Supporting Information

1. Heat shock protein 70 inhibitors: 2,5'-thiodipyrimidine and 5-(phenylthio)pyrimidine acrylamides as irreversible binders to an allosteric site on heat shock protein 70

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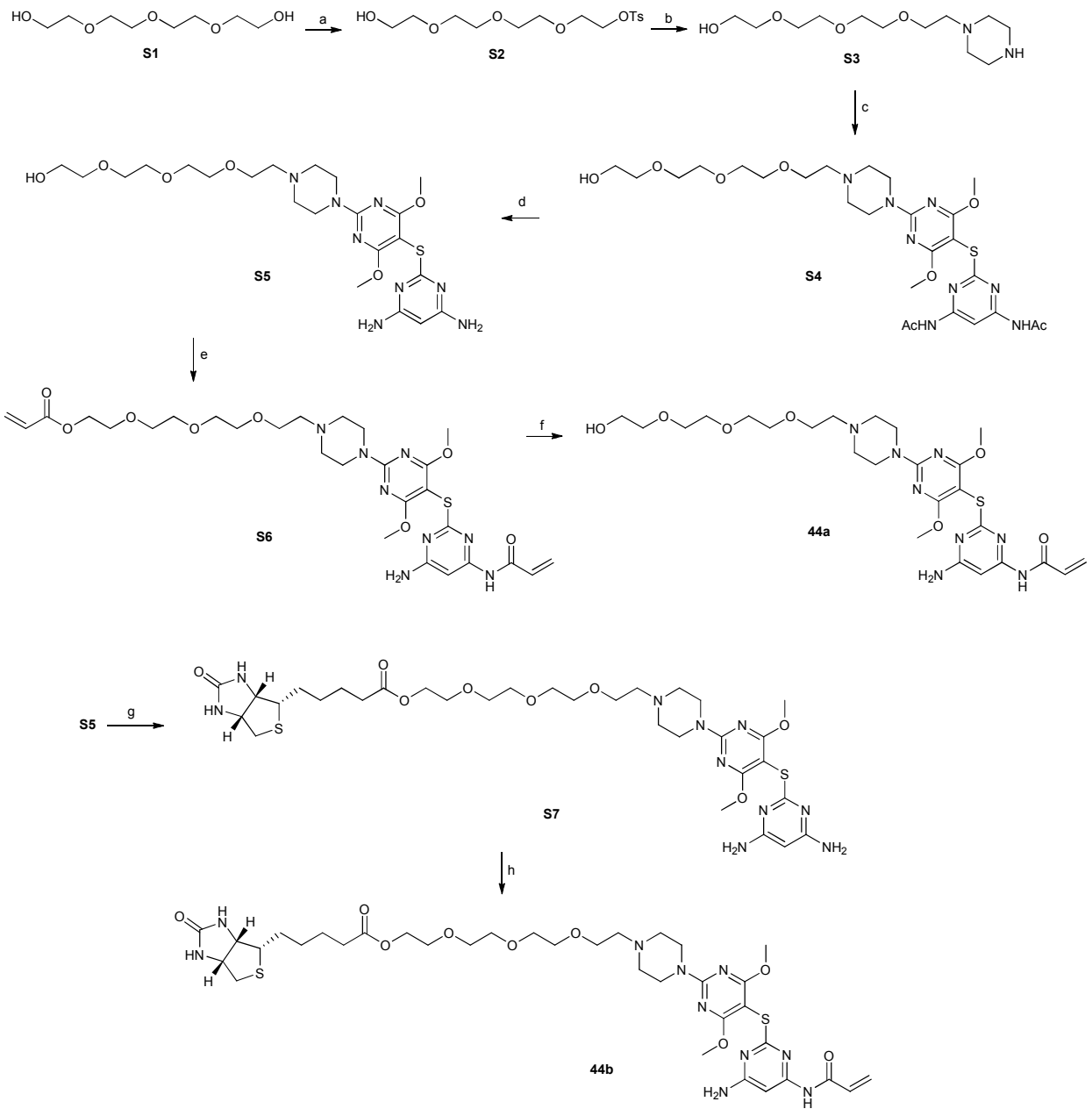
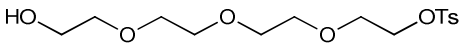
General. NMR spectra were recorded on a Bruker AV-III-500 MHz NMR spectrometer. Chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard. ^{1}H data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constant (Hz), integration. ^{13}C chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard. High resolution mass spectra were recorded on a Waters LCT Premier system. Low resolution mass spectra were obtained on Waters Acquity Ultra Performance LC with electrospray ionization and SQ detector. Analytical HPLC was performed on a Waters Autopurification system with PDA, MicroMass ZQ and ELSD detector. Analytical thin layer chromatography was performed on 250 μM silica gel F_{254} plates. Preparative thin layer chromatography was performed on 1000 μM silica gel F_{254} plates. Flash column chromatography was performed employing 230-400 mesh silica gel. Solvents were HPLC grade. All reagents were purchased from either Aldrich or Acros Organics and used without purification. All reactions were performed under argon protection.

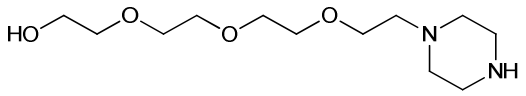


Scheme S1. Synthesis of **44a** and **44b**.

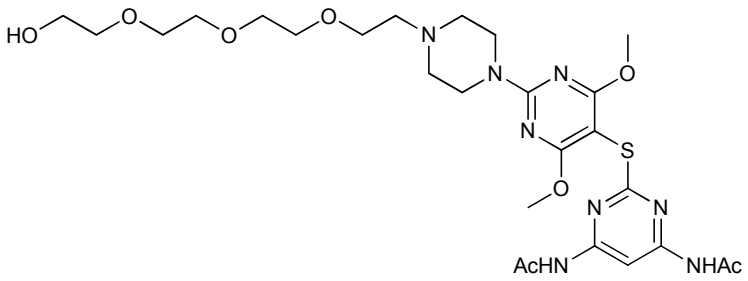
Reagents and conditions: (a) TsCl, NaOH, THF, H_2O, 0°C, 2 h, 78%; (b) piperazine, CH_3CN, 75°C, 12 h, 66%; (c) **14a**, DMF, 90°C, 2 h, 79%; (d) NaOH, MeOH, H_2O, 60°C, 2 h, 98%; (e) acryloyl chloride, Et_3N, CH_2Cl_2, 0°C, 7 h, 36%; (f) NaOH, THF, H_2O, 3 h, 75%; (g) D-biotin, DCC, DMAP, CH_2Cl_2, sonication, 13 h, 85%; (h) acryloyl chloride, Et_3N, CH_2Cl_2, 0°C, 6 h, 41%.



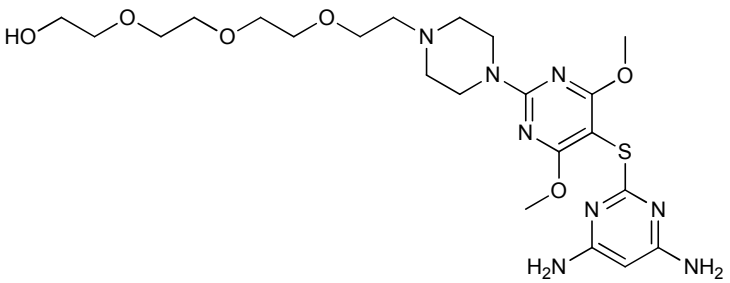
2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (S2). 2.0 g (10.3 mmol) of tetraethylene glycol **(S1)** in 10 ml of THF was cooled to 0°C. 0.200 g (5 mmol) of NaOH in 2 ml of distilled water was added and it was stirred for 30 minutes. Then 0.491 g (2.58 mmol) of *p*-toluenesulfonyl chloride was added slowly and stirring continued at 0°C for 2 h. Solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane:EtOAc, 50:50 to 10:90) to give 0.705 g (78%) of an oil **S2**. TLC (hexane:EtOAc, 10:90 v/v): R_f = 0.26; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 4.16 (t, *J* = 4.9 Hz, 2H), 3.59-3.74 (m, 14H), 2.45 (s, 3H); MS (*m*/*z*): $[M+Na]^+$ 371.3.



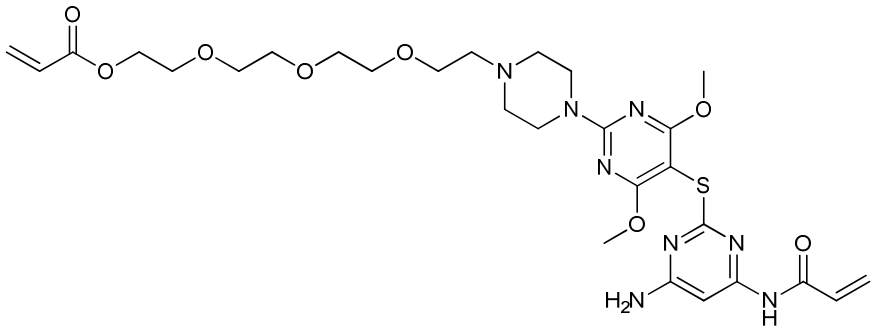
2-(2-(2-(2-(Piperazin-1-yl)ethoxy)ethoxy)ethoxy)ethanol (S3). 0.705 g (2.02 mmol) of **S2** and 0.697 g (8.09 mmol) of piperazine in 45 ml of CH_3CN was heated at 75°C for 12 h. Solvent and excess reagent were removed under reduced pressure and the oily residue was purified by column chromatography (CH_2Cl_2:MeOH:MeOH-NH_3 (7N), 90:5:5 to 90:0:10) to give 0.350 g (66%) of an oil **S3**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.72 (m, 2H), 3.58-3.70 (m, 12H), 2.91 (m, 4H), 2.59 (br m, 2H), 2.49 (m, 4H); MS (*m*/*z*): $[M+H]^+$ 263.3.



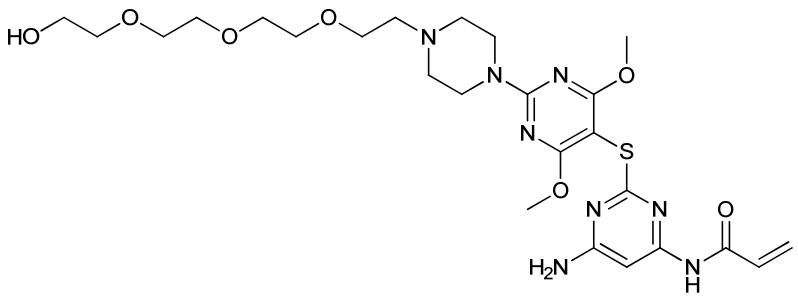
N,N'-(2-(2-(4-(2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-4,6-dimethoxypyrimidin-5-ylthio)pyrimidine-4,6-diyl)diacetamide (S4). To 0.430 g (1.12 mmol) of **14a** in 27 ml of DMF was added 0.310 g (1.18 mmol) of **S3** and was heated at 90°C for 2 h. Solvent was removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2:MeOH, 10:1) to give 0.552 g (79%) of **S4**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (br s, 2H), 8.13 (s, 1H), 3.88 (br s, 10H), 3.57-3.74 (m, 14H), 2.66 (br s, 2H), 2.58 (br s, 4H), 2.15 (s, 6H); MS (*m*/*z*): $[M+H]^+$ 625.5.



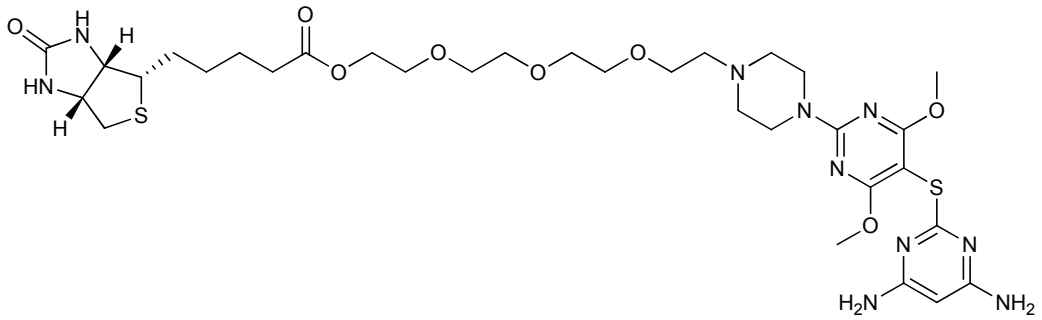
2-(2-(2-(2-(4-(5-(4,6-Diaminopyrimidin-2-ylthio)-4,6-dimethoxypyrimidin-2-yl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)ethanol (S5). To 0.520 g (0.832 mmol) of **S4** was added 25 ml of MeOH and 7 ml of 10% NaOH (*aq.*) and the suspension was stirred at 60°C for 2 h. Solvent was removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2:MeOH, 15:1) to give 0.440 g (98%) of **S5**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.17 (s, 1H), 4.60 (br s, 4H), 3.88 (br s, 10H), 3.57-3.77 (m, 14H), 2.67 (br s, 2H), 2.59 (br s, 4H); MS (*m*/*z*): $[M+H]^+$ 541.4.



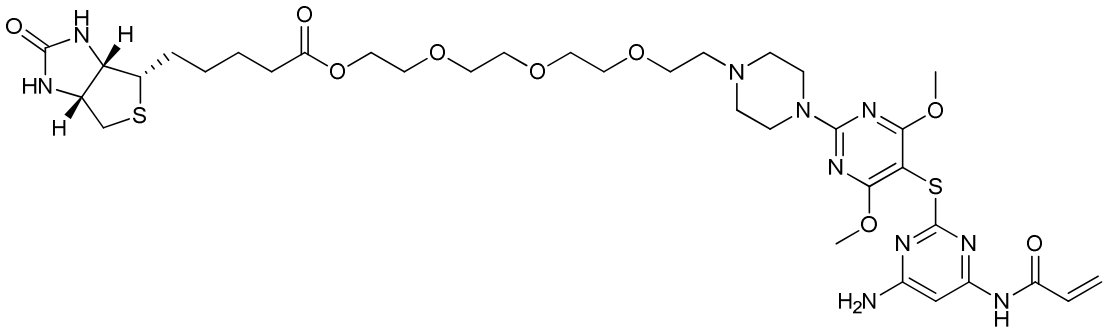
2-(2-(2-(2-(4-(5-(4-Acrylamido-6-aminopyrimidin-2-ylthio)-4,6-dimethoxypyrimidin-2-yl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)ethyl acrylate (S6). To 22.6 mg (0.042 mmol) of **S5** in 3 ml of CH_2Cl_2 at 0°C was added 83.6 mg (116 µl, 0.836 mmol) of Et_3N. 11.4 mg (10.2 µl, 0.126 mmol) of acryloyl chloride was added at 0°C. After 1 h an additional 11.4 mg (10.2 µl, 0.126 mmol) of acryloyl chloride was added. This was repeated five more times for a total reaction time of 7 h (total acryloyl chloride, 79.8 mg, 71.7 µl, 0.882 mmol). The reaction mixture was concentrated under reduced pressure and the residue purified by preparatory TLC (CH_2Cl_2:MeOH, 10:1) to yield 9.8 mg (36%) of **S6**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (br s, 1H), 7.05 (s, 1H), 6.35-6.45 (m, 2H), 6.10-6.20 (m, 2H), 5.73-5.85 (m, 2H), 4.90 (br s, 2H), 4.32 (br s, 2H), 3.89 (br s, 10H), 3.60-3.70 (m, 12H), 2.69 (br s, 2H), 2.61 (br s, 4H); MS (*m*/*z*): $[M+H]^+$ 649.5.



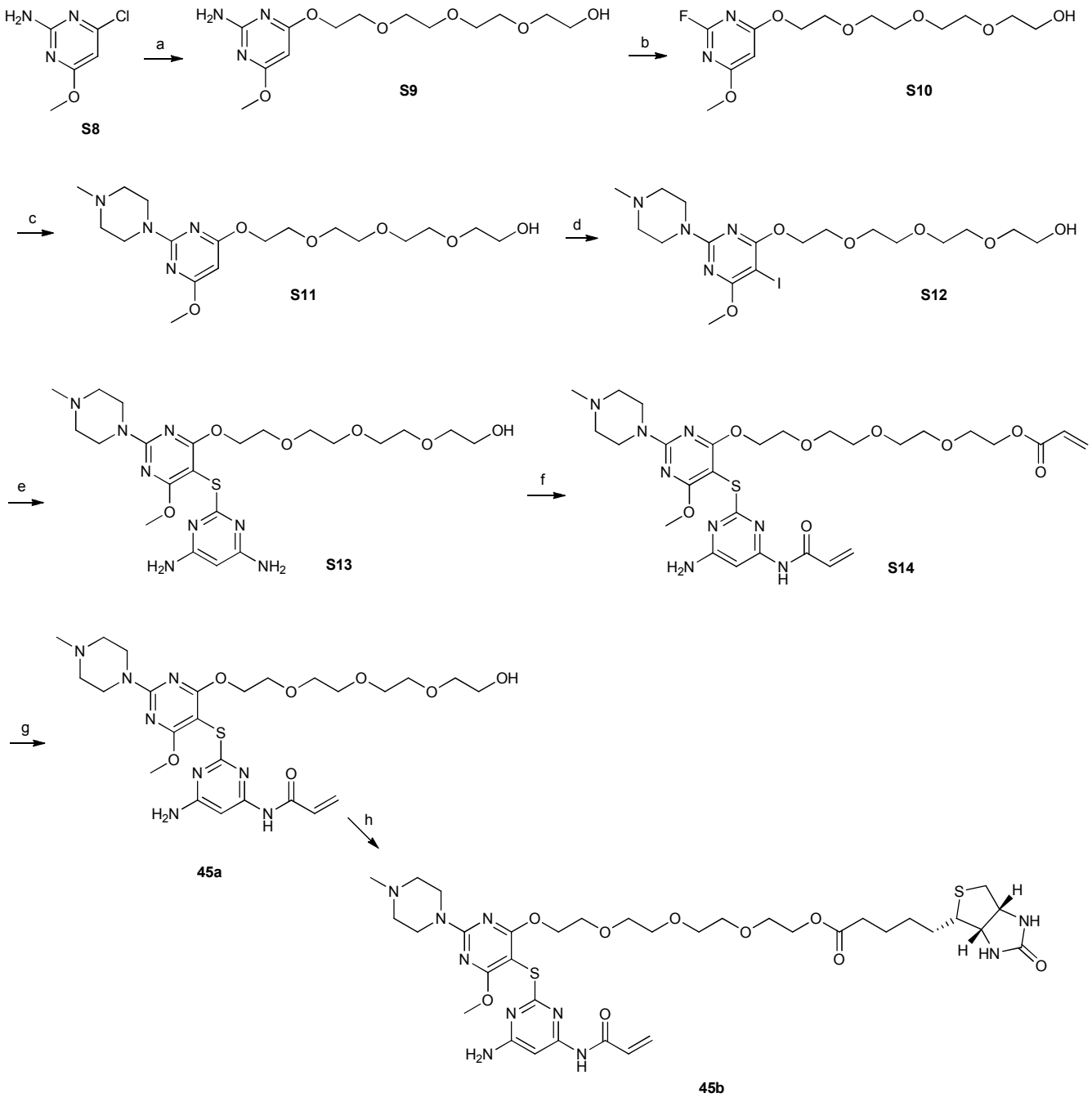
N-(6-Amino-2-(2-(4-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-4,6-dimethoxypyrimidin-5-ylthio)pyrimidin-4-yl)acrylamide (44a). To 8.0 mg (0.012 mmol) of **S6** dissolved in 1.6 ml of THF was added 0.4 mL of 0.5 N NaOH (*aq.*) at rt and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and the residue purified by preparatory TLC (CH_2Cl_2:MeOH, 10:1) to yield 5.5 mg (75%) of **44a**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.03 (s, 1H), 6.36 (d, *J* = 16.2 Hz, 1H), 6.11 (m, 1H), 5.70 (d, *J* = 10.1 Hz, 1H), 5.14 (br s, 2H), 3.88 (br s, 4H), 3.85 (s, 6H), 3.57-3.75 (m, 15H), 2.70 (br s, 2H), 2.62 (br s, 4H); HRMS (*m*/*z*): $[M+H]^+$ calcd. for $C_{25}H_{39}N_8O_7S$, 595.2662; found, 595.2684. HPLC: (a) H_2O + 0.1 % TFA (b) ACN + 0.1% TFA (5 to 95% b in 10 min.) R_t = 6.05 min.



2-(2-(2-(2-(4-(5-(4,6-Diaminopyrimidin-2-ylthio)-4,6-dimethoxypyrimidin-2-yl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)ethyl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (S7). 50.0 mg (0.0925 mmol) of **S5**, 90.0 mg (0.37 mmol) D-(+)-biotin, 11.3 mg (0.0925 mmol) DMAP, 153.0 mg (0.74 mmol) DCC in 15 ml of CH_2Cl_2 was sonicated for 13 h in a sealed tube. The reaction mixture was evaporated to dryness and the residue column chromatographed (CH_2Cl_2:MeOH-NH_3 (7N), 20:1 to 10:1) to give impure **S7** which was purified by preparatory TLC ($CHCl_3$:MeOH-NH_3 (7N), 10:1) to yield 60.0 mg (85%) of **S7**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.27 (br s, 1H), 5.18 (s, 1H), 4.82 (br s, 1H), 4.59 (br s, 4H), 4.49 (m, 1H), 4.32 (m, 1H), 4.22 (m, 2H), 3.88 (br s, 10H), 3.60-3.75 (m, 12H), 3.15 (m, 1H), 2.92 (m, 1H), 2.90 (m, 1H), 2.69 (m, 2H), 2.60 (m, 4H), 2.34 (t, *J* = 5.9 Hz, 2H), 1.37-1.78 (m, 6H); HRMS (*m*/*z*): $[M+H]^+$ calcd. for $C_{32}H_{51}N_{10}O_8S_2$, 767.3333; found 767.3361.

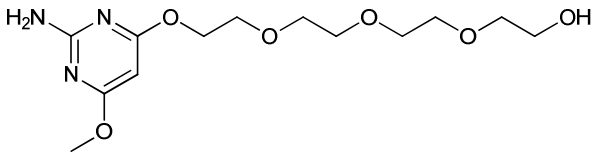


2-(2-(2-(2-(4-(5-(4-Acrylamido-6-aminopyrimidin-2-ylthio)-4,6-dimethoxypyrimidin-2-yl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)ethyl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (44b). To 50.0 mg (0.065 mmol) of **S7** in 10 ml of CH_2Cl_2 at 0°C was added 195.4 mg (271 µl, 1.953 mmol) of Et_3N. Then 17.7 mg (15.9 µl, 0.196 mmol) of acryloyl chloride was added at 0°C. After 1 hour an additional 17.7 mg (15.9 µl, 0.196 mmol) of acryloyl chloride was added. This was repeated four more times for a total reaction time of 6 hours (total acryloyl chloride, 106.2 mg, 95.4 µl, 1.17 mmol). The reaction mixture was concentrated under reduced pressure and the residue purified by preparatory TLC ($CHCl_3$:MeOH-NH_3 (7N), 10:1) to yield 22.0 mg (41%) of **44b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.99 (s, 1H), 7.09 (s, 1H), 6.40 (d, *J* = 16.8 Hz, 1H), 6.31 (dd, *J* = 16.7, 9.9 Hz, 1H), 5.79 (br s, 1H), 5.74 (d, *J* = 10.8 Hz, 1H), 5.09 (s, 2H), 5.08 (s, 1H), 4.50 (m, 1H), 4.36 (m, 1H), 4.21 (t, *J* = 6.8 Hz, 2H), 3.87 (s, 10H), 3.6-3.75 (m, 12H), 3.16 (m, 1H), 2.91 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.74 (d, *J* = 12.8 Hz, 1H), 2.70 (t, *J* = 5.4 Hz, 2H), 2.61 (t, *J* = 4.8 Hz, 4H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.37-1.8 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.9, 170.4, 169.5, 164.1, 164.0, 163.2, 159.3, 156.3, 130.3, 127.9, 88.1, 78.9, 69.9, 69.8, 69.7, 68.5, 68.0, 62.8, 61.4, 59.6, 57.7, 57.1, 54.9, 53.5, 52.5, 42.9, 39.9, 33.1, 27.8, 27.6, 24.0; HRMS (*m*/*z*): $[M+H]^+$ calcd. for $C_{35}H_{53}N_{10}O_9S_2$, 821.3438; found 821.3455; HPLC: (a) H_2O + 0.1 % TFA (b) ACN + 0.1% TFA (5 to 95% b in 10 min.) R_t = 6.98 min.

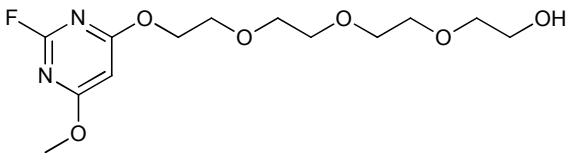


Scheme S2. Synthesis of **45a** and **45b**.

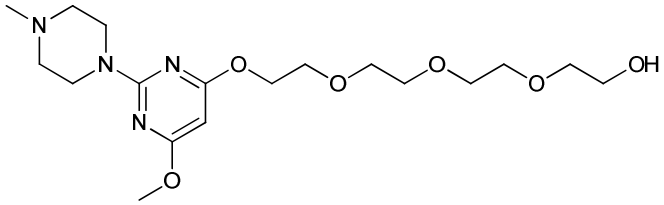
Reagents and conditions: (a) $H(OCH_2CH_2)_4OH$, NaH, DMF, 80°C, 3 h, 83%; (b) HF/pyridine, $NaNO_2$, 0°C, 42%; (c) 1-methylpiperazine, DMF, 90°C, 1 h, 91%; (d) NIS, CH_3CN, rt, 1.5 h, 85%; (e) 4,6-diamino-2-mercaptopyrimidine, neocuproine, CuI, K_3PO_4, DMSO, 150°C, 2.5 h, 73%; (f) acryloyl chloride, Et_3N, CH_2Cl_2, 0°C to rt, 8 h, 38%; (g) NaOH, THF, H_2O, rt, 6 h, 52%; (h) D-biotin, DCC, DMAP, CH_2Cl_2, sonicate, 8 h, 72%.



2-(2-(2-(2-(2-Amino-6-methoxypyrimidin-4-yloxy)ethoxy)ethoxy)ethoxy)ethanol (S9). To 7.28 g (37.5 mmol) of tetraethylene glycol dissolved in 20 mL of DMF was added 0.900 g (37.5 mmol) of NaH and the resulting suspension was stirred for 10 minutes at rt. Then 2.0 g (12.5 mmol) of 2-amino-4-chloro-6-methoxypyrimidine **(S8)** was added and the reaction mixture heated at 80°C for 3 h. Solvent was removed under reduced pressure and the oily residue was purified by column chromatography (EtOAc:MeOH, 100:0 to 95:5) to give 3.30 g (83%) of an oil **S9**. TLC (EtOAc:MeOH, 95:5 v/v): R_f = 0.24; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.48 (s, 1H), 5.08 (br s, 2H), 4.39 (t, *J* = 4.8 Hz, 2H), 3.83 (s, 3H), 3.79 (t, *J* = 4.8 Hz, 2H), 3.60-3.73 (m, 12H), 3.21 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.6, 172.0, 162.4, 80.2, 72.8, 70.86, 70.80, 70.77, 70.57, 69.7, 65.6, 61.8, 53.9; MS (*m*/*z*): $[M+H]^+$ 318.1.

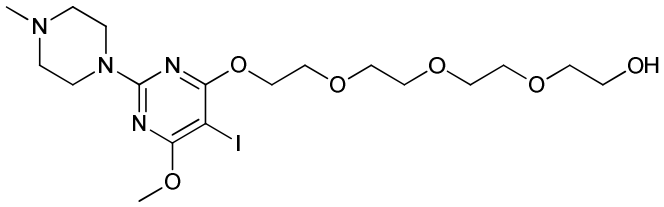


2-(2-(2-(2-(2-Fluoro-6-methoxypyrimidin-4-yloxy)ethoxy)ethoxy)ethoxy)ethanol (S10). 1.55 g (4.88 mmol) of **S9** was added to a plastic tube fitted with a stir bar and cooled to 0°C. Then a solution of HF/pyridine (1.22 ml, 48.8 mmol) was added. After several minutes 0.505 g (7.32 mmol) of $NaNO_2$ was added in portions over a period of 20 minutes with stirring. It was vigorously stirred for an additional 70 minutes at 0°C and at rt for 3 h. Then 15 ml of CH_2Cl_2 and 4.88 g of $CaCO_3$ (48.8 mmol) were added and the mixture was stirred for 5 h at rt. It was then filtered over a cintered disc funnel and the solid washed with EtOAc (4 x 25 ml). The combined filtrate was filtered over celite, concentrated under reduced pressure and the oily residue was purified by column chromatography (EtOAc:MeOH, 100:0 to 95:5) to give 0.65 g (42%) of an oil **S10**. TLC (EtOAc): R_f = 0.19; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.99 (s, 1H), 4.48-4.50 (m, 2H), 3.95 (s, 3H), 3.80-3.84 (m, 2H), 3.58-3.75 (m, 12H), 2.56 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.7 (d, *J* = 15.7 Hz), 173.0 (d, *J* = 15.7 Hz), 161.6 (d, *J* = 215.9 Hz), 88.0 (d, *J* = 6.8 Hz), 72.5, 70.70, 70.68, 70.57, 70.37, 69.2, 66.6, 61.8, 54.7; MS (*m*/*z*): $[M+H]^+$ 321.2.

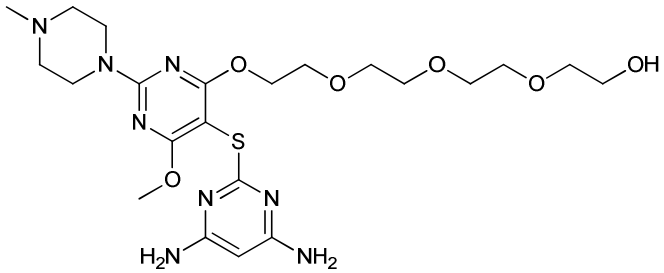


2-(2-(2-(2-(6-Methoxy-2-(4-methylpiperazin-1-yl)pyrimidin-4-yloxy)ethoxy)ethoxy)ethoxy)ethanol (S11). 0.55 g (1.72 mmol) of **S10** was dissolved in 40 ml of DMF and 1.72 g (17.2 mmol) of 1-methylpiperazine was added and heated at 90°C for 1 h. Solvent and excess reagent were removed under reduced pressure and the oily residue was

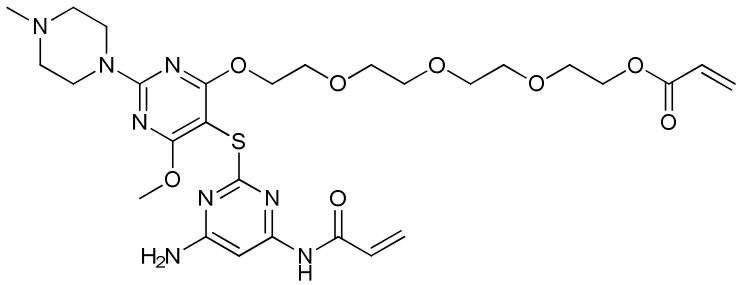
purified by column chromatography (CH_2Cl_2:MeOH-NH_3 (7N), 20:1) to give 0.63 g (91%) of an oil **S11**. TLC (CH_2Cl_2:MeOH-NH_3 (7N), 20:1): R_f = 0.24; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.40 (s, 1H), 4.42 (t, *J* = 5.0 Hz, 2H), 3.85 (s, 3H), 3.80 (t, *J* = 5.1 Hz, 4H), 3.65-3.73 (m, 12H), 3.60 (t, *J* = 4.5 Hz, 2H), 2.44 (t, *J* = 5.1 Hz, 4H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.0, 171.3, 160.7, 78.5, 72.6, 71.8, 70.64, 70.55, 70.33, 69.5, 65.1, 61.7, 54.9, 53.5, 46.2, 43.7; MS (*m*/*z*): $[M+H]^+$ 401.3.



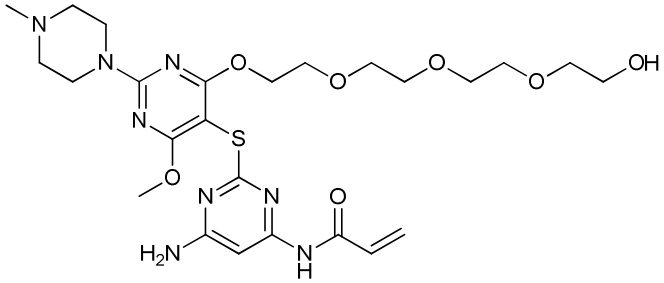
2-(2-(2-(2-(5-Iodo-6-methoxy-2-(4-methylpiperazin-1-yl)pyrimidin-4-yloxy)ethoxy)ethoxy)ethoxy)ethanol (S12). To 0.600 g (1.50 mmol) of **S11** dissolved in 20 ml CH_3CN was added 0.581 g (2.58 mmol) of N-iodosuccinimide and the solution was stirred for 1.5 h at rt. Solvent was removed under reduced pressure and the oily residue was purified by column chromatography ($CHCl_3$:MeOH:Et_3N, 90:10:2) to give 0.670 g (85%) of an oil **S12**. TLC ($CHCl_3$:MeOH:Et_3N, 90:10:2): R_f = 0.29; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.46 (t, *J* = 4.7 Hz, 2H), 3.92 (s, 3H), 3.86 (m, 4H), 3.57-3.83 (m, 14H), 2.46 (m, 4H), 2.33 (s, 3H); MS (*m*/*z*): $[M+H]^+$ 527.2.



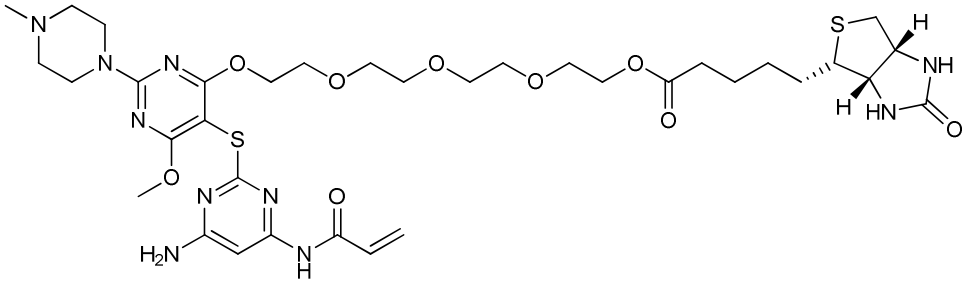
2-(2-(2-(2-(5-(4,6-Diaminopyrimidin-2-ylthio)-6-methoxy-2-(4-methylpiperazin-1-yl)pyrimidin-4-yloxy)ethoxy)ethoxy)ethoxy)ethanol (S13). 0.620 g (1.18 mmol) of **S12**, 0.501 g (2.36 mmol) of K_3PO_4, 0.053 g (0.236 mmol) of neocuproine, 0.045 g (0.236 mmol) of CuI, and 0.184 g (1.30 mmol) of 4,6-diamino-2-mercaptopyrimidine in 14 ml DMSO was heated at 150°C for 2.5 h. Solvent was removed under reduced pressure and the residue was purified by column chromatography ($CHCl_3$:MeOH:Et_3N, 99:1:2 to 95:5:2) to give 0.465 g (73%) of **S13**. TLC ($CHCl_3$:MeOH:Et_3N, 85:15:2): R_f = 0.35; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.18 (s, 1H), 4.94 (br s, 4H), 4.45 (t, *J* = 4.2 Hz, 2H), 3.87 (s, 3H), 3.80-3.86 (br s, 4H), 3.74 (t, *J* = 4.2 Hz, 2H), 3.69 (t, *J* = 4.7 Hz, 2H), 3.51-3.63 (m, 10H), 2.46 (t, *J* = 4.6 Hz, 4H), 2.35 (s, 3H); MS (*m*/*z*): $[M+H]^+$ 541.4.



2-(2-(2-(2-(5-(4-Acrylamido-6-aminopyrimidin-2-ylthio)-6-methoxy-2-(4-methylpiperazin-1-yl)pyrimidin-4-yloxy)ethoxy)ethoxy)ethoxy)ethyl acrylate (S14). To 0.100 g (0.185 mmol) of **S13** in 2 ml of CH_2Cl_2 at 0°C was added 0.037 g (51 µl, 0.370 mmol) of Et_3N. Then 0.117 g (105 µl, 1.30 mmol) of acryloyl chloride was added at 0°C. After 5 minutes the ice-bath was removed and stirring continued at rt. After 2 h additional 0.037 g (51 µl, 0.370 mmol) of Et_3N and 0.050 g (45 µl, 0.555 mmol) of acryloyl chloride were added and stirring continued for an additional 6 h. The reaction mixture was concentrated under reduced pressure and the residue purified by preparatory TLC (CH_2Cl_2:MeOH-NH_3 (7N), 12:1) to yield 0.049 g (38%) of **S14**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (br s, 1H), 7.06 (s, 1H), 6.39-6.42 (m, 2H), 6.10-6.27 (m, 2H), 5.76-5.83 (m, 2H), 4.92 (s, 2H), 4.49 (br s, 2H), 4.29 (br s, 2H), 3.89 (s, 3H), 3.86 (s, 4H), 3.51-3.74 (m, 12H), 2.48 (s, 4H), 2.37 (s, 3H); MS (*m*/*z*): $[M+H]^+$ 649.4.



N-(6-Amino-2-(4-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)-6-methoxy-2-(4-methylpiperazin-1-yl)pyrimidin-5-ylthio)pyrimidin-4-yl)acrylamide (45a). To 0.042 g (0.0647 mmol) of **S14** dissolved in 0.8 ml of THF was added 0.2 mL of 0.5 N NaOH (*aq.*) at rt and stirred for 6 h. The reaction mixture was concentrated under reduced pressure and the residue purified by preparatory TLC ($CHCl_3$:MeOH-NH_3 (7N), 10:1) to yield 0.020 g (52%) of **45a**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.07 (s, 1H), 6.42 (d, *J* = 16.7 Hz, 1H), 6.24 (dd, *J* = 16.7, 10.3 Hz, 1H), 5.77 (d, *J* = 10.3 Hz, 1H), 4.95 (br s, 2H), 4.49 (br s, 2H), 3.90 (s, 3H), 3.86 (br s, 4H), 3.51-3.74 (m, 14H), 2.47 (br s, 4H), 2.36 (s, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd. for $C_{25}H_{39}N_8O_7S$, 595.2662; found, 595.2658; HPLC: (a) H_2O + 0.1 % TFA (b) ACN + 0.1% TFA (5 to 95% b in 10 min.) R_t = 5.05 min.



2-(2-(2-(2-(5-(4-Acrylamido-6-aminopyrimidin-2-ylthio)-6-methoxy-2-(4-methylpiperazin-1-yl)pyrimidin-4-yloxy)ethoxy)ethoxy)ethoxy)ethyl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (45b). 5.0 mg (0.0084 mmol) of **45a**, 7.0 mg (0.0287 mmol) D-(+)-biotin, 1.0 mg (0.0084 mmol) DMAP, 14.0 mg (0.0679 mmol) DCC in 2 ml of CH_2Cl_2 was sonicated for 8 hours in a sealed tube. The reaction mixture was concentrated under reduced pressure and the residue purified by preparatory TLC ($CHCl_3$:MeOH-NH_3 (7N), 10:1) to yield 5.0 mg (72%) of **45b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.58 (s, 1H), 7.15 (s, 1H), 6.41-6.43 (m, 2H), 6.19 (br s, 1H), 5.77 (br s, 1H), 5.73 (dd, *J* = 7.3, 4.3 Hz, 1H), 5.31 (br s, 2H), 4.5-4.6 (m, 2H), 4.41-4.47 (m, 1H), 4.35- 4.4 (m, 1H), 4.15-4.25 (m, 2H), 3.85-3.93 (br s, 7H), 3.5-3.75 (m, 12H), 3.14-3.19 (m, 1H), 2.93 (dd, *J* = 12.9, 4.9 Hz, 1H), 2.84 (d, *J* = 12.7 Hz, 1H), 2.56 (br s, 4H), 2.41 (s, 2H), 2.28 (t, *J* = 7.5 Hz, 3H), 1.37-1.78 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.2, 170.7, 170.2, 169.4, 164.6, 164.3, 163.7, 159.5, 156.6, 130.6, 128.3, 88.3, 77.2, 70.5, 70.2, 70.1, 69.3, 68.8, 65.8, 63.1, 61.8, 59.9, 55.2, 54.2, 53.8, 45.4, 42.9, 40.1, 33.4, 29.3, 27.9, 27.8, 24.4; HRMS (*m*/*z*): $[M+H]^+$ calcd. for $C_{35}H_{53}N_{10}O_9S_2$, 821.3438; found, 821.3439; HPLC: (a) H_2O + 0.1 % TFA (b) ACN + 0.1% TFA (5 to 95% b in 10 min.) R_t = 7.10 min.