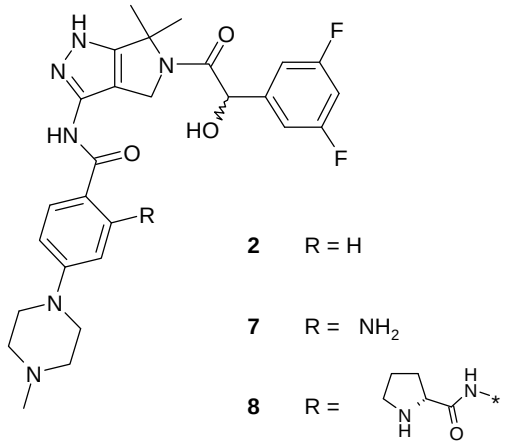
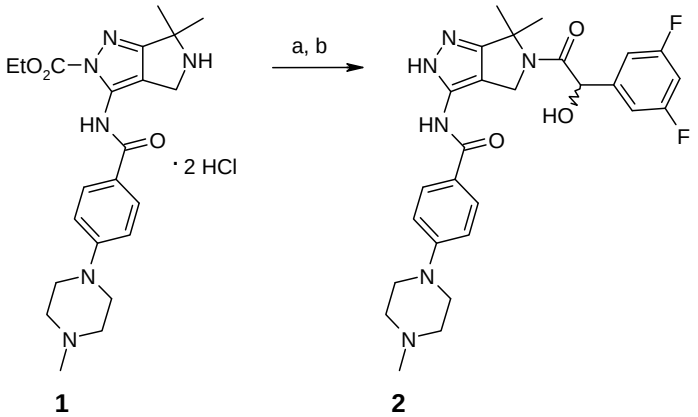
Supporting Information for 'Crystal Structures of Anaplastic Lymphoma Kinase in Complex with ATP-competitive Inhibitors'

Synthesis of PHA-E589, NMS-E107 and NMS-E828



The synthesis of the compound **1** (Scheme 1) has been reported in the International Patent Application WO2007099171 and the preparation of scaffold **3** has been described by Brasca and coworkers (*1*). The carboxylic acid derivative (4-(4-methylpiperazin-1-yl)-2-nitrobenzoic acid) is commercially available (Tyger) and the corresponding acyl chloride **A** was prepared according to the procedure reported by Fancelli and coworkers (*2*) for the analog (4-(4-methylpiperazin-1-yl)benzoyl chloride. The 3,5-difluoromandelic acid is commercially available (Sigma-Aldrich); the corresponding acetyl derivative **I** [(acetyloxy)(3,5-difluorophenyl)acetic acid] and the corresponding acylchloride **II** [(acetyloxy)(3,5-difluorophenyl)acetyl chloride] were prepared analogously to acetylmandelic acid and acetylmandelyl chloride as described by Thayer (*3*).
Acyl chloride of FMOC-D-Proline **III** is commercially available (3B Scientific Corp).

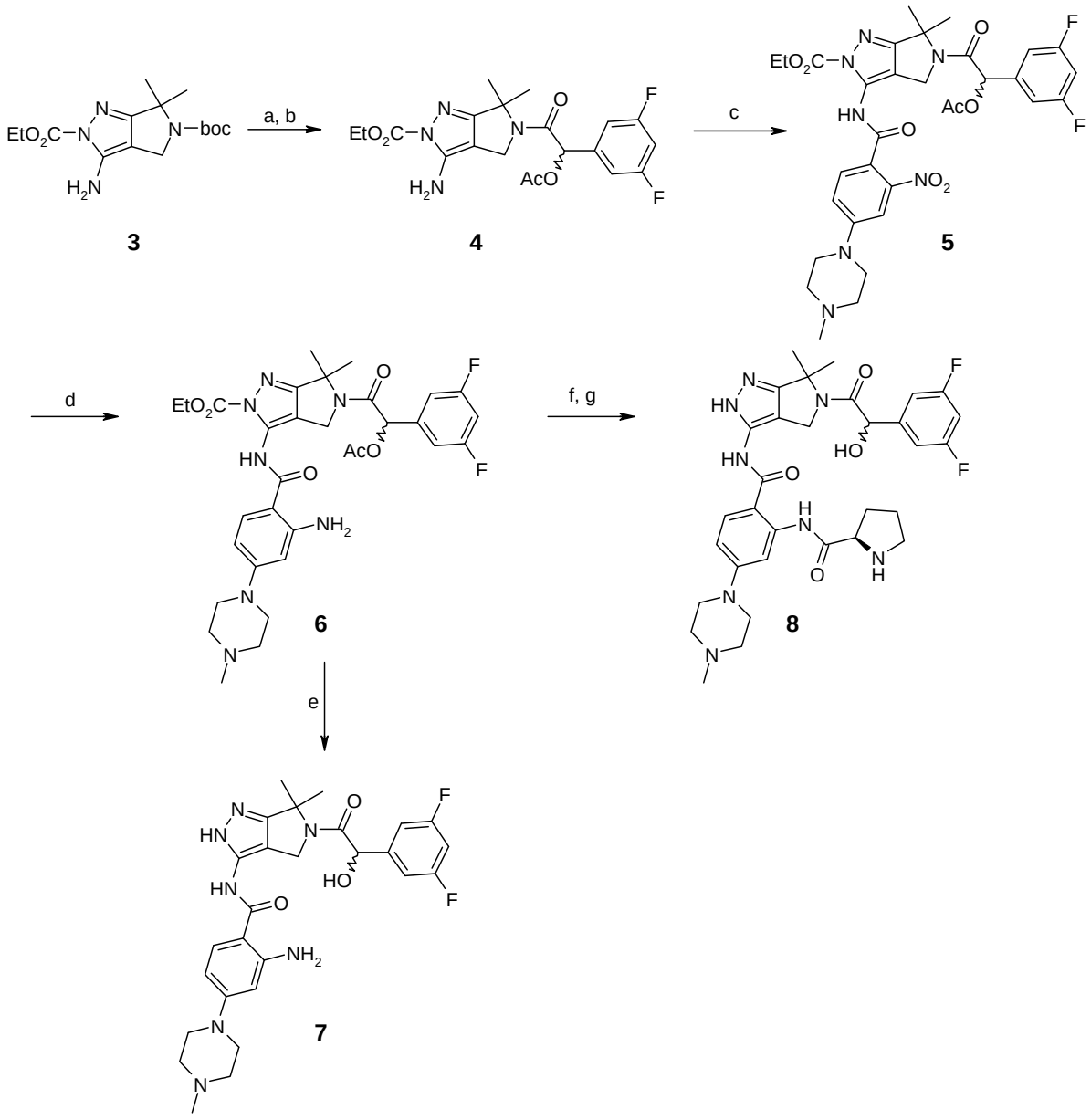
Scheme 1. Preparation of compound **2** (PHA-E429)



1 **2**

a: DIEA, DCM anhydrous, **II**, room-temperature, overnight; b: MeOH, TEA, 60 °C, 4 h (38% yield over two steps)

Scheme 2. Preparation of compounds **7** (NMS-E107) and **8** (NMS-E828)



a: TFA, DCM, rt, 6 h; b: TBTU, DIEA, DCM, **I**, rt, overnight, 74%; c: DIEA, DCM, **A**, 50 °C, 24 h, 61%; d: 10% Pd/C, cyclohexene, THF, EtOH, H_2O, 23% HCl, 70 °C, 4 h, 88%; e: LiOH, THF/H_2O, rt, 4 h, 67%; f: PS-TEA, DCM, **III**, rt, overnight, 36%; g: MeOH : piperidine 8:2, rt, 72 h, 80%.

ESI(+) high-resolution mass spectra (HRMS) were obtained on a Waters Q-Tof Ultima directly connected with micro HPLC 1100 Agilent.

^{1}H NMR spectra were acquired at 25° C in DMSO-d6 on a Varian Inova Inova 400 spectrometer operating at 400 MHz and equipped with a 5 mm ^{1}H{^{15}N-^{31}P} Z-axis-PFG

Indirect Detection Probe. Residual not-deuterated solvent signal was used as reference with δ = 2.50 ppm for DMSO-d5. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, bs = broad singlet, bd = broad doublet, dd = doublet of doublet, td = triplet of doublet, m = multiplet), coupling constants, and number of protons.

Compound **2** (PHA-E429)

HRMS (ESI): calcd for $C_{27}H_{30}F_2N_6O_3 + H^+$ 525.2420 found 525.2406
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (br. s., 1H), 10.55 (br. s., 1H), 7.87 (br. s., 2H), 7.16 (tt, *J* = 2.41, 9.36 Hz, 1H), 7.05 - 7.12 (m, 2H), 6.98 (br. s., 2H), 5.93 (d, *J* = 7.07 Hz, 1H), 5.31 (d, *J* = 7.19 Hz, 1H), 4.54 - 4.95 (m, 2H), 3.22 - 3.38 (m, 4H), 2.42 - 2.56 (m, 4H), 2.26 (br. s., 3H), 1.71 (br. s., 3H), 1.65 (br. s., 3H)

Compound **7** (NMS-E107)

HRMS (ESI): calcd for $C_{27}H_{31}F_2N_7O_3 + H^+$ 540.2529 found 540.2532
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 - 12.43 (m, 1H), 10.02 - 10.35 (m, 1H), 7.42 - 7.70 (m, 1H), 7.16 (t, *J* = 9.08 Hz, 1H), 7.02 - 7.12 (m, 2H), 6.43 - 6.66 (m, 2H), 6.08 - 6.34 (m, *J* = 7.68 Hz, 2H), 5.90 (d, *J* = 6.34 Hz, 1H), 5.30 (d, *J* = 6.95 Hz, 1H), 4.35 - 4.87 (m, 2H), 3.18 (br. s., 4H), 2.36 - 2.46 (m, 4H), 2.21 (s, 3H), 1.53 - 1.77 (m, 6H)

Compound **8** (NMS-E828)

HRMS (ESI): calcd for $C_{32}H_{38}F_2N_8O_4 + H^+$ 637.3057 found 637.3051
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.46 (br. s., 1H), 12.06 (br. s., 1H), 10.64 (br. s., 1H), 8.16 - 8.32 (m, 1H), 7.58 - 7.81 (m, 1H), 7.12 - 7.23 (m, 1H), 7.07 (t, *J* = 5.85 Hz, 2H), 6.58 - 6.81 (m, 1H), 5.85 - 6.00 (m, 1H), 5.28 (dd, *J* = 2.87, 7.26 Hz, 1H), 4.41 - 4.97 (m, 2H), 3.70 (td, *J* = 4.86, 9.18 Hz, 1H), 3.25 (br. s., 4H), 2.75 - 3.12 (m, 2H), 2.37 - 2.47 (m, 4H), 2.22 (s, 3H), 1.75 - 2.10 (m, 2H), 1.73 (br. s., 3H), 1.66 - 1.70 (m, 3H), 1.54 - 1.71 (m, 2H)

REFERENCES.

1. Brasca, M.G., Albanese, C., Amici, R., Ballinari, D., Corti, L., Croci, V., Fancelli, D., Fiorentini, F., Nesi, M., Orsini, P., Orzi, F., Pastori, W., Perrone, E., Pesenti, E., Pevarello, P., Riccardi-Sirtori, F., Roletto, F., Roussel, P., Varasi, M., Vulpetti, A. and Mercurio C. (2007) 6-Substituted pyrrolo[3,4-c]pyrazoles: an improved class of CDK2 inhibitors. *ChemMedChem. 2(6)*, 841-52.

2. Fancelli, D., Berta, D., Bindi, S., Cameron, A., Cappella, P., Carpinelli, P., Catana, C., Forte, B., Giordano, P., Giorgini, M.L., Mantegani, S., Marsiglio, A., Meroni, M., Moll, J., Pittalà, V., Roletto, F., Severino, D., Soncini, C., Storici, P., Tonani, R., Varasi, M., Vulpetti, A. and Vianello, P. (2005) Potent and selective Aurora inhibitors identified by the expansion of a novel scaffold for protein kinase inhibition. *J. Med. Chem. 48(8),* 3080-3084.

3. Thayer, F.K. (1941) Acetylmandelic acid and Acetylmandelyl chloride, in **Organic Synthesis Collective Vol. 1** (Gilman, H., Ed.) 2nd ed., pp 12-13, John Wiley & Sons, New York.