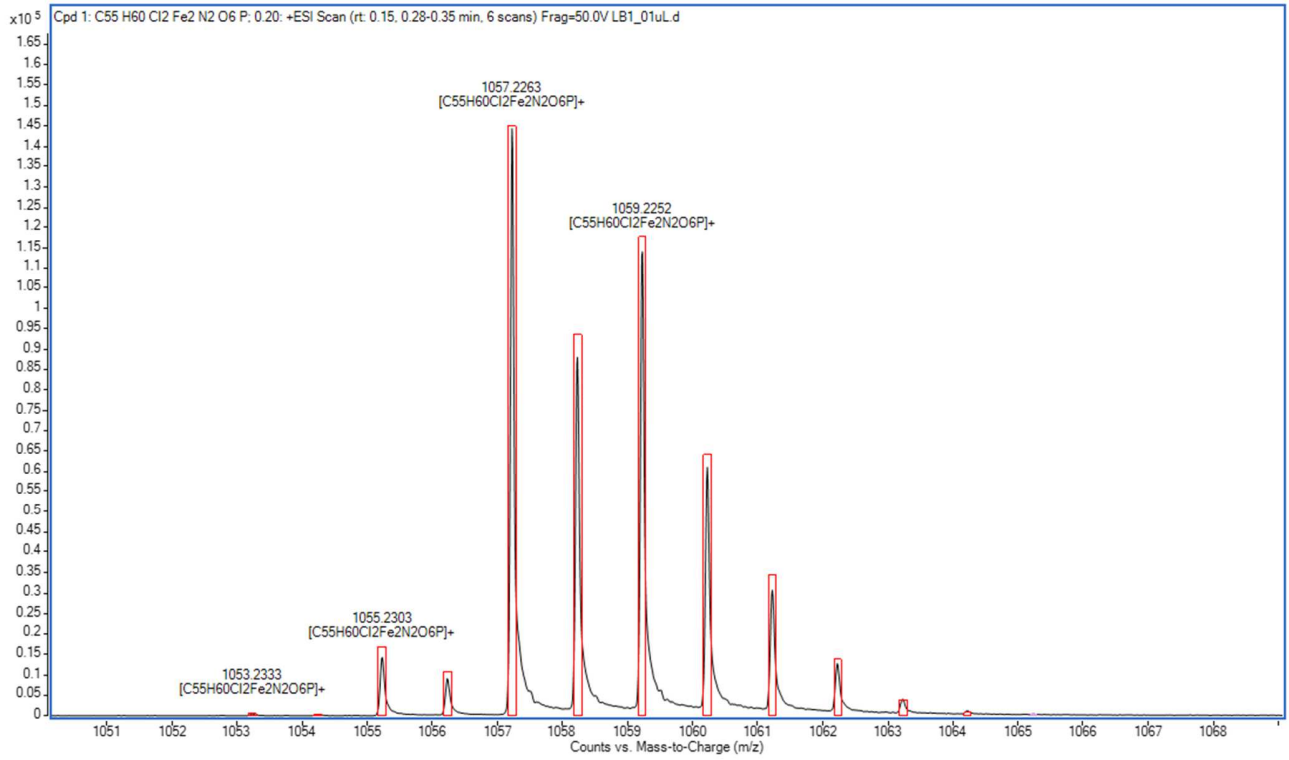


Figure S28. FIA-ESI(+)-MS spectrum of [**4**]CF_3SO_3 in MeOH (black line) and calculated isotopic pattern (red boxes). Calcd. base peak for [**4**]$^+$ ($C_{55}H_{60}Cl_2Fe_2N_2O_6P$): 1057.2358 Da.



HPLC analyses of bionconjugated diiron complexes

Figure S29. Chromatogram of [**3**]CF_3SO_3 recorded on a C_{18} capillary column using $^{i}PrOH/H_2O$ 4:1 *V*/*V* as eluent (isocratic elution) and an UV-Vis detector at 280 nm. t_r = 2.90 min. Chromatographic purity 92 % (integration: 2.0-4.5 min).

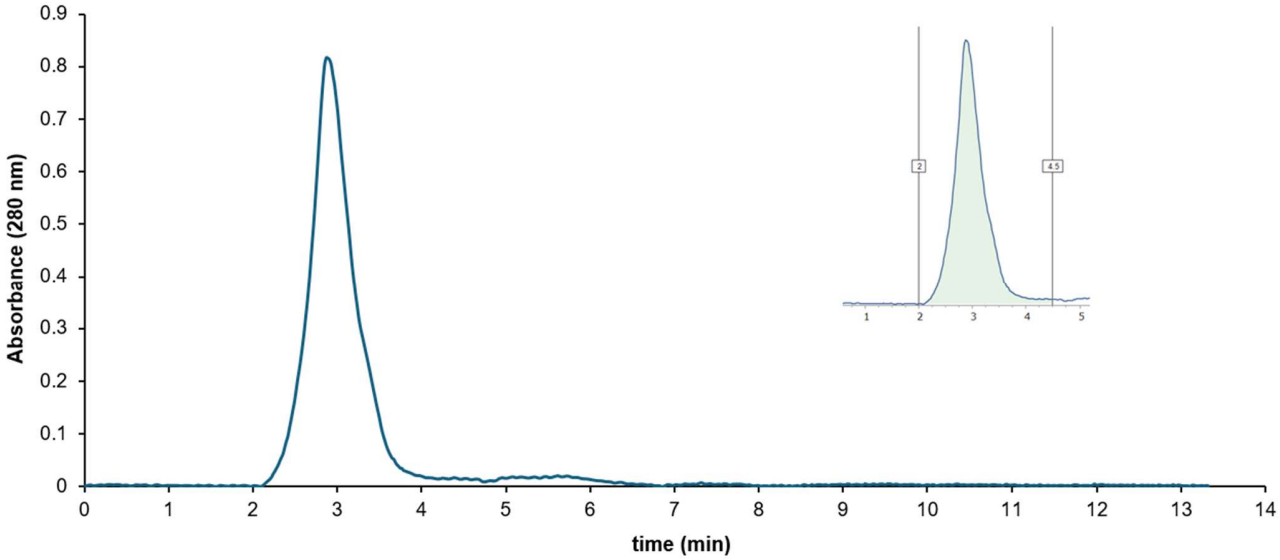
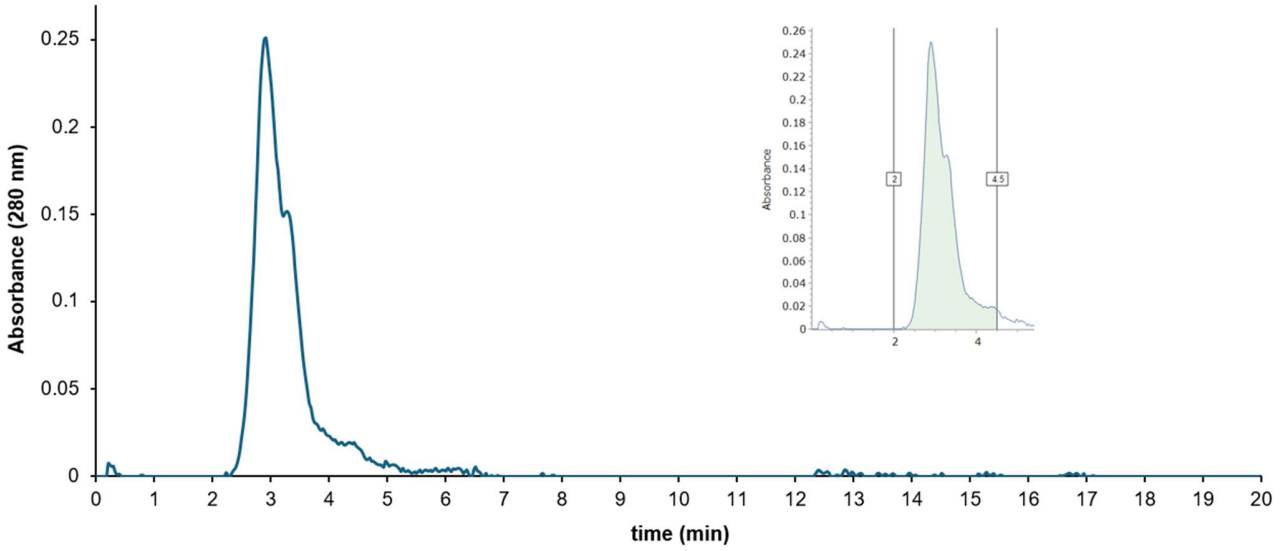


Figure S30. Chromatogram of [**4**]CF_3SO_3 recorded on a C_{18} capillary column using $^{i}PrOH/H_2O$ 4:1 *V*/*V* as eluent (isocratic elution) and an UV-Vis detector at 280 nm. t_r = 2.90 and 3.29 min, ascribable to *E*/*Z* diastereomers. Chromatographic purity 93 % (integration: 2.0-4.5 min).



NMR spectra of bionconjugated phosphane oxides

Figure S31. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of 2-hydroxyethyl 4-(diphenylphosphoryl)benzoate, **O=P^{OH}**.

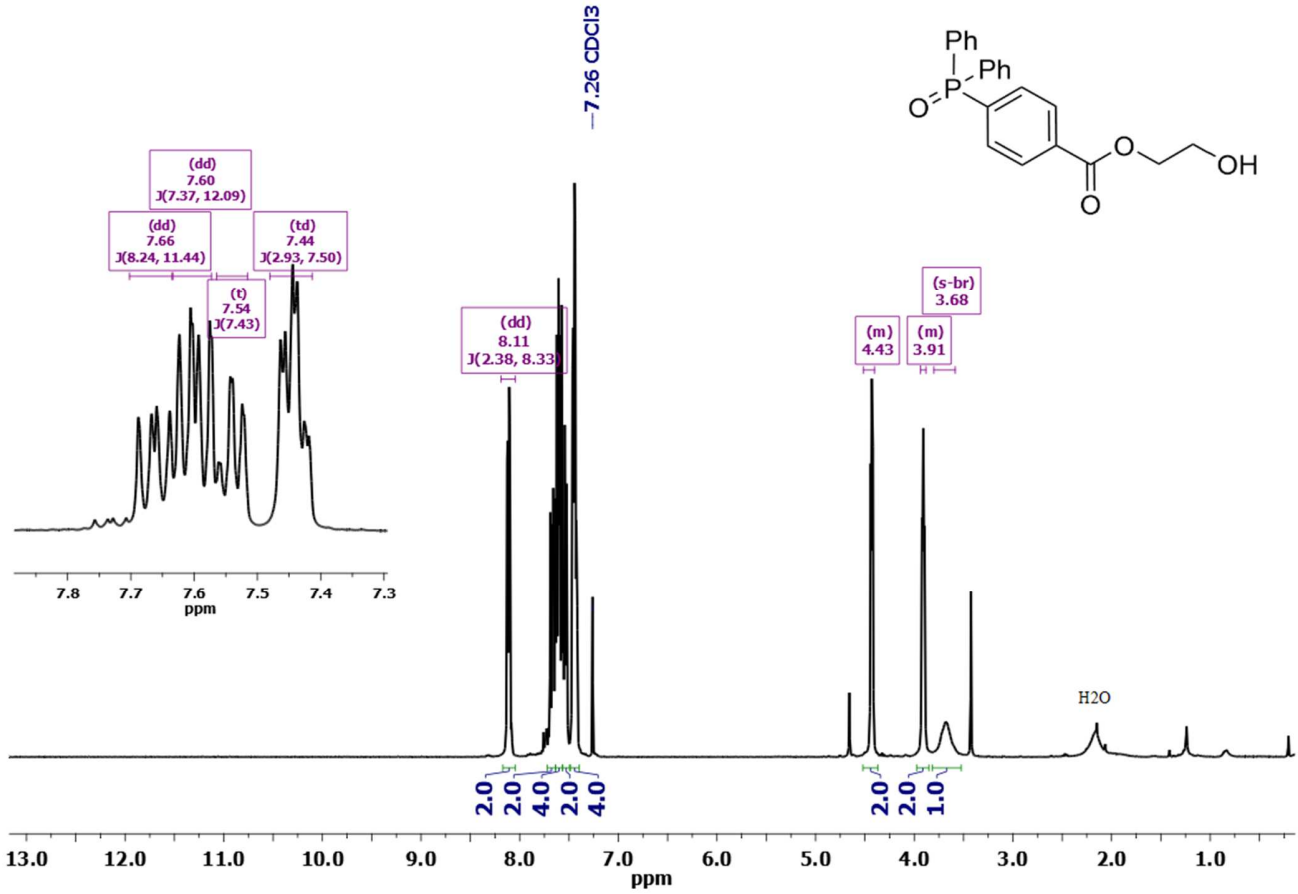


Figure S32. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of **O=P^{OH}**.

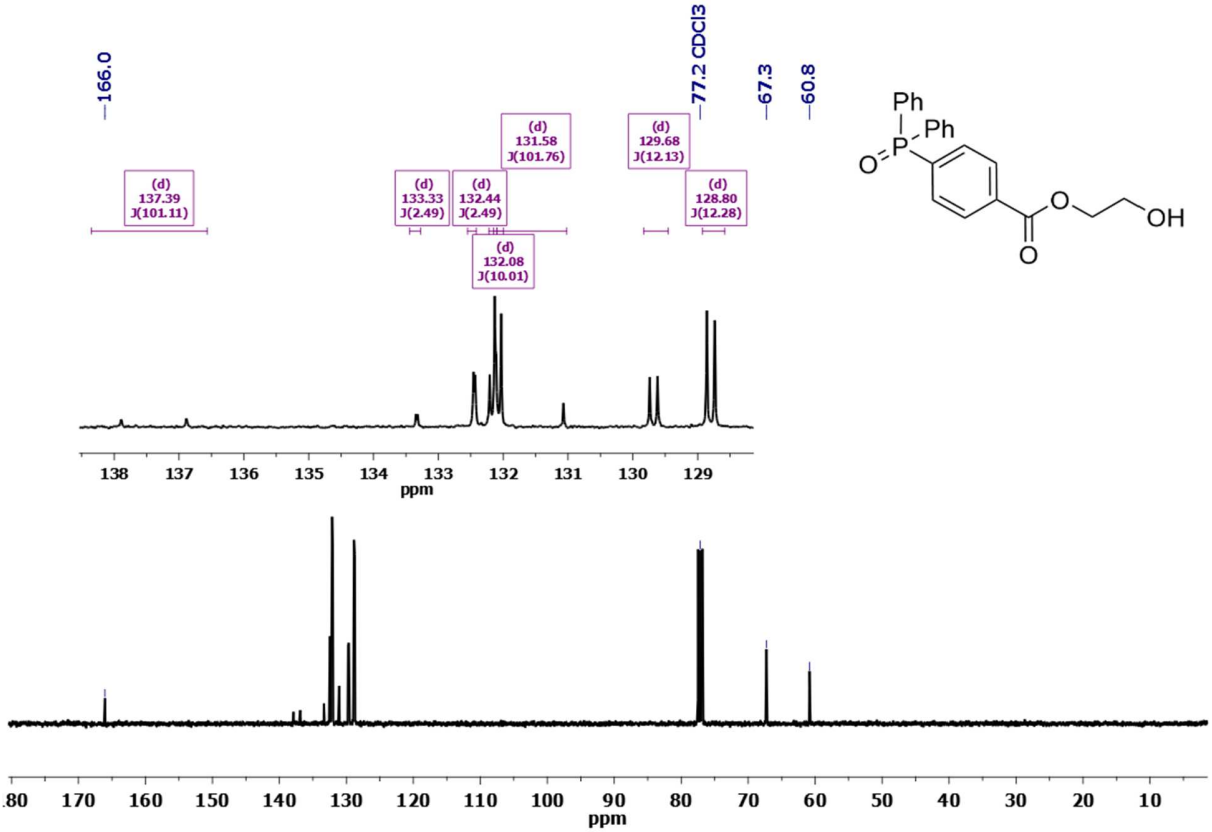


Figure S33. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, $CDCl_3$) of **O=P^{OH}**.

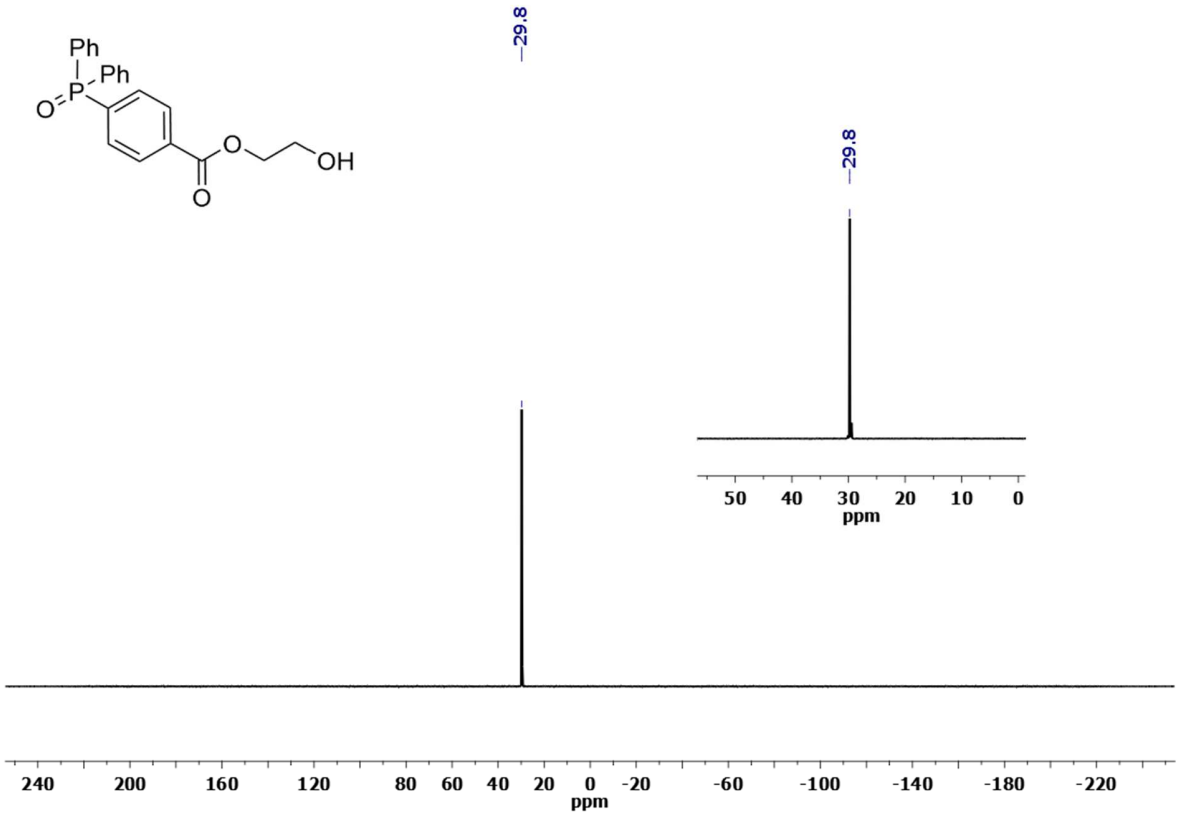


Figure S34. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of Flurbiprofen, 2-hydroxyethyl 4-(diphenylphosphoryl)benzoate, **O=P^{FLU}**.

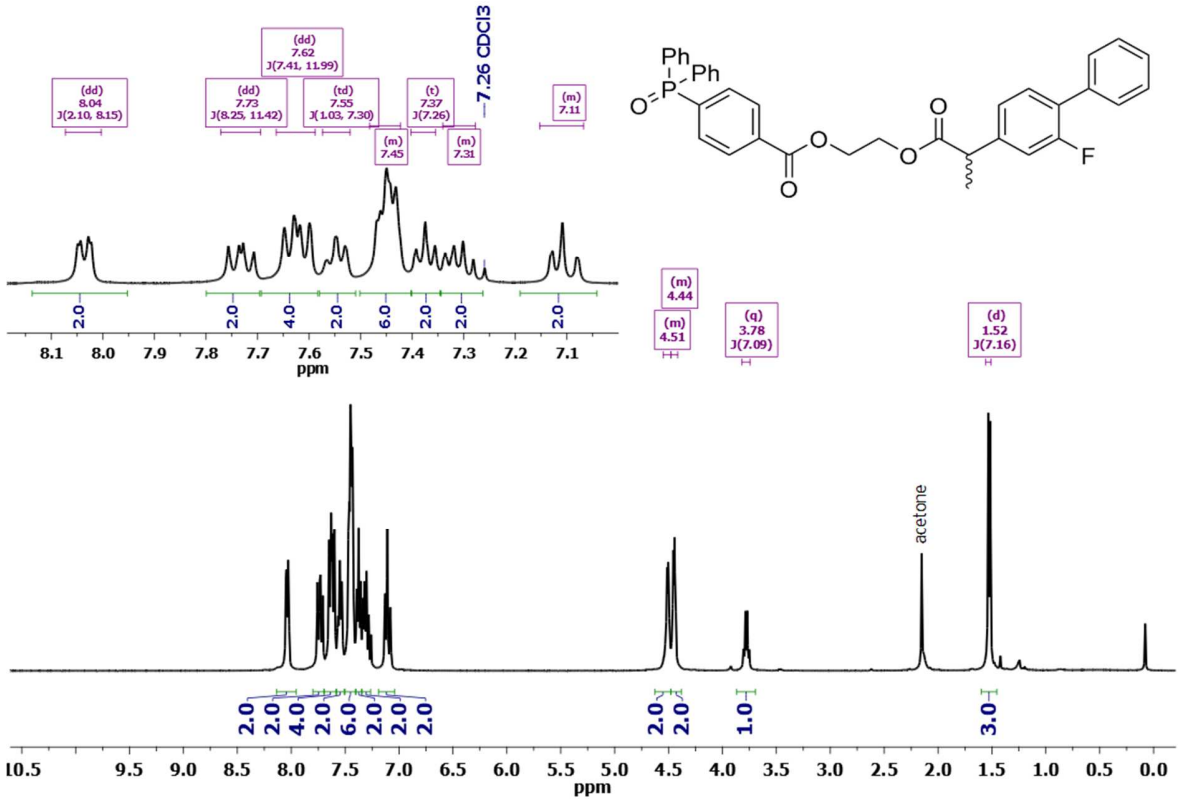


Figure S35. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of **O=P^{FLU}**.

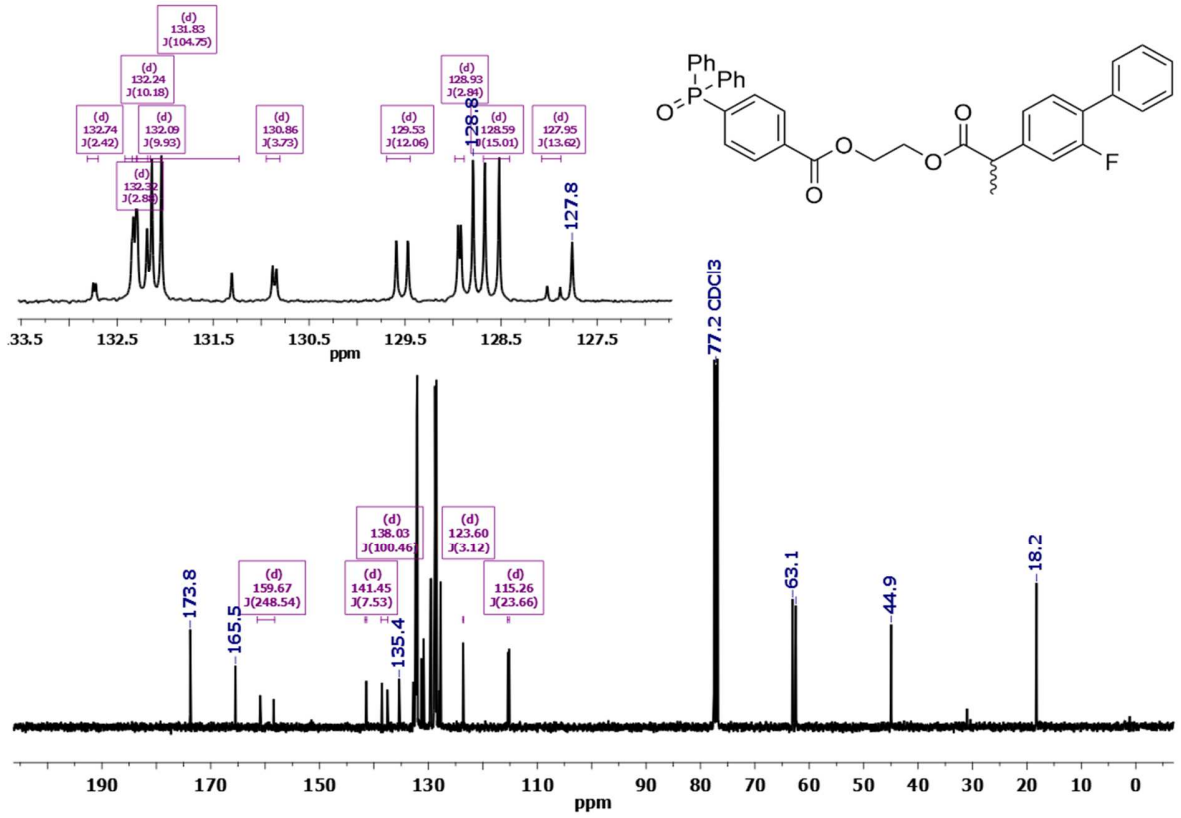


Figure S36. ^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **O=P^{FLU}**.

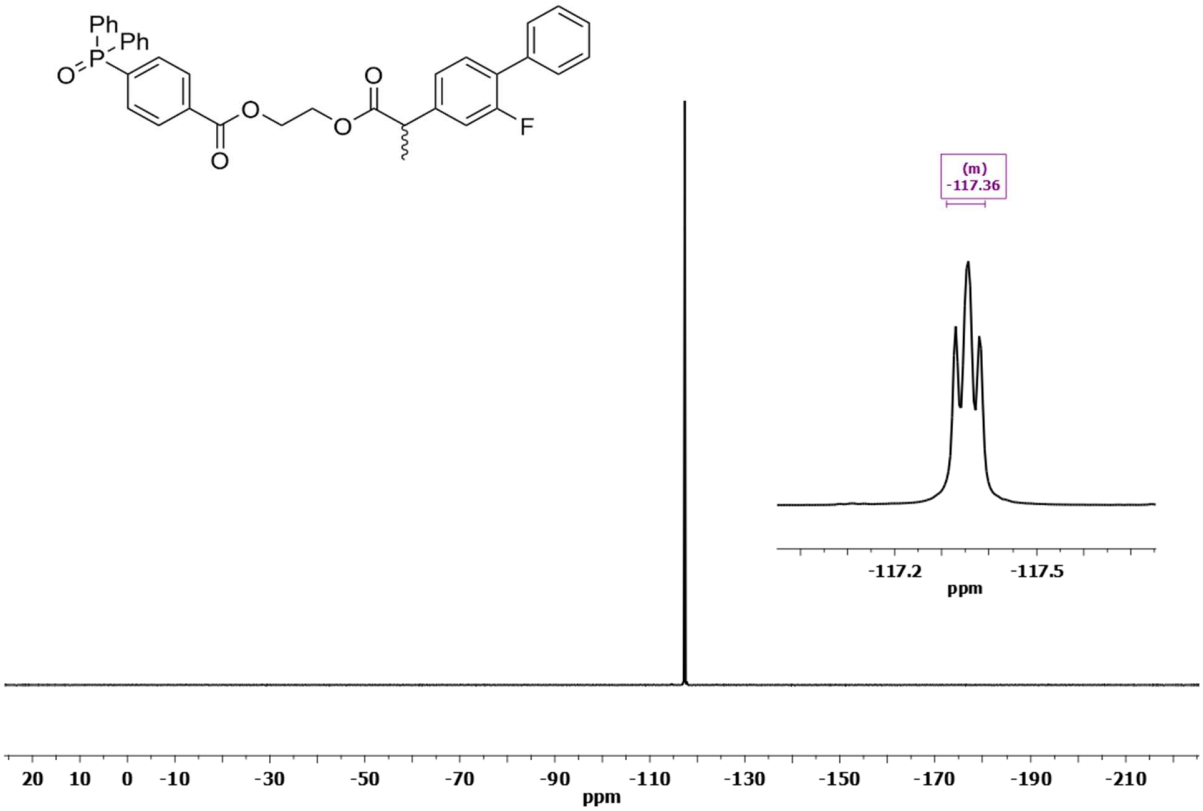


Figure S37. $^{31}P\{^1H\}$ NMR spectrum (162 MHz, $CDCl_3$) of **O=P^{FLU}**.

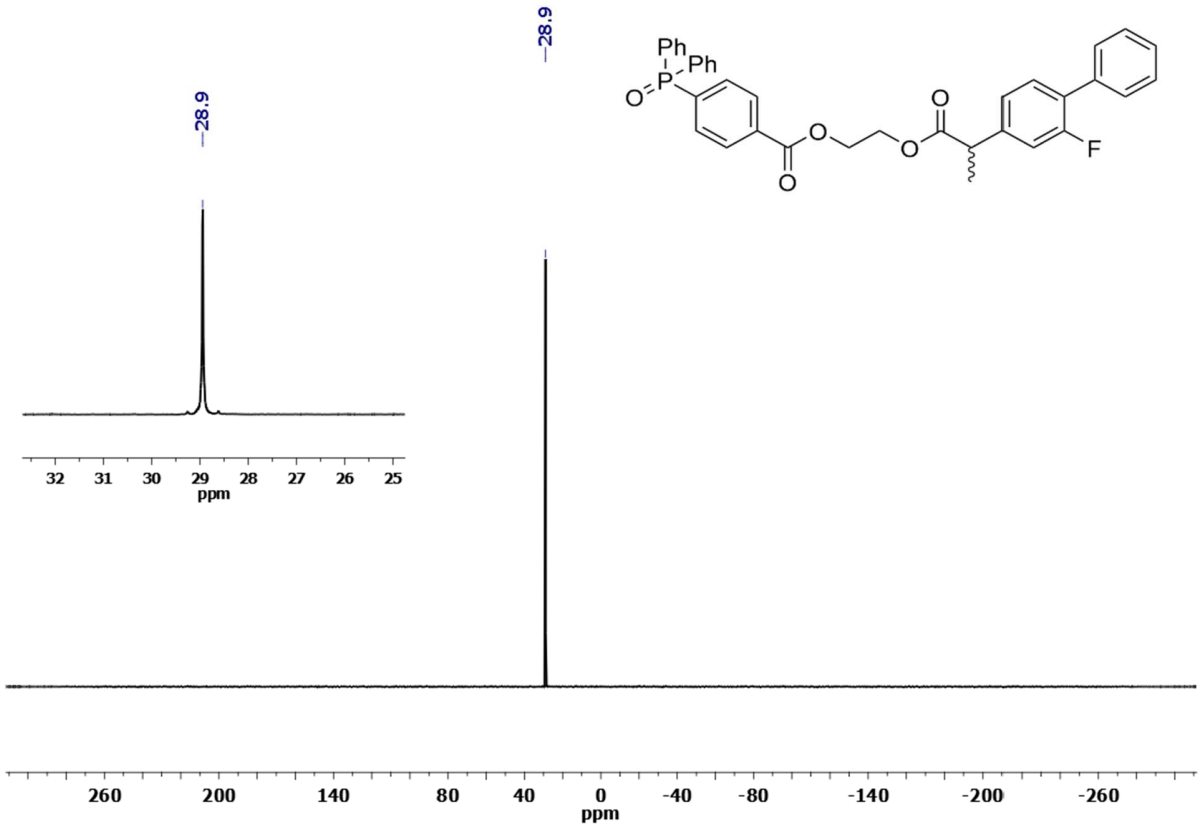


Figure S38. ^{1}H-^{13}C HMBC NMR spectrum of ($CDCl_3$) of **O=P^{FLU}**.

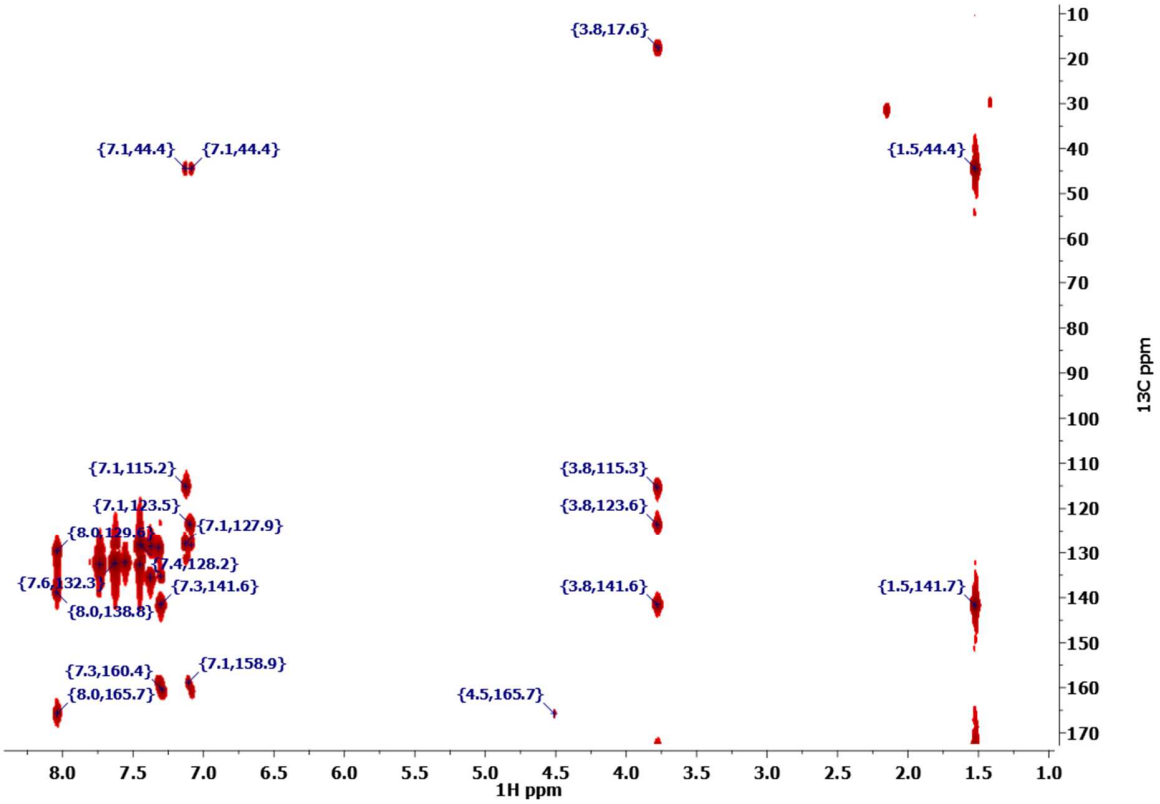


Figure S39. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of Chlorambucil, 2-hydroxyethyl 4-(diphenylphosphoryl)benzoate, **O=P^{CMB}**.

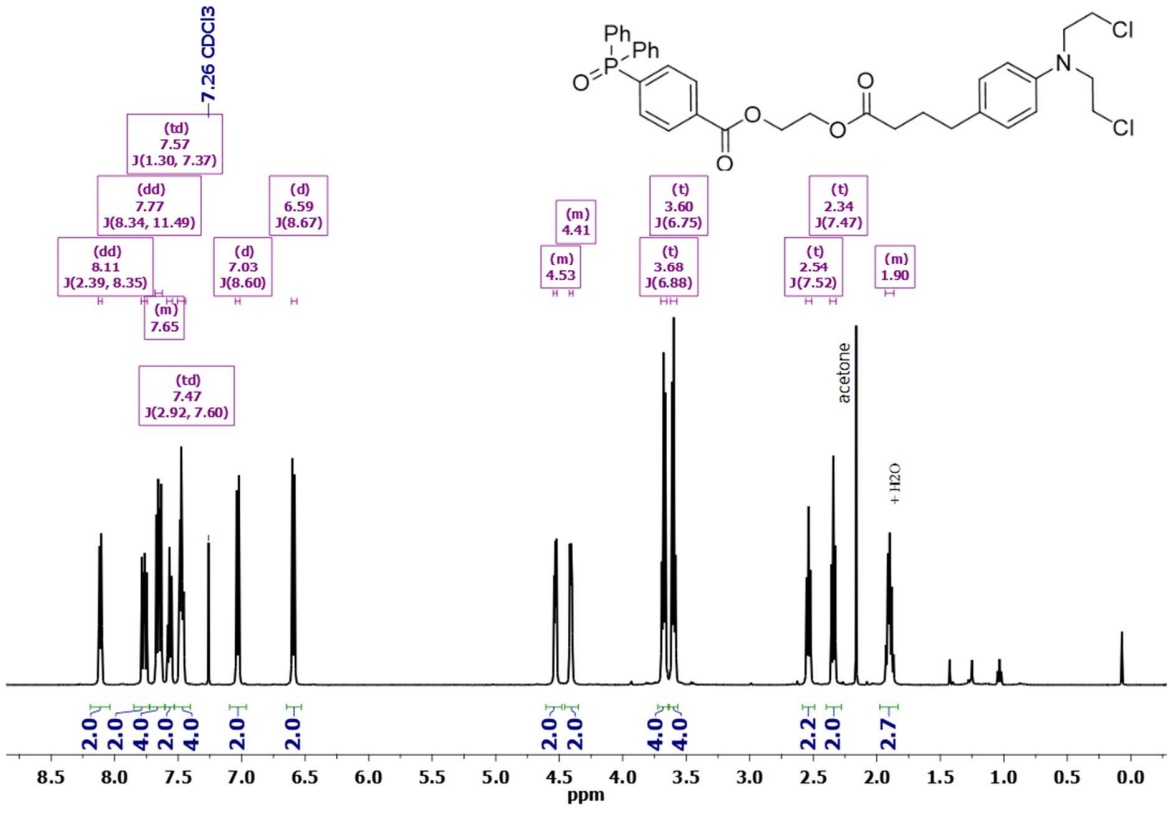


Figure S40. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of **O=P^{CMB}**.

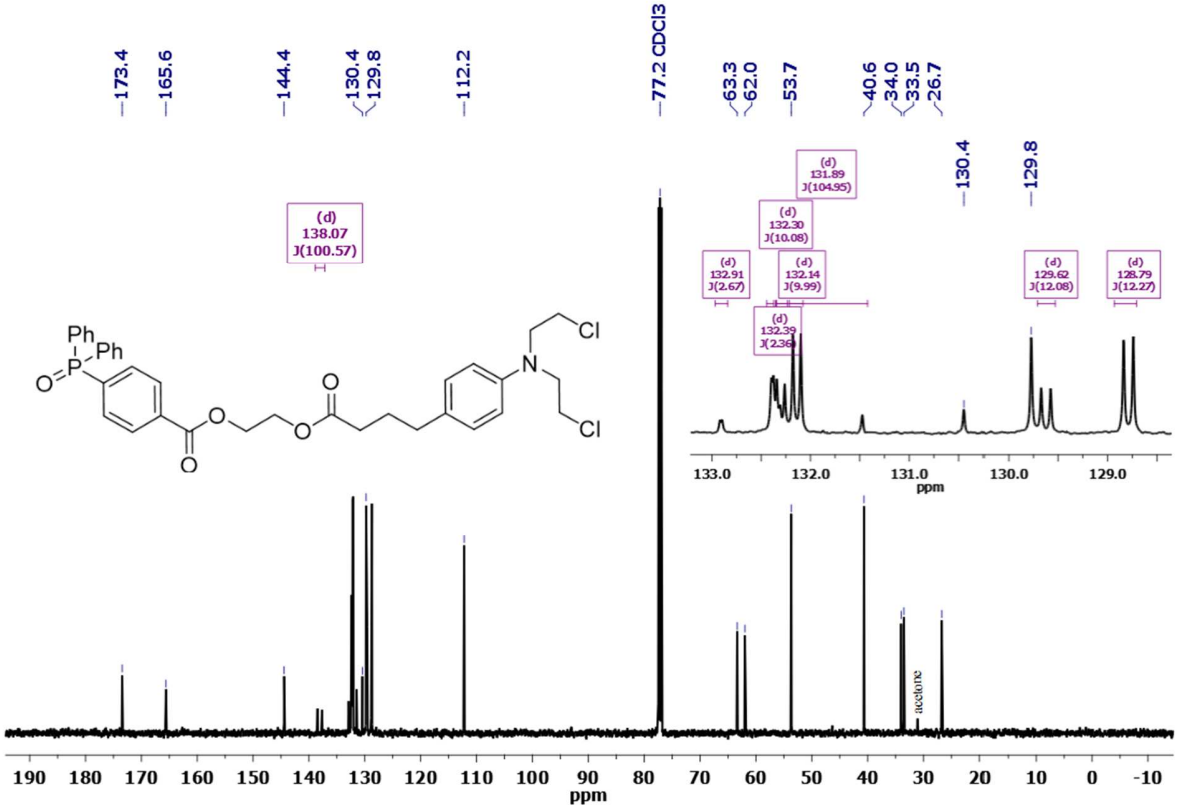


Figure S41. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, $CDCl_3$) of **O=P^{CMB}**.

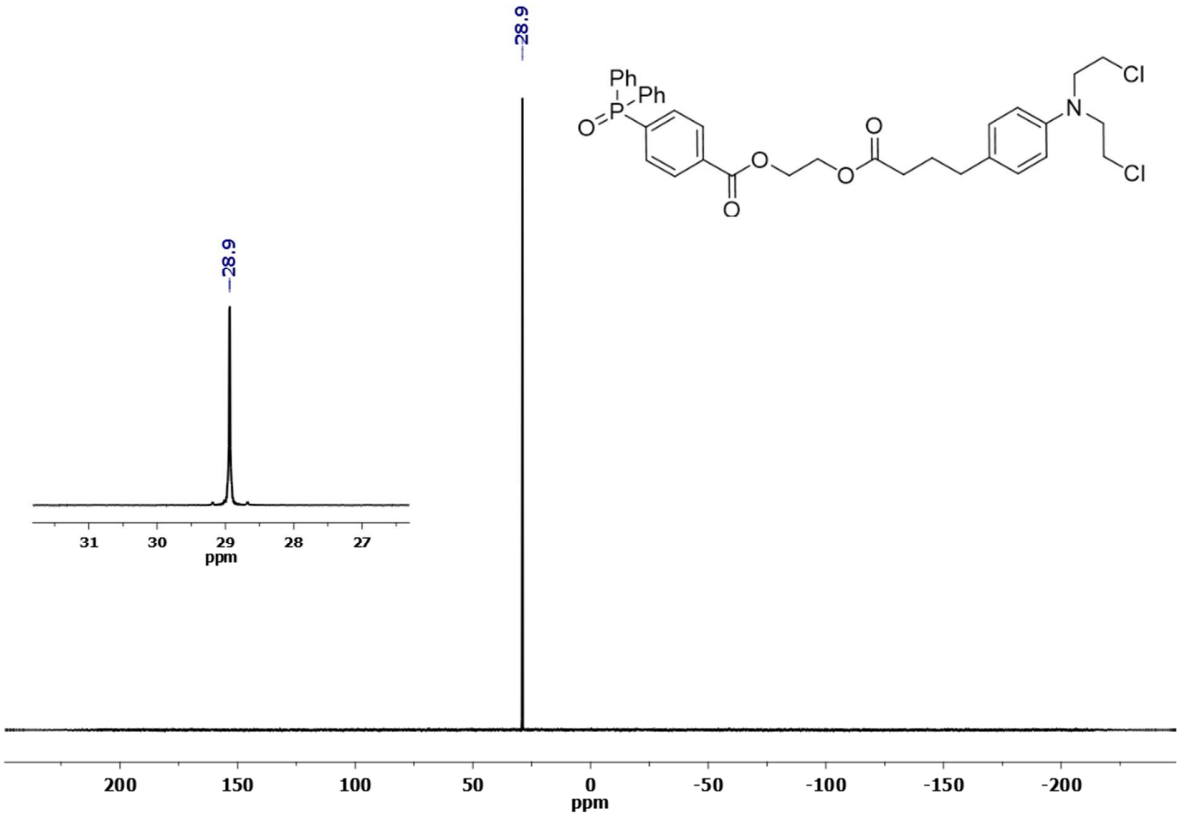
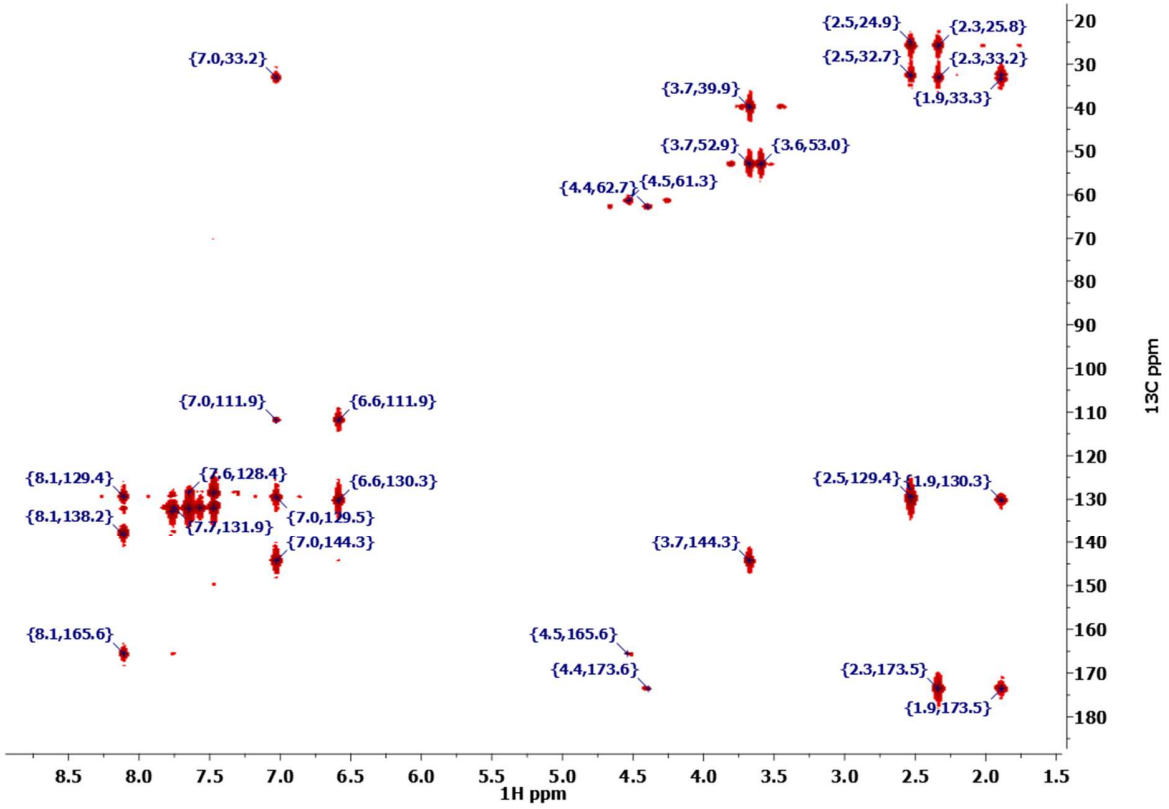


Figure S42. ^{1}H-^{13}C HMBC NMR spectrum of ($CDCl_3$) of **O=P^{CMB}**.



Inertness/speciation in aqueous media: NMR data and other details

Chemical shifts in ^{1}H NMR spectra are referenced to Me_2SO_2 [δ/ppm = 3.14]

[2]CF_3SO_3. Olive green-brown solution. ^{1}H NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 7.99–7.88 (m, 2H); 7.76–7.15 (m, 12H); 5.15, *5.14* (s, 5H); 5.10, *5.00* (s, 5H); 4.09, *4.02* (s, 3H); 2.28–1.29 (m, 10H). ^{31}P{^{1}H} NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 60.4, *60.0*. Isomer (*Z*/*E*) ratio = 2.0 (0-72 h).

Other species detected in the final solution. **O=PPh_2(4-$C_6H_4CO_2H$)**. ^{1}H NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 8.09−8.04 (m). ^{31}P{^{1}H} NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 32.9.

2^{-H}. Olive green-brown solution. ^{1}H NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 7.87 (d, *J* = 7.0 Hz, 2H); 7.57–7.12 (m, 12H); 5.15, *5.14* (s, 5H); 5.10, *5.00* (s,*); 4.09, *4.01* (s, 3H); 2.14–1.28 (m, 10H). ^{31}P{^{1}H} NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 60.2, *59.8*. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.97–7.91 (m, 2H); 7.59–7.34 (m, 9H); 7.26 (s-br, 3H); 5.18, *5.17* (s, 5H); 5.12, *5.03* (s,*); 4.13, *4.06* (s, 3H). ^{31}P{^{1}H} NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 59.8, *59.4*. Isomer (*Z*/*E*) ratio = 2.0 (0-72 h, for both solutions). *Over HDO resonance.

Other species detected in the final solutions. **O=PPh_2(4-$C_6H_4CO_2$)$^-$**. ^{1}H NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 8.07−8.02 (m). ^{31}P{^{1}H} NMR (D_2O/CD_3OD 1:1 *V*/*V*): δ/ppm = 35.7. The same set of signals (for **2^{-H}** and **O=PPh_2(4-$C_6H_4CO_2$)$^-$**) were observed in the DMEM-d/CD_3OD 1:1 *v*/*v* solution of [**2**]CF_3SO_3.

[3]CF_3SO_3. Olive green-brown solution. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.76 (app. t, *J* = 7.6 Hz, 2H), 7.60–7.07 (m, 20H); 5.15, *5.13*, 5.01, *4.94* (s,*); 4.61–4.38 (m, 4H); 4.06, *3.97* (s, 3H); 3.93 (q, *J* = 7.3 Hz, 1H); 2.37–1.37 (m, 10H); 1.53 (app. dd, *J* = 6.7 Hz, 3H). *Over HDO resonance. ^{31}P {^{1}H} NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 60.2, *59.8*. Isomer (*Z*/*E*) ratio = 2.0 (0-72 h).

Other species detected in the final solution: **O=P^{FLU}.**

[4]CF_3SO_3. Olive green-brown solution. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.99 (app. t, *J* = 8.7 Hz, 2H); 7.64–7.15 (m, 12H); 7.01–6.94 (m, 2H); 6.60 (app. t, *J* = 9.0 Hz, 2H); 5.16, *5.14*, 4.99 (s,*); 4.66–4.59 (m, 2H); 4.56–4.49 (m, 2H); 4.09, *4.01* (s, 3H); 3.75–3.64 (m, 8H); 2.46 (t, *J* = 7.3 Hz, 2H); 2.38 (t, *J* = 6.7 Hz, 2H); 2.31–2.06, 1.97–1.80, 1.76–1.20 (m, 12H). *Over HDO resonance. ^{31}P{^{1}H} NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 60.2, *59.8*. Isomer (*Z*/*E*) ratio = 2.0 (0-72 h).

Other species detected in the final solution: **O=P^{CMB}** and/or related species; diiron compounds derived from [**4**]$^+$ by rearrangements/hydrolysis of the chlorambucil backbone. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 5.17 (s), 5.15 (s), 5.10 (s), 4.10 (s), 4.03 (s), 3.57−3.47 (m).

Reference compounds.

PPh_2(4-$C_6H_4CO_2H$). ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 8.00 (d, *J* = 7.2 Hz, 2H), 7.51–7.41 (m, 6H), 7.35 (app. q, *J* = 7.2 Hz, 6H). ^{31}P{^{1}H} NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = − 5.2.

O=PPh_2(4-$C_6H_4CO_2H$). ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 8.20 (dd, *J* = 8.3, 2.5 Hz, 2H), 7.80–7.61 (m, 12H). ^{31}P{^{1}H} NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 33.5.

O=P^{FLU}. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.93 (dd, *J* = 8.2, 2.2 Hz, 2H), 7.74 (t, *J* = 7.2 Hz, 2H), 7.68–7.52 (m, 9H), 7.43–7.30 (m, 4H), 7.28–7.17 (m, 2H), 7.13 (d, *J* = 12.0 Hz, 1H), 4.69 (m*), 4.60–4.55 (m, 2H), 4.45 (dt, *J* = 7.8, 3.6 Hz, 1H), 3.93 (q, *J* = 7.0 Hz, 1H), 1.54 (d, *J* = 7.1 Hz, 3H); *shoulder of the HDO signal. ^{31}P{^{1}H} NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 32.9.

O=P^{CMB}. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 8.20 (dd, *J* = 8.2, 2.2 Hz, 2H), 7.85–7.73 (m, 4H), 7.70–7.58 (m, 8H), 6.98 (d, *J* = 8.5 Hz, 2H), 6.61 (d, *J* = 8.5 Hz, 2H), 4.68–4.63 (m*), 4.55–4.50 (m, 2H), 3.74– 3.67 (m, 4H), 3.67–3.62 (m, 4H), 2.49 (t, *J* = 7.4 Hz, 2H), 2.38 (t, *J* = 7.0 Hz, 2H), 1.87 (q, *J* = 7.4 Hz, 2H); *shoulder of the HDO signal. ^{31}P{^{1}H} NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 32.9.

FLU-CO_2^- (from **FLU**-CO_2H in the buffered DMEM-d solution). ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.59–7.54 (m, 2H), 7.53–7.42 (m, 4H), 7.28 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.22 (dd, *J* = 12.0, 1.3 Hz, 1H), 3.84 (q, *J* = 7.1 Hz, 1H), 1.54 (d, *J* = 7.1 Hz, 3H).

FLU-$CO_2(CH_2)_2OH$. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.59–7.55 (m, 2H), 7.54–7.48 (m, 3H), 7.47–7.42 (m, 1H), 7.29 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.23 (dd, *J* = 11.9, 1.7 Hz, 1H), 4.34–4.26 (m, 1H), 4.21–4.15 (m, 1H), 3.97 (q, *J* = 7.1 Hz, 1H), 3.85–3.76 (m, 2H), 1.58 (d, *J* = 7.2 Hz, 3H).

CMB-CO_2^- (from **CMB**-CO_2H in the buffered DMEM-d solution). ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.13 (d, *J* = 8.6 Hz, 2H), 6.75 (d, *J* = 8.6 Hz, 2H), 3.79–3.76 (m, 4H), 3.74–3.69 (m, 4H), 2.59 (t, *J* = 7.5 Hz, 2H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.98–1.81 (m, 2H).

CMB-$CO_2(CH_2)_2OH$. ^{1}H NMR (D_2O/CD_3OD 2:5 *V*/*V*): δ/ppm = 7.13 (d, *J* = 8.6 Hz, 2H), 6.75 (d, *J* = 8.6 Hz, 2H), 4.21–4.16 (m, 2H), 3.83–3.75 (m, 6H), 3.74–3.70 (m, 4H), 2.60 (t, *J* = 7.4 Hz, 2H), 2.43 (t, *J* = 7.0 Hz, 2H), 1.93 (quint, *J* = 7.4 Hz, 2H).

References

1 L. Biancalana, L. K. Batchelor, S. A. P. Pereira, P.-J. Tseng, S. Zacchini, G. Pampaloni, L. M. F. S. Saraiva, P. J. Dyson, F. Marchetti, Bis-conjugation of Bioactive Molecules to Cisplatin-like Complexes through (2,2′-Bipyridine)-4,4′-Dicarboxylic Acid with Optimal Cytotoxicity Profile Provided by the Combination Ethacrynic Acid/Flurbiprofen, *Chem. Eur. J.* 2020, **26**, 17525-17535.

2 (a) J. Liu, W. Liu, I. Weitzhandler, J. Bhattacharyya, X. Li, J. Wang, Y. Qi, S. Bhattacharjee, A. Chilkoti, Ring-Opening Polymerization of Prodrugs: A Versatile Approach to Prepare Well-Defined Drug-Loaded Nanoparticles, *Angew. Chem. Int. Ed.* 2015, **54**, 1002-1006. (b) M. D. Urbaniak, J. P. Bingham, J. A. Hartley, D. N. Woolfson, S. Caddick, Design and Synthesis of a Nitrogen Mustard Derivative Stabilized by Apo-neocarzinostatin, *J. Med. Chem*. 2004, **47**, 4710-4715.