# Supporting Information

# Versatile Synthesis and Rational Design of Caged Morpholinos

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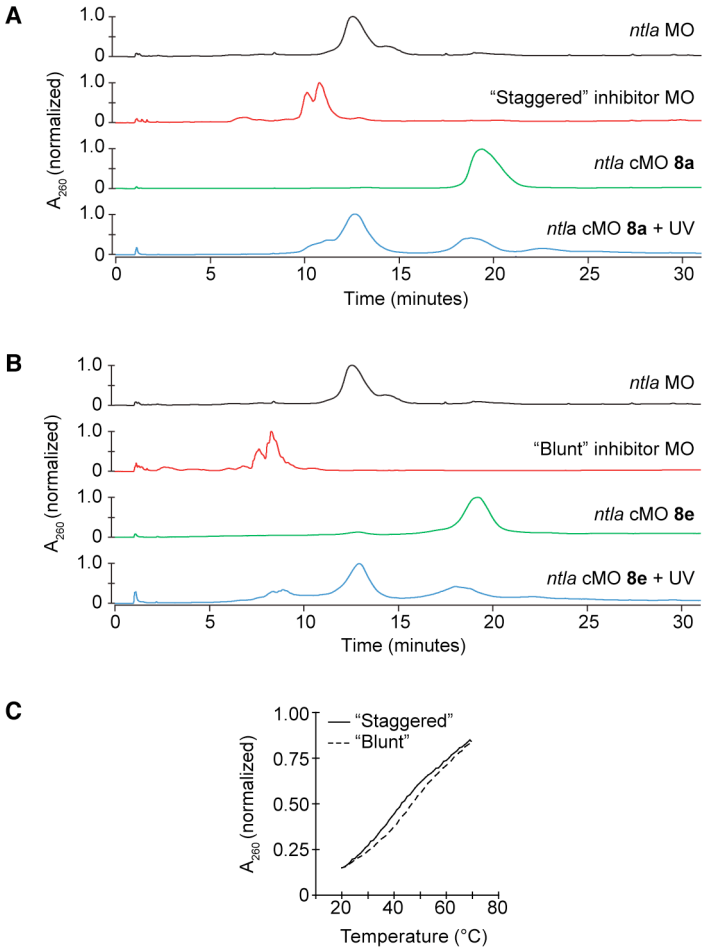



**Figure S1. HPLC and thermal melt analyses of the *ntla* cMO photocleavage products.** (A and B) HPLC analyses of the *ntla* cMOs **8a** and **8e** and their photolysis reactions. Peak areas for the *ntla* MO, inhibitor, and cMO oligomers after UV irradiation indicate that **8a** and **8e** are photocleaved with yields of 74% and 75%, respectively. (C) Representative thermal denaturation curves for the photocleavage products of *ntla* cMOs **8a** and **8e**.

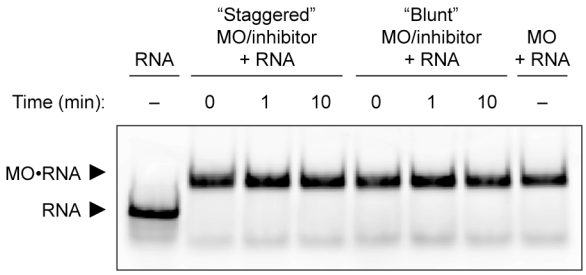



**Figure S2. Gel-shift analysis of MO/inhibitor exchange with RNA.** Intermolecular "staggered" and "blunt" MO/inhibitor duplexes corresponding to *ntla* cMOs **8a** and **8e**, respectively, were incubated with complementary 25-base RNA labeled with fluorescein. RNA exchange was allowed to occur for the indicated lengths of time, and the resulting products were then rapidly resolved by polyacrylamide gel electrophoresis. RNA exchange was complete for all MO/inhibitor duplexes within the time frame of each experimental condition.

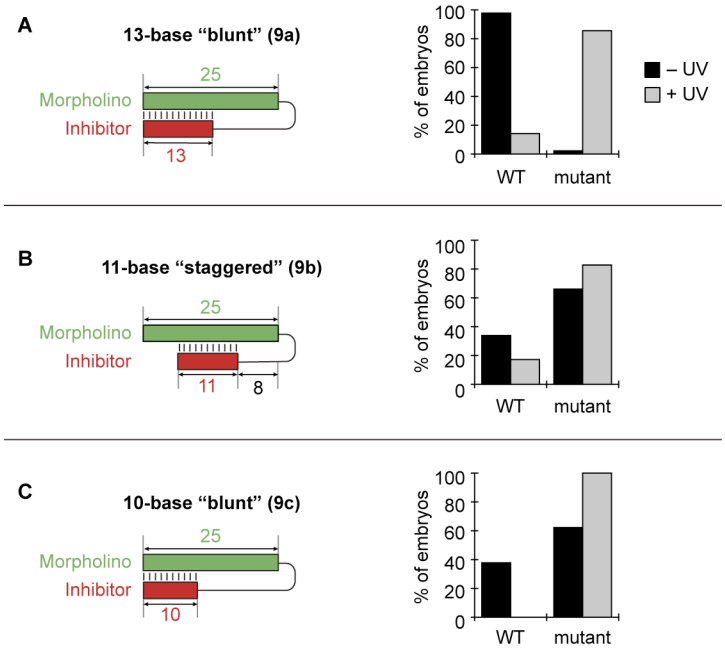



**Figure S3. Activity profiles of *heg* cMOs of different structures.** Schematic representations of *heg* cMOs **9a** (A), **9b** (B), **9c** (C) and the distribution of phenotypes for each cMO configuration (460 fmol/embryo) are shown. Phenotypes were categorized as either wildtype (WT) or mutant (heart defects resulting in a lack of blood circulation and cardiac edema).

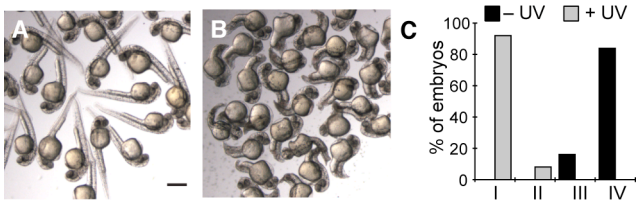



**Figure S4**. **One-photon uncaging of the BHQ-based *ntla* cMO *in vivo*.** One-cell stage embryos were microinjected with 230 fmol of *ntla* cMO **22a** and either cultured in the dark (A) or irradiated with 360-nm light at 3 hpf (B). Phenotypes at 24 hpf are shown. Scale bar = 500 μm. (C) Distribution of the resulting phenotypes, according to the morphological classes described in **Figure 3**.

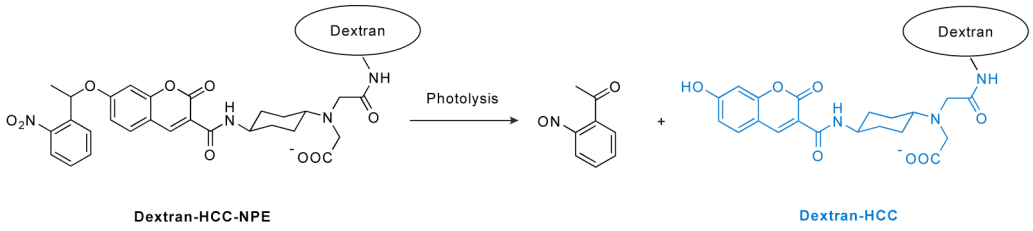
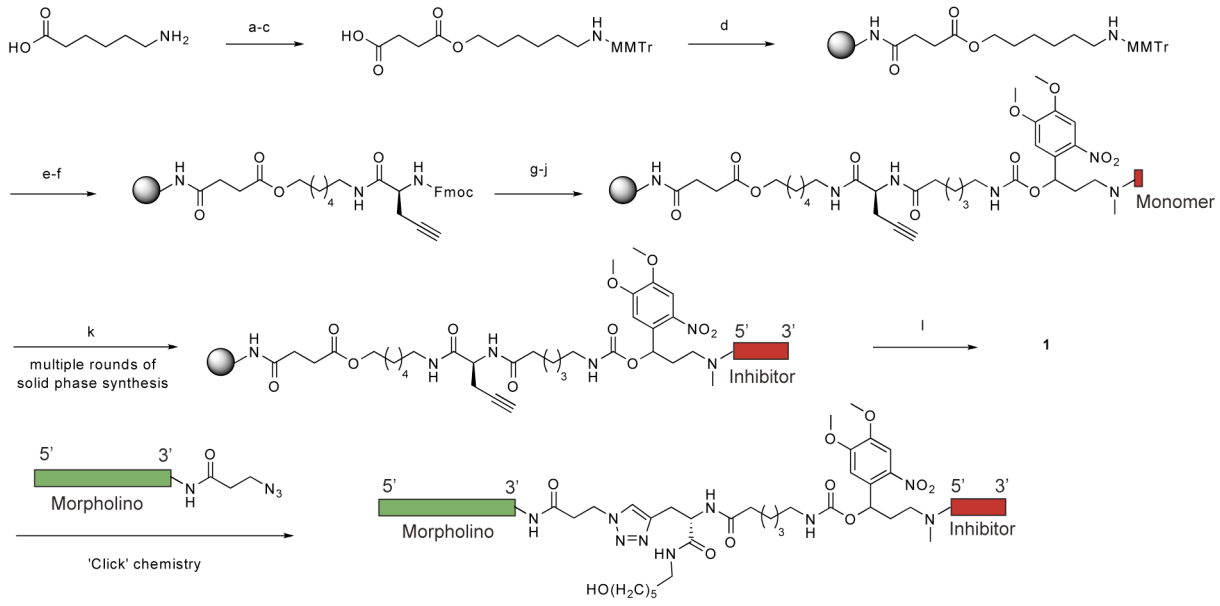



**Figure S5. Structure and photolysis of dextran-HCC-NPE, a photoactivatable fluorophore.**[1]




**Scheme S1. cMO assembly through solid-phase synthesis and "click chemistry."**[2] Reagents and conditions: (a) MMTrCl, TEA, DCM, 41%; (b) $LiAlH_4$, THF, 99%; (c) succinic anhydride, DMAP, pyridine, 49%; (d) amine-functionalized resin, HBTU, HOBt, N-ethylmorpholine, NMP, 63%; (e) HOAc, trifluoroethanol; (f) Fmoc-propargylglycine, HBTU, HOBt, N-ethylmorpholine, NMP; (g) piperidine, DMF; (h) MMTr-6-aminocaproic acid, HBTU, HOBt, N-ethylmorpholine, NMP, 85% over four steps; (i) HOAc, trifluoroethanol; (j) linker-conjugated MO monomer, N-ethylmorpholine, NMP, 67% over four steps; (k) solid-phase synthesis of morpholino oligomer using morpholino monomers; (l) $NH_4OH$, 49% from first monomer for oligomer synthesis.

**Table S1. Thermodynamic parameters of duplexes generated by *ntla* cMO photolysis**

| Cleaved hairpin[a] | $T_m$ (°C) | $\Delta T_m$ [b] (°C) | $\Delta G$ [c] (kcal/mol) | $\Delta\Delta G$ [d] (kcal/mol) |
|---|---|---|---|---|
| **8a** | 36.8 ± 1.8 | + 0.5 | – 11.2 ± 0.7 | – 0.5 |
| **8e** | 41.6 ± 1.4 | – 4.1 | – 11.9 ± 0.4 | + 0.4 |

[a] Dimers of MO and inhibitory MO oligomers generated by photolysis of hairpins with 360-nm light. [b] Difference in melting temperature between cleaved hairpins and unmodified dimers (**Table 2**). [c] Binding free energy of the hairpin photolysis products at 28 °C, [d] Difference in duplex free energy between cleaved hairpins and unmodified dimers (**Table 2**).

**Description of Supplementary Movies 1 and 2**

**Supplementary Movie 1**. Time-lapse videomicroscopy of a 2.5-dpf zebrafish embryo that had been injected with an *etv2* cMO at the one-cell stage and then irradiated with 360-nm light for 10 sec at the sphere stage (4 hpf). Due to *etv2* silencing, blood vessel formation has been disrupted and blood circulation is nearly absent.

**Supplementary Movie 2.** Time-lapse videomicroscopy of a 2.5-dpf zebrafish embryo that had been injected with an *etv2* cMO at the one-cell stage and then cultured in the dark. Note that the vasculature is properly patterned and blood circulation is evident.

**Derivation of equations describing non-photoactivated cMO/RNA binding *in vitro***

$$K_{hairpin} = \frac{[\mathrm{cMO_{open}}]}{[\mathrm{cMO_{closed}}]} \qquad \text{(Eq. 1)}$$

$$K_d^{MO \bullet RNA} = \frac{[\mathrm{cMO_{open}}][\mathrm{RNA}]}{[\mathrm{cMO_{open}} \bullet \mathrm{RNA}]}$$

$$[\mathrm{cMO}]_t = [\mathrm{cMO_{open}}] + [\mathrm{cMO_{closed}}] + [\mathrm{cMO_{open}} \bullet \mathrm{RNA}] \approx [\mathrm{cMO_{open}}] + [\mathrm{cMO_{closed}}]$$

$$[\mathrm{RNA}]_t = [\mathrm{cMO_{open}} \bullet \mathrm{RNA}] + [\mathrm{RNA}]$$

$$[\mathrm{RNA}]_t = [\mathrm{cMO_{open}} \bullet \mathrm{RNA}] + \frac{K_d^{MO \bullet RNA}[\mathrm{cMO_{open}} \bullet \mathrm{RNA}]}{[\mathrm{cMO_{open}}]}$$

$$\frac{[\mathrm{cMO_{open}} \bullet \mathrm{RNA}]}{[\mathrm{RNA}]_t} = \frac{[\mathrm{cMO_{open}}]}{[\mathrm{cMO_{open}}] + K_d^{MO \bullet RNA}}$$

$$[\mathrm{cMO}]_t \approx [\mathrm{cMO_{open}}] + \frac{[\mathrm{cMO_{open}}]}{K_{hairpin}}$$

$$[\mathrm{cMO_{open}}] \approx \frac{[\mathrm{cMO}]_t K_{hairpin}}{1 + K_{hairpin}}$$

$$\frac{[\mathrm{cMO_{open}} \bullet \mathrm{RNA}]}{[\mathrm{RNA}]_t} \approx \frac{[\mathrm{cMO}]_t K_{hairpin}}{[\mathrm{cMO}]_t K_{hairpin} + K_{hairpin} K_d^{MO \bullet RNA} + K_d^{MO \bullet RNA}} \qquad \text{(Eq. 2)}$$

**Derivation of equations describing photoactivated cMO/RNA binding *in vitro***

$$K_d^{MO\bullet RNA} = \frac{[\mathrm{MO}][\mathrm{RNA}]}{[\mathrm{MO}\bullet\mathrm{RNA}]}$$

$$K_d^{MO\bullet INH} = \frac{[\mathrm{MO}][\mathrm{INH}]}{[\mathrm{MO}\bullet\mathrm{INH}]} = \frac{[\mathrm{MO}]^2}{[\mathrm{MO}\bullet\mathrm{INH}]}$$

$$[\mathrm{cMO}]_t = [\mathrm{MO}] + [\mathrm{MO}\bullet\mathrm{INH}] + [\mathrm{MO}\bullet\mathrm{RNA}] \approx [\mathrm{MO}] + [\mathrm{MO}\bullet\mathrm{INH}]$$

$$[\mathrm{RNA}]_t = [\mathrm{MO}\bullet\mathrm{RNA}] + [\mathrm{RNA}]$$

$$[\mathrm{RNA}]_t = [\mathrm{MO}\bullet\mathrm{RNA}] + \frac{K_d^{MO\bullet RNA}[\mathrm{MO}\bullet\mathrm{RNA}]}{[\mathrm{MO}]}$$

$$\frac{[\mathrm{MO}\bullet\mathrm{RNA}]}{[\mathrm{RNA}]_t} = \frac{[\mathrm{MO}]}{K_d^{MO\bullet RNA} + [\mathrm{MO}]} \qquad \text{(Eq. 4)}$$

$$[\mathrm{MO}]^2 = K_d^{MO\bullet INH}[\mathrm{MO}\bullet\mathrm{INH}] \approx K_d^{MO\bullet INH}([\mathrm{cMO}]_t - [\mathrm{MO}])$$

$$[\mathrm{MO}]^2 + K_d^{MO\bullet INH}[\mathrm{MO}] - K_d^{MO\bullet INH}[\mathrm{cMO}]_t \approx 0$$

$$[\mathrm{MO}] \approx \frac{-K_d^{MO\bullet INH} + \sqrt{(K_d^{MO\bullet INH})^2 + 4K_d^{MO\bullet INH}[\mathrm{cMO}]_t}}{2} \qquad \text{(Eq. 3)}$$

**Derivation of equations modeling functional MO/RNA interactions *in vivo***

$$K_{app}^{MO\bullet RNA} = \frac{[\mathrm{MO}][\mathrm{RNA}]}{[\mathrm{MO}\bullet\mathrm{RNA}]}$$

$$[\mathrm{MO}]_t = [\mathrm{MO}] + [\mathrm{MO}\bullet\mathrm{RNA}] \approx [\mathrm{MO}]$$

$$[\mathrm{RNA}]_t = [\mathrm{RNA}] + [\mathrm{MO}\bullet\mathrm{RNA}]$$

$$[\mathrm{RNA}]_t = \frac{K_{app}^{MO\bullet RNA}[\mathrm{MO}\bullet\mathrm{RNA}]}{[\mathrm{MO}]} + [\mathrm{MO}\bullet\mathrm{RNA}]$$

$$\frac{[\mathrm{MO}\bullet\mathrm{RNA}]}{[\mathrm{RNA}]_t} = \frac{[\mathrm{MO}]}{K_{app}^{MO\bullet RNA} + [\mathrm{MO}]} \approx \frac{[\mathrm{MO}]_t}{K_{app}^{MO\bullet RNA} + [\mathrm{MO}]_t}$$

$$\frac{\mathrm{RNA}_{\mathrm{MO}}^{\mathrm{Act}}}{\mathrm{RNA}_{\mathrm{WT}}^{\mathrm{Act}}} = 1 - \frac{[\mathrm{MO}\bullet\mathrm{RNA}]}{[\mathrm{RNA}]_t} \approx 1 - \frac{[\mathrm{MO}]_t}{K_{app}^{MO\bullet RNA} + [\mathrm{MO}]_t} = \frac{K_{app}^{MO\bullet RNA}}{K_{app}^{MO\bullet RNA} + [\mathrm{MO}]_t} \qquad \text{(Eq. 5)}$$

**Derivation of equations modeling functional MO/inhibitor interactions *in vivo***

$$K_{app}^{MO \bullet INH} = \frac{[\mathrm{MO}][\mathrm{INH}]}{[\mathrm{MO} \bullet \mathrm{INH}]}$$

$$K_{app}^{MO \bullet RNA} = \frac{[\mathrm{MO}][\mathrm{RNA}]}{[\mathrm{MO} \bullet \mathrm{RNA}]}$$

$$[\mathrm{MO}]_t = [\mathrm{MO}] + [\mathrm{MO} \bullet \mathrm{INH}] + [\mathrm{MO} \bullet \mathrm{RNA}] \approx [\mathrm{MO}] + [\mathrm{MO} \bullet \mathrm{INH}]$$

$$[\mathrm{RNA}]_t = [\mathrm{RNA}] + [\mathrm{MO} \bullet \mathrm{RNA}]$$

$$[\mathrm{INH}]_t = [\mathrm{INH}] + [\mathrm{MO} \bullet \mathrm{INH}]$$

$$[\mathrm{RNA}]_t = \frac{K_{app}^{MO \bullet RNA}[\mathrm{MO} \bullet \mathrm{RNA}]}{[\mathrm{MO}]} + [\mathrm{MO} \bullet \mathrm{RNA}]$$

$$\frac{[\mathrm{MO} \bullet \mathrm{RNA}]}{[\mathrm{RNA}]_t} = \frac{[\mathrm{MO}]}{K_{app}^{MO \bullet RNA} + [\mathrm{MO}]}$$

$$\frac{\mathrm{RNA}_{\mathrm{MO,INH}}^{\mathrm{Act}}}{\mathrm{RNA}_{\mathrm{WT}}^{\mathrm{Act}}} = 1 - \frac{[\mathrm{MO} \bullet \mathrm{RNA}]}{[\mathrm{RNA}]_t} = 1 - \frac{[\mathrm{MO}]}{K_{app}^{MO \bullet RNA} + [\mathrm{MO}]} = \frac{K_{app}^{MO \bullet RNA}}{K_{app}^{MO \bullet RNA} + [\mathrm{MO}]} \qquad \text{(Eq. 6)}$$

$$[\mathrm{MO}] \approx [\mathrm{MO}]_t - [\mathrm{MO} \bullet \mathrm{INH}]$$

$$[\mathrm{INH}]_t = \frac{K_{app}^{MO \bullet INH}[\mathrm{MO} \bullet \mathrm{INH}]}{[\mathrm{MO}]} + [\mathrm{MO} \bullet \mathrm{INH}]$$

$$[\mathrm{MO{\bullet}INH}] = \frac{[\mathrm{INH}]_t[\mathrm{MO}]}{K_{app}^{MO{\bullet}INH} + [\mathrm{MO}]}$$

$$[\mathrm{MO}] \approx [\mathrm{MO}]_t - \frac{[\mathrm{INH}]_t[\mathrm{MO}]}{K_{app}^{MO{\bullet}INH} + [\mathrm{MO}]}$$

$$[\mathrm{MO}]^2 + (K_{app}^{MO{\bullet}INH} - [\mathrm{MO}]_t + [\mathrm{INH}]_t)[\mathrm{MO}] - K_{app}^{MO{\bullet}INH}[\mathrm{MO}]_t \approx 0$$

$$[\mathrm{MO}] \approx \frac{-(K_{app}^{MO{\bullet}INH} - [\mathrm{MO}]_t + [\mathrm{INH}]_t) + \sqrt{(K_{app}^{MO{\bullet}INH} - [\mathrm{MO}]_t + [\mathrm{INH}]_t)^2 + 4K_{app}^{MO{\bullet}INH}[\mathrm{MO}]_t}}{2} \quad \text{(Eq. 7)}$$

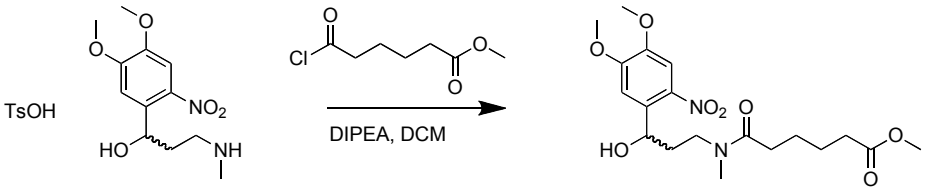
For a cMO, upon photoactivation $[\mathrm{MO}]_t = [\mathrm{INH}]_t = [\mathrm{cMO}]_t$. Eqs. 6 and 7 then become:

$$\frac{\mathrm{RNA}_{\mathrm{cMO}}^{\mathrm{Act}}}{\mathrm{RNA}_{\mathrm{WT}}^{\mathrm{Act}}} = \frac{K_{app}^{MO{\bullet}RNA}}{K_{app}^{MO{\bullet}RNA} + [\mathrm{MO}]} \quad \text{(Eq. 8)}$$

$$[\mathrm{MO}] \approx \frac{-(K_{app}^{MO{\bullet}INH}) + \sqrt{(K_{app}^{MO{\bullet}INH})^2 + 4K_{app}^{MO{\bullet}INH}[\mathrm{cMO}]_t}}{2} \quad \text{(Eq. 9)}$$

**Synthetic procedures**

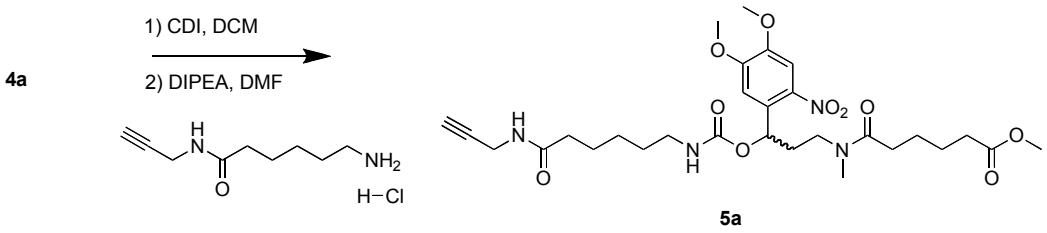
**General synthetic procedures.** All reactions were carried out in flame-dried glassware under an argon atmosphere using commercial reagents without further purification, unless otherwise indicated. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC), using glass-backed silica gel $60_{F254}$ (Merck, 250 μm thickness). Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. $SiO_2$ chromatography was carried out with EM Science silica gel (60 Å, 70-230 mesh) as a stationary phase. $^1$H NMR and $^{13}$C NMR spectra were acquired on Varian 300, 400, and 500 MHz spectrometers and standardized to the NMR solvent peak. Electrospray (ESI) mass spectra were obtained using a Micromass ZQ single quadrupole liquid chromatography-mass spectrometer (LC-MS) and a Micromass Q-TOF hybrid quadrupole LC-MS.




**3a** **4a**

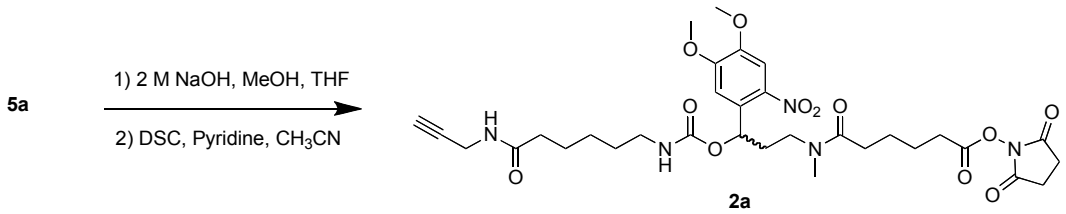
**Methyl 6-((3-(4,5-dimethoxy-2-nitrophenyl)-3-hydroxypropyl)(methyl)amino)-6-oxohexanoate (4a).** 1-(4,5-Dimethoxy-2-nitro-phenyl)-3-methylamino-propan-1-ol tosylate salt **3a**[2] (600 mg, 1.35 mmol) and N,N-diisopropylethylamine (476 μL, 2.7 mmol) were dissolved in anhydrous DCM (5 mL), and the solution was cooled to 0 °C. Methyl adipoyl chloride (241 mg, 1.35 mmol) was added over 10 min, and the reaction mixture was stirred for 6 h at room temperature under nitrogen. After the reaction solvent was removed *in vacuo*, the resulting residue was dissolved in EtOAc, washed twice with saturated aq. $NaHCO_3$ and then dried over anhydrous $Na_2SO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography (EtOAc) to yield **4a** as a yellow oil (480 mg, 86%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.42 (s, 1H), 5.21 (d, 1H, $J$ = 3.5 Hz), 5.15, (d, 1H, $J$ = 7.0 Hz), 4.51 (m, 1H), 4.02 (s, 3H), 3.94 (s, 3H), 3.66 (s, 3H), 3.15 (s, 3H), 2.84 (m, 1H), 2.46 (m,

2H), 2.36 (m, 2H), 2.19 (m, 2H), 1.72 (m, 4H), 1.49 (m, 1H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 174.93, 173.94, 154.09, 147.47, 138.70, 136.20, 108.96, 107.58, 65.28, 56.57, 56.37, 51.65, 44.66, 36.09, 35.58, 33.86, 33.06, 24.70, 24.64. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{19}H_{29}N_2O_8$, 413.2; observed, 413.2. $[M + Na]^+$ calculated for $C_{19}H_{28}N_2NaO_8$, 435.2; observed, 435.2.



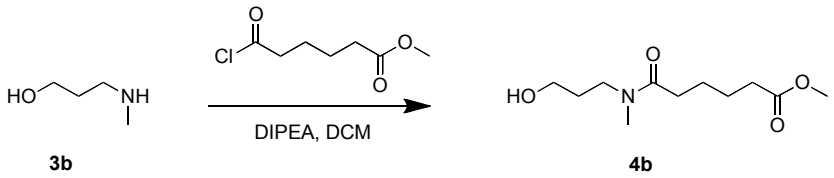

**Methyl 14-(4,5-dimethoxy-2-nitrophenyl)-17-methyl-5,12,18-trioxo-13-oxa-4,11,17-triaza-tricos-1-yn-23-oate (5a).** Compound **4a** (150 mg, 0.364 mmol) was dissolved in anhydrous DCM (1 mL) and added to 1,1'-carbonyl diimidazole (145 mg, 0.894 mmol) in anhydrous DCM (1.5 mL). The reaction mixture was stirred for 4 h at room temperature under nitrogen, diluted with DCM, washed two times with water, and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo* to yield crude imidazole carbamate as a yellow gum (164 mg, 66%). MS-ESI (m/z): $[M + H]^+$ calculated for $C_{23}H_{31}N_4O_9$, 507.2; observed, 507.0. Without further purification, the imidazole carbamate (121 mg, 0.239 mmol) was dissolved in anhydrous DCM (1.5 mL) and N,N-diisopropylethylamine (330 μL, 1.91 mmol). To this mixture was added 6-oxo-6-(prop-2-ynylamino)hexan-1-aminium hydrochloride salt[3] (145 mg, 0.708 mmol) in anhydrous DMF (1.4 mL). The reaction mixture was stirred overnight at room temperature under nitrogen. Solvent was then removed *in vacuo*, and the crude material was re-dissolved in toluene and evaporated to dryness again. The resulting yellow gum was then dissolved in $CHCl_3$, washed once with 1 M HCl, washed once with 5% saturated aq. $NaHCO_3$, washed once with brine, and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography ($CHCl_3$/acetone, stepwise gradient from 4/1 to 1/1) to yield **5a** as a thick yellow gum (126 mg, 57% from **4a**). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.63 (m, 1H), 6.98 (m, 1H),

6.24-5.88 (m, 2H), 5.24-5.00 (m, 1H), 4.03 (m, 1H), 3.98-3.93 (m, 6H), 3.67 (m, 3H), 3.56 (m, 1H), 3.16 (m, 1H), 3.06-2.95 (m, 3H), 2.34 (m, 3H), 2.17 (m, 4H), 1.95 (m, 1H), 1.67 (m, 6H), 1.50 (m, 2H), 1.34 (m, 2H). $^{13}$C NMR (75 MHz, DMSO-d$^6$) δ 172.53, 171.01, 154.59, 153.18, 147.60, 139.35, 131.53, 124.28, 109.01, 108.09, 80.88, 78.57, 78.30, 71.59, 68.34, 66.48, 55.93, 50.35, 34.57, 34.50, 32.76, 29.99, 28.57, 27.33, 25.38, 24.57, 24.16, 23.73, 23.68. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{29}H_{43}N_4O_{10}$, 607.3; observed, 607.3. $[M + Na]^+$ calculated for $C_{29}H_{42}N_4NaO_{10}$, 629.3; observed, 629.3.




**2,5-Dioxopyrrolidin-1-yl 14-(4,5-dimethoxy-2-nitrophenyl)-17-methyl-5,12,18-trioxo-13-oxa 4,11,17-triazatricos-1-yn-23-oate (2a).** Compound **5a** (121 mg, 0.200 mmol) was dissolved in a mixture of MeOH (2 mL), THF (2 mL) and 6 M aq. NaOH (2 mL). The reaction mixture was stirred for 3 h at room temperature. After the reaction solvent was removed *in vacuo*, the resulting residue was dissolved in EtOAc, washed once with 1 M HCl, and dried over anhydrous $Na_2SO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography (MeOH/EtOAc = 1/9) to yield the carboxylic acid as a light yellow oil (110 mg, 93%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.63 (m, 1H), 7.03 (br, s, 1H), 6.50 (br, s, 1H), 6.23-6.15 (m, 2H), 4.04-3.93 (m, 7H), 3.20-2.97 (m, 4H), 2.39 (m, 3H), 2.24 (m, 4H), 1.72 (m, 6H), 1.51 (br, s, 2H), 1.36 (br, s, 2H), 1.27-1.22 (m, 6H). $^{13}$C NMR (75 MHz, DMSO-d$^6$) δ 173.42, 171.10, 154.61, 153.19, 147.60, 139.35, 135.59, 131.53, 109.01, 108.10, 80.89, 78.57, 71.60, 68.36, 55.92, 34.57, 34.37, 33.34, 33.12, 31.57, 28.58, 27.35, 25.38, 24.17, 23.84, 23.78, 23.74. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{28}H_{41}N_4O_{10}$, 593.3; observed, 593.4. $[M + Na]^+$ calculated for $C_{28}H_{40}N_4NaO_{10}$, 615.3; observed, 615.4. $[M - H]^-$ calculated for $C_{28}H_{39}N_4O_{10}$, 591.3; observed, 591.7.

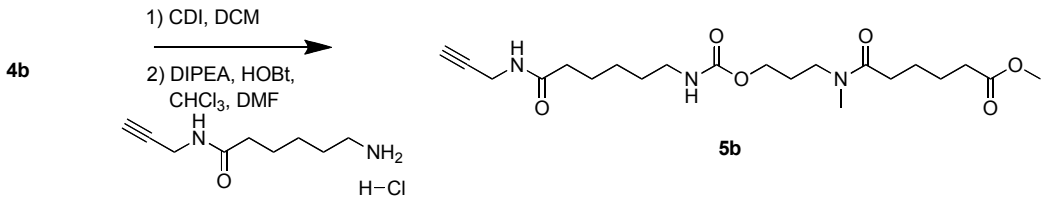
The carboxylic acid precursor (80 mg, 0.13 mmol), DSC (173 mg, 0.675 mmol) and pyridine (53 mg, 0.67 mmol) were dissolved in $CH_3CN$ (2 mL) and reacted at room temperature for 16 h. Solvent was then removed *in vacuo*, and the crude material was re-dissolved in toluene and evaporated to dryness again. The remaining residue was dissolved in EtOAc, washed once with 0.1 M aq. HCl, washed once with saturated aq. $NaHCO_3$ and dried over anhydrous $Na_2SO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography ($CHCl_3$/acetone = 1/1) to yield **2a** as a light yellow oil (70 mg, 75%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.61 (m, 1H), 6.98 (m, 1H), 6.17 (m, 2H), 5.12 (m, 1 H), 4.01 (m, 2H), 3.95 (m, 6H), 3.69 (m, 1H), 3.54 (m, 1H), 3.15 (m, 1H), 3.07 (s, 3H), 2.94 (s, 1H), 2.84 (m, 4H), 2.64 (m, 2H), 2.37 (m, 2H), 2.17 (m, 4H), 1.95 (m, 2H), 1.79 (m, 3H), 1.62 (m, 2H), 1.47 (m, 2H), 1.29 (m, 2H). HRMS (TOF MS ES+) (m/z): $[M + Na]^+$ calculated for $C_{32}H_{43}N_5NaO_{12}$, 712.2806; observed, 712.2802.



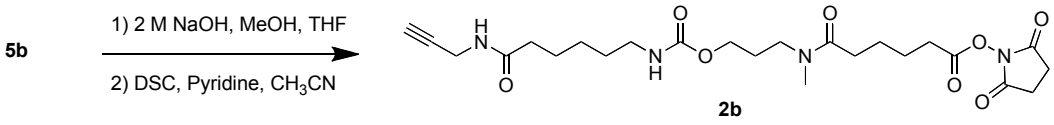

**3b** **4b**

**Methyl 6-((3-hydroxypropyl)(methyl)amino)-6-oxohexanoate (4b).** 3-(methylamino)propan-1-ol (**3b**, 660 μL, 6.96 mmol) was dissolved in anhydrous DCM, and the solution was cooled to -78 °C. Methyl adipoyl chloride (490 μL, 3.15 mmol) was added, and the reaction mixture was stirred for 2 h at 0 °C under nitrogen. Solvent was removed *in vacuo* and the residue was purified by $SiO_2$ column chromatography (EtOAc/$CHCl_3$, stepwise gradient from 1/1 to 1/0) to yield **4b** as a colorless oil (303 mg, 42%). $^1$H NMR (400 MHz, $CDCl_3$) δ 4.00 (t, 1H, $J$ = 7.0 Hz), 3.67 (d, 3H, $J$ = 3.2 Hz), 3.53 (t, 2H, $J$ = 3.0 Hz), 3.47 (dt, 2H, $J$ = 7.2 Hz, 5.4 Hz), 2.99 (s, 3H), 2.36 (m, 4H), 1.69 (m, 4H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.08, 173.94, 57.85, 51.60, 43.90, 35.42, 33.81, 32.99, 29.41, 24.63, 24.55. MS-

ESI (m/z): $[M + H]^+$ calculated for $C_{11}H_{22}NO_4$, 232.1; observed, 232.1. $[M + Na]^+$ calculated for $C_{11}H_{21}NNaO_4$, 254.1; observed, 254.1.

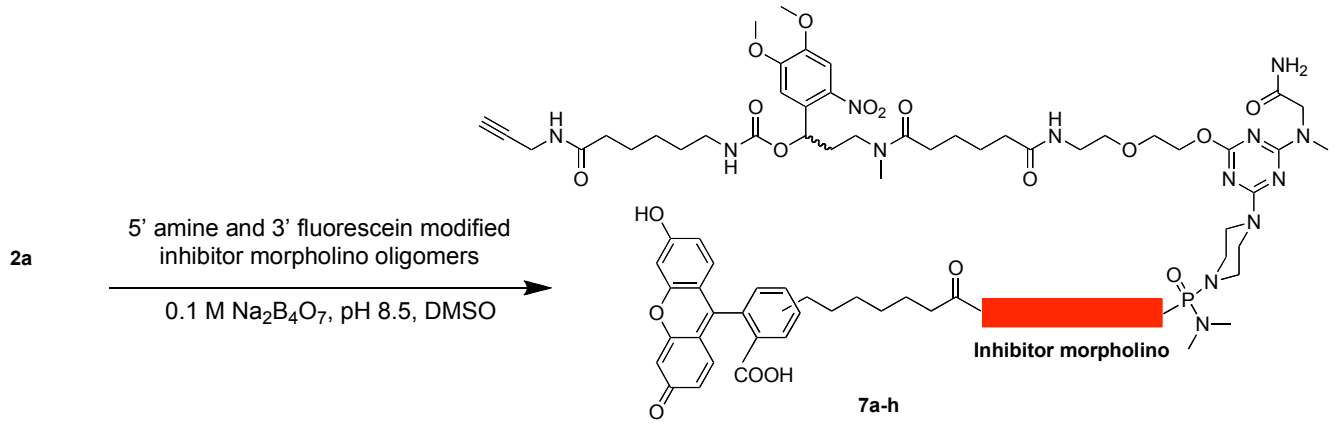



**Methyl 17-methyl-5,12,18-trioxo-13-oxa-4,11,17-triazatricos-1-yn-23-oate (5b).** Compound **4b** (50.4 mg, 0.218 mmol) was dissolved in anhydrous DCM (0.75 mL) and added to 1,1'-carbonyl diimidazole (88.7 mg, 0.547 mmol) in anhydrous DCM (1 mL). The reaction mixture was stirred for 1.5 h at room temperature under nitrogen, diluted with $CHCl_3$, washed two times with water, and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo* to yield crude imidazole carbamate as a yellow gum (62.5 mg, 88%). Without further purification, the imidazole carbamate (60.2 mg, 0.092 mmol) was dissolved in $CHCl_3$ (0.4 mL), and to this mixture was added 6-oxo-6-(prop-2-ynylamino)hexan-1-aminium trifluoroacetate salt (62.7 mg, 0.222 mmol), N,N-diisopropylethylamine (155 μL, 0.888 mmol), and *N*-hydroxybenzotriazole (19.0 mg, 0.141 mmol) in anhydrous DCM (0.5 mL). The reaction was stirred overnight at room temperature under nitrogen, diluted with $CHCl_3$, washed once with 1 M HCl, washed once with 0.5 M aqueous bicarbonate, washed once with brine, and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography ($CHCl_3$/acetone, stepwise gradient from 1/0 to 5/1, then $CHCl_3$/MeOH = 9/1) to yield **5b** as a thick white gum (19.9 mg, 45% from **4b**). $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.16 (m, 1H), 4.98 (m, 1H), 4.06 (m, 4H), 3.67 (s, 3H), 3.41 (m, 2H), 3.17 (q, 2H, $J$ = 6.3 Hz), 2.95 (m, 3H), 2.34 (m, 4H), 2.22 (m, 3H), 1.85 (m, 2H), 1.66 (m, 6H), 1.51 (q, 2H, $J$ = 7.1 Hz), 1.35 (q, 2H, $J$ = 7.2 Hz). MS-ESI (m/z): $[M + H]^+$ calculated for $C_{21}H_{36}N_3O_6$, 426.3; observed, 426.1. $[M + Na]^+$ calculated for $C_{21}H_{35}N_3NaO_4$, 448.2; observed, 448.2.

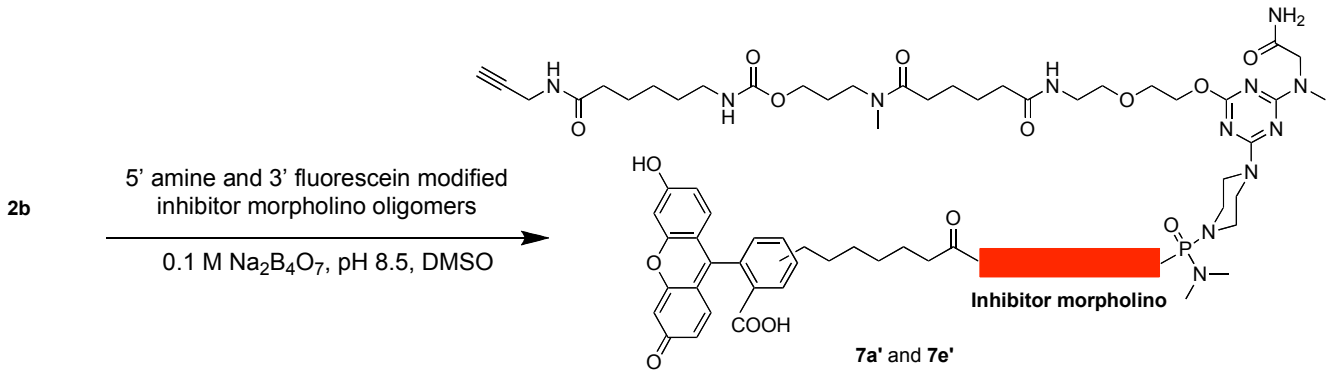



**2,5-dioxopyrrolidin-1-yl-17-methyl-5,12,18-trioxo-13-oxa-4,11,17-triazatricos-1-yn-23-oate (2b).** Compound **5b** (18.3 mg, 43.1 μmol) was dissolved in a mixture of MeOH (1.5 mL), THF (1.5 mL) and 6 M aq. NaOH (1.5 ml). The reaction mixture was stirred for 3 h at room temperature and organic solvent was removed *in vacuo*. The remaining aqueous solution was acidified with 1 M HCl and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and solvent was removed *in vacuo* to yield the carboxylic acid as a colorless oil (16.0 mg, 90%). $^1$H NMR (400 MHz, $CDCl_3$) δ 6.32 (m, 1H), 5.21 (m, 1H), 4.08 (m, 1H), 4.05 (m, 2H), 3.44 (m, 2H), 3.17 (s, 2H), 2.96 (m, 3H), 2.38 (m, 4H), 2.24 (m, 4H), 1.89 (m, 2H), 1.69 (m, 6H), 1.52 (m, 2H), 1.36 (m, 2H). MS-ESI (m/z): $[M + H]^+$ calculated for $C_{20}H_{34}N_3O_6$, 412.2; observed, 412.2. $[M + Na]^+$ calculated for $C_{20}H_{33}N_3NaO_6$, 434.2; observed, 434.2. $[M - H]^-$ calculated for $C_{20}H_{32}N_3O_6$, 410.2; observed, 410.3.

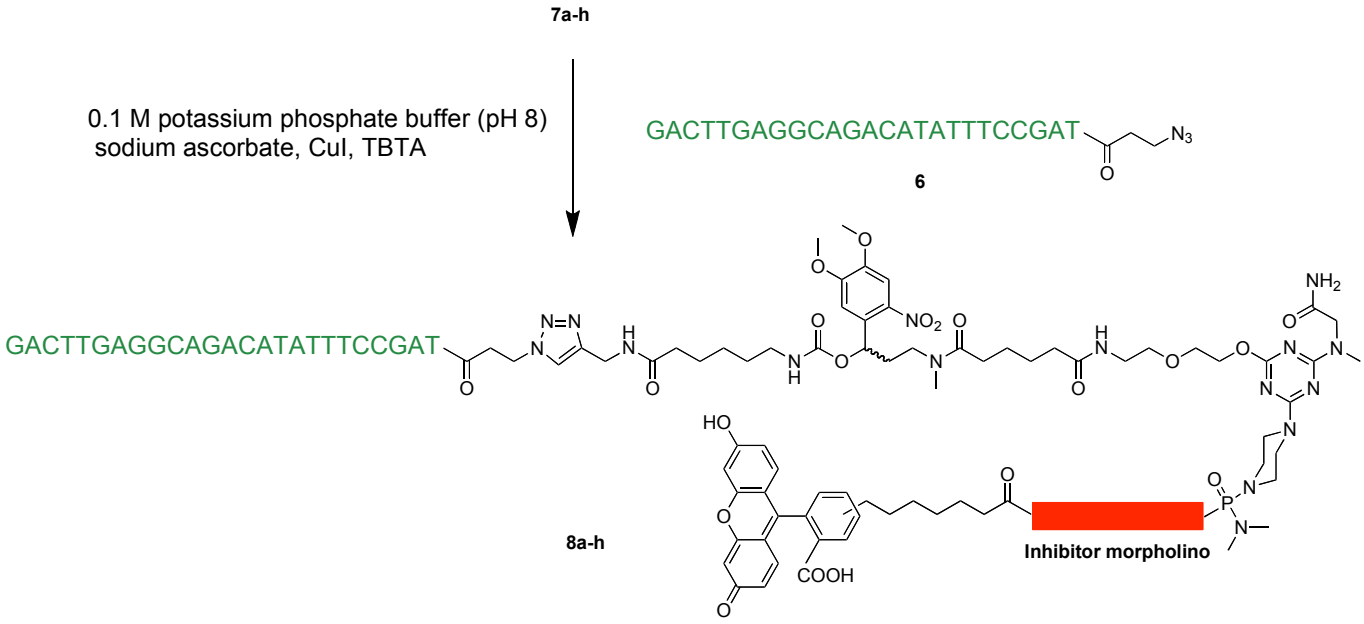
The carboxylic acid (18.5 mg, 44.9 μmol), DSC (28.5 mg, 111 μmol) and pyridine (39.2 mg, 0.496 mmol) were dissolved in $CH_3CN$ (0.5 mL) and reacted at room temperature for 16 h. Solvent was then removed *in vacuo*, and the crude material was re-dissolved in toluene and evaporated to dryness again. The remaining residue was dissolved in EtOAc, washed once with 0.1 M aq. HCl, washed once with saturated aq. $NaHCO_3$ and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo* to yield **2b** as a pale yellow gum (14.7 mg, 64 %). $^1$H NMR (400 MHz, $CDCl_3$) δ 6.03 (m, 1H), 4.90 (m, 1H), 4.06 (m, 4H), 3.41 (m, 2H), 3.14 (m, 3H), 2.95 (m, 3H), 2.85 (s, 4H), 2.65 (t, 2H, $J$ = 7.0 Hz), 2.35 (t, 2H, $J$ = 6.4 Hz), 2.21 (m, 2H), 1.81 (m, 6H), 1.67 (q, 2H, $J$ = 3.7 Hz), 1.52 (q, 2H, $J$ = 7.2 Hz), 1.35 (m, 2H). HRMS (TOF MS ES+) (m/z): $[M + Na]^+$ calculated for $C_{24}H_{36}N_4NaO_8$, 531.2431; observed, 531.2416.




**DMNB-conjugated *ntla* MO inhibitory oligomers (7a-h).** Synthetic procedures for the *ntla* MO inhibitors were analogous to those described for **7e**. MO sequences were: **7a** (5'-TATGTCTGCC-3'), **7b** (5'-TATGTCTGCCTC-3'), **7c** (5'-TATGTCTGCCTCAA-3'), **7d** (5'-TATGTCTGCCTCAAGT-3'), **7e** (5'-GCCTCAAGTC-3'), **7f** (5'-CTGCCTCAAGTC-3'), **7g** (5'-GTCTGCCTCAAGTC-3'), **7h** (5'-ATGTCTGCCTCAAGTC-3'). Compounds **7a-h** were recovered as yellow solids (70-90 nmol, 70-90%). MS-ESI (m/z): $[M + H]^+$ calculated for photolyzed **7a** $C_{185}H_{266}N_{65}O_{64}P_{10}$, 4734; observed, 4736. $[M + H]^+$ calculated for **7b** $C_{198}H_{287}N_{72}O_{70}P_{12}$, 5167; observed, 5170. $[M + H]^+$ calculated for photolyzed **7c** $C_{232}H_{339}N_{88}O_{79}P_{14}$, 6058; observed, 6060. $[M + H]^+$ calculated for **7d** $C_{246}H_{360}N_{97}O_{85}P_{16}$, 6532; observed, 6534. $[M + H]^+$ calculated for **7e** $C_{184}H_{264}N_{69}O_{61}P_{10}$, 4728; observed, 4728. $[M + H]^+$ calculated for photolyzed **7f** $C_{197}H_{285}N_{76}O_{67}P_{12}$, 5162; observed, 5165. $[M + H]^+$ calculated for **7g** $C_{231}H_{338}N_{89}O_{79}P_{14}$, 6059; observed, 6064. $[M + H]^+$ calculated for **7h** $C_{255}H_{375}N_{100}O_{87}P_{16}$, 6729; observed, 6729.

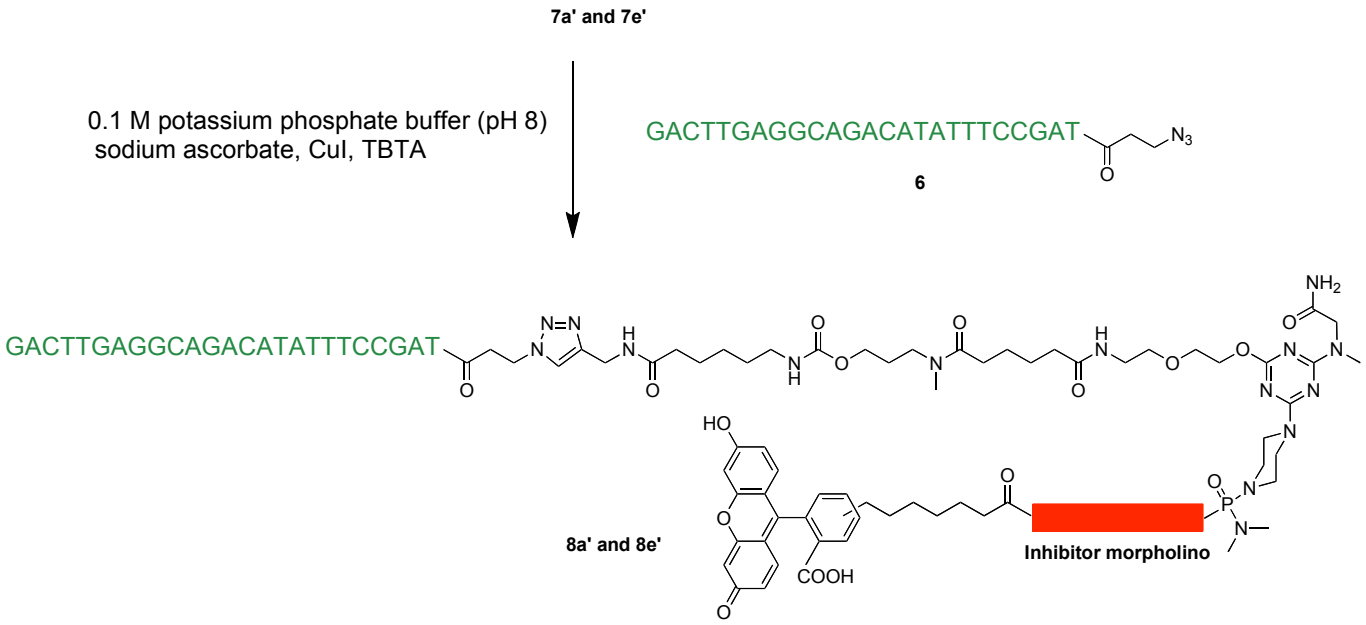


**Non-cleavable *ntla* MO inhibitors (7a' and 7e').** Synthetic procedures for non-photocleavable versions of the *ntla* cMOs were analogous to those described for **7e**. Inhibitor MO sequences were identical to **7a** and **7e**. Each MO oligomer (100 nmol) was dissolved in 0.1 M $Na_2B_4O_7$, pH 8.5 (100 µL), and combined with **2b** (0.76 mg, 1.5 µmol) in DMSO (15 µL). The remaining synthetic procedures were identical to those of **7a-h**. Yield: 70-90 nmol, 70-90%. MS-ESI (m/z): $[M + H]^+$ calculated for **7a'** $C_{177}H_{259}N_{64}O_{60}P_{10}$, 4553; observed, 4552. $[M + H]^+$ calculated for **7e'** $C_{176}H_{257}N_{68}O_{57}P_{10}$, 4547; observed, 4547.




**DMNB-based *ntla* cMOs (8a-h).** Synthetic procedures for the *ntla* cMOs were analogous to those described for **8e**. Compounds **8a-h** were recovered as yellow solids (5.6-10.5 nmol, 6-10% overall). MS-ESI (m/z): $[M + H]^+$ calculated for **8a** $C_{489}H_{739}N_{215}O_{167}P_{35}$, 13385; observed, 13384. $[M + H]^+$ calculated for **8b** $C_{512}H_{776}N_{224}O_{176}P_{37}$, 14031; observed, 14032. $[M + H]^+$ calculated for **8c** $C_{536}H_{812}N_{238}O_{182}P_{39}$, 14709; observed, 14705. $[M + H]^+$ calculated for **8d** $C_{560}H_{849}N_{249}O_{191}P_{41}$, 15395; observed, 15391. $[M + H]^+$ calculated for **8e** $C_{488}H_{737}N_{219}O_{164}P_{35}$, 13379; observed, 13380. $[M + H]^+$ calculated for **8f** $C_{511}H_{774}N_{228}O_{173}P_{37}$, 14025; observed, 14025. $[M + H]^+$ calculated for **8g**

$C_{535}H_{811}N_{239}O_{182}P_{39}$, 14711; observed, 14713. $[M + H]^+$ calculated for **8h** $C_{559}H_{848}N_{250}O_{190}P_{41}$, 15380; observed, 15379.




**Non-cleavable *ntla* MO hairpins (8a' and 8e').** The functionalized oligomers **7a'** and **7e'** (50 nmol) were conjugated with azide-functionalized *ntla* MO **6** (50 nmol). The synthetic procedures and yields were identical to those of **8a-h**. MS-ESI (m/z): $[M + H]^+$ calculated for **8a'** $C_{481}H_{732}N_{214}O_{163}P_{35}$, 13204; observed, 13204. $[M + H]^+$ calculated for **8e'** $C_{480}H_{730}N_{218}O_{160}P_{35}$, 13198; observed, 13199.

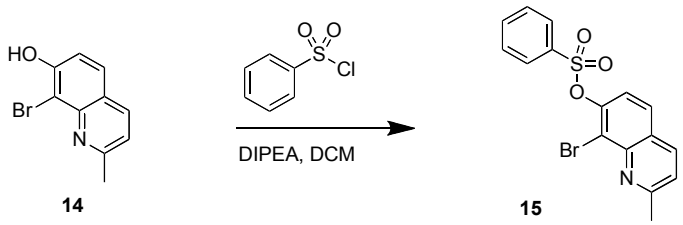
***heg* cMO (9a-c).** Synthetic procedures identical to those for *ntl* cMOs **8a-h** were utilized, with the following modifications. The inhibitory oligomers contained 5' amine but not 3' fluorescein modifications, and each oligonucleotide (100 nmol) was dissolved in 0.1 M $Na_2B_4O_7$, pH 8.5 (100 µL) and combined with DMNB linker **2a** (0.76 mg, 1.5 µmol) in DMSO (15 µL). The reaction was shaken overnight in the dark. The reaction was diluted to 500 µL with water and passed through a NAP™5 size exclusion column (GE Healthcare) according to the manufacturer's instructions. Product-containing fractions (~1 mL) were pooled and concentrated to 400 µL by lyophilization, acidified with 4 µL of

HOAc, and washed with $CHCl_3$ (3 x 400 μL) and EtOAc (2 x 400 μL). The remaining aqueous solution was neutralized with $NH_4OH$ (10%, 20 μL) and lyophilized to give the linker-modified inhibitors as white solids. After conjugation of each synthetic intermediate (50 nmol) and azide-functionalized *heg* MO (50 nmol) by Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition, the reaction supernatant was diluted to 800 μL, split and passed through two NAP™5 size exclusion columns (GE Healthcare) according to the manufacturer's instructions. Further purification of the cMOs by ion-exchange HPLC then yielded the final products as white solids (**9a**, 16 nmol, 16% overall; **9b**, 15.6 nmol, 16% overall; **9c**, 12 nmol, 12% overall). MS-ESI (m/z): $[M + H]^+$ calculated for **9a** $C_{501}H_{776}N_{241}O_{165}P_{38}$, 13992; observed, 13993. $[M + H]^+$ calculated for **9b** $C_{477}H_{738}N_{230}O_{158}P_{36}$, 13338; observed, 13340. $[M + H]^+$ calculated for **9c** $C_{466}H_{721}N_{222}O_{155}P_{35}$, 12997; observed, 12999.

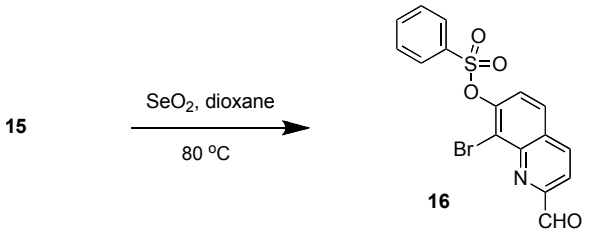
***flh* cMO (10).** Synthetic procedures identical to those for *ntla* cMOs **8a-h** were utilized. Final product was recovered as a yellow solid (5.6 nmol, 6% overall). MS-ESI (m/z): $[M + H]^+$ calculated for **10** $C_{489}H_{737}N_{218}O_{168}P_{35}$, 13441; observed, 13441.

***etv2* cMO (11).** Synthetic procedures identical to those for *ntla* cMOs **8a-h** were utilized. Final product was recovered as a yellow solid (8.7 nmol, 9% overall). MS-ESI (m/z): $[M + H]^+$ calculated for **11** $C_{488}H_{740}N_{210}O_{168}P_{35}$, 13320; observed, 13322.

***spt* cMO (12).** Synthetic procedures identical to those for *ntla* cMOs **8a-h** were utilized. Final product was recovered as a yellow solid (10.5 nmol, 10% overall). MS-ESI (m/z): $[M + H]^+$ calculated for **12** $C_{487}H_{736}N_{217}O_{166}P_{35}$, 13370; observed, 13369.

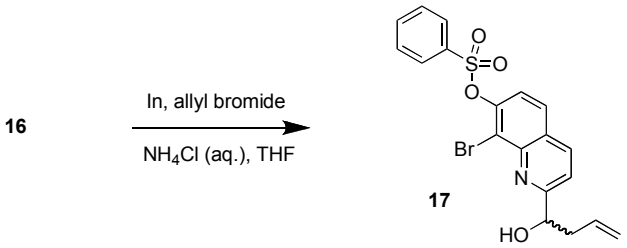



**8-bromo-2-methylquinolin-7-yl benzenesulfonate (15).** 8-bromo-2-methylquinolin-7-ol[4] (**14**, 1.10 g, 4.62 mmol) and N,N-diisopropylethylamine (1.19 g, 9.24 mmol) were dissolved in anhydrous DCM (10 mL), and the solution was cooled to 0 °C. Benzenesulfonyl chloride (0.90 g, 5.10 mmol) in DCM (5 mL) was added over 10 min, and the reaction mixture was stirred for 14 h at room temperature under argon. Solvent was removed *in vacuo*, and residue was dissolved in EtOAc, washed twice with saturated aq. $NaHCO_3$, and dried over anhydrous $Na_2SO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography (hexanes/EtOAc = 1/1) to yield **15** as a white solid (1.70 g, 4.49 mmol, 97%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 1H, *J* = 8.4 Hz), 7.98 (m, 1H), 7.96 (m, 1H), 7.76 (d, 1H, *J* = 8.8 Hz), 7.70-7.66 (m, 1H), 7.60 (d, 1H, *J* = 8.8 Hz), 7.55-7.51 (m, 2H), 7.35 (d, 1H, *J* = 8.0 Hz), 2.79 (s, 3H). $^{13}$C NMR (500 MHz, $CDCl_3$) δ 161.59, 148.07, 145.80, 136.57, 135.92, 134.83, 129.44, 128.91, 128.03, 126.12, 123.24, 121.95, 118.04, 25.92. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{16}H_{13}BrNO_3S$, 378.0 ($^{79}$Br) and 380.0 ($^{81}$Br); observed 378.0 ($^{79}$Br) and 379.9 ($^{81}$Br).

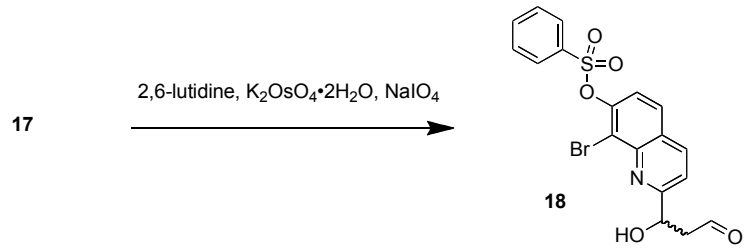



**8-Bromo-2-formylquinolin-7-yl benzenesulfonate (16).** A mixture of $SeO_2$ (500 mg, 4.51 mmol) and 1,4-dioxane (10 mL) was heated to over 80 °C. 8-Bromo-2-methylquinolin-7-yl benzenesulfonate (**15**, 1.70 g, 4.49 mmol) in 1,4-dioxane (5 mL) was added. After stirring at 80 °C for 24 h, the reaction was cooled and vacuum filtered. The filtrate was collected and concentrated to yield a

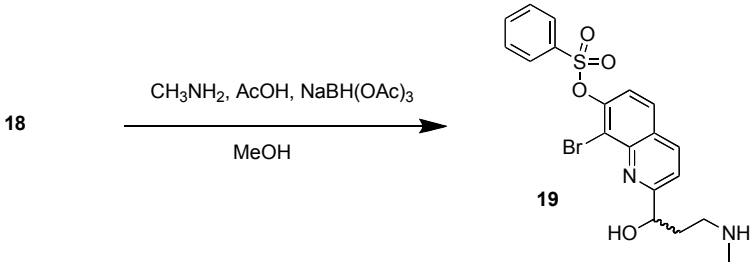
yellow solid. Purification by $SiO_2$ column chromatography ($CHCl_3$) gave **16** as a white solid (1.60 g, 4.08 mmol, 91% yield). $^1$H NMR (500 MHz, $CDCl_3$) δ 10.25 (s, 1H), 8.37 (d, 1H, *J* = 7.5 Hz), 8.09 (d, 1H, *J* = 8.5 Hz), 7.99 (d, 2H, *J* = 7.0 Hz), 7.91 (d, 1H, *J* = 9.0 Hz), 7.79 (d, 1H, *J* = 9.0 Hz), 7.72 (t, 1H, *J* = 7.5 Hz), 7.57 (t, 2H, *J* = 8.0 Hz). $^{13}$C NMR (500 MHz, $CDCl_3$) δ 193.33, 153.80, 149.08, 146.10, 138.35, 135.82, 135.17, 129.79, 129.66, 128.99, 128.37, 125.61, 119.93, 118.51. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{16}H_{11}BrNO_4S$, 392.0 ($^{79}$Br) and 394.0 ($^{81}$Br); observed 391.8 ($^{79}$Br) and 393.8 ($^{81}$Br).




**8-Bromo-2-(1-hydroxybut-3-enyl)quinolin-7-yl benzenesulfonate (17).** A mixture of compound **16** (448 mg, 1.14 mmol), indium powder (150 mg, 1.31 mmol) and allyl bromide (160 μL, 1.87 mmol) were stirred in a mixture of 10 mL THF and 10 mL aq. $NH_4Cl$ for 3 hours. THF was removed *in vacuo*, and residue was extracted with EtOAc and then dried over anhydrous $Na_2SO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography (hexanes/EtOAc = 2/1) to yield **17** as a colorless oil (478 mg, 1.10 mmol, 96%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, 1H, *J* = 8.0 Hz), 7.96-7.93 (m, 2H), 7.76 (d, 1H, *J* = 9.0 Hz), 7.68 (t, 1H, *J* = 8.5 Hz), 7.54-7.50 (m, 3H), 7.42 (d, 1H, *J* = 8.5 Hz), 5.89-5.80 (m, 1H), 5.11-5.03 (m, 2H), 4.97 (s, 2H), 2.73-2.69 (m, 1H), 2.54-2.48 (m, 1H). $^{13}$C NMR (500 MHz, $CDCl_3$) δ 163.40, 148.17, 144.08, 137.34, 135.61, 134.86, 133.88, 129.40, 128.67, 128.01, 126.84, 122.47, 119.53, 118.22, 118.12, 72.24, 42.34. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{19}H_{17}BrNO_4S$, 434.01 ($^{79}$Br) and 436.00 ($^{81}$Br); observed 434.11 ($^{79}$Br) and 436.10 ($^{81}$Br).

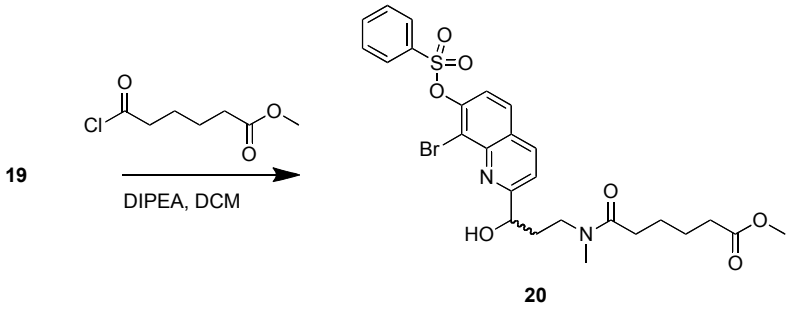



**8-Bromo-2-(1-hydroxy-3-oxopropyl)quinolin-7-yl benzenesulfonate (18).** To a solution of compound **17** (330 mg, 0.760 mmol) in dioxane-water (3:1, 8 mL) were added 2,6-lutidine (0.177 mL, 1.73 mmol), $K_2OsO_4$•2 $H_2O$ (6 mg, 0.016 mmol), and $NaIO_4$ (655 mg, 3.06 mmol). The reaction was stirred at 25 °C and monitored by TLC. After the reaction was complete, water (10 mL) and $CH_2Cl_2$ (20 mL) were added. The organic layer was separated, and the aqueous layer was extracted by DCM (10 mL) three times. The organic layers were pooled, washed with brine, and dried over $Na_2SO_4$. The solvent was removed, and the product was purified with $SiO_2$ column chromatography (hexanes/EtOAc = 2/3) to afford aldehyde **18** (250 mg, 0.573 mmol, 75%) as a colorless oil. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{18}H_{15}BrNO_5S$, 435.99 ($^{79}$Br) and 437.98 ($^{81}$Br); observed 436.04 ($^{79}$Br) and 437.97 ($^{81}$Br).

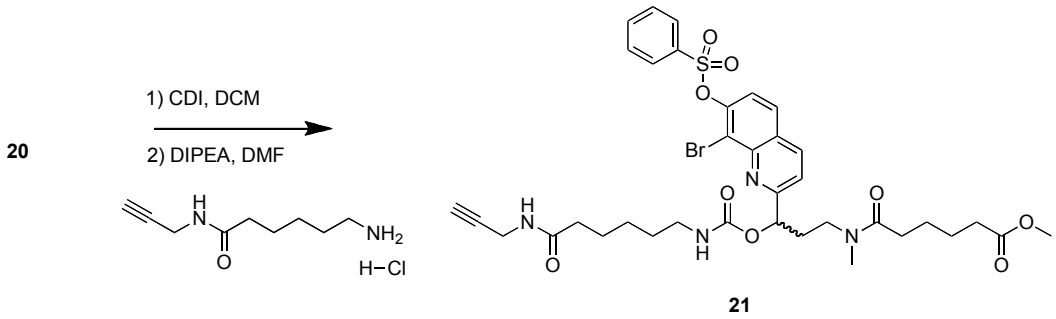



**8-Bromo-2-(1-hydroxy-3-(methylamino)propyl)quinolin-7-yl benzenesulfonate (19).** To a solution of compound **18** (190 mg, 0.435 mmol) in MeOH (1 mL) were added methyl amine (40.0 μL, 0.462 mmol), HOAc (0.005 mL), and $NaBH(OAc)_3$ (200 mg, 0.943 mmol). The reaction was stirred at 25 °C for 20 h. After the reaction was complete, 1 M HCl (0.1 mL) was added to the reaction mixture and then neutralized with saturated aq. $NaHCO_3$. The resulting mixture was extracted with EtOAc, and the organic layers were pooled, washed with brine, and dried over $Na_2SO_4$. Solvent was removed *in*

*vacuo* to afford **19** (160 mg, 0.355 mmol, 81%) as a colorless oil, which was used without further purification. MS-ESI (m/z): $[M + H]^+$ calculated for $C_{19}H_{20}BrN_2O_4S$, 451.03 ($^{79}Br$) and 453.03 ($^{81}Br$); observed 450.89 ($^{79}Br$) and 453.01 ($^{81}Br$).

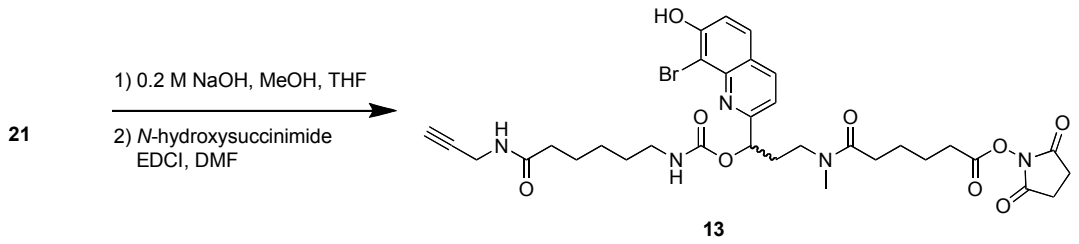



**Methyl 6-((3-(8-bromo-7-(phenylsulfonyloxy)quinolin-2-yl)-3-hydroxypropyl)methyl) amino)-6-oxohexanoate (20).** Compound **19** (160 mg, 0.355 mmol) was dissolved in anhydrous DCM (5 mL), and the solution was cooled to 0 °C. Methyl adipoyl chloride (66 mg, 0.37 mmol) was added over 5 min, and the reaction mixture was stirred for 6 h at room temperature under nitrogen. Solvent was removed *in vacuo*, and residue was dissolved in EtOAc, washed twice with saturated aq. $NaHCO_3$, and dried over anhydrous $Na_2SO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography ($CHCl_3$/acetone = 1/1) to yield **20** as a colorless oil (124 mg, 0.209 mmol, 59%). $^1H$ NMR (500 MHz, $CDCl_3$) δ 8.23-8.16 (m, 1H), 7.99-7.96 (m, 2H), 7.84-7.79 (m, 1H), 7.73-7.68 (m, 2H), 7.62-7.45 (m, 3H), 5.40-5.16 (m, 1H), 4.92-4.85 (m, 1H), 3.78-3.72 (m, 1H), 3.66 (m, 3H), 3.46-3.40 (m, 1H), 2.96 (m, 3H), 2.35-2.28 (m, 3H), 2.23-2.04 (m, 3H), 1.67-1.54 (m, 4H). MS-ESI (m/z): $[M + H]^+$ calculated for $C_{26}H_{30}BrN_2O_7S$, 593.10 ($^{79}Br$) and 595.09 ($^{81}Br$); observed 593.11 ($^{79}Br$) and 595.10 ($^{81}Br$).




**Methyl 14-(8-bromo-7-(phenylsulfonyloxy)quinolin-2-yl)-17-methyl-5,12,18-trioxo-13-oxa-4,11,17-triazatricos-1-yn-23-oate (21).** Compound **20** (114 mg, 0.192 mmol) was dissolved in anhydrous DCM (1 mL) and added to 1,1'-carbonyl diimidazole (46.7 mg, 0.288 mmol) in anhydrous DCM (1.5 mL). The reaction mixture was stirred for 4 h at room temperature under nitrogen, diluted with DCM, washed two times with water, and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography ($CHCl_3$/acetone = 1/1) to yield the imidazole carbamate as a colorless gum (116 mg, 0.169 mmol, 88%). MS-ESI (m/z): $[M + H]^+$ calculated for $C_{30}H_{32}BrN_4O_8S$, 687.1 ($^{79}Br$) and 689.1 ($^{81}Br$); observed 687.2 ($^{79}Br$) and 689.2 ($^{81}Br$). The imidazole carbamate (66 mg, 0.096 mmol) was then dissolved in anhydrous DMF (1.5 mL) and N,N-diisopropylethylamine (33 μL, 0.190 mmol). To this mixture was added 6-oxo-6-(prop-2-ynylamino)hexan-1-aminium hydrochloride salt (36 mg, 0.177 mmol) in anhydrous DMF (1.4 mL). The reaction mixture was stirred overnight at room temperature under nitrogen. Solvent was then removed *in vacuo*, and the crude material was re-dissolved in toluene and evaporated to dryness again. The resulting yellow gum was then dissolved in $CHCl_3$, washed once with 1 M HCl, once with 5% saturated aq. $NaHCO_3$, once with brine, and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography ($CHCl_3$/acetone, stepwise gradient from 4/1 to 2/1) to yield **21** as a viscous colorless gum (70 mg, 0.089 mmol, 93%). $^1H$ NMR (500 MHz, CDCl3) δ 8.20-8.15 (m, 1H), 7.99-7.97 (m, 2H), 7.81-7.77 (m, 1H), 7.72-7.71 (m, 1H), 7.61-7.52 (m, 4H), 6.12 (m, 1H), 5.95-5.88 (m, 1H), 5.31-5.14 (m, 1H), 4.03 (m, 2H), 3.66 (s, 3H), 3.59-3.55 (m, 1H), 3.49 (m,

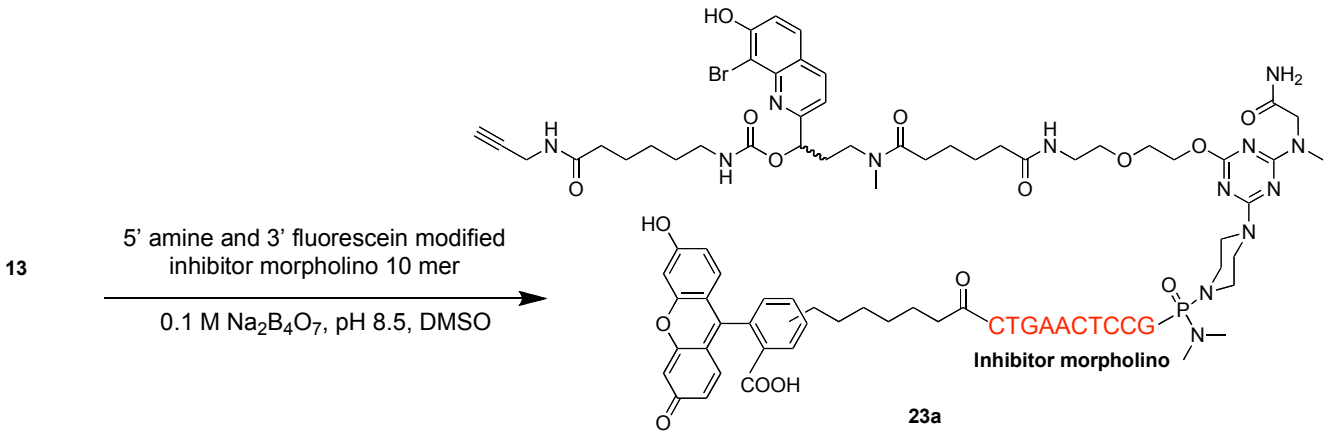
2H), 3.25-3.12 (m, 2H), 2.99-2.91 (m, 3H), 2.40-2.18 (m, 8H), 1.68-1.25 (m, 10H). MS-ESI (m/z): $[M + H]^+$ calculated for $C_{36}H_{44}BrN_4O_9S$, 787.20 ($^{79}Br$) and 789.20 ($^{81}Br$); observed 787.30 ($^{79}Br$) and 789.30 ($^{81}Br$).



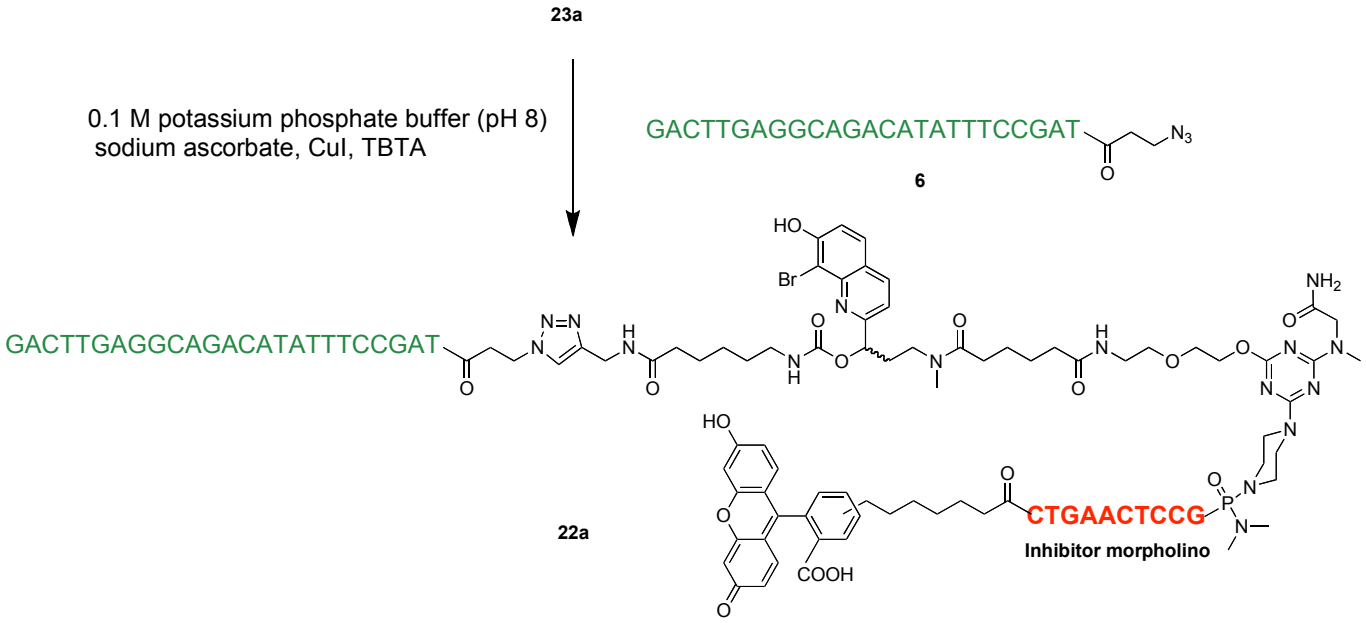
13

**2,5-Dioxopyrrolidin-1-yl 14-(8-bromo-7-hydroxyquinolin-2-yl)-17-methyl-5,12,18-trioxo-13-oxa-4,11,17-triazatricos-1-yn-23-oate (13).** Compound **21** (26 mg, 0.033 mmol) was dissolved in MeOH (0.25 mL) and THF (0.25 mL) and added to 0.4 M NaOH (0.5 mL). The reaction was monitored by TLC, and upon completion, MeOH and THF were removed *in vacuo*. The residual solution was loaded onto Toyopearl Super-Q resin (1 mL), washed three times with wash solution (0.4 M NaOH, 50% $CH_3CN$) and two times with water. The carboxylic acid was eluted from the resin with 1 mL of aq. 5% HOAc/50% $CH_3CN$. The eluent was lyophilized to give the carboxylic acid as a colorless gum (17 mg, 0.027 mmol, 82%). MS-ESI (m/z): $[M + H]^+$ calculated for $C_{29}H_{38}BrN_4O_7$, 633.2 ($^{79}Br$) and 635.2 ($^{81}Br$); observed 633.3 ($^{79}Br$) and 635.3 ($^{81}Br$). To synthesize compound **13**, the carboxylic acid (16 mg, 0.025 mmol) was dissolved in 0.5 mL DMF, and EDCI (10 mg, 0.052 mmol) and *N*-hydroxysuccinimide (6 mg, 0.052 mmol) were then added. The resulting mixture was stirred in the dark for 48 h. Solvent was then removed *in vacuo*, and the crude material was re-dissolved in toluene and evaporated to dryness again. The resulting yellow gum was then dissolved in $CHCl_3$, washed once with aq. 15% citric acid, and dried over anhydrous $MgSO_4$. Solvent was removed *in vacuo*, and the residue was purified by $SiO_2$ column chromatography ($CHCl_3$/acetone, stepwise gradient from 3/1 to 3/2) to yield **13** as a thick colorless gum (9.0 mg, 0.012 mmol, 48%). $^1H$ NMR (500 MHz, $CDCl_3$) δ 8.12-8.07 (dd, 1H, $J_1$ = 8.5 Hz, $J_2$ = 19.0 Hz), 7.69 (t, 1H, $J$ = 8.5 Hz), 7.40-7.37 (dd, 1H, $J_1$ = 5.0 Hz, $J_2$ = 8.0 Hz),

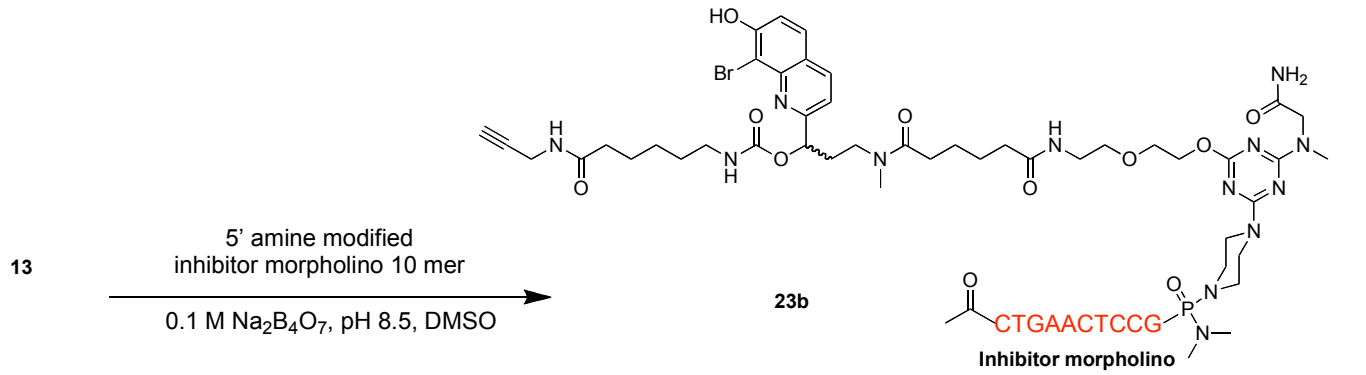
7.32-7.29 (dd, 1H, $J_1$ = 3.0 Hz, $J_2$ = 9.0 Hz), 6.56-6.30 (m, 1H), 6.61-5.80 (m, 2H), 5.08 (m, 1H), 4.04 (m, 2H), 3.58-3.41 (m, 2H), 3.27-3.13 (m, 2H), 2.99-2.95 (m, 3H), 2.84 (br, 4H), 2.63 (t, 1H, $J$ = 7.0 Hz), 2.56 (t, 1H, $J$ = 7.5 Hz), 2.45-2.34 (m, 2H), 2.24-2.17 (m, 4H), 1.79-1.25 (m, 11H). HRMS (TOF MS ES+) (m/z): $[M + Na]^+$ calculated for $C_{33}H_{40}BrN_5NaO_9$, 752.1907 ($^{79}$Br); observed 752.1898 ($^{79}$Br).




**BHQ-conjugated, fluorescent *ntla* MO inhibitor (23a).** Synthetic procedures for the BHQ-conjugated *ntla* MO inhibitors were analogous to those described for **7e**, using the identical fluorescinated oligomer (5'-GCCTCAAGTC-3'). Compounds **23a** was recovered as a yellow solid (75 nmol, 75%). MS-ESI (m/z): $[M + H]^+$ calculated for **23a,** $C_{185}H_{261}N_{69}O_{58}P_{10}Br$, 4769; observed, 4772.

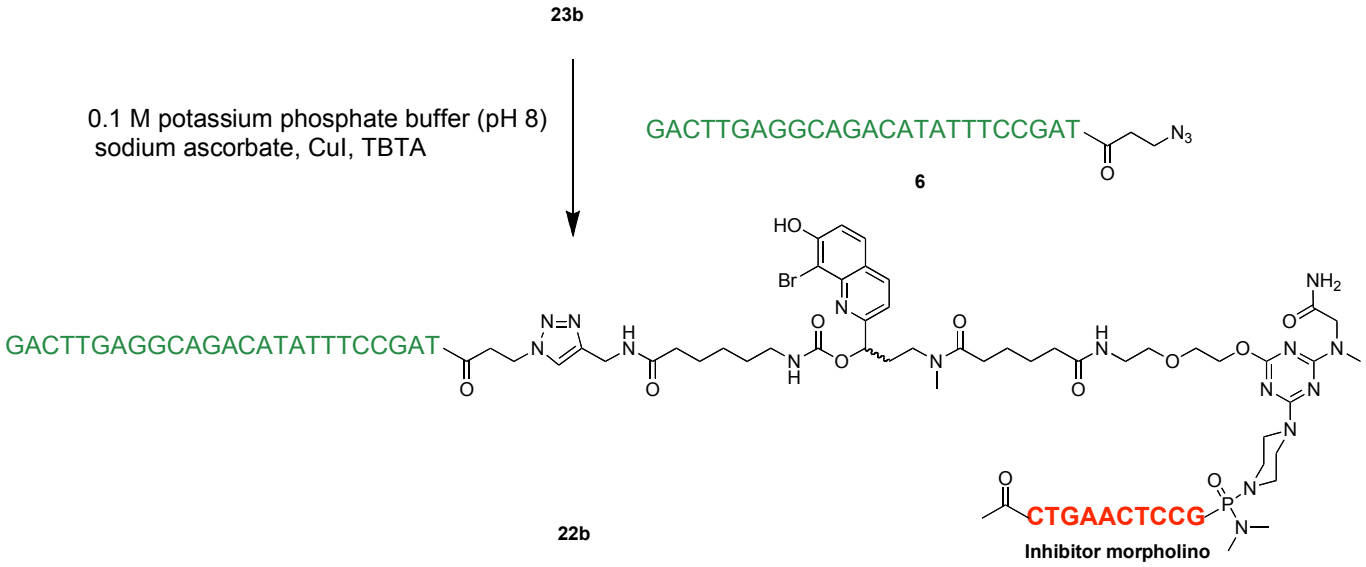



**BHQ-based, fluorescent *ntla* cMO (22a).** Synthetic procedures for the BHQ-based *ntla* cMO were analogous to those described for **8e**, using the BHQ functionalized inhibitor oligomer **23a** (75 nmol) and the azide-functionalized *ntla* MO **6** (75 nmol). cMO **22a** was recovered as a yellow solid (10 nmol, 10% overall). MS-ESI (m/z): $[M + H]^+$ calculated for **22a**, $C_{489}H_{734}N_{219}O_{161}P_{35}Br$, 13417; observed, 13422.




**BHQ-conjugated, non-fluorescent *ntla* MO inhibitor (23b).** Synthetic procedures for the BHQ-conjugated *ntla* MO inhibitors were analogous to those described for **7e** with the following modifications. An identical inhibitor MO sequence (5'-GCCTCAAGTC-3') was used, except the oligomer contained 5' amine but not 3' fluorescein modifications. The inhibitory oligomer (100 nmol)

was dissolved in 0.1 M $Na_2B_4O_7$, pH 8.5 (100 µL) and combined with BHQ linker **13** (0.80 mg, 1.5 µmol) in DMSO (15 µL). The reaction was shaken overnight in the dark. The reaction was diluted to 500 µL with water and passed through a NAP™5 size exclusion column (GE Healthcare) according to the manufacturer's instructions. Product-containing fractions (~1 mL) were pooled and concentrated to 400 µL by lyophilization, acidified with 4 µL of HOAc, and washed with $CHCl_3$ (3 x 400 µL) and EtOAc (2 x 400 µL). The remaining aqueous solution was neutralized with $NH_4OH$ (10%, 20 µL) and lyophilized to give **23b** as a white solid (45 nmol, 45%). MS-ESI (m/z): $[M + H]^+$ calculated for **23b** $C_{161}H_{246}N_{68}O_{50}P_{10}Br$, 4324; observed, 4324.




**BHQ-based, non-fluorescent *ntla* cMO (22b).** The inhibitory oligomer **23b** (45 nmol) and azide-functionalized *ntla* MO **6** (45 nmol) were dissolved in phosphate buffer ($KH_2PO_4$, pH 8.0, 230 µL). To this mixture was added sodium ascorbate (99.0 µg, 500 nmol) in 25 µL of water, followed by TBTA (265 µg, 500 nmol), and CuI (95.2 µg, 500 nmol) in 50 µL of DMSO. The reaction mixture was briefly sonicated and stirred overnight at room temperature in the dark. Precipitate was removed from reaction mixture by centrifugation, and the supernatant was diluted to 800 µL, split and passed through two NAP™5 size exclusion columns (GE Healthcare) according to the manufacturer's

instructions. The desired product was purified from the reaction mixture by adjusting the solution pH to 11.5 with aq. 1 M NaOH and loading it onto a DNAPac PA-100 ion-exchange HPLC column (Dionex, 9 mm x 250 mm). Aqueous running buffers were A: 0.02 M NaOH, 1% $CH_3CN$; B: 0.375 M $NaClO_4$ in 0.02 M NaOH and 1% CAN, and a step-wise gradient was used to separate the product and starting materials, with specific conditions determined by column capacity. A representative purification gradient is: 7 to 15% B in 5 min, 15 to 17% B in 10 min, 17 to 50% B in 1 min, and 50% B for 9 min (flow rate of 4 mL/min). Elution fractions were collected with the UV-VIS flow-cell lamp turned off to prevent photolysis. Fractions (1 mL) were collected every 15 sec and buffered with aq. 1 M $NH_4OAc$, pH 5 (40 μL). Product-containing fractions were identified by absorbance using a Nanodrop spectrophotometer (Thermo Scientific), combined, and lyophilized to dryness. The residue was redissolved in 400 μL of water and passed through a NAP™5 size exclusion column (GE Healthcare). Eluent volume was reduced *in vacuo* to 50 μL and the MOs were precipitated with acetone (400 μL). After centrifugation, the supernatant was discarded and the MO pellet was washed with $CH_3CN$ (100 μL) and lyophilized to dryness. cMO **22b** was recovered as a white solid (7 nmol, 7% overall). MS-ESI (m/z): $[M + H]^+$ calculated for **22b** $C_{465}H_{719}BrN_{218}O_{153}P_{35}$, 12972; observed, 12971.

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