A synthesis of new 7-amino-substituted 4-aminopyrazolo[1,5-*a*][1,3,5]triazines *via* a selective three-component triazine ring annulation [§]

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Abstract

3-Amino-substituted 5-aminopyrazoles were found to be suitable substrates for the synthesis of new 4-aminopyrazolo[1,5-*a*][1,3,5]triazines (5-aza-9-deaza-adenines) when used in the one-pot, three-component reaction with cyanamide and triethyl orthoformate under microwave irradiation. The reaction proceeded selectively and its scope was demonstrated by the preparation of a library of 4-aminopyrazolo[1,5-*a*][1,3,5]triazines. Some structural aspects of the prepared compounds were investigated using dynamic NMR spectroscopy and X-ray crystallography. The operational simplicity, short reaction time, and good reproducibility are attractive features of the developed robust and practical approach for the synthesis of 7-amino-substituted 4-aminopyrazolo[1,5-*a*][1,3,5]triazines.

[§] Part 34 in the series "Fused heterocyclic systems with an *s*-triazine ring." For part 33 see ref.[1]

1. Introduction

The ubiquitous presence of compounds with the purine scaffold in nature and their regulatory role in many biological processes[2] inspired work on the development of new bioactive compounds resembling the structures of natural purines. Applied to the 1,3,5-triazine based purine analogues,[3] this strategy has been successfully implemented with the 5-aza-9-deazapurine (pyrazolo[1,5-*a*][1,3,5]triazine) skeleton.[4] Compounds with this scaffold often exhibit superior biological activity compared to their purine counterpart. For example, the replacement of purine scaffold with the 5-aza-9-deazapurine improved inhibitory activity[5,6] and the increased affinity to the enzyme[7] for cyclin-dependent kinase inhibitors designed as cancer therapeutics. A similar strategy was reported[8] for the development of a Cav2 voltage gated calcium channel agonist with the 5-aza-9-deazapurine skeleton. It was significantly more effective than its purine analogue.

In our program on the development of efficient methods for the preparation of new pyrazolo[1,5-*a*][1,3,5]triazines,[9] we recently reported a microwave-assisted approach for the synthesis of novel 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates **1**.[9a] Later we found that some of these compounds exhibited promising biological activities (Fig. 1). For example, compounds **1a-d** effectively inhibited the *Trypanosoma cruzi* growth with selectivity for myocardiocyte parasites (Fig 1).[10]
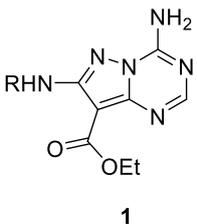
| Compound	R	*T.cruzi* growth, % inhibition at 5 μM	*T.cruzi* myocardiocyte parasites per cell, % inhibition at 5 μM
1a	2-MeOC$_6$H$_4$	90	92
1b	3-MeOC$_6$H$_4$	58	56
1c	3-ClC$_6$H$_4$	65	32
1d	4-ClC$_6$H$_4$	61	53

Figure 1. Bioactive 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates **1a-d**.

Continuing our research in this direction, we decided to explore importance of the ester group for the observed biological activities. Therefore, we designed decarboxylated analogues, which more closely resemble the adenine structure. Herein, we describe our results on the development of an efficient synthesis of new 4-aminopyrazolo[1,5-*a*][1,3,5]triazines (5-aza-9-deaza-adenines).

2 Results and discussion

### 2.1. Attempts to decarboxylate 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates 1

Having alkyl 4-aminopyrazolo[1,5-a][1,3,5]triazine-8-carboxylates **1** prepared earlier,[9a] we initially attempted to involve ethyl 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylate (**1e**) into a hydrolytic decarboxylation of the ester group to prepare the corresponding adenine analogue **4a**. However, heating **1e** in aqueous hydrochloric acid resulted in the formation of **2** as a product of hydrolytic deamination at the triazine ring, while the ester group remained intact (Scheme 1). A similar outcome (formation of **2**, 29%) was achieved when hydrochloric acid was replaced with sulphuric acid of the same concentration. The peaks of the amino group of the starting **1e** were replaced in ^{1}H NMR spectrum of **2** by a downfield shifted signal of the triazine ring N-H at 13.04 ppm, which together with the carbonyl group peak at 163.8 ppm in the ^{13}C NMR spectrum, confirmed the structure assignments. The ethoxycarbonyl group signals in the NMR spectra of **2** remained present.
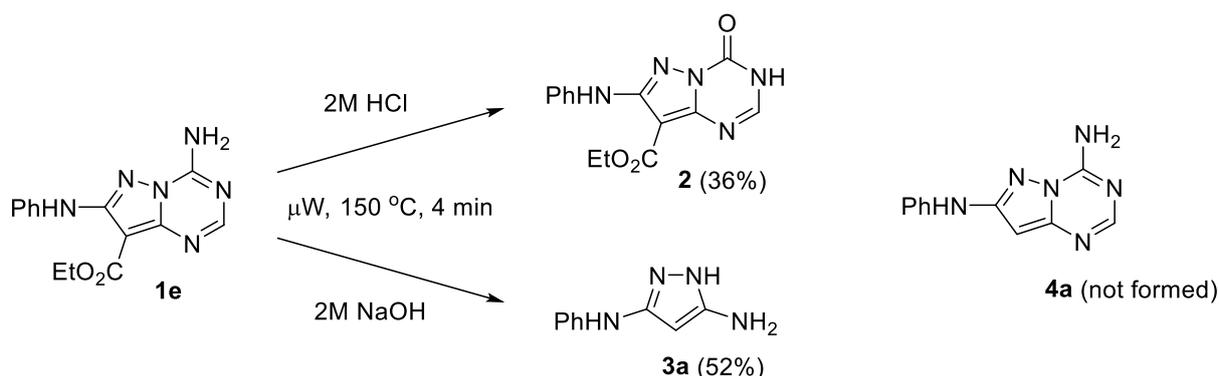
Scheme 1. Acid and base-catalysed hydrolytic decomposition of ethyl 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylate **1e**.

More complex transformations were observed when **1e** was treated with alkali under similar microwave conditions (Scheme 1). The hydrolysis and decarboxylation of **1e** were achieved under these conditions, but they were also destructive for the triazine ring affording 5-amino-3-phenylaminopyrazole (**3a**). We concluded that high electrophilicity of the triazine ring in 4-aminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylates **1** made them less suitable for the initially proposed decarboxylation pathway. However, the isolated 5-amino-

3-phenylaminopyrazole (**3a**) appeared to be a suitable substrate for our previously developed three-component reaction of aminopyrazoles with cyanamide and orthoformate. This reaction could potentially lead to the formation of the desired **4a**. Moreover, once **1e** was effectively converted to **3a**, the pyrazole precursor of **1e** *viz*. 5-amino-3-phenylaminopyrazole-4-carboxylate **5a**[11] was also explored as a substrate and was successfully involved in the hydrolytic decarboxylation under similar conditions.[12] Therefore, we decided to explore this pathway for the synthesis of adenine analogues **4** (Scheme 2).
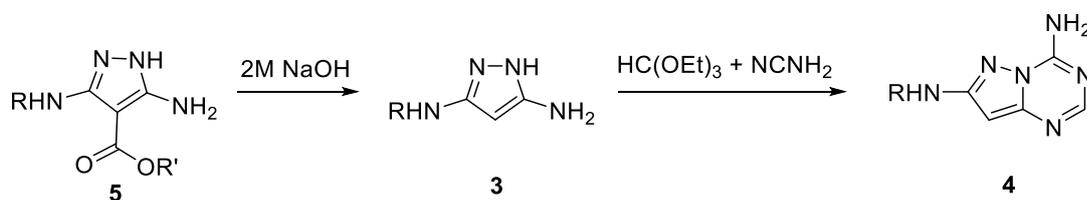
Scheme 2. Proposed approach for the synthesis of 5-aza-9-deaza-adenines **4**.

2.2 Synthesis of 5-aza-9-deaza-adenines 4

We prepared 3-amino-substituted 5-aminopyrazoles (**3**)[12] and attempted to use them as substrates for the one-pot multicomponent reaction (MCR) with cyanamide and triethyl orthoformate. Unlike 4-substituted 5-aminopyrazoles, compounds **3** have one more nucleophilic centre at C-4, which is additionally activated by two amino groups at the neighbouring carbon atoms. This centre increases the complexity of heterocyclizations for 5-aminopyrazoles in the proposed reaction, potentially resulting in the formation of four products **4** and **6-8** (Scheme 3). However, an attempt to carry out this reaction under microwave irradiation at 150 °C for 20 min in methanol using **3a** as a substrate led to the successful formation of the desired 4-amino-7-phenylaminopyrazolo[1,5-*a*][1,3,5]triazine (**4a**) and no products of the pyrimidine ring annulation (**7** and **8**) were detected. Their structures were excluded on the basis of spectral data, particularly by the presence of characteristic H-8 signal at 5.56-6.09 ppm in ^{1}H NMR spectra and HMBC data for representative compounds. The X-ray crystallographic data (*vide infra*) excluded regioisomeric structure **6** and unambiguously confirmed formation of compounds **4**.
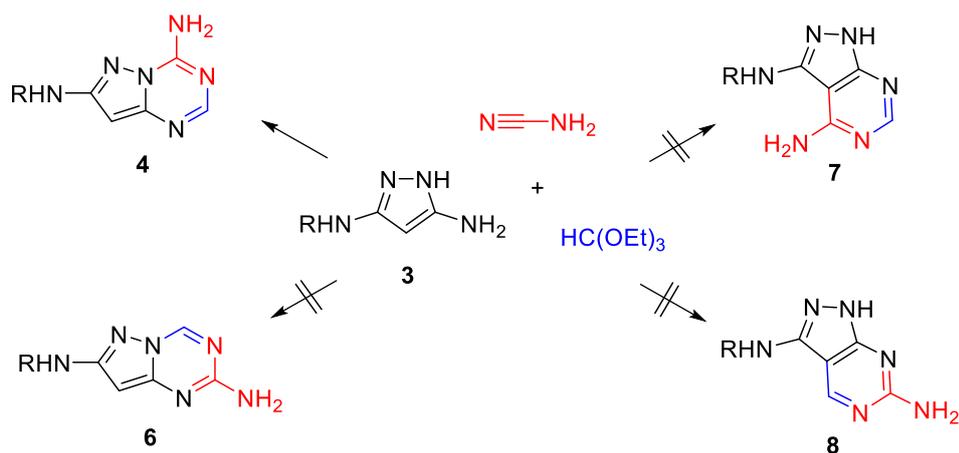
Scheme 3. Potential outcomes of the MCR of **3** with triethyl orthoformate and cyanamide.

The initial attempt of the MCR with 5-amino-3-phenylaminopyrazole (**3a**), triethyl orthoformate and cyanamide afforded **4a** in 49% yield (Table 1). Despite the moderate yield, product **4a** was isolated in pure form without a need of chromatographic purification. Further optimising of the reaction conditions, we found that through manipulation with reaction time (Table 1, entries 1-3), the yield could be improved to 60% by performing the reaction at 150 °C for 25 min (Entry 2). However, conducting the reaction in different solvents (Entries 4 and 5) did not increase in yield. Attempts to manipulate with the ratio of reagents (Entries 6 and 7) and temperature of reaction (Entries 8 and 9) also did not lead to further improvements in the reaction outcome.

The developed method was optimised using Discover SP (CEM) microwave synthesizer and was further tested using another microwave reactor, Monowave 400 (Anton Paar). The three-component reaction of **3a**, triethyl orthoformate and cyanamide under the optimized conditions resulted in the formation of **4a** in similar yields in the both microwave systems (60% and 56%, respectively). To further assess the importance of microwave irradiation for the reaction outcome, we also attempted to carry out the MCR of **3a**, triethyl orthoformate and cyanamide in sealed vessels under pressurized, fast, conventional heating. The Monowave 50 (Anton Paar) reactor, imitating the heating pattern of the earlier optimised microwave irradiation conditions, was found to be suitable for this purpose. It led to the successful formation of equally pure **4a** was obtained in 53% yield. Therefore, we could exclude any significant contribution of non-thermal microwave effects in promoting this MCR.

Table 1. Optimization conditions for the synthesis of 4-amino-7-phenylaminopyrazolo[1,5-a][1,3,5]triazine (**4a**)[a]
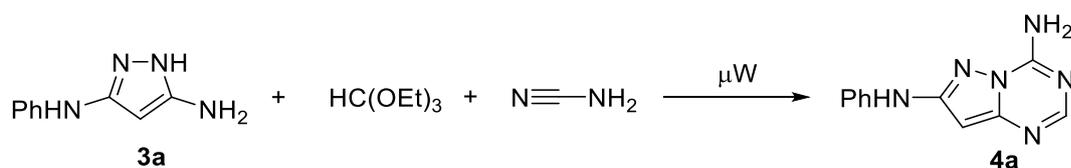

Entry	Solvent	Ratio of 3a: HC(OEt)$_3$: NCNH$_2$	Temperature (°C)	Time (min)	Isolated yield (%)
1	MeOH	1:1.8:1.2	150	20	49
2	MeOH	1:1.8:1.2	150	25	60
3	MeOH	1:1.8:1.2	150	30	52
4	EtOH	1:1.8:1.2	150	25	55
5	AcOEt	1:1.8:1.2	150	25	21
6	MeOH	1:2:2	150	25	60
7	MeOH	1:1.5:1.2	150	25	52
8	MeOH	1:1.8:1.2	140	25	49
9	MeOH	1:1.8:1.2	160	25	35

[a] The reaction was performed using a Discover SP CEM microwave synthesizer with 1 mmol of 5-amino-3-phenylaminopyrazole (**3a**) in 2 mL of the specified solvent.

With the optimized conditions in hand, the scope of our MCR was explored using substrates **3a**-**l**. The one-pot MCR of 5-aminopyrazoles (**3a**-**l**), with triethyl orthoformate and cyanamide proceeded efficiently with the formation of the desired 4-aminopyrazolo[1,5-a][1,3,5]triazines **4** in high purity and similar yields (Table 2). The reaction was found to be highly chemo- and regioselective affording 1,3,5-triazine ring annulation onto 5-aminopyrazole **3a**-**l**. No products of reactions involving a potential nucleophilic attack of the C-4 pyrazole carbon atom or the secondary amino group were detected. The regioisomeric

2-aminopyrazolo[1,5-*a*][1,3,5]triazines **6** with an alternative position of the primary amino group were not found in the reaction mixture.

Table 2. Microwave-assisted synthesis of 8-amino-substituted 5-aza-9-deaza-adenines (**4**)[a]
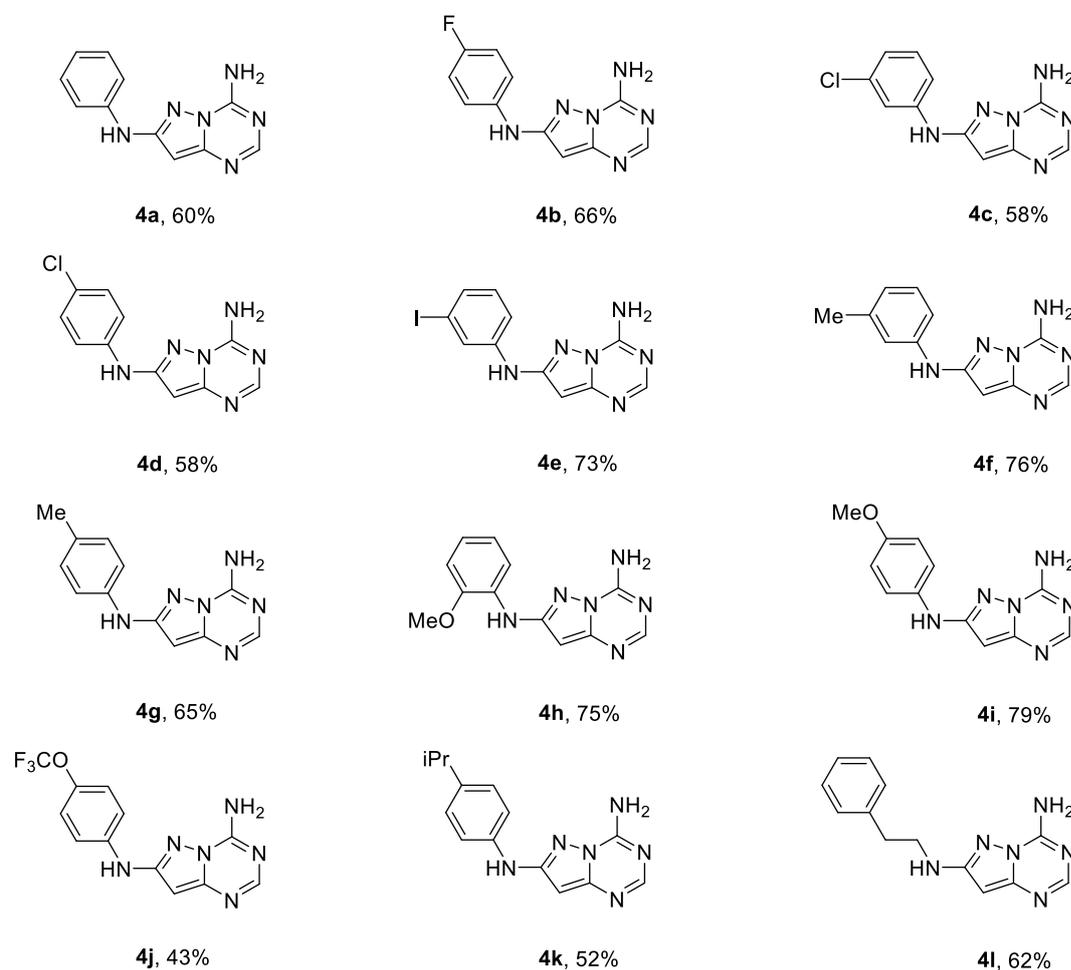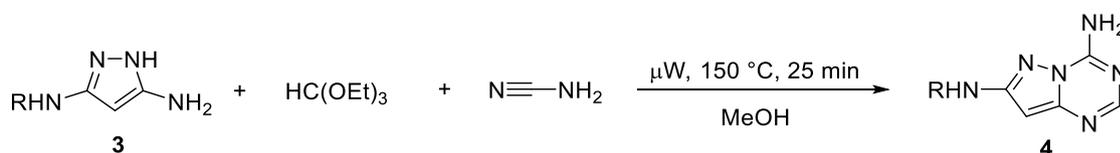
4a, 60%

4b, 66%

4c, 58%

4d, 58%

4e, 73%

4f, 76%

4g, 65%

4h, 75%

4i, 79%

4j, 43%

4k, 52%

4l, 62%

[a] The reaction was performed using a Discover SP CEM microwave synthesizer with 1 mmol of 5-amino-3-phenylaminopyrazoles (**3**), 1.8 mmol of triethyl orthoformate and 1.2 mmol of cyanamide at 150 °C for 25 min in 2 mL of MeOH.

2.3 Restricted rotation around the primary amino group of 5-aza-9-deaza-adenines

Similarly to the previously reported 5-aza-9-deaza-adenine analogues,[9] the primary amino group of the prepared compounds **4** was observed to possess a hindered rotation around the C-N bond due to the delocalization of electrons over the electron-deficient triazine ring. This restricted rotation made the protons on the amino group magnetically non-equivalent resulting in the appearance of two broad signals at 7.51 - 8.04 ppm and 8.38 - 8.56 ppm in the ^{1}H NMR spectra. The signals were found to coalesce at higher temperature (Fig. 2).

The dynamic NMR experiments for **4a** were performed to assess the restricted rotation parameters. The activation enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) were calculated using the rate constant values estimated *via* the line-shape analysis of the spectra. The $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values calculated using Eyring equation were 3.89 kJ mol^{-1} and -180.2 J K^{-1} mol^{-1}, respectively.
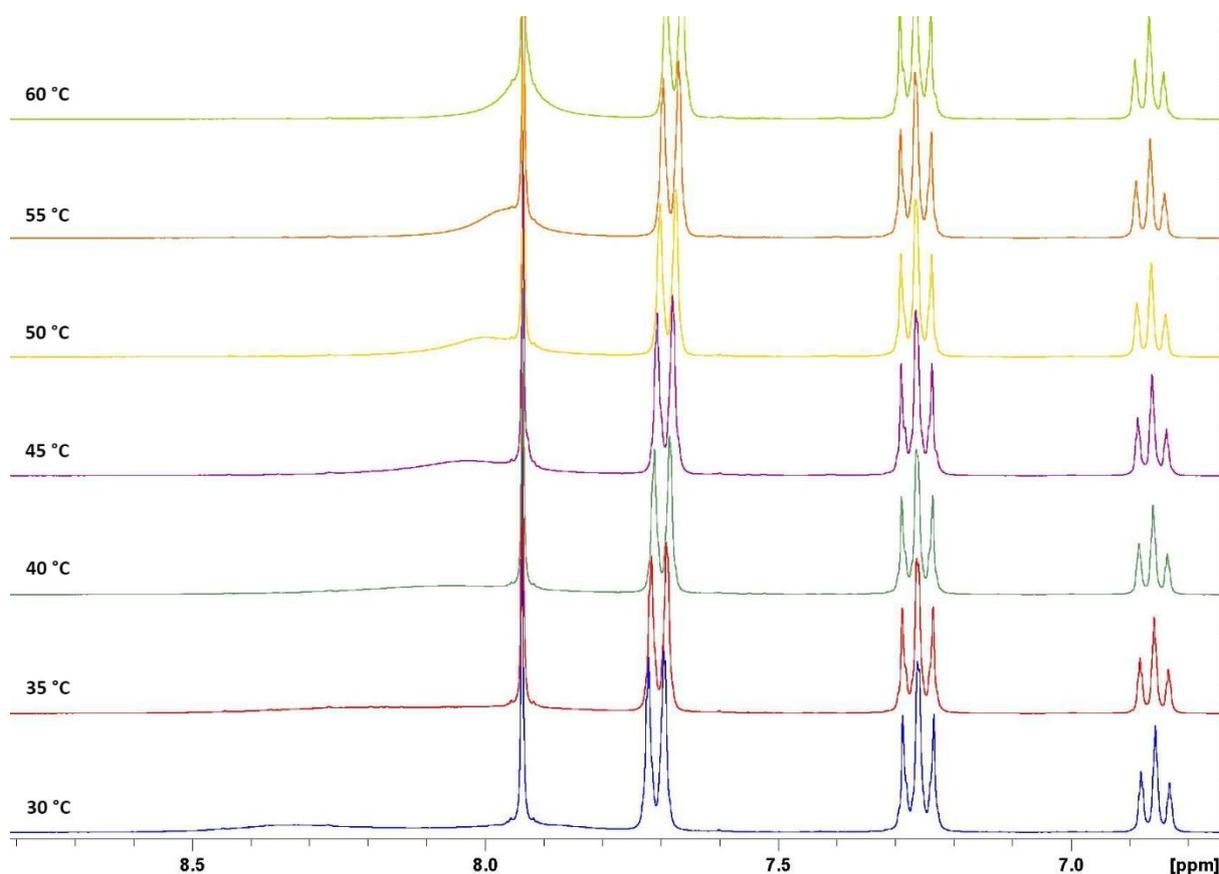

Figure 2. ^{1}H NMR spectra of **4a** in DMSO-d_6 at range of temperatures.

2.4 X-ray crystallography of 4-aminopyrazolo[1,5-*a*][1,3,5]triazine 4l

The crystallographic asymmetric unit comprises two independent molecules with the molecular structure of first of these shown in Fig. 3(a) and the second in ESI Fig. S2[†]. The pyrazolo[1,5-*a*][1,3,5]triazine ring is effectively planar with the r.m.s. deviation for the nine fitted atoms being 0.0203 Å [0.0234 Å for the second independent molecule]; the maximum deviation from the least-squares plane of 0.0306(14) Å was found for the C8 atom [0.0361(14) Å for atom C8a]. The appended primary-N4 and secondary-N7 amine atoms lie 0.0765(24) and 0.0632(22) Å to either side of the plane [0.0364(25) and 0.0539(22) Å]. With a N7–C71–C72–C73 torsion angle of -61.11(2)° [-62.4(2)°], the ethylene group linking the amine-N7 and phenyl ring has a –syn-clinal conformation. The major difference between the two independent molecules relates to the relative orientation of the pendent aromatic residues as seen in the values of the C71–C72–C73–C74 torsion angles of 104.0(2) *cf*. 115.8(2)°, Fig. 3(b), nevertheless the dihedral angles between the planes of 88.93(4) *cf*. 89.17(4)° are similar and indicate an orthogonal relationship.
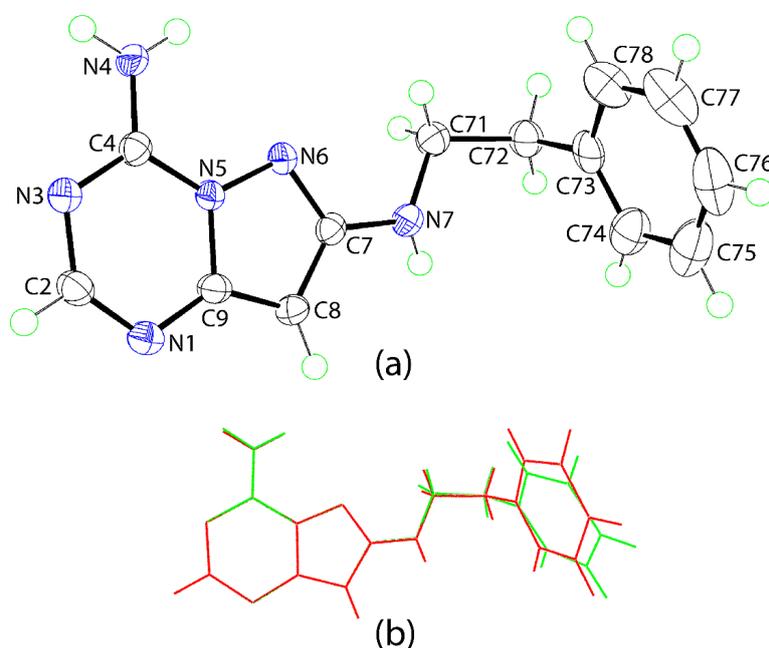

Figure 3. (a) Molecular structure of **4l** showing atom labelling scheme and 70% anisotropic displacement parameters and (b) an overlay diagram of the two independent molecules comprising the asymmetric unit: red image, the molecule shown in (a) and green image, molecule "a". The molecules are overlapped so that the triazine rings are coincident.

As expected from the molecular structure of **4l**, there is significant N–H$\cdots$N hydrogen bonding operating in the crystal; each of the independent molecules engages in analogous hydrogen bonding. Thus, supramolecular ribbons with a corrugated topology are formed along the *c*-axis comprising one type of molecule only, as illustrated for the first independent molecule in Fig. 4; the geometric characteristics of the hydrogen bonding are given in ESI Table S2[†]. The hydrogen bonding involves the primary amine-N4–H hydrogen atoms functioning as the donors to triazine-N3 and pyrazole-N6 as acceptors to form two different centrosymmetric synthons, being, respectively, eight-membered {$\cdots$HNCN}$_2$ and 10-membered {$\cdots$HNCN$_2$}$_2$. The ribbons stack in layers of like-molecules along the *a*-axis and are offset with respect to each (along the *b*-axis) other allowing their connection into a three-dimensional architecture *via* weaker, secondary amine-N7–H$\cdots$N1(triazine) hydrogen bonds (between unlike molecules). A view of the unit cell contents for **4l** is given in ESI Fig. S3[†].
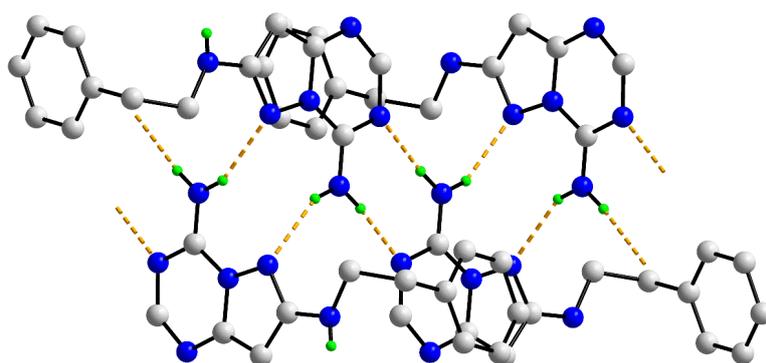

Figure 4. Primary amine-N4–H…N(triazine-N3, pyrazole-N6) hydrogen bonding (see ESI Table S2 and Fig. S3[†]) in the crystal of **4l** leading to supramolecular ribbons along the *c*-axis. The N–H$\cdots$N hydrogen bonding is represented as orange dashed lines, and non-participating hydrogen atoms have been omitted.

3. Conclusion

The MCR of 3-amino-substituted 5-aminopyrazoles **3** with cyanamide and triethyl orthoformate proceeded smoothly *via* a selective 1,3,5-triazine ring closure thus generating a representative library of new 5-aza-9-deaza-adenines **4**. The developed approach is practical and robust and could be attractive for organic and medicinal chemists aiming to

the preparation of purine-like compounds for the drug discovery process. The method is reproducible in different microwave reactors and under microwave-like heating. The rotation of the primary amino group in **4** is restricted and activation parameters for this process are determined using dynamic NMR spectroscopy.

4. Experimental

4.1 General information

Melting points (uncorrected) were determined on a Stuart™ SMP40 automatic melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Fourier NMR spectrometer (300 MHz) using DMSO-d_6 as a solvent and TMS as an internal reference. IR spectra were recorded in KBr pellets using Varian 640-IR spectrophotometer. Microwave-assisted reactions were carried out in the closed vessel focused single mode using a Discover SP microwave synthesizer (CEM, USA) monitoring reaction temperature by equipped IR sensor. For the method validation, the model reaction was also carried out using Monowave 400 and Monowave 50 (Anton Paar, Austria) reactors.

4.2 Synthesis of 4-oxo-7-(phenylamino)-3,4-dihydropyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**2**) from 4-aminopyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**1**)

An ethyl 4-amino-7-phenylaminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylate (**1b**, 0.335 mmol, 100 mg) in aqueous 2M HCl (2.0 mL) was irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W (Discover SP, CEM) at 150 °C for 4 min. After cooling, the precipitated product **2** was filtered and washed with cold water. An analytical sample was recrystallised from DMF & MeOH. Yield 36%; mp 329-331 °C (DMF & MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, ^3J = 7.1 Hz, CH$_3$), 4.36 (2H, q, ^3J = 7.1 Hz, CH$_2$), 6.98 (1H, t, ^3J = 7.4 Hz, H-4'), 7.35 (2H, t, ^3J = 8.0 Hz, H-3' and H-5'), 7.74 (2H, d, ^3J = 7.7 Hz, H-2' and H-6'), 8.24 (1H, s, H-2), 8.74 (1H, s, NH), 13.04 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.2 (CH$_3$), 60.1 (CH$_2$), 90.6 (C-8), 117.4 (C-2' and C-6'), 121.2 (C-4'), 128.9 (C-3' and C-5'), 140.0 (C-1'), 142.7 (C-8a), 148.7 (C-2), 149.7 (C-4), 154.8

(C-7), 163.8 (C=O). Anal. calcd. for $C_{14}H_{13}N_5O_3$: C, 56.2; H, 4.4; N, 23.4. Found: C, 55.8; H, 4.5; N, 23.2.

4.3 Synthesis of 5-amino-3-phenylaminopyrazole (3a) from ethyl 4-amino-7-phenylaminopyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (1b)

An ethyl 4-amino-7-phenylaminopyrazolo[1,5-*a*][1,3,5]triazine-8-carboxylate (**1b**, 0.335 mmol, 100 mg) in aqueous 2M NaOH (2.0 mL) was irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W (Discover SP, CEM) at 150 °C for 4 min. After cooling, the precipitated product **3a** was filtered and washed with cold water. An analytical sample was recrystallised from MeOH to give the product identical to the one obtained according to the earlier described method.[12] Yield 52%; mp 166-168 °C (MeOH).

4.5 General method for the microwave-assisted synthesis of 5-aza-9-deaza-adenines 4

A mixture of 3-amino-substituted 5-aminopyrazoles **5** (1.0 mmol), cyanamide (50.4 mg, 1.20 mmol) and triethyl orthoformate (0.30 mL, 1.8 mmol) in MeOH (2.0 mL) was irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W (Discover SP, CEM) or 850 W (Monowave 450, Anton Paar) at 150 °C for 25 min. After cooling, the precipitated product **4** was filtered and washed with cold MeOH and recrystallised from a suitable solvent.

4.5.1 4-Amino-7-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine (4a)

Yield 60%; mp 277-278 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.80 (1H, s, H-8), 6.86 (1H, t, ^3J = 7.3 Hz, H-4'), 7.26 (2H, t, ^3J = 8.0 Hz, H-3' and H-5'), 7.71 (2H, d, ^3J = 7.7 Hz, H-2' and H-6'), 7.89 (1H, br s, NH), 7.93 (1H, s, H-2), 8.38 (1H, br s, NH), 9.16 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 82.5 (C-8), 116.5 (C-2' and C-6'), 119.5 (C-4'), 128.7 (C-3' and C-5'), 141.7 (C-1'), 148.4 (C-8a), 149.6 (C-4), 154.1 (C-7), 156 (C-2). IR (KBr): ν 3467 (N-H), 3305 (N-H), 3245 (N-H), 3053 (C-H), 1637, 1557, 1491, 1373, 1267, 906 cm^{-1}. Anal. calcd. for $C_{11}H_{10}N_6$: C, 58.4; H, 4.5; N, 37.15. Found: C, 58.2; H, 4.6; N, 37.0.

4.5.2 4-Amino-7-[(4-fluorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (4b)

Yield 66%; mp 278-280 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.78 (1H, s, H-8), 7.07 (2H, dd, ^3J = 8.9 Hz, $^3J_{HF}$ = 8.9 Hz H-3' and H-5'), 7.74 (2H, dd, ^3J = 9.2 Hz, $^4J_{HF}$ = 4.8 Hz, H-2' and H-6'), 7.93 (1H, s, H-2), 7.95 (1H, br s, NH), 8.39 (1H, br s, NH), 9.20 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 82.3 (C-8), 115.0 (d, $^2J_{CF}$ = 21.6 Hz, C-3' and C-5'), 117.8 (d, $^3J_{CF}$ = 7.5 Hz, C-2' and C-6'), 138.3 (d, $^4J_{CF}$ = 2.2 Hz, C-1'), 148.4 (C-8a), 149.6 (C-4), 154.2 (C-7), 156.0 (d, $^1J_{CF}$ = 235.8 Hz, C-4'), 156.0 (C-2). IR (KBr): ν 3469 (N-H), 3310 (N-H), 3234 (N-H), 3062 (C-H), 1685, 1578, 1508, 1372, 1283, 1208 cm^{-1}. Anal. calcd. for $C_{11}H_9FN_6$: C, 54.1; H, 3.7; N, 34.4. Found: C, 53.9; H, 3.9; N, 34.15.

4.5.3 4-Amino-7-[(3-chlorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (4c)

Yield 58%; mp 261-263 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.82 (1H, s, H-8), 6.88 (1H, ddd, ^4J = 0.8 Hz, ^4J = 2.0 Hz, ^3J = 7.9 Hz, H-4'), 7.27 (1H, t, ^3J = 8.1 Hz, H-5'), 7.64 (1H, t, ^4J = 2.1 Hz, H-2'), 7.76 (1H, ddd, ^4J = 0.8 Hz, ^4J = 2.1 Hz, ^3J = 8.3 Hz, H-6'), 7.95 (1H, s, H-2), 8.04 (1H, br s, NH), 8.42 (1H, br s, NH), 9.39 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 82.8 (C-8), 115.1 (C-6'), 115.6 (C-2'), 119.2 (C-4'), 130.4 (C-5'), 133.2 (C-3'), 143.1 (C-1'), 148.5 (C-8a), 149.7 (C-4), 154.2 (C-7), 155.4 (C-2). IR (KBr): ν 3443 (N-H), 3304 (N-H), 3053 (C-H), 1680, 1619, 1591, 1558, 1447, 1371, 1271 cm^{-1}. Anal. calcd. for $C_{11}H_9ClN_6$: C, 50.7; H, 3.5; N, 32.2. Found: C, 50.5; H, 3.55; N, 32.1.

4.5.4 4-Amino-7-[(4-chlorophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (4d)

Yield 58%; mp 283-285 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.79 (1H, s, H-8), 7.26 (2H, d, ^3J = 8.9 Hz, H-3' and H-5'), 7.76 (2H, d, ^3J = 9.0 Hz, H-2' and H-6'), 7.94 (1H, s, H-2), 7.99 (1H, br s, NH), 8.43 (1H, br s, NH), 9.35 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 82.6 (C-8), 118.1 (C-2' and C-6'), 122.9 (C-4'), 128.4 (C-3' and C-5'), 140.6 (C-1'), 148.4 (C-8a), 149.7 (C-4), 154.2 (C-7), 155.6 (C-2). IR (KBr): ν 3634 (N-H), 3445 (N-H), 3307 (N-H), 3092 (C-H), 1680, 1593, 1559, 1443, 1373, 1249 cm^{-1}. Anal. calcd. for $C_{11}H_9ClN_6$: C, 50.7; H, 3.5; N, 32.2. Found: C, 50.5; H, 3.6; N, 32.0.

4.5.5 4-Amino-7-[(3-iodophenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (4e)

Yield 73%; mp 246-248 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.81 (1H, s, H-8), 7.06 (1H, t, ^3J = 8.0 Hz, H-5'), 7.20 (1H, ddd, ^4J = 1.0 Hz, ^4J = 1.4 Hz, ^3J = 7.8 Hz, H-4'), 7.74 (1H, t, ^4J = 1.9

Hz, H-2'), 7.95 (1H, s, H-2), 7.98 (1H, br s, NH), 8.02 (1H, ddd, ^4J = 0.8 Hz, ^4J = 2.2 Hz, ^3J = 8.3 Hz, H-6'), 8.41 (1H, br s, NH), 9.29 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 82.8 (C-8), 94.5 (C-3'), 115.8 (C-6'), 124.2 (C-2'), 128.1 (C-4'), 130.9 (C-5'), 143.2 (C-1'), 148.5 (C-8a), 149.7 (C-4), 154.2 (C-7), 155.4 (C-2). IR (KBr): ν 3377 (N-H), 3300 (N-H), 3242 (N-H), 3059 (C-H), 1658, 1544, 1491, 1370, 1275, 1231 cm^{-1}. Anal. calcd. for $C_{11}H_9IN_6$: C, 37.5; H, 2.6; N, 23.9. Found: C, 37.3; H, 2.7; N, 23.7.

4.5.6 4-Amino-7-[(3-methylphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (*4f*)

Yield 76%; mp 223-225 °C (EtOH); ^1H NMR (300 MHz, DMSO-d_6): δ 2.31 (3H, s, CH$_3$), 5.79 (1H, s, H-8), 6.67 (1H, d, ^3J = 7.5 Hz, H-4'), 7.14 (1H, t, ^3J = 7.8 Hz, H-5'), 7.42 (1H, s, H-2'), 7.54 (1H, d, ^3J = 8.3 Hz, H-6'), 7.88 (1H, br s, NH), 7.92 (1H, s, H-2), 8.38 (1H, br s, NH), 9.06 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.2 (CH$_3$), 82.6 (C-8), 113.8 (C-6'), 116.9 (C-2'), 120.4 (C-4'), 128.6 (C-5'), 137.8 (C-3'), 141.6 (C-1'), 148.4 (C-8a), 149.6 (C-4), 154.0 (C-7), 156.1 (C-2). IR (KBr): ν 3466 (N-H), 3286 (N-H), 3140 (N-H), 3107 (C-H), 1654, 1562, 1499, 1368, 1327, 1279 cm^{-1}. Anal. calcd. for $C_{12}H_{12}N_6$: C, 60.0; H, 5.0; N, 35.0. Found: C, 59.7; H, 5.2; N, 34.8.

4.5.7 4-Amino-7-[(4-methylphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (*4g*)

Yield 65%; mp 270-272 °C (EtOH); ^1H NMR (300 MHz, DMSO-d_6): δ 2.24 (3H, s, CH$_3$), 5.76 (1H, s, H-8), 7.06 (2H, d, ^3J = 8.1 Hz, H-3' and H-5'), 7.59 (2H, d, ^3J = 8.5 Hz, H-2' and H-6'), 7.83 (1H, br s, NH), 7.91 (1H, s, H-2), 8.36 (1H, br s, NH), 9.03 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.2 (CH$_3$), 82.3 (C-8), 116.6 (C-2' and C-6'), 128.1 (C-4'), 129.1 (C-3' and C-5'), 139.3 (C-1'), 148.4 (C-8a), 149.6 (C-4), 154.0 (C-7), 156.2 (C-2). IR (KBr): ν 3462 (N-H), 3286 (N-H), 3029 (C-H), 1659, 1554, 1495, 1372, 1332, 1269 cm^{-1}. Anal. calcd. for $C_{12}H_{12}N_6$: C, 60.0; H, 5.0; N, 35.0. Found: C, 59.8; H, 5.2; N, 34.8.

4.5.8 4-Amino-7-[(2-methoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (*4h*)

Yield 75%; mp 225-227 °C (EtOH); ^1H NMR (300 MHz, DMSO-d_6): δ 3.89 (3H, s, OCH$_3$), 6.09 (1H, s, H-8), 6.86 (1H, ddd, ^4J = 1.8 Hz, ^3J = 7.5 Hz, ^3J = 7.5 Hz, H-4'), 6.92 (1H, ddd, ^4J = 1.8 Hz, ^3J = 7.6 Hz, ^3J = 7.6 Hz, H-5'), 6.98 (1H, dd, ^4J = 1.7 Hz, ^3J = 7.7 Hz, H-6'), 7.00 (1H, s, H-2), 7.91 (1H, br s, NH), 7.94 (1H, s, NH), 8.36 (1H, br s, NH), 8.55 (1H, dd, ^4J = 1.7 Hz, ^3J = 7.8 Hz, H-3'); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.6 (OCH$_3$), 83.7 (C-8), 110.1 (C-3'), 116.9 (C-6'), 119.8 (C-4')

120.8 (C-5'), 130.6 (C-1'), 146.9 (C-2'), 148.1 (C-8a), 149.7 (C-4), 154.0 (C-7), 156.1 (C-2). IR (KBr): ν 3427 (N-H), 3331 (N-H), 3050 (C-H), 1672, 1598, 1555 1464, 1373, 1312, 1250 cm^{-1}. Anal. calcd. for $C_{12}H_{12}N_6O$: C, 56.2; H, 4.7; N, 32.8. Found: C, 56.0; H, 4.95; N, 32.5.

4.5.9 4-Amino-7-[(4-methoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (4i)

Yield 79%; mp 278-280 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (3H, s, OCH$_3$), 5.72 (1H, s, H-8), 6.85 (2H, d, ^3J = 9.0 Hz, H-3' and H-5'), 7.63 (2H, d, ^3J = 9.1 Hz, H-2' and H-6'), 7.80 (1H, br s, NH), 7.90 (1H, s, H-2), 8.36 (1H, br s, NH), 8.93 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.0 (OCH$_3$), 82.0 (C-8), 114.0 (C-3' and C-5'), 117.9 (C-2' and C-6'), 135.3 (C-1'), 148.4 (C-8a), 149.5 (C-4), 152.8 (C-4'), 154.0 (C-7), 156.3 (C-2). IR (KBr): ν 3467 (N-H), 3243 (N-H), 3057 (N-H), 3032 (C-H), 1638, 1557, 1493, 1266, 1245, 1034 cm^{-1}. Anal. calcd. for $C_{12}H_{12}N_6O$: C, 56.2; H, 4.7; N, 32.8. Found: C, 56.1; H, 4.8; N, 32.7.

4.5.10 4-Amino-7-[(4-trifluoromethoxyphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (4j)

Yield 43%; mp 241-243 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.81 (1H, s, H-8), 7.22 (2H, d, ^3J = 9.0 Hz, H-3' and H-5'), 7.83 (2H, d, ^3J = 9.2 Hz, H-2' and H-6'), 7.95 (1H, s, H-2), 7.97 (1H, br s, NH), 8.45 (1H, br s, NH), 9.43 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 82.6 (C-8), 115.2 (C-2' and C-6'), 120.2 (q, $^1J_{CF}$ = 254.8 Hz, CF$_3$), 121.6 (C-3' and C-5'), 140.9 (q, $^3J_{CF}$ = 1.9 Hz, C-4'), 141.0 (C-1'), 148.4 (C-8a), 149.7 (C-4), 154.2 (C-7), 155.6 (C-2). IR (KBr): ν 3467 (N-H), 3307 (N-H), 3249 (N-H), 3067 (C-H), 1639, 1561, 1493, 1320, 1268, 1204 cm^{-1}. Anal. calcd. for $C_{12}H_9FN_6O$: C, 46.5; H, 2.9; N, 27.1. Found: C, 46.3; H, 3.15; N, 26.9.

4.6 Synthesis of 4-amino-7-[(4-isopropylphenyl)amino]pyrazolo[1,5-a][1,3,5]triazine (4k)

A mixture of 5-amino-3-(4-isopropylphenyl)aminopyrazole 3k (216 mg, 1.00 mmol), cyanamide (50.4 mg, 1.20 mmol) and triethyl orthoformate (0.30 mL, 1.8 mmol) in MeOH (2.0 mL) was irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W (Discover SP, CEM) at 150 °C for 25 min. Product was purified by column chromatography on silica gel using ethyl acetate : hexane (6:4) as an eluent. An analytical sample was recrystallised from MeOH.

Yield 52%; mp 248-250 °C (MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.19 (6H, d, ^3J = 7.0 Hz, (CH$_3$)$_2$), 2.83 (1H, m, ^3J = 6.9 Hz, CH), 5.77 (1H, s, H-8), 7.12 (2H, d, ^3J = 8.5 Hz, H-3' and H-5'),

7.61 (2H, d, ^3J = 8.6 Hz, H-2' and H-6'), 7.80 (1H, br s, NH), 7.91 (1H, s, H-2), 8.35 (1H, br s, NH), 9.03 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.0 (2 x CH$_3$), 32.6 (CH), 82.4 (C-8), 116.6 (C-2' and C-6'), 126.4 (C-3' and C-5'), 139.4 and 139.6 (C-1' and C-4'), 148.4 (C-8a), 149.6 (C-4), 154.0 (C-7), 156.2 (C-2). IR (KBr): ν 3466 (N-H), 3246 (N-H), 3057 (N-H), 2959 (C-H), 1637, 1552, 1492, 1371, 1267, 1185 cm^{-1}. Anal. calcd. for C$_{14}$H$_{16}$N$_6$: C, 62.7; H, 6.0; N, 31.3. Found: C, 62.5; H, 6.2; N, 31.05.

4.7 Synthesis of 4-amino-7-(phenethylamino)pyrazolo[1,5-a][1,3,5]triazine (4l)

A mixture of 5-amino-3-phenylethylaminopyrazole **3l** (202 mg, 1.00 mmol), cyanamide (50.4 mg, 1.20 mmol) and triethyl orthoformate (0.30 mL, 1.8 mmol) in MeOH (2.0 mL) was irradiated in a 10 mL seamless pressure vial using microwave system operating at maximal microwave power up to 150 W (Discover SP, CEM) at 150 °C for 25 min. Product was purified by column chromatography on silica gel using ethyl acetate : hexane (6:4) as an eluent. Analytical sample was recrystallised from MeCN.

Yield 62%; mp 166-168 °C (MeCN); ^{1}H NMR (300 MHz, DMSO-d_6): δ ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.88 (2H, t, ^3J = 7.5 Hz, CH$_2$), 3.43 (2H, q, ^3J = 7.1 Hz, CH$_2$), 5.56 (1H, s, H-8), 6.25 (1H, t, ^3J = 5.8 Hz, NH), 7.17-7.30 (5H, m, H-1', H-2', H-3', H-4', and H-5'), 7.52 (1H, br s, NH), 7.83 (1H, s, H-2), 8.10 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 35.3 (CH$_2$), 44.4 (CH$_2$), 80.4 (C-8), 125.9 (C-4'), 128.2 (C-2' and C-6'), 128.7 (C-3' and C-5'), 139.8 (C-1'), 149.3 (C-8a and C-4), 153.5 (C-7), 160.5 (C-2). IR (KBr): ν 3306 (N-H), 3023 (N-H), 2923 (C-H), 1680, 1564, 1487, 1379, 1321, 1266, 1009 cm^{-1}. Anal. calcd. for C$_{13}$H$_{14}$N$_6$: C, 61.4; H, 5.55; N, 33.05. Found: C, 61.2; H, 5.8; N, 32.8.

4.8 Dynamic NMR analysis

Dynamic NMR experiments and lineshape analysis for **4a** were conducted on a Bruker Fourier spectrometer (300 MHz) using DMSO-d_6 as a solvent and TMS as an internal reference at a temperature range of 27 - 60 °C. A temperature below the coalescence point (27 °C) was chosen to fix parameters for the dynamic experiments (Fix Line Broadening at 0.4 Hz). Reaction rate parameters of exchange process at 30 - 60 °C were obtained using the

DNMR Lineshape Fitting module[13] and plotting a graph of ln(k/T) vs 1/T. The activation parameters for the rotation around the C-NH$_2$ bond were calculated using the Eyring equation.

4.9 X-ray crystallographic analysis

Intensity data for crystals (0.03 x 0.09 x 0.13 mm) of **4l** were measured at 100 K on an Rigaku/Oxford Diffraction XtaLAB Synergy diffractometer (Dualflex, AtlasS2) fitted with CuKα radiation (λ = 1.54178 Å) so that θ_{max} = 67.1°. Data reduction and Gaussian absorption corrections were by standard methods.[14] The structures were solved by direct methods[15] and refined[16] on F^2 with anisotropic displacement parameters and C-bound H atoms included in the riding model approximation. The N-bound H atoms were refined freely. A weighting scheme of the form w = 1/[$\sigma^2(F_o^2)$ + $(aP)^2$ + bP] where P = $(F_o^2 + 2F_c^2)/3$) was introduced in each case. The molecular structure diagrams showing 70% probability displacement ellipsoids were generated by ORTEP for Windows[17] and the packing diagrams with DIAMOND.[18] Additional data analysis was made with PLATON.[19]

4.9.1 Crystal data for 4l: C$_{13}$H$_{14}$N$_6$, *M* = 254.30, monoclinic, *P2/c*, *a* = 21.6171(4), *b* = 9.5233(2), *c* = 12.2819(2) Å, β = 104.881(2)°, *V* = 2443.63(8) Å^3, *Z* = 8, D_x = 1.382 g cm^{-3}, *F*(000) = 1072, μ = 0.725 mm^{-1}, no. reflns meas. = 29918, no. unique reflns = 4377 (R_{int} = 0.037), no. reflns with *I* ≥ 2σ(*I*) = 4112, no. parameters = 367, *R*(obs. data) = 0.048, *a* and *b* in weighting scheme = 0.057 and 2.237, *wR2*(all data) = 0.130. CCDC deposition number: 1866663.

Appendix A. Supplementary data

† Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.tet.XXXX.XX.XXXX

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