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Conformational Preferences and Dynamics of 4-Isoxazolyl-1,4-dihydropyridine Calcium Channel Antagonists as Determined by Variable-Temperature NMR and NOE Experiments

Robert B. Palmer†
Department of Chemistry, University of Washington, Seattle, Washington 98195, USA

Tina M. Andro and Nicholas R. Natale
Department of Chemistry, University of Idaho, Moscow, Idaho 83843, USA

Niels H. Andersen*
Department of Chemistry, University of Washington, Seattle, Washington 98195, USA

A series of 14 4-(3′,5′-disubstituted isoxazolyl)-1,4-dihydropyridine calcium channel antagonists were examined using variable-temperature proton nuclear magnetic resonance spectroscopy and nuclear Overhauser effect (NOE) experiments. Two of these compounds, the 1,4-dihydro-2,6-dimethyl-4-[5′-methyl-3′-(4″-fluorophenyl)isoxazol-4′-yl]-3,5-pyridinedicarboxylic acid dimethyl ester (3a) and 1,4-dihydro-2,6-dimethyl-4-[5′-methyl-3′-(4″-bromophenyl)isoxazol-4′-yl]-3,5-pyridinedicarboxylic acid dimethyl ester (5a), were synthesized to assist in the clarification of ambiguities discovered in the low-temperature spectra of 1,4-dihydro-2,6-dimethyl-4-(5′-methyl-3′-phenylisoxazol-4′yl)-3,5-pyridinedicarboxylic acid diethyl ester (2b). The solid-state structure of 3a is also reported. The solution-state rotameric preferences of the 14 compounds are reported and compared with those calculated at the AM1 level. C-4—C-4′ bond rotation barriers were also calculated at the AM1 level for nine of the systems examined. Several species failed to display temperature-dependent signals associated with hindered rotation owing to highly biased rotameric equilibria at the temperatures required to freeze out the rotation. The seven experimental rotation barriers ($\Delta G^{\neq}$ from $\leqslant$26 to 40.4 kJ mol^{-1}) reported are 1–6.8 kJ mol^{-1} higher than $\Delta H^{\neq}$ calculated at the AM1 level).

KEY WORDS 4-isoxazolyl-1,4-dihydropyridine calcium channel antagonists; conformation; dynamics; variable-temperature NMR; NOE

INTRODUCTION

Structure–activity relationships remain a central theme in medicinal chemistry. The specific conformational features of a pharmacologically active compound which relate to biological potency and the agonism–antagonism balance as well as the energetics of receptor binding are active areas of research. The degree of similarity expected for the receptor, solution- and solid-state structures of drugs is often difficult to assess; structure–activity correlations often employ solid-state structures from x-ray crystal data.[1] Numerous x-ray structures of 1,4-dihydropyridines have been reported[2–6] and a correlation between the planarity of the dihydropyridine ring in the solid state and biological activity has been drawn.[7] Dihydropyridines (DHPs) with the 4-aryl substituent replaced by a 3,5-disubstituted isoxazolyl moiety have demonstrated biological activity as calcium channel antagonists.[8] In fact, the 3′-phenyl-5′-methylisoxazolyldihydropyridine **2b** was shown to be nearly as potent pharmacologically as nifedipine (**9**).[9]

We have previously studied the rotational barriers and rotameric preferences about the C-4—aryl bond in a series of both symmetric and asymmetric 4-phenyl-1,4-DHPs bearing ester and cyano functions at C-3 and C-5,[6,10–13] including a detailed re-analysis of the mesityl diester **10**. It was established that in order to observe experimentally the freezing out of ring junction rotation in phenyl-DHPs, it is necessary to have substituents in both positions 2′ and 6′ and that 3,5-dinitriles consistently had barriers that were 8.7–11.6 kJ mol^{-1} larger than those of the corresponding 3,5-diesters. These results prompted us to examine other series of biologically active 4-aryl-1,4-DHPs.[8,14] Here, we report a study in which the barriers to ring junction rotation for a series of 4-(3′,5′-disubstituted isoxazolyl)-1,4-dihydropyridine calcium channel antagonists (Fig. 1) were determined experimentally using very low-temperature ^{1}H NMR. The rotameric preferences were also deduced using nuclear Overhauser effect (NOE) experiments.

* Author to whom correspondence should be addressed.
† Present address: Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195, USA.

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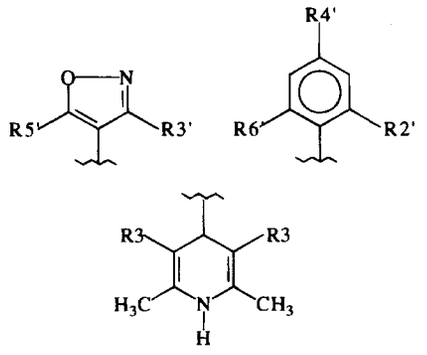

	4-Aryl Group	R3′	R5′	R3
1a	isoxazole	Me	Me[25]	CO_2Me
1b	isoxazole	Me	Me[8]	CO_2Et
1c	isoxazole	Me	Me	CN
2a	isoxazole	Ph	Me	CO_2Me
2b	isoxazole	Ph	Me[9]	CO_2Et
3a	isoxazole	4″-F-Ph	Me	CO_2Me
4a	isoxazole	4″-Cl-Ph	Me	CO_2Me
5a	isoxazole	4″-Br-Ph	Me	CO_2Me
5c	isoxazole	4″-Br-Ph	Me	CN
6a	isoxazole	Ph	Et	CO_2Me
6b	isoxazole	Ph	Et[14]	CO_2Et
7a	isoxazole	Ph	*i*-Pr	CO_2Me
7b	isoxazole	Ph	*i*-Pr[14]	CO_2Et
8b	isoxazole	Me	1″-phenylprop-2″-yl[22]	CO_2Et

		R2′	R4′	R6′	R3
9	phenyl	NO_2	H	H	CO_2Me
10	phenyl	Me	Me	Me	CO_2Me

Figure 1. The 4-isoxazolyl-1,4-dihydropyridines studied. The superscript numbers indicate references where these compounds have been reported previously.

EXPERIMENTAL

General

All, 3,5-diester compounds were prepared via the Hantzsch pyridine synthesis from the appropriately substituted isoxazolyl-4-carboxaldehyde and methyl acetoacetate in ammonia and methanol. The 3,5-dinitrile compounds were prepared by condensing the appropriate aldehyde with 3-aminocrotonitrile in glacial acetic acid. The details of the synthetic transformations, purification procedures and additional analytical data have been reported elsewhere.[11] The interatomic distances reported in Fig. 3 were obtained on a Silicon Graphics INDIGO using structures minimized with the program SPARTAN 2.0 (Wavefunction, Irvine, CA, USA). AM1 calculations were performed using the program SPARTAN 2.0 with no modification to the default parameters.

The x-ray crystal structure studies of **3a** were performed at Bristol-Myers Squibb (Princeton, NJ, USA) using the same apparatus and method described by Rovnyak *et al.*[10]

NMR spectroscopy

NMR spectra were recorded (16 K time domain points) on either a Bruker AMX500 spectrometer with a sweep width of 6024 Hz or a Bruker AF300 spectrometer with a sweep width of 4000 Hz. The 300 MHz instrument was used for structural proof and sample purity assessment and all variable-temperature and NOE experiments were performed using the higher field instrument equipped with an Aspect 3000 computer. Data transformations and analyses were performed on a Silicon Graphics (Personal IRIS) 4D/25TG using FELIX 2.0 (Hare Research, Woodinville, WA, USA). Chemical shifts are reported in ppm downfield from internal $(CH_3)_4Si$. All NMR spectra were recorded in '100%' $CDCl_3$ containing 0.03% (v/v) $(CH_3)_4Si$ (Cambridge Isotope Laboratories, Woburn, MA, USA) or Freon-21 synthesized in-house.[15] Typical sample concentrations were 20–30 mM. For the variable-temperature experiments, the probe was cooled using liquid N_2 and allowed to equilibrate for 15 min at each temperature before each acquisition. All temperatures were corrected using a CH_3OH standard. Solubility, particularly of the 3,5-dinitrile compounds, was notably diminished at the low-temperature extremes.

The estimates of rotational barriers were based on the equation

$$\Delta G^{\neq} = 19.14T_c[9.97 + \log(T_c/\Delta\nu)]\text{J mol}^{-1} \quad (1)$$

where $\Delta\nu$ is the chemical shift difference between the individual rotameric signals and T_c is the temperature of coalescence; this equation assumes equal populations of rotamers.[16]

Transient (selective inversion) ΔNOE spectra were collected directly into memory using the pulse sequence described by Andersen *et al.*:[17]

$$\text{PD}-^{s}180(t_1)_{on/off}-\tau-^{ns}90-t_2(\text{acquire})$$

Typically, experiments with eight 'on' and 'off' resonance scans per difference cycle were parameterized with a preparatory delay (PD) of 1.0 s, an NOE build-up time (τ) incrementally varied from 75 to 3000 ms and a t_2 acquisition time of 2.05 s. The time t_1 for a selective 180° decoupler pulse was 30–45 ms. A 9.5 μs non-selective read pulse was employed. A typical NOE experiment collected 16 difference cycles for each values of τ.

RESULTS

Rotameric preference determinations

There are two principal trends for the x-ray structures of 4-aryl-DHPs:[18] (1) the DHP ring adopts a flattened boat conformation and (2) the 4-aryl group adopts a pseudo-axial orientation with respect to, and nearly bisecting, the plane of the DHP ring. Calculations have shown this perpendicular disposition to be the low-energy form.[19] In the case of an asymmetric ring system, such as an isoxazole, there are two possible rotamers. These have been denoted 'O-*endo*' (the isoxazolyl ring oxygen over the DHP ring) and 'O-*exo*' (the isoxazolyl ring oxygen away from the DHP ring). X-ray crystal structures of both rotameric forms have been reported, but there are no reported cases of rotameric mixtures for the solid-state structure of a single compound.

X-ray structures are available in the literature for **1b**, **2b**, **6b**, **7b** and **8b**. Of these five structures, only that for **7b** exhibits an O-*exo* rotameric preference in the solid state. There is no apparent steric basis for the rotameric preference exhibited by the symmetrically substituted system (**1b**). Although the bulkier phenyl group is positioned away from the DHP ring in **2b** and **6b**, the simple steric argument does not apply for the structures of **7b** and **8b**. Indeed, it seems counterintuitive that these solid-state structures would be such that the exclusively populated rotamer is that with the sterically larger group over the DHP ring. It might initially be reasoned that the solid-state structures are not a result of thermodynamic stability but rather of crystal packing forces.

To this database, we add two crystal structures for the 3′-*p*-fluorophenyl-5′-methylisoxazolyl **3a** determined at 295 and 243 K (Fig. 2 and Table 1). The only significant differences between the two determinations were that the lower temperature study had smaller isotropic temperature factors and molecular volume. For the present discussion, the two crystal structures are considered to be equivalent. Compound **3a** shows the expected perpendicular disposition of the isoxazole and DHP rings and the flattened boat geometry of the DHP. The isoxazole is 2–3° off the perpendicular with respect to the DHP (for which the sum of the six interior torsion angles is 66.9°). For comparison, the analogous sum for nifedipine (**9**) is 72.1°.[8] Both ester groups in **3a** are synperiplanar (*sp/sp*, specifying the relationship between the carbonyl and the DHP C—C double bond). The 3′-phenyl ring is approximately 17° out of the perpendicular plane with respect to the isoxazole.

Surprisingly, the replacement of the *para*-hydrogen of the phenyl ring in **2a** with a fluorine had a dramatic effect on the rotameric preference in **3a**. [The solid-state structure of **2a** has not been reported but that of **2b** has,[9] and the solution-state preferences of **2a** and **2b** are identical (see below).] The O-*exo* rotamer is exclusively populated in the solid-state structure of **3a** whereas only the O-*endo* rotamer appears in the x-ray crystal structure of **2b**. Although the distance between the N-1 H and C4″ fluorine is large (4.41 Å), a hydrogen-bond-like interaction is the only explanation that we can provide for this alteration in rotameric preference. The interaction must be intramolecular as no evidence for an intermolecular hydrogen bond exists in the crystal lattice. The acquisition parameters and pertinent torsion angles are reported in Table 1. The coordinates and errors for both x-ray crystal structure determinations of **3a** are available from the authors upon request.

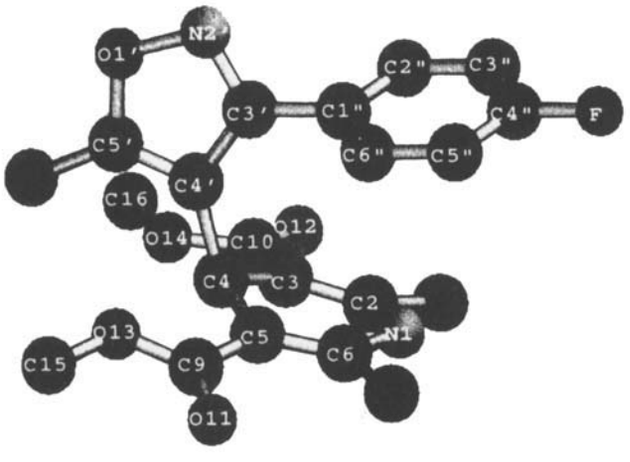

Figure 2. X-ray crystal structure of the 3′-(4″-fluorophenyl)-5′-methylisoxazolyl dimethyl ester compound **3a** at 243 K.

Table 1. Acquisition parameters and selected torsion angles for the x-ray crystal structures of 3a[a]

Parameter	295 K	243 K
a (Å)	13.637 (1)	13.544 (2)
b (Å)	26.608 (2)	26.560 (3)
c (Å)	10.765 (1)	10.742 (1)
Space group	P_{bca}	P_{bca}
NUNI[b]	3641	3606
NOBS[c]/NV[d]	1992/262	2194/262
R/R_w	0.049/0.058	0.050/0.061
Torsions:[e]		
N1—C2—C3—C4	1.0	2.8
C2—C3—C4—C5	−15.7	−16.8
C3—C4—C5—C6	19.0	19.6
C4—C5—C6—N1	−7.5	−8.5
C5—C6—N1—C2	−10.1	−8.2
C6—N1—C2—C3	13.4	11.3
C2—C3—C10—O12	−9.1	−6.3
C6—C5—C9—O11	−8.0	−8.6
C3—C10—O14—C16	176.5	176.4
C5—C9—O13—C15	177.2	177.5
C4′—C3′—C1″—C2″	107.5	108.0
C3—C4—C4′—C5′	−62.3	−61.9

[a] The crystals of **3a** were colorless rods recrystallized from absolute ethanol–ethyl acetate using slight heating with slow evaporation. Both studies gave a cell containing eight molecules with formulae $C_{21}H_{21}N_2O_5F$. The hydrogens were not refined and are excluded for clarity.
[b] Number of symmetry-independent reflections.
[c] Number of reflections with $I \geq 3\sigma(I)$ used in least-squares refinement.
[d] Number of refined variables.
[e] Angles are reported in degrees.

Attempts to derive solution-state rotameric preferences from chemical shifts

At room temperature, the signals in the NMR spectra of all species examined in the present study are population weighted averages of the rotamers contributing to the rapid conformational equilibrium. The diamagnetic anisotropy of the DHP ring should produce differential shielding of substituents placed in the 3′ and 5′ positions of the isoxazolyl moiety. If rotameric preferences are sufficiently large, the weighted average signals will show diagnostic changes from their expected chemical shifts. A chemical shift analysis for the aromatic protons of compounds **3a**, **4a** and **5a** was performed using monohalobenzenes as models for the chemical shift changes due to halogen effects[20,21] and 3-phenyl-5-methylisoxazole-4-carbinol as the model for chemical shift changes associated with the isoxazolyl substituent. An upfield movement of the aromatic resonances (particularly for the protons *meta* to the C-4″ X group) from their predicted shifts based on the models should

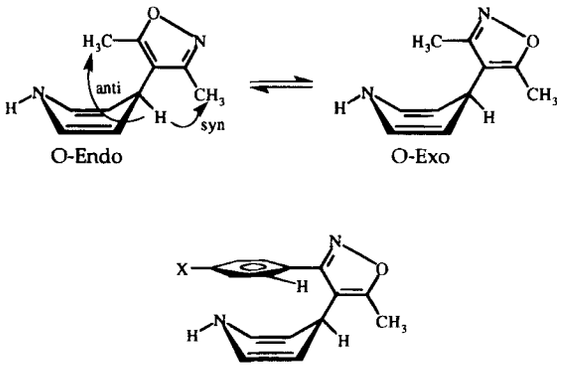

Figure 3. Illustrations of the rotameric distinction terms 'O-*endo*' and 'O-*exo*'. According to modeling results, in the 3',5'-dimethylisoxazolyl compound **1a** the distances from the C-4—H to the methyl group are 2.3 and 5.2 Å for the *syn* and *anti* dispositions, respectively. The additional substituents on the dihydropyridine ring have been omitted for clarity. The O-*exo* conformer of a 3'-phenyl species is also illustrated.

be indicative of a significant O-*exo* population (see Fig. 3).

A downfield shift from the 7.27 ppm of benzene is observed for all five phenyl protons when the phenyl ring is attached to an isoxazole (as in 3-phenyl-5-methylisoxazole-4-carbinol). Those protons *ortho* to the isoxazole shift by + 0.48 ppm whereas those *meta* and *para* to the isoxazole shift by + 0.18 ppm [where a positive sign indicates movement to a larger chemical shift (downfield)]. These effects are additive with those of the halogen substitution and chemical shifts in the absence of anisotropic effects of the DHP ring can therefore be predicted. The differences in chemical shift between the observed and predicted values for the aromatic protons *ortho* to the isoxazolyl moiety range from −0.26 ppm (**3a**) to −0.33 ppm (**4a**). In all three cases (**3a**, **4a** and **5a**), the differences between observed and predicted chemical shifts for the protons *meta* to the isoxazole are −0.08 ppm. The differences in the predicted chemical shift differences between the protons *ortho* and *meta* to the isoxazole ring are ($\delta_{ortho} - \delta_{meta}$) are +0.55, +0.25 and +0.04 ppm for **3a**, **4a** and **5a**, respectively. The experimentally observed values are +0.37, 0.0 and −0.18 ppm, respectively. The smaller experimentally observed $\Delta\delta$s indicate a net shielding. We believe the source of this effect to be the diamagnetic anisotropy of the DHP ring and, since this effect can happen only in the O-*exo* conformation, a significant portion of that rotamer must be present.

The shift comparisons above were only valid for compounds **3a**, **4a** and **5a**. To obtain definitive rotameric preference determinations for all of the compounds, we turned to NOE experiments. The key NOEs used to address this problem are between the substituents in the 3' and 5' positions of the isoxazole moiety and the C-4 H of the dihydropyridine ring. Molecular modeling indicates a distance of approximately 2.3 Å between a methyl hydrogen *syn* to the C-4 H and 5.2 Å in the *anti* case (Fig. 3). A strong NOE would be expected from a substituent *syn* to the C-4 H whereas for the substituent in the *anti* position the NOE would be absent or very weak. The isoxazolyl DHPs were examined using transient difference NOEs at various temperatures.

The 3',5'-dimethylisoxazolyl-DHPs (**1a** and **b**), showed NOEs of essentially equal intensity from the bridgehead C-4 H to the 3' and 5'-methyl signals at both ambient temperature and 220 K. Thus, **1a** and **1b** exhibit both ring juncture rotation that is fast on the NMR time-scale (even at the lower temperature) and a nearly equal population of rotamers. (With regard to the latter conclusion, it is noted that negative NOEs were observed even at the low temperature. Therefore, saturation transfer from the other rotameric site would have the opposite sign. If the populations of the two rotameric forms were not equal, that site experiencing the larger direct NOE could only diminish the size of the net transfer peak for the other site upon site exchange by rotation. Hence apparent NOE ratios would always accentuate any small difference in rotameric population ratio.) In distinct contrast to the NOE data, the solid-state structure of **1b** shows *exclusively* the O-*endo* form of the compound.[8] The same NOE analysis showed that **1c** displayed the same C-4—C-4' bond rotation equilibrium as the diester compounds (**1a** and **b**) (i.e. NOEs of equal intensity to both the 3'- and 5'-methyl groups from the C-4 H).

A similar NOE analysis applies when the substituents in the 3' and 5' positions are not identical. The expectation NOE ratios for each pure rotamer can be calculated (taking into account spin population and distance differences) and those expected for rotamer mixtures were derived as population weighted averages. For most of the compounds studied it was clear that two rotamers are required to rationalize the NOEs and the major and minor rotamers could be assigned. Compound **8b** showed only the NOEs expected for a single rotamer (see below). One compound, **2b**, gave experimental data of sufficient accuracy to quantitate the relative rotameric populations; a second compound, **6a**, provided data that allowed for an approximate ratio determination. The method of quantitation is detailed below.

The 3'-phenyl-5'-methylisoxazolyl-DHP **2b** showed large NOEs from the C-4 H to the *o*-phenyl protons and also small NOEs to the methyl group at the 5' position. For compound **2b**, distances extracted from x-ray structure data show that *one* *o*-phenyl proton would have a distance of approximately 2.2 Å from the C-4 H in the *syn* conformer and a distance of 4.9 Å in the *anti* case. This distance alone argues against an NOE from the C-4 H to an *anti* phenyl ring. In compound **2b**, the ratio of integrals (in the growth phase) of the NOEs from the C-4 H is approximately 3:1 in favor of the 'effectively single-spin' *o*-phenyl *vs.* the three-spin 5'-methyl.

The pertinent distance between the aromatic and C-4 H for **2b** was obtained from the x-ray crystal structure and that expected for the same interaction and for a *syn* methyl–C-4 H interaction (2.3 Å) was estimated from the x-ray structure of **3a** as it has an O-*exo* rotameric conformation. If the population ratio were 1:1, an NOE growth rate ratio of 1:2.29 in favor of the C-5' methyl group is calculated. Therefore, a 3:1 relationship of NOEs corresponds to a rotameric population ratio of 6.87:1 in favor of O-*endo* (i.e. the phenyl group *syn* to the C-4 H). This information confirms that there are two solution-state conformers and that the most

highly populated is the O-*endo* form, identical with the reported solid-state structure.[9] In the case of the 5′-ethyl species, as the dimethyl ester (**6a**), a similar NOE analysis indicates a 3.7–6.7 : 1 ratio favoring the O-*endo* form at 300 K.

NOE experiments on compound **6b** confirmed that the rotameric preference is that in which the 5′-ethyl group is over the DHP ring (O-*endo*), although a smaller NOE was also noted to the methylene of the 5′-ethyl group, indicating that the O-*exo* rotamer is significantly populated. NOE studies of **7b** at both 220 K and ambient temperature showed large NOEs from the C-4 H to the methine of the isopropyl substituent and much smaller NOEs to the *ortho*-aromatic protons (Fig. 4). Thus, the solution-state and solid-state rotameric preferences are the same, but both rotameric forms are populated in solution.

Additional NOE results confirmed that the rotameric preferences exhibited by the 3′-phenyl-5′-methyl series (**2a** and **b**) were identical. Likewise, the solution-state rotameric preferences in the 3′-phenyl-5′-isopropyl series (**7a** and **b**) were the same. The NOE results for **5c** indicated an identical rotameric preference in solution to the analogous 3,5-dimethyl ester compound (**5a**).

Whereas the solid-state structure of **6a** has not been reported, that of its 3,5-diethyl ester analogue (**6b**) has.[14] When NOE studies of both the 3,5-dimethyl and 3,5-diethyl ester forms of several of these DHPs were performed, they revealed no change in rotameric preference with the 3,5-diester alteration. Furthermore, the x-ray structure reported for the 3,5-diethyl ester compound (**6b**) has the same rotameric preference indicated by the NOE studies of both the 3,5-dimethyl and 3,5-diethyl ester analogues. Hence it appears safe to assume that the rotameric preference from the solid-state structure of a 3,5-diethyl ester compound also applies for the corresponding 3,5-dimethyl ester.

The 3′-methyl-5′-(1″-phenylprop-2″)-yl compound **8b** gave analogous results to the 3′-*para*-fluorophenyl-5′-methyl compound **3a** in that the solid-state structure showed exclusively one rotameric form.[22] When the NOE experiments were performed at 300 K on **8b**, irradiation of the C-4 H showed an NOE *only* to the 3′-methyl group, confirming the rotameric preference in the x-ray data and showing that a single rotameric site is populated in solution even at this relatively elevated temperature. This is in contrast to an earlier report by Mirzaei *et al.*,[22] where a highly symmetrized room temperature NOESY analysis was said to indicate the presence of both rotamers. In both **3a** and **8b**, the NOE and solid-state data are consistent. Both solution- and solid-state data for the 3′-phenyl-5′-methyl compound **2b** indicate an O-*endo* rotameric preference. NOE studies confirmed the rotameric preferences in solution to be the same as those in the solid-state structures for **3a** and **2b**. The rotameric preferences of the 3′-(4″-fluorophenyl)-5′-methylisoxazolyl-3,5-dimethyl ester compound **3a** and the 3′-phenyl-5′-methylisoxazolyl-3,5-dimethyl ester compound **2a** differ in the solution and solid states. We can offer no fully satisfactory explanation for this. It is noteworthy, since 11 of the 14 compounds had clearly defined rotameric preferences in solution. The 3′,5′-dimethyl series of compounds (**1a–c**) display no rotameric preference in solution.

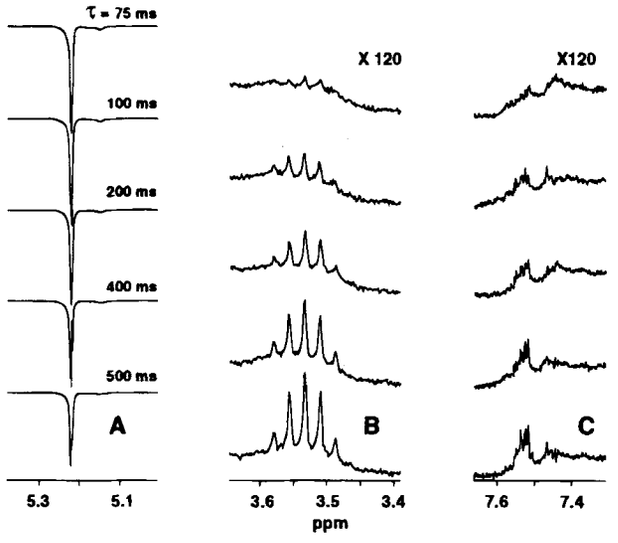

Figure 4. Transient difference NOEs of the 5′-isopropyl-3′-phenylisoxazolyl dihydropyridine **7b** at the isopropyl methine (B) and the *ortho*-aromatics (C) when the C-4—H is irradiated (A). The intensity scales in (B) and (C) are 120 times that of the spectra in (A). The NOE build-up time is indicated in (A) by τ.

Computational results

The question of 4-aryl-DHP rotameric preference has been addressed computationally in the literature. Holtje and Marrar[23] used MNDO optimized geometries to conclude a rotameric preference for 4-phenyl-DHPs with the *ortho* (C-2′) substituent *syn* to the C-4 H was favored by 12–16 kJ mol^{-1}, but Rovnyak *et al.*[6] concluded on the basis of AM1 calculations that there was little intrinsic difference between *syn* and *anti* rotamers. The rotameric preference of the 3′,5′-dimethylisoxazolyl-3,5-diethyl ester **1b** has also been the subject of a prior computational investigation.[8] At the MM2 level, a 7.5 kJ mol^{-1} preference for the O-*endo* geometry was reported. At the INDO/I level, the reported preference was reversed, favoring the O-*exo* rotamer by 4.4 kJ mol^{-1}.

In our study, the geometries were first optimized at the AM1 level using SPARTAN 2.0. The isoxazole ring was then rotated 180° from the minimized geometry about the C-4—C-4′ bond. This angle was then constrained and the rest of the molecule was allowed to relax. Comparison of the two heats of formation yielded the calculated rotameric preferences. For compound **1b** our study resulted in a preference of only 2.2 kJ mol^{-1} for the O-*endo* rotamer (Table 2). Changing from a diethyl ester (**1b**) to a dimethyl ester (**1a**) results in an increase in rotameric preference to 2.7 kJ mol^{-1} while the dinitrile (**1c**) had a smaller preference.

In contrast to the O-*endo* preference in the 3′,5′-dimethylisoxazolyl compounds, the 3′-phenyl-5′-methylisoxazolyl-3,5-diester compounds all showed a calculated O-*exo* preference. The 3,5-diethyl ester derivative of this series (**2b**) shows an O-*exo* preference that is 0.4 kJ mol^{-1} greater than for the corresponding dimethyl ester compound (**2a**). The addition of a halide in the *para* position of the 3′-phenyl group resulted in

Table 2. Comparison of rotameric preferences

Compound	AM1 rotameric preference (kJ mol^{-1})	x-ray preference[a]	NMR preference[b]
1a	2.67 (O-*endo*)	n.d.[c]	None[d]
1b	2.18 (O-*endo*)	O-*endo*[8]	None[d]
1c	1.98 (O-*endo*)	n.d.	None[d]
2a	1.84 (O-*exo*)	n.d.	O-*endo*
2b	2.25 (O-*exo*)	O-*endo*[9]	O-*endo*
3a	1.13 (O-*exo*)	O-*exo*	O-*exo*
4a	1.67 (O-*exo*)	n.d.	O-*endo*
5a	0.91 (O-*exo*)	n.d.	O-*endo*
5c	3.36 (O-*endo*)	n.d.	O-*endo*
6a	n.d.	n.d.	O-*endo*
6b	n.d.	O-*endo*[14]	O-*endo*
7a	n.d.	n.d.	O-*exo*
7b	n.d.	O-*exo*[14]	O-*exo*
8b	n.d.	O-*endo*[22]	O-*endo*[e]

[a] The rotameric form in the x-ray crystal structure.
[b] The more highly populated rotamer as determined using the transient difference NOE protocol.
[c] n.d. = Not determined.
[d] Equal populations of both rotamers present.
[e] Exclusively populated rotamer in solution.

an apparent lowering of rotational preference by 0.2–0.9 kJ mol^{-1}, relative to the unsubstituted phenyl-3,5-dimethyl ester (**2a**) case, although the calculated preference is still O-*exo*. The *p*-bromophenyl-3,5-dimethyl ester compound **5a** shows the lowest preference over all at only 0.9 kJ mol^{-1}. An increase of 0.8 kJ mol^{-1} was calculated for the *p*-chlorophenyl compound **4a** relative to **5a**. Contrary to the 3′,5′-dimethylisoxazolyl case, the replacement of the 3,5-diesters by nitriles resulted in a rotameric preference of 3.4 kJ mol^{-1}, but in the O-*endo* direction.

Of the reported x-ray crystal structures, the rotameric preference is the same as the AM1 prediction for the 3′,5′-dimethyl (**1b**) and 3′-*p*-fluorophenyl-5′-methyl (**3a**) compounds, but different for the 3′-phenyl-5′-methylisoxazolyl-DHP (**2b**). It should be noted that all of the calculated rotameric preferences may well be meaningless as they rely on conformational energy differences of <4 kJ mol^{-1}. Furthermore, there is clear experimental evidence that the 3′,5′-dimethylisoxazolyl-3,5-diester compounds (**1a** and **b**) exhibit no rotameric preference in solution, even though one is computationally predicted.

As a prelude to the NMR experiments, the rotational barriers were also examined at the AM1 level. These calculations were also performed using SPARTAN 2.0, constraining the C-4-H—C-4—C-4′—C-3′ dihedral angle in 30° increments and optimizing each geometry first at the MM2 and then at the AM1 level. Using an IRIS Indigo, we were able to obtain results for nine of the 14 systems under investigation; the others had too many atoms for calculations at the desired level. The members of the 3′,5′-dimethylisoxazolyl series (**1a–c**) have the lowest ring junction rotational barriers. The rotational barrier of the diethyl ester compound **1b** has been calculated previously by Natale *et al.*[8] At the MM2 level, they reported a barrier of 38.5 kJ mol^{-1} and at the INDO/I level a barrier of 18.9 kJ mol^{-1}, the latter fairly close to our AM1 value of 19.9 kJ mol^{-1}. We obtained larger calculated barriers for the corresponding dimethyl ester (**1a**) ($\Delta\Delta G^{\neq} = 1.6$ kJ mol^{-1}) and dinitrile (**1c**) ($\Delta\Delta G^{\neq} = 3.9$ kJ mol^{-1}).

The lowest calculated barrier for the 3′-phenyl-5′-methylisoxazolyl series belonged to the *p*-bromophenyl diester compound **5a**, which was 1.6 kJ mol^{-1} lower than the barrier calculated for the unsubstituted 4-phenyl diester. In this series, $\Delta\Delta G^{\neq}$ for the dimethyl ester to dinitrile substitution was 6 kJ mol^{-1}. The complete set of barrier calculations appears in Table 4.

Low-temperature NMR

Initial studies employed $CDCl_3$ as the solvent. Proton spectra were collected for five compounds: 3′,5′-dimethyl- (**1a**), 3′-*p*-fluorophenyl-5′-methyl- (**3a**), 3′-methyl-5′-(1″-phenylprop-2″)-yl- (**8b**), 3′-phenyl-5′-methyl- (**2b**) and 3′-phenyl-5′-isopropyl- (**7b**) isoxazolyl-DHPs. No spectral changes suggesting hindered C-4—C-4′ rotation were evident down to the freezing point of the solvent (205 K). The only changes noted were in the aromatic region of the 3′-phenyl-5′-methyl compound (**2b**) (Fig. 5). The two possible interpretations of this were (1) slowed rotation about the C-3—C-1″ (isoxazole–phenyl) bond or (2) differences in the chemical shift temperature gradients of the *ortho*, *meta* and *para* protons on the phenyl ring. Additional studies (see below) support the latter rationale.

The NOE studies and computational results suggest that some of these compounds should have C-4—C-4′ rotational barriers that are experimentally observable. We turned to the use of deuterated Freon-21 ($CDFCl_2$) as the solvent and a specially designed probe and nitrogen cascade cooling system to lower the range of temperatures available. With Freon-21 samples, the low-temperature limit was 149 K.[24] The resulting spectra display broader lines but remain interpretable.

The 3′,5′-dimethyl compounds (**1a–c**) showed no additional peaks in the spectra even at 149 K. Some additional broadening was, however, observed for dinitrile **1c**. The most reasonable explanation for this is that the barriers to rotation are, indeed, too small to be detected. Assuming that chemical shift differences for the rotamers would be comparable to those which we observed in other series, an estimated $T_c < 139$ K leads

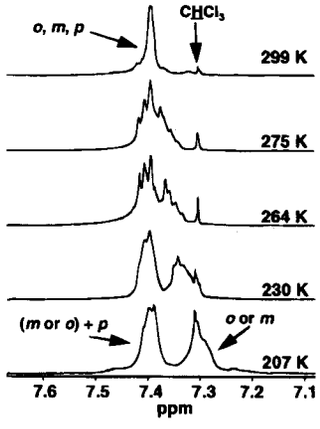

Figure 5. Temperature-dependent changes in the aromatic signals of the 3′-phenyl-5′-methylisoxazolyl compound **2b** in $CDCl_3$.

to a barrier estimate of ≤ 26.4 kJ mol^{-1} for the dinitrile **1c** (which is predicted to have the highest barrier in the series, 23.8 kJ mol^{-1}, by AM1). The two species with a 5′-isopropyl group (**7a** and **b**) also failed to show interpretable changes. Given that NOE studies at 220 K indicate that rotation is occuring and that one of the rotamers is a minor contributor to the equilibrium, this failure may reflect the very low equilibrium population of the minor form at the low-temperature limit. Compound **8b**, which has the very large 1″-phenylprop-2″yl substituent at the 5′ position of the isoxazolyl ring, also failed to show any evidence of a frozen rotameric equilibrium (recall that NOEs were observed from the C-4 H only to the C-3′ methyl group).

The first indications of hindered C-4—C-4′ rotation which we found were for the 3′-*p*-fluorophenyl-5′-methyl compound as the dimethyl ester (**3a**). Consistent temperature-dependent spectral changes were observed. However, the unfortunate chemical shift coincidence of the downfield signal from the aromatic ring with one of the residual proteo-Freon-21 signals prevented unambiguous assignment; the possibility of restricted rotation about the isoxazole–phenyl (C-3′—C-1″) bond remained. This was impetus for the synthesis of corresponding *p*-chloro and *para*-bromo compounds (**4a** and **5a**).

The effects of halogen substitution on the phenyl hydrogen *ortho* and *meta* to the halogen are well known,[20,21] $\Delta\delta_{(ortho-meta)} = -0.25$ ppm (F), $+0.05$ ppm (Cl) and $+0.26$ ppm (Br). These effects, combined with the $\Delta\delta$ values associated with the isoxazole substituent, should, in at least one case, avoid the previously mentioned shift coincidence and produce an interpretable AA′BB′ pattern for the phenyl ring protons, the latter being essential for the detection of hindered rotation about the C-3′—C-1″ bond. The *p*-bromo series proved to be best suited for our studies. The aromatic protons of **5a** gave the expected pair of doublets for a *para*-substituted phenyl ring as well resolved signals [Fig. 6A)]. Compound **5a** showed temperature-dependent changes similar to those for **3a** in the non-aromatic signals. More important, however, the changes in the aromatic signals were finally interpretable. No evidence for slowing of isoxazole–phenyl (C-3′—C-1″) rotation was detected. The temperature-dependent changes (in addition to the aromatic hydrogen resolution) reflecting C-4—C-4′ bond rotamers that were observed included the splitting of the C-4 H, ester methyls, 5′-methyl and 2/6-methyls each into two signals and are illustrated in Fig. 6(B). Remaining signal overlaps at the low-temperature limit precluded a definitive determination of rotamer populations *and* identity. The relative heights of the non-overlapping peaks at the low-temperature extreme suggest rotameric ratios of *ca.* 3 : 1. The minor rotamer is characterized by downfield shifts for the aromatic hydrogen and the C-4 H and by an upfield shift of the 5′-methyl, all of which suggest that the major rotamer has, in agreement with the NOE results, the O-*endo* conformation.

With the exception of the 5′-isopropyl species (**7a**), the resolution of two ester methyl signals at low temperatures was observed for all of the 3′-phenyl dimethyl esters. These species also displayed at least three of the other signal resolutions (due to hindered C-4—C-4′ rotation) listed above. The diagnostic resolutions of the C-4 H and C-2/C-6 Me signals were also observed for the 3,5-dinitrile **5c**. The $\Delta\nu$ values and T_c estimates are given in Table 3. Barrier estimations were performed using Eqn (1). In those cases where the T_c values for different signals varied, the trend, if any, in the derived barriers suggests a small negative $T\Delta S^{\neq}$ value.

The unsubstituted 3′-phenyl species **2a** exhibits the lowest barrier of the 3′-phenyl-5′-methyl-3,5-dimethyl ester series, 30.0 kJ mol^{-1}. The introduction of a 4″-chlorine atom (**4a**) raises the barrier by 1.8 kJ mol^{-1} over that of **2a**. The barriers for compounds **3a** and **5a** were determined to be 2.8 and 3.2 kJ mol^{-1} greater than for **2a**, respectively. The change of the 5′-methyl group of **2a** to an ethyl substituent (**6a**) resulted in an increase in the rotational barrier of 1.7 kJ mol^{-1}.

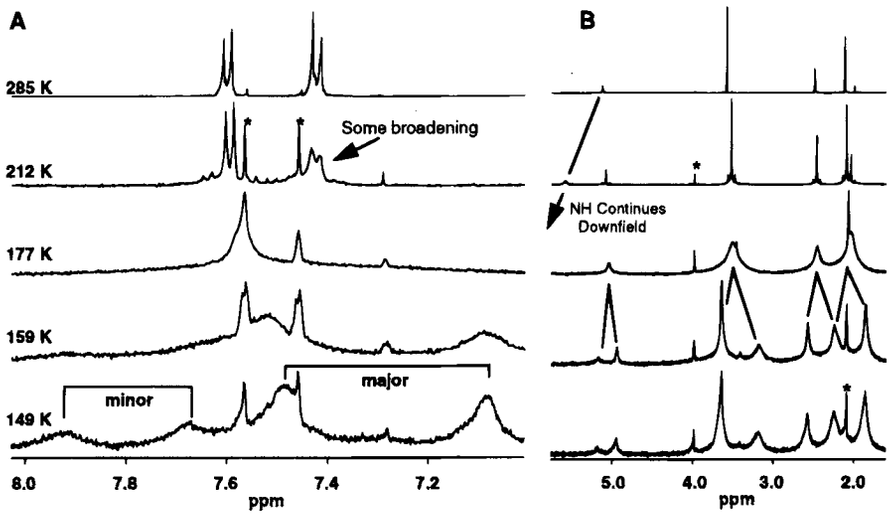

Figure 6. (A) Temperature-dependent changes in the aromatic signals of the 3′-(4″-bromophenyl)-5′-methylisoxazolyl dimethyl ester compound **5a** in Freon-21. The asterisks indicate residual signals from proteo-Freon-21. (B) Upfield region of the variable-temperature ^{1}H spectrum of compound **5a** in Freon-21 showing the temperature-dependent behavior used to determine $\Delta G^{\neq}$ for the isoxazole–dihydropyridine ring juncture rotation. The asterisks indicate impurities from sample decomposition. Lines indicate the resolution of population weighted average single peaks into individual rotamer peaks.

Table 3. Experimentally determined rotational barriers for 4-isoxazolyl-1,4-dihydropyridines[a]

Compound	Parameter[b]	Aromatics	C-4 H	Ester Me	C-5′ substituent	C-2/C-6 Me	Average $\Delta G^{\neq}$[c]
2a	$\Delta\nu$	88.5	n.d.[d]	238.4	206.2	104.5	
	T_c	155	n.d.	162	155	155	
	$\Delta G^{\neq}$	30.3	n.d.	30.4	29.2	30.1	30.0
3a	$\Delta\nu$	n.d.	110.5	234.7	170.1	156.5	
	T_c	n.d.	166	177	172	172	
	$\Delta G^{\neq}$	n.d.	32.2	33.4	32.8	33.0	32.8
4a	$\Delta\nu$	n.d.	116.3	241.6	179.2	195.5	
	T_c	n.d.	166	170	166	166	
	$\Delta G^{\neq}$	n.d.	32.2	31.9	31.6	31.5	31.8
5a	$\Delta\nu$	n.d.	115.2	231.0	163.5	194.2	
	T_c	n.d.	170	175	175	175	
	$\Delta G^{\neq}$	n.d.	33.0	33.0	33.5	33.2	33.2
5c	$\Delta\nu$	n.d.	69.4	n.d.	n.d.	170.2	
	T_c	n.d.	200	n.d.	n.d.	212	
	$\Delta G^{\neq}$	n.d.	39.9	n.d.	n.d.	40.8	40.4
6a	$\Delta\nu$	171.2	114.0	250.7	122.3	131.5	
	T_c	166	163	172	163	163	
	$\Delta G^{\neq}$	31.6	31.6	32.3	31.5	31.4	31.7

[a] Those compounds not shown on this list did not have kinetically isolated states at low temperature.
[b] $\Delta\nu$ in Hz, T_c in K, $\Delta G^{\neq}$ in kJ mol^{-1}.
[c] Errors in the determination of $\Delta\nu$, given the broadness and overlap of some signals, may be as large as 15 Hz but these do not contribute to the error in the barrier estimate as significantly as the 5–10 K error in the T_c estimate. The errors in individual $\Delta G^{\neq}$ estimates are 1–2 kJ mol^{-1}.
[d] n.d. = Not determined.

Significantly, although spectral changes consistent with rotation were observed in compounds **2a** and **6a**, analogous changes were not observed in the spectra of **2b** and **6b**. This raises the possibility of a substantial energy barrier effect in changing from a 3,5-dimethyl ester compound to a 3,5-diethyl ester compound. Two possible rationales for this observation are that (1) the barrier is much lower in the 3,5-diethyl ester compounds than in the 3,5-dimethyl ester compounds or, less likely, (2) there is a much larger thermodynamic conformational dominance in the case of the 3,5-diethyl ester compounds. Both of these possibilities could result from different preferred conformations of the esters, although this cannot be easily proved.

Since it was possible to isolate C-4—C-4′ rotation in the 3′-phenyl-5′-methyl-3,5-dimethyl ester species **2a** but not in the case of the 3′-phenyl-5′-isopropyl-3,5-dimethyl ester species **7a**, it was reasoned that the size of the 5′-substituent may influence both the rotamer populations and rotational barrier. In order to probe this possibility, the 3′-phenyl-5′-*ethyl*-3,5-dimethyl ester compound **6a** was examined. The rotational barrier in **6a**, which could be determined from the Freon-21 spectra by the isolation of signals corresponding to the two rotameric forms of the compound at low temperature, was only *ca.* 1.7 kJ mol^{-1} higher than that observed for **2a**. The low-temperature spectra suggest a *ca.* 3:1 ratio of rotamers, which is, surprisingly, less skewed than the population ratio (3.7–6.7:1) at 300 K derived from NOE data. Given these observations, we attribute the failure to detect two rotamers of **7a** to a highly skewed rotamer population ratio rather than a very large barrier.

Some critical questions remained with respect to the 3,5-diester functionalities. Although the spectral changes for the isolable dynamics of the Freon-21 spectra of the isoxazolyl-DHPs were consistent with rotation about the C-4—C-4′ ring juncture, they could potentially be interpreted as changes in the ester configuration in an *ap/sp* equilibrium since comparable barriers have been reported for phenyl ester rotations.[11] The 3,5-dicyano-substituted compound **5c** provides a clear resolution of this question by eliminating ester dynamics as a possibility. The low-temperature spectra for the 3′-*p*-bromophenyl-5′-methyl-3,5-dicyano compound **5c** displayed the same changes (with a much higher T_c) as observed for the corresponding 3,5-dimethyl ester (**5a**); these spectral changes are, without a doubt, due to rotation about the C-4—C-4′ bond, not a manifestation of an ester conformational equilibrium.

DISCUSSION

Of the five x-ray crystal structures reported in the literature, four have the same rotameric preference in solution as is observed in the solid state. The only exception is compound **1b** for which, although the published x-ray crystal structure shows exclusively the O-*endo* form, NOE evidence shows equal populations of both rotamers, even at 206 K in $CDCl_3$. The rotameric preferences at the AM1 level and as determined by x-ray crystallography and NOE methods appear in Table 2.

With regard to the rotational barrier, the prior computational results of Natale *et al.*[8] and our own AM1 calculations (Table 4) appear to be in accord with the experimental results. At the AM1 level, the switch from a 3,5-dimethyl ester to a 3,5-dinitrile for the 3′,5′-dimethylisoxazolyl compound (**1a** → **1c**) resulted in an increase in barrier of 2.3 kJ mol^{-1} and the same 3,5-alteration results in an increase of 6.0 kJ mol^{-1} for the

Table 4 Comparison of the rotational barriers[a]

Compound	AM1 $\Delta H^{\neq}$ (kJ mol^{-1})	NMR $\Delta G^{\neq}$ (kJ mol^{-1})
1a	21.5	$\ll 26.3$[b]
1b	19.9	n.d.[c]
1c	23.8	≤ 26.4[b]
2a	29.2	30.0
2b	29.5	n.d.
3a	29.7	32.8
4a	28.5	31.8
5a	27.6	33.2
5c	33.6	40.4

[a] Assuming a small entropy term, the AM1 $\Delta H^{\neq}$ and the experimentally determined $\Delta G^{\neq}$ may be compared directly.
[b] The NMR $\Delta G^{\neq}$ assumes $T_c \leq 139$ K for **1c** and $T_c \ll 139$ K for **1a** and that comparable $\Delta\nu$ values would be observed for the kinetically isolated rotamers.
[c] n.d. = Not determined.

p-bromophenyl case. Since the 3′,5′-dimethylisoxazolyl-DHP barriers were too low to measure, it was necessary to turn to the *p*-bromophenyl compounds for a comparison with an experimental determination. In this case, an increase of 7.2 kJ mol^{-1} ($\Delta\Delta G^{\neq}$) was observed experimentally. Whether this difference, calculated $\Delta H^{\neq}$ *vs.* experimental $\Delta G^{\neq}$, is large enough to argue for a $\Delta\Delta S^{\neq}$ term is unclear.

Only five of the 12, 3,5-diester isoxazolyl-DHPs had Freon-21 spectra that showed temperature-dependent changes consistent with C-4—C-4′ rotation. These compounds were the 3′-phenyl-5′-methyl (**2a**), 3′-phenyl-5′-ethyl (**3a**), 3′-(4″-fluorophenyl)-5′-methyl (**5a**), 3′-(4″-chlorophenyl)-5′-methyl (**6a**) and the 3′-(4″-bromophenyl)-5′-methyl (**7a**), all of which are 3,5-dimethoxycarbonyl compounds.

Allowing for the difficulties in accurately determining T_c and $\Delta\nu$, the agreement in the estimates of $\Delta G^{\neq}$ for these compounds is very good (Table 3). With the available experiments, we were able to determine barriers as low as *ca.* 30.0 kJ mol^{-1}. The lowest barrier is for a 3′,5′-dimethyl-substituted isoxazole (**1c**), and this is an estimate of the upper limit based on minimal differential line broadening. The barrier for the dimethyl ester **1a** must be significantly lower. The mutation of the 3′-methyl substituent to a phenyl group results in an increase in the barrier of at least 4 kJ mol^{-1}. The replacement of the 3,5-diesters with 3,5-dinitriles results in a higher rotational barrier on the case of isoxazolyl-DHPs, just as was observed in the phenyl-DHP cases.[11] Experimentally and in AM1 calculations, the change in barrier (calculated $\Delta\Delta H^{\neq}$ *vs.* measured $\Delta\Delta G^{\neq}$) for the diester to dinitrile substitution is less in the 4-isoxazol-4′-yl series [AM1 2.3–6.0 kJ mol^{-1}; experimental 7.2 kJ mol^{-1} (in the case of **5a** and **c**)] than in the *ortho*-disubstituted 4-phenyl series (AM1 8.9–11.5 kJ mol^{-1}; experimental 8.7–11.6 kJ mol^{-1}).[11] If the experimentally measured $\Delta\Delta G^{\neq}$ for the 3′-*p*-bromophenyl-5′-methyl series is an appropriate approximation for the 3′,5′-dimethylisoxazolyl series, the 3,5-dimethyl ester compound **1a** could have a barrier as low as 20 kJ mol^{-1}.

CONCLUSION

In order to observe experimentally the freezing out of C-4—C-4′ bond rotation in isoxazolyl-DHPs, groups with very large steric demands are required in positions 3′ and 5′. Lower temperatures were required to isolate the rotamers than in *ortho*-disubstituted phenyl-DHPs. In a number of cases, the addition of very large substituents in positions 3′ and/or 5′ of the isoxazolyl ring appears to result in large rotamer population differences that preclude the experimental determination of rotational barriers by the methods we employed. The studies reported confirm the C-4—C-4′ rotation barrier increase associated with the 3,5-diester to 3,5-dinitrile mutation.[11] In addition, the present study refutes (for the solution state) the previously reported O-*endo* rotameric preference reported for 4-isoxazolyl-DHPs bearing 3′ and 5′ substituents of essentially equal size.[8]

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