N-Inversion-Associated Conformational Dynamics Is Unusually Rapid in Morphine Alkaloids

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^{13}C DNMR studies of codeine and sinomenine (derivatives of *N*-Me morphinan) indicated that N-inversion−C−N rotation (NIR) is unusually fast for these substituted piperidines when compared with other *N*-Me piperidines. Since only broadening, but no signal splitting, was reached at low temperatures and the difference of chemical shifts ($\Delta\delta$) for individual conformers with the equatorially and axially oriented *N*-Me substituent was unavailable, the limits of the NIR barrier for these amines were determined by line shape analysis using $\Delta\delta$ values provided by ab initio calculations. On the basis of the comparison of experimentally determined ^{13}C NMR chemical shifts for tropane conformers with the ones calculated at different theory levels for this *N*-Me piperidine, the B3LYP/6-31G(p)/GIAO level was chosen as a sufficiently accurate method for calculations of $\Delta\delta$. By this new "semiempirical" procedure of line shape analysis the NIR barrier for the studied morphinans lies within a 25−27 kJ mol^{-1} (6.0−6.5 kcal mol^{-1}) range. A low NIR barrier for morphine alkaloids is supposed to be an important factor in the activation of morphine receptor.

A concerted nitrogen inversion−C−N rotation (NIR) is a common intramolecular dynamic process in alkylamines.[1a−f] In general, NIR is a fast conformational transformation: NIR barriers lie in a 20−40 kJ mol^{-1} (∼5−9 kcal mol^{-1}) range for a majority of nonfunctionalized alkylamines. Only certain changes in amine skeleton (without functionalization) raise the barrier to 45−105 kJ mol^{-1} (high NIR barrier alkylamines).[1c,2−4]

Experimental NIR barriers for chair-shaped *N*-Me piperidines are relatively high among usual amine barriers: they concentrate in or near the 30−36 kJ mol^{-1} (∼7.5−8.5 kcal mol^{-1}) range and do not cross the lower border of 29 kJ mol^{-1} (∼7 kcal mol^{-1}). For instance, these barriers for *N*-Me piperidine[5a] and *N*-Me 9-azabicylo[3.3.1]nonane[5b] are 36.4 and 29.7 kJ mol^{-1}, respectively.[5c] Of course, tropane (**1**; see Figure 1; 46.4 kJ mol^{-1} for the measured NIR barrier[2]) belongs to high NIR barrier systems.[6]

The situation with morphine (**2**), an alkaloid with the piperidine ring adopting a chair conformation also in solution[7a−c] (Figure 1), has been surprisingly unclear. An attempt[8] to measure NIR rate in **2** by DNMR was methodologically incorrect.[7c] The conclusion that a slow NIR occurs in this piperidine[8] is consequently nonrelevant. In contrast, the MM3-provided estimate of NIR barrier for *N*-Me amine **2** (27.6 kJ mol^{-1})[2a] lies below the above-mentioned lower limit. This barrier value relates this alkaloid to piperidines possessing the most rapid NIR among a family of *N*-Me piperidines. Also the *amine geometry−NIR barrier* correlation, which is capable of prediction of the barrier range in cyclic *N*-Me amines,[2a] indicates a rapid NIR for **1** (30.5 kJ mol^{-1} for the NIR barrier[2a]). Taking in mind these intriguing estimates and in view of the importance of morphine alkaloids, we have undertaken a computation-supported DNMR study of NIR in codeine (**3**) and sinomenine (**4**).

Results and Discussion

General Methodology. Of course, ab initio calculations of the NIR barriers at a high theory level could in principle lead to reasonable values for morphinans.[2b] For instance, the MP2/6-31G* level turned out to be sufficiently accurate in prediction of NIR barriers of relatively small bicyclic *N*-Me amines while the B3LYP/6-31G* level failed.[2a] Unfortunately, the accuracy of such ab initio calculations is questionable when applied to polycyclic alkaloids. Our calculations at the MP2/6-31G* level for non-H-bonded **2** gave the NIR barrier of 37.7 kJ mol^{-1} (this work; gas phase; no correction to the zero-point energy). This estimate differs from the above-mentioned estimates and is in a sharp contradiction with the no-decoalescence behavior of related compounds **3** and **4** at low temperatures (see below). The ab initio derived barrier should be obviously considered as not reliable for NIR in alkaloid **2**.

We consider this compound as problematic for NMR-based kinetic studies of NIR. In aprotic solvents amines and phenols form equilibrating mixtures of complexes of different strength and composition.[9a−d] Therefore one should expect such intermolecular equilibrium for **2** due to the presence of both amino and phenolic moieties in the morphine structure. An exchange between free amines and their protonated forms in solution is described as a multistep kinetic process of proton transfer.[9e−g] Therefore, the extraction of the barrier of NIR for a *free* amino group from experimental DNMR data for a system of complex kinetics, H-bonded amine **2**, would be unreliable (see ref 9h for a more detailed discussion of assignment problems of DNMR-measured barriers in the related case of H-bonding of aliphatic alcohols and alkylamines).

To avoid these difficulties, we have chosen for our DNMR study compounds **3** and **4**. Alkaloid **3**, an *O*-methylated parent opiate **2**, has no free phenolic function and therefore cannot form H-bonded complexes with amines. Also, the nonmethylated phenol group in analogue **4** is scarcely capable of such a complexation. The chemical shift of the proton of this group indicates that this moiety in **4** is H-bonded with the neighboring methoxy substituent, simi-

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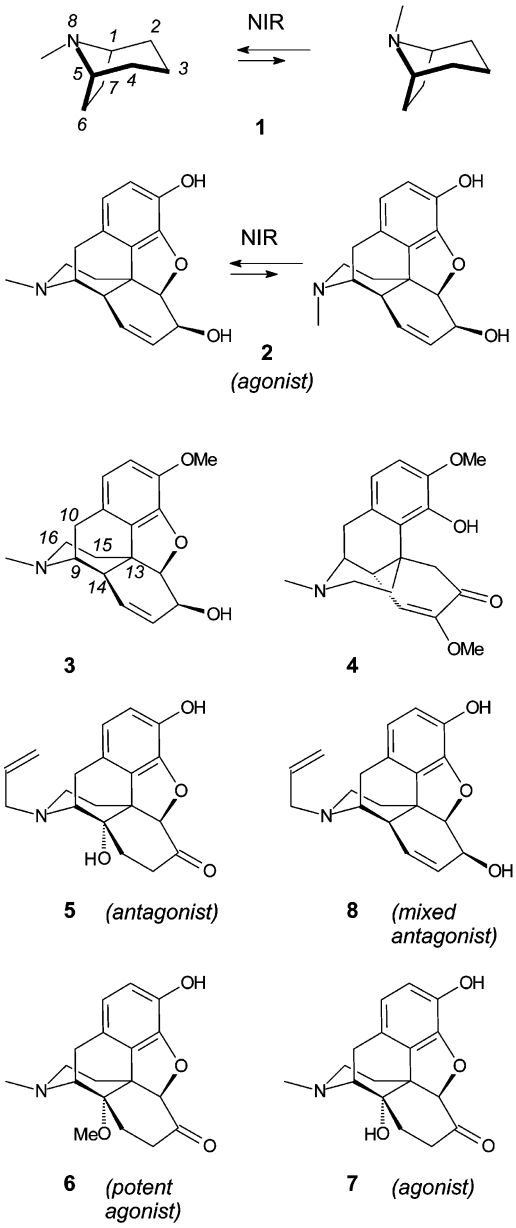

Figure 1. Alkaloids **1**–**8** and NIR in **1** and **2** (for **1** and **3** the numbering of the backbone atoms is shown). For morphinans **2** and **5**–**8** their activity toward morphine receptor is indicated.

larly to intramolecular H-bonding of these groups in *o*-methoxyphenol **5**[10a–c] (δ_{OH} = 5.5 ppm for **4** vs 5.7 ppm for **5**; in $CDCl_3$). In addition, ab initio calculations (see below) establish the presence of this H-bond: the distance between the proton of the OH group and the oxygen atom of the methoxy group is 0.199 nm for the minimal energy conformer of **4**. This intramolecular H-bonding should be sufficiently strong to provide NMR detection of the equilibrium only between the conformers with a free, not protonated amino group (as the lowest energy conformers): the enthalpy of the formation of this OH···OMe bond[10] in analogue **5** is 20.0 kJ mol^{-1}.

DNMR Studies. For the determination of NIR barriers in alkaloids **3** and **4** we have used line shape analysis of ^{13}C signals in variable-temperature ^{13}C NMR spectra of the these amines, based on iterative fitting of simulated line shapes to experimental ones. Aprotic solvents were used to prevent formation of moderate- or high-energy H-bonds of the *N*-Me group with solute molecules. Signal assignment was performed according to the assignment of ^{13}C signals of alkaloids **3**[11] and **4**[12] in $CDCl_3$ (see Figure 2 for ^{13}C shifts in CD_2Cl_2). Lowering the temperature of a solution of **3** or **4** in CD_2Cl_2 or in a 1:1 mixture of methylcyclohexane-d_{14}–THF-d_4 we observed significant line broadening at low temperatures.[13a] This broadening is most probably due to the slowing of conformational dynamics in *N*-Me amines **3** and **4**, since the signals of different backbone carbons are broadened appreciably differently with the temperature decrease (see Figure 2). Indeed, this assumption is confirmed by the fact that experimental signal width correlates with the difference of chemical shifts ($\Delta\delta$; see below for ab initio calculations of δ), e.g., of conformers with an equatorial and an axial *N*-Me group in compound **3**. For instance, methylene carbons C-10 and C-15 have larger $\Delta\delta$ values than the methylene C-16 (see below). Broad lines at 20.2 and 35.3 ppm (at 147.5 K) correspond to C-10 and C-15, respectively, while a narrower signal at 46.2 ppm belongs to the resonance of C-16.

Unfortunately, the signal decoalescence was not reached because of freezing of the sample at ~144 K and at ~138 K for the pure solvent and the solvent mixture, respectively. As a result, it was impossible to determine NIR rate for the studied compounds using only the obtained DNMR data because they do not provide necessary information for line shape analysis: the $\Delta\delta$ values for ^{13}C signals of individual conformers with equatorially and axially oriented *N*-Me substituents as well as the ratio of these conformers (in other words, their free energy difference $\Delta G°$).[13b] The lack of signal splitting at temperatures that are lower than the usual temperatures of signal dichotomy for other piperidines was the first experimental indication supporting our "morphine low barrier" suggestion.

For instance, we were able to measure ^{13}C chemical shifts for the conformers with different orientation of the *N*-substituent of piperidine **1** in CD_2Cl_2 at 182 K (this work; Figure 1 and Table 1) as well as of 7-*tert*-butyl-7-azabicyclo-[2.2.1]heptane (an amine of relatively low NIR barrier) in CD_2Cl_2 at 157 K.[3b] The conformational equilibrium in **1** is frozen even at higher temperatures,[2a] and ^{13}C signals of individual conformers are observable.

Combined DNMR–ab Initio Calculations Approach for Estimation of Conformational Barriers. Nevertheless, while the DNMR observations are insufficient for an accurate determination of NIR barriers for alkaloids **3** and **4**, satisfactory tight limits of these barriers can be established quite reliably combining the obtained DNMR data and quantum mechanical ab initio calculations. This approach employs *calculated* $\Delta\delta$ and different test values of the constant *K* for the equilibrium *N*-Me_{ax} conformer–*N*-Me_{eq} conformer. Then these values are used in traditional simulation of line shapes for variable-temperature NMR spectra, which provides desired kinetic parameters via the fitting of simulated spectra to their experimental line shapes.[13b] Indeed, the value of $K = 1$ (K_{min}; i.e., the case of the equal conformer content) obviously puts the lowest limit for *K* values. On the other hand, the maximal *K* value for $K > 1$ (K_{max}) that satisfies the fitting of experimental and simulated spectra for the given $\Delta\delta$ (in our case the ab initio-derived $\Delta\delta$) is the upper limit for *K* values. In other words, no *K* value higher than a certain value of K_{max} (i.e., too low a content of the minor conformer) can provide the experimentally observed signal line broadening *lw* upon the given value of $\Delta\delta$. Therefore, for this $\Delta\delta$, a pair $K_{min}/\Delta\delta$ actually determines the lowest limit of the DNMR-measured NIR barrier, while a pair $K_{max}/\Delta\delta$ actually determines the upper barrier limit.

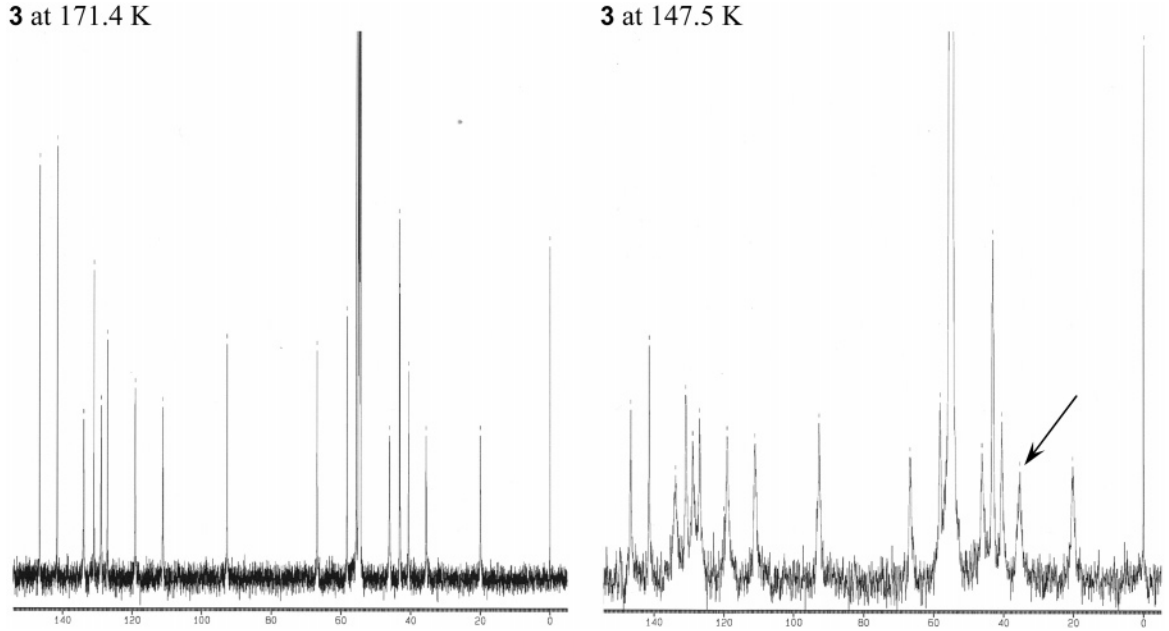

Figure 2. ^{13}C NMR spectra of **3** taken at two different temperatures. The signals are appreciably broadened differently for different carbon atoms as the temperature is decreased, while the TMS signal remains sharp. Line shape analysis was performed for the signal indicated by an arrow (the signal of the C-15 atom).

Table 1. Experimental and Calculated ^{13}C NMR Chemical Shifts for *N*-Me Amine **1**

	experimental ^{13}C NMR data[a]			B3LYP/6-31G(d); GIAO[b]			B3LYP/6-31G(d); CSGT[c]			B3LYP/6-31G(d,p); GIAO[d]			B3LYP/6-311G(d,p); GIAO[e]		
C atom	δ_{eq}	δ_{ax}	$\Delta\delta$	δ_{eq}	δ_{ax}	$\Delta\delta$	δ_{eq}	δ_{ax}	$\Delta\delta$	δ_{eq}	δ_{ax}	$\Delta\delta$	δ_{eq}	δ_{ax}	$\Delta\delta$
C-1,5	62.1	57.5	4.6	63.0	58.7	4.3	59.8	55.6	4.2	64.2	59.9	4.3	65.5	64.3	1.2
C-2,4	32.7	22.2	10.5	33.8	23.5	10.3	31.4	22.2	9.2	34.4	24.1	10.3	36.4	26.3	10.1
C-3	16.5	17.3	−0.8	19.3	20.3	−1.0	17.7	18.7	−1.0	19.8	20.7	−0.9	19.8	22.7	−2.9
C-6,7	25.6	29.0	−3.4	27.9	31.2	−3.3	27.7	30.1	−2.4	28.4	31.6	−3.2	27.6	33.8	−6.2
N-Me	42.0	33.0	9.0	41.5	33.3	8.2	42.8	34.8	8.0	41.7	33.4	8.7	41.9	35.1	6.8

[a] In CD_2Cl_2 at 182 K. $\Delta\delta = \delta_{eq} - \delta_{ax}$. [b−e] The calculated isotropic ^{13}C shifts for TMS are *b*: 189.7; *c*: 188.6; *d*: 191.8; and *e*: 184.5 ppm.

Of course, an accurate DNMR-independent estimation of both $\Delta\delta$ and $\Delta G°$ would lead to a certain satisfactory value of the NIR barrier for **3** or **4** (and not to a barrier range) when combined with DNMR data analysis (see above). However, computational methods for an accurate estimation of the conformer ratio for organic molecules that contain several dozens of atoms (i.e., calculations of relative Gibbs energy of the conformers) are not a reliable tool. Concerning the accuracy of such estimates of the relative stability of the conformers, a ±2−4 kJ mol^{-1} deviation from real values is a good result for ab initio as well as molecular mechanics calculations.[13c]

Such an accuracy is not suitable to be applied to DNMR-assisted studies of conformational kinetics. The range of $\Delta G°$ values for the equilibrium lowest energy conformer−higher energy conformer, which can cause measurable dynamic effects in NMR spectra at lower than room temperature, is less than 8.4 kJ mol^{-1} for a ∼50−99% content of one of the conformers. Due to the exponential character of the $\Delta G°-K$ relationship, a range of relative stability of only 0−2 kJ mol^{-1} covers a range of ∼50−77% population for a conformer at 300 K or ∼50−83% population at 200 K; that is, the ±2 kJ mol^{-1} accuracy in the determination of the relative stability of two conformers corresponds to approximately a ±30% deviation of absolute values for the content of the each conformer. Then, in relative error terms, an increase of the conformer ratio from 1:1 to 9:1 corresponds to an increase of this error from ∼120 to ∼600% for the population of the minor conformer. Of course, a *K* value that is estimated with a relative error of several hundred percent[13d] is quite useless in line shape analysis. Hence, the accuracy of $\Delta G°$ estimates should be significantly higher than 2 kJ mol^{-1} in order to provide a reasonable accuracy of determination of barriers for intra- or intermolecular processes *by DNMR*. This accuracy level means that the solvation contribution to $\Delta G°$ cannot be neglected. However, it is dubious that computational solvation models are as accurate as required above.

In contrast, a very good prediction of chemical shifts for different organic molecules by ab initio methods[14a−e] would provide the possibility to estimate $\Delta\delta$ successfully. This technique was even used to reproduce experimental chemical shifts for equilibrating conformer mixtures (i.e., time-averaged values), calculating the shifts for individual conformers for which relative stability had been determined independently.[15a−f] Regarding nonfunctionalized cyclic tertiary amines, calculations of δ turned out to be sufficiently accurate to allow quite reliable assignment of experimental ^{13}C signals for individual N-invertomers (observed under conditions of a slow NIR) of some simple azabicycles.[15g]

The values of $\Delta\delta$ are determined using GIAO[16a−c] as well as CSGT[17a,b] ab initio methodology (implemented in the Gaussian98 package[18]) for calculations of chemical shifts. To employ an adequate calculation method for piperidines **3** and **4** bearing an axial α-substituent, we examined the accuracy of ab initio-derived chemical shifts for the major and minor conformers of model piperidine **1**, which were obtained using different levels of theory (Table 1) for geometries optimized at the corresponding theory level. The piperidine ring in this cyclic amine adopts a chair confor-

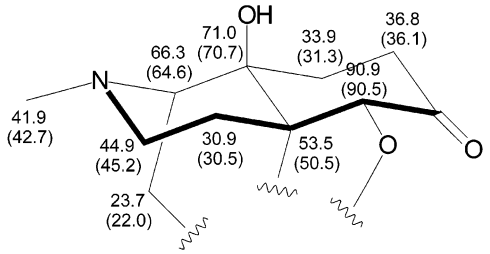

Figure 3. ^{13}C NMR chemical shifts calculated for sp^3-hybridized carbon atoms of oxymorphone **7** at the B3LYP/6-31G(d) level using the GIAO method (the corresponding experimental values[19b] are shown in parentheses). The geometry of **7** was optimized at the same level of theory.

mation (as the piperidine cycle in morphine alkaloids[7a−c]), and the minor stable conformation (i.e., the *exo*-conformer; the *N*-Me group is axially oriented with respect to the six-membered ring) is sufficiently populated at low temperatures to be NMR-detectable.[2a,19a]

Calculations at the B3LYP/6-31G(d) level (gas phase) using the GIAO methodology provide sufficiently accurate estimates of ^{13}C NMR chemical shifts as well as $\Delta\delta$ for the conformers of **1**, when compared with the experimental data. Regression analysis shows a very good correlation (a) between the calculated and experimental chemical shifts for the both conformers ($R^2 = 0.995$ at the 98% confidence level, standard error is 1.1 ppm) and (b) between the calculated and experimental $\Delta\delta$ values ($R^2 = 0.996$ at the 98% confidence level, standard error is 0.4 ppm). Statistical characteristics of the other used calculation methods are poorer. Only calculations at the B3LYP/6-31G(d,p) level provide a good estimation of $\Delta\delta$, but the shifts δ deviate more from the experimental values.

In addition, to be more confident of the reliability of the B3LYP/6-31G(d) level for estimation of ^{13}C NMR chemical shifts *in morphinans* we tested this methodology by calculating δ values for oxymorphone **7**. This compound adopts predominantly only one conformation (with an equatorial *N*-Me substituent) due to stabilization of this form via an intramolecular H-bonding; see below. There is therefore a convenient model for the comparison of experimental[19b] and calculated (this work) δ values of ^{13}C atoms in morphinans. A good correspondence was observed for these values (see Figure 3). The average deviation is 0.9 ppm for the calculated chemical shifts of the sp^3-hybridized carbons of **7** (standard error of linear correlation is 1.1 ppm; $R^2 = 0.999$ at the 98% confidence level). Moreover, since the parameter we need to predict is the *difference* of chemical shifts $\Delta\delta$ for the invertomers of morphinans **3** or **4**, the accuracy of such calculations is actually somewhat higher than the quality of the estimates of δ values themselves. Thus, we have chosen the above ab initio level for the estimation of $\Delta\delta$ for these alkaloids.

The low-energy conformers among the family with an equatorial as well as axial orientation of the *N*-Me group of **3** and **4** were located by Monte Carlo conformational search followed by MM3*-based energy minimization (Macromodel6.5 package;[20a−c] see Experimental Section). The geometry of the two lowest energy conformers (conformers **E** and **A** for **3** in Figure 4) was optimized at the B3LYP/6-31(d) level (gas phase; no correction to the energy zero point), and ^{13}C NMR chemical shifts for these structures were calculated at the B3LYP/6-31(d)/GIAO level. Conformers with an equatorially oriented *N*-Me group (e.g., conformer **E** for **3**) predominate over the corresponding conformers with an axial *N*-Me group (e.g., **A** for **3**). Relative stability (in terms of electron energy difference, ΔE) of these conformers for **3** and **4** is 8.8 and 10.0 kJ mol^{-1}, respectively. Ab initio calculations at the MP2/6-31(d) level are more successful for amines.[2a] Using this theory level we obtained the ΔE difference of 8.0 kJ mol^{-1} for **2** and 7.5 kJ mol^{-1} for **3**. The MM3 version with special parameters for amines[21a−c] gives a difference of 4.6 and 4.1 kJ mol^{-1} (in terms of steric energy) for conformers **A** and **E** of alkaloids **2** and **3**, respectively.

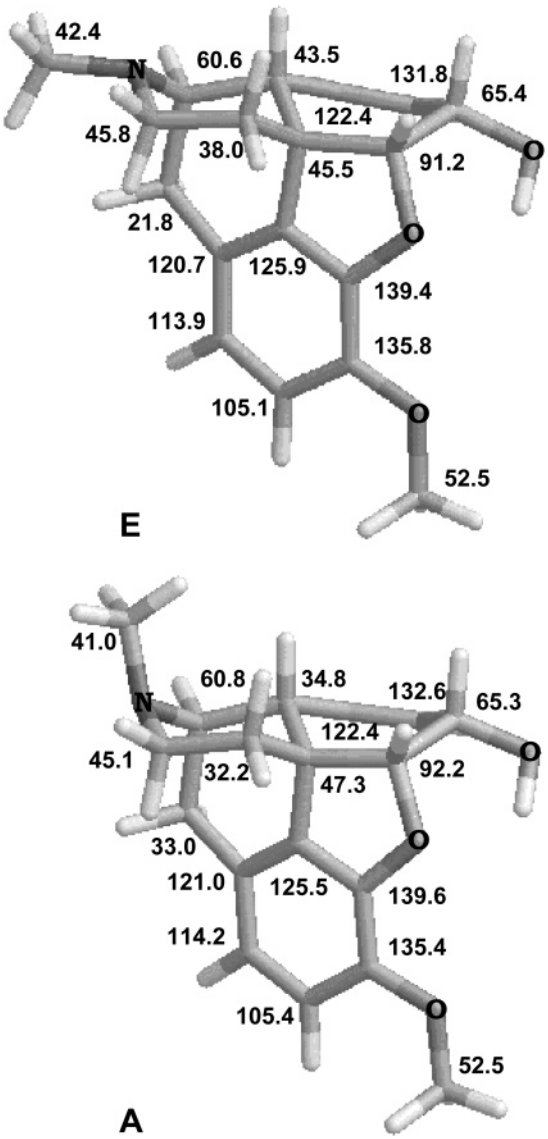

Figure 4. Conformers **E** and **A** of *N*-Me amine **3** (optimized geometry) and ^{13}C NMR chemical shifts (δ values) calculated by the GIAO method.

From the viewpoint of computation, this 3.4 kJ mol^{-1} difference between estimates obtained by two independent methods for the N-invertomers of **2** or **3** indicates a satisfactory calculation accuracy. Nevertheless, since a substantially higher accuracy of estimates is required for DNMR-studied kinetics (see above), we are unable to utilize these apparently good values as a quantitative measure of conformational equilibrium of **A** and **E**. In addition, the ab initio estimates do not correspond to the observed significant broadening of NMR signals at low temperatures for **3** or **4** (see below), since a low content of the minor conformer could not lead to an appreciable change in the variable-temperature spectra.

The observed signals at 35.31 ppm at 147.5 K for **3** and 34.73 at 152.9 K for **4** in CD_2Cl_2 at room temperature

belong to C-15 of **3** and **4** (Figures 1 and 2), respectively. The calculated values $\Delta\delta_{calc}$ for these signals (5.8 and 6.6 ppm or 875 and 996 Hz corresponding to our 14.1 T instrument for **3** and **4**, respectively) were used in the determination of the limits of the NIR barrier.

We note that these $\Delta\delta$ estimates for chemical shifts of carbon atoms, which are γ-positioned to the exocyclic Me substituent of the piperidine ring, are in excellent agreement with the 5−6 ppm value of the well-known γ-effect[22a−c] in six-membered cycles. Calculations of chemical shifts at the B3LYP/6−31(d) level using the CSGT method supply $\Delta\delta_{calc}$ of 5.0 ppm for C-15 of the conformers of **3**. Indeed, our choice of the GIAO method (see above for the test compound **1**) and thus the use of the 5.8 ppm value of $\Delta\delta_{calc}$ for **3** and 6.6 ppm for **4** is favored: the γ-effect of an axial *N*-Me substituent in the methiodide of α-metazocine[22d] (a benzomorphan derivative) is 6.8 ppm at C-4 (the structural analogue of the C-15 structural unit in morphines).

The fitting procedure demonstrated excellent agreement between experimental and simulated line shapes for **3** [T = 147.5 K, $\Delta\delta_{calc}$ = 875 Hz, line width = ∼180 Hz (corrected by the line width of the TMS line, 13 Hz)] and **4** (T = 152.9 K, $\Delta\delta_{calc}$ = 996 Hz, line width = ∼130 Hz (TMS line: 11 Hz)] starting from K = 4 (i.e., K_{max} = 4) and K = 6 (i.e., K_{max} = 6), respectively, down to K = 1. A higher K cannot provide the desired fit using the above parameters, as the maximum line broadening becomes smaller than the experimentally observed value. Thus, the range of NIR barrier ($\Delta G^{\#}$) for alkaloid **3** may be represented as 24.7 ≤ $\Delta G^{\#}$ ≤ 26.8 kJ mol^{-1} (0 ≤ $\Delta G°$ ≤ 1.7 kJ mol^{-1} at 147.5 K). The barrier range for alkaloid **4** is 24.7 ≤ $\Delta G^{\#}$ ≤ 26.9 kJ mol^{-1} (0 ≤ $\Delta G°$ ≤ 2.2 kJ mol^{-1} at 152.9 K). These barrier ranges are quite narrow. According to the ab initio as well as molecular mechanics estimates, **E** type conformers are somewhat favored over the corresponding **A** type conformers (i.e., K_{min} > 1). This reliable *qualitative* conclusion means that the ranges are even narrower, since the lower limit of the barrier range is actually higher than its minimal limit (the case of K_{min} = 1). Thus, the NIR barriers for *N*-Me alkaloids of the **2** series at low temperatures may be considered as a satisfactory accurate value of 25−27 kJ mol^{-1} (6.0−6.5 kcal mol^{-1}).

The correlation *amine geometry−NIR barrier*,[2a] which uses the average CNC angle (α_{av}) and the average CCN angle (β_{av}), predicts the barrier height for *N*-Me amines **3** and **4** to be 29.3 and 28.5 kJ mol^{-1}, respectively (α_{av} is 111.4° and 111.3° and β_{av} is 113.3° and 113.9° for the optimized geometry of **3** and **4**, respectively). We can conclude that similar results for these piperidines, which are supplied by the "semiempirical" *NMR−ab initio calculations* estimation and by the above correlation, provide additional support for our orbital model,[2a] explaining the dependence *amine geometry−NIR barrier* in cyclic amines. This means that the observed decrease of NIR barrier in morphines relative to other known piperidines is due to an unusual reciprocal orientation of the C_α−C_β bonds belonging to two different α-C atoms of the piperidine ring: one α-substituent of a piperidine chair is present and covalently locked axially in a unique benzomorphan skeleton (a structural component of morphinans).

Possible Biological Importance of Fast NIR in Morphines. NIR in *N*-Me morphinan derivatives indeed turns out to be unusually fast compared to other *N*-Me piperidines. Is the revealed morphinan-specific increase of NIR rate associated with a certain molecular mechanism of neurophysiological activity of opiates? It seems that the answer is rather positive.[23] For instance, it was demonstrated that alkaloid **2** undergoes conformational change (probably NIR) upon binding to UDP-glucuronosyl-transferase.[24a] Computational modeling of the post-uptake activation of the morphine receptor concluded that proton removal from the protonated amino group of **2** initiates the activation.[24b] It also means that the NIR-provided transformation *equatorial N-Me* → *axial N-Me* in the receptor-bound opiate is enabled. There is an indication that this conformational transformation takes place. Etorphine derivatives bearing a covalently fixed axial *N*-substituent are agonists of the morphine receptor and are devoid of antagonistic activity.[25a] Both stereoisomeric β- and α-*S*-methyl morphinans (sulfonium analogues of *N*-Me morphinan with the axially and equatorially oriented *S*-Me group, respectively) are recognized by the μ-opioid receptor; the β-isomer is more active than the α-compound in producing analgesia in rats.[25b]

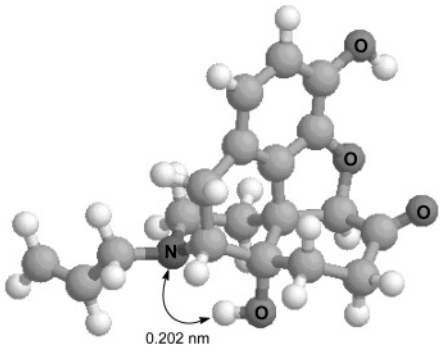

Figure 5. Intramolecular H-bonding in **5** [the B3LYP/6-31G(d,p) derived geometry is shown].

H-bonding with participation of the nitrogen lone pair increases NIR barriers in amines.[9h] Our calculations (this work) show that in the lowest energy conformer of naloxone **5** the 14-positioned OH group is H-bonded with the *N*-Me amino group (see Figures 1 and 5).[26] The role of this hydroxy group in diminishing the agonist activity is well-known[2b,25c,d] for many morphinans (e.g., **5** is a potent antagonist; compound **6**, with a methylated 14-OH group, possesses much higher agonist activity than the parent oxymorphone **7**[25e]). Such 14-hydroxy compounds should undergo relatively slow NIR owing to the intramolecular H-bonding, while morphinans without a H-bonded amino group possess a fast NIR similar to the measured one for alkaloid **3**. Thus, NIR slowing in opiates correlates with a decrease of their agonistic activity.

The agonism[25c] of opiates **2** as well as **6** and the antagonism[25d] of intramolecularly H-bonded morphinans are in line with the suggestion that a post-uptake NIR in receptor-bound opiates (i.e., the conformational transformation *equatorial N-Me* → *axial N-Me* for a free *N*-Me group) is necessary for activation of the receptor. In other words, we assume that the relative heights of three barriers, receptor activation, receptor deactivation, and opiate NIR (more accurately, the high energy points; see Figure 6), determine the mode of the receptor action. For instance, if two barriers, that of NIR for the *N*-Me group of morphinans (e.g. in **2**) and that of activation for conformational reorganization of the protein chain in the receptor−alkaloid complex, are lower than the barrier of another (deactivating) conformational change of the protein, the antagonism of the opiate is prevented. Consequently, the presence of a high barrier of NIR in the receptor-bound guest is sufficient to provide receptor deactivation.

Current models of the morphine receptor (reviewed in refs 25c,d; see also ref 27a) are actually thermodynamic

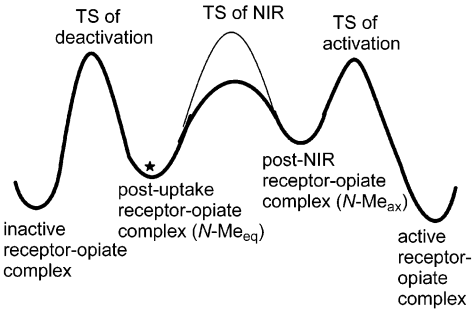

Figure 6. Kinetic control of activation−deactivation of morphine receptor (TS, transition state; *N*-Me$_{eq}$, receptor-bound morphinan with equatorially oriented *N*-Me group; *N*-Me$_{ax}$, receptor-bound morphinan with axially oriented *N*-Me group; asterisk indicates the initial complex receptor−opiate). In general, the relative height of the three barriers may be different for different opiates, and it determines their agonistic, antagonistic, or mixed mode of action. Bold contour shows an agonist case (e.g., **2**): the NIR barrier is the lowest one and the deactivation barrier is higher than that of the activation. The thin line shows the effect of the NIR barrier increase: the corresponding opiate is an antagonist of the receptor.

models of host−guest recognition, and activity of different opioids is considered from the viewpoint of their relativity affinity to the receptor. "One common recognition site" models seem quite reasonable for binding of morphinans. Indeed, a specific uptake of very similar molecules, for instance, agonist−antagonist pairs oxymorphone **7** versus antagonist **5** or agonist **2** versus nalorphine **8** (see Figure 1), by two different hypothetic "agonist-" and "antagonist-binding" receptor domains is scarcely possible. Accepting this point, our hypothesis of the receptor functioning is nevertheless not thermodynamic: the receptor response is considered to be under kinetic control (a conformational one) of post-uptake stages for opiates occupying one common receptor domain.

Decrease of internal conformational mobility (estimated measuring ^{13}C NMR relaxation times) in **2**, **5**, **7**, and **8** as well as some other analogues correlates with increase of antagonistic activity.[28a,b] This observation resulted in a similar conclusion regarding the role of conformational kinetics in the morphine receptor: the rate of conformational changes in opiates regulates its functioning.[28b] However, this correlation was found for the rotational freedom of the *N*-alkyl substituent and not for NIR. We studied the conformational dynamics of the nitrogen-containing fragment in *N*-allyl compound **8** in order to compare it with that of the *N*-Me analogue **2**.

MM3-provided barriers of NIR in alkylamines correspond well to experimental values[1b,e,9h] (e.g., see above for our DNMR estimate and the calculated value for **2**[2a]). Therefore this force field was used for the estimation of conformational barriers for **8** (see Experimental Section for details). The calculations have demonstrated that conformational kinetics in *N*-allyl compound **8** is as fast as in the *N*-Me analogue **2** (Figure 7). All three conformers ***A***, ***B***, and ***C*** with the axially oriented *N*-substituent are accessible from the lowest energy conformer with the equatorial *N*-allyl group passing through low-energy conformational pathways: via a low barrier NIR (conformer ***A***) or via this NIR followed by isolated rotation (ISR) of the axial substituent (conformers ***B*** and ***C***).

However, these results represent NIR and ISR rates for a free amine **8**. These barriers have to be higher in receptor-bound **8** due to steric interactions of a semirigid *N*-allyl substituent with the surrounding protein chain during a high-amplitude rotational motion of the substituent (NIR as a concerted process includes rotation of the *N*-substituent). For instance, *N*-methyl-*N*-ethyl-*N*-isopropylamine, the N pyramid of which is inverted in solution rapidly, undergoes a slow NIR inside a macromolecular cavity.[29] Thus, it is reasonable to consider the *N*-Me compound **2** and the *N*-allyl compound **8** as amines of different conformational kinetics in the receptor-bound form.

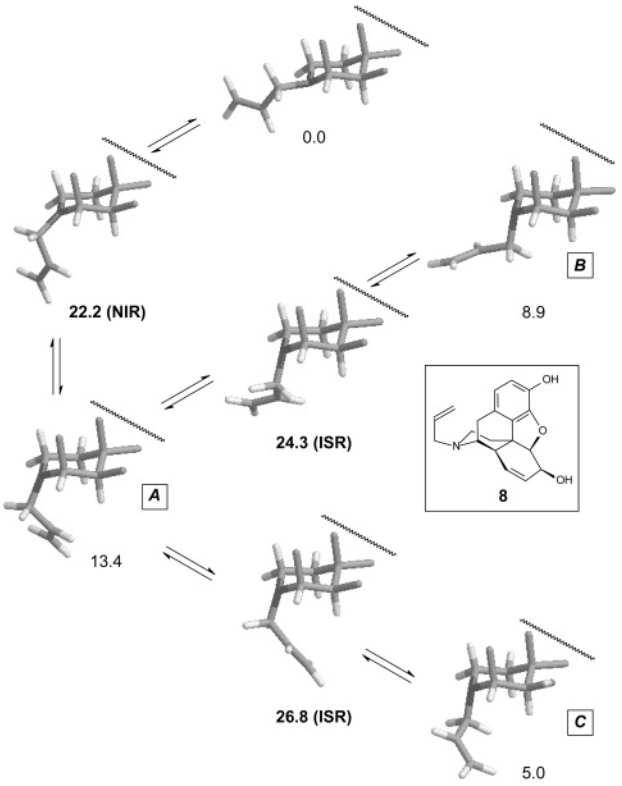

Figure 7. Low-energy conformational pathways from the minimal energy conformer with the equatorial *N*-allyl group of morphinan **8** to conformers ***A***, ***B***, and ***C*** with the axially oriented *N*-substituent (by MM3; for clarity only the piperidine cycle of **8** is shown). Numbers indicate relative steric energy (kJ mol^{-1}; in bold for transition states).

We realize that the present "three-barrier model" is only a hypothesis. Nevertheless, this model is capable of revealing the previously not understood agonist−antagonist dichotomy for a variety of morphine alkaloids combining differently functionalized opiates (morphinans with and without a 14-hydroxy group) and isofunctional opiates (different only by the structure of the *N*-alkyl substituent). The unexpected structure−activity relationship of these structurally quite different morphinans becomes understandable. In light of this model, the rate of NIR regulates the receptor response, and this regulator is dependent on H-bonding of the amino moiety as well as on the bulk of the *N*-substituent. It also explains the presence of mixed agonistic/antagonistic activity of some morphinan derivatives: this dual activity appears for an opiate for which the difference between the NIR activation and deactivation barrier is small. Finally, it leads to a new principle of rational design of new CNS active agents on the basis of the morphinan skeleton: such amine compounds have to possess decreased or increased NIR barriers to be receptor agonists or antagonists, respectively.

Experimental Section

General Experimental Procedures. ^{13}C NMR spectra were obtained on a Bruker DMX-600 spectrometer, with TMS as internal standard. Samples (~20 mg in 0.5 mL of CD_2Cl_2 and in a 1:1 mixture of methylcyclohexane-d_{14}−THF-d_4) were equilibrated ~10 min at each temperature before measuring.

Temperatures were measured with a calibrated Eurotherm 840/T digital thermometer and are believed to be accurate to 0.5 K. For the complete line shape analysis a modified version of a program that solves the exchange matrix, written by R. E. D. McClung, University of Alberta, Edmonton, Canada T6J 2G2, was used with visual fitting (the program was derived from the general equation for NMR line shape[30]). The activation parameters were calculated using the Eyring equation.

Codeine (3). ^{13}C NMR (CD_2Cl_2, 150.9 MHz, 171.4 K): δ 146.7 (C-4), 141.4 (C-3), 133.9 (C-7), 131.0 (C-12), 129.0 (C-8), 127.1 (C-11), 119.1 (C-1), 111.1 (C-2), 92.8 (C-5), 66.7 (C-6), 58.1 (C-9), 55.7 (*O*-Me), 46.2 (C-16), 43.1 (C-13), 43.1 (*N*-Me), 40.5 (C-14), 35.3 (C-15), 20.2 (C-10).

Sinomenine (4). ^{13}C NMR (CD_2Cl_2, 150.9 MHz, 171.4 K): δ 195.4 (C-6), 151.5 (C-7), 146.2 (C-4), 145.7 (C-3), 130.3 (C-11), 124.0 (C-12), 117.8 (C-1), 116.1 (C-8), 108.2 (C-2), 55.8[a] (C-6), 55.0[b] (3-*O*-Me), 54.4 (7-*O*-Me), 48.6 (C-5), 47.4 (C-14), 47.4 (C-16), 42.4 (*N*-Me), 40.9 (C-13), 34.7 (C-15), 24.2 (C-10); [a,b]a possible assignment reversal.

Molecular Mechanics Calculations. These were performed using Amber*, OPLS*, and MM3* force fields implemented into the Macromodel 6.5 package.[20a−c] The Amber* and OPLS* force fields were used for the geometry optimization of conformers of **2** and **5**, while MM3* was employed for compounds **3** and **4**. The *no solvent* as well as *distance-dependent dielectric electrostatics* options of Macromodel were employed for the energy minimization by these force fields. The *Monte Carlo* option was used for conformational search for polycycles **3**−**5** and **8** (generation of 10^4 structures for each *N*-Me conformer family with the energy upper limit of 3 kcal mol^{-1} from the lowest energy conformer found).

The 1996 version of the MM3 program[21a−c] with explicit parametrization for amines was used for conformational analysis of **3** and **8**. Energy minimization for the minima and maxima of steric energy was performed without restriction for the structural elements (full matrix minimization option). For calculations of NIR barriers the following procedure sequence was used: structures with a planar amino fragment were built via orientation of the minimum energy structure (located by Macromodel-mediated conformational search) to place the N atom and the two ring C_α atoms in the *xy*-plane; the *z*-coordinate of the third C_α atom was changed to zero; block diagonal minimization of this structure with no motion along the *z*-coordinate for the N atom and three C_α atoms was performed; the final step was full matrix minimization for the resulting structures. For location of rotational transition states the driver option was employed (rotation step of 1°) followed by full matrix minimization in the highest energy points. Coordinates derived from the eigen vectors (produced by option 5) of vibrational modes with imaginary frequency were employed as starting coordinates for minimization in the establishment of the relationship between transition states of NIR or ISR and the corresponding stable conformations.

Ab Initio Calculations. The geometry of molecular mechanics-minimized structures was used as the starting geometry for ab initio calculations (Gaussian98 package[18]) for the gas phase. Initial ab initio geometry optimization was performed at the restricted Hartree−Fock level using the 3-21G basis set. The resulting geometry was optimized at the RHF/6-31G(d) level and then at the B3LYP/6-31G(d) (for **1**, **3**, **4**, **7**, and **8**), B3LYP/6-31G(d,p) (for **1** and **5**), B3LYP/6-311G(d,p) (for **1**), or MP2/6-31G(d) (for **2** and **3**) level. Values of electron energy are not corrected to the zero-point energy. For each structure isotropic chemical shifts were calculated by the GIAO[16a−c] (for **1**, **3**−**5**, **7**, and TMS) or CSGT[17a,b] (for **1**, **3**, and TMS) method (implemented in Gaussian98) at the same theory level that was used for the geometry optimization.

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Supporting Information Available: Absolute electron energies (au) or steric energies (kcal mol^{-1}) as well as optimized geometry (Cartesian coordinates or Z-matrixes) for conformations of studied compounds; frequencies for transition states. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) We have chosen these solvents because of (i) satisfactory solubility of polar compounds **3** and **4** even at low temperatures (Freons or neat methylcyclohexane-d_{14}, which are often used in NMR studies for variable-temperature experiments do not provide appreciable solubility of these functionalized polycycles at low temperatures), (ii) the relatively low freezing point of individual solvent components, (iii) the relatively low polarity, and (iv) the absence of the ability to form high- or moderate-energy H-bonds with an amino group [in contrast to $CDFCl_2$, usually a convenient NMR solvent for low-temperature measurements (see Siegel, J. S.; Anet, F. A. L. *J. Org. Chem.* **1988**, *53*, 2629−2630), or $CDCl_3$]. (b) Parameters $\Delta\delta$, equilibrium constant *K*, line width in the absence of exchange (usually determined experimentally), as well as the rate constant *k* (a probe value to be fitted) are necessary for the simulation of the line shape for the equilibrium of two conformers (e.g., in the case of amines **3** and **4**). (c) Molecular mechanics cannot reproduce barriers very accurately since the calculated steric energy may be considered only as a rough approximation of the Gibbs energy. In contrast, the use of the combination of a higher theory level (e.g., MP4), diffuse functions, and an extended basis set would substantially improve the accuracy

of the estimation of the Gibbs energy by ab initio calculations for vacuum. In fact, computation facilities of most universities and other research centers do not permit applying such calculations to organic molecules containing more than 30−40 atoms. (d) Small relative errors for physical values are summed for the result of the algebraic division/product of these values. Accordingly to the definition, $K = p_2/p_1$ (p_j is the population of conformer j). Therefore the relative error for K is approximately the sum of relative errors for p_1 and p_2 if $\Delta p_1/p_1$ and $\Delta p_2/p_2$ are small. For bigger relative errors $\Delta p_1/p_1$ and $\Delta p_2/p_2$ (as in the case of an inaccurate estimation of p_1 and p_2 by calculation methods) the relative error for K is not expressed by a simple algebraic expression, but it is obviously substantially higher than relative errors $\Delta p_1/p_1$ and $\Delta p_2/p_2$ for the population of individual conformers.

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