

Supporting Information

Control of STING agonistic/antagonistic activity using amine skeleton-based c-di-GMP analogues

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Supplementary Results

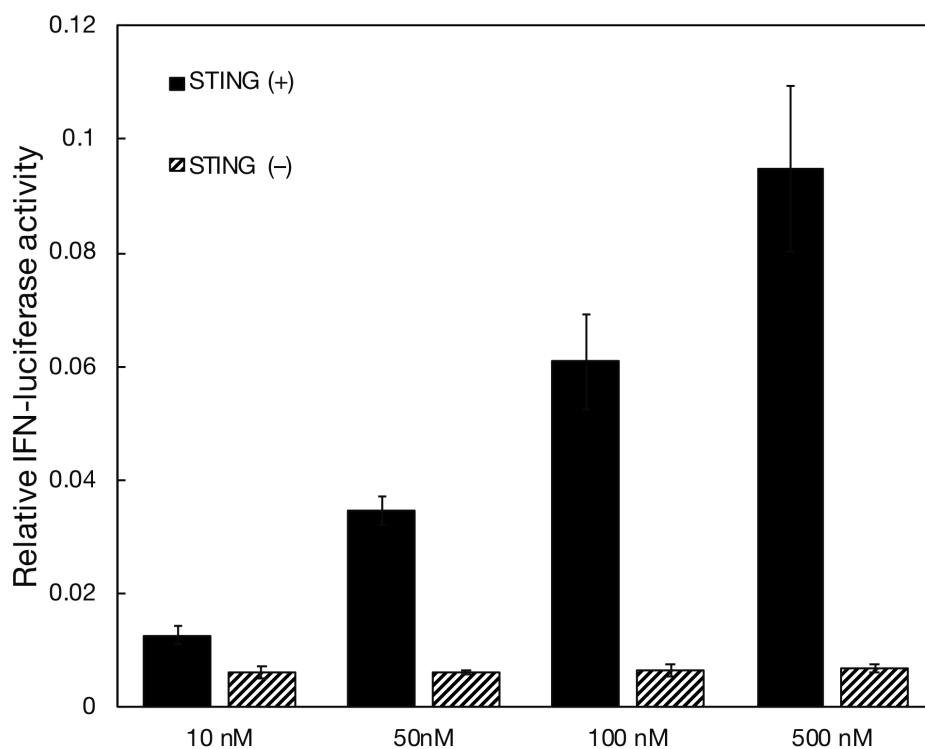


Figure S1. The evaluation of the IFN-β induction activity of cGAMP with/without STING transfection. HEK293T cells were transfected with vectors encoding Flag-STING or Flag-Empty as indicated, together with an IFN-luciferase reporter, and luciferase activity was measured 6 h after stimulation. Data are presented as mean ± SEM (*n* = 3).

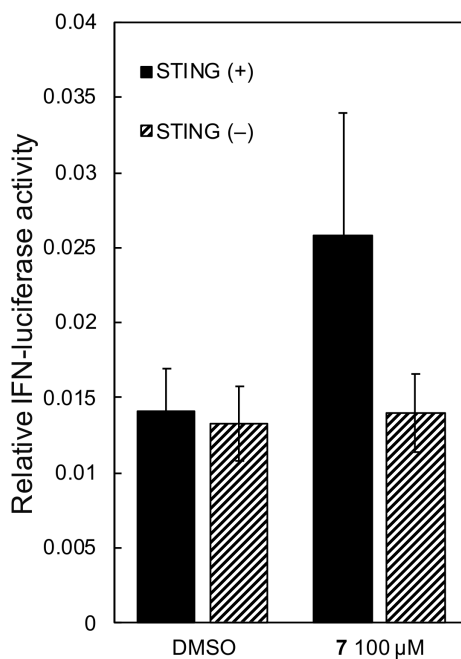


Figure S2. The evaluation of the IFN-β induction activity of **7** with/without STING transfection. HEK293T cells were transfected with vectors encoding Flag-STING or Flag-Empty as indicated, together with an IFN-luciferase reporter, and luciferase activity was measured 6 h after stimulation. Data are presented as mean ± SEM (*n* = 3).

Table S1. The evaluation of the IFN- β induction activity of cGAMP and **amine skeleton compounds** with digitonin permeabilization. HEK293T cells were transfected with vectors encoding STING as indicated, together with an IFN-luciferase reporter, and luciferase activity was measured at 6 h after stimulation.

Cpd. (100 μ M)	Agonistic Activity	Antagonistic Activity
DMSO (1%)	0.040	N.A.
cGAMP (500 nM)	1.217	N.A.
7	0.075	0.97
9	0.045	1.41
11	0.039	1.53
12	0.045	0.78
13	0.048	1.42
14	0.043	1.24
16	0.040	1.40
17	0.042	1.29
21	0.045	1.74
26	0.047	1.12
28	0.038	1.52

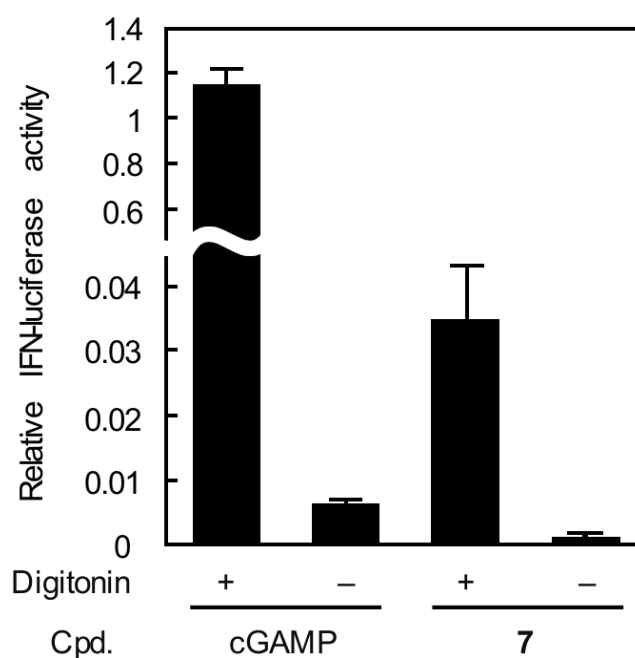


Figure S3. The evaluation of the IFN- β induction activity of cGAMP and **7** with/without digitonin permeabilization. HEK293T cells were transfected with vectors encoding STING as indicated, together with an IFN-luciferase reporter, and luciferase activity was measured 6 h after stimulation. IFN-luciferase activities were standardized by subtract the DMSO-treated control as background. Data are presented as mean \pm SEM ($n = 3$).

Table S2. The evaluation of the IFN- β induction activity of cGAMP and **7** with/without digitonin permeabilization. HEK293T cells were transfected with vectors encoding STING as indicated, together with an IFN-luciferase reporter, and luciferase activity was measured 6 h after stimulation. IFN-luciferase activities were standardized by subtract the DMSO-treated control as background.

	cGAMP	Compound 7
Digitonin -/+ ^[a]	0.0056	0.037

[a] Relative value of luciferase activity of digitonin (-) to luciferase activity of digitonin (+)

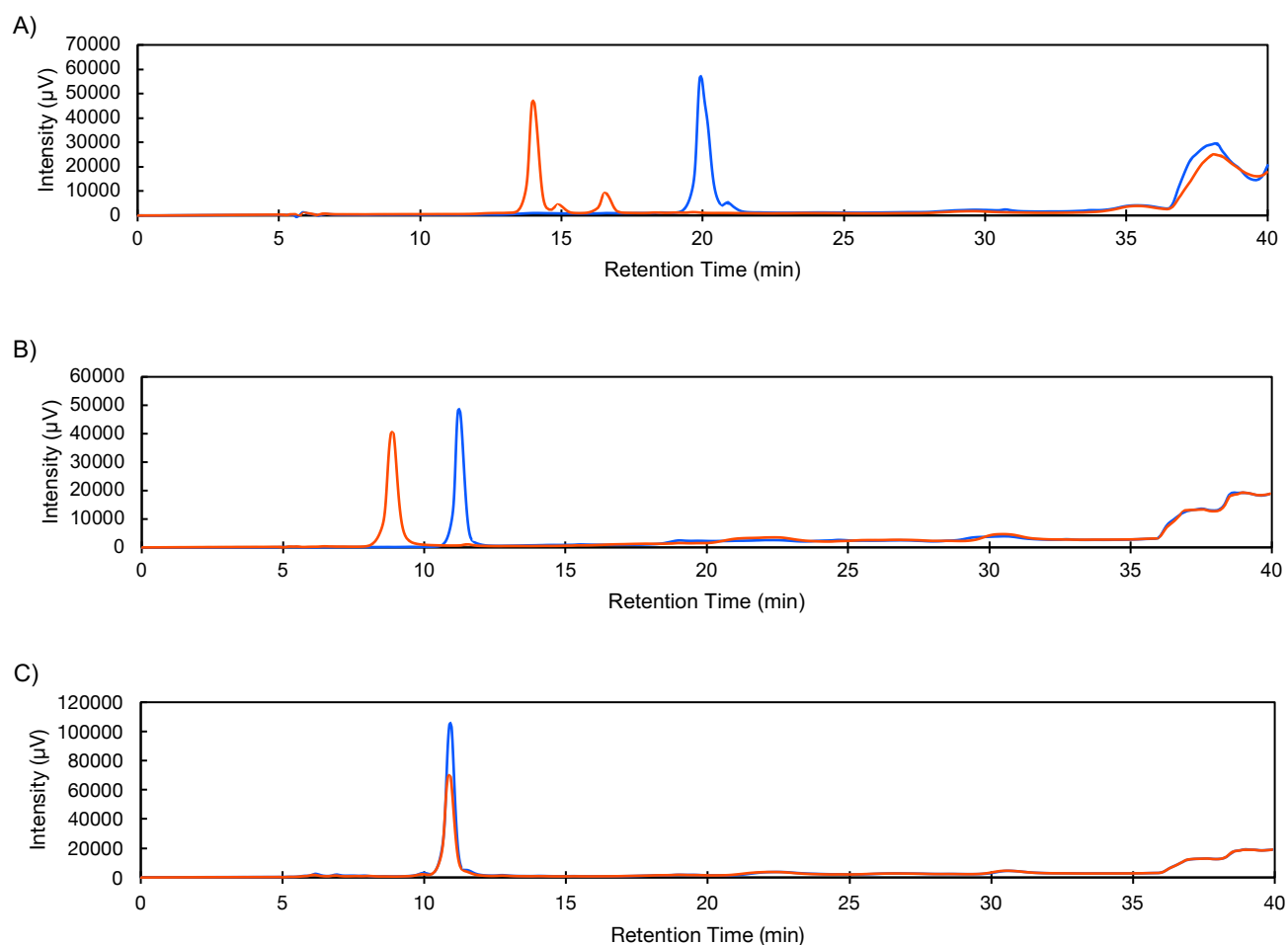


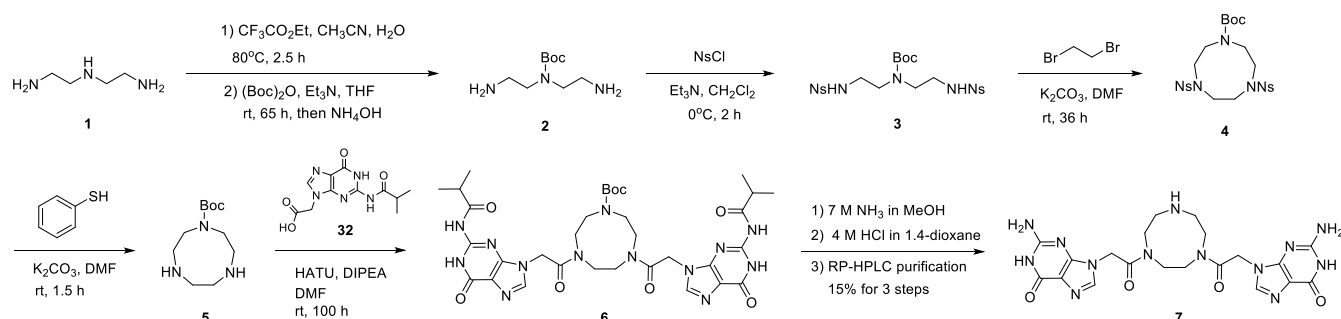
Figure S4. HPLC profile of the enzymatic digestion products of A) c-di-GMP, B) cGAMP, C) compound **7**. Red: NP1 (+), Blue: NP1(-).

Experimental procedures

Chemistry^[S1]

General information

All chemicals were obtained from Sigma-Aldrich, FUJIFILM Wako Pure Chemical, Tokyo Chemical Industry Co., Ltd., and were used as received without further purification. TLC analysis, which was used to monitor the progress of reactions, was conducted using Merck silica gel 60 F254 pre-coated plates, visualizing with a 254/365 nm UV lamp and staining with iodine or ninhydrin. Column chromatography was performed using silica gel (spherical, neutral) purchased from Kanto Chemical or NH silica gel (CHROMATOREX NH-DM1020) purchased from Fuji Silysia, or medium pressure chromatography (Smart Flash; YAMAZEN) equipped with a Hi-Flash column and an Inject column (YAMAZEN). HPLC was performed using LC-2000Plus (JASCO). ¹H and ¹³C NMR spectra were recorded on an ECZ 600R spectrometer (JEOL), and measurements were carried out using deuterated solvents. Chemical shift values (ppm) were calibrated using tetramethylsilane (TMS, 0.00 ppm) or residual non-deuterated solvent peaks as an internal reference (CDCl₃: 7.26 for ¹H NMR, 77.0 for ¹³C NMR; CD₃OD: 3.30 for ¹H NMR, 49.0 for ¹³C NMR; DMSO-*d*₆: 2.50 for ¹H NMR, 39.5 for ¹³C NMR). Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint.), septet (sept.), double of doublets (dd), doublet of triplet (dt), multiplet (m), broad (br.). High-resolution mass spectrum (HRMS) were measured using a Shimadzu IT-TOF MS equipped with an electrospray ionization source in positive mode or negative mode.

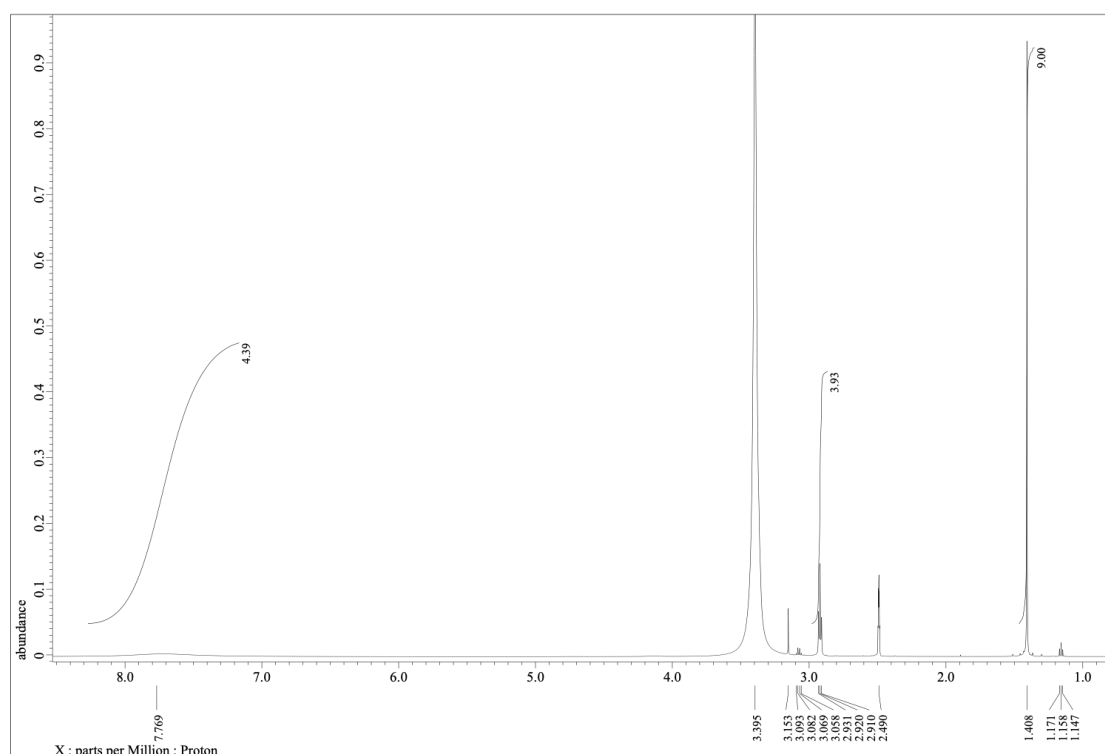


Scheme S1. Synthesis of compound 7

tert-Butyl bis(2-(2,2,2-trifluoroacetamido)ethyl)carbamate (2)^[S2]

Diethylenetriamine (1.09 mL, 10 mmol) was dissolved in ACN (20 mL) containing water (200 µL). Ethyl trifluoroacetate (4.04 mL, 3.5 eq) was added to the solution, then the solution was refluxed for 14 h at 80°C. The solvents were removed under reduced pressure, the residue was added 1:1 ACN – Et₂O (20 mL). The solvents were removed under reduced pressure, dried under vacuum. The resulting residue was dissolved in THF (7 mL). Et₃N (2.09 mL, 1.5 eq) and (Boc)₂O (3.31 g, 1.5 eq) in THF (3 mL) was added dropwise into the solution under stirring at 0°C. The mixture was stirred over weekend at rt. Almost solvents were removed under reduced pressure. The resulting residue was dissolved in 28% NH₄OH (10 mL) and MeOH (5 mL), stirred over weekend at 50°C. The reaction mixture was concentrated under reduced pressure, purified by flash silica gel column chromatography (ϕ = 42 mm, h = 145 mm, CH₂Cl₂/MeOH/28% NH₄OH = 20:5:1) to afford a pale yellowish oil including 2 (4.63 g). This oil was used next reaction without further purification.

¹H NMR (600 MHz, DMSO-*d*₆) δ 7.77 (br.s, 4H), 2.92 (t, *J* = 7.5 Hz, 4H), 1.41 (s, 9H). ESI-HRMS calcd for C₉H₂₂N₃O₂ [M+H]⁺: 204.1707, found: 204.1716.

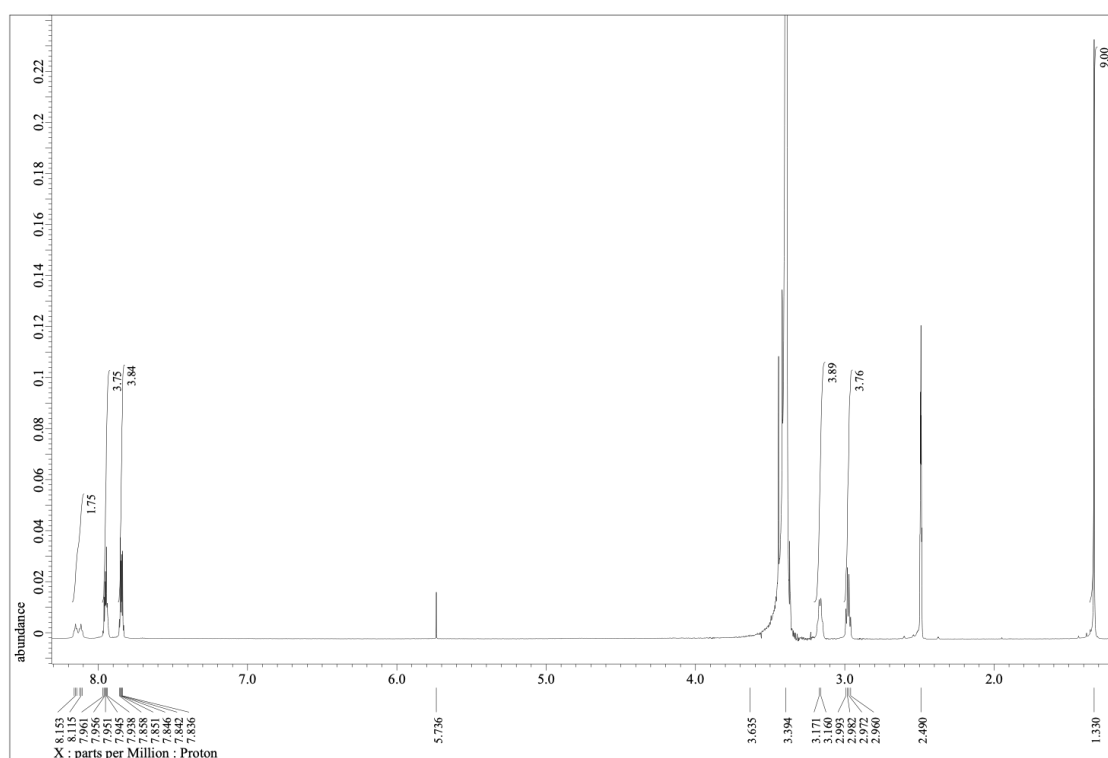


¹H NMR spectrum of **2**

tert-Butyl bis(2-(2-nitrophenylsulfonamido)ethyl)carbamate (3**)**

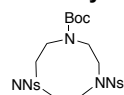
The oil including **2** (203 mg) was dissolved in CH₂Cl₂ (5 mL), added Et₃N (613 μL, 4.4 eq) at rt. 2-Nitrobenzensulfonyl chloride (487.5 mg, 2.2 eq) was added into the mixture under stirring at 0°C, the mixture was continue stirring for 2 h at 0°C. The reaction mixture was diluted with CH₂Cl₂, washed with KHSO₄ aq. (2 M), brine. The organic layer was dried over Na₂SO₄, filtered, concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (Yamazen inject column size: M, Yamazen Hi-flash column size: M, CH₂Cl₂/MeOH = 100:0 to 97:3) to afford **3** (169 mg, 0.3 mmol, 68% yield for 4 steps) as a white foam solid.

¹H NMR (600 MHz, DMSO-*d*₆) δ 8.13 (dt, *J* = 5.7 Hz, 24 Hz, 2H), 7.94-7.97 (m, 4H), 7.84-7.86 (m, 4H), 3.17 (d, *J* = 6.6 Hz, 4H), 2.98 (q, *J* = 6.6 Hz, 4H), 1.33 (s, 9H). ESI-HRMS calcd for C₂₁H₂₈N₅O₁₀S₂ [M-H]⁻: 572.1126, found: 572.1082.



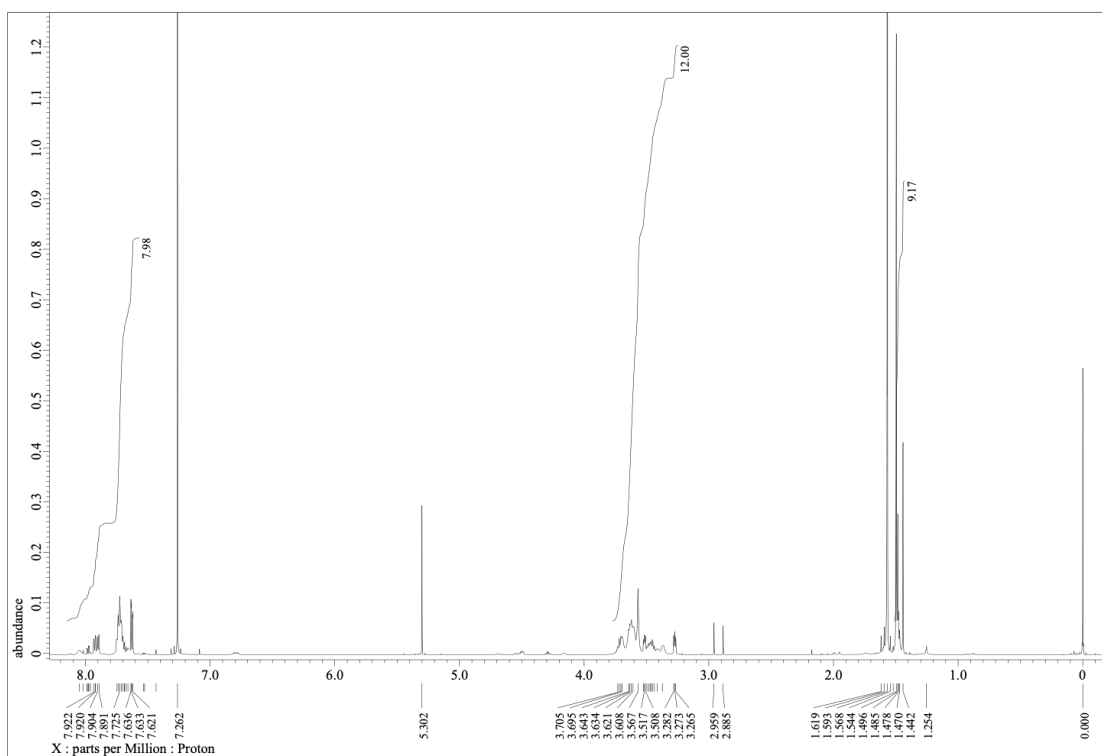
¹H NMR spectrum of **3**

tert-Butyl 4,7-bis((2-nitrophenyl)sulfonyl)-1,4,7-triazonane-1-carboxylate (4**)**



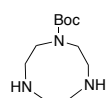
Compound **3** (229 mg, 0.4 mmol) was dissolved in DMF (1 mL), added K₂CO₃ (110 mg, 2 eq) at rt then stirred at 0°C. 1,2-dibromoethane (75 mg, 1 eq) in DMF (1 mL) was added dropwise into the mixture at 0°C. The mixture was stirred for overnight at rt. K₂CO₃ (55 mg, 1 eq) and 1,2-dibromoethane (75 mg, 1 eq) was added into the mixture, stirred for over weekend. The reaction mixture was diluted with EtOAc, washed with HCl (2 M) twice, sat. aq. NaHCO₃, brine. The organic layer was dried over Na₂SO₄, filtered, concentrated under reduced pressure. The residue was purified by NH silica gel column chromatography (ϕ = 15 mm, h = 105 mm, CH₂Cl₂/MeOH = 100:0 to 98:2) to afford **4** (150 mg, 0.25 mmol, 63% yield) as a pale yellow foam.

¹H NMR (600 MHz, CDCl₃) δ 7.62-8.05 (m, 8H), 3.27-3.73 (br.m, 12H), 1.44-1.50 (m, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 155.5, 148.6, 148.6, 134.0, 133.9, 131.8, 131.7, 130.6, 130.5, 124.2, 80.8, 53.4, 52.7, 51.7, 51.3, 50.9, 45.7, 28.3. ESI-HRMS calcd for C₁₁H₂₂NO₂ [M-H]⁻: 598.1283, found: 598.1246.



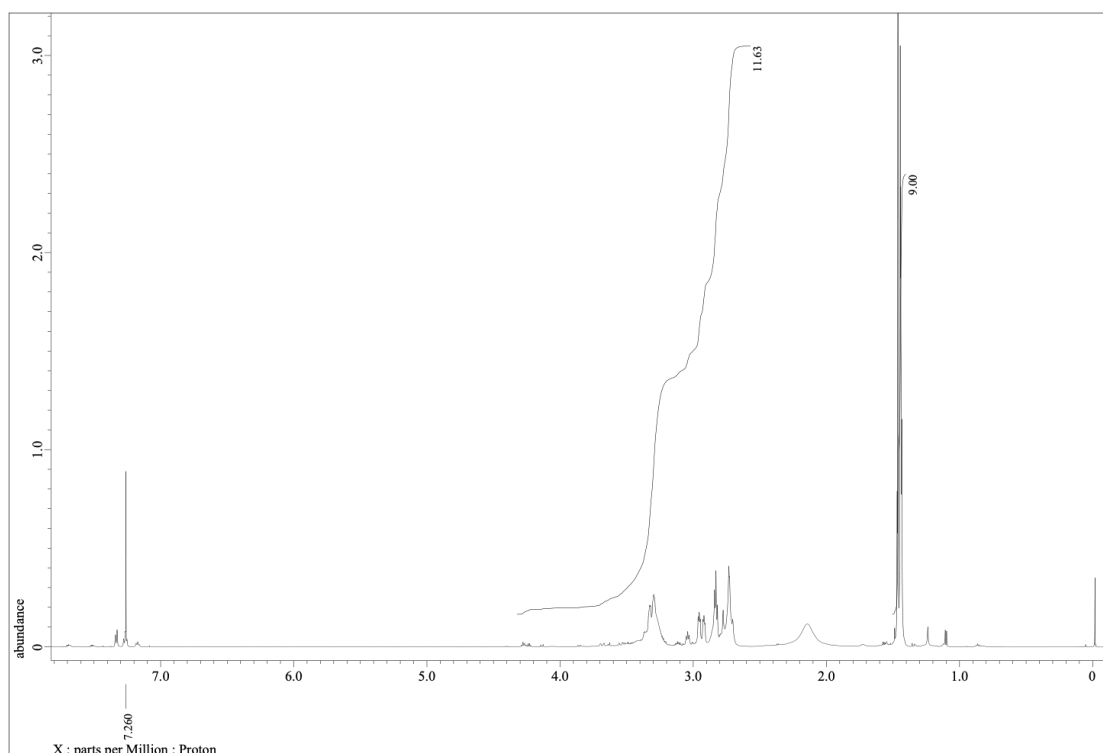
¹H NMR spectrum of **4**

***tert*-Butyl 1,4,7-triazonane-1-carboxylate (**5**)**



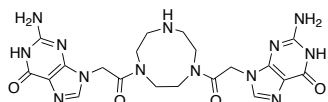
Compound **4** (150 mg, 0.25 mmol) was dissolved in DMF (1.2 mL), added K₂CO₃ (111 mg, 3.2 eq) at rt then stirred at 0°C. Thiophenol (82 µL, 3.2 eq) was added into the mixture at 0°C, stirred for 1.5 h at rt. The reaction mixture was diluted with CH₂Cl₂, filtered, concentrated under reduced pressure, dried under vacuum. The residue was purified by NH silica gel column chromatography (ϕ = 25 mm, h = 70 mm, CH₂Cl₂/MeOH = 100:0 to 92:8) to afford **5** (47.4 mg, 0.21 mmol, 82% yield) as a yellow foam.

¹H NMR (600 MHz, CDCl₃) δ 4.29-2.70 (m, 12H), 1.49-1.43 (m, 9H). ESI-HRMS calcd for C₁₁H₂₄N₃O₂ [M+H]⁺: 230.1863, found: 230.1863.



¹H NMR spectrum of **5**

2-Amino-9-(2-(4-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetyl)-1,4,7-triazonan-1-yl)ethyl)-1*H*-purin-6(9*H*)-one (7**)**

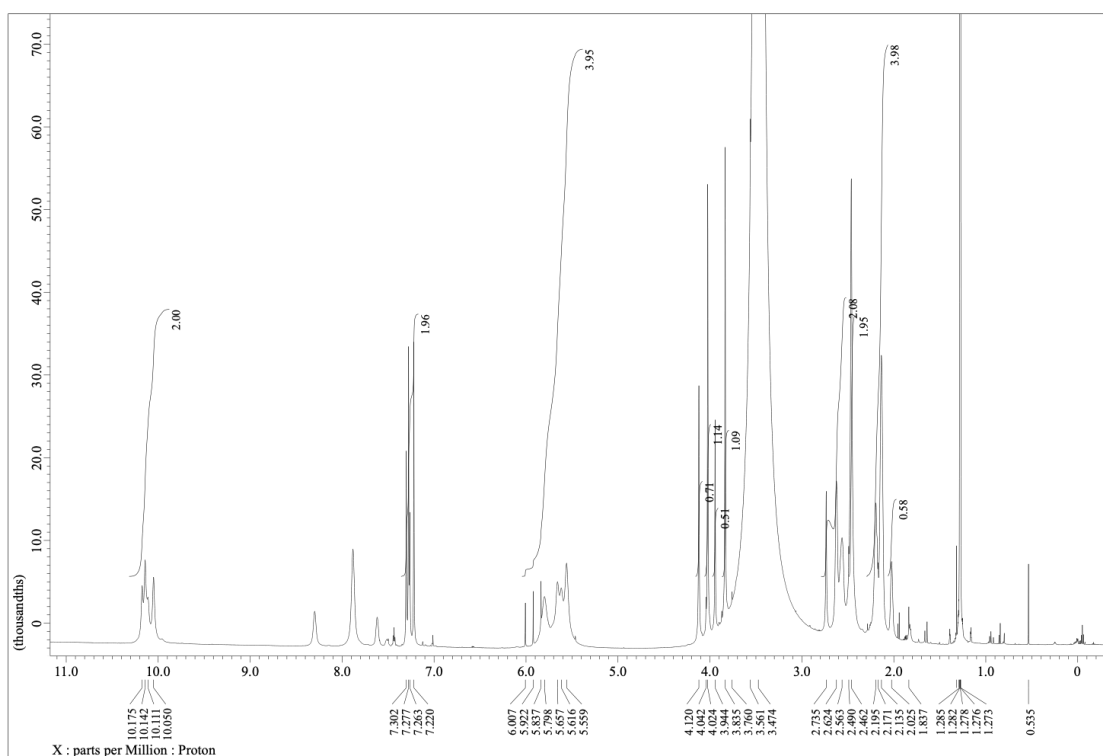


Compound **5** (47.4 mg, 0.21 mmol) was dissolved in DMF (1 mL) and stirred at 0°C. Compound **32** (126 mg, 2.2 eq), HATU (236 mg, 3 eq) and DIPEA (216 µL, 6 eq) was added into the above mixture, stirred for over weekend at rt. The reaction mixture was concentrated under reduced pressure.

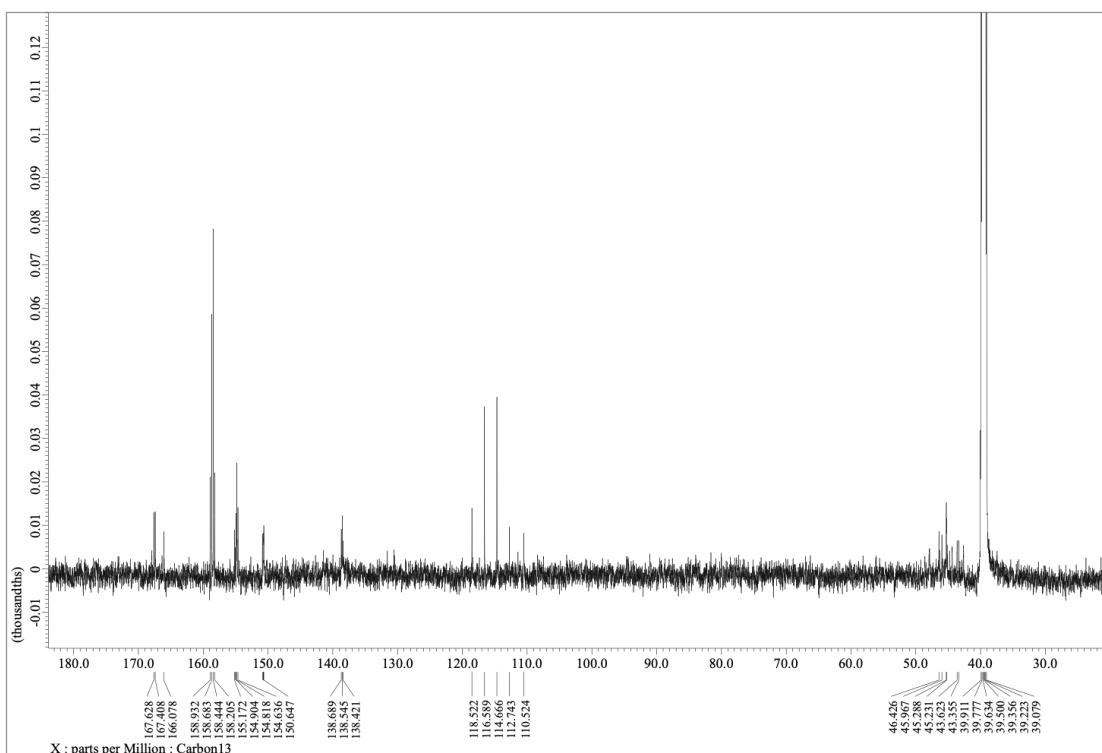
The resulting residue was added NH₃ in MeOH (7 M, 1 mL), stirred for overnight at rt. The mixture was added additional NH₃ in MeOH (7 M, 2 mL), stirred for over weekend at rt. The reaction mixture was concentrated under reduced pressure, dried under vacuum.

The resulting residue was added HCl in 1,4-dioxane (4 M, 3 mL), stirred for 2 h at rt. The reaction mixture was concentrated under reduced pressure, the residue was dried by codistillation with MeOH twice. The residue was purified by HPLC (LC-2000Plus (JASCO); Discovery BIO Wide Pore C18 HPLC Column (Supelco), 21.2 × 250 mm; Flow rate: 10 mL/min; 40°C; Mobile phase A: 0.1% TFA in H₂O, B: 0.1% TFA in ACN; Gradient: B 0% to 10 % (20 min); Detection: 260 nm) to afford **7** (15.8 mg, 0.031 mmol, 14.7% yield for 3 steps) as a white solid.

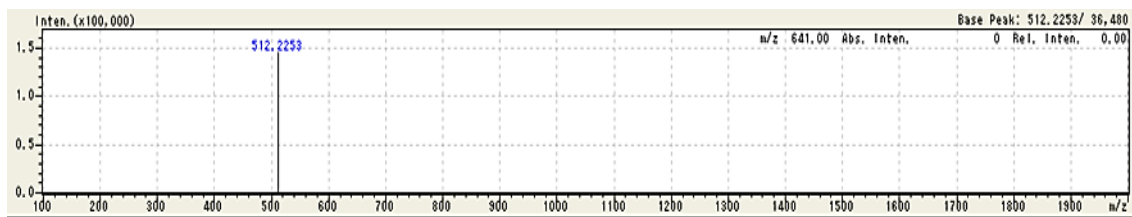
¹H NMR (600 MHz, DMSO-*d*₆) δ 10.18-10.05 (m, 2H), 7.30-7.22 (m, 2H), 6.01-5.56 (m, 4H), 4.12 (s, 1H), 4.02 (s, 1H), 3.94 (s, 1H), 3.83 (s, 1H), 2.74-2.56 (m, 2H), 2.46 (s, 2H), 2.20-2.13 (m, 4H), 2.02 (s, 1H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 167.6, 167.4, 166.1, 158.9, 158.7, 158.4, 158.2, 155.2, 155.0, 154.9, 154.8, 154.6, 150.9, 150.8, 150.7, 150.7, 150.6, 138.7, 138.5, 138.4, 118.5, 116.6, 114.7, 112.7, 110.5, 46.4, 46.0, 45.3, 45.2, 43.6, 43.4. ESI-HRMS calcd for C₂₀H₂₆N₁₃O₄ [M+H]⁺: 512.2225, found: 512.2253.



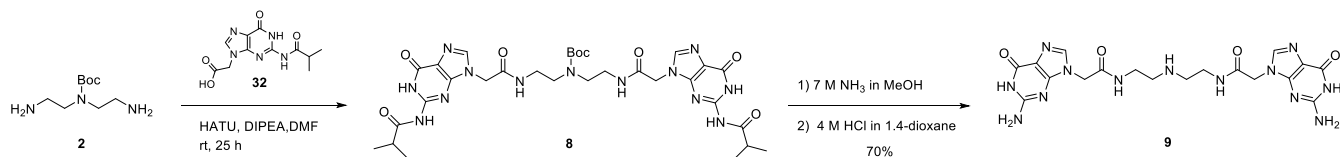
¹H NMR spectrum of 7



¹³C NMR spectrum of 7



ESI-MS spectrum of **7**



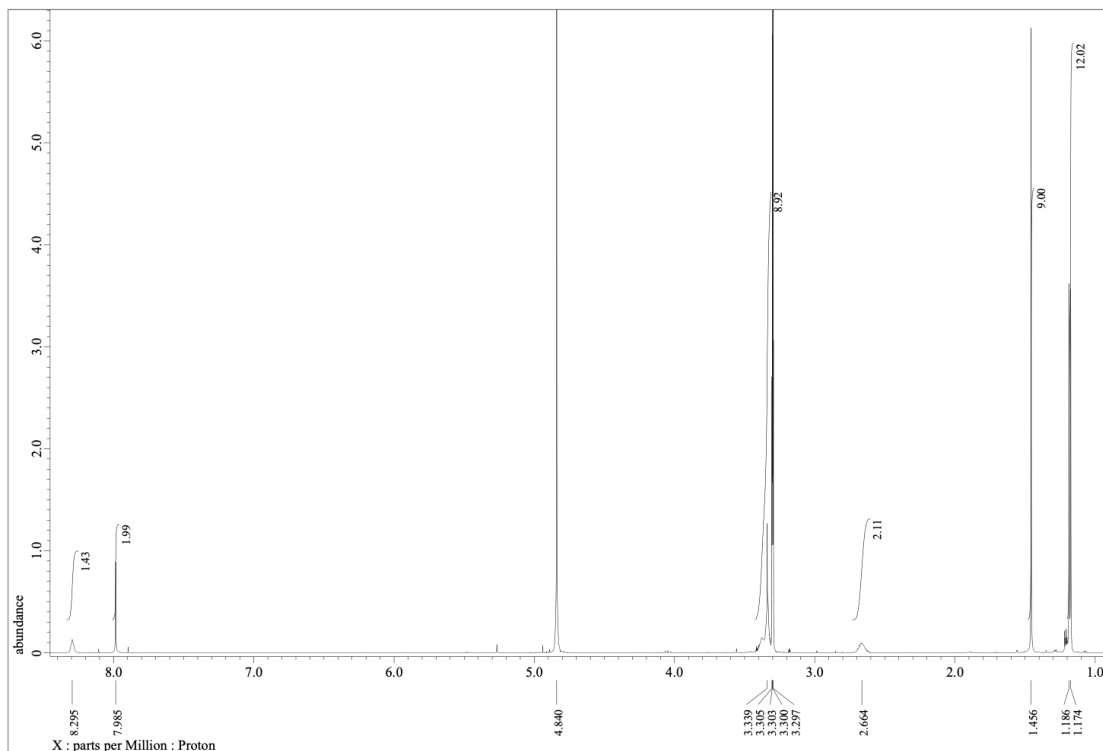
Scheme S2. Synthesis of compound **9**

tert-Butyl bis(2-(2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)acetamido) ethyl)carbamate (8**)**

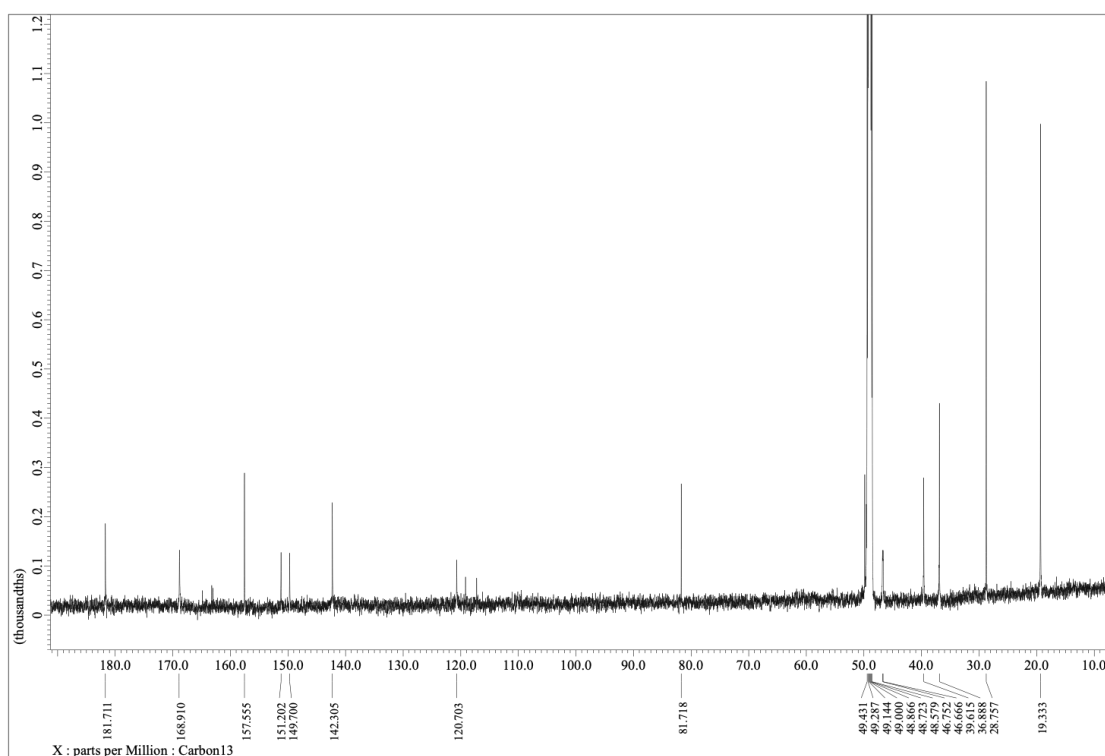
The oil including **2** (203 mg) was dissolved in DMF (5 mL) and stirred at 0°C. Compound **32** (614 mg, 2.2 eq), HATU (1.14 g, 3 eq) and DIPEA (1 mL, 6 eq) was added into the mixture, stirred for 23 h at rt. The reaction mixture was diluted with EtOAc, washed with HCl (1 M), brine. The aqueous layer was extracted 3 times with CH₂Cl₂. The organic layers were dried over Na₂SO₄, filtered, concentrated under reduced pressure, dried under vacuum. The crude mixture was absorbed on silica gel (Yamazen inject column size: M, h = 2 cm).

Purified by flash silica gel column chromatography (Yamazen Hi-flash column size: M, CH₂Cl₂/MeOH = 99:1 to 50:50) to afford **8** (117 mg, 0.16 mmol, 37% yield for 4 steps) as a pale yellow foam solid.

¹H NMR (600 MHz, CD₃OD) δ 8.30 (br.s, 1H), 7.99 (s, 2H), 3.42-3.31 (br.m, 8H), 2.66 (br.s, 2H), 1.46 (s, 9H), 1.18 (d, J = 6.9 Hz, 12H); ¹³C NMR (151 MHz, CD₃OD) δ 181.7, 168.9, 157.6, 151.2, 149.7, 142.3, 120.7, 81.7, 39.6, 36.9, 28.8, 19.3. ESI-HRMS calcd for C₃₁H₄₄N₁₃O₈ [M+H]⁺: 726.3430, found: 726.3423.

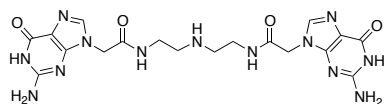


¹H NMR spectrum of **8**



¹³C NMR spectrum of **8**

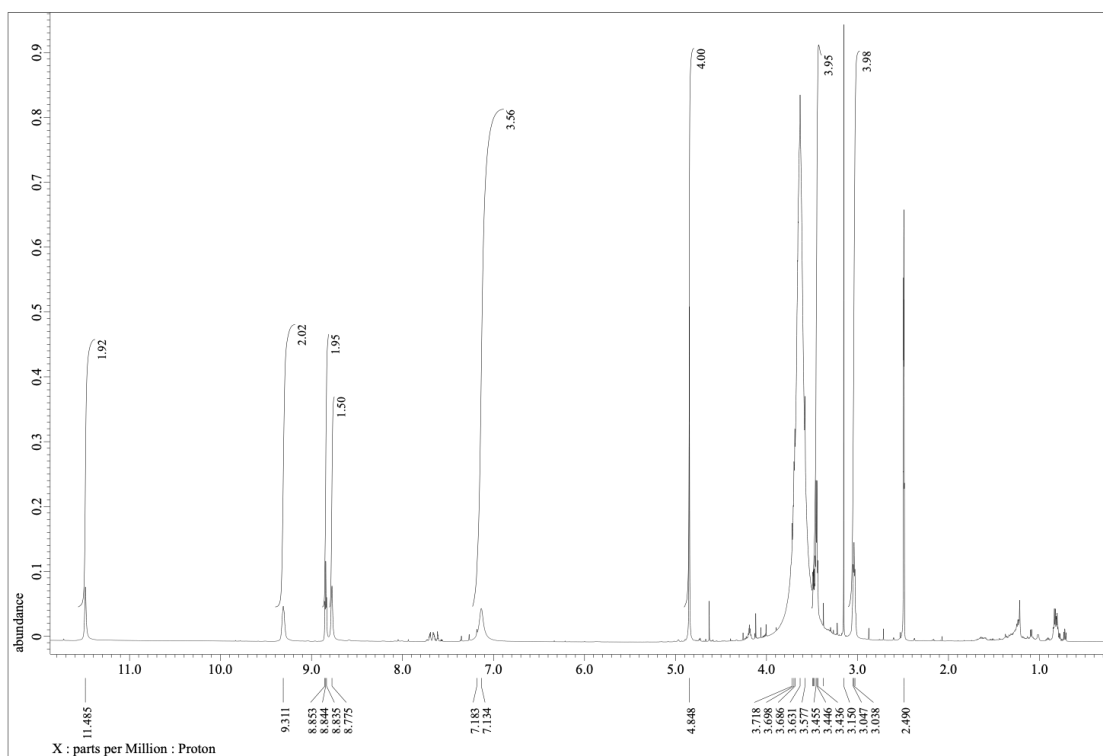
***N,N'*-(Azanediylobis(ethane-2,1-diyl))bis(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamide) (**9**)**



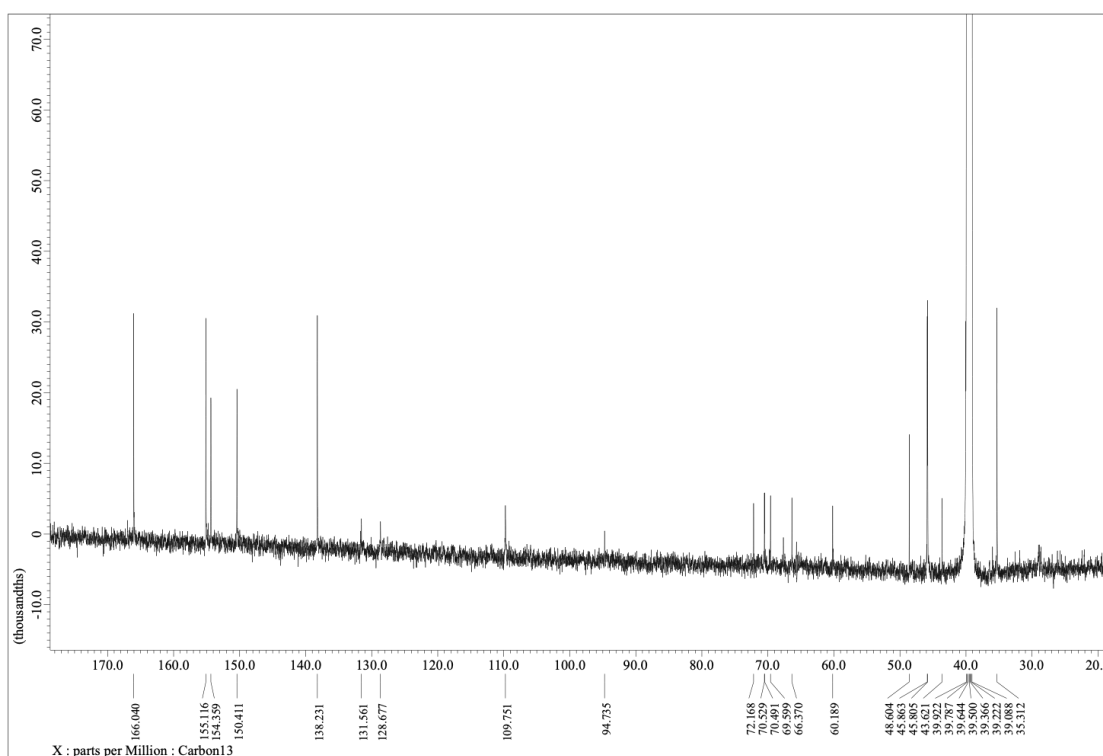
Compound **8** (10 mg, 0.014 mmol) was dissolved in NH₃ in MeOH (7 M, 1 mL), stirred over weekend at rt. The resulting precipitate was collected by centrifugation, washed with ACN, dried under vacuum.

The residue was added HCl in 1,4-dioxane (4 M, 1 mL), stirred for 2 h at rt. The reaction mixture was concentrated under reduced pressure and the residue was dried by codistillation with MeOH to afford **9** (5.8 mg, 9.8 μmol, 70% yield) as a pale yellow solid.

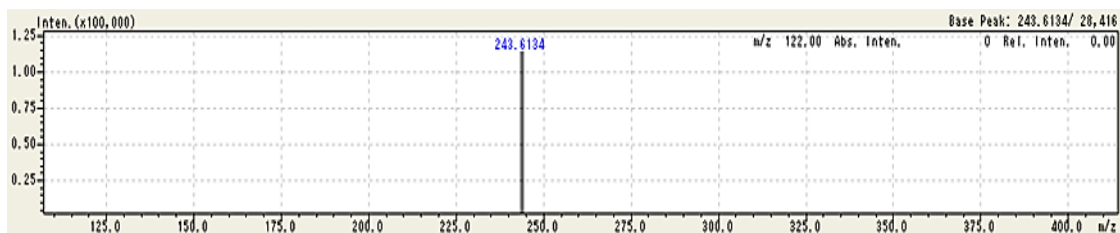
¹H NMR (600 MHz, DMSO-*d*₆) δ 11.49 (s, 2H), 9.31 (s, 2H), 8.84 (t, *J* = 5.5 Hz, 2H), 8.78 (s, 2H), 7.13 (s, 4H), 4.85 (s, 4H), 3.49-3.44 (m, 4H), 3.04 (t, *J* = 5.9 Hz, 4H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.0, 155.1, 154.4, 150.4, 138.2, 131.6, 128.7, 109.8, 94.7, 72.2, 70.5, 70.5, 69.6, 66.4, 60.2, 48.6, 45.9, 45.8, 43.6, 35.3. ESI-HRMS calcd for C₁₈H₂₄N₁₃O₄ [M+2H]²⁺: 243.6071, found: 243.6134.



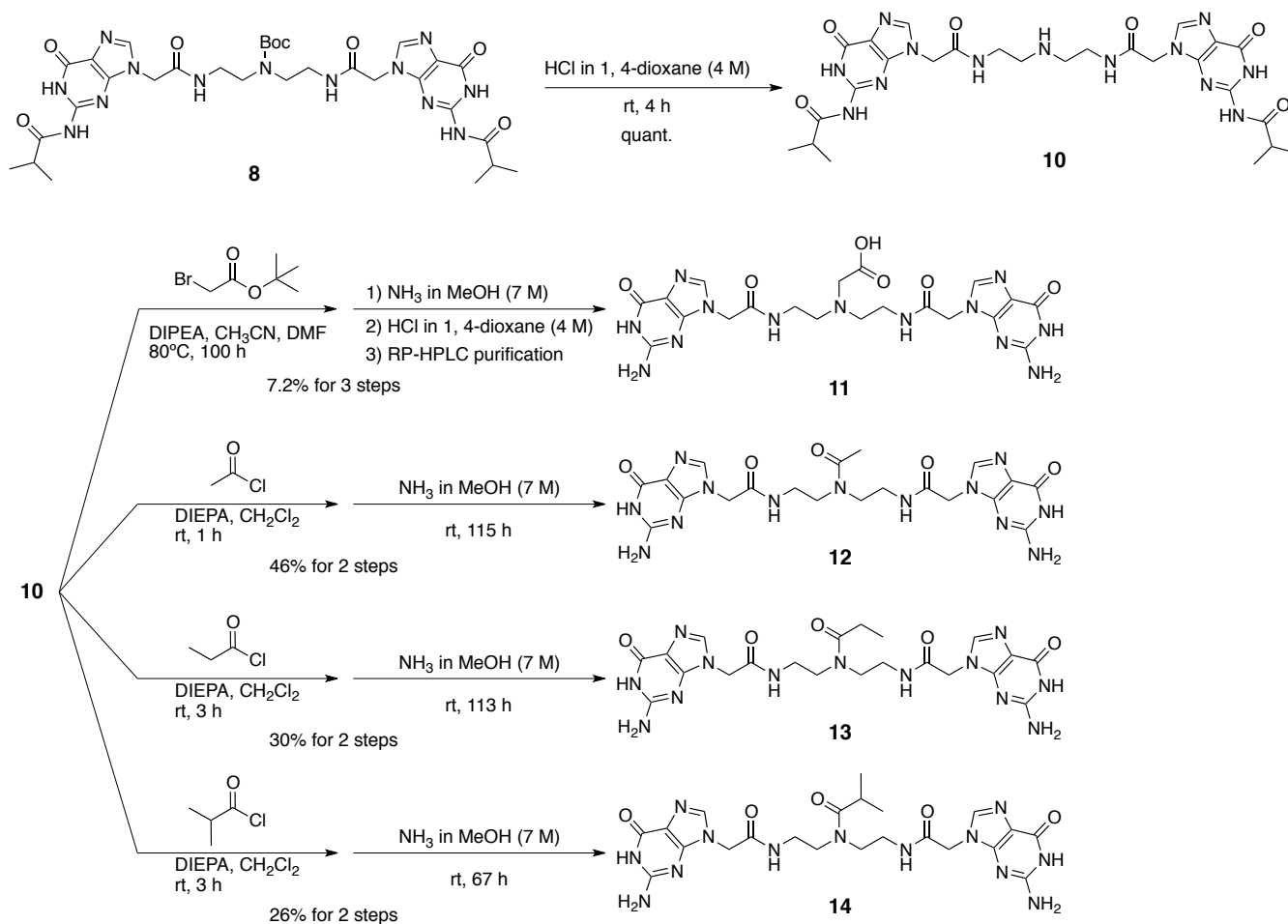
¹H NMR spectrum of **9**



¹³C NMR spectrum of **9**

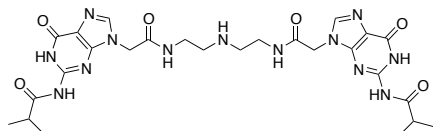


ESI-MS chromatogram of **9**



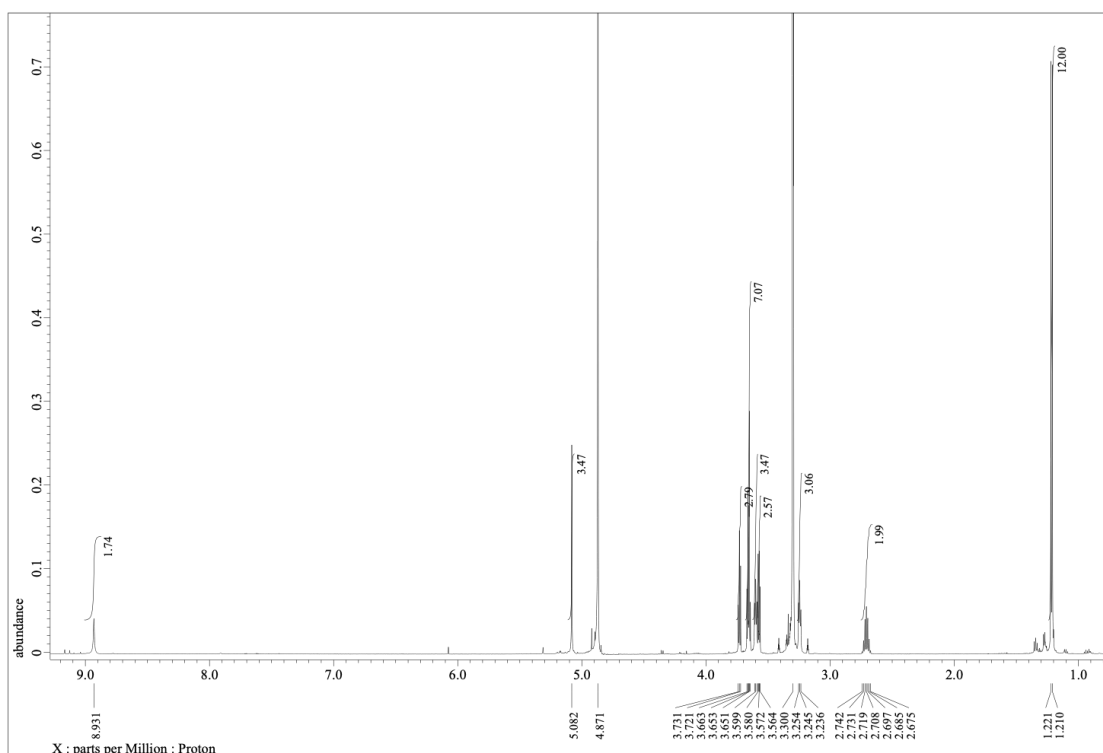
Scheme S3. Synthesis of compounds **11**, **12**, **13** and **14**

***N,N'*-(9,9'-(((Azanediylobis(ethane-2,1-diyl))bis(azanediylobis(2-oxoethane-2,1-diyl))bis(6-oxo-6,9-dihydro-1*H*-purine-9,2-diyl))bis(2-methylpropanamide) (**10**))**



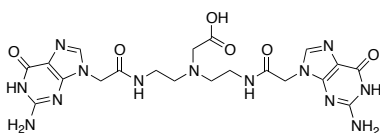
Compound **8** (63 mg, 0.087 mmol) was dissolved in HCl in 1,4-dioxane (4 M, 10 mL), stirred for 1.5 h at rt. The reaction mixture was concentrated under reduced pressure and the residue was dried by codistillation with MeOH to afford **10** (59.7 mg, 0.090 mmol, quant.) as a yellow solid.

^1H NMR (600 MHz, CD_3OD) δ 8.93 (s, 2H), 5.08 (s, 3H), 3.73 (t, $J = 5.9$ Hz, 3H), 3.67-3.64 (m, 7H), 3.60 (t, $J = 5.5$ Hz, 3H), 3.58-3.56 (m, 3H), 3.24 (t, $J = 5.5$ Hz, 3H), 2.71 (sept., $J = 6.7$ Hz, 2H), 1.22 (d, $J = 6.9$ Hz, 12H). ESI-HRMS calcd for $\text{C}_{26}\text{H}_{36}\text{N}_{13}\text{O}_6$ $[\text{M}+\text{H}]^+$: 626.2906, found: 626.2862.



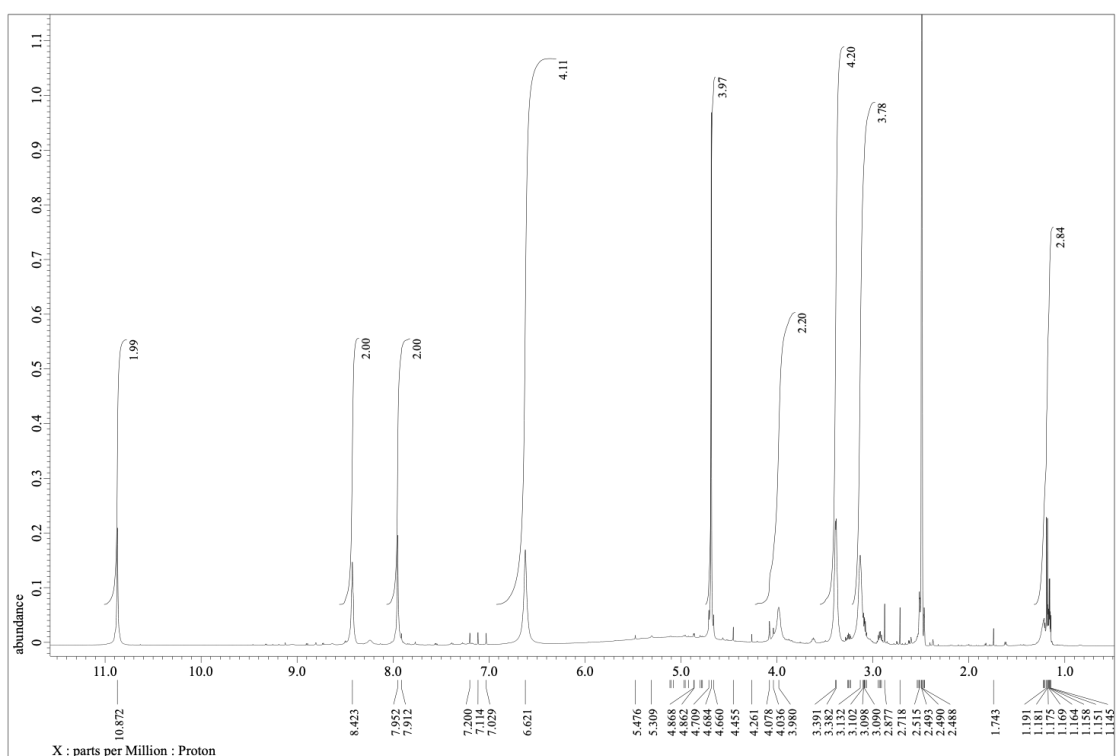
¹H NMR spectrum of **10**

2-(Bis(2-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl)amino) acetic acid (11)

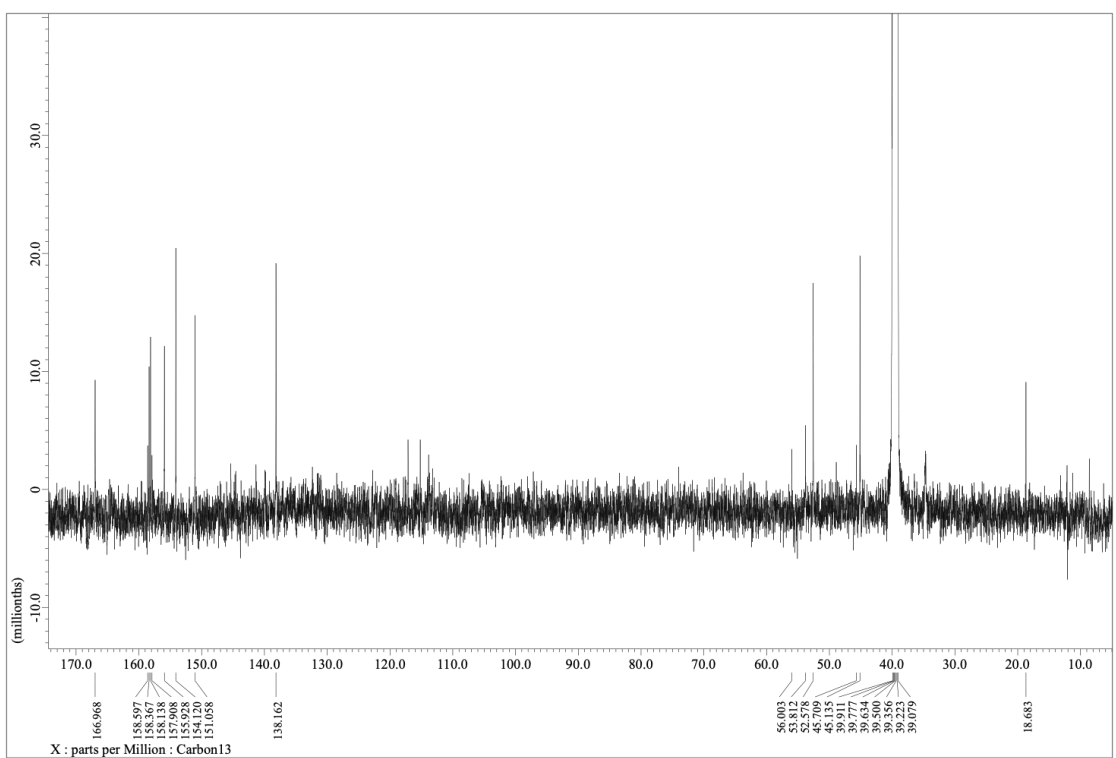


Compound **10** (91 mg, 0.14 mmol) was dissolved in ACN (1.4 mL) and DMF (1.4 mL), then stirred at rt. DIPEA (141 μ L, 5.8 eq) and *tert*-butyl 2-bromoisobutyrate (50 μ L, 2.4 eq) was added into the mixture, stirred for over weekend at 80°C. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc, washed with HCl (1 M), sat. NaHSO₄ aq., brine. The combined aqueous layers were concentrated under reduced pressure, dried by codistillation with ACN. The residue was added NH₃ in MeOH (7 M, 10 mL), stirred for overnight at 40°C. The reaction mixture was concentrated under reduced pressure. The residue was added HCl in 1,4-dioxane (4 M, 10 mL), stirred for 5 h at rt. The reaction mixture was concentrated under reduced pressure, the residue was dried by codistillation with ACN. The residue was purified by HPLC (LC-2000Plus (JASCO); Discovery BIO Wide Pore C18 HPLC Column (Supelco), 21.2 \times 250 mm, 10 μ m; Flow rate: 10 mL/min; rt; Mobile phase A: 0.1% TFA in H₂O, B: 0.1% TFA in ACN; Gradient: B 0% to 30 % (20 min); Detection: 260 nm) to afford **11** (8.5 mg, 0.016 mmol, 7.2% yield for 3 steps) as a pale yellow solid.

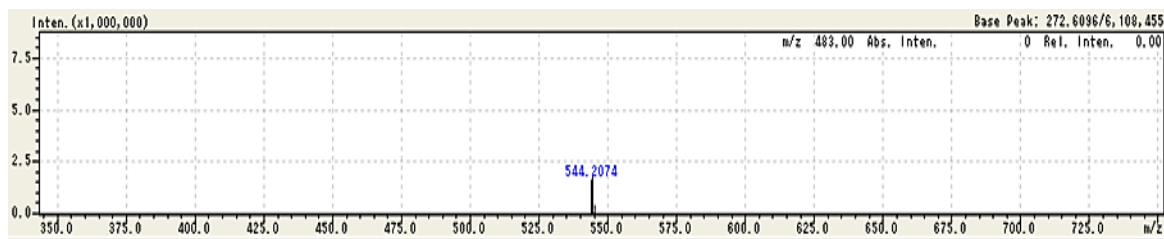
¹H NMR (600 MHz, DMSO-*d*₆) δ 10.87 (s, 2H), 8.42 (s, 2H), 7.95 (s, 2H), 6.62 (s, 4H), 4.68 (s, 4H), 3.98 (br. s, 2H), 3.39 (br. d, *J* = 5.5 Hz, 4H), 3.13 (s, 4H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 167.0, 158.6, 158.4, 158.1, 157.9, 155.9, 154.1, 151.1, 138.2, 56.0, 53.8, 52.6, 45.7, 45.1, 18.7. ESI-HRMS calcd for C₂₀H₂₆N₁₃O₆ [M+H]⁺: 544.2124, found: 544.2074.



¹H NMR spectrum of **11**

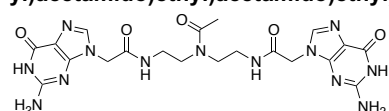


¹³C NMR spectrum of **11**



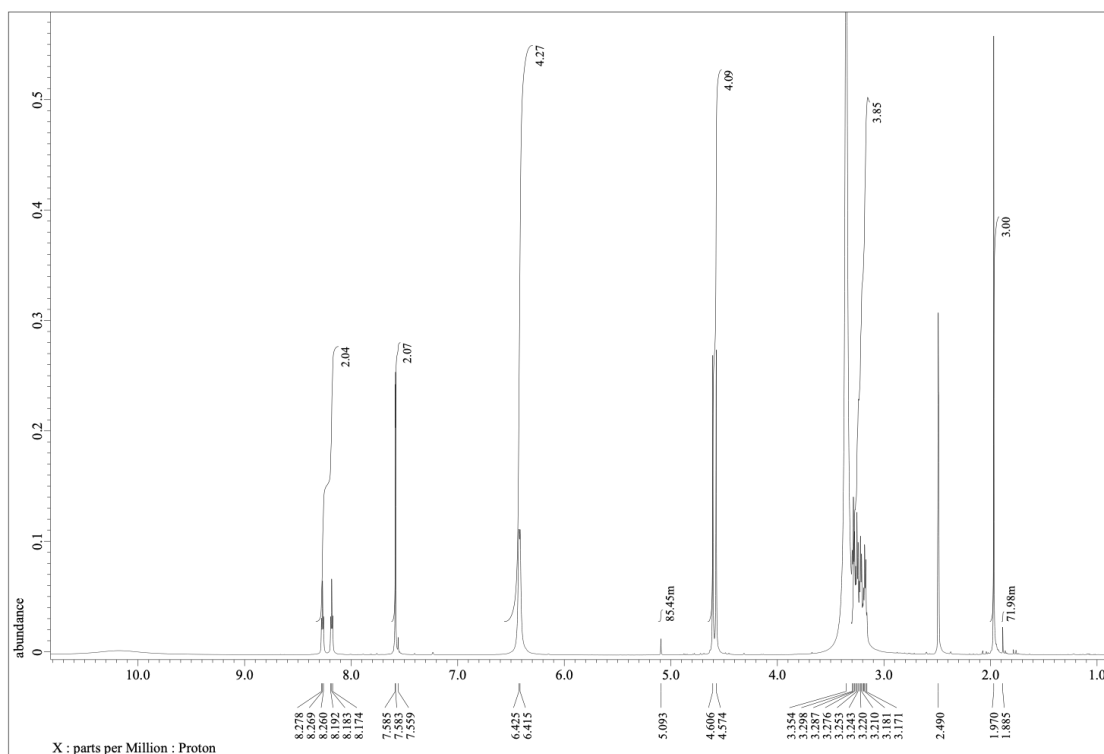
ESI-MS chromatogram of **11**

2-(2-Amino-6-oxo-1*H*-purin-9(6*H*)-yl)-*N*-(2-(*N*-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl)acetamido)ethyl)acetamide (12**)**

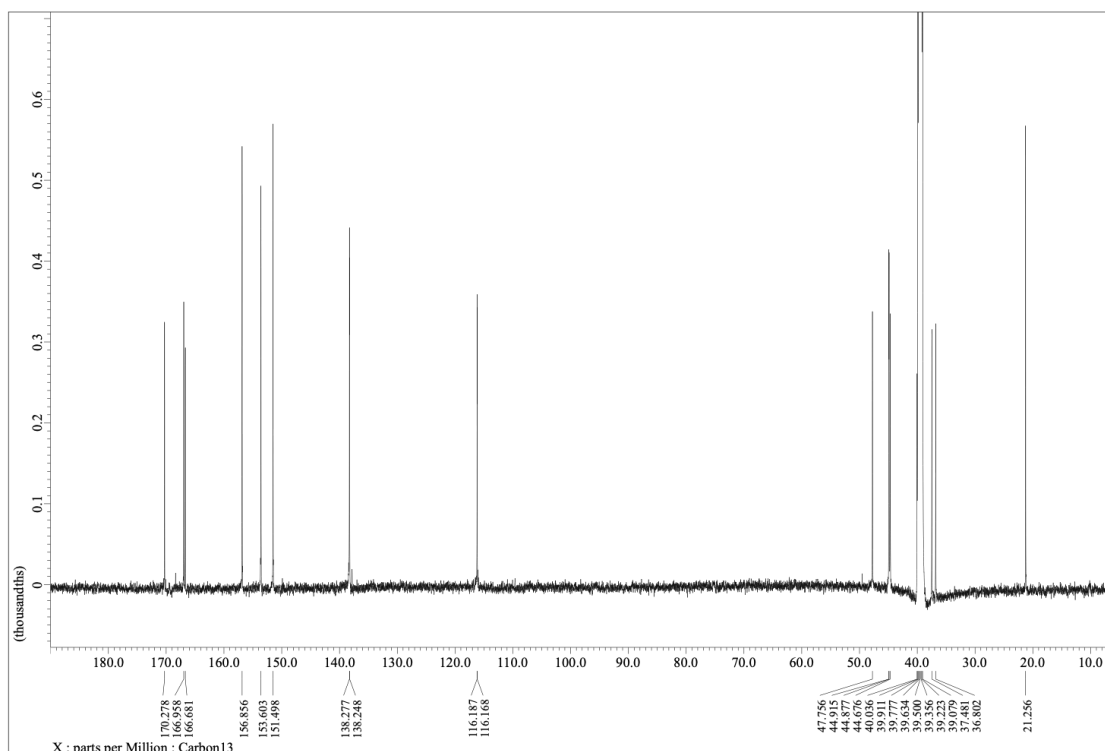


Compound **10** (72 mg, 0.1 mmol) was dissolved in CH₂Cl₂ (1 mL) and DIPEA (95 μ L, 5 eq), then stirred at 0°C. Acetyl chloride (16 μ L, 2 eq) was added into the mixture, stirred for 1.5 h at rt. The reaction mixture was concentrated under reduced pressure, dried under vacuum. The residue was added NH₃ in MeOH (7 M, 10 mL), stirred over weekend at rt, added additional NH₃ in MeOH (7 M, 5 mL), stirred for 6 h at 40°C. The reaction mixture was concentrated under reduced pressure. The residue was added NH₃ in MeOH (7 M, 15 mL), stirred for 23 h at 50°C. The reaction mixture was concentrated under reduced pressure. The residue was added MeOH, filtered, washed with MeOH, dried under vacuum to afford **12** (24.1 mg, 0.046 mmol, 46% yield for 2 steps) as a white solid.

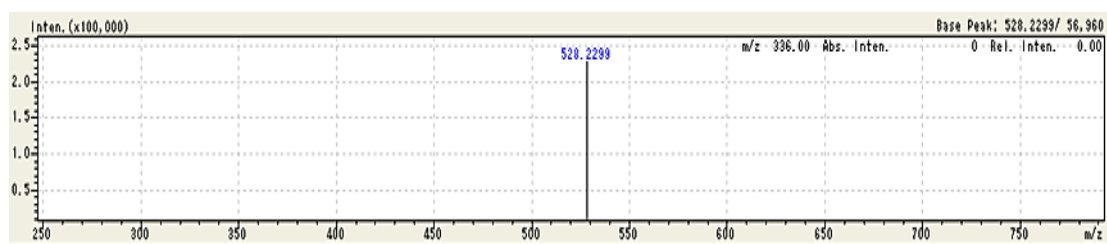
¹H NMR (600 MHz, DMSO-*d*₆) δ 8.23 (dt, *J* = 51.6, 5.5 Hz, 2H), 7.58 (d, *J* = 1.4 Hz, 2H), 6.42 (d, *J* = 6.2 Hz, 4H), 4.59 (d, *J* = 19.3 Hz, 4H), 3.30-3.16 (m, 4H), 1.97 (s, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 170.3, 167.0, 166.7, 156.9, 153.6, 151.5, 138.3, 138.2, 116.2, 116.2, 47.8, 44.9, 44.9, 44.7, 37.5, 36.8, 21.3. ESI-HRMS calcd for C₂₀H₂₆N₁₃O₅ [M+H]⁺: 528.2174, found: 528.2299.



¹H NMR spectrum of **12**

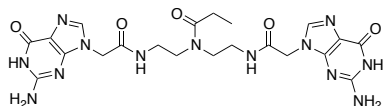


¹³C NMR spectrum of 12



ESI-MS chromatogram of 12

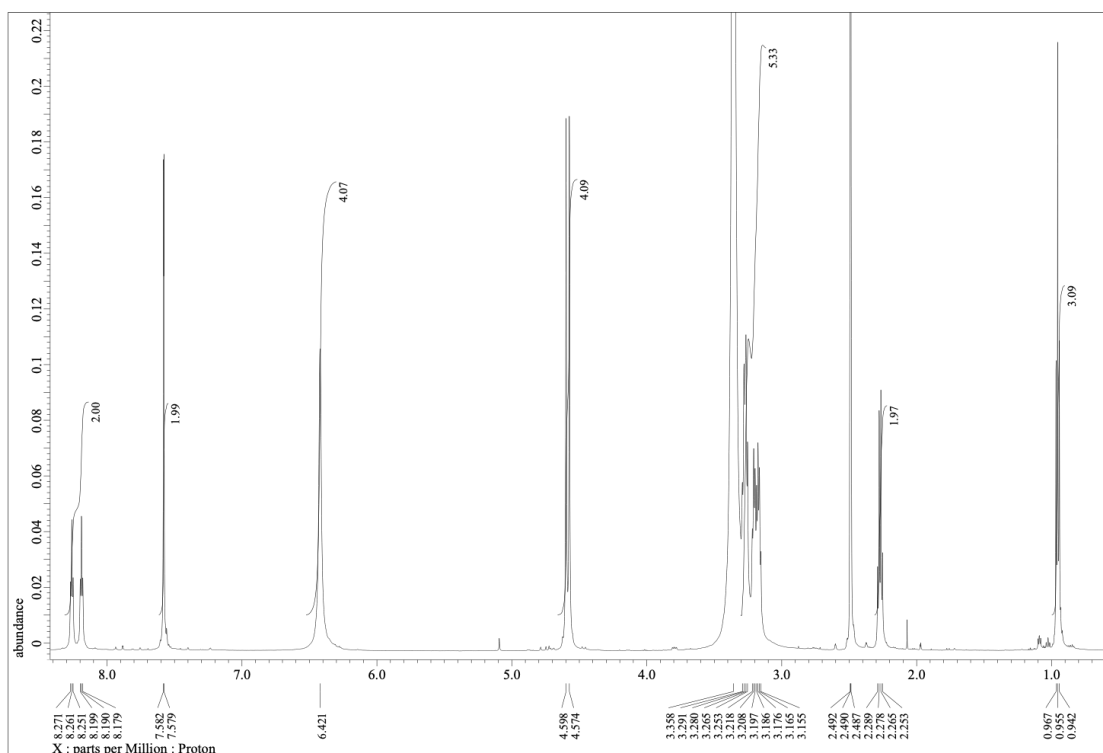
***N,N*-Bis(2-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl) propionamide (13)**



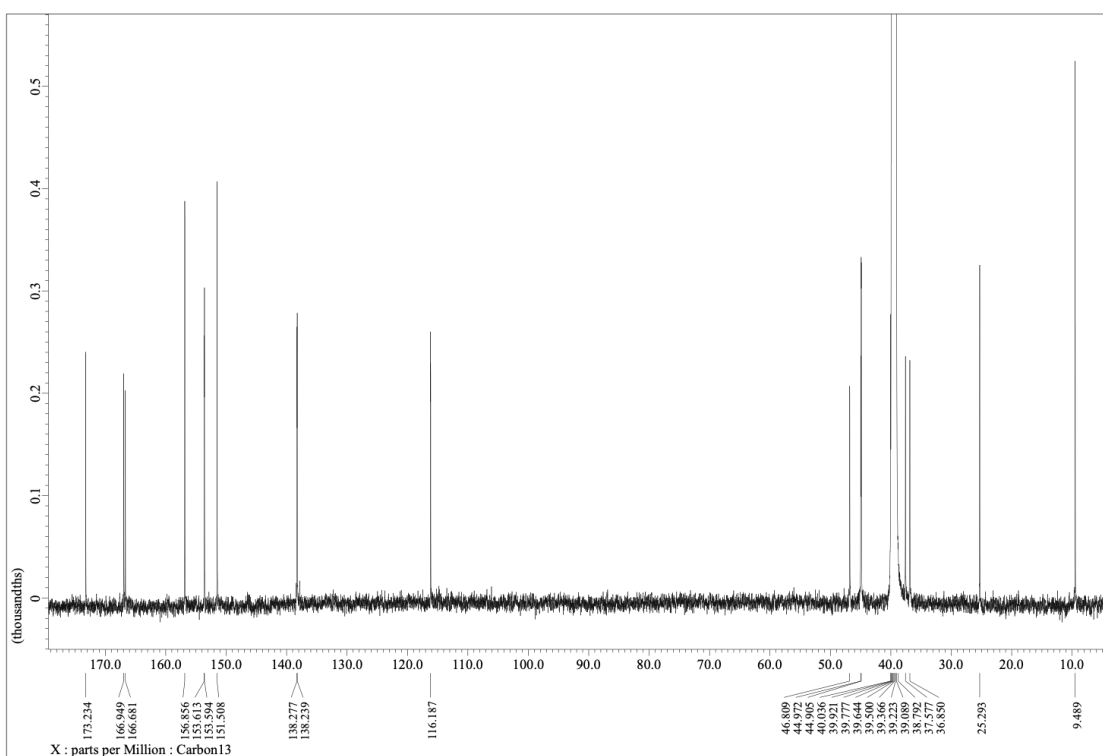
Compound **10** (40 mg, 0.06 mmol) was dissolved in CH₂Cl₂ (600 μ L) and DIPEA (52.6 μ L, 5 eq), then stirred at 0°C. Propionyl chloride (10.5 μ L, 2 eq) was added into the mixture, stirred for 2 h at rt. DIPEA (1 eq) and propionyl chloride (1 eq) was added into the mixture, stirred for 1 h at rt. The reaction mixture was concentrated under reduced pressure, dried under vacuum.

The residue was added NH₃ in MeOH (7 M, 5 mL), stirred for over weekend at rt. The reaction mixture was concentrated under reduced pressure. The residue was added NH₃ in MeOH (7 M, 5 mL), stirred over weekend at rt. The reaction mixture was concentrated under reduced pressure. The residue was added MeOH, the precipitate was collected by centrifugation, washed with MeOH, dried under vacuum to afford **13** (9.7 mg, 0.018 mmol, 30% yield for 2 steps) as a pale yellow solid.

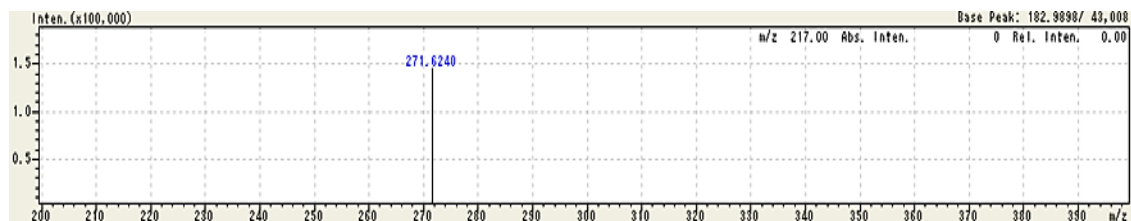
¹H NMR (600 MHz, DMSO-*d*₆) δ 8.23 (dt, *J* = 43.2, 5.9 Hz, 2H), 7.58 (d, *J* = 2.1 Hz, 2H), 6.42 (s, 4H), 4.59 (d, *J* = 14.5 Hz, 4H), 3.29-3.15 (m, 5H), 2.27 (q, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.2, 166.9, 166.7, 156.9, 153.6, 153.6, 151.5, 138.3, 138.2, 116.2, 46.8, 45.0, 44.9, 40.0, 39.9, 39.8, 39.6, 39.5, 39.4, 39.2, 39.1, 38.8, 37.6, 36.8, 25.3, 9.5. ESI-HRMS calcd for C₂₁H₂₈N₁₃O₅ [M+2H]²⁺: 271.6202, found: 271.6240.



^1H NMR spectrum of **13**

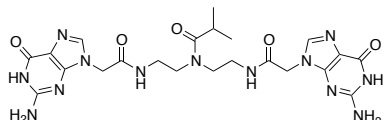


^{13}C NMR spectrum of **13**



ESI-MS chromatogram of **13**

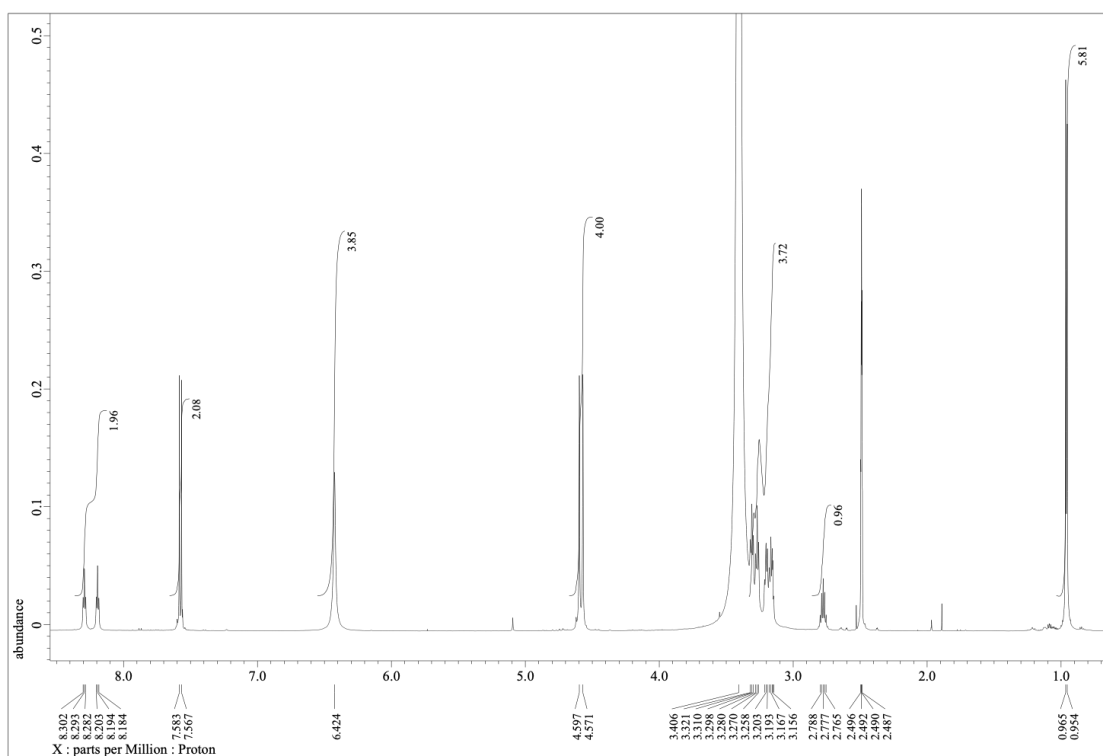
***N,N*-Bis(2-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl) isobutyramide (**14**)**



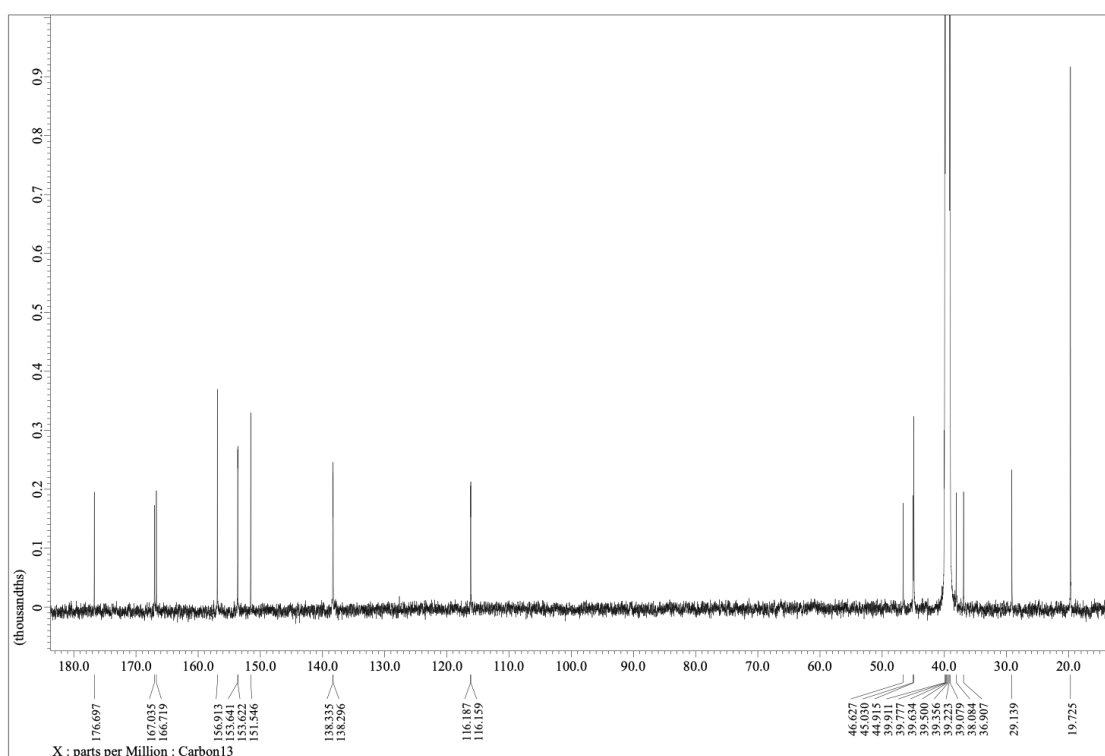
Compound **10** (40 mg, 0.06 mmol) was dissolved in CH₂Cl₂ (600 μ L) and DIPEA (52.6 μ L, 5 eq), then stirred at 0°C. Isobutyryl chloride (13 μ L, 2 eq) was added into the mixture, stirred for 2 h at rt. DIPEA (1 eq) and isobutyryl chloride (1 eq) was added into the mixture, stirred for 1 h at rt. The reaction mixture was concentrated under reduced pressure, dried under vacuum.

The residue was added NH₃ in MeOH (7 M, 5 mL), stirred over weekend at rt. The reaction mixture was concentrated under reduced pressure. The residue was added MeOH, the precipitate was collected by centrifugation, washed with MeOH, dried under vacuum to afford **14** (8.5 mg, 0.015 mmol, 26% yield for 2 steps) as a pale yellow solid.

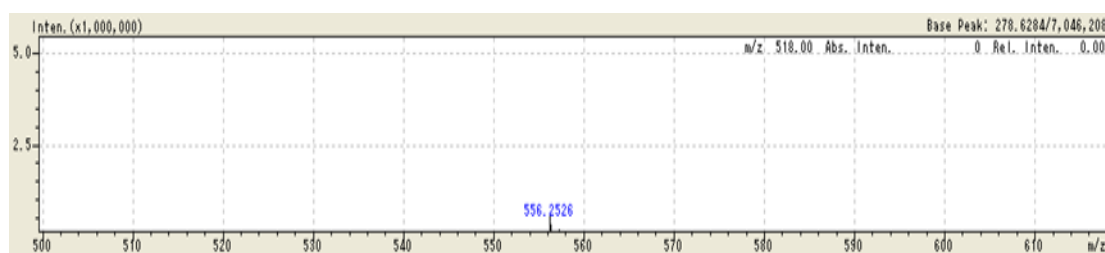
¹H NMR (600 MHz, DMSO-*d*₆) δ 8.24 (dt, *J* = 59.2, 5.9 Hz, 2H), 7.58 (d, *J* = 9.6 Hz, 2H), 6.42 (s, 4H), 4.58 (d, *J* = 15.8 Hz, 4H), 3.32–3.15 (m, 4H), 2.78 (sept., *J* = 6.7 Hz, 1H), 0.96 (d, *J* = 6.9 Hz, 6H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 176.7, 167.0, 166.7, 156.9, 153.6, 151.5, 138.3, 138.3, 116.2, 46.6, 45.0, 44.9, 40.0, 38.1, 36.9, 29.1, 19.7. ESI-HRMS calcd for C₂₂H₃₀N₁₃O₅ [M+H]⁺: 556.2487, found: 566.2526.



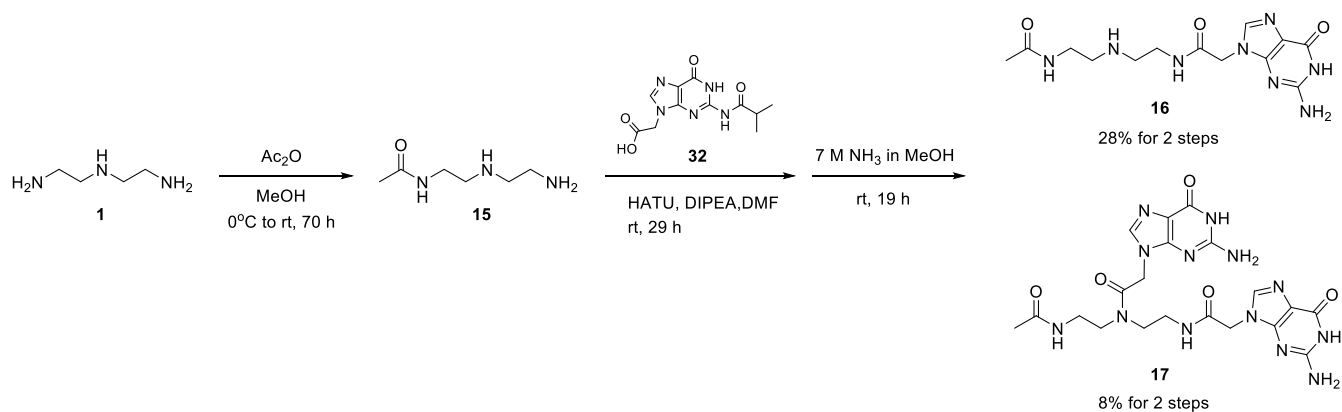
¹H NMR spectrum of **14**



¹³C NMR spectrum of **14**

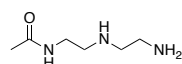


ESI-MS chromatogram of **14**



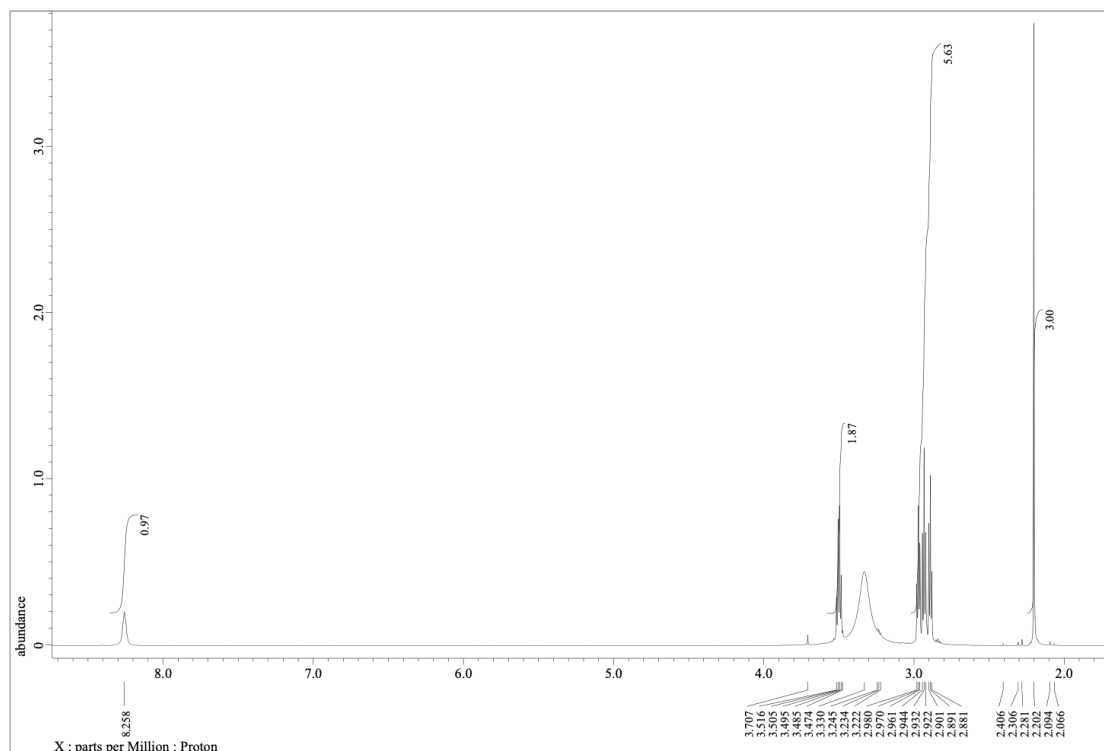
Scheme S4. Synthesis of compounds **16** and **17**

***N*-(2-((2-Aminoethyl)amino)ethyl)acetamide (15)**



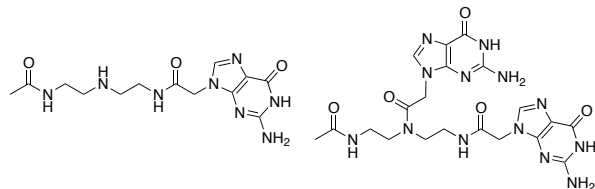
Compound **1** (1 mL, 0.01 mmol) was dissolved in MeOH (100 mL), stirred at 0°C. Acetic anhydride (5.7 mL, 0.2 eq) dissolved in MeOH (100 mL) was added dropwise over 4.5 h using a dropping funnel, stirred over weekend at 0°C to rt. The reaction mixture was concentrated under reduced pressure to remove solvent, diluted with CH₂Cl₂, extracted with H₂O. The aqueous layer was concentrated under reduced pressure, dried under vacuum. The residue was purified by flash silica gel column chromatography (ϕ = 4 cm, h = 13 cm, CH₂Cl₂/MeOH/28% NH₄OH = 20:5:1 to 18:7:1), followed by purified by flash silica gel column chromatography (ϕ = 4 cm, h = 11 cm, CH₂Cl₂/MeOH/28% NH₄OH = 60:30:0 to 57:33:0 to 57:33:10) to afford **15** (1.14 g, 7.85 mmol, 79% yield) as a pale yellow oil.

¹H NMR (600 MHz, DMSO-*d*₆) δ 8.26 (s, 1H), 3.50 (q, *J* = 6.2 Hz, 2H), 2.97 (t, *J* = 5.9 Hz, 2H), 2.93 (t, *J* = 6.5 Hz, 2H), 2.89 (t, *J* = 6.2 Hz, 2H), 2.20 (s, 3H).



¹H NMR spectrum of **15**

***N*-(2-((2-Acetamidoethyl)amino)ethyl)-2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamide (16) and *N*-(2-Acetamidoethyl)-2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-*N*-(2-((2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl)acetamide (17)**



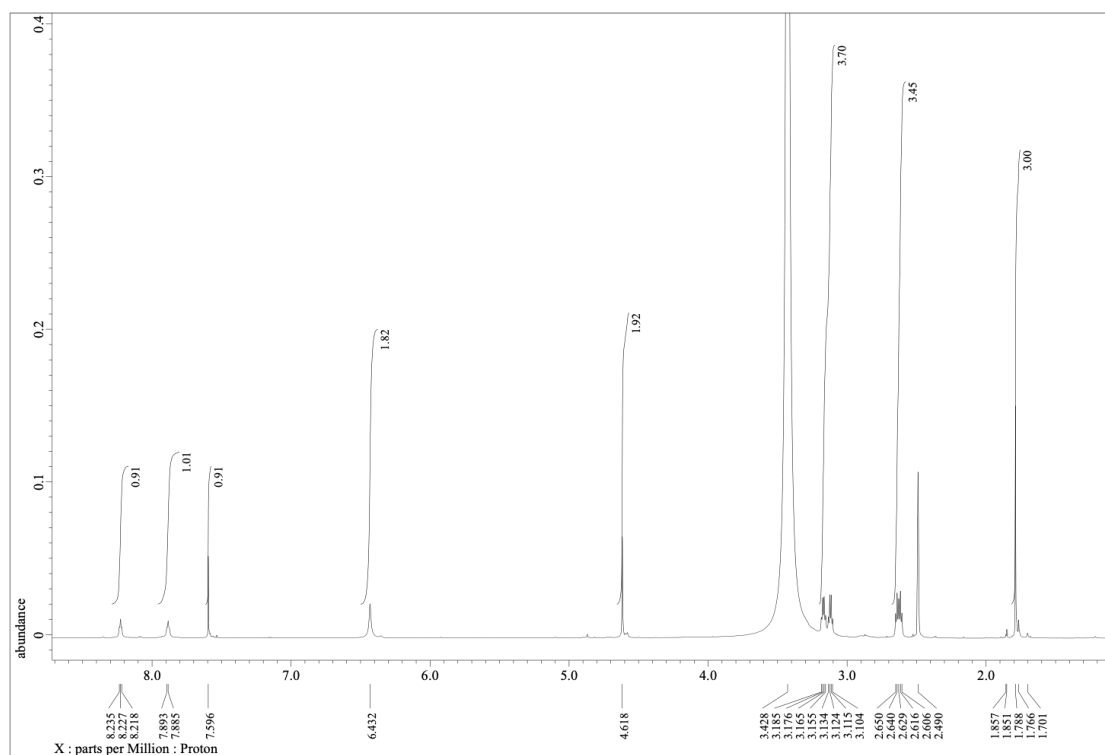
Compound **15** (145 mg, 1 mmol) was dissolved in DMF (5 mL) and stirred at 0°C. Compound **32** (279 mg, 1 eq), HATU (570 mg, 1.5 eq) and DIPEA (523 μ L, 3 eq) was added into the mixture, stirred overnight at rt. The reaction mixture was diluted with EtOAc, extracted with H₂O. The aqueous layer was concentrated under reduced pressure, dried codistillation with MeOH. A half of the residue was added NH₃ in MeOH (7 M, 15 mL), stirred over weekend at rt. The reaction mixture was

concentrated under reduced pressure, dried under vacuum. The residue was purified by flash silica gel column chromatography (ϕ = 2.5 cm, h = 10.5 cm, CH₂Cl₂/MeOH/28% NH₄OH = 85:15:0 to 40:50:10) to afford **16** (47 mg, 0.14 mmol, 28% yield for 2 steps) as a pale orange solid and **17** (20 mg, 0.038 mmol, 8% yield for 2 steps) as a pale orange solid.

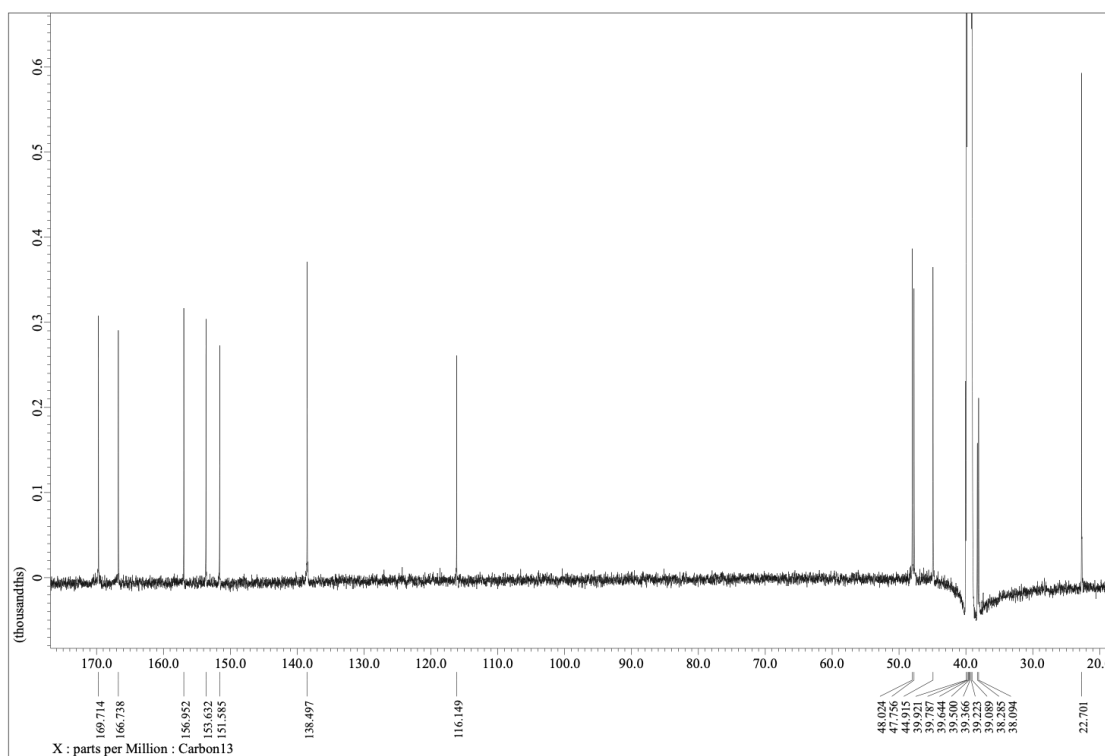
For **16**: ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.23 (t, *J* = 5.2 Hz, 1H), 7.89 (d, *J* = 4.8 Hz, 1H), 7.60 (s, 1H), 6.43 (s, 2H), 4.62 (s, 2H), 3.14 (dq, *J* = 30.5, 6.0 Hz, 4H), 2.65-2.61 (dt, *J* = 13.6 Hz, 6 Hz, 3H), 1.79 (s, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 169.7, 166.7, 157.0, 153.6, 151.6, 138.5, 116.1, 48.0, 47.8, 44.9, 38.3, 38.1, 22.7. ESI-HRMS calcd for C₁₃H₂₁N₈O₃ [M+H]⁺: 337.1731, found: 337.1850.

For **17**: ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.35 (t, *J* = 5.5 Hz, 1H), 8.21 (t, *J* = 5.9 Hz, 1H), 8.08 (t, *J* = 5.9 Hz, 1H), 7.89 (t, *J* = 5.9 Hz, 1H), 7.60 (s, 1H), 7.56 (d, *J* = 1.4 Hz, 2H), 7.54 (s, 1H), 6.43-6.35 (m, 10H), 4.88 (s, 4H), 4.70 (s, 2H), 4.59 (s, 2H), 3.41 (q, *J* = 6.9 Hz, 4H), 3.17 (dq, *J* = 34.1, 6.5 Hz, 4H), 1.85 (s, 3H), 1.76 (s, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 170.0, 169.5, 167.2, 166.7, 166.7, 166.6, 156.8, 153.6, 153.5, 151.6, 151.5, 138.5, 138.4, 138.3, 138.2, 116.2, 116.1, 116.0, 46.3, 46.0, 45.4, 45.3, 45.0, 44.8, 43.6, 43.5,

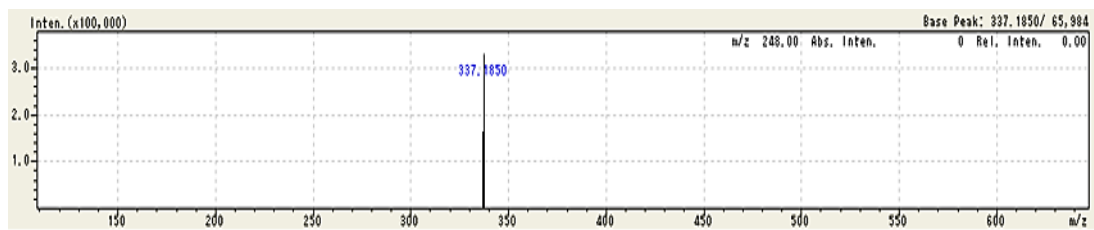
40.1, 37.4, 37.3, 36.5, 36.3, 22.7, 22.6. ESI-HRMS calcd for $C_{20}H_{26}N_{13}O_5$ $[M+H]^+$: 528.2174, found: 528.2177.



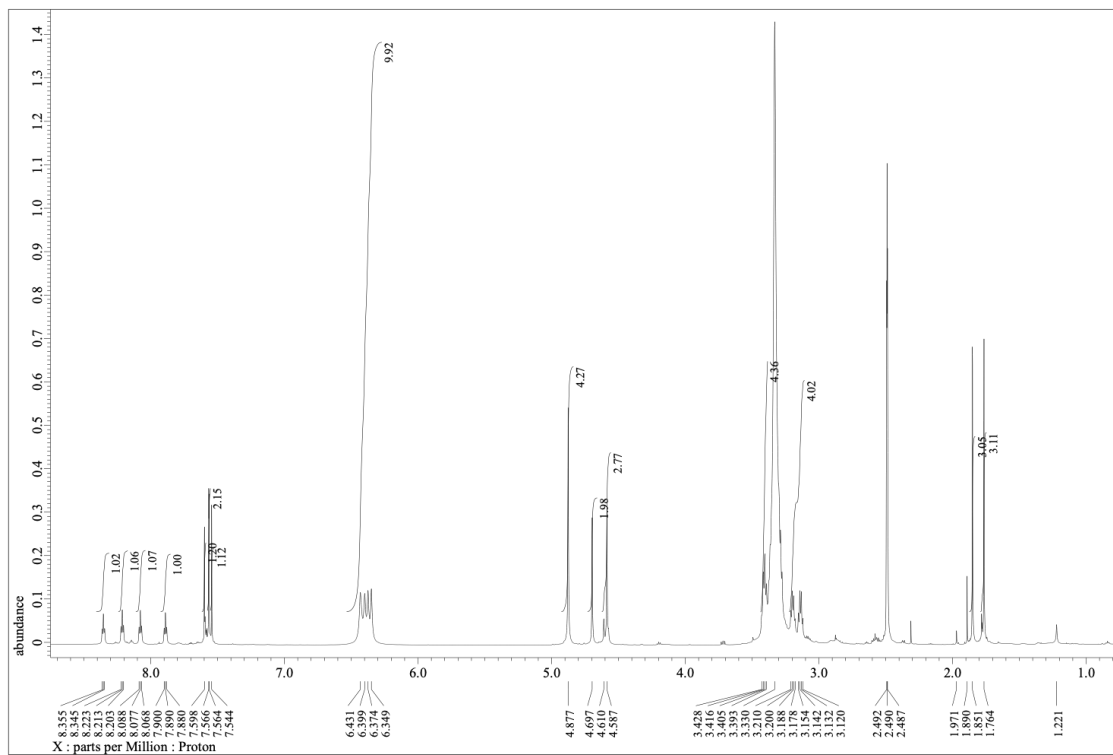
1H NMR spectrum of **16**



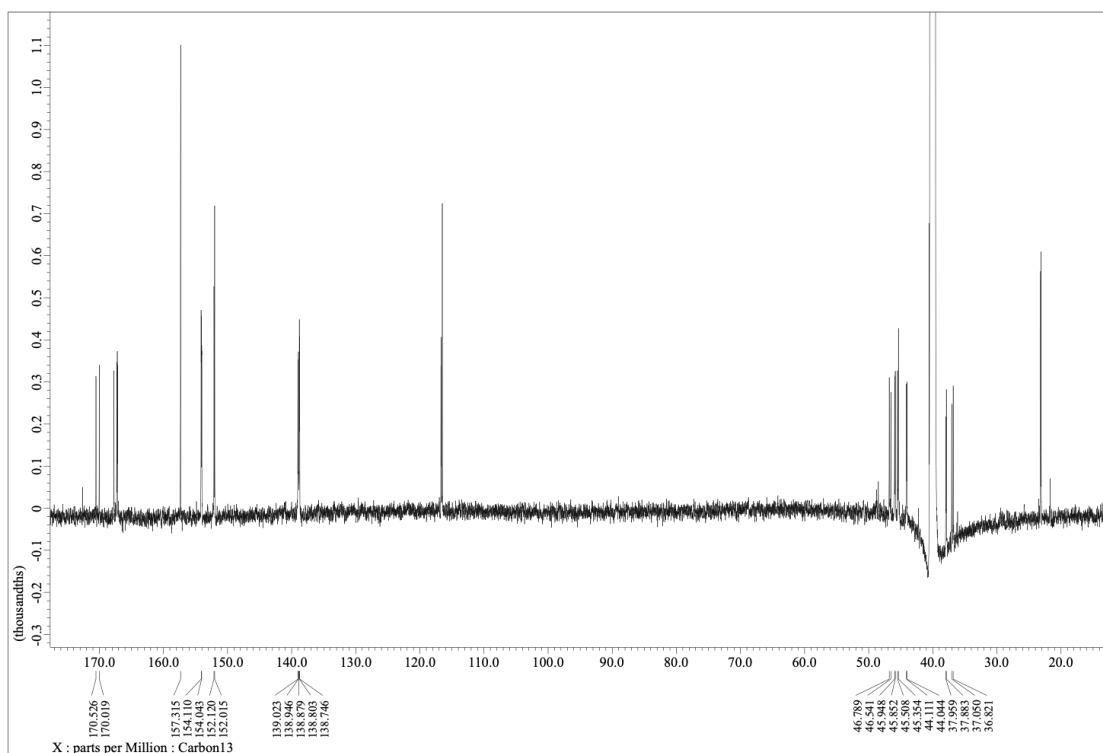
^{13}C NMR spectrum of **16**



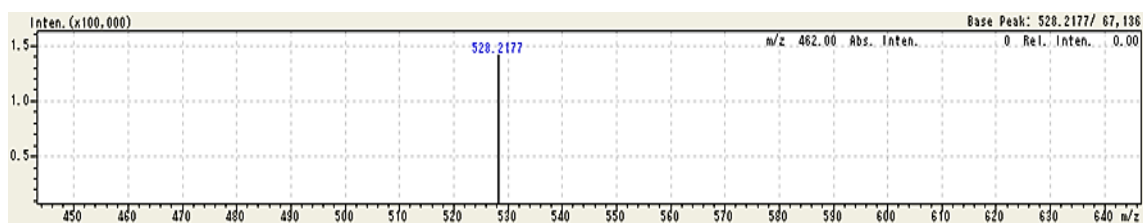
ESI-MS chromatogram of 16



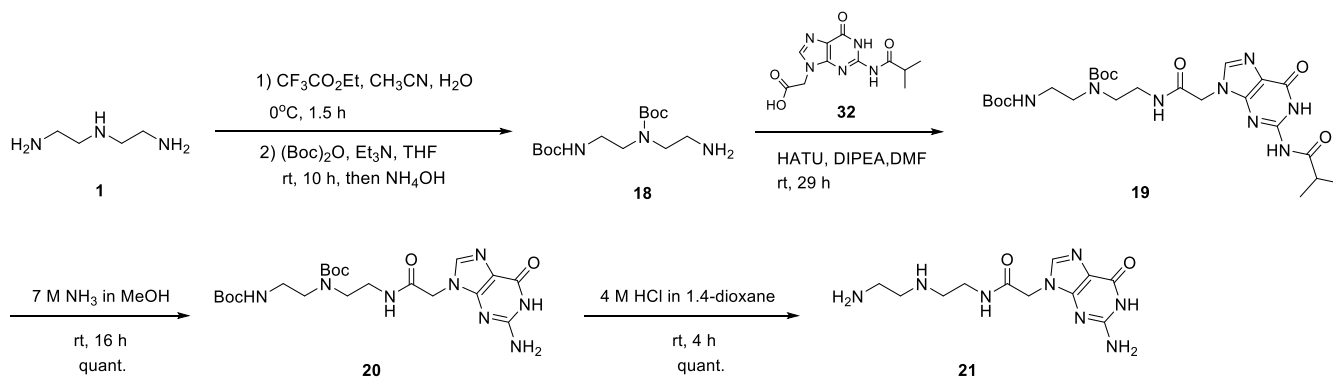
^1H NMR spectrum of 17



¹³C NMR spectrum of **17**

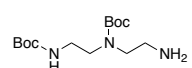


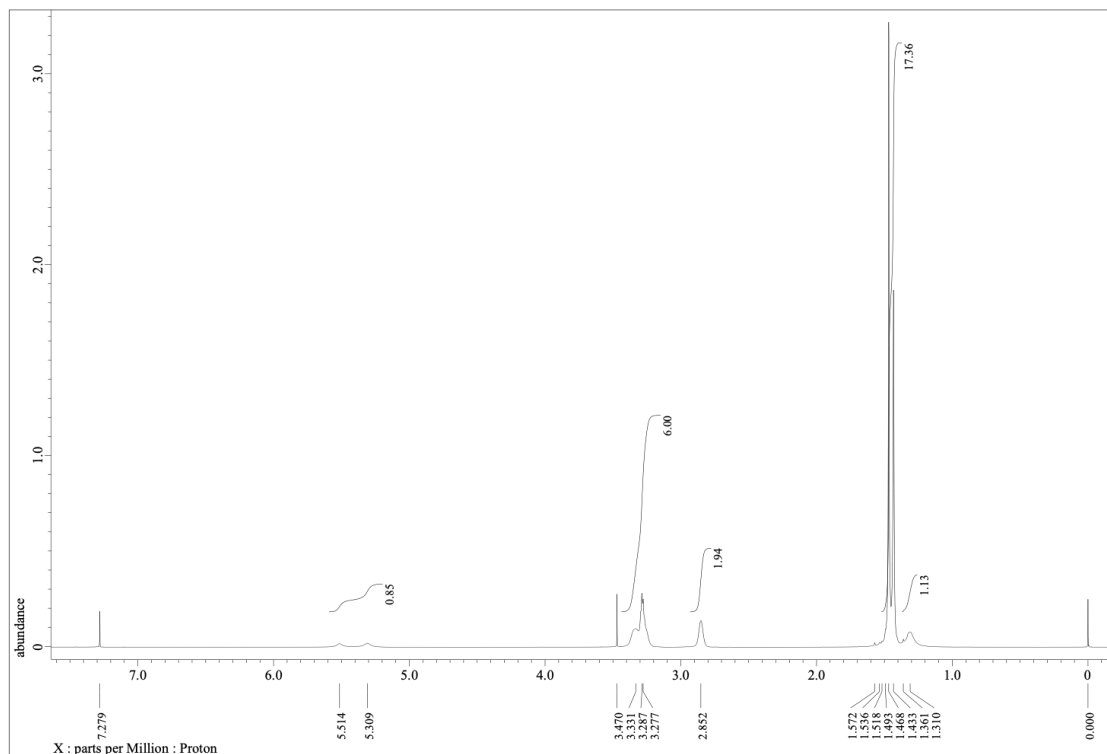
ESI-MS chromatogram of **17**



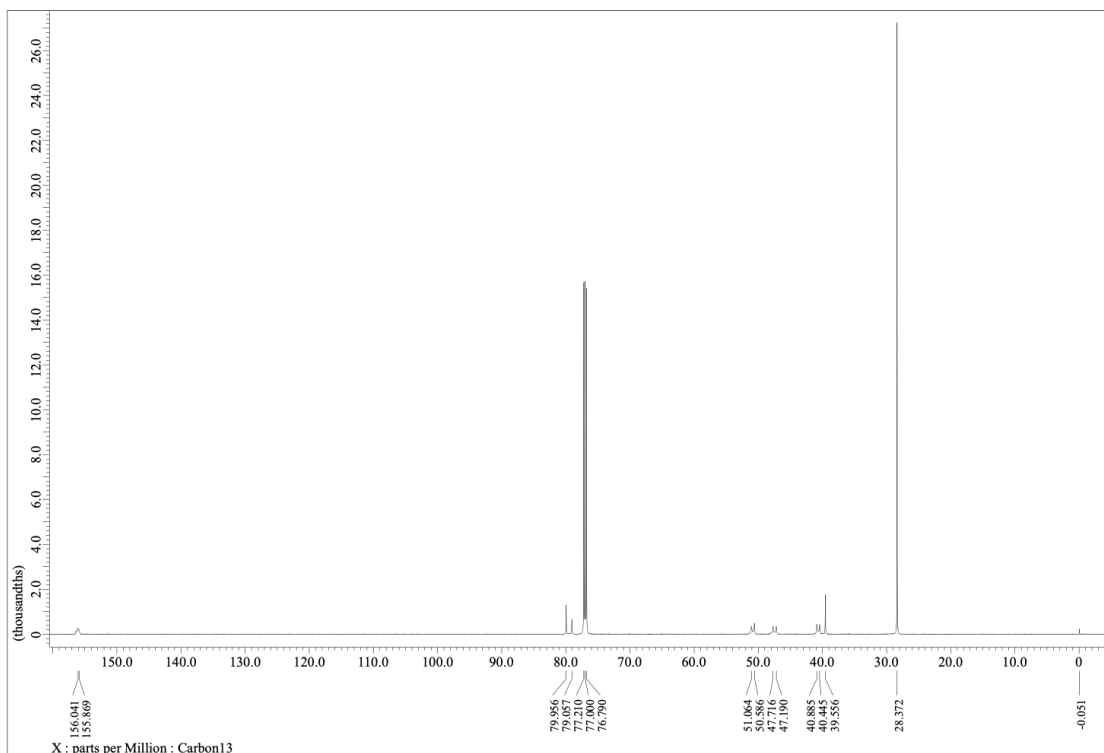
Scheme S5. Synthesis of compound **21**

***tert*-Butyl (2-aminoethyl)(2-((*tert*-butoxycarbonyl)amino)ethyl)carbamate (**18**)**


Compound **1** (1.03 g, 10 mmol) was dissolved in MeOH (40 mL), stirred at -78°C. Ethyl trifluoroacetate (1.25 mL, 1.05eq) in MeOH (50 mL) was added dropwise into the mixture over 30 min using a dropping funnel, then stirred 1.5 h at 0°C. (Boc)₂O (6.89 g, 3 eq) in MeOH (10 mL) was added into the mixture, stirred for 10 h at rt. The reaction mixture was concentrated under reduced pressure to give a colorless oil. The oil was dissolved in MeOH (15 mL), added 28% NH₄OH (15 mL) at rt, stirred for 24 h at 50°C. After cooling to rt, the reaction mixture was concentrated under reduced pressure to remove the volatiles. The residue was added 1 M NaOH aq., and extracted with CH₂Cl₂ (100 mL) 3 times. The combined organic extracts were dried over Na₂SO₄, filtered, concentrated under reduced pressure. Purified by flash silica gel column chromatography (φ = 2.5 cm, h = 15 cm, CH₂Cl₂/MeOH/28% NH₄OH = 100:5:1 to 100:10:1) to afford **18** (1.82 g, 6.0 mmol, 60% yield) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 5.41 (d, J = 123.3 Hz, 1H), 3.33-3.28 (m, 6H), 2.85 (br.s, 2H), 1.47 (s, 9H), 1.43 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 156.0, 155.9, 80.0, 79.1, 51.1, 50.6, 47.7, 47.2, 40.9, 40.4, 39.6, 28.4. ESI-HRMS calcd for C₁₄H₃₀N₃O₄ [M+H]⁺: 304.2231, found: 304.2239.

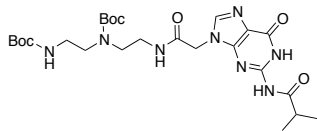


¹H NMR spectrum of **18**



¹³C NMR spectrum of **18**

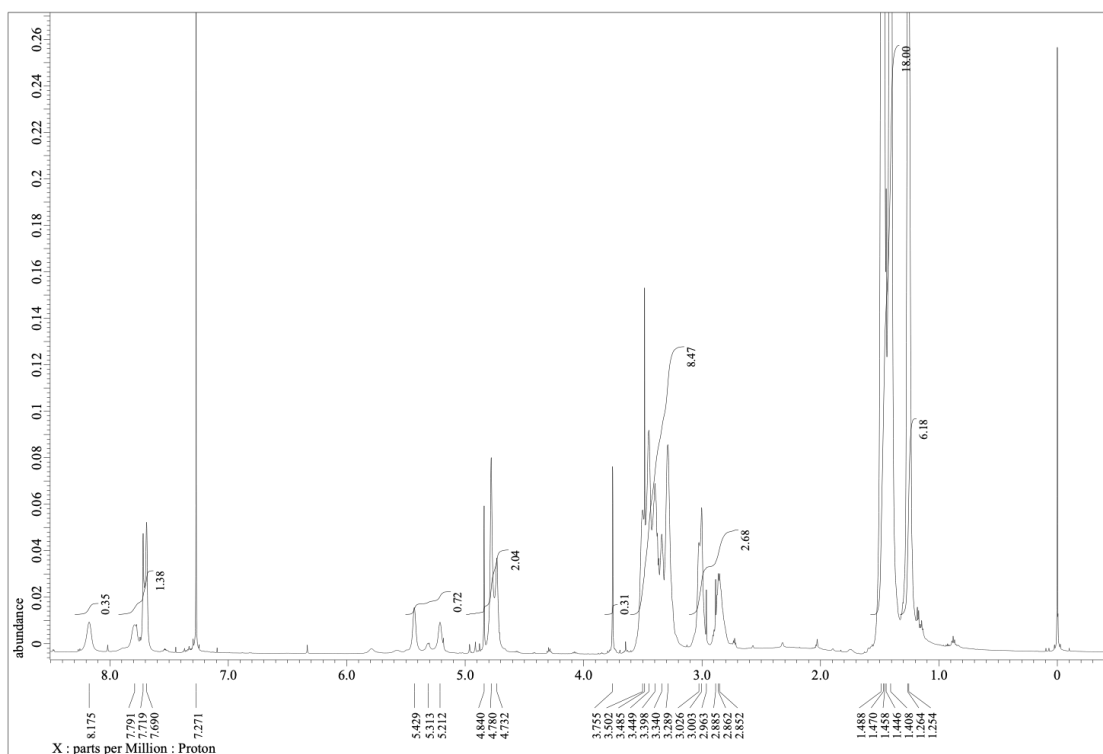
***tert*-Butyl (2-(((*tert*-butoxycarbonyl)amino)ethyl)(2-(2-(2-isobutyramido-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl)carbamate (**19**)**



Compound **18** (30 mg, 0.1 mmol) was dissolved in DMF (500 μ L) and stirred at 0°C. Compound **32** (31 mg, 1.1 eq), HATU (57 mg, 1.5 eq) and DIPEA (52 μ L, 3 eq) was added into the mixture, stirred over weekend at rt. The reaction mixture was diluted with EtOAc, washed with HCl (1 M), brine. The aqueous layer was extracted with CH₂Cl₂ 3 times. The organic layer was dried over Na₂SO₄, filtered, concentrated under reduced pressure. The residue was purified by flash NH silica gel column

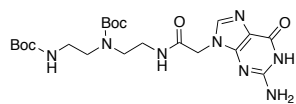
chromatography (ϕ = 1.5 cm, h = 10 cm, CH₂Cl₂/MeOH = 100:0 to 85:15) to afford **19** (24 mg, 0.043 mmol, 43% yield) as a pale yellow solid.

¹H NMR (600 MHz, CDCl₃) δ 7.70 (d, J = 17.9 Hz, 1H), 5.43-5.21 (m, 1H), 4.84-4.73 (m, 2H), 3.50-3.29 (m, 8H), 3.03-2.96 (m, 2H), 2.89-2.85 (m, 1H), 1.49-1.41 (m, 18H), 1.25 (s, J = 6.2 Hz, 6H). ESI-HRMS calcd for C₂₅H₄₁N₈O₇ [M+H]⁺: 565.3093, found: 565.3116.



¹H NMR spectrum of **19**

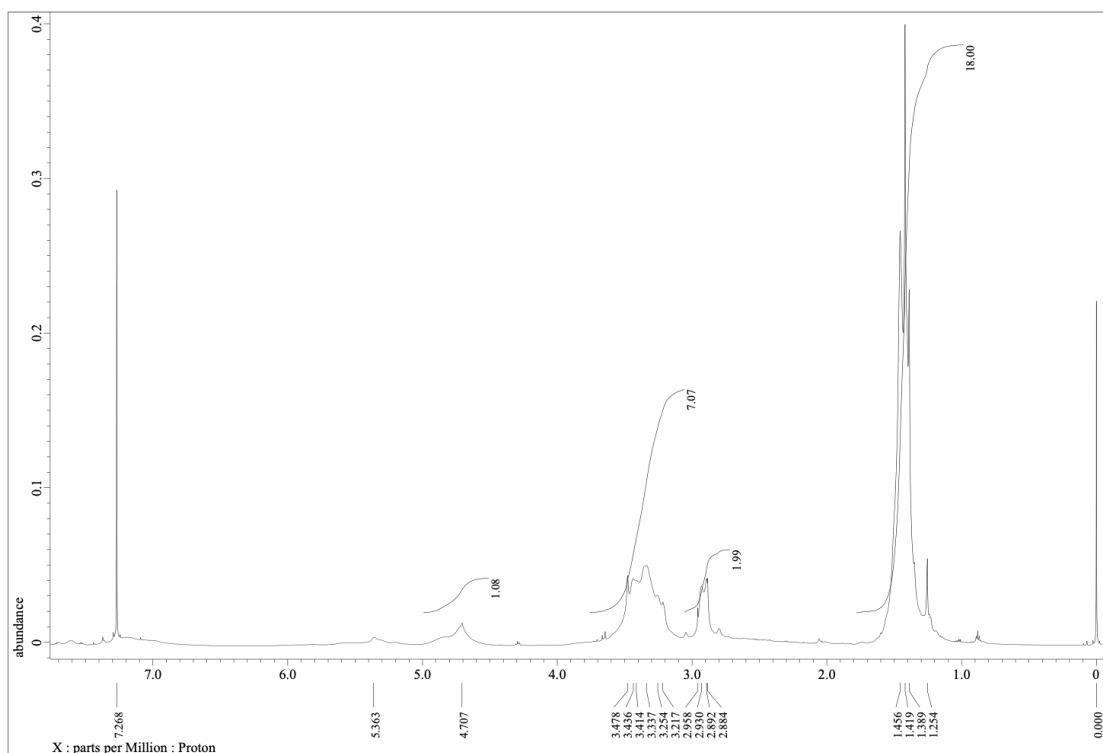
***tert*-Butyl (2-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl)(2-((*tert*-butoxycarbonyl)amino)ethyl)carbamate (**20**)**



Compound **19** (24 mg, 0.043 mmol) was added NH₃ in MeOH (7 M, 10 mL), stirred for 18 h at rt. The reaction mixture was concentrated under reduced pressure, dried under vacuum to afford **20** (24 mg, 0.049 mmol, quant.) as a pale yellow solid.

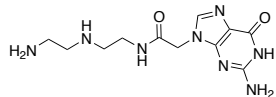
¹H NMR (600 MHz, CDCl₃) δ 4.71 (br.s, 1H), 3.48-3.22 (m, 7H), 2.96-2.88 (m, 2H), 1.46-1.25 (m, 18H).

ESI-HRMS calcd for C₂₁H₃₅N₈O₆ [M+H]⁺: 495.2674, found: 495.2709.



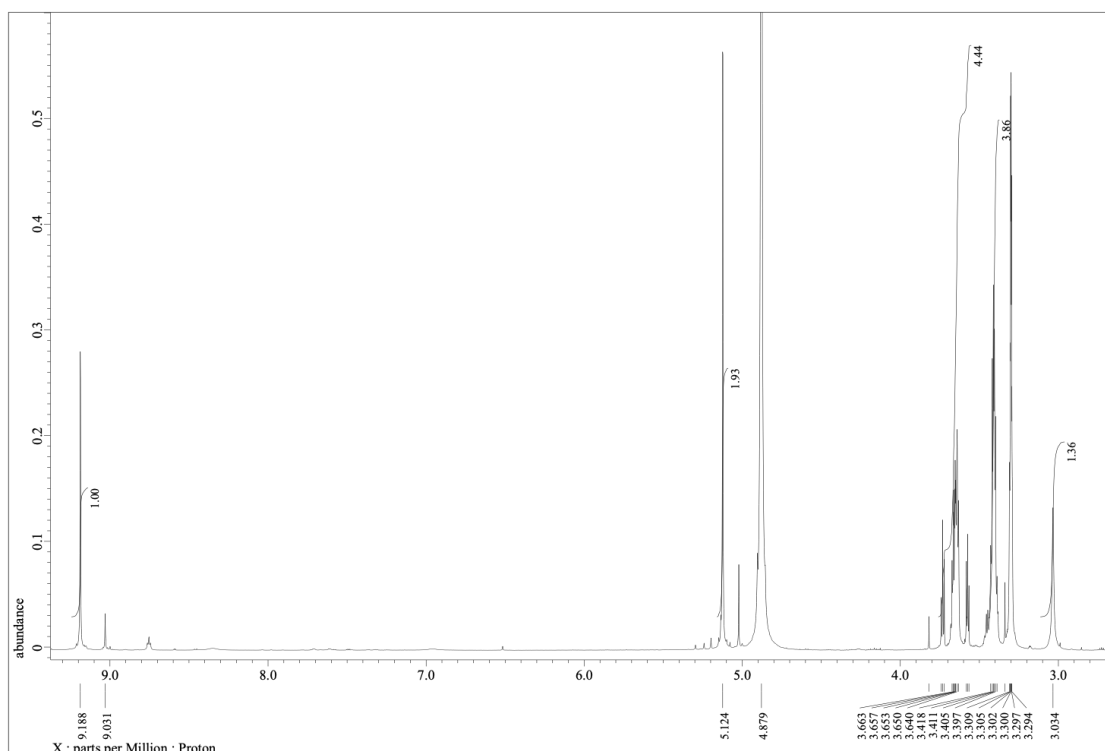
¹H NMR spectrum of **20**

2-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-N-((2-aminoethyl)amino)ethyl acetamide (21**)**

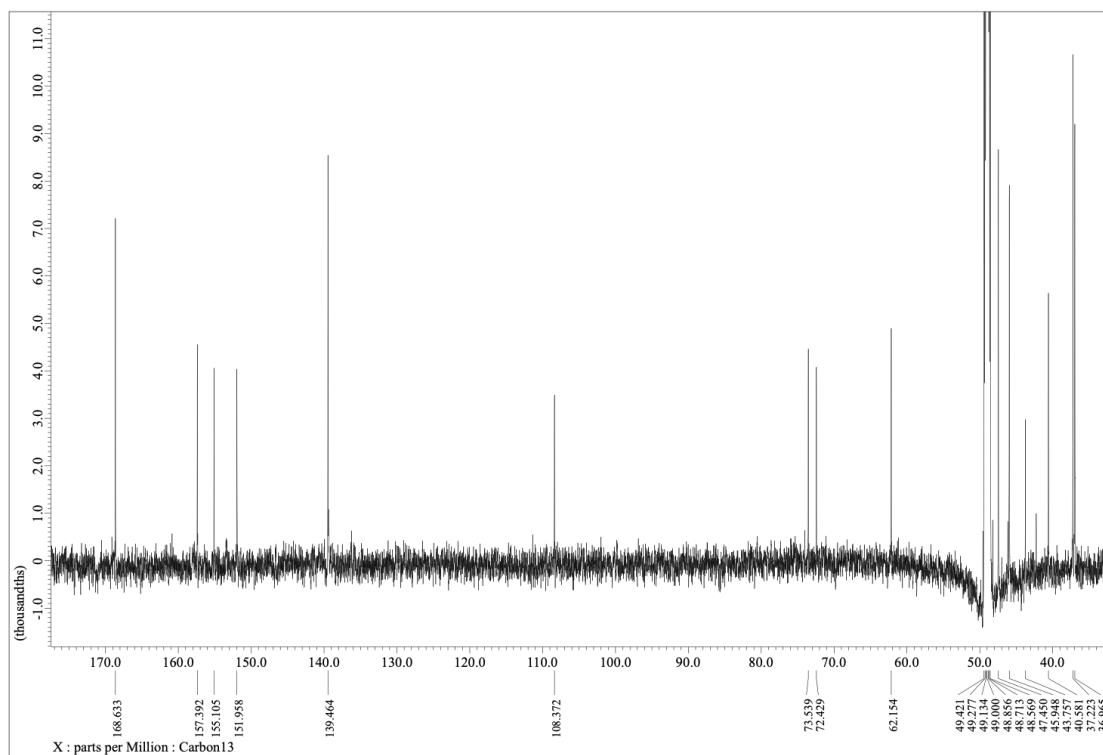


Compound **20** (24 mg, 0.049 mmol) was added HCl in 1,4-dioxane (4 M, 10 mL), stirred for 1 h at rt. The reaction mixture was concentrated under reduced pressure. The residue was added ACN, the precipitate was collected by centrifugation, washed with ACN 2 times, dried under vacuum to afford **21** (15.5 mg, 0.053 mmol, quant.) as a white solid.

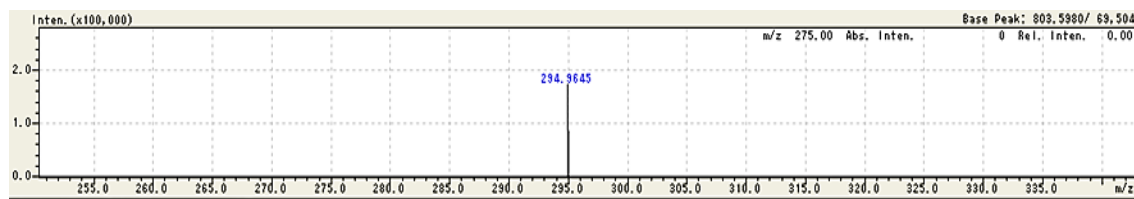
¹H NMR (600 MHz, CD₃OD) δ 9.19 (s, 1H), 5.12 (s, 2H), 3.74-3.57 (m, 4H), 3.41 (dt, *J* = 13.3, 5.2 Hz, 4H), 3.03 (s, 1H); ¹³C NMR (151 MHz, CD₃OD) δ 168.6, 157.4, 155.1, 152.0, 139.5, 108.4, 73.5, 72.4, 62.2, 47.5, 45.9, 43.8, 40.6, 37.2, 37.0. ESI-HRMS calcd for C₁₁H₁₉N₈O₂ [M+H]⁺: 295.1625, found: 294.9645.



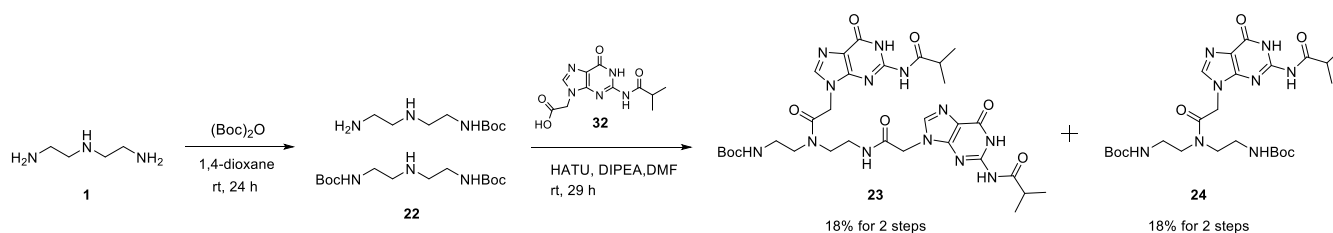
^1H NMR spectrum of **21**



^{13}C NMR spectrum of **21**

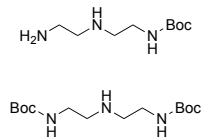


ESI-MS chromatogram of **21**



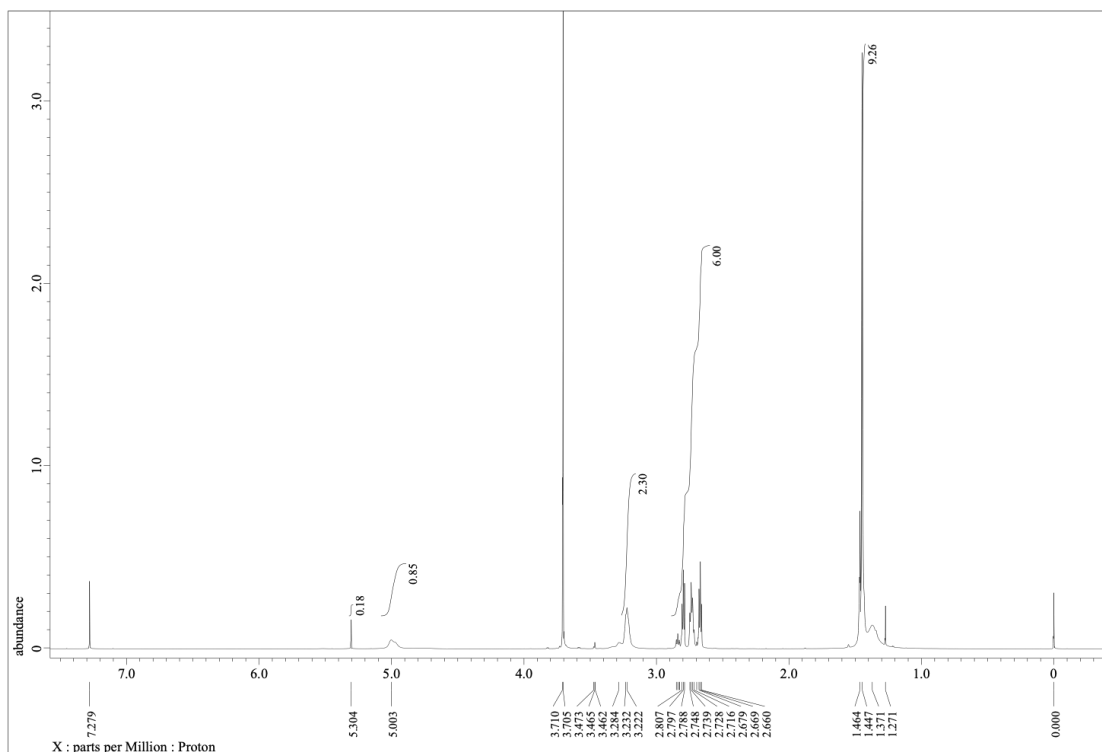
Scheme S7. Synthesis of compounds **23** and **24**

***tert*-Butyl (2-((2-aminoethyl)amino)ethyl)carbamate (**22**)**^[S3]



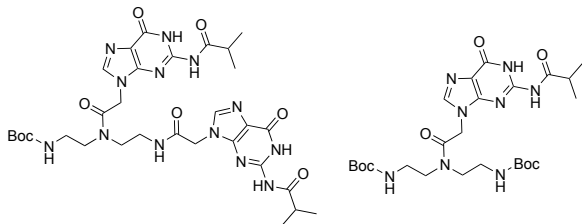
Compound **1** (10.9 mL, 100 mmol) was dissolved in 1,4-dioxane (17 mL), stirred at rt. (Boc)₂O (3.1 g, 0.14 eq) in 1,4-dioxane (20 mL) was added dropwise over 1 h using a dropping funnel, stirred for 24 h. The reaction mixture was concentrated under reduced pressure at 50°C. The residue was added H₂O (15 mL) then filtered. The filtrate was extracted with CH₂Cl₂ (9 mL) 5 times. The combined extracts were dried over Na₂SO₄, filtered, concentrated under reduced pressure, dried under vacuum to afford mixture **22** (1.98 g) as a colorless oil.

¹H NMR (600 MHz, CDCl₃) δ 5.00 (br.s, 1H), 3.22 (s, 2H), 2.85-2.66 (m, 6H), 1.45 (s, 9H). ESI-HRMS calcd for C₉H₂₂N₃O₂ [M+H]⁺: 204.1707, found: 204.1698.



¹H NMR spectrum of **22**

tert-Butyl (2-(2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)-N-(2-(2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)acetamido)ethyl)acetamido)ethyl) carbamate (23) and Di-tert-butyl (((2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)acetyl)azanediyl)bis(ethane-2,1-diyl))dicarbamate (24)

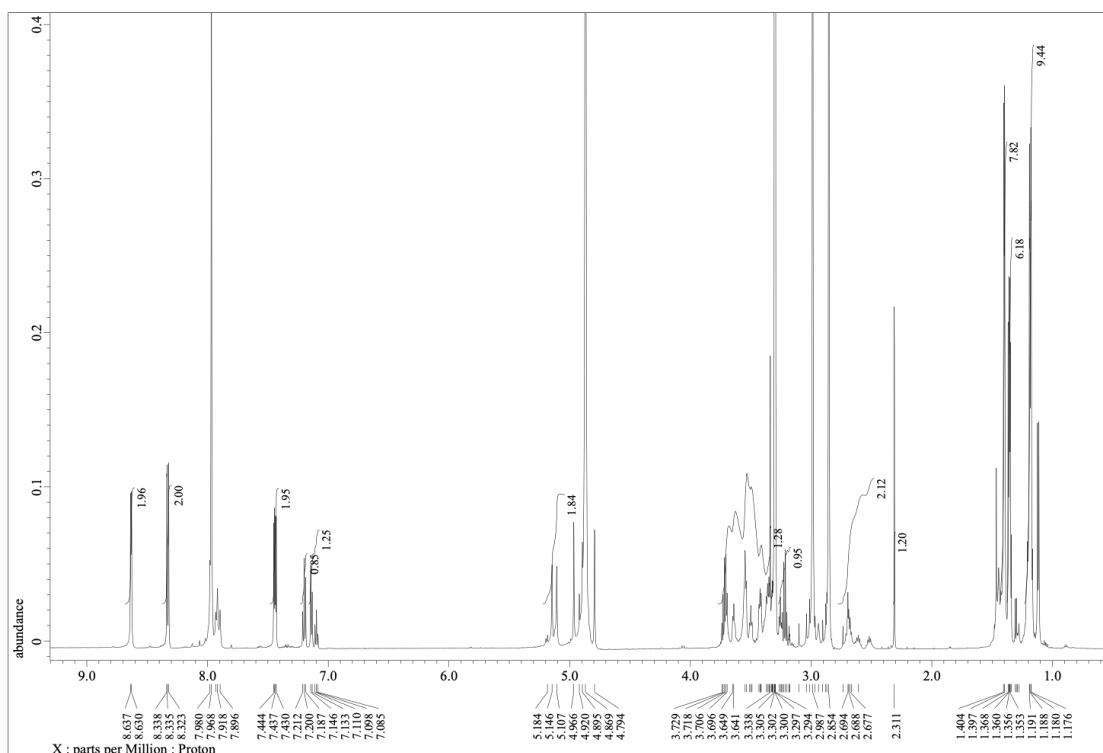


Mixture **22** (102 mg) was dissolved in DMF (2.5 mL) and stirred at 0°C. Compound **32** (305 mg, 2.2 eq), HATU (570 mg, 3 eq) and DIPEA (523 μ L, 6 eq) was added into the mixture, stirred for 29 h at rt. The reaction mixture was diluted with EtOAc, washed with HCl (1 M), brine. The organic layer was dried over Na₂SO₄, filtered, concentrated under reduced pressure, dried under vacuum. The residue was purified by flash silica gel column chromatography (Yamazen inject column size: M, Yamazen Hi-flash column size: M, CH₂Cl₂/MeOH = 99:1 to 80:20) to afford **23** (91 mg,

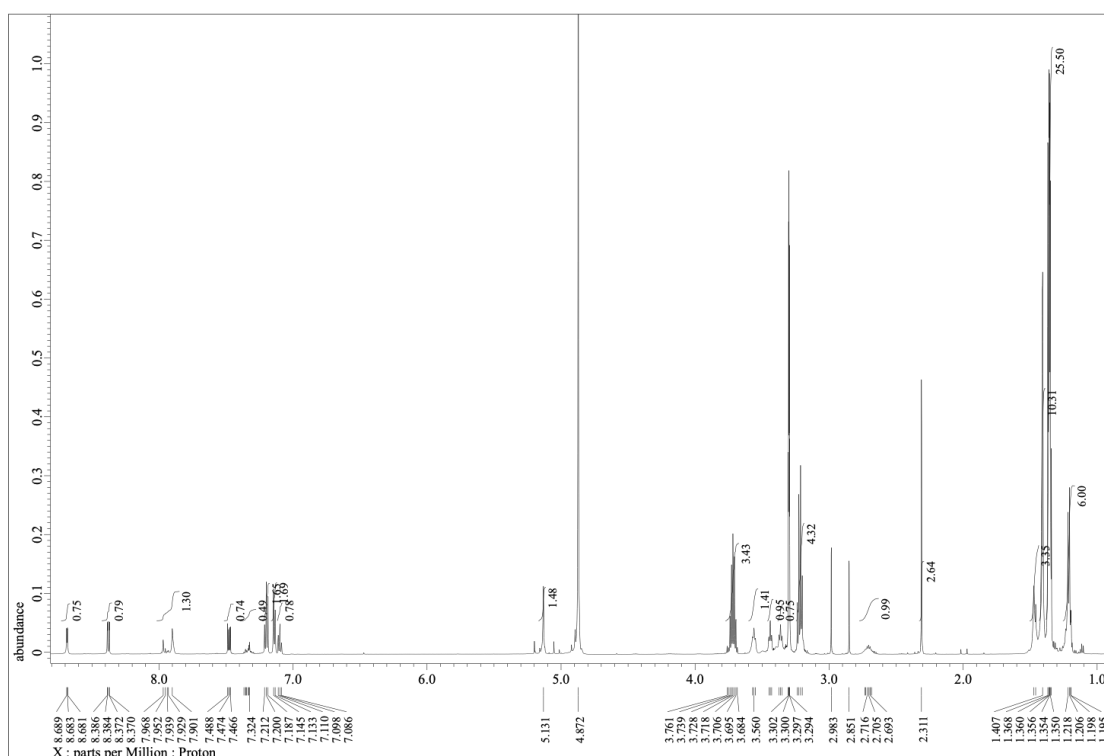
0.13 mmol, 18% yield for 2 steps) as a pale orange solid and **24** (72 mg, 0.13 mmol, 18% for 2 steps) as a dull orange gum.

For **23** : ¹H NMR (600 MHz, CD₃OD) δ 8.63 (d, J = 4.1 Hz, 2H), 8.34-8.32 (d, J = 7.2 Hz, 2H), 7.44 (q, J = 4.4 Hz, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.15-7.08 (m, 1H), 5.15 (t, J = 23.1 Hz, 2H), 3.74-3.34 (m, 1H), 3.26-3.18 (m, 1H), 2.74-2.61 (m, 2H), 2.31 (s, 1H), 1.40 (d, J = 4.1 Hz, 8H), 1.37-1.34 (m, 6H), 1.19-1.18 (m, 9H). ESI-HRMS calcd for C₃₁H₄₄N₁₃O₈ [M+H]⁺: 726.3430, found: 726.3393.

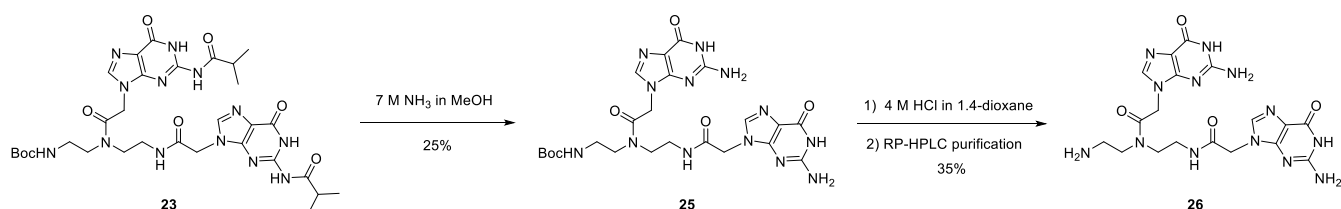
For **24** : ¹H NMR (600 MHz, CD₃OD) δ 8.68 (t, J = 2.4 Hz, 1H), 8.38 (dd, J = 8.3, 1.4 Hz, 1H), 7.97-7.90 (m, 1H), 7.48 (q, J = 4.4 Hz, 1H), 7.20 (t, J = 7.6 Hz, 2H), 7.14 (d, J = 6.9 Hz, 2H), 7.10 (t, J = 7.2 Hz, 1H), 5.13 (s, 1H), 3.72 (sept., J = 6.5 Hz, 3H), 3.56 (t, J = 6.2 Hz, 1H), 3.44 (t, J = 6.2 Hz, 1H), 3.36 (t, J = 6.5 Hz, 1H), 3.22 (q, J = 7.3 Hz, 4H), 2.73-2.68 (m, 1H), 2.31 (s, 3H), 1.41 (s, 10H), 1.37-1.34 (m, 25H), 1.22-1.19 (m, 6H). ESI-HRMS calcd for C₂₅H₄₁N₈O₇ [M+H]⁺: 565.3093, found: 565.3061.



¹H NMR spectrum of **23**

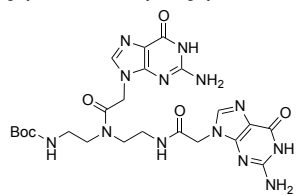


¹H NMR spectrum of **24**

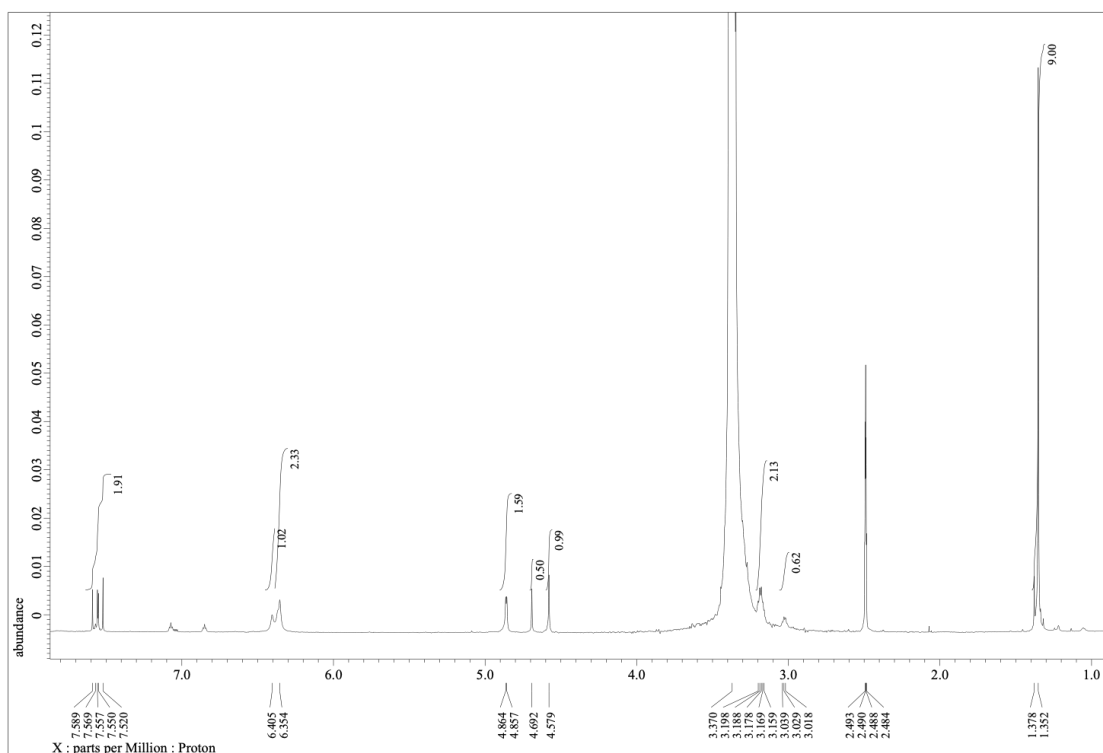


Scheme S8. Synthesis of compound **26**

***tert*-Butyl (2-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-*N*-(2-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl)acetamido)ethyl)carbamate (**25**)**

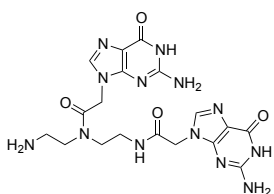


Compound **23** (91 mg, 0.13 mmol) was added NH₃ in MeOH (7 M, 5 mL), stirred for 20 h at rt. The reaction mixture was concentrated under reduced pressure. The residue was added NH₃ in MeOH (7 M, 5 mL), stirred for 23 h at 30°C. The resulting precipitate was collected by centrifugation, washed with ACN 2 times, dried under vacuum to afford **25** (18.6 mg, 0.032 mmol, 25% yield) as a pale orange solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.59-7.52 (m, 2H), 6.40 (br.s, 1H), 6.35 (br.s, 2H), 4.86 (d, *J* = 4.1 Hz, 2H), 4.69 (s, 1H), 4.58 (s, 1H), 3.20-3.16 (m, 2H), 3.03 (t, *J* = 6.2 Hz, 1H), 1.36 (d, *J* = 15.8 Hz, 9H). ESI-HRMS calcd for C₂₃H₃₂N₁₃O₆ [*M*+*H*]⁺: 586.2593, found: 586.2638.



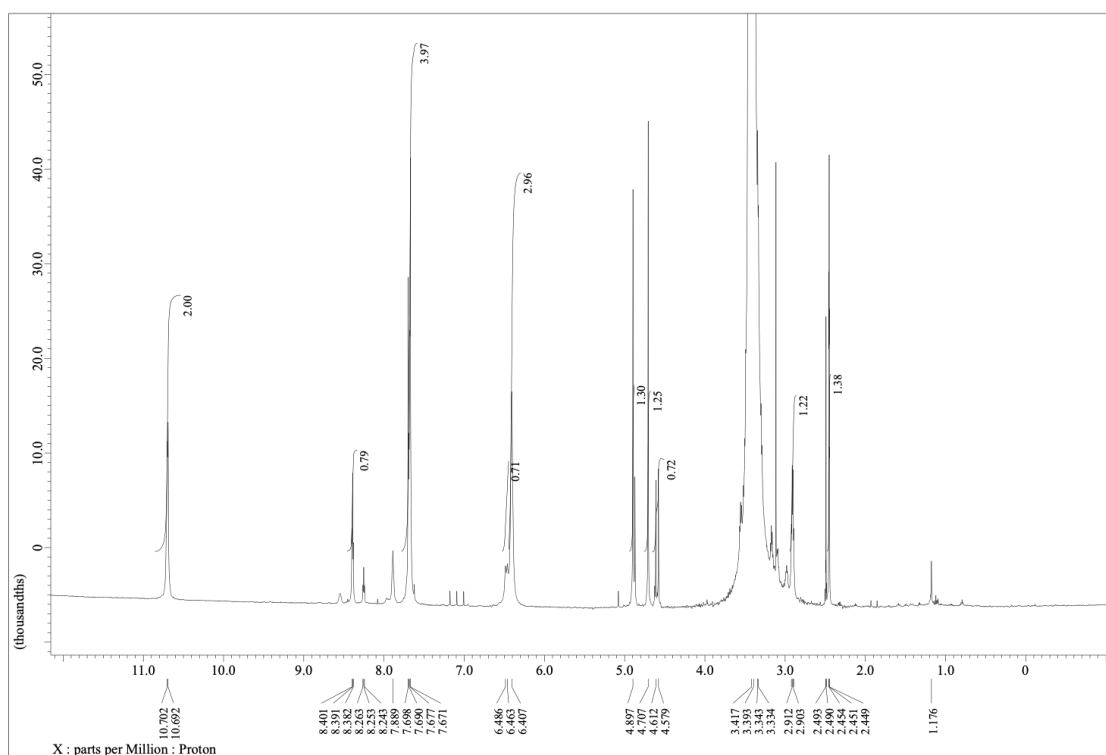
¹H NMR spectrum of **25**

2-(2-Amino-6-oxo-1*H*-purin-9(6*H*)-yl)-*N*-(2-(2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)acetamido)ethyl)-*N*-(2-aminoethyl)acetamide
(26)

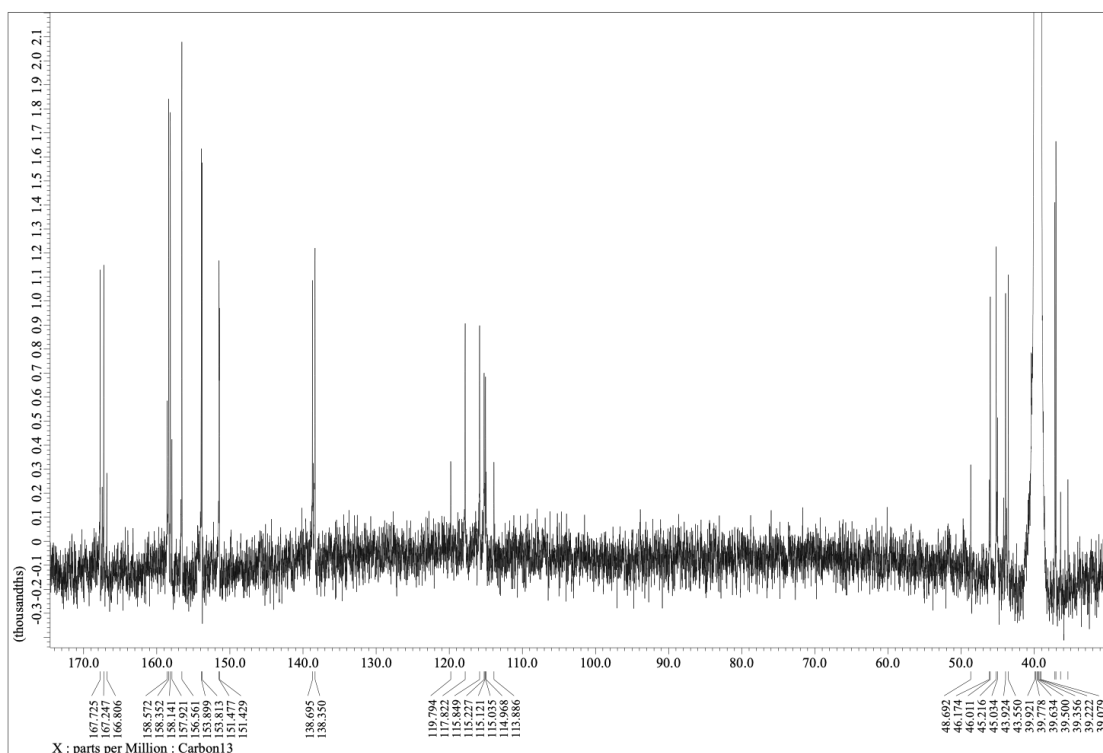


Compound **25** (15 mg, 0.026 mmol) was added HCl in 1,4-dioxane (4 M, 5 mL) and stirred at rt for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was dried by codistillation with MeOH. The residue was purified by HPLC (LC-2000Plus (JASCO); Discovery BIO Wide Pore C18 HPLC Column (Supelco), 21.2 × 250 mm, 10 μm; Flow rate: 10 mL/min; rt; Mobile phase A: 0.1% TFA in H₂O, B: 0.1% TFA in ACN; B 10% to 60 % (30 min); Detection : 260 nm) to afford **26** (4.4 mg, 9.1 μmol, 35% yield) as a pale yellow solid.

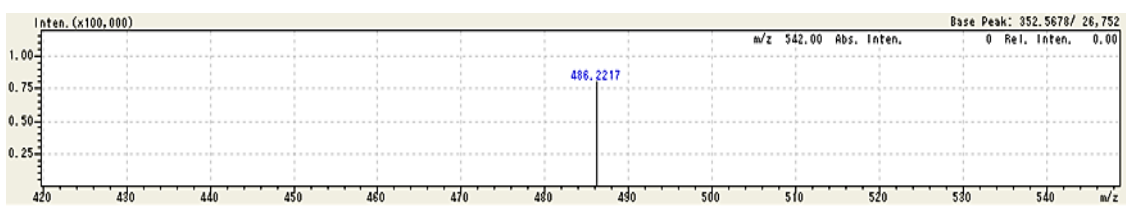
¹H NMR (600 MHz, DMSO-*d*₆) δ 10.70 (d, *J* = 6.2 Hz, 2H), 8.39 (t, *J* = 5.5 Hz, 1H), 7.68 (dd, *J* = 11.7, 4.1 Hz, 4H), 6.47 (d, *J* = 13.8 Hz, 1H), 6.41 (s, 3H), 4.90 (s, 1H), 4.71 (s, 1H), 4.60 (d, *J* = 20.0 Hz, 1H), 2.91 (q, *J* = 6.0 Hz, 1H), 2.45 (t, *J* = 1.7 Hz, 1H); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 167.7, 167.2, 166.8, 158.6, 158.4, 158.1, 157.9, 156.6, 153.9, 153.8, 151.5, 151.4, 138.7, 138.3, 119.8, 117.8, 115.8, 115.2, 115.1, 115.0, 115.0, 113.9, 48.7, 46.2, 46.0, 45.2, 45.0, 43.9, 43.6, 37.2, 37.0, 36.4, 35.4. ESI-MS : 486.2217 [M+H]⁺.



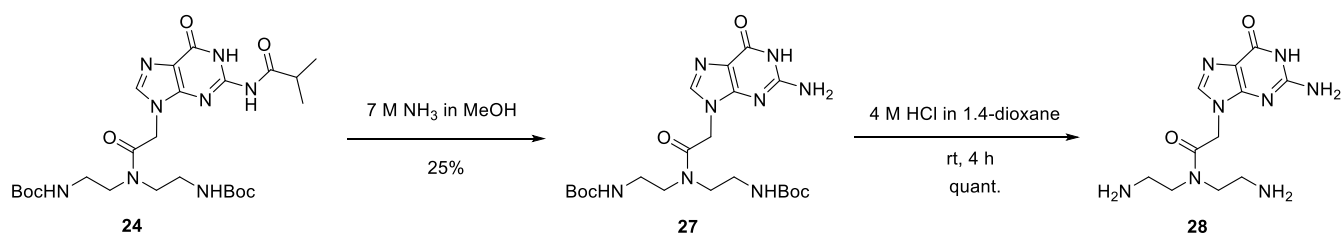
¹H NMR spectrum of 26



¹³C NMR spectrum of 26

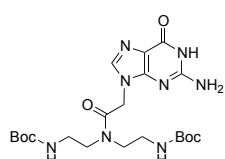


ESI-MS spectrum of **26**



Scheme S9. Synthesis of compound **28**

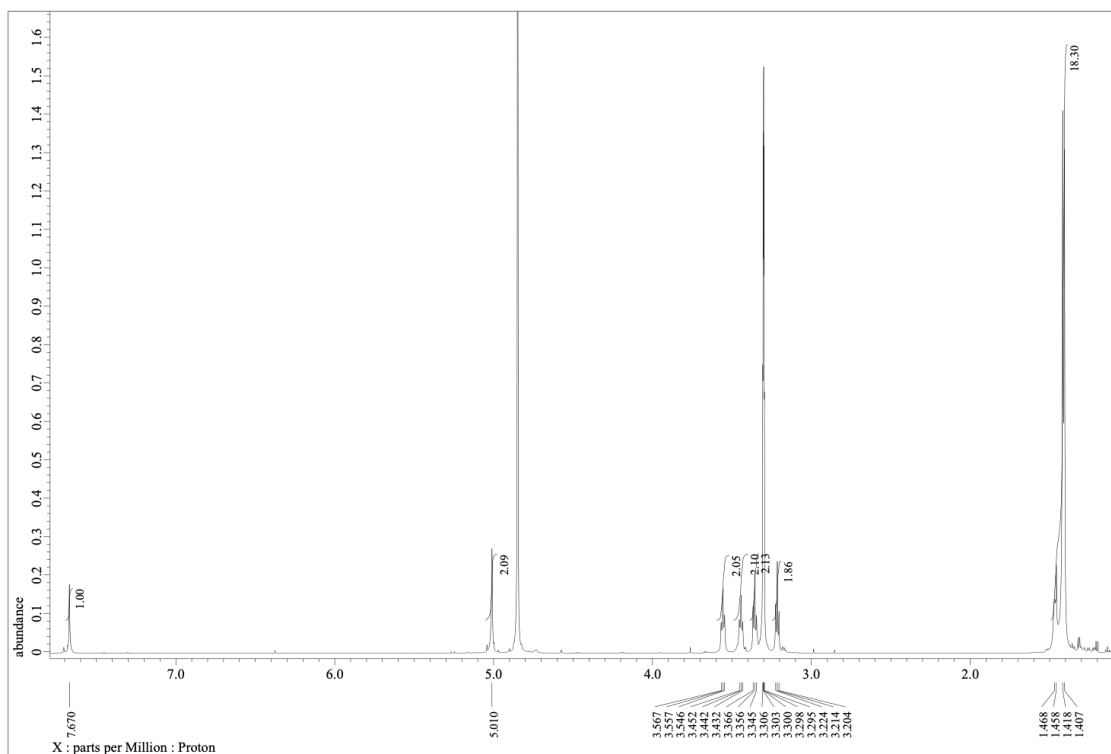
Di-tert-butyl (((2-(2-amino-6-oxo-1H-purin-9(6H)-yl)acetyl)azanediyl)bis(ethane-2,1-diyl))dicarbamate (27**)**



Compound **24** (72 mg, 0.13 mmol) was added NH_3 in MeOH (7 M, 5 mL), stirred for 38 h at rt. The reaction mixture was concentrated under reduced pressure, dried under vacuum. The residue was purified by flash silica gel column chromatography (Yamazen inject column size: S, Yamazen Hi-flash column size: M, $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100:0$ to $50:50$) to afford **27** (23.6 mg, 0.048 mmol, 37% yield) as an orange solid.

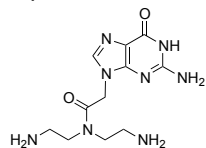
^1H NMR (600 MHz, CD_3OD) δ 7.67 (s, 1H), 5.01 (s, 2H), 3.56 (t, $J = 6.2$ Hz, 2H), 3.44 (t, $J = 6.2$ Hz, 2H), 3.36 (t, $J = 6.5$ Hz, 2H), 3.21 (t, $J = 6.2$ Hz, 2H), 1.47-1.41 (m, 18H). ESI-HRMS calcd for $\text{C}_{21}\text{H}_{35}\text{N}_8\text{O}_6$ $[\text{M}+\text{H}]^+$:

495.2674, found: 495.2683.



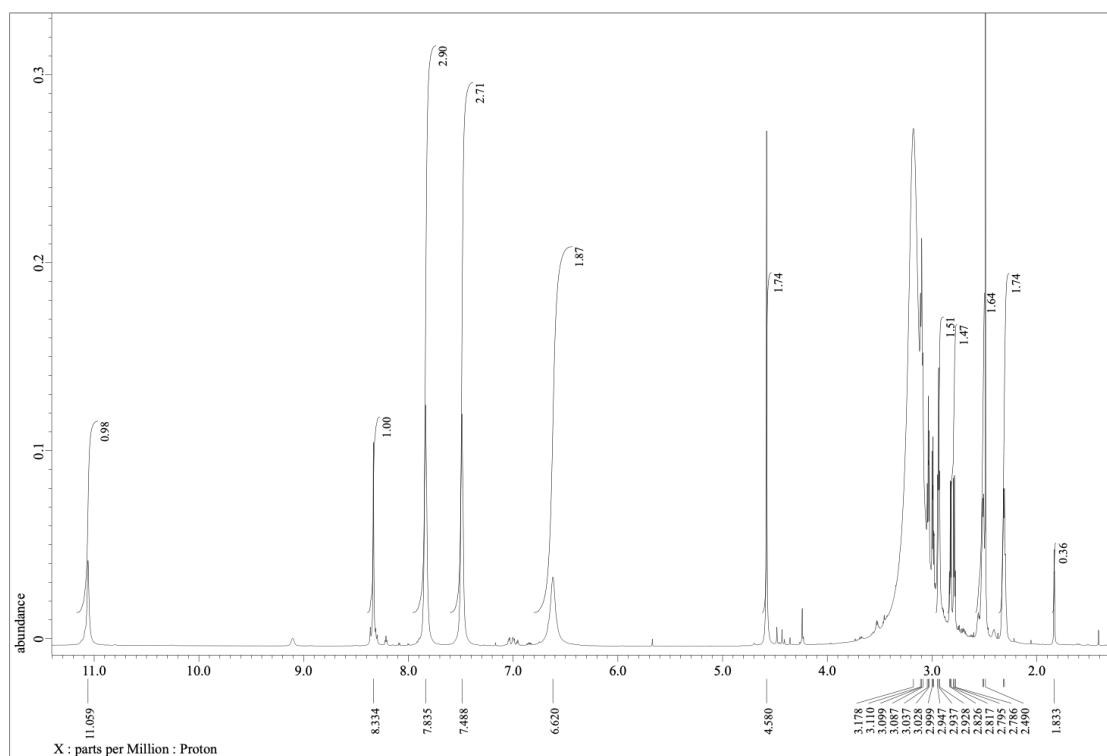
^1H NMR spectrum of **27**

2-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-N,N-bis(2-aminoethyl)acetamide trihydrochloride (28**)**

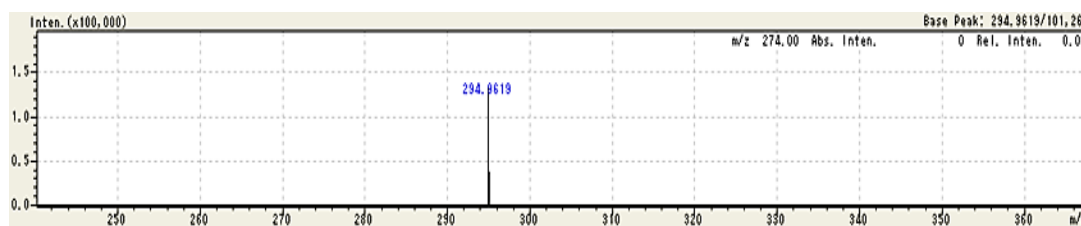


Compound **27** (24 mg, 0.049 mmol) was added HCl in 1,4-dioxane (4 M, 5 mL) and stirred for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was dried by codistillation with MeOH to afford **28** (25.3 mg, 0.069 mmol, quant.) as a pale orange solid.

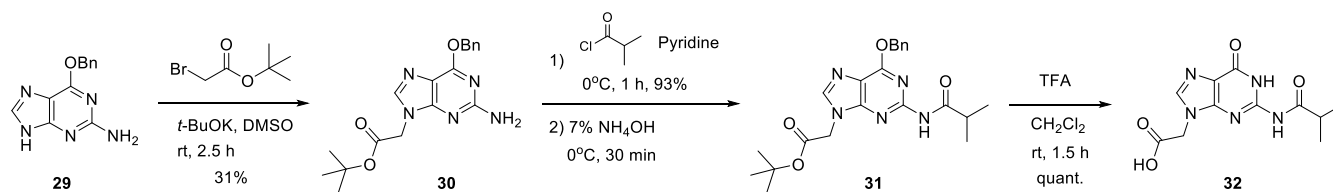
^1H NMR (600 MHz, DMSO- d_6) δ 11.06 (s, 1H), 8.33 (s, 1H), 7.84 (s, 3H), 7.49 (s, 3H), 6.62 (s, 2H), 4.58 (s, 2H), 2.94 (t, J = 5.9 Hz, 2H), 2.81 (dt, J = 23.9, 5.3 Hz, 1H), 2.51 (d, J = 5.5 Hz, 2H), 2.31 (d, J = 5.5 Hz, 2H). ESI-MS: 294.9619 $[\text{M}+\text{H}]^+$.



^1H NMR spectrum of **28**



ESI-MS chromatogram of **28**



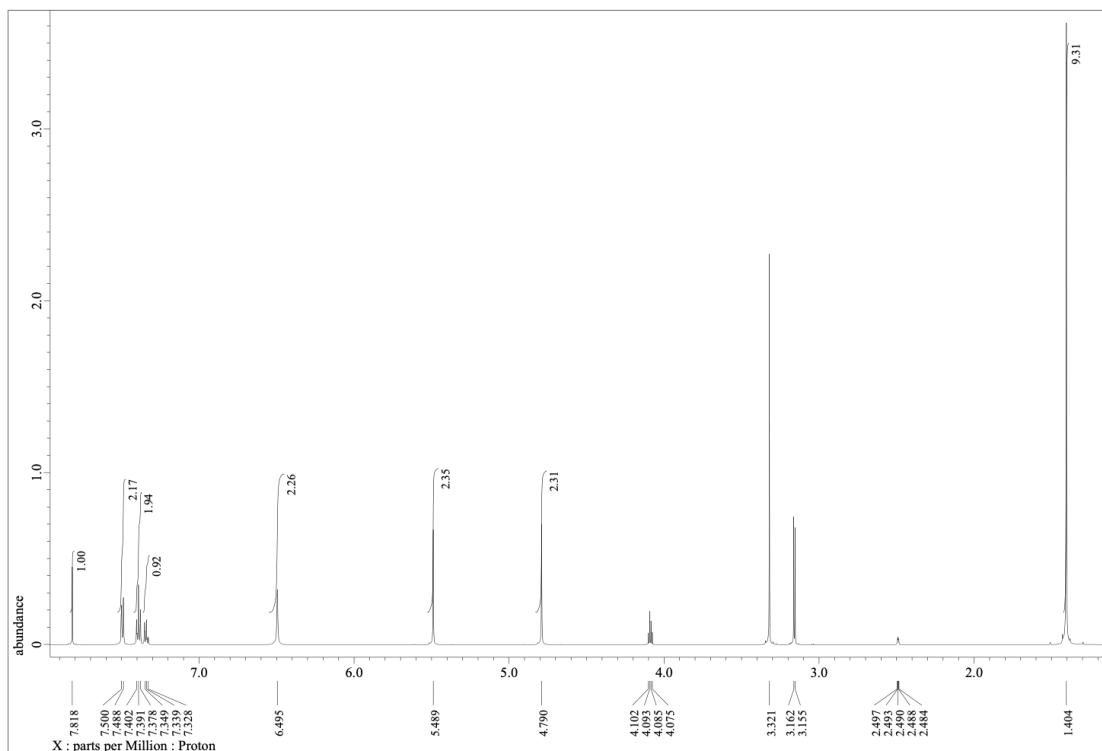
Scheme S10. Synthesis of compound **32**

***tert*-Butyl 2-(2-amino-6-(benzyloxy)-9*H*-purin-9-yl)acetate (**30**)**

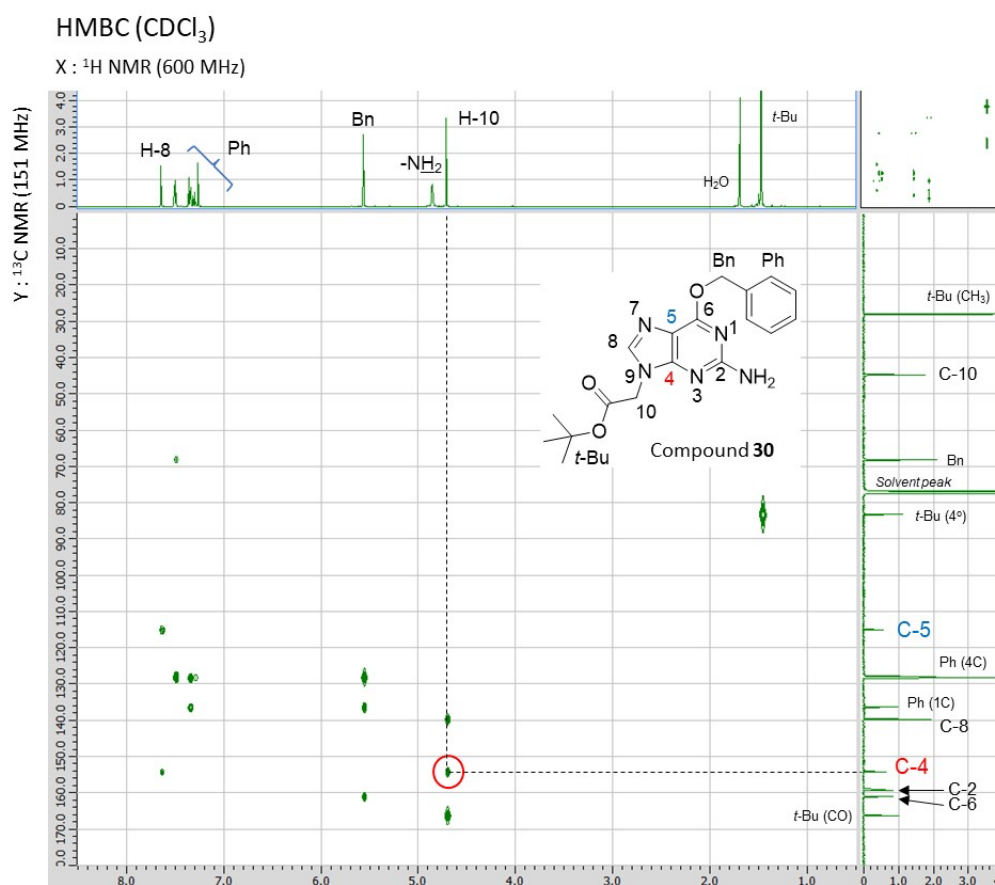
2-Amino-6-benzoyloxypurine **29** (10 g, 41 mmol) was dissolved in DMSO (82 mL) and stirred at 0°C. The mixture was added *t*-BuOK (5.58 g, 1.2 eq) slowly and stirred for 30 min at 0°C. *tert*-Butyl bromoacetate (8.5 mL, 1.4 eq) was added dropwise and stirred for 1 h at rt. The reaction mixture was diluted with EtOAc, washed with sat. NH₄Cl aq., H₂O and brine. The organic layer was dried over Na₂SO₄, filtered, concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (Yamazen inject column size: 2L, Hi-flash column size: 3L, CH₂Cl₂/MeOH = 100:0 to 98:2) to afford **30** (4.6 g, 13 mmol, 31% yield) as a white solid.

¹H NMR (600 MHz, DMSO-*d*₆) δ 7.82 (s, 1H), 7.49 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.34 (t, *J* = 6.5 Hz, 1H), 6.50 (s, 2H), 5.49 (s, 2H), 4.79 (s, 2H), 1.40 (s, 9H). ESI-HRMS calcd for C₁₈H₂₂N₅O₃ [M+H]⁺: 356.1717, found: 356.1733.

The position of *N*-alkylation was confirmed by 2D-NMR (HMBC) measurements (Correlation between C-4 and H-10).

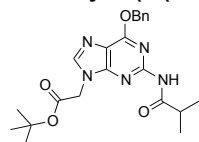


¹H NMR spectrum of **30**



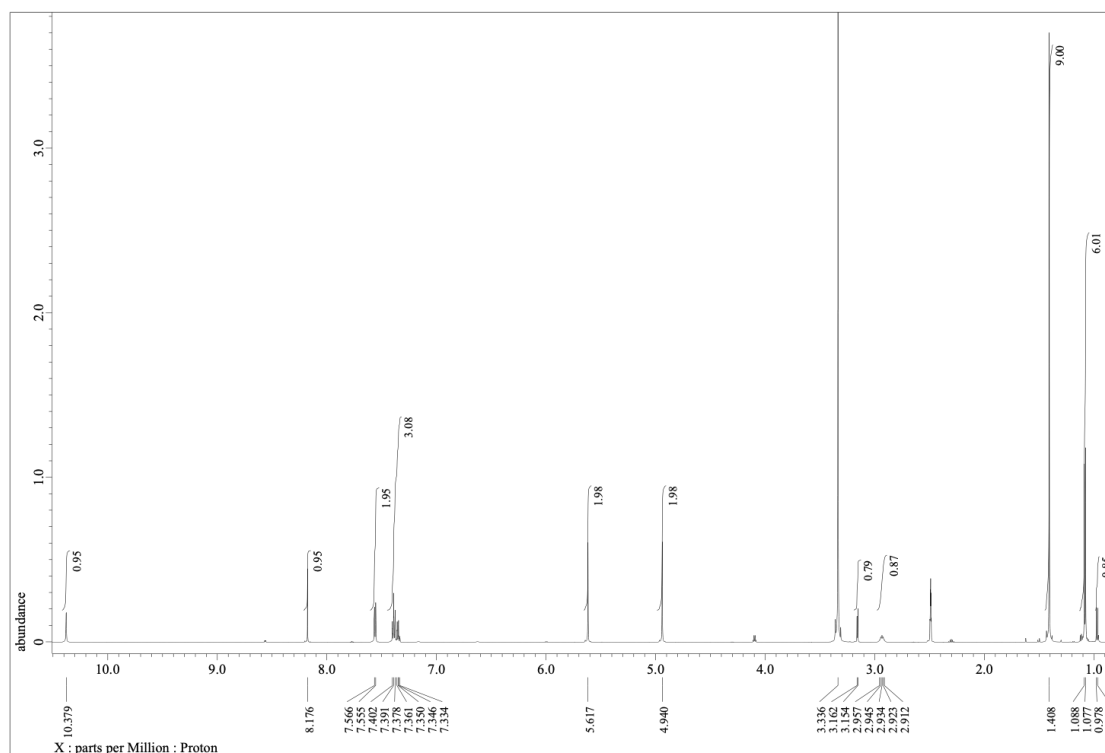
HMBC spectrum of **30**

tert-Butyl 2-(6-(benzyloxy)-2-isobutyramido-9H-purin-9-yl)acetate (31**)**



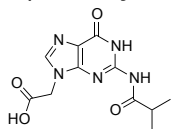
Compound **30** (4.6 g, 13 mmol) was dissolved in pyridine (65 mL) and stirred at 0°C. Isobutyryl chloride (2.7 mL, 2 eq) was added dropwise then stirred for 2 h at rt. After stirring, the reaction mixture was added isobutyryl chloride (1.4 mL, 1 eq) at 0°C, then stirred for 1 h at rt. After cooling to 0°C, the reaction mixture was added dropwise ice-cold 7% NH₄OH (60 mL) over 3 min under well-stirring. The reaction mixture was kept stirring for 30 min at 0°C. The reaction mixture was diluted with EtOAc, washed with sat. NaHCO₃ aq. and brine. The organic layer was dried over Na₂SO₄, filtered, concentrated under reduced pressure, dried under vacuum to afford **31** (5.1 g, 12 mmol, 93%) as a white foam solid.

¹H NMR (600 MHz, DMSO-*d*₆) δ 10.38 (s, 1H), 8.18 (s, 1H), 7.56 (d, *J* = 6.9 Hz, 2H), 7.40-7.33 (m, 3H), 5.62 (s, 2H), 4.94 (s, 2H), 3.16 (d, *J* = 4.8 Hz, 1H), 2.96-2.91 (quint., *J* = 6.8 Hz, 1H), 1.41 (s, 9H), 1.08 (d, *J* = 6.9 Hz, 6H), 0.97 (d, *J* = 6.9 Hz, 1H). ESI-HRMS calcd for C₂₂H₂₈N₅O₄ [M+H]⁺: 426.2136, found: 426.2144.



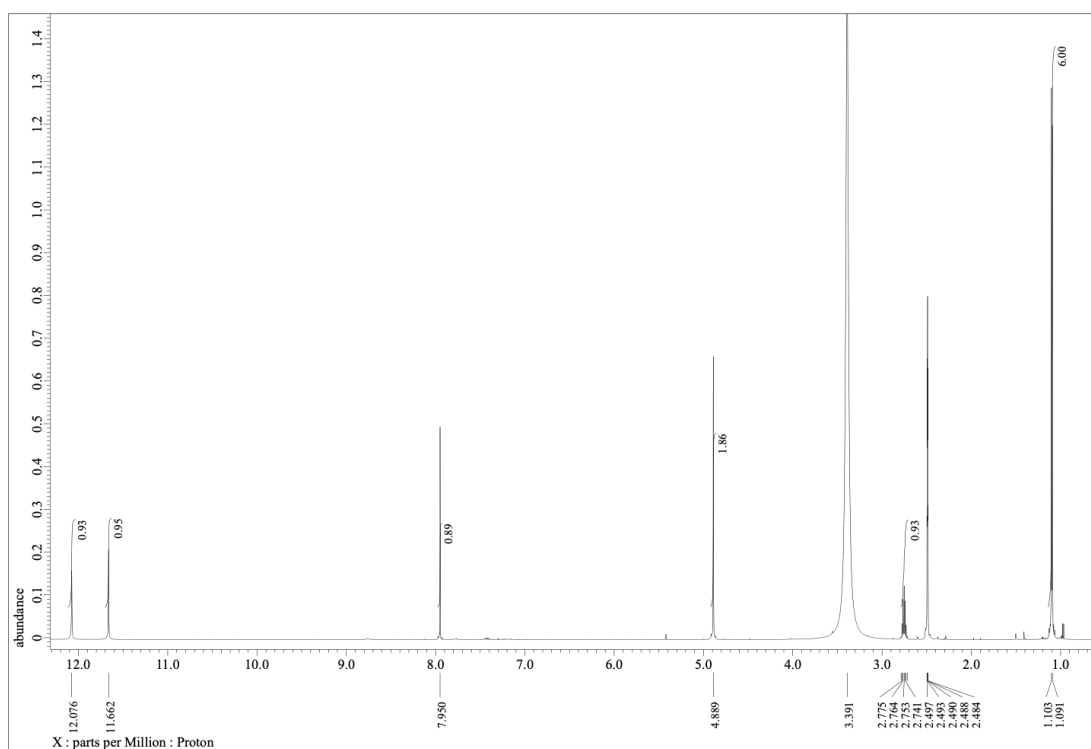
¹H NMR spectrum of **31**

2-(2-Isobutyramido-6-oxo-1*H*-purin-9(6*H*)-yl)acetic acid (**32**)



Compound **31** (5.1 g, 12 mmol) was dissolved in CH₂Cl₂ (10 mL) and TFA (40 mL) then stirred for 2 h at rt. The reaction mixture was diluted with toluene, concentrated under reduced pressure to remove the volatiles. The residue was added Et₂O, and triturated to solidify. The resulting precipitate was collected by filtration, washed with Et₂O, dried under vacuum to afford **32** (3.9 g, 14 mmol, quant.) as a white powder.

¹H NMR (600 MHz, DMSO-*d*₆) δ 12.08 (s, 1H), 11.66 (s, 1H), 7.95 (s, 1H), 4.89 (s, 2H), 2.78-2.73 (sept., *J* = 6.9 Hz, 1H), 1.10 (d, *J* = 6.9 Hz, 6H). ESI-HRMS calcd for C₁₁H₁₄N₅O₄ [M+H]⁺: 280.1040, found: 280.1040.



^1H NMR spectrum of **32**

References

- [S1] K. Ikeda, Y. Yanase, K. Hayashi, Y. Hara-Kudo, G. Tsuji, and Y. Demizu, *Bioorg. Med. Chem. Lett.* **2021**, 32, 127713, DOI: 10.1016/j.bmcl.2020.127713.
- [S2] Y. Yang, X. Wu, N. Busschaert, H. Furuta, P. A Gale, *Chem. Commun.* **2017**, 53, 9230-9233, DOI: 10.1039/c7cc04912a.
- [S3] D. D. N'Da, E. Neuse, M. Nell, C. E. J. van Rensburg, *S. Afr. J. Chem.* **2003**, 59, 33-42.