SUPPLEMENTARY INFORMATION

Reversible targeting of noncatalytic cysteines with chemically tuned electrophiles

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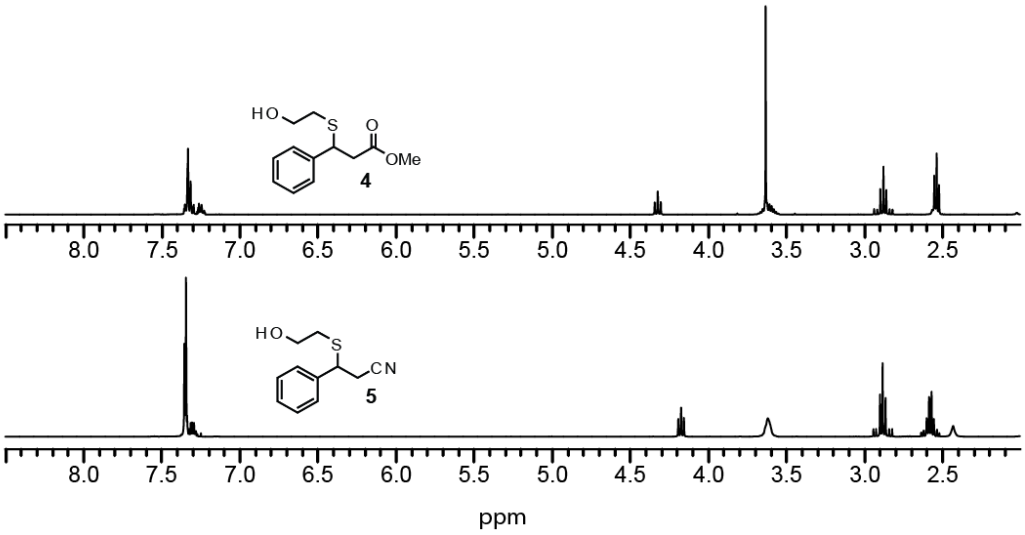
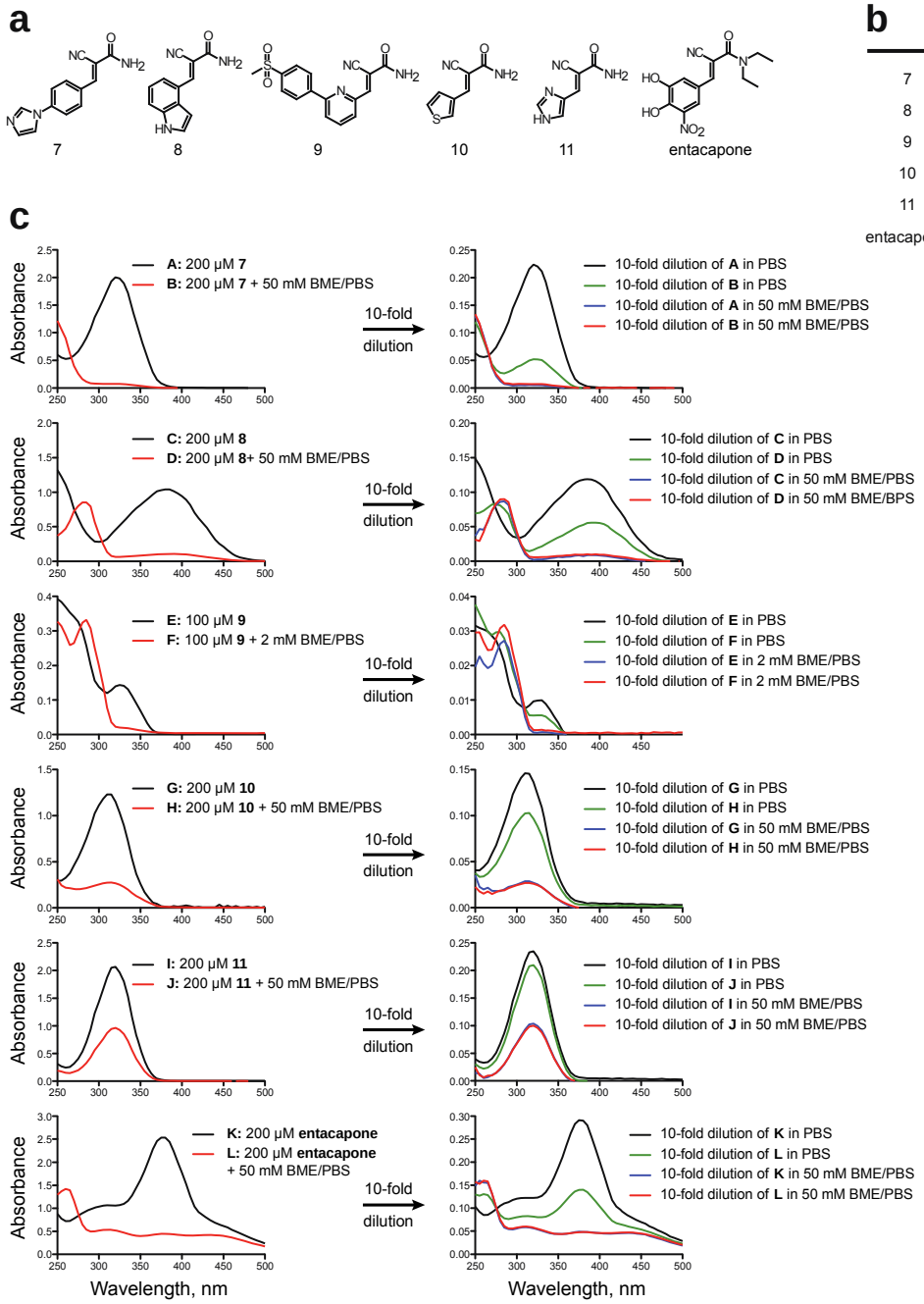
SUPPLEMENTARY RESULTS

Supplementary Figure 1



Supplementary Figure 1. ^{1}H NMR spectra ($CDCl_3$) of stable BME adducts **4** and **5** (see Methods for additional characterization data).

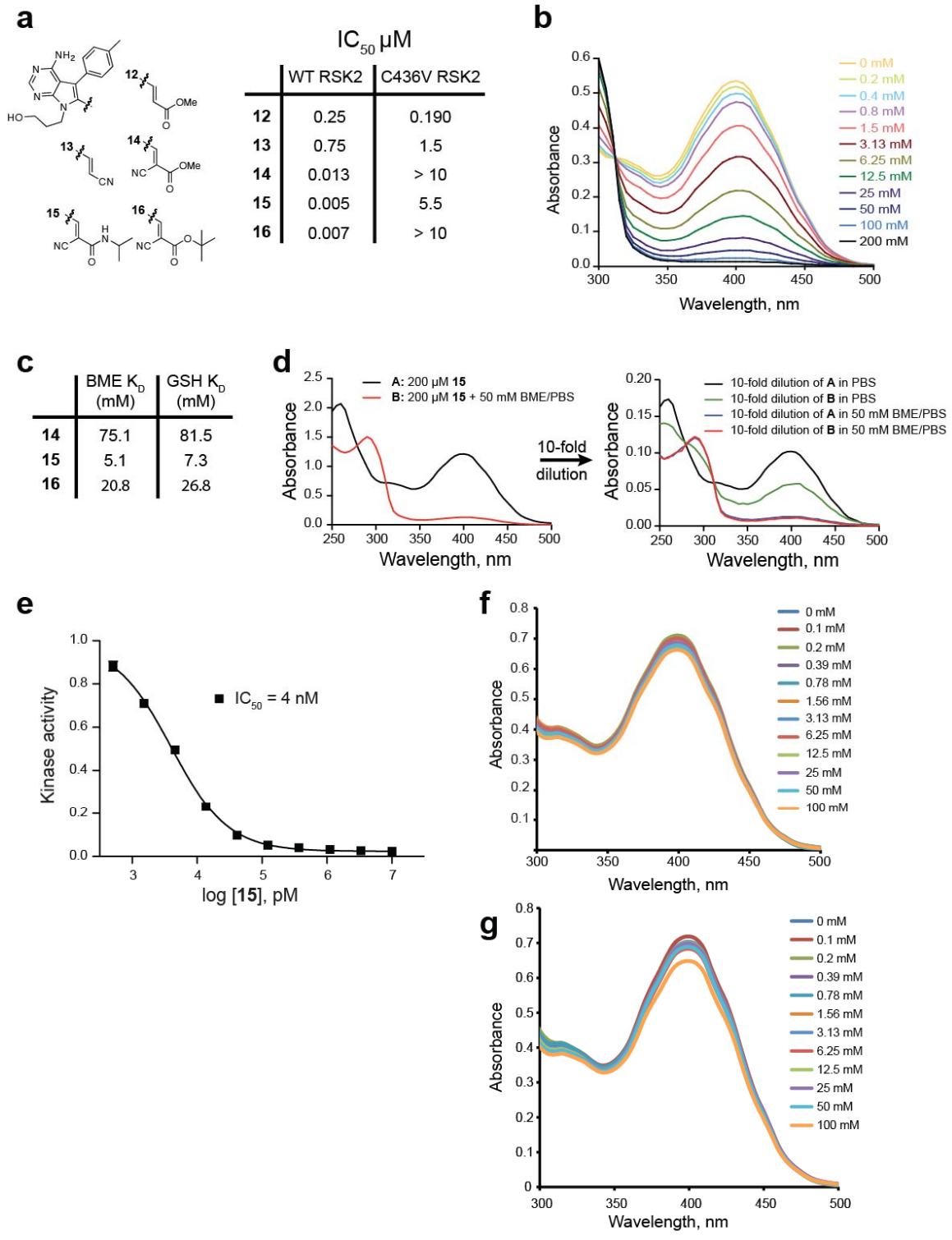
Supplementary Figure 2



Supplementary Figure 2. BME reacts rapidly and reversibly with diverse cyanoacrylamides. **a**, Chemical structures of cyanoacrylamides tested for BME reactivity (for synthesis and characterization data, see Methods). **b**, Equilibrium dissociation constants of

BME/cyanoacrylamide adducts determined by BME titration and UV-visible spectroscopy. **c**, UV-visible spectroscopy assay showing reversible formation of BME adducts with cyanoacrylamides. Cyanoacrylamides in PBS (1-2% DMSO) were treated with or without the indicated concentration of BME for 10 min, after which UV-visible absorption spectra were recorded (left panels). Each reaction was then diluted 10-fold into PBS or PBS/BME as indicated, and the absorption spectra were recorded again (right panels). Reversibility is demonstrated by the reappearance or increase of the cyanoacrylamide absorption peak after dilution of BME-containing samples into PBS (compare red and green curves in right panels). The extent of cyanoacrylamide recovery varies depending on the equilibrium constant and the concentration of BME after dilution.

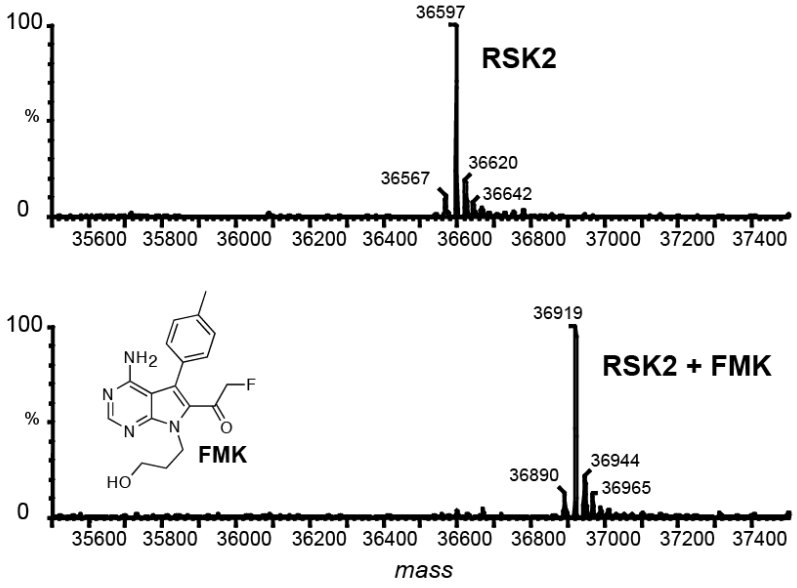
Supplementary Figure 3



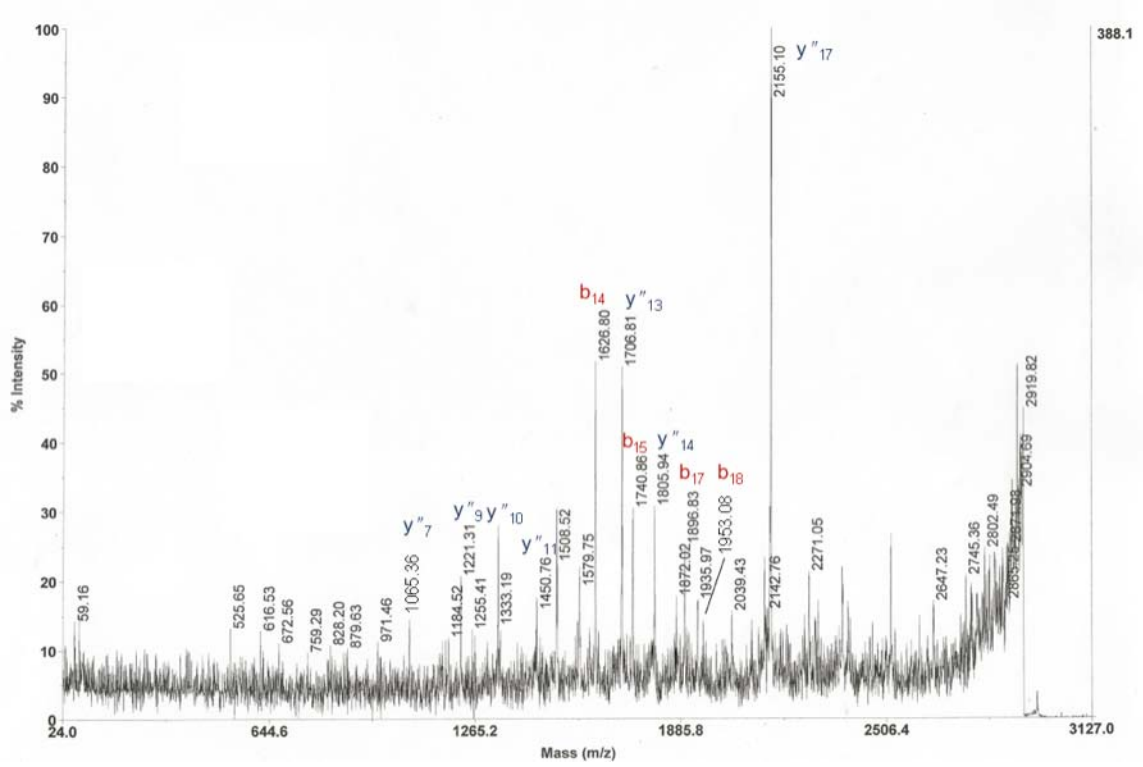
Supplementary Figure 3. In vitro RSK2-CTD potency and thiol reactivity of electrophilic pyrrolopyrimidines. **a**, Chemical structures of pyrrolopyrimidine Michael acceptors and their half-maximum inhibitory concentrations (IC_{50} in μM) against wild type (WT) or C436V RSK2-CTD. **b**, Absorption spectra of cyanoacrylamide **15** (100 μM in PBS, pH 7.4) in the presence of increasing concentrations of BME (0.2–200 mM, PBS, pH 7.4). Similar spectra were obtained by titration with glutathione (GSH, data not shown). **c**, Equilibrium dissociation constants determined by titrating BME or GSH vs. the doubly activated Michael acceptors **14**–**16** (see Fig. 1B and Methods for details). **d**, UV-visible absorption assay depicting the rapid reversion upon dilution of the BME/**15** adduct (see fig. S2C and Methods for detailed assay description). **e**, Kinase assay inhibition curve showing that pretreatment of cyanoacrylamide **15** with 10 mM GSH does not affect its potency toward RSK2-CTD. This experiment was performed identically to the one shown in Fig. 2B, except that 10 mM GSH was included during the 30 min pre-incubation period. **f**, Absorption spectra of cyanoacrylamide **15** (100 μM in PBS, pH 7.4) in the presence of increasing concentrations of ethanolamine (0.1–100 mM, PBS, pH 7.4). **g**, Absorption spectra of cyanoacrylamide **15** (100 μM in PBS, pH 7.4) in the presence of increasing concentrations of lysine (0.1–100 mM, PBS, pH 7.4).

Supplementary Figure 4

a



b



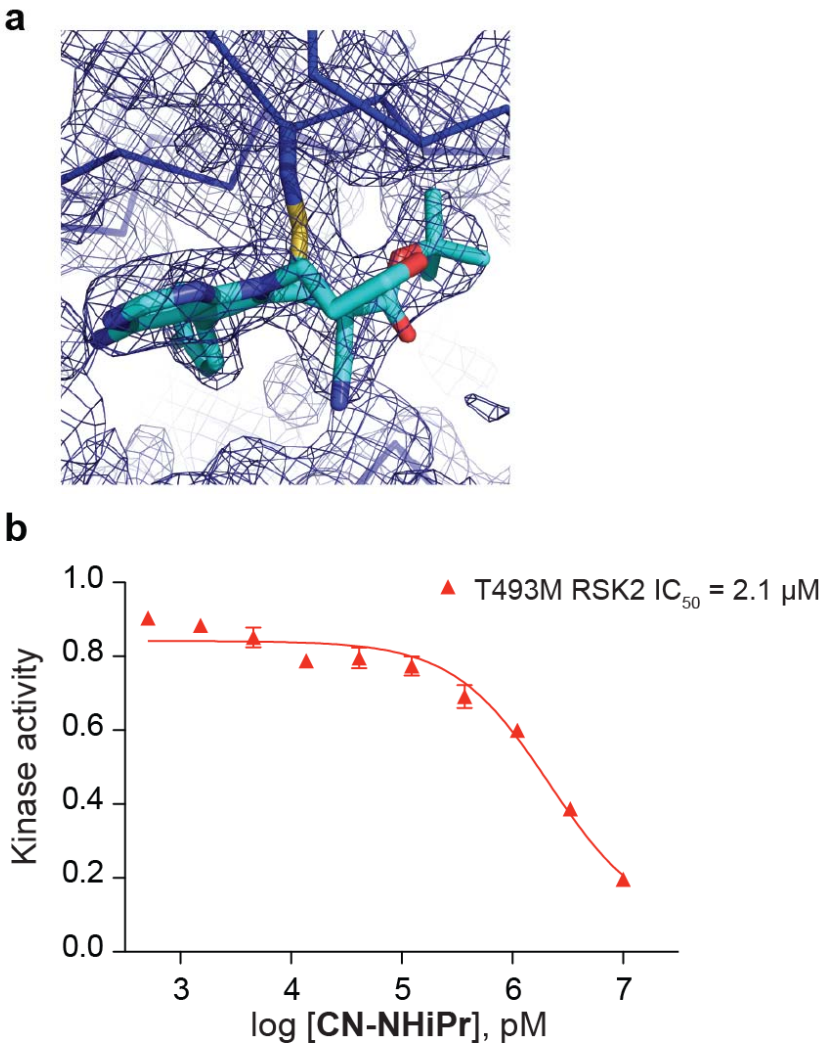
Supplementary Figure 4. Mass spectra showing irreversible modification of RSK2-CTD by FMK (+322 Da, with loss of HF). **a**, RSK2-CTD (5 μM) was incubated with FMK (100 μM) for 10 min, followed by LC-MS analysis of the intact protein. **b**, FMK-treated RSK2-CTD was digested with trypsin and analyzed first by ESI-Q-FTICR mass spectrometry (Bruker Apex IV, 9.4T). Only two modified peptides, corresponding to residues 414-437 and 414-438 (both containing Cys436), were detected (not shown). Sequence ions (ABI 4800 MALDI-TOF/TOF) of the modified 414-437 peptide gave partial sequence information consistent with modification of Cys436. For example, calculated mass of modified $y\bullet_7$ ion (residues 431-437, GSYSVCK): 1065.50; observed mass: 1065.36. Calculated mass of unmodified b_{18} ion (residues 414-431, NSIQFTDGYEVKEDIGVG): 1952.92; observed mass: 1953.08.

Supplementary Figure 5

Kinase	**CN-NHiPr** K_D (nM)
RSK1	0.54
RSK4	1.2
MAP3K1	45
STK16	230
RIPK2	310
RET	360
MEK5	2300
PDGFRB	2800

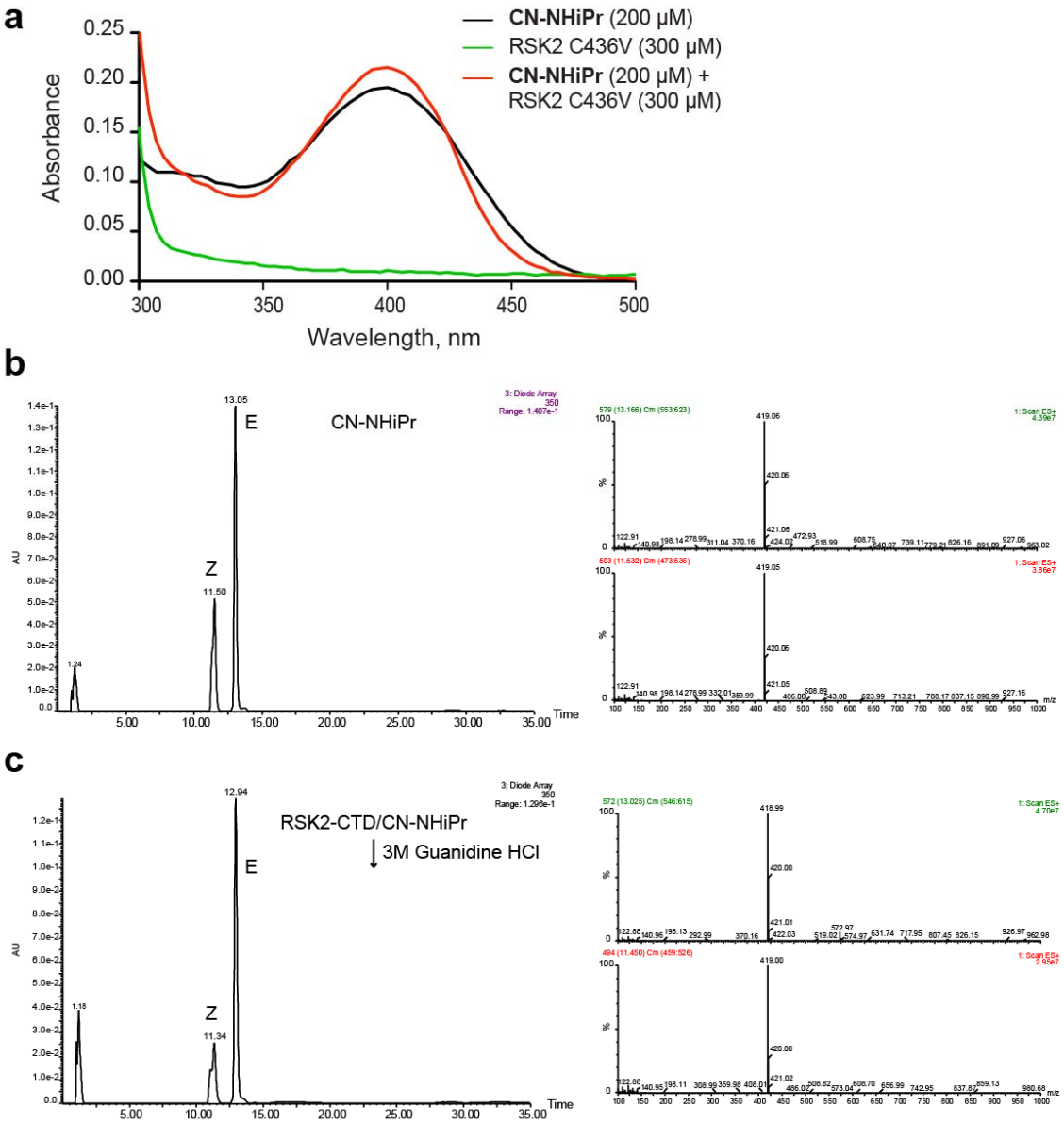
Supplementary Figure 5. Kinase profiling of CN-NHiPr using the Ambit competitive binding platform (now administered by DiscoverRx). In the initial screen, CN-NHiPr (1 μM) was profiled against the entire 442-kinase panel (data provided in Supplementary Table). Only 8 kinases (including RSK1/4 CTD) showed greater than 70% inhibition by CN-NHiPr in this single-point determination. K_D values, depicted above, were determined from dose-response curves for these 8 kinases. RSK3-CTD, which has a Met gatekeeper but is otherwise 85% identical to RSK1/2-CTD, was not significantly inhibited by 1 μM CN-NHiPr in the initial screen. RSK2-CTD was not on the panel.

Supplementary Figure 6



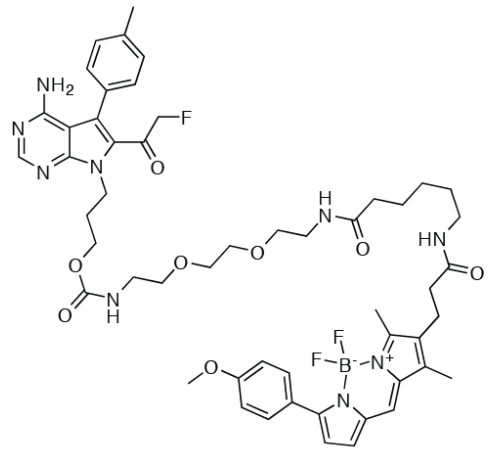
Supplementary Figure 6. Specific noncovalent interactions drive covalent bond formation. **a,** $2F_0$-F_c electron density map (contoured at 1.0 σ) showing the covalent bond between Cys436 and the electrophilic beta-carbon of compound **16**. **b,** Mutation of the RSK2-CTD gatekeeper (T493M) confers ~1000-fold resistance to CN-NHiPr as evident from kinase assays. Kinase assays with T493M RSK2-CTD were performed as described for WT RSK2-CTD.

Supplementary Figure 7

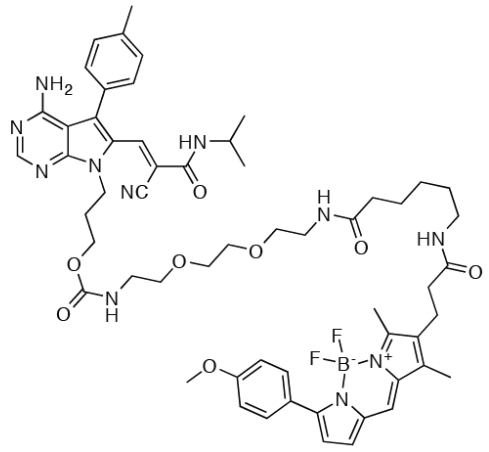


Supplementary Figure 7. Covalent binding of CN-NHiPr to RSK2-CTD requires Cys436 and is fully reversed upon protein unfolding. **a**, UV-visible spectroscopy experiment similar to Fig. 4B, but with higher concentrations of CN-NHiPr (200 μM) and C436V RSK2-CTD (300 μM). Even at high micromolar concentrations, covalent bond formation is undetectable, despite the presence of four additional cysteines (total [Cys] = 1.2 mM). In this experiment, spectra were recorded with a NanoDrop 1000 Spectrophotometer (Thermo Scientific). **b**, LC-MS chromatogram of CN-NHiPr (62.5 μM in PBS:6M guanidine-HCl:MeCN, 25:25:50) showing separation of the E and Z isomers (UV chromatogram, 350 nm, left panel) and the corresponding mass spectra (right panel). **c**, LC-MS chromatogram and mass spectra showing quantitative recovery of CN-NHiPr after unfolding the RSK2-CTD/CN-NHiPr complex with 3 M guanidine-HCl. RSK2-CTD (300 μM) in PBS was treated with CN-NHiPr (250 μM) to form the covalent RSK2-CTD/CN-NHiPr complex (confirmed by disappearance of the cyanoacrylamide absorption peak at 400 nm). An equal volume of 6 M guanidine-HCl was then added. After 1 min, acetonitrile (MeCN) was added to final concentration of 50% (resulting in a final [CN-NHiPr] of 62.5 μM). The mixture was then ultrafiltered (10 kDa MWCO) and analyzed by LC-MS as in Supplementary Fig. 7b.

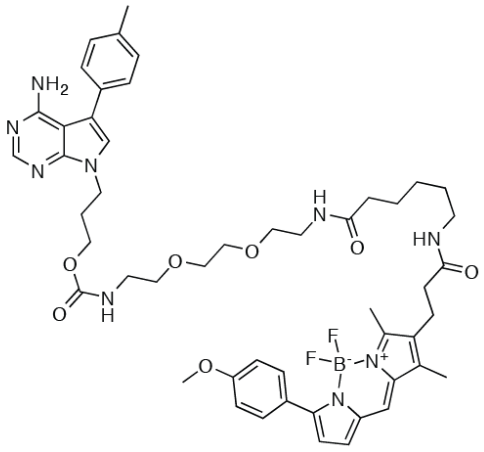
Supplementary Figure 8



17 (FMK-BODIPY)



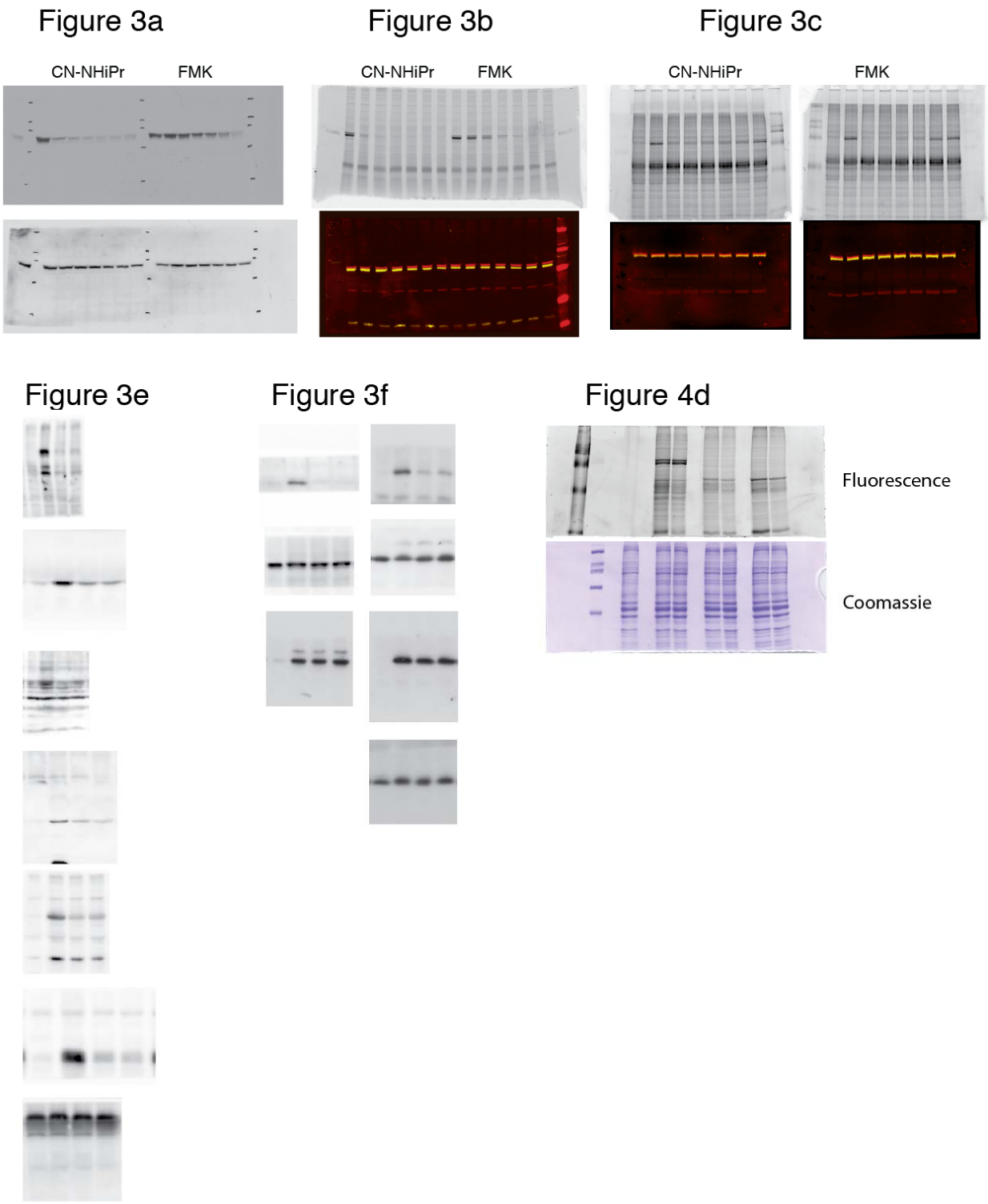
18 (CN-NHiPr-BODIPY)



19 (scaffold-BODIPY)

Supplementary Figure 8. Chemical structures of the BODIPY conjugates **17**-**19** (see Methods for synthesis and characterization data).

Supplementary Figure 9



Supplementary Figure 9. Original images of gels and Western blots.

SUPPLEMENTARY METHODS

Determination of equilibrium dissociation constants (K_D) for thiol/Michael acceptor adducts

Reactions of cyanoacrylates and cyanoacrylamides with BME, GSH, ethanolamine, and lysine were monitored with a Spectramax M5 plate reader (Molecular Devices, Sunnyvale CA). Reactions were initiated by mixing equal volumes of the Michael acceptor (200–400 μM in PBS, pH 7.4) with the thiol (0–200 mM BME or GSH in PBS pH 7.4, two-fold dilution series). Final solutions (Costar flat-bottom clear 96-well plate, 100 μL per well), containing 100–200 μM Michael acceptor and increasing concentrations of BME/GSH, were incubated for 10 min at room temp prior to acquiring absorption spectra (250–500 nm). Equilibration of these doubly activated Michael acceptors with BME or GSH occurred within seconds or less (as determined by sequential absorbance measurements, data not shown). Formation of the thiol adduct was quantified by monitoring the disappearance of the absorbance peak (λ_{max}) relative to the no-thiol control sample. Data were fit using PRISM 4.0 to obtain equilibrium dissociation constants. Test reactions of **CN-NHiPr** with ethanolamine or lysine were also carried out. Reactions were initiated as described above by mixing equal volumes of **CN-NHiPr** (100 μM in PBS, pH 7.4) with solutions of ethanolamine or lysine (0.1–100 mM amine solution in PBS pH 7.4). Negligible reaction was observed after 5 hours of incubation

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Reversible BME addition to cyanoacrylate 3 monitored by ^{1}H NMR

To a solution of cyanoacrylate **3** (17.1 mg, 91.4 μmol) in 0.75 mL of DMSO-d6 was added a solution of 400 mM BME in deuterated PBS (0.25 mL). Analysis of the reaction mixture by ^{1}H NMR after 30 minutes indicated an 85:15 ratio of the thiol adduct **6** to the starting cyanoacrylate **3**. The reaction mixture was then diluted 10-fold by addition of 100 μL to 900 μL of 3:1 v/v DMSO-d6:deuterated PBS. Analysis of the solution after 30 minutes by ^{1}H NMR indicated a 55:45 ratio of the thiol adduct **6** to the starting cyanoacrylate **3**.

In a second experiment, a solution of cyanoacrylate **3** (5.1 mg, 27.3 μmol) in 0.75 mL of DMSO-d6 was added to 0.25 mL of 400 mM BME in deuterated PBS. Analysis of the reaction mixture by ^{1}H NMR indicated a >95:5 ratio of the thiol adduct **6** to the starting cyanoacrylate **3**. The sample was then allowed to stand at ambient temperature. The ratio of adduct **6** to starting cyanoacrylate **3** changed with time as the excess BME in solution underwent air oxidation to the corresponding disulfide. After 17 days, the ratio of **6**:**3** was <5:95 (data not shown).

Characterization of the BME adduct 6.

For the purpose of characterization, a solution of cyanoacrylate 3 (13.5 mg) in DMSO-*d*6 (0.75 mL) was treated with 1.2 M BME in deuterated PBS (0.25 mL) to drive the reaction towards the adduct 6 (final concentrations of cyanoacrylate 3 and BME were 72 mM and 300

mM respectively). ^{1}H and ^{13}C spectra were acquired under these conditions and are reported for the mixture of diastereomeric adducts in solution containing excess BME.

^{1}H NMR (400 MHz, 3:1 v/v DMSO-*d*6: deuterated PBS): 7.40 (m, 1H, minor diastereomer), 7.39 (m, 1H, major diastereomer), 7.33-7.23 (m, 4H), 4.51 (s, 1H), 3.63 (s, 3H, minor diastereomer), 3.54 (s, 3H, major diastereomer), 3.47 (m, 2H, minor diastereomer), 3.38 (m, 2H, major diastereomer), 2.64–2.40 (m, 2H); ^{13}C NMR (100 MHz, 3:1 v/v DMSO-*d*6: deuterated PBS): 166.2, 139.0, 138.1, 129.9, 129.8, 129.6, 128.92, 128.87, 116.77, 116.74, 61.4, 61.2, 54.8, 54.6, 49.2, 48.3, 34.9, 34.8.

LC-MS detection of RSK2-CTD adducts with electrophilic pyrrolopyrimidines.

Purified RSK2-CTD (5 μM) was incubated with the indicated compounds (25 μM, 5 equiv) for 1 h at room temp in buffer (20 mM Hepes pH 8.0, 100 mM NaCl, 10 mM $MgCl_2$). The reaction was quenched by adding an equal volume of 0.4% formic acid, and the samples were analyzed by liquid chromatography (Microtrap C18 Protein column [Michrom Bioresources], 5% MeCN, 0.2% formic acid, 0.25 mL/min; eluted with 95% MeCN, 0.2% formic acid) and in-line ESI mass spectrometry (LCT Premier, Waters). Mass/charge envelope data were deconvolved with MassLynx software to provide molecular masses. Experimentally determined molecular masses for RSK2-CTD and derived adducts were in accord with predicted values.

In vitro kinase assays

WT or mutant RSK2-CTD (10 μM) was first activated by His_6-ERK2 (10 μM in 20 mM Hepes pH 8.0, 10 mM $MgCl_2$, 2.5 mM TCEP, 0.2 mg/mL BSA, 200 μM ATP) for 30 min at

room temp. Active RSK2-CTD (5 nM in 20 mM Hepes pH 8.0, 10 mM $MgCl_2$, 2.5 mM TCEP, 0.25 mg/mL BSA, 100 μM ATP) was then treated with inhibitors (ten concentrations, in duplicate) for 30 min. Kinase reactions were initiated by the addition of 5 μCi of [γ-^{32}P]ATP (6000 Ci/mmol, NEN) and 167 μM peptide substrate (RRQLFRGFSFVAK, CTD-tide) and incubated at room temp for 30 min. Kinase activity was determined by spotting 5 μL of each reaction onto P81 cation exchange paper (Whatman). Each blot was washed once with 1% AcOH solution, twice with 0.1% H_3PO_4 solution, and once with MeOH (5-10 minutes per wash). Dried blots were exposed for 30 min to a storage phosphor screen and scanned by a Typhoon imager (GE Life Sciences). The data were quantified using ImageQuant 5.2 software and fit using PRISM 4.0.

Dialysis of RSK2-CTD after treatment with electrophilic pyrrolopyrimidines

Activated RSK2-CTD (50 nM in 20 mM Hepes pH 8.0, 10 mM $MgCl_2$, 2.5 mM TCEP, 0.25 mg/mL BSA, 100 μM ATP) was treated with 1 μM inhibitor for 60 min at room temp. The reactions were transferred to a dialysis cassette (0.1–0.5 mL Slide-A-Lyzer, 10 kDa MWCO, Pierce) and dialyzed vs. 2 L buffer (20 mM Hepes pH 8.0, 10 mM $MgCl_2$, 1 mM DTT) at 4˚C. Buffer was exchanged after 2 h of dialysis, and then daily until the end of the experiment. 50 μL aliquots were removed each day for kinase assays. Kinase reactions (in triplicate for each time point) were initiated by adding 10 μL of 5 μCi of [γ-^{32}P]ATP (6000 Ci/mmol, NEN) and 167 μM peptide substrate (RRQLFRGFSFVAK, CTD-tide) to 10 μL of each time point aliquot and were incubated at room temp for 30 min. Kinase activity was determined as described above.

Estimation of inhibitor dissociation rates using FMK as a kinetic trap

RSK2-CTD (5 μM in 20 mM Hepes pH 8.0, 10 mM $MgCl_2$, 100 mM NaCl, 2.5 mM TCEP, 0.2 mg/mL BSA) was preincubated with 10 μM of **14** or **15** (or DMSO) for 60 min at room temp. FMK (100 μM) was then added, and 50 μL aliquots were removed at various times after FMK addition. Each time point reaction was stopped by adding an equal volume of 0.4% formic acid, and the samples were analyzed by by liquid chromatography (Microtrap C18 Protein column [Michrom Bioresources], 5% MeCN, 0.2% formic acid, 0.25 mL/min; eluted with 95% MeCN, 0.2% formic acid) and in-line ESI mass spectrometry (LCT Premier, Waters). After charge envelope deconvolution, relative amounts of unmodified RSK2-CTD and the FMK adduct were determined by quantifying the mass peak intensities using MassLynx software (peak areas provided nearly identical results). These data (percentage of FMK adduct vs. time) were fit to a single exponential function (assuming pseudo-first order kinetics) using PRISM 4.0 to estimate the dissociation half-times of **14** and **15**. This assumes that dissociation of **14** and **15** from RSK2-CTD is rate-limiting and that the rebinding rate of free **14** and **15** is negligible given the large excess of FMK competitor.

pS386 RSK inhibition in MDA-MB-231 cells

MDA-MB-231 cells were seeded into 6-well plates at a density of 300,000 cells/well in DMEM supplemented with 10% FBS. After 48 h, the media was exchanged with serum-free DMEM, and the cells were treated for 2 h with the indicated concentrations of inhibitors. Following inhibitor treatment, the cells were stimulated for 20 min with PMA (100 ng/mL), then washed with 2 mL of cold PBS and frozen onto the plate at –80˚C. The cells were thawed in the presence of 70 μL CelLytic M lysis buffer (Sigma) supplemented with protease (Complete,

Roche) and phosphatase (PhoStop, Roche) inhibitors, and mechanically scraped from the wells. The lysates were cleared by centrifugation at 14K rpm for 10 min at 4˚C, and normalized by Bradford assay. Laemmli sample buffer was added to the lysates and the proteins were separated by 7.5% SDS-PAGE and analyzed by immunoblot using phospho-Ser386 RSK (1:500 dilution, rabbit Ab, Cell Signaling #9341) and RSK2 (E-1) (1:500 dilution, mouse monoclonal, Santa Cruz cs-9986) antibodies. Immunoblots were developed and imaged as described [1].

Cellular RSK1/2 occupancy assay using FMK-BODIPY

MDA-MB-231 cells were seeded into 6-well plates at 300,000 cells/well. After 48 h, the media was exchanged with serum-free DMEM, and the cells were treated for 2 h with the indicated concentrations of inhibitors, followed by 1 h incubation with 3 μM of the FMK-BODIPY probe. The media was aspirated and the cells were washed with 2 mL of cold PBS and lysed with 70 μL CelLytic M lysis buffer (Sigma) supplemented with protease (Complete, Roche) and phosphatase (PhoStop, Roche) inhibitors. The lysates were cleared by centrifugation at 14K rpm for 10 min at 4˚C and normalized by Bradford assay. Laemmli sample buffer was added to the lysates and the proteins were separated by 7.5% SDS-PAGE and detected by in-gel fluorescence scanning with a Typhoon 9400 flatbed laser-induced scanner, followed by immunoblot analysis using RSK1 (C-21, Santa Cruz sc-231) and RSK2 (E-1, Santa Cruz sc-9986) antibodies. After incubation with primary antibodies, immunoblots were incubated with infrared dye-labeled secondary antibodies (IR680 or IR800) and visualized using the LI-COR Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

Cellular RSK1/2 occupancy after inhibitor washout

MDA-MB-231 cells were seeded into 6-well plates at 300,000 cells/well. After 24 h, the cells were treated with 1 μM of the indicated inhibitors for 1 h in DMEM supplemented with 10% serum. The media was then exchanged with inhibitor-free media (with 10% serum). At the indicated time points (post-washout), the cells were trypsinized, pelleted and frozen in liquid nitrogen. The cell pellets were lysed with 70 μL PBS supplemented with protease (Complete, Roche) and phosphatase (PhoStop, Roche) inhibitors. The lysates were cleared by centrifugation at 14K rpm for 10 min at 4˚C, normalized by Bradford assay and treated with 5 μM fmk-BODIPY for 1 h. Laemmli sample buffer was added to the lysates and the proteins were separated by 7.5% SDS-PAGE and detected by in-gel fluorescence scanning (Typhoon 9400), followed by immunoblot analysis using RSK1 (C-21, Santa Cruz sc-231) and RSK2 (E-1, Santa Cruz sc-9986) antibodies. After incubation with primary antibodies, immunoblots were incubated with infrared dye-labeled secondary antibodies (IR680 or IR800) and visualized using the LI-COR Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

Polarized epithelial cell multilayering assay

MDCK-RAF:ER cells [2] were plated onto 0.4 μm pore size Transwell polyester filters (Corning Inc., New York; #3460), and experiments were initiated 3 days after the cells reached confluency. Cells were treated for 24 h with the indicated inhibitors (2 μM) in the presence or absence of 4-hydroxytamoxifen (1 μM), after which time the cells were fixed for 10 min with Lillies fixative (4% phosphate-buffered formaldehyde [pH 7.0]) and thereafter permeabilized with 0.5% Triton X-100. Actin filaments were stained with rhodamine-phalloidin (Invitrogen) and imaged with a Zeiss LSM 510 confocal microscope.

Matrigel invasion assay

MDCK-RAF1:ER and MCF10A-RAF1:ER cells [2] were seeded at 8×10^4 cells/cm^2 in 9.6 cm^2 wells. The next day, medium was changed to complete medium with or without indicated treatment (2 μM RSK inhibitors, 1 μM 4HT). After 24 h, cells were trypsinized, and 100,000 cells were seeded in serum-free medium in the top chamber of 8 μm pore-size Transwell polyester filters (Corning, #3422) coated with growth factor-reduced Matrigel (BD Biosciences, #356231). The cells were allowed to invade against a chemotactic agent (MDCK-RAF1:ER cells, 8 nM HGF; MCF10A-RAF1:ER , 10% FBS) for 24 h with or without the indicated treatment (2 μM RSK inhibitors, 1 μM 4HT). Invading cells (attached to the filter facing the bottom chamber) were fixed in methanol, stained with 0.1% crystal violet, and quantified as described previously [3].

RSK2-CTD expression and purification

RSK2-CTD (mouse RSK2 399-740) was expressed in E. coli strain BL21 (DE3)-RIL (pET-46 Ek/LIC His6-fusion vector was kindly provided by M. Malakhova and Zigang Dong, University of Minnesota). Cells were lysed in lysis buffer (50 mM Tris pH 8.0, 0.5 M NaCl, 10% glycerol, 15 mM imidazole) using a homogenizer (EmulsiFlex-C5, Avestin) operated at 15000 psi at 4˚C for 15 min (continuous flow lysis). Soluble His-tagged RSK2-CTD was purified by Ni/NTA affinity chromatography (50 mM Tris pH 8.0, 0.5 M NaCl, 10 mM imidazole) using a 10 mL column at 2 mL/min flow rate with gradient elution (10–500 mM imidazole), followed by cleavage of the His_6-tag with enterokinase (1U per 1 mg protein) (EMD Biosciences) at 4˚C. The cleaved RSK2-CTD was further purified by HiLoad 16/60 Superdex-75 size exclusion

chromatography (20 mM Tris pH 8.0, 50 mM NaCl, 1 mM DTT) and the protein eluted as a single peak at 55 mL, corresponding to a monomer. Purified protein was concentrated and flash frozen in liquid nitrogen in 20 mM Tris pH 8.0, 50 mM NaCl, 1 mM DTT, 12% glycerol and stored at -80˚C. Protein concentrations were determined using the calculated extinction coefficients at 280 nm measured with a NanoDrop1000 spectrophotometer (Thermo Scientific).

RSK2-CTD crystallization and data collection

Initial RSK2-CTD crystallization conditions were determined through conventional screens. Purified RSK2-CTD was concentrated to 5–10 mg/mL in 20 mM Tris pH 8.0, 50 mM NaCl, 1 mM DTT, and then incubated with one molar equivalent of *tert*-butyl cyanoacrylate **16**. Drops of 1 µl RSK2-CTD/**16** and 1 µL precipitant solution were deposited in hanging drop 24-well plates using the commercial Classics II Suite (Qiagen) crystallization screen. Several conditions produced crystals, the best quality of which were under condition 45 of the Classics II Suite (Qiagen) screen, composed of 0.1 M Tris pH 8.5, 25% PEG 3350. Crystals were cryoprotected in mother liquor with 30% ethylene glycol and flash frozen by plunging in liquid nitrogen. Crystallographic data acquisition was performed at the Advanced Light Source (ALS, Berkeley, CA) Beamline 8.3.1. Two data sets were collected for cyanoacrylate **16**, one with the detector at 300 mm and the other at 450 mm with an oscillation of 0.5 degrees per frame in order to maximize the resolution and minimize spot overlap. The data sets were indexed, integrated, and scaled using the XDS suite of programs [4]. All of the data sets fit the 4_12_12 space group, with unit cell dimensions of approximately 46 x 46 x 295 Å. The length of this unit cell caused two problems: significant overlapping or spots, and highly anisotropic data sets. Overlap was minimized as described above and by reorienting the crystal in the beamline. We were unable to

improve on the anisotropy of the crystal, which subsequently resulted in higher than expected B-factors.

Structure determination

Prior to solving the liganded RSK2-CTD structure, we first re-solved the structure of apo-RSK2-CTD. We expressed the protein as described above, recrystallized it, and collected data sets on ALS Beamline 8.3.1, with the exception that we flash froze the crystals in Paratone oil (Hampton Research). These crystals exhibited significantly higher resolution (~1.9 Å), with less mosaicity. After processing the data sets using XDS, we solved the structure by molecular replacement using 2qr8 as a search model [5]. Interestingly, our data sets enabled us to build residues N-terminal to the reported structure out to residue 406 (data not shown). We then used this structure as a model for molecular replacement in solving the liganded crystal structures.

The liganded complexes were modeled by several rounds of manual rebuilding and restrained refinement with programs Coot [6] and Phenix.refine [7]. Ligands were then built in ChemDraw (CambridgeSoft), and exported in SMILES string format and read into PhenixELBOW to generate both a coordinate and restraint file that were used during model building and refinement. Before adding the ligand to the model, it was clear from the electron density map where the ligand would be placed in the pocket, and that it was covalently linked to Cys436. After ligand placement in this density, the ligand was redrawn with a tetrahedral carbon at the C11 attachment position. The previous ligand was then replaced with the newer one, and the structure refined with Phenix. The placement of the ligand was then confirmed by composite omit maps. The models were validated using Molprobity [8] from within Phenix.

Data collection and refinement statistics

	4d9u (Rsk2-CTD_**16)**
Data collection	
Space group	$P4_12_12$
Cell dimensions	
a, *b*, *c* (Å)	46.7, 46.7, 293.0
α, β, γ (°)	90, 90, 90
Resolution (Å)	50-2.4 (2.5-2.4)*
R_{sym} or R_{merge}	0.110(0.753)
$I/\sigma I$	14.7(2.06)
Completeness (%)	99.7(99.2)
Redundancy	8.0(3.7)
Refinement	
Resolution (Å)	50-2.4
No. reflections	13,695 (unique)
$R_{work/}$ R_{free}	20.4/27.6
No. atoms	2464
Protein	2403
Ligand/ion	32/1
Water	31
B-factors	
Protein	67.0
Ligand/ion	74.7
Water	52.1
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.10

*Highest resolution shell is shown in parenthesis.

Formation of covalent RSK2-CTD/CN-NHiPr complex; covalent bond dissociation upon RSK2-CTD unfolding or proteolysis

RSK2-CTD (25 μM) in PBS was added to 20 μM CN-NHiPr (or DMSO control) in a final reaction volume of 0.7 mL. UV-visible absorption spectra (250– 500 nm) were obtained for CN-NHiPr before (control) and after addition of RSK2-CTD using Spectramax M5 Spectrophotometer (1 cm path). SDS (2% w/v final) or guanidine-HCl (3 M final) was added,

followed by acquisition of absorption spectra within ~1 min. Alternatively, trypsin (32 μg total) or Proteinase K (32 μg total) were added to the covalent RSK2-CTD/CN-NHiPer complex for 1 h at 37˚C, followed by acquisition of absorption spectra.

LC-MS detection of recovered CN-NHiPr after guanidine-induced unfolding of the RSK2-CTD/CN-NHiPr covalent complex

RSK2-CTD (300 μM, PBS) was added to CN-NHiPr (250 μM, PBS) in a total volume of 50 μL. The covalent complex was then unfolded by treatment with 50 μL of 6 M guanidine-HCl for 1 min, after which time acetonitrile was added to a final concentration of 50%. The mixture was centrifuged, filtered through a 0.2 μm filter, and finally filtered through a Microcon device (10 kDa MWCO). The filtrate was then analyzed by LC-MS (20 μL injection, Waters XTerra MS C18 column, 20 min gradient, 5–70% MeCN/0.1% formic acid; Waters 2695 Alliance Separations Module; Waters Micromass ZQ mass spectrometer), as shown in fig. S7C. A control CN-NHiPr sample without RSK2-CTD was processed identically (fig. S7B).

Treatment of MDA-MB-231 cells with electrophilic and non-electrophilic BODIPY conjugates

MDA-MB-231 cells were seeded into 6-well plates at 300,000 cells/well. After 24 h, cells were treated with 10 μM or 3 μM of the indicated BODIPY conjugates **17**-**19** for 1 h in serum-free media. The media was aspirated and the cells washed with 2 mL of cold PBS and lysed with 70 μL CelLytic lysis buffer (Sigma) supplemented with protease (Complete, Roche) and phosphatase (PhoStop, Roche) inhibitors. The lysates were cleared by centrifugation at 14K rpm for 10 min at 4˚C and normalized by Bradford assay. Laemmli sample buffer was added to

the lysates and the proteins were separated by 10% SDS-PAGE and detected by in-gel fluorescence scanning (Typhoon 9400), followed by Coomassie blue staining.

Kinase profiling

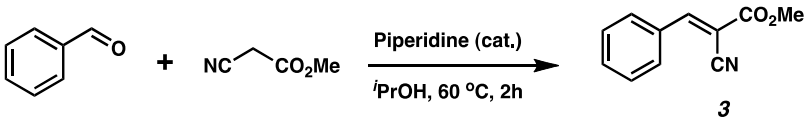
Supplementary Table 1 lists the results of profiling CN-NHiPr (1 μM) vs. 442 kinases, reported as "% DMSO Ctrl", where lower numbers indicate stronger hits. Kinase profiling of CN-NHiPr (1 μM) by competitive binding assays was performed at Ambit Biosciences (kinase profiling with this platform is now administered by DiscoveRx) [9]. The eight kinases showing >70% inhibition relative to the DMSO control are highlighted in the Supplementary Table. K_D values were subsequently determined for these kinases.

Synthetic chemistry methods

Except as otherwise indicated, reactions were carried out under dry argon or nitrogen with dry solvents. Dry solvents were dispensed from a delivery system that passes the solvents through packed columns (tetrahydrofuran, acetonitrile, and methylene chloride: dry neutral alumina; dimethylformamide: activated molecular sieves). All other reagents were purchased from commercial sources and used as received.

Yields of reactions refer to chromatographically and spectroscopically pure compounds. Reactions were monitored by thin layer chromatography (TLC) using glass plates precoated with Merck silica gel 60 F_{254}. Visualization was by the quenching of UV fluorescence (λ_{max} = 254 nm) or by staining with ceric ammonium molybdate, *p*-anisaldehyde, potassium permanganate and Iodoplatinate stain. Retention factors (R_f) are quoted to 0.01. Proton magnetic resonance spectra were recorded on Varian Inova (400 MHz) spectrometer. Chemical shifts (δ_H) are quoted in ppm and are referenced to tetramethylsilane (internal). Coupling constants (*J*) are reported in Hertz to the nearest 0.1 Hz. Data are reported as follows: chemical shift, integration, multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant(s) and number of protons. Carbon magnetic resonance spectra were recorded on a Varian Inova (100 MHz) spectrometer. Chemical shifts (δ_C) are quoted in ppm to the nearest 0.1 ppm, and are referenced to tetramethylsilane (internal). Low resolution electron impact (EI) mass spectra were obtained with a Waters AutoSpec mass spectrometer with an Agilent 7890A gas chromatograph; JEOL AX-505H, SX-102A (CI/EI), Micromass Platform II and LCT (APCI/ES/LCMS) spectrometers. Only molecular ions, fractions from molecular ions and other major peaks are reported. High-resolution mass spectra (HRMS) was obtained with a Thermo

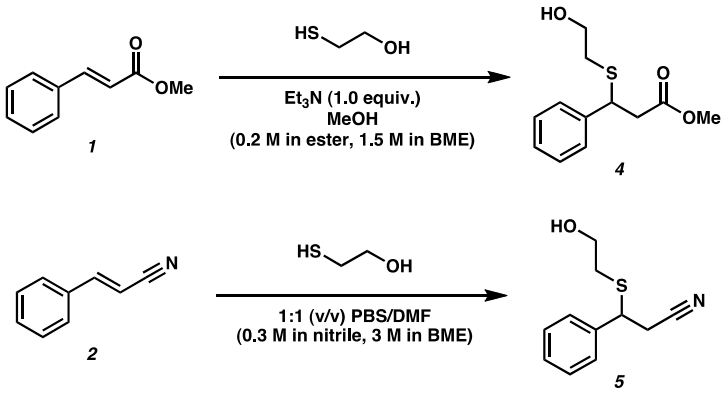
Electron Corporation LTQFT spectrometer using electrospray ionization with FT resolution set to 30000, and reported mass values are within the error limits of ± 5 ppm mass units.



3

Methyl 2-cyano-3-phenylacrylate 3

To a solution of benzaldehyde (1.28 g, 12.06 mmol) and methyl cyanoacetate (1.21 g, 12.21 mmol, 1.01 equiv) in 2-propanol (5 mL) was added piperidine (150 μL, 1.52 mmol, 0.13 equiv). The reaction mixture was heated to 60˚C for 2 h and DI water (15 mL) was added dropwise. The resulting slurry was cooled to 0–5˚C for 15 min and filtered. The filter cake was washed with 2-propanol:DI water (1:3 v/v, 30 mL) and dried in vacuo to afford a white solid (ca. 7:1 of the desired methyl ester to the corresponding isopropyl ester formed by transesterification. Purification by silica gel chromatography (5–10% EtOAc in Hexanes) afforded 1.54 g (68% yield) of the desired methyl ester **3** as a white solid [10]. R_f 0.27 (9:1 Hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ□ □ □s, 1H), 7.95 (m, 2H), 7.53–7.44 (m, 3H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 155.1, 133.3, 131.3, 131.0, 129.2, 115.3, 102.4, 53.3, MS (EI): 187.2 (M^+)



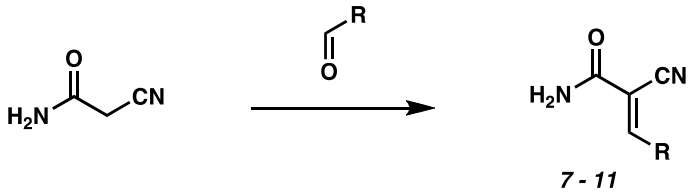
Methyl 3-(2-hydroxyethylthio)-3-phenylpropanoate 4

To a solution of *trans*-methylcinnamate **1** (133.5 mg, 0.823 mmol) and triethylamine (115 μL, 1.0 equiv) in MeOH (3 mL) was added BME (173 μL, 3.0 equiv). The reaction mixture was maintained at 20–25˚C and after 2.5 h additional BME (200 μL, 3.5 equiv) was added. The reaction mixture was maintained at 20–25 ºC for an additional 45.5 h, at which point complete conversion of the cinnamate was observed. The reaction mixture was concentrated and the residue was purified by silica gel chromatography (3:1 Hexanes:EtOAc) to afford 197 mg (>99% yield) of ester **4** as a colorless oil. R_f 0.20 (3:1 Hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.22 (m, 5H), 4.32 (t, J = 7.7 Hz, 1H), 3.62 (s, 3H), 3.61 (m, 2H), 2.88 (m, 2H), 2.56–2.52 (m, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 141.2, 128.7, 127.6, 127.5, 60.6, 51.9, 44.9, 41.3, 34.3; IR (film, cm^{-1}): 3429 (b, s), 3028 (s), 2952 (s), 1734 (s), 1436 (s), 1150 (s), 1044 (s); MS (EI): 240.1 (M^+).

3-(2-hydroxyethylthio)-3-phenylpropanenitrile 5

To a solution of cinnamonitrile **2** (204.6 mg, 1.58 mmol) in DMF (2 mL) was added 3 mL of a solution of 2-mercaptoethanol (BME, 5M) in phosphate buffered saline (PBS). The reaction mixture was maintained at 20–25˚C for 11 days at which point complete conversion was observed. The reaction mixture was diluted with EtOAc (50 mL) and washed with DI water (4 x

25 mL). The combined aqueous washes were extracted with EtOAc (50 mL). The combined EtOAc extracts were washed with brine (50 mL), dried (Na_2SO_4) and concentrated to afford a colorless oil. Purification by silica gel chromatography (3:1→5:2 Hexanes:EtOAc) afforded 201.7 mg (61% yield) of nitrile **5** as a colorless solid. R_f 0.10 (3:1 Hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ ; 7.39–7.28 (m, 5H), 4.19 (t, J = 7.3 Hz, 1H), 3.63 (m, 2H), 2.89 (m, 2H), 2.59 (m, 2H), 2.44 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.1, 129.0, 128.4, 127.3, 117.3, 60.9, 45.2, 34.5, 25.7; IR (film, cm^{-1}): 3426 (b, s), 2251 (s), 1494 (m), 1291 (m), 1046 (s); MS (EI): 207.1 (M^+).



7 - 11

General procedure for the preparation of 2-cyanoacrylamides 7-11

In a 2 mL vial fitted with a magnetic stir bar was combined the aldehyde (0.1 mmol, purchased from Aldrich), 2-cyanoacetamide (16 mg, 0.2 mmol), piperidine (0.01 mL, 0.1 mmol), and 2-propanol (0.3 mL). The mixture was stirred at room temp or 60˚C for 1–18 h. Products that precipitated from the reaction mixture were isolated by filtration, washed with water, and dried *in vacuo*. Products that did not precipitate were extracted with EtOAc and water. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was then purified by silica gel chromatography (elution with EtOAc).

3-(4-(1*H*-Imidazol-1-yl)phenyl)-2-cyanoacrylamide (7). Yield: 23 mg (97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (s, 1H), 8.22 (s, 1H), 8.08 (d, 2H, J = 7.5), 7.95-7.88 (m, 4H), 7.79

(broad s, 1H), 7.16 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.6, 149.4, 139.3, 135.7, 131.8, 130.9, 130.4, 130.0, 120.2, 119.6, 117.6, 116.5, 106.3; HRMS (ESI) found 239.0943, calcd for $C_{13}H_{11}N_4O$ (MH^+) 239.0927.

2-Cyano-3-(1*H*-indol-4-yl)acrylamide (8). Yield: 15 mg (67%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 8.52 (s, 1H), 8.04 (broad s, 1H), 7.96 (d, 1H, J = 7.7), 7.72 (broad s, 1H), 7.63 (d, 1H, J = 8.0), 7.57 (t, 1H, J = 2.2), 7.26 (t, 1H, J = 7.5 Hz), 6.81 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 147.6, 136.2, 129.1, 127.7, 122.8, 121.0, 118.9, 117.1, 115.9, 105.4, 99.5; HRMS (ESI) found 234.0649, calcd for $C_{12}H_9N_3O$ (M Na^+) 234.0638.

2-Cyano-3-(6-(4-(methylsulfonyl)phenyl)pyridin-2-yl)acrylamide (9). Yield: 12 mg (37%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.59 (d, 2H, J = 8.5), 8.30 (d, 1H, J = 8.5), 8.25 (s, 1H), 8.16 (t, 1H, J = 7.8), 8.04 (d, 2H, J = 8.5), 7.84 (d, 1H, J = 7.8), 3.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.6, 154.5, 149.9, 148.3, 142.3, 141.5, 139.2, 127.8, 127.39, 127.37, 123.4, 116.5, 109.8, 43.3; HRMS (ESI) found 328.0767, calcd for $C_{16}H_{14}N_3O_3S$ (MH^+) 328.0750.

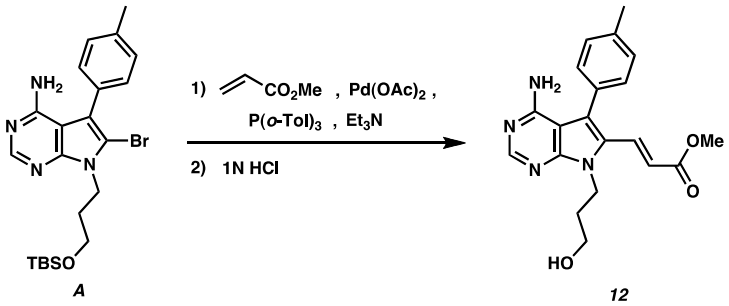
2-Cyano-3-(thiophen-3-yl)acrylamide (10).

In a 20 mL vial fitted with a magnetic stir bar was dissolved 2-cyanoacetamide (80 mg, 1 mmol) in 10% $NaHCO_3$ (3 mL). To the solution was added 3-thiophene carboxaldehyde (101 mg, 0.9 mmol) and the resulting mixture was stirred vigorously for 3 h at room temp. The product was isolated by filtration, washed with water and dried *in vacuo*. Yield: 123 mg (69%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.37 (broad s, 1H), 8.19 (s, 1H), 7.82 (broad s, 1H), 7.79 (m,

2H), 7.69 (broad s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 146.9, 135.4, 127.9, 127.4, 117.5, 101.6; MS (ES^+): 179.1 (MH^+), calcd for $C_8H_6N_2OS$ 178.0.

2-Cyano-3-(1*H*-imidazol-5-yl)acrylamide (11).

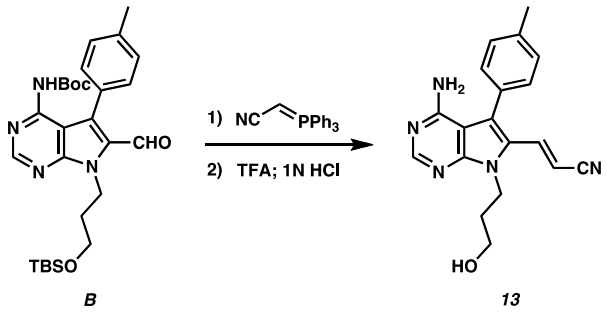
In a 20 mL vial fitted with a magnetic stir bar was dissolved 2-cyanoacetamide (80 mg, 1 mmol) in 10% $NaHCO_3$ (3 mL). To the solution was added 4(5)-imidazole carboxaldehyde (86 mg, 0.9 mmol) and the resulting mixture was stirred vigorously for 3 h at room temp. The product was isolated by filtration, washed with water and dried *in vacuo*. Yield: 58 mg (39%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.78 (s, 1H), 8.03 (s, 1H), 7.95 (s, 1H), 7.92 (s, 1H), 7.64 (broad s, 1H), 7.52 (broad s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 143.5, 138.1, 134.7, 124.6, 117.0, 100.6; HRMS (ESI) found 163.0622, calcd for $C_7H_7N_4O$ (MH^+) 163.0614



(*E*)-methyl 3-(4-amino-7-(3-hydroxypropyl)-5-*p*-tolyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)acrylate 12

Bromide **A** was synthesized as previously reported [1]. In a 10 mL glass microwave tube containing 2mL DMF were added bromide **A** (50 mg, 0.1 mmol), $Pd(OAc)_2$ (7 mg, 0.3 equiv), tri-*o*-tolyl phosphine (20 mg, 0.6 equiv), Et_3N (47 μL, 3 equiv) and methyl acrylate (95 μL, 10

equiv). The reaction mixture was heated by microwave irradiation at 100˚C with stirring for two 10 min cycles at a power of 250W. The crude reaction was diluted with 5 mL EtOAc and the organic layer was washed with saturated aqueous sodium bicarbonate (2 x 5 mL), brine (2 x 5 mL), dried over $MgSO_4$. The mixture was then concentrated to a residue and absorbed on silica and purified by column chromatography (50% EtOAc/hexanes isocratic) to afford the TBS-protected methyl ester. The TBS-protected methyl ester (28 mg) was dissolved in THF (2 mL) and cooled to 0˚C, then 1N HCl (0.3 mL) was added and the reaction was allowed to warm to room temperature (20–25˚C). After 2.5 h the deprotection was complete and the reaction was diluted with 10 mL EtOAc. The organic phase was washed with saturated aqueous sodium bicarbonate (2 x 15 mL), brine (2 x 15 mL), dried over $MgSO_4$ and concentrated. The residue was absorbed on silica and purified by column chromatography (3% MeOH in EtOAc, isocratic) to yield 12.3 mg (33%) of methyl acrylate **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 7.61 (d, *J* = 17.6 Hz, 1H), 7.28—7.21 (m, 4H), 6.05 (d, *J* = 17.6 Hz, 1H), 4.53 (t, *J* = 5.4 Hz, 2H), 3.73 (s, 3H), 3.479 (t, *J* = 5.1 Hz, 2H), 2.44 (s, 3H), 2.01 (m, 2H), 1.67 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.5, 157.7, 157.2, 153.8, 151.9, 138.8, 131.7, 130.3, 128.1, 126.9, 121.2, 118.1, 102.4, 57.4, 51.9, 38.7, 32.9, 21.5 HRMS (ESI) found 367.1775, calcd for $C_{20}H_{22}N_4O_3$ (MH^+) 367.1765

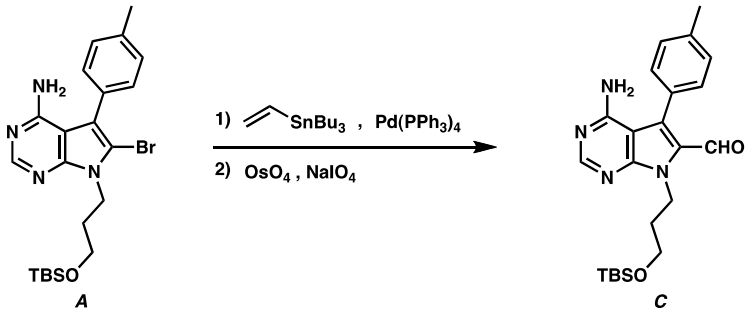


B *13*

(*E*)-3-(4-amino-7-(3-hydroxypropyl)-5-*p*-tolyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)acrylonitrile 13

A flame dried 50 mL round bottom flask was charged with aldehyde **B** (218 mg, 0.41 mmol; prepared under contract by Albany Molecular Research Inc., Albany, NY; details available upon request) and (triphenylphosphoranylidine)acetonitrile (500 mg, 4 equiv). CH_2Cl_2 (10 mL) was added and the reaction mixture was stirred at room temp. After 16 h the reaction was complete and the crude mixture after concentration was adsorbed on silica and purified by column chromatography (20% EtOAc/hexanes isocratic) to yield the protected nitrile, which was dissolved in CH_2Cl_2 (2 mL) and was cooled to 0˚C. TFA (2 mL) was added dropwise and the reaction mixture was allowed to warm to room temp. After 12 h the reaction mixture was concentrated and the residue redissolved in THF (3 mL). The solution was cooled to 0˚C and 1N HCl (1 mL) was added after which the reaction mixture was allowed to warm to room temp. After 4.5 h the deprotection was complete and the reaction mixture was diluted with 10 mL of EtOAc. The organic phase was washed with saturated aqueous sodium bicarbonate (2 x 15 mL), brine (2 x 15 mL), dried over $MgSO_4$ and concentrated. The residue was adsorbed on silica and purified by column chromatography (3% MeOH in EtOAc, isocratic) to yield 9.1 mg (11.4%) of acrylonitrile **13**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.36—7.29 (m, 4H), 7.24 (d, J = 16.8 Hz, 1H), 5.42 (d, J = 16.8 Hz, 1H), 5.15 (s, broad, 2H), 4.80 (s, broad, 1H), 4.48 (t, J = 5.8

Hz, 2H), 3.48 (m, 2H), 2.46 (s, 3H), 1.96 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.9, 154.4, 151.9, 139.4, 136.9, 130.7, 129.9, 127.2, 124.4, 121.6, 118.7, 102.5, 96.4, 57.4, 38.7, 32.9, 21.5 HRMS (ESI) found 334.1677, calcd for $C_{19}H_{20}N_5O$ (MH^+) 334.1662.

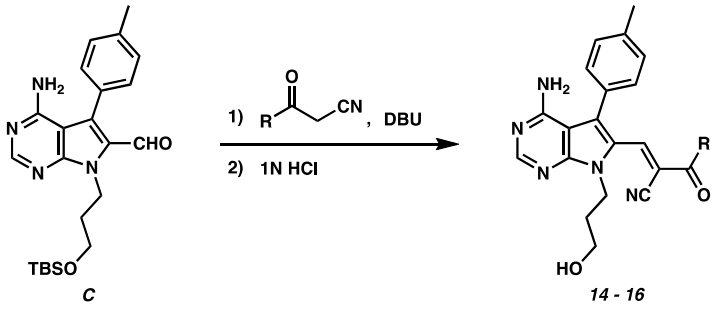


4-amino-7-(3-(*tert*-butyldimethylsilyloxy)propyl)-5-*p*-tolyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carbaldehyde C

To a solution of bromide **A** [1] (1.0 g, 2.1 mmol) in toluene (30 ml) was added tributylvinyltin (0.8 ml, 2.73 mmol). The solution was sparged with argon for 10 min. Tetrakis(triphenylphosphine)palladium (244 mg, 0.21 mmol) was quickly added and the reaction mixture was sparged with argon for a further 10 min, then heated to reflux for 3 h. The reaction mixture was filtered through celite and the filtrate was concentrated. The residue was purified by flash column chromatography in 50% EtOAc/hexanes to give a light yellow residue that was lyophilized from benzene to afford the corresponding vinyl pyrrolopyrimidine as a light yellow powder.

To a solution of the vinyl pyrrolopyrimidine (760 mg, 1.8 mmol) in 3:1 v/v THF:H_2O (11.3 ml) under argon was added, dropwise, osmium tetroxide solution (1.75 ml, 0.18 mmol, 2.5% in *t*-BuOH). The reaction was stirred at room temperature (20–25 °C) under argon for 20 min. Sodium periodate (860 mg, 3.6 mmol, dissolved in 2.4 mL of warm water) was added

dropwise to the reaction over a period of 30 min. The reaction mixture was stirred for 1.5 h at room temp and was then diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium thiosulfate and the aqueous layer was back extracted with ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography in 50% EtOAc/hexanes to give 417 mg (55% yield) of aldehyde **C** as a yellowish oil that solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.63 (s, 1H), 8.34 (s, 1H), 7.39—7.32 (m, 4H), 4.72 (m, 2H), 3.72 (m, 2H), 2.45 (s, 3H), 2.043 (m, 2H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.1, 158.8, 156.0, 151.8, 139.1, 130.5, 130.3, 129.7, 128.9, 128.4, 101.8, 60.9, 41.2, 33.7, 25.9, 21.3, 18.3, -5.4 MS (ES^+): 425.0 (MH^+).



C *14 - 16*

General procedure for the preparation of cyanoacrylates and cyanoacrylamides 14 – 16

To a solution of aldehyde **C** (0.3 mmol) in THF (2 ml) were added DBU (1.2 equiv) and the appropriate cyanoacrylate or cyanoacetamide (1.2 equiv). The reaction was stirred at room temp until all the starting material had been consumed. The reaction mixture was concentrated and the residue was purified by flash column chromatography to give the TBS-protected products.

To a solution of the TBS-protected cyanoacrylates or cyanoacrylamides (0.1 mmol) in THF (1 ml) was added 1N aqueous HCl. The reaction mixture was stirred at room temp for 1.5 h and then diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate followed by brine and then dried over sodium sulfate, filtered and concentrated. The residues were purified by flash column chromatography in 100% EtOAc → 5% MeOH/EtOAc to give the desired compounds **14–16**.

Methyl 3-(4-amino-7-(3-hydroxypropyl)-5-*p*-tolyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)-2-cyanoacrylate 14

Cyanoacrylate **14** was prepared in 22% yield from methyl 2-cyanoacetate and aldehyde **C**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.24 (s, 1H), 7.35—7.29 (m, 4H), 5.47 (s, broad, 2H), 4.49 (t, *J* = 6.0 Hz, 2H), 3.86 (s, 3H), 3.44 (t, *J* = 5.0 Hz, 2H), 2.47 (s, 3H), 1.95 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 158.5, 154.9, 152. 8, 142.1, 139.1, 130.9, 130.3, 129.6, 125.4, 114.2, 102.7, 57.6, 53.7, 39.3, 32.9, 21.6 HRMS (ESI) found 392.1723, calcd for $C_{21}H_{22}N_5O_3$ (MH^+) 392.1717.

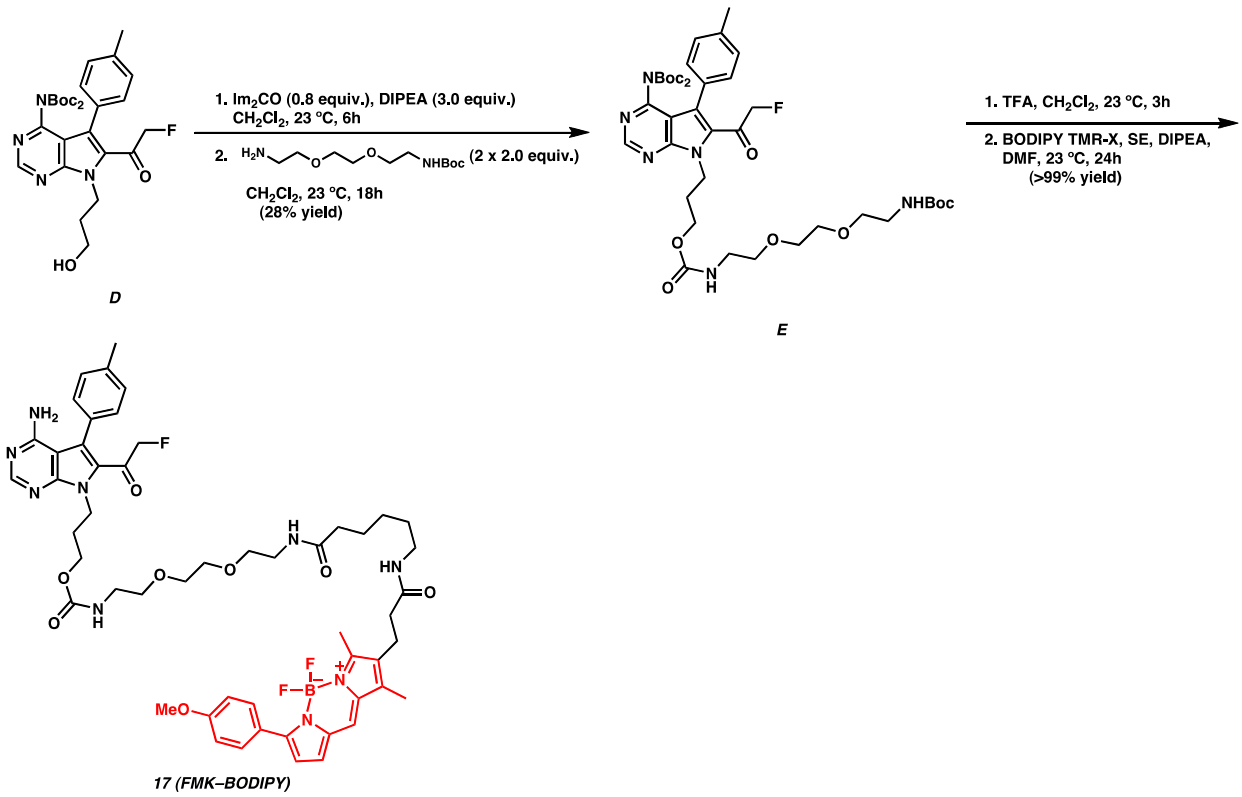
3-(4-amino-7-(3-hydroxypropyl)-5-*p*-tolyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)-2-cyano-*N*-isopropylacrylamide 15 (CN-NHiPr)

Cyanoacrylamide **16** was prepared in 13% yield from *N*-isopropylcyanoacetamide [11] and aldehyde **C**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.30 (s, 1H), 7.35—7.29 (m, 4H), 5.94 (d, *J* = 7.7 Hz, 1H), 5.51 (broad s, 1H), 4.46 (t, *J* = 5.8 Hz, 2H), 4.12 (m, 1H), 3.45 (t, *J* = 5.5 Hz, 2H), 2.43 (s, 3H), 1.95 (m, 2H), 1.20 (d, *J* = 6.6, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9, 158.3, 154.4, 152.4, 139.4, 138.7, 130.8, 130.6, 129.7, 125.8, 123.3, 115.6, 108.1, 102.5, 57.5,

43.1, 39.1, 33.0, 22.6, 21.6 HRMS (ESI) found 419.2193, calcd for $C_{23}H_{27}N_6O_2$ (MH^+) 419.2190.

tert-butyl 3-(4-amino-7-(3-hydroxypropyl)-5-_p_-tolyl-7_H_-pyrrolo[2,3-_d_]pyrimidin-6-yl)-2-cyanoacrylate 16

Cyanoacrylate **16** was prepared in 16% yield from *tert*-butyl 2-cyanoacetate and aldehyde **C**. ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.37 (s, 1H), 8.15 (s, 1H), 7.34—7.28 (m, 4H), 5.34 (broad s, 2H), 4.95 (t, J = 5.8 Hz, 2H), 3.43 (m, 2H), 2.43 (s, 3H), 1.95 (m, 2H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.1, 158.4, 156.7, 154.7, 152.1, 141.1, 138.9, 136.4, 130.7, 129.7, 124.4, 114.4, 107.9, 102.6, 57.6, 39.3, 33.0, 28.1, 27.7, 21.6 HRMS (ESI) found 434.2181, calcd for $C_{24}H_{28}N_5O_3$ (MH^+) 434.2187.



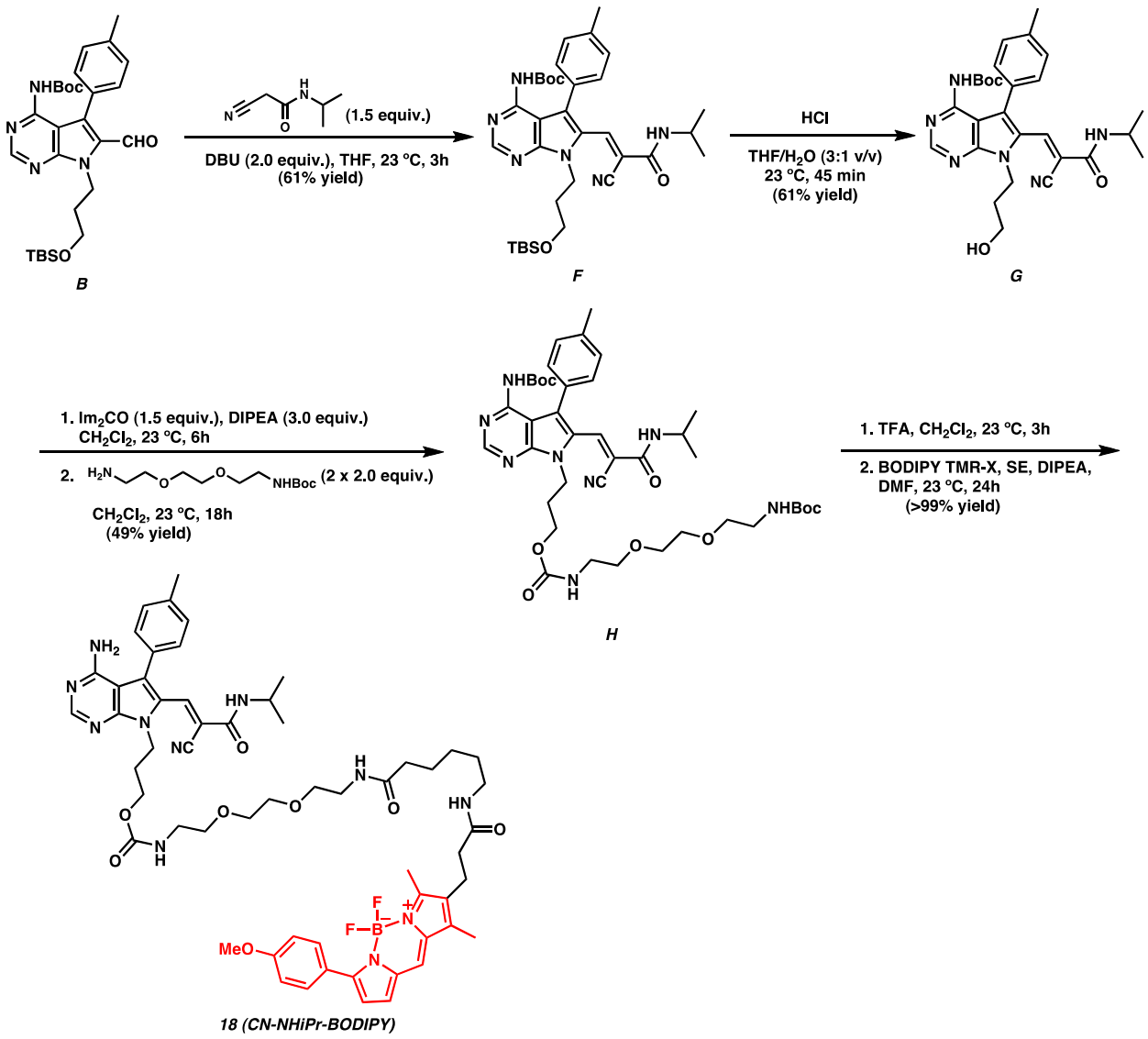
Carbamate E

To a solution of alcohol **D** [1] (65.5 mg, 0.121 mmol) and dicarbonylimidazole (15.7 mg, 0.8 equiv) in CH_2Cl_2 (1 mL) was added DIPEA (16.8 μL, 0.8 equiv). The reaction mixture was maintained at 20–25˚C for 2 h and *N*-Boc-2,2'-(ethylenedioxy)diethylamine (37.3 μL, 1.3 equiv) was added. After 3 h, additional diamine (37.3 μL, 1.3 equiv) was added. After 2 h, the reaction mixture was diluted with EtOAc (50 mL) and washed with 0.5 M aqueous HCl (20 mL) and brine (30 mL). The organic phase was dried (Na_2SO_4) and concentrated to afford a colorless oil. Purification by preparative TLC (1:2 Hexanes:EtOAc x 5 elutions) afforded unreacted alcohol **D** (15.7 mg, 24% yield), the acylimidazole of the alcohol (10.1 mg, 16% yield) and the desired carbamate **E** (20.9 mg , 28% yield) as a colorless oil. R_f 0.32 (1:1 Hexanes/EtOAc x 3 elutions); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (s, 1H), 7.25 (m, 4H), 5.17 (bs, 1H), 5.09 (bs, 1H), 4.80 (app. t, J = 7.0 Hz, 2H), 4.63 (d, J = 46.7 Hz, 2H), 4.14 (m, 2H), 3.61 (s, 4H), 3.54 (app. q, J =

5.3 Hz, 4H), 3.34 (m, 4H), 2.46 (s, 3H), 2.18 (m, 2H), 1.44 (s, 9H), 1.33 (s, 18H); MS (ESI): 817.4 (MH^+).

17 (FMK-BODIPY)

To a solution of carbamate **L** (10.1 mg, 12.4 μmol, 1.5 equiv) in CH_2Cl_2 (0.9 mL) was added TFA (0.9 mL) at 20–25˚C. The reaction mixture was maintained at ambient temperature for 3 h, then concentrated under reduced pressure to afford a colorless oil. A solution of BODIPY-TMR-X, SE (5.0 mg, 8.2 μmol, 1.0 equiv) in DMF (1.5 mL) was then added to the oil followed by DIPEA (14.3 μL, 20.0 equiv). The reaction mixture was maintained at ambient temperature for 24 h while protected from light, then concentrated under reduced pressure to afford a purple solid. Purification by preparative TLC (10:1 CH_2Cl_2:MeOH x 2 elutions) afforded FMK-BODIPY **17** (7.6 mg, 92% yield based on BODIPY-TMR-X, SE; 94.4:5.6 of fluoromethyl ketone: methyl ketone) as a purple solid. R_f 0.47 (10:1 CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.86 (d, J = 9.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 7.08 (s, 1H), 7.00–6.94 (m, 3H), 6.53 (d, J = 4.2 Hz, 1H), 6.21 (bs, 1H), 5.93 (bs, 1H), 5.36 (bs, 1H), 4.89 (bs, 2H), 4.73 (t, J = 6.9 Hz, 2H), 4.61 (d, J = 46.8 Hz, 2H), 4.09 (m, 2H), 3.86 (s, 3H), 3.64–3.51 (m, 8H), 3.41 (q, J = 5.3 Hz, 2H), 3.36 (m, 2H), 3.20 (q, J = 6.4 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.51 (s, 3H), 2.47 (s, 3H), 2.28 (t, J = 7.4 Hz, 2H), 2.20 (s, 3H), 2.15–2.08 (m, 4H), 1.57 (m, 2H), 1.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9, 159.1, 156.1, 140.2, 130.90, 130.86, 130.82, 130.7, 130.5, 129.6, 128.0, 126.9, 125.7, 123.0, 113.9, 84.9, 83.1, 70.5, 70.47, 70.38, 70.36, 70.2, 70.17, 70.15, 70.12, 70.1, 62.6, 55.5, 41.5, 41.0, 39.5, 39.4, 39.3, 36.6, 36.3, 30.1, 29.1, 26.4, 25.1, 21.6, 20.4, 14.0, 13.3, 9.8; HRMS (ESI) found 1010.4952, calcd for $C_{52}H_{64}BF_3N_9O_8$ (MH^+): 1010.



Cyanoacrylamide F.

To a solution of aldehyde **B** (247.3 mg, 0.471 mmol; prepared under contract by Albany Molecular Research Inc., Albany, NY; details available upon request) and *N*-isopropylcyanoacetamide [11] (89.2 mg, 0.707 mmol, 1.5 equiv) in THF (5 mL) was added DBU (141 μL, 0.943 mmol, 2.0 equiv). The reaction mixture was maintained at 20–25˚C for 2.5 h, quenched with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (4 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was purified by silica gel chromatography (4:1→3:1 Hexanes:EtOAc) to afford 181 mg (61% yield) of the

cyanoacrylamide **F** as a yellow oil. R_f 0.38 (3:1 Hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ□□□s, 1H□□□□s, 1H, 7.36 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 5.95 (d, *J* = 7.7 Hz, 1H), 4.49 (t, *J* = 7.3 Hz, 2H), 4.14 (sextet, *J* = 6.6 Hz, 1H), 3.63 (t, *J* = 5.9 Hz, 2H), 2.44 (s, 3H), 2.01 (m, 2H), 1.42 (s, 9H), 1.21 (d, *J* = 6.6 Hz, 6H), □□ (s, 9H), 0.36 (s, 6H)□ ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.1, 153.8, 152.71, 152.67, 149.4, 140.0, 138.8, 130.5, 130.0, 129.6, 127.8, 120.9, 115.5, 109.7, 105.4, 81.6, 60.1, 42.9, 41.0, 33.1, 28.0, 25.9, 22.5, 21.3, 18.3, –5.4; MS (ESI): 633 (MH^+)

Alcohol G

To a solution of Boc pyrrolopyrimidine **F** (181 mg, 0.286 mmol) in THF (3 mL) was added 1 M aqueous HCl (1 mL). The reaction mixture was maintained at 20–25˚C for 45 min, then quenched with saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to afford a colorless oil, which was purified by preparative TLC (EtOAc) to afford the alcohol **G** as an orange-yellow foam, 89.7 mg (61% yield). R_f 0.24 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H), 8.35 (s, 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 7.28 (d, *J* = 7.9 Hz, 2H), 5.95 (d, *J* = 7.7 Hz, 1H), 4.48 (t, *J* = 6.1 Hz, 2H), 4.11 (m, 1H), 4.05 (t, *J* = 6.9 Hz, 2H), 3.45 (q, *J* = 5.8 Hz, 2H), 2.43 (s, 3H), 1.96 (m, 2H), 1.40 (s, 9H), 1.19 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.2, 153.9, 153.0, 152.6, 149.3, 139.2, 139.0, 130.7, 129.9, 129.6, 127.5, 121.0, 115.1, 110.2, 105.0, 81.9, 57.7, 43.1, 39.3, 32.8, 28.0, 22.4, 21.4; MS (ESI): 519.6 (MH^+).

Carbamate H

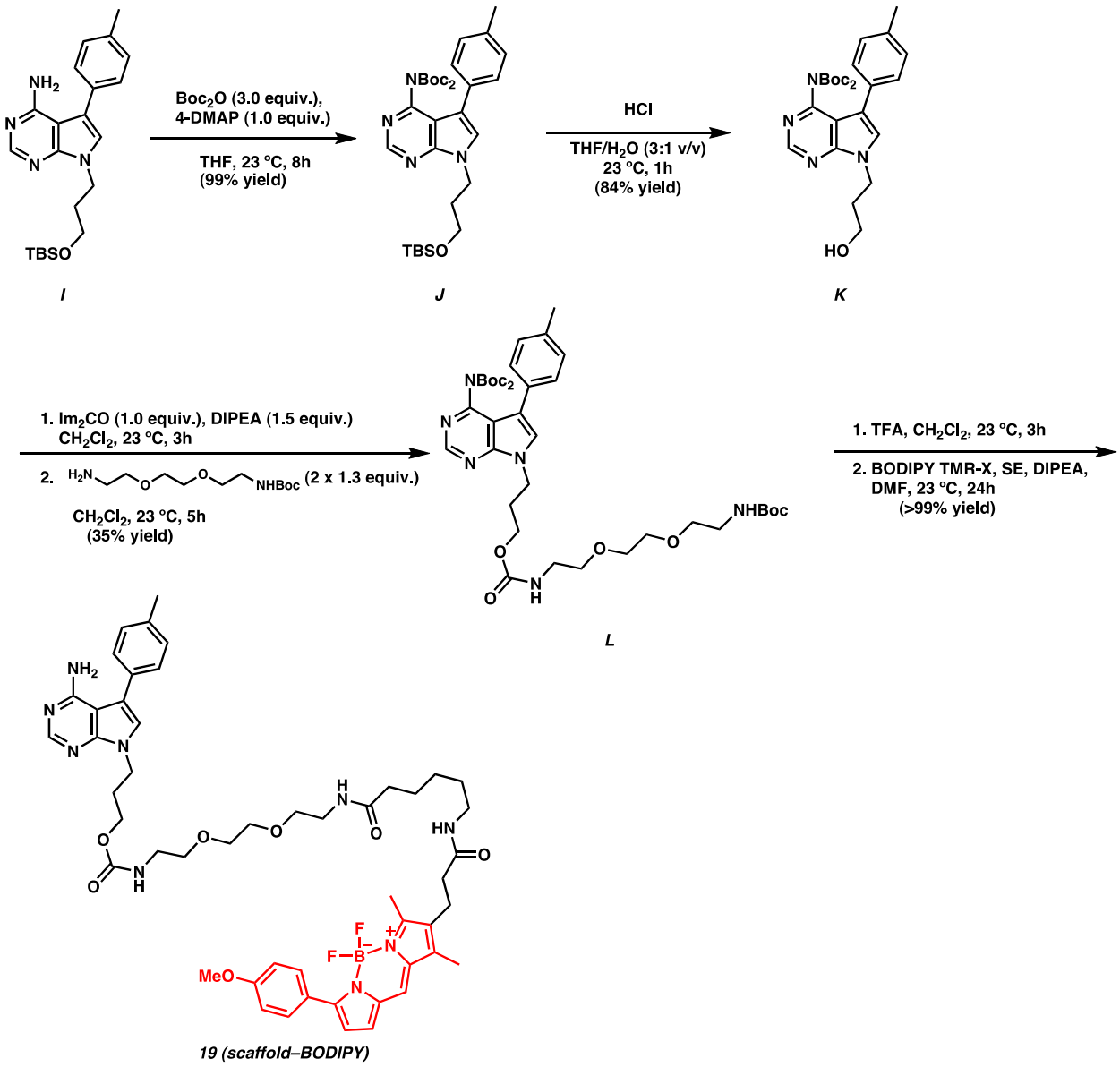
To a solution of alcohol **G** (72.5 mg, 0.140 mmol) and dicarbonylimidazole (34 mg, 0.21 mmol, 1.5 equiv) in CH_2Cl_2 (2 mL) was added DIPEA (73 μL, 0.42 mmol, 3.0 equiv). The reaction mixture was maintained at 20–25˚C for 6 h and *N*-Boc-2,2'-(ethylenedioxy)diethylamine

(66.4 μL, 0.28 mmol, 2.0 equiv) was added. After 6 h, additional diamine (66.4 μL, 0.28 mmol, 2.0 equiv) was added and the reaction mixture was maintained at 20–25˚C for 12 further hours, after which it was diluted with EtOAc (10 mL) and saturated aqueous NH_4Cl (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2 x 5 mL). The combined organic extracts were concentrated to afford a colorless oil. Purification by preparative TLC (EtOAc x 2 elutions) afforded carbamate **H** (53.9 mg , 49% yield, *E*:*Z* = 85:15) as a yellow foam. Characterized as a mixture of geometric isomers (chemical shifts in italics represent peaks due to the minor isomer), R_f 0.30 (1:2 Hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, 1H), *8.69* (s, 1H), 8.57 (s, 1H), 7.40–7.21 (m, 4H), 6.06 (bs, 1H), 5.99 (d, *J* = 7.6 Hz, 1H), 5.06 (bs, 1H), 4.44 (t, *J* = 6.7 Hz, 2H), *4.32* (t, *J* = 6.8 Hz, 2H), 4.11 (m, 1H), 4.05 (m, *J* = 5.6 Hz, 2H), 3.63–3.51 (m, 8H), 3.38 (q, *J* = 5.6 Hz, 2H), 3.31 (m, 2H), 2.43 (s, 3H), *2.41* (s, 3H), 2.18 (m, 2H), 1.85 (bs, 1H), 1.42 (s, 9H), 1.40 (s, 9H), *1.37* (s, 9H), 1.20 (d, *J* = 6.6 Hz, 6H), *1.04* (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.7, 156.3, *156.0*, 154.0, *153.2*, 152.8, *152.53*, 152.47, 152.1, *149.6*, 149.4, 140.2, *139.5*, 138.9, 130.6, *130.1*, 130.0, 129.9, *129.7*, 127.6, 120.9, 115.1, 109.2, 105.5, 81.7, 70.34, 70.29, 70.25, 70.1, 60.9, 43.1, *42.6*, 40.8, *40.6*, 40.4, 40.1, 29.4, 22.4, 22.1, 21.4, *21.3*; MS(ESI): 793.4 (MH^+).

18 (CN-NHiPr-BODIPY)

To a solution of carbamate **H** (5.2 mg, 6.6 μmol, 1.5 equiv) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL) at 20–25˚C. The reaction mixture was maintained at ambient temperature for 3 h, then concentrated under reduced pressure to afford a yellow oil. A solution of BODIPY-TMR-X, SE (2.5 mg, 4.1 μmol, 1.0 equiv) in DMF (1 mL) was then added to the oil followed by DIPEA (7.2 μL, 10.0 equiv). The reaction mixture was maintained at ambient temperature for 24 h while protected from light, then concentrated under reduced pressure to afford a purple oil.

Purification by preparative TLC (10:1 CH_2Cl_2:MeOH x 2 elutions) afforded CN-NHiPr-BODIPY **18** (4.5 mg, E:Z = 3.9:1, >99% yield based on BODIPY-TMR-X, SE) as a purple solid. R_f 0.50 (9:1 CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$), chemical shifts of peaks from the minor isomer are in italics: δ 8.53 (s, 1H), 8.32 (s, 1H), *8.31* (s, 1H), 7.86 (d, *J* = 9.0 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.08 (s, 1H), 7.00–6.94 (m, 3H), 6.53 (d, *J* = 4.0 Hz, 1H), 6.17 (bs, 1H), 6.12 (t, *J* = 5.6 Hz, 1H), 5.99 (d, *J* = 7.7 Hz, 1H), 5.84 (t, *J* = 5.7 Hz, 1H), 5.23 (bs, 2H), *5.12* (bs, 2H), 4.41 (t, *J* = 6.8 Hz, 2H), *4.28* (t, *J* = 6.9 Hz, 2H), 4.11 (m, 1H), 4.03 (t, *J* = 5.5 Hz, 2H), *3.95* (t, *J* = 6.2 Hz, 2H), 3.85 (s, 3H), 3.61–3.56 (m, 6H), 3.52 (t, *J* = 5.1 Hz, 2H), 3.42–3.34 (m, 4H), 3.20 (m, 2H), 2.75 (t, *J* = 7.3 Hz, 2H), 2.52 (s, 3H), 2.43 (s, 3H), *2.41* (s, 3H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.21 (s, 3H), 2.17–2.09 (m, 4H), 1.59 (m, 2H), 1.44 (m, 2H), 1.25 (m, 2H), 1.20 (d, *J* = 6.6 Hz, 6H), *1.05* (d, *J* = 6.5Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 171.6, 154.5, 140.3, 130.6, 130.5, 130.0, 129.6, 127.8, 122.7, 118.2, 113.7, 70.3, 70.0, 69.90, 69.88, 55.3, 43.0, 39.2, 39.1, 39.05, 36.4, 36.0, 29.7, 29.4, 28.9, 26.2, 24.8, 22.4, 21.4, 20.1, 13.2, 9.6; HRMS (ESI) found 1086.5583, calcd for $C_{57}H_{71}BF_2N_{11}O_8$ (MH^+): 1086.5543.



DiBoc pyrrolopyrimidine J

To a solution of pyrrolopyrimidine **I** [1] (116.4 mg, 0.293 mmol) and DMAP (7.2 mg, 0.2 equiv) in MeCN/THF (2:1 v/v, 6 mL) was added Boc_2O (160.1 mg, 2.5 equiv). The reaction mixture was maintained at 20–25˚C for 2 h, at which point additional Boc_2O (160.1 mg, 2.5 equiv) and DMAP (36 mg, 1.0 equiv) was added. The reaction mixture was maintained at 20–25˚C for an additional 8 h, then concentrated to afford a brown solid. Purification by preparative TLC (3:1 Hexanes:EtOAc) afforded the DiBoc pyrrolopyrimidine **J** as a colorless foam, 173.8

mg (99% yield). R_f 0.38 (3:1 Hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.27 (s, 1H), 7.18 (d, J = 8.1 Hz, 2H), 4.43 (t, J = 6.9 Hz, 2H), 3.65 (t, J = 5.8 Hz, 2H), 2.38 (s, 3H), 2.09 (app. q, J = 6.4 Hz, 2H), 1.27 (s, 18H), 0.91 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.4, 151.4, 150.9, 150.7, 136.5, 130.6, 129.2, 128.6, 127.8, 116.0, 113.3, 83.0, 59.7, 41.8, 32.8, 27.7, 26.0, 21.2, 18.3, –5.3; MS (ESI): 597.3 (MH^+).

Alcohol K

To a solution of DiBoc pyrrolopyrimidine **H** (113.1 mg, 0.190 mmol) in THF (3 mL) was added 1M aqueous HCl (1 mL). The reaction mixture was maintained at 20–25˚C for 1 h, then quenched with saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to afford a colorless oil, which was purified by preparative TLC (1:2 Hexanes:EtOAc) to afford the alcohol **K** as a colorless oil, 76.2 mg (84% yield). R_f 0.17 (1:1 Hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 7.35 (d, J = 7.9 Hz, 2H), 7.24 (s, 1H), 7.19 (d, J = 7.9 Hz, 2H), 4.46 (t, J = 6.2 Hz, 2H), 3.77 (t, J = 6.3 Hz, 1H), 3.49 (m, 2H), 2.38 (s, 3H), 2.03 (m, 2H), 1.29 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.6, 151.8, 150.9, 150.6, 136.8, 130.2, 129.3, 128.6, 127.5, 116.7, 113.2, 83.2, 58.0, 41.1, 33.0, 27.6, 21.2; MS (ESI): 483 (MH^+).

Carbamate L

To a solution of alcohol **I** (54 mg, 0.112 mmol) and dicarbonylimidazole (18.1 mg, 1.0 equiv) in CH_2Cl_2 (2 mL) was added DIPEA (29.2 μL, 1.5 equiv). The reaction mixture was maintained at 20–25˚C for 3 h and *N*-Boc-2,2'-(ethylenedioxy)diethylamine (34.5 μL, 1.3 equiv) was added. After 3 h, further diamine (34.5 μL, 1.3 equiv) was added. The reaction mixture was maintained at ambient temperature for a further 2 h and then diluted with EtOAc (10 mL) and saturated aqueous NH_4Cl (5 mL). The phases were separated and the aqueous phase was

extracted with EtOAc (2 x 5 mL). The combined organic extracts were concentrated to afford a colorless oil. Purification by preparative TLC (1:2 Hexanes:EtOAc x 5 elutions) afforded carbamate **L** (30 mg , 35% yield) as a colorless oil. R_f 0.30 (1:2 Hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.26 (s, 1H), 7.18 (d, J = 8.1 Hz, 2H), 5.20 (bs, 1H), 5.05 (bs, 1H), 4.42 (t, J = 6.9 Hz, 2H), 4.14 (t, J = 5.7 Hz, 2H), 3.59 (s, 4H), 3.53 (app. q, J = 4.7 Hz, 4H), 3.33 (m, 4H), 2.38 (s, 3H), 2.22 (m, 2H), 1.43 (s, 9H), 1.28 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.4, 156.1, 153.5, 151.5, 151.1, 150.8, 136.7, 130.5, 129.3, 128.7, 127.3, 116.4, 113.4, 83.2, 70.33, 70.28, 70.1, 62.1, 42.3, 40.8, 40.4, 29.7, 28.5, 28.1, 27.8, 21.2; MS (ESI): 757.9 (MH^+).

19 (scaffold-BODIPY)

To a solution of carbamate **L** (10.8 mg, 14.3 μmol, 3.5 equiv) in CH_2Cl_2 (1 mL) was added TFA (1 mL) at 20–25˚C. The reaction mixture was maintained at ambient temperature for 3 h, then concentrated under reduced pressure to afford a colorless oil. A solution of BODIPY-TMR-X, SE (2.5 mg, 4.1 μmol, 1.0 equiv) in DMF (1 mL) was then added to the oil followed by DIPEA (7.2 μL, 10.0 equiv). The reaction mixture was maintained at ambient temperature for 24 h while protected from light, then concentrated under reduced pressure to afford a purple oil. Purification by preparative TLC (10:1 CH_2Cl_2:MeOH x 2 elutions) afforded scaffold-BODIPY **19** (4.1 mg, >99% yield based on BODIPY-TMR-X, SE) as a purple oil. R_f 0.40 (10:1 CH_2Cl_2:MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 7.86 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.08 (s, 1H), 7.00–6.93 (m, 4H), 6.53 (d, J = 4.1 Hz, 1H), 6.19 (bs, 1H), 5.91 (bs, 1H), 5.33 (bs, 1H), 5.14 (bs, 2H), 4.30 (t, J = 6.5 Hz, 2H), 4.08 (t, J = 5.8 Hz, 2H), 3.85 (s, 3H), 3.59 (bs, 4H), 3.55–3.50 (m, 4H), 3.41 (q, J = 5.2 Hz, 2H), 3.34 (m, 2H), 3.19 (q, J = 6.5 Hz, 2H), 2.75 (t, J = 7.4 Hz, 2H), 2.51 (s, 3H), 2.40 (s, 3H), 2.27 (t, J = 7.3

Hz, 2H), 2.19 (s, 3H), 2.18 (m, 2H), 2.09 (t, J = 7.2 Hz, 2H), 1.56 (m, 2H), 1.42 (m, 2H), 1.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 160.4, 157.0, 151.8, 139.9, 137.0, 134.9, 134.4, 133.3, 131.7, 130.7, 129.7, 128.7, 127.8, 125.5, 122.7, 122.5, 118.3, 118.2, 116.4, 116.3, 113.7, 70.2, 70.0, 69.9, 61.9, 55.3, 41.5, 40.8, 39.9, 39.6, 36.4, 36.0, 29.7, 28.8, 26.2, 24.8, 21.1, 20.1, 14.1, 13.2, 9.6; HRMS (ESI) found 950.4940, calcd for $C_{50}H_{63}BF_2N_9O_7$ (MH^+): 950.4906.

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