

Supporting Information: **A New Strategy for Site-Specific Alkylation of DNA using Oligonucleotide Containing an Abasic site and Alkylating Probes**

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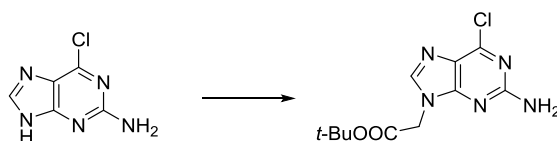
General

All air sensitive or water sensitive reactions were carried out under argon atmosphere using a high-vacuum line, dry equipment, and dry solvents.

^1H (400 MHz) NMR spectra were recorded on a Bruker 400 spectrometer. ^{13}C (125 MHz) NMR spectra were recorded on a Bruker AVANCE III 500 spectrometer. ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker AVANCE III 600 spectrometer. Chemical shifts of ^1H NMR were reported in ppm using deuterated solvents as internal standard (CDCl_3 , δ 7.26; $\text{DMSO}-d_6$, δ 2.50; $\text{MeOD}-d_4$, δ 3.31). Multiplicity was described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shifts of ^{13}C NMR were reported in ppm using deuterated solvents as internal standard (CDCl_3 , δ 77.16; $\text{DMSO}-d_6$, δ 39.52; $\text{MeOD}-d_4$, δ 49.00). High resolution electrospray mass analysis was performed on a Bruker MicrOTOFQ II. MALDI-TOF mass analysis was performed on a Bruker autoflex speed mass spectrometer. Thin layer chromatography was performed on Silica gel 60 F254 (Merck-Millipore). Column chromatography was performed with Silica gel 60N [40-50 μm or 100-210 μm; KANTO CHEMICAL CO. INC. (Tokyo, Japan)]. Reverse phased high performance liquid chromatography

was performed on JASCO HPLC system [PU-2089 as the pump, UV-2075 as the UV-detector, FP-2025 as the fluorescence detector, and CO-2065 as the column oven] purchased by JASCO Corporation (Tokyo, Japan) and COSMOSIL ODS columns [5C18-AR-II (4.6 or 10 x 250 mm) for organic compound or 5C18-MS-II (4.6 or 10 x 250 mm) for oligonucleotide] purchased by Nacalai tesque (Kyoto, Japan). Ultraviolet-visible (UV-Vis) spectra were recorded on a BECKMAN COULTER DU800 using 1 cm path length micro cell. Fluorescence spectra were recorded on a JASCO FP-5200 spectrometer using 1 cm path length quartz cell. pH measurement was performed on Seven Easy pH meter (Mettler Toledo), using ORION 8220BNWP (Thermo Fisher Scientific) as the electrode. Densitometric analysis of the gel was carried out on 20% denaturing polyacrylamide gel plates, and visualized and quantified by FLA-5100 Fluor Imager (Fujifilm Co., Tokyo, Japan). Reagents and organic solvents were purchased by Wako Pure Chemical Industries (Osaka, Japan), Tokyo Chemical Industry (Tokyo, Japan), Sigma-Aldrich, Nacalai tesque (Kyoto, Japan), and Junsei Chemical (Tokyo, Japan). Reagents for solid phase peptide synthesis were purchased by Novabiochem and Peptide Institute, Inc. (Osaka, Japan). DNAs were purchased by Japan Bio Services Co., LTD. (Saitama, Japan). dNTPs were purchased by Takara Bio (Shiga, Japan). Klenow fragment was purchased by New England Biolabs.

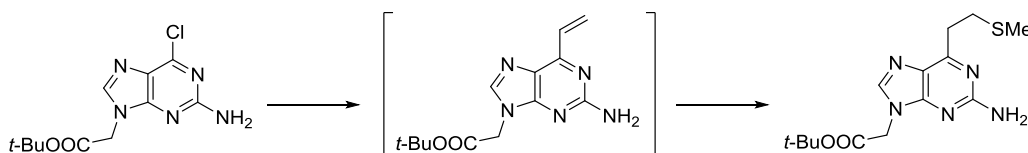
Synthesis of *tert*-butyl (6-chloro-2-aminopurin-9-yl)acetate (**5**)



To a solution of 2-amino-6-chloropurine (4.0 g, 23.66 mmol) in DMSO (16 mL), was added *tert*-BuOK (3.18 g, 28.3 mmol) in DMSO (40 mL). After stirred at room temperature for 1 h, *tert*-butyl bromoacetate (4.84 mL, 33.0 mmol) was added drop-wise and stirred at the same temperature. After 2 h, the reaction mixture was diluted with EtOAc, and the reaction was quenched with sat. NH₄Cl/water = 1/1 (100 mL). The extracted organic layer was washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated to afford yellow solid. The solid was washed with hexane/Et₂O = 1/1 (100 mL) and concentrated to gain pale yellow solid (4.9 g, 73%).

¹H NMR (CDCl₃, 400 MHz) δ 7.82 (1H, s), 5.10 (2H, brs), 4.73 (2H, s), 1.48 (9H, s)

Synthesis of *tert*-butyl 2-(2-amino-6-(2-methylthio)ethyl)-9H-purin-9-yl)acetate

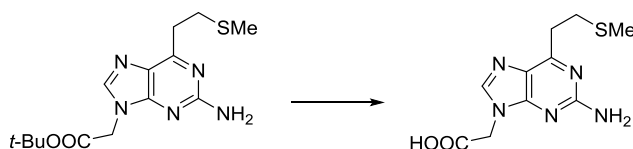


To a suspension of **5** (2.0 g, 7.05 mmol) in 1,4-dioxane (40 mL), were added CH₂CHSnBu₃ (2.46 mL, 8.50 mmol), PdCl₂(PPh₃)₂ (0.247 g, 0.352 mmol) and stirred at 120 °C. After 10 h, the mixture was cooled to room temperature and extracted with EtOAc (100 mL x 2). Each organic layer was washed with sat. NaHCO₃/water = 1/2 (100 mL), water (100 mL), and brine (100 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was added to hexane (100 mL) and the

resulting precipitation was collected. The precipitation was washed with hexane (100 mL) and dried under reduced pressure to afford a pale orange solid. The resulting solid was suspended in acetonitrile (17 mL), and to this suspension was added 40% CH₃SNa (1.77 g, 10.1 mmol) in water (2 mL). After stirred at room temperature for 30 min, the reaction mixture was extracted with EtOAc (100 mL). The organic layer was washed with sat. NH₄Cl (30 mL), water (30 mL) and brine (30 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (CHCl₃/EtOAc = 3/1 to 1/1 to 0/1) to give the desired product (1.17 g, 54% in 2 steps) as a pale yellow solid.

¹H NMR (CDCl₃, 600 MHz) δ 7.76 (1H, s), 5.05 (2H, brs), 4.72 (2H, s), 3.30 (2H, t, $J = 7.5$ Hz), 3.01 (2H, t, $J = 7.5$ Hz), 2.16 (3H, s), 1.46 (9H, s); ¹³C NMR (CDCl₃, 150 MHz) δ 166.4, 161.5, 159.9, 153.0, 141.7, 126.5, 83.6, 44.5, 33.1, 32.2, 28.1, 15.6; HRMS-ESI (m/z): [M+H]⁺ calcd for C₁₄H₂₁N₅O₂S, 324.1489; found 324.1490

Synthesis of 2-(2-amino-6-(2-(methylthio)ethyl)-9H-purin-9-yl)acetic acid (6)



To a solution of *tert*-butyl 2-(2-amino-6-(2-(methylthio)ethyl)-9H-purin-9-yl)acetate (17.6 mg, 54.4 μ mol) in CH₂Cl₂ (0.1 mL), was added triethylsilane (9.0 μ L, 56.3 μ mol) and trifluoroacetic acid (0.4 mL). After stirred at room temperature for 3 h, the mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (0.5 mL) and was added to Et₂O (2.5 mL) to afford the desired product (12.0 mg, 83%) as a pale yellow precipitation.

¹H NMR (DMSO-*d*₆, 600 MHz) δ 7.99 (1H, s), 6.55 (2H, brs), 4.85 (2H, s), 3.14 (2H, t, $J = 7.5$ Hz), 2.95 (2H, t, $J = 7.5$ Hz), 2.09 (3H, s); ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 169.4, 160.1, 159.8, 153.0, 142.3, 125.1, 43.7, 32.2, 31.2, 14.6; HRMS-ESI (m/z): [M-H]⁻ calcd for C₁₀H₁₃N₅O₂S 266.0717; found 266.0718

Synthesis of (Arg)₅ probe (7)

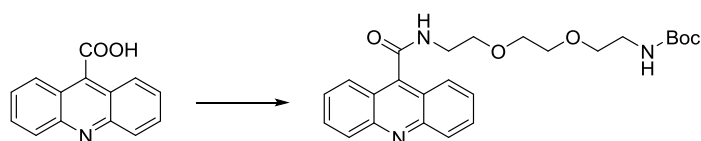
The peptides elongation and coupling of AVP unit (6) were performed manually by Fmoc-SPPS procedures.

The Rink Amide AM resin (0.71 mmol/g, 25.8 mg; Novabiochem) was washed with *N*-methylpyrrolidone (3 mL x 5) for every 1 min. The Fmoc group was deprotected with 20% (v/v) piperidine in *N*-methylpyrrolidone (3 mL x 2), and shaken for 1 min and again for 15 min using fresh reagents. After deprotection, the resin was washed with *N*-methylpyrrolidone (3 mL x 5) for every 1 min. The coupling reaction was performed with Fmoc-Arg(Pbf) (3 equiv.), HBTU (3 equiv.), HOBT (3 equiv.), and DIPEA (6 equiv.) in *N*-methylpyrrolidone (2.0 mL) and shaken at room temperature for 1 h. After coupling reaction, the reaction mixture was removed and the resin was washed with *N*-methylpyrrolidone (3.0 mL x 5) for every 1 min. Elongation of another four Arginine residues and AVP unit (6) were carried out with the same conditions. After synthesis, the (Arg)₅-AVP was cleaved from the resin with concomitant removal of the protecting group of (Arg)₅ by treatment with a

cleavage cocktail containing water/triisopropylsilane/TFA (7.5/7.5/285 μL) for 30 min. The resin was filtered off and the crude mixture was added to cooled Et_2O (4 mL) to afford white precipitation. The precipitation was purified with reversed phase HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C₁₈-AR-II, 10 x 250 mm) by a liner gradient of 0-30%/30min acetonitrile in 0.1% TFA buffer at a flow rate of 4 mL/min at 40.0 $^\circ\text{C}$, and monitored by UV detection at $\lambda = 254$ nm to afford a white solid (1.67 mg). The product was characterized by MALDI-TOF mass spectrometry. The concentration was determined by UV absorption at 260 nm in water using the extinction coefficient: $963.1 \text{ M}^{-1} \text{ cm}^{-1}$ [6-(2-(methylthio)ethyl)-9H-purin-2-amine].

MALDI-TOF MS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{40}\text{H}_{74}\text{N}_{26}\text{O}_6\text{S}$, 1047.61; found 1047.57

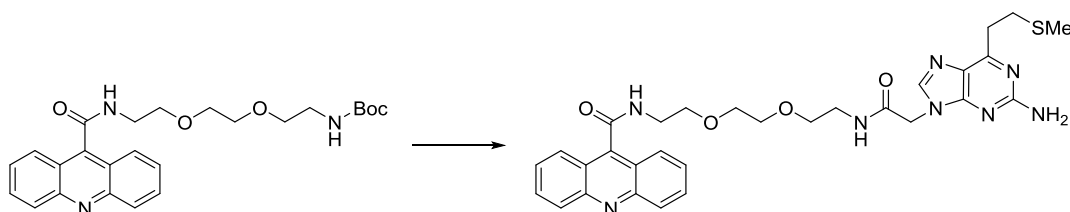
Synthesis of *N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)acridine-9-carboxamide (**8**)



To a suspension of acridine-9-carboxylic acid n-hydrate (100 mg, 0.41 mmol as monohydrate) and HOBt (73 mg, 0.54 mmol) in dry DMF (1.5 mL), was added EDC-HCl (103 mg, 0.54 mmol) and stirred at room temperature for 5 min. To this mixture, was added *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (134 mg, 0.54 mmol) in dry DMF (1 mL) and stirred at room temperature. After 5 h, the reaction mixture was diluted with EtOAc (15 mL), washed with sat NaHCO_3 (x 3 times) and brine, and evaporated under reduced pressure to afford pale brown oil. The residue was purified by column chromatography ($\text{CHCl}_3/\text{MeOH} = 100/0$ to $100/1$) to give pale yellow oil (128 mg, 68 %).

^1H NMR (CDCl_3 , 400 MHz) δ 8.22 (2H, d, $J = 8.8$ Hz), 8.10 (2H, d, $J = 8.4$ Hz), 7.79 (2H, dd, $J = 7.2, 8.0$ Hz), 7.58 (2H, dd, $J = 7.2, 8.0$ Hz), 4.77 (1H, brs), 3.86-3.94 (4H, m), 3.70 (2H, brs), 3.60 (2H, brs), 3.39 (2H, brs), 3.06 (2H, brs), 1.32 (9H, s); ^{13}C NMR (CDCl_3 , 125 MHz) δ 167.2, 155.7, 148.5, 140.9, 130.3, 129.6, 126.7, 125.3, 122.2, 79.2, 70.4, 70.1, 69.7, 40.1, 40.0, 28.4, 28.3; HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{31}\text{N}_3\text{O}_5$, 454.2336; found 454.2352

Synthesis of *N*-(2-(2-(2-(2-(2-amino-6-(2-(methylthio)ethyl)-9H-purin-9-yl)acetamido)ethoxy)ethyl)acridine-9-carboxamide (**9**)

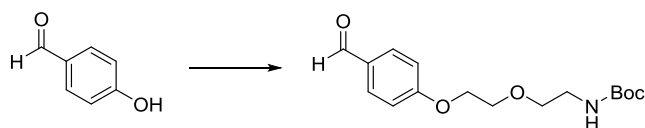


To a solution of **8** (55 mg, 0.12 mmol) in CH_2Cl_2 (0.1 mL), was added TFA (0.4 mL) and stirred at room temperature for 2 h. The volatile compound was removed by evaporation under vacuum to afford a light yellow solid. To above mixture, was added DIPEA (211 μL , 1.2 mmol), followed by a pre-stirred (0°C , 1 h) solution of **6** (42 mg, 0.16 mmol), EDC-HCl (30 mg, 0.16 mmol), HOBt (21 mg, 0.16 mmol) in dry DMF (1.2 mL), and stirred at room temperature. After 3 h, the reaction

mixture was diluted with CH₂Cl₂, washed with sat. NaHCO₃ and brine, dried over Na₂SO₄, filtered, and evaporated under reduced pressure to afford orange oil. This residue was purified by column chromatography (CHCl₃/MeOH = 50/1 to 30/1) to give pale yellow oil. The sample was further purified by reversed phase HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C₁₈-AR-II, 10 x 250 mm) by a liner gradient of 0-45%/30min acetonitrile in 0.1% TFA buffer at a flow rate of 4 mL/min at 40.0 °C, and monitored by UV detection at λ = 254 nm and fluorescence detection (λ_{ex} = 357 nm, λ_{em} = 482 nm) to afford a pale yellow solid (33.0 mg, 38% as a mono TFA salt). The product was characterized by MALDI-TOF mass spectrometry. The concentration of **9** was determined with quantitative NMR using maleic acid as an internal standard.

¹H NMR (MeOD-*d*₄, 400 MHz) δ 8.15 (2H, d, *J* = 8.8 Hz), 8.13 (2H, d, *J* = 8.0 Hz), 7.87 (1H, s), 7.84 (2H, dd, *J* = 7.6, 8.8 Hz), 7.63 (2H, dd, *J* = 7.6, 8.0 Hz), 4.67 (2H, s), 3.82-3.86 (4H, m), 3.72-3.75 (2H, m), 3.66-3.68 (2H, m), 3.54 (2H, t, *J* = 5.2 Hz), 3.31 (2H, t, *J* = 5.2 Hz), 3.18 (2H, t, *J* = 8.0 Hz), 2.92 (2H, t, *J* = 8.0 Hz), 2.12 (3H, s); ¹³C NMR (MeOD-*d*₄, 125 MHz) δ 168.0, 167.0, 158.0, 156.5, 154.4, 151.5, 150.1, 144.0, 137.2, 129.6, 128.1, 126.5, 123.9, 123.6, 71.5, 71.3, 70.4, 70.3, 46.2, 41.0, 40.6, 32.7, 30.5, 15.1; HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₃₀H₃₄N₈O₄S, 603.2496; found 603.2499

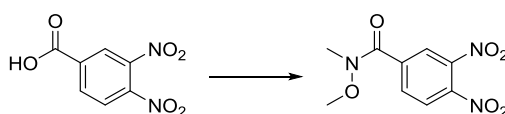
Synthesis of *tert*-butyl (2-(2-(4-formylphenoxy)ethoxy)ethyl)carbamate (**10**)



To a solution of 4-hydroxybenzaldehyde (366 mg, 3.0 mmol) in dry THF (7.5 mL), were added triphenylphosphine (865 mg, 3.3 mmol) and *tert*-butyl (2-(2-hydroxyethoxy)ethyl)carbamate (800 mg, 3.9 mmol) and cooled to 0°C under argon atmosphere. To the above mixture, was added DIAD (649 μL, mmol) drop-wise under stirring. After addition was completed, the reaction mixture was stirred at room temperature. After 5 h, the mixture was diluted with EtOAc (20 mL), and washed with 1N NaOH, H₂O, and brine sequentially. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure to afford white solid crude material. This residue was purified by column chromatography (Hexane/EtOAc = 3/1 to 2/1) to give colorless oil (458 mg, 49 %).

¹H NMR (CDCl₃, 400 MHz) δ 9.89 (1H, s), 7.83 (2H, d, *J* = 8.8 Hz), 7.03 (2H, d, *J* = 8.8 Hz), 4.92 (1H, brs), 4.20 (2H, t, *J* = 4.8 Hz), 3.85 (2H, t, *J* = 4.8 Hz), 3.61 (2H, t, *J* = 5.2 Hz), 3.35 – 3.34 (2H, m), 1.44 (9H, s); ¹³C NMR (CDCl₃, 125 MHz) δ 190.7, 163.7, 155.9, 131.9, 130.0, 114.8, 79.2, 70.4, 69.1, 67.6, 40.2, 28.3, 21.9; HRMS-ESI (*m/z*): [M+Na]⁺ calcd for C₁₆H₂₃NO₅Na⁺, 332.1468; found 332.1474

Synthesis of *N*-methoxy-*N*-methyl-3,4-dinitrobenzamide

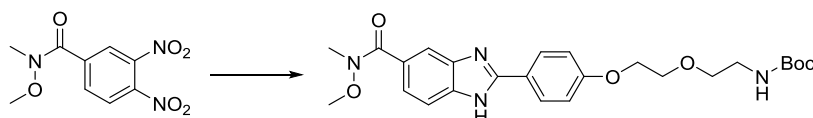


A mixture of 3,4-dinitrocarboxylic acid (2.0 g, 9.43 mmol) and thionyl chloride (5.5 mL, 75.8 mmol) was stirred at 80 °C. After 4 h, the excess thionyl chloride was removed by evaporation and

co-evaporated with toluene three times to give a yellow solid. This crude product was used in next reaction without further purification.

The crude material was dissolved in dry CH_2Cl_2 (10 mL), and cooled to 0 °C. To this mixture, was slowly added pyridine (2.1 mL), followed by *N,O*-dimethylamine hydrochloride (1.26 g, 12.9 mmol) and stirred at room temperature. After 8 h, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 10% HCl aq. and sat. NaHCO_3 aq., three times. The organic layer was dried over anhydrous Na_2SO_4 , filtered, and evaporated under reduced pressure to afford pale yellow solid (ca 1.70 g). This product was used in next step without further purification.

Synthesis of *tert*-butyl (2-(2-(4-(5-methoxy(methyl)carbamoyl)-1*H*-benzimidazol-2-yl)phenoxy)ethoxy)ethyl) carbamate

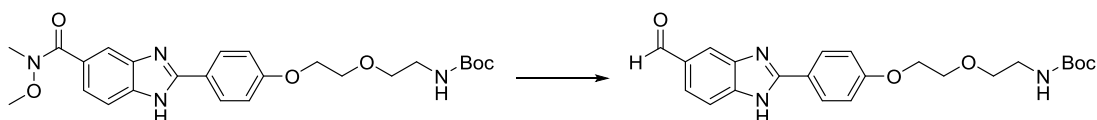


To a suspension of 3,4-dinitro weinreb amide (510 mg, 2.00 mmol) in EtOH (30 mL) was added 10% Pd/C (100 mg), and the reaction mixture was stirred at room temperature for 7 h under hydrogen atmosphere. To this mixture, were directly added $\text{Na}_2\text{S}_2\text{O}_5$ (190 mg, 1.00 mmol) in H_2O (0.8 mL) and *tert*-butyl (2-(2-(4-formylphenoxy)ethoxy)ethyl)carbamate (680 mg, 2.20 mmol) in EtOH (2 mL) and stirred at 85 °C for 14 h. After cooled to room temperature, the mixture was filtered through Celite, and filtrate was concentrated under reduced pressure to afford reddish brown solid. This residue was purified by column chromatography ($\text{CHCl}_3/\text{MeOH} = 100/1, 50/1, \text{ to } 20/1$) to give reddish brown foam (411.1 mg, 42 %).

^1H NMR ($\text{MeOD-}d_4$, 600 MHz) δ 7.99 (2H, d, $J = 8.4$ Hz), 7.92 (1H, s), 7.59-7.54 (2H, m), 7.04 (2H, d, $J = 8.4$ Hz), 4.12 (2H, t, $J = 4.5$ Hz), 3.78 (2H, t, $J = 4.5$ Hz), 3.60 (3H, s), 3.56 (2H, t, $J = 5.7$ Hz), 3.38 (3H, s), 3.24 (2H, t, $J = 5.7$ Hz), 1.41 (9H, s); ^{13}C NMR ($\text{MeOD-}d_4$, 150 MHz) δ 171.9, 162.3, 158.4, 155.3, 129.5, 129.0, 124.1, 123.0, 116.1, 80.0, 71.2, 70.3, 68.7, 61.5, 41.2, 34.7, 28.7; HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{32}\text{N}_4\text{O}_6$, 485.2395; found 485.2395

In ^{13}C NMR spectra, some carbons derived from benzimidazole were not observed due to tautomerization.^{S1}

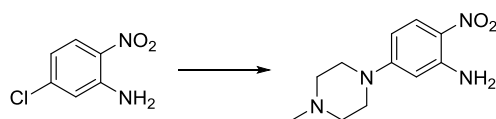
Synthesis of *tert*-butyl (2-(2-(4-(5-formyl-1*H*-benzimidazole-2-yl)phenoxy)ethoxy)ethyl) carbamate (11)



To a solution of *tert*-butyl (2-(2-(4-(5-methoxy(methyl)carbamoyl)-1*H*-benzimidazole-2-yl)phenoxy)ethoxy)carbamate (90 mg, 0.19 mmol) in THF(3 mL)/Et₂O(1 mL), was added LiAlH_4 (36 mg, 0.95 mmol) at 0°C under argon atmosphere, followed by stirred at room temperature for 30 min. The reaction was quenched by the addition of sat. NH_4Cl aq. at 0 °C, and extracted with EtOAc (5 mL x 2). The combined organic layer was washed with brine, dried anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to afford pale brown oil. This crude product was used in next

step without further purification.

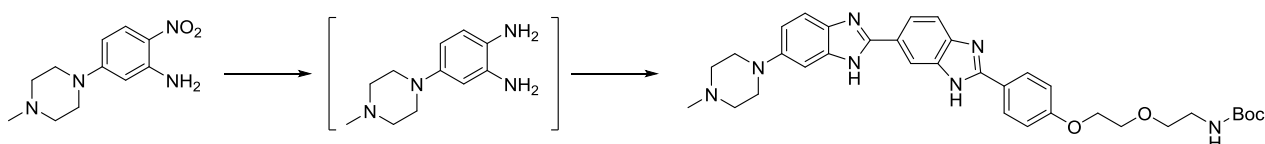
Synthesis of 5-(4-methylpiperazin-1-yl)-2-nitroaniline



To a suspension of 5-chloro-2-nitroaniline (3.45 g, 20.0 mmol) and K_2CO_3 (15.3 g, 100 mmol), was added *N*-methylpiperazine (11.1 mL, 100 mmol) and stirred at 50 °C. After stirred for 48 h, the reaction mixture was cooled to 0 °C and the mixture was poured into excess crushed-ice to afford precipitation. The resulting precipitate was collected by filtration and washed with water successively, and dried under vacuum to afford a yellow powder solid (4.13 g, 88%). This crude product was used in next step without further purification.

1H NMR ($CDCl_3$, 400 MHz) δ 8.02 (1H, d, $J = 10.0$ Hz), 6.28 (1H, dd, $J = 2.4, 10.0$ Hz), 6.15 (2H, brs), 5.95 (1H, d, $J = 2.4$ Hz), 3.37 (4H, t, $J = 5.2$ Hz), 2.52 (4H, t, $J = 5.2$ Hz), 2.34 (3H, s)

Synthesis of *tert*-butyl (2-(2-(4-(6-(4-methylpiperazin-1-yl)-2,5'-bibenzimidazol-2'-yl)phenoxy)ethoxy)ethyl)carbamate (12)

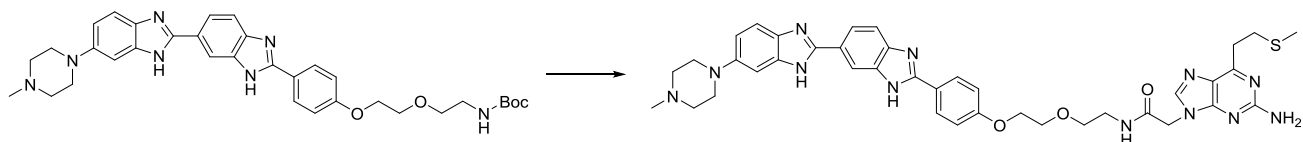


To a solution of 5-(4-methylpiperazin-1-yl)-2-nitroaniline (45 mg, 0.19 mmol) in EtOH (3.8 mL), was added 10% Pd/C (25 mg) and stirred under hydrogen atmosphere at room temperature for 5 h. To this reaction mixture, were directly added $Na_2S_2O_5$ (18 mg, 0.09 mmol) in H_2O (0.2 mL) and followed by *tert*-butyl (2-(2-(4-(5-formyl-1H-benzimidazole-2-yl)phenoxy)ethoxy)ethyl)carbamate (as 0.19 mmol), and stirred at 80 °C for 5 h. After cooled to room temperature, the mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure to afford reddish brown solid. This residue was purified by column chromatography ($CHCl_3/MeOH = 40/1, 20/1, to 5/1$) to give pale brown solid (30.0 mg, 26 %).

1H NMR ($MeOD-d_4$, 600 MHz) δ 8.12 (1H, s), 7.89 (2H, d, $J = 8.4$ Hz), 7.84 (1H, d, $J = 8.4$ Hz), 7.57 (1H, d, $J = 8.4$ Hz), 7.44 (1H, d, $J = 8.4$ Hz), 7.03 (1H, s), 6.92-6.95 (3H, m), 3.99 (2H, brs), 3.70 (2H, brs), 3.51 (2H, d, $J = 5.4$ Hz), 3.22 (2H, d, $J = 5.4$ Hz), 3.14 (4H, brs), 2.63 (4H, brs), 2.35 (3H, s), 1.41 (9H, s); ^{13}C NMR ($MeOD-d_4$, 150 MHz) δ 162.1, 158.4, 155.0, 153.6, 149.4, 140.1, 136.0, 129.4, 125.5, 123.0, 122.3, 116.6, 116.2, 115.9, 102.1, 80.1, 71.2, 70.3, 68.6, 56.0, 51.5, 45.9, 41.2, 28.8; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{34}H_{41}N_7O_4$, 612.3293; found 612.3293

In ^{13}C NMR spectra, some carbons derived from benzimidazole were not observed due to tautomerization.^{S1}

Synthesis of Hoechst probe (13)

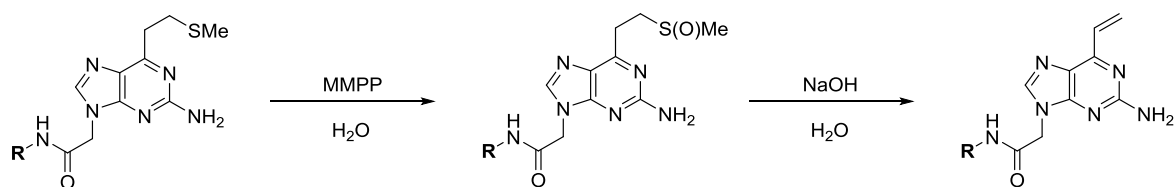


To a solution of *tert*-butyl (2-(2-(4-(6-(4-methylpiperazin-1-yl)-2,5'-bibenzimidazol-2'-yl)phenoxy)ethoxy)ethyl)carbamate (8.0 mg, 13 μ mol) in CH_2Cl_2 (0.1 mL), was added trifluoroacetic acid (0.4 mL) and stirred at room temperature for 2 h. The volatile was concentrated and co-evaporated with acetonitrile (5 mL x 3) under reduced pressure to afford pale brown oil. This residue was dissolved in dry DMF (0.5 mL), and the mixture of HBTU (6.4 mg, 0.017 mmol), HOBt (2.3 mg, 0.017 mmol), DIPEA (23 μ L, 0.13 mmol), and 2-(2-amino-6-(2-(methylthio)ethyl)-9*H*-purin-9-yl)acetic acid (4.3 mg, 0.017 mmol) was slowly added and stirred at room temperature. After 3 h, the mixture was concentrated under reduced pressure to afford reddish brown solid. This residue was purified by column chromatography ($\text{CHCl}_3/\text{MeOH} = 9/1$ to $5/1$) to give pale brown solid (7 mg). This product was further purified by reverse phased HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C₁₈-AR-II, 10 x 250 mm) by a linear gradient of 0-45%/30min acetonitrile in 0.1% TFA buffer at a flow rate of 1 mL/min at 40.0 $^\circ\text{C}$, and monitored by UV detection at $\lambda = 254$ nm and fluorescence detection ($\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 461$ nm), to afford the desired product (3.6 mg, 25% as a tri TFA salt) as a pale yellow solid.

^1H NMR (MeOD-*d*₄, 400 MHz) δ 8.39 (1H, s), 8.24 (1H, s), 8.11 (2H, d, $J = 8.4$ Hz), 8.02 (1H, d, $J = 8.4$ Hz), 7.88 (1H, d, $J = 8.8$ Hz), 7.71 (1H, d, $J = 9.2$ Hz), 7.38 (1H, d, $J = 9.2$ Hz), 7.31 (1H, s), 7.18 (2H, d, $J = 8.8$ Hz), 4.88 (2H, s), 4.26 (2H, t, $J = 4.0$ Hz), 3.89 (2H, t, $J = 4.0$ Hz), 3.67 (2H, t, $J = 5.2$ Hz), 3.46 (2H, t, $J = 5.2$ Hz), 3.36-3.32 (12H, m), 2.99 (3H, s), 2.13 (3H, s); ^{13}C NMR (MeOD-*d*₄, 150 MHz) δ 168.5, 163.2, 158.9, 157.8, 156.5, 156.4, 151.6, 150.4, 147.6, 135.4, 130.2, 129.4, 126.5, 123.4, 121.7, 119.7, 119.1, 116.5, 116.4, 115.9, 115.6, 101.4, 70.6, 70.5, 69.1, 54.7, 49.6, 46.1, 43.6, 40.7, 32.9, 32.0, 15.2; HRMS-ESI (m/z): $[\text{M}+2\text{H}]^{2+}$ calcd for C₃₉H₄₄N₁₂O₃S, 381.1763; found 381.1763

The concentration of Hoechst probe (13) was determined with quantitative ^1H -NMR using a maleic acid as an internal standard.

Synthesis of (Arg)₅-AVP (2) and Acridine-AVP (3)

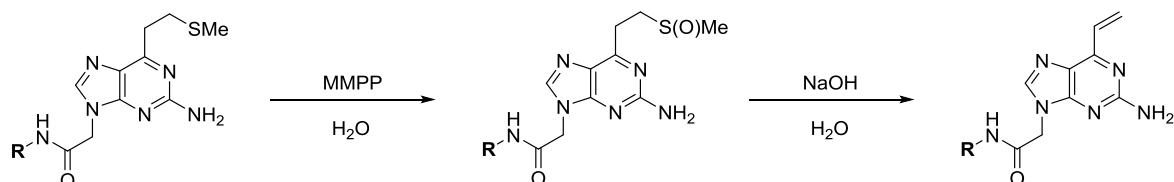


R = penta-arginine or acridine

To a solution of precursors (**7** or **9**, 500 μM), was added 25 mM MMPP solution (0.6 eq.) dissolved in water and the mixture was incubated at 37 $^\circ\text{C}$ for 10 min to oxidize the sulfide group. To this mixture, was added 1 M NaOH solution (50 eq.) and incubated at 37 $^\circ\text{C}$ for 1 h. After alkali treatment, pH was neutralized by adding 1 M or 0.1 M HCl solution. Reaction proceedings in each step were monitored with reverse phased HPLC with C-18 column (Nacalai tesque: COSMOSIL

5C₁₈-AR-II, 4.6 x 250 mm) by a liner gradient of 0-30%/30min acetonitrile in 0.1% TFA buffer at a flow rate of 1 mL/min at 40.0 °C, and monitored by UV detection at $\lambda = 254$ nm and the structure was characterized by MALDI-TOF MS measurement.

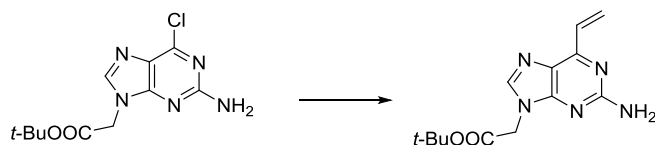
Synthesis of Hoechst-AVP (4)



R = Hoechst

To a solution of precursors (**13**, 500 μ M), was added 25 mM MMPP solution (0.6 eq.) dissolved in water and the mixture was incubated at 37 °C for 10 min to oxidize the sulfide group. To this mixture, were added DMSO (5 v/v%) and 1 M NaOH solution (50 eq.) and incubated at 37 °C for 2 h. After alkali treatment, pH was neutralized by adding 1 M or 0.1 M HCl solution. Reaction proceedings in each step were monitored with reverse phased HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C₁₈-AR-II, 4.6 x 250 mm) by a liner gradient of 0-45%/30min acetonitrile in 0.1% TFA buffer at a flow rate of 1 mL/min at 40.0 °C, and monitored by UV detection at $\lambda = 254$ nm and the structure was characterized by MALDI-TOF MS measurement.

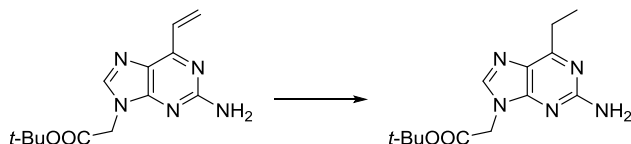
Synthesis of *tert*-butyl-2-(2-amino-6-vinyl-9H-purin-9-yl)acetate



To a suspension of *tert*-butyl (6-chloro-2-aminopurin-9-yl)acetate (107.1 mg, 0.377 mmol) in 1,4-dioxane (2.8 mL) and water (0.9 mL), were added potassium carbonate (53.9 mg, 0.224 mmol), vinylboronic anhydride-pyridine complex (47.8 mg, 0.199 mmol), and Pd(PPh₃)₄ (46.1 mg, 10 mol%) under Ar atmosphere. The reaction mixture was stirred at 110 °C for 3 h. After cooled to room temperature, the mixture was diluted and extracted with EtOAc (10 mL x 4). Each organic layer was washed with sat. NH₄Cl (10 mL), water (10 mL), and brine (10 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue purified by column chromatography (Hexane/EtOAc = 1/3) to gain yellow solid containing Pd catalysis (82.6 mg). The residue was dissolved in CHCl₃ (1.0 mL), and this solution was slowly added to hexane (5.0 mL) to afford pink precipitation. The precipitation was collected and washed with MeOH (0.3 mL) to gain pale yellow solid (42.2 mg, 41%).

¹H NMR (CDCl₃, 600 MHz) δ 7.79 (1H, s), 7.11 (1H, dd, $J = 10.8, 17.4$ Hz), 6.88 (1H, dd, $J = 1.8, 17.4$ Hz), 5.85 (1H, dd, $J = 1.8, 10.8$ Hz), 5.12 (2H, brs), 4.73 (2H, s), 1.45 (9H, s); ¹³C NMR (CDCl₃, 150 MHz) δ 166.3, 159.9, 154.6, 154.1, 142.2, 132.1, 125.8, 125.2, 83.5, 44.5, 28.1; HRMS-ESI (m/z): [M+H]⁺ calcd for C₁₃H₁₇N₅O₂ 276.1455; found 276.1456

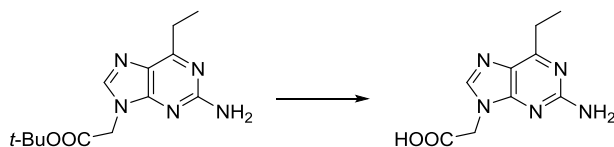
Synthesis of *tert*-butyl 2-(2-amino-6-ethyl-9H-purin-9-yl)acetate



NaBH₄ (9.0 mg, 0.238 mmol) was suspended in MeOH (0.3 mL) and cooled to 0 °C. To this suspension, was added *tert*-butyl-2-(2-amino-6-vinyl-9H-purin-9-yl)acetate (30.2 mg, 0.110 mmol) in MeOH (0.8 mL) drop-wise and stirred at room temperature. After stirring for 1.5 h, the reaction was quenched by addition of sat. NH₄Cl (10 mL) slowly, and extracted with EtOAc (10 mL x 2). The each organic layer was washed with water (10 mL) and brine (10mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (Hexane/EtOAc = 1/5, to EtOAc only) to afford the desired product (29.8 mg, 98%) as pale yellow solid.

¹H NMR (CDCl₃, 600 MHz) δ 7.74 (1H, s), 5.13 (2H, brs), 4.72 (2H, s), 3.01 (2H, q, *J* = 7.8 Hz), 1.46 (9H, s), 1.36 (3H, t, *J* = 7.8 Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 166.4, 165.1, 160.0, 152.8, 141.3, 126.0, 83.5, 44.5, 28.19, 26.6, 12.8; HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₁₃H₁₉N₅O₂, 278.1612; found 278.1613

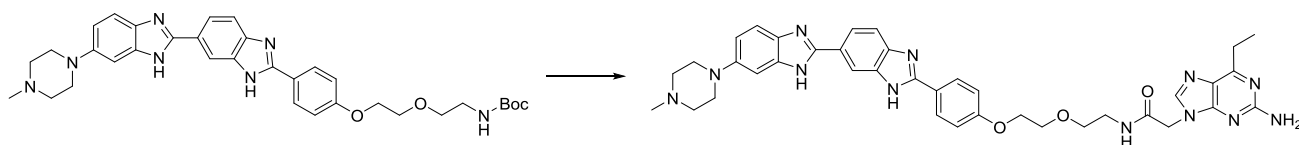
Synthesis of 2-(2-amino-6-ethyl-9H-purin-9-yl)acetic acid



A solution of *tert*-butyl 2-(2-amino-6-ethyl-9H-purin-9-yl)acetate (280 mg) in CH₂Cl₂(0.2 mL), was added TFA (0.8 mL). After stirred at room temperature for 2 h, the volatile was removed by evaporation. The residue was triturated in Et₂O(2 mL)/Hexane(8 mL), and the resulting precipitate was collected by filtration to give pale yellow solid. The obtained solid was suspended in hot-EtOH(80°C), and filtered, washed with EtOH, dried under reduced pressure to afford a white solid (148 mg) as a pure product.

¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.35 (1H, s), 4.92 (2H, s), 3.02 (2H, q, *J* = 7.5 Hz), 1.36 (3H, t, *J* = 7.5 Hz); ¹³C NMR (DMSO-*d*₆, 150 MHz) d 168.8, 157.6, 155.8, 155.5, 147.0, 124.1, 44.2, 23.4, 12.4; HRMS-ESI (*m/z*): [M-H]⁻ calcd for C₉H₁₁N₅O₂, 220.0840; found 220.0841

Synthesis of Hoechst-AEP (14)



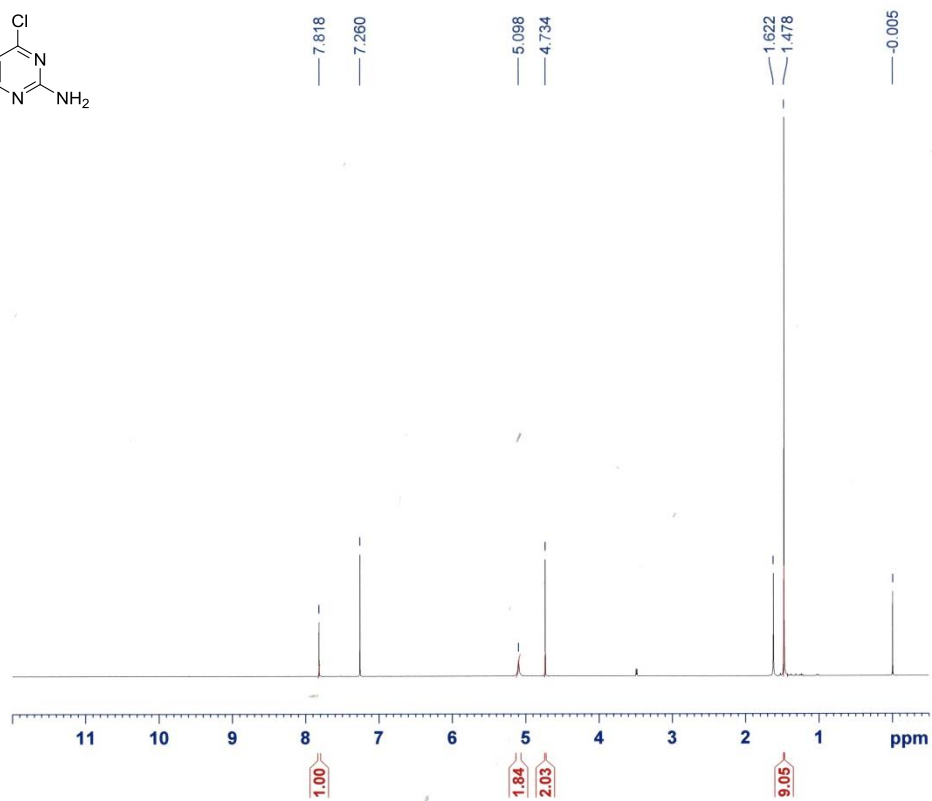
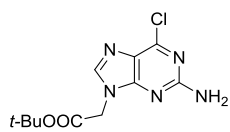
To a solution of **12** (3.8 mg, 6.21 μmol) in CH₂Cl₂ (0.10 mL), was added trifluoroacetic acid (0.20 mL) and stirred at room temperature for 1 h. After stirring, the reaction mixture was concentrated and co-evaporated with methanol (0.10 mL x 3) three times to give brown solid. The solid was dissolved in DMF (0.25 mL), followed by addition of the mixture **S10** in

2-(2-amino-6-ethyl-9*H*-purin-9-yl)acetic acid (2.0 mg, 9.0 μmol), HBTU (3.3 mg, 8.7 μmol), HOBt (1.2 mg, 8.9 μmol), and DIPEA (11 μL , 63 μmol), and stirred at room temperature for 3 h. The mixture was concentrated and purified by column chromatography ($\text{CHCl}_3/\text{MeOH} = 29/1, 9/1, 5/1, \text{ to } 3/1$). Further purification was performed with reversed phase HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C₁₈-AR-II, 10 x 250 mm) by a liner gradient of 0-45%/30min acetonitrile in 0.1% TFA buffer at a flow rate of 1 mL/min at 40.0 °C, and monitored by UV detection at $\lambda = 254$ nm and fluorescence detection ($\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 461$ nm), to afford the desired product (2.8 mg, 43% in two steps as a tri-TFA salt) as a pale yellow solid.

¹H NMR ($\text{MeOD-}d_4$, 400 MHz) δ 8.38 (1H, d, $J = 1.2$ Hz), 8.24 (1H, s), 8.13 (2H, d, $J = 8.8$ Hz), 8.02 (1H, dd, $J = 1.2, 8.8$ Hz), 7.88 (1H, d, $J = 8.8$ Hz), 7.71 (1H, d, $J = 8.8$ Hz), 7.37 (1H, d, $J = 8.8$ Hz), 7.31 (1H, d, $J = 2.0$ Hz), 7.20 (2H, d, $J = 8.8$ Hz), 4.79 (2H, s), 4.27 (2H, t, $J = 4.4$ Hz), 3.90 (2H, t, $J = 4.4$ Hz), 3.68 (2H, t, $J = 5.6$ Hz), 3.46-3.49 (2H, m), 3.09 (2H, q, $J = 7.6$ Hz), 3.02 (3H, s), 1.42 (3H, t, $J = 7.6$ Hz); HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for C₃₈H₄₂N₁₂O₃, 715.3576; found 715.3583

The concentration of Hoechst-AEP (17) was determined by UV absorption at 260 nm using the molar extinction coefficients: 37,110 M⁻¹ cm⁻¹.

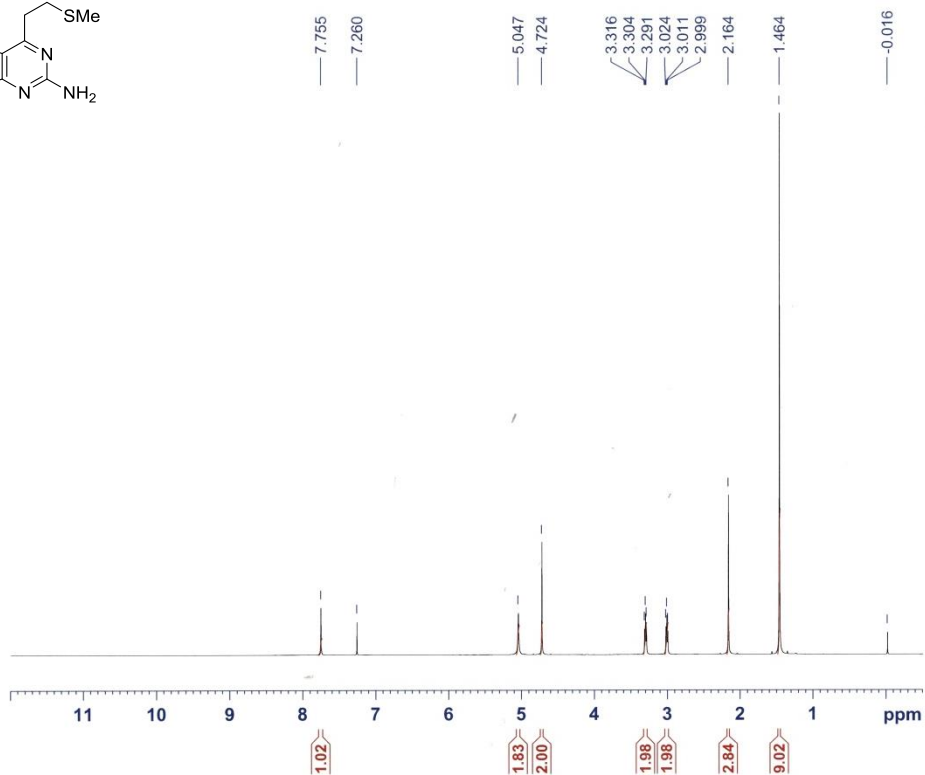
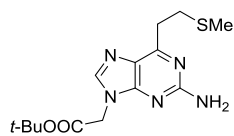
^1H NMR: (CDCl_3 , 400 MHz)



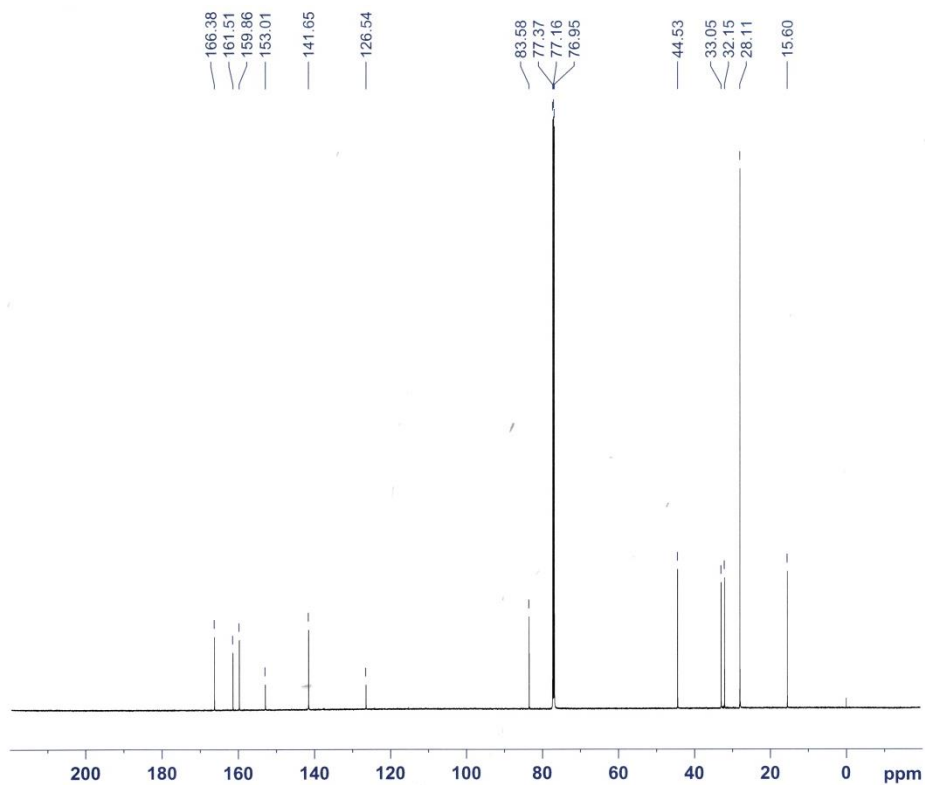
S12

tert-butyl 2-(2-amino-6-(2-methylthio)ethyl)-9H-purin-9-yl)acetate

^1H NMR: (CDCl_3 , 600 MHz)



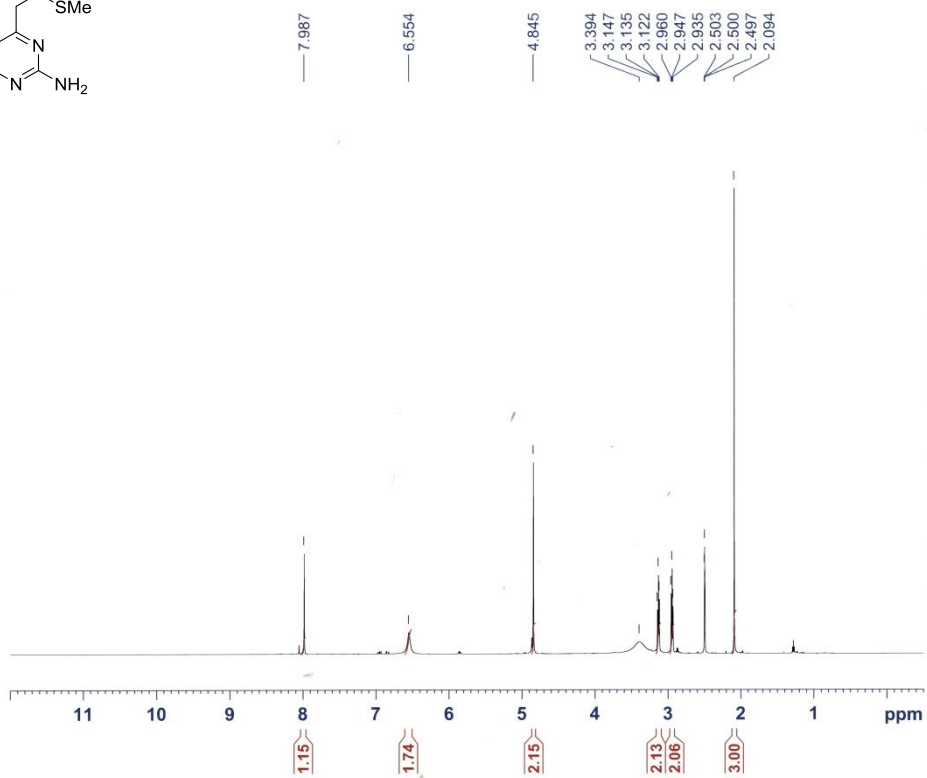
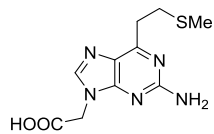
^{13}C NMR: (CDCl_3 , 150 MHz)



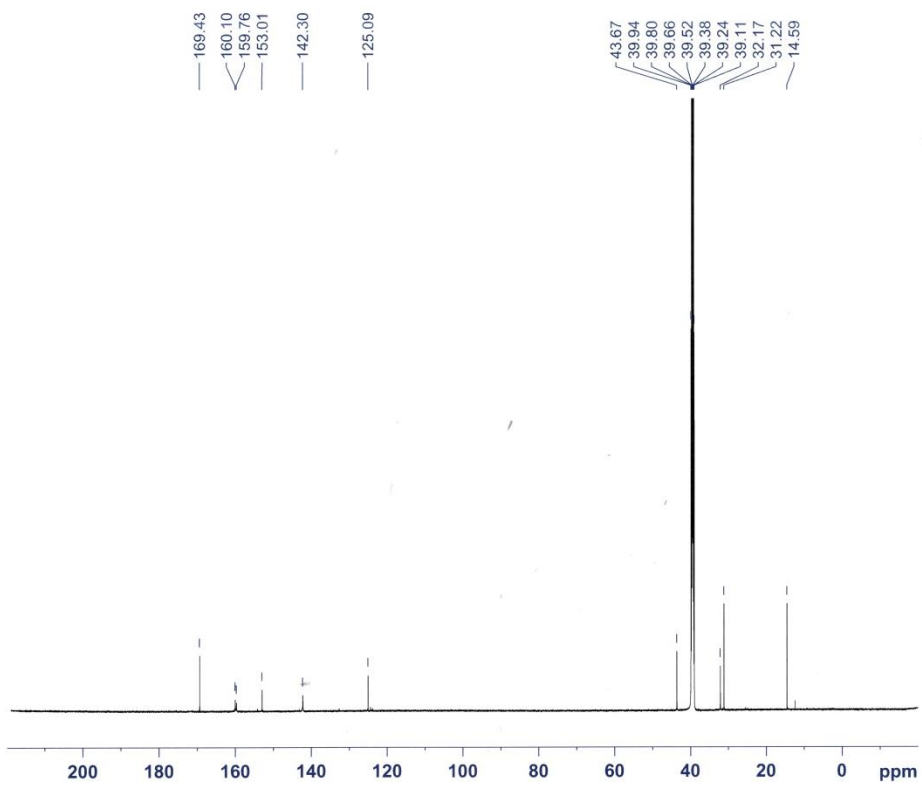
S13

2-(2-amino-6-(2-(methylthio)ethyl)-9H-purin-9-yl)acetic acid (**6**)

^1H NMR: (DMSO- d_6 , 600 MHz)

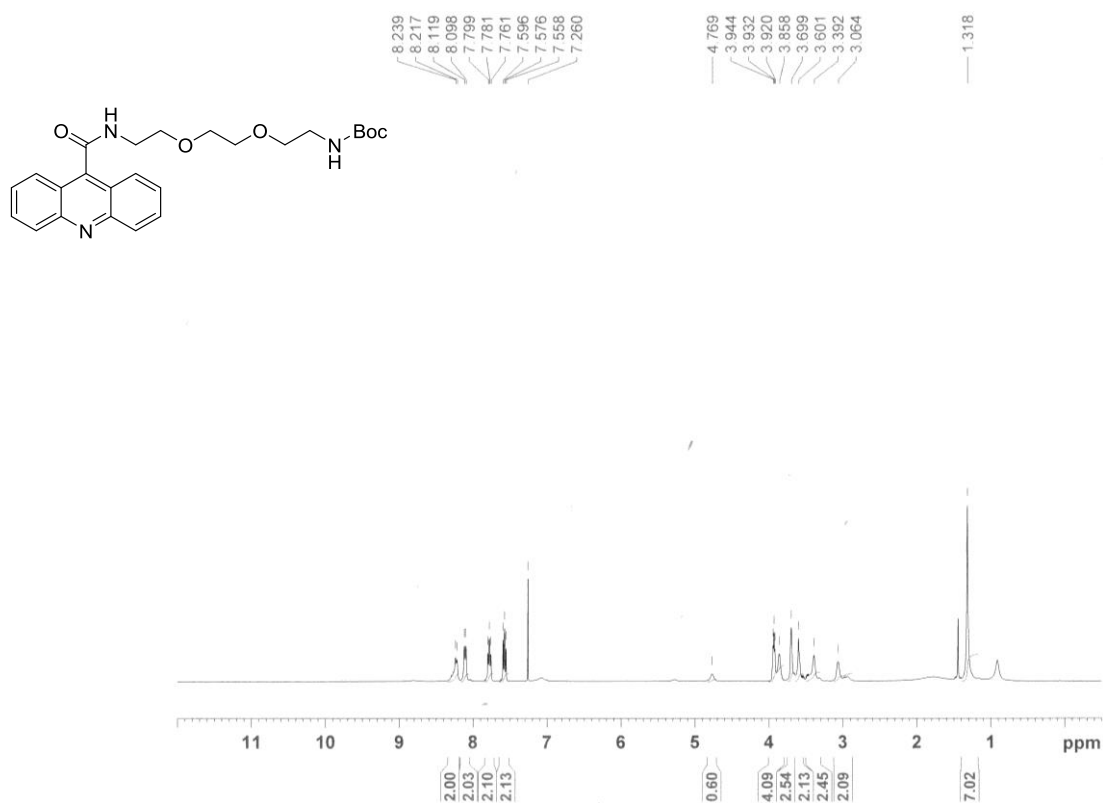


^{13}C NMR: (DMSO- d_6 , 150 MHz)

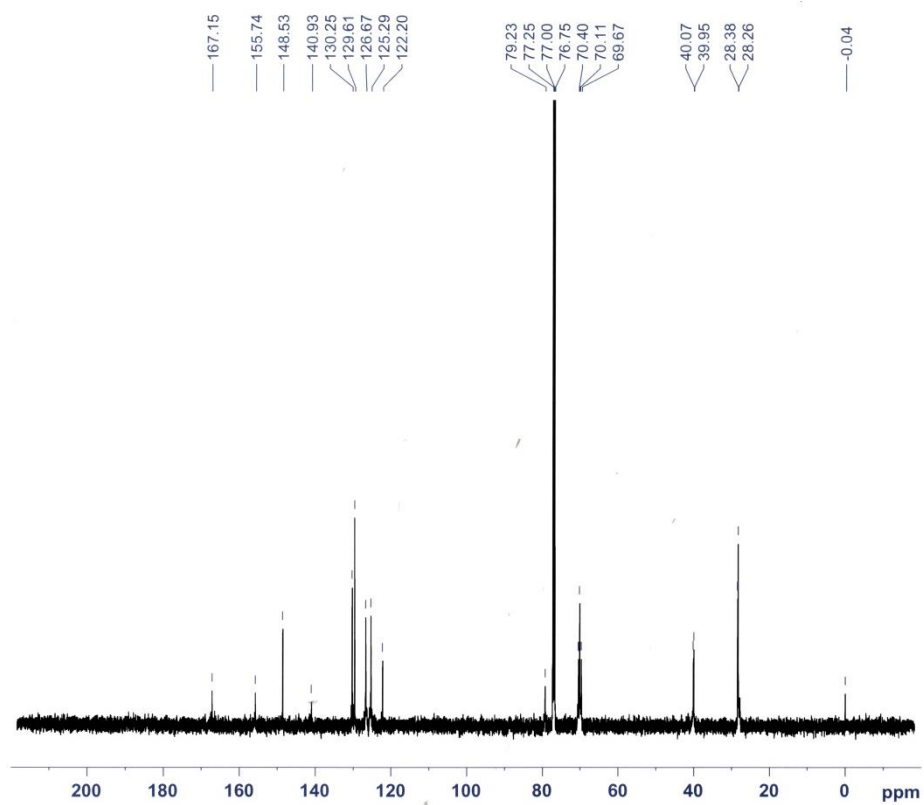


N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)acridine-9-carboxamide (**8**)

¹H NMR: (CDCl₃, 400 MHz)

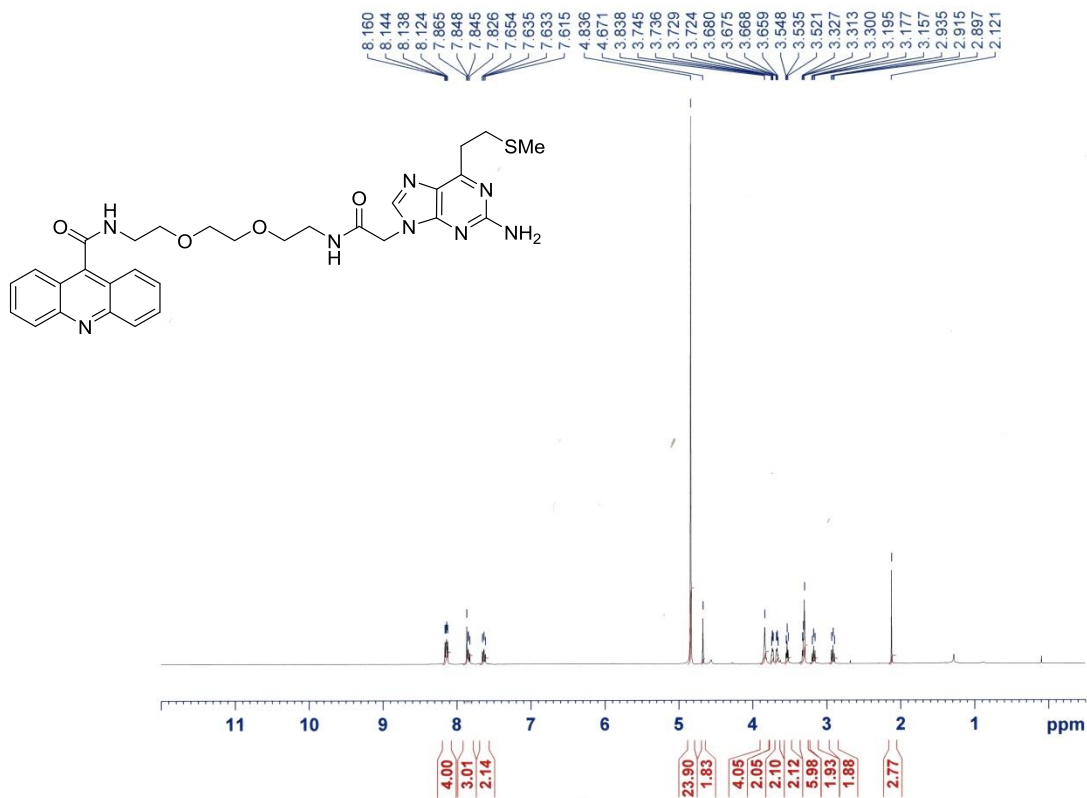


¹³C NMR: (CDCl₃, 125 MHz)

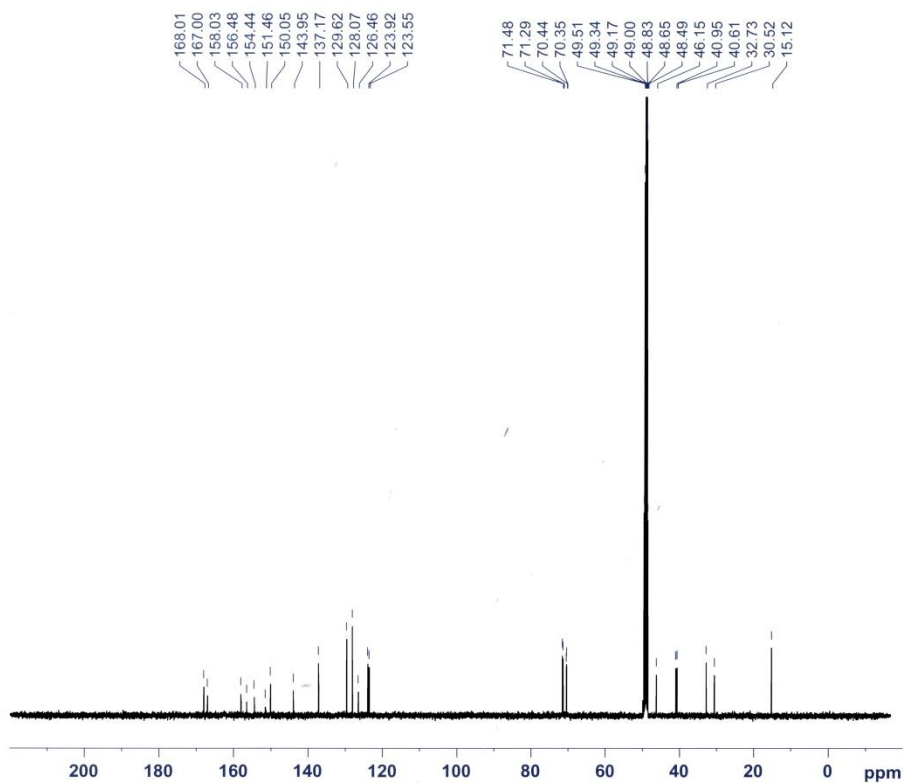


N-(2-(2-(2-(2-(2-amino-6-(2-(methylthio)ethyl)-9*H*-purin-9-yl)acetamido)ethoxy)ethyl)acridine-9-carboxamide (**9**))

¹H NMR (MeOD-*d*₄, 400 MHz)

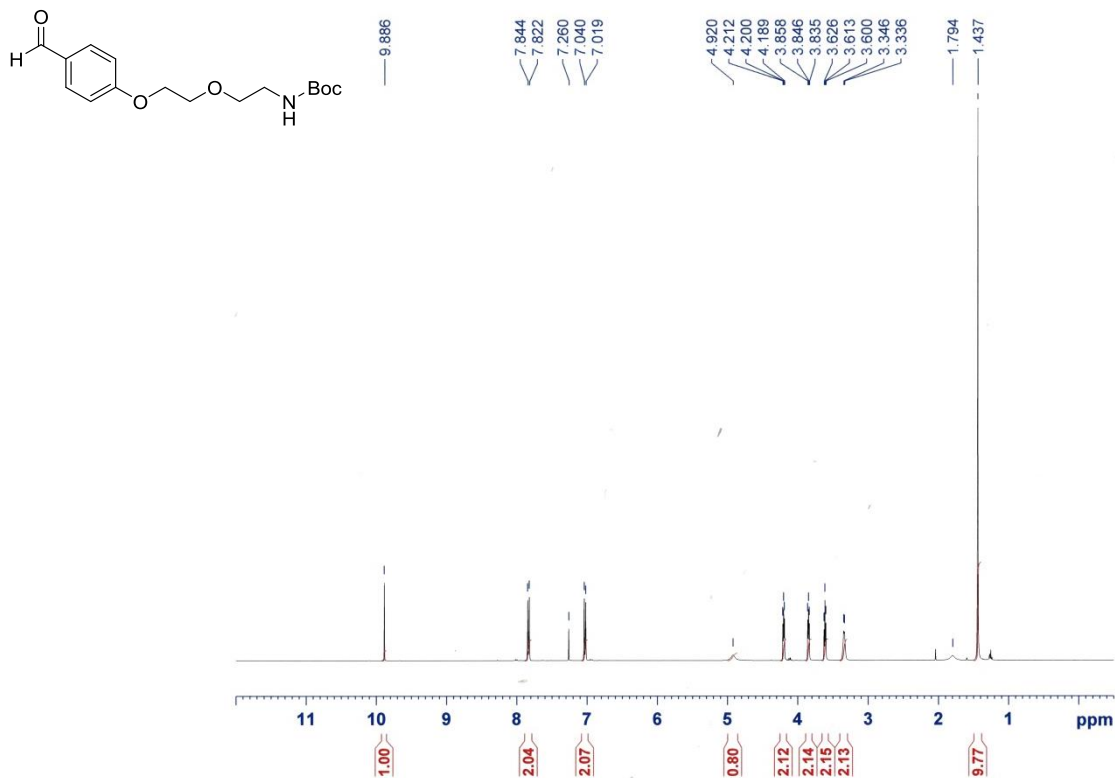


¹³C NMR: (MeOD-*d*₄, 125 MHz)

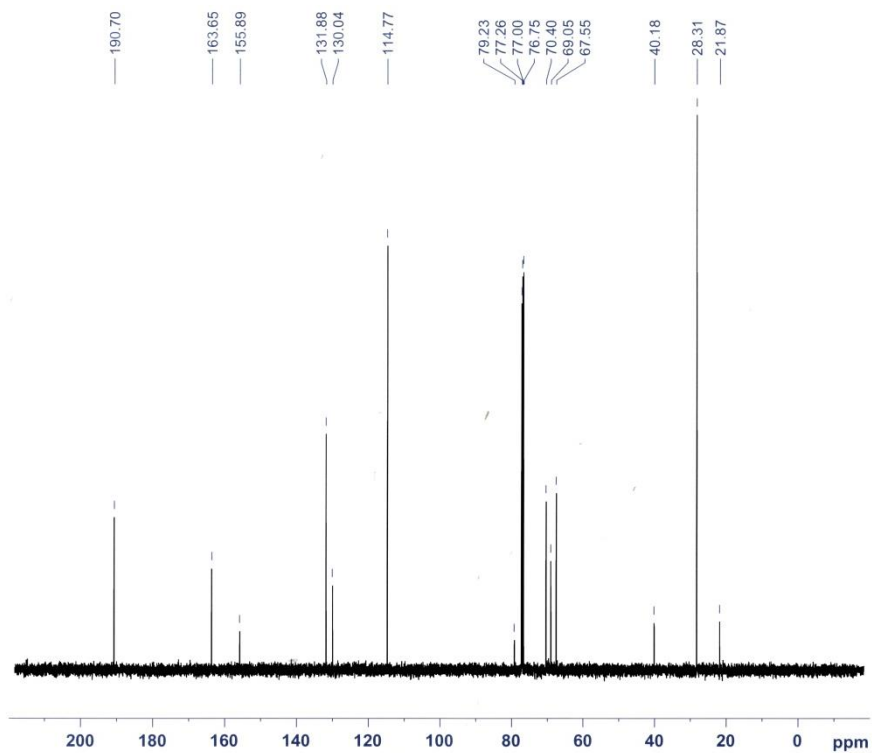


tert-butyl (2-(2-(4-formylphenoxy)ethoxy)ethyl)carbamate (**10**)

^1H NMR: (CDCl_3 , 400 MHz)

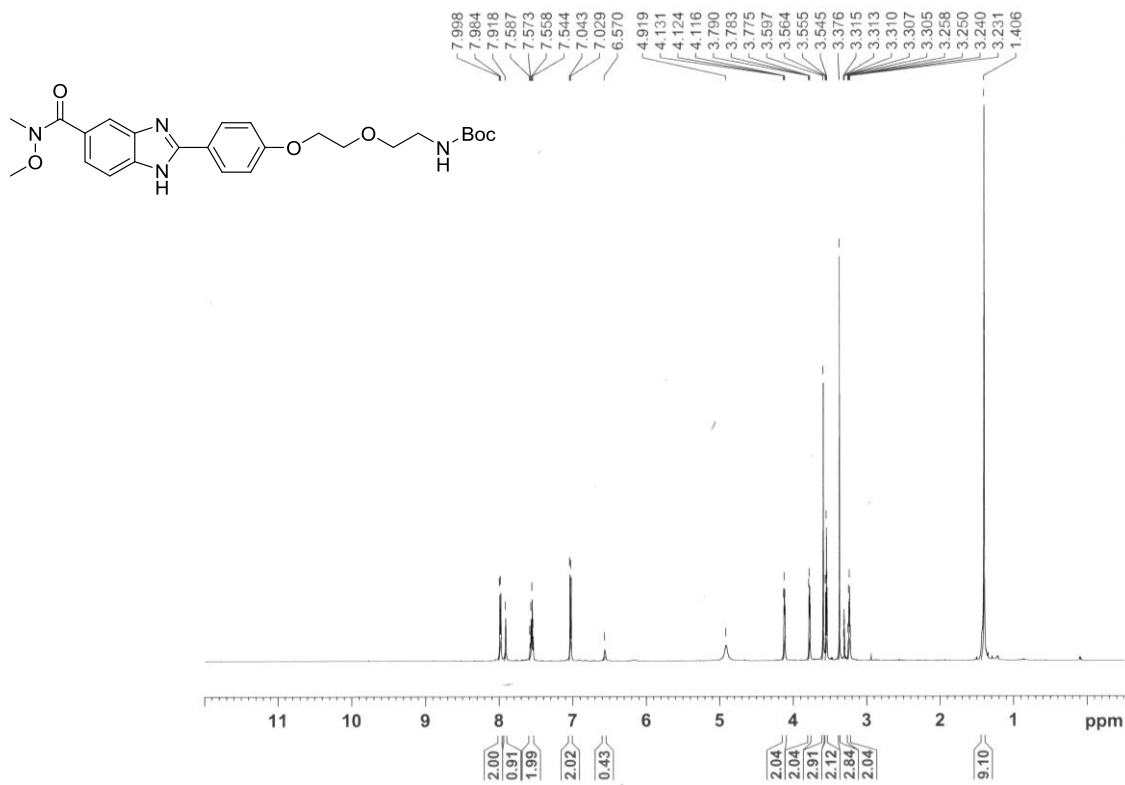


^{13}C NMR: (CDCl_3 , 125 MHz)

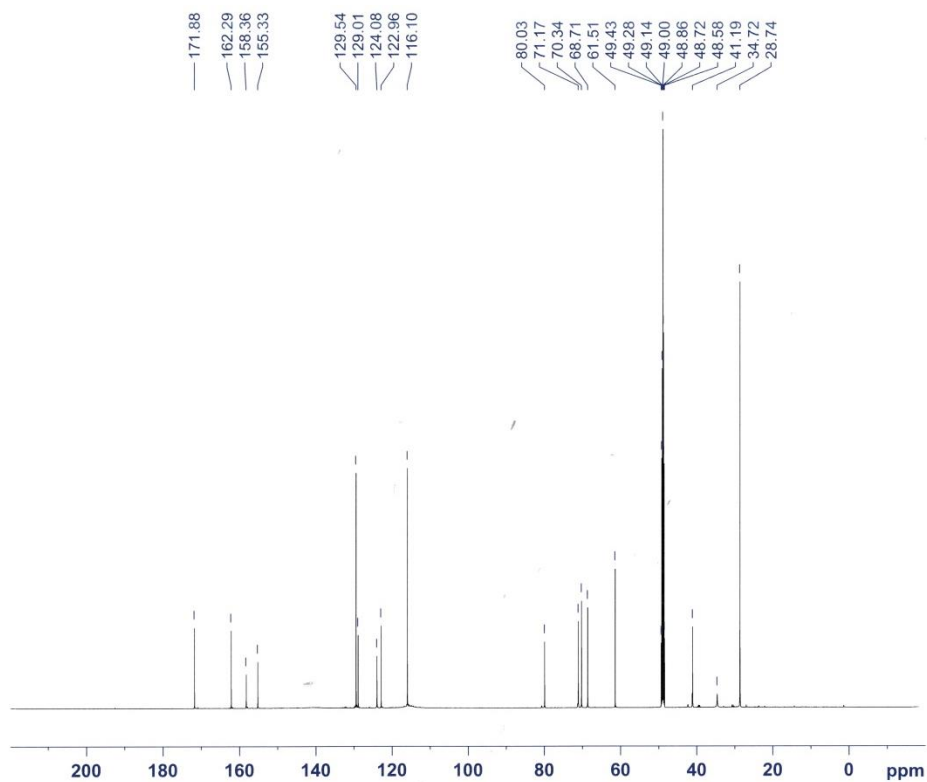


tert-butyl (2-(2-(4-(5-methoxy(methyl)carbamoyl)-1*H*-benzimidazol-2-yl)phenoxy)ethoxy)ethoxy)carbamate

¹H NMR: (MeOD-*d*₄, 600 MHz)

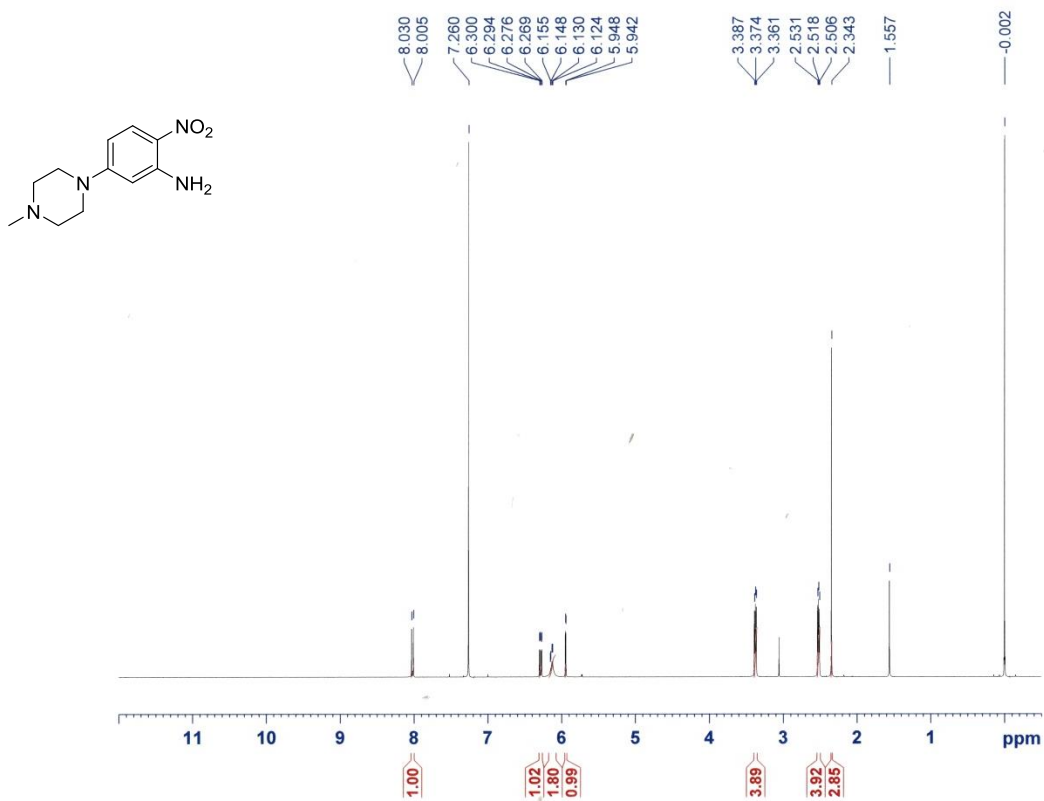


¹³C NMR: (MeOD-*d*₄, 150 MHz)



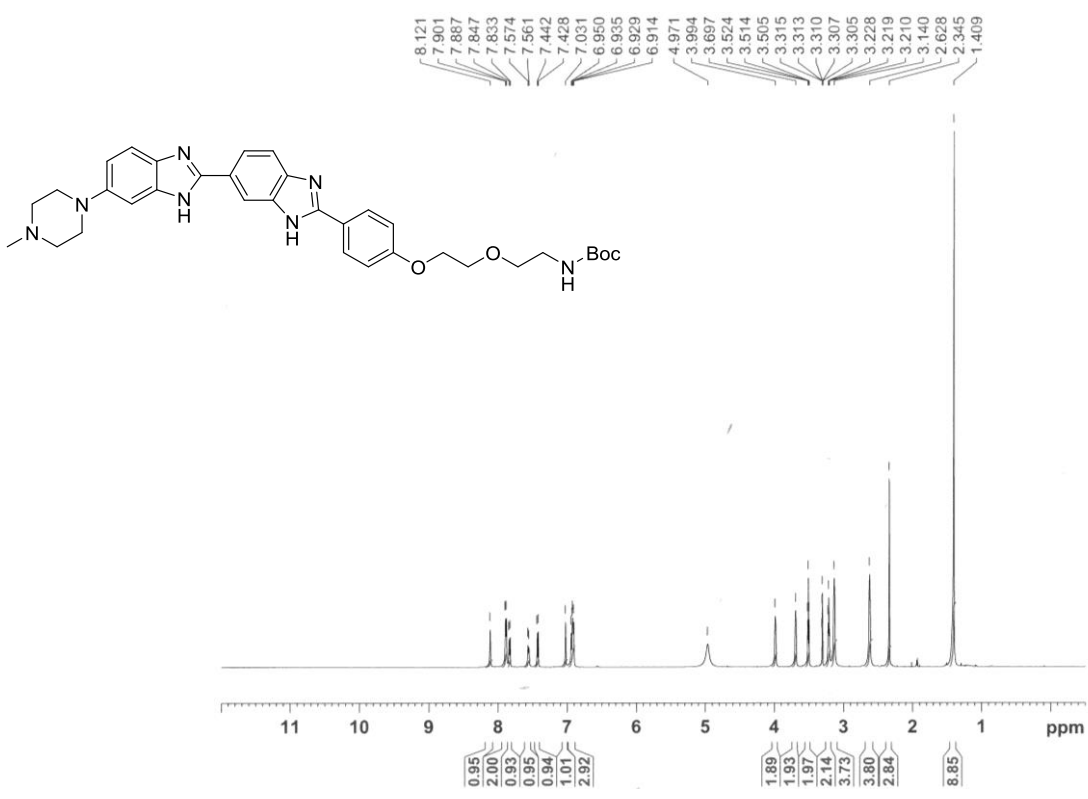
5-(4-methylpiperazin-1-yl)-2-nitroaniline

^1H NMR: (CDCl_3 , 400 MHz)

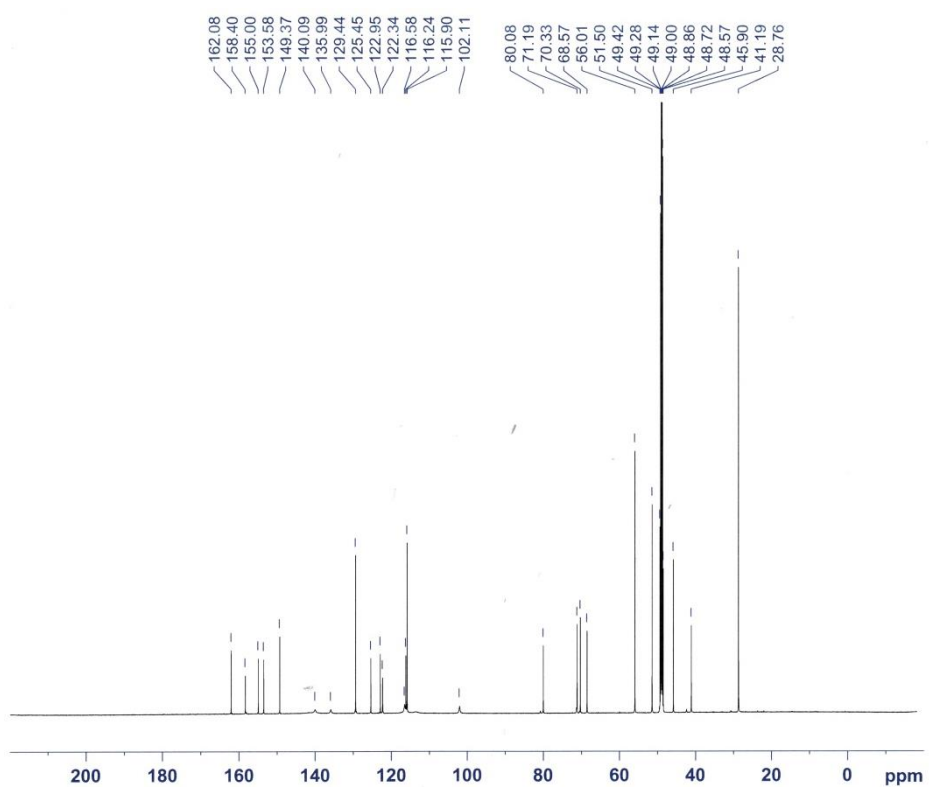


tert-butyl (2-(2-(4-(6-(4-methylpiperazin-1-yl)-2,5'-bibenzimidazol-2'-yl)phenoxy)ethoxy)ethyl)carbamate (**12**)

¹H NMR: (MeOD-*d*₄, 600 MHz)

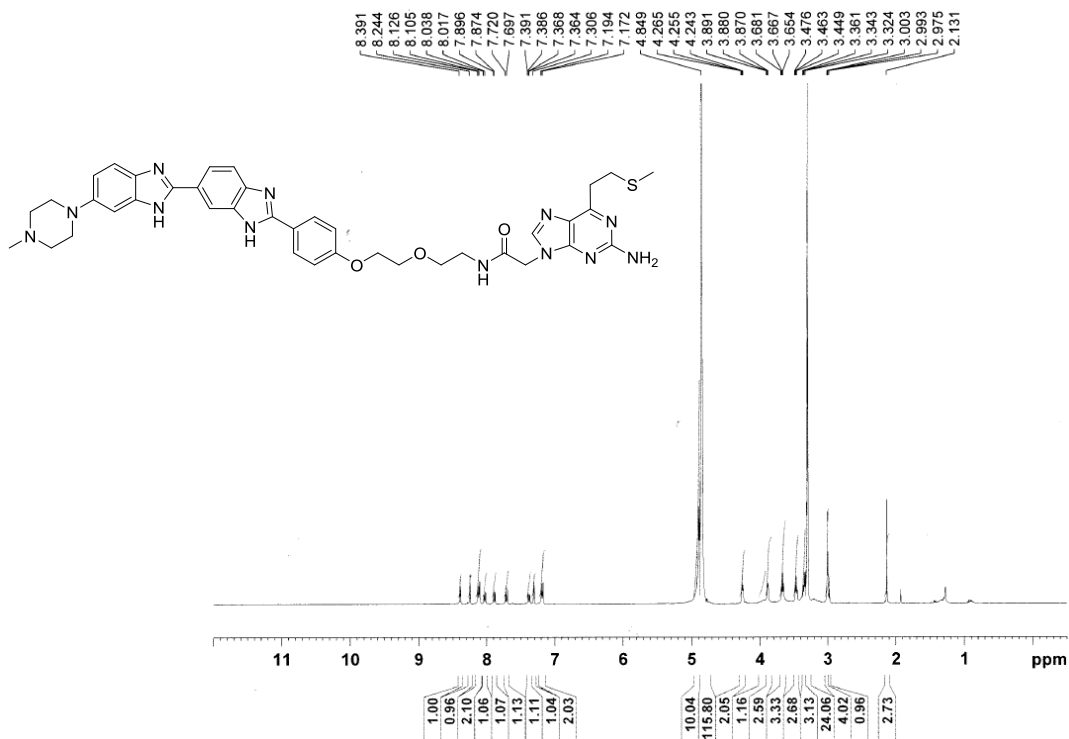


¹³C NMR: (MeOD-*d*₄, 150 MHz)

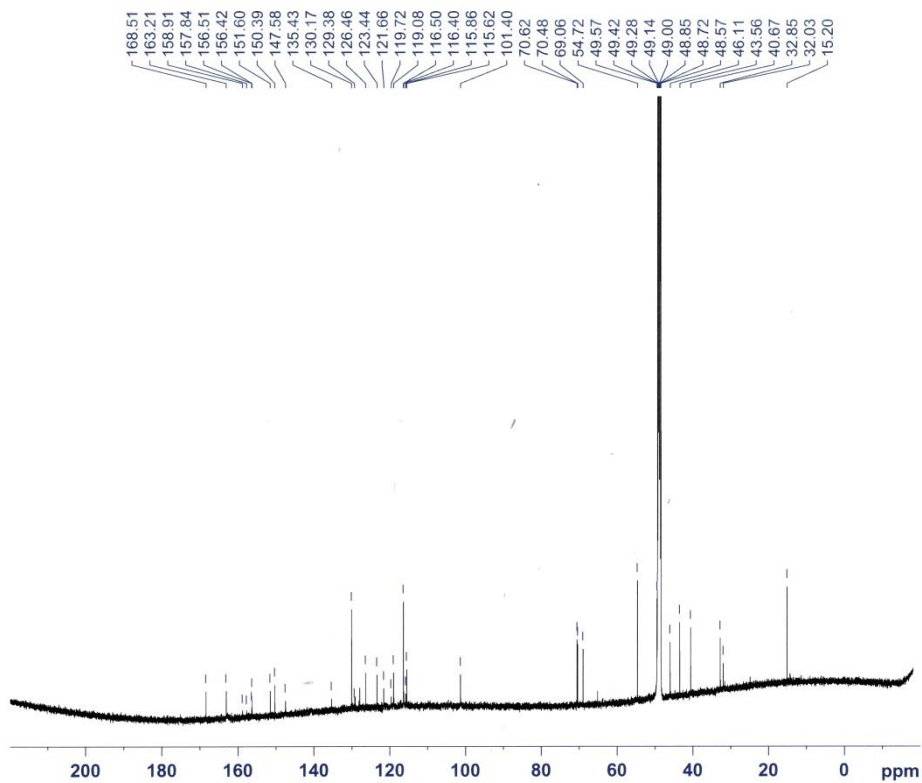


2-(2-amino-6-(2-(methylthio)ethyl)-9H-purin-9-yl)-N-(2-(2-(4-(6-(4-methylpiperazin-1-yl)-1H,3'-H-[2,5'-bibenzo[d]imidazol]-2'-yl)phenoxy)ethoxy)ethyl)acetamide; Hoechst probe (**13**)

¹H NMR: (MeOD-*d*₄, 400 MHz)

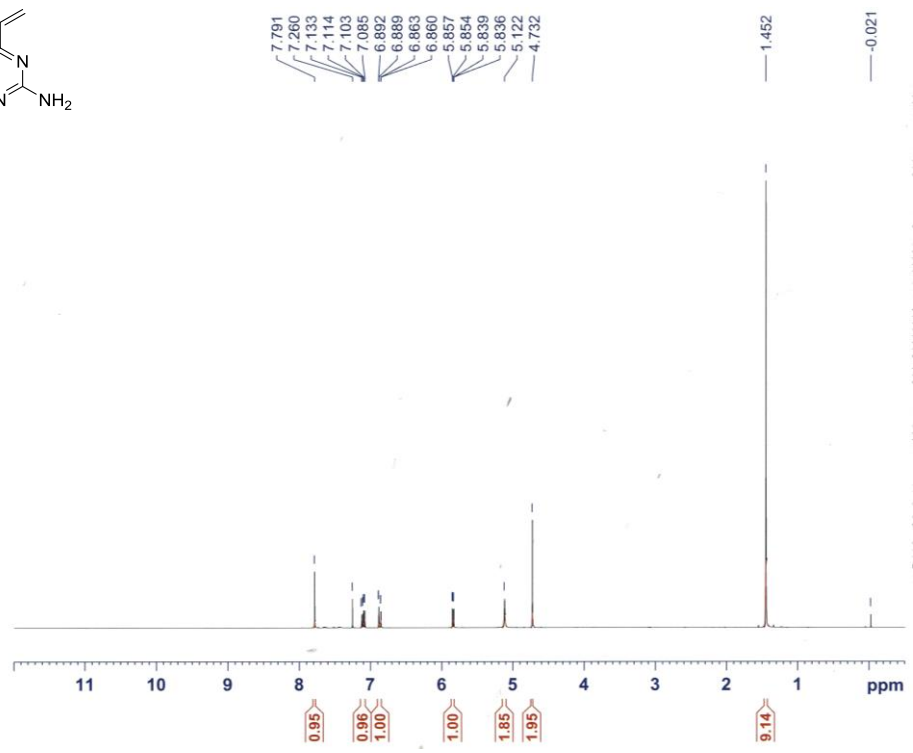
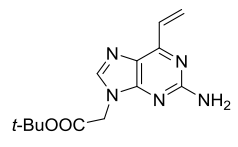


¹³C NMR: (MeOD-*d*₄, 600 MHz)

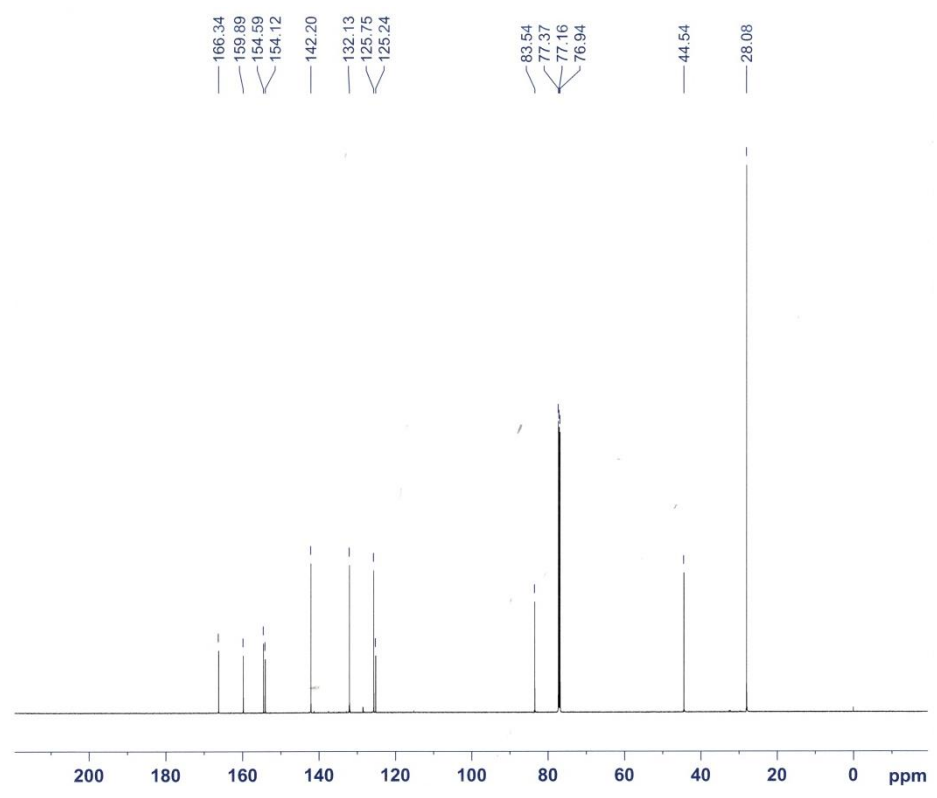


tert-butyl 2-(2-amino-6-vinyl-9*H*-purin-9-yl)acetate

¹H NMR: (CDCl₃, 600 MHz)

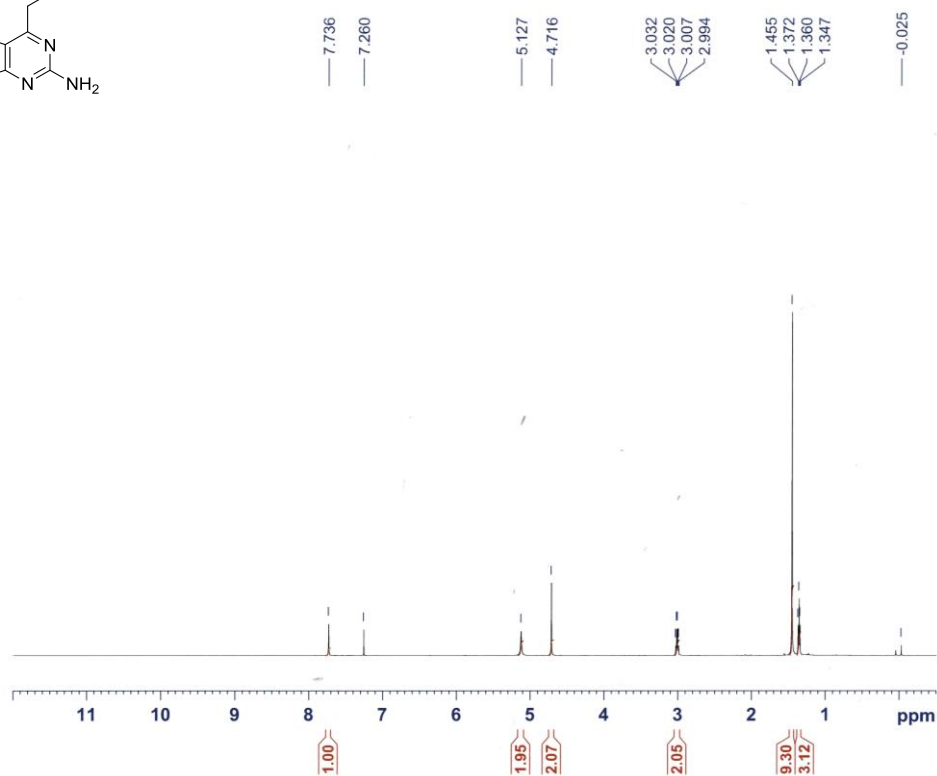
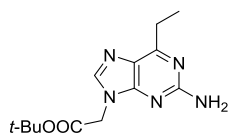


¹³C NMR: (CDCl₃, 150 MHz)

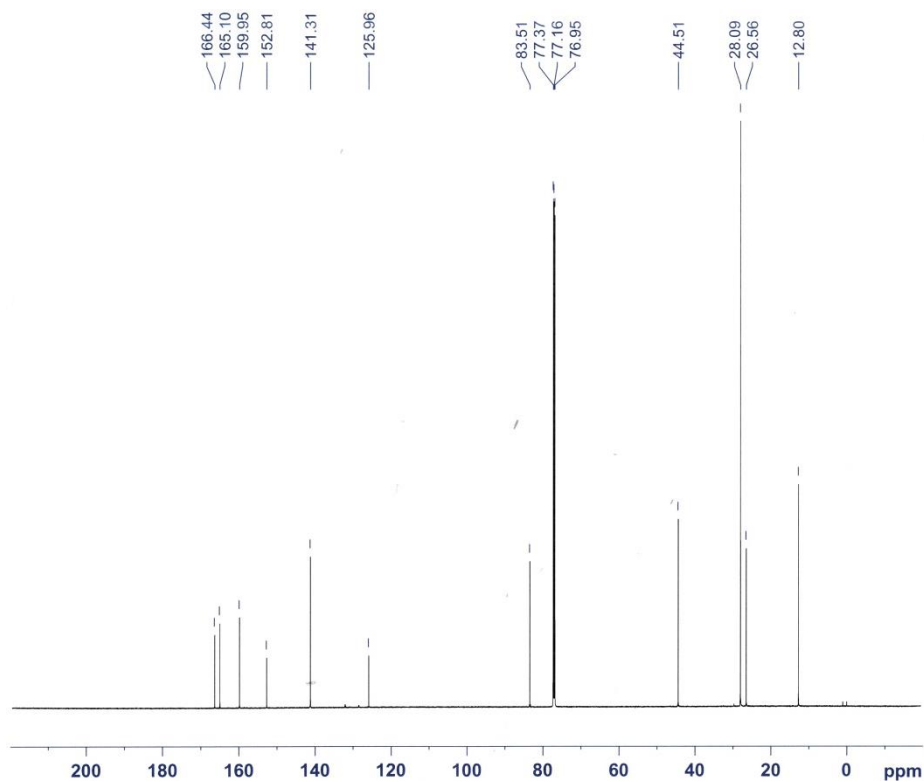


tert-butyl 2-(2-amino-6-ethyl-9*H*-purin-9-yl)acetate

¹H NMR: (CDCl₃, 600 MHz)

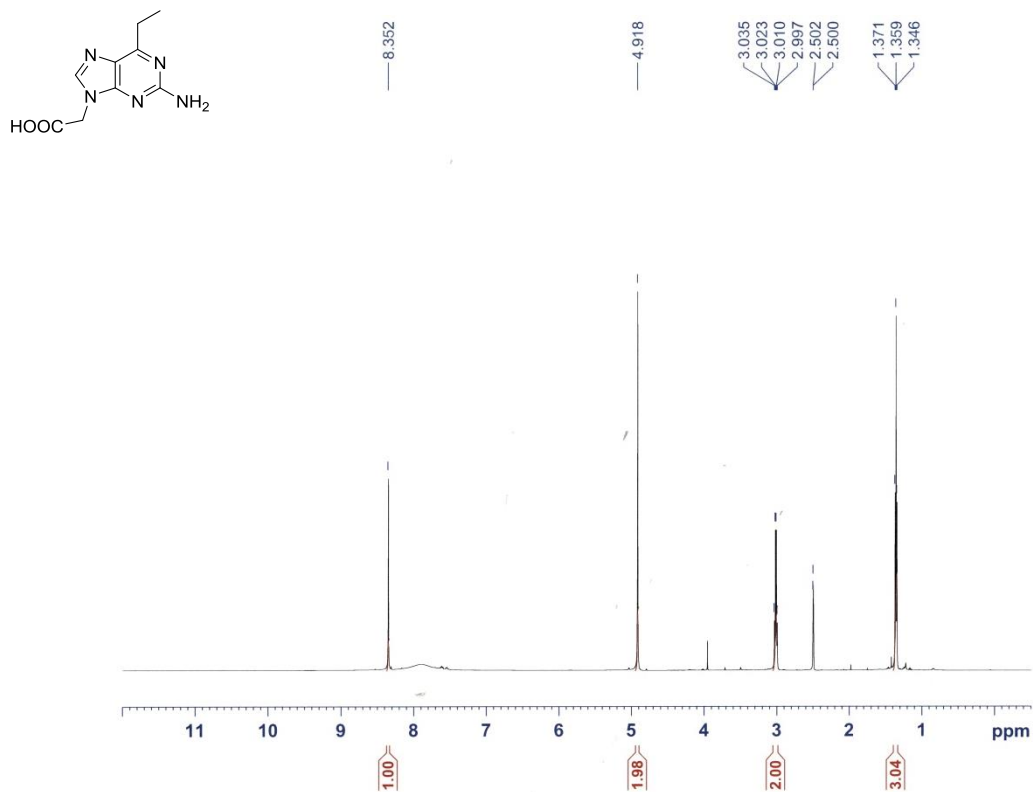


¹³C NMR: (CDCl₃, 150 MHz)

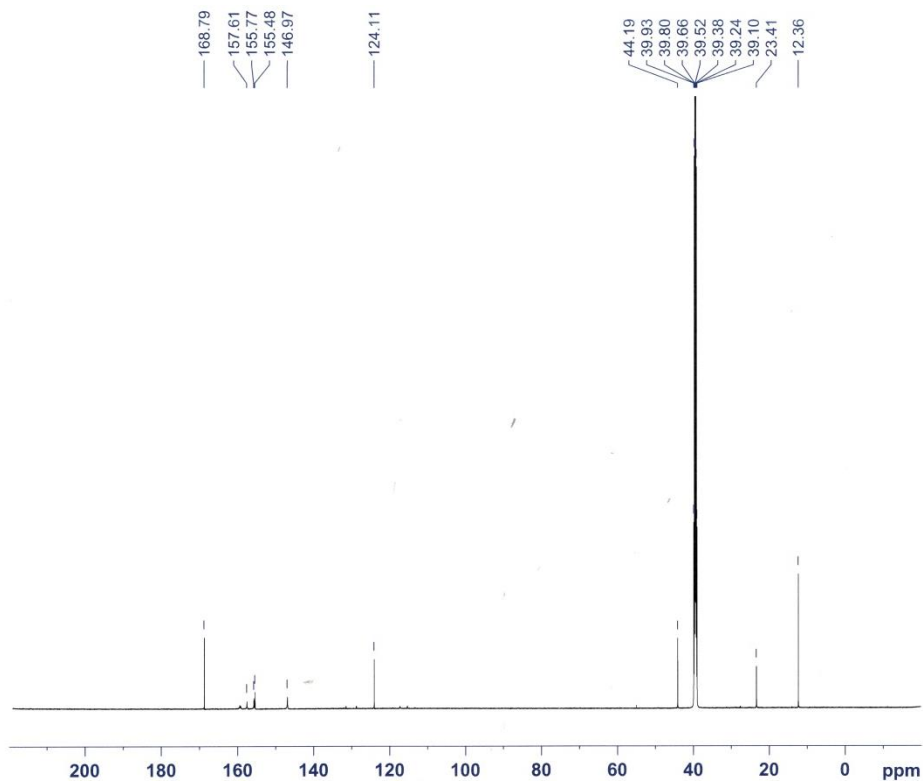


2-(2-amino-6-ethyl-9H-purin-9-yl)acetic acid

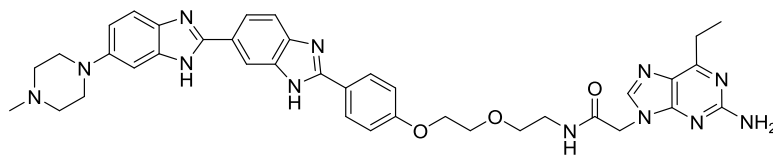
^1H NMR: (DMSO- d_6 , 600 MHz)



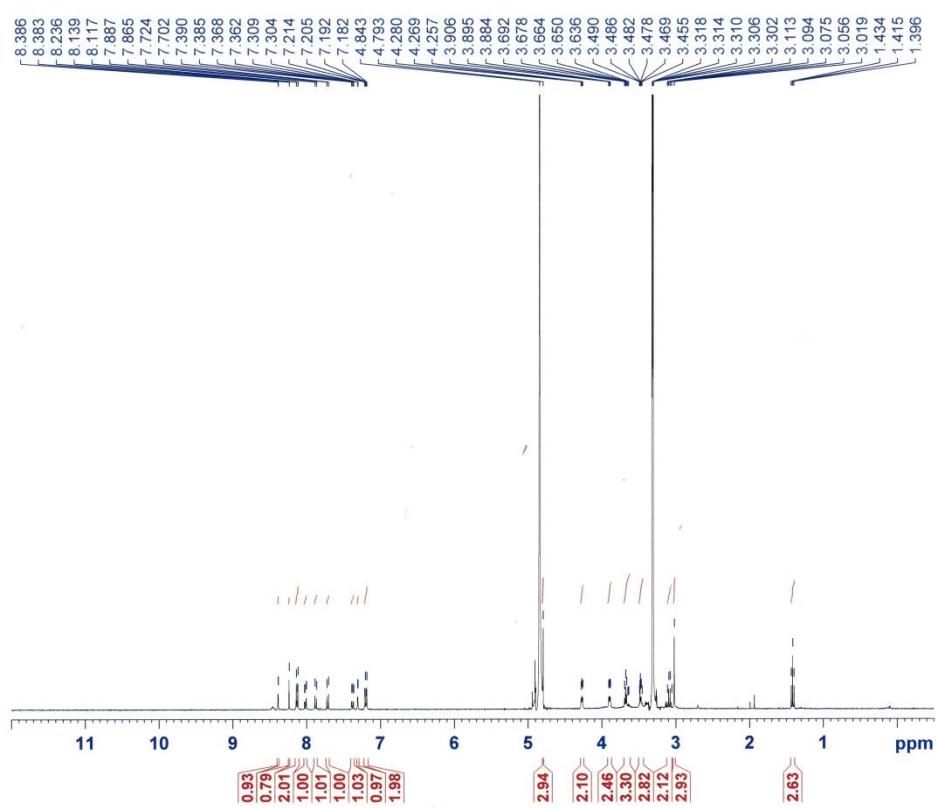
^{13}C NMR: (DMSO- d_6 , 150 MHz)



2-(2-amino-6-ethyl-9H-purin-9-yl)-N-(2-(2-(4-(6-(4-methylpiperazin-1-yl)-1H,3'H-[2,5'-bibenzimidazol]-2'-yl)phenoxy)ethoxy)ethyl)acetamide (**14**)



¹H NMR: (MeOD-*d*₄, 400MHz)



HPLC profile for (Arg)₅-AVP (2)

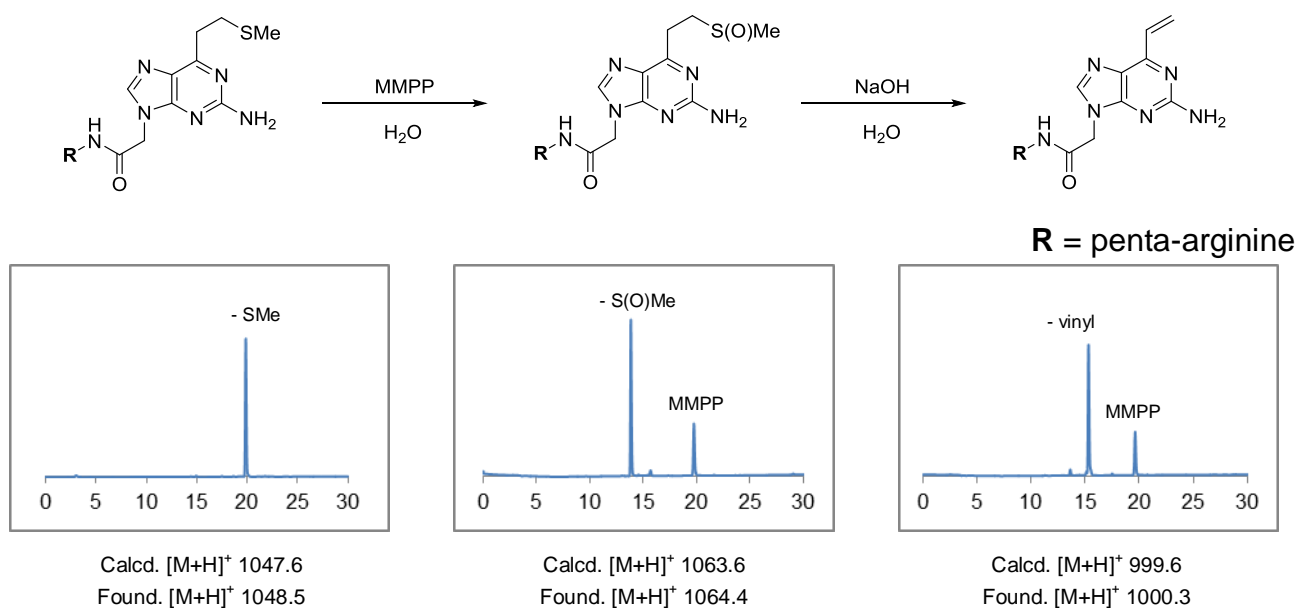


Fig S1 HPLC analysis for synthesis of (Arg)₅-AVP. The analysis was performed with reversed-phase HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C18-AR-II, 4.6 x 250 mm) by a linear gradient of 0-30%/30min acetonitrile in 0.1% TFA buffer (pH 7.0) at a flow rate of 1 mL/min at 40.0 °C, and monitored by UV detection at $\lambda = 254$ nm and the structures were characterized by MALDI-TOF mass spectrum measurement.

Synthesis of Acridine-AVP (3)

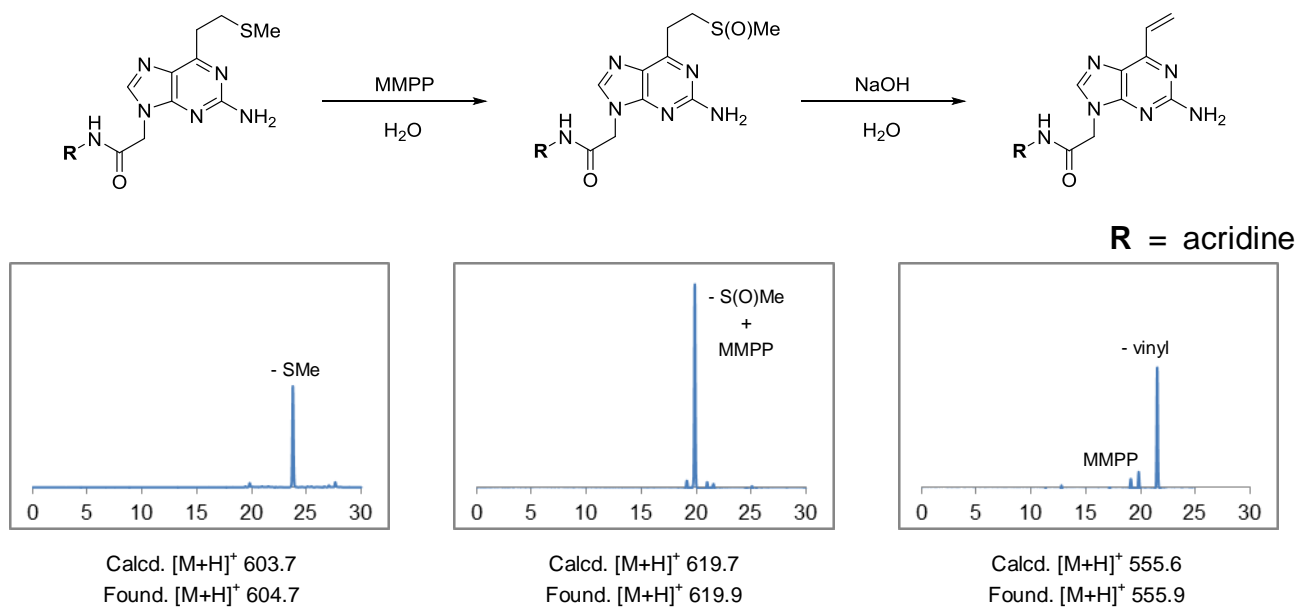


Fig S2 HPLC analysis for synthesis of Acridine-AVP. The analysis was performed with reversed-phase HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C18-AR-II, 4.6 x 250 mm) by a linear gradient of 0-30%/30min acetonitrile in 0.1% TFA buffer (pH 7.0) at a flow rate of 1 mL/min at 40.0 °C, and monitored by UV detection at $\lambda = 254$ nm or by fluorescence detection ($\lambda_{ex} = 357$ nm, $\lambda_{em} = 482$ nm), and the structures were characterized by MALDI-TOF mass spectrum measurement.

Synthesis of Hoechst-AVP (4)

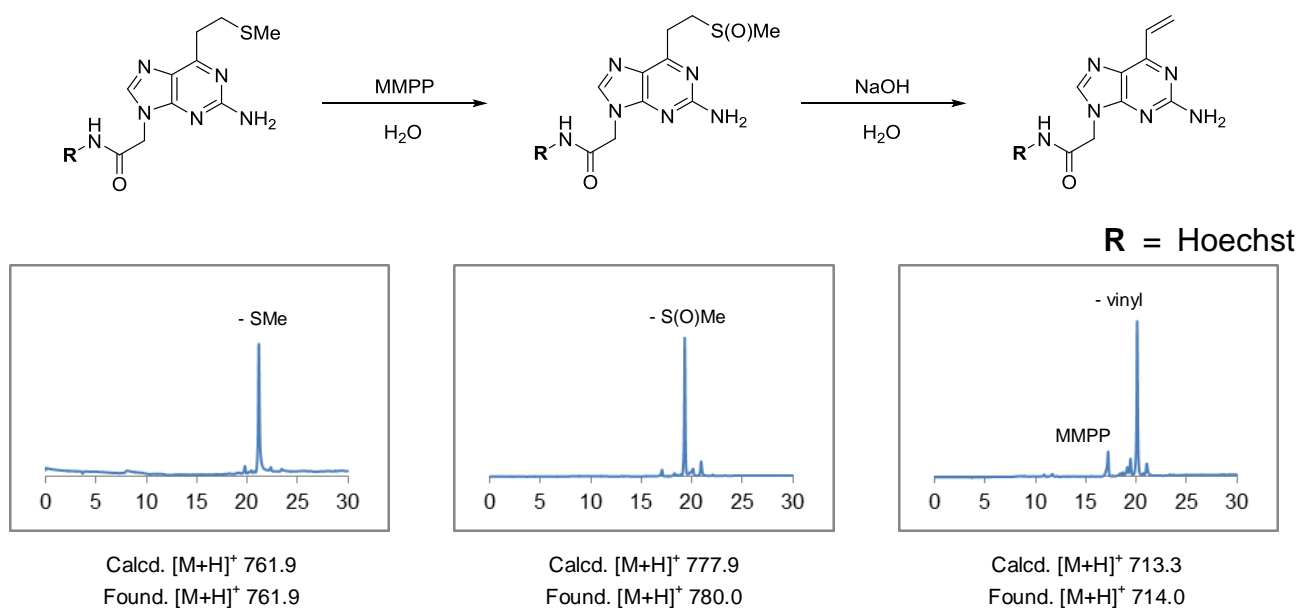


Fig S3 HPLC analysis for synthesis of Hoechst-AVP. The analysis was performed with reversed-phase HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C18-AR-II, 4.6 x 250 mm) by a linear gradient of 0-45%/30min acetonitrile in 0.1% TFA buffer (pH 7.0) at a flow rate of 1 mL/min at 40.0 °C, and monitored by UV detection at $\lambda = 254$ nm or by fluorescence detection ($\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 461$ nm), and the structures were characterized by MALDI-TOF mass spectrum measurement.

DNA2: 5'6-FAM-d(CAG CGC TAA TTC GCG AGT)-3'

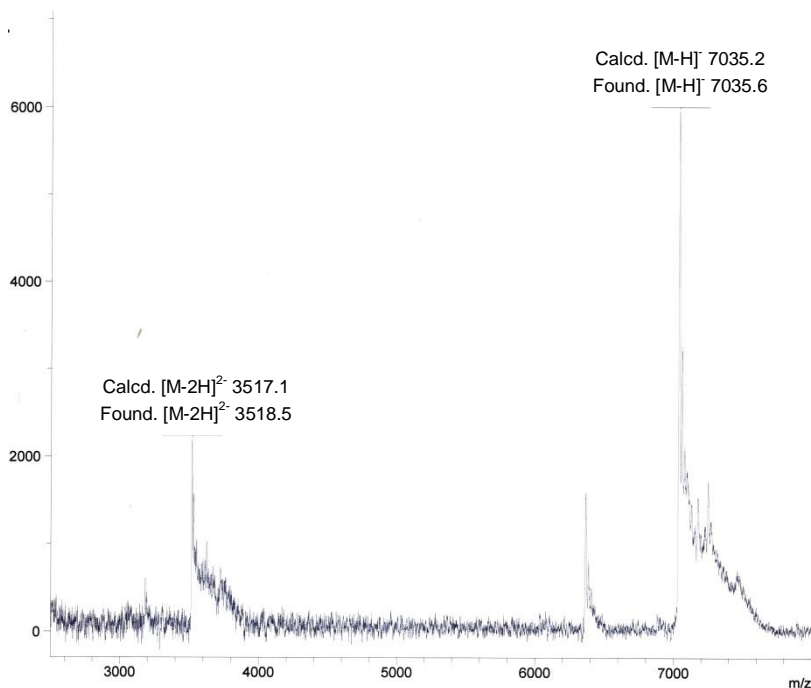


Fig S4 MALDI-spectrum of DNA2 alkylated with (Arg)₅-AVP.

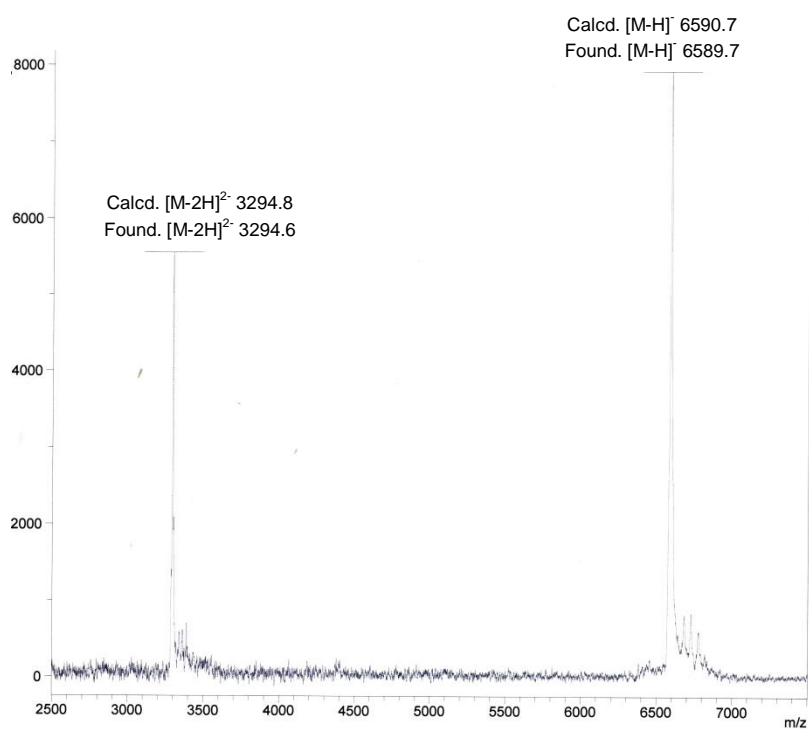


Fig S5 MALDI-spectrum of **DNA2** alkylated with acridine-AVP.

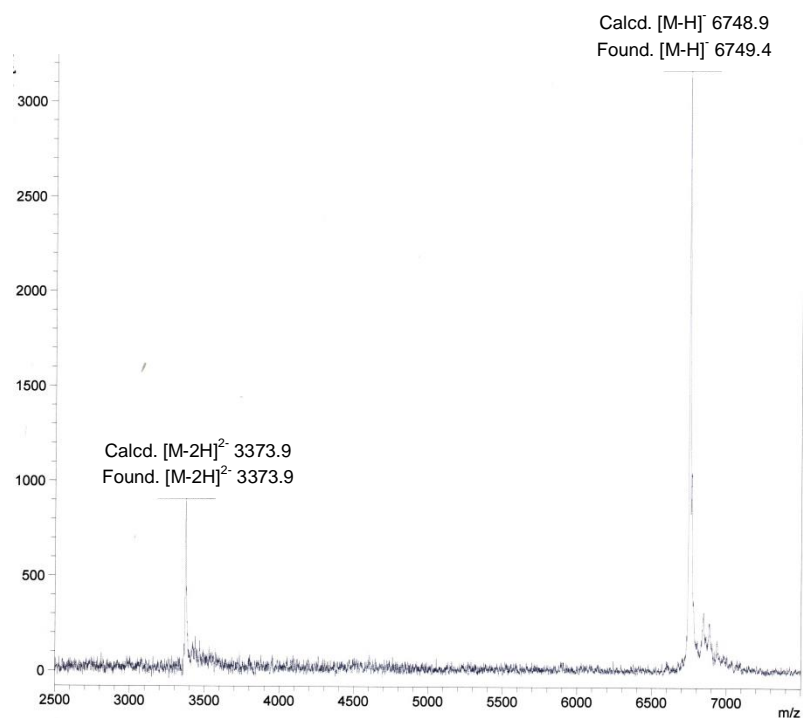


Fig S6 MALDI-spectrum of **DNA2** alkylated with Hoechst-AVP.

General procedure of alkylating reaction within AP site

The alkylation reaction was performed with the target duplex DNA [5'-fluorescein labelled target strand (5.0 μM) and opposite strand containing abasic site or full-match (7.5 μM)] or the target single strand DNA [5'-fluorescein labelled target strand (5.0 μM)] and the 2-AVP probe [(Arg)₅-AVP (100 μM), Acridine-AVP (100 μM), or Hoechst-AVP (25 μM)] were incubated in a buffer of 50 mM MES (pH 7.0) and 100 mM NaCl at 37 °C. A part of the reaction mixture was collected and 3 equiv. of loading dye (95% formamide, 0.05% BPB) was added to quench the reaction. The alkylation yield was analyzed by denaturing PAGE using 20% polyacrylamide gel containing 20% formamide and 7 M urea, and fluorescence labelled DNAs were visualized and quantified with Fujifilm FLA-5100 (Tokyo, Japan). The slowly migrating bands were cleaved from the gel and extracted by shaking in a buffer of 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. The elution was filtered, freeze-dried, treated with Zip-tip, and thereafter MALDI-TOF mass spectroscopy was measured.

Isolation of Hoechst-AVP adducted single strand DNA

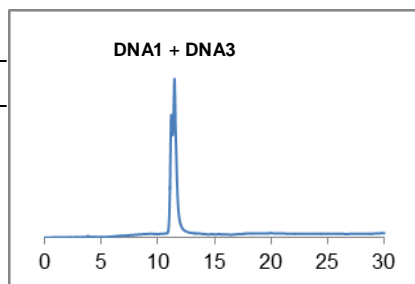
The duplex DNA containing an abasic site [target strand (5.0 μM) and opposite strand containing abasic site (7.5 μM)] and Hoechst-AVP probe (25 μM) were incubated in a buffer of 50 mM MES (pH 7.0) and 100 mM NaCl at 37 °C for 72 h. After incubation, excess ligand was filtered off by centrifugation using amicon-ultra (membrane for 3 kDa; Merck-Millipore). Then, the crude mixture was purified by using reversed-phase HPLC with C-18 column (Nacalai tesque: COSMOSIL 5C₁₈-MS-II, 4.6 x 250 mm) by a liner gradient of 5-35%/30min acetonitrile in 0.1 M TEAA buffer (pH 7.0) at a flow rate of 1 mL/min at 60.0 °C, and monitored by UV detection at $\lambda = 254$ nm. The isolated DNA was freeze-dried, treated with Zip-tip, and thereafter MALDI-TOF mass spectroscopy was measured. The concentration of Hoechst-adducted DNA was determined by UV absorption at 260 nm using the molar extinction coefficients: 212,510 $\text{M}^{-1} \text{cm}^{-1}$ [175,400 $\text{M}^{-1} \text{cm}^{-1}$ (target ss-DNA) and 37,110 $\text{M}^{-1} \text{cm}^{-1}$ (**14**)]

AP0bp (Non-modified)

DNA1: 3' -d (GTCGCG X TTAA GCGCTCA) -

DNA3: 5' -d (CAGCGC T AATT CGCGAGA) -

25 mM Hoechst-AVP (16)
50 mM MES (pH 7.0), 100 mM NaCl
37 °C for 72 h

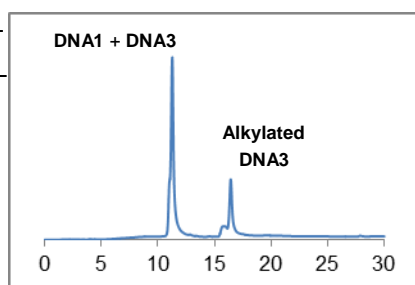


AP0bp (Alkylated)

DNA1: 3' -d (GTCGCG X TTAA GCGCTCA) -

DNA3: 5' -d (CAGCGC N AATT CGCGAGA) -

HPLC purification



Hoechst-AVP adducted ss-DNA

DNA3: 5' -d (CAGCGC T AATT CGCGAGA) -

Hoechst-AVP

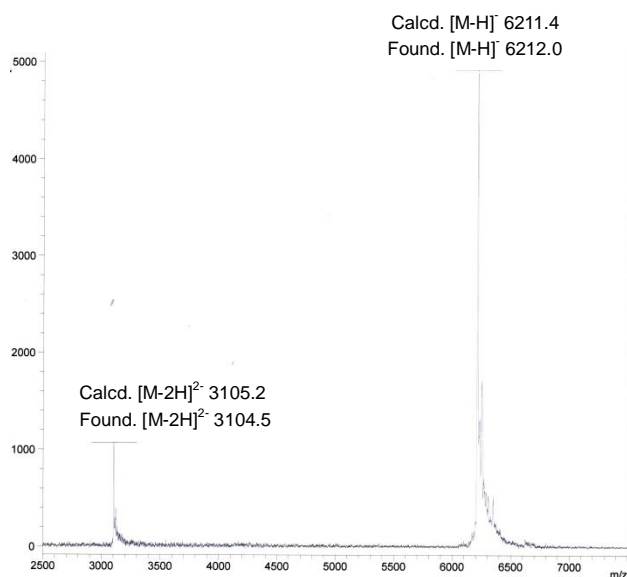
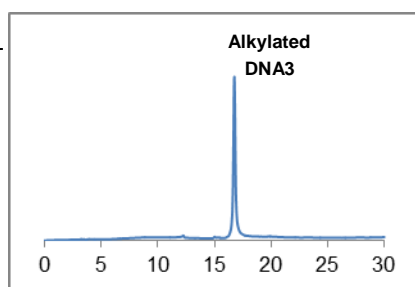


Fig S7 HPLC purification of Hoechst-AVP adducted DNA. a) HPLC purification for isolation of **DNA 3** alkylated with Hoechst-AVP. The purification was performed with C-18 column (Nacalai tesque: COSMOSIL 5C18-MS-II, 4.6 x 250 mm) by a linear gradient of 5-35%/30min acetonitrile in 0.1 M TEAA buffer (pH 7.0) at a flow rate of 1 mL/min at 60.0 °C, and monitored by UV detection at $\lambda = 254$ nm or by fluorescence detection ($\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 461$ nm). b) MALDI-TOF mass spectrum measurement of **DNA3** alkylated with Hoechst-AVP.

Estimation of the stability of the adduct between AVP-Hoechst and DNA by HPLC

After purification of the adduct between AVP-Hoechst and DNA by HPLC, the adduct was treated with the indicated conditions.

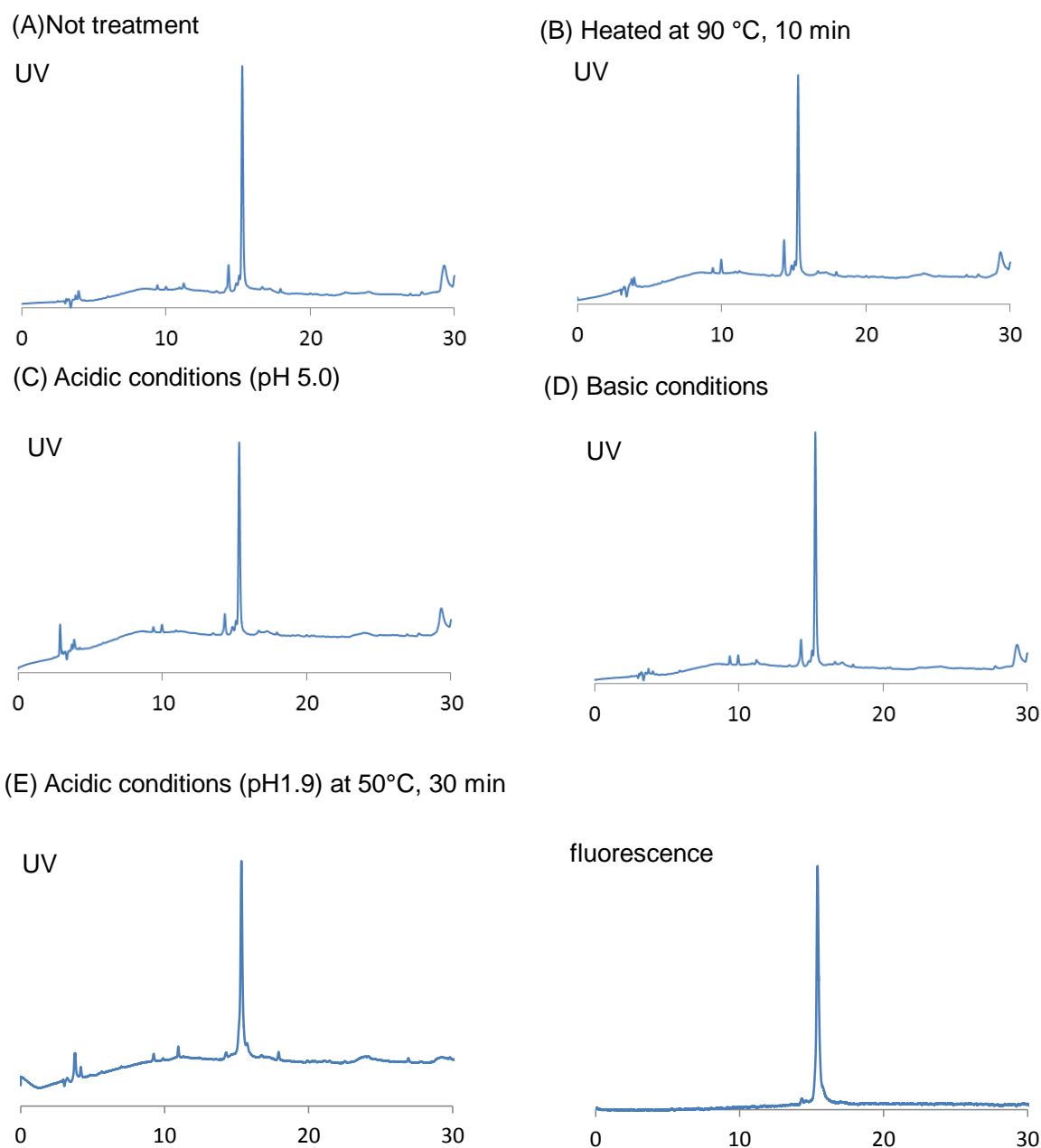


Fig S8 The stability of Hoechst-AVP adducted DNA was evaluated by HPLC. HPLC was performed with C-18 column (Nacalai tesque: COSMOSIL 5C18-MS-II, 4.6 x 250 mm) by a linear gradient of 5-35%/30 min acetonitrile in 0.1 M TEAA buffer (pH 7.0) at a flow rate of 1 mL/min at 40.0 °C and monitored by UV detection at $\lambda = 254$ nm or by fluorescence detection ($\lambda_{\text{ex}} = 350$ nm, $\lambda_{\text{em}} = 461$ nm).

(A) No treatment (B) Heated at 90 °C for 10 min in 50 mM MES, pH 7.0, 100 mM NaCl (C) Standing at 37 °C for 24 hr in 50 mM MES pH 5.0, 100 mM NaCl (D) Standing at 37 °C for 24 hr in 50 mM Tris-HCl, pH 9.0, 100 mM NaCl. (E) Heated at 50 °C for 30 min in 20 % acetic acid (pH 1.9)

Primer extension reaction

The template DNA [18-mer ssDNA alkylated with Hoechst-AVP (0.5 μM) or non-alkylated 18-mer ssDNA (0.5 μM)] and the primer DNA [5'-fluorescein labelled 9-mer or 11-mer ssDNA (0.25 μM)] were dissolved in a buffer of 1x NEB2 buffer [50 mM NaCl, 10 mM MgCl_2 , 1 mM DTT, and 10 mM Tris-HCl (pH 7.9)], followed by thermal annealing from 95 $^\circ\text{C}$ to room temperature slowly. To the mixtures, were added dNTPs (10 μM) and Klenow fragment (3' \rightarrow 5' exo^- , 0.25 U). After incubation at 37 $^\circ\text{C}$ for 5 min, the reaction was quenched by addition of 3 equiv. of loading dye (90% formamide, 50 mM EDTA, 0.05% BPB). The extended products were analyzed by 20% denaturing polyacrylamide gel containing 20% formamide and 7 M urea.

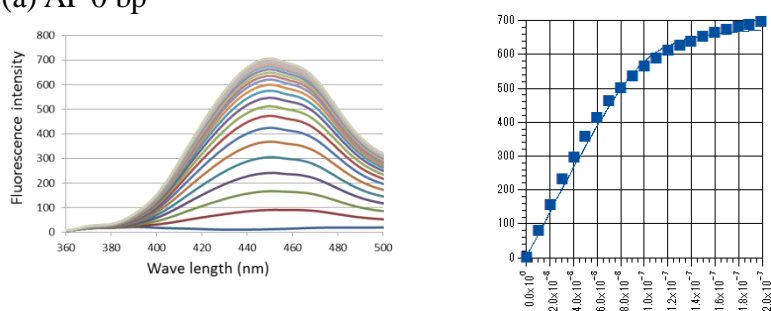
Fluorescence titration measurement

Fluorescence spectra were recorded on JASCO FP-6500 fluorescence spectrometer (Hachioji, Japan) using 1 cm quartz cell, and performed at 25 $^\circ\text{C}$. To a solution of Hoechst 33258 (0.1 μM) or Hoechst-AVP (0.1 μM) in a buffer (2.5 mL) containing 50 mM MES (pH 7.0) and 100 mM NaCl, was titrated dsDNA (25 μM , 1 μL per injection). The solution was excited at 351 nm and fluorescence spectra were recorded in the 360-500 nm wavelength range. The increase of fluorescence intensity at each point of injection ($F - F^0$) was plotted versus the concentration of titrated ds-DNA $[D]$. The obtained profiles were fitted by one-to-one binding equilibrium using Deltagraph (Nihon Poladigital K. K., Tokyo, Japan).^{S2-S3}

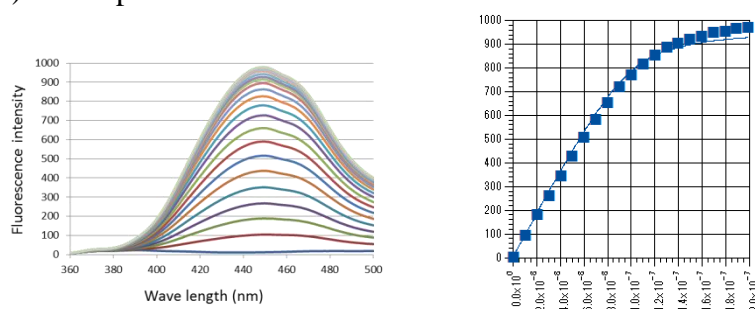
$$(F - F^0) = \frac{1}{[L]} (F^b - F^0) \left(\frac{[L]}{2} + \frac{[D]}{2} + \frac{1}{2K_s} - \sqrt{\frac{1}{4K_s^2} + \frac{[L]^2}{4} + \frac{[D]^2}{4} + \frac{[L]}{2K_s} + \frac{[D]}{2K_s} - \frac{[L][D]}{2}} \right)$$

where F is the fluorescence intensity at each injection, F^0 is the fluorescence intensity of the free ligand, F^b is the fluorescence intensity at end point of injection, $[L]$ is the total concentration of Hoechst33258 or Hoechst-AVP regarded as a constant value through the titration, $[D]$ is the total concentration of ds-DNA at each point of injection, K_s is the binding constant.

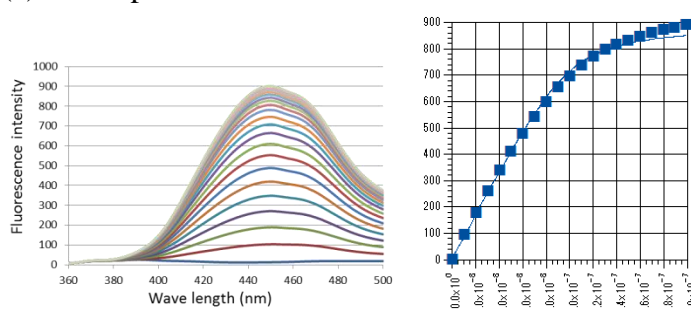
(a) AP 0 bp



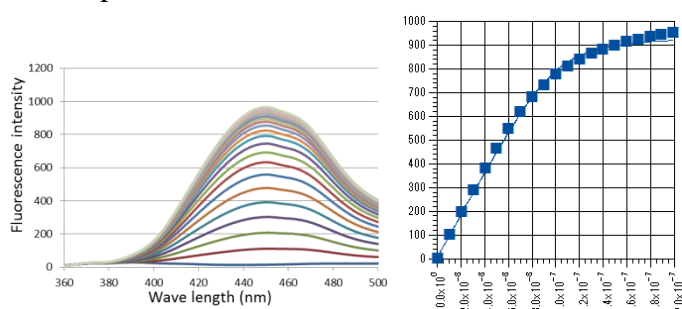
(b) AP 1 bp



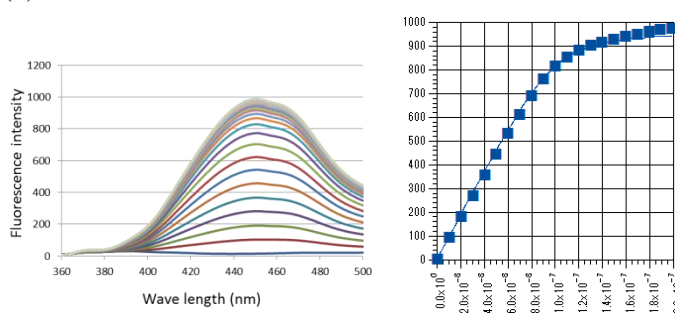
(c) AP 2 bp



(d) AP 3 bp



(e) Reverse AP



(f) w/o AATT

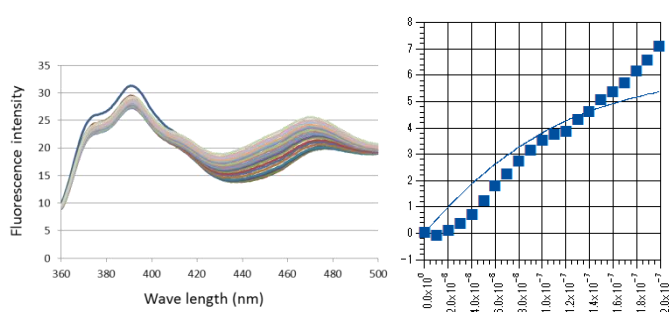


Fig.S9. Changes in fluorescence spectra of **14** (0.1 μ M) in 50 mM MES (pH 7.0), 100 mM NaCl with increasing duplex DNA concentration and changes in fluorescence intensity at 450 nm are plotted versus the DNA concentration.

References

- S1. V. Sridharan, S. Saravanan, S. Muthusubramanian, and S. Sivasubramanian, *Magn. Reson. Chem.* **2005**, *43*, 551-556
- S2. F. Han, N. Taulier, T. V. Chalikian, *Biochemistry* **2005**, *44*, 9785-9794
- S3. D. Beckett, *Methods in Enzymology* **2011**, *488*, 1-16