Supporting Information

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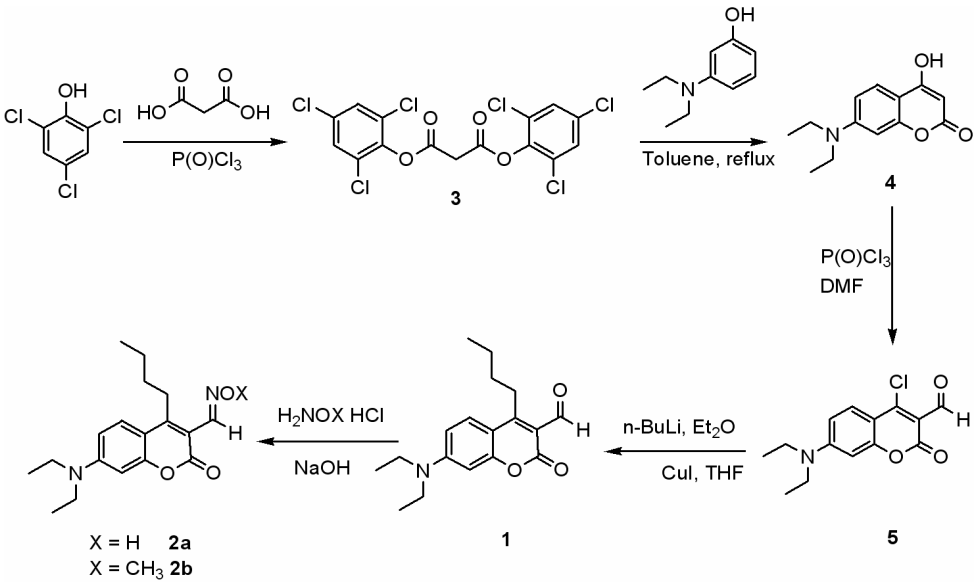
Synthetic Procedure

General: ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 300 spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (0 ppm) as the internal standard and coupling constants (*J*) are recorded in Hertz (Hz). The multiplicities in the ^{1}H NMR are reported as (br) broad, (s) singlet, (d) doublet, (dd) doublet of doublets, (ddd) doublet of doublet of doublets, (t) triplet, (sp) septet, (m) multiplet. All spectra are recorded at ambient temperatures. UV-vis experiments were performed on a Beckman DU-70 UV-Vis spectrometer and Fluorescence experiments were carried out on a PTI fluorimeter. Low and High-resolution mass spectra were measured with a Finnigan TSQ70 and VG Analytical ZAB2-E instruments, respectively. Stopped-flow Experiments were carried out on A KinTek Stopped-Flow apparatus (Model SF-2001, KinTek Corp., Austin, TX).



Preparation of Magic Malonate (3)[1]: To a 100 mL RBF were added malonic acid (0.52 g, 5.0 mmol), 2,4,6-trichlorophenol (1.58 g, 8.0 mmol) and $P(O)Cl_3$ (1 mL, 1.645 g, 10.5 mmol). The flask was topped with a $CaSO_4$ drying tube and was heated to 100 °C for 4 h with constant stirring. After this time, the reaction was slowly poured into 75 mL CH_2CL_2 and washed with iced, saturated $NaHCO_3$ solution (3 × 100 mL). The organic layer was then dried over Na_2SO_4 and concentrated to yield a white solid (1.70 g, 92% yield). ^{1}H NMR ($CDCl_3$) δ 7.27 (s, 2H), 4.13 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 161.7, 142.6, 133.0, 129.7, 129.0, 40.0.

Preparation of 7-(Diethylamino)-4-hydroxy-coumarin (4)[1]: To a 500 mL RBF were added **1** (4.63 g, 10 mmol) and 3-diethylaminophenol (1.65 g, 10 mmol). Dry toluene (10 ml) was added and the reaction was brought to reflux with constant stirring for 2 h. After this period, the reaction was allowed to cool to room temperature and was filtered. The resultant grey solid was washed with toluene and dried under high vacuum. (1.28 g, 55% yield) ^{13}C NMR ($CDCl_3$) δ 168.2, 166.9, 156.6, 151.6, 124.6, 108.8, 104.3, 97.1, 44.8, 12.5; LRMS (CI) 234 (100%) M+1.

Preparation of 7-(Diethylamino)-4-chloro-3-formyl-coumarin (5)[1]: To a 50 mL RBF was added was added **2** (1.05 g 4.5mmol) and anhydrous DMF (3.2 mL) The reaction was heated at 100 °C until homogenous and the solution was then stored at –4 °C for 3 h. After this time, large pale grey crystals had formed in the bottom of the flask. The reaction was set stirring under argon and $P(O)Cl_3$ (1.1 mL, 1.82 g) was added dropwise over a period of 5 min. After 30 min, the reaction was transferred to a sepratory funnel and 100 mL CH2Cl2 was added. The organic layer was washed with saturated $NaHCO_3$ solution (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated to give a dark oil. This was subjected to column chromatography (silica 1:1 hexanes:EtOAc) to give an orange solid (689 mg, 55% yield) ^{1}H NMR ($CDCl_3$) δ 10.27 (2, 1H), 7.82 (d, J = 9.3 Hz, 2H), 6.69 (d, J = 10.2 Hz, 1H), 6.42 (d, J = 2.4 Hz, 1H), 3.50 (q, J = 7.2 Hz, 4H) 1.27 (t, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 187.2, 160.1, 156.7, 154.3, 129.5, 111.1, 110.8, 107.9, 96.8, 45.6, 12.7.

Preparation of 7-(Diethylamino)-4-butyl-3-formyl-coumarin (1)[1]: A suspension of CuI (99.99%) (0.30g, 1.8 mmol) was added to dry diethyl ether (20 mL) and cooled to 0 °C, to which n-BuLi (1.7 mL in 2 M pentane, 3.4 mmol) was added slowly over a 15 min period. The solution was then cooled to –78 °C (acetone/dry ice) and dry THF was

added. In a separate flask 4-chloro-7-diethylamino-3-formylcoumarin (**X**) (0.50 g, 1.8 mmol) was dissolved in dry THF (15 mL) and was added drop wise via a cannula over a 30 min period to th n-BuLi solution. The solution was continuously stirred and allowed to warm to room temperature. The solution was quenched with saturated NH_4Cl. The aqueous layer was extracted several times into chlorofor (3 × 75 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent removed. The orange oil was the subjected to column chromatography (EtOAc/Hex 10:90) that gave coumarin **2a** as am orange solid (0.25 g, 55 % yield). ^{1}H NMR ($CDCl_3$) δ 10.37 (s, 1H), 7.62 (d, *J* = 9.3 Hz, 1H), 6.66 (dd, *J* = 2.6, 9.3.2 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 3.46 (q, *J* = 7.2 Hz, 4H), 3.26 (t, *J* = 7.2 Hz, 2H), 1.65 (m, 4H) 1.23 (t, *J* = 7.2 Hz, 6H) 0.99 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 190.8, 164.2, 164.1, 163.2, 157.2, 157.5, 152.5, 128.5, 111.7, 109.8, 108.5, 97.3, 45.0, 32.6, 27.5, 23.3, 13.8, 12.5.

General preparation of Coumarin derivatives (2a) and (2b). A suspension of coumarin **1** (200 mg, 0.7 mmol) in ethanol (15 mL) was added to a solution of either hydroxylamine hydrochloride or methoxylamine hydrochloride (1.4 mmol) and sodium hydroxide (1.4 mmol) in water (10 mL) and stirred at room temperature for 7 h. The solution was adjusted to pH 6 by the addition of glacial acetic acid and placed into an ice bath for 1 h. The yellow solid was filtered, washed once with a mixture of glacial acetic acid and water (50 mL, 10 : 1) and recrystallized from ethanol–water.

Characterization of **2a**: ^{1}H NMR ($CDCl_3$) δ 8.58 (brs, 1H) disappears on D_2O shake, 8.41 (s, , 1H), 7.52 (d, *J* = 9.22 Hz, 1H), 6.63 (dd, *J* = 2.6, 9.22 Hz, 1H), 6.48 (d, *J* = 2.8 Hz, 1H), 3.44 (m, 4H), 3.01 (m, 2H) 1.62-1.43 (m, 4H) 1.22 (t, *J* = 6.92 Hz, 4H); 0.94 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 168.2, 164.1, 153.8, 146.2, 129.5, 119.0, 118.3, 109.9, 106.8, 51.0, 36.5, 31.6, 25.5, 16.1, 15.3; UV-Vis (DMSO, 409 nm λ=409 nm); CI-MS: m/z =$[M]^+$ 316; CI-HRMS: calcd for $C_{18}H_{24}N_2O_3$ 316.178, found $C_{18}H_{24}N_2O_3$ 316.177. X-Ray quality crystals where grown from slow evaporation of MeOH (*vide infra*).

Characterization of **2b:** ^{1}H NMR ($CDCl_3$) 8.38s, (s, 1H), 7.51 (d, *J* = 9.22 Hz, 1H), 6.63 (dd, *J* = 2.6, 9.22 Hz, 1H), 6.48 (d, *J* = 2.8 Hz, 1H), 3.93 (s, 3H) 3.43 (m, 4H), 3.07 (m, 2H) 1.51-1.70 (m, 4H) 1.22 (t, *J* = 6.92 Hz, 6H); 1.0 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 169.0,

165.0, 165.0, 152.7, 146.1, 129.6, 119.5, 118.0, 111.4, 107.3, 52.1, 49.9, 35.5, 31.6, 24.5, 16.1, 13.3; UV-Vis (DMSO, λ = 410 nm); CI-MS: m/z =$[M+H]^+$ 331; CI-HRMS: calcd for $C_{19}H_{27}N_2O_3$ 331.194, found $C_{18}H_{24}N_2O_3$ 331.194.

General UV-Vis spectroscopic methods: Solutions of **2a** and **2b** (2.5×10^{-5} mol dm^{-3}) were prepared in DMSO solution. Aliquots of DFP 6.00 mol dm^{-3} were added to a 1 mL UV-Vis cell. The UV-Vis spectrum was recorded after each addition. Figure S1 shows the UV-Vis spectra of A) coumarin 2a and 2a (oximate) and B) œumarin 2b on the addition of DFP. The spectra over lay indicating that the protected coumarin does not undergo phosphorylation.

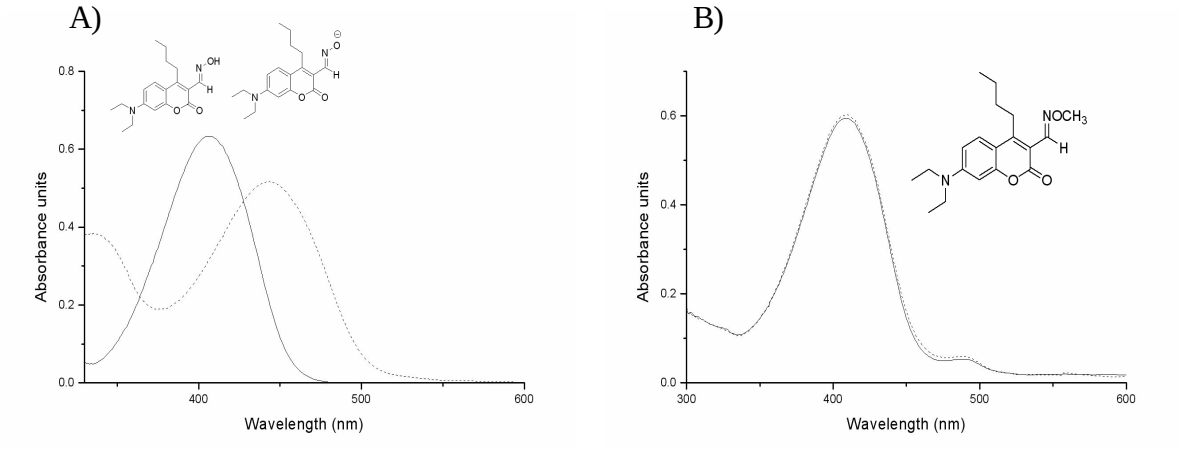


Figure S1: UV-Vis Spectra of A) 2a and 2a(oximate) B) 2b model coumarin.

General fluorescence spectroscopic methods: Solutions of **2a** and **2b** (2.5×10^{-6} mol dm^{-3}) were prepared in DMSO solution. The solution was excited at 410 nm and emission spectra were recorded between 425 and 600 nm. An excess of DFP 6.00 mol dm^{-3} was added to a 2 mL fluorescence cell. Notice that the fluorescence intensity is high and no quenching is seen on the addition of base and no spectral change is seen upon phosphorylation, (Figure S2).

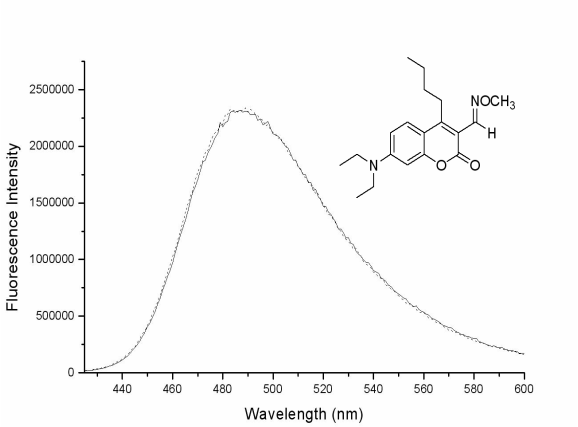


Figure S2: Coumarin model compound 2a and the addition of excess DFP

General Stop-Flow Method: A 2.5×10^{-5} mol dm^{-3} solution of either coumarin **2a** or **2b** was prepared in DMSO (containingg P4 base) and 1 mL was transferred to syringe A (Figure S3) and a 1.25×10^{-4} mol dm^{-3} solution of DFP was prepared in DMSO and 1 mL transferred to syringe B (Figure S3). Upon mixing equal volumes the fluorescence spectra was recorded for 4 seconds (λ_{ex} = 410 nm). The straight line kinetic trace was seen when equal volumes of 2b and DFP were mixed (Figure S4).

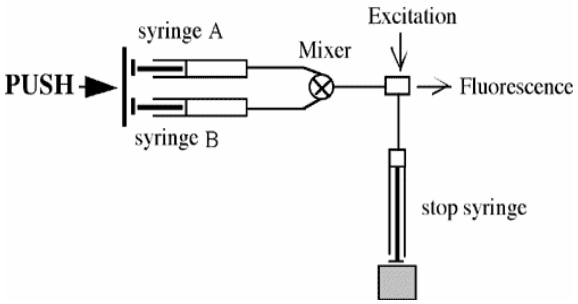


Figure S3: A typical experimental set up for stopped-flow kinetics

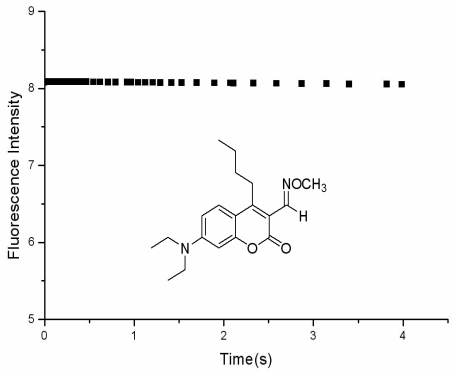


Figure S4 Kinetic-trace of model coumarin 2b on phosphorylation with DFP

X-Ray Crystallography for $C_{18}H_{24}N_2O_3$: Crystals grew as orange lathes by slow evaporation from methanol. The data crystal was cut from a larger crystal and had approximate dimensions; 0.27 x 0.20 x 0.04 mm. The data were collected on a Nonius Kappa CCD diffractometer using a graphite monochromator with MoKα radiation (λ = 0.71073Å). A total of 270 frames of data were collected using ω-scans with a scan range of 1° and a counting time of 216 seconds per frame. The data were collected at 153 K using an Oxford Cryostream low temperature device. Details of crystal data, data collection and structure refinement are listed in Table 1. Data reduction were performed using DENZO-SMN.[2] The structure was solved by direct methods using SIR97[3] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-H atoms using SHELXL-97.[4] The hydrogen atoms on carbon were calculated in ideal positions with isotropic displacement parameters set to 1.2xUeq of the attached atom (1.5xUeq for methyl hydrogen atoms). The hydrogen atoms on the oxime oxygen atoms were observed in a ΔF map and refined with isotropic displacement parameters. The function, $\Sigma w(|F_o|^2 - |F_c|^2)^2$, was minimized, where $w = 1/[(\sigma(F_o))^2 + (0.02*P)^2]$ and $P = (|F_o|^2 + 2|F_c|^2)/3$. $R_w(F^2)$ refined to 0.141, with R(F) equal to 0.0786 and a goodness of fit, S, = 1.14. The data were corrected for secondary extinction effects. The correction takes the form: $F_{corr} = kF_c/[1 + (2.6(3)x10^{-6})* F_c^2 \lambda^3/(\sin 2\theta)]^{0.25}$ where k is the overall scale factor. Neutral atom scattering factors and values used to calculate the linear

absorption coefficient are from the International Tables for X-ray Crystallography (1992).[5]

References:

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(5) Wilson, J. C. In *International Tables for X-ray Crystallography*: Boston, 1992, pp Tables 4.2.6.8 and 6.1.1.4,.