Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

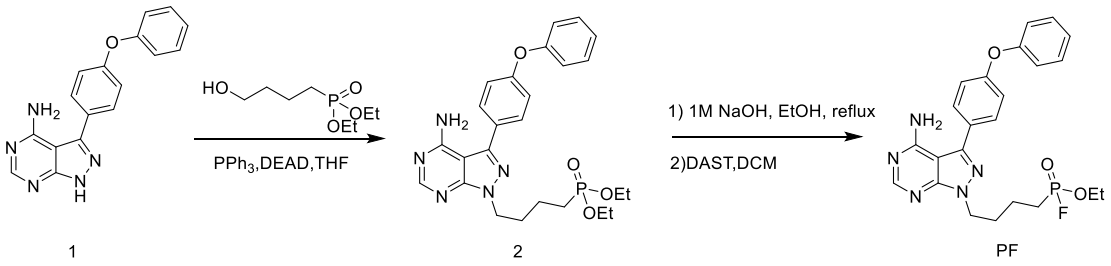
Covalent and selective immobilization of GST fusion proteins with fluorophosphonate-based probes

Contents **Page No.**

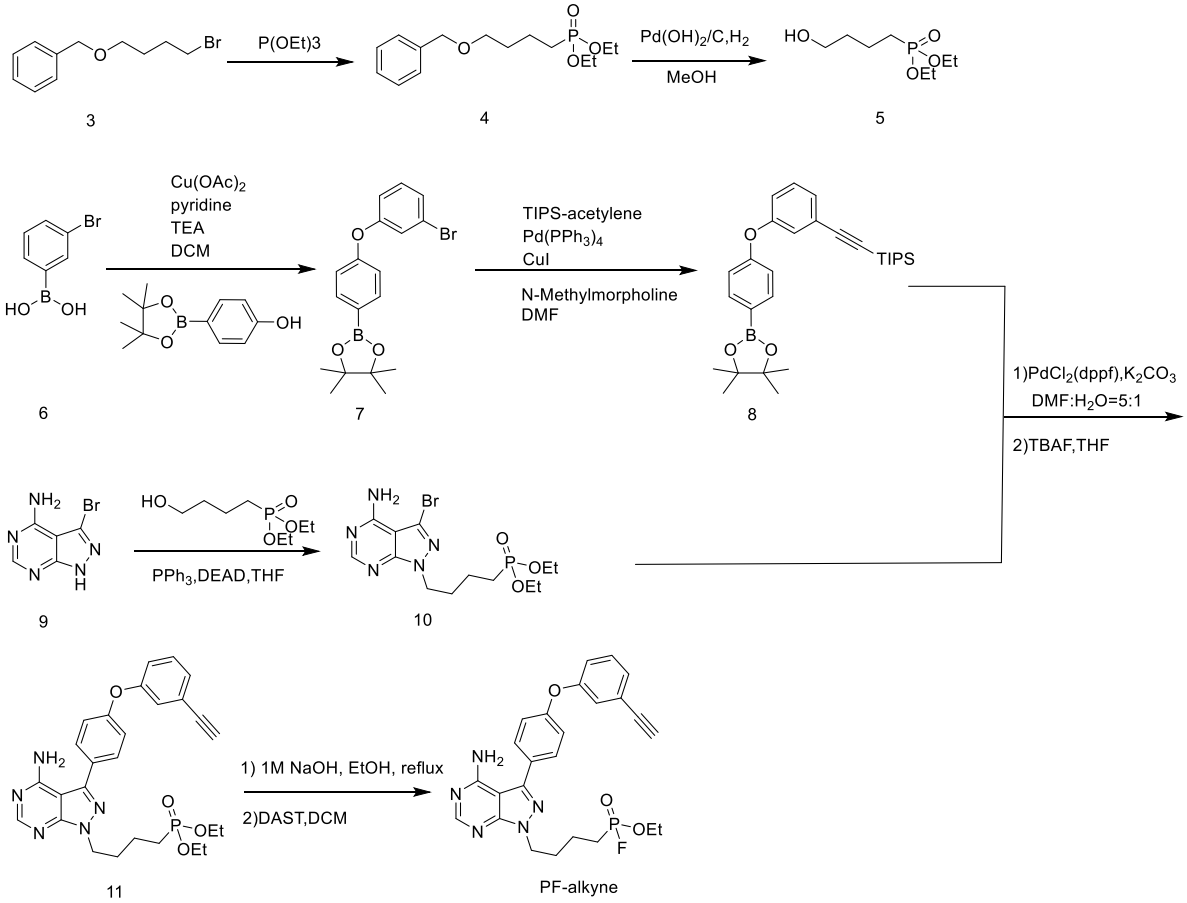
1. General Information

All the reagents were purchased commercially and used without further purification. Anhydrous DCM was distilled from calcium hydride. Anhydrous tetrahydrofuran (THF) was distilled from clastic Sodium. Other reagents were used directly without special drying operation. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Jiangyou silica gel plates (HSGF254) using UV light as visualizing agent . Flash column chromatography was carried out using Haiyang silica (ZCX-II). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Advance 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) with chemical shift values in ppm relative to TMS (δ_H 0.00 and δ_C 0.00),residual chloroform (δ_H 7.26 and δ_C 77.16) as standard. ^{19}F-NMR spectra were recorded on a Bruker Advance 400 with $CFCl_3$ (δ_F 0.00) as a standard. ^{32}P-NMR spectra were recorded on a Bruker Advance 400 with 85% Phosphoric acid solution (δ_P 0.00) as a standard. HR-MS were obtained using Bruker Apex IV RTMS. Purity of compounds was determined by HPLC chromatograms acquired on an Agilent 1260 HPLC. The gel was scanned by Pharos FX imaging system (Bio-rad). Kinase enzymatic activity was measured by Multi-detection microplate reader (PerkinElmer). Western blot analysis was performed by Universal gel imaging analyzer (Bio-rad).

2. Synthetic Routes of PF and PF-Alkyne



Scheme 1. Synthetic Route of PF



Scheme 2. Synthetic Route of PF-alkyne

3. Experimental Procedures and Spectroscopy Data

Compound 2

Compound 1 (216 mg, 0.71mmol), compound 5 (180 mg, 0.86mmol) and 281mg triphenylphosphine (281mg, 1.1mmol) were dissolved in 3mL tetrahydrofuran (THF). Then diethyl azodicarboxylate (DEAD) (0.16ml, 1.1mmol) was added slowly to the solution which had already been cooled to 0℃ with an ice bath. Then the mixture was warmed to room temperature and stirred overnight. After quenching by water, the mixture was extracted with ethyl acetate three times. The organic layers were collected, washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and purified by flash chromatograph column to get the product as yellow oil (324mg, yield 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.31 (dd, *J* = 20.8, 12.7 Hz, 2H), 7.18 – 6.98 (m, 5H), 6.19 (s, 2H), 4.40 (t, *J* = 6.9 Hz, 2H), 4.14 – 3.92 (m, 4H), 2.12 – 1.89 (m, 2H), 1.85 – 1.69 (m, 2H), 1.62 (td, *J* = 15.5, 8.9 Hz, 2H), 1.23 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 158.30, 156.39, 155.70, 154.32, 143.81, 129.93, 127.87, 123.97, 119.46, 119.12, 98.29, 61.48, 46.29, 30.32, 25.82, 24.42, 19.72, 16.43.

HRMS (m/z): $[M+H]^+$ calcd. for $C25H31N5O4P^+$, 496.2108; found: 496.2104.

Compound PF

Compound 1 (80mg, 0.16mmol) was dissolved in 1ml ethanol, then 1ml 1M NaOH was added, and the mixture was refluxed in 100℃ for 4 hour. The reaction was quenched by water, adjusted to pH=1 with 1M HCl and extracted with ethyl acetate three times. Then the organic layers were collected, washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and used directly in the next step.

The crude compound was dissolved in 1.5ml DCM, then cooled to -78℃. 63uL DAST was slowly added to the solutions. The reaction mixture was warmed to room temperature and stirred for another 30min, after which the reaction was quenched by adding 5ml H_2O and extracted with ethyl acetate three times. The organic layer was collected, washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and purified by flash chromatograph column to get the desire product as pale yellow solid (31mg, yield 41% of two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.64 (d, *J* = 8.6 Hz, 2H), 7.37 (t, *J* = 8.0 Hz, 2H), 7.22 – 7.10 (m, 3H), 7.07 (d, *J* = 7.7 Hz, 2H), 5.89 (s, 2H), 4.45 (t, *J* = 6.8 Hz, 2H), 4.32 – 4.09 (m, 2H), 2.14 – 2.03 (m, 2H), 2.04 – 1.84 (m, 2H), 1.70 (dt, *J* = 15.6, 7.7 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 158.60, 158.11, 156.47, 155.97, 154.56, 144.02, 130.05, 127.86, 124.13, 119.62, 119.25, 98.44, 63.30, 46.11, 30.11,

24.71, 24.48, 23.28, 23.05, 19.30, 16.42.

^{19}F NMR (376 MHz, $CDCl_3$) δ -62.83, -65.67.

^{31}P NMR (162 MHz, $CDCl_3$) δ 34.14, 27.54.

HRMS (m/z): $[M+H]^+$ calcd. for $C_{23}H_{26}FN_5O_3P^+$, 470.1752; found: 470.1754.

Compound 5

Commercially available compound 3 (2ml, 11mmol) was mixed with triethyl phosphite (3.6ml, 21mmol) and heated at 160℃for 4.5 h. Then the temperature of flask was cooled down to 100℃and 5ml saturated aq. $NaHCO_3$ was added and the mixture was stirred another 30min. After cooled to room temperature, the mixture was extracted with ethyl acetate three times. The organic layers were combined and washed with saturated aq. $NaHCO_3$ solution once, then dried over $MgSO_4$ and concentrated in vacuo. The crude product used directly without any other purification.

The crude product was dissolved in 30ml MeOH and the solution was added with a spoon of $Pd(OH)_2/C$, stirred overnight under hydrogen atmosphere (balloon) at room temperature. The mixture was filtered by Celatom and the filtrate was concentrated in vacuo and purified by flash chromatograph column to get the desire product as pale yellow oil (1.9 g, yield 87% of two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 – 3.91 (m, 4H), 3.61 (t, *J* = 6.0 Hz, 2H),

2.85 (s, 1H), 1.81 – 1.51 (m, 6H), 1.28 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 61.94 – 61.52, 33.31, 25.97, 24.57, 18.90, 16.52.

HRMS (m/z): $[M+H]^+$ calcd. for $C8H20O4P^+$, 211.1094; found: 211.1099.

Compound 7

Two round-bottom flasks were charged with anhydrous 4 Å molecular sieves (10 g each) and anhydrous CH_2Cl_2 (200 mL each). One flask was added with $Cu(OAc)_2$ (7.1 g, 39 mmol), Et3N (13.9mL, 100 mmol), and 4-(4,4,5,5-ktetramethyl-1,3,2-dioxaborolan-2-yl)phenol (12.1 g, 55 mmol), and the other flask was added with 3-bromophenylboronic acid (10 g, 50 mmol) and pyridine (3.9 mL, 50 mmol). Both flasks were stirred at room temperature under nitrogen atmosphere for 4 h. The 3-bromophenylboronic acid/pyridine solution was then added to the other flask using a canula. The reaction was stirred for 16h at room temperature. The reaction mixture was filtered by SiO_2 using 10:1 hexanes/ethyl acetate and the filtrate was concentrated in vacuo and purified by flash chromatograph column to get the desire product as yellow oil (2.4g, yield 13%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.5 Hz, 2H), 7.35 – 7.20 (m, 3H), 7.08 (t, *J* = 9.2 Hz, 2H), 7.04 (ddd, *J* = 8.0, 2.2, 1.0 Hz, 1H), 1.43 (s, 12H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.26, 157.79, 136.88, 130.93, 126.57,

122.95, 122.26, 118.31, 117.76, 83.86, 24.96.

HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{21}BBrO_3^+$, 375.0762; found: 375.0771.

Compound 8[1]

Compound 7 (1.0 g, 2.7 mmol) was dissolved in DMF (5 ml). The solution was then treated with N-methylmorpholine (2.9 mL, 27 mmol) and ethynyltriisopropylsilane (2.4ml, 11mmol) and Pd(PPh3)4 (185mg, 0.16 mmol) and CuI (10 mg, 0.053 mmol). The mixture was then thoroughly degassed using a stream of Ar and stirred under Ar at 90 °C for 5 h. The reaction was diluted with Et_2O and washed with H_2O. The organic layer was collected, washed once with saturated aq. NaCl, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatograph column to get compound 8 as yellow oil (1.0 g, yield 82%).

^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (t, *J* = 8.1 Hz, 2H), 7.28 – 7.21 (m, 2H), 7.14 (s, 1H), 7.01 – 6.90 (m, 3H), 1.33 (s, 12H), 1.11 (s, 18H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 160.04, 156.38, 136.82, 129.77, 127.74, 125.28, 122.97, 119.94, 117.79, 106.31, 91.53, 83.91, 25.01, 18.80, 11.42.

HRMS (m/z): $[M+H]^+$ calcd. for $C_{29}H_{42}BO_3Si^+$, 477.2991; found: 477.2983.

Compound 10

Commercially available compound 9 (205mg, 0.96mmol) was dissolved in 4ml tetrahydrofuran. The solution was added with compound

5 (402mg, 1.9mmol) and triphenylphosphine (498mg, 1.9mmol). Then diethyl azodicarboxylate (DEAD) (0.3ml, 1.9mmol) was added slowly to the solution which had already been cooled to 0℃ with an ice bath. The mixture was warmed to room temperature and stirred overnight. After quenched by water, the mixture was extracted with ethyl acetate three times. The organic layers were collected, washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and purified by flash chromatograph column to get the desire product as yellow solid (405mg, yield >99%).

1H NMR (500 MHz, $CDCl_3$) δ 8.27 – 8.18 (m, 1H), 4.30 (t, J = 6.9 Hz, 2H), 4.07 – 3.94 (m, 4H), 1.99 – 1.90 (m, 2H), 1.77 – 1.67 (m, 2H), 1.60 – 1.48 (m, 2H), 1.23 (q, J = 7.0 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 157.70, 156.59, 154.17, 117.45, 100.30, 61.57, 46.70, 30.13, 25.67, 24.55, 19.64, 16.45.

HRMS (m/z): $[M+H]^+$ calcd. for $C13H22BrN5O3P^+$, 406.0638; found: 460.0637

Compound 11

Compound 8 (152mg, 0.32mmol), compound 10 (108mg, 0.27mmol), $PdCl_2$(dppf)(20mg, 0.027mmol) and K_2CO_3(112mg, 0.81mmol) was charged in a glass tube and then dissolved in 1.5ml DMF and 0.3ml H_2O. The tube was degassed using a stream of Ar, capped and heated at 120℃

in microwave for 1h. The mixture was diluted by ethyl acetate and washed with H_2O. Then the water layer was extracted by ethyl acetate twice. Collected ethyl acetate layers were washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and purified by flash chromatograph column to get an intermediate product as brown oil (120mg, yield 66%).

The intermediate was then dissolved in 1ml tetrahydrofuran and the solution was added with TBAF(0.54ml, 0.54mmol) and stirred at room temperature for 3h. The mixture was diluted with ethyl acetate and washed with H_2O. Collected ethyl acetate layers were washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and purified by flash chromatograph column to get the desire product as pale yellow solid (84mg, yield 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.71 – 7.63 (m, 2H), 7.37 – 7.26 (m, 2H), 7.20 – 7.11 (m, 3H), 7.08 (ddd, *J* = 8.0, 2.4, 1.3 Hz, 1H), 5.61 (s, 2H), 4.45 (t, *J* = 7.0 Hz, 2H), 4.18 – 3.93 (m, 4H), 3.10 (s, 1H), 2.14 – 2.01 (m, 2H), 1.88 – 1.74 (m, 2H), 1.72 – 1.57 (m, 2H), 1.31 – 1.24 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 157.91, 156.61, 155.90, 154.54, 143.76, 130.15, 128.52, 127.83, 123.95, 122.70, 120.22, 119.72, 98.48, 82.90, 78.13, 61.64, 46.52, 30.48, 26.01, 24.60, 19.89, 16.58, 0.13.

HRMS (m/z): $[M+H]^+$ calcd. for $C27H31N5O4P^+$, 520.2108; found: 520.2102

Compound PF-alkyne

Compound 11(124mg, 0.24mmol) was dissolved in 1.5ml ethanol and the solution was added with 1M NaOH (1ml), refluxed in 100℃ for 4 hour. The reaction was quenched by water, adjusted to pH=1 with 1M HCl (aq.) and extracted with ethyl acetate three times. Then the organic layer was collected, washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and used directly in the next step.

The crude compound was dissolved in 2ml DCM, then cooled to -78℃. DAST(95ul, 0.72mmol) was slowly added to the solution. The mixture was warmed to room temperature and stirred for another 40min, after which the reaction was quenched by adding 5ml H_2O and extracted with ethyl acetate three times. The organic layers were collected, washed with saturated aq. NaCl, dried over anhydrous $MgSO_4$, concentrated in vacuo and purified by flash chromatograph column to get the desire product as pale yellow solid (35mg, yield 30% of two steps)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.31 (dt, *J* = 15.3, 7.7 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 3H), 7.08 (d, *J* = 7.9 Hz, 1H), 5.58 (s, 2H), 4.47 (t, *J* = 6.8 Hz, 2H), 4.31 – 4.15 (m, 2H), 3.10 (s, 1H), 2.15 – 2.06 (m, 2H), 1.98 (ddd, *J* = 15.7, 12.5, 7.5 Hz, 2H), 1.71 (d, *J* = 7.5 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 157.96, 156.59, 156.07, 154.66, 143.89,

130.19, 130.11, 128.46, 127.85, 123.97, 122.73, 120.24, 119.71, 98.51, 82.91, 78.13, 63.36, 46.21, 30.14, 24.65, 23.21, 19.35, 16.46, 0.13.

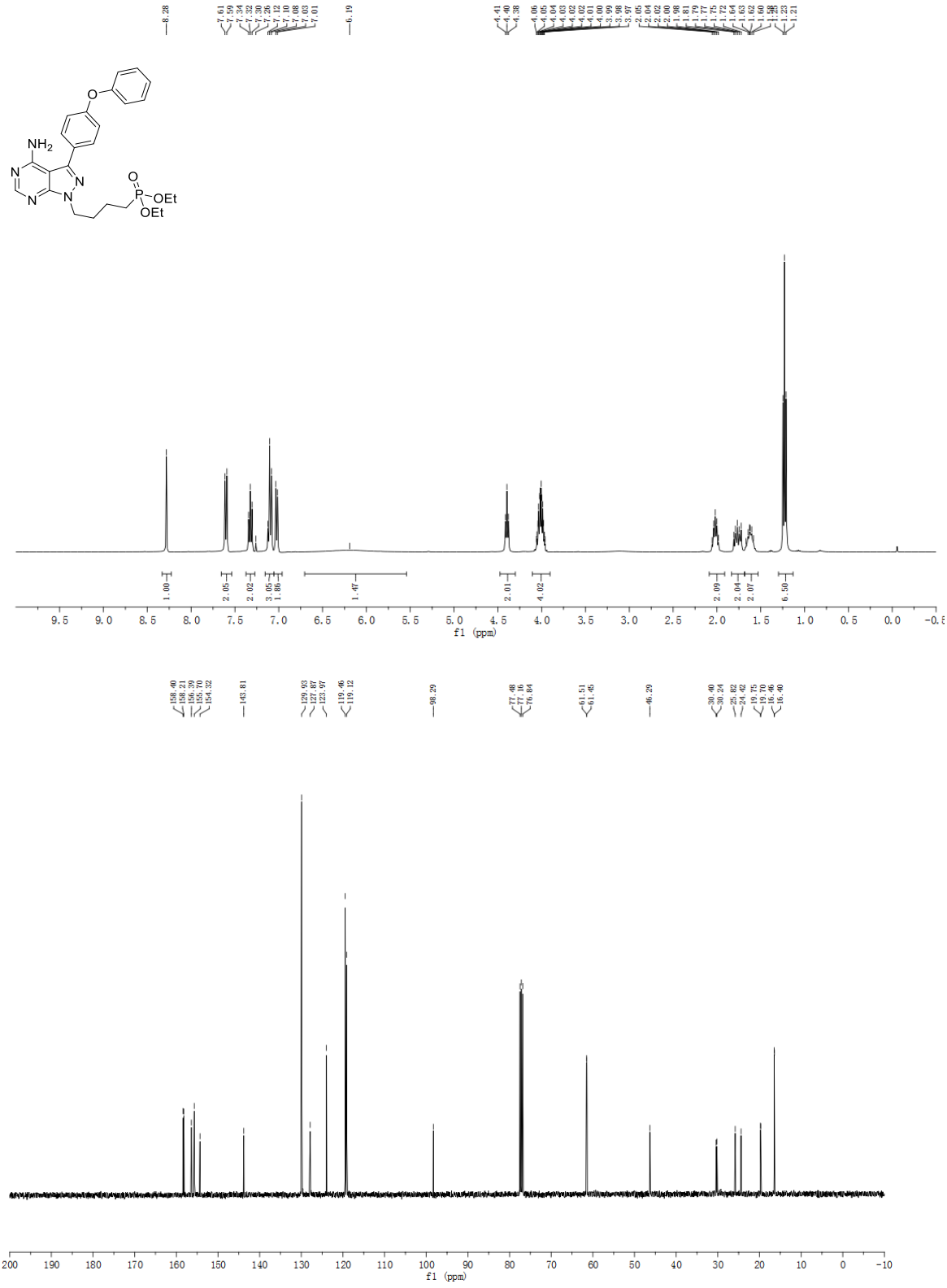
^{19}F NMR (376 MHz, $CDCl_3$) δ -62.82, -65.66.

^{31}P NMR (162 MHz, $CDCl_3$) δ 34.11, 27.51.

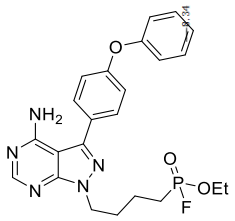
HRMS (m/z): $[M+H]^+$ calcd. for $C_{25}H_{26}FN_5O_3P^+$, 494.1752; found: 494.1757.

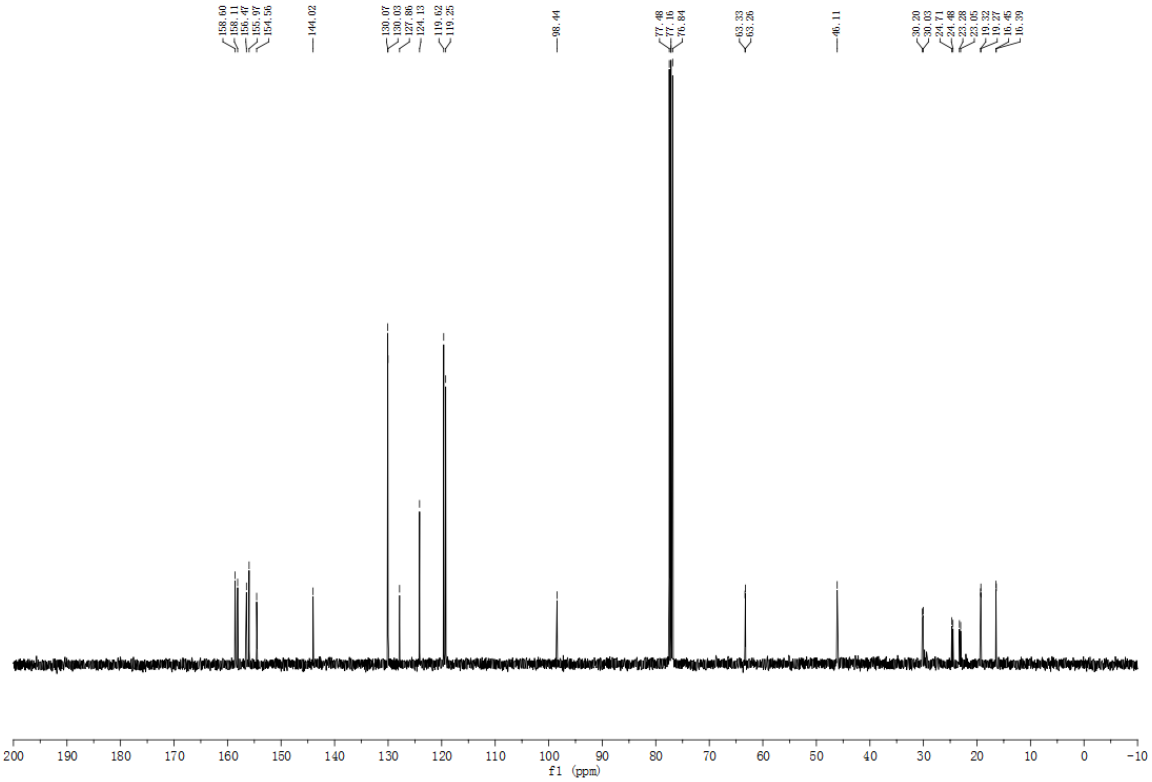
4. NMR Spectra of Compounds

Compound 1

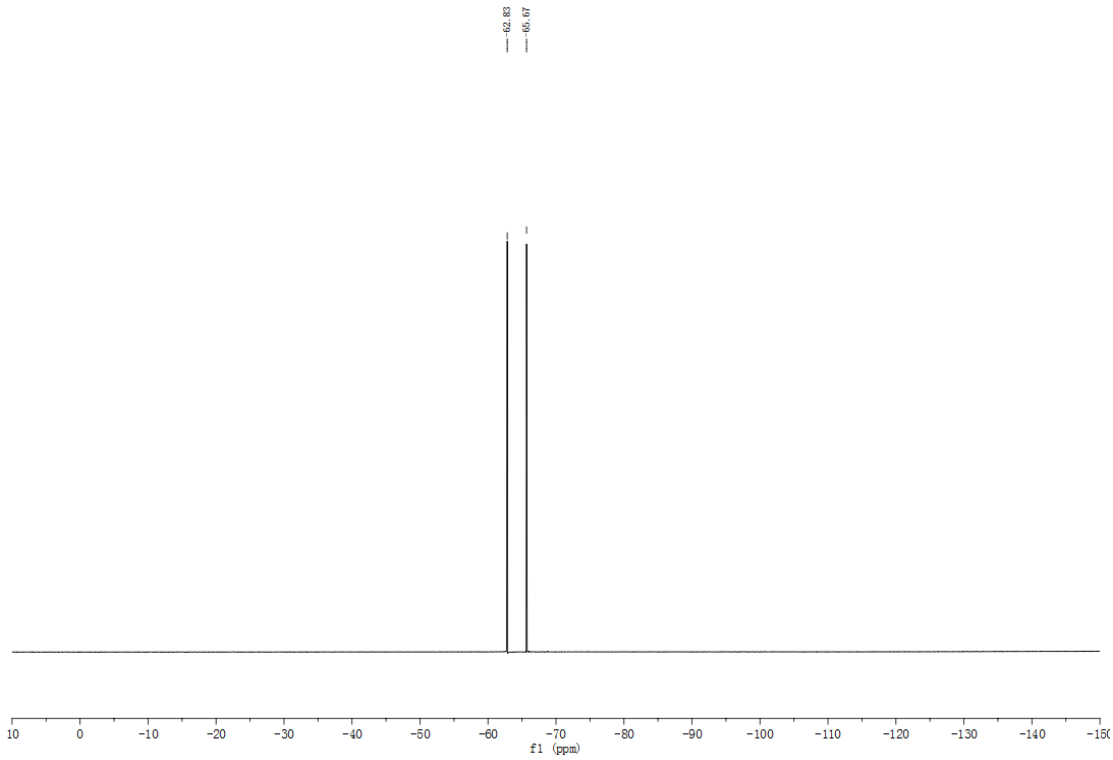


Compound PF

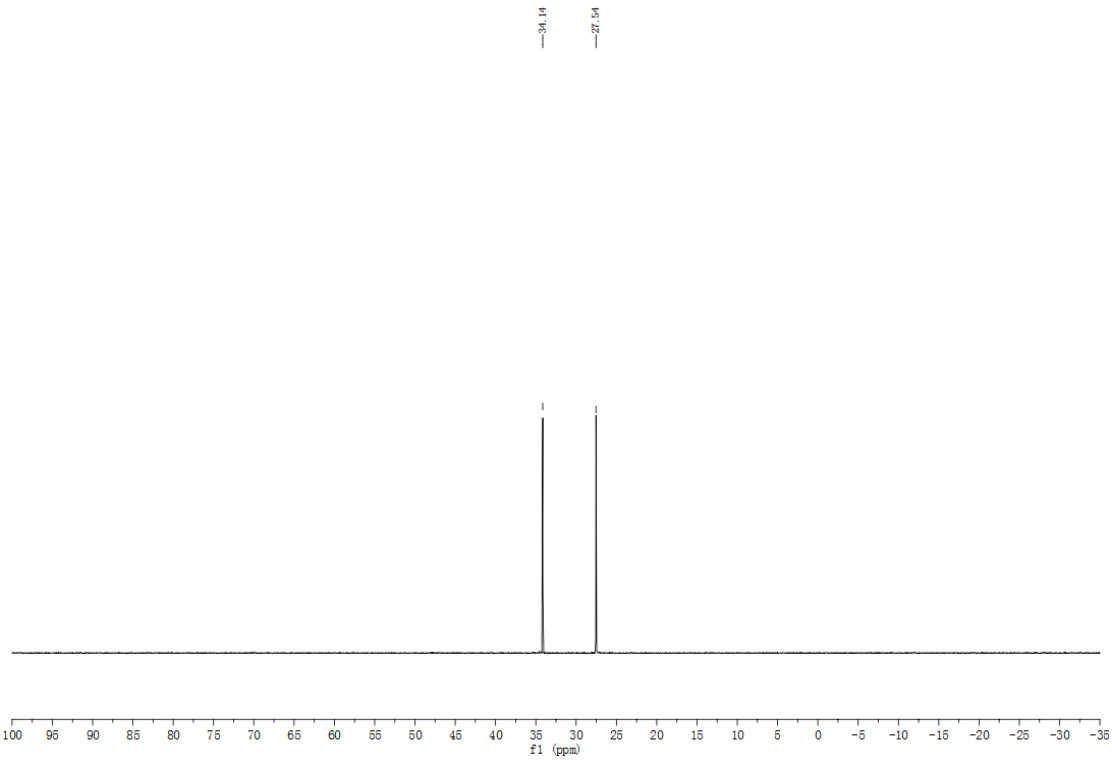




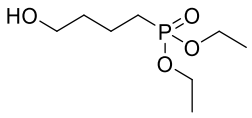
^{19}F-NMR



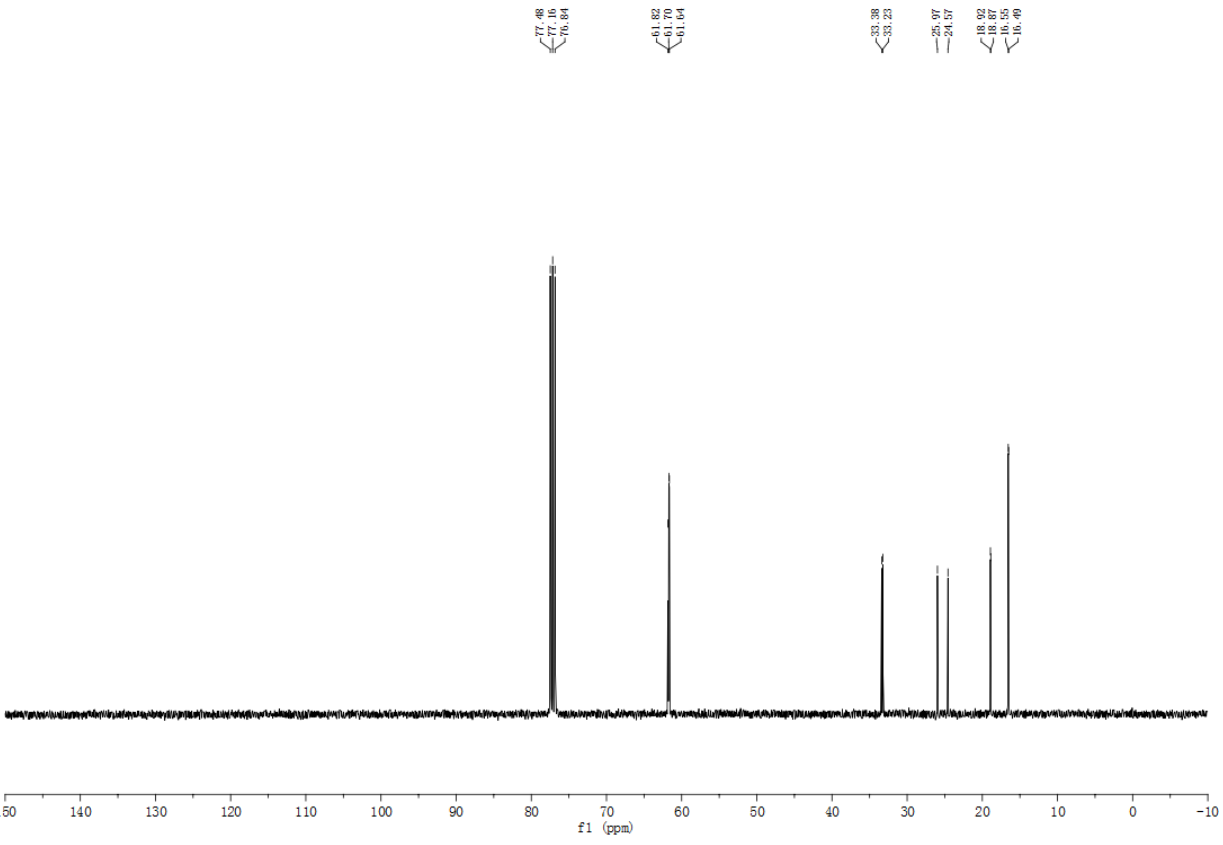
^{31}P- NMR



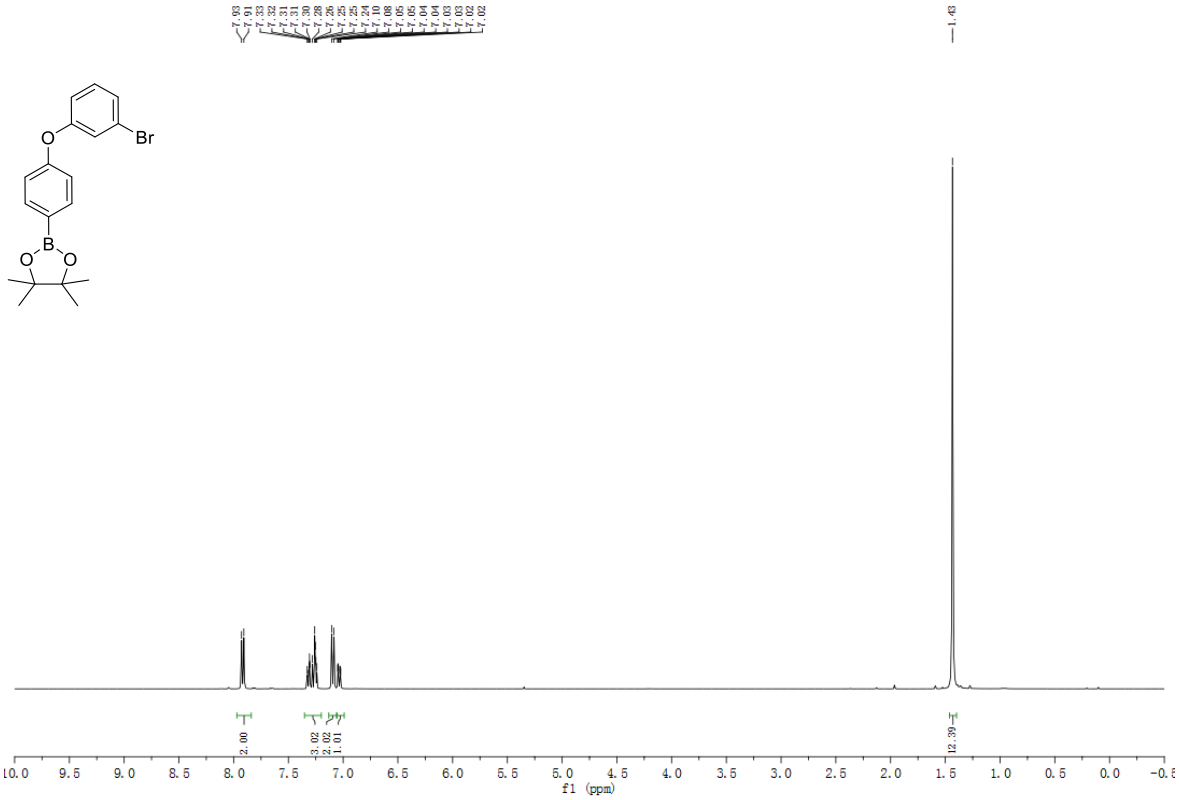
Compound 5

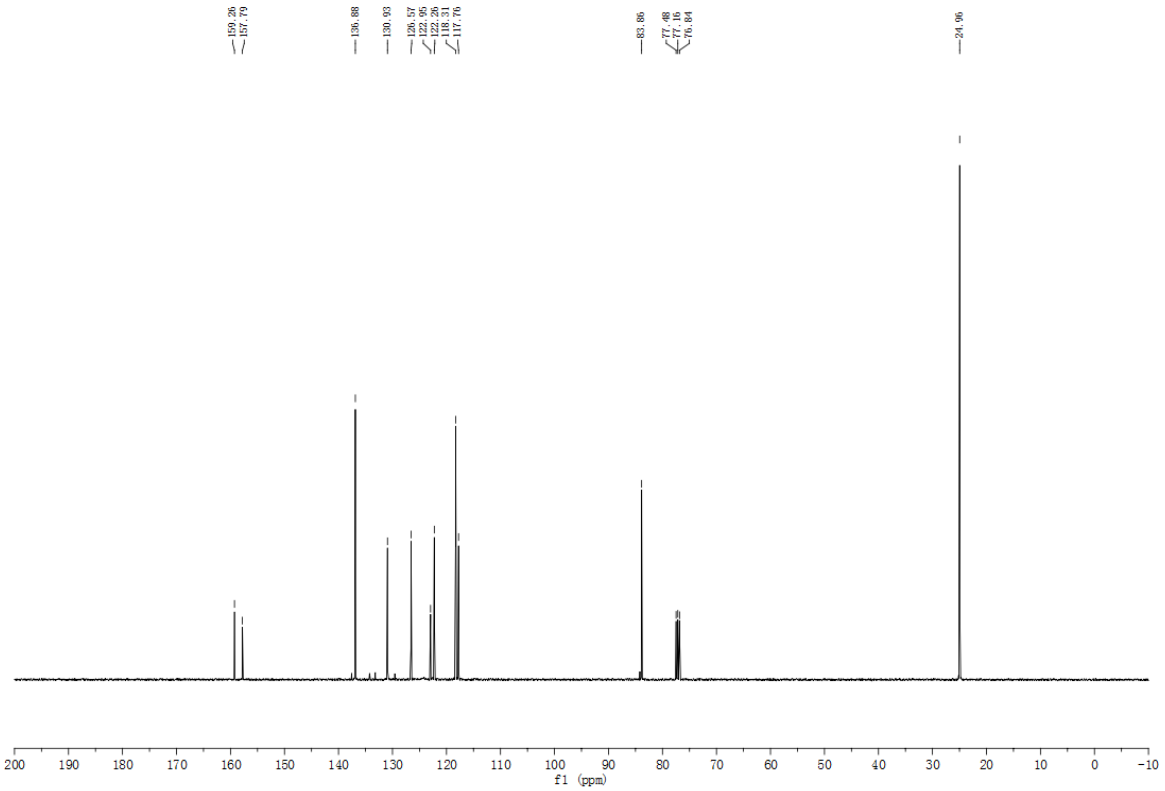

HO
P
O
O
O

7.26
4.12
4.10
4.08
4.06
4.05
4.04
4.03
4.02
4.01
4.00
3.98
3.62
3.61
3.59
2.85
1.76
1.74
1.71
1.70
1.69
1.68
1.66
1.65
1.64
1.63
1.62
1.28
1.27
4.00
2.04
1.21
6.12
6.02
f1 (ppm)

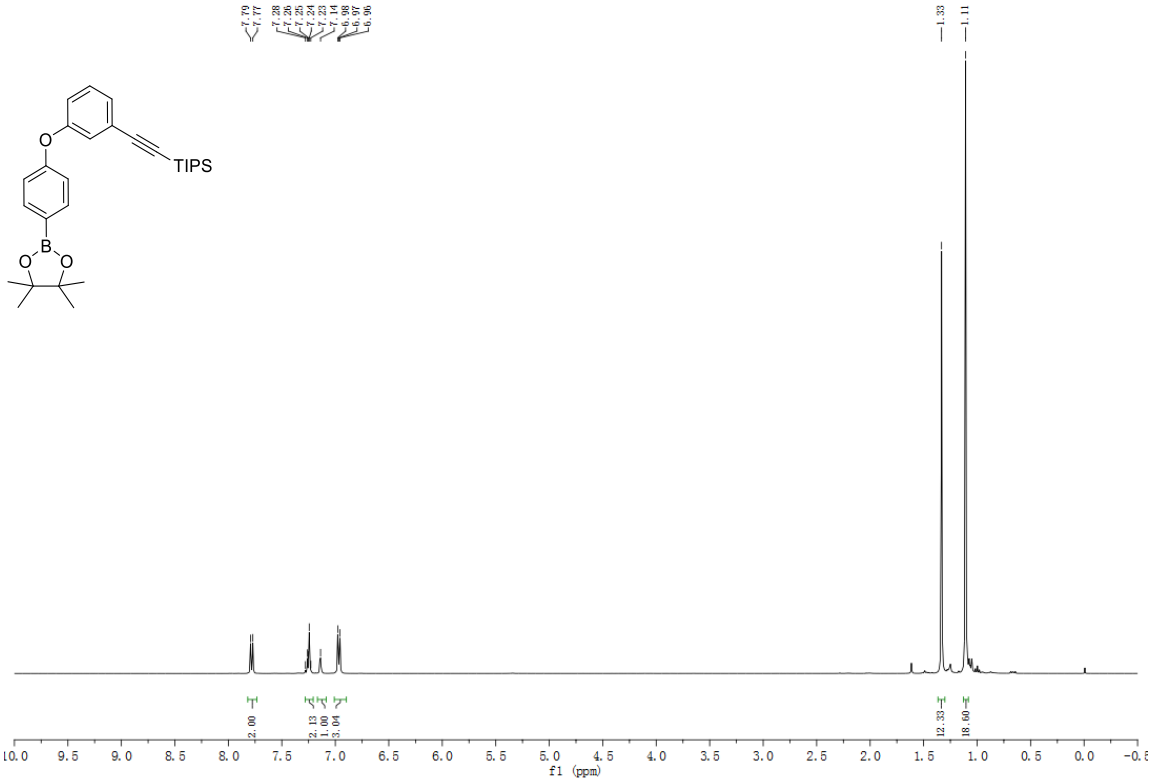

77.48
77.16
76.84
61.82
61.70
61.64
33.38
33.23
25.97
24.57
18.92
18.87
16.55
16.49
f1 (ppm)

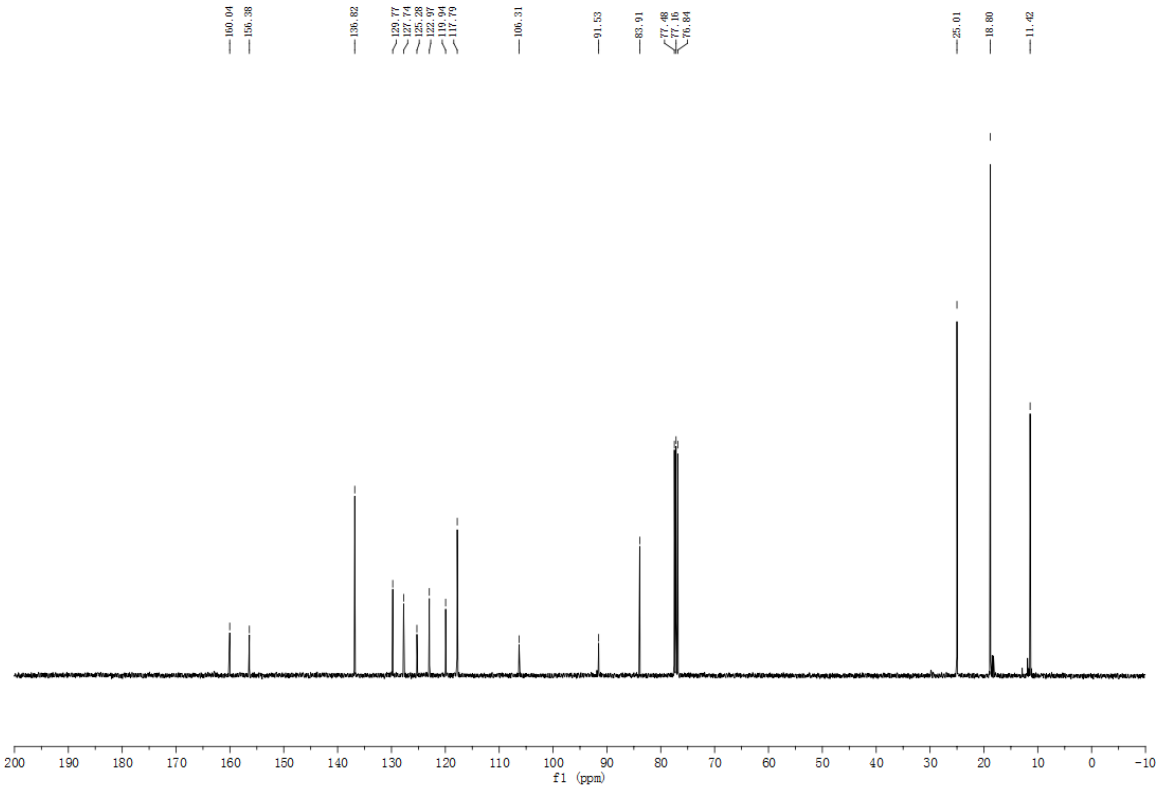
Compound 7



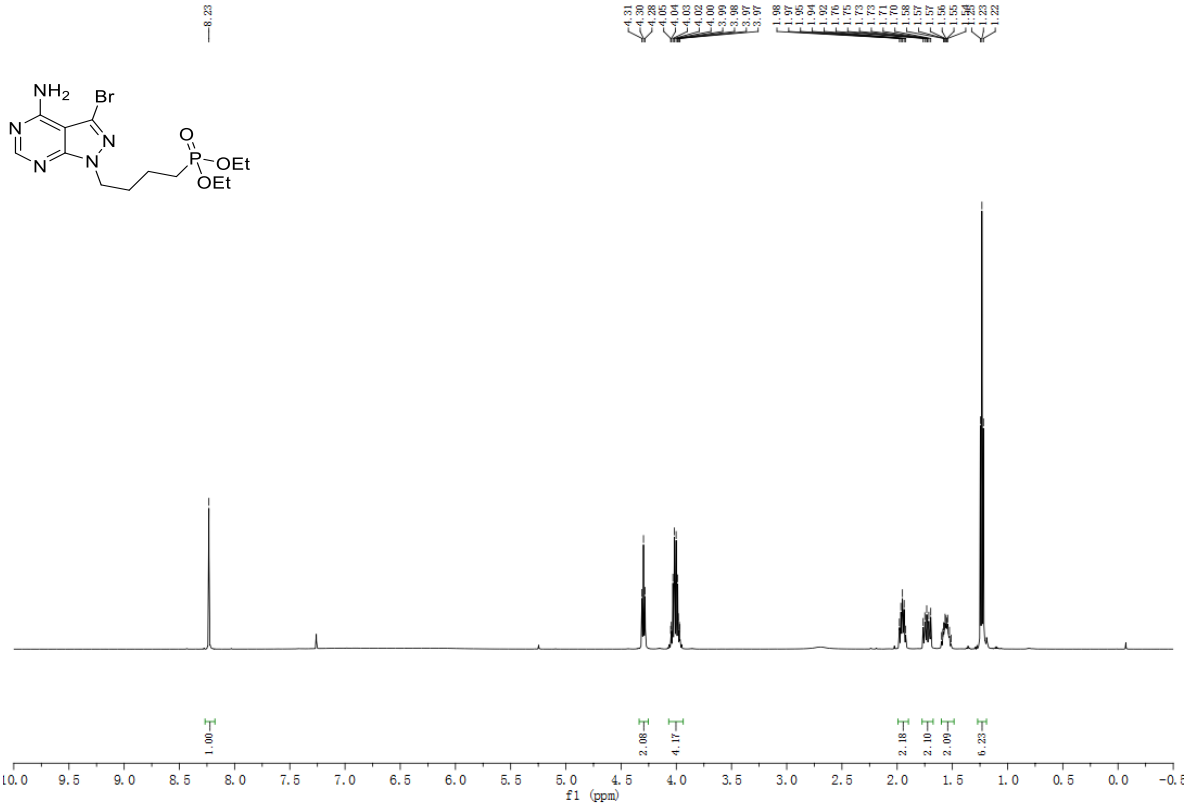


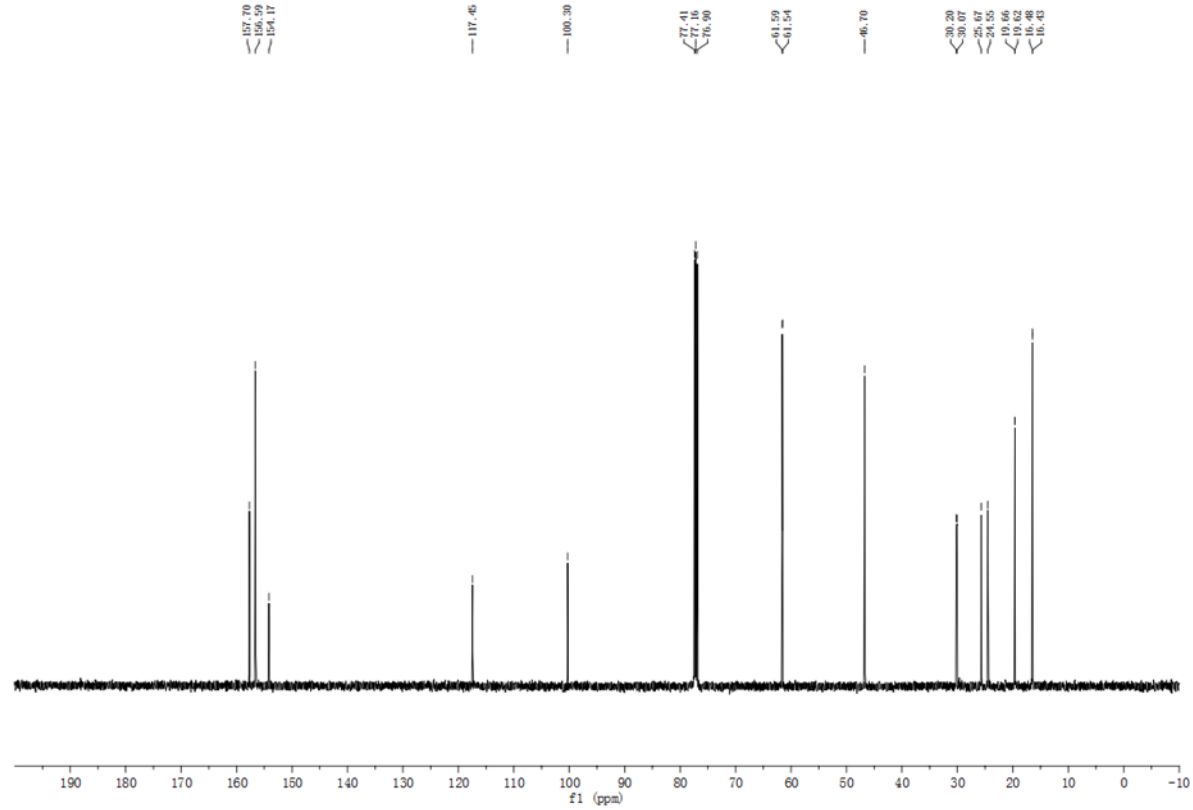
Compound 8


7.79
7.77
7.28
7.26
7.25
7.24
7.23
7.14
6.98
6.97
6.96
1.33
1.11
O
TIPS
B
O
O
2.00
2.13
1.00
3.04
12.33
18.60
f1 (ppm)

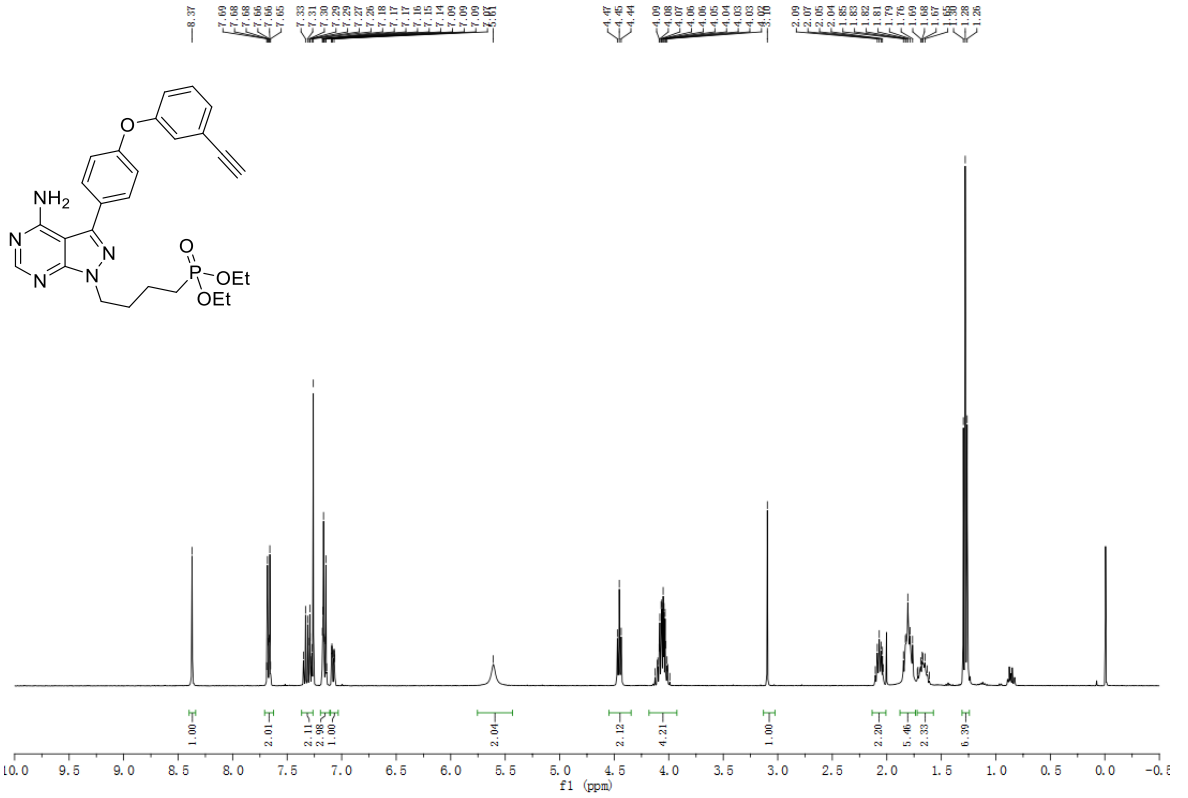

160.04
156.38
136.82
129.77
127.74
125.28
122.97
119.94
117.79
106.31
91.53
83.91
77.48
77.16
76.84
25.01
18.80
11.42
f1 (ppm)

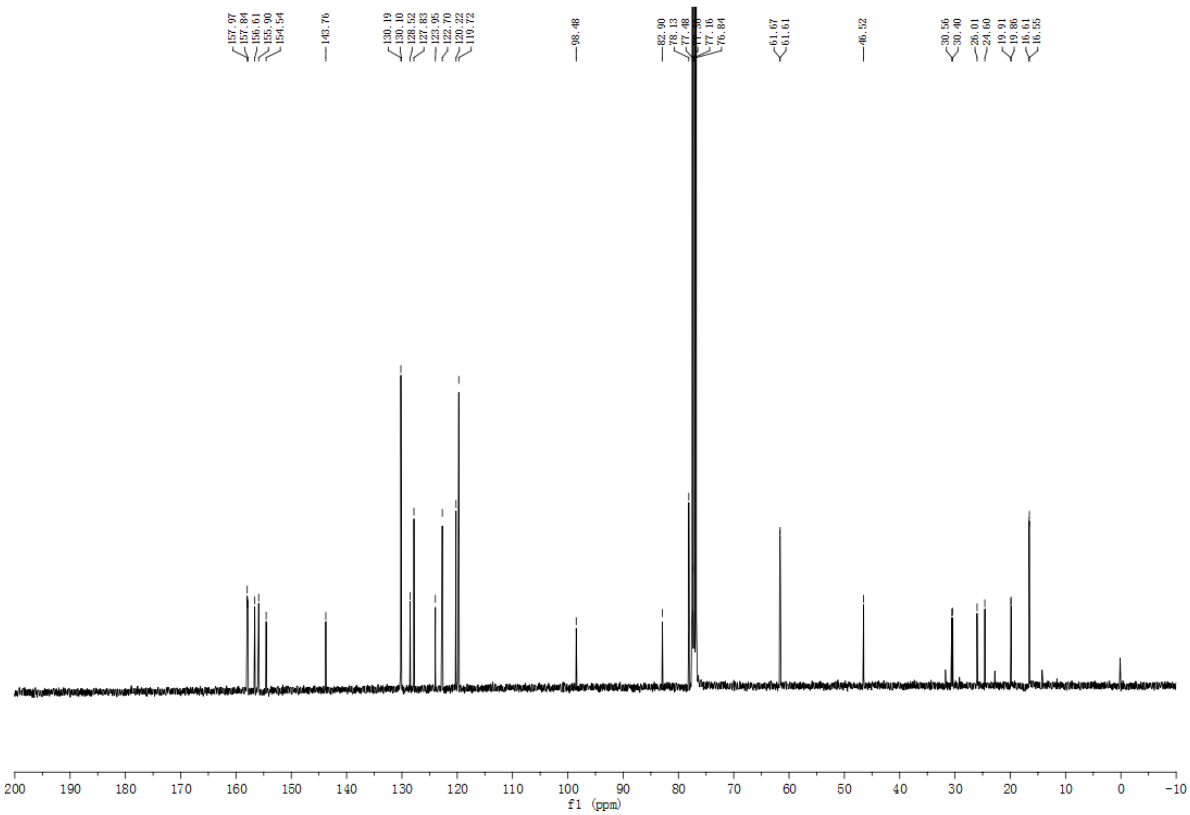
Compound 10



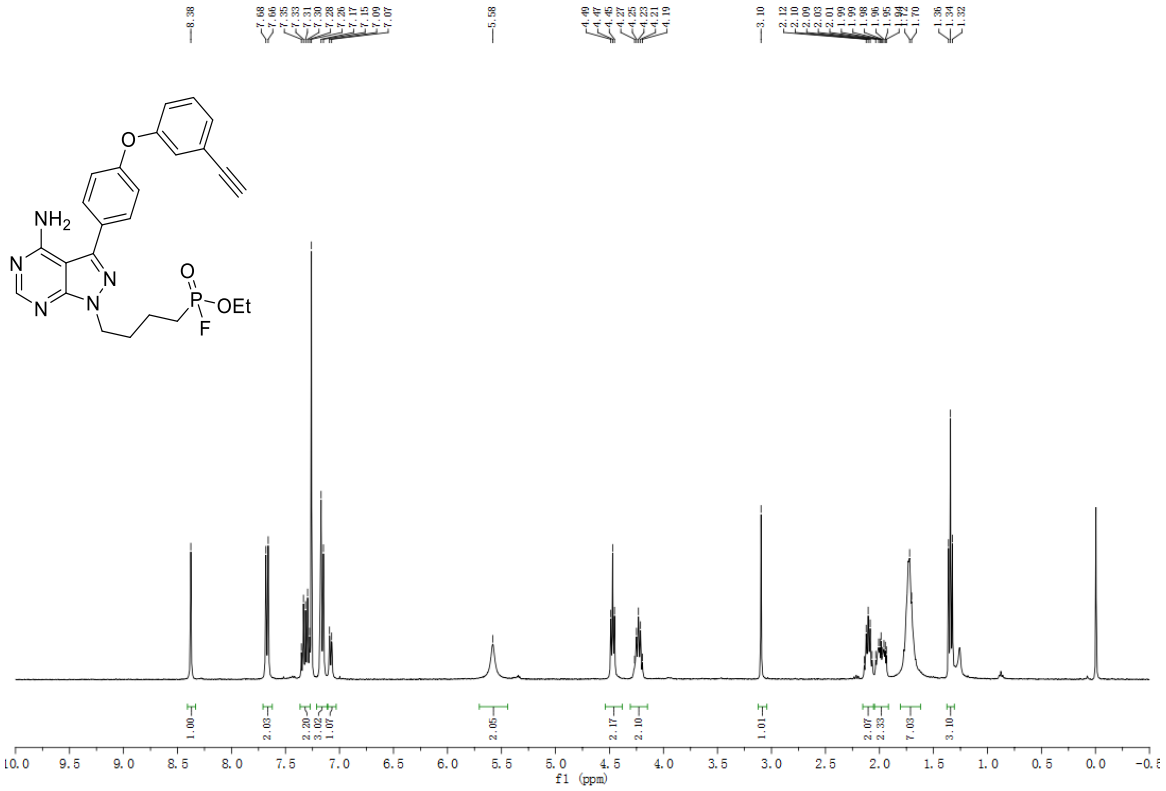


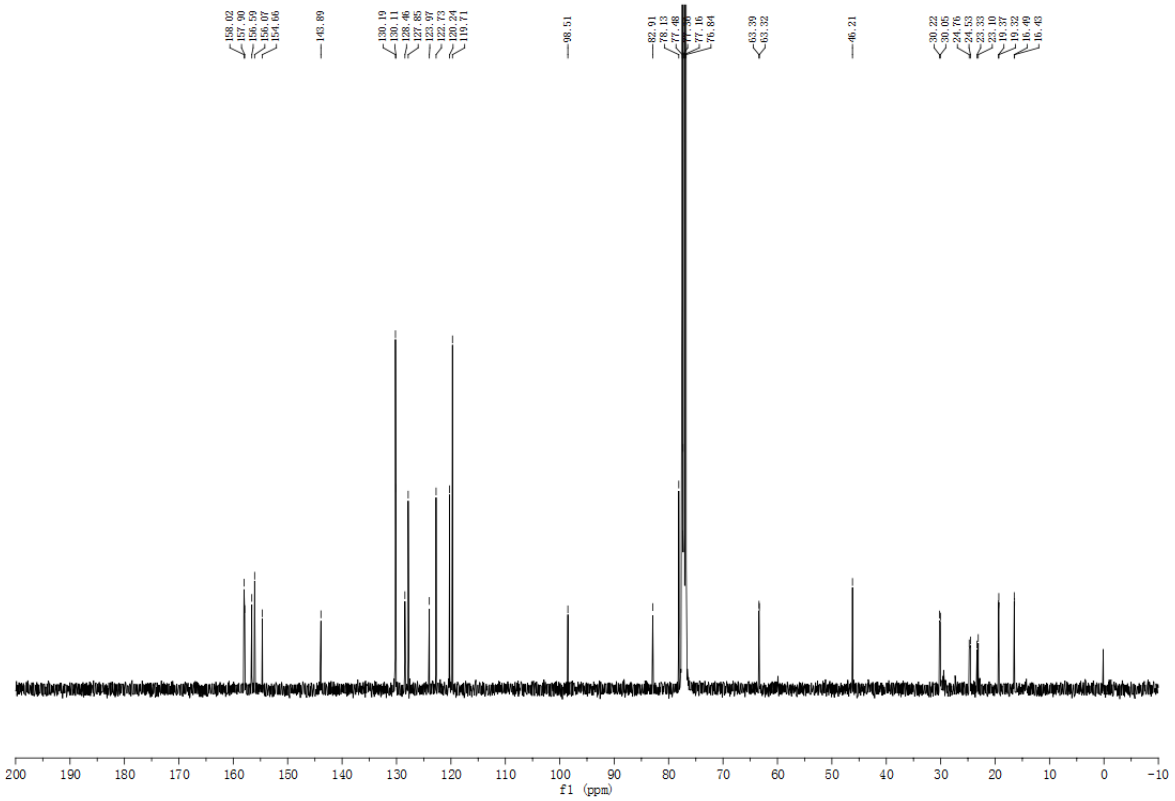
Compound 11



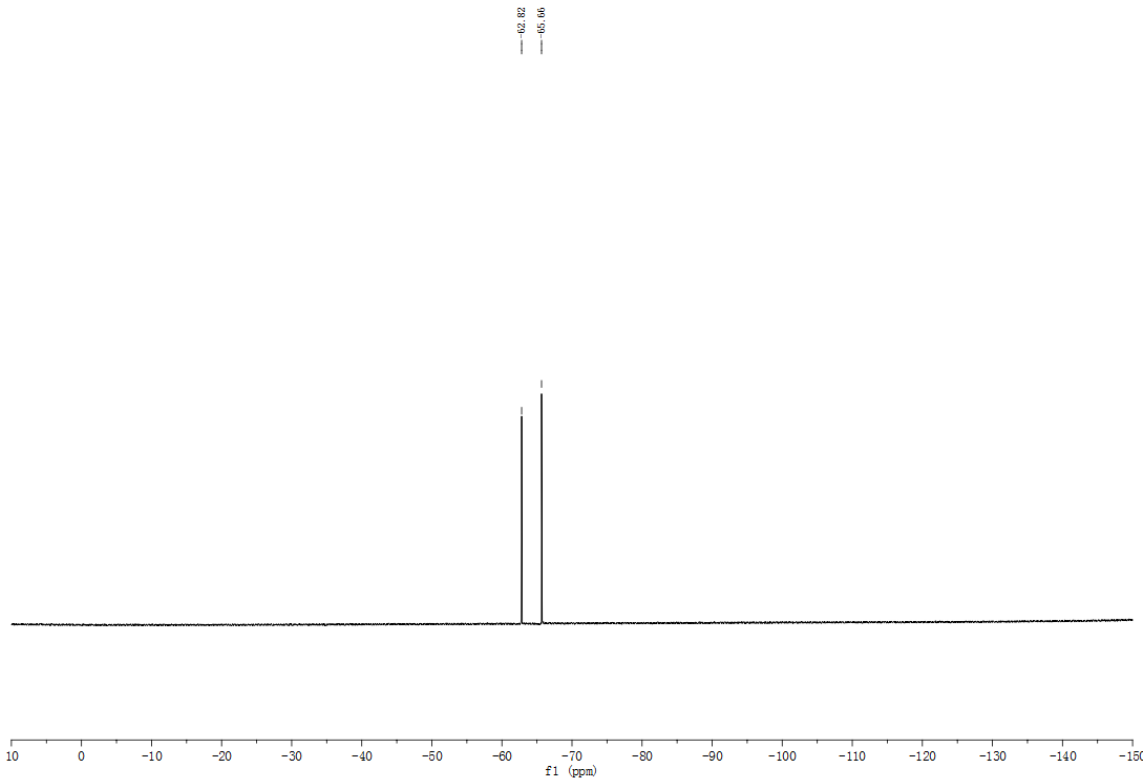


Compound PF-alkyne

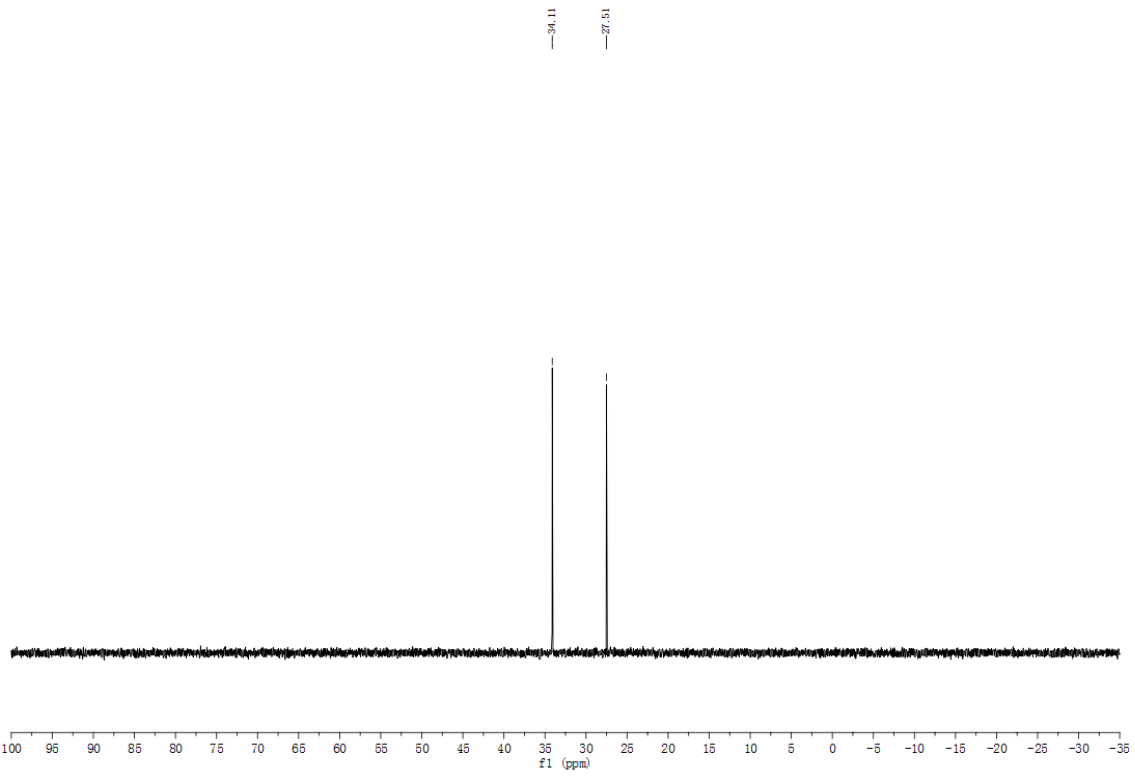




^{19}F-NMR



^{31}P-NMR



5. Procedures of Biological Experiments

5.1 Recombinant sjGST protein labeling

Recombinant sjGST protein which was purchased from SinoBiological was diluted to a final concentration of 0.1 μg/μL using phosphate buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM MgCl2) and incubated with 5 μM PF-alkyne, unless otherwise indicated, for 1.5 hour at room temperature. The protein samples reacted with TAMRA-N3 by a Cu(I)-catalyzed Huisgen [3 + 2] cycloaddition ("click") reaction. For each reaction, 5 μL of protein samples were added with 0.2 μl mixture (each was 0.05μl) of TAMRA-N3 (10 mM stock in DMSO, Lumiprobe), $CuSO_4$(100 mM stock in H_2O, Sigma), TBTA (10 mM stock in t-butanol:DMSO 4:1, Sigma) and ascorbic sodium (100 mM stock in H_2O, Sigma). The sample was shaken in dark at room temperature for 1h. The mixture was then added with 5ul(2x) loading buffer with 2% β mercaptoethanol (ME) and boiled at 100℃ for 10 min. Samples were loaded to 10% Tris - glycine SDS-PAGE polyacrylamide gel and ran about 2h. The gel was scanned by Pharos FX imaging system (Bio-rad), following which the gel was dealt with sliver staining.

5.2 Selective labeling of sjGST and seven classes of human GST proteins

Recombinant human GSTM1, GSTO1 and GSTP1 were purchased from Genecopoedia. GSTK1, GSTZ1 were purchased from SinoBiological. GSTT1

was purchased from ProSpec. GSTA1 was purchased from BioVision. The labeling procedure was same as sjGST labeling in section 5.1.[2]

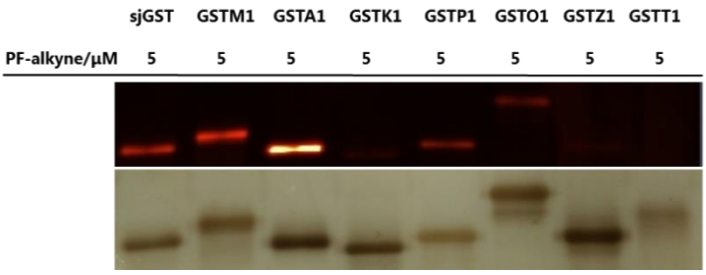


Fig. S1: PF-alkyne labeling of sjGST and seven classes of human GSTs

5.3 Immobilization of GSTA1 and GST-eGFP on Streptavidin-functionalized polymer beads

Commercially available GSTA1 (1 μg/μL) was diluted to final concentration of 0.1 μg/μL and incubated with 100μm PF-alkyne or DMSO (1%) for 1.5h at room temperature. For competition experiments, the GSTA1 was firstly incubated with 100μM PF or 20mM GSH for 1.5h and then incubated with 100μM PF-alkyne for 1.5h, or firstly incubated with 100μM PF-alkyne for 1.5h and then incubated with 100μM PF or 20mM GSH for 1.5h. After incubation, the mixture was "clicked" with N_3-PEG_4-biotin for 1h with continuous shaking. Then the mixture was added with beads, blended and rocked overnight at 4℃. The beads must be equilibrated by binding buffer three times before use. After binding, the beads was washed with binding buffer three times and added with (2x) loading buffer with 2% β ME and boiled at 100℃ for 10min. Samples were loaded to 10% Tris - glycine SDS-PAGE polyacrylamide gel and ran about 2h followed by silver staining.

As for immobilization of GST-eGFP, after washing, the beads in EP tube was firstly scanned by Pharos FX imaging system (Bio-rad, excitation wavelength is 488nm) and then deal with SDS-PAGE polyacrylamide gel and silver staining.

5.4 Immobilization of GST-eGFP on glass slides

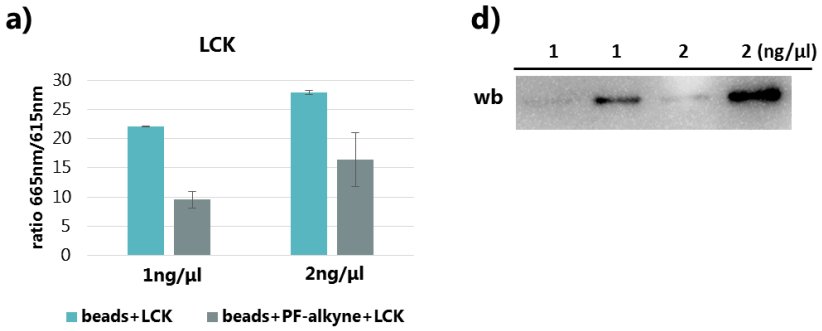
N,N'-Disuccinimidyl carbonate (DSC, 1g, 4mmol) and 0.7 mL of DIEA (4 mmol) was dissolved in 9.7ml of DMF, and then added onto a glass slide in a reaction chamber. The chamber was purged with Ar, sealed, and shaken at r.t. for 8h at 80−90 rpm. The DSC−DIEA solution was removed, and the glass slide was washed twice with DMF. The slide was added with a mixture of N_3-PEG-NH_2 (MW2000, 100mg, 0.05mmol) and 35 µL of DIEA in 3 mL of DMF. The slide was shaken for 15h at 80−90 rpm, and then added with 204 µL (2mmol) of ethanolamine, 35ul (2mmol) of DIEA, shaken another 4h, then washed with DMF three times and dried in air.

4ul GST-eGFP (1.4µg/µL) was incubated with 4ul PF (200µm) and 4µL PF-alkyne (200µm) for 1.5h then "clicked" with the azido-modified glass slide with shaking at 80-90rpm for 1h in a humid chamber. The slide was washed with PBST in a wash station and scanned by Pharos FX imaging system (Bio-rad, excitation wavelength is 488nm).

5.5 Immobilization of kinases on CH-sepharose 4B beads and the kinase activity assay

42mg commercially available activated CH-sepharose 4B beads, 8.4mg N_3-PEG-NH_2 (MW=2000) and 5μl DIEA were added into 500μl CH_2Cl_2, then shaken at room temperature for 2h. Then the beads were washed with CH_2Cl_2 three times and dried.

2.1mg beads was added into 10μl PF-alkyne, 90μl CH_3CN, 3μl "click" reagent and shaken at room temperature for 1h, then the beads was washed with CH_3CN three times and dried. The beads were diluted with the kinase buffer into 0.16ng/μL. 4μL beads was incubated with 2μL different concentrations of kinases in 4℃ for 2h, then after centrifugation, the supernatant was used for western blot analysis. 6μL kinase buffer was added again to beads. As a control, the same amount of beads not treated with PF-alkyne were also diluted into 0.16ng/μL. 4μL beads was incubated with 2μL different concentration of kinase in 4℃ for 2h. After incubation, kinase activity was measured by a homogeneous time-resolved fluorescence (HTRF)-based kinase assay sold by Cisbio Bioassays. Data analysis was performed with GraphPad™ Prism 5.0.



c)

	Apparent Activity (%)	Immobilization (%)	Retained Kinase Activity (%)
LCK(1ng/µl)	43%	91%	47%
LCK(2ng/µl)	59%	90%	66%

Fig. S2: Immobilization of GST-lymphocyte-specific protein tyrosine kinase **(LCK)** on CH-sepharose 4B beads then its kinase's activity for phosphorylating substrates was examined. a) Value of LCK's apparent activity (ratio 665nm/615nm) without (light blue) or with (dark blue) immobilizing by PF-alkyne at two different concentrations of LCK; b) Estimation of the amounts of eluted LCK (lane 1, 3) compared with the loading amount of LCK (lane 2, 4) by western blot (**wb**) analysis. c) Retained BLK's activity.

References

1. B. R. Lanning, L. R. Whitby, M. M Dix, J. Douhan, A. M Gilbert, E. C. Hett, T. O. Johnson, C. Joslyn, J. C. Kath, S. Niessen, L. R. Roberts, M. E. Schnute, C. Wang, J. J. Hulce, B. Wei, L. O. Whiteley, M. M. Hayward and B. F. Cravatt, *Nat. Chem. Biol.*, 2014, **10**, 760–767.
2. Y. Zhou, T. Guo, G. Tang, H. Wu, N. K. Wong, Z. Pan, *Bioconjugate Chem.*, 2014, **25**, 1911−1915.