Supporting Information

Design, Synthesis, and Kinetic Analysis of Potent Protein N-terminal Methyltransferase 1 Inhibitors

Gang Zhang, Stacie Lynn Richardson, Yunfei Mao and Rong Huang*

Department of Medicinal Chemistry, and the Institute of Structural Biology & Drug Discovery, Virginia Commonwealth University, Richmond, VA 23219, USA

*Corresponding author. Fax: +01-804-827-3664; Tel.: +01-804-828-5619; E-mail: rhuang@vcu.edu

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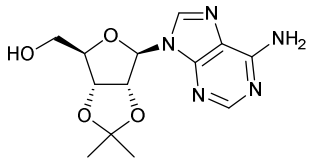
I. General

Unless otherwise noted, reagents and materials were obtained from commercial suppliers and used without further purification. Anhydrous tetrahydrofuran and dichloromethane were obtained from commercial sources. All solvents used for routine isolation of products and for chromatography were reagent grade. Moisture- and air-sensitive reactions were carried out under an atmosphere of nitrogen. Reaction flasks were flame-dried under a stream of nitrogen.

Reactions involving nitrogen or hydrogen were performed using gas filled balloons. Solvents were removed under reduced pressure using a Büchi rotary evaporator with an Edwards RV5 pump. All reactions were monitored by thin layer chromatography (TLC) and column chromatography purification was performed using 230-400 mesh silica gel. NMR spectra were measured on Bruker AV400 spectrometer at 400 MHz for ^{1}H spectra and at 100 MHz for ^{13}C spectra using $CDCl_3$, CD_3OD and D_2O, and calibrated from the residual solvent signal. Peptides were prepared using standard Fmoc chemistry with a CEM Liberty microwave peptide synthesizer. Peptides and peptide conjugates were purified by reverse phase HPLC (Waters) and quantified. Mass spectra were obtained with an Applied Biosystems Voyager MALDI time-of-flight (TOF) mass spectrometer in reflector mode or Perkin Elmer AxION 2 Time-Of-Flight mass spectrometer. All known compounds were characterized by ^{1}H NMR and HRMS analyses. All new small molecule compounds were also characterized by ^{13}C NMR and final peptide conjugates were confirmed by HRMS.

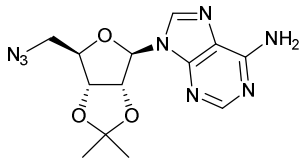
II. Synthesis of NAM-TZ-SKPRIA, NAM-TZ and TZ-SPKRIA

Synthesis of [(3*aR*,4*R*,6*R*,6*aR*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyl-tetrahydro-furo[3,4-d]-1,3-dioxol-4-yl]-methanol (1)[1]



To a suspension of adenosine (5.0 g, 18.7 mmol, 1 eq.) in dry acetone (1 L) was added p-TsOH monohydrate (32.2 g, 187.0 mmol, 10 eq.) in one portion. The mixture was stirred under an atmosphere of N_2 and turned to a yellow solution upon dissolution. After 3 hours, ice-cold saturated $NaHCO_3$ solution (1 L) was added to the above mixture with stirring over 5 minutes. The volatiles were removed under reduced pressure and the residue was dried. The resulting solid was dissolved in acetone (1 L), stirred for 1 hour, and filtered. The volatiles were removed and the crude product was purified by column chromatography (dichloromethane: methanol 20:1, silica). After dried under high vacuum, a white solid (5.7 g, quantitative yield) was obtained. R_f = 0.2 (CH_2Cl_2 : MeOH = 10:1) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1 H), 7.84 (s, 1 H), 6.54 (d, 1 H, *J* = 11.2 Hz), 5.94 (brs, 2 H), 5.86 (d, 1 H, *J* = 4.8 Hz), 5.21 (m, 1 H), 5.12 (m, 1 H), 4.55 (s, 1 H), 3.98 (m, 1 H), 3.79 (m, 1 H), 1.65 (s, 3 H), 1.38 (s, 3 H). HRMS (ESI$^+$) calcd for $C_{13}H_{18}N_5O_4$ $(M + H)^+$ m/z 308.1353, found m/z 308.1347.

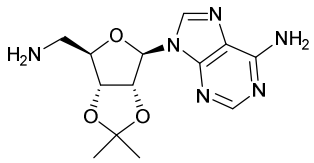
Synthesis of 9-((3*aR*,4*R*,6*R*,6*aR*)-6-Azidomethyl-2,2-dimethyl-tetrahydro-furo[3,4-d]-1,3-dioxol-4-yl)-9*H*-purin-6-ylamine (2)[2]



To a suspension of 2',3'-O-isopropylideneadenosine (1.00 g, 3.25 mmol, 1.00 eq.) in 1,4-dioxane (10 mL) was added dppa (1.40 mL, 6.51 mmol, 2.00 eq.) and DBU (1.46 mL, 9.77 mmol, 3.00 eq.) at room temperature under N_2. The solution was stirred for 2 hours after which NaN_3 (1.06 g, 16.30 mmol, 5.00 eq.) and 15-crown-5 (6.5 L, 33 mol, 0.01 eq.) were added and the reaction mixture was heated to reflux.[2] After 1 hour the solid was removed by filtration. The solvent was evaporated and the crude product was purified by column chromatography (ethyl acetate/Hexane 1:1, to Methanol/Dichloromethane 1:50, silica). After dried under high vacuum, a light yellow solid (1.03 g, 95 %) was obtained. R_f = 0.2 (EtOAc : Hexanes = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1 H), 7.91 (s, 1 H), 6.10 (d, 1 H, *J* = 2.2 Hz), 6.05 (brs, 2 H), 5.46 (m, 1 H), 5.05 (m, 1 H), 5.12 (m, 1 H),

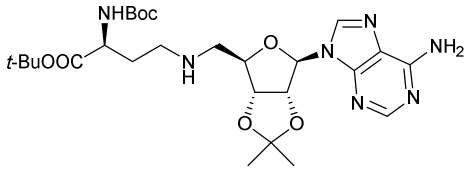
4.37 (m, 1 H), 3.56 (m, 2 H), 1.60 (s, 3 H), 1.38 (s, 3 H). HRMS (ESI^+) calcd for $C_{13}H_{17}N_8O_3$ $(M + H)^+$ m/z 333.1418, found m/z 333.1420.

Synthesis of 9-((3*aR*,4*R*,6*R*,6*aR*)-6-Aminomethyl-2,2-dimethyl-tetrahydro-furo[3,4-d]-1,3-dioxol-4-yl)-9*H*-purin-6-ylamine (3)[3]



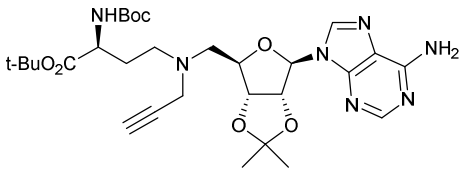
To the adenosine azide derivative (1.08 g, 3.25 mmol) in ethanol was added Palladium on activated charcoal (0.1 g). The reaction mixture was placed under vacuum and then under an atmosphere of H_2 overnight. The reaction mixture was filtered through a short pad of celite. The solvent was evaporated and the crude product (0.99 g, quantitative yield) was obtained. R_f = 0.2 (CH_2Cl_2 : MeOH = 20:1). ^1H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1 H), 7.84 (s, 1 H), 5.85 (m, 1 H), 5.79 (brs, 2 H), 5.21 (m, 1 H), 5.11 (m, 1 H), 4.54 (m, 1 H), 3.65 (m, 1 H), 3.58 (m, 2 H), 1.65 (s, 3 H), 1.38 (s, 3 H). HRMS (ESI^+) calcd for $C_{13}H_{19}N_6O_3$ $(M + H)^+$ m/z 307.1513, found m/z 307.1528.

Synthesis of 5′-*N*-[4-{(2S)-2-(N-*tert*-Butoxycarbonyl)amino-butyric acid}]*tert*-butyl ester-5′-deoxy-2′,3′-O,O-(1-methyl ethylidene) adenosine (4)[4]



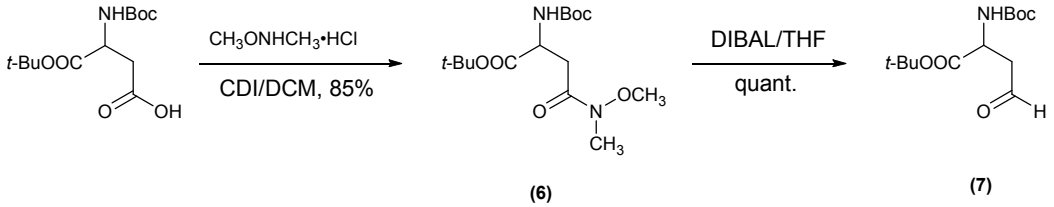
A solution of aldehyde **8** (1.0 g, 3.66 mmol, 1.0 eq.) in CH_2Cl_2 (15 mL) was added into a solution of amine **3** (1.18 g, 4.02 mmol, 1.1 eq.) in CH_2Cl_2 (15 mL) at room temperature. The mixture was stirred for 20 min. After TEA (0.55 g, 5.49 mmol, 1.5 eq.) was added into the solution, sodium triacetoxyborohydride (1.09 g, 5.12 mmol, 1.4 eq.) was added in portions and the mixture stirred at room temperature for 3 h. The reaction solution was quenched with saturated aqueous Na_2CO_3 (30 mL), extracted with CH_2Cl_2 (2 X 50 mL), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica flash column chromatography to give secondary amine as a white solid (1.48 g, 2.63 mmol, 72 %). R_f = 0.4 (CH_2Cl_2 : MeOH = 20:1). ^1H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1 H), 7.90 (s, 1 H), 6.08 (s, 1 H), 5.95 (m, 1 H), 5.70 (brs, 2 H), 5.47 (m, 1 H), 5.05 (m, 1 H), 4.34 (m, 1 H), 4.26 (m, 1 H), 2.92 (m, 1 H), 2.75 (m, 2 H), 2.61 (m, 1 H), 1.90 (m, 1 H), 1.80 (m, 1 H), 1.59 (s, 3 H), 1.43 (s, 9 H), 1.37 (s, 3 H), 1.36 (s, 9 H). HRMS (ESI^+) calcd for $C_{26}H_{42}N_7O_7$ $(M + H)^+$ m/z 564.3140, found m/z 564.3156.

Synthesis of 5′-*N*-propynylamino-*N*′-[4-{(2S)-2-(*N*-*tert*-butoxycarbonyl)amino-butyric acid}] *tert*-butyl ester-5′-deoxy-2′,3′-O,O-(1-methylethylidene)adenosine (5)[4]



Propargyl bromide (80 % in toluene, 40 mg, 275.44 µmol, 1.1 eq.) was added to a solution of secondary amine (135 mg, 239.51 µmol, 1.0 eq.) and anhydrous K_2CO_3 (37 mg, 275.44 µmol, 1.1 eq.) in DMF (2 mL) at room temperature and stirred for 3 hours. The reaction mixture was diluted with ethyl acetate (10 mL), washed with brine (3 X 10 mL), dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 30:1) to give desired compound (123 mg, 85 %) as a solid. R_f = 0.3 (CH_2Cl_2 : MeOH = 30:1). ^1H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1 H), 7.96 (s, 1 H), 6.07 (s, 1 H), 5.56 (m, 1 H), 5.49 (brs, 2 H), 5.00 (m, 1 H), 4.35 (m, 1 H), 4.22 (m, 1 H), 3.43 (s, 2 H), 2.80 (m, 1 H), 2.73 (m,

1 H), 2.57 (m, 2 H), 2.10 (s, 1 H), 1.91 (m, 1 H), 1.78 (m, 1 H), 1.62 (s, 3 H), 1.40 (s, 18 H), 1.40 (s, 3 H). HRMS (ESI^+) calcd for $C_{29}H_{44}N_7O_7$ $(M + H)^+$ m/z 602.3297, found m/z 602.3295.



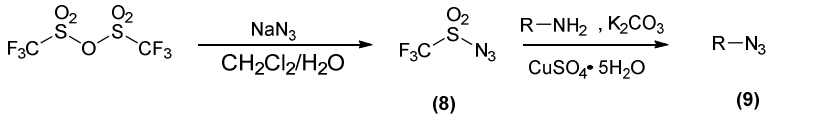
(6) **(7)**

Synthesis of *N*-*t*-Boc-*L*-aspartic Acid 1-(*tert*-Butyl ester)-*N*-Methoxy-*N*-methylamide (6)[5]

Weinreb amid **6** was synthesized as previously described.[5] To a stirred solution of Boc-aspartic acid- O-*t*-Bu (1.0 g, 3.46 mmol, 1.0 eq.) in dichloromethane at room temperature was added 1,1'-carbonyldiimidazole (0.62 g, 3.82 mmol, 1.1 eq.). After 1 h, N,O-dimethylhydroxylamine hydrochloride (0.38 g, 3.82 mmol, 1.1 eq.) was added and stirred for 18 hr. The reaction was diluted with ethyl acetate and washed with HCl (1M, 10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried and concentrated to afford crude product, and then the crude product was purified via chromatography to afford the target product in 85% yield. ^1H NMR (400 MHz, $CDCl_3$): δ 5.66 (s, 1 H), 4.44 (m, 1 H), 3.69 (s, 3 H), 3.16 (s, 3 H), 3.14 (dd, 1 H, J = 3.4 Hz, 17.0 Hz), 2.87 (dd, 1 H, J = 3.4 Hz, 17.0 Hz), 1.46 (s, 9 H), 1.44 (s, 9 H). HRMS (ESI^+) calcd for $C_{15}H_{27}N_2O_6Na$ $(M + Na)^+$ m/z 355.1840, found m/z 355.1853.

1-*tert*-Butyl L-2-(t-Boc-amino)-4-oxobutanoate (7)[5]

A solution of diisobutylaluminum hydride (DIBAL) in hexane (1 M, 1.62 mmol, 1.62 mL, 1.5 eq.) was added dropwise into a stirred solution of starting material (0.36 g, 1.08 mmol, 1.0 eq.) in anhydrous THF at -75 °C. Upon addition the mixture continued to stir at -75 °C for 2 h. The reaction mixture was partitioned between 0.35 M $NaHSO_4$ aqueous solution (20 mL) and ether (30 mL) and the aqueous layer was extracted with ether (3X10 mL). The combined ethereal solutions were washed with 1 M HCl (3 X 10 mL), 1M $NaHCO_3$ (3 X 10 mL) and brine (3 X 10 mL) and dried with anhydrous Na_2SO_4. After filtering, the filtrate was concentrated under vacumm to afford the crude product 0.3g in quantitative yield. R_f = 0.19 (Hex : EtOAc = 4:1). ^1H NMR (400 MHz, $CDCl_3$): δ 9.73 (s, 1 H), 5.35 (s, 1 H), 4.47 (d, 1 H, J = 6.92 Hz), 2.95 (m, 2 H), 1.44 (s, 9 H), 1.43 (s, 9 H). HRMS (ESI^+) calcd for $C_{13}H_{24}NO_5$ $(M + H)^+$ m/z 274.1649, found m/z 274.1647.



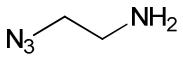
(8) **(9)**

R = SPKRIA on resin

General procedure for synthesis of Azido compound (9)[6]

A solution of sodium azide (1.78 g, 27.45 mmol) was dissolved in distilled H_2O (4.5 mL) with CH_2Cl_2 (7.5 mL) and cooled on an ice bath. Trifluoromethanesulfonic anhydride (0.93 mL, 5.55 mmol) was added slowly over 5 min while stirring continued for 2 h. The mixture was placed in a separatory funnel and the CH_2Cl_2 phase removed. The aqueous portion was extracted with CH_2Cl_2 (2 X 3 mL). The organic fractions, containing the triflyl azide **8**, were pooled and washed once with saturated Na_2CO_3 and used without further purification. SPKRIA on resin (0.03 mmol, 1.0 eq.) with N-terminal free amino group was suspended in the mixture of CH_3OH (1 mL), K_2CO_3 (16 mg, 0.09 mmol, 3.0 eq.), Copper (II) sulfate pentahydrate (1 mg, 0.003 mmol, 0.1 eq.) and distilled H_2O (1 mL) and the above triflyl azide in CH_2Cl_2 (0.09 mmol, 3.0 eq.) was added. The mixture was shaken at ambient temperature overnight. Once reaction was complete, the suspension was filtered, and the resin was washed (3 X 2 mL H_2O, MeOH, CH_2Cl_2), and air-dried. The product on resin can be used directly in next step.

2-Azidoethanamine (10) [7]

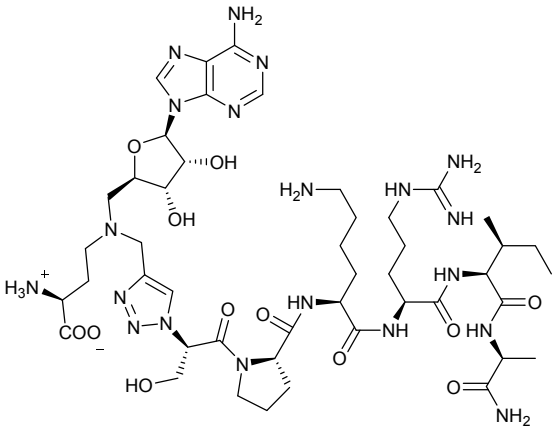


Synthesis of compound 10 was prepared as previously described.[7] To a solution of NaN_3 (0.57 g, 8.77 mmol, 3.0 eq.) in 5 mL of water was added 2-bromoethylamine hydrobromide (0.6 g, 2.93 mmol, 1.0 eq.) and this solution was heated to 75 ^oC. The reaction mixture was stirred for 30 h, then cooled in an ice-water bath. To the cooled solution was added NaOH (0.68 g, 17.1 mmol, 5.8 eq.) and the mixture was stirred until NaOH was fully dissolved. The aqueous phase was extracted twice with Et_2O and the combined organic phases were washed with brine and dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to afford 2-azidoethanamine as a clear oil in 85% yield. ^1H NMR (400 MHz, $CDCl_3$): δ 3.35 (t, 2 H), 2.86 (t, 2 H), 1.47 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 54.7, 41.4.

General Procedure for Synthesis of Bisubstrate inhibitors

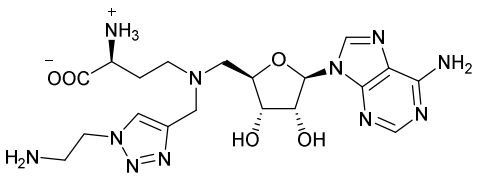
Intermediate **5** (87.25 µmol, 1.05 eq.), azido intermediate **10** or **11** (83.10 µmol, 1.0 eq.), copper (II) sulfate pentahydrate (1.0 mg, 4.15 µmol, 0.05 eq.), Sodium ascorbate (3.3 mg, 16.6 µmol, 0.2 eq.) were mixed in 3 mL of *t*-BuOH/H_2O (2:1). The mixture was shaken at ambient temperature overnight. For NAM-TZ-SPKRIA, once reaction time was complete, the suspension was filtered, and the resin was washed (3 X 2 mL H_2O, MeOH, CH_2Cl_2), and air-dried. The peptide was cleaved from the resin with concomitant removal of side chain-protecting groups by treatment with 6 mL of trifluoroacetic acid (TFA)/3,6-dioxa-1,8-octane-dithiol (DODT)/H_2O/triisopropylsilane (TIPS) (94:2.5:2.5:1 v/v) for 4 h at room temperature. After filtering, the filtrate was condensed and the residue was mixed with 10 mL of ice-cold ether and then pelleted by centrifugation. The supernatant was discarded and the pellet was washed well with chilled ether, air-dried. The crude product was purified by HPLC. For NAM-TZ, the mixture was diluted by ethyl acetate, then washed by brine for three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated under vacumm. The residue was dissolved in 4N HCl in 1,4-dioxane and stirred at room temperature. After 1 h, the solution was concentrated under vacumm. The residue was purified by HPLC.

NAM-TZ-SPKRIA



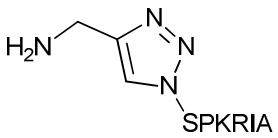
HRMS MALDI-TOF (positive) *m/z*: calcd for $C_{46}H_{77}N_{20}O_{12}$ $(M + H)^+$ m/z 1101.6024, found m/z 1101.9032.

NAM-TZ



^{1}H NMR (400 MHz, D_2O): δ 8.42 (s, 1 H), 8.40 (s, 1 H), 8.28 (s, 1 H), 6.17 (d, 1 H, *J* = 4.0 Hz), 4.78 (m, 3 H), 4.67 (m, 1 H), 4.57 (m, 2 H), 4.47 (m, 1 H), 3.76 (m, 2 H), 3.67 (m, 1 H), 3.59 (m, 2 H), 3.50 (m, 2 H), 2.40 (m, 1 H), 2.21 (m, 1 H). ^{13}C NMR (100 MHz, D_2O): δ 172.3, 163.1, 150.2, 148.2, 144.6, 143.5, 136.3, 128.4, 119.3, 90.1, 78.4, 73.1, 71.7, 55.3, 52.3, 51.7, 47.6, 38.9, 24.8. HRMS (ESI$^+$) calcd for $C_{19}H_{30}N_{11}O_5$ $(M + H)^+$ m/z 492.2426, found m/z 492.2434.

Synthesis of TZ-SPKRIA (11)



The intermediate **9** (0.4 mmol, 1.0 eq.) was suspended in 3 mL of *t*-BuOH/H_2O (2:1). The propargylamine (66.1 mg, 1.2 mmol, 3.0 eq.), Copper (II) sulfate pentahydrate (20 mg, 0.08 mmol, 0.2 eq.) and sodium ascorbate (63 mg, 0.32 mmol, 0.8 eq.) were added. The reaction mixture was shaken at ambient temperature overnight. Once reaction time was complete, the suspension was filtered, and the resin was washed (3 X 2 mL H_2O, MeOH, CH_2Cl_2), and air-dried. The peptide was cleaved from the resin with concomitant removal of side chain-protecting groups by treatment with 6 mL of trifluoroacetic acid (TFA)/3,6-dioxa-1,8-octane-dithiol (DODT)/H_2O/triisopropylsilane (TIPS) (94:2.5:2.5:1 v/v) for 4 h at room temperature. After filtering, the filtrate was condensed and the residue was mixed with 10 mL of ice-cold ether and then pelleted by centrifugation. The supernatant was discarded and the pellet was washed well with chilled ether, air-dried. The crude product was purified by HPLC. HRMS (ESI$^+$) calcd for $C_{32}H_{59}N_{14}O_7$ $(M + H)^+$ m/z 751.4686, found m/z 751.6788.

III. NTMT1, PRMT1 and G9a inhibition assays

NTMT1, G9a, and PRMT1 were expressed in *E. Coli* BL21 (DE3) codon plus RIL cells in LB medium in the presence of 50 μg/ml of kanamycin, respectively.[8-10] All inhibitory activities were determined with both substrates at their K_m values at 37 ^{o}C. NTMT1 assay was performed as described by Richardson, *et al.* under the following condition: pH 7.4, 25 mM Tris, pH 7.4, 50 mM KCl, 0.2 μM His-NTMT1, 10 μM SsSAHH, 8 μM SAM, 15 μM ThioGlo 1, various concentration of inhibitor. The assay was initiated by the addition of the peptide substrate RCC1-10 (SPKRIAKRRS).[8] Fluorescence was then measured in a FlexStation 3 Muti-Mode Microplate Reader using 370 nm excitation and 500 nm emission. Mass spectrometry (MS) based methylation assays were performed and analyzed via an Applied Biosystems Voyager matrix assisted laser desorption/ionization time-of-flight mass spectrometer.[8] The IC_{50} values were determined for all four inhibitors by fitting the activity data with GraphPad. Similar experiments were carried out for inhibitory effects on PRMT1 and G9a. [9,10]

IV. NAM-TZ-SPKRIA docking study

The X-ray crystal structure of human protein N-terminal RCC1 methyltransferase bound to S-adenosyl homocysteine (SAH) (PDB 2EX4, 1.75 Å) was obtained from the Protein Data Bank. Docking study was performed using Gold 5.2. The protonation state of the protein and the ligand were calculated using the default settings. The active site was defined by a sphere of 6.0 Å from the native ligand in the crystal structure. The ligand for docking was prepared using SYBYL X2.1, and the energy was minimized using the external Tripos force field to a constant of 20.781 kcal/mol. The docked poses were scored using CHEMPLP scoring function. Molecules used for the docking experiments were constructed in ChemBio3D Ultra 13.0 and minimized using the MMFF94x force field to a constant of -9.639 kcal/mol. The best docked pose of the ligand was visualized using Pymol Version 1.3 and its score was shown in the table below.

Supplementary Table 1. The score of the best docked pose of NAM-TZ-SPKRIA

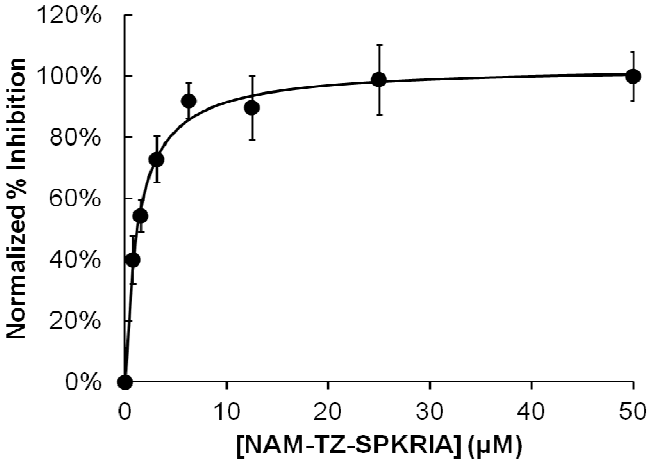
Score	S(PLP)	S(hbond)	DE(clash)	DE(tors)	intcor
111.14	-98.94	7.48	12.71	5.05	12.58

(Note: PLP: Piecewise Linear Potential; hbond: hydrogen bond contribution; DE(clash): Protein-ligand clash penalty to the PLP value; DE(tors): Internal ligand torsional strain penalty to the PLP value; intcor: Internal ligand energy offset)

V. References

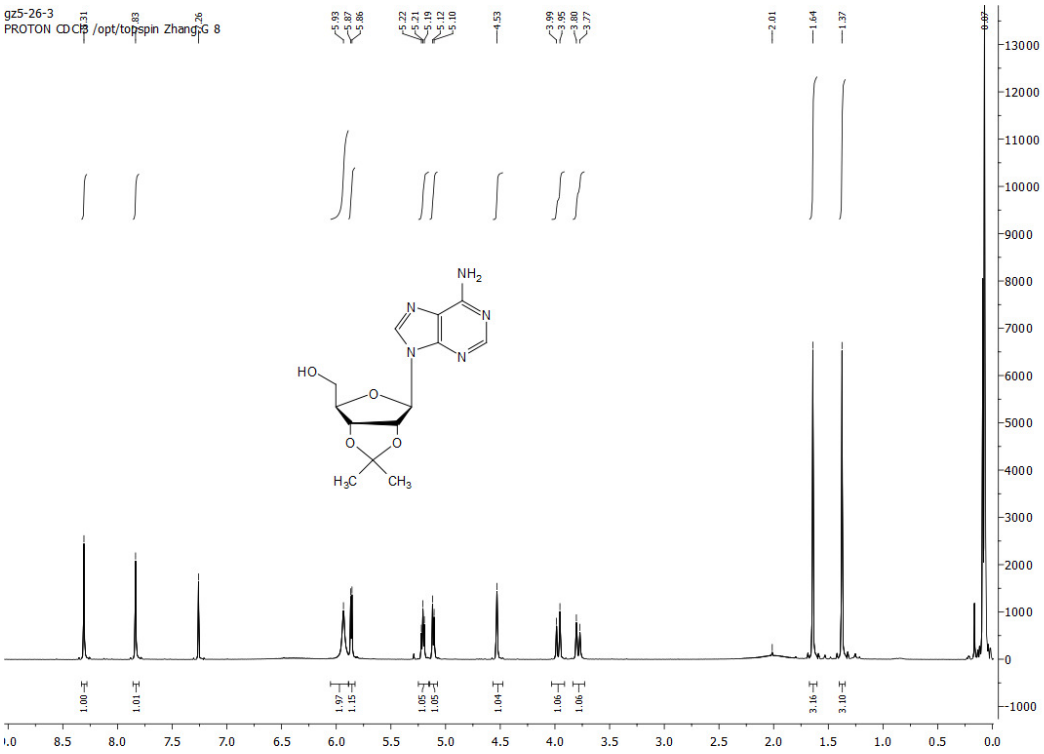
[1] A. Hampton, *J. Am. Chem. Soc*., 1961, **83**, 3640.
[2] F. Liu, D. J. Austin, *J. Org. Chem.*, 2001, **66**, 8643.
[3] P. Ciuffreda, A. Loseto, E. Santaniello,*Tetrahedron*, 2002, **58**, 5767.
[4] J. Dowden, W. Hong, R. V. Parry, R. A. Pike, S. G. Ward, *Bioorg. Med. Chem. Lett*., 2010, **20**, 2103.
[5] D. Wernic, J. Dimaio, J. Adams, *J. Org. Chem*., 1989, **54**, 4224.
[6] J. T. Lundquist, J. C. Pelletier, *Org. Lett*., 2001, **3**, 781.
[7] T. Mayer, M. E. Maier, *Eur. J. Org. Chem*. 2007, **2007**, 4711.
[8] S. L. Richardson, Y. Mao, G. Zhang, P. Hanjra, D. L. Peterson, R. Huang. *J. Biol. Chem.*, 2015, submitted
[9] Y. Feng, N. Xie, M. Jin, M. R. Stahley, J. T. Stivers, Y. G. Zheng, *Biochemistry.*, 2011, **50**, 7033.
[10] F. Liu, X. Chen, A. Allali-Hassani, A. M. Quinn, T. J. Wigle, G. A. Wasney, A. Dong, G. Senisterra, I. Chau, A. Siarheyeva, J. L. Norris, D. B. Kireev, A. Jadhav, J. M. Herold, W. P. Janzen, C. H. Arrowsmith, S. V. Frye, P. J. Brown, A. Simeonov, M. Vedadi, J. Jin, *J. Med. Chem.*, 2010, **53**, 5844.

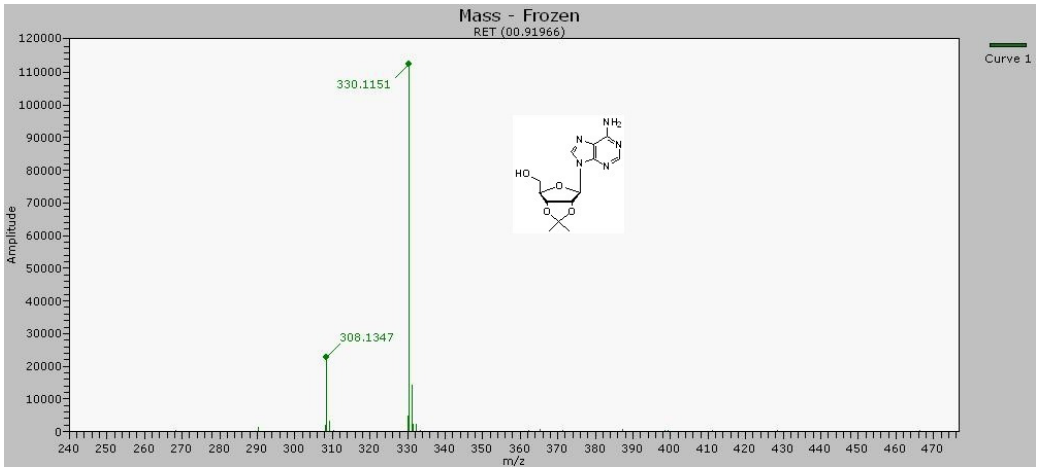
VI. IC_{50} curve of NAM-TZ-SPKRIA

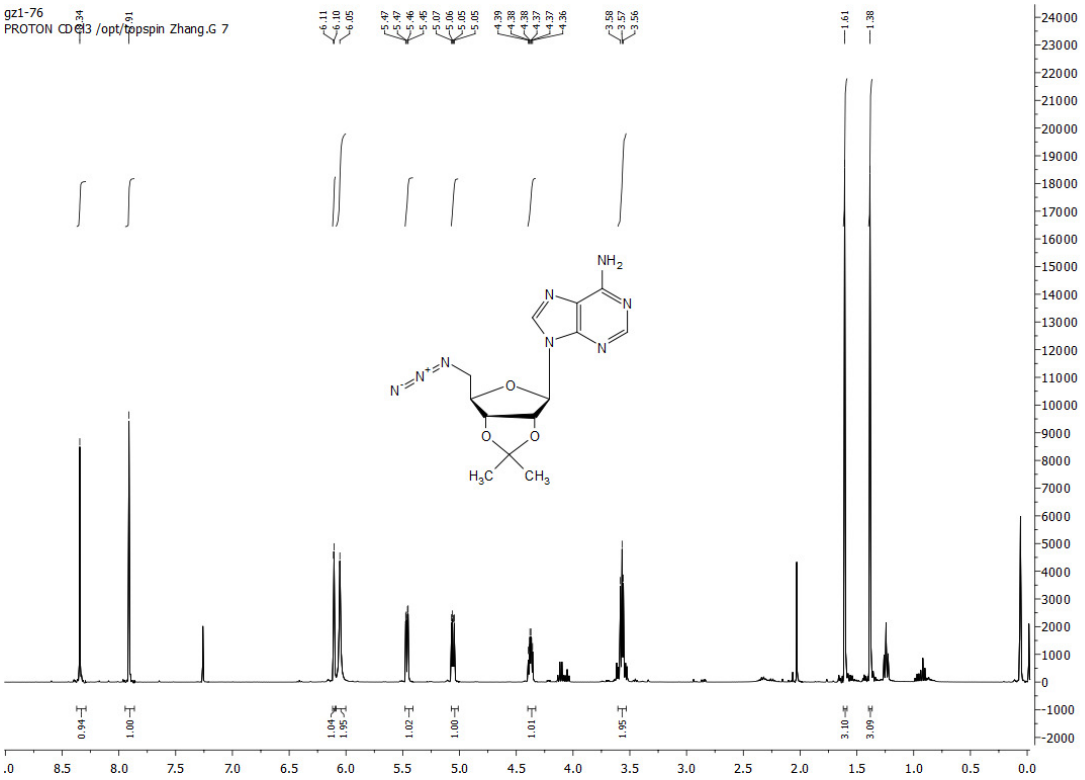


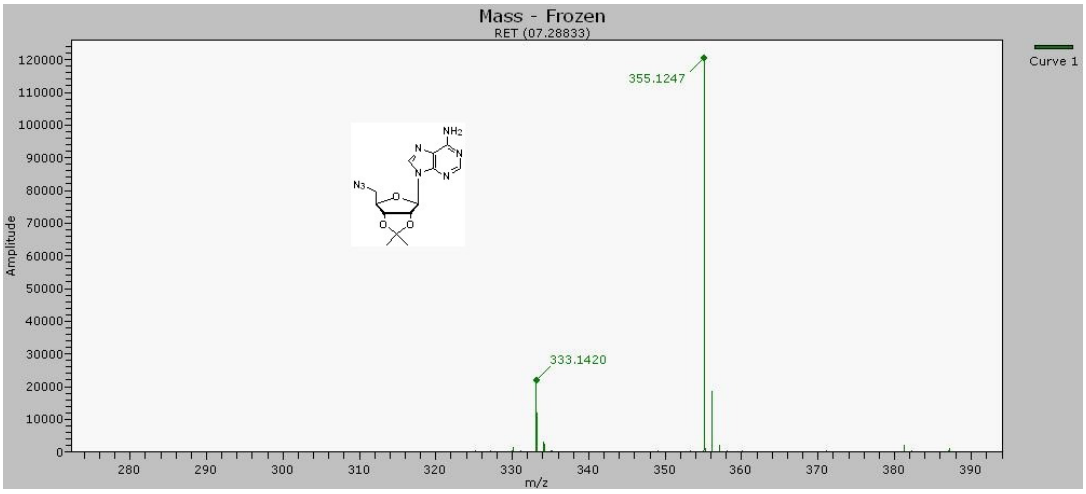
Supplementary Figure 1. Steady-state inhibition of NTMT1 by NAM-TZ-SPKRIA in the MALDI-MS assay.

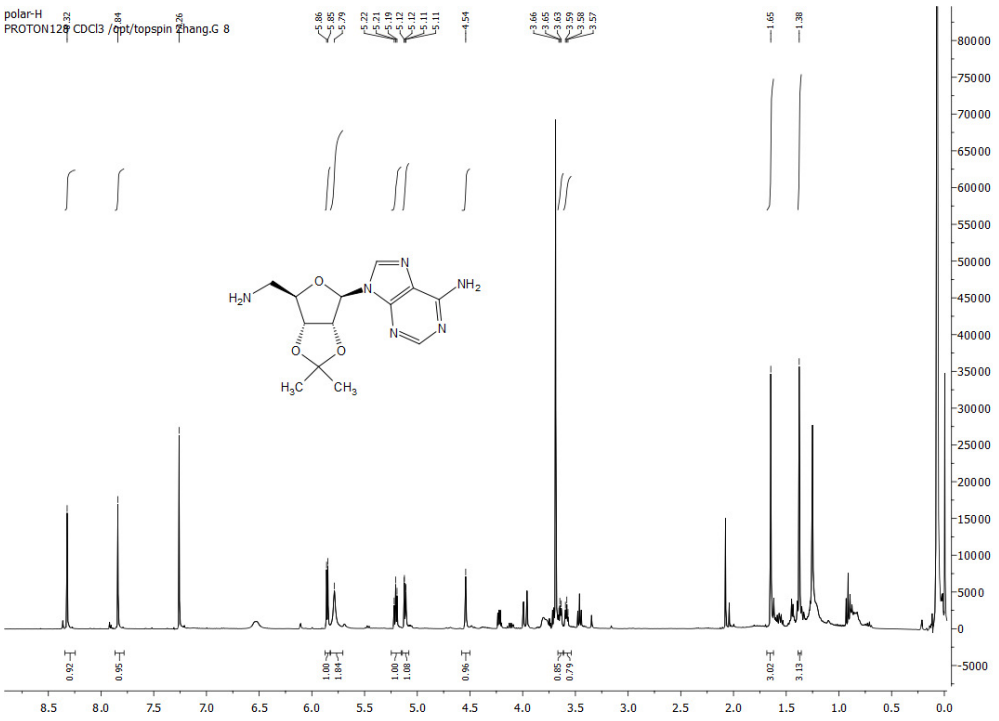
VII. Original Spectra (^{1}H NMR, ^{13}C NMR, MS and HPLC)

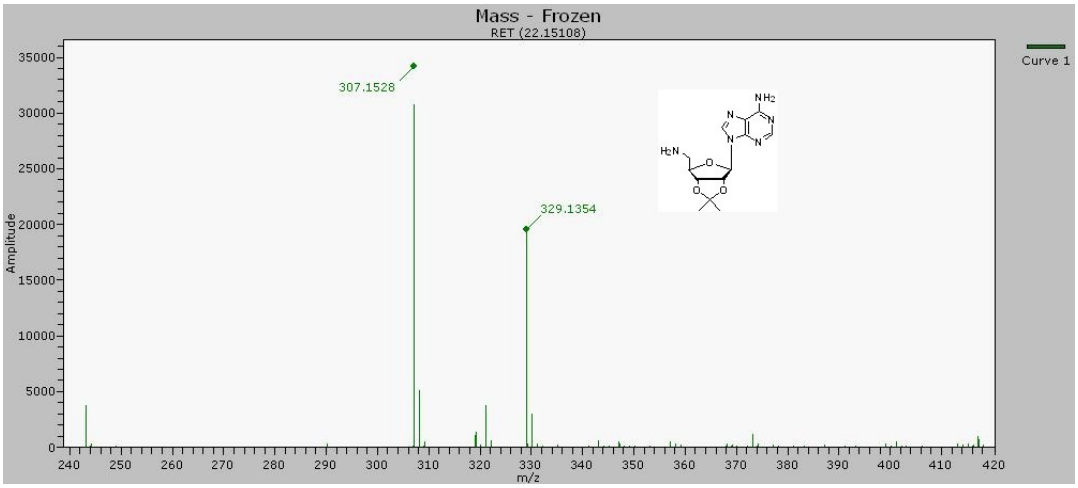


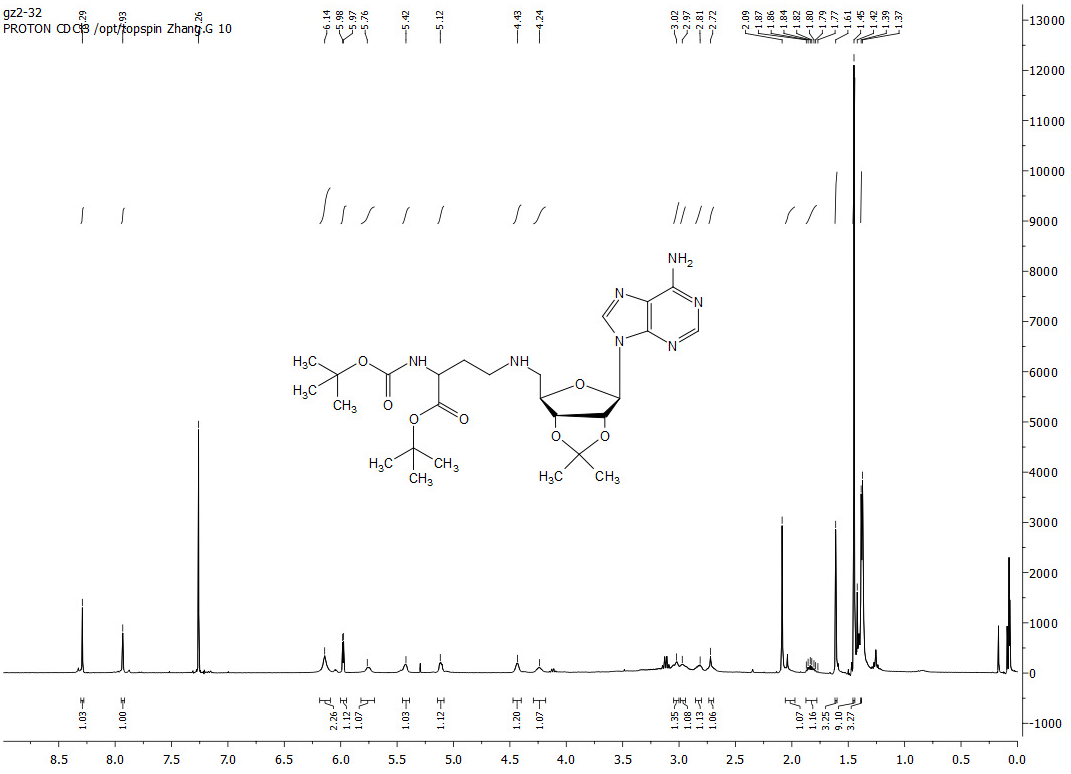


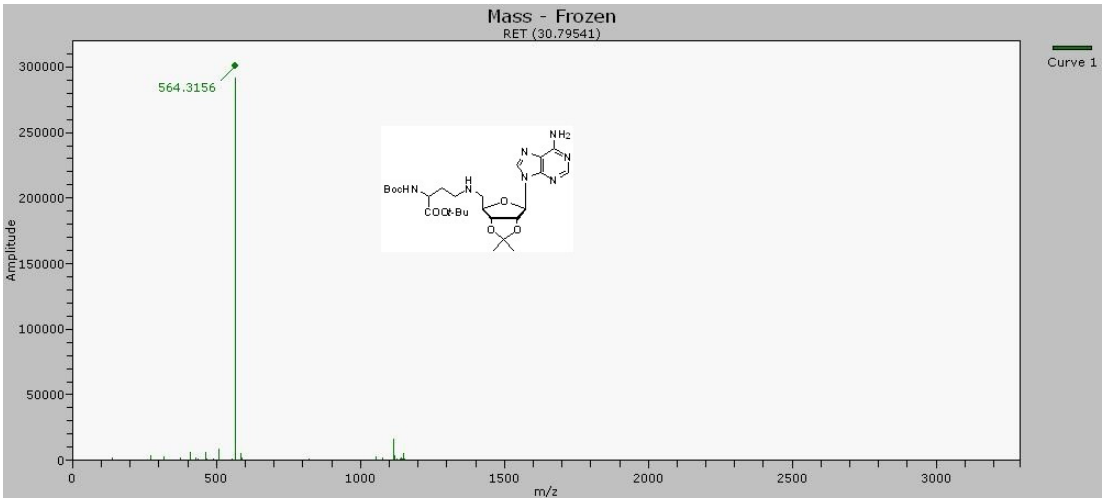

gz1-76
PROTON CDCl3 /opt/topspin Zhang.G 7
6.11
6.10
6.05
5.47
5.47
5.46
5.46
5.07
5.06
5.05
5.05
4.39
4.38
4.38
4.37
4.37
4.36
3.58
3.57
3.56
1.61
1.38
0.94
1.00
1.04
1.95
1.02
1.00
1.01
1.95
3.10
3.09

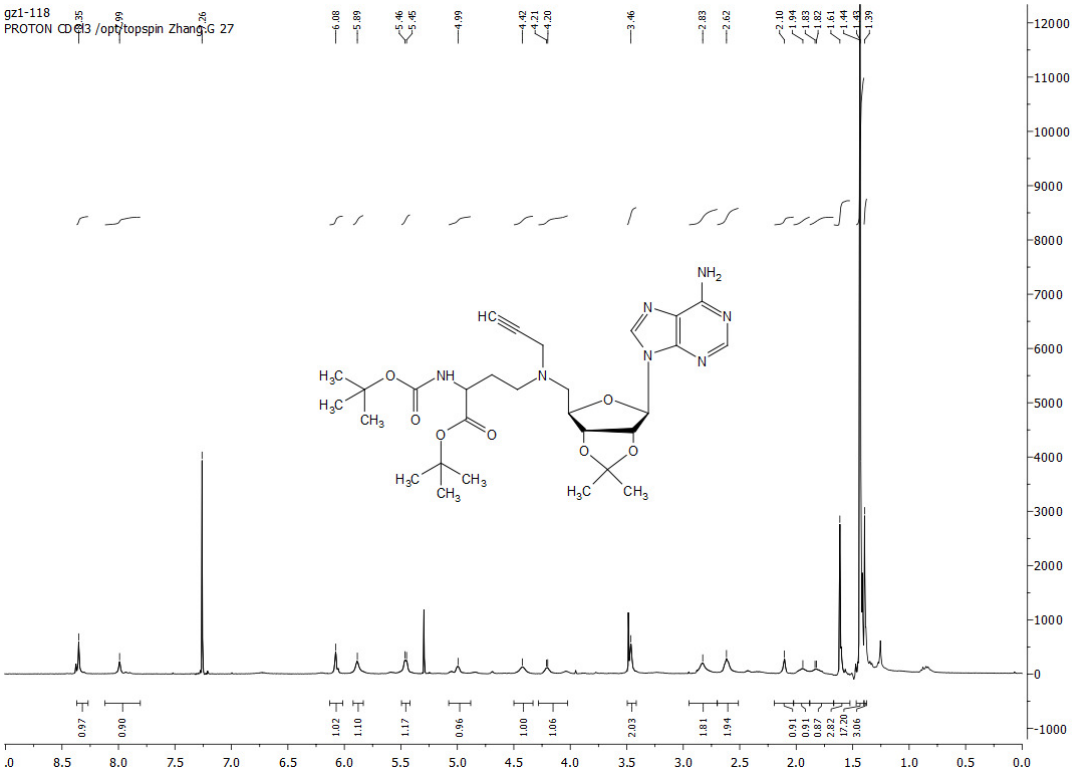

Mass - Frozen
RET (07.28833)
Curve 1
355.1247
333.1420
Amplitude
m/z

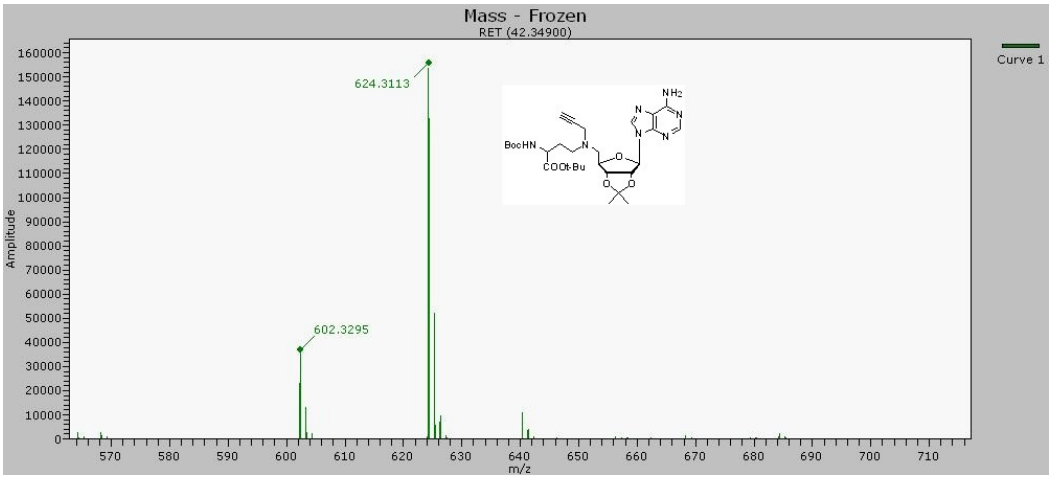

polar-H
PROTON128 CDCl3 /opt/topspin Zhang.G 8
1.65
1.38
4.54
NH2
H2N
H3C
CH3

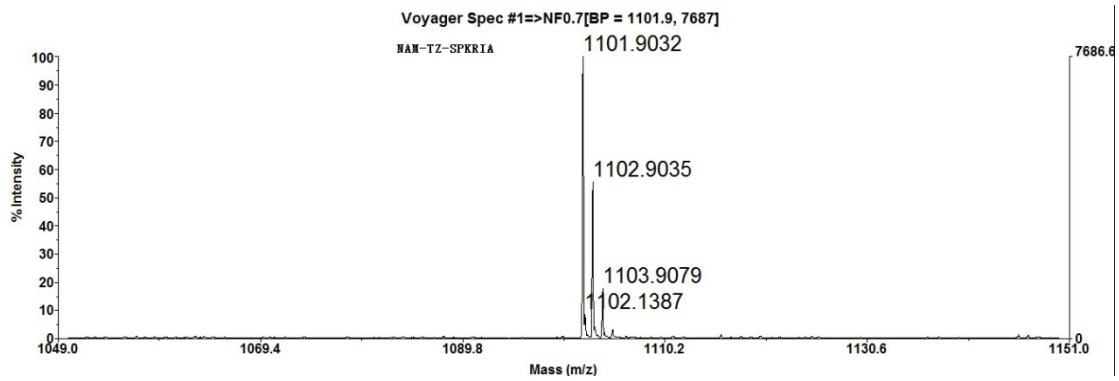

Mass - Frozen
RET (22.15108)
Curve 1
307.1528
329.1354
Amplitude
m/z

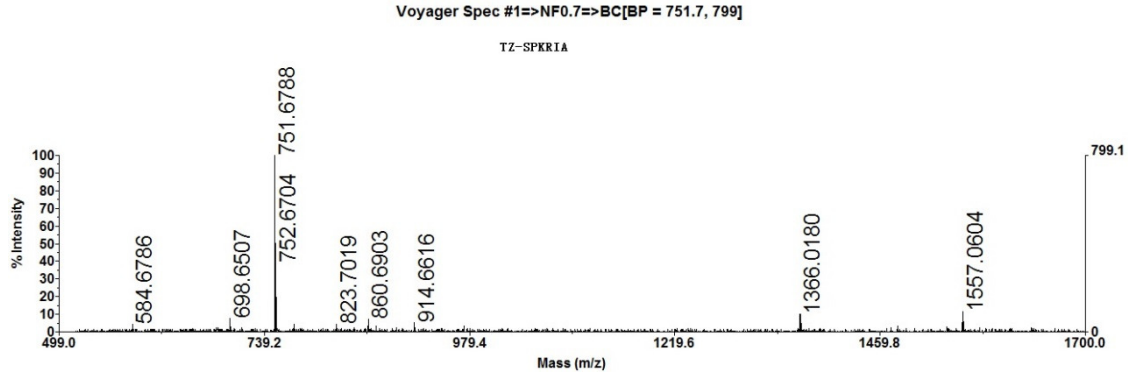

gz2-32
PROTON CDCl3 /opt/topspin Zhang.G 10

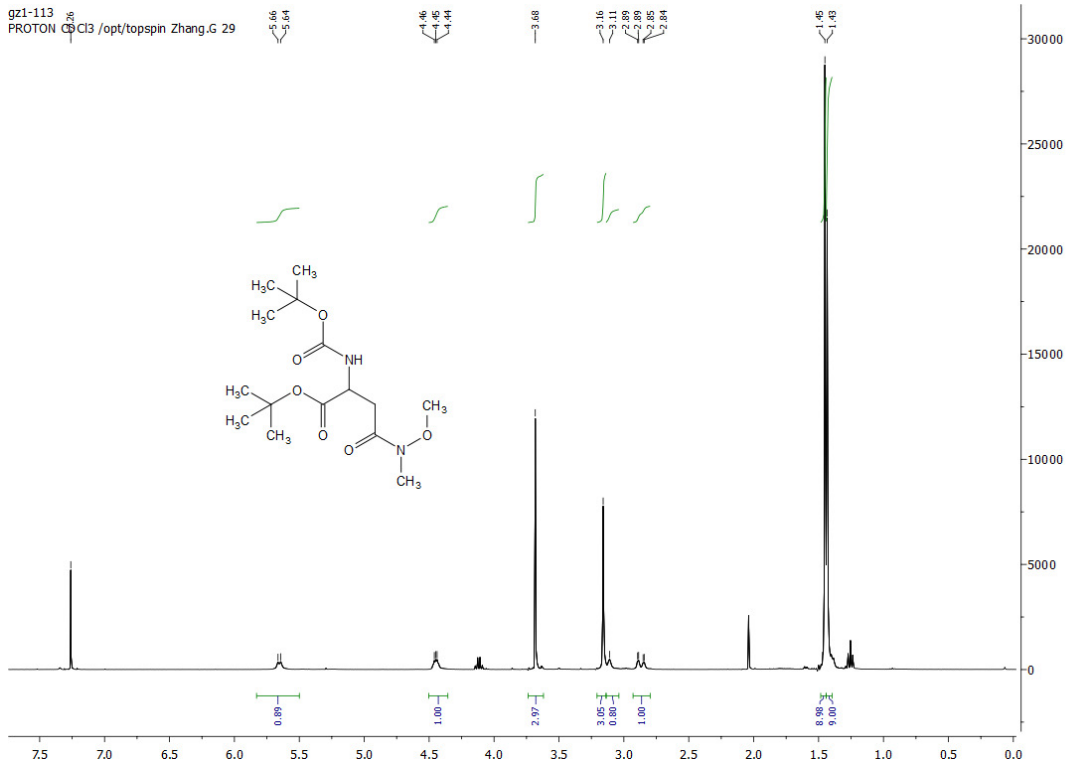

Mass - Frozen
RET (30.79541)
Curve 1
564.3156
Amplitude
m/z

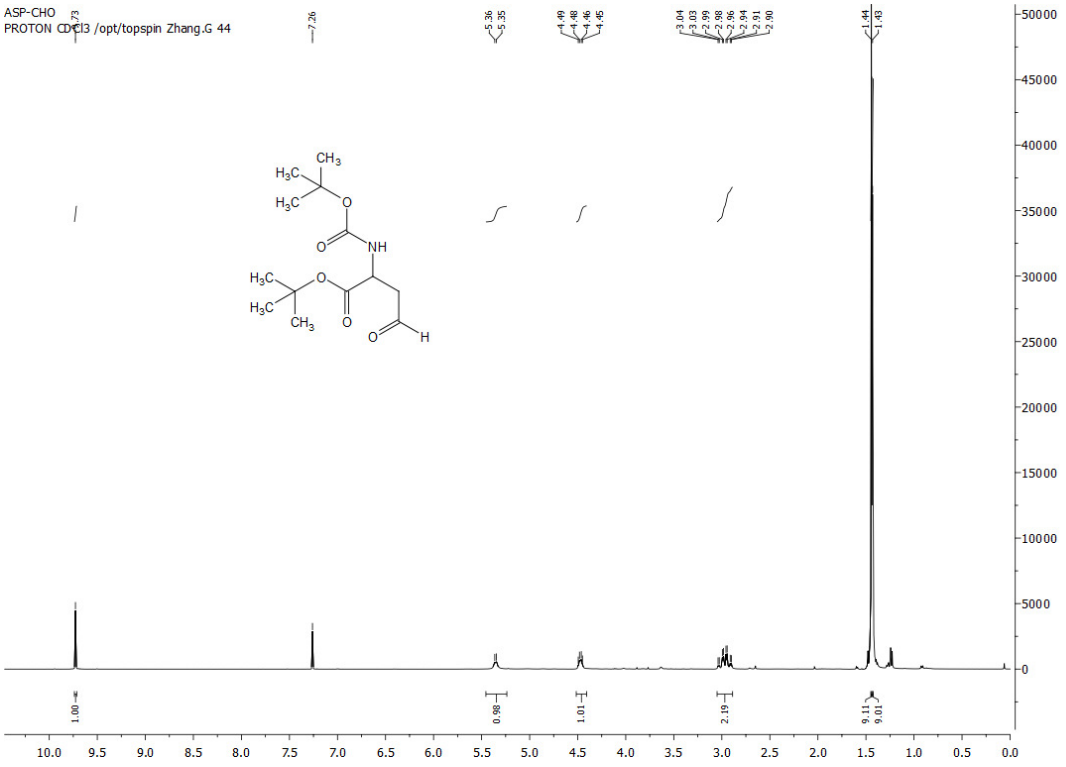

gz1-118
PROTON CDCl3 /opt/topspin Zhang-G 27

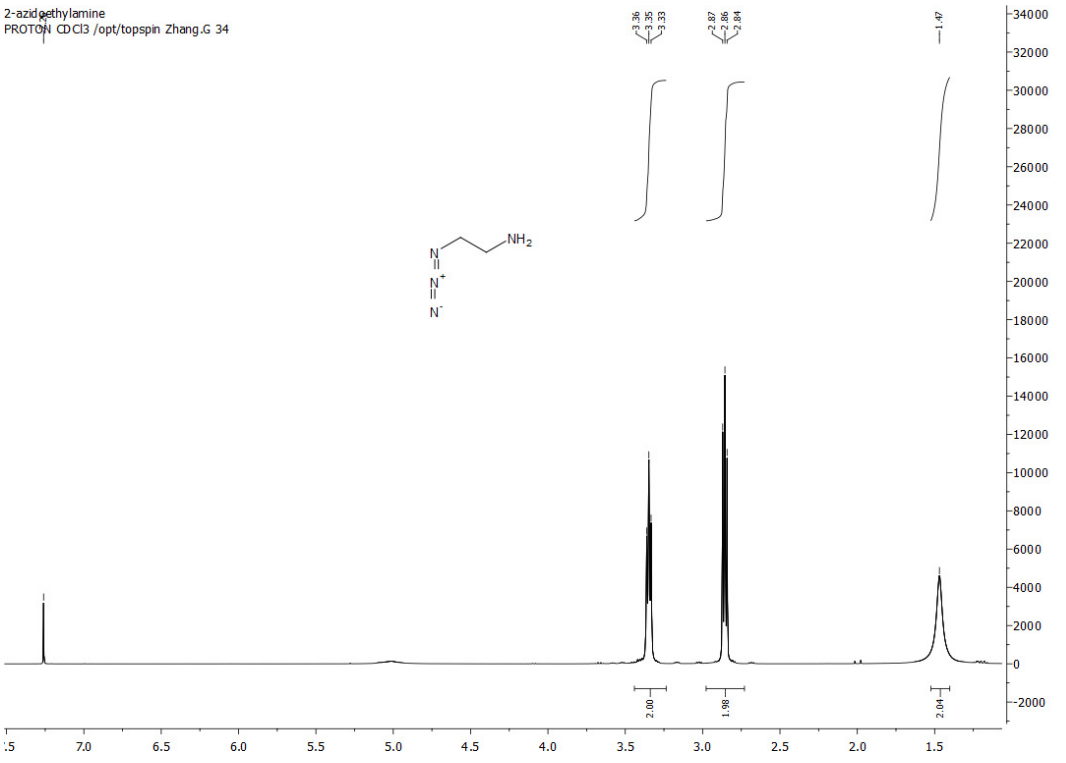

Mass - Frozen
RET (42.34900)
Curve 1
624.3113
602.3295
Amplitude
m/z


Voyager Spec #1=>NF0.7[BP = 1101.9, 7687]
NAM-TZ-SPKRIA
1101.9032
1102.9035
1103.9079
1102.1387
7686.6
% Intensity
Mass (m/z)
1049.0
1069.4
1089.8
1110.2
1130.6
1151.0


Voyager Spec #1=>NF0.7=>BC[BP = 751.7, 799]
TZ-SPKRIA
751.6788
752.6704
584.6786
698.6507
823.7019
860.6903
914.6616
1366.0180
1557.0604
799.1
% Intensity
Mass (m/z)
499.0
739.2
979.4
1219.6
1459.8
1700.0


gz1-113
PROTON CDCl3 /opt/topspin Zhang.G 29
7.26
5.66
5.64
4.46
4.45
4.44
3.68
3.16
3.11
2.89
2.85
2.84
1.45
1.43
0.89
1.00
2.97
3.05
0.80
1.00
8.98
9.00
NH
CH3
H3C
O
N
30000
25000
20000
15000
10000
5000
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


ASP-CHO
PROTON CDCl3 /opt/topspin Zhang.G 44
9.73
7.26
5.36
5.35
4.49
4.48
4.46
4.45
3.04
3.03
2.99
2.98
2.96
2.94
2.91
2.90
1.44
1.43
1.00
0.88
1.01
2.19
9.11
9.01


2-azidoethylamine
PROTON CDCl3 /opt/topspin Zhang.G 34
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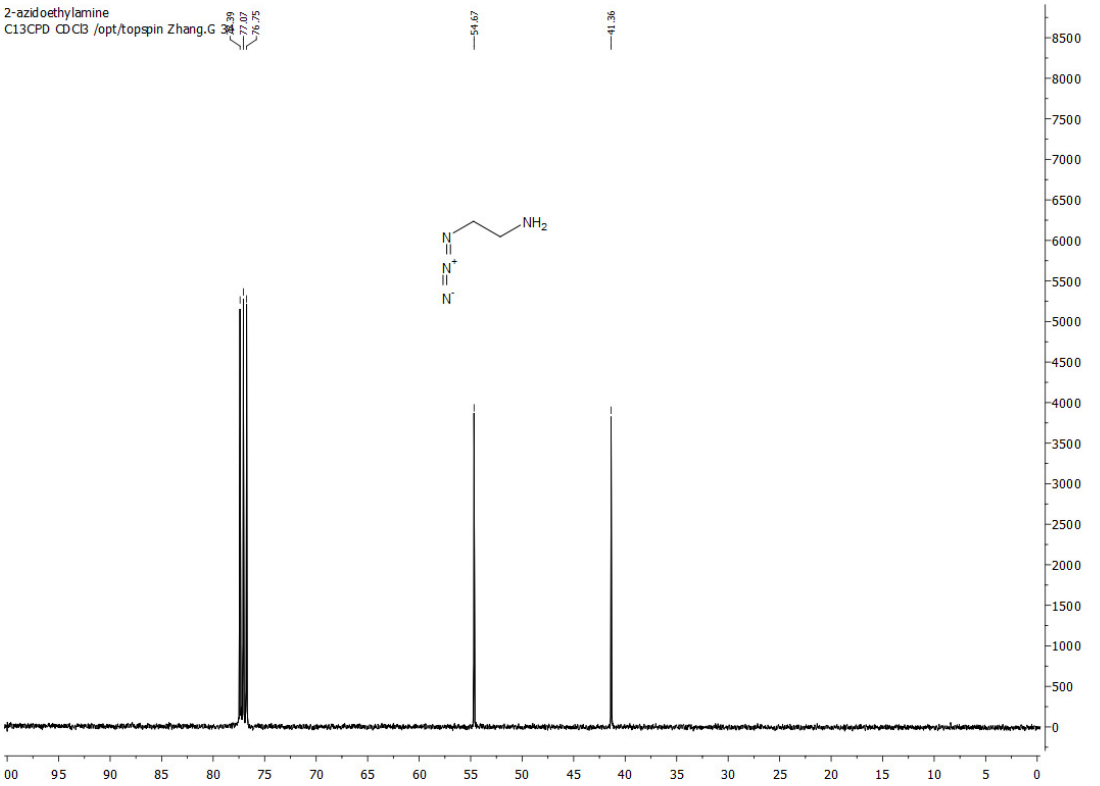
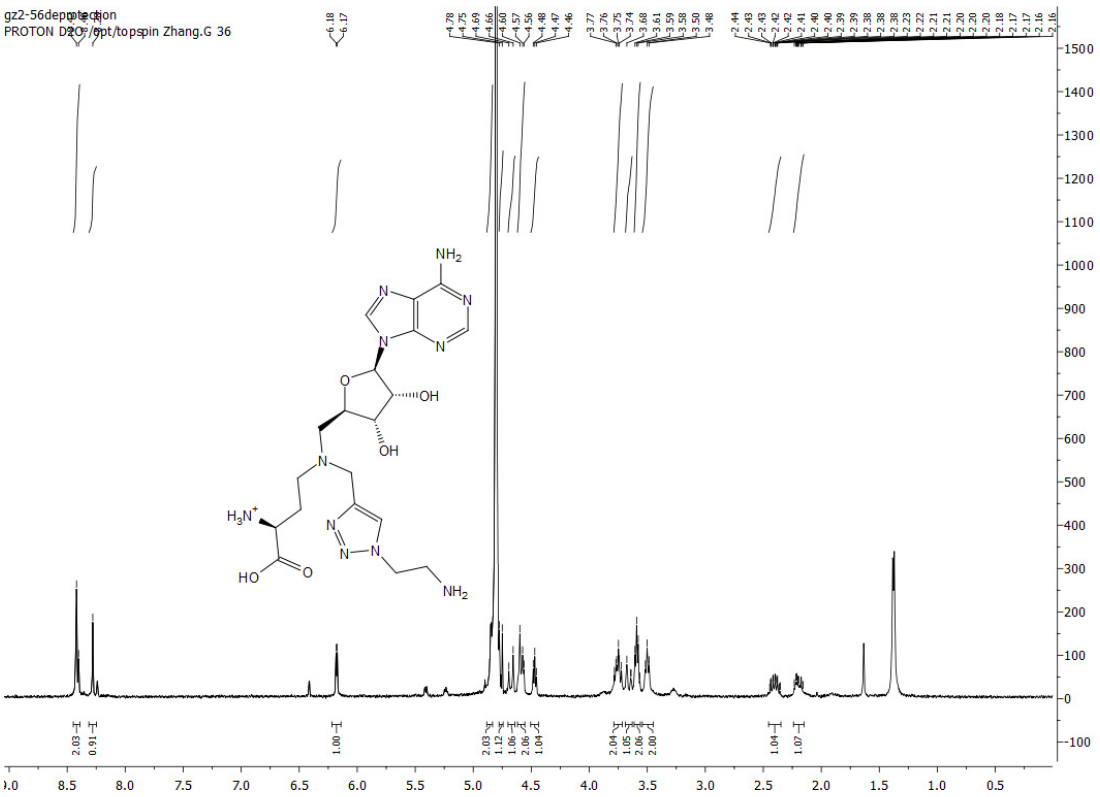
3.35
3.33
2.87
2.86
2.84
1.47
2.00
1.98
2.04


2-azidoethylamine
C13CPD CDCl3 /opt/topspin Zhang.G 36
77.39
77.07
76.75
54.67
41.36
NH2
N
N+
N-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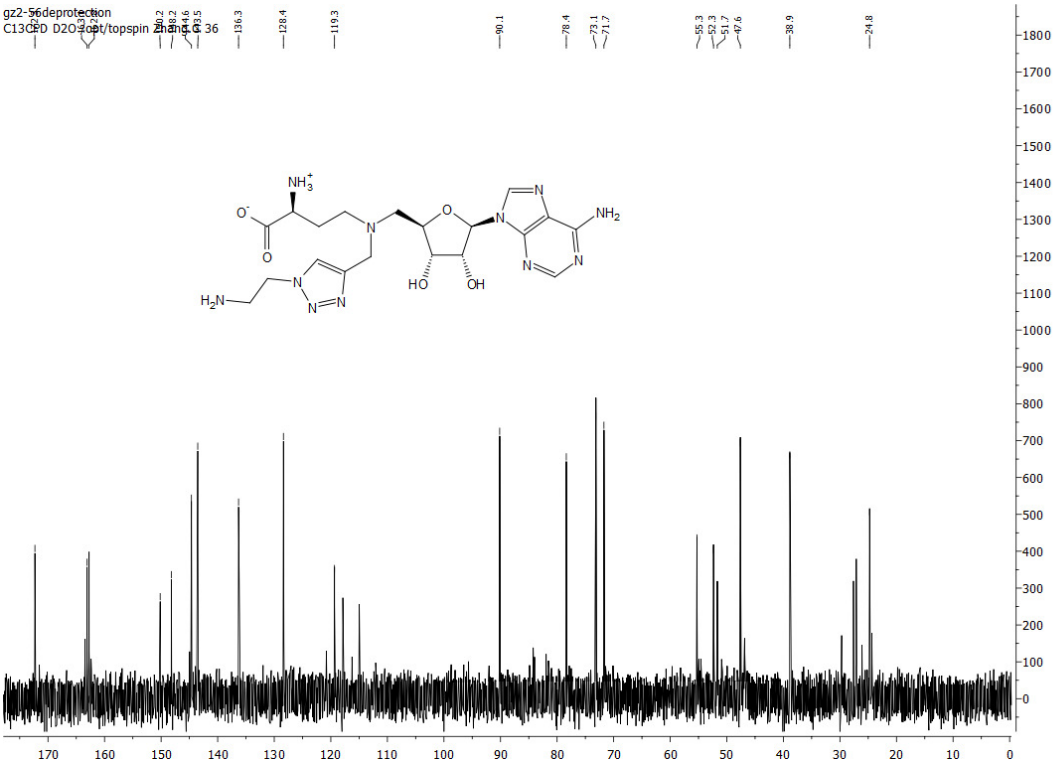
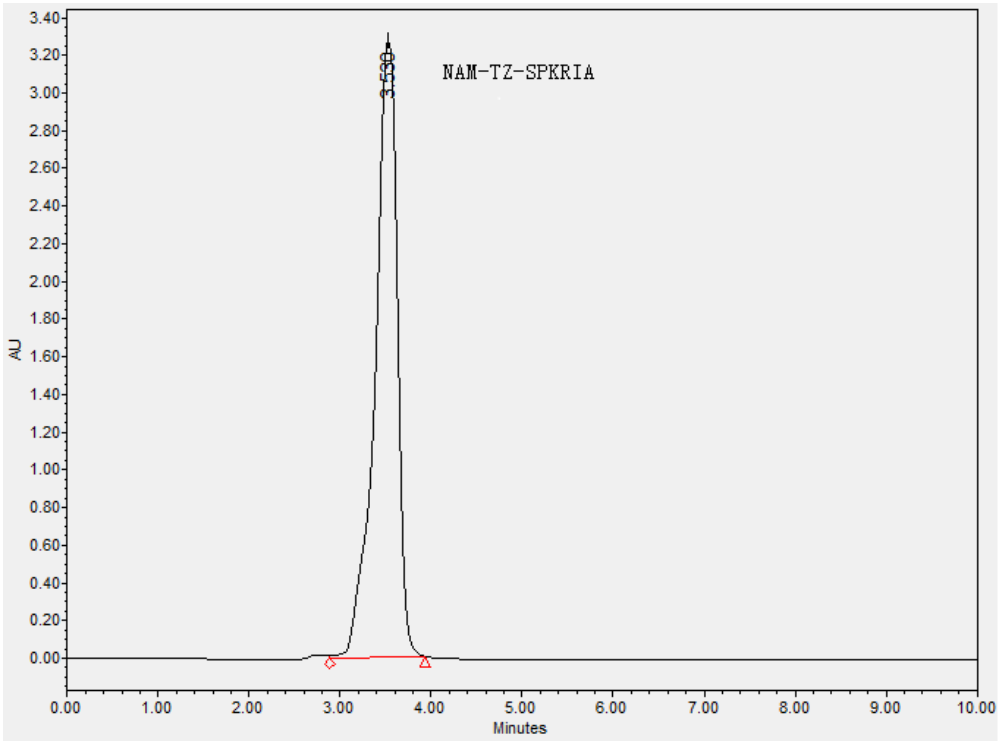
PROTON D2O /opt/topspin Zhang.G 36
6.18
6.17
4.78
4.75
4.69
4.66
4.60
4.57
4.56
4.48
4.47
4.46
3.77
3.76
3.75
3.74
3.68
3.61
3.59
3.58
3.50
3.48
2.44
2.43
2.43
2.42
2.42
2.41
2.40
2.39
2.39
2.38
2.38
2.23
2.22
2.21
2.21
2.20
2.20
2.20
2.18
2.17
2.17
2.16
2.16
NH2
OH
OH
H3N+
HO
O
2.03
0.91
1.00
2.03
1.12
1.06
2.06
1.04
2.04
1.05
2.06
2.00
1.04
1.07


gz2-56deprotection
C13CPD D2O+cat/topspin 2ha nLG 36
136.3
128.4
119.3
90.1
78.4
73.1
71.7
55.3
52.3
51.7
47.6
38.9
24.8
NH3+
O-
O
N
O
N
N
N
N
NH2
HO
OH
H2N
N
N
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0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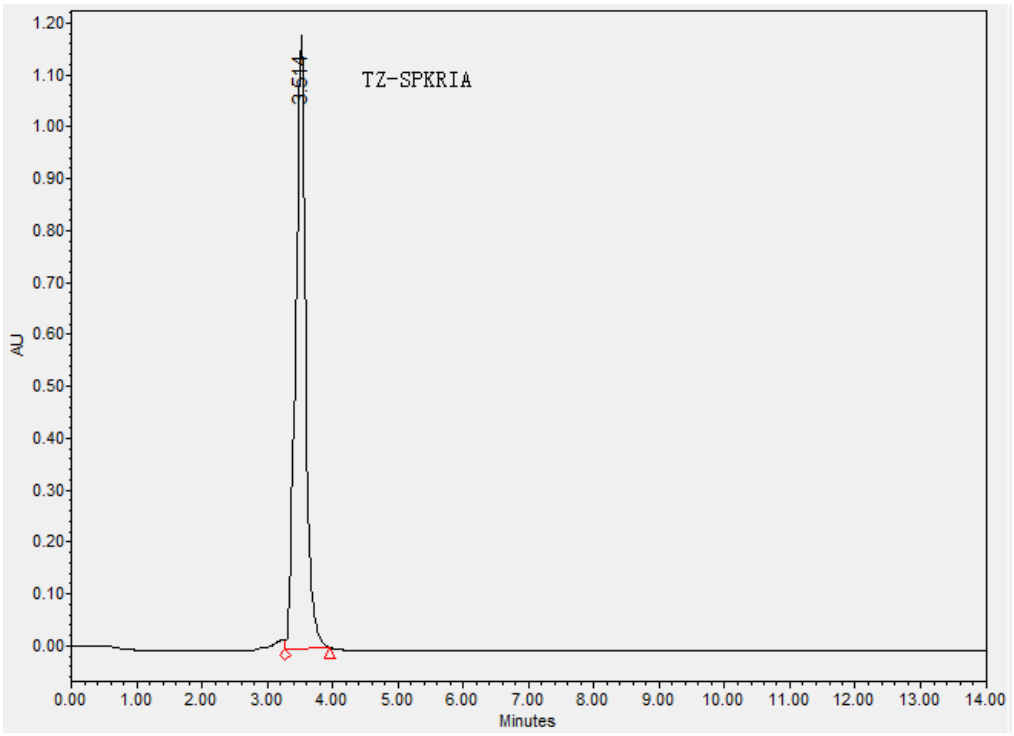
3.550
AU
Minutes
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00


TZ-SPKRIA
3.514
AU
Minutes
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00