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Differentiation between [1,2,4]triazolo[1,5-a] pyrimidine and [1,2,4]triazolo[4,3-a]-pyrimidine regioisomers by $^{1}H-^{15}N$ HMBC experiments

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The condensation of malonoaldehyde derivatives with either a 3-amino-[1,2,4]-triazole or a 3,5-diamino-[1,2,4]-triazole precursor was studied. In agreement with previous reports, two different bicycles, namely, bearing the regioisomeric [1,2,4]triazolo[1,5-a]pyrimidine (1) or[1,2,4] triazolo [4,3-a]pyrimidine (2) structural surrogates, could be obtained. We found that, depending on the triazole precursor, only one regioisomer resulted, either of the 1 or 2 series. We also observed that these two structural surrogates could be unambiguously differentiated by indirectly measuring their ^{15}N chemical shifts by $^{1}H-^{15}N$ HMBC experiments. The occasional conversion of [1,2,4]triazolo[4,3-a]pyrimidines to the [1,2,4]triazolo[1,5-a]pyrimidine counterparts could be unequivocally determined by ^{15}N NMR data. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: NMR; ^{1}H; ^{13}C; ^{15}N; HMBC; [1,2,4]triazolo[1,5-a]pyrimidines; [1,2,4]triazolo[4,3-a]pyrimidines; regioisomerism; isomerisation

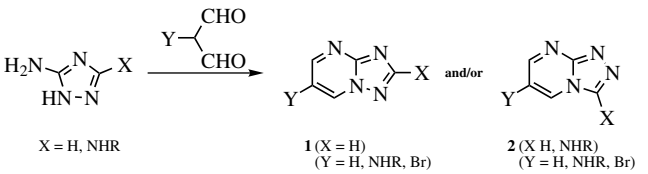
Introduction

[1,2,4]-Triazolo[1,5-a]pyrimidines (**1**, Fig. 1) can be regarded as the aza-analogues of purines.[1] They have received a growing interest in the past years due to their important pharmaceutical properties.[1–5] For instance, the anti-ischaemic drug Trapidil[2] and the recently isolated antibiotic Essramycin[3] have been found to have the [1,2,4]-triazolo[1,5-a]pyrimidine parent structure (**1**). Most of the reported syntheses of these compounds are based on the reaction of a 1,3-divalent synthon with either a 3-amino-[1,2,4]-triazole[6] or a 2-hydrazinopyrimidine precursor.[7] In both cases, it is commonly accepted that the [1,2,4]triazolo[4,3-a]pyrimidine system (**2**) is formed first, but isomerises to the thermodynamically more stable [1,5-a] isomer (**1**), either thermally or under acidic or alkaline conditions. This reaction, known as the *Dimroth rearrangement*, has been reported to occur via ring opening of the six-membered cycle in a formal [3,5] sigmatropic shift.[8–10] It should be noted that less work has been devoted to [1,2,4]-triazolo[4,3-a]pyrimidines (**2**), perhaps due to their intrinsic lower stability.[11–17]

Results and Discussion

As part of our medicinal chemistry programme, we decided to prepare compounds of the parent structure [1,2,4]triazolo[4, 3-a]pyrimidine (**2**). It had been described previously that direct condensation of malonoaldehyde derivatives with either a 3-amino-[1,2,4]-triazole or a 3,5-diamino-[1,2,4]-triazole precursor invariably gives a mixture of the two possible regioisomers **1** and **2** (Scheme 1).[8,13,18]

When we attempted this condensation with either mono- or diaminotriazoles and malonate-type compounds, we found that always a single compound was obtained. Yet, some doubt arose when it came to distinguishing between the two possible parent structures **1** and **2**. We found that one- and two-dimensional ^{1}H and ^{13}C chemical shift data would be difficult to assess, as $^{1}H-^{13}C$ HMBC experiments for comparatively small cyclic systems can be rather deceitful. Furthermore, as ^{1}H and ^{13}C chemical shifts strongly depend on the substituents attached to the core scaffold, these data can be sometimes complicated to interpret. On top of that, in fused, rigid aromatic systems like the title compounds, nuclear Overhauser effect (NOE) experiments may not afford conclusive results for those bearing few hydrogen atoms. More useful NMR experiments would be those that yield information about carbon–carbon or even carbon–nitrogen connectivities. These experiments are of very low sensitivity (INADEQUATE) or present processing problems, such as covariance methods.[19,20]



Scheme 1. Condensation between malonoaldehydes and 3-amino-[1,2,4]-triazole or 3,5-diamino-[1,2,4]-triazole precursors.[1]

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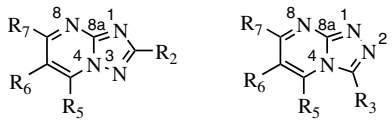


1 2

Figure 1. Parent structures of 1,2,4-triazolo[1,5-a]pyrimidines (**1**) and 1,2,4-triazolo[4,3-a]pyrimidines (**2**).

In these cases, it may become mandatory to resort to X-ray crystal analyses, which may not always be feasible.

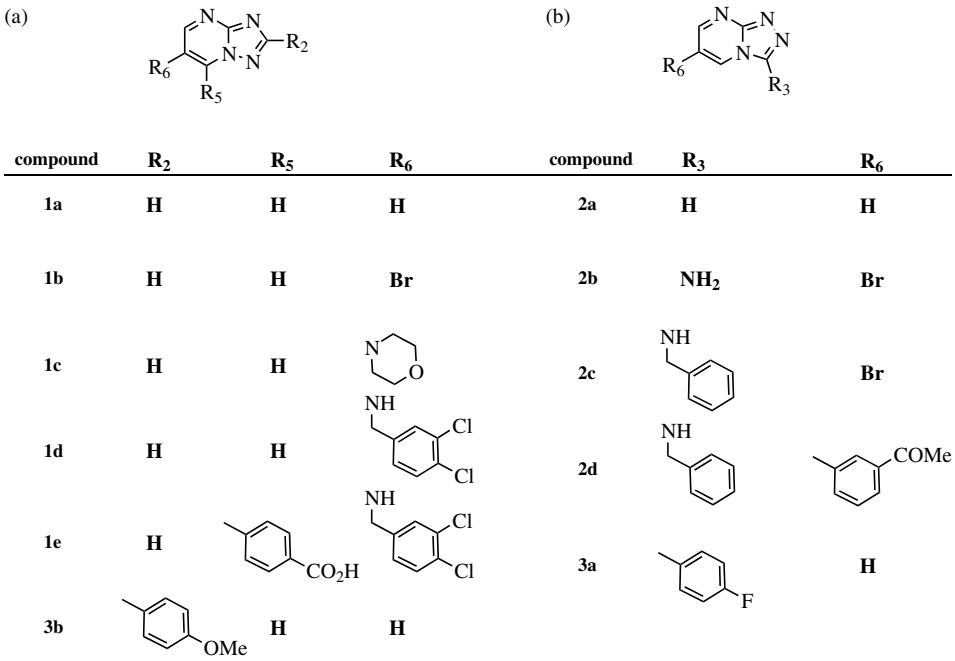
Some differentiation studies between [1,2,4]triazolo[4,3-a]pyrimidines and [1,2,4]triazolo[1,5-a]pyrimidines had been carried out in as early as 1975.[12] The authors based their structural analysis on ^{13}C NMR data stating that C-2 in the [1,5-a] series (**1**) should be more deshielded than C-3 in the [4,3-a] analogues (**2**). Other authors distinctly identified [1,2,4]-triazolo[1,5-a]pyrimidines and [1,2,4]-triazolo[4,3-a]pyrimidines from their ^{1}H data.[13] In another study, the structures of 2-methyl-[1,2,4]triazolo[1,5-a]pyrimidine and 3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine were distinguished on the basis of their ^{13}C NMR data.[21] Again, the C-3 carbon of 3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine showed a lower frequency than C-2 of 2-methyl-[1,2,4]triazolo[1,5-a]pyrimidine. Nevertheless, these authors pointed out that isomer differentiation on the basis of spectroscopic data was by no means straightforward and that in many cases disagreement had been encountered among the published data.

^{15}N NMR spectroscopy, in particular long-range $^{1}H-^{15}N$ heteronuclear multiple-bond correlation ($^{1}H-^{15}N$ HMBC) experiments, can constitute a useful alternative.[22,23] Unlike the ^{13}C counterparts, these experiments have been rarely employed due to the low gyromagnetic ratio and natural abundance of the ^{15}N nuclide. Yet, despite these shortcomings, ^{15}N NMR spectroscopy has the advantage that ^{15}N chemical shifts span over quite a large range (up to 1500 ppm). With the advent of high-field magnets, cryoprobes and the use of pulsed gradients for coherence selection, indirect ^{15}N detection methods with natural isotopic abundance have become more common.[22] It is interesting to note that some regioisomerism problems have been resolved *via* $^{1}H-^{15}N$ HMBC.[24–30]

In order to determine whether our compounds have the [1,2,4]triazolo[1,5-a]pyrimidine (**1**) or the [1,2,4]triazolo[4,3-a]pyrimidine (**2**) structure, we decided to obtain their ^{15}N NMR data from $^{1}H-^{15}N$ HMBC experiments. ^{15}N NMR data of [1,2,4]triazolo[1,5-a]pyrimidine (**1**) derivatives have been reported,[1,8,31,32] but to the best of our knowledge, no ^{15}N NMR data of [1,2,4]triazolo[4,3-a]pyrimidine derivatives other than the bare compound **2a** have been published.[31]

The structures of the prepared compounds are shown in Fig. 2. Compound **1a** was synthesised as described by Breitmaier *et al*.,[8] and compound **2a** was prepared by following the method of Paudler[33] with minor modifications. Compound **1b** had already been described by Rusinov *et al*.[34] All other compounds are new and gave satisfactory mass spectrometry (MS) and NMR data. The obtained ^{1}H and ^{13}C NMR data are shown in Tables 1 and 2. HSQC, HMBC and NOE experiments were run when appropriate to confirm the structures.

As it can be seen from Tables 1 and 2, ^{1}H and ^{13}C NMR data do not really allow us to clearly distinguish between both isomers, as chemical shifts can substantially vary (up to 30 ppm in ^{13}C NMR) depending on the substituents. Nevertheless, some regularity can be seen with the ^{1}H NMR chemical shifts in both regioisomeric series. For compounds **1c** and **1d**, the presence of an amine at C-6 shields H-5 as compared to the parent compound **1a** (1.03 ppm difference for **1d**). For the 6-bromo derivative **1b**, H-5 also resonates at higher field than **1a** (0.45 ppm difference). The chemical shift of H-2 and H-6 hydrogen atoms of compounds **1b–d** varies upon substitution at C-6 but to a lesser extent than H-5. The presence of a second substituent at C-5 (**1e**) shields H-2 and H-7. In the [1,2,4]triazolo[4,3-a]pyrimidine series, the amino group at



compound	R_2	R_5	R_6
1a	H	H	H
1b	H	H	Br
1c	H	H	
1d	H	H	
1e	H		
3b		H	H

compound	R_3	R_6
2a	H	H
2b	NH_2	Br
2c		Br
2d		
3a		H

Figure 2. (a) Structure of compounds **1a** to **1e** and **3b**. (b) Structure of compounds **2a** to **2d** and **3a**.

Table 1. ^{1}H chemical shifts of **1a–e, 2a–d** and **3a–b**

	^{1}H chemical shifts (ppm), $^{n}J(^{1}H\text{-}^{1}H)$ (Hz)					
	H-2	H-3	H-5	H-6	H-7	Other H
1a	8.69 (*s*)	–	9.43 (*dd*) ^{3}J 6.7, ^{4}J 4.2	7.38 (*dd*) ^{3}J 4.3, 1.8	8.91 (*dd*) ^{3}J 4.2, ^{4}J 1.8	–
1b[a]	8.46 (*s*)	–	8.98 (*s*)	–	8.81 (*s*)	–
1c	8.50 (*s*)	–	8.83 (*s*)	–	8.98 (*s*)	3.19 (4H, *m*), 3.78 (4H, *m*)
1d	8.43 (*s*)	–	8.40 (*d*) ^{4}J 2.6	–	8.63 (*d*) ^{4}J 2.6	4.10–4.60 (1H, br s), 4.35 (2H, *s*), 7.42 (1H, *d*, ^{2}J 8.1), 7.61 (1H, *d*, ^{2}J 8.1), 7.71 (1H, *s*)
1e[b]	8.26 (*s*)	–	–	–	8.56 (*s*)	4.33 (2H, *d*, ^{2}J 6.5), 5.88 (1H, *t*, ^{2}J 6.6), 7.25 (1H, *d*, ^{2}J 8.2), 7.49 (1H, *d*, ^{2}J 8.3), 7.52 (1H, *s*), 7.62 (2H, *d*, ^{2}J 8.2), 8.08 (2H, *d*, ^{2}J 8.2)
2a	–	9.25 (*s*)	9.02 (*dd*) ^{3}J 6.9, ^{4}J 1.9	7.13 (*dd*) ^{3}J 6.9, ^{4}J 3.8	8.77 (*dd*) ^{3}J 3.8, ^{4}J 1.9	–
2b	–	–	9.39 (*d*) ^{4}J 1.9	–	8.55 (*d*) ^{4}J 1.9	6.55 (2H, broad *s*)
2c	–	–	9.44 (*d*) ^{4}J 2.2	–	8.58 (*d*) ^{4}J 2.2	4.47 (2H, *d*, ^{2}J 6.3), 7.22 (1H, *t*, ^{2}J 7.2), 7.30 (2H, *dd*, ^{2}J 7.5, 7.2), 7.35 (2H, *d*, ^{2}J 7.5), 7.71 (1H, *t*, ^{2}J 6.3)
2d	–	–	9.51 (*d*) ^{4}J 2.4	–	8.95 (*d*) ^{4}J 2.4	2.67 (3H, *s*), 4.51 (2H, *d*, ^{2}J 6.3), 7.23 (1H, *t*, ^{2}J 7.2), 7.31 (1H, *dd*, ^{2}J 7.6, 7.2), 7.38 (2H, *d*, ^{2}J 7.6), 7.63 (1H, *t*, ^{2}J 7.6), 7.68 (1H, *t*, ^{2}J 6.3), 7.95 (1H, *d*, ^{2}J 7.6), 8.05 (1H, *d*, ^{2}J 7.6), 8.33 (1H, *s*)
3a	–	–	9.01 (*dd*) ^{3}J 6.9, ^{4}J 1.6	7.17 (*dd*) ^{3}J 6.9, 4.0	8.78 (*dd*) ^{3}J 6.9, ^{4}J 4.0	7.49 (2H, *dd*, ^{2}J 8.3, $^{3}J_{HF}$ 8.3), 8.01 (2H, *dd*, ^{2}J 8.3, $^{4}J_{HF}$ 5.5)
3b[a]	–	–	8.91 (*s*) ^{3}J 5.5	7.16 (dd) ^{3}J 5.5, 3.2	8.76 (*s*) ^{3}J 3.2	3.86 (3H, s), 7.01 (2H, *d*, ^{2}J 8.2), 8.19 (2H, *d*, ^{2}J 8.2)

[a] In $CDCl_3$/MeOD (4 : 1).
[b] Structure confirmed by X-ray crystal analysis.

Table 2. ^{13}C chemical shifts of **1a–e, 2a–d** and **3a–b**

	^{13}C chemical shifts (ppm)						
	C-2	C-3	C-5	C-6	C-7	C-8a	Other C
1a	156.3	–	138.1	111.3	156.0	155.1	–
1b[a]	156.3	–	135.3	105.7	155.4	153.3	–
1c	155.5	–	121.3	138.8	150.6	151.0	49.5, 66.1
1d	154.2	–	114.1	135.6	149.2	148.8	45.1, 48.5, 127.8, 129.5, 130.5, 131.0, 139.6
1e[b]	154.5	–	129.7	131.5	145.6	150.7	45.7, 127.4, 128.7, 129.3, 129.8, 130.1, 130.5, 131.0, 137.9, 140.8, 145.5, 169.3
2a	–	135.8	133.9	109.9	155.4	152.8	–
2b	–	168.0	134.8	106.2	152.0	153.7	–
2c	–	168.2	135.2	102.5	152.7	154.2	45.2, 126.7, 127.1, 128.3, 139.9
2d	–	170.5	135.1	123.2	153.8	157.1	29.63, 48.15, 129.3, 129.7, 129.9, 130.9, 132.1, 133.8, 134.7, 136.7, 140.3, 142.8, 200.5
3a	–	144.8	133.6	110.8	155.5	154.2	116.3 (*d*, $^{2}J_{C-F}$ = 22 Hz), 122.7 (*d*, $^{4}J_{C-F}$ = 3 Hz), 130.6 (*d*, $^{3}J_{C-F}$ = 9 Hz), 163.1 (*d*, $^{1}J_{C-F}$ = 248 Hz)
3b[a]	165.6	–	135.7	110.2	154.8	155.7	54.2, 114.7, 122.1, 128.9, 161.8

[a] In $CDCl_3$ – MeOD 4 : 1.
[b] Structure confirmed by X-ray crystal analysis.

C-3 (**2b–d**) shifts the NMR signal of H-5 to higher frequencies as compared to **2a** (0.49 ppm difference for **2d**). On the other hand, in compounds **2a–d**, the H-7 signal is less sensitive to the presence of the amino group at C-3.

The ^{13}C NMR signals that show greater regularity in their chemical shifts are C-2 for **1a–e** and C-3 for **2b–d**, respectively, resonating at $\delta = 154–156$ and 167–170 ppm. Interestingly, the measured ^{13}C chemical shift of C-3 of the fully unsubstituted compound **2a** is smaller ($\delta = 135.8$ ppm). Other carbon atoms are found to be more sensitive to the presence and nature of the substituents. In the [1,2,4]triazolo[1,5-a]pyrimidine series, C-5 and C-6 resonate between $\delta = 135–138$ and 105–111 ppm for derivatives **1a** and **1b**, respectively, but for those derivatives having an amino group at C-6 (**1c–e**), C-5 and C-6 are shielded and deshielded, respectively. In the [1,2,4]triazolo[4,3-a]pyrimidine series, the carbon atom showing greater sensitivity towards substitution at C-6 is C-5, which resonates between $\delta = 133$ and 135 ppm. As to C-6, it can be seen (Table 2) that in compounds **2a–c**, all those having an amino group at C-3 or no substitution at all (**2a**), C-6 resonates between $\delta = 101$ and 110 ppm. In the case of **2d**, with an aryl substituent at C-6 and an amino function at C-3, the measured chemical shift for C-6 is noticeably larger ($\delta = 123$ ppm). The carbon atoms C-7 and C-8a followed a more definite pattern, appearing at $\delta =$ *ca* 155 ppm in both series. Consequently, the comparatively large dispersion of ^{13}C chemical shift values made regioisomer differentiation difficult.

With these results in hand, we found that structural assignment based only on ^{1}H and ^{13}C NMR data could be rather deceitful. Thus, we turned our attention to $^{1}H-^{15}N$ HMBC experiments. We expected that valuable structural information about the regioisomeric systems **1** and **2** would be obtained from long-range $^{1}H-^{15}N$ correlations. Traditionally, ^{15}N chemical shifts in nitrogen-containing aromatic systems have been discussed in terms of two different kinds of nitrogen atoms, the so-called pyrrole-like and pyridine-like.[29,35] It is well known that, due to their higher π-electron density, pyrrole-like nitrogen atoms are substantially more shielded compared to their pyridine-like counterparts (*ca* 50–100 ppm).[36]

$^{1}H-^{15}N$ HMBC experiments were run to measure the ^{15}N chemical shifts of compounds **1a–e** and **2a–d** indirectly (Table 3). We observed that the ^{15}N chemical shifts of all compounds followed a far more distinctive and regular pattern than those due to the ^{13}C signals. As expected, four ^{15}N signals for the aromatic nitrogen atoms could be detected for each compound. Surprisingly, we observed that, depending on the triazole precursor employed in the syntheses (Scheme 1), the chemical shift values of these four aromatic ^{15}N NMR signals were distinctly and uniformly distributed (Fig. 3a and 3b).

When 3-amino-[1,2,4]-triazole was used, the four aromatic ^{15}N resonances of **1b–e** came in two narrow frequency regions, at $\delta = 225–230$ ppm (N-1 and N-4) and at $\delta = 275–278$ ppm (N-3 and N-8). The values of the encountered ^{15}N chemical shifts were very similar to those reported for **1a** and for other [1,2,4]triazolo[1,5-a]pyrimidine derivatives.[8,37] This ^{15}N chemical shift distribution pattern permitted us to conclude that **1b–e** had the [1,2,4]triazolo[1,5-a]pyrimidine parent structure. The X-ray crystal analysis of **1e** (Fig. 4) fully corroborated the [1,2,4]triazolo[1,5-a]pyrimidine structure. (CCDC 766900 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.) On the other hand, for compounds **2b–d**, which were synthesised from 3,5-diamino-[1,2,4]-triazole precursors, the four aromatic ^{15}N signals were distributed differently to **1b–e**, with one signal at *ca* $\delta = 195$ ppm (N-4), two other signals at *ca* $\delta = 225–230$ ppm (N-1 and N-2), and the fourth signal (N-8) at *ca* $\delta = 270$ ppm. It was assumed that this other ^{15}N chemical shift distribution pattern corresponded to the [1,2,4]triazolo[4,3-a]pyrimidine series. That was supported by a 1D NOESY experiment of **2d**. Unequivocal NOE interaction between H-5 ($\delta = 9.51$ ppm) and the methylene of the benzylamino group linked at C-3 ($\delta = 4.51$ ppm) confirmed the [1,2,4]triazolo[4,3-a]pyrimidine structure of **2d** (Figs 3 and 4).

Table 3. ^{15}N chemical shifts of **1a–e, 2a–d** and **3a–b**

	^{15}N chemical shifts (ppm)					
	N-1	N-2	N-3	N-4	N-8	Other N
1a	231	–	275	227	278	–
1b[a]	227	–	271	223	278	–
1c	230	–	276	225	278	58 (C6–N)
1d	228	–	274	225	278	57 (C3–NH)
1e[b]	229	–	273	225	270	53 (C6–NH)
2a	296	331	–	183	277	–
2b	228	231	–	198	266	57 (C3–NH)[c]
2c	227	229	–	195	266	66 (C3–NH)
2d	224	227	–	196	264	66 (C3–NH)
3a	n.d.	n.d.	–	179	277	–
3b[a]	n.d.	–	n.d.	226	274	–

[a] In $CDCl_3$–MeOD 4 : 1.
[b] Structure confirmed by X-ray crystal analysis.
[c] Determined with an HMQC experiment.

In the [1,2,4]triazolo[1,5-a]pyrimidine series (**1a–e**), we observed that the N-8 chemical shift ranged from $\delta = 270$ to 278 ppm. This value fits to a pyridine-like nitrogen atom in a six-membered ring.[36] The N-1 atoms also gave a signal with a very uniform chemical shift ($\delta = 227–231$ ppm), consistent with a pyridine-like nitrogen atom but in a five-membered ring. The bridge-head N-4 atoms, which contribute with a full electron pair to the π system, are clearly pyrrole-like, although they are substantially deshielded ($\delta = 223–227$ ppm) when compared to what would be expected for a common pyrrole nitrogen. This was assumed to be due to the effect of the neighbouring N-3 atoms, which resonate between $\delta = 271$ and 276 ppm. As to compounds **2b–d**, the ^{15}N chemical shift values also show remarkable uniformity, although some differences with the [1,2,4]triazolo[1,5-a]pyrimidines **1a–e** are noticed. The N-4 atoms of **2b–d** are comparatively more shielded ($\delta = 195–198$ ppm vs $\delta = 223–227$ ppm for **1a–e**), in agreement with a pyrrole-like nitrogen without adjacent nitrogen atoms. The N-8 atoms give signals ranging from $\delta = 264$ to 266 ppm, slightly more shielded than in **1a-e**. The chemical shifts of N-1 and N-2 are very similar in magnitude ($\delta = 223–231$ ppm), both fitting to imino nitrogens. It should be noticed that the presence of an adjacent nitrogen atom (N-2) in **2b–d** does not result in a substantial modification of the chemical shifts of the N-1 atoms, when compared to **1a–e**.

Unlike the ^{13}C counterparts, it is noteworthy that these two ^{15}N signal distribution patterns are essentially not dependent on the substituents attached to the core scaffold. This emphasises the relevance of ^{15}N NMR as a tool for regioisomer differentiation. The only exception to this trend was the unsubstituted compound

2a whose N-1 and N-2 ^{15}N chemical shifts (δ = 296 and 331 ppm, respectively) differed greatly from the rest of the series (δ = 223 and 224 ppm for **2b**). This can be attributed to the increased π-electron density of the triazole ring due to the exocyclic amino group. The N-8 nucleus (δ = 277 ppm) was also deshielded when compared to the rest of the series (δ = 266 ppm for **2b**), whereas N-4 was more shielded (δ = 183 ppm vs δ = 198 ppm for **2b**). This could be due to the plausible electron-donating effect of the substituents at C-3 of the [1,2,4]triazolo[4,3-a]pyrimidine core scaffold. To explore further the ^{15}N chemical shifts of other [1,2,4]triazolo[4,3-a]pyrimidines devoid of an exocyclic amino group, compounds **3a** and **3b** were prepared from 2-hydrazinopyrimidine precursors (Scheme 2).[17,38] These reactions are expected to give the [1,2,4]triazolo[4,3-a]pyrimidine system exclusively and, consequently, their ^{15}N chemical shifts should be similar to those of **2a**.

The ^{13}C and ^{15}N NMR data of these two compounds are shown in Tables 2 and 3. The ^{1}H–^{15}N HMBC experiment of **3a** permitted to spot two ^{15}N resonances at δ = 179 and 277 ppm. The magnitude of the chemical shift of these two signals agreed with pyrrole-type and a pyridine-type nitrogen atoms, respectively. These values are also very similar in magnitude to those of N-4 and N-8 of **2a** and support the [1,2,4]triazolo[4,3-a]pyrimidine structure of **3a**. Additional evidence comes from the ^{13}C NMR data, as the resonances due to the C-5, C-6, C-7 and C-8a atoms of compounds **2a** and **3a** are essentially identical. Some differences are seen only with the chemical shifts of C-3 (δ = 135.8 ppm for **2a** and δ = 144.8 ppm for **3a**) due to the substitution in **3a**. On the other hand, intense NOE interaction between the ^{1}H resonances at δ = 8.80 and 7.75 ppm, respectively, attributed to H-5 and H-2,6 of the *p*-fluorophenyl substituent at C-3, corroborate the [1,2,4]triazolo[4,3-a]pyrimidine structure of **3a**.

Yet, the encountered ^{15}N chemical shift values of **3b** (δ = 226 and 274 ppm) are not consistent with a [1,2,4]triazolo[4,3-a]pyrimidine derivative such as **2a** or **3a**. Instead, these chemical shift values are much like those of the [1,2,4]triazolo[1,5-a]pyrimidine derivatives **1a–e**. The ^{13}C chemical shifts of **3b** are also quite similar to those of **1a**, with the exception of the signal due to the C-2 atom (δ = 156.3 ppm for **1a** and δ = 165.6 ppm for **3b**), probably caused by the substitution at C-2 of **3b**. Moreover, 1D NOESY experiments irradiating at δ = 8.91 ppm (H-5) did not result in any NOE interaction with the *p*-methoxyphenyl ring of **3b**. All these results support the fact that **3b** belongs to the [1,2,4]triazolo[1,5-a]pyrimidine series. The presence of the [1,2,4]triazolo[1,5-a]pyrimidine structure instead of the expected [4,3-a] counterpart can be explained by a Dimroth rearrangement that gives rise to the more stable [1,2,4]triazolo[1,5-a]pyrimidine scaffold.[11] These findings illustrate how ^{15}N NMR can

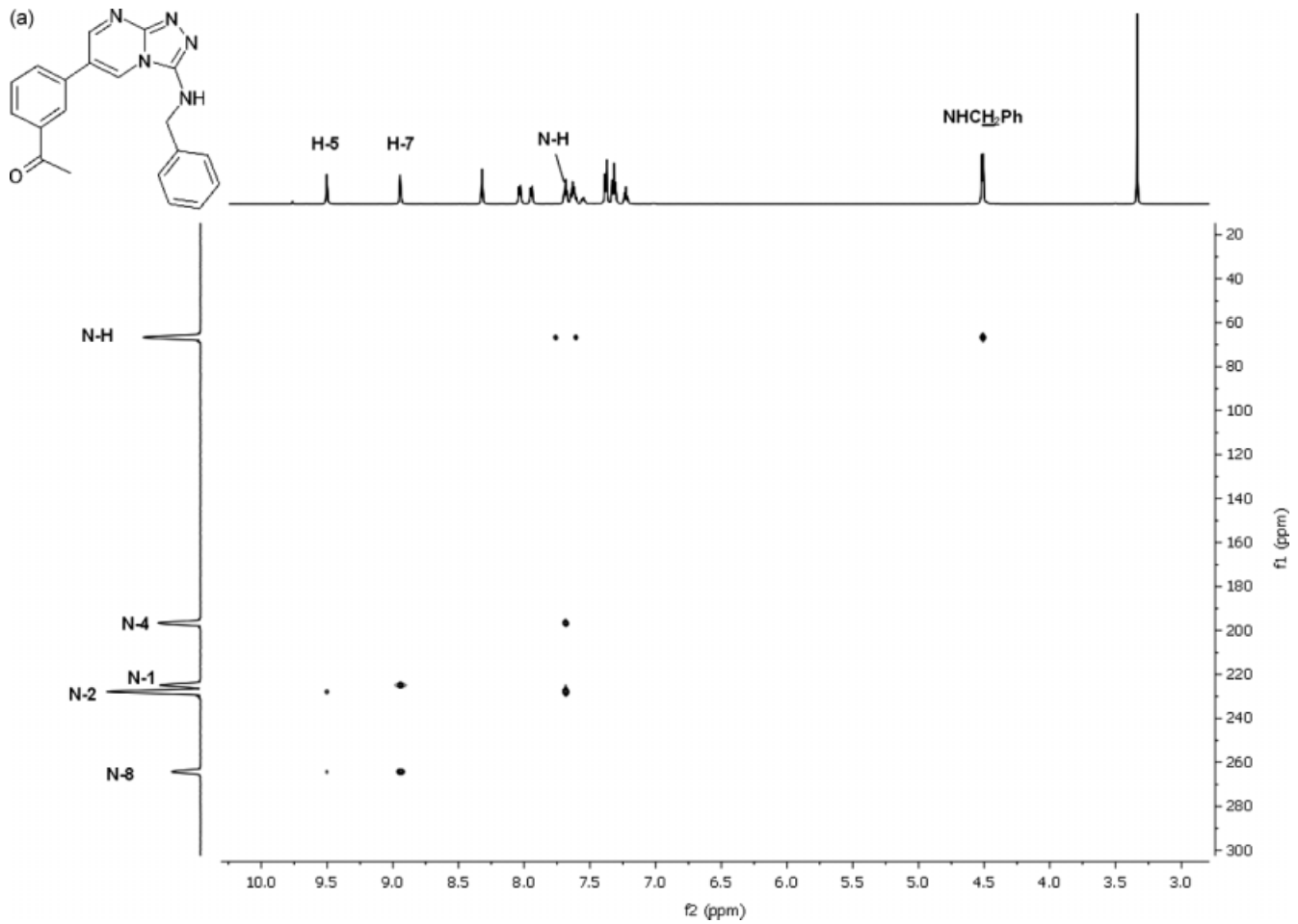


Figure 3. (a) ^{1}H–^{15}N HMBC spectrum of compound **2d** (DMSO-d_6, 700 MHz) recorded with an evolution delay τ of 62.5 ms for the optimal detection of long-range J_{H-N} of 8 Hz. (b) ^{1}H–^{15}N HMBC spectrum of compound **1d** (DMSO-d_6, 700 MHz) recorded with an evolution delay τ of 166 ms for the optimal detection of long-range J_{H-N} of 3 Hz. A ^{1}H–^{15}N four-bond correlation between H-7 and N-1 can be seen.

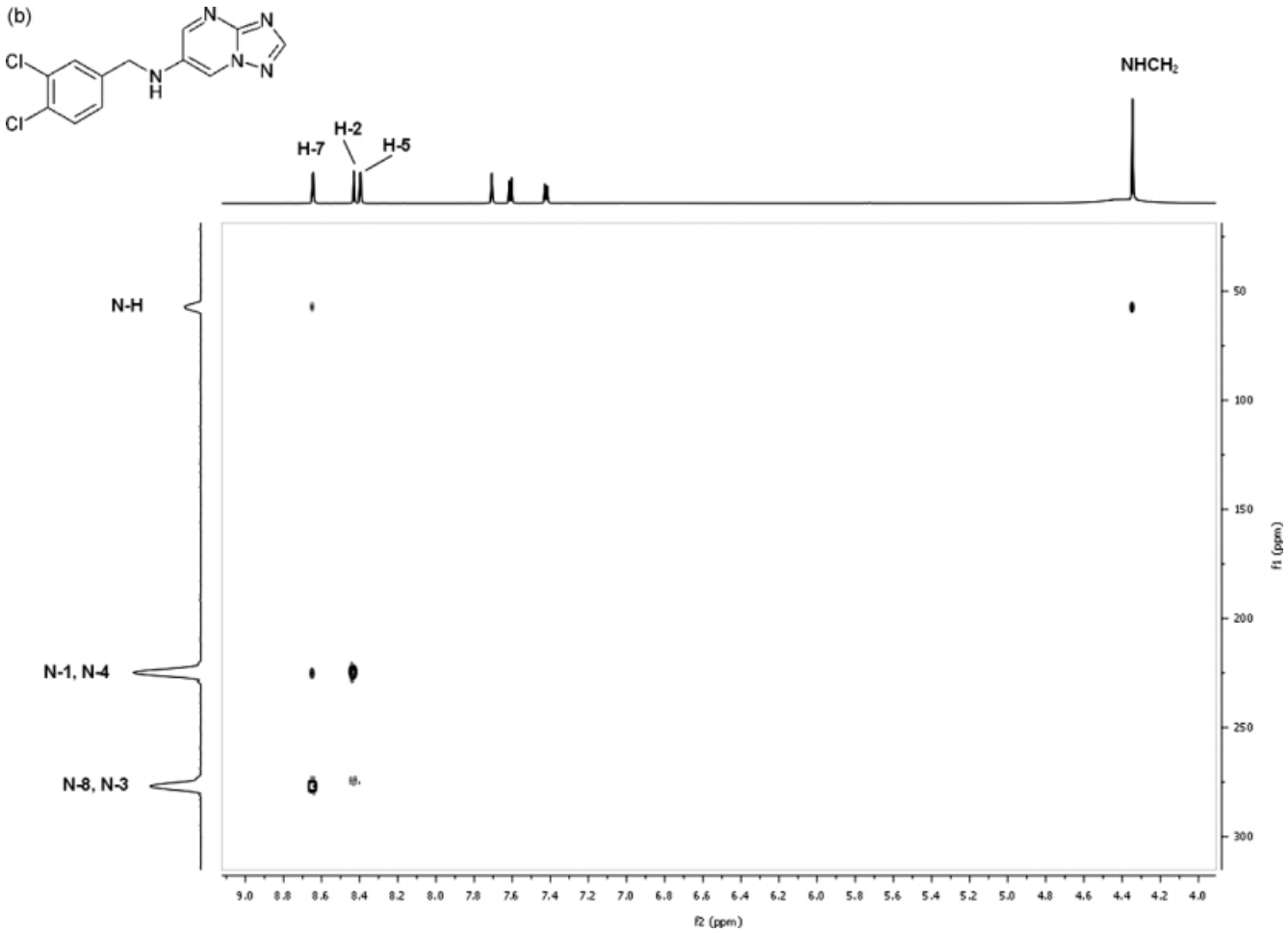


Figure 3. (*Continued*).

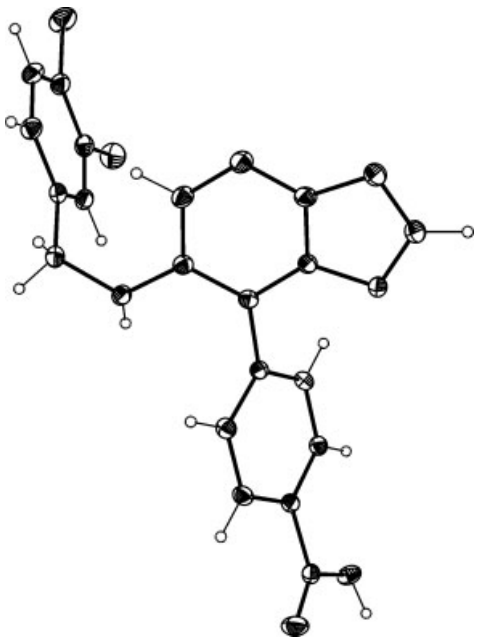
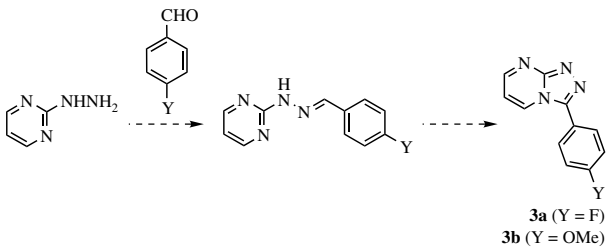


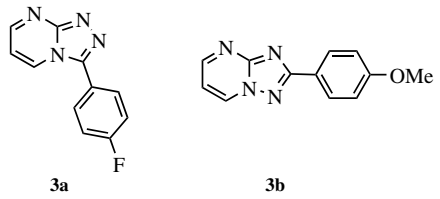
Figure 4. X-ray crystal analysis of compound **1e**. (CCDC 766900 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif).



Scheme 2. Planned synthesis for compounds **3a** and **3b**.[17,38]

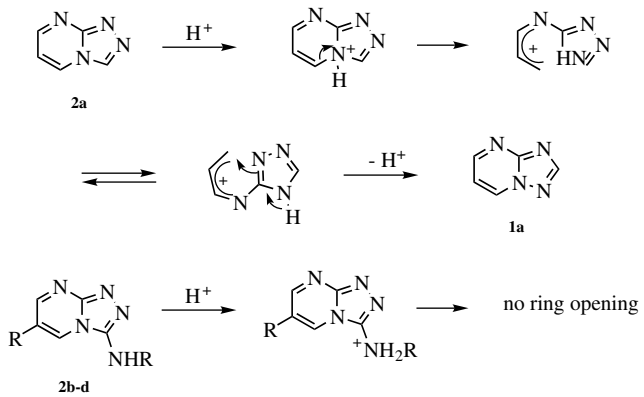
be used to verify the structure of some reported [1,2,4]triazolo[4,3-a]pyrimidine compounds,[12,14–17] in particular those cases prone to undergo Dimroth-type isomerisation. As we have seen in the case of **3b**, in which a rearrangement to a [1,2,4]triazolo[1,5-a]pyrimidine had occurred, ^{15}N NMR data can easily settle this point (Fig. 5).

Finally, from the regioselectivity found in the condensation reactions of malonoaldehydes with either 3-amino-[1,2,4]-triazole or 3,5-diamino-[1,2,4]-triazole precursors (Scheme 1), we rationalised that structures **1** result after an acid-mediated Dimroth-type rearrangement of a previously formed derivative of the parent structure **2** (Scheme 3). For **2b–d**, however, protonation occurs at the exocyclic amino group, and thus no isomerisation to **1** takes place. It should be mentioned at this point that other authors



3a 3b

Figure 5. Structure of compounds **3a** and **3b**.



Scheme 3. Rearrangement of [1,2,4]-triazolo[4,3-a]pyrimidines.

isolated 3-amino-[1,2,4]triazolo[4,3-a]pyrimidines from the condensation of a 3,5-diamino-[1,2,4]-triazole and pentane-2,4-dione, and that conversion to the corresponding [1,2,4]triazolo[1,5-a]pyrimidine was achieved in another step.[39]

Conclusions

The structural determination of some [1,2,4]triazolo[4,3-a]pyrimidine and [1,2,4]triazolo[1,5-a]pyrimidine derivatives was unambiguously achieved from ^{15}N NMR. The observed isomerisation of [1,2,4]triazolo[4,3-a]pyrimidine structure to the regioisomeric [1,2,4]triazolo[1,5-a]pyrimidine scaffold was rationalised on the basis of a Dimroth rearrangement. The result of this isomerisation was clarified by measuring the ^{15}N chemical shifts. The obtained results validate $^{1}H-^{15}N$ HMBC as a useful tool for regioisomer differentiation. Further studies, including comparison of experimental ^{15}N chemical shifts to theoretical data, are currently in progress.

Experimental

All samples were dissolved in DMSO-d_6 unless otherwise stated. The concentration ranged from 60 to 200 mM. Spectra were recorded at 25 °C on a Bruker AVANCE 700 spectrometer fitted with a QXI 700 MHz S4 probe and operating at 700.13, 176.05 or 70.94 MHz (^{1}H, ^{13}C and ^{15}N frequencies, respectively). Chemical shifts are referred to external TMS (^{1}H and ^{13}C, 0.00 ppm) or liquid NH_3 (^{15}N, 0.00 ppm). The ^{1}H 90° hard pulse was calculated for every sample. No loss of signal resolution due to tentative aggregation effects was observed in the concentration range used here. The standard Bruker pulse sequence *hmbcgplpndqf* was used for the $^{1}H-^{13}C$ and $^{1}H-^{15}N$ HMBC experiments. The spectral sizes for the $^{1}H-^{13}C$ HMBC experiments were 8389.26 Hz (11.98 ppm) × 39 062.50 Hz (221.86 ppm) with 128 increments in f_1 and 16 transients per increment (total experiment time 45 min). Delays were adjusted for one-bond ($^{1}J_{HC}$) couplings of 145 Hz and multiple-bond ($^{n}J_{HC}$) couplings of 10 Hz. The $^{1}H-^{15}N$ HMBC experiments were carried out in duplicate, with delays adjusted for one-bond ($^{1}J_{HN}$) couplings of 90 Hz and multiple-bond ($^{n}J_{HN}$) couplings of either 8 or 3 Hz. In both cases, the spectral sizes were 8389.26 Hz (11.98 ppm) × 39 062.50 Hz (550.4 ppm) with 512 increments in f_1 and 32 transients per increment (total experiment time 6 h 28 min). All HMBC spectra were presented in magnitude mode. Proton-decoupled ^{13}C NMR spectra were recorded on a Bruker AVANCE II 300 spectrometer fitted with a QNP 300 MHz S1 probe and operating at 300.13 and 75.47 MHz (^{1}H and ^{13}C frequencies, respectively). All NMR data were processed with the MestReNova software (version 6.0.4–5850, Mestrelab Research S. L., Santiago de Compostela, Spain). ESI+ high-resolution mass spectra (HRMS) were obtained with a Bruker Daltonics maXis UHR-TOF system.

Preparation of [1,2,4]triazolo[1,5-a]pyrimidine (1a)

3-Amino-[1,2,4]-triazole (1.0 g, 11.89 mmol), 1,1,3,3-tetramethoxypropane (2.16 ml, 13.08 mmol) and AcOH (10 ml) were refluxed for 1 h.[8] Evaporation of excess acetic acid and column chromatography (silica, 2–20% MeOH in CH_2Cl_2) gave **1a** as a yellow solid (84%). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1).

Preparation of 6-bromo-[1,2,4]triazolo[1,5-a]pyrimidine (1b)

3-Amino-[1,2,4]-triazole (1.0 g, 11.89 mmol) and bromomalonaldehyde (2.33 g, 15.46 mmol) were refluxed in EtOH (10 ml) for 4 h.[35] Solvent evaporation and column chromatography (silica, 0–20% MeOH in CH_2Cl_2) gave **1b** as a pale yellow solid (84%). ^{1}H NMR (700 MHz, $CDCl_3$-MeOD 4 : 1) (Table 1). ^{13}C NMR (75 MHz, $CDCl_3$–MeOD 4 : 1) (Table 2).

Preparation of 6-(morpholin-4-yl)-[1,2,4]triazolo[1,5-a]pyrimidine (1c)

1b (0.1 g, 0.5 mmol) was refluxed in morpholine (2.5 ml) for 3 h. Removal of excess morpholine and column chromatography (silica, 0–20% MeOH in CH_2Cl_2) gave **1c** as a white solid (73%). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1). ^{13}C NMR (75 MHz, DMSO-d_6) (Table 2). HRMS (ESI+): $C_9H_{11}N_5O^+$ requires *m/z* 205.0964, $C_9H_{12}N_5O^+$ ($M + H^+$) requires *m/z* 206.1036; found 206.1017.

Preparation of 6-(3,4-dichlorophenylmethyl)amino-[1,2,4]triazolo[1,5-a]pyrimidine (1d)

1b (0.05 g, 0.25 mmol) and 3,4-dichlorobenzylamine (0.15 ml, 1.13 mmol) were stirred at 100 °C for 10 min. After cooling, conventional work-up (CH_2Cl_2) and column chromatography (silica, 0–20% MeOH in CH_2Cl_2) gave **1d** as a white solid (23%). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1). ^{13}C NMR (75 MHz, DMSO-d_6) (Table 2). HRMS (ESI+): $C_{12}H_9Cl_2N_5^+$ requires *m/z* 293.0235, $C_{12}H_{10}Cl_2N_5^+$ ($M + H^+$) requires *m/z* 294.0308; found 294.0330.

Preparation of 5-(4-carboxyphenyl)-6-(3,4-dichlorophenylmethyl)amino-[1,2,4]triazolo[1,5-a]pyrimidine (1e)

To **1d** (100 mg, 0.34 mmol) in $CHCl_3$ (2 ml) was added N-bromo Succinimide (61 mg, 0.34 mmol). After 10 min stirring at room temperature (RT) for 10 min, the mixture was extracted with $CHCl_3$:^{i}PrOH (3 × 10 ml). Purification by column chromatography

(silica, 0–20% MeOH in CH_2Cl_2) gave a pale yellow solid (50 mg). This solid was immediately suspended in water (1 ml), and 3-acetylphenylboronic acid (0.029 g, 0.17 mmol), $Pd(PPh_3)_4$ (0.015 g, 0.013 mmol), sodium carbonate (0.043 g, 0.40 mmol) and TBAB (0.043 g, 0.13 mmol) were added. Microwave irradiation (120 °C, 200 W) for 1 h and column chromatography (silica, 0–20% MeOH in CH_2Cl_2) afforded **2e** as a yellowish solid (4% overall yield). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1). ^{13}C NMR (75 MHz, DMSO-d_6) (Table 2). HRMS (ESI+): $C_{19}H_{13}Cl_2N_5O_2^+$ requires *m/z* 413.0446, $C_{19}H_{14}Cl_2N_5O_2^+$ ($M + H^+$) requires *m/z* 414.0519; found 414.0531.

Preparation of [1,2,4]triazolo[4,3-a]pyrimidine (2a)

2-Hydrazinopyrimidine (0.5 g, 4.54 mmol, 1 equiv.) was suspended in triethyl orthoformate (4 ml) and the reaction mixture was heated at 110 °C for 45 min (open to the air to allow evaporation of EtOH).[33] On cooling, the resulting yellow suspension was filtered and rinsed with EtOH to give **2a** (66%) as a yellow solid. ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1).

Preparation of 3-amino-6-bromo-[1,2,4]triazolo[4, 3-a]pyrimidine (2b)

3,5-Diamino-[1,2,4]-triazole (0.05 g, 0.51 mmol) and bromomalonaldehyde (0.1 g, 0.66 mmol) in EtOH (1 ml) were irradiated with microwaves for 30 min (200 W, 130 °C). Column chromatography (silica, 0–30% MeOH in CH_2Cl_2) gave **2b** as a yellowish solid (33%). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1). ^{13}C NMR (75 MHz, DMSO-d_6) (Table 2). HRMS (ESI+): $C_5H_4BrN_5^+$ requires *m/z* 212.9650, $C_5H_5BrN_5^+$ ($M + H^+$) requires *m/z* 213.9723; found 213.9710.

Preparation of 3-benzylamino-6-bromo-[1,2,4]triazolo[4, 3-a]pyrimidine (2c)

5-Amino-3-benzylamino-[1,2,4]triazole (0.5 g, 2.64 mmol), bromomalonaldehyde (0.5 g, 3.17 mmol) and EtOH (5 ml) in acetonitrile (20 ml) were refluxed for 15 h. Solvent evaporation and column chromatography (silica, 0–30% MeOH in CH_2Cl_2) gave **2c** as a beige solid (51%). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1). ^{13}C NMR (75 MHz, DMSO-d_6) (Table 2). HRMS (ESI+): $C_{12}H_{10}BrN_5^+$ requires *m/z* 303.0120, $C_{12}H_{11}BrN_5^+$ ($M + H^+$) requires *m/z* 304.0192; found 304.0180.

Preparation of 6-(3-acetylphenyl)-3-benzylamino-[1,2,4]triazolo[4,3-a]pyrimidine (2d)

2c (0.2 g, 0.66 mmol), 3-acetylphenylboronic acid (0.14 g, 0.86 mmol), $Pd(PPh_3)_4$ (0.076 g, 0.066 mmol), sodium carbonate (0.21 g, 1.97 mmol) and tetrabutylammonium bromide (0.21 g, 0.66 mmol) in H_2O (4 ml) were irradiated with microwaves (120 °C, 200 W) for 50 min. Conventional work-up (CH_2Cl_2) and column chromatography (silica, 0–20% MeOH in CH_2Cl_2) gave **2d** as a white solid (11%). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1). ^{13}C NMR (75 MHz, DMSO-d_6) (Table 2) HRMS (ESI+): $C_{20}H_{17}N_5O^+$ requires *m/z* 343.1433, $C_{20}H_{18}N_5O^+$ ($M + H^+$) requires *m/z* 344.1506; found 344.1495.

Preparation of 3-(4-fluorophenyl)-[1,2,4]triazolo[4, 3-a]pyrimidine (3a)

2-Hydrazinopyrimidine (0.5 g, 4.54 mmol) and 4-fluorobenzaldehyde (0.51 ml, 4.77 mmol) were stirred in absolute EtOH (15 ml) for 3 h at RT. A white solid (255 mg) was collected by filtration and dissolved in dichloromethane (8 ml). Iodobenzene diacetate (380 mg, 1.17 mmol) was added portion-wise over 5 min. Stirring for 1 h at RT, solvent evaporation and trituration of the solid residue with petroleum ether afforded **3a** as a pale yellow solid (51%). ^{1}H NMR (700 MHz, DMSO-d_6) (Table 1). ^{13}C NMR (75 MHz, DMSO-d_6) (Table 2). HRMS (ESI+): $C_{11}H_7FN_4^+$ requires *m/z* 214.0655, $C_{11}H_8FN_4^+$ ($M + H^+$) requires *m/z* 215.0727; found 215.0745.

Preparation of 3-(4-methoxyphenyl)-[1,2,4]triazolo[1, 5-a]pyrimidine (3b)

2-Chloropyrimidine (0.5 g, 4.37 mmol, 1.0 equiv.) and 4-methoxybenzhydrazide (0.725 g, 4.37 mmol) were suspended in absolute EtOH (7 ml) and refluxed for 24 h. After cooling, a pale yellow solid (530 mg) was collected by filtration. This compound was suspended in xylene (5 ml), phosphorus oxychloride (1 ml) was added and the mixture was refluxed for 3 h. Careful basification with 32% ammonia, extraction with Et_2O and column chromatography (silica, 2–15% MeOH in CH_2Cl_2) afforded **3b** as a white solid (19%, combined yield). ^{1}H NMR (700 MHz, $CDCl_3$–MeOD 4 : 1) (Table 1). ^{13}C NMR (75 MHz, $CDCl_3$–MeOD 4 : 1) (Table 2). HRMS (ESI+): $C_{12}H_{10}N_4O^+$ requires *m/z* 226.0855, $C_{12}H_{11}N_4O^+$ ($M + H^+$) requires *m/z* 227.0927; found 227.0935.

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References

[1] G. Fischer, *Adv. Heterocycl. Chem.* **2008**, *95*, 143.
[2] A. Maresta, M. Balducelli, L. Cantini, A. Casari, R. Chioin, M. Fabbri, A. Fontanelli, P. A. Monici Preti, S. Repetto, S De Servi, E. Varani, *Circulation* **1994**, *20*, 2710.
[3] M. M. A. El-Gendy, M. Shaaban, K. A. Shaaban, A. M. El-Bondkly, H. Laatsch, *J. Antibiot.* **2008**, *61*, 149.
[4] M. R. Shaaban, T. S. Saleh, A. M. Farag, *Heterocycles* **2007**, *71*, 1765.
[5] A. M. Farag, *Heterocycles* **2008**, *75*, 3005.
[6] S. A. Yamashkin, N. Y. Kucherenko, M. A. Yurovskaya, *Chem. Heterocycl. Compd.* **1997**, *33*, 499.
[7] E. M. Gizatullina, V. G. Kartsev, *Chem. Heterocycl. Compd.* **1993**, *29*, 1369.
[8] M. Künstlinger, E. Breitmaier. *Synthesis* **1983**, 44.
[9] D. J. Brown, T. Nagamatsu, *Aust. J. Chem.* **1977**, *30*, 2515.
[10] E. S. H. El Ashry, Y. El Kilany, N. Rashed, H. Assafir, *Adv. Heterocycl. Chem.* **1999**, *75*, 79.
[11] M. A. E. Shaban, A. E. A. Morgaan, *Adv. Heterocycl. Chem.* **1999**, *73*, 131.
[12] T. Novinson, T. Okabe, R. K. Robins, P. Dea, *J. Heterocycl. Chem.* **1975**, *12*, 1187.
[13] T. La Noce, A. M. Giuliani, *Tetrahedron* **1978**, *34*, 2927.
[14] A. S. Shawali, A. H. Elghandour, A. R. Sayed, *Synth. Commun.* **2001**, *31*, 731.
[15] M. I. MušiŠ, B. VerŠek, *Synth. Commun.* **2001**, *31*, 1511.
[16] M. R. Shaaban, T. M. A. Eldebss, A. F. Darweesh, A. M. Farag, *J. Heterocycl. Chem.* **2008**, *45*, 1739.
[17] O. Prakash, V. Bhardwaj, R. Kumar, P. Tyagi, A. R. Aneja, *Eur. J. Med. Chem.* **2004**, *39*, 1073.
[18] C. Bulow, K. Hass, *Berichte.* **1909**, *42*, 4638.
[19] G. E. Martin, P. A. Irish, B. D. Hilton, K. A. Blinov, A. J. Williams, *Magn. Reson. Chem.* **2007**, *45*, 624.

[20] G. E. Martin, B. D. Hilton, K. A. Blinov, A. J. Williams, *J. Nat. Prod.* **2007**, *70*, 1966.
[21] H. S. El Khadem, J. Kawai, D. L. Swartz, *Heterocycles* **1989**, *28*, 239.
[22] G. E. Martin, C. E. Hadden, *J. Nat. Prod.* **2000**, *63*, 543.
[23] R. Marek, A. Lycka, E. Kolehmainen, E. Sieväne, J. Tousek, *Curr. Org. Chem.* **2007**, *11*, 1154.
[24] G. E. Martin, A. J. Williams, *Annu. Rep. NMR Spectrosc.* **2005**, *55*, 1.
[25] I. Delso, T. Tejero, *Tetrahedron Lett.* **2007**, *48*, 4101.
[26] M. Köck, J. Junker, T. Lindel, *Org. Lett.* **1999**, *1*, 2041.
[27] S. Fukuzawa, S. Matsunaga, N. Fusetami, *Tetrahedron Lett.* **1996**, *37*, 1447.
[28] P. A. McDonnell, A. D. Gauthier, M. P. Ferro, *Magn. Reson. Chem.* **1998**, *36*, 35.
[29] N. Kawamura, R. Sawa, Y. Takahashi, K. Isshiki, T. Sawa, H. Naganawa, T. Takeuchi, *J. Antibiot.* **1996**, *49*, 651.
[30] P. M. Chaudhary, S. R. Chavan, M. Kavitha, S. P. Maybhate, S. R. Deshpande, A. P. Likhite, P. R. Rajamohanan, *Magn. Reson. Chem.* **2008**, *46*, 1168.
[31] L. Stefaniak, M. Witanowski, U. Mahmoud, J. D. Roberts, G. A. Webb, *J. Crystallogr. Spectrosc. Res.* **1989**, *19*, 159.
[32] E. Szlyk, A. Grodzicki, L. Pazderski, E. Bednarek, B. Kamienski, *Polyhedron* **2000**, *19*, 965.
[33] W. W. Paudler, L. S. Helmick, *J. Heterocycl. Chem.* **1966**, *3*, 269.
[34] G. L. Rusinov, P. V. Plekhanov, A. U. Ponomareva, O. N. Chupakhin, *Mendeleev Commun*. **1999**, 233.
[35] M. Witanowski, L. Stefaniak, G. A. Webb, *Ann. Rep. NMR Spectrosc.* **1993**, *25*, 1.
[36] G. C. Levi, R. L. Lichter, in *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*, Wiley: New York, **1979**, pp 114.
[37] E. Szlyk, L. Pazderski, I. Lakomska, L. Kozerski, J. Sitkowski, *Magn. Reson. Chem.* **2002**, *40*, 529.
[38] M. Ciesielski, D. Pufky, M. Döring, *Tetrahedron* **2005**, *61*, 5942.
[39] B. C. Bishop, H. Marley, P. N. Preston, S. H. B. Wright. *J. Chem. Soc., Perkin Trans. 1*, **1999**, 1527.

