Supporting Information

Cancer selective target degradation by folate-caged PROTACs

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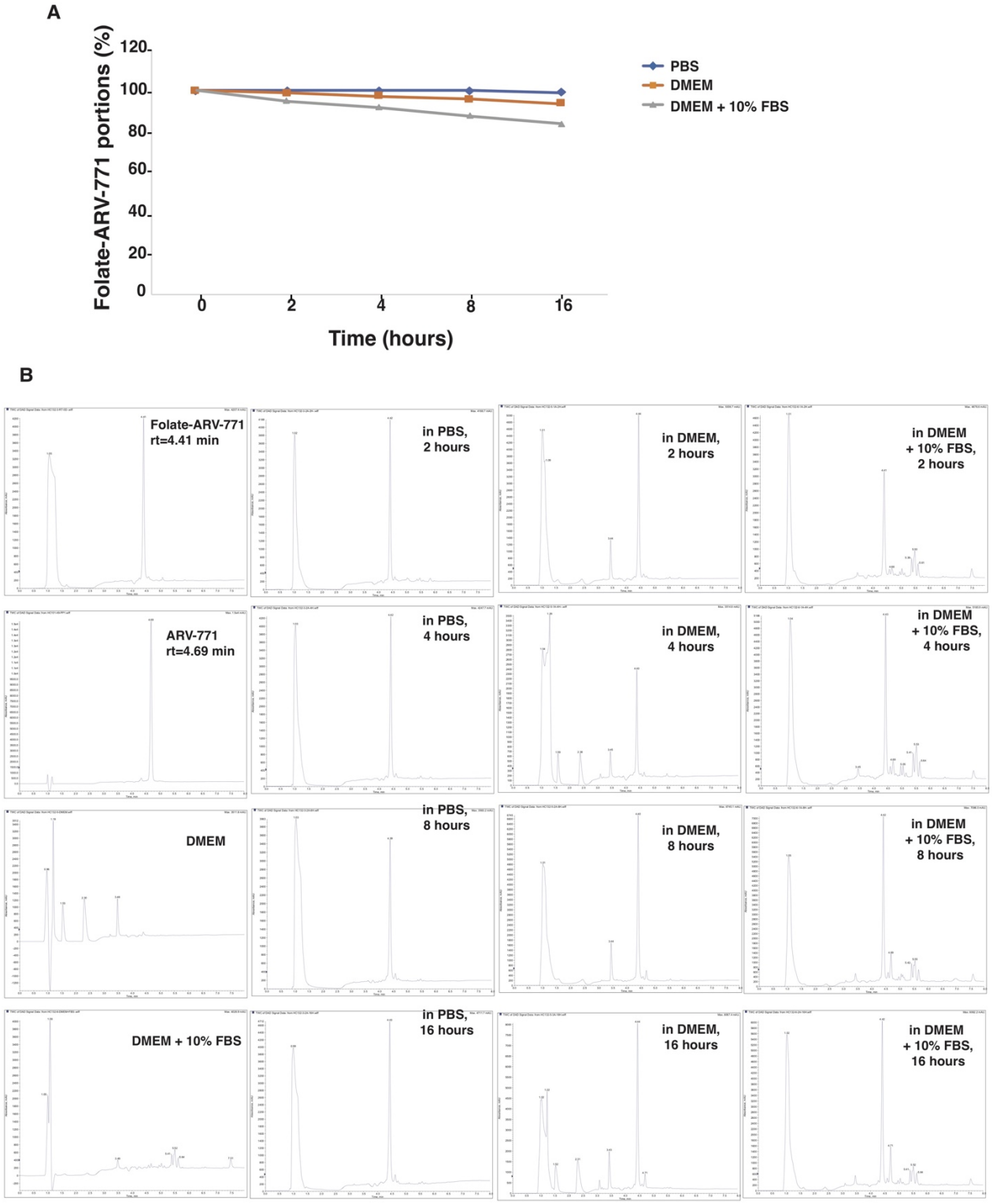
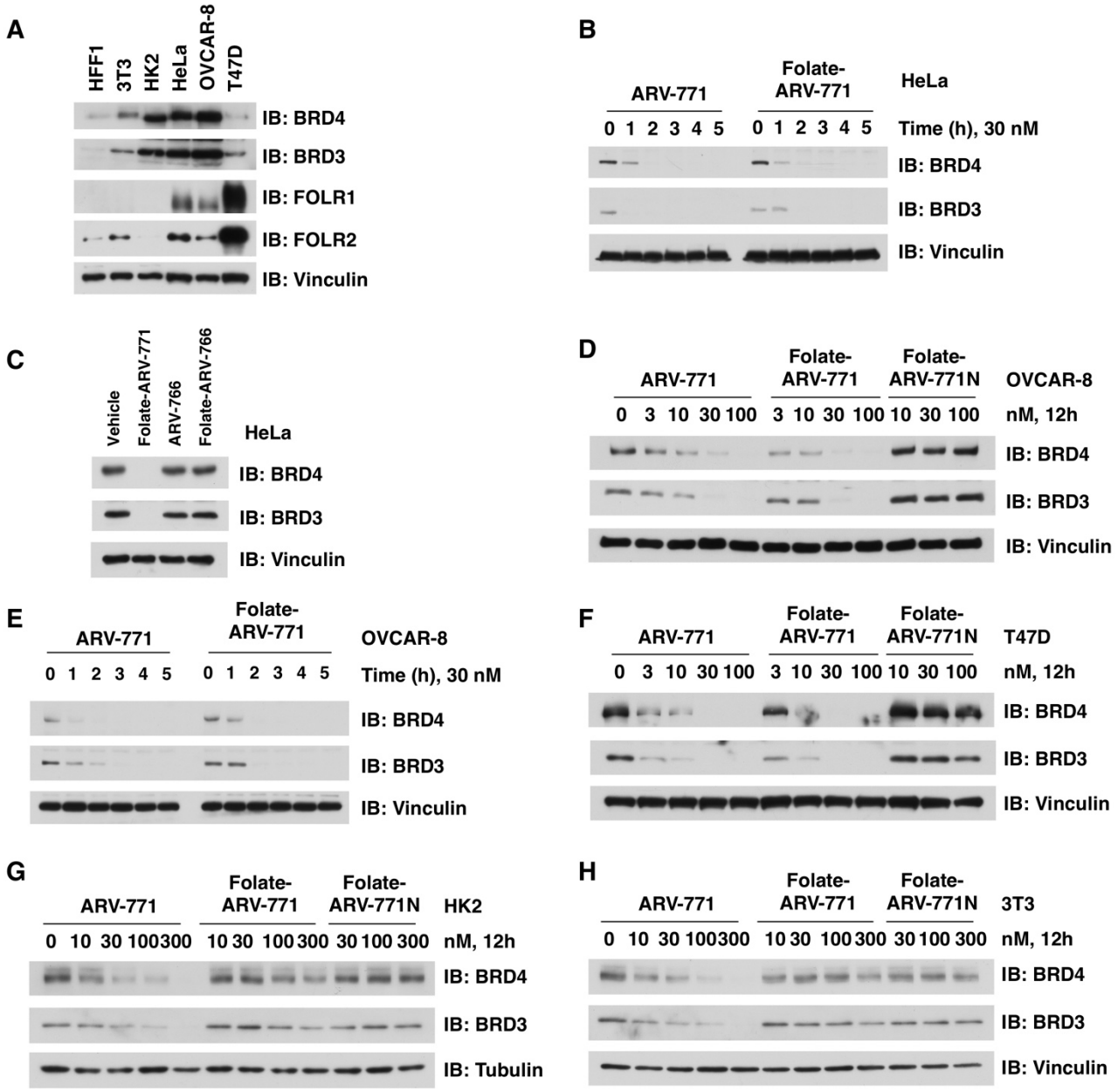
Supporting Figures

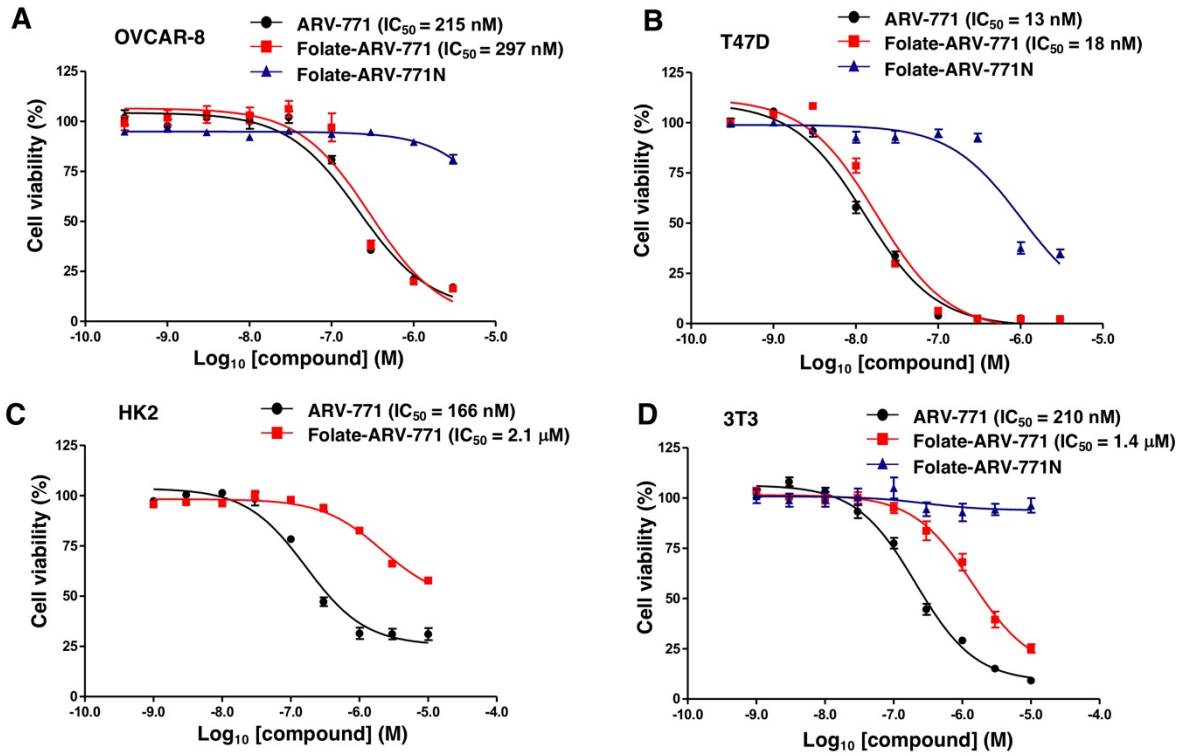


Supporting Figure S1. Folate-ARV-771 is relatively stable in physiological conditions. (A-B) Stability assay of folate-ARV-771 in PBS, or DMEM, or DMEM+10% FBS with HPLC spectra.

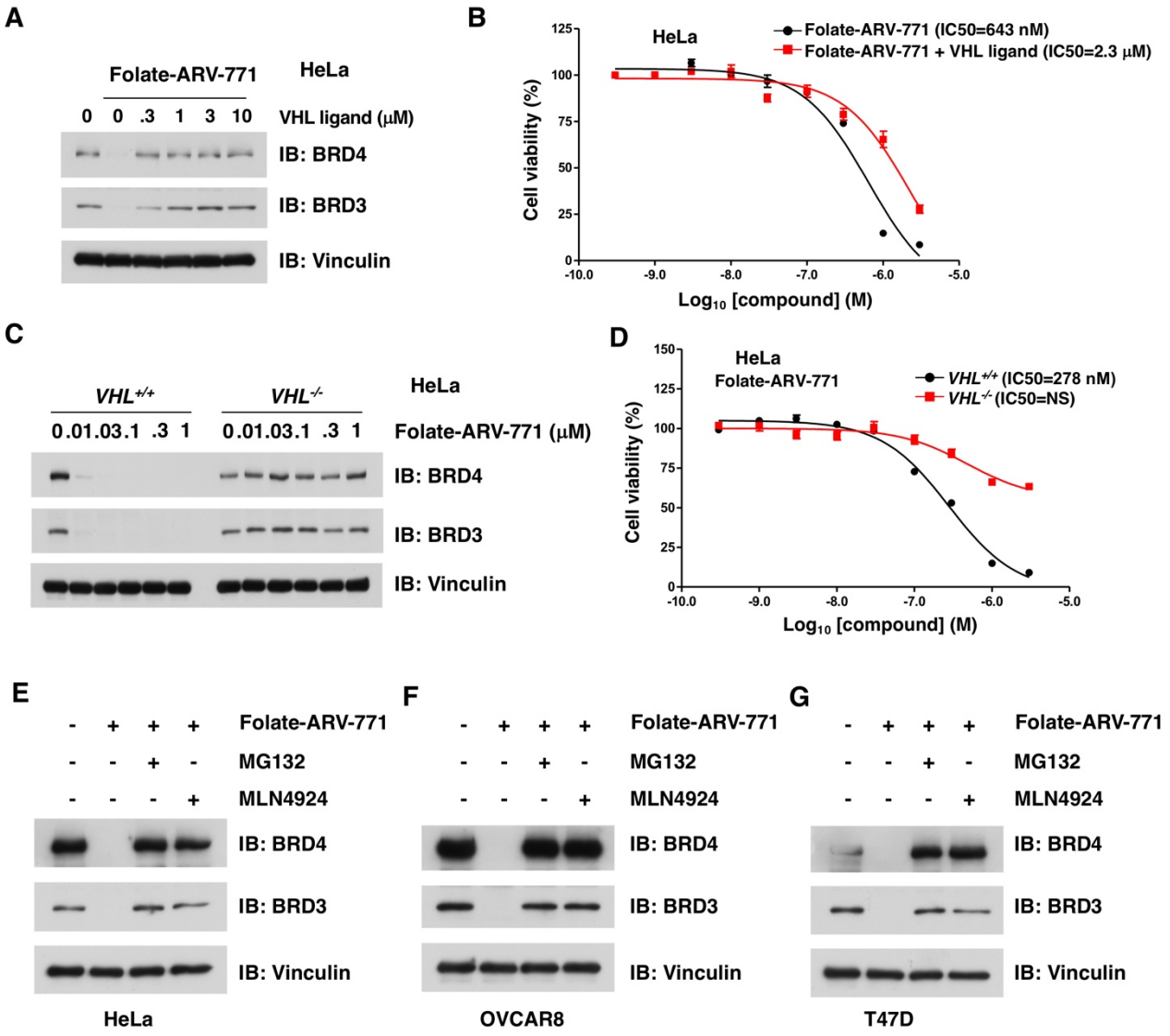


Supporting Figure S2. Folate-ARV-771 preferentially degrades BRDs proteins in FOLR1-expressing cancer cells. (A) Western blot analysis for BRDs and FOLR1/2 in noncancerous normal cells and cancer cells. (B) Folate-ARV-771 degraded BRDs in a time-dependent manner in HeLa cells. HeLa cells were treated with 30 nM of ARV-771 or folate-ARV-771 for indicated time, and then harvested for Western blot analysis for BRDs. (C) The stereochemistry negative control PROTAC, ARV-766 and its folate conjugate folate-ARV-766, were incapable of degrading BRDs. HeLa cells were treated with 30 nM of folate-ARV-771, or 100 nM of ARV776 or 100 nM of folate-ARV-776 for 12 hours, and then

harvested for Western blot analysis for BRDs. (D) Folate-ARV-771 degraded BRDs in a dose-dependent manner in OVCAR-8 cells. OVCAR-8 cells were treated with indicated dose of ARV-771, folate-ARV-771 or folate-ARV-771N for 12 hours, and then harvested for Western blot analysis for BRDs. (E) Folate-ARV-771 degraded BRDs in a time-dependent manner in OVCAR-8 cells. OVCAR-8 cells were treated with 30 nM of ARV-771 or folate-ARV-771 for indicated time, and then harvested for Western blot analysis for BRDs. (F) Folate-ARV-771 degraded BRDs in a dose-dependent manner in T47D cells. T47D cells were treated with indicated dose of ARV-771, folate-ARV-771 or folate-ARV-771N for 12 hours, and then harvested for Western blot analysis for BRDs. (G, H) ARV-771 and folate-ARV-771 degraded BRDs in a dose-dependent manner in HK2 epithelial cells and 3T3 fibroblasts. HK2 and 3T3 cells were treated with indicated dose of ARV-771, folate-ARV-771 or folate-ARV-771N for 12 hours, and then harvested for Western blot analysis for BRDs.

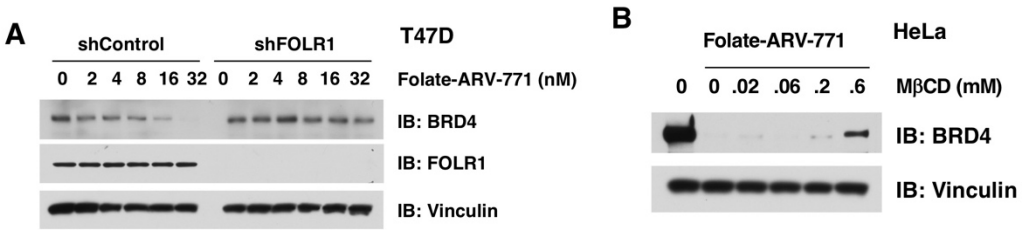


Supporting Figure S3. Folate-ARV-771 preferentially inhibits the proliferation of cancer cells. (A, B) Cell viability of OVCAR-8 and T47D cancer cells after treatment with ARV-771, folate-ARV-771 or folate-ARV-771N for 72 hours. (C) Cell viability of HK2 noncancerous normal cells after treatment with ARV-771 or folate-ARV-771 for 72 hours. (D) Cell viability of 3T3 noncancerous, normal cells after treatment with ARV-771, folate-ARV-771 or folate-ARV-771N for 72 hours.

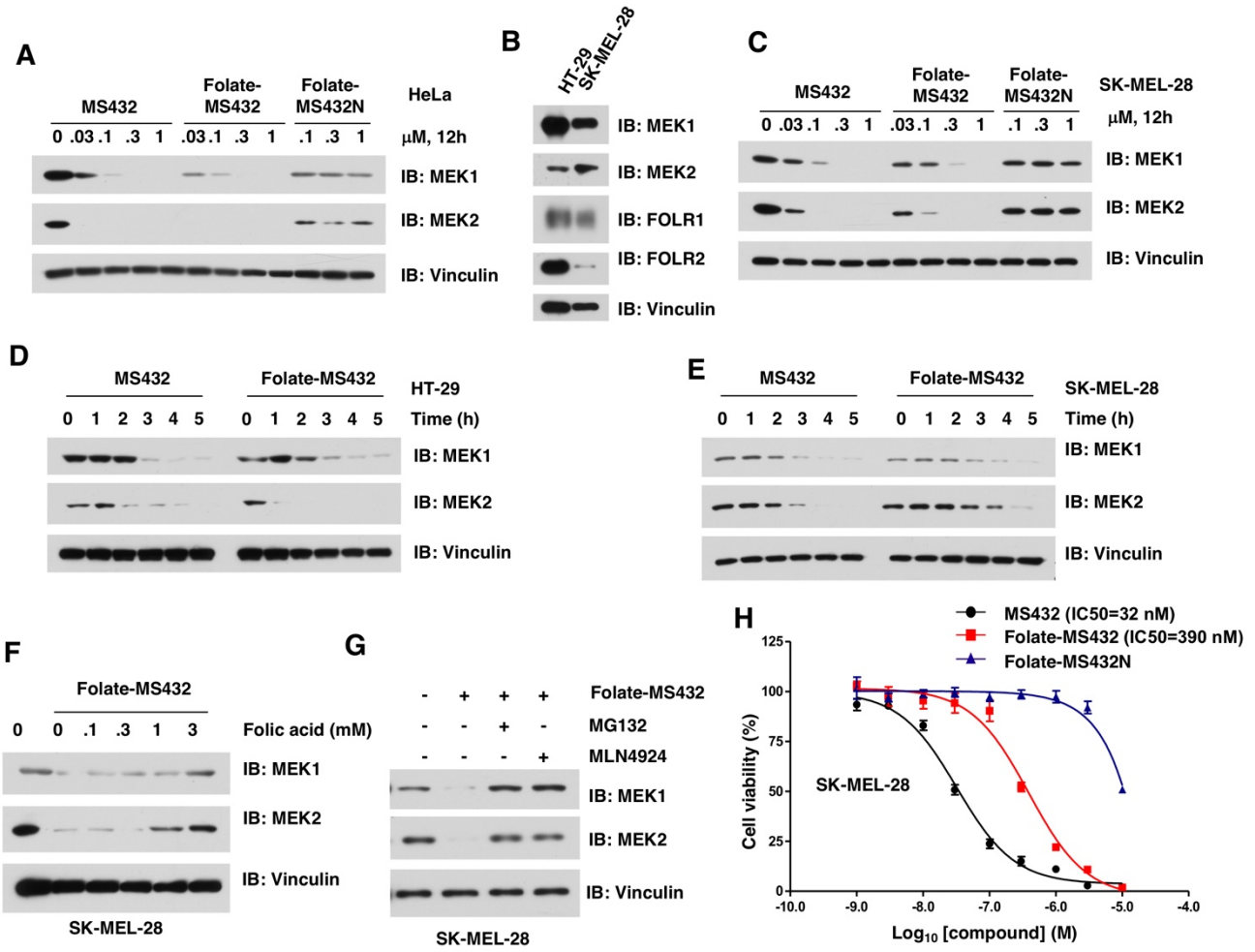


Supporting Figure S4. Folate-ARV-771 degrades BRDs proteins in VHL- and proteasome-dependent manners. (A) Free VHL ligand (VH-032) blocked the effect of folate-ARV-771 in degrading BRDs in HeLa cells. HeLa cells were treated with indicated dose of VHL ligand together with 10 nM of folate-ARV-771 for 12 hours, and then harvested for Western blot analysis for BRDs. (B) Cell viability of HeLa cells after treatment with folate-ARV-771 for 72 hours, with or without co-treatment of VHL ligand (VH-032). (C) Knockout of *VHL* abolished the effect of folate-ARV-771 in degrading BRDs in HeLa cells. HeLa cells were infected with sgVHL virus and selected with puromycin for 72 hours. The selected cells were treated with indicated dose of folate-ARV-771 for 12 hours, and then harvested for Western blot analysis for BRDs. (D) Cell viability of HeLa-*VHL*$^{+/+}$ or HeLa-*VHL*$^{-/-}$ cells after treatment

with folate-ARV-771 for 72 hours. NS: not sensitive. (E-G) Proteasome inhibition and CRL neddylation inhibition blocked the effect of folate-ARV-771 in degrading BRDs in HeLa, OVCAR-8 and T47D cancer cells. HeLa (E), OVCAR-8 (F) and T47D (G) cancer cells were co-treated with 30 nM of folate-ARV-771 and 10 μM of the proteasome inhibitor MG132 or 1 μM of the CRL neddylation inhibitor MLN4924 for 12 hours, and then harvested for Western blot analysis for BRDs.

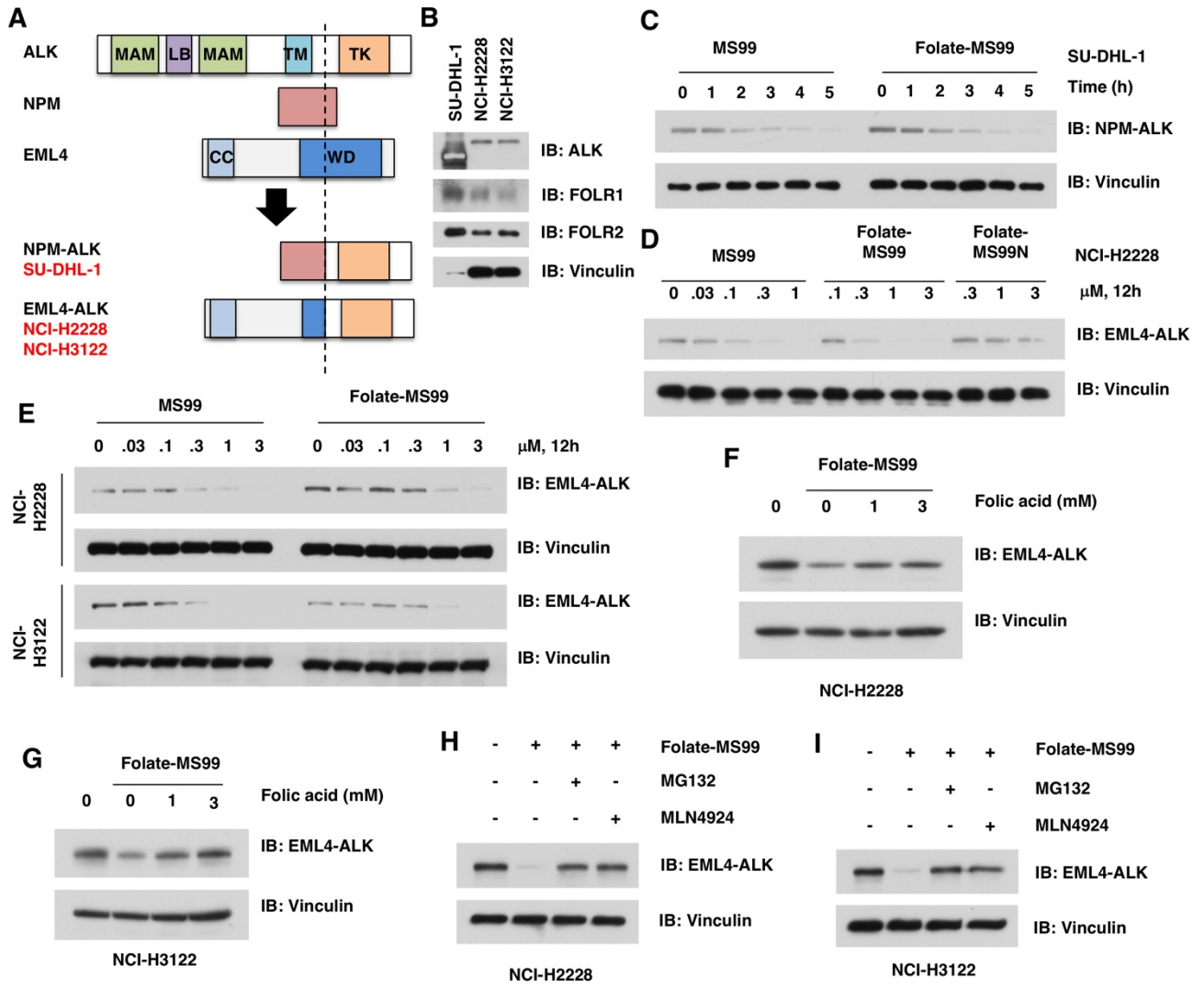


Supporting Figure S5. Folate-ARV-771 degrades BRD4 protein in a FOLR1-dependent manner. (A) Knockdown of *FOLR1* abolished the effect of folate-ARV-771 in degrading BRD4 in T47D cells. T47D cells were infected with shFORL1 virus and selected with puromycin for 72 hours. The selected cells were treated with indicated dose of folate-ARV-771 for 2 hours, and then harvested for Western blot analysis. (B) Endocytosis inhibitor MβCD blocked the effect of folate-ARV-771 in degrading BRDs in HeLa cells. HeLa cells were treated with indicated doses of MβCD together with 30 nM of folate-ARV-771 for 12 hours, and then harvested for Western blot analysis for BRD4.



Supporting Figure S6. Folate-MS432 degrades MEK1/2 in a FOLR1-dependent manner. (A) Folate-MS432 degraded MEK1/2 in a dose-dependent manner in HeLa cells. HeLa cells were treated with indicated dose of MS432, folate-MS432, or folate-MS432N for 12 hours, and then harvested for Western blot analysis for MEK1/2. (B) Western blot analysis for MEK1/2 and FOLR1/2 in HT-29 and SK-MEL-28 cells. (C) Folate-MS432 degraded MEK1/2 in a dose-dependent manner in SK-MEL-28 cells. SK-MEL-28 cells were treated with indicated dose of MS432, folate-MS432, or folate-MS432N for 12 hours, and then harvested for Western blot analysis for MEK1/2. (D-E) Folate-MS432 degraded MEK1/2 in a time-dependent manner in HT-29 cells and SK-MEL-28 cells. HT-29 cells and SK-MEL-28 cells were treated with 0.3 μM of MS432 or folate-MS432 for indicated time, and then harvested for Western blot analysis for MEK1/2. (F) Free folic acid antagonized the effect of folate-MS432 in degrading MEK1/2 in SK-MEL-28 cells. SK-MEL-28 cells were treated with indicated doses of folic acid together with 0.3 μM of folate-MS432 for 12 hours, and then harvested for Western blot analysis

for MEK1/2. (G) Proteasome inhibition and CRL neddylation inhibition blocked the effect of folate-MS432 in degrading MEK1/2 in SK-MEL-28 cells. SK-MEL-28 cells were co-treated with 0.3 μM of folate-MS432 and 10 μM of the proteasome inhibitor MG132 or 1 μM of the CRL neddylation inhibitor MLN4924 for 12 hours, and then harvested for Western blot analysis for MEK1/2. (H) Cell viability of SK-MEL-28 cells after treating with MS432, folate-MS432, or folate-MS432N for 72 hours.

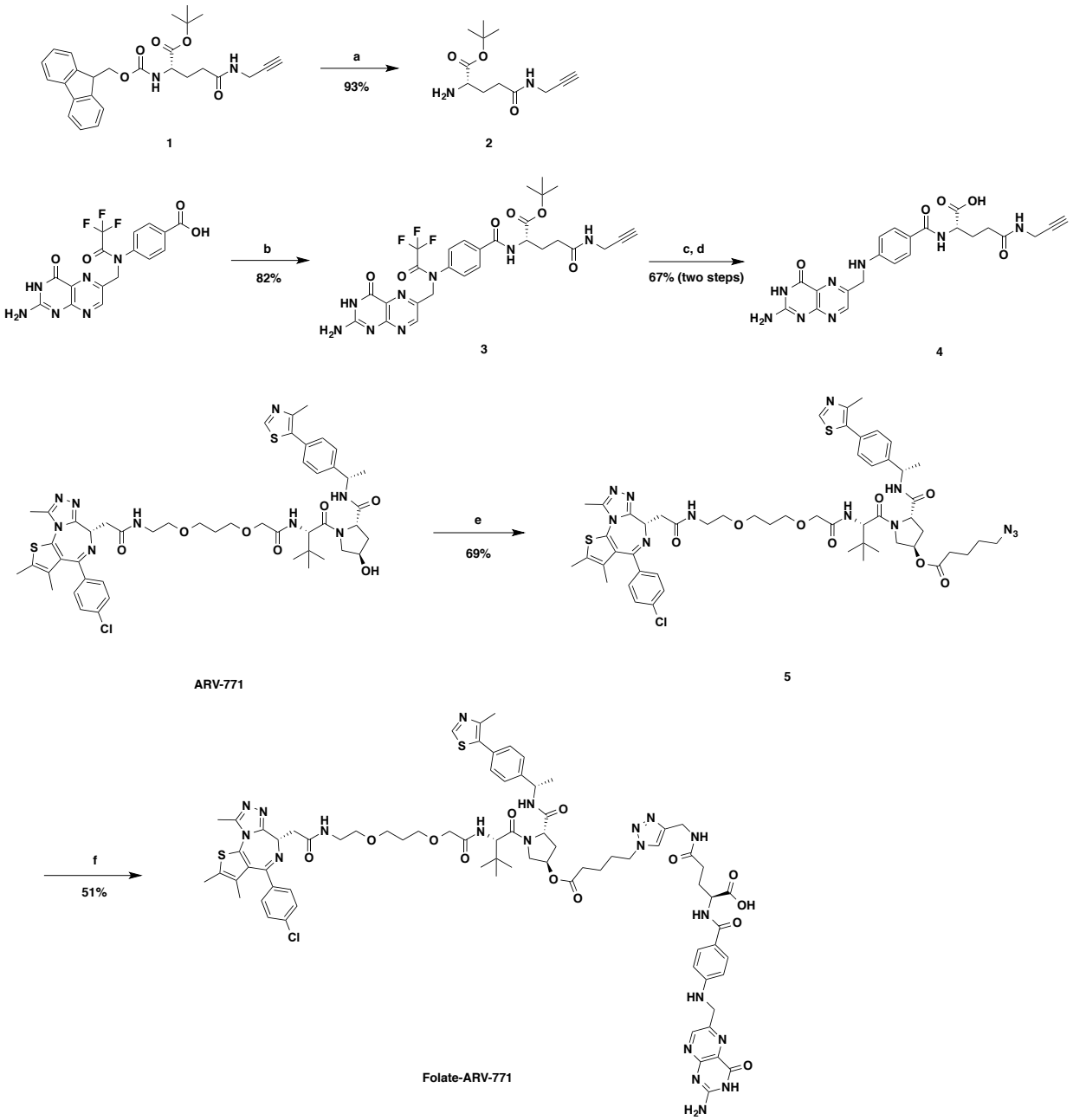


Supporting Figure S7. Folate-MS99 degrades ALK fusion proteins in a FOLR1-dependent manner. (A) Schematic representation of ALK fusion proteins in cancer cells. (B) Western blot analysis for ALK fusion proteins and FOLR1/2 in SU-DHL-1 and NCI-H2228 and NCI-H3122 non-small cell lung cancer (NSCLC) cells. (C) Folate-MS99 degraded the NPM-ALK fusion protein in a time-dependent manner in SU-DHL-1 cells. SU-DHL-1 cells were treated with 0.1 μM of MS99, or folate-MS99 for indicated hours, and then harvested for Western blot analysis for NPM-ALK. (D-E) Folate-MS99 degraded the EML4-ALK fusion protein in a dose-dependent manner in the two NSCLC cell lines. NCI-H2228 and NCI-H3122 NSCLC cells were treated with indicated doses of MS99, folate-MS99, or folate-MS99N for 12 hours, and then harvested for Western blot analysis for EML4-ALK. (F-G) Free folic acid antagonized the effect of folate-MS99 in degrading the EML4-ALK fusion protein in the two NSCLC

cell lines. NCI-H2228 and NCI-H3122 cells were treated with indicated doses of folic acid together with 1 μM of folate-MS99 for 12 hours, and then harvested for Western blot analysis for EML4-ALK. (H-I) Proteasome inhibition and CRL neddylation inhibition blocked the effect of folate-MS99 in degrading ALK fusion proteins the two NSCLC cell lines. NCI-H2228 and NCI-H3122 cells were co-treated with 1 μM of folate-MS99 and 10 μM of the proteasome inhibitor MG132 or 1 μM of the CRL neddylation inhibitor MLN4924 for 12 hours, and then harvested for Western blot analysis for ALK fusion proteins.

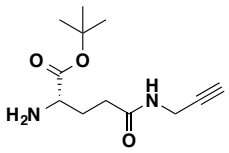
Chemical synthesis

Supporting Scheme S1. Synthesis of folate-ARV-771.



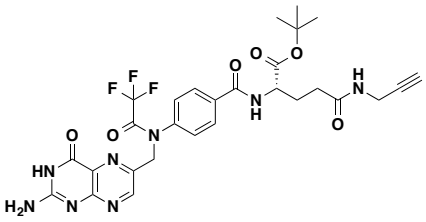
Reaction conditions: (a) dimethylamine, DMF, rt, 30 min; (b) **2**, EDCI, HOAt, NMM, DMSO, rt, 1 h; (c) TFA/CH_2Cl_2, rt, 2 h; (d) K_2CO_3, MeOH/H_2O, rt, 1 h; (e) 5-azidopentanoic acid, DCC, DMAP, TEA, CH_2Cl_2, rt, 4 h; (f) **4**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, DMF/H_2O, 50 °C, 2 h.

Synthesis of *tert*-butyl N^5-(prop-2-yn-1-yl)-*L*-glutaminate (2)



To a solution of compound **1** (see reference[1] for the details of synthesis) (300 mg, 0.65 mmol, 1.0 equiv) in DMF (2 mL) was added dimethylamine (1.62 mL, 2M THF solution, 3.25 mmol, 5.0 equiv) at room temperature. The reaction mixture was stirred at room temperature for 30 min. Then the reaction solution was diluted with ethyl acetate (30 mL) and washed with water (2 x 30 mL) and brine (30 mL). The organic layer was concentrated under reduced pressure. The crude product was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **2** as colorless solid in TFA salt form (213 mg, 93%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 4.05 – 3.93 (m, 3H), 2.59 (t, J = 2.5 Hz, 1H), 2.54 – 2.42 (m, 2H), 2.24 – 2.09 (m, 2H), 1.54 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 172.18, 167.92, 84.03, 79.47, 70.88, 52.61, 30.59, 28.14, 26.73, 25.68. ESI m/z =241.3 $[M + H]^+$.

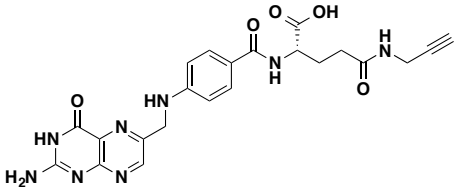
Synthesis of *tert*-butyl N^2-(4-(*N*-((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)-2,2,2-trifluoroacetamido)benzoyl)-N^5-(prop-2-yn-1-yl)-*L*-glutaminate (3)



To a solution of N^{10}-(Trifluoroacetyl)pteroic acid (204.2 mg, 0.5 mmol, 1.0 equiv) in DMSO (4 mL) were added compound **2** (212.6 mg, 0.6 mmol, 1.2 equiv), EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (191.7 mg, 1.0 mmol, 2.0 equiv), HOAt (1-hydroxy-7-azabenzo-triazole) (136.1 mg, 1.0 mmol, 2.0 equiv) and NMM (N-Methylmorpholine) (202.2 mg, 2.0 mmol, 4.0 equiv). After being stirred at room temperature for 1 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated

to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **3** as brown solid (258.5 mg, 82%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.77 (d, J = 7.4 Hz, 1H), 8.73 (s, 1H), 8.31 (t, J = 5.5 Hz, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 5.18 (s, 2H), 4.31 – 4.22 (m, 1H), 3.92 – 3.78 (m, 2H), 3.07 (t, J = 2.5 Hz, 1H), 2.34 – 2.19 (m, 2H), 2.08 – 2.02 (m, 1H), 1.98 – 1.88 (m, 1H), 1.41 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.13, 171.02, 165.71, 159.88, 155.72 (q, J = 34.7 Hz), 153.27, 152.31, 148.95, 145.91, 141.62, 134.54, 128.70, 128.49, 128.13, 116.12 (q, J = 288.4 Hz), 81.19, 80.68, 72.91, 53.82, 53.16, 31.49, 27.84, 27.68, 26.21. ESI m/z =631.3 $[M + H]^+$.

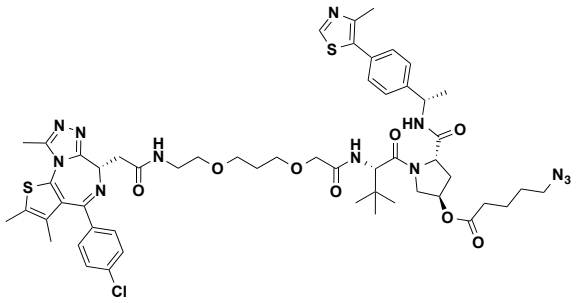
Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-(prop-2-yn-1-yl)-*L*-glutamine (4)



To a suspension of compound **3** (258.5 mg, 0.41 mmol) in dichloromethane (2 mL) was added TFA (trifluoroacetic acid) (2 mL) at room temperature. After being stirred at room temperature for 2 h, the resulting mixture was concentrated under reduced pressure and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O) to afford the intermediate as brown solid. ESI m/z =575.3 $[M + H^+]$. To a suspension of the obtained brown solid in methanol (3 mL) was dropwise added a solution of K_2CO_3 (169.7 mg) in water (1 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to remove methanol. The resulting solution was diluted with water (3 mL) and then pH was adjusted with hydrochloric acid (HCl, 3N) to 2-3. The suspension was filtered and the solid cake was washed with water. After being dried under reduced pressure, afforded compound **4** as brown solid (132.0 mg, 67% yield for two step). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.46 (s, 1H), 11.41 (s, 1H), 8.65 (s, 1H), 8.27 (t, J = 5.5 Hz, 1H), 8.16 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 8.8 Hz, 2H), 6.93 (s, 1H), 6.64 (d, J = 8.8 Hz, 2H), 4.52 – 4.45 (m, 2H), 4.27 (ddd, J = 9.8, 7.5, 4.7 Hz, 1H), 3.88 – 3.77 (m, 2H), 3.06 (t, J = 2.5 Hz, 1H), 2.26 – 2.15 (m, 2H), 2.10 – 1.99 (m, 1H),

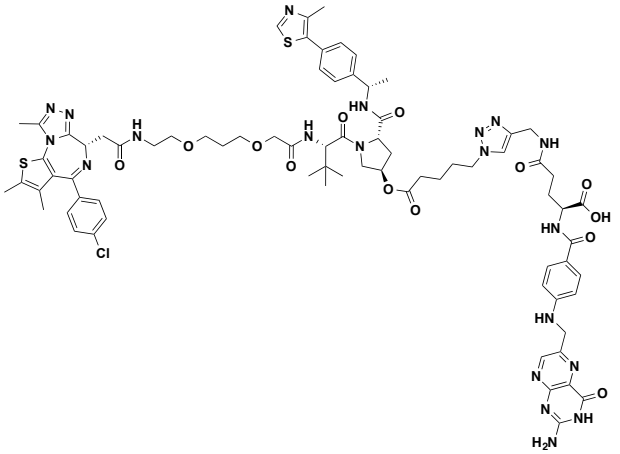
1.95 – 1.84 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.27, 171.82, 166.83, 161.53, 156.55, 154.25, 151.23, 149.14, 149.05, 129.46, 128.41, 121.80, 111.66, 81.68, 73.34, 52.63, 46.37, 32.18, 28.29, 26.87. ESI m/z =479.4 $[M + H]^+$.

Synthesis of (3*R*,5*S*)-1-((*S*)-2-(*tert*-butyl)-15-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-diazapentadecanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl 5-azidopentanoate (5)



To a solution of ARV-771 (29.0 mg, 0.03 mmol, 1.0 equiv) in dichloromethane (2 mL) were added 5-azidopentanoic acid (8.6 mg, 0.06 mmol, 2.0 equiv), DCC (N,N'-Dicyclohexylcarbodiimide) (9.3 mg, 0.045 mmol, 1.5 equiv), DMAP (4-Dimethylaminopyridine) (0.4 mg, 0.003 mmol, 0.1 equiv) and TEA (triethylamine) (3 mg, 0.03 mmol, 1.0 equiv). After being stirred at room temperature for 4 h, the resulting mixture was concentrated to remove CH_2Cl_2, then dissolved in DMSO and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **5** as white solid (23.0 mg, 69%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.19 (s, 1H), 7.53 – 7.41 (m, 8H), 5.35 (t, J = 4.3 Hz, 1H), 5.02 (q, J = 7.1, 6.7 Hz, 1H), 4.73 (dd, J = 8.8, 5.2 Hz, 1H), 4.67 – 4.58 (m, 2H), 4.13 (d, J = 12.0 Hz, 1H), 4.06 – 3.96 (m, 2H), 3.90 (dd, J = 11.9, 4.0 Hz, 1H), 3.74 – 3.63 (m, 4H), 3.62 (t, J = 5.4 Hz, 2H), 3.55 – 3.45 (m, 3H), 3.41 – 3.36 (m, 1H), 3.30 (t, J = 6.6 Hz, 2H), 2.77 (s, 3H), 2.52 (s, 3H), 2.47 (s, 3H), 2.43 – 2.35 (m, 3H), 2.14 (ddd, J = 14.0, 9.4, 4.7 Hz, 1H), 1.97 – 1.90 (m, 2H), 1.71 (s, 3H), 1.69 – 1.63 (m, 2H), 1.61 – 1.56 (m, 2H), 1.51 (d, J = 7.0 Hz, 3H), 1.06 (s, 9H). ESI m/z =1111.3 $[M + H]^+$.

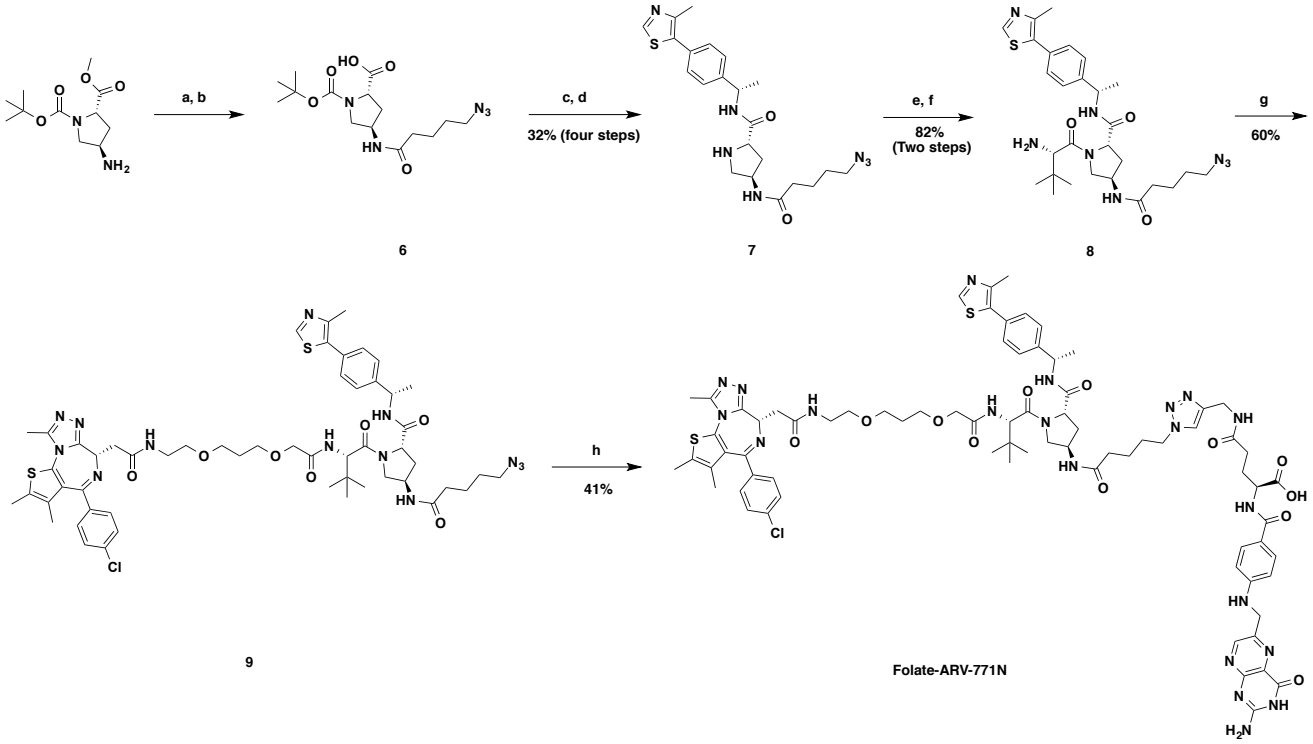
Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-((1-(5-(((3*R*,5*S*)-1-((*S*)-2-(2-(3-(2-(2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)ethoxy)propoxy)acetamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*L*-glutamine (folate-ARV-771)



To a solution of compound **5** (23.0 mg, 0.02 mmol, 1.0 equiv) in DMF (1.2 mL)/water (0.6 mL) were added compound **4** (12 mg, 0.024 mmol, 1.2 equiv), sodium ascorbate (1.6 mg, 0.008 mmol, 0.4 equiv) and $CuSO_4.5H_2O$ (1.0 mg, 0.004 mmol, 0.2 equiv) at room temperature. The reaction mixture was heated to 50 °C. After being stirred at 50 °C for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford **folate-ARV-771** as light yellow solid (16.1 mg, 51%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.73 (s, 1H), 8.52 (d, J = 7.7 Hz, 1H), 8.38 (t, J = 5.6 Hz, 1H), 8.32 (t, J = 5.7 Hz, 1H), 8.24 (d, J = 7.6 Hz, 1H), 7.91 (s, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.3 Hz, 2H), 7.49 – 7.37 (m, 7H), 6.68 (d, J = 8.4 Hz, 2H), 5.26 (s, 1H), 4.98 – 4.92 (m, 1H), 4.61 – 4.54 (m, 3H), 4.51 (t, J = 8.4 Hz, 1H), 4.47 (d, J = 9.4 Hz, 1H), 4.39 – 4.26 (m, 5H), 4.00 – 3.90 (m, 3H), 3.85 – 3.78 (m, 1H), 3.61 – 3.51 (m, 4H), 3.46 (t, J = 6.0 Hz, 2H), 3.40 – 3.23 (m, 4H), 2.64 (s, 3H), 2.49 (s, 3H), 2.45 (s, 3H), 2.39 – 2.23 (m, 5H), 2.18 – 2.08 (m, 1H), 2.06 – 1.92 (m, 2H), 1.89 – 1.78 (m, 4H), 1.66 (s, 3H), 1.55 – 1.45 (m, 2H), 1.41 (d, J = 7.0 Hz, 3H), 0.99 (s, 9H). ^{13}C NMR (151 MHz, DMSO) δ 174.29, 172.74, 172.03, 170.22, 170.14, 169.68, 169.34, 166.80, 163.63, 160.03, 155.58, 153.19, 152.04, 151.63, 151.07, 150.81, 148.42, 148.14, 145.41,

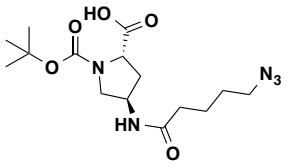
145.06, 137.10, 135.79, 132.64, 131.66, 131.37, 130.65, 130.36, 130.16, 130.10, 129.46, 129.39, 129.33, 128.92, 128.42, 126.79, 123.10, 121.94, 111.73, 73.45, 69.82, 69.36, 68.47, 67.52, 58.71, 56.53, 54.23, 54.13, 52.58, 49.30, 48.35, 46.23, 39.08, 37.87, 35.59, 35.00, 34.72, 33.16, 32.27, 29.89, 29.41, 26.93, 26.61, 22.91, 21.64, 16.38, 14.49, 13.13, 11.73. HRMS (ESI-TOF) calcd for $C_{76}H_{90}ClN_{20}O_{13}S_2^+$ [M + H]$^+$ 1589.6121, found 1589.6103. HPLC > 98%, t_R= 4.29 min.

Supporting Scheme S2. Synthesis of folate-ARV-771N.



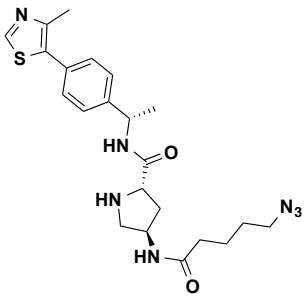
Reaction conditions: (a) 5-azidopentanoic acid, EDCI, HOAt, NMM, DMSO, rt, 3 h; (b) LiOH, MeOH/H_2O, rt, 2 h; (c) (*S*)-1-(4-(4-methylthiazol-5-yl)-phenyl)ethan-1-amine hydrochloride, EDCI, HOAt, NMM, DMSO, rt, 3 h; (d) TFA/CH_2Cl_2, rt, 30 min; (e) (*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoic acid, EDCI, HOAt, NMM, DMSO, rt, 2 h; (f) TFA/CH_2Cl_2, rt, 30 min; (g) (*S*)-2-(3-(2-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno-[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)ethoxy)propoxy)acetic acid,EDCI, HOAt, NMM, DMSO, rt, 3 h ;(h) **4**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, DMF/H_2O, 50 ^oC, 2 h.

Synthesis of (2*S*,4*R*)-4-(5-azidopentanamido)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (6)



To a solution of 1-(*tert*-butyl) 2-methyl (2*S*,4*R*)-4-aminopyrrolidine-1,2-dicarboxylate (146.6 mg, 0.6 mmol, 1.5 equiv) in DMSO (3 mL) were added 5-azidopentanoic acid (57.3 mg, 0.4 mmol, 1.0 equiv), EDCI (153.4 mg, 0.8 mmol, 2.0 equiv), HOAt (108.8 mg, 0.8 mmol, 2.0 equiv) and NMM (161.6 mg, 1.6 mmol, 4.0 equiv). After being stirred at room temperature for 3 h, the resulting mixture was diluted with ethyl acetate (30 mL) and washed with water (2 x 30 mL) and brine (30 mL). The organic layer was concentrated under reduced pressure and telescoped to next step. ESI m/z =392.2 $[M + Na]^+$. The resulting oil was dissolved in MeOH (4 mL) and then a solution of lithium hydroxide (LiOH) (28.8 mg, 1.2 mmol, 3.0 equiv) in water (2 mL) was added. After being stirred at room temperature for 2 h, the reaction mixture was acidified with hydrochloric acid (1N, 2 mL) and then extracted with ethyl acetate (3 x 20 mL). The organic layers were combined and concentrated to afford compound **6** as colorless oil. ESI m/z = 378.4 $[M + Na]^+$. The resulting oil was telescoped to next step without further purification.

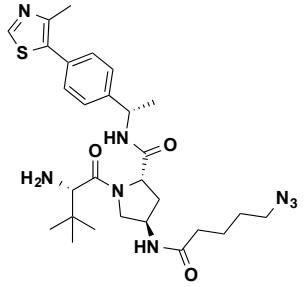
Synthesis of (2*S*,4*R*)-4-(5-azidopentanamido)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (7)



To a solution of the obtained compound **6** in DMSO (3 mL) were added (*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethan-1-amine hydrochloride (101.9 mg, 0.4 mmol, 1.0 equiv), EDCI (153.4 mg, 0.8 mmol, 2.0 equiv), HOAt (108.8 mg, 0.8 mmol, 2.0 equiv) and NMM (161.6 mg, 1.6 mmol, 4.0 equiv). After being stirred at room temperature for 3 h, the resulting mixture was purified by preparative HPLC (10%-

100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford colorless oil. ESI m/z = 556.3 $[M + H]^+$. The obtained oil was dissolved in dichloromethane (2 mL) and TFA (trifluoroacetic acid) (2 mL) was added at room temperature. After being stirred at room temperature for 30 min, the resulting mixture was concentrated and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O) to afford the compound **7** as colorless oil (72.0 mg, 32% for four steps) ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.08 (s, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 5.03 – 4.97 (m, 1H), 4.50 (t, J = 8.1 Hz, 1H), 4.36 – 4.30 (m, 1H), 3.56 (dd, J = 12.1, 6.7 Hz, 1H), 3.26 – 3.19 (m, 3H), 2.43 (s, 3H), 2.38 (ddd, J = 13.4, 8.4, 4.8 Hz, 1H), 2.23 – 2.13 (m, 3H), 1.67 – 1.56 (m, 2H), 1.55 – 1.48 (m, 2H), 1.44 (d, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.59, 166.89, 152.49, 146.08, 144.22, 132.89, 129.54, 129.27, 126.35, 58.89, 50.71, 49.84, 49.24, 48.89, 35.20, 34.74, 27.99, 22.47, 20.87, 13.75. ESI m/z =456.4 $[M + H]^+$.

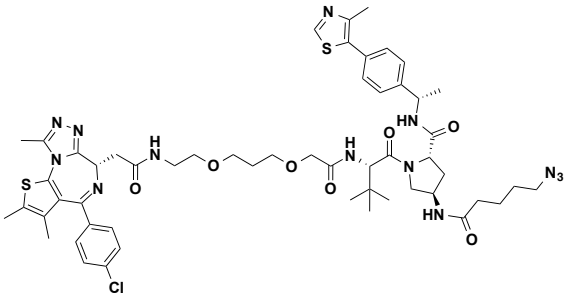
Synthesis of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-(5-azidopentanamido)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (8)



To a solution of compound **7** (72.0 mg, 0.13 mmol, 1.0 equiv) in DMSO (2 mL) were added (*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoic acid (43.8 mg, 0.19 mmol, 1.5 equiv), EDCI (49.8 mg, 0.26 mmol, 2.0 equiv), HOAt (35.4 mg, 0.26 mmol, 2.0 equiv) and NMM (52.5 mg, 0.52 mmol, 4.0 equiv). After being stirred at room temperature for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford colorless oil. ESI m/z = 669.5 $[M + H]^+$. The obtained oil was dissolved in dichloromethane (2 mL) and TFA (trifluoroacetic acid) (2 mL) was added at room temperature. After being stirred at room temperature for

30 min, the resulting mixture was concentrated and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O) to afford the compound **8** as white oil (72.8 mg, 82% for two steps). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.17 (s, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H), 5.01 (q, J = 7.5 Hz, 1H), 4.68 (t, J = 7.6 Hz, 1H), 4.46 (p, J = 5.2 Hz, 1H), 4.05 (s, 1H), 3.91 (dd, J = 10.8, 5.9 Hz, 1H), 3.74 (dd, J = 10.8, 3.9 Hz, 1H), 3.32 (t, J = 6.7 Hz, 2H), 2.53 (s, 3H), 2.31 (ddd, J = 13.3, 8.3, 5.0 Hz, 1H), 2.26 (t, J = 7.4 Hz, 2H), 2.17 – 2.07 (m, 1H), 1.77 – 1.65 (m, 2H), 1.65 – 1.58 (m, 2H), 1.52 (d, J = 7.0 Hz, 3H), 1.17 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.43, 170.91, 167.10, 152.41, 146.08, 144.71, 133.00, 129.31, 129.18, 126.33, 59.08, 58.91, 53.06, 50.73, 49.24, 48.82, 34.75, 34.55, 34.31, 28.00, 25.28, 22.58, 20.97, 13.72. ESI m/z =569.3 $[M + H]^+$.

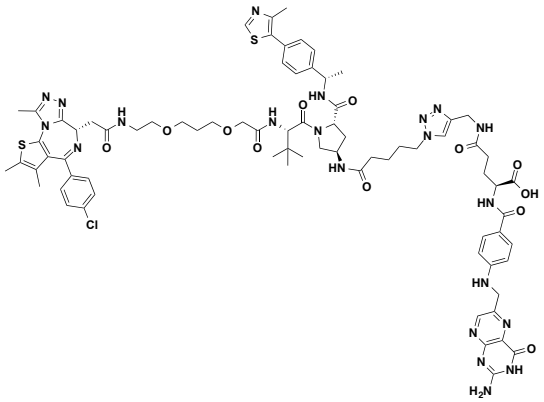
Synthesis of (2*S*,4*R*)-4-(5-azidopentanamido)-1-((*S*)-2-(*tert*-butyl)-15-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-diazapentadecanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (9)



To a solution of compound **8** (25.0 mg, 0.044 mmol, 1.1 equiv) in DMSO (2 mL) were added (*S*)-2-(3-(2-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)ethoxy)propoxy)acetic acid (see reference[2] for the details of synthesis) (22.4 mg, 0.040 mmol, 1.0 equiv), EDCI (15.3 mg, 0.08 mmol, 2.0 equiv), HOAt (10.9 mg, 0.08 mmol, 2.0 equiv) and NMM (16.2 mg, 0.16 mmol, 4.0 equiv). After being stirred at room temperature for 3 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford the compound **9** as white solid (26.6 mg, 60%). ^{1}H NMR (600 MHz,

Methanol-d_4) δ 9.07 (s, 1H), 7.42 – 7.30 (m, 8H), 4.89 (q, J = 7.0 Hz, 1H), 4.61 (dd, J = 8.8, 5.3 Hz, 1H), 4.51 (t, J = 7.6 Hz, 1H), 4.48 (s, 1H), 4.34 (p, J = 5.0 Hz, 1H), 3.92 (d, J = 15.4 Hz, 1H), 3.86 (d, J = 15.4 Hz, 1H), 3.81 (dd, J = 10.9, 5.7 Hz, 1H), 3.74 (dd, J = 10.9, 3.7 Hz, 1H), 3.60 – 3.45 (m, 6H), 3.42 – 3.33 (m, 3H), 3.29 – 3.23 (m, 1H), 3.17 (t, J = 6.7 Hz, 2H), 2.65 (s, 3H), 2.40 (s, 3H), 2.36 (s, 3H), 2.17 – 2.06 (m, 3H), 2.03 – 1.96 (m, 1H), 1.83 – 1.77 (m, 2H), 1.60 (s, 3H), 1.58 – 1.51 (m, 2H), 1.50 – 1.43 (m, 2H), 1.38 (d, J = 7.0 Hz, 3H), 0.95 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.29, 171.18, 170.80, 170.40, 165.26, 152.33, 146.14, 144.60, 137.02, 136.14, 132.56, 132.02, 130.91, 130.64, 130.23, 130.16, 129.35, 129.17, 129.14, 128.49, 126.37, 126.23, 69.44, 68.86, 68.56, 67.32, 58.75, 57.02, 53.49, 53.24, 50.70, 49.17, 48.76, 39.18, 35.15, 34.78, 34.59, 29.45, 27.97, 25.57, 22.82, 22.61, 20.97, 13.79, 13.04, 11.59, 10.17. ESI m/z =1110.2 $[M + H]^+$.

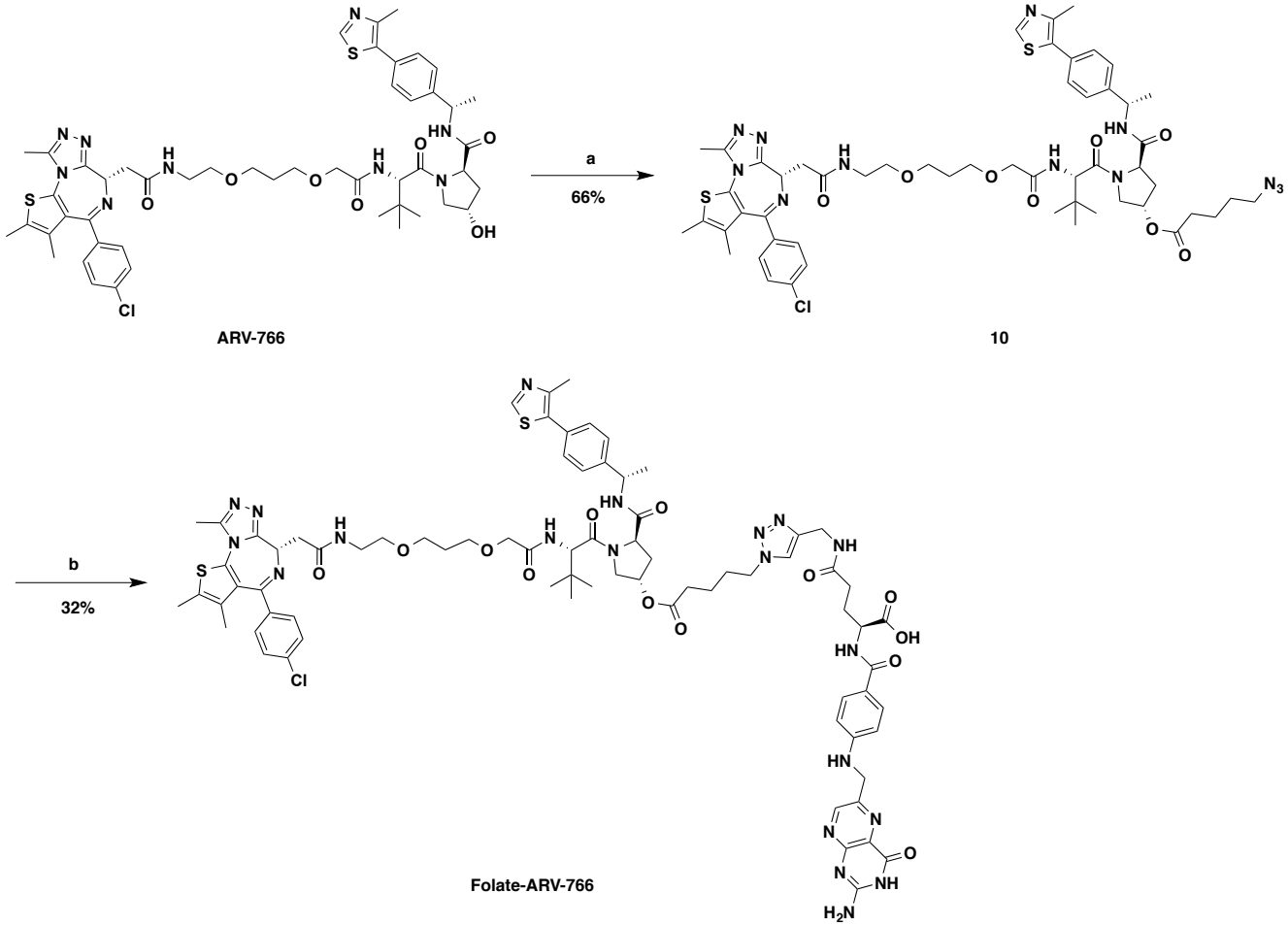
Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-((1-(5-(((3*R*,5*S*)-1-((*S*)-2-(2-(3-(2-(2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)ethoxy)propoxy)acetamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)amino)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*L*-glutamine (folate-ARV-771N)



To a solution of compound **9** (26.6 mg, 0.024 mmol, 1.0 equiv) in DMF (1.0 mL)/water (0.5 mL) were added compound **4** (13.8 mg, 0.029 mmol, 1.2 equiv), sodium ascorbate (1.9 mg, 0.01 mmol, 0.4 equiv) and $CuSO_4.5H_2O$ (1.3 mg, 0.005 mmol, 0.2 equiv) at room temperature. The reaction mixture was heated to 50 °C. After being stirred at 50 °C for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to

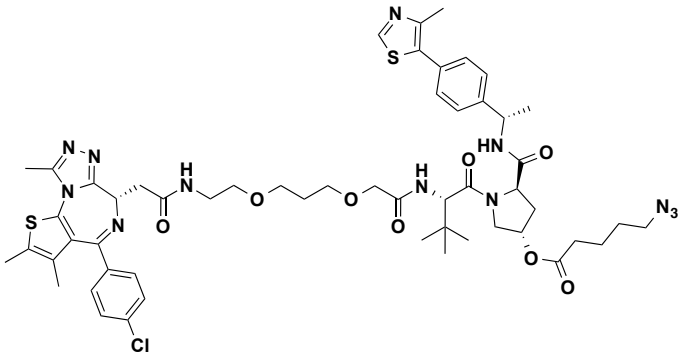
remove the organic solvent under reduced pressure and then dried by lyophilization to afford **folate-ARV-771N** as light yellow solid (15.8 mg, 41%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.67 (s, 1H), 8.35 (d, J = 7.6 Hz, 1H), 8.28 (t, J = 5.7 Hz, 1H), 8.22 (t, J = 5.7 Hz, 1H), 8.15 (d, J = 7.5 Hz, 1H), 8.03 (d, J = 6.9 Hz, 1H), 7.80 (s, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.39 – 7.33 (m, 4H), 7.33 – 7.26 (m, 3H), 6.59 (d, J = 8.4 Hz, 2H), 4.84 (p, J = 6.9 Hz, 1H), 4.53 – 4.38 (m, 5H), 4.32 – 4.12 (m, 6H), 3.93 – 3.80 (m, 2H), 3.75 – 3.69 (m, 1H), 3.48 (t, J = 6.4 Hz, 2H), 3.46 – 3.41 (m, 3H), 3.36 (t, J = 5.9 Hz, 2H), 3.29 – 3.12 (m, 4H), 2.54 (s, 3H), 2.38 (s, 3H), 2.33 (s, 3H), 2.23 – 2.12 (m, 2H), 2.08 – 1.94 (m, 4H), 1.90 – 1.78 (m, 2H), 1.77 – 1.62 (m, 4H), 1.55 (s, 3H), 1.43 – 1.33 (m, 2H), 1.30 (d, J = 6.9 Hz, 3H), 0.88 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.30, 172.25, 172.01, 170.53, 170.12, 169.54, 169.13, 166.78, 163.65, 159.85, 155.57, 153.11, 152.03, 151.05, 150.50, 150.08, 148.33, 148.11, 145.39, 145.11, 137.09, 135.81, 132.63, 131.68, 131.39, 130.66, 130.37, 130.15, 130.12, 129.47, 129.40, 129.34, 128.92, 128.42, 126.79, 123.08, 121.97, 111.75, 69.88, 69.37, 68.50, 67.49, 58.59, 56.22, 54.22, 53.29, 52.58, 49.45, 48.52, 48.30, 46.22, 39.09, 37.86, 35.87, 35.05, 34.85, 34.73, 32.27, 29.91, 29.76, 26.93, 26.66, 22.87, 22.52, 16.37, 14.49, 13.12, 11.73. HRMS (ESI-TOF) calcd for $C_{76}H_{91}ClN_{21}O_{12}S_2^+$ $[M + H]^+$ 1588.6281, found 1588.6280. HPLC > 98%, t_R= 4.21 min.

Supporting Scheme S3. Synthesis of folate-ARV-766.



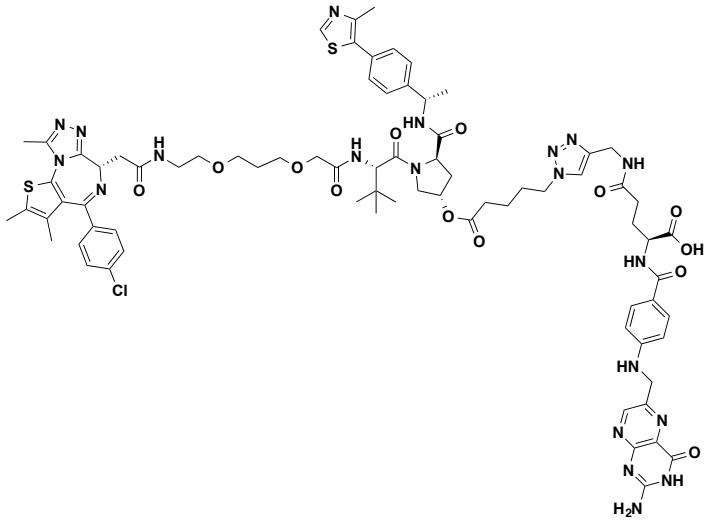
Reaction conditions: (a) 5-azidopentanoic acid, DCC, DMAP, TEA, CH_2Cl_2, rt, 4 h; (b) **4**, $CuSO_4 \cdot 5H_2O$, Sodium ascorbate, DMF/H_2O, 50 ºC, 2 h.

Synthesis of (3*S*,5*R*)-1-((*S*)-2-(*tert*-butyl)-15-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-diazapentadecanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl 5-azidopentanoate (10)



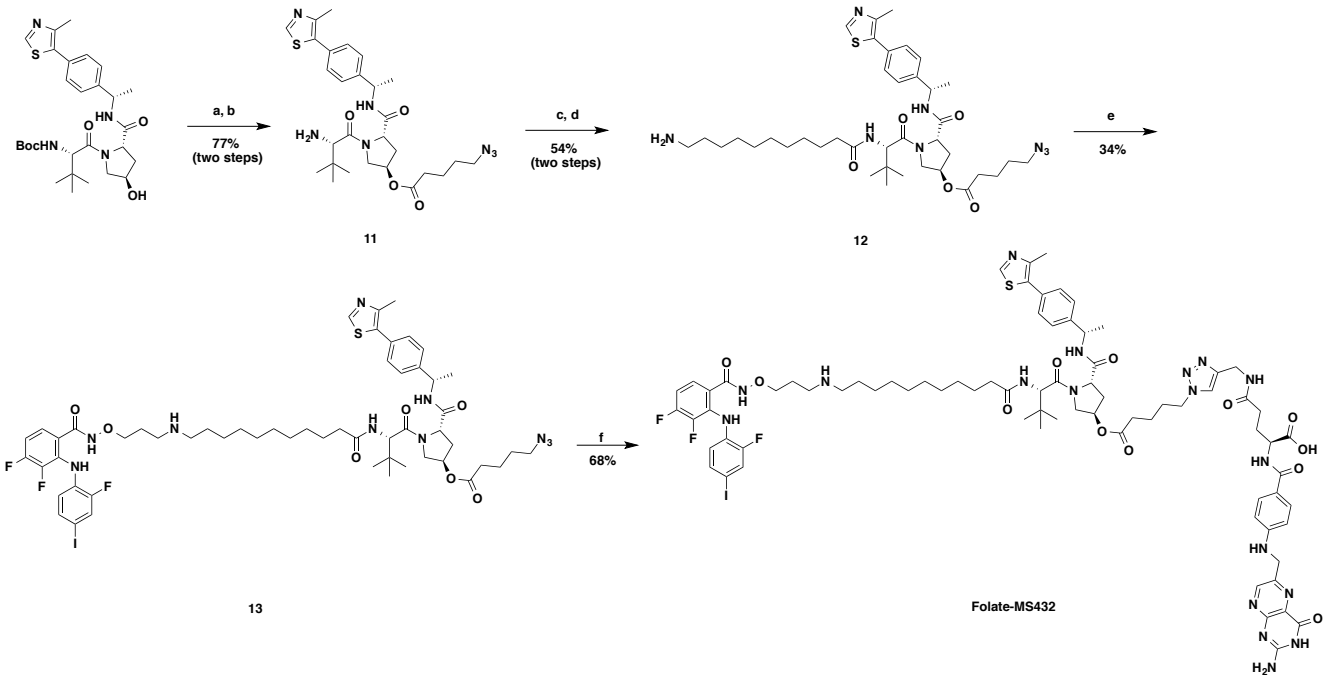
To a solution of ARV-766 (22.4 mg, 0.023 mmol, 1.0 equiv) (see reference[2] for the details of synthesis) in dichloromethane (2 mL) were added 5-azidopentanoic acid (6.6 mg, 0.046 mmol, 2.0 equiv), DCC (9.5 mg, 0.046 mmol, 2.0 equiv), DMAP (0.3 mg, 0.002 mmol, 0.1 equiv) and TEA (4.6 mg, 0.046 mmol, 2.0 equiv). After being stirred at room temperature for 4 h, the resulting mixture was concentrated to remove CH_2Cl_2, then dissolved in DMSO and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **10** as white solid (17.0 mg, 66%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.96 (s, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.49 – 7.44 (m, 4H), 7.41 (d, J = 8.5 Hz, 2H), 5.32 – 5.25 (m, 1H), 5.08 (p, J = 7.1 Hz, 1H), 4.69 (d, J = 8.7 Hz, 1H), 4.65 – 4.56 (m, 2H), 4.08 – 3.94 (m, 4H), 3.62 (t, J = 6.0 Hz, 2H), 3.59 – 3.49 (m, 4H), 3.49 – 3.43 (m, 1H), 3.43 – 3.32 (m, 2H), 3.28 (t, J = 6.6 Hz, 2H), 3.18 (dd, J = 15.3, 4.8 Hz, 1H), 2.73 (s, 3H), 2.49 (s, 3H), 2.46 (s, 3H), 2.44 – 2.38 (m, 1H), 2.35 – 2.18 (m, 3H), 1.87 – 1.79 (m, 2H), 1.71 (s, 3H), 1.67 – 1.59 (m, 2H), 1.59 – 1.53 (m, 2H), 1.50 (d, J = 7.0 Hz, 3H), 1.05 (s, 9H). ESI m/z = 1111.7 $[M + H]^+$.

Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-((1-(5-(((3*S*,5*R*)-1-((*S*)-2-(2-(3-(2-(2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)ethoxy)propoxy)acetamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*L*-glutamine (folate-ARV-766)



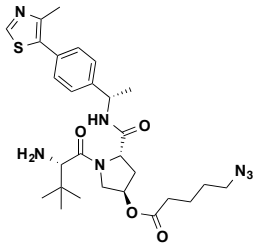
To a solution of compound **10** (12.2 mg, 0.011 mmol, 1.1 equiv) in DMF (1.0 mL)/water (0.5 mL) were added compound **4** (4.8 mg, 0.01 mmol, 1.0 equiv), sodium ascorbate (3.0 mg, 0.015 mmol, 1.5 equiv) and $CuSO_4.5H_2O$ (2.5 mg, 0.01 mmol, 1.0 equiv) at room temperature. The reaction mixture was heated to 50 °C. After being stirred at 50 °C for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure at room temperature and then dried by lyophilization to afford **folate-ARV-766** as light yellow solid (5.1 mg, 32%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.61 (s, 1H), 8.26 (t, J = 5.7 Hz, 1H), 8.21 (d, J = 7.9 Hz, 1H), 8.17 (t, J = 5.6 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.80 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.44 – 7.32 (m, 10H), 6.57 (d, J = 8.4 Hz, 2H), 5.21 – 5.15 (m, 1H), 4.86 – 4.80 (m, 1H), 4.47 – 4.41 (m, 4H), 4.37 (t, J = 7.6 Hz, 1H), 4.26 – 4.14 (m, 5H), 3.88 – 3.73 (m, 4H), 3.41 (t, J = 6.3 Hz, 2H), 3.33 (t, J = 6.4 Hz, 2H), 3.29 (t, J = 5.9 Hz, 2H), 3.24 – 3.09 (m, 4H), 2.52 (s, 3H), 2.37 (s, 3H), 2.33 (s, 3H), 2.28 – 2.13 (m, 5H), 2.07 – 1.96 (m, 2H), 1.92 – 1.79 (m, 1H), 1.77 – 1.67 (m, 2H), 1.63 (p, J = 6.5 Hz, 2H), 1.54 (s, 3H), 1.38 (p, J = 7.5 Hz, 2H), 1.26 (d, J = 7.0 Hz, 3H), 0.85 (s, 9H). HRMS calcd for $C_{76}H_{90}ClN_{20}O_{13}S_2^+$ $[M + H]^+$ 1589.6121, found 1589.6113. HPLC > 98%, t_R = 4.28 min.

Supporting Scheme S4. Synthesis of folate-MS432.



Reaction conditions: (a) 5-azidopentanoic acid, DCC, DMAP, CH_2Cl_2, rt, 18 h; (b) TFA/CH_2Cl_2, rt, 1 h; (c) 11-((*tert*-butoxycarbonyl)amino)undecanoic acid, EDCI, HOAt, NMM, DMSO, rt, 1 h; (d) TFA/CH_2Cl_2, rt, 1 h; (e) 3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-*N*-(3-oxopropoxy)benzamide, $NaBH_3CN$, MeOH/CH_2Cl_2, rt, 3 h ; (f) **4**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, DMF/H_2O, 50 °C, 2 h.

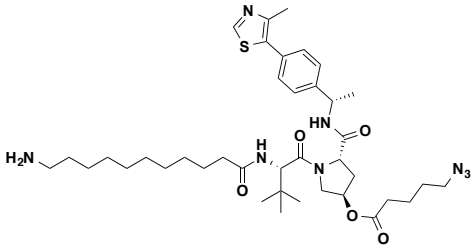
Synthesis of (3*R*,5*S*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl 5-azidopentanoate (11)



To a solution of *tert*-butyl ((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (see reference[2] for the details of synthesis) (247 mg, 0.454 mmol, 1.0 equiv) in dichloromethane (5 mL) were added 5-azidopentanoic acid (97.4 mg, 0.68 mmol, 1.5 equiv), DCC (N,N'-Dicyclohexylcarbodiimide) (140.1 mg, 0.68 mmol, 1.5 equiv) and DMAP (4-Dimethylaminopyridine) (5.5 mg, 0.045 mmol, 0.1 equiv.

After being stirred at room temperature for 18 h, the resulting mixture was concentrated to remove CH_2Cl_2, and then dissolved in DMSO and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford intermediate compound as white solid. ESI m/z =692.4 $[M + Na]^+$. Dissolved the white solid in CH_2Cl_2 (2 mL) and then TFA (2 mL) was added to the reaction at room temperature. After being stirred at room temperature for 1 h, the resulting mixture was concentrated and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **11** as white solid in TFA salt form (238 mg, 77% for two steps). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.00 (s, 1H), 7.36 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 5.27 (t, J = 4.3 Hz, 1H), 4.90 (q, J = 6.9 Hz, 1H), 4.56 (dd, J = 9.4, 7.7 Hz, 1H), 3.97 (s, 1H), 3.87 (d, J = 12.1 Hz, 1H), 3.75 (dd, J = 12.0, 4.1 Hz, 1H), 3.22 (t, J = 6.6 Hz, 2H), 2.40 (s, 3H), 2.35 – 2.29 (m, 1H), 2.27 (t, J = 7.3 Hz, 2H), 2.01 (ddd, J = 14.1, 9.5, 4.7 Hz, 1H), 1.64 – 1.55 (m, 2H), 1.55 – 1.48 (m, 2H), 1.41 (d, J = 7.0 Hz, 3H), 1.04 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 172.69, 170.76, 167.06, 152.24, 146.37, 144.56, 132.78, 129.48, 129.17, 126.31, 73.11, 59.37, 58.99, 54.03, 50.71, 48.87, 34.83, 34.44, 32.93, 27.85, 25.25, 21.64, 20.99, 13.84. ESI m/z =570.3 $[M + H]^+$.

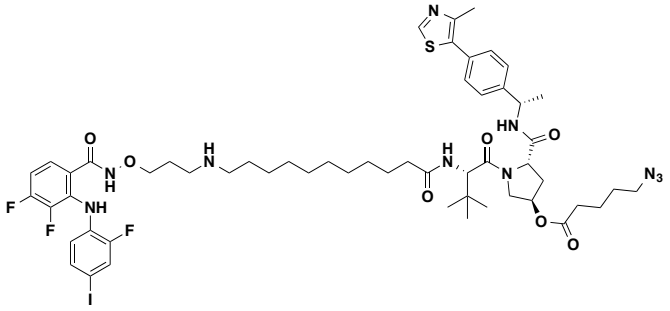
Synthesis of (3*R*,5*S*)-1-((*S*)-2-(11-aminoundecanamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl 5-azidopentanoate (12)



To a solution of compound **11** (50.0 mg, 0.073 mmol, 1.0 equiv) in DMSO (2 mL) were added 11-((*tert*-butoxycarbonyl)amino)undecanoic acid (26.4 mg, 0.088 mmol, 1.2 equiv), EDCI (28.0 mg, 0.146 mmol, 2.0 equiv), HOAt (19.9 mg, 0.146 mmol, 2.0 equiv) and NMM (29.3 mg, 0.29 mmol, 4.0 equiv). After being stirred at room temperature for 1 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to

remove the organic solvent under reduced pressure and then dried by lyophilization to afford colorless oil. ESI m/z = 753.4 [M - Boc + 2H] $^{+}$. The obtained oil was dissolved in dichloromethane (2 mL) and TFA (1 mL) was added at room temperature. After being stirred at room temperature for 1 h, the resulting mixture was concentrated and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O) to afford the compound **12** as white solid (34.0 mg, 54% for two steps). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.23 (s, 1H), 7.49 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 5.34 (d, J = 4.4 Hz, 1H), 5.09 – 5.02 (m, 1H), 4.59 (t, J = 8.5 Hz, 1H), 4.52 (s, 1H), 4.18 (d, J = 11.8 Hz, 1H), 3.87 (dd, J = 11.8, 4.0 Hz, 1H), 3.33 (t, J = 6.6 Hz, 2H), 2.93 (t, J = 7.7 Hz, 2H), 2.53 (s, 3H), 2.46 – 2.21 (m, 5H), 2.13 (ddd, J = 13.9, 9.3, 4.8 Hz, 1H), 1.75 – 1.57 (m, 6H), 1.53 (d, J = 7.0 Hz, 3H), 1.45 – 1.30 (m, 14H), 1.07 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.76, 172.87, 171.28, 171.07, 152.57, 145.74, 144.82, 133.26, 129.27, 129.16, 126.41, 73.14, 59.02, 57.90, 53.97, 50.73, 48.82, 39.36, 35.14, 34.64, 34.62, 32.99, 29.11, 29.07, 29.05, 28.96, 28.81, 27.90, 27.19, 26.06, 25.67, 25.64, 21.61, 20.98, 13.60. ESI m/z = 753.6 [M + H]$^{+}$.

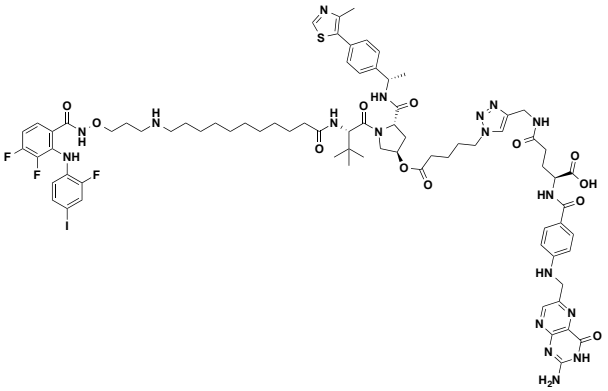
Synthesis of (3*R*,5*S*)-1-((*S*)-20-(*tert*-butyl)-1-(3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)phenyl)-1,18-dioxo-3-oxa-2,7,19-triazahenicosan-21-oyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl 5-azidopentanoate (13)



To a solution of compound **12** (34.0 mg, 0.04 mmol, 1.0 equiv) in MeOH/CH_2Cl_2 (1/2 mL) were added 3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-*N*-(3-oxopropoxy)benzamide (see reference[3] for the details of synthesis) (18.6 mg, 0.04 mmol, 1.0 equiv) and $NaBH_3CN$ (3.8 mg, 0.06 mmol, 1.5 equiv). After being stirred at room temperature for 3 h, the resulting mixture was quenched by water (0.5 mL) and then purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product

containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **13** as light yellow solid (18.1 mg, 34%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.98 (s, 1H), 7.44 – 7.20 (m, 7H), 6.95 (td, J = 9.2, 6.9 Hz, 1H), 6.54 (td, J = 8.8, 4.4 Hz, 1H), 5.25 – 5.18 (m, 1H), 4.91 (q, J = 7.1 Hz, 1H), 4.50 – 4.44 (m, 1H), 4.40 (s, 1H), 4.06 (d, J = 11.5 Hz, 1H), 4.00 – 3.95 (m, 2H), 3.75 (dd, J = 11.8, 4.0 Hz, 1H), 3.19 (t, J = 6.6 Hz, 2H), 3.14 (t, J = 5.9 Hz, 2H), 2.94 (dd, J = 9.0, 6.7 Hz, 2H), 2.40 (s, 3H), 2.32 – 2.22 (m, 3H), 2.22 – 2.10 (m, 2H), 2.01 (ddd, J = 13.9, 9.3, 4.8 Hz, 1H), 1.97 – 1.90 (m, 2H), 1.67 – 1.54 (m, 4H), 1.53 – 1.46 (m, 4H), 1.41 (d, J = 7.0 Hz, 3H), 1.34 – 1.26 (m, 2H), 1.26 – 1.14 (m, 10H), 0.94 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.71, 172.86, 171.26, 171.08, 153.12 (d, J = 247.6 Hz), 152.15, 144.55, 133.26 (d, J = 4.5 Hz), 131.10 (d, J = 10.6 Hz), 129.54, 129.15, 126.33, 126.18, 124.40, 123.93 (d, J = 21.1 Hz), 119.68, 118.69, 110.22 (d, J = 18.1 Hz), 81.18(d, J = 7.6 Hz), 76.36, 73.15, 59.03, 57.89, 53.98, 50.73, 48.81, 48.02, 47.23, 35.16, 34.63, 33.00, 29.18, 29.10, 29.01, 28.84, 27.91, 26.23, 26.04, 25.68, 25.65, 25.60, 24.33, 21.61, 20.97, 13.92. ESI m/z = 1201.8 $[M + H]^+$.

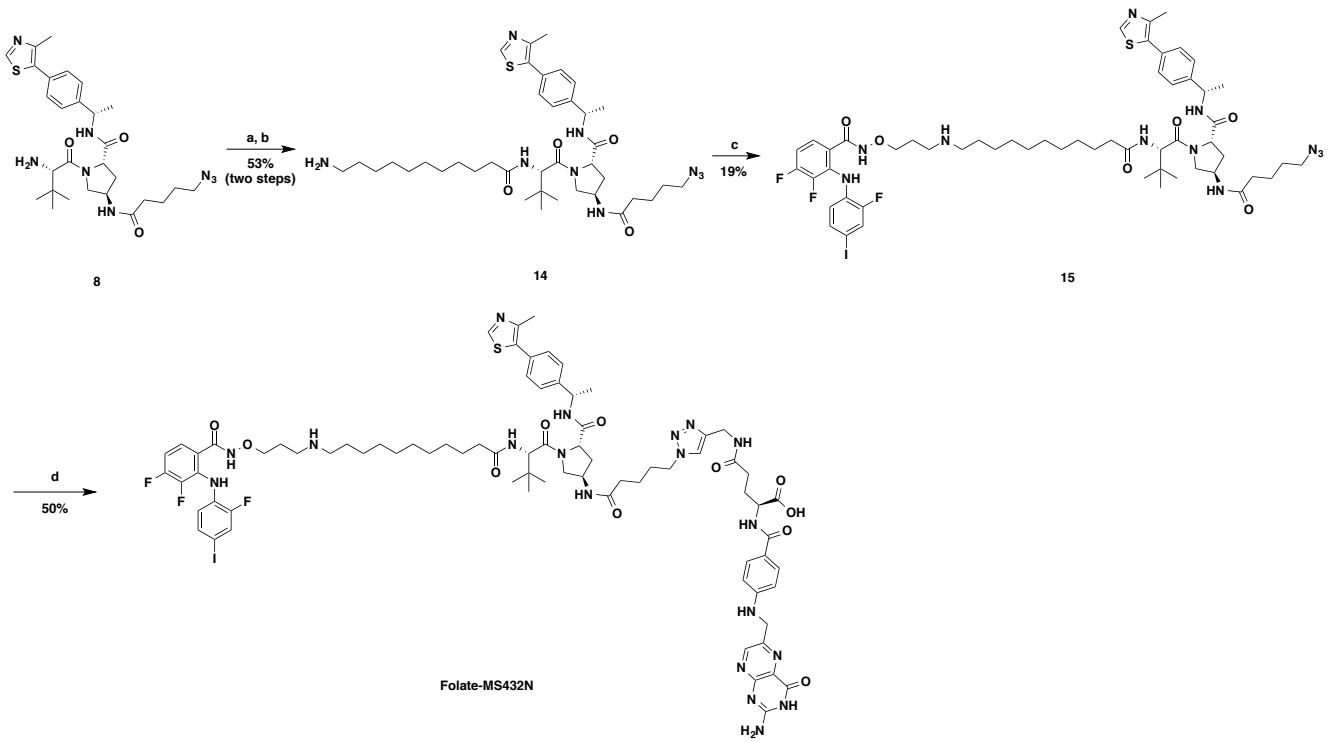
Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-((1-(5-(((3*R*,5*S*)-1-((*S*)-2-(11-((3-((3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)benzamido)oxy)propyl)amino)undecanamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*L*-glutamine (folate-MS432)



To a solution of compound **13** (18.1 mg, 0.014 mmol, 1.0 equiv) in DMF (1.0 mL)/water (0.5 mL) were added compound **4** (10.0 mg, 0.021 mmol, 1.5 equiv), sodium ascorbate (1.1 mg, 0.006 mmol, 0.4

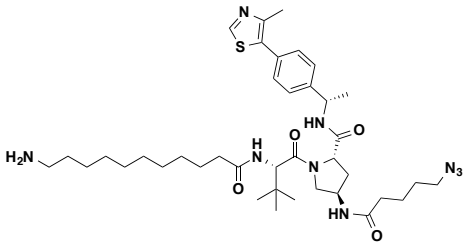
equiv) and $CuSO_4.5H_2O$ (0.7 mg, 0.003 mmol, 0.2 equiv) at room temperature. The reaction mixture was heated to 50 °C. After being stirred at 50 °C for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure at room temperature and then dried by lyophilization to afford **folate-MS432** as light yellow solid (17.0 mg, 68%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.11 (s, 1H), 8.91 (s, 1H), 8.70 – 8.58 (m, 2H), 8.48 (s, 2H), 8.36 (d, J = 7.7 Hz, 1H), 8.28 (t, J = 5.7 Hz, 1H), 8.14 (d, J = 7.6 Hz, 1H), 7.80 (s, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.59 (d, J = 8.5 Hz, 2H), 7.54 – 7.46 (m, 1H), 7.36 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 7.21 – 7.11 (m, 1H), 6.68 – 6.53 (m, 3H), 5.14 (s, 1H), 4.85 (p, J = 7.1 Hz, 1H), 4.45 (s, 2H), 4.39 (t, J = 8.4 Hz, 1H), 4.32 – 4.15 (m, 4H), 3.97 – 3.84 (m, 3H), 3.67 (dd, J = 11.7, 4.0 Hz, 1H), 3.07 – 2.96 (m, 2H), 2.89 – 2.77 (m, 2H), 2.38 (s, 3H), 2.31 – 2.11 (m, 6H), 2.07 – 1.97 (m, 2H), 1.97 – 1.89 (m, 1H), 1.88 – 1.81 (m, 3H), 1.75 – 1.69 (m, 2H), 1.54 – 1.35 (m, 6H), 1.31 (d, J = 7.0 Hz, 3H), 1.26 – 1.06 (m, 14H), 0.88 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.30, 172.98, 172.71, 172.01, 170.41, 170.32, 166.83, 165.37, 160.86, 153.91, 153.85, 152.21, 151.97, 151.17, 150.24, 148.75, 148.22, 145.45, 145.01, 133.69, 132.17, 131.61, 130.21, 129.46, 129.32, 128.42, 126.83, 125.26, 124.17 (d, J = 21.1 Hz), 123.05, 121.86, 121.02, 120.58, 111.69, 110.95 (d, J = 18.1 Hz), 82.57 (d, J = 6.0 Hz), 74.32, 73.53, 58.68, 57.49, 54.00, 52.55, 49.33, 48.30, 47.46, 46.32, 45.50, 40.86, 35.19, 34.96, 34.74, 33.19, 32.26, 29.49, 29.37, 29.29, 29.24, 29.12, 28.98, 26.95, 26.86, 26.40, 26.02, 25.90, 24.83, 22.88, 21.62, 16.42. HRMS (ESI-TOF) calcd for $C_{77}H_{95}F_3IN_{18}O_{12}S^+$ $[M + H]^+$ 1679.6089, found 1679.6082. HPLC > 97%, t_R = 4.59 min.

Supporting Scheme S5. Synthesis of folate-MS432N.



Reaction conditions: (a) 11-((*tert*-butoxycarbonyl)amino)undecanoic acid, EDCI, HOAt, NMM, DMSO,rt, 1 h; (b) TFA/CH_2Cl_2, rt, 1 h; (c) 3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-*N*-(3-oxopropoxy)benzamide, $NaBH_3CN$, MeOH/CH_2Cl_2, rt, 18 h ; (d) **4**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, DMF/H_2O, 50 ºC, 2 h.

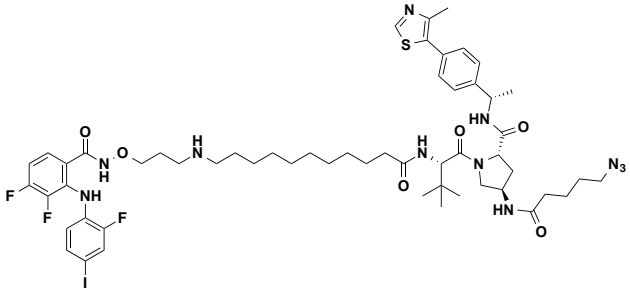
Synthesis of (2*S*,4*R*)-1-((*S*)-2-(11-aminoundecanamido)-3,3-dimethylbutanoyl)-4-(5-azidopentanamido)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (14)



To a solution of compound **8** (34.1 mg, 0.05 mmol, 1.0 equiv) in DMSO (1 mL) were added 11-((*tert*-butoxycarbonyl)amino)undecanoic acid (30.1 mg, 0.10 mmol, 2.0 equiv), EDCI (19.2 mg, 0.10 mmol, 2.0 equiv), HOAt (13.6 mg, 0.10 mmol, 2.0 equiv) and NMM (20.2 mg, 0.20 mmol, 4.0 equiv). After being stirred at room temperature for 1 h, the resulting mixture was purified by preparative HPLC (10%-

100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford colorless oil. ESI m/z = 852.5 $[M + H]^+$. The obtained oil was dissolved in dichloromethane (2 mL) and TFA (1 mL) was added at room temperature. After being stirred at room temperature for 1 h, the resulting mixture was concentrated and purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O) to afford the compound **14** as white solid (23.0 mg, 53% for two steps). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.00 (s, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 5.02 (q, J = 6.9 Hz, 1H), 4.59 (t, J = 7.7 Hz, 1H), 4.49 – 4.40 (m, 2H), 4.00 – 3.92 (m, 1H), 3.88 (dd, J = 10.9, 5.4 Hz, 1H), 3.37 – 3.32 (m, 2H), 2.93 (t, J = 7.7 Hz, 2H), 2.51 (s, 3H), 2.39 – 2.21 (m, 5H), 2.14 – 2.06 (m, 1H), 1.77 – 1.57 (m, 6H), 1.52 (d, J = 7.0 Hz, 3H), 1.45 – 1.31 (m, 14H), 1.09 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 175.12, 174.30, 171.37, 171.23, 152.63, 145.73, 144.84, 133.30, 129.18, 129.10, 126.40, 58.51, 53.15, 50.71, 49.27, 48.77, 39.37, 34.89, 34.86, 34.58, 34.16, 29.13, 29.10, 28.97, 28.83, 28.02, 27.20, 26.08, 25.66, 25.54, 22.64, 20.99, 13.58. ESI m/z = 752.6 $[M + H]^+$.

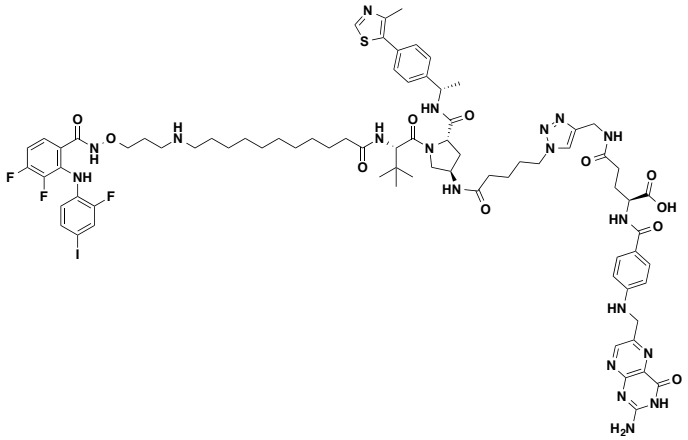
Synthesis of (2*S*,4*R*)-4-(5-azidopentanamido)-1-((*S*)-20-(*tert*-butyl)-1-(3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)phenyl)-1,18-dioxo-3-oxa-2,7,19-triazahenicosan-21-oyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (15)



To a solution of compound **14** (17.3 mg, 0.02 mmol, 1.0 equiv) in MeOH/CH_2Cl_2 (0.5/1.0 mL) were added 3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-*N*-(3-oxopropoxy)benzamide (9.2 mg, 0.02 mmol, 1.0 equiv) and $NaBH_3CN$ (2.5 mg, 0.04 mmol, 2.0 equiv). After being stirred at room temperature for 18 h, the resulting mixture was quenched by water (0.5 mL) and then purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound

15 as light yellow solid (5.0 mg, 19%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.97 (s, 1H), 7.54 – 7.36 (m, 7H), 7.13 – 7.05 (m, 1H), 6.66 (td, J = 8.8, 4.4 Hz, 1H), 5.02 (q, J = 6.8 Hz, 1H), 4.59 (t, J = 7.7 Hz, 1H), 4.46 – 4.41 (m, 2H), 4.13 – 4.07 (m, 2H), 3.96 (dd, J = 10.7, 3.5 Hz, 1H), 3.87 (dd, J = 10.9, 5.5 Hz, 1H), 3.31 (t, J = 6.7 Hz, 2H), 3.26 (t, J = 5.8 Hz, 2H), 3.10 – 3.03 (m, 2H), 2.51 (s, 3H), 2.38 – 2.22 (m, 5H), 2.16 – 2.08 (m, 1H), 2.07 – 2.02 (m, 2H), 1.79 – 1.56 (m, 8H), 1.52 (d, J = 7.0 Hz, 3H), 1.45 – 1.28 (m, 12H), 1.08 (s, 9H). ESI m/z = 1200.2 $[M + H]^+$.

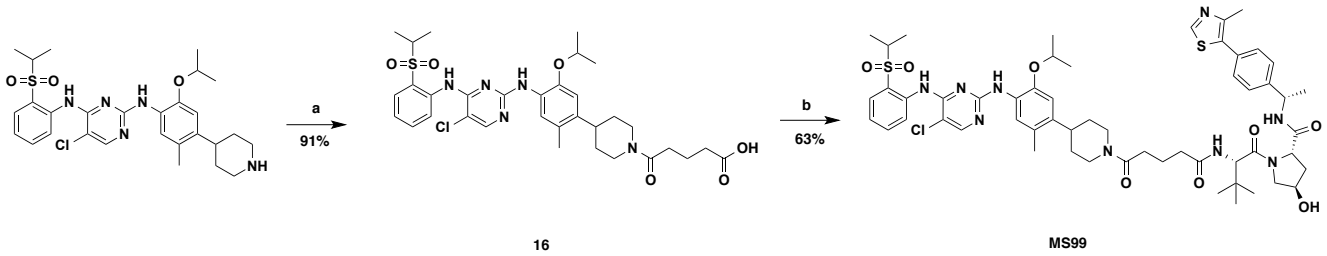
Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-((1-(5-(((3*R*,5*S*)-1-((*S*)-2-(11-((3-((3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)benzamido)oxy)propyl)amino)undecanamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)amino)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*L*-glutamine (folate-MS432N)



To a solution of compound **15** (5.0 mg, 0.004 mmol, 1.0 equiv) in DMF (0.8 mL)/water (0.4 mL) were added compound **4** (2.8 mg, 0.006 mmol, 1.5 equiv), sodium ascorbate (1.2 mg, 0.006 mmol, 1.5 equiv) and $CuSO_4.5H_2O$ (1.0 mg, 0.004 mmol, 1.0 equiv) at room temperature. The reaction mixture was heated to 50 °C. After being stirred at 50 °C for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford **folate-MS432N** as light yellow solid (3.6 mg, 50%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.14 (s, 1H), 8.99 (s, 1H), 8.66 (s, 1H), 8.46 – 8.37 (m, 2H), 8.35 – 8.29 (m, 2H), 8.20 (d, J = 7.6 Hz, 1H), 8.03 (d, J = 6.7 Hz,

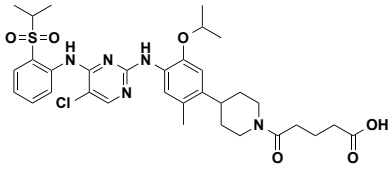
1H), 7.89 – 7.81 (m, 2H), 7.73 – 7.63 (m, 2H), 7.58 (dd, *J* = 10.8, 2.0 Hz, 1H), 7.49 – 7.33 (m, 6H), 7.25 (q, *J* = 8.7 Hz, 1H), 6.71 – 6.61 (m, 3H), 4.91 (p, *J* = 7.2 Hz, 1H), 4.56 – 4.46 (m, 3H), 4.40 (d, *J* = 6.0 Hz, 1H), 4.33 – 4.22 (m, 5H), 3.93 (t, *J* = 5.7 Hz, 2H), 3.81 – 3.73 (m, 1H), 3.12 – 3.04 (m, 2H), 2.94 – 2.86 (m, 2H), 2.45 (s, 3H), 2.31 – 2.19 (m, 3H), 2.15 – 1.86 (m, 9H), 1.80 – 1.72 (m, 2H), 1.61 – 1.40 (m, 6H), 1.37 (d, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 8.8 Hz, 14H), 0.96 (s, 9H). HRMS (ESI-TOF) calcd for $C_{77}H_{96}F_3IN_{19}O_{11}S^+$ $[M + H]^+$ 1678.6249, found 1678.6233. HPLC > 97%, t_R = 4.61 min.

Supporting Scheme S6. Synthesis of MS99.



Reaction conditions: (a) glutaric acid, EDCI, HOAt, NMM, DMSO, rt, 1 h; (b) (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide hydrochloride, EDCI, HOAt, NMM, DMSO, 18 h.

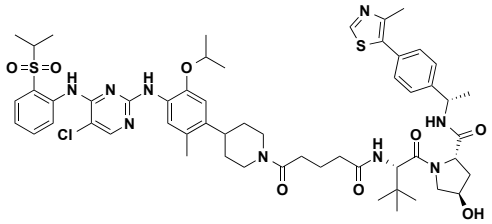
Synthesis of 5-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-5-oxopentanoic acid (16)



To a solution of 5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (Ceritinib) (111.6 mg, 0.2 mmol, 1.0 equiv) in DMSO (3 mL) were added glutaric acid (132.0 mg, 1.0 mmol, 5.0 equiv), EDCI (76.7 mg, 0.4 mmol, 2.0 equiv), HOAt (54.4 mg, 0.4 mmol, 2.0 equiv) and NMM (202.0 mg, 2.0 mmol, 10 equiv). After being stirred at room temperature for 1 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **16** as white solid (123.0 mg, 91%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.36 (d, J = 8.1 Hz, 1H), 8.21 (s, 1H), 7.98 (dd, J = 7.9, 1.6 Hz, 1H), 7.81 – 7.65 (m, 1H), 7.54 – 7.46 (m, 1H), 7.45 (s, 1H), 6.90 (s, 1H), 4.77 – 4.70 (m, 1H), 4.68 – 4.59 (m, 1H), 4.20 – 4.10 (m, 1H), 3.45 – 3.34 (m, 1H), 3.25 (td, J = 13.2, 2.6 Hz, 1H), 3.05 (tt, J = 12.1, 3.5 Hz, 1H), 2.75 (td, J = 13.0, 2.7 Hz, 1H), 2.60 – 2.47 (m, 2H), 2.42 (t, J = 7.2 Hz, 2H), 2.22 (s, 3H), 1.94 (p, J = 7.3 Hz, 2H), 1.89 – 1.77 (m, 2H), 1.69 (qd, J = 12.6, 4.1 Hz, 1H), 1.59 (qd, J = 12.7, 4.2 Hz, 1H), 1.30 – 1.25 (m, 12H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 175.48, 171.82, 160.85, 157.25, 153.60, 148.12, 145.42, 141.60, 136.48, 134.84, 131.29, 127.33, 127.12, 125.58, 125.20,

124.02, 111.68, 105.56, 71.25, 55.61, 46.26, 42.34, 38.20, 32.79, 32.72, 32.09, 31.90, 21.00, 20.51, 17.61, 14.13. ESI m/z = 672.4 $[M + H]^+$.

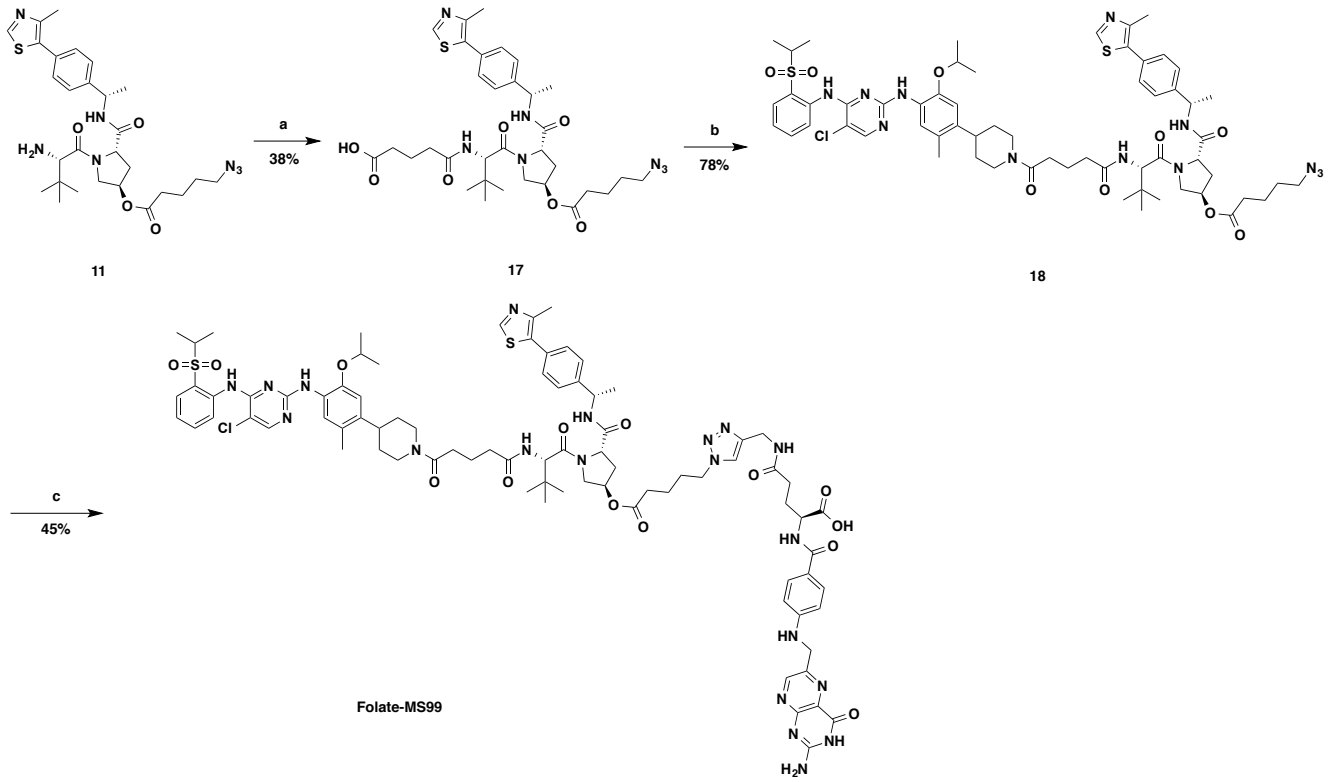
Synthesis of (2*S*,4*R*)-1-((*S*)-2-(5-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-5-oxopentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (MS99)



To a solution of compound **16** (25.0 mg, 0.037 mmol, 1.0 equiv) in DMSO (2 mL) were added (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide hydrochloride (132.0 mg, 0.042 mmol, 1.1 equiv), EDCI (14.2 mg, 0.074 mmol, 2.0 equiv), HOAt (10.1 mg, 0.074 mmol, 2.0 equiv) and NMM (15.0 mg, 0.149 mmol, 4 equiv). After being stirred at room temperature for 18 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford **MS99** as white solid (25.6 mg, 63%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.02 (s, 1H), 8.32 (s, 1H), 8.23 (s, 1H), 8.01 (dd, J = 8.0, 1.6 Hz, 1H), 7.75 – 7.70 (m, 1H), 7.54 (t, J = 7.7 Hz, 1H), 7.46 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.41 – 7.31 (m, 1H), 6.92 (s, 1H), 5.06 – 4.98 (m, 1H), 4.73 (d, J = 13.0 Hz, 1H), 4.68 – 4.62 (m, 2H), 4.60 – 4.56 (m, 1H), 4.45 (s, 1H), 4.13 (d, J = 14.1 Hz, 1H), 3.91 (d, J = 11.0 Hz, 1H), 3.77 (dd, J = 11.0, 4.0 Hz, 1H), 3.40 (p, J = 6.8 Hz, 1H), 3.25 (t, J = 12.6 Hz, 1H), 3.06 (t, J = 12.1 Hz, 1H), 2.76 (t, J = 12.7 Hz, 1H), 2.50 (s, 3H), 2.44 – 2.34 (m, 3H), 2.27 – 2.12 (m, 4H), 2.06 – 1.92 (m, 3H), 1.87 (d, J = 12.9 Hz, 1H), 1.82 (d, J = 13.2 Hz, 1H), 1.75 – 1.64 (m, 1H), 1.63 – 1.57 (m, 2H), 1.56 – 1.47 (m, 3H), 1.33 – 1.24 (m, 12H), 1.08 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 173.96, 171.84, 171.15, 170.93, 157.67, 152.98, 151.98, 148.66, 146.83, 145.03, 144.56, 143.76, 142.29, 136.22, 134.83, 132.57, 131.36, 129.64, 129.10, 129.01, 127.79, 127.52, 126.31, 126.03,

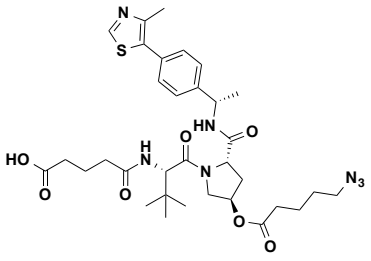
125.67, 111.84, 105.63, 71.24, 69.58, 59.18, 57.77, 56.58, 55.60, 48.74, 48.47, 46.25, 42.28, 38.26, 37.39, 35.03, 34.49, 32.80, 32.06, 32.00, 25.71, 21.45, 20.99, 20.93, 17.50, 14.07. HRMS (ESI-TOF) calcd for $C_{56}H_{73}ClN_9O_8S_2^+$ $[M + H]^+$ 1098.4707, found 1098.4723.

Supporting Scheme S7. Synthesis of folate-MS99.



Reaction conditions: (a) glutaric acid, EDCI, HOAt, NMM, DMSO, rt, 1 h; (b) Ceritinib, EDCI, HOAt, NMM, DMSO, 2 h. (c) **4**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, DMF/H_2O, 50 °C, 2 h.

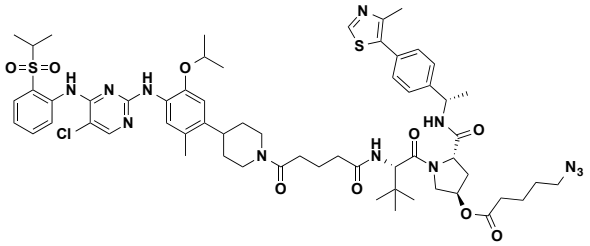
Synthesis of 5-(((*S*)-1-((2*S*,4*R*)-4-((5-azidopentanoyl)oxy)-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-5-oxopentanoic acid (17)



To a solution of compound **11** (50.0 mg, 0.073 mmol, 1.0 equiv) in DMSO (2.5 mL) were added glutaric acid (58.1 mg, 0.44 mmol, 6.0 equiv), EDCI (21.1 mg, 0.11 mmol, 1.5 equiv), HOAt (15.0 mg,

0.11 mmol, 1.5 equiv) and NMM (73.7 mg, 0.73 mmol, 10 equiv). After being stirred at room temperature for 1 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **17** as white solid (19.0 mg, 38%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.19 (s, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 5.38 – 5.30 (m, 1H), 5.03 (q, J = 7.1 Hz, 1H), 4.66 – 4.57 (m, 1H), 4.52 (s, 1H), 4.20 (d, J = 11.7 Hz, 1H), 3.87 (dd, J = 11.8, 4.0 Hz, 1H), 3.33 (t, J = 6.6 Hz, 2H), 2.53 (s, 3H), 2.46 – 2.29 (m, 7H), 2.14 (ddd, J = 13.9, 9.3, 4.8 Hz, 1H), 1.90 (p, J = 7.4 Hz, 2H), 1.75 – 1.66 (m, 2H), 1.66 – 1.59 (m, 2H), 1.52 (d, J = 7.0 Hz, 3H), 1.07 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 175.35, 173.90, 172.97, 171.28, 171.11, 152.50, 145.92, 144.79, 133.10, 129.20, 129.16, 126.41, 73.17, 59.02, 58.04, 53.99, 50.74, 48.81, 34.64, 34.53, 34.11, 33.01, 32.74, 27.89, 25.66, 22.85, 21.62, 20.97, 13.71. ESI m/z = 684.5 $[M + H]^+$.

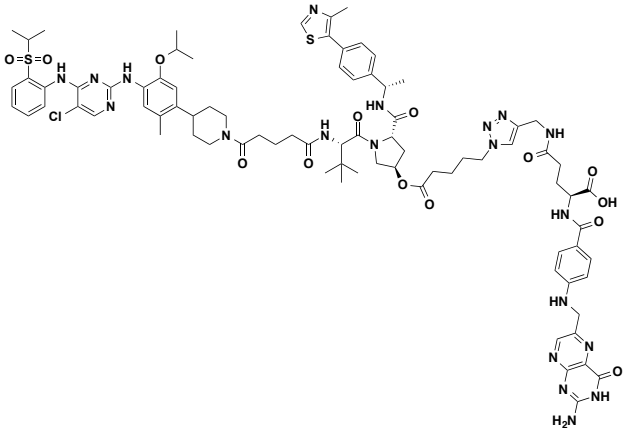
Synthesis of (3*R*,5*S*)-1-((*S*)-2-(5-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-5-oxopentanamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl 5-azidopentanoate (18)



To a solution of compound **17** (19.0 mg, 0.028 mmol, 1.0 equiv) in DMSO (1 mL) were added Ceritinib (18.6 mg, 0.033 mmol, 1.2 equiv), EDCI (10.7 mg, 0.056 mmol, 2.0 equiv), HOAt (7.6 mg, 0.056 mmol, 2.0 equiv) and NMM (11.3 mg, 0.112 mmol, 4 equiv). After being stirred at room temperature for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **18** as white solid (27.0 mg, 78%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.85 (s, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.11 (s, 1H), 7.88 (dd, J = 8.0, 1.6 Hz, 1H), 7.60 (t, J = 7.9 Hz, 1H), 7.40 (t, J = 7.7 Hz, 1H), 7.37 – 7.26 (m, 5H), 6.79 (d, J =

6.4 Hz, 1H), 5.27 – 5.18 (m, 1H), 4.96 – 4.86 (m, 1H), 4.66 – 4.60 (m, 1H), 4.56 – 4.50 (m, 1H), 4.50 – 4.46 (m, 1H), 4.40 (s, 1H), 4.08 (d, *J* = 11.8 Hz, 1H), 4.02 (d, *J* = 13.9 Hz, 1H), 3.83 – 3.73 (m, 1H), 3.32 – 3.25 (m, 1H), 3.20 (t, *J* = 6.5 Hz, 2H), 3.15 (tdd, *J* = 13.2, 5.3, 2.6 Hz, 1H), 2.95 (tt, *J* = 12.1, 3.5 Hz, 1H), 2.65 (tt, *J* = 12.9, 2.7 Hz, 1H), 2.39 (s, 3H), 2.33 – 2.22 (m, 6H), 2.11 (s, 3H), 2.08-2.00 (m, 2H), 1.88 – 1.79 (m, *J* = 7.2 Hz, 2H), 1.78 – 1.68 (m, 2H), 1.63 – 1.54 (m, 3H), 1.53 – 1.44 (m, 3H), 1.44 – 1.37 (m, 3H), 1.23 – 1.14 (m, 12H), 0.98 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.00, 172.89, 171.73, 171.22, 171.08, 160.30, 157.49, 153.11, 152.01, 148.44, 146.87, 144.96, 144.41, 142.02, 136.30, 134.84, 132.67, 131.35, 129.69, 129.13, 129.07, 127.44, 126.35, 125.86, 125.41, 123.81, 111.74, 105.59, 73.19, 71.26, 59.05, 58.12, 55.62, 53.98, 50.76, 48.78, 46.25, 42.30, 38.25, 34.59, 34.56, 34.41, 33.02, 32.89, 32.82, 32.12, 32.06, 27.91, 25.74, 21.63, 21.06, 20.98, 17.61, 14.17, 14.13. ESI m/z = 1223.6 $[M + H]^+$.

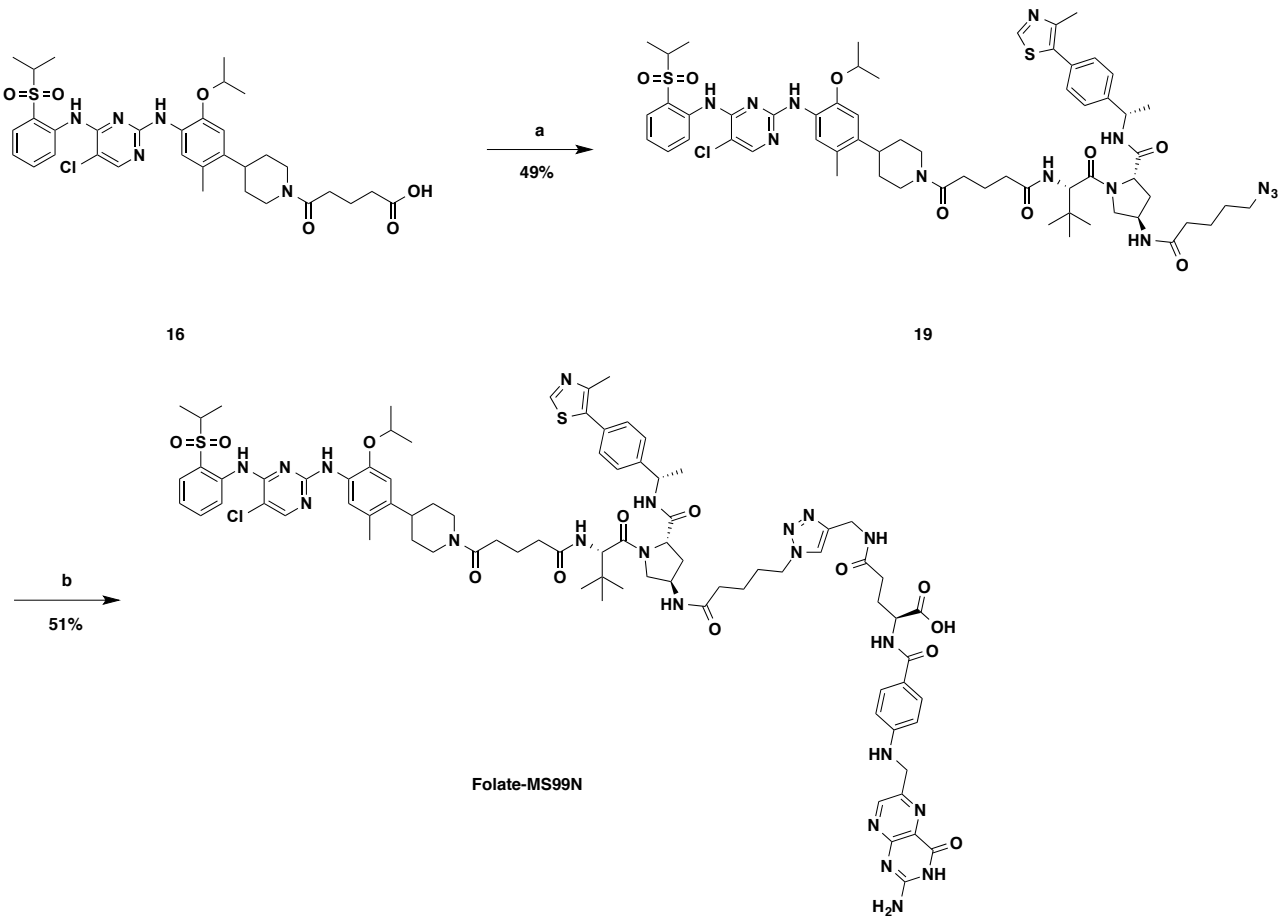
Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-((1-(5-(((3*R*,5*S*)-1-((*S*)-2-(5-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-5-oxopentanamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*L*-glutamine (folate-MS99)



To a solution of compound **18** (31.0 mg, 0.025 mmol, 1.0 equiv) in DMF (2.0 mL)/water (1.0 mL) were added compound **4** (14.4 mg, 0.030 mmol, 1.2 equiv), sodium ascorbate (2.0 mg, 0.010 mmol, 0.4 equiv) and $CuSO_4.5H_2O$ (1.2 mg, 0.005 mmol, 0.2 equiv) at room temperature. The reaction mixture

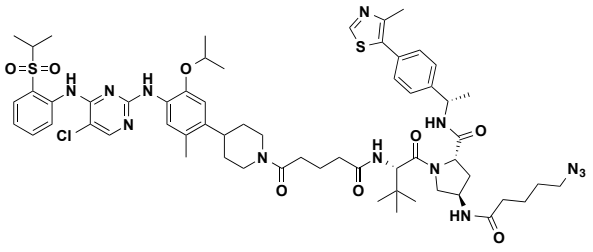
was heated to 50 °C. After being stirred at 50 °C for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford **folate-MS99** as light yellow solid (19.2 mg, 45%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.92 (s, 1H), 8.64 (s, 1H), 8.38 – 8.21 (m, 5H), 8.14 (d, J = 7.6 Hz, 1H), 7.85 – 7.76 (m, 3H), 7.68 – 7.56 (m, 3H), 7.40 (s, 1H), 7.39 – 7.26 (m, 5H), 6.75 (d, J = 8.7 Hz, 1H), 6.58 (d, J = 8.4 Hz, 2H), 5.13 (s, 1H), 4.86 – 4.81 (m, 1H), 4.53 – 4.44 (m, 5H), 4.39 (t, J = 8.4 Hz, 1H), 4.32 – 4.14 (m, 5H), 3.92 (d, J = 11.7 Hz, 1H), 3.88 (d, J = 12.9 Hz, 1H), 3.71 – 3.63 (m, 1H), 3.37 (p, J = 6.8 Hz, 1H), 3.03 (t, J = 12.7 Hz, 1H), 2.87 – 2.76 (m, 1H), 2.52 (t, J = 12.8 Hz, 1H), 2.38 (s, 3H), 2.33 – 2.10 (m, 8H), 2.10 – 1.98 (m, 5H), 1.96 – 1.81 (m, 2H), 1.78 – 1.56 (m, 6H), 1.55 – 1.34 (m, 4H), 1.34 – 1.26 (m, 3H), 1.17 – 1.05 (m, 12H), 0.89 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.29, 172.86, 172.75, 172.00, 170.46, 170.40, 170.35, 166.79, 160.37, 157.31, 155.90, 153.75, 153.49, 152.00, 151.11, 151.01, 148.56, 148.19, 147.21, 145.43, 145.02, 139.85, 138.11, 135.35, 131.65, 131.48, 130.18, 129.45, 129.32, 128.45, 127.04, 126.97, 126.82, 126.73, 125.82, 124.85, 124.71, 124.42, 123.07, 121.91, 112.30, 111.70, 104.82, 73.56, 71.14, 58.70, 57.71, 55.24, 54.02, 52.57, 49.35, 48.29, 46.27, 46.11, 42.19, 40.49, 38.21, 34.88, 34.85, 34.72, 33.20, 33.10, 32.47, 32.27, 29.47, 26.94, 26.86, 26.78, 22.88, 22.31, 21.88, 21.62, 18.88, 16.40, 15.28. HRMS (ESI-TOF) calcd for $C_{83}H_{102}ClN_{20}O_{14}S_2^+$ $[M + H]^+$ 1701.7009, found 1701.7003. HPLC > 98%, t_R = 4.90 min.

Supporting Scheme S8. Synthesis of folate-MS99N.



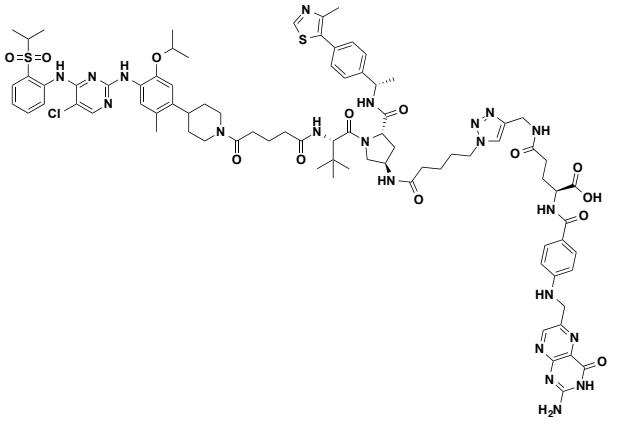
Reaction conditions: (a) **8**, EDCI, HOAt, NMM, DMSO, rt, 18 h; (b) **4**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, DMF/H_2O, 50 ºC, 2 h.

Synthesis of (2*S*,4*R*)-4-(5-azidopentanamido)-1-((*S*)-2-(5-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-5-oxopentanamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (19)



To a solution of compound **16** (33.6 mg, 0.05 mmol, 1.0 equiv) in DMSO (2.5 mL) were added compound **8** (35.8 mg, 0.053 mmol, 1.05 equiv), EDCI (19.2 mg, 0.10 mmol, 2.0 equiv), HOAt (13.6 mg, 0.10 mmol, 2.0 equiv) and NMM (20.2 mg, 0.20 mmol, 4 equiv). After being stirred at room temperature for 18 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford compound **19** as white solid (30.1 mg, 49%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.96 (s, 1H), 8.34 (s, 1H), 8.21 (s, 1H), 8.03 – 7.92 (m, 1H), 7.74 – 7.64 (m, 1H), 7.55 – 7.33 (m, 6H), 6.88 (d, J = 5.6 Hz, 1H), 5.04 – 4.95 (m, 1H), 4.74 (d, J = 12.7 Hz, 1H), 4.67 – 4.56 (m, 2H), 4.45 (s, 1H), 4.14 (d, J = 13.4 Hz, 1H), 4.01 – 3.92 (m, 1H), 3.92 – 3.82 (m, 1H), 3.44 – 3.20 (m, 4H), 3.10 – 2.99 (m, 1H), 2.75 (t, J = 12.5 Hz, 1H), 2.60 – 2.33 (m, 8H), 2.32 – 2.16 (m, 6H), 2.12 (dt, J = 13.4, 7.2 Hz, 1H), 2.02 – 1.90 (m, 2H), 1.89 – 1.77 (m, 2H), 1.73 – 1.54 (m, 6H), 1.52 – 1.44 (m, 3H), 1.35 – 1.18 (m, 12H), 1.10 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.45, 174.25, 171.78, 171.26, 171.21, 160.36, 157.25, 153.83, 151.76, 147.25, 145.82, 144.27, 144.22, 141.22, 136.57, 134.80, 131.30, 129.91, 129.58, 129.12, 129.07, 127.31, 126.26, 125.55, 125.35, 111.70, 105.52, 71.27, 58.69, 58.47, 55.56, 53.15, 50.72, 49.33, 48.72, 48.23, 46.24, 42.29, 38.21, 34.89, 34.51, 34.06, 32.84, 32.02, 28.00, 25.68, 22.64, 21.43, 21.05, 21.00, 20.95, 17.58, 14.27, 14.09. ESI m/z = 1222.5 $[M + H]^+$.

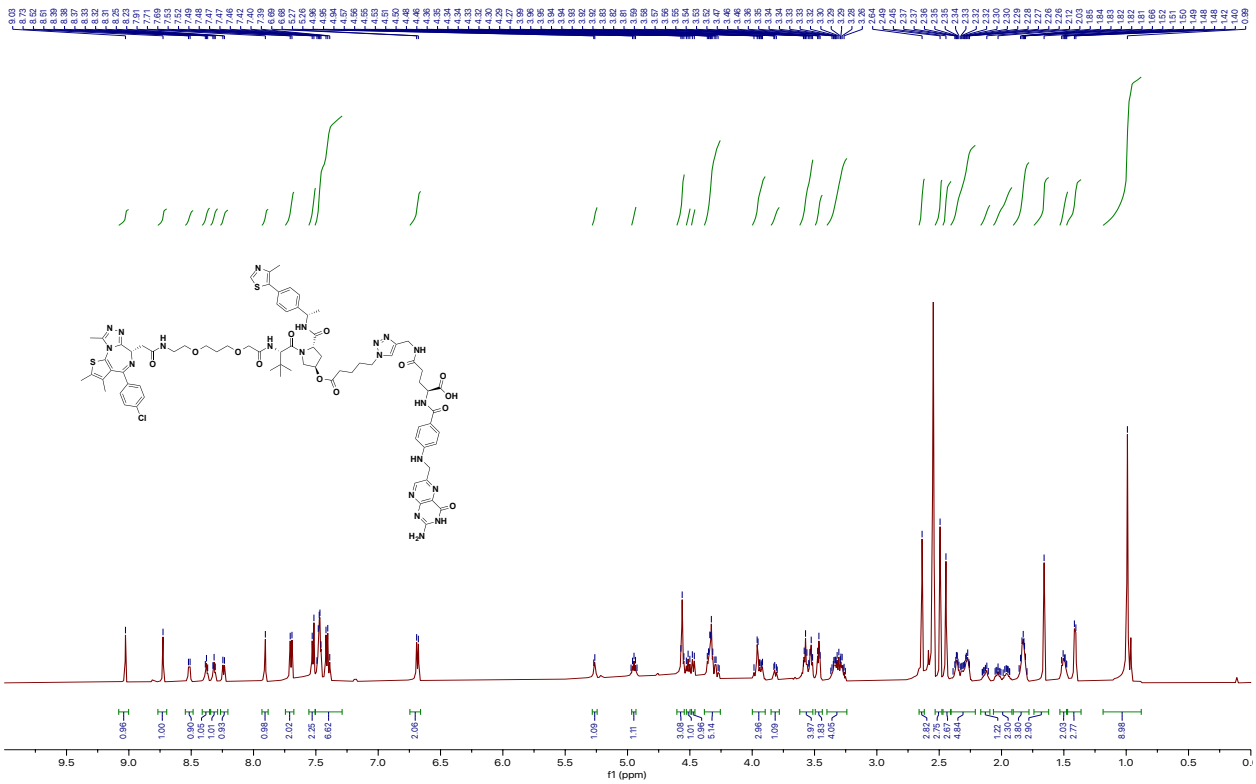
Synthesis of N^2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-N^5-((1-(5-(((3*R*,5*S*)-1-((*S*)-2-(5-(4-(4-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-5-isopropoxy-2-methylphenyl)piperidin-1-yl)-5-oxopentanamido)-3,3-dimethylbutanoyl)-5-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)amino)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*L*-glutamine (folate-MS99N)



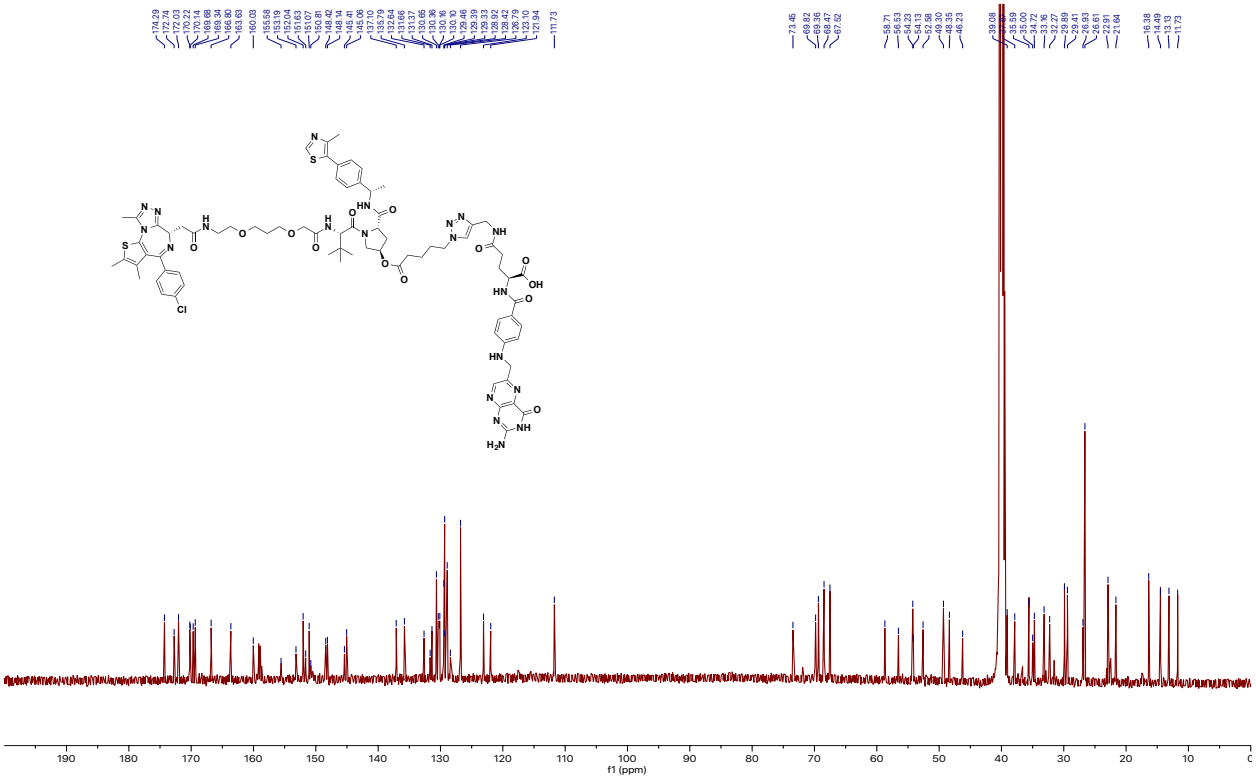
To a solution of compound **19** (24.4 mg, 0.02 mmol, 1.0 equiv) in DMF (2.0 mL)/water (1.0 mL) were added compound **4** (14.4 mg, 0.03 mmol, 1.5 equiv), sodium ascorbate (5.9 mg, 0.03 mmol, 1.5 equiv) and $CuSO_4.5H_2O$ (5.0 mg, 0.02 mmol, 1.0 equiv) at room temperature. The reaction mixture was heated to 50 °C. After being stirred at 50 °C for 2 h, the resulting mixture was purified by preparative HPLC (10%-100% acetonitrile / 0.1% TFA in H_2O). The product containing fractions were concentrated to remove the organic solvent under reduced pressure and then dried by lyophilization to afford **folate-MS99N** as light yellow solid (17.5 mg, 51%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.92 (s, 1H), 8.63 (s, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.29 – 8.17 (m, 4H), 8.13 (d, J = 7.6 Hz, 1H), 7.96 (dd, J = 6.7, 2.5 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 7.81 – 7.74 (m, 2H), 7.66 – 7.53 (m, 3H), 7.41 (s, 1H), 7.38 – 7.27 (m, 5H), 6.76 (d, J = 6.5 Hz, 1H), 6.58 (d, J = 8.7 Hz, 2H), 4.88 – 4.79 (m, 1H), 4.54 – 4.47 (m, 2H), 4.46 (s, 2H), 4.44 – 4.41 (m, 1H), 4.34 (dd, J = 8.3, 4.5 Hz, 1H), 4.26 – 4.12 (m, 6H), 3.92 – 3.84 (m, 1H), 3.72 – 3.64 (m, 1H), 3.55 – 3.49 (m, 1H), 3.42 – 3.31 (m, 1H), 3.04 (t, J = 12.7 Hz, 1H), 2.89 – 2.76 (m, 1H), 2.58 – 2.51 (m, 1H), 2.38 (s, 3H), 2.31 – 2.12 (m, 6H), 2.09 – 1.94 (m, 7H), 1.90 – 1.79 (m, 2H), 1.74 – 1.57 (m, 6H), 1.55 – 1.45 (m, 1H), 1.43 – 1.33 (m, 3H), 1.32 – 1.25 (m, 3H), 1.18 – 1.05 (m, 12H), 0.90 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.29, 173.02, 172.26, 172.02, 170.69, 170.48, 170.26, 166.80, 159.91, 156.38, 153.17, 152.03, 151.84, 151.06, 150.28, 148.36, 148.13, 147.33, 145.39, 145.05, 140.24, 137.82, 135.37, 131.69, 131.54, 130.16, 129.58, 129.46, 129.32, 128.42, 127.06, 127.02, 126.81, 126.52, 126.26, 125.38, 125.20, 124.57, 123.08, 121.96, 112.31, 111.74, 104.88, 71.19, 71.17, 58.38, 57.55, 57.49, 55.21, 53.17, 52.58, 49.46, 48.63, 48.25, 46.23, 46.11, 42.20, 40.45, 38.23, 34.91, 34.87, 34.73, 33.08, 32.45, 32.28, 29.78, 26.93, 26.85, 22.83, 22.54, 22.27, 21.82, 18.85, 16.36,

15.26. HRMS calcd for $C_{83}H_{103}ClN_{21}O_{13}S_2^+$ $[M + H]^+$ 1700.7169, found 1700.7161. HPLC > 97%, t_R = 4.87 min.

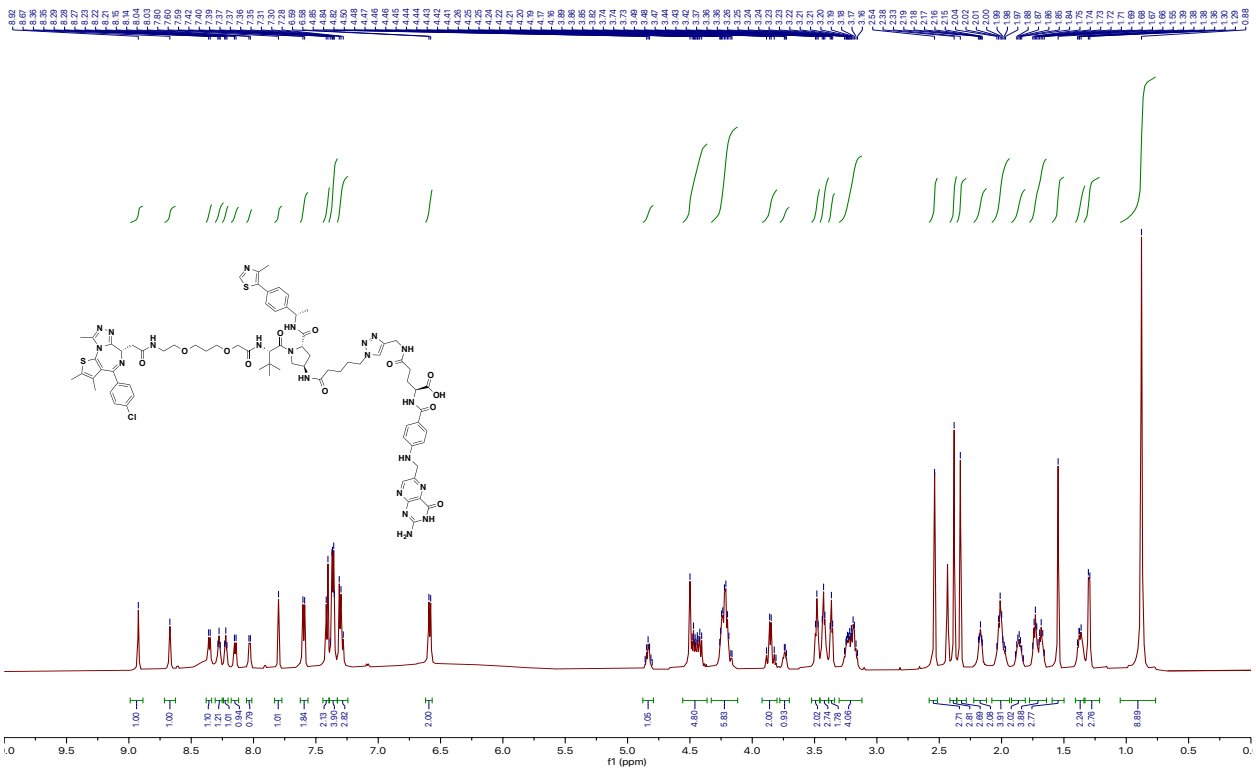
^{1}H-NMR of folate-ARV-771 in DMSO-d_6



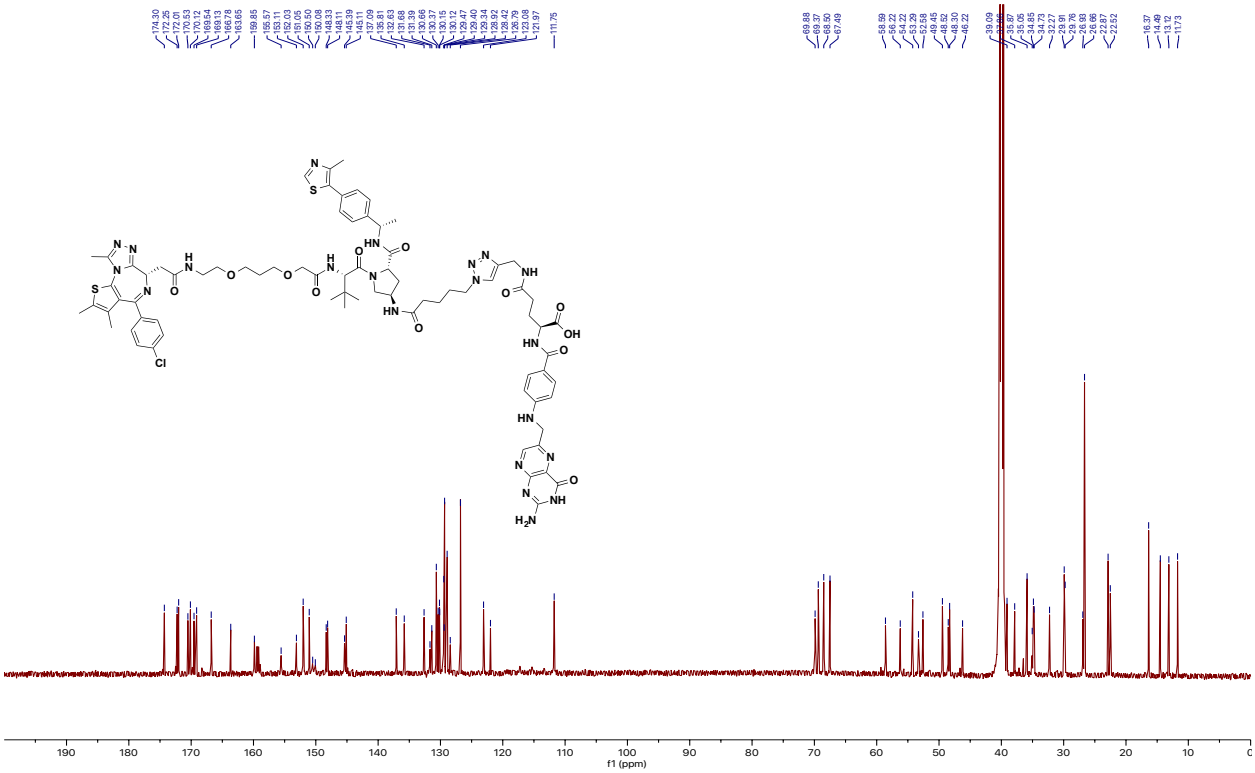
^{13}C-NMR of folate-ARV771 in DMSO-d_6



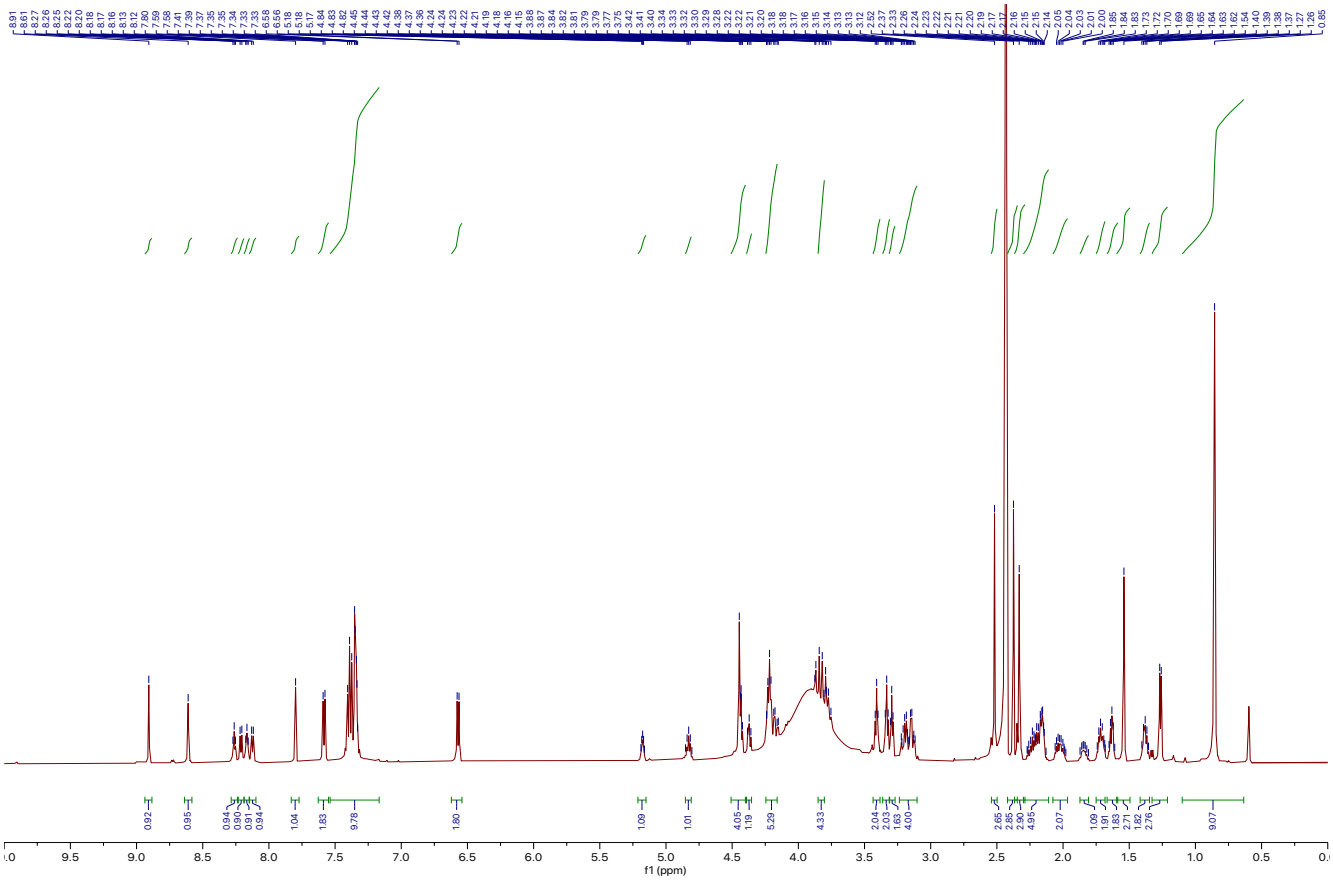
^{1}H-NMR of folate-ARV-771N in DMSO-d_6



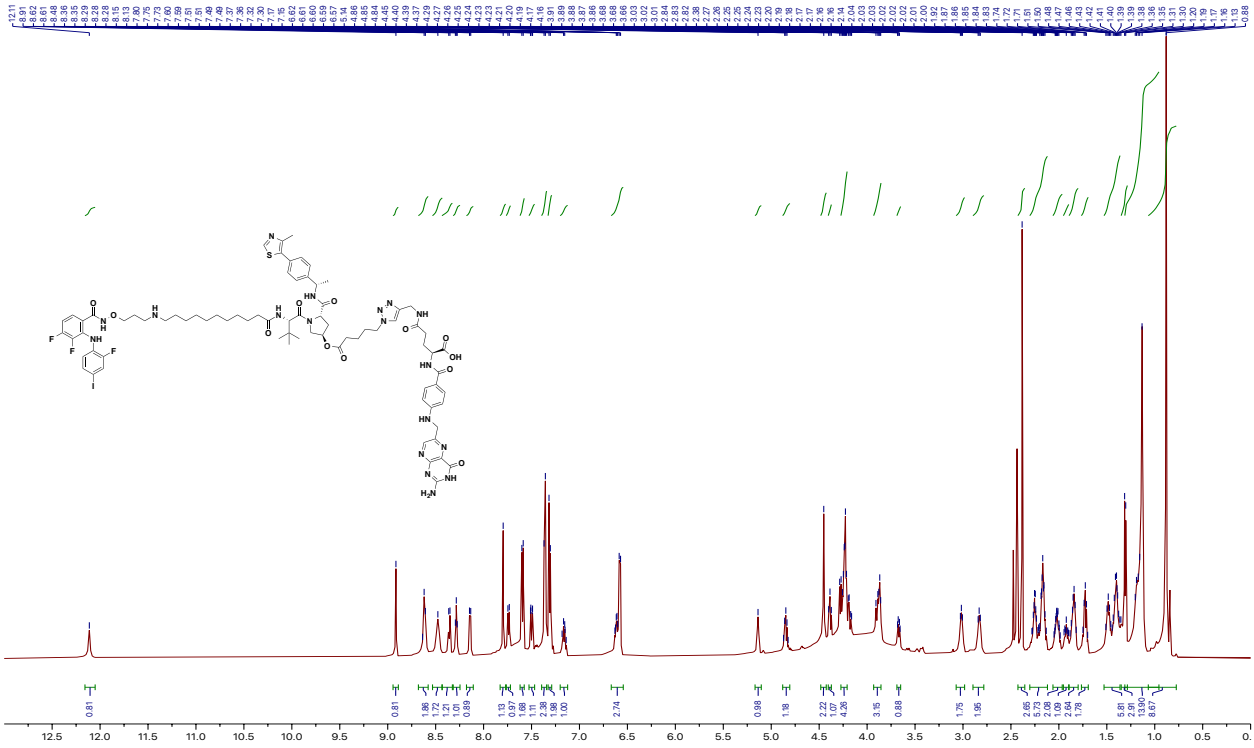
^{13}C-NMR of folate-ARV-771N in DMSO-d_6



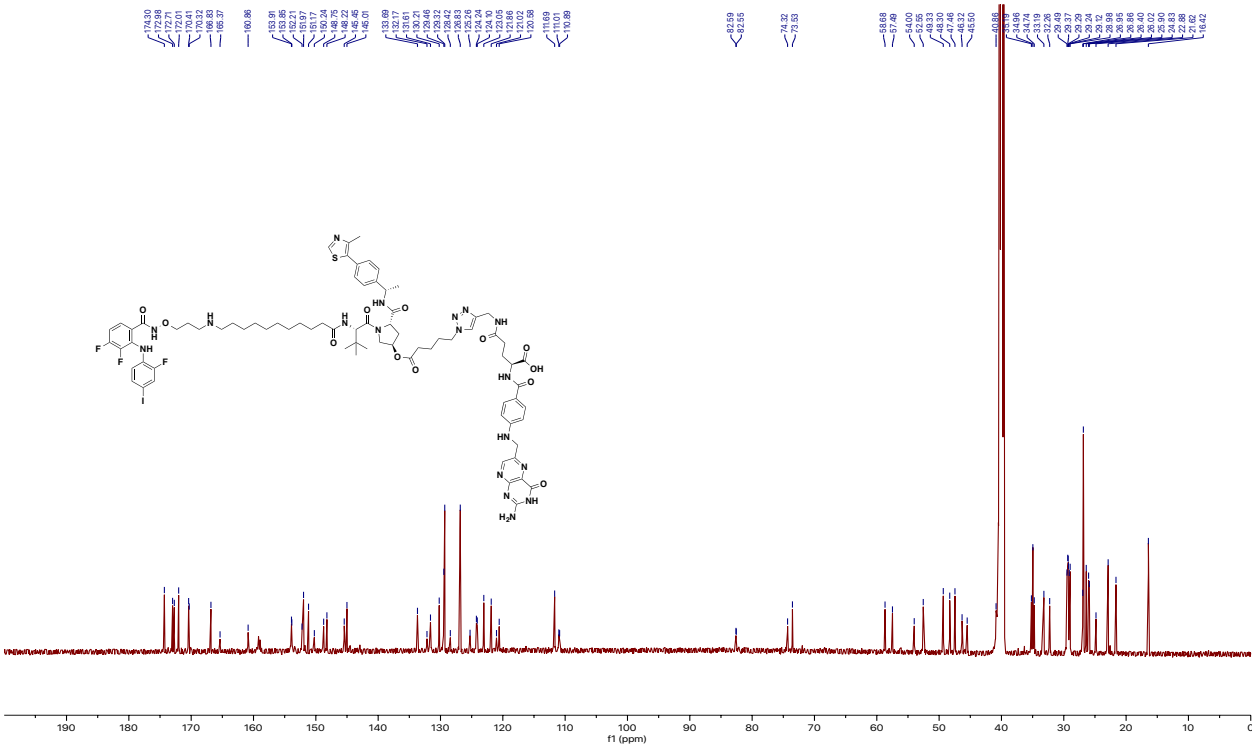
^{1}H-NMR of folate-ARV-766 in DMSO-d_6



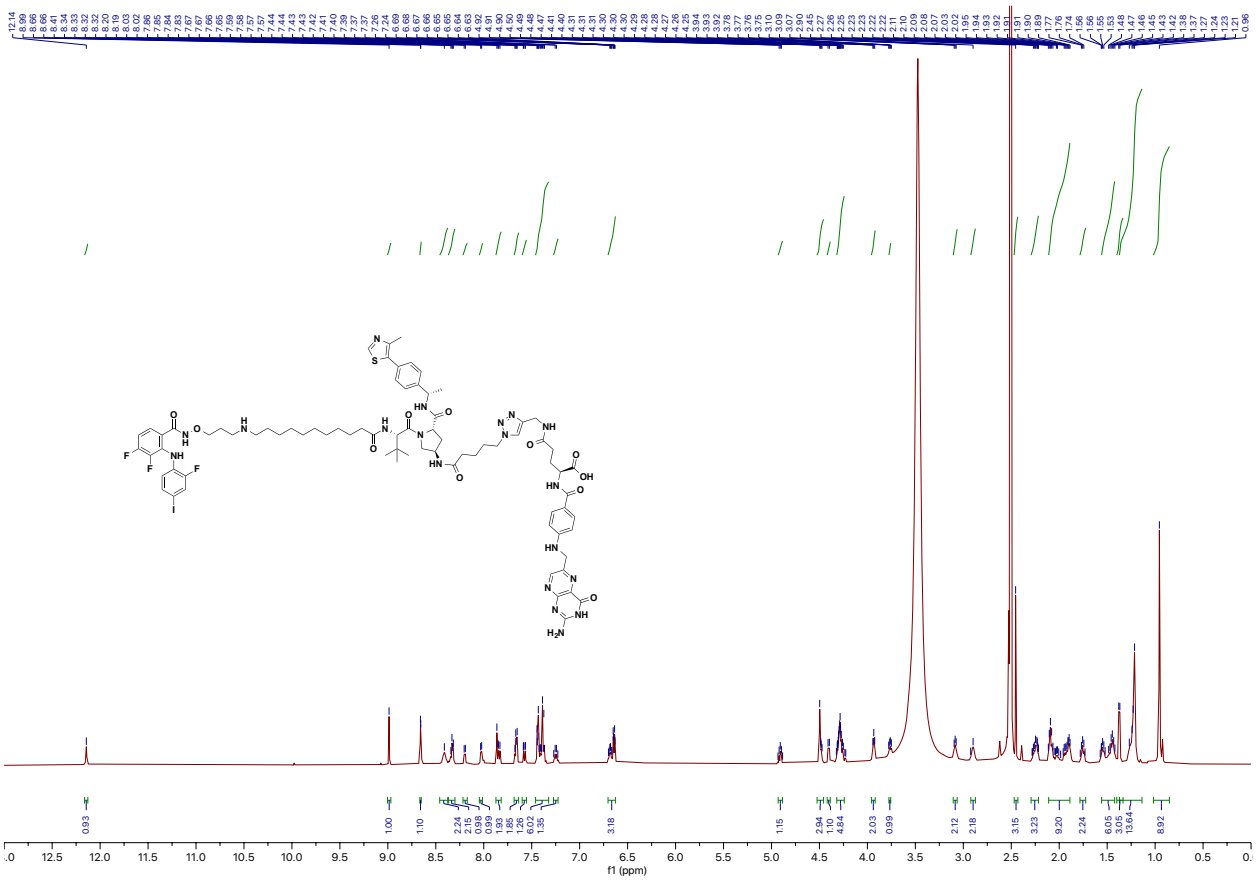
^{1}H-NMR of folate-MS432 in DMSO-d_6



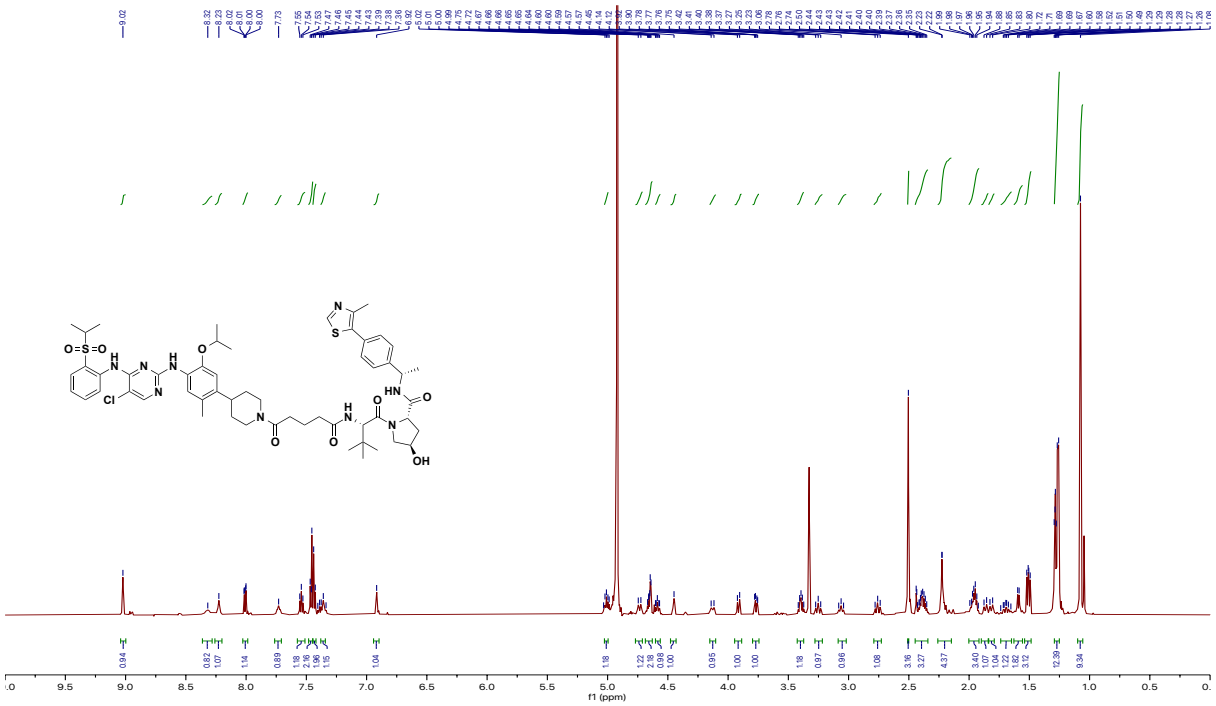
^{13}C-NMR of folate-MS432 in DMSO-d_6



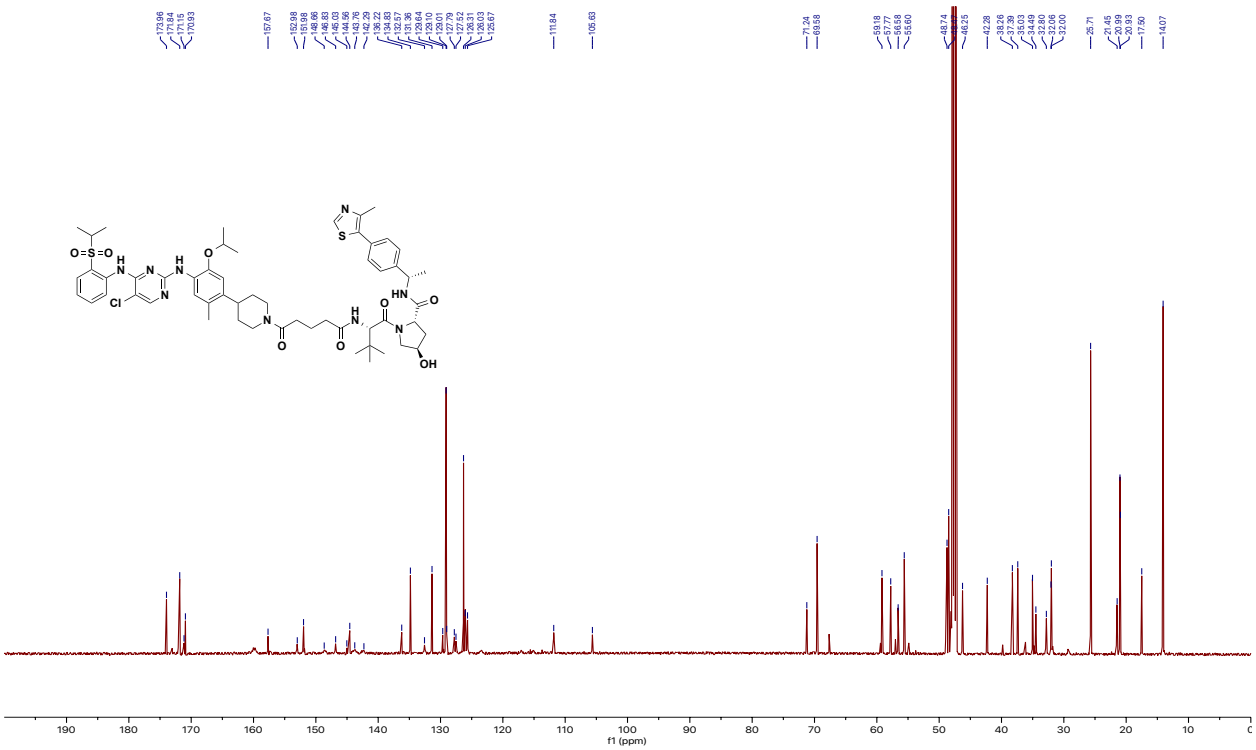
^{1}H-NMR of folate-MS432N in DMSO-d_6



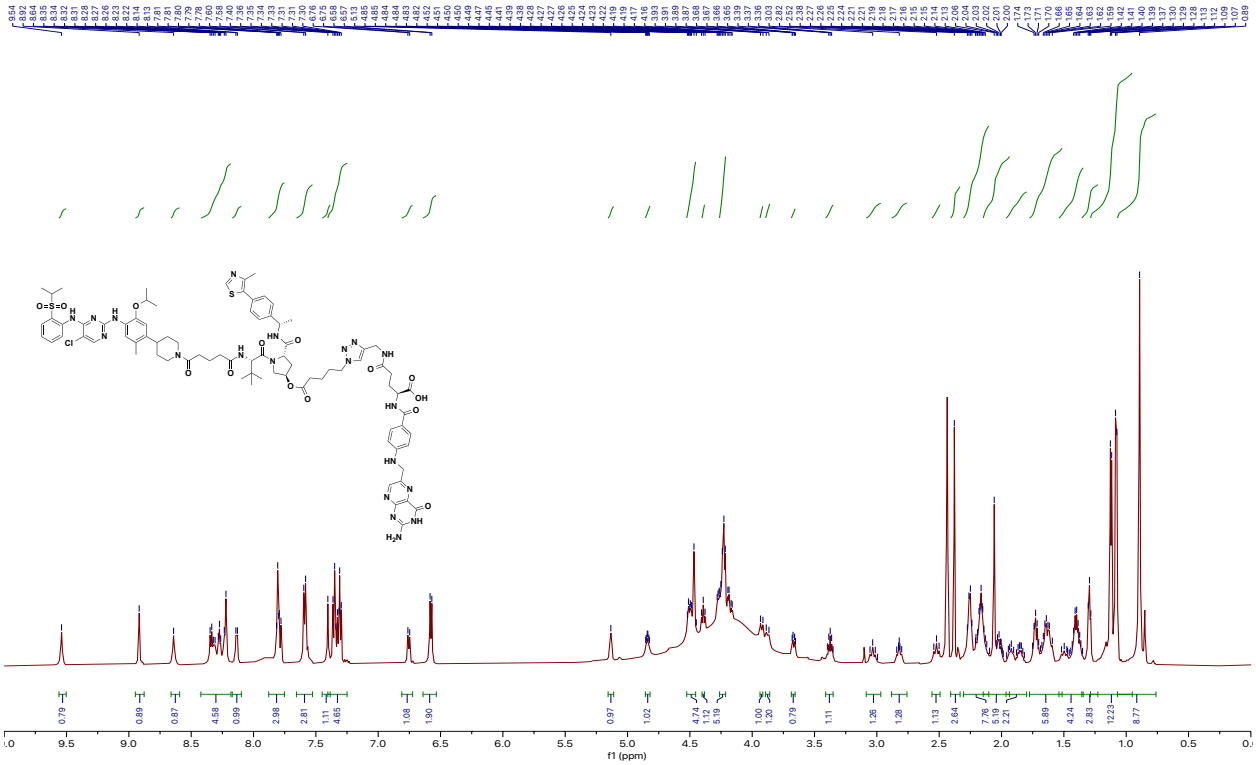
^{1}H-NMR of MS99 in Methanol-*d*4



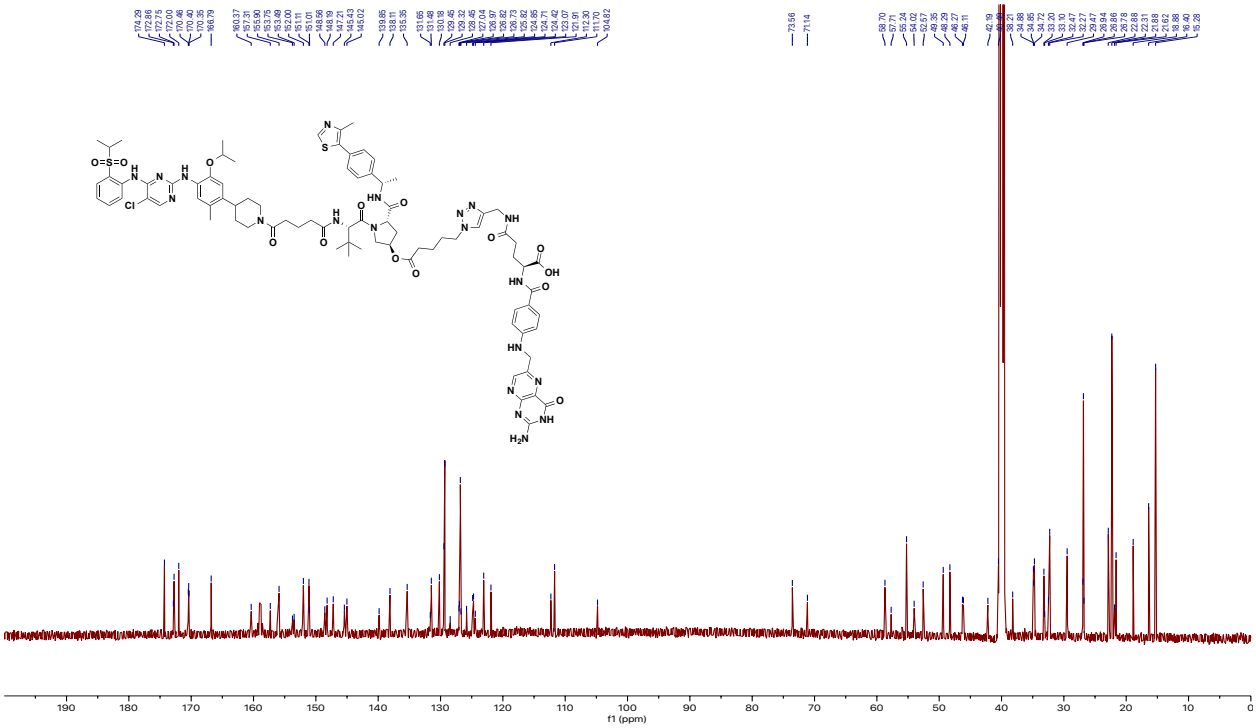
^{13}C-NMR of MS99 in Methanol-*d*4



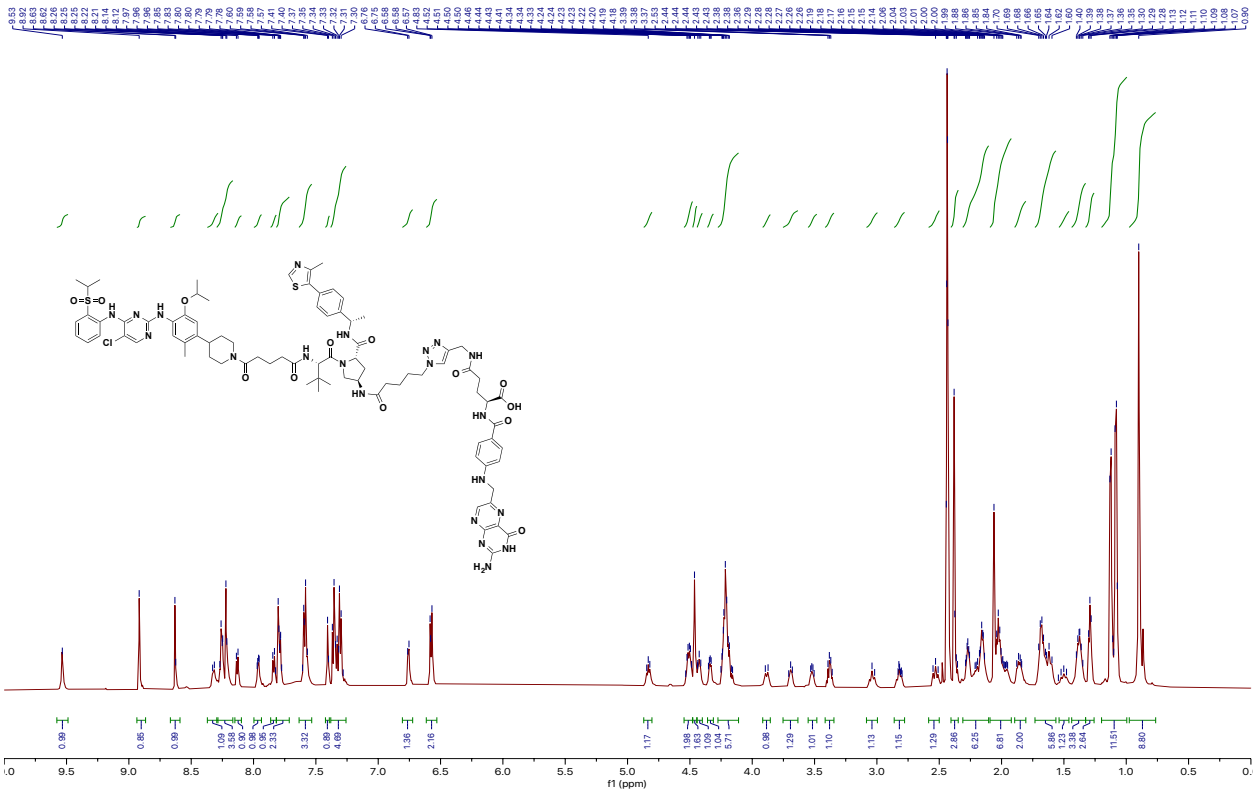
^{1}H-NMR of folate-MS99 in DMSO-d_6



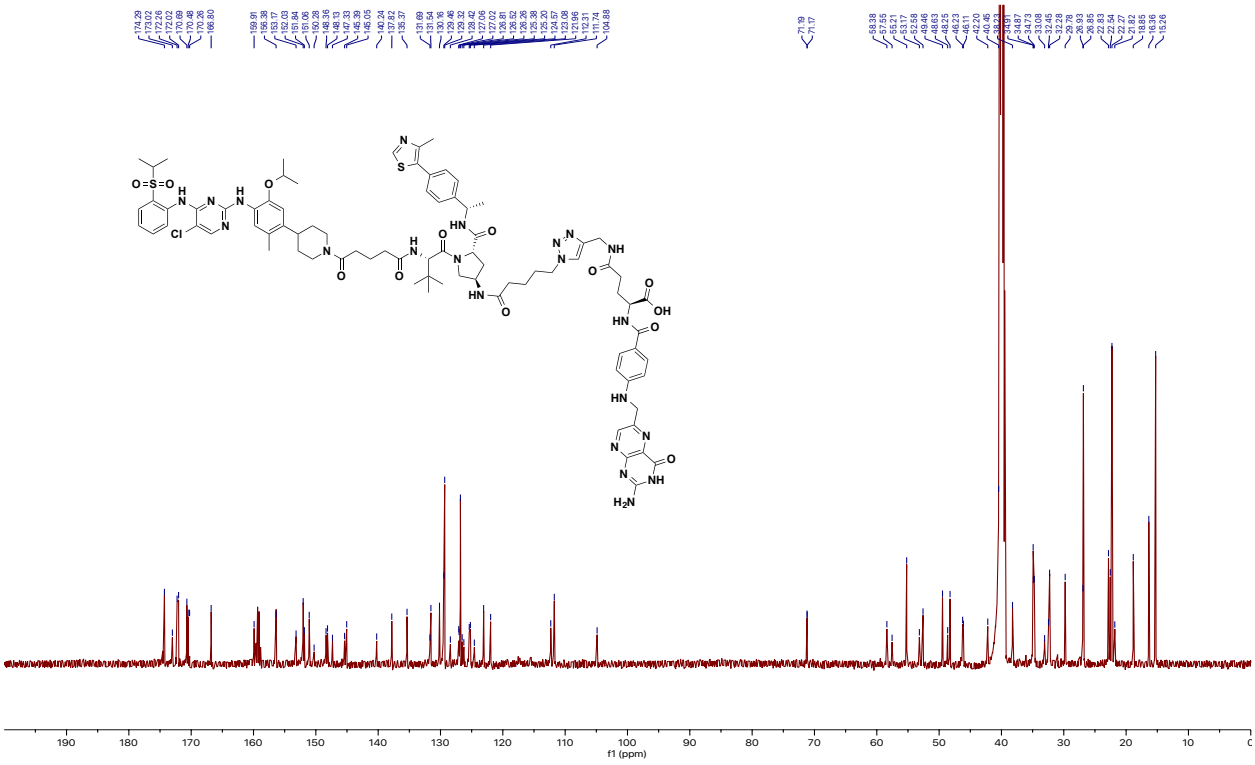
^{13}C-NMR of folate-MS99 in DMSO-d_6



^{1}H-NMR of folate-MS99N in DMSO-d_6



^{13}C-NMR of folate-MS99N in DMSO-d_6



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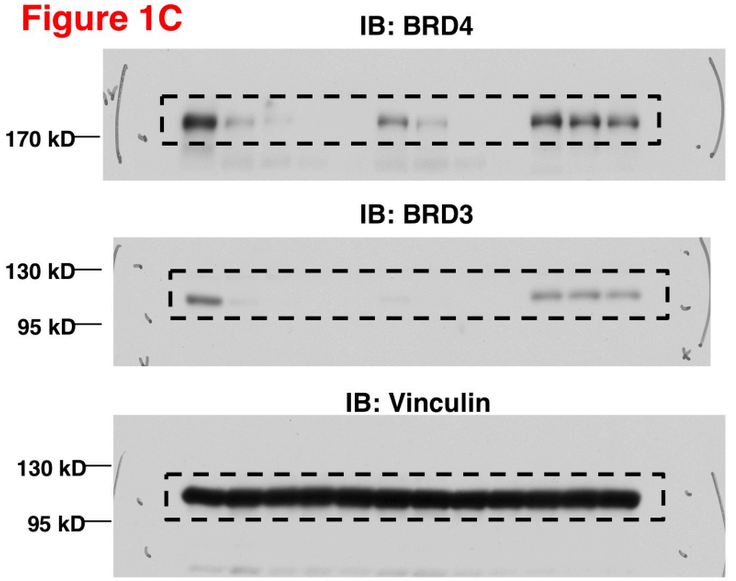
(1) Candelon, N.; Hădade, N. D.; Matache, M.; Canet, J. L.; Cisnetti, F.; Funeriu, D. P.; Nauton, L.; Gautier, A. Luminogenic "Clickable" Lanthanide Complexes for Protein Labeling. *Chem. Commun.* **2013**, *49* (80), 9206–9208.

(2) Raina, K.; Lu, J.; Qian, Y.; Altieri, M.; Gordon, D.; Rossi, A. M. K.; Wang, J.; Chen, X.; Dong, H.; Siu, K.; Winkler, J. D.; Crew, A. P.; Crews, C. M.; Coleman, K. G. PROTAC-Induced BET Protein Degradation as a Therapy for Castration-Resistant Prostate Cancer. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (26), 7124–7129.

(3) Wei, J.; Hu, J.; Wang, L.; Xie, L.; Jin, M. S.; Chen, X.; Liu, J.; Jin, J. Discovery of a First-in-Class Mitogen-Activated Protein Kinase Kinase 1/2 Degrader. *J. Med. Chem.* **2019**, *62* (23), 10897–10911.

Figure 1C



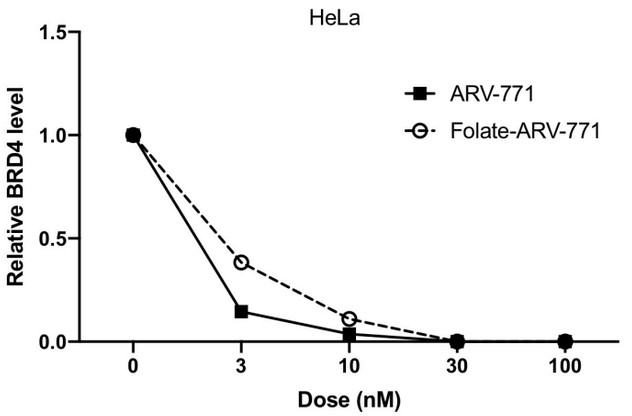
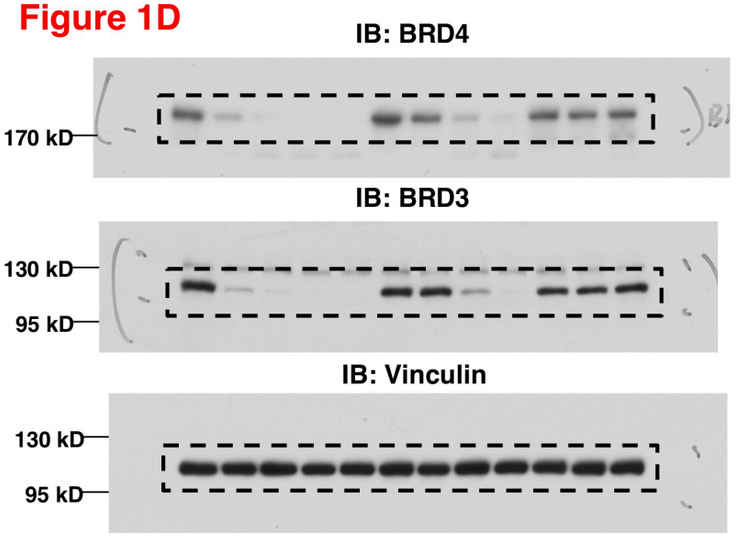


Figure 1D



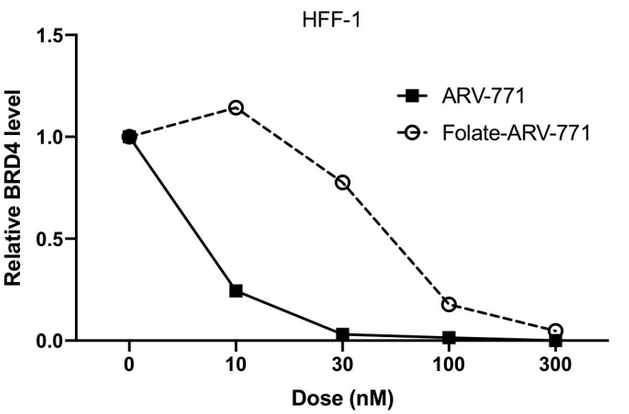


Figure 2A

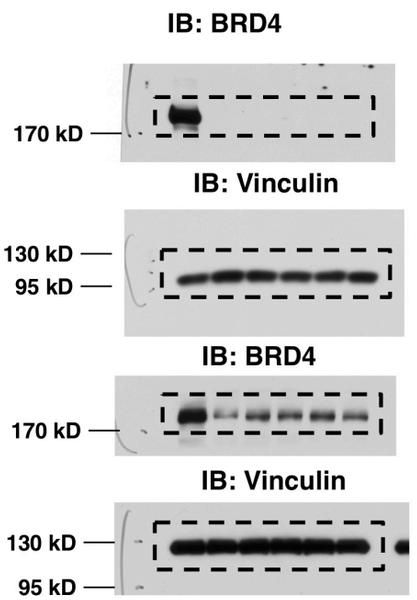


Figure 2C

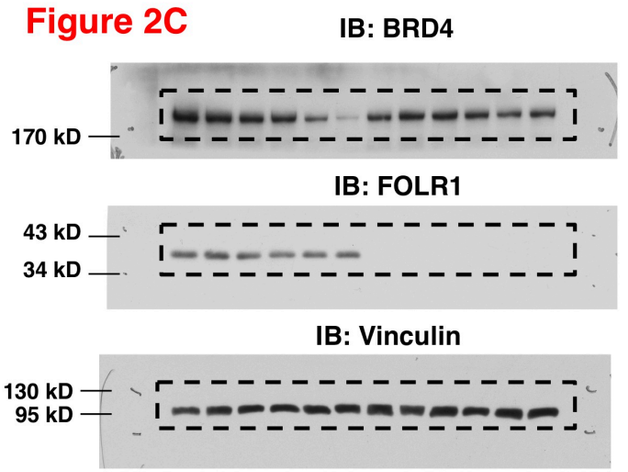


Figure 2D

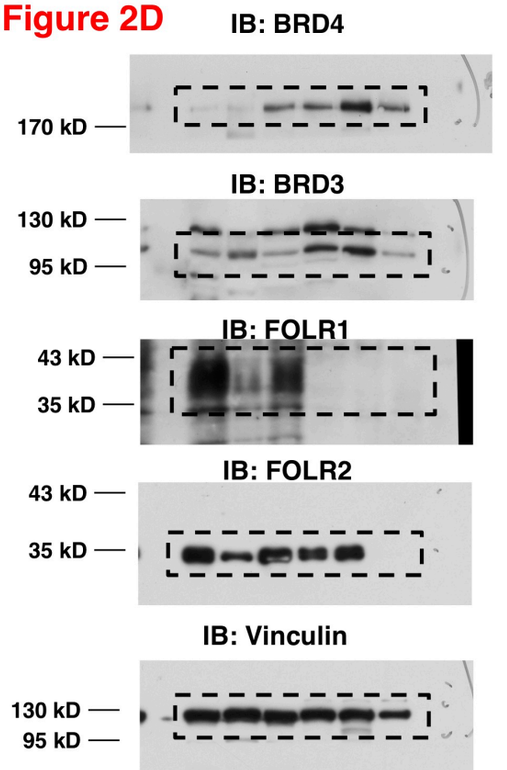
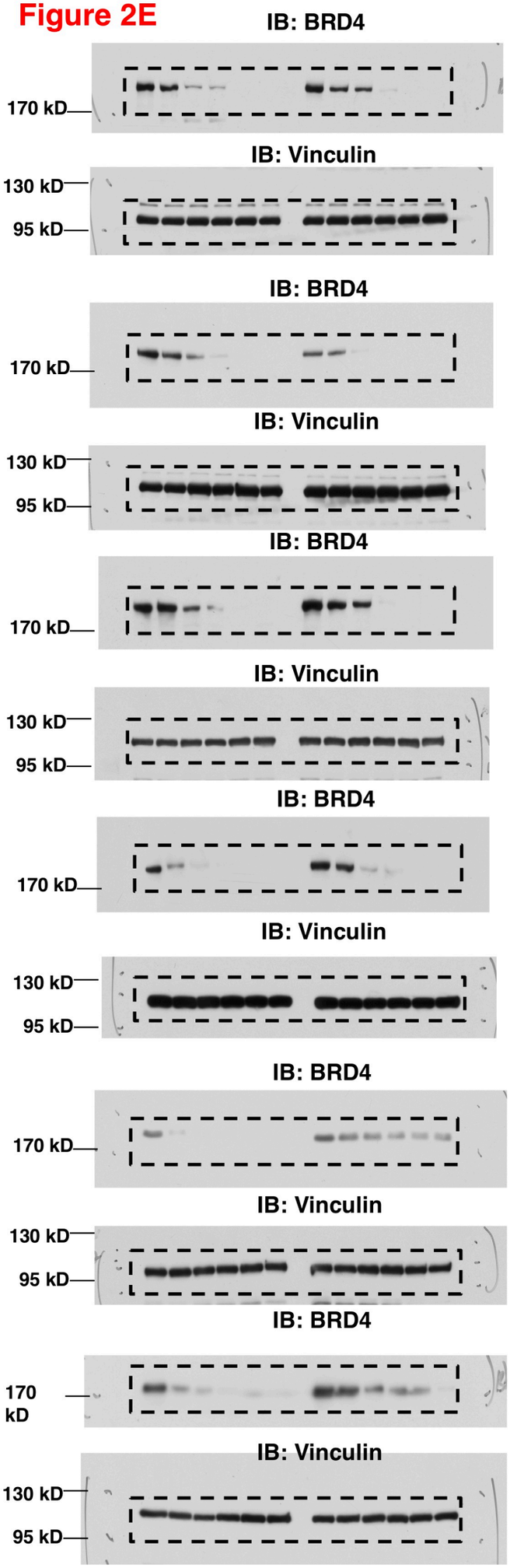
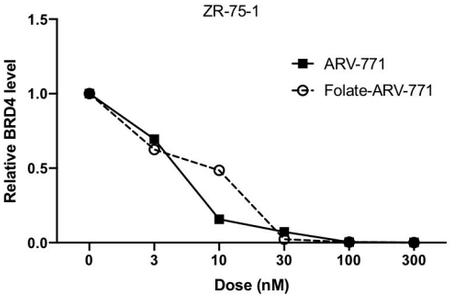
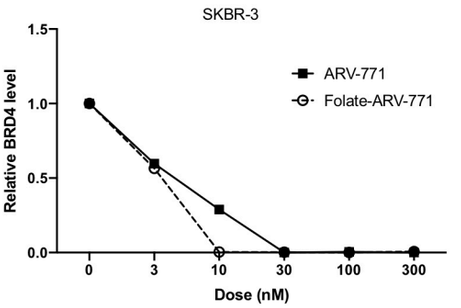
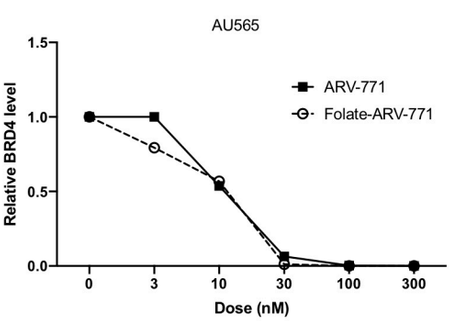


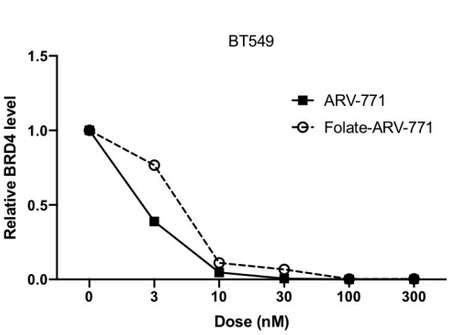
Figure 2E

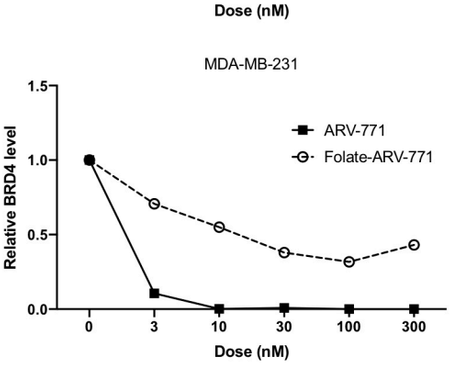

IB: BRD4
170 kD
IB: Vinculin
130 kD
95 kD
IB: BRD4
170 kD
IB: Vinculin
130 kD
95 kD
IB: BRD4
170 kD
IB: Vinculin
130 kD
95 kD
IB: BRD4
170 kD
IB: Vinculin
130 kD
95 kD
IB: BRD4
170 kD
IB: Vinculin
130 kD
95 kD
IB: BRD4
170 kD
IB: Vinculin
130 kD
95 kD


ZR-75-1
ARV-771
Folate-ARV-771
Relative BRD4 level
1.5
1.0
0.5
0.0
0
3
10
30
100
300
Dose (nM)


SKBR-3
ARV-771
Folate-ARV-771
Relative BRD4 level
1.5
1.0
0.5
0.0
0
3
10
30
100
300
Dose (nM)


AU565
ARV-771
Folate-ARV-771
Relative BRD4 level
1.5
1.0
0.5
0.0
0
3
10
30
100
300
Dose (nM)


BT549
ARV-771
Folate-ARV-771
Relative BRD4 level
1.5
1.0
0.5
0.0
0
3
10
30
100
300
Dose (nM)


MDA-MB-231
ARV-771
Folate-ARV-771
Relative BRD4 level
1.5
1.0
0.5
0.0
0
3
10
30
100
300
Dose (nM)

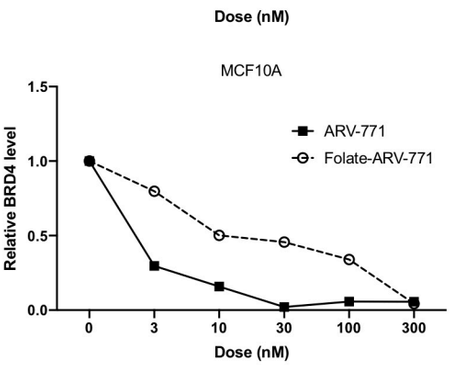
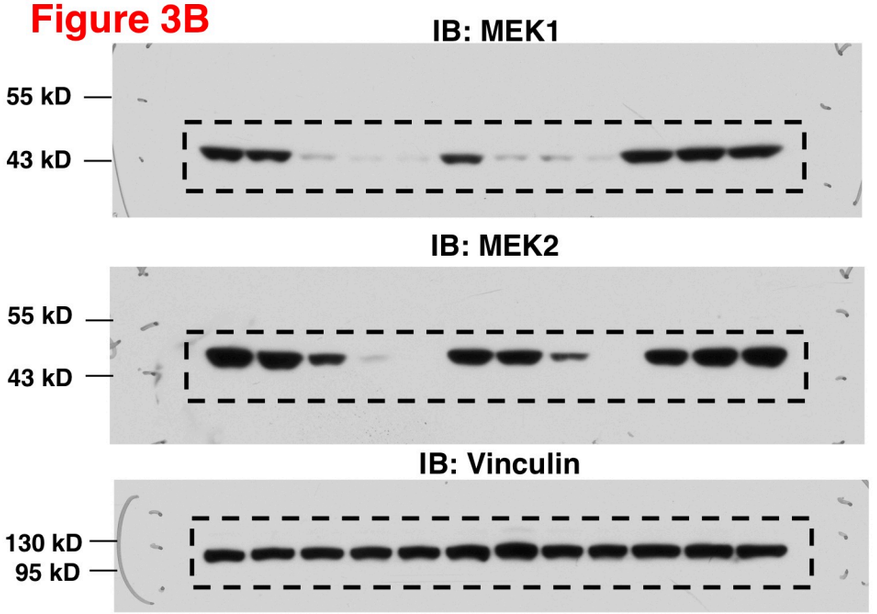

MCF10A
ARV-771
Folate-ARV-771
Relative BRD4 level
1.5
1.0
0.5
0.0
0
3
10
30
100
300
Dose (nM)

Figure 3B



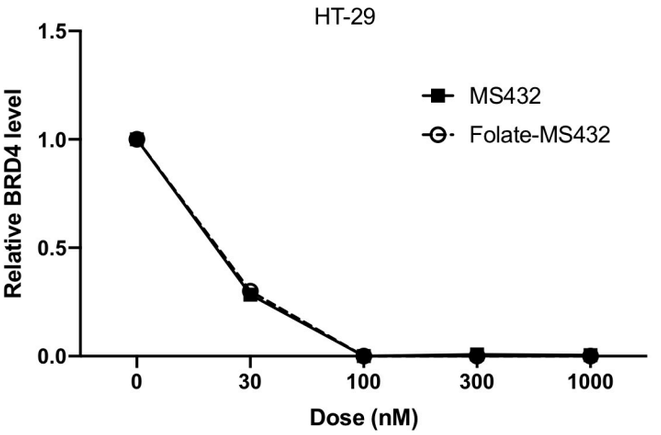


Figure 3C

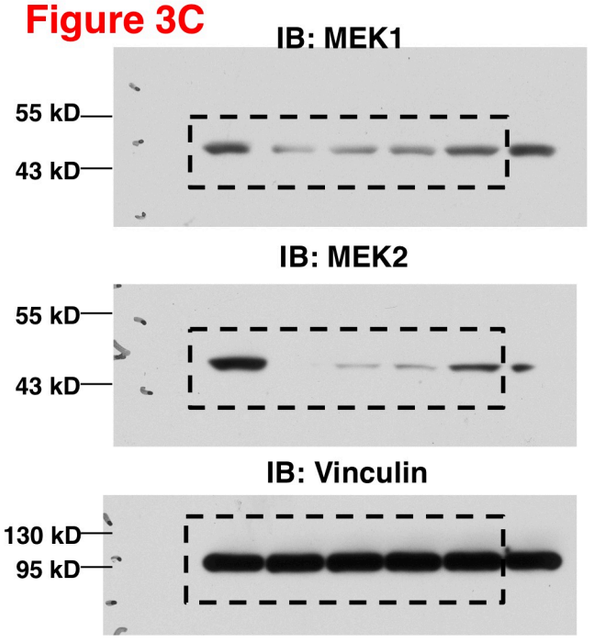


Figure 3D

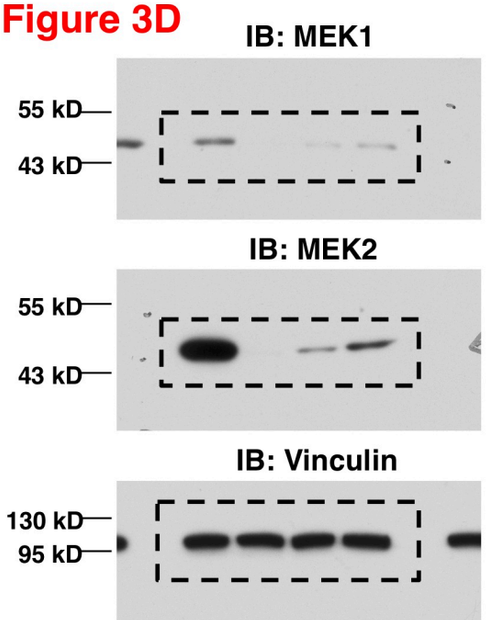


Figure 3E

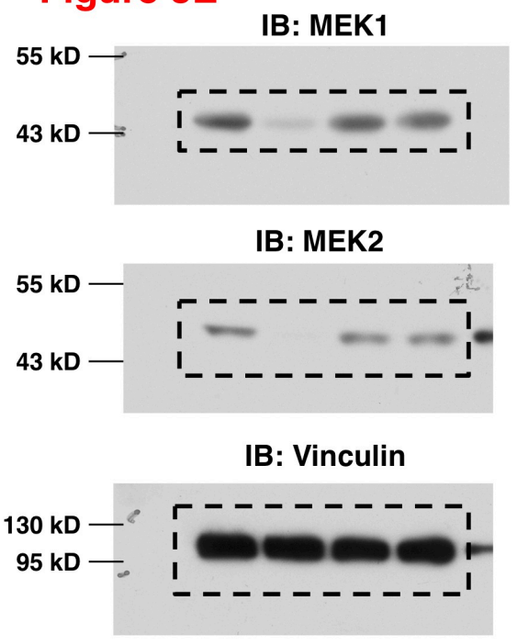
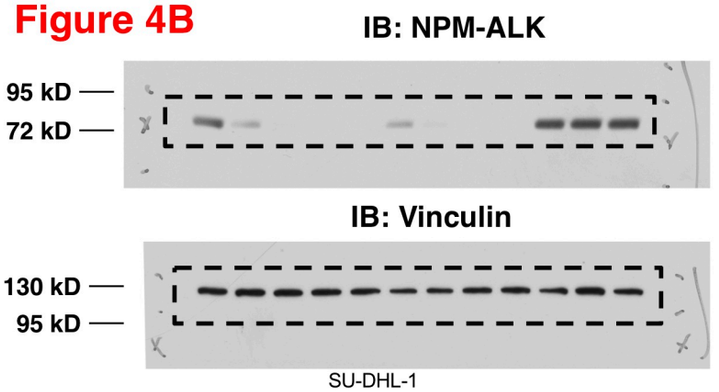


Figure 4B



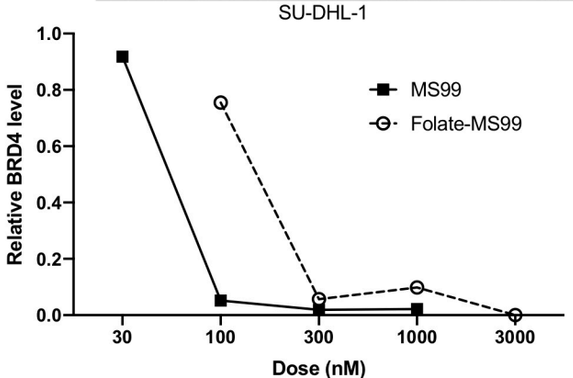


Figure 4C

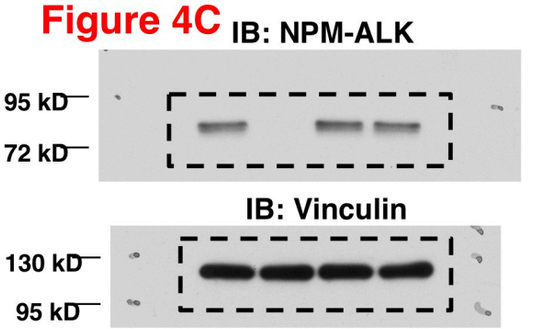


Figure 4D

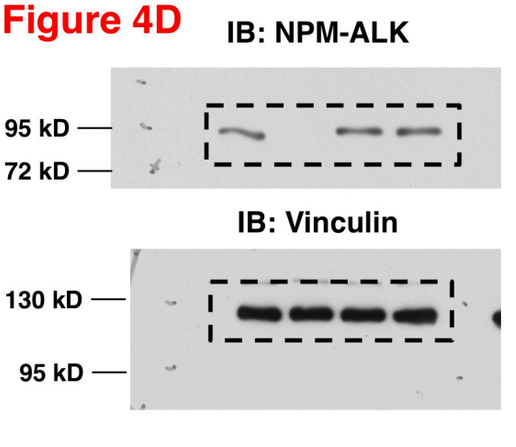


Figure S2A

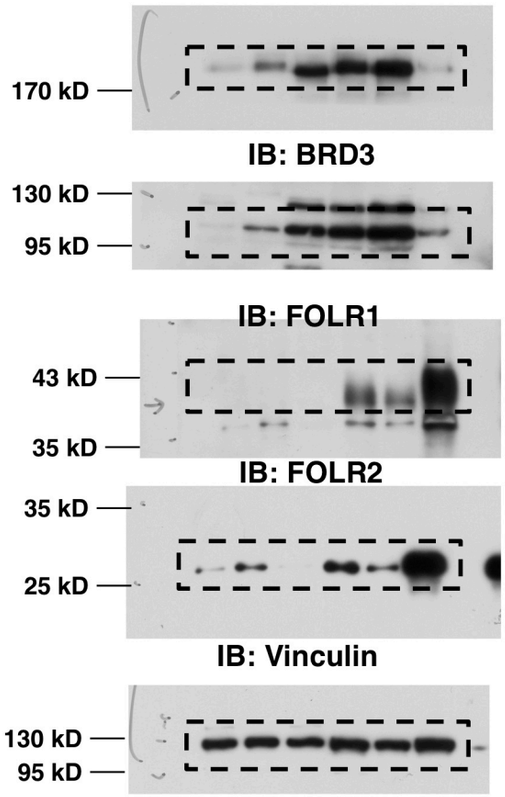


Figure S2B

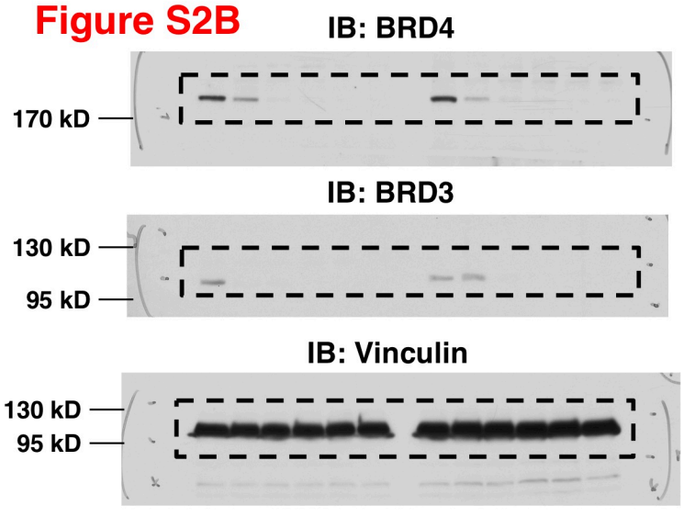


Figure S2C

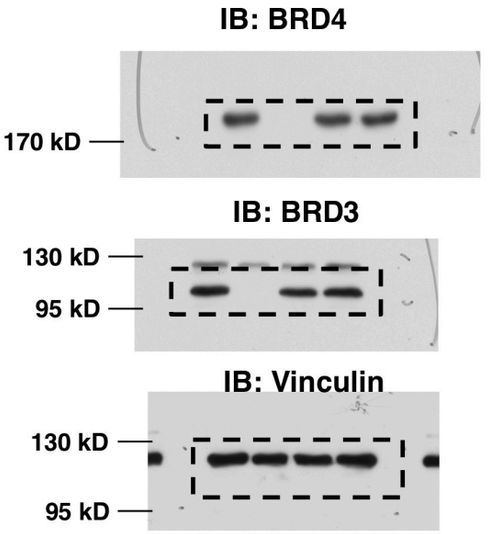


Figure S2D

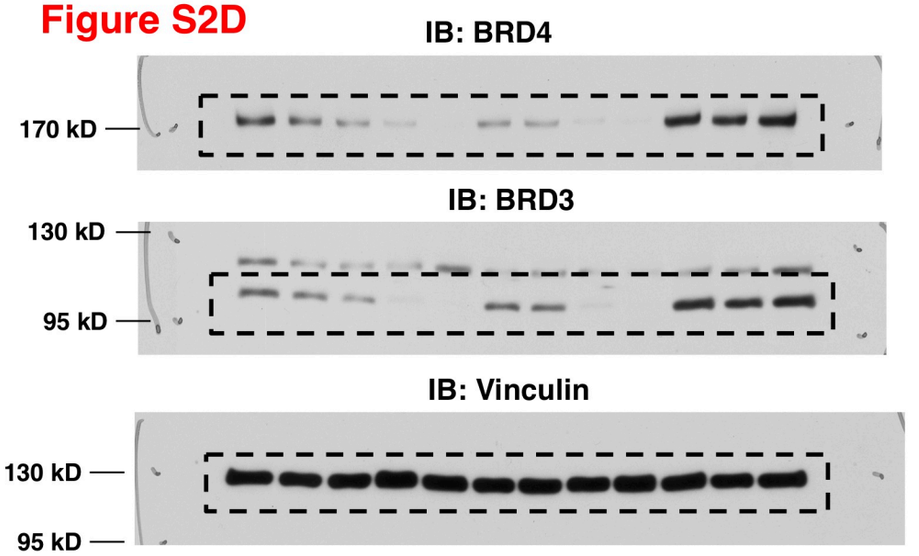


Figure S2E

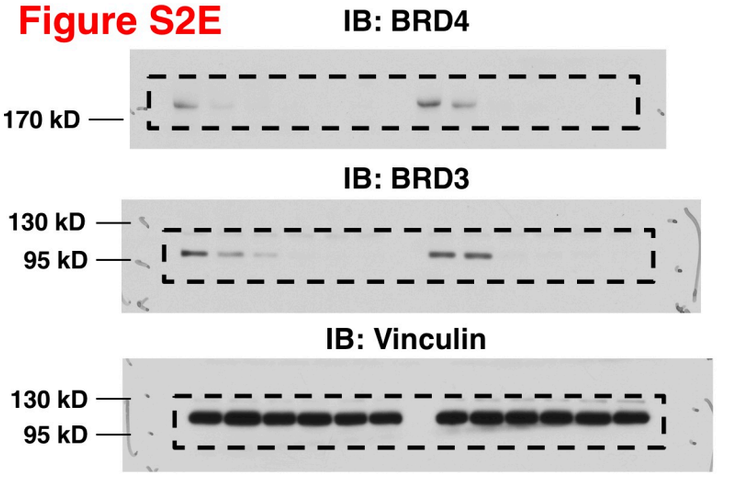


Figure S2F

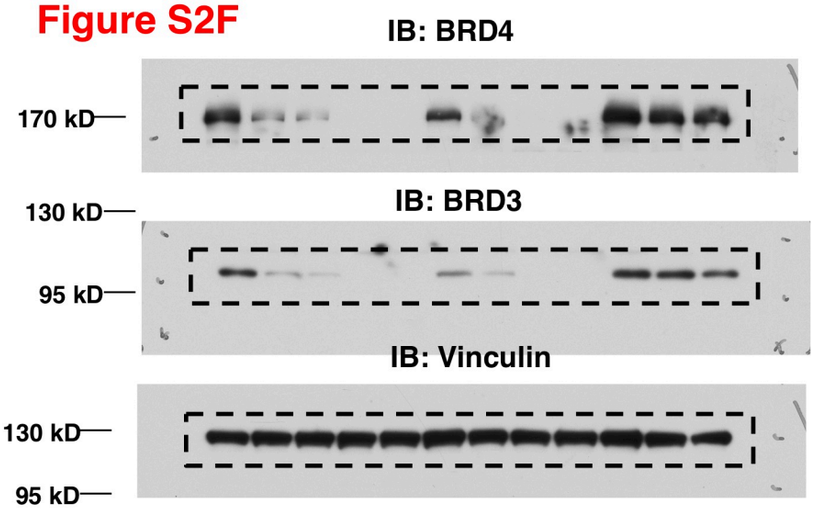


Figure S2G

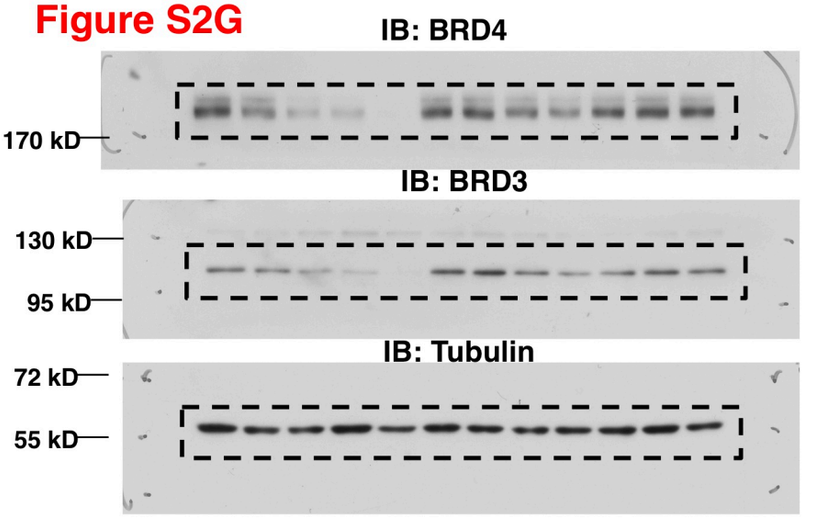


Figure S2H

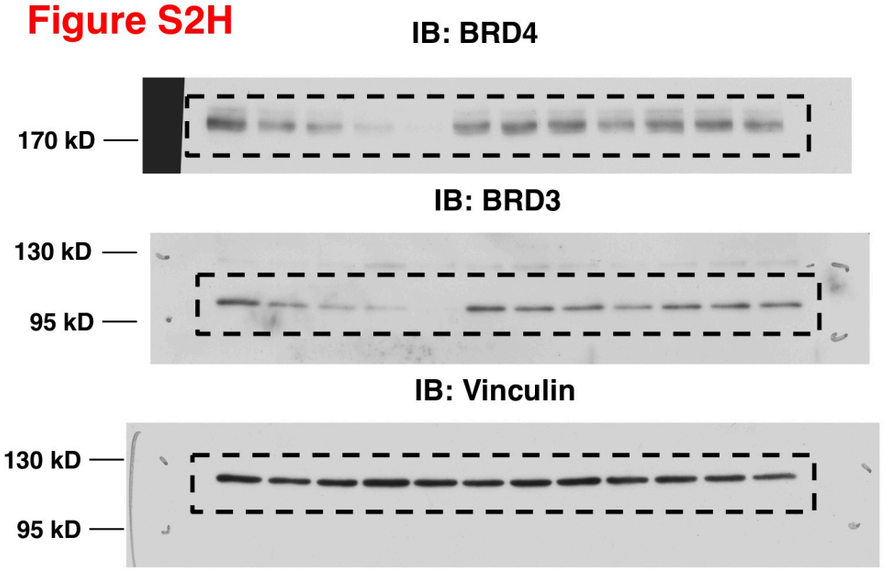


Figure S4A

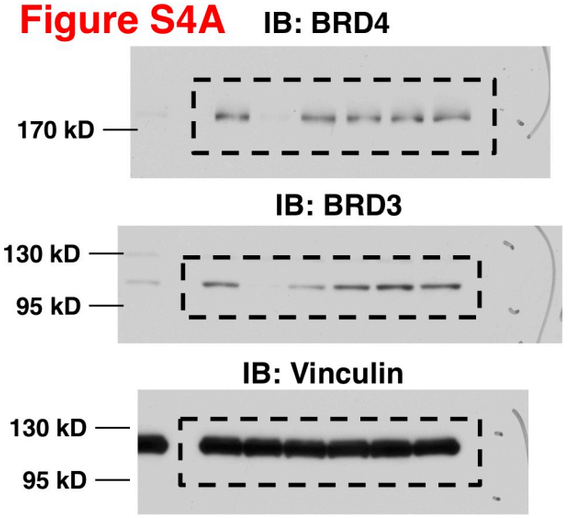


Figure S4C

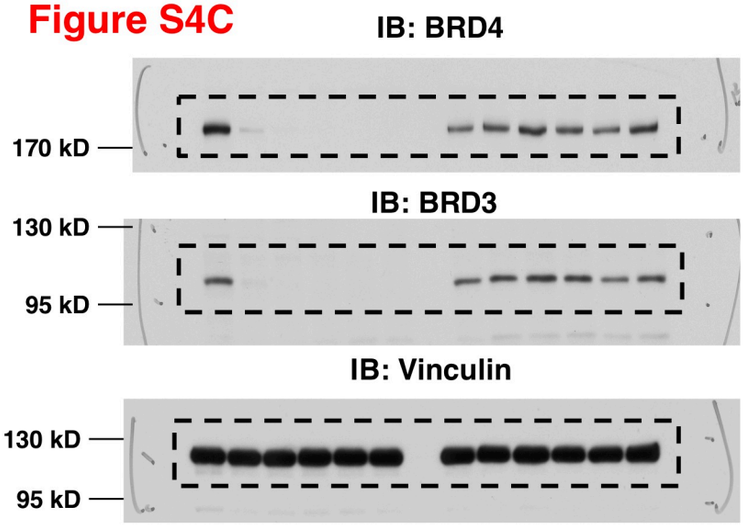


Figure S4E

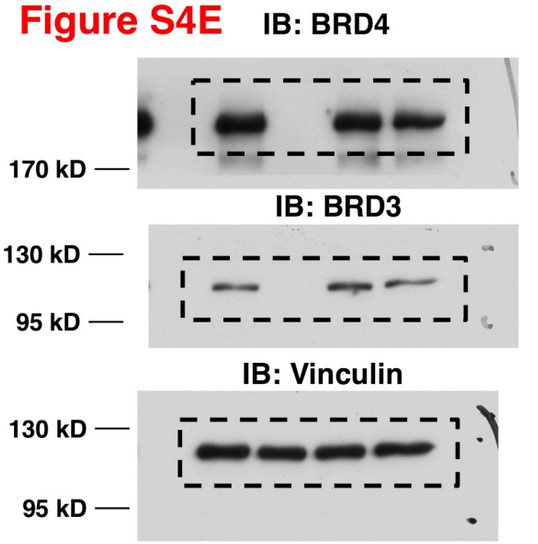


Figure S4F

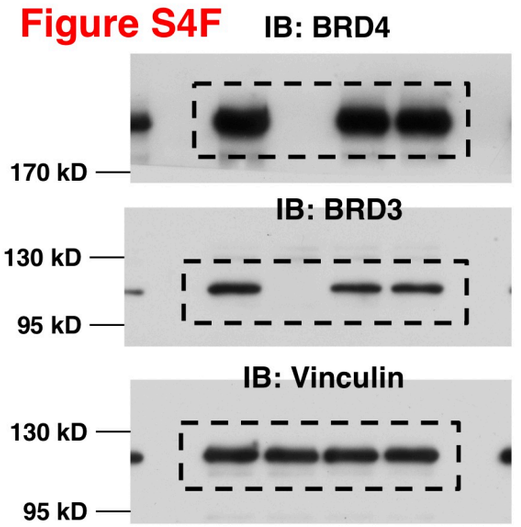


Figure S4G

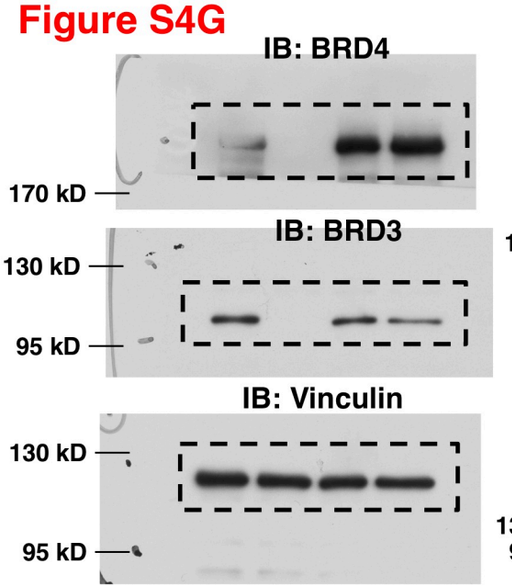


Figure S5A

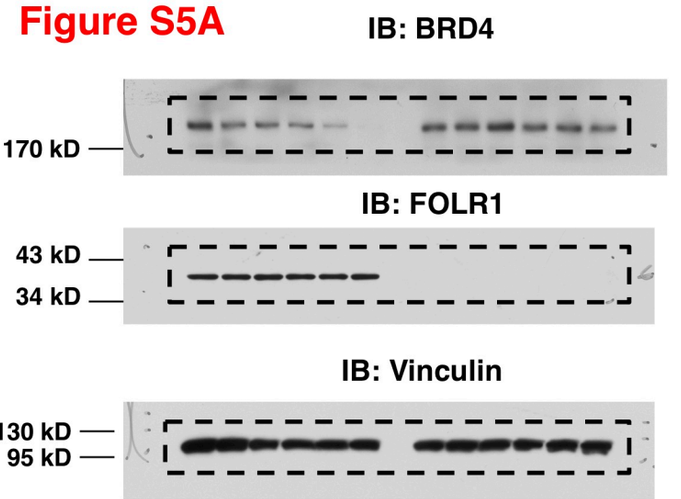


Figure S5B

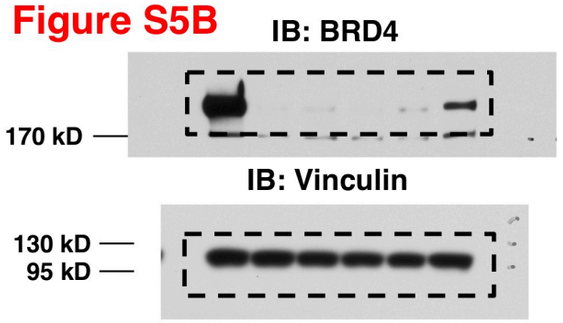


Figure S6A

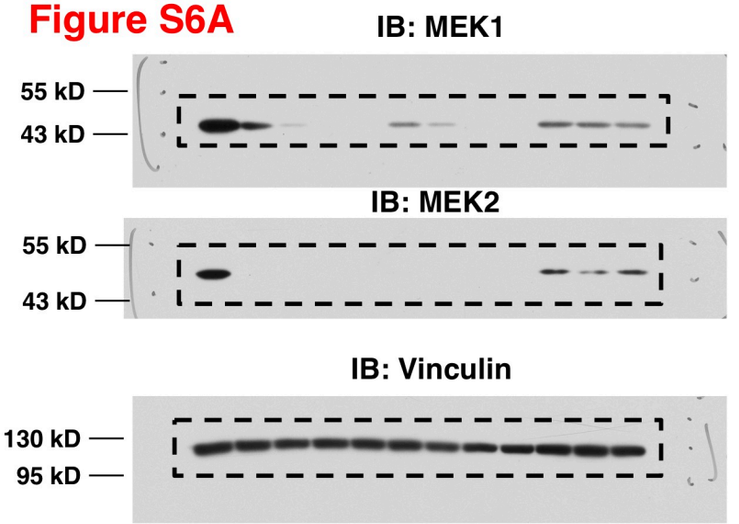


Figure S6B

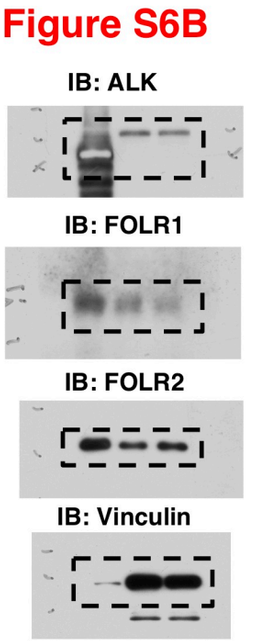


Figure S6C

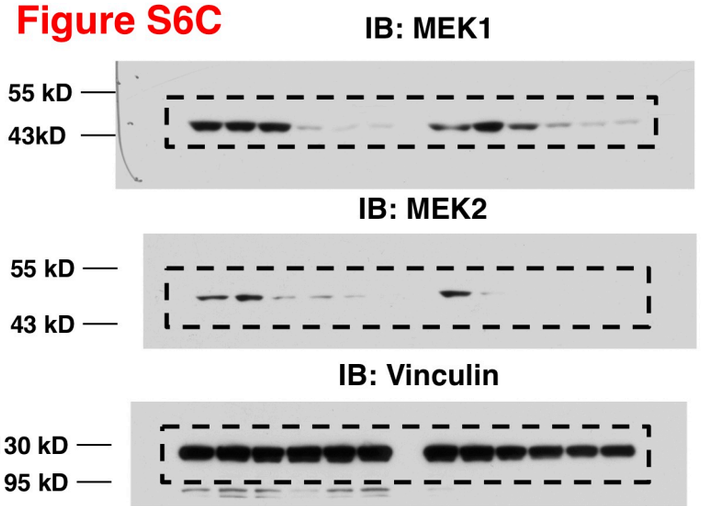


Figure S6D

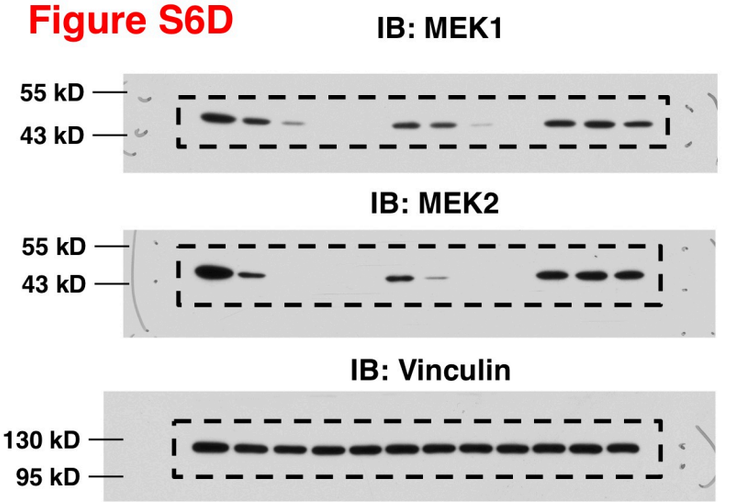


Figure S6E

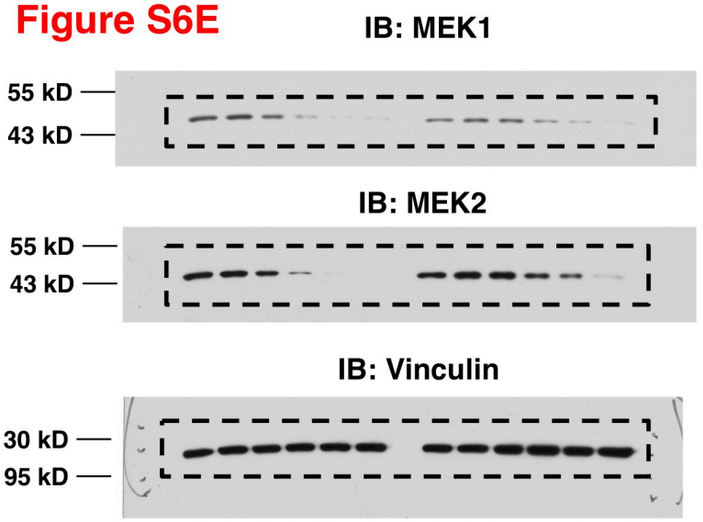


Figure S6F

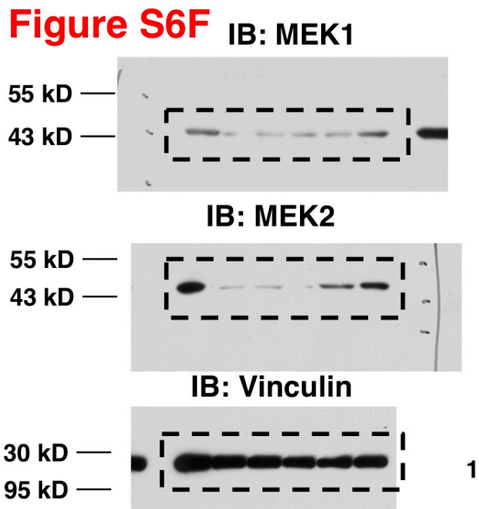


Figure S6G

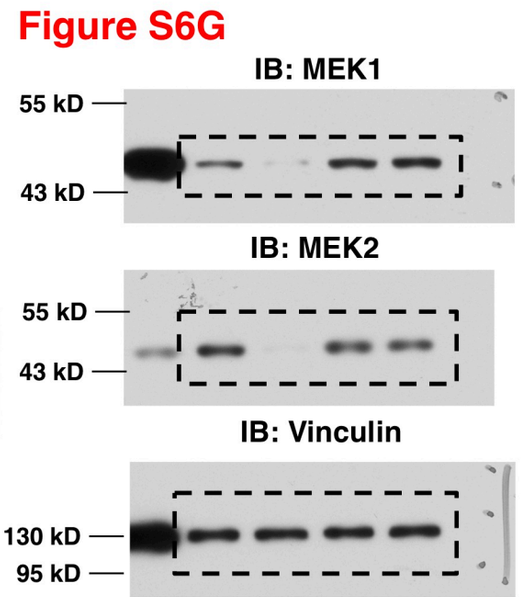


Figure S7B

Figure S7C

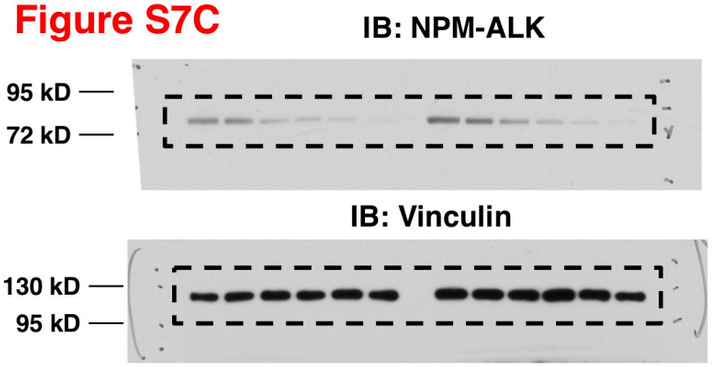


Figure S7D

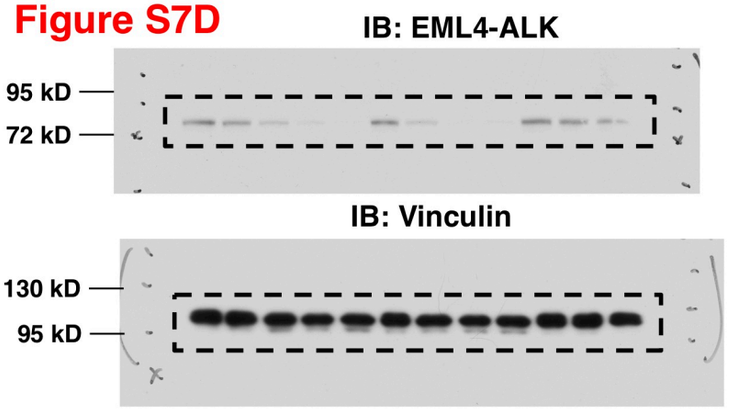


Figure S7E

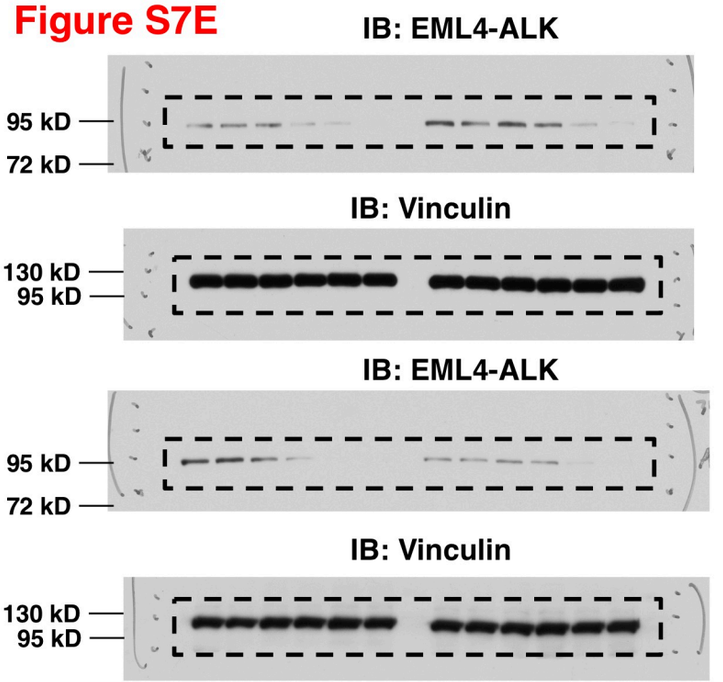


Figure S7F

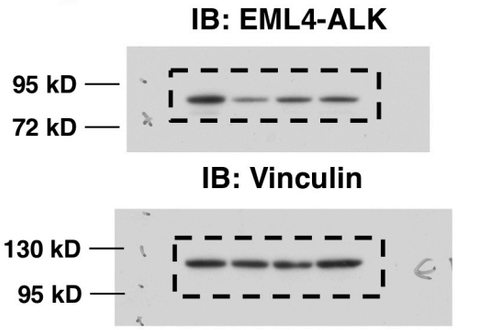


Figure S7G

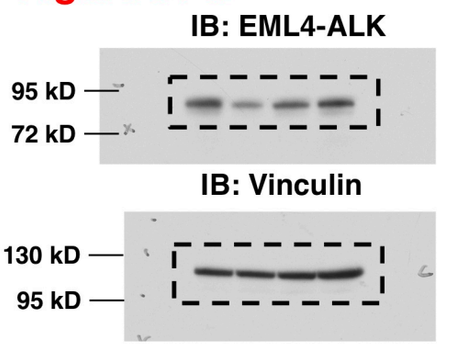


Figure S7H

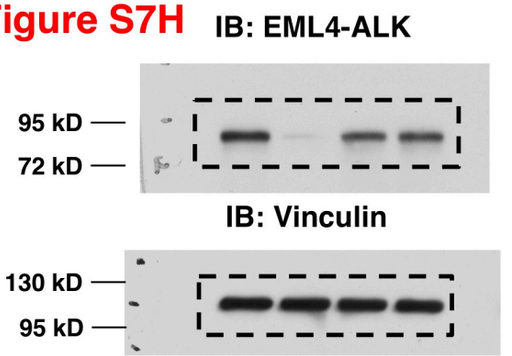


Figure S7I