Thermodynamic Analysis of mRNA Cap Binding by the Human Initiation Factor eIF4E via Free-Energy Perturbations

*Cristiano R. W. Guimarães [a], David J. Kopecky [b], Jeff Mihalic [b], Shanling Shen [c], Shawn Jeffries [c], Steve Thibault [a], Xiaoqi Chen [b], Nigel Walker [a], and Mario Cardozo [a]**

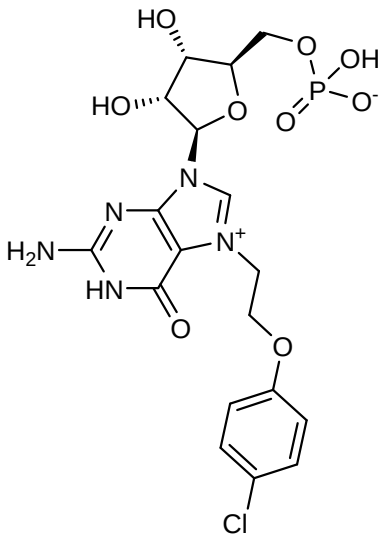
Contribution from the Departments of Molecular Structure [a], Medicinal Chemistry [b], and Oncology [c], Amgen, Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080.

Experimental Section

General Procedure. All commercial reagents were used as received. Reverse phase preparatory HPLC was performed on a Varian Prostar instrument with a Waters 30 x 150 mm Sunfire Prep C_{18} OBD 10 □m column. NMR spectra were recorded on Bruker Avance 400 and Bruker Avance 500 FT-NMR instruments. Proton NMR spectra were recorded in ppm and referenced to residual solvent: DMSO-d_6 (2.50 ppm). Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet), integration and coupling constant in Hertz (Hz). Multiplets (m) are reported over the range (ppm) at which they appear at the indicated field strength. Carbon NMR spectra were recorded in ppm relative to the solvent signal: DMSO-d_6 (39.5 ppm). Mass spectral

data was obtained on an Agilent 6210 LC-MS TOF spectrometer in either positive or negative modes with electrospray ionization.

Synthesis of compound **1**

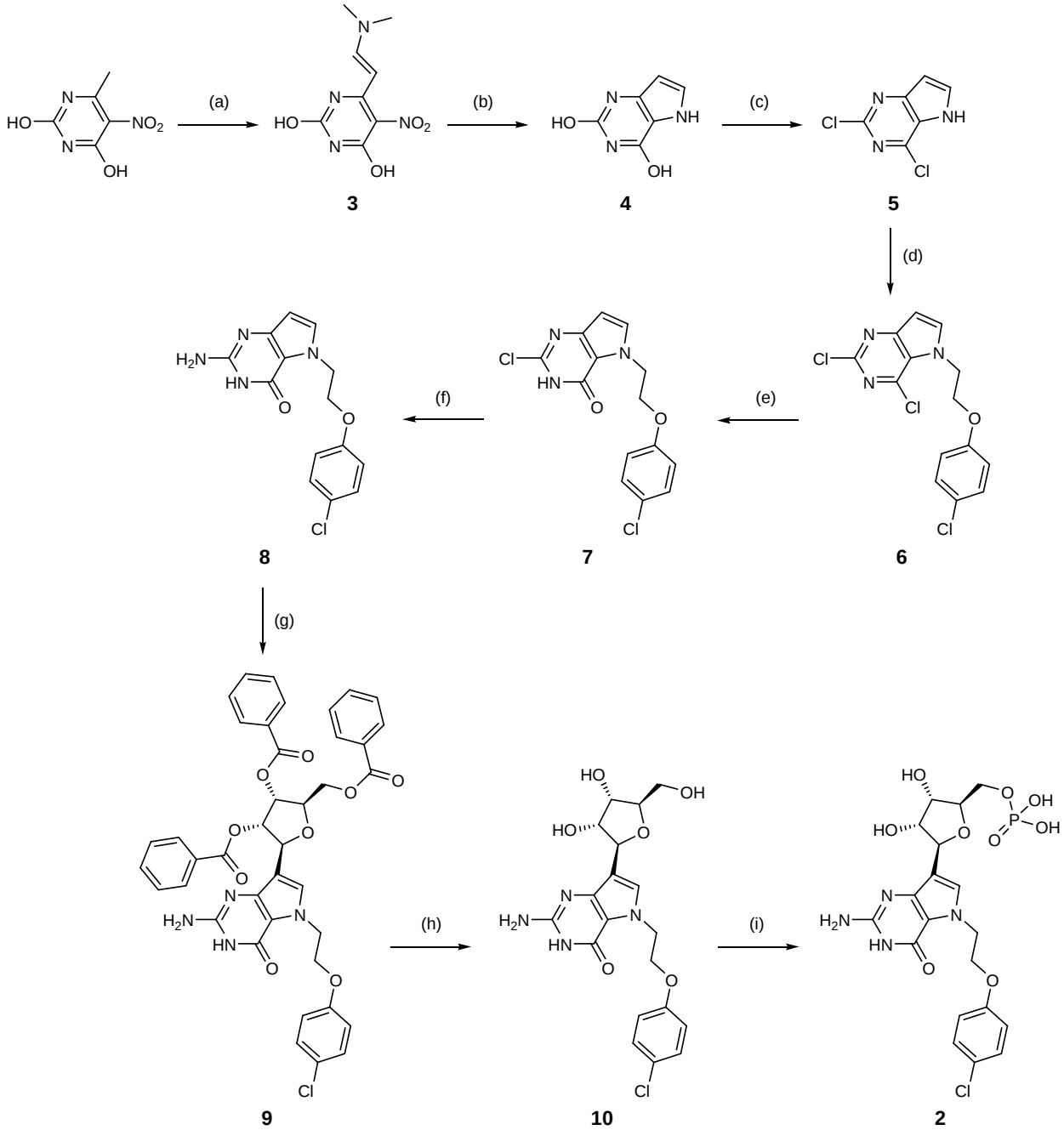


7-(4-chlorophenoxyethyl)guanosine-5'-monophosphate (1).

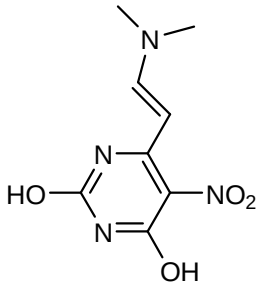
To a slurry of guanosine-5'-monophosphate, disodium salt hydrate (380 mg, 0.933 mmol) in DMSO (4.3 mL) added 4-chlorophenyl-2-bromoethyl ether (879 mg, 3.73 mmol). The resulting white slurry was heated 55 °C for 3.5 d; during this time all solids dissolved. The reaction mixture was purified directly by reverse phase prep. HPLC (gradient elution of 10% MeCN in water to 30% MeCN in water over a 30 min period, where both solvents contain 0.1% TFA). After discarding mixed fractions, 23 mg (4%) of the trifluoroactetate salt of 7-(4-chlorophenoxyethyl)guanosine-5'-monophosphate was isolated as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.95-4.05 (m, 2 H), 4.09-4.18 (m, 2 H), 4.20 (t, *J* = 4.7 Hz, 1 H), 4.40 (t, *J* = 5.1 Hz, 2 H), 4.47 (t, *J* = 3.9 Hz, 1 H), 4.77 (br s, 2 H), 5.86 (d, *J* = 3.9 Hz, 1 H), 6.93 (d, *J* = 9.0 Hz, 2 H), 7.26 (d, *J* = 8.6 Hz, 2

H), 7.47 (br s, 1 H), 9.65 (s, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.2, 156.5, 154.1, 150.0, 137.4, 129.9, 125.5, 117.0, 107.6, 89.9, 84.5, 74.7, 70.1, 66.1, 64.5, 48.8; HRMS-ESI (neg) calcd for $C_{18}H_{20}ClN_5O_9P$ [$\tilde{M}H^-$] 516.0687, found 516.0692.

Scheme 1. Synthesis of compound **2**.

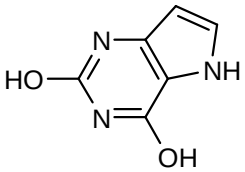


Reagents and conditions: (a) DMF-DMA, 100 °C; (b) Zn, HOAc, 100 °C; (c) phenylphosphonic dichloride, neat, 180 °C; (d) NaH, 4-chlorophenyl-2-bromoethyl ether, MeCN, 55 °C; (e) aq.1 M NaOH, 1,4-dioxane, reflux; (f) 7 N NH_3 in MeOH, 170 °C; (g) $SnCl_4$, beta-d-ribofuranose 1-acetate 2,3,5-tribenzoate, $MeNO_2$, 60 °C; (h) NaOMe, MeOH, 23 °C; (i) $POCl_3$, $PO(MeO)_3$; 0 °C.



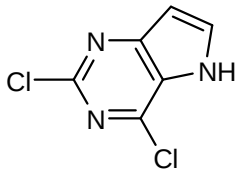
(E)-6-(2-(dimethylamino)vinyl)-5-nitropyrimidine-2,4-diol (3).

Dimethoxy-N,N-dimethylmethanamine (13.0 g, 109 mmol) was added to a DMF solution (75 mL) containing 6-methyl-5-nitropyrimidine-2,4-diol (15.5 g, 91 mmol), and the resulting mixture was stirred for 3 h at 100 °C. Excess DMF was then removed using reduced pressure, and water was added to the residue to produce a brown precipitate. This solid (13.4 g, 65%) was isolated and used in next step without purification: ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.90 (br s, 3 H), 3.16 (br s, 3 H), 5.38 (d, *J* = 12.8 Hz, 1 H), 8.09 (d, *J* = 12.8 Hz, 1 H), 10.69 (br s, 1 H), 10.99 (br s, 1 H).



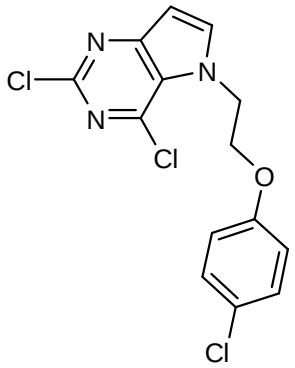
5H-pyrrolo[3,2-*d*]pyrimidine-2,4-diol (4).

Zinc (1.6 mL, 176 mmol) was added to a acetic acid solution (300 mL) containing (E)-6-(2-(dimethylamino)vinyl)-5-nitropyrimidine-2,4-diol (13.4 g, 59 mmol). The resulting mixture was stirred at 0 °C for 2 hours then heated at 100 °C for 15 h. The solution was then concentrated, and the resulting solid was washed with water and dried with ether. 8.7 g (97%) of 5H-pyrrolo[3,2-*d*]pyrimidine-2,4-diol was obtained: ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.84 (d, *J* = 2.44 Hz, 1 H), 7.13 (d, *J* = 2.44 Hz, 1 H), 10.55 (br. s, 1 H), 10.63-10.92 (m, 1 H), 11.59-12.00 (m, 1 H).



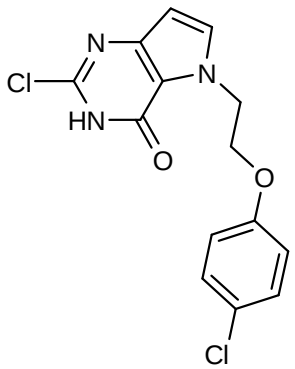
2,4-dichloro-5H-pyrrolo[3,2-*d*]pyrimidine (5).

5H-pyrrolo[3,2-*d*]pyrimidine-2,4-diol (8.0 g, 52.9 mmol) was added to phenylphosphonic dichloride (39.3 mL, 280.6 mmol), and the resulting mixture was stirred at 180 °C. After 3 h., the reaction was poured onto ice and extracted with ethyl acetate (2 X 500 mL). The organic layers were then combined and washed with 1 N NaOH, dried over sodium sulfate, and concentrated. The remaining solid (5.24 g, 53%) was collected and washed with a small amount of toluene. This material was used in the next step without further purification: ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.73 (d, *J* = 3.05 Hz, 1 H), 8.11 (d, *J* = 3.05 Hz, 1 H), 12.76 (br. s, 1 H); MS-ESI (neg) calcd for $C_6H_2Cl_2N_3$ [$\tilde{M}\bar{H}$] 186, found 186.



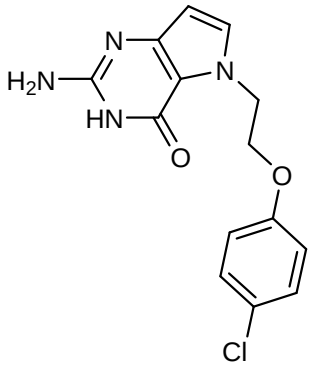
2,4-dichloro-5-(2-(4-chlorophenoxy)ethyl)-5H-pyrrolo[3,2-*d*]pyrimidine (6).

Sodium hydride (60 % in mineral oil) (0.28 mL, 6.7 mmol) was added to an acetonitrile solution (75 mL) containing 2,4-dichloro-5H-pyrrolo[3,2-*d*]pyrimidine (1.00 g, 5.3 mmol). After stirring for 20 min. at room temperature, 4-chlorophenyl 2-bromoethyl ether (1.5 g, 6.4 mmol) was added, and the resulting mixture was stirred overnight at 55 °C. The solution was then concentrated and purified on silica gel (gradient elution of 10% EtOAc in hexanes to pure EtOAc) to give 1.7 g (94%) of 2,4-dichloro-5-(2-(4-chlorophenoxy)ethyl)-5H-pyrrolo[3,2-*d*]pyrimidine. MS-ESI (pos) calcd for $C_{14}H_{11}Cl_3N_3O$ [MH] 342, found 342.



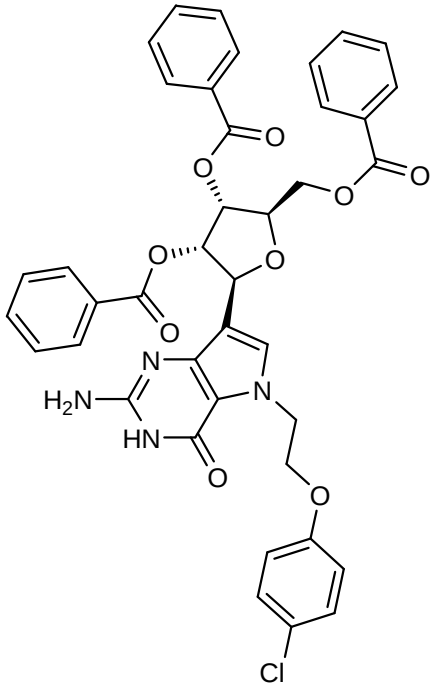
2-Chloro-5-(2-(4-chlorophenoxy)ethyl)-5H-pyrrolo[3,2-*d*]pyrimidin-4-ol (7).

Aqueous 1 M sodium hydroxide (6 mL, 6 mmol) was added to a dioxane solution (60 mL) containing 2,4-dichloro-5-(2-(4-chlorophenoxy)ethyl)-5H-pyrrolo[3,2-*d*]pyrimidine (2.13 g, 6 mmol). The resulting mixture was stirred at reflux for 3 h, then cooled to room temperature, and neutralized with acetic acid. After cooling the mixture on ice, a precipitate formed and was collected (1.98 g, 99%). This material was used in the next step without further purification. MS-ESI (neg) calcd for $C_{14}H_{10}Cl_2N_3O_2$ [M$\bar{H}$] 322, found 322.



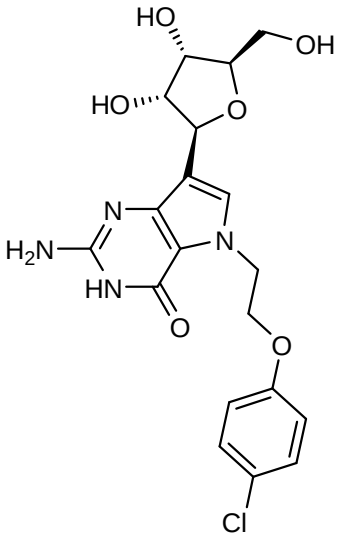
7-(4-chlorophenoxyethyl)-9-deazaguanine (8).

2-Chloro-5-(2-(4-chlorophenoxy)ethyl)-5H-pyrrolo[3,2-*d*]pyrimidin-4-ol (2 g, 6 mmol) was suspended in a methanolic ammonia solution (7 N, 50 mL), sealed in a Parr steel reactor, and heated at 170 °C overnight. The reaction was cooled to room temperature and purified on silica gel (gradient elution of pure DCM to 20% DCM in MeOH) to give 1.56 g (84%) of 7-(4-chlorophenoxyethyl)-9-deazaguanine: MS-ESI (pos) calcd for $C_{14}H_{14}ClN_4O_2$ [M$\dot{H}$] 305, found 305.



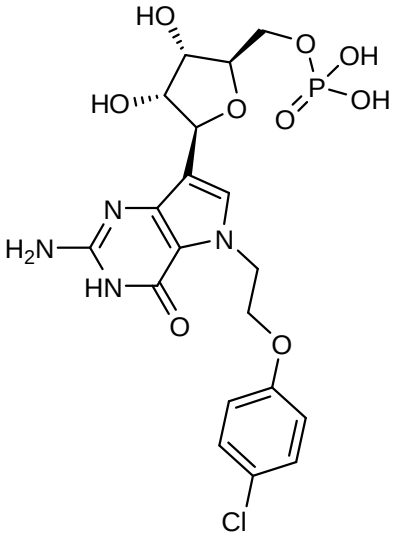
7-(4-chlorophenoxyethyl)-9-deazaguanosine-3',4',5'-tribenzoate (9).

Tin(IV)chloride (190 µL, 1.6 mmol) was added to a nitromethane (20 mL) solution containing beta-d-ribofuranose 1-acetate 2,3,5-tribenzoate (350 mg, 0.69 mmol) and 7-(4-chlorophenoxyethyl)-9-deazaguanine (180 mg, 0.58 mmol). The resulting mixture was stirred for 3 h at 60 °C. Excess solvent was then removed using reduced pressure, and the remaining residue was purified on silica gel (gradient elution of pure DCM to 20% DCM in MeOH) to give 100 mg (26%) of 7-(4-chlorophenoxyethyl)-9-deazaguanosine-3',4',5'-tribenzoate: MS-ESI (pos) calcd for $C_{40}H_{34}ClN_4O_9$ [MH] 749, found 749.



7-(4-chlorophenoxyethyl)-9-deazaguanosine (10).

Sodium methoxide (81 mg, 1.5 mmol) was added to a methanol solution (10 mL) containing 7-(4-chlorophenoxyethyl)-9-deazaguanosine-3',4',5'-tribenzoate (110 mg, 0.15 mmol). The resulting mixture was stirred overnight at room temperature and purified directly by reverse phase prep. HPLC (gradient elution of 5% MeCN in water to 70% MeCN in water over a 30 min period, where both solvents contain 0.1% TFA). After discarding mixed fractions, 40 mg (61%) of 7-(4-chlorophenoxyethyl)-9-deazaguanosine was obtained: MS-ESI (pos) calcd for $C_{19}H_{22}ClN_4O_6$ [MH] 437, found 437.



7-(4-chlorophenoxyethyl)-9-deazaguanosine-5'-monophosphonic acid (2).

Phosphorus oxychloride (29 µL, 309 µmol) and trimethylphosphate (12 µl, 103 µmol) were premixed at 0 °C for 10 min then added to 7-(4-chlorophenoxyethyl)-9-deazaguanosine (45 mg, 103 µmol). The mixture was stirred for 4 h then diluted with water and purified directly by reverse phase prep. HPLC (gradient elution of 5% MeCN in water to 50% MeCN in water over a 30 min period, where both solvents contain 0.1% TFA). After discarding mixed fractions, 12 mg (23%) of 7-(4-chlorophenoxyethyl)-9-deazaguanosine-5'-monophosphonic acid was obtained: ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.81-3.89 (m, 1 H), 3.89-4.06 (m, 4 H), 4.28 (t, *J* = 5.5 Hz, 2 H), 4.56-4.64 (m, 2 H), 4.66 (d, *J* = 7.3 Hz, 1 H), 6.96 (d, *J* = 9.2 Hz, 2 H), 7.31 (d, *J* = 9.2 Hz, 2 H), 7.42 (s, 1 H), 7.69-7.97 (m, 2 H). MS-ESI (pos) calcd for $C_{19}H_{23}ClN_4O_9P$ [MH] 517, found 517.