Tetrahedron 69 (2013) 8857–8864

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis and photophysical characterization of 1- and 4-(purinyl) triazoles☆

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ARTICLE INFO

Article history:
Received 31 March 2013
Received in revised form 30 July 2013
Accepted 12 August 2013
Available online 15 August 2013

Keywords:
Purinyltriazole
CuAAc
Fluorescent purines

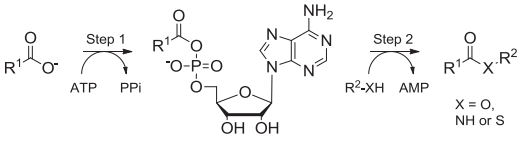
ABSTRACT

Fluorescent adenine mimetics are useful tools for studying DNA, RNA and enzyme functions. Herein we describe the synthesis of eight 1-(purinyl)triazoles and two 4-(purinyl)triazoles utilizing the 1,4-regioselective copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction as the key step. The fluorescence properties of five of the synthesized 1-(purinyl)triazoles are also presented. The five measured compounds displayed low quantum yields. The results, when compared to previously published data, suggest a high influence of the substitution pattern of the triazole on the fluorescence properties.

1. Introduction

Adenylate-forming enzymes are involved in a range of different essential biological processes, such as ribosomal and non-ribosomal peptide synthesis, fatty acid oxidation, and enzyme regulation.[1]

In the first step catalyzed by adenylate-forming enzymes, a carboxylate reacts with ATP to afford an acyl-adenylate intermediate (acyl-AMP) with concomitant release of pyrophosphate (PP_i) (Scheme 1, Step 1). In the second step the reactive intermediate reacts with a nucleophile to form the final product together with a release of AMP (Scheme 1, Step 2). Some of the adenylate-forming enzymes have been regarded as potential drug targets, such as aminoacyl-tRNA synthetases,[2] *Mycobacterium tuberculosis* pantothenate synthetase,[3,4] and aryl acid adenylating enzymes involved in siderophore biosynthesis in *M. tuberculosis*.[5,6] Since the acyl-AMP is assumed to bind tightly to the active site of adenylate-forming enzymes it is envisaged that non-reactive analogues of acyl-AMP could potentially serve as inhibitors of the enzymes. There are several examples in the literature where non-reactive



Scheme 1. The two-step adenylation process catalyzed by adenylate-forming enzymes, which results in the formation of thioesters, amides, and esters.

analogues of acyl-AMP have been synthesized and evaluated as inhibitors of specific adenylate-forming enzymes.[1,2,7–16] One such example is the use of sulfamoyl-adenylate analogues as inhibitors of aminoacyl-tRNA synthetases (Fig. 1, A).[2,16] We have previously reported the design and synthesis of several non-hydrolyzable sulfamoyl analogues of acyl-AMP, which have been used in structural studies of a number of tRNA synthetases.[17–21]

Fluorescence is a useful technique to study macromolecules, such as DNA and RNA. For this purpose artificial base analogues with intrinsic fluorescence are potentially useful tools.[22,23] We have previously reported the synthesis of 4-(adenosinyl)triazoles as fluorescent base analogues utilizing the 1,4-regioselective copper-catalyzed azide–alkyne cycloaddition (CuAAC).[24,25] The use of CuAAC facilitates the variation of substituents in the 1- and 4-positions of the 1,2,3-triazole through the use of a variety of azides/acetylenes. Small fluorescent compounds capable of detecting specific enzyme targets have been shown to be useful for studying enzymatic activation and regulation within the cell. Such

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0040-4020/$ – see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2013.08.023

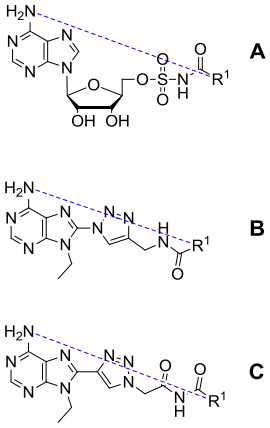


Fig. 1. A. Structures of sulfamoyloxy- and purinyltriazole-based (B and C) analogues of acyl-AMP. Blue dashed lines indicate similarities in the end groups of the compounds in their extended conformations.

tools have been used in enzyme function studies of kinases,[26] ATPase,[27] and glutathione transferase.[28]

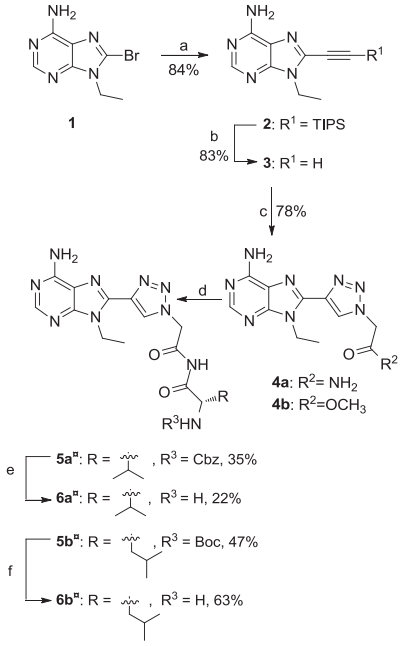
We hypothesized that purinyltriazoles (Fig. 1B and C) may fit into the adenylate-binding site of tRNA synthetases in a manner similar to the sulfamoyl analogues of acyl-AMP (Fig. 1A). Exchanging the ribose unit for a small lipophilic substituent would eliminate the need for the rather extensive protecting group strategies required for ribose-containing structures. These compounds could potentially be used as fluorescent probes to study adenylate-forming enzymes. In this paper we present the synthesis and photophysical characterization of a small series of 1- and 4-purinyltriazoles.

2. Results and discussion

2.1. Synthesis

The initial strategy to obtain aminoacyl bearing 4-purinyltriazoles utilized our previously described route to 8-(1*H*-1,2,3-triazole-4-yl) adenosine derivatives.[24] 8-Bromo-9-ethyladenine (**1**)[29] was synthesized in two steps from adenine. A Sonogashira cross-coupling was performed on **1** to introduce (triisopropylsilyl)acetylene in the 8-position using $Pd(PPh_3)_2Cl_2$ and CuI as catalysts with Amberlite IRA-67 as base (Scheme 2). The desired product **2** was obtained in 84% yield. The TIPS protecting group was removed with polymer-supported fluoride, which enabled work-up by filtration and compound **3** was isolated in 83% yield after purification. Initial attempts to synthesize **3** using (trimethylsilyl)acetylene resulted in in situ deprotection and low yields. The 1,2,3-triazole ring was synthesized using a copper-catalyzed [3+2]-cycloaddition between the alkyne **3** and an appropriate azide. Compound **4a** was isolated by precipitation from water in 78% yield and was used in the next step without further purification.

Initial attempts to prepare imides from **4a** using nitrophenyl activated esters and *n*-BuLi as base, were unsuccessful.[30] Changing the base to sodium hydride (NaH) resulted in acylation of **4a** using Cbz-L-valine 4-nitrophenyl ester or Boc-L-leucine 4-nitrophenyl ester affording **5a** and **5b** in 35% and 47% yield, respectively. The benzyl carbamate (Cbz) protecting group in **5a** was removed using a continuous-flow catalytic hydrogenation reactor (H-cube®) with Pd/C (10 wt % catalyst cartridge) and MeOH as solvent, affording **6a** in 22% yield after purification by preparative HPLC. The low isolated yield of **6a** can be partly attributed to the formation of the methyl ester **4b** (identified by NMR and LC/MS) by nucleophilic attack of MeOH on the imide functionality of **5a**. Compound **5a** could not be



Scheme 2. Synthesis of imide-based compounds. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (20 mol %), Amberlite IRA-67 (5 equiv), ethynyltriisopropylsilane (3.3 equiv), THF, MW 120 °C, 30 min. (b) PS-fluoride (2.4–3.6 equiv, 2–3 mmol/g loading), THF, rt, N_2, 24 h. (c) 1. NaN_3 (1.2 equiv), 2-bromoacetamide (1.1 equiv), DMF, MW 80 °C, 20 min. 2. **3** (1.0 equiv), sodium ascorbate (0.6 equiv), CuI (0.2 equiv), *N*,*N*′-dimethylenediamine (0.3 equiv), MW 80 °C, 2 h. (d) **4a**, NaH (2.0 equiv), R^2-amino acid-ONp (1.1 equiv), THF, 0 °C 15 min then at rt, 3–5 h. (e) H_2/Pd/C (10% CatCart, 30×4 mm, H-cube®, 21 °C, 25 min, MeOH, flow rate: 1 ml/min). (f) 50% TFA in DCM, 1–1.5 h. ¤Unstable compounds.

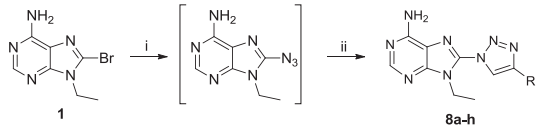
purified by flash chromatography using an eluent containing MeOH, since the same side reaction occurred on the column. It was however not possible to identify a suitable replacement eluent system for this purification. The Boc-protecting group in **5b** was removed using TFA (50% in DCM), resulting in the isolation of **6b** in 63% yield after purification by preparative HPLC. Although, these compounds degraded within days when stored at −10 °C.

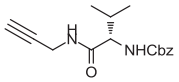
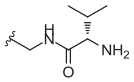
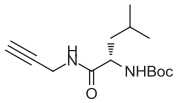
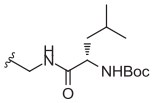
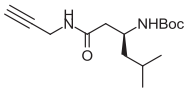
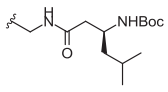
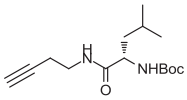
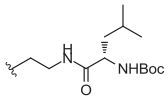
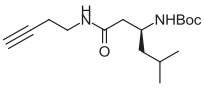
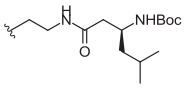
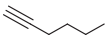
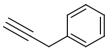
Due to the instability of **6a** and **6b** in the presence of nucleophiles like MeOH as well as on storage, it was decided to prepare compounds in which the imide was replaced by the more stable amide functionality. The existing route to the 4-(purinyl)triazoles involved a Sonogashira coupling on the 8-bromopurine derivative and subsequent desilylation followed by CuAAC with different azides to obtain the purinyltriazoles. Inverting the triazole would enable the cyclization on a range of more easily accessible alkynes. Aminoacyl substituted 1-(purinyl)triazoles would be accessible from a two-step azide formation/cyclization reaction with the alkynylamide of the amino acid and 8-bromo-9-alkyladenines, such as **1**.[29]

As in the case of the 4-(purinyl)triazoles, the synthesis of 1-(purinyl)triazoles started from 8-bromo-9-ethyladenine (**1**).[29] Propargylamides **7a**–**c** and β-alkynylamides **7d**,**e** were synthesized by amide coupling of the corresponding Boc-protected amino acids with propargylamine and 4-butynylamine, respectively (Table 1). Heating **1** and sodium azide at 90 °C for 22 h in DMF with the exclusion of light resulted in the formation of 8-azido-9-ethyladenine (observed by LC/MS). Existing protocols for similar transformations using DMSO failed to provide satisfactory conversions in our hands.[31,32]

Without isolation of the formed azide intermediate, copper(I) iodide (CuI), sodium ascorbate (NaAsc), *N*,*N*′-dimethylethylenediamine (DMEDA), and Cbz-valine-propargylamide were added to the reaction mixture and heated at 90 °C for 24 h. Although ^1H NMR

Table 1
Synthesis of compounds **8b**–**h**



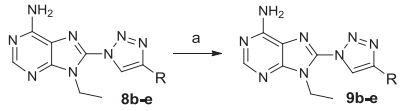
Entry	Alkyne	Alkyne	Compd	R	Yield, %
1	**7a**		**8a**		—[a]
2	**7b**		**8b**		40
3	**7c**		**8c**		40
4	**7d**		**8d**		45
5	**7e**		**8e**		38
6	**7f**		**8f**		48
7	**7g**		**8g**		50
8	**7h**		**8h**		50

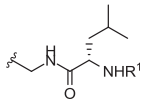
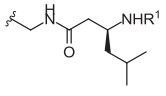
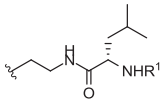
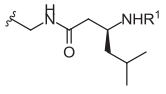
Compounds **8b**–**e** were purified by preparative HPLC while compounds **8f**–**h** were purified by flash chromatography on silica. (i) NaN_3 (1.8 equiv), DMF, 90 °C, 22 h. (ii) Alkyne (1.4 equiv), sodium ascorbate (0.4 equiv), CuI (20 mol %), DMEDA (0.3 equiv), rt, 24 h, in the dark.
[a] No product isolated.

indicated formation of debenzoylated product **8a**, the HRMS data did not correspond with the calculated value. Likewise, the corresponding cyclization using Cbz-leucine-propargylamide (data not shown) did not produce the expected product in satisfying yield. These poor results prompted us to consider an alternative to Cbz-protected propargylamides. Changing the protecting group to Boc and running the cyclization step at room temperature overnight afforded **8b**–**e** in 40–66% yield (Table 1). The protecting group of **8b**–**e** was subsequently removed with TFA in DCM to afford the corresponding trifluoroacetate salts **9b**–**e** in high yields (Table 2). To access substrates more comparable with our previously published fluorescent purinyltriazoles,[24] **1** was reacted with alkynes **7f**–**h** under the same reaction conditions utilized for **7b**–**e**. Triazoles **8f**–**h** were isolated in 48–50% yield. When the reaction was attempted with *N*,*N*-dimethylpropargylamine, low conversion was observed (LCMS) and only trace amounts could be isolated even after prolonged reaction times (data not shown).

The modest yields in these reactions can in part be attributed to the reduction of the azidopurine to the corresponding amine under the cyclization conditions. The amine was observed on LC/MS (data not shown). In situ reduction of azides by excess NaN_3 in the presence of copper has previously been reported.[33] Procedures for synthesis of 8-azido purines reported in the literature generally utilize a large excess of NaN_3[31,32,34] and reducing the amount of NaN_3 below 1.8 equiv resulted in lower conversion of **1** to 8-azidopurine in our case.

Table 2
Deprotection of **8b**–**e**



Entry	R	Compd		Yield, %
1		**8b** R^1=Boc	**9b** R^1=H	94
2		**8c** R^1=Boc	**9c** R^1=H	99
3		**8d** R^1=Boc	**9d** R^1=H	99
4		**8e** R^1=Boc	**9e** R^1=H	71

Reaction conditions: a: TFA/DCM 1:1 (v/v), rt, 1 h.

2.2. Photophysical characterization

Molar absorptivities and quantum yields for five of the synthesized compounds were determined in MeOH (Table 3). All derivatives have similar absorption maxima (between 282 nm and 291 nm) and emission maxima (401–409 nm). The absorption maximum for the derivative with the phenyl group conjugated with the triazole moiety (**8h**) is red-shifted (9 nm) compared to the aliphatic derivatives (**9b**, **9d**, **8f**, **8g**). Furthermore the quantum yield is significantly lower for **8h** compared to the aliphatic and benzylic derivatives. This result is in agreement with our previous study of 8-(1*H*-1,2,3-triazole-4-yl)-adenosine derivatives.[24]

Table 3
Photophysical characterization of absorption and fluorescence properties of compounds **9b**, **d**, **f**, **g**, **h** in MeOH

Compound	Abs_{max} (nm)	Em_{max} (nm)	Φ [%]
9b	283	408	0.55
9d	284	409	0.64
8f	282	401	0.60
8h	282, 291	401	0.21
8g	283	404	0.57

However the aliphatic and benzylic 1-(purinyl)triazole derivatives (**9b**, **9d**, **8f**, **8g**) have significantly lower quantum yields (below 1%) compared to previously published 4-(adenosinyl)triazole derivatives (49–64%).[24] Most notably **8f** and **8g** have the same substituent on the triazole as the 4-(adenosinyl)triazoles, which showed some of the highest quantum yields (62 and 64%, respectively) in our previous study.[24] These results indicate that the position of the nitrogen atoms in the triazole has a significant influence on the quantum yields of these types of compounds. Purines substituted with a triazole in the 2-postion[35] were recently reported to have fluorescent properties with quantum yields of up to 53%, which indicates that the position of the triazole on the purine is also important for the fluorescent properties. As discussed above, the 4-(purinyl)triazoles **6a** and **6b** were not stable in MeOH and their fluorescent properties were therefore not measured.

3. Conclusion

We have synthesized two 4-(purinyl)triazoles and eight 1-(purinyl)triazoles. The key step in the synthesis is the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction. The presented synthesis provides access to the desired products in a two-step reaction from 8-bromopurine and terminal alkynes. The photophysical properties of five of the 1-(purinyl)triazoles were investigated. The compounds display fluorescence properties, albeit with significantly lower quantum yields than previously reported for 4-(adenosinyl)triazoles. A deeper understanding of the results will require further studies and such are currently being undertaken in our laboratory.

4. Experimental section

4.1. General

All commercial chemicals were used without prior purification. DCM was distilled from calcium hydride. THF was distilled from sodium/benzophenone. All reactions were monitored by TLC (Merck silica gel $60F_{254}$) and analyzed under UV (254 nm). Microwave reactions were performed in a Biotage Initiator reactor with fixed hold time. Column chromatography was performed by manual flash chromatography (wet-packed silica, 0.04–0.063 mm) or by automated column chromatography on a Biotage SP-4 instrument using pre-packed silica columns. Analytical high-performance liquid chromatography (HPLC) analysis was carried out on a Waters separation module 2690 connected to a Waters photodiode array detector 996 using an Atlantis® 5 μm C18 AQ (250×4.6 mm) column. Preparative HPLC was carried out on a Waters 600 controller connected to a Waters 2487 Dual λ Absorbance detector using an Atlantis® Prep T3 5 μm C-18 (250×19 mm) column, unless otherwise stated. ^1H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, using a Varian 400/54 spectrometer. IR absorption was measured on a Biotools ChiralIR-2X instrument, optical rotations were measured on a Perkin–Elmer 341LC Polarimeter, and melting points were recorded with a Mettler FP82 heater connected to a Mettler FP80 processor.

4.2. Photophysical measurements

All photophysical measurements were performed in MQ-water or MeOH. Absorption spectra were measured with a Varian Cary 4000 spectrophotometer. Solutions of samples containing adenine analogues in MeOH, which gave absorption of approximately 0.06 at the excitation wavelength (290 nm) were used in all experiments. Steady-state fluorescent measurements were performed with a Spex Fluorolog 3 spectrofluorimeter (JY Horiba). The quantum yields of the adenine analogues were determined relative to 2-aminopyridine (Φ=0.60)[36] in 0.05 M H_2SO_4 at 25 °C with the excitation wavelength of 290 nm.

4.3. Synthesis

*4.3.1. 6-Amino-8-bromo-9-ethyl-9H-purine (**1**).* Following a modified literature procedure,[37] 6-amino-9-ethyl-9*H*-purine was synthesized by the addition of ethyl iodide (3.6 ml, 45 mmol) to a white suspension of adenine (5.00 g, 37.0 mmol) and cesium carbonate (14.5 g, 44.4 mmol) in dry DMF (60 ml) under nitrogen. The suspension was heated at 60 °C for 7 h. A white solid was filtered off, the yellow solution was quenched with water (5 ml) and the solvents were removed under reduced pressure. The yellow solid was partially dissolved in $CHCl_3$ and MeOH and the insoluble material was removed by filtration. Removal of the solvents under reduced pressure resulted in a crude yellow solid that was purified by flash column chromatography (gradient, 2–10% v/v MeOH in $CHCl_3$). 6-Amino-9-ethyl-9*H*-purine was isolated as a white solid (3.97 g, 24.3 mmol, 66%). Mp 191–194 °C (lit. 192–193 °C,[38] 185–187 °C[39]). The ^1H NMR data were consistent with that in the literature.[39] ^1H NMR (DMSO-d_6): 8.14 (s, 1H), 8.13 (s, 1H), 7.16 (br s, 2H), 4.16 (q, *J* 7.3 Hz, 2H), 1.39 (t, *J* 7.3 Hz, 3H); ^{13}C NMR (DMSO-d_6, **1**): 155.9, 152.3, 149.3, 140.4, 118.8, 38.0, 15.3; ν_{max} (DMSO) 3250, 2250, 2120, 1640 cm^{-1}; HRMS *m*/*z* $[M+H]^+$ calculated for $C_7H_9N_5$: 164.0936. Found: 164.0935. The regioisomer (6-amino-7-ethyl-7*H*-purine) was also isolated as a white solid (0.60 g, 3.68 mmol, 10%).

Following a modified literature procedure,[37] bromine (15 ml) was slowly added to an ice-cold solution of 6-amino-9-ethyl-9*H*-purine (5.86 g, 35.9 mmol) in a HOAc buffer (pH ~ 4, 20 ml), THF (20 ml), and MeOH (20 ml). The reaction mixture was stirred at room temperature for 24 h, then cooled on ice, and quenched with an aqueous sodium metabisulfite and sodium thiosulfate solution (10 wt %). The mixture was neutralized with an aqueous NaOH solution (3 M), which resulted in the precipitation of a solid. The solid was filtered off, washed with water, and dried overnight yielding a pale yellow solid, which was purified by column chromatography (gradient, 0–10% MeOH in $CHCl_3$), which gave **1** as an off-white solid (5.39 g, 62%). Mp 221–225 °C (lit. 218–220 °C[39]). The ^1H NMR data were consistent with that in the literature.[39] ^1H NMR (DMSO-d_6): 8.13 (s, 1H), 7.36 (br s, 2H), 4.15 (q, *J* 7.2 Hz, 2H),

1.31 (t, *J* 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6): 154.7, 152.8, 150.4, 125.9, 119.1, 38.9, 14.6; ν_{max} (DMSO) 3400, 3250, 3000, 2900, 2250, 2120, 1710 cm^{-1}; HRMS *m*/*z* [M+H]$^+$ calculated for $C_7H_8N_5Br$: 242.0041. Found: 242.0042.

*4.3.2. 6-Amino-9-ethyl-8-(2-triisopropylsilylethynyl)-9H-purine (**2**).* Compound **1** (100 mg, 0.41 mmol), $Pd(PPh_3)_2Cl_2$ (14.7 mg, 0.0209 mmol), CuI (15.7 mg, 0.0824 mmol) were added to a microwave vial followed by THF (2 ml) and Amberlite IRA-67 (362 mg, 2.03 mmol). The vial was capped and nitrogen was bubbled through the reaction mixture. (Triisopropylsilyl)acetylene (300 μl, 1.34 mmol) was added and the reaction mixture was heated in a microwave reactor (120 °C, 30 min). The reaction mixture was filtered through a short plug of silica, eluted with 10% MeOH in $CHCl_3$ and the solvents were removed to produce a pale pink solid, which was purified by automated flash column chromatography (gradient, 2–10% MeOH in $CHCl_3$). Compound **2** was isolated as a white solid (119 mg, 0.35 mmol, 84%). Mp 167 °C; ^{1}H NMR ($CDCl_3$): 8.34 (s, 1H), 6.17 (br s, 2H), 4.34 (q, *J* 7.2 Hz, 2H), 1.46 (t, *J* 7.2 Hz, 3H), 1.27–1.05 (m, 21H); ^{13}C NMR ($CDCl_3$): 155.5, 153.9, 149.5, 134.5, 119.4, 100.2, 94.7, 38.8, 18.7, 15.3, 11.3; ν_{max}(DMSO) 3250, 3000, 2250, 2120 cm^{-1}; HRMS *m*/*z* [M+H]$^+$ calculated for $C_{18}H_{29}N_5Si$: 344.2271. Found: 344.2273.

*4.3.3. 6-Amino-9-ethyl-8-ethynyl-9H-purine (**3**).* Polymer-supported fluoride (2.0 g, 2–3 mmol/g loading) was added to a solution of **2** (0.580 g, 1.69 mmol) in THF (10 ml). The reaction mixture was stirred at room temperature for 24 h, after which a white precipitate had formed. The reaction mixture was diluted in MeOH (the precipitate dissolved), and the polymer was filtered off and washed with MeOH. Removal of the solvents yielded a yellow solid that was purified by automated flash column chromatography (gradient, 2–10% MeOH in $CHCl_3$). Compound **3** was isolated as a white solid (261 mg, 1.39 mmol, 83%). The ^{1}H NMR data were consistent with published data for this compound.[40] Mp decomposes at 210 °C (lit.>200 °C ignition[40]); ^{1}H NMR (DMSO-d_6): 8.18 (s, 1H), 7.46 (br s, 2H), 4.92 (s, 1H), 4.22 (q, *J* 7.2 Hz, 2H), 1.35 (t, *J* 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6): 155.9, 153.8, 148.9, 132.0, 118.5, 86.4, 73.0, 38.1, 14.9; ν_{max} (DMSO) 3250, 3000, 2250, 2120 cm^{-1}. HRMS *m*/*z* [M+H]$^+$ calculated for $C_9H_9N_5$: 188.0936. Found: 188.0930.

*4.3.4. 2-(4-(6-Amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-1-yl) acetamide (**4a**).* NaN_3 (49 mg, 0.75 mmol), DMF (4 ml), and 2-bromoacetamide (97 mg, 0.70 mmol) were added to a microwave vial that was flushed with nitrogen and the reaction mixture was heated in a microwave reactor (80 °C, 20 min). Compound **3** (120 mg, 0.64 mmol), sodium ascorbate (52 mg, 0.41 mmol), and CuI (24 mg, 0.13 mmol) were added followed by *N*,*N*′-dimethylenediamine (18 μl, 0.17 mmol). The reaction mixture was heated in a microwave reactor (80 °C, 2 h) and allowed to reach room temperature before being poured into water (10 ml). The product precipitated and was filtered off, washed thoroughly with ice-cold water, and dried under vacuum to yield **4a** as an off-white solid (143 mg, 0.50 mmol, 78%). Mp 299–300 °C; ^{1}H NMR (DMSO-d_6): 8.61 (s, 1H), 8.19 (br s, 1H), 7.82 (s, 1H), 7.49 (s, 1H), 7.29 (br s, 2H), 5.24 (s, 2H), 4.63 (q, *J* 6.9 Hz, 2H), 1.38 (t, *J* 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6): 167.0, 155.6, 152.6, 150.5, 140.9, 138.8, 127.2, 118.7, 51.7, 38.5, 15.3; ν_{max} (DMSO) 3250, 3000, 2910, 2250, 2120 cm^{-1}; HRMS *m*/*z* [M+H]$^+$ calculated for $C_{11}H_{13}N_9O$: 288.1321. Found: 288.1317.

*4.3.5. N-(2-(4-(6-Amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-1-yl)acetyl)-2-benzyloxycarbonylamino-3-methylbutanamide (**5a**).* NaH (14.5 mg, 0.35 mmol, 60% mineral dispersion) was added to **4a** (50.0 mg, 0.17 mmol), which was suspended in THF (6 ml) at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for 15 min, then allowed to reach room temperature and stirred for 45 min. Cbz-L-valine 4-nitrophenyl ester (71.3 mg, 0.19 mmol) was dissolved in THF (1.5 ml) and added dropwise to the bright yellow reaction mixture at 0 °C under nitrogen, the mixture was stirred at that temperature for 15 min and then at room temperature for 5 h. The reaction was quenched by the addition of 1 M HCl (1 ml), the layers were separated and the aqueous layer was extracted with EtOAc (2×5 ml). The combined organic layers were washed with aqueous saturated $NaHCO_3$ (5 ml), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified using automated flash chromatography (gradient, 2–20% MeOH in $CHCl_3$) and preparative HPLC (H_2O/AcCN (0.1% TFA) 100:0 to 0:100 for 36 min, then at 0:100 for 15 min, with a flow of 14 ml/min). The desired compound **5a** was obtained as a white crystalline material (32.0 mg, 0.06 mmol, 35%). However, **5a** was unstable and ^{13}C NMR could not be obtained. ^{1}H NMR ($CDCl_3$/MeOH-d_4): 8.46 (s, 1H), 8.26 (s, 1H), 7.30–7.15 (m, 5H), 5.72 (s, 2H), 5.03 (s, 2H), 4.97–4.72 (m, 2H), 4.30–3.71 (m, 3H), 2.53–1.72 (m, 1H), 1.45 (t, *J* 6.6 Hz, 3H), 1.01–0.81 (m, 6H).

*4.3.6. N-(2-(4-(6-Amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-1-yl)acetyl)-2-tert-butoxycarbonylamino-4-methylpentanamide (**5b**).* NaH (14.5 mg, 0.35 mmol) was added to **4a** (50 mg, 0.17 mmol), which was suspended in THF (5 ml) at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for 15 min then allowed to reach room temperature and stirred for another 45 min. Boc-L-leucine 4-nitrophenyl ester (67.5 mg, 0.19 mmol) was dissolved in THF (1.5 ml) and added dropwise to the bright yellow reaction mixture at 0 °C under nitrogen, and stirred at that temperature for 15 min and then at room temperature for 3 h. The reaction was quenched by the addition of 1 M HCl (1 ml), the phases were separated and the aqueous layer was extracted with EtOAc (2×5 ml). The combined organic phases were washed with an aqueous saturated $NaHCO_3$ (5 ml), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified using automated flash chromatography (gradient, 2–20% MeOH in $CHCl_3$) and preparative HPLC (H_2O/AcCN (0.1%TFA) 100:0 to 0:100 for 36 min, then at 0:100 for 15 min, with a flow of 14 ml/min). The desired compound **5b** was obtained as a white crystalline material (41.0 mg, 0.08 mmol, 47%). However, **5b** was unstable and ^{13}C NMR could not be obtained. ^{1}H NMR ($CDCl_3$/MeOH-d_4): 8.37 (s, 1H), 8.15 (s, 1H), 5.62 (s, 2H), 4.71 (q, *J* 7.1 Hz, 2H), 4.15–4.06 (m, 1H), 1.62–1.53 (m, 1H), 1.41–1.38 (m, 2H), 1.35 (t, *J* 7.1 Hz, 3H), 1.28 (s, 9H), 0.80 (t, *J* 6.5 Hz, 6H).

*4.3.7. 2-Amino-N-(2-(4-(6-amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylbutanamide (**6a**).* Compound **5a** (47 mg, 0.09 mmol) was dissolved in MeOH (5 ml) and catalytic hydrogenation was carried out in a continuous-flow hydrogenation reactor (H-cube®) (flow rate: 1 ml/min; temperature: 21 °C; catalyst: 10% Pd/C CatCart, 30×4 mm; time: 25 min). The solvent was removed under reduced pressure and the desired product was purified by preparative HPLC (H_2O/AcCN (0.1%TFA) 100:0 to 0:100 for 36 min, then at 0:100 for 15 min, with a flow of 14 ml/min). Compound **6a** was obtained as a white crystalline material (8.0 mg, 0.02 mmol, 22%). However, **6a** was unstable and ^{13}C NMR could not be obtained. ^{1}H NMR (MeOH-d_4): 8.66 (s, 1H), 8.39 (br s, 2H), 5.98—5.80 (m, 6H), 5.09 (br s, 1H), 4.66–4.55 (m, 2H), 1.90–1.80 (m, 1H), 1.50 (d, *J* 7.2 Hz, 3H), 0.92 (d, *J* 7.6 Hz, 3H), 0.66 (d, *J* 6.5 Hz, 3H).

*4.3.8. 2-Amino-N-(2-(4-(6-amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-1-yl)acetyl)-4-methylpentanamide (**6b**).* Compound **5b** (30.0 mg, 0.06 mmol) was dissolved in DCM (1 ml) and TFA (1 ml) was added dropwise. The reaction mixture was stirred for 1 h at room temperature. TFA was removed using a stream of nitrogen, and the solvent was removed under reduced pressure. The crude

product was purified by preparative HPLC (H_2O/AcCN (0.1%TFA) at 100:0 to 0:100 for 36 min, then at 0:100 for 15 min, with a flow of 14 ml/min). The desired compound **6b** was obtained as a white crystalline material (15.0 mg, 0.04 mmol, 63%). However, **6b** was unstable and ^{13}C NMR could not be obtained. ^1H NMR (DMSO-d_6): 8.40 (s, 1H), 8.38 (br s, 2H), 8.07 (s, 1H), 5.92 (s, 2H), 4.71–4.61 (m, 2H), 3.72–3.62 (m, 1H), 1.67–1.62 (m, 1H), 1.56–1.50 (m, 3H), 1.45–1.35 (m, 2H), 0.91–0.88 (m, 6H).

*4.3.9. N-Benzyloxycarbonyl-L-valylpropargylamide (**7a**).*[41] Propargylamine (0.14 ml, 2.19 mmol), *N*-Cbz-L-valine (509 mg, 2.03 mmol), HOBt (489 mg, 3.19 mmol), EDC·HCl (588 mg, 3.07 mmol), and Et_3N (0.6 ml, 4.30 mmol) were added to DMF (8.5 ml) and the reaction mixture was stirred under nitrogen at room temperature for 3 days. The reaction mixture was partitioned between water (50 ml) and EtOAc (50 ml). The aqueous phase was extracted with EtOAc (2×50 ml), and the organic phases were collected and washed with 1 M HCl (2×10 ml), water (10 ml), aqueous saturated $NaHCO_3$ (2×10 ml), and brine. The solution was dried over Na_2SO_4 and the solvents were removed to yield an off-white solid (515 mg, 1.79 mmol, 89%), which was recrystallized from MeOH to yield the expected product as white, needle-shaped crystals (321 mg, 1.11 mmol, 55%). Mp 171–174 °C (lit. ref. not found); $[\alpha]_D^{20}$ −9.6 (*c* 0.4, $CHCl_3$) ^1H NMR ($CDCl_3$): 7.42–7.28 (m, 5H), 6.39 (br s, 1H), 5.40 (br d, 1H), 5.13 (d, *J* 12.4 Hz, 1H), 5.10 (d, *J* 12.3 Hz, 1H), 4.15–3.94 (m, 3H), 2.21 (t, 1H), 2.14 (app. h, *J* 6.7 Hz, 1H), 0.97 (d, *J* 6.8 Hz, 3H), 0.93 (d, *J* 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): 171.2, 156.6, 136.2, 128.7, 128.4, 128.2, 79.3, 71.9, 67.3, 60.5, 31.2, 29.3, 19.3, 18.0. ν_{max} ($CHCl_3$) 3435, 3252, 3070, 2999, 2912, 2251, 2124, 1996, 1832, 1767, 1677, 1620 cm^{-1}. HRMS *m*/*z* $[M+H]^+$ calculated for $C_{16}H_{21}N_2O_3$: 289.1547 Found: 289.1551.

*4.3.10. N-tert-Butoxycarbonyl-L-leucinylpropargylamide (**7b**).*[42] Compound **7b** was synthesized according to a literature procedure and analytical data were in agreement with the literature.

*4.3.11. General procedure A for N-tert-butoxycarbonyl-leucinyl and homoleucinyl alkyne derivatives (**7c**–**e**).* Alkynylamine (1.5 equiv) was added to *N*-Boc-L-leucine or *N*-Boc-L-β-homoleucine (1 equiv) and HATU (1.1 equiv) in DMF at room temperature under nitrogen. The solutions were cooled on ice and Et_3N (3 equiv) was added, the reaction mixture was then allowed to reach room temperature and stirred for 22 h. The reaction mixture was diluted with EtOAc (30 ml), washed with 0.1 M HCl (2×10 ml), saturated $NaHCO_3$ (10 ml), brine (20 ml), dried over Na_2SO_4 and the solvents were removed. The crude product was purified by automated flash chromatography (gradient, 0–100% EtOAc in heptane).

*4.3.12. N-tert-Butoxycarbonyl-L-homoleucinylpropargylamide (**7c**).* Following general procedure A using propargylamine (40 μl, 0.62 mmol) and *N*-Boc-L-homoleucine (100 mg, 0.62 mmol) as substrates in DMF (4 ml). *N-tert*-Butoxycarbonyl-L-homoleucinylpropargylamide (**7c**) was isolated as a white solid (102 mg, 0.36 mmol, 88%). Mp 114–116 °C; $[\alpha]_D^{20}$ −47.7 (*c* 0.03, DMSO); ^1H NMR ($CDCl_3$): 6.92 (br s, 1H), 5.19 (br d, *J* 9.2 Hz, 1H), 3.96 (dd, *J* 5.3, 2.6 Hz, 2H), 3.93–3.84 (m, 1H), 2.46–2.28 (m, 2H), 2.16 (t, *J* 2.5 Hz, 1H), 1.68–1.51 (m, 1H), 1.37 (s, 9H), 1.29–1.17 (m, 1H), 0.85 (dd, *J* 6.6, 2.6 Hz, 6H). ^{13}C NMR ($CDCl_3$): 171.1, 155.9, 79.7, 79.3, 71.3, 46.3, 43.9, 41.8, 29.1, 28.4, 24.9, 23.0, 22.1; ν_{max} (DMSO) 3261, 2957, 1703, 1668, 1535 cm^{-1}; HRMS *m*/*z* $[M+H]^+$ calculated for $C_{15}H_{26}N_2O_3$: 283.2021. Found: 283.2013.

*4.3.13. N-tert-Butoxycarbonyl-L-leucinylbutynylamide (**7d**).* Following general procedure A using 3-butynylamine (0.16 ml, 2.0 mmol) and *N*-Boc-L-leucine (300 mg, 1.3 mmol) as substrates in DMF (6 ml). *N-tert*-Butoxycarbonyl-L-leucinylbutynylamide (**7d**) was isolated as a white solid (0.24 g, 0.85 mmol, 66%). Mp 106 °C; $[\alpha]_D^{20}$ −43.0 (*c* 0.1, DMSO); ^1H NMR ($CDCl_3$): 6.85 (br s, 1H), 5.16 (br s, 1H), 4.11 (br s, 1H), 3.47–3.25 (m, 2H), 2.35 (br s, 2H), 1.95 (br s, 1H), 1.73–1.31 (m, 12H), 0.89 (t, *J* 5.7 Hz, 6H); ^{13}C NMR ($CDCl_3$): 173.0, 155.8, 81.4, 80.0, 70.0, 53.1, 41.5, 38.1, 28.4, 24.8, 23.0, 22.1, 19.4; ν_{max} (DMSO) 3370, 2940, 2820 cm^{-1}; HRMS *m*/*z* $[M+H]^+$ calculated for $C_{15}H_{26}N_2O_3$: 283.2021. Found: 283.2016.

*4.3.14. N-tert-Butoxycarbonyl-L-homoleucinylbutynylamide (**7e**).* Following general procedure A using 3-butynylamine (0.15 ml, 1.8 mmol) and *N*-Boc-L-homoleucine (300 mg, 1.2 mmol) as substrates in DMF (6 ml). *N-tert*-Butoxycarbonyl-L-homoleucinylbutynylamide (**7e**) was isolated as a white solid (0.24 g, 0.81 mmol, 66%). Mp 121 °C; $[\alpha]_D^{20}$ −80.0 (*c* 0.1, DMSO); ^1H NMR ($CDCl_3$): 6.69 (br s, 1H), 5.22 (br s, 1H) 3.96–3.83 (m, 1H),3,38–3.28 (m, 2H), 2.41–2.29 (m, 4H), 1.95 (t, *J* 2.5 Hz, 1H), 1.67–1.33 (m, 11H), 1.29–1.17 (m, 1H), 0.89 (dd, *J* 6.6, 1.7 Hz, 6H); ^{13}C NMR ($CDCl_3$): 171.5, 155.9, 81.6, 79.3, 70.0, 46.4, 43.9, 42.1, 38.2, 28.4, 25.0, 23.0, 22.1, 19.4; ν_{max} (DMSO) 3260, 2360, 1650, 1560, 1510 cm^{-1}; HRMS *m*/*z* $[M+H]^+$ calculated for $C_{16}H_{28}N_2O_3$: 297.2178. Found: 297.2174.

*4.3.15. General procedure B 1-(Purinyl)triazoles (**8b**–**h**).* NaN_3 (1.8–2.0 equiv) was added to **1** (1 equiv) in DMF in a microwave vial. The vial was capped, flushed with nitrogen and the reaction mixture was stirred for 22 h at 90 °C with exclusion of light. The reaction mixture was allowed to reach room temperature and opened. Compounds **7b**–**h** (1.2–1.4 equiv) in DMF, CuI (20 mol %), sodium ascorbate (0.4 equiv), and *N,N′*-dimethylenediamine (0.3 equiv) were added. The microwave vial was recapped, flushed with nitrogen, and stirred at room temperature for 22–24 h with exclusion of light. The reaction mixture was then poured into water (20 ml) and extracted with EtOAc (3×30 ml) (Caution! Should be kept slightly basic to avoid hydrazoic acid formation from excess NaN_3). The organic phases were collected and dried over Na_2SO_4 before the solvents were removed and the crude product was purified by preparative HPLC (isocratic H_2O/AcCN 50:50, 0.1% TFA) unless otherwise mentioned.

*4.3.16. N-(1(1-(6-Amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)methyl)-2-tert-butoxycarbonylamino-4-methynylpentanamide (**8b**).* Following general procedure B, using NaN_3 (20 mg, 0.31 mmol), **1** (50 mg, 0.21 mmol), and **7b** (70 mg, 0.25 mmol) as substrates in DMF (3 ml). Compound **8b** was isolated as a white solid (31 mg, 40%). Mp 97–102 °C; $[\alpha]_D^{20}$ −22.1 (*c* 0.03, DMSO); ^1H NMR ($CDCl_3$): 8.40 (s, 1H), 8.33 (s, 1H), 7.96 (br s, 1H), 7.61 (br s, 1H), 5.10 (d, *J* 8.0 Hz, 1H), 4.89 (dd, 1H), 4.71 (q, *J* 7.1 Hz, 2H), 4.46 (d, 1H), 4.27 (m, 1H), 1.79–1.51 (m, 3H), 1.48 (t, *J* 7.1 Hz, 3H), 1.36 (s, 9H), 0.96 (d, 3H), 0.93 (d, 3H); ^{13}C NMR ($CDCl_3$): 173.6, 156.4, 151.7, 149.7, 145.0, 141.7, 123.1, 116.9, 80.8, 77.4, 53.5, 41.5, 35.3, 28.4, 24.9, 23.1, 21.9, 15.2; ν_{max} (DMSO) 3431, 3262, 3001, 2959, 2914, 1695, 1606, 1541 cm^{-1}; HRMS *m*/*z* $[M+H]^+$ calculated for $C_{21}H_{32}N_{10}O_3$: 473.2737. Found: 473.2737.

*4.3.17. N-(2(1-(6-Amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)methyl)-tert-butoxycarbonylamino-5-methylhexanamide (**8c**).* Following general procedure B using NaN_3 (28 mg, 0.43 mmol), **1** (70 mg, 0.29 mmol), and **7c** (90 mg, 0.32 mmol) as substrates in DMF (3 ml). Compound **8c** was isolated as a white solid (56 mg, 0.12 mmol, 40%). Mp 206–207 °C; $[\alpha]_D^{20}$ −21.0 (*c* 0.1, DMSO); ^1H NMR ($CDCl_3$): 8.41 (s,1H), 8.39 (s, 1H), 7.02 (br s, 1H), 6.03 (br s, 2H), 5.14 (d, *J* 8.8 Hz, 1H), 4.69–4.56 (m, 4H), 4.01–3.87 (m, 1H), 2.57–2.38 (m, 2H), 1.64 (sept, *J* 6.8 Hz, 1H), 1.52–1.20 (m, 14H), 0.89 (d, *J* 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$): 171.7, 156.1, 155.2,

153.2, 150.7, 145.3, 140.1, 123.5, 117.4, 79.7, 46.5, 44.1, 42.4, 40.2, 35.0, 28.5, 25.0, 23.1, 22.1, 15.3; ν_{max} (DMSO) 3447, 3275, 1701, 1654, 1604, 1533 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{22}H_{34}N_{10}O_3$: 487.2893. Found: 487.2877.

*4.3.18. N-(1(1-(6-Amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)ethyl)-2-tert-butoxycarbonylamino-4-methylpentanamide (**8d**).* Following general procedure B using NaN_3 (27 mg, 0.41 mmol), **1** (50 mg, 0.21 mmol), and **7d** (70 mg, 0.25 mmol) as substrates in DMF (3 ml). Compound **8d** was isolated as a white solid (45 mg, 0.10 mmol, 45%). Mp 128 °C; $[\alpha]_D^{20}$ −27.0 (*c* 0.1, DMSO); ^1H NMR ($CDCl_3$): 10.84 (br s, 1H), 8.31 (s, 1H), 8.21 (s, 1H), 7.82 (br s, 1H), 7.41 (br s, 1H), 5.24 (br s, 1H) 4.77–4.61 (m, 2H), 4.08–3.99 (m, 1H), 3.88–3.70 (m, 1H), 3.54–3.39 (m, 1H), 3.07 (t, *J* 5.9 Hz, 2H), 1.68–1.26 (m, 15H), 0.89 (dd, *J* 15.9, 6.3 Hz, 6H); ^{13}C ($CDCl_3$): 174.0, 156.3, 151.2, 149.4, 146.0, 144.4, 122.9, 116.7, 80.5, 53.3, 41.7, 41.6, 38.8, 28.4, 25.6, 24.8, 23.0, 21.8, 15.4; ν_{max} (DMSO) 3370, 2940, 2820, 1700 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{22}H_{34}N_{10}O_3$: 487.2810. Found: 487.2878.

*4.3.19. N-(2(1-(6-Amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)ethyl)-3-tert-butoxycarbonylamino-5-methylhexanamide (**8e**).* Following general procedure B using NaN_3 (27 mg, 0.41 mmol), **1** (51 mg, 0.21 mmol), and **7e** (75 mg, 0.25 mmol) as substrates in DMF (3 ml). Compound **8e** was isolated as a white solid (40 mg, 0.10 mmol, 38%). Mp 143 °C; $[\alpha]_D^{20}$ −30.0 (*c* 0.1, DMSO); ^1H NMR ($CDCl_3$): 8.40 (s, 1H), 8.24 (s, 1H), 6.57 (br s, 1H), 5.80 (br s, 2H), 5.18 (br s, 1H), 4.63 (q, *J* 14.0, 7.0 Hz, 2H) 3.94–3.83 (m, 1H), 3.73–3.59 (m, 2H), 3.04 (t, *J* 6.5 Hz, 1H), 2.50–2.33 (m, 2H), 1.67–1.53 (m, 1H), 1.52–1.19 (m, 14H), 0.86 (d, *J* 6.4 Hz, 6H); ^{13}C NMR ($CDCl_3$): 171.4, 156.0, 155.4, 153.8, 150.8, 145.7, 140.2, 122.5, 117.4, 79.5, 46.6, 44.0, 42.2, 40.2, 38.4, 28.5, 25.8, 25.1, 23.1, 22.2, 15.3; ν_{max} (DMSO) 3370, 2940, 2820, 1700 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{23}H_{36}N_{10}O_3$: 501.3050. Found: 501.3036.

*4.3.20. 9-Ethyl-8-(4-butyl-1H-1,2,3-triazol-1-yl)-9H-purin-6-amine (**8f**).* Following general procedure B using NaN_3 (28 mg, 0.43 mmol), **1** (70 mg, 0.29 mmol), and 1-hexyne (36 μl, 26 mg, 0.32 mmol) as substrates in DMF (3 ml). Compound **8f** was isolated as a white solid (40 mg, 0.14 mmol, 48%) after purification by automated flash chromatography (gradient, 0–10% MeOH in $CHCl_3$). Mp 112 °C; ^1H NMR ($CDCl_3$): 8.39 (s, 1H), 8.10 (t, *J* 0.8 Hz, 1H), 6.21 (br s, 2H), 4.63 (q, *J* 7.1 Hz, 2H), 2.80 (t, *J* 7.7 Hz, 2H), 1.76–1.67 (m, 2H), 1.48–1.35 (m, 5H), 0.93 (t, *J* 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$): 155.2, 153.2, 150.8, 148.7, 140.6, 121.4, 117.4, 40.3, 31.2, 25.2, 22.4, 15.3, 13.9; ν_{max} (DMSO) 3450, 3250, 3070, 3000, 2250, 2120, 2000, 1830, 1770, 1620 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{13}H_{18}N_8$: 287.1649. Found: 287.1719.

*4.3.21. 9-Ethyl-8-(4-benzyl-1H-1,2,3-triazol-1-yl)-9H-purin-6-amine (**8g**).* Following general procedure B using NaN_3 (28 mg, 0.44 mmol), **1** (70 mg, 0.29 mmol), and 3-phenyl-1-propyne (37 mg, 0.32 mmol) as substrates in DMF (3 ml). Compound **8g** was isolated as a white solid (46 mg, 0.14 mmol, 50%) after purification by automated flash chromatography (gradient, 0–10% MeOH in $CHCl_3$). Mp 193 °C; ^1H NMR ($CDCl_3$): 8.39 (br s, 1H), 8.01 (br s, 1H), 7.32–7.23.61 (m, 5H), 6.16 (s, 2H), 4.63 (q, *J* 7.1 Hz, 2H), 4.18 (br s, 2H) 1.46 (t, *J* 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): 155.2, 153.1, 150.7, 148.1, 140.3, 138.1, 128.9, 128.9, 127.0, 122.3, 117.3, 40.3, 32.1, 25.6, 15.3; ν_{max} (DMSO) 3250, 2250, 2120, 2000, 1770, 1710, 1620 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{16}H_{16}N_8$: 321.1492. Found: 321.1560.

*4.3.22. 9-Ethyl-8-(4-phenyl-1H-1,2,3-triazol-1-yl)-9H-purin-6-amine (**8h**).* Following general procedure B using NaN_3 (28 mg, 0.44 mmol), **1** (70 mg, 0.29 mmol), and phenylacetylene (32 mg, 0.32 mmol) as substrates in DMF (3 ml). Compound **8h** was isolated as a white solid (44 mg, 0.14 mmol, 50%) after purification by automated flash chromatography (gradient, 0–10% MeOH in $CHCl_3$). Mp 178 °C; ^1H NMR (DMSO-d_6): 9.28 (br s, 1H), 8.31 (s, 1H), 8.02 (d, *J* 7.3 Hz, 2H), 7.61 (br s, 1H), 7.51 (t, *J* 7.6 Hz, 2H), 7.43 (t, *J* 7.4 Hz, 1H), 4.32 (q, *J* 7.2 Hz, 2H), 1.36 (t, *J* 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6): 155.8, 155.7, 153.5, 149.9, 146.7, 139.0, 129.3, 129.1, 128.7, 122.7, 169.5, 38.9, 14.9; ν_{max} (DMSO) 3250, 2250, 2120, 2000, 1770, 1710, 1620 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{15}H_{14}N_8$: 307.1336. Found: 307.1407.

*4.3.23. General procedure C for Boc deprotection of **8b**–**e**.* To a suspension of **8b**–**e** in DCM (1 ml), TFA (1 ml) was added. The solutions were stirred at room temperature for 1 h. The solvents and TFA were then removed and the product was freezedried ×3 from MQ-water to yield the trifluoroacetate salt of **9b**–**e**.

*4.3.24. 2-Amino-N-((1-(6-amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)methyl)-4-methylpentanamide (**9b**).* Following procedure C with **8b** (14.4 mg, 0.031 mmol) **9b** was obtained as a white solid (14.0 mg, 0.029 mmol, 94%). Mp 123 °C; $[\alpha]_D^{20}$ −18.8 (*c* 0.03, DMSO); ^1H NMR (CD_3CN): 8.42 (s, 1H), 8.34 (s, 1H), 8.25 (br t, *J* 5.7 Hz, 1H),7.82 (br s, 4H), 4.64 (dd, *J* 6.1, 15.5 Hz, 1H), 4.49 (q, *J* 7.1 Hz, 2H), 4.43 (dd, *J* 5.3, 15.6 Hz, 1H), 4.09 (t, *J* 7.0 Hz, 1H), 1.78–1.63 (m, 3H), 1.39 (t, *J* 7.2 Hz, 3H), 0.93 (d, *J* 2.8 Hz, 3H), 0.91 (d, *J* 2.8 Hz, 3H); ^{13}C NMR (CD_3CN): 170.5, 154.2, 151.0, 149.2, 146.3, 142.1, 124.7, 53.2, 41.3, 41.2, 35.8, 25.1, 22.8, 22.2, 15.2; ν_{max} (DMSO) 3447, 3263, 1693, 1653, 1558, 1541 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{16}H_{24}N_{10}O$: 373.2213. Found: 373.2188.

*4.3.25. 3-Amino-N-(2-(1-(6-amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)methyl)-5-methylhexanamide (**9c**).* Following procedure C with **8c** (17 mg, 0.035 mmol), **9c** was obtained as white solid (18 mg, 0.035 mmol, 99%). Mp 96 °C; $[\alpha]_D^{20}$ −62.8 (*c* 0.03, DMSO); ^1H NMR (CD_3CN): 8.42 (s, 1H), 8.35 (s, 1H), 7.90–7.62 (m, 4H), 4.62–4.45 (m, 4H), 3.65–3.55 (m, 1H), 2.68 (dd, *J* 16.4, 3.7 Hz, 1H), 2.59 (dd, *J* 16.4, 8.8 Hz, 1H), 1.69 (sept, *J* 6.7 Hz, 1H), 1.62–1.44 (m, 2H), 1.40 (t, *J* 7.2 Hz, 3H), 0.90 (t, *J* 6.4 Hz, 6H); ^{13}C NMR (CD_3CN): 172.6, 162.5, 153.7, 150.9, 148.4, 146.4, 142.4, 124.7, 49.0, 42.2, 41.4, 36.6, 35.3, 24.9, 22.7, 22.2, 15.2. ν_{max} (DMSO) 3449, 3298, 1696, 1653, 1558, 1541 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{17}H_{26}N_{10}O$: 387.2369. Found: 387.2374.

*4.3.26. 2-Amino-N-(2-(1-(6-amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)ethyl)-4-methylpentanamide (**9d**).* Following procedure C with **8d** (40 mg, 0.08 mmol), **9d** was obtained as white solid (38 mg, 0.08 mmol, 99%). Mp 173 °C; $[\alpha]_D^{20}$ −23.0 (*c* 0.1, DMSO); ^1H NMR (CD_3CN): 8.40 (s, 1H), 8.36 (s, 1H), 7.89 (br s, 3H), 4.52 (q, *J* 7.1 Hz, 2H), 3.93 (br s, 1H), 3.67–3.49 (m, 2H), 3.00 (t, *J* 6.4 Hz, 2H), 1.71–1.54 (m, 3H), 1.41 (t, *J* 7.1 Hz, 3H), 0.87 (d, *J* 3.6 Hz, 6H); ^{13}C NMR ($CDCl_3$): 170.4, 152.7, 150.7, 146.6, 146.4, 142.9, 124.5, 117.7, 53.2, 41.6, 41.1, 39.6, 25.9, 25.1, 22.7, 22.2, 15.2; ν_{max} (DMSO) 3360, 2940, 2820, 2510, 1870, 1830, 1770, 1690, 1650, 1610, 1540, 1510 cm^{-1}; HRMS *m/z* $[M+H]^+$ calculated for $C_{17}H_{26}N_{10}O$: 387.2286. Found: 387.2377.

*4.3.27. 3-Amino-N-(2-(1-(6-amino-9-ethyl-9H-purine-8-yl)-1H-1,2,3-triazol-4-yl)ethyl)-5-methylhexanamide (**9e**).* Following procedure C with **8e** (35 mg, 0.07 mmol), **9e** was obtained as white solid (34 mg, 0.05 mmol, 71%). Mp 183 °C; $[\alpha]_D^{20}$ −50.0 (*c* 0.1, DMSO); ^1H NMR (CD_3CN): 8.36 (s, 2H), 7.82 (s, 1H), 7.82 (br s, 1H), 7.74 (s, 1H), 7.30 (br s, 1H) 4.51 (q, *J* 7.0 Hz, 2H), 3.63–3.39 (m, 3H), 3.00 (br s, 2H), 2.59–2.29 (m, 2H), 1.71–1.46 (m 3H), 1.41 (t, *J* 7.1 Hz, 3H), 0.87 (t, *J* 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$): 153.3, 150.8, 147.4, 146.6, 142.7, 138.9, 126.2, 124.4, 49.0, 42.0, 41.4, 39.2, 36.7, 26.0, 24.9, 22.7, 22.2, 15.2; ν_{max} (DMSO) 3390, 3000, 2930, 2820, 1770, 1690,

1670, 1510 cm^{-1}; HRMS m/z $[M+H]^+$ calculated for $C_{18}H_{28}N_{10}O$: 401.2442. Found: 401.2521.

Acknowledgements

We are thankful to the Department of Chemistry and Molecular Biology, University of Gothenburg, the Swedish Academy of Pharmaceutical Sciences and the Swedish Research Council (project # 62120083533) for their financial support.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.08.023.

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