1 *P. aeruginosa* autoinducer-antibiotic conjugates

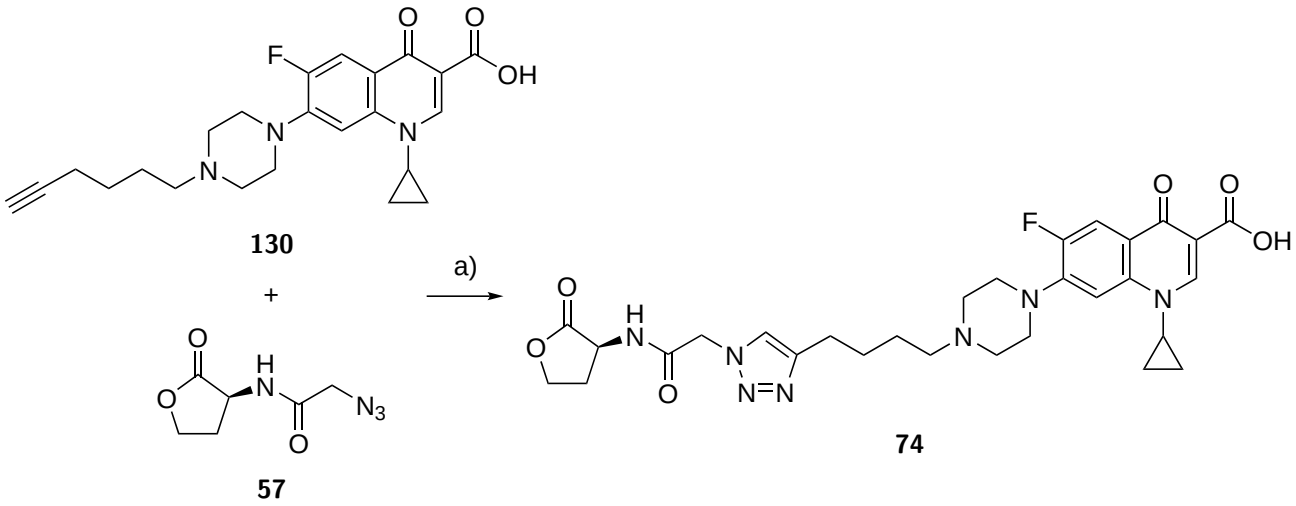
1.1 Synthesis of autoinducer-antibiotic conjugate 74

Test reactions using C_4-HSL analogue **57** and ciprofloxacin analogue **130** were performed to find conditions for the click reactions between the azido autoinducers and the alkynyl antibiotics (see Scheme 1 and Table 1). Stirring at r.t. had no effect even with an extended reaction time. Heating to 50 °C did lead to slow formation of the product, but a mixture of the 1,4 **74** and 1,5 **131** isomers was observed . Use of the ligand tris[(1-benzyl-*1H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) **132** lead to some conversion at room temperature, however the reaction stopped before completion, probably due to oxidation of the Cu(I) catalytic species. When degassed solvent and an argon atmosphere were used the reaction proceeded to completion at room temperature in around 3 h.

check and include ratio from NMR? diagram, isomers diagram

Conditions	**Outcome**
$CuSO_4 \cdot H_2O$, sodium ascorbate, H_2O, *t*-BuOH, air, r.t., 7 d.	No reaction
$CuSO_4 \cdot H_2O$, sodium ascorbate, H_2O, *t*-BuOH, air, 50 °C, 5 d.	1,3-Triazole product **74** and 1,5 triazole impurity **133**
$CuSO_4 \cdot H_2O$, sodium ascorbate, TBTA, H_2O, *t*-BuOH, air, r.t., 3 h.	1,3-Triazole product **74** and starting materials **57** and **130**
$CuSO_4 \cdot H_2O$, sodium ascorbate, TBTA, H_2O, *t*-BuOH, Ar, r.t., 3 h.	1,3-Triazole product **74**

Table 1: Conditions attempted for the synthesis of **74** (see Scheme 1).



Scheme 1: Synthesis of **74**. a) see Table 1.

1.2 Synthesis of the initial triazole-linked library

Once conditions had been found for the click reaction, the synthesis of other conjugates was attempted. Synthesis of some conjugates proved more difficult than expected; AHLs hydrolysed upon HPLC purification, the 3-oxo-C_{12}-HSL conjugate degraded when subjected to column chromatography, and quinolones coordinated copper, thus inhibiting the click reactions. Nonetheless, several conjugates were produced for testing. The results of the reactions are shown in Table 2, Table 3, Table 4 and Table 5.

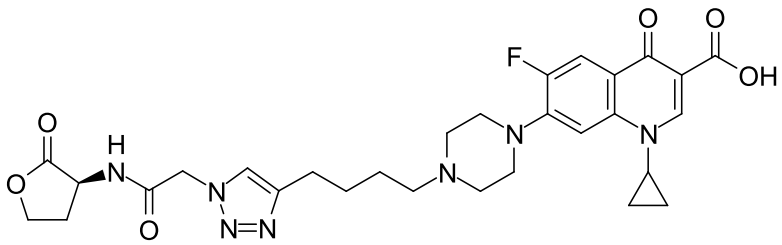
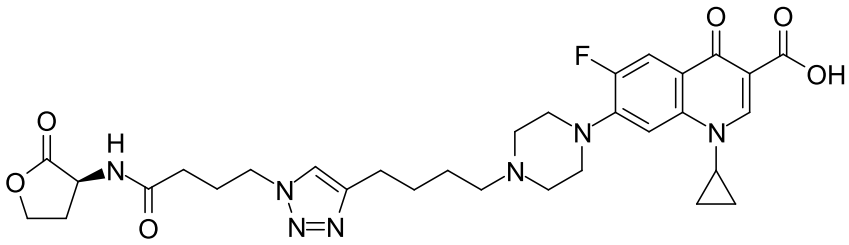
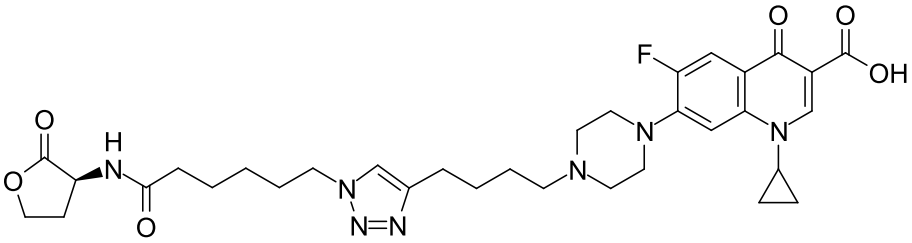
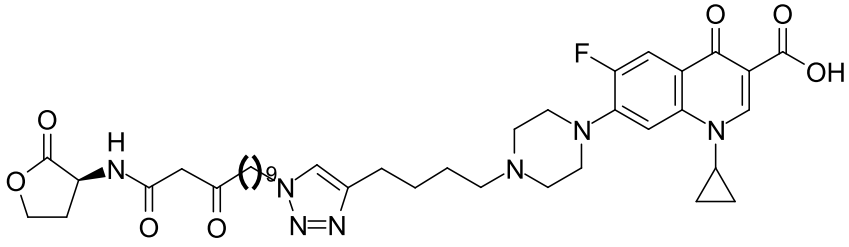
Product	Outcome
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2, 0 - 20 % $MeOH/CH_2Cl_2$).
	✗ Reaction complete by LCMS in 3.5 h, but product degraded when subjected to column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).

Table 2: Click reactions attempted.

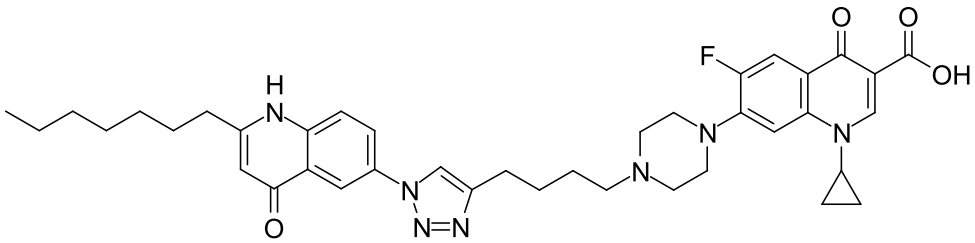
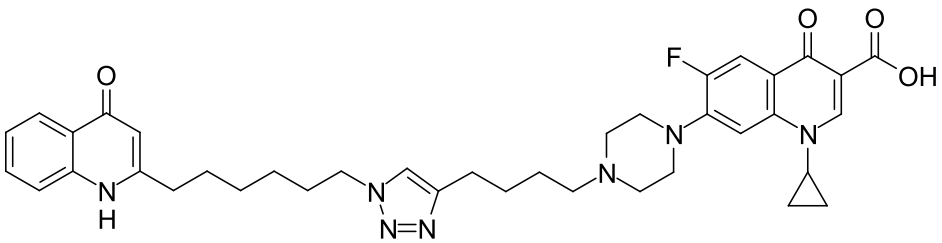
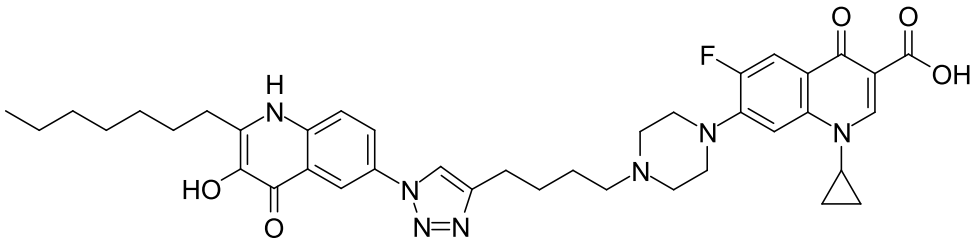
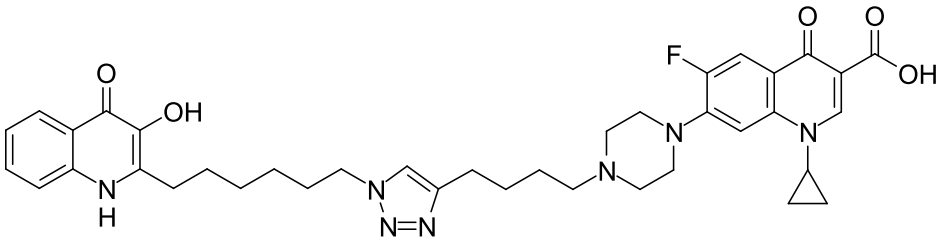
Product	Outcome
	✓ Reaction complete by LCMS. Purified by prep. HPLC.
	✗ Reaction not attempted due to lack of starting material.
	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.
	✗ No reaction.

Table 3: Click reactions attempted.

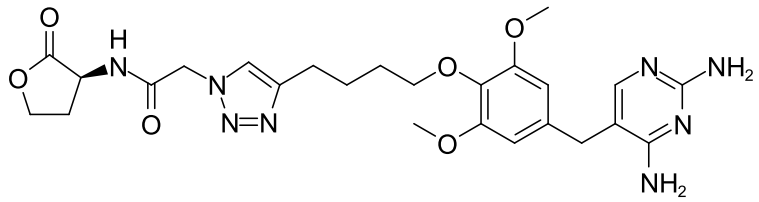
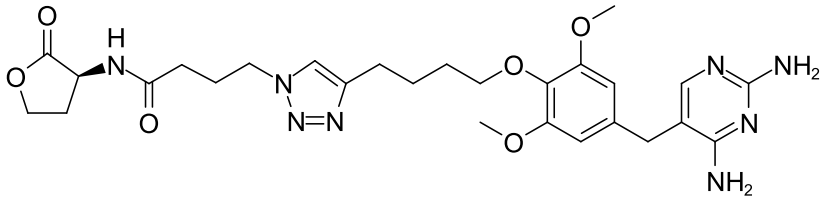
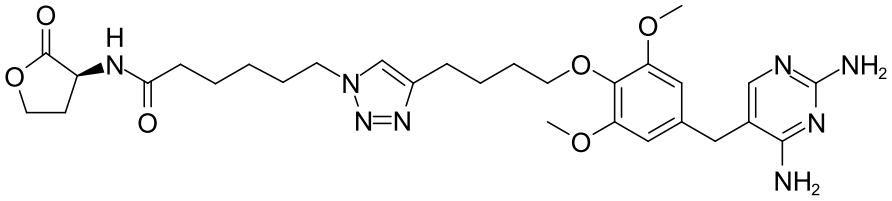
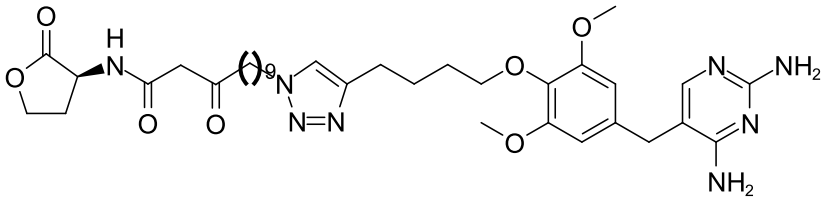
Product	Outcome
	✗ Reaction complete by LCMS in 2 h, but lactone hydrolysed on HPLC column in acidic conditions.
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✗ Degraded.

Table 4: Click reactions attempted.

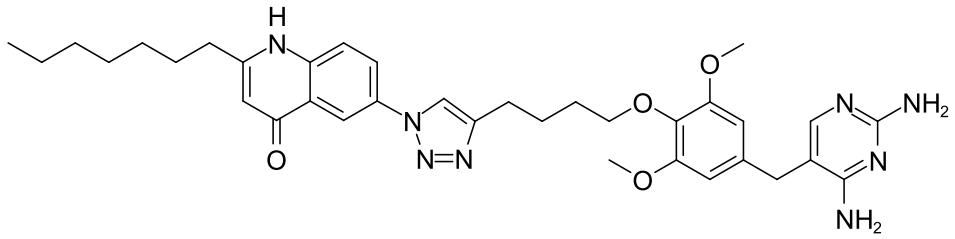
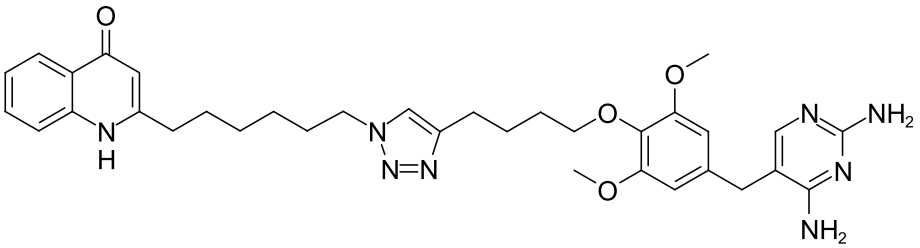
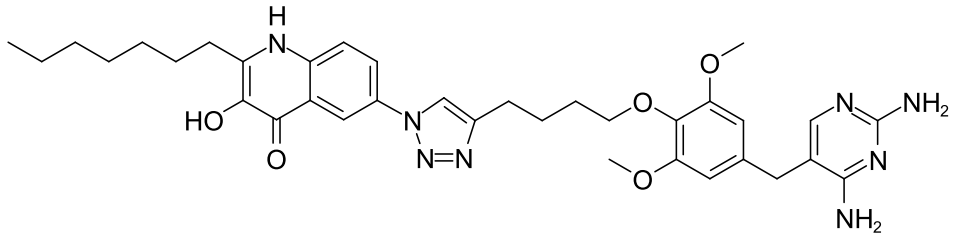
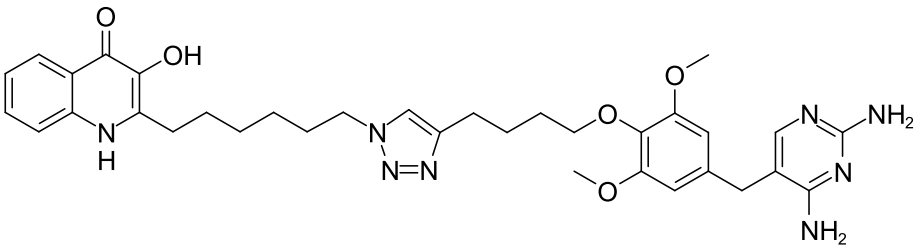
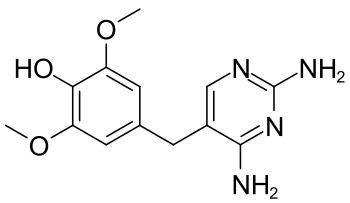
Product	Outcome
	✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.
	✗ Reaction not attempted due to lack of starting material.
	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.
	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$)

Table 5: Click reactions attempted.

1.3 4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol 71



Hydrobromic acid (48 % w/w, aq., 50 ml) was heated to 100 °C. Trimethoprim **30** (5.00 g, 17.2 mmol) was added, and the suspension was stirred for 40 min under Ar. The mixture was removed from the heat, and NaOH (50 % w/w, aq., 15 ml) was added dropwise. The reaction mixture was then cooled slowly to 0 °C, and the resulting crystals were filtered out and washed with cold water. The crystals were then dissolved in hot water

diagrams, discussion, saying what I got from Yssy, why didn't I do them all

(80 ml), neutralized with NH_4OH (sat., aq.) and cooled slowly to 0 °C. The resulting crystals were filtered out, washed with cold water and dried under vacuum. **71** was obtained as pale pink prisms (2.06 g, 7.46 mmol, 43.4 %).

TLC R_f = 0.04 (5 % MeOH/$CHCl_2$)

mp T / °C = 238 (H_2O, decomposes)

IR (neat) ν_{max} / cm^{-1} = 3314.0 (N-H), 3137.4 (N-H), 3045.3 (C-H), 3000.9 (C-H), 2938.1 (C-H), 2838.7 (C-H), 1662.9 (pyrimidine), 1645.2 (pyrimidine), 1626.6 (pyrimidine)

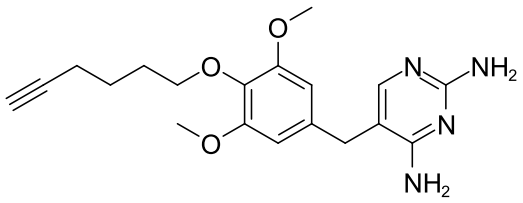
^{1}H NMR (400 MHz, MeOD) δ / ppm = 7.21 (s, 1 H, C<u>H</u>N), 6.54 (s, 2 H, *meta* to OCH_2), 4.87 (br s, 5 H, OH, NH_2 × 2), 3.82 (s, 6 H, OC<u>H</u>$_3$), 3.63 (s, 2 H, CC<u>H</u>$_2$C)

^{13}C NMR (101 MHz, MeOD) δ / ppm = 166.4 ($CH_2C\underline{C}NH_2$), 162.0 (CHN<u>C</u>NH_2), 156.2 (<u>C</u>HNCNH_2), 149.8 (*ipso* to OCH_3), 135.9 (*ipso* to OH), 128.2 (*para* to OH), 111.7 ($CH_2\underline{C}CNH_2$), 107.5 (*meta* to OH), 57.0 (O<u>C</u>H_3), 33.9 (C<u>C</u>H_2C)

HRMS (ESI^+) m/z / Da = 277.1295, $[M+H]^+$ found, $[C_{13}H_{17}N_4O_3]^+$ requires 277.1301

The data are consistent with the literature.[1]

1.4 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 73



4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol **71** (1.00 g, 3.62 mmol, 1 eq.), 6-chloro-1-hexyne **72** (0.524 ml, 0.420 g, 4.34 mmol, 1.2 eq.), Cs_2CO_3 (2.36 g, 7.24 mmol, 2 eq.) and anhydrous DMF (30 ml) were stirred at 70 °C for 7 h. The solvent was removed under reduced pressure, then CH_2Cl_2 (30 ml) was added and the mixture filtured. The filtrate was concentrated under reduced pressure and purified by column chromatography using a Combiflash (SiO_2, 5 % MeOH/CH_2Cl_2). **73** was obtained as a pale cream amorphous solid (0.253 g, 0.709 mmol, 19.6 %). weigh

TLC R_f = 0.14 (5 % MeOH/CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 3451.4 (alkyne C-H), 3313.4 (N-H), 3136.7 (N-H), 3113.9 (N-H), 2944.2 (C-H), 2839.0 (C-H), 1635.1 (pyrimidine)

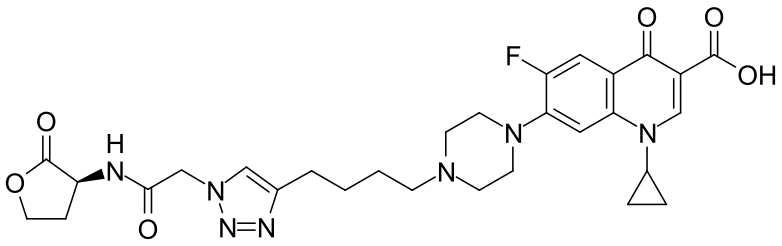
^{1}H NMR (400 MHz, MeOD) δ / ppm = 7.77 (s, 1 H, C<u>H</u>N), 6.37 (s, 2 H, *meta* to OCH_2), 4.83 (br s, 2 H, CHNCN<u>H</u>$_2$), 4.63 (br s, 2 H, $CH_2CCN\underline{H}_2$), 3.95 (t, J = 6.3 Hz, 2 H, C<u>H</u>$_2$O), 3.79 (s, 6 H, OC<u>H</u>$_3$), 3.65 (s, 2 H, CC<u>H</u>$_2$C), 2.28 (td, J = 7.1, 2.6 Hz, 2 H, HC≡CC<u>H</u>$_2$), 1.94 (t, J = 2.7 Hz, 1 H, <u>H</u>C≡C), 1.81 - 1.90 (m, 2 H, C<u>H</u>$_2$$CH_2$O), 1.71 - 1.80 (m, 2 H, C<u>H</u>$_2$$CH_2$$CH_2$O)

^{13}**C NMR** (101 MHz, MeOD) δ / ppm = 162.7 ($CH_2C\underline{C}NH_2$), 162.0 ($CHN\underline{C}NH_2$), 156.4 ($\underline{C}HNCNH_2$), 153.8 (*ipso* to OCH_3), 136.0 (*ipso* to OCH_2), 133.6 (*para* to OCH_2), 106.5 ($CH_2\underline{C}CNH_2$), 105.0 (*meta* to OCH_2), 84.5 ($HC{\equiv}\underline{C}$), 72.6 ($\underline{C}H_2O$), 68.3 ($H\underline{C}{\equiv}C$), 56.1 ($O\underline{C}H_3$), 34.7 ($C\underline{C}H_2C$), 29.1 ($\underline{C}H_2CH_2O$), 24.9 ($\underline{C}H_2CH_2CH_2O$), 18.0 ($HC{\equiv}C\underline{C}H_2$)

HRMS (ESI$^+$) m/z / Da = 357.1920, $[M+H]^+$ found, $[C_{19}H_{25}N_4O_3]^+$ requires 357.1927

The compound has not been reported previously.

1.5 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 74



50 % water/*t*-BuOH (2 ml) was degassed by bubbling N_2 through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **70** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-2-azido-*N*-(2-oxotetrahydrofuran-3-yl)acetamide **57** (9.2 mg, 50.0 μmol, 1 eq.). A similarly degassed solution of $CuSO_4 \cdot 5\,H_2O$ (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in 50 % water/*t*-BuOH (50 μl) was then added. The mixture was stirred at r.t. under argon for 3 h. On observation that the reaction had stalled, the reaction was degassed again, and a further portion of cataylst solution (50 μl) was added. After a further 3 h the reaction mixture was dry-loaded onto SiO_2 and purified by column chromatography using a Combiflash (SiO_2, 0-20 % MeOH/CH_2Cl_2 over 15 min). The combined pure fractions were dried with $MgSO_4$ and evaporated under reduced pressure. **74** was obtained as a white amorphous solid (8.8 mg, 14.8 μmol, 29.6 %).

IR (neat) ν_{max} / cm^{-1} = 3266.3 (N-H), 2949.0 (C-H), 2934.8 (C-H), 2827.2 (C-H), 1778.0 (lactone C=O), 1724.9 (carboxylic acid C=O), 1665.0 (amide C=O), 1625.5 (quinolone C=O)

^{1}**H NMR** (400 MHz, DMSO d_6) δ / ppm = 15.23 (s, 1 H, C(=O)O$\underline{H}$), 8.84 (d, J = 7.9 Hz, 1 H, N$\underline{H}$), 8.66 (s, 1 H, *ortho* to C(=O)OH), 7.90 (d, J = 13.3 Hz, 1 H, *ortho* to F), 7.82 (s, 1 H, C$\underline{H}$=CCH_2), 7.57 (d, J = 7.6 Hz, 1 H, *meta* to F), 5.13 (s, 1 H, C(=O)C$\underline{H}$HN), 5.12 (s, 1 H, C(=O)CH$\underline{H}$N), 4.64 (ddd, J = 10.9, 9.0, 7.8 Hz, 1 H, C$\underline{H}$NH), 4.36 (td, J = 8.9, 1.7 Hz, 1 H, OC$\underline{H}$H), 4.23 (ddd, J = 10.6, 8.8, 6.4 Hz, 1 H, OCH$\underline{H}$), 3.83 (tt, J = 7.0, 4.0 Hz, 1 H, NC$\underline{H}$$(CH_2)_2$), 3.32 (br s, 4 H, $CH_2CH_2CH_2N(CH_2C\underline{H}_2)CH_2C\underline{H}_2$), 2.67 (t, J = 7.4 Hz, 2 H, CH=$CC\underline{H}_2$), 2.58 (br t, J = 5.0 Hz, 4 H, $CH_2CH_2CH_2N(C\underline{H}_2)C\underline{H}_2$), 2.42 - 2.49 (m, 1 H, $OCH_2C\underline{H}H$), 2.40 (t, J = 7.1 Hz, 1 H, CH=$CCH_2CH_2CH_2C\underline{H}_2$), 2.17 (dtd, J = 11.7, 10.8, 10.8, 9.0 Hz, 1 H, $OCH_2CH\underline{H}$), 1.66 (quin, J = 7.2 Hz, 1 H, CH=$CCH_2C\underline{H}_2$), 1.53 (quin, J = 7.2 Hz, 1 H, CH=$CCH_2CH_2C\underline{H}_2$), 1.28 - 1.35 (m, 1 H, NCH$(C\underline{H}H)_2$), 1.16 - 1.21 (m, 1 H, NCH$(CH\underline{H})_2$)

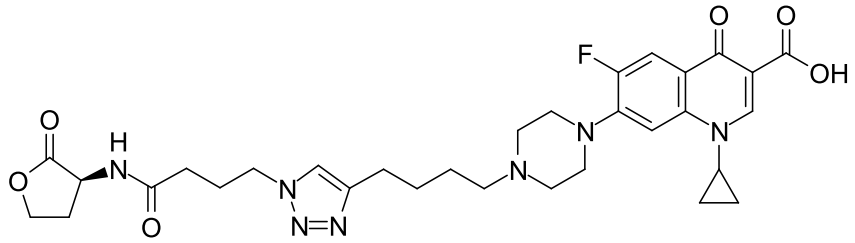
^{13}**C NMR** (101 MHz, DMSO d_6) δ / ppm = 176.4 ($\underline{C}$(=O)CC(=O)OH), 174.9 (O$\underline{C}$(=O)), 166.0 ($\underline{C}$(=O)OH),

165.9 (NH<u>C</u>(=O)), 153.1 (d, J = 250.8 Hz, *ipso* to F), 148.0 (<u>C</u>H=CC(=O)OH), 146.6 (CH=<u>C</u>CH_2), 145.3 (d, J = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 123.4 (<u>C</u>H=CCH_2), 118.5 (d, J = 7.5 Hz, *para* to piperazine), 110.9 (d, J = 23.5 Hz, *ortho* to C=O and *ortho* to F), 106.7 (<u>C</u>C(=O)OH), 106.4 (d, J = 3.2 Hz, *meta* to C=O and *meta* to F), 65.4 (O<u>C</u>H_2), 57.3 (CH=C$CH_2CH_2CH_2$<u>C</u>H_2N), 52.4 ($CH_2CH_2CH_2$N(<u>C</u>H_2)<u>C</u>H_2), 51.2 (C(=O)<u>C</u>H_2N), 49.5 ($CH_2CH_2CH_2$N(CH_2<u>C</u>H_2)CH_2CH_2), 49.5 ($CH_2CH_2CH_2$N(CH_2CH_2)CH_2<u>C</u>H_2), 48.2 (<u>C</u>HNH), 35.9 (N<u>C</u>H(CH_2)$_2$), 28.2 (<u>C</u>H_2CHNH), 26.8 (CH=CCH_2<u>C</u>H_2), 25.7 (CH=CCH_2CH_2<u>C</u>H_2), 24.9 (CH=C<u>C</u>H_2), 7.6 (NCH(<u>C</u>H_2)$_2$)

HRMS (ESI$^+$) m/z / Da = 596.2627, $[M+H]^+$ found, $[C_{29}H_{35}FN_7O_6]^+$ requires 596.2633

$[\alpha]_D^{20}$ / $^\circ 10^{-1}$cm^{2}g^{-1} = -3.5 (c / g(100 ml)$^{-1}$ = 0.0575 , MeOH)

1.6 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 75



50 % water/*t*-BuOH (2 ml) was degassed by bubbling N_2 through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **70** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **60** (10.6 mg, 50.0 μmol, 1 eq.). A similarly degassed solution of $CuSO_4 \cdot 5\,H_2O$ (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in 50 % water/*t*-BuOH (50 μl) was then added. The mixture was stirred at r.t. under argon for 3 h, then dry-loaded onto SiO_2 and purified by column chromatography using a Combiflash (SiO_2, 0-20 % MeOH/CH_2Cl_2 over 15 min). The combined pure fractions were dried with $MgSO_4$ and evaporated under reduced pressure. **75** was obtained as a white amorphous solid (14.6 mg, 23.4 μmol, 46.8 %).

IR (neat) ν_{max} / cm^{-1} = 3286.7 (N-H), 2949.7 (C-H), 2820.6 (C-H), 2778.0 (C-H), 1778.1 (lactone C=O), 1725.6 (carboxylic acid C=O), 1663.7 (amide C=O), 1625.8 (quinolone C=O)

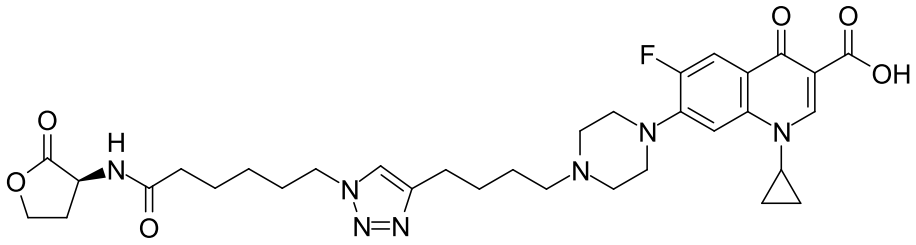
^{1}H NMR (500 MHz, DMSO d_6) δ / ppm = 15.22 (br s, 1 H, C(=O)O<u>H</u>), 8.65 (s, 1 H, *ortho* to C(=O)OH), 8.40 (d, J = 8.0 Hz, 1 H, N<u>H</u>), 7.88 (d, J = 13.4 Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, C<u>H</u>=CCH_2), 7.55 (d, J = 7.5 Hz, 1 H, *meta* to F), 4.53 (ddd, J = 10.9, 9.0, 8.1 Hz, 1 H, C<u>H</u>NH), 4.33 (td, J = 8.9, 1.8 Hz, 1 H, OC<u>H</u>H), 4.31 (t, J = 7.0 Hz, 2 H, C<u>H</u>$_2$NCH=C), 4.20 (ddd, J = 10.5, 8.8, 6.5 Hz, 1 H, OCH<u>H</u>), 3.82 (tt, J = 6.9, 4.0 Hz, 1 H, NC<u>H</u>(CH_2)$_2$), 3.32 (br. t, J = 4.2, 4.2 Hz, 4 H, $CH_2CH_2CH_2$N(CH_2C<u>H</u>$_2$)CH_2C<u>H</u>$_2$), 2.64 (t, J = 7.4 Hz, 2 H, CH=CC<u>H</u>$_2$), 2.57 (br. t, J = 5.0, 5.0 Hz, 2 H, $CH_2CH_2CH_2$N(C<u>H</u>$_2$)C<u>H</u>$_2$), 2.34 - 2.42 (m, 3 H, OCH_2C<u>H</u>H and CH=C$CH_2CH_2CH_2$C<u>H</u>$_2$), 2.09 - 2.19 (m, 3 H, OCH_2CH<u>H</u> and C(=O)C<u>H</u>$_2$), 2.02 (quin, J = 7.2 Hz, 2 H, C(=O)CH_2C<u>H</u>$_2$), 1.64 (quin, J = 7.6 Hz, 2 H, CH=CCH_2C<u>H</u>$_2$), 1.52 (quin, J = 7.2 Hz, 2 H, CH=CCH_2CH_2C<u>H</u>$_2$), 1.29 - 1.34 (m, 2 H, NCH(C<u>H</u>H)$_2$), 1.15 - 1.21 (m, 2 H, NCH(CH<u>H</u>)$_2$)

^{13}C NMR (126 MHz, DMSO d_6) δ / ppm = 176.3 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 171.2 (NHC(=O)), 166.0 (C(=O)OH), 153.0 (d, J = 248.6 Hz, *ortho* to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=$\underline{C}CH_2$), 145.2 (d, J = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.7 (CH=CCH_2), 118.5 (d, J = 7.5 Hz, *para* to piperazine), 110.9 (d, J = 22.4 Hz, *ortho* to C=O and *ortho* to F), 106.7 ($\underline{C}C(=O)OH$), 106.3 (d, J = 3.2 Hz, *meta* to C=O and *meta* to F), 65.3 ($O\underline{C}H_2$), 57.3 ($CH=CCH_2CH_2CH_2\underline{C}H_2N$), 52.4 ($CH_2CH_2CH_2N(\underline{C}H_2)\underline{C}H_2$), 49.5 ($CH_2CH_2CH_2N(CH_2\underline{C}H_2)CH_2CH_2$), 49.4 ($CH_2CH_2CH_2N(CH_2CH_2)CH_2\underline{C}H_2$), 48.6 ($\underline{C}H_2NCH=C$), 47.9 ($OC(=O)\underline{C}HNH$), 35.9 ($N\underline{C}H(CH_2)_2$), 31.7 ($NHC(=O)\underline{C}H_2$), 28.2 ($\underline{C}H_2CHNH$), 26.9 ($CH=CCH_2\underline{C}H_2$), 25.8 ($NHC(=O)CH_2\underline{C}H_2$ and $CH=CCH_2CH_2\underline{C}H_2$), 24.9 ($CH=C\underline{C}H_2$), 7.6 ($NCH(\underline{C}H_2)_2$)

HRMS (ESI$^+$) m/z / Da = 624.2928, $[M+H]^+$ found, $[C_{31}H_{39}FN_7O_6]^+$ requires 624.2946

$[\alpha]_D^{20}$ / $^\circ 10^{-1} cm^2 g^{-1}$ = -10.6 (c / g(100 ml)$^{-1}$ = 0.094 , MeOH)

1.7 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 76



50 % water/*t*-BuOH (2 ml) was degassed by bubbling N_2 through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **70** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **63** (12.0 mg, 50.0 μmol, 1 eq.). A similarly degassed solution of $CuSO_4 \cdot 5\,H_2O$ (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in 50 % water/*t*-BuOH (50 μl) was then added. The mixture was stirred at r.t. under argon for 3 h, then dry-loaded onto SiO_2 and purified by column chromatography using a Combiflash (SiO_2, 0-20 % MeOH/CH_2Cl_2 over 15 min) The combined pure fractions were dried with $MgSO_4$ and evaporated under reduced pressure. **76** was obtained as a white amorphous solid (12.4 mg, 19.0 μmol, 38.0 %).

TLC R_f = 0.30 (30 % MeOH/CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 3301.8 (N-H), 2939.7 (C-H), 2857.5 (C-H), 1784.6 (lactone C=O), 1728.5 (carboxylic acid C=O), 1658.2 (amide C=O), 1625.5 (quinolone C=O)

^{1}H NMR (500 MHz, DMSO d_6) δ / ppm = 15.22 (br s, 1 H, C(=O)O$\underline{H}$), 8.65 (s, 1 H, *ortho* to C(=O)OH), 8.32 (d, J = 8.0 Hz, 1 H, N$\underline{H}$), 7.89 (d, J = 13.3 Hz, 1 H, *ortho* to F), 7.84 (s, 1 H, C$\underline{H}$=CCH_2), 7.55 (d, J = 7.6 Hz, 1 H, *meta* to F), 4.51 (ddd, J = 10.9, 9.1, 7.9 Hz, 1 H, C$\underline{H}$NH), 4.33 (td, J = 8.8, 1.8 Hz, 1 H, OC$\underline{H}$H), 4.28 (t, J = 7.1 Hz, 2 H, C$\underline{H}_2$NCH=C), 4.19 (ddd, J = 10.5, 8.7, 6.6 Hz, 1 H, OCH$\underline{H}$), 3.82 (tt, J = 7.0, 4.0 Hz, 1 H, NC$\underline{H}(CH_2)_2$), 3.32 (br t, J = 4.5, 4 H, $CH_2CH_2CH_2N(CH_2C\underline{H}_2)CH_2C\underline{H}_2$), 2.63 (t, J = 7.5 Hz, 2 H, CH=CC$\underline{H}_2$), 2.57 (br t, J = 4.2, 4.2 Hz, 4 H, $CH_2CH_2CH_2N(C\underline{H}_2)C\underline{H}_2$), 2.33 - 2.41 (m, 3 H, $OCH_2C\underline{H}H$ and $CH=CCH_2CH_2CH_2C\underline{H}_2$), 2.06 - 2.16 (m, 3 H, $OCH_2CH\underline{H}$ and C(=O)C$\underline{H}_2$), 1.79 (quin, J = 7.4 Hz, 2 H,

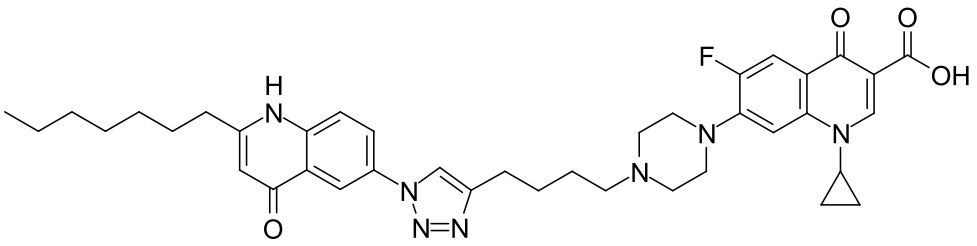
C(=O)$CH_2CH_2CH_2C\underline{H}_2$), 1.63 (quin, J = 7.5 Hz, 2 H, CH=$CCH_2C\underline{H}_2$), 1.45 - 1.56 (m, 4 H, C(=O)$CH_2C\underline{H}_2$ and CH=$CCH_2CH_2C\underline{H}_2$), 1.29 - 1.34 (m, 2 H, NCH(C$\underline{H}$H)$_2$), 1.19 - 1.25 (m, 2 H, C(=O)$CH_2CH_2C\underline{H}_2$), 1.15 - 1.19 (m, 2 H, NCH(CH$\underline{H}$)$_2$)

^{13}C NMR (126 MHz, DMSO d_6) δ / ppm = 176.4 ($\underline{C}$(=O)CC(=O)OH), 175.4 (O$\underline{C}$(=O)), 172.1 (NH$\underline{C}$(=O)), 166.0 ($\underline{C}$(=O)OH), 153.0 (d, J = 250.2 Hz, *ipso* to F), 148.0 ($\underline{C}$H=CC(=O)OH), 146.8 (CH=$\underline{C}CH_2$), 145.2 (d, J = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.6 ($\underline{C}$H=CCH_2), 118.5 (d, J = 8.0 Hz, *para* to piperazine), 110.9 (d, J = 23.5 Hz, *ortho* to C=O and *ortho* to F), 106.7 ($\underline{C}$C(=O)OH), 106.3 (d, J = 2.1 Hz, *meta* to C=O and *meta* to F), 65.3 (O$\underline{C}H_2$), 57.4 (CH=$CCH_2CH_2CH_2\underline{C}H_2$N), 52.4 ($CH_2CH_2CH_2N(\underline{C}H_2)\underline{C}H_2$), 49.5 ($CH_2CH_2CH_2N(CH_2\underline{C}H_2)CH_2CH_2$), 49.5 ($CH_2CH_2CH_2N(CH_2CH_2)CH_2\underline{C}H_2$), 49.0 ($\underline{C}H_2$NCH=C), 47.8 ($\underline{C}$HNH), 35.9 (N$\underline{C}$H($CH_2$)$_2$), 34.8 (NHC(=O)$\underline{C}H_2$), 29.5 ($\underline{C}H_2CH_2$NCH=C), 28.3 ($\underline{C}H_2$CHNH), 26.9 (CH=C $CH_2\underline{C}H_2$), 25.7 (CH=$CCH_2CH_2\underline{C}H_2$), 25.4 (NHC(=O)$CH_2CH_2\underline{C}H_2$), 24.9 (CH=C$\underline{C}H_2$), 24.5 (NHC(=O)$CH_2\underline{C}$ H_2), 7.6 (NCH($\underline{C}H_2$)$_2$)

HRMS (ESI$^+$) m/z / Da = 652.3254, $[M+H]^+$ found, $[C_{33}H_{43}FN_7O_6]^+$ requires 652.3248

$[\boldsymbol{\alpha}]_D^{20}$ / °10^{-1}cm^{2}g^{-1} = -8.5 (c / g(100 ml)$^{-1}$ = 0.106 , MeOH)

1.8 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 77



50 % water/*t*-BuOH (1 ml) was degassed by bubbling N_2 through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **70** (4.1 mg, 10.0 μmol, 1 eq.) and 6-azido-2-heptylquinolin-4(1*H*)-one **40** (2.8 mg, 10.0 μmol, 1 eq.). A similarly degassed solution of $CuSO_4 \cdot 5\,H_2O$ (125 μg, 0.5 μmol, 0.05 eq. 50 mM), THPTA (218 μg, 0.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (198 μg, 1 μmol, 0.1 eq., 100 mM) in 50 % water/*t*-BuOH (10 μl) was then added. The mixture was stirred at r.t. under argon for 1.5 h, then the reaction mixture was evaporated under reduced pressure. The residue was purified by preparatory HPLC (50-100 % acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between $NaHCO_3$ (aq., sat., 10 ml) and 10 % *i*-PrOH/$CHCl_3$ (10 ml). The organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. **77** was obtained as a white amorphous solid (8.6 mg, 2.7 μmol, 27.0 %).

IR (neat) ν_{max} / cm^{-1} = 2927.0 (C-H), 2865.5 (C-H), 1715.5 (carboxylic acid C=O), 1631.0 (ciprofloxacin quinolone C=O and HHQ C=O)

^{1}H NMR (500 MHz, DMSO d_6) 15.12 (br s, $\underline{C}$(=O)OH), 11.79 (s, 1 H, N$\underline{H}$), 8.75 (s, 1 H, NC$\underline{H}$=CCH_2), 8.71 (s, 1 H, *ortho* to C(=O)OH), 8.40 (d, J = 2.7 Hz, 1 H, *ortho* to C(=O) and *ortho* to N), 8.18 (dd, J = 8.9, 2.6 Hz, 1 H, *para* to C(=O) and *ortho* to N), 7.99 (d, J = 13.0 Hz, 1 H, *ortho* to F), 7.75 (d, J = 9.0 Hz, 1 H,

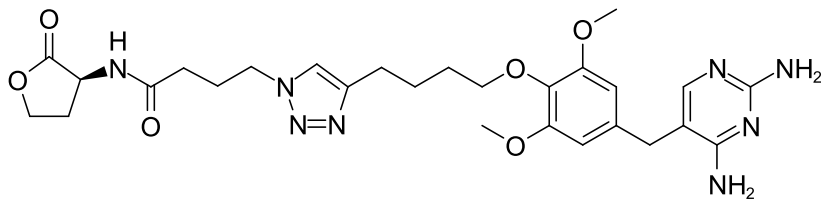
meta to C(=O) and *meta* to N), 7.62 (d, J = 7.8 Hz, 1 H, *meta* to F), 6.02 (s, 1 H, NHC=C<u>H</u>C(=O)), 3.85 (tt, J = 7.0, 4.0 Hz, 1 H, NC<u>H</u>$(CH_2)_2$), 3.23 - 3.30 (m, 6 H, C<u>H</u>$_2$N(C<u>H</u>$_2$C<u>H</u>$_2$)C<u>H</u>$_2$C<u>H</u>$_2$), 2.82 (t, J = 5.9 Hz, 2 H, NCH=CC<u>H</u>$_2$), 2.63 (t, J = 7.9 Hz, 2 H, C<u>H</u>$_2$C=CHC(=O)), 1.76 - 1.81 (m, 4 H, NCH=CCH$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.70 (quin, J = 7.2 Hz, 2 H, C<u>H</u>$_2$CH$_2$C=CHC(=O)), 1.15 - 1.38 (m, 12 H, CH$_3$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$, NCH(C<u>H</u>H)$_2$ and NCH(CH<u>H</u>)$_2$), 0.87 (t, J = 6.9 Hz, 3 H, C<u>H</u>$_3$)

^{13}C NMR (126 MHz, DMSO d$_6$) δ / ppm = 176.4 (<u>C</u>(=O)CC(=O)OH), 176.3 (CH<u>C</u>(=O)), 165.8 (<u>C</u>(=O)OH), 154.3 (CCH<u>C</u>(=O)), 152.9 (d, J = 240.1 Hz, *ipso* to F), 148.3 (<u>C</u>H=CC(=O)OH), 147.5 (NCH<u>C</u>CH$_2$), 143.3 (d, J = 8.5 Hz, *ortho* to F and *ipso* to N), 139.6 (*ipso* to NH), 139.0 (*para* to F), 132.0 (*para* to NH), 124.9 (*ipso* to C(=O) and *ortho* to NH), 123.6 (*para* to C(=O) and *meta* to NH), 120.5 (N<u>C</u>H=CCH$_2$), 120.0 (*meta* to C(=O) and *meta* to N), 119.6 (d, J = 9.6 Hz, *ipso* to C(=O) and *para* to N), 115.1 (*ortho* to C(=O) and *ortho* to N), 111.3 (d, J = 28.8 Hz, *ortho* to F and *ortho* to C(=O)), 107.9 (*meta* to F and *meta* to C(=O)), 107.2 (<u>C</u>HC(=O)), 106.9 (<u>C</u>C(=O)OH), 55.4 (CH=CCH$_2$CH$_2$CH$_2$<u>C</u>H$_2$N), 50.6 (CH$_2$CH$_2$CH$_2$N(<u>C</u>H$_2$)<u>C</u>H$_2$), 46.5 (CH$_2$CH$_2$CH$_2$N(CH$_2$<u>C</u>H$_2$)CH$_2$CH$_2$), 46.5 (CH$_2$CH$_2$CH$_2$N(CH$_2$CH$_2$)CH$_2$<u>C</u>H$_2$), 36.0 (N<u>C</u>H(CH$_2$)$_2$), 33.2 (<u>C</u>H$_2$CNH), 31.2 (CH$_3$CH$_2$<u>C</u>H$_2$), 28.3 - 28.5 (CH$_3$CH$_2$CH$_2$<u>C</u>H$_2$<u>C</u>H$_2$<u>C</u>H$_2$), 25.6 (CH=CCH$_2$<u>C</u>H$_2$), 24.4 (CH=C <u>C</u>H$_2$), 22.7 (CH=CCH$_2$CH$_2$<u>C</u>H$_2$), 22.0 (CH$_3$<u>C</u>H$_2$), 13.9 (<u>C</u>H$_3$), 7.6 (NCH(<u>C</u>H$_2$)$_2$)

^{19}F NMR (376.45 MHz, MeOD) δ / ppm = ??

HRMS (ESI$^+$) m/z / Da = 696.3667, [M+H]$^+$ found, [$C_{39}H_{47}FN_7O_4$]$^+$ requires 696.3668

1.9 (*S*)-4-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy) butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)butanamide 78



50 % water/*t*-BuOH (2 ml) was degassed by bubbling N_2 through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **73** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **60** (15.9 mg, 75.0 μmol, 1.5 eq.). Similarly degassed solutions of $CuSO_4 \cdot 5\,H_2O$ (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in water (50 μl) were then added. An extra portion of **60** (10.6 mg, 50.0 μmol, 1 eq.) was added after 4 d. Extra portions of the catalysts were added after 9 d. After 2 weeks, the reaction mixture was extracted with CH_2Cl_2 (6×10 ml) then dry-loaded onto SiO_2 and purified by column chromatography using a Combiflash (SiO_2, 0-20 % MeOH/CH_2Cl_2).The combined pure fractions were dried with $MgSO_4$ and evaporated under reduced pressure. **78** was obtained as a pale brown gum (4.8 mg, 8.4 μmol, 16.8 %).

TLC R_f = 0.30 (30 % MeOH/CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 3340.5 (N-H), 3303.3 (N-H), 3182.5 (N-H), 2933.8 (C-H), 1774.2 (lactone C=O), 1659.7 (amide C=O and pyrimidine)

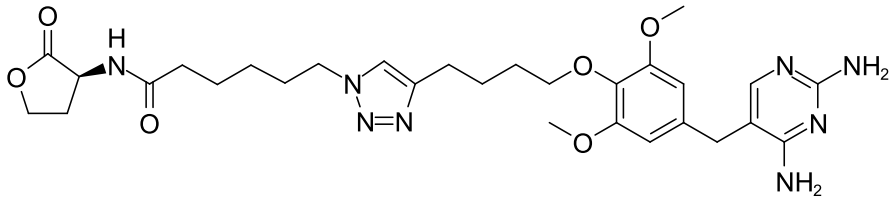
^{1}H NMR (500 MHz, DMSO d_6) δ / ppm = 8.43 (d, J = 8.0 Hz, 1 H, NC<u>H</u>), 7.80 (s, 1 H, NC<u>H</u>=CCH$_2$), 7.46 (s, 1 H, C<u>H</u>N=CNH$_2$), 6.68 (br s, 2 H, CH$_2$CCN<u>H</u>$_2$), 6.53 (s, 2 H, *meta* to CH$_2$), 6.21 (br s, 2 H, CHN=CN<u>H</u>$_2$), 4.49 (dt, J = 10.7, 8.6 Hz, 1 H, C<u>H</u>NH), 4.32 (td, J = 8.7, 1.6 Hz, 1 H, C<u>H</u>HOC(=O)), 4.29 (t, J = 6.8 Hz, 2 H, C<u>H</u>$_2$N), 4.19 (ddd, J = 10.6, 8.7, 6.5 Hz, 1 H, CH<u>H</u>OC(=O)), 3.79 (t, J = 6.2 Hz, 2 H, CH$_2$CH$_2$C<u>H</u>$_2$O), 3.68 (s, 6 H, C<u>H</u>$_3$), 3.53 (br s, 2 H, CC<u>H</u>$_2$C), 2.63 (t, J = 7.5 Hz, 2 H, CH=CC<u>H</u>$_2$), 2.37 (dddd, J = 12.2, 8.9, 6.7, 1.8 Hz, 1 H, C<u>H</u>HCHNH), 2.08 - 2.15 (m, 3 H, CH<u>H</u>CHNH and C(=O)C<u>H</u>$_2$), 2.00 (quin, J = 7.2 Hz, 2 H, C<u>H</u>$_2$CH$_2$N), 1.72 (quin, J = 7.3 Hz, 2 H, CH=CCH$_2$C<u>H</u>$_2$), 1.61 (quin, J = 6.7 Hz, 2 H, C<u>H</u>$_2$CH$_2$O)

^{13}C NMR (126 MHz, DMSO d_6) δ / ppm = 175.8 (O<u>C</u>=O), 171.9 (NH<u>C</u>=O), 163.1 (C<u>C</u>(NH$_2$)N), 159.7 (br s, N<u>C</u>(NH$_2$)N), 153.2 (*ipso* to OCH$_3$), 150.5 (br s, <u>C</u>HNC(NH$_2$)N), 147.3 (NCH=<u>C</u>CH$_2$CH$_2$), 135.2 (*para* to CH$_2$O), 135.0 (*ipso* to CH$_2$O), 122.1 (<u>C</u>H=CCH$_2$CH$_2$), 107.3 (CH$_2$<u>C</u>C(NH$_2$)=N), 106.2 (*meta* to CH$_2$O), 72.3 (CH$_2$CH$_2$<u>C</u>H$_2$O), 65.7 (O<u>C</u>H$_2$CH$_2$CHNH), 56.2 (O<u>C</u>H$_3$), 48.9 (<u>C</u>H$_2$N), 48.3 (<u>C</u>HNH), 32.9 (C<u>C</u>H$_2$C), 32.0 (C(=O)<u>C</u>H$_2$), 29.3 (CH$_2$<u>C</u>H$_2$CH$_2$O), 28.4 (OCH$_2$<u>C</u>H$_2$CHNH), 26.0 (<u>C</u>H$_2$CH$_2$N), 25.7 (CH=CCH$_2$<u>C</u>H$_2$), 24.9 (CH=C<u>C</u>H$_2$CH$_2$)

HRMS (ESI$^+$) m/z / Da = 569.2834, [M+H]$^+$ found, [$C_{27}H_{37}N_8O_6$]$^+$ requires 569.2836

$[\boldsymbol{\alpha}]_D^{20}$ / °10^{-1}cm^{2}g^{-1} = -4.6 (c / g(100 ml)$^{-1}$ = 0.0433 , MeOH)

1.10 (*S*)-6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy) butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 79



50 % water/*t*-BuOH (2 ml) was degassed by bubbling N_2 through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **73** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **63** (18.0 mg, 75.0 μmol, 1.5 eq.). Similarly degassed solutions of $CuSO_4 \cdot 5\,H_2O$ (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in water (50 μl) were then added. An extra portion of **63** (12.0 mg, 50.0 μmol, 1 eq.) was added after was added after 4 d. Extra portions of the catalysts were added after 9 d. After 2 weeks, the After 2 weeks, the reaction mixture was extracted with CH_2Cl_2 (6×10 ml) then dry-loaded onto SiO_2 and purified by column chromatography using a Combiflash (SiO_2, 0-20 % MeOH/CH_2Cl_2).The combined pure fractions were dried with $MgSO_4$ and evaporated under reduced pressure. **79** was obtained as a clear gum (8.0 mg, 13.4 μmol, 26.8 %).

TLC R_f = 0.35 (30 % MeOH/CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 3336.0 (N-H), 3208.7 (N-H), 2941.1 (C-H), 2869.2 (C-H), 1775.2 (lactone C=O), 1657.3 (amide C=O and pyrimidine)

^{1}H NMR (500 MHz, DMSO d_6) δ / ppm = 8.34 (d, J = 8.0 Hz, 1 H, NC<u>H</u>), 7.83 (s, 1 H, NC<u>H</u>=CCH$_2$), 7.50 (s, 1 H, C<u>H</u>N=CNH$_2$), 6.54 (s, 2 H, *meta* to CH$_2$), 6.17 (br s, 2 H, CH$_2$CCN<u>H</u>$_2$), 5.77 (br s, 2 H, CHN=CN<u>H</u>$_2$),

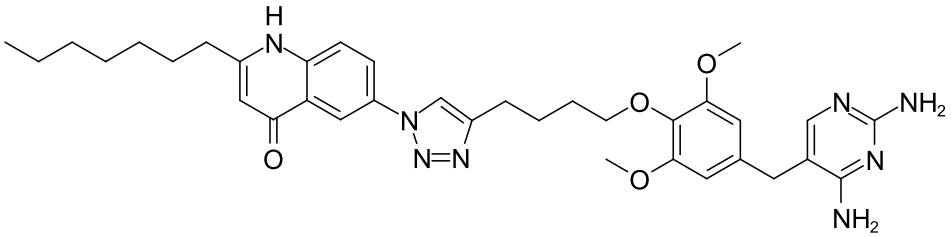
4.51 (ddd, J = 11.0, 9.0, 8.1 Hz, 1 H, C<u>H</u>NH), 4.33 (td, J = 8.8, 1.9 Hz, 1 H, C<u>H</u>HOC(=O)), 4.27 (t, J = 7.1 Hz, 2 H, C<u>H$_2$</u>N), 4.19 (ddd, J = 10.5, 8.7, 6.5 Hz, 1 H, CH<u>H</u>OC(=O)), 3.80 (t, J = 6.3 Hz, 2 H, CH$_2$CH$_2$C<u>H$_2$</u>O), 3.70 (s, 6 H, C<u>H$_3$</u>), 3.52 (s, 2 H, CC<u>H$_2$</u>C), 2.64 (t, J = 7.5 Hz, 2 H, CH=CC<u>H$_2$</u>), 2.36 (dddd, J = 12.1, 8.9, 6.7, 1.8 Hz, 1 H, C<u>H</u>HCHNH), 2.06 - 2.16 (m, 3 H, CH<u>H</u>CHNH and C(=O)C<u>H$_2$</u>), 1.78 (quin, J = 7.4 Hz, 2 H, C<u>H$_2$</u>CH$_2$N), 1.73 (quin, J = 7.7 Hz, 2 H, CH=CCH$_2$C<u>H$_2$</u>), 1.63 (quin, J = 6.8 Hz, 2 H, C<u>H$_2$</u>CH$_2$O), 1.52 (quin, J = 7.5 Hz, 2 H, C(=O)CH$_2$C<u>H$_2$</u>), 1.17 - 1.27 (m, 2 H, C(=O)CH$_2$CH$_2$C<u>H$_2$</u>)

^{13}C NMR (125 MHz, DMSO d$_6$) δ / ppm = 175.4 (O<u>C</u>=O), 172.0 (NH<u>C</u>=O), 162.2 (C<u>C</u>(NH$_2$)N), 161.8 (N<u>C</u>(NH$_2$)N), 154.8 (<u>C</u>HNC(NH$_2$)N), 152.8 (*ipso* to OCH$_3$), 146.7 (CH=<u>C</u>CH$_2$CH$_2$), 135.5 (*para* to CH$_2$O), 134.8 (*ipso* to CH$_2$O), 121.6 (<u>C</u>H=CCH$_2$CH$_2$), 105.9 (CH$_2$<u>C</u>C(NH$_2$)=N), 105.8 (*meta* to CH$_2$O), 71.9 (CH$_2$CH$_2$ <u>C</u>H$_2$O), 65.2 (O<u>C</u>H$_2$CH$_2$CHNH), 55.8 (O<u>C</u>H$_3$), 49.0 (<u>C</u>H$_2$N), 47.8 (<u>C</u>HNH), 34.8 (C(=O)<u>C</u>H$_2$), 32.9 (C<u>C</u>H$_2$C), 29.4 (<u>C</u>H$_2$CH$_2$N), 29.1 (CH$_2$<u>C</u>H$_2$CH$_2$O), 28.2 (OCH$_2$<u>C</u>H$_2$CHNH), 25.5 (CH=CCH$_2$<u>C</u>H$_2$), 25.3 (C(=O)CH$_2$CH$_2$ <u>C</u>H$_2$), 24.7 (CH=C<u>C</u>H$_2$CH$_2$), 24.4 (C(=O)CH$_2$<u>C</u>H$_2$)

HRMS (ESI$^+$) m/z / Da = 597.3149, [M+H]$^+$ found, [C$_{29}$H$_{41}$N$_8$O$_6$]$^+$ requires 597.3144

$[\boldsymbol{\alpha}]_D^{20}$ / °10^{-1}cm^{2}g^{-1} = -3.6 (c / g(100 ml)$^{-1}$ = 0.11 , MeOH)

1.11 6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-2-heptylquinolin-4(1*H*)-one 80



50 % water/*t*-BuOH (1 ml) was degassed by bubbling N$_2$ through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **73** (3.6 mg, 10.0 μmol, 1 eq.) and 6-azido-2-heptylquinolin-4(1*H*)-one **40** (2.8 mg, 10.0 μmol, 1 eq.). A similarly degassed solution of $CuSO_4 \cdot 5\,H_2O$ (125 μg, 0.5 μmol, 0.05 eq. 50 mM), THPTA (218 μg, 0.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (198 μg, 1 μmol, 0.1 eq., 100 mM) in water (10 μl) was then added. The mixture was stirred at r.t. under argon for 1.5 h, then evaporated under reduced pressure. The residue was purified by preparatory HPLC (5-100 % acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between $NaHCO_3$ (aq., sat., 10 ml) and 10 % *i*-PrOH/$CHCl_3$ (10 ml). The organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. **80** was obtained as a clear gum (2.6 mg, 4.1 μmol, 41.0 %).

TLC R_f = 0.17 (20 % MeOH/CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 2927.7 (C-H), 2855.5 (C-H), 1664.1 (pyrimidine), 1645.4 (pyrimidine and HHQ C=O),

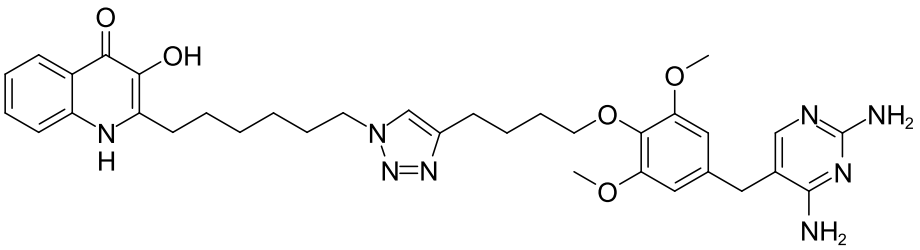
^{1}H NMR (500 MHz, DMSO d$_6$) δ / ppm = 11.80 (s, 1 H, N<u>H</u>), 8.69 (s, 1 H, NC<u>H</u>=CCH$_2$), 8.41 (d, J = 2.7 Hz, 1 H, *ortho* to C=O), 8.17 (dd, J = 9.0, 2.6 Hz, 1 H, *para* to C=O), 7.73 (d, J = 9.0 Hz, 1 H, <u>ortho</u> to NH), 7.51 (br s, 4 H, NH$_2$), 7.41 (s, 1 H, C<u>H</u>N=CNH$_2$), 6.61 (s, 2 H, *meta* to CH$_2$), 6.02 (d, J = 1.8 Hz, 1 H,

C(=O)C<u>H</u>), 3.86 (t, J = 6.3 Hz, 2 H, C<u>H</u>$_2$O), 3.73 (s, 6 H, OC<u>H</u>$_3$), 3.57 - 3.62 (m, 2 H, CC<u>H</u>$_2$C), 2.78 (t, J = 7.5 Hz, 2 H, CH=CC<u>H</u>$_2$), 2.63 (t, J = 7.3 Hz, 2 H, HNCC<u>H</u>$_2$), 1.85 (quin, J = 7.5 Hz, 2 H, CH=CCH$_2$C<u>H</u>$_2$), 1.61 - 1.78 (m, 4 H, HNCCH$_2$C<u>H</u>$_2$ and CH=CCH$_2$CH$_2$C<u>H</u>$_2$), 1.31 - 1.40 (m, 4 H, HNCCH$_2$CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.25 - 1.31 (m, 4 H, CH$_3$C<u>H</u>$_2$C<u>H</u>$_2$), 0.86 (t, J = 7.2 Hz, 3 H, C<u>H</u>$_3$CH$_2$)

^{13}C NMR (125 MHz, DMSO d$_6$) δ / ppm = 176.4 (<u>C</u>=O), 164.1 (C<u>C</u>(NH$_2$)N), 154.3 (HN<u>C</u>), 154.2 (N<u>C</u>(NH$_2$)N), 153.1 (*ipso* to OCH$_3$), 148.3 (CH=<u>C</u>CH$_2$CH$_2$), 140.2 (<u>C</u>HNC(NH$_2$)N), 139.6 (*ipso* to NH), 135.4 (*ipso* to CH$_2$O), 132.8 (*para* to CH$_2$O), 132.1 (*para* to NH), 124.9 (*ipso* to C=O), 123.7 (*para* to C=O), 120.3 (<u>C</u>H=CCH$_2$CH$_2$), 120.0 (*meta* to C=O and *ortho* to NH), 115.1 (*ortho* to C=O and *meta* to NH), 109.0 (CH$_2$<u>C</u>C(NH$_2$)=N), 108.0 (C(=O)<u>C</u>H), 106.3 (*meta* to CH$_2$O), 72.0 (CH$_2$CH$_2$<u>C</u>H$_2$O), 56.0 (O<u>C</u>H$_3$), 33.3 (HNC<u>C</u>H$_2$), 32.1 (C<u>C</u>H$_2$C), 31.2 (CH$_3$CH$_2$<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$CH$_2$O), 28.3 - 28.6 (CH$_3$CH$_2$CH$_2$<u>C</u>H$_2$<u>C</u>H$_2$<u>C</u>H$_2$), 25.3 (<u>C</u>H$_2$CH$_2$CH$_2$O), 24.7 (CH=C<u>C</u>H$_2$), 22.1 (CH$_3$<u>C</u>H$_2$), 14.0 (<u>C</u>H$_3$CH$_2$)

HRMS (ESI$^+$) m/z / Da = 641.3557, [M+H]$^+$ found, [C$_{35}$H$_{45}$N$_8$O$_4$]$^+$ 641.3558

1.12 2-(6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(1*H*)-one 81



50 % water/*t*-BuOH (1 ml) was degassed by bubbling N$_2$ through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **73** (14.2 mg, 39.8 μmol, 1 eq.) and 2-(6-azidohexyl)-3-hydroxyquinolin-4(1H)-one **19** (11.4 mg, 39.8 μmol, 1 eq.). A similarly degassed solution of $CuSO_4 \cdot 5\,H_2O$ (1.25 mg, 5 μmol, 0.125 eq. 50 mM), THPTA (2.18 mg, 5 μmol, 0.125 eq. 50 mM) and sodium ascorbate (1.98 mg, 10 μmol, 0.25 eq., 100 mM) in water (100 μl) was then added. The mixture was stirred at r.t. under argon for 3 h, then MeOH (1 ml) was added and the reaction mixture was dry-loaded onto SiO_2 and purified by column chromatography (SiO_2, 0-20 % MeOH/CH_2Cl_2). The combined pure fractions were dried with $MgSO_4$ and evaporated under reduced pressure. **81** was obtained as a pale brown amorphous solid (4.7 mg, 7.3 μmol, 18.3 %).

TLC R_f = 0.21 (20 % MeOH/CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 2924.8 (C-H), 2853.4 (C-H), 1660.0 (pyrimidine), 1638.8 (pyrimidine and PQS C=O),

^{1}H NMR (500 MHz, DMSO d$_6$) δ / ppm = 11.53 (br s, 1 H, N<u>H</u>), 8.09 (d, J = 8.0 Hz, 1 H, *ortho* to C=O), 7.83 (s, 1 H, NC<u>H</u>=CCH$_2$), 7.48 - 7.57 (m, 3 H, *para* to C=O, *ortho* to NH and C<u>H</u>N=CNH$_2$), 7.21 (ddd, J = 8.0, 6.3, 1.5 Hz, 1 H, *para* to NH), 6.55 (s, 2 H, *meta* to CH$_2$), 4.28 (t, J = 7.1 Hz, 2 H, C<u>H</u>$_2$N), 3.80 (t, J = 6.2 Hz, 2 H, C<u>H</u>$_2$O), 3.70 (s, 6 H, C<u>H</u>$_3$), 3.53 (d, J = 0.3 Hz, 2 H, CC<u>H</u>$_2$C), 2.73 (t, J = 7.5 Hz, 2 H, HNCC<u>H</u>$_2$), 2.64 (t, J = 7.4 Hz, 2 H, CH=CC<u>H</u>$_2$), 1.80 (quin, J = 7.4 Hz, 2 H, C<u>H</u>$_2$CH$_2$N), 1.73 (quin, J = 7.5 Hz, 2 H, CH=CCH$_2$C<u>H</u>$_2$), 1.66 (quin, J = 7.2 Hz, 2 H, HNCCH$_2$C<u>H</u>$_2$), 1.62 (quin, J = 6.8 Hz, 2 H, C<u>H</u>$_2$CH$_2$O), 1.33 - 1.40 (m, 2 H, HNCCH$_2$CH$_2$C<u>H</u>$_2$), 1.27 - 1.32 (m, 2 H, HNCCH$_2$CH$_2$CH$_2$C<u>H</u>$_2$)

^{13}C NMR (125 MHz, DMSO d_6) δ / ppm = 168.9 (C=O), 162.5 (CC(NH_2)N), 162.5 (NC(NH_2)N), 152.9 (CHNC(NH_2)N), 152.8 (*ipso* to OCH_3), 146.8 (CH=CCH$_2$CH$_2$), 137.7 (COH), 137.3 (*para* to OH), 135.4 (HNC), 135.1 (*para* to CH_2O), 134.8 (*ipso* to CH_2O), 129.9 (*para* to C=O), 124.4 (*ortho* to C=O and *meta* to NH), 122.1 (*ipso* to C=O), 121.5 (*para* to NH), 121.4 (CH=CCH$_2$CH$_2$), 117.7 (*meta* to C=O and *ortho* to NH), 106.2 (CH_2CC(NH_2)=N), 105.8 (*meta* to CH_2O), 71.9 (CH_2CH_2CH$_2$O), 55.8 (OCH_3), 49.0 (CH_2N), 32.8 (CCH_2C), 29.5 (CH_2CH_2N), 29.0 (CH_2CH_2O), 28.1 (HNC$CH_2CH_2CH_2$), 27.9 (HNCCH_2), 27.6 (HNCCH_2CH_2), 25.6 ($CH_2CH_2CH_2$N), 25.4 ($CH_2CH_2CH_2$O), 24.6 (CH=CCH_2CH_2)

HRMS (ESI$^+$) m/z / Da = 643.3365, $[M+H]^+$ found, $[C_{34}H_{43}N_8O_5]^+$ requires 643.3351

2 References

[1] C. Jing and V. W. Cornish. A fluorogenic TMP-tag for high signal-to-background intracellular live cell imaging. *ACS Chemical Biology*, 8(8):1704–12. 2013.

Todo list