nature portfolio

https://doi.org/10.1038/s41557-021-00662-w

Supplementary information

Automated synthesis of prexasertib and derivatives enabled by continuous-flow solid-phase synthesis

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SUPPLEMENTARY INFORMATION

Automated Synthesis of Prexasertib and Derivatives Enabled by Continuous-Flow Solid-Phase Synthesis

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Materials and Methods
Supplementary Text
Supplementary Figures 1 to 64
Supplementary Tables 1 to 11

Other Supplementary Materials for this manuscript include the following:

Supplementary Movie

Contents

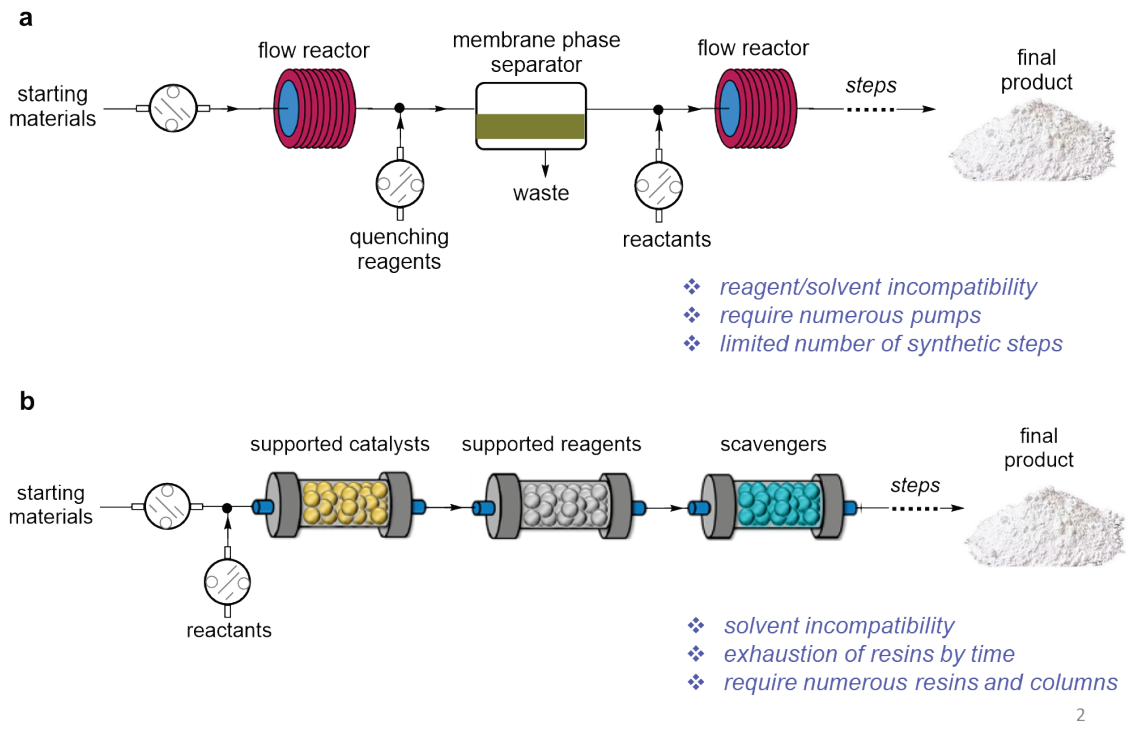
1. General Information

Unless otherwise indicated, all commercially available reagents were used directly from the supplier without further purification. Tetrahydrofuran (THF), dichloromethane (DCM), dimethylformamide (DMF), 1,4-dioxane, diethyl ether (Et_2O) and dimethyl sulfoxide (DMSO) were high-performance liquid chromatography (HPLC) grade and anhydrous. Ethyl acetate (EtOAc), Methanol (MeOH) and ethanol (EtOH) were analytical grade, and directly used without further purification.

All microwave reactions were performed on a CEM™ Discover SP Microwave System. Infrared spectra were recorded in potassium bromide (KBr) pellets using a Nicolet AVATER FTIR380 spectrometer. All high-resolution mass spectra (HRMS) were obtained on a Finnigan/MAT 95XL-T spectrometer. Optical rotation was obtained on an Anton Paar MCP-100 digital polarimeter using a 1 cm^3 glass cell. The melting points were measured by Buchi B-540 melting point apparatus. Analytical-HPLC was performed using Agilent reversed phase column (4.6 x 150 mm, pore size: 95 Å, particle size: 5 µm, phase: eclipse Plus C18, part number: 959993-902).^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature in DMSO-d_6 (2.50 ppm for ^{1}H NMR, 39.52 ppm for ^{13}C NMR) or methanol-d_4 (3.34 ppm for ^{1}H NMR, 49.0 ppm for ^{13}C NMR) using Bruker 400 MHz and Bruker 500 MHz spectrometers. Chemical shift values are expressed as parts per million (ppm), and *J*-values are in hertz (Hz). Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, q: quartet, p: pentet, or combination, brs: broad singlet, or m: multiplet. Analytic HPLC was performed using a Shimadzu Prominence System equipped with a Shim Pack GVP-ODS 2.0 mm C18 column (5 µM, 120Å, 250 mm x 4.60 mm i.d.) at room temperature. Preparative HPLC was performed using a Waters e2695 Separations Module equipped with a C18 column (Luna 5µM, C18(2) 100 Å, LC Column 250 × 10 mm) with a PDA detector at room temperature.

The human lymphocytic leukemia (non-T, non-B) cell line (REH) and human T-lymphoblastic leukemia (MOLT4) were cultured in RPMI 1640 media (HyClone, CA, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, CA, USA) and 2 mM L-glutamine (Gibco, CA, USA). Cells were seeded in 96 well plates, followed by treatment of prexasertib derivatives for 48 hours. The Cell Counting Kit-8 colorimetric assay (CCK8, Dojindo, Japan) was used to determine the percentage of cell viability.

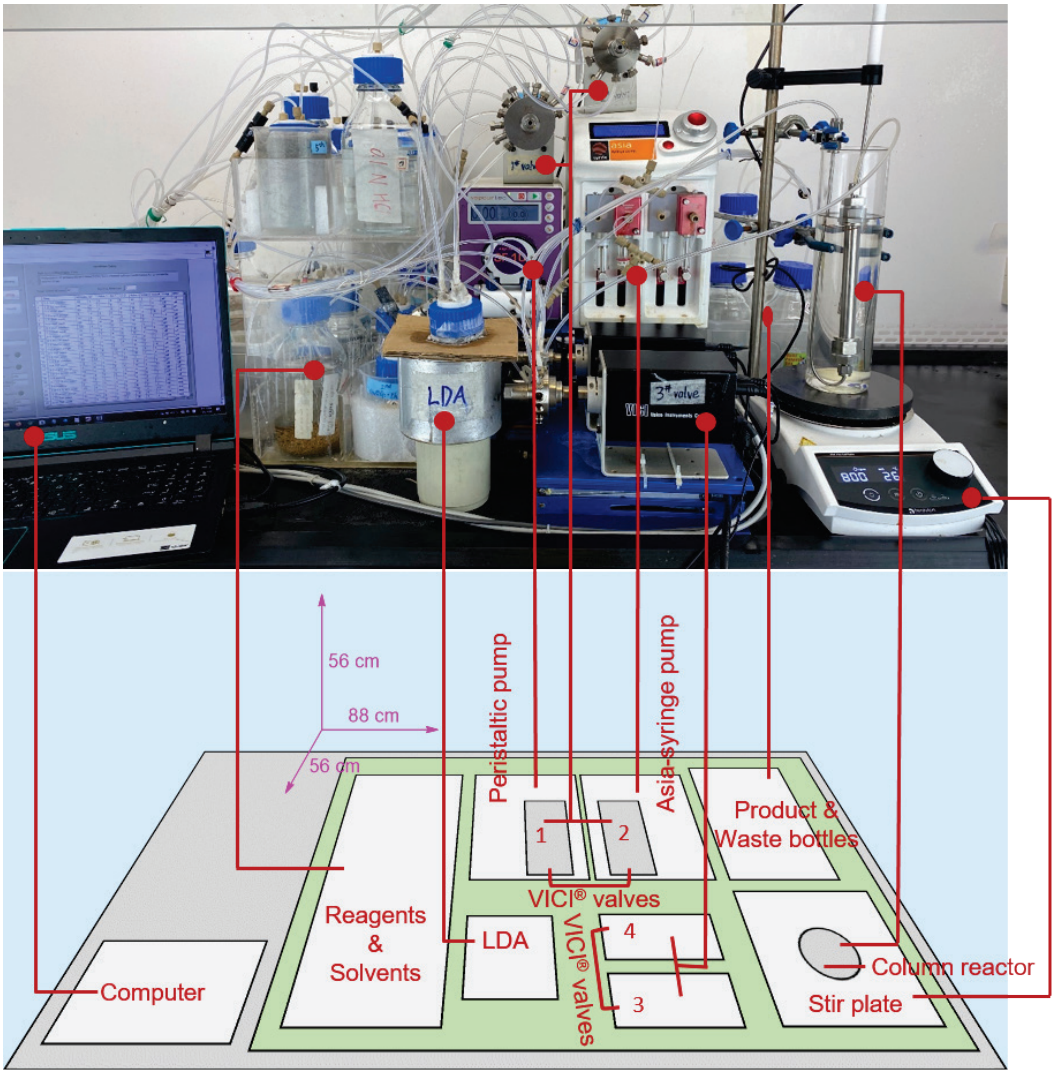
2. State of the art for multistep continuous-flow synthesis



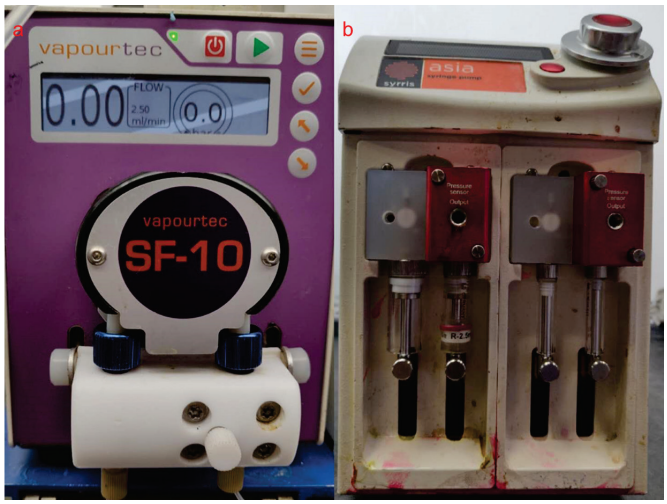
Supplementary Figure 1. State of the art for multistep continuous-flow synthesis of APIs. a, Based on inline membrane-based phase separators. **b**, Based on polymer supported reagents and scavengers.

3. Description of the automated synthesizer and its components

Images of the automated SPS-flow platform and components are shown in Supplementary Figs. 2 to 7. Back pressure regulators (BPRs), high-purity perfluoroalkoxy polymer (HPFA) tubing, fittings (except stainless steel fittings), and bottom filters were purchased from IDEX Health & Science Technologies. Stainless steel tubing and fittings were purchased from Swagelok Ltd. The SF-10 peristaltic pump was purchased from Vaportec Ltd. The Asia syringe pump was purchased from Syrris Ltd. Selection valves were purchased from Valco Instruments Co. Inc. A remote-controllable magnetic stirrer was purchased from Heidolph Instruments GmbH & CO. KG.

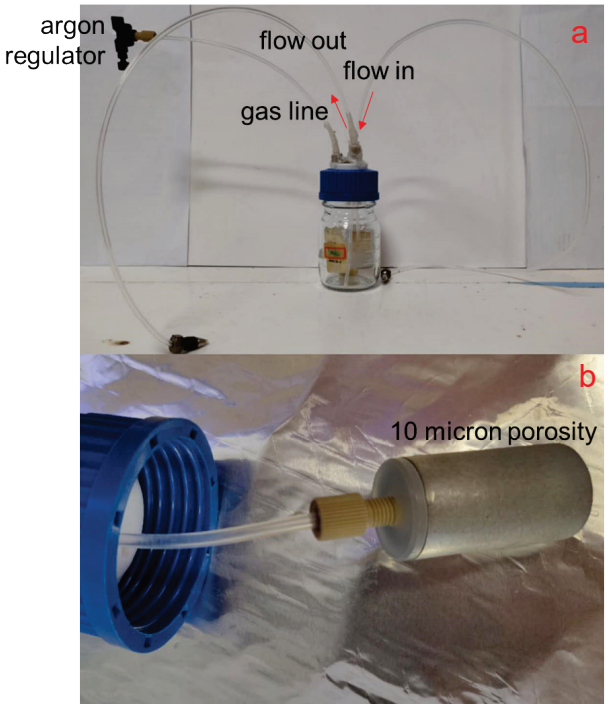


Supplementary Figure 2. Photograph and interpretation of the assembled automated SPS-flow system.

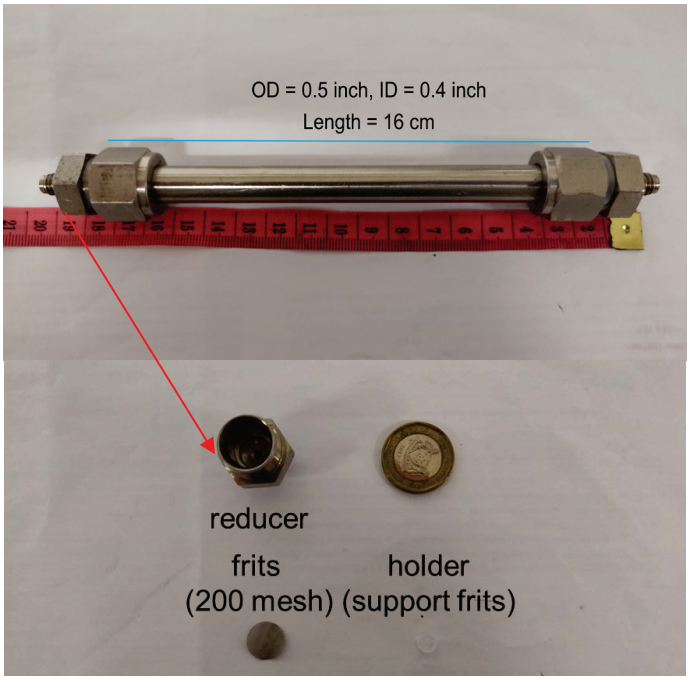


Supplementary Figure 3. Photograph of the pumps. a, Pump #1, Vaportec SF-10 peristaltic pump, 10 bar max. back pressure, 10 mL/min max. flow rate. **b**, Pump #2, Syrris Asia syringe pump, 20 bar max. back pressure, 10 mL/min max. flow rate for the left channel, 2.5 mL/min max. flow rate for the right channel.

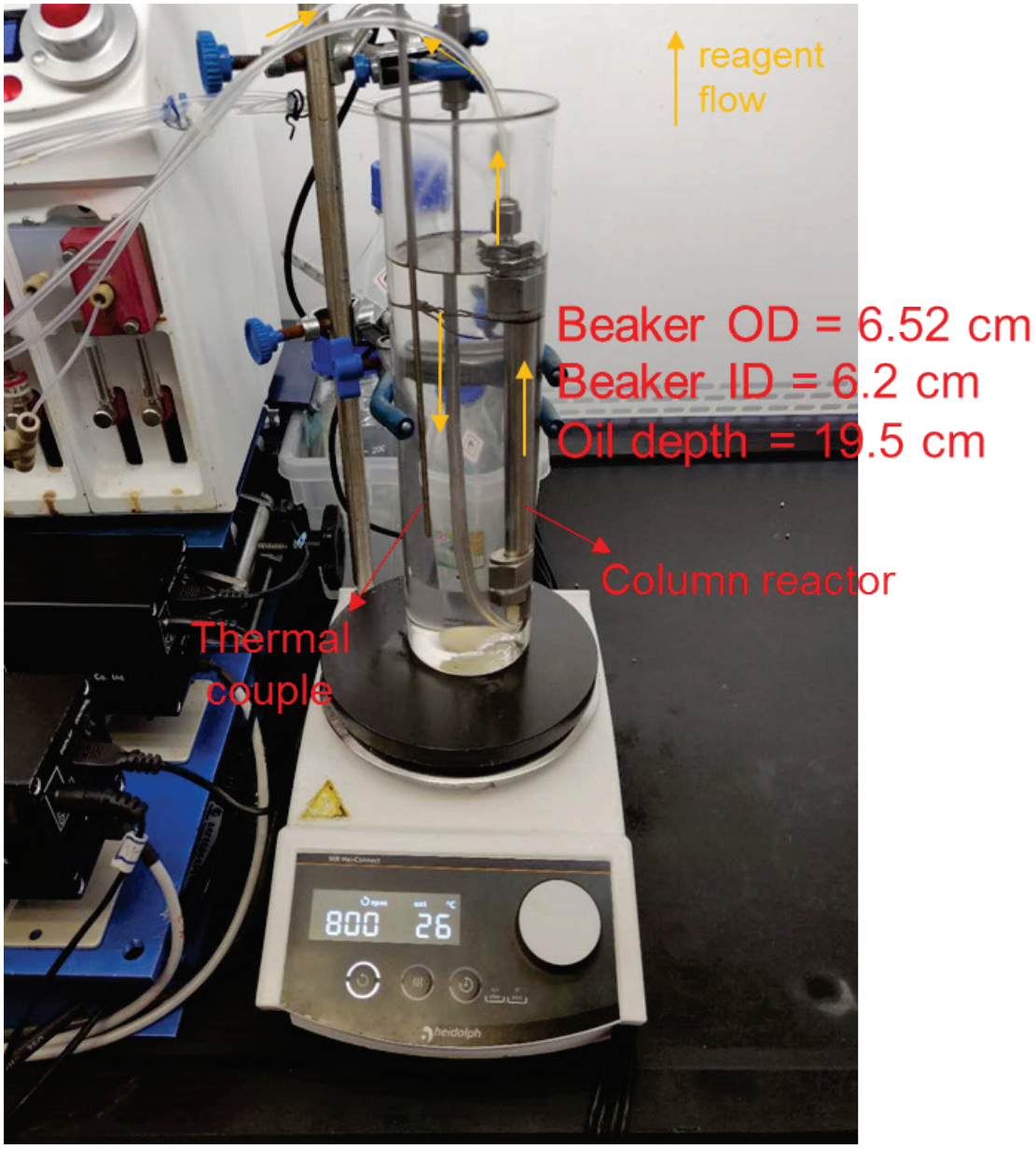
Supplementary Figure 4. Photograph of the multiway selector valves. a, 10-position selector valve, VICI EUTA-2SD10MWE. **b**, 16-position selector valve, VICI EUTA-2SD16MWE. **c**, Profile view of the selector valve.



Supplementary Figure 5. Photograph of the reagent storage bottles. a, 100 mL 3-port GL45 screw-capped bottle. **b**, Solvent inlet filter for the 2nd step.

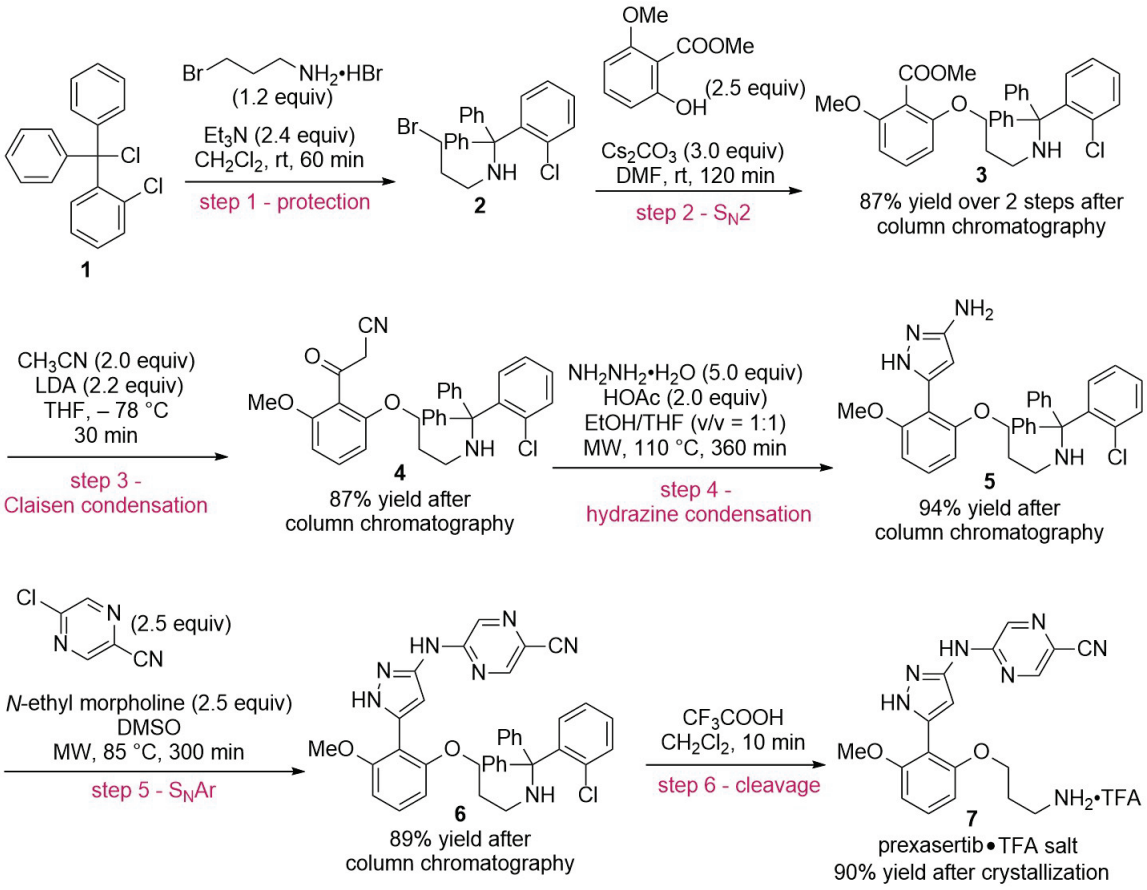


Supplementary Figure 6. Photograph of the column reactor.



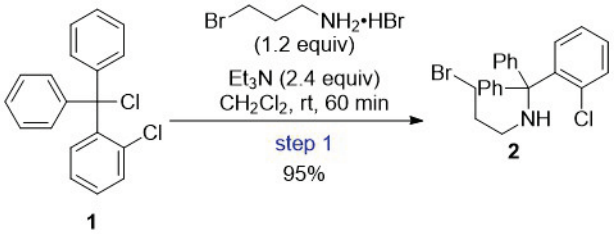
Supplementary Figure 7. Photograph of the oil bath and the heating plate.

4. Solution-batch synthesis of prexasertib TFA salt



Supplementary Figure 8. Scheme for the solution batch synthesis of prexasertib TFA salt.

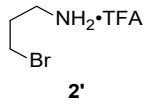
Step 1-protection



To a dry flask containing **1** (2-chlorotrityl chloride) (9.2 mmol, 2.87 g) and amine hydrobromide (11.0 mmol, 2.40 g) was added CH_2Cl_2 (300 mL). Under stirring, Et_3N (22.0 mmol, 3.1 mL) was added dropwise to the resulting mixture over 5 min. The amine hydrobromide salt gradually disappeared within 2 min. The resulting solution was stirred at room temperature for 60 min. Then, saturated aqueous NH_4Cl (200 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (100 mL × 2). The combined organic phase was washed with water (100 mL) and brine (100 mL),

dried over Na_2SO_4, and concentrated in vacuum. **2** was obtained as a white solid (3.62 g, 95%) without purification and was ready for use in the following step.

To a solution of **2** (200 mg) in 10 mL CH_2Cl_2 was added 0.1 mL CF_3COOH. The resulting mixture was stirred at room temperature for 10 min and then concentrated under vacuum. The residue was purified by flash column chromatography over silica gel (CH_2Cl_2/CH_3OH 10:1) to afford **2'** (103 mg).

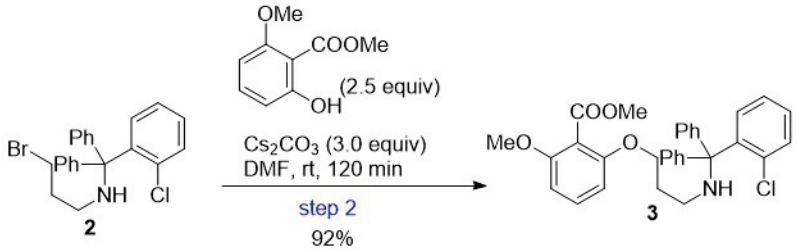


2'

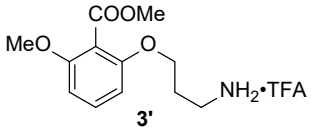
^{1}H NMR (500 MHz, methanol-d_4) δ 3.53 (t, J = 6.4 Hz, 2H), 3.08 (t, J = 7.6 Hz, 2H), 2.23 – 2.16 (m, 2H); ^{13}C NMR (125 MHz, methanol-d_4) δ 39.4, 31.4, 29.8.

*See Supplementary Fig. 24 for the NMR spectra of compound **2'**.*

Step 2-S_N2



To a dry flask containing methyl 2-hydroxy-6-methoxybenzoate (18.1 mmol, 3.3 g) and Cs_2CO_3 (21.8 mmol, 7.08 g) was added anhydrous DMF (150 mL). The resulting mixture was stirred at rt for 30 min and then kept stationary for 10 min. Then, the upper clear solution was transferred to another dry flask containing **2** (7.26 mmol, 3.0 g) via a cannula. The reaction mixture was stirred at room temperature for 120 min and then quenched by the addition of aqueous 0.1 N HCl (50 mL) and extracted with EtOAc (100 mL × 3). The combined organic layer was washed successively with H_2O (150 mL × 3) and brine (200 mL) and then dried over Na_2SO_4 and concentrated under vacuum. Purification of the residue via flash chromatography on silica gel (*n*-hexane/EtOAc = 8:1) afforded **3** as a solid (3.45 g, 92% yield). Then, 200 mg **3** and CH_2Cl_2 (20 mL) was stirred at room temperature for 2 min, followed by the addition of CF_3COOH (0.1 mL). The resulting mixture was stirred at room temperature for 5 min and then concentrated under vacuum. The residue was purified by flash column chromatography over silica gel (CH_2Cl_2/CH_3OH = 10:1) to afford **3'** (110 mg).



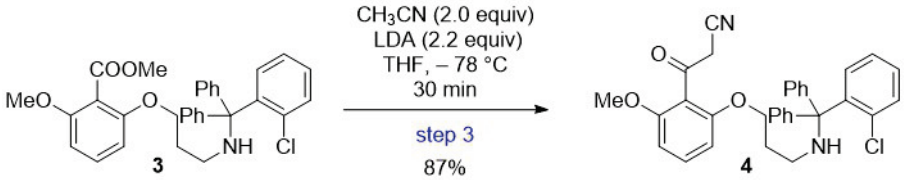
3'

^{1}H NMR (500 MHz, methanol-d_4) δ 7.35 (t, J = 8.4 Hz, 1H), 6.71 (d, J = 6.8, 1H), 6.69 (d, J = 6.8, 1H), 4.16 (t, J = 5.6 Hz, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.11 (t, J = 7.0 Hz, 2H), 2.12 – 2.07 (m, 2H); ^{13}C NMR (125 MHz, methanol-d_4) δ 169.1, 163.3 (q, J = 35 Hz, 1C), 159.1, 157.6, 132.9,

118.0 (q, J = 290 Hz, 1C), 114.0, 106.0, 105.7, 67.5, 56.5, 52.8, 38.8, 28.0; HRMS calculated for $C_{12}H_{18}NO_4^+$ ($M+H^+$) 240.1230, found 240.1231.

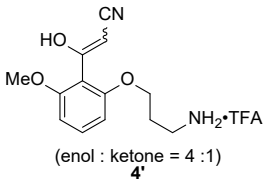
*See Supplementary Fig. 25 for the NMR spectra of compound **3'**.*

Step 3-Claisen condensation



A solution of *i*-Pr_2NH (12.8 mmol, 1.79 mL) in THF (150 mL) was cooled to –78 °C, followed by the dropwise addition of *n*-BuLi (2 M in cyclohexane, 6.4 mL). The reaction solution was stirred at –78 °C for 5 min, followed by the dropwise addition of CH_3CN (11.6 mmol, 0.61 mL), and then stirred at –78 °C for 10 min. A solution of **3** (5.82 mmol, 3.00 g) in 10 mL THF was added to the above reaction solution. The resulting mixture continued to be stirred at –78 °C for 30 min, after which it was allowed to warm to room temperature and quenched by the addition of 0.5 N HCl (100 mL). The mixture was extracted with EtOAc (100 mL × 2). The combined organic layer was washed successively with H_2O (150 mL) and brine (150 mL), dried over Na_2SO_4, and concentrated under vacuum. Purification of the residue via flash chromatography over silica gel (*n*-hexane/EtOAc = 8:1 to 4:1) afforded **4** (2.65 g, 87%) as a solid.

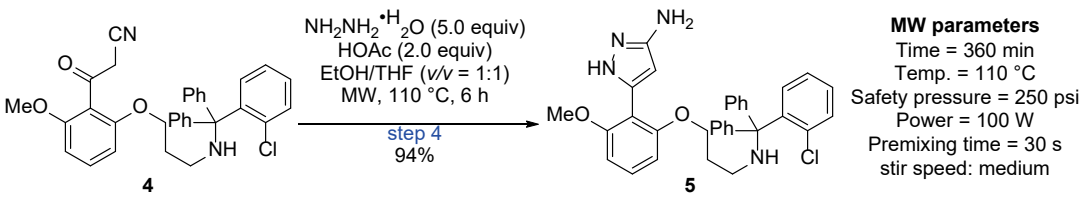
Then, 100 mg **4** and CH_2Cl_2 (20 mL) were stirred at room temperature for 2 min, followed by the addition of CF_3COOH (0.1 mL). The resulting mixture was stirred at room temperature for 5 min and then concentrated under vacuum. Purification of the residue via preparative HPLC afforded **4'** (65 mg). Preparative HPLC separation was carried out at a flow rate of 5 mL/min (solvent A = 0.1% v/v TFA in water; solvent B = 0.1% v/v TFA in acetonitrile).



^{1}H NMR (400 MHz, methanol-d_4) δ 7.49 (t, J = 8.6 Hz, 0.8H), 7.44 – 7.37 (m, 0.32H), 6.82 (d, J = 8.6 Hz, 0.8H), 6.78 (d, J = 8.6 Hz, 0.8H), 6.76 – 6.71 (m, 0.4H), 4.22 (t, J = 5.6 Hz, 1.6H), 4.20 – 4.15 (m, 0.4H), 3.93 (s, 2.2H), 3.88 (s, 0.33H), 3.86 (s, 0.21H), 3.23 – 3.16 (m, 2H), 2.19 – 2.13 (m, 2H); ^{13}C NMR (125 MHz, methanol-d_4) δ 193.4, 159.7, 159.7, 157.84, 157.83, 134.8, 133.0, 132.9, 117.5, 115.9, 106.3, 106.0, 105.9, 105.7, 105.6, 105.5, 67.9, 67.0, 66.8, 56.6, 56.5, 56.5, 39.0, 38.7, 38.6, 28.3, 28.3, 27.9; HRMS calculated for $C_{13}H_{17}N_2O_3^+$ ($M+H^+$) 249.1234, found 249.1236.

*See Supplementary Fig. 26 for the NMR spectra of compound **4'**.*

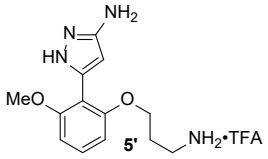
Step 4-hydrazine condensation



4 5

To a microwave tube (35 mL volume) containing **4** (3.81 mmol, 2.00 g) and a magnetic stirrer were added 8 mL THF and 8 mL EtOH followed by the addition of $NH_2NH_2•H_2O$ (19.0 mmol, 0.93 mL) and HOAc (7.62 mmol, 0.45 mL). The tube was capped and subjected to microwave irradiation at 110 °C for 6 h. Then, the mixture was transferred to a flask and concentrated under vacuum. Purification of the residue via chromatography over silica gel (CH_2Cl_2/MeOH/Et_3N = 120:3.5:1) afforded **5** as an amorphous foam (1.93 g, 94%).

Then, 200 mg **5** and CH_2Cl_2 (20 mL) was stirred at room temperature for 2 min, followed by the addition of CF_3COOH (0.1 mL). The resulting mixture was stirred at room temperature for 10 min and then concentrated under vacuum. Purification of the residue via flash chromatography over silica gel (CH_2Cl_2/CH_3OH = 10:1) afforded **5'** (104 mg).

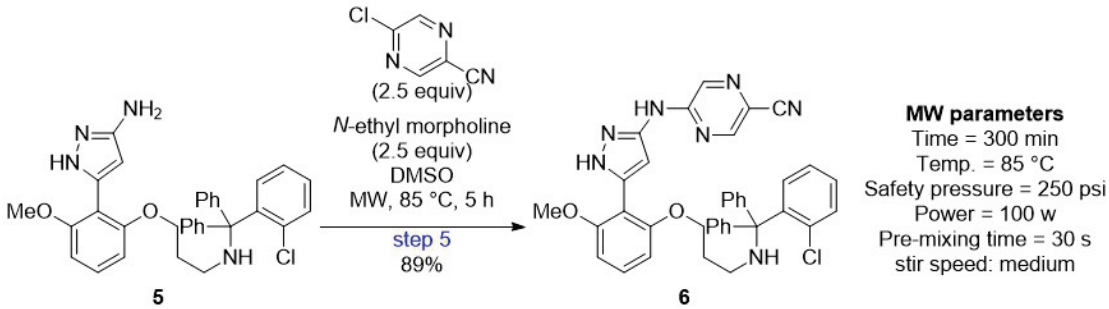


5'

^{1}H NMR (400 MHz, methanol-d_4) δ 7.37 (t, J = 8.4 Hz, 1H), 6.97 (s, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 4.20 (t, J = 5.8 Hz, 2H), 3.91 (s, 3H), 3.20 (t, J = 8.2, 7.8 Hz, 2H), 2.24 – 2.13 (m, 2H); ^{13}C NMR (125 MHz, methanol-d_4) δ 159.5, 158.2, 156.7, 137.6, 131.5, 108.4, 106.4, 105.7, 100.7, 67.0, 56.4, 38.6, 28.4; HRMS calculated for $C_{13}H_{19}N_4O_2^+$ ($M+H^+$) 263.1503, found 263.1504.

*See Supplementary Fig. 27 for the NMR spectra of compound **5'**.*

Step 5-S_NAr



5 6

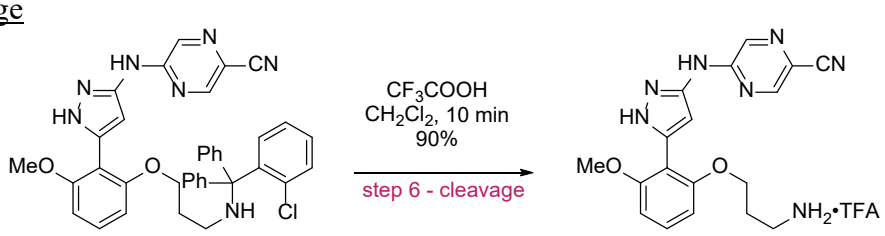
To a microwave tube (35 mL volume) containing **5** (2.78 mmol, 1.50 g) and a magnetic stirrer were added 15 mL DMSO followed by the addition of 5-chloropyrazine-2-carbonitrile (7.0 mmol, 973 mg) and *N*-ethyl morpholine (7.0 mmol, 0.88 mL). The reaction mixture was subjected to microwave irradiation at 85 °C for 5 h. The mixture was transferred to a separating funnel followed by the addition of H_2O (50 mL). The aqueous phase was extracted with EtOAc (50 mL × 3), dried

over Na_2SO_4 and concentrated under vacuum. Purification of the residue via flash chromatography over silica gel (*n*-hexane/EtOAc = 1:1 to CH_2Cl_2/MeOH =10:1) afforded **6** as an amorphous foam (1.58 g, 89%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.34 (s, 1H), 8.24 (s, 1H), 7.43 – 7.27 (m, 7H), 7.19 (td, J = 7.6, 1.7 Hz, 1H), 7.14 – 6.99 (m, 8H), 6.86 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 8.7 Hz, 1H), 4.35 (t, J = 5.6 Hz, 2H), 3.97 (s, 3H), 2.38 (t, J = 6.4 Hz, 2H), 2.17 – 2.05 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.5, 156.8, 151.9, 147.7, 146.3, 144.9, 140.8, 135.3, 134.9, 133.5, 131.5, 131.4, 129.7, 129.6, 128.6, 127.6, 126.3, 125.9, 117.7, 115.8, 107.0, 105.3, 104.1, 98.6, 70.5, 66.2, 55.8, 40.7, 29.7; HRMS calculated for $C_{37}H_{32}ClN_7O_2$ ($M+H^+$) 642.2379, found 642.2377.

See Supplementary Fig. 28 for the NMR spectra of compound ***6****.*

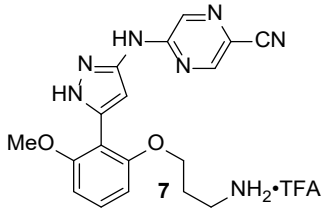
Step 6-cleavage



6 **7**

All of the purified **6** (1.58 g) and CH_2Cl_2 (100 mL) were added to a flask, followed by the addition of CF_3COOH (0.5 mL). The resulting mixture was stirred at room temperature for 10 min and then concentrated under vacuum. The residue was obtained as a brown solid.

Protocol for crystallization: MeOH was gradually added to a 50 mL round-bottom flask containing the crude product from step 6 with gentle shaking until all crude product dissolved completely. In total, 5 mL MeOH was consumed. Then, EtOAc (50 mL) was added to the resulting solution with shaking. White solids gradually precipitated overnight. Filtration and washing with MeOH/EtOAc (*v*/*v* = 1:20, 30 mL) and subsequent drying under vacuum at room temperature afforded **7** as a white powder (1.06 g, 90%).

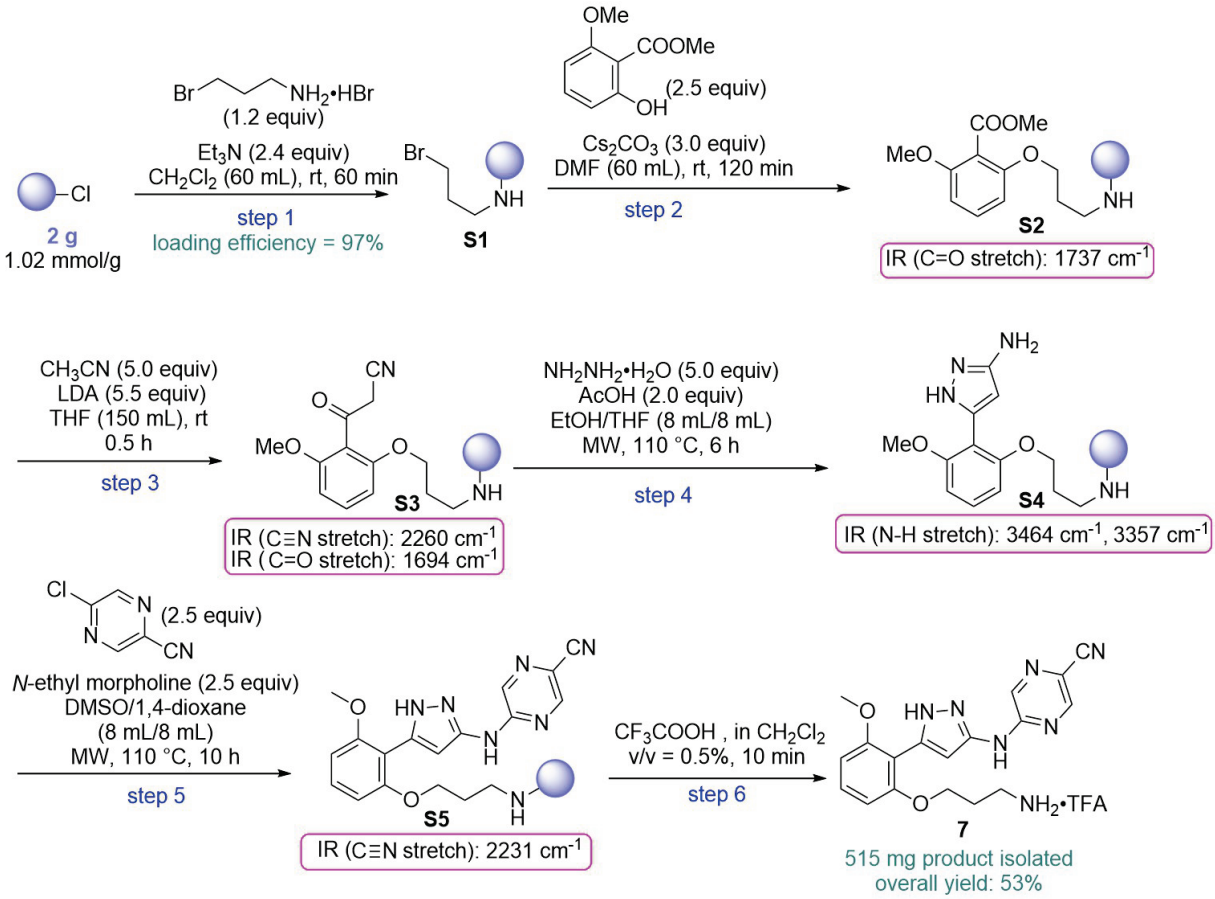


7

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.34 (s, 1H), 10.76 (s, 1H), 8.69 (d, J = 1.3 Hz, 1H), 8.54 (s, 1H), 8.00 (s, 3H), 7.34 (t, J = 8.4 Hz, 1H), 6.87 (s, 1H), 6.78 (d, J = 8.4, 3.8 Hz, 1H), 6.77 (d, J = 8.4, 1H), 4.11 (t, J = 6.0 Hz, 2H), 3.81 (s, 3H), 2.99 – 2.90 (m, 2H), 2.08 – 2.00 (m, 2H);^{13}C NMR (125 MHz, DMSO-d_6) δ 158.8, 157.7, 156.5, 152.1, 148.1, 146.5, 135.4, 134.7, 130.0, 120.8, 118.4, 117.9, 116.1, 113.6, 107.2, 105.4, 104.5, 98.4, 65.2, 55.9, 36.2, 26.9; HRMS calculated for $C_{18}H_{20}N_7O_2^+$ ($M+H^+$) 366.1673, found 366.1675; FT-IR (cm^{-1}): 3428, 3168, 2983, 2227, 1668, 1612, 1571, 1540, 1501, 1464, 1163, 1128, 1096, 1022, 799, 698. Melting point: 225.1 – 226.4 °C.

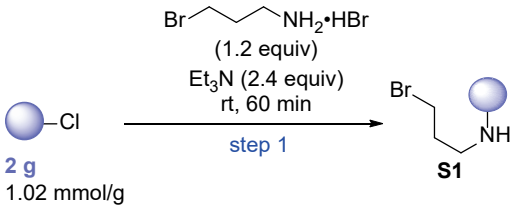
*See Supplementary Fig. 29 for the NMR spectra of compound **7**.*

5. SPS-batch synthesis of prexasertib TFA salt



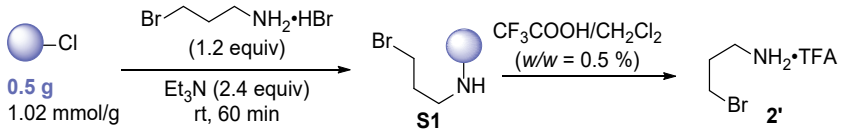
Supplementary Figure 9. Scheme for the SPS-batch synthesis of prexasertib TFA salt.

Step 1-attachment of the starting compound to the resin



A mixture of 2-Cl trityl chloride resin (2.0 g, 1.02 mmol/g) and amine hydrobromide (2.45 mmol, 534 mg) in 60 mL CH_2Cl_2 was stirred at room temperature for 10 min, followed by the addition of Et_3N (4.9 mmol, 0.68 mL) dropwise over 5 min. The amine hydrobromide salt gradually disappeared within 2 minutes. The resulting mixture was stirred at room temperature for 60 min, and then quenched with 3 mL MeOH. The resin **S6** was successively washed with CH_2Cl_2 (50 mL), MeOH (50 mL), CH_2Cl_2 (50 mL), and MeOH (50 mL), and dried under vacuum at room temperature for 60 min, after which it was ready for the next step.
FT-IR (cm^{-1}) of resin **S1**: 3567, 3441, 3059, 3024, 2920, 2850, 1600, 1492, 1452, 1062, 1040, 905, 828, 756, 697.

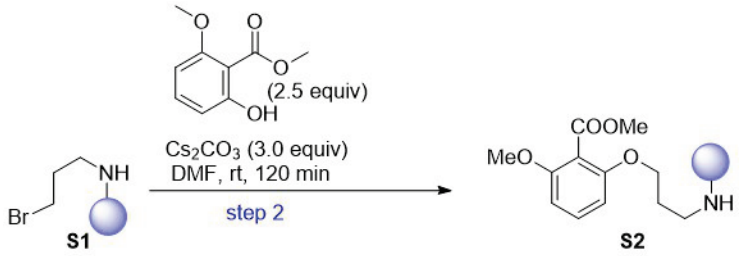
To identify the loading efficiency of the resin:



$$\text{loading efficiency} = \frac{m_{2'}/M_{2'}}{1.02 \times 0.5}$$

First, 500 mg 2-Cl trityl chloride resin was converted to resin **S1** by step 1, and then, a mixture of resin **S1** and CH_2Cl_2 (20 mL) was stirred at room temperature for 10 min, followed by the addition of CF_3COOH (0.1 mL). The resulting mixture was stirred at room temperature for 10 min, filtered off, and washed with CH_2Cl_2 (20 mL) and MeOH (20 mL). The filtrate was concentrated using a rotary evaporator, and then dried under vacuum for 5 h. The resulting **2'** was obtained, and the loading efficiency was identified (97%, 255 mg), the crude NMR spectra of which coincided with that of **2'** from solution-batch synthesis.

Step 2-S_N2



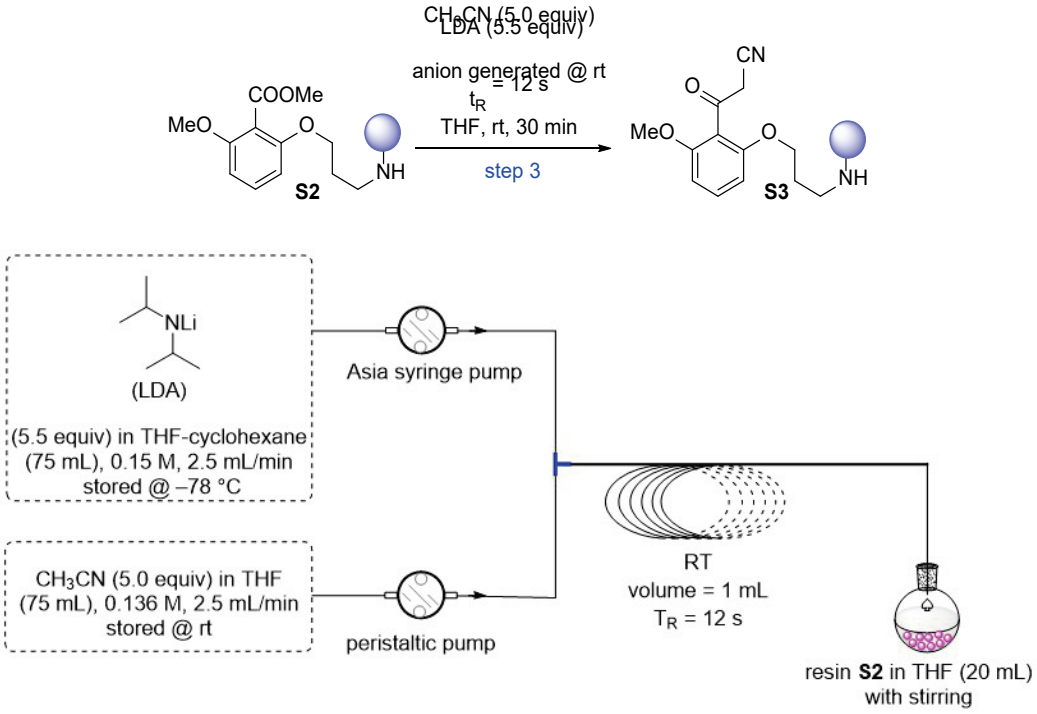
To a dry flask containing methyl 2-hydroxy-6-methoxybenzoate (2.55 mmol, 930 mg) and Cs_2CO_3 (6.12 mmol, 2.0 g) was added anhydrous DMF (30 mL). The resulting mixture was stirred at room

temperature for 30 min, and then kept stationary for another 30 min. The upper clear solution was transferred to another dry flask containing resin **S1** (swelled in 30 mL DMF) from step 1. The resulting reaction mixture was stirred at room temperature for 120 min and then quenched by the addition of 0.1 N HCl (50 mL). The resin **S2** was filtered off and washed successively with H_2O (100 mL), THF (50 mL), MeOH (50 mL), THF (50 mL), and MeOH (50 mL), and then dried under vacuum at room temperature for 60 min, after which it was ready for the next step.

FT-IR (cm^{-1}) of resin **S2**: 3567, 3058, 3024, 2920, 2848, **1737**, 1597, 1493, 1464, 1453, 1254, 1108, 1072, 756, 697.

Then, resin **S2** (100 mg) was treated with TFA in CH_2Cl_2 (v/v = 0.5%, 50 mL) at room temperature for 10 min. The mixture was subsequently filtered off and washed with CH_2Cl_2 (10 mL) and MeOH (10 mL). The filtrate was concentrated under vacuum, the crude NMR data of which coincided with that of **3'** from solution-batch synthesis.

Step 3-Claisen condensation



LDA solution: To a 100 mL dry 3-port GL45 screw-capped bottle containing a stir bar were added 70 mL anhydrous THF and *i*-Pr_2NH (5.5 equiv., 1.57 mL). The solution was cooled to − 78 °C, followed by addition of *n*-BuLi (5.5 equiv., 2.0 M in cyclohexane, 5.6 mL). The resulting mixture was vigorously stirred at − 78 °C for 5 min and then kept in a dry-ice bath, after which it was ready for use (delivered by Asia syringe pump channel #2).

CH_3CN solution: To another 100 mL dry 3-port GL45 screw capped bottle were added 75 mL anhydrous THF and CH_3CN (5.0 equiv., 0.53 mL). The resulting solution was mixed well and was ready for use (delivered by the peristaltic pump).

To a dry flask with argon protection containing resin **S2** from step 2 was added THF (20 mL). The flask and two pumps were connected by PFA tubing. Both pumps were set at the flow rate = 2.5 mL/min. After 30 min of pumping, to the flask containing the resin was added 0.5 N HCl (100 mL). The resin **S3** was filtered off and washed successively with 0.5 N HCl (100 mL), THF (50 mL), MeOH (50 mL), THF (50 mL), and MeOH (50 mL), and then dried under vacuum at room temperature for 60 min, after which it was ready for the next step.

FT-IR (cm^{-1}) of resin **S3**: 3314, 3081, 3060, 3018, 2958, 2945, 2841, **2260**, **1694**, 1595, 1491, 1467, 1437, 1258, 1095, 1020, 755, 732, 705, 697.

Next, resin **S3** (100 mg) was treated with TFA in CH_2Cl_2 (v/v = 0.5%, 50 mL) at room temperature for 10 min. Then, the mixture was filtered off and washed with CH_2Cl_2 (10 mL) and MeOH (10 mL). The filtrate was concentrated under vacuum, the crude NMR data of which coincided with that of **4'** from solution-batch synthesis.

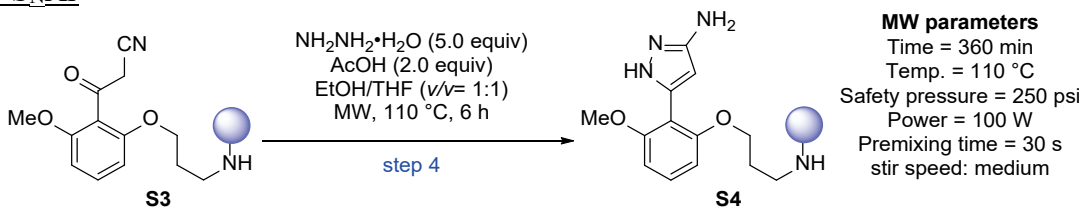
Step 4-hydrazine condensation

To a microwave tube (35 mL volume) containing resin **S3** and a magnetic stirrer were added 8 mL THF and 8 mL EtOH. The resulting mixture was stirred for 10 min, followed by the addition of $NH_2NH_2•H_2O$ (10.2 mmol, 0.5 mL) and HOAc (4.08 mmol, 0.24 mL). The tube was capped and subjected to microwave irradiation for 6 h. The resulting resin **S4** was washed successively with THF (50 mL), MeOH (50 mL), THF (50 mL), and MeOH (50 mL), and then dried under vacuum at room temperature for 60 min, after which it was ready for the next step.

FT-IR (cm^{-1}) of resin **S4**: **3464**, **3357**, 3058, 3024, 2920, 2850, 1597, 1492, 1452, 1438.

Next, resin **S4** (100 mg) was treated with TFA in CH_2Cl_2 (v/v = 0.5%, 5 mL) at room temperature for 10 min. Then, the mixture was filtered off and washed with CH_2Cl_2 (10 mL) and MeOH (10 mL). The filtrate was concentrated under vacuum, the crude NMR data of which coincided with that of **5'** from solution-batch synthesis.

Step 5-S_NAr

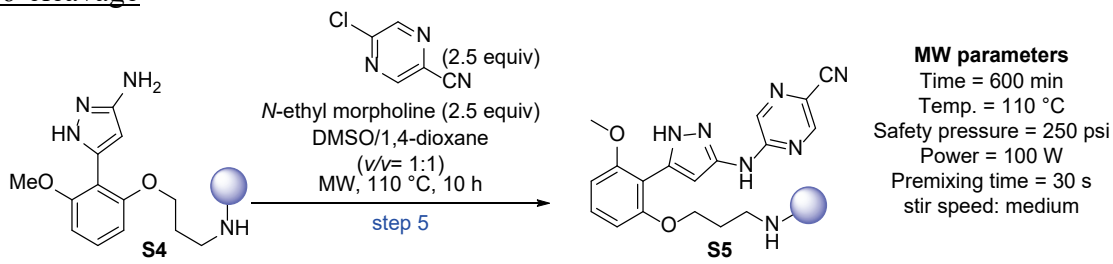


To a microwave tube (35 mL volume) containing resin **S4** and a magnetic stirrer were added 8 mL 1,4-dioxane and 8 mL DMSO. The resulting mixture was stirred for 10 min, followed by the

addition of 5-chloropyrazine-2-carbonitrile (5.1 mmol, 709 mg) and *N*-ethyl morpholine (5.1 mmol, 0.64 mL). The tube was capped and subjected to microwave irradiation at 110 °C for 10 h. Resin **S5** was washed successively with THF (50 mL), MeOH (50 mL), THF (50 mL), and MeOH (50 mL), and then dried under vacuum at room temperature for 60 min, after which it was ready for the next step.

FT-IR (cm^{-1}) of resin **S5**: ν 3430, 3234, 3038, 2969, **2231**, 1673, 1630, 1600, 1542, 1441, 1262, 1206, 1134, 1103, 814, 722.

Step 6-cleavage



All resin **S5** from step 5 was treated with TFA in CH_2Cl_2 (v/v = 0.5%, 50 mL) at room temperature for 10 min. Then, the mixture was filtered off and washed with CH_2Cl_2 (50 mL) and MeOH (50 mL). The filtrate was concentrated under vacuum for 10 min. The crude prexasertib TFA salt was obtained.

Protocol for crystallization: To a 50 mL round-bottom flask containing the crude product was added MeOH (2 mL) gradually with gentle shaking until all crude product dissolved completely. Then to the resulting solution was added EtOAc (20 mL) with shaking. White solids gradually precipitated overnight. Filtration and washing with MeOH/EtOAc (*v/v* = 1:20, 20 mL) and subsequent drying under vacuum at room temperature furnished a white powder (515 mg, 53% overall yield), the NMR data of which coincided with that of prexasertib TFA salt **7** from solution-batch synthesis.

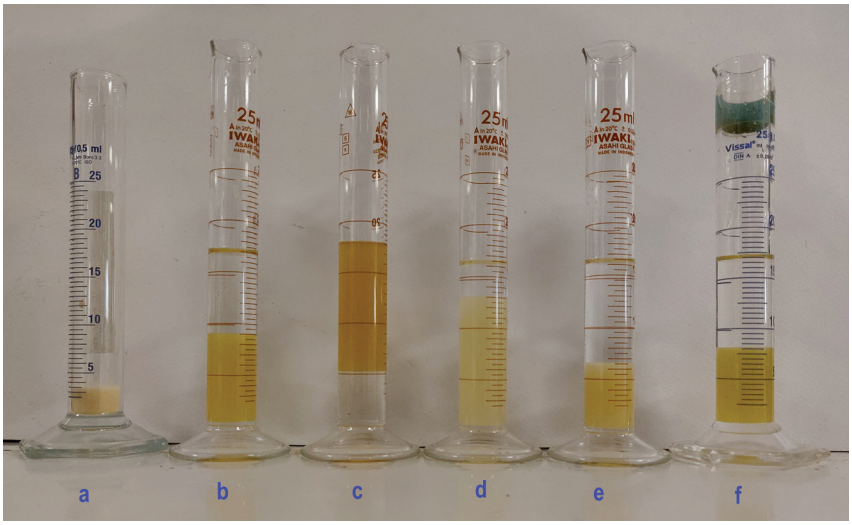
6. SPS-flow synthesis of prexasertib TFA salt in a column reactor

6.1 Determination of the size of the column reactor

- The matrix of the resin employed is polystyrene crosslinked with 1% divinylbenzene, the swelling factor of which varies with the kinds of solvents. We tested the swelling effect in the solvents involved in the synthesis:

Supplementary Table 1: Identifying the volumes of totally-swollen resins in different solvents.

Protocol for identification			The solvent (15 mL) and 2-Cl trityl chloride resin (2 g) were added in a 25 mL graduated cylinder, followed by sufficient shaking, and the mixture was left stationary for 20 min. Then, the volume was recorded.		
Solvent for involved	DMF	CH_2Cl_2	THF	EtOH-THF (*v*/*v*= 1:1)	DMSO-dioxane (*v*/*v*= 1:1)
Volume (mL) recorded	9.2	13.0	13.0	6.8	8.5
Conclusion: The volume of the column was determined to be **13 mL**, avoiding excessive back pressure caused by the swelling, which may exceed the max. pressure of pumps.					



Supplementary Figure 10. 2.0 g resin swollen in different solvents: (**a**) dry resin, (**b**) DMF, (**c**) CH_2Cl_2, (**d**) THF, (**e**) EtOH-THF (*v*/*v* = 1:1), (**f**) DMSO-dioxane (*v*/*v* = 1:1).

- The resin particles have a size of 100 to 200 mesh. Thus, frits with a porosity of 200 mesh (75 μm) can meet the requirement.

Based on the analysis, we customized the stainless steel column (Supplementary Fig. 6): outer-diameter (OD) = 0.5 inch, inner-diameter (ID) = 0.4 inch, length = 16 cm, volume = 13 mL, fitted with 200 mesh frits.

6.2 Establishing the solvent washing protocol

Supplementary Table 2: Establishing the washing protocol between each step.

Step	Washing protocol	Purpose
1 → 2	CH_2Cl_2 (5 mL/min for 20 min)	To wash away the reagent residue from the 1st step
	Argon purging (5 min)	To purge the CH_2Cl_2 residue
	DMF (2 mL/min for 10 min, 4 mL/min for 20 min)	To ensure that the resin is thoroughly swollen for the 2nd step
2 → 3	DMF (5 mL/min for 20 min)	To wash away the reagent residue from the 2nd step
	Argon purging (10 min)	To purge the DMF residue
	MeOH (2 mL/min for 10 min, 4 mL/min for 20 min)	To thoroughly replace DMF from the resins using a solvent with a poor swelling effect
	Argon purging (30 min)	To thoroughly purge the MeOH
	THF (2 mL/ 10 min, 6 mL/ 20 min)	To ensure that the resin is thoroughly swollen for the 3rd step
3 → 4	THF (5 mL/min for 15 min)	To wash away the reagent residue from the 3rd step
	Argon purging (3 min)	To purge the THF
	0.1 N HCl (2 mL/min for 10 min, 5 mL/min for 20 min)	To dissolve and wash away the lithium salt residue
	Heated to 60 °C, 0.1 N HCl (5 mL/min for 20 min)	To thoroughly dissolve and wash away the lithium salt residue
	Argon purging (3 min, 60 °C)	To purge the aqueous solution
	THF wash (2 mL/min for 10 min, 5 mL/min for 15 min, 60 °C)	To replace the aqueous solution and ensure that the resin is swollen
	EtOH-THF (*v/v* = 1:1, 2.5 mL/min for 20 min, 60 °C)	To switch to the solvent for the 4th step
	Heated to 110 °C	To reach the target temperature of the 4th step
4 → 5	EtOH-THF (*v/v* = 1:1, 2.5 mL/min for 20 min, 110 °C)	To wash out the reagent residue of the 4th step
	THF (5 mL/min for 20 min, 110 °C)	To replace EtOH
	Argon purging (20 min, 110 °C)	To purge the EtOH and THF residue
	Dioxane-DMSO (*v/v* = 1:1, 5 mL/min for 20 min, 110 °C)	To change to the solvent for the 5th step and ensure that the resin is thoroughly swollen
5 → 6	Cooled to 35 °C	To approach the target temperature of the 6th step
	MeOH (2.5 mL/min for 20 min); THF (2.5 mL/min for 20 min)	To wash out and replace DMSO
	Argon purging (20 min)	To purge the DMSO, THF, and MeOH residue
	CH_2Cl_2 (2 mL/min for 15 min, 5 mL/min for 20 min)	To replace the solvent for the 6th step and ensure that the resin is thoroughly swollen
6	CH_2Cl_2 (5 mL/min for 10 min)	To thoroughly flush out the product

7. Description of LabVIEW control

7.1 Description of the hardware

7.1.1 Instrument model number

SF-10 Reagent pump
Manufacturer: Vapourtec Ltd

Asia Flow Chemistry Syringe Pump
Manufacturer: Syrris Ltd

Valco Dead-end Selector with Universal Actuator serial interface: RS-232
Manufacturer: VICI Valco Instruments Co. Inc

Hei-Connect Magnetic Stirrer
Manufacturer: Heidolph Instruments

7.1.2 Communication with the computer

All hardware was controlled via a computer through an RS232 cable interface (refer to instrument manuals for the communication setups).

7.1.3 Serial Commands List

For the SF-10 reagent pump, Valco dead-end selector valve, and Hei-connect stir plate, the lists of serial commands can be found in their respective operation manuals or acquired from the manufacturers. Syrris Ltd provides a free application programming interface in C language for controlling the Asia pump from a computer. We used AsiaPumpInterface.h (Version 1.2, Oct 2017). The relevant provided DLL file was placed in the work directory. The functions in the DLL were converted to LabVIEW VI by using the share library converter in LabVIEW.

Below is a list of all communication commands utilized, organized by hardware component.

SF-10 Reagent pump:
STOP
SETFLOW X: Sets flow rate to X ml/min (flow rate range 0.02ml/min to 10 ml/min)
START

Valco Dead-end Selector:
CP: Displays the current position
GOnn: Sends the actuator to position, nn (from 1 to NP), via the shortest route

Asia Flow Chemistry Syringe Pump:

scanForAsiaPumps(): Returns the number of Asia Pumps attached to the PC. This command was sent before other functions.
enterRemoteMode(): Put pump into remote mode. This locks the front screen of the pump (shows EXTERNAL CONTROL)
This mode will automatically exit if the PC does not communicate with the pump at least once every 10 seconds.
pumpStatus(): Returns status of a given pump.
Error = -1
Uninitialised = 0
Initialising = 1
Idle = 2
Filling = 3
Full = 4
Emptying = 5
Empty = 6
Pumping = 7
fill(): Tells target pump/channel to fill at specified fill rate.
pump(): Start pumping on the specified pump/channel at the specified rate.
pumpStop(): Halt asia control on the pump and stops the pump.
empty(): Tell pump to empty. Drives both syringes on the target pump/channel to the top.
clearUp(): Release all pumps and ensures everything is stopped. Always call this function as the last call before exiting.
exitRemoteMode(): Stops the pump, puts it back into manual mode and unlocks the menu system on the pump.

Hei-connect stir plate:
RESET: Reset all: activate old interface protocol, heating off, motor off, deactivate remote control
OUT_MODE_4 Y: Set temperature control mode Y: 0 = Precise-Mode; 1 = Fast-Mode
OUT_SP_1 Y: Set temperature to Y °C
OUT_SP_3 Y: Set rotating speed to Y rpm
START_1: Start heating: Remote active; "PC" blinking in display MR
START_2: Start rotation: Remote active; "PC" blinking in display MR
STOP_1: Stop heating
STOP_2: Stop rotation
IN_PV_1: Display the current sensor temperature

7.2 Software and programming

Code availability: The LabVIEW™ code for operating the SPS-flow automated synthesis in this study is available at https://github.com/nus-automated-flow-system/auto-SPS-Flow-Supplementary-Software.

7.2.1 Software version
For our control software, we utilized NI LabVIEW 2018 (32-bit) NI LabVIEW Runtime 2018

NI-Serial Runtime 17.5
NI-VISA Runtime 18.0

(The application installer includes required runtime engines.)

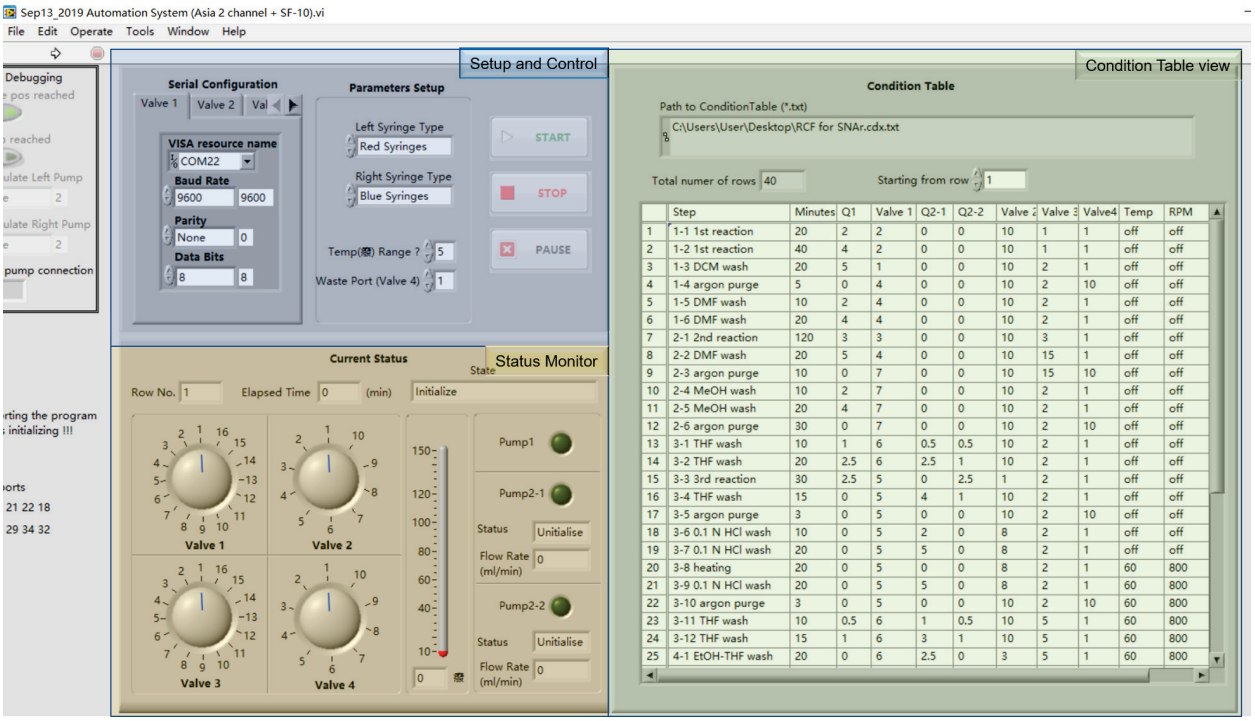
Below, we describe the different aspects of the control program that we developed.

7.2.2 Program overview

The SPF Flow System Automation program was written in LabVIEW 2018. The front panel contains three parts: user control panel, user monitor panel and condition table panel. The following paragraphs and subsections describe the functionalities available in the program interface shown in Supplementary Fig. 11.

The main process of the automation step is to move the valves to set port positions, fill the Asia pump if necessary, start both the SF-10 and Asia pumps simultaneously and start a built-in timer. Once the target duration has elapsed, the two pumps are stopped simultaneously, and the program will determine if the Asia pump needs to empty the syringes for the next step. Once the emptying process is finished, the program will read the next row conditions and repeat the whole process, starting from the setting of the valve port positions.

One highlight of the program's functionality is the temperature adjustment. The Hei-Connect stir plate uses a built-in proportional-integral-derivative (PID) controller to adjust the temperature. This controller has two modes, namely, precise-mode, which avoids an overshoot in the sample temperature, and fast-mode, in which the heater will heat rapidly but in our testing the overshoot could be as large as 15 °C. We chose to use the stir plate's precise-mode in our program, and in addition to this built-in control, we added the functionality that any pumps that are pumping will be paused whenever the measured temperature of the oil bath is out of a user-defined acceptable temperature range. After the pumps pause, the program will wait for the temperature to return to a different defined range of the set temperature plus or minus one. By setting two temperature ranges, one for pausing pumps (±5 °C) and the other for resuming pumps (±1 °C), the pumps do not rapidly switch on and off when the temperature is at the edge of the user set temperature range. This approach results in a reliable temperature and more precise pumping times.
In addition to the temperature adjustment functionality, another highlight of the program is the ability for users to pause the pumps whenever they are pumping. The pause functionality was vital during the developmental stage of the chemical process; it allowed a chemist user much flexibility in modifying the process.



Supplementary Figure 11. SPS flow system automation LabVIEW™ user interface. This user interface contains three parts: (a) the setup and control panels (labeled Serial Configuration and Parameters Setup) are used to set communication configurations and process parameters and to control the process; (b) the current status is a real-time monitor showing the current settings of the instruments; and (c) the condition table displays the user-defined table of conditions for keeping track of steps.

Condition Table

The condition table is a user-defined table containing the information of duration, flow rate of pump1 and pump2, port position of valve 1-4, hot plate temperature and rotation speed. We modified the condition table in Microsoft Excel and saved it as a tab delimited text file. To correctly interpret the condition table in LabVIEW™, the titles and units in the text file match the table titles shown in the LabVIEW™ program user interface window. For the heating and cooling steps, we set the duration to "x" or "0" in the condition table, since the duration of this step depends on the stir plate heating speed and cooling speed.

Start from row number

Set the program to start from a user-defined step. The skipped steps are grayed out, and the current step is highlighted with a yellow background.

Serial Configuration

Set the serial communication between the instrument and computer, including COM port number, baud rate, parity and data bits. The automation program has default serial parameters corresponding to each instrument, although the COM port number is dependent on the user's

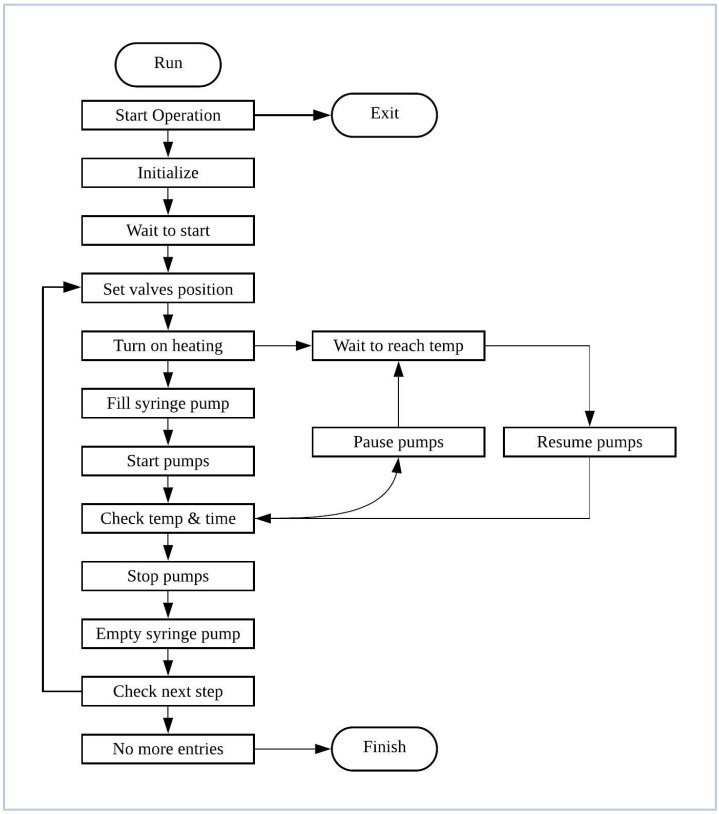
computer. The Syrris Asia pump hardware did not require serial configuration. After installing the driver, the pump was automatically detected by the computer.

Syringe Type
Set the syringe types loaded into the left and right channels of the Syrris Asia pump to calculate the maximum filling and emptying speed. Note that it is required to set the syringe types directly on the pump interface and to ensure that the same syringe types are specified in the automation program.

Temp Range
Set the acceptable temperature range for the sample. Since the PID heating control system inevitably has fluctuations in temperature, this program includes the functionality of running the process only within the acceptable temperature range to ensure a good outcome of the product. Once the temperature measured by the sensor is out of this range, the automation program will pause the timer and pumps and resume once the temperature is accurate again.

7.2.3 State machine

The program follows a state machine structure for executing the flow system process. Here, we describe the state machine logic that the control program is built around.



Supplementary Figure 12. SFS flow system automation program state machine chart.

Start operation (default):
This state is the default beginning state when the program enters the state machine structure. It checks if the condition table has entries and sends the initialize command to the Syrris Asia pump.

```
1. if the row number is greater than 0:
       send initialize command to Syrris Asia pump
       go to next state "Initialize"
   else:
       stay in the current state
       prompt: no entries

2. Reset pumps indicators
```

Initialize:
This state is used to wait for the Syrris Asia pump to finish the initializing process for both channels. This process will take several minutes. During this process, the operation buttons (START, STOP, PAUSE) are disabled in order to prevent intervention in initializing Syrris Asia pump. Intervening in this process may cause the pump to inaccurately determine its home position.

```
1. if both the left channel state and right channel state are at
   initialized:
       go to next state "Wait to start"
   else:
       stay in the current state
```

Wait to start:
After completion of the "Initialize" state, the START and STOP buttons are enabled in this state. The "Wait to start" state is used to wait for the user to start the process by pressing the START button, or to exit the program by pressing the STOP button.

```
1. Enable Start and STOP button
2. if user press the START button:
       go to next state "Set valve position"
   else:
       stay in the current state
```

Set valve positions:
This state sends a command to the valves to go the set port position via the **shortest route** and highlights the current step in the condition table.

```
1. set the current row in the condition table with lime
   background
2. send commands to the actuator to set the valves port
```

```
3. check the current valve position
   if all four valves have reached the set positions:
       go to next state "Turn on heating"
   else:
       stay in the current state
```

Turn on heating:
This state sends a command to turn on the heater and motor of the stir plate if the temperature and rotation speed are set in the condition table. The heating function of the Hei-Connect stir plate has two modes: precise-mode and fast-mode. We set the **precise-mode** as default to enable heating without overshooting for temperature-sensitive samples.

```
1. if a temperature is set in the current row:
       send command to Hei-connect to set the temperature
       send command to Hei-connect to turn on the heater
   else:
       send command to Hei-connect to turn off the heater
2. if a rotation speed is set in the current row:
       send command to Hei-connect to set the rpm
       send command to Hei-connect to turn on the motor
   else:
       send command to Hei-connect to turn off the motor
3. if the duration is not set in the current row:
   # meaning the current row is a heating or cooling step, the
   actual duration of this row depends on the stir plate heating
   performance
       go to state "Wait to reach temperature"
   else:
       go to state "Fill syringe pump"
```

Wait to reach temp:
This state is used to wait for the oil bath to reach the set temperature. During this state, the timer will be paused.

```
1. pause the timer
2. if the current temperature is within 1 degree around the set
   temperature:
       go to state "Resume pumps"
   else:
       stay in the current state
```

Fill syringe pump:
In this state, the Syrris Asia pump will fill the syringes if needed. There are two cases where the syringes need to be filled before pumping:

1. The set flow rate is nonzero, and the valve 2 position has changed from the previous row
2. The set flow rate is nonzero, and the valve 2 position is the same as the previous row, but the set flowrate from the previous row is zero; i.e., the pump did not run in the previous row and was not filled with solution.

```
1. if set flow rate of the left channel is not zero and either
   valve2 set position is different from previous row or set
   flow rate of the left channel is different from previous row:
       if the left channel status is not full:
           fill the left channel
       else:
           return left channel filling finished as TRUE
   else:
       return left channel filling finished as TRUE
2. if set flow rate of the right channel is not zero and either
   valve 2 set position is different from previous row or set
   flow rate of the right channel is different from previous row:
       if the right channel status is not full:
           fill the right channel
       else:
           return right channel filling finished as TRUE
   else:
       return right channel filling finished as TRUE
3. if both left and right channel filling finished Booleans are
   TRUE:
       go to state "Start pumps"
   else:
       stay in the current state
```

Start pumps:
This state sends commands to the corresponding pumps to start pumping.

```
1. if the set flow rate of pump1 (SF-10) is not zero:
       send command to SF-10 to set flow rate
       send command to SF-10 to start pumping
   else:
       send command to SF-10 to stop pumping
2. if the set flow rate of pump2 (Syrris Asia) left channel is
   not zero:
       send command to Syrris Asia left channel to set flow rate
       and start pumping
   else:
       send command to Syrris Asia left channel to stop pumping
3. if the set flow rate of pump2 (Syrris Asia) right channel is
   not zero:
       send command to Syrris Asia right channel to set flow rate
```

```
        and start pumping
    else:
        send command to Syrris Asia right channel to stop pumping
4. start the timer
5. go to state "Check temp & time"
```

Check temp & time:
This state continually updates the timer and measured temperature in cycles. The two cases that will cause the process to pause are as follows: 1. The measured temperature is not within the user-defined temperature range; 2. The user presses the PAUSE button. If either of the two cases occur, the process will go to the state "Pause pumps"; otherwise, it will stay in the current state until the set duration has elapsed and then go to state "Stop pumps".

```
1. if set temperature is nonzero but the measured temperature is
   not in the range around set temperature, or user presses PAUSE
   button:
       go to state "Pause pumps"
   else if the elapsed time has reached the set duration:
       go to state "Stop pumps"
   else:
       stay in the current state
```

Pause pumps:
This state pauses the running pumps and timer. If the user pressed the PAUSE button, this state will wait for the user to press the PAUSE button again to resume the process. Otherwise, if the pause state was triggered by an out-of-range measured temperature, this state will lead to the state "Wait to reach temp".

```
1. if any pump is pumping:
       send command to SF-10 to stop pumping
       send command to Syrris Asia left and right channels to
   stop pumping
2. pause the timer
3. if user pressed PAUSE button:
       stay in the current state
   else:
       go to state "Wait to reach temp"
```

Resume pumps:
This state resumes the pumps to pump with the current flow rate.

```
1. if set flow rate of pump1 (SF-10) is nonzero:
       send command to SF-10 to set the flow rate
       send command to SF-10 to start pumping
```

```
    else:
        send command to SF-10 to stop pumping
2. if set flow rate of pump2 (Syrris Asia) left channel is
   nonzero:
        send command to Syrris Asia left channel to set the flow
        rate
        send command to Syrris Asia left channel to start pumping
   else:
        send command to Syrris Asia left channel to stop pumping
3. if set flow rate of pump2 (Syrris Asia) right channel is
   nonzero:
        send command to Syrris Asia right channel to set the flow
        rate
        send command to Syrris Asia right channel to start pumping
   else:
        send command to Syrris Asia right channel to stop pumping
4. go to state "Check temp & time"
```

Stop pumps:
This state determines whether the Syrris Asia pump syringes need to be emptied before going to the next step. It will wait until the emptying process is completed and then go to the next state.

There are two cases where the syringes need to be emptied:
1. The flow rate of the finished step is nonzero, but the flow rate of the next step is zero, i.e. the pump does not need to pump in the next step;
2. The flow rate of the finished step is nonzero, but the valve 2 port position needs to change for the next row.

If either one or both syringes need to be emptied, the process will stay in this state and wait for both syringes to get ready for the next state. If neither of the syringes need to be emptied, it will go to the next state "Check next step".

```
1. if the flow rate of Syrris Asia left channel was nonzero and
   either the flow rate of left channel in next row is zero or
   the valve 2 position in the next row is different from the
   current row:
        if valve 4 is at the waste port:
            if the left channel status is not empty yet:
                send empty command to Syrris Asia left channel
            else:
                return left channel emptying finished as TRUE
        else:
            send command to valve 4 to go to the waste port
   else:
        # No need to empty the left channel
        return left channel emptying finished as TRUE
```

```
2. if the flow rate of Syrris Asia right channel was nonzero and
   either the next flow rate of right channel is zero or the
   valve 2 position in the next row is different from the current
   row:
       if valve 4 is at the waste port:
           if the right channel status is not empty yet:
               send empty command to Syrris Asia right channel
           else:
               return right channel emptying finished as TRUE
       else:
           send command to valve 4 to go to the waste port
   else:
       # No need to empty the right channel
       return right channel emptying finished as TRUE
3. if both left and right channel emptying finished Booleans are
   TRUE:
       go to state "Check next step"
   else:
       stay in the current state
```

Check the next step:
This state checks if the next row in the condition table has entries. If it does, the program will repeat the process from the state "Set valve position"; otherwise, the whole process has finished, and the program will clear indicators in the interface, close the communcation and exit

```
1. if the set duration, flow rates of two pumps, temperature and
   rotation speed of stir plate are all zero:
       go to state "No more entries"
   else:
       go to state "Set valves position"
```

7.3 Suggested operation procedure

In this chapter, we walk through an operation procedure for using the SPS Flow System Automation program to conduct a production process.

- Prepare the condition table: Fill in the condition table (in the form of an Excel file, **Supplementary Table 3**), and save as a **Tab delimited .txt file**
  Note: The heating and cooling steps require entering "x" or "0" under Duration (Minutes); otherwise, the system may not reach the expected temperature. In the next row, enter the duration and conditions that you would like the system to perform at the expected temperature.
  Note: For steps that do not need to be conducted at a specific temperature, enter "off" under Temperature (Celsius)

- Setup hardware: Setup the instruments, connect the system tubing and prepare the solutions

- Connect to computer: Connect instruments to the computer via USB cable and USB hub. Contact the instrument manufacturers for detailed connection information.

- Setup the communication: Open the SPS Flow System Automation program. Select the COM port corresponding to each instrument, except the Syrris Asia pump, which is automatically connected (refer to Syrris Asia pump Interface Library Reference Version 1.2). Refer to the communication configurations stated in each instrument's user manual.

- Input parameters: Select the syringe types used in the Syrris Asia pump. Set the acceptable temperature range in degree Celsius.

- Run the program: Click the run arrow  to run the program

- Load the condition table: In the prompt window, choose the path to the condition table that you created in the first step. Check if the loaded condition table is correct by viewing the Condition Table on the user interface. The program will start initializing the Syrris Asia pump. Once the initialization is completed and the START button is enabled, it is ready to start the process

- Start the process by clicking the START button

- Pause/Stop the process if needed: During the process, if users need to pause or terminate the procedure, they can press the PAUSE or STOP button. If the procedure is paused, all pumps will stop pumping, the program timer will pause timing, and these will be resumed after the user resumes the process by pressing the PAUSE button again. If the procedure is terminated by pressing the STOP button, the program will stop and exit after closing communication with the instruments. Note: If the program is stopped when the Syrris Asia pump syringes are not empty, they will not be emptied automatically; they will be emptied in the initialization process when you run the program again.

  Note: It is not recommended to abort the program using the Abort button  , since the program will not stop pumps if it is aborted. Use the STOP button instead.

- Wait for the process to finish automatically.

Supplementary Table 3: The condition table in the form of an Excel file for automated synthesis of prexasertib.

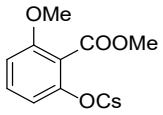
Step	Substep	Time	Vapourtec Flowrate	Valve1	Asia-Left Flowrate	Asia-Right Flowrate	Valve2	Valve3	Valve 4	Temperature	Stirring speed
Unit		min	ml/min	position	ml/min	ml/min	position	position	position	°C	rpm
1	1-1 1st reaction	20	2	2	0	0	10	1	1	off	off
	1-2 1st reaction	40	4	2	0	0	10	1	1	off	off
	1-3 DCM wash	20	5	1	0	0	10	2	1	off	off
	1-4 argon purge	5	0	4	0	0	10	2	10	off	off
	1-5 DMF wash	10	2	4	0	0	10	2	1	off	off
	1-6 DMF wash	20	4	4	0	0	10	2	1	off	off
2	2-1 2nd reaction	120	3	3	0	0	10	3	1	off	off
	2-2 DMF wash	20	5	4	0	0	10	15	1	off	off
	2-3 argon purge	10	0	7	0	0	10	15	10	off	off
	2-4 MeOH wash	10	2	7	0	0	10	2	1	off	off
	2-5 MeOH wash	20	4	7	0	0	10	2	1	off	off
	2-6 argon purge	30	0	7	0	0	10	2	10	off	off
3	3-1 THF wash	10	1	6	0.5	0.5	10	2	1	off	off
	3-2 THF wash	20	2.5	6	2.5	1	10	2	1	off	off
	3-3 3rd reaction	30	2.5	5	0	2.5	1	2	1	off	off
	3-4 THF wash	15	0	5	4	1	10	2	1	off	off
	3-5 argon purge	3	0	5	0	0	10	2	10	off	off
	3-6 0.1 N HCl wash	10	0	5	2	0	8	2	1	off	off
	3-7 0.1 N HCl wash	20	0	5	5	0	8	2	1	off	off
	3-8 heating	x	0	5	0	0	8	2	1	60	800
	3-9 0.1 N HCl wash	20	0	5	5	0	8	2	1	60	800
	3-10 argon purge	3	0	5	0	0	10	2	10	60	800
	3-11 THF wash	10	0.5	6	1	0.5	10	5	1	60	800
	3-12 THF wash	15	1	6	3	1	10	5	1	60	800
4	4-1 EtOH-THF wash	20	0	6	2.5	0	3	5	1	60	800
	4-2 heating	x	0	6	0	0	3	5	1	110	800
	4-3 4th reaction	360	0	6	2.5	0	2	4	1	110	800
	4-4 EtOH-THF wash	20	0	6	2.5	0	3	5	1	110	800
	4-5 THF wash	20	1.5	6	2.5	1	10	5	1	110	800
	4-6 argon purge	20	0	6	0	0	10	2	10	110	800
5	5-1 Dioxane-DMSO wash	20	2.5	15	2.5	0	4	5	1	110	800
	5-2 5th mixing	12	2.5	14	2.5	0	5	6	1	110	800
	5-3 5th reaction	600	0	14	2.5	0	6	6	1	110	800
	5-4 cooling	x	0	14	0	0	6	6	1	35	800
	5-5 MeOH & THF wash	20	2.5	7	2.5	0	10	5	1	off	off
	5-6 argon purge	20	0	7	0	0	10	2	10	off	off
6	6-1 DCM wash	15	2	1	0	0	10	2	1	off	off
	6-2 DCM wash	20	5	1	0	0	10	2	1	off	off
	6-4 6th reaction	120	3	16	0	0	10	16	1	off	off
	6-5 DCM wash	10	5	1	0	0	10	16	1	off	off

8. Automated SPS-flow synthesis of prexasetib and analogs

8.1 Automated synthesis of prexasetib and analogs (5th step as the nucleophilic aromatic substitution reaction)

8.1.1 Preparation and storage of reagents

a. 1st reagent solution: To a 100 mL dry 3-port GL45 screw capped bottle containing a stir bar were added 60 mL CH_2Cl_2 and 3-bromo-propan-*1*-amine hydrobromide (1.2 equiv., 533 mg). To the resulting mixture was added Et_3N (2.4 equiv., 0.68 mL) dropwise. The amine hydrobromide salt gradually dissolved. The resulting solution was used as a stock solution for automated synthesis *(connecting to valve 1)*.

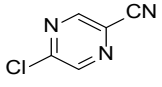
b. 

for the 2nd step: To a 100 mL dry 3-port GL45 screw capped bottle containing a stir bar were added methyl 2-hydroxy-6-methoxybenzoate (2.5 equiv., 930 mg) and Cs_2CO_3 (3.0 equiv., 1990 mg), followed by the addition of anhydrous DMF (60 mL). The blue screw cap was then sealed. The resulting mixture was stirred at rt for 30 min, and kept stationary for 5 min. A GL45 screw cap equipped with a bottom filter (Supplementary Fig. 5b) was used to replace the blue cap. The resulting mixture was ready for use in automated synthesis *(connecting to valve 1).*

c. LDA solution for the 3rd step: To a 100 mL dry 3-port GL45 screw capped bottle containing a stir bar were added 70 mL anhydrous THF and *i*-Pr_2NH (5.5 equiv., 1.57 mL). Then, the solution was cooled to −78 °C, followed by the addition of *n*-BuLi (5.5 equiv., 2.0 M in cyclohexane, 5.6 mL). The resulting mixture was stirred at − 78 °C for 5 min, and then kept in a dry-ice bath for use in automated synthesis *(connecting to valve 2).*

d. CH_3CN solution for the 3rd step: To a 100 mL dry 3-port GL45 screw capped bottle were added 75 mL anhydrous THF and CH_3CN (5.0 equiv., 0.53 mL). The resulting solution was mixed well and was ready for use as a stock solution for automated synthesis *(connecting to valve 1).*

e. 4th reagent solution: To a 100 mL 3-port GL45 screw capped bottle were added 30 mL THF, 30 mL EtOH, $NH_2NH_2 \bullet H_2O$ (5.0 equiv., 0.5 mL) and HOAc (2.0 equiv., 0.24 mL). The resulting solution was mixed well and was ready for use as a stock solution for automated synthesis *(connecting to valve 2).*

f. 

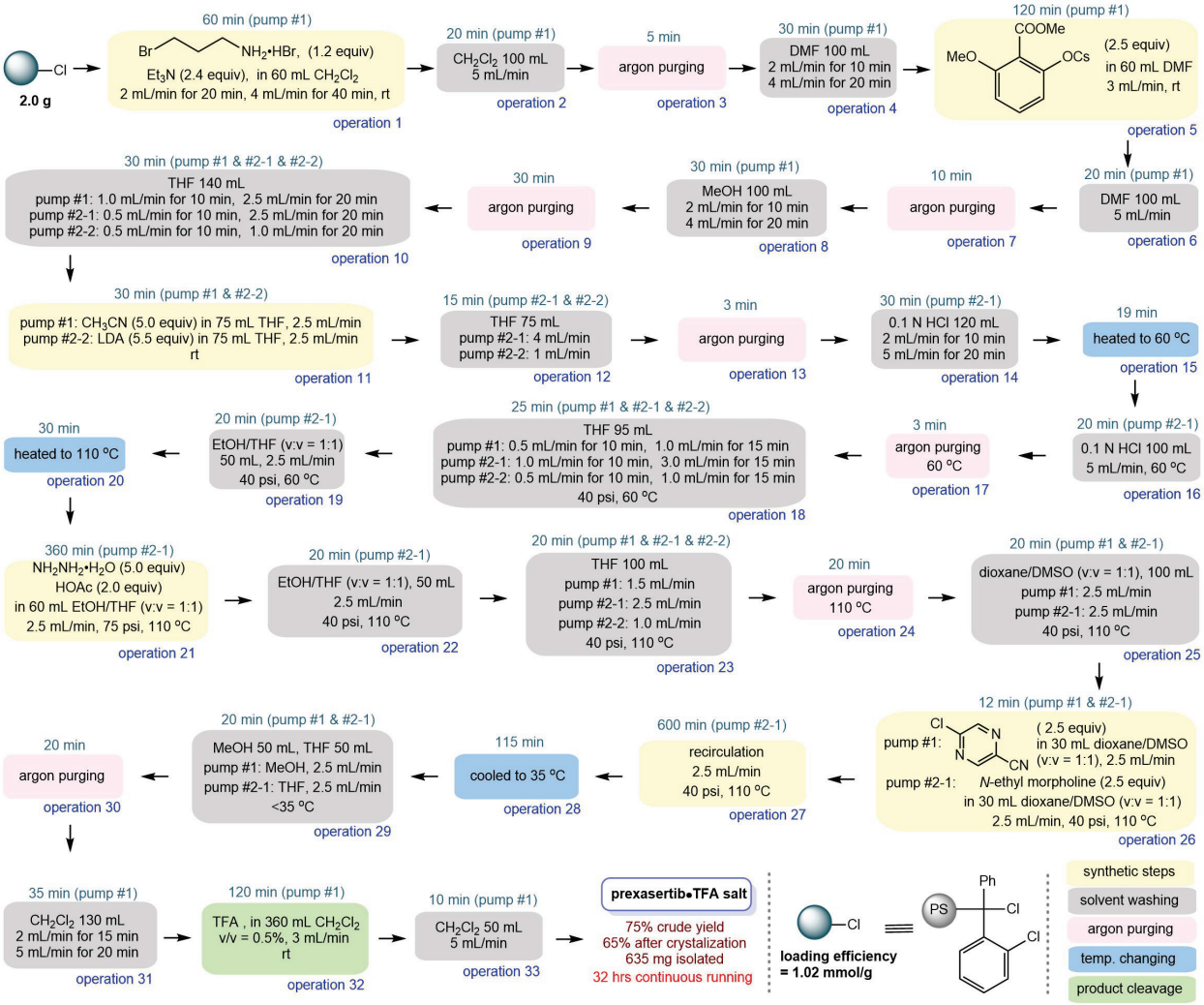
solution for the 5th step: To a 100 mL 3-port GL45 screw capped bottle were added 15 mL DMSO, 15 mL 1,4-dioxane, and 5-chloropyrazine-2-carbonitrile (2.5 equiv., 708 mg). The resulting solution was mixed well and was ready for use as a stock solution for automated synthesis *(connecting to valve 1).*

g. *N*-Ethyl morpholine solution for the 5th step: To a 100 mL 3-port GL45 screw capped bottle were added 15 mL DMSO, 15 mL 1,4-dioxane, and 4-ethyl morpholine (2.5 equiv., 0.64 mL). The resulting solution was mixed well and was ready for use as a stock solution for automated synthesis *(connecting to valve 2).*

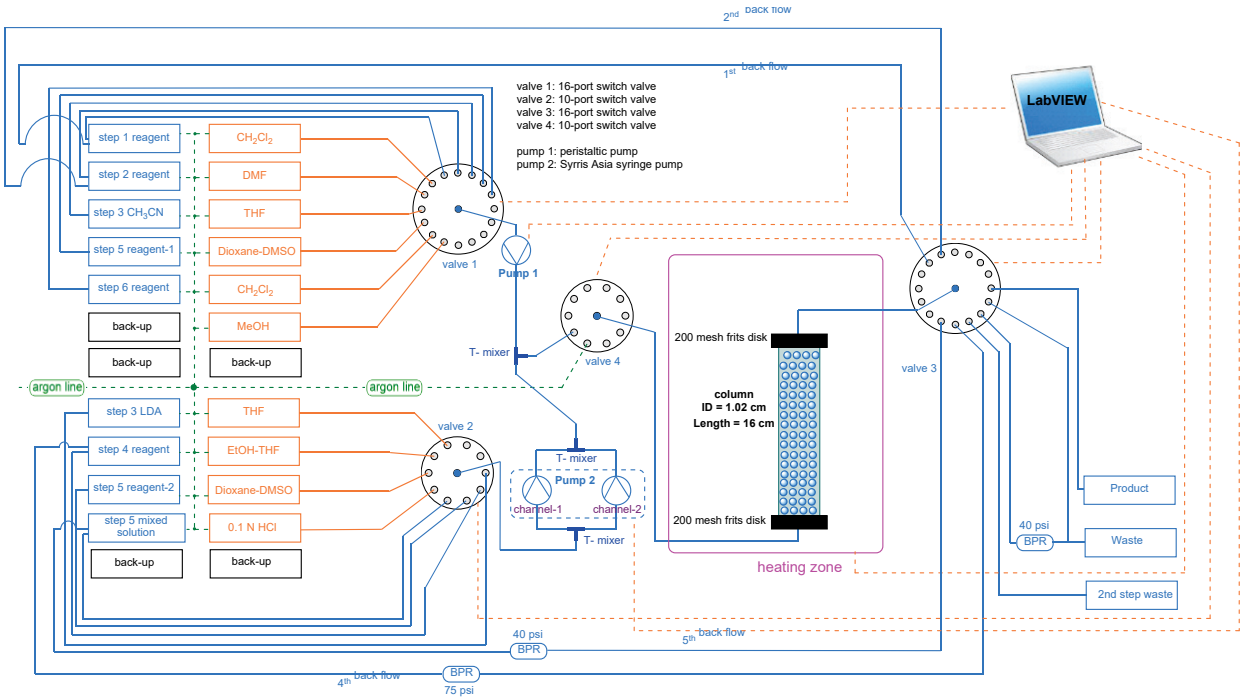
h. CF_3COOH solution (*v/v* = 0.5%) for the 6th step: To a 500 mL 3-port GL45 screw capped bottle were added 360 mL CH_2Cl_2 and 1.6 mL CF_3COOH. The resulting solution was mixed well and was ready for use as a stock solution for automated synthesis (*connecting to valve 1).*

8.1.2 Establishing the chemical recipe file (CRF) #1 for automated synthesis (5th step as the nucleophilic aromatic substitution reaction)

For all parameters of CRF #1, see Supplementary Table 4.



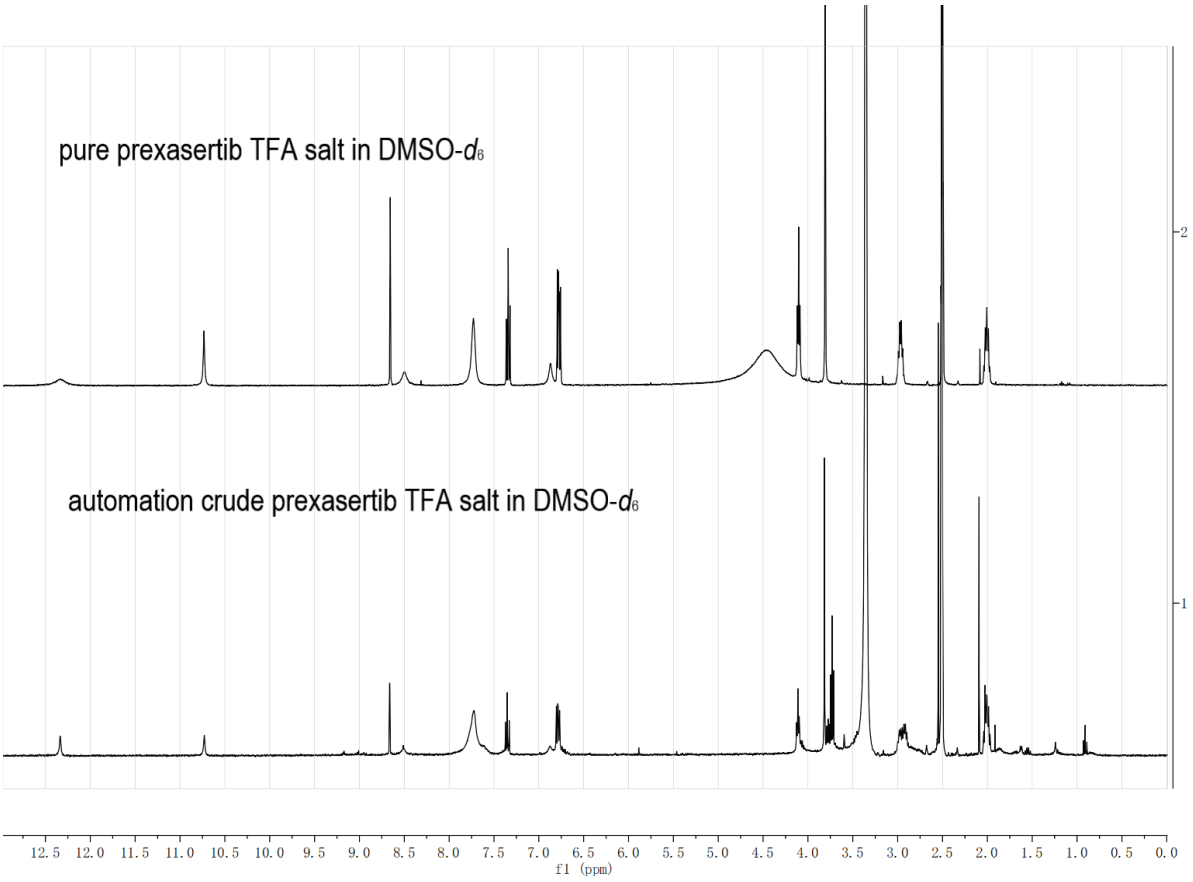
Supplementary Figure 13. CRF #1 (5th step as the nucleophilic aromatic substitution reaction).



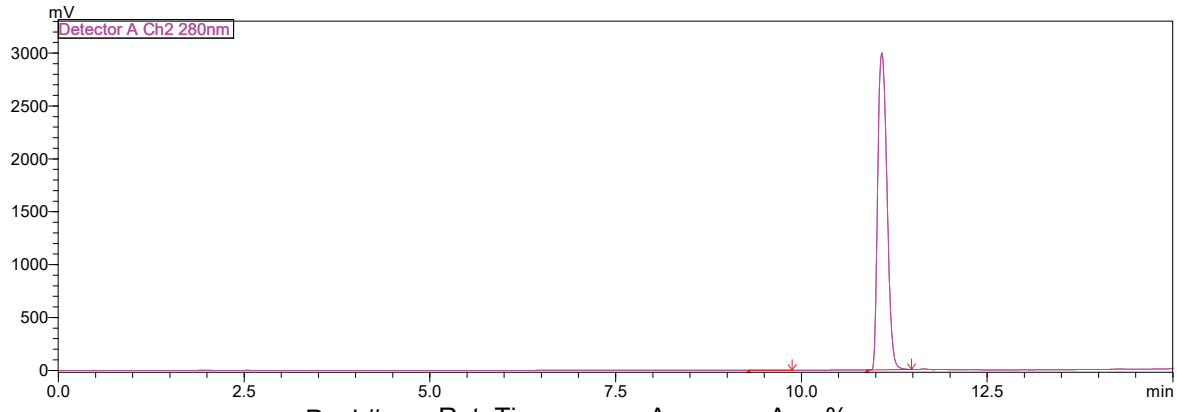
Supplementary Figure 14. Schematic drawing of automated synthesizer for the synthesis of prexasetib.

Supplementary Table 4: Reagents and solvents corresponding to the storage slots and connection to the multiway valves for the synthesis of prexasertib.

valve / port	1#	2#	3#	4#
1	CH_2Cl_2	3rd -CH_3CN	1st back	reagent/solvent
2	1st reagent	4th reagent	waste	argon
3	2nd reagent	EtOH-THF	2nd recirculation	
4	DMF	Dioxane-DMSO	4th recirculation	
5	3rd -LDA	5th-2 reagent	waste+40 psi	
6	THF	5th recirculation	5th recirculation	
7	MeOH			
8		0.1 N HCl		
9				
10		THF		
11				
12				
13				
14	5th-1 reagent			
15	Dioxane-DMSO		2nd Waste	
16	6th reagent		product	



Supplementary Figure 15. Comparison of ^{1}H NMR spectra between pure prexasertib TFA salt and the crude product obtained from automated multistep synthesis.



Peak#	Ret. Time	Area	Area%
1	9.400	9088	0.035
2	11.082	26128156	99.965
Total		26137243	100.000

Supplementary Figure 16. Chromatogram of prexasertib TFA salt produced automatically using the SPS-flow system after purification by crystallization (HPLC conditions:

temperature: 25 °C; solvent A = 0.1% (v/v) TFA in water; solvent B = 0.1% (v/v) TFA in acetonitrile; 5-80% solvent B over 15 min, flow rate: 1 mL/min). The HPLC analysis showed the purity of the product was >99.9%.

8.1.3 Comparison of waste generation for synthesis of 1 gram prexasertib TFA salt via solution-batch synthesis *vs* automated SPS-flow synthesis

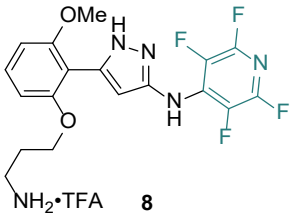
Supplementary Table 5: Waste generation in solution-batch synthesis vs automated SPS-flow synthesis.

Waste-generation in **solution phase synthesis in batch**:				
Based on the 57% overall yield, 3.66 mmol 2-Cl trityl chloride need to be used to produce 1g prexasertib·TFA salt				
Operation / Step	Reaction (mL)	washing& extraction (mL)	Purification (mL)	Sub-total (mL)
1st step	120	240		360
2nd step	75	505	1200	1780
3rd step	88	330	1100	1518
4th step	12		1400	1412
5th step	14	144	1000	1158
6th step	95		80	175
Total（ mL)				6403

Waste-generation in **Automated SPS-flow synthesis**:				
Based on the 65% overall yield, 3.2 g 2-Cl trityl chloride resin need to be used to produce 1 g prexasertib·TFA salt				
Operation / Step	Reaction (mL)	Washing (mL)	Purification (mL)	Sub-total (mL)
1st step	96	320		416
2nd step	96	544		640
3rd step	240	704		944
4th step	96	400		496
5th step	96	368		464
6th step	576	80	80	736
Total (mL)				3696

The CRF for the automated synthesis of prexasertib was also applied to automated synthesis of compound **8** without any modification.

Compound **8**



S_NAr @ 5th step

52% yield, 545 mg, 32 hrs

Protocol for Purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatograph (CH_2Cl_2/MeOH = 5:1), and recrystallization with CH_2Cl_2/EtOAc. A pale-yellow solid was obtained (545 mg, 52% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.32 (s, 1H), 9.39 (s, 1H), 7.91 (brs, 3H), 7.34 (t, J = 8.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.32 (s, 1H), 4.11 (t, J = 6.0 Hz, 2H), 3.81 (s, 3H), 2.95 – 2.85 (m, 2H), 2.05 – 1.95 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.4 (q, J = 31.3 Hz), 157.6, 156.5, 146.3, 144.6 (d, J = 231.2, 15.7 Hz), 136.2-136.0 (m), 134.9, 132.1 (app. dd, J = 250.6 Hz, 34.6 Hz), 130.0, 107.2, 105.4, 104.5, 100.7, 65.2, 55.9, 36.1, 26.8; ^{19}F NMR (376 MHz, DMSO-d_6) δ -73.78 (s, 3F), -95.62 to -95.81 (m, 2F), -156.26 to -156.45 (m, 2F); HRMS calculated for $C_{18}H_{18}F_4N_5O_2^+$ ($M+H^+$) 412.1391, found 412.1395; FTIR (cm^{-1}) 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465; Melting point: 218.1 – 219.0 °C.

*See Supplementary Fig. 30 and Fig. 30 for the NMR spectra of compound **8**.*

8.2 Automated synthesis with the 5th step as the amide coupling (CRF #2)

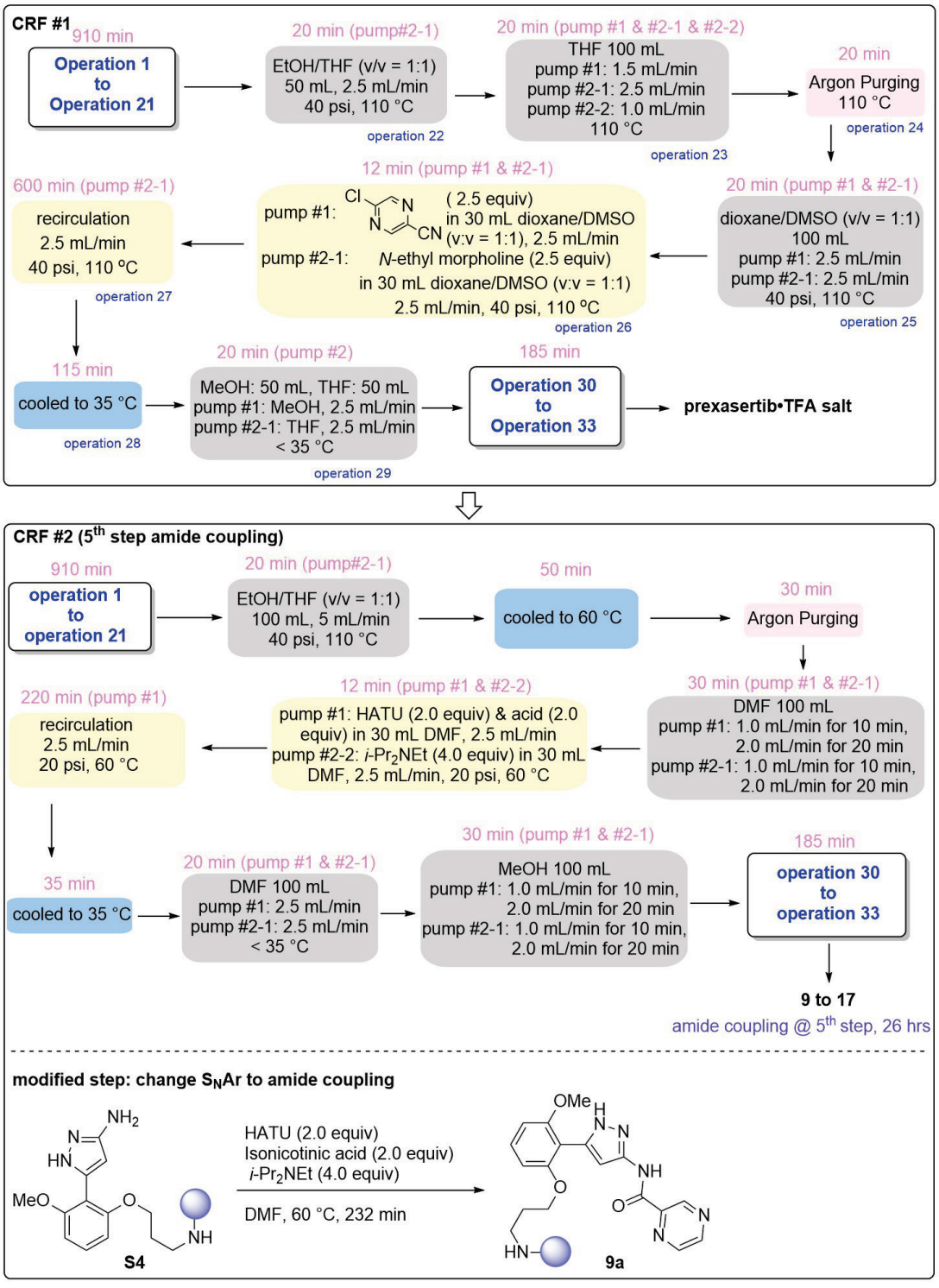
8.2.1 Preparation and storage of reagents for CRF #2

The 1st, 2nd, 3rd, 4th, and 6th reagent solutions were the same as those in CRF #1.

Solutoion-1 for the 5th step: To a 100 mL dry 3-port GL45 screw capped bottle containing HATU (2.0 equiv., 1550 mg) and *iso*-nicotinic acid (2.0 equiv., 500 mg) was added 30 mL anhydrous DMF. The resulting solution was ready for the use as a stock solution for automation synthesis (*connecting to valve 1*).

Solutoion-2 for the 5th step: To a 100 mL dry 3-port GL45 screw capped bottle was added 30 mL DMF and (i-Pr)$_2$NEt (4.0 equiv., 1.42 mL). The resulting solution was ready for use as a stock solution for automation synthesis (*connecting to valve 2*).

8.2.2 Establishing the CRF #2 (5th step as the amide coupling reaction)

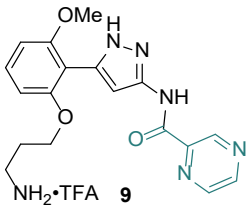


Supplementary Figure 17. CRF #2 (5th step as the amide coupling reaction). HATU = 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-Oxide Hexafluorophosphate.

Supplementary Table 6: The condition table in the form of an Excel file for CRF #2.

Step		Substep	Time	Vapourtec Flowrate	Valve1	Asia-Left Flowrate	Asia-Right Flowrate	Valve2	Valve3	Valve 4	Temperature	Stirring speed
Unit			min	ml/min	position	ml/min	ml/min	position	position	position	℃	rpm
1		1-1 1st reaction	20	2	2	0	0	10	1	1	off	off
		1-2 1st reaction	40	4	2	0	0	10	1	1	off	off
		1-3 DCM wash	20	5	1	0	0	10	2	1	off	off
		1-4 argon purge	5	0	4	0	0	10	2	10	off	off
		1-5 DMF wash	10	2	4	0	0	10	2	1	off	off
		1-6 DMF wash	20	4	4	0	0	10	2	1	off	off
2		2-1 2nd reaction	120	3	3	0	0	10	3	1	off	off
		2-2 DMF wash	20	5	4	0	0	10	15	1	off	off
		2-3 argon purge	10	0	7	0	0	10	15	10	off	off
		2-4 MeOH wash	10	2	7	0	0	10	2	1	off	off
		2-5 MeOH wash	20	4	7	0	0	10	2	1	off	off
		2-6 argon purge	30	0	7	0	0	10	2	10	off	off
3		3-1 THF wash	10	1	6	0.5	0.5	10	2	1	off	off
		3-2 THF wash	20	2.5	6	2.5	1	10	2	1	off	off
		3-3 3rd reaction	30	2.5	5	0	2.5	1	2	1	off	off
		3-4 THF wash	15	0	5	4	1	10	2	1	off	off
		3-5 argon purge	3	0	5	0	0	10	2	10	off	off
		3-6 0.1 N HCl wash	10	0	5	2	0	8	2	1	off	off
		3-7 0.1 N HCl wash	20	0	5	5	0	8	2	1	off	off
		3-8 heating	x	0	5	0	0	8	2	1	60	800
		3-9 0.1 N HCl wash	20	0	5	5	0	8	2	1	60	800
		3-10 argon purge	3	0	5	0	0	10	2	10	60	800
		3-11 THF wash	10	0.5	6	1	0.5	10	5	1	60	800
		3-12 THF wash	15	1	6	3	1	10	5	1	60	800
4		4-1 EtOH-THF wash	20	0	6	2.5	0	3	5	1	60	800
		4-2 heating	x	0	6	0	0	3	5	1	110	800
		4-3 4th reaction	360	0	6	2.5	0	2	4	1	110	800
		4-4 EtOH-THF wash	20	0	6	5	0	3	5	1	110	800
		4-5 cooling	x	0	6	0	0	3	5	1	60	800
		4-6 argon purge	30	0	6	0	0	3	5	10	60	800
5		5-1 DMF wash	10	1	15	1	0	4	2	1	60	800
		5-2 DMF wash	20	2	15	2	0	4	2	1	60	800
		5-3 5th mixing	12	2.5	14	2.5	0	5	6	1	60	800
		5-4 5th reaction	220	0	14	2.5	0	6	6	1	60	800
		5-5 cooling	x	0	14	0	0	6	6	1	35	800
		5-6 DMF wash	20	2.5	15	2.5	0	4	2	1	off	off
		5-7 MeOH wash	10	1	7	1	0	9	2	1	off	off
		5-8 MeOH wash	20	2	7	2	0	9	2	1	off	off
		5-9 argon purge	20	0	7	0	0	9	2	10	off	off
6		6-1 DCM wash	15	2	1	0	0	10	2	1	off	off
		6-2 DCM wash	20	5	1	0	0	10	2	1	off	off
		6-4 6th reaction	120	3	16	0	0	10	16	1	off	off
		6-5 DCM wash	10	5	1	0	0	10	16	1	off	off
											Modified operations	

Compound **9**



9

amide coupling @ 5th step

55% yield, 540 mg, 26 hrs

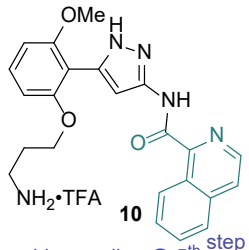
Protocol for purification: The collection was concentrated under vacuum. The resulted crude product was dissolved in CH_2Cl_2 (5 mL), followed by addition of EtOAc (10 mL) slowly. Overnight crystallization following by washing with EtOAc-CH_2Cl_2 (*v*/*v* = 4:1, 20 mL) and drying under vacuum at room temperature afforded the pure compound as a yellow powder (540 mg, 55% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.62 (s, 1H), 9.34 (d, J = 1.0 Hz, 1H), 8.92 (d, J = 2.5 Hz, 1H), 8.80 (t, J = 1.5 Hz, 1H), 7.91 (s, 3H), 7.34 (t, J = 8.5 Hz, 1H), 6.97 (s, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.77 (d, J = 8.5 Hz, 1H), 4.13 (t, J = 6.0 Hz, 2H), 3.81 (s, 3H), 3.06 – 2.96 (m, 2H), 2.08 – 2.00 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.8, 158.7 (q, J = 35.1 Hz), 157.8, 156.6, 147.9, 145.1, 144.6, 144.0, 143.5, 135.1, 130.1, 116.1 (q, J = 291.1 Hz), 107.5, 105.4, 104.6, 98.9, 65.4,

55.9, 36.4, 27.0; HRMS calculated for $C_{18}H_{21}N_6O_3^+$ ($M+H^+$) 369.1670, found 369.1673; FTIR (cm^{-1}): 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465; Melting point: 218.0 – 219.1 °C.

*See Supplementary Fig. 32 for the NMR spectra of compound **9**.*

Compound **10**



amide coupling @ 5th step

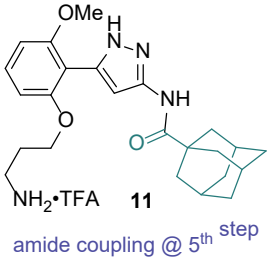
56% yield, 605 mg, 26 hrs

Protocol for purification: The collection was concentrated under vacuum. The resulted crude product was dissolved in CH_2Cl_2 (5 mL), followed by addition of EtOAc (10 mL) slowly. Overnight crystallization with EtOAc-CH_2Cl_2 (*v/v* = 4:1) washing (20 mL) and drying under vacuum at room temperature afforded the pure compound as a pale-yellow powder (605 mg, 56% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 1H), 9.05 (d, J = 8.5 Hz, 1H), 8.62 (d, J = 5.5 Hz, 1H), 8.12 – 8.05 (m, 2H), 7.93 (brs, 3H), 7.90 – 7.84 (m, 1H), 7.80 – 7.75 (m, 1H), 7.35 (t, J = 8.5 Hz, 1H), 7.03 (s, 1H), 6.80 (d, J = 8.5 Hz, 1H), 6.80 (d, J = 8.5 Hz, 1H), 4.14 (t, J = 6.2 Hz, 2H), 3.83 (s, 3H), 3.07 – 2.96 (m, 1H), 2.11 – 1.99 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.6, 158.7 (q, J = 35.0 Hz), 157.8, 156.7, 150.2, 145.6, 140.9, 136.8, 135.0, 130.9, 130.1, 128.8, 127.3, 126.6, 125.8, 123.9, 116.1 (q, J = 291.1 Hz), 107.6, 105.4, 104.6, 98.6, 65.4, 55.9, 36.4, 27.0; HRMS calculated for $C_{23}H_{24}N_5O_3$ ($M+H^+$) 418.1874, found 418.1875; FTIR (cm^{-1}): 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465; Melting point: 190.4 – 191.8 °C.

*See Supplementary Fig. 33 for the NMR spectra of compound **10**.*

Compound **11**



amide coupling @ 5th step

70% yield, 768 mg, 26 hrs

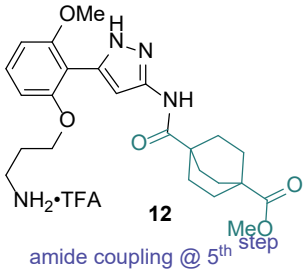
Protocol for purification: The collection was concentrated under vacuum. To a 50 mL flask containing the crude solid was added MeOH (10 mL). Then resulted suspension was stirred vigorously at 40 °C for 10 min, followed by addition of CH_2Cl_2 (5 mL). The mixture was kept stationary at room temperature for 10 min, filtrated, washed with cooled MeOH (10 mL) and

CH_2Cl_2 (5 mL). Drying under vacuum at room temperature furnished a white solid (768 mg, 70% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (s, 1H), 7.84 (brs, 3H), 7.31 (t, J = 8.4 Hz, 1H), 6.76 (d, J = 8.4 Hz, 1H), 6.75 (d, J = 8.4 Hz, 2H), 6.68 (brs, 1H), 4.08 (t, J = 6.0 Hz, 2H), 3.77 (s, 3H), 2.95 (q, J = 6.5 Hz, 2H), 2.01 – 1.96 (m, 5H), 1.92 (d, J = 2.9 Hz, 6H), 1.72 – 1.67 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.3, 158.4 (q, J = 33.9 Hz), 157.7, 156.5, 146.1, 134.6, 129.8, 116.4 (q, J = 295.7 Hz), 108.0, 105.3, 104.5, 98.8, 65.3, 55.8, 40.6, 38.3, 36.4, 36.0, 27.7, 26.8; HRMS calculated for $C_{18}H_{21}N_6O_3$ ($M+H^+$) 425.2547, found 425.2548; FTIR (cm^{-1}): 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465; Melting point: 212.5 – 214.1 °C.

*See Supplementary Fig. 34 for the NMR spectra of compound **11**.*

Compound **12**

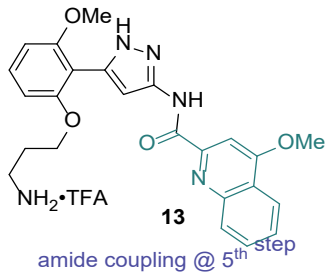


Protocol for purification: The solution was concentrated under vacuum. To a 50 mL flask containing the crude solid was added MeOH (10 mL). Then resulted suspension was stirred vigorously at 40 °C for 10 min, followed by addition of CH_2Cl_2 (5 mL). The mixture was kept stationary at room temperature for 10 min, filtrated, washed with cooled MeOH (10 mL) and CH_2Cl_2 (5 mL). Drying under vacuum at room temperature furnished a white solid (790 mg, 68% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.76 (s, 1H), 7.83 (brs, 3H), 7.31 (t, J = 8.5 Hz, 1H), 6.75 (d, J = 8.5 Hz, 1H), 6.74 (d, J = 8.5 Hz, 1H), 6.66 (brs, 1H), 4.08 (t, J = 6.0 Hz, 2H), 3.77 (s, 3H), 3.59 (s, 3H), 2.94 (q, J = 6.5 Hz, 2H), 2.02 – 1.95 (m, 2H), 1.85 – 1.72 (m, 12H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.2, 174.8, 158.5 (q, J = 34.9 Hz), 157.7, 156.5, 146.1, 134.7, 129.9, 116.4 (q, J = 291.6 Hz), 107.8, 105.3, 104.5, 98.8, 65.3, 55.8, 51.6, 38.8, 38.2, 36.4, 27.7, 27.2, 26.8; HRMS calculated for $C_{18}H_{21}N_6O_3$ ($M+H^+$) 457.2446, found 457.2453; FTIR (cm^{-1}) 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465. Melting point: 219.8 – 222.1 °C.

*See Supplementary Fig. 35 for the NMR spectra of compound **12**.*

Compound **13**



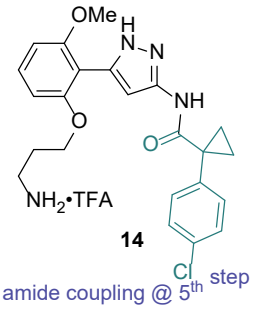
63% yield, 720 mg, 26 hrs

Protocol for purification: The collection was concentrated under vacuum. The resulted crude product was dissolved in CH_2Cl_2 (6 mL), followed by addition of EtOAc (10 mL) slowly. Overnight crystallization with EtOAc-CH_2Cl_2 (*v/v* = 4:1) washing (20 mL) and drying under vacuum at room temperature afforded the pure compound as a pale-yellow powder (720 mg, 63% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.5 Hz, 1H), 7.91 – 7.87 (m, 1H), 7.83 (brs, 3H), 7.74 (s, 1H), 7.73 – 7.69 (m, 1H), 7.36 (t, J = 8.4 Hz, 1H), 7.00 (s, 1H), 6.80 (d, J = 8.4 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 4.18 (s, 3H), 4.13 (t, J = 6.0 Hz, 2H), 3.82 (s, 3H), 3.05 – 2.94 (m, 2H), 2.08 – 1.97 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.5, 161.3, 158.3 (q, J = 34.8 Hz), 157.7, 156.5, 150.9, 146.9, 145.3, 135.0, 131.0, 130.1, 129.1, 127.6, 121.7, 121.4, 116.1 (q, J = 292.1 Hz), 107.5, 105.4, 104.5, 98.2, 98.0, 65.3, 56.5, 55.9, 36.3, 26.9; HRMS calculated for $C_{24}H_{26}N_5O_4$ ($M+H^+$) 448.1979, found 448.1987; FTIR (cm^{-1}) 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465. Melting point: 220.9 – 221.4 °C.

*See Supplementary Fig. 36 for the NMR spectra of compound **13**.*

Compound **14**



54% yield, 610 mg, 26 hrs

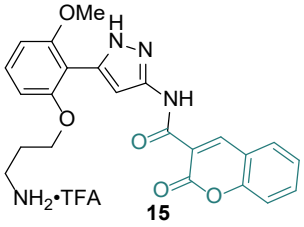
Protocol for purification: The collection was concentrated under vacuum. The resulted crude product was dissolved in CH_2Cl_2 (4 mL), followed by addition of *n*-Hexane/EtOAc (*v/v* = 1:2) (5 mL) slowly. Overnight crystallization with *n*-hexane/EtOAc (*v/v* = 1:2) washing (5 mL) and drying under vacuum at room temperature afforded the pure compound as a yellow powder (610 mg, 54% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (s, 1H), 7.83 (brs, 3H), 7.48 – 7.41 (m, 4H), 7.31 (t, J = 8.4 Hz, 1H), 6.75 (d, J = 8.4, 1H), 6.74 (d, J = 8.4, 1H), 6.66 (s, 1H), 4.07 (t, J = 6.0 Hz, 2H), 3.76 (s, 3H), 2.97-2.87 (m, 2H), 2.01 – 1.92 (m, 2H), 1.48 (dd, J = 6.8 Hz, 4.2 Hz, 2H), 1.10 (dd, J = 6.8

Hz, 4.2 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 158.5 (q, J = 34.8 Hz), 157.7, 156.5, 145.8, 139.0, 134.7, 132.0, 131.8, 130.0, 128.6, 116.1 (q, J = 291.8 Hz), 107.7, 105.3, 104.5, 98.5, 65.3, 55.8, 36.3, 30.6, 26.8, 15.0; HRMS calculated for $C_{23}H_{26}ClN_4O_3$ ($M+H^+$) 441.1688, found 441.1689; FTIR (cm^{-1}) 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465. Melting point: 179.6 – 181.5 °C.

*See Supplementary Fig. 37 for the NMR spectra of compound **14**.*

Compound **15**



15

amide coupling @ 5th step

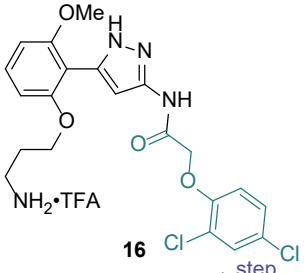
61% yield, 681 mg, 26 hrs

Protocol for purification: The collection was concentrated under vacuum. The crude compound was dissolved in a mixture solvent of CH_2Cl_2/MeOH (5 mL/1mL), followed by addition of EtOAc (2 mL) slowly. Overnight crystallization with EtOAc washing (5 mL) and drying under vacuum at room temperature afforded the pure compound as a chartreuse powder (681 mg, 61% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.38 (s, 1H), 10.93 (s, 1H), 9.00 (s, 1H), 8.02 (dd, J = 7.9, 1.6 Hz, 1H), 7.83 – 7.79 (m, 1H), 7.78 (s, 3H), 7.58 (d, J = 8.4 Hz, 1H), 7.50 (td, J = 7.6, 1.1 Hz, 1H), 7.35 (t, J = 8.4 Hz, 1H), 6.93 (s, 1H), 6.79 (d, J = 8.3 Hz), 6.78 (d, J = 8.3 Hz), 4.11 (t, J = 6.0 Hz, 2H), 3.81 (s, 3H), 3.35 (s, 5H), 3.00 – 2.91 (m, 2H), 2.05– 1.95 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.0, 158.4, 158.1 (q, J = 31 Hz), 157.7, 156.6, 154.0, 147.9, 145.5, 134.8, 134.5, 130.3, 130.1, 125.4, 118.9, 118.6, 117.8 (q, J = 300 Hz), 116.3, 107.4, 105.4, 104.5, 98.5, 65.2, 55.9, 36.3, 26.9; HRMS calculated for $C_{24}H_{26}N_5O_4$ ($M+H^+$) 435.1663, found 435.1668; FTIR (cm^{-1}) 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465; Melting point: 248.1 – 249.6 °C.

*See Supplementary Fig. 38 for the NMR spectra of compound **15**.*

Compound **16**



16

amide coupling @ 5th step

60% yield, 707 mg, 26 hrs

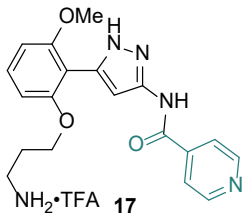
Protocol for purification: The collection was concentrated under vacuum. The crude solid was dissolved in CH_2Cl_2/MeOH (5 mL/1 mL) followed by addition of Et_2O (5 mL). Overnight

crystallization with Et_2O washing (10 mL) and drying under vacuum at room temperature furnished a white powder (707 mg, 60% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 7.83 (s, 3H), 7.60 (d, J = 2.6 Hz, 1H), 7.38 (dd, J = 8.9, 2.6 Hz, 1H), 7.32 (t, J = 8.4 Hz, 1H), 7.12 (d, J = 8.9 Hz, 1H), 6.78 – 6.73 (m, 3H), 4.87 (s, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.78 (s, 3H), 3.01 – 2.85 (m, 2H), 2.04 – 1.94 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.7, 158.5 (q, J = 35.4 Hz), 157.7, 156.5, 152.7, 145.6, 134.6, 129.9, 129.4, 128.1, 125.0, 122.4, 116.0 (q, J = 292.9 Hz), 115.2, 107.5, 105.4, 104.5, 98.2, 67.5, 65.2 , 55.8, 36.2, 26.8; HRMS calculated for $C_{21}H_{23}Cl_2N_4O_4^+$ ($M+H^+$) 465.1091, found 465.1088; FT-IR (cm^{-1}): 3447, 3181, 3099, 2982, 2946, 2893, 2854, 1675, 1634, 1595, 1481, 1430, 1287, 1247, 1207, 1174, 1131, 1105, 834, 725; Melting point: 190.4 – 192.7 °C.

*See Supplementary Fig. 39 for the NMR spectra of compound **16**.*

Compound **17**



amide coupling @ 5th step
65% yield, 637 mg, 26 hrs

Protocol for purification: The collection was concentrated under vacuum. The crude solid was dissolved in CH_2Cl_2 (5 mL), followed by addition of Et_2O (3 mL). Overnight crystallization with Et_2O washing (20 mL) and dying under vacuum at room temperature furnished a pale-yellow powder (637 mg, 65% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.28 (s, 1H), 8.87 (dd, J = 4.4 Hz, 1.9 Hz, 2H), 8.10 (dd, J = 4.4 Hz, 1.9 Hz, 2H), 7.87 (s, 3H), 7.34 (t, J = 8.5 Hz, 1H), 6.94 (s, 1H), 6.79 (d, J = 8.5 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 4.13 (t, J = 6.0 Hz, 2H), 3.82 (s, 3H), 3.05 – 2.94 (m, 2H), 2.07 – 1.98 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.4, 158.6 (q, J = 35.3 Hz), 157.7, 156.6, 148.5, 146.0, 143.3, 134.6, 130.0, 122.6, 116.0 (q, J = 290.9 Hz), 107.5, 105.4, 104.5, 99.4, 65.3, 55.9, 36.4, 26.9; HRMS calculated for $C_{19}H_{22}N_5O_3$ ($M+H^+$) 368.1717, found 368.1714; FTIR (cm^{-1}) 3407, 3239, 3081, 2970, 1611, 1563, 1542, 1496, 1465; Melting point: 184.2 – 186.3 °C.

*See Supplementary Fig. 40 for the NMR spectra of compound **17**.*

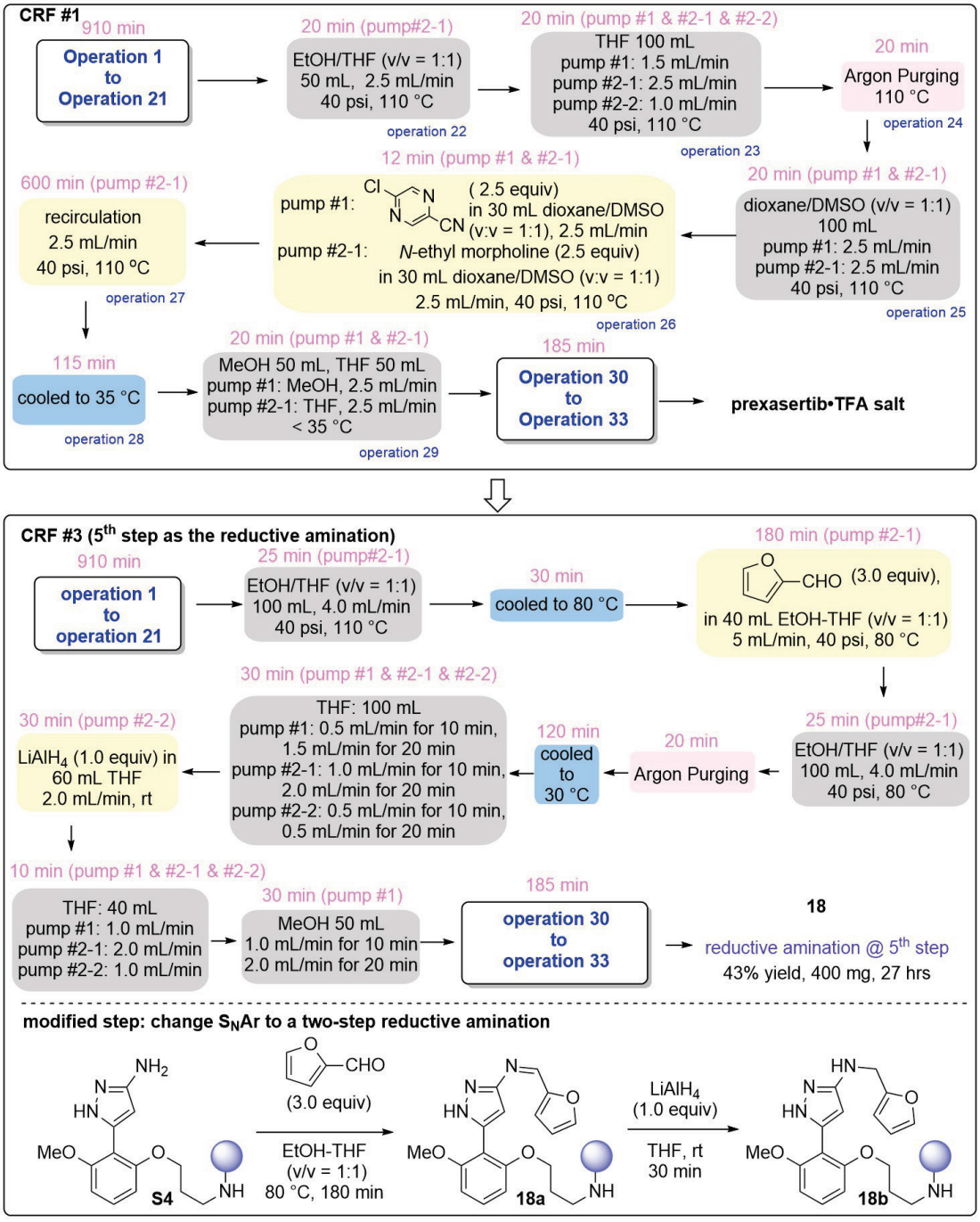
8.3 Automated synthesis with the 5th step as the reductive amination (CRF #3)

8.3.1 Preparation and storage of reagents for CRF #3:
The 1st, 2nd, 3rd, 4th, and 6th reagent solutions were the same as those in CRF #1.

Reagent solution for the 5th step (imine generation): To a 100 mL dry 3-port GL45 screw capped bottle were successively added 20 mL EtOH, 20 mL THF and furfural (3.0 equiv., 0.51 mL). The resulting solution was ready for use as a stock solution for automated synthesis (*connecting to valve 2*).

Reagent solution for the 5th step (imine reduction): To a 100 mL dry 3-port GL45 screw capped bottle were added 60 mL anhydrous THF and $LiAlH_4$ (1.0 M in THF, 2 mL). The resulting solution was ready for use as a stock solution for automated synthesis (*connecting to valve 2*).

8.3.2 Establishing the CRF #3 (5th step as the reductive amination reaction)

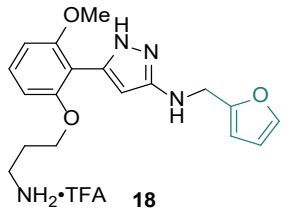


Supplementary Figure 18. CRF #3 (5th step as the reductive amination reaction).

Supplementary Table 7: The condition table in form of an Excel file for CRF #3.

Step		Substep	Time	Vapourtec Flowrate	Valve1	Asia-Left Flowrate	Asia-Right Flowrate	Valve2	Valve3	Valve 4	Temperature	Stirring speed
Unit			min	ml/min	position	ml/min	ml/min	position	position	position	°C	rpm
1		1-1 1st reaction	20	2	2	0	0	10	1	1	off	off
		1-2 1st reaction	40	4	2	0	0	10	1	1	off	off
		1-3 DCM wash	20	5	1	0	0	10	2	1	off	off
		1-4 argon purge	5	0	4	0	0	10	2	10	off	off
		1-5 DMF wash	10	2	4	0	0	10	2	1	off	off
		1-6 DMF wash	20	4	4	0	0	10	2	1	off	off
2		2-1 2nd reaction	120	3	3	0	0	10	3	1	off	off
		2-2 DMF wash	20	5	4	0	0	10	15	1	off	off
		2-3 argon purge	10	0	7	0	0	10	15	10	off	off
		2-4 MeOH wash	10	2	7	0	0	10	2	1	off	off
		2-5 MeOH wash	20	4	7	0	0	10	2	1	off	off
		2-6 argon purge	30	0	7	0	0	10	2	10	off	off
3		3-1 THF wash	10	1	6	0.5	0.5	10	2	1	off	off
		3-2 THF wash	20	2.5	6	2.5	1	10	2	1	off	off
		3-3 3rd reaction	30	2.5	5	0	2.5	1	2	1	off	off
		3-4 THF wash	15	0	5	4	1	10	2	1	off	off
		3-5 argon purge	3	0	5	0	0	10	2	10	off	off
		3-6 0.1 N HCl wash	10	0	5	2	0	8	2	1	off	off
		3-7 0.1 N HCl wash	20	0	5	5	0	8	2	1	off	off
		3-8 heating	x	0	5	0	0	8	2	1	60	800
		3-9 0.1 N HCl wash	20	0	5	5	0	8	2	1	60	800
		3-10 argon purge	3	0	5	0	0	10	2	10	60	800
		3-11 THF wash	10	0.5	6	1	0.5	10	5	1	60	800
		3-12 THF wash	15	1	6	3	1	10	5	1	60	800
4		4-1 EtOH-THF wash	20	0	6	2.5	0	3	5	1	60	800
		4-2 heating	x	0	6	0	0	3	5	1	110	800
		4-3 4th reaction	360	0	6	2.5	0	2	4	1	110	800
		4-4 EtOH-THF wash	25	0	6	4	0	3	5	1	110	800
		4-5 cooling	x	0	6	4	0	3	5	1	80	800
5		5-1 imine generation	180	0	6	5	0	4	6	1	80	800
		5-2 EtOH-THF wash	25	0	6	4	0	3	5	1	80	800
		5-3 argon purge	20	0	6	0	0	3	5	10	80	800
		5-4 cooling	x	0	14	0	0	3	5	1	30	800
		5-5 THF wash	10	0.5	6	1	0.5	10	2	1	off	off
		5-6 THF wash	20	1.5	6	2	0.5	10	2	1	off	off
		5-7 imine reduction	30	0	6	0	2	9	2	1	off	off
		5-8 THF wash	10	1	6	2	1	10	2	1	off	off
		5-9 MeOH wash	10	1	7	0	0	10	2	1	off	off
		5-10 MeOH wash	20	2	7	0	0	10	2	1	off	off
		5-6 argon purge	20	0	7	0	0	10	2	10	off	off
6		6-1 DCM wash	15	2	1	0	0	10	2	1	off	off
		6-2 DCM wash	20	5	1	0	0	10	2	1	off	off
		6-4 6th reaction	120	3	16	0	0	10	16	1	off	off
		6-5 DCM wash	10	5	1	0	0	10	16	1	off	off
											Modified operations	

Compound **18**



reductive amination @ 5th step

43% yield, 400 mg, 27 hrs

Protocol for purification: The solution was concentrated under vacuum. Purification of the residue over silica gel chromatography (CH_2Cl_2 : MeOH = 20 : 1) afforded a pale-yellow powder (400 mg, 43% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (brs, 1H), 8.15 (s, 3H), 7.53 (dd, J = 1.9, 0.9 Hz, 1H), 7.25 (t, J = 8.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 2H), 6.36 (dd, J = 3.2, 1.9 Hz, 1H), 6.26 (dd, J = 3.2, 0.9 Hz, 1H), 5.83 (s, 1H), 5.47 (brs, 1H), 4.24 (s, 2H), 4.08 (t, J = 6.1 Hz, 2H), 3.76 (s, 3H), 2.95 (t, J = 7.3 Hz, 2H), 2.08 – 1.97 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.4 (q, J = 31.9 Hz), 157.8, 156.6, 155.4, 154.6, 141.7, 135.2, 129.4, 117.3 (q, J = 298 Hz), 110.5, 108.5, 106.5, 105.6, 104.7, 93.0, 65.5, 55.9, 41.2, 36.5, 26.9; HRMS calculated for $C_{18}H_{23}N_4O_3^+$ ($M+H^+$) 343.1765, found 343.1773; FT-IR (cm^{-1}): 3420, 1683, 1507, 1470, 1439, 1207, 1141, 1103, 802, 725; Melting point: 119.8 – 120.9 °C.

*See supplementary Fig. 41 for the NMR spectra of compound **18**.*

8.4 Automated synthesis (5th step as *N*-triflation)

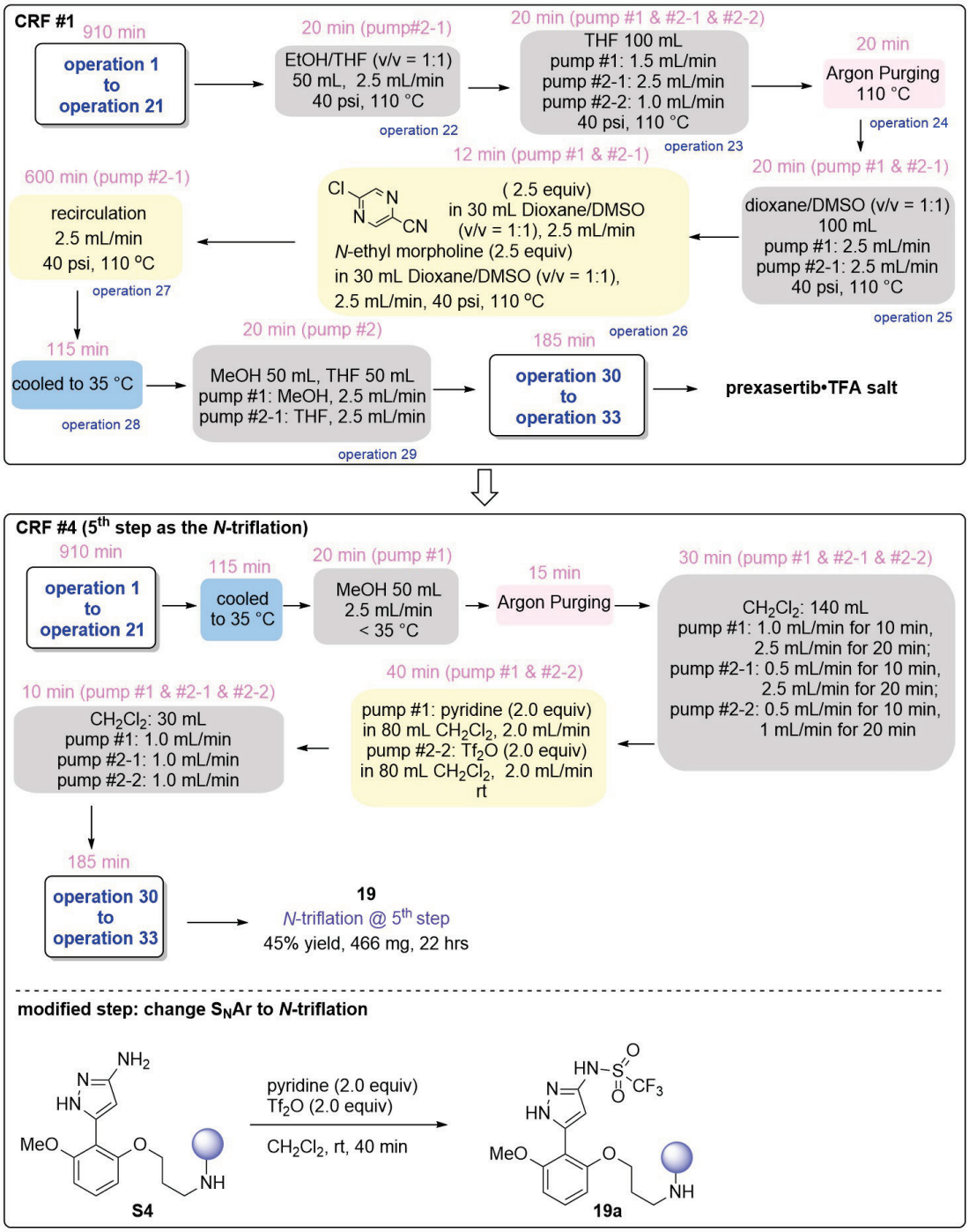
8.4.1 Preparation and storage of reagents for CRF #4 (5th step as *N*-triflation):

The 1st, 2nd, 3rd, 4th, and 6th reagent solutions were the same as those in CRF #1.

Reagent solution-1 for the 5th step: To a 100 mL dry 3-port GL45 screw capped bottle were added CH_2Cl_2 (80 mL) and pyridine (2.0 equiv., 0.33 mL). The resulting solution was ready for use as a stock solution for automated synthesis (*connecting to valve 1*).

Reagent solution-2 for the 5th step: To a 100 mL dry 3-port GL45 screw capped bottle were added CH_2Cl_2 (80 mL) and Tf_2O (2.0 equiv., 0.69 mL). The resulting solution was ready for use as a stock solution for automation synthesis (*connecting to valve 2*).

8.4.2 Establishing the CRF #4 (5th step as the *N*-triflation reaction)



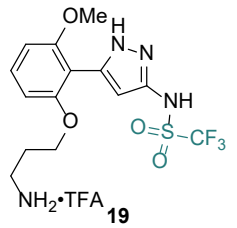
Supplementary Figure 19. CRF #4 (5th step as the *N*-triflation reaction). Tf_2O = trifluoromethanesulfonic anhydride.

Supplementary Table 8: The condition table in form of an Excel file for CRF #4.

Step		Substep	Time	Vapourtec Flowrate	Valve1	Asia-Left Flowrate	Asia-Right Flowrate	Valve2	Valve3	Valve 4	Temperature	Stirring speed
Unit			min	ml/min	position	ml/min	ml/min	position	position	position	°C	rpm
1		1-1 1st reaction	20	2	2	0	0	10	1	1	off	off
		1-2 1st reaction	40	4	2	0	0	10	1	1	off	off
		1-3 DCM wash	20	5	1	0	0	10	2	1	off	off
		1-4 argon purge	5	0	4	0	0	10	2	10	off	off
		1-5 DMF wash	10	2	4	0	0	10	2	1	off	off
		1-6 DMF wash	20	4	4	0	0	10	2	1	off	off
2		2-1 2nd reaction	120	3	3	0	0	10	3	1	off	off
		2-2 DMF wash	20	5	4	0	0	10	15	1	off	off
		2-3 argon purge	10	0	7	0	0	10	15	10	off	off
		2-4 MeOH wash	10	2	7	0	0	10	2	1	off	off
		2-5 MeOH wash	20	4	7	0	0	10	2	1	off	off
		2-6 argon purge	30	0	7	0	0	10	2	10	off	off
3		3-1 THF wash	10	1	6	0.5	0.5	10	2	1	off	off
		3-2 THF wash	20	2.5	6	2.5	1	10	2	1	off	off
		3-3 3rd reaction	30	2.5	5	0	2.5	1	2	1	off	off
		3-4 THF wash	15	0	5	4	1	10	2	1	off	off
		3-5 argon purge	3	0	5	0	0	10	2	10	off	off
		3-6 0.1 N HCl wash	10	0	5	2	0	8	2	1	off	off
		3-7 0.1 N HCl wash	20	0	5	5	0	8	2	1	off	off
		3-8 heating	x	0	5	0	0	8	2	1	60	800
		3-9 0.1 N HCl wash	20	0	5	5	0	8	2	1	60	800
		3-10 argon purge	3	0	5	0	0	10	2	10	60	800
		3-11 THF wash	10	0.5	6	1	0.5	10	5	1	60	800
		3-12 THF wash	15	1	6	3	1	10	5	1	60	800
4		4-1 EtOH-THF wash	20	0	6	2.5	0	3	5	1	60	800
		4-2 heating	x	0	6	0	0	3	5	1	110	800
		4-3 4th reaction	360	0	6	2.5	0	2	4	1	110	800
		4-4 cooling	x	0	6	0	0	2	4	1	35	800
		4-5 MeOH wash	20	2.5	7	0	0	2	2	1	off	off
		4-6 argon purge	15	0	1	0	0	4	2	10	off	off
5		5-1 DCM wash	10	1	1	0.5	0.5	4	2	1	off	off
		5-2 DCM wash	20	2.5	1	2.5	1	4	2	1	off	off
		5-3 5th reaction	40	2	15	2	0	5	2	1	off	off
		5-4 DCM wash	10	1	1	1	1	4	2	1	off	off
		5-5 argon purge	20	0	1	0	0	4	2	10	off	off
6		6-1 DCM wash	15	2	1	0	0	4	2	1	off	off
		6-2 DCM wash	20	5	1	0	0	4	2	1	off	off
		6-4 6th reaction	120	3	16	0	0	4	16	1	off	off
		6-5 DCM wash	10	5	1	0	0	4	16	1	off	off

Modified operations

Compound **19**



N-triflation @ 5th step

45% yield, 466 mg, 22 hrs

Protocol for purification: The collection was concentrated under vacuum. Purified over silica gel chromatography (CH_2Cl_2 : MeOH = 8 : 1) afforded a pale-yellow amorphous foam (466 mg, 45% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (s, 3H), 7.34 (t, J = 8.4 Hz, 1H), 6.71 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.48 (s, 2H), 5.48 (s, 1H), 4.01 (t, J = 6.1 Hz, 1H), 3.69 (s, 3H), 2.85 (t, J = 7.3 Hz, 2H), 1.92 – 1.83 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) 158.4, 158.2 (q, J = 32 Hz), 157.2, 155.2, 152.9, 131.1, 119.2 (q, J = 320 Hz), 117.4 (q, J = 300 Hz), 109.9, 105.4, 104.6, 91.7, 65.4, 56.0, 36.2, 26.9; ^{19}F NMR (377 MHz, DMSO-d_6) δ -73.70, -73.81;
HRMS calculated for $C_{14}H_{18}F_3N_4O_4S^+$ ($M+H^+$) 395.0995, found 395.0988; FT-IR (cm^{-1}): 3446, 2948, 2846, 1676, 1603, 1488, 1475, 1436, 1411, 1235, 1207, 1142, 1098, 1030, 803, 723, 630.

*See Supplementary Fig. 42 and Fig. 43 for the NMR spectra of compound **19**.*

8.5 Automated early-stage diversification (2nd step as the Mitsunobu reaction)

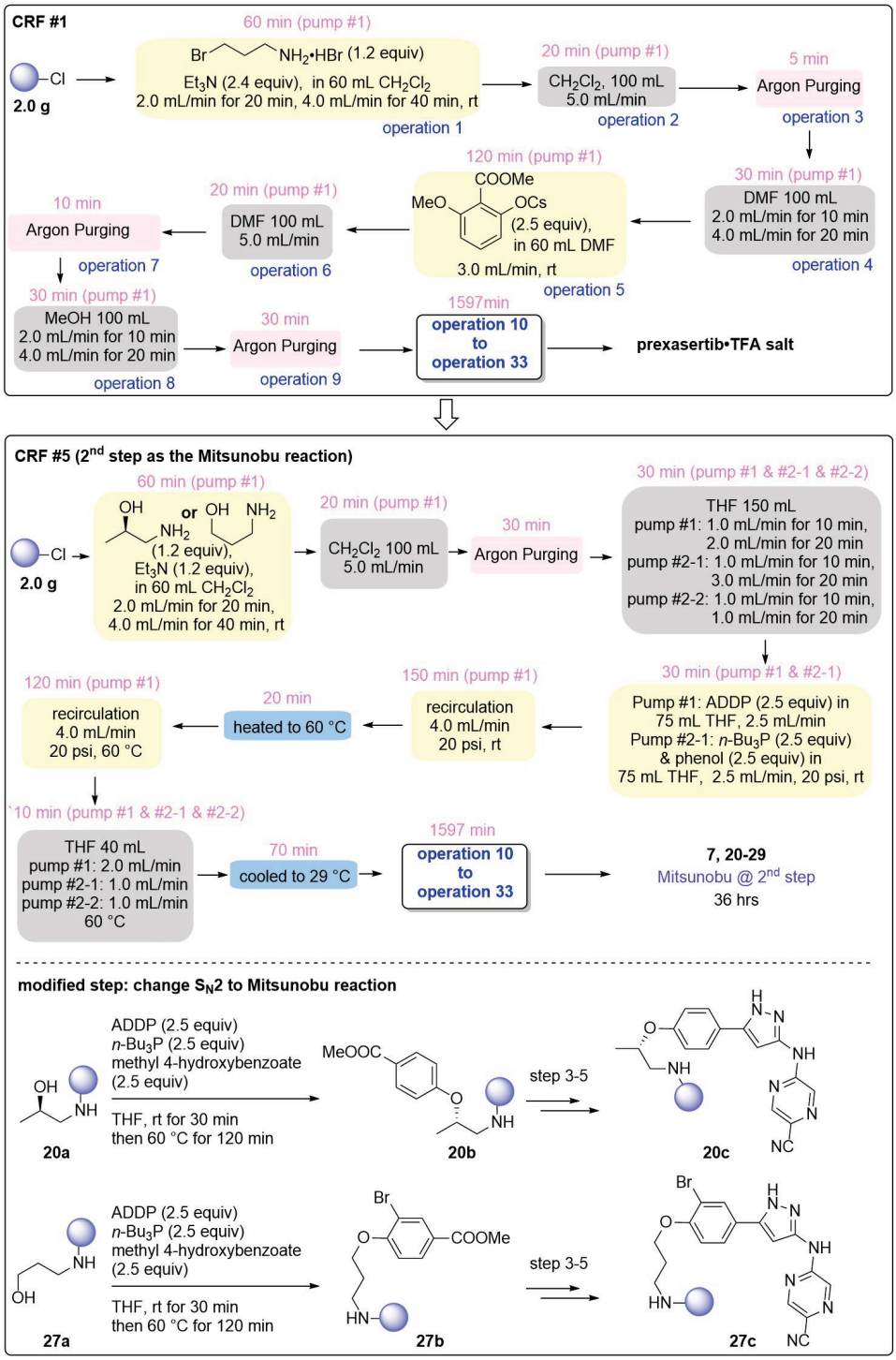
8.5.1 Preparation and storage of reagents for CRF #5 (2nd step as the Mitsunobu reaction)

The preparation of the 3rd, 4th, 5th, and 6th reagent solutions was the same as those in CRF #1.

Reagent solution-1 for the 2nd step: To a 100 mL dry 3-port GL45 screw capped bottle were added 1,1'-(azodicarbonyl) dipiperidine (*ADDP*, 2.5 equiv., 1285 mg) and 75 mL THF. The resulting solution was ready for use as a stock solution for automated synthesis (*connecting to valve 1*).

Reagent solution-2 for 2nd step: To a 100 mL dry 3-port GL45 screw capped bottle were added methyl *2*-hydroxy-*6*-methoxybenzoate (2.5 equiv., 928 mg), *n*-Bu_3P (2.5 equiv., 1.27 mL) and 75 mL THF. The resulting solution was ready for automated synthesis (*connecting to valve 2*).

8.5.2 Establishing the CRF #5 (2nd step as the Mitsunobu reaction)

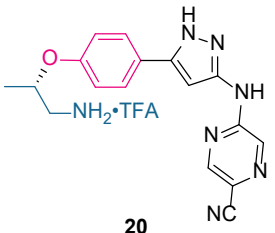


Supplementary Figure 20. CRF #5 (2nd step as the Mitsunobu reaction). ADDP = 1,1-(azodicarbonyl)dipiperidine.

Supplementary Table 9: The condition table in form of an Excel file for CRF #5.

Step		Substep	Time	Vapourtec Flowrate	Valve1	Asia-Left Flowrate	Asia-Right Flowrate	Valve2	Valve3	Valve 4	Temperature	Stirring speed
Unit			min	ml/min	position	ml/min	ml/min	position	position	position	°C	rpm
1		1-1 1st reaction	20	2	2	0	0	10	1	1	off	off
		1-2 1st reaction	40	4	2	0	0	10	1	1	off	off
		1-3 DCM wash	20	5	1	0	0	10	2	1	off	off
		1-4 argon purge	30	0	1	0	0	10	2	10	off	off
		1-5 THF wash	10	1	6	1	1	10	2	1	off	off
		1-6 THF wash	20	2	6	3	1	10	2	1	off	off
2		2-1 2nd mixing	30	2.5	3	2.5	0	9	3	1	off	off
		2-2 2nd reaction	150	4	4	0	0	9	3	1	off	off
		2-3 heating	x	0	4	0	0	9	3	1	60	800
		2-4 2nd reaction	120	4	4	0	0	9	3	1	60	800
		2-5 THF wash	10	2	6	1	1	10	5	1	60	800
		2-6 cooling	x	0	6	0	0	10	2	1	29	800
3		3-1 THF wash	10	1	6	0.5	0.5	10	2	1	off	off
		3-2 THF wash	20	2.5	6	2.5	1	10	2	1	off	off
		3-3 3rd reaction	30	2.5	5	0	2.5	1	2	1	off	off
		3-4 THF wash	15	0	5	4	1	10	2	1	off	off
		3-5 argon purge	3	0	5	0	0	10	2	10	off	off
		3-6 0.1 N HCl wash	10	0	5	2	0	8	2	1	off	off
		3-7 0.1 N HCl wash	20	0	5	5	0	8	2	1	off	off
		3-8 heating	x	0	5	0	0	8	2	1	60	800
		3-9 0.1 N HCl wash	20	0	5	5	0	8	2	1	60	800
		3-10 argon purge	3	0	5	0	0	10	2	10	60	800
		3-11 THF wash	10	0.5	6	1	0.5	10	5	1	60	800
		3-12 THF wash	15	1	6	3	1	10	5	1	60	800
4		4-1 EtOH-THF wash	20	0	6	2.5	0	3	5	1	60	800
		4-2 heating	x	0	6	0	0	3	5	1	110	800
		4-3 4th reaction	360	0	6	2.5	0	2	4	1	110	800
		4-4 EtOH-THF wash	20	0	6	2.5	0	3	5	1	110	800
		4-5 THF wash	20	1.5	6	2.5	1	10	5	1	110	800
		4-6 argon purge	20	0	6	0	0	10	2	10	110	800
5		5-1 Dioxane-DMSO wash	20	2.5	15	2.5	0	4	5	1	110	800
		5-2 5th mixing	12	2.5	14	2.5	0	5	6	1	110	800
		5-3 5th reaction	600	0	14	2.5	0	6	6	1	110	800
		5-4 cooling	x	0	14	0	0	6	6	1	35	800
		5-5 MeOH & THF wash	20	2.5	7	2.5	0	10	5	1	off	off
		5-6 argon purge	20	0	7	0	0	10	2	10	off	off
6		6-1 DCM wash	15	2	1	0	0	10	2	1	off	off
		6-2 DCM wash	20	5	1	0	0	10	2	1	off	off
		6-4 6th reaction	120	3	16	0	0	10	16	1	off	off
		6-5 DCM wash	10	5	1	0	0	10	16	1	off	off
											Modified operations	

Compound **20**



20

Mitsunobu @ 2nd step

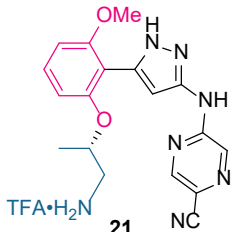
50% yield, 457 mg, 36 hrs

Protocol for purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatography (CH_2Cl_2 : MeOH = 8:1 to 3:1) followed by recrystallization with EtOAc/Et_2O, furnishing a pale-yellow powder (457 mg, 50% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.65 (d, J = 1.3 Hz, 1H), 8.50 (s, 1H), 8.11 (s, 3H), 7.70 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 6.85 (s, 1H), 4.76 – 4.67 (m, 1H), 3.16 (dt, J = 14.4, 4.5 Hz, 1H), 3.11 – 2.98 (m, 1H), 1.29 (d, J = 6.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.8, 152.0, 147.8, 147.5, 142.5, 135.4, 126.6, 122.8, 117.7, 116.6, 116.4, 93.1, 70.8, 43.3, 16.9; HRMS calculated for $C_{17}H_{18}N_7O^+$ ($M+H^+$) 336.1567, found 336.1572; FT-IR (cm^{-1}): 3474, 3135, 2982, 2236, 1680, 1607, 1535, 1491, 1377, 1247, 1181, 1134, 1022, 833, 798, 722; Melting point: 142.1 – 144.5 °C (decomposed); $[\alpha]^D_{25}$ = +8 (c = 0.1, MeOH).

*See Supplementary Fig. 44 for the NMR spectra of compound **20**.*

Compound **21**



21

Mitsunobu @ 2nd step

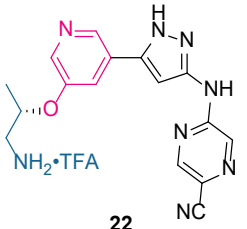
58% yield, 566 mg, 36 hrs

Protocol for purification: The collection was concentrated under vacuum. The residue was dissolved in MeOH (5 mL), followed by addition of EtOAc (10 mL) slowly. Overnight crystallization with EtOAc washing (20 mL) and vacuum drying at room temperature furnished a yellow powder (566 mg, 58% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H), 10.71 (s, 1H), 8.65 (d, J = 1.4 Hz, 1H), 8.55 (s, 1H), 8.13 (s, 3H), 7.35 (t, J = 8.4 Hz, 1H), 6.89 (s, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 4.80 – 4.67 (m, 1H), 3.82 (s, 3H), 3.15 (dd, J = 13.3, 3.7 Hz, 1H), 3.06 (dd, J = 13.3, 7.9 Hz, 1H), 1.16 (d, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.3 (q, J = 31.0 Hz), 157.7, 154.7, 152.1, 148.0, 146.3, 135.2, 134.4, 129.9, 117.7, 117.5 (q, J = 299 Hz), 116.1, 109.1, 107.9, 105.3, 98.8, 71.9, 55.9, 43.4, 16.8; HRMS calculated for $C_{18}H_{20}N_7O_2^+$ ($M+H^+$) 366.1673, found 366.1672; FT-IR (cm^{-1}): 3395, 3175, 2981, 2224, 1684, 1617, 1569, 1542, 1506, 1475, 1206, 1182, 1131, 1092, 1023, 799, 722; Melting point: 165.2 – 166.5 °C; $[\alpha]^D_{25}$ = +10 (c = 0.1, MeOH).

*See Supplementary Fig. 45 for the NMR spectra of compound **21**.*

Compound **22**



22

Mitsunobu @ 2nd step

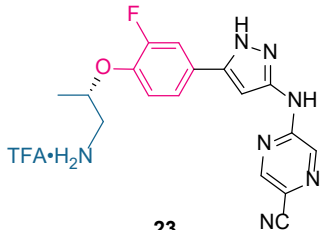
18% yield, 165 mg, 36 hrs

Protocol for purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatography (CH_2Cl_2 : MeOH = 10:1 to 2:1) followed by recrystallization with EtOAc/Et_2O, furnishing a pale-yellow powder (165 mg, 18% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.65 (s, 1H), 8.57 (s, 1H), 8.42 (s, 1H), 8.33 (s, 3H), 7.88 (s, 1H), 7.03 (s, 1H), 4.94 – 4.81 (m, 1H), 3.32 – 3.19 (m, 1H), 3.18 – 2.98 (m, 1H), 1.36 (d, J = 6.3 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.4 (q, J = 32.5 Hz), 154.0, 152.0, 147.8, 147.0, 140.2, 138.1, 137.7, 135.7, 127.3, 119.7, 117.8, 116.9, 116.8 (q, J = 293 Hz), 94.6, 72.0, 43.5, 17.0; HRMS calculated for $C_{16}H_{17}N_8O^+$ ($M+H^+$) 337.1520, found 337.1527; FT-IR (cm^{-1}): 3396, 3190, 2956, 2849, 2227, 1682, 1646, 1539, 1491, 1418, 1385, 1285, 1192, 1017, 800, 748, 722; Melting point: 230.2 – 233.1 °C (decomposed); $[\alpha]^D_{25}$= +14 (c = 0.1, MeOH)

*See Supplementary Fig. 46 for the NMR spectra of compound **22**.*

Compound **23**



23

Mitsunobu @ 2nd step

30% yield, 285 mg, 36 hrs

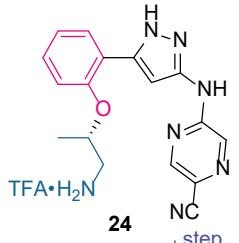
Protocol for purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatography (CH_2Cl_2 : MeOH = 6 : 1) followed by recrystallization with EtOAc/Et_2O to afford a yellow powder (285 mg, 30% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.68 (d, J = 1.4 Hz, 1H), 8.51 (s, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.52 (dd, J = 8.9, 2.4 Hz, 1H), 7.23 (d, J = 8.9 Hz, 1H), 7.08 (s, 1H), 4.90 – 4.81 (m, 1H), 3.28 – 3.15 (m, 2H), 1.24 (d, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.4 (q, J = 32.0 Hz), 153.9, 152.0, 147.8, 144.4 (d, J = 10.0 Hz), 141.4, 135.4, 124.0, 121.6 (d, J =

3.6 Hz), 118.3, 117.6, 117.5 (q, J = 300.0 Hz), 116.5, 113.3, 113.1, 93.7, 73.1, 43.4, 17.0; HRMS calculated for $C_{17}H_{17}FN_7O^+$ ($M+H^+$) 354.1473, found 354.1476; FT-IR (cm^{-1}): 3172, 3121, 3055, 2984, 2231, 1679, 1624, 1538, 1515, 1275, 1204, 1135, 1016, 800, 723; Melting point: 202.1 – 204.9 °C (decomposed); $[\alpha]^{D}_{25}$ = +8 (c = 0.1, MeOH).

*See supplementary Fig. 47 for the NMR spectra of compound **23**.*

Compound **24**



Mitsunobu @ 2nd step

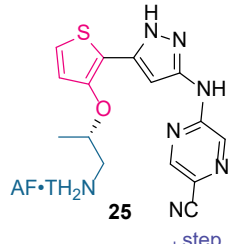
30% yield, 274 mg, 36 hrs

Protocol for purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatography (CH_2Cl_2 : MeOH = 8:1 to 3:1), followed by recrystallization with EtOAc/Et_2O, furnishing a pale-yellow powder (274 mg, 30% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.64 (s, 1H), 8.55 (s, 1H), 7.68 (d, J = 7.7 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.07 (t, J = 7.5 Hz, 2H), 6.99 (s, 1H), 4.90 (s, 1H), 3.32 – 3.18 (m, 2H), 1.23 (d, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.0 (q, J = 32.5 Hz), 153.1, 152.0, 147.8, 146.9, 139.3, 135.4, 129.5, 128.4, 121.8, 119.7, 117.6, 117.2 (q, J = 294 Hz), 116.4, 115.3, 96.3, 71.7, 43.3, 16.7; HRMS calculated for $C_{17}H_{18}N_7O^+$ ($M+H^+$) 336.1567, found 336.1565; FT-IR (cm^{-1}): 3421, 2961, 2928, 2230, 1683, 1617, 1539, 1499, 1386, 1207, 1140, 1019, 801, 724; Melting point: 214.6 – 217.0 °C; $[\alpha]^{D}_{25}$= +22 (c = 0.1, MeOH).

*See Supplementary Fig. 48 for the NMR spectra of compound **24**.*

Compound **25**



Mitsunobu @ 2nd step

55% yield, 510 mg, 36 hrs

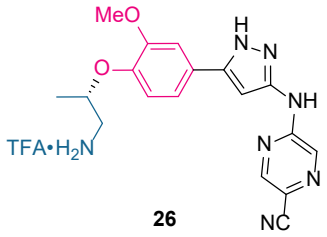
Protocol for purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatography (CH_2Cl_2 : MeOH = 10 : 1) followed by recrystallization with EtOAc/Et_2O to afford a yellow powder (510 mg, 55% yield).

^{1}H NMR (500 MHz, Methanol-d_4) δ 8.55 (d, J = 1.4 Hz, 1H), 8.42 (s, 1H), 7.42 (d, J = 5.6 Hz, 1H), 7.07 (d, J = 5.6 Hz, 1H), 6.66 (s, 1H), 4.72 – 4.63 (m, 1H), 3.27 – 3.22 (m, 2H), 1.35 (d, J =

6.2 Hz, 3H); ^{13}C NMR (125 MHz, Methanol-d_4) δ 163.1 (q, J = 34.8 Hz), 153.4, 152.9, 148.5, 146.6, 139.7, 136.5, 125.2, 120.3, 119.1, 118.1 (q, J = 293 Hz), 118.0, 114.8, 94.7, 76.9, 45.7, 17.8; HRMS calculated for $C_{15}H_{16}N_7OS^+$ ($M+H^+$) 342.1132, found 342.1141; FT-IR (cm^{-1}): 3368, 3344, 3112, 3085, 2220, 1682, 1605, 1535, 1489, 1240, 1197, 1139, 1022, 775, 724; Melting point: 185.9 – 188.3 °C; $[\alpha]^D_{25}$ = +59 (c = 0.1, MeOH).

See Supplementary Fig. 49 for the NMR spectra of compound ***25***.

Compound **26**



26

Mitsunobu @ 2nd step

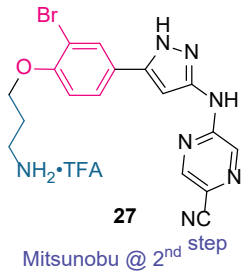
63% yield, 615 mg, 36 hrs

Protocol for purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatography (CH_2Cl_2 : MeOH = 4 : 1) followed by recrystallization with MeOH/EtOAc to afford a yellow powder (615 mg, 63% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.96 (s, 1H), 10.80 (s, 1H), 8.66 (d, J = 1.4 Hz, 1H), 8.49 (s, 1H), 7.97 (s, 3H), 7.42 (d, J = 2.2 Hz, 1H), 7.29 (dd, J = 8.3, 2.2 Hz, 1H), 7.14 (d, J = 8.3 Hz, 1H), 6.91 (s, 1H), 4.60 – 4.52 (m, 1H), 3.87 (s, 3H), 3.13 (dd, J = 13.3, 3.6 Hz, 1H), 3.05 (dd, J = 13.3, 8.1 Hz, 1H), 1.23 (d, J = 6.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.0, 150.9, 147.8, 145.7, 142.2, 135.4, 124.1, 118.6, 118.4, 117.7, 117.6, 116.4, 109.5, 93.4, 72.7, 55.8, 43.4, 17.0; HRMS calculated for $C_{18}H_{20}N_7O_2^+$ ($M+H^+$) 366.1673, found 366.1674; FT-IR (cm^{-1}): 3094, 3061, 2983, 2944, 2227, 1680, 1610, 1594, 1536, 1507, 1398, 1260, 1198, 1130, 1053, 1024, 800, 722; Melting point: 198.9 – 201.2 °C (decomposed); $[\alpha]^D_{25}$= +14 (c = 0.1, MeOH).

See Supplementary Fig. 50 for the NMR spectra of compound ***26***.

Compound **27**

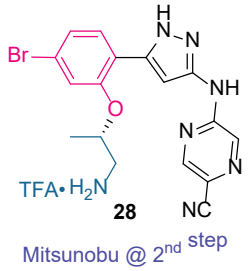


Protocol for purification: The collection was concentrated under vacuum. The residue was dissolved in MeOH (1.5 mL), after that, EtOAc (2 mL) was slowly added to the solution. The compound was crystallized as a yellow powder (140 mg, 13% yield) overnight.

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.99 (s, 1H), 10.82 (s, 1H), 8.65 (d, J = 1.4 Hz, 1H), 8.48 (s, 1 H), 8.03 (brs, 3H), 8.00 (d, J = 2.2 Hz, 1H), 7.74 (dd, J = 8.6, 2.2 Hz, 1H), 7.19 (d, J = 8.6 Hz, 1H), 6.90 (s, 1H), 4.20 (t, J = 6.0 Hz, 2H), 3.03 (t, J = 7.4 Hz, 2H), 2.14 – 1.99 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.4 (q, J = 31 Hz), 154.3, 152.0, 147.8, 140.9, 140.8, 135.4, 129.3, 125.8, 123.6, 117.6, 117.2 (q, J = 300 Hz), 116.4, 114.1, 111.6, 93.5, 65.8, 36.2, 26.8; HRMS calculated for $C_{17}H_{17}BrN_7O^+$ ($M+H^+$) 414.0673, found 414.0668; FT-IR (cm^{-1}): 3392, 3184, 3115, 2963, 2228, 1678, 1616, 1538, 1494, 1394, 1288, 1253, 1203, 1136, 1015, 800, 723; Melting point: 225.1 – 226.4 °C (decomposed).

*See Supplementary Fig. 51 for the NMR spectra of compound **27**.*

Compound **28**

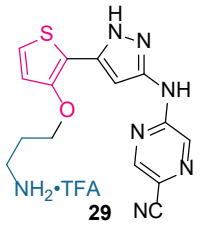


Protocol for purification: The collection was concentrated under vacuum. The residue was purified over silica gel chromatography (CH_2Cl_2 : MeOH = 10:1) followed by recrystallization with EtOAc/Et_2O to afford a yellow powder (462 mg, 43% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (d, J = 1.4 Hz, 1H), 8.51 (s, 1H), 7.88 (d, J = 2.5 Hz, 1H), 7.52 (dd, J = 9.0, 2.5 Hz, 1H), 7.23 (d, J = 9.0 Hz, 1H), 7.08 (s, 1H), 4.91 – 4.81 (m, 1H), 3.28 – 3.18 (m, 2H), 1.24 (d, J = 6.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.9 (appear s, 1C), 152.9, 152.5, 148.3, 147.2, 138.7, 136.0, 132.2, 130.6, 122.6, 118.1, 118.0, 117.2 (q, J = 300 Hz, 1C), 116.9, 113.7, 97.6, 72.8, 43.8, 17.1; HRMS calculated for $C_{17}H_{17}BrN_7O^+$ ($M+H^+$) 414.0673, found 414.0668; FT-IR (cm^{-1}): 3391, 3172, 3118, 2986, 2229, 1680, 1611, 1542, 1499, 1475, 1385, 1237, 1204, 1139, 1015, 802, 723; Melting point: 225.1 – 226.4 °C (decomposed); $[\alpha]^{D}_{25}$ = +13 (c = 0.1, MeOH).

*See Supplementary Fig. 52 for the NMR spectra of compound **28**.*

Compound **29**



Mitsunobu @ 2nd step

25% yield, 232 mg, 36 hrs

Purification: The collection was concentrated under vacuum. The residue was dissolved in MeOH (3 mL), followed by addition of EtOAc (3 mL) slowly. Overnight crystallization with EtOAc washing (5 mL) and vacuum drying at room temperature for 60 min afforded a pale-yellow powder (232 mg, 25% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.57 (s, 1H), 10.81 (s, 1H), 8.68 (d, J = 1.3 Hz, 1H), 8.46 (brs, 1H), 7.95 (brs, 3H), 7.52 (s, 1H), 7.11 (d, J = 5.5 Hz, 1H), 6.83 (s, 1H), 4.23 (t, J = 5.9 Hz, 2H), 3.05 (t, J = 7.4 Hz, 2H), 2.11 – 2.00 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.4 (q, J = 31.3 Hz), 153.3, 151.9, 147.9, 147.6, 135.5, 135.2, 124.3, 117.8, 117.7, 117.2 (q, J = 300.8 Hz), 116.3, 108.0, 94.1, 68.0, 36.3, 27.2; HRMS calculated for $C_{15}H_{16}N_7OS^+$ ($M+H^+$) 342.1132, found 342.1140. Melting point: 223.0 – 225.6 °C (decomposed).

*See Supplementary Fig. 53 for the NMR spectra of compound **29**.*

8.6 Automated early-stage diversifications (2nd step as the click reaction)

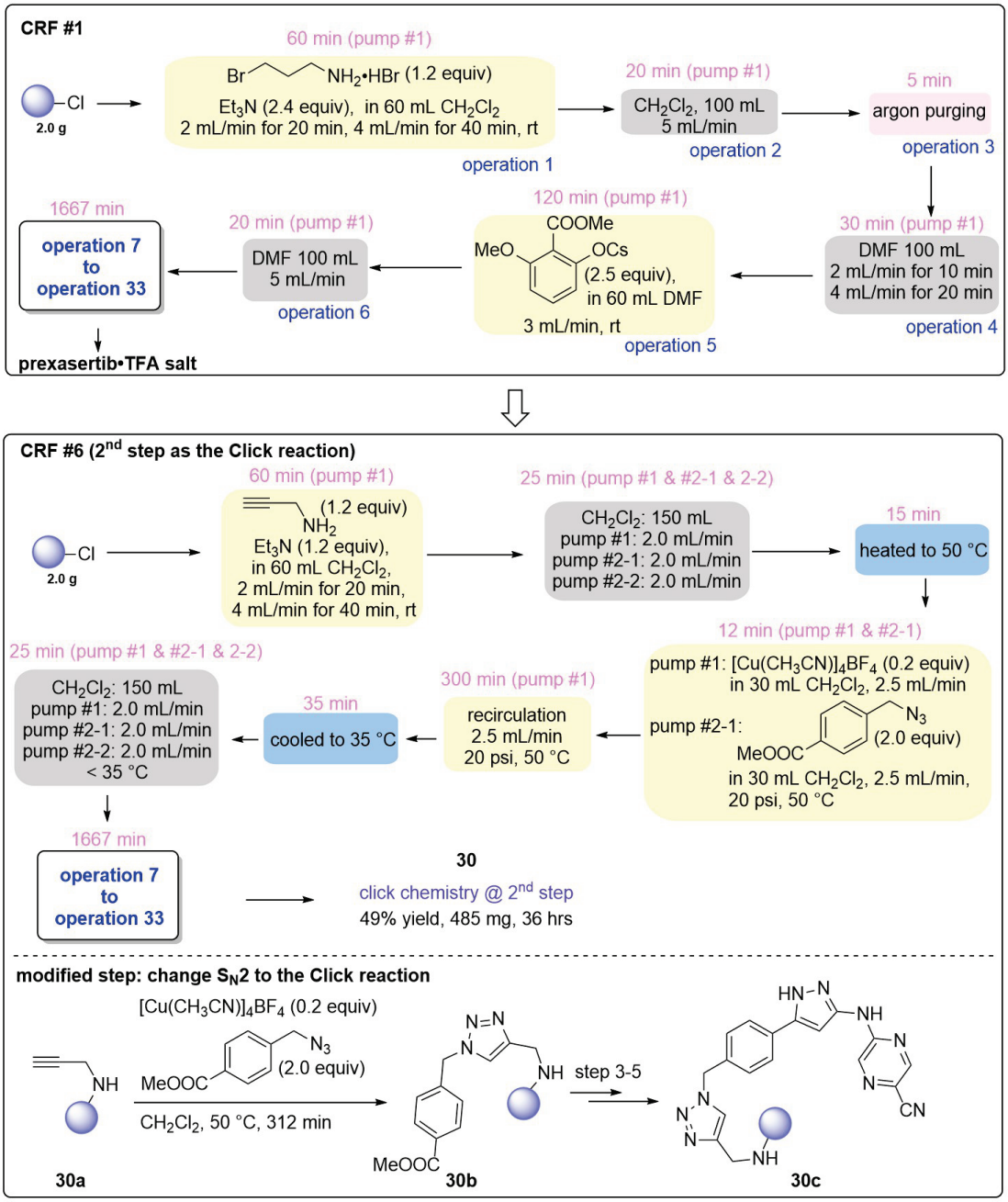
8.6.1 Preparation and storage of reagents for CRF #6 (2nd step as the click reaction)

The 1st, 3rd, 4th, 5th, and 6th reagent solutions were the same as those in CRF #1.

Reagent solution-1 for the 2nd step: To a 100 mL dry 3-port GL45 screw capped bottle were added $[Cu(CH_3CN)_4]BF_4$ (0.2 equiv., 128 mg) and 30 mL CH_2Cl_2. The resulting solution was ready for use as a stock solution for automated synthesis (*connecting to valve 1*).

Reagent solution-2 for the 2nd step: To a 100 mL dry 3-port GL45 screw capped bottle was added methyl *4*-(azidomethyl) benzoate (2.0 equiv., 780 mg). The resulting solution was ready for use as a stock solution for automated synthesis (*connecting to valve 2*).

8.6.2 Establishing the CRF #6 (2nd step as the click reaction)

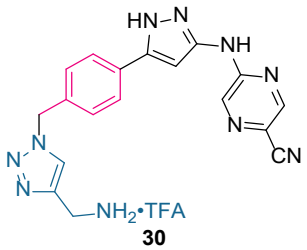


Supplementary Figure 21. CRF #6 (2nd step as the Click reaction).

Supplementary Table 10: The condition table in form of an Excel file for CRF #6.

Step		Substep	Time	Vapourtec Flowrate	Valve1	Asia-Left Flowrate	Asia-Right Flowrate	Valve2	Valve3	Valve 4	Temperature	Stirring speed
Unit			min	ml/min	position	ml/min	ml/min	position	position	position	°C	rpm
1		1-1 1st reaction	20	2	2	0	0	9	1	1	off	off
		1-2 1st reaction	40	4	2	0	0	9	1	1	off	off
		1-3 DCM wash	25	2	1	2	2	9	2	1	off	off
		1-4 heating	x	0	1	0	0	9	3	1	50	800
2		2-1 2nd mixing	12	2.5	3	2.5	0	8	3	1	50	800
		2-2 2nd reaction	300	2.5	4	0	0	8	3	1	50	800
		2-3 cooling	x	0	4	0	0	8	3	1	35	800
		2-4 DCM wash	25	2	1	2	2	9	2	1	off	off
		2-5 argon purge	10	0	1	0	0	9	2	10	off	off
		2-6 MeOH wash	10	2	7	0	0	10	2	1	off	off
		2-7 MeOH wash	20	4	7	0	0	10	2	1	off	off
		2-8 argon purge	30	0	7	0	0	10	2	10	off	off
3		3-1 THF wash	10	1	6	0.5	0.5	10	2	1	off	off
		3-2 THF wash	20	2.5	6	2.5	1	10	2	1	off	off
		3-3 3rd reaction	30	2.5	5	0	2.5	1	2	1	off	off
		3-4 THF wash	15	0	5	4	1	10	2	1	off	off
		3-5 argon purge	3	0	5	0	0	10	2	10	off	off
		3-6 0.1 N HCl wash	10	0	5	2	0	8	2	1	off	off
		3-7 0.1 N HCl wash	20	0	5	5	0	8	2	1	off	off
		3-8 heating	x	0	5	0	0	8	2	1	60	800
		3-9 0.1 N HCl wash	20	0	5	5	0	8	2	1	60	800
		3-10 argon purge	3	0	5	0	0	10	2	10	60	800
		3-11 THF wash	10	0.5	6	1	0.5	10	5	1	60	800
		3-12 THF wash	15	1	6	3	1	10	5	1	60	800
4		4-1 EtOH-THF wash	20	0	6	2.5	0	3	5	1	60	800
		4-2 heating	x	0	6	0	0	3	5	1	110	800
		4-3 4th reaction	360	0	6	2.5	0	2	4	1	110	800
		4-4 EtOH-THF wash	20	0	6	2.5	0	3	5	1	110	800
		4-5 THF wash	20	1.5	6	2.5	1	10	5	1	110	800
		4-6 argon purge	20	0	6	0	0	10	2	10	110	800
5		5-1 Dioxane-DMSO wash	20	2.5	15	2.5	0	4	5	1	110	800
		5-2 5th mixing	12	2.5	14	2.5	0	5	6	1	110	800
		5-3 5th reaction	600	0	14	2.5	0	6	6	1	110	800
		5-4 cooling	x	0	14	0	0	6	6	1	35	800
		5-5 MeOH & THF wash	20	2.5	7	2.5	0	10	5	1	off	off
		5-6 argon purge	20	0	7	0	0	10	2	10	off	off
6		6-1 DCM wash	15	2	1	0	0	10	2	1	off	off
		6-2 DCM wash	20	5	1	0	0	10	2	1	off	off
		6-4 6th reaction	120	3	16	0	0	10	16	1	off	off
		6-5 DCM wash	10	5	1	0	0	10	16	1	off	off
											Modified operations	

Compound **30**



30

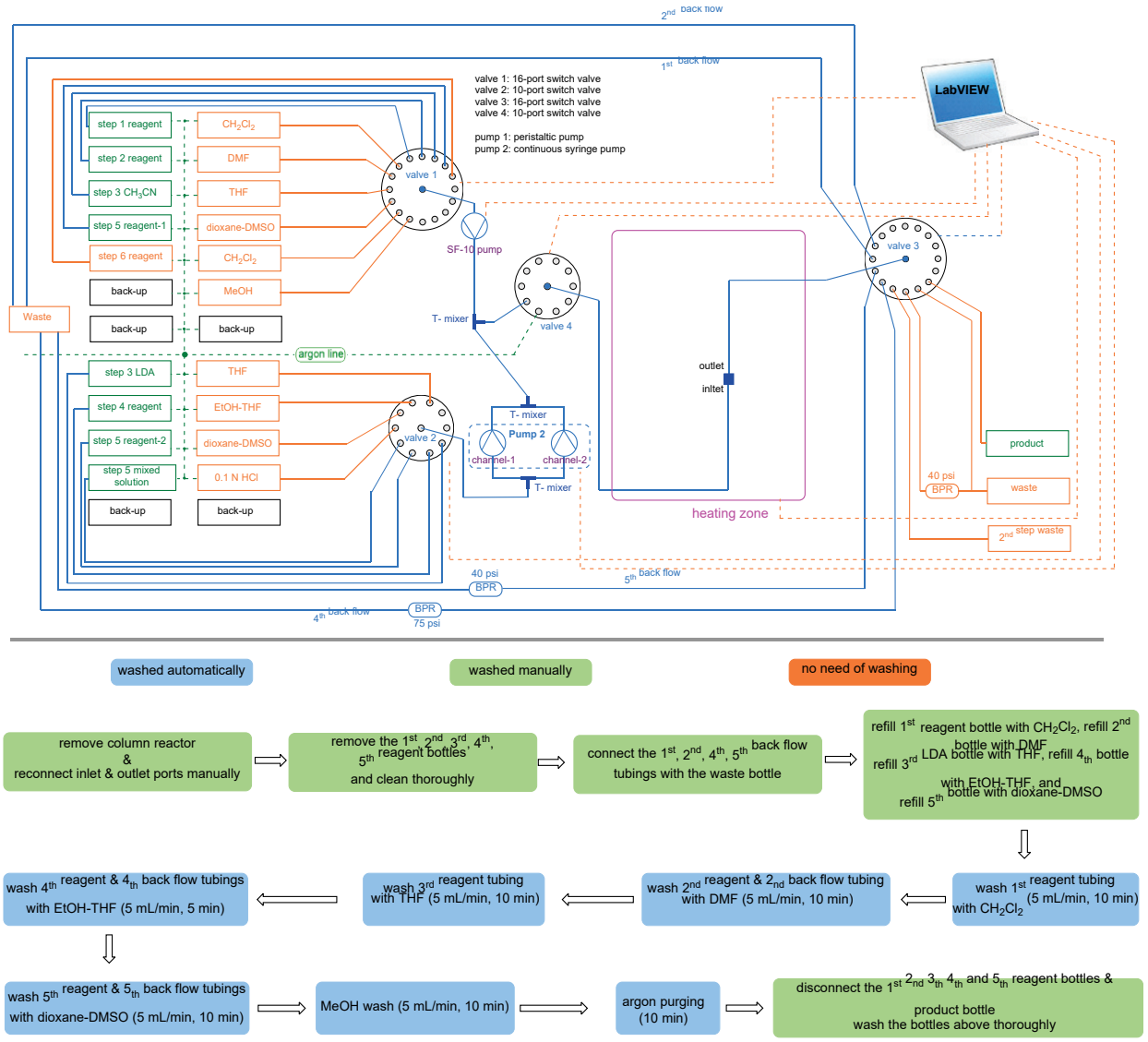
click chemistry @ 2nd step

49% yield, 485 mg, 36 hrs

Protocol for purification: The collection was concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (5 mL), followed by addition of EtOAc (5 mL) slowly. Overnight crystallization with EtOAc washing (5 mL) and vacuum drying at room temperature afforded a white solid (485 mg, 49% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 13.10 (s, 1H), 10.85 (s, 1H), 8.66 (d, J = 1.4 Hz, 1H), 8.48 (s, 1H), 8.32 (s, 3H), 8.22 (s, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 6.94 (s, 1H), 5.69 (s, 2H), 4.14 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.2(q, J = 30 Hz), 151.9, 147.8, 141.9, 140.5, 135.7, 135.4, 129.3, 129.0, 128.8, 125.4, 124.5, 117.6, 116.5, 93.9, 52.6, 33.9; HRMS calculated for $C_{18}H_{17}N_{10}^+$ ($M+H^+$) 373.1632, found 373.1628; FT-IR (cm^{-1}): 3412, 3130, 3060, 2954, 2228, 1728, 1684, 1540, 1501, 1203, 1134, 801, 723; Melting point: 191.2 – 193.7 °C (decomposed).

*See Supplementary Fig. 54 for the NMR spectra of compound **30**.*

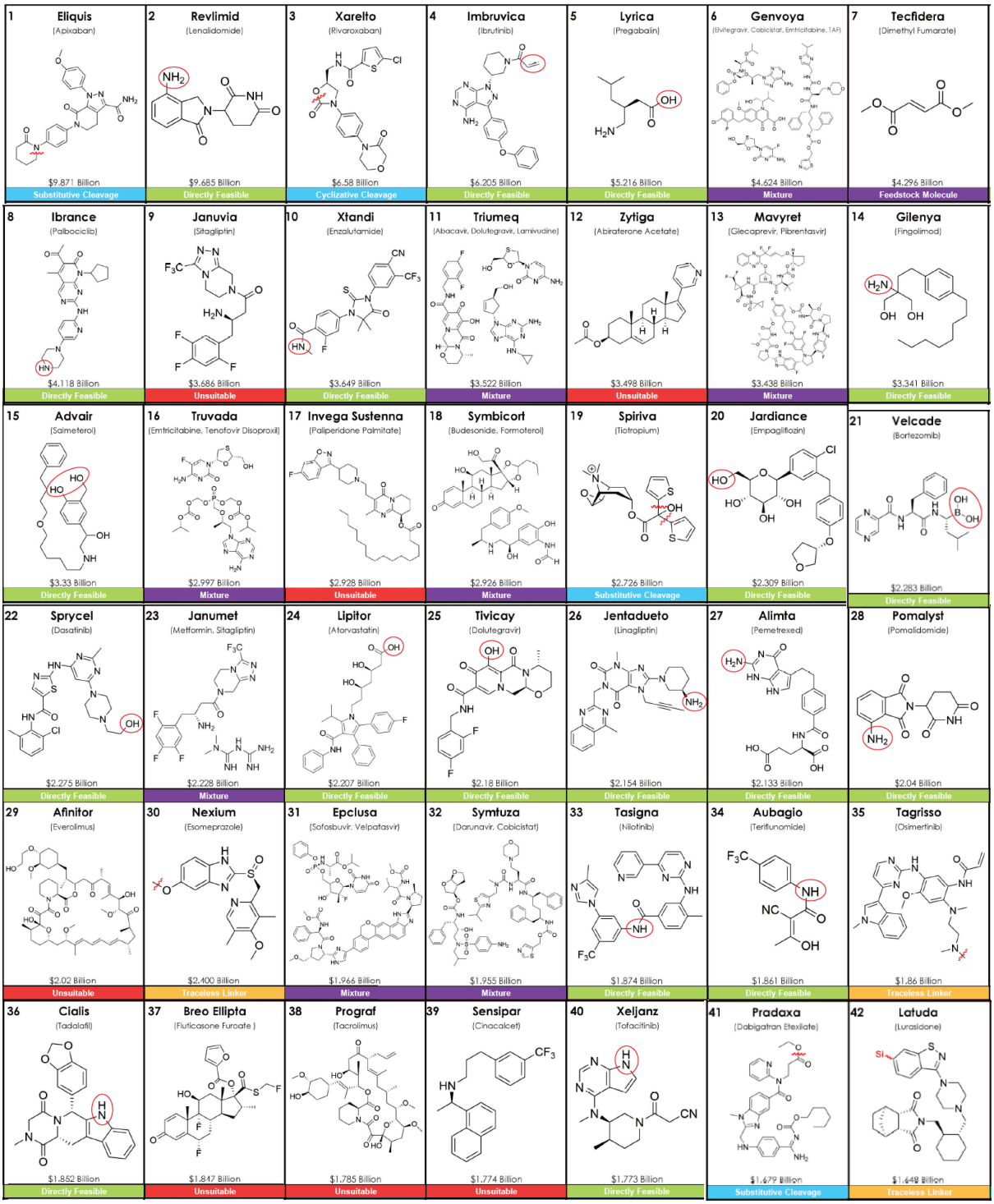


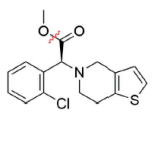
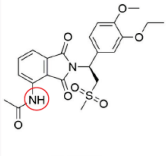
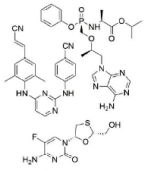
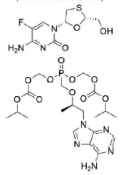
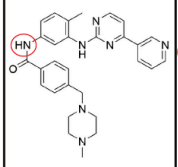
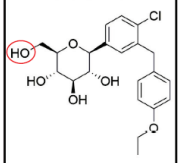
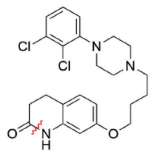
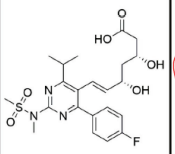
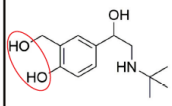
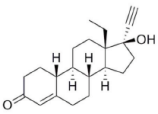
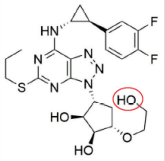
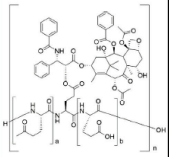
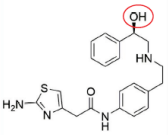
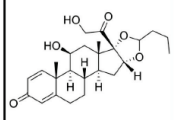
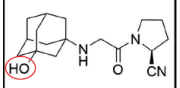
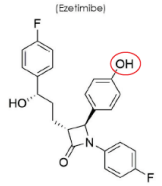
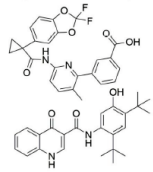
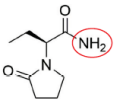
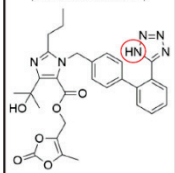
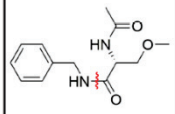
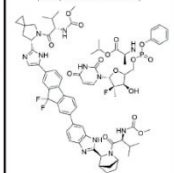
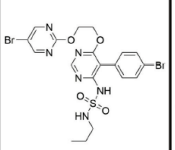
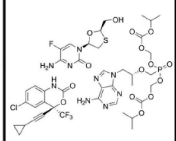
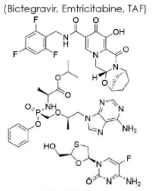
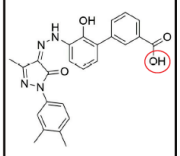
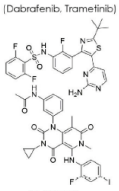
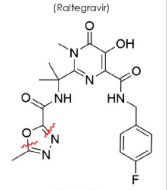
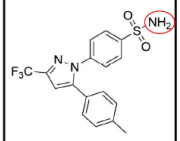
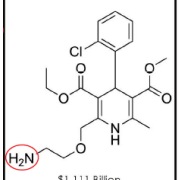
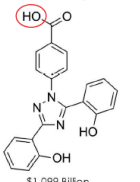
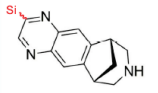
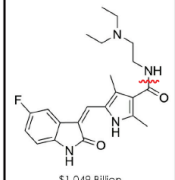
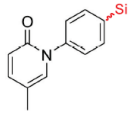
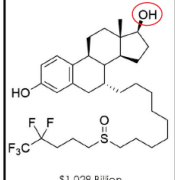
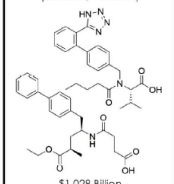
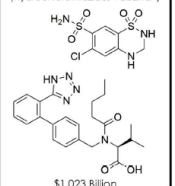
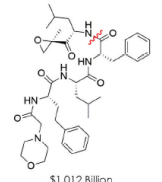
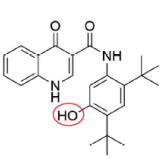
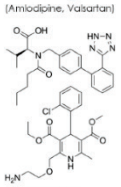
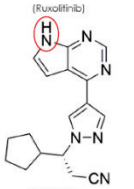
Supplementary Figure 22. Schematic drawing for the cleaning protocol between each molecule synthesis implementation.

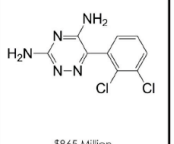
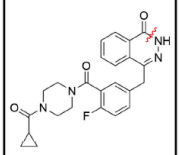
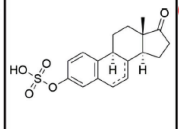
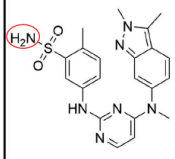
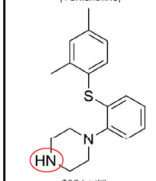
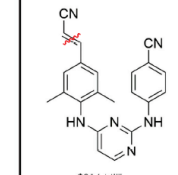
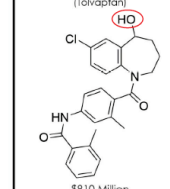
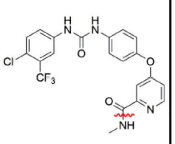
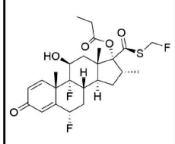
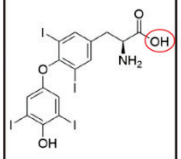
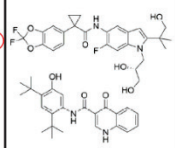
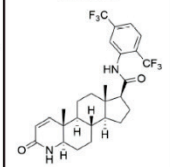
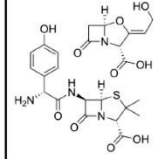
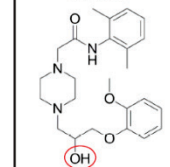
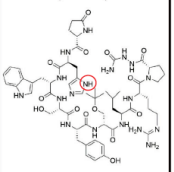
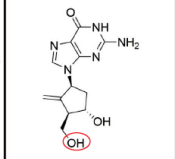
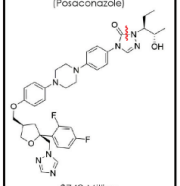
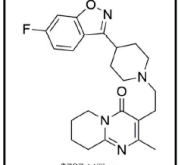
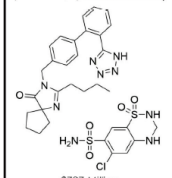
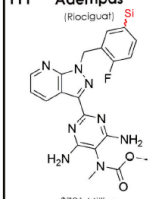
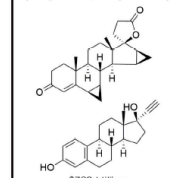
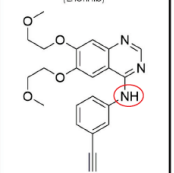
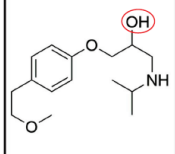
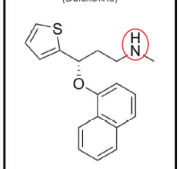
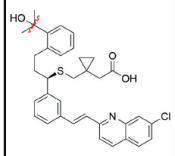
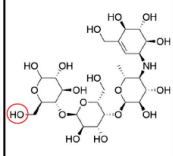
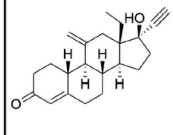
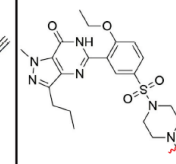
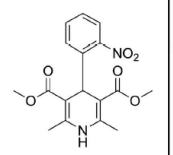
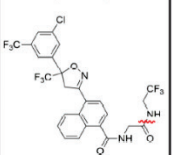
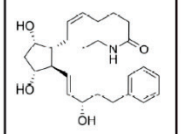
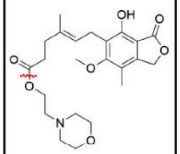
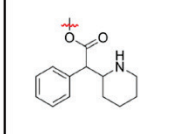
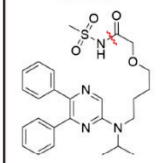
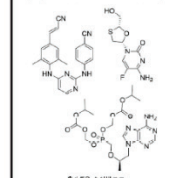
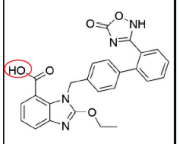
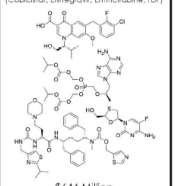
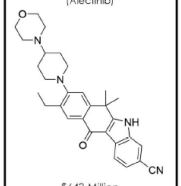
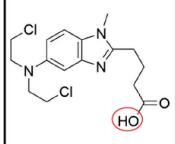
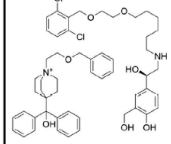
9. Analysis of the feasibility of synthesizing top-selling drugs using SPS-flow technology

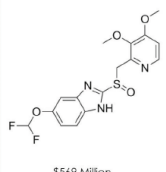
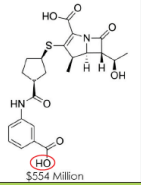
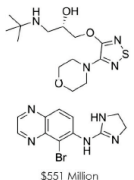
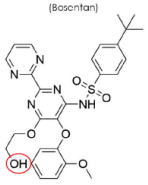
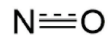
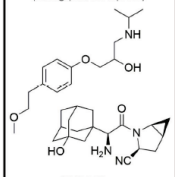
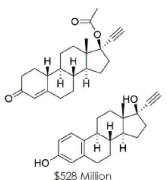
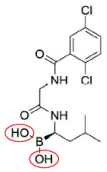
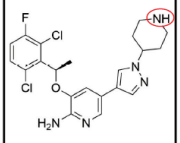
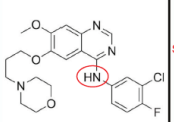
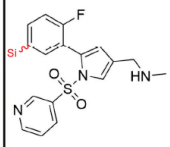
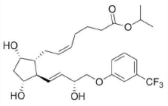
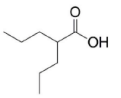
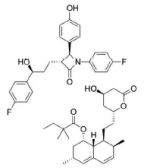
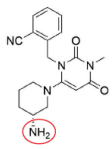
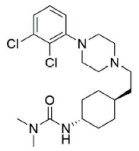
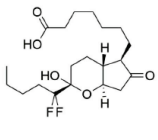
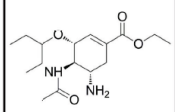
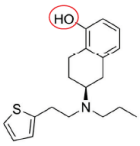
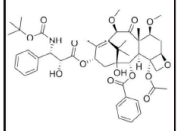
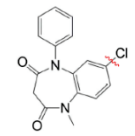
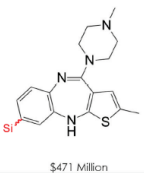
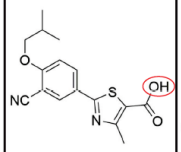
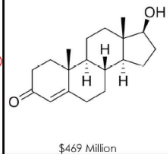
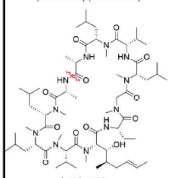
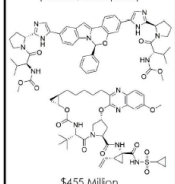
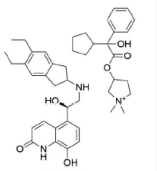
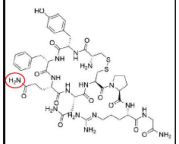
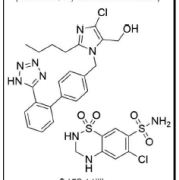
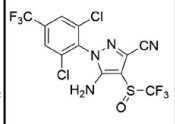
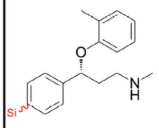
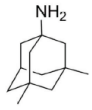
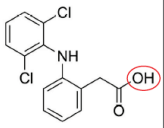
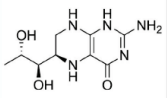
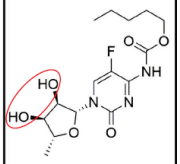
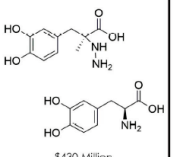
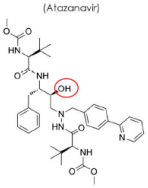
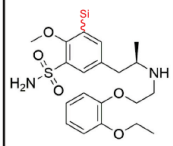
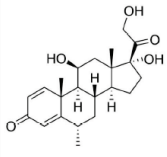
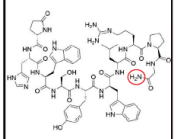
Upon close analysis of the top 200 small-molecule pharmaceuticals (by retail sales in 2018), we envision that 73 out of 155 single small molecules (among the top 200 pharmaceuticals, 39 are mixtures of molecules and 6 are feedstock compounds) possess functional group handles suitable for attachment to solid supports, 28 molecules can be achieved by cyclization-cleavage or substitution-cleavage strategies, and 12 molecules can potentially be approached through SPS assisted by a traceless linker. Taken together, 73% of these single pharmaceutical molecules can potentially be synthesized by SPS:

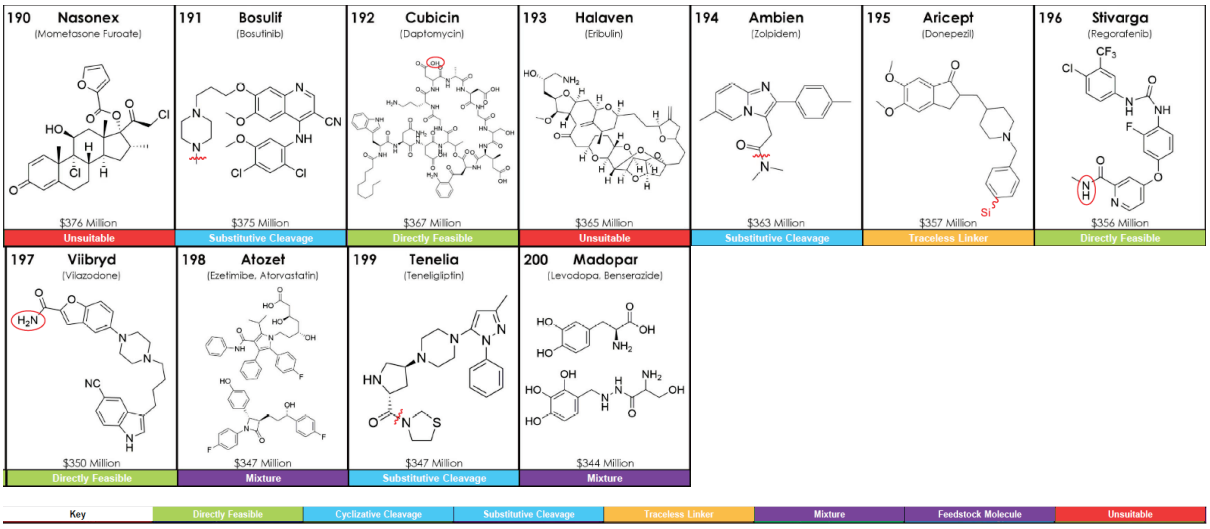
Supplementary Table 11: SPS Feasibility Analysis for Top 200 Small Molecule Pharmaceuticals (by Retail Sales in 2018)



43 Plavix (Clopidogrel)  $1.627 Billion Substitutive Cleavage	44 Otezla (Apremilast)  $1.608 Billion Directly Feasible	45 Odefsey (Emtricitabine, Rilpivirine, TAF)  $1.598 Billion Mixture	46 Descovy (Emtricitabine, TAF)  $1.581 Billion Mixture	47 Gleevec (Imatinib)  $1.561 Billion Directly Feasible	48 Farxiga (Dapagliflozin)  $1.514 Billion Directly Feasible	49 Abilify Maintena (Aripiprazole)  $1.491 Billion Cyclizative Cleavage
50 Crestor (Rosuvastatin)  $1.433 Billion Unsuitable	51 Ventolin (Albuterol)  $1.377 Billion Directly Feasible	52 Mirena Family (Levonorgestrel)  $1.342 Billion Unsuitable	53 Brilinta (Ticagrelor)  $1.321 Billion Directly Feasible	54 Abraxane (Paclitaxel Protein Bound)  $1.292 Billion Unsuitable	55 Myrbetriq (Mirabegron)  $1.287 Billion Directly Feasible	56 Pulmicort (Budesonide)  $1.286 Billion Unsuitable
57 Galvus (Vildagliptin)  $1.284 Billion Directly Feasible	58 Zetia (Ezetimibe)  $1.271 Billion Directly Feasible	59 Orkambi (Lumacaftor, Ivacaftor)  $1.262 Billion Mixture	60 Keppra (Levetiracetam)  $1.254 Billion Directly Feasible	61 Benicar (Olmesartan Medoxomil)  $1.242 Billion Directly Feasible	62 Vimpat (Lacosamide)  $1.241 Billion Substitutive Cleavage	63 Harvoni (Ledipasvir, Sofosbuvir)  $1.222 Billion Mixture
64 Opsumit (Macitentan)  $1.215 Billion Unsuitable	65 Atripla (Efavirenz, Emtricitabine, TDF)  $1.206 Billion Mixture	66 Biktarvy (Bictegravir, Emtricitabine, TAF)  $1.184 Billion Mixture	67 Promacta (Eltrombopag)  $1.174 Billion Directly Feasible	68 Tafinlar + Mekinist (Dabrafenib, Trametinib)  $1.155 Billion Mixture	69 Isentress (Raltegravir)  $1.14 Billion Substitutive Cleavage	70 Celebrex (Celecoxib)  $1.129 Billion Directly Feasible
71 Norvasc (Amlodipine)  $1.111 Billion Directly Feasible	72 Exjade (Deferasirox)  $1.099 Billion Directly Feasible	73 Chantix (Varenicline)  $1.084 Billion Traceless Linker	74 Sutent (Sunitinib)  $1.049 Billion Substitutive Cleavage	75 Esbriet (Pirfenidone)  $1.041 Billion Traceless Linker	76 Faslodex (Fulvestrant)  $1.028 Billion Directly Feasible	77 Entresto (Sacubitril, Valsartan)  $1.028 Billion Mixture
78 Co-Diovan (Hydrochlorothiazide, Valsartan)  $1.023 Billion Mixture	79 Kyprolis (Carfilzomib)  $1.012 Billion Substitutive Cleavage	80 Kalydeco (Ivacaftor)  $1.008 Billion Directly Feasible	81 Exforge (Amlodipine, Valsartan)  $1.002 Billion Mixture	82 Jakavi (Ruxolitinib)  $977 Million Directly Feasible	83 Letairis (Ambrisentan) $943 Million Directly Feasible	84 Bridion (Sugammadex) $917 Million Unsuitable
85 Doliprane (Acetaminophen) $911 Million Directly Feasible	86 Nuvaring (Ethinyl Estradiol, Etonogestrel) $902 Million Mixture	87 Lupron (Leuprolide) $892 Million Directly Feasible	88 Rexulti (Brexpiprazole) $884 Million Cyclizative Cleavage	89 Vesicare (Solifenacin) $884 Million Unsuitable	90 Lixiana (Edoxaban) $881 Million Unsuitable	91 Invokamet (Canagliflozin, Metformin) $881 Million Mixture

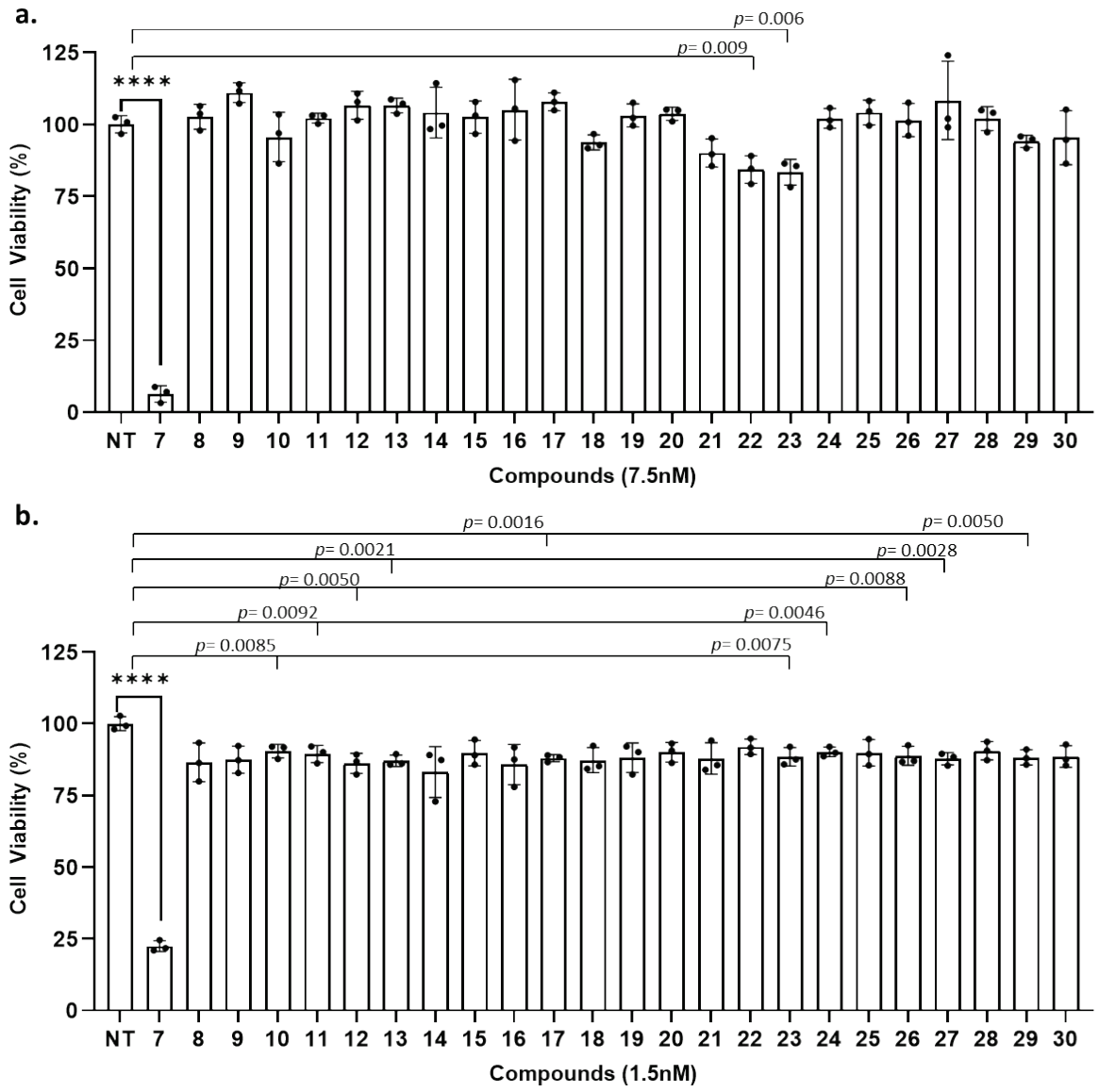
92 Lamictal (Lamotrigine)  $865 Million Unsuitable	93 Lynparza (Olaparib)  $834 Million Cyclizative Cleavage	94 Premarin Family (Conjugated Estrogens)  $832 Million Unsuitable	95 Votrient (Pazopanib)  $828 Million Directly Feasible	96 Brintellix (Vortioxetine)  $826 Million Directly Feasible	97 Edurant (Rilpivirine)  $816 Million Substitutive Cleavage	98 Samsca (Tolvaptan)  $810 Million Directly Feasible
99 Nexavar (Sorafenib)  $805 Million Substitutive Cleavage	100 Flovent (Fluticasone Propionate)  $791 Million Unsuitable	101 Synthroid (Levothyroxine)  $776 Million Directly Feasible	102 Symdeko (Tezacaftor, Ivacaftor)  $769 Million Mixture	103 Avodart (Dutasteride)  $761 Million Unsuitable	104 Augmentin (Amoxicillin, Clavulanate)  $758 Million Mixture	105 Ranexa (Ranolazine)  $758 Million Directly Feasible
106 Zoladex (Goserelin)  $752 Million Directly Feasible	107 Baraclude (Entecavir)  $744 Million Directly Feasible	108 Noxafil (Posaconazole)  $742 Million Cyclizative Cleavage	109 Risperdal Consta (Risperidone)  $737 Million Unsuitable	110 Aprovel (Irbesartan, Hydrochlorothiazide)  $737 Million Mixture	111 Adempas (Riociguat)  $731 Million Traceless Linker	112 YAZ (Drospirenone, Ethinyl Estradiol)  $722 Million Mixture
113 Tarceva (Erlotinib)  $714 Million Directly Feasible	114 Toprol (Metoprolol)  $712 Million Directly Feasible	115 Cymbalta (Duloxetine)  $708 Million Directly Feasible	116 Singulair (Montelukast)  $708 Million Substitutive Cleavage	117 Glucobay (Acarbose)  $704 Million Directly Feasible	118 Nexplanon (Etonogestrel)  $703 Million Unsuitable	119 Viagra (Sildenafil)  $692 Million Substitutive Cleavage
120 Adalat (Nifedipine)  $690 Million Unsuitable	121 Nexgard (Afoxolaner)  $689 Million Substitutive Cleavage	122 Ganfort (Bimatoprost)  $684 Million Unsuitable	123 Cellcept (Mycophenolate Mofetil)  $676 Million Substitutive Cleavage	124 Concerta (Methylphenidate)  $663 Million Substitutive Cleavage	125 Uptravi (Selexipag)  $663 Million Substitutive Cleavage	126 Complera (Rilpivirine, Emtricitabine, TDF)  $653 Million Mixture
127 Azilva (Azilsartan)  $647 Million Directly Feasible	128 Stribild (Cobicistat, Elvitegravir, Emtricitabine, TDF)  $644 Million Mixture	129 Alecensa (Alectinib)  $643 Million Unsuitable	130 Bendeka (Bendamustine)  $642 Million Directly Feasible	131 Anoro Ellipta (Umeclidinium, Vilanterol)  $633 Million Mixture	132 Aspirin Cardio (Acetylsalicylic Acid) $629 Million Directly Feasible	133 Vidaza (Azacitidine) $617 Million Directly Feasible
134 Lenvima (Lenvatinib) $617 Million Directly Feasible	135 Dexilant (Dexlansoprazole) $617 Million Unsuitable	136 Emend (Aprepitant) $615 Million Unsuitable	137 Sulperazon (Cefoperazone, Sulbactam) $613 Million Mixture	138 Allegra (Fexofenadine) $588 Million Directly Feasible	139 Bystolic (Nebivolol) $586 Million Directly Feasible	140 Ambisome (Amphotericin B) $571 Million Unsuitable

141 Pantoprazole (Pantoprazole)  $569 Million Unsuitable	142 Invanz (Ertapenem)  $554 Million Directly Feasible	143 Combigan (Brimonidine, Timolol)  $551 Million Mixture	144 Tracleer (Bosentan)  $546 Million Directly Feasible	145 Inomax (Nitric Oxide)  $543 Million Feedstock Molecule	146 Kombiglyze (Saxagliptin, Metoprolol)  $543 Million Mixture	147 Lo Loestrin (Norethindrone Acetate, Ethinyl Estradiol)  $528 Million Mixture
148 Ninlaro (Ixazomib)  $526 Million Directly Feasible	149 Xalkori (Crizotinib)  $524 Million Directly Feasible	150 Iressa (Gefitinib)  $518 Million Directly Feasible	151 Takecab (Vonoprazan)  $518 Million Traceless Linker	152 Travoprost Group (Travoprost)  $517 Million Unsuitable	153 Depakine (Valproate)  $511 Million Feedstock Molecule	154 Vytorin (Ezetimibe, Simvastatin)  $497 Million Mixture
155 Nesina (Alogliptin)  $496 Million Directly Feasible	156 Vraylar (Cariprazine)  $487 Million Unsuitable	157 Amitiza (Lubiprostone)  $479 Million Unsuitable	158 Tamiflu (Oseltamivir)  $478 Million Unsuitable	159 Neupro (Rotigotine)  $478 Million Directly Feasible	160 Jevtana (Cabazitaxel)  $477 Million Unsuitable	161 Onfi (Clobazam)  $475 Million Substitutive Cleavage
162 Zyprexa (Olanzapine)  $471 Million Traceless Linker	163 Uloric (Febuxostat)  $471 Million Directly Feasible	164 Androgel (Testosterone)  $469 Million Unsuitable	165 Sandimmune (Dexmethylphenidate)  $463 Million Substitutive Cleavage	166 Zepatier (Elbasvir, Grazoprevir)  $455 Million Mixture	167 Ultibro Breezhaler (Indacaterol, Glycopyrronium)  $454 Million Mixture	168 Vasostrict (Vasopressin)  $454 Million Directly Feasible
169 Hyzaar (Losartan, Hydrochlorothiazide)  $453 Million Mixture	170 Frontline (Fipronil)  $451 Million Unsuitable	171 Strattera (Atomoxetine)  $451 Million Traceless Linker	172 Memary (Memantine)  $450 Million Feedstock Molecule	173 Voltaren (Diclofenac)  $445 Million Directly Feasible	174 Kuvan (Sapropterin)  $434 Million Unsuitable	175 Xeloda (Capecitabine)  $431 Million Directly Feasible
176 Duodopa (Carbidopa, Levodopa)  $430 Million Mixture	177 Reyataz (Atazanavir)  $427 Million Directly Feasible	178 Flomax (Tamsulosin)  $427 Million Traceless Linker	179 Medrol (Methylprednisolone)  $426 Million Unsuitable	180 Decapeptyl (Triptorelin)  $421 Million Directly Feasible	181 Seroquel (Quetiapine) $404 Million Traceless Linker	182 Vosevi (Sofosbuvir, Velpatasvir, Voxilaprevir) $396 Million Mixture
183 Multaq (Dronedarone) $396 Million Substitutive Cleavage	184 Canasa (Mesalazine) $394 Million Feedstock Molecule	185 Vfend (Voriconazole) $393 Million Unsuitable	186 Sevoflurane (Sevoflurane) $391 Million Feedstock Molecule	187 Injectafer (Ferric Carboxymaltose) $387 Million Unsuitable	188 Amaryl (Glimepiride) $379 Million Substitutive Cleavage	189 Incruse Ellipta (Umeclidinium) $378 Million Unsuitable


190 Nasonex
(Mometasone Furoate)
$376 Million
Unsuitable
191 Bosulif
(Bosutinib)
$375 Million
Substitutive Cleavage
192 Cubicin
(Daptomycin)
$367 Million
Directly Feasible
193 Halaven
(Eribulin)
$365 Million
Unsuitable
194 Ambien
(Zolpidem)
$363 Million
Substitutive Cleavage
195 Aricept
(Donepezil)
$357 Million
Traceless Linker
196 Stivarga
(Regorafenib)
$356 Million
Directly Feasible
197 Viibryd
(Vilazodone)
$350 Million
Directly Feasible
198 Atozet
(Ezetimibe, Atorvastatin)
$347 Million
Mixture
199 Tenelia
(Teneligliptin)
$347 Million
Substitutive Cleavage
200 Madopar
(Levodopa, Benserazide)
$344 Million
Mixture
Key
Directly Feasible
Cyclizative Cleavage
Substitutive Cleavage
Traceless Linker
Mixture
Feedstock Molecule
Unsuitable

10. Bioactivity evaluation

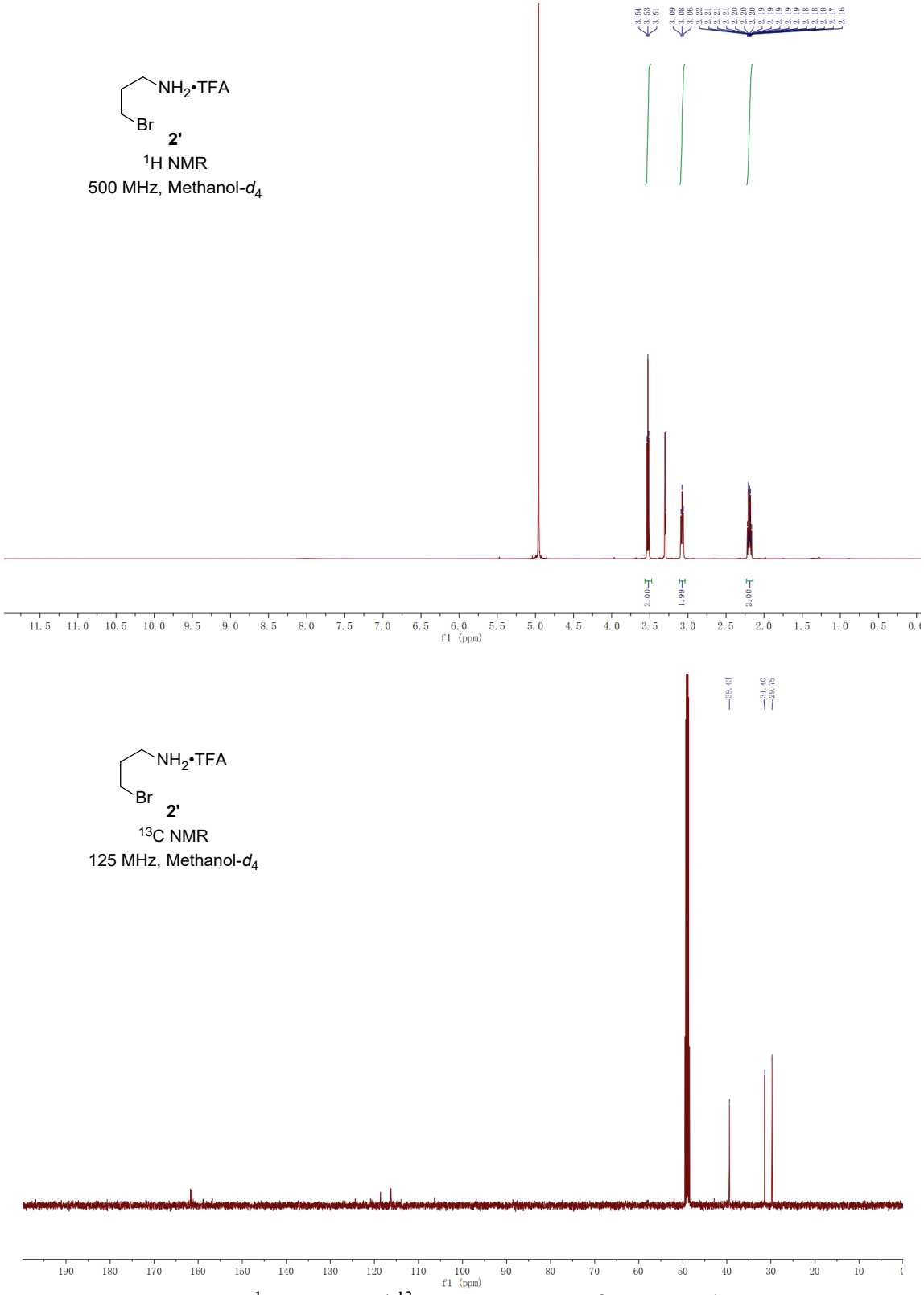
The half maximal inhibitory concentration (IC_{50}) of prexasertib was first determined in a human lymphocytic leukemia REH cell line (IC_{50}= 4.57 nM) and a T-lymphoblastic leukemia Molt-4 cell line (IC_{50}=0.89 nM). The anti-cancer activity of a series of prexasertib analogs was evaluated by CCK8 assay using concentrations of 7.5 nM and 1.5 nM in REH and Molt4, respectively. Preliminary evaluation suggests that prexasertib **7** remains the most potent compound among all the tested derivatives.



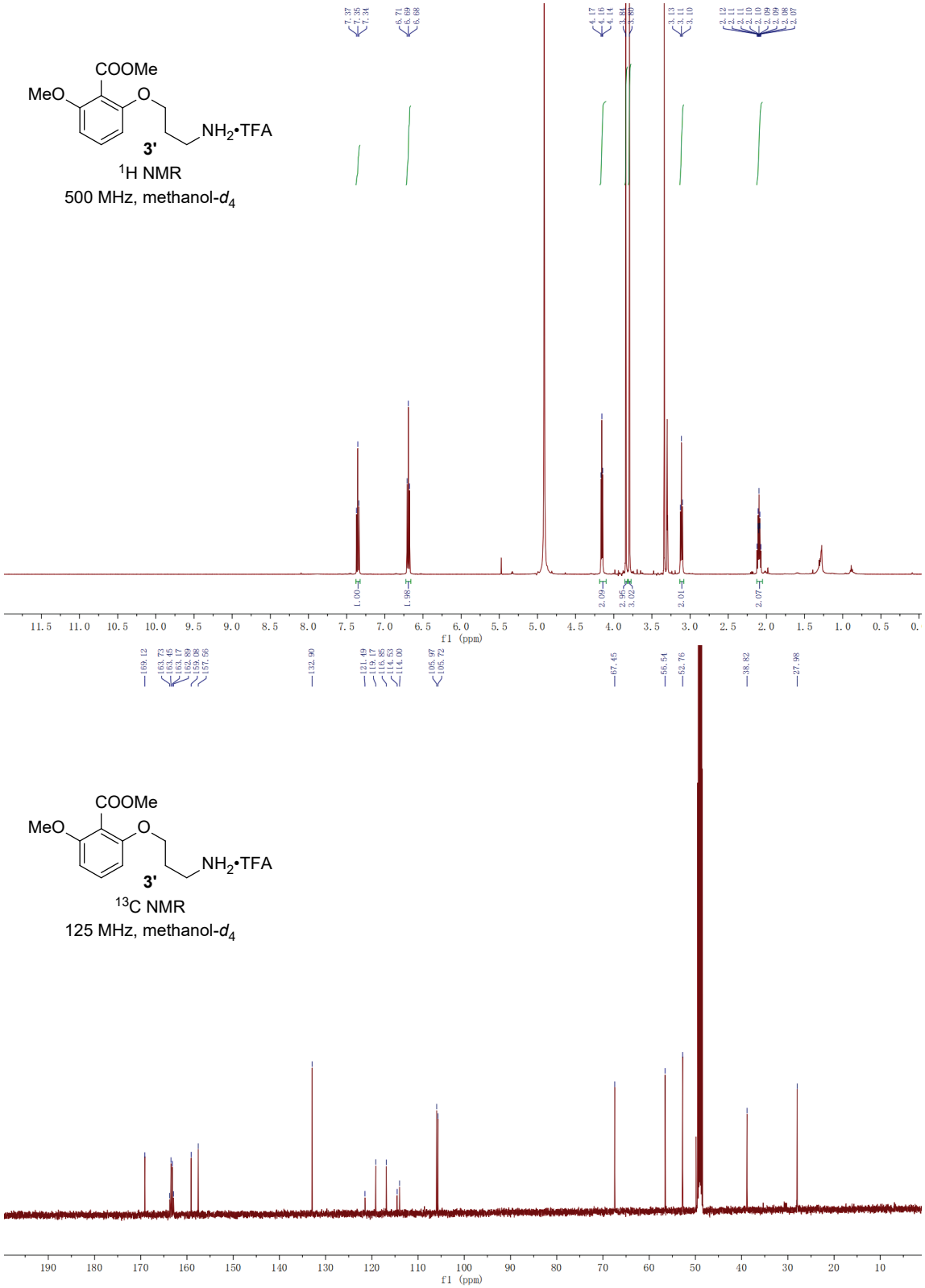
Supplementary Fig. 23. Cell viability of compounds at a) 7.5 nM on REH cell line and b) 1.5nM on Molt4 cell line after treatment for 48 hr. The experiment was conducted in n=3 biologically independent samples. Two independent experimental replicates were performed and showed similar results. Error bars, mean +/- s.d. Differences between NT vs compound treatment with *p*

value < 0.01 are indicated; ****, $p < 0.0001$. Two-tailed unpaired t-test statistical analysis was used.

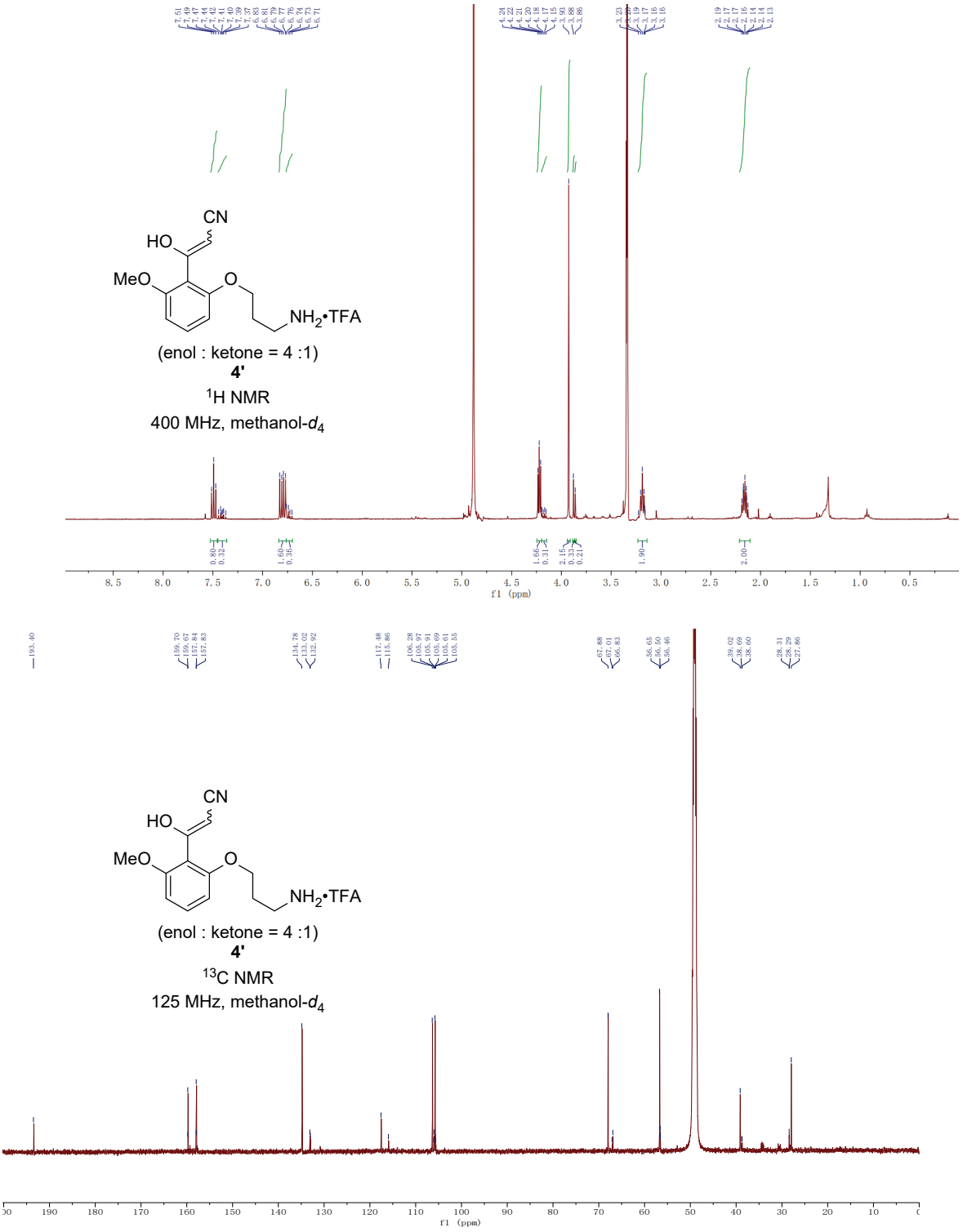
11. NMR spectra of new compounds



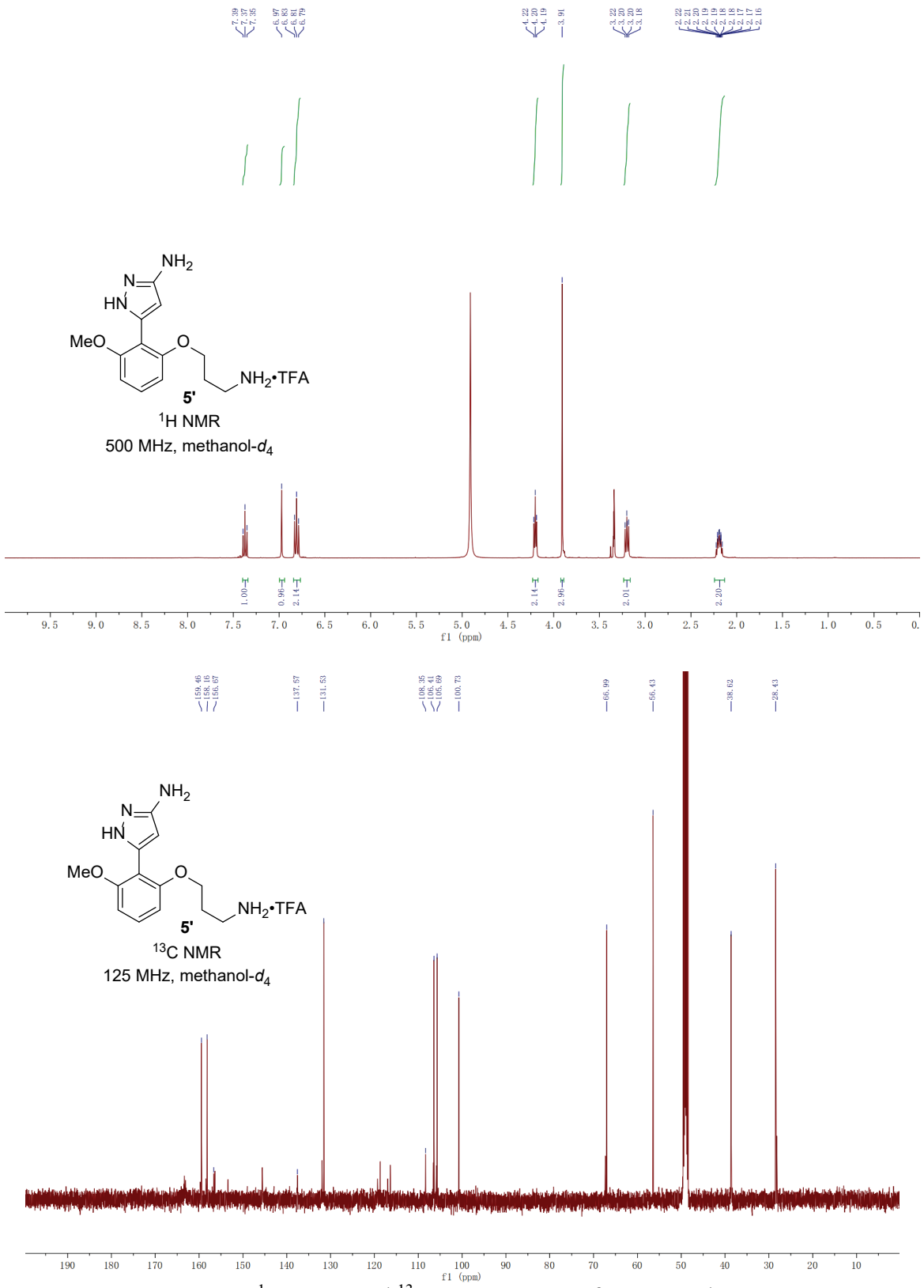
Supplementary Figure 24. ^{1}H NMR and ^{13}C NMR spectra of compound **2'**.



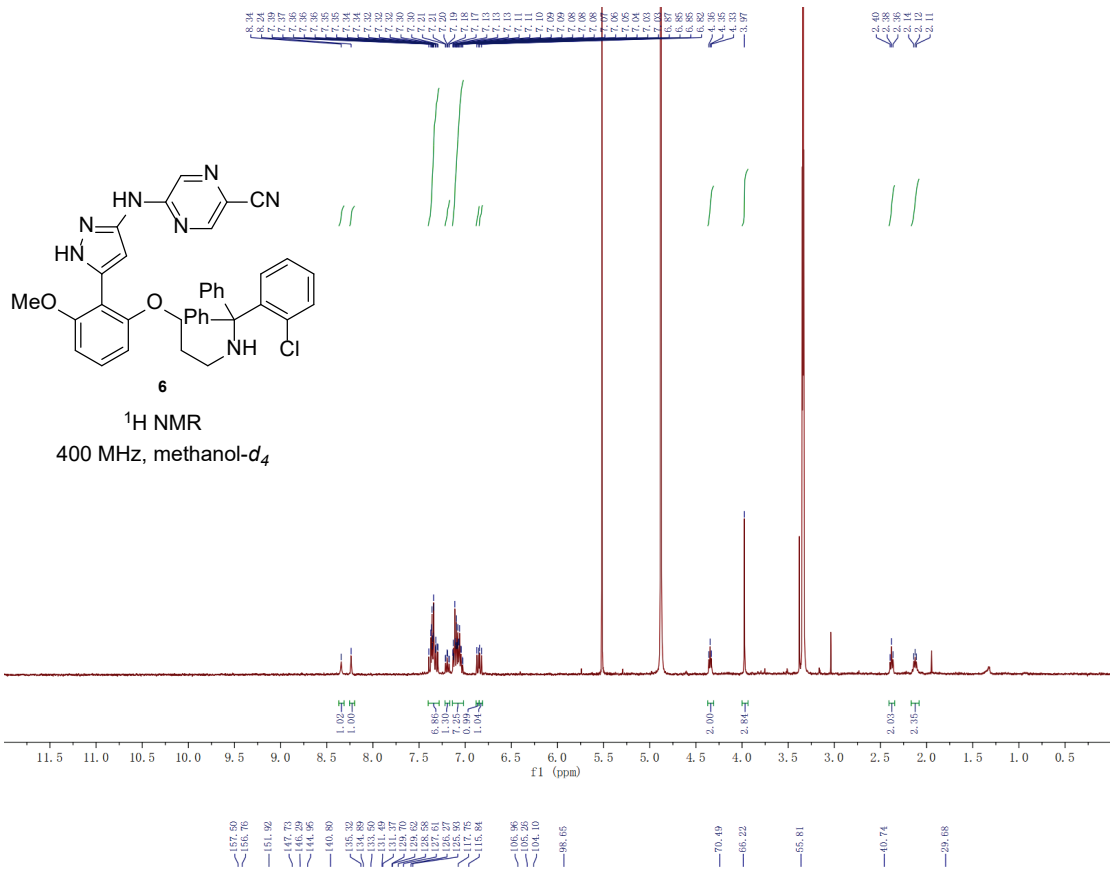
Supplementary Figure 25. ^{1}H NMR and ^{13}C NMR spectra of compound **3'**.

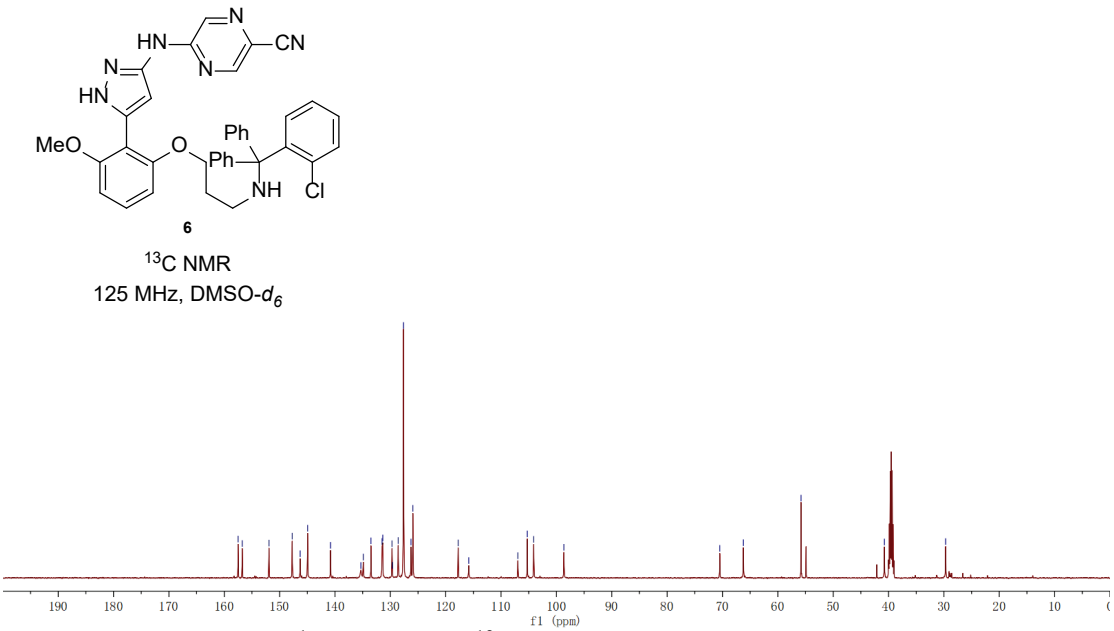


Supplementary Figure 26. ^{1}H NMR and ^{13}C NMR spectra of compound **4'**.

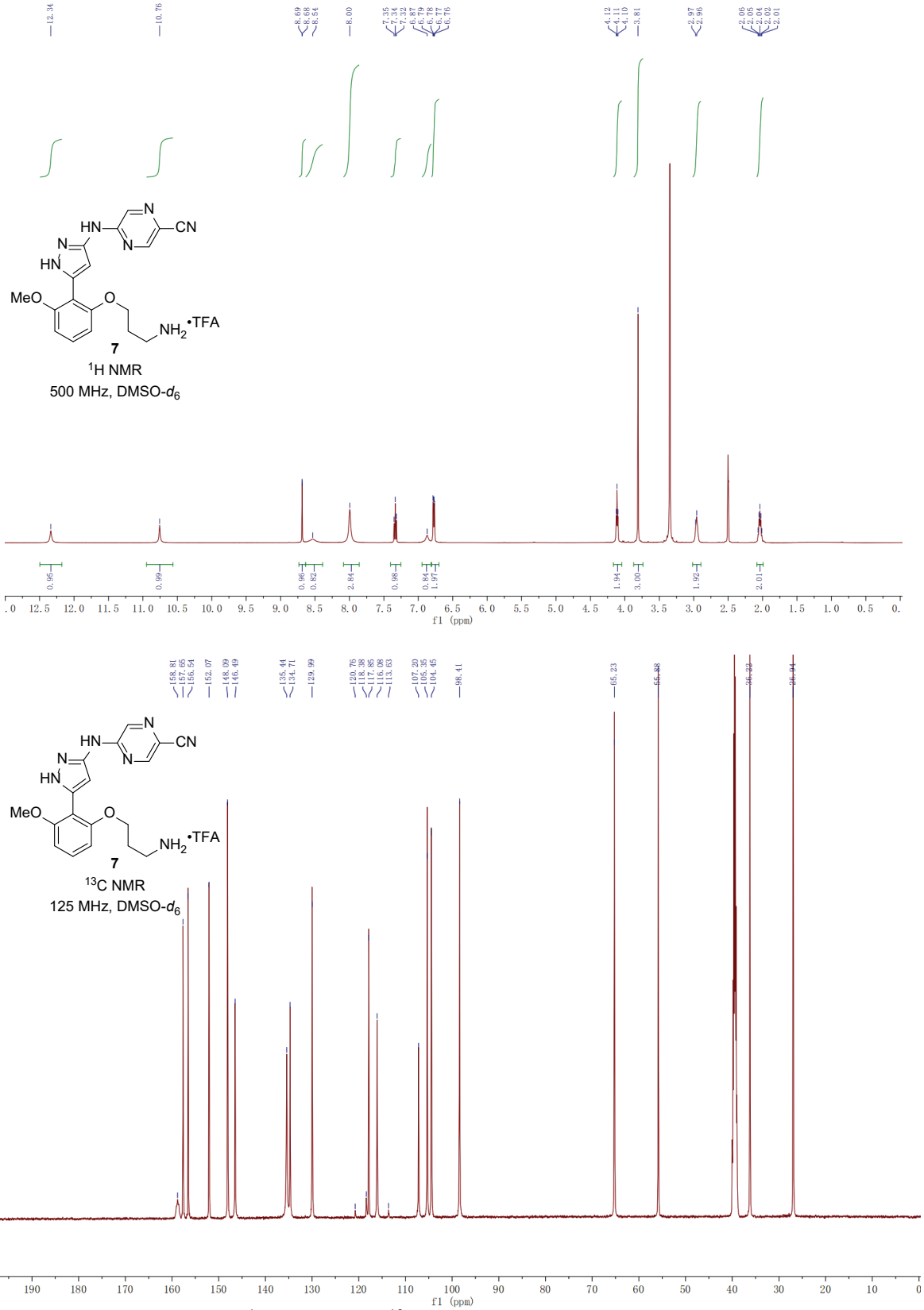


Supplementary Figure 27. ^{1}H NMR and ^{13}C NMR spectra of compound **5'**.

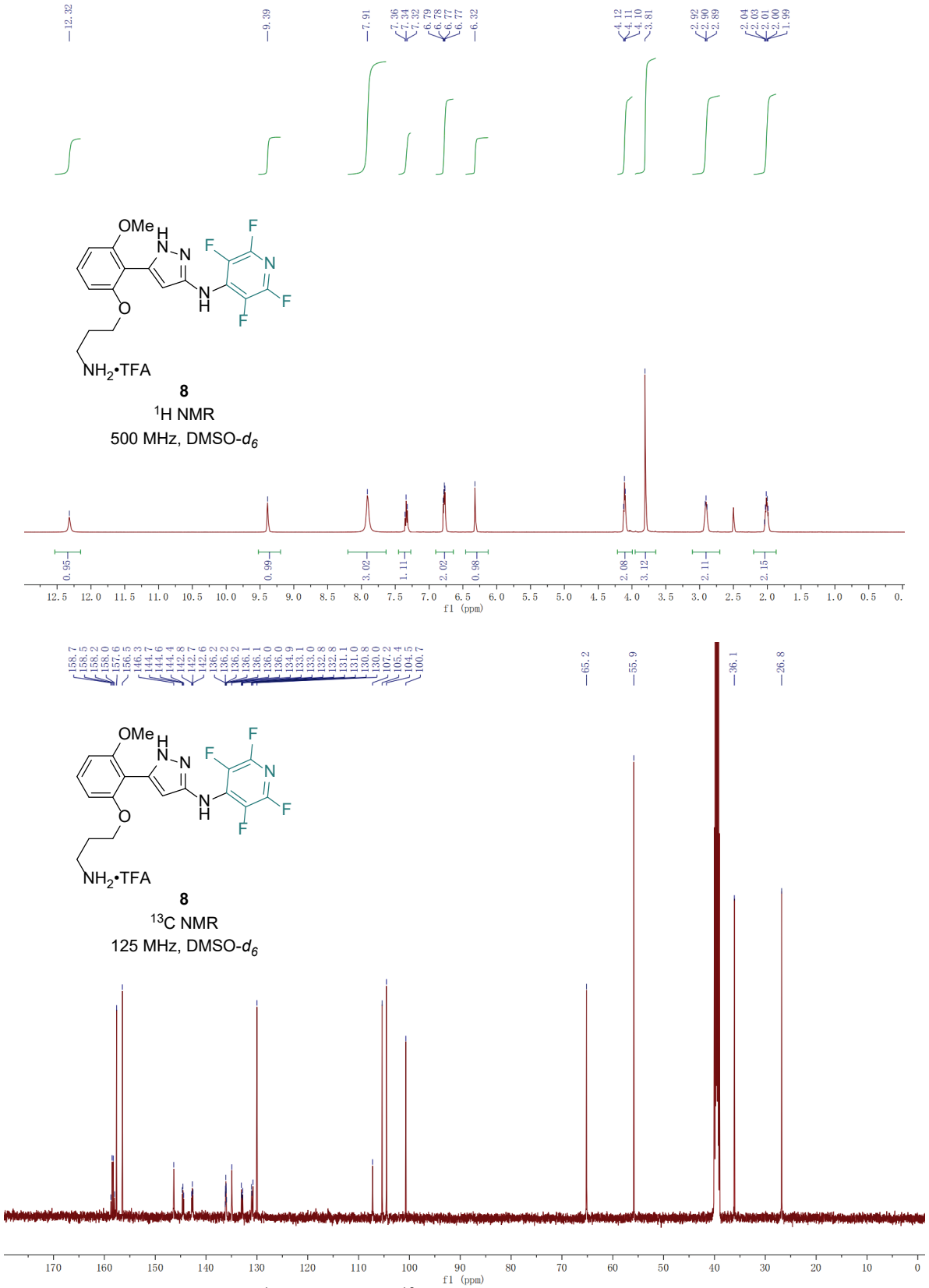




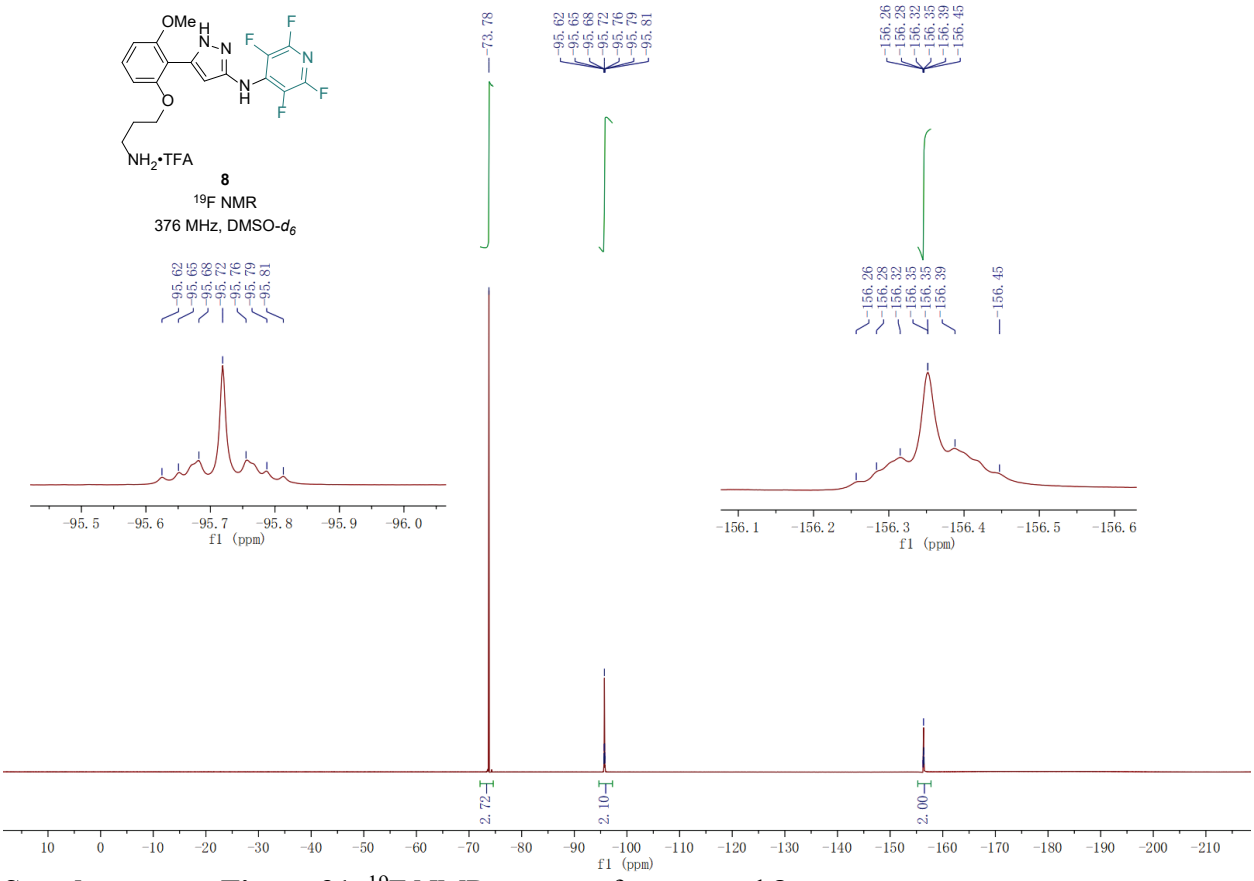
Supplementary Figure 28. ^{1}H NMR and ^{13}C NMR spectra of compound **6**.



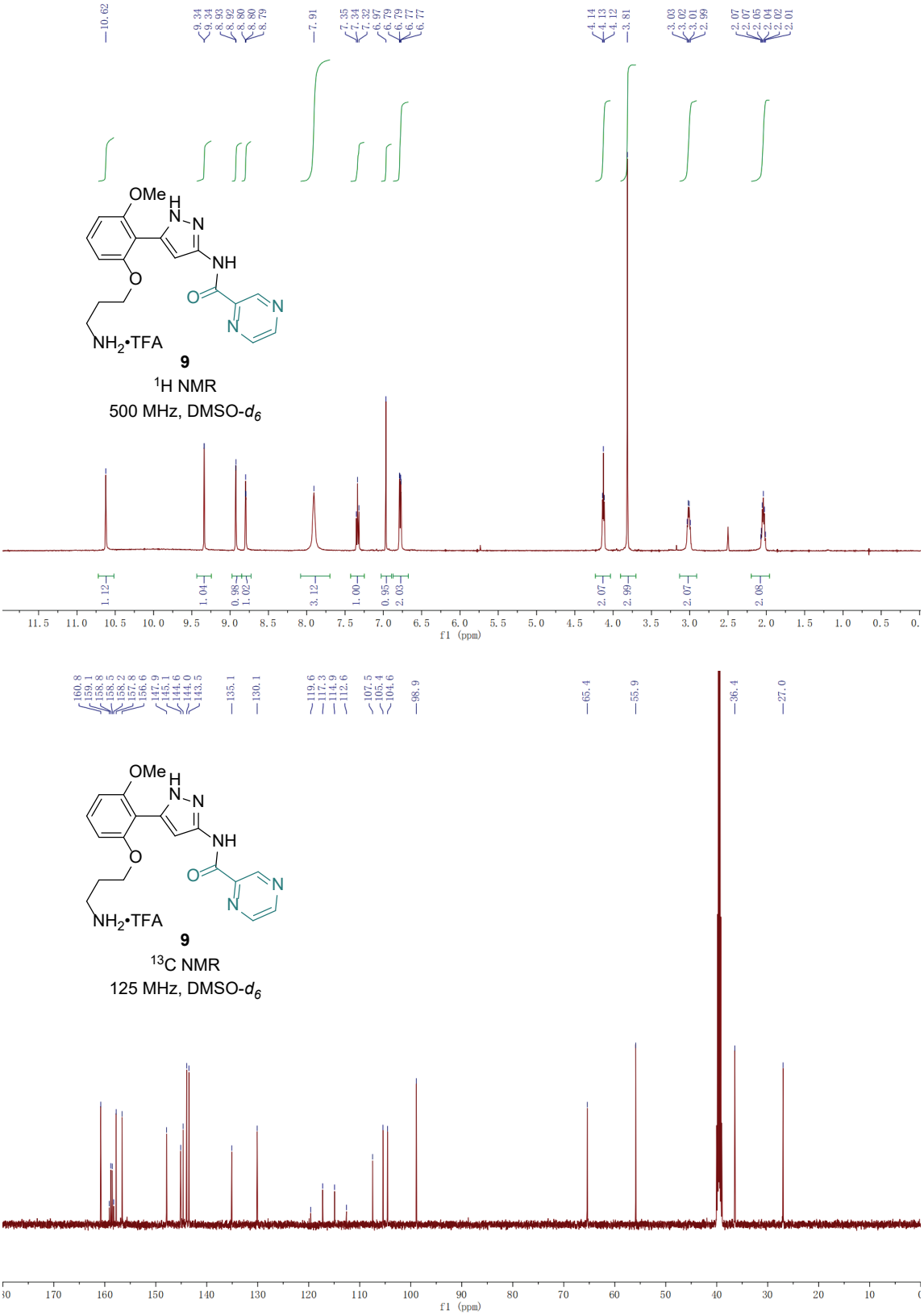
Supplementary Figure 29. ^{1}H NMR and ^{13}C NMR spectra of compound **7**.



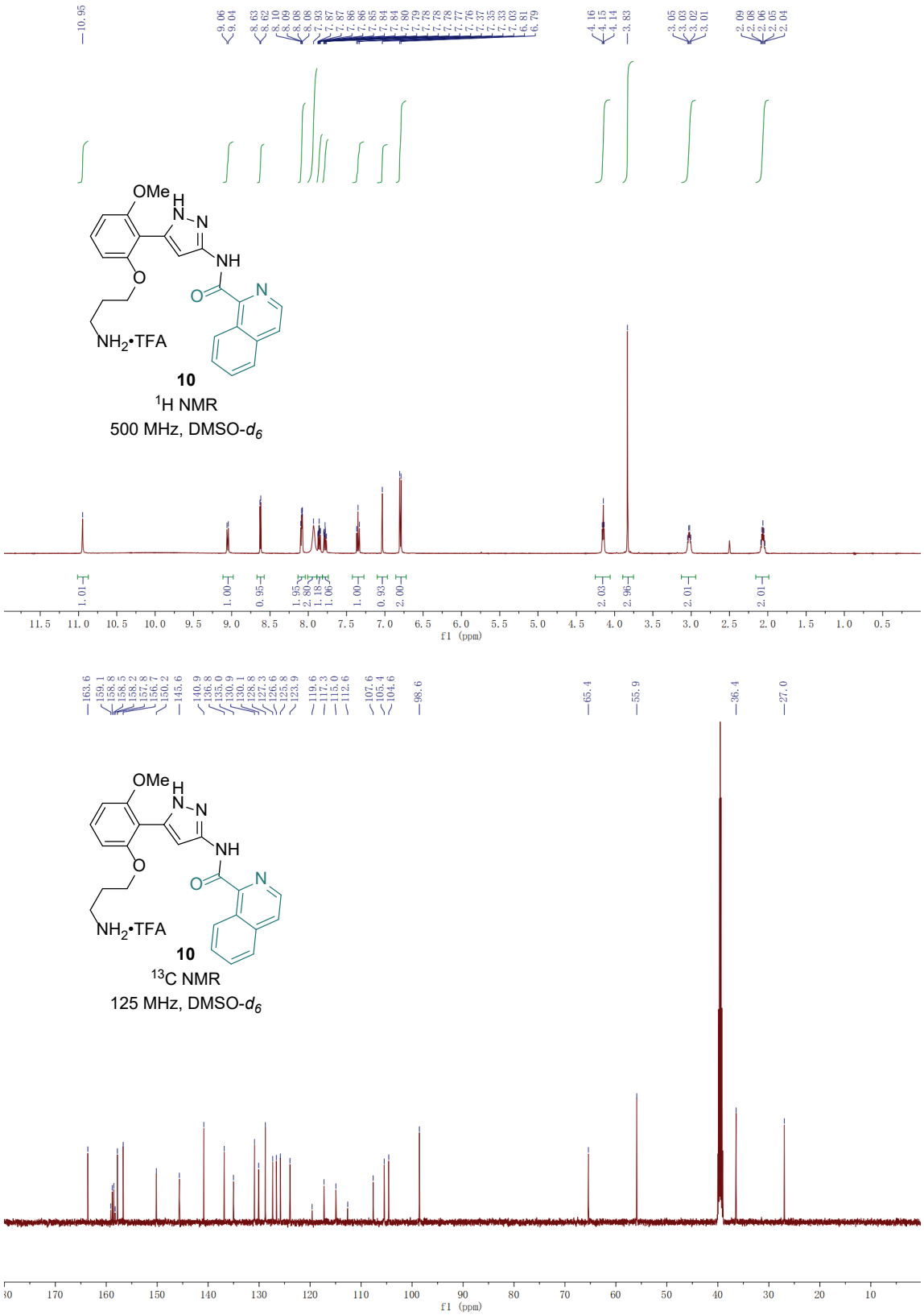
Supplementary Figure 30. ^{1}H NMR and ^{13}C NMR spectra of compound **8**.



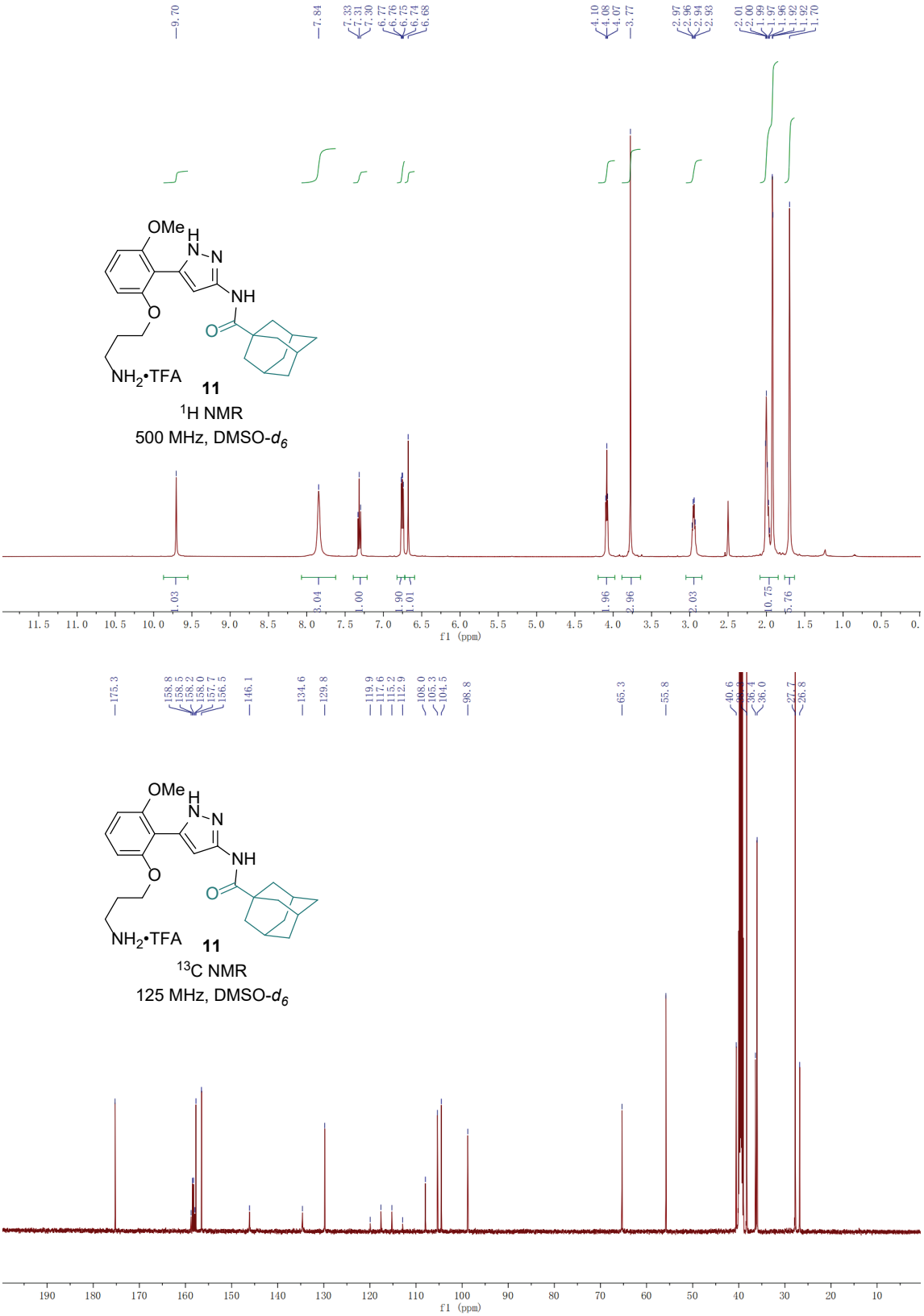
Supplementary Figure 31. ^{19}F NMR spectra of compound **8**.



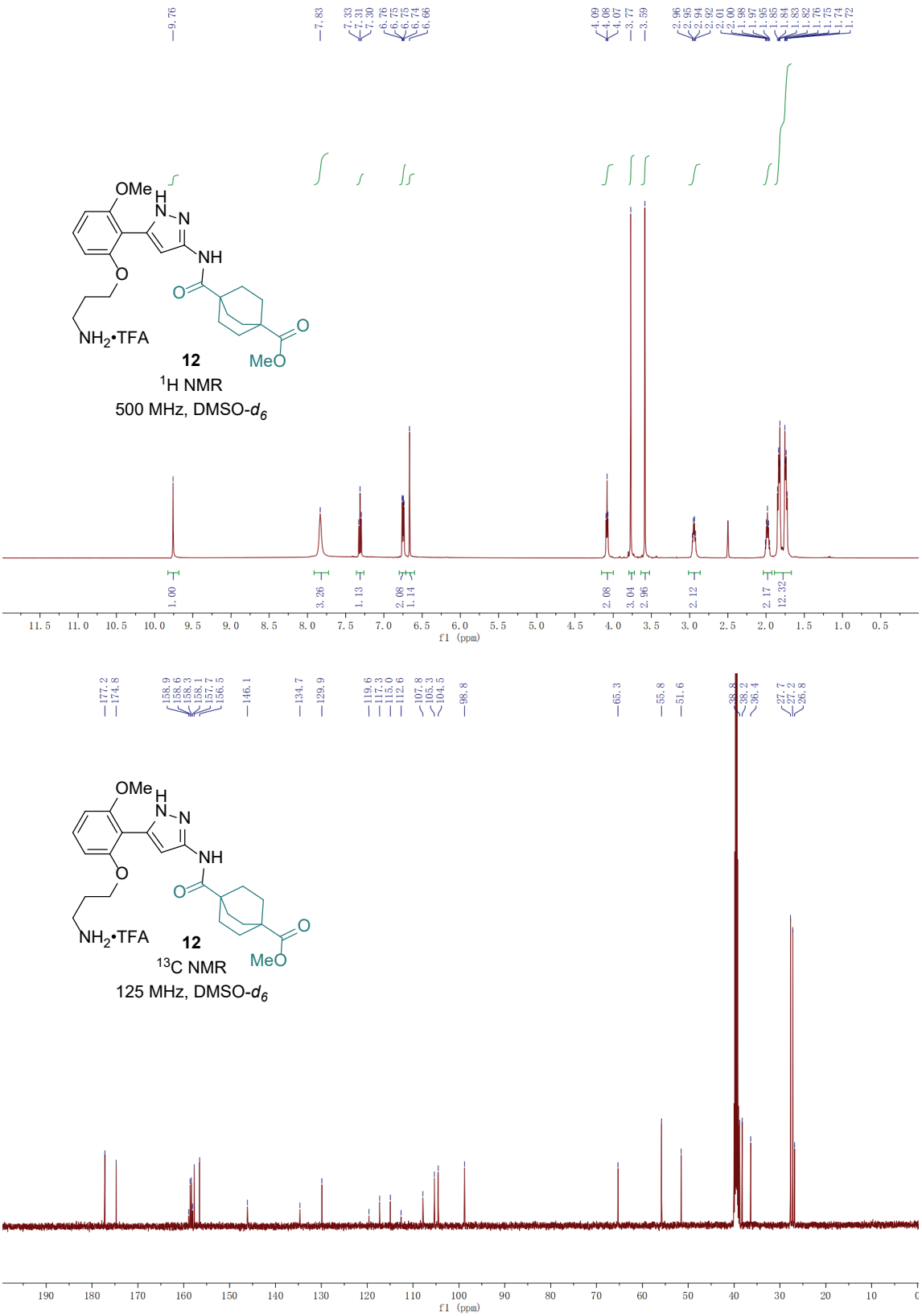
Supplementary Figure 32. ^{1}H NMR and ^{13}C NMR spectra of compound **9**.



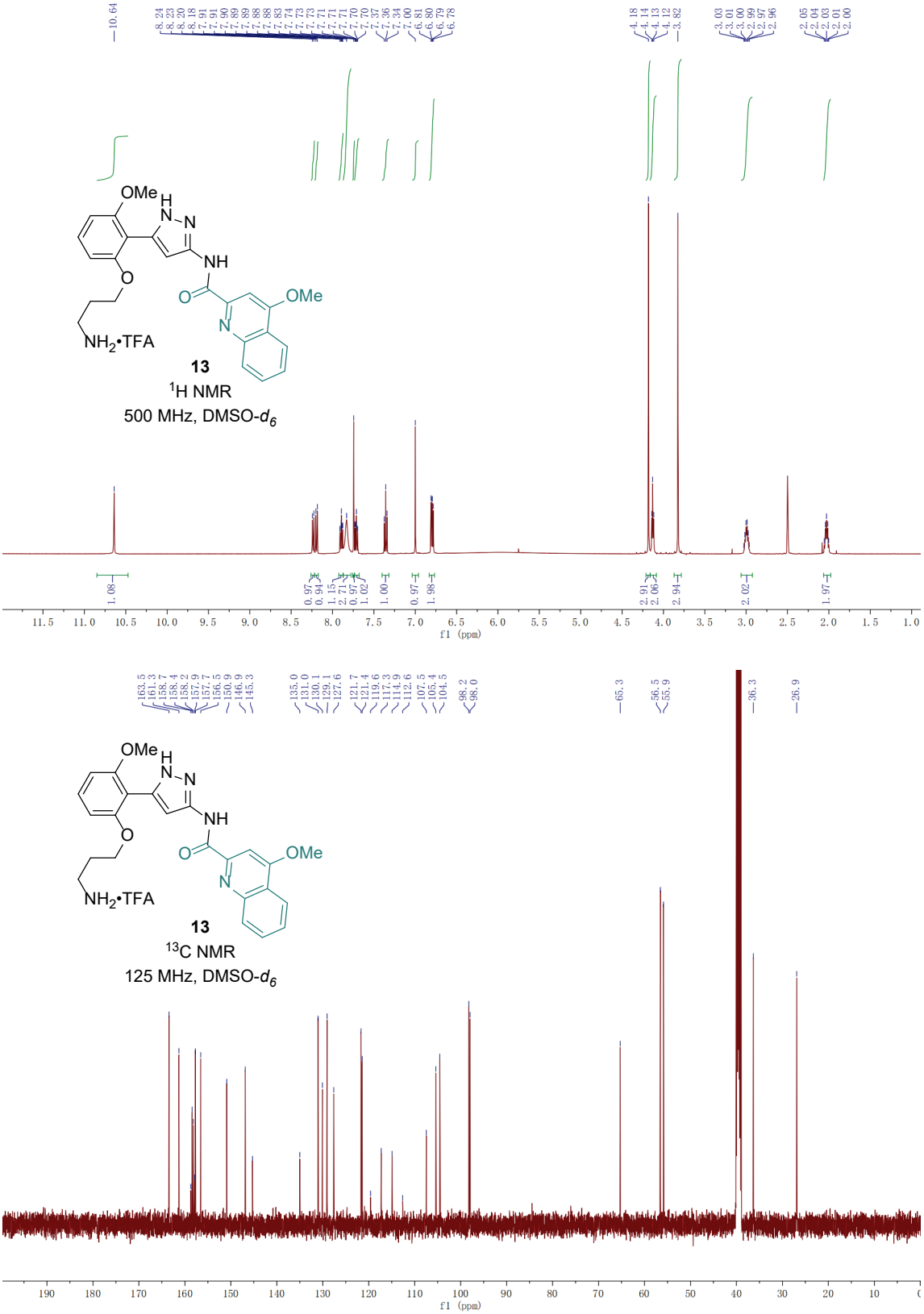
Supplementary Figure 33. ^{1}H NMR and ^{13}C NMR spectra of compound **10**.



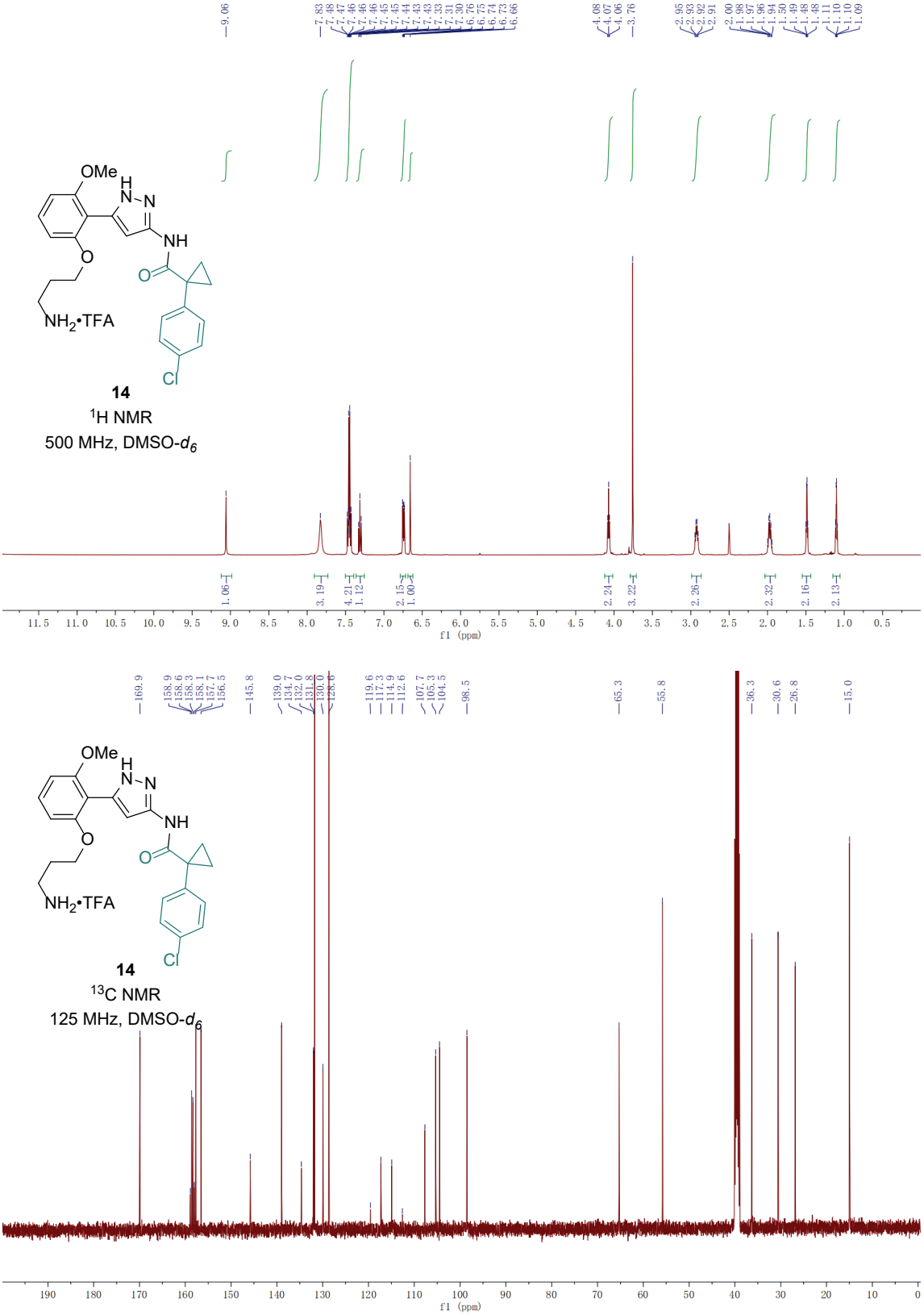
Supplementary Figure 34. ^{1}H NMR and ^{13}C NMR spectra of compound **11**.



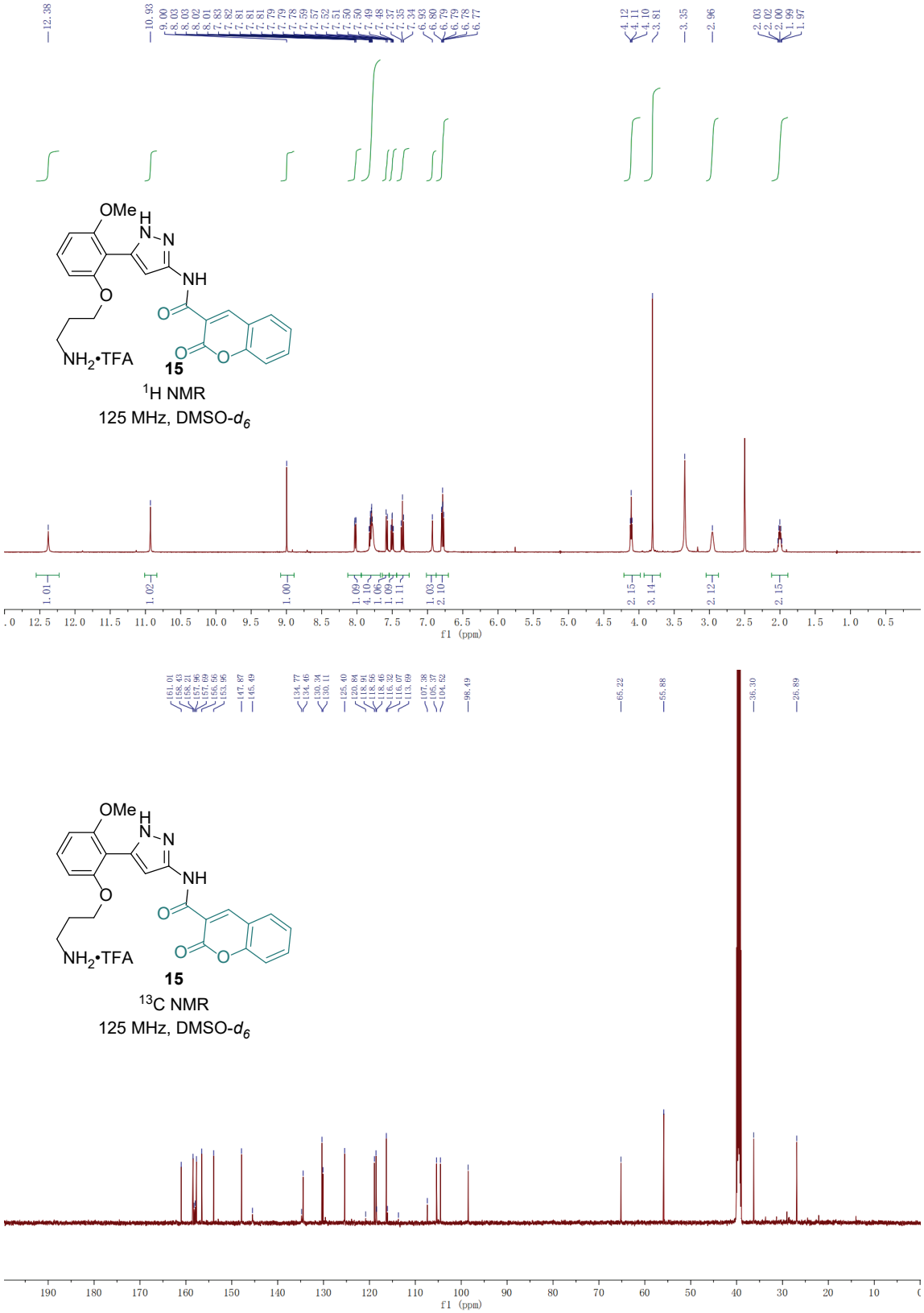
Supplementary Figure 35. ^{1}H NMR and ^{13}C NMR spectra of compound **12**.



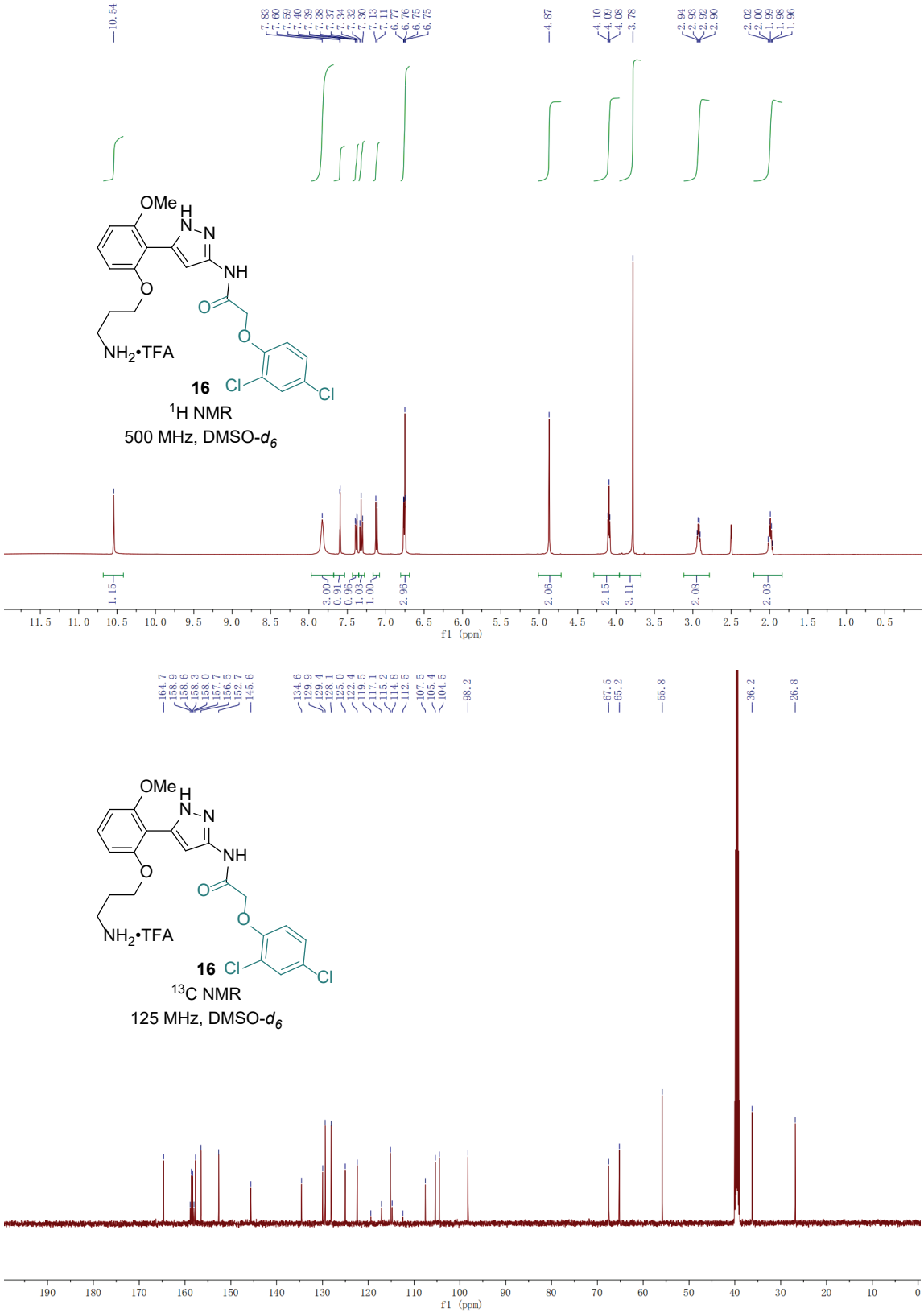
Supplementary Figure 36. ^{1}H NMR and ^{13}C NMR spectra of compound **13**.



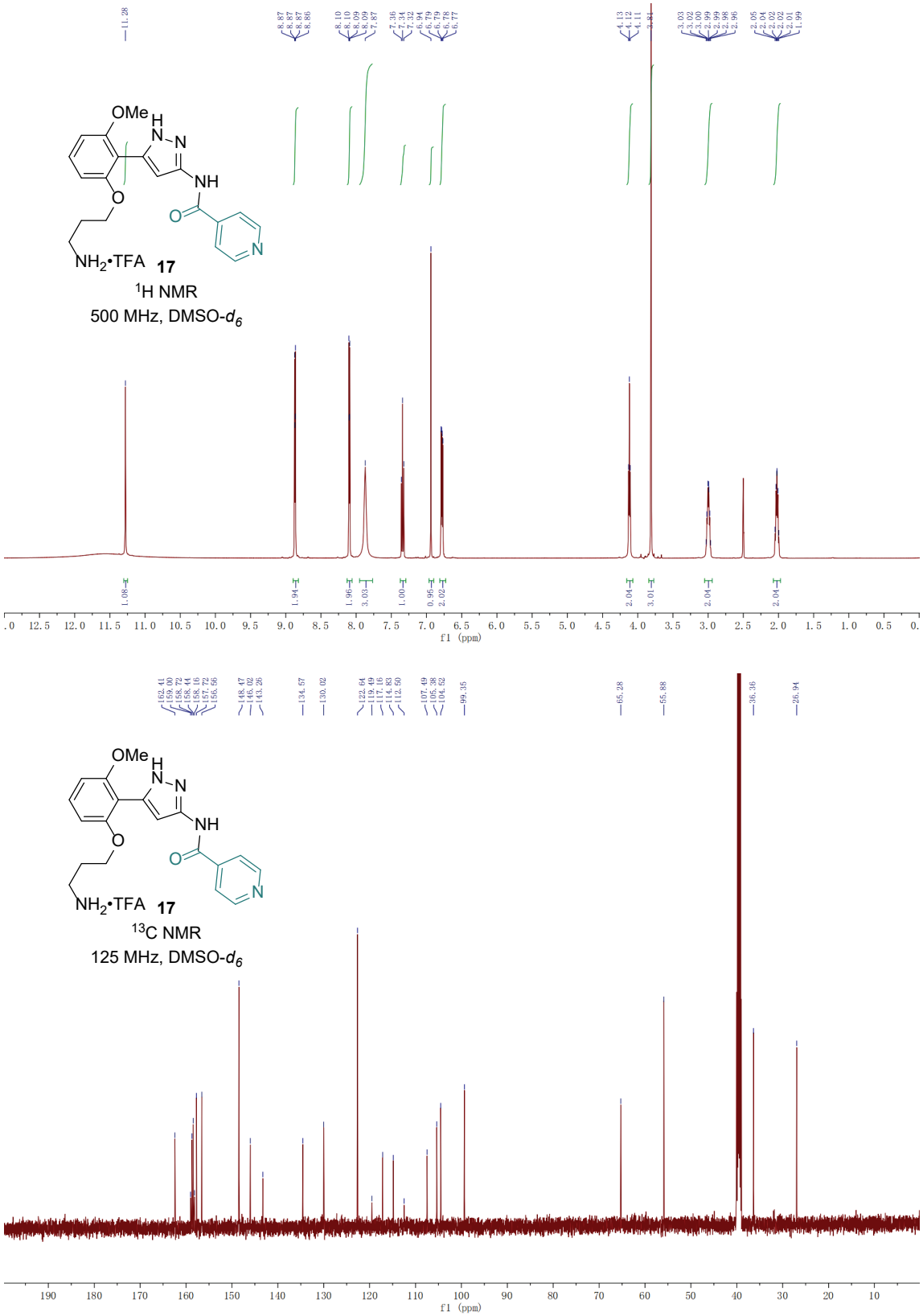
Supplementary Figure 37. ^{1}H NMR and ^{13}C NMR spectra of compound **14**.



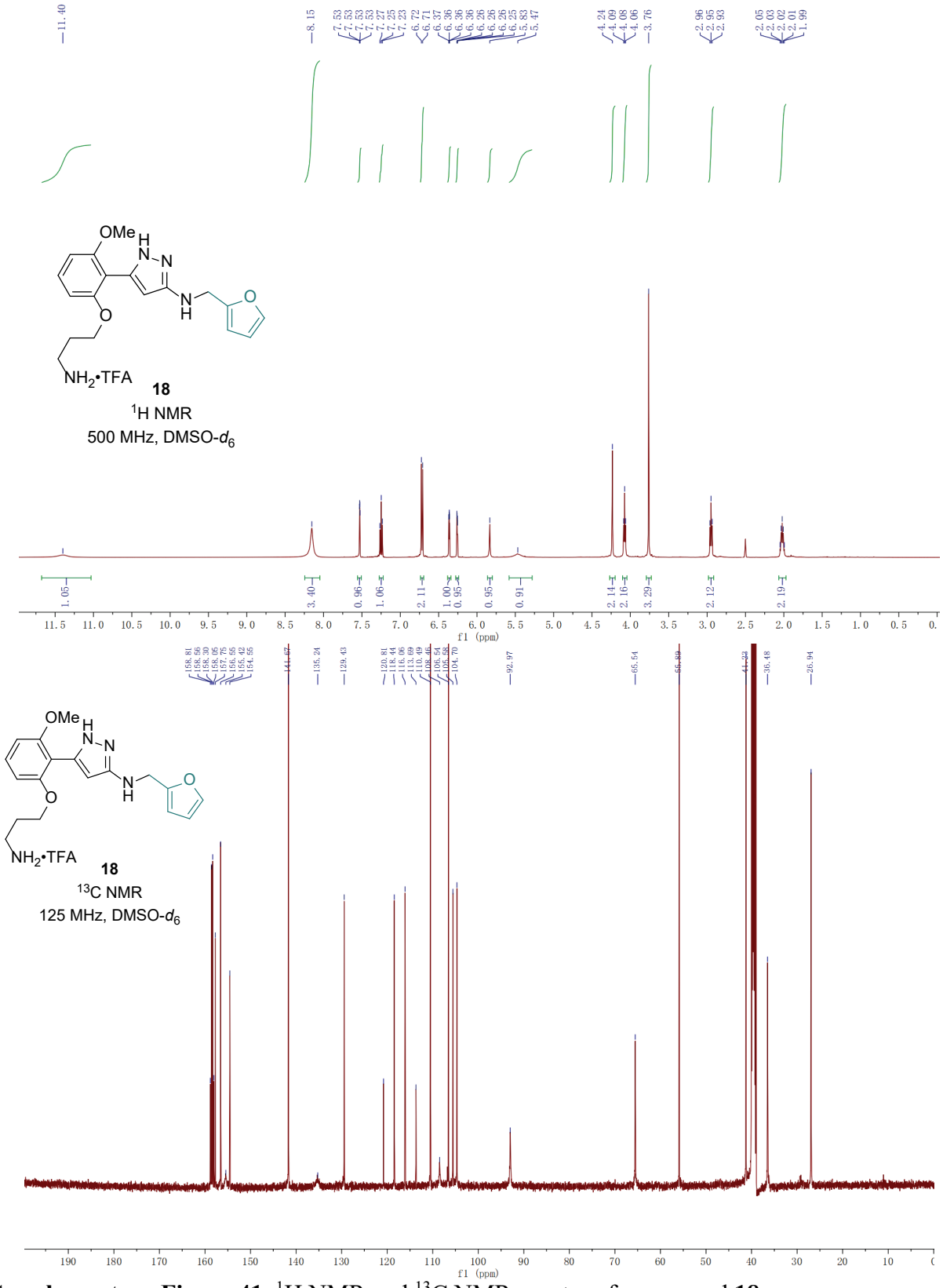
Supplementary Figure 38. ^{1}H NMR and ^{13}C NMR spectra of compound **15**.



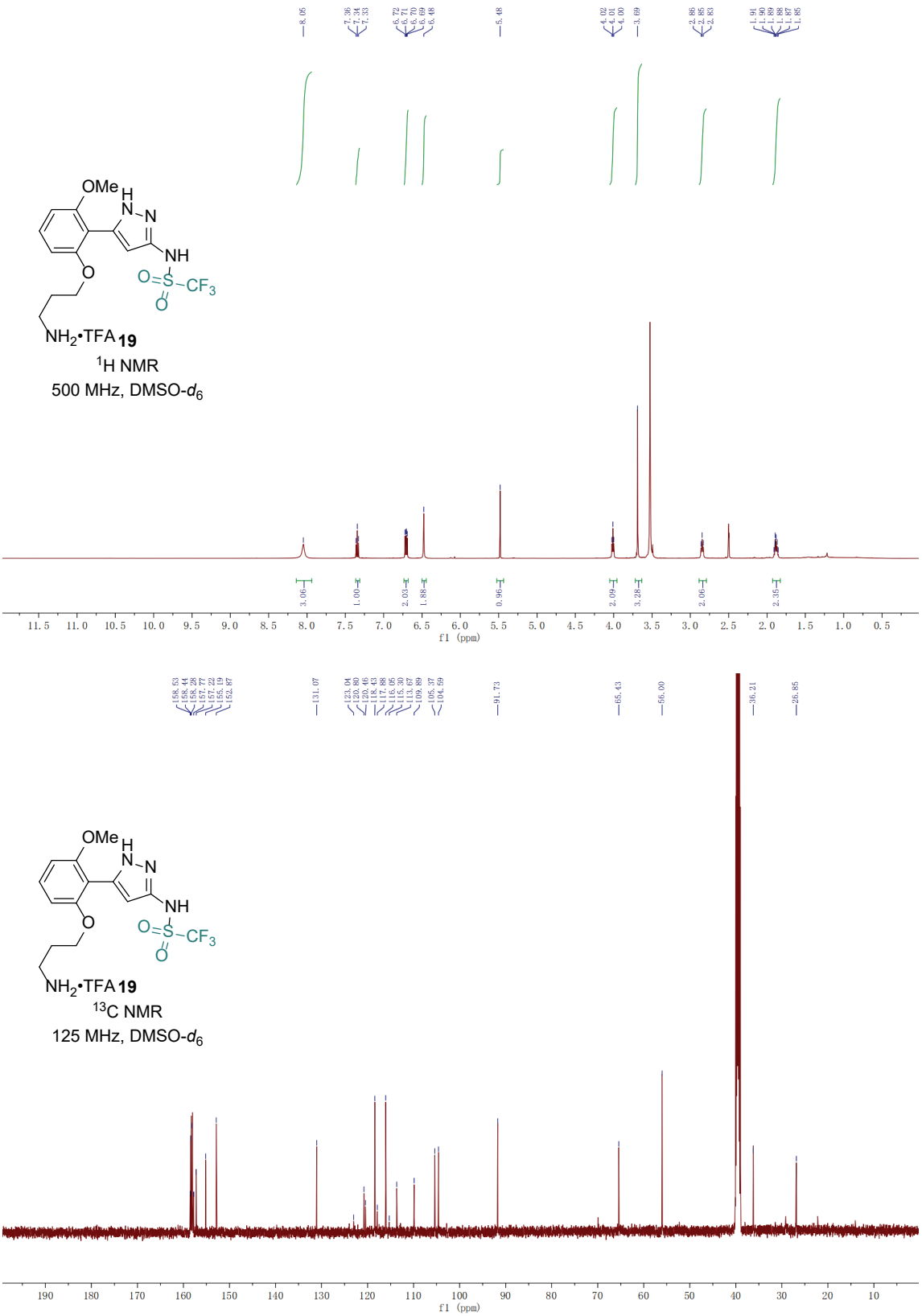
Supplementary Figure 39. ^{1}H NMR and ^{13}C NMR spectra of compound **16**.



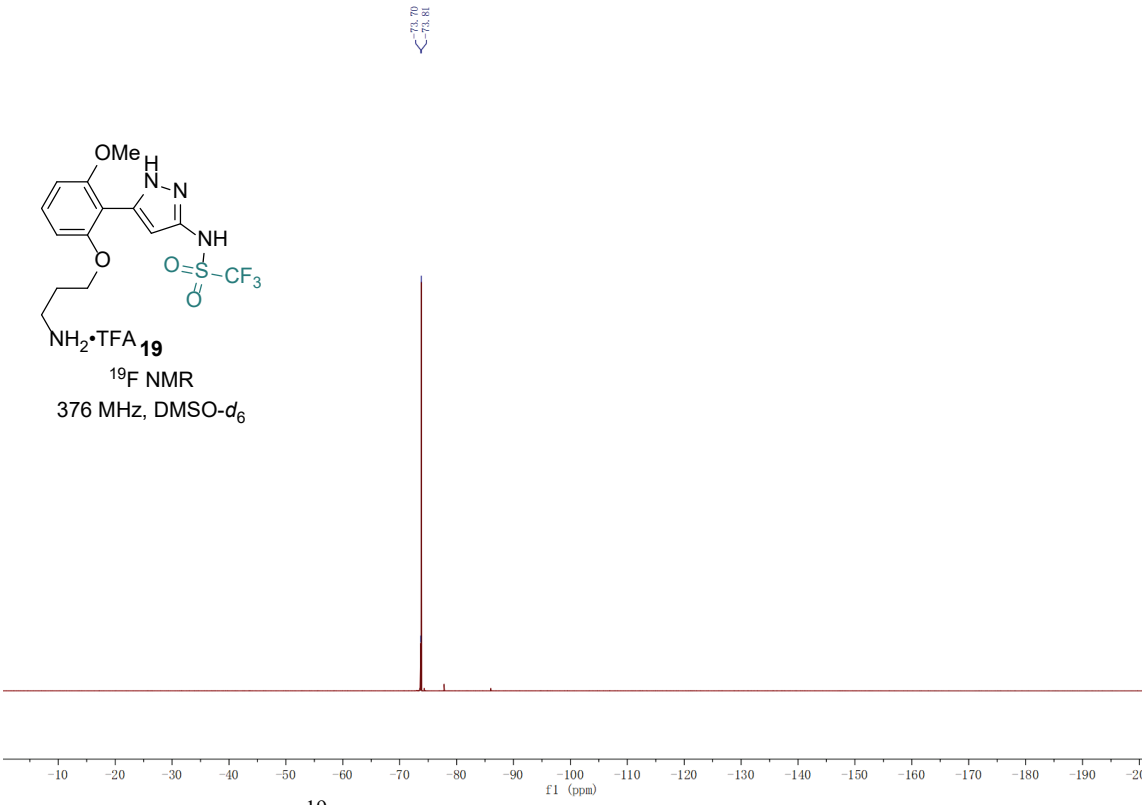
Supplementary Figure 40. ^{1}H NMR and ^{13}C NMR spectra of compound **17**.



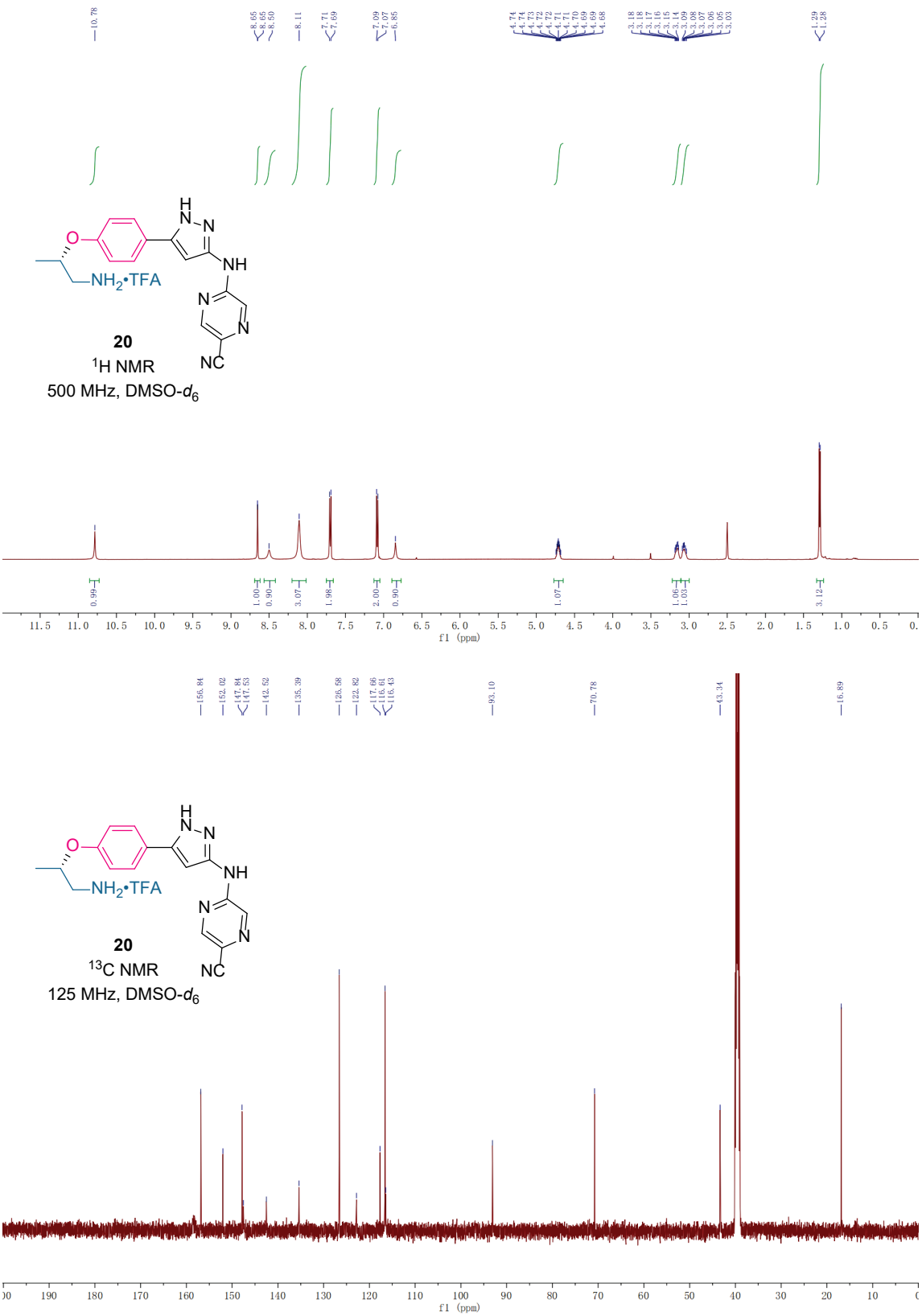
Supplementary Figure 41. ^{1}H NMR and ^{13}C NMR spectra of compound **18**.



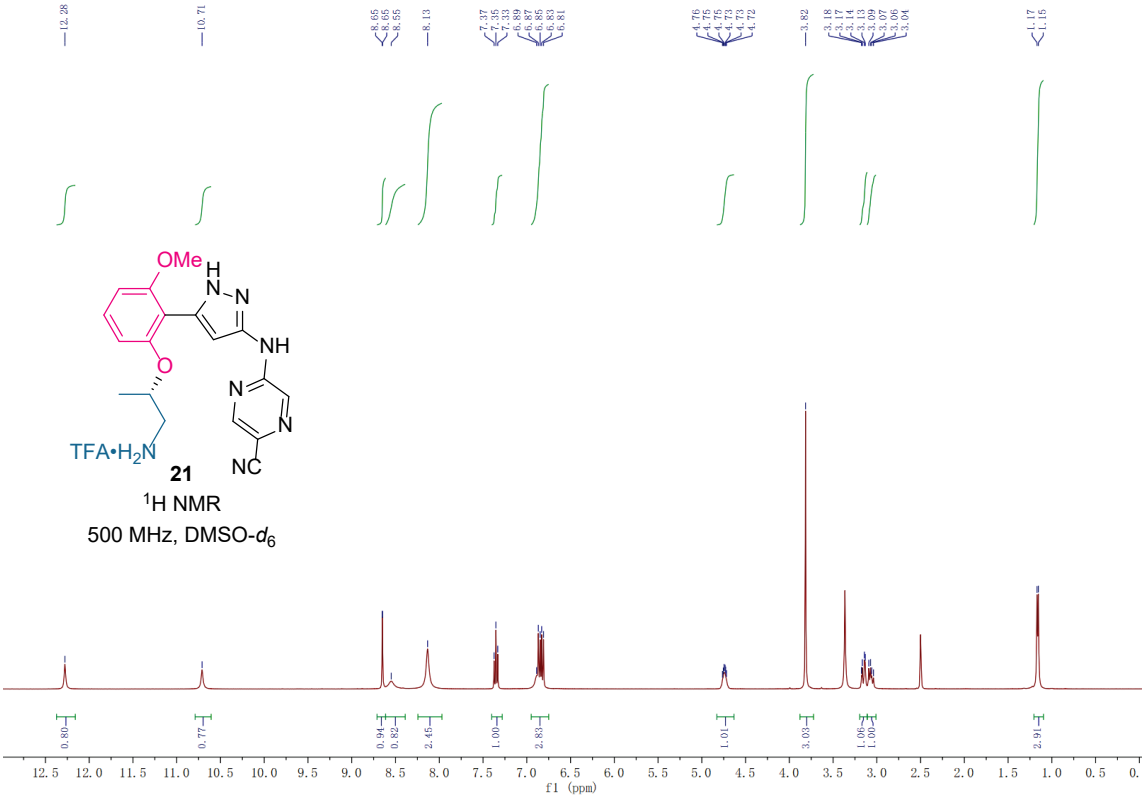
Supplementary Figure 42. ^{1}H NMR and ^{13}C NMR spectra of compound **19**.

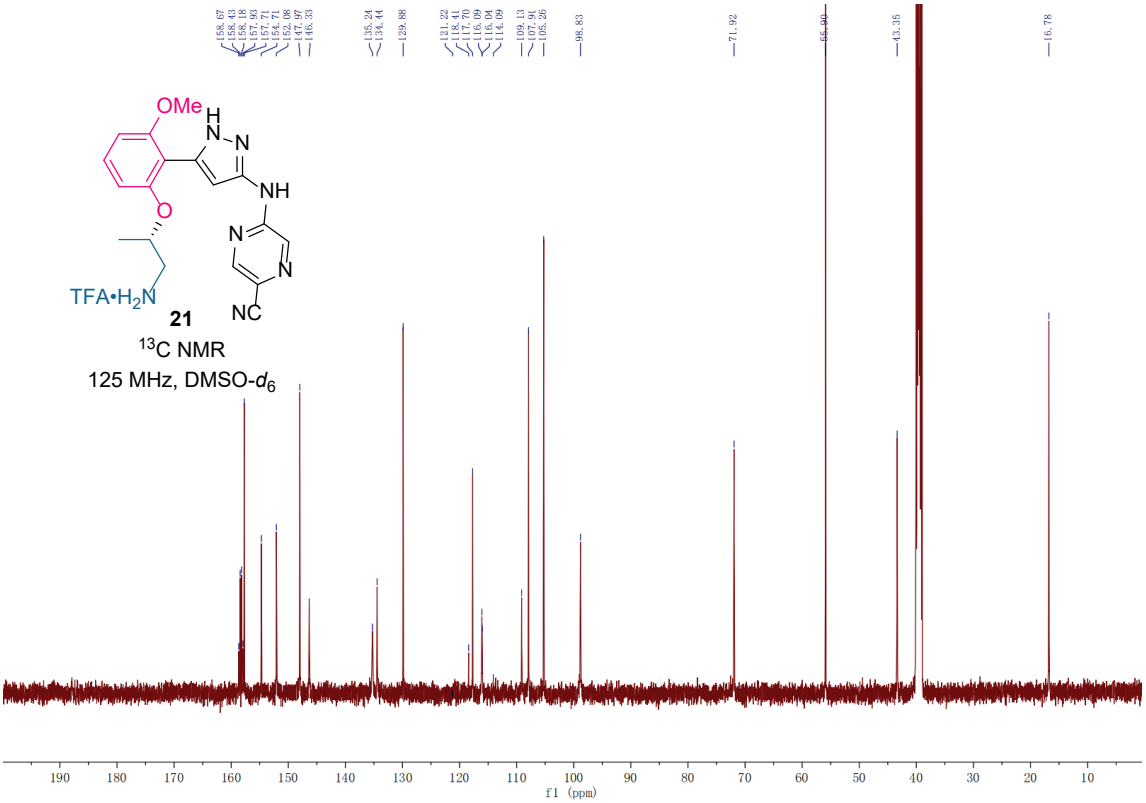


Supplementary Figure 43.^{19}F NMR spectra of compound **19**.

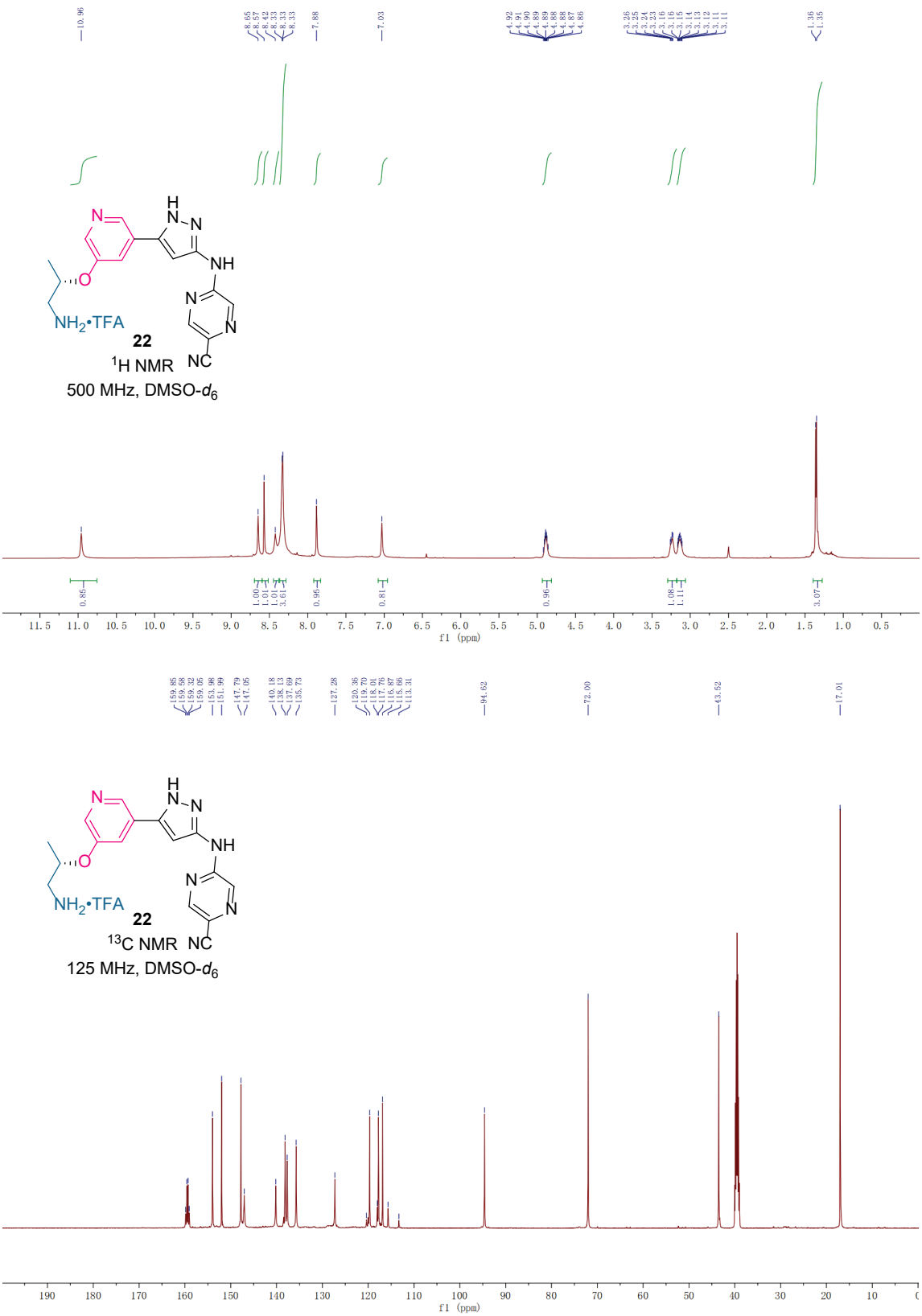


Supplementary Figure 44. ^{1}H NMR and ^{13}C NMR spectra of compound **20**.

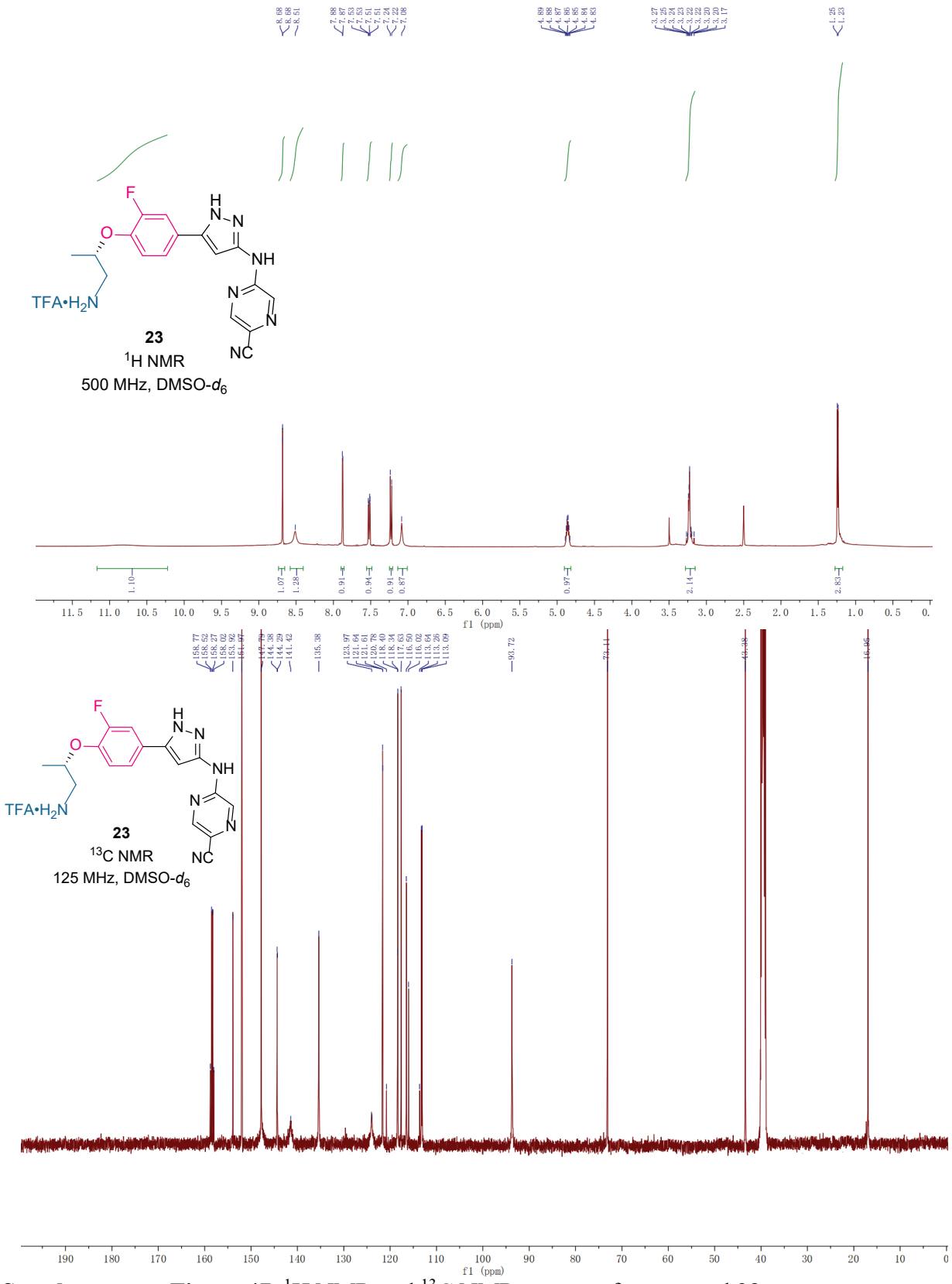




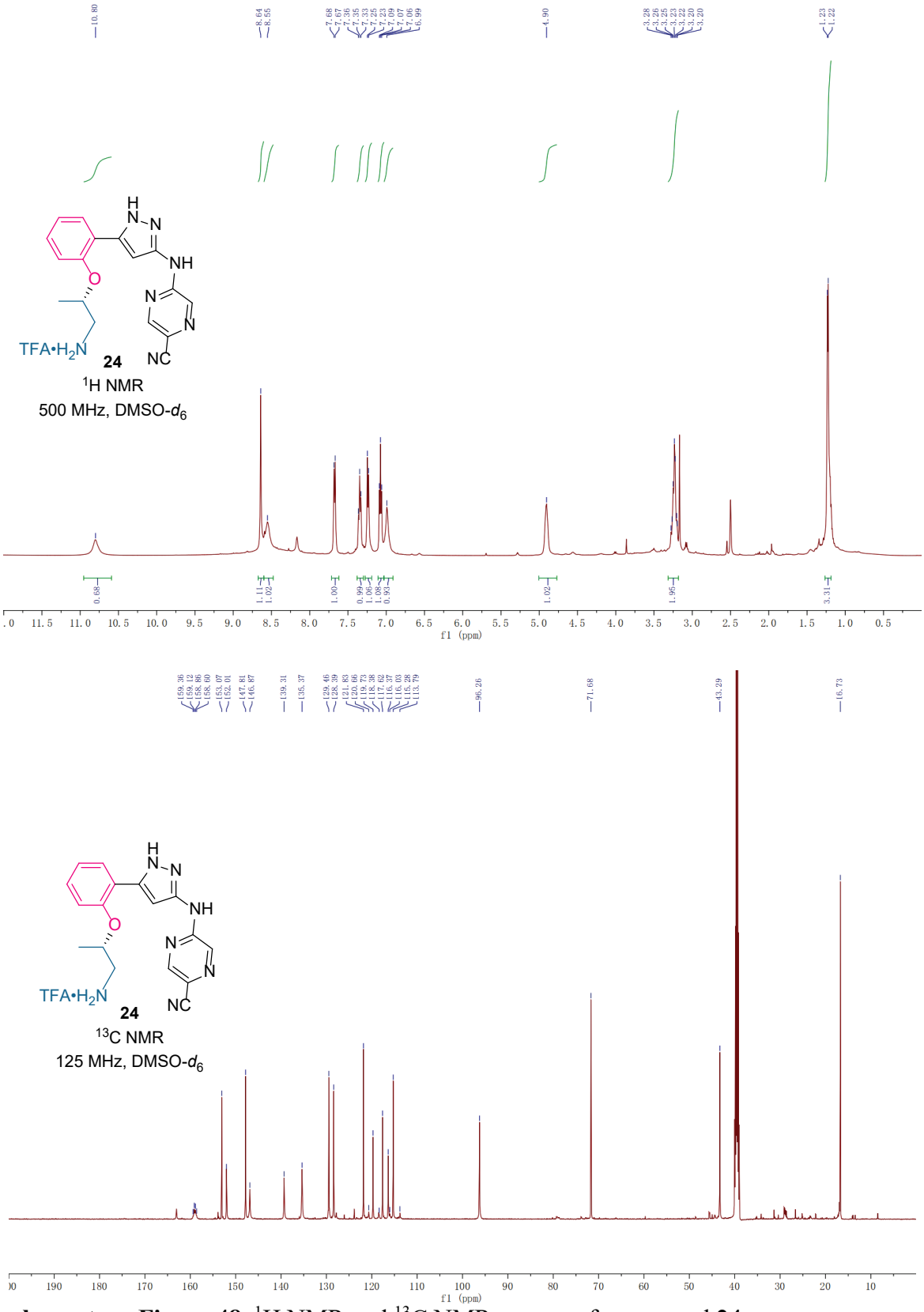
Supplementary Figure 45. ^{1}H NMR and ^{13}C NMR spectra of compound **21**.



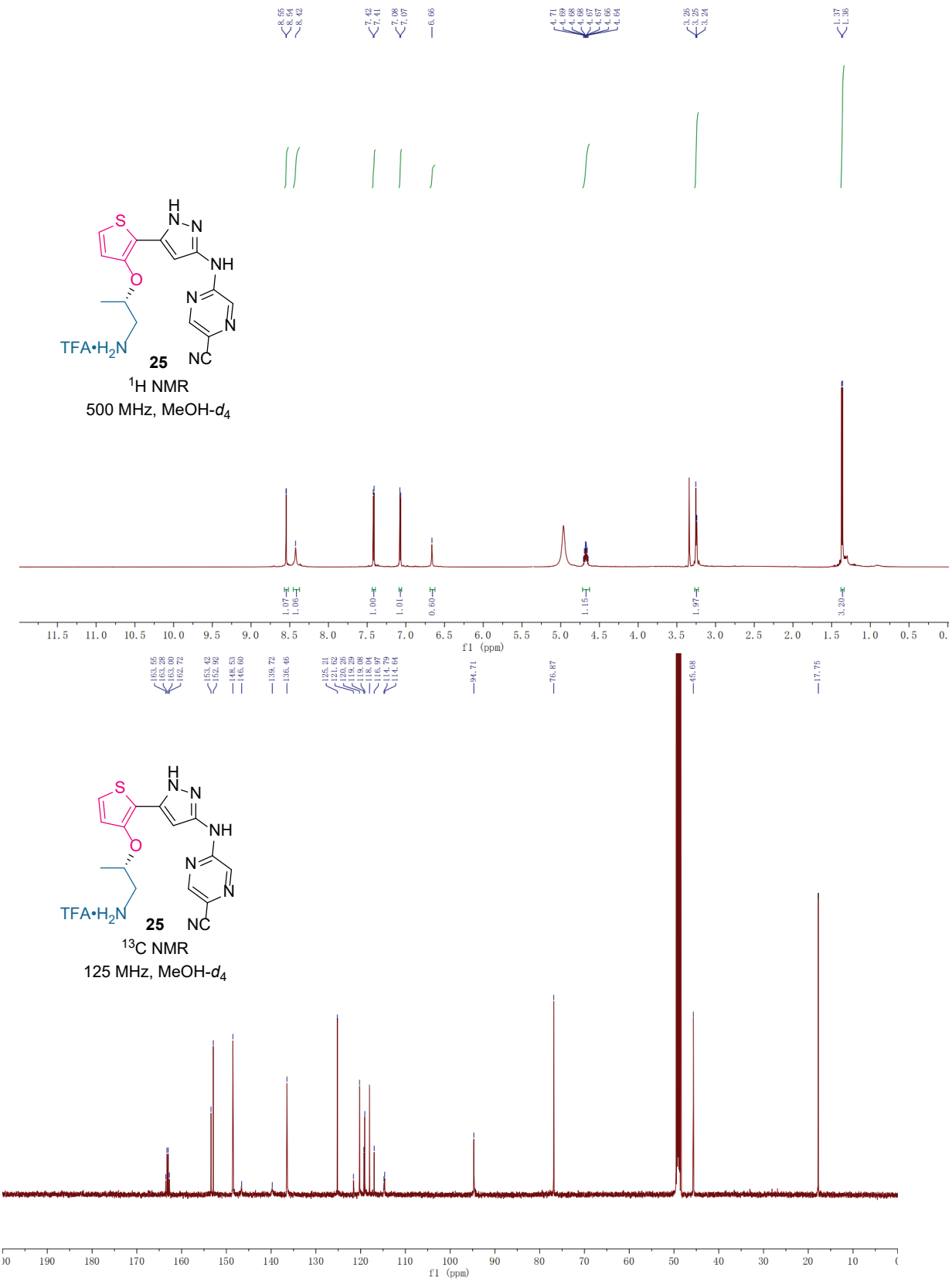
Supplementary Figure 46. ^{1}H NMR and ^{13}C NMR spectra of compound **22**.



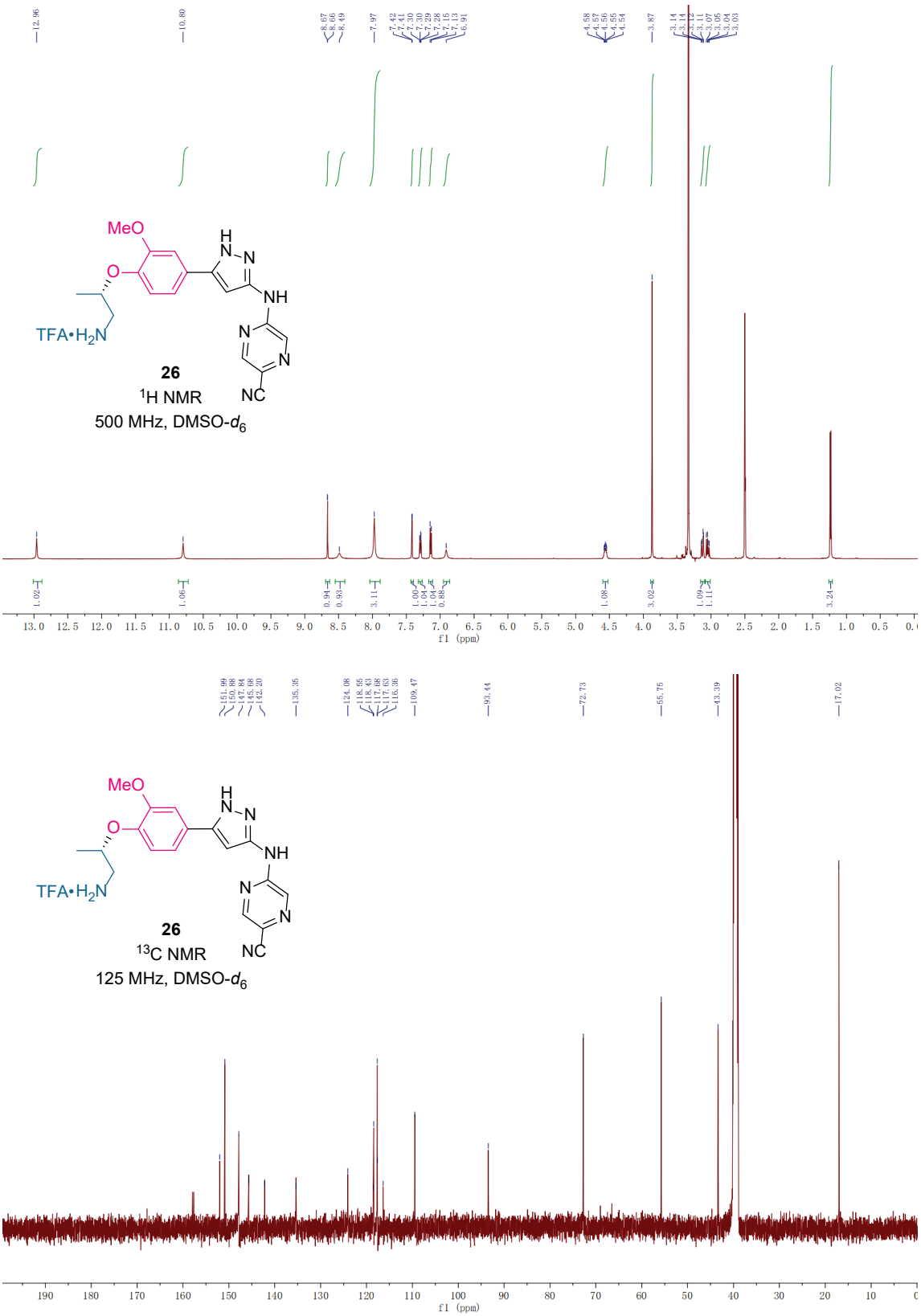
Supplementary Figure 47. ^{1}H NMR and ^{13}C NMR spectra of compound **23**.



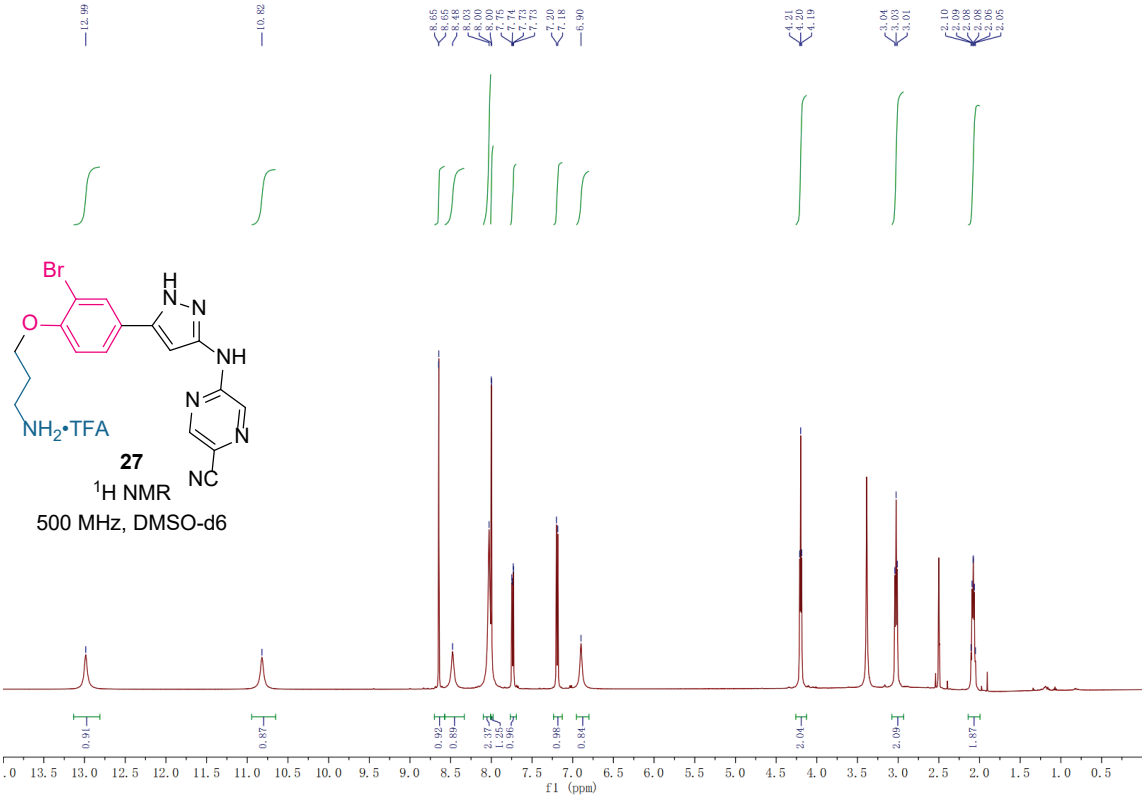
Supplementary Figure 48. ^{1}H NMR and ^{13}C NMR spectra of compound **24**.

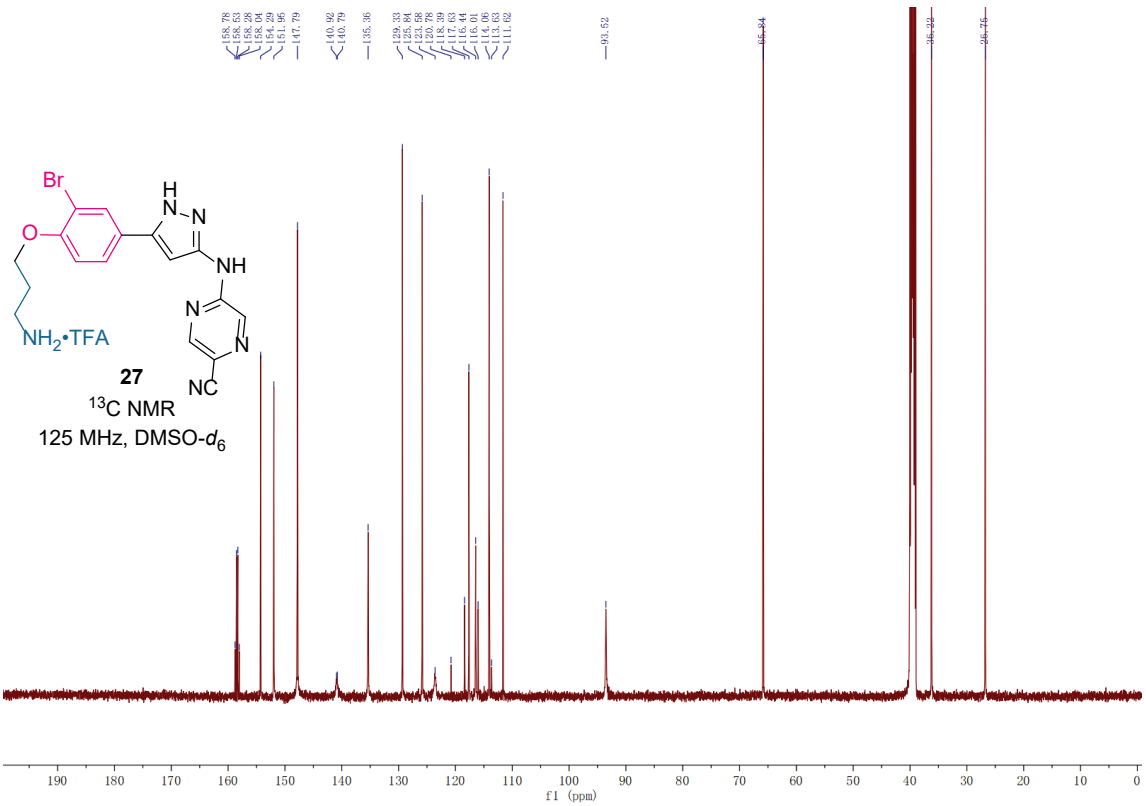


Supplementary Figure 49. ^{1}H NMR and ^{13}C NMR spectra of compound **25**.

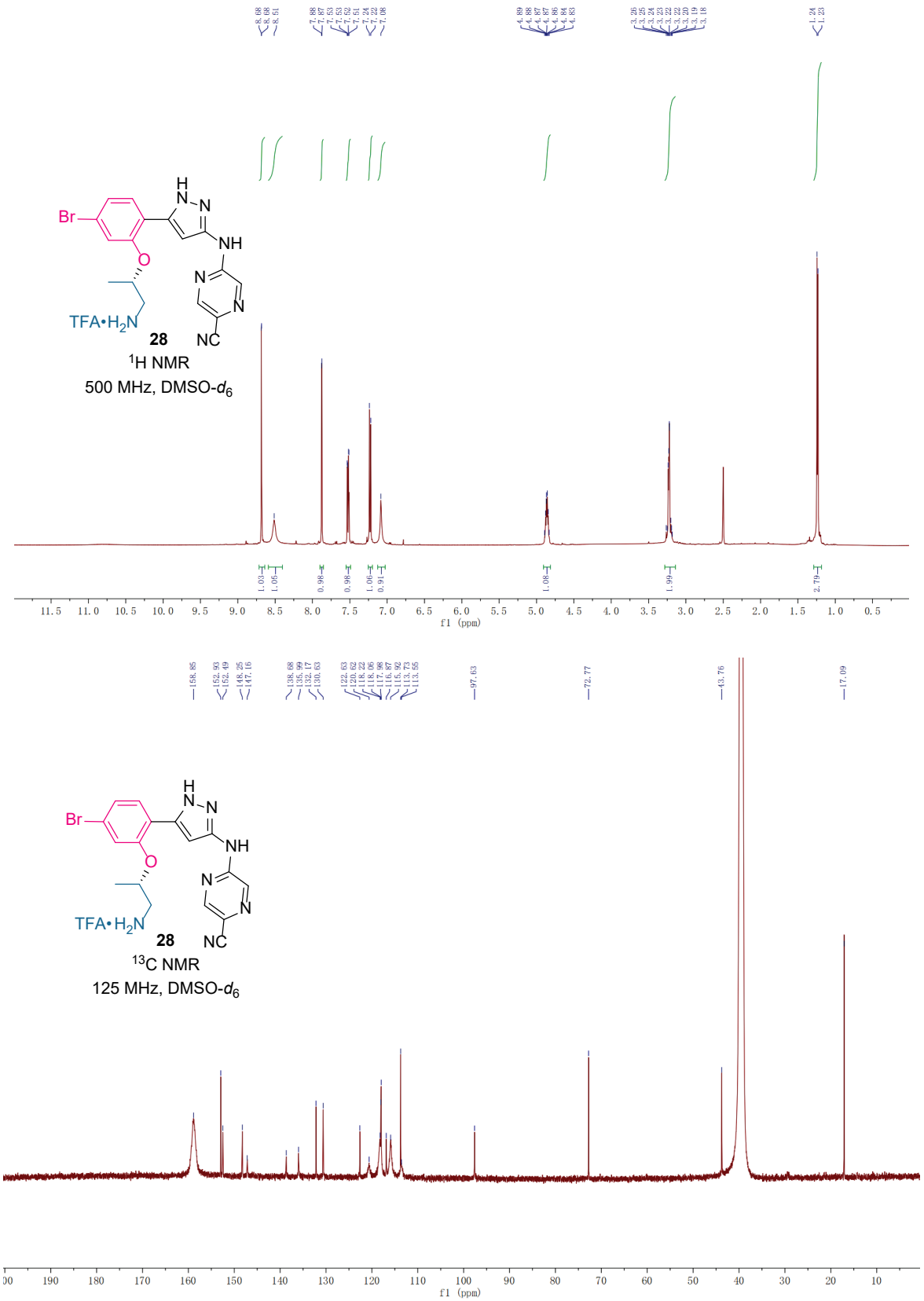


Supplementary Figure 50. ^{1}H NMR and ^{13}C NMR spectra of compound **26**.

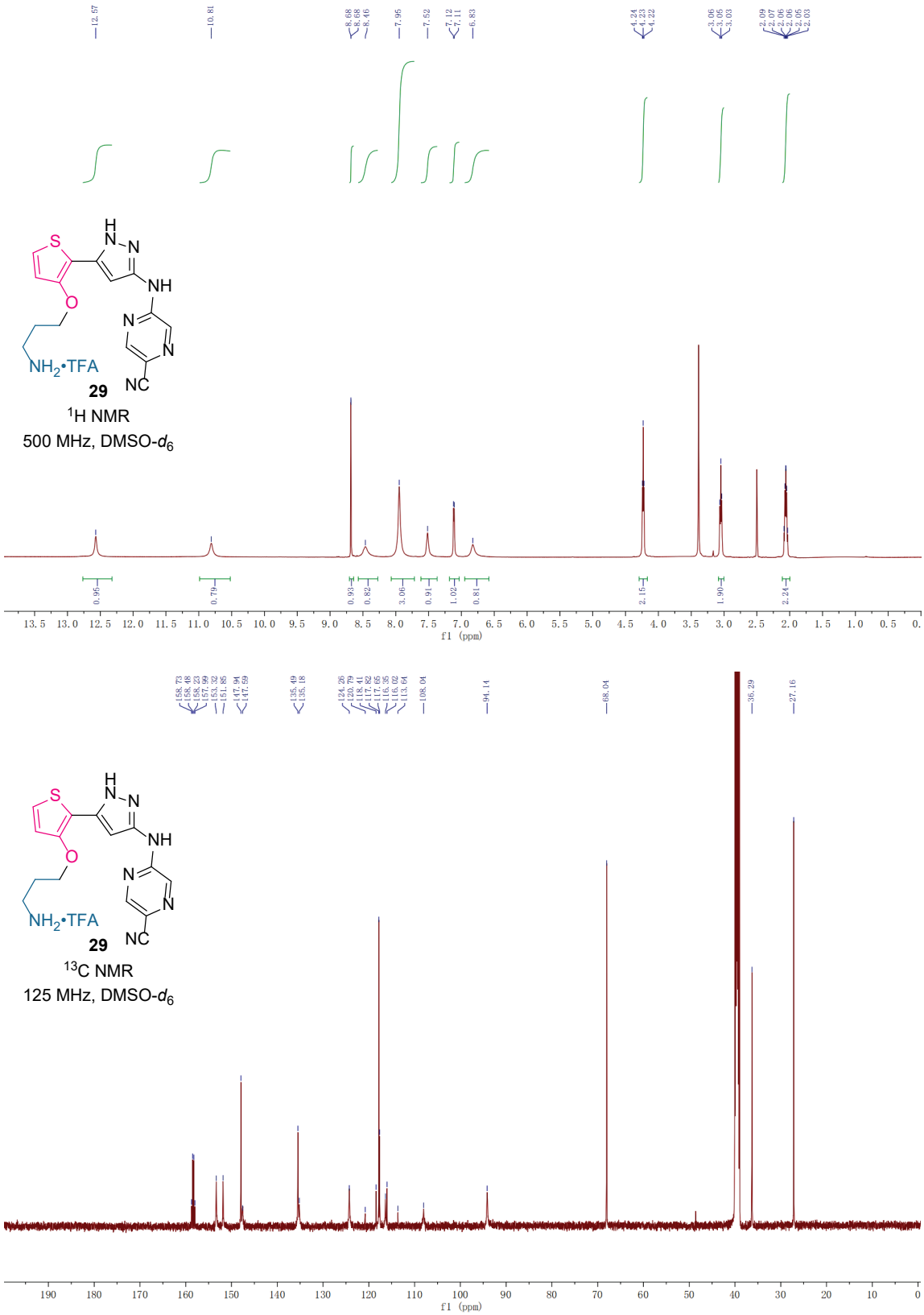




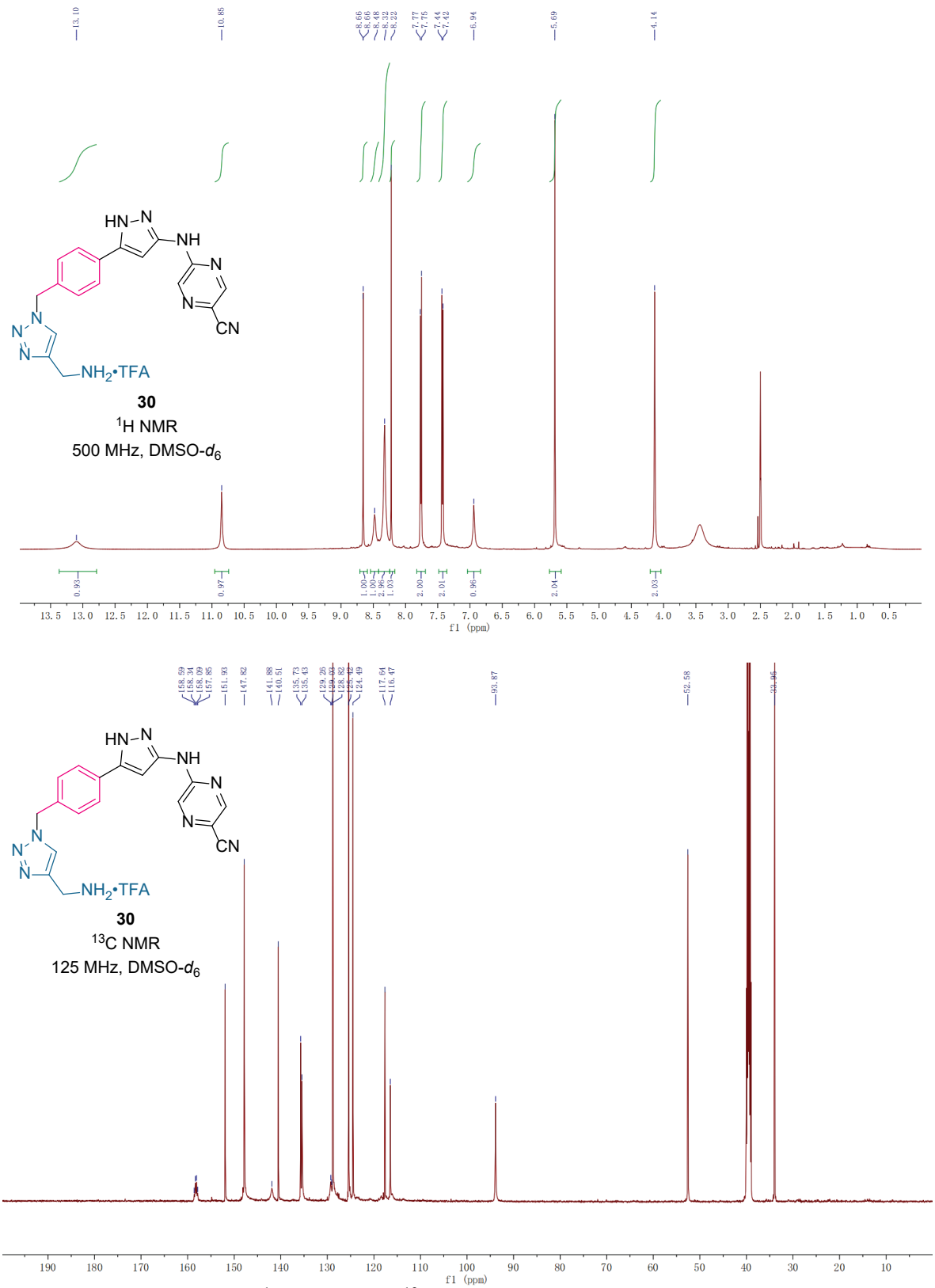
Supplementary Figure 51. ^{1}H NMR and ^{13}C NMR spectra of compound **27**.



Supplementary Figure 52. ^{1}H NMR and ^{13}C NMR spectra of compound **28**.



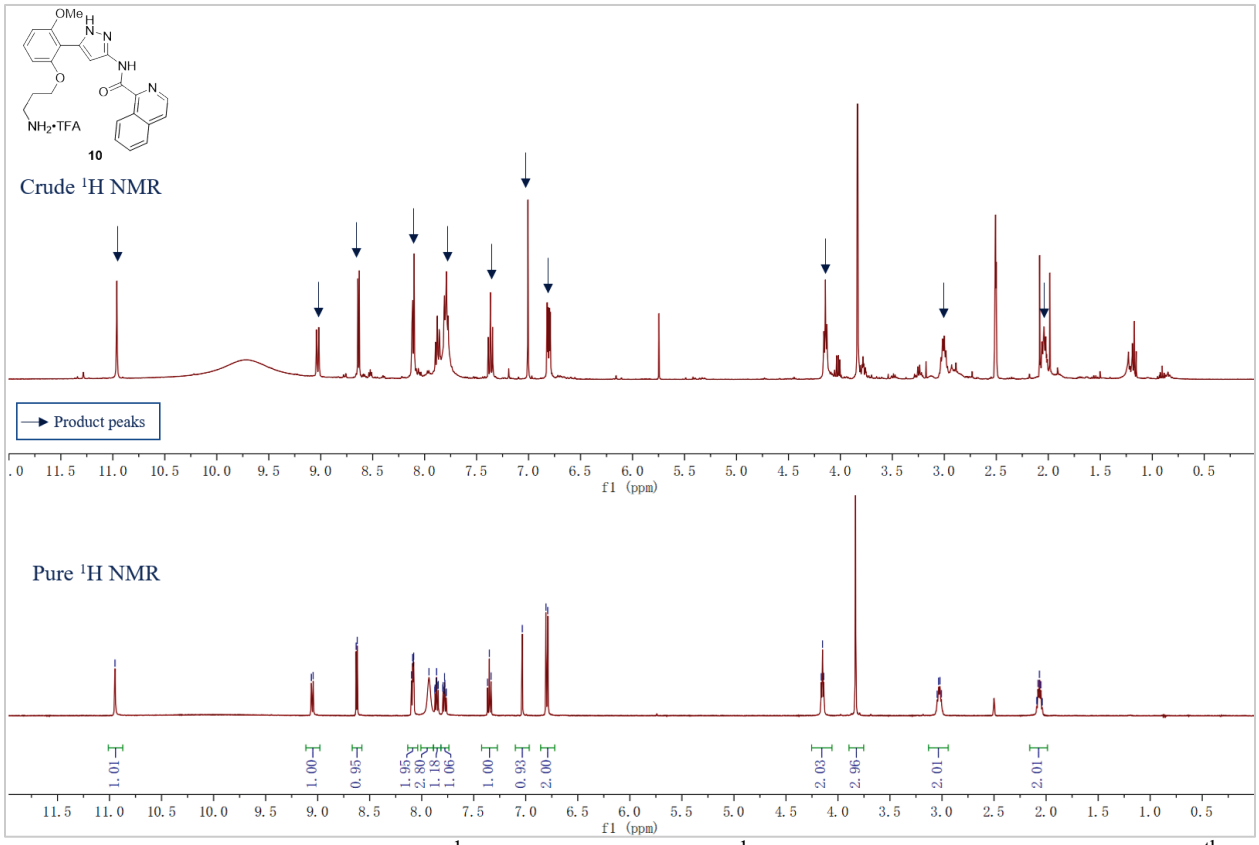
Supplementary Figure 53. ^{1}H NMR and ^{13}C NMR spectra of compound **29**.



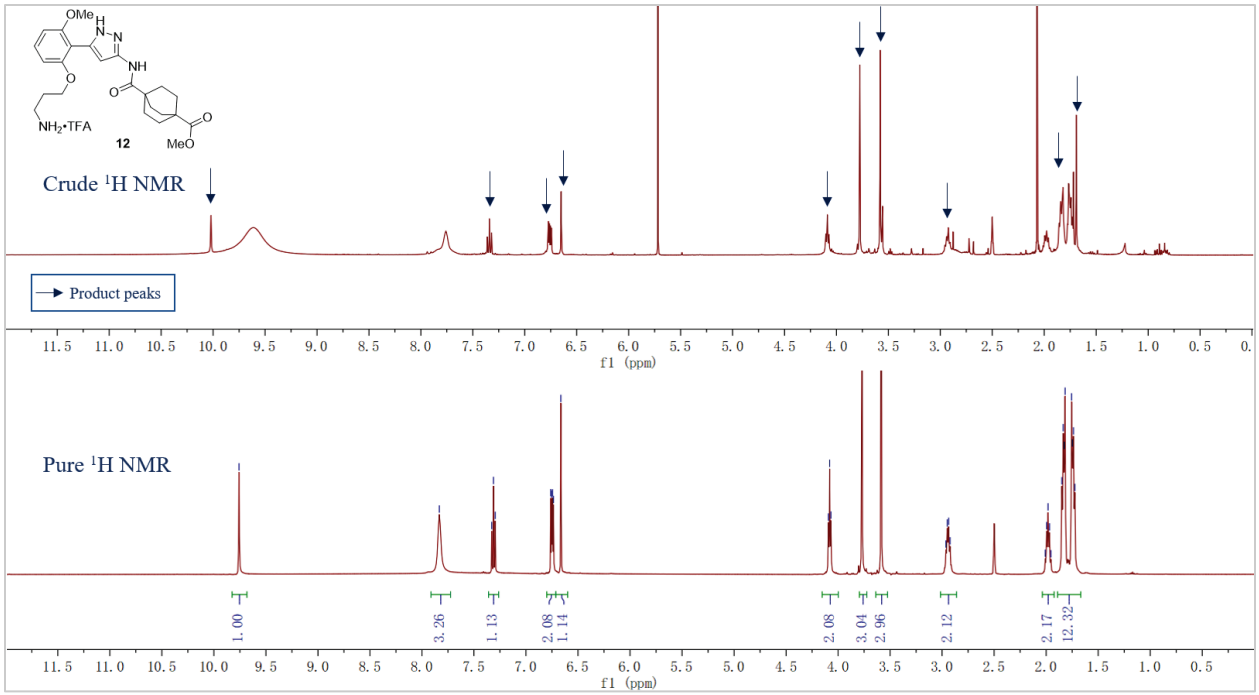
Supplementary Figure 54. ^{1}H NMR and ^{13}C NMR spectra of compound **30**.

12. Representative crude ^{1}H NMR spectra of the cleaved derivatives

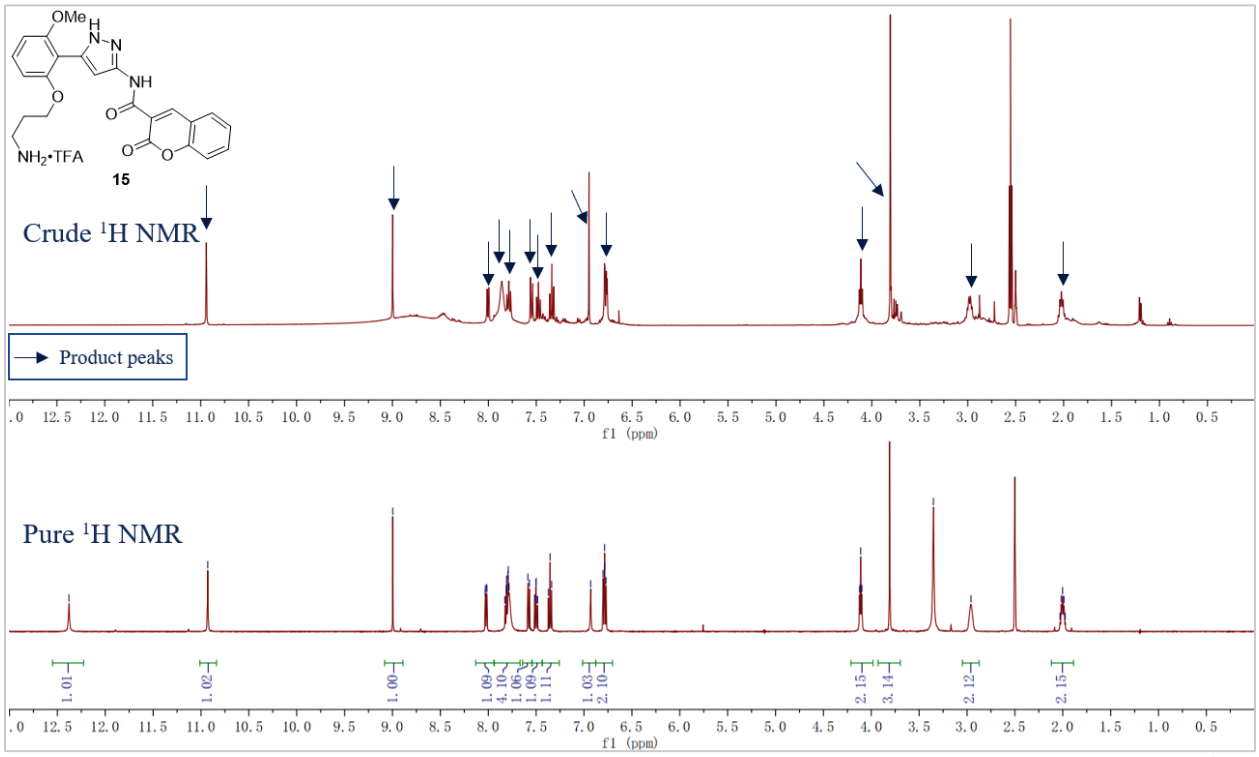
Notes: The rude ^{1}H NMR spectra of derivatives were acquired without any purification after cleaving the products from the solid resin in automated SPS-flow synthesis.



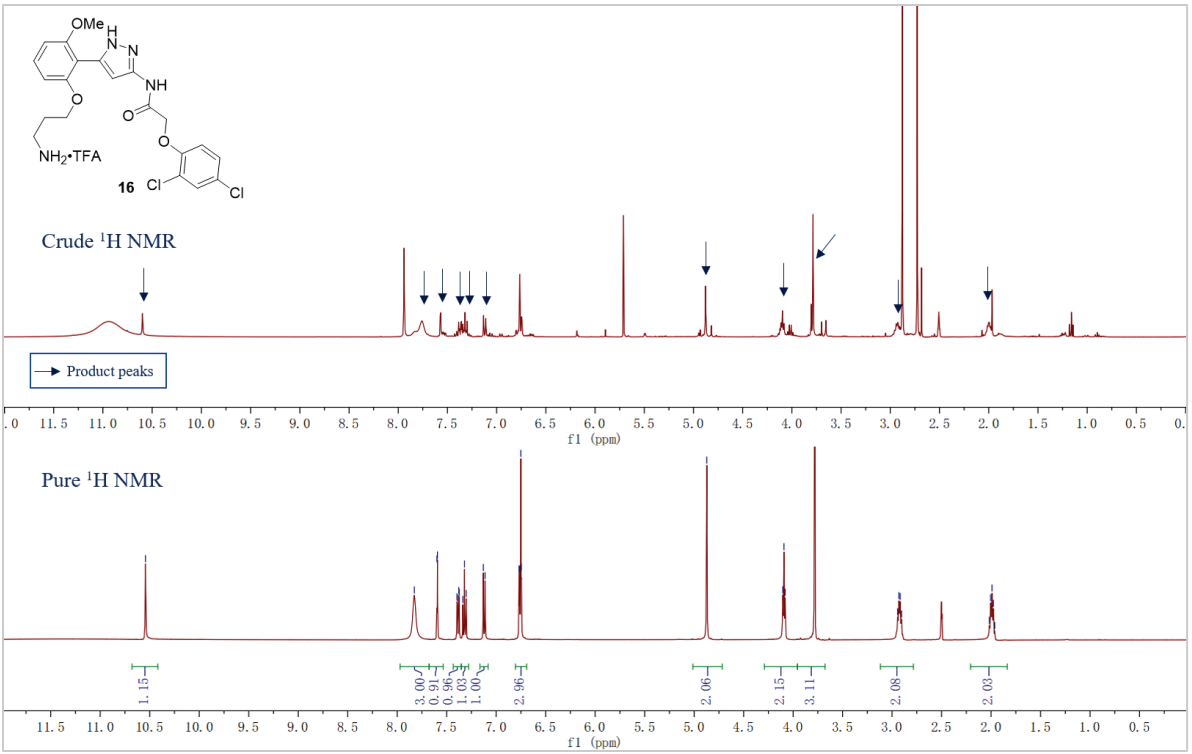
Supplementary Figure 55. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **10** (5th step amide coupling)



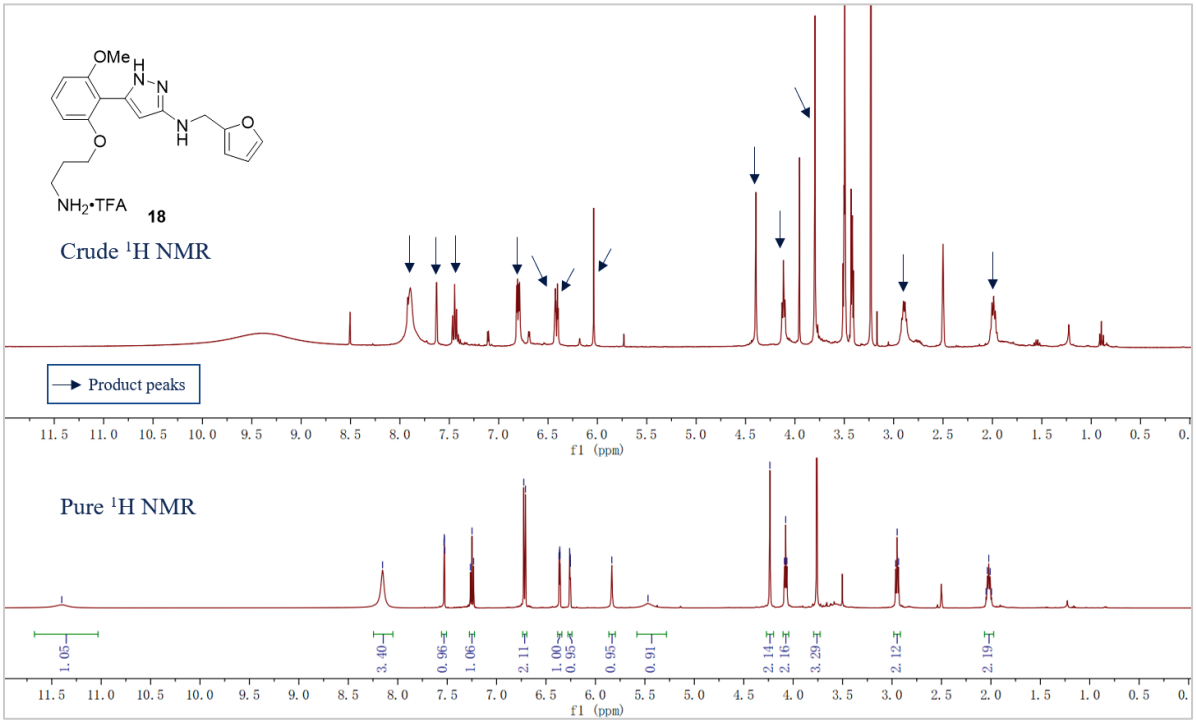
Supplementary Figure 56. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **12** (5th step amide coupling).



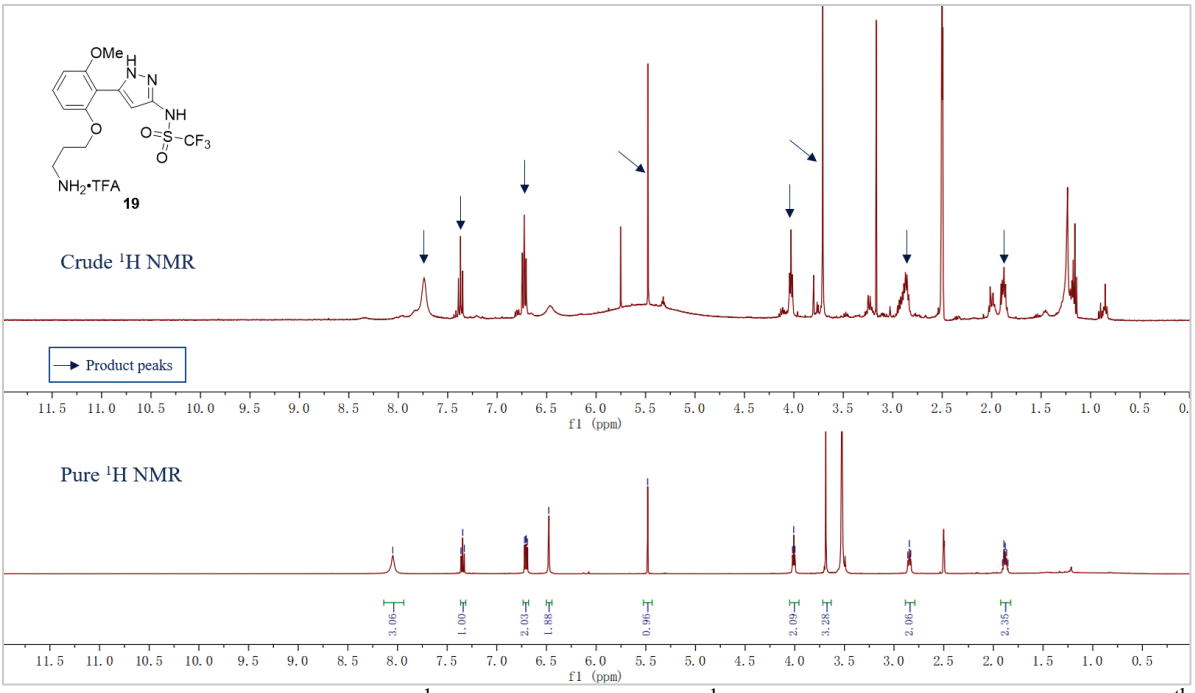
Supplementary Figure 57. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **15** (5th step amide coupling).



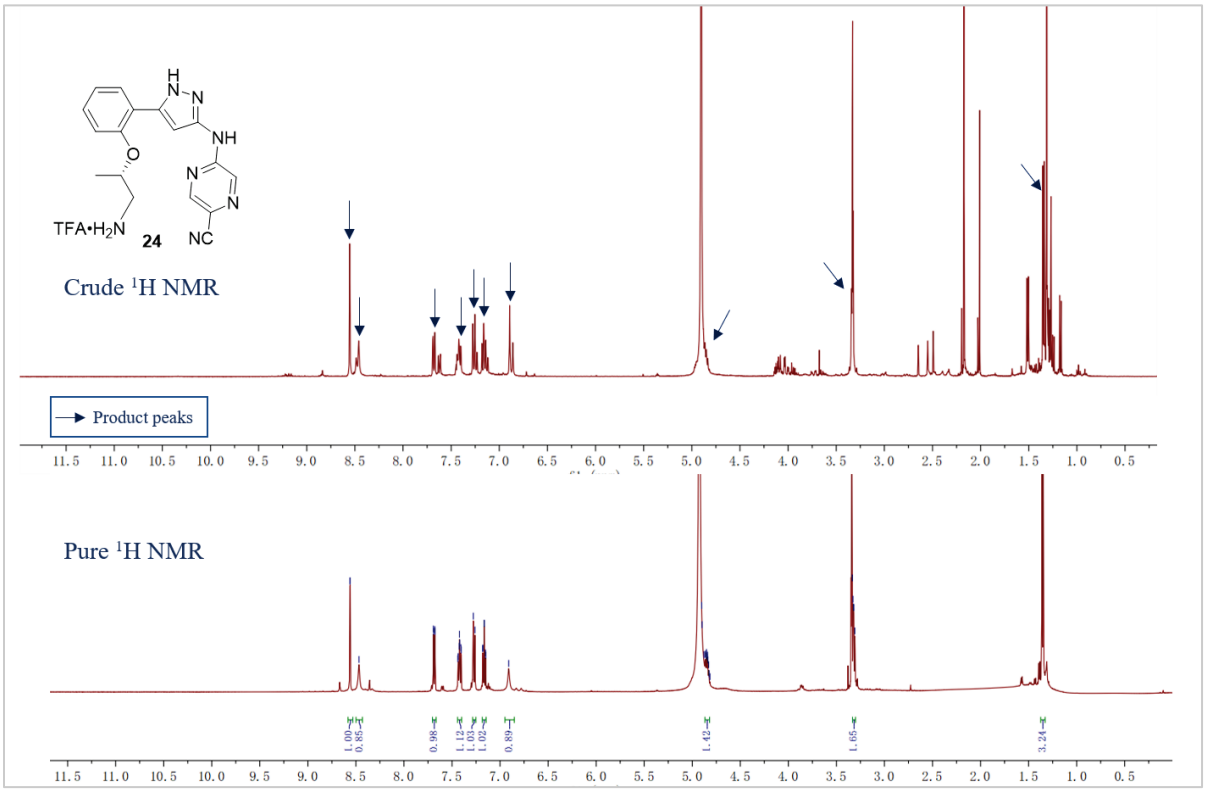
Supplementary Figure 58. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **16** (5th step amide coupling).



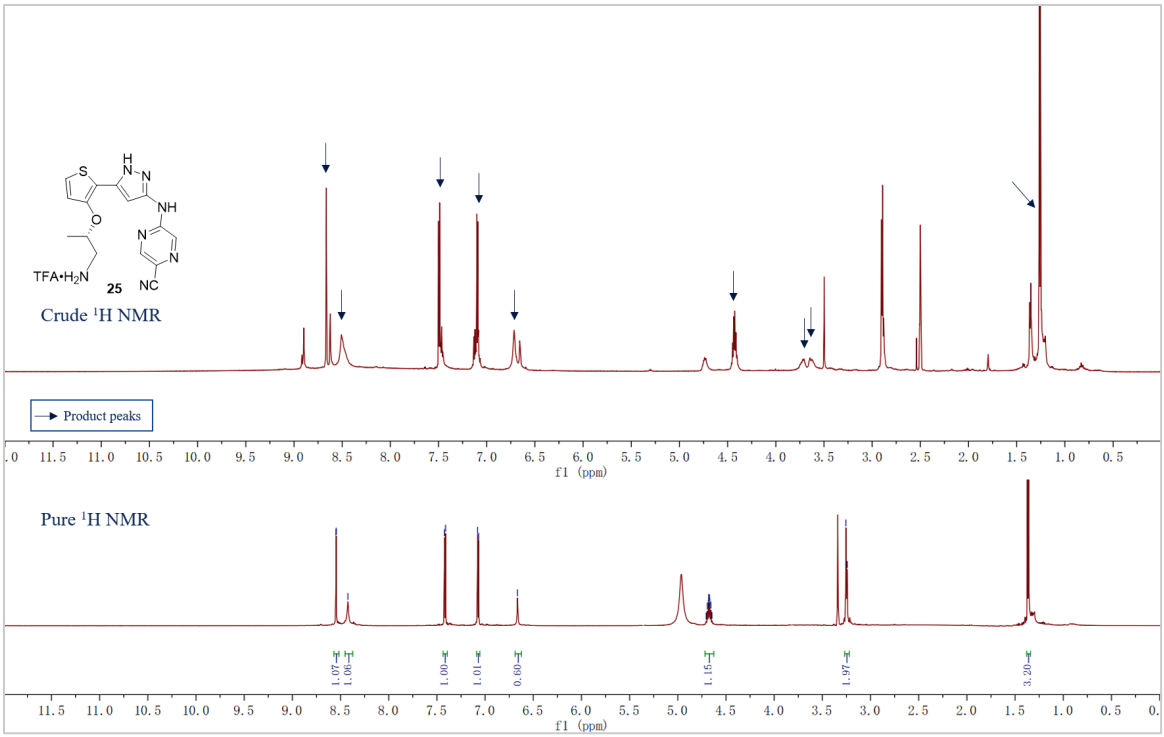
Supplementary Figure 59. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **18** (5th step reductive amination).



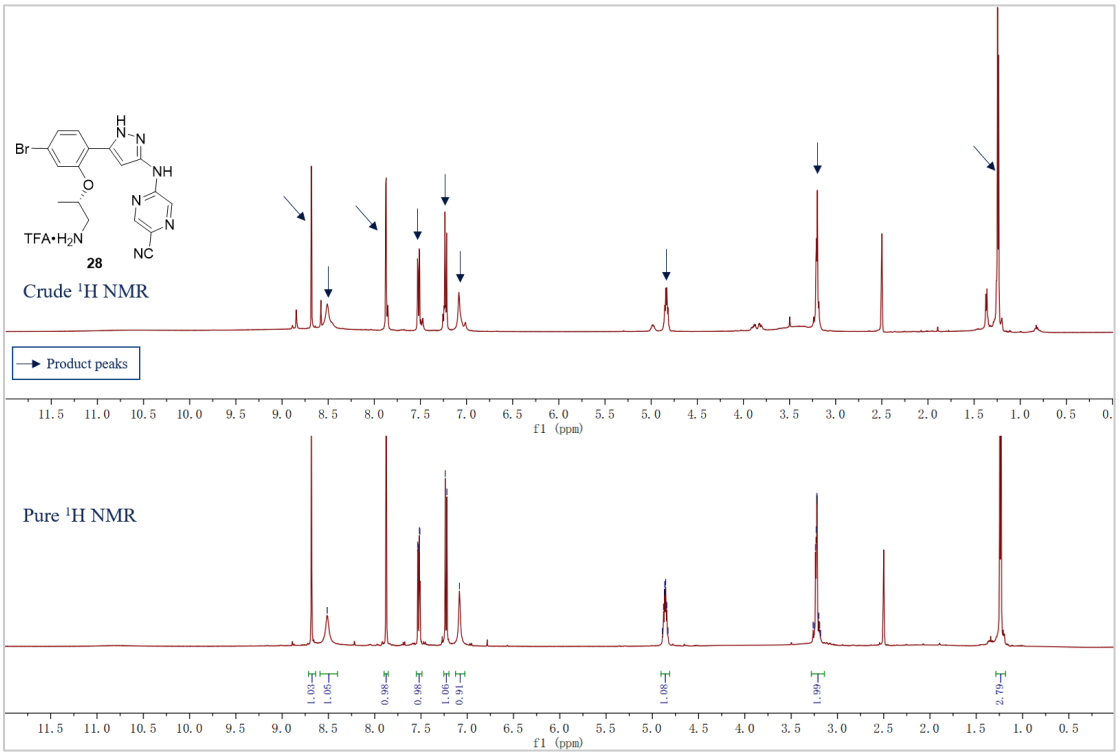
Supplementary Figure 60. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **19** (5th step *N*-triflation).



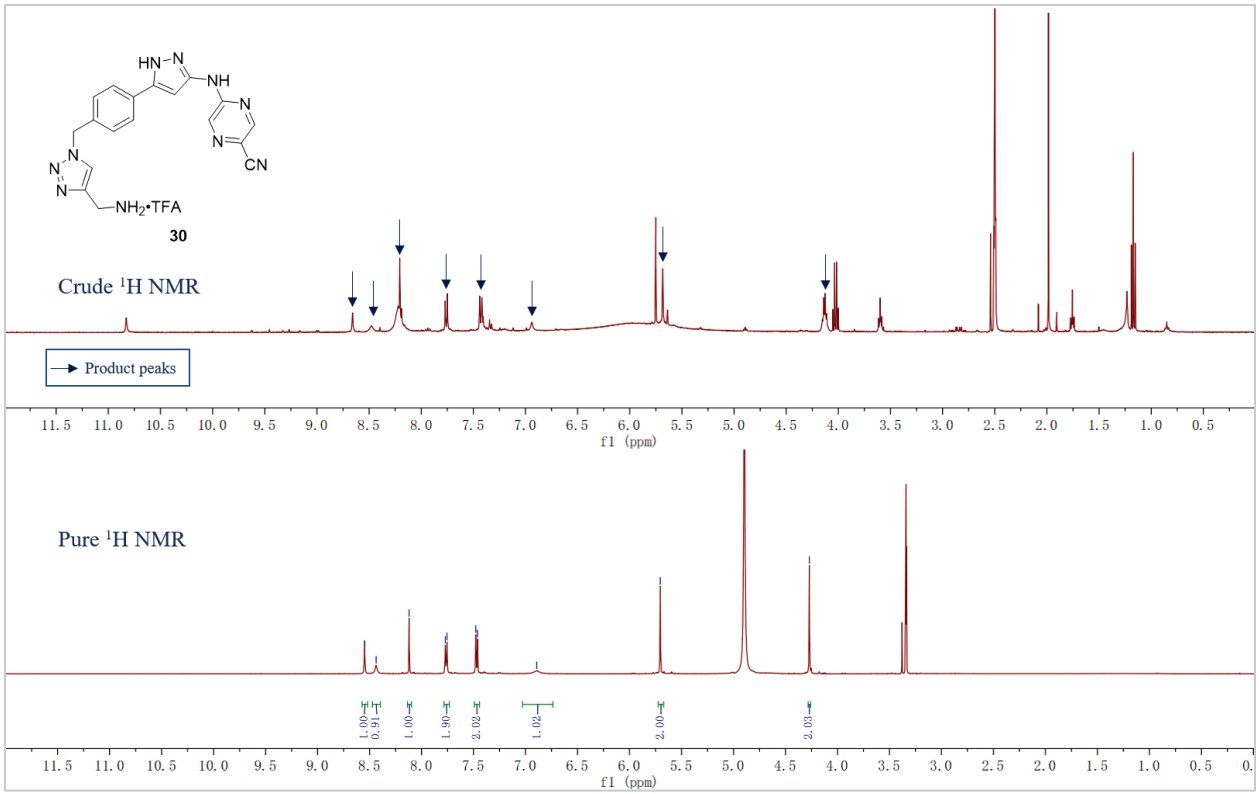
Supplementary Figure 61. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **24** (2nd step Mitsunobu reaction).



Supplementary Figure 62. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **25** (2nd step Mitsunobu reaction).



Supplementary Figure 63. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **28** (2nd step Mitsunobu reaction).



Supplementary Figure 64. Crude ^{1}H NMR and purified ^{1}H NMR spectra of compound **30** (2nd step the Click reaction).