Optimization of Brigatinib as New Wild-Type Sparing Inhibitors of EGFR$^{T790M/C797S}$ Mutants

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Supporting Information

Table S1. The raw kinase inhibitory rate data of 18k against 28 different kinases at concentration of 100 nM (ELISA assay).

Kinase	Inhibition Rate (%)			
	Raw Data		Corrected Data*	
	Group 1	Group 2	Mean*	SD*
VEGFR1	5.6	7.1	6.4	1.1
VEGFR2	-10.3	1.5	0.8	1.1
VEGFR3	74.7	58.9	66.8	11.2
PDGFR-α	-5.2	-14.4	0	0
PDGFR-β	3.1	28.9	16.0	18.2
RET	36.9	-1.5	18.4	26.1
Kit	9.4	-14.3	4.7	6.6
Flt-3	21.6	25	23.3	2.4
ErbB2	20	-10	10.0	14.1
ErbB4	26.5	40.2	33.4	9.7
Src	27	26.1	26.6	0.6
Abl	10	-5.8	5.0	7.1
EPH-A2	7	9.6	8.3	1.8
EPH-B2	10	2.4	6.2	5.4
ACK1	19.4	39.8	29.6	14.4
IGF1R	21.5	6.4	14.0	10.7
IR	7.1	15.8	11.5	6.2
FGFR1	26.1	-8.4	13.0	18.4
FGFR2	11.5	-18.1	5.8	8.1
FGFR3	27	17.2	22.1	6.9
FGFR4	31.4	15.5	23.5	11.2
BTK	3.8	-7.3	1.9	2.7
CSF1R	22.4	10.7	16.6	8.3
FAK	87.7	82.1	84.9	4.0
ITK	4.4	2	3.2	1.7
RON	6.4	17.1	11.8	7.6
ALK	0.4	0.7	0.6	0.2
BLK	62	65.8	63.9	2.7

* To calculate the mean value and SD value, negative data will be corrected to 0.

EXPERIMENTAL SECTION

General Methods for Chemistry

All reagents and solvents were purchased from commercial sources and used without further purification. The reactions were monitored by thin-layer chromatography (TLC) on GF254 silica 367 gel coated plates and visualized by UV light visualization (254 nm and 365 nm). Column chromatography was performed using 200-300 mesh silica gel. The ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AV- 400 spectrometer at 400 MHz, Brucker AV-500 spectrometer at 125 MHz or Brucker AV-600 spectrometer at 151 MHz using deuterated solvents as internal standard (TMS). Coupling constants (*J*) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to the internal standard. The high resolution ESI-MS results were recorded on Applied Biosystems Q-STAR Elite ESILC-MS/MS mass spectrometer. Purity of compounds was determined by reverse-phase high-performance liquid chromatography (HPLC) analysis. A flow rate of 1.0 mL/min was used with mobile phase of methanol in water with 0.1% triethylamine (*v/v*).

General procedure for the preparation of 20. To a solution of 2-iodoaniline (**19**, 5.06 g, 23 mmol) in 50 mL DMF was added dimethylphosphine oxide (2.20 g, 27.2 mmol), $Pd(OAc)_2$ (0.26 g, 1.2 mmol), xantphos (0.67 g, 1.2 mmol) and K_3PO_4 (5.40 g, 25.4 mmol). The suspension was degassed with argon for three times. The reaction mixture was stirred at 120 °C overnight. After cooling down and filtering over Celite, the filtrate was dissolved in ethyl acetate, washed twice with water, dried with saturated brine and Na_2SO_4, and concentrated in *vacuo*. The residue was purified by column chromatography to afford **20** as off-white solid (yield 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26-7.13 (m, 2H), 6.68-6.61 (m, 1H), 6.59-6.52 (m, 1H), 6.14 (s, 2H), 1.64 (d, J = 13.2 Hz, 6H).

General procedure for the preparation of 21. A mixture of intermediate **20** (1.0 eq), R_1 substituted pyrimidine (1.0 eq), K_2CO_3 (1.2 eq) and n-Bu_4NHSO_4 (0.1 eq) in DMF was heated up to 65 ºC and stirred overnight. Upon cooling, the solution was evaporated and purified by column chromatography to get the desired compound **21**.

(2-((2,5-Dichloropyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**21a**) White solid, yield 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.46 (s, 1H), 8.43 (dd, J = 8.3 Hz, 4.2 Hz, 1H), 7.71-7.56 (m, 2H), 7.31-7.21 (m, 1H), 1.83 (s, 3H), 1.80 (s, 3H).

(2-((5-Bromo-2-chloropyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**21g**) Off-white solid, yield 71%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.27 (s, 1H), 8.56 (dd, J = 8.5, 4.4 Hz, 1H), 8.33 (s, 1H), 7.59 (dd, J = 8.5, 7.4 Hz, 1H), 7.37-7.26 (m, 1H), 7.22-7.11 (m, 1H), 1.84 (d, J = 13.2 Hz, 6H).

(2-((2-Chloro-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**21h**) Off-white solid, yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.68 (s, 1H), 8.64 (dd, J = 8.4, 4.4 Hz, 1H), 8.59 (s, 1H), 7.56 (dd, J = 8.5, 7.3 Hz, 1H), 7.33-7.33 (m, 1H), 7.20-7.09 (m, 1H), 1.84 (d, J = 13.2 Hz, 6H).

General procedure for the preparation of compounds 18. R_2 substituted aniline was commercially available from several sources, which could also be conveniently

prepared by successively undergoing nucleophilic substitution reaction of 4-fluoronitrobenzene with aliphatic amine and nitro reduction reaction mediated by iron powder. To a solution of **21** (1.1 eq) and R_2 substituted aniline (1.0 eq) in 2-methoxyethanol was added 2.5 M HCl in ethanol (2.5 eq). The resulting mixture was heated at 120 °C overnight in a sealed tube under stirring. Upon cooling, the reaction mixture was evaporated, and purified by column chromatography to yield the target compound **18**.

(2-((5-Chloro-2-((3-methoxy-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18a**) Yield 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.89 (s, 1H), 8.61 (dd, *J* = 8.4, 4.4 Hz, 1H), 8.07 (s, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.32-7.22 (m, 1H), 7.13-6.96 (m, 4H), 6.87 (d, *J* = 8.5 Hz, 1H), 3.74 (s, 3H), 3.51 (t, *J* = 16.4 Hz, 2H), 2.73-2.35 (m, 10H), 2.33-2.08 (m, 5H), 1.90-1.76 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.91 (s), 155.93 (s), 154.86 (s), 152.53 (s), 143.85 (d, *J* = 2.6 Hz), 137.55 (s), 134.74 (s), 132.47 (d, *J* = 2.0 Hz), 129.56 (d, *J* = 11.2 Hz), 122.66 (d, *J* = 7.1 Hz), 122.42 (d, *J* = 12.1 Hz), 119.93 (d, *J* = 95.7 Hz), 118.34 (s), 112.81 (s), 106.50 (s), 105.13 (s), 61.94 (s), 55.50 (s), 51.27 (s), 48.83 (s), 46.03 (s), 28.36 (s), 18.63 (d, *J* = 71.6 Hz), 1.02 (s). HRMS (ESI) for $C_{29}H_{40}ClN_7O_2P$ $[M + H]^+$, calcd: 584.2664; found 584.2658. HPLC analysis: MeOH-H_2O (80:20), RT = 6.12 min, 98.8% purity.

(2-((5-Chloro-2-((3-methyl-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18b**) Yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.89 (s, 1H), 8.62 (dd, *J* = 8.4, 4.4 Hz, 1H), 8.07 (s, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.36-7.27 (m, 3H), 7.14-7.05 (m, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.80 (s, 1H), 3.14 (d, *J* = 11.9 Hz, 2H), 2.86-2.38 (m, 10H), 2.37-2.29 (m, 4H), 2.27 (s, 3H), 2.00-1.90 (m, 2H), 1.83 (d, *J* = 13.1 Hz, 6H), 1.78-1.69 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.79 (s), 155.88 (s), 154.59 (s), 147.38 (s), 143.31 (d, *J* = 2.5 Hz), 134.38 (s), 133.28 (s), 132.59 (d, *J* = 2.0 Hz), 129.60 (d, *J* = 11.2 Hz), 123.12 (s), 123.01 (d, *J* = 6.9 Hz), 122.72 (d, *J* = 12.5 Hz), 120.11 (d, *J* = 96.5 Hz), 119.15 (s), 118.72 (s), 105.83 (s), 61.86 (s), 54.97 (s), 52.11 (s), 48.79 (s), 45.68 (s), 28.90 (s), 18.18 (d, *J* = 71.7 Hz), 17.80 (s). HRMS (ESI) for $C_{29}H_{40}ClN_7OP$ $[M + H]^+$, calcd: 568.2715; found 568.2711. HPLC analysis: MeOH-H_2O (80:20), RT = 9.93 min, 98.6% purity.

(2-((5-Chloro-2-((4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)-3-(trifluoromethoxy)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18c**) Yield 52%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.87 (s, 1H), 8.51 (dd, *J* = 8.4, 4.4 Hz, 1H), 8.09 (s, 1H), 7.55-7.44 (m, 2H), 7.33-7.26 (m, 2H), 7.13 (td, *J* = 7.4, 1.4 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 1H), 6.84 (s, 1H), 3.38 (d, *J* = 11.8 Hz, 2H), 2.96-2.33 (m, 10H), 2.36-2.23 (m, 4H), 1.94 (d, *J* = 11.4 Hz, 2H), 1.83 (d, *J* = 13.2 Hz, 6H), 1.77-1.68 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.50 (s), 156.02 (s), 154.78 (s), 143.56 (d, *J* = 2.6 Hz), 142.39 (d, *J* = 1.5 Hz), 140.84 (s), 134.47 (s), 132.49 (s), 129.56 (d, *J* = 11.1 Hz), 122.83 (d, *J* = 7.4 Hz), 122.72 (d, *J* = 12.3 Hz), 120.49 (q, *J* = 257.5 Hz), 120.36 (d, *J* = 95.6 Hz), 120.17 (s), 119.00 (s), 114.59 (s), 106.94 (s), 61.74 (s), 55.45 (s), 51.25 (s), 49.18 (s), 46.02 (s), 28.88 (s), 18.56 (d, *J* = 71.6 Hz). HRMS (ESI) for $C_{29}H_{37}ClF_3N_7O_2P$ $[M + H]^+$, calcd: 638.2381; found 638.2377. HPLC analysis: MeOH-

H_2O (80:20), RT = 11.82 min, 99.3% purity.

(2-((5-Chloro-2-((4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)-3-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18d**) Yield 71%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (s, 1H), 8.53 (dd, *J* = 8.4, 4.4 Hz, 1H), 8.10 (s, 1H), 7.75 (d, *J* = 2.5 Hz, 1H), 7.67 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.45 (t, *J* = 7.9 Hz, 1H), 7.33-7.27 (m, 2H), 7.16-7.08 (m, 1H), 6.96 (s, 1H), 3.07 (d, *J* = 11.4 Hz, 2H), 2.86-2.31 (m, 11H), 2.30 (s, 3H), 1.98-1.87 (m, 2H), 1.85 (s, 3H), 1.82 (s, 3H), 1.78-1.70 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.38 (s), 156.02 (s), 154.77 (s), 147.60 (s), 143.56 (d, *J* = 2.7 Hz), 136.24 (s), 132.55 (d, *J* = 2.2 Hz), 129.61 (d, *J* = 10.9 Hz), 127.75 (q, *J* = 28.5 Hz), 124.38 (s), 123.94 (s), 123.77 (q, *J* = 273.6 Hz), 122.70 (d, *J* = 12.2 Hz), 122.55 (d, *J* = 7.0 Hz), 120.11 (d, *J* = 95.6 Hz), 118.59 (q, *J* = 6.3 Hz), 107.27 (s), 61.78 (s), 55.48 (s), 53.86 (s), 49.17 (s), 46.02 (s), 29.01 (s), 18.63 (d, *J* = 71.6 Hz). HRMS (ESI) for $C_{29}H_{37}ClF_3N_7OP$ $[M + H]^+$, calcd: 622.2432; found 622.2431. HPLC analysis: MeOH-H_2O (80:20), RT = 10.72 min, 98.6% purity.

(2-((5-Chloro-2-((3-(difluoromethyl)-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18e**) Yield 46%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.44 (dd, *J* = 8.3, 4.5 Hz, 1H), 8.10 (s, 1H), 7.73 (d, *J* = 2.3 Hz, 1H), 7.66 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.64-7.57 (m, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.26 (td, *J* = 7.5, 1.3 Hz, 1H), 7.19 (s, 1H), 7.04 (t, *J* = 51.6 Hz, 1H), 3.10 (d, *J* = 11.6 Hz, 2H), 3.00-2.17 (m, 14H), 1.99 (d, *J* = 11.8 Hz, 2H), 1.85 (d, *J* = 13.5 Hz, 6H), 1.70 (qd, *J* = 12.0, 3.7 Hz, 2H). HRMS (ESI) for $C_{29}H_{38}ClF_2N_7OP$ $[M + H]^+$, calcd: 604.2527; found 604.2525. HPLC analysis: MeOH-H_2O (80:20), RT = 8.83 min, 99.1% purity.

(2-((5-Chloro-2-((3-chloro-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18f**) Yield 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.94 (s, 1H), 8.58 (dd, *J* = 8.4, 4.4 Hz, 1H), 8.08 (s, 1H), 7.76 (d, *J* = 2.5 Hz, 1H), 7.55 (t, *J* = 7.9 Hz, 1H), 7.33-7.28 (m, 1H), 7.21 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.16-7.08 (m, 1H), 6.98 (d, *J* = 8.7 Hz, 1H), 6.84 (s, 1H), 3.40 (d, J = 11.7 Hz, 2H), 2.84-2.46 (m, 9H), 2.46-2.37 (s, 1H), 2.33 (s, 3H), 2.05-1.89 (m, 3H), 1.84 (d, *J* = 13.1 Hz, 6H), 1.81-1.73 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.36 (s), 155.91 (s), 154.60 (s), 144.65 (s), 143.14 (d, *J* = 2.2 Hz), 135.13 (s), 133.02 (s), 129.63 (d, *J* = 10.8 Hz), 129.02 (s), 122.91 (d, *J* = 2.5 Hz), 122.82 (d, *J* = 2.6 Hz), 122.08 (s), 120.34 (s), 120.16 (d, *J* = 96.4 Hz), 119.10 (s), 106.44 (s), 61.75 (s), 54.98 (s), 51.65 (s), 48.63 (s), 45.66 (s), 28.43 (s), 18.28 (d, *J* = 71.7 Hz). HRMS (ESI) for $C_{28}H_{37}Cl_2N_7OP$ $[M + H]^+$, calcd: 588.2169; found 588.2166. HPLC analysis: MeOH-H_2O (80:20), RT = 9.37 min, 99.2% purity.

(2-((5-Bromo-2-((3-chloro-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18g**) Yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.71 (s, 1H), 8.49 (dd, *J* = 8.4, 4.3 Hz, 1H), 8.21 (s, 1H), 7.76 (d, *J* = 2.5 Hz, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.37-7.29 (m, 1H), 7.23 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.16 (td, *J* = 7.4, 1.5 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 1H), 6.87 (s, 1H), 3.41 (d, *J* = 11.8 Hz, 2H), 2.99-2.19 (m, 14H), 1.95 (d, *J* = 11.7 Hz, 2H), 1.90-1.74 (m, 8H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.74 (s), 157.58 (s), 156.64 (s), 144.56 (s), 142.97 (d, *J* = 2.2 Hz), 135.05 (s), 132.97 (s), 129.61 (d, *J* = 10.9 Hz), 128.98 (s), 123.27

(d, *J* = 7.2 Hz), 123.08 (d, *J* = 12.2 Hz), 121.93 (s), 120.57 (d, *J* = 96.5 Hz), 120.31 (s), 118.98 (s), 94.87 (s), 61.71 (s), 54.98 (s), 51.67 (s), 48.67 (s), 45.75 (s), 28.39 (s), 18.16 (d, *J* = 71.7 Hz). HRMS (ESI) for $C_{28}H_{37}BrClN_7OP$ $[M + H]^+$, calcd: 632.1664; found 632.1662. HPLC analysis: MeOH-H_2O (80:20), RT = 9.53 min, 99.2% purity.

(2-((2-((3-Chloro-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18h**) Yield 37%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.01 (s, 1H), 8.41 (dd, *J* = 8.4, 4.4 Hz, 1H), 8.33 (s, 1H), 7.60 (d, *J* = 2.3 Hz, 1H), 7.41-7.30 (m, 2H), 7.25-7.14 (m, 1H), 7.11-6.97 (m, 2H), 6.75 (s, 1H), 3.45 (d, *J* = 11.8 Hz, 2H), 2.97-2.27 (m, 14H), 1.96 (d, *J* = 13.0 Hz, 2H), 1.85-1.78 (m, 8H). HRMS (ESI) for $C_{29}H_{37}ClF_3N_7OP$ $[M + H]^+$, calcd: 622.2432; found 622.2424. HPLC analysis: MeOH-H_2O (80:20), RT = 10.29 min, 99.0% purity.

(2-((5-Chloro-2-((5-methyl-6-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)pyridin-3-yl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18i**) Yield 67%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.45-8.35 (m, 1H), 8.18 (d, *J* = 2.5 Hz, 1H), 8.08 (s, 1H), 7.84 (d, *J* = 2.5 Hz, 1H), 7.62 (ddd, *J* = 14.1, 7.7, 1.4 Hz, 1H), 7.54 (t, *J* = 7.9 Hz, 1H), 7.32-7.20 (m, 1H), 3.39 (d, *J* = 12.4 Hz, 2H), 3.09-2.65 (m, 9H), 2.64-2.32 (m, 5H), 2.22 (s, 3H), 2.08-1.93 (m, 2H), 1.87 (s, 3H), 1.83 (s, 3H), 1.76-1.65 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.84 (s), 157.66 (s), 156.00 (s), 154.83 (s), 143.74 (d, *J* = 2.6 Hz), 137.53 (s), 132.46 (d, *J* = 2.2 Hz), 132.08 (s), 131.30 (s), 129.64 (d, *J* = 11.0 Hz), 125.28 (s), 122.57 (d, *J* = 5.0 Hz), 122.48 (s), 120.08 (d, *J* = 95.5 Hz), 106.92 (s), 62.17 (s), 54.54 (s), 49.65 (s), 48.27 (s), 45.29 (s), 28.43 (s), 18.67 (d, *J* = 71.7 Hz), 18.17 (s). HRMS (ESI) for $C_{28}H_{39}ClN_8OP$ $[M + H]^+$, calcd: 569.2667; found 569.2662. HPLC analysis: MeOH-H_2O (80:20), RT = 5.77 min, 92.1% purity.

(2-((5-Chloro-2-((3,5-dichloro-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18j**) Yield 50%.^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (s, 1H), 8.52 (dd, *J* = 8.4, 4.4 Hz, 1H), 8.10 (s, 1H), 7.67-7.48 (m, 2H), 7.44 (d, *J* = 2.3 Hz, 1H), 7.35-7.26 (m, 1H), 7.14 (t, *J* = 6.7 Hz, 1H), 6.90 (s, 1H), 3.34 (t, *J* = 11.1 Hz, 2H), 3.03 (d, *J* = 11.8 Hz, 2H), 2.90-2.25 (m, 11H), 2.09-1.92 (m, 1H), 1.95-1.69 (m, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.96 (s), 156.02 (s), 154.78 (s), 143.46 (d, *J* = 2.6 Hz), 140.12 (s), 137.07 (s), 136.02 (s), 134.63 (s), 133.12 (s), 129.68 (d, *J* = 10.9 Hz), 122.75 (d, *J* = 12.2 Hz), 122.52 (d, *J* = 7.1 Hz), 120.24 (d, *J* = 95.6 Hz), 119.71 (s), 119.19 (s), 107.56 (s), 62.14 (s), 55.54 (s), 49.72 (s), 49.11 (s), 46.06 (s), 29.37 (s), 18.65 (d, *J* = 71.6 Hz). HRMS (ESI) for $C_{28}H_{36}Cl_3N_7OP$ $[M + H]^+$, calcd: 622.1779; found 622.1782. HPLC analysis: MeOH-H_2O (80:20), RT = 22.15 min, 100% purity.

(2-((5-Chloro-2-((2,5-dichloro-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**18k**) Yield 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (s, 1H), 8.56 (dd, *J* = 8.1, 4.3 Hz, 1H), 8.41 (s, 1H), 8.15 (s, 1H), 7.61 (dd, *J* = 8.5, 7.4 Hz, 1H), 7.36-7.30 (m, 1H), 7.23 (s, 1H), 7.21-7.11 (m, 1H), 7.08 (s, 1H), 3.41 (d, *J* = 11.9 Hz, 2H), 2.79-2.30 (m, 14H), 1.96 (d, *J* = 11.9 Hz, 2H), 1.90-1.72 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.92 (s), 155.99 (s), 154.85 (s), 144.75 (s), 143.48 (d, *J* = 2.6 Hz), 133.19 (d, *J* = 2.1 Hz), 131.70 (s), 129.64 (d, *J* = 10.9 Hz), 127.60 (s), 122.67 (d, *J* = 12.2 Hz), 122.45 (s), 122.42 (d, *J* = 6.7 Hz), 121.38 (s), 120.89 (s), 120.11 (d, *J* = 95.5 Hz), 107.58 (s), 61.54 (s), 55.45 (s),

51.63 (s), 49.02 (s), 46.00 (s), 28.55 (s), 18.64 (d, J = 71.6 Hz). HRMS (ESI) for $C_{28}H_{36}Cl_3N_7OP$ $[M + H]^+$, calcd: 622.1779; found 622.1782. HPLC analysis: MeOH-H_2O (80:20), RT = 19.50 min, 96.8% purity.

Biological assays

Cell culture.

BaF3 cell line was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), and cultured in RPMI1640 containing 10% FBS and 10 ng/mL IL3. BaF3-EGFR$^{L858R/T790M/C797S}$ and BaF3-EGFR$^{19Del/T790M/C797S}$ cells were built by Jian Ding's laboratory and cultured in RPMI1640 with 10% FBS without IL3. NCI-H1975, PC9 lung cancer cell lines were purchased from ATCC (American type culture collection) or ECACC (European Collection of Authenticated Cell Cultures), respectively. PC9-EGFR$^{T790M/C797S}$ cell line was constructed using CRISPR knock-in technology. Tumor cells were cultured in standard culture medium and all cell lines were maintained at 37 °C in 5% CO_2 incubator.

Compounds and antibodies.

Osimertinib (#T2490) was purchased from Topscience. Brigatinib (#S8229) was obtained from Selleck. Antidodies phospho-EGFR (Tyr1068; #3777), EGFR (#4267), p-AKT (Ser473, #4060) and AKT (#9272S) were purchased from Cell Signaling Technologies and β-actin (#P60709) was purchased from Abgent.

In Vitro Kinase Assay.

EGFR^{WT} and EGFR$^{L858R/T790M}$ kinase proteins were purchased from Eurofins Scientific. EGFR$^{L858R/T790M/C797S}$ kinase was purchased from BPS Bioscience. Kinase inhibition activities of compounds were evaluated using an enzyme-linked-immunosorbent assay (ELISA) according to the standard procedures as previously described[1]. Absorbance was measured at 492 nm using a multi-well spectrophotometer (VERSAmax™). The inhibitory rate (%) was calculated with the formula [1- (A492 treated/A492 control)] ×100%. IC_{50} values were calculated by SoftMax Pro from inhibitory curves.

Cellular Proliferation Assays.

CCK-8 (Cell Counting Kit-8,) assay: Cells were cultured in 96-well plates overnight and treated with indicated concentration of compounds for 72 h. 10 μL of CCK-8 solution (#AC11L057, Life iLab) was added to each well and incubated with cells for 1-2 h, then the absorbance was measured using a multiwell spectrophotometer (VERSAmax™) at 450 nm. The growth inhibition rate (%) was calculated as [1-(A450 treated/A450 control)] × 100% and IC_{50} values were determined using Logit method. SRB assay: H1299 cells were plated in a 96-well culture plate and cultured overnight. The test compounds in different concentrations were added, and then the cells were cultured for 72 hours. Then, sulforhodamine B (SRB, #S9012, Sigma-Aldrich) assay was performed according to standard protocols, and the results were acquired using a multiwall spectrophotometer (VERSA max™, Molecular Devices) at an absorbance of 560 nm.

Western Blot Analysis.

Cells were cultured in 12-well plates and starved in serum-free RPMI1640 for 20 h. Then cells were treated with indicated compounds or DMSO control for 2 h and

stimulated with 50 ng/ml EGF during the last 15 minutes. The cells were washed with cold PBS for 3 times, and then lysed in SDS lysis buffer and heated for 30 min at 100 °C for the following Western Blot analysis. Cell lysis were loaded on SDS-PAGE gels, then target proteins were separated and transferred from gels to nitrocellulose membranes After blocked in a 5% non-fat milk-TBST for 40 minutes, the membranes were incubated in primary antibodie diluent overnight at 4 °C. Then the membranes were washed with TBST for 3 times (5 min per wash) and incubated with diluted secondary antibody for 60 minutes at room temperature. After washed with TBST for 3 times, target proteins in membranes were analyzed by chemiluminescence using Amersham Imager 600.

Pharmacokinetic Studies

The pharmacokinetic study was carried out by Shanghai Medicilon Inc., according to the protocols and guidelines of the institutional care and use committee. All the procedures related to animal handling, care, and treatment in this article were performed in compliance with Agreement of the Ethics Committee on Laboratory Animal Care and the Guidelines for the Care and Use of Laboratory Animals in Shanghai, China. Compound **18f** and **18k** were dissolved in mixed solvents PEG300/EtOH/NaCl (40/10/50, v/v/v) for intravenous injection and 0.5% HPMC for oral administration. SD rats (male, 3 animals per group) were fasted for 12 h before administration and remained fasting for 2 hours. After intravenous injection (0.5 mg/kg) or oral administration (3 mg/kg) of **18f** or **18k**, blood would be taken *via* jugular vein or other suitable vein, 0.2 mL/time point. Blood samples were placed in tubes containing K2-EDTA and stored on ice until centrifuged. The blood samples were centrifuged at 6800 g/min for 6 minutes at 2-8 °C within 1h after collected and stored frozen at approximately -80 °C until further use. The analytical results were confirmed using quality control samples for intra-assay variation. The accuracy of >66.7% of the quality control samples should be between 80-120% of the known value(s). Standard set of parameters including Area Under the Curve ($AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$), elimination half-live ($T_{1/2}$), maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}) will be calculated using noncompartmental analysis modules in FDA certified pharmacokinetic program Phoenix WinNonlin 7.0 (Pharsight, USA) by the Study Director.

Protein expression, crystallization and structure determination

Constructs spanning residues 696-1022 of the mutant human $EGFR^{T790M/C797S}$ were expressed and purified using the baculovirus/insect cell system as described[2]. To improve the resolution of the crystals, we introduced three A-loop mutations E865A/E866A/K867A to the construct as in the previous report[3]. Crystals used in this study were prepared by hanging drop vapor diffusion. For co-crystallization, the compound **18f** was added to 7.0 mg/mL proteins to a final concentration of 1mM and incubated at 4 °C for two hours before setting up the crystallization tray. The reservoir solutions to grow crystals were 0.1 M HEPES pH 7.8, 40% PEG400, 0.15 M NaCl, 5 mM tris(2-carboxyethyl)-phosphine (TCEP). The diffraction data were collected on

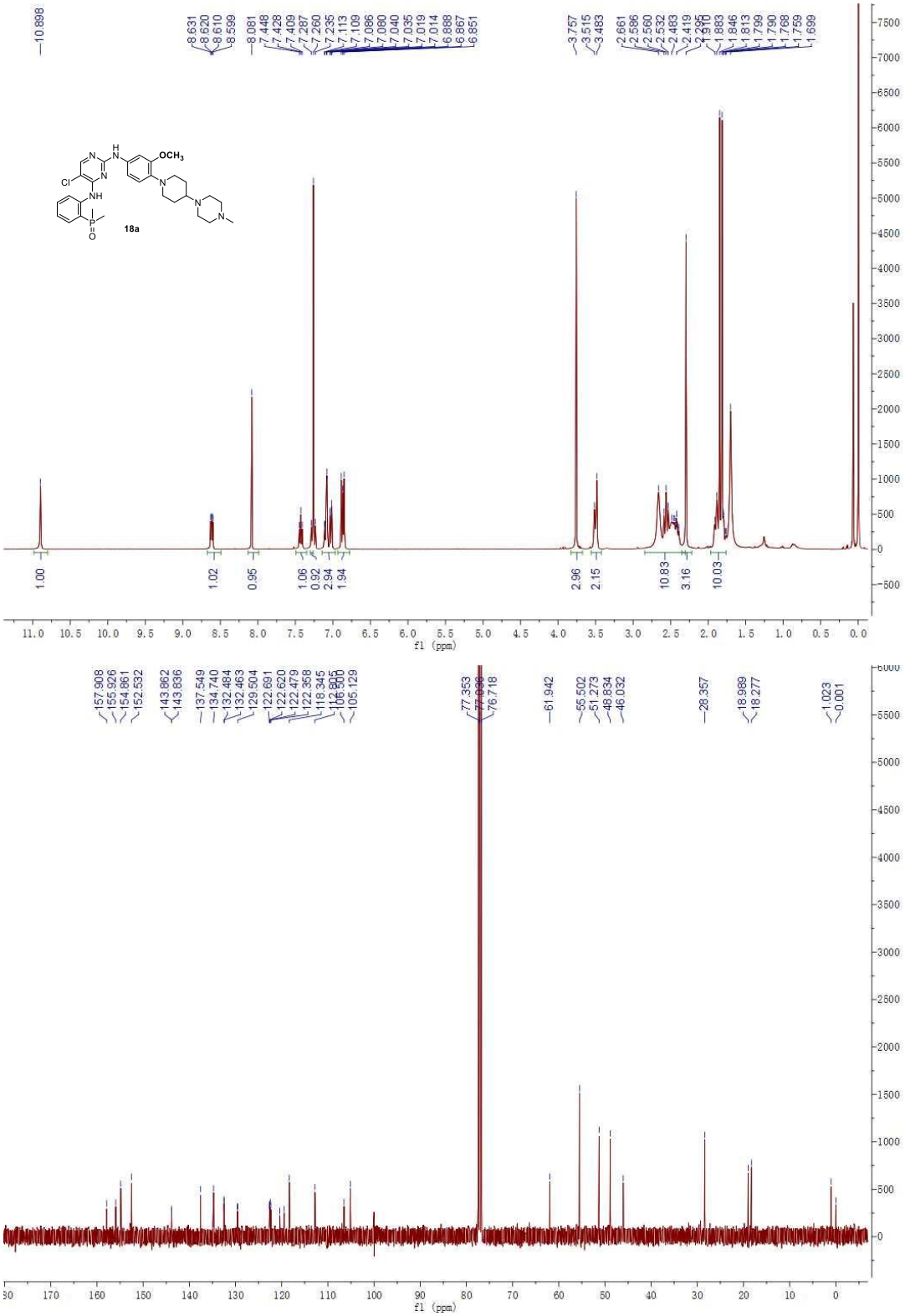
beamline BL18U1 at Shanghai Synchrotron Radiation Facility (SSRF). The diffraction data were processed using HKL3000[4]. The structures were all solved by molecular replacement with Phenix[5] using the previously determined EGFR 696-1022 T790M/C797S in complex with D3003 (PDB 5ZTO) as the search model. Repeated rounds of manual refitting and crystallographic refinement were then performed using COOT[6] and Phenix. The inhibitor was modeled into the closely fitting positive Fo-Fc electron density and included in following refinement cycles. Topology and parameter files for the inhibitor were generated using Phenix. The diffraction data collection and refinement statistics were summarized in Supplemental Table S2.

Table S2. Co-crystal structure data collection and refinement statistics

	TMCS_**18f**
PDB ID	**7ER2**
Data collection*	
Space group	I23
Cell dimensions	
a, *b*, *c* (Å)	145.93, 145.93, 145.93,
α, β, γ (°)	90.0,90.0,90.0
Resolution (Å)	50.0-2.65(2.74-2.65)
R_{pim}	0.056(0.541)
$I/\sigma I$	21(2.5)
Completeness (%)	100(100)
Redundancy	36.8(27.2)
Refinement	
Resolution (Å)	28.62-2.65
No. reflections	14931
R_{work} / R_{free}	0.195/0.242
No. atoms	
Protein	2269
Ligand/ion	39
Water	16
B-factors	
Protein	57.1
Ligand/ion	71.13
Water	58.49
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.024
Ramachandran Plot	
Favored region (%)	98.2
Allowed region (%)	1.8
Outliers (%)	0.0

* Values in parentheses are for highest-resolution shell.

Copies of NMR Spectra and HPLC Purity Data



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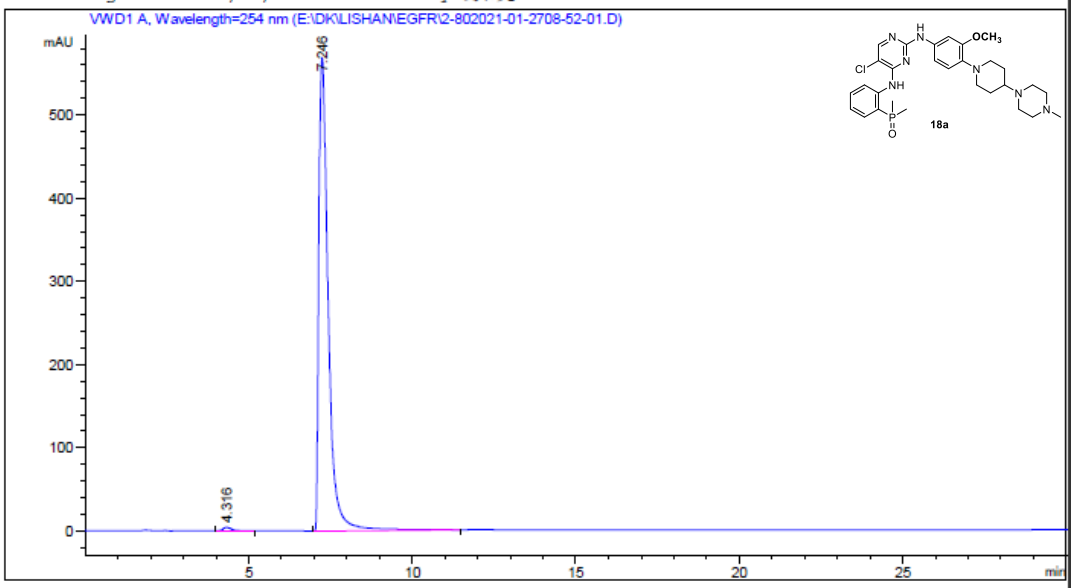


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Dilution              :      1.0000
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Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

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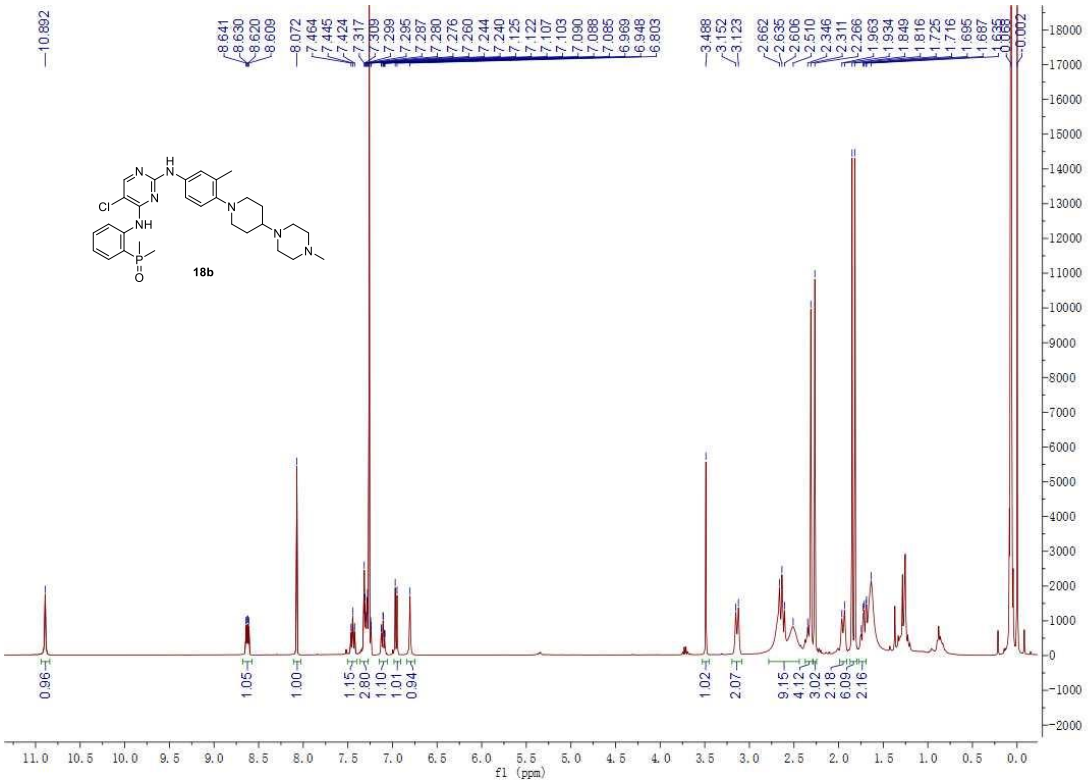
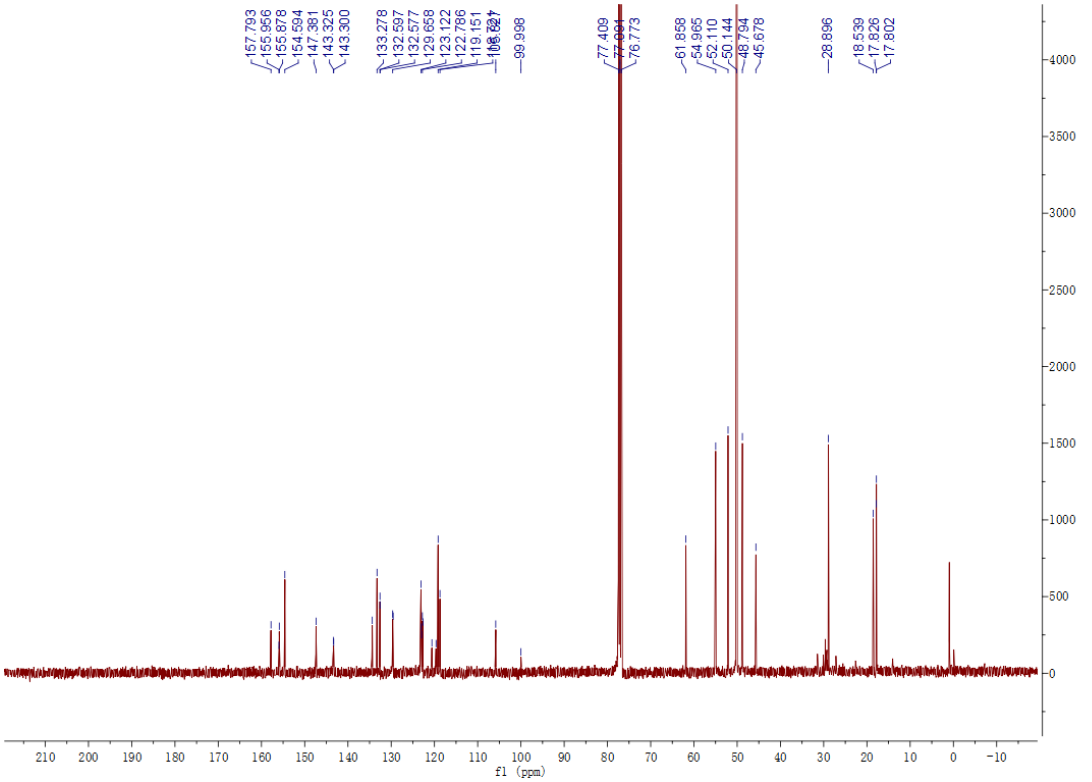

18b
fl (ppm)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Last changed    : 06/04/2021 10:23:35 by 系统
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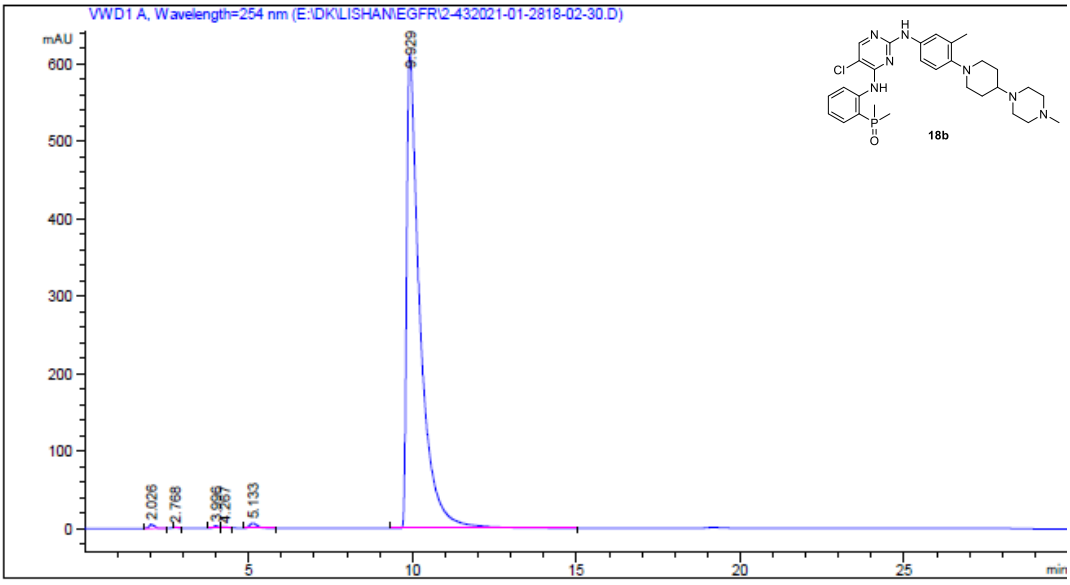


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Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
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   2   2.768 VB    0.1023   13.20130    1.76338   0.0747
   3   3.996 BV    0.1631   34.71921    3.04300   0.1966
   4   4.267 VV    0.1981   27.72350    2.04582   0.1570
   5   5.133 BB    0.2471  108.44701    6.65446   0.6140
   6   9.929 BB    0.4049 1.74196e4   612.32159  98.6174
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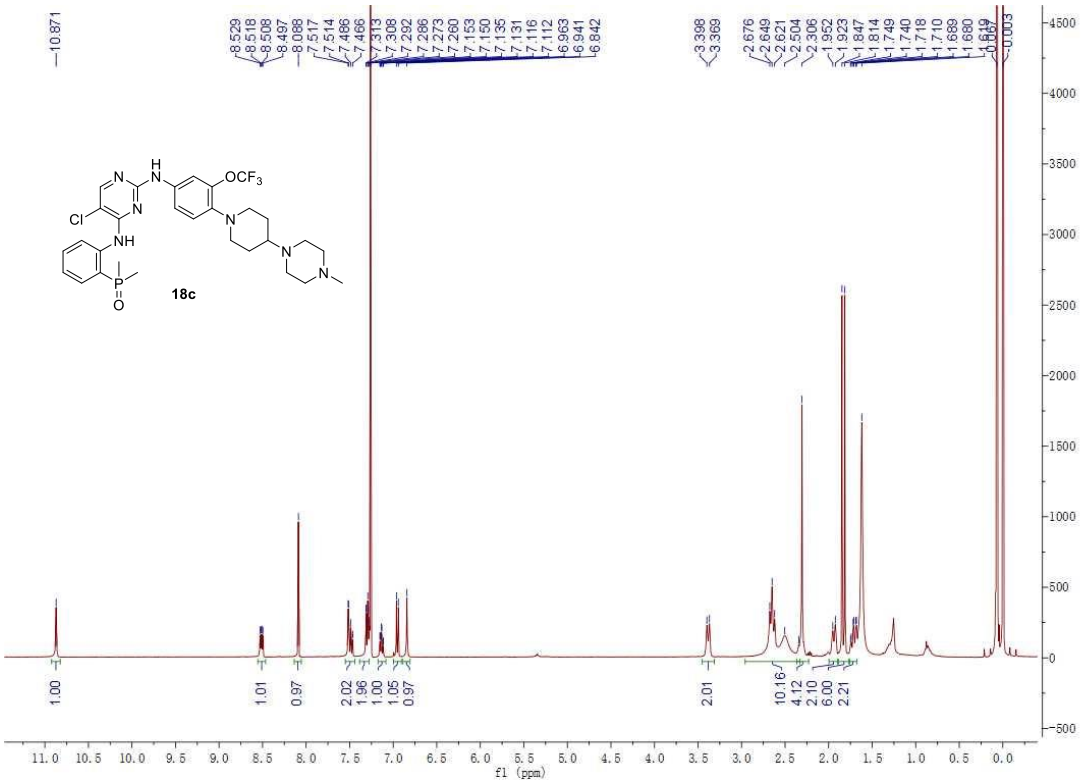
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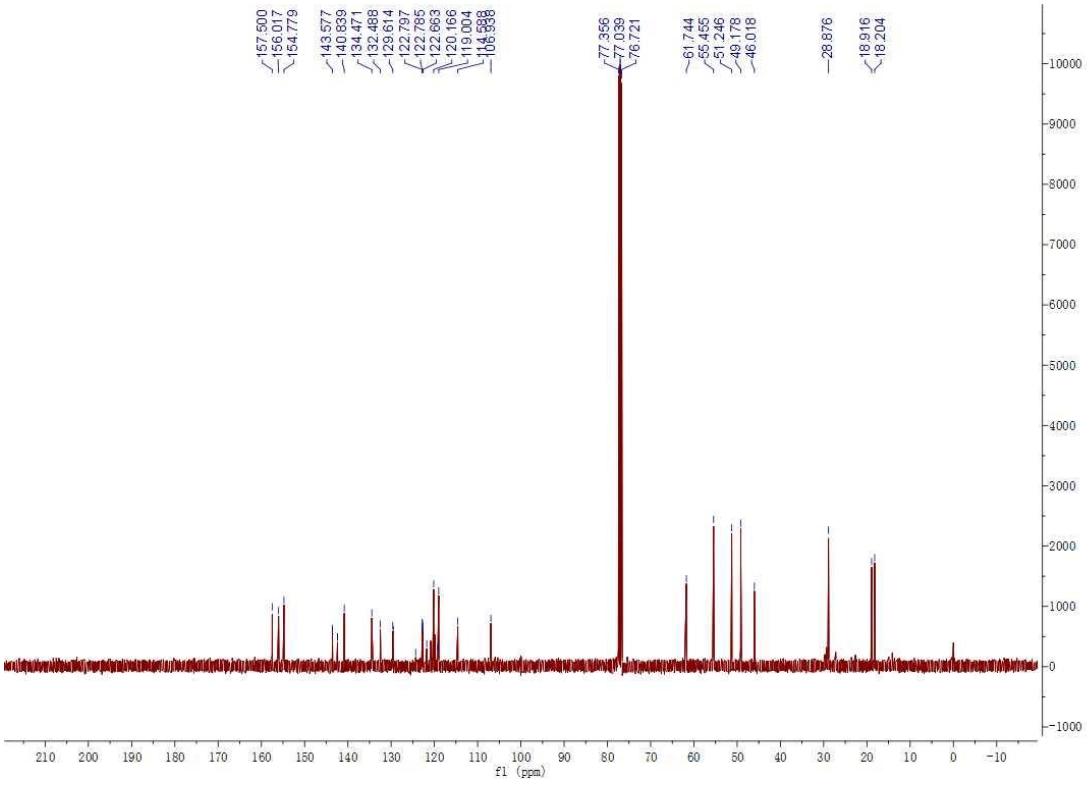

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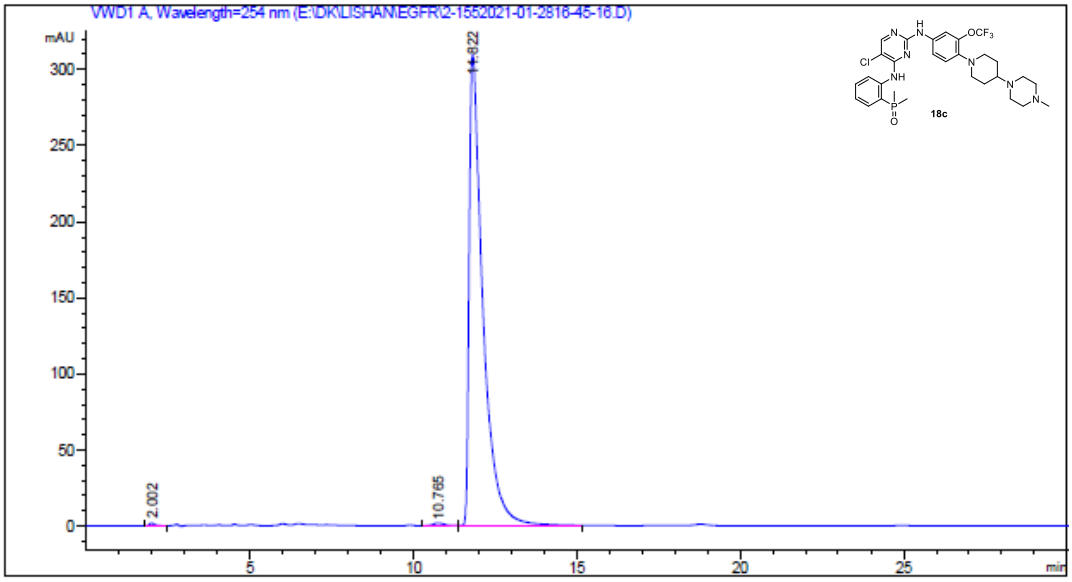


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Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

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   1   2.002 BB    0.1641   22.51513    1.97370   0.2468
   2  10.765 BB    0.3587   40.68840    1.73431   0.4460
   3  11.822 BB    0.4212 9060.61035  309.85352  99.3073

Totals :                  9123.81388  313.56152
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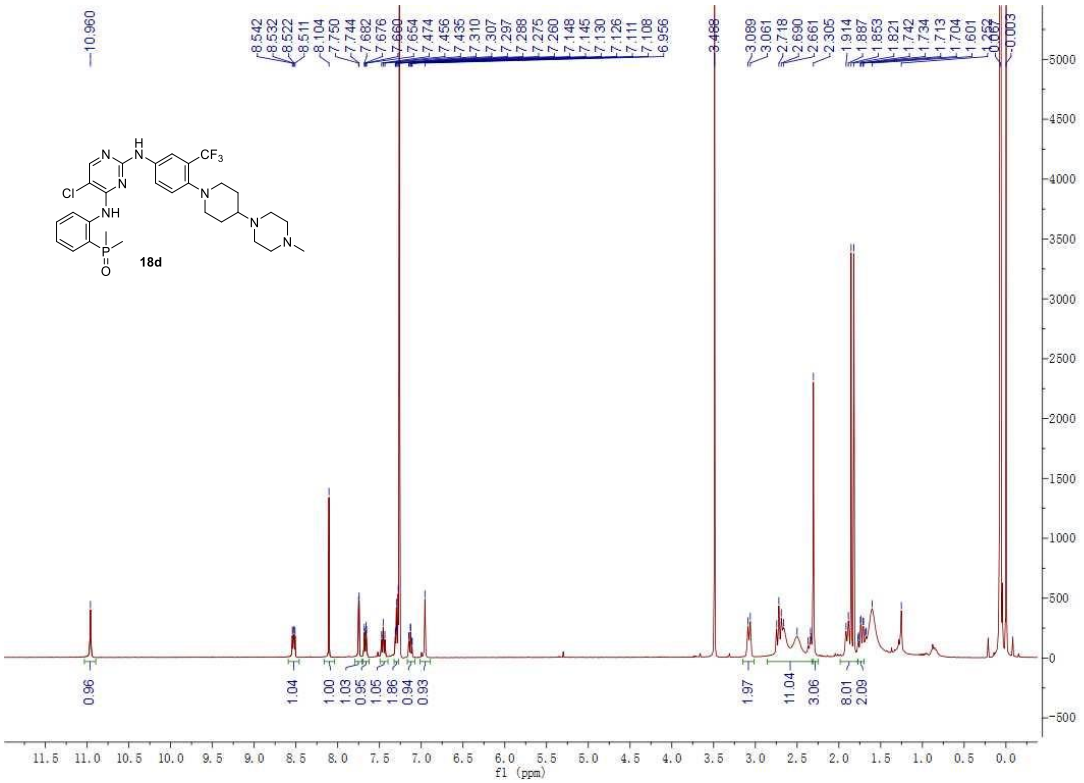
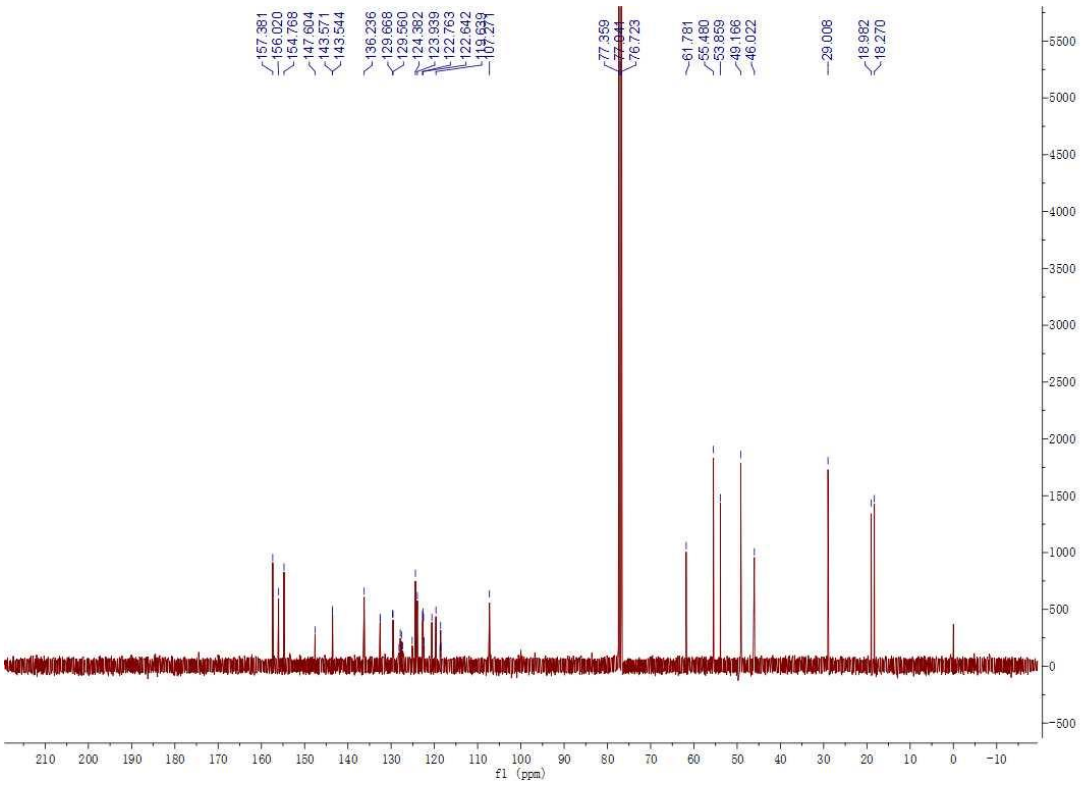

18d



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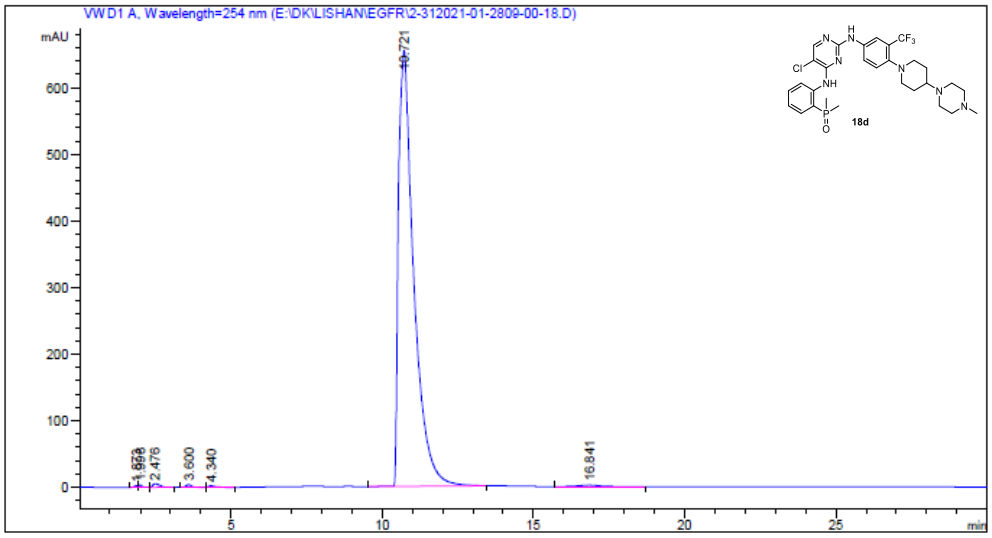


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Dilution              :      1.0000
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Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

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   2   1.998 VV    0.1075   26.40466    3.52252   0.1139
   3   2.476 VB    0.2207   84.48629    5.19726   0.3644
   4   3.600 BV R  0.1903   51.37792    3.96267   0.2216
   5   4.340 VB    0.2768   40.02019    2.01426   0.1726
   6  10.721 VB R  0.4666 2.28632e4   654.35315  98.6166

   7  16.841 BB    0.6485   99.32301    2.09715   0.4284

Totals :                  2.31839e4   673.41852


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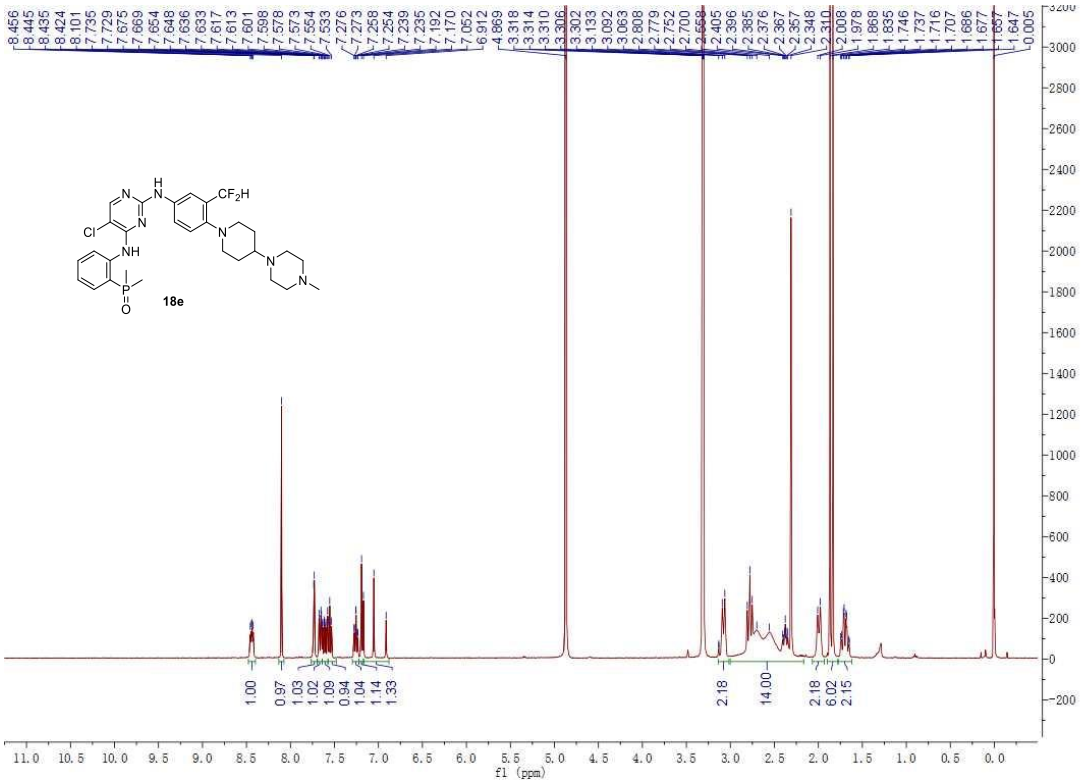
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1.02
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1.33
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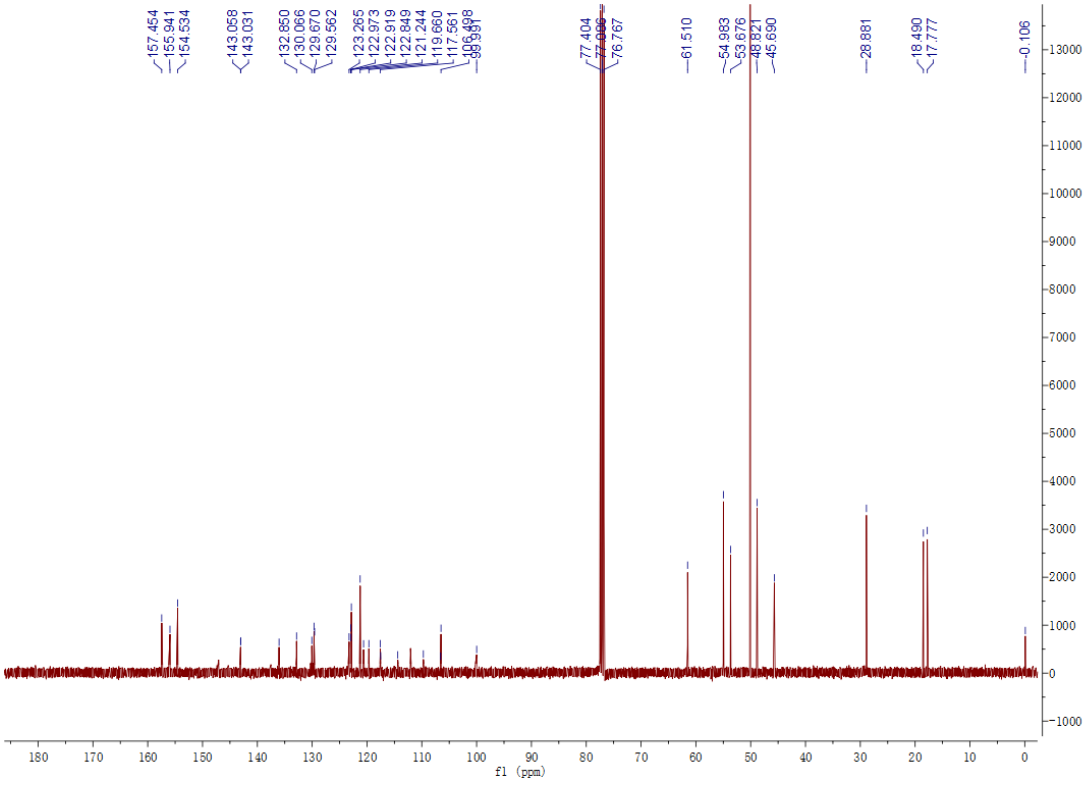
14.00
2.18
6.02
2.15
f1 (ppm)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122.849
121.244
119.660
117.561
77.404
76.767
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f1 (ppm)

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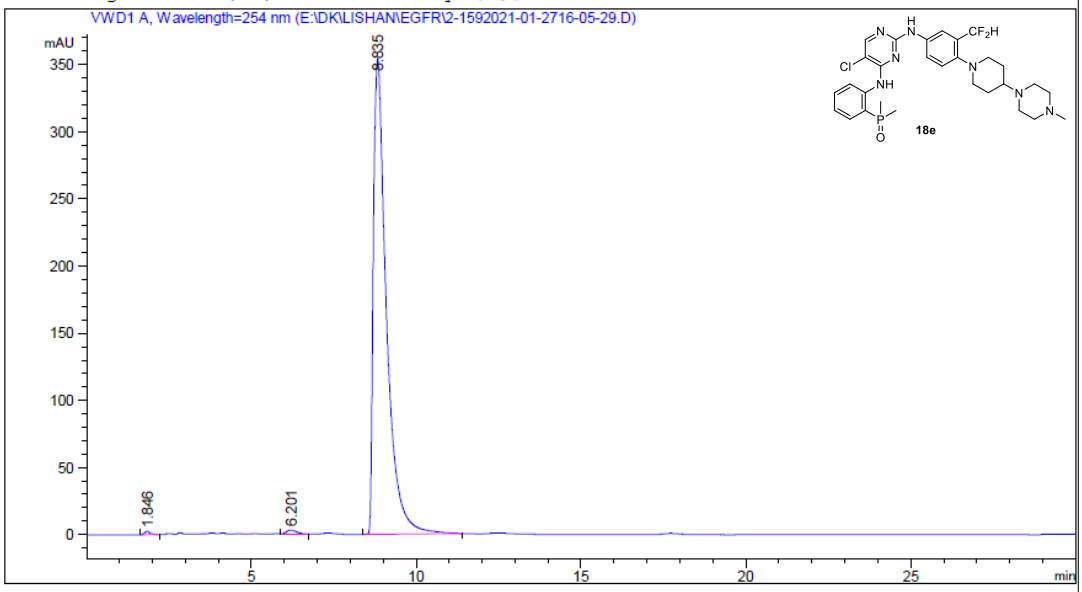


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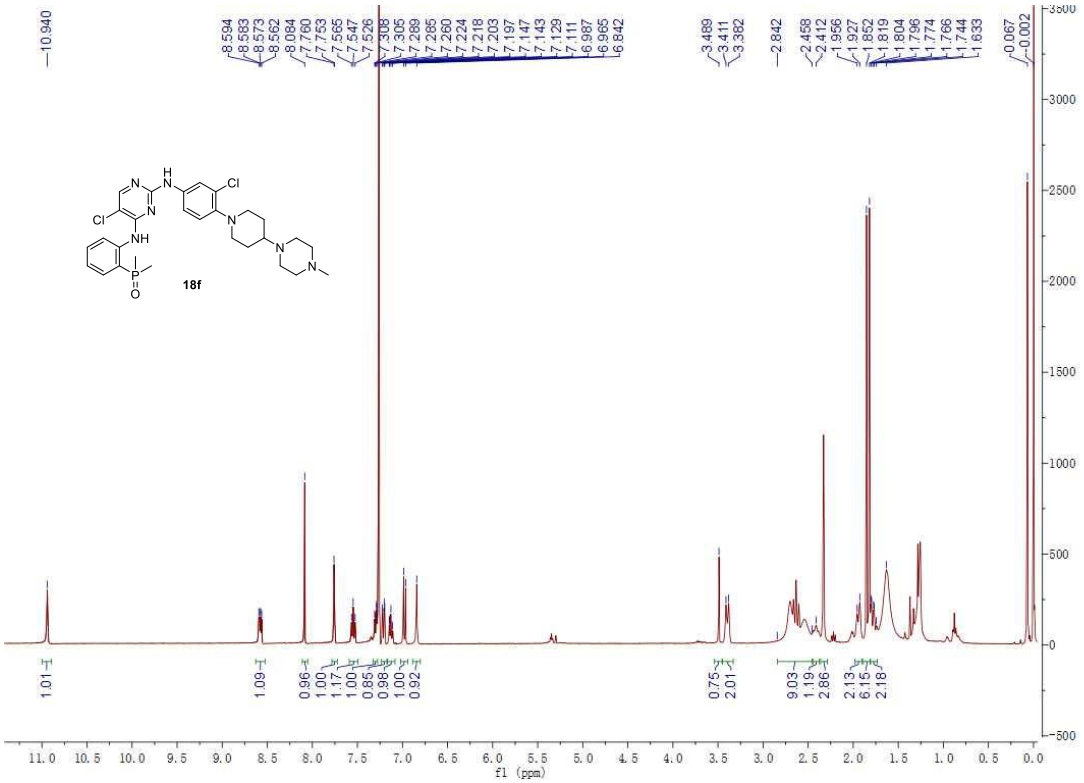
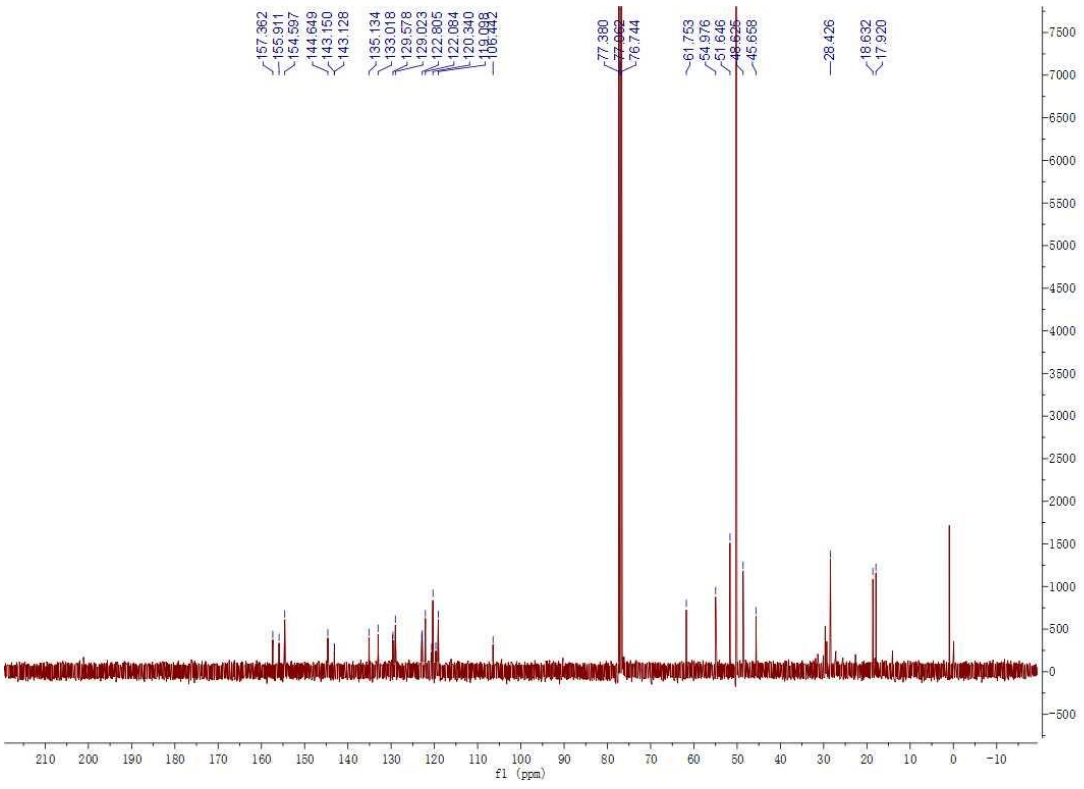
Dilution              :      1.0000
Sample Amount:                :     20.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

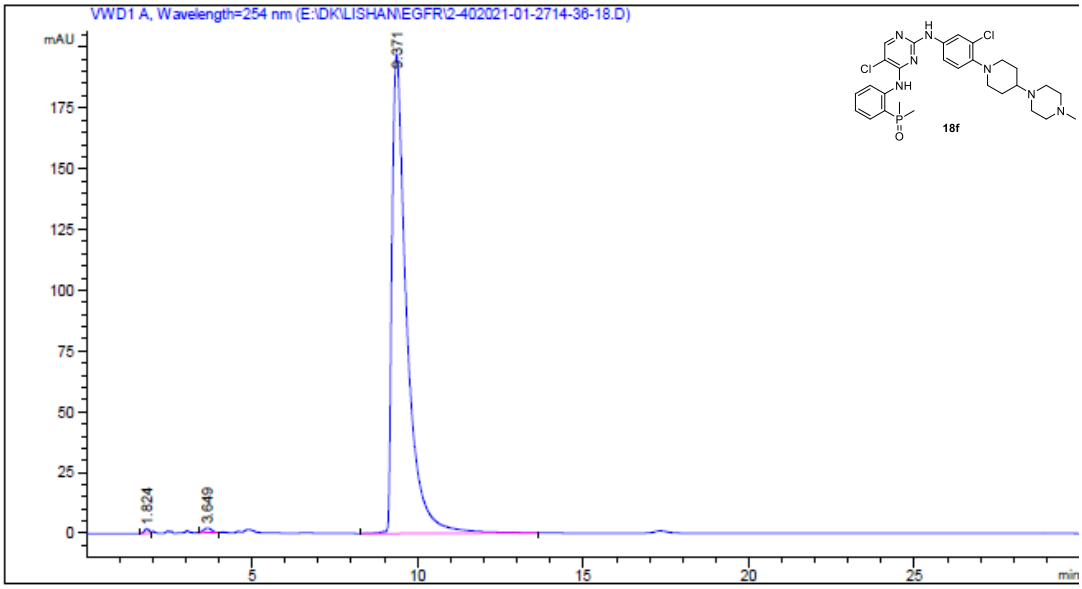
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.846 BV    0.1588   29.64580    2.66400   0.3024
   2   6.201 BB    0.3241   57.58860    2.76406   0.5874
   3   8.835 BB    0.4069 9716.12109  354.35516  99.1102

Totals :                   9803.35549  359.78322
```


18f
fl (ppm)


fl (ppm)

```
==================================================================
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   1
Injection Date  : 27/01/2021 14:37:02
                                                Inj Volume : 15.000 µl
Acq. Method     : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 27/01/2021 13:57:19 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 06/04/2021 10:23:35 by 系统
```



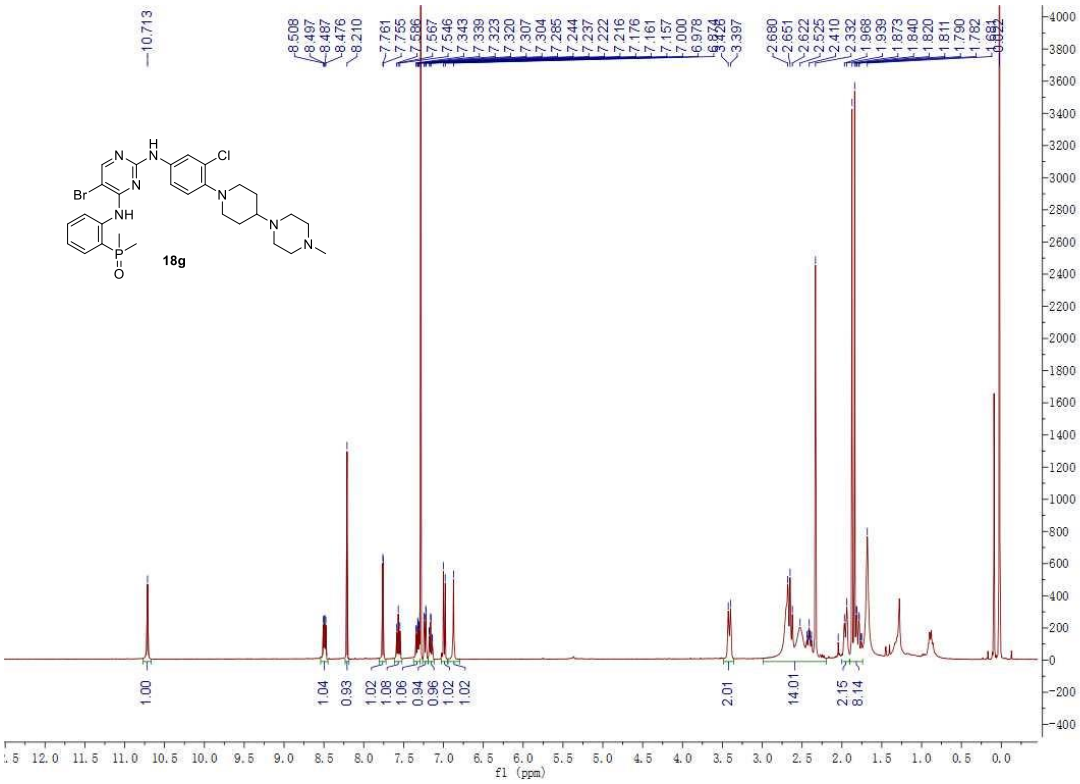

```
==================================================================
                        Area Percent Report
==================================================================

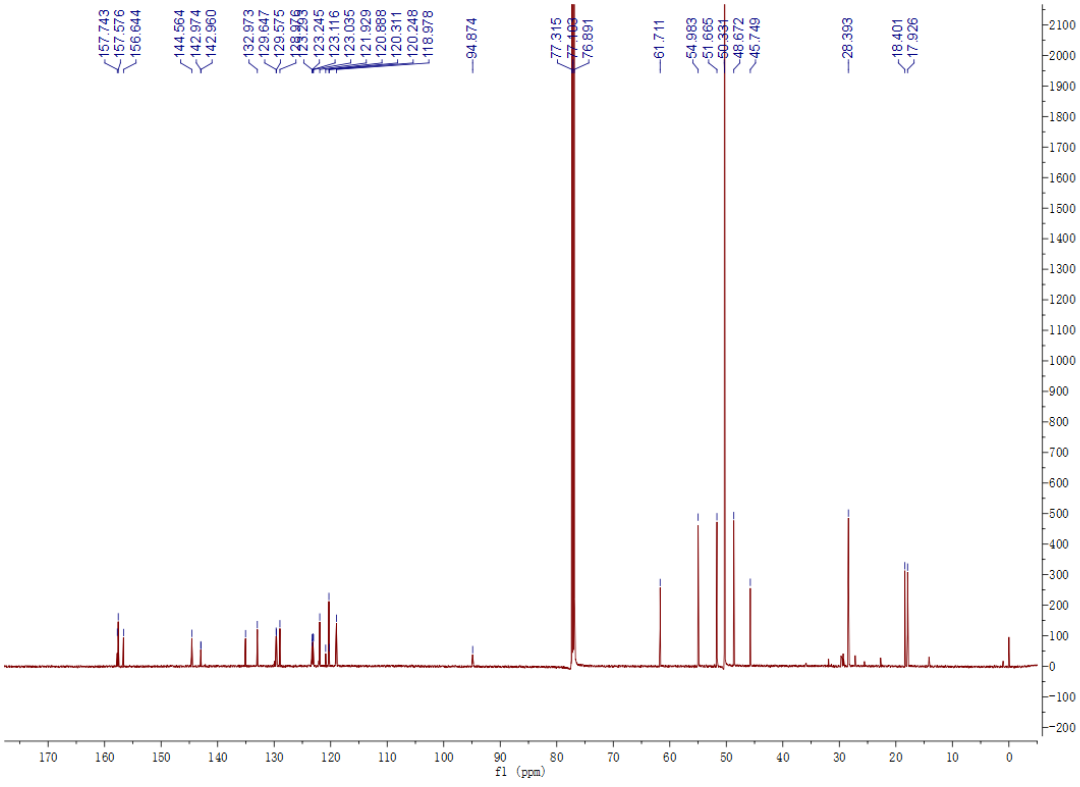
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:        :     20.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

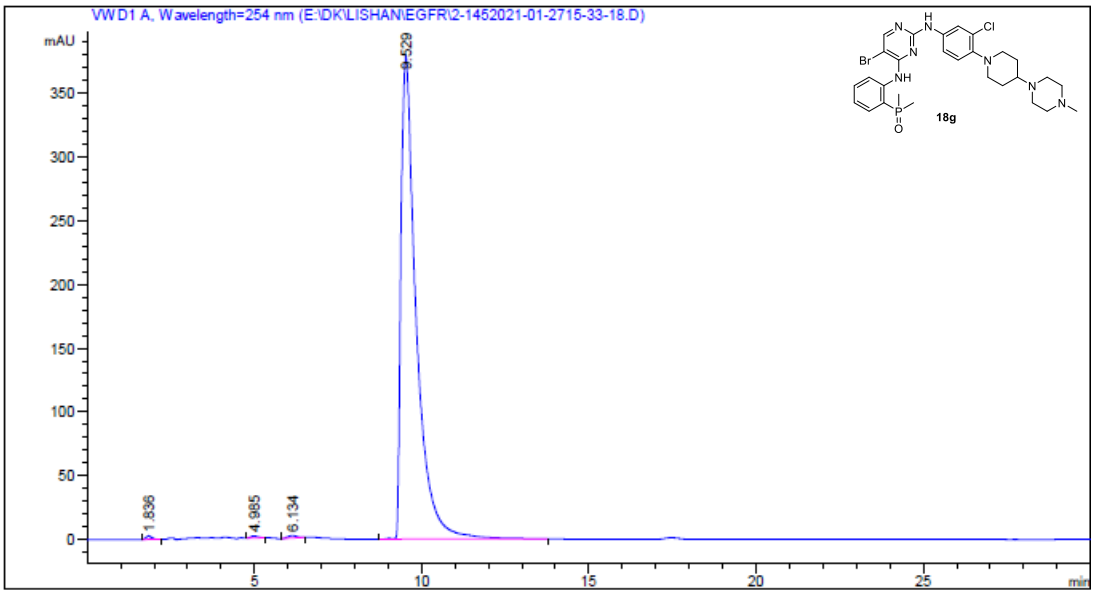
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.824 BV    0.1409   18.95191    1.99387   0.3095
   2   3.649 BB    0.2465   29.13995    1.84247   0.4759
   3   9.371 VB R  0.4543 6075.28857  196.73708  99.2146

Totals :                   6123.38043  200.57341
```


18g
10.713
8.508
8.497
8.487
8.476
8.210
7.761
7.755
7.586
7.567
7.546
7.343
7.339
7.323
7.320
7.307
7.304
7.285
7.244
7.237
7.222
7.216
7.176
7.161
7.157
7.000
6.978
3.428
3.397
2.680
2.651
2.622
2.525
2.410
2.332
1.988
1.939
1.873
1.840
1.820
1.811
1.790
1.782
1.681
0.822
1.00
1.04
0.93
1.02
1.08
1.06
0.94
0.96
1.02
1.02
2.01
14.01
2.15
8.14
f1 (ppm)


157.743
157.576
156.644
144.564
142.974
142.960
132.973
129.647
129.575
123.245
123.116
123.035
121.929
120.888
120.311
120.248
118.978
94.874
77.315
77.000
76.891
61.711
54.983
51.665
50.331
48.672
45.749
28.393
18.401
17.926
f1 (ppm)

```
================================================================================
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                          Location :   1
Injection Date  : 27/01/2021 15:34:07
                                                  Inj Volume : 15.000 µl
Acq. Method     : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 27/01/2021 13:57:19 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 06/04/2021 10:23:35 by 系统
```



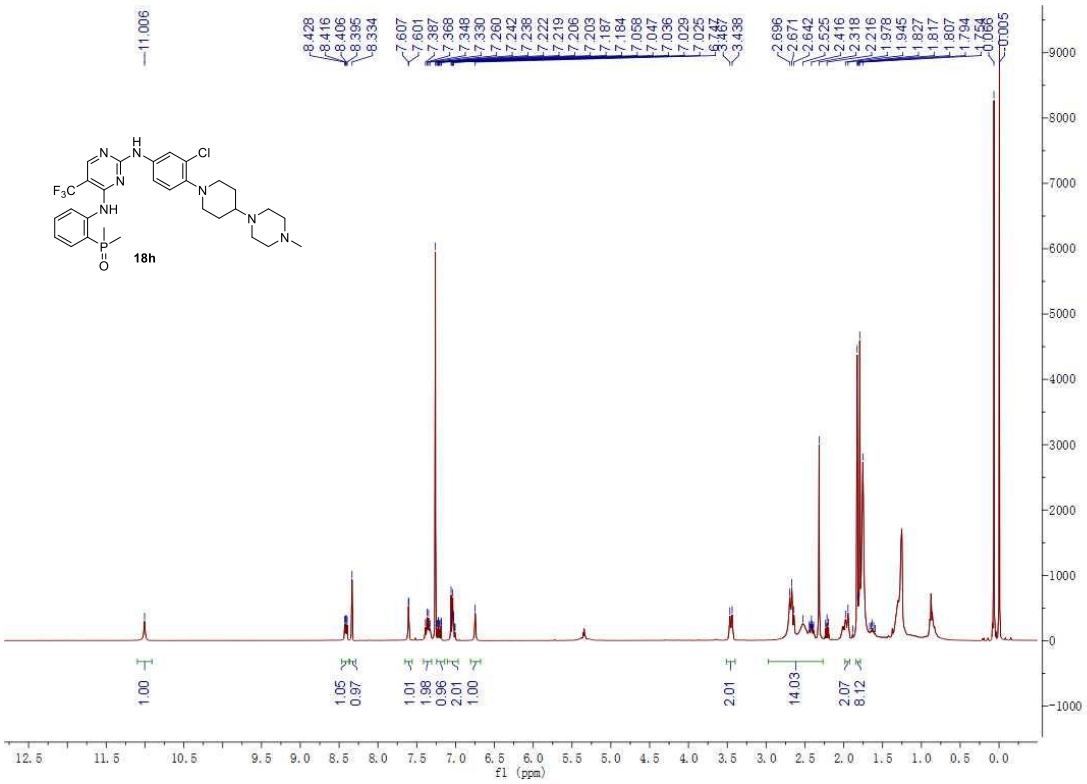

```
================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :     20.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

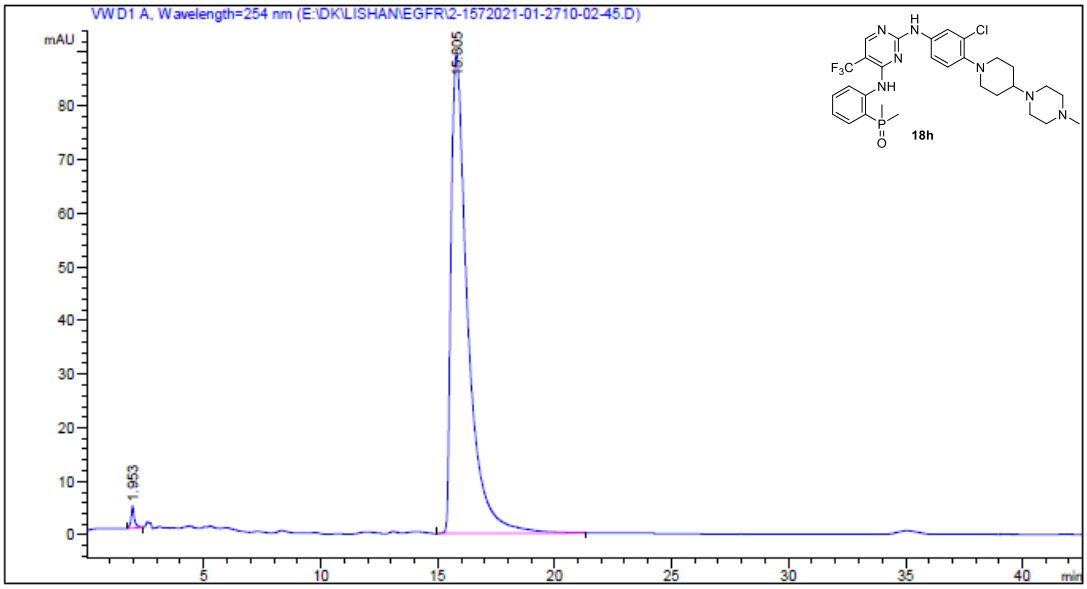

Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.836 BB    0.1559   31.56513    2.88000   0.2604
   2   4.985 VB    0.2246   24.88697    1.73206   0.2053
   3   6.134 BB    0.2928   38.20140    2.05600   0.3152
   4   9.529 VB R  0.4663 1.20263e4   378.90887  99.2191

Totals :                  1.21210e4   385.57694
```


18h
11.006
8.428
8.416
8.406
8.395
8.334
7.607
7.601
7.387
7.368
7.348
7.330
7.260
7.242
7.238
7.222
7.219
7.206
7.203
7.187
7.184
7.058
7.047
7.036
7.029
7.025
6.747
3.467
3.438
2.696
2.671
2.642
2.525
2.416
2.318
2.216
1.978
1.945
1.827
1.817
1.807
1.794
1.754
0.066
0.005
1.00
1.05
0.97
1.01
1.98
0.96
2.01
1.00
2.01
14.03
2.07
8.12
f1 (ppm)

```
==================================================================
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                          Location :   1
Injection Date  : 27/01/2021 10:03:29
                                                  Inj Volume : 15.000 µl
Acq. Method     : E:\DK\TL\方法75C-25A-60MIN.M
Last changed    : 27/01/2021 10:00:19 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 06/04/2021 10:23:35 by 系统
```



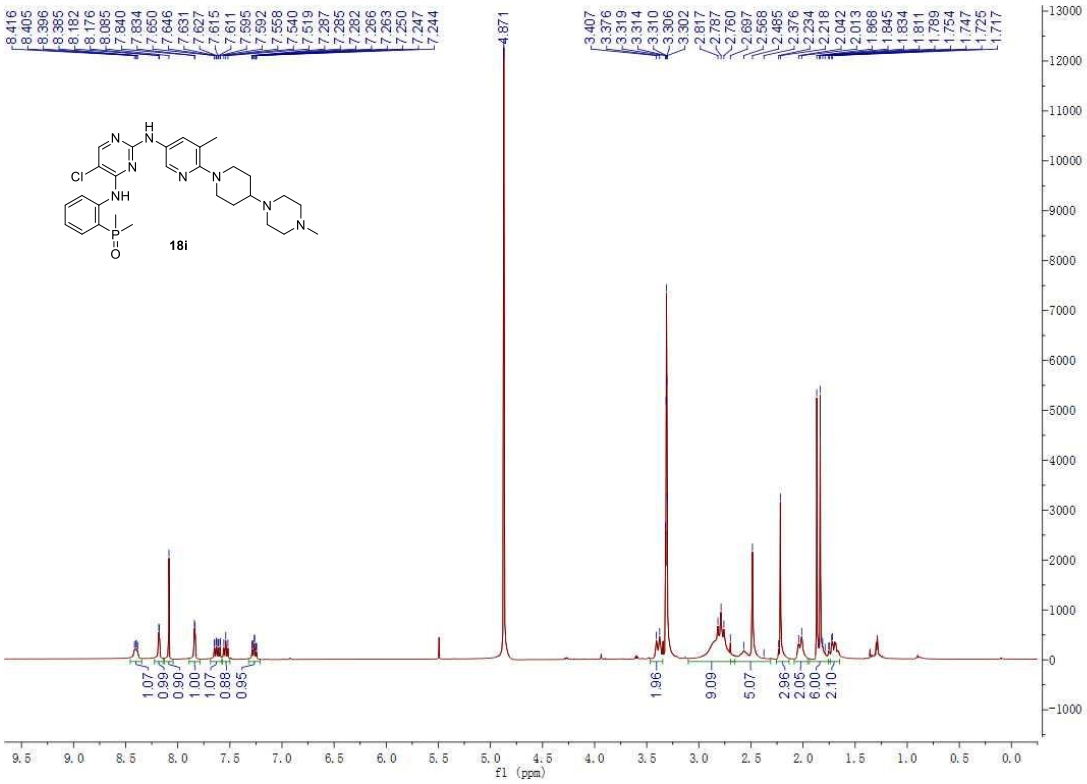

```
==================================================================
                        Area Percent Report
==================================================================

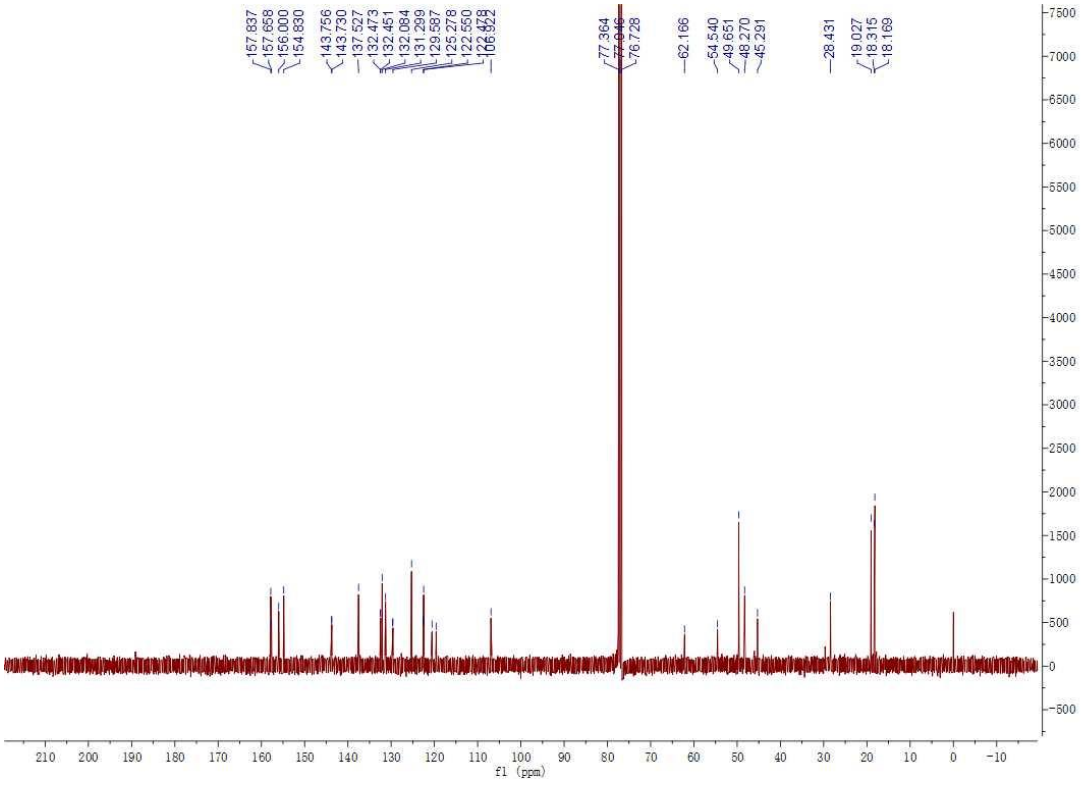
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :     20.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

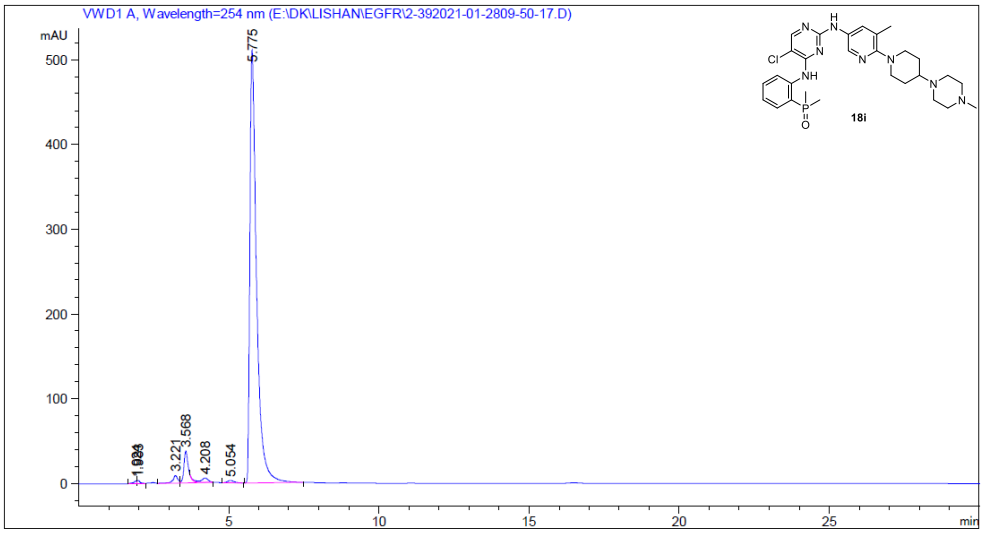
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.953 BB    0.1402   42.04947    4.25991   0.9390
   2  15.805 BB    0.7356 4436.13184   89.29628  99.0610

Totals :                  4478.18131   93.55619
```


18i



```
==========================================================================
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                          Location :   1
Injection Date  : 28/01/2021 09:51:02
                                                  Inj Volume : 15.000 µl
Acq. Method     : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 28/01/2021 09:44:07 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 06/04/2021 10:23:35 by 系统
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :     20.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

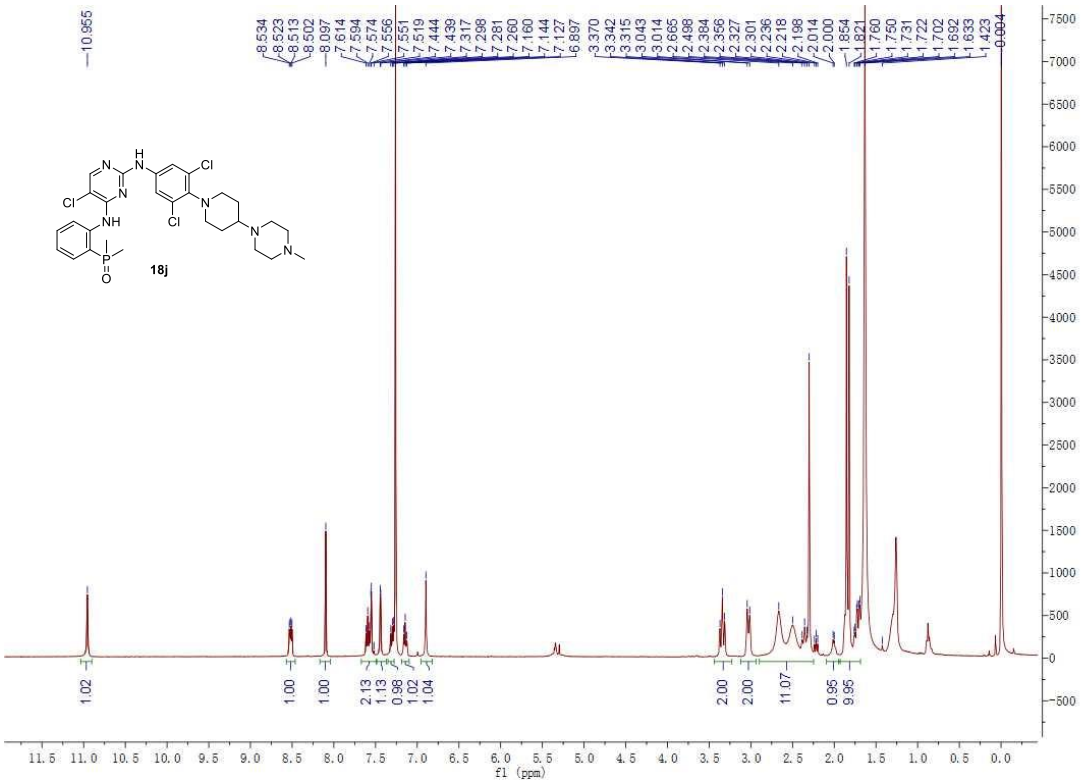
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.924 BV    0.0975   23.87279    3.18393   0.2767
   2   1.983 VB    0.0993   24.35547    3.58136   0.2823
   3   3.221 BV    0.1652  105.38245    8.95810   1.2214
   4   3.568 VV R  0.1560  397.07452   37.36277   4.6020
   5   4.208 VB E  0.2451   88.56344    5.11211   1.0264
   6   5.054 BB    0.2249   40.30576    2.67399   0.4671

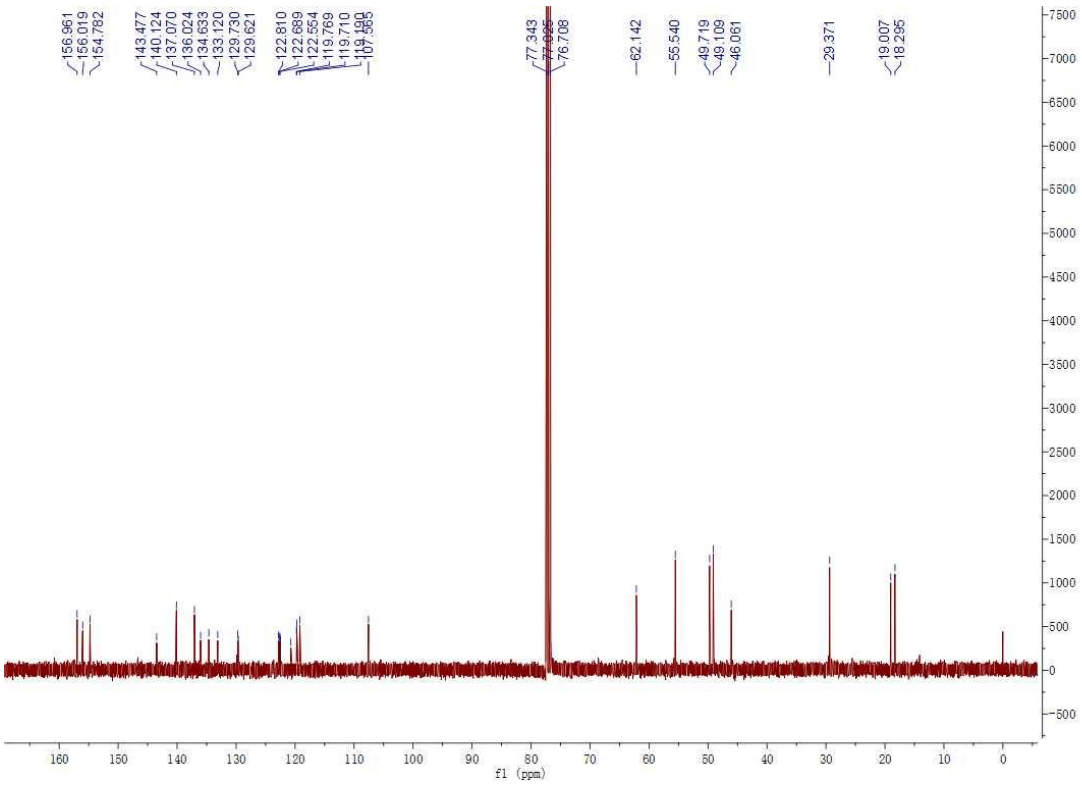
1260LC 22/04/2021 11:05:37 系统                                  Page   1 of 2
```

```
Data File E:\DK\LISHAN\EGFR\2-392021-01-2809-50-17.D
Sample Name: 2-39

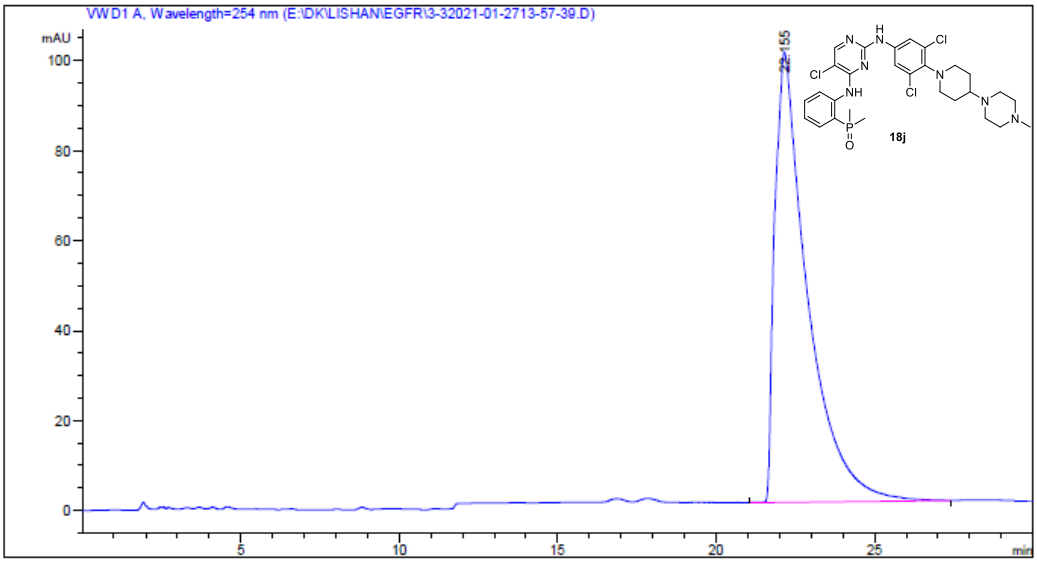
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   7   5.775 BB    0.2306 7948.70361  510.79099  92.1241

Totals :                  8628.25804  571.66324
```


18j
f1 (ppm)


f1 (ppm)

```
==================================================================
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   1
Injection Date  : 27/01/2021 13:58:23
                                                Inj Volume : 15.000 µl
Acq. Method     : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 27/01/2021 13:57:19 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 06/04/2021 10:23:35 by 系统
```



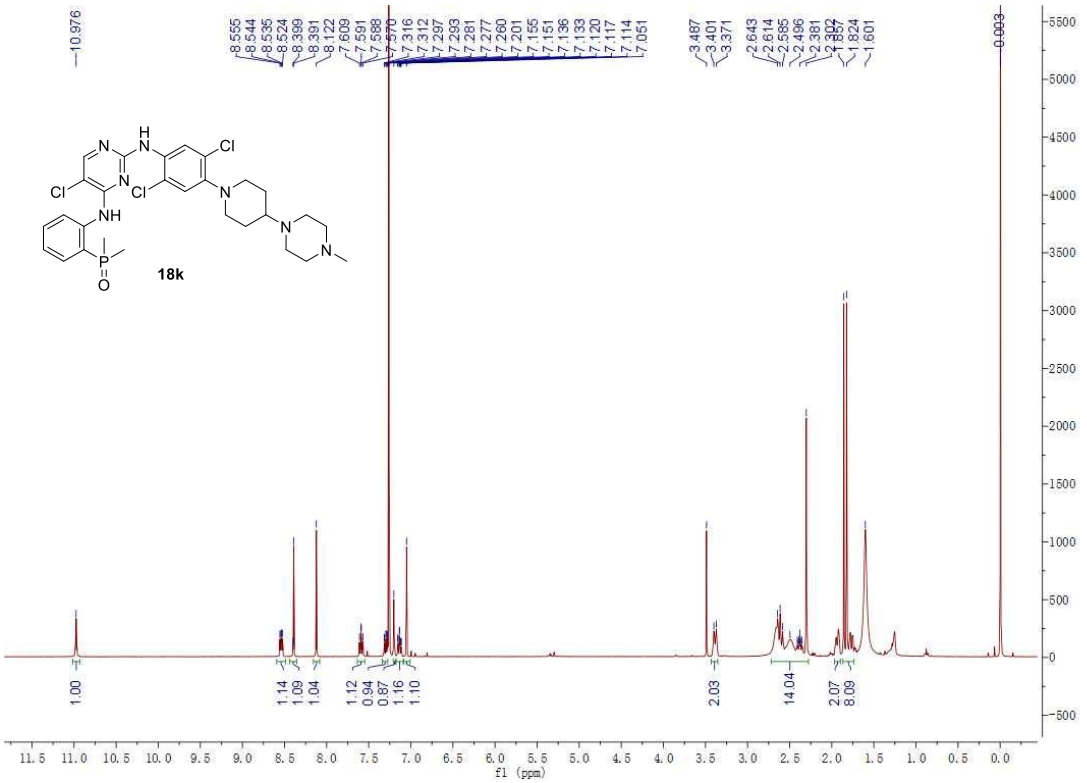

```
==================================================================
                      Area Percent Report
==================================================================

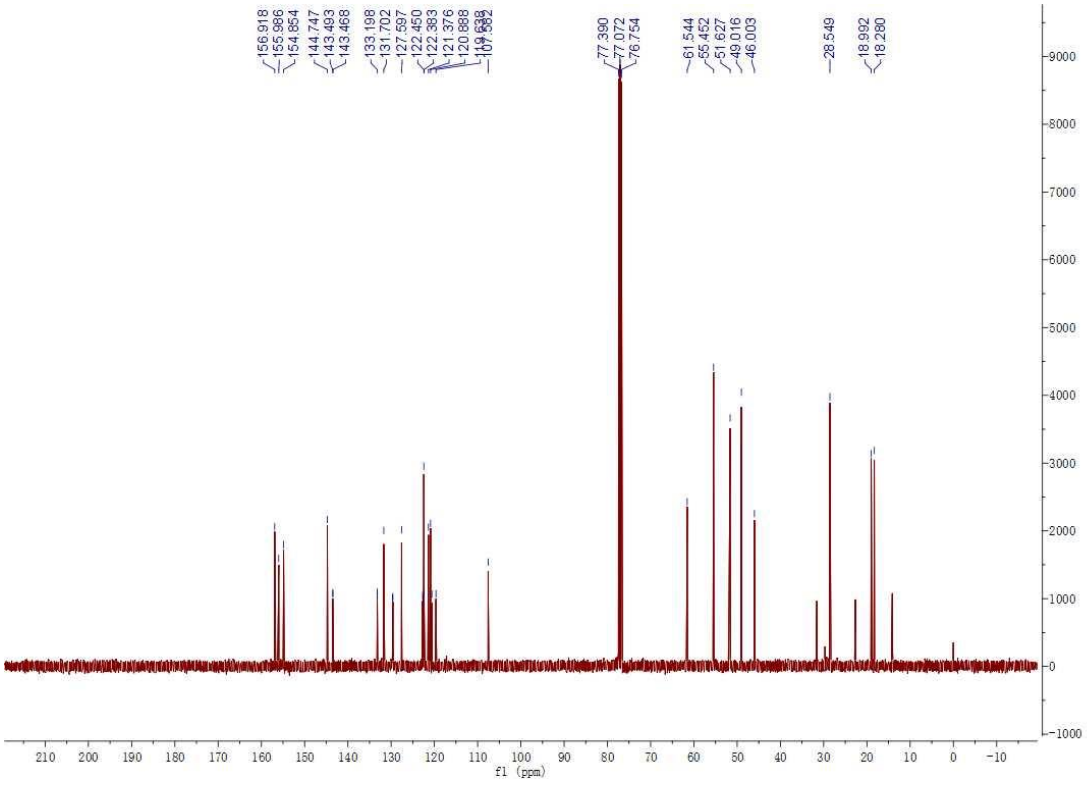
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :     20.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

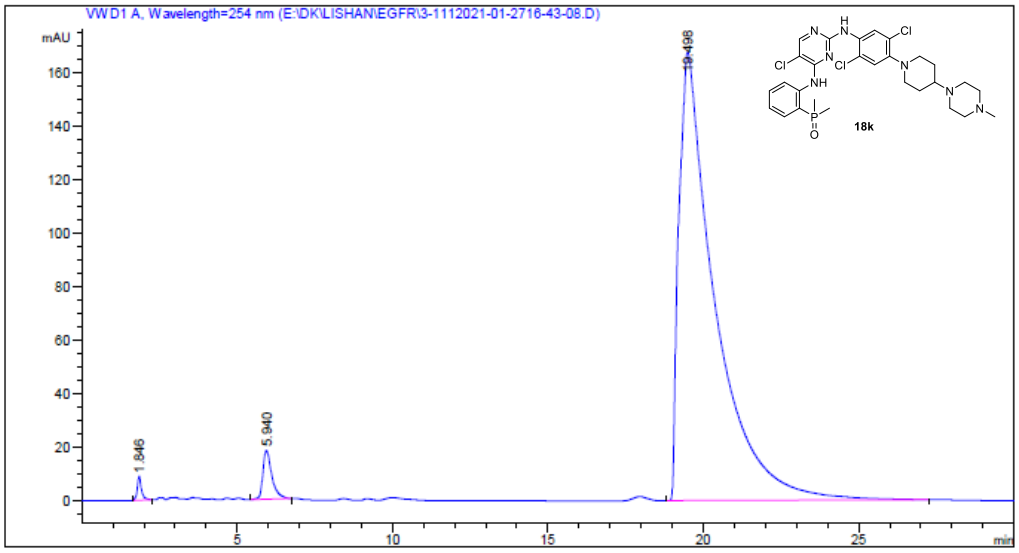
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  22.155 BB    0.9403 7156.07959   99.95277 100.0000

Totals :                  7156.07959   99.95277
```


18k



```
================================================================================
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                          Location :   1
Injection Date  : 27/01/2021 16:43:53
                                                  Inj Volume : 15.000 µl
Acq. Method     : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 27/01/2021 13:57:19 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法80C-20A-30min-1u.M
Last changed    : 06/04/2021 10:23:35 by 系统
```




```
================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :     20.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.846 BB    0.1367   83.74465    8.98485   0.6020
   2   5.940 BB    0.3020  370.19730   18.30449   2.6613
   3  19.498 BB    1.0413 1.34565e4   167.66962  96.7367

Totals :                  1.39104e4   194.95896
```

References

(1) Chen, Z.; Tong, L. J.; Tang, B. Y.; Liu, H. Y.; Wang, X.; Zhang, T.; Cao, X. W.; Chen, Y.; Li, H. L.; Qian, X. H.; Xu, Y. F.; Xie, H.; Ding, J., C11, a novel fibroblast growth factor receptor 1 (FGFR1) inhibitor, suppresses breast cancer metastasis and angiogenesis. *Acta Pharmacol Sin* **2019**, *40* (6): 823-832.

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(4) Minor, W.; Cymborowski, M.; Otwinowski, Z.; Chruszcz, M., HKL-3000: the integration of data reduction and structure solution--from diffraction images to an initial model in minutes. *Acta Crystallogr D Biol Crystallogr* **2006**, *62* (Pt 8): 859-866.

(5) Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H., PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* **2010**, *66* (Pt 2): 213-221.

(6) Emsley, P.; Cowtan, K., Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* **2004**, *60* (Pt 12 Pt 1): 2126-2132.