Organic & Biomolecular Chemistry

Accepted Manuscript

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Organic & Biomolecular Chemistry

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ARTICLE

Synthesis and photophysical characterization of new fluorescent triazole adenine analogues

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Fluorescent nucleic acid base analogues are powerful probes of DNA structure. Here we describe the synthesis and photo-physical characterization of a series of 2-(4-amino-5-(1*H*-1,2,3-triazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) and 2-(4-amino-3-(1*H*-1,2,3-triazol-4-yl)-1H-pyrazolo[3,4-*d*]pyrimidin-1-yl) analogues *via* Sonogashira cross-coupling and [3+2]-cycloaddition reactions as the key steps in the synthesis. Compounds with a nitrogen atom in position 8 showed an approximately ten-fold increase in quantum yield and decreased Stokes shift compared to analogues with a carbon atom in position 8. Furthermore, the analogues containing nitrogen in the 8-position showed a more red-shifted and structured absorption as opposed to those which have a carbon incorporated in the same position. Compared to the previously characterised C8-triazole modified adenine, the emissive potential was significantly lower (tenfold or more) for this new family of triazoles-adenine compounds. However, three of the compounds have photophysical properties which will make them interesting to monitor inside DNA.

Introduction

Fluorescence has been shown to be an extremely valuable and versatile tool for the investigation of biological systems.[1,2] It complements other techniques such as NMR spectroscopy and X-crystallography which may sometimes be limited by the resolution which can be achieved.[3] However most importantly, fluorescence allows for real time observation/tracking of the labelled molecules in living systems.[4-6] Furthermore, it is straightforward and requires very little sample. The virtually non fluorescent nature of naturally occurring nucleic acid bases highlights the need for synthetically labelled nucleobases as tools for the investigation of nucleic acid systems. These are typically synthesised by attaching a fluorophore to naturally existing nucleic acid bases.[7,8] However, the most commonly used fluorophores tend to suffer from certain limitations. Most dye molecules are large and hydrophobic which tends to impact negatively on the aqueous solubility of the resultant nucleosides. The fluorescent tags also tend to project outwards from the nucleic acid chains and can therefore interfere with the mobility and geometry of their targets, affecting their critical biological functions. For that reason, fluorescent base analogues (FBAs), which possess intrinsic fluorescence and can be incorporated into DNA/RNA causing only minimal perturbations of the delicate balance of biochemical function are potentially of great value.

Over the years FBAs have been shown to be extremely powerful tools with a wide range of applications in biology and biotechnology as molecular probes and reporters for nucleic acids.[7,9,10] Extensively studied FBAs include the pteridines developed by Hawkins *et al.*,[11] the cytosine analogue pyrrolo dC[12] and derivatives thereof[13-16], the environmental less sensitive tricyclic cytosines tC^{O}[17] and tC[18] as well as the emissive RNA alphabet designed by Tor and colleagues,[19] to name but a few (for recent reviews see for example Sinkeldam *et al.*,[7] Wilhelmsson[9] and Dodd *et al.*[20]). The adenine analogue 2-aminopurine, one of the most widely applied FBAs, has also been thoroughly characterized.[7,21]

Purine analogues are commonly modified on the C8 position of natural adenine/guanine.[22-31] Interest in these compounds stems from their utility as fluorescent markers, biomolecular probes, supramolecular building blocks, conformational probes and the use they have in therapeutics. Even though small modifications are reported to insignificantly perturb the DNA duplex,[25,31] bulky C8-substituents have shown to be destabilizing[22,23,29,30] and can shift the conformation around the glycosidic bond from *anti* to *syn*.[32,33] On the other hand, modifications on the purine 7-position have been shown

to be well tolerated by the DNA helix.[34-37] Recently, we reported the synthesis and characterisation of the photophysical and base-mimicking properties of a novel C8-modified fluorescent adenine analogue, triazole adenine (A^T), in DNA.[27] It showed promising photophysical properties, both as a monomer as well as inside DNA. However, as for other C8-modified adenines, A^T destabilizes the DNA structure, most likely because of the bulky C8-triazole group.[30]

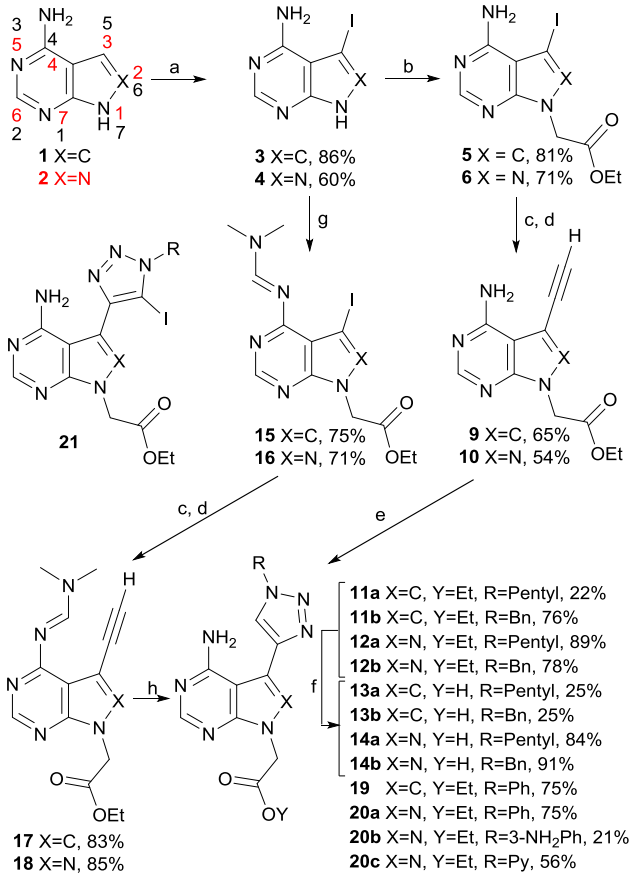
Pursuant to our interest in this area, we herein present the synthesis and photo-physical characterization of a model series of 2-(4-amino-5-(1*H*-1,2,3-triazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) and 2-(4-amino-3-(1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl) analogues which were designed in an attempt to further minimize the structural perturbations to B-DNA seen with the adenine analogue A^T by shifting the position of the triazole substituent to the adenine 7-position.[30] We report dramatic changes of the emissive properties of this new family of triazole adenine compounds compared to the previously characterized C8-modified series that could be of potential use in future applications in nucleic acid systems. Our photophysical characterization of this family of adenine analogues also helps to create a better understanding of the structure-fluorescence relationship, which is poorly understood at the moment.

Results and discussion

Synthetic procedures

The key intermediates **9** and **10** were readily accessed in 3 to 4 steps utilizing a common protocol. Starting from commercially available 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1**), *N*-iodosuccinimide (NIS) mediated iodination in acetonitrile under microwave irradiation afforded **3** in good yields (X = C 86%) (Scheme 1).[38]

Similar conditions were applied to the synthesis of **4** with comparable results from commercially available 4-aminopyrazolo [3, 4-*d*] pyrimidine (**2**) (X = N 71%). Treatment of DMF solutions of both **3** and **4** respectively with ethyl bromoacetate, at 0°C afforded the desired protected compounds **5** an d **6** respectively in reasonably good yields (X = C 81%, X = N 71%).[39] Sonogashira couplings with TMS-acetylene afforded the respective TMS acetylenes in excellent yields (X = C 91%, X = N 90%).[30,40] Subsequent deprotection with polymer supported fluoride afforded the key intermediates **9** and **10** in good yields (X = C 70%, X = N 61%).[30] A copper catalysed [3+2] cycloaddition reaction afforded the desired compounds in good yields.[40] A more detailed inspection of the LCMS data however revealed the presence of a small quantity of a contaminant, (< 5%), which is believed to arise from the insertion of iodine from the copper catalyst into the triazole ring depicted as **21**.[41] Therefore in a slight modification to the previously applied protocol for the synthesis of the alkyl analogues, CuI was replaced with Cu/C to avoid this contaminant.[42] Aryl analogues were synthesised utilising a protocol previously reported by Klein *et al.* utilising azides generated *in situ* from the corresponding aryl iodides (Table 1).[40] In initial attempts to synthesise the aryl analogues utilising this protocol, the competing amination reaction afforded the Ullman product as the major product. An alternate strategy to avoid this competing reaction required the synthesis of the N-protected acetylenes which were obtained by initial protection of the exocyclic amino group on **5** and **6** respectively with N,N-Dimethylformamide dimethyl acetal affording the protected iodides **15** and **16** in good yields (X = C 75%, X = N 71%).



Scheme 1. General synthetic pathway, conditions. Systematic numbering shown in red for X = N and in black for X = C. Red numbering was used for the NMR assignment for consistency: (a) NIS (1.5 eq), DMF μλ 120 °C, 20 min, (b) Cs_2CO_3, (1.2 eq), ethyl bromoacetate, (1.1 eq), DMF 0°C to RT, o/n, (c) TMS-acetylene, (1.5 eq), $Pd(PPh_3)_2Cl_2$ (10 mol%) and CuI (25 mol%), ETN/DMF (1:4), RT, o/n, (d) Polymer supported fluoride (Amberlite® IRA 900 F^- form), (2.0 eq), $CHCl_3$, (e) Alk-Br, (16.1 eq), NaN_3, (32.6 eq), H_2O μλ 140 °C, 30 min, then Et_3N (1.0 eq), RT o/n, EtOAc, (f) LiOH/THF 50%, (2.0 eq), (g) Cs_2CO_3, (1.2 eq), ethyl bromoacetate, (1.1 eq), DMF 0°C to RT then DMF-DMA, (5.0 eq), DMF RT o/n, (h) Ar-I, NaN_3, (1.2 eq), sodium ascorbate (40 mol%), sodium carbonate (20 mol %), L-proline (20 mol%), Cu/C (40 mol%), then NH_3/MeOH.

Sonogashira couplings, followed by subsequent deprotection with polymer supported fluoride afforded the key intermediates **17** and **18** in good yields over the 2 steps (X = C 61%, X = N

85%). The 1,2,3- triazole rings were then synthesized utilising the appropriate copper catalysed [3+2] cycloaddition reaction protocol between the respective alkynes **9**, **10**, **17** or **18** and the corresponding azides.[40,42,43] Deprotection with methanolic ammonia afforded the desired aryl analogues. Treatment with LiOH in THF converted **11** and **12** into **13** and **14** respectively.

Table 1: Synthesis of triazole analogues, conditions; Alk-Br, (16.1 eq), NaN_3, (32.6 eq), H_2O μλ 140 °C, 30 min, then Et_3N (1.0 eq), RT o/n, EtOAc, or Ar-I, NaN_3, (1.2 eq), sodium ascorbate (40 mol%), sodium carbonate (20 mol%), L-proline (20 mol%), Cu/C (40 mol%).Then LiOH/THF 50%, (2.0 eq), to obtain the corresponding acids where synthesised.

Compound #	Alkyne #	Azide	Product (yield %)	Acid (Yield %)
11a	**9**	Pent	22	25(**13a**)
12a	**10**	Pent	76	54(**14a**)
11b	**9**	Benz	89	84(**13b**)
12b	**10**	Benz	78	91(**14b**)
19	**17**	Ph	75	-[a]
20a	**18**	Ph	21	-[a]
20b	**18**	3-NH_2 Ph	56	-[a]
20c	**18**	Py	56	-[a]

[a] Not synthesised

Photophysical properties

The previously characterised sister compound of **11a** and **12a**, A^T, containing the same triazole-pentyl modification on C8 instead, exhibits a high quantum yield in both water (61%) and methanol (49%). Also inside DNA, A^T shows promising fluorescence properties, but displays a destabilising effect on the B-DNA helix as a consequence of the C8-triazole group, which is suggested to shift the equilibrium of the glycosidic bond from *anti* to *syn*.[30] On the other hand, purine analogues modified on the 7-position are known to be well accommodated in the DNA duplex.[34-37] In an attempt to obtain an analogue that retains the advantageous photophysical properties of A^T while incorporating the non-perturbing nature of 7-modiefied purine analogues, the current series of 7-modified triazole adenines has been synthesised and here we investigate its fluorescence properties.

Absorption and emission envelopes of all triazole adenine compounds (Scheme 1) in methanol are shown in Figure 1 and 2, respectively. Their characteristic photophysical properties are summarised in Table 2.

As for many adenine analogues, all compounds presented in Figure 1 show a red-shifted absorption (lowest energy A_{max}= 280-293 nm) compared to natural adenine (A_{max}= 260 nm), allowing for specific excitation in the red absorption tail outside the absorbance of the natural nucleobases. Our previous series of triazole adenines with similar substituents but having the triazole group placed on C8 of adenine showed absorption maxima in the same region in THF (289-296 nm)[27], methanol (286 nm, pentyl)[30] and water (282 nm, pentyl,[30] isopentyl[27]). Also the C8-modified 8-vinyldeoxyadenosine (8vdA) in methanol (294 nm)[25] has its maximum absorption in this wavelength range. As a comparison the absorbance of the most commonly applied FBA, 2-AP in water (303-304 nm)[21] or methanol (309 nm)[45], is situated in a similar region.

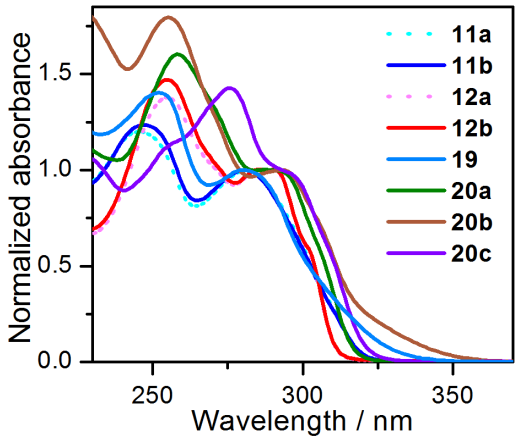


Figure 1: Absorption spectra of the triazole adenine analogues (Scheme 1) normalized to the lowest energy absorption maximum. Compounds were dissolved in methanol at room temperature.

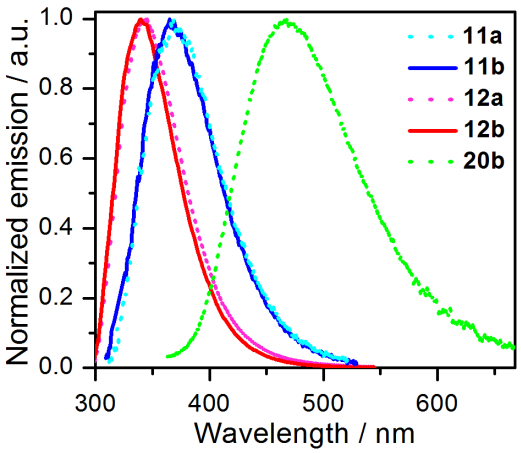


Figure 2: Normalized emission of triazole adenine analogues (with Φ_f > 0.2%) (Scheme 1) dissolved in methanol at 25°C.

As shown in Figures 1, 2 and Table 2, compounds **11a** and **11b** exhibit very similar absorptive and emissive properties, which is also the case for compounds **12a** and **12b**. This finding is expected as the conjugated π-system does not extend further than the triazole ring in these analogues, implying that the non-conjugated benzyl and pentyl-moieties should have a minimal effect on the photophysical characteristics. Similar observations were made for the previously characterised analogous set of triazole adenine compounds bearing the triazole ring on C8 of adenine, for which those containing aliphatic substituents showed very similar properties.[27] The differences that are found between both pairs of analogues, **11** and **12**, must be explained by the incorporation of the nitrogen on C8 of adenine. This yields an approximately ten-fold increase in quantum yield and decreased Stokes shift for compounds **12a** and **12b** (Φ_f~5%,

$\Delta\tilde{\nu}$~5200 cm^{-1}, $\Delta\lambda$~50 nm) compared to the two sister compounds, **11a** and **11b** (Φ_f~0.5%, $\Delta\tilde{\nu}$~8300 cm^{-1}, $\Delta\lambda$~85 nm) lacking this feature. However, these four compounds show similar extinction coefficients (10700-11800 $M^{-1}cm^{-1}$, Table 2), indicating no dramatic differences in their radiative rate constants according to the Strickler-Berg equation.[46] This suggests that the significant differences in emissive properties are mainly due to more efficient non-radiative processes depopulating the excited state for **11a** and **11b**.

Table 2: Fluorescence quantum yield (Φ_f), absorption maximum of the lowest energy absorption band ($\lambda_{A,max}$), corresponding extinction coefficients (ε_{max})(also at 260 nm, ε_{260nm}) and emission maximum ($\lambda_{Em,max}$) of each compound dissolved in methanol.

Compound[a]	Φ_f (%)[b]	$\lambda_{A,max}$ (nm)	$\lambda_{Em,max}$ (nm)	ε_{max} ($M^{-1}cm^{-1}$)	ε_{260nm} ($M^{-1}cm^{-1}$)
11a	0.75	282	369	11200	9700
11b	0.39	281	366	11800	10900
12a	5.22	290	343	10700	13800
12b	4.38	290	340	10800	14800
19	<0.1	280	-[d]	14500	17200
20a	<0.2	288	345	13600	21500
20b	0.62	293	468	14000	24100
20c	<0.1	293[c]	359	15300[e]	17800[e]

[a] Compounds are shown in Scheme 1. [b] Quantum yields were determined relative to 2-aminopyridine (Φ_f = 60%) in 0.05 M H_2SO_4 at 25°C.[44] [c] Absorption maximum estimated for lowest energy absorption shoulder. [d] Emission maximum omitted due to the weak fluorescence. [e] Extinction coefficient only determined once due to a limited amount of substance.

Generally, the analogues containing a nitrogen on the 8-position of adenine, (**12a**, **12b**, **20a**, **20b**, **20c**, λ_{max} 288-293 nm) show a more redshifted and structured absorption as opposed to those which have a carbon incorporated in this position (**11a**, **11b**, **19**, λ_{max} 280-282; Table 2). Within each group of compounds, the lowest energy absorption tail extends further into the lower energy wavelength region for compounds with aromatic extensions past the triazole ring (**19**, **20a-c**; Figure 1). They also exhibit the highest absorption coefficients which may be due to the larger conjugated system for these compounds compared to those with aliphatic substituents. Moreover, these aromatic derivatives show the lowest quantum yields within their groups. This finding is in line with the related C8-modified family.[27] As can be seen in Figure 1, analogues **20b** and **19** exhibit an extended absorption tail, which may raise concerns about aggregation. However, no changes in the shape of the absorption envelope could be detected upon dilution of concentrated samples of these compounds, nor by heating the solutions (Supporting information Figure S1-S4).

Interestingly, compound **20b** shows an extensive Stokes shift of ~12800 cm^{-1} ($\Delta\lambda$ = 175 nm), due to an emission maximum ~100-125 nm red-shifted compared to the other compounds, including the related conjugated analogues **20a** and **20c**. This large shift in emission wavelength ($\lambda_{Em,max}$= 468 nm) could be due to intramolecular charge transfer (ICT) in the excited state from the electron donating amino group. Since ICT would result in a larger dipole moment in the excited state compared to the ground state, the emission energy is expected to decrease with solvent polarity.[47] Moreover, hydrogen bonding can also play an important role in stabilizing the ICT-state.[48] Therefore, the emission of **20b** and for comparison also of **20c** was recorded in dichloromethane (DCM), an apolar and aprotic solvent. Whereas the emission properties of **20c** (Φ_f<0.1%, $\lambda_{Em,max}$ = 359 nm) remain unaltered, the quantum yield of **20b** increases dramatically in DCM (Φ_f~21%) and its emission maximum is shifted to 393 nm. Also in acetonitrile (ACN), a polar aprotic solvent, the emission of **20b** remains intense (Φ_f~24%) with the emission maximum found at 428 nm. Hence, the emission energy of **20b** seems to decrease both with polarity and protic character of the solvent, whereas no significant changes can be detected in the absorption spectra (See Figure S5, Supporting information). These findings are in accordance to the suggested character of the excited state ICT.

Apart from observed shifts in emission energy, **20b** seems to be efficiently quenched by protic solvents, as seen from the large decrease in fluorescence quantum yield in methanol (Φ_f = 0.62%) compared to ACN (Φ_f~24%) and DCM (Φ_f~21%). This may be due to a more efficient internal conversion in case of hydrogen bonding with solvent molecules.[47] Also for N^6,N^6-dimethyladenosine, significant quenching of the fluorescence of the putative twisted intramolecular charge transfer (TICT)-state was observed by protic solvents.[49] Similar as for **20b** in DCM, the 3-aminophenyl modification also yielded a significantly higher fluorescence quantum yield among the conjugated compounds (38% versus 3-5%) in an aprotic apolar solvent (THF) in a previous study concerning C8-modified triazole adenine analogues.[27] However, we do not know whether the behaviour of the latter compound would be similar as for **20b** in a polar protic solvent.

Compared to the previously characterised C8-modified A^T (Φ_f = 49% in methanol), the emissive potential is decreased tenfold for sister compound **12a** (5%) and even more dramatically for **11a** (0.75%) presented here. Within each group, distinguished by a nitrogen or carbon in position 8, all other compounds have even lower fluorescence quantum yields in methanol (Table 2). It is unclear what causes these major differences in emissive performance, since it remains challenging to predict fluorescence behaviour based on chemical structure.

Conclusions

A series of adenine derivatives have been synthesised with Sonogashira cross-coupling and [3+2]-cycloaddition reactions being the key steps in the synthesis. The cycloaddition reaction provides a means for rapid tuning of the fluorescence properties of the compounds. In general, derivatives with nitrogen in position 8 have a higher quantum yield and a more red-shifted absorption compared to

compounds with carbon in position 8. It is difficult to rationalise these differences. However, the design and characterisation of new FBAs remains important to the field due to the large potential for brighter and more photostable isomorphic probes. Although most FBAs show a decrease in fluorescence upon incorporation into nucleic acid systems,[7,11,12,20,22,26,31,34] increasing quantum efficiencies have been reported for some analogues[50]. Therefore it will be interesting to investigate the potential of **12a** or **12b** inside DNA as it is likely that the C7-triazole modification will be well accommodated in the major groove, potentially accompanied by interesting and applicable fluorescence properties. In addition the emission of **20b**, which shows a high quantum yield in aprotic environments, will be interesting to monitor inside DNA where it is more shielded from protic solvent molecules.

Acknowledgements

The authors thank Carl Tryggers Stiftelse, Stiftelsen Olle Engkvist Byggmästare and the Swedish Research Council for financial support and Professor Bo Albinsson and Munefumi Tokugawa for helpful discussions.

Experimental

Chemistry

General methods: Reagents and solvents were either used as received or purified according to standard techniques. Microwave reactions were performed using Biotage Initiator Microwave synthesiser, using single mode microwave irradiation with temperature and pressure control with fixed hold time on. Reactions were monitored by LC-MS (ESI/UV), using a PerkinElmer PE Sciex API 150 EX mass spectrometer equipped with a C8 column (Genesis Light C8 4 µm, length 50 mm, ID 4.6 mm) and H_2O-MeCN (95:5) to H_2O-MeCN (5:95) eluent, H_2O containing 1 % formic acid. The chromatograms were analysed with Analyst 1.5.1 software. TLC was carried out on silica gel plates (Merck 60 F_{254}) and analyzed under UV (254 nm). Column chromatography was performed by automated column chromatography on a Biotage SP-4 instrument using pre-packed silica columns or NH silica columns. ^{1}H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, using a Varian 400/54 spectrometer.

5-Iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (3)
To a microwave vial (2-5 mL) fitted with a magnetic stirrer was added 4-Amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1**) (0.60 g, 4.47 mmol, 1.0 eq) and *N*-iodosuccinimide (1.51 g, 6.71 mmol, 1.5 eq). The vial was then sealed and flushed with nitrogen. Acetonitrile (3.5 mL) was added and the resulting mixture heated at 120 °C by microwave irradiation for 20 minutes using the fixed hold time setting. The resultant precipitate was filtered off, washed with ice cold acetonitrile (3 X 1 mL) and dried under reduced pressure to afford the crude product (1.00 g, 3,85 mmol, 86%) as a dark brown solid. This was used in the following synthesis without further purification. LRMS (ES^+): *m/z* (%) 261 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.95 (N<u>H</u>), 8.05 (1H, s, C6-<u>H</u>), 7.35 (1H, s, C2-<u>H</u>), 6.52 (N<u>H</u>$_2$); ^{13}C NMR (101 MHz, d_6-DMSO) δ 157.5 (C4), 152.3 (C6), 151.0 (C7a), 127.1 (C2), 103.1 (C3a), 50.5 (C3).

3-Iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (4)[38]
To a microwave vial (20 mL) fitted with a magnetic stirrer was added 1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2**) (2.0 g, 14.81 mmol, 1.0 eq) and *N*-iodosuccinimide (3.99 g, 17.73 mmol, 1.2 eq). The vial was then sealed and flushed with nitrogen. DMF (10 mL) was added and the resulting mixture heated at 100 ºC by microwave irradiation for 20 minutes using the fixed hold time setting. The solvent was removed under reduced pressure and the resultant crude product washed with hot ethanol (30 mL) to afford the desired product (2.75 g, 10.53 mmol, 71%) as a pink solid. This was used in the following synthesis without further purification. LRMS (ES^+): *m/z* (%) 262 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.15 (1H, s, C6-<u>H</u>); ^{13}C NMR (101 MHz, d_6-DMSO) δ 157.8 (C4), 156.2 (C6), 155.3 (C7a), 102.9 (C3a), 90.2 (C3).

General Procedure A for preparation of ethyl propionate protected pyrimidines (5 & 6)
To a mixture of the respective iodopyrimidine (**3** or **4**, 1.0 eq) and cesium carbonate (1.2 eq) in a sealed flask flushed with nitrogen, was added DMF (5 mL) and cooled to 0°C in an ice bath. Ethylbromoacetate (1.1 eq) was then slowly added to the slurry and the resulting mixture allowed to warm up to room temperature and stirred at room temperature for 18 hours. Water (50 mL) was then added and the resultant precipitate filtered off washed and dried under reduced pressure. Purification by flash column chromatography on silica gel eluting with a gradient from 0% to 3% MeOH in $CHCl_3$ afforded the desired compound.

Ethyl 2-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-acetate (5)
Using General procedure A, **3** (1.02 g, 3.92 mmol, 1.0 eq) afforded the title compound (1.10 g, 3.18 mmol, 81%) as a cream coloured solid. LRMS (ES^+): *m/z* (%) 347 (100) $[M+H]^+$, 693 (2) $[2M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.09 (1H, s, C6-<u>H</u>), 7.44 (1H, s, C2-<u>H</u>), 6.66 (2H, br NH_2), 4.98 (2H, s, C<u>H</u>$_2$), 4.17-4.11 (2H, q, *J* = 7.1 Hz, C<u>H</u>$_2$-OEt), 1.20 (3H, t, *J* = 7.1 Hz, C<u>H</u>$_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 168.7 (<u>C</u>O$_2$Et), 157.6 (C4), 152.5 (C6), 150.4 (C7a),130.6 (C2), 103.1 (C3a), 61.6 <u>C</u>H$_2$-OEt, 50.7 (C3), 45.5 N-<u>C</u>H$_2$, 14.5 <u>C</u>H$_3$-OEt.

Ethyl 2-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-acetate (6)
Using General procedure A, **4** (2.00 g, 7.66 mmol, 1.0 eq) afforded the title compound (1.90 g, 5.47 mmol, 71%) as an off white solid. LRMS (ES^+): *m/z* (%) 348 (100) $[M+H]^+$, 695 (5) $[2M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.19 (1H, s, C6-<u>H</u>), 5.16 (2H, s, C<u>H</u>$_2$), 4.16-4.10 (2H, q, *J* = 7.1 Hz, C<u>H</u>$_2$-OEt), 1.18 (3H, t, *J* = 7.1 Hz, C<u>H</u>$_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 168.0 (<u>C</u>O$_2$Et), 158.1 (C4), 156.7 (C6), 154.9 (C7a),

103.5 (C3a), 90.6 (C3), 61.8 $\underline{C}H_2$-OEt, 48.4 N-$\underline{C}H_2$, 14.4 $\underline{C}H_3$-OEt.

General Procedure B for preparation of amidine protected ethyl propionate pyrimidines (15 & 16)

To the respective iodopyrimidines (**5** or **6**, 1.0 eq) in a sealed flask flushed with nitrogen, was added DMF (5 mL). N,N-Dimethylformamide dimethyl acetal (5.0 eq) was then added and the resulting mixture stirred at 60°C for 18 hours. Solvents were removed under reduced pressure and purification by flash column chromatography on silica gel eluting with a gradient from 0% to 3% MeOH in $CHCl_3$ afforded the desired compound.

Ethyl (E)-2-(4-(((dimethylamino)methylene)amino)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)acetate (15)

Using General procedure B, **5** (1,10 g, 3.18 mmol, 1.0 eq) afforded the title compound (0.95 g, 2.37 mmol, 75%) as a light brown solid. LRMS (ES^+): *m/z* (%) 402 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, s, N=C$\underline{H}$), 8.41 (1H, s, C6-$\underline{H}$), 7.12 (1H, s, C2-$\underline{H}$), 4.90 (2H, s, C$\underline{H}_2$), 4.21-4.16 (2H, q, *J* = 7.1 Hz, C$\underline{H}_2$-OEt), , 3.27 (3H, s, NC$\underline{H}_3$), 3.14 (3H, s, NC$\underline{H}_3$), 1.23 (3H, t, *J* = 7.1 Hz, C$\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 167.9 ($\underline{C}O_2$Et), 160.6 (C4), 155.9 N=$\underline{C}$H, 152.0 (C6), 151.4 (C7a), 130.9 (C2), 110.8 (C3a), 61.8 $\underline{C}H_2$-OEt, 52.6 (C3), 45.3 N-$\underline{C}H_2$, 40.8 (N-CH_3), 35.3 (N-CH_3), 14.4 $\underline{C}H_3$-OEt.

Ethyl (E)-2-(4-(((dimethylamino)methylene)amino)-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (16)

Using General procedure B, **6** (2.87 g, 8.27 mmol, 1.0 eq) afforded the title compound (2.41 g, 5.99 mmol, 71%) as a light brown solid. LRMS (ES^+): *m/z* (%) 403 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (1H, s, N=C$\underline{H}$), 8.49 (1H, s, C6-$\underline{H}$), 5.15 (2H, s, C$\underline{H}_2$), 4.24-4.19 (2H, q, *J* = 7.1 Hz, C$\underline{H}_2$-OEt), , 3.34 (3H, s, NC$\underline{H}_3$), 3.24 (3H, s, NC$\underline{H}_3$), 1.25 (3H, t, *J* = 7.1 Hz, C$\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 167.3 ($\underline{C}O_2$Et), 157.4(C4), 159.9 N=$\underline{C}$H, 155.8(C6), 154.9 (C7a), 109.9 (C3a), 90.7 (C3), 61.8 $\underline{C}H_2$-OEt, 48.4 N-$\underline{C}H_2$, 41.3 (N-CH_3), 35.6 (N-CH_3), 14.4 $\underline{C}H_3$-OEt.

General Procedure C for preparation of Silylethynyl pyrimidines (7 & 8)

To a mixture of the respective iodopyrimidine (**5** or **6**, 1.0 eq) $Pd(PPh_3)_2Cl_2$ (10 mol%) and CuI (25 mol%) in a sealed flask flushed with nitrogen, was added a degassed mixture of DMF:Et_3N (4:1) followed by Ethynyl-TMS (1.5 eq) and the resulting mixture stirred at room temperature for 18 hours. The reaction mixture was then absorbed onto celite under reduced pressure and purified by flash column chromatography on silica gel eluting with a gradient from 0% to 2% MeOH in $CHCl_3$ to afford the desired compound.

Ethyl 2-(4-amino-5-((trimethylsilyl)ethynyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-7-yl)acetate (7)

Using General procedure C, **5** (0.50 g, 1.45 mmol, 1.0 eq) afforded the title compound (0.42 g, 1.33 mmol, 91%) as a brown solid. LRMS (ES^+): *m/z* (%) 317 (100) $[M+H]^+$, 633 (5) $[2M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (1H, s, C6-$\underline{H}$), 7.17 (1H, s, C2-$\underline{H}$), 5.65 (2H, s, NH_2), 4.91 (2H, s, C$\underline{H}_2$), 4.25-4.20 (2H, q, *J* = 7.1 Hz, C$\underline{H}_2$-OEt), 1.26 (3H, t, *J* = 7.1 Hz, C$\underline{H}_3$-OEt), 0.26 (9H, s, Si(C$\underline{H}_3$)$_3$; ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7 ($\underline{C}O_2$Et), 157.5 (C4), 153.3 (C6), 150.0 (C7a),129.0 (C2), 103.1 (C3a), 98.4 (C3), 97.4 (C≡$\underline{C}$-Si), 96.7($\underline{C}$≡C-Si), 61.6 ($\underline{C}H_2$-OEt), 45.4 (N-$\underline{C}H_2$), 14.1 $\underline{C}H_3$-OEt), 0.1 (Si($\underline{C}H_3$)$_3$.

Ethyl 2-(4-amino-3-((trimethylsilyl)ethynyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidin-1-yl)acetate (8)

Using General procedure C, **6** (1.18 g, 3.40 mmol, 1.0 eq) afforded the title compound (0.97 g, 3.06 mmol, 90%) as a brown solid. LRMS (ES^+): *m/z* (%) 318 (100) $[M+H]^+$, 635 (3) $[2M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (1H, s, C6-$\underline{H}$), 6.02 (2H, s, NH_2), 5.13 (2H, s, C$\underline{H}_2$), 4.25-4.19 (2H, q, *J* = 7.1 Hz, C$\underline{H}_2$-OEt), 1.26 (3H, t, *J* = 7.1 Hz, C$\underline{H}_3$-OEt), 0.30 (9H, s, Si(C$\underline{H}_3$)$_3$; ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0 ($\underline{C}O_2$Et), 157.7 (C4), 156.9 (C6), 154.0 (C7a), 127.4 (C3a), 101.8 (C≡$\underline{C}$-Si), 101.6 ($\underline{C}$≡C-Si), 95.9 (C3), 62.0 ($\underline{C}H_2$-OEt), 48.4 (N-$\underline{C}H_2$), 14.1($\underline{C}H_3$-OEt), 0.4 (Si($\underline{C}H_3$)$_3$.

General Procedure D for preparation of ethynyl pyrimidines (9 & 10)

To a solution of either (**7** or **8**, 1.0 eq) respectively, in chloroform was added Fluoride on polymer support (2.0 eq) and the resulting mixture flushed with nitrogen then stirred at room temperature for 18 hours. The reaction mixture was then filtered washed with THF and $CHCl_3$, (3 X 20 mL), absorbed onto celite under reduced pressure and purified by flash column chromatography on silica gel eluting with a gradient from 0% to 5% MeOH in $CHCl_3$ to afford the desired compound.

Ethyl 2-(4-amino-5-ethynyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)acetate (9)

Using General procedure D, **7** (0.46 g, 1.33 mmol, 1.0 eq) afforded the title compound (0.23 g, 0.94 mmol, 70%) as a light brown solid. LRMS (ES^+): *m/z* (%) 245 (100) $[M+H]^+$, 489 (10) $[2M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (1H, s, C6-$\underline{H}$), 7.21 (1H, s, C2-$\underline{H}$), 5.72 (2H, s, NH_2), 4.92 (2H, s, C$\underline{H}_2$), 4.26-4.21 (2H, q, *J* = 7.1 Hz, C$\underline{H}_2$-OEt), 3.25 (1H, s, $\underline{H}$C≡C), 1.29 (3H, t, *J* = 7.1 Hz, C$\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, $CDCl_3$δ 167.7 ($\underline{C}O_2$Et), 157.4 (C4), 153.3 (C6), 150.0 (C7a),129.6 (C2), 103.0 (C3a), 94.7 (C3) 79.9 (C≡$\underline{C}$-H), 77.4 ($\underline{C}$≡C-H), 62.0 $\underline{C}H_2$-OEt, 45.4 N-$\underline{C}H_2$, 14.1 $\underline{C}H_3$-OEt.

Ethyl 2-(4-amino-3-ethynyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (10)

Using General procedure D, **8** (0.97 g, 2.79 mmol, 1.0 eq) afforded the title compound (0.42 g, 1.71 mmol, 61%) as a light brown solid. LRMS (ES^+): *m/z* (%) 246 (100) $[M+H]^+$, 491 (15) $[2M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (1H, s, C6-$\underline{H}$), 6.00 (2H, s, NH_2), 5.16 (2H, s, C$\underline{H}_2$), 4.27-4.21 (2H, q, *J* = 7.1 Hz, C$\underline{H}_2$-OEt), 3.50 (1H, s, $\underline{H}$C≡C), 1.27 (3H, t, *J* = 7.1 Hz, C$\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0 ($\underline{C}O_2$Et),

157.7 (C4), 156.9 (C6), 154.1 (C7a),126.4 (C3), 101.9 (C3a), 82.9 (C≡C-H), 75.5 (C≡C-H), 62.0 CH_2-OEt, 48.4 N-CH_2, 14.0 CH_3-OEt.

Ethyl (E)-2-(4-(((dimethylamino)methylene)amino)-3-ethynyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (17)

Using General procedure C, **15** (0.40 g, 1.00 mmol, 1.0 eq) afforded the title compound (0.15 g, 0.50 mmol, 50%) as a cream solid. LRMS (ES^+): *m/z* (%) 300 (100) $[M+H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (1H, s, N=CH), 8.32 (1H, s, C6-H), 7.63 (1H, s, C2-H), 5.03 (2H, s, CH_2), 4.15-4.14 (2H, q, *J* = 7.5 Hz, CH_2-OEt), 3.94 (1H, s, HC≡C), 3.17 (3H, s, NCH_3), 3.14 (3H, s, NCH_3), 1.2 (3H, t, *J* = 7.5 Hz, CH_3-OEt); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2 (CO_2Et), 160.8 N=CH, 156.4 (C4), 152.0 (C6), 150.9 (C7a), 132.4 (C2), 110.9 (C3), 109.6 (C3a), 95.1 (C≡C-H), 80.5 (C≡C-H), 61.1 CH_2-OEt, 45.1 N-CH_2, 34.4 (N-CH_3), 13.9 CH_3-OEt.

Ethyl (E)-2-(4-(((dimethylamino)methylene)amino)-3-ethynyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (18)

Using General procedure C, **16** (2.35 g, 5.845 mmol, 1.0 eq) afforded the title compound (1.81 g, 4.86 mmol, 85%) as a light brown solid. 1.25 g of this was treated with polymer supported Fluoride according to general procedure D affording the target compound as a cream solid (0.85 g, 2.83 mmol, 85%). LRMS (ES^+): *m/z* (%) 301 (100) $[M+H]^+$, 601 (10) $[2M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87-8.82 (1H, s, N=CH), 8.47(1H, s, C6-H), 5.13 (2H, s, CH_2), 4.19-4.14 (2H, q, *J* = 7.1 Hz, CH_2-OEt), 3.33 (1H, s, HC≡C), 3.22 (3H, s, NCH_3), 3.17 (3H, s, NCH_3), 1.19 (3H, t, *J* = 7.1 Hz, CH_3-OEt); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (CO_2Et), 162.6 N=CH, 157.3 (C4), 156.1 (C6), 154.9 (C7a),128.3(C3), 108.6 (C3a), 80.8 (C≡C-H), 76.0 (C≡C-H), 61.8 CH_2-OEt, 48.4 N-CH_2, , 41.3 (N-CH_3), 35.6 (N-CH_3), 14.0 CH_3-OEt.

General Procedure D for preparation of triazoles from alkyl bromides (11 & 12)

To a mixture of sodium azide (32.6 eq) and the corresponding bromide respectively (16.1 eq) in a microwave vial was added water (3 mL) and the resulting solution heated at 140 °C by microwave irradiation for 1 hour using the fixed hold time setting. The reaction was cooled, extracted with ethyl acetate (2 X 3 mL) and dried (*via* fritted syringe containing $MgSO_4$). The combined organic fractions were then added to a mixture of either (**9** or **10**, 1.0 eq) respectively, Cu/C (40 mol%) and Et_3N (1.0 eq) in a sealed flask flushed with nitrogen and the resulting mixture stirred at room temperature for 18 hours. The reaction mixture was then filtered and absorbed onto celite under reduced pressure and purified by flash column chromatography on silica gel eluting with a gradient from 0% to 5% MeOH in $CHCl_3$ to afford the desired compound.

Ethyl 2-(4-amino-5-(1-pentyl-1H-1,2,3-triazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)acetate (11a)

Using General procedure D, **9** (70.00 mg, 0.28 mmol, 1.0 eq) and 1-bromopentane (0.69 g, 0.57 mL, 4.62 mmol, 16.1 eq) afforded the title compound (22.51 mg, 6.30 Exp^{-5} mol, 22%) as a white solid. LRMS (ES^+): *m/z* (%) 358(100) $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.77 (1H, s, NH), 10.15 (1H, s, NH), 7.99 (1H, s, C6-H), 7.77 (1H, s, C5'-H), 7.39 (1H, s, C2-H), 4.99 (2H, s, CH_2), 4.39 (2H, t, *J* = 7.2 Hz, C1''-CH_2); 4.29-4.24 (2H, q, *J* = 7.1 Hz, CH_2-OEt), 1.99-1.93 (2H, m, C2''-CH_2), 1.37-1.29 (7H, m, C3''-CH_2, C4''-CH_2, CH_3-OEt), 0.91 (t, *J* = 7.0 Hz, 3H, C5''-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2 (CO_2Et), 152.9 (C4), 147.9 (C6), 142.8 (C7a),140.4 (C1'), 123.4 (C5'), 119.4 (C2), 109.5 (C3), 99.7 (C3a), 62.4 (CH_2-OEt), 50.9 (C1''), 45.8 (N-CH_2), 29.8 (C2''), 28.5 (C3''), 22.0 (C4''), 14.0 (CH_3-OEt), 13.8 (5'')

Ethyl 2-(4-amino-3-(1-pentyl-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (12a)

Using General procedure D, **10** (90.00 mg, 0.37 mmol, 1.0 eq) and 1-bromopentane (0.79 g, 0.55 mL, 5.91 mmol, 16.1 eq) afforded the title compound (118.0 mg, 0.33 mmol, 89%) as a white solid. LRMS (ES^+): *m/z* (%) 359(100) $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (1H, br s, NH), 8.42 (1H, br s, C6-H), 8.10 (1H, s, C5'-H), 6.11 (1H, br s, NH), 5.15 (2H, br, s, CH_2), 4.42 (2H, t, *J* = 8.0 Hz, C1''-CH_2); 4.26-4.21 (2H, q, *J* = 8.0 Hz, CH_2-OEt), 1.99-1.95 (2H, m, C2''-CH_2), 1.39-1.25 (7H, m, C3''-CH_2, C4''-CH_2, CH_3-OEt), 0.93-0.89 (3H, m, C5''-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 (CO_2Et), 158.7 (C4), 158.8 (C7a),155.3 (C6), 141.9 (C3) 137.1 (C1'), 123.4 (C5'), 109.5 (C3a), 62.9 (CH_2-OEt), 50.8 (C1''), 48.1 (N-CH_2), 29.8 (C2''), 28.5 (C3''), 22.0 (C4''), 14.0 (CH_3-OEt), 13.8 (5'').

Ethyl 2-(4-amino-5-(1-benzyl-1H-1,2,3-triazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)acetate (11b)

Using General procedure D, **9** (70.00 mg, 0.29 mmol, 1.0 eq) and benzyl bromide (0.89 g, 0.73 mL, 4.62 mmol, 16.1 eq) afforded the title compound (81.00 mg, 0.22 mmol, 76%) as a white solid.

LRMS (ES^+): *m/z* (%) 378 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (1H, br s, C6-H), 7.57 (1H, s, C5'-H), 7.39-7.37 (3H, m, C4'', C5'', C6''), 7.30-7.26 (2H, m, C3'', C7''), 7.12 (1H, s, C2-H), 5.52 (2H, s, C1''-CH_2), 4.90 (2H, s, CH_2) 4.23-4.17 (2H, q, *J* = 7.1 Hz, CH_2-OEt), 1.25 (3H, t, *J* = 7.1 Hz, CH_3-OEt); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2 (CO_2Et), 157.9 (C4), 152.6 (C7a), 151.1 (C6), 142.9 (C1'), 134.2 (C2"), 129.2 (C3'', 7"), 128.9 (C4'', C6''), 128.1 (C5''), 121.5 (C5'), 118.9 (C2), 106.7 (C3a), 61.9 (CH_2-OEt), 54.4 (C1"), 45.2 (N-CH_2), 14.1 (CH_3-OEt).

Ethyl 2-(4-amino-3-(1-benzyl-1H-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (12b)

Using General procedure D, **10** (90.00 mg, 0.37 mmol, 1.0 eq) and 1-bromopentane (0.79 g, 0.55 mL, 5.91 mmol, 16.1 eq) afforded the title compound (110.90 mg, 0.29 mmol, 78%) as a white solid. LRMS (ES^+): *m/z* (%) 379 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (1H, br s, NH), 8.33 (1H, s, C6-H), 8.02 (1H, s, C5'-H), 7.42-7.36 (3H, m, C4'', C5'', C6''), 7.34-7.31 (2H, m, C3'', C7''), δ 6.14 (1H, br s, NH), 5.58 (2H, s,

$C\underline{H}_2$), 5.11 (2H, s, $C\underline{H}_2$), 4.23-4.18 (2H, q, J = 7.1 Hz, $C\underline{H}_2$-OEt), 1.24 (3H, t, J = 7.0 Hz, 3H, $C\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 ($\underline{C}O_2Et$), 158.5 (C4), 156.9 (C6), 155.3 (C7a),142.3 (C3), 136.9 (C1"), 133.7 (C1'), 129.3 (C3'', C7''), 129.3 (C5''), 128.3 (C4'', C6''), 121.2 (C5'), 98.5 (C3a), 61.8 ($\underline{C}H_2$-OEt), 54.7 (C1"), 48.0 (N-$\underline{C}H_2$), 14.1 ($\underline{C}H_3$-OEt).

General Procedure E for preparation of triazoles from aryl iodides (20)

To a mixture of sodium azide (1.2 eq), the corresponding iodide (1.2 eq), sodium ascorbate (40 mol%), sodium carbonate (20 mol %), L-proline (20 mol%), Cu/C (40 mol%) **17**, (1.0 eq) in a microwave vial was added DMSO:H_2O (3 mL) and the resulting solution heated at 130 ºC by microwave irradiation for 30 minutes using the fixed hold time setting. The reaction mixture was then cooled Ammonia in ethanol (7M 2mL) added and stirred for 2 hours and poured into aqueous ammonia solution (10 mL, 20% *v*/*v*) and extracted with ethyl acetate (3 X 10 mL) and. The combined organic layers were washed with brine then dried ($MgSO_4$) and absorbed onto celite under reduced pressure. Purification by flash column chromatography on silica gel eluting with a gradient from 0% to 5% MeOH in $CHCl_3$ afforded the desired compound.

Ethyl 2-(4-amino-3-(1-phenyl-1H-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (19)

Using General procedure E, **17** (100.0 mg, 0.34 mmol, 1.0 eq) and Iodobenzene (81.8 mg, 44.9 µL, 0.40 mmol, 1.2 eq) afforded the title compound (92.6 mg, 0.25 mmol, 75%) as a brown solid. LRMS (ES^+): *m/z* (%) 364 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.27 (1H, s, C 6-$\underline{H}$), 8.07 (1H, s, C5'), 7.73-7.72 (2H, d, J = 7.4 Hz, C2'', C6''), 7.54 (2H, t, J = 7.4 Hz, C3'', C5''), 7.46 (1H, s, C4''), 7.27 (H, s, C2), 4.97 (2H, s, $C\underline{H}_2$), 4.26-4.22 (2H, q, J = 7.2 Hz, $C\underline{H}_2$-OEt), 1.28 (3H, t, J = 7.2 Hz, $C\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 168.3 ($\underline{C}O_2Et$), 158.1 (C4), 153.7 (C6), 151.3 (C7a), 142.3 (C3), 136.7 (C1"), 129.8 (C1'), 129.7 (C4"), 121.7 (C3'', C5''), 120.5 (C5'), 116.9(C2", C6"), 106.2 (C2), 100.3 (C3a), 61.89 ($\underline{C}H_2$-OEt), 45.2 (N-$\underline{C}H_2$), 14.1 ($\underline{C}H_3$-OEt).

Ethyl 2-(4-amino-3-(1-phenyl-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (20a)

Using General procedure E, **18** (100.0 mg, 0.34 mmol, 1.0 eq) and Iodobenzene (81.8 mg, 44.9 µL, 0.40 mmol, 1.2 eq) afforded the title compound (92.6 mg, 0.25 mmol, 75%) as a brown solid. LRMS (ES^+): *m/z* (%) 365 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.54 (1H, NH_2), 9.44 (1H, s, C5'), 9.04 (1H, br s, NH), 8.26 (1H, s, C6-$\underline{H}$), 8.20 (1H, br s, NH), 8.07-8.04 (2H, qd, J = 3.0 Hz, J = 1.7 Hz, C2'', C6''), 7.65-7.60 (2H, dd, J = 7.2 Hz, J = 2.4 Hz, C3'', C5''), 7.56-7.55 (1H, qd, J = 3.0 Hz, J = 1.7 Hz, C4''), 5.24 (2H, s, $C\underline{H}_2$), 4.19-4.13 (2H, q, J = 7.1 Hz, $C\underline{H}_2$-OEt), 1.20 (3H, t, J = 7.1 Hz, $C\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 168.1 ($\underline{C}O_2Et$), 158.7 (C4), 157.2 (C6), 155.3 (C7a), 142.3 (C3), 136.7 (C1"), 136.0 (C1'), 130.4 (C2'', C6''), 129.7 (C4"), 121.3 (C5'), 120.95 (C3", C5"), 97.9 (C3a), 61.8 ($\underline{C}H_2$-OEt), 48.4 (N-$\underline{C}H_2$), 14.1 ($\underline{C}H_3$-OEt).

Ethyl-2-(4-amino-3-(1-(3-aminophenyl)-1*H*-1,2,3-triazol-4-yl)-1H-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (20b))

Using General procedure E, **18** (100.0 mg, 0.34 mmol, 1.0 eq) and 3-Iodoaniline (87.8 mg, 0.40 mmol, 1.2 eq) afforded the title compound (27.0 mg, 0.07 mmol, 21%) as a white solid. LRMS (ES^+): *m/z* (%) 380 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.24 (1H, s, C5'-$\underline{H}$), 9.07 (1H, br s, NH), 8.26 (1H, s, C6-$\underline{H}$), 8.19 (1H, br s, NH), 7.22-7.20 (2H, dt, J = 8.0 Hz, J = 4.0 Hz, C5'', C6''), 7.11-7.08 (1H, d, J = 12 Hz, *C2"*), 6.71-6.69 (1H, d, J = 8 Hz, *C4"*), 5.54 (2H,br s, NH_2), 5.24 (2H, s, N-$C\underline{H}_2$) 4.19-4.13 (2H, q, J = 8.0 Hz, $C\underline{H}_2$-OEt), 1.20 (3H, t, J = 8.0 Hz, $C\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 168.1 ($\underline{C}O_2Et$), 158.7 (C4), 157.2 (C6), 155.3 (C7a), 150.6 (C3"), 142.0 (C3), 137.5 (C1"), 136.1 (C1'), 130.7 (C5"), 121.6 (C5'), 115.0 (C6"), 109.9 (C3a), 107.8 (C4"), 105.7 (C2"), 61.8 ($\underline{C}H_2$-OEt), 48.4 (N-$\underline{C}H_2$), 14.4 ($\underline{C}H_3$-OEt).

Ethyl 2-(4-amino-3-(1-(pyridin-2-yl)-1*H*-1,2,3-triazol-4-yl)-1H-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate (20c)

Using General procedure E, **18** (100.0 mg, 0.34 mmol, 1.0 eq) and 2-Iodopyridine (82.2 mg, 42.6 µL, 0.40 mmol, 1.2 eq) afforded the title compound (69.3 mg, 0.19 mmol, 56%) as a cream solid. LRMS (ES^+): *m/z* (%) 366 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.24 (1H, d, J = 8.0 Hz, C5'-$\underline{H}$), 8.89 (1H, br s, NH), 8.65-8.63 (1H, m, C3"-$\underline{H}$), 8.24 (1H, s, C6-$\underline{H}$), 8.20-8.14 (2H, m, C5'', C6''), 7.26-7.59 (1H, m, *C4"*), 5.23 (2H, s, N-$C\underline{H}_2$) 4.17-4.12 (2H, q, J = 7.1, $C\underline{H}_2$-OEt), 1.20 (3H, t, J = 8.0 Hz, $C\underline{H}_3$-OEt); ^{13}C NMR (101 MHz, d_6-DMSO) δ 168.1 ($\underline{C}O_2Et$), 158.6 (C4), 157.2 (C6), 155.3 (C7a), 149.6 (C3"), 142.1 (C3), 140.7 (C5"), 135.6 (C1'), 125.58 (C4"), 119.6 (C5'), 114.9 (C6"), 98.0 (C3a), 61.8 ($\underline{C}H_2$-OEt), 48.5 (N-$\underline{C}H_2$), 14.4 ($\underline{C}H_3$-OEt).

General Procedure F for preparation of acids (13& 14)

To a solution of the respective triazole in THF (2 mL) was added LiOH (2.0 eq as a solution in H_2O 2 mL) and stirred at room temperature for 18 hours. The pH of the reaction mixture was then adjusted to approximately pH3 with HCl (1M). The resultant precipitate was filtered off and washed with H_2O to afford the desired compound.

2-(4-amino-5-(1-pentyl-1*H*-1,2,3-triazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-7-yl)acetic acid (13a)

Using General procedure F, **11a** (22.00 mg, 6.16 Exp^{-5} mol, 1.0 eq) afforded the title compound (5.00 mg, 1.51 Exp^{-5} mol, 25%) as a white solid. LRMS (ES^+): *m/z* (%) 330 (100) $[M+H]^+$; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.54 (1H, s, C6-$\underline{H}$), 8.20 (1H, s, C5'-$\underline{H}$), 7.74 (1H, C2-$\underline{H}$), 4.98 (2H, s, $C\underline{H}_2$), 4.42 (2H, t, ^3J = 7.1 Hz, C1''-$C\underline{H}_2$); 1.89-1.85 (2H, m, C$\underline{2}$''-$C\underline{H}_2$), 1.34-1.22 (4H, m, C3''-$C\underline{H}_2$, C4''-$C\underline{H}_2$), 0.86 (t, ^3J = 7.1 Hz, 3H, C5''-$C\underline{H}_3$).

2-(4-amino-3-(1-pentyl-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo-[3,4-*d*]pyrimidin-1-yl)acetic acid (14a)

Using General procedure F, **12a** (58.80 mg, 1.56 mmol, 1.0 eq) afforded the title compound (46 mg, 1.31 mmol, 84%) as a white solid. LRMS (ES^+): *m/z* (%) 331 (100) $[M+H]^+$; ^1H NMR (400 MHz, d_6-DMSO) δ 13.22 (1H, S COO<u>H</u>), 9.15 (1H, N<u>H</u>$_2$), 8.76 (1H, s, C5'-<u>H</u>), 8.23 (1H, s, C6-<u>H</u>), 8.08 (1H, N<u>H</u>$_2$), 5.10 (2H, s, C<u>H</u>$_2$), 4.47 (2H, t, ^3J = 7.1 Hz, C1''-C<u>H</u>$_2$); 1.93-1.89 (2H, m, C2''-C<u>H</u>$_2$), 1.34-1.18 (4H, m, C3''-C<u>H</u>$_2$, C4''-C<u>H</u>$_2$), 0.86 (t, ^3J = 7.1 Hz, 3H, C5''-C<u>H</u>$_3$.

2-(4-amino-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-7-yl)acetic acid (13b)

Using General procedure F, **11b** (80.00 mg, 0.22 mmol, 1.0 eq) afforded the title compound (40.00 mg, 0.12 mmol, 54%) as a white solid. LRMS (ES^+): *m/z* (%) 350 (100) $[M+H]^+$; ^1H NMR (400 MHz, d_6-DMSO) δ 8.53 (1H, s, C6-<u>H</u>), 8.10 (1H, s, C5'-<u>H</u>), 7.67 (1H, s, C6-<u>H</u>), 7.42-7.36 (5H, m, Ar-<u>H</u>), 5.68 (2H, s, C1''-<u>H</u>), 4.93 (2H, s, C<u>H</u>$_2$).

2-(4-amino-3-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo-[3,4-*d*]pyrimidin-1-yl)acetic acid (14b)

Using General procedure F, **12b** (81.5 mg, 0.22 mmol, 1.0 eq) afforded the title compound (69.00 mg, 0.20 mmol, 91%) as a white solid. LRMS (ES^+): *m/z* (%) 351 (100) $[M+H]^+$; ^1H NMR (400 MHz, d_6-DMSO) δ 9.09 (1H, N<u>H</u>$_2$), 8.81 (1H, s C5'-<u>H</u>), 8.22 (1H, s, C6-<u>H</u>), 8.11 (1H, N<u>H</u>$_2$), 7.41-7.36 (5H, m, Ar-<u>H</u>), 5.71 (2H, s, C1''-<u>H</u>), 5.09 (2H, s, C<u>H</u>$_2$).

Photophysical measurements

The triazole adenine analogues were dissolved in methanol, acetonitrile (**20b**) or dichloromethane (**20b**, **20c**) for all photophysical measurements. Absorption spectra were recorded on a Varian Cary 4000 or 5000 spectrophotometer. Extinction coefficients were determined by dissolving small amounts of the compounds (typically 1.5-3 mg) in a known volume of methanol. Extinction coefficient values reported are averages of at least two measurements (except for **20c**). Emission spectra were recorded on a Horiba Spex fluorolog 3 and corrected for Raman scattering. Quantum yields were determined relative to 2-aminopyridine (Φ_f = 60%) in 0.05 M H_2SO_4 (aq.) at 25°C and an excitation wavelength of 290 nm.[44] Values reported were determined by at least two independent measurements.

Notes and references

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Electronic Supplementary Information (ESI) available: NMR spectra of all new compounds as well as additional photophysical characterisation. See DOI: 10.1039/b000000x/

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