Supplementary Data

Structure-based design of selective high-affinity telomeric quadruplex-binding ligands

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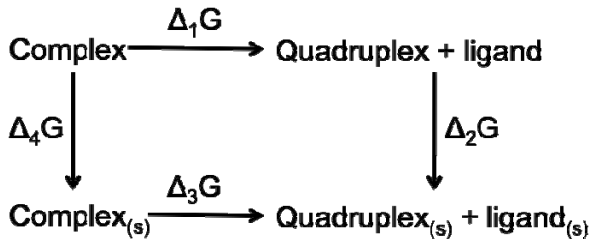
Molecular modeling

The crystal structure of the parallel-stranded propeller-loop topology (PDB 1KF1) human telomeric G-quadruplex complexed with BRACO-19 wasobtained from the Protein Data Bank and used as a starting point to examine plausible interactions with the ligands. The molecular models for the triazole series of ligands were constructed, minimized and partial charges calculated semi-empirically using the MOPAC program[1] from the Insight suite software (www.accelrys.com). The ligands were minimized and docked using the AFFINITY docking program (www.accelrys.com), employing the grid docking method available with AFFINITY. This approach has been validated in previous studies from this laboratory (see reference list) on the rational design of quadruplex ligands. The automated docking process identifies and ranks positions based on high interaction energies within the binding site. The complexes were then subjected to 1000 steps of molecular mechanics minimization and 200ps of molecular dynamics simulations in explicit solvent at 300K using the DISCOVER3 program in the Insight II suite software. The data was visualized by means of the VMD program.[2] Electrostatic contributions to the overall binding energy were calculated using the APBS software, employing an implicit solvent model.[3] The docked complexes were prepared using the PDB2PQR server (http://agave.wustl.edu/pdb2pqr). Charges, atom types and radii were assigned to each ligand atom based on the AMBER force field. The complexes were then subjected to APBS electrostatic calculations. A dielectric constant of 2, a solvent dielectric constant of 80 were used, together with grid spacing that were all optimally chosen such that the grid was always finer than 0.5 Å. The remaining parameters were kept at default

values. The binding free energies were calculated on the basis of the simple thermodynamics cycle



where [complex, quadruplex, ligand] and [$complex_{(s)}$, $quadruplex_{(s)}$, $ligand_{(s)}$] represent the non-solvated and solvated states of the system respectively. The program APBS calculates binding free energies using the equation:

$$\Delta G_{binding} = -\Delta_3 G = \Delta_4 G - d\Delta_1 G - \Delta_2 G$$

This binding free energy cycle illustrates binding in terms of the transfer of free energies from a homogenous dielectric environment to an inhomogenous dielectric environment with differing internal and external dielectric constants.[3]

1. J. J. P. Stewart. *J. Comput. Aided Mol. Des*. 1990, **4**, 100.
2. W. Humphrey, A. Dalke and K. Schulten. *J. Mol. Graph*. 1996, **14**, 33.
3. N. A. Baker, D. Sept, S. Joseph, M. Holst and A.J. McCammon.. *Proc. Natl. Acad. Sci. USA* 2001, **98**, 10037.

Electrospray mass spectrometry

Materials and methods

All the oligonucleotides were purchased from Eurogentec (Belgium) and used without any further purification. The sequences analysed were: duplex $(dCGCGAATTCGCG)_2$ (MW= 7354.8), Telo-22 ($d[AGGG(TTAGGG)_3]$) (MW = 6966.6) and TG4T ($[dTG_4T]_4$) (MW = 7639). The sequence T6 (dT_6) (MW = 1763.2) was used in all the injections as an internal standard. The DNAs were annealed as 50μM stock solutions in 150mM ammonium acetate (obtained by dilution of a 5 M solution, molecular biology grade from Fluka) buffer, heating to 90°C for 10 minutes, then slowly cooling down to room temperature. The annealed solutions were stored at 4°C for at least 24 hours before the use. The final concentration of all the oligonucleotides in each sample was 5 μM, except for the standard T6, whose concentration was 2 μM. The drugs were stored at 4 °C as 10 mM stock solutions in DMSO

and were diluted in ammonium acetate 150 mM buffer to the required concentrations. 10 % MeOH was added to all the samples prior to the injection to improve the quality of the signal.

ESI-MS studies were conducted on a Q-TOF Ultima Global mass spectrometer (Waters, Manchester, UK). All samples were infused at 4 µL/min in the electrospray source that was operated in negative ion mode (capillary voltage = -2.2 kV). Temperatures of ESI source and nitrogen desolvation gas were 40 °C and 60 °C, respectively. The pressure of the source was 3.45 mbar. For each sample MS spectra were recorded using different sets of acceleration voltages: cone voltage was varied from 50 to 100 V; RF Lens 1 voltage was varied from 50 to 125 V; collision energy (acceleration voltage before the collision hexapole) was always set to 10 V. The lowest voltages give the softest source condition at the chosen source pressure, and have been used, for example, to preserve the ammonium cations inside the tel22 G-quadruplex. We checked that in the voltage range described above, the ligand-DNA complexes did not dissociate.

Determination of the equilibrium dissociation constants (K_d) of the 1:1 complex between the tel22 DNA sequence dAGGG(TTAGGG)$_3$ and the ligands

To determine the K_ds of ligand binding to the telomeric G-quadruplex tel22, we recorded ESI-MS spectra of mixtures of reference (2 µM) + 5 µM tel22 + 5 or 10 µM ligand. The reference was added to control that the sum of areas of free and bound quadruplex ($A(tel22)^{5-} + A(tel22+L)^{5-}$) was constant relative to the area of T6 ($A(dT_6)^{2-}$). This was the case, as very commonly found for DNA-ligand complexes, despite the fact that ligands are positively charged. As discussed elsewhere (F. Rosu, E. De Pauw and V. Gabelica, *Biochimie* 2008, **90**, 1074), it seems that the final charge state distribution depends more on the surface area of the ionized molecule than on the location of each charge in solution. We can therefore safely assume that the response factors of the free and bound quadruplex are equal at charge state 5-, and the peak areas were used to calculate the concentration ratios, as follows:

$$[tel22]_{free} = 5\ \mu M \times \frac{A(tel22)^{5-}}{A(tel22)^{5-} + A(tel22+L)^{5-}} \qquad \text{(Eq. S1)}$$

$$[tel22+L] = 5\ \mu M \times \frac{A(tel22+L)^{5-}}{A(tel22)^{5-} + A(tel22+L)^{5-}} \qquad \text{(Eq. S2)}$$

$$[L]_{free} = [L]_{tot} - [tel22 + L] \qquad \text{(Eq. S3)}$$

$$K_d = \frac{[L]_{free}.[tel22]_{free}}{[tel22 + L]} \qquad \text{(Eq. S4)}$$

The data were recorded at a cone voltage of 50 V, and three different RF lens voltages (75V, 100V and 125 V). The spectra shown consist in a sum of at least 150 scans, processed as follows using MassLynx 4.0 software: smoothing (2 x mean, 20 channels), background-subtraction (polynomial order = 10, 1% below curve). For peak area determination, the spectra were converted to centroid.

Typical spectra are shown in Figure S1. The 1:1 complex was the only complex detectable. The K_ds reported in main text Table 1 are the averages of the K_ds determined from measurements at the two ligand concentrations (5 and 10 µM) and three voltage settings each.

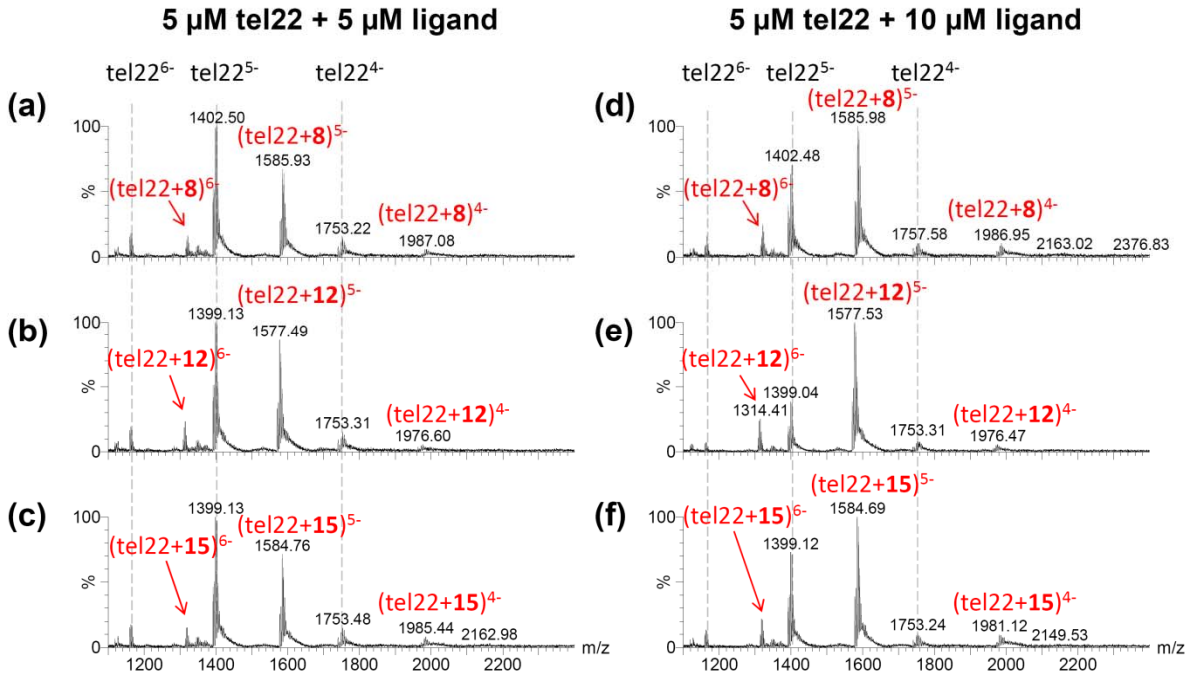


Figure S1: Representative ESI-MS spectra of mixtures between 5 µM telomeric G-quadruplex dAGGG(TTAGGG)$_3$ (tel22) and 5 µM ligand **8** (a), **12** (b), **15** (c), or 10 µM of the same ligand (d-f). The free tel22 and the complexes were detected at charge states 6- to 4-. The most intense charge state (5-) was used for determining the K_d's.

Competition experiment

For the competition experiment, the mixtures injected contained 5 µM of duplex $(dCGCGAATTCGCG)_2$, 5 µM of intramolecular telomeric quadruplex $dAGGG(TTAGGG)_3$, 5 µM of tetramolecular quadruplex $[dTGGGGT]_4$, 10 µM of ligand, and 2 µM of single strand dT_6 which is added as an intensity reference. The data were recorded at 3 different sets of voltages: [cone 50 V, RF lens 60 V]; [cone 100 V, RF lens 60 V]; [cone 100 V, RF lens 100 V]. The spectra shown consist in a sum of at least 150 scans, processed as follows using MassLynx 4.0 software: smoothing (2 x mean, 8 channels), background-subtraction (polynomial order = 50, 1% below curve). For peak area determination, the spectra were converted to centroid.

Typical spectra are shown in Figure S2. The spectrum with no ligand (Fig. S2(a)) shows peaks of the reference at *m/z* = 880.15. The intensity changes of free quadruplex or duplex upon ligand addition will be monitored compared to the intensity of the peak of dT_6^{2-}. The duplex is detected at charge states 5- and 4- (the peak at m/z = 1214.31 corresponds to the single strand with three charges, as deduced from the isotope pattern, not shown). The quadruplexes are detected at charge states 6-, 5- and 4-. The charge state 5- is the most intense for all target DNAs, and was used for the quantitation of free DNA.

Each day, a spectrum of the mixture without ligand was recorded, and the areas of the peaks of DNA (DNA stands for the duplex or one of the quadruplexes) and the peak of the reference strand were recorded. The experiments were then conduced with each ligand at 10 µL and the peak areas are measured. The data presented in main text Figure 1 is the fraction of free duplex or quadruplex after ligand addition, which was calculated as follows:

$$Fraction\ free\ DNA = \frac{\frac{A(DNA)^{5-}_{[L]=10\mu M}}{A(dT_6)^{2-}_{[L]=10\mu M}}}{\frac{A(DNA)^{5-}_{[L]=0}}{A(dT_6)^{2-}_{[L]=0}}} \quad \text{(Eq. S5)}$$

The fraction of free DNA is therefore deduced solely from the peak areas of the free species. The mass spectra however provide further information on the nature of the complexes responsible for the diminishing of the peak areas of the free species. For example, in the case of ligand **14** (Fig. S2(b)), the tel22 quadruplex is consumed by forming a 1:1 complex with the ligand. In contrast, the $[TG_4T]_4$ quadruplex forms not only 1:1 complex with the ligand, but also dimers (2Q + 2 to 4 ligands), detected at charge state 7-. The formation of these higher-order aggregates of $[TG_4T]_4$ was detected with all ligands that significantly deplete the amount of free $[TG_4T]_4$ quadruplex (ligands **6**, **9**, **10**, **13** and **14**). Note that all these ligands have *para* substituents.

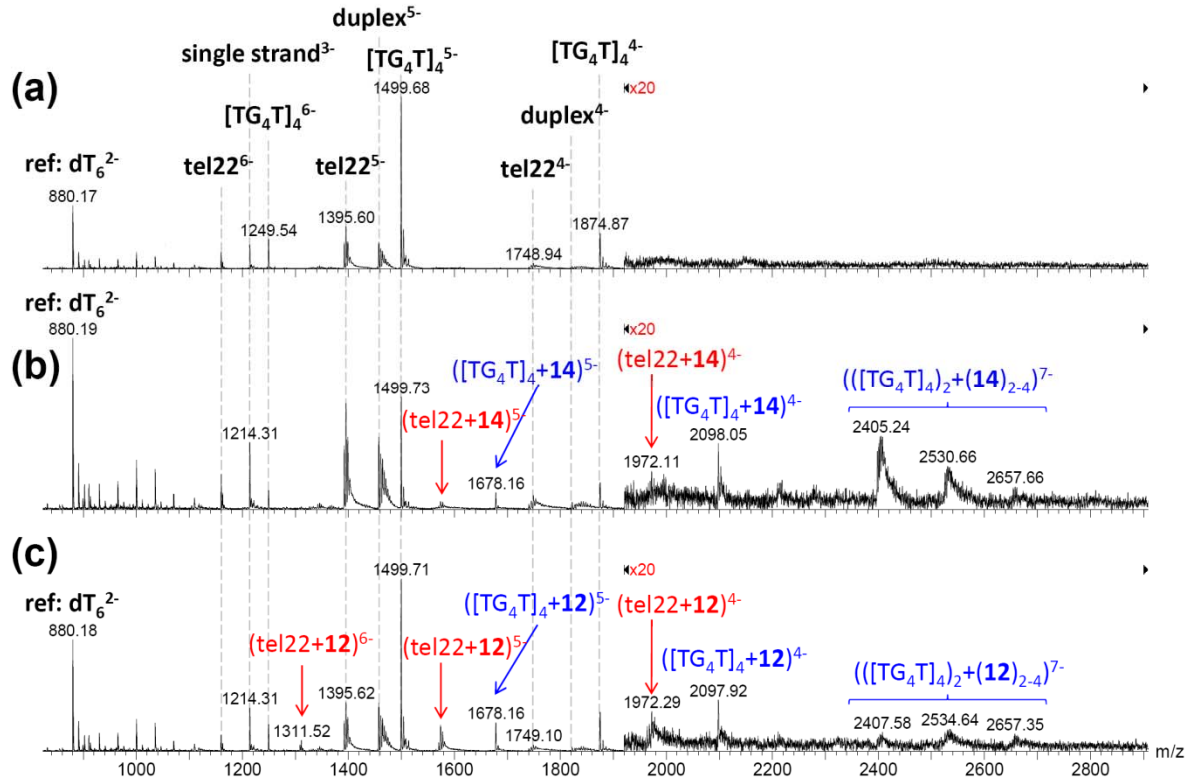
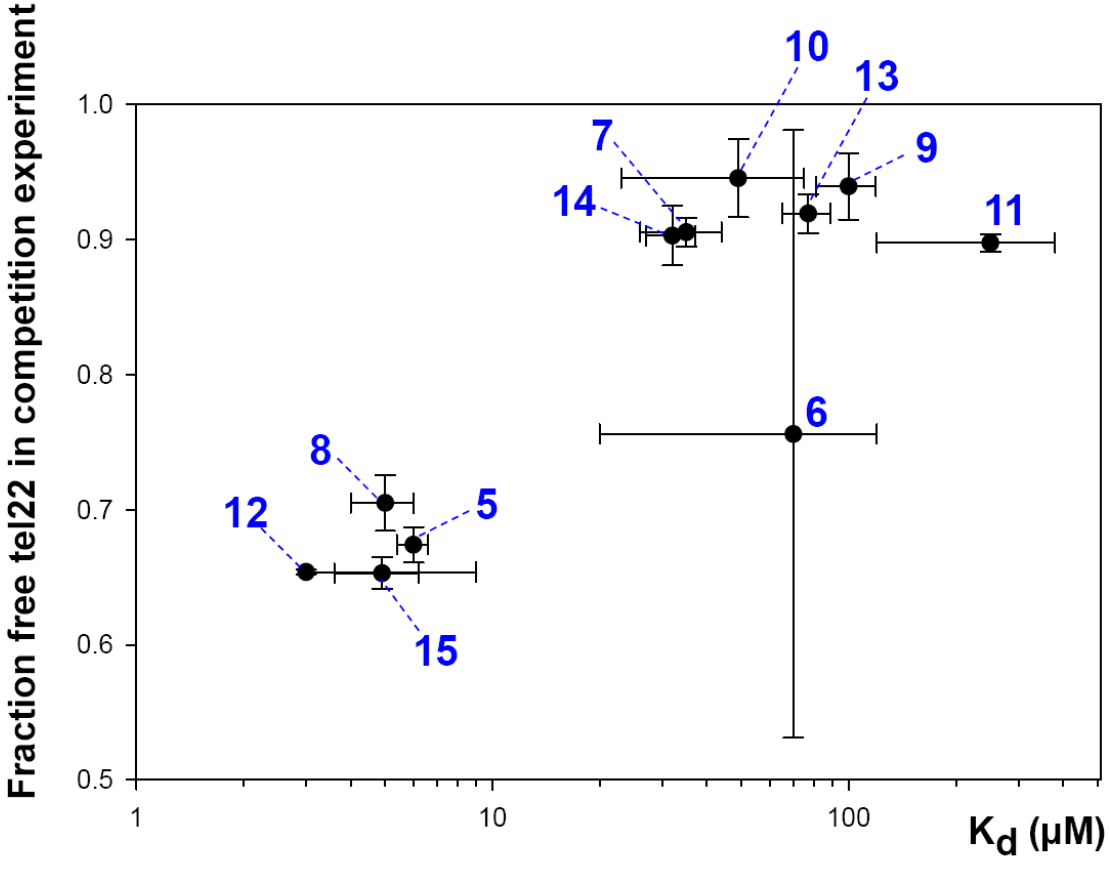


Figure S2: Competition experiment between the telomeric G-quadruplex $dAGGG(TTAGGG)_3$ (tel22), the duplex $(dCGCGAATTCGCG)_2$, and the tetrameric G-quadruplex $[dTG_4T]_4$, 5 μM each. The single strand dT_6 is added (2 μM) as an intensity reference. Ammonium acetate concentration was 100 mM. **(a)** No ligand added, **(b)** addition of 10 μM ligand **14**, **(c)** addition of 10 μM ligand **12**. Note the 20 × intensity zoom in *m/z* range [1920-2950].



F igure S3: The excellent correlation between the K_d for ligand binding to tel22 and determination and the fraction free tel22 found in the competition experiment shows that the competition experiment is a reliable way to assess relative ligand binding affinities for several DNA structures at the same time.

Absence of binding to the duplex $(dCGCGAATTCGCG)_2$

We attempted to determine the K_d values of ligand binding to the duplexes in order to calculate the selectivity of the ligands for the telomeric quadruplex. We ran mixtures of reference + 5 µM $(dCGCGAATTCGCG)_2$ duplex + 40 µM ligand. A typical spectrum is shown in Figures S4. We could detect no complex between the duplex and any of the ligands. The presence of the ligand in the stock solutions was verified by testing their binding to the $[TG_4T]_4$ quadruplex. We note a slight decrease of the signal of the duplex compared to the reference in the presence of 40 µM ligand. Because of the absence of detectable complex, we conclude that this change in relative intensities can be due to a change in the relative responses of dT_6 vs. duplex due to the presence of excess ligand.

In the absence of detectable complex, only a minimum limit value of the K_d can be obtained from the background signal. From the noise level at m/z = 1635.90, we can estimate the minimum K_d that would lead to a detectable complex, i.e. the K_d that would lead to signal-to-noise ratio of 3 for the complex (assuming equal response factors for the duplex and the complex). By this procedure we deduce that all K_d values for duplex binding are higher than 3000 µM. This gives a quadruplex/duplex selectivity ratio > 1000 for ligand **12**, for example.

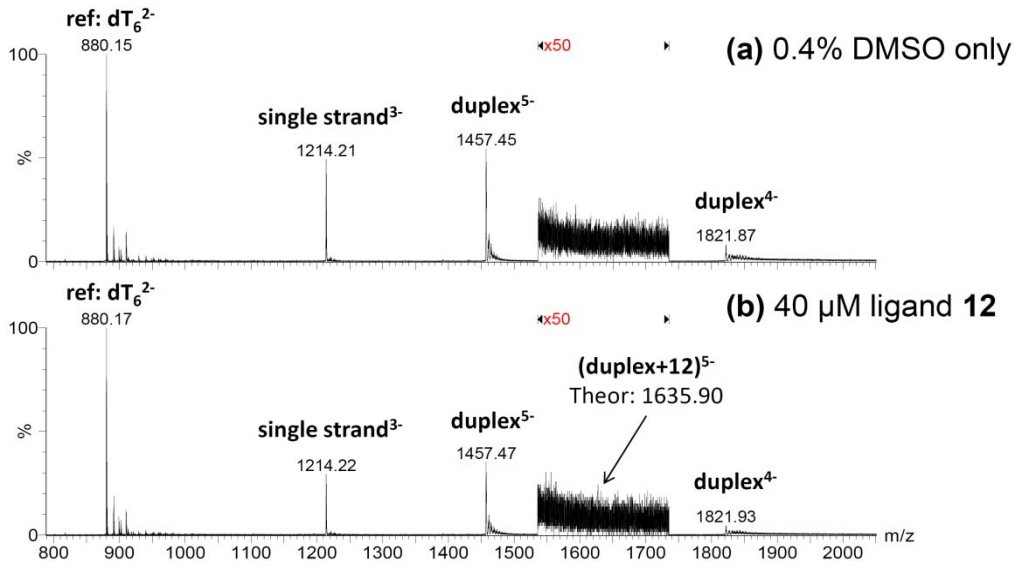


Figure S4: ESI-MS spectra of (a) 5 µM duplex $(dCGCGAATTCGCG)_2$ alone with 2 µM reference strand dT_6, and of (b) 5 µM duplex $(dCGCGAATTCGCG)_2$ with 2 µM reference strand dT_6 and 40 µM of ligand **12**. The 1:1 complex between the duplex and the ligand

should have been detected at an *m/z* of 1635.90 for charge state 5- (arrow). The background was magnified 50 times in the *m/z* region [1535-1735] to show the absence of detectable peak.

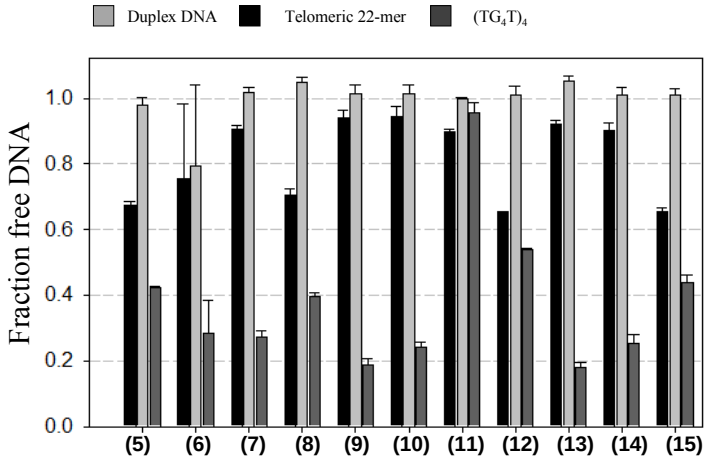


Figure S5: Results of ESI-MS competition experiments for the series of ligands reported here, showing in each instance the fraction of free DNA for three oligonucleotides. Ligands are defined in Table 1.

Chemistry

General methods

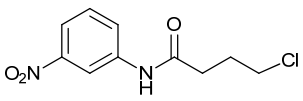
Reagents, solvents and chemicals were purchased from Sigma-Aldrich, Alfa Aesar, GOSS, Lancaster Synthesis, Novabiochem, Avocado Organics or ABCR GMBH & CO (1,3,5-triethynylbenzene) and were used as supplied without further purification. All the organic solvents used for the reactions were purchased from VWR and Fisher scientific. Microwave irradiation was performed with an Initiator microwave (Biotage, Sweden). Flash chromatography was carried out with BDH silica gel (BDH 153325P).

HPLC analyses were performed using a Gilson apparatus combining a 322 PUMP and an Agilent 1100 SERIES detector. The column used was semi-prep reversed phase YMC-Pack ODS-A, phase C18, particle 5µm, pore 12 nm, ID 30 mm, length 100 mm, (AA12S05-1030WT, YMC, Japan).

LC/MS were performed using a Waters system combining a 2695 separation module, a Micromass ZQ spectrometer and a 2996 photodiode array detector. Method description:

mode: electrospray positive (ES+); MS running conditions: 5 min run time; cone: 25; offset: 1; skimmer: 1.5; RF lens: 0.1; source heater: 150°C; gas: 400 l/h; method used [Solvents: A = H_2O, 0.1% formic acid; B = CH_3CN, 0.1% formic acid. 0 min (95% A, 5%B), 1 min (95% A, 5%B), 3 min (50% A, 50%B), 5 min (95% A, 5%B)]. Melting points (mp) were recorded on a Stuart Scientific SMP1 melting point apparatus and are uncorrected; dc refers to observed decomposition. IR spectra were recorded using a Perkin Elmer SPECTRUM 1000 FT-IR spectrometer. NMR spectra were recorded at 400 or 500 MHz (1H NMR) and 100 or 125 MHz (13C NMR) on a Bruker spectrometer in $CDCl_3$, d4-methanol or d6-DMSO using the residual solvent peaks as internal standards. Coupling constant *J* values are given in hertz (Hz) designated as s (singlet), br s (broad singlet), d (doublet), t (triplet), dd (doublet of doublets), td (triple of doublets), tt (triplet of triplets) and m (multiplet). High resolution mass spectra (HRMS) and elemental analysis (CHN) services were provided by The School of Pharmacy. HRMS were conducted upon a Micromass Q-TTOF Ultima Global tandem mass spectrometer run under electrospray ionisation (ESI) or matrix assisted laser desorption/ionisation (MALDI) modes. CHN were conducted upon a Carlo Erba CHN1108 elemental analyser.

Procedure for the synthesis of compound (1)



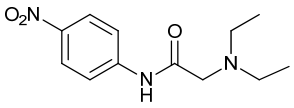
3-Nitroaniline (1.00 g, 7.24 mmol) was cooled in ice bath and treated with an excess of 4-chlorobutyryl chloride (4.23 g, 30.00 mmol); this mixture was allowed to warm up to room temperature and stirred overnight. Once the completion was reached (TLC 5% MeOH in DCM), the reaction was poured into ice, slowly basified with saturated aqueous $NaHCO_3$ solution until pH 9 and stirred for 3 hours. The precipitate formed was filtered and washed with water several time, then with diethyl ether, obtaining a pale yellow solid. The $NaHCO_3$ solution was also extracted with diethyl ether 3 times (100 ml), dried ($MgSO_4$) and taken to dryness *in vacuo*. The pale yellow solid overall obtained was compound **(1)** (1.52 g, 86%); Rf 0.65 [5% MeOH in DCM]; mp 82 °C (lit. 65-67 °C[1]); δ_H ($CDCl_3$, 400 MHz) 8.41 (1H, s, N*H*), 7.96-7.93 (1H, m, Ar*H*), 7.94-7.93 (1H, m, Ar*H*), 7.83 (1H, broad s, Ar*H*), 7.48 (1H, t, *J* = 8.0 Hz, Ar*H*), 3.66 (2H, t, *J* = 6.0 Hz, *C*H_2), 2.63 (2H, t, *J* = 7.2 Hz, C*H*$_2$), 2.25-2.18 (2H, m, C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 170.65 (*C*=O), 148.49 (Ar-*C*), 138.83 (Ar-*C*), 129.85 (Ar-*C*H), 125.54 (Ar-*C*H), 118.95 (Ar-*C*H), 114.61 (Ar-*C*H), 44.25 (*C*H_2), 34.03 (*C*H_2), 27.66 (*C*H_2); LC-MS (5 min) m/z 243.16 $[C_{10}H_{11}{}^{35}ClN_2O_3+H]^+$ (75), 245.13 $[C_{10}H_{11}{}^{37}ClN_2O_3+H]^+$ (25), Rt 2.78 min; HRMS *m/z* calc.

$C_{10}H_{12}ClN_2O_3$ $[M+H]^+$ 243.0531, found $[M+H]^+$ 243.0535; anal**.** CHN calc. $C_{10}H_{11}ClN_2O_3$. C 49.5%, H 4.6%, N 11.5, found C 49.3%, H 4.5%, N 11.5%.

General procedure for the synthesis of nitrocompounds (2) a-k

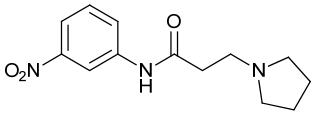
The required nitroaniline (1 eq) was dissolved in THF and cooled down to 4 °C in ice bath. To this stirred mixture first TEA (2 eq), then the appropriate acid chloride (1.2 eq) were added. The reaction was allowed to warm up to room temperature and after 2,30 hours the mixture was cooled down in ice bath, the appropriate amine was added (3 eq) and the reaction was stirred overnight at room temperature. After approx 14 hr (TLC 5% MeOH in DCM), the solvent was evaporated *in vacuo*. The crude was dissolved in DCM (75 ml), washed 3 times with saturated $NaHCO_3$ solution (50 ml), dried ($MgSO_4$) and taken to dryness. The crude obtained was purified by silica gel chromatography (0%-5% MeOH in DCM).

2-(diethylamino)-N-(4-nitrophenyl)acetamide (2a)



4-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol), chloroacetyl chloride (0.98 g, 8.70 mmol) and diethylamine (1.59 g, 21.7 mmol) reacted in THF (20 ml) to afford **(2a)** as a yellow oil (0.63 g, 36%); Rf 0.26 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.80 (1H, s, N*H*), 8.21-8.19 (2H, m, 2 x Ar*H*), 7.79-7.71 (2H, m, 2 x Ar*H*), 3.18 (2H, s, CH_2), 2.66 (4H , quartet, *J* = 7.2 Hz, 2 x CH_2), 1.09 (6H, t, *J* = 7.2 Hz, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 170.74 (*C*=O), 143.39 (2 x Ar-*C*), 125.08 (2 x Ar-*C*H), 118.66 (2 x Ar-*C*H), 58.03 (*C*H_2), 48.89 (2 x *C*H_2), 12.33 (2 x *C*H_3); LC-MS (5 min) m/z 252.37 $[C_{12}H_{17}N_3O_3+H]^+$ (100), Rt 0.8 min; HRMS calc $C_{12}H_{18}N_3O_3$ $[M+H]^+$ 252.1353, found $[M+H]^+$ 252.1345. Spectroscopic data are in accordance with those reported in literature[2].

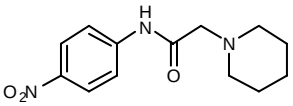
N-(3-nitrophenyl)-3-(pyrrolidin-1-yl)propanamide (2b)



3-Nitroaniline (3.00 g, 21.72 mmol), TEA (4.44 g, 43.96 mmol), 3-chloropropionyl chloride (2.77 g, 21.81 mmol) and pyrrolidine (4.70 g, 66.20 mmol) reacted in THF (40 ml) to afford **(2b)** as a yellow solid (lit. brown oil[1]) (5.55 g, 96%); Rf 0.24 [5% MeOH in DCM]; mp 125 °C; δ_H ($CDCl_3$, 400 MHz) 11.71 (1H, s, N*H*), 8.33-8.32 (1H, m, Ar*H*), 7.88-7.87 (1H, m, Ar*H*), 7.86-7.85 (1H, m, Ar*H*), 7.42 (1H, m, Ar*H*), 2.90-2.87 (2H, m, CH_2), 2.73-2.70 (4H, m, 2 x

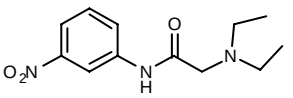
C*H*$_2$), 2.59-2.56 (2H, m, C*H*$_2$), 1.93-1.90 (4H, m, 2 x C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 171.21 (*C*=O), 148.52 (Ar-*C*), 139.92 (Ar-*C*), 129.55 (Ar-*C*H), 125.24 (Ar-*C*H), 117.99 (Ar-*C*H), 114.20 (Ar-*C*H), 53.08 (*C*H$_2$), 51.09 (2 x *C*H$_2$), 34.38 (*C*H$_2$), 23.67 (2 x *C*H$_2$); LC-MS (5 min) m/z $[C_{13}H_{17}N_3O_3+H]^+$ 263.35 (100), Rt 0.8 min; HRMS calc $C_{13}H_{18}N_3O_3$ $[M+H]^+$ 264.1343, found $[M+H]^+$ 264.1350; anal. CHN calc. $C_{13}H_{17}N_3O_3$. C 59.3%, H 6.5%, N 16.0%, found C 59.5%, H 6.6%, N 15.8%.

N-(4-nitrophenyl)-2-(piperidin-1-yl)acetamide (2c)



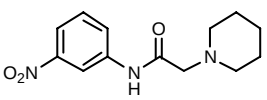
4-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol), chloroacetyl chloride (0.98 g, 8.70 mmol) and piperidine (1.85 g, 21.7 mmol) reacted in THF (20 ml) to afford **(2c)** as a yellow oil (0.88 g, 46%); Rf 0.24 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.66 (1H , s, N*H*), 8.17-8.13 (2H, m, 2 x Ar*H*), 7.73-7.69 (2H, m, 2 x Ar*H*), 3.08 (2H, s, C*H*$_2$), 2.53-2.50 (4H, m, 2 x C*H*$_2$), 1.65-1.59 (4H, m, 2 x C*H*$_2$), 1.48-1.44 (2H, m, C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 169.52 (*C*=O), 143.40 (Ar-*C*), 143.21 (Ar-*C*), 124.88 (2 x Ar-*C*H), 118.63 (2 x Ar-*C*H), 62.55 (*C*H$_2$), 54.77 (2 x *C*H$_2$), 26.10 (2 x *C*H$_2$), 23.34 (*C*H$_2$); LC-MS (5 min) m/z 264.54 $[C_{13}H_{17}N_3O_3+H]^+$ (100), Rt 1.13 min; HRMS calc $C_{13}H_{18}N_3O_3$ $[M+H]^+$ 264.1343, found $[M+H]^+$ 264.1340. Spectroscopic data are in accordance with those reported in literature[2].

2-(diethylamino)-N-(3-nitrophenyl)acetamide (2d)



3-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol), chloroacetyl chloride (0.98 g, 8.70 mmol) and diethylamine (1.59 g, 21.7 mmol) reacted in THF (20 ml) to afford **(2d)** as a yellow semisolid (0.92 g, 50%); Rf 0.26 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.68 (1H , s, N*H*), 8.36-8.35 (1H, m, Ar*H*), 8.05-8.03 (1H, m, Ar*H*), 7.93 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, Ar*H*), 7.51-7.47(1H, m, Ar*H*), 3.18 (2H, s, C*H*$_2$), 2.67 (4H, quartet, J = 7.2 Hz, 2 x C*H*$_2$), 1.10 (6H , t, J = 7.2 Hz, 2 x C*H*$_3$); δ_C ($CDCl_3$, 100 MHz) 169.95 (*C*=O), 147.27 (Ar-*C*), 138.64 (Ar-*C*), 129.61 (Ar-*C*H), 110.74 (Ar-*C*H), 109.17 (Ar-*C*H), 105.92 (Ar-*C*H), 58.14 (*C*H$_2$) , 48.79 (2 x *C*H$_2$), 12.39 (2 x *C*H$_3$); LC-MS (5 min) m/z 252.37 $[C_{12}H_{17}N_3O_3+H]^+$ (100), Rt 0.83 min; HRMS *m/z* calc. $C_{12}H_{18}N_3O_3$ $[M+H]^+$, found $[M+H]^+$; anal. CHN calc. $C_{12}H_{17}N_3O_3$. C 57.4%, H 6.8%, N 16.7%, found C 57.3%, H 7.1%, N 16.6%.

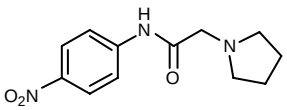
N-(3-nitrophenyl)-2-(piperidin-1-yl)acetamide (**2e**)



3-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol),

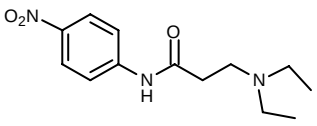
chloroacetyl chloride (0.98 g, 8.70 mmol) and piperidine (1.85 g, 21.7 mmol) reacted in THF (20 ml) to afford **(2e)** as a orange solid (1.47 g, 93%); Rf 0.25 [5% MeOH in DCM]; mp 101 °C; δ_H ($CDCl_3$, 400 MHz) 9.55 (1H , s, N*H*), 8.35-8.34 (1H, m, Ar*H*), 8.05 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz, Ar*H*), 7.96-7.93 (1H, m, Ar*H*), 7.51-7.48 (1H, m, Ar*H*), 3.11 (2H, s, CH_2), 2.57-2.55 (4H, m, 2 x CH_2), 1.67 (4H, quintet, J = 5.6 Hz, 2 x CH_2), 1.54-1.49 (2H, m, CH_2); δ_C ($CDCl_3$, 100 MHz) 169.50 (*C*=O), 148.63 (Ar-*C*), 138.85 (Ar-*C*), 129.86 (Ar-*C*H), 125.06 (Ar-*C*H), 118.60 (Ar-*C*H), 114.01 (Ar-*C*H), 62.64 (*C*H_2), 54.98 (2 x *C*H_2), 26.26 (2 x *C*H_2), 23.52 (*C*H_2); LC-MS (5 min) m/z 264.39 $[C_{13}H_{17}N_3O_3+H]^+$ (100), Rt 1.42 min; HRMS *m/z* calc. $C_{13}H_{18}N_3O_3$ $[M+H]^+$ 264.1343, found $[M+H]^+$ 264.1335; anal. CHN calc. $C_{13}H_{17}N_3O_3$. C 59.3%, H 6.5%, N 16.0%, found C 59.2%, H 6.4%, N 15.7%.

N-(4-nitrophenyl)-2-(pyrrolidin-1-yl)acetamide (**2f**)



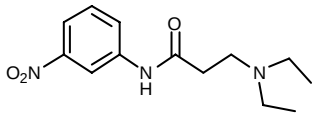
4-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol), chloroacetyl chloride (0.98 g, 8.70 mmol) and pyrrolidine (1.59 g, 21.7 mmol) reacted in THF (20 ml) to afford (**2f**) as a yellow solid (1.47 g, 91%); Rf 0.23 [5% MeOH in DCM]; mp 77 °C; δ_H ($CDCl_3$, 400 MHz) 9.51 (1H, s, N*H*), 8.20 (2H, d, J = 8.8 Hz, 2 x Ar*H*), 7.76 (2H, d, J = 8.8 Hz, 2 x Ar*H*), 3.13 (2H, s, CH_2), 2.71-2.69 (4H, m, 2 x CH_2), 1.87 (4H, quintet, J = 3.2 Hz, 2 x CH_2); δ_C ($CDCl_3$, 100 MHz) 169.78 (*C*=O), 143.49 (Ar-*C*), 143.43 (Ar-*C*), 125.04 (2 x Ar-*C*H), 118.83(2 x Ar-*C*H), 59.72 (*C*H_2), 54.67 (2 x *C*H_2), 24.09 (2 x *C*H_2); LC-MS (5 min) m/z 250.21 $[C_{12}H_{15}N_3O_3+H]^+$ (100), Rt 1.35 min; HRMS *m/z* calc. $C_{12}H_{16}N_3O_3$ $[M+H]^+$ 250.1186, found $[M+H]^+$ 250.1192. Spectroscopic data are in accordance with those reported in the literature[2].

3-(diethylamino)-N-(4-nitrophenyl)propanamide (**2g**)



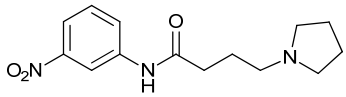
4-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol), 3-chloropropionyl chloride (1.10 g, 8.70 mmol) and diethylamine (1.59 g, 21.7 mmol) reacted in THF (20 ml) to afford (**2g**) as a yellow solid (1.14 g, 60%); Rf 0.15 [5% MeOH in DCM]; mp 91 °C; δ_H ($CDCl_3$, 400 MHz) 12.00 (1H , s, N*H*), 8.19-8.16 (2H, m, 2 x Ar*H*), 7.68-7.64 (2H, m, 2 x Ar*H*), 2.81-2.78 (2H, m, CH_2), 2.70 (4H, quartet , J = 7.2 Hz, 2 x CH_2), 2.55-2.52 (2H, m, CH_2), 1.15 (6H, t, J = 7.2, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 171.43 (*C*=O), 144.72 (Ar-*C*) , 142.94 (Ar-*C*), 125.12 (2 x Ar-*C*H), 118.74 (2 x Ar-*C*H), 48.67 (*C*H_2), 45.90 (2 x *C*H_2), 33.06 (*C*H_2), 11.46 (2 x *C*H_3); LC-MS (5 min) m/z 266.35 $[C_{13}H_{19}N_3O_3+H]^+$ (100), Rt 0.8 min; HRMS calc $C_{13}H_{20}N_3O_3$ $[M+H]^+$ 266.1505, found $[M+H]^+$ 266.1486; anal. CHN calc. $C_{13}H_{19}N_3O_3$. C 58.8%, H 7.2%, N 15.8%, found C 58.9%, H 7.3%, N 15.8%.

3-(diethylamino)-N-(3-nitrophenyl)propanamide (**2h**)



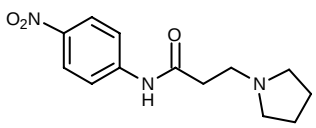
3-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol), 3-chloropropionyl chloride (1.10 g, 8.70 mmol) and diethylamine (1.59 g, 21.7 mmol) reacted in THF (20 ml) to afford (**2h**) as a yellow solid (0.74 g, 63%); Rf 0.12 [5% MeOH in DCM]; mp 44 °C; δ_H ($CDCl_3$, 400 MHz) 11.79 (1H , s, N*H*), 8.35 (1H, t, *J* = 2.0 Hz, Ar*H*), 7.91-7.87 (2H, m, 2 x Ar*H*), 7.46-7.41(1H, m, Ar*H*), 2.82-2.80 (2H, m, CH_2), 2.13 (4H, quartet , *J* = 7.2 Hz, 2 x CH_2), 2.56-2.53 (2H, m, CH_2), 1.15 (6H, t, *J* = 7.2, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 171.26 (*C*=O), 148.61 (Ar-*C*), 139.87 (Ar-*C*), 129.65 (Ar-*C*H), 125.09 (Ar-*C*H), 118.02 (Ar-*C*H), 114.03 (Ar-*C*H), 48.70 (*C*H_2), 45.95 (2 x *C*H_2), 32.89 (*C*H_2), 11.37 (2 x *C*H_3); LC-MS (5 min) m/z 266.43 $[C_{13}H_{19}N_3O_3+H]^+$ (100), Rt 0.9 min; HRMS calc $C_{13}H_{20}N_3O_3$ $[M+H]^+$ 266.1505, found $[M+H]^+$ 266.1492.

N-(3-nitrophenyl)-4-(pyrrolidin-1-yl)butanamide (2i)



Compound **(1)** (1.45 g, 6.00 mmol) was stirred overnight in excess of pyrrolidine (2.12 g, 30.0 mmol). Work up and purification were performed as reported in the general procedure **(2 a-k)**, to afford **(2i)** as a pale yellow oil (0.66 g, 79%); Rf 0.17 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 11.14 (1H , s, N*H*), 8.20-8.19 (1H, m, Ar*H*), 8.07-8.05(1H, m, Ar*H*), 7.90-7.87 (1H, m, Ar*H*), 7.47-7.43 (1H, m, Ar*H*), 2.69-2.64 (6H, m, 3 x CH_2), 2.60-2.57 (2H, m, CH_2), 1.93-1.87 (6H, m, 3 x CH_2); δ_C ($CDCl_3$, 100 MHz) 172.22 (*C*=O), 148.47 (Ar-*C*), 140.30 (Ar-*C*), 129.72 (Ar-*C*H), 125.34 (Ar-*C*H), 117.97 (Ar-*C*H), 113.96 (Ar-*C*H), 56.64 (*C*H_2), 54.08 (2 x *C*H_2), 38.06 (*C*H_2), 23.60 (*C*H_2), 23.47 (2 x *C*H_2); LC-MS (5 min) m/z 278.08 $[C_{14}H_{19}N_3O_3+H]^+$ (5), 207.06 $[(C_{14}H_{19}N_3O_3+H) - 71]^+$ (60), Rt 1.85 min; HRMS *m/z* calc. $C_{14}H_{20}N_3O_3$ $[M+H]^+$ 278.1505, found $[M+H]^+$ 278.1500. Spectroscopic data are in accordance with those reported in the literature[1].

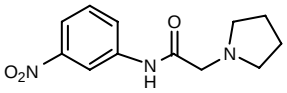
N-(4-nitrophenyl)-3-(pyrrolidin-1-yl)propanamide (2j)



4-Nitroaniline (2.00 g, 14.48 mmol), TEA (2.94 g, 29.11 mmol), 3-chloropropionyl chloride (2.19 g, 17.38 mmol) and pyrrolidine (2.90 g, 40.84 mmol) reacted in THF (35 ml) to afford **(2j)** as a yellow oil (lit. yellow solid[2]) (3.60 g, 97%); Rf 0.18 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 11.99 (1H , s, N*H*), 8.19-8.15 (2H, m, 2 x Ar*H*),7.63-7.59 (2H, m, 2 x Ar*H*), 2.89-2.86 (2H, m, CH_2), 2.73-2.69 (4H, m, 2 x CH_2), 2.58-2.55 (2H, m, CH_2), 1.95-1.91 (4H, m, 2 x

C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 171.42 (*C*=O), 144.86 (Ar-*C*), 142.93 (Ar-*C*), 125.07 (2 x Ar-*C*H), 118.92 (2 x Ar-*C*H), 53.04 (*C*H$_2$), 51.03 (2 x *C*H$_2$), 34.55 (*C*H$_2$), 23.74 (2 x *C*H$_2$); LC-MS (5 min) m/z 264.27 $[C_{13}H_{17}N_3O_3+H]^+$ (100), Rt 1.50 min; HRMS calc $C_{13}H_{18}N_3O_3$ $[M+H]^+$ 264.1343 , found $[M+H]^+$ 264.1348.

N-(3-nitrophenyl)-2-(pyrrolidin-1-yl)acetamide (2k)

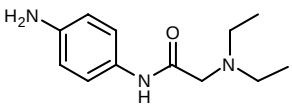


3-Nitroaniline (1.00 g, 7.24 mmol), TEA (1.47 g, 14.50 mmol), chloroacetyl chloride (0.98 g, 8.70 mmol) and pyrrolidine (1.51 g, 21.7 mmol) reacted in THF (20 ml) to afford **(2k)** as a yellow oil (1.76 g, 97%); Rf 0.23 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.40 (1H , s, N*H*), 8.35 (1H, t, *J* = 2.0 Hz, Ar*H*), 8.09 (1H, dd, J_1 = 8.0, J_2 = 2.0 Hz, Ar*H*), 7.96-7.93(1H, m, Ar*H*), 7.51-7.47 (1H, m, Ar*H*), 3.32 (2H, s, C*H*$_2$), 2.73-2.71 (4H, m, 2 x C*H*$_2$), 1.90-1.87 (4H, m, 2 x C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 169.67 (*C*=O), 148.59 (Ar-*C*), 138.88 (Ar-*C*), 129.85 (Ar-*C*H), 125.15 (Ar-*C*H), 118.59 (Ar-*C*H), 114.08 (Ar-*C*H), 59.65 (*C*H$_2$), 54.69 (2 x *C*H$_2$), 24.11(2 x *C*H$_2$); LC-MS (5 min) m/z 250.14 $[C_{12}H_{15}N_3O_3+H]^+$ (100), Rt 1.27 min; HRMS *m/z* calc. $C_{12}H_{16}N_3O_3$ $[M+H]^+$ 250.1186, found $[M+H]^+$ 250.1190. Spectroscopic data are in accordance with those reported in the literature[1]

General procedure for the synthesis of amino-compounds (3) a-k

The required compound **(2)** (1 eq) was dissolved in anhydrous THF in atmosphere of N_2 and 10% Pd/C (10% m/m) was added. The atmosphere was then saturated with H_2 and the mixture was stirred overnight. The crude was filtered on celite washing with EtOAc and the organic solution was evaporated *in vacuo*. The crude was dissolved in DCM (75 ml), washed 3 times with 5N aqueous NH_4OH solution (50 ml), dried ($MgSO_4$) and taken to dryness. The product obtained was utilised in the next step without any further purification.

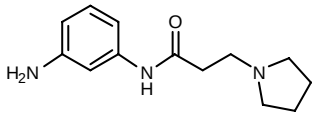
N-(4-aminophenyl)-2-(diethylamino)acetamide (3a)



Compound (**2a**) (0.39 g, 1.55 mmol) was dissolved in anhydrous THF (18 ml) in atmosphere of N_2, then Pd/C (0.039 g) and H_2 were added, to afford (**3a**) as a yellow oil (lit. yellow solid[2]) (0.26 g, 78%); Rf 0.44 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.16 (1H, s, N*H*), 7.36-7.31 (2H, m, 2 x Ar*H*), 6.68-6.63 (2H, m, 2 x Ar*H*), 3.57 (2H, broad s, N*H*$_2$), 3.10 (2H, s, C*H*$_2$), 2.62 (4H, m, 2 x C*H*$_2$), 1.07 (6H, t,

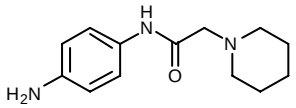
J = 7.2 Hz, 2 x C*H*$_3$); δ_C ($CDCl_3$, 100 MHz) 169.60 (*C*=O), 142.99 (Ar-*C*), 129.33 (Ar-*C*), 121.09 (2 x Ar-*C*H), 115.43 (2 x Ar-*C*H), 58.02 (*C*H$_2$), 48.83 (2 x *C*H$_2$), 12.42 (2 x *C*H$_3$); HRMS *m/z* calc. $C_{12}H_{20}N_3O$ $[M+H]^+$ 222.1601, found $[M+H]^+$ 222.1605.

N-(3-aminophenyl)-3-(pyrrolidin-1-yl)propanamide (**3b**)



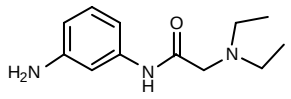
Compound (**2b**) (0.27 g, 1.16 mmol) was dissolved in anhydrous THF (18 ml) in atmosphere of N_2, then Pd/C (0.027 g) and H_2 were added, to afford (**3b**) as a yellow oil (lit. brown oil[1]) (0.29 g, 68%); Rf 0.45 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 11.05 (1H, s, N*H*), 7.18 (1H, m, Ar*H*), 7.04 (1H, t, *J* = 8.0, Ar*H*), 6.60 (1H, dd, *J* = 8.0, 1.20 Hz, Ar*H*), 6.38 (1H, dd, J_1 = 7.6, J_2 1.6 Hz, Ar*H*), 3.68 (2H, broad s, N*H*$_2$), 2.83-2.80 (2H, m, C*H*$_2$), 2.69-2.61 (4H, m, 2 x C*H*$_2$), 2.51-2.48 (2H, t, *J* = 6.0 Hz, C*H*$_2$), 1.89-1.85 (4H, m, 2 x C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 170.79 (*C*=O), 147.16 (Ar-*C*), 139.83 (Ar-*C*), 129.49 (Ar-*C*H), 110.33 (Ar-*C*H), 109.60 (Ar-*C*H), 106.44 (Ar-*C*H), 53.04 (*C*H$_2$), 51.32 (2 x *C*H$_2$), 34.73 (*C*H$_2$), 23.66 (2 x *C*H$_2$); HRMS *m/z* calc. $C_{13}H_{20}N_3O$ $[M+H]^+$ 234.1601, found 234.1615 $[M+H]^+$.

N-(4-aminophenyl)-2-(piperidin-1-yl)acetamide (**3c**)



Compound (**2c**) (0. 88 g, 3.34 mmol) was dissolved in anhydrous THF (22 ml) in atmosphere of N_2, then Pd/C (0.088 g) and H_2 were added, to afford (**3c**) as a yellow oil (lit. yellow solid[2]) (0.59 g, 76%); Rf 0.42 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.03 (1H , s, N*H*), 7.35-7.31 (2H, m, 2 x Ar*H*), 6.67-6.63 (2H, m, 2 x Ar*H*), 3.58 (2H, broad s, N*H*$_2$), 3.03 (2H, s, C*H*$_2$), 2.53-2.50 (4H, m, 2 x C*H*$_2$), 1.65-1.59 (4H, m, 2 x C*H*$_2$), 1.49-1.45 (2H, m, C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 168.45 (*C*=O), 143.02 (Ar-*C*), 129.25 (Ar-*C*), 121.19 (2 x Ar-*C*H), 115.39 (2 x Ar-*C*H), 62.68 (*C*H$_2$), 54.87 (2 x *C*H$_2$), 26.26 (2 x *C*H$_2$), 23.62 (*C*H$_2$); HRMS *m/z* calc. $C_{13}H_{20}N_3O$ $[M+H]^+$ 234.1601, found $[M+H]^+$ 234.1606.

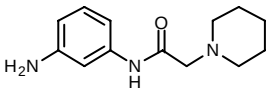
N-(3-aminophenyl)-2-(diethylamino)acetamide (**3d**)



Compound (**2d**) (0.73 g, 2.90 mmol) was dissolved in anhydrous THF (20 ml) in atmosphere of N_2, then Pd/C (0.073 g) and H_2 were added, to afford (**3d**) as a yellow oil (0.58g, 90%); Rf 0.44 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.28 (1H , s, N*H*), 7.26-7.20 (1H, m, Ar*H*), 7.07 (1H, t, *J* = 8.0 Hz, Ar*H*), 6.74-6.72 (1H, m, Ar*H*), 6.43-6.40 (1H, m, Ar*H*), 3.71 (2H, broad s, N*H*$_2$), 3.11 (2H, s, C*H*$_2$), 2.65-2.59 (4H, m, 2 x C*H*$_2$), 1.69 (6H , t, *J* = 7.2 Hz, 2 x C*H*$_3$); δ_C ($CDCl_3$, 100 MHz) 169.95 (*C*=O), 147.27 (Ar-*C*), 138.64 (Ar-*C*), 129.61 (Ar-*C*H), 110.74 (Ar-*C*H), 109.17 (Ar-*C*H),

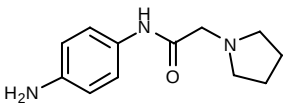
105.92 (Ar-*C*H), 58.14 (*C*H_2), 48.79 (2 x *C*H_2) , 12.39 (2 x *C*H_3); HRMS *m/z* calc. $C_{12}H_{20}N_3O$ $[M+H]^+$ 222.1601, found $[M+H]^+$.222.1610.

N-(3-aminophenyl)-2-(piperidin-1-yl)acetamide (**3e**)



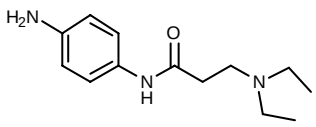
Compound (**2e**) (1.08 g, 4.10 mmol) was dissolved in anhydrous THF (22 ml) in atmosphere of N_2, then Pd/C (0.108 g) and H_2 were added, to afford (**3e**) as a dark yellow oil (0.94 g, 99%); Rf 0.44 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.16 (1H , s, N*H*), 7.21-7.20 (1H, m, Ar*H*), 7.10-7.06 (1H, m, Ar*H*), 6.73-6.71 (1H, m, Ar*H*), 6.42 (1H, dd, J_1 = 8.0, J_2= 2.4 Hz, Ar*H*), 3.71 (2H, broad s, NH_2), 3.04 (2H, s, CH_2), 2.54-2.53 (4H, m, 2 x CH_2), 1.63 (4H, quintet, J = 6.8 Hz, 2 x CH_2), 1.50-1.46 (2H, m, CH_2); δ_C ($CDCl_3$, 100 MHz) 168.84 (*C*=O), 147.26 (Ar-*C*), 138.72 (Ar-*C*), 129.65 (Ar-*C*H), 110.80 (Ar-*C*H), 109.32 (Ar-*C*H), 106.04 (Ar-*C*H), 62.82 (*C*H_2), 54.86 (2 x *C*H_2), 26.29 (2 x *C*H_2), 23.61 (*C*H_2); HRMS *m/z* calc. $C_{13}H_{20}N_3O$ $[M+H]^+$ 234.1601, found 234.1607 $[M+H]^+$.

N-(4-aminophenyl)-2-(pyrrolidin-1-yl)acetamide (**3f**)



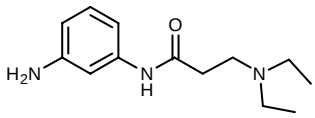
Compound (**2f**) (1.48 g, 5.93 mmol) was dissolved in anhydrous THF (25 ml) in atmosphere of N_2, then Pd/C (0.148 g) and H_2 were added, to afford (**3f**) as a yellow oil (0.91 g, 70%); Rf 0.19 [10% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 8.87 (1H , s, N*H*), 7.36-7.33 (2H, m, 2 x Ar*H*), 6.69-6.62 (2H, m, 2 x Ar*H*), 3.58 (2H, broad s, NH_2), 3.25 (2H, s, CH_2), 2.69-2.66 (4H, m, 2 x CH_2), 1.85-1.82 (4H, m, 2 x CH_2); δ_C ($CDCl_3$, 100 MHz) 168.67 (*C*=O), 143.05 (Ar-*C*), 129.24 (Ar-*C*), 121.33 (2 x Ar-*C*H), 115.38 (2 x Ar-*C*H), 59.64 (*C*H_2), 54.54 (2 x *C*H_2), 24.02 (2 x *C*H_2); HRMS *m/z* calc. $C_{12}H_{18}N_3O$ $[M+H]^+$ 220.1444, 220.1447 found $[M+H]^+$; anal. CHN calc. $C_{12}H_{17}N_3O$ C 65.7%, H 7.8%, N 19.2%, found C 65.5%, H 7.7%, N 19.0%. Spectroscopic data are in accordance with those reported in literature[1]

N-(4-aminophenyl)-3-(diethylamino)propanamide (**3g**)



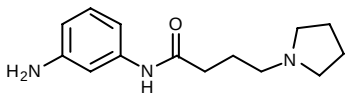
Compound (**2g**) (1.02 g, 3.86 mmol) was dissolved in anhydrous THF (20 ml) in atmosphere of N_2, then Pd/C (0.102 g) and H_2 were added, to afford (**3g**) as a yellow oil (0.49 g, 54%); Rf 0.10 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 10.90 (1H , s, N*H*), 7.32-7.29 (2H, m, 2 x Ar*H*), 6.65-6.62 (2H, m, 2 x Ar*H*), 3.54 (2H, broad s, NH_2), 2.76-2.73 (2H, m, CH_2), 2.64 (4H, quartet, J = 7.2 Hz, 2 x CH_2), 2.48-2.45 (2H, m, CH_2), 1.11 (6H, t, J = 7.2 Hz, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 170.46 (*C*=O), 142.49 (Ar-*C*), 130.50 (Ar-*C*), 121.14 (2 x Ar-*C*H), 115.47 (2 x Ar-*C*H), 49.04 (*C*H_2), 45.95 (2 x *C*H_2), 33.01 (*C*H_2), 11.55 (2 x *C*H_3); HRMS *m/z* calc. $C_{13}H_{22}N_3O$ $[M+H]^+$ 235.1685, found $[M+H]^+$ 235.1686.

N-(3-aminophenyl)-3-(diethylamino)propanamide (3h)



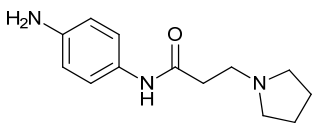
Compound (**2h**) (0.67 g, 2.54 mmol) was dissolved in anhydrous THF (20 ml) in atmosphere of N_2, then Pd/C (0.067 g) and H_2 were added, to afford (**3h**) as a yellow oil (0.31 g, 51%); Rf 0.10 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 11.11 (1H , s, N*H*), 7.23-7.22 (1H, m, Ar*H*), 7.05 (1H, t, *J* = 8.0 Hz, Ar*H*), 6.66-6.64 (1H, m, Ar*H*), 6.40-6.37 (1H, m, Ar*H*), 3.67 (2H, broad s, NH_2), 2.75 (2H, t, *J* = 6.0 Hz, CH_2), 2.65 (4H, quartet , *J* = 7.2 Hz, 2 x CH_2), 2.48-2.45 (2H, m, CH_2), 1.12 (6H, t, *J* = 7.2, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 170.88 (*C*=O), 147.18 (Ar-*C*), 139.83 (Ar-*C*), 129.54 (Ar-*C*H), 110.33 (Ar-*C*H), 109.50 (Ar-*C*H), 106.38 (Ar-*C*H), 49.00 (*C*H_2), 45.92 (2 x *C*H_2), 33.24 (*C*H_2), 11.52 (2 x *C*H_3); HRMS *m/z* calc. $C_{13}H_{22}N_3O$ $[M+H]^+$ 235.1685, found $[M+H]^+$ 235.1680.

N-(3-aminophenyl)-4-(pyrrolidin-1-yl)butanamide (3i)



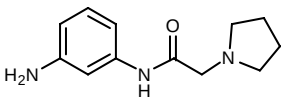
Compound (**2i**) (0.95 g, 3.40 mmol) was dissolved in anhydrous THF (20 ml) in atmosphere of N_2, then Pd/C (0.095 g) and H_2 were added, to afford (**3i**)as a pale yellow oil (0.45 g, 53%); Rf 0.10 [5% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 9.25 (1H , s, N*H*), 7.23-7.20 (1H, m, Ar*H*), 7.04 (1H, t, *J* = 8.0 Hz, Ar*H*), 6.60 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, Ar*H*), 6.40-6.37 (1H, m, Ar*H*), 3.68 (2H, broad s, NH_2), 2.59-2.44 (6H, m, 3 x CH_2), 2.47-2.44 (2H, m, CH_2), 1.91-1.81 (6H, m, 3 x CH_2); δ_C ($CDCl_3$, 100 MHz) 171.07 (*C*=O), 147.19 (Ar-*C*), 139.71 (Ar-*C*), 129.50 (Ar-*C*H), 110.49 (Ar-*C*H), 109.37 (Ar-*C*H), 106.44 (Ar-*C*H), 55.61 (*C*H_2), 53.93 (2 x *C*H_2), 36.86 (*C*H_2), 24.07 (*C*H_2), 23.56 (2 x *C*H_2); HRMS *m/z* calc. $C_{14}H_{22}N_3O$ $[M+H]^+$ 248.1757, found $[M+H]^+$ 248.1754. Spectroscopic data are in accordance with those reported in the literature[1]

N-(4-aminophenyl)-3-(dimethylamino)propanamide (3j)



Compound (**2j**) (3.00 g, 13.00 mmol) was dissolved in anhydrous THF (35 ml) in atmosphere of N_2, then Pd/C (0.30 g) and H_2 were added, to afford (**3j**) as a dark brown oil (0.78 g, 29%); Rf 0.20 [10% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 10.51 (1H , s, N*H*), 7.32-7.29 (2H, m, 2 x Ar*H*), 6.67-6.65 (2H, m, 2 x Ar*H*), 3.59 (2H, broad s, NH_2), 2.67-2.63 (2H, m, CH_2), 2.51-2.48 (2H, m, CH_2), 2.39-2.37 (6H, m, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 170.18 (*C*=O), 142.67 (Ar-*C*), 130.19 (Ar-*C*), 121.55 (2 x Ar-*C*H), 115.36 (2 x Ar-*C*H), 49.04 (*C*H_2), 55.17 (*C*H_2), 44.41 (2 x *C*H_3), 33.29 (*C*H_2); HRMS *m/z* calc. $C_{13}H_{20}N_3O$ $[M+H]^+$ 234.1601, found $[M+H]^+$ 234.1601. Spectroscopic data are in accordance with those reported in the literature[2].

N-(3-aminophenyl)-2-(pyrrolidin-1-yl)acetamide (**3k**)

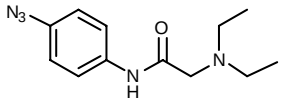


Compound (**2k**) (1.26 g, 5.00 mmol) was dissolved in anhydrous THF (28 ml) in atmosphere of N_2, then Pd/C (0.126 g) and H_2 were added, to afford (**3k**) as a yellow oil (0.84 g, 76%); Rf 0.50 [20% MeOH in DCM]; δ_H ($CDCl_3$, 400 MHz) 8.99 (1H , s, N*H*), 7.22 (1H, t, *J* = 2.0 Hz, Ar*H*), 7.08 (1H, t, *J* = 4.0 Hz, Ar*H*), 6.74-6.72 (1H, m, Ar*H*), 6.42 (1H, dd, J_1 = 2.5 Hz, J_2 = 8.0 Hz, Ar*H*), 3.70 (2H, broad s, NH_2), 3.25 (2H, s, CH_2), 2.69-2.66 (4H, m, 2 x CH_2), 1.86-1.83 (4H, m, 2 x CH_2); δ_C ($CDCl_3$, 100 MHz) 169.06 (*C*=O), 147.23 (Ar-*C*), 138.74 (Ar-*C*), 129.64 (Ar-*C*H), 110.84 (Ar-*C*H), 109.46 (Ar-*C*H), 106.18 (Ar-*C*H), 59.82 (*C*H_2), 54.54 (2 x *C*H_2), 24.07 (2 x *C*H_2); HRMS *m/z* calc. $C_{12}H_{18}N_3O$ $[M+H]^+$ 220.1444, found $[M+H]^+$ 220.1446. Spectroscopic data are in accordance with those reported in the literature[1].

General procedure for the synthesis of azides (4) a-k

The required compound **(3)** (1 eq) was dissolved in THF and cooled down in ice bath. This stirred mixture was treated first with HCl conc (5.5 eq), then with ^{t}BuONO (2.5 eq). The reaction was stirred in ice bath for 1.5 hours and after that time NaN_3 (3 eq) was added, followed by water, added very carefully until the fizz disappeared. The reaction was allowed to warm to room temperature and stirred overnight. The mixture was carefully neutralised with aqueous $NaHCO_3$ solution and THF was evaporated *in vacuo*. The aqueous solution was extracted three times with EtOAc (75 ml), dried ($MgSO_4$) and taken to dryness. The product obtained was utilised in the final step without any further purification.

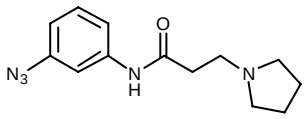
N-(4-azidophenyl)-2-(diethylamino)acetamide (4a)



Compound **(3a)** (0.35 g, 1.60 mmol) was dissolved in THF (6 ml), then HCl conc (0.69 ml, 8.80 mmol), ^{t}BuONO (0.39 g, 3.8 mmol) and NaN_3 (0.29 g, 4.50 mmol) were added, to afford **(4a)** as a orange oil (0.33 g, 83%); Rf 0.24 [5% MeOH in DCM]; IR (film): 3280.78, 2969.49, 2116.96, 1681.97, 1506.48 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 9.41 (1H, s, N*H*), 7.60-7.55 (2H, m, 2 x Ar*H*), 7.00-6.98 (2H, m, 2 x Ar*H*), 3.14 (2H, s, CH_2), 2.65 (4H, quartet, *J* = 7.2 Hz, 2 x CH_2), 1.09 (6H, t, *J* = 7.2 Hz, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 170.07 (*C*=O), 135.46 (Ar-*C*), 134.84 (Ar-*C*), 120.71 (2 x Ar-*C*H), 119.50 (2 x Ar-*C*H), 58.04 (*C*H_2), 48.88 (2 x *C*H_2) , 12.41 (2 x *C*H_3); LC-MS (5 min) m/z 248.24 $[C_{12}H_{17}N_5O +H]^+$ (60), 220.21 $[(C_{12}H_{17}N_5O +H) - 28]^+$ (100) Rt 2.07 min; HRMS

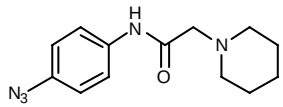
m/z calc. $C_{12}H_{18}N_5O$ $[M+H]^+$ 248.1506, found $[M+H]^+$ 248.1508. Spectroscopic data are in accordance with those reported in the literature[2].

N-(3-azidophenyl)-3-(pyrrolidin-1-yl)propanamide (4b)



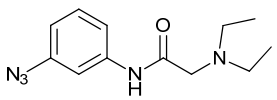
Compound **(3b)** (1.07 g, 4.6 mmol) was dissolved in THF (10 ml), then HCl conc (2.10 ml, 25.3 mmol), ^{t}BuONO (1.19 g, 11.5 mmol) and NaN_3 (0.90 g, 13.80 mmol) were added, to afford **(4b)** as a brown oil (1.15 g, 96%); Rf 0.22 [10% MeOH in DCM]; IR (film): 3319.22 , 2936.15, 2116.10, 1659.31, 1542.96 cm^{-1}. δ_H ($CDCl_3$, 400 MHz) 11.31 (1H, s, N*H*), 7.36 (1H, t, *J* = 2.0, Ar*H*), 7.25 (t, *J* = 8.0 Hz, 1H), 7.14 (1H, m, Ar*H*), 6.73 (1H, m, Ar*H*), 2.88-2.86 (2H, m, CH_2), 2.72-2.69 (4H, m, 2 x CH_2), 2.56-2.54 (2H, m, CH_2), 1.92-1.89 (4H, m, 2 x CH_2); δ_C ($CDCl_3$, 100 MHz) 170.88 (*C*=O), 140.73 (Ar-*C*), 140.31 (Ar-*C*), 130.02 (Ar-*C*H), 115.93 (Ar-*C*H), 113.98 (Ar-*C*H), 110.25 (Ar-*C*H), 53.14 (*C*H_2), 51.25 (2 x *C*H_2), 34.54 (*C*H_2), 23.69 (2 x *C*H_2); LC-MS (5 min) m/z 260.09 $[C_{13}H_{17}N_5O$ +H$]^+$ (60), 232.06 $[(C_{13}H_{17}N_5O$ +H) - 28$]^+$ (100) Rt 2.10 min; HRMS *m/z* calc. $C_{13}H_{18}N_5O$ $[M+H]^+$ 260.1506, found $[M+H]^+$260.1520. Spectroscopic data are in accordance with those reported in the literature[1].

N-(4-azidophenyl)-2-(piperidin-1-yl)acetamide (4c)



Compound **(3c)** (0.59 g, 2.50 mmol) was dissolved in THF (6 ml), then HCl conc (1.15 ml, 13.75 mmol), ^{t}BuONO (0.64 g, 6.25 mmol) and NaN_3 (0.49 g, 7.50mmol) were added, to afford **(4c)** as a orange solid (0.56g, 85%); Rf 0.23 [10% MeOH in DCM]; IR (film): 3283.09, 2934.63, 2116.65, 1648.31,1516.84 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 9.28 (1H , s, N*H*), 7.59-7.55 (2H, m, 2 x Ar*H*), 7.00-6.97 (2H, m, 2 x Ar*H*), 3.06 (2H, s, CH_2), 2.54 (4H, t, *J* = 4.8 Hz, 2 x CH_2), 1.67-1.61 (4H, m, 2 x CH_2), 1.51-1.47 (2H, m, CH_2); δ_C ($CDCl_3$, 100 MHz) 168.91 (*C*=O), 135.45 (Ar-*C*), 134.85 (Ar-*C*), 120.78 (2 x Ar-*C*H), 119.48 (2 x Ar-*C*H), 62.69 (*C*H_2), 54.92 (2 x *C*H_2), 26.29 (2 x *C*H_2), 23.58 (*C*H_2); LC-MS (5 min) m/z 260.09 $[C_{13}H_{17}N_5O$ +H$]^+$ (60), 232.13 $[(C_{13}H_{17}N_5O$ +H) - 28$]^+$ (100) Rt 2.20 min; HRMS *m/z* calc. $C_{12}H_{18}N_5O$ $[M+H]^+$ 260.1506, found $[M+H]^+$260.1500. Spectroscopic data are in accordance with those reported in the literature[2].

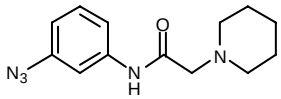
N-(3-azidophenyl)-2-(diethylamino)acetamide (4d)



Compound **(3d)** (0.52 g, 2.35 mmol) was dissolved in THF (6 ml), then HCl conc (1.08 ml, 12.92 mmol), ^{t}BuONO (0.61 g, 5.87 mmol) and NaN_3 (0.46 g, 7.05 mmol) were added, to afford **(4d)** as a dark brown oil (0.56 g, 85%); Rf 0.24 [10% MeOH in DCM]; IR (film): 3279.67, 2934.63, 2110.57, 1687.86, 1515.73 cm^{-1};

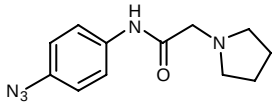
δ_H ($CDCl_3$, 400 MHz) 9.43 (1H, s, N*H*), 7.41 (1H, m, Ar*H*), 7.30-7.23 (2H, m, 2 x Ar*H*), 6.76 (1H, td, J_1 = 8.0, J_2 = 2.0 Hz, Ar*H*), 3.13 (2H, s, CH_2), 2.64 (4H, m, 2 x CH_2), 1.08 (6H, m, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 170.25 (*C*=O), 140.95 (Ar-*C*), 139.18 (Ar-*C*), 130.14 (Ar-*C*H), 115.60 (Ar-*C*H), 114.48 (Ar-*C*H), 109.96 (Ar-*C*H), 58.11 (*C*H_2), 48.92 (2 x *C*H_2), 12.40 (2 x *C*H_3); LC-MS (5 min) m/z 248.49 $[C_{12}H_{17}N_5O_3 + H]^+$ (25), 220.07 $[(C_{12}H_{17}N_5O_3 + H) - 28]^+$ (40) Rt 2.05 min; HRMS *m/z* calc. $C_{12}H_{18}N_5O$ $[M+H]^+$ 248.1506, found $[M+H]^+$248.1501

N-(3-azidophenyl)-2-(piperidin-1-yl)acetamide (**4e**)



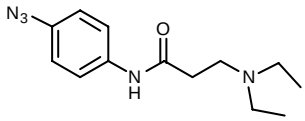
Compound (**3e**) (0.96 g, 4.12 mmol) was dissolved in THF (8 ml), then HCl conc (1.88 ml, 22.66 mmol), ^{t}BuONO (1.06 g, 10.30 mmol) and NaN_3 (0.80 g, 12.36 mmol) were added, to afford (**4e**) as a dark brown solid (0.80 g, 75%). Rf 0.25 [10% MeOH in DCM]; IR (film): 3232.92, 2942.05, 2107.98, 1667.82, 1519.43 cm^{-1}. δ_H ($CDCl_3$, 400 MHz) 9.31 (1H , s, N*H*), 7.42-7.41 (1H, m, Ar*H*), 7.74-7.31 (2H, m, 2 x Ar*H*), 6.79-6.76 (1H, m, Ar*H*), 3.07 (2H, s, CH_2), 2.55-2.53 (4H, m, 2 x CH_2), 1.68-1.62 (4H, m, 2 x CH_2), 1.52-1.48 (2H, m, CH_2). δ_C ($CDCl_3$, 100 MHz) 169.12 (*C*=O), 140.96 (Ar-*C*), 139.22 (Ar-*C*), 130.15 (Ar-*C*H), 115.71 (Ar-*C*H), 114.50 (Ar-*C*H), 110.07 (Ar-*C*H), 62.76 (*C*H_2), 54.94 (2 x *C*H_2), 26.30 (2 x *C*H_2), 23.60 (*C*H_2); LC-MS (5 min) m/z 259.14 $[C_{13}H_{17}N_5O + H]^+$ (50), 231.14 $[(C_{12}H_{15}N_5O_3 + H) - 28]^+$ (50) Rt 2.20 min; HRMS *m/z* calc. $C_{13}H_{18}N_5O$ $[M+H]^+$ 260.1506, found $[M+H]^+$260.1509.

N-(4-azidophenyl)-2-(pyrrolidin-1-yl)acetamide (4f)



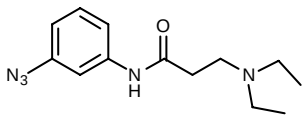
Compound **(3f)** (0.57 g, 2.61 mmol) was dissolved in THF (5 ml), then HCl conc (1.20 ml, 14.35 mmol), ^{t}BuONO (0.64 g, 6.26 mmol) and NaN_3 (0.51 g, 7.80 mmol) were added, to afford **(4f)** as a yellow solid (0.45 g, 71%); Rf 0.55 [10% MeOH in DCM]; IR (film): 3280.50, 2962.81, 2115.57, 1683.85, 1504.89 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 9.12 (1H , s, N*H*), 7.60-7.56 (2H, m, 2 x Ar*H*), 7.00-6.96 (2H, m, 2 x Ar*H*), 3.28 (2H, s, CH_2), 2.72-2.68 (4H, m, 2 x CH_2), 1.88-1.84 (4H, m, 2 x CH_2); δ_C ($CDCl_3$, 100 MHz) 169.05 (*C*=O), 135.50 (Ar-*C*), 134.86 (Ar-*C*), 120.91 (2 x Ar-*C*H), 119.46 (2 x Ar-*C*H), 59.69 (*C*H_2), 54.62 (2 x *C*H_2), 24.08 (2 x *C*H_2); LC-MS (5 min) m/z 246.15 $[C_{12}H_{15}N_5O + H]^+$ (60), 218.13 $[(C_{12}H_{15}N_5O + H) - 28]^+$ (100) Rt 2.03 min; HRMS *m/z* calc. $C_{12}H_{16}N_5O$ $[M+H]^+$ 246.1349, found $[M+H]^+$ 246.1354. Spectroscopic data are in accordance with those reported in the literature[2].

N-(4-azidophenyl)-3-(diethylamino)propanamide (4g)



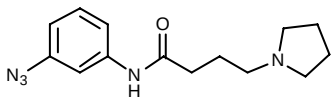
Compound **(3g)** (0.45 g, 1.89 mmol) was dissolved in THF (15 ml), then HCl conc (0.87 ml, 10.40 mmol), ^{t}BuONO (0.48 g, 4.70 mmol) and NaN_3 (0.37 g, 5.70 mmol) were added, to afford **(4g)** as a yellow oil (0.42 g, 86%); Rf 0.23 [10% MeOH in DCM]; IR (film): 3303.30, 2969.68, 2112.62, 1683.85, 1504.78 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 11.31 (1H , s, N*H*), 7.54-7.51 (2H, m, 2 x Ar*H*), 6.98-6.94 (2H, m, 2 x Ar*H*), 2.78 (2H, t, *J* = 6.0 Hz, CH_2), 2.70-2.65 (4H, m, 2 x CH_2), 2.52-2.49 (2H, m, CH_2), 1.14 (6H, t, *J* = 7.2, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 170.82 (*C*=O), 135.95 (Ar-*C*), 134.85 (Ar-*C*), 120.80 (2 x Ar-*C*H), 119.45 (2 x Ar-*C*H), 48.93 (*C*H_2), 45.96 (2 x *C*H_2), 33.00 (*C*H_2), 11.45 (2 x *C*H_3); LC-MS (5 min) m/z 262.51 $[C_{13}H_{19}N_5O$ +H$]^+$ (40), 235.24 $[(C_{13}H_{19}N_5O$ +H) - 28$]^+$ (90) Rt 2.08 min; HRMS *m/z* calc. $C_{13}H_{20}N_5O$ $[M+H]^+$ 262.1668, found $[M+H]^+$262.1660.

N-(3-azidophenyl)-3-(diethylamino)propanamide (4h)



Compound **(3h)** (0.30 g, 1.27 mmol) was dissolved in THF (5 ml), then HCl conc (0.58 ml, 6.35 mmol), ^{t}BuONO (0.33 g, 3.17 mmol) and NaN_3 (0.25 g, 3.80 mmol) were added, to afford **(4h)** as a dark yellow oil (0.27 g, 81%); Rf 0.25 [10% MeOH in DCM]; IR (film): 3312.79, 2968.39, 2109.20, 1664.21, 1595.29 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 11.45 (1H , s, N*H*), 7.43-7.42 (1H, m, Ar*H*), 7.30-7.28 (1H, m, Ar*H*), 7.22-7.19 (1H, m, Ar*H*), 6.77-6.74 (1H, m, Ar*H*), 2.81-2.78 (2H, m, CH_2), 2.69 (4H, quartet , *J* = 7.2 Hz, 2 x CH_2), 2.54-2.51 (2H, m, CH_2), 1.15 (6H, t, *J* = 7.2, 2 x CH_3); δ_C ($CDCl_3$, 100 MHz) 171.07 (*C*=O), 140.73 (Ar-*C*), 140.25 (Ar-*C*), 130.02 (Ar-*C*H), 115.72 (Ar-*C*H), 113.90 (Ar-*C*H), 110.11 (Ar-*C*H), 48.87 (*C*H_2), 45.91 (2 x *C*H_2), 33.06 (*C*H_2), 11.47(2 x *C*H_3); LC-MS (5 min) m/z 263.21 $[C_{13}H_{19}N_5O$+H$]^+$ (60), 234.11 $[(C_{13}H_{19}N_5O$+H) - 28$]^+$ (100) Rt 2.25 min; HRMS *m/z* calc. $C_{13}H_{20}N_5O$ $[M+H]^+$ 262.1506, found $[M+H]^+$262.1509.

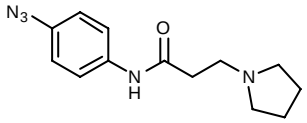
N-(3-azidophenyl)-4-(pyrrolidin-1-yl)butanamide (4i)



Compound **(3i)** (0.42 g, 1.70 mmol) was dissolved in THF (6 ml), then HCl conc (0.80 ml, 9.30 mmol), ^{t}BuONO (0.43 g, 4.20 mmol) and NaN_3 (0.33 g, 5.10 mmol) were added, to afford **(4i)** as a brown oil (0.36 g, 76%); Rf 0.25 [20% MeOH in DCM]; IR (film): 3192.73, 2958.15, 2113.17, 1681.90, 1594.15 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 10.10 (1H , s, N*H*), 7.36-7.35 (1H, m, Ar*H*), 7.27-7.19 (2H, m, 2 x Ar*H*), 6.74-6.71 (1H, m, Ar*H*), 2.65-2.61 (6H, m, 3 x CH_2), 2.54-2.51 (2H, m, CH_2), 1.93-1.85 (6H, m, 3 x CH_2); δ_C ($CDCl_3$, 100 MHz) 171.78 (*C*=O), 140.72 (Ar-*C*), 140.33 (Ar-*C*),

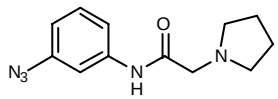
130.02 (Ar-*C*H), 115.79 (Ar-*C*H), 114.13 (Ar-*C*H), 110.08 (Ar-*C*H), 55.91 (*C*H_2), 53.96 (2 x *C*H_2), 37.25 (*C*H_2), 23.70 (*C*H_2), 23.60 (2 x *C*H_2); LC-MS (5 min) m/z 274.33 [$C_{14}H_{19}N_5O$ +H]$^+$ (5), 246.32 [($C_{14}H_{19}N_5O$ +H) - 28]$^+$ (80) Rt 1.95 min; HRMS *m/z* calc. $C_{14}H_{20}N_5O$ [M+H]$^+$ 274.1668, found [M+H]$^+$274.1673. Spectroscopic data are in accordance with those reported in the literature[1]

N-(4-azidophenyl)-3-(pyrrolidin-1-yl)propanamide (4j)



Compound **(3j)** (0.20 g, 0.86 mmol) was dissolved in THF (5 ml), then HCl conc (0.39 ml, 4.70 mmol), ^{t}BuONO (0.22 g, 2.10 mmol) and NaN_3 (0.17 g, 2.60 mmol) were added, to afford **(4j)** as an orange solid (0.3 6g, 99%); Rf 0.36 [20% MeOH in DCM]; IR (film): 3239.99, 2962.10, 2116.20, 1669.39, 1505.43 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 10.70 (1H , s, N*H*), 7.56 (2H, d, *J* = 8.0 Hz, 2 x Ar*H*), 6.95 (2H, d, *J* = 8.0 Hz, 2 x Ar*H*), 3.13-3.10 (2H, m, C*H*$_2$), 2.97-2.92 (4H, m, 2 x C*H*$_2$), 2.78-2.77 (2H, m, C*H*$_2$), 1.95-2.00 (4H, m, 2 x C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 169.46 (*C*=O), 135.74 (Ar-*C*), 135.18 (Ar-*C*), 121.13 (2 x Ar-*C*H), 119.38 (2 x Ar-*C*H), 53.60 (*C*H_2), 51.42 (2 x *C*H_2), 34.06 (*C*H_2), 23.55 (2 x *C*H_2); LC-MS (5 min) m/z 260.17 [$C_{13}H_{17}N_5O$ +H]$^+$ (60), 232.14 [($C_{13}H_{17}N_5O$ +H) - 28]$^+$ (100) Rt 2.17 min; HRMS *m/z* calc. $C_{13}H_{18}N_5O$ [M+H]$^+$ 260.1511, found [M+H]$^+$260.1506. Spectroscopic data are in accordance with those reported in the literature[1].

N-(3-azidophenyl)-2-(pyrrolidin-1-yl)acetamide (4k)

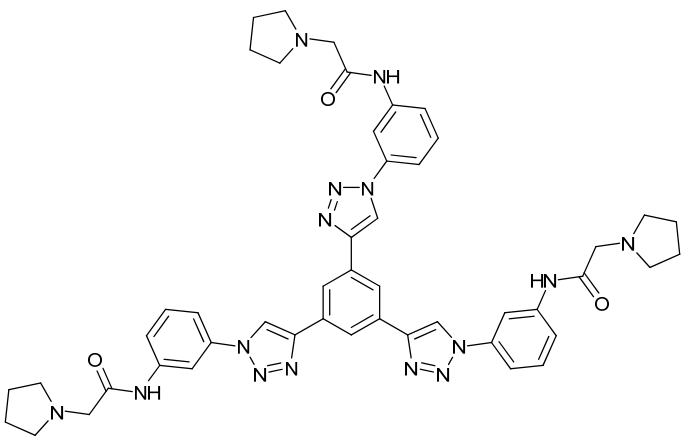


Compound **(3k)** (0.66 g, 3.03 mmol) was dissolved in THF (10 ml), then HCl conc (1.39 ml, 16.67 mmol), ^{t}BuONO (0.78 g, 7.57 mmol) and NaN_3 (0.59 g, 9.10 mmol) were added, to afford **(4k)** as a dark brown solid (0.55 g, 74%); Rf 0.35 [20% MeOH in DCM]; IR (film): 3208.78, 2964.28, 2112.00, 1660.71, 1519.43 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 9.14 (1H , s, N*H*), 7.42-7.41 (1H, m, Ar*H*), 7.30-7.28 (2H, m, 2 x Ar*H*), 6.79-6.76 (1H, m, Ar*H*), 3.27 (2H, s, C*H*$_2$), 2.72-2.69 (4H, m, 2 x C*H*$_2$), 1.88-1.85 (4H, m, 2 x C*H*$_2$); δ_C ($CDCl_3$, 100 MHz) 169.34 (*C*=O), 140.89 (Ar-*C*), 139.19 (Ar-*C*), 130.13 (Ar-*C*H), 115.80 (Ar-*C*H), 114.54 (Ar-*C*H), 110.12 (Ar-*C*H), 59.77 (*C*H_2), 54.63 (2 x *C*H_2), 24.10 (2 x *C*H_2); LC-MS (5 min) m/z 246.45 [$C_{12}H_{15}N_5O_3$ +H]$^+$ (50), 218.46 [($C_{12}H_{15}N_5O$ +H) - 28]$^+$ (50) Rt 2.12 min; HRMS *m/z* calc. $C_{12}H_{16}N_5O$ [M+H]$^+$ 246.1349, found [M+H]$^+$ 246.1347.

General procedure for the synthesis of tri-click compounds (5-15)

1,3,5-triethynylbenzene (1 eq) was dissolved in the appropriate volume of solvent (50% H_2O-50% ^tBuOH), followed by the required azide **(4a-k)** (4 eq), then by the catalytic mixture of $CuSO_4$ x $5H_2O$ (0.05 eq) and sodium ascorbate (0.2 eq). An extra catalyst, bathophenanthrolinedispufonic acid disodium salt hydrate (click catalyst, 0.1 eq) was added and in presence of azides with n = 2, 3 (compounds **(4-b, g, h, i, j)** the reaction was performed in excess of the required amine (6 eq), to avoid elimination. The mixture reacted under microwave irradiation for 15 minutes at 110°C and was monitored by LC-MS [Solvents: A = H_2O, 0.1% formic acid; B = CH_3CN, 0.1% formic acid. 0 min (95% A, 5%B), 1 min (95% A, 5%B), 3 min (50% A, 50%B), 5 min (95% A, 5%B)]. After completion, the reaction was filtered *in vacuo* and the solid obtained was dried for further purification. NH_4 (aq) 5N (20 ml) was added to the aqueous phase and the solution was extracted 3 times with DCM (30 ml), dried ($MgSO_4$) and taken to dryness. The solids obtained both via filtration and extraction were purified by HPLC [(Solvents: A = H_2O, 0.1% formic acid; B = CH_3CN, 0.1% formic acid. 0 min (100%A, 0%B), 6 min (70%A, 30%B),19 min (60% A, 40%B)] to afford the final compounds **(5-15)** with purities ≥ 95%. The yields were calculated from the integrations of the crude LC-MS peaks.

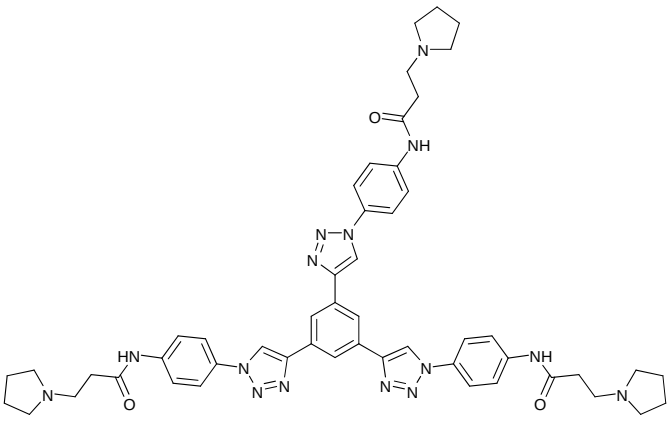
N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-3,1-diyl))tris(2-(pyrrolidin-1-yl)acetamide) (5)



1,3,5-triethynylbenzene (31 mg, 0.21 mmol) was dissolved in H_2O (2 ml) and ^tBuOH (2 ml), then (**4k**) (157 mg, 0.62 mmol), $CuSO_4$ (3 mg, 0.005 mmol), sodium ascorbate (8 mg, 0.04 mmol) and click catalyst (11 mg, 0.02 mmol) were added, to afford compound (**5**) as a brown crude solid (117 mg, 63%). Prep HPLC was conducted to yield a white solid; HPLC Rt 10.89 minutes, purity 95%; mp (dc) 174°C; δ_H (d_6-DMSO, 400 MHz) 10.10 (3H, s, 3 x N*H*), 9.46 (3H, s, 3 x C=C*H*), 8.47-8.45 (3H, m, 3 x Ar*H* , 8.18 (3H, s, 3 x Ar*H*), 7.78 (3H, d, *J* = 8.0 Hz, 3 x Ar*H*), 7.70 (3H, d, *J* = 8.0 Hz, 3 x Ar*H*), 7.61-7.57 (3H, m, 3 x Ar*H*), 3.36 (6H, s, 3 x CH_2), 2.67-2.65 (12H, m, 6 x CH_2), 1.81-1.75 (12H, m, 6 x CH_2); δ_C (d_6-DMSO, 100 MHz) 169.02 (3 x *C*=O), 146.70 (3 x *C*=CH), 139.95 (3 x Ar-*C*), 136.74 (3 x Ar-*C*), 131.62 (3 x Ar-*C*), 130.11 (3 x Ar-*C*H), 121.90 (3 x Ar-*C*H), 120.16 (3 x C=*C*H), 119.42 (3 x Ar-*C*H), 114.67 (3 x Ar-*C*H), 110.98 (3 x Ar-*C*H),

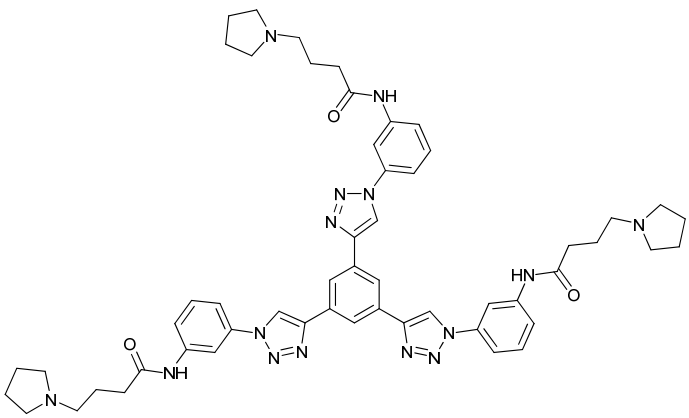
59.26 (3 x *C*H_2), 53.62 (6 x *C*H_2), 23.38 (6 x *C*H_2); HRMS *m/z* calc. $C_{48}H_{54}N_{15}O_3$ $[M+3H]^{3+}$ 296.1511, found 296.1498.

N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-4,1-diyl))tris(3-(pyrrolidin-1-yl)propanamide) (6)



1,3,5-triethynylbenzene (13 mg, 0.09 mmol) was dissolved in H_2O (2 ml) and ^{t}BuOH (2 ml), then (**4j**) (69 mg, 0.27 mmol), $CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (4 mg, 0.02 mmol), click catalyst (5 mg, 0.009 mmol) and pyrrolidine (38 mg, 0.54 mmol) were added, to afford compound **(6)** as a brown solid crude (101 mg, 76%). Prep HPLC was conducted to yield a brown solid; HPLC Rt 9.83 min, purity 100%; mp (dc) 132 °C; δ_H (d_6-DMSO, 400 MHz) 10.40 (3H, s, 3 x N*H*), 9.42 (3H, s, 3 x C=C*H*), 8.56 (3H, s, 3 x Ar*H*), 7.95 (6H, d, *J* = 8.8 Hz, 6 x Ar*H*), 7.85 (6H, d, *J* = 8.8 Hz, 6 x Ar*H*), 2.85 (6H, t, *J* = 6.9 Hz, 3 x CH_2), 2.59 (18H, m, 9 x CH_2), 1.74 (12H, m, 6 x CH_2); δ_C (d_6-DMSO, 100 MHz) 170.08 (3 x *C*=O), 146.57 (3 x *C*=CH), 139.51 (3 x Ar-*C*), 131.70 (3 x Ar-*C*), 131.55 (3 x Ar-*C*), 120.58 (3 x Ar-*C*H), 119.94 (3 x Ar-*C*H), 119.70 (3 x C=*C*H), 105.46 (3 x Ar-*C*H), 53.24 (3 x *C*H_2), 51.08 (6 x *C*H_2), 35.46 (3 x *C*H_2), 22.99 (6 x *C*H_2); HRMS *m/z* calc. $C_{51}H_{59}N_{15}O_3$ $[M+2H]^{2+}$ 464.7463, found 474.7451.

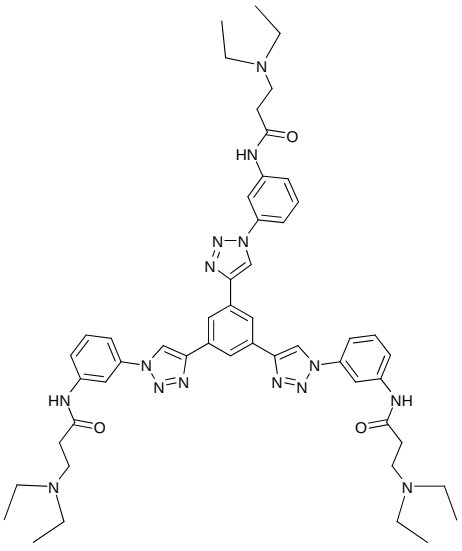
N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-3,1-diyl))tris(4-(pyrrolidin-1-yl)butanamide) (7)



1,3,5-triethynylbenzene (14 mg, 0.09 mmol) was dissolved in H_2O (2 ml) and ^{t}BuOH (2 ml), then (**4i**) (102 mg, 0.37 mmol), $CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (4 mg, 0.02 mmol), click catalyst (5 mg, 0.009 mmol) and pyrrolidine (39 mg, 0.55 mmol) were added, to afford compound (**7**) as a yellow solid crude (86.3 mg, 72%). Prep HPLC was conducted to yield a yellow solid; HPLC Rt 8.82 min, purity 100%; mp (dc) 129 °C; δ_H (d_6-DMSO, 400 MHz)

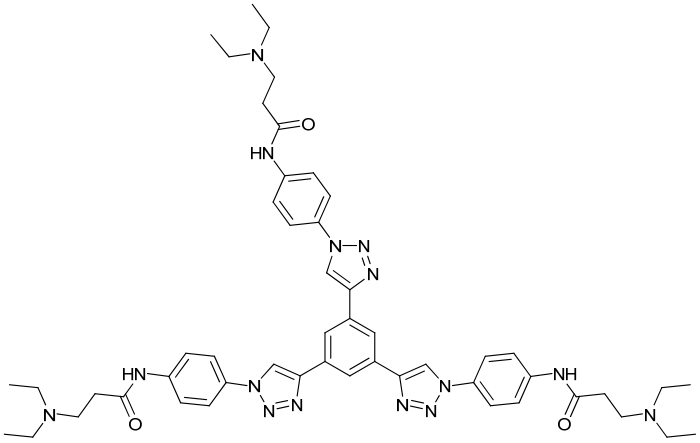
10.30 (3H, s, 3 x N*H*), 9.46 (3H, s, 3 x C=C*H*), 8.61 (3H, s, 3 x Ar*H*), 8.46 (3H, broad s, 3 x Ar*H*), 7.65 (6H, d, *J* = 7.7 Hz, 6 x Ar*H*), 7.57 (3H, t, *J* = 8.0 Hz, 3 x Ar*H*), 2.56 (18H, m, 9 x CH_2), 2.44 (6H, t, *J* = 7.2 Hz, 3 x CH_2), 1.83 (6H, quintet, *J* = 7.2 Hz, 3 x CH_2), 1.74-1.70 (12H, m, 6 x CH_2); δ_C (d_6-DMSO, 100 MHz): 171.41 (3 x *C*=O), 146.72 (3 x *C*=CH), 140.56 (3 x Ar-*C*), 136.77 (3 x Ar-*C*), 131.61 (3 x Ar-*C*), 130.13 (3 x Ar-*C*H), 121.88 (3 x Ar-*C*H), 120.14 (3 x C=*C*H), 118.90 (3 x Ar-*C*H), 114.26 (3 x Ar-*C*H), 110.42 (3 x Ar-*C*H), 54.66 (3 x *C*H_2), 53.27 (6 x *C*H_2), 34.15 (3 x *C*H_2), 23.57 (3 x *C*H_2), 22.93 (6 x *C*H_2); HRMS *m/z* calc. $C_{54}H_{65}N_{15}O_3$ $[M+2H]^{2+}$, theoretical mass 485.7697, found 485.7684.

N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-3,1-diyl))tris(3-(diethylamino)propanamide) (8)



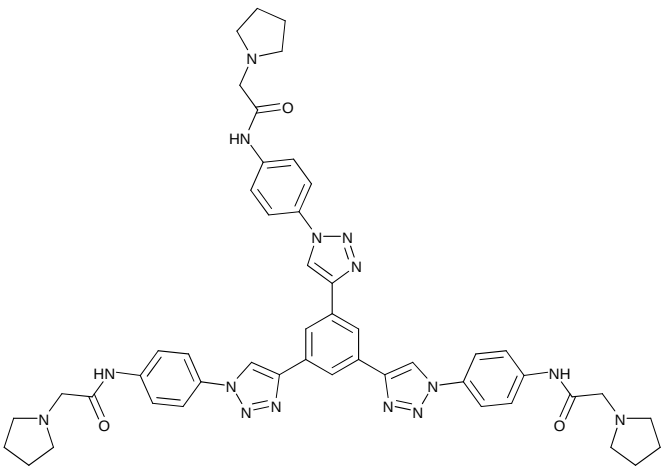
1,3,5-triethynylbenzene (15 mg, 0.10 mmol) was dissolved in H_2O (2.5 ml) and ^{t}BuOH (2.5 ml), then (**4h**) (103 mg, 0.40 mmol), $CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (4 mg, 0.02 mmol), click catalyst (5 mg, 0.009 mmol) and diethylamine (43 mg, 0.59 mmol) were added, to afford compound (**8**) as a brown crude solid (86.3 mg, 84%). Prep HPLC was conducted to yield a light yellow solid; HPLC Rt 10.56 min, purity 97%; mp (dc) 132 °C; δ_H (d_6-DMSO, 400 MHz) 10.49 (3H, s, 3 x N*H*), 9.46 (3H, s, 3 x C=C*H*), 8.61 (3H, s, 3 x Ar*H*), 8.45 (3H, s, 3 x Ar*H*), 7.65 (6H, m, 6 x Ar*H*), 7.58 (3H, m, 3 x Ar*H*), 2.90-2.87 (6H, m, 3 x CH_2), 2.65- 2.60 (12H, m, 6 x CH_2), 2.57-2.54 (6H, m, 3 x CH_2), 1.03 (18H, m, 6 x CH_3); δ_C (d_6-DMSO, 100 MHz) 170.50 (3 x *C*=O), 146.74 (3 x *C*=CH), 140.44 (3 x Ar-*C*), 136.82 (3 x Ar-*C*), 131.62 (3 x Ar-*C*), 130.21 (3 x Ar-*C*H), 121.93 (3 x Ar-*C*H), 120.15 (3 x C=*C*H), 118.91 (3 x Ar-*C*H), 114.37 (3 x Ar-*C*H), 110.44 (3 x Ar-*C*H), 47.89 (3 x *C*H_2), 46.05 (6 x *C*H_2), 33.55 (3 x *C*H_2), 11.13 (6 x *C*H_3); HRMS *m/z* calc. $C_{51}H_{67}N_{15}O_3$ $[M+2H]^{2+}$ 467.7697, found 467.7679.

N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-4,1-diyl))tris(3-(diethylamino)propanamide) (9)



1,3,5-triethynylbenzene (15 mg, 0.10 mmol) was dissolved in H_2O (2.5 ml) and ^{t}BuOH (2.5 ml), then (**4g**) (116 mg, 0.40 mmol), $CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (4 mg, 0.02 mmol), click catalyst (5 mg, 0.009 mmol) and diethylamine (43 mg, 0.59 mmol) were added, to afford compound **(9)** as a off-white crude solid (132 mg, 94%). Prep HPLC was conducted to yield an off white solid; HPLC Rt 10.07 min, purity 100%; mp (dc) 132 °C; δ_H (d_6-DMSO, 500 MHz) 10.43 (3H, s, 3 x N*H*), 9.41 (3H, s, 3 x C=C*H*), 8.55 (3H, s, 3 x Ar*H*), 7.95 (6H, d, *J* = 8.9 Hz, 6 x Ar*H*) 7.84 (6H, d, *J* = 8.9 Hz, 6 x Ar*H*), 2.82 (6H, t, *J* = 7.0 Hz, 3 x CH_2), 2.59-2.55 (12H, m, 6 x CH_2), 3 x CH_2 (triplet, 6H) of the side chain is hidden underneath DMSO peak, 1.01 (18H, t, *J* = 7.1Hz , 6 x *C*H_3); δ_C (d_6-DMSO, 125 MHz) 170.54 (3 x *C*=O), 146.59, (3 x *C*=CH), 139.56 (3 x Ar-*C*), 131.72 (3 x Ar-*C*), 131.53 (3 x Ar-*C*), 121.74 (3 x Ar-*C*H), 120.63 (6 x Ar-*C*H), 119.97 (3 x C=*C*H), 119.67 (6 x Ar-*C*H), 48.04 (3 x *C*H_2), 46.04 (6 x *C*H_2), 33.83 (3 x *C*H_2), 11.42 (6 x *C*H_3); HRMS *m/z* calc. $C_{51}H_{65}N_{15}O_3$ $[M+2H]^{2+}$ 485.7697, found 485.7678.

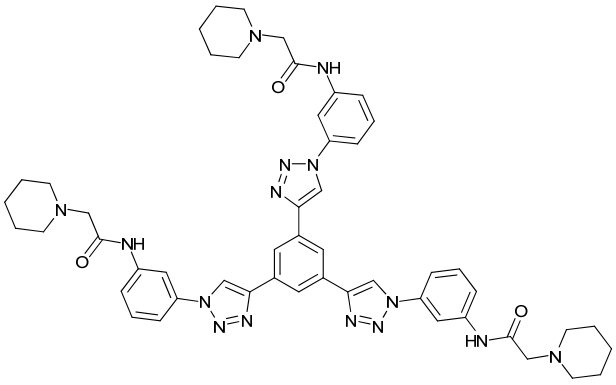
N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-4,1-diyl))tris(2-(pyrrolidin-1-yl)acetamide) (10)



1,3,5-triethynylbenzene (15 mg, 0.10 mmol) was dissolved in H_2O (2.5 ml) and ^{t}BuOH (2.5 ml), then **(4f)** (101 mg, 0.40 mmol), $CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (4 mg, 0.02 mmol) and click catalyst (5 mg, 0.009 mmol) were added, to afford compound **(10)** as a beige crude solid (93 mg, 75%). Prep HPLC was conducted to yield a white solid; HPLC Rt 9.31 min, purity 96%; mp (dc) 128 °C; δ_H (d_6-DMSO, 500 MHz) 10.05 (3H, s, 3 x N*H*), 9.43 (3H, s, 3 x C=C*H*), 8.56 (3H, s, 3 x Ar*H*), 7.96 (6H, d, *J* = 9.1 Hz, 6 x Ar*H*), 7.93 (6H, d, *J* = 9.1 Hz, 6

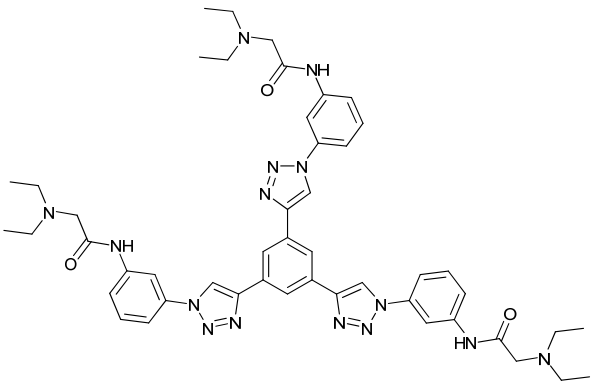
x Ar*H*), 3.34 (6H, s, 3 x CH_2), 2.65 (12H, m, 6 x CH_2), 1.78 (12H, m, 6 x CH_2); δ_C (d_6-DMSO, 125 MHz) 168.90 (3 x *C*=O), 146.61 (3 x *C*=CH), 139.04 (3 x Ar-*C*), 131.79 (3 x Ar-*C*), 131.71 (3 x Ar-*C*), 121.75 (3 x Ar-*C*H), 120.45 (3 x C=*C*H), 120.23 (6 x Ar-*C*H), 119.96 (6 x Ar-*C*H), 59.30 (3 x *C*H_2), 53.62 (6 x *C*H_2), 23.38 (6 x *C*H_2); HRMS *m/z* calc. $C_{48}H_{52}N_{15}O_3$ $[M+H]^+$ 886.4377, found 886.4388.

N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-3,1-diyl))tris(2-(piperidin-1-yl)acetamide) (11)



1,3,5-triethynylbenzene (15 mg, 0.10 mmol) was dissolved in H_2O (2.5 ml) and ^{t}BuOH (2.5 ml), then **(4e)** (107 mg, 0.40 mmol), $CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (4 mg, 0.02 mmol) and click catalyst (5 mg, 0.009 mmol) were added, to afford compound **(11)** as a beige solid crude (89 mg, 96%). Prep HPLC was conducted to yield a white solid; HPLC Rt 10.14 min, purity 96%; mp (dc) 149 °C; δ_H (d_6-DMSO, 500 MHz) 10.03 (3H, s, 3 x N*H*), 9.48 (3H, s, 3 x C=C*H*), 8.62 (3H, s, 3 x Ar*H*), 8.47-8.45 (3H, m, 3 x Ar*H*), 7.80-7.78 (3H, m, 3 x Ar*H*) 7.72- 7.70 (3H, m, 3 x Ar*H*), 7.62-7.59 (3H, m, 3 x Ar*H*), 3.15 (6H, s, 3 x CH_2), 1.63-1.58 (12H, s, 6 x CH_2), group of 6 C$H_2$of pyrrolidine hidden underneath DMSO peak, 1.44-1.43 (6H, m, 3 x CH_2); δ_C (d_6-DMSO, 125 MHz) 169.10 (3 x *C*=O), 146.72 (3 x *C*=CH), 139.88 (3 x Ar-*C*), 136.78 (3 x Ar-*C*), 131.6 (3 x Ar-*C*), 130.15 (3 x Ar-*C*H), 121.91 (3 x Ar-*C*H), 120.19 (3 x C=*C*H), 119.39 (3 x Ar-*C*H), 114.72 (3 x Ar-*C*H), 110.91 (3 x Ar-*C*H), 62.60 (3 x *C*H_2), 53.99 (6 x *C*H_2), 25.31 (6 x *C*H_2), 23.44 (3 x *C*H_2); HRMS *m/z* calc. $C_{51}H_{59}N_{15}O_3$ $[M+2H]^{2+}$464.7463, found 464.7455.

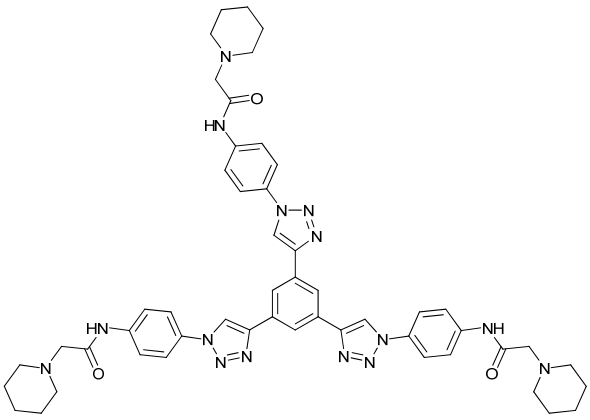
N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-3,1-diyl))tris(2-(diethylamino)acetamide) (12)



1,3,5-triethynylbenzene (17 mg, 0.11 mmol) was dissolved in H_2O (2.5 ml) and ^{t}BuOH (2.5 ml), then **(4d)** (110 mg, 0.45 mmol),

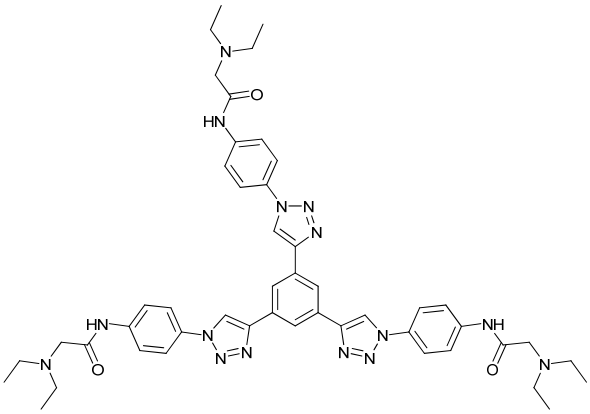
$CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (5 mg, 0.024 mmol) and click catalyst (6 mg, 0.01 mmol) were added, to afford compound **(12)** as a beige crude solid (98 mg, 93%). Prep HPLC was conducted to yield a white solid; HPLC Rt 9.75 min, purity 95%; mp (dc) 130 °C; δ_H (d_6-DMSO, 500 MHz) 9.99 (3H, s, 3 x N*H*), 9.48 (3H, s, 3 x C=C*H*), 8.61 (3H, s, 3 x Ar*H*), 8.45 (3H, t, *J* = 2.0 Hz, 3 x Ar*H*), 7.84-7.82 (3H, m, 3 x Ar*H*), 7.71-7.70 (3H, m, 3 x Ar*H*), 7.61-7.58 (3H, m, 3 x Ar*H*), 3.22 (6H, s, 3 x CH_2), 2.67-2.62 (12H, m, 6 x CH_2) 1.07-1.04 (18H, m, 6 x CH_3); δ_C (d_6-DMSO, 125 MHz) 170.46 (3 x *C*=O), 146.72 (3 x *C*=CH), 139.77 (3 x Ar-*C*), 136.81 (3 x Ar-*C*), 131.66 (3 x Ar-*C*), 130.21 (3 x Ar-*C*H), 121.92 (3 x Ar-*C*H), 120.24 (3 x C=*C*H), 119.32(3 x Ar-*C*H), 114.73 (3 x Ar-*C*H), 110.84 (3 x Ar-*C*H), 57.28 (3 x *C*H_2), 47.81 (6 x *C*H_2), 11.85 (6 x *C*H_3); HRMS *m/z* calc. $C_{48}H_{59}N_{15}O_3$ $[M+2H]^{2+}$ 446.7463, found 446.7446.

N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-4,1-diyl))tris(2-(piperidin-1-yl)acetamide) (13)



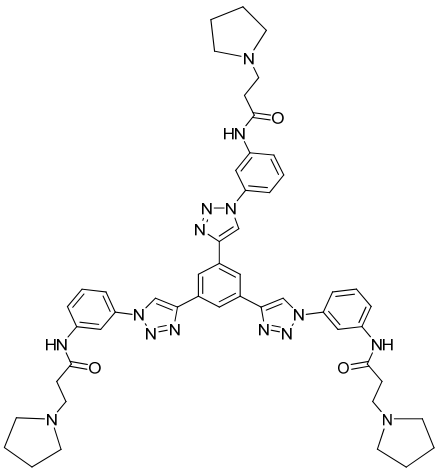
1,3,5-triethynylbenzene (27 mg, 0.11 mmol) was dissolved in H_2O (2.5 ml) and ^tBuOH (2.5 ml), then **(4c)** (183 mg, 0.78 mmol), $CuSO_4$ (2 mg, 0.009 mmol), sodium ascorbate (7 mg, 0.034 mmol) and click catalyst (9 mg, 0.02 mmol) were added, to afford compound **(13)** as a light brown crude solid (146 mg, 93%). Prep HPLC was conducted to yield a light orange solid; HPLC Rt 9.31 min, purity 95%; mp (dc) 150 °C; δ_H (d_6-DMSO, 500 MHz) 9.96 (3H, s, 3 x N*H*), 9.43 (3H, s, 3 x C=C*H*), 8.56 (3H, s, 3 x Ar*H*), 7.97 (6H, d, *J* = 9.0 Hz, 6 x Ar*H*), 7.92 (6H, d, *J* = 9.0 Hz, 6 x Ar*H*), 3.13 (6H, s, 3 x CH_2), group of 6 CH_2 of piperidine hidden underneath DMSO peak, 1.61-1.58 (12H, m, 6 x CH_2), 1.45-1.37 (6H, m, 3 x CH_2); δ_C (d_6-DMSO, 125 MHz) 168.91 (3 x *C*=O), 146.62 (3 x *C*=CH), 138.92 (3 x Ar-*C*), 131.83 (3 x Ar-*C*), 131.72 (3 x Ar-*C*), 121.77 (3 x Ar-*C*H), 120.48 (3 x C=*C*H), 120.19 (6 x Ar-*C*H), 119.96 (6 x Ar-*C*H), 62.56 (3 x *C*H_2), 53.99 (6 x *C*H_2), 25.32 (6 x *C*H_2), 23.45 (3 x *C*H_2); HRMS *m/z* calc. $C_{51}H_{59}N_{15}O_3$ $[M+2H]^{2+}$ 464.7463, found 464.7446.

N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-4,1-diyl))tris(2-(diethylamino)acetamide) (14)



1,3,5-triethynylbenzene (16 mg, 0.11 mmol) was dissolved in H_2O (2.5 ml) and ^tBuOH (2.5 ml), then **(4a)** (106 mg, 0.43 mmol), $CuSO_4$ (1 mg, 0.005 mmol), sodium ascorbate (4 mg, 0.021 mmol) and click catalyst (6 mg, 0.01 mmol) were added, to afford compound **(14)** as a beige crude solid (114mg, 93%). Prep HPLC was conducted to yield a pale yellow solid; HPLC Rt 9.89 min, purity 95%; mp (dc) 134 °C; δ_H (d_6-DMSO, 500 MHz) 9.93 (3H, s, 3 x N*H*), 9.44 (3H, s, 3 x C=C*H*), 8.60 (3H, s, 3 x Ar*H*), 7.97 (6H, d, *J* = 9.2 Hz, 6 x Ar*H*), 7.94 (6H, d, *J* = 9.2 Hz, 6 x Ar*H*), 3.21 (6H, s, 3 x CH_2), 2.63 (12H, quartet, *J* = 7.1 Hz, 6 x CH_2), 1.05 (18H, t, *J* = 7.1 Hz, 6 x CH_3); δ_C (d_6-DMSO, 125 MHz) 170.14 (3 x *C*=O), 146.60 (3 x *C*=CH), 138.75 (3 x Ar-*C*), 131.83 (3 x Ar-*C*), 131.69 (3 x Ar-*C*), 121.74 (3 x Ar-*C*H), 120.44 (3 x C=*C*H), 120.13 (6 x Ar-*C*H), 119.91 (6 x Ar-*C*H), 57.26 (3 x *C*H_2), 47.74 (6 x *C*H_2), 11.78 (6 x *C*H_3); HRMS *m/z* calc. $C_{48}H_{58}N_{15}O_3$ $[M+H]^+$ 892.4847, found 892.4823.

N,N',N''-((4,4',4''-(benzene-1,3,5-triyl)tris(1H-1,2,3-triazole-4,1-diyl))tris(benzene-3,1-diyl))tris(3-(pyrrolidin-1-yl)propanamide) (**15**)



1,3,5-triethynylbenzene (33 mg, 0.22 mmol) was dissolved in H_2O (2.5 ml) and ^tBuOH (2.5 ml), then (**4a**) (224 mg, 0.86 mmol), $CuSO_4$ (3 mg, 0.01 mmol), sodium ascorbate (8 mg, 0.04 mmol), click catalyst (12 mg, 0.02 mmol) and pyrrolidine (94 mg, 1.32 mmol) were added, to afford compound (**15**) as a brown crude solid (202 mg, 81%). Prep HPLC was conducted to yield a pale yellow solid; HPLC Rt 9.86 min, purity 98%; mp (dc) 131 °C; δ_H (d_6-DMSO, 500 MHz) 10.46 (3H, s, 3 x N*H*), 9.46 (3H, s, 3 x C=C*H*), 8.61 (3H, s, 3 x Ar*H*), 8.46 (3H, s, 3 x Ar*H*), 7.70-7.62 (6H, m, 6 x Ar*H*), 7.60-7.56 (3H, s, 3 x Ar*H*), 2.90-2.70 (6H, m, 3 x CH_2), 2.68-2.54 (18H, m, 9 x CH_2), 1.75-1.65 (12H, m, 6 x CH_2); δ_C (d_6-DMSO, 125 MHz) 170.28 (3 x *C*=O), 146.75 (3 x *C*=CH), 140.46 (3 x Ar-*C*), 136.81 (3 x Ar-*C*), 131.62 (3 x Ar-*C*), 130.22 (3 x Ar-*C*H), 121.93 (3 x Ar-*C*H), 120.17 (3 x C=*C*H), 118.93 (3 x Ar-*C*H), 114.39 (3 x Ar-*C*H), 110.44 (3 x Ar-*C*H),

53.26 (3 x CH_2), 51.07 (6 x CH_2), 35.48 (3 x CH_2), 23.01 (6 x CH_2); HRMS *m/z* calc. $C_{51}H_{58}N_{15}O_3$ $[M+H]^+$ 928.4847, found 928.4829.

[1] W. Drewe, *The Synthesis and Biological Evaluation of Novel Urea-Based Ligands as G-Quadruplex Interacting Agents,* PhD thesis, The School of Pharmacy, University of London, **2008**.
[2] A. D., Moorhouse, S., Haider, M., Gunaratnam, D., Munnur, S., Neidle, and J. E. Moses. *Mol. BioSyst.*, **2008**, 4, 629

Circular dichroism

Simultaneous UV & CD spectra of the 23mer DNA d[TA(GGGTTA)$_2$GGG] alone and with compound **15** in sodium and potassium phosphate buffers (100 mM, pH = 7.4) were measured on the Applied Photophysics Ltd (Leatherhead, UK) Chirascan Plus spectrometer. The UV absorbance & CD spectra were acquired between 400-220 nm in a rectangular 10mm and 1mm cell pathlengths. The instrument was flushed continuously with pure evaporated nitrogen throughout the measurements. Spectra were recorded with a 1.0nm step size, a 1.0s measurement time-per-point and a spectral bandwidth of 2.0nm. All spectra were buffer baseline corrected and measured at 20^{o}C. All CD spectra were smoothed using the Savitzky-Golay method with a window factor of 4 for a better presentation. The appropriate volume of ligand **15** in the required buffer was added to a 2.2 μM DNA solution in the same buffer, in order to obtain DNA:drug ratios from 1:1 to 1:6. These mixtures were heated at 85 °C, and then gradually cooled to room temperature. The collected UV and CD spectra of these solutions are reported in Figure S5.

UV and CD spectra of 23-mer telomeric DNA annealed in the presence of **15** in Na^+

UV and CD spectra of 23-mer telomeric DNA annealed in the presence of **15** in K^+

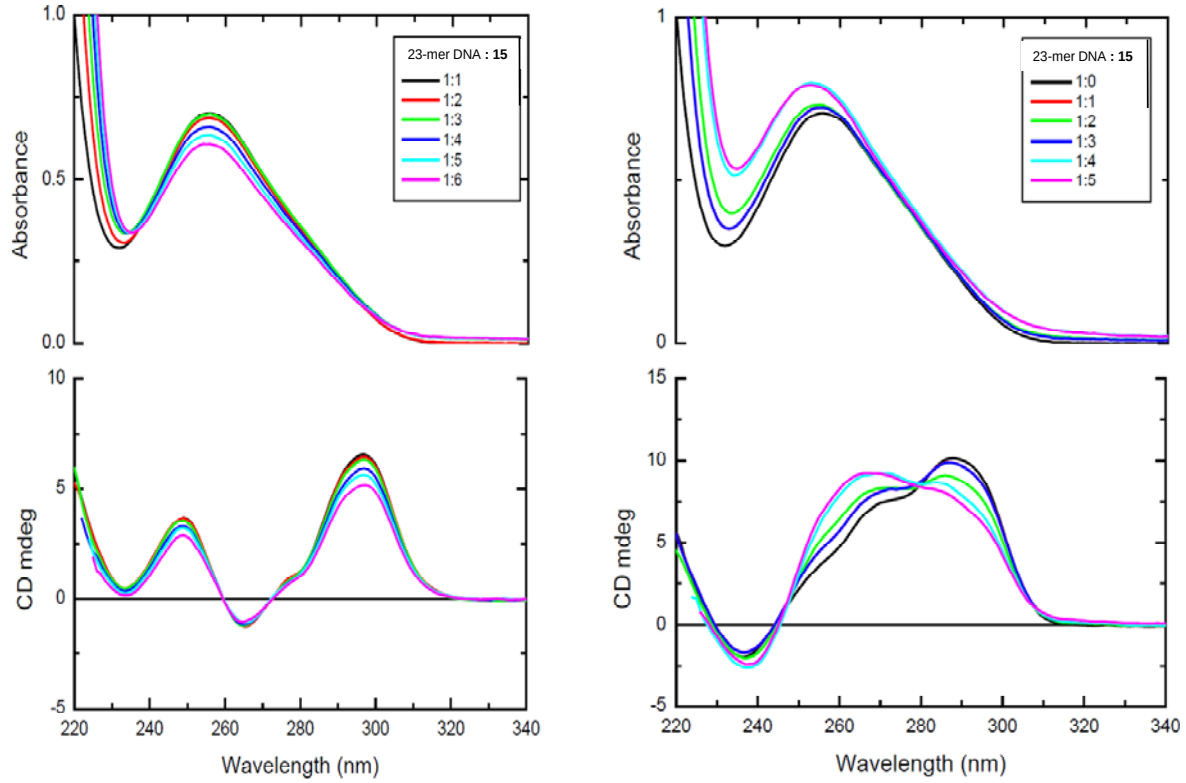


Figure S5. CD and related UV spectra of DNA annealed in the presence of increasing concentrations of ligand **15** in sodium (left) and potassium (right) buffer. Although in sodium the structure did not display any significant changes at increasing drug concentrations, the curve in potassium showed a clear shift towards a parallel arrangement.