Separation of planar rotamers through intramolecular hydrogen bonding in polysubstituted 5-nitrosopyrimidines†‡

L. Čechová,§ab E. Procházková,§ac I. Cisařová,cc M. Dračinský*ad and Z. Janeba*a

While purifying new polysubstituted 5-nitrosopyrimidines, the unique separation of pairs of rotamers as chemical species, stabilized by a single intramolecular hydrogen bond and differing only in nitroso group orientation, was achieved. This interesting stereochemical phenomenon is compared to the well-known atropisomerism.

The ability to form strong hydrogen bonds is one of the most important properties of biologically active compounds. Intramolecular hydrogen bonding is crucial for encoding genetic information (Watson–Crick base pairs); intramolecular hydrogen bonding is pivotal to the formation of biologically relevant threedimensional structures of macromolecules, such as nucleic acids and proteins. Nevertheless, the ability to form intramolecular hydrogen bonds (IMHBs) also has a great impact on the molecular structure and physico-chemical properties of small organic molecules, making the closed form (with an IMHB) more lipophilic and the open form more water-soluble.1 Furthermore, the cyclic system formed due to the presence of an IMHB can mimic the structural motifs of other biologically relevant molecules. Thus, replacing the real ring with a “pseudo” ring in drug-like molecules has become an important medicinal chemistry strategy.

The high stability of IMHBs has often been observed in planar systems where a six-membered ring is formed and the linker atoms are sp²-hybridized (as in amides, enols of β-diketones and β-enaminones, heteroaromatic rings, etc.).1 Some researchers suggest that the stability of such systems is due to stabilization by a resonance-assisted hydrogen bond (RAHB).3 Others believe that RAHB is not the primary reason behind the strength of the IMHBs in a series of enols of β-diketones and β-enaminones, but rather that the molecular structure (c-skeleton) keeps the hydrogen donor and hydrogen acceptor coplanar and, therefore, closer to each other.4

An effect of the internal hydrogen bond on the conformational isomerism of 2-amino-2-methylpropanol was theoretically studied by Andrade et al.5 Recently, conformers of C-nitroso derivatives of indolizines and azaindolizines with restricted rotation about the C-NO bond were studied by the Katritzky group using solution NMR methods.6 Conjugated nitrosamines can evidently form planar systems with enhanced σ-delocalization and, thus, belong to a class of compounds able to form strong IMHBs.7 During recent studies of 5-nitrosopyrimidines bearing distinct amino substituents in the C4 and C6 positions, two possible rotamers, stabilized by strong IMHBs, were identified as two sets of signals in 1H NMR spectra.8 In our previous paper,9d the ratio of the two rotamers was found to be significantly substituent-dependent with a broad range of conformation ratios. NMR spectroscopy showed that the interconversion barriers of these rotamers were as high (>20 kcal mol⁻¹) as those reported for atropisomers,9 which are conformational isomers isolable through sterically hindered rotation. The high interconversion barriers of these rotamers led us to wonder: could the use of low temperatures with appropriately substituted amino groups in positions C4 and/or C6 potentially lead to the separation of a pair of IMHB-stabilized rotamers? As non-covalent interactions (e.g. hydrogen bonds) are considerably less stable compared to covalent bonds, it has been suggested by Szymańska10 that separation of isomers based on a single hydrogen bond is not possible, unless additional auxiliaries are introduced. Indeed, the isolation of such isomers has so far been successful either in relatively complex molecules with an ability to form cooperative robust systems of many hydrogen bonds,11 or in the case of relatively stable formamide rotamers.12

†‡ Electronic supplementary information (ESI) available: Supplementary figures and Table, X-ray diffraction data, kinetics, DFT calculations, synthesis and characterization of the prepared compounds, CCDC 1001296 and 1001297. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4cc06978a

§ L.C. and E.P. contributed equally to this work.
Although spatial separation using an electric field has recently been developed to enable the study of small molecule conformers,\textsuperscript{13} the isolation and full characterization of small molecule rotamers of aromatic compounds as separate chemical species stabilized by a single resonance-assisted IMHB has not yet been reported.

In this paper, we present unique examples of the separation and full characterization of such planar rotamers isolable through a single intramolecular hydrogen bond.

Our previously described methodology\textsuperscript{8d} (see ESI\textsuperscript{‡}) was used to prepare a new set of polysubstituted 5-nitrosopyrimidines bearing two different hydrogen-bond donors in positions C4 and C6 of the pyrimidine moiety. While purifying compounds 1–4 (Fig. 1), two products were observed as partially separated UPLC peaks and as two different hydrogen-bond donors in positions C4 and C6 of the pyrimidine moiety.

We were quite astonished by the fact that we were able to separate rotamers A and B (Fig. 1) at room temperature and characterize them using just standard spectroscopic methods. Initially, we considered this phenomenon to be a special case of atropisomerism because atropisomers are, by definition,\textsuperscript{9} stereochemically different species. However, we quickly realized that there were significant differences between typical atropisomers and our rotamers, the most obvious being their mode of stabilization. Steric hindrance stabilizes atropisomers, but our planar rotamers were stabilized by intramolecular hydrogen bonding (Fig. 2).

Having made this distinction, we explored further similarities and differences between atropisomers and our planar rotamers. We found that there are two necessary preconditions for both the phenomena. The first precondition is a rotationally stable axis, which, in our case, lies along the C5–NO bond. Analogous to the axis of the most common class of atropisomers represented by biaryl compounds (the sp\textsuperscript{2}–sp\textsuperscript{2} family),\textsuperscript{9} the herein reported rotamers are characterized by a rotationally stable bond between sp\textsuperscript{2} carbon (C5) and sp\textsuperscript{2} nitrogen (nitroso group) atoms. The second precondition involves the presence of different substituents on both sides of the axis; in our case, there are differently substituted amino groups in the C4 and C6 positions, and an oxygen and a free electron pair on the nitrosyl nitrogen. However, unlike atropisomers,\textsuperscript{9} which contain an element of chirality (usually through the axis), our planar rotamers (shortly planaromers), precisely because they are planar, lack any element of chirality.

The solid-state \textsuperscript{13}C cross polarization-magic angle spinning (CP-MAS) NMR spectra of rotamers 1A and 1B obtained after gentle evaporation of the solvents from the chromatographic fractions are depicted in Fig. 3 and Fig. S1 (ESI\textsuperscript{†}). According to these spectra, both rotamers were pure; the amount of the second one in the pair was lower than the solid-state NMR detection limit (ca. 5\%). The \textsuperscript{13}C NMR signals of rotamer 1A are sharp, indicating long-range order in the solid, while the spectrum of rotamer 1B exhibits the features typical of amorphous structures; namely, broad signals with multiple overlapped resonances. Interestingly, the solid-state \textsuperscript{13}C NMR spectra show that while the crystalline rotamer 1A was perfectly stable at room temperature, the solid amorphous rotamer 1B slowly re-crystallized, over a period of months, into the crystalline rotamer 1A (Fig. 3 and Fig. S1 in ESI\textsuperscript{†}).

The solid-state IR spectra of rotamers 1A and 1B were acquired on samples dispersed in KBr pellets. Similar to the NMR spectra, there were significant differences between the rotamers. For example, a difference of up to 40 cm\textsuperscript{-1} was observed between their antisymmetric N–H vibrations. Furthermore, as with the NMR spectra, the signals of the amorphous isomer 1B were slightly...
broader than those of the crystalline isomer 1A, with no visible contamination by each other (Fig. S2 in ESI†).

After the re-crystallization of compound 1 (a mixture of 1A and 1B) from dry acetone, a monocrystal suitable for X-ray diffraction (XRD) was obtained (Fig. 4A). XRD confirmed that the orientation of the nitroso group corresponded to that of pure rotamer 1A. The solid-state 13C NMR spectrum of this recrystallized material was identical to the spectrum of 1A obtained by column chromatography. However, following re-crystallization from an acetone–water mixture, a monohydrate of compound 1 was obtained. In this monohydrate (Fig. 4B), both rotamers were present, 1A being predominant (ca. 80%). Clearly, in a solid state, the conformational stability of a rotamer depends on the polymorphic/crystalline form of the parent compound. In this way, our planamers meet Oki’s criterion for atropisomers:14 physically separable species at a given temperature, having a half-life (τ) of at least 1000 s (~16.7 min), which is the time considered to be the minimum lifetime for a molecule to be isolable.

Immediately after dissolution of the separated rotamers A or B in DMSO-d6 or CD3OD, their 1H and 13C NMR spectra contained one major set of signals together with the low intensity signals of its corresponding second rotamer, B or A, respectively. Gradually, the A/B signal ratio changed until, after several hours, equilibrium was reached. The kinetics of these transformations were determined by solution-state 1H NMR spectroscopy (Fig. 5 and Fig. S3 in ESI†). The NH hydrogen signals of the two rotamers were usually well separated and, thus, their integration enabled the mixture composition to be determined at various times after dissolution. Table S1 (see ESI†) summarizes the rate constants (calculated according to the procedure in ESI†) and the concentrations of rotamers 1A–4A in equilibrium in various solvents. It is evident that the rotamer equilibrium ratio was not only strongly substituent dependent, but also solvent dependent.

The signals of the NH hydrogens involved in the hydrogen bond were shifted downfield (10–13 ppm) with respect to the signals of the free NH hydrogens (7–9 ppm). The position and orientation of the hydrogen-bond donor atom was confirmed both by the splitting of the signal, which resulted from indirect spin–spin interactions with neighbouring hydrogen atoms, and by the two- and three-bond correlations observed in the HMBC spectra. We also observed four-bond correlations between the

NH hydrogen atoms and the pyrimidine carbon atoms. These correlations were observed when the atoms involved were arranged in the form of a W-like coupling path (Fig. S4 in ESI†). Furthermore, the orientation of the nitroso group had a significant influence on the chemical shifts of C4 and C6: by ca. 12 ppm, the C6 carbon was more shielded in rotamer A and the C4 carbon more shielded in rotamer B.

Fig. 4 X-ray crystallographic analysis of pure rotamer 1A (A) and of the monohydrate of compound 1 (B). Carbon is shown in grey, nitrogen in blue, oxygen in red and phosphorus in yellow. The less occupied position of the nitroso group of rotamer 1B is drawn with dashed lines (B). The displacement ellipsoids are drawn at a 30% probability level. The disordered atoms of the phosphonate moiety and solvating water molecule are omitted for clarity.

Fig. 5 Interconversion of rotamers 1A and 1B. Interconversion was monitored by 1H NMR spectroscopy in a DMSO-d6 solution. R1 = CH2–CH2–O–CH2–P(O)(OEt)2.

Fig. 6 The progress of the H–D exchange of hydrogen atoms of the amino group in position C6 in the equilibrium mixture of rotamers 1A and 1B in a DMSO-d6–CD3OD mixture (9 : 1).
bond. This hypothesis is supported by the density functional theory (DFT) calculations for the C4–NH$_2$ rotation barrier, which was found to be 5.6 kcal mol$^{-1}$ lower than the rotation of the nitroso group around the C5–NO bond (see ESI†).

In summary, we succeeded in preparation of planar rotamers which, like atropisomers, are characterized by a high interconversion barrier that enables their separation by column chromatography at room temperature. Herein, we have isolated and fully characterized planar rotamers structurally derived from poly-substituted 5-nitrosopyrimidines. Unlike atropisomers, whose separation is achieved through steric hindrance (and display chirality), the isolation of our planar rotamers is achieved through intramolecular hydrogen bonding (and are achiral). To make a distinction from relatively common atropisomerism, we suggest a term ‘planamerism’ and define ‘planamers’ as small aromatic molecule rotamers with a planar conjugated moiety that are isolated as chemical species. It is possible that the reported stereochemical phenomenon has a role to play in disciplines involving weak and reversible non-covalent interactions, such as supramolecular assemblies, host molecules, or in the development of novel drug-like molecules. Therefore, to more clearly understand the scale and scope of this phenomenon, we are currently synthesizing other substituted (hetero)aromatic derivatives likely to be capable of forming strong intramolecular hydrogen bonds and, consequently, separable rotamers.

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Notes and references