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Identification of Aminopyrimidine Regioisomers via Line Broadening Effects in ^{1}H and ^{13}C NMR Spectroscopy

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Substituted mono- and diamino-pyrimidines were synthesized as part of our medicinal chemistry programmes. Primary amines substituted at the 4-position exhibited room-temperature line broadening effects in both ^{1}H and ^{13}C NMR spectroscopy due to the presence of rotamers, but these effects were not observed for substituents in the 2-position. This provided a simple diagnostic tool for the identification of regioisomers, a determination which would otherwise have required two-dimensional experiments.

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Introduction

Substituted pyrimidines are an important class of compounds among medicinal chemistry programmes due to their relative ease of manipulation and prevalence as effective antagonists/agonists in biological systems.[1,2] These heterocycles are amongst the most extensively investigated organic molecules. We are currently engaged in varied medicinal chemistry research programmes requiring simple mono- and disubstituted aminopyrimidines. For example, we have investigated their utility as competitive antagonists of corticotropin releasing hormone.[3,4]

Crucial to these synthetic studies was the development of simple and rapid protocols for complete characterization of the different regioisomers that might form during our synthetic transformations. Surprisingly, there are few literature precedents to facilitate rapid assignment of regiochemistry. The majority of NMR investigations of simple pyrimidines were performed during the early era of NMR structure elucidation on low-resolution instruments, and were usually without reported carbon spectra.[5–7] These investigations focussed on compound sets that provided detailed data on tautomeric pyrimidines. Dynamic NMR studies have in some instances indicated varying degrees of spectral line broadening, an effect that is almost exclusively reported for large protein fragments and nucleoside-derived structures.[8,9] Herein we report on a surprisingly simple and ubiquitous methodology for the assignment of regiochemistry in a small library of aminopyrimidines.

Results and Discussion

The majority of compounds examined in this study were synthesized by a 5 M lithium perchlorate–diethyl ether (LPDE) mediated displacement.[10–12] Briefly, dichloropyrimidines **A**–**D** (Table 1) were stirred overnight at room temperature in 5 M LPDE with excess aliphatic amine (5–10 equiv.); extractive workup gave, in all instances, monosubstituted products in moderate-to-good yields (25–64%). Regioisomers of asymmetrical pyrimidines (**C** and **D**) afforded products with a higher selectivity of C4 substitution over C2, typically in ratios of 7–10 : 1. Cyclic aliphatic amines did not react in this system; however, these derivatives were synthesized by nucleophilic substitution using NaH in tetrahydrofuran (THF). Disubstituted products were synthesized by refluxing in THF, and required separation from monosubstituted products, affording only moderate yields.[13]*

C4-monosubstituted derivatives of **A**, **B**, and **C** exhibited significant room-temperature line broadening in both the ^{13}C NMR (at C5) and ^{1}H NMR spectra (at the α-carbon of the amine substituent). No such effects were observed, with the C2 regioisomer facilitating unambiguous assignment of the relative regiochemistry (Scheme 1), thus obviating HMBC and HMQC experiments.† Line broadening was noted for various linear and cyclic aliphatic amines and benzylamine. Derivatives of secondary amines, anilines, e.g., 2,4,6-trichloroaniline, and the derivatives of compound **D** did not exhibit any line broadening irrespective of substitution pattern. Diaminopyrimidines did not exhibit line broadening

* We have previously reported the experimental details for compounds **A**–**D**, **1**–**3**, **5**–**7**, **20**–**25**, **27**–**33**, **39**, and **40**. The experimental details for all other compounds may be found in the Accessory Materials.

† Assignment of C2 or C4 substituents were confirmed by HMBC and HMQC experiments, and in all instances confirmed the above assignments based on line broadening effects.

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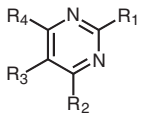
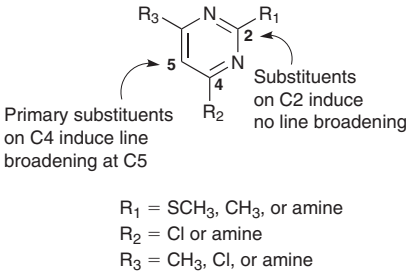
Table 1. Pyrimidines synthesized including their parent compounds and method of synthesis
Methods: (*a*) 5 M LPDE, room temp., 24 h; (*b*) neat, room temp., 24 h; (*c*) NaH/THF, reflux, 18 h; (*d*) EtOH, sealed tube, 160°C, 18 h; (*e*) THF, sealed tube, 160°C, 18 h; (*f*) H_2O, reflux, 72 h; (*g*) THF, 50°C, 24 h



	Parent	R_1	R_2	R_3	R_4	Method	Broad
			Parent compounds				
A	—	CH_3	Cl	H	Cl	—	—
B	—	SCH_3	Cl	H	Cl	—	—
C	—	Cl	Cl	H	CH_3	—	—
D	—	Cl	Cl	CH_3	H	—	—
			Derivatives of symmetrical pyrimidines				
1	**A**	CH_3	Pr^nNH	H	Cl	*a*	yes
2	**A**	CH_3	Bu^iNH	H	Cl	*a*	yes
3	**A**	CH_3	$Pr^nNCH_2Pr^c$	H	Cl	*a*	no
4	**B**	SCH_3	Et^nNH	H	Cl	*d*	yes
5	**B**	SCH_3	Pr^nNH	H	Cl	*a*	yes
6	**B**	SCH_3	Bu^iNH	H	Cl	*a*	yes
7	**B**	SCH_3	$Pr^nNCH_2Pr^c$	H	Cl	*a*	no
8	**B**	SCH_3	*c*-hexylNH	H	Cl	*c*	yes
9	**B**	SCH_3	*c*-pentylNH	H	Cl	*c*	yes
10	—	H	*c*-hexylNH	H	Cl	*f*	yes
11	—	H	*c*-hexylNH	H	$Pr^nNCH_2Pr^c$	*f*	yes
			Derivatives of asymmetrical pyrimidines (diagnostic)				
12	**C**	$(Me)_2NCH_2CH_2NH$	$(Me)_2NCH_2CH_2NH$	H	CH_3	*e*	yes
13	**C**	$HOCH_2CH_2NH$	Cl	H	CH_3	*g*	no
14	**C**	Cl	$HOCH_2CH_2NH$	H	CH_3	*g*	yes
15	**C**	$HOCH_2CH_2NH$	$HOCH_2CH_2NH$	H	CH_3	*e*	yes
16	**C**	*c*-hexylNH	Cl	H	CH_3	*f*	no
17	**C**	Cl	*c*-hexylNH	H	CH_3	*f*	yes
18	**C**	*n*-hexylNH	Cl	H	CH_3	*a*	no
19	**C**	Cl	*n*-hexylNH	H	CH_3	*a*	yes
20	**C**	Pr^nNH	Cl	H	CH_3	*a*	no
21	**C**	Cl	Pr^nNH	H	CH_3	*a*	yes
22	**C**	Bu^iNH	Cl	H	CH_3	*a*	no
23	**C**	Cl	Bu^iNH	H	CH_3	*a*	yes
24	**C**	BnNH	Cl	H	CH_3	*e*	no
25	**C**	Cl	BnNH	H	CH_3	*e*	yes
			Other compounds				
26	**C**	*N*-morpholino	*N*-morpholino	H	CH_3	*b*	no
27	**C**	Cl	$Pr^nNCH_2Pr^c$	H	CH_3	*a*	no
28	**C**	$Pr^nNCH_2Pr^c$	Cl	H	CH_3	*a*	no
29	**D**	Cl	Pr^nNH	CH_3	H	*a*	no
30	**D**	Cl	Bu^iNH	CH_3	H	*a*	no
31	**D**	Bu^iNH	Cl	CH_3	H	*a*	no
32	**D**	Cl	$Pr^nNCH_2Pr^c$	CH_3	H	*a*	no
33	**D**	$Pr^nNCH_2Pr^c$	Cl	CH_3	H	*a*	no
34	**C**	$Pr^nNCH_2Pr^c$	*c*-hexylNH	H	CH_3	*e*	yes
35	**C**	$(Et)_2N$	*c*-hexylNH	H	CH_3	*e*	yes
36	**C**	Cl	$(2,3\text{-}Me_2)$*c*-hexylNH	H	CH_3	*f*	yes
37	**C**	$Pr^nNCH_2Pr^c$	$(2,3\text{-}Me_2)$*c*-hexylNH	H	CH_3	*e*	no
38	**C**	$(Et)_2N$	$(2,3\text{-}Me_2)$*c*-hexylNH	H	CH_3	*e*	no
39	**C**	Cl	2,4,6-TCA	H	CH_3	*c*	no
40	**C**	2,4,6-TCA	Cl	H	CH_3	*c*	no

from a primary amine derivative if the other amine was an aniline, but it was observed when the group was an alicyclic or aliphatic amine.

While simple pyrimidines with tautomeric groups have been studied extensively in the last 20 years, there is no evidence that pyrimidines with simple amine substituents will tautomerize except via protonation, a process usually facilitated by the addition of trifluoroacetic acid or methanesulfonic acid.[2] We therefore hypothesized that the observed line broadening was a result of the presence of rotamers.



Scheme 1. Requirements for line broadening with amino-substituted pyrimidines.

A previous structural and conformational study of large substituted triazines with allylamine and piperazine functional groups observed line broadening effects analogous to that observed in the pyrimidines.[14,15] Restricted rotation of the Ar–N bond was observed at room temperature in the ^{1}H, ^{13}C, and ^{15}N NMR spectra. In contrast to the pyrimidines, ^{13}C NMR line broadening was only observed from the α-carbon of the amine substituent. Line broadening was also observed from the ^{15}N NMR spectrum at N3 and N5. These nitrogens in the triazine ring are spatially analogous to C5 of the pyrimidine ring and the results were in parallel to our line broadening observations in the ^{13}C NMR spectrum. Coupled with crystal structures of the mono- and bis-methanesulfonates, it was determined that the line broadening was a result of the equilibrium between two (or more) rotamer conformations.[14,15]

As with other rotameric studies, variation of temperature (up or down) affected NMR peak shape. The most significant changes were observed in the ^{13}C NMR spectrum for the C5 peak. Broad doublets indicating a high- and low-energy conformation were often observed at temperatures below 298 K (Fig. 1*a*). At 313 K almost all the compounds examined had passed the coalescence temperature (T_c)[‡] and exhibited a sharp peak due to the averaged environment. Only large amine substituents induced ^{13}C NMR line broadening of the α-carbon of the amine substituent, but resolution into two peaks was never observed over the temperature range available (**8**–**11**, **17**, **35**, **36**). A small degree of line broadening was occasionally observed from C4 and C6 of the pyrimidine ring.

There was also a strong contribution to the rotamer equilibrium by a C2 or C6 methyl (or SMe) substitution in our compound set. Simple H substitution at C2 (**10**, **11**) resulted in coalescence temperatures that were significantly lower (<283 K) than all other derivatives. Changing the solvent to $(CD_3)_2SO$ (Fig. 1*b*), using **4** as a model compound, significantly perturbed the system so that an increased T_c of C5 was observed. Additionally, the two C5 peaks at low temperature were well resolved, and conversion into one sharp peak at higher temperatures occurred over a narrower range than in $CDCl_3$. In our attempts to explore our hypothesis, detailed conformational analysis using *Spartan 02* on regioisomers **22** and **23** was conducted. We believed that this would aid in explaining the line broadening observed.[§]

As illustrated in Fig. 2, regioisomer **22**, which did not exhibit line broadening, has fewer distinct conformations (Fig. 2, top) than **23**, which displays significant line broadening (Fig. 2, bottom). There was only a small difference between the calculated maximum and minimum energy conformers (approx. 7 kJ mol^{-1}) of **22**. In each conformation the amine N–H bond was calculated to be in the plane of the pyrimidine ring, while the rest of the alkyl chain exhibited substantial variation between each conformation.

The energy difference of 20 kJ mol^{-1} between the highest and lowest energy conformers of **23** represents a reasonable barrier to rotation at the temperatures over which line broadening is observed. While more conformers were calculated, they could be classified into three sets with distinctive features. The high-energy conformers (**11**–**14**) always oriented the amine N–H towards CH(5) and in the same plane as the aryl ring (Fig. 2*b*, bottom). The low-energy conformers (**1**–**5**) have the amine N–H oriented away from CH(5) and the α-carbon of the amine in close proximity (Fig. 2*a*, bottom). All other conformations orient the N–H towards CH(5), though slightly out of plane with the aryl ring, with large differences in the orientation of the alkyl chain. The relative populations of each of the conformations enabling a clearer picture of conformational mobility could not be calculated within the program.

Primary aniline derivatives [e.g., 2,4,6-trichloroaniline (2,4,6-TCA) **39**] do not exhibit line broadening at room temperature. This is explained by observations of a preferred stable orthogonal orientation of the aryl ring of the aniline to the plane of the pyrimidine ring.[16] Conformer distribution calculations of **39** (data not shown) resulted in a 1.8 kJ mol^{-1} difference in energy between the highest and lowest energy conformer. In all conformations the aniline ring was orthogonal to the pyrimidine ring; this provided a strong argument to the previously reported single preferred conformation.

Analysis of the conformations calculated by *Spartan 02* revealed that the high- and low-energy conformations were analogous to the triazine crystal structures previously reported.[14,15] The key difference, we propose, is that it is the interactions between CH(5) of the pyrimidine ring and the N–H group and α-carbon of the amine substituent that induces the line broadening observed. *Spartan 02* was able to calculate bond lengths and angles with a high degree of accuracy at high levels of theory, especially for relatively simple compounds such as our set of pyrimidines. Calculated hydrogen distances between the highest and lowest

[‡] Coalescence temperature, for the purpose of qualitative analysis, is reported as the temperature at which coalescence is actually observed; that is, a very low, broad baseline hump is observed. Alternatively, T_c is estimated by extrapolating between the two measurements at which it occurred.

[§] Compounds were geometry-optimized using the Møller–Plesset function at MP2 level of theory. Conformer distributions were calculated using molecular mechanics at the MMFF level of theory. Compounds were rotated by 30° increments for all available torsion points of the C(aryl)–N bond of the amine substituent. The program allowed for a maximum of 20000 conformations and a maximum ΔE of 100 kJ mol^{-1} difference. Conformations that had similar energies and van der Waals overlap were treated as identical by the programme, which reduced the set of 'unique' conformers reported. Each conformer can be regarded as representative of a number of conformations with the same energy and similar structural space.

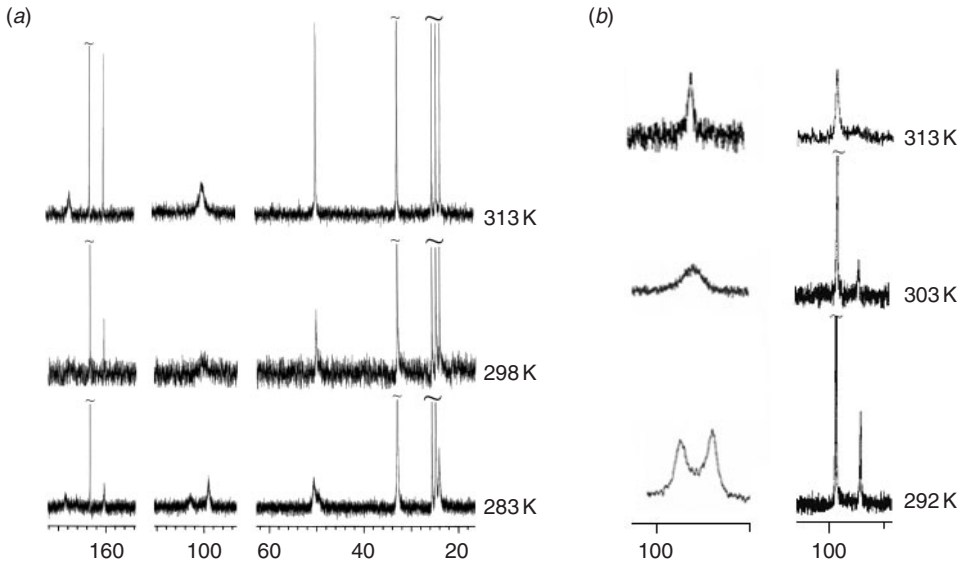


Fig. 1. Spectra of **17**. (*a*) Effect of temperature on line broadening for C5 (approx. 100 ppm), α-carbon of amine (approx. 50 ppm), and other pyrimidine ring carbon (approx. 160 ppm) signals in $CDCl_3$. (*b*) Effect of temperature increase on C5 signal of **4** in $CDCl_3$ (left) and $(CD_3)_2SO$ (right).

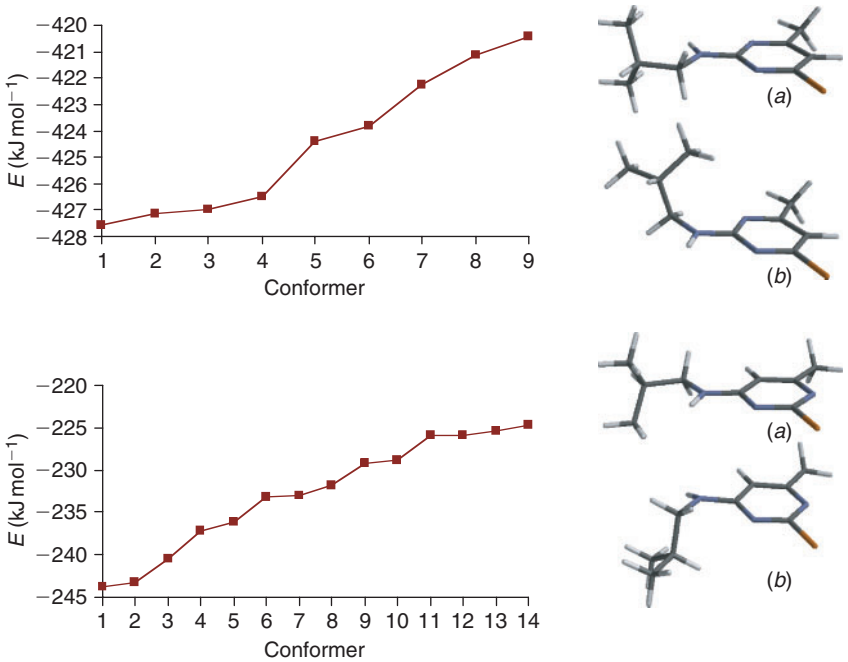
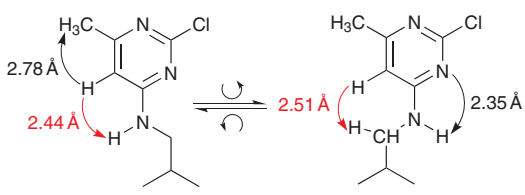


Fig. 2. Top image depicts a graph showing the energy distribution of the conformers of **22**: (*a*) the lowest energy conformer; (*b*) the highest energy conformer. The bottom image depicts a graph showing the energy distribution of the conformers of **23**: (*a*) the lowest energy conformer; (*b*) the highest energy conformer.

energy conformers predicted a change from 2.44 to 2.51 Å between H5 and the closest hydrogens (Scheme 2). While the variation in distance is small, the hydrogens approach significantly closer than those observed by Amm et al. (from 3.0 to 3.8 Å).[14,15] The closer proximity is suggestive of why significant room-temperature line broadening is observed for such small molecules, compared to the much larger compounds in which restricted rotation could be expected.



Scheme 2. Calculated distances from the highest and lowest energy conformers of **23**.

Conclusions

C4-substituted pyrimidines with small primary aliphatic and alicyclic functional groups exhibit line broadening in ^{1}H and ^{13}C NMR spectra at room temperature, with significant broadening observed from C5 of the pyrimidine ring. This effect is usually reported for bulky pyrimidines with molecular weights greater than 1000, and it is surprising that instances of room-temperature line broadening of low molecular weight pyrimidines have been overlooked, considering their preponderance in the literature. We believe such broadening is due to rotamers arising from the hindered rotation occurring between the CH(5) of the pyrimidine ring and amine-substituent hydrogens. These observations have allowed us to develop a simple and effective diagnostic tool to determine the substitution pattern of different regioisomers isolated during synthesis.

Accessory Materials

General experimental methods, details for the syntheses of compounds **4**, **8**–**19**, **26**, and **34**–**38**, and a typical example of the identification of regioisomers by two-dimensional NMR are available from the author or, until November 2009, the *Australian Journal of Chemistry*.

Acknowledgments

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