

# Click chemistry assembly of G-quadruplex ligands incorporating a diarylurea scaffold and triazole linkers

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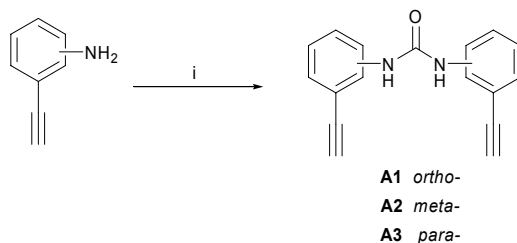
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## **Contents**

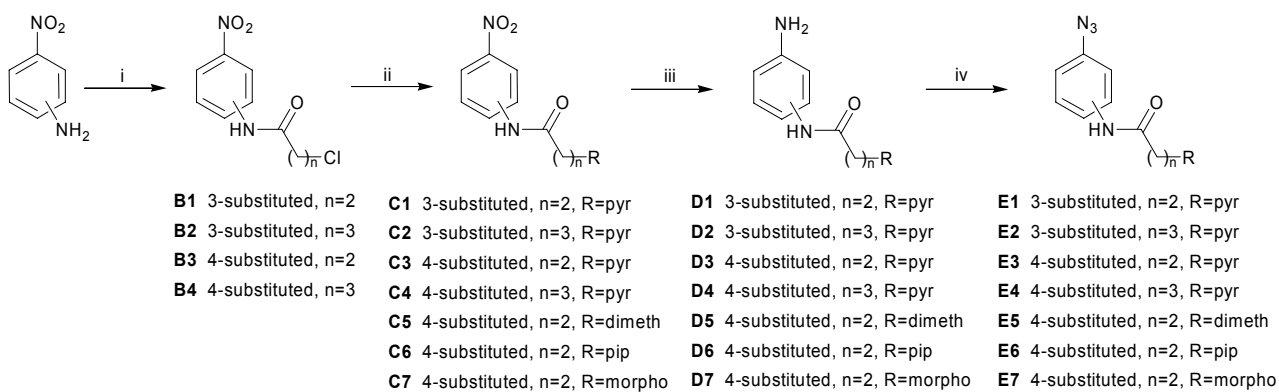
<b>1.0 Synthetic Schemes</b> .....	2
<b>2.0 Chemistry</b> .....	3
2.1 General procedure for the synthesis of the alkyne building blocks (A1-3) .....	4
2.2 General procedure for the synthesis of the azide side chains (E1-7) .....	5
2.3 General procedure for the synthesis of the target ligands 1 – 15 .....	8
<b>3.0 HPLC Purity Analysis</b> .....	20
<b>4.0 Fluorescence Resonance Energy Transfer (FRET) Assays</b> .....	21
4.1 FRET Melting Curves .....	22
<b>5.0 Cell Culture</b> .....	23
<b>6.0 References</b> .....	24

## 1.0 Synthetic Schemes



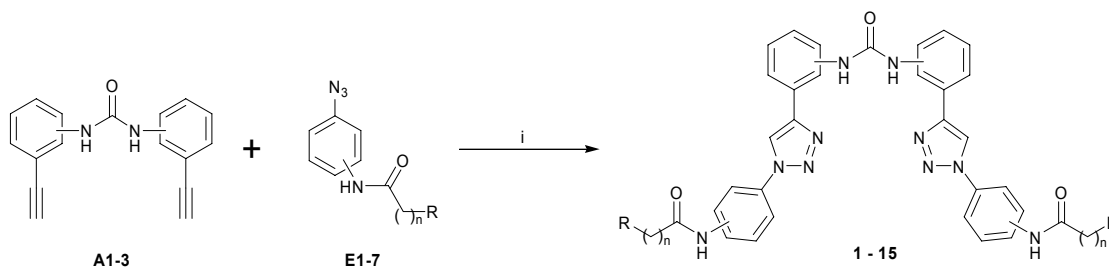
**Scheme 1 - The synthesis of the 1,3-bis(ethynylphenyl)urea building blocks (A1-3).**

i) CDI, THF, reflux (93 – 100%).



**Scheme 2 - The synthesis of the azide side chains (E1-7).**

i)  $\text{Cl}(\text{CH}_2)_{n=2/3}\text{COCl}$ , 50°C (62-100%); (ii) pyrrolidine (pyr)/dimethylamine (dimeth)/piperidine (pip)/morpholino (morpho), neat rt or THF/MeOH 30°C (60-100%); (iii) ammonium formate, 10% Pd/C, MeOH, rt (40-100%); iv)  $^t\text{BuONO}$ , HCl, THF, 0°C then  $\text{NaN}_3$ ,  $\text{dH}_2\text{O}$ , 0°C to rt (26-93%).



**Scheme 3 – The synthesis of the target ligands (1-13).**

i)  $^t\text{BuOH}/\text{dH}_2\text{O}$ ,  $\text{CuSO}_4$ , sodium ascorbate,  $\mu\text{W}$  130°C or rt (56-95%).

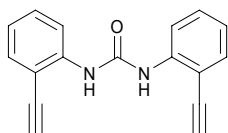
## 2.0 Chemistry

All reagents were reagent grade and purchased from Sigma-Aldrich, Alfa Aesar, Avocado Organics and Lancaster Synthesis and were used as supplied without further purification. Solvents were supplied by BDH, Sigma-Aldrich (anhydrous) and Fisher Scientific (HPLC grade). Microwave reactions were conducted upon a Biotage Initiator Microwave (software v1.1).  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at 295K upon on a Bruker Avance 400 spectrometer at 400 MHz and 100 MHz respectively using the specified deuterated solvent purchased from GOSS Scientific or Sigma-Aldrich. NMR spectra were analysed in MestReC 4.5.6.0 and reported as observed with coupling constants ( $J$ ) in hertz (Hz). Melting points (Mp.) were conducted upon a Bibby Stuart Scientific SMP3 melting point apparatus, where 'dec' indicates decomposition. High resolution accurate mass spectra (HRMS) were conducted upon a Micromass Q-TOF Ultima Global Tandem Mass Spectrometer using electrospray ionization mode and 50% acetonitrile in water and 0.1% formic acid as solvent, and processed using the MassLab 3.2 software. Infrared spectra (IR) were recorded from neat samples upon a Nicolet Smart Golden Gate spectrometer (Avatar 360 FT-IR E.S.P.) and processed using the software OMNIC E.S.P. 5.1. All ligands were purified by semi-preparative reversed-phase HPLC (Semi-Prep HPLC) on a Gilson Chromatograph with a Gilson 215 Liquid Handler, a Gilson 845Z injection module coupled to a Gilson UV/VIS 155 detector and a YMC C18  $5\mu$  (100 x 20 mm) column, using 0.1% formic acid in acetonitrile and 0.1% aqueous formic acid, 5% - 50% organic over 28 minutes (10 ml/min), injected from 5% acetonitrile in 0.1% aqueous formic acid (3 ml). Compounds were isolated by reduction of fraction volumes (to  $\sim 5$  ml), precipitation with 5%  $\text{NH}_3$  (aq.), centrifugation to concentrate the precipitate and freeze drying upon a Savant A160 SpeedVac Concentrator co-evaporating with EtOH (1 ml). This afforded ligand samples of suitable purity for biological assessment as assessed by analytical HPLC (HPLC) which were conducted upon a Gilson Chromatograph with a YMC C18  $5\mu$  (100 x 4.6 mm) column and an Agilent 1100 series Photo Diode array detector using the solvent system specified. Spectra were processed using the Unipoint 5.11 software package, with compound purity and retention time (RT) assessed at 254 nm.

## 2.1 General procedure for the synthesis of the alkyne building blocks (A1-3)

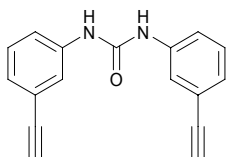
To anhydrous THF under N<sub>2</sub> was added the required ethynylbenzenamine (conc. = 0.4 M) followed by CDI (0.6 equiv.) and the mixture heated at reflux for 4 hours. The solvent was evaporated and the solid residue dissolved in EtOAc (75 ml), washed with 1 M HCl (aq.) (2 x 50 ml), brine (50 ml) and dried over MgSO<sub>4</sub>. If the reaction was incomplete by TLC, a further portion of CDI (0.4 equiv.) was added and reflux continued overnight prior to workup.

### The synthesis of 1,3-bis(2-ethynylphenyl)urea (A1).



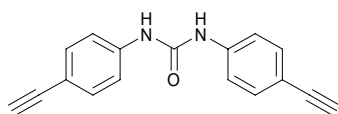
2-ethynylbenzenamine (531 mg, 0.52 ml, 4.52 mmol) was reacted with CDI (692 mg, 4.27 mmol) to yield a beige solid (588 mg, 2.26 mmol, quant.). R<sub>f</sub> 0.41 [20% EtOAc in Hexane]. Mp. 188 – 190°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 9.01 (2H, s, 2NH), 7.94 (2H, d, *J*=7.6 Hz, 2ArH), 7.45 (2H, dd, *J*=7.6, 1.4 Hz, 2ArH), 7.35 (2H, dd, *J*=7.6, 1.4 Hz, 2ArH), 7.03 (2H, dt, *J*=7.6, 1.4 Hz, 2ArH), 4.56 (2H, s, 2C≡CH) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 152.4 (C=O), 140.5 (2ArC), 132.6 (2ArCH), 129.3 (2ArCH), 122.5 (2ArCH), 121.0 (2ArCH), 112.0 (2ArC), 86.7 (2C≡CH), 79.9 (2C≡CH) ppm. IR (neat) ν<sub>max</sub> 3282 (-C≡C) cm<sup>-1</sup>. HRMS *m/z* calc. C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 261.1022, found [M+H]<sup>+</sup> 261.1017.

### The synthesis of 1,3-bis(3-ethynylphenyl)urea (A2).



3-ethynylbenzenamine (1.00 g, 0.89 ml, 8.54 mmol) was reacted with CDI (831 mg, 5.12 mmol) to yield a white solid (1.04 g, 3.98 mmol, 93%). R<sub>f</sub> 0.27 [20% EtOAc in Hexane]. Mp. 222 – 224°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 8.82 (2H, s, 2NH), 7.67 (2H, s, 2ArH), 7.41 (2H, d, *J*=7.9 Hz, 2ArH), 7.29 (2H, t, *J*=7.9 Hz, 2ArH), 7.09 (2H, d, *J*=7.9 Hz, 2ArH), 4.14 (2H, s, 2C≡CH) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 152.3 (C=O), 139.7 (2ArC), 129.1 (2ArCH), 125.1 (2ArC), 122.0 (2ArCH), 121.0 (2ArCH), 118.9 (2ArCH), 83.4 (2C≡CH), 80.2 (2C≡CH) ppm. IR (neat) ν<sub>max</sub> 3284 (-C≡C) cm<sup>-1</sup>. HRMS *m/z* calc. C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 261.1022, found [M+H]<sup>+</sup> 261.1035.

### The synthesis of 1,3-bis(4-ethynylphenyl)urea (A3).



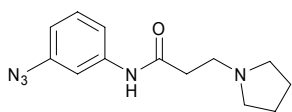
4-ethynylbenzenamine (500 mg, 4.27 mmol) was reacted with CDI (415 mg, 2.56 mmol) to yield a pale yellow solid (556 mg, 2.14 mmol, quant.).  $R_f$  0.19 [20% EtOAc in Hexane]. Mp. > 315°C dec.  $^1\text{H}$  NMR (400 MHz,  $d_6$ -DMSO)  $\delta$  8.92 (2H, s, 2NH), 7.47 (4H, d,  $J=8.7$  Hz, 4ArH), 7.39 (4H, d,  $J=8.7$  Hz, 4ArH), 4.30 (2H, s, 2C $\equiv$ CH) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $d_6$ -DMSO)  $\delta$  151.9 (C=O), 140.0 (2ArC), 132.3 (4ArCH), 117.9 (4ArCH), 114.7 (2ArC), 83.6 (2C $\equiv$ CH), 79.3 (2C $\equiv$ CH) ppm. IR (neat)  $\nu_{\text{max}}$  3270 (-C $\equiv$ C)  $\text{cm}^{-1}$ . HRMS  $m/z$  calc.  $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}$  [M+H] $^+$  261.1022, found [M+H] $^+$  261.1026.

The synthesis of the amine side chains D1-7 and precursors B1-4/C1-7 was achieved by methods analogous to those previously described.<sup>1-3</sup>

### 2.2 General procedure for the synthesis of the azide side chains (E1-7)

The required aniline (D1-7) was dissolved in THF (100 ml) and cooled to 0°C prior to HCl (12 M, 5.5 equiv.) and  $^t\text{BuONO}$  (2.5 equiv.) being added and the mixture stirred at 0°C for 1 hour.  $\text{NaN}_3$  (3 equiv.) was added followed by CAUTIOUS addition of distilled water (10 ml), and the mixture allowed to warm to room temperature and stirred overnight. The reaction was quenched with sat.  $\text{NaHCO}_3$  (aq.) (60 ml) and the THF evaporated before extraction of the product into EtOAc (3 x 100 ml), washing with brine and drying over  $\text{MgSO}_4$ .

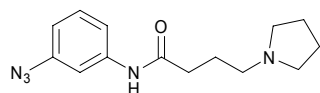
### The synthesis of *N*-(3-azidophenyl)-3-(pyrrolidin-1-yl)propanamide (E1).



*N*-(3-aminophenyl)-3-(pyrrolidin-1-yl)propanamide (D1) (1.70 g, 7.29 mmol) was reacted to yield an orange oil (1.75 g, 6.74 mmol, 93%).  $R_f$  0.28 [10% MeOH in DCM].  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  11.35 (1H, s, NH), 7.35 (1H, t,  $J=2.1$  Hz, ArH), 7.25 (1H, t,  $J=8.1$  Hz, ArH), 7.14 (1H, ddd,  $J=8.1, 2.1, 0.9$  Hz, ArH), 6.73 (1H, ddd,  $J=8.1, 2.1, 0.9$  Hz, ArH), 2.86 (2H, t,  $J=5.9$  Hz, CH $_2$ ), 2.69 (4H, m, 2CH $_2$ ), 2.53 (2H, t,  $J=5.9$  Hz, CH $_2$ ), 1.90 (4H, m, 2CH $_2$ ) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.9 (C=O), 140.6 (ArC), 140.3 (ArC), 129.9 (ArCH), 115.9 (ArCH), 113.9 (ArCH), 110.2 (ArCH), 53.0 (2CH $_2$ ), 51.2 (CH $_2$ ),

34.5 ( $\underline{\text{C}}\underline{\text{H}}_2$ ), 23.6 ( $2\underline{\text{C}}\underline{\text{H}}_2$ ) ppm. IR (neat)  $\nu_{\text{max}}$  2106 ( $-\text{N}_3$ )  $\text{cm}^{-1}$ . HRMS  $m/z$  calc.  $\text{C}_{13}\text{H}_{17}\text{N}_5\text{O}$   $[\text{M}+\text{H}]^+$  260.1511, found  $[\text{M}+\text{H}]^+$  260.1512.

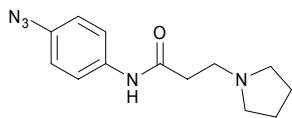
**The synthesis of *N*-(3-azidophenyl)-4-(pyrrolidin-1-yl)propanamide (E2).**



*N*-(3-aminophenyl)-4-(pyrrolidin-1-yl)propanamide (D2)

(2.05 g, 8.30 mmol) was reacted to yield a yellow oil (1.80 g, 6.60 mmol, 80%).  $R_f$  0.15 [10% MeOH in DCM].  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  10.08 (1H, s,  $\underline{\text{N}}\underline{\text{H}}$ ), 7.37 (1H, s,  $\text{Ar}\underline{\text{H}}$ ), 7.31 (1H, t,  $J=7.8$  Hz,  $\text{Ar}\underline{\text{H}}$ ), 7.28 (1H, d,  $J=7.8$  Hz,  $\text{Ar}\underline{\text{H}}$ ), 6.73 (1H, d,  $J=7.8$  Hz,  $\text{Ar}\underline{\text{H}}$ ), 2.64 (6H, m,  $3\underline{\text{C}}\underline{\text{H}}_2$ ), 2.52 (2H, t,  $J=6.6$  Hz,  $\underline{\text{C}}\underline{\text{H}}_2$ ), 1.90 (2H, m,  $\underline{\text{C}}\underline{\text{H}}_2$ ), 1.86 (4H, m,  $2\underline{\text{C}}\underline{\text{H}}_2$ ) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  171.8 ( $\underline{\text{C}}=\underline{\text{O}}$ ), 140.7 ( $\text{Ar}\underline{\text{C}}$ ), 140.4 ( $\text{Ar}\underline{\text{C}}$ ), 130.0 ( $\text{Ar}\underline{\text{C}}\underline{\text{H}}$ ), 115.9 ( $\text{Ar}\underline{\text{C}}\underline{\text{H}}$ ), 114.2 ( $\text{Ar}\underline{\text{C}}\underline{\text{H}}$ ), 110.2 ( $\text{Ar}\underline{\text{C}}\underline{\text{H}}$ ), 56.0 ( $\underline{\text{C}}\underline{\text{H}}_2$ ), 54.0 ( $2\underline{\text{C}}\underline{\text{H}}_2$ ), 37.1 ( $\underline{\text{C}}\underline{\text{H}}_2$ ), 23.8 ( $\underline{\text{C}}\underline{\text{H}}_2$ ), 23.6 ( $2\underline{\text{C}}\underline{\text{H}}_2$ ) ppm. IR (neat)  $\nu_{\text{max}}$  2104 ( $-\text{N}_3$ )  $\text{cm}^{-1}$ . HRMS  $m/z$  calc.  $\text{C}_{14}\text{H}_{19}\text{N}_5\text{O}$   $[\text{M}+\text{H}]^+$  274.1668, found  $[\text{M}+\text{H}]^+$  274.1661.

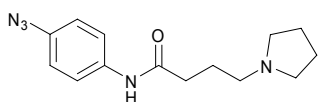
**The synthesis of *N*-(4-azidophenyl)-3-(pyrrolidin-1-yl)propanamide (E3).**



*N*-(4-aminophenyl)-3-(pyrrolidin-1-yl)propanamide (D3)

(1.70 g, 7.29 mmol) was reacted to yield a brown solid (1.49 g, 5.74 mmol, 79%).  $R_f$  0.33 [10% MeOH in DCM]. Mp. 67 – 69°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  11.19 (1H, s,  $\underline{\text{N}}\underline{\text{H}}$ ), 7.50 (2H, d,  $J=8.9$  Hz,  $2\text{Ar}\underline{\text{H}}$ ), 6.96 (2H, d,  $J=8.9$  Hz,  $2\text{Ar}\underline{\text{H}}$ ), 2.88 (2H, t,  $J=6.0$  Hz,  $\underline{\text{C}}\underline{\text{H}}_2$ ), 2.71 (4H, m,  $2\underline{\text{C}}\underline{\text{H}}_2$ ), 2.56 (2H, t,  $J=6.0$  Hz,  $\underline{\text{C}}\underline{\text{H}}_2$ ), 1.91 (4H, m,  $2\underline{\text{C}}\underline{\text{H}}_2$ ) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.5 ( $\underline{\text{C}}=\underline{\text{O}}$ ), 135.9 ( $\text{Ar}\underline{\text{C}}$ ), 134.8 ( $\text{Ar}\underline{\text{C}}$ ), 120.9 ( $2\text{Ar}\underline{\text{C}}\underline{\text{H}}$ ), 119.3 ( $2\text{Ar}\underline{\text{C}}\underline{\text{H}}$ ), 53.1 ( $2\underline{\text{C}}\underline{\text{H}}_2$ ), 51.2 ( $\underline{\text{C}}\underline{\text{H}}_2$ ), 34.4 ( $\underline{\text{C}}\underline{\text{H}}_2$ ), 23.6 ( $2\underline{\text{C}}\underline{\text{H}}_2$ ) ppm. IR (neat)  $\nu_{\text{max}}$  2106 ( $-\text{N}_3$ )  $\text{cm}^{-1}$ . HRMS  $m/z$  calc.  $\text{C}_{13}\text{H}_{17}\text{N}_5\text{O}$   $[\text{M}+\text{H}]^+$  260.1511, found  $[\text{M}+\text{H}]^+$  260.1516.

**The synthesis of *N*-(4-azidophenyl)-4-(pyrrolidin-1-yl)propanamide (E4).**

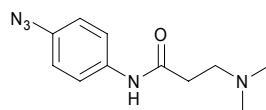


*N*-(4-aminophenyl)-4-(pyrrolidin-1-yl)propanamide (D4)

(1.89 g, 7.64 mmol) was reacted to yield a brown oil which solidified on standing (1.44 g, 5.27 mmol, 69%).  $R_f$  0.20 [10% MeOH in DCM]. Mp 53 – 55°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.90 (1H, s,  $\underline{\text{N}}\underline{\text{H}}$ ),

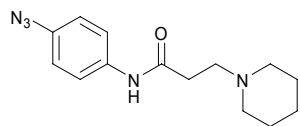
7.52 (2H, d,  $J=8.8$  Hz, ArH), 6.95 (2H, d,  $J=8.8$  Hz, ArH), 2.65 (6H, m, 3CH<sub>2</sub>), 2.52 (2H, t,  $J=6.6$  Hz, CH<sub>2</sub>), 1.91 (2H, m, CH<sub>2</sub>), 1.86 (4H, m, 2CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.4 (C=O), 135.9 (ArC), 135.0 (ArC), 121.0 (2ArCH), 119.3 (2ArCH), 55.8 (CH<sub>2</sub>), 53.9 (2CH<sub>2</sub>), 36.8 (CH<sub>2</sub>), 23.7 (CH<sub>2</sub>), 23.5 (2CH<sub>2</sub>) ppm. IR (neat)  $\nu_{\max}$  2110 (-N<sub>3</sub>) cm<sup>-1</sup>. HRMS  $m/z$  calc. C<sub>14</sub>H<sub>19</sub>N<sub>5</sub>O [M+H]<sup>+</sup> 274.1668, found [M+H]<sup>+</sup> 274.1671.

**The synthesis of *N*-(4-azidophenyl)-3-(dimethylamino)propanamide (E5).**



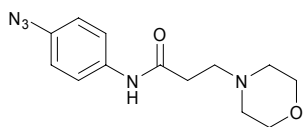
*N*-(4-aminophenyl)-3-(dimethylamino)propanamide (**D5**) (1.59 g, 7.66 mmol) was reacted to yield a brown oil (1.52 g, 6.52 mmol, 85%).  $R_f$  0.19 [MeOH]. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.92 (1H, s, NH), 7.50 (2H, d,  $J=8.9$  Hz, 2ArH), 6.94 (2H, d,  $J=8.9$  Hz, 2ArH), 2.65 (2H, m CH<sub>2</sub>), 2.49 (2H, m, CH<sub>2</sub>), 2.37 (6H, s, 2CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 170.6 (C=O), 135.9 (ArC), 135.0 (ArC), 121.2 (2ArCH), 119.4 (2ArCH), 55.1 (CH<sub>2</sub>), 44.4 (2CH<sub>3</sub>), 33.4 (CH<sub>2</sub>) ppm. IR (neat)  $\nu_{\max}$  2107 (-N<sub>3</sub>) cm<sup>-1</sup>. HRMS  $m/z$  calc. C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O [M+H]<sup>+</sup> 234.1349, found [M+H]<sup>+</sup> 234.1358.

**The synthesis of *N*-(4-azidophenyl)-3-(piperidin-1-yl)propanamide (E6).**



*N*-(4-aminophenyl)-3-(piperidin-1-yl)propanamide (**D6**) (1.75 g, 7.09 mmol) was reacted to yield a brown oil (1.38 g). Purification was achieved by flash chromatography [5% - 20% MeOH in DCM] to yield a brown oil (500 mg, 1.83 mmol, 26%).  $R_f$  0.32 [MeOH]. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 11.40 (1H, s, NH), 7.54 (2H, d,  $J=8.8$  Hz, 2ArH), 6.97 (2H, d,  $J=8.8$  Hz, 2ArH), 2.66 (2H, m CH<sub>2</sub>), 2.54 (4H, m, 2CH<sub>2</sub>), 2.50 (2H, m, CH<sub>2</sub>), 1.69 (4H, m, 2CH<sub>2</sub>), 1.55 (2H, m, CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 170.7 (C=O), 136.1 (ArC), 134.8 (ArC), 120.8 (2ArCH), 119.4 (2ArCH), 54.3 (CH<sub>2</sub>), 53.6 (2CH<sub>2</sub>), 32.4 (CH<sub>2</sub>), 26.2 (2CH<sub>2</sub>), 24.2 (CH<sub>2</sub>) ppm. IR (neat)  $\nu_{\max}$  2115 (-N<sub>3</sub>) cm<sup>-1</sup>. HRMS  $m/z$  calc. C<sub>14</sub>H<sub>19</sub>N<sub>5</sub>O [M+H]<sup>+</sup> 274.1662, found [M+H]<sup>+</sup> 274.1673.

**The synthesis of *N*-(4-azidophenyl)-3-morpholinopropanamide (**E7**).**



*N*-(4-aminophenyl)-3-morpholinopropanamide (**D7**) (1.77 g, 7.09 mmol) was reacted to yield a brown oil (1.63 g). Purification was achieved by flash chromatography [1% - 5% MeOH in DCM] to yield a brown oil which solidified on standing (841 mg, 3.06 mmol, 43%).  $R_f$  0.50 [5% MeOH in DCM]. Mp. 67 – 69°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  10.76 (1H, s, NH), 7.52 (2H, d,  $J=8.8$  Hz, 2ArH), 6.97 (2H, d,  $J=8.8$  Hz, 2ArH), 3.81 (4H, t,  $J=4.6$  Hz, 2CH<sub>2</sub>), 2.73 (2H, m, CH<sub>2</sub>), 2.61 (4H, m, 2CH<sub>2</sub>), 2.53 (2H, m, CH<sub>2</sub>) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.1 (C=O), 135.7 (ArC), 135.1 (ArC), 120.8 (2ArCH), 119.5 (2ArCH), 67.0 (2CH<sub>2</sub>), 54.1 (CH<sub>2</sub>), 52.8 (2CH<sub>2</sub>), 32.2 (CH<sub>2</sub>) ppm. IR (neat)  $\nu_{\text{max}}$  2109 ( $-\text{N}_3$ )  $\text{cm}^{-1}$ . HRMS  $m/z$  calc.  $\text{C}_{13}\text{H}_{17}\text{N}_5\text{O}_2$   $[\text{M}+\text{H}]^+$  276.1455, found  $[\text{M}+\text{H}]^+$  276.1469.

**2.3 General procedure for the synthesis of the target ligands 1 – 13**

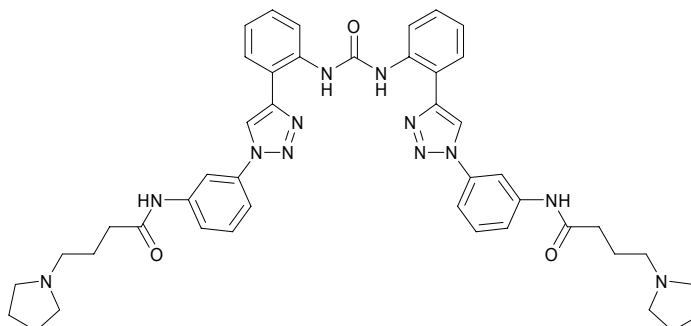
**a) Microwave irradiation.** The required azide (**E1-7**, 0.58 mmol, 3 equiv.) was dissolved in  $t$ BuOH (2.5 ml) and distilled water (2.5 ml) and to this was added the required 1,3-bis(ethynylphenyl)urea (**A1-3**, 50 mg, 0.2 mmol), sodium ascorbate (19 mg, 0.10 mmol, 0.5 equiv.) and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (2 mg, 10  $\mu\text{mol}$ , 5 mol%) and the mixture heated at 130°C under microwave irradiation for 30 minutes. The reaction was cooled to 45°C, diluted with distilled water (5 ml) and cooled on ice for 10 minutes. The crude product was isolated by filtration, washed with ice cold distilled water (2 ml),  $\text{Et}_2\text{O}$  (2 x 2 ml) and oven dried overnight, unless stated otherwise.

**b) Ambient Temperature.** The reaction was conducted as described above except that  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (4 mg, 20  $\mu\text{mol}$ , 10 mol%) was added and the reaction stirred at room temperature overnight.



**The synthesis of 1,3-bis-(2-(1-(3-(4-(pyrrolidin-1-yl)butanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (1).**

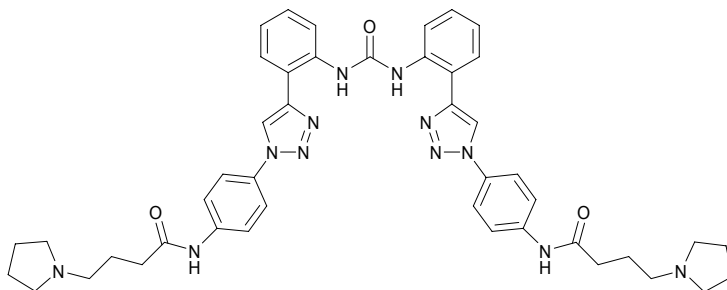
*N*-(3-azidophenyl)-4-(pyrrolidin-1-yl)propanamide (**E2**) (157 mg, 0.58 mmol) was reacted with 1,3-bis(2-ethynylphenyl)urea (**A1**) (50 mg, 0.19 mmol). No precipitation was observed, hence 5% NH<sub>3</sub> (aq.) (10 ml) was



added and the precipitate isolated by filtration and washed with ice cold distilled water (2 ml) and Et<sub>2</sub>O (2 x 2 ml) prior to oven drying as a brown solid (86 mg, 0.11 mmol, 56%). Semi-Prep HPLC was conducted to yield a brown solid. HPLC (method A) 98%, RT 22.35 minutes. Mp. 128 – 130°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.29 (2H, s, 2NH; amide), 9.78 (2H, s, 2NH; urea), 9.16 (2H, s, 2C=CH), 8.37 (2H, s, 2ArH), 8.00 (2H, d, *J*=7.8 Hz, 2ArH), 7.85 (2H, dd, *J*=7.8, 1.2 Hz, 2ArH), 7.63 (2H, d, *J*=8.1 Hz, 2ArH), 7.54 (2H, d, *J*=8.1 Hz, 2ArH), 7.48 (2H, t, *J*=8.1 Hz, 2ArH), 7.37 (2H, dt, *J*=7.8, 1.2 Hz, 2ArH), 7.20 (2H, t, *J*=7.8 Hz, 2ArH), 2.60 (8H, m, 4CH<sub>2</sub>), 2.58 (4H, t, *J*=7.5 Hz, 2CH<sub>2</sub>), 2.41 (4H, t, *J*=7.3 Hz, 2CH<sub>2</sub>), 1.80 (4H, m, 2CH<sub>2</sub>), 1.71 (8H, m, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 171.3 (2C=O; amide), 153.1 (C=O; urea), 145.9 (2C=CH), 140.5 (2ArC), 136.6 (2ArC), 136.2 (2ArC), 130.0 (2ArCH), 128.5 (2ArCH), 128.2 (2ArCH), 123.2 (2ArCH), 123.2 (2ArCH), 121.1 (2C=CH), 120.4 (2ArC), 119.0 (2ArCH), 114.5 (2ArCH), 110.7 (2ArCH), 54.5 (2CH<sub>2</sub>), 53.2 (4CH<sub>2</sub>), 34.0 (2CH<sub>2</sub>), 23.3 (2CH<sub>2</sub>), 22.9 (4CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 807.4202, found [M+H]<sup>+</sup> 807.4196.

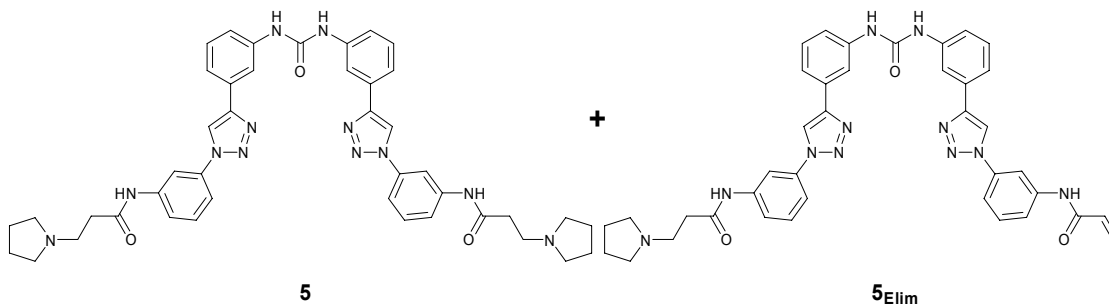
**The synthesis of 1,3-bis-(2-(1-(4-(4-(pyrrolidin-1-yl)butanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (2).**

*N*-(4-azidophenyl)-4-(pyrrolidin-1-yl)propanamide (**E4**) (154 mg, 0.58 mmol) was reacted with 1,3-bis(2-ethynylphenyl)urea (**A1**) (50 mg, 0.19 mmol). The



precipitate was isolated by centrifugation and washed with ice cold distilled water (2 ml) prior to freeze drying to yield a red solid (91 mg, 0.11 mmol, 59%). Semi-Prep HPLC was conducted to yield a red solid. HPLC (method A) 97%, RT 23.05 minutes. Mp. 130 – 132°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.24 (2H, s, 2NH; amide), 9.79 (2H, s, 2NH; urea), 9.14 (2H, s, 2C=CH), 8.02 (2H, d, *J*=7.8 Hz, 2ArH), 7.86 (4H, d, *J*=9.1 Hz, 4ArH), 7.84 (6H, m, 6ArH), 7.37 (2H, t, *J*=7.8 Hz, 2ArH), 7.19 (2H, t, *J*=7.8 Hz, 2ArH), 2.63 (8H, m, 4CH<sub>2</sub>), 2.60 (4H, t, *J*=7.2 Hz, 2CH<sub>2</sub>), 2.42 (4H, t, *J*=7.1 Hz, 2CH<sub>2</sub>), 1.82 (4H, m, 2CH<sub>2</sub>), 1.73 (8H, m, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 171.1 (2C=O; amide), 153.0 (C=O; urea), 145.8 (2C=CH), 139.7 (2ArC), 136.2 (2ArC), 131.2 (2ArC), 128.4 (2ArCH), 128.1 (2ArCH), 123.1 (2ArCH), 123.0 (2ArCH), 120.9 (2C=CH), 120.7 (4ArCH), 120.3 (2ArC), 119.6 (4ArCH), 54.5 (2CH<sub>2</sub>), 53.2 (4CH<sub>2</sub>), 34.0 (2CH<sub>2</sub>), 23.3 (2CH<sub>2</sub>), 22.9 (4CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 807.4202, found [M+H]<sup>+</sup> 807.4202.

The synthesis of 1,3-bis(3-(1-(3-(3-(pyrrolidin-1-yl)propanamido)phenyl)-1*H*-1,2,3-triazol-4-yl)phenyl)urea (**3**) and 1-(3-(1-(3-(3-(pyrrolidin-1-yl)propanamido)phenyl)-1*H*-1,2,3-triazol-4-yl)phenyl)-3-(3-(1-(3-(acrylamido)phenyl)-1*H*-1,2,3-triazol-4-yl)phenyl)urea (**3<sub>Elim</sub>**).



*N*-(3-azidophenyl)-3-(pyrrolidin-1-yl)propanamide (**E1**) (150 mg, 0.58 mmol) was reacted with 1,3-bis(3-ethynylphenyl)urea (**A2**) (50 mg, 0.19 mmol) to yield a yellow powder (129 mg, 0.17 mmol, 86%). Semi-Prep HPLC was conducted to yield (**3**) as a cream powder. HPLC (method A) 97%, RT 22.26 minutes. Mp. 227 – 229°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.42 (2H, s, 2NH; amide), 9.27 (2H, s, 2NH; urea), 9.25 (2H, s, 2C=CH), 8.38 (2H, s, 2ArH), 8.24 (2H, s, 2ArH), 7.63 (4H, m, 4ArH), 7.55 (4H, m, 4ArH), 7.47 (2H, d, *J*=8.2 Hz, 2ArH), 7.40 (2H, t, *J*=8.2 Hz, 2ArH), 2.80 (4H, t, *J*=7.0 Hz, 2CH<sub>2</sub>), 2.56 (12H, m, 6CH<sub>2</sub>), 1.70 (8H, m, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 170.5 (2C=O; amide), 152.7 (C=O; urea), 147.4 (2C=CH), 140.5 (2ArC), 140.4 (2ArC), 136.9 (2ArC), 130.7 (2ArC), 130.2 (2ArCH), 129.4 (2ArCH), 119.6 (2C=CH), 119.2 (2ArCH), 118.9 (2ArCH), 118.2 (2ArCH), 115.1 (2ArCH), 114.4 (2ArCH), 110.5 (2ArCH), 53.4 (4CH<sub>2</sub>), 51.3 (2CH<sub>2</sub>), 35.9 (2CH<sub>2</sub>), 23.1 (4CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 779.3894, found [M+H]<sup>+</sup> 779.3875; (**3<sub>Elim</sub>**) as a cream solid. HPLC (method A) 87%, RT 26.17 minutes. Mp. > 297°C dec. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.49 (1H, s, NH; amide), 10.40 (1H, s, NH; amide), 9.24 (1H, s, NH; urea), 9.22 (1H, s, NH; urea), 9.13 (2H, s, 2C=CH triazole), 8.43 (1H, t, *J*=1.9 Hz, ArH), 8.37 (1H, t, *J*=1.9 Hz, ArH), 8.22 (2H, m, 2ArH), 7.75 (1H, d, *J*=8.0 Hz, ArH), 7.65 (2H, 2ArH), 7.60 (1H, s, ArH), 7.55 (4H, m, 4ArH), 7.45 (2H, d, *J*=8.0 Hz, 2ArH), 7.41 (2H, t, *J*=8.0 Hz, 2ArH), 6.48 (1H, dd, *J*=17.0, 10.1 Hz, CH=CH<sub>2</sub>), 6.33 (1H, dd, *J*=17.0, 1.9 Hz, CH=CH<sub>2</sub>), 5.82 (1H, dd, *J*=10.1, 1.9 Hz, CH=CH<sub>2</sub>), 2.82 (2H, t, *J*=7.0 Hz, CH<sub>2</sub>),

2.57 (6H, m, 3CH<sub>2</sub>), 1.71 (4H, m, 2CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>39</sub>H<sub>37</sub>N<sub>11</sub>O<sub>3</sub> [M+H]<sup>+</sup> 708.3159, found [M+H]<sup>+</sup> 708.3160.

**The synthesis of 1,3-bis(3-(1-(3-(4-(pyrrolidin-1-yl)butanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (4).**

*N*-(3-azidophenyl)-4-

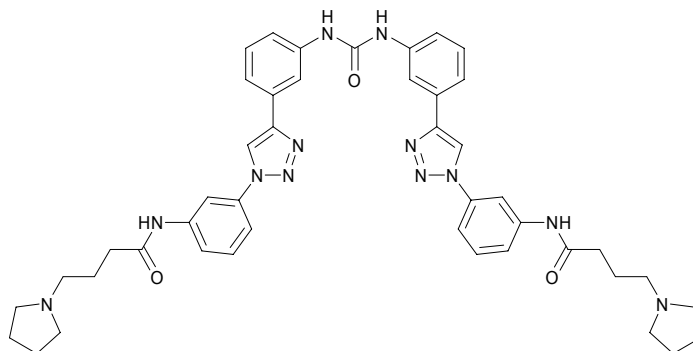
(pyrrolidin-1-yl)propanamide

(E2) (157 mg, 0.58 mmol) was

reacted with 1,3-bis(3-

ethynylphenyl)urea (A2) (50

mg, 0.19 mmol) to yield a



yellow solid (133 mg, 0.17 mmol, 86%). Semi-Prep HPLC was conducted to yield a

cream powder. HPLC (method A) 94%, RT 22.83 minutes. Mp. 232 – 234°C. <sup>1</sup>H NMR

(400 MHz, *d*<sub>6</sub>-DMSO) δ 10.29 (2H, s, 2NH; amide), 9.45 (2H, s, 2NH; urea), 9.24 (2H, s,

2C=CH), 8.39 (2H, s, 2ArH), 8.25 (2H, s, 2ArH), 7.66 (2H, d, *J*=8.5 Hz, 2ArH), 7.62

(2H, d, *J*=8.5 Hz, 2ArH), 7.55 (4H, m, 4ArH), 7.48 (2H, d, *J*=7.7 Hz, 2ArH), 7.41 (2H, t,

*J*=7.7 Hz, 2ArH), 2.54 (12H, m, 6CH<sub>2</sub>), 2.43 (4H, t, *J*=7.3 Hz, 2CH<sub>2</sub>), 1.81 (4H, m,

2CH<sub>2</sub>), 1.70 (8H, m, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 171.5 (2C=O;

amide), 152.7 (C=O; urea), 147.4 (2C=CH), 140.6 (2ArC), 140.5 (2ArC), 136.9 (2ArC),

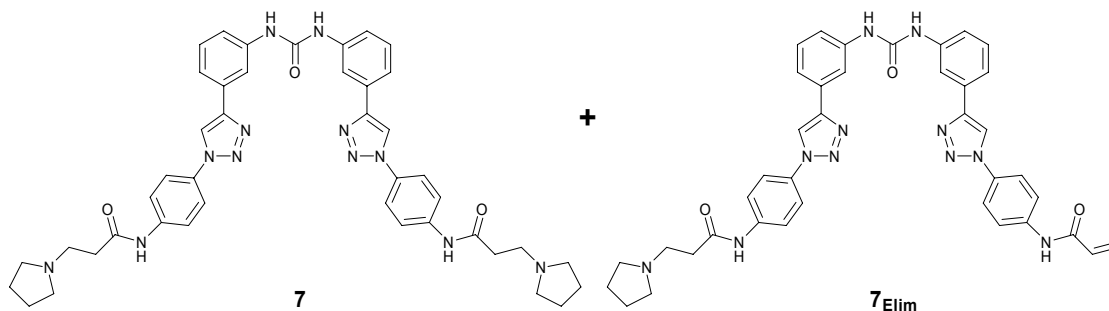
130.7 (2ArC), 130.1 (2ArCH), 129.3 (2ArCH), 119.5 (2C=CH), 119.1 (2ArCH), 118.9

(2ArCH), 118.2 (2ArCH), 115.1 (2ArCH), 114.3 (2ArCH), 110.5 (2ArCH), 54.8 (2CH<sub>2</sub>),

53.4 (4CH<sub>2</sub>), 34.3 (2CH<sub>2</sub>), 23.8 (2CH<sub>2</sub>), 23.0 (4CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>45</sub>H<sub>50</sub>N<sub>12</sub>O<sub>3</sub>

[M+H]<sup>+</sup> 807.4207, found [M+H]<sup>+</sup> 807.4209.

The synthesis of 1,3-bis(3-(1-(4-(3-(pyrrolidin-1-yl)propanamido)phenyl)-1*H*-1,2,3-triazol-4-yl)phenyl)urea (**5**) and 1-(3-(1-(4-(3-(pyrrolidin-1-yl)propanamido)phenyl)-1*H*-1,2,3-triazol-4-yl)phenyl)-3-(3-(1-(4-(acrylamido)phenyl)-1*H*-1,2,3-triazol-4-yl)phenyl)urea (**5<sub>Elim</sub>**).



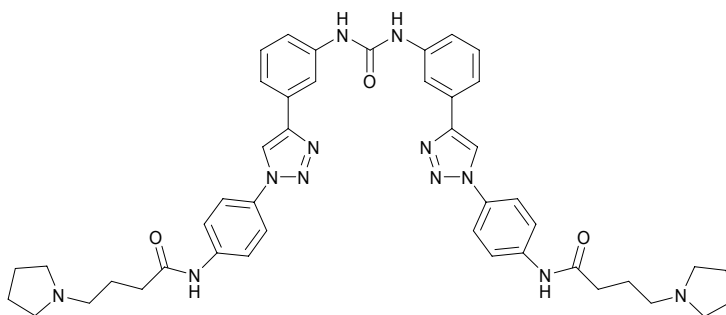
*N*-(4-azidophenyl)-3-(pyrrolidin-1-yl)propanamide (**E3**) (150 mg, 0.58 mmol) was reacted with 1,3-bis(3-ethynylphenyl)urea (**A2**) (50 mg, 0.19 mmol) to yield a dark green solid (130 mg, 0.17 mmol, 87%). Semi-Prep HPLC was conducted to yield (**5**) as a pale yellow solid. HPLC (method A) 99%, RT 22.24 minutes. Mp. 228 – 230°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.38 (2H, s, 2NH; amide), 9.25 (2H, s, 2NH; urea), 9.20 (2H, s, 2C=CH), 8.20 (2H, s, 2ArH), 7.92 (4H, d, *J*=9.0 Hz, 4ArH), 7.82 (4H, d, *J*=9.0 Hz, 4ArH), 7.53 (2H, d, *J*=7.9 Hz, 2ArH), 7.47 (2H, d, *J*=7.9 Hz, 2ArH), 7.40 (2H, t, *J*=7.9 Hz, 2ArH), 2.80 (4H, t, *J*=7.0 Hz, 2CH<sub>2</sub>), 2.55 (12H, m, 6CH<sub>2</sub>), 1.71 (8H, m, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 170.3 (2C=O; amide), 152.6 (C=O; urea), 147.2 (2C=CH), 140.4 (2ArC), 139.5 (2ArC), 131.7 (2ArC), 130.8 (2ArC), 129.4 (2ArCH), 120.7 (4ArCH), 119.7 (4ArCH), 119.4 (2C=CH), 119.2 (2ArCH), 118.1 (2ArCH), 115.0 (2ArCH), 53.4 (4CH<sub>2</sub>), 51.3 (2CH<sub>2</sub>), 35.8 (2CH<sub>2</sub>), 23.1 (4CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 779.3894, found [M+H]<sup>+</sup> 779.3898; (**5<sub>Elim</sub>**) as a cream solid. HPLC (method A) 95%, RT 25.91 minutes. Mp. > 259°C dec. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.42 (1H, s, NH; amide), 10.35 (1H, s, NH; amide), 9.20 (1H, s, NH; urea), 9.18 (1H, s, NH; urea), 9.14 (2H, s, 2C=CH triazole), 8.19 (2H, s, 2ArH), 7.95 (2H, d, *J*=9.2 Hz, 2ArH), 7.91 (2H, d, *J*=9.0 Hz, 2ArH), 7.91 (2H, d, *J*=9.2 Hz, 2ArH), 7.82 (2H, d, *J*=9.0 Hz, 2ArH), 7.53 (2H, d, *J*=7.7 Hz, 2ArH), 7.47 (2H, d, *J*=7.7 Hz, 2ArH), 7.41 (2H, t, *J*=7.7 Hz, 2ArH), 6.48 (1H, dd, *J*=17.0, 10.1 Hz, CH=CH<sub>2</sub>), 6.31 (1H, dd, *J*=17.0, 2.0 Hz, CH=CH<sub>2</sub>), 5.81 (1H, dd, *J*=10.1, 2.0 Hz, CH=CH<sub>2</sub>), 2.81 (2H, t, *J*=6.9 Hz, CH<sub>2</sub>),

2.56 (6H, m, 3CH<sub>2</sub>), 1.72 (4H, m, 2CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>39</sub>H<sub>37</sub>N<sub>11</sub>O<sub>3</sub> [M+H]<sup>+</sup> 708.3159, found [M+H]<sup>+</sup> 708.3142.

**The synthesis of 1,3-bis(3-(1-(4-(4-(pyrrolidin-1-yl)butanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (6).**

*N*-(4-azidophenyl)-4-(pyrrolidin-1-yl)propanamide (**E4**) (157 mg, 0.58 mmol)

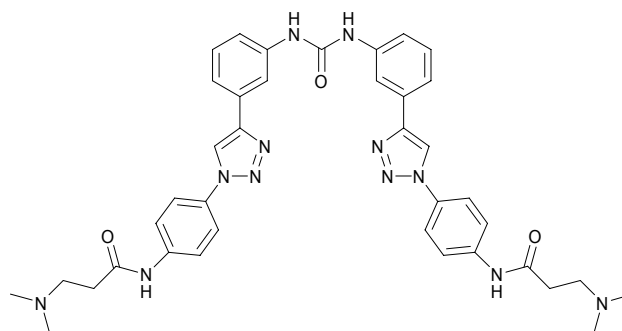
was reacted with 1,3-bis(3-ethynylphenyl)urea (**A2**) (50 mg, 0.19 mmol) to yield a brown solid (142 mg, 0.18



mmol, 92%). Semi-Prep HPLC was conducted to yield a cream powder. HPLC (method A) 99%, RT 22.61 minutes. Mp. 234 – 236°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.23 (2H, s, 2NH; amide), 9.42 (2H, s, 2NH; urea), 9.20 (2H, s, 2C=CH), 8.22 (2H, s, 2ArH), 7.92 (4H, d, *J*=9.0 Hz, 4ArH), 7.83 (4H, d, *J*=9.0 Hz, 4ArH), 7.53 (2H, d, *J*=8.0 Hz, 2ArH), 7.48 (2H, d, *J*=8.0 Hz, 2ArH), 7.41 (2H, t, *J*=8.0 Hz, 2ArH), 2.53 (12H, m, 6CH<sub>2</sub>), 2.42 (4H, t, *J*=7.3 Hz, 2CH<sub>2</sub>), 1.81 (4H, m, 2CH<sub>2</sub>), 1.71 (8H, m, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 171.4 (2C=O; amide), 152.7 (C=O; urea), 147.3 (2C=CH), 140.5 (2ArC), 139.6 (2ArC), 131.6 (2ArC), 130.8 (2ArC), 129.3 (2ArCH), 120.6 (4ArCH), 119.7 (4ArCH), 119.3 (2C=CH), 119.1 (2ArCH), 118.1 (2ArCH), 115.1 (2ArCH), 54.9 (2CH<sub>2</sub>), 53.4 (4CH<sub>2</sub>), 34.3 (2CH<sub>2</sub>), 23.8 (2CH<sub>2</sub>), 23.0 (4CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>45</sub>H<sub>50</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 807.4207, found [M+H]<sup>+</sup> 807.4179.

**The synthesis of 1,3-bis(3-(1-(4-(3-(dimethylamino)propanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (7).**

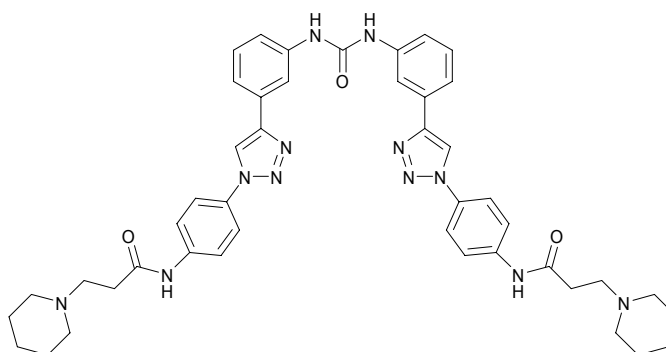
*N*-(4-azidophenyl)-3-(dimethylamino)propanamide (**E5**) (135 mg, 0.58 mmol) was reacted with 1,3-bis(3-ethynylphenyl)urea (**A2**) (50 mg, 0.19 mmol) to yield a brown solid (127 mg, 0.17 mmol,



87%). Semi-Prep HPLC was conducted to yield an off white solid. HPLC (method A) 98%, RT 20.76 minutes. Mp. 265 – 267°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.31 (2H, s, 2NH; amide), 9.19 (2H, s, 2C=CH), 9.00 (2H, s, 2NH; urea), 8.19 (2H, s, 2ArH), 7.92 (4H, d, *J*=9.0 Hz, 4ArH), 7.83 (4H, d, *J*=9.0 Hz, 4ArH), 7.54 (2H, d, *J*=7.9 Hz, 2ArH), 7.47 (2H, d, *J*=7.9 Hz, 2ArH), 7.42 (2H, t, *J*=7.9 Hz, 2ArH), 2.61 (4H, t, *J*=6.8 Hz, 2CH<sub>2</sub>), 2.51 (4H, t, *J*=6.8 Hz, 2CH<sub>2</sub>), 2.21 (12H, s, 4CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 170.5 (2C=O; amide), 152.6 (C=O; urea), 147.2 (2C=CH), 140.3 (2ArC), 139.5 (2ArC), 131.7 (2ArC), 130.9 (2ArC), 129.4 (2ArCH), 120.6 (4ArCH), 119.7 (4ArCH), 119.4 (2C=CH), 119.2 (2ArCH), 118.1 (2ArCH), 115.1 (2ArCH), 54.9 (2CH<sub>2</sub>), 44.9 (4CH<sub>3</sub>), 34.7 (2CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>39</sub>H<sub>42</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 727.3575, found [M+H]<sup>+</sup> 727.3610.

**The synthesis of 1,3-bis(3-(1-(4-(3-(piperidin-1-yl)propanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (8).**

*N*-(4-azidophenyl)-3-(piperidin-1-yl)propanamide (**E6**) (158 mg, 0.58 mmol) was reacted with 1,3-bis(3-ethynylphenyl)urea (**A2**) (50 mg, 0.19 mmol) to yield a brown solid (148 mg, 0.18 mmol,



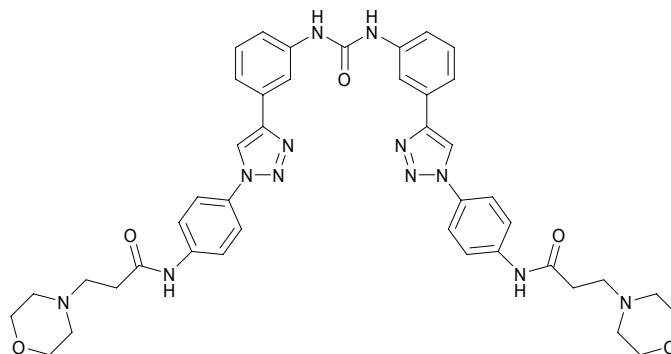
95%). Semi-Prep HPLC was conducted to yield an off white solid as the 0.12 formate salt (by <sup>1</sup>H NMR). HPLC (method A) 98%, RT 22.51 minutes. Mp. 164 – 166°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.35 (2H, s, 2NH; amide), 9.11 (2H, s, 2C=CH), 8.93 (2H, s,

2NH<sub>2</sub>; urea), 8.28 (0.12H, s, formic acid, HCOOH), 8.11 (2H, s, 2ArH), 7.84 (4H, d, *J*=9.0 Hz, 4ArH), 7.74 (4H, d, *J*=9.0 Hz, 4ArH), 7.47 (2H, d, *J*=7.9 Hz, 2ArH), 7.39 (2H, d, *J*=7.9 Hz, 2ArH), 7.37 (2H, t, *J*=7.9 Hz, 2ArH), 2.57 (4H, t, *J*=7.0 Hz, 2CH<sub>2</sub>), 2.44 (4H, m, 2CH<sub>2</sub>), 2.35 (8H, m, 4CH<sub>2</sub>), 1.45 (8H, m, 4CH<sub>2</sub>), 1.33 (4H, m, 2CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 170.6 (2C=O; amide), 152.6 (C=O; urea), 147.2 (2C=CH), 140.3 (2ArC), 139.5 (2ArC), 131.7 (2ArC), 130.9 (2ArC), 129.4 (2ArCH), 120.7 (4ArCH), 119.7 (4ArCH), 119.4 (2C=CH), 119.2 (2ArCH), 118.1 (2ArCH), 115.1 (2ArCH), 54.3 (2CH<sub>2</sub>), 53.6 (4CH<sub>2</sub>), 34.0 (2CH<sub>2</sub>), 25.5 (4CH<sub>2</sub>), 23.9 (2CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>45</sub>H<sub>50</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 807.4202, found [M+H]<sup>+</sup> 807.4229.

**The synthesis of 1,3-bis(3-(1-(4-(3-(morpholino)propanamido)phenyl)-1*H*-1,2,3-triazol-4-yl)phenyl)urea (9).**

*N*-(4-azidophenyl)-3-morpholinopropanamide (E7)

(159 mg, 0.58 mmol) was reacted with 1,3-bis(3-ethynylphenyl)urea (A2) (50 mg, 0.19 mmol) to yield a brown

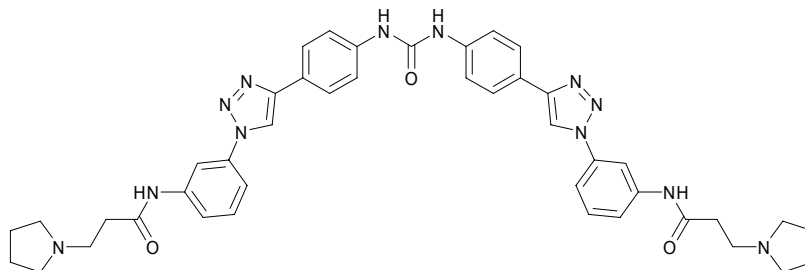


solid (120 mg, 0.15 mmol, 77%). Semi-Prep HPLC was conducted to yield an off white solid. HPLC (method A) 98%, RT 20.96 minutes. Mp. 230 – 232°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.30 (2H, s, 2NH<sub>2</sub>; amide), 9.19 (2H, s, 2C=CH), 8.89 (2H, s, 2NH<sub>2</sub>; urea), 8.18 (2H, s, 2ArH), 7.92 (4H, d, *J*=9.0 Hz, 4ArH), 7.83 (4H, d, *J*=9.0 Hz, 4ArH), 7.55 (2H, dt, *J*=7.8, 1.5 Hz, 2ArH), 7.47 (2H, dt, *J*=7.8, 1.5 Hz, 2ArH), 7.42 (2H, t, *J*=7.8 Hz, 2ArH), 3.59 (8H, t, *J*=4.5 Hz, 4CH<sub>2</sub>), 2.66 (4H, t, *J*=7.0 Hz, 2CH<sub>2</sub>), 2.54 (4H, t, *J*=7.0 Hz, 2CH<sub>2</sub>), 2.43 (8H, t, *J*=4.5 Hz, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 170.4 (2C=O; amide), 152.5 (C=O; urea), 147.2 (2C=CH), 140.3 (2ArC), 139.5 (2ArC), 131.7 (2ArC), 130.9 (2ArC), 129.4 (2ArCH), 120.7 (4ArCH), 119.8 (4ArCH), 119.4 (2C=CH), 119.2 (2ArCH), 118.1 (2ArCH), 115.1 (2ArCH), 66.2 (4CH<sub>2</sub>), 54.1 (2CH<sub>2</sub>), 53.0 (4CH<sub>2</sub>), 34.0 (2CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>5</sub> [M+H]<sup>+</sup> 811.3787, found [M+H]<sup>+</sup> 811.3784.



**The synthesis of 1,3-bis(4-(1-(3-(3-(pyrrolidin-1-yl)propanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (10).**

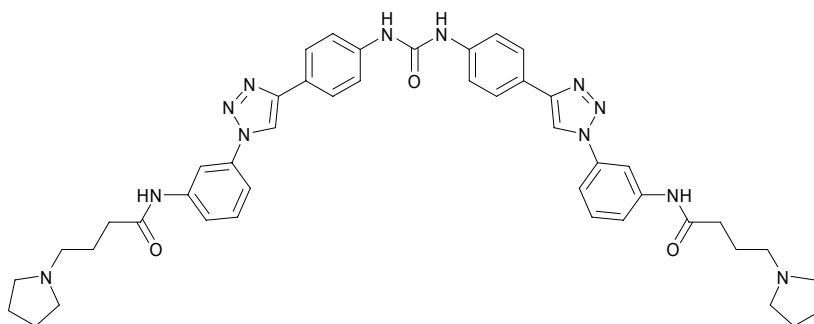
*N*-(3-azidophenyl)-3-(pyrrolidin-1-yl)propanamide (**E1**) (150 mg, 0.58 mmol) was reacted with 1,3-



ethynylphenyl)urea (**A3**) (50 mg, 0.19 mmol) to yield a yellow solid (97 mg, 0.13 mmol, 65%). Semi-Prep HPLC was conducted to yield a cream solid. HPLC (method A) 94%, RT 21.73 minutes. Mp. 235 – 237°C. <sup>1</sup>H NMR (400 MHz, *d*<sub>6</sub>-DMSO) δ 10.39 (2H, s, 2NH; amide), 9.17 (2H, s, 2NH; urea), 9.15 (2H, s, 2C=CH), 8.36 (2H, s, 2ArH), 7.89 (4H, d, *J*=8.5 Hz, 4ArH), 7.62 (6H, m, 6ArH), 7.56 (4H, m, 4ArH), 2.79 (4H, t, *J*=7.0 Hz, 2CH<sub>2</sub>), 2.55 (12H, m, 6CH<sub>2</sub>), 1.71 (8H, m, 4CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (100 MHz, *d*<sub>6</sub>-DMSO) δ 170.4 (2C=O; amide), 152.4 (C=O; urea), 147.2 (2C=CH), 140.4 (2ArC), 139.8 (2ArC), 136.9 (2ArC), 130.1 (2ArCH), 125.9 (4ArCH), 123.7 (2ArC), 118.7 (2C=CH), 118.6 (2ArCH), 118.3 (4ArCH), 114.2 (2ArCH), 110.3 (2ArCH), 53.3 (4CH<sub>2</sub>), 51.2 (2CH<sub>2</sub>), 35.8 (2CH<sub>2</sub>), 23.0 (4CH<sub>2</sub>) ppm. HRMS *m/z* calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 779.3889, found [M+H]<sup>+</sup> 779.3892.

**The synthesis of 1,3-bis(4-(1-(3-(4-(pyrrolidin-1-yl)butanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (11).**

*N*-(3-azidophenyl)-4-(pyrrolidin-1-yl)propanamide (**E2**) (157 mg, 0.58 mmol) was reacted with 1,3-



ethynylphenyl)urea (**A3**) (50 mg, 0.19 mmol) to yield a brown solid (133 mg, 0.16 mmol, 86%). Semi-Prep HPLC was conducted to yield a pale yellow solid. HPLC (method A) 90%, RT 21.80

minutes. Mp. 240 – 242°C.  $^1\text{H}$  NMR (400 MHz,  $d_6$ -DMSO)  $\delta$  10.24 (2H, s, 2NH; amide), 9.31 (2H, s, 2NH; urea), 9.15 (2H, s, 2C=CH), 8.36 (2H, s, 2ArH), 7.88 (4H, d,  $J=8.6$  Hz, 4ArH), 7.63 (6H, m, 6ArH), 7.55 (4H, m, 4ArH), 2.50 (12H, m, 6ArH), 2.42 (4H, t,  $J=7.4$  Hz, 2CH<sub>2</sub>), 1.80 (4H, m, 2CH<sub>2</sub>), 1.69 (8H, m, 4CH<sub>2</sub>) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $d_6$ -DMSO)  $\delta$  171.5 (2C=O; amide), 152.4 (C=O; urea), 147.3 (2C=CH), 140.5 (2ArC), 139.8 (2ArC), 136.8 (2ArC), 130.0 (2ArCH), 125.9 (4ArCH), 123.6 (2ArC), 118.7 (2ArCH), 118.5 (2C=CH), 118.3 (4ArCH), 114.1 (2ArCH), 110.3 (2ArCH), 54.8 (2CH<sub>2</sub>), 53.4 (4CH<sub>2</sub>), 34.3 (2CH<sub>2</sub>), 23.8 (2CH<sub>2</sub>), 23.0 (4CH<sub>2</sub>) ppm. HRMS  $m/z$  calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 807.4202, found [M+H]<sup>+</sup> 807.4211.

**The synthesis of 1,3-bis(4-(1-(4-(3-(pyrrolidin-1-yl)propanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (12).**

*N*-(4-azidophenyl)-3-

(pyrrolidin-1-yl)propanamide

(E3) (150 mg, 0.58 mmol)

was reacted with 1,3-bis(4-

ethynylphenyl)urea (A3) (50

mg, 0.19 mmol) to yield a

beige solid (142 mg, 0.18

mmol, 85%). Semi-Prep HPLC was conducted to yield a pale brown powder as the 1.84

formate salt (by  $^1\text{H}$  NMR). HPLC (method A) 94%, RT 21.41 minutes. Mp. > 315°C dec.

$^1\text{H}$  NMR (400 MHz,  $d_6$ -DMSO)  $\delta$  10.38 (2H, s, 2NH; amide), 9.60 (2H, s, 2NH; urea),

9.11 (2H, s, 2C=CH), 8.28 (1.84H, s, formic acid, HCOOH), 7.88 (4H, d,  $J=9.1$  Hz,

4ArH), 7.85 (4H, d,  $J=8.7$  Hz, 4ArH), 7.82 (4H, d,  $J=9.1$  Hz, 4ArH), 7.64 (4H, d,  $J=8.7$

Hz, 4ArH), 2.92 (4H, t,  $J=7.1$  Hz, 2CH<sub>2</sub>), 2.70 (8H, m, 4CH<sub>2</sub>), 2.62 (4H, t,  $J=7.1$  Hz,

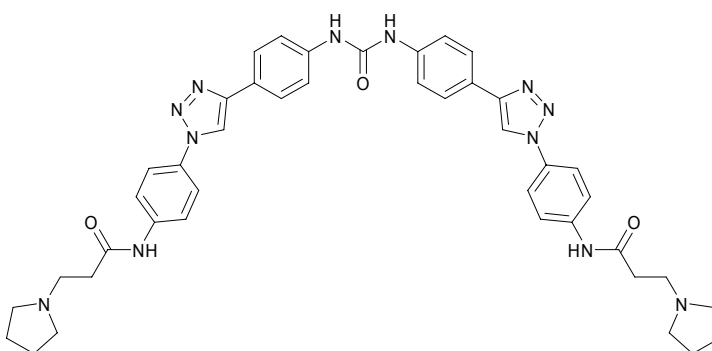
2CH<sub>2</sub>), 1.76 (8H, m, 4CH<sub>2</sub>) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $d_6$ -DMSO)  $\delta$  169.8 (2C=O;

amide), 164.3 (formic acid, HCOOH), 152.5 (C=O; urea), 147.2 (2C=CH), 140.0 (2ArC),

139.3 (2ArC), 131.7 (2ArC), 125.8 (4ArCH), 123.6 (2ArC), 120.4 (4ArCH), 119.7

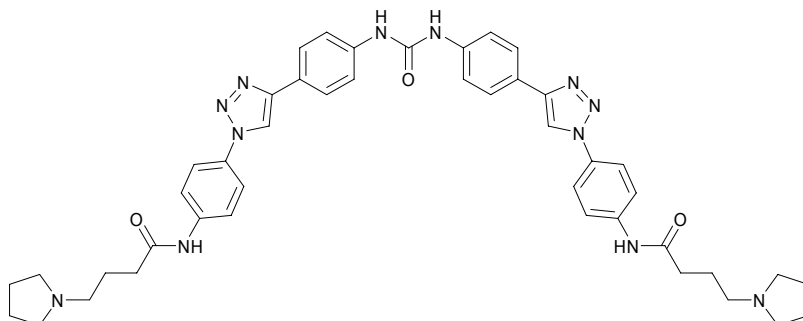
(4ArCH), 118.3 (4ArCH), 118.3 (2C=CH), 53.2 (4CH<sub>2</sub>), 50.9 (2CH<sub>2</sub>), 35.0 (2CH<sub>2</sub>), 22.9

(4CH<sub>2</sub>) ppm. HRMS  $m/z$  calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 779.3889, found [M+H]<sup>+</sup> 779.3904.



**The synthesis of 1,3-bis(4-(1-(4-(4-(pyrrolidin-1-yl)butanamido)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)urea (13).**

*N*-(4-azidophenyl)-4-(pyrrolidin-1-yl)propanamide (**E4**) (157 mg, 0.58 mmol) was reacted with 1,3-bis(4-ethynylphenyl)urea



(**A3**) (50 mg, 0.19 mmol) to yield a yellow solid (110 mg, 0.14 mmol, 71%). Semi-Prep HPLC was conducted to yield a pale yellow solid as the 1.60 formate salt (by  $^1\text{H}$  NMR). HPLC (method A) 91%, RT 22.03 minutes. Mp. > 315°C dec.  $^1\text{H}$  NMR (400 MHz,  $d_6$ -DMSO)  $\delta$  10.22 (2H, s, 2NH; amide), 9.83 (2H, s, 2NH; urea), 9.10 (2H, s, 2C=CH), 8.33 (1.60H, s, formic acid, HCOOH), 7.85 (12H, m, 12ArH), 7.65 (4H, d,  $J=8.7$  Hz, 4ArH), 2.72 (8H, m, 4CH<sub>2</sub>), 2.68 (4H, t,  $J=7.4$  Hz, 2CH<sub>2</sub>), 2.43 (4H, t,  $J=7.3$  Hz, 2CH<sub>2</sub>), 1.84 (4H, m, 2CH<sub>2</sub>), 1.76 (8H, m, 4CH<sub>2</sub>) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $d_6$ -DMSO)  $\delta$  171.1 (2C=O; amide), 165.1 (formic acid, HCOOH), 152.6 (C=O; urea), 147.2 (2C=CH), 140.0 (2ArC), 139.4 (2ArC), 131.6 (2ArC), 125.7 (4ArCH), 123.6 (2ArC), 120.4 (4ArCH), 119.6 (2C=CH), 119.6 (4ArCH), 118.3 (4ArCH), 54.5 (2CH<sub>2</sub>), 53.2 (4CH<sub>2</sub>), 34.0 (2CH<sub>2</sub>), 23.3 (2CH<sub>2</sub>), 22.9 (4CH<sub>2</sub>) ppm. HRMS  $m/z$  calc. C<sub>43</sub>H<sub>46</sub>N<sub>12</sub>O<sub>3</sub> [M+H]<sup>+</sup> 807.4202, found [M+H]<sup>+</sup> 807.4217.

### 3.0 HPLC Purity Analysis

Ligand	HPLC Method	% Purity	HPLC Method	% Purity	Ave. % Purity
1	A	98	B	99	99
2	A	97	B	99	98
3	A	97	B	92	95
3 <sub>Elim</sub>	A	87	B	91	89
4	A	94	B	96	95
5	A	99	B	99	99
5 <sub>Elim</sub>	A	95	B	Insoluble	95
6	A	99	B	95	97
7	A	98	B	98	98
8	A	98	B	99	99
9	A	98	B	98	98
10	A	94	B	94	94
11	A	90	B	95	93
12	A	94	B	97	96
13	A	91	B	92	92

**Method A** - 0.1% formic acid in acetonitrile and 0.1% aqueous formic acid, 5% - 50% organic over 28 minutes (1 ml/min); **Method B** - 0.1% formic acid in MeOH and 0.1% aqueous formic acid, 25% - 75% organic over 18 minutes (1 ml/min).

#### 4.0 Fluorescence Resonance Energy Transfer (FRET) Assays

FRET oligonucleotides (Eurogentec Ltd., U.K.) have the following sequences (where FAM is 6-carboxyfluorescein and TAMRA 6-carboxytetramethylrhodamine):

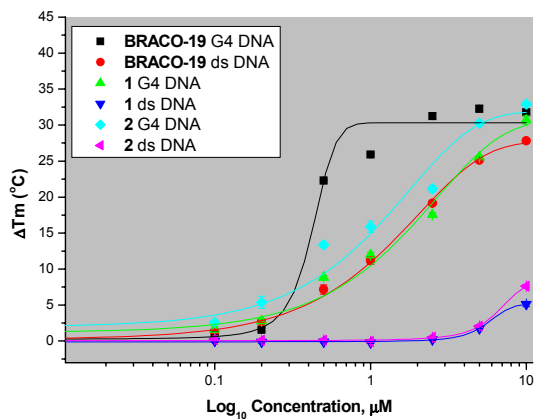
**G4 DNA** 5'FAM-d[G<sub>3</sub>(T<sub>2</sub>AG<sub>3</sub>)<sub>3</sub>]-TAMRA<sup>3'</sup>;

**ds DNA** 5'FAM-d[(TA)<sub>2</sub>GC(TA)<sub>2</sub>T<sub>6</sub>(TA)<sub>2</sub>GC(TA)<sub>2</sub>]-TAMRA<sup>3'</sup>;

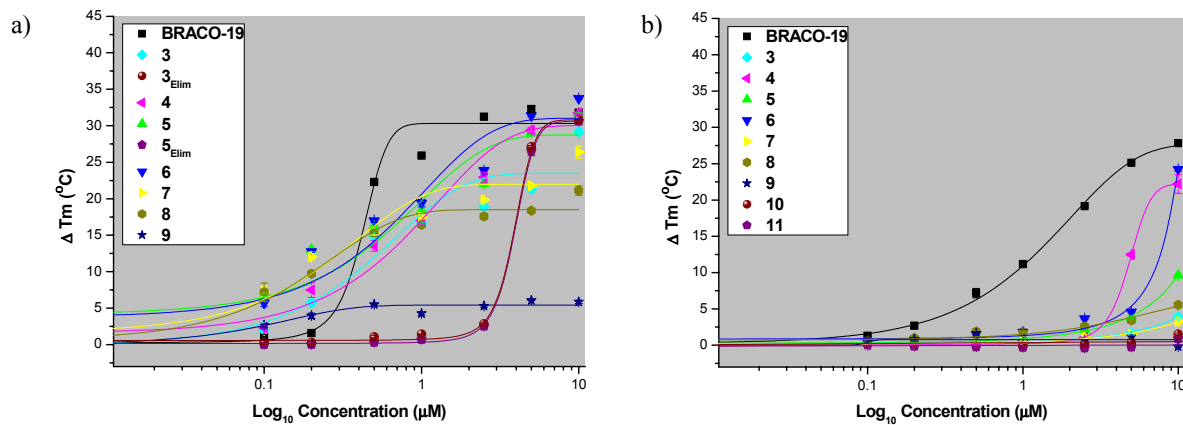
**FRET Melting Assay:** The required oligonucleotide was suspended in FRET buffer (60mM KCl, Kcacodylate, pH 7.4; 400nM DNA) and heated to 85°C for 10 minutes prior to cooling to room temperature. DNA was distributed (50µl) across a 96 well RT-PCR plate (Bio-Rad) to which ligand was added (50µl; stored as a 20mM DMSO stock, -20°C; diluted to 1mM in HPLC grade DMSO) to afford the required concentration. FRET buffer was used as a negative control. DNA melting was assessed upon a MJ Research Opticon DNA Engine Continuous Fluorescence Detector exciting at 450 – 495nm. Fluorescence values were recorded at 515 – 545nm at 0.5°C intervals as the plate was heated from 30 – 100°C. The data was analysed in the Origin 7.0 software package (Origin Lab Corp., Northampton, MA). The change in melting temperature at 1µM ligand concentration ( $\Delta T_{m_{1\mu M}}$ ) was calculated from four experiments by subtraction of the averaged negative control from the averaged 1µM ligand melting temperature.

**FRET Competition Assay:** To G4 DNA (50µl) was added calf thymus DNA (CT-DNA; 25µl; 533.3µM CT-DNA bp stock in 0.5mM EDTA/30mM Kcacodylate buffer) to afford the required CT-DNA bp concentration. To this was added ligand (25µl, 4µM) to afford the required ligand concentration (1µM). FRET buffer represented no competitor. The percentage retained stabilisation was calculated from three experiments, and normalised to the  $\Delta T_{m_{1\mu M}}$  for that ligand with no CT-DNA competitor (100%)  $\pm$  normalised sd. BRACO-19 was prepared in-house to a HPLC purity of >98%.

#### 4.1 FRET Melting Curves

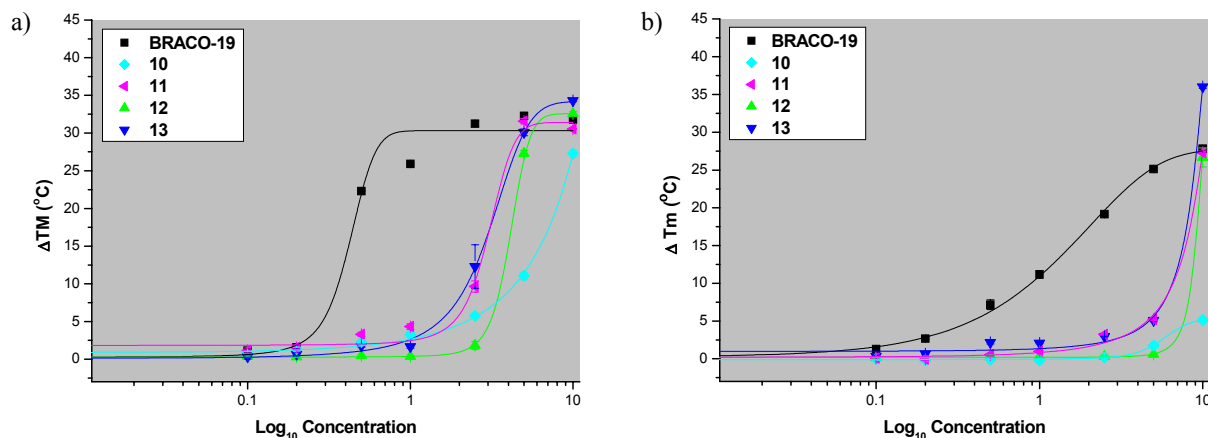


**Figure 1** – The melting curves of ligands 1 and 2.



**Figure 2** – The melting curves of ligands 3 – 9.

Against a) G4 DNA and b) ds DNA oligonucleotides.



**Figure 3 – The melting curves of ligands 10 – 13.**

Against a) G4 DNA and b) ds DNA oligonucleotides.

## 5.0 Cell Culture

Cell lines were supplied by ATCC-LGC Promochem and viability maintained in a Heraeus Hera Cell 240 incubator (37°C, 5% CO<sub>2</sub>; 75 cm<sup>2</sup> plates supplied by TPP). Cells were removed for experimentation as required. Sterile work was conducted in a Heraeus Hera Safe hood. Dulbecco's Modified Eagles Media (DMEM; Invitrogen) supplemented with foetal bovine serum (10% v/v; Invitrogen), hydrocortisone (0.5µg/ml; Acros Organics), L-glutamine (2mM; Invitrogen) and non-essential amino acids (1x; Invitrogen) was used for the MCF7 and A549 cell lines, and Minimal Essential Medium (MEM; Sigma-Aldrich) supplemented with foetal bovine serum (10% v/v; Invitrogen), L-glutamine (2mM; Invitrogen) and non-essential amino acids (1x; Invitrogen) was used for the WI38 cell line. **Sulforhodamine B (SRB) Cytotoxicity Assay:** Short term growth inhibition was measured using the SRB assay as described previously.<sup>4</sup> Briefly, cells were seeded (4000 cells/well MCF7 and WI38; 1000 cells/well A549) into the wells of 96 well-plates in appropriate medium and incubated overnight to allow the cells to attach. Subsequently cells were exposed to freshly-made solutions of drug at increasing concentrations between 0.1 – 25 µM in quadruplicate and incubated for a further 96h. Following this the cells were fixed with ice cold trichloroacetic acid (TCA) (10% w/v)

for 30 min and stained with 0.4% SRB dissolved in 1% acetic acid for 15 min. All incubations were carried out at room temperature. The IC<sub>50</sub> value, the concentration required to inhibit cell growth by 50%, was determined from the mean absorbance at 540nm for each drug concentration expressed as a percentage of the well absorbance in untreated control cells.

## 6.0 References

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3. A. D. Moorhouse, S. Haider, M. Gunaratnam, D. Munnur, S. Neidle, and J. E. Moses, *Mol. Biosyst.*, 2008, **4**, 629.
4. M. Gunaratnam, O. Greciano, C. Martins, A. P. Reszka, C. M. Schultes, H. Morjani, J. F. Riou, and S. Neidle, *Biochem. Pharmacol.*, 2007, **74**, 679.