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Supplemental Material to:

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Supplemental materials:

Synthesis and screening of 3-MA derivatives for autophagy inhibitors

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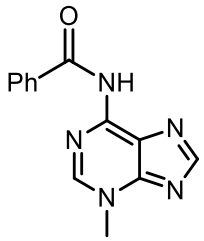
Methods and materials:

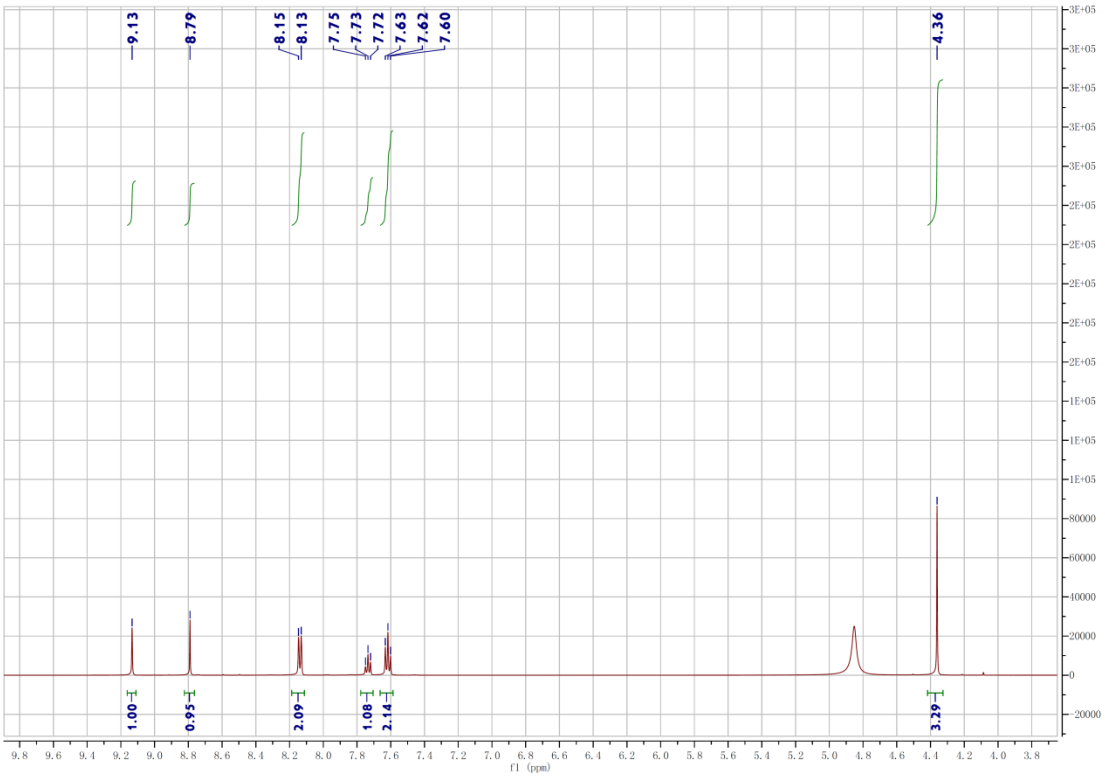
The structural formula for each compound is shown, followed by a description of the method of synthesis, and ^{1}H NMR and ^{13}C NMR spectroscopy signals.

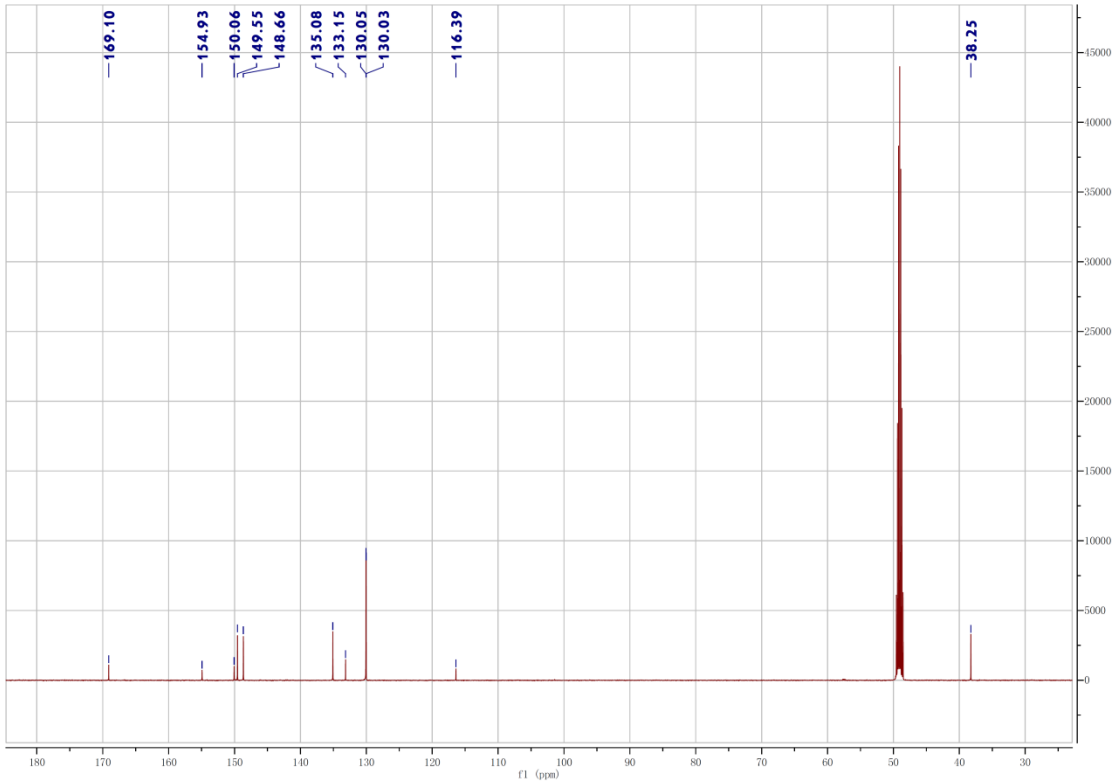


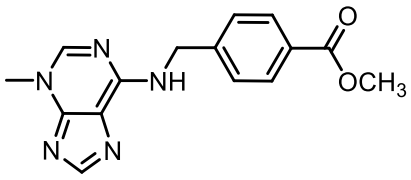
1

Preparation of 1 (N-(3-methyl-3H-purin-6-yl)benzamide)

A solution of 3-methyladenine (100.0 mg, 0.67 mmol) and benzoyl chloride (188.5 mg, 1.34 mmol) in pyridine (3.0 ml) was stirred at 117°C for 24 h. After that, the solution was cooled to room temperature. The precipitated white solid was filtered and dried at 100°C in a drying oven (yield: 34%).^{1}H NMR (400 MHz, CD_3OD): δ 9.13 (s, 1H), 8.79 (s, 1H), 8.15-8.13 (d, 2H), 7.75-7.72 (t, 1H), 7.63-7.60 (t, 2H), 4.36 (s, 3H).^{13}C NMR (100 MHz, CD_3OD) δ 169.1, 154.9, 149.6, 148.7, 135.1, 133.2, 130.1, 130.0, 116.4, 38.3, 17.2; HRMS calculated for $C_{13}H_{11}N_5O$ exact mass: 254.1036, found 254.1038; mp: 250°C.







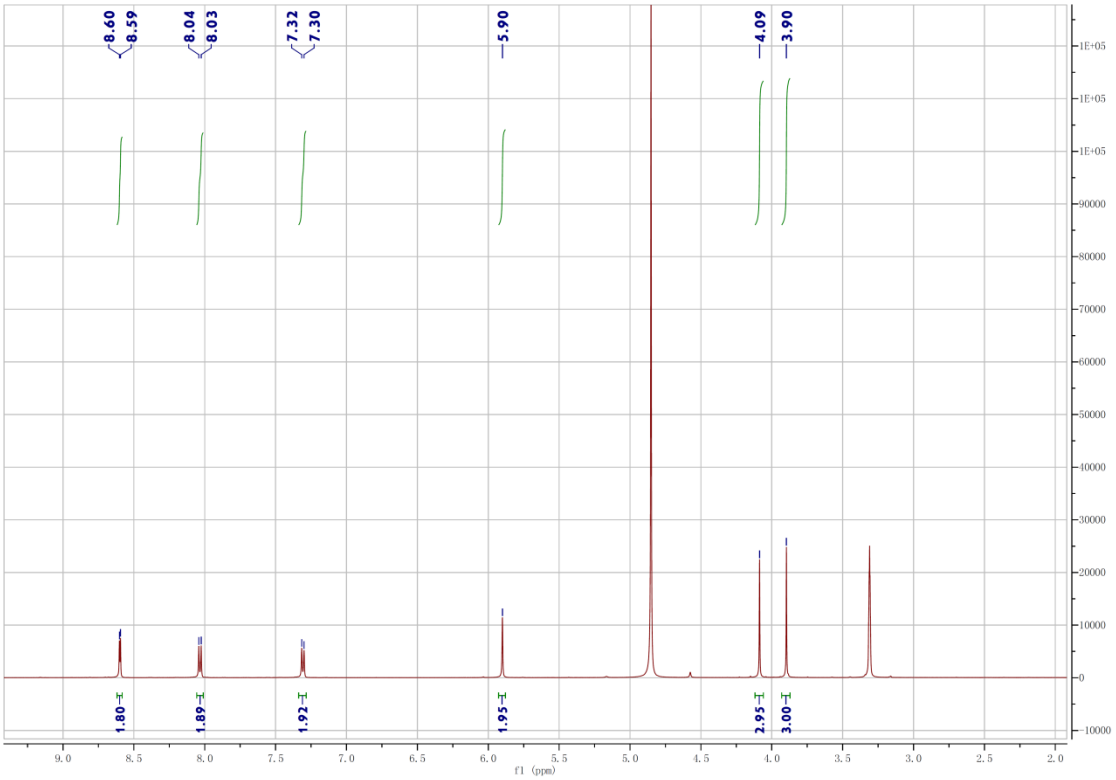
2

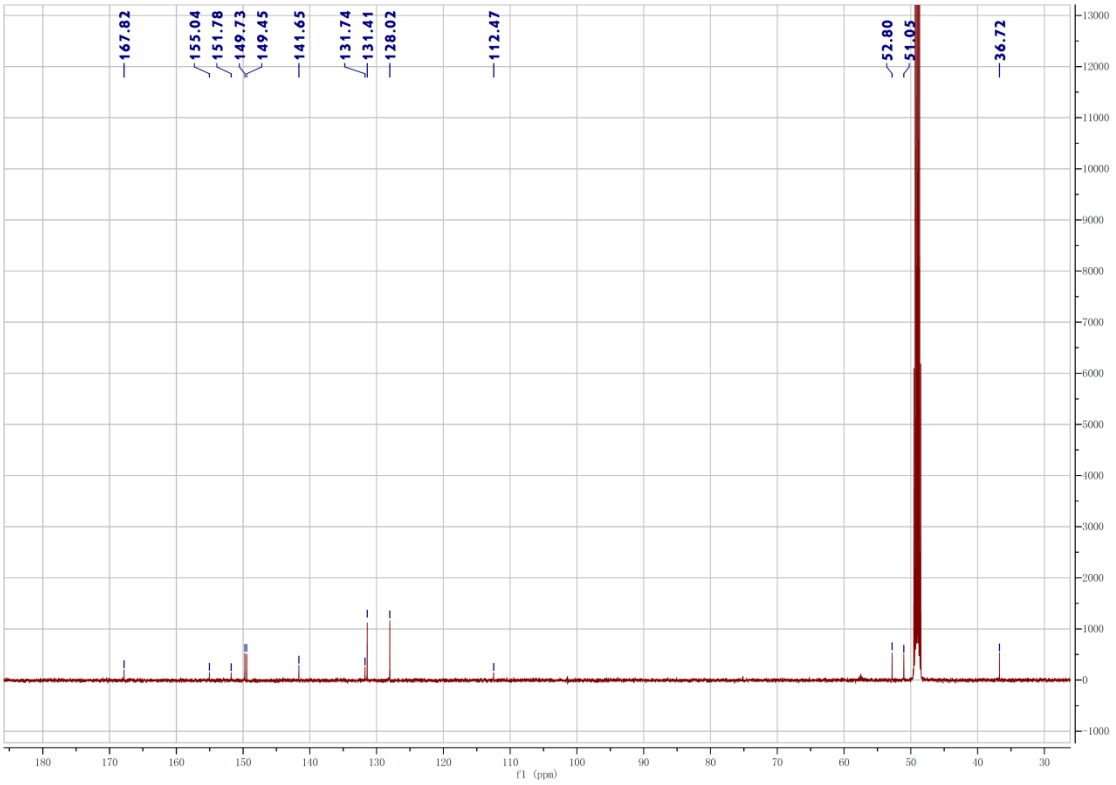
Preparation of 2

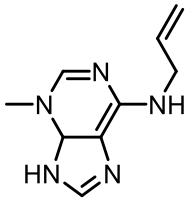
(methyl 4-(((3-methyl-3H-purin-6-yl)amino)methyl)benzoate)

Methyl 4-(bromomethyl)benzoate (307.0 mg, 1.34 mmol) , 3-methyladenine (100.0 mg, 0.67 mmol) and in dimethylformamide (DMF) (3.0 ml) was stirred at 100°C for 24 h, the solvent was removed under reduced pressure. The residue was washed with CH_2Cl_2 and further purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent to generate the desired product (yield: 43%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.60-8.59 (d, 2H), 8.04-8.03 (d, 2H), 7.32-7.30 (d, 2H), 5.90 (s,

2H), 4.09 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.8, 155.0, 151.8, 149.7, 149.5, 131.7, 131.4, 128.0, 112.5, 57.5, 52.8, 51.1, 36.7; HRMS calculated for $C_{15}H_{16}N_5O_2$ exact mass: 298.1297, found 298.1299; mp: 253°C.



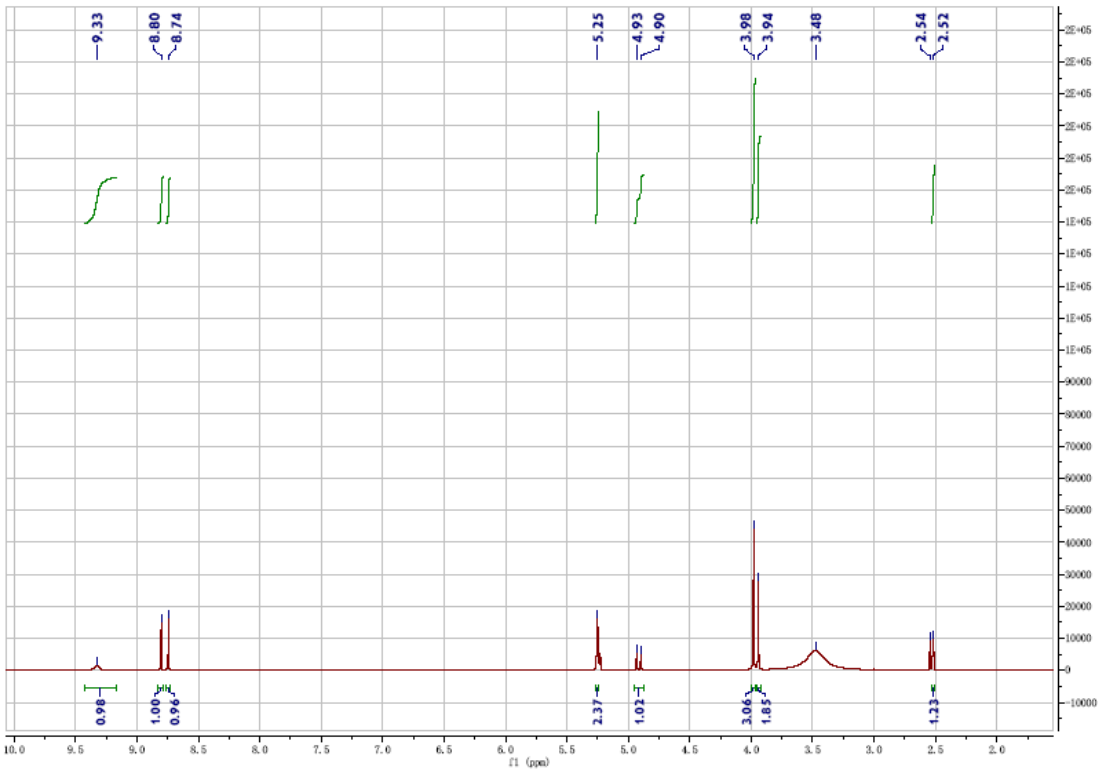




3

Preparation of 3 (N-allyl-3-methyl-3H-purin-6-amine)

3-bromoprop-1-ene (241.0 mg, 1.34 mmol), 3-methyladenine (100.0 mg, 0.67 mmol) and in DMF (3.0 ml) was stirred at 100°C for 24 h, the solvent was removed under reduced pressure. The residue was washed with CH_2Cl_2 and further purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent to generate the desired product (yield: 31%). ^{1}H NMR (400 MHz, DMSO-d6): δ 9.33 (s, 1H), 8.80 (s, 1H), 8.74 (s, 1H), 5.25 (s, 2H), 4.93-4.90 (d, 1H), 3.98 (s, 3H), 3.94 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d6): δ 154.3, 152.7, 148.4, 147.7, 133.7, 117.0, 110.5, 48.4, 35.9, 35.8; HRMS calculated for $C_9H_{11}N_5$ exact mass: 190.1087, found 190.1084; mp: 243°C.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8.74
5.25
4.93
4.90
3.98
3.94
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2.52
0.98
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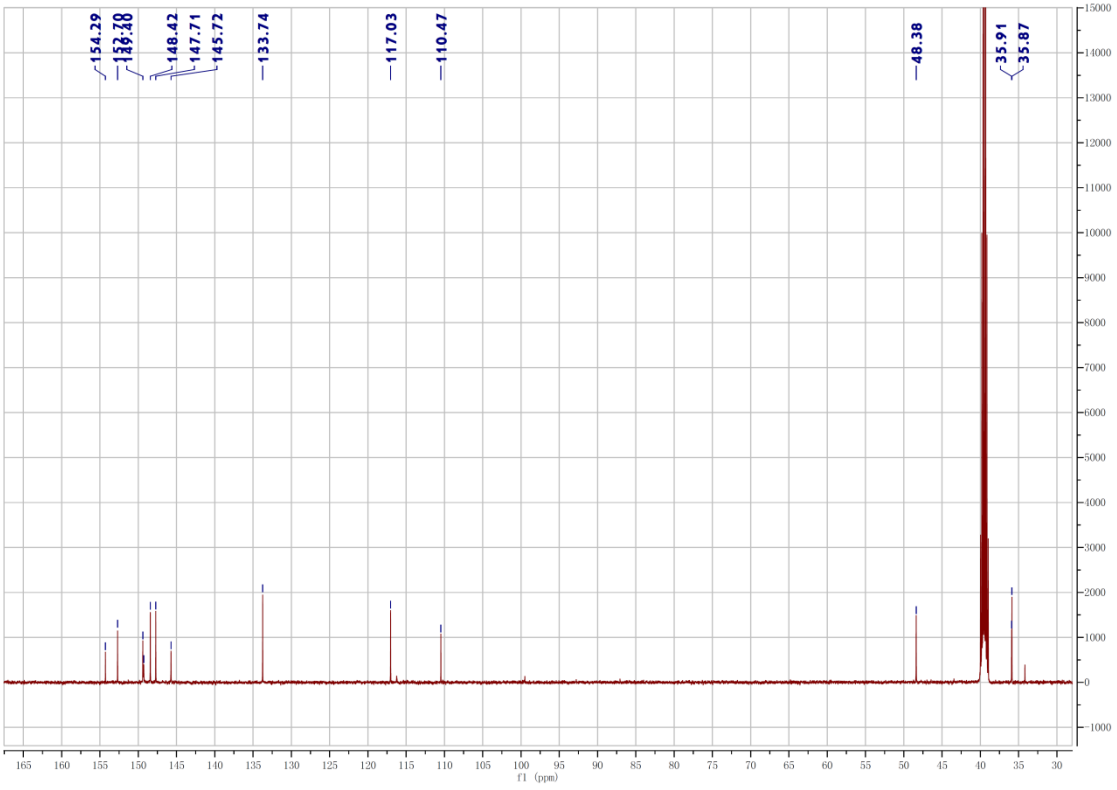
0.96
2.37
1.02
3.06
1.85
1.23
f1 (ppm)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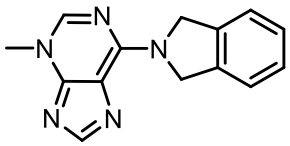
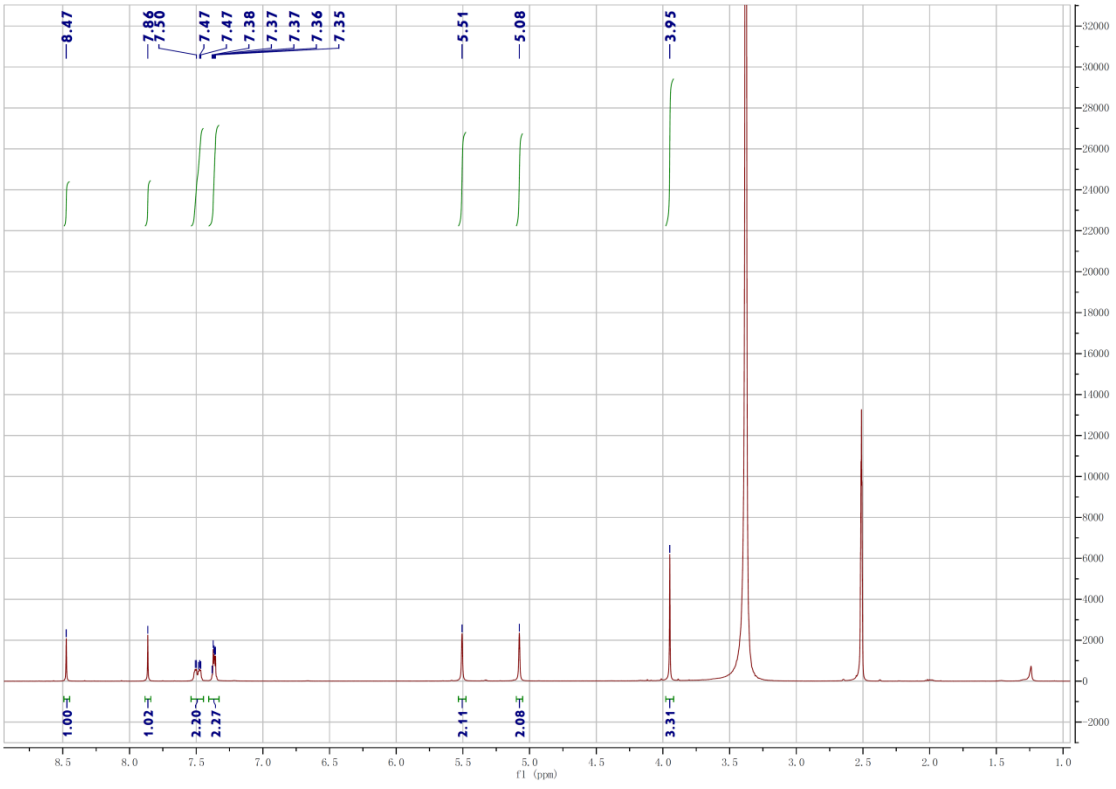
f1 (ppm)



4

Preparation of 4 (6-(isoindolin-2-yl)-3-methyl-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 3-methyladenine (149.2 mg, 1.0 mmol), and 1,2-Bis(bromomethyl)benzene (316.7 mg, 1.2 mmol), NaH (143.9 mg, 6.0 mmol), and DMF (3.0 ml) were added. The mixture was kept stirring at 30°C for 24 h, Then the solvent was removed smoothly with a cold trap in vacuum, The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (8:1) the desired product was generated as a solid. The product was recrystallized from MeOH to give the desired product (yield: 38 %).^{1}H NMR (400 MHz, DMSO-d6): δ 8.47 (s, 1H), 7.86 (s, 1H), 7.49-7.47 (m, 2H), 7.37-7.35 (m, 2H), 5.51 (s, 2H), 5.08 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d6): δ 152.7, 151.0, 150.6, 143.5, 137.0, 135.6, 127.4, 122.9, 54.9, 53.6, 35.9; HRMS calculated for $C_{14}H_{14}N_5$ exact mass: 252.1244, found 252.1243; mp: 291°C.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7.38
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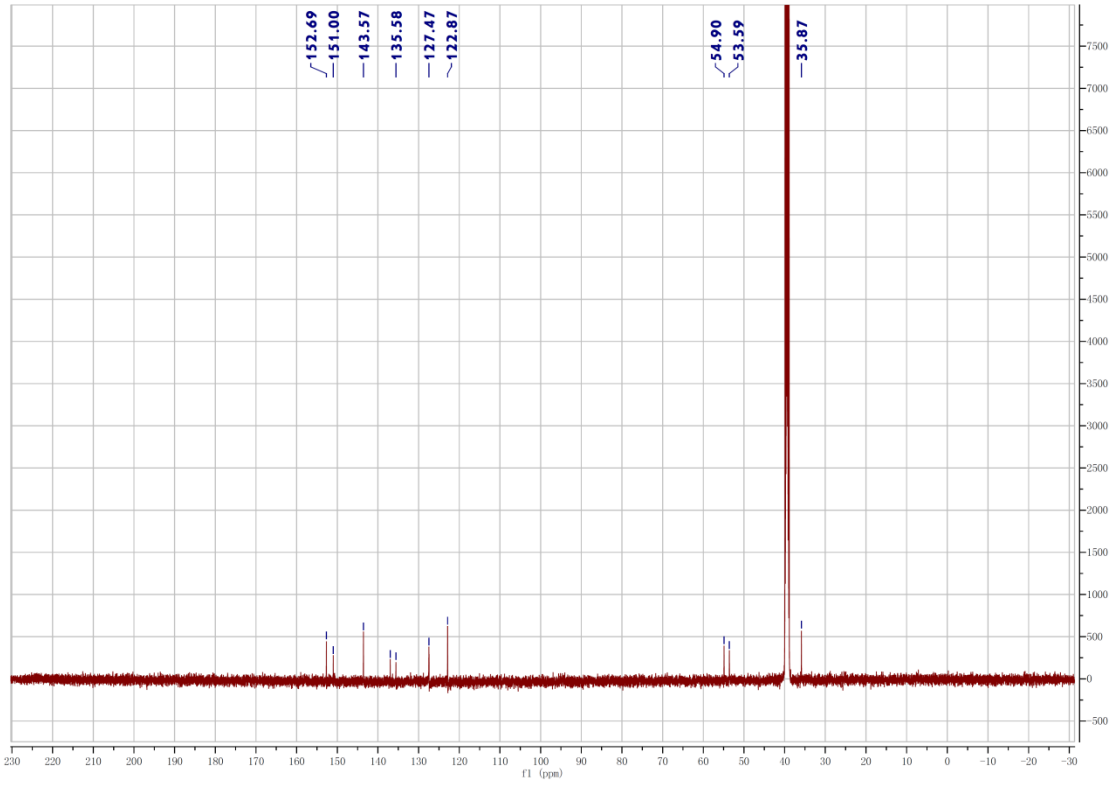
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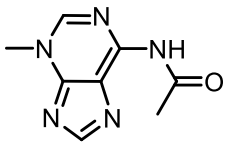
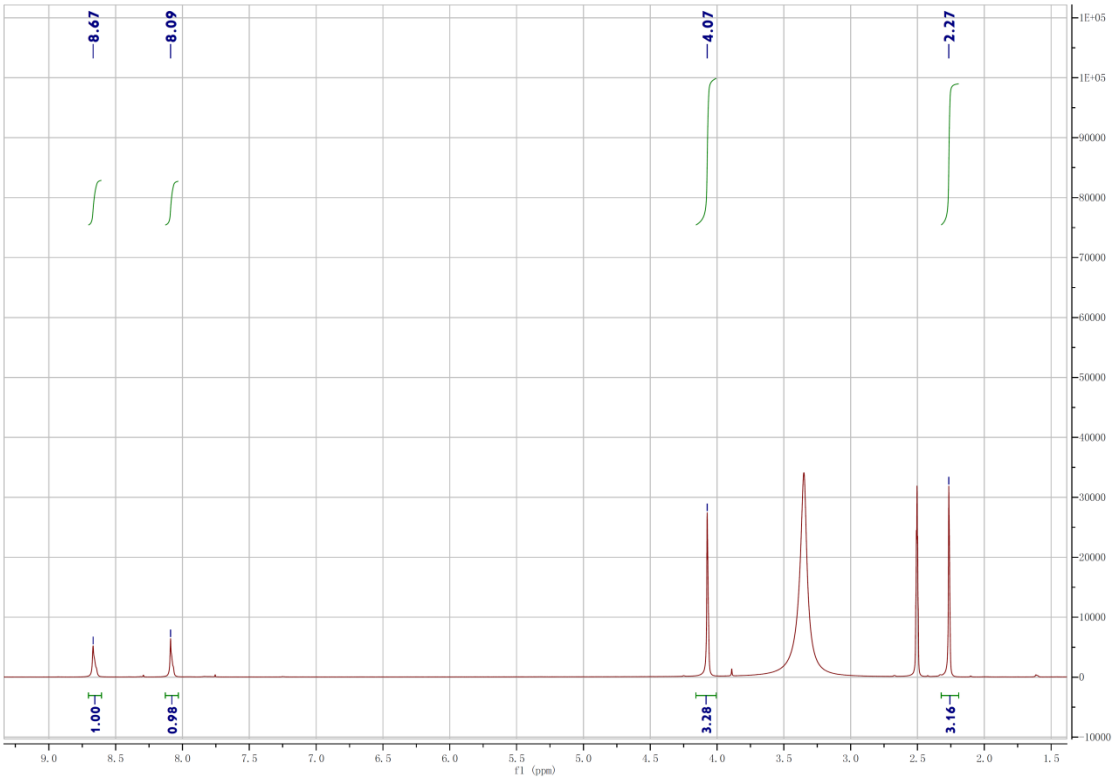


5

Preparation of 5 (N-(3-methyl-3H-purin-6-yl)acetamide)

A solution of 6-Chloropurine (1.55 g, 10.0 mmol) and acetamide (0.89 g, 15.0 mmol) in MeOH (10 ml) was stirred at 80°C for 2 h. After that, the solution was cooled to room temperature. The precipitated solid was filtered and dried at 100°C in a drying oven to deliver N-(9H-purin-6-yl)acetamide (yield: 67%).

N-(9H-purin-6-yl)acetamide (176.0 mg, 1.0 mmol) was added to a flame-dried Schlenk tube. This tube was degassed and refilled with nitrogen 3 times and 2.0 ml of freshly distilled DMF was added by syringe. Then, 1.0 ml of MeI was added dropwise to the stirred mixture at 0°C. After stirring at room temperature for an additional 24 h under N_2, the solvent was removed under reduced pressure. The residue was washed with CH_2Cl_2 and further purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent to generate the desired product (yield: 76%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.67 (s, 1H), 8.09(s, 1H), 4.07(s, 3H), 2.27(s, 3H); ^{13}C NMR (100 MHz, DMSO-d6) δ 169.9, 156.4, 154.0, 148.1, 142.6, 125.9, 36.6, 24.9; HRMS calculated for $C_8H_{10}N_5O$ exact mass: 192.0879, found 192.0875; mp: 240°C.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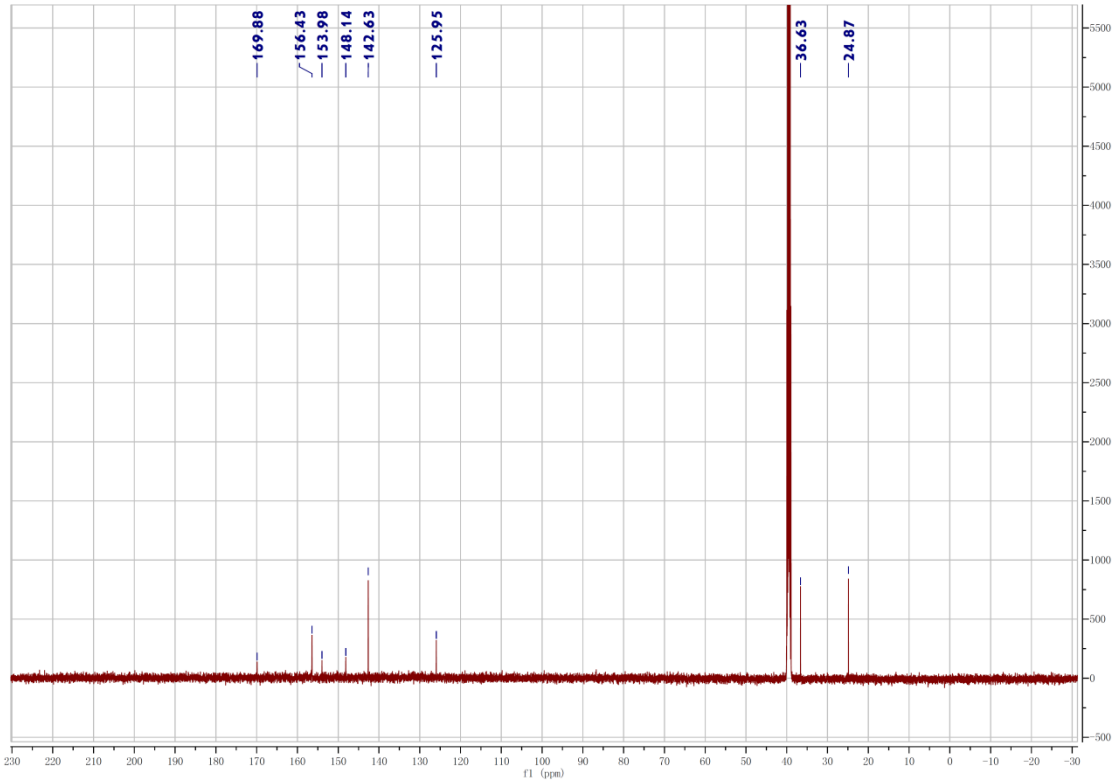
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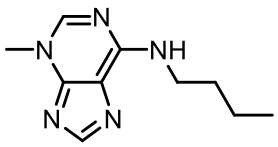
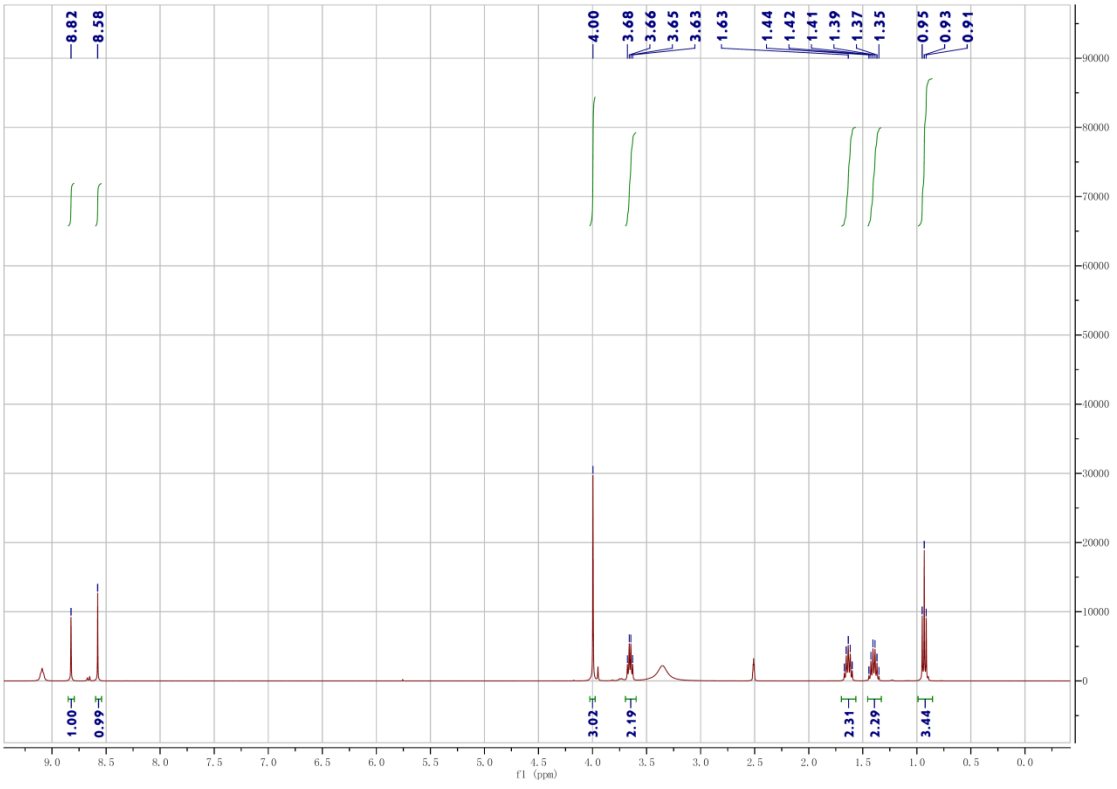


6

Preparation of 6 (N-butyl-3-methyl-3H-purin-6-amine)

A solution of 6-chloropurine (1.55 g, 10.0 mmol) and butylamine (1.10 g, 15.0 mmol) in MeOH (10 ml) was stirred at 80°C for 2 h. After that, the solution was cooled to room temperature. The precipitated solid was filtered and dried at 100°C in a drying oven to deliver N-butyl-9H-purin-6-amine (yield: 55%).

`N-butyl-9H-purin-6-aminee (191.0 mg, 1.0 mmol) was added to a flame-dried Schlenk tube. This tube was degassed and refilled with nitrogen 3 times and 2.0 mL of freshly distilled DMF was added by syringe. Then, 1.0 ml of MeI was added dropwise to the stirred mixture at 0°C. After stirring at room temperature for an additional 24 h under N_2, the solvent was removed under reduced pressure. The residue was washed with CH_2Cl_2 and further purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent to generate the desired product (yield: 74%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.82(s, 1H), 8.58(s, 1H), 4.00 (s, 3H), 3.68-3.63(m, 2H), 1.67-1.60 (m, 2H), 1.41-1.35 (m, 2H), 0.95-0.91 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.2, 148.6, 147.1, 144.4, 42.7, 40.6, 36.2, 30.5, 19.4, 13.6; HRMS calculated for $C_{10}H_{16}N_5$ exact mass: 206.1400, found 206.1399; mp: 160°C.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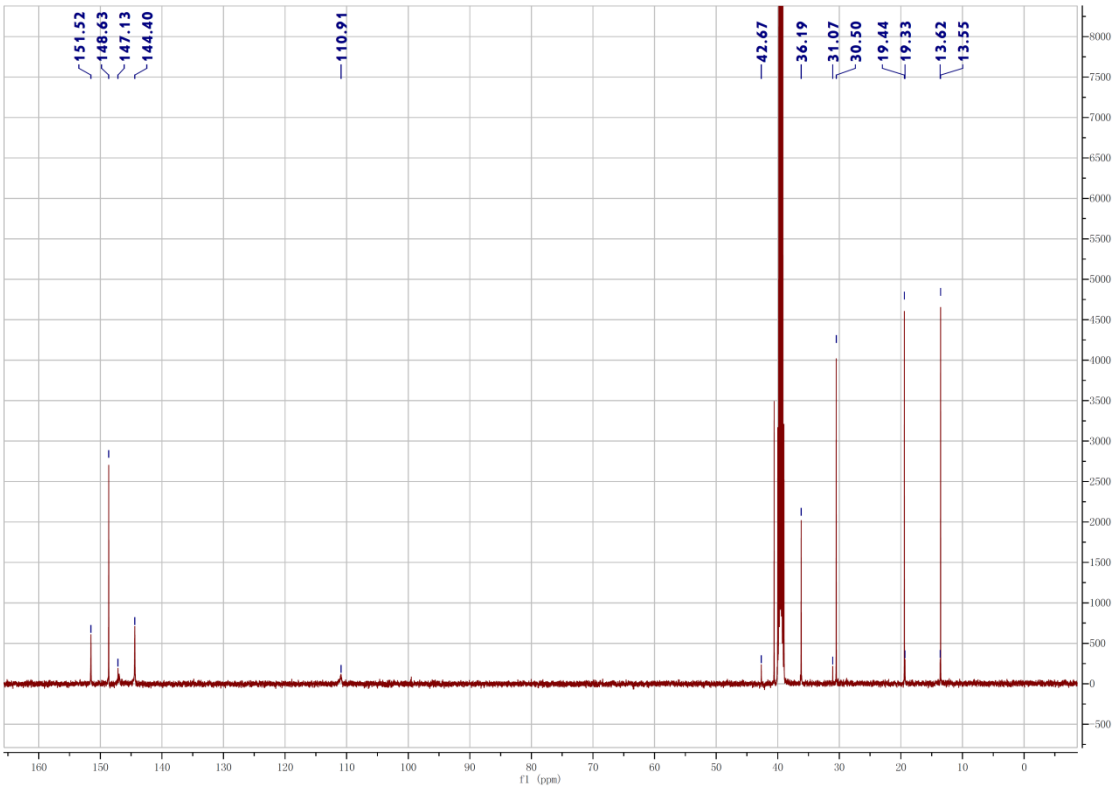
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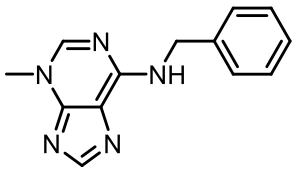
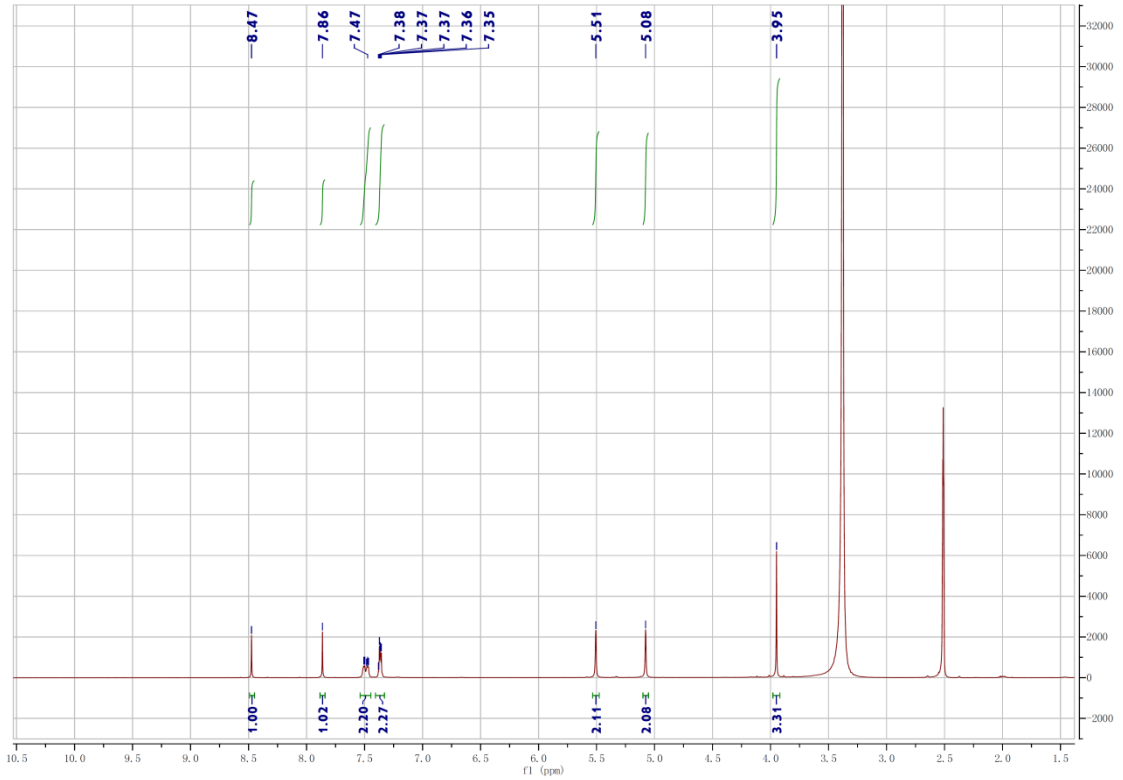


7

Preparation of 7 (N-benzyl-3-methyl-3H-purin-6-amine)

A solution of 6-chloropurine (1.55 g, 10.0 mmol) and benzylamine (1.60 g, 15.0 mmol) in MeOH (10 ml) was stirred at 80°C for 2 h. After that, the solution was cooled to room temperature. The precipitated solid was filtered and dried at 100°C in a drying oven to deliver N-benzyl-9H-purin-6-amine (yield: 55%).

N-benzyl-9H-purin-6-amine (225.0 mg, 1.0 mmol) was added to a flame-dried Schlenk tube. This tube was degassed and refilled with nitrogen 3 times and 2.0 ml of freshly distilled DMF was added by syringe. Then, 1.0 ml of MeI was added dropwise to the stirred mixture at 0°C. After stirring at room temperature for an additional 24 h under N_2, the solvent was removed under reduced pressure. The residue was washed with CH_2Cl_2 and further purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent to generate the desired product (yield: 70%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.47 (s, 1H), 7.86 (s, 1H), 7.44-7.35 (m, 5H), 5.51 (s, 2H), 5.08 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.4, 148.7, 144.7, 137.1, 128.7, 127.9, 127.7, 44.3, 36.3; HRMS calculated for $C_{13}H_{13}N_5$ exact mass: 240.1244, found 240.1240; mp: 240°C.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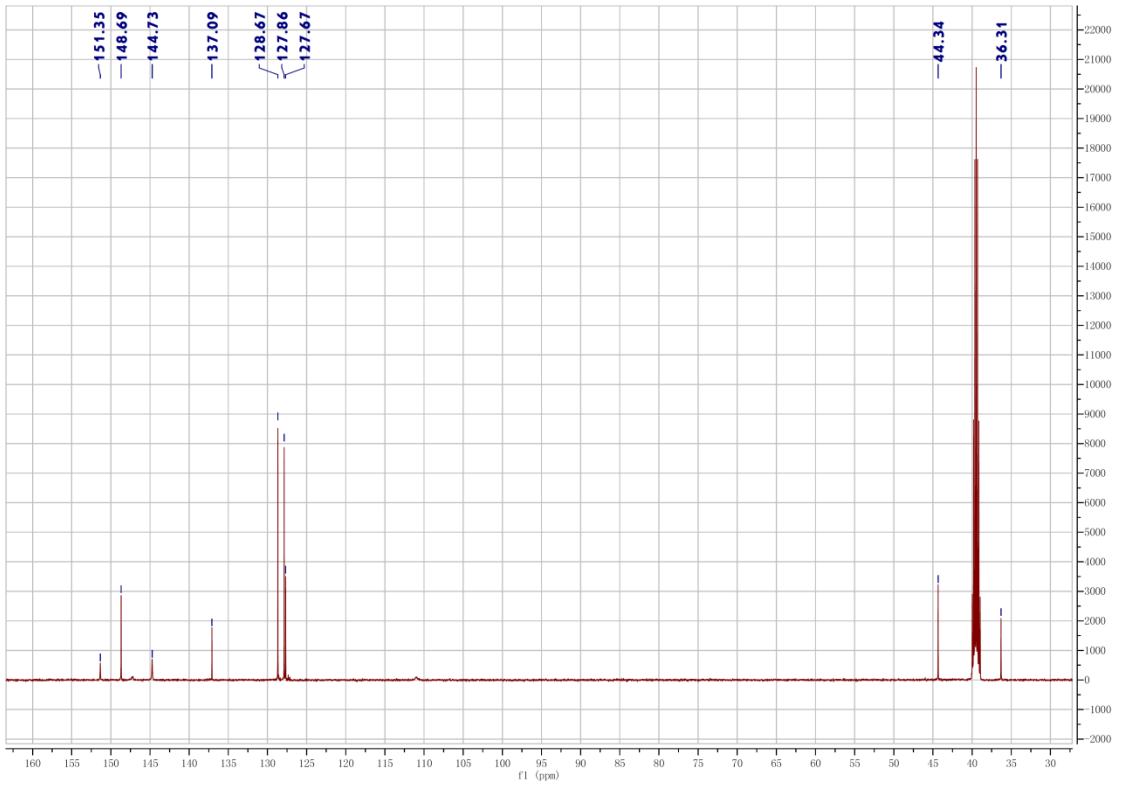
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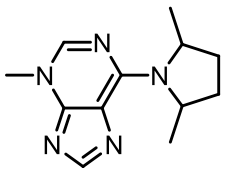


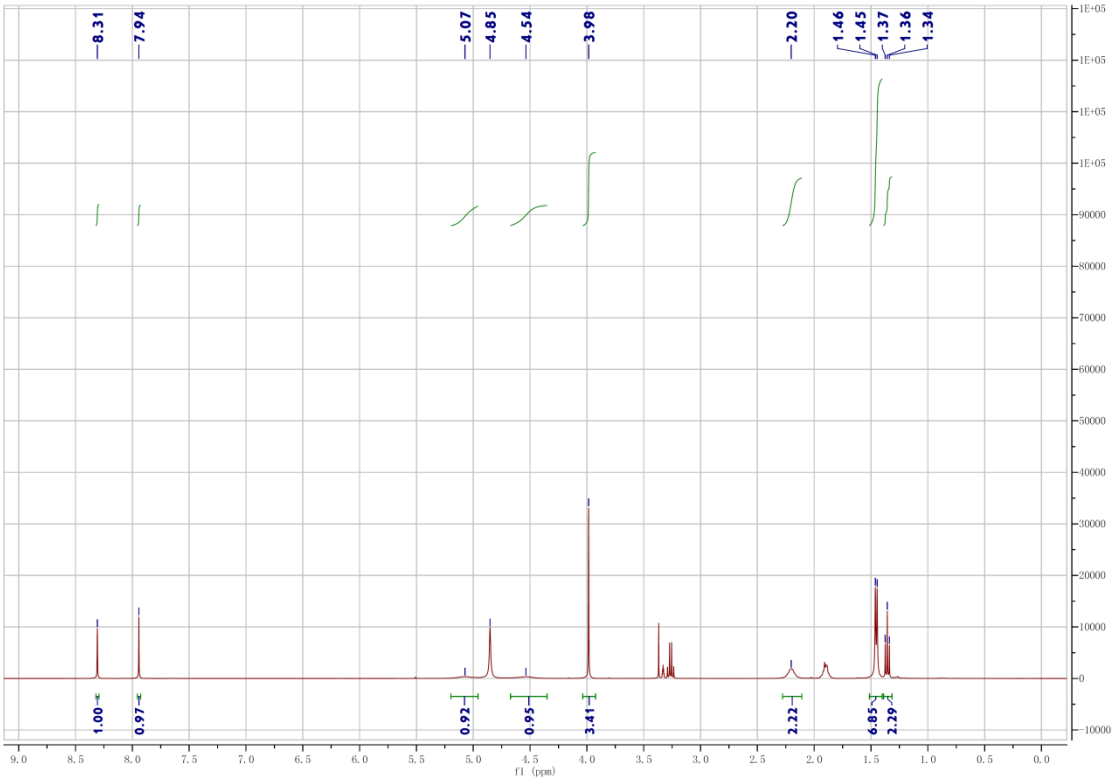
8

Preparation of 8

(6-(2,5-dimethylpyrrolidin-1-yl)-3-methyl-3H-purine)

A solution of 6-chloropurine (1.55 g, 10.0 mmol) and 2,5-dimethylpyrrolidine (1.49 g, 15.0 mmol) in MeOH (10 ml) was stirred at 80°C for 2 h. After that, the solution was cooled to room temperature. The precipitated solid was filtered and dried at 100°C in a drying oven to deliver 6-(2,5-dimethylpyrrolidin-1-yl)-9H-purine (yield: 65%).

6-(2,5-dimethylpyrrolidin-1-yl)-9H-purine (225.0 mg, 1.0 mmol) was added to a flame-dried Schlenk tube. This tube was degassed and refilled with nitrogen 3 times and 2.0 ml of freshly distilled DMF was added by syringe. Then, 1.0 ml of MeI was added dropwise to the stirred mixture at 0°C. After stirring at room temperature for an additional 24 h under N_2, the solvent was removed under reduced pressure. The residue was washed with CH_2Cl_2 and further purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent to generate the desired product (yield: 70%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.31 (s, 1H), 7.94 (s, 1H), 5.07 (s, 1H), 4.54 (s, 1H), 3.98 (s, 3H), 2.20 (s, 2H), 1.46-1.45 (d, 6H), 1.37-1.34 (t, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 154.3, 152.7, 149.4, 149.3, 148.4, 147.7, 145.7, 133.7, 117.0, 110.5, 48.4, 35.9; HRMS calculated for $C_{12}H_{18}N_5$ Exact Mass: 232.1557, found 232.1553; mp: 250°C.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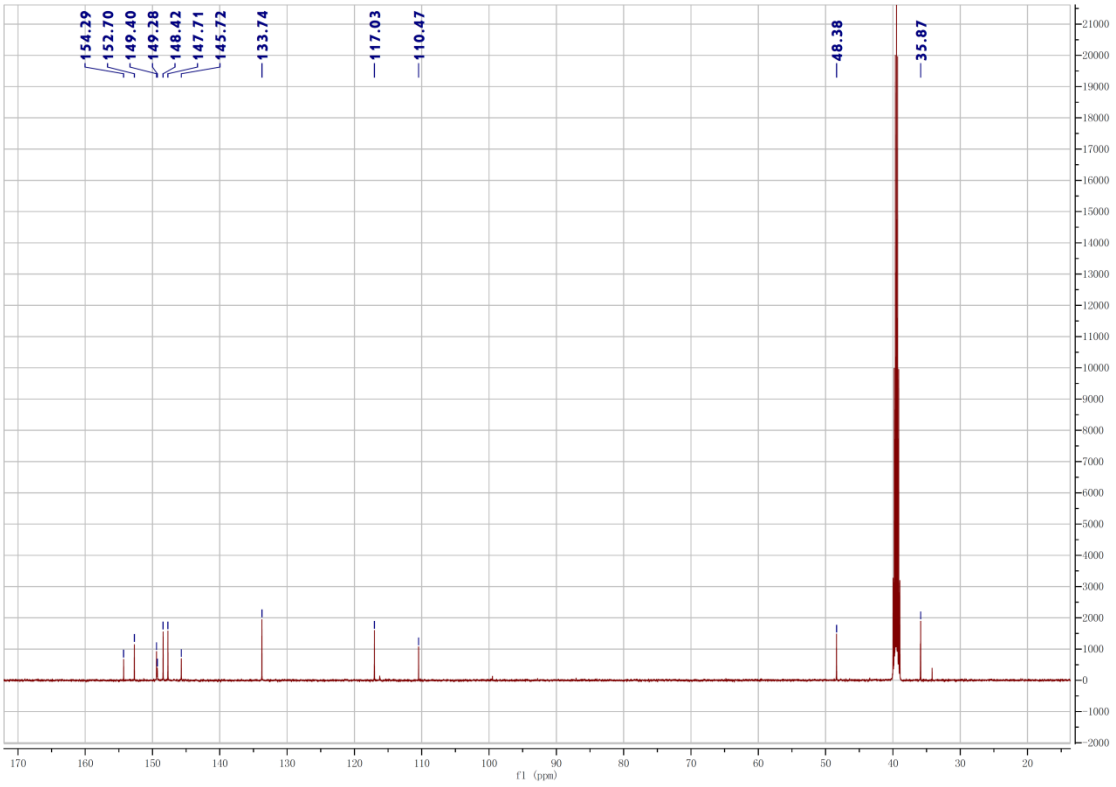
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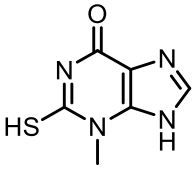
35.87
f1 (ppm)

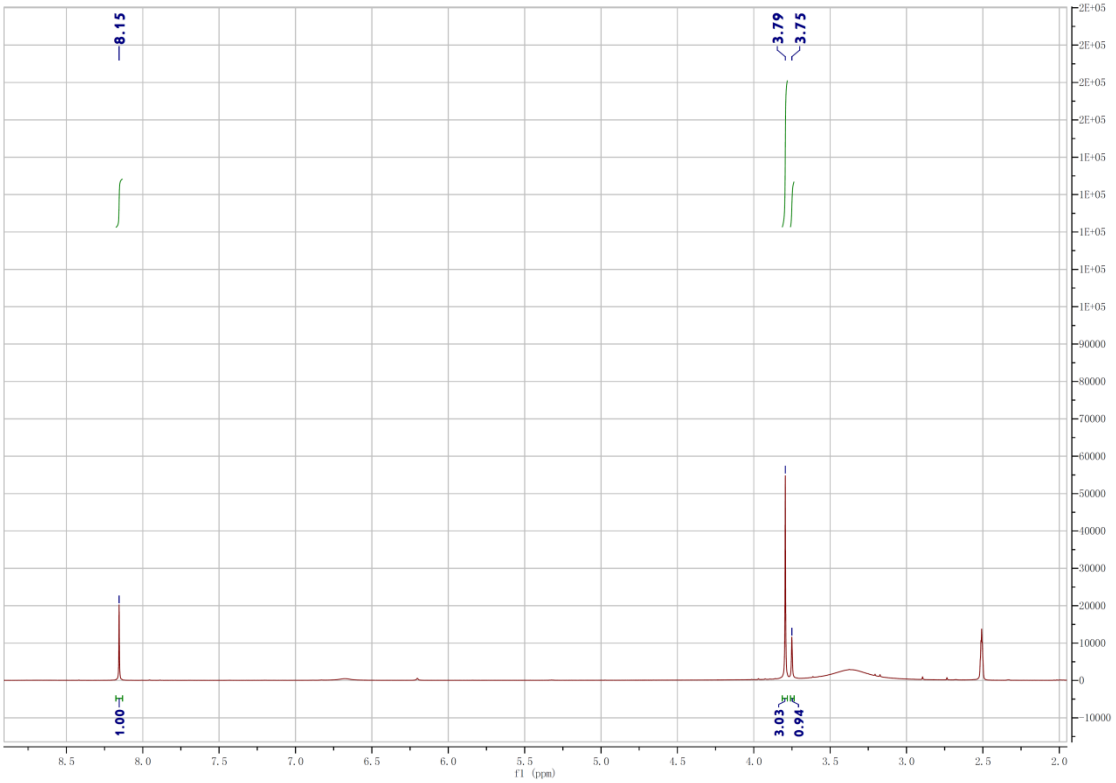

O
N
N
HS
N
N
H

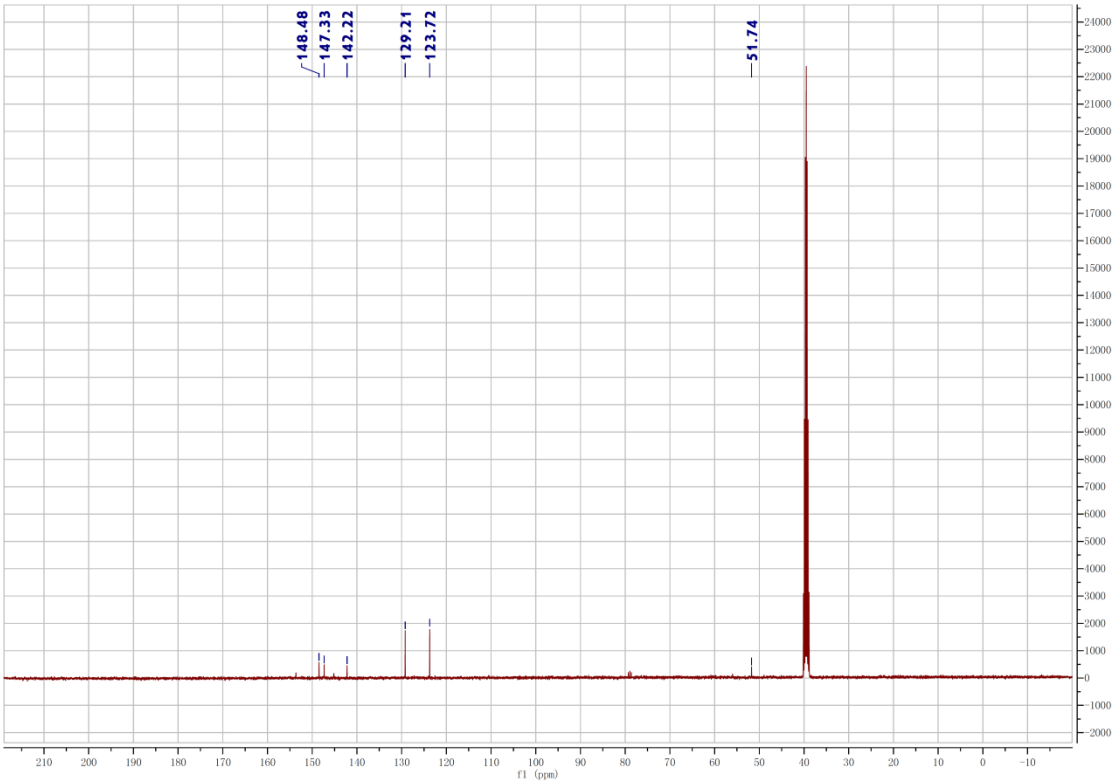
9

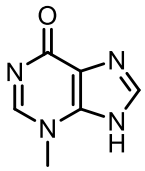
Preparation of 9 (3-Methyl-3H-purin-6(9H)-one)

Sodium metal (1.47 g, 64.0 mmol, 2 equiv) was dissolved in absolute EtOH (60.0 ml) to prepare 1.0 M sodium ethoxide in EtOH. (Z)-ethyl 2-cyano-2-(hydroxyimino)acetate (4.55 g, 32.0 mmol) was added to the solution at room temperature and stirred until all material dissolved. A solution of N'-methylcar-bamimidothioic acid (2.88 g, 32.0 mmol) in 42 ml of 2-methoxyethanol was combined with the reaction mixture, and the resulting mix was refluxed for 5.5 h. After cooling to 0°C, 1.0 M HCl was added dropwise until a pH of 2 was obtained. A blue-gray solid formed, which was filtered, washed with a small portion of ice-cold H_2O, and dried in an oven overnight to yield 5.10 g (86%) of 6-amino-2-mercapto-1-methyl-5-nitrosopyrimidin-4(1H)-one.The compound was used without purification.
6-amino-2-mercapto-1-methyl-5-nitrosopyrimidin-4(1H)-one (5.10 g, 27.4 mmol) was dissolved in 100 ml of 1.0 M NaOH, and sodium dithionite (17.65 g, 101.4 mmol, 3.7 equiv) was added. After 1.5 h at room temperature, the solids were filtered off and dried in an oven to obtain 4.7 g of 5,6-diamino-2-mercapto-1-methylpyrimidin-4(1H)-one in quantitative yield. These steps were repeated to provide sufficient material for the last stage of the synthesis of 13.

5,6-Diamino-2-mercapto-1-methylpyrimidin-4(1H)-one (7.74 g, 44.95 mmol) was dissolved in DMF (10 ml) and trimethyl orthoformate (73.76 ml, 674.18 mmol, 15.0 equiv). The mixture was heated at reflux for 5 days. After full conversion of the starting material was achieved (LC-MS analysis) the reaction mixture was concentrated and the residual material was recrystallized from water to obtain 8.19 g (100%) of compound 13: ^{1}H NMR (400 MHz, DMSO-d6) δ 8.17 (s, 1H), 3.78 (s, 3H); 3.75(s, 1H); ^{13}C NMR (100 MHz, DMSO-*d*6) *δ* 148.5, 147.3.7, 142.2, 129.2, 123.7, 51.7; HRMS calculated for $C_6H_7N_4OS$ exact mass: 183.0335, found 183.0332; mp: 384°C


8.15
3.79
3.75
1.00
3.03
0.94
f1 (ppm)

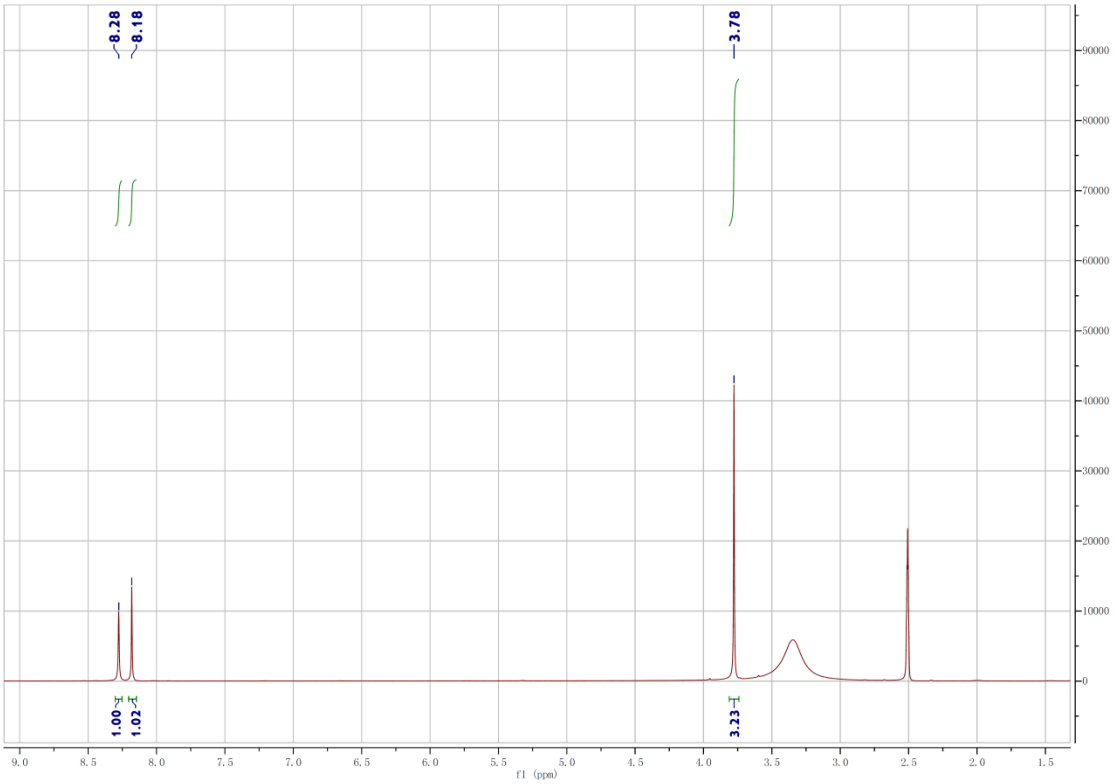

148.48
147.33
142.22
129.21
123.72
51.74
f1 (ppm)

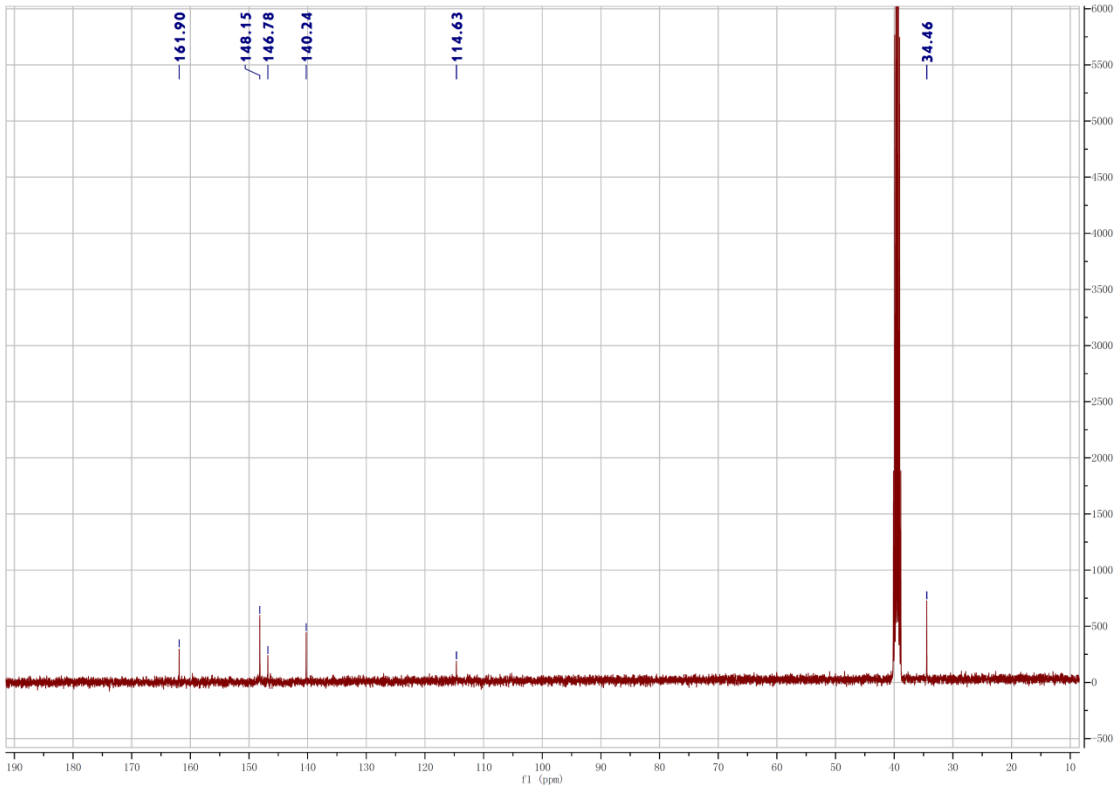


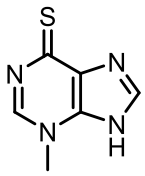
10

Preparation of 10 (3-Methyl-3H-purin-6(9H)-one)

Raney nickel (16.0 g) was washed three times with 2-methoxyethanol and then suspended in minimal H_2O. A solution of purinone 13 (8.20 g, 45.0 mmol) in 1.0 M NaOH (55.0 ml) was added to the Raney nickel slurry. The reaction mixture was heated to 80°C for 3 h until all starting material was consumed as determined by TLC. The room-temperature mixture was filtered through a pad of Celite, which was washed with H_2O and 2-methoxyethanol. The filtrate was concentrated in vacuo. A total of 6.76 g of 14 was obtained and was used without purification (yield: 82 %) : ^{1}H NMR (400 MHz, DMSO-d6) δ 8.28 (s, 1H), 8.18 (s, 1H), 3.78 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) *δ* 161.9, 148.2, 146.8, 140.2, 114.7, 34.5; HRMS calculated for $C_6H_7N_4O$ exact mass: 151.0614, found 151.0611; mp: 326^{o}C



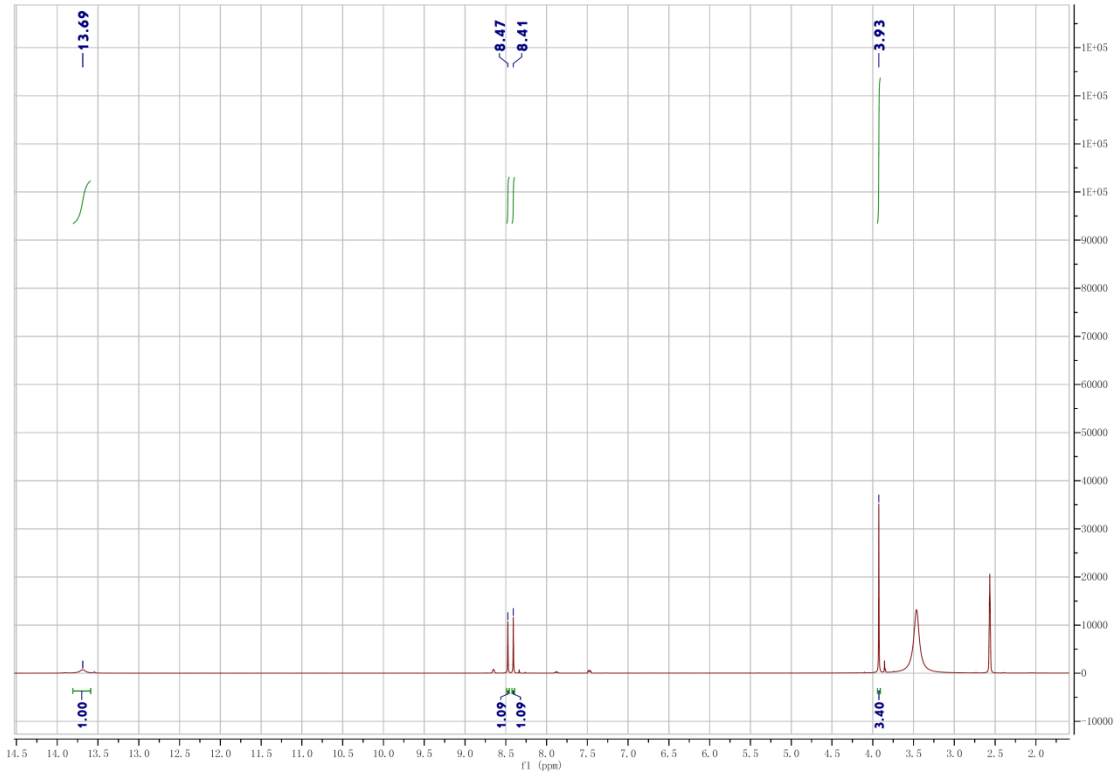




11

Preparation of 11 (3-Methyl-3H-purine-6(9H)-thione)

To purinone 14 (100 mg, 666 µmol) was added pyridine (5.45 ml) and phosphorus pentasulfide (454.5 mg, 2.04 mmol, 3.1 equiv), and the reaction was refluxed for 4 h and then concentrated in vacuo. To the resulting brown solid was added boiling water. A suspension formed, which was allowed to cool to room temperature. The solids were filtered off and dried in an oven overnight to deliver 56 mg (51%) of the corresponding 15: ^{1}HNMR (400 MHz, DMSO-d6) δ 13.69 (br s, 1H), 8.47 (s, 1H), 8.41 (s, 1H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) *δ* 183.6, 143.7, 143.3, 141.4, 128.5, 35.3; HRMS calculated for $C_6H_7N_4S$ exact mass: 167.0386, found 167.0382; mp: 286°C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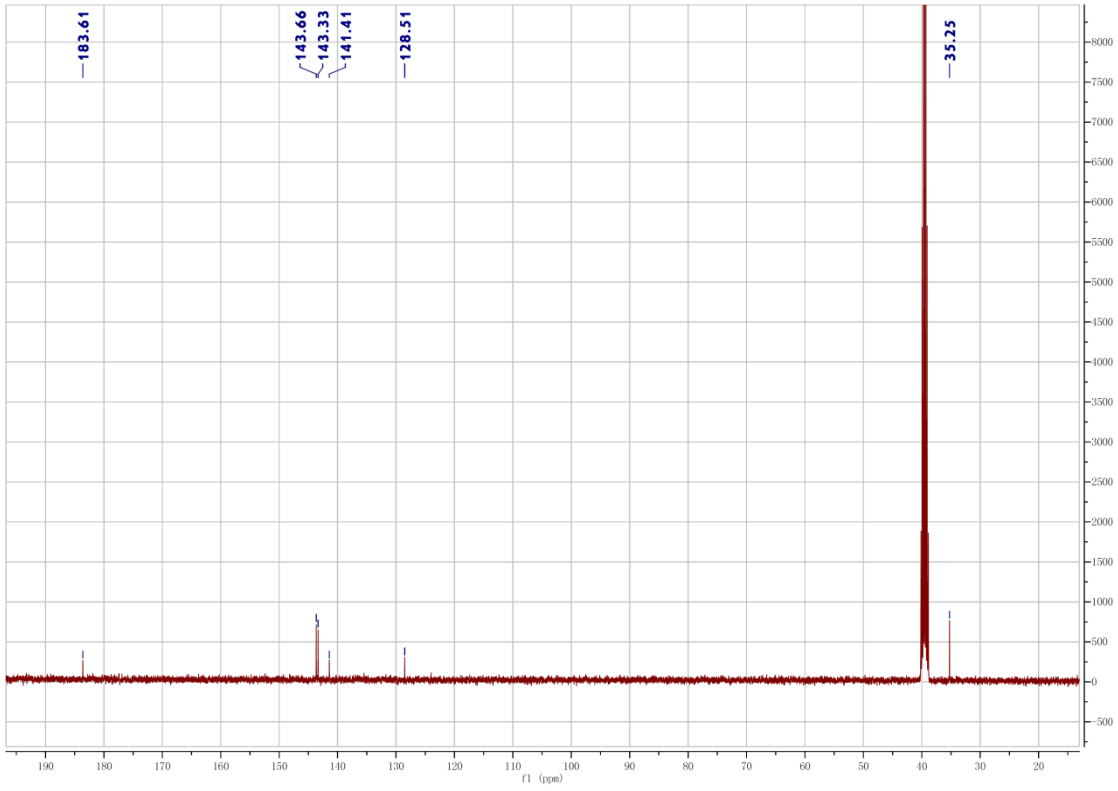
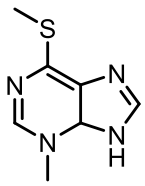
8.47
8.41
3.93
1.00
1.09
1.09
3.40
f1 (ppm)

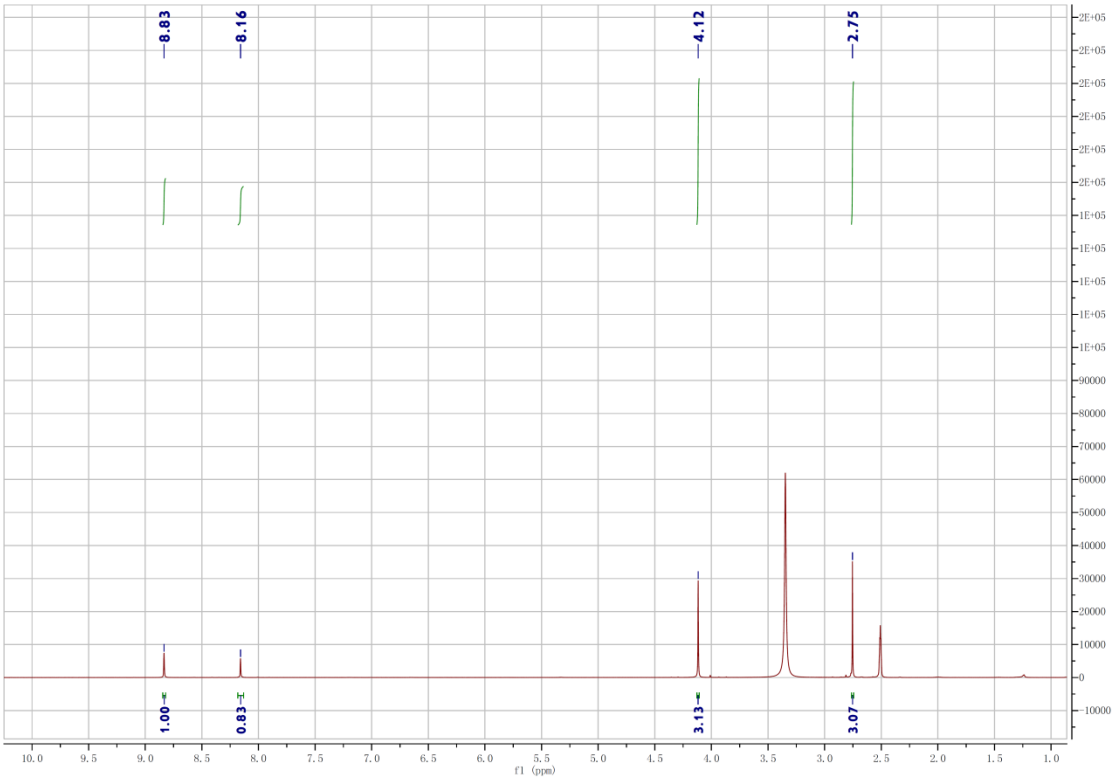




12

Preparation of 12 (3-methyl-6-(methylthio)-3H-purine)

3-Methyl-3H-purine-6(9H)-thione 15 (5.34 g, 32.13 mmol) was combined with 2.5% aqueous NaOH (66.74 ml, 1.3 equiv) and iodomethane (4.00 ml, 64.26 mmol, 2.0 equiv) at room temperature. The resulting mixture was stirred for 2 h at room temperature, during which time a white solid precipitated. The precipitate was filtered off and dried to deliver 3.56 g (61%) of the methylthio product 16: ^{1}H NMR (400 MHz,DMSO-d6) δ 8.83 (s, 1H), 8.16 (s, 1H), 4.12 (s, 3H), 2.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) *δ* 158.9, 157.7, 150.1, 142.25, 135.2, 36.9, 12.0; HRMS calculated for $C_7H_9N_4S$ exact mass: 181.0542, found 181.0540; mp: 124°C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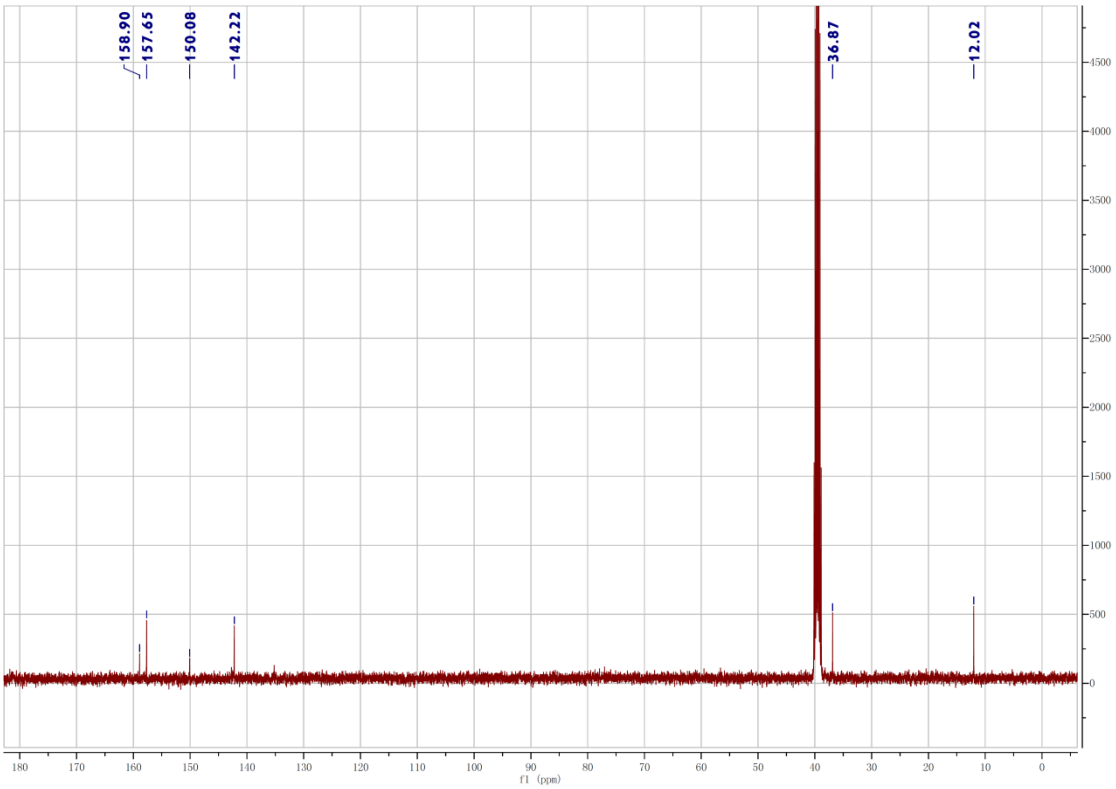
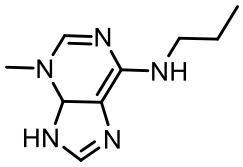
8.16
4.12
2.75
1.00
0.83
3.13
3.07
f1 (ppm)

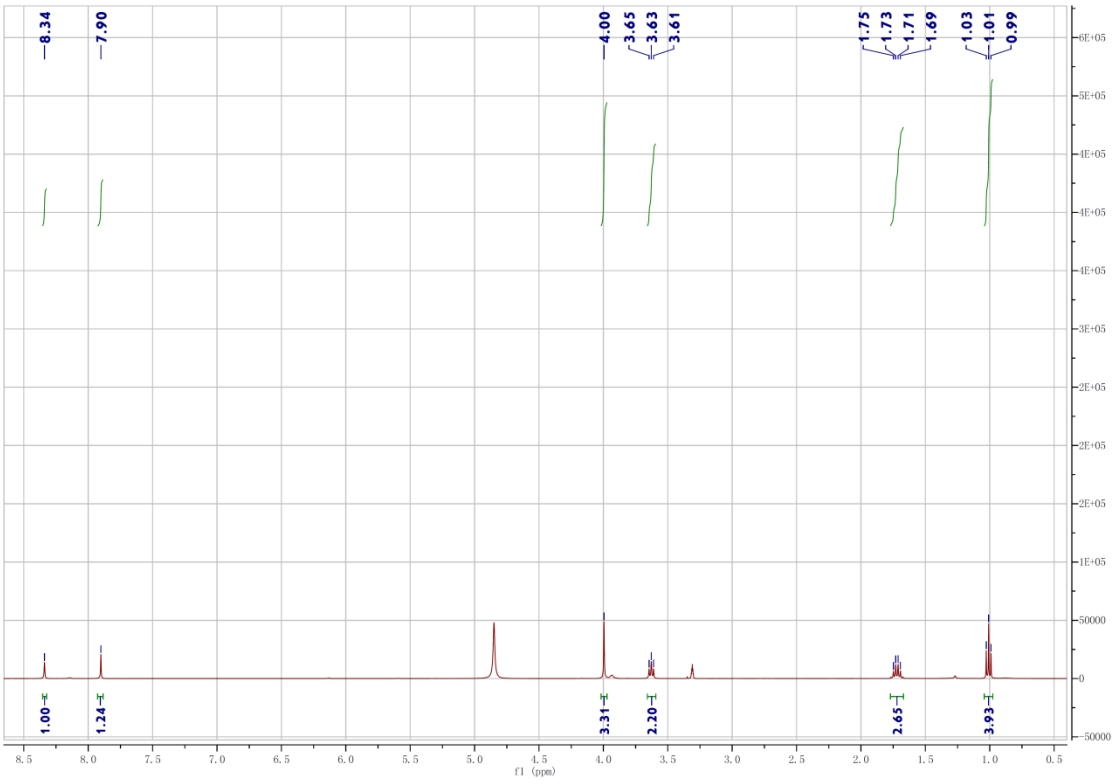

158.90
157.65
150.08
142.22
36.87
12.02
f1 (ppm)

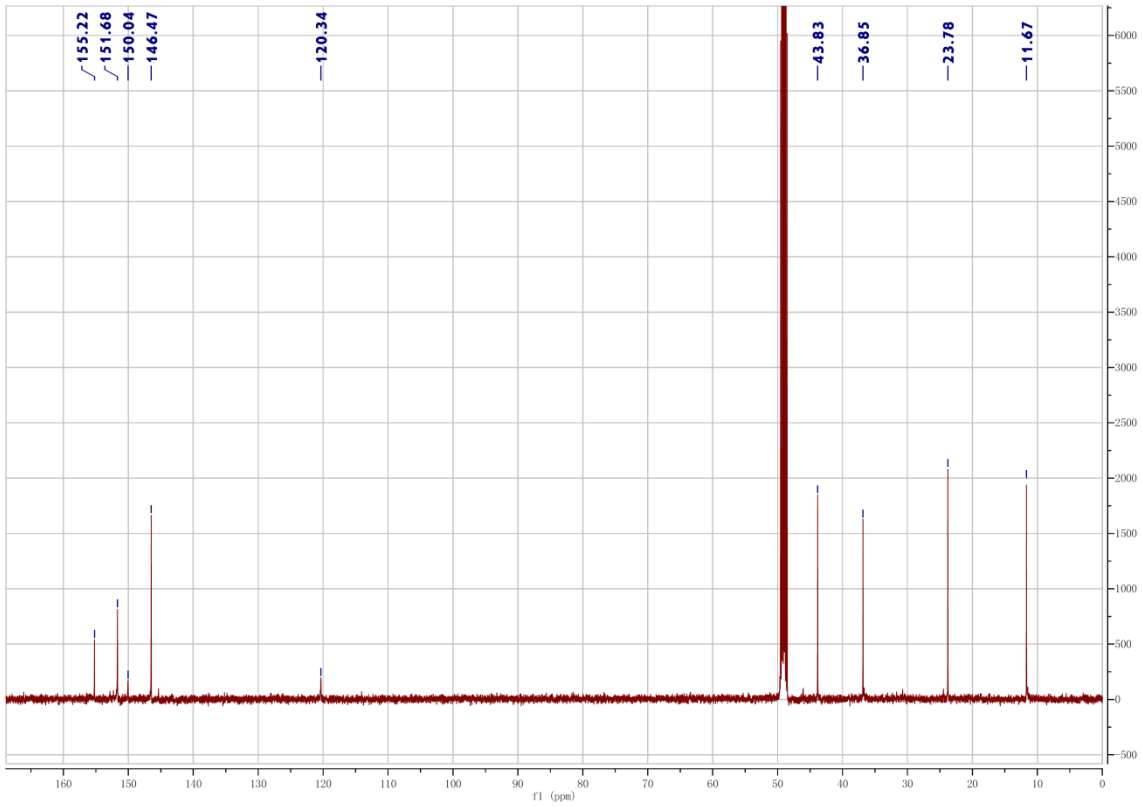


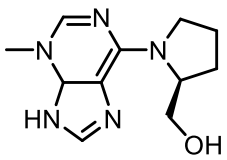
13

Preparation of 13 (3-methyl-N-propyl-3H-purin-6-amine)

A solution of 3-methyl-6-(methylthio)-3H-purine (180.0 mg, 1.0 mmol) in propylamine (3.0 ml) was stirred at 100°C for 24 h, the solvent was removed under reduced pressure. The residue was washed with CH_2Cl_2 and further purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent to generate the desired product (yield: 42%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.34(s, 1H), 7.90(s, 1H), 4.00 (s, 3H), 3.65-3.61 (t, 2H), 1.75-1.68 (m, 2H), 1.03-0.99 (t, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 155.2, 151.7, 150.1, 146.5, 120.3, 43.8, 36.9, 23.8, 11.7; HRMS calculated for $C_9H_{13}N_5$ exact mass: 192.1244, found 192.1238; mp: 147°C







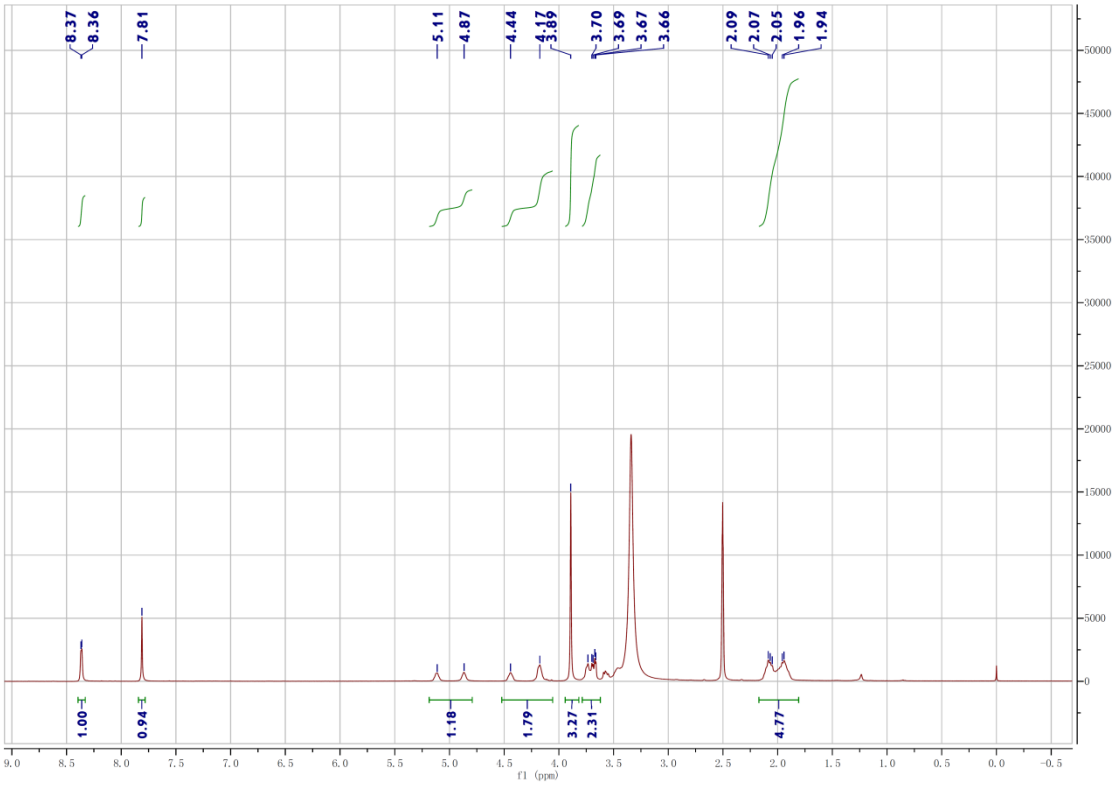
14

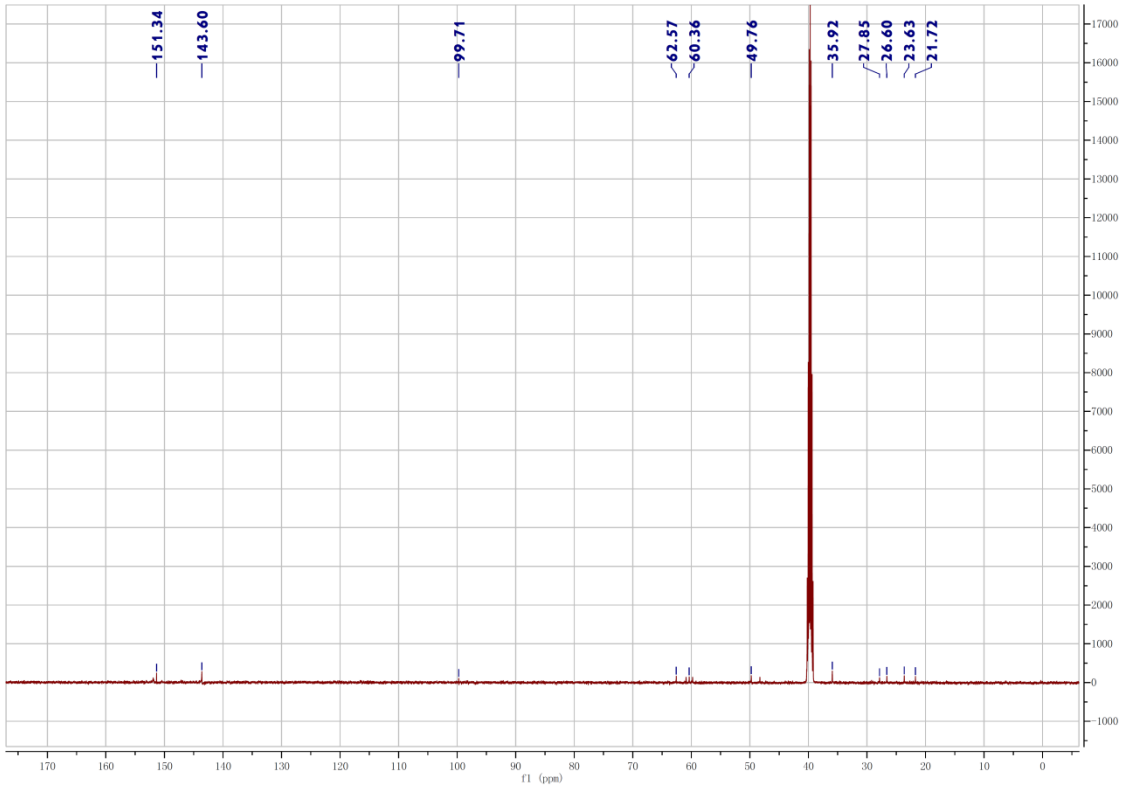
Preparation of 14

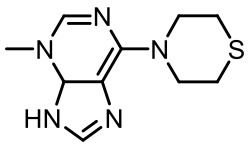
((S)-(1-(3-methyl-3H-purin-6-yl)pyrrolidin-2-yl)methanol)

To an oven-dried Schlenk tube containing a stir bar, L-(+)-prolinol (202.3 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The vessel was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed smoothly with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) the desired product was generated as a white solid (yield: 48%). ^{1}H NMR (400

MHz,DMSO-d6) δ 8.37-8.36 (d, 1H), 7.81 (s, 1H), 5.11-4.87 (d, 1H), 4.44-17 (d, 2H), 3.89 (s, 3H), 3.70-3.66 (t, 3H), 2.04-1.92 (d, 5H); ^{13}C NMR (100 MHz, DMSO-d6): δ 151.4, 143.6, 99.7, 62.6, 60.3, 49.8, 35.9, 27.8, 26.6, 23.6, 21.7; HRMS calculated for $C_{11}H_{16}N_5O$ exact mass: 234.1249, found 234.1250; mp: 187°C.





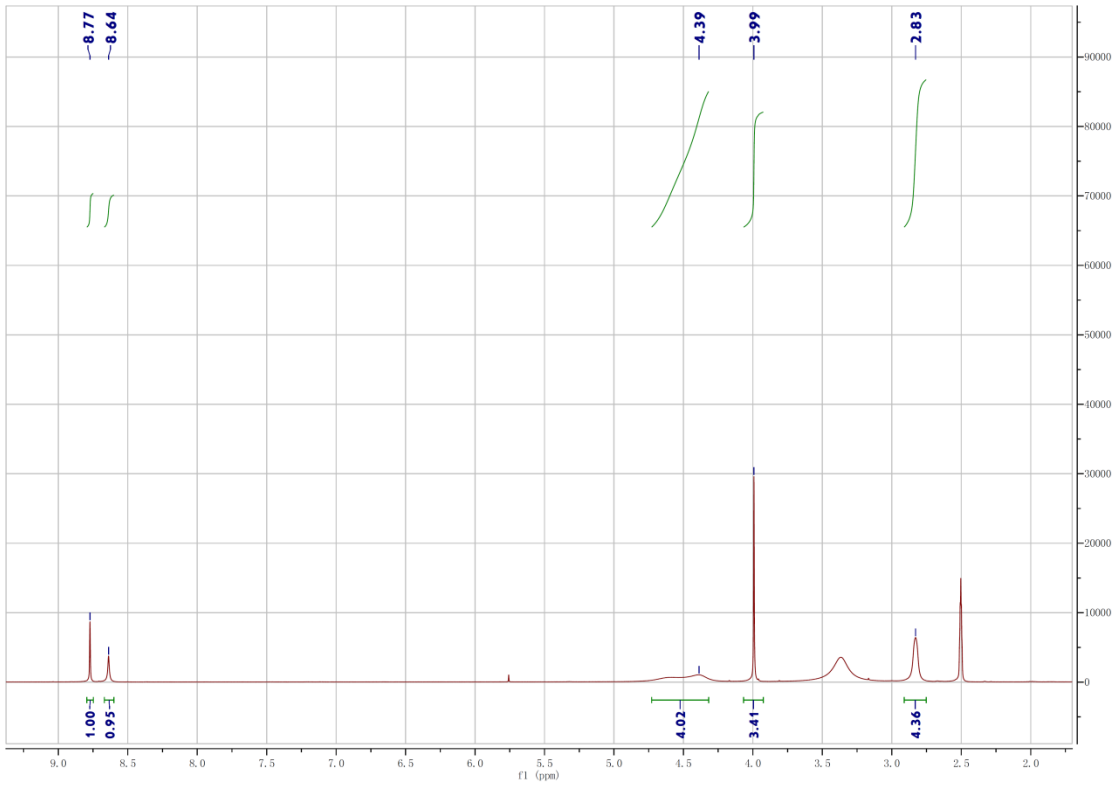


15

Preparation of 15 (4-(3-methyl-3H-purin-6-yl)thiomorpholine)

To an oven-dried Schlenk tube containing a stir bar, thiomorpholine (206.4 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The vessel was degassed for 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed smoothly with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) the desired product was generated as a white solid (yield: 62%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.77 (s, 1H), 8.64 (s, 1H), 4.39 (m, 4H), 3.99 (s, 3H), 2.83 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d6) δ 153.6, 152.4, 152.3, 144.8, 136.3, 125.5, 121.2, 36.8, 28.5, 17.1; HRMS calculated for $C_{10}H_{14}N_5S$ exact mass: 236.0964, found 236.0963;

mp: 267°C.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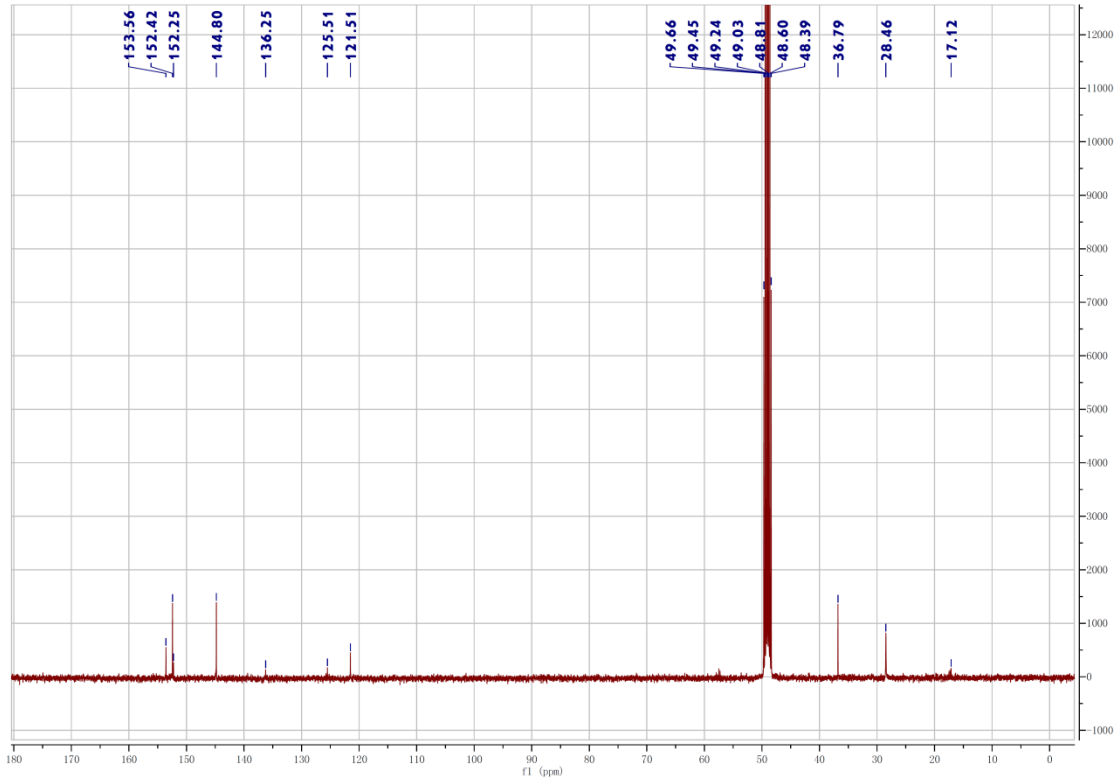
3.99
2.83
1.00
0.95
4.02
3.41
4.36
f1 (ppm)


153.56
152.42
152.25
144.80
136.25
125.51
121.51
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49.24
49.03
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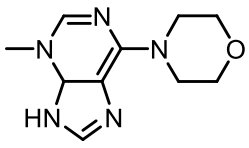
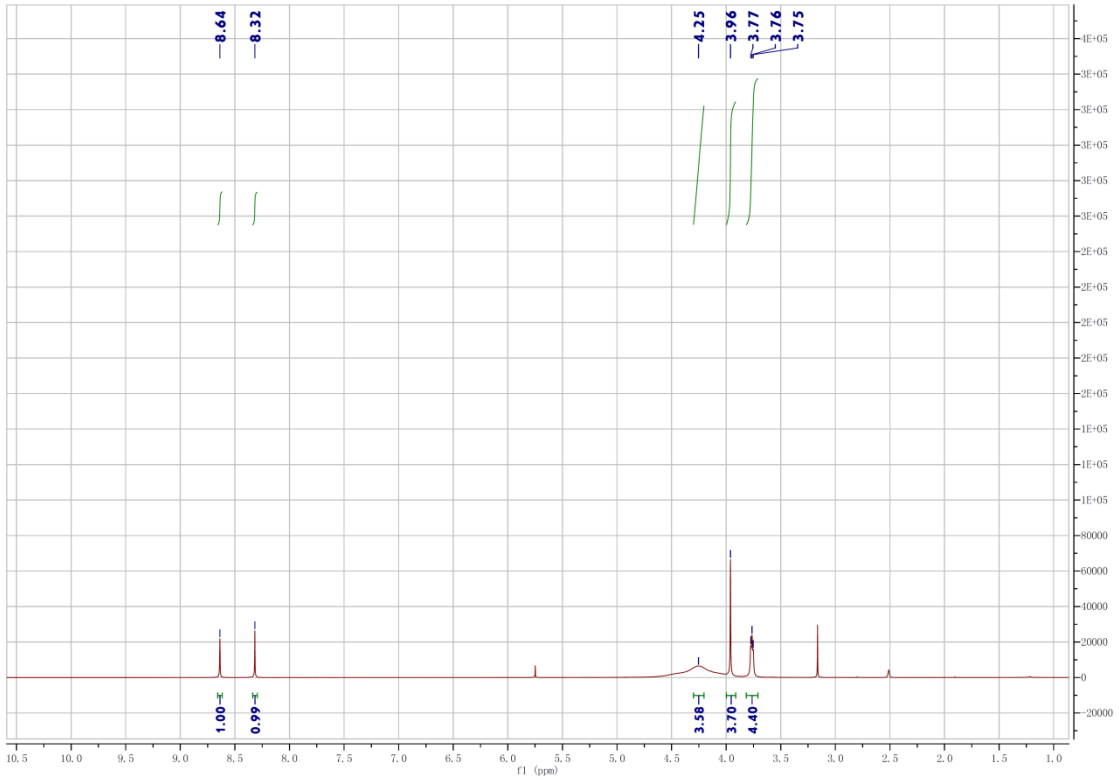
48.60
48.39
36.79
28.46
17.12
f1 (ppm)



16

Preparation of 16 (4-(3-methyl-3H-purin-6-yl)thiomorpholine)

To an oven-dried Schlenk tube containing a stir bar, morpholine (174.3 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The vessel was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed smoothly with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) the desired product was generated as a white solid (yield:54%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.64 (s, 1H), 8.32 (s, 1H), 4.25 (m, 4H), 3.96 (s, 3H), 3.77-3.75 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.5, 147.5, 146.0, 145.4, 116.6, 66.0, 54.9, 44.8, 36.6; HRMS calculated for $C_{10}H_{13}N_5O$ exact mass: 220.1193, found 220.1192; mp: 255°C.


8.64
8.32
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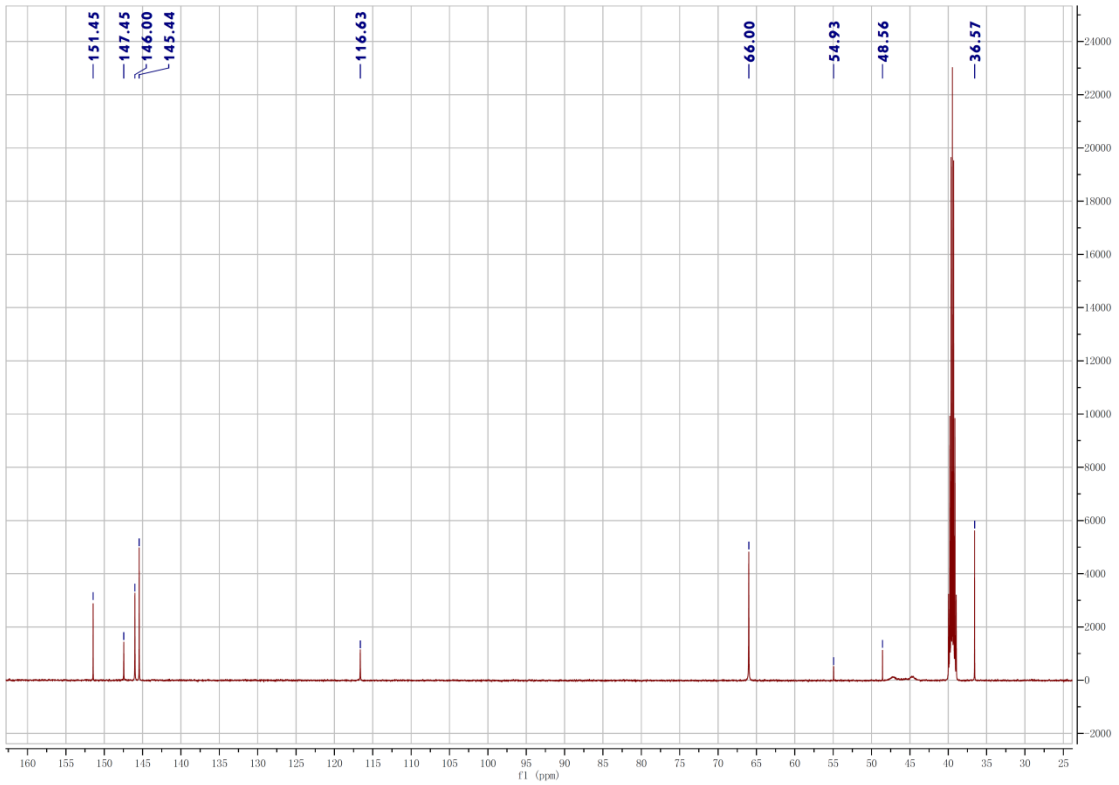
3.96
3.77
3.76
3.75
1.00
0.99
3.58
3.70
4.40
f1 (ppm)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48.56
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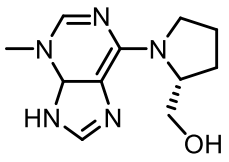
f1 (ppm)

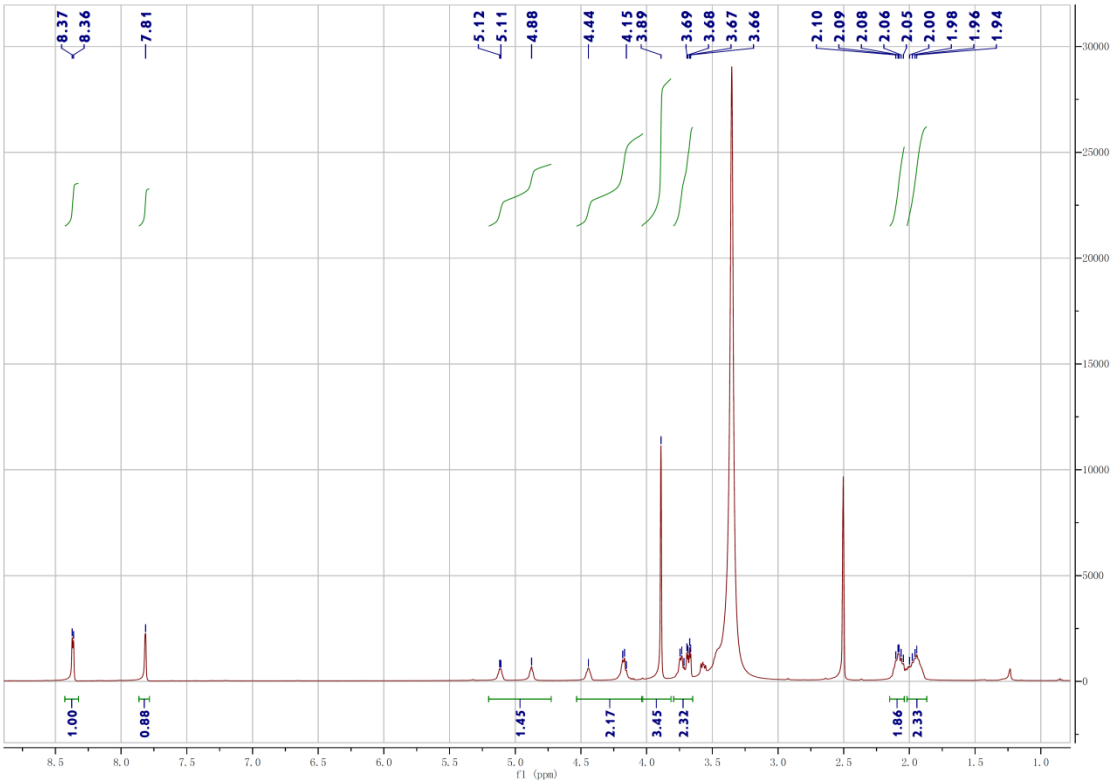


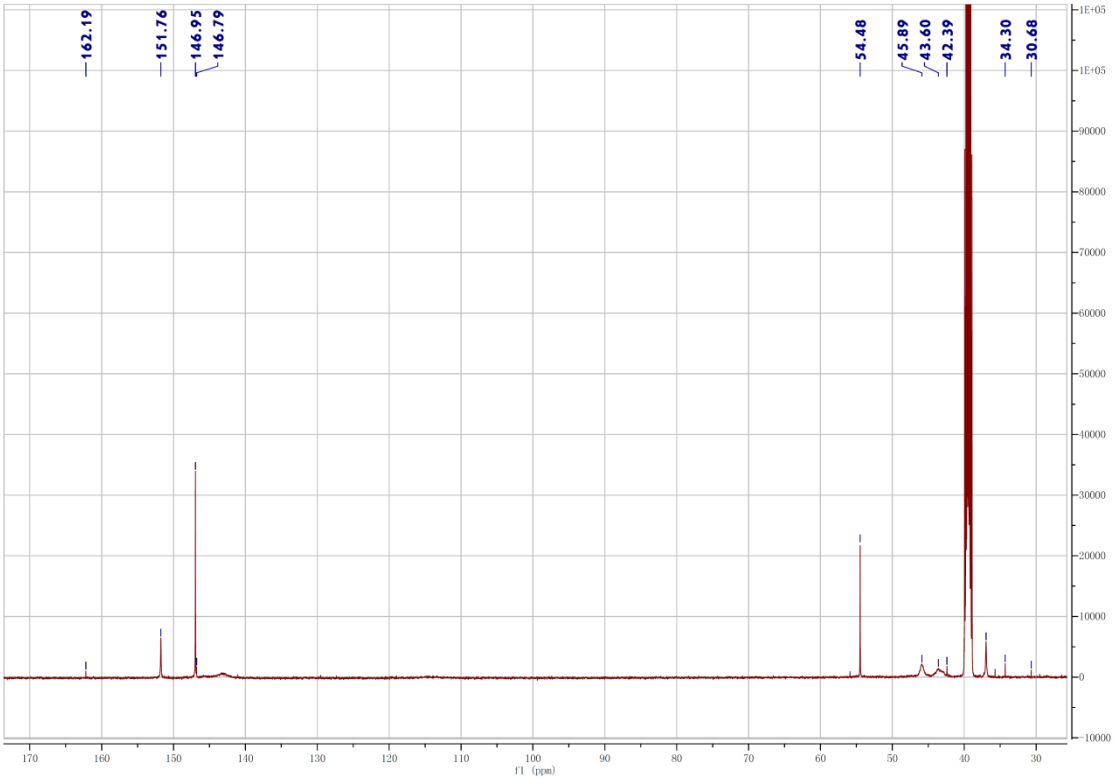
17

Preparation of 17

((R)-(1-(3-methyl-3H-purin-6-yl)pyrrolidin-2-yl)methanol)

To an oven-dried Schlenk tube containing a stir bar, D-(-)-prolinol (202.3 mg, 2.0 mmol), and 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The vessel was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed smoothly with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) the desired product was generated as a white solid (yield: 47%) ^{1}H NMR (400 MHz, DMSO-d6): δ 8.37-8.36 (d, 1H), 7.81 (s, 1H), 5,12-5.11 (d, 1H), 4.88 (s, 1H), 4.44 (s, 1H), 4.15 (s, 1H), 3.89 (s, 3H), 3.73-3.66 (m, 2H), 2.21-1.94 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d6): δ 151.1, 143.4, 99.5, 62.4, 49.5, 48.1, 35.7, 27.6, 26.4, 23.4, 21.5; HRMS calculated for $C_{11}H_{16}N_5O$ exact mass: 234.1349, found 234.1351; mp: 181°C.


8.37
8.36
7.81
5.12
5.11
4.88
4.44
4.15
3.89
3.69
3.68
3.67
3.66
2.10
2.09
2.08
2.06
2.05
2.00
1.98
1.96
1.94
1.00
0.88
1.45
2.17
3.45
2.32
1.86
2.33
f1 (ppm)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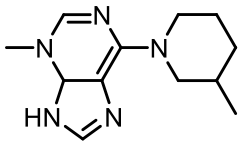
45.89
43.60
42.39
34.30
30.68
f1 (ppm)

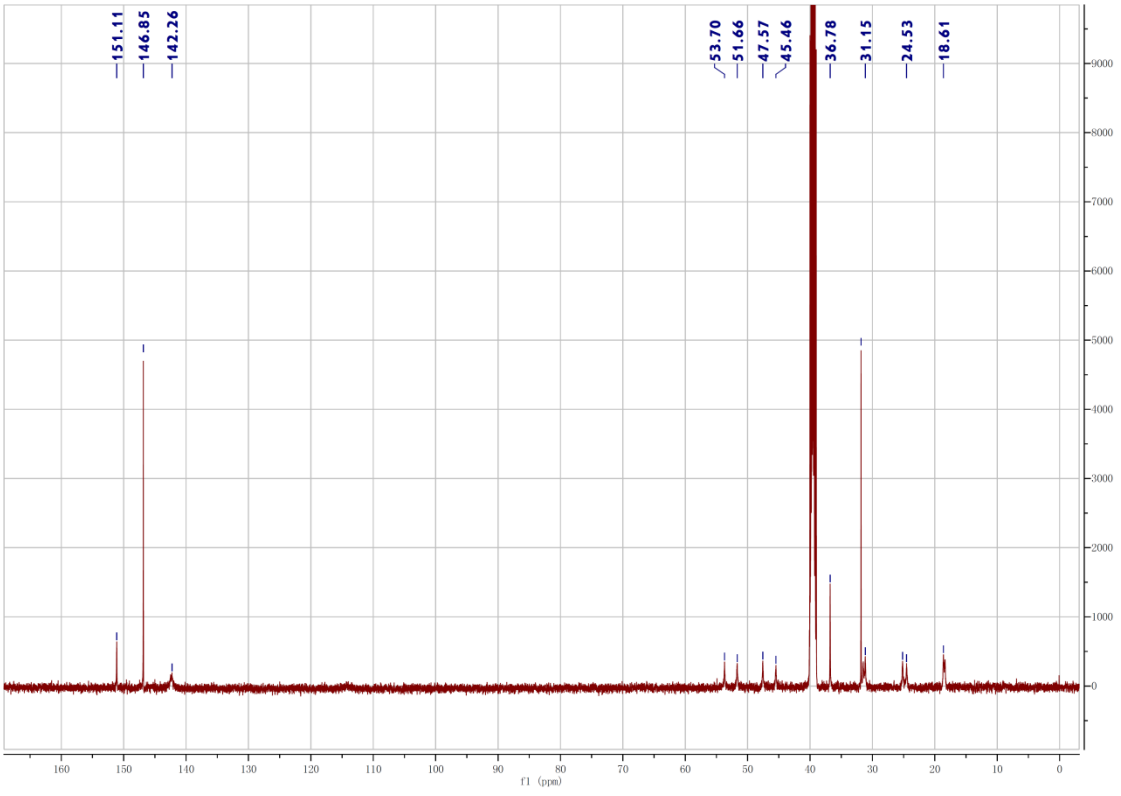


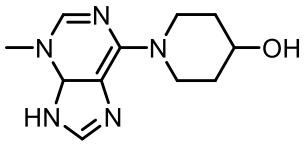
18

Preparation of 18 (3-methyl-6-(3-methylpiperidin-1-yl)-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 3-methylpiperidine (198.4 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol), and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 61%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.70 (s, 1H), 8.60 (s, 1H), 4.81 (s, 2H), 3.96 (s, 3H), 3.57 (s, 1H), 2.97 (s, 1H), 1.84 (m, 2H), 1.35-1.23 (m, 3H), 0.97-0.96 (d, 3H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.1, 146.8, 142.2, 53.7, 51.6, 47.6, 45.4, 36.8, 31.2, 24.5, 18.6; HRMS calculated for $C_{12}H_{17}N_5$ exact mass: 232.1556, found 232.1555; mp: 194°C.


8.70
8.60
4.81
3.96
3.57
2.97
2.51
2.50
2.50
1.84
1.35
1.33
1.30
1.23
0.97
0.96
1.00
0.91
2.04
3.19
1.19
0.97
3.19
2.11
1.11
1.12
1.12
3.12
f1 (ppm)

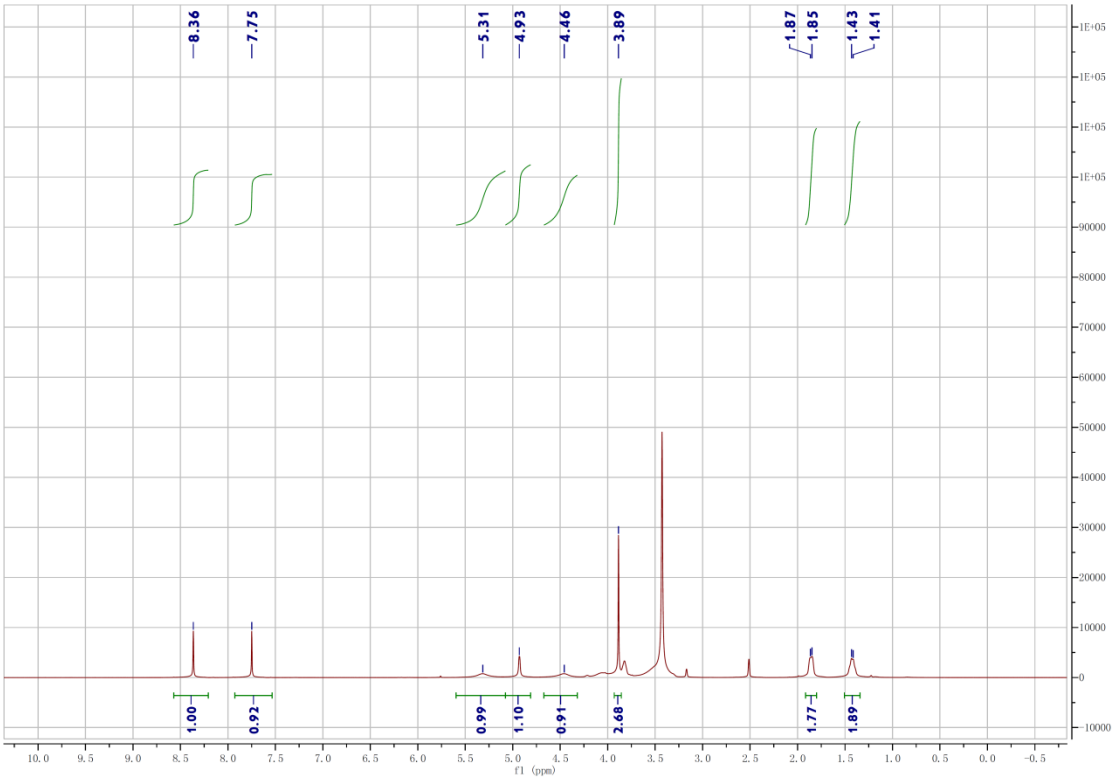

151.11
146.85
142.26
53.70
51.66
47.57
45.46
36.78
31.15
24.53
18.61
f1 (ppm)

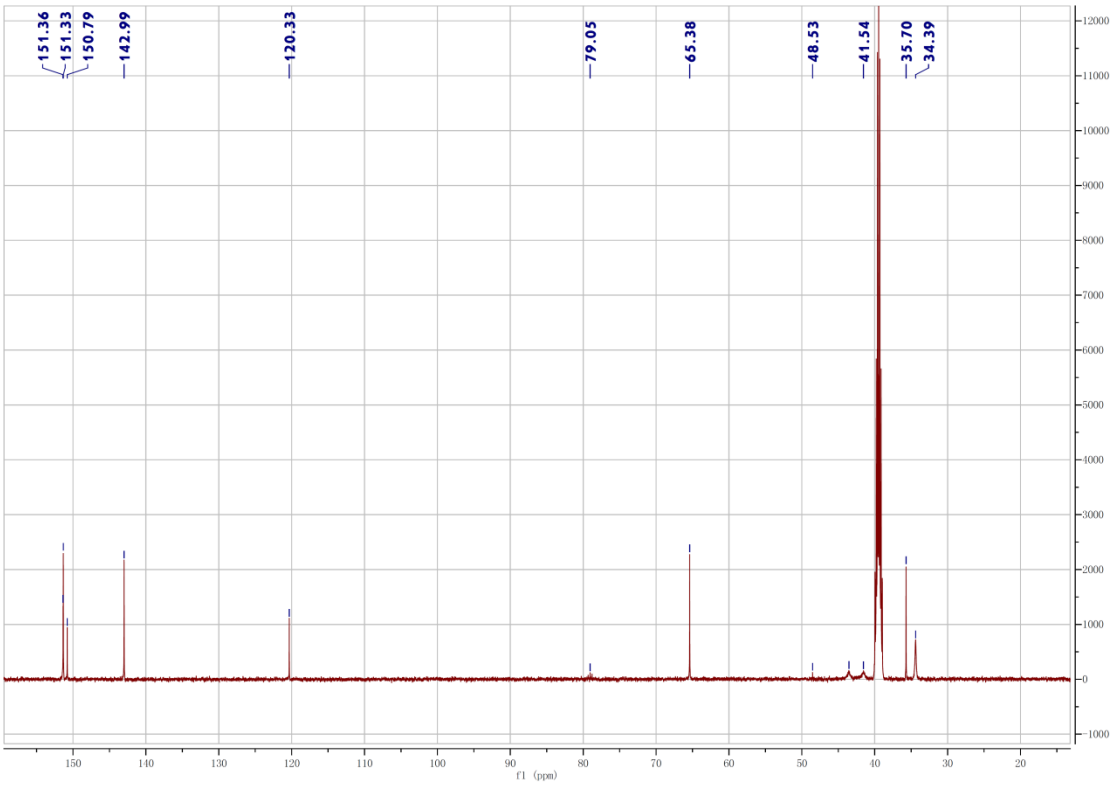


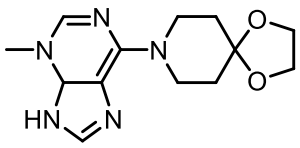
19

Preparation of 19 (1-(3-methyl-3H-purin-6-yl)piperidin-4-ol)

To an oven-dried Schlenk tube containing a stir bar, 4-hydroxypiperidine (202.3 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol), and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 62%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.36 (s, 1H), 7.75 (s, 1H), 5.31 (s, 1H), 4.93 (s, 2H), 4.46 (s, 1H), 3.89 (s, 3H), 1.87-1.85 (d, 2H), 1.43-1.41 (d, 2H); ^{13}C NMR (100 MHz, DMSO-d6): δ 151.4, 151.3, 150.8, 143.0, 120.3, 79.1, 65.4, 48.3, 41.6, 35.7, 34.4; HRMS calculated for $C_{11}H_{16}N_5O$ exact mass: 234.1349, found 234.1348; mp: 240°C.


8.36
7.75
5.31
4.93
4.46
3.89
1.87
1.85
1.43
1.41
1.00
0.92
0.99
1.10
0.91
2.68
1.77
1.89
f1 (ppm)


151.36
151.33
150.79
142.99
120.33
79.05
65.38
48.53
41.54
35.70
34.39
f1 (ppm)

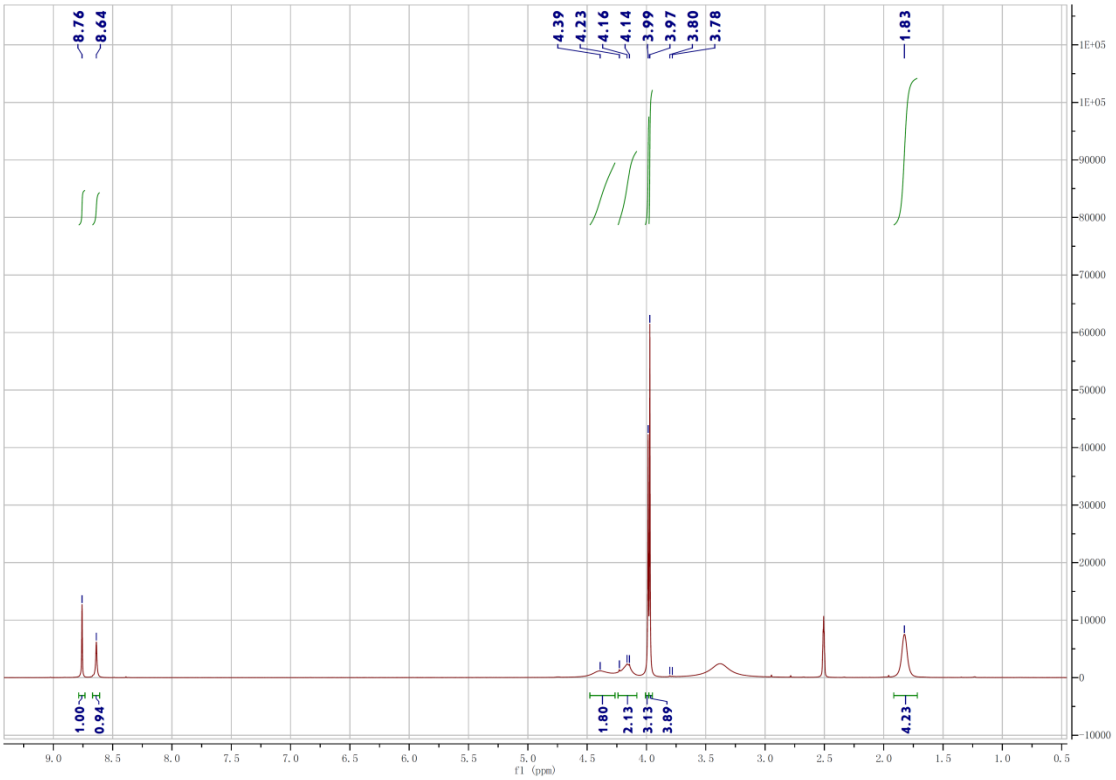


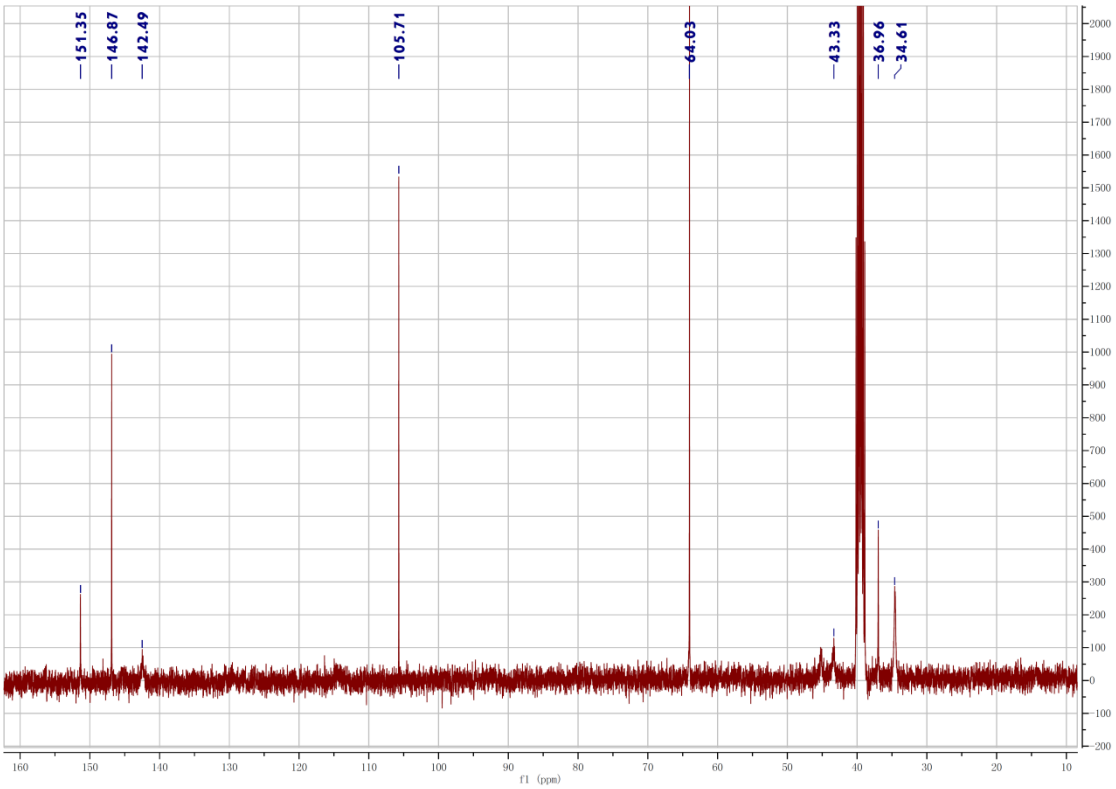
20

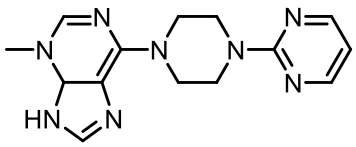
Preparation of 20

(8-(3-methyl-3H-purin-6-yl)-1,4-dioxa-8-azaspiro[4.5]decane)

To an oven-dried Schlenk tube containing a stir bar, 1,4-Dioxa-8-azaspiro[4.5]decane (287.6 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 68%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.76 (s, 1H), 8.64 (s, 1H), 4.39-4.23 (m, 2H), 4.16-4.14 (m, 2H), 3.99 (s, 3H), 3.97-3.78 (m, 4 H), 1.83 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.4, 148.4, 146.9, 142.5, 116.3, 114.9, 105.7, 64.0, 45.2, 43.3, 37.0, 34.6; HRMS calculated for $C_{13}H_{18}N_5O_2$ exact mass: 276.1452, found 276.1455; mp: 266°C.


8.76
8.64
4.39
4.23
4.16
4.14
3.99
3.97
3.80
3.78
1.83
1.00
0.94
1.80
2.13
3.13
3.89
4.23
f1 (ppm)


151.35
146.87
142.49
105.71
64.03
43.33
36.96
34.61
f1 (ppm)

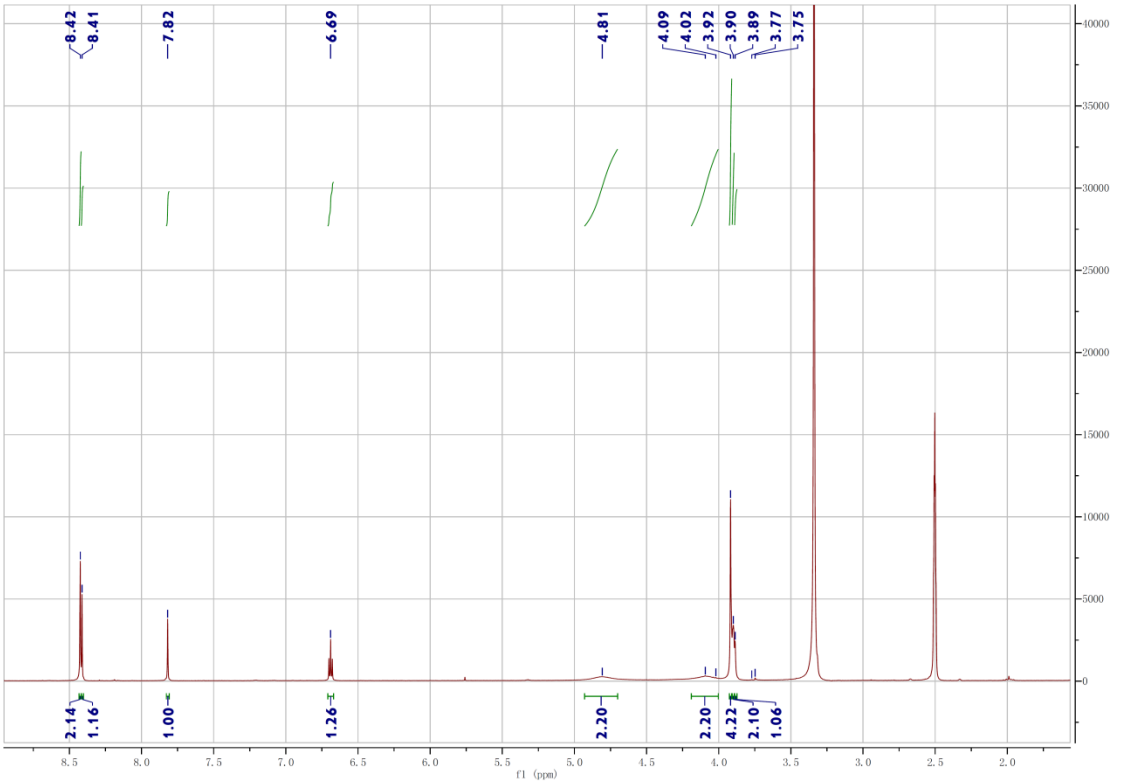


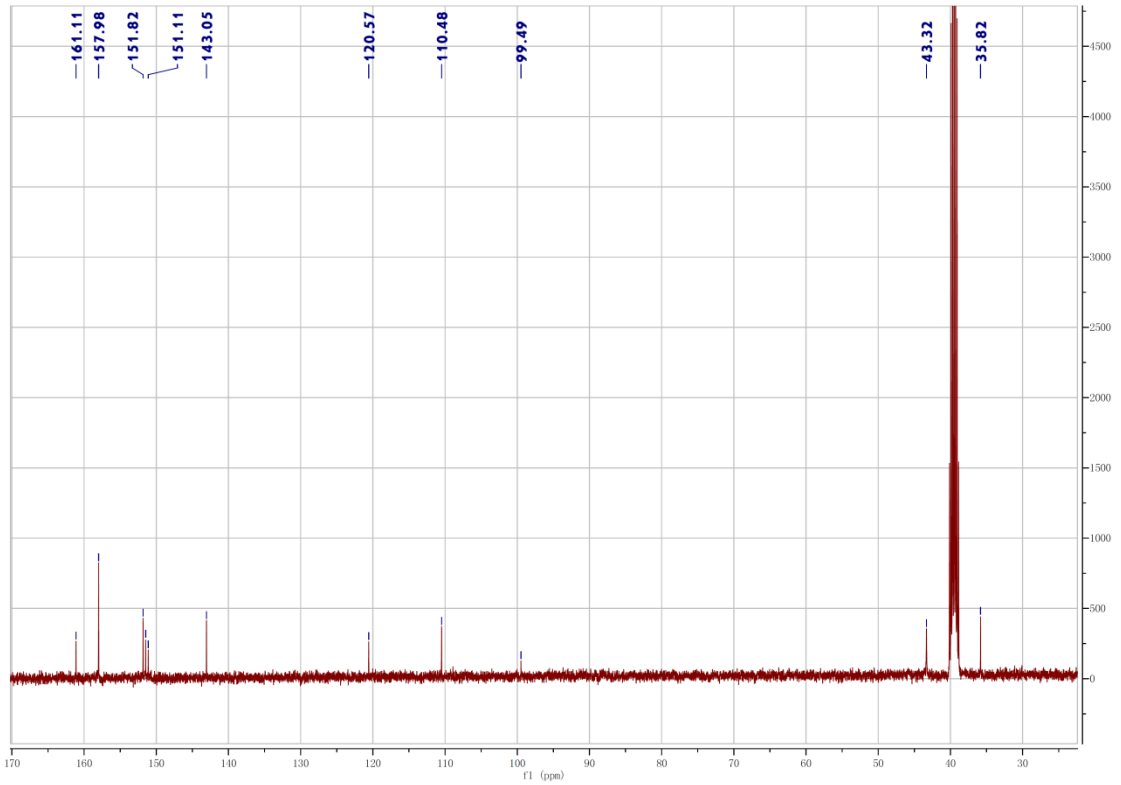
21

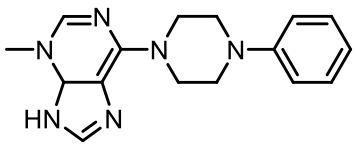
Preparation of 21

(3-methyl-6-(4-(pyrimidin-2-yl)piperazin-1-yl)-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 2-(1-Piperazinyl)pyrimidine (328.4 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 55%). 1H NMR (400 MHz, DMSO-d6): δ 8.42 (m, 2H), 8.41 (s, 1H), 7.82 (s, 1H), 6.69 (s, 1H), 4.81(m, 2H), 4.09 (m, 2H), 3.92 (s, 3H), 3.90 (m, 2H), 3.89 (s, 1H), 1.22 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d6) δ 161.1, 158.0, 151.8, 151.5, 151.1, 143.1, 120.6, 110.5, 99.5, 43.3, 35.8; HRMS calculated for $C_{14}H_{17}N_8$ exact mass: 297.1563, found 297.1570; mp: 223°C.


8.42
8.41
7.82
6.69
4.81
4.09
4.02
3.92
3.90
3.89
3.77
3.75
2.14
1.16
1.00
1.26
2.20
2.20
4.22
2.10
1.06
f1 (ppm)

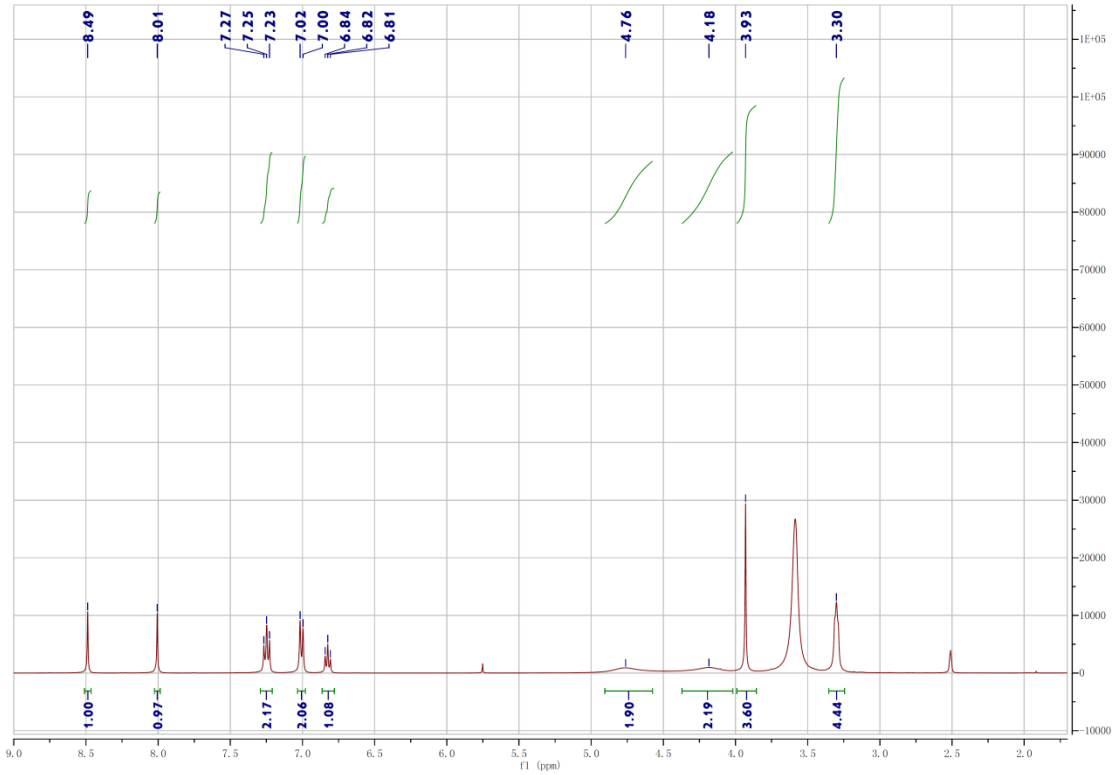

161.11
157.98
151.82
151.11
143.05
120.57
110.48
99.49
43.32
35.82
f1 (ppm)

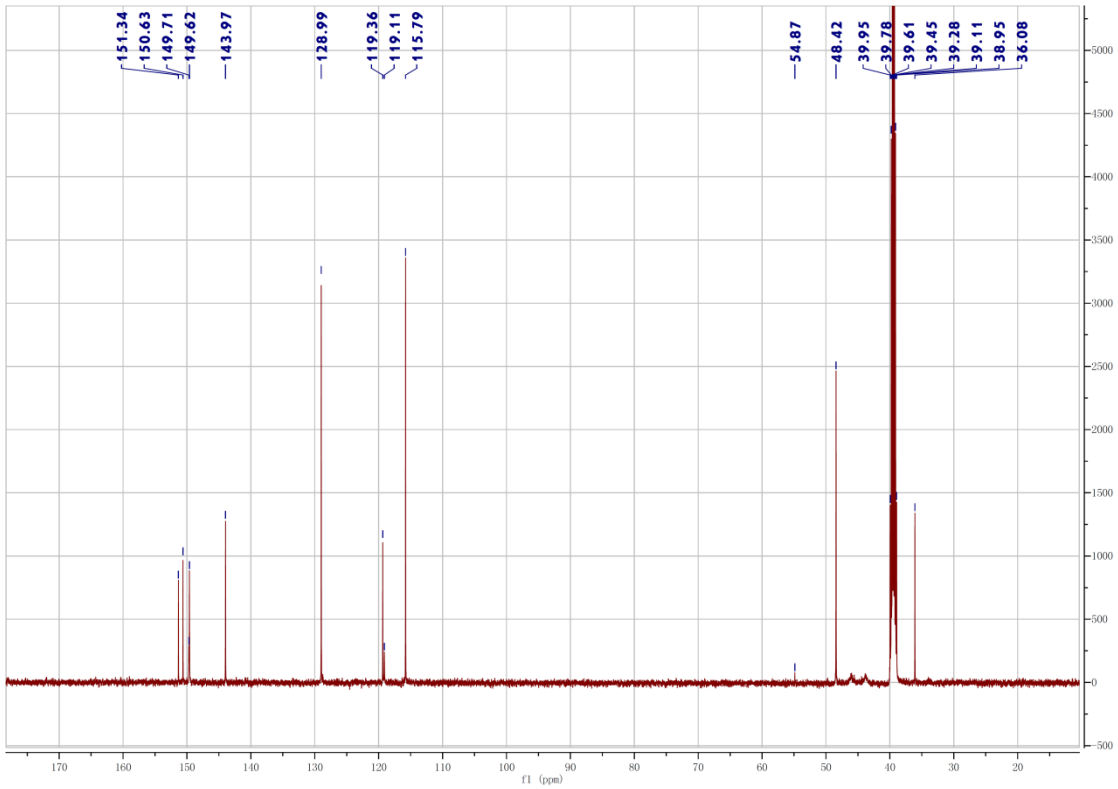


22

Preparation of 22 (3-methyl-6-(4-phenylpiperazin-1-yl)-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 1-phenylpiperazine (324.2 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 58%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.49 (s, 1H), 8.01 (s, 1H), 7.27-7.23 (m, 2H), 7.02-7.00 (m, 2H), 6.84-6.81 (m, 1H), 4.76 (m, 2H), 4.18 (m, 2H), 3.93 (s, 3H), 3.30 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.3, 150.6, 149.7, 149.6, 144.0, 129.0, 119.4, 119.1, 115.8, 54.9, 48.4, 36.0; HRMS calculated for $C_{16}H_{18}N_6$ exact mass: 295.1666, found 295.1665; mp:164°C.


8.49
8.01
7.27
7.25
7.23
7.02
7.00
6.84
6.82
6.81
4.76
4.18
3.93
3.30
1.00
0.97
2.17
2.06
1.08
1.90
2.19
3.60
4.44
f1 (ppm)


151.34
150.63
149.71
149.62
143.97
128.99
119.36
119.11
115.79
54.87
48.42
39.95
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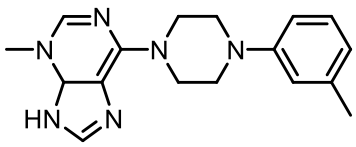
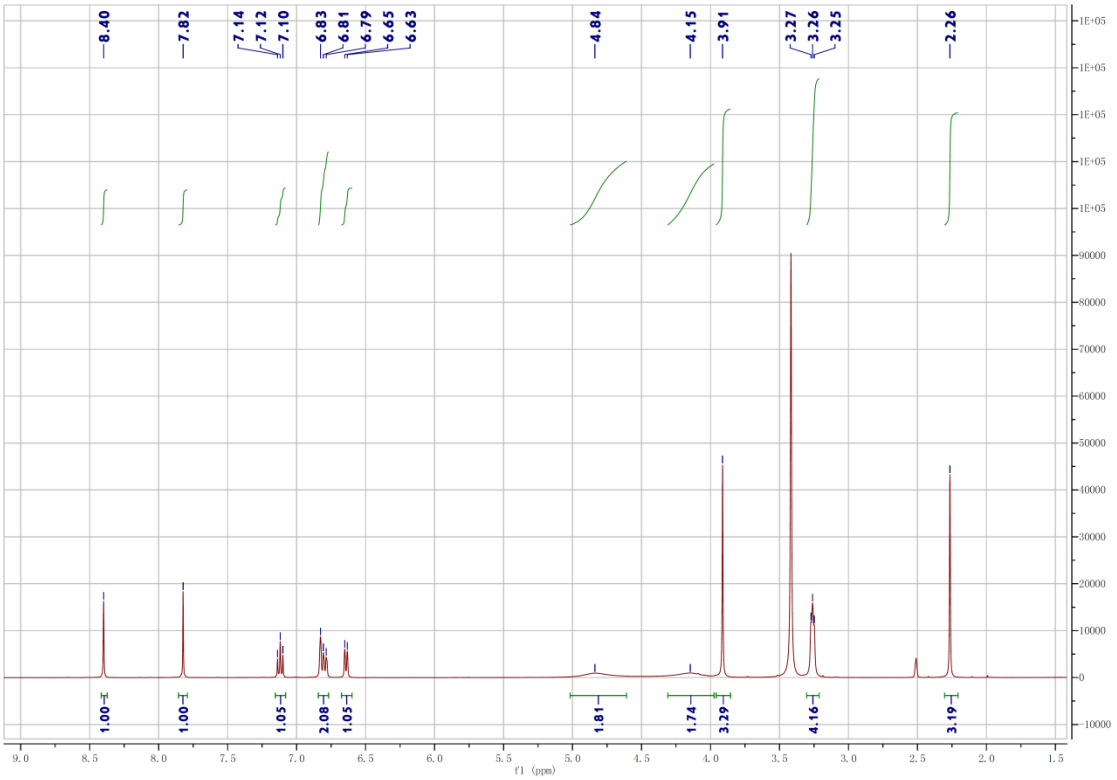
39.28
39.11
38.95
36.08
f1 (ppm)



23

Preparation of 23 (3-methyl-6-(4-(m-tolyl)piperazin-1-yl)-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 1-(m-tolyl)piperazine (352.5 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 56%). 1H NMR (400 MHz, DMSO-d6): δ 8.40 (m, 1H), 7.82 (s, 1H), 7.14-7.10 (m, 1H), 6.83-6.79 (s, 2H), 6.65-6.63(m, 1H), 4.84 (m, 2H), 4.15 (m, 2H), 3.91 (s, 3H), 3.27-3.25 (m, 4H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.7, 151.3, 151.0, 150.8, 143.1, 138.1, 128.8, 120.5, 120.2, 116.6, 113.1, 48.7, 35.8, 21.4; HRMS calculated for $C_{17}H_{20}N_6$ exact mass: 309.1822, found 309.1823; mp: 207°C.


8.40
7.82
7.14
7.12
7.10
6.83
6.81
6.79
6.65
6.63
4.84
4.15
3.91
3.27
3.26
3.25
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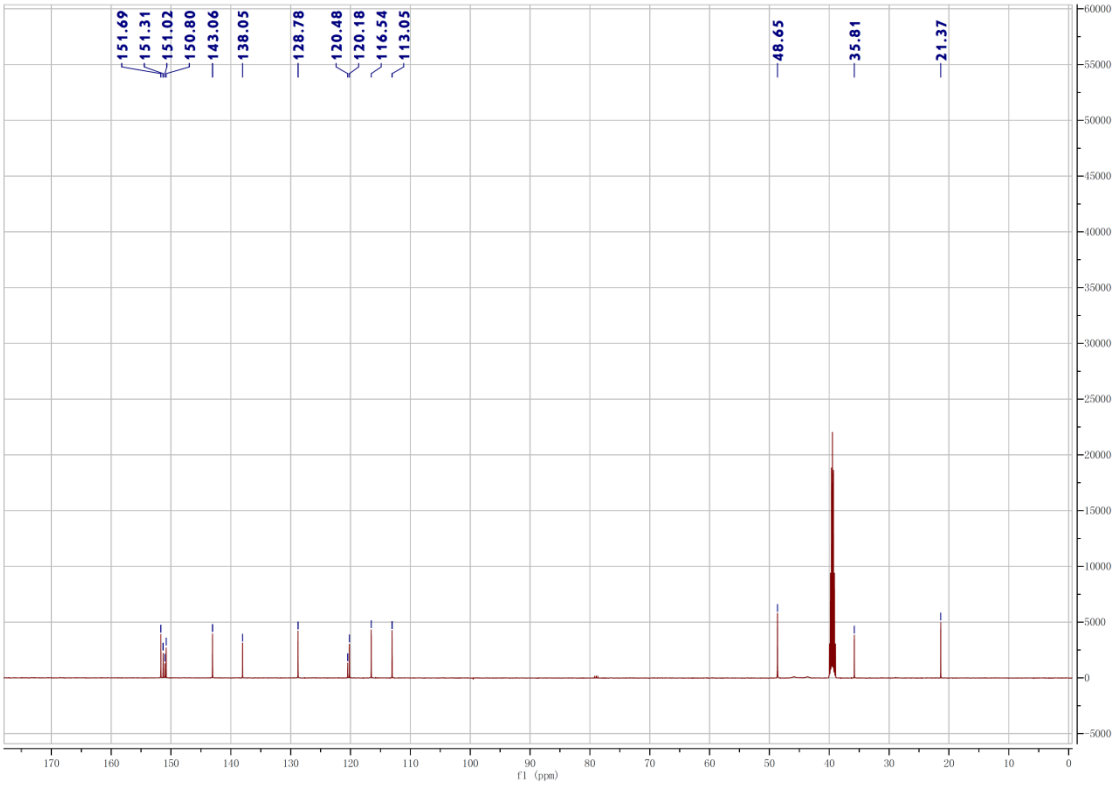
1.81
1.74
3.29
4.16
3.19
f1 (ppm)


151.69
151.31
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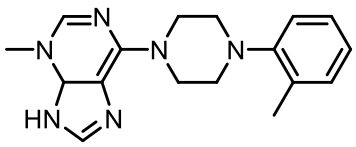
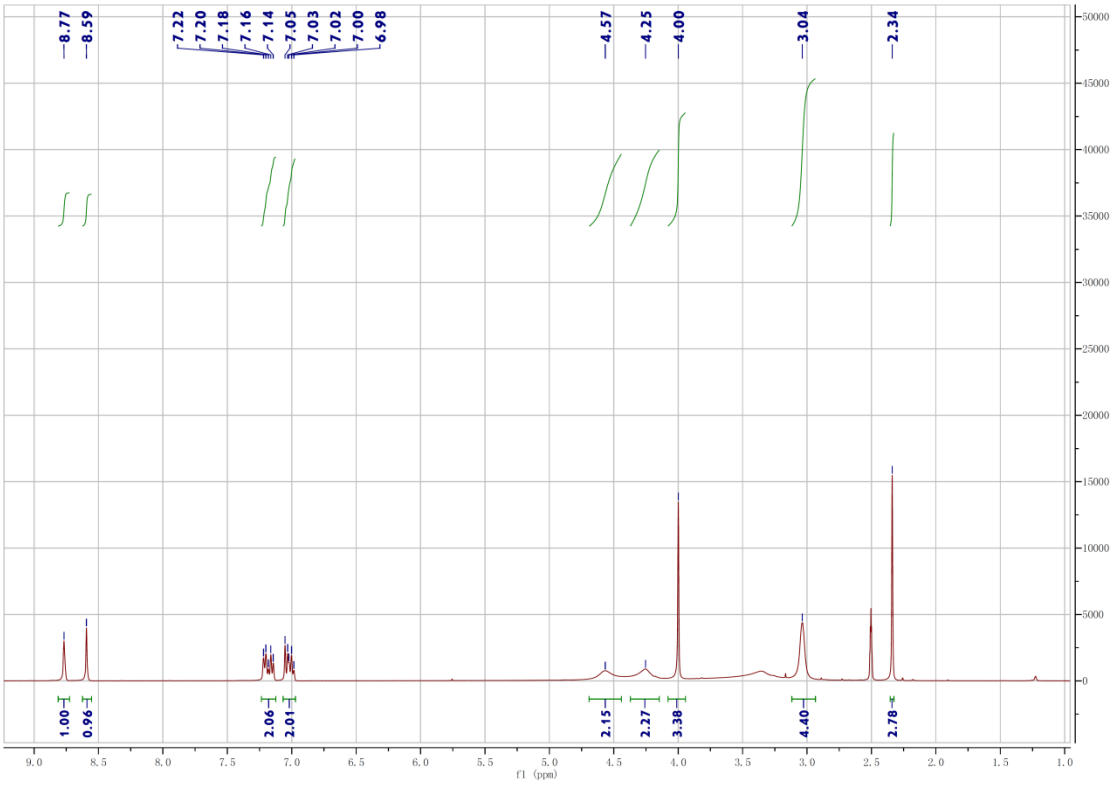
150.80
143.06
138.05
128.78
120.48
120.18
116.54
113.05
48.65
35.81
21.37
f1 (ppm)



24

Preparation of 24 (3-methyl-6-(4-(o-tolyl)piperazin-1-yl)-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, and 1-(o-tolyl)piperazine (352.5 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The vessel was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed smoothly with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) the desired product was generated as a white solid (yield: 57%). 1H NMR (400 MHz, DMSO-d6): δ8.49 (s, 1H), 8.05 (s, 1H), 7.18-7.17 (m, 1H), 7.13-7.11 (m, 1H), 7.01-6.99 (m, 1H), 6.97-6.95(m, 3 H), 4.72 (m, 2H), 4.22-4.17 (m, 2H), 3.92 (s, 3H), 2.96 (s, 4H), 2.31 (s, 3H), ^{13}C NMR (100 MHz, DMSO-d6) δ 151.6, 150.4, 146.6, 143.3, 132.1, 130.1, 123.5, 119.2, 51.2, 45.2, 36.9, 17.6; HRMS calculated for $C_{17}H_{21}N_6$ exact mass: 309.1822, found 309.1819, mp: 210°C.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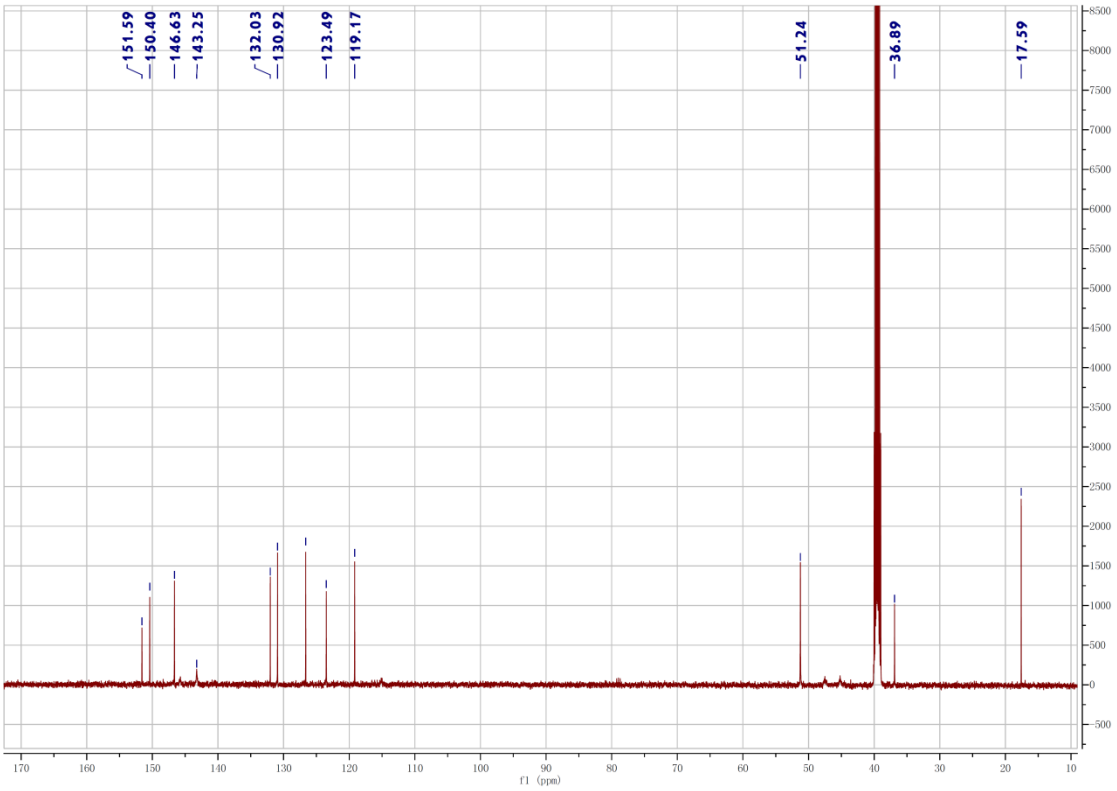
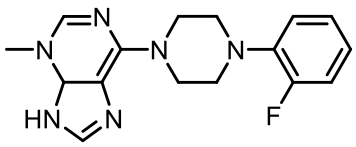
8.59
7.22
7.20
7.18
7.16
7.14
7.05
7.03
7.02
7.00
6.98
4.57
4.25
4.00
3.04
2.34
1.00
0.96
2.06
2.01
2.15
2.27
3.38
4.40
2.78
f1 (ppm)


151.59
150.40
146.63
143.25
132.03
130.92
123.49
119.17
51.24
36.89
17.59
f1 (ppm)

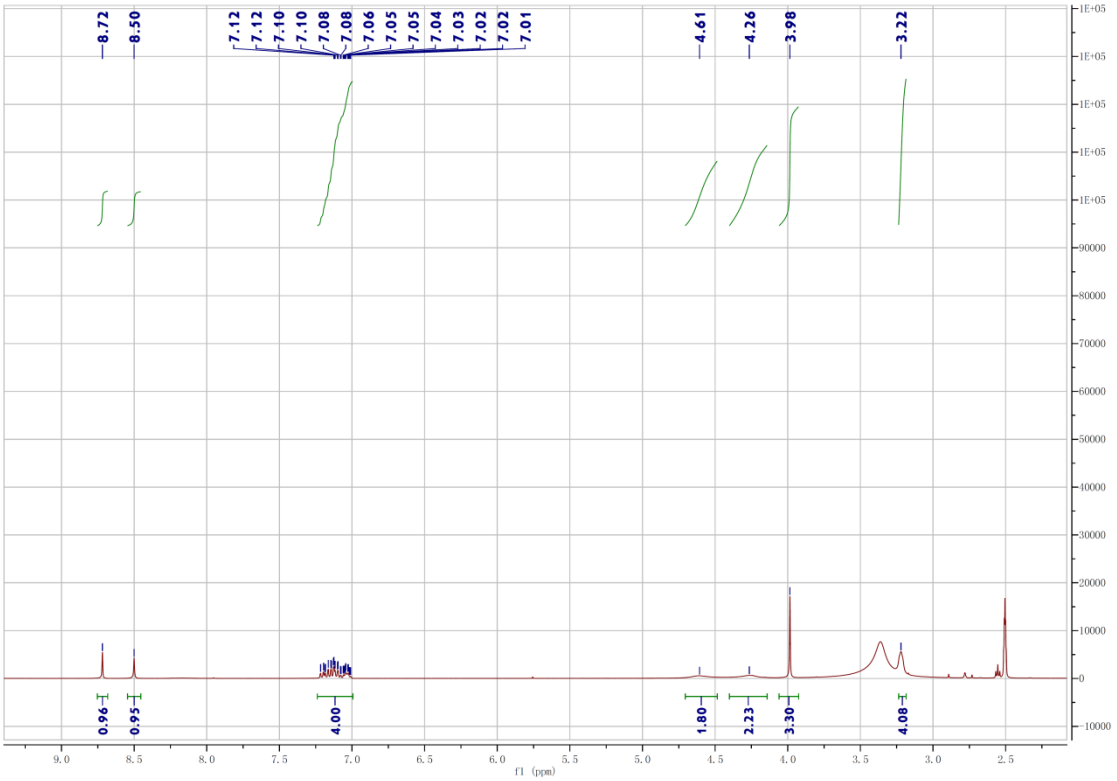


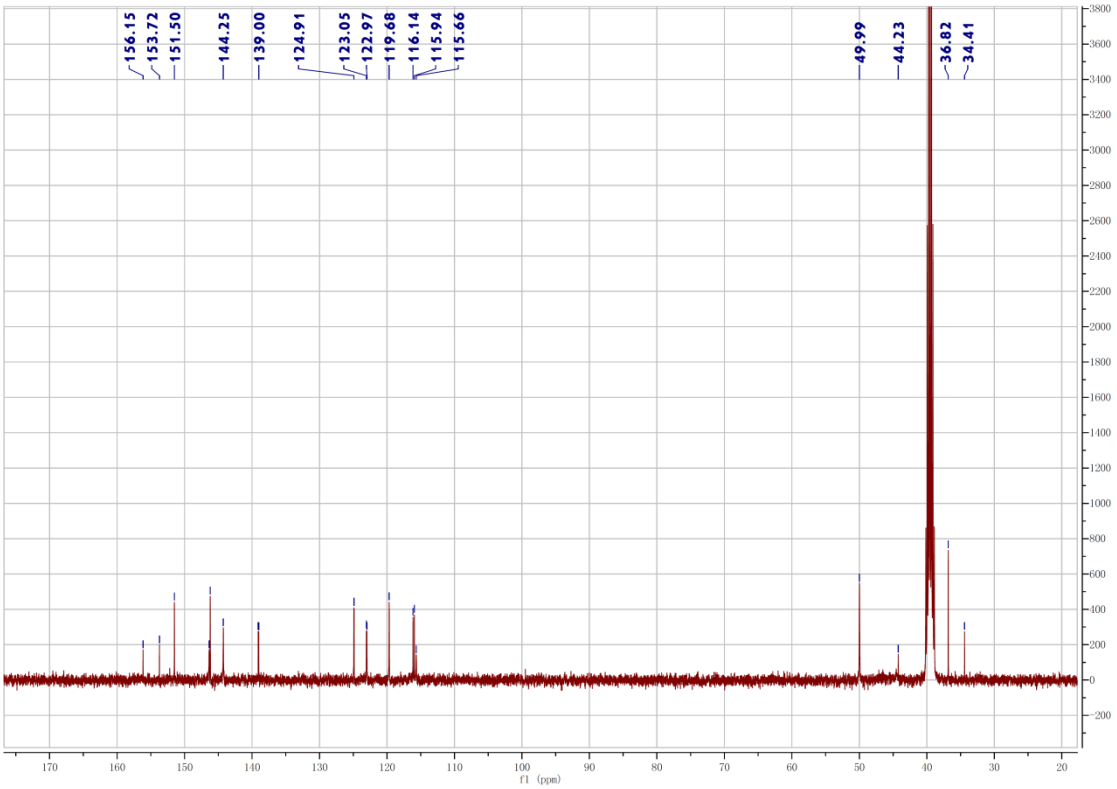
25

Preparation of 25

(6-(4-(2-fluorophenyl)piperazin-1-yl)-3-methyl-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 1-(2-fluorophenyl)piperazine (360.4 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 48 %). 1H NMR (400 MHz, DMSO-d6): δ8.72 (s, 1H), 8.50(s, 1H), 7.22-7.19 (m, 1H), 7.16-7.14 (m, 1H), 7.12-7.10 (m, 1H), 7.05-7.02 (m, 1H), 4.61 (s, 2H), 4.26 (s, 2H), 3.98 (s, 3H), 3.22 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d6) δ 155.9, 153.9, 144.2, 139.1, 139.0, 124.9, 124.8, 123.1, 123.0, 119.7, 116.1, 115.9, 115.6, 49.9, 44.2, 36.8, 34.4; HRMS calculated for $C_{16}H_{18}FN_6$ exact mass: 313.1571, found 313.1569; mp: 216°C.


8.72
8.50
7.12
7.12
7.10
7.10
7.08
7.08
7.06
7.05
7.05
7.04
7.03
7.02
7.02
7.01
4.61
4.26
3.98
3.22
0.96
0.95
4.00
1.80
2.23
3.30
4.08
f1 (ppm)


156.15
153.72
151.50
144.25
139.00
124.91
123.05
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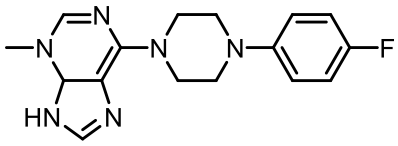
119.68
116.14
115.94
115.66
49.99
44.23
36.82
34.41
f1 (ppm)

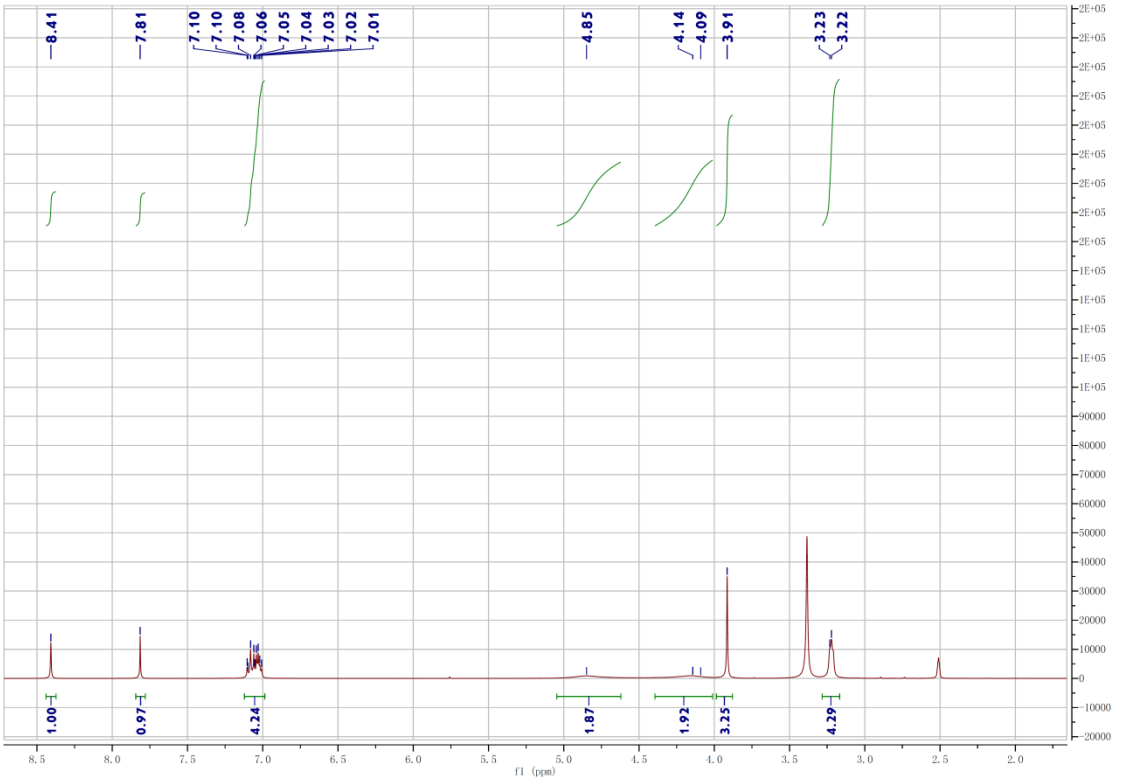


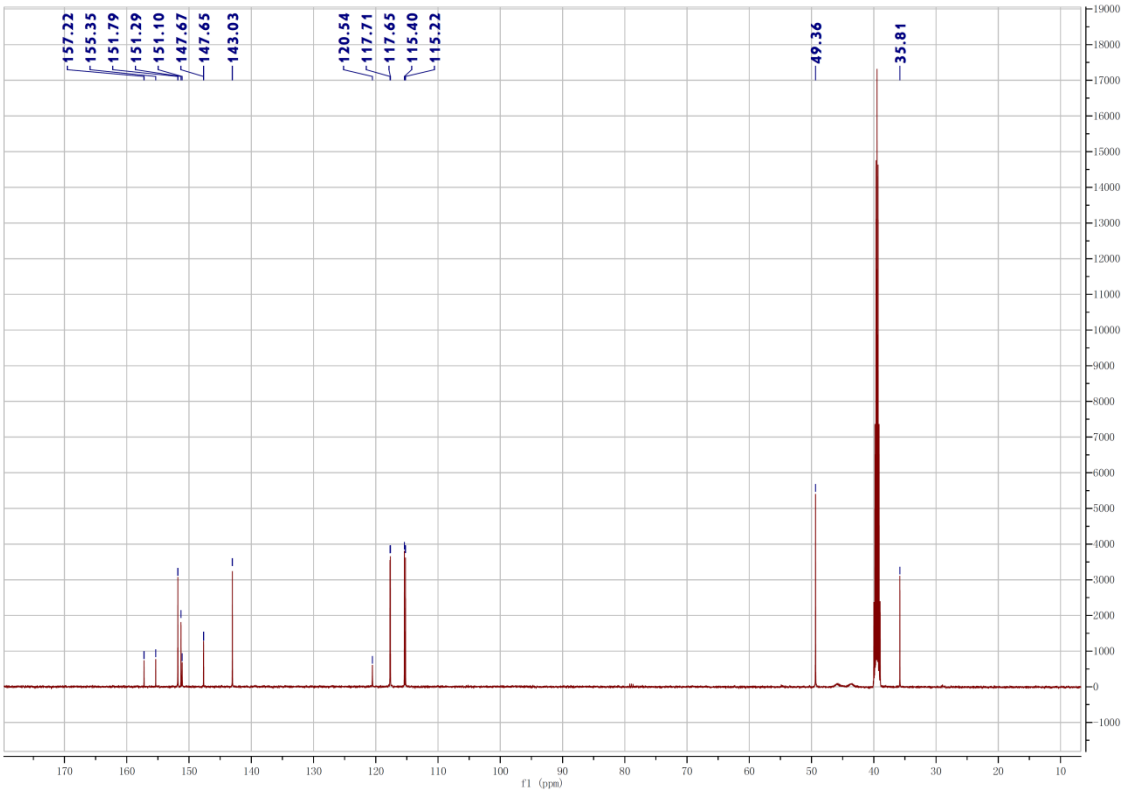
26

Preparation of 26

(6-(4-(4-fluorophenyl)piperazin-1-yl)-3-methyl-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 1-(4-fluorophenyl)piperazine (360.4 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 51%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.41 (s, 1H), 7.81 (s, 1H), 7.10-7.08 (m, 2H), 7.06-7.01(m, 2H), 4.85(m, 2H), 4.14-4.09 (m, 2H), 3.91 (s, 3H), 3.23-3.22 (d, 4H), ^{13}C NMR (100 MHz, DMSO-d6) δ 157.2, 155.3, 151.8, 151.3, 151.1, 147.7, 147.6, 143.0, 120.6, 117.7, 117.6, 115.4, 115.2, 49.4, 35.8. HRMS calculated for $C_{16}H_{18}FN_6$ exact mass: 313.1571, found 313.1569, mp: 216°C.


8.41
7.81
7.10
7.10
7.08
7.06
7.05
7.04
7.03
7.02
7.01
4.85
4.14
4.09
3.91
3.23
3.22
1.00
0.97
4.24
1.87
1.92
3.25
4.29
f1 (ppm)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151.79
151.29
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117.65
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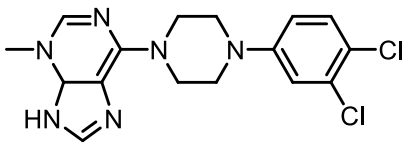
115.22
49.36
35.81
f1 (ppm)

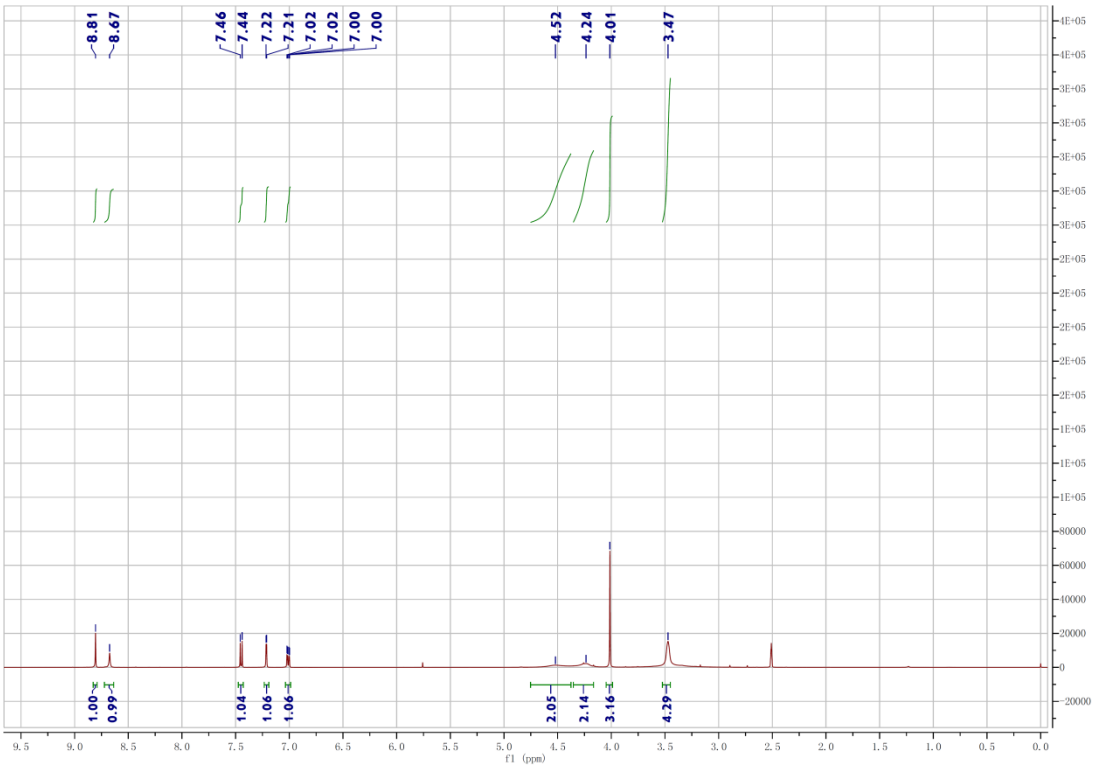


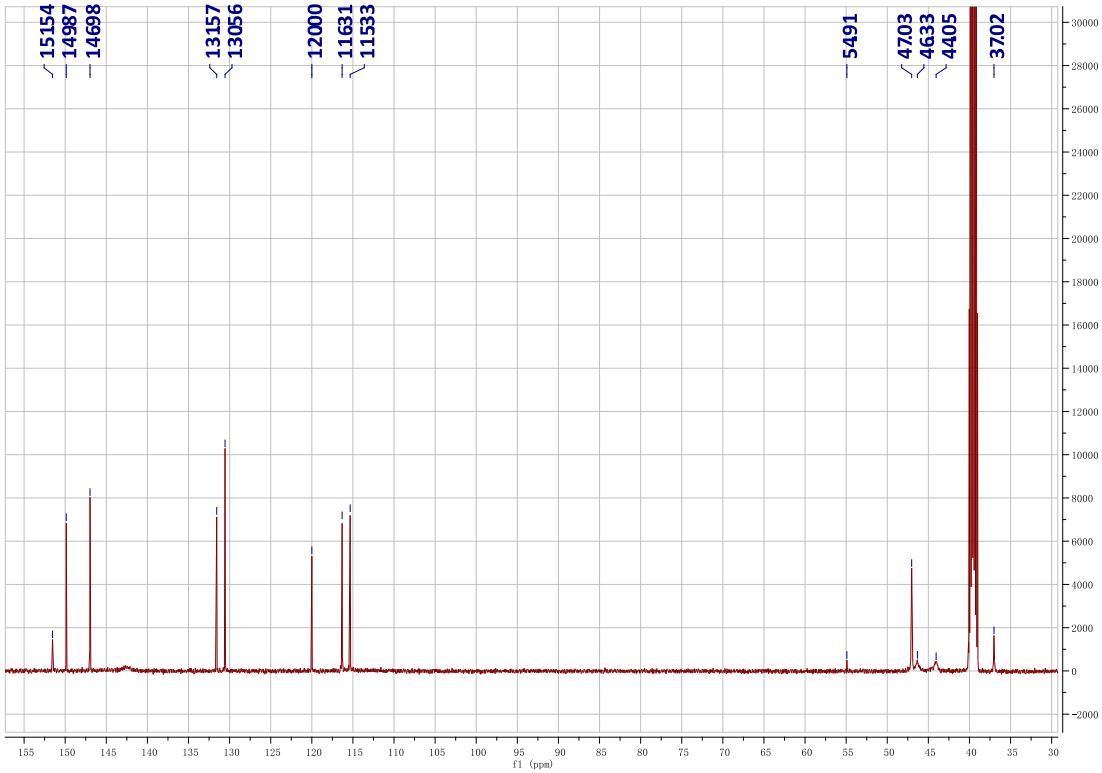
27

Preparation of 27

(6-(4-(3,4-dichlorophenyl)piperazin-1-yl)-3-methyl-3H-purine)

To an oven-dried Schlenk tube containing a stir bar, 1,2-dichloro-4-cyclohexylbenzene (458.3 mg, 2.0 mmol), 3-methyl-6-(methylthio)-3H-purine (180.2 mg, 1.0 mmol) and pyridine (158.2 mg, 2.0 mmol) were added. The tube was degassed 3 times and then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 160°C for 24 h under an atmosphere of N_2. Then the solvent was removed with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) generated the desired product as a white solid (yield: 57%). 1H NMR (400 MHz, DMSO-d6: δ 8.81 (s,1H), 8.67 (s, 1H), 7.46-7.44 (d, 1H), 7.22-7.21(d, 1H), 7.02-7.00 (m, 1H), 4.52 (s, 2H), 4.24 (s, 2H), 4.01 (s, 3H), 3.47 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d6) δ 151.6, 149.9, 146.9, 131.6, 130.6, 120.0, 116.3, 115.3, 54.9, 47.0, 46.3, 44.0, 37.0; HRMS calculated for $C_{16}H_{16}Cl_2N_6$ exact mass: 362.0822, found 362.0823. mp: 218°C.


8.81
8.67
7.46
7.44
7.22
7.21
7.02
7.02
7.00
7.00
4.52
4.24
4.01
3.47
1.00
0.99
1.04
1.06
1.06
2.05
2.14
3.16
4.29
f1 (ppm)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115.33
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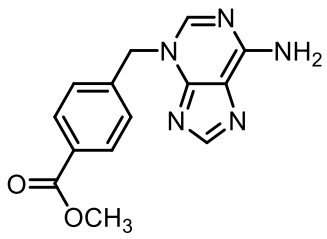
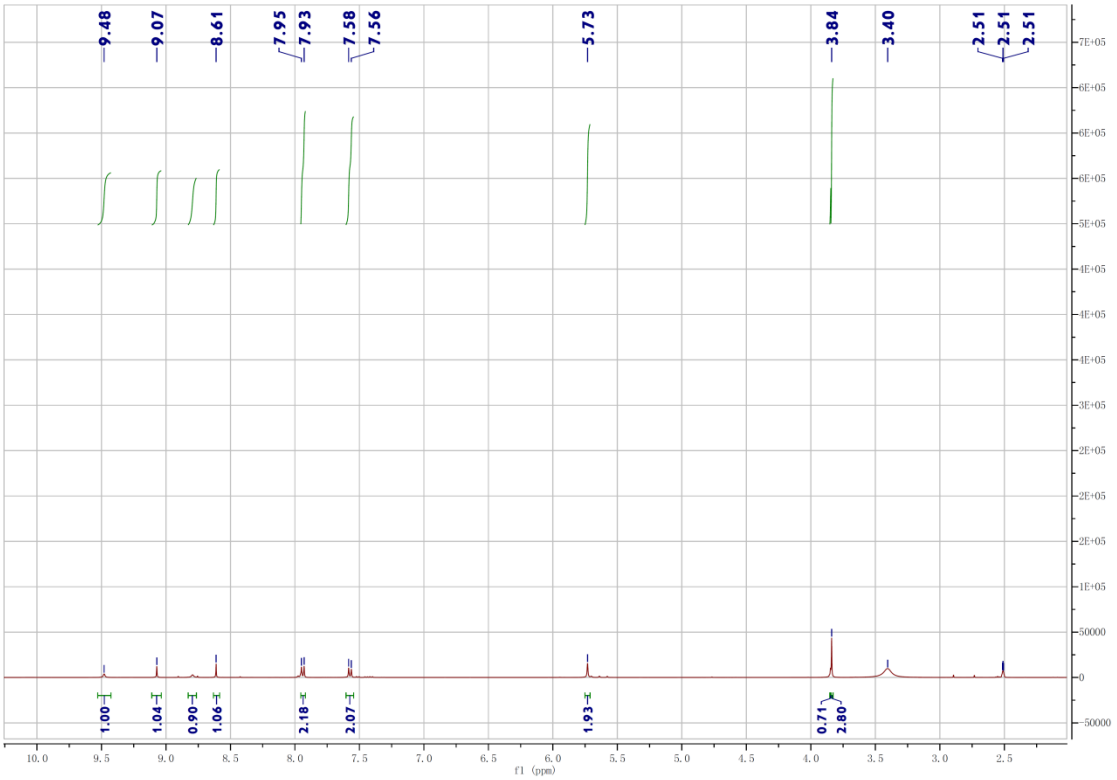
47.03
46.33
44.05
37.02
f1 (ppm)



28

Preparation of 28 (methyl 4-((6-amino-3H-purin-3-yl)methyl)benzoate)

To an oven-dried Schlenk tube containing a stir bar, adenine (135.1 mg, 1.0 mmol), and methyl 4-(bromomethyl)benzoate (458.1 mg, 2.0 mmol) were added. Then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 100°C for 12 h in air. Then the solvent was removed smoothly with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) the desired product was generated as a white solid (yield: 47%). ^{1}H NMR (400 MHz, DMSO-d6): δ 9.48 (s, 1H), 9.07 (s, 1H), 8.79 (s, 1H), 8.61 (s, 1H), 7.95-7.93 (t, 2H), 7.58-7.56(t, 2 H), 5.73 (s, 2H), 3,84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d6) δ 154.6, 153.9, 153.8, 153.1, 145.7, 134.2, 133.1, 123.3, 122.6, 119.2, 118.2, 115.7, 50.3, 38.5; HRMS calculated for $C_{14}H_{13}N_5O_2$ exact mass: 284.1136, found 284.1142; mp: 279°C.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9.07
8.61
7.95
7.93
7.58
7.56
5.73
3.84
3.40
2.51
2.51
2.51
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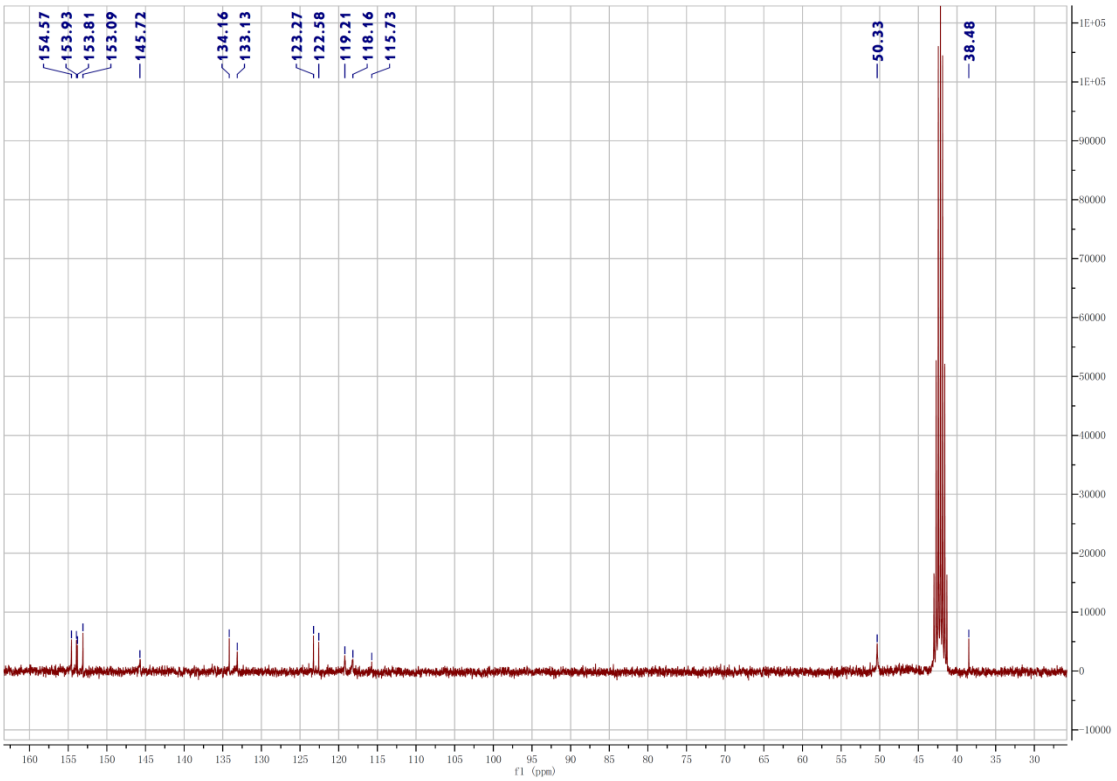
1.04
0.90
1.06
2.18
2.07
1.93
0.71
2.80
f1 (ppm)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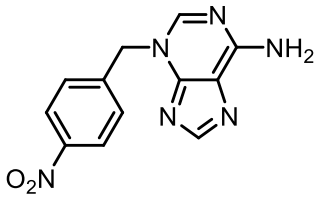
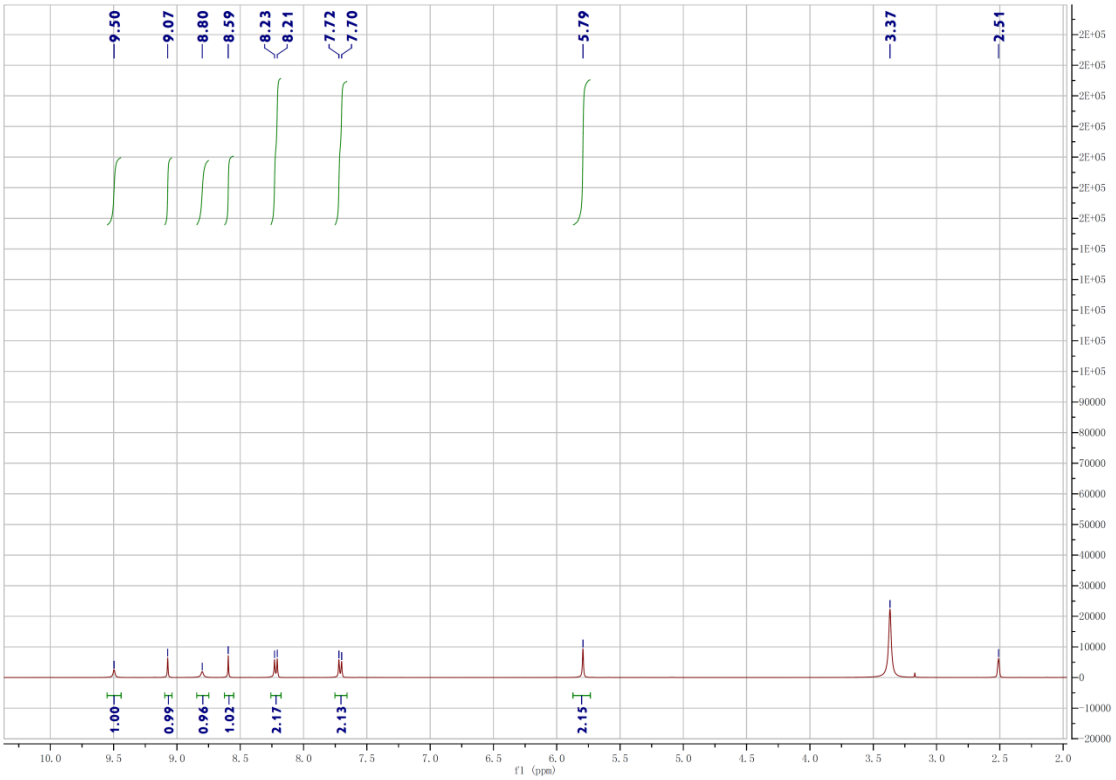
123.27
122.58
119.21
118.16
115.73
50.33
38.48
f1 (ppm)

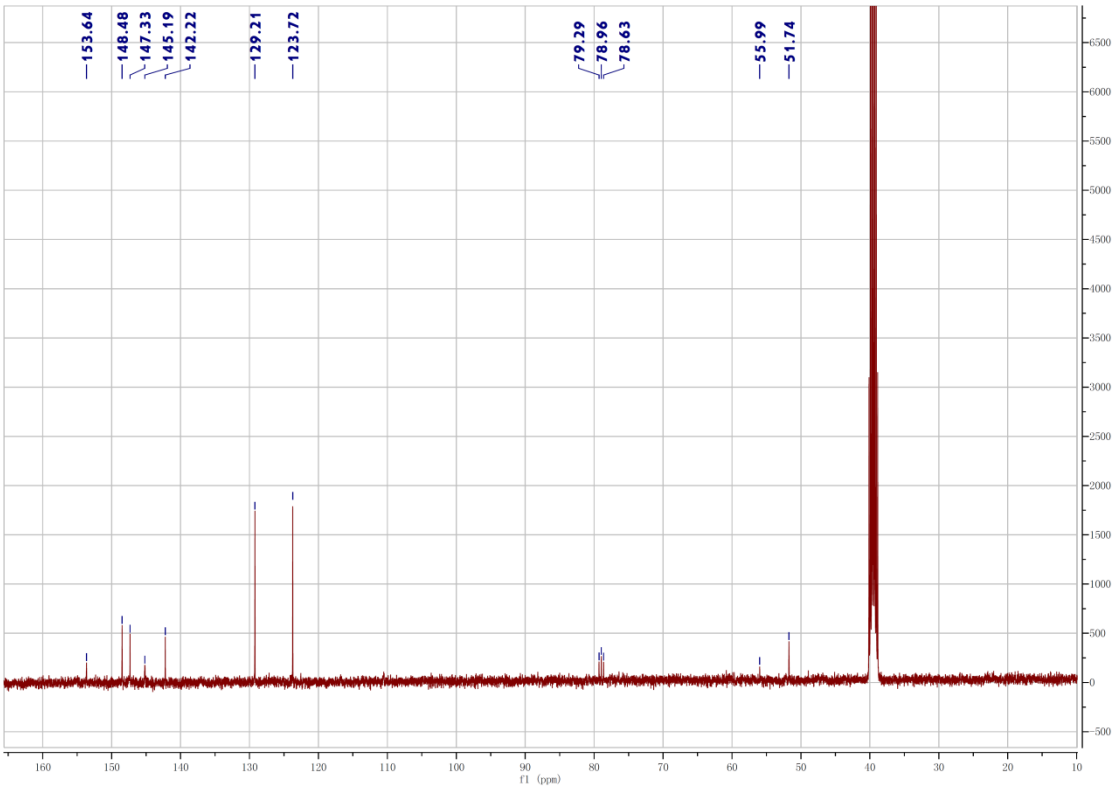


29

Preparation of 29 (4-((6-amino-3H-purin-3-yl)methyl)phenyl nitrate)

To an oven-dried Schlenk tube containing a stir bar, adenine (135.1 mg, 1.0 mmol), and 4-nitrobenzyl bromide (432.1 mg, 2.0 mmol) were added. Then 2.0 ml of freshly distilled DMF was added by syringe. The mixture was kept stirring at 100°C for 12 h in air. Then the solvent was removed smoothly with a cold trap in vacuum. The residue was washed with CH_2Cl_2 and dried in vacuum. Further purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (15:1) the desired product was generated as a white solid (yield: 43%). ^{1}H NMR (400 MHz, DMSO-d6): δ 9.50 (s, 1H), 9.07 (s, 1H), 8.80 (s, 1H), 8.59 (s, 1H), 8.23-8.21 (t, 2H), 7.72-7.70 (t, 2H), 5.79 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d6) δ 153.6, 148.4, 145.2, 142.2, 129.2, 123.7, 79.3, 78.9, 78.6, 55.9, 51.7; HRMS calculated for $C_{12}H_{10}N_6O_2$ exact mass: 271.0933, found 271.0938; mp: 259°C.


9.50
9.07
8.80
8.59
8.23
8.21
7.72
7.70
5.79
3.37
2.51
1.00
0.99
0.96
1.02
2.17
2.13
2.15
f1 (ppm)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148.48
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145.19
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129.21
123.72
79.29
78.96
78.63
55.99
51.74
f1 (ppm)

Figure S1

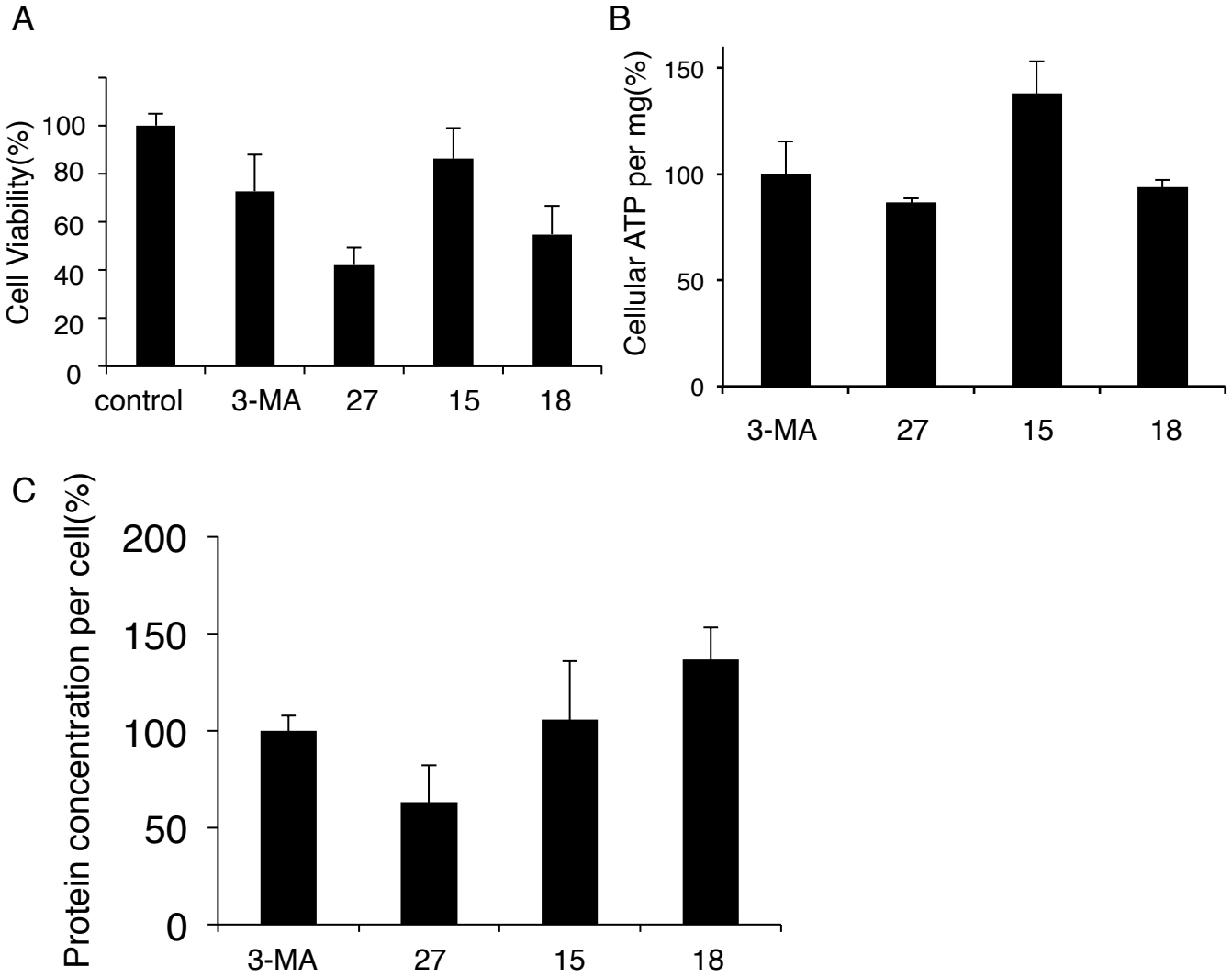

Figure S1. The effect of 3-MA derivatives 15, 18 and 27 on cell function. (**A**) NRK cells were treated with 10 mM 3-MA, 0.1 mM 27, 1 mM 15, or 1 mM 18 for 12 h and cell viability was tested with the MTT method. (**B**) NRK cells were treated as in (**A**) and the ATP levels were detected with an ATP Determination Kit (Invitrogen A22066). The data was normalized with the same concentration of protein in different treated groups. (**C**) NRK cells were treated as in (**A**) and the protein levels were determined with a BCA protein assay kit. The number of cells in each group was determined by light microscopy using a cell counter and the protein level per cell for each treatment was calculated.

Figure S2

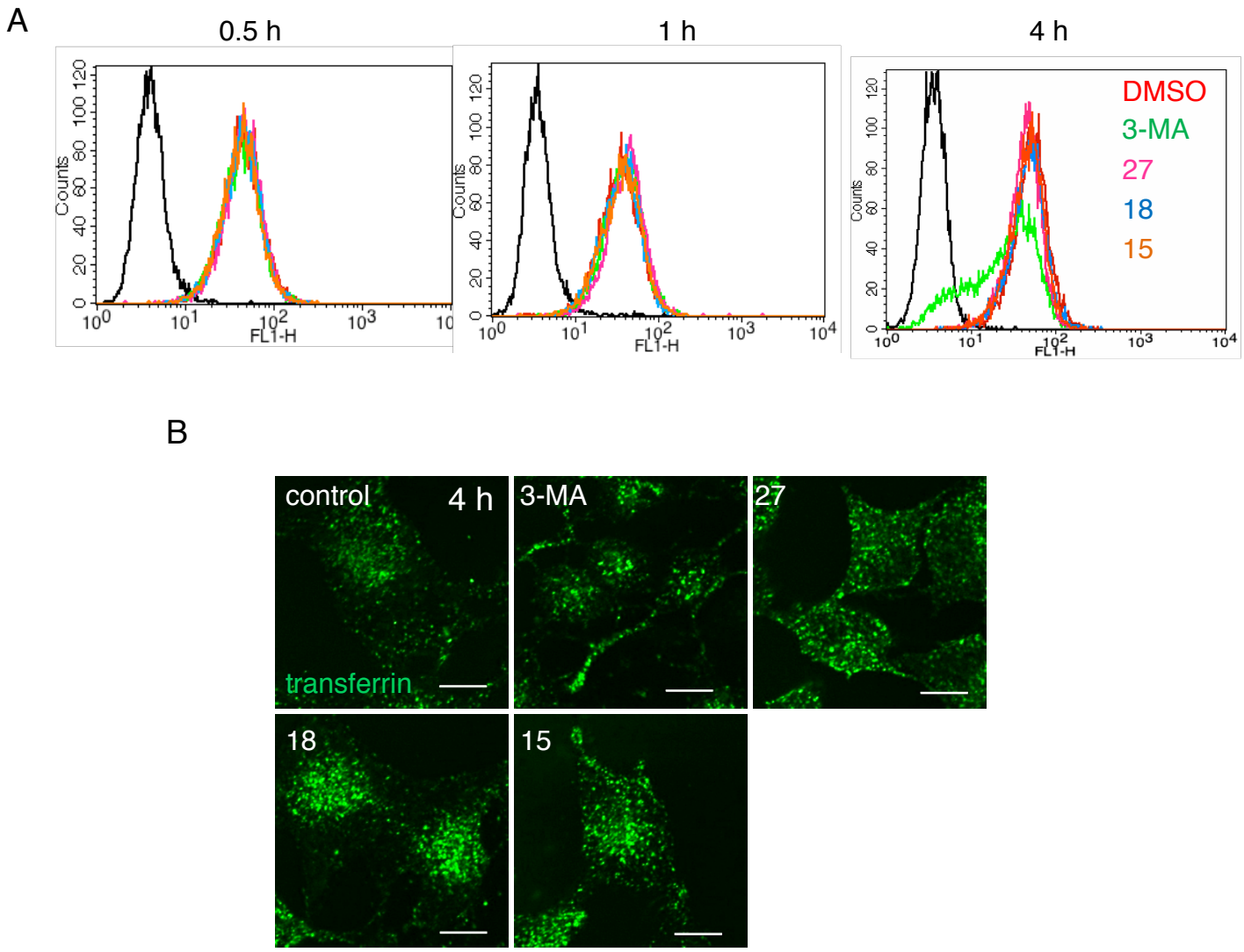

Figure S2. 3-MA derivatives 15,18 and 27 affect endocytosis. (**A**) NRK cells were treated with 10 mM 3-MA, 0.1 mM 27, 1 mM 18, or 1 mM 15 for 0.5, 1 or 4 h, washed with DMEM without serum three times, then incubated with Alexa 488 TF in DMEM without serum for 15 min. Surface TF was removed by washing with pH 5.0 PBS, then the fluorescence intensity was tested with flow cytometry. (**B**) NRK cells were treated as in (**A**). After washing with pH 2.0 buffer three times to remove surface transferrin, images were then taken.

Figure S3

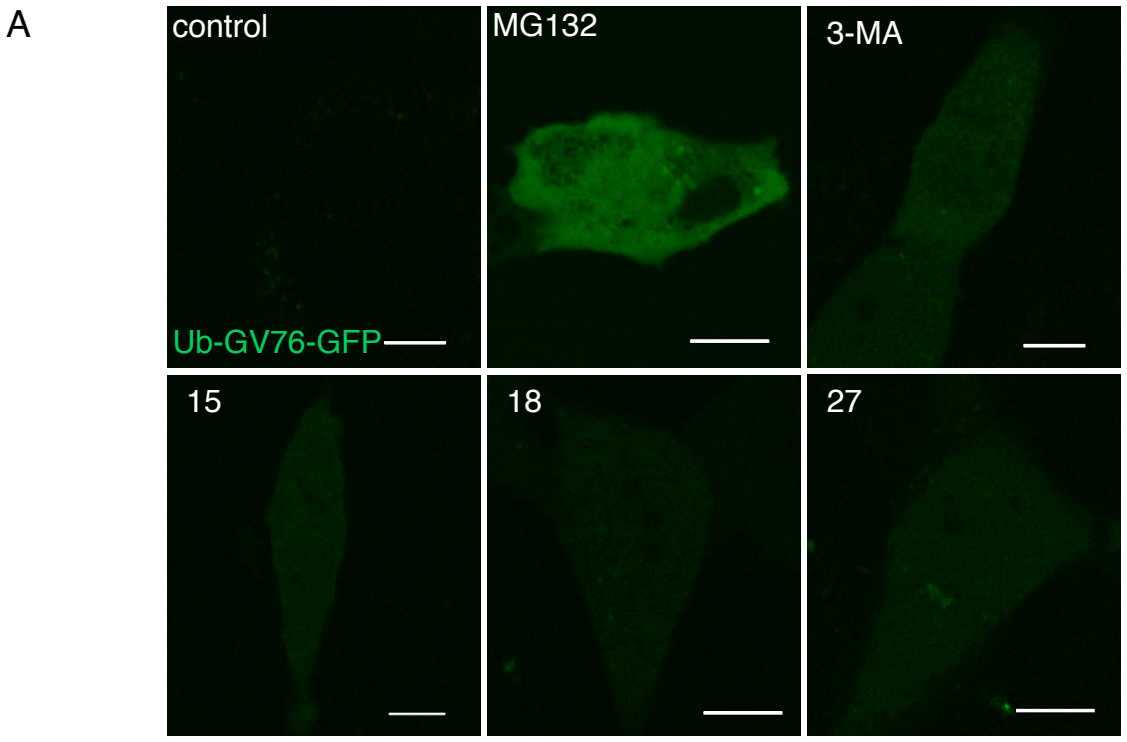

Figure S3. 3-MA derivatives 15, 18 and 27 inhibit proteasome-mediated proteolysis. (**A**) NRK cells were transfected with Ub-G76V-GFP. After 24 h the cells were treated with 10 mM 3-MA, 0.1 mM 27, 1 mM 15, 5 μ M MG132, or 1 mM 18 for 4 h and images were taken. (**B**) NRK cells were treated as in (**A**) and the levels of GFP protein were tested with anti-GFP antibody.

Figure S1. The effect of 3-MA derivatives 15, 18 and 27 on cell function. (**A**) NRK cells were treated with 10 mM 3-MA, 0.1 mM 27, 1 mM 15, or 1 mM 18 for 12 h and cell viability was tested with the MTT method. (**B**) NRK cells were treated as in (**A**) and the ATP levels were detected with an ATP Determination Kit (Invitrogen A22066). The data was normalized with the same concentration of protein in different treated groups. (**C**) NRK cells were treated as in (**A**) and the protein levels were determined with a BCA protein assay kit. The number of cells in each group was determined by light microscopy using a cell counter and the protein level per cell for each treatment was calculated.

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18 for 4 h and images were taken. (**B**) NRK cells were treated as in (**A**) and the levels of GFP protein were tested with anti-GFP antibody.