

Supporting Information

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Table of Contents

S1	Title Page
S2	Biological Methods
S3	Preparation of analogs
S12	Preparation of additional compounds
S20	Table S1
S22	Table S2
S23	Table S3
S24	Table S4
S25	Table S5
S27	Table S6
S28	References

Biological Methods

Assays of other kinases

Assays of 22 protein kinases were performed by the Wyeth Screening Sciences Kinase Profiling group (Wyeth Research, Collegeville, PA). The assays employed the commercially purchased enzymes ABL1, Aurora B, CDK1, CDK2, CHK1, CK1gamma1, ERK2, FYN, GCK, HCK, LYN, MET, MK2, p38alpha, PDGFRalpha, PKA, PKCalpha, ROCK1, RSK1, SRC, VEGFR2 (Invitrogen) and IKKalpha (Upstate Biotechnology) and various fluorescein-labeled substrate peptides. The assays were performed in 384-well plate format with enzyme, 1.5 μ M substrate peptide, ATP (2x K_m value), and various inhibitors for 1 h, and results were detected by the Caliper LabChip 3000 chip-based mobility shift electrophoresis assay platform (Caliper LifeSciences).

Other tumor cell lines growth assays

Cell proliferation inhibition assays were performed as previously described.¹

Preparation of analogs

6-(1H-indol-5-yl)-4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (6a): Yield: 29 mg (0.049 μ mol, 41%). LCMS: 93% pure, RT 2.52 min, m/z 397.2 ([M+H]⁺). HRMS: m/z 397.17685 ([M+H]⁺). For [M+H]⁺ mass error = -0.29 mmu.

3-(4-morpholino-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline (6b): Yield: 32 mg (0.087 μ mol, 72%). LCMS: 87% pure, RT 2.19 min, m/z 373.2 ([M+H]⁺).

6-(4-morpholino-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)pyridin-2-amine (6e): Yield: 12 mg (0.031 μ mol, 31%). LCMS: 99.8% pure, RT 1.90 min, m/z 374.2 ([M+H]⁺).

6-(4-morpholino-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)pyridin-3-amine (6f): Yield: 14 mg (0.037 μ mol, 37%). LCMS: 100% pure, RT 1.93 min, m/z 374.2 ([M+H]⁺).

N'-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-N,N-dimethylurea (16b):

To commercially available 4-isocyanatophenyl boronic acid, pinacol ester (49 mg, 0.2 mmol) in a microwave vial was added a 2N solution of dimethylamine in THF (1 mL) and the mixture was stirred for 0.5 h. The mixture was concentrated and to the resulting ureidophenyl boronic acid, pinacol ester **15b** was added 4-(1-(1-benzylpiperidin-4-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-yl)morpholine hydrochloride (**12**, 50 mg, 0.12 mmol) and 0.25 mL of a 2M aqueous solution of sodium carbonate. Tetrakis(triphenylphosphine)palladium(0) (14 mg, 0.1 eq) was added and the mixture was heated for 10 min at 185 °C under microwave irradiation. The mixture was concentrated, dissolved in DMSO, filtered and purified by reversed phase

HPLC (TFA buffers) to give the target compound as the trifluoroacetate salt (50 mg, 0.076 mmol, 63%). LCMS: 100% pure, RT 1.98 min, m/z 541.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-3-methoxyurea (16c): methoxylamine.HCl (167 mg, 2.0 mmol) was stirred in 2 mL DCM and 2 mL of a 1N aqueous solution of sodium hydroxide prior to addition of 4-isocyanatophenylboronic acid, pinacol ester. The mixture was diluted with dichloromethane and water and the organic phase was transferred to a microwave vial and concentrated. The ureidophenyl boronic acid, pinacol ester was dissolved in dimethoxyethane (2 mL) prior to Suzuki-Miyaura coupling. Yield: 12 mg (18 µmol, 15%). LCMS: 93% pure, RT 1.94 min, m/z 543.3 ([M+H]⁺).

N-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-2,2-dimethylhydrazinecarboxamide (16d): 1,1-dimethylamine (152 µL, 2.0 mmol) was stirred in 2 mL DCM with 4-isocyanatophenylboronic acid, pinacol ester for 1.5 h. The mixture was diluted with dichloromethane and water and the organic phase was transferred to a microwave vial and concentrated. The ureidophenyl boronic acid, pinacol ester was dissolved in dimethoxyethane (2 mL) prior to Suzuki-Miyaura coupling. Yield: 15 mg (19 µmol, 15%). LCMS: 98% pure, RT 1.99 min, m/z 556.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}urea (16e): 2 mL of a 0.5N solution of ammonia in dioxane was used. Excess amine and solvent were removed under a stream of nitrogen and the ureidophenyl boronic acid, pinacol ester was dissolved in dimethoxyethane (2 mL) prior to Suzuki-Miyaura coupling. Yield: 5 mg (8 µmol, 7%). LCMS: 100% pure, RT 1.81 min, m/z 513.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl}-3-ethylurea (16f): 1 mL of a 2N solution of ethylamine in THF was used. Excess amine and solvent were removed under a stream of nitrogen and the ureidophenyl boronic acid, pinacol ester was dissolved in dimethoxyethane (2 mL) prior to Suzuki-Miyaura coupling. Yield: 60 mg (92 μ mol, 76%). LCMS: 98% pure, RT 1.91 min, m/z 541.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl}-3-(2-fluoroethyl)urea (16g): 2-fluoroethylamine.HCl (199 mg, 2.0 mmol) was stirred in 2 mL DCM and 2 mL of a 1N aqueous solution of sodium hydroxide prior to addition of 4-isocyanatophenylboronic acid, pinacol ester. The mixture was diluted with dichloromethane and water and the organic phase was transferred to a microwave vial and concentrated. The ureidophenyl boronic acid, pinacol ester was dissolved in dimethoxyethane (2 mL) prior to Suzuki-Miyaura coupling. Yield: 7 mg (11 μ mol, 9%). LCMS: 100% pure, RT 1.94 min, m/z 559.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl}-3-isopropylurea (16h): isopropylamine (42 μ L, 0.5 mmol) in 1 mL dimethoxyethane was used. Excess amine and solvent were removed under a stream of nitrogen and the ureidophenyl boronic acid, pinacol ester was dissolved in dimethoxyethane (2 mL) prior to Suzuki-Miyaura coupling. Yield: 25 mg (35 μ mol, 29%). LCMS: 87% pure, RT 1.97 min, m/z 555.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl}-3-(2-hydroxyethyl)urea (16i): A 1N stock solution of amine was prepared by diluting 1 mmol of ethanolamine (60 μ L) with dimethoxyethane to a total volume of 1.0 mL. 0.2 mL of the stock solution (0.2 mmol amine), diluted with 1 mL of dimethoxyethane, was

used to react with the isocyanatophenylboronate. The crude solution of ureidophenyl boronic acid, pinacol ester was used in the Suzuki-Miyaura coupling. Yield: 40 mg (59 μ mol, 49%).

LCMS: 100% pure, RT 1.79 min, m/z 557.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-3-(2-methoxyethyl)urea (16j): A 1N stock solution of amine was prepared by diluting 1 mmol of 2-methoxyethylamine (87 μ L) with dimethoxyethane to a total volume of 1.0 mL. 0.2 mL of the stock solution (0.2 mmol amine), diluted with 1 mL of dimethoxyethane, was used to react with the isocyanatophenylboronate. The crude solution of ureidophenyl boronic acid, pinacol ester was used in the Suzuki-Miyaura coupling. Yield: 38 mg (56 μ mol, 47%). LCMS: 99% pure, RT 1.88 min, m/z 571.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-3-[2-(dimethylamino)ethyl]urea (16k): A 1N stock solution of amine was prepared by diluting 1 mmol of N,N-dimethylethylenediamine (109 μ L) with dimethoxyethane to a total volume of 1.0 mL. 0.2 mL of the stock solution (0.2 mmol amine), diluted with 1 mL of dimethoxyethane, was used to react with the isocyanatophenylboronate. The crude solution of ureidophenyl boronic acid, pinacol ester was used in the Suzuki-Miyaura coupling. Yield: 39 mg of the bis-trifluoroacetate salt (48 μ mol, 40%). LCMS: 100% pure, RT 1.65 min, m/z 584.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-3-(1-methylpiperidin-4-yl)urea (16l): A 1N stock solution of amine was prepared by diluting 1 mmol of 4-amino-1-methylpiperidine (114 mg) with dimethoxyethane to a total volume of 1.0 mL. 0.2 mL of the stock solution (0.2 mmol amine), diluted with 1 mL of dimethoxyethane, was used to react with the isocyanatophenylboronate. The crude solution of

ureidophenyl boronic acid, pinacol ester was used in the Suzuki-Miyaura coupling. Yield: 33 mg of the bis-trifluoroacetate salt (39 μ mol, 33%). LCMS: 100% pure, RT 1.68 min, m/z 610.4 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-3-phenylurea (16m): aniline (18 μ L, 0.2 mmol) in 1 mL dimethoxyethane was used. The crude ureidophenyl boronic acid, pinacol ester was diluted with dimethoxyethane (1 mL) prior to Suzuki-Miyaura coupling. Yield: 15 mg (22 μ mol, 18%). LCMS: 92% pure, RT 2.10 min, m/z 589.3 ([M+H]⁺).

1-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenyl}-3-pyridin-3-ylurea (16n): 3-aminopyridine (19 mg, 0.2 mmol) in 1 mL dimethoxyethane was used. The crude ureidophenyl boronic acid, pinacol ester was diluted with dimethoxyethane (1 mL) prior to Suzuki-Miyaura coupling. Yield: 4 mg (6 μ mol, 5%). LCMS: 92% pure, RT 1.81 min, m/z 590.3 ([M+H]⁺).

2-hydroxyethyl (4-{4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl)carbamate (22b): Prepared using the same method as described for **22a**, using 0.77 mmol of 4-(4-morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline hydrochloride with 20 eq (15 mmol) of ethylene glycol. The product was obtained after HPLC purification (TFA buffers) as the TFA salt. Yield 63 mg. LCMS: 100% pure, RT 1.68 min, m/z 559.3 ([M+H]⁺).

1-methyl-3-(4-(4-morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenyl)urea (22c):

To 4-(4-Morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline hydrochloride (**21**, 685 mg, 1.35 mmol) was added dichloromethane (10 mL) and triethylamine (0.75 mL) to give a yellow solution. Triphosgene (201 mg) was added and the mixture was stirred at ambient temperature for 5 min. A 2N solution of methylamine in THF was added (13.5 mL, 27 mmol) and the mixture was stirred for 30 min. The solvents were concentrated. The crude product was applied to a silica gel column and eluted with a gradient of methanol (0-20%) in ethyl acetate containing 1% triethylamine to give the title compound as 216 mg (0.41 mmol, 30%) of an off-white solid. LCMS: 100% pure, RT 1.77 min, m/z 528.3 ([M+H]⁺). HRMS: m/z 528.28165 ([M+H]⁺). Exptl - Calc'd = -1.35 mmu. ¹H-NMR (DMSO-d₆): δ 11.62 (1H, bs), 9.18 (1H, bs), 9.10 (1H, bs), 8.97 (1H, d, J=4.4 Hz), 8.75 (1H, d, J=7.2 Hz), 8.32 (3H, t, J=8.0 Hz), 8.06 (1H, t, J=6.6 Hz), 7.52 (2H, d, J=8.8 Hz), 5.09 (1H, m), 4.60 (2H, s), 3.99 (4H, m), 3.78 (4H, m), 3.59 (2H, m), 3.36 (2H, m), 2.66 (3H, s), 2.60 (2H, d, J=12.8 Hz), 2.16 (2H, d, J=12.8 Hz).

1-methoxy-3-(4-{4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl)urea (22d): Prepared using the same method as described for **22a**, using 49 mg (0.11 mmol) of 4-(4-morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline (**21**) and 160 mg (1.92 mmol) methoxylamine.HCl in 1.92 mL 1N NaOH. The product was obtained after HPLC purification (TFA buffers) as the TFA salt. Yield: 44 mg. LCMS: 100% pure, RT 1.81 min, m/z 544.3 ([M+H]⁺).

methyl 4-[6-(4-[(2-fluoroethyl)carbamoyl]amino)phenyl]-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carboxylate (25b): a solution of 2-fluoroethylamine.HCl (99 mg) in 1N NaOH (1 mL) was used. Yield: 22 mg (42 μ mol, 35%). LCMS: 100% pure, RT 2.19 min, m/z 527.2 ([M+H]⁺).

methyl 4-(6-{4-[(cyclopropylcarbamoyl)amino]phenyl}-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (25c): a solution of cyclopropylamine (70 μ L) in dichloromethane (1 mL) was used. Yield: 5 mg (10 μ mol, 8%). LCMS: 100% pure, RT 2.23 min, m/z 521.3 ([M+H]⁺).

methyl 4-(6-{4-[(anilino)carbonyl]amino}phenyl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (25d): a solution of aniline (93 μ L) in dichloromethane (1 mL) was used. Yield: 15 mg (27 μ mol, 23%). LCMS: 100% pure, RT 2.43 min, m/z 557.3 ([M+H]⁺).

methyl 4-(6-(4-(3-(4-(4-methylpiperazin-1-yl)phenyl)ureido)phenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (25e): Using the general conditions described above, methyl 4-(6-(4-aminophenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (**24a**) was treated with 4-(4-methylpiperazin-1-yl)aniline to provide **25e**. Yield: 0.012 g, 14%, white solid. LCMS: 99% pure, RT 1.97 min, m/z 655.3 ([M+H]⁺).

methyl 4-(6-(4-(3-(4-(2-hydroxyethyl)phenyl)ureido)phenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (25f): Using the general conditions described above, methyl 4-(6-(4-aminophenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (**24a**) was treated with 2-(4-aminophenyl)ethanol to provide **25f**. Yield: 0.052 g, 34%, white solid. LCMS: 99% pure, RT 2.22 min, m/z 601.3 ([M+H]⁺).

isopropyl 4-[6-(4-[(2-fluoroethyl)carbamoyl]amino)phenyl]-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carboxylate (26b): a solution of 2-fluoroethylamine.HCl (99 mg) in 1N NaOH (1 mL) was used. Yield: 45 mg (80 μ mol, 67%). LCMS: 100% pure, RT 2.38 min, m/z 555.3 ([M+H]⁺).

isopropyl 4-(6-{4-[(cyclopropylcarbamoyl)amino]phenyl}-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (26c): a solution of cyclopropylamine (70 μ L) in dichloromethane (1 mL) was used. Yield: 49 mg (89 μ mol, 74%). LCMS: 100% pure, RT 2.43 min, m/z 549.3 ([M+H]⁺).

tert-butyl 4-[6-(4-[(2-fluoroethyl)carbamoyl]amino)phenyl]-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carboxylate (27b): a solution of 2-fluoroethylamine.HCl (99 mg) in 1N NaOH (1 mL) was used. Yield: 45 mg (78 μ mol, 65%). LCMS: 94% pure, RT 2.46 min, m/z 569.3 ([M+H]⁺).

tert-butyl 4-(6-{4-[(cyclopropylcarbamoyl)amino]phenyl}-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (27c): a solution of cyclopropylamine (70 μ L) in dichloromethane (1 mL) was used. Yield: 49 mg (87 μ mol, 73%). LCMS: 95% pure, RT 2.52 min, m/z 563.3 ([M+H]⁺).

methyl 4-(6-(4-(3-(4-(2-(4-methylpiperazin-1-yl)ethyl)phenyl)ureido)phenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (25h): A procedure analogous to that used for the preparation of methyl 4-(4-morpholino-6-(4-(3-(4-(2-(pyrrolidin-1-yl)ethyl)phenyl)ureido)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (**25g**) was used, substituting N-methyl-piperazine for pyrrolidine. Purification by

HPLC provided **25h**. Yield: 0.011 g, 22%, off-white solid. LCMS: 100% pure, RT 1.95 min, m/z 683.4 ([M+H]⁺).

methyl 4-(4-morpholino-6-(4-(3-(4-(2-morpholinoethyl)phenyl)ureido)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (25i): A procedure analogous to that used for the preparation of methyl 4-(4-morpholino-6-(4-(3-(4-(2-(pyrrolidin-1-yl)ethyl)phenyl)ureido)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (**25g**) was used, substituting morpholine for pyrrolidine. Purification by HPLC provided **25i**. Yield: 0.010 g, 20%, off-white solid. LCMS: 100% pure, RT 2.00 min, m/z 670.3 ([M+H]⁺).

Preparation of additional compounds

The following compounds were all prepared according to Scheme 2 – 4.

5-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]pyridin-2-amine (S1a): Yield: 33 mg (57 μ mol, 47%). LCMS: 100% pure, RT 1.62 min, m/z 471.3 ([M+H]⁺).

5-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]pyrimidin-2-amine (S1b): Yield: 33 mg (56 μ mol, 46%). LCMS: 100% pure, RT 1.82 min, m/z 472.2 ([M+H]⁺).

5-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]pyridin-2-ol (S1c): Yield: 33 mg (56 μ mol, 46%). LCMS: 100% pure, RT 1.79 min, M+H=472.2. IR: 1653 cm⁻¹: carbonyl C=O stretch. ¹H-NMR (acetone-d₆): δ 8.63 (1H, d, J=2.0 Hz), 8.47 (1H, dd, J=2.4 Hz, 9.6Hz), 8.16 (1H, bs), 7.65 (2H, m), 7.47 (3H, t, J=3.0 Hz), 6.41 (1H, d, J=9.6 Hz), 5.09 (1H, m), 4.41 (2H, bs), 4.05 (4H, t, J=5.0 Hz), 3.83 (4H, t, J=5.0 Hz), 3.64 (2H, bs), 3.26 (2H, m), 2.74 (2H, m), 2.09 (2H, m).

1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-6-pyridin-3-yl-1H-pyrazolo[3,4-d]pyrimidine (S1d): Yield: 30 mg (53 μ mol, 44%). LCMS: 100% pure, RT 1.78 min, m/z 456.2 ([M+H]⁺).

3-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenol (S1e): purified using NH₄OH buffers to give the free base of the target compound. Yield: 23 mg (49 μ mol, 41%). LCMS: 94% pure, RT 1.97 min, m/z 471.2 ([M+H]⁺). ¹H-NMR (DMSO-d₆): δ 9.51 (1H, s), 8.30 (1H, s), 7.87 (2H, m), 7.35 (4H, m), 7.27 (2H, m), 6.87 (1H, ddd,

J=1.2Hz, 2.8 Hz, 8.0 Hz), 4.78 (1H, m), 4.00 (4H, m), 3.79 (4H, t, J=4.8 Hz), 3.57 (2H, s), 2.97 (2H, m), 2.21 (4H, m), 1.90 (2H, m).

4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]phenol

(S1f): Yield: 32 mg (54 μ mol, 45%). LCMS: 100% pure, RT 1.90 min, m/z 471.2 ([M+H]⁺).

3-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]aniline

(S1g): Yield: 40 mg (69 μ mol, 57%). LCMS: 100% pure, RT 1.80 min, m/z 470.3 ([M+H]⁺).

N-{3-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-

yl]phenyl}formamide (S1h): Yield: 36 mg (59 μ mol, 49%). LCMS: 100% pure, RT 1.98 min, m/z 498.3 ([M+H]⁺).

N-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-

yl]phenyl}formamide (S1i): Yield: 46 mg (74 μ mol, 62%). LCMS: 100% pure, RT 1.89 min, m/z 498.3 ([M+H]⁺).

N-{3-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-

yl]phenyl}acetamide (S1j): Yield: 8 mg (12 μ mol, 10%). LCMS: 98% pure, RT 2.00 min, m/z 512.3 ([M+H]⁺).

N-{4-[1-(1-benzylpiperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-

yl]phenyl}acetamide (S1k): Yield: 42 mg (67 μ mol, 55%). LCMS: 99% pure, RT 1.98 min, m/z 512.3 ([M+H]⁺).

5-[1-(1-Benzyl-piperidin-4-yl)-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidin-6-yl]-1,3-

dihydro-benzoimidazol-2-one (S1l): 53 mg (0.2 mmol) 4-amino-3-nitrophenylboronic acid,

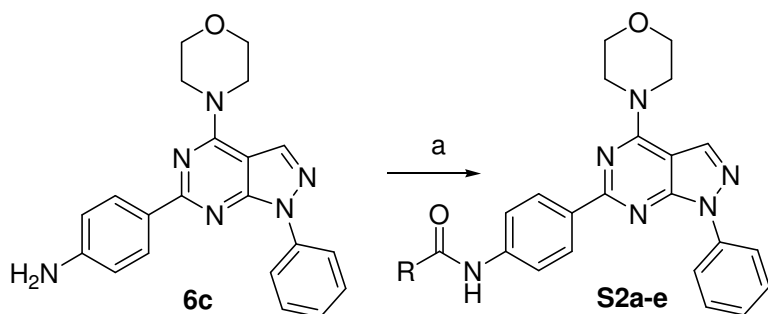
pinacol ester was suspended in 2 mL DME. A catalytic amount of Pd/C was added and the

nitro group was reduced under an atmosphere of hydrogen over 4 days. 130 μ L NEt₃ was

added followed by 30 mg triphosgene. The mixture was stirred for 15 min and the resulting

1,3-dihydro-benzoimidazol-2-one boronate was reacted with 50 mg 1-(1-Benzyl-piperidin-4-yl)-6-chloro-4-morpholin-4-yl-1H-pyrazolo[3,4-d]pyrimidine according to the general procedure. Yield: 35 mg (55 μ mol, 55%). LCMS: 100% pure, RT 1.84 min, m/z 511.2 ([M+H]⁺).

Scheme S1^a



^a Reagents and conditions: (a) RCOOH, IIDQ

General procedure for IIDQ acylation of 4-(4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline **6c**:

To a solution of 4-(4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline (**6c**, 40 mg, 0.1 mmol) in DMF (600 μ L) was added 0.2 – 0.6 mmol of carboxylic acid and 0.2 – 0.6 mmol of IIDQ (60 – 180 μ L). The reaction mixture was stirred for 72 h at RT or 50° C. The reactions were worked up by concentration followed by HPLC purification.

N-[4-(4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenyl]glycinamide (S2a): N-(tert-butoxycarbonyl)glycine (35 mg, 0.2 mmol) and IIDQ (60 μ L, 0.2 mmol) were used at room temperature. The Boc group in the resulting product was removed by addition of

TFA (0.6 mL). HPLC purification was done using NH₄OH buffers. Yield: 15 mg (0.035 μ mol, 35%). LCMS: 75% pure, RT 2.07 min, m/z 430.2 ([M+H]⁺).

N2,N2-dimethyl-N-[4-(4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenyl]glycinamide (S2b): N,N-dimethylglycine (63 mg, 0.6 mmol) and IIDQ (180 μ L, 0.6 mmol) were used in DMA at 50 °C. HPLC purification was done using TFA buffers. Yield: 22 mg (0.048 μ mol, 48%). LCMS: 100% pure, RT 2.04 min, m/z 458.2 ([M+H]⁺).

N-[4-(4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenyl]-beta-alaninamide (S2c): Boc-beta-alanine (38 mg, 0.2 mmol) and IIDQ (60 μ L, 0.2 mmol) were used at room temperature. The Boc group in the resulting product was removed by addition of TFA (0.6 mL) and heating to 50 °C. HPLC purification was done using NH₄OH buffers. Yield: 8 mg (0.019 μ mol, 19%). LCMS: 85% pure, RT 2.05 min, m/z 444.2 ([M+H]⁺).

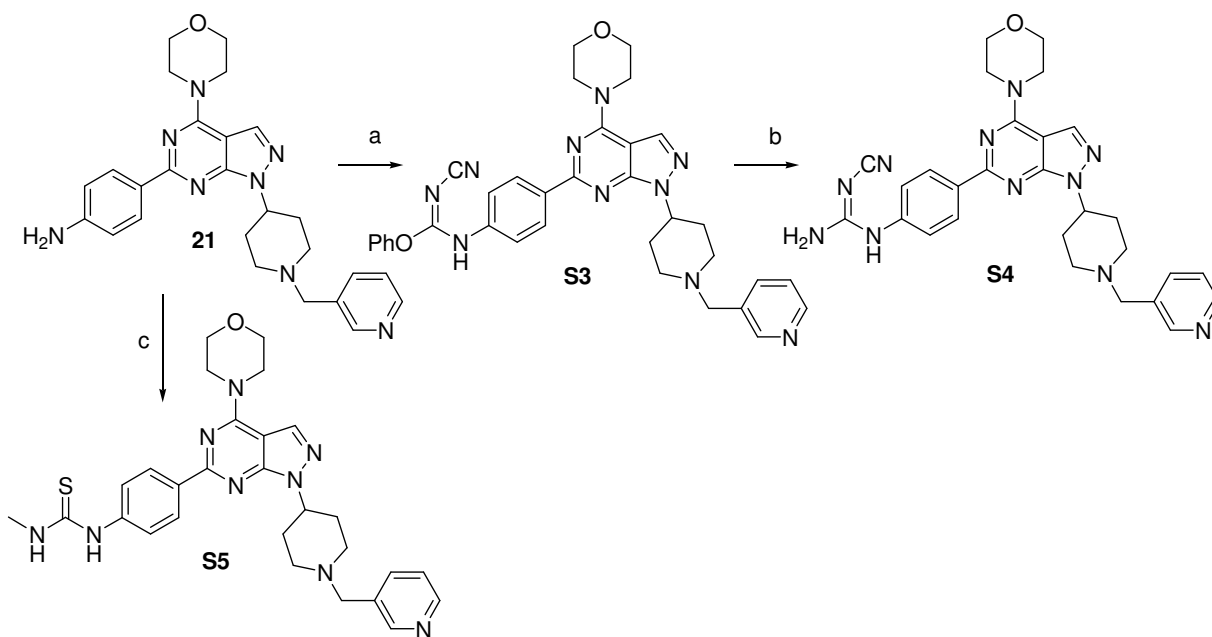
1-methyl-N-[4-(4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenyl]piperidine-4-carboxamide (S2d): 1-Me-piperidine-4-carboxylic acid.HCl (36 mg, 0.2 mmol) and IIDQ (60 μ L, 0.2 mmol) were used at 50 °C. HPLC purification was done using NH₄OH buffers. Yield: 50 mg (0.10 μ mol, 100%). LCMS: 93% pure, RT 2.20 min, m/z 498.3 ([M+H]⁺).

3-methoxy-N-[4-(4-morpholin-4-yl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenyl]propanamide (S2e): 3-methoxypropionic acid (21 mg) and IIDQ (60 μ L, 0.2 mmol) were used at room temperature. HPLC purification was done using NH₄OH buffers. Yield: 16 mg (0.036 μ mol, 36%). LCMS: 99.5% pure, RT 2.42 min, m/z 459.2 ([M+H]⁺).

Preparation of urea isosteres is outlined in Schemes S2 and S3. As shown in Scheme S2, condensation of aniline **21** with diphenyl cyanocarbonimide, followed by reaction of

resulting **S3** with ammonia, gave cyanoguanidine **S4**. Condensation of **21** with methylisothiocyanate gave thiourea **S5**. As shown in Scheme S3, methylsulfamoyl chloride **S8** was prepared by adaptation of known methods.^{2,3} Thus, acylation of methylamine with chlorosulfonic acid, followed by chlorination of resulting **S6** led to methylsulfamoyl chloride **S7**. Acylation of **24c** with methylsulfamoyl chloride **S7** gave sulfamate **S8**.

Scheme S2^a



^a Reagents and conditions: (a) NEt₃, PhOC(NCN)OPh; (b) NH₃; (c) MeNCS.

2-cyano-1-(4-{4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl)guanidine (S4):

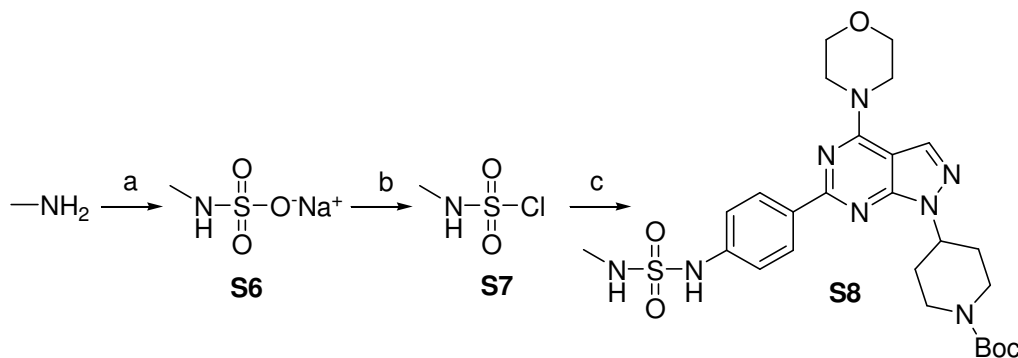
4-(4-morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline (**21**, 50 mg, 0.09 mmol) was dissolved in DCM (1 mL). 1 equivalent of diphenyl cyanocarbonimidate (22 mg, 0.09 mmol) and 2 equivalents (26 μ L) triethylamine were added.

The reaction was stirred at room temperature until LCMS revealed complete conversion into **S3**. The mixture was concentrated and a 0.5 N solution of ammonia in dioxane (10 mL) was added. The mixture was heated in a sealed vial at 70 °C overnight. The solvents were evaporated and the product was purified by RP-HPLC (TFA buffers) to give the title compound as the TFA salt. Yield: 28 mg (43 μmol, 48%). LCMS: 100% pure, RT 1.74 min, m/z 538.3 ([M+H]⁺).

1-methyl-3-(4-{4-morpholin-4-yl-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]-1H-pyrazolo[3,4-d]pyrimidin-6-yl}phenyl)thiourea (S5**):**

4-(4-morpholino-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)aniline (**23**, 50 mg) was dissolved in dichloromethane (1 mL) and triethylamine was added (65 μL). Methylisothiocyanate (15 μL, 0.2 mmol) was added and the mixture was heated at 40 °C overnight. The solvents were evaporated and the crude product was dissolved in DMSO, filtered and purified by HPLC (TFA buffers) to give the title compound as TFA salt. Yield: 26 mg (0.039 mmol, 39%). LCMS: 84% pure, RT 1.78 min, m/z 544.3 ([M+H]⁺).

Scheme S3^a



^a Reagents and conditions: (a) 1. NEt₃, ClSO₃H, 2. NaOH, H₂O; (b) POCl₃; (c) **24c**.

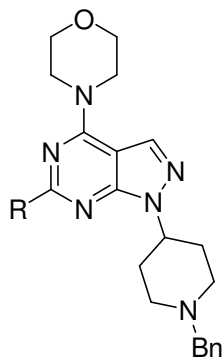
methylsulfamoyl chloride (S7):

A solution of methylamine hydrochloride (2.00 g, 29.62 mmol) in triethylamine (41.3 mL, 296.2 mmol) was stirred at room temperature for 25 min. Chloroform (60 mL) was then added and the solution was cooled to 0 °C. Chlorosulfonic acid (1.98 mL, 29.62 mmol) was slowly added over 15 min. The suspension was concentrated under reduced pressure, then treated with 2N aqueous sodium hydroxide (45 mL, 90 mmol). Water was removed under reduced pressure and warm ethanol (100 mL) was added. The suspension was filtered and the filtrate was concentrated to provide the sodium salt of methylsulfamic acid (**S6**). The crude product was dissolved in 1,2-dichloroethane (200 mL), and phosphorus oxychloride (5.42 mL, 59.24 mmol) was added. The solution was heated to 80 °C for 64 h, then cooled to room temperature and concentrated under reduced pressure to provide methylsulfamoyl chloride **S7** (1.42 g, 11.01 mmol) as a light yellow oil.

tert-butyl 4-(6-(4-(N-methylsulfamoylamino)phenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate (S8)

tert-Butyl 4-(6-(4-aminophenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carboxylate **24c** was treated with methyl sulfamoyl chloride (**S7**, 2 eq) and pyridine (4 eq) in DMF and stirred at RT for 3.5 h. Concentration and purification by reverse phase preparative high performance liquid chromatography provided *tert*-butyl 4-(6-(4-(N-methylsulfamoylamino)phenyl)-4-morpholino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-

carboxylate **S8**. Yield: 0.068 g, 0.12 mmol, 16%. HPLC: 74% pure, RT 15.3 min. ^1H -NMR (CDCl_3): δ 8.40 (2H, d, $J=9.2$ Hz), 7.95 (1H, s), 7.3 (2H, m), 6.8 (1H, bs), 5.04 (1H, m), 4.30 (2H, m), 4.07 (4H, t, $J=4.6$ Hz), 3.91 (4H, t, $J=4.8$ Hz), 2.99 (2H, m), 2.72 (3H, s), 2.23 (2H, m), 2.00 (2H, m), 1.50 (9H, s).

Table S1. Aminopyridine analogs.

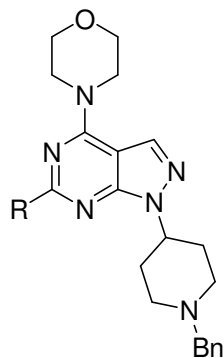
<i>Cmpd.</i>	<i>R</i>	<i>mTOR</i>	<i>PI3K-α</i>	<i>Selectivity^b</i>
		<i>IC₅₀ (nM)^a</i>	<i>IC₅₀ (nM)^a</i>	
S1a		17 +/- 3.0	107 +/- 30	6
S1b		21 +/- 2.5	18 +/- 3.0	0.9
S1c		8,000 +/- 200	7,810 +/- 589	1.0
S1d		695 +/- 25	244 +/- 64	0.4

^aMean +/- SEM. ^bSelectivity (IC₅₀ PI3K-α/IC₅₀ mTOR).

Similar activity was observed for aminopyridine **S1a** and aminopyrimidine **S1b**. The latter compound was an equipotent inhibitor of mTOR and PI3K and may function as an interesting lead for the development of dual mTOR/PI3K inhibitors. Replacement of the amine in **S1a** with a hydroxyl group (leading to **S1c**) led to a more than 400-fold decrease in potency. This

observation may reflect the fact that **S1c** exists predominantly in the pyridinone tautomeric form,⁴ which will not be able to form the same H-bonds as the aminopyrimidine. Finally, the requirement for a 4-amine group was confirmed by the greatly decreased activity of pyridine **S1d**, reflecting the loss of the interaction between the exocyclic amine and Asp2195 and Glu2190.

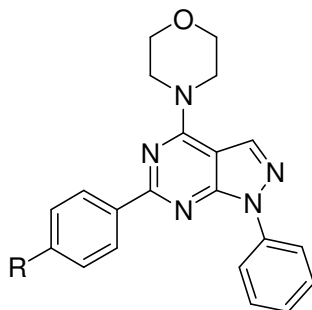
Table S2. Comparison of *meta* vs. *para*-substitution.



<i>Cmpd.</i>	<i>R</i>	<i>m/p</i>	<i>mTOR</i>	<i>PI3K-α</i>	<i>Selectivity^b</i>
			<i>IC</i> ₅₀ (nM) ^a	<i>IC</i> ₅₀ (nM) ^a	
S1e		m	83 +/- 4.4	31 +/- 6.8	0.4
S1f		p	170 +/- 30	720 +/- 194	4
S1g		m	325 +/- 32	989 +/- 79	3
13a		p	200 +/- 20	1,368 +/- 137	7
S1h		m	1,230 +/- 270	2,862 +/- 360	2
S1i		p	106 +/- 15	684 +/- 40	7
S1j		m	2,450 +/- 450	2,005 +/- 162	0.8
S1k		p	13 +/- 3.1	420 +/- 122	32

^aMean +/- SEM. ^bSelectivity (*IC*₅₀ PI3K-α/*IC*₅₀ mTOR).

Table S3. Introduction of water-solubilizing groups through acylated anilines.



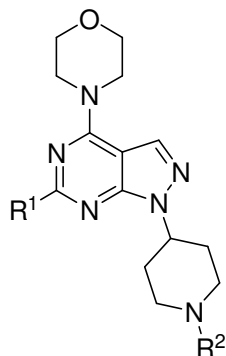
<i>Cmpd.</i>	<i>R</i>	<i>mTOR</i>	<i>PI3K-α</i>	<i>Selectivity^b</i>
		<i>IC₅₀ (nM)^a</i>	<i>IC₅₀ (nM)^a</i>	
S2a		19 +/- 3.8	49 +/- 15	3
S2b		62 +/- 7.5	325 +/- 83	5
S2c		24 +/- 5.5	64 +/- 4.0	3
S2d		77 +/- 5.5	209 +/- 10	3
S2e		34 +/- 2.0	303 +/- 38	9

^aMean +/- SEM. ^bSelectivity (IC₅₀ PI3K-α/IC₅₀ mTOR).

Table S4. Antiproliferative activity (IC₅₀'s, nM) of ATP-competitive mTOR inhibitors against a broad panel of tumor cell lines.

Cmpd.	Sensitive cell lines				
	LNCap	U87MG	PC3MM2	MDA361	MCF7
	prostate	glioma	prostate	breast	breast
	(PTEN -/-)	(PTEN -/-)	(PTEN -/-)	(PIK3CA)	(PIK3CA)
13o	500	1,000	800	1,050	280
24a	213	600	180	240	13
18a	1.5	30	1.2	8	<0.7
24c	3.4	12	0.7	2.8	0.7

Cmpd.	Less sensitive lines			
	MDA435	MDA231	DU145	HT29
	breast	breast	prostate	colon
13o	2,700	3,800	4,000	5,000
24a	1,050	1,600	1,250	10,000
18a	65	320	120	2,800
24c	40	180	90	3,000

Table S5. Ureidophenyl analogs and isosteres.

<i>Cmpd.</i>	<i>R¹</i>	<i>R²</i>	<i>mTOR</i> <i>IC₅₀ (nM)^a</i>	<i>PI3K-α</i> <i>IC₅₀ (nM)^a</i>	<i>Selectivity^b</i>	<i>LNCaP cell</i> <i>IC₅₀ (nM)^c</i>
S5			1.8 +/- 0.05	124 +/- 9.5	69	70
S4			4.6 +/- 1.3	260 +/- 50	56	1,400
S11			195 +/- 5.0	4,394	23	4,000
S8			85 +/- 3.5	3,423	41	4,000

^aMean +/- SEM. ^bSelectivity (IC₅₀ PI3K-α/IC₅₀ mTOR). ^cThe average error for LNCaP IC₅₀ determinations was <25%.

A further investigation of isosteric replacements for the ureidophenyl group is presented in Table S5. Methylthiourea **S5** was less potent and selective than the corresponding methyl urea.

A more pronounced decrease in activity is observed for cyanoguanidine **S4**. Compound **S1k**, in which the ureido group was cyclized onto the phenyl ring, was much less potent than the corresponding methyl urea. Finally, sulfamate **S8** was significantly less potent than the corresponding methyl urea.

Table S6. Activity and selectivity profile of ATP-competitive mTOR inhibitors against a panel of lipid and protein kinases. IC₅₀'s (μM) against the various kinases are listed.

Cmpd.	PIKKs			
	<i>mTOR</i>	<i>PI3Kα</i>	<i>PI3Kγ</i>	<i>ATR</i>
24a	0.0046	0.801	6.224	>50
24c	0.0004	0.041	0.495	11.4
29a	0.0007	0.080	0.409	11.7

Cmpd.	<i>Non-PIKKs</i>											
	<i>ABL1</i>	<i>AuroraB</i>	<i>CDK1</i>	<i>CDK2</i>	<i>CHK1</i>	<i>CK1γ1</i>	<i>ERK2</i>	<i>FYN</i>	<i>GCK</i>	<i>HCK</i>	<i>LYN A</i>	<i>MET</i>
24a	>50	>50	>50	>50	>50	17.9	>50	>50	>50	>50	>50	>50
24c	>50	45.1	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
29a	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

Cmpd.	<i>Non-PIKKs (cntd.)</i>									
	<i>VEGFR2</i>	<i>MK2</i>	<i>P38α</i>	<i>PDGFRα</i>	<i>PKA</i>	<i>ROCK1</i>	<i>RSK1</i>	<i>SRC</i>	<i>PKCα</i>	<i>IKKα</i>
24a	>50	>50	28.9	>50	>50	>50	>50	>50	>50	>50
24c	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
29a	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

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4. LigPrep (version 2.2, Schrödinger, LLC, New York, NY, 2005) was used to predict the predominant tautomeric state(s) for **13c**. Using LigPrep with Epik, states were determined at pH 7.4 +/- 1.0 and at pH 7.4. In both cases, the only state returned by LigPrep was that of the keto-form. In addition, the IR spectrum for **S1c** showed a strong absorption at a wavenumber of 1653 cm^{-1} , ascribed to a carbonyl C=O stretch, consistent with the keto-form.