



Research paper

Restricted amide rotation with steric hindrance induced multiple conformations

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ABSTRACT

The C–N bond character is dependent directly upon the resonance-contributor structure population driven by the delocalized nitrogen lone-pair of electrons. In the case of *N,N*-dibenzyl-*ortho*-toluamide (*o*-DBET), the molecule adopts subpopulations of conformers with distinct NMR spectral features, particularly at low temperatures. This conformational adaptation is unique to *o*-DBET, while the corresponding *meta*- and *para*- forms do not show such behavior. Variable-temperature (VT) NMR, two-dimensional exchange spectroscopy (EXSY), and qualitative molecular modeling studies are used to demonstrate how multiple competing interactions such as restricted amide rotation and steric hindrance effects can lead to versatile molecular adaptations in the solution state.

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1. Introduction

The origin of the rotational barrier around the C–N bond of amides is associated with its partial double bond character, which arises from resonance interaction between the lone pair of electrons on the nitrogen atom and the carbonyl π system. Dynamic nuclear magnetic resonance spectroscopy (DNMR) is often the chosen technique to investigate the partial double bond characteristics of the amide bond. With experimental demonstration using NMR spectroscopy in *N,N*-dimethylformamide, [1,2], there is sustaining interest in the field [3–10].

In the case of *N,N*-diethyl-*o*-toluamide (*o*-DEET), we recently demonstrated that the steric interactions between the methyl group at the *ortho*- position and the two *N*-ethyl groups modulate the restricted rotation of the amide bond kinetics [11]. In particular, the steric hindrance induced by the methyl group introduces an additional third higher-energy barrier by altering both the enthalpic and entropic contributions to rotational energy barrier as determined by DNMR measurements. The energy barrier to the restricted amide rotation is primarily determined by the π -bond order of the C–N bond, and therefore the electronic effect of substituent groups on either side of the bond will influence the rotational barrier. The third high-energy conformation can

be eliminated either by moving the methyl group to the *meta*-*para* positions of DEET [11] or by reducing the size of the nitrogen side-chain from the ethyl to a methyl group [12]. We explored the kinetic aspects of *N,N*-dibenzyl-*o*-toluamide (*o*-DBET), where the ethyl group is replaced by a benzyl group. The motivation behind this series of molecules (*ortho*, *meta* and *para* forms of DBET) is to investigate how the molecules adapt to competing interactions between restricted amide rotation, steric hindrance, increased size of the side-chain, as well as the relative juxtaposition of the two benzyl groups and the phenyl ring in the molecule.

Variable temperature NMR experiments performed over a temperature range (1 °C–55 °C) did provide characteristic DNMR band shapes for the *m*-DBET and *p*-DBET molecules. However, in the case of *o*-DBET, to our surprise, the conformational states divided into two distinct sets of populations. The DNMR results show these are two independent sets of molecular conformations introduced due to the combination of geometric constraints induced by both the methyl group at the *ortho* position as well as the relative juxtaposition of the aromatic moieties present in the two benzyl groups. The presence of the two sets of conformational states is demonstrated experimentally using two-dimensional exchange spectroscopy (EXSY), particularly at low temperatures. Thermodynamic parameters are estimated using variable temperature NMR experiments, and the molecular mechanics calculations further elucidate the complex conformational energy landscape for *o*-DBET in comparison with *m*-DBET and *p*-DBET.

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2. Materials and methods

2.1. Synthesis

All chemicals were purchased from Sigma Aldrich or MERCK and were used as received without further purification. *Ortho*-DBET: *N, N*-dibenzyl-*o*-methyl benzamide: To a solution of *o*-toluic acid (2.08 g, 15.3 mmol) in methylene chloride (28 mL), a catalytic amount of DMF (2 drops) was added with stirring. Freshly distilled thionyl chloride (2.3 mL, 31.0 mmol) was added to the flask, and the resulting solution was gently refluxed for two hours. The reaction mixture was concentrated under reduced pressure to remove excess thionyl chloride using a warm water bath (55 °C). The crude acid chloride was redissolved in methylene chloride (28 mL), and dibenzyl amine (5.88 mL, 30.6 mmol) was carefully added to the flask, dropwise, over the course of 2–3 min (Note: exothermic, produces gaseous HCl). The solution was allowed to stir at room temperature overnight. The organic layer was washed with water (30 mL) followed by 5% HCl (30 mL), 10% NaOH (30 mL), finally with brine (30 mL), and dried with anhydrous Na₂SO₄. The solution was filtered and concentrated under reduced pressure to yield a granular, slightly yellow solid before being dissolved in a minimum amount of hot hexanes. The solution was allowed to cool, and crystals were collected and dried to yield 1.98 g (41% overall) of the pure *N, N*-dibenzyl-*o*-toluamide.

Each DEET analog was synthesized by following the above method and purified by crystallization (hot hexanes) or column chromatography (10% ethyl acetate in hexanes and 230–400 mesh silica gel) as required. Approximately 10 mg of each sample was dissolved in CDCl₃ (total volume of 600 μL) for the NMR experiment. Each NMR tube was glass-sealed by first applying the freeze-thaw technique using liquid nitrogen to expel any dissolved air/oxygen, followed by sealing them using a small butane torch.

2.2. NMR spectroscopy

All the NMR experiments were performed in a 400 MHz (¹H resonance frequency) VNMR spectrometer (Varian-Agilent) and using a one-NMR probe. The probe temperature was calibrated using MeOH [13]. The probe temperature was varied from 1 °C to 55 °C (in steps of 3 °C). One-dimensional, variable-temperature experiments were performed with 16 transients over 16 K complex points after calibrating the 90° pulse at each temperature. Samples were equilibrated for 20 min at each temperature, and a relaxation delay of 30 s was used between the transients. WinDNMR [14,15] was used to estimate the exchange rates (k_{ex} s⁻¹). A three-site exchange model was used to fit the variable temperature line shape data of *o*-DBET, and a two-site exchange model was used to fit for *m*-DBET and *p*-DBET molecules. The constant line width of 6 Hz was used for all the fits. In the three-site model with a slow exchange between the third spin to first two spins was assumed. It is necessary to adopt a three-site model for *o*-DBET, due to chemical shift overlap between the spins in the up-field part of the exchange spectrum. The activation energy was estimated following the Eyring analysis (exchange rates vs. inverse of temperature in K).

Two-dimensional exchange spectroscopy (EXSY) [16] was performed at three different temperatures (1 °C, 25 °C, and 55 °C) using a standard Nuclear Overhauser Effect Spectroscopy (NOESY) pulse sequence [17] using the procedure described previously [11].

Molecular mechanics calculations were performed using Avogadro as a function of two dihedral angles: α (C–C–O/Aromatic–CO) and β (O–C–N–C) [18]. The dihedral angle α was changed from –180° to +180° in steps of 5 °C. The calculations can only be considered qualitative or at most semi-quantitative.

For each value of α calculated, the dihedral angle β was changed –180° to +180° in steps of 5 °C, leading a matrix of 33 × 33.

3. Results

Fig. 1 shows the variable temperature NMR experiments of the *ortho* (*o*), *meta* (*m*) and *para* (*p*) – DBET molecules over the temperature range recorded by the variable temperature, between 1 °C and 51 °C. At low temperature (1 °C) the chemical shifts of the chemically exchanging methylene protons at the two sites are of *m*-DBET are at 5.45 ppm and 5.15 ppm, which reaches an intermediate exchange region at the high temperature (51 °C) (Fig. 1, panel m). The *p*-DBET also shows a similar chemical exchange behavior with the two distinct sites at 5.65 ppm and 5.38 ppm at low temperature (1 °C) and a coalesced single peak at higher temperatures (51 °C) (Fig. 1, panel p). At high temperature (51 °C), *o*-DBET molecule shows a broad resonance (5.32 ppm) representing an intermediate exchange and a sharp resonance (4.77 ppm) similar to a third high-energy conformation observed previously in the case *o*-DEET [11]. Upon lowering the temperature, a complex peak pattern emerges (Fig. 1, panel o). When the probe temperature reaches approximately below 10 °C, in addition to the splitting of down-field resonance (5.78 ppm), the upfield peak shows a complex line shape (centered at 4.81 ppm).

The two distinct set of exchange peaks from *o*-DBET are seen in the two-dimensional exchange spectrum (Fig. 2) recorded at 1 °C with a mixing time of 300 ms. Two sets of spin systems identified are as follows: an (AX)₂ spins system ($\delta_{AX} = 400.2$ Hz and $J_{AX} = 12$ Hz) and an (AB)₂ spin system ($\delta_{AB} = 56.2$ Hz and $J_{AB} = 16$ Hz). These NMR parameters are determined by simulating the 1D NMR spectrum in the absence of chemical exchange (Fig. S1). Contribution from zero-quantum coherences in the EXSY experiment is expected to be significantly reduced due to long mixing time (300 ms) and the effect of solvent viscosity over the temperature range (Chloroform viscosity 0.699 mPa s at 0 °C–0.389 mPa s at 60 °C) is also expected to be insignificant [11].

Increasing the temperature alters the kinetics of the sub-spin systems separately (Fig. 1). Fig. 3 shows variable temperature NMR experiments of *o*-DBET (red lines) along with the corresponding line shape fitted using a three-site exchange model (black lines). At temperatures below 11 °C, a three-site model was unable to reproduce the line shape (Fig. 3). In contrast, the temperature-dependence of the *m*-DBET and *p*-DBET spectra follows the characteristic features corresponding to a two-site chemical exchange process. Fig. 4 shows the Eyring analysis plot ($\ln(k_{ex}/T)$ vs. 1000/

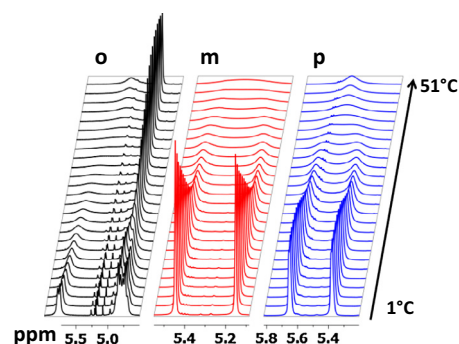


Fig. 1. Variable temperature DNMR spectra. (o) Temperature dependence of the NMR line shapes of the methylene protons in *N, N*-dibenzyl-*o*-toluamide (*o*-DBET, black), (m) *N, N*-dibenzyl-*m*-toluamide (*m*-DBET) and *N, N*-diethyl-*m*-toluamide (*m*-DEET, red) and (p) *N, N*-dibenzyl-*p*-toluamide (*p*-DBET, blue). The stacked plot for each molecule was recorded from 1 °C to 51 °C and in steps of 2 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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