Supporting Information

Synthesis of Hydrophilic Caged DAG-lactones for Chemical Biology Applications

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I. General information

I-I. General methods

All reactions were performed using commercially supplied reagents and solvents in dried glassware under an atmosphere of nitrogen unless otherwise noted. CH$_2$Cl$_2$ was used after being distilled from CaH$_2$. Analytical thin-layer chromatography (TLC, Merck 60F$_{254}$) was performed on precoated silica-gel plates and was visualized by fluorescence quenching under UV light and staining with phosphomolybdic acid and ninhydrin. Flash column chromatography was conducted using Silica-gel 60 N (Kanto Chemical Co.).

I-II. Characterization Data

$^1$H NMR (400 or 500 MHz) and $^{13}$C NMR (126 MHz) spectra were obtained with a Bruker Avance II spectrometer with a CryoProbe. All NMR measurements were performed at 25 ºC using DMSO-$d_6$, CDOD, CDCl$_3$ and THF-$d_8$ as solvents. Chemical shifts are reported in $\delta$ (ppm) relative to Me$_4$Si or the solvent peak as an internal reference. Infrared (IR) spectra were measured on a JASCO FT/IR 4100, and are recorded as wavelength (cm$^{-1}$). The absorbance spectra (UV/Vis) and fluorescence spectra (FL) were measured with a JASCO V-650 spectrophotometer and a JASCO FP-750 spectrofluorophotometer, respectively. High-resolution mass spectra (HRMS) were recorded on a Bruker Daltonics microOTOF (ESI-MS) spectrometer under positive (ESI$^+$) and negative (ESI$^-$) electrospray ionization.
II. Experimental procedures

(2-(((7-Hydroxy-6-iodo-2-oxo-2H-pyrano[2,3-b]pyridin-4-yl)oxy)carbonyl)oxy)methyl)-5-oxo-4-(prop-2-ylidene)tetrahydrofuran-2-yl)methyl decanoate (2): DAG-lactone 1 (24.4 mg, 0.0717 mmol) was added at 0 °C to a solution (A) of CDI (11.4 mg, 0.0703 mmol) in THF (0.400 mL), and the mixture was stirred at 0 °C for 1 h. A solution (B) of 8-aza-Ihc-CH$_2$OH (22.5 mg, 0.0705 mmol) and Et$_3$N (30.0 μL, 0.215 mmol) in THF (0.6 mL) was prepared in a separate flask. After 1 h, solution A was added to solution B by syringe. The mixture was then stirred at room temperature for 9 h. The reaction mixture was concentrated with reduced pressure to give the crude compound. Purification by flash column chromatography over silica gel with CHCl$_3$-MeOH (7:1) gave the title compound (2) (22.2 mg, 46% yield) as a white solid; $^1$H NMR (500 MHz, CDCl$_3$) δ 0.88 (m, 3H), 1.28 (m, 12H), 1.61 (m, 2H), 1.91 (s, 3H), 2.28 (s, 3H), 2,34 (m, 2H), 2.78 (m, 2H), 4.22 (m, 2H), 4.33 (s, 1H), 5.22 (s, 2H), 6.29 (s, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 14.1, 20.1, 22.7, 24.7, 24.8, 29.1, 29.2, 29.4, 31.8, 32.5, 34.0, 64.4, 65.0, 69.2, 78.4, 80.0, 102.0, 108.8, 117.7, 144.3, 147.3, 152.9, 153.9, 155.7, 157.2, 160.7, 168.1, 173.1; HRMS (ESI), m/z calcd for C$_{29}$H$_{35}$INO$_{10}$ [M-H]$^-$ 684.1311, found 684.1309.

(2-(((6-Bromo-7-hydroxy-2-oxo-2H-pyrano[2,3-b]pyridin-4-yl)oxy)carbonyl)oxy)methyl)-5-oxo-4-(propan-2-ylidene)tetrahydrofuran-2-yl)methyl decanoate (3): The DAG-lactone 1 (23.8 mg, 0.0700 mmol) was added to a solution of CDI (11.4 mg, 0.0705 mmol) in THF (0.400 mL), and the mixture was stirred at 0 °C for 1 h. Subsequently, 8-aza-B(BuO)c-CH$_2$OH (22.9 mg, 0.0700 mmol) and Et$_3$N (24.4 μL, 0.175 mmol) in THF (0.6 mL) were added to the above solution, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under
reduced pressure, and purification by flash column chromatography over silica gel with hexane-EtOAc (2:1) gave the compound S1 (29.4 mg, 61% yield). TFA (0.420 mL) was added to a solution of compound S1 (29.4 mg, 0.0424 mmol) in CH2Cl2 (0.140 mL), and the mixture was stirred at room temperature for 1 h. Purification by flash column chromatography over silica gel with CHCl3-MeOH (7:1) gave the title compound (3) (16.5 mg, 61% yield) as a white solid; 1H NMR (500 MHz, CDCl3) δ 0.88 (m, 3H), 1.27 (m, 12H), 1.60 (m, 2H), 1.90 (s, 3H), 2.28 (s, 3H), 2.32 (m, 2H), 2.78 (m, 2H), 4.19 (m, 2H), 4.33 (s, 1H), 5.21 (s, 2H), 6.31 (s, 1H), 7.98 (s, 1H); 13C NMR (126 MHz, CDCl3) δ 14.1, 20.1, 22.6, 24.7, 24.8, 29.1, 29.2, 29.4, 31.8, 32.5, 34.0, 64.4, 65.0, 69.2, 78.4, 99.5, 101.1, 109.2, 117.7, 137.5, 152.9, 153.9, 154.6, 168.0, 173.1; HRMS (ESI), m/z calcd for C29H35BrNO10 [M−H]− 636.1450, found 636.1451.

III. References
IV. Translocation observation of PKCδ with GFP (another data for the reproducibility)

V. Translocation data using DAG-lactone (1) (10 μM and 5 μM)
VI. $^1$H NMR and $^{13}$C NMR charts
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IBB-nmr Analysis

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