SUPPORTING INFORMATION

2-Arylamino-6-Ethynylpurines Are Cysteine-Targeting Irreversible Inhibitors of Nek2 Kinase

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1. Synthesis and Characterisation of Compounds

1.1. General Experimental Details

Chemicals and Solvents

All chemical reagents were purchased from the Aldrich Chemical Company, Apollo Scientific or Alfa Aesar Chemicals and were of the highest available purity. Chemicals were used as supplied with no further treatment. If chemicals used were stated as dry/anhydrous, they were stored in SureSeal™ septum-sealed bottles and removed under an inert nitrogen environment, with the reaction being carried out under the relevant inert atmosphere. Palladium catalysts were stored and measured out under an inert atmosphere.

Chromatography

Reaction monitoring and compound identification was aided using Thin Layer Chromatography (TLC) and Retardation factor (Rf) values. TLC was conducted with Merck aluminium backed Si F_{254}, NH_{2} F_{254s} and RP-18 F_{254s} plates. Fluorescent compounds were
visualised under short wave (254 nm) UV irradiation. Compound purification was achieved using medium pressure ‘Flash’ column chromatography, with the use of Davisil silica 40-60 μm as the stationary phase, or Biotage automated chromatography using pre-packed silica cartridges. A Biotage SP4 automated flash purification system was used with UV monitoring at 298 nm and compound collection at 254 nm. Biotage KP-NH cartridges were employed for the separation of secondary, tertiary, and heterocyclic amines; using a primary amine (propyl amine) bonded silica. When stated, compounds were purified via semi-preparative HPLC, using an ACE 5 Phenyl 150 x 21.2 mm column using an Agilent 1200 Modular Preparative HPLC system.

### Analytical Techniques

All melting points were determined using a Stuart Scientific SMP3 or a Stuart Scientific SMP40 melting point apparatus and are uncorrected. \(^1\)H and \(^{13}\)C nuclear magnetic resonance (NMR) spectra were obtained as solutions in deuterated solvents DMSO-\(d_6\), MeOD or CDCl\(_3\) using a Bruker Avance III 500 spectrometer recording at 500 MHz. Chemical shifts (\(\delta\)) are reported in parts per million (ppm) and the spin-multiplicity abbreviated as: s (singlet), d (doublet), t (triplet), q (quartet), quin (quinet), sept (septet), m (multiplet), or br (broad), with coupling constants (\(J\)) given in Hertz (Hz). Liquid Chromatography – Mass Spectrometry (LC-MS) was carried out on a Micromass Platform LC running in both positive and negative electrospray mode with a PDA 240-400 nm detector using a Waters Symmetry Shield RP18 3 μm, 4.6 x 20 mm column with a flow rate of 3.0 mL/min. Alternatively, a Waters Acquity UPLC system was used, with a Waters SQD ESCi source using an Acquity UPLC BEH C18 1.7 μm, 2.1 x 50 mm column with a flow rate of 0.6 mL/min. The mobile phase used was 0.1% v/v formic acid (aq.)/MeCN. Fourier Transform Infrared (FTIR) spectra were obtained using a Bio-Rad FTS 3000MX diamond ATR as a neat sample. Ultraviolet (UV) absorption data were collected using a Hitachi U-2800A spectrophotometer in ethanol. High-resolution mass spectra were performed by the ESPRC UK National Mass Spectrometry Facility, Swansea University, Singleton Park, Swansea, SA2 8PP. The purity of final compounds was assessed by reversed-phase HPLC; all tested compounds were >95% purity. HPLC instrument, Agilent 1200 equipped with a photodiode array detector (190-400 nm). Sample temperature, ambient; injection volume, 5 μL; flow rate, 1 mL/min. 5% to 100% MeCN gradient over 9 min and an isocratic hold at 100% MeCN for 2.5 min, before returning to initial conditions. Mobile phase A = 0.1% ammonia in water or 0.1% formic acid in water, mobile phase B = MeCN. Column: Waters XSELECT CSH C18, 3.5 μm, 4.6 mm x 150 mm or Waters XTerra RP18, 5 μm, 4.6 mm x 150 mm. Column maintained at ambient temperature.
Microwave Assisted Synthesis

When stated, reactions were carried out under microwave irradiation, in sealed vessels, using a Biotage Initiator Sixty with robotic sample bed. Samples were irradiated at 2.45 GHz, able to reach temperatures of 60 - 250 °C with a rate of heating at 2-5 °C/sec, and pressures of up to 20 bar.

1.2. General Synthetic Procedures

General Procedure A: TFA/TFE coupling of anilines with 2-fluoropurines using conventional heating

TFA (2.5-5.0 equiv.) was added to a solution of the purine substrate (1.0 equiv.) and the required aniline (2.0 equiv.) in TFE (10 mL/mmol). The reaction mixture was heated at reflux for 24 h unless otherwise stated, after which the solution was cooled and evaporated to dryness. The resulting residue was dissolved in EtOAc (10 mL/mmol) and washed with a saturated aqueous solution of NaHCO₃ (5 mL/mmol) and brine (5 mL/mmol). The combined aqueous layers were extracted with EtOAc (10 mL/mmol), and the combined organic extracts were dried (MgSO₄) and concentrated in vacuo to give the crude product for chromatographic purification.

General Procedure B: TFA/TFE coupling of anilines with 2-fluoropurines using microwave heating

The purine substrate (1.0 equiv.), the required aniline (2.0 equiv.) and TFA (2.5-5.0 equiv.) were dissolved in TFE (10 mL/mmol) and heated under microwave irradiation at 140 °C for 2 h, unless otherwise stated. Following removal of the solvent in vacuo, the resulting residue was dissolved in EtOAc (10 mL/mmol) and washed with a saturated aqueous solution of NaHCO₃ (5 mL/mmol) and brine (5 mL/mmol). The combined aqueous layers were extracted with EtOAc (10 mL/mmol), and the combined organic extracts were dried (MgSO₄) and concentrated in vacuo to give the crude product for chromatographic purification.

General Procedure C: Removal of TIPS-protecting groups using TBAF

TBAF (1.2 equiv.) was added to a solution of the TIPS-protected substrate (1.0 equiv.) in THF (10-20 mL/mmol). The reaction mixture was stirred at RT for 5 min before being concentrated in vacuo and the crude residue purified by chromatography to afford the target compound.

General Procedure D: CDI mediated amide coupling reactions
CDI (2.0 equiv.) and DIPEA (2.0 equiv.) were added to a solution of the carboxylic acid substrate (1.0 equiv.) in dry DMF (10 mL/mmol). The mixture was stirred at RT for 1.5 h, at which point the required amine (4.0 equiv.) was added. Following a further 18 h stirring at RT, the solvent was removed in vacuo and the resulting residue was purified by chromatography to give the desired product.

**General Procedure E: Removal of PMB protecting groups using TFA**

The PMB-protected substrate (1.0 equiv.) was dissolved in TFA (10-20 mL/mmol) and the resulting solution was heated at reflux for 24 h, unless stated otherwise. The reaction mixture was evaporated to dryness and the resulting residue was dissolved in EtOAc (20 mL/mmol) and washed with a saturated aqueous solution of NaHCO$_3$ (2 × 10 mL/mmol) and brine (10 mL/mmol). The combined aqueous layers were extracted with EtOAc (20 mL/mmol) and the combined organic extracts were dried (MgSO$_4$), concentrated in vacuo, and the residue purified by chromatography to give the desired compound.

**General Procedure F: Removal of TIPS-protecting groups using KF and 18-crown-6**

KF (1.2 equiv.) and 18-crown-6 (0.1 equiv.) were added to a solution of the TIPS-protected substrate (1.0 equiv.) in THF (10 mL/mmol) and the reaction mixture was stirred at RT for 24 h. The solvent was removed in vacuo and the crude product was purified by chromatography.

**General Procedure G: Removal of TIPS-protecting groups using TBAF followed by removal of TBAF contaminant**

TBAF (1.2 equiv.) was added to a solution of the TIPS-protected substrate (1.0 equiv.) in THF (10-20 mL/mmol). The reaction mixture was stirred at RT for 5 min before being diluted with THF (100-200 mL/mmol) and the TBAF scavenger resin (10 × w/w) added. The resulting suspension was agitated at RT for 48 h, before being filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography to afford the target compound.

**General Procedure H: Sulfonamide synthesis by treatment of trifluoroethyl sulfonate esters with amines**

The trifluoroethyl sulfonate ester substrate (1.0 equiv.), the required amine (1.3 equiv.) and DBU (2.0 equiv.) were combined in dry THF (10 mL/mmol) in a sealed vial. The reaction mixture was heated under microwave irradiation at 160 °C for 15 min, before being evaporated to dryness. The resulting residue was dissolved in DCM (10 mL/mmol) and washed with a saturated aqueous solution of NaHCO$_3$ (10 mL/mmol), after which the biphasic mixture was passed through an Isolute® phase separator and the organic phase
was concentrated in vacuo. The crude residue was purified via chromatography to give the target compound.

**TBAF scavenger resin**

![TBAF scavenger resin](image)

Amberlite 15 Ion exchange resin (SO$_3$H, 100 mL) was loaded into a column and washed with water (400 mL). The column was eluted with sat. calcium hydroxide solution whilst the pH of the eluent was monitored. Once the initially pH neutral eluent became strongly basic, the column was eluted with water until the pH of the eluent returned to neutral. The resin was washed with DCM (300 mL), THF (300 mL) and Et$_2$O (300 mL), before being removed from the column and dried in a vacuum oven at 40 °C.

### 1.3. Compound Data

**6-Chloro-2-fluoro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (10)**

![Chemical structure](image)

Part 1. To a stirred solution of HBF$_4$ (48% aqueous, 120 mL) at 0 °C, was added 2-amino-6-chloropurine (6.0 g, 35.0 mmol). Over 20 min, a solution of NaNO$_2$ (4.9 g, 70.0 mmol) in water (200 mL) was added dropwise, ensuring the temperature remained close to 0 °C. The pale yellow solution was raised to RT and stirred for 18 h. The resulting solution was neutralised to pH 7 in an ice bath at 0 °C by addition of Na$_2$CO$_3$ (6.00 g) in water (200 mL). The crude material was purified by chromatography on silica (10% MeOH/DCM) to afford 6-chloro-2-fluoropurine as a white crystalline solid (4.52 g, 75%); m.p. 171-173 °C (lit., m.p. 174 °C); $\lambda_{\text{max}}$ (EtOH/nm) 393; IR (cm$^{-1}$) 2964, 2785, 1735, 1581; $^1$H NMR (500 MHz, DMSO-d$_6$) 8.60 (1H, s, H-8), 13.9 (1H, s, NH-9); LRMS (ES$^+$) $m/z$ 172.6 [M+H]$^+$.  

Part 2. 3,4-Dihydropyran (60 µL, 0.58 mmol) was added dropwise over 10 min to a vigorously stirred solution of 6-chloro-2-fluoropurine (100 mg, 0.58 mmol) and (rac)-camphorsulfonic acid (5 mg, 0.02 mmol) in EtOAc (50 mL) at 65 °C. The temperature was maintained at 65 °C for 18 h. The resulting bright yellow solution was neutralised to pH 7 by careful addition of aqueous NH$_3$ solution, until a cloudy suspension persisted. The crude
mixture was washed with brine (2 × 30 mL) and the aqueous phase was re-extracted with EtOAc (2 × 30 mL). The combined organic extracts were dried (Na$_2$SO$_4$) and purified by chromatography on silica (30% EtOAc/Petrol). The desired compound was isolated as a pale yellow oil which solidified on refrigeration (110 mg, 75%); m.p. 69-70 °C (lit. 6 m.p. not available); $\lambda_{\text{max}}$ (EtOH/nm) 269; IR (cm$^{-1}$) 3125, 2954, 2872, 2028, 1577; $^1$H NMR (300 MHz, DMSO-$d_6$) 1.60 (2H, m, CH$_2$), 1.74 (1H, m, CH), 2.01 (2H, m, CH$_2$), 2.32 (1H, s, CH), 3.71 (1H, t, $J = 12.0$ Hz, CH), 4.01 (1H, d, $J = 12.0$ Hz, CH), 5.64 (1H, d, $J = 12.0$ Hz, CH), 8.25 (1H, s, H-8); LRMS (ES$^+$) m/z 257.7 [M+H]$^+$. 

2-Fluoro-9-(tetrahydro-2H-pyran-2-yl)-6-((triisopropylsilyl)ethynyl)-9H-purine (11)

\[ \text{An oxygen-free solution of 6-chloro-2-fluoro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (10) (50 mg, 1.95 mmol), bis(triphenylphosphine)palladium (II) chloride (41 mg, 3.00 mol%) and copper iodide (7 mg, 2.00 mol%) in THF (10 mL) was degassed by bubbling nitrogen through the solution in a sealed Biotage microwave vial for 5 min. Trisopropylsilylacetylene (0.50 mL, 2.20 mmol) and triethylamine (0.70 mL, 4.90 mmol) were added to the mixture which was again degassed for 15 min. The solution quickly became dark red and stirring was continued at room temperature for 18 h. The black-brown suspension was filtered through Celite®, eluting with MeOH (3 × 20 mL). The product was purified by chromatography on silica (10% EtOAc/Petrol) and isolated as a viscous yellow oil (78 mg, 99%); $\lambda_{\text{max}}$ (EtOH/nm) 303.5; IR (cm$^{-1}$) 3433, 2945, 2865, 2705, 1702; $^1$H NMR (500 MHz, DMSO-$d_6$) 1.15 (21H, m, Si(CH(CH$_3$)$_2$)$_3$) and Si(CH(CH$_3$)$_2$)$_3$, 1.60 (2H, m, CH$_2$), 1.75 (1H, m, CH), 1.99 (2H, m, CH$_2$), 2.19 (1H, s, CH), 3.74 (1H, t, $J = 12.0$ Hz, CH), 4.16 (1H, d, $J = 12.0$ Hz, CH), 5.69 (1H, d, $J = 12.0$ Hz, CH), 8.30 (1H, s, H-8); LRMS (ES$^+$) m/z 403.4 [M+H]$^+$.} 

2-Fluoro-6-(2-(triisopropylsilyl)ethynyl)-9H-purine (12)
TFA (3 mL) was added to a solution of THP-protected purine 11 (0.644 g, 1.60 mmol) in IPA (15 mL). Water (3 mL) was added and the solution was heated to reflux for 2 h. The mixture was cooled and neutralised (conc. NH₃) before being extracted with EtOAc (3 × 50 mL) and the combined organic extracts dried (MgSO₄) and concentrated. The resulting residue was purified by chromatography on silica (30% EtOAc/Petrol) to give the desired product as a pale yellow oil (0.461 g, 91%); Rf 0.25 (7:3 Petrol/EtOAc); λmax (EtOH/nm) 302; IR (cm⁻¹) 2945, 2866, 2361, 2000, 1584; ¹H NMR (500 MHz, DMSO-d₆) 1.12-1.21 (21H, m, Si(CH(CH₃)₂)₃), 8.68 (1H, s, H-8), 13.89 (1H, br, NH-9); HRMS calcd. for C₁₆H₂₄FN₄Si (ES⁺) m/z 319.1749 [M+H]+, found 319.1752.

**N-Phenyl-6-((triisopropylsilyl)ethynyl)-9H-purin-2-amine (13)**

According to general procedure A, the title compound was prepared using: 2-fluoro-6-((triisopropylsilyl)ethynyl)-9H-purine (12) (0.70 g, 2.2 mmol) and aniline (0.40 mL, 4.4 mmol). The compound was isolated after chromatography (silica: 5% MeOH/DCM) followed by reversed phase column chromatography (C18 silica; 25% to 95% MeCN/water + 0.1% HCOOH), as a yellow oil (0.44 g, 47%); λmax (EtOH/nm) 276; IR (cm⁻¹) 3389, 2361, 2021; ¹H NMR (500 MHz, CDCl₃) 1.13 (21H, m, Si(CH(CH₃)₂)₃), 7.02 (1H, t, J = 7.5 Hz, H-4'), 7.33 (2H, dd, J = 7.4, 7.5 Hz, H-3' and H-5'), 7.80 (2H, d, J = 7.4 Hz, H-2' and H-6'), 10.42 (1H, s, NH); LRMS (ES+) m/z 392.0 [M+H]^+.

**4-(6-((Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)benzenesulfonamide (14)**
The title compound was synthesised following **general procedure A** using: 2-fluoro-6-((triisopropylsilyl)ethynyl)-9H-purine (12) (0.156 g, 0.49 mmol) and 4-aminobenzenesulfonamide (0.17 g, 0.98 mmol). The compound was purified using reversed phase column chromatography (C18 silica; 25% to 95% MeCN/water + 0.1% HCOOH), followed by trituration of the resulting oil using DCM, to obtain the product as a yellow solid (70 mg, 30%); m.p.163-165 °C; λ\textsubscript{max} (EtOH/nm) 361.0, 291.0, 286.5, 215.5; IR (cm\textsuperscript{-1}) 3327, 2944, 2867, 1569, 1531, 1368; \textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) 1.15 (21H, m, Si(C\textsubscript{6}H\textsubscript{5})(C\textsubscript{3}H\textsubscript{5})\textsubscript{2})\textsubscript{3}), 7.17 (2H, s, SO\textsubscript{2}NH\textsubscript{2}), 7.70 (2H, d, J = 9.0 Hz, H-2' and H-6'), 8.33 (1H, s, H-8); HRMS calcd for C\textsubscript{22}H\textsubscript{31}N\textsubscript{6}O\textsubscript{2}SSi [M+H]\textsuperscript{+} 471.19874, found 471.19420.

6-(2-(Triisopropylsilyl)ethynyl)-N-p-tolyl-9H-purin-2-amine (15)

2-Fluoropurine intermediate 12 (0.357 g, 1.12 mmol) and 4-methylaniline (0.241 g, 2.25 mmol) were reacted with TFA (432 μL, 5.61 mmol) in TFE (6 mL) according to **general procedure A**. The resulting crude residue was purified by chromatography on reverse phase silica (19:1 MeOH/H\textsubscript{2}O + 0.1% HCOOH) to give the desired compound as a yellow oil (0.215 g, 47%); R\textsubscript{f} 0.27 (19:1 MeOH/H\textsubscript{2}O + 0.1% HCOOH, C18); λ\textsubscript{max} (EtOH/nm) 276; IR (cm\textsuperscript{-1}) 2942, 2865, 2361, 2336, 1605; \textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) 1.13-1.22 (21H, m, Si(CH(\textsubscript{6}H\textsubscript{5})\textsubscript{3}))\textsubscript{3}), 2.31 (3H, s, CH\textsubscript{3}), 7.14 (2H, d, J = 8.4 Hz, H-3' and H-5'), 7.74 (2H, d, J = 8.4 Hz, H-2' and H-6'), 8.28 (1H, s, H-8), 9.65 (1H, s, NH), 13.11 (1H, br, NH-9); HRMS calcd. for C\textsubscript{23}H\textsubscript{32}N\textsubscript{5}Si (ES+) m/z 406.2421 [M+H]\textsuperscript{+}, found 406.2423.

N-(4-Ethylphenyl)-6-(2-(triisopropylsilyl)ethynyl)-9H-purin-2-amine (16)
2-Fluoropurine intermediate 12 (0.394 g, 1.24 mmol) and 4-ethylaniline (310 μL, 2.48 mmol) were reacted with TFA (477 μL, 6.19 mmol) in TFE (6 mL) according to general procedure A. The resulting residue was purified by chromatography on reverse phase silica (19:1 MeOH/H₂O + 0.1% HCOOH) to give the desired compound as a yellow oil/gum (0.281 g, 54%); Rf 0.27 (19:1 MeOH/H₂O + 0.1% HCOOH, C18); λ max (EtOH/nm) 277; IR (cm⁻¹) 2941, 2865, 2361, 2338, 2160, 1605; ¹H NMR (500 MHz, DMSO-d6) 1.13-1.21 (21H, m, Si(CH₃)₂), 1.23 (3H, t, J = 7.6 Hz, CH₂CH₃), 2.61 (2H, q, J = 7.6 Hz, CH₂CH₃) 7.17 (2H, d, J = 8.4 Hz, H-3’ and H-5’), 7.75 (2H, d, J = 8.4 Hz, H-2’ and H-6’), 8.27 (1H, s, H-8), 9.66 (1H, s, NH), 13.11 (1H, br, NH-9); HRMS calcd. for C₂₄H₃₄N₅Si (ES+) m/z 420.2578 [M+H]⁺, found 420.2579.

N-(4-Isopropylphenyl)-6-(2-(triisopropylsilyl)ethynyl)-9H-purin-2-amine (17)

2-Fluoropurine intermediate 12 (0.395 g, 1.24 mmol) and 4-isopropylaniline (353 μL, 2.48 mmol) were reacted with TFA (478 μL, 6.21 mmol) in TFE (6 mL) according to general procedure A. The resulting residue was purified by chromatography on reverse phase silica (19:1 MeOH/H₂O + 0.1% HCOOH) to give the desired compound as a yellow oil/gum (0.314 g, 58%); Rf 0.23 (19:1 MeOH/H₂O + 0.1% HCOOH, C18); λ max (EtOH/nm) 277; IR (cm⁻¹) 2956, 2866, 2360, 2157, 1607; ¹H NMR (500 MHz, DMSO-d6) 1.12-1.21 (21H, m, Si(CH(CH₃)₂)₃), 1.25 (6H, d, J = 7.0 Hz, CH(CH₃)₂), 2.89 (1H, sept, J = 7.0 Hz, CH(CH₃)₂), 7.19 (2H, d, J = 8.6 Hz, H-3’ and H-5’), 7.74 (2H, d, J = 8.6 Hz, H-2’ and H-6’), 8.26 (1H, s, H-8), 9.65 (1H, s, NH), 13.10 (1H, br, NH-9); HRMS calcd. for C₂₅H₃₆N₅Si (ES+) m/z 434.2734 [M+H]⁺, found 434.2735.

6-((Triisopropylsilyl)ethynyl)-N-(4-((triisopropylsilyl)oxy)phenyl)-9H-purin-2-amine (18)
2-Fluoropurine intermediate 12 (0.379 g, 1.19 mmol) and aniline 105 (0.610 g, 2.38 mmol) were reacted with TFA (460 μl, 5.96 mmol) in TFE (8 mL) according to general procedure A. Purification by chromatography on silica (7:3 Petrol/EtOAc) gave the target compound as an orange oil (0.181 g, 0.32 mmol, 27%); Rf 0.44 (7:3 Petrol/EtOAc); λmax (EtOH/nm) 277; IR (cm⁻¹) 2944, 2866, 2367, 2343, 2187, 1607; ¹H NMR (500 MHz, DMSO-d₆) 1.08 (18H, d, J = 7.3 Hz, OSi(CH(CH₃)₂)₃), 1.12-1.24 (24H, m, OSi(CH(CH₃)₂)₃ and Si(CH(CH₃)₂)₃), 6.80 (2H, d, J = 9.3 Hz, H-3' and H-5'), 7.64 (2H, d, J = 9.3 Hz, H-2' and H-6'), 8.20 (1H, s, H-8), 9.54 (1H, s, NH), 13.01 (1H, br, NH-9); HRMS calcd. for C₃₁H₅₀N₅O₃Si₂ (ES⁺) m/z 564.3548 [M+H]⁺, found 564.3543.

N-(4-methoxyphenyl)-6-((triisopropylsilyl)ethynyl)-9H-purin-2-amine (19)

The title compound was prepared according to general procedure A using: 2-fluoro-6-((triisopropylsilyl)ethynyl)-9H-purine (12) (0.20 g, 0.63 mmol) and para-anisidine (0.155 g, 1.26 mmol). Purification by chromatography on silica (50% EtOAc/petrol) followed by chromatography on reverse phase silica (25% to 95% MeCN/water + 0.1% HCOOH) gave the compound as a brown glassy solid (0.14 g, 43%); m.p. 101-103 °C; λmax (EtOH) 277.0, 210.0; IR (cm⁻¹) 2943, 2862, 1607, 1574, 1510; ¹H NMR (500 MHz, DMSO-d₆) 1.01 (21H, s, Si(CH(CH₃)₂)₃), 3.59 (3H, s, OCH₃), 6.72-6.74 (2H, d, J = 10.0 Hz, H-2' and H-6'), 7.52-7.54 (2H, d, J = 10.0 Hz, H-3' and H-5'), 8.08 (1H, s, H-8), 9.39 (1H, br s, NH); LRMS (ES⁺) m/z 422.3 [M+H]⁺.

6-(2-(Triisopropylsilyl)ethynyl)-N-(3-methoxyphenyl)-9H-purin-2-amine (20)
2-Fluoropurine intermediate 12 (0.421 g, 1.32 mmol) and 3-methoxyaniline (298 µL, 2.65 mmol) were reacted with TFA (510 µL, 6.62 mmol) in TFE (6 mL) according to general procedure A. Chromatography on reverse phase silica (19:1 MeOH/H₂O + 0.1% HCOOH) afforded the target compound as a yellow oil/gum (0.297 g, 53%); Rᵣ 0.33 (19:1 MeOH/H₂O + 0.1% HCOOH, C₁₈); λ_max (EtOH/nm) 274; IR (cm⁻¹) 3147, 2943, 2865, 2360, 1600; ¹H NMR (500 MHz, DMSO-d₆) 1.14-1.23 (21H, m, Si(CH(CH₃)₂)₃), 3.76 (3H, s, OCH₃), 6.52 (1H, ddd, J = 2.3, 2.5 and 8.5 Hz, H-4′), 7.17 (1H, dd, J = 8.1 and 8.2 Hz, H-5′), 7.29-7.33 (1H, m, H-6′), 7.64-7.67 (1H, m, H-2′), 8.26 (1H, s, H-8), 9.71 (1H, s, NH), 13.12 (1H, br, NH-9); HRMS calcd. for C₂₃H₃₂N₅O₅Si (ES⁺) m/z 422.2371 [M+H]⁺, found 422.2372.

6-(2-(Triisopropylsilyl)ethyl)-N-(4-chlorophenyl)-9H-purin-2-amine (21)

2-Fluoropurine intermediate 12 (0.200 g, 0.62 mmol), 4-chloroaniline (0.161 g, 1.26 mmol), TFA (242 µL, 3.14 mmol) and TFE (6 mL) were reacted according to general procedure A. The dried (MgSO₄) and concentrated crude material was purified by chromatography on silica (7:3 Petrol/EtOAc) to give the desired product as a yellow oil (0.165 g, 61%); Rᵣ 0.25 (7:3 Petrol/EtOAc); λ_max (EtOH/nm) 289; IR (cm⁻¹) 3118, 2945, 2866, 2361, 1541; ¹H NMR (500 MHz, DMSO-d₆) 1.13-1.22 (21H, m, Si(CH(CH₃)₂)₃), 7.33 (2H, d, J = 8.9 Hz, H-2‘ and H-6′), 7.85 (2H, d, J = 8.9 Hz, H-3‘ and H-5‘), 8.27 (1H, s, H-8), 9.91 (1H, s, NH), 13.17 (1H, br, NH-9); LRMS (ES⁺) m/z 426.2 [M⁺Cl+H]⁺, 428.2 [M⁺²Cl+H]⁺.

N-(3-Chlorophenyl)-6-(2-(triisopropylsilyl)ethyl)-9H-purin-2-amine (22)
2-Fluoropurine intermediate 12 (0.354 g, 1.11 mmol) and 3-chloroaniline (234 μL, 2.23 mmol) were reacted with TFA (429 μL, 5.57 mmol) in TFE (6 mL) according to general procedure A. Purification of the crude residue by chromatography on reverse phase silica (19:1 MeOH/H₂O + 0.1% HCOOH) gave the desired compound as a yellow oil/gum (0.281 g, 59%); Rₓ 0.27 (19:1 MeOH/H₂O + 0.1% HCOOH, C18); λₓ max (EtOH/nm) 276; IR (cm⁻¹) 3278, 2944, 2866, 2363, 1596; ¹H NMR (500 MHz, DMSO-d₆) 1.13-1.22 (21H, m, Si(CH₃)₂S), 6.96 (1H, ddd, J = 2.0, 2.2 and 8.0 Hz, H-4'), 7.29 (1H, dd, J = 8.0 and 8.1 Hz, H-5'), 7.64 (1H, ddd, J = 2.0, 2.1 and 8.1 Hz, H-6'), 8.11-8.14 (1H, m, H-2'), 8.30 (1H, s, H-8), 9.96 (1H, s, NH), 13.21 (1H, br, NH-9); HRMS calcd. for C₂₂H₂₉ClN₅Si (ES⁺) m/z 426.1875 [M+H]⁺, found 426.1877.

6-Ethynyl-N-phenyl-9H-purin-2-amine (23)

The TIPS-protected purine 13 (0.330 g, 0.84 mmol) and TBAF (1M in THF, 930 μL, 0.93 mmol) were reacted in THF (10 mL) according to general procedure C, with purification by chromatography on silica (EtOAc) to give the desired product as a yellow solid (0.201 g, 100%); Rₓ 0.27 (EtOAc); m.p. 140-160 °C (decomposed); λₓ max (EtOH/nm) 243; IR (cm⁻¹) 3414, 3111, 3072, 2920, 2110, 1704; ¹H NMR (500 MHz, DMSO-d₆) 4.86 (1H, s, C≡CH), 6.91-6.96 (1H, m, H-4'), 7.29 (2H, dd, J = 7.6 and 8.0 Hz, H-3' and H-5'), 7.80 (2H, dd, J = 2.0 and 8.0 Hz, H-2' and H-6'), 8.30 (1H, s, H-8), 9.70 (1H, s, NH), 13.17 (1H, br, NH-9); HRMS calcd. for C₁₃H₁₀N₅ (ES⁺) m/z 236.0934 [M+H]⁺, found 236.0931.

4-(6-Ethynyl-9H-purin-2-ylamino)benzenesulfonamide (24)
Following general procedure C, the title compound was prepared using: TBAF solution (1.0 M in THF, 170 μL, 0.17 mmol) and 4-(6-((triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)benzenesulfonamide (14) (50 mg, 0.11 mmol) in THF (3 mL) to complete the deprotection after 5 min. The title compound was purified by chromatography on silica (10% MeOH/DCM) and isolated as a beige solid (18 mg, 50%); m.p. 156-158 °C; λ_{max} (EtOH/nm) 356.0, 292.5, 215.5; IR (cm\(^{-1}\)) 3347, 3255, 2920, 2848, 2118, 1568, 1529, 1477, 1128; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 4.90 (1H, s, C≡C−H), 7.16 (2H, s, SO\(_2\)NH\(_2\)), 7.17 (2H, d, \(J = 9.0\) Hz, H-2' and H-6'), 7.93 (2H, d, \(J = 9.0\) Hz, H-3' and H-5'), 8.36 (1H, s, H-8); HRMS calcd for C\(_{13}\)H\(_{11}\)N\(_6\)O\(_2\)S [M+H]+ 315.0664, found 315.0687.

6-Ethynyl-N-p-tolyl-9H-purin-2-amine (25)

The TIPS-protected purine 15 (0.208 g, 0.51 mmol) was reacted with TBAF (1M in THF, 770 μL, 0.77 mmol) in THF (5 mL) according to general procedure C. Purification by chromatography on silica (1:4 Petrol/EtOAc) afforded the desired compound as a yellow solid (0.102 g, 80%); R\(_f\) 0.33 (EtOAc); m.p. 110-120 °C (decomposed); \(\lambda_{max}\) (EtOH/nm) 271; IR (cm\(^{-1}\)) 3409, 3268, 2852, 2721, 2110; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.26 (3H, s, CH\(_3\)), 4.82 (1H, s, C≡C-H), 7.10 (2H, d, \(J = 8.4\) Hz, H-2' and H-6'), 7.67 (2H, d, \(J = 8.4\) Hz, H-3' and H-5'), 8.24 (1H, s, H-8), 9.57 (1H, s, NH), 13.09 (1H, br, NH-9); HRMS calcd. for C\(_{14}\)H\(_{12}\)N\(_5\) (ES+) m/z 250.1087 [M+H]+, found 250.1084.

N-(4-Ethylphenyl)-6-ethynyl-9H-purin-2-amine (26)

The TIPS-protected purine 16 (0.258 g, 0.61 mmol) was reacted with TBAF (1M in THF, 920 μL, 0.92 mmol) in THF (6 mL) according to general procedure C. Purification by
chromatography on silica (1:4 Petrol/EtOAc) gave the desired compound as a yellow solid (0.119 g, 0.45 mmol, 74%); Rf 0.36 (EtOAc); m.p. 120-140 °C (decomposed); λmax (EtOH/nm) 274; IR (cm⁻¹) 3408, 3109, 2961, 2923, 2110, 1748; ¹H NMR (500 MHz, DMSO-d₆) 1.17 (3H, t, J = 7.7 Hz, CH₂CH₃), 2.56 (2H, q, J = 7.7 Hz, CH₂CH₃), 4.83 (1H, s, C≡CH), 7.13 (2H, d, J = 8.5 Hz, H-3' and H-5'), 7.68 (2H, d, J = 8.5 Hz, H-2' and H-6'), 8.26 (1H, s, H-8), 9.57 (1H, s, NH), 13.11 (1H, br, NH-9); HRMS calcd. for C₁₅H₁₄N₅ (ES+) m/z 264.1246 [M+H]⁺, found 264.1244.

N-(4-Isopropylphenyl)-6-ethynyl-9H-purin-2-amine (27)

The TIPS-protected purine 17 (0.259 g, 0.60 mmol) was reacted with TBAF (1M in THF, 900 μL, 0.90 mmol) in THF (6 mL) according to general procedure C. Purification by chromatography on silica (1:4 Petrol/EtOAc) afforded the target compound as a yellow solid (0.104 g, 0.37 mmol, 62%); Rf 0.40 (EtOAc); m.p. 120-140 °C (decomposed); λmax (EtOH/nm) 277; IR (cm⁻¹) 3408, 3275, 2957, 2814, 2366, 2111; ¹H NMR (500 MHz, DMSO-d₆) 0.97 (6H, d, J = 7.1 Hz, CH(C₃H₃)₂), 2.61 (1H, sept, J = 7.1 Hz, C(CH₃)₂), 4.59 (1H, s, C≡CH), 6.92 (2H, d, J = 8.5 Hz, H-3' and H-5'), 7.44 (2H, d, J = 8.5 Hz, H-2' and H-6'), 8.02 (1H, s, H-8), 9.32 (1H, s, NH), 12.88 (1H, br, NH-9); HRMS calcd. for C₁₆H₁₆N₅ (ES+) m/z 278.1404 [M+H]⁺, found 278.1400.

4-(6-Ethynyl-9H-purin-2-ylamino)phenol (28)

The TIPS-protected purine 18 (0.167 g, 0.30 mmol) was reacted with TBAF (1M in THF, 0.66 mL, 0.66 mmol) in THF (3 mL) according to general procedure C. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a beige solid (36 mg, 48%); Rf 0.36 (9:1 DCM/MeOH); m.p. 275-290 °C (decomposed); λmax (EtOH/nm) 274; IR (cm⁻¹) 3391, 3257, 3059, 2921, 2849, 2567, 2114, 1623; ¹H NMR (500 MHz, DMSO-d₆) 4.60 (1H, s, C≡CH), 6.51 (2H, d, J = 8.9 Hz, H-2' and H-6'), 7.32 (2H, d, J = 8.9 Hz, H-3' and H-5'), 8.00 (1H, s, H-8), 8.83 (1H, s, NH), 9.12 (1H, s, OH), 12.80 (1H, s, NH-9); HRMS calcd. for C₁₃H₁₀N₅O (ES+) m/z 252.0886 [M+H]⁺, found 252.0880.
6-Ethynyl-N-(4-methoxyphenyl)-9H-purin-2-amine (29)

The title compound was prepared according to general procedure C using: N-phenyl-6-((triisopropylsilyl)ethynyl)-9H-purin-2-amine (19) (70 mg, 0.17 mmol) and TBAF solution (1.0 M in THF, 0.25 mL, 0.25 mmol) in THF (10 mL). The compound was isolated after purification by chromatography on silica (5% MeOH/DCM) as a dark brown solid (29 mg, 63%); m.p. 129-131 °C; \( \lambda_{\text{max}} \) (EtOH/nm) 340.5, 291.0; IR (cm\(^{-1}\)) 3347, 2919, 2831, 2108, 1607, 1576; \(^1\)H NMR (500 MHz, DMSO-\text{d}_6) 4.89 (1H, s, C≡C−H), 3.59 (3H, s, OCH\(_3\)), 6.93 (2H, d, J = 6.5 Hz, H-2' and H-6'), 7.70 (2H, d, J = 6.5 Hz, H-3' and H-5'), 8.26 (1H, s, H-8), 9.51 (1H, br s, NH), 13.09 (1H, br s, NH-9); HRMS calcd for C\(_{14}\)H\(_{12}\)N\(_5\)O [M+H]\(^+\) 266.0664, found 266.0687.

6-Ethynyl-N-(3-methoxyphenyl)-9H-purin-2-amine (30)

The TIPS-protected purine 20 (0.271 g, 0.64 mmol) was reacted with TBAF (1M in THF, 960 μL, 0.96 mmol) in THF (6 mL) according to general procedure C. Purification by chromatography on silica (1:4 Petrol/EtOAc) gave the target compound as a yellow solid (0.119 g, 70%); R\(_f\) 0.30 (EtOAc); m.p. 130-150 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 272; IR (cm\(^{-1}\)) 3410, 3264, 3108, 2958, 2868, 2112, 1703; \(^1\)H NMR (500 MHz, DMSO-\text{d}_6) 3.76 (3H, s, OCH\(_3\)), 4.86 (1H, s, C≡CH), 6.53 (1H, ddd, J = 2.1 and 8.1 Hz, H-4'), 7.18 (1H, dd, J = 8.2 Hz, H-5'), 7.30-7.34 (1H, m, H-6'), 7.59-7.62 (1H, m, H-2'), 8.30 (1H, s, H-8), 9.68 (1H, s, NH), 13.20 (1H, s, NH-9); HRMS calcd for C\(_{14}\)H\(_{12}\)N\(_5\)O (ES+) \( \text{m/z} \) 266.1036 [M+H]\(^+\), found 266.1038.

N-(4-Chlorophenyl)-6-ethynyl-9H-purin-2-amine (31)
The TIPS-protected purine 21 (0.125 g, 0.29 mmol) and TBAF (1M in THF, 440 µL, 0.44 mmol) were reacted in THF (5 mL) according to general procedure C, with purification by chromatography on silica (EtOAc) to give the desired product as a yellow solid (77 mg, 81%); Rf 0.34 (EtOAc); m.p. 180-200 °C (decomposed); λmax (EtOH/nm) 241; IR (cm⁻¹) 3414, 3278, 2921, 2848, 2108, 1576, 1522; ¹H NMR (500 MHz, DMSO-d₆) 4.87 (1H, s, C≡CH), 7.34 (2H, d, J = 8.9 Hz, H-2’ and H-6’), 7.84 (2H, d, J = 8.9 Hz, H-3’ and H-5’), 8.32 (1H, s, H-8), 9.85 (1H, s, NH), 13.21 (1H, br, NH-9); HRMS calcd. for C₁₃H₉N₅Cl (ES+) m/z 270.0541 [M+H]^+, found 270.0540.

N-(3-Chlorophenyl)-6-ethynyl-9H-purin-2-amine (32)

The TIPS-protected purine 22 (0.255 g, 0.60 mmol) was reacted with TBAF (1M in THF, 900 µL, 0.90 mmol) in THF (6 mL) according to general procedure C. Purification by chromatography on silica (1:4 Petrol/EtOAc) afforded the desired compound as a yellow solid (0.126 g, 78%); Rf 0.35 (EtOAc); m.p. 130-150 °C (decomposed); λmax (EtOH/nm) 273; IR (cm⁻¹) 3407, 3282, 3078, 2926, 2812, 2110, 1706; ¹H NMR (500 MHz, DMSO-d₆) 4.93 (1H, s, C≡CH), 7.00 (1H, ddd, J = 2.0, 2.1 and 8.0 Hz, H-6’), 7.34 (1H, dd, J = 8.0 Hz, H-5’), 7.71 (1H, ddd, J = 2.0, 2.1 and 8.1 Hz, H-4’), 8.09 (1H, br, H-2’), 8.37 (1H, s, H-8), 9.97 (1H, s, NH), 13.31 (1H, s, NH-9); HRMS calcd. for C₁₃H₉N₅Cl (ES+) m/z 270.0541 [M+H]^+, found 270.0546.

3-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)benzamide (33)

Part 1. 2-Fluoropurine intermediate 12 (0.265 g, 0.83 mmol), aniline 112 (0.428 g, 1.67 mmol) and TFA (320 µL, 4.17 mmol) were reacted in TFE (5 mL) according to general procedure A. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) followed by normal phase silica (1:1 Petrol/EtOAc) gave the desired compound as a yellow oil (88 mg, 9%); Rf
0.42 (1:1 Petrol/EtOAc); \( \lambda_{\text{max}} \) (EtOH/nm) 279, 364; IR (cm\(^{-1}\)) 2864, 2159, 1739, 1641; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.13-1.18 (21H, m, Si(CH\((CH_3)_2\))_3), 3.74 (3H, s, OCH\(_3\)), 4.40 (2H, d, \( J = 5.9 \) Hz, CH\(_2\)NH), 6.90 (2H, d, \( J = 8.9 \) Hz, H-3" and H-5"), 7.27 (2H, d, \( J = 8.9 \) Hz, H-2" and H-6"), 7.35 (1H, m, H-5'), 7.41 (1H, m, H-6'), 7.95 (1H, m, H-4'), 8.22-8.25 (2H, m, H-8 and H-2'), 8.89 (1H, t, \( J = 5.9 \) Hz, CH\(_2\)N\(_\text{H}\)), 9.83 (1H, s, NH); HRMS calcd. for C\(_{31}\)H\(_{39}\)N\(_6\)O\(_2\)Si (ES+) \( m/z \) 555.2898 [M+H]+, found 555.2893.

Part 2. PMB-carboxamide (82 mg, 0.15 mmol) and TFA (2 mL) were reacted according to general procedure E over 24 h. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) afforded the target compound as a yellow oil (55 mg, 87%); \( R_f \) 0.41 (9:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 263, 365; IR (cm\(^{-1}\)) 3295, 2964, 2943, 1660; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.14-1.19 (21H, m, Si(CH\((CH_3)_2\))_3), 7.30-7.36 (2H, m, CON\(_\text{H}H'\) and H-5'), 7.40-7.42 (1H, m, H-6'), 7.85 (1H, s, CONH\(_\text{H}H'\)), 7.90-7.93 (1H, m, H-4'), 8.23 (1H, br, H-2'), 8.31 (1H, s, H-8), 9.83 (1H, s, NH); HRMS calcd. for C\(_{23}\)H\(_{31}\)N\(_6\)OSi (ES+) \( m/z \) 435.2323 [M+H]+, found 435.2325.

3-(3-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)phenyl) propanamide (34)

Part 1. 2-Fluoropurine intermediate 12 (0.273 g, 0.86 mmol), aniline 113 (0.490 g, 1.72 mmol) and TFA (330 \( \mu \)L, 4.29 mmol) were reacted in TFE (5 mL) according to general procedure A. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the target compound as a yellow oil (0.205 g, 41%); \( R_f \) 0.35 (19:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 277, 363; IR (cm\(^{-1}\)) 3278, 3082, 2944, 2865, 2359, 1644; \(^1\)H NMR (500 MHz, CDCl\(_3\)) 1.17-1.31 (21H, m, Si(CH\((CH_3)_2\))_3), 2.57 (2H, t, \( J = 7.3 \) Hz, CH\(_2\)CH\(_2\)), 2.99 (2H, t, \( J = 7.3 \) Hz, CH\(_2\)CH\(_2\)), 3.73 (3H, s, OCH\(_3\)), 4.31 (2H, d, \( J = 5.5 \) Hz, CH\(_2\)NH), 6.12 (1H, br, CH\(_2\)NH), 6.72 (2H, d, \( J = 8.8 \) Hz, H-3" and H-5"), 6.82-6.86 (1H, m, H-6'), 7.01 (2H, d, \( J = 8.8 \) Hz, H-2" and H-6"), 7.17-7.23 (2H, m, H-4' and H-5'), 7.37 (1H, s, NH), 7.79 (1H, s, H-8), 7.83 (1H, br, H-2'), 11.94 (1H, br, NH-9); HRMS calcd. for C\(_{33}\)H\(_{43}\)N\(_6\)OSi (ES+) \( m/z \) 583.3211 [M+H]+, found 583.3212.

Part 2. PMB-carboxamide (0.178 g, 0.31 mmol) and TFA (3 mL) were reacted according to general procedure E. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH)

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gave the desired compound as a yellow oil (81 mg, 58%); Rf 0.38 (19:1 DCM/MeOH, KP-NH); $\lambda_{\text{max}}$ (EtOH/nm) 260, 368; IR (cm$^{-1}$) 3185, 2942, 2864, 2159, 2030, 1660; $^1$H NMR (500 MHz, DMSO-$d_6$) 1.10-1.23 (21H, m, Si(CH(CH$_3$)$_2$)$_2$)$_3$), 2.37 (2H, t, $J$ = 7.6 Hz, CH$_2$CH$_2$), 2.78 (2H, t, $J$ = 7.6 Hz, CH$_2$CH$_2$), 6.77-6.80 (2H, m, CONH$'$ and H-6'), 7.17 (1H, dd, $J$ = 7.8 and 7.9 Hz, H-5'), 7.31 (1H, s, CONH$'$), 7.60-7.64 (1H, m, H-4'), 7.66-7.69 (1H, m, H-2'), 8.25 (1H, s, H-8), 9.63 (1H, s, NH), 13.08 (1H, br, NH-9); HRMS calcd. for C$_{25}$H$_{35}$N$_5$OSi (ES+) $m/z$ 463.2636 [M+H]$^+$, found 463.2634.

$2$-[(3-[(6-((triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)phenyl]-N-methylacetamide

(35)

2-Fluoropurine intermediate 12 (0.127 g, 0.40 mmol), aniline 118 (0.130 g, 0.80 mmol) and TFA (155 μL, 2.00 mmol) were reacted in TFE (2 mL) according to general procedure A. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) afforded the desired compound as a yellow oil (70 mg, 38%); Rf 0.41 (19:1 DCM/MeOH, KP-NH); $\lambda_{\text{max}}$ (EtOH/nm) 264, 368; IR (cm$^{-1}$) 2942, 2864, 2159, 1641; $^1$H NMR (500 MHz, DMSO-$d_6$) 1.13-1.17 (21H, m, Si(CH(CH$_3$)$_2$)$_2$)$_3$), 2.59 (3H, d, $J$ = 4.6 Hz, NHCH$_3$), 3.36 (2H, s, CH$_2$), 6.83-6.85 (1H, m, H-6'), 7.20 (1H, dd, $J$ = 7.9 and 7.9 Hz, H-5'), 7.57 (1H, br, H-2'), 7.74-7.77 (1H, m, H-4'), 7.90 (1H, br, NHCH$_3$), 8.25 (1H, s, H-8), 9.66 (1H, s, NH), 13.08 (1H, s, NH-9); HRMS calcd. for C$_{25}$H$_{35}$N$_5$OSi (ES+) $m/z$ 463.2636 [M+H]$^+$, found 463.2640.

$N$-Methyl-2-[(4-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl)acetamide

(36)

2-Fluoropurine 12 (0.200 g, 0.63 mmol), aniline 121 (0.206 g, 1.26 mmol) and TFA (120 μL, 1.57 mmol) were reacted in TFE (6 mL) according to general procedure B. Purification by
chromatography on KP-NH silica (19:1 DCM/MeOH) afforded the target compound as a yellow oil/gum (0.137 g, 47%); Rf 0.37 (19:1 DCM/MeOH, KP-NH); λmax (EtOH/nm) 279, 379; IR (cm⁻¹) 3275, 2942, 2864, 1604, 1573, 1523; ¹H NMR (500 MHz, DMSO-d₆) 1.13-1.23 (21H, m, Si(CH(CH₃)₂)₃), 2.58 (3H, d, J = 4.7 Hz, NHCH₃), 3.33 (2H, s, COCH₂), 7.15 (2H, d, J = 8.6 Hz, H-2' and H-6'), 7.70 (2H, d, J = 8.6 Hz, H-3' and H-5'), 7.84 (1H, q, J = 4.7 Hz, NHCH₃), 8.23 (1H, s, H-8), 9.61 (1H, s, NH), 13.04 (1H, br, NH-9); HRMS calcd. for C₂₅H₃₅N₆O₅Si (ES+) m/z 463.2636 [M+H]⁺, found 463.2631.

*N*,*N*-Dimethyl-2-(4-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl)acetamide (37)

2-Fluoropurine 12 (73 mg, 0.23 mmol), aniline 124 (96 mg, 0.46 mmol) and TFA (45 µL, 0.58 mmol) were reacted in TFE (3 mL) according to general procedure B. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil/gum (69 mg, 61%); Rf 0.32 (19:1 DCM/MeOH, KP-NH); λmax (EtOH/nm) 278, 379; IR (cm⁻¹) 3275, 3104, 2941, 2864, 1603, 1570; ¹H NMR (500 MHz, DMSO-d₆) 1.13-1.23 (21H, m, Si(CH(CH₃)₂)₃), 2.83 (3H, s, NCH₃), 3.00 (3H, s, NCH₃), 3.62 (2H, s, COCH₂), 7.12 (2H, d, J = 8.6 Hz, H-2' and H-6'), 7.71 (2H, d, J = 8.6 Hz, H-3' and H-5'), 8.22 (1H, s, H-8), 9.60 (1H, s, NH), 13.04 (1H, br, NH-9); HRMS calcd. for C₂₆H₃₇N₆O₅Si (ES+) m/z 477.2793 [M+H]⁺, found 477.2779.

*N*,*N*-Dimethyl-2-(4-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl)malonamide (38)

2-Fluoropurine 12 (0.198 g, 0.63 mmol), aniline 126 (0.277 g, 1.26 mmol) and TFA (120 µL, 1.57 mmol) were reacted in TFE (6 mL) according to general procedure B. Purification by
chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil/gum (0.167 g, 51%); \( R_f \) 0.35 (19:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 280, 354; IR (cm\(^{-1}\)) 3287, 3085, 2943, 2864, 1663, 1603, 1573, 1521; \( ^1\text{H NMR} \) (500 MHz, DMSO-\(d_6\) ) 1.13-1.23 (21H, m, Si(CH(CH\(_3\))\(_2\))\(_3\)), 2.62 (6H, d, \( J = 4.7 \) Hz, (2 x NHCH\(_3\)), 4.28 (1H, s, COCHCO), 7.26 (2H, d, \( J = 8.7 \) Hz, H-2' and H-6'), 7.71 (2H, d, \( J = 8.7 \) Hz, H-3' and H-5'), 8.02 (2H, q, \( J = 4.7 \) Hz, 2 x NHCH\(_3\)), 8.23 (1H, s, H-8), 9.68 (1H, s, NH), 13.05 (1H, br, NH-9); HRMS calcd. for C\(_{27}\)H\(_{38}\)N\(_7\)O\(_2\)Si (ES+) m/z 520.2851 [M+H]\(^+\), found 520.2847.

\( N\)-Methyl-3-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino) benzamide (39)

2-Fluoropurine 12 (0.202 g, 0.63 mmol), aniline 130 (0.190 g, 1.26 mmol) and TFA (120 \( \mu \)L, 1.57 mmol) were reacted in TFE (6 mL) according to general procedure B over 3 h. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the target compound as a yellow oil/gum (0.101 g, 37%); \( R_f \) 0.30 (19:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 276; IR (cm\(^{-1}\)) 3069, 2942, 2865, 2161, 1580; \( ^1\)H NMR (500 MHz, DMSO-\(d_6\) ) 1.10-1.25 (21H, m, Si(CH(CH\(_3\))\(_2\))\(_3\)), 2.78 (3H, d, \( J = 4.5 \) Hz, NHCH\(_3\)), 7.32-7.36 (2H, m, H-5' and H-6'), 7.89-7.93 (1H, m, H-4'), 8.19-8.21 (1H, m, H-2'), 8.24 (1H, s, H-8), 8.30 (1H, q, \( J = 4.5 \) Hz, NHCH\(_3\)), 9.81 (1H, s, NH), 13.14 (1H, br, NH-9); HRMS calcd. for C\(_{24}\)H\(_{33}\)N\(_6\)OSi (ES+) m/z 449.2480 [M+H]\(^+\), found 449.2480.

\( N,N\)-Dimethyl-3-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino) benzamide (40)

2-Fluoropurine 12 (0.121 g, 0.38 mmol), aniline 131 (0.125 g, 0.76 mmol) and TFA (73 \( \mu \)L, 0.95 mmol) were reacted in TFE (4 mL) according to general procedure B over 3 h. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the target
compound as a yellow oil/gum (81 mg, 47%); Rf 0.34 (19:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 228, 317; IR (cm\(^{-1}\)) 3079, 2942, 2864, 2057, 1576, 1538; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.13-1.23 (21H, m, Si(C(\(CH(\(CH_3)\))_2)_3)), 2.96 (3H, s, NCH\(_3\)), 3.00 (3H, s, NCH\(_3\)), 6.93 (1H, ddd, \( J = 1.1, 1.2 \) and 7.7 Hz, H-6'), 7.33 (1H, dd, \( J = 7.7 \) and 7.9 Hz, H-5'), 7.76-7.80 (1H, m, H-4'), 7.95 (1H, m, H-2'), 8.27 (1H, s, H-8), 9.82 (1H, s, NH), 13.13 (1H, br, NH-9); HRMS calcd. for C\(_{25}\)H\(_{35}\)N\(_6\)Si (ES+) \( m/z \) 463.2636 [M+H]\(^+\), found 463.2632.

4-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)benzamide (41)

![Chemical structure of 4-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)benzamide](image)

2-Fluoropurine intermediate 12 (0.216 g, 0.68 mmol), aniline 119 (0.185 g, 1.36 mmol) and TFA (262 \( \mu \)L, 3.40 mmol) were reacted in TFE (4 mL) according to general procedure A. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) afforded the desired compound as a yellow oil (0.114 g, 38%); Rf 0.29 (9:1 DCM/MeOH); \( \lambda_{\text{max}} \) (EtOH/nm) 224, 301, 363; IR (cm\(^{-1}\)) 3113, 2942, 2864, 2160, 1653; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.14-1.18 (21H, m, Si(C(\(CH(\(CH_3)\))_2)_3)), 7.16 (1H, br, CONHH'), 7.79-7.82 (3H, m, CONHH', H-3' and H-5'), 7.89 (2H, d, \( J = 8.9 \) Hz, H-2' and H-6'), 8.31 (1H, s, H-8), 10.01 (1H, s, NH), 13.17 (1H, br, NH-9); HRMS calcd. for C\(_{23}\)H\(_{31}\)N\(_6\)Si (ES+) \( m/z \) 435.2323 [M+H]\(^+\), found 435.2323.

4-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)-N-methyl benzamide (42)

![Chemical structure of 4-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)-N-methyl benzamide](image)

2-Fluoropurine intermediate 12 (0.270 g, 0.85 mmol), aniline 120 (0.255 g, 1.70 mmol) and TFA (330 \( \mu \)L, 4.25 mmol) were reacted in TFE (4 mL) according to general procedure A. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) afforded the desired compound as a yellow oil (0.117 g, 31%); Rf 0.51 (9:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm)
230, 283, 362; IR (cm⁻¹) 3278, 3082, 2942, 2864, 2154, 2027, 1602; ¹H NMR (500 MHz, DMSO-ｄ₆) 1.14-1.19 (21H, m, Si(CH₃(CH₃)₂)₃), 2.78 (3H, d, J = 4.6 Hz, NHCH₃), 7.77 (2H, d, J = 8.8 Hz, H-3' and H-5'), 7.89 (2H, d, J = 8.8 Hz, H-2' and H-6'), 8.23 (1H, q, J = 4.6 Hz, NHCH₃), 8.31 (1H, s, H-8), 10.00 (1H, s, NH), 13.19 (1H, br, NH-9); HRMS calcd. for C₂₄H₃₃N₆O Si (ES⁺) m/z 449.2480 [M+H]⁺, found 449.2480.

4-(6-(2-(Triisopropylsilyl)ethyl)n)-9H-purin-2-ylamino)-N,N-dimethyl benzamide (43)

2-Fluoropurine intermediate 12 (0.205 g, 0.64 mmol), aniline 132 (0.210 g, 1.28 mmol) and TFA (248 µL, 3.22 mmol) were reacted in TFE (4 mL) according to general procedure A. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) afforded the desired compound as a yellow oil (79 mg, 27%); Rₗ 0.48 (19:1 DCM/MeOH, KP-NH); λ_max (EtOH/nm) 283, 364; IR (cm⁻¹) 2942, 2862, 2160, 2028, 1601; ¹H NMR (500 MHz, DMSO-ｄ₆) 1.14-1.18 (21H, m, Si(CH₃(CH₃)₂)₃), 2.04 (3H, s, CH₃), 7.21-7.25 (1H, m, H-6'), 7.53-7.57 (1H, m, H-4'), 7.76-7.80 (1H, m, H-2'), 8.23 (1H, s, H-8), 9.96 (1H, s, NH), 13.19 (1H, br, NH-9); HRMS calcd. for C₂₅H₃₅N₆O Si (ES⁺) m/z 463.2636 [M+H]⁺, found 463.2641.

N-(3-((6-(Triisopropylsilyl)ethyl)-9H-purin-2-yl)amino)phenyl) acetamide (44)

2-Fluoropurine 12 (0.230 g, 0.72 mmol), aniline 135 (0.216 g, 1.44 mmol) and TFA (140 µL, 1.80 mmol) were reacted in TFE (7 mL) according to general procedure B. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) afforded the target compound as a yellow oil/gum (0.200 g, 62%); Rₗ 0.29 (19:1 DCM/MeOH, KP-NH); λ_max (EtOH/nm) 276, 375; IR (cm⁻¹) 3109, 2942, 2862, 1667, 1577, 1534; ¹H NMR (500 MHz, DMSO-ｄ₆) 1.13-1.19 (21H, m, Si(CH₃(CH₃)₂)₃), 2.04 (3H, s, CH₃), 7.17 (1H, dd, J = 7.9 and 8.0 Hz, H-5'), 7.21-7.25 (1H, m, H-6'), 7.53-7.57 (1H, m, H-4'), 7.76-7.80 (1H, m, H-2'), 8.23 (1H, s, H-8), 9.96 (1H, s, NH), 13.19 (1H, br, NH-9); HRMS calcd. for C₂₅H₃₅N₆O Si (ES⁺) m/z 463.2636 [M+H]⁺, found 463.2641.
9.65 (1H, s, CONH), 9.85 (1H, s, NH), 13.05 (1H, br, NH-9); HRMS calcd. for C_{24}H_{33}N_{6}OSi (ES+) m/z 449.2480 [M+H]^+, found 449.2479.

2-(3-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)phenyl)-2-methylpropanamide (45)

2-Fluoropurine intermediate 12 (0.197 g, 0.62 mmol), aniline 140 (0.220 g, 1.24 mmol) and TFA (240 μL, 3.10 mmol) were reacted in TFE (4 mL) according to general procedure A. Purification by chromatography on silica (19:1 DCM/MeOH) afforded the desired compound as a yellow oil (0.127 g, 43%); Rf 0.28 (19:1 DCM/MeOH); λ_{max} (EtOH/nm) 270, 368; IR (cm\(^{-1}\)) 3094, 2964, 2943, 2162, 1970, 1659; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.13-1.19 (21H, m, Si(CH(CH\(_3\))_2)_3), 1.45 (6H, s, C(CH\(_3\))_2), 6.80 (1H, s, CONH'H'), 6.89 (1H, s, CONH'H'), 7.04-7.07 (1H, m, H-6'), 7.21 (1H, dd, J = 7.9 and 8.1 Hz, H-5'), 7.65-7.69 (1H, m, H-4'), 7.86-7.88 (1H, m, H-2'), 8.25 (1H, s, H-8), 9.65 (1H, s, NH), 13.07 (1H, br, NH-9); HRMS calcd. for C_{26}H_{37}N_{6}OSi (ES+) m/z 477.2793 [M+H]^+, found 477.2796.

3-(6-Ethynyl-9H-purin-2-ylamino)benzamide (46)

TIPS-protected purine 33 (33 mg, 0.076 mmol), KF (22 mg, 0.38 mmol) and 18-crown-6 (3 mg, 0.008 mmol) were reacted in THF (1 mL) according to general procedure F. Purification by chromatography on silica (17:3 DCM/MeOH) afforded the desired compound as a yellow solid (20 mg, 94%); Rf 0.38 (17:3 Petrol/EtOAc); m.p. 170-180 °C (decomposed); λ_{max} (EtOH/nm) 274; IR (cm\(^{-1}\)) 3254, 3085, 2830, 2775, 2551, 2162, 2117, 1659; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 4.89 (1H, s, C≡CH), 7.35 (1H, s, CONH'H'), 7.39 (1H, dd, J = 7.8 and 7.9 Hz, H-5'), 7.43-7.46 (1H, m, H-6'), 7.90 (1H, s, CONH'H'), 7.97-7.80 (1H, m, H-4'), 8.20-
8.23 (1H, m, H-2'), 8.32 (1H, s, H-8), 9.80 (1H, s, NH), 13.21 (1H, br, NH-9); HRMS calcd. for C_{14}H_{11}NO (ES+) \text{m/z} 279.0993 [M+H]^+, found 279.0989.

3-(3-(6-Ethynyl-9H-purin-2-ylamino)phenyl)propanamide (47)

TIPS-protected purine 34 (45 mg, 0.097 mmol) and TBAF (1M in THF, 106 μL, 0.106 mmol) were reacted in THF (2 mL) according to \textit{general procedure G}. Upon completion of the reaction, the solution was diluted with THF (10 mL) and treated with solid supported TBAF scavenger (0.20 g, 4 x w/w) at RT for 18 h. The resin was removed via filtration and the solvent removed \textit{in vacuo}. The crude residue was purified by chromatography on silica (17:3 DCM/MeOH) to give the desired compound as a yellow solid (16 mg, 46%); Rf 0.45 (17:3 Petrol/EtOAc); m.p. 135-145 °C (decomposed); \(\lambda_{\text{max}}\) (EtOH/nm) 273; IR (cm\(^{-1}\)) 3380, 3249, 3093, 2828, 2779, 2160, 2116, 2030, 1653; \(^1\)H NMR (500 MHz, DMSO-\text{d}_6) 2.37 (2H, t, \(J = 7.6\) Hz, COCH\(_2\)CH\(_2\)), 2.78 (2H, t, \(J = 7.6\) Hz, COCH\(_2\)CH\(_2\)), 4.84 (1H, s, C≡CH), 6.77-6.81 (2H, m, CONH\(_H'\) and H-6'), 7.19 (1H, dd, \(J = 7.9\) and 8.0 Hz, H-5'), 7.32 (1H, s, CONH\(_H'\)), 7.57-7.60 (1H, br, H-2'), 7.66-7.69 (1H, m, H-4'), 8.27 (1H, s, H-8), 9.60 (1H, s, NH), 13.13 (1H, br, NH-9); HRMS calcd. for C_{16}H_{15}NO (ES+) \text{m/z} 307.1305 [M+H]^+, found 307.1302.

2-(3-(6-Ethynyl-9H-purin-2-ylamino)phenyl)-N-methylacetamide (48)

TIPS-protected purine 35 (50 mg, 0.11 mmol), KF (31 mg, 0.54 mmol) and 18-crown-6 (3 mg, 0.011 mmol) were reacted in THF (1 mL) according to \textit{general procedure F}. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a yellow solid (24 mg, 73%); Rf 0.41 (9:1 DCM/MeOH); Mp 170-180 °C (decomposed); \(\lambda_{\text{max}}\) (EtOH/nm) 274; IR (cm\(^{-1}\)) 3270, 3088, 2158, 2025, 1596; \(^1\)H NMR (500 MHz, DMSO-\text{d}_6) 2.59 (3H, d, \(J = 4.7\) Hz, NHCH\(_3\)), 3.36 (2H, s, CH\(_2\)CO), 4.84 (1H, s, C≡CH), 6.81-6.84 (1H, m, H-6'), 7.21 (1H, dd, \(J = 7.8\) and 7.9 Hz, H-5'), 7.52-7.55 (1H, m, H-2'), 7.74-7.77 (1H, m, H-4'), 7.92 (1H, br,
CONH), 8.27 (1H, s, H-8), 9.62 (1H, s, NH), 13.12 (1H, br, NH-9); HRMS calcd. for C16H15N6O (ES+) m/z 307.1305 [M+H]+, found 307.1302.

2-(4-((6-Ethynyl-9H-purin-2-yl)amino)phenyl)-N-methylacetamide (49)

The TIPS-protected purine 36 (0.130 g, 0.28 mmol), TBAF (1M in THF, 0.34 mL, 0.34 mmol) and TBAF scavenger resin (1.30 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Chromatography on KP-NH silica (9:1 DCM/MeOH) gave the target compound as a yellow solid (50 mg, 58%); Rf 0.26 (9:1 DCM/MeOH, KP-NH); m.p. 140-160 °C (decomposed); $\lambda_{\text{max}}$ (EtOH/nm) 276.0, 371.0; IR (cm$^{-1}$) 3414, 3092, 2531, 2112, 1611, 1577, 1530; $^1$H NMR (500 MHz, DMSO-d$_6$) 2.58 (3H, d, $J$ = 4.6 Hz, NHC$_3$), 3.33 (2H, s, COCH$_2$), 4.82 (1H, s, N=CH), 7.16 (2H, d, $J$ = 8.5 Hz, H-2' and H-6'), 7.68 (2H, d, $J$ = 8.5 Hz, H-3' and H-5'), 7.86 (1H, q, $J$ = 4.6 Hz, NHCH$_3$), 8.25 (1H, s, H-8), 9.60 (1H, s, NH), 13.09 (1H, br, NH-9); HRMS calcd. for C$_{16}$H$_{15}$N$_6$O (ES+) m/z 307.1302 [M+H]+, found 307.1306.

2-(4-((6-Ethynyl-9H-purin-2-yl)amino)phenyl)-N,N-dimethylacetamide (50)

The TIPS-protected purine 37 (56 mg, 0.12 mmol), TBAF (1M in THF, 0.14 mL, 0.14 mmol) and TBAF scavenger resin (0.60 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Chromatography on KP-NH silica (9:1 DCM/MeOH) afforded the desired compound as a yellow solid (27 mg, 69%); Rf 0.33 (9:1 DCM/MeOH, KP-NH); m.p. 120-130 °C (decomposed); $\lambda_{\text{max}}$ (EtOH/nm) 276.0, 378.0; IR (cm$^{-1}$) 2932, 2538, 2110, 1607, 1575, 1530; $^1$H NMR (500 MHz, DMSO-d$_6$) 2.84 (3H, s, N-CH$_3$), 3.01 (3H, s, NCH$_3$), 3.62 (2H, s, COCH$_2$), 4.83 (1H, s, C=CH), 7.13 (2H, d, $J$ = 8.6 Hz, H-2' and H-6'), 7.69 (2H, d, $J$ = 8.6 Hz, H-3' and H-5'), 8.26 (1H, s, H-8), 9.60 (1H, s, NH), 13.10 (1H, br, NH-9); HRMS calcd. for C$_{17}$H$_{17}$N$_6$O (ES+) m/z 321.1458 [M+H]+, found 321.1462.

2-(4-((6-Ethynyl-9H-purin-2-yl)amino)phenyl)-N',N'-dimethylmalonamide (51)
The TIPS-protected purine 38 (0.120 mg, 0.23 mmol), TBAF (1M in THF, 0.28 mL, 0.28 mmol) and TBAF scavenger resin (1.20 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) afforded the target compound as a yellow solid (29 mg, 35%); Rf 0.26 (9:1 DCM/MeOH, KP-NH); m.p. 160-180 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 277.0, 368.5; IR (cm\(^{-1}\)) 3274, 2550, 2160, 2111, 2030, 1660; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.61 (6H, d, \( J = 4.6 \) Hz, 2 x NHC\( \text{H}_3 \)), 4.28 (1H, s, COC\( \text{H} \)), 4.84 (1H, s, C≡CH), 7.27 (2H, d, \( J = 8.7 \) Hz, H-2' and H-6'), 7.69 (2H, d, \( J = 8.7 \) Hz, H-3' and H-5'), 8.04 (2H, q, \( J = 4.6 \) Hz, 2 x NHCH\( \text{H}_3 \)), 8.28 (1H, s, H-8), 9.65 (1H, s, NH), 13.10 (1H, br, NH-9); HRMS calcd. for C\(_{18}\)H\(_{18}\)N\(_7\)O\(_2\) (ES+) \( m/z \) 364.1516 [M+H]\(^+\), found 364.1520.

3-((6-Ethynyl-9H-purin-2-yl)amino)-N-methylbenzamide (52)

The TIPS-protected purine 39 (72 mg, 0.16 mmol), TBAF (1M in THF, 0.19 mL, 0.19 mmol) and TBAF scavenger resin (0.70 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) gave the target compound as a yellow solid (29 mg, 63%); Rf 0.24 (9:1 DCM/MeOH, KP-NH); m.p. 200-220 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 274.0, 363.5; IR (cm\(^{-1}\)) 3302, 3252, 3113, 1607; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.79 (3H, d, \( J = 4.5 \) Hz, NHCH\( \text{H}_3 \)), 4.86 (1H, s, C≡CH), 7.33-7.39 (2H, m, H-5' and H-6'), 7.92-7.97 (1H, m, H-4'), 8.16-8.18 (1H, m, H-2'), 8.29 (1H, s, H-8), 8.36 (1H, q, \( J = 4.5 \) Hz, NHCH\( \text{H}_3 \)), 9.78 (1H, s, NH), 13.20 (1H, br, NH-9); HRMS calcd. for C\(_{15}\)H\(_{13}\)N\(_6\)O (ES+) \( m/z \) 293.1145 [M+H]\(^+\), found 293.1149.

3-((6-Ethynyl-9H-purin-2-yl)amino)-N,N-dimethylbenzamide (53)
The TIPS-protected purine 40 (52 mg, 0.11 mmol), TBAF (1M in THF, 0.13 mL, 0.13 mmol) and TBAF scavenger resin (0.50 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) gave the target compound as a yellow solid (23 mg, 69%); Rf 0.33 (9:1 DCM/MeOH, KP-NH); m.p. 175-190 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 274.5, 362.5; IR (cm\(^{-1}\)) 3281, 2558, 2160, 2031, 1976, 1575; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.96 (3H, s, N\(\text{CH}_3\)), 3.00 (3H, s, N\(\text{CH}_3\)), 4.86 (1H, s, C\(\equiv\)CH), 6.94 (1H, ddd, \( J = 1.3, 2.0 \text{ and } 7.6 \text{ Hz}, \text{H-6}'\)), 7.34 (1H, dd, \( J = 7.6 \text{ and } 7.7 \text{ Hz}, \text{H-5}'\)), 7.80-7.83 (1H, m, H-4'), 7.88 (1H, dd, \( J = 1.3 \text{ and } 1.4 \text{ Hz}, \text{H-2}'\)), 8.30 (1H, s, H-8), 9.82 (1H, s, NH), 13.17 (1H, br, NH-9); HRMS calcd. for C\(_{16}\)H\(_{15}\)N\(_6\)O (ES+) \( m/z \) 307.1302 [M+H]\(^+\), found 307.1306.

4-(6-Ethynyl-9H-purin-2-ylamino)benzamide (54)

![4-(6-Ethynyl-9H-purin-2-ylamino)benzamide (54)](image)

TIPS-protected purine 41 (86 mg, 0.20 mmol), KF (57 mg, 0.99 mmol) and 18-crown-6 (5 mg, 0.020 mmol) were reacted in THF (2 mL) according to general procedure F. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a yellow solid (41 mg, 74%); Rf 0.25 (9:1 DCM/MeOH); m.p. 180-190 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 220, 302; IR (cm\(^{-1}\)) 3267, 2164, 2024, 1653, 1602; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 4.89 (1H, s, C\(\equiv\)CH), 7.17 (1H, s, CON\(\text{H}_3\)), 7.70 (1H, s, CONNH\(\text{H}_3\)), 7.83 (2H, d, \( J = 8.7 \text{ Hz}, \text{H-2}' \text{ and } \text{H-6}'\)), 7.87 (2H, d, \( J = 8.7 \text{ Hz}, \text{H-3}' \text{ and } \text{H-5}'\)), 8.35 (1H, s, H-8), 10.00 (1H, s, NH), 13.23 (1H, br, NH-9); HRMS calcd. for C\(_{14}\)H\(_{11}\)N\(_6\)O (ES+) \( m/z \) 279.0994 [M+H]\(^+\), found 279.0989.

4-(6-Ethynyl-9H-purin-2-ylamino)-N-methylbenzamide (55)

![4-(6-Ethynyl-9H-purin-2-ylamino)-N-methylbenzamide (55)](image)

TIPS-protected purine 42 (0.105 g, 0.23 mmol), KF (67 mg, 1.15 mmol) and 18-crown-6 (6 mg, 0.023 mmol) were reacted in THF (2.5 mL) according to general procedure F. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a yellow solid (46 mg, 69%); Rf 0.24 (9:1 DCM/MeOH); m.p. 180-190 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 221, 301; IR (cm\(^{-1}\)) 3275, 3109, 2163, 2111, 2022, 1605; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.78 (3H, d, \( J = 4.5 \text{ Hz}, \text{NHCH}_3\)), 4.89 (1H, s, C\(\equiv\)CH), 7.78 (2H, d, \( J = 8.8 \text{ Hz}, \text{H-2}'\)
and H-6’), 7.87 (2H, d, J = 8.8 Hz, H-3’ and H-5’), 8.24 (1H, q, J = 4.5 Hz, CONH), 8.34 (1H, s, H-8), 9.99 (1H, s, NH), 13.25 (1H, br, NH-9); HRMS calcd. for C_{15}H_{13}N_{6}O (ES+) m/z 293.1148 [M+H]^+, found 293.1145.

4-(6-Ethynyl-9H-purin-2-ylamino)-N,N-dimethylbenzamide (56)

TIPS-protected purine 43 (63 mg, 0.14 mmol) and 18-crown-6 (4 mg, 0.014 mmol) were reacted in THF (1.5 mL) according to general procedure F. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a yellow solid (27 mg, 63%); Rf 0.29 (9:1 DCM/MeOH); m.p. 190-210 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 292; IR (cm\(^{-1}\)) 3101, 2111, 1599; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.98 (6H, s, N(CH\(_3\))\(_2\)), 4.89 (1H, s, C≡CH), 7.38 (2H, d, J = 8.4 Hz, H-2’ and H-6’), 7.86 (2H, d, J = 8.4 Hz, H-3’ and H-5’), 8.33 (1H, s, H-8), 9.95 (1H, s, NH), 13.23 (1H, s, NH-9); HRMS calcd. for C_{16}H_{15}N_{6}O (ES+) m/z 307.1302 [M+H]^+, found 307.1302.

N-(3-((6-Ethynyl-9H-purin-2-yl)amino)phenyl)acetamide (57)

The TIPS-protected purine 44 (0.151 mg, 0.33 mmol), TBAF (1M in THF, 0.40 mL, 0.40 mmol) and TBAF scavenger resin (1.50 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) gave the desired compound as a yellow solid (48 mg, 48%); Rf 0.29 (9:1 DCM/MeOH, KP-NH); m.p. 160-180 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 251.5, 275.5, 362.0; IR (cm\(^{-1}\)) 3266, 2546, 2112, 1668, 1605, 1581, 1536; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.04 (3H, s, COCH\(_3\)), 4.84 (1H, s, C≡CH), 7.19 (1H, dd, J = 7.8 and 7.9 Hz, H-5’), 7.24 (1H, m, H-6’), 7.55 (1H, m, H-4’), 7.77 (1H, m, H-2’), 8.27 (1H, s, H-8), 9.63 (1H, s, NH), 9.88 (1H, s, CONH), 13.12 (1H, br, NH-9); HRMS calcd. for C_{15}H_{13}N_{6}O (ES+) m/z 293.1145 [M+H]^+, found 293.1149.

2-(3-(6-Ethynyl-9H-purin-2-ylamino)phenyl)-2-methylpropanamide (58)
TIPS-protected purine 45 (0.108 g, 0.23 mmol), KF (65 mg, 1.13 mmol) and 18-crown-6 (6 mg, 0.023 mmol) were reacted in THF (2.5 mL) according to general procedure F. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) afforded the desired compound as a yellow solid (37 mg, 51%); Rf 0.29 (9:1 DCM/MeOH, KP-NH); m.p. 140-160 °C (decomposed); λmax (EtOH/nm) 274; IR (cm⁻¹) 3270, 2975, 2853, 2155, 1979, 1659, 1599; ¹H NMR (500 MHz, DMSO-d₆) 1.45 (6H, s, C(CH₃)₂), 4.82 (1H, s, C≡CH), 6.80 (1H, s, CONH), 6.84 (1H, s, CONH'), 6.90-6.92 (1H, m, H-6'), 7.22 (1H, dd, J = 8.1 and 8.2 Hz, H-5'), 7.74-7.76 (2H, m, H-2' and H-4'), 8.27 (1H, s, H-8), 9.60 (1H, s, NH), 13.11 (1H, br, NH-9); HRMS calcd. for C₁₇H₁₇N₆O (ES⁺) m/z 321.1463 [M+H]^+, found 321.1458.

2-(3-(6-(2-(Triisopropylsilyl)ethyl)-9H-purin-2-ylamino)phenyl)acetic acid (59)

2-Fluoropurine intermediate 12 (1.06 g, 3.32 mmol) and 3-aminophenyl acetic acid (1.00 g, 6.64 mmol) were reacted with TFA (1.28 mL, 16.6 mmol) in TFE (25 mL) according to general procedure A. Upon completion of the reaction, the reaction solvent was removed in vacuo and the resultant residue was dissolved in THF (20 mL) and 1M NaOH solution (15 mL). The mixture was stirred at RT for 18 h before the THF was removed in vacuo. The aqueous solution was then taken to pH 3 with 4M HCl solution and extracted with EtOAc (3 × 75 mL). The combined organic extracts were dried (Na₂SO₄) and concentrated, and the resulting orange residue was purified by chromatography on silica (9:1 DCM/MeOH) followed by chromatography on reverse phase silica (9:1 MeOH/H₂O + 0.1% HCOOH). The desired product was obtained as an orange oil/gum (0.952 g, 64%); Rf 0.32 (9:1 DCM/MeOH); λmax (EtOH/nm) 275; IR (cm⁻¹) 2972, 2360, 2340, 1777, 1702; ¹H NMR (500 MHz, DMSO-d₆) 1.13-1.22 (21H, m, Si(CH(CH₃)₂)₃), 3.51 (2H, s, CH₂CO₂H), 6.82-6.85 (1H, m, H-6'), 7.22 (1H, dd, J = 7.7 and 7.8 Hz, H-5'), 7.65-7.68 (1H, m, H-2'), 7.72-7.76 (1H, m,
H-4'), 8.25 (1H, s, H-8), 9.71 (1H, s, NH), 12.33 (1H, br, CO_2H), 13.10 (1H, br, NH-9); HRMS calcd. for C_{24}H_{30}N_{5}O_{2}Si (ES+) m/z 450.2320 [M+H]^+, found 450.2320.

2-(4-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)phenyl)acetic acid (60)

![Image of the molecule](image_url)

2-Fluoropurine intermediate 12 (0.485 g, 1.53 mmol), (4-aminophenyl)acetic acid (0.460 g, 3.05 mmol) and TFA (588 μL, 7.63 mmol) were reacted in TFE (15 mL) according to general procedure A. Upon completion of the reaction, the residue was dissolved in THF (15 mL) and 1M NaOH solution (10 mL). The mixture was stirred at RT for 18 h before the THF was removed in vacuo. The aqueous solution was then taken to pH 3 with 4M HCl solution and extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated, and the resulting orange residue was purified by chromatography on reverse phase silica (9:1 MeOH/H_2O + 0.1% HCOOH). The desired product was obtained as an orange oil (0.365 g, 53%); R_f 0.44 (9:1 MeOH/H_2O, 0.1% + HCOOH, C18); λ_max (EtOH/nm) 263, 368; IR (cm⁻¹) 2942, 2866, 2540, 2161, 2037, 1636; ^1H NMR (500 MHz, DMSO-d_6) 1.16 (21H, m, Si(CH(CH_3)_2)_3), 3.34 (2H, s, CH2), 7.16 (2H, d, J = 8.6 Hz, H-2' and H-6'), 7.73 (2H, d, J = 8.6 Hz, H-3' and H-5'), 8.24 (1H, s, H-8), 9.66 (1H, s, NH), 13.10 (1H, br, NH-9); HRMS calcd. for C_{24}H_{30}N_{5}O_{2}Si (ES-) m/z 448.2174 [M-H] - found 448.2163.

N-(4-Methoxybenzyl)-2-(3-(6-(2-(triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)phenyl)acetamide (61)

![Image of the molecule](image_url)
Carboxylic acid 59 (0.193 g, 0.43 mmol), CDI (0.140 g, 0.86 mmol), DIPEA (150 μL, 0.86 mmol) and 4-methoxybenzylamine (223 μL, 1.72 mmol) were reacted in dry DMF (5 mL) according to **general procedure D**. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil/gum (0.238 g, 99%); Rf 0.48 (19:1 DCM/MeOH, KP-NH); λ_{max} (EtOH/nm) 277; IR (cm^{-1}) 2360, 2153, 2120, 1980; ^1H NMR (500 MHz, DMSO-d_6) 1.12-1.25 (21H, m, Si(CH(CH_3)_2)_3), 3.42 (2H, s, COCH_2), 3.71 (3H, s, OCH_3), 4.20 (2H, d, J = 5.8 Hz, NHCH_2), 6.85 (2H, d, J = 8.8 Hz, H-3'' and H-5''), 6.86-6.91 (1H, m, H-6'), 7.17 (2H, d, J = 8.8 Hz, H-2'' and H-6''), 7.20 (1H, dd, J = 7.9 and 8.0 Hz, H-5'), 7.59-7.62 (1H, m, H-2'), 7.74-7.78 (1H, m, H-4'), 8.24 (1H, s, H-8), 8.44 (1H, t, J = 5.8 Hz, NHCH_2), 9.65 (1H, s, NH), 12.06 (1H, br, NH-9); HRMS calcd. for C_{32}H_{41}N_6O_2Si (ES+) m/z 569.3055 [M+H]^+, found 569.3057.

**N-(4-Methoxybenzyl)-2-(4-(6-(2-(triisopropylsilyl)ethyl)-9H-purin-2-ylamino)phenyl) acetamide (62)**

![Structure](image)

The carboxylic acid 60 (0.160 g, 0.36 mmol), CDI (0.115 g, 0.71 mmol), DIPEA (125 μL, 0.71 mmol) and 4-methoxybenzylamine (185 μL, 1.43 mmol) were reacted in dry DMF (2 mL) according to **general procedure D**. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil (0.159 g, 78%); Rf 0.48 (19:1 DCM/MeOH, KP-NH); λ_{max} (EtOH/nm) 278, 370; IR (cm^{-1}) 3102, 2965, 2943, 2364, 2154, 1646; ^1H NMR (500 MHz, CDCl_3) 1.14-1.23 (21H, m, Si(CH(CH_3)_2)_3), 3.62 (2H, s, CH_2CO), 3.80 (3H, s, OCH_3), 4.41 (2H, d, J = 5.8 Hz, CH_2NH), 6.13 (1H, br, CH_2NH), 6.86 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.18 (2H, d, J = 8.8 Hz, H-3' and H-5'), 7.21 (2H, d, J = 8.5 Hz, H-3'' and H-5''), 7.56 (2H, d, J = 8.5 Hz, H-2'' and H-6''), 8.08 (1H, s, NH), 8.23 (1H, s, H-8); HRMS calcd. for C_{32}H_{41}N_6O_2Si (ES+) m/z 569.3055 [M+H]^+, found 569.3051.

**2-(3-(6-(2-(Triisopropylsilyl)ethyl)-9H-purin-2-ylamino)phenyl) acetamide (63)**
PMB-amide 61 (0.232 g, 0.41 mmol) was reacted in TFA (6 mL) according to **general procedure E** over 72 h. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil/gum (0.170 g, 90%); Rf 0.24 (19:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 276; IR (cm\(^{-1}\)) 3282, 2965, 2943, 2362, 1669; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.13-1.21 (21H, m, Si(C\(\text{H}(\text{C})\text{H}_3\)\(\text{H}\)_2\)_3)), 3.34 (2H, s, COC\(\text{H}_2\)), 6.83-6.86 (1H, m, H-6'), 6.88 (1H, s, CON\(\text{H}H'\)), 7.19 (1H, dd, \( J = 8.5 \) and 8.7 Hz, H-5'), 7.42 (1H, s, CON\(\text{H}H'\)), 7.57-7.60 (1H, m, H-2'), 7.72-7.76 (1H, m, H-4'), 8.23 (1H, s, H-8), 9.65 (1H, s, NH); 13.08 (1H, s, NH-9); HRMS calcd. for C\(\text{C}_{24}\)H\(\text{C}_{33}\)N\(\text{O}_{6}\)Si (ES+) m/z 449.2480 [M+H]\(^{+}\), found 449.2480.

2-(4-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)phenyl) acetamide (64)

PMB-carboxamide 62 (87 mg, 0.15 mmol) and TFA (2 mL) were reacted according to **general procedure E**. Purification by chromatography on KP-NH silica (9:1 DCM/MeOH) gave the target compound as a yellow oil (60 mg, 87%); Rf 0.37 (9:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 278, 370; IR (cm\(^{-1}\)) 3472, 2943, 2865, 2040, 1659; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.13-1.23 (21H, m, Si(C\(\text{H}(\text{C})\text{H}_3\)\(\text{H}\)_2\)_3)), 3.31 (2H, s, COCH\(\text{H}_2\)), 6.83 (1H, s, CON\(\text{H}H'\)), 7.16 (2H, d, \( J = 8.6 \) Hz, H-2' and H-6'), 7.38 (1H, s, CON\(\text{H}H'\)), 7.71 (2H, d, \( J = 8.6 \) Hz, H-3' and H-5'), 8.22 (1H, s, H-8), 9.64 (1H, s, NH); 13.06 (1H, br, NH-9); HRMS calcd. for C\(\text{C}_{24}\)H\(\text{C}_{33}\)N\(\text{O}_{6}\)Si (ES+) m/z 449.2480 [M+H]\(^{+}\), found 449.2480.

2-(3-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)phenyl)-\(N,N\)-dimethylacetamide (65)
Carboxylic acid 59 (54 mg, 0.12 mmol), CDI (40 mg, 0.24 mmol), DIPEA (125 μL, 0.72 mmol) and dimethylamine hydrochloride (40 mg, 0.48 mmol) were reacted in dry DMF (1 mL) according to general procedure D. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil (51 mg, 92%); Rf 0.38 (19:1 DCM/MeOH, KP-NH); λ_{max} (EtOH/nm) 275, 368; IR (cm^{-1}) 3117, 2965, 2943, 2363, 2160, 1711, 1598; ^1H NMR (500 MHz, CDCl_3) 1.14-1.18 (21H, m, Si(CH_3)_2), 2.96 (3H, s, NCH_3), 3.01 (3H, s, NCH_3), 3.68 (2H, s, CH_2), 6.82-6.85 (1H, m, H-6'), 7.16 (1H, dd, J = 7.9 and 8.0 Hz, H-5'), 7.38-7.38 (1H, m, H-4'), 7.61 (1H, s, H-8), 7.65 (1H, br, H-2'), 7.86 (1H, s, NH); HRMS calcd. for C_{26}H_{37}N_{6}OSi (ES+) m/z 477.2793 [M+H]^+, found 477.2797.

2-(3-(6-Ethynyl-9H-purin-2-ylamino)phenyl)acetamide (66)

The TIPS-protected purine 63 (0.165 g, 0.37 mmol) was reacted with TBAF (1M in THF, 0.55 mL, 0.55 mmol) in THF (4 mL) according to general procedure C. The residue was purified by semi-preparative HPLC (17:3 H_2O/MeCN), to give the desired compound as a yellow solid (0.084 g, 0.28 mmol, 76%); Rf 0.32 (17:3 DCM/MeOH, KP-NH); m.p. 250-270 °C (decomposed); λ_{max} (EtOH/nm) 274; IR (cm^{-1}) 3359, 3170, 2921, 2107, 1658; ^1H NMR (500 MHz, DMSO-d_6) 4.76 (1H, s, C≡CH), 6.77-6.81 (1H, m, H-6'), 6.83 (1H, s, CONH-H'), 7.15 (1H, dd, J = 8.0 and 8.1 Hz, H-5'), 7.38 (1H, s, CONHH'), 7.47-7.50 (1H, m, H-2'), 7.68-7.73 (1H, m, H-4'), 8.19 (1H, s, H-8), 9.52 (1H, s, NH), 13.06 (1H, s, NH-9); HRMS calcd. for C_{15}H_{13}N_{6}O (ES+) m/z 293.1149 [M+H]^+, found 293.1145.

2-(4-(6-Ethynyl-9H-purin-2-ylamino)phenyl)acetamide (67)
TIPS-protected purine 64 (53 mg, 0.12 mmol) and TBAF (1M in THF, 130 μL, 0.13 mmol) were reacted in THF (2 mL) according to general procedure G. Upon completion of the reaction, the solution was diluted with THF (10 mL) and treated with the solid supported TBAF scavenger (0.20 g, 4 x w/w) at RT for 18 h. The resin was removed via filtration and the solvent removed in vacuo. The crude residue was purified by chromatography on silica (9:1 DCM/MeOH) to give the desired compound as a yellow solid (16 mg, 46%); Rf 0.24 (9:1 DCM/MeOH); m.p. 140-150 °C (decomposed); λ\text{max} (EtOH/nm) 275; IR (cm\(^{-1}\)) 3375, 3287, 3193, 3127, 2157, 2119, 2030, 1653, 1604; ¹H NMR (500 MHz, DMSO-\text{d}_6) 3.31 (2H, s, COCH\(_2\)), 4.84 (1H, s, C≡CH), 6.85 (1H, s, CONH\(_H'\)), 7.17 (2H, d, \(J = 8.6\) Hz, H-2' and H-6'), 7.40 (1H, s, CONH\(_H\)), 7.69 (2H, d, \(J = 8.6\) Hz, H-3' and H-5'), 8.27 (1H, s, H-8), 9.62 (1H, s, NH), 13.12 (1H, br, NH-9); HRMS calcd. for C\(_{15}\)H\(_{13}\)N\(_6\)O (ES+) m/z 293.1148 [M+H]\(^+\), found 293.1145.

2-(3-(6-Ethynyl-9H-purin-2-ylamino)phenyl)-\(N,N\)-dimethylacetamide (68)

TIPS-protected purine 65 (48 mg, 0.10 mmol) and TBAF (1M in THF, 110 μL, 0.11 mmol) were reacted in THF (2 mL) according to general procedure C. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a yellow solid (28 mg, 87%); Rf 0.45 (9:1 DCM/MeOH); m.p. 120-130 °C (decomposed); \(λ\text{max} (\text{EtOH}/\text{nm})\) 274; IR (cm\(^{-1}\)) 3084, 2818, 2161, 2110, 2029, 1596; ¹H NMR (500 MHz, DMSO-\text{d}_6) 2.65 (3H, s, N\text{CH}_3), 2.83 (3H, s, N\text{CH}_3), 3.45 (2H, s, CH\(_2\)CO), 4.65 (1H, s, C≡CH), 6.57-6.60 (1H, m, H-6'), 7.02 (1H, dd, \(J = 7.8\) and 8.0 Hz, H-5'), 7.31-7.34 (1H, m, H-2'), 7.54 (1H, ddd, \(J = 1.0, 1.2\) and 7.8 Hz, H-4'), 8.08 (1H, s, H-8), 9.44 (1H, s, NH), 12.93 (1H, br, NH-9); HRMS calcd. for C\(_{17}\)H\(_{17}\)N\(_6\)O (ES+) m/z 321.1462 [M+H]\(^+\), found 321.1458.

5-(6-(2-(Triisopropylsilyl)ethylene)-9H-purin-2-ylamino)indolin-2-one (69)
2-Fluoropurine intermediate 12 (0.319 g, 1.00 mmol), 5-amino-1,3-dihydro-2H-indol-2-one (0.298 g, 2.01 mmol) and TFA (390 μL, 5.02 mmol) were reacted in TFE (5 mL) according to **general procedure A**. Purification by chromatography on KP-NH silica (17:3 DCM/MeOH) afforded the desired compound as an orange oil (0.143 g, 32%); Rf 0.45 (17:3 DCM/MeOH, KP-NH); λ_max (EtOH/nm) 255, 300, 374; IR (cm\(^{-1}\)) 2941, 2864, 2154, 1685; \(^1\)H NMR (500 MHz, DMSO-d\(_6\)) 1.14-1.19 (21H, m, Si(CH(CH\(_3\))\(_2\))\(_3\)), 3.47 (2H, s, COCH\(_2\)), 6.74 (1H, d, J = 8.4 Hz, H-3'), 7.51-7.54 (1H, m, H-4'), 7.72-7.75 (1H, br, H-6'), 8.19 (1H, s, H-8), 9.54 (1H, s, indole NH), 10.23 (1H, s, NH), 13.03 (1H, br, NH-9); HRMS calcd. for C\(_{24}\)H\(_{31}\)N\(_6\)OSi (ES+) m/z 447.2323 [M+H]+, found 447.2322.

7-(6-(2-(Triisopropylsilyl)ethynyl)-9H-purin-2-ylamino)indolin-2-one (70)

2-Fluoropurine intermediate 12 (0.120 g, 0.37 mmol), aminoindolinone 144 (0.110 g, 0.75 mmol) and TFA (142 μL, 1.85 mmol) were reacted in TFE (4 mL) according to **general procedure A**. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) afforded the desired compound as an orange oil (54 mg, 32%); Rf 0.36 (19:1 DCM/MeOH); λ_max (EtOH/nm) 255, 359; IR (cm\(^{-1}\)) 3351, 3251, 3070, 2943, 2866, 2159, 1704; \(^1\)H NMR (500 MHz, DMSO-d\(_6\)) 1.14-1.19 (21H, m, Si(CH(CH\(_3\))\(_2\))\(_3\)), 3.52 (2H, s, CH\(_2\)), 6.90-7.00 (2H, m, H-4' and H-5'), 7.58-7.62 (1H, m, H-6'), 8.20 (1H, s, H-8), 9.00 (1H, s, indole NH), 10.08 (1H, s, NH), 13.05 (1H, br, NH-9); HRMS calcd. for C\(_{24}\)H\(_{31}\)N\(_6\)OSi (ES+) m/z 447.2323 [M+H]+, found 447.2323.
2-Methyl-7-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)-3,4-dihydroisoquinolin-1(2H)-one (71)

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2-Fluoropurine 12 (0.225 g, 0.71 mmol), aniline 149 (0.250 g, 1.41 mmol) and TFA (136 µl, 1.77 mmol) were reacted in TFE (7 mL) according to \textit{general procedure B}. Chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired product as a yellow oil/gum (0.142 g, 42%); Rf 0.37 (19:1 DCM/MeOH, KP-NH); \(\lambda_{\text{max}}\) (EtOH/nm) 279; IR (cm\(^{-1}\)) 3390, 3136, 2941, 2864, 2167, 1609, 1577, 1538; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.12-1.23 (21H, m, Si(CH\(_3\))\(_3\)), 2.91 (2H, t, \(J = 6.6\) Hz, NCH\(_2\)CH\(_2\)), 3.04 (3H, s, NCH\(_3\)), 3.53 (2H, t, \(J = 6.6\) Hz, NCH\(_2\)CH\(_2\)), 7.19 (1H, d, \(J = 8.3\) Hz, H-5'), 7.90 (1H, dd, \(J = 2.3\) and 8.3 Hz, H-6'), 8.25 (1H, s, H-8), 8.27 (1H, d, \(J = 2.3\) Hz, H-8'), 9.77 (1H, s, NH), 13.14 (1H, br, NH-9); HRMS calcd. for C\(_{26}\)H\(_{35}\)N\(_6\)OSi (ES+) m/z 475.2636 [M+H]\(^+\), found 475.2635.

2-Methyl-7-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)-1,2-dihydroisoquinolin-3(4H)-one (72)

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2-Fluoropurine 12 (0.145 g, 0.45 mmol), aniline 152 (0.160 g, 0.91 mmol) and TFA (87 µL, 1.14 mmol) were reacted in TFE (5 mL) according to \textit{general procedure B}. Purification through chromatography on KP-NH silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil/gum (0.138 g, 64%); Rf 0.39 (19:1 DCM/MeOH, KP-NH); \(\lambda_{\text{max}}\) (EtOH/nm) 277; IR (cm\(^{-1}\)) 2943, 2864, 1605, 1577, 1537; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.13-1.22 (21H, m, Si(CH\(_3\))\(_3\)), 2.98 (3H, s, NCH\(_3\)), 3.46 (2H, s, COCH\(_2\)), 4.48 (2H, s, NCH\(_2\)), 7.10 (1H, d, \(J = 8.4\) Hz, H-5'), 7.66 (1H, dd, \(J = 2.1\) and 8.4 Hz, H-6'), 7.73-7.77 (1H, m, H-8'), 8.25 (1H, s, H-8), 9.71 (1H, s, NH), 13.08 (1H, br, NH-9); HRMS calcd. for C\(_{26}\)H\(_{35}\)N\(_6\)OSi (ES+) m/z 475.2636 [M+H]\(^+\), found 475.2631.
5-((6-Ethynyl-9H-purin-2-yl)amino)indolin-2-one (73)

TIPS-protected purine 69 (66 mg, 0.15 mmol), TBAF (1M in THF, 0.18 mL, 0.18 mmol) and TBAF scavenger resin (0.60 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on silica (9:1 DCM/MeOH) gave the desired compound as an orange solid (29 mg, 67%); Rf 0.38 (9:1 DCM/MeOH); m.p. 130-160 °C (decomposed); λ\text{max} (EtOH/nm) 285.0, 375.5; IR (cm\textsuperscript{-1}) 2835, 2161, 2112, 2028, 1977, 1668; \textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) 3.48 (2H, s, CH\textsubscript{2}), 4.83 (1H, s, C≡CH), 6.74 (1H, d, J = 8.4 Hz, H-7'), 7.53 (1H, dd, J = 1.4 and 8.4 Hz, H-6'), 7.69-7.71 (1H, m, H-4'), 8.24 (1H, s, H-8), 9.51 (1H, s, lactam-NH), 10.26 (1H, s, NH), 13.08 (1H, br, NH-9); HRMS calcd. for C\textsubscript{15}H\textsubscript{11}N\textsubscript{6}O (ES\textsuperscript{+}) m/z 291.0990 [M+H]\textsuperscript{+}, found 291.0989.

7-((6-Ethynyl-9H-purin-2-yl)amino)indolin-2-one (74)

TIPS-protected purine 70 (99 mg, 0.22 mmol), TBAF (1M in THF, 0.27 mL, 0.27 mmol) and TBAF scavenger resin (1.00 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the target compound as a pale orange solid (43 mg, 67%); Rf 0.27 (9:1 DCM/MeOH); m.p. 195-225 °C (decomposed); λ\text{max} (EtOH/nm) 244.0, 361.0; IR (cm\textsuperscript{-1}) 3248, 2363, 2169, 2013, 1978, 1662; \textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) 3.52 (2H, s, CH\textsubscript{2}), 4.81 (1H, s, C≡CH), 6.93 (1H, dd, J = 7.4 and 7.7 Hz, H-5'), 6.95-6.98 (1H, m, H-4'), 7.61-7.67 (1H, m, H-6'), 8.23 (1H, s, H-8), 8.96 (1H, s, lactam-NH), 10.11 (1H, s, NH), 13.08 (1H, br, NH-9); HRMS calcd. for C\textsubscript{15}H\textsubscript{11}N\textsubscript{6}O (ES\textsuperscript{+}) m/z 291.0990 [M+H]\textsuperscript{+}, found 291.0989.
7-((6-Ethynyl-9H-purin-2-yl)amino)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (75)

TIPS-protected purine 71 (98 mg, 0.21 mmol), TBAF (1M in THF, 0.25 mL, 0.25 mmol) and TBAF scavenger resin (1.00 g, 10 x w/w) were reacted in THF (5 mL) according to **general procedure G**. Purification by chromatography on silica (9:1 DCM/MeOH) gave the target compound as a yellow solid (36 mg, 54%); Rf 0.34 (9:1 DCM/MeOH); m.p. 280-300 °C (decomposed); λ_max (EtOH/nm) 276; IR (cm⁻¹) 3117, 2945, 2108, 1641, 1608, 1575, 1540; ¹H NMR (500 MHz, DMSO-d₆) 2.92 (2H, t, J = 6.6 Hz, NCH₂C₆H₂), 3.04 (3H, s, NCH₃), 3.54 (2H, t, J = 6.6 Hz, NC₆H₂CH₂), 4.85 (1H, s, C≡CH), 7.20 (1H, d, J = 8.3 Hz, H-5'), 7.89 (1H, dd, J = 2.3 and 8.3 Hz, H-6'), 8.26 (1H, d, J = 2.3 Hz, H-8'), 8.28 (1H, s, H-8), 9.73 (1H, s, NH), 13.2 (1H, br, NH-9); HRMS calcd. for C₁₇H₁₅N₆O (ES⁺) m/z 319.1302 [M+H]⁺, found 319.1307.

7-((6-Ethynyl-9H-purin-2-yl)amino)-2-methyl-1,2-dihydroisoquinolin-3(4H)-one (76)

TIPS-protected purine 72 (0.112 g, 0.24 mmol), TBAF (1M in THF, 0.28 mL, 0.28 mmol) and TBAF scavenger resin (1.10 g, 10 x w/w) were reacted in THF (5 mL) according to **general procedure G**. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the target compound as a yellow solid (41 mg, 54%); Rf 0.41 (9:1 DCM/MeOH); m.p. 200-220 °C (decomposed); λ_max (EtOH/nm) 274.5, 346.5; IR (cm⁻¹) 3292, 3086, 2963, 2774, 2104, 1610, 1551, 1494; ¹H NMR (500 MHz, DMSO-d₆) 2.98 (3H, s, NCH₃), 3.46 (2H, s, Ar-CH₂), 4.49 (2H, s, Ar-CH₂), 4.84 (1H, s, C=CH), 7.11 (1H, d, J = 8.5 Hz, H-5'), 7.65-7.71 (2H, m, H-6' and H-8'), 8.28 (1H, s, H-8), 9.69 (1H, s, NH), 13.13 (1H, br, NH-9); HRMS calcd. for C₁₇H₁₅N₆O (ES⁺) m/z 319.1302 [M+H]⁺, found 319.1301.

3-((2-Methyl-1,3-dithian-2-yl)methyl)aniline (77)
Tin(II) chloride (2.81 g, 14.8 mmol) was added to a mixture of aromatic nitro substrate 154 (1.00 g, 3.71 mmol) in ethanol (40 mL). The reaction mixture was heated at reflux for 1.5 h, after which the solvent was removed in vacuo. The resulting residue was dissolved in EtOAc (300 mL) and a saturated aqueous solution of NaHCO$_3$ was added until the aqueous phase was at pH 9-10. The resulting precipitate was removed by filtration through Celite$^\circledR$ and the organic phase was collected, washed with brine (100 mL) and evaporated to dryness. Purification by chromatography on silica (7:3 Petrol/EtOAc) gave the desired compound as an off-white solid on cooling (0.760 g, 85%); $R_f$ 0.41 (7:3 Petrol/EtOAc); m.p. 73-75 °C; $\lambda_{max}$ (EtOH/nm) 235, 291; IR (cm$^{-1}$) 3444, 3349, 2941, 2913, 1612, 1585; $^1$H NMR (500 MHz, DMSO-$d_6$) 1.48 (3H, s, CH$_3$), 1.80-1.92 (2H, m, C(SCH$_2$)$_2$CH$_2$), 2.82-2.99 (4H, m, C(SC$_2$H$_2$)$_2$CH$_2$), 3.02 (2H, s, Ar-CH$_2$), 6.41 (1H, ddd, $J = 1.2$, 1.6 and 7.6 Hz, H-6), 6.44 (1H, ddd, $J = 1.2$, 2.2 and 7.7 Hz, H-4), 6.46-6.48 (1H, m, H-2), 6.91 (1H, dd, $J = 7.6$ and 7.7 Hz, H-5); HRMS calcd. for C$_{12}$H$_{18}$NS$_2$ (ES+) $m/z$ 240.0875 [M+H]$^+$, found 240.0878.

$N$-(3-((2-methyl-1,3-dithian-2-yl)methyl)phenyl)-6-((triisopropylsilyl)ethynyl)-9H-purin-2-amine (78)

2-Fluoropurine 12 (0.195 g, 0.61 mmol), aniline 77 (0.220 g, 0.92 mmol) and TFA (117 µl, 1.53 mmol) were reacted in TFE (6 mL) according to general procedure B. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the target compound as a yellow oil/gum (0.182 g, 56%); $R_f$ 0.41 (19:1 DCM/MeOH, KP-NH); $\lambda_{max}$ (EtOH/nm) 276, 379; IR (cm$^{-1}$) 3072, 2942, 2863, 1595, 1575, 1533; $^1$H NMR (500 MHz, CDCl$_3$) 1.17-1.26 (21H, m, Si(CH(CH$_3$)$_2$)$_3$), 1.58 (3H, s, CH$_3$), 1.97-2.03 (2H, m, C(SCH$_2$)$_2$CH$_2$), 2.87-2.93 (4H, m, C(SCH$_2$)$_2$CH$_2$), 3.19 (2H, s, Ar-CH$_2$), 7.00-7.04 (1H, m, H-6'), 7.28 (1H, dd, $J = 7.7$ and 7.8 Hz, H-5'), 7.38 (1H, s, H-8), 7.39 (1H, s, NH), 7.44-7.47 (1H, m, H-2'), 7.49-7.54 (1H, m, H-4'), 11.80 (1H, s, NH-9); HRMS calcd. for C$_{28}$H$_{40}$N$_8$S$_2$Si (ES+) $m/z$ 538.2489 [M+H]$^+$, found 538.2484.
1-(3-((6-((Triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl)propan-2-one (79)

[Bis(trifluoroacetoxy)iodo]benzene (0.158 g, 0.37 mmol) was added to a solution of dithiane protected purine 78 (0.132 g, 0.25 mmol) in MeOH/H$_2$O (9:1, 25 mL). After stirring at RT for 10 min, the solution was diluted with sat. NaHCO$_3$ solution (30 mL) and the resultant suspension extracted with DCM (2 x 20 mL). The combined organic extracts were dried through a phase separator, evaporated to dryness, and the resultant residue was purified by chromatography on silica (19:1 DCM/MeOH). The desired compound was obtained as a yellow oil/gum (98 mg, 88%); R$_f$ 0.33 (19:1 DCM/MeOH); λ$_{max}$ (EtOH/nm) 277; IR (cm$^{-1}$) 3402, 2943, 2865, 2363, 2160, 1705, 1597, 1577, 1542; $^1$H NMR (500 MHz, CDCl$_3$) 1.14-1.25 (21H, m, Si(CH$_3$)$_2$), 2.16 (3H, s, CH$_3$), 3.67 (2H, s, CH$_2$), 6.85-6.89 (1H, m, H-6'), 7.27 (1H, dd, J = 7.7 and 7.9 Hz, H-5'), 7.40-7.45 (2H, m, H-4' and NH), 7.52-7.56 (1H, m, H-2'), 7.70 (1H, s, H-8), 11.44 (1H, br, NH-9); HRMS calcd. for C$_{25}$H$_{34}$N$_5$OSi (ES+) m/z 448.2527 [M+H]$^+$, found 448.2527.

1-(3-((6-Ethynyl-9H-purin-2-yl)amino)phenyl)propan-2-one (80)

TIPS-protected purine 79 (90 mg, 0.20 mmol), TBAF (1M in THF, 0.24 mL, 0.24 mmol) and TBAF scavenger resin (0.90 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on silica (9:1 DCM/MeOH) gave the desired compound as a yellow solid (43 mg, 70%); R$_f$ 0.43 (9:1 DCM/MeOH); m.p. 90-110 °C (decomposed); λ$_{max}$ (EtOH/nm) 273; IR (cm$^{-1}$) 3260, 3082, 2964, 2827, 2113, 1705, 1604, 1578, 1537; $^1$H NMR (500 MHz, DMSO-$d_6$) 2.15 (3H, s, CH$_3$), 3.71 (2H, s, CH$_2$), 4.84 (1H, s, C≡CH), 6.77-6.80 (1H, m, H-6'), 7.24 (1H, dd, J = 7.8 and 7.9 Hz, H-5'), 7.52 (1H, dd, J = 1.4 and 1.6 Hz, H-2'), 7.75-7.78 (1H, m, H-4'), 8.28 (1H, s, H-8), 9.64 (1H, s, NH), 13.14 (1H, br, NH-9); HRMS calcd. for C$_{16}$H$_{14}$N$_5$O (ES+) m/z 292.1193 [M+H]$^+$, found 292.1197.
**N-(3-((Methylsulfonyl)methyl)phenyl)-6-((triisopropylsilyl)ethynyl)-9H-purin-2-amine (81)**

2-Fluoropurine intermediate 12 (0.213 g, 0.67 mmol), aniline 157 (0.248 g, 1.34 mmol) and TFA (130 µL, 1.67 mmol) were reacted in TFE (7 mL) according to **general procedure B**. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) gave the target compound as a yellow oil/gum (0.134 g, 42%); Rf 0.36 (19:1 DCM/MeOH, KP-NH); \( \lambda_{\text{max}} \) (EtOH/nm) 277, 369; IR (cm\(^{-1}\)) 3338, 2942, 2864, 1599, 1577, 1539; \(^{1}\)H NMR (500 MHz, DMSO-\(d_6\)) 1.11-1.20 (21H, m, Si(CH\(\text{CH}_3\)_2)\_3), 2.94 (3H, s, CH\_3), 4.40 (2H, s, Ar-CH\_2), 6.96-7.01 (1H, m, H-4'), 7.27-7.31 (1H, dd, \( J = 7.9 \) and 8.1 Hz, H-5'), 7.77-7.80 (1H, m, H-2'), 7.85-7.90 (1H, m, H-6'), 8.26 (1H, s, H-8), 9.79 (1H, s, NH), 13.11 (1H, br, NH-9); HRMS calcd. for C\(_{24}\)H\(_{34}\)N\(_5\)O\(_2\)SSi (ES+) \( m/z \) 484.2197 [M+H]\(^{+}\), found 484.2197.

**6-Ethynyl-N-(3-((methylsulfonyl)methyl)phenyl)-9H-purin-2-amine (82)**

TIPS-protected purine 81 (0.111 g, 0.23 mmol), TBAF (1M in THF, 0.28 mL, 0.28 mmol) and TBAF scavenger resin (1.10 g, 10 x w/w) were reacted in THF (5 mL) according to **general procedure G**. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a pale yellow solid (52 mg, 66%); Rf 0.36 (9:1 DCM/MeOH); m.p. 180-200 °C (decomposed); \( \lambda_{\text{max}} \) (EtOH/nm) 346.5, 275.0; IR (cm\(^{-1}\)) 3330, 3257, 2982, 2919, 2116, 1607, 1548, 1492; \(^{1}\)H NMR (500 MHz, DMSO-\(d_6\)) 2.97 (3H, s, SO\(\text{CH}_3\)), 4.43 (2H, s, SO\(_2\)CH\(_2\)), 4.83 (1H, s, C\(\equiv\)CH), 6.98-7.02 (1H, m, H-4'), 7.32 (1H, dd, \( J = 7.9 \) and 8.0 Hz, H-5'), 7.69-7.73 (1H, m, H-2'), 7.85-7.89 (1H, m, H-6'), 8.27 (1H, s, H-8), 9.77 (1H, s, NH), 13.11 (1H, br, NH-9); HRMS calcd. for C\(_{15}\)H\(_{14}\)N\(_5\)O\(_2\)S (ES+) \( m/z \) 328.0863 [M+H]\(^{+}\), found 328.0868.
2,2,2-Trifluoroethyl (3-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl) methanesulfonate (83)

2-Fluoropurine intermediate 12 (0.416 g, 1.31 mmol) and aniline 160 (0.704 g, 2.62 mmol) were reacted with TFA (504 μL, 6.54 mmol) in TFE (10 mL) according to general procedure A. The resulting orange oil was purified by chromatography on reverse phase silica (19:1 MeOH/H2O + 0.1% HCOOH) followed by chromatography on silica (7:3 Petrol/EtOAc) to give the desired compound as a yellow/orange oil/gum (0.506 g, 68%); Rf 0.34 (9:1 MeOH/H2O, + 0.1% HCOOH, C18); λmax (EtOH/nm) 368, 277; IR (cm⁻¹) 2950, 2365, 2161, 2011, 1967, 1601; 1H NMR (500 MHz, DMSO-d6) 1.13-2.23 (21H, m, Si(CH3)3), 4.85 (2H, s, Ar-CH2), 4.95 (2H, q, J = 8.7 Hz, F3CCCH3O), 7.00-7.04 (1H, m, H-6'), 7.34 (1H, dd, J = 7.9 and 8.1 Hz, H-5'), 7.74-7.77 (1H, m, H-2'), 7.96-8.00 (1H, m, H-4'), 8.28 (1H, s, H-8), 9.84 (1H, s, NH), 13.12 (1H, br, NH-9); HRMS calcd. for C25H33F3N5O3SSi (ES+) m/z 568.2020 [M+H]+, found 568.2015.

N-(4-Methoxybenzyl)-1-(3-((6-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl)methanesulfonamide (84)

Trifluoroethylsulfonate ester 83 (0.215 g, 0.38 mmol), 4-methoxybenzylamine (64 μl, 0.49 mmol) and DBU (115 μL, 0.76 mmol) were reacted in dry THF (3 mL) according to general procedure H. Purification by chromatography on silica (19:1 DCM/MeOH) gave the desired compound as a yellow oil (0.224 g, 97%); Rf 0.44 (19:1 DCM/MeOH); λmax (EtOH/nm) 276; IR (cm⁻¹) 2361, 2341, 2162, 1992, 1969, 1609; 1H NMR (500 MHz, DMSO-d6) 1.13-1.22 (21H, m, Si(CH(CH3)2)3), 3.72 (3H, s, OCH3), 4.05 (2H, d, J = 6.0 Hz, NHCH3), 4.23 (2H, s,
Ar-CH₂), 6.87 (2H, d, J = 8.7 Hz, H-3" and H-5"), 6.91-6.95 (1H, m, H-6'), 7.23 (2H, d, J = 8.7 Hz, H-2" and H-6''), 7.28 (1H, dd, J = 8.0 and 8.2 Hz, H-5'), 7.55 (1H, t, J = 6.0 Hz, NHCH₂), 7.72-7.75 (1H, m, H-2'), 7.87-7.91 (1H, m, H-4'), 8.26 (1H, s, H-8), 9.76 (1H, s, NH), 13.06 (1H, s, NH-9); HRMS calcd. for C₃₁H₃₇N₆O₃SSi (ES+) m/z 605.2725 [M+H]*, found 605.2723.

(3-((6-((Triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl) methanesulfonamide (85)

PMB-sulfonamide 84 (0.221 g, 0.37 mmol) was reacted in TFA (6 mL) according to general procedure E over 3 h. The crude product was purified by chromatography on KP-NH silica (19:1 DCM/MeOH) to give the desired compound as a yellow oil/gum (0.108 g, 60%); Rf 0.21 (19:1 DCM/MeOH, KP-NH); λmax (EtOH/nm) 276; IR (cm⁻¹) 3367, 2943, 2865, 2160, 2021, 1606; ¹H NMR (500 MHz, DMSO-ｄ₆) 1.13-1.23 (21H, m, Si(CH(CH₃)₂)₃), 4.21 (2H, s, Ar-CH₂), 6.84 (2H, s, SO₂NH₂), 6.95-6.98 (1H, br, H-6'), 7.13 (1H, dd, J = 7.9 and 8.0 Hz, H-5'), 7.68-7.71 (1H, m, H-2'), 7.90 (1H, ddd, J = 1.0, 1.9 and 8.0 Hz, H-4'), 8.25 (1H, s, H-8), 9.75 (1H, s, NH), 13.07 (1H, s, NH-9); HRMS calcd. for C₂₃H₂₃N₆O₂SSi (ES+) m/z 485.2149 [M+H]*, found 485.2147.

N,N-Dimethyl-1-(3-((triisopropylsilyl)ethynyl)-9H-purin-2-yl)amino)phenyl methanesulfonamide (86)

Trifluoroethylsulfonate ester 83 (0.164 g, 0.29 mmol), dimethylamine (2M in THF, 0.29 mL, 0.58 mmol) and DBU (86 µL, 0.58 mmol) were reacted in dry THF (3 mL) according to general procedure H. Purification by chromatography on KP-NH silica (19:1 DCM/MeOH) afforded the target compound as a yellow oil/gum (0.148 g, 100%); Rf 0.50 (19:1 DCM/MeOH).
DCM/MeOH, KP-NH; $\lambda_{\text{max}}$ (EtOH/nm) 276, 366; IR (cm$^{-1}$) 3084, 2942, 2864, 2160, 1599, 1577, 1539; $^1$H NMR (500 MHz, DMSO-$d_6$) 1.13-1.23 (21H, m, Si(CH$_3$)$_2$), 2.74 (6H, s, N(CH$_3$)$_2$), 4.34 (2H, s, SO$_2$CH$_2$), 6.97-7.01 (1H, m, H-6$'$), 7.29 (1H, dd, $J$ = 7.9 and 8.0 Hz, H-5$'$), 7.80 (1H, dd, $J$ = 1.6 and 1.7 Hz, H-2$'$), 7.87 (1H, ddd, $J$ = 1.0, 1.7 and 8.0 Hz, H-4$'$), 8.27 (1H, s, H-8), 9.78 (1H, s, NH), 12.25 (1H, br, NH-9); HRMS calcd. for C$_{25}$H$_{37}$N$_6$O$_2$SSi (ES$^+$) m/z 513.2462 [M+H]$^+$, found 513.2451.

(3-((6-Ethynyl-9H-purin-2-yl)amino)phenyl)methanesulfonamide (87)

The TIPS-protected purine 85 (60 mg, 0.12 mmol), TBAF (1M in THF, 0.15 mL, 0.15 mmol) and the TBAF scavenger resin (0.60 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on silica (9:1 DCM/MeOH) gave the target compound as a pale yellow solid (23 mg, 58%); $R_f$ 0.29 (9:1 DCM/MeOH); m.p. 190-210 °C (decomposed); $\lambda_{\text{max}}$ (EtOH/nm) 273.5, 359.0; IR (cm$^{-1}$) 3361, 3248, 2976, 2809, 2707, 2114, 1701, 1610, 1583, 1491; $^1$H NMR (500 MHz, DMSO-$d_6$) 4.22 (2H, s, SO$_2$CH$_2$), 4.85 (1H, s, C≡CH), 6.86 (2H, s, SO$_2$NH$_2$), 6.94-6.98 (1H, m, H-6$'$), 7.30 (1H, dd, $J$ = 7.7 and 8.0 Hz, H-5$'$), 7.64-7.68 (1H, m, H-2$'$), 7.88-7.92 (1H, m, H-4$'$), 8.29 (1H, s, H-8), 9.72 (1H, s, NH), 13.13 (1H, br, NH-9); HRMS calcd. for C$_{14}$H$_{13}$N$_6$O$_2$S (ES$^+$) m/z 329.0815 [M+H]$^+$, found 329.0821.

1-(3-((6-Ethynyl-9H-purin-2-yl)amino)phenyl)-N,N-dimethylmethane sulfonamide (88)

The TIPS-protected purine 86 (0.139 g, 0.27 mmol), TBAF (1M in THF, 0.33 mL, 0.33 mmol) and TBAF scavenger resin (1.40 g, 10 x w/w) were reacted in THF (5 mL) according to general procedure G. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the target compound as a yellow solid (56 mg, 58%); $R_f$ 0.42 (9:1 DCM/MeOH); m.p. 90-110 °C (decomposed); $\lambda_{\text{max}}$ (EtOH/nm) 275, 367; IR (cm$^{-1}$) 3253, 3126, 2966, 2926, 2852, 2113, 1599, 1580, 1540; $^1$H NMR (500 MHz, DMSO-$d_6$) 2.76 (6H, s, N(CH$_3$)$_2$), 4.36 (2H, s, SO$_2$CH$_2$), 4.85 (1H, s, C≡CH), 6.98-7.01 (1H, m, H-6$'$), 7.31 (1H, dd, $J$ = 7.8 and 8.0 Hz, H-
5'), 7.73-7.76 (1H, m, H-2'), 7.84-7.89 (1H, m, H-4'), 8.29 (1H, s, H-8), 9.75 (1H, s, NH), 13.1 (1H, br, NH-9); HRMS calcd. for C_{16}H_{17}N_{6}O_{2}S (ES+) m/z 357.1128 [M+H]^+, found 357.1134.

(E)-6-(2-(azepan-1-yl)vinyl)-N-phenyl-9H-purin-2-amine (89)\(^6\)

A solution of 6-ethynyl-N-phenyl-9H-purin-2-amine (23) (50 mg, 0.21 mmol) and homopiperidine (480 μL, 4.23 mmol) in anhydrous THF (2 mL) was subjected to microwave heating at 100 °C for 10 minutes in a sealed nitrogen flushed microwave vial (2-5 mL capacity). The cooled solution was partitioned between EtOAc (20 mL) and saturated NaHCO\(_3\) solution (20 mL). The organic extract was concentrated in vacuo to a yellow/orange syrup which was subjected to purification by chromatography on KP-NH silica (9.5:0.5 DCM/MeOH), yielding the title compound as a yellow solid (69 mg, 98%); m.p. 135-137 °C (lit.,\(^6\) m.p. 135-137 °C); λmax (EtOH/nm) 360, 238, 254; IR (cm\(^{-1}\)) 3030, 2921, 2850, 1629, 1559; \(^1\)H NMR (500 MHz, CDCl\(_3\)) 1.49 (4H, m, homopiperidine CH\(_2\)), 1.64-1.71 (4H, m, homopiperidine CH\(_2\)), 3.28-3.38 (4H, m, homopiperidine CH\(_2\)), 5.49-5.52 (1H, d, J = 15.0 Hz, CH=CH), 6.95-6.99 (1H, t, J = 10.5 Hz, H-4'), 7.19 (1H, s, H-8), 7.22-7.26 (2H, dd, J = 9.5, 10.5 Hz, H-3' and H-5'), 7.32 (1H, br s, NH), 7.44-7.46 (2H, d, J = 9.5 Hz, H-2' and H-6'), 8.23-8.26 (1H, d, J = 15.0 Hz, CH=CH), 12.89 (1H, br s, NH-9); HRMS calcd for C\(_{19}\)H\(_{22}\)N\(_6\) [M+H]^+ 335.1975, found 335.1979.

6-(2-(Azepan-1-yl)ethyl)-N-phenyl-9H-purin-2-amine (90)

To a stirred solution of (E)-6-(2-(azepan-1-yl)vinyl)-N-phenyl-9H-purin-2-amine (89) (130 mg, 0.39 mmol) in THF (3 mL) was added sodium cyanoborohydride solution (1 M, in THF, 1.95 mL, 1.95 mmol) followed by TFA (3 µL, 0.04 mmol) or HCl (aq) (1 M, 40 µL, 0.04 mmol). Stirring was continued for 2 h (TFA) or 5 h (HCl), after which the solution was partitioned
between EtOAc (20 mL) and saturated NaHCO₃ solution (10 mL). The organic extract was dried (Na₂SO₄) before purification by chromatography on KP-NH silica (9.5:0.5 DCM/MeOH) to yield a pale yellow solid (41 mg, 30%); m.p. 47-49 °C; λmax (EtOH/nm) 326, 277, 238; IR (cm⁻¹) 2928, 2858, 1582; ¹H NMR (500 MHz, DMSO-d₆) 1.32-1.33 (4H, m, homopiperidine CH₂), 1.39-1.40 (4H, m, homopiperidine CH₂), 2.53-2.56 (4H, t, J = 5.5 Hz, homopiperidine NCH₂), 2.86-2.91 (2H, t, J = 8.0 Hz, CH₂), 2.92-2.98 (2H, d, J = 8.5 Hz, H-4'), 7.05-7.08 (2H, dd, J = 7.5 and 8.5 Hz, H-3' and H-5'), 7.65-7.66 (2H, d, J = 7.5 Hz, H-2' and H-6'), 7.97 (1H, s, H-8), 9.21 (1H, br s, NH); HRMS calcd for C₁₉H₂₅N₆ [M+H]+ 337.2135, found 337.2138.

**N-phenyl-6-vinyl-9H-purin-2-amine (92)**

\[
\begin{align*}
N & \quad H \\
H_b & \quad \text{H}_b \\
N & \quad \text{H}_a \\
H & \quad \text{H} \\
O & \quad \text{O}
\end{align*}
\]

m-CPBA (titrated as 62%, 30 mg, 0.108 mmol) was added in one portion to a stirred solution of 6-(2-(azepan-1-yl)ethyl)-N-phenyl-9H-purin-2-amine (90) (30 mg, 0.09 mmol) in anhydrous DCM (2 mL). The colourless solution instantly became bright yellow. After 2 h at room temperature the reaction was diluted with DCM (5 mL) and washed with saturated NaHCO₃ solution (4 mL). The crude product was purified by chromatography on silica (9.5:0.5 DCM/MeOH) to yield a yellow solid (6.4 mg, 30%); m.p. 121-123 °C; UV λmax (EtOH) 270, 207; IR (cm⁻¹) 1578, 1532, 1496, 1439, 1392, 1349; ¹H NMR (500 MHz, DMSO-d₆) 5.84-5.86 (1H, dd, J = 1.5 and 12.5 Hz, alkene Hₐ), 6.86-6.89 (1H, dd, J = 1.5 and 17.5 Hz, alkene Hₐ), 7.01-7.04 (1H, d, J = 8.5 Hz, H-4'), 7.08-7.14 (1H, dd, J = 12.5 and 17.5 Hz, alkene Hₐ), 7.24 (1H, s, H-8), 7.27-7.30 (2H, dd, J = 8.5 and 7.5 Hz, H-3' and H-5'), 7.52-7.54 (2H, d, J = 7.5 Hz, H-2' and H-6'); HRMS calcd for C₁₅H₁₂N₅ [M+H]+ 238.0509, found 238.0511.

**6-Ethynyl-2-fluoro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (93)**

The TIPS-protected purine 11 (68 mg, 0.169 mmol) and TBAF (1M in THF, 203 µL, 0.203 mmol) were reacted in THF (3 mL) according to general procedure C, with purification by
chromatography on silica (15-100% EtOAc/petrol) to give the desired product as an off-white solid (41 mg, 100%); Rf 0.32 (50% EtOAc/petrol); 1H NMR (500 MHz, CDCl3) 1.58-1.63 (1H, m, CH), 1.63-1.78 (2H, m, CH2), 1.90-1.99 (1H, m, CH), 1.99-2.06 (1H, m, CH), 2.07-2.12 (1H, m, CH), 3.68-3.74 (1H, m, CH), 3.71 (1H, s, C≡C), 4.09-4.14 (1H, m, CH), 5.65 (1H, dd, J = 2.6 and 10.8 Hz, NCH), 8.26 (1H, s, H-8); LRMS (ES+) m/z 247.0 [M+H]+.

6-Ethyl-2-fluoro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (94)

Lindlar’s catalyst (10 mg, 20% w/w) was suspended in a stirred solution of 93 (50 mg, 0.20 mmol) and quinoline (20 μL, 0.16 mmol) in EtOAc (5 mL) under a balloon of H2. After 2 h at room temperature the reduction was complete and the suspension was filtered through a plug of Celite, eluting with methanol (30 mL). Volatiles were removed in vacuo and the crude residue purified by chromatography on silica (50% EtOAc/petrol). The pure compound was isolated as a colourless oil (50 mg, 99%); λmax (EtOH/nm) 264; IR (cm⁻¹) 2946, 2860, 2364, 2338, 1604; 1H NMR (500 MHz, DMSO-d6) 1.35 (3H, t, J = 7.5 Hz, CH3), 1.59 (2H, m, tetrahydropyran CH2), 1.97 (1H, m, tetrahydropyran CH), 1.99 (2H, d, J = 10.5 Hz, tetrahydropyran CH2), 2.50 (1H, m, tetrahydropyran CH), 3.01 (2H, q, J = 7.5 Hz, CH2), 3.73 (1H, m, tetrahydropyran CH), 4.04 (1H, d, J = 10.5 Hz, tetrahydropyran CH), 5.69 (1H, d, J = 10.5 Hz, tetrahydropyran CH), 8.74 (1H, s, H-8); LRMS (ES+) m/z 251.0 [M+H]+.

6-Ethyl-2-fluoro-9H-purine (95)

TFA (3 mL) was added to a solution of 6-ethyl-2-fluoro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (94) (0.18 g, 0.72 mmol) in IPA (15 mL). Water (3 mL) was added and the solution was heated to reflux for 2 h. The mixture was cooled and neutralised (conc. NH3) before being extracted with EtOAc (3 x 50 mL) and the combined organic extracts dried (MgSO4) and concentrated. The resulting residue was purified by chromatography on silica (25% EtOAc/petrol). The compound was isolated as a white solid (0.117 g, 98%); m.p. 146-148 °C; λmax (EtOH/nm) 269; IR (cm⁻¹) 1676, 1616, 1573; 1H NMR (400 MHz, DMSO-d6) 1.30 (3H, t, J
= 7.5 Hz, CH₃), 3.00 (2H, q, J = 7.5 Hz, CH₂), 8.17 (1 H, s, H-8); LRMS (ES⁺) m/z = 167.7 [M+H]⁺.

4-(6-Ethyl-9H-purin-2-ylamino)benzenesulfonamide (96)

The title compound was synthesised according to general procedure A using: 6-ethyl-2-fluoro-9H-purine (95) (81 mg, 0.49 mmol) and 4-aminobenzenesulfonamide (0.17 g, 0.98 mmol). The compound was isolated after purification by chromatography on silica (50% EtOAc/petrol) as a white solid (33 mg, 21%); m.p. 291-293 ºC; λmax (EtOH/nm) 318, 287, 212; IR (cm⁻¹) 3377, 3060, 2852, 1388, 1158; ¹H NMR (500 MHz, DMSO-d₆) 1.35 (3H, t, J = 6.0 Hz, CH₃), 3.00 (2H, q, J = 6.0 Hz, CH₂), 7.14 (2H, br s, SO₂NH₂), 7.69 (2H, d, J = 7.5 Hz, H-2' and H-6'), 7.99 (2H, d, J = 7.5 Hz, H-3' and H-5'), 8.17 (1H, s, H-8), 9.89 (1H, br s, NH); HRMS calcd for C₁₃H₁₅N₆O₂ [M+H]⁺ 319.0971, found 319.0979.

N²-Phenylguanine 2,2,2-trifluoroacetate salt (98)

To a suspension of 2-bromohypoxanthine (1.00 g, 4.7 mmol) and aniline (0.9 mL, 9.40 mmol) in TFE (40 mL) was added TFA (1.80 mL, 23.5 mmol). The mixture was heated to reflux under nitrogen for 24 h. The mixture was filtered hot, washed with EtOH (3 × 50 mL/g), and air-dried for 30 min. The filtrate was evaporated in vacuo and the solid was recrystallised from EtOH to obtain the title compound as a white solid (1.17 g, 73%); m.p. 229-231 ºC; λmax (EtOH/nm) 272; IR (cm⁻¹) 3332, 3128, 2943, 2756, 2555, 2387, 1678, 1572; ¹H NMR (300 MHz, DMSO-d₆) 7.07 (1H, t, J = 7.5 Hz, H-4'), 7.36 (2H, dd, J = 7.5, 8.0 Hz, H-3' and H-5'), 7.62 (2 H, d, J = 8.0 Hz, H-2' and H-6'), 7.94 (1H, s, H-8), 8.46 (1H, br s, NH), 9.00 (1H, br s, NH); HRMS calcd for C₁₃H₁₅N₅O₂ [M+H]⁺ 319.0971, found 319.0979.

6-Chloro-N-phenyl-9H-purin-2-amine (99)¹⁰
**N^2-Phenylguanine trifluoroacetate salt (98)** (2.00 g, 5.87 mmol) and *N,N*-diethylaniline (1.9 mL, 11.73 mmol) was suspended in neat POCl₃ (30 mL) at room temperature. The reaction mixture was heated at 115 °C for 60 min under a nitrogen atmosphere. The resulting yellow solution was carefully added dropwise on to crushed ice in an ice bath with rapid stirring [CAUTION – VERY EXOTHERMIC]. Once addition was complete and the ice had melted, the homogeneous solution was neutralised to pH 7 by slow addition of NaOH solution (1.0 M), maintaining rapid stirring in an ice bath. The aqueous mixture was extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried (Na₂SO₄) and purified by chromatography on silica (50% EtOAc/petrol). The title compound was isolated as a white solid (0.67 g, 46%); m.p. 172-174 °C (lit. 155-160 °C); λ_max (EtOH/nm) 329, 272; IR (cm⁻¹) 3399, 3289, 1627, 1601, 1571, 1540; ¹H NMR (500 MHz, DMSO-d₆) 6.89-6.92 (1H, t, J = 7.5 Hz, H-4’), 7.23-7.26 (2H, dd, J = 7.4 and 7.5 Hz, H-3’ and H-5’), 7.71-7.73 (2H, d, J = 7.4 Hz, H-2’ and H-6’), 8.20 (1H, s, H-8), 9.81 (1H, br s, NH), 13.20 (1H, br s, NH-9); LRMS (ES⁺) m/z 246.06 [M+H]⁺.

**6-Chloro-9-(4-methoxybenzyl)-N-phenyl-9H-purin-2-amine (100)**

4-Methoxybenzylchloride (0.33 mL, 2.43 mmol) was added dropwise to a stirred solution of 6-chloro-*N*-phenyl-9H-purin-2-amine (99) (0.15 g, 0.6 mmol) and K₂CO₃ (0.25 g, 1.83 mmol) in anhydrous DMF (15 mL). The resulting solution was gently warmed to 60 °C under nitrogen for 18 h. Upon addition of water (50 mL) and brine (6 mL) to the mixture, a white precipitate was observed. The mixture was extracted with DCM (2 × 30 mL) and the combined organics dried (Na₂SO₄). The N-9 regioisomer was separated by column chromatography on silica (50% EtOAc/petrol) as a white crystalline solid (114 mg, 52%); m.p. 166-168 °C; λ_max (EtOH/nm) 274, 225; IR (cm⁻¹) 3286, 2834, 1599, 1511; ¹H NMR (500 MHz, DMSO-d₆) 3.74 (3H, s, OCH₃), 5.35 (2H, s, CH₂), 6.93-6.95 (2H, d, J = 9.0 Hz, H-3" and H-5"), 6.98-7.01 (1H, t, J = 8.5 Hz, H-4’), 7.30-7.34 (2H, dd, J = 8.0 and 8.5 Hz, H-3’ and
H-5'), 7.35-7.38 (2H, d, \( J = 9.0 \) Hz, H-2'' and H-6''), 7.76-7.79 (2H, d, \( J = 8.0 \) Hz, H-2' and H-6'), 8.04 (1H, s, H-8), 9.80 (1H, br s, NH); LRMS (ES') \( m/z \) 365.1 [M+1]+.

6-Cyano-9-(4-methoxybenzyl)-2-(phenylamino)-9H-purine-6 (101)

6-Chloro-9-(4-methoxybenzyl)-N-phenyl-9H-purin-2-amine (100) (0.10 g, 0.27 mmol) was suspended in anhydrous MeCN (10 mL) and stirred at room temperature. Addition of tetraethylamonium cyanide (86 mg, 0.55 mmol) followed by DABCO (61 mg, 0.55 mmol) afforded a yellow homogenous solution which was stirred for a further 18 h under nitrogen. Excess cyanide was hydrolysed by addition of aqueous ammonium hydroxide solution (32% v/v, 30 mL) with stirring for an additional 1 h. The crude mixture was partitioned between DCM (20 mL) and brine (20 mL). The organic layer was isolated and dried (Na\(_2\)SO\(_4\)) before purification by column chromatography on silica (50% EtOAc/petrol). The required compound was obtained as a bright yellow solid (62 mg, 62%); m.p. 242-244 °C; \( \lambda_{max} \) (EtOH) 356, 276; IR (cm\(^{-1}\)) 4000, 3470, 3293, 3180, 2258, 1611, 1584, 1511, 1471, 1245; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 3.73 (3H, s, OCH\(_3\)), 5.36 (2H, s, CH\(_2\)), 6.93-6.95 (2H, dd, \( J = 8.0, 8.5 \) Hz, H-3' and H-5'), 6.98-7.01 (1H, t, \( J = 8.5 \) Hz, H-4'), 7.34-7.39 (4H, m, H-2", H-3", H-5" and H-6"), 7.73-7.75 (2H, d, \( J = 8.0 \) Hz, H-2' and H-6'), 8.66 (1H, s, H-8), 10.11 (1H, br s, NH); LRMS (ES') \( m/z \) 357.2 [M+H]+.

6-Cyano-2-(phenylamino)-9H-purine (102)
6-Cyano-9-(4-methoxybenzyl)-2-(phenylamino)-9H-purine (101) (50 mg, 0.14 mmol) was dissolved in TFA (2 mL). The deep orange solution was heated at 70 °C for 5 h. The reaction mixture was concentrated in vacuo and the resulting orange oil was redisolved in EtOAc (2 mL). Residual TFA was neutralised by washing the organic phase with aqueous NaHCO₃ (2 × 2 mL). The organic extract was dried (Na₂SO₄) and purified using reversed phase column chromatography on silica (70% MeOH/H₂O + 0.1% HCOOH) to obtain a bright yellow solid (13 mg, 40%); m.p. 247–249 °C (decomposed); λmax (EtOH/nm) 387, 272; IR (cm⁻¹) 3389, 2255, 1601, 1537, 1496, 1396, 1348; ¹H NMR (500 MHz, DMSO-d₆) 7.01 (1H, t, J = 8.5 Hz, H-4'), 7.34 (2H, dd, J = 8.0 and 8.5 Hz, H-3' and H-5'), 7.76 (2H, d, J = 8.0 Hz, H-2' and H-6'), 8.52 (1H, s, H-8), 9.99 (1H, br s, NH), 13.60 (1H, br s, NH-9); HRMS calcd for C₁₂H₈N₆ [M+]⁺ 236.0738, found 236.0734.

2. Synthesis of Aniline Precursors

![Scheme A](image)

**Scheme A. Reagents and conditions:** (a) TIPSCI, imidazole, DCM, RT, 2 h; (b) Zn, AcOH, RT, 2.5 h.

**(4-Nitrophenoxo)triisopropylsilane (104)**¹¹

Imidazole (0.735 g, 10.8 mmol) was added to a solution of 103 (0.502 g, 3.60 mmol) in dry DCM (10 mL). Triisopropylsilyl chloride (1.5 mL, 7.20 mmol) was added, and the reaction mixture was stirred at RT for 2 h. The mixture was washed with sat. brine solution (2 × 10 mL) and the combined aqueous washings were extracted with DCM (3 × 10 mL). The combined organic extracts were dried (Na₂SO₄) and concentrated, and the resulting residue was purified by chromatography on silica (Petrol). The desired compound was obtained as a colourless oil (0.998 g, 3.38 mmol, 94%); Rf 0.40 (Petrol); λmax (EtOH/nm) 245, 295; IR (cm⁻¹) 2980, 2930, 2156; ¹H NMR (500 MHz, DMSO-d₆) 1.13 (18H, d, J = 7.6 Hz, Si(CH(CH₃)₂)₃),
1.38 (3H, sept, J = 7.6 Hz, Si(CH(CH$_3$)$_2$)$_3$), 7.14 (2H, d, J = 9.2 Hz, H-2 and H-6), 8.24 (2H, d, J = 9.2 Hz, H-3 and H-5); LRMS (ES+) m/z 296.2 [M+H]$^+$. 

4-Triisopropylsilyloxyaniline (105)$^{12}$

![Image of 4-Triisopropylsilyloxyaniline](image)

Zinc (0.454 g, 6.94 mmol) was added to a solution of the nitro compound 104 (0.205 g, 0.69 mmol) in acetic acid (10 mL) and the mixture was stirred at RT for 2.5 h. The solvent was removed in vacuo and the residue was dissolved in water. The solution was adjusted to pH 8 (conc. NH$_3$) and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried (Na$_2$SO$_4$) and concentrated, and the residue was purified by chromatography on silica (9:1 Petrol/EtOAc). The desired compound was obtained as an orange oil (0.132 g, 0.50 mmol, 72%); R$_f$ 0.76 (7:3 Petrol/EtOAc); $\lambda_{\text{max}}$ (EtOH/nm) 236, 301; IR (cm$^{-1}$) 2944, 2866, 1613; $^1$H NMR (500 MHz, DMSO-d$_6$) 0.94 (18H, d, J = 7.3 Hz, Si(CH(CH$_3$)$_2$)$_3$), 1.07 (3H, sept, J = 7.3 Hz, Si(CH(CH$_3$)$_2$)$_3$), 4.64 (2H, s, Ar-NH$_2$), 6.36 (2H, d, J = 8.9 Hz, H-2 and H-6), 6.47 (2H, d, J = 8.9 Hz, H-3 and H-5); LRMS (ES+) m/z 266.2 [M+H]$^+$. 

Scheme B. Reagents and conditions: (a) Boc$_2$O, 1,4-dioxane, NaOH, H$_2$O, RT, 18 h; (b) PMB-NH$_2$, CDI, DIPEA, DMF, RT, 18 h; (c) TFA, DCM, RT, 18 h.

3-((tert-Butoxycarbonyl)amino)benzoic acid (108)$^{13}$

![Image of 3-((tert-Butoxycarbonyl)amino)benzoic acid](image)

3-Aminobenzoic acid 106 (1.00 g, 7.29 mmol) and di-tert-butyl dicarbonate (1.75 g, 8.02 mmol) were dissolved in 1,4-dioxane (15 mL), water (7.5 mL) and 0.5 M NaOH (15 mL). The reaction mixture was stirred at RT for 18 h, after which the volume was reduced by half in vacuo and the solution taken to pH 3 with 2 M KHSO$_4$ solution. The aqueous mixture was extracted with EtOAc (2 × 70 mL) and the combined organic extracts were dried (MgSO$_4$)
and concentrated in vacuo. The crude residue was purified by chromatography on silica and was obtained as a white waxy solid (1.61 g, 6.77 mmol, 93%); Rf 0.15 (1:1 Petrol/EtOAc); m.p. 183-185 °C (lit.13 189-190 °C); λmax (EtOH/nm) 247, 301; IR (cm⁻¹) 3352, 3002, 2971, 1690; ¹H NMR (500 MHz, DMSO-d₆) 1.49 (9H, s, C(CH₃)₃), 7.37 (1H, d, J = 7.9 and 8.0 Hz, H-5), 7.54 (1H, ddd, J = 1.2 and 1.3 and 7.9 Hz, H-6), 7.61-7.65 (1H, m, H-4), 8.13-8.16 (1H, m, H-2), 9.55 (1H, br, Ar-NH), 12.88 (1H, br, COOH); LRMS (ES-) m/z 236.2 [M-H]⁻.

3-(3-(tert-Butoxycarbonylamino)phenyl)propanoic acid (109)

(3-Aminophenyl)propionic acid 107 (1.00 g, 6.06 mmol) and di-tert-butyl dicarbonate (1.45 g, 6.66 mmol) were dissolved in 1,4-dioxane (12 mL), 1 M NaOH (6 mL) and water (6 mL). The reaction mixture was stirred at RT for 18 h, after which the volume was reduced by half in vacuo and the solution taken to pH 3 with 2 M KHSO₄ solution. The aqueous mixture was extracted with EtOAc (2 × 60 mL) and the combined organic extracts were dried (MgSO₄) and concentrated in vacuo. The crude residue was purified by chromatography on silica to give the desired product, which was obtained as an off-white waxy solid (1.42 g, 5.35 mmol, 88%); Rf 0.18 (1:1 Petrol/EtOAc); m.p. 120-123 °C; λmax (EtOH/nm) 249, 276; IR (cm⁻¹) 3307, 2927, 1690; ¹H NMR (500 MHz, DMSO-d₆) 1.48 (9H, s, C(CH₃)₃), 2.50 (2H, t, J = 7.7 Hz, COCH₂CH₂), 2.76 (2H, t, J = 7.7 Hz, COCH₂CH₂), 6.81-6.85 (1H, m, H-6), 7.11-7.16 (1H, m, H-5), 7.22-7.26 (1H, m, H-4), 7.34-7.37 (1H, m, H-2), 9.27 (1H, s, Ar-NH), 12.13 (1H, s, COOH); HRMS calcd. for C₁₄H₁₈NO₄ (ES-) m/z 264.1241 [M-H]⁻, found 264.1233.

tert-Butyl 3-(4-methoxybenzylcarbamoyl)phenyl carbamate (110)

Carboxylic acid 108 (0.601 g, 2.53 mmol), CDI (0.823 g, 5.07 mmol) and DIPEA (885 μL, 5.07 mmol) were combined in dry DMF (13 mL) and stirred at RT for 1.5 h. 4-Methoxybenzylamine (1.31 mL, 10.1 mmol) was added to the mixture. Following a further 18 h stirring at RT, the solvent was removed in vacuo and the resulting residue was purified by chromatography on silica (7:3 Petrol/EtOAc). The desired compound was obtained as a
white solid (0.841 g, 2.36 mmol, 93%); Rf 0.27 (7:3 Petrol/EtOAc); m.p. 166-170 °C; λmax (EtOH/nm) 234; IR (cm⁻¹) 3349, 3274, 3062, 2836, 1697, 1644; 1H NMR (500 MHz, DMSO-d6) 1.49 (9H, s, C(CH₃)₃), 3.74 (3H, s, OCH₃), 4.38 (2H, d, J = 6.0 Hz, CH₂NH), 6.89 (2H, d, J = 8.4 Hz, H-3’ and H-5’), 7.24 (2H, d, J = 8.4 Hz, H-2’ and H-6’), 7.33 (1H, dd, J = 7.9 and 8.1 Hz, H-5), 7.43-7.47 (1H, m, 1H, m, H-6), 7.98-8.00 (1H, m, H-2), 8.89 (1H, t, J = 6.0 Hz, CH₂N), 9.48 (1H, s, Ar-NH); HRMS calcd. for C₂₀H₂₅N₂O₄ (ES+) m/z 357.1809 [M+H]+, found 357.1812.

tert-Butyl-3-(2-(4-methoxybenzylcarbamoyl)ethyl)phenyl carbamate (111)

Carboxylic acid 109 (0.762 g, 2.87 mmol), CDI (0.932 g, 5.74 mmol) and DIPEA (1.00 mL, 5.74 mmol) were combined in dry DMF (15 mL) and stirred at RT for 1.5 h. 4-methoxybenzylamine (1.50 mL, 11.5 mmol) was added to the mixture. Following a further 18 h stirring at RT, the solvent was removed in vacuo and the resulting residue was purified by chromatography on silica (1:1 Petrol/EtOAc) to afford the desired compound as a colourless oil (1.08 g, 2.80 mmol, 98%); Rf 0.48 (1:1 Petrol/EtOAc); λmax (EtOH/nm) 232, 274; IR (cm⁻¹) 3363, 3271, 2971, 2933, 1698, 1643; 1H NMR (500 MHz, DMSO-d6) 1.47 (9H, s, C(CH₃)₃), 2.40 (2H, t, J = 7.6 Hz, CH₂CH₂), 2.78 (2H, t, J = 7.6 Hz, CH₂CH₂), 3.72 (3H, s, OCH₃), 4.18 (2H, d, J = 5.9 Hz, CH₂NH), 6.79-6.83 (1H, m, H-4), 6.84 (2H, d, J = 8.4 Hz, H-3’ and H-5’), 7.07 (2H, d, J = 8.4 Hz, H-2’ and H-6’), 7.14 (1H, dd, J = 8.1 and 8.2 Hz, H-5), 7.21-7.24 (1H, m, H-6), 7.35-7.38 (1H, m, H-2), 8.25 (1H, t, J = 5.9 Hz, CH₂N), 9.27 (1H, s, Ar-NH); HRMS calcd. for C₂₂H₂₉N₂O₄ (ES+) m/z 385.2122 [M+H]+, found 385.2126.

N-(4-Methoxybenzyl)-3-aminobenzamide (112)

Boc-protected aniline 110 (0.831 g, 2.33 mmol) was treated with TFA (1.80 mL, 23.3 mmol) in DCM (25 mL), which was stirred at RT for 18 h, before diluting with DCM (25 mL). The crude mixture was washed with sat. aqueous NaHCO₃ (25 mL). The resulting biphasic
mixture was passed through an Isolute® phase separator, and the organic phase concentrated to dryness. The resulting crude residue was purified by chromatography on silica (1:1 Petrol/EtOAc), giving the target compound as a white waxy solid (0.472 g, 1.84 mmol, 79%); Rf 0.30 (1:1 Petrol/EtOAc); m.p. 90-93 °C; λmax (EtOH/nm) 242, 306; IR (cm⁻¹) 3349, 3274, 2972, 2930, 1697, 1643; ¹H NMR (500 MHz, DMSO-d₆) 3.73 (3H, s, OCH₃), 4.36 (2H, d, J = 6.1 Hz, CH₂NH), 5.22 (2H, s, Ar-NH₂), 6.89 (1H, ddd, J = 2.3 and 2.4 and 7.9 Hz, H-4), 6.88 (2H, d, J = 8.8 Hz, H-3' and H-5'), 6.97-7.00 (1H, m, H-6), 7.04-7.09 (2H, m, H-2 and H-5), 7.23 (2H, d, J = 8.8 Hz, H-2' and H-6'), 8.73 (1H, t, J = 6.1 Hz, CH₂NH); HRMS calcd. for C₁₅H₁₇N₂O₂ (ES+) m/z 257.1285 [M+H]⁺, found 257.1289.

**N-(4-Methoxybenzyl)-3-(3-aminophenyl)propanamide (113)**

![Chemical structure](image)

Boc-protected aniline 111 (1.00 g, 2.60 mmol) was treated with TFA (2.00 mL, 26.0 mmol) in DCM (25 mL), which was stirred at RT for 18 h, before diluting with DCM (25 mL). The crude mixture was washed with satu. aqueous NaHCO₃ (25 mL). The resulting biphasic mixture was passed through an Isolute® phase separator, and the organic phase concentrated to dryness. The resulting crude residue was purified by chromatography on silica (1:1 Petrol/EtOAc) affording the desired compound as a white solid (0.643 g, 2.26 mmol, 87%); Rf 0.46 (1:1 Petrol/EtOAc); m.p. 88-91 °C; λmax (EtOH/nm) 231, 274; IR (cm⁻¹) 3424, 3337, 3241, 3063, 2945, 2904, 1629; ¹H NMR (500 MHz, DMSO-d₆) 2.36 (2H, t, J = 7.8 Hz, CH₂CH₂), 2.67 (2H, t, J = 7.8 Hz, CH₂CH₂), 3.73 (3H, s, OCH₃), 4.18 (2H, d, J = 5.9 Hz, CH₂NH), 4.95 (2H, s, Ar-NH₂), 6.33-6.40 (3H, m, H-2, H-4 and H-6), 6.86 (2H, d, J = 7.9 Hz, H-3' and H-5'), 6.90 (1H, dd, J = 7.4 and 7.5 Hz, H-5'), 7.11 (2H, d, J = 7.9 Hz, H-2' and H-6'), 8.25 (1H, t, J = 5.9 Hz, CH₂NH); HRMS calcd. for C₁₇H₂₁N₂O₂ (ES+) m/z 285.1598 [M+H]⁺, found 285.1603.

![Chemical reactions](image)

114; R = 3-CH₂CO₂H  
115; R = 4-CO₂H  
116; R = 3-CH₂CO₂Me  
117; R = 4-CO₂Me  
118; R = 3-CH₂CONHMe  
119; R = 4-CONH₂  
120; R = 4-CONHMe
**Scheme C: Reagents and conditions:** (a) SOCl₂, MeOH, reflux, 1 h; (b) Aq. RNH₂, RT to 60 °C, 18 h.

**Methyl 2-(3-aminophenyl)acetate (116)**

![Structure](image)

(3-Aminophenyl)acetic acid (1.00 g, 6.62 mmol) and thionyl chloride (970 μL, 13.2 mmol) were combined in dry MeOH (35 mL). The resulting solution was heated at reflux for 1 h, after which the solvent was removed *in vacuo*. The crude residue was purified by chromatography on silica (7:3 Petrol/EtOAc) to give the target compound as a pale yellow oil (0.927 g, 5.61 mmol, 85%); Rf 0.32 (7:3 Petrol/EtOAc); λ<sub>max</sub> (EtOH/nm) 221, 285; IR (cm<sup>-1</sup>) 3369, 2952, 1724; <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) 3.50 (2H, s, CH₂), 3.61 (3H, s, OCH₃), 5.70 (2H, br, Ar-NH₂), 6.45-6.48 (1H, m, H-4), 6.51-6.54 (2H, m, H-2 and H-6), 6.97-7.01 (1H, m, H-5); LRMS (ES+) m/z 166.2 [M+H]<sup>+</sup>.

**Methyl 4-aminobenzoate (117)**

![Structure](image)

4-Aminobenzoic acid (0.500 g, 3.65 mmol) and thionyl chloride (0.53 mL, 7.29 mmol) were combined in dry methanol (20 mL). The resulting solution was heated at reflux for 1 h, after which the solvent was removed *in vacuo*. The crude residue was purified by chromatography on silica (7:3 Petrol/EtOAc) to afford the desired compound as a white solid (0.520 g, 3.44 mmol, 94%); Rf 0.42 (7:3 Petrol/EtOAc); m.p. 111-114 °C (lit.<sup>5</sup> 109-110 °C); λ<sub>max</sub> (EtOH/nm) 255, 309; IR (cm<sup>-1</sup>) 3407, 3336, 3298, 2944, 1681; <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) 3.74 (3H, s, OCH₃), 5.97 (2H, s, Ar-NH₂), 6.57 (2H, d, J = 8.8 Hz, H-3 and H-5), 7.64 (2H, d, J = 8.8 Hz, H-2 and H-6); LRMS (ES+) m/z 152.2 [M+H]<sup>+</sup>.

**2-(3-Aminophenyl)-N-methylacetamide (118)**

![Structure](image)

Methyl ester 116 (0.250 g, 1.51 mmol) was suspended in a concentrated aqueous solution of methylamine (40%, 8 mL) and the resulting mixture was stirred at RT for 24 h. Chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a pale yellow
oil (0.233 g, 1.42 mmol, 94%); Rf 0.38 (9:1 DCM/MeOH); λmax (EtOH/nm) 220; IR (cm⁻¹) 3331, 3091, 2943, 1629; ¹H NMR (500 MHz, DMSO-d₆) 2.56 (3H, d, J = 4.8 Hz, NHCH₃), 3.20 (2H, s, CH₂), 4.98 (2H, s, Ar-NH₂), 6.37-6.41 (2H, m, H-4 and H-6), 6.42-6.45 (1H, br, H-2), 6.91 (1H, dd, J = 7.7 and 7.9 Hz, H-5), 7.82 (1H, br, NHCH₃); LRMS (ES+) m/z 165.2 [M+H]+.

4-Aminobenzamide (119)¹⁷

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H₂N
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NH₂
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Methyl ester 117 (0.408 g, 2.70 mmol) was dissolved in concentrated ammonium hydroxide (10 mL) and the resulting solution was heated at 60 °C for 18 h. The solvent was removed in vacuo and the crude product was purified by chromatography on KP-NH silica (19:1 DCM/MeOH) to give the desired compound as a white solid (0.214 g, 1.57 mmol, 58%); Rf 0.31 (19:1 DCM/MeOH); m.p. 178-181 °C (lit. 171-175 °C); λmax (EtOH/nm) 230, 262; IR (cm⁻¹) 3463, 3317, 3205, 1591; ¹H NMR (500 MHz, DMSO-d₆) 5.59 (2H, s, Ar-NH₂), 6.52 (2H, d, J = 8.6 Hz, H-3 and H-5), 6.82 (1H, br, CONH'), 7.50 (1H, br, CONHH'), 7.58 (2H, d, J = 8.6 Hz, H-2 and H-6); LRMS (ES+) m/z 137.1 [M+H]+.

4-Amino-N-methylbenzamide (120)¹⁷

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Methyl ester 117 (0.413 g, 2.70 mmol) was suspended in a concentrated aqueous solution of methylamine (40%, 10 mL) and the reaction mixture was stirred at RT for 24 h. The solvent was removed in vacuo and the crude residue was purified by chromatography on silica (19:1 DCM/MeOH) to afford the desired compound as a pale yellow solid (0.396 g, 2.64 mmol, 98%); Rf 0.36 (19:1 DCM/MeOH); m.p. 173-176 °C (lit. 178-180 °C); λmax (EtOH/nm) 236, 270; IR (cm⁻¹) 3399, 3336, 3228, 2934, 1627; ¹H NMR (500 MHz, DMSO-d₆) 2.72 (3H, d, J = 4.6 Hz, NHCH₃), 5.57 (2H, s, Ar-NH₂), 6.53 (2H, d, J = 8.6 Hz, H-3 and H-5), 7.55 (2H, d, J = 8.6 Hz, H-2 and H-6), 7.94 (1H, br, NHCH₃); LRMS (ES+) m/z 151.2 [M+H]+.
Scheme D. Reagents and conditions: (a) SOCl₂, MeOH, reflux, 1 h; (b) Aq. MeNH₂, RT, 18 h.

2-(4-Aminophenyl)-N-methylacetamide (121)

4-Aminophenylacetic acid (0.601 g, 3.97 mmol) and thionyl chloride (0.58 mL, 7.94 mmol) were combined in methanol (20 mL) and heated at reflux for 1 h, after which the solvent was removed in vacuo. A concentrated aqueous solution of methylamine (40% aqueous solution, 20 mL) was added to the residue and the reaction mixture was stirred at RT for 24 h. The solvent was removed in vacuo and the crude residue was purified by chromatography on silica (19:1 DCM/MeOH), affording the desired product as a pale orange oil (0.573 g, 3.49 mmol, 88%); Rf 0.39 (19:1 DCM/MeOH); λmax (EtOH/nm) 242, 291; IR (cm⁻¹) 3444, 3347, 3308, 3098, 1618, 1556, 1515; ¹H NMR (500 MHz, DMSO-d₆) 2.55 (3H, d, J = 4.6 Hz, NHC₃H₃), 3.18 (2H, s, COCH₂), 4.89 (2H, s, Ar-NH₂), 6.49 (2H, d, J = 8.4 Hz, H-3 and H-5), 6.89 (2H, d, J = 8.4 Hz, H-2 and H-6), 7.74 (1H, br, NHCH₃); LRMS (ES+) m/z 165.1 [M+H]+.

Scheme E. Reagents and conditions: (a) i) CDI, DIPEA, DMF, RT, 1.5 h, ii) MeRNH, THF, RT, 18 h. (b) Fe, AcOH, 50 °C, 15 min.

N,N-Dimethyl-2-(4-nitrophenyl)acetamide (123)

4-Nitrophenylacetic acid (0.600 g, 3.31 mmol), CDI (1.07 g, 6.62 mmol) and DIPEA (1.15 mL, 6.62 mmol) were combined in dry DMF (30 mL) and stirred at RT for 1.5 h. Dimethylamine (2M in THF, 6.60 mL, 13.2 mmol) was added to the mixture and stirring was continued at RT for a further 18 h. The solvent was removed in vacuo and the resulting residue was purified by chromatography on silica (7:3 Petrol/EtOAc), affording the target compound as an off-white solid (0.204 g, 0.98 mmol, 30%); Rf 0.38 (7:3 Petrol/EtOAc); m.p. 80-83 °C (lit.¹⁸ 87 °C);
\( \lambda_{\text{max}} \) (EtOH/nm) 270; IR (cm\(^{-1}\)) 2937, 2449, 1637, 1512; \(^1\)H NMR (500 MHz, CDCl\(_3\)) 3.01 (3H, s, NCH\(_3\)), 3.08 (3H, s, NCH\(_3\)), 3.83 (2H, s, COCH\(_2\)), 7.45 (2H, d, \( J = 8.5 \) Hz, H-2 and H-6), 8.21 (2H, d, \( J = 8.5 \) Hz, H-3 and H-5); LRMS (ES+) \( m/z \) 209.1 [M+H]\(^+\).

2-(4-Aminophenyl)-\(N,N\)-dimethylacetamide (124)

Iron powder (0.355 g, 6.39 mmol) was added to a solution of nitro compound 123 (0.133 g, 0.64 mmol) in acetic acid (6 mL) and the resulting mixture was heated at 50 °C for 15 min, before being filtered through Celite\textsuperscript{®} and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO\(_3\) (2 × 200 mL) and brine (200 mL). The combined aqueous layers were extracted with EtOAc (200 mL) and the combined organic extracts were dried (MgSO\(_4\)) and evaporated to dryness. Chromatography on silica (9:1 DCM/MeOH) gave the desired compound as a pale red oil (0.105 g, 0.58 mmol, 92%); \( R_f \) 0.40 (9:1 DCM/MeOH); \( \lambda_{\text{max}} \) (EtOH/nm) 242, 291; IR (cm\(^{-1}\)) 3414, 3342, 3230, 2933, 1611, 1515; \(^1\)H NMR (500 MHz, DMSO-d\(_6\)) 2.81 (3H, s, NCH\(_3\)), 2.96 (3H, s, NCH\(_3\)), 3.46 (2H, s, COCH\(_2\)), 4.89 (2H, s, Ar-NH\(_2\)), 6.50 (2H, d, \( J = 8.4 \) Hz, H-3 and H-5), 6.86 (2H, d, \( J = 8.4 \) Hz, H-2 and H-6); LRMS (ES+) \( m/z \) 179.1 [M+H]\(^+\).

Scheme F. Reagents and conditions: (a) i) CDI, DIPEA, DMF, RT, 1.5 h, ii) Methylamine, THF, RT, 18 h. (b) Fe, AcOH, 50 °C, 15 min.

\( N',N^6\)-Dimethyl-2-(4-nitrophenyl)malonamide (125)

4-Nitrophenylacetic acid (0.598 g, 3.31 mmol), CDI (1.07 g, 6.62 mmol) and DIPEA (1.15 mL, 6.62 mmol) were combined in dry DMF (30 mL) and stirred at RT for 1.5 h. Methylamine (2M in THF, 6.60 mL, 13.2 mmol) was added to the mixture and stirred at RT for a further 18 h.
The solvent was removed in vacuo and the resulting residue was purified by chromatography on silica (7:3 Petrol/EtOAc) to give the desired compound as an off-white solid (0.608 g, 2.42 mmol, 73%); Rf 0.33 (7:3 Petrol/EtOAc); m.p. 221-224 °C; \( \lambda_{\text{max}} \) (EtOH/nm) 273; IR (cm\(^{-1}\)) 3405, 3311, 3111, 2932, 1655, 1512; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.62 (6H, d, \( J = 4.6 \) Hz, 2 x NHC\(H_3\)), 4.57 (1H, s, COC\(H\)), 7.65 (2H, d, \( J = 8.8 \) Hz, H-2 and H-6), 8.11 (2H, q, \( J = 4.6 \) Hz, 2 x NH), 8.21 (2H, d, \( J = 8.8 \) Hz, H-3 and H-5); HRMS calcd. for C\(_{11}\)H\(_{14}\)N\(_3\)O\(_4\) (ES+) \( m/z \) 252.0979 [M+H]\(^+\), found 252.0983.

2-(4-Aminophenyl)-\(N^1, N^8\)-dimethylmalonamide (126)

Iron powder (1.70 g, 30.5 mmol) was added to a solution of nitro compound 125 (0.593 g, 3.05 mmol) in acetic acid (30 mL). The reaction mixture was heated at 50 °C for 15 min, before being filtered through Celite\(^\circledR\) and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO\(_3\) (2 x 200 mL) and brine (200 mL). The combined aqueous layers were extracted with EtOAc (200 mL) and the combined organic extracts were dried (MgSO\(_4\)) and evaporated to dryness. The resulting residue was purified by chromatography on silica (9:1 DCM/MeOH) to give the desired compound as a beige waxy solid (0.380 g, 2.32 mmol, 76%); Rf 0.35 (9:1 DCM/MeOH); m.p. 148-150 °C; \( \lambda_{\text{max}} \) (EtOH/nm) 248, 292; IR (cm\(^{-1}\)) 3414, 3273, 3100, 2937, 1663, 1650, 1515; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.59 (6H, d, \( J = 4.6 \) Hz, 2 x NHC\(H_3\)), 4.11 (1H, s, COC\(H\)), 5.00 (2H, s, Ar-NH\(_2\)), 6.49 (2H, d, \( J = 8.5 \) Hz, H-3 and H-5), 7.00 (2H, d, \( J = 8.5 \) Hz, H-2 and H-6), 7.94 (2H, q, \( J = 4.6 \) Hz, 2 x NHCH\(_3\)); HRMS calcd. for C\(_{11}\)H\(_{16}\)N\(_3\)O\(_2\) (ES+) \( m/z \) 222.1237 [M+H]\(^+\), found 222.1239.
Scheme G: *Reagents and conditions:* (a) R$_2$NH, PCl$_3$, MeCN, MW 150 °C, 5 min; (b) Fe, AcOH, 50 °C, 15 min.

*N-Methyl-3-nitrobenzamide (128)\textsuperscript{20}*

![N-Methyl-3-nitrobenzamide](image)

Phosphorus trichloride (288 µL, 3.29 mmol) was added to a solution of 3-nitrobenzoic acid (0.502 g, 2.99 mmol) and methylamine (2M in THF, 3.74 mL, 7.48 mmol) in dry MeCN (15 mL) in a sealed vial. The reaction mixture was heated under microwave irradiation at 150 °C for 5 min, before being concentrated to dryness. The resulting residue was dissolved in DCM (30 mL) and washed with a sat. aqueous solution of NaHCO$_3$ (30 mL). The biphasic mixture was passed through an Isolute® phase separator and the organic phase was concentrated *in vacuo*. Purification by chromatography on silica (7:3 Petrol/EtOAc) gave the desired compound as a beige solid (0.535 g, 2.97 mmol, 99%); R$_f$ 0.33 (7:3 Petrol/EtOAc); m.p. 175-177 °C (lit.\textsuperscript{20} 175 °C); $\lambda_{\text{max}}$ (EtOH/nm) 241; IR (cm$^{-1}$) 3352, 3285, 3093, 1637, 1619, 1559, 1524; $^1$H NMR (500 MHz, DMSO-d$_6$) 2.83 (3H, d, $J$ = 4.6 Hz, NCH$_3$), 7.79 (1H, dd, $J$ = 7.9 and 8.0 Hz, H-5), 8.28 (1H, ddd, $J$ = 1.0, 1.6 and 7.9 Hz, H-6), 8.38 (1H, ddd, $J$ = 1.0, 2.4 and 8.0 Hz, H-4), 8.65-8.68 (1H, m, H-2), 8.84 (1H, br, NCH$_3$); LRMS (ES+) m/z 181.1 [M+H]$^+$.  

*N,N-Dimethyl-3-nitrobenzamide (129)\textsuperscript{21}*

![N,N-Dimethyl-3-nitrobenzamide](image)

Phosphorus trichloride (288 µL, 3.29 mmol) was added to a solution of 3-nitrobenzoic acid (0.499 g, 2.99 mmol) and dimethylamine (2M in THF, 3.74 mL, 7.48 mmol) in dry MeCN (15 mL) in a sealed vial. The reaction mixture was heated under microwave irradiation at 150 °C for 5 min, before being concentrated to dryness. The resulting residue was dissolved in DCM (30 mL) and washed with a sat. aqueous solution of NaHCO$_3$ (30 mL). The biphasic mixture was passed through an Isolute® phase separator and the organic phase was concentrated *in vacuo*. Chromatography on silica (7:3 Petrol/EtOAc) gave the target compound as a beige solid (0.560 g, 2.88 mmol, 96%); R$_f$ 0.36 (7:3 Petrol/EtOAc); m.p. 81-84 °C (lit.\textsuperscript{21} 83-84 °C); $\lambda_{\text{max}}$ (EtOH/nm) 243; IR (cm$^{-1}$) 3083, 2941, 1626, 1527; $^1$H NMR (500 MHz, CDCl$_3$) 3.03 (3H, s, NCH$_3$), 3.18 (3H, s, NCH$_3$), 7.64 (1H, dd, $J$ = 7.7 and 8.0 Hz, H-5), 7.80 (1H, ddd, $J$ = 1.2, 1.4 and 7.7 Hz, H-6), 8.29-8.33 (2H, m, H-2/H-4); LRMS (ES-) m/z 195.1 [M+H]$^+$.  

S61
Iron powder (1.30 g, 23.3 mmol) was added to a solution of nitro compound 128 (0.420 g, 2.33 mmol) in acetic acid (20 mL) and was heated at 50 °C for 15 min, before being filtered through Celite® and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO₃ (2 × 200 mL) and brine (200 mL). The combined aqueous layers were extracted with EtOAc (200 mL) and the combined organic extracts were dried (MgSO₄) and evaporated to dryness. Purification by chromatography on silica (19:1 DCM/MeOH) gave the desired compound as a beige solid (0.276 g, 1.84 mmol, 79%); Rf 0.38 (19:1 DCM/MeOH); m.p. 98-100 °C (lit. m.p. not available); λmax (EtOH/nm) 217, 313; IR (cm⁻¹) 3465, 3412, 3294, 3221, 1634, 1580, 1547; ¹H NMR (500 MHz, DMSO-d₆) 2.74 (3H, d, J = 4.6 Hz, NHC₃H₃), 5.19 (2H, s, Ar-NH₂), 6.67 (1H, ddd, J = 1.0, 2.4 and 7.9 Hz, H-4), 6.92 (1H, ddd, J = 1.0, 1.6 and 7.7 Hz, H-5), 7.02 (1H, dd, J = 1.6 and 2.4 Hz, H-2), 7.06 (1H, dd, J = 7.7 and 7.9 Hz, H-5), 8.15 (1H, q, J = 4.6 Hz, NHCH₃); LRMS (ES+) m/z 151.1 [M+H]+.

3-Amino-N,N-dimethylbenzamide (131)²¹

Iron powder (0.57 g, 10.3 mmol) was added to a solution of nitro compound 129 (0.200 g, 1.03 mmol) in acetic acid (10 mL). The reaction mixture was heated at 50 °C for 15 min, before being filtered through Celite® and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO₃ (2 × 200 mL) and brine (200 mL). The combined aqueous layers were extracted with EtOAc (200 mL) and the combined organic extracts were dried (MgSO₄) and evaporated to dryness. Purification by chromatography on silica (19:1 DCM/MeOH) gave the target compound as a golden oil (0.142 g, 0.86 mmol, 84%); Rf 0.41 (19:1 DCM/MeOH); λmax (EtOH/nm) 243, 302; IR (cm⁻¹) 3413, 3342, 3232, 2930, 1598, 1579; ¹H NMR (500 MHz, DMSO-d₆) 2.90 (3H, s, NCH₃), 2.94 (3H, s, NCH₃), 5.21 (2H, s, Ar-NH₂), 6.46 (1H, ddd, J = 1.1, 1.4 and 7.4 Hz, H-4), 6.54 (1H, dd, J = 1.4 and 2.3 Hz, H-2), 6.59 (1H, ddd, J = 1.1, 2.3 and 8.0 Hz, H-6), 7.05 (1H, dd, J = 7.4 and 8.0 Hz, H-5); LRMS (ES+) m/z 165.1 [M+H]+.
Scheme H. Reagents and conditions: (a) SOCl₂, DMF, reflux, 1 h; (b) Me₂NH, Et₃N, THF, RT, 18 h.

4-Amino-\(N,N\)-dimethylbenzamide (132)²³

4-Aminobenzoic acid (0.500 g, 3.65 mmol) was dissolved in thionyl chloride (5 mL) and 1 drop of DMF was added. The solution was refluxed for 1 h then concentrated to dryness. Remaining thionyl chloride was removed through addition of toluene (5 mL) and removal of the azeotrope in vacuo. The resultant orange residue was suspended in dry THF (4 mL) and triethylamine (530 μL, 3.65 mmol), and dimethylamine (2M in THF, 5.50 mL, 10.9 mmol) was added under nitrogen. The reaction mixture was stirred at RT for 18 h, after which the solvent was removed in vacuo and the resultant residue was purified by chromatography on silica (19:1 DCM/MeOH). The desired compound was obtained as an off-white solid (0.348 g, 2.12 mmol, 58%); \(R_f \) 0.40 (19:1 DCM/MeOH); m.p. 150-153 °C (lit.²³ 153 °C); \(\lambda_{\text{max}} \) (EtOH/nm) 220, 272; IR (cm⁻¹) 3428, 3335, 3236, 2925, 1642; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 2.94 (6H, s, \(N(CH_3)_2\)), 5.47 (2H, s, Ar-NH₂), 6.54 (2H, d, \(J = 8.6\) Hz, H-3 and H-5), 7.14 (2H, d, \(J = 8.6\) Hz, H-2 and H-6); LRMS (ES+) \(m/z\) 165.2 [M+H]⁺.

Scheme I: Reagents and conditions: (a) Ac₂O, RT, 30 min; (b) Fe, AcOH, 50 °C, 15 min.

\(N\)-(3-Nitrophenyl)acetamide (134)²⁴
3-Nitroaniline (0.503 g, 3.62 mmol) was added to acetic anhydride at RT. The yellow solution was stirred at RT for 30 mins, over which time a white precipitate formed. Water (20 mL) was added, and the mixture was extracted with DCM (2 × 40 mL). The combined organic extracts were washed with 1M HCl (20 mL), sat. NaHCO₃ solution (20 mL) and brine (20 mL). The organic phase was dried (MgSO₄) and concentrated in vacuo, and the resultant crude was triturated with Et₂O (20 mL). The desired product was obtained as a white solid, collected by filtration (0.334 g, 1.85 mmol, 51%); Rf 0.27 (7:3 Petrol/EtOAc); m.p. 153-156 °C (lit.24 152-153 °C); λmax (EtOH/nm) 241, 329; IR (cm⁻¹) 3261, 3193, 3129, 3097, 1672, 1599, 1547, 1526; ¹H NMR (500 MHz, DMSO-d₆) 2.10 (3H, s, CH₃), 7.60 (1H, dd, J = 8.2 and 8.3 Hz, H-5), 7.88-7.91 (2H, m, H-4 and H-6), 8.62 (1H, dd, J = 2.1 and 2.2 Hz, H-2); LRMS (ES+) m/z 181.1 [M+H]+.

N-(3-Aminophenyl)acetamide (135)²⁵

Iron powder (0.845 g, 15.1 mmol) was added to a solution of nitro compound 134 (0.272 g, 1.51 mmol) in acetic acid (15 mL). The reaction mixture was heated at 50 °C for 15 min, before being filtered through Celite® and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO₃ (2 × 200 mL) and brine (200 mL). The combined aqueous layers were extracted with EtOAc (200 mL/mmol) and the combined organic extracts were dried (MgSO₄) and evaporated to dryness. Purification by chromatography on silica (9:1 DCM/MeOH) afforded the desired compound as a pale red oil (0.206 g, 1.37 mmol, 91%); Rf 0.46 (9:1 DCM/MeOH); λmax (EtOH/nm) 223, 297; IR (cm⁻¹) 3245, 3074, 2621, 1658, 1608, 1545; ¹H NMR (500 MHz, DMSO-d₆) 1.99 (3H, s, CH₃), 5.01 (2H, s, Ar-NH₂), 6.21-6.25 (1H, m, H-4), 6.63-6.67 (1H, m, H-6), 6.89 (1H, dd, J = 7.9 and 8.0 Hz, H-5), 6.92 (1H, dd, J = 1.8 and 1.9 Hz, H-2), 9.58 (1H, s, NH); LRMS (ES+) m/z 151.1 [M+H]+.
Scheme J: Reagents and conditions: (a) SOCl₂, MeOH, reflux, 1 h; (b) NaH, Mel, THF, 0 °C to RT, 18 h; (c) Conc. NH₄OH, 60 °C, 18 h; (d) i) SOCl₂, DMF, DCM, RT, 15 min, ii) Conc. NH₄OH, THF, RT, 18 h; (e) Pd/C, NH₄HCOO, MeOH, RT, 18 h.

Methyl 2-(3-nitrophenyl)acetate (137)

Thionyl chloride (400 μL, 5.52 mmol) was added to a solution of 3-nitrophenylacetic acid (0.500 g, 2.76 mmol) in methanol (25 mL). The resulting solution was heated at reflux for 1 h, after which the solvent was removed in vacuo. The crude product was purified by chromatography on silica (17:3 Petrol/EtOAc) to give the desired compound as a colourless oil (0.501 g, 2.56 mmol, 93%); R\textsubscript{f} 0.36 (17:3 Petrol/EtOAc); \(\lambda_{\text{max}}\) (EtOH/nm) 272, 320; IR (cm\(^{-1}\)) 2985, 2359, 1728, 1530; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 3.65 (3H, s, OCH\(_3\)), 3.93 (2H, s, CH\(_2\)), 7.64 (1H, dd, \(J = 7.9\) and 8.1 Hz, H-5), 7.75-7.78 (1H, m, H-6), 8.15 (1H, ddd, \(J = 0.9, 2.3\) and 7.9 Hz, H-4), 8.18-8.21 (1H, m, H-2); LRMS (ES\(^+\)) \textit{m/z} 196.3 [M+H]\(^+\).

Methyl 2-methyl-2-(3-nitrophenyl)propanoate (138)

Sodium hydride (60% dispersion in mineral oil, 90 mg, 2.25 mmol) was added portion-wise to a solution of 137 (0.200 g, 1.02 mmol) and methyl iodide (160 μL, 2.56 mmol) in dry THF (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, before being warmed to RT and stirred for a further 18 h. The reaction was quenched with acetic acid (1 mL) and the solvent was removed in vacuo. The crude residue was purified by chromatography on silica (17:3 Petrol/EtOAc) to give the desired compound as a pale orange oil (0.124 g, 0.56 mmol, 55%); R\textsubscript{f} 0.45 (17:3 Petrol/EtOAc); \(\lambda_{\text{max}}\) (EtOH/nm) 270, 308; IR (cm\(^{-1}\)) 2983, 2954, 2361,
2341, 1730, 1527; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.59 (6H, s, C(CH\(_3\)_2)), 3.63 (3H, s, OCH\(_3\)), 7.67 (1H, dd, \(J = 7.9\) and 8.1 Hz, H-5), 7.82 (1H, ddd, \(J = 1.0, 1.9\) and 7.9 Hz, H-6), 8.12 (1H, dd, \(J = 1.9\) and 2.3 Hz, H-2), 8.15 (1H, ddd, \(J = 1.0, 2.3\) and 8.1 Hz, H-4); LRMS (ES+) \(m/z\) 224.2 [M+H]\(^+\).

2-Methyl-2-(3-nitrophenyl)propanamide (139)

\[
\text{H}_2\text{N} \quad \begin{array}{c}
\text{O} \\
\text{NO}_2
\end{array} \\
\text{CH}_3
\]

Methyl ester 138 (0.110 g, 0.49 mmol) was dissolved in concentrated ammonium hydroxide (5 mL) and the resultant solution was heated in a sealed vessel at 60 °C for 18 h. The solvent was removed \textit{in vacuo} and the residue was dissolved in dry DCM (5 mL) and treated with thionyl chloride (72 μL, 0.98 mmol) and 1 drop of DMF. The solution was stirred at RT under nitrogen for 15 min, after which point the solvent was removed \textit{in vacuo}. The residue was dissolved in dry THF (5 mL) and the solution was added drop-wise to concentrated ammonium hydroxide (3 mL). The mixture was stirred at RT for 18 h, before the solvent was removed \textit{in vacuo}. The crude residue was purified via chromatography on KP-NH silica (7:3 Petrol/EtOAc) to give the desired compound as a white solid (72 mg, 0.35 mmol, 71%); \(R_f\) 0.29 (7:3 Petrol/EtOAc, KP-NH); m.p. 116-118 °C; \(\lambda_{\text{max}}\) (EtOH/nm) 220, 270; IR (cm\(^{-1}\)) 3391, 3208, 2965, 1650, 1535; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.51 (6H, s, C(CH\(_3\)_2)), 7.06 (1H, s, CONH\(_H\)'), 7.11 (1H, s, CONHH'), 7.65 (1H, dd, \(J = 8.0\) and 8.0 Hz, H-5), 7.81 (1H, ddd, \(J = 1.0, 1.8\) and 8.0 Hz, H-6), 8.12 (1H, ddd, \(J = 1.0, 2.3\) and 8.0 Hz, H-4), 8.15 (1H, dd, \(J = 1.8\) and 2.3 Hz, H-2); HRMS calcd. for C\(_{10}\)H\(_{13}\)N\(_2\)O\(_3\) (ES+) \(m/z\) 209.0921 [M+H]\(^+\), found 209.0924.

2-(3-Aminophenyl)-2-methylpropanamide (140)

\[
\text{H}_2\text{N} \quad \begin{array}{c}
\text{O} \\
\text{NH}_2
\end{array} \\
\text{CH}_3
\]

Palladium on carbon (40 mg, 10% w/w) and ammonium formate (1.19 g, 18.8 mmol) were added to a solution of 139 (0.392 g, 1.88 mmol) in methanol (20 mL). The reaction mixture was stirred at RT for 18 h, before being filtered through Celite®. The solvent was removed \textit{in vacuo} and chromatography on silica (19:1 DCM/MeOH) gave the desired compound as an off-white solid (0.311 g, 1.75 mmol, 93%); \(R_f\) 0.21 (19:1 DCM/MeOH); m.p. 117-120 °C; \(\lambda_{\text{max}}\) (EtOH/nm) 243; IR (cm\(^{-1}\)) 3410, 3337, 3172, 2982, 1665; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 1.36 (6H, s, C(CH\(_3\)_2)), 5.10 (2H, br, Ar-NH\(_2\)), 6.40 (1H, ddd, \(J = 0.8, 2.1\) and 7.9 Hz, H-6), 6.47-
6.50 (1H, m, H-4), 6.54-6.56 (1H, m, H-2), 6.72 (1H, s, CONHH'), 6.78 (1H, s, CONHH'), 6.94 (1H, dd, J = 7.9 and 8.0 Hz, H-5); HRMS calcd. for C_{18}H_{15}N_{2}O (ES+) m/z 179.1179 [M+H]^+, found 179.1180.

Scheme K: Reagents and conditions: (a) Di-tert-butylmalonate, K_{2}CO_{3}, DMF, 60 °C, 18 h; (b) PMB-NH_{2}, THF, 80 °C, 18 h; (c) i) Pd/C, NH_{4}HCOO, AcOH, MW 100 °C, 80 min, ii) AcCl, MeOH, RT, 1 h.

Di-tert-butyl 2-(3-fluoro-2-nitrophenyl)malonate (142)

2,6-Difluoronitrobenzene (0.501 g, 3.14 mmol), di-tert-butyl malonate (775 μL, 3.46 mmol) and potassium carbonate (0.780 g, 5.65 mmol) were combined in dry DMF (9 mL) and heated at 60 °C for 18 h, after which the reaction mixture was neutralised with 1M HCl, and extracted with Et_{2}O (3 x 20 mL). The combined organic extracts were dried (Na_{2}SO_{4}), concentrated in vacuo, and purified via chromatography on silica (9:1 Petrol/EtOAc) to give the desired compound as a yellow oil (0.681 g, 1.92 mmol, 61%); R_{f} 0.56 (9:1 Petrol/EtOAc); \lambda_{max} (EtOH/nm) 230; IR (cm^{-1}) 2981, 2937, 1727; \textsuperscript{1}H NMR (500 MHz, CDCl_{3}) 1.50 (18H, s, (OC(CH_{3})_{3})_{2}), 4.66 (1H, s, CH), 7.22-7.26 (1H, m, H-4), 7.42-7.45 (1H, m, H-6), 7.50-7.55 (1H, m, H-5); LRMS (ES-) m/z 354.2 [M-H]^-.

Di-tert-butyl 2-(3-(4-methoxybenzylamino)-2-nitrophenyl)malonate (143)
Malonate 142 (0.527 g, 1.48 mmol) was combined with 4-methoxybenzylamine (575 μl, 4.44 mmol) in THF (15 mL). The reaction mixture was heated at 80 °C for 18 h, after which the solvent was removed in vacuo. The crude residue was purified via chromatography on silica (9:1 Petrol/EtOAc) to give the desired product as a dark orange oil (0.460 g, 1.04 mmol, 70%); Rf 0.25 (9:1 Petrol/EtOAc); λmax (EtOH/nm) 241; IR (cm⁻¹) 3409, 2980, 2360, 1727; ¹H NMR (500 MHz, DMSO-d₆) 1.49 (18H, s, (OC(CH₃))₂), 3.81 (3H, s, OCH₃), 4.38 (2H, d, J = 4.7 Hz, CΗ₂NH), 4.83 (1H, s, CH), 6.73 (1H, dd, J = 1.0 and 7.5 Hz, H-4), 6.80 (1H, dd, J = 1.0 and 8.7 Hz, H-6), 6.89 (2H, d, J = 8.8 Hz, H-3’ and H-5’), 7.25 (2H, d, J = 8.8 Hz, H-2’ and H-6’), 7.31 (1H, dd, J = 7.5 and 8.7 Hz, H-5); HRMS calcd. for C₂₅H₃₃N₂O₇ (ES+) m/z 473.2282 [M+H]⁺, found 473.2281.

7-Aminoindolin-2-one (144)²⁸

Ammonium formate (1.07 g, 17.0 mmol) and palladium on carbon (0.080 g, 10% w/w) were added to a solution of 143 (0.802 g, 1.70 mmol) in acetic acid (17 mL). The mixture was heated under microwave irradiation at 100 °C for 80 min, then filtered through Celite® and the solvent removed in vacuo. The resulting residue was dissolved in EtOAc (30 mL) and washed with sat. NaHCO₃ solution (3 × 30 mL). The organic extract was dried (Na₂SO₄) and concentrated, and the crude residue was purified via chromatography on silica (9:1 DCM/MeOH) to give the N-acetylated product as an off-white solid (0.146 g, 0.77 mmol, crude yield 45%). The solid was suspended in methanol (8 mL) and treated with acetyl chloride (330 μL, 4.62 mmol) at RT for 1 h. The solvent was removed in vacuo, the residue partitioned between EtOAc (10 mL) and sat. NaHCO₃ solution (10 mL) and the organic extract dried (Na₂SO₄) and concentrated to dryness. Purification by chromatography on silica (1:4 Petrol/EtOAc) gave the desired compound as a beige solid (91 mg, 0.61 mmol, 36%); Rf 0.44 (1:4 Petrol/EtOAc); m.p. 249-251 °C (lit.¹⁸ 247-249 °C); λmax (EtOH/nm) 255; IR (cm⁻¹) 3425, 3363, 3247, 1694; ¹H NMR (500 MHz, DMSO-d₆) 3.41 (2H, s, CH₂), 4.81 (2H, s, Ar-
NH₂), 6.47-6.49 (1H, m, H-4), 6.50 (1H, dd, J = 1.0 and 8.0 Hz, H-6), 6.69 (1H, ddd, J = 8.0 and 8.1 Hz, H-5); LRMS (ES+) \textit{m/z} 149.1 [M+H]+.

Scheme L: Reagents and conditions: (a) MeCO₂Cl, K₂CO₃, Et₂O, H₂O, RT, 1 h; (b) Eaton’s reagent, MW 120 °C, 15 min; (c) HNO₃, H₂SO₄, 0 °C, 30 min; (d) Fe, AcOH, 50 °C, 15 min.

Methyl methyl(phenethyl) carbamate (146)

A solution of methyl chloroformate (1.59 mL, 20.6 mmol) in Et₂O (5 mL) was added dropwise over 30 min to the biphasic mixture of \textit{N}-methyl-phenethylamine (2.00 mL, 13.8 mmol) and K₂CO₃ (5.71 g, 41.3 mmol) in Et₂O (20 mL) and water (20 mL). The mixture was stirred at RT for 1 h, before the organic phase was separated and washed with 1M HCl (20 mL), dried (MgSO₄) and evaporated to dryness. The crude residue was purified by chromatography on silica (4:1 Petrol/EtOAc) to give the target compound as a colourless liquid (2.44 g, 11.9 mmol, 86%); Rf 0.44 (4:1 Petrol/EtOAc); λ_max (EtOH/nm) 259; IR (cm⁻¹) 3027, 2950, 1697; \textit{¹}H NMR (500 MHz, DMSO-d₆, 348 K) 2.79 (2H, t, \textit{J} = 7.6 Hz, NCH₂C₃H₂), 2.80 (3H, s, N-CH₃), 3.45 (2H, t, \textit{J} = 7.6 Hz, NCH₂CH₂), 3.56 (3H, s, OCH₃), 7.19-7.23 (3H, s, NCH₂CH₂), 7.28-7.32 (2H, m, H-3/H-5); LRMS (ES+) \textit{m/z} 194.2 [M+H]+.

2-Methyl-3,4-dihydroisoquinolin-1(2\textit{H})-one (147)
Methyl carbamate 146 (1.77 g, 9.16 mmol) was heated in Eaton’s reagent (10 mL) under microwave irradiation conditions at 120 °C for 15 min. The resultant brown oil was dissolved in EtOAc (100 mL) and slowly added to a stirred sat. NaHCO₃ solution (100 mL). The organic phase was separated, washed with brine (50 mL), dried (MgSO₄) and concentrated in vacuo. Chromatography on silica (4:1 Petrol/EtOAc) gave the desired compound as a colourless oil (1.30 g, 8.09 mmol, 88%); Rf 0.26 (4:1 Petrol/EtOAc); λmax (EtOH/nm) 229; IR (cm⁻¹) 2941, 2871, 1641, 1604, 1578; ¹H NMR (500 MHz, CDCl₃) 3.02 (2H, t, J = 6.7 Hz, NCH₂C₂H₂), 3.17 (3H, s, N-CH₃), 3.58 (2H, t, J = 6.7 Hz, NC₂H₂CH₂), 7.17-7.20 (1H, m, H-5), 7.34 (1H, ddd, J = 1.1, 7.5 and 7.6 Hz, H-7), 7.42 (1H, ddd, J = 1.4, 7.4 and 7.5 Hz, H-6), 8.10 (1H, dd, J = 1.4 and 7.6 Hz, H-8); LRMS (ES+) m/z 162.1 [M+H]+.

2-Methyl-7-nitro-3,4-dihydroisoquinolin-1(2H)-one (148)

Fuming HNO₃ (42 µL, 1.01 mmol) was added to conc. H₂SO₄ (2 mL) at 0 °C. A solution of isoquinolinone 147 (0.135 g, 0.84 mmol) in conc. H₂SO₄ (0.5 mL) was added dropwise, and the solution was stirred at 0 °C for 30 min. The mixture was poured onto ice water (15 mL) and the resulting precipitate collected by filtration and washed with cold water (5 mL) before being dried in a vacuum oven to give the target compound as a white solid (100 mg, 0.48 mmol, 57%); Rf 0.29 (19:1 DCM/MeOH); m.p. 137-140 °C; λmax (EtOH/nm) 220, 255; IR (cm⁻¹) 2925, 2868, 1647, 1610, 1518; ¹H NMR (500 MHz, DMSO-d₆) 3.07 (3H, s, N-CH₃), 3.14 (2H, t, J = 6.7 Hz, NCH₂CH₂), 3.62 (2H, t, J = 6.7 Hz, NCH₂CH₂), 7.61 (1H, d, J = 8.3 Hz, H-5), 8.31 (1H, dd, J = 2.5 and 8.3 Hz, H-6), 8.56 (1H, d, J = 2.5 Hz, H-8); HRMS calcd. for C₁₀H₁₁N₂O₃ (ES+) m/z 207.0764 [M+H]+, found 207.0765.

7-Amino-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (149)

Iron powder (2.56 g, 45.9 mmol) was added to a solution of the nitro compound 148 (0.947 g, 4.59 mmol) in acetic acid (45 mL). The reaction mixture was heated at 50 °C for 15 min, before being filtered through Celite® and the filtrate concentrated in vacuo. The residue was
dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO$_3$ (2 × 200 mL) and brine (200 mL). The combined aqueous layers were extracted with EtOAc (200 mL) and the combined organic extracts were dried (MgSO$_4$) and evaporated to dryness. Purification by chromatography on silica (19:1 DCM/MeOH) afforded the desired compound as a pale yellow solid (0.678 g, 3.85 mmol, 84%); R$_f$ 0.24 (19:1 DCM/MeOH); m.p. 131-133 °C; $\lambda_{\text{max}}$ (EtOH/nm) 224, 330; IR (cm$^{-1}$) 3439, 3359, 3330, 3234, 2931, 1635, 1599, 1576, 1498; $^1$H NMR (500 MHz, DMSO-$d_6$) 2.77 (2H, t, $J = 6.7$ Hz, NCH$_2$C$\equiv$H), 2.99 (N-CH$_3$), 3.45 (2H, t, $J = 6.7$ Hz, NCH$_2$CH$_2$), 5.12 (2H, s, Ar-NH$_2$), 6.66 (1H, dd, $J = 2.5$ and 8.0 Hz, H-6), 6.91 (1H, d, $J = 8.0$ Hz, H-5), 7.15 (1H, d, $J = 2.5$ Hz, H-8); HRMS calcd. for C$_{10}$H$_{13}$N$_2$O (ES+) m/z 177.1022 [M+H$^+$], found 177.1021.

Scheme M: Reagents and conditions: (a) i) SOCl$_2$, MeOH, reflux, 1 h, ii) Aq. MeNH$_2$, RT, 72 h; (b) (CH$_2$O)$_n$, Eaton’s reagent, 80 °C, 6 h; (c) Fe, AcOH, 50 °C, 15 min.

$N$-Methyl-2-(4-nitrophenyl)acetamide (150)$^{31}$

Thionyl chloride (0.81 mL, 11.0 mmol) was added to a solution of 4-nitrophenylacetic acid (1.00 g, 5.52 mmol) in methanol (50 mL). The resulting solution was heated at reflux for 1 h, after which the solvent was removed in vacuo. A concentrated aqueous solution of methylamine (40% aqueous solution, 50 mL) was added to the residue and the reaction mixture was stirred at RT for 72 h. The solvent was removed in vacuo and purification by chromatography on silica (19:1 DCM/MeOH) gave the target compound as an off-white solid (0.916 g, 5.08 mmol, 92%); R$_f$ 0.31 (19:1 DCM/MeOH); m.p. 157-160 °C (lit.$^{31}$ 159 °C); $\lambda_{\text{max}}$ (EtOH/nm) 271; IR (cm$^{-1}$) 3260, 3084, 2943, 2844, 1638, 1565, 1505; $^1$H NMR (500 MHz, DMSO-$d_6$) 2.60 (3H, d, $J = 4.7$ Hz, NHC$_2$H$_5$), 3.58 (2H, s, CH$_2$), 7.53 (2H, d, $J = 9.0$ Hz, H-2
and H-6), 8.09 (1H, br, NHCH₃), 8.18 (2H, d, J = 9.0 Hz, H-3 and H-5); LRMS (ES+) m/z 195.2 [M+H]+.

2-Methyl-7-nitro-1,2-dihydroisoquinolin-3(4H)-one (151)

Paraformaldehyde (19 mg, 0.62 mmol) was added to a suspension of 150 (102 mg, 0.51 mmol) in Eaton’s reagent (1 mL). The mixture was heated in a sealed vial at 80 °C for 6 h, after which point the brown solution was cooled to RT, diluted with ice water (5 mL) and neutralised with 50% NaOH solution. The resultant suspension was extracted with EtOAc (3 × 10 mL) and the combined organic extracts were dried (MgSO₄) and concentrated in vacuo. The crude residue was purified by chromatography on silica gel (19:1 DCM/MeOH) to give the target compound as a pale orange solid (78 mg, 0.38 mmol, 75%); Rf 0.30 (19:1 DCM/MeOH); m.p. 151-154 °C; λ_max (EtOH/nm) 273; IR (cm⁻¹) 3041, 2875, 1637, 1597, 1500; ¹H NMR (500 MHz, DMSO-d₆) 2.98 (3H, s, N-CH₃), 3.70 (2H, s, N-CH₂), 4.64 (2H, s, CH₂), 7.50 (1H, d, J = 8.4 Hz, H-5), 8.13 (1H, dd, J = 2.3 and 8.4 Hz, H-6), 8.20 (1H, d, J = 2.3 Hz, H-8); LRMS (ES+) m/z 207.1 [M+H]+.

7-Amino-2-methyl-1,2-dihydroisoquinolin-3(4H)-one (152)

Iron powder (0.943 g, 16.9 mmol) was added to a solution of 151 (0.348 g, 1.69 mmol) in acetic acid (17 mL). The reaction mixture was heated at 50 °C for 15 min, before being filtered through Celite® and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO₃ (2 × 200 mL) and brine (20 mL). The combined aqueous layers were extracted with EtOAc (200 mL) and the combined organic extracts were dried (MgSO₄) and evaporated to dryness. Purification via chromatography on silica gel (19:1 DCM/MeOH) gave the desired compound as a pale orange solid (0.196 g, 1.11 mmol, 66%); Rf 0.25 (19:1 DCM/MeOH); m.p. 167-170 °C; λ_max (EtOH/nm) 241, 296; IR (cm⁻¹) 3426, 3343, 3241, 3023, 2871, 1612, 1502; ¹H NMR (500 MHz, DMSO-d₆) 2.94 (3H, s, N-CH₃), 5.03 (2H, s, CH₂), 6.42 (1H, d, J = 2.2 Hz, H-8), 6.46 (1H, dd, J = 2.2 and 8.0 Hz, H-6), 6.82 (1H, d, J = 8.0 Hz, H-5); HRMS calcd. for C₁₀H₁₄N₂O (ES+) m/z 177.1022 [M+H]+, found 177.1019.
Scheme N: Reagents and conditions: (a) Ac₂O, pyridine, reflux, 4 h; (b) 1,3-propanedithiol, BF₃·Et₂O, DCM, RT, 18 h; (c) SnCl₂·H₂O, EtOH, reflux, 1.5 h.

1-(3-Nitrophenyl)propan-2-one (153)

Acetic anhydride (10.4 mL, 110 mmol) was added to a solution of 3-nitrophenylacetic acid (2.00 g, 11.0 mmol) in dry pyridine (4.50 mL, 55.2 mmol). The reaction mixture was heated at reflux under N₂ for 4 h, before being concentrated in vacuo. The brown residue was suspended in a mixture of conc. HCl (1 mL) and EtOH (8 mL), and the suspension was heated at reflux for 1 h. The resultant solution was poured onto ice water (50 mL) and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried (MgSO₄), concentrated in vacuo, and the crude product purified by chromatography on silica (4:1 Petrol/EtOAc). The target compound was obtained as a pale yellow oil which crystallised on standing (1.33 g, 7.43 mmol, 68%); Rf 0.26 (4:1 Petrol/EtOAc); m.p. 62-65 °C (lit. 33 62 °C); λ_max (EtOH/nm) 263; IR (cm⁻¹) 3076, 1719, 1518; ¹H NMR (500 MHz, CDCl₃) 2.28 (3H, s, CH₃), 3.88 (2H, s, CH₂), 7.53-7.56 (2H, m, H-4 and H-5), 8.07-8.10 (1H, m, H-2), 8.14-8.18 (1H, m, H-6); LRMS (ES-) m/z 178.1 [M-H⁻].

2-Methyl-2-(3-nitrobenzyl)-1,3-dithiane (154)

1,3-Propanedithiol (0.77 mL, 7.70 mmol) and boron trifluoride diethyl etherate (1.58 mL, 12.8 mmol) were added to a solution of 153 (1.15 g, 6.42 mmol) in dry DCM (30 mL) at 0 °C. The solution was stirred under N₂ at RT for 18 h, before being washed with sat. NaHCO₃ solution (20 mL). The organic phase was dried through a phase separator, concentrated in vacuo, and the crude residue purified via chromatography on silica (4:1 Petrol/EtOAc). The desired compound was obtained as an off-white crystalline solid on cooling (1.50 g, 5.56 mmol, 87%); Rf 0.44 (4:1 Petrol/EtOAc); m.p. 75-78 °C; λ_max (EtOH/nm) 261; IR (cm⁻¹) 2933, 2907, 1525; ¹H NMR (500 MHz, CDCl₃) 1.56 (3H, s, CH₃), 1.97-2.11 (2H, m, C(SCH₂)₂CH₂), 2.88-3.05
(4H, m, C(SCH₂)₂CH₂), 3.37 (2H, s, Ar-CH₂), 7.50 (1H, dd, J = 7.6 and 7.7 Hz, H-5), 7.61-7.65 (1H, m, H-6), 8.15-8.18 (2H, m, H-2 and H-4); LRMS (ES+) m/z 270.2 [M+H]^+.

Scheme O: Reagents and conditions: (a) NaSO₂CH₃, EtOH, reflux, 2 h; (b) Fe, AcOH, 50 °C, 15 min.

1-((Methylsulfonyl)methyl)-3-nitrobenzene (156)³⁴

Sodium methanesulfinate (0.306 g, 3.01 mmol) in ethanol (2 mL) was added to a solution of 3-nitrobenzyl bromide (0.502 g, 2.31 mmol) in ethanol (6 mL). The mixture was heated at 80 °C for 2 h, before being cooled and concentrated in vacuo. The resultant residue was partitioned between DCM (15 mL) and water (10 mL), and the organic phase was dried through a phase separator. The solvent was removed and the crude residue purified by chromatography on silica (1:1 Petrol/EtOAc) to give the target compound as a white solid (0.467 g, 2.17 mmol, 94%); Rf 0.39 (1:1 Petrol/EtOAc); m.p. 119-121 °C (lit.³⁴ 105-106 °C); λₘₐₓ (EtOH/nm) 260; IR (cm⁻¹) 3018, 2989, 2935, 1521; ¹H NMR (500 MHz, CDCl₃) 2.91 (3H, s, CH₃), 4.40 (2H, s, CH₂), 7.66 (1H, dd, J = 7.6 and 7.7 Hz, H-5), 7.83 (1H, dd, J = 1.3, 1.5 and 7.7 Hz, H-6), 8.29-8.33 (2H, m, H-2 and H-4); LRMS (ES-) m/z 214.1 [M-H]⁻.

3-((Methylsulfonyl)methyl)aniline (157)³⁵

Iron powder (1.80 g, 32.3 mmol) was added to a solution of 156 (0.695 g, 3.23 mmol) in acetic acid (30 mL). The reaction mixture was heated at 50 °C for 15 min, before being filtered through Celite® and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with a sat. aqueous solution of NaHCO₃ (2 x 200 mL) and brine (200 mL). The combined aqueous layers were extracted with EtOAc (200 mL) and the combined organic extracts were dried (MgSO₄) and evaporated to dryness. Purification by
chromatography on silica (9:1 DCM/MeOH) afforded the target compound as an off-white solid (0.532 g, 2.87 mmol, 89%); Rf 0.42 (9:1 DCM/MeOH); m.p. 123-126 °C (lit.35 126 °C); λ_max (EtOH/nm) 243, 298; IR (cm⁻¹) 3463, 3373, 3221, 3011, 2970, 2930, 1625, 1603; ¹H NMR (500 MHz, DMSO-d₆) 2.87 (3H, s, CH₃), 4.27 (2H, s, CH₂), 5.16 (2H, s, Ar-NH₂), 6.52-6.55 (1H, m, H-6), 6.56 (1H, ddd, J = 1.0, 2.2 and 8.0 Hz, H-4), 6.58-6.60 (1H, m, H-2), 7.02 (1H, dd, J = 7.8 and 8.0 Hz, H-5); LRMS (ES+) m/z 186.1 [M+H]+.

Scheme P: Reagents and conditions: (a) TFE, DMAP, Et₃N, RT, 3 h; (b) H₂, Pd/C, TFE, EtOAc, RT, 18 h.

2,2,2-Trifluoroethyl (3-nitrophenyl)methanesulfonate (159)³⁶

3-Nitro-α-toluenesulfonyl chloride 158 (1.00 g, 4.24 mmol) was added to a solution of DMAP (0.052 g, 0.42 mmol) and triethylamine (1.77 mL, 12.7 mmol) in TFE (10 mL). The mixture was stirred at RT for 3 h, after which the solvent was removed in vacuo. The residue was dissolved in DCM (60 mL) and washed with 0.05 M HCl (60 mL) and water (60 mL), and the organic phase was passed through a phase separator and concentrated. The resulting oil was sonicated with water (5 mL), resulting in the formation of the product as a white precipitate which was collected via filtration (1.25 g, 4.19 mmol, 99%); Rf 0.73 (7:3 Petrol/EtOAc); m.p. 89-91 °C (lit.36 84-85 °C); λ_max (EtOH/nm) 257; IR (cm⁻¹) 2998, 2950, 2160, 1977, 1531; ¹H NMR (500 MHz, DMSO-d₆) 5.00 (2H, q, J = 8.4 Hz, F₃CCCH₂), 5.19 (2H, s, Ar-CH₂), 7.76 (1H, dd, J = 8.0 and 8.2 Hz, H-5), 7.90-7.95 (1H, m, H-6), 8.30 (1H, ddd, J = 2.3, 2.4 and 8.2 Hz, H-4), 8.37-8.39 (1H, m, H-2); LRMS (ES-) m/z 298.1 [M-H]⁻.

2,2,2-Trifluoroethyl (3-aminophenyl)methanesulfonate (160)³⁶

Palladium on carbon (0.30 g, 10% w/w) was added to a solution of 159 (1.00 g, 3.34 mmol) in TFE (10 mL) and EtOAc (3 mL). The mixture was hydrogenated at RT for 18 h before being passed through Celite® and concentrated. The crude oil was purified via chromatography on silica (7:3 Petrol/EtOAc) to give an oil, which was triturated with petrol
(10 mL) to give the desired compound as a white solid (0.789 g, 2.93 mmol, 88%); Rf 0.36 (7:3 Petrol/EtOAc); m.p. 74-76 °C (lit.36 77-78 °C); \( \lambda_{\text{max}} \) (EtOH/nm) 243; IR (cm\(^{-1}\)) 3502, 3397, 2945, 2359, 1619; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) 4.76 (2H, s, Ar-NH\(_2\)), 4.95 (2H, q, \( J = 8.7 \) Hz, F\(_3\)CCH\(_2\)O), 5.28 (2H, s, Ar-CH\(_2\)), 6.59-6.64 (2H, m, H-4/H-6), 6.65-6.68 (1H, m, H-2), 7.09 (1H, dd, \( J = 7.7 \) and 7.9 Hz, H-5); LRMS (ES+) \( m/z \) 270.0 [M+H]^+.

3. \(^1\)H NMR Spectra for Key Compounds

![NMR Spectra](image-url)
4. Biological Evaluation

Synthesised inhibitors were evaluated for Nek2 inhibitory activity and counter-screened by Kathy Boxall, Sam Burns, Yvette Newblatt and Maura Westlake under the supervision of Dr. Wynne Aherne in the Analytical Screening and Technology Laboratory of the CR UK Centre for Cancer Therapeutics, The Institute for Cancer Research, Sutton, Surrey, UK, SM2 5NG. The determined inhibitory concentrations are reported as inhibition coefficients for 50% inhibition (IC_{50}) or percentage inhibition as appropriate.

Evaluation of synthesised inhibitors for CDK2/cyclin A3 inhibitory activity was conducted by Lan-Zhen Wang at The Northern Institute for Cancer Research, Paul O’Gorman Building,
Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH. Details are given below. Enamine stability studies were undertaken by Huw D. Thomas, also of the above address.

All animal experiments performed were conducted in compliance with the relevant laws and institutional guidelines.

**Nek2 Biochemical Assay**

Nek2 inhibitory activities were determined using a Caliper EZ Reader II instrument with either a 4 or 12 sipper microfluidic chip (Caliper Life Sciences Ltd, Runcorn, UK). The assay format employs an electrophoretic method which separates a phosphorylated peptide substrate of Nek2 from the non-phosphorylated substrate after incubation in the assay medium for a defined time in the presence of a chemical inhibitor. The separation is based on charge, with the negatively charged phosphate peptide migrating more rapidly towards a positively charged terminal. The extent of phosphorylation is proportional to the extent of functional Nek2 enzymatic activity in the assay solution and its ability to bind ATP to phosphorylate the peptide substrate. The Caliper instrument readout gives an estimate of the proportion of peptide substrate which is phosphorylated by uninhibited Nek2. Therefore, the greater the affinity of an inhibitor for Nek2, the lower the proportion of phosphate peptide as detected by a fluorescence sensor.

The assay was conducted by 1:4 dilution of a 10 mM stock solution of an inhibitor in DMSO by taking 15 µL of inhibitor solution and adding this to 45 µL of DMSO in the first row of a 384 well polypropylene assay plate (Greiner). Seven further successive 1:3 dilutions were performed by taking 20 µL of the inhibitor solution and adding this to 60 µL of 100% DMSO in the well directly below, to give a 250 µM top concentration in DMSO. The 100% DMSO solutions were each diluted 20-fold by adding 2.5 µL of each well to 47.5 µL of a stock kinase buffer (25 mL, consisting of: stock Cisbio buffer (5 mL), 1,4-dithiothreitol (25 µL, 1 mM), MgCl₂ (125 µL, 5 mM solution in water), Tween₂₀ (25 µL, 0.1%) and HPLC grade water (20 mL)) to give a top concentration of 125 µM in 5% DMSO. To a second 384 well assay plate was added 4 µL of 5% DMSO solution from each well of the first assay plate to give a top concentration of 50 µM in 2% DMSO after a 1:2.5 dilution. To each well was added Nek2 enzyme (2 µL of 0.28 mg/mL aqueous solution, giving a 4 nM final concentration, PV3360 Invitrogen), substrate ‘peptide-11’ (5-FAM-KKLNRTLSVA-COOH, 2 µL of 1.5 mM aqueous solution, giving a 1 µM final concentration, #760355 Caliper Life Sciences) and finally ATP (2 µL aqueous 10 mM solution, giving a 30 µM final concentration) was added to initiate the...
reaction. The plate was immediately sealed and centrifuged for 1 minute to mix all of the reagents before incubation at room temperature for 60 min.²

The reaction was stopped after the required time by addition of 90 µL of separation buffer (#760367 Caliper Life Sciences). The amount of peptide 11 phosphorylation was then determined using the Caliper EZ Reader II instrument (1.5 psi, 1750 ΔV). The percentage conversion of substrate protein was measured and the percentage inhibition was thus calculated relative to blank wells, which contained no enzyme and 2% DMSO, and totals wells which contained all reagents and 2% DMSO replacing the inhibitor.

IC₅₀ values were determined in duplicate over a range of 8 concentrations using GraphPad Prism 5, employing a non-linear regression fit of log[inhibitor] versus response (% inhibition) with a variable slope equation. It is noteworthy that IC₅₀ values presented for the irreversible inhibitor series are the values obtained as above, after 60 minute incubation of the inhibitor with Nek2.

**CellTiter-Blue Assay for Growth Inhibition**³

U2OS human osteosarcoma cells (American Type Culture Collection, Manassas, Virginia, United States) were grown in McCoy’s 5A medium supplemented with 1.5 mM L-glutamine, 25 mM HEPES, 2% penicillin/streptomycin (Invitrogen, Paisley, United Kingdom) and 10% fetal bovine serum (Biosera, Ringmer, East Sussex, United Kingdom). MDA-MB-231 human breast cancer cells (American Type Culture Collection, Manassas, Virginia, United States) were grown in RPMI 1640 medium (Invitrogen) supplemented with 2 mM L-glutamine, 25 mM HEPES, 2% penicillin/streptomycin and 10% fetal bovine serum. HeLa cells were grown in Dulbecco’s Modified Eagle Medium (D-MEM) (Invitrogen) supplemented with 2% penicillin/streptomycin and 10% fetal bovine serum. All three cell lines were maintained in a humidified atmosphere of 5% CO₂ at 37°C. The medium was aspirated and the cells were washed with PBS (Invitrogen), trypsinized (Internal supply, 0.25% versene trypsin with EDTA), neutralized and counted. Cells were seeded into 384-well clear tissue culture treated microtiter plates (Corning B.V. Life Sciences, Amsterdam, The Netherlands) at 200 cells per well in a 45 µL volume of the respective media. Columns 1 and 24 had no cells added and were plated with 45 µL of media alone. Cells were incubated at 37°C / 5% CO₂. At 24 hours after plating, compounds were three-fold serially diluted in large volume V-shape 384-well microplates (Greiner Bio-One, Stonehouse, Gloucestershire, United Kingdom) using an Evolution plate handling system (PerkinElmer Life Sciences, Waltham, Massachusetts, USA). Then 5 µL of diluted test compounds, Etoposide as positive control (Sigma-Aldrich,
Gillingham, Dorset, United Kingdom), or DMSO at 1% v/v final concentration (Fisher Scientific, Loughborough, Leicestershire, United Kingdom) were added to the wells using a MiniTrack V plate handling system (PerkinElmer Life Sciences). There were four replicates of each compound concentration, 32 replicates of DMSO wells, and 32 replicates of wells containing no cells. Test compounds were screened at final concentrations of 100 µM, 33.33 µM, 11.11 µM, 3.70 µM, 0.41 µM, 0.14 µM, and 0.05 µM. Etoposide was screened at final concentrations of 10 µM, 3.33 µM, 1.11 µM, 0.37 µM, 0.12 µM, 0.041 µM, 0.014 µM, and 0.005 µM. After 92 hours, 5 µL of CellTiter-Blue Reagent (Promega, Southampton, United Kingdom) was added to the cells using a Multidrop dispenser (Thermo Electron, Basingstoke, Hants, United Kingdom) and incubated for 4 hours in a humidified atmosphere of 5% CO₂ at 37°C. After the incubation, the plates were placed at room temperature for 40 minutes before fluorescence was recorded (560Ex/590Em) on an EnVision 2103 plate reader (PerkinElmer Life Sciences). Data were plotted as percentage of DMSO control against compound concentration using GraphPad Prism 5 Software. The 50% growth inhibition (GI₅₀) was calculated as the compound concentration required to reduce the cell number by 50% compared with the DMSO control.

**CDK2/cyclin A3 Biochemical Assay**

Inhibition of human CDK2/Cyclin A3 was assayed as previously described using recombinant CDK2/cyclin A3 (10 µL) with 1 mg/mL histone H1 (150 µL, Sigma type III-S), in the presence of [gamma-³²P] ATP (1-5 µL, 3000 Ci/mmol, Cat number NEG002A Perkin Elmer) and cold ATP (13.13 µL, 1 mM) in a final volume of 30 µl. The assay buffer (500 µL total volume) contained Tris-HCl pH 7.5 (50 mM) and MgCl₂ (5 mM). The final DMSO concentration in the assay was 1% (V/V), after inhibitors stocks in 100% DMSO were diluted 1:10 in the appropriate assay buffer (3 µl + 27 µl buffer), followed by addition of 3 µl of 10% inhibitor solution to a total assay volume of 30 µl. Therefore, the final DMSO concentration was 1%, final inhibitor concentration was 1/100 of the original stock solution and the final ATP concentration in the assay was 12.5 µM. After incubation for 10 min at 30 °C, 25 µl aliquots were spotted onto 2.5 cm × 3 cm pieces of Whatman P81 phosphocellulose paper, and after 20 s, the filters were washed five times (> 5 min each time) in 1% phosphoric acid. The dry filters were transferred into 6 ml plastic scintillation vials, 5ml scintillation fluid (Amersham) was added, and the radioactivity was measured using a scintillation counter.

**5. Kinase profiling**

**Table 1:** Kinase inhibition following treatment with 1 µM 66 for ProfilerPro® plates. *IC₅₀ determination for kinases inhibited >50%.
| Enzyme | MAPK APK2 | AurA | PKCz | Rsk1 | PRAK | Erk1 | PKD2 | CK1d | Chk1 |
|--------|------------|------|------|------|------|------|------|------|------|
| % inhib. | 6          | 0.078* | 9    | 8    | -3   | 8    | 3    | 8    | 11   |

| Enzyme | ABL FYN LYN Chk2 MET LCK SRC GSK3β Erk2 |
|--------|-----------------------------------------|
| % inhib. | 39          | 10   | 9    | 13   | 6    | 17   | 22   | 9    | 2    |

| Enzyme | PKA AKT2 INSR P38α AKT1 Msk1 Msk2 P38γ PKD1 |
|--------|------------------------------------------|
| % inhib. | 1            | 3    | -3   | -1   | 13   | 6    | -6   | -1   | 31   |

| Enzyme | MARK 2 BMX CSNK 1A1 PKD3 BRSK1 Nek2 PIM1 SGK2 SGK3 |
|--------|-----------------------------------------------|
| % inhib. | 27           | 0.83* | 5    | 26   | 12   | 0.088* | 18   | 7    | 11   |

| Enzyme | ARG DCAM KL2 Rsk2 Rsk3 BRSK1 PKC-α PKC-β1 PKC-γ PKC-δ |
|--------|------------------------------------------------------|
| % inhib. | 32          | -2   | 12   | 14   | 5    | 15   | 2    | 29   | 29   |

| Enzyme | PKC-ε PKC-η PKC-θ |
|--------|-------------------|
| % inhib. | 8         | 17   | 35   |

**Table 2:** Kinase inhibition data for 23 and 66 and the literature irreversible Nek2 inhibitor 7 from the National Centre for Protein Kinase Profiling, Dundee University.

| Kinase | 23 (%) | 66 (%) | Inhibitor 7 (%) | Inhibitor 7 (%) |
|--------|--------|--------|-----------------|-----------------|
| MKK1   | 95     | 46     | 2               | 16              |
| MKK2   | 103    | 75     | 8               | 15              |
| MKK6   | 105    | 109    | 4               | 54              |
| ERK1   | 102    | 105    | 13              | 79              |
| ERK2   | 91     | 92     | 4               | 70              |
| JNK1   | 82     | 67     | 10              | 85              |
| JNK2   | 96     | 92     | 3               | 101             |
| JNK3   | 89     | 111    | 18              | 91              |
| p38α MAPK | 110  | 103    | 12              | 106             |
| p38β MAPK | 85   | 94     | 2               | 86              |
| p38γ MAPK | 99   | 100    | 18              | 97              |
| p38δ MAPK | 96   | 129    | 14              | 105             |
| ERK8   | 86     | 108    | 2               | 14              |
| RSK1   | 95     | 84     | 5               | 7               |

Percentage activity at 1 μM Inhibitor
| Protein   | Data 1 | Data 2 | Data 3 | Data 4 | Data 5 | Data 6 |
|-----------|--------|--------|--------|--------|--------|--------|
| RSK2      | 72     | 2      | 88     | 19     | 16     | 0      |
| PDK1      | 87     | 12     | 63     | 1      | 35     | 10     |
| PKBa      | 91     | 2      | 116    | 6      | 124    | 18     |
| PKBb      | 75     | 27     | 98     | 4      | 110    | 23     |
| SGK1      | 103    | 14     | 93     | 16     | 64     | 1      |
| S6K1      | 98     | 5      | 102    | 16     | 25     | 2      |
| PKA       | 90     | 10     | 108    | 0      | 100    | 10     |
| ROCK2     | 91     | 6      | 89     | 2      | 23     | 1      |
| PRK2      | 103    | 14     | 99     | 6      | 16     | 3      |
| PKCa      | 95     | 5      | 108    | 3      | 86     | 2      |
| PKCy      | 105    | 10     | 98     | 4      | 114    | 5      |
| PKCz      | 104    | 7      | 104    | 1      | 94     | 6      |
| GCK       | 78     | 3      | 71     | 8      | 14     | 1      |
| MINK1     | 87     | 6      | 92     | 0      | 13     | 0      |
| MLK1      | 54     | 24     | 40     | 11     | 9      | 2      |
| MLK3      | 33     | 5      | 29     | 0      | 17     | 2      |
| TAO1      | 115    | 13     | 101    | 7      | 95     | 5      |
| ASK1      | 96     | 1      | 128    | 10     | 68     | 0      |
| TAK1      | 50     | 0      | 37     | 4      | 31     | 16     |
| IRAK1     | 83     | 9      | 92     | 4      | 33     | 6      |
| IRAK4     | 86     | 6      | 85     | 3      | 24     | 1      |
| RIPK2     | 89     | 3      | 93     | 1      | 44     | 1      |
| OSR1      | 93     | 4      | 103    | 2      | 84     | 6      |
| TTK       | 80     | 5      | 92     | 5      | 35     | 4      |
| MPSK1     | 101    | 7      | 92     | 21     | 100    | 6      |
| Src       | 91     | 7      | 100    | 5      | 56     | 10     |
| Lck       | 110    | 16     | 113    | 1      | 59     | 5      |
| CSK       | 102    | 12     | 79     | 7      | 82     | 2      |
| YES1      | 108    | 7      | 92     | 6      | 29     | 3      |
| ABL       | 87     | 8      | 70     | 16     | 87     | 21     |
| BTK       | 63     | 11     | 95     | 8      | 87     | 10     |
| JAK2      | 75     | 1      | 30     | 0      | 40     | 4      |
| SYK       | 88     | 14     | 117    | 7      | 83     | 2      |
| ZAP70     | 114    | 9      | 119    | 2      | 88     | 4      |
| FGFR1     | 97     | 3      | 74     | 9      | 84     | 17     |
| HER4      | 61     | 14     | 87     | 10     | 126    | 11     |
| IGF-1R    | 97     | 6      | 115    | 5      | 59     | 16     |
| IR        | 85     | 17     | 86     | 1      | 9      | 0      |
| IRR       | 87     | 3      | 92     | 5      | 67     | 1      |
| TrkA      | 109    | 7      | 74     | 7      | 31     | 2      |
| VEGFR     | 97     | 22     | 64     | 5      | 9      | 1      |
| EPH-A2    | 105    | 17     | 122    | 2      | 110    | 7      |
| EPH-A4    | 106    | 15     | 107    | 8      | 105    | 7      |
| EPH-B1    | 93     | 1      | 95     | 1      | 91     | 14     |
| EPH-B2    | 106    | 13     | 104    | 15     | 108    | 9      |
| EPH-B3    | 99     | 2      | 88     | 8      | 122    | 10     |
| Protein     | S6 Phosphorylation | S2 Phosphorylation | S1 Phosphorylation | T8 Phosphorylation | T4 Phosphorylation | Total Phosphorylation |
|-------------|--------------------|--------------------|--------------------|---------------------|--------------------|----------------------|
| EPH-B4      | 104                | 22                 | 108                | 12                  | 118                | 11                   |
| BRSK1       | 93                 | 17                 | 110                | 8                   | 12                 | 3                    |
| BRSK2       | 95                 | 2                  | 103                | 11                  | 12                 | 0                    |
| MELK        | 93                 | 6                  | 115                | 1                   | 10                 | 5                    |
| NUAK1       | 72                 | 26                 | 17                 | 3                   | 10                 | 3                    |
| CK1d        | 82                 | 9                  | 91                 | 5                   | 82                 | 27                   |
| CK2         | 93                 | 5                  | 94                 | 13                  | 28                 | 1                    |
| DFR1A       | 93                 | 2                  | 107                | 11                  | 3                  | 0                    |
| DFR2        | 90                 | 4                  | 112                | 12                  | 6                  | 2                    |
| DFR3        | 89                 | 6                  | 113                | 4                   | 6                  | 1                    |
| NEK2a       | 4                  | 1                  | 11                 | 0                   | 9                  | 2                    |
| NEK6        | 101                | 15                 | 103                | 10                  | 79                 | 3                    |
| IKKb        | 72                 | 13                 | 118                | 10                  | 84                 | 1                    |
| IKKe        | 84                 | 0                  | 60                 | 1                   | 14                 | 2                    |
| TBK1        | 91                 | 23                 | 51                 | 7                   | 10                 | 0                    |
| PIM1        | 101                | 5                  | 108                | 0                   | 38                 | 4                    |
| PIM2        | 103                | 15                 | 106                | 7                   | 95                 | 13                   |
| PIM3        | 92                 | 4                  | 114                | 12                  | 27                 | 3                    |
| SRPK1       | 75                 | 26                 | 94                 | 5                   | 67                 | 4                    |
| EF2K        | 106                | 12                 | 107                | 6                   | 96                 | 4                    |
| EIF2AK3     | 96                 | 6                  | 100                | 7                   | 96                 | 8                    |
| HIPK1       | 105                | 29                 | 57                 | 7                   | 32                 | 5                    |
| HIPK2       | 94                 | 8                  | 115                | 6                   | 22                 | 4                    |
| HIPK3       | 99                 | 15                 | 107                | 6                   | 29                 | 7                    |
| CLK2        | 77                 | 11                 | 63                 | 3                   | 6                  | 2                    |
| PAK2        | 96                 | 9                  | 102                | 6                   | 92                 | 19                   |
| PAK4        | 70                 | 7                  | 71                 | 15                  | 44                 | 4                    |
| PAK5        | 93                 | 13                 | 84                 | 6                   | 103                | 27                   |
| PAK6        | 97                 | 1                  | 102                | 0                   | 81                 | 2                    |
| MST2        | 79                 | 11                 | 90                 | 5                   | 30                 | 4                    |
| MST3        | 92                 | 4                  |                    |                    | 103                | 16                   |
| MST4        | 96                 | 4                  | 94                 | 4                   | 41                 | 2                    |
| PKD1        | 104                | 18                 | 120                | 13                  | 43                 | 4                    |
| STK33       | 74                 | 9                  | 72                 | 10                  | 34                 | 7                    |
| MSK1        | 87                 | 5                  | 129                | 20                  | 60                 | 0                    |
| MNK1        | 91                 | 18                 | 110                | 1                   | 116                | 45                   |
| MNK2        | 101                | 4                  | 82                 | 1                   | 71                 | 4                    |
| MAPKAP-K2   | 102                | 13                 | 78                 | 9                   | 68                 | 3                    |
| MAPKAP-K3   | 89                 | 15                 | 94                 | 3                   | 105                | 14                   |
| PRAK        | 101                | 15                 | 103                | 8                   | 30                 | 5                    |
| CAMKKb      | 101                | 19                 | 103                | 4                   | 23                 | 2                    |
| CAMK1       | 95                 | 14                 | 138                | 18                  | 55                 | 5                    |
| SmMLCK      | 97                 | 15                 | 107                | 1                   | 9                  | 0                    |
| PHK         | 89                 | 9                  | 126                | 6                   | 6                  | 2                    |
| DAPK1       | 93                 | 5                  | 85                 | 4                   | 13                 | 1                    |
6. Crystallographic Analysis

Crystal structures of purine inhibitors co-crystallised with Nek2 were solved by Dr Richard Bayliss and Dr Corine Mas-Droux at the Section of Structural Biology, The Institute for Cancer Research, 237 Fulham Road, London, SW3 6JB. The structures were re-refined and deposited to the PDB by Dr Mark Richards and Dr Richard Bayliss at the Astbury Centre for Structural Molecules Biology at the University of Leeds, Woodhouse Lane, Leeds, LS2 9JT.

To examine the binding mode of ethynylpurines to Nek2 and observe the covalent bond directly, we solved X-ray co-crystal structures of Nek2 with compounds 24 and 66, and also with the competitively-binding control compounds 96 and 102 (Table 3).

Table 3. Summary of crystallographic analysis.

| Protein     | Kd (μM) | IC50 (nM) | KIC (nM) | IC50 (nM) | KIC (nM) |
|-------------|---------|-----------|----------|-----------|----------|
| CHK1        | 77      | 18        | 38       | 4         | 15       |
| CHK2        | 73      | 10        | 59       | 4         | 31       |
| GSK3b       | 87      | 0         | 100      | 10        | 30       |
| CDK2-Cyclin A| 83     | 7         | 96       | 10        | 46       |
| PLK1        | 89      | 11        | 115      | 6         | 116      |
| Aurora A    | 95      | 9         | 69       | 4         | 55       |
| Aurora B    | 77      | 13        | 67       | 7         | 103      |
| TLK1        | 95      | 5         | 96       | 0         | 90       |
| LKB1        | 100     | 3         | 95       | 11        | 91       |
| AMPK        | 83      | 2         | 60       | 1         | 6        |
| MARK1       | 92      | 10        | 87       | 4         | 14       |
| MARK2       | 93      | 12        | 95       | 3         | 100      |
| MARK3       | 101     | 24        | 96       | 10        | 8        |
| MARK4       | 90      | 3         | 89       | 9         | 27       |
| TIE2        | 100     | 3         | 111      | 1         | 111      |
| BRK         | 74      | 1         | 103      | 13        | 117      |
| MEKK1       | 85      | 7         | 104      | 6         | 105      |
| TTKB1       | 81      | 21        | -        | -         | 86       |
| TESK1       | 89      | 15        | -        | -         | 97       |
| WNK1        | 109     | 12        | -        | -         | 117      |
| DDR2        | -       | -         | -        | -         | 107      |
| CDK9-cyclin T1 | 79 | 4    | -        | -         | 106      |
| SIK2        | 78      | 12        | -        | -         | 63       |
| SIK3        | 88      | 0         | -        | -         | 33       |
| TSSK1       | 79      | 8         | -        | -         | 16       |
| CK1y2       | 83      | 0         | -        | -         | 56       |
|                  | 24 PDB: 6SGD | 66 PDB: 6SGH | 96 PDB: 6SGI | 102 PDB: 6SGK |
|------------------|-------------|-------------|-------------|-------------|
| **Data collection** |             |             |             |             |
| Space group      | C 1 2 1     | C 1 2 1     | C 1 2 1     | C 1 2 1     |
| **Cell dimensions** |     |     |     |     |
| a (Å)            | 99.67       | 101.02      | 99.39       | 98.73       |
| b (Å)            | 56.69       | 56.28       | 57.04       | 56.86       |
| c (Å)            | 73.78       | 74.01       | 78.40       | 73.19       |
| α (°)            | 90          | 90          | 90          | 90          |
| β (°)            | 129.71      | 129.46      | 133.07      | 128.28      |
| γ (°)            | 90          | 90          | 90          | 90          |
| **Resolution**   | 49.75 - 2.00| 57.14 - 3.00| 38.79 - 2.30| 30.76 - 2.00|
| **R_pim**        | 0.067 (0.424)| 0.104 (0.336)| 0.128 (0.731)| 0.130 (0.353)|
| **Completeness** | 99.6 (99.9) | 97.3 (98.3) | 99.6 (99.2) | 98.9 (98.5) |
| **Multiplicity** | 3.6 (3.7)   | 2.3 (2.4)   | 3.0 (2.6)   | 3.2 (3.2)   |
| **Refinement**   |             |             |             |             |
| Resolution (Å)   | 30.36 - 2.00| 57.14 - 3.00| 38.79 - 2.30| 28.43 - 2.00|
| Completeness (%) | 99.56       | 97.16       | 99.36       | 98.65       |
| No. reflections  | 21455       | 6371        | 14349       | 21392       |
| R work / R free | 19.77 / 23.38| 22.79 / 28.73| 21.29 / 26.58| 19.54 / 22.27|
| **Mean B-factors** |             |             |             |             |
| Protein          | 37.38       | 47.38       | 42.51       | 33.64       |
| Ligand           | 36.07       | 40.62       | 42.48       | 32.03       |
| Solvent          | 39.79       | 34.04       | 41.98       | 36.87       |
| **r.m.s. deviations** |             |             |             |             |
| bond lengths (Å) | 0.005       | 0.003       | 0.003       | 0.006       |
| bond angles (°)  | 0.773       | 0.654       | 0.592       | 0.799       |
| **MolProbity analysis** |         |             |             |             |
| All-atom clash-score | 13.83       | 12.12       | 4.73        | 11.84       |
| Rotamers outliers (%) | 0.00      | 0.00        | 0.00        | 0.00        |
| Ramachandran outliers (%) | 0.00    | 0.00        | 0.00        | 0.42        |
| Ramachandran favoured (%) | 95.34  | 95.95       | 96.31       | 97.88       |

7. References

1. For more information see: [http://www.caliperls.com/products/labchip-systems/ez-reader-ii.htm](http://www.caliperls.com/products/labchip-systems/ez-reader-ii.htm).
2. Note: for time-dependence experiments reaction was sampled in the Caliper EZ Reader II instrument at different times throughout the duration of the reaction.
3. Cell-based assays conducted and protocol (see experimental section) provided courtesy of Maura Westlake, CR UK Centre for Cancer Therapeutics, ICR, Sutton.
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