SUPPLEMENTARY NOTES

Abbreviations

CDI 1,1'-Carbonyldiimidazole
DBU 2,3,4,6,7,8,9,10-Octahydropyrimidol[1,2-a]azepine
DCM Dichloromethane
DIEA or DIPEA N,N-Diisopropylethylamine or Hüning’s base
DMAP 4-(Dimethylamino)pyridine
DMF N,N-Dimethylformamide
DMSO Dimethyl sulfoxide
HATU 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxid hexafluorophosphate or N-[(Dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide
NVOC 6-Nitroveratryloxycarbonyl chloride or 4,5-Dimethoxy-2-nitrobenzyl chloroformate
RT Room temperature (25°C)
TEA Triethylamine
TFA Trifluoroacetic acid

General information

All commercially available reagents and solvents were used as received. 2-(2-aminoethoxy)ethanol, NaH (60% in mineral oil), and 48% aqueous HBr solution were purchased from Acros Organics. 6-chloro-1-bromohexane, 4,5-dimethoxy-2-nitrobenzyl chloroformate (NVOC), tert-Butyl-3-iodopropylcarbamate was purchased from Ace Synthesis LLC. Trimethoprim was purchased from Astatech, Inc. HATU was purchased from GenScript, and the rest of the chemicals were purchased from Sigma Aldrich. All chemicals were used without further purification.
**Instrumentation**

- Thin-layer chromatography (TLC) was performed on Sorbent Technologies silica plates (250 μm thickness).
- Flash column chromatography was performed using Silicycle silica gel (55–65 Å pore diameter).
- Automated flash column chromatography was performed using RediSep Rf silica gel on CombiFlash Rf+ system with internal UV detector. The instrument is available from Teledyne Isco, Inc., NE., USA.
- Ultraviolet-Visible (UV-Vis) absorption spectrophotometry was performed on a JASCO V-650 spectrophotometer with a PAC-743R multichannel Peltier using quartz cells with a 1 cm cell path length. Infrared (IR) spectra were obtained on Jasco FT-IR Spectrum BX system and reported as wavenumber of the absorption maxima between 4000 cm$^{-1}$ and 800 cm$^{-1}$ of only major peaks.
- Proton nuclear magnetic resonance spectroscopy ($^1$H NMR) and Carbon nuclear magnetic resonance spectroscopy ($^{13}$C NMR) spectra were recorded on a Bruker UNI 400 MHz, AVII 500 MHz and Biodrx 600 MHz NMR and processed by MestReNova or Topspin software.
- Low-resolution mass spectra were obtained using Liquid-Chromatography-Mass-Spectrometry (LCMS) on Waters instrument, electrospray ionization in either positive or negative mode. High-resolution mass spectra (HRMS) were obtained at the University of Pennsylvania’s Mass Spectrometry Service Center on Waters LC-TOF mass spectrometer (model LCT-XE Primer) using electrospray ionization in positive or negative mode, depending on the analytes. HRMS data analysis was performed using the automated Waters software.
Chemical structures and numbering of the dimerizers used in this study.

Reagents and conditions: (a) i) SeO$_2$, p-xylene, reflux, 3 days; ii) NaBH$_4$, THF:EtOH (1:1), 25 °C, 4 h, 10% (two steps) (b) 48% HBr$_{aq}$, 100 °C, 30 min, 48% (c) tert-butyl-3-bromopropylcarbamate, DBU, DMSO, 25 °C, 16 h, 56% (d) i) CDI, DIEA, DMAP, DCM, reflux, 16 h; ii) 8, DIEA, DMAP, DCM, 25 °C, 16 h, 12% (two steps) (e) i) TFA:DCM (1:3), 25 °C, 2 h; ii) 6*, HATU, DIEA, DMF, 25 °C, 16 h, 64% (two steps)

*Note that compound 6 was previously reported [S7].
**Supplementary Scheme 2.** Synthesis of the photocleavable TMP-NVOC-Halo ligand, TNH, 3.

**Reagents and conditions:**

(a) NaH, 1-bromo-6-chlorohexane, THF:DMF (2:1), 0 °C to 25 °C, 16 h, 40%;
(b) PPh₃, CBr₄ THF, 25 °C, 16 h, 50%;
(c) K₂CO₃, benzyl bromide, DMF, 80 °C, 16 h 99%;
(d) i) Fuming HNO₃, 1,2-dichloroethane, -30 °C to 25 °C, 3 h ii) 48% HBrₐq, 90 °C, 3 h, 23% (two steps);
(e) 15, K₂CO₃, DMF, 60 °C, 16 h, 30%;
(f) NaBH₄, MeOH:Dioxane (1:1) 0 °C to 25 °C, 2 h, quantitative yield;
(g) i) 4-Nitrophenyl chloroformate, DMAP, DCM, 25 °C, 16 h ii) Deprotected-11 (TMP-NH₂TFA salt) DIEA, DCM, 25 °C, 16 h 16% (two steps).
Dimerizer synthesis and characterization

Compound 1, 4, 5, 6 and 10 were synthesized using previously reported procedure [S7].

Synthesis details for CTH

Synthesis of 4-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)amino)-4-oxobutanoic acid, Halo-COOH (6)

Compound 6 was synthesized by a modified procedure described by Passemard et al [S1].

To a solution of 5 (1.00 g, 3.21 mmol) in 10 mL anhydrous DCM at 0 °C was slowly added TFA (10 mL, 130 mmol). Thereafter, reaction mixture was warmed to room temperature (RT) and stirred for 2 h. After completion of reaction as evident by TLC analysis, indicating the complete consumption of protected amine starting material, the solvent was removed under high vacuum to obtain crude product as a TFA salt, which was used without further purification.

To a solution of the above deprotected amine product (0.72 g, 3.21 mmol) in DCM (5 mL) were slowly added triethylamine (19.3 mmol, 1.95 g) and subsequently succinic anhydride (9.63 mmol, 0.96 g). The solution was stirred at RT overnight. Then the reaction mixture was diluted with DCM (10 mL), washed with 1 M aq. HCl (3×5 mL) and brine. The organic layer was dried over MgSO₄ and concentrated under vacuum to afford the product, 6, as brown oil (90% over two steps). The spectral data were in agreement with the reported data [S7].
Synthesis of 7-(diethylamino)-4-(hydroxymethyl)-2H-chromen-2-one, Coumarin-OH (8)

Compound 8 was synthesized following a modified procedure described by Schönleber et al [S2]. Selenium dioxide (3.33 g, 30.0 mmol) was added to a solution of commercially available 4-methyl-7-diethylaminocoumarin (4.63 g, 20.0 mmol) in p-xylene (120 mL), and heated to reflux under inert atmosphere with vigorous stirring. After 24 h, the mixture was cooled down to room temperature (RT) and added additional selenium dioxide (1.50 g, 13.5 mmol). The crude mixture was continued refluxing for another 48 h. After refluxing for 3 days, the crude black mixture was filtered through celite and silica gel followed by concentration under reduced pressure at 50 °C. The dark brown residual oil was redissolved in EtOH:THF (1:1, 130 mL) in a 500 mL round-bottom flask, sodium borohydride (760 mg, 20.0 mmol) was added portionwise while stirring, and the solution was stirred for 4 h at RT. Thereafter, the crude mixture was carefully quenched and hydrolyzed with 1 M HCl (20 mL) and diluted with H2O (50 mL). The organic solvents (EtOH and THF) were partially removed under reduced pressure before the aqueous solution was extracted with CH2Cl2 (3×100 mL). The organic phase was washed with H2O and brine, dried over MgSO4, and then concentrated in vacuo. The resulting crude mixture was purified by column chromatography (Acetone:DCM, 1:5) to obtain 494 mg (10% over two steps) of the alcohol product as a dark yellow semi-solid. The spectral data were in agreement with the reported data [S2].

Rf = 0.30 (5% MeOH:DCM).

1H NMR (500 MHz, Chloroform-d): 7.27 (d, J = 9.0 Hz, 1H), 6.51 (dd, J = 9.0, 2.6 Hz, 1H), 6.39 (d, J = 2.6 Hz, 1H), 6.25 (t, J = 1.4 Hz, 1H), 4.80 - 4.76 (m, 2H), 3.75 - 3.71 (m, 1H), 3.34 (q, J = 7.1 Hz, 4H), 1.15 (t, J = 7.1 Hz, 6H).

13C NMR (126 MHz, Chloroform-d): 163.2, 156.0, 155.8, 150.6, 124.5, 108.8, 106.4, 105.1, 97.6, 60.7, 44.7, 12.5.

Synthesis of tert-butyl (3-(4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)propyl)carbamate, TMP-NBoc (11)

Compound 11 was synthesized using modified procedure reported by Colloway et al [S3].

Compound 10, TMP-OH (4.14 g, 15.0 mmol) was dissolved in DMSO (45 mL) and DBU (2.51 g, 16.5 mmol) was added at room temperature (RT). After TMP-OH was completely dissolved and the solution turned deep red after about 10 minutes, tert-butyl-3-bromopropylcarbamate (3.93 g, 16.5 mmol) was added. The reaction mixture was stirred at RT overnight. Distilled water (150 mL) was added and the mixture was extracted with EtOAc (4×100 mL). The combined EtOAc solution was washed with water (100 mL) and brine, dried over MgSO₄, and evaporated. The crude mixture was purified by column chromatography with silica gel (10% to 20% MeOH:DCM) to yield 3.67 g (56%) of product 11 as light brown amorphous solid. The spectral data were in agreement with the reported data [S7].

R_f = 0.70 (20% MeOH:DCM).

UV-Vis (λ_max in DCM): 239, 282 nm.

^1H NMR (500 MHz, MeOD): 7.51 (s, 1H), 6.51 (s, 2H), 3.94 (t, J = 5.9 Hz, 2H), 3.78 (s, 6H), 3.63 (s, 2H), 3.28 (d, J = 6.7 Hz, 2H), 1.88 - 1.76 (m, 2H), 1.43 (s, 9H).

^13C NMR (126 MHz, MeOD): 164.3, 163.1, 158.4, 155.9, 154.6, 136.4, 108.0, 106.6, 80.0, 72.3, 56.5, 39.1, 39.0, 34.4, 30.8, 28.8.

HRMS (ESI, m/z): Calcd. for C_{21}H_{31}N_{5}O_{5} [M+H]^+: 434.2403. Found: 434.2413.
**Synthesis of tert-butyl (3-(4-amino-2-(((7-(diethylamino)-2-oxo-2H-chromen-4-yl)methoxy)carbonyl)amino)pyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)propyl)carbamate, Coumarin-TMP-NBoc (12)**

In a 50 mL round-bottom flask equipped with nitrogen, compound 11 (315 mg, 0.730 mmol) was dissolved in anhydrous and degassed DCM (5 mL), DIEA (150 μL, 0.730 mmol), DMAP (45 mg, 0.36 mmol). The resulting light brown suspension was stirred for 15 minutes at room temperature (RT). After the mixture became less cloudy, 1,1′-Carbonyldiimidazole (177 mg, 1.09 mmol, CDI, ≥98% purity, purchased from Chem Impex Int, Inc. and kept dried in a desiccator) was added to the mixture. Thereafter, the suspension was heated to reflux and the solution became transparent. Heating was continued to reflux, under nitrogen atmosphere, overnight. The reaction mixture was then cooled to RT. The reaction progression was monitored by LCMS as follows: a small amount of the solution was taken, dissolved in ~500 μL of MeOH, injected on LCMS (Waters, ESI+ mode). The formation of CDI-TMP-NBoc could be seen as a MeOH adduct of MeOH-trapped CDI-TMP-NBoc intermediate, [M+H⁺]: 550.24, the structure shown below.

In a separate small vial, Coumarin-OH, compound 8 (180 mg, 0.730 mmol), was dissolved in DCM (2 mL), DIEA (150 μL, 0.730 mmol), DMAP (45 mg, 0.36 mmol) and then added into the above solution. Subsequently, the mixture was heated to reflux overnight and reaction progression was monitored by TLC and LCMS. A small amount of the solution was taken, dissolved in ~500 μL of MeOH, injected on LCMS (Waters, ESI+ mode) for analysis. The addition of one Coumarin-OH was observed with two
isomers. However, the major isomer was Coumarin-TMP-NBoc, compound 12, LCMS (ESI+) [M+H\(^+\)]: 707.33. The reaction mixture was then cooled to RT, was quenched with water (20 mL) and diluted with DCM (30 mL) and separated. The organic layer was washed with saturated aqueous solution of NH\(_4\)Cl once and then brine, dried with anhydrous Na\(_2\)SO\(_4\), concentrated to get dark brown oil. The crude mixture was purified by column chromatography with silica gel (2% to 20% MeOH:DCM) to yield 60 mg (12% over two steps) of product 12 as dark brown amorphous solid.

\(R_f=0.40\) (10% MeOH:DCM).

**UV-Vis** (\(\lambda_{\text{max}}\) in 20% DMSO/PBS buffer pH 7.4): 239, 282, 381 nm.

**IR** (NaCl, thin film): \(\nu\) 3309, 2934, 1717, 1605, 1527, 1506, 1424, 1356, 1206, 1126 cm\(^{-1}\).

**\(^1\)H NMR** (500 MHz, DMSO-\(d_6\)): 9.99 (s, 1H), 7.80 (s, 1H), 7.47 (d, \(J=9.0\) Hz, 1H), 6.78 - 6.51 (m, 7H), 6.19 (d, \(J=1.5\) Hz, 1H), 5.30 (d, \(J=1.5\) Hz, 2H), 3.81 (t, \(J=6.3\) Hz, 2H), 3.72 (s, 6H), 3.64 (s, 2H), 3.43 (q, \(J=7.0\) Hz, 4H), 3.09 (q, \(J=6.5\) Hz, 2H), 1.70 (p, \(J=6.6\) Hz, 2H), 1.37 (s, 9H), 1.12 (t, \(J=7.0\) Hz, 6H).

**\(^{13}\)C NMR** (126 MHz, DMSO-\(d_6\)): 155.7, 155.5, 155.0, 152.8, 151.4, 150.4, 134.5, 125.3, 111.8, 108.7, 106.0, 105.2, 104.6, 96.8, 77.4, 70.5, 61.1, 55.9, 44.0, 39.1, 37.32, 32.9, 30.0, 28.2, 12.3.

**HRMS** (ESI, m/z): Calcd. for C\(_{36}\)H\(_{47}\)N\(_6\)O\(_9\) [M+H]\(^+\): 707.3405 Found: 707.3405
(7-(diethylamino)-2-oxo-2H-chromen-4-yl)methyl (4-amino-5-(4-((21-chloro-5,8-dioxo-12,15-dioxo-4,9-diazahenicosyl)oxy)-3,5-dimethoxybenzyl)pyrimidin-2-yl)carbamate, Coumarin-TMP-Halo (CTH, 2)

Compound 12 (60 mg, 0.085 mmol) was deprotected by directly dissolving in 2 mL of TFA:DCM (1:3) and stirring at room temperature (RT) for 2 h. Next, excess TFA was removed and co-evaporated with DCM and DMF several times under high vacuum to yield the TFA salt as a light brown semi-solid. *R*<sub>f</sub> = 0.02 in 10% MeOH:DCM. The primary amine salt can be stained on TLC using Ninhydrin solution, a pink spot close to baseline appeared upon heated. The crude product was used in the next step without further purification.

The crude mixture above was dissolved in 1 mL of DMF followed by addition of DIEA (~200 μL) and stirring at room temperature for 10 minutes. In a separate flask, a 1 mL solution of 6 (28 mg, 0.085 mmol) in DMF, DIEA (~200 μL), and HATU (65 mg, 0.17 mmol) were stirred at RT for 10 minutes and then added to the above stirring mixture of deprotected Coumarin-TMP-NH₂ TFA salt. The mixture was then allowed to stir at RT for another 16 h before being concentrated under high vacuum. The crude brown oil was dissolved in DCM (50 mL) and washed with saturated NH₄Cl solution and brine, dried with anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography using 2% to 15% MeOH:DCM to yield pure product 2 (64% over two steps) as a brown oil. 

*R*<sub>f</sub> = 0.40 (10% MeOH:DCM).

**UV-Vis** (λ<sub>max</sub> in 20% DMSO/PBS buffer pH 7.4): 238, 280, 381 nm.

*Coumarin-TMP- Halo (CTH, 2)* (To Be Continued)
**Coumarin-TMP- Halo (CTH, 2)** (Continued)

$^1$H NMR (500 MHz, DMSO-$d_6$): 10.03 (s, 1H), 7.85 (t, $J = 5.6$ Hz, 1H), 7.81 - 7.72 (m, 2H), 7.44 (d, $J = 9.0$ Hz, 1H), 6.66 (m, 3H), 6.58 (s, 2H), 6.52 (d, $J = 2.5$ Hz, 1H), 6.17 (d, $J = 1.4$ Hz, 1H), 5.28 (d, $J = 1.5$ Hz, 2H), 3.80 (t, $J = 6.3$ Hz, 2H), 3.70 (s, 6H), 3.64 - 3.55 (m, 4H), 3.50 - 3.28 (m, 14H), 3.16 (m, 4H), 2.27 (m, 4H), 1.67 (m, 4H), 1.50 - 1.40 (m, 2H), 1.38 - 1.22 (m, 4H), 1.09 (t, $J = 7.0$ Hz, 6H).

$^{13}$C NMR (126 MHz, DMSO-$d_6$): 171.4, 171.2, 162.4, 160.8, 155.8, 155.7, 155.0, 152.91, 151.5, 150.4, 134.5, 125.3, 108.7, 106.0, 105.2, 104.6, 96.9, 70.5, 70.2, 69.6, 69.4, 69.1, 61.1, 55.9, 45.4, 44.0, 39.3, 38.6, 35.9, 32.9, 32.0, 30.9, 30.8, 29.8, 29.1, 26.1, 24.9, 12.3.

HRMS (ESI, m/z): Calcd. for C$_{45}$H$_{63}$ClN$_7$O$_{11}$ [M+H]$^+$: 912.4274 Found: 912.4276

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**Synthesis details for TNH**

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Compound 13 – 19 (except compound 18) were synthesized using modified procedures reported by Erhart et al and Zimmermann et al [S4-S5].

**Synthesis of 18-chloro-3,6,9,12-tetraoxaoctadecan-1-ol, 4EG-Halo (14)**

![Chemical Structure](image)

Tetraethyleneglycol, 13 (1.45 mL, 8.40 mmol) was dissolved in 18 mL of THF:DMF (2:1). Sodium hydride (60% in mineral oil, 368 mg, 9.20 mmol) was added portionwise at 0 °C. After stirring for 30 min at room temperature (RT), 1-bromo-6-chlorohexane (1.25 mL, 8.40 mmol) was added dropwise. The mixture was stirred at RT for 16 h. The excess of sodium hydride was carefully quenched with water and the crude mixture was poured into water (50 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude oil was purified by flash column chromatography using 2% to 5% MeOH:DCM to yield compound 14 as a light yellow oil (1.05 g, 40%). The spectral data were in agreement with the reported data [S4].

**Rf** = 0.55 (5% MeOH:DCM).

**1H NMR** (500 MHz, Chloroform-d): 3.72 (t, J = 4.5 Hz, 2H), 3.69 - 3.55 (m, 14H), 3.53 (t, J = 6.7 Hz, 2H), 3.45 (t, J = 6.6 Hz, 2H), 2.67 (s, 1H), 1.77 (p, J = 6.9 Hz, 2H), 1.59 (p, J = 6.9 Hz, 2H), 1.49 - 1.31 (m, 4H).

**13C NMR** (126 MHz, Chloroform-d): 72.7, 71.4, 70.8, 70.7, 70.7, 70.5, 70.2, 61.9, 45.2, 32.7, 29.6, 26.8, 25.5.

**HRMS** (ESI, m/z): Calcd. for C₁₄H₃₀ClO₅ [M+H]⁺: 313.1782 Found: 313.1783

**Synthesis of 1-bromo-18-chloro-3,6,9,12-tetraoxaoctadecane, Br-4EG-Halo (15)**
Compound 14 (2.10 g, 6.71 mmol) was dissolved in THF (20 mL). Triphenylphosphine (2.05 g, 7.82 mmol) and carbon tetrabromide (2.59 g, 7.82 mmol) were added portionwise at 0°C. The resulting mixture was stirred at room temperature (RT) for 16 h. The solvent was evaporated under reduced pressure and the crude oil was purified by flash column chromatography using 10% to 50% EtOAc:Hexanes to yield compound 15 as a light yellow oil (1.24 g, 50%). The spectral data were in agreement with the reported data [S4].

$R_f = 0.60$ (40% EtOAc:Hexanes).

$^1$H NMR (500 MHz, Chloroform-$d$): 3.80 (t, $J = 6.3$ Hz, 2H), 3.68 - 3.61 (m, 10H), 3.59 - 3.55 (m, 2H), 3.52 (t, $J = 6.7$ Hz, 2H), 3.46 (q, $J = 6.5$ Hz, 4H), 1.77 (m, 2H), 1.59 (m, 2H), 1.49 - 1.30 (m, 4H).

$^{13}$C NMR (126 MHz, Chloroform-$d$): 71.4, 70.8, 70.8, 70.8, 70.7, 70.7, 70.3, 45.2, 32.7, 30.4, 29.6, 26.8, 25.6.

HRMS (ESI, m/z): Calcd. for C$_{14}$H$_{29}$BrClO$_4$ [M+H]$^+$: 375.0938 Found: 375.0939

**Synthesis of 4-(benzyloxy)-3-methoxybenzaldehyde (17)**

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Compound 17 was synthesized using the same procedure described by Critchley et al. [S6]. In brief, commercially available vanillin 16 (10.0 g, 65.7 mmol) and benzylbromide (11.2 g, 65.7 mmol) were dissolved in 100 mL of DMF. K$_2$CO$_3$ (9.08 g, 65.7 mmol) was added and the mixture was stirred at 80°C for 16 h. The reaction was concentrated under high vacuum, quenched with saturated NH$_4$Cl (500 mL) and extracted with EtOAc three times. The combined organic layers were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting product 17 (15.76 g, 99%) was used without further purification. The spectral data were in agreement with the reported data [S6].

$R_f = 0.60$ (25% EtOAc:Hexanes).

$^1$H NMR (500 MHz, Chloroform-$d$): 9.84 (s, 1H), 7.44 (m, 3H), 7.41 - 7.36 (m, 3H), 7.36 - 7.29 (m, 1H), 6.99 (d, $J = 8.2$ Hz, 1H), 5.25 (s, 2H), 3.95 (s, 3H).

$^{13}$C NMR (126 MHz, Chloroform-$d$): 191.1, 153.7, 150.2, 136.1, 130.4, 128.7, 128.3, 127.3, 126.8, 112.5, 109.4, 71.0, 56.2.

HRMS (ESI, m/z): Calcd. for C$_{15}$H$_{15}$O$_3$ [M+H]$^+$: 243.1021 Found: 243.1018
Synthesis of 4-(benzyl氧)-5-methoxy-2-nitrobenzaldehyde (17a)

Compound 17a was synthesized using the same procedure described by Critchley et al [S6]. In brief, the benzylated vanillin 17 (1.00 g, 4.13 mmol) and 1,2-dichloroethane (5 mL) were cooled to -30 °C under argon. Fuming nitric acid (highly corrosive) (2 mL) was added dropwise with caution, and the temperature was maintained at -15 °C for 3 h (with proper shielding equipment in a fume hood). Thereafter, the reaction mixture was poured into water and extracted with EtOAc three times. Removal of the solvent gave a bright yellow amorphous solid, which was used in the next step without further purification.

\[ R_f = 0.50 \text{ (25\% EtOAc:Hexanes).} \]

\[ ^1H \text{ NMR (500 MHz, Chloroform-d): 10.44 (s, 1H), 7.67 (s, 1H), 7.48 -7.31 (m, 6H), 5.27 (s, 2H), 4.02 (s, 3H).} \]

\[ ^{13}C \text{ NMR (126 MHz, Chloroform-d): 187.9, 153.9, 151.6, 143.8, 135.0, 129.0, 128.8, 127.7, 125.9, 110.2, 109.1, 71.7, 56.9.} \]

\[ \text{HRMS (ESI, m/z): Calcd. for } C_{15}H_{14}NO_5 [M+H]^+: 288.0872 \text{ Found: 288.0878} \]
Synthesis of 4-hydroxy-5-methoxy-2-nitrobenzaldehyde, Nitro-Vanillin (18)

6-Nitro-o-benzylvanillin 17a (4.12 g, 14.3 mmol) was dissolved in acetic acid (30 mL, 99%), and heated to 85 °C. 48% HBr\textsubscript{aq} (15 mL) was added to the mixture and stirred for 3 h. The mixture was poured into ice water and extracted with EtOAc three times. The combined organic layers were washed with saturated NaHCO\textsubscript{3}, brine and dried over Na\textsubscript{2}SO\textsubscript{4}. The solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography using 20% to 60% EtOAc:Hexanes to yield the desired product 18 as dark brown liquid (0.65 g, 23% over two steps). The spectral data were in agreement with the reported data [S6].

R\textsubscript{f} = 0.50 (40% EtOAc:Hexanes).

HRMS (ESI, m/z): Calcd. for C\textsubscript{8}H\textsubscript{6}NO\textsubscript{5} [M-H]: 196.0246 Found: 196.0245
Synthesis of 4-((18-chloro-3,6,9,12-tetraoxaoctadecyl)oxy)-5-methoxy-2-nitrobenzaldehyde (19)

\[ \text{o-Hydroxy-6-nitrovanillin (18) (880 mg, 4.46 mmol) and K}_2\text{CO}_3\ (617 mg, 4.46 mmol) were dissolved in DMF (15 mL). Compound 5 (1.68 g, 4.46 mmol) was added to the above solution. The reaction mixture was stirred at 60°C overnight before being concentrated. Then, the crude mixture was poured into a solution of saturated NH}_4\text{Cl (300 mL) and extracted with EtOAc three times and dried over Na}_2\text{SO}_4. The solvent was evaporated under reduced pressure and the crude product was purified by flash column chromatography using 40% to 80% EtOAc:Hexanes to yield the desired product 19 as brown oil (659 mg, 30%).} \]

\[ R_f = 0.50 \text{ (75% EtOAc:Hexanes).} \]

\[ ^1\text{H NMR (500 MHz, Chloroform-}d\text{): 10.43 (s, 1H), 7.70 (s, 1H), 7.39 (s, 1H), 4.35 - 4.29 (m, 2H), 3.99 (s, 3H), 3.95 - 3.90 (m, 2H), 3.75 - 3.70 (m, 2H), 3.69 - 3.59 (m, 8H), 3.59 - 3.54 (m, 2H), 3.52 (t, } J = 6.7 \text{ Hz, 2H), 3.44 (t, } J = 6.6 \text{ Hz, 2H), 1.76 (m, 2H), 1.62 - 1.53 (m, 2H), 1.48 - 1.30 (m, 4H).} \]

\[ ^{13}\text{C NMR (126 MHz, Chloroform-}d\text{): 187.9, 153.7, 152.0, 143.81, 125.7, 110.1, 108.9, 77.4, 71.4, 71.1, 70.8, 70.7, 70.2, 69.5, 69.5, 56.8, 45.2, 32.7, 29.6, 26.8, 25.6.} \]

\[ \text{HRMS (ESI, m/z): Calcd. for C}_{22}\text{H}_{35}\text{ClNO}_9\ [M+H]^+: 492.2000 \text{ Found: 492.1985} \]
**Synthesis of (4-((18-chloro-3,6,9,12-tetraoxaoctadecyl)oxy)-5-methoxy-2-nitrophenyl)methanol (20)**

Compound 19 (610 mg, 1.24 mmol) was dissolved in MeOH:Dioxane (1:1, 16 mL). NaBH$_4$ (85.3 mg, 2.25 mmol) was added portionwise at 0°C. The mixture was stirred at room temperature for 2 h. Then, the mixture was poured into water, neutralized with a 1 M solution of HCl$_{aq}$ and extracted with DCM three times. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography using 3% to 5% MeOH:DCM to yield compound 20 as brown oil in quantitative yield (613 mg).

$R_f$ = 0.55 (5% MeOH:DCM).

$^1$H NMR (500 MHz, Chloroform-$d$): 7.76 (s, 1H), 7.16 (s, 1H), 4.94 (s, 2H), 4.24 (m, 2H), 3.96 (s, 3H), 3.93 - 3.88 (m, 2H), 3.75 - 3.59 (m, 10H), 3.56 (m, 2H), 3.52 (t, $J$ = 6.7 Hz, 2H), 3.44 (t, $J$ = 6.7 Hz, 2H), 2.81 (s, 1H), 1.80 - 1.70 (m, 2H), 1.58 (p, $J$ = 6.8 Hz, 2H), 1.46 - 1.31 (m, 4H).

$^{13}$C NMR (126 MHz, Chloroform-$d$): 154.5, 147.3, 139.6, 132.8, 111.2, 110.2, 71.4, 71.0, 70.8, 70.7, 70.2, 69.6, 69.2, 62.8, 56.5, 45.2, 32.7, 29.6, 26.8, 25.5.

HRMS (ESI, m/z): Calcd. for C$_{22}$H$_{36}$ClNNaO$_9$ [M+Na]$^+$: 516.1976 Found: 516.1971
Synthesis of 4-((18-chloro-3,6,9,12-tetraoxaoctadecyl)oxy)-5-methoxy-2-nitrobenzyl (3-(4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)propyl)carbamate, TMP-NVOC-Halo (TNH, 3)

![Chemical Structure]

Compound 20 (430 mg, 0.870 mmol), 4-nitrophenyl chloroformate (263 mg, 1.31 mmol), and DMAP (210 mg, 1.74 mmol) were dissolved in DCM (5 mL). The solution was stirred at room temperature (RT) under nitrogen atmosphere overnight.

Compound 11, TMP-NHBoc (430 mg, 0.992 mmol) was deprotected by directly dissolving in 4 mL of TFA/DCM (1:3) and stirring at RT for 2 h. Then, excess TFA was removed and co-evaporated with DCM several times under high vacuum to yield the TMP-NH$_2$ TFA salt as a light brown solid. $R_f = 0.02$ in 10% MeOH:DCM. The primary amine salt can be stained on TLC using Ninhydrin solution, a pink spot close to baseline appeared upon heating. The crude TMP-NH$_2$ TFA salt was dissolved in a mixture of DIEA (150 μL) and DCM (2 mL) and subsequently added to the above solution containing compound 20, which had been stirred overnight. The reaction mixture was stirred at RT under nitrogen atmosphere overnight. The reaction was quenched with water (10 mL), concentrated under high vacuum before being extracted with DCM three times. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography using 2% to 20% MeOH:DCM to yield compound 3 as brown viscous oil (120 mg, 16% over two steps).

$R_f = 0.70$ (15% MeOH:DCM).

**TMP-NV-HTag (TNH, 3)** (To Be Continued)
**TMP-NV-HTag (TNH, 3)** (Continued)

$^{1}$H NMR (500 MHz, Chloroform-d): 7.71 (s, 1H), 7.50 (s, 1H), 6.99 (s, 1H), 6.38 (m, 3H), 5.79 (s, broad, 1H), 5.48 (s, 2H), 4.20 (t, $J = 4.8$ Hz, 2H), 4.02 (t, $J = 5.5$ Hz, 2H), 3.88 (m, 3H), 3.84 (s, 3H), 3.77 (s, 6H), 3.69 (m, 2H), 3.66 - 3.57 (m, 10H), 3.54 (m, 2H), 3.48 (m, 4H), 3.42 (t, $J = 6.6$ Hz, 2H), 1.90 (p, $J = 5.7$ Hz, 2H), 1.78 - 1.68 (m, 2H), 1.55 (p, $J = 6.8$ Hz, 2H), 1.46 - 1.27 (m, 4H).

$^{13}$C NMR (126 MHz, Chloroform-d): 163.2, 156.2, 154.1, 153.6, 147.3, 139.6, 135.6, 133.3, 129.1, 110.5, 110.1, 105.2, 71.9, 71.3, 70.9, 70.6, 70.6, 70.6, 70.1, 69.45, 69.0, 63.3, 56.3, 56.1, 45.1, 39.4, 34.4, 32.6, 29.5, 26.7, 25.5.

HRMS (ESI, m/z): Calcd. for $\text{C}_{39}\text{H}_{58}\text{ClN}_{6}\text{O}_{13}$ [M+H]$^{+}$: 853.3750 Found: 853.3748
NMR Spectra
$^1$H NMR spectrum of 8 in CDCl$_3$ (500 MHz).
$^{13}\text{C}$ NMR spectrum of 8 in CDCl$_3$ (126 MHz).
\(^1\)H NMR spectrum of 12 in DMSO-\(d_6\) (500 MHz).
$^{13}$C NMR spectrum of 12 in DMSO-$d_6$ (126 MHz).
$^1$H - $^{15}$N HMBC NMR spectrum of 12 in DMSO-$d_6$ (126 MHz).
$^1$H NMR spectrum of 2 in DMSO-$d_6$ (500 MHz).
$^{13}$C NMR spectrum of 2 in DMSO-$d_6$ (126 MHz).
$^1$H- $^{15}$N HMBC NMR spectrum of 2 in DMSO-$d_6$ (126 MHz).
\( ^1\text{H NMR spectrum of 14 in CDCl}_3 \) (500 MHz).
$^{13}$C NMR spectrum of 14 in CDCl$_3$ (126 MHz).
$^1$H NMR spectrum of 15 in CDCl$_3$ (500 MHz).
$^{13}$C NMR spectrum of 15 in CDCl$_3$ (126 MHz).
$^1$H NMR spectrum of 17 in CDCl$_3$ (500 MHz).
\( ^{13}\text{C} \) NMR spectrum of 17 in CDCl\(_3\) (126 MHz).
$^1$H NMR spectrum of 17a in CDCl$_3$ (500 MHz).
$^{13}$C NMR spectrum of 17a in CDCl$_3$ (126 MHz).
$^1$H NMR spectrum of 19 in CDCl$_3$ (500 MHz).
$^{13}$C NMR spectrum of 19 in CDCl$_3$ (126 MHz).
$^1$H NMR spectrum of 20 in CDCl$_3$ (500 MHz).
$^{13}$C NMR spectrum of 20 in CDCl$_3$ (126 MHz).
$^1$H NMR spectrum of 3 in CDCl$_3$ (500 MHz).

TNH, 3

TMP-NVOC-Halo
$^{13}$C NMR spectrum of 3 in CDCl$_3$ (126 MHz).
**Supplementary references**


