Supplementary Information

Practical Synthesis of Immucillins BCX-1777 and BCX-4430

Minjie Zhang, [†a] Fei Xue,[†a] Jingdan Ou,[a] Yuan Huang,[a] Fei Lu,[a] Bingqing Zhou,[a] Zhibing Zheng,[b] Xiao-Yu Liu,[a] Wu Zhong,*[b] and Yong Qin*[a]

[a]*Key Laboratory of Drug-Targeting and Drug Delivery System of the Education Ministry, Sichuan Engineering Laboratory for Plant-Sourced Drug and Sichuan Research Center for Drug Precision Industrial Technology, West China School of Pharmacy, Sichuan University, Chengdu 610041, China.*

[b] *National Engineering Research Center for the Emergence Drugs, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China.*

21 Dec 2020

Note added after first publication:
This Supplementary Information file replaces that originally published on 09 Oct 2020. There was an error on page S8, where compound 13 was written as compound 14. This has now been corrected, and the text now reads, "Then a solution of nitrone **13** (100.0 g, 0.240 mol, 1.00 equiv) in anhydrous MTBE (500 mL) was added dropwise, and the resulting mixture was stirred for 15 h at –20 °C."

Table of Contents

1. General Information…… 3
2. Experimental Procedures and Characterization Data……...4
3. References…….....29
4. NMR Spectra…….....30

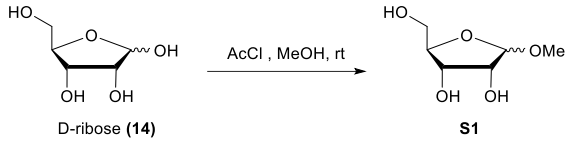
1. General Information

All reactions that require anhydrous conditions were performed in flame-dried glassware under Ar atmosphere and all reagents were purchased from commercial suppliers without further purification. Solvent purification was conducted according to Purification of Laboratory Chemicals 2nd edn (Perrin, D. D., Armarego, W. L. F. and Perrin, D. R., Pergamon Press: Oxford, 1980). Reactions were monitored by thin layer chromatography (TLC, 0.2 mm, HSGF254) supplied by Yantai Chemicals (China). Visualization was accomplished with UV light, exposure to iodine, stained with basic solution of $KMnO_4$. The products were purified by flash column chromatography on silica gel (200 – 300 meshes) from the Anhui Liangchen Silicon Material Company (China). ^1H NMR and ^{13}C NMR spectra were recorded on Varian INOVA-400/54 or Agilent DD2-600/54 instruments and calibrated by using deuterated chloroform ($CDCl_3$, ^1H NMR: δ = 7.260, ^{13}C NMR δ = 77.0), deuterated methanol (Methanol-d_4, ^1H NMR: δ = 3.35, ^{13}C NMR: δ = 49.8), deuterated water (D_2O, ^1H NMR: δ = 4.79), deuterated DMSO: (DMSO-d_6, ^1H NMR δ = 2.50, ^{13}C NMR = 39.50). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, br = broad, tt = triple triple, m = multiplet, and coupling constants (*J*) are reported in Hertz (Hz). Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum Two FT-IR spectrometer. High-resolution mass spectra (HRMS) were recorded on Bruker Apex IV FTMS or Agilent LC-MSD TOF ESI mass spectrometers. The specific optical rotation was obtained from Rudolph Research Analytical Autopol VI automatic polarimeter. HPLC analysis was performed on HP Agilent 1260 apparatus (ZORBAX SB-C18 Column, 4.6 × 150 mm, 5 μm).

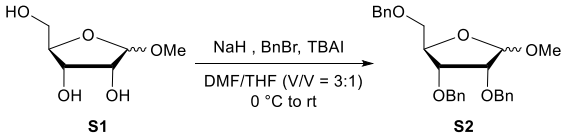
2. Experimental Procedures and Characterization Data

1) Preparation of nitrone 13

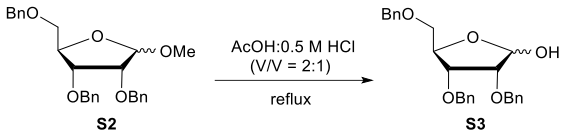
*Nitrone **13** was prepared according to the reported procedure with slight modifications.[1] The procedures are shown below:*



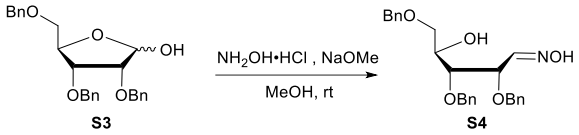
Synthesis of Compound S1[1a]**:** D-Ribose (**14**) (500.0 g, 3.33 mol, 1.00 equiv) was added to a solution of acetyl chloride (71.0 mL, 1.00 mol, 0.30 equiv) in MeOH (2500 mL) at 25 °C. The resulting mixture was stirred at room temperature for 2 h before being quenched by addition of $NaHCO_3$ (90.0 g). Then the mixture was filtered and the filtrate was concentrated under reduced pressure to afford the crude compound **S1** as a yellow oil, which was directly used in next step without further purification.



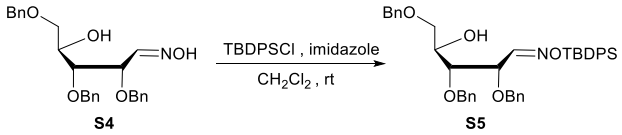
Synthesis of Compound S2[1a]**:** To a vigorously stirred mixture of NaH (60% suspension in mineral oil, 440.0 g, 11.0 mol, 3.30 equiv) in anhydrous DMF (1250 mL) and THF (1250 mL) at 0 °C, was slowly added a solution of the crude **S1** in anhydrous DMF (2500 mL) under Ar atmosphere. After being stirred at the same temperature for 30 min, Bu_4NI (18.5 g, 50.0 mmol, 0.015 equiv) was added in one portion. Then, a small fraction (approximately 5 mL) of the total volume of BnBr (1306 mL, 11.0 mol, 3.30 equiv) was added dropwise first to prevent the reaction from becoming violent. And the rest of the total volume of BnBr was added slowly afterwards. The resulting mixture was stirred for 12 h at room temperature before being quenched carefully with saturated NH_4Cl (3000 mL) at 0 °C. The layers were separated and the aqueous layer was extracted with EtOAc (1000 mL × 3). The combined organic layers were washed with water (1500 mL × 1) and brine (1500 mL × 1), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product **S2**, which was subjected to the next step without further purification.



Synthesis of Compound S3[1a]**:** The crude compound **S2** resulting from the last step was treated with a solution of 0.5 M HCl (1200 mL) in AcOH (2400 mL) and heated under reflux for 5 h. Then the mixture was cooled to 0 °C and the pH value was adjusted to 8 by 12 M aqueous NaOH solution under stirring. The layers were separated, and the aqueous layer was extracted with EtOAc (2000 mL × 1, 1500 mL × 1). The combined organic layers were washed with brine (1500 mL × 1), dried over anhydrous Na_2SO_4 and filtered. The solvent was removed *in vacuo* to afford crude compound **S3**, which was used in the next step without purification.

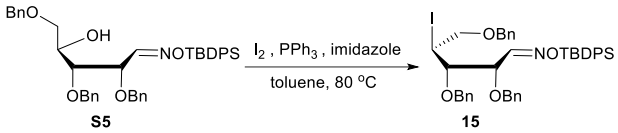


Synthesis of Compound S4[1b]**:** The crude compound **S3** resulting from the last step was dissolved in methanol (2500 mL). Hydroxylamine hydrochloride (1619 g, 23.3 mol, 7.00 equiv) was added followed by slow addition of sodium methoxide (2340 mL, 5 M in MeOH, 11.7 mol, 3.50 equiv). After being stirred for 3 h at room temperature, the solvent of the above mixture was removed under reduced pressure. The residue was dissolved in DCM (5000 mL) and washed with water (2000 mL × 1). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford the crude compound **S4**, which was used directly in the next step without further purification.

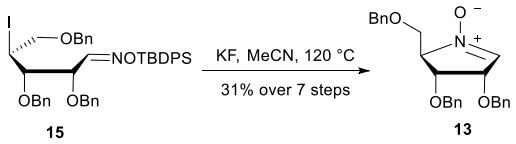


Synthesis of Compound S5[1b]: The crude compound **S4** derived from the last step was dissolved in anhydrous DCM (2500 mL). Imidazole (340.4 g, 5.00 mol, 1.50 equiv) and TBDPSCl (1082 mL, 4.16 mol, 1.25 equiv) were added successively under stirring. The resulting mixture was stirred for 1.5 h at room temperature before being quenched with water (1000 mL). The layers were separated, and the organic layer was washed with brine (1000 mL × 1), dried over anhydrous Na_2SO_4, filtered and the concentrated *in vacuo* to give crude compound **S5**, which was used

directly in the next step without further purification.



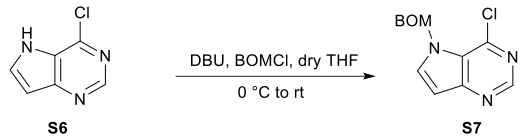
Synthesis of Compound 15[1b]: To a stirred solution of crude **S5** in anhydrous toluene (2500 mL), imidazole (226.7 g, 3.33 mol, 1.00 equiv), triphenylphosphine (873.4 g, 3.33 mol, 1.00 equiv) and iodine (675.1 g, 2.66 mol, 0.80 equiv) were added successively. The resulting brown mixture was heated at 80 °C for 2 h. After completion of the reaction, the mixture was slowly cooled to 0 °C. Then 30% aqueous solution of H_2O_2 (100 mL) was added dropwisely. The resulting mixture was stirred for 5 min at 0 °C before saturated aqueous Na_2SO_3 solution (3000 mL) was added. The organic phase was separated, dried over anhydrous Na_2SO_4 and filtered, then anhydrous $MgCl_2$ (952.0 g, 10.0 mol, 3.00 equiv) was added.[2] The resulting mixture was stirred for 24 h at room temperature and filtered by Celite. The filtrate was concentrated under reduced pressure to give crude compound **15**, which was used in the next step directly without purification.



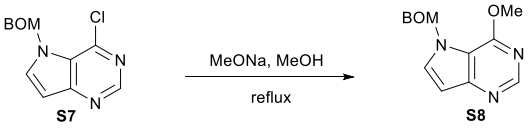
Synthesis of Compound 13: To a solution of the crude compound **15** in anhydrous MeCN (2500 mL), KF (581.0 g, 1.00 mol, 3.00 equiv) was added in one portion under stirring. The resulting mixture was heated at 120 °C for 10 h. Then the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (EA / petroleum ether 1:1 to 2:1, v/v) to afford nitrone **13** (431.0 g, 31 % yield over 7 steps) as a pale-yellow oil. **Optical rotation**: $[\alpha]_D^{25}$ = +115 (*c* = 0.8, $CHCl_3$). **^{1}H NMR**: (400 MHz, $CDCl_3$) δ 7.38 – 7.22 (m, 13H), 7.19 (d, *J* = 4.8 Hz, 2H), 6.92 (s, 1H), 4.66 – 4.56 (m, 4H), 4.53 (dd, *J* = 8.0, 2.0 Hz, 2H), 4.44 (t, *J* = 3.6 Hz, 1H), 4.39 (d, *J* = 8.0 Hz, 1H), 4.12 (dd, *J* = 7.2, 1.6 Hz, 1H), 4.08 (brs, 1H), 3.60 (dd, *J* = 7.2, 1.2 Hz, 1H). **^{13}C NMR**: (100 MHz, $CDCl_3$) δ 137.4, 137.2, 137.0, 133.2, 128.4, 128.4, 128.3, 128.0, 128.0, 127.9, 127.7, 127.5, 76.2, 75.2, 74.3, 73.2, 72.2, 71.9, 64.6. *The ^{1}H and ^{13}C NMR data were consistent with those reported in the literature.*[1b] **IR** (neat): ν_{max} = 3029, 2921, 2863, 1566, 1453, 1260, 1108, 1026, 801, 727, 697 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{26}H_{27}NO_4$ $[M+H]^+$ 418.2013, found 418.2018.

2) Preparation of 9-deazapurine derivative 16.

*Compound **16** was prepared according the reported procedure with slight modifications.[3] The procedures are shown below:*



Synthesis of Compound S7: To a solution of 4-chloro-*5H*-pyrrolo[*3,2-d*]pyrimidine (**S6**) (200 g, 1.31 mol, 1.00 equiv) in anhydrous THF (1000 mL) was added DBU (486 mL, 2.60 mol, 2.00 equiv) at 0 °C. After being stirred at the same temperature for 10 min, BOMCl (361 mL, 2.60 mol, 2.00 equiv) was added dropwise. Then the resulting mixture was allowed to warm to room temperature and stirred for 15 h. The solvent was removed under reduced pressure to give crude compound **S7**, which was used directly in the next step without purification.

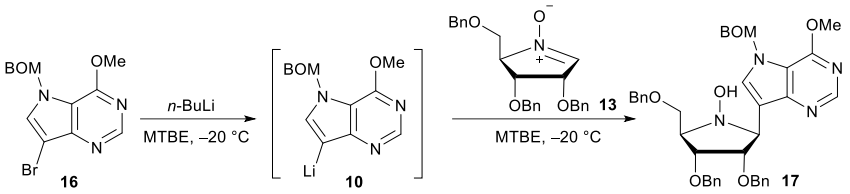


Synthesis of Compound S8: The crude compound **S7** resulting from the last step was dissolved in MeOH (1000 mL). NaOMe (520 mL, 2.60 mol, 5 M in MeOH, 2.00 equiv) was added slowly under stirring. The reaction was heated under reflux for 4 h. Then the solvent was evaporated under reduced pressure to give crude compound **S8**, which was used directly in the next step without purification.

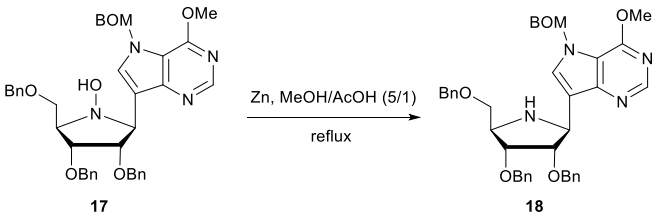
Synthesis of Compound 16: The crude compound **S8** resulting from the last step was dissolved in anhydrous DMF (1000 mL). NBS (231 g, 1.30 mol, 1.00 equiv) was added in small portions at 0 °C under stirring. The resulting mixture was stirred for 30 min at the same temperature before a saturated aqueous solution of $Na_2S_2O_3$ (1000 mL) was added. Then the resulting precipitation was filtered. The filter cake was washed with water (50 mL × 3) and collected. Next, the light brown solid was dissolved in EA (1000 mL); the solution was washed successively with water (500 mL × 1), saturated aqueous solution of $NaHCO_3$ (500 mL × 2) and brine (500 mL × 1). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The solid residue was

purified by recrystallization from an EA-petroleum ether mixture (v/v = 1:3, 600 mL), affording compound **16** as a white solid (339 g, 75% yield over 3 steps). **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.44 (s, 1H), 7.34 – 7.22 (m, 5H), 5.71 (s, 2H), 4.48 (s, 2H), 4.11 (s, 3H). **^{13}C NMR:** (100 MHz, $CDCl_3$) δ 156.3, 151.0, 148.4, 136.5, 131.5, 128.5, 128.1, 127.6, 115.5, 92.4, 70.4, 53.8. *The ^{1}H and ^{13}C NMR data were consistent with those reported in the literature.*[3a] **IR** (neat): ν_{max} = 3077, 3003, 2945, 1601, 1539, 1486, 1355, 1089, 1074, 778, 733.696, 655 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{15}H_{15}BrN_3O_2$ $[M+H]^+$ 348.0342, found 348.0345.

3) Synthesis of BCX-1777 (1)

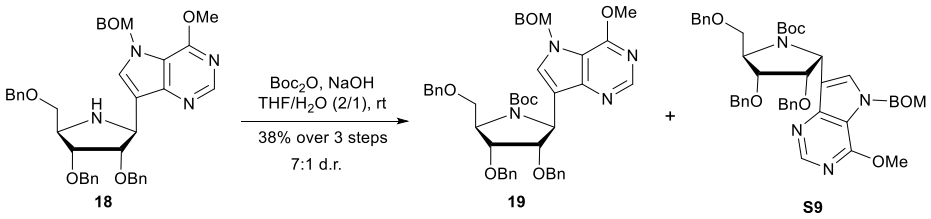


Synthesis of Compound 17: Under an argon atmosphere, to a solution of compound **16** (150.4 g, 0.432 mol, 1.80 equiv) in anhydrous MTBE (3000 mL), *n*-BuLi (2.5 M in *n*-hexane, 207 mL, 0.518 mol, 2.16 equiv,) was added dropwise at −20 °C and the mixture was allowed to stir at −20 °C for an additional 30 min. Then a solution of nitrone **13** (100.0 g, 0.240 mol, 1.00 equiv) in anhydrous MTBE (500 mL) was added dropwise, and the resulting mixture was stirred for 15 h at −20 °C. After completion of the reaction, it was quenched with a saturated aqueous solution of NH_4Cl (2000 mL) and warmed to room temperature. The resulting mixture was diluted with EA (3000 mL); the layers were separated and the aqueous layer was extracted with EA (1000 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4 and filtered, then the solvent was removed *in vacuo* to give crude compound **17**, which was used directly in the next step without further purification.



Synthesis of Compound 18: The crude compound **17** resulting from the last step was dissolved in a mixture of MeOH and AcOH (V/V = 5:1, 400 mL). Zinc powder (312 g, 4.80 mol, 20.0 equiv) was added in one portion under vigorously stirring. The reaction was heated under reflux for 2 h. After being cooled to room temperature, the mixture was filtered by Celite; the filtrate was

concentrated *in vacuo* to give crude compound **18**, which was immediately used in the next step without purification.



Synthesis of Compound 19: The crude compound from the last step was dissolved in a mixture of THF and water (V/V = 2:1, 2100 mL), of which the pH was adjusted to 9-10 with 3 M aqueous NaOH at 0 °C under stirring. Boc_2O (105 g, 0.480 mmol, 2.00 equiv) was then added and the reaction mixture was stirred at room temperature for 15 h. The resulting mixture was filtered by Celite; the filtrate was evaporated under reduced pressure to remove volatiles. Next, the residue was diluted with EA (1000 mL) and the layers were separated. The aqueous layer was extracted with EA (300 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The dr value of the products was determined by HPLC analysis of the crude mixture (dr = 7:1). The residue was purified by silica gel flash column chromatography (EA / petroleum ether 1:5, v/v) to afford a pair of separable diastereoisomers **19** (70.3 g, 38 % yield in 3 steps, a yellow oil) and **S9** (9.2 g, 5% yield in 3 steps, a yellow oil). Major isomer **19**: **Optical rotation**: $[\alpha]_D^{25}$ = +56.2 (*c* = 0.55, $CHCl_3$). **^{1}H NMR**: (400 MHz, $CDCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 8.51 (s, 1H), 7.57 – 7.36 (m, 3H), 7.32 – 7.15 (m, 18H), 5.60 – 5.11 (m, 3H), 4.96 – 4.80 (m, 2H), 4.52 – 4.40 (m, 4H), 4.31 – 4.00 (m, 9H), 3.70 (m, 1H), 1.45 (s, 4H), 1.24 (s, 5H). **^{13}C NMR** (100 MHz, $CDCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 156.0, 155.3, 155.0, 149.6, 149.6, 148.9, 138.3, 138.0, 137.1, 137.0, 132.3, 132.2, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 117.1, 116.1, 115.7, 115.5, 79.9, 79.5, 79.0, 77.6, 76.2, 73.3, 73.3, 71.4, 71.2, 71.0, 69.7, 68.0, 67.6, 60.7, 58.2, 58.1, 53.4, 28.4, 28.3. **IR:** (neat) ν_{max} = 3035, 2963, 2927, 2863, 1695, 1609, 1391, 1358, 1259, 1089, 1018, 798, 737, 697 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{46}H_{51}N_4O_7$ $[M+H]^+$ 771.3752, found 771.3759. Minor isomer **S9: Optical rotation**: $[\alpha]_D^{25}$ = +21.7 (*c* = 0.35, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 8.55 (s, 1H), 7.62 (s, 1H), 7.42 – 7.08 (m, 18H), 7.05 – 6.97 (m, 2H), 5.73 (d, *J* = 8.0 Hz, 1H), 5.63 (d, *J* = 10.8 Hz, 1H), 5.37 (d, *J* = 10.8 Hz, 1H), 4.75 – 4.60 (m, 2H), 4.57 – 4.40 (m, 5H), 4.39 – 4.34 (m, 1H), 4.27 – 4.16 (m, 3H), 4.09 (s, 2H), 4.06 (s, 1H), 3.71 – 3.61 (m, 2H), 1.32 (s, 2H), 1.01 (s, 7H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 156.1, 154.3, 151.2, 149.6, 138.3, 138.3, 138.2, 137.0, 134.2, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 127.5, 127.5,

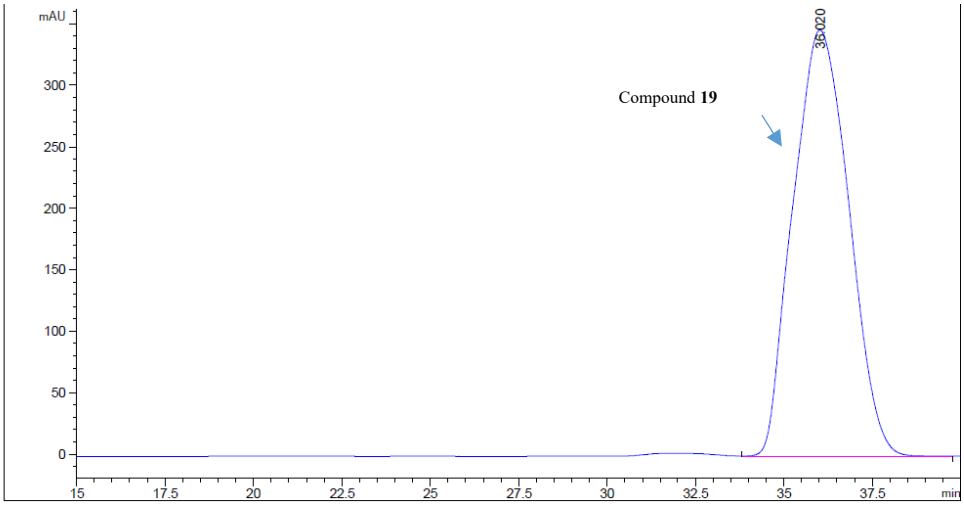
127.4, 127.3, 127.2,116.0, 114.5, 80.1, 79.5, 78.7, 73.3, 72.3, 72.0, 69.7, 68.7, 62.3, 53.4, 53.1, 28.4, 28.1. **IR:** (neat) ν_{max} = 3032, 2929, 2863, 1689, 1609, 1536, 1454, 1381, 1363, 1107, 1069, 772, 737, 697 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{46}H_{51}N_4O_7$ $[M+H]^+$ 771.3752, found 771.3759.

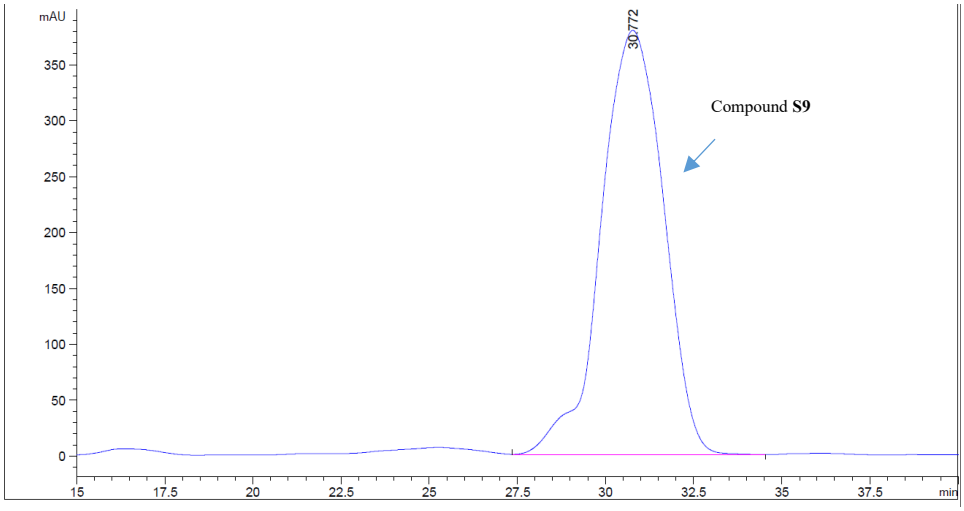
HPLC Analysis for measuring the dr value of the above reaction

Run Information:

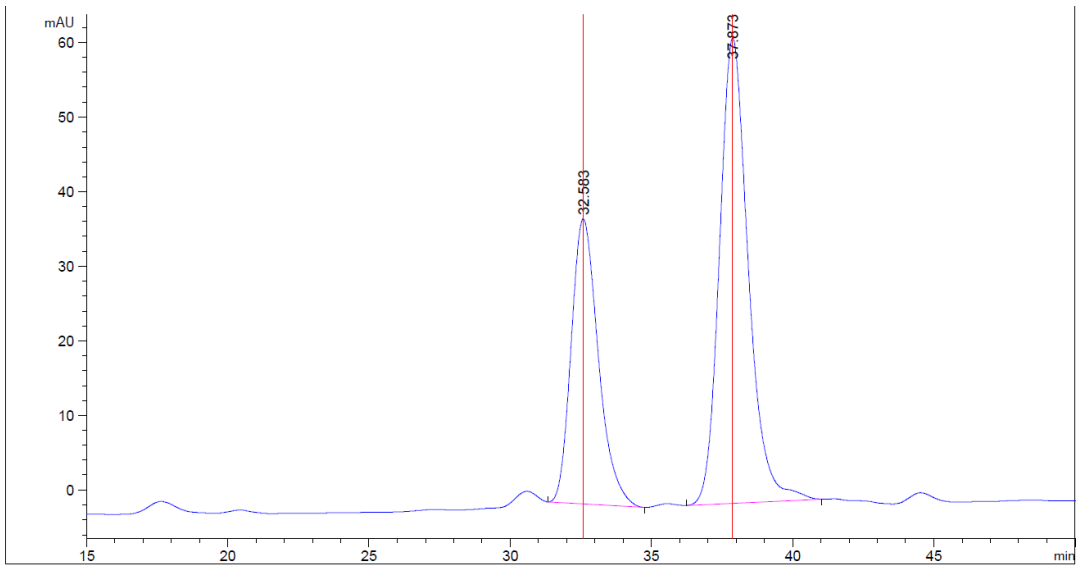
Analytical HPLC: Agilent 1260 Series HPLC; Column: ZORBAX SB-C18 (4.6 * 150 mm, 5 μm); Solvent: H_2O/CH_3OH (the gradient of CH_3OH is 85% during 0-3 min, from 85% to 80% during 3-8 min, 80% during 8-15 min, from 80% to 85% during 15-45 min; 85% during 45-60 min); Flow = 0.8 mL/min; Detected by UV at 254 nm; Retention time for the major isomer **19** and minor isomer **S9** in HPLC: 36.020 min (compound **19**, major), 30.772 min (compound **S9**, minor).

The HPLC spectra of 19 and S9:

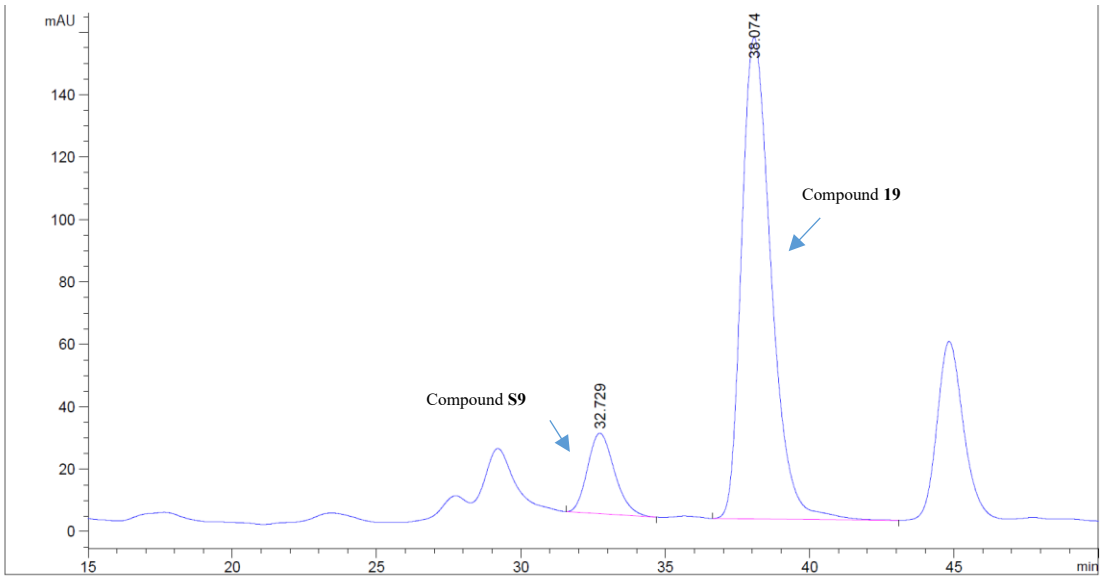




The HPLC analysis of the mixture of 19 and S9:



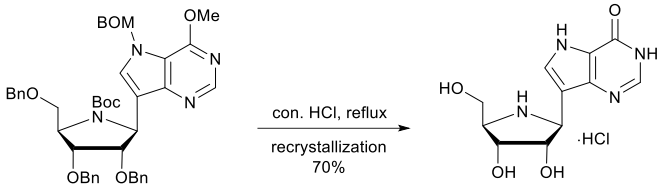
The HPLC analysis of the crude mixture resulting from the above reaction:



Peak information (wavelength: 254 nm):

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	32.729	BB	1.0031	1677.25562	25.84555	13.2399
2	38.074	BB	1.1129	1.09909e4	154.32454	86.7601

dr value = 7:1

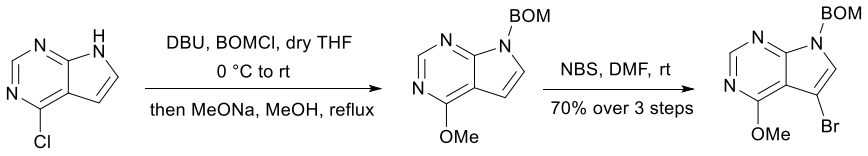


19 **BCX-1777 (1)**

Synthesis of BCX-1777 (**1**)**:** To a solution of compound **19** (60.0 g, 77.8 mmol, 1.00 equiv) in MeOH (50 mL) was added concentrated hydrochloric acid (12 M, 300 mL). The reaction mixture was heated under reflux for 72 h. After the completion of reaction, the solvent was removed under reduced pressure to afford a brownish red solid. This solid was dissolved in water (90 mL), then ethanol (500 mL) was added slowly. The mixture was cooled to 0 ºC and maintained at this temperature for 4 h. The resulting solid was obtained by filtration, and it was decolorized by ion-exchange resin (Na^+ form) and then purified by recrystallization from a water-ethanol mixture, affording BCX-1777 (**1**) (16.5 g, 70% yield) as a white solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.38 (d, J = 2.8 Hz, 1H), 12.18 (s, 1H), 10.47 (s, 1H), 8.45 (s, 1H), 7.89 (s, 1H), 7.65 (d, J = 2.8 Hz, 1H), 5.61 (d, J = 6.0 Hz, 1H), 5.51 (d, J = 5.2 Hz, 1H), 5.46 (t, J = 5.2 Hz, 1H), 4.63 (d, J = 7.6 Hz, 1H), 4.49 – 4.35 (m, 1H), 4.17 (q, J = 4.8 Hz, 1H), 3.73 (t, J = 4.8 Hz, 2H), 3.48 (q, J = 4.4 Hz, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6) δ 153.5, 143.0, 142.2, 127.5, 118.0, 109.3, 74.0, 70.3, 65.2, 58.4, 56.5. *The ^{1}H and ^{13}C NMR data were consistent with those reported in the literature.*[4] **IR:** (neat) ν_{max} = 3333, 3087, 3041, 1688, 1660, 1590, 1562, 1424, 1328, 1160, 1065, 796, 697 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{11}H_{15}N_4O_4$ $[M+H]^+$ 267.1088; found 267.1091.

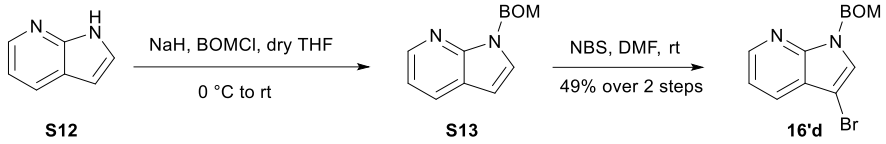
4) Reactions of nitrone 13 with other lithiated aromatic heterocycles

A. Synthesis of aromatic heterocycles 16’



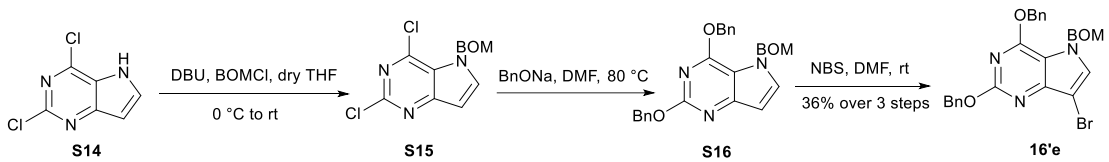
S10 **S11** **16'a**

Compound **16’a** was synthesized from 4-chloro-*7H*-pyrrolo[*2,3-d*]pyrimidine **S10** according to the procedure for synthesis of compound **16**. **16’a** (White solid, 70% yield from **S10** over three steps**)** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.34 − 7.26 (m, 6H), 7.20 (s, 1H), 5.66 (s, 2H), 4.50 (s, 2H), 4.15 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 152.0, 151.7, 136.7, 128.4, 128.0, 127.8, 125.1, 104.9, 88.5, 73.0, 71.0, 54.0. **IR:** (neat) ν_{max} = 3129, 3032, 2927, 2853, 1591, 1558, 1478, 1316, 1220, 1087, 1031, 790, 698 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{15}H_{15}BrN_3O_2$ $[M+H]^+$ 348.0342, found 348.0344.



Synthesis of compound 16'd: To a solution of *1H*-pyrrolo[2,3-*b*]pyridine **S12** (2.40 g, 20.3 mmol, 1.00 equiv) in anhydrous THF (25 mL) was added NaH (2.03g, 60% in oil, 50.8 mmol, 2.50 equiv) at 0 °C. After being stirred at the same temperature for 20 min, BOMCl (5.65 mL, 40.6 mmol, 2.00 equiv) was added dropwise. Then the resulting mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was quenched carefully with saturated NH_4Cl (30mL) at 0 °C. The layers were separated and the aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with water (30 mL × 1) and brine (30 mL × 1), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude **S13**, which was used directly in the next step without purification.

The crude compound **S13** resulting from the last step was dissolved in anhydrous DMF (25 mL). NBS (3.61 g, 20.3 mmol, 1.00 equiv) was added in small portions at 0 °C under stirring. The resulting mixture was stirred for 30 min at the same temperature before a saturated aqueous solution of $Na_2S_2O_3$ (25 mL) was added. The resulting mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with water (50 mL × 2) and brine (50 mL × 1), dried over anhydrous Na_2SO_4 and filtered. The residue was purified by flash column chromatography on silica gel (EA / petroleum ether 1:10 to 1:5, v/v) to afford compound **16'd** (3.2 g, 49 % yield over 2 steps) as a pale-yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40 (d, J = 6.0 Hz, 1H), 7.87 (d, J = 9.6 Hz, 1H), 7.39 (s, 1H), 7.35 – 7.26 (m, 5H), 7.20 (dd, J = 7.8, 4.8 Hz, 1H), 5.76 (s, 2H), 4.52 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.2, 144.5, 137.1, 128.4, 127.8, 127.7, 126.7, 120.1, 117.1, 90.4, 72.8, 70.9. **IR:** (neat) ν_{max} = 3031, 2942, 2882, 1730, 1454, 1425, 1379, 1314, 1074, 1045, 1027, 945, 736, 697 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{15}H_{13}BrN_2NaO$ $[M+Na]^+$ 341.0083, found 341.0079.



Synthesis of compound 16'e: To a solution of 2,4-dichloro-*5H*-pyrrolo[3,2-*d*]pyrimidine **S14** (5 g, 26.6 mmol, 1.00 equiv) in anhydrous THF (100 mL) was added DBU (7.95 mL, 53.2 mmol, 2.00 equiv) at 0 °C. After being stirred at the same temperature for 10 min, BOMCl (7.40 mL, 53.2 mmol, 2.00 equiv) was added dropwise. Then the resulting mixture was allowed to warm to room

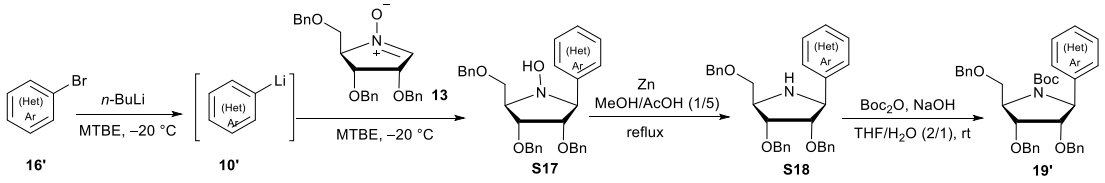
temperature and stirred overnight. The solvent was removed under reduced pressure to give crude **S15**, which was used directly in the next step without purification.

The crude compound **S15** resulting from the last step was dissolved in dry DMF (50 mL). Newly prepared BnONa (53.2 mL, 53.2 mmol, 1 M in BnOH and DMF, 2.00 equiv) was added slowly under stirring. The reaction was heated under 80 °C for 4 h. After being cooled to room temperature, water was added and the resulting mixture was extracted with EtOAc (100 mL × 3). The combined organic layers were washed with water (100 mL × 2) and brine (100 mL × 1), dried over anhydrous Na_2SO_4 and filtered. Then the solvent was evaporated under reduced pressure to give crude compound **S16,** which was used directly in the next step without purification.

The crude compound **S16** resulting from the last step was dissolved in anhydrous DMF (50 mL). NBS (4.73 g, 26.6 mmol, 1.00 equiv) was added in small portions at 0 °C under stirring. The resulting mixture was stirred for 30 min at the same temperature before a saturated aqueous solution of $Na_2S_2O_3$ (50 mL) was added. The resulting mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with water (50 mL × 2) and brine (50 mL × 1), dried over anhydrous Na_2SO_4 and filtered. Then the solvent was removed *in vacuo.* The residue was purified by flash column chromatography on silica gel (EA / petroleum ether 1:10 to 1:5, v/v) to afford compound **16'e** (5.3 g, 36 % yield over 3 steps) as a pale-yellow solid.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 7.2 Hz, 2H), 7.44 – 7.21 (m, 11H), 7.17 – 7.07 (m, 2H), 5.61 (s, 2H), 5.57 (s, 2H), 5.52 (s, 2H), 4.37 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 157.3, 150.1, 137.0, 136.6, 135.8, 132.0, 128.7, 128.6, 128.4, 128.4, 128.3, 128.2, 128.0, 127.9, 127.5, 112.3, 91.2, 77.3, 70.3, 69.3, 68.7. **IR:** (neat) ν_{max} = 3031, 2918, 1610, 1555, 1474, 1454, 1352, 1298, 1127, 1070, 738, 697 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{28}H_{24}BrN_3NaO_3$ $[M+Na]^+$ 554.0873, found 554.0863.

B. Reactions of nitrone 13 with other lithiated aromatic heterocycles

General procedures for synthesis 19' via nucleophilic addition of 16' with nitrone 13



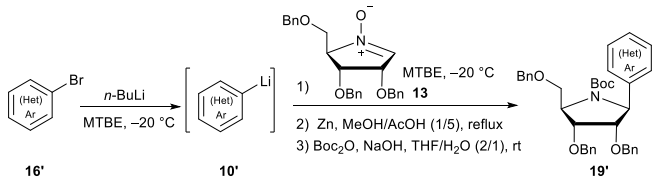
Synthesis of compound 19': Under an argon atmosphere, to a solution of compound **16'** (4.32 mmol, 1.80 equiv) in anhydrous MTBE (43 mL), *n*-BuLi (2.5 M in n-hexane, 2.1 mL, 5.18 mmol, 2.16 equiv) was added dropwise at –20 °C and the mixture was allowed to stir at –20 °C for an additional 30 min. Then a solution of nitrone **13** (1.0 g, 2.40 mmol, 1.00 equiv) in anhydrous

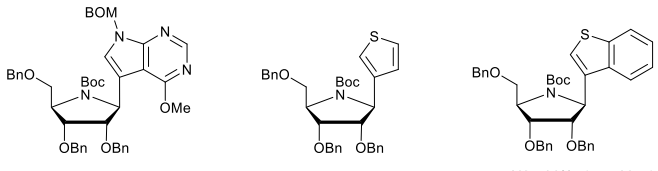
MTBE (5 mL) was added dropwise, and the resulting mixture was stirred for 15 h at –20 °C. After completion of the reaction, it was quenched with a saturated aqueous solution of NH_4Cl (20 mL) and warmed to room temperature. The resulting mixture was diluted with EA (30 mL); the layers were separated and the aqueous layer was extracted with EA (10 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4 and filtered, then the solvent was removed *in vacuo* to give crude compound **S17**, which was used directly in the next step without further purification.

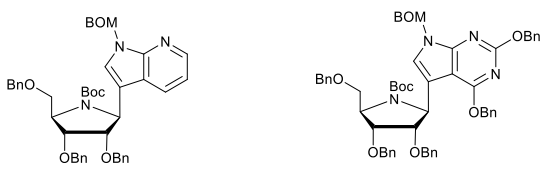
The crude compound **S17** resulting from the last step was dissolved in a mixture of MeOH and AcOH (V/V = 5:1, 60 mL). Zinc powder (3.12 g, 48.0 mmol, 20.0 equiv) was added in one portion under vigorously stirring. The reaction was heated under reflux for 2 h. After being cooled to room temperature, the mixture was filtered by Celite; the filtrate was concentrated *in vacuo* to give crude compound **S18**, which was immediately used in the next step without purification.

The crude compound **S18** from the last step was dissolved in a mixture of THF and water (V/V = 2:1, 90 mL), of which the pH was adjusted to 9-10 with 3 M aqueous NaOH at 0 °C under stirring. Boc_2O (1.05 g, 4.80 mmol, 2.00 equiv) was then added and the reaction mixture was stirred at room temperature for 15 h. The resulting mixture was filtered by Celite; the filtrate was evaporated under reduced pressure to remove volatiles. Next, the residue was diluted with EA (50 mL) and the layers were separated. The aqueous layer was extracted with EA (30 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography to give **19'**.

Table S1 Reactions of nitrone **13** with other lithiated aromatic heterocycles[a]







[a]Reactions were conducted on 2.4 mmol scale of **13** through a three-step synthetic sequence with one isolation operation and the yields of **19'** were calculated according to isolated materials through column over three steps from **16'.** The d.r. value was determined by LC-MS analysis of the crude materials.

Compound 19'a was obtained according to the general procedure in 40% yield from **16'a**.as colorless oil. **Optical rotation**: $[\alpha]_D^{25}$ = + 29.9 (*c* = 0.23, $CHCl_3$).**^{1}H NMR** (600 MHz, $CDCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 8.45 (s, 1H), 7.55 (s, 1H), 7.40 – 7.21 (m, 19H), 5.62 (s, 1H), 5.41 – 5.30 (m, 1H), 5.21 (m, *J* = 10.8 Hz, 1H), 4.96 (m, 1H), 4.74 (m, 2H), 4.57 – 4.37 (m, 4H), 4.29 (m, 4H), 4.12 (s, 3H), 4.06 (s, 1H), 3.94 (d, *J* = 8.4 Hz, 1H), 3.72 (m, 1H), 1.45 (s, 5H), 1.20 (s, 4H). **^{13}C NMR** (150 MHz, $CDCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 163.1, 155.6, 152.8, 151.0, 138.6, 138.1, 137.9, 137.4, 128.5, 128.4, 128.3, 128.3, 128.1, 128.0, 127.8, 127.7, 127.7, 127.5, 127.4, 125.1, 125.0, 103.7, 80.4, 80.2, 79.4, 79.0, 76.4, 73.5, 73.3, 73.2, 72.9, 71.8, 71.2, 70.9, 70.6, 70.4, 67.5, 60.4, 60.2, 60.05, 59.7, 53.4, 28.4, 28.2. **IR:** (neat) ν_{max} = 3030, 2966, 2926, 2867, 1695, 1595, 1570, 1474, 1454, 1390, 1364, 1315, 1091, 1026, 800, 737, 697 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{46}H_{50}N_4NaO_7$ $[M+Na]^+$793.3572, found 793.3541.

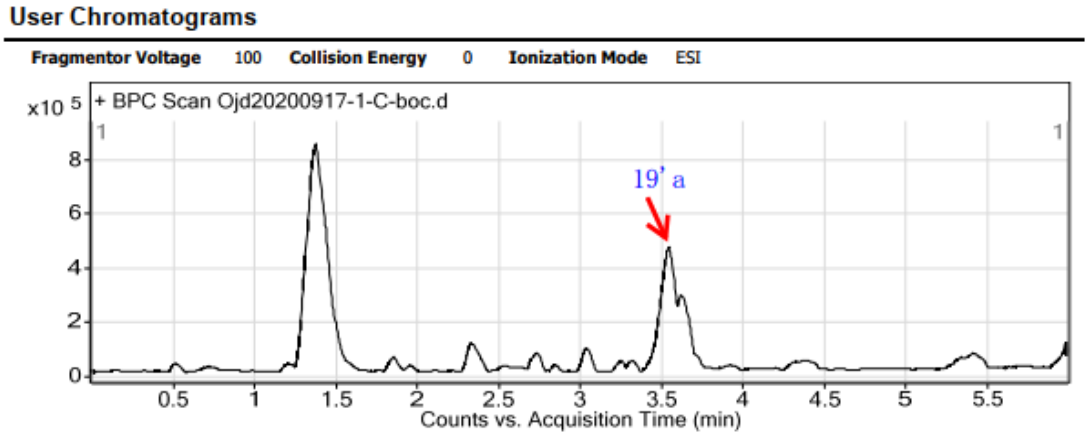
LC-MS Analysis of the crude product

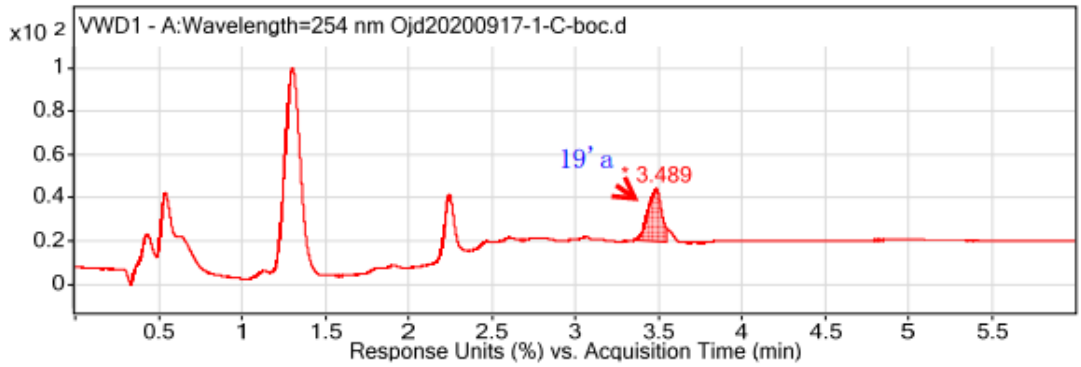
Run Information:

Aglient LC-MS QQQ 6420A; Column: Ultimate UHPLC XB-C18 (1.8 μm, 2.1*50 mm); Solvent: H_2O/CH_3CN (the gradient of CH_3CN is from 60% to 98% during 0-1.5 min, 98% during 1.5-5.5 min, 98% to 85% during 5.5-6 min); Flow = 0.5 mL/min; Detected by UV at 254 nm;

Retention time for **19'a** in LC-MS: 3.489 min (compound **19'a**, major);

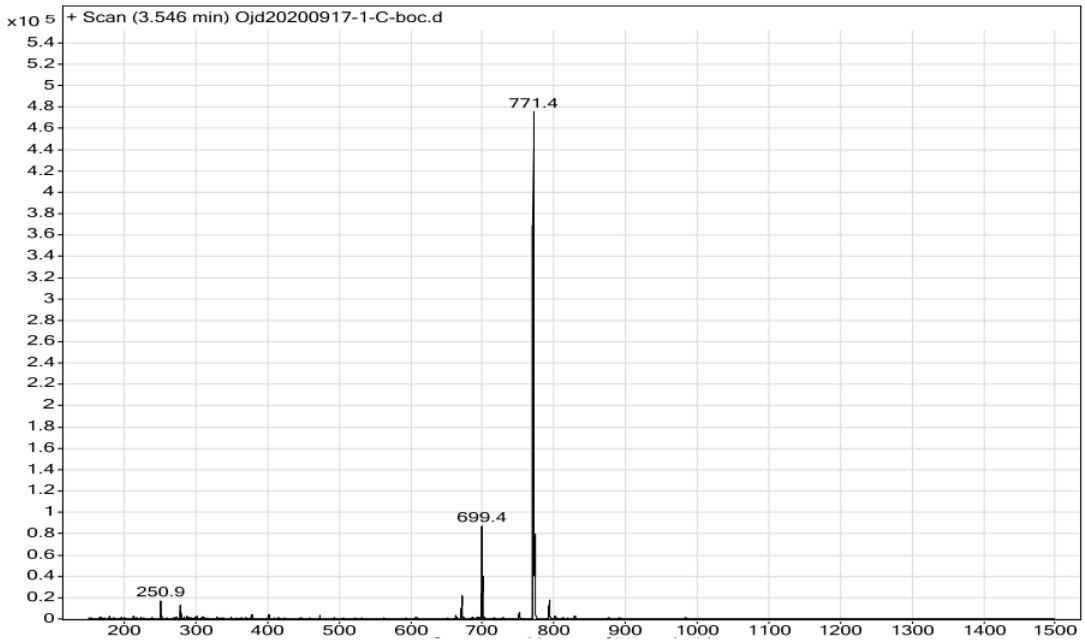
Base peak chromatogram: 3.546 min (compound **19'a**, $[M+H]^+$ = 771.4).





Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	3.356	3.489	3.55	2.02	11.39	100



Compound 19'b was obtained according to the general procedure in 25% yield from 2-bromothiophene (**16'b)** as colorless oil. **Optical rotation**: $[\alpha]_D^{25}$ = –43.5 (*c* = 1.0, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 7.32 – 7.13 (m, 16H), 6.99 (s, 1H), 6.88 – 6.86 (m, 1H), 5.23 – 5.01 (m, 1H), 4.66 – 4.38 (m, 6H), 4.23 – 4.04 (m, 3H), 3.70 (s, 2H), 1.39 (s, 2H), 1.24 (s, 7H). **^{13}C NMR** (100 MHz, $CDCl_3$,, some signals exist as a pair due to the presence of amide rotamers) δ 155.3, 146.8, 138.3, 137.8, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.6, 126.3, 124.6, 123.5, 84.7, 80.2, 73.3, 72.1, 69.5, 61.1, 28.1. **IR:** (neat) ν_{max} = 3030, 2972, 2922, 2862, 1693, 1388, 1363, 1155, 1100, 735, 695 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{35}H_{39}NNaO_5S$ $[M+Na]^+$ 608.2441, found 608.2466.

LC-MS Analysis of the crude product

Run Information:

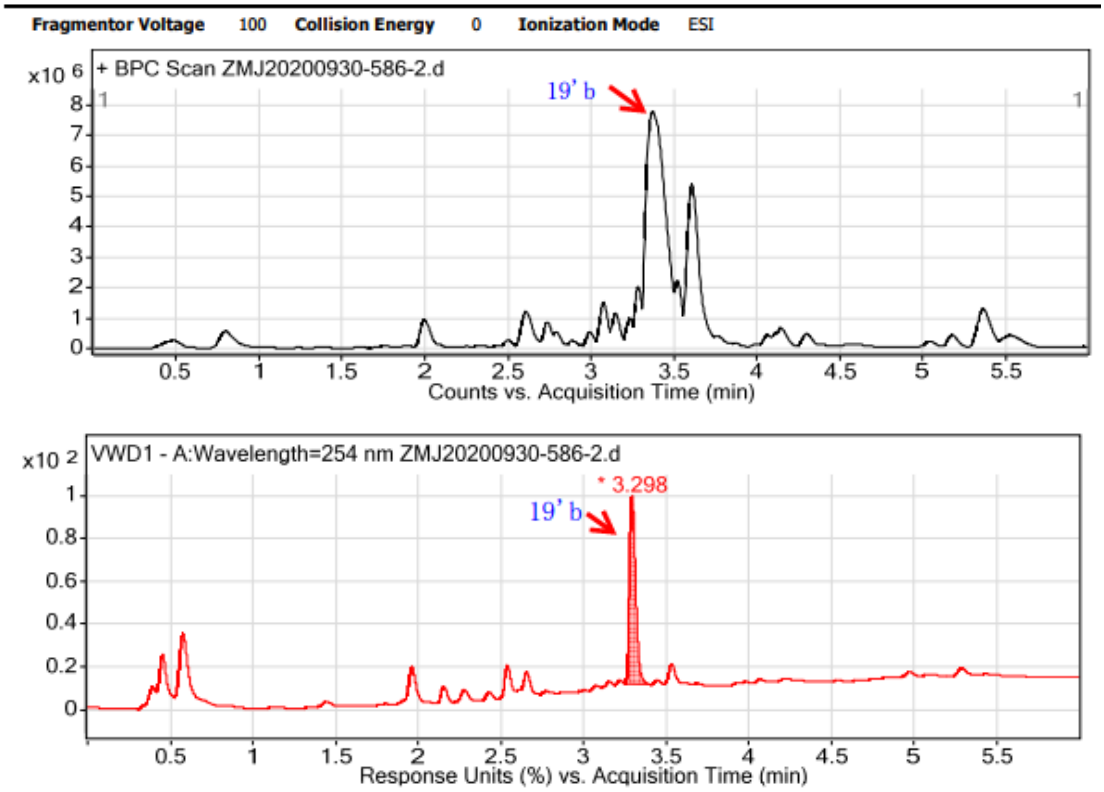
Aglient LC-MS QQQ 6420A; Column: Ultimate UHPLC XB-C18 (1.8 μm, 2.1*50 mm); Solvent:

H_2O/CH_3CN (the gradient of CH_3CN is from 60% to 98% during 0-1.5 min, 98% during 1.5-5.5 min, 98% to 85% during 5.5-6 min); Flow = 0.5 mL/min; Detected by UV at 254 nm;

Retention time for **19'b** in LC-MS: 3.298 min (compound **19'b**, major)

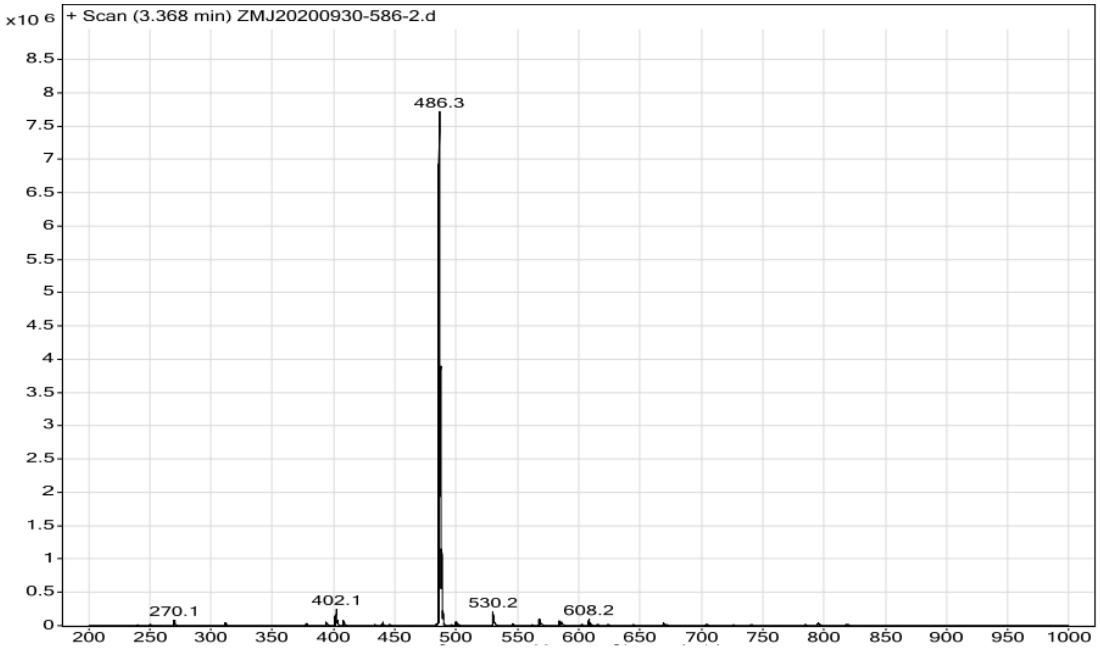
Base peak chromatogram: 3.368 min (compound **19'b**, major, $[M\text{-Boc}+H]^+$ = 486.3).

User Chromatograms



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	3.253	3.298	3.391	59.68	154.71	100



Compound 19'c was obtained according to the general procedure in 36% yield from 3-bromo-1-benzothiophene (**16'c)** as colorless oil. **Optical rotation**: $[\alpha]_D^{25}$ = – 36.0 (*c* = 0.38, $CHCl_3$). **^{1}H NMR** (400 MHz, $CHCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 7.80 – 7.21 (m, 20H), 5.50-5.06 (m, 1H), 4.76-4.40 (m, 6H), 4.25 – 4.11 (m, 3H), 3.79 – 3.75 (m, 2H), 1.43 (s, 4H), 1.21 (s, 5H). **^{13}C NMR** (100 MHz, $CHCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 155.2, 147.6, 139.7, 139.1, 138.2, 138.0, 137.8, 137.6, 128.4, 128.2, 127.8, 127.6, 127.6, 123.9, 123.7, 123.1, 122.2, 121.1, 83.9, 81.7, 80.4, 73.3, 72.2, 72.1, 71.7, 71.4, 69.4, 61.9, 61.6, 61.4, 60.5, 28.0, 27.9. **IR:** (neat) ν_{max} = 2964, 2919, 2856, 1695, 1454, 1364, 1260, 1096, 1023, 800, 738, 698 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{39}H_{41}NNaO_5S[M+Na]^+$ 658.2598, found 658.2571.

LC-MS Analysis of the crude product

Run Information:

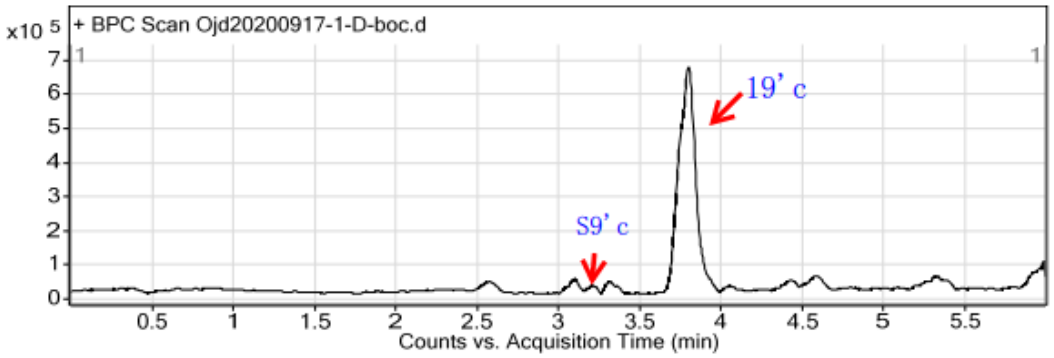
Aglient LC-MS QQQ 6420A; Column: Ultimate UHPLC XB-C18 (1.8 μm, 2.1*50 mm); Solvent: H_2O/CH_3CN (the gradient of CH_3CN is from 60% to 98% during 0-1.5 min, 98% during 1.5-5.5 min, 98% to 85% during 5.5-6 min); Flow = 0.5 mL/min; Detected by UV at 254 nm;

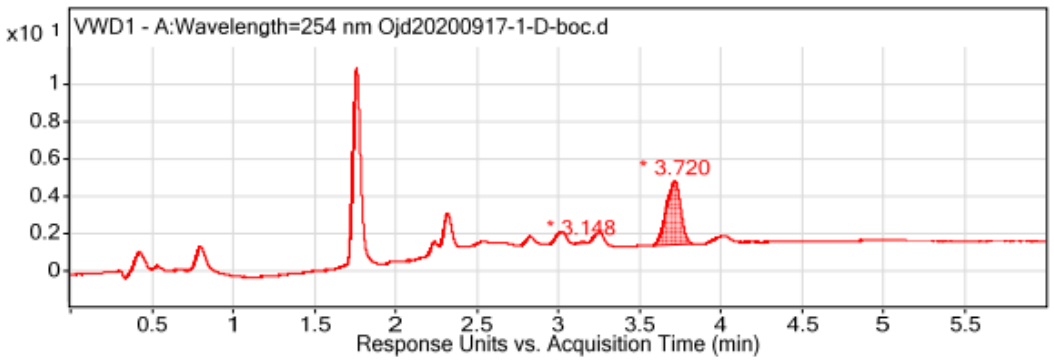
Retention time for major isomer **19'c** and minor isomer **S9'c** in LC-MS: 3.720 min (compound **19'c**, major), 3.148 min (compound **S9'c**, minor);

Base peak chromatogram: 3.802 min (compound **19'c**, major, $[M\text{-}Boc+H]^+$ = 536.2), 3.210 min (compound **S9'c**, minor, $[M\text{-}Boc+H]^+$ = 536.3).

User Chromatograms

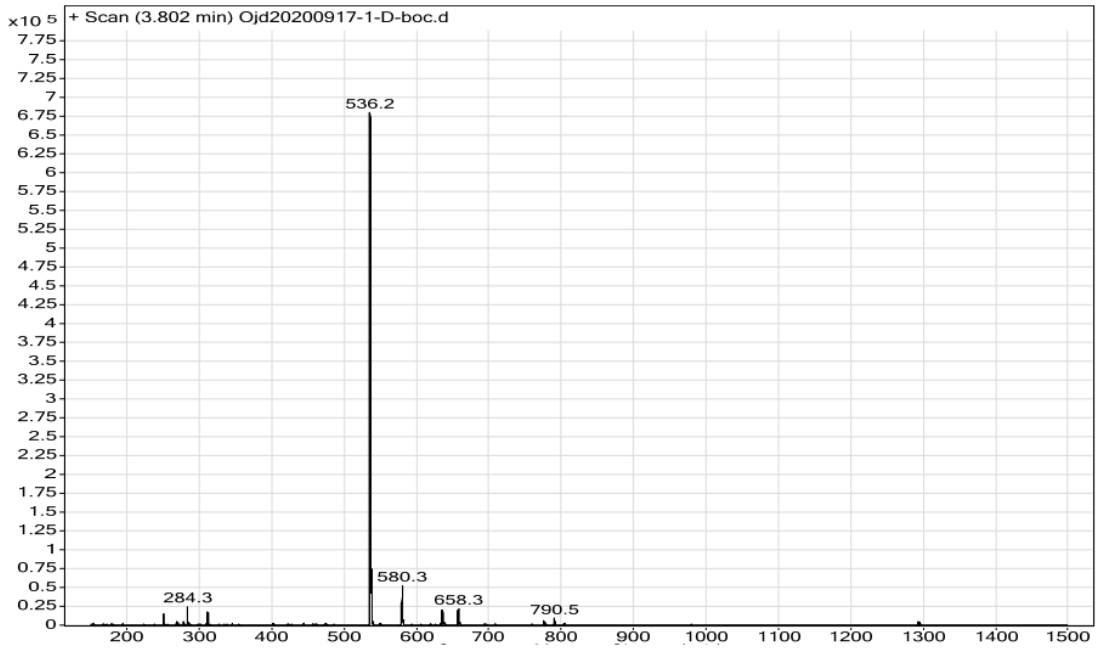
Fragmentor Voltage 100 **Collision Energy** 0 **Ionization Mode** ESI

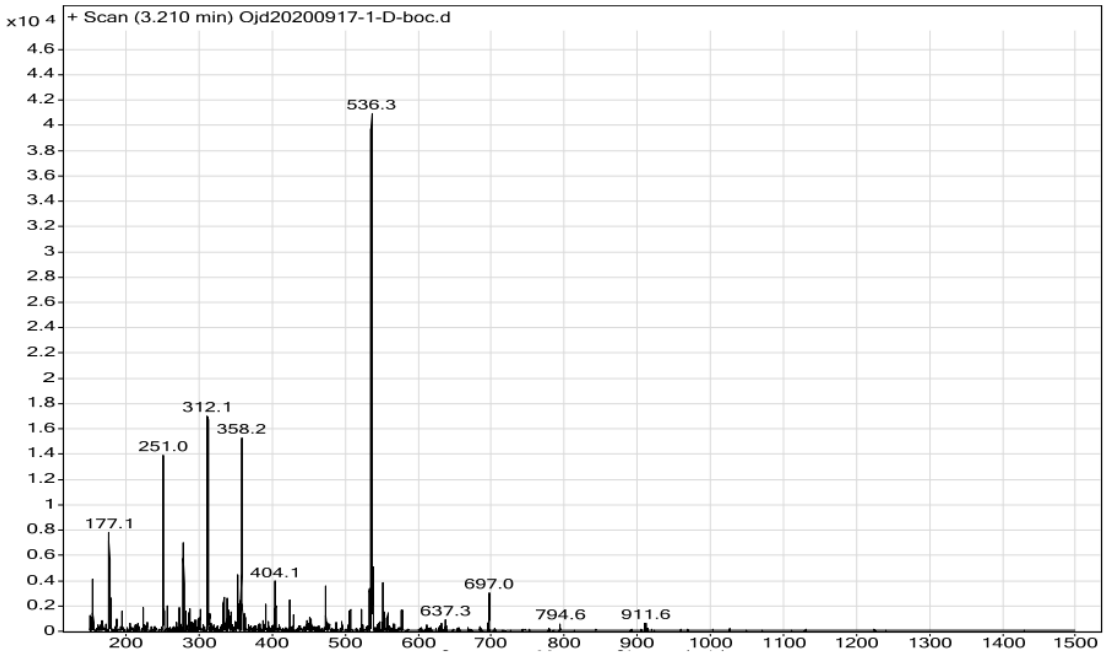




Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	3.099	3.148	3.185	0.08	0.22	1.05
2	3.585	3.72	3.83	3.39	20.74	100





Compound 19'd was obtained according to the general procedure in 39% yield from **16'd** as colorless oil. **Optical rotation**: $[\alpha]_D^{25}$ = – 13.2 (*c* = 0.64, $CHCl_3$). **^{1}H NMR** (400 MHz, $CHCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 8.30 (d, *J* = 4.0 Hz, 1H), 7.81 (d, *J* = 16.0 Hz, 1H), 7.29 – 6.96 (m, 21H), 5.60 (s, 1H), 5.38 (d, *J* = 10.4 Hz, 2H), 5.06 (s, 1H), 4.59 – 4.34 (m, 8H), 4.20 – 4.03 (m, 4H), 3.71 (d, *J* = 8.8 Hz, 1H), 1.42 (s, 4H), 1.09 (s, 5H). **^{13}C NMR** (150 MHz, $CHCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 155.5, 148.4, 148.2, 144.3, 143.1, 138.1, 137.8, 137.5, 134.6, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 126.1, 125.6, 119.0, 117.3, 115.9, 82.3, 79.7, 73.4, 72.8, 72.0, 71.9, 71.9, 71.8, 71.6, 71.0, 70.4, 69.3, 61.7, 61.0, 58.8, 58.6, 28.4, 28.1. **IR:** (neat) ν_{max} = 3030, 2924, 2861, 1691, 1453, 1389, 1364, 1089, 1026, 736, 696 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{46}H_{49}N_3NaO_6[M+Na]^+$ 762.3514, found 762.3501.

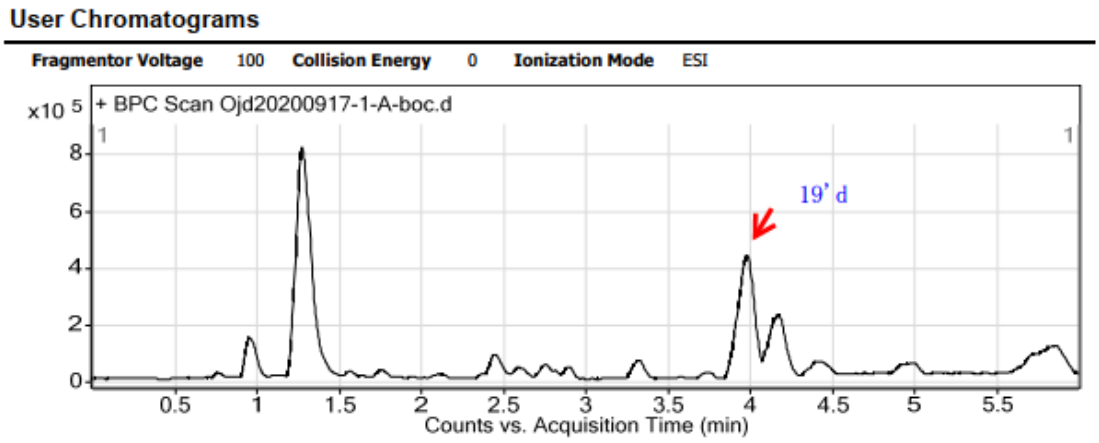
LC-MS Analysis of the crude product

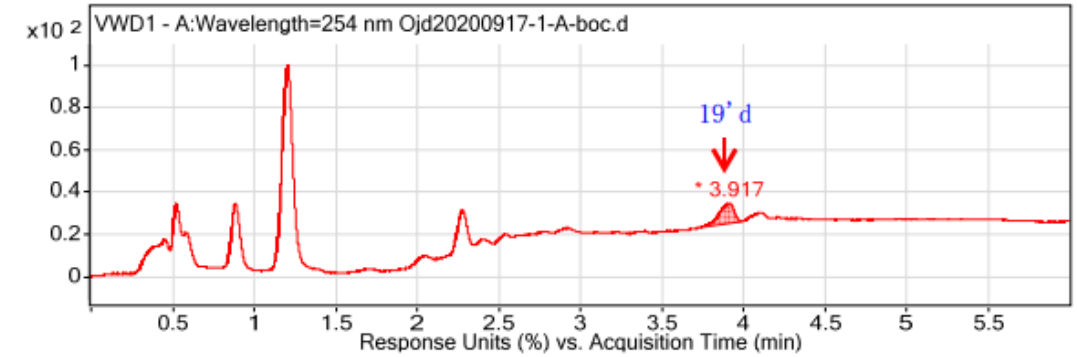
Run Information:

Aglient LC-MS QQQ 6420A; Column: Ultimate UHPLC XB-C18 (1.8 μm, 2.1*50 mm); Solvent: H_2O/CH_3CN (the gradient of CH_3CN is from 60% to 98% during 0-1.5 min, 98% during 1.5-5.5 min, 98% to 85% during 5.5-6 min); Flow = 0.5 mL/min; Detected by UV at 254 nm;

Retention time for major isomer **19'd** in LC-MS: 3.917 min (compound **19'd**, major);

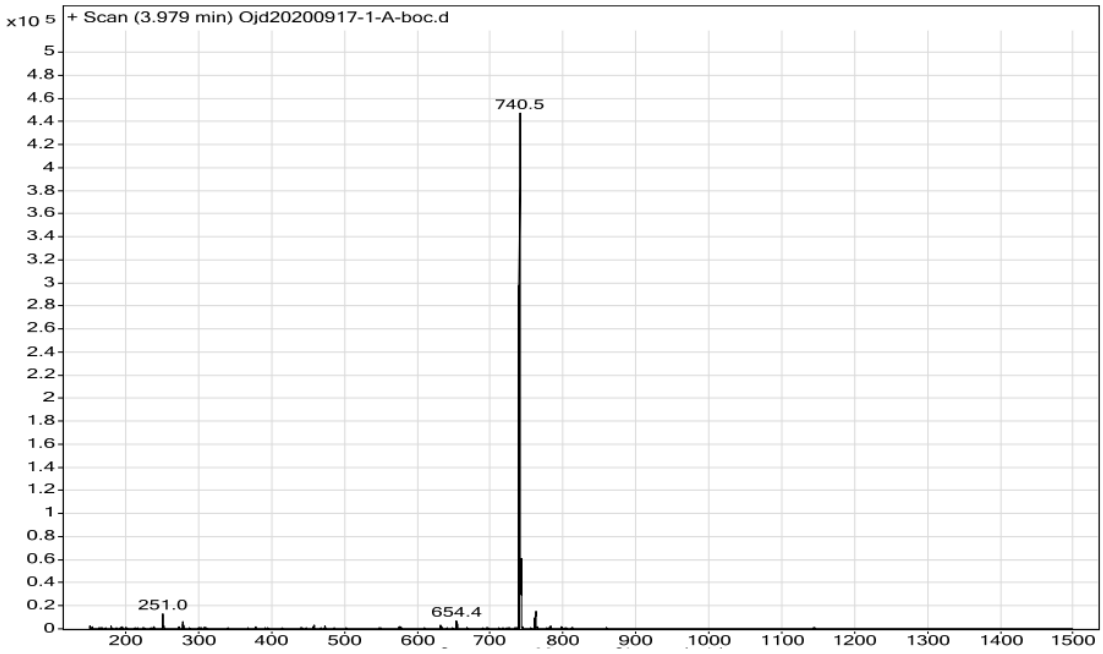
Base peak chromatogram: 3.979 min (compound **19'd**, major, $[M+H]^+$ = 740.5).





Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	3.743	3.917	4	0.52	3.24	100



Compound 19'e was obtained according to the general procedure in 40% yield from **16'e** as colorless oil. **Optical rotation**: $[\alpha]_D^{25}$ = +38.9 (*c* = 0.60, $CHCl_3$). **^{1}H NMR** (400 MHz, $CHCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 7.46 – 7.43 (m, 4H), 7.35 –

7.20 (m, 25H), 7.10 (s, 2H), 5.60-5.53 (m, 2H), 5.47-5.40 (s, 2H), 5.31 – 5.17 (m, 2H), 4.94-4.84 (m, 1H), 4.74-4.67 (m, 1H), 4.52 – 3.99 (m, 10H), 3.76-3.67 (m, 1H), 1.45 (s, 4H), 1.24 (s, 5H).**^{13}C NMR** (150 MHz, $CHCl_3$, some signals exist as a pair due to the presence of amide rotamers) δ 159.0, 157.0, 155.3, 155.1, 150.2, 138.5, 138.3, 138.3, 138.0, 137.3, 137.2, 137.1, 136.3, 136.2, 133.0, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 115.8, 115.0, 112.4, 112.2, 79.9, 79.4, 79.0, 76.2, 73.3, 73.2, 73.2, 71.5, 71.3, 71.1, 69.7, 68.8, 68.1, 68.0, 67.8, 60.6, 58.1, 28.4, 28.3. **IR:** (neat) ν_{max} = 3031, 2922, 2867, 1693, 1614, 1546, 1453, 1391, 1351, 1298, 1128, 1091, 1026, 734, 696 cm^{-1}. **HRMS (ESI)**: *m/z* calcd. for $C_{59}H_{60}N_4NaO_8[M+Na]^+$ 975.4303, found 975.4285.

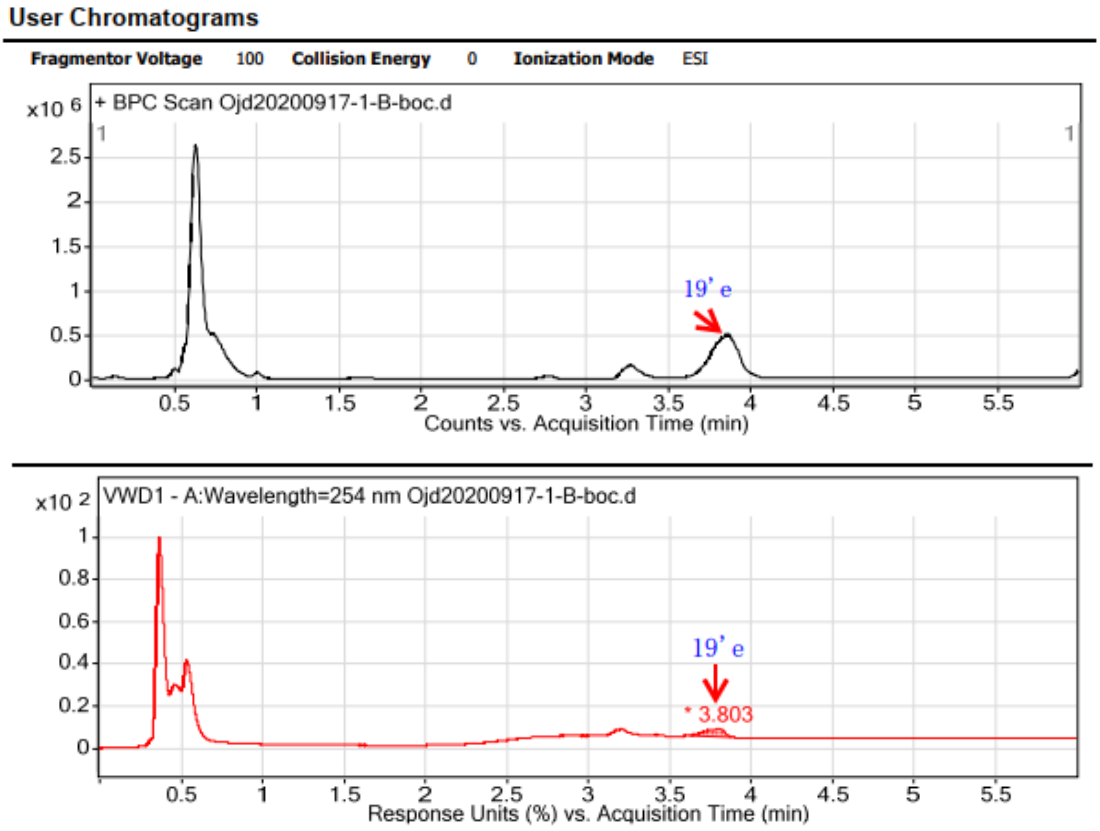
LC-MS Analysis of the crude product

Run Information:

Aglient LC-MS QQQ 6420A; Column: Ultimate UHPLC XB-C18 (1.8 μm, 2.1*50 mm); Solvent: H_2O/CH_3CN (the gradient of CH_3CN is from 60% to 98% during 0-1.5 min, 98% during 1.5-5.5 min, 98% to 85% during 5.5-6 min); Flow = 0.5 mL/min; Detected by UV at 254 nm;

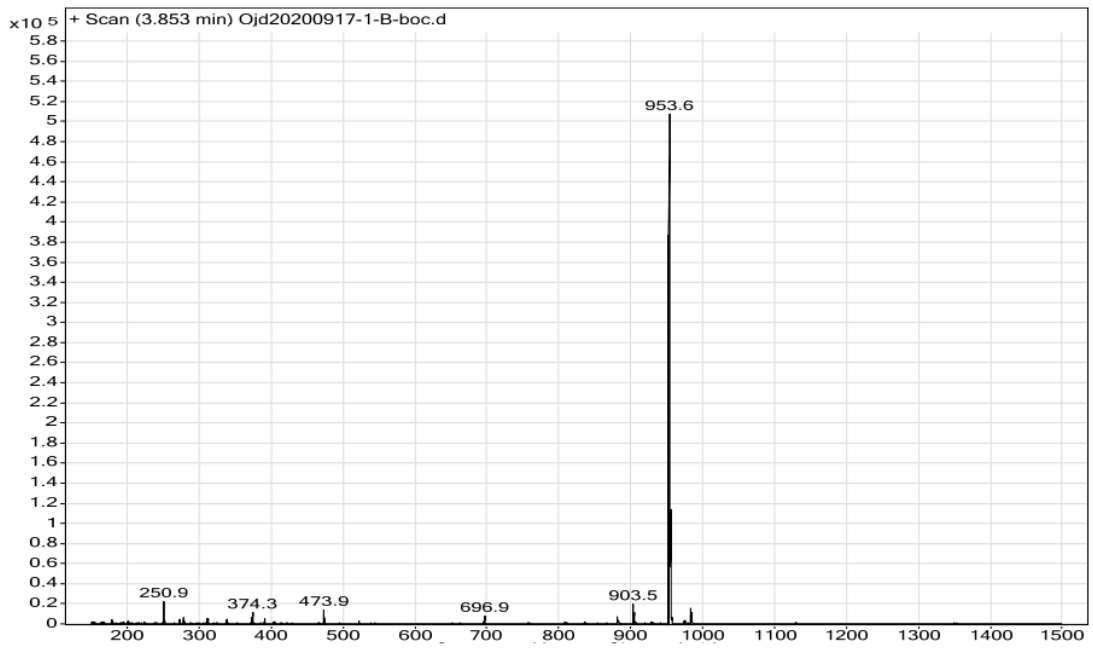
Retention time for major isomer **19'e** in LC-MS: 3.803 min (compound **19'e**, major);

Base peak chromatogram: 3.853 min (compound **19'e**, major, $[M+H]^+$ = 953.6).

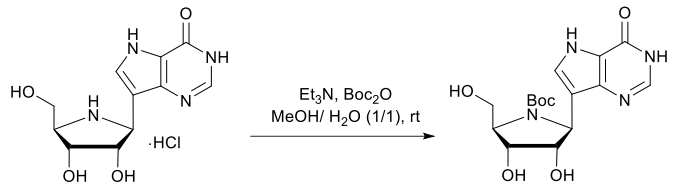


Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	3.62	3.803	3.889	1.15	9.29	100

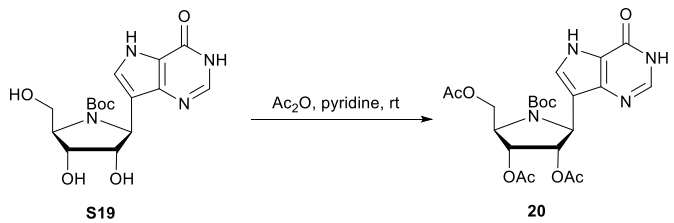


5) Synthesis of BCX-4430 (2)



BCX-1777 (**1**) **S19**

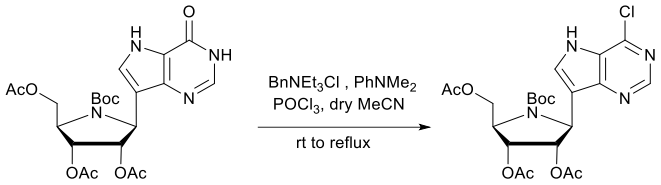
Synthesis of Compound S19[4]**:** *Compound **S19** was prepared according to the literature method.*[4] To a solution of BCX-1777 (**1**) (4.80 g, 15.9 mmol, 1.00 equiv) in MeOH and water (100 mL, v/v = 1:1), Et_3N (6.60 mL, 47.7 mmol, 3.00 equiv) and Boc_2O (11.3 mL, 49.3 mmol, 3.50 equiv) was added successively at room temperature under stirring. The mixture was stirred at room temperature overnight. The resulting solid was filtered, washed with water (50 mL × 1), dried under high vacuum at 50 °C to afford crude **S19** as a white solid, which was used directly in the next step without further purification.



S19 **20**

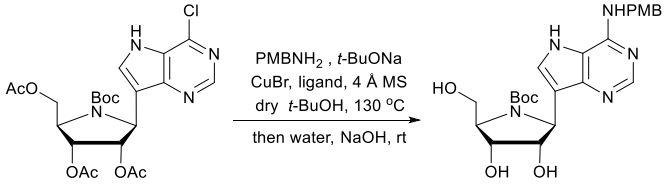
Synthesis of Compound 20[4]**:** *Compound **20** was prepared according to a modified literature*

method.[4] The crude compound **S19** from the last step was dissolved in dry pyridine (40 mL), and Ac_2O (5.26 mL, 55.7 mmol, 3.50 equiv) was added at 0 °C under stirring. The reaction was then allowed to warm to room temperature and stirred overnight. Next, the mixture was diluted with chloroform (180 mL) and deionized water (120 mL). The layers were separated and the organic layer was washed with water (120 mL × 1). The aqueous layers were combined and extracted with chloroform (100 mL × 2). The organic layers were combined and washed successively with aqueous 2 M HCl (150 mL × 2), water (150 mL × 1), saturated aqueous $NaHCO_3$ (150 mL × 2) and brine (150 mL), then dried over Na_2SO_4, filtered and concentrated in vacuo to give crude product **20**, which was used for next step without further purification.



20 **11**

Synthesis of Compound 11[4]**:** *Compound **11** was prepared according to a modified literature method.*[4] To a solution of crude **20** in anhydrous MeCN (160 mL) was added $BnNEt_3Cl$ (7.23 g, 31.7 mmol, 2.00 equiv), $PhNMe_2$ (6.10 mL, 47.6 mmol, 3.00 equiv) and $POCl_3$ (8.90 mL , 95.2 mmol, 6.00 equiv) successively. The reaction mixture was slowly heated to reflux and stirred for 1 h. After completion of the reaction, it was allowed to cool to room temperature and slowly poured into the saturated aqueous solution of $NaHCO_3$ (1000 mL). Then the resulting mixture was evaporated under reduce pressure to remove the volatiles. The layers were separated and the aqueous phase was extracted with chloroform (500 mL × 1, 200 mL × 2). The organic layers were combined, washed with aqueous 2 N HCl (200 mL × 2), water (200 mL), saturated aqueous $NaHCO_3$ (200 mL × 2) and brine (200 mL), then dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford the crude product of **11**. The crude product could be use in next step without further purification. *Note: Compound **11** used in the amination optimization studies to prepare **22** was purified through silica gel column chromatography (70% yield over three steps from BCX-1777, a white foamy solid); the ^1H and ^{13}C NMR data were consistent with those reported in the literature.*[4]

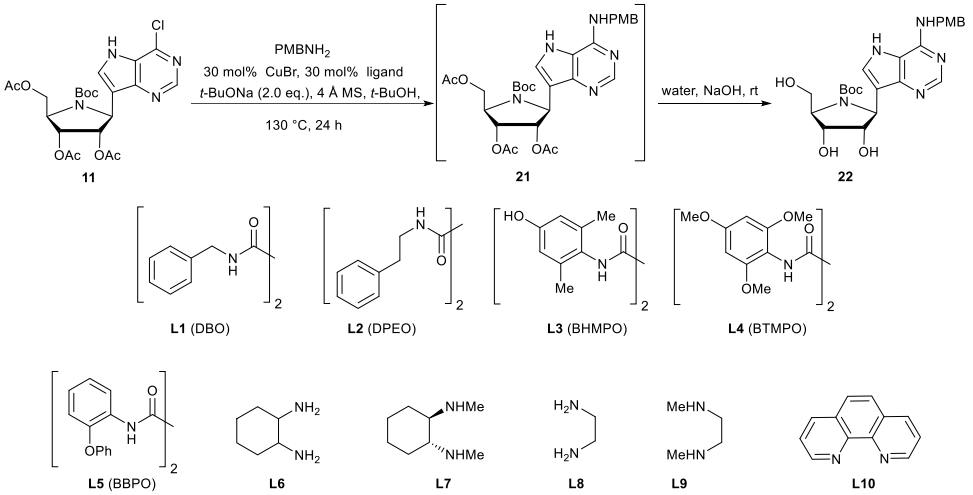


11 **22**

Synthesis of Compound 22:

A. Screening of the ligands for the Ullmann-type amination of 11 to prepare 22

Table S2 The copper-catalyzed Ullmann-type amination of chloride **11**[a,c]



Entry	Ligand	Yield of **22**[b]	Entry	Ligand	Yield of **22**[b]
1	L1	83%	6	L6	54%
2	L2	73%	7	L7	80%
3	L3	77%	8	L8	46%
4	L4	80%	9	L9	30%
5	L5	81%	10	L10	65%

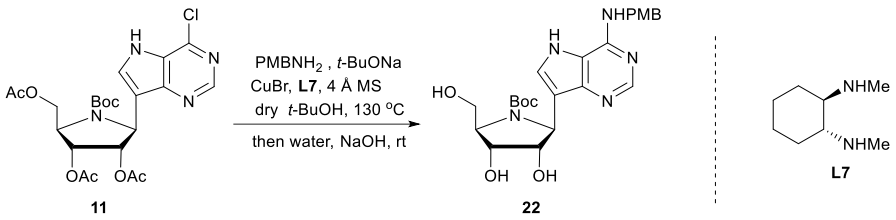
[a] Reactions were conducted on 0.2 mmol scale of **11** (1.0 equiv) in 4.0 mL *t*-BuOH, with 2.0 equiv. of $PMBNH_2$, 0.30 equiv. of CuBr, 0.30 equiv. of ligands and 2.0 equiv. of *t*-BuONa. [b] Yields were calculated based on the materials isolated through column. [c] Compound **11** used in the amination optimization studies (ligands screening) was purified through silica gel column chromatography.

B. General Procedure for Synthesis of Compound 22 by Using Different ligands.

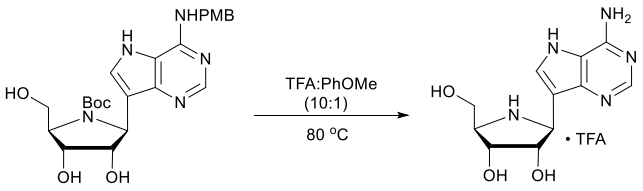
To an oven-dried reaction tube with a stir bar was added compound **11** (100 mg, 0.200 mmol, 1.00 equiv), *t*-BuONa (38.4 mg, 0.400 mmol, 2.00 equiv), CuBr (8.6 mg, 60 μmol, 0.30 equiv), ligand (60 μmol, 0.30 equiv) and 4Å molecular sieve (100 mg). The reaction tube was evacuated and backfilled with argon for three times before dry *t*-BuOH (4 mL) and $PMBNH_2$ (52.0 μL, 0.400 mmol, 2.00 equiv) was added. The resulting mixture was heated at 130 °C for 24 h under vigorous stirring. Next, the reaction was cooled to about 50 °C, water (4 mL) was added followed by addition of solid NaOH (48.0 mg, 1.20 mmol, 6.00 equiv). The resulting mixture was stirred for 1 h at room temperature. The solution was diluted with EA (5 mL) and aqueous saturated ammonium chloride solution (5 mL), then filtered by Celite. The layers were separated and the aqueous layer was extracted with EA (5 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/ MeOH/NH_4OH = 100:5:0.5 v/v) to afford

compound **22** as a pale-yellow foamy solid.

C. Synthesis of Compound 22 under the optimized reaction conditions

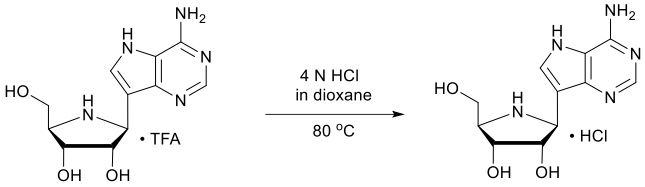


To an oven-dried round-bottom flask with a stir bar was added the crude compound **11** (from 4.80 g BCX-1777, without purification of intermediates **S19** and **20**), *t*-BuONa (3.05 g, 31.7 mmol, 2.00 equiv), CuBr (0.683 g, 4.76 mmol, 0.300 equiv), ligand **L7** (0.677 g ,4.76 mmol, 0.300 equiv) and 4Å molecular sieve (8.00 g) were placed into an oven-dried round-bottom flask (250 mL) with a magnetic stir bar. The reaction vessel was evacuated and backfilled with argon three times, and dry *t*-BuOH (100 mL) was added afterwards. Then $PMBNH_2$ (4.14 mL, 31.7 mmol, 2.00 equiv) was added and the reaction mixture was heated at 130 °C for 24 h under vigorous stirring. The reaction was cooled to about 50 °C and water (100 mL) was added followed by addition of solid NaOH (3.81 g, 95.2 mmol, 6.00 equiv). The resulting mixture was stirred for 1 h at room temperature. The solution was diluted with EA (50 mL) and aqueous saturated ammonium chloride solution (50 mL), then filtered by Celite. The layers were separated and the aqueous layer was extracted with EA (100 mL × 3). The organic layers were combined and dried over anhydrous Na_2SO_4 and filtered, then the solvent was removed *in vacuo*. The residue was used directly in next step without further purification. An analytical sample of compound **22** was prepared by silica gel chromatography (CH_2Cl_2/ MeOH/NH_4OH = 100:5:0.5 v/v) as a pale-yellow foamy solid. **Optical rotation**: $[\alpha]_D^{25}$ = –114 (*c* = 0.12, MeOH). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 8.16 (s, 1H), 7.38 (s, 1H), 7.32 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 4.72 – 4.64 (m, 3H), 4.58 – 4.50 (m, 1H), 4.26 – 4.15 (m, 2H), 3.87 (s, 1H), 3.78 (s, 3H), 3.70 (d, *J* = 11.6 Hz, 1H), 1.39 (s, 3H), 1.05 (s, 6H). **^{13}C NMR**：(100 MHz, Methanol-d_4) δ 160.7, 157.2, 151.1, 150.2, 143.9, 131.8, 130.3, 128.4, 117.4, 116.0, 115.1, 81.2, 80.7, 78.0, 77.7, 74.3, 69.0, 63.1, 63.0, 61.2, 61.1, 55.7, 45.1, 28.7, 28.3. **IR:** (neat) ν_{max} = 3336, 3023, 2977, 2930, 1632, 1541, 1408, 1367, 1214, 1146,746, 666 cm^{-1}. **HRMS (ESI)**: *m/z* calcd for $C_{24}H_{32}N_5O_6$ $[M+H]^+$: 486.2347, found 486.2349.



22 23

Synthesis of Compound 23: The crude compound **22** from the last step was dissolved in a mixture of TFA (150 mL) and anhydrous anisole (15 mL). Then the reaction was heated to 80 °C. After being stirred for 2 h at the same temperature, the solvent was removed under reduce pressure. The residue was dissolved in water (100 mL) and washed with EA (50 mL). The aqueous layer was concentrated to dryness *in vacuo*, giving the crude compound **23** as a yellow solid.



23 BCX-4430 (**2**)

Synthesis of BCX-4430 (**2**): The suspension of crude **23** in a solution of 4 N HCl in dioxane (150 mL) was vigorously stirred at 80 °C for 24 h. The solvent was removed *in vacuo* and the residue was dispersed in EtOH (150 mL). The resulting mixture was heated at 80 °C for 30 min with vigorously stirring. Then the suspension was slowly cooled to 0 °C and maintained at this temperature for 1 h without stirring. A white solid of crude BCX-4430 was obtained by filtration, the solid was purified by C18 column chromatography (0.001 M HCl) to afford BCX-4430 (**2**) as a white solid (1.20 g, 25% yield in 6 steps) .**^{1}H NMR** (400 MHz, D_2O) δ 8.32 (s, 1H), 7.94 (s, 1H), 4.90 (d, J = 8.9 Hz, 1H), 4.68 (s, 1H), 4.37 (dd, J = 4.8, 3.5 Hz, 1H), 3.88 (d, J = 4.7 Hz, 2H), 3.81 (q, J = 4.6 Hz, 1H). **^{13}C NMR** (100 MHz, D_2O) δ 149.4, 143.5, 138.3, 132.4, 112.8, 105.1, 73.4, 70.5, 65.6, 58.5, 55.6. **IR:** (neat): ν_{max} = 3297, 3243, 3114, 2966, 1670, 1606, 1586, 1476, 1402, 1344, 1112, 1016, 961, 825, 655 cm^{-1}; **HRMS (ESI)**: *m/z* calcd for $C_{11}H_{16}N_5O_3[M+H]^+$ 266.1248, found 266.1251.

3. Reference

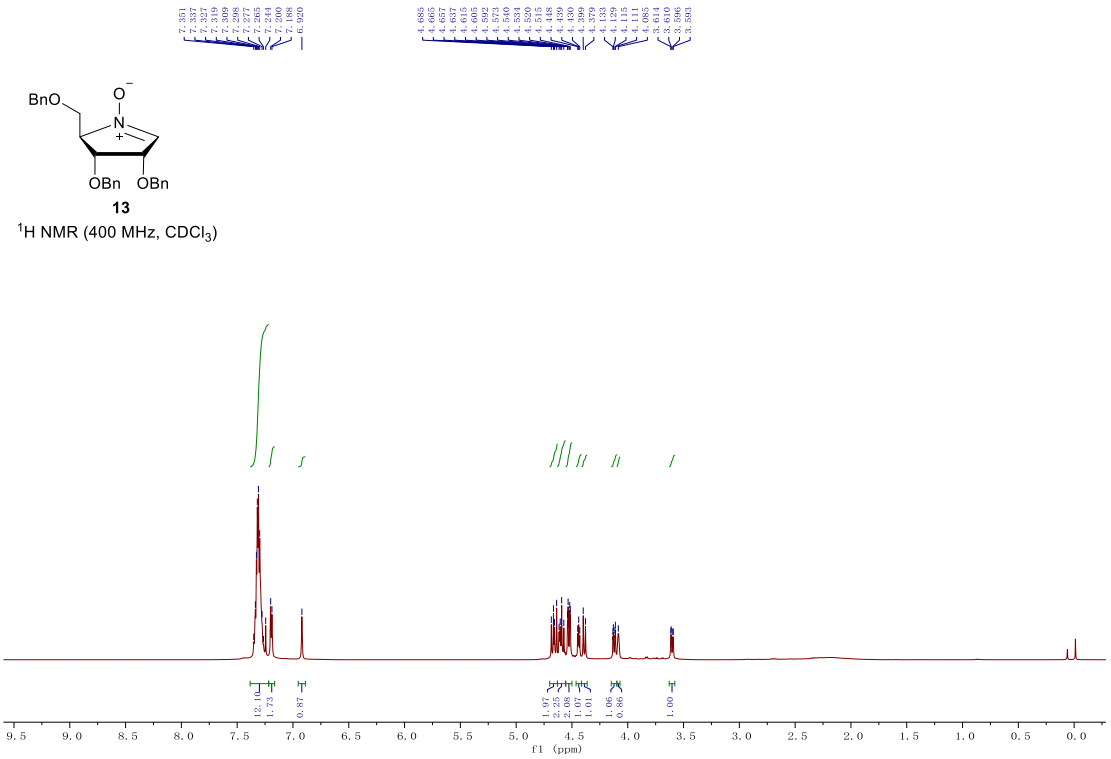
[1] (a) E. R. van Rijssel, P. van Delft, G. Lodder, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov and J. D. C. Codée, Furanosyl Oxocarbenium Ion Stability and Stereoselectivity, *Angew. Chem. Int. Ed.*, 2014, **53**, 10381–10385; (b) E.-L. Tsou, Y.-T. Yeh, P.-H. Liang and W.-C. Cheng, A convenient approach toward the synthesis of enantiopure isomers of DMDP and ADMDP, *Tetrahedron*, 2009, **65**, 93–100.

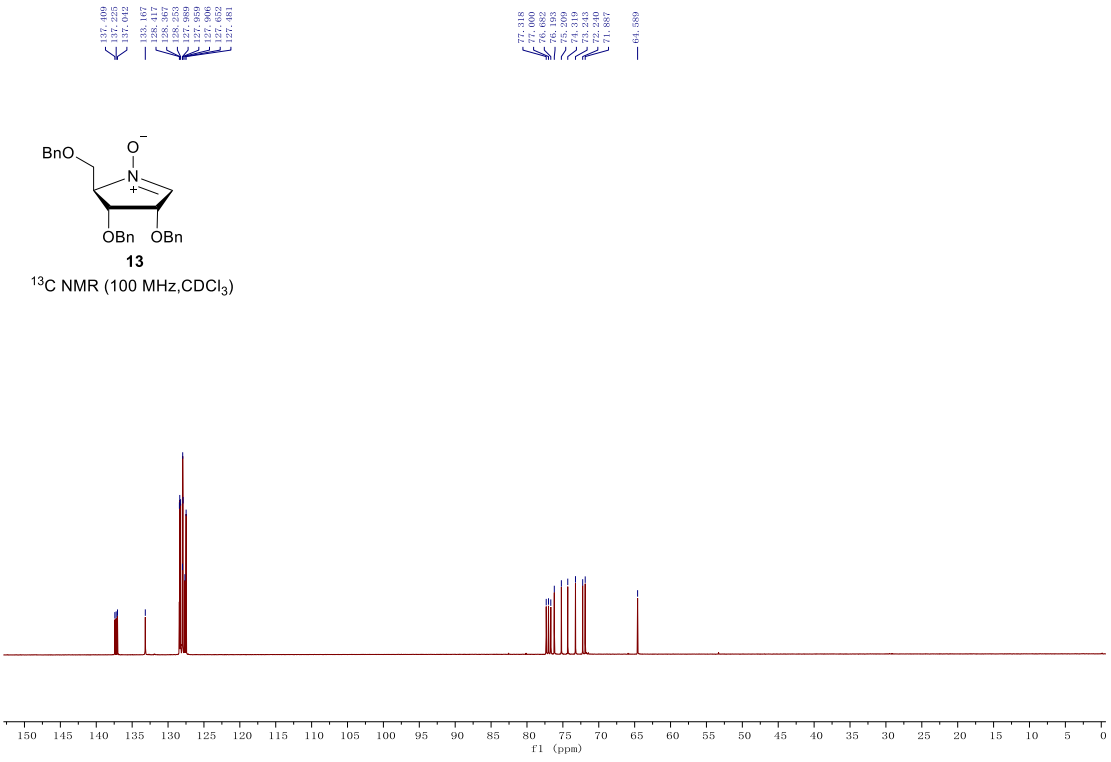
[2] K. Lukin, V. Kishore and T. Gordon, Development of a Scalable Synthesis of Oxadiazole Based S1P1 Receptor Agonists, *Org. Process Res. Dev.*, 2013, **17**, 666−671.

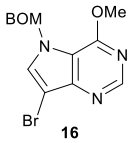
[3] (a) G. B. Evans, R. H. Furneaux, T. L. Hutchison, H. S. Kezar, P. E. Morris, Jr., V. L. Schramm and P. C. Tyler, Addition of lithiated 9-deazapurine derivatives to a carbohydrate cyclic imine: convergent synthesis of the aza-C-nucleoside immucillins. *J. Org. Chem.*, 2001, **66**, 5723–5730; (b) F. Bass, R. Lyons, N. Reilly, US Pat., 20180258091A1, 2018.

[4] T. K. Warren, J. Wells, R. G. Panchal, K. S. Stuthman, N. L. Garza, S. A. Van Tongeren, L. Dong., C. J. Retterer, B. P. Eaton, G. Pegoraro, et al, Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430, *Nature*, 2014, **508**, 402–405

4. NMR Spectra

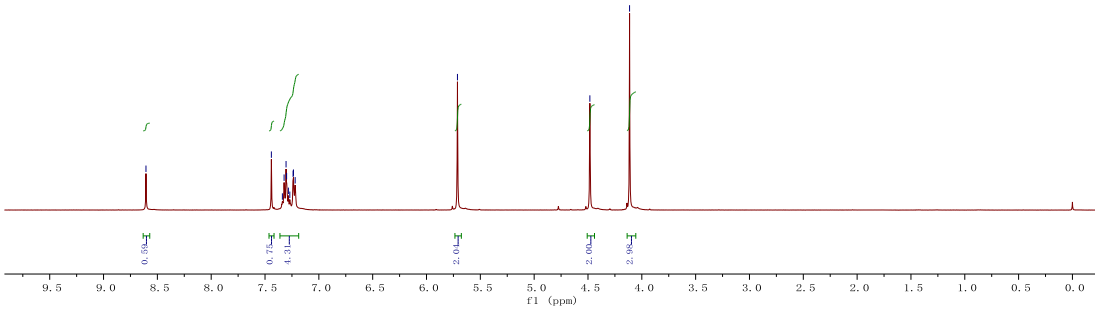


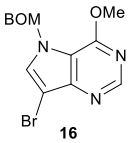




16

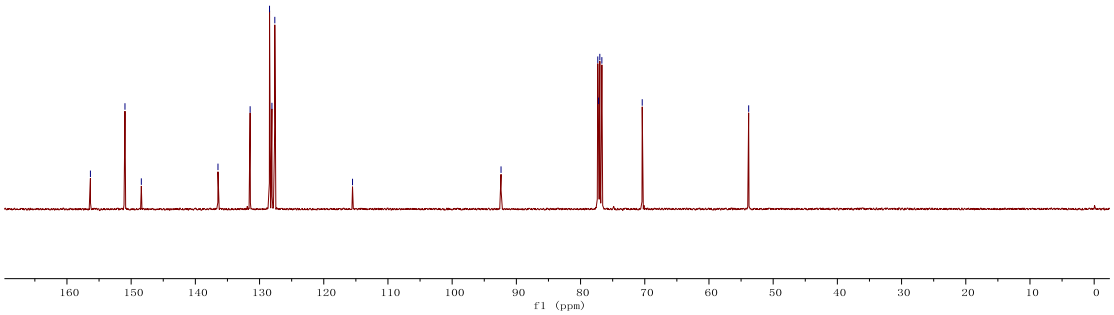
^{1}H NMR (400 MHz, $CDCl_3$)

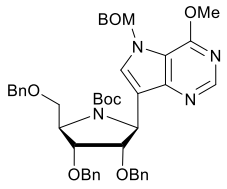




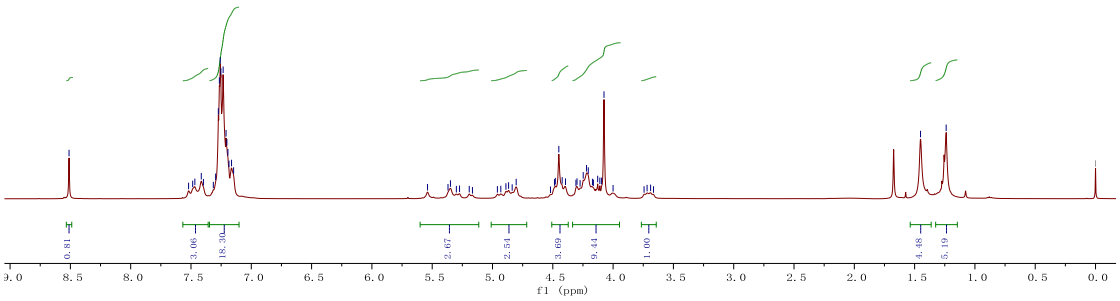
16

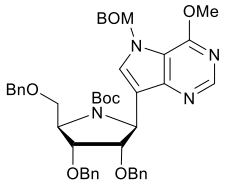
^{13}C NMR (100 MHz, $CDCl_3$)



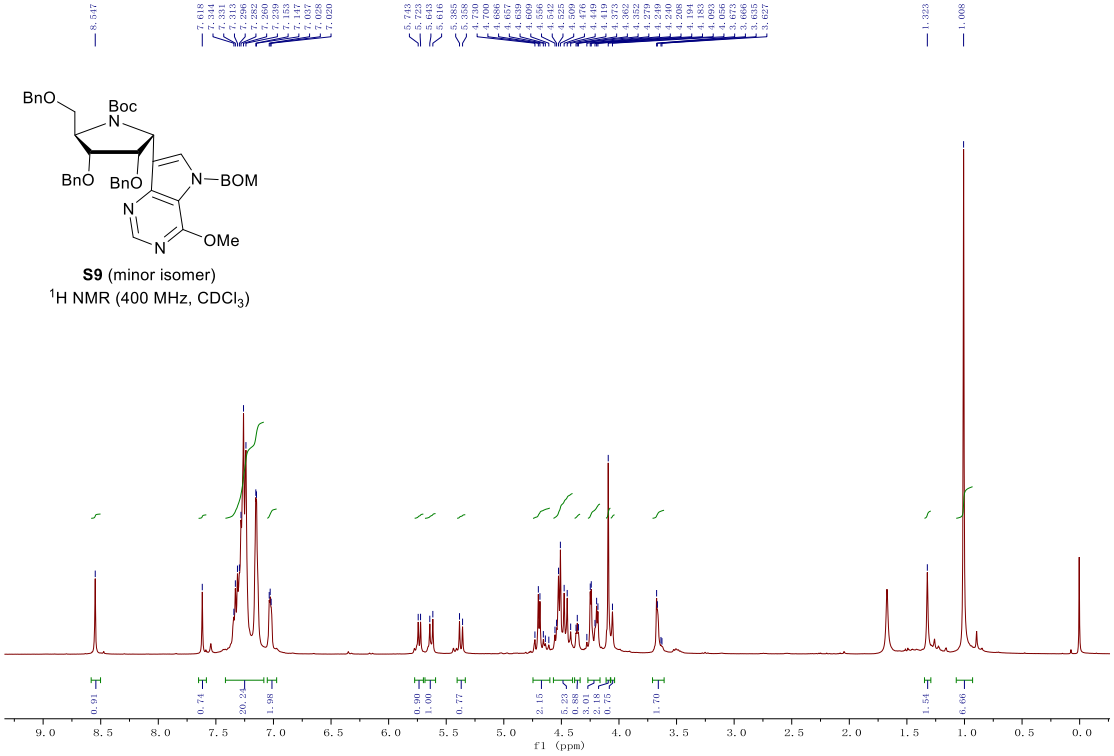


19 (major isomer)
^{1}H NMR (400 MHz, $CDCl_3$)

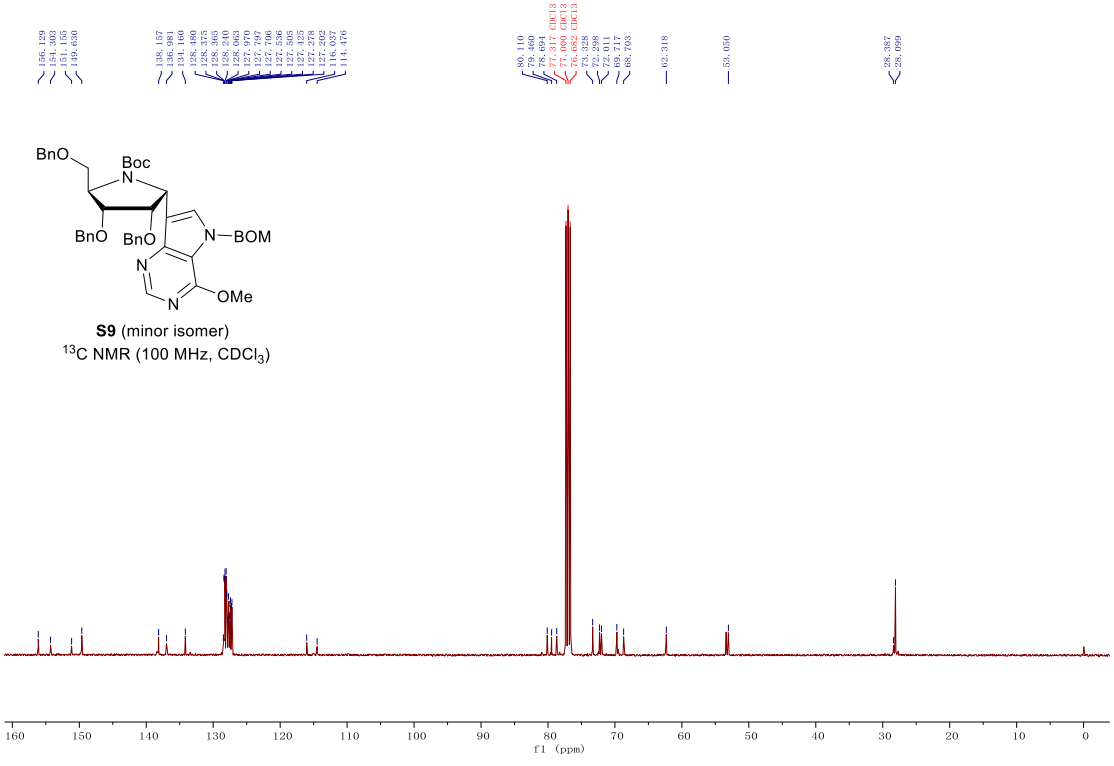




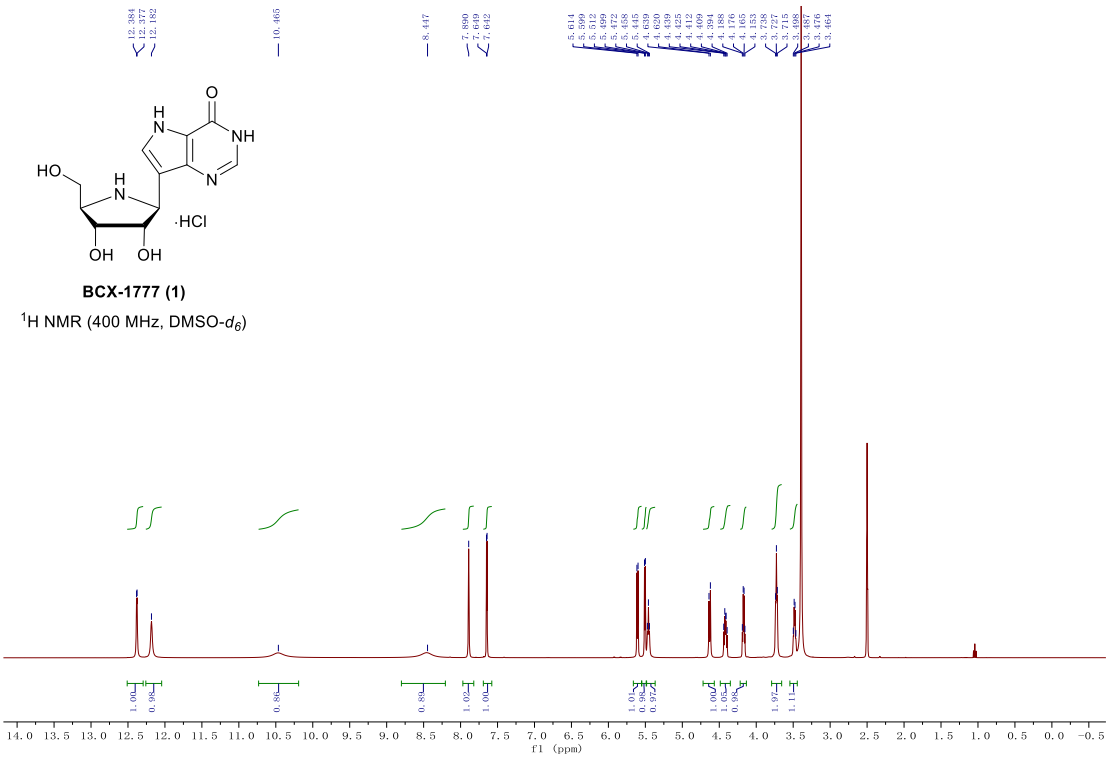
19 (major isomer)
^{13}C NMR (100 MHz, $CDCl_3$)



S9 (minor isomer)
^{1}H NMR (400 MHz, $CDCl_3$)

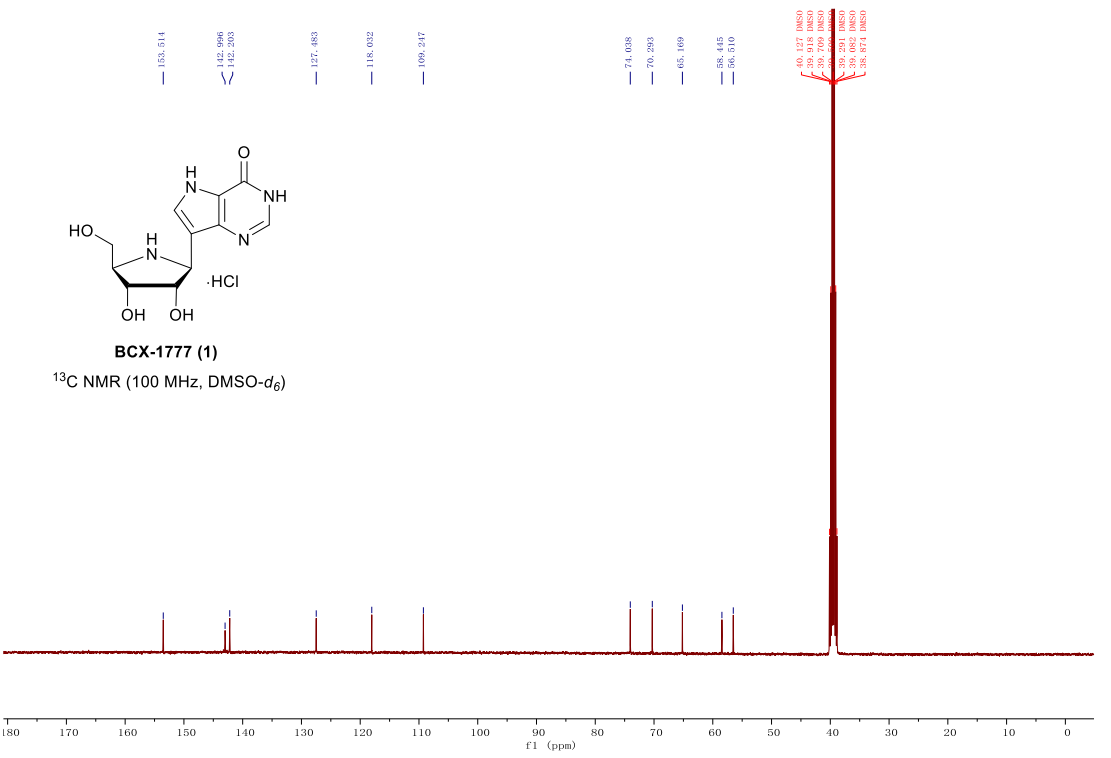


S9 (minor isomer)
^{13}C NMR (100 MHz, $CDCl_3$)



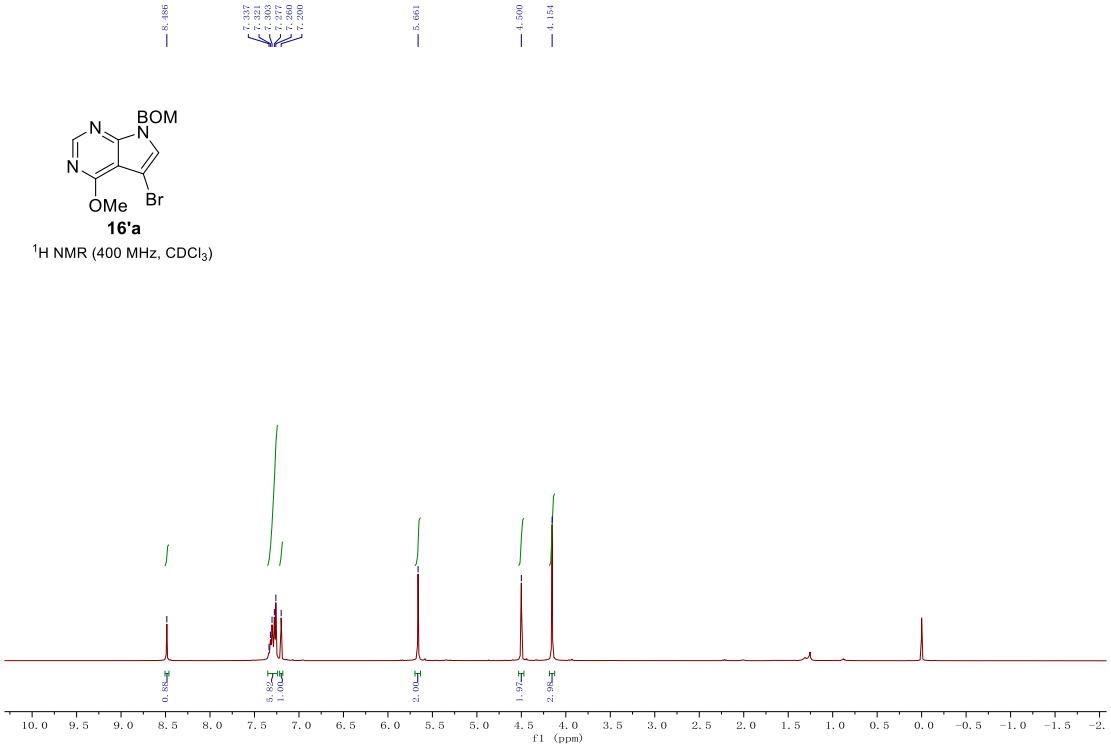
BCX-1777 (1)

^{1}H NMR (400 MHz, DMSO-d_6)



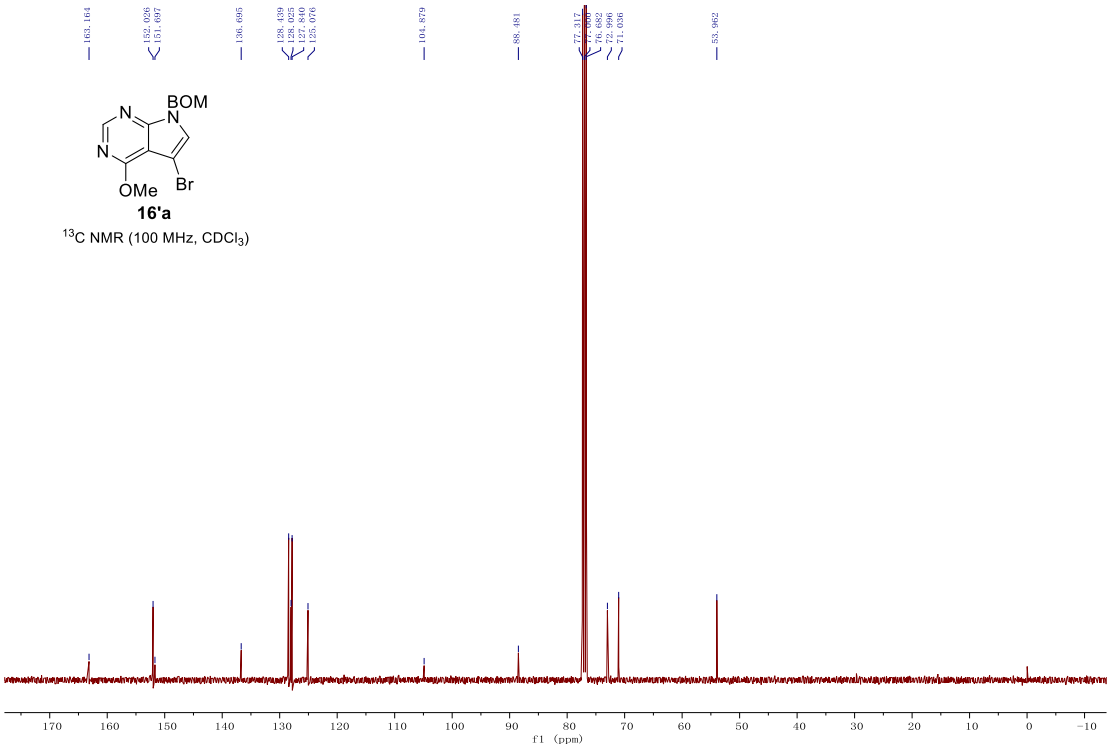
BCX-1777 (1)

^{13}C NMR (100 MHz, DMSO-d_6)



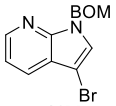
16'a

^{1}H NMR (400 MHz, $CDCl_3$)



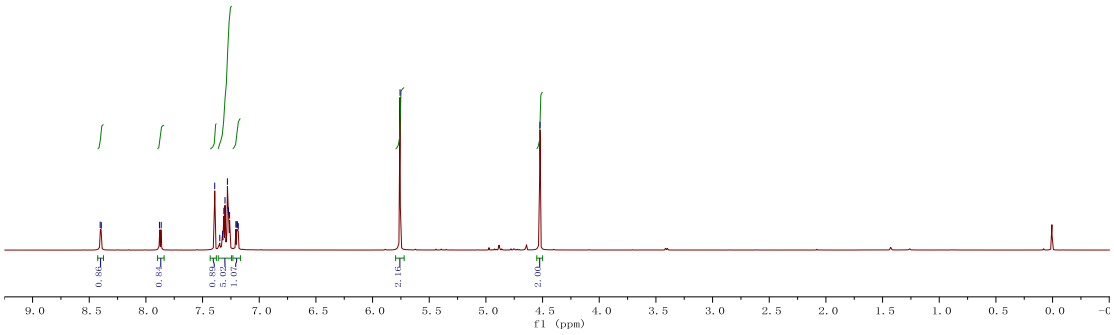
16'a

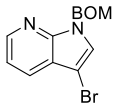
^{13}C NMR (100 MHz, $CDCl_3$)



16'd

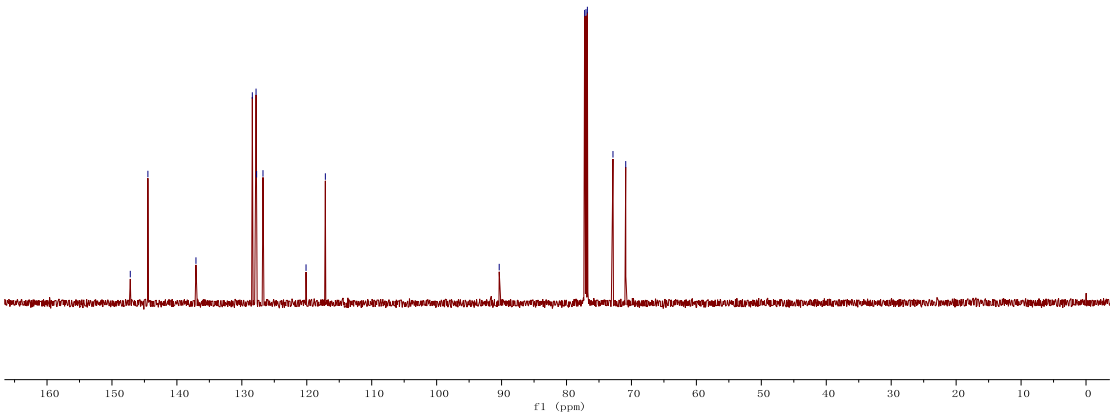
^{1}H NMR (600 MHz $CDCl_3$)

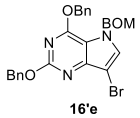




16'd

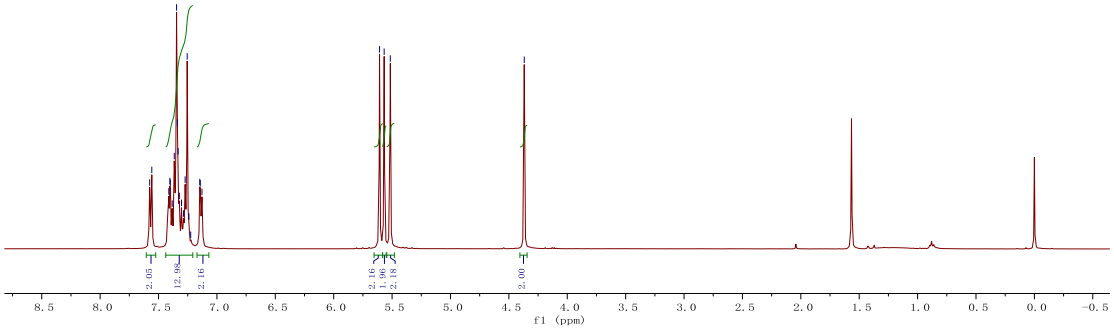
^{13}C NMR (150 MHz $CDCl_3$)

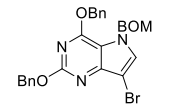




16'e

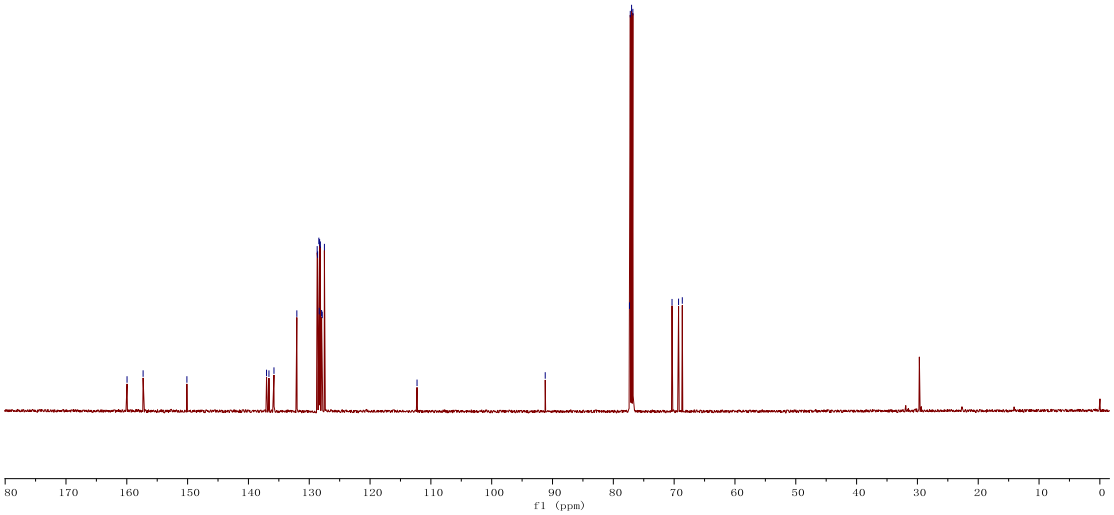
^{1}H NMR (400 MHz, $CDCl_3$)

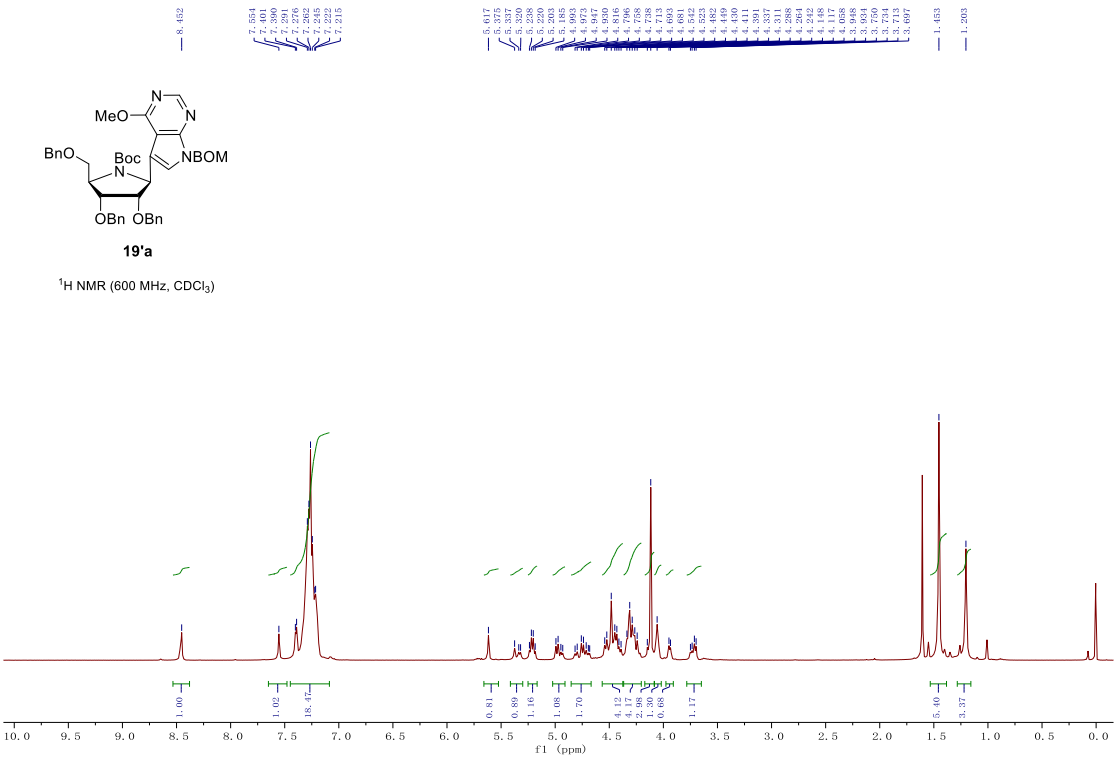


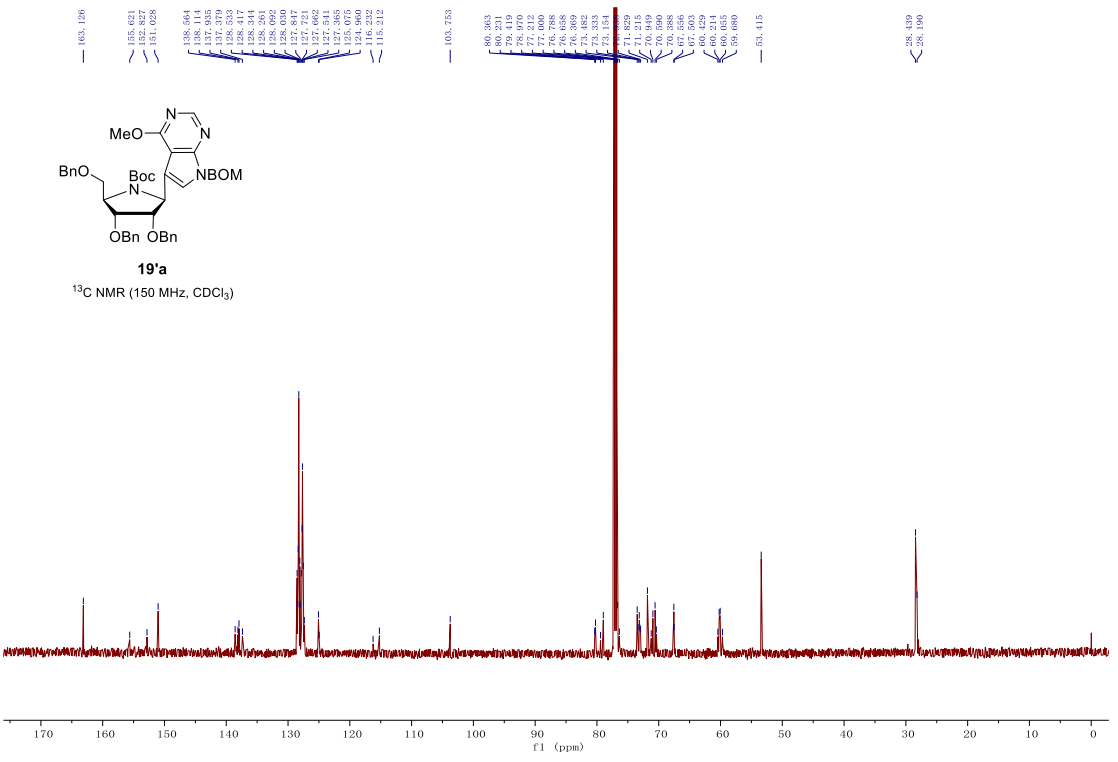


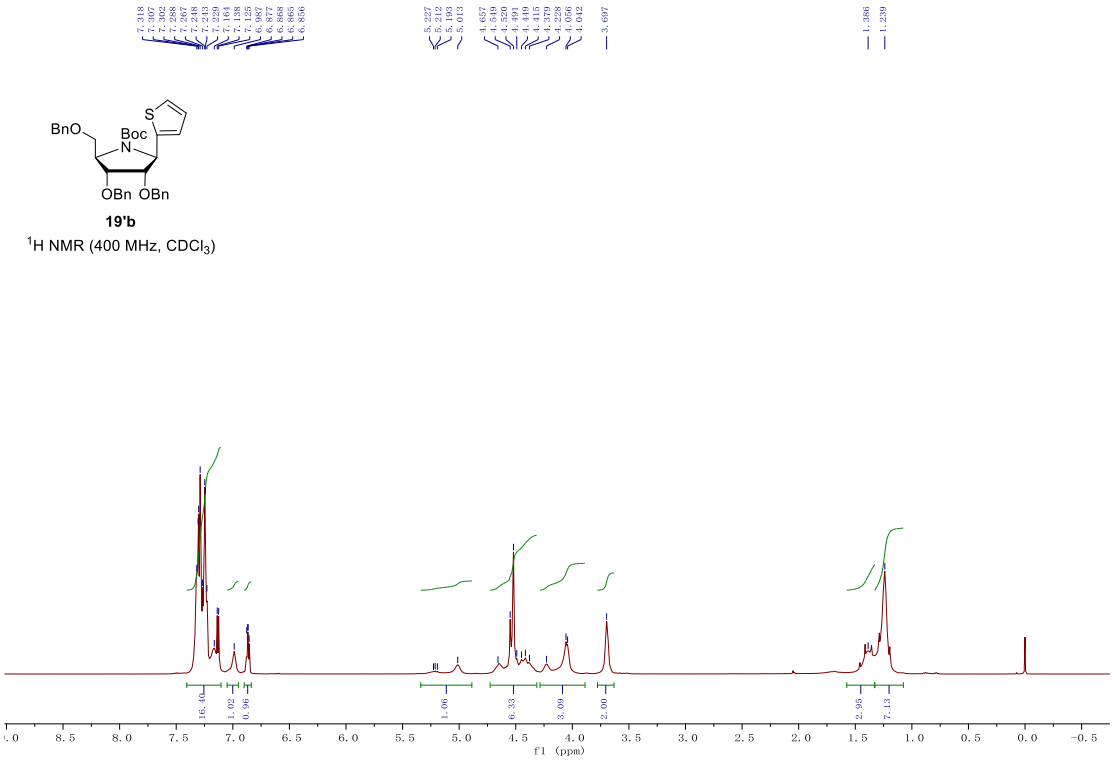
16'e

^{13}C NMR (100 MHz, $CDCl_3$)



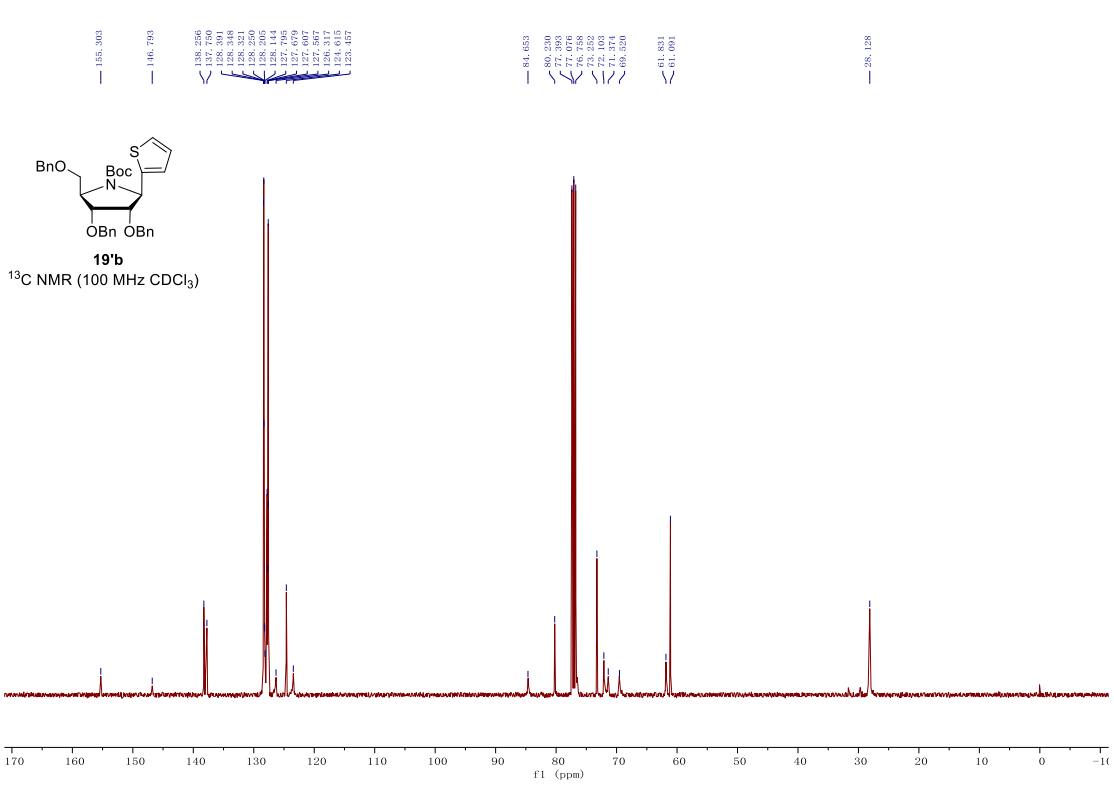

MeO
N
N
BnO
Boc
N
NBOM
OBn OBn
19'a
1H NMR (600 MHz, CDCl3)
f1 (ppm)


MeO
N
N
BnO
Boc
N
NBOM
OBn OBn
19'a
13C NMR (150 MHz, CDCl3)
f1 (ppm)



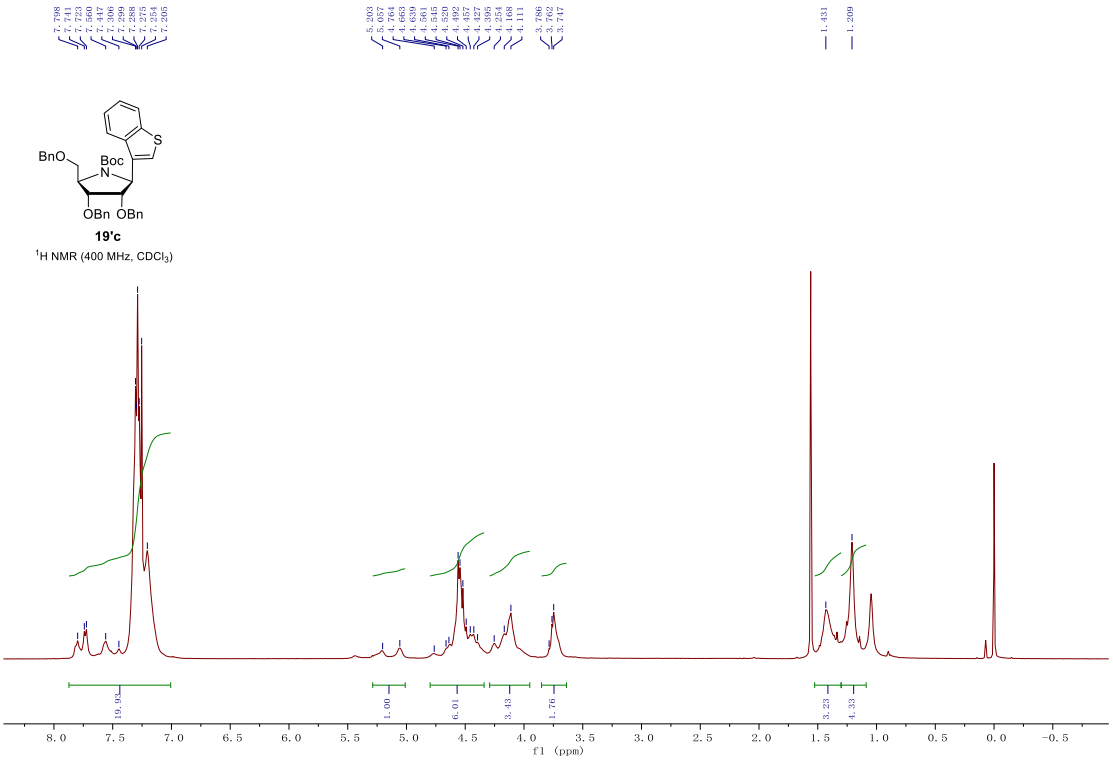
19'b

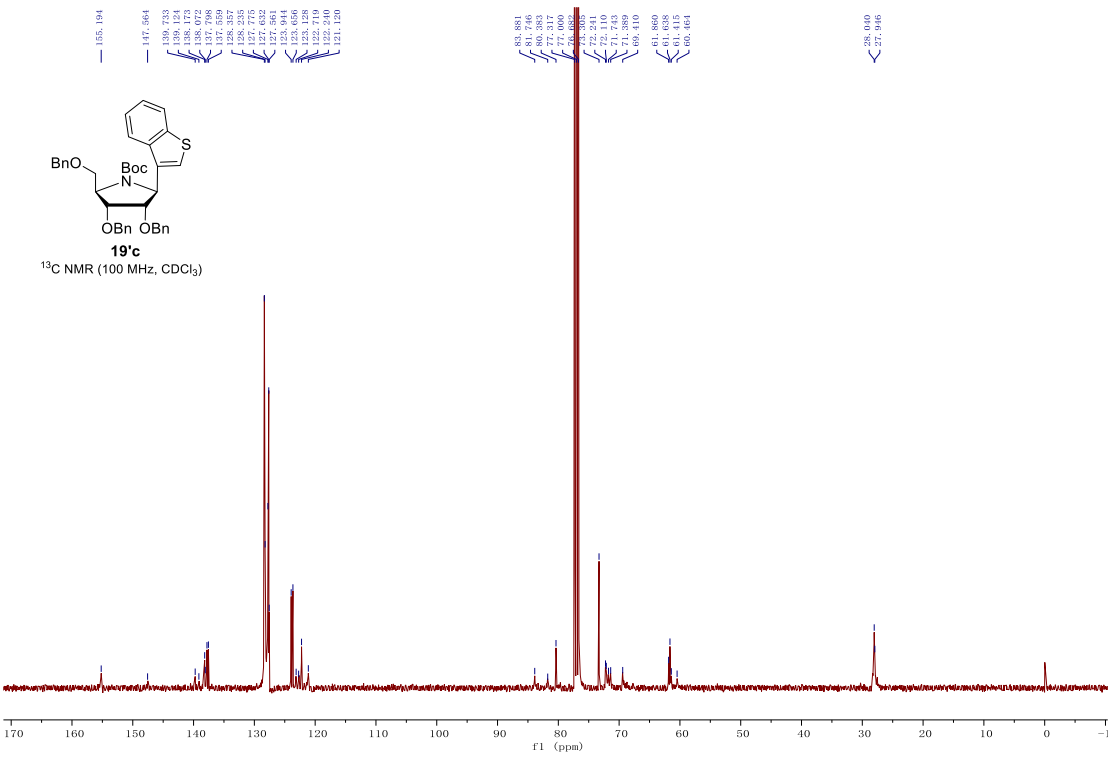
^{1}H NMR (400 MHz, $CDCl_3$)

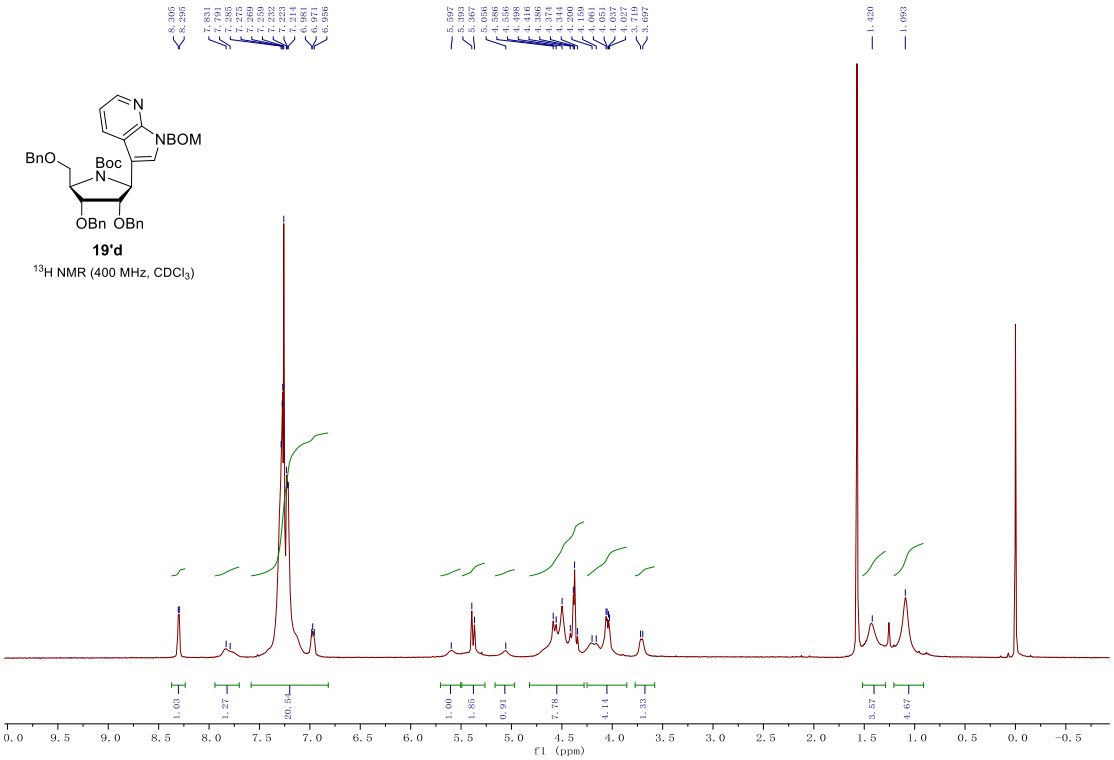


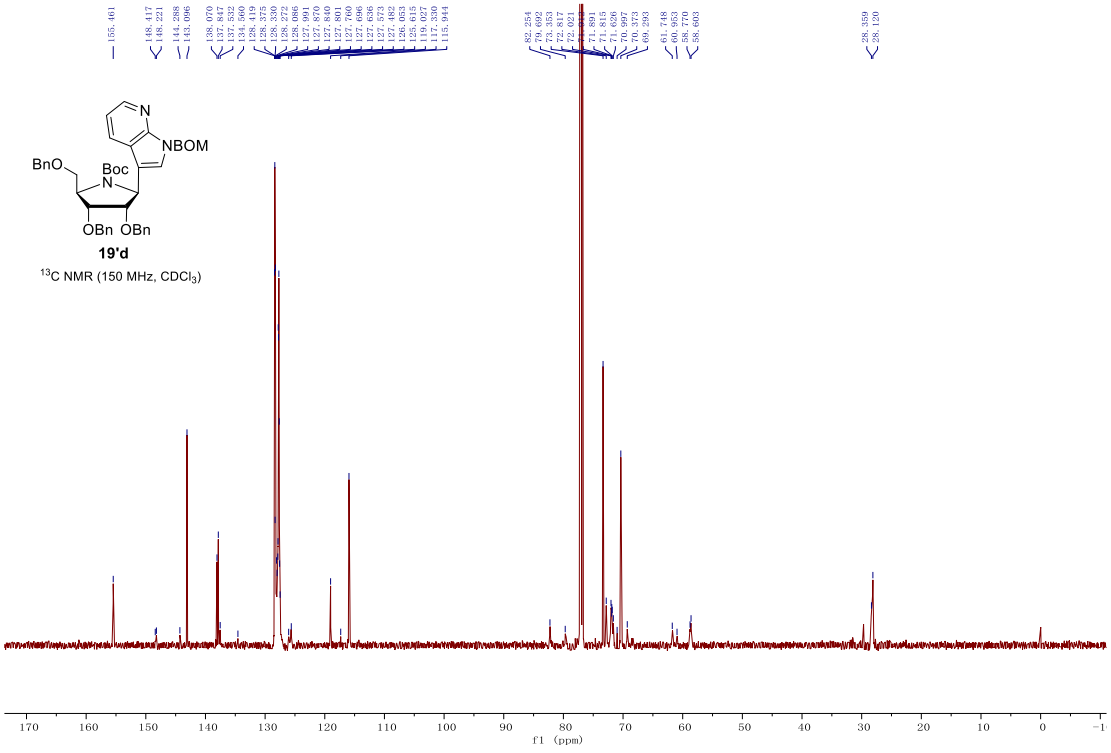
19'b

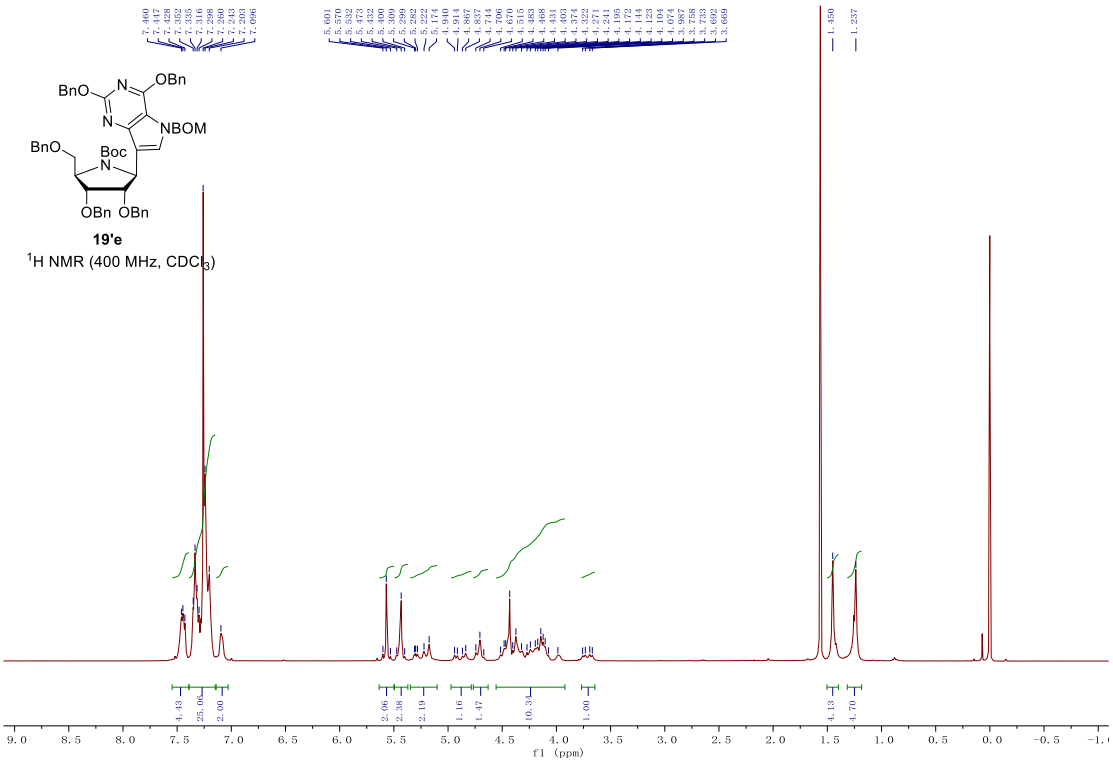
^{13}C NMR (100 MHz $CDCl_3$)

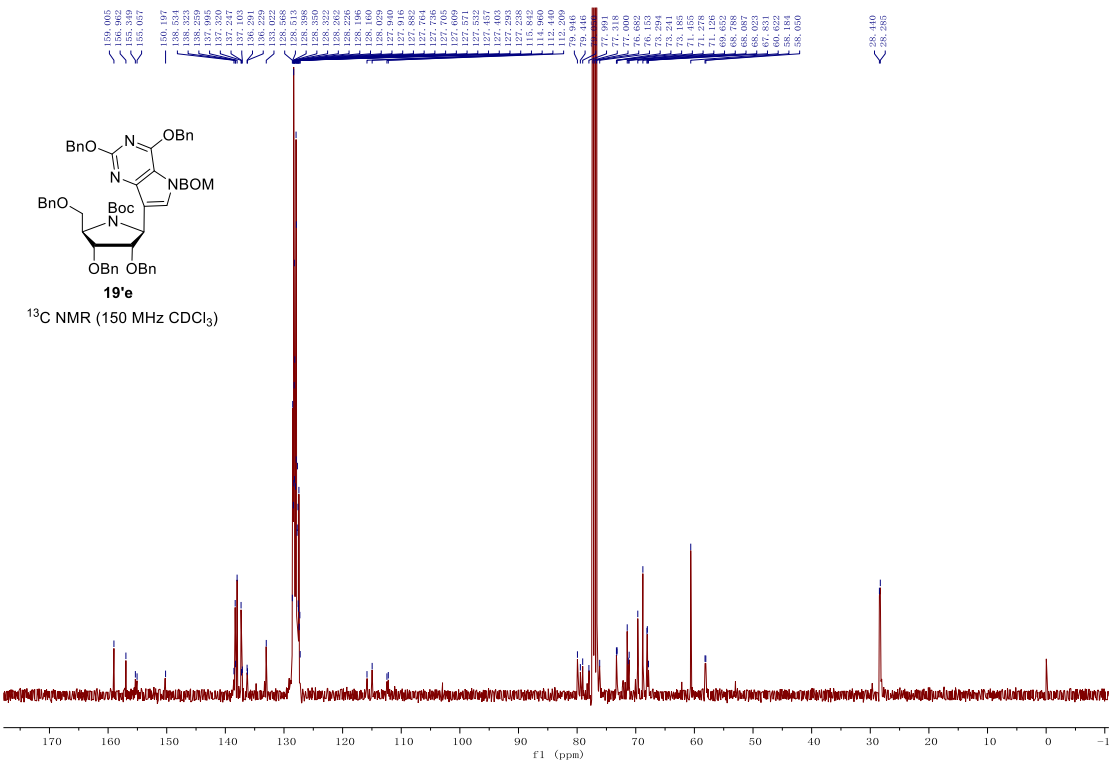

BnO
Boc
N
S
OBn OBn
19'c
1H NMR (400 MHz, CDCl3)
f1 (ppm)

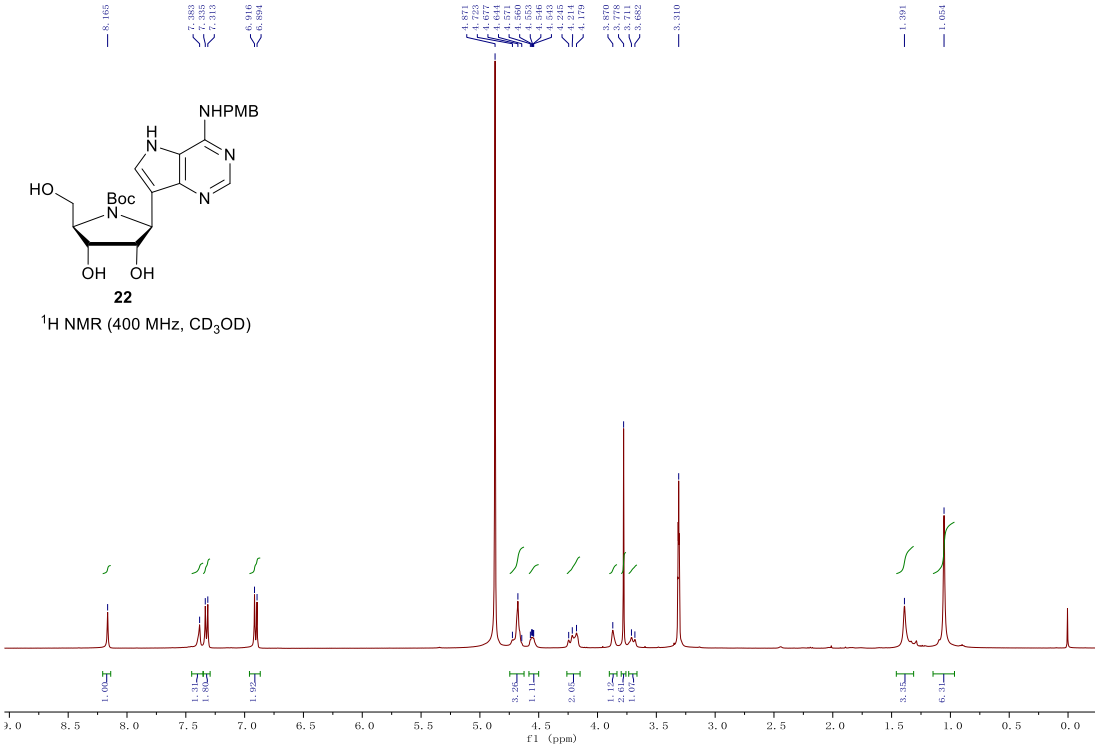

BnO
Boc
N
S
OBn OBn
19'c
13C NMR (100 MHz, CDCl3)
f1 (ppm)

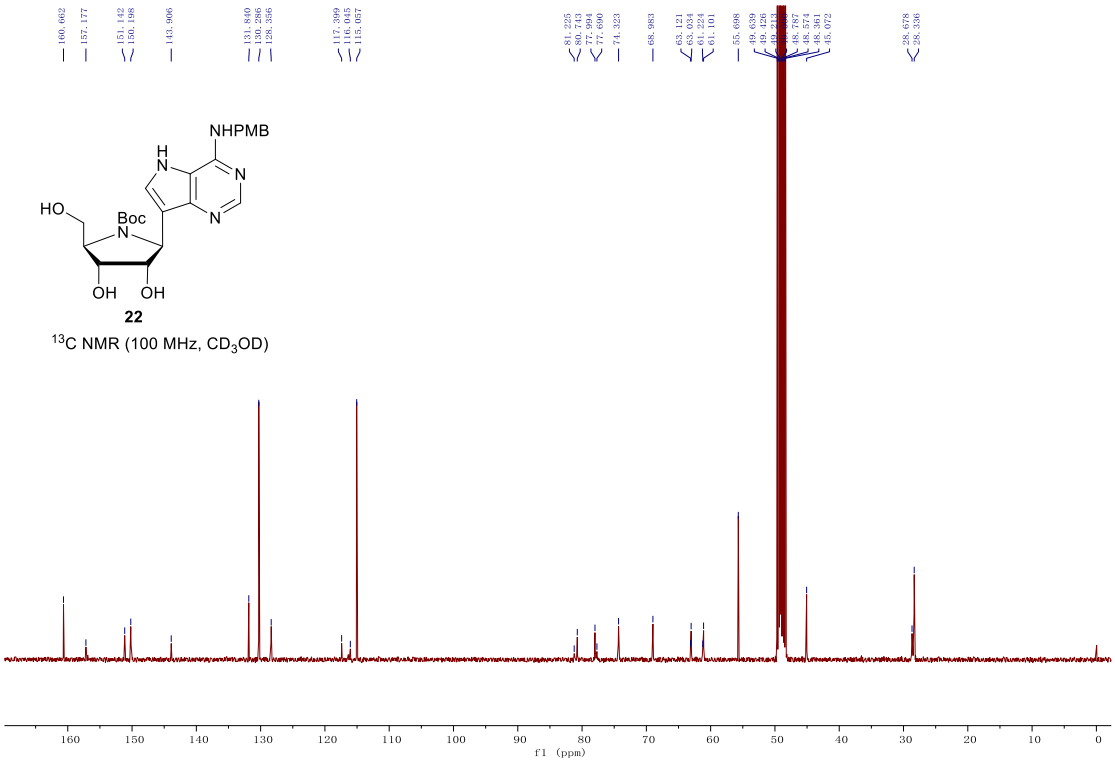

BnO
Boc
N
NBOM
OBn OBn
19'd
13H NMR (400 MHz, CDCl3)
f1 (ppm)

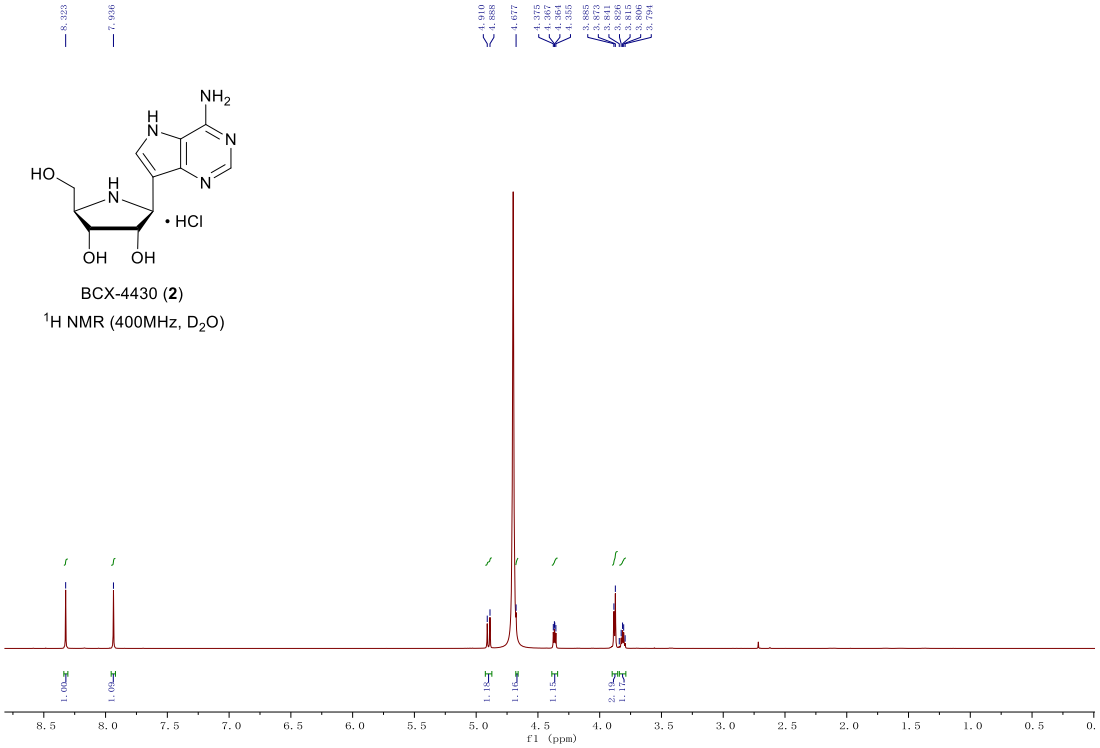

BnO
Boc
N
NBOM
OBn OBn
19'd
13C NMR (150 MHz, CDCl3)
f1 (ppm)


BnO
OBn
N
NBOM
Boc
OBn OBn
19'e
1H NMR (400 MHz, CDCl3)
f1 (ppm)


BnO
OBn
N
NBOM
Boc
OBn OBn
19'e
13C NMR (150 MHz CDCl3)
f1 (ppm)

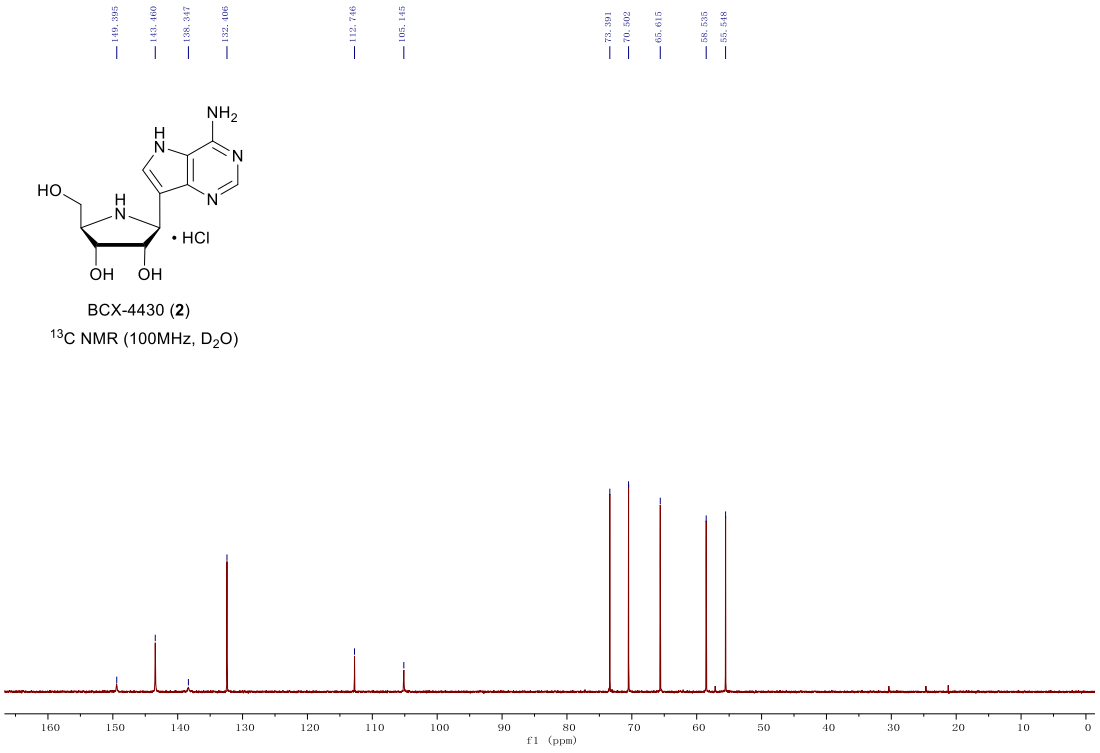

NHPMB
HO
Boc
OH
OH
22
1H NMR (400 MHz, CD3OD)
f1 (ppm)


NHPMB
HO
Boc
OH
OH
22
13C NMR (100 MHz, CD3OD)
f1 (ppm)



BCX-4430 (**2**)

^{1}H NMR (400MHz, D_2O)



BCX-4430 (**2**)

^{13}C NMR (100MHz, D_2O)