Alteration of the *cis*:trans Isomer Ratio in *N*-Methylformamide using Lanthanide Shift Reagents. Determination of the Equilibrium Constants for Complexation

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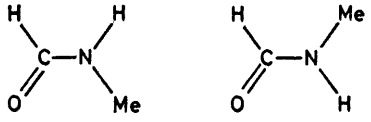
The *cis–trans* conformational equilibrium in a secondary amide is usually disrupted upon the binding of a lanthanide shift reagent (LSR). In this work, n.m.r. spectroscopy is used to monitor the changes in the *cis* : *trans* population ratio as well as to measure the lanthanide-induced shifts for each isomer at each concentration of LSR. A theory is developed which allows the determination of equilibrium constants and the limiting shifts for the 1 : 1 amide–LSR complexes from the experimental data. Application to the *N*-methylformamide–Eu(fod)$_3$–CCl_4 system reveals that the equilibrium constant for LSR binding to the *cis*-conformation of *N*-methylformamide is much larger than for binding to the *trans*-conformation. This causes the population of the *cis*-isomer to change from 8% in the absence of LSR to 44% when the molar ratio of LSR to amide is 0.5.

ONE of the assumptions usually made in the analysis of molecular conformations *via* lanthanide-induced shifts (LIS) is that the binding of the lanthanide shift reagent (LSR) does not alter the molecular conformation.[1] For certain substrates, however, for example secondary amides which can exist as a mixture of *cis* and *trans* rotational conformers, the presence of LSR can greatly perturb the *cis* : *trans* isomer ratio.[2-4] *N*-Methylformamide (NMF) was studied in this work since it has frequently served as a model compound for theoretical calculations of the relative energies of the *cis*- and *trans*-conformations of the peptide bond.[5]

Although most secondary amides, with a torsional barrier of *ca.* 70 kJ mol^{-1} about the central C–N bond, exist predominantly or exclusively in the *trans*-conformation, *N*-methylformamide exists, both neat [6] and



(1a) *trans* (1b) *cis*

in CCl_4 solution, as a mixture of 8% *cis* : 92% *trans* isomers. We have recently determined [2] that the addition of the lanthanide shift reagent Eu(fod)$_3$ to a solution of NMF in benzene or CCl_4 causes the percentage of *cis*-isomer to increase to *ca.* 44% at a molar ratio of Eu(fod)$_3$: NMF of 0.5. The continual increase in the *cis* : *trans* NMF isomer ratio as the amount of Eu(fod)$_3$ increases makes it impossible to analyse the data by published methods.

Equations have therefore been developed which treat the isomer ratio as well as the lanthanide-induced shifts as a function of LSR concentration. Assuming a 1 : 1 association model between NMF and the LSR, and taking into account LSR dimerization, estimates of the limiting shifts for the *N*-methyl protons of the *cis*- and *trans*-isomers allow the determination of the individual equilibrium constants for complexation of the LSR to the *cis*- and to the *trans*-isomer of NMF.

THEORY

1. *The* 1 : 1 *Complex.* cis- *and* trans-*Isomers complexing to the LSR.*—(a) *The equilibrium expressions.* Let c_1 and t_1 be isomers of a substrate capable of associating with the LSR. Let the equilibrium between *cis* (c_1) and *trans* (t_1) isomers be represented by equation (1) with equilibrium

$$t_1 \overset{K_x}{\rightleftharpoons} c_1 \qquad (1)$$

constant K_x. Upon the addition of LSR, each isomer can associate with the LSR to form 1 : 1 complexes [reactions (2) and (3)]. Furthermore, the LSR dimerizes to L_2

$$t_1 + L \overset{K_t}{\rightleftharpoons} t_1L \qquad (2)$$

$$c_1 + L \overset{K_c}{\rightleftharpoons} c_1L \qquad (3)$$

readily in inert solvents [reaction (4)].[7] Application of this

$$L + L \overset{K_L}{\rightleftharpoons} L_2 \qquad (4)$$

method to n.m.r. spectroscopy requires that there be at least one group in the n.m.r. spectrum of the molecule for which a *cis*-isomer signal can be distinguished from *trans*.

Let I_c and I_t be the relative intensity of the *cis*- and *trans*-isomer signals. Let S_0 be the total stoicheiometric amount of substrate in the solution (in M). Then I_c and I_t can be used to find C_c and C_t, where C_c is the molar concentration of *cis*-isomer, both bonded to LSR and unbonded and C_t is the corresponding concentration of

$$\begin{aligned} C_c + C_t &= S_0 \\ C_c &= c_1 + c_1L \end{aligned} \qquad (5)$$

trans-isomer [equations (5) and (6)]. The ratio (C_c/

$$C_t = t_1 + t_1L \qquad (6)$$

$C_t)_{L\to 0} = c_1/t_1 = K_x$. The conservation of mass equation for the LSR is (7) where L_0 is the stoicheiometric amount of

$$L_0 = L + c_1L + t_1L + 2L_2 \qquad (7)$$

LSR.

(b) *The n.m.r. chemical shift expression.* The lanthanide-induced shift of an n.m.r. signal from a *trans*-isomer is given by equation (8) where Δ_t is the limiting lanthanide-

$$\delta_t = [t_1L]\Delta_t/C_t \qquad (8)$$

induced shift (LIS) in the *trans*-1 : 1 complex and $[t_1L]/C_t$ is the mole fraction of *trans*-isomers bonded to LSR.

Replacing $[t_1L]$ in (8) by (2) results in equation (9). In a

$$\delta_t = [t_1][L]K_t\Delta_t/C_t \quad (9)$$

similar manner, the lanthanide-induced shift of an n.m.r. signal from a *cis*-isomer is given by equation (10) where Δ_c

$$\delta_c = [c_1L]\Delta_c/C_c = [c_1][L]K_c\Delta_c/C_c \quad (10)$$

is the limiting LIS in the *cis* 1 : 1 complex.

By taking the ratio of (9) to (10), as in equation (11) the

$$\delta_t/\delta_c = K_t/K_xK_c \cdot \Delta_t/\Delta_c \cdot C_c/C_t \quad (11)$$

inter-relationship among the parameters to be determined is evident. A plot of δ_t/δ_c *versus* C_c/C_t yields a straight line of slope $(K_t/K_xK_c)\cdot(\Delta_t\Delta_c)$ for the 1 : 1 association model.

(c) *Obtaining the equilibrium parameters.* The equations given above may be combined in various ways in order to obtain K_t, K_c, Δ_t and Δ_c. Eliminating $[t_1]$ from (6) and (9) yields equations (12) and (13). Substituting (2)—(4)

$$L = \frac{\delta_t}{K_t(\Delta_t - \delta_t)} \quad (12)$$

$$t_1 = \frac{c_t\,(\Delta_t - \delta_t)}{\Delta_t} \quad (13)$$

into (7) and rearranging gives the set of equations to be minimized for each experimental measurement [equation (14)]. In (14), $c_1 = K_xt_1$, K_x may be found from the

$$L_o - L(1 + K_cc_1 + K_tt_1 + 2K_1L) = 0 \quad (14)$$

intercept of a plot of C_c *versus* L_0/S_0, and K_L may be estimated, or literature values used. The values of [L] and $[t_1]$ are given by (12) and (13). The experimental data at each value of L_0/S_0 are the lanthanide-induced shifts of a selected group in the *cis*- and *trans*-isomers (for NMF, the *N*-methyl proton resonances). This leaves the values of K_t, K_c, Δ_t, and Δ_c to be determined. The value of the slope of the line resulting from a least-squares fitting of experimental data to (11) together with K_x and an estimate of Δ_t/Δ_c may be used to establish a relationship between K_c and K_t. (This was preferable to allowing all four parameters to vary, since an inverse relationship between K_t and Δ_t and between K_c and Δ_c was apparent.) Therefore, $K_c = (\Delta_t/\Delta_c)\cdot(K_t/K_x\cdot\text{slope})$.

This leaves only two unknowns to be determined for equations (14), K_t and Δ_t. Estimating a value for Δ_t/Δ_c, Δ_c and K_c may be found.

The ' goodness of fit ' of the experimental data to the theoretical model is given by an agreement factor, defined here by equation (15).

$$AF = \frac{\sum_i[(L_o)_{calc} - (L_o)_{obs}]^2}{\sum_i[(L_o)_{obs}]^2} \quad (15)$$

2. *The* 2 : 1 *Substrate* : *LSR Complex.*—(a) *The equilibrium expressions.* It is known that 2 : 1 substrate–$Eu(fod)_3$ complexes are in equilibrium with 1 : 1 complexes and in fact predominate in solutions where the L_0/S_0 ratio is small.[8] This leads to additional equilibria, for example (16).

$$t_1L + t_1 \overset{K_{t_2}}{\rightleftharpoons} t_1Lt_1 \quad (16a)$$

$$c_1L + c_1 \overset{K_{c_2}}{\rightleftharpoons} c_1Lc_1 \quad (16b)$$

(b) *The n.m.r. chemical shift expression.* The lanthanide-induced shift of the n.m.r. signal from the *trans*-isomer is given by equation (17) where $C_t = t_1 + t_1L + 2t_1Lt_1$.

$$\delta_t = ([t_1L]\Delta_1 + 2[t_1Lt_1]\Delta_{2t})/C_t \quad (17)$$

A similar expression is found for δ_c. Each 2 : 1 complex may have a unique value of Δ_2, the limiting shift, and mixed isomer complexes such as t_1Lc_1 are also expected to form. It is readily seen that many new parameters are introduced by considering the 2 : 1 complex for a substance which exists as a mixture of isomers. For this reason the NMF–$Eu(fod)_3$–CCl_4 system was assumed to undergo 1 : 1 complexation only.

RESULTS

The experimentally measured lanthanide-induced shifts for the *N*-methyl protons are shown in Figure 1 for a 0.100M-NMF in CCl_4 solution. The line of larger slope is due to the *N*-methyl protons of the *trans*-isomer (1a). It will be noted that the lines of Figure 1 are not straight as was observed for a number of dimethylamides, $RCON(CH_3)_2$, in both benzene and CCl_4 solution.[2] Instead, a definite

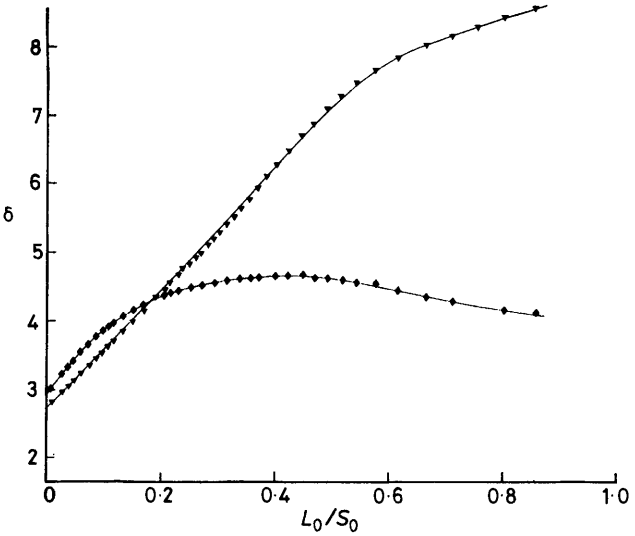


FIGURE 1 The chemical shift of the methyl protons in *cis*- and *trans*-NMF *versus* L_0/S_0 in CCl_4 solution: ▲, Protons of the *trans* isomer; ♦, Protons of the *cis*-isomer. The peaks cross at L_0/S_0 0.2. Curvature is apparent in the plots of each isomer. For clarity, partially overlapping symbols were omitted from the graph

curvature is observed, making it impossible to determine the slope of the line in the region $L_0/S_0 \longrightarrow 0$. This is due to the changing concentrations of the *cis*- and *trans*-NMF isomers with concentration of LSR, as shown in Figure 2. Due to the inaccuracies in determining the peak intensities, the relative peak heights were used to compute the percentage of *cis*-isomer present in 20 spectra selected over the concentration range $0 \leqslant L_0/S_0 \leqslant 0.545$ by a least squares analysis of % *cis versus* L_0/S_0. The results were used to calculate the percentage of *cis*- and *trans*-isomers at each value of L_0/S_0 at which an n.m.r. spectrum was run. The extrapolated *cis* : *trans* isomer ratio is 0.104 in the absence of LSR.

Equation (11) indicates that a plot of δ_t/δ_c *versus* C_c/C_t should be a straight line with a slope of $(K_t/K_xK_c)\cdot(\Delta_t/\Delta_c)$.

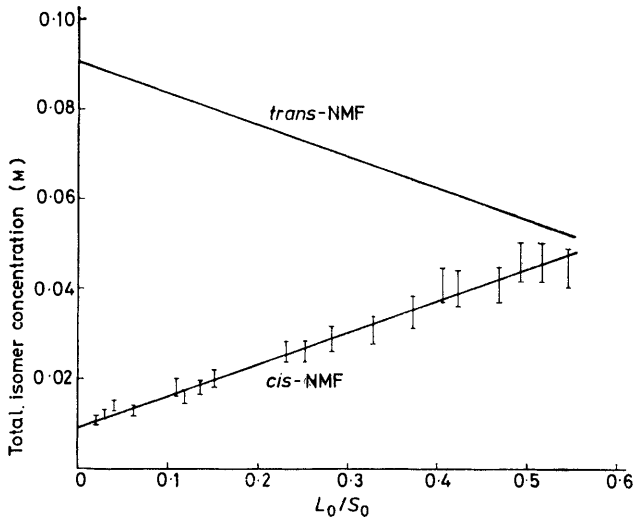


FIGURE 2 The molar concentrations of the *cis*- and *trans*-NMF isomers as determined from peak height measurements of the *N*-methyl protons *versus* L_0/S_0 in CCl_4 solution. The straight lines result from a least squares analysis of 20 spectra selected in the region $0 < L_0/S_0 < 0.545$ (see Results section). A $\pm 10\%$ error is assumed in the concentrations as L_0/S_0 increases due to LSR-induced line broadening

A least-squares analysis of this data yielded a straight line on the first 37 points (up to L_0/S_0 0.468) after which a positive curvature is obtained. The slope of the line is 2.88 ± 0.01. For further computer analysis, the 37 points falling in the range $0.020 \leqslant L_0/S_0 \leqslant 0.468$ were used.

Using a value of K_x of 0.104, which was obtained from Figure 2 at L_0/S_0 0, and a value of K_L of 100 l mol^{-1},[7] a Powell minimization [9] of equation (14) was used to find the best values of Δ_t and K_t at various values of the ratio Δ_t/Δ_c. The Table shows the results of the minimization. The

Values of the parameters for different initial values of Δ_t/Δ_c [a]

Δ_t/Δ_c	K_t/l mol^{-1}	K_c/l mol^{-1}	Δ_t	Δ_c [b]	AF [c]
1.80	95.4	573	11.3	5.0	0.0167
2.00	88.2	589	11.8	4.7	0.0163
2.20	82.1	602	12.4	4.4	0.0159
2.40	76.7	614	12.9	4.3	0.0156
2.60	71.9	624	13.4	4.1	0.0154
2.80	67.7	634	14.0	3.9	0.0151
3.00	64.0	640	14.5	3.8	0.0149

[a] *N* 37, K_L 100 l mol^{-1}. [b] After computer minimization on K_t and Δ_t, a linear regression was used to find the best value of Δ_c to fit the experimental values of δ_c (see text). [c] The agreement factor was computed using equation (15). At smaller values of Δ_t/Δ_c the value of AF continued to increase.

agreement factor is only slightly sensitive to the value of K_L due to the small fraction of free [L] in solution in this concentration range. In order to show how the concentrations $[t_1]$, $[c_1]$, $[t_1L]$ and $[c_1L]$ change as L_0/S_0 changes (Figure 3), a value of Δ_t/Δ_c of 1.8 was selected.

The values of $[t_1]$, $[t_1L]$, $[c_1]$, and $[c_1L]$ in Figure 3 are obtained directly from the minimization which yielded values of 95.4 l mol^{-1} for K_t and 11.3 for Δ_t. The values of Δ_c shown in the Table are obtained *via* a linear regression technique [10] using (10) and the experimental δ_c values. The values of [L] range from 1.25×10^{-3}M at L_0/S_0 0.020 to 6.02×10^{-2}M at 0.468. This corresponds to 86% of the LSR bound to NMF at L_0/S_0 0.020 and 53% bound at 0.468. Using K_L 100 l mol^{-1}, the LSR dimer concentration is 1.6×10^{-6}M at L_0/S_0 0.020 and 3.6×10^{-3}M at 0.468.

The formyl proton resonance was observed up to the last measurement at L_0/S_0 0.92. It did not separate into *cis*- and *trans*-peaks, and the lanthanide-induced shift roughly paralleled the *N*-methyl peak from the *trans*-isomer with a slope approximately twice as large.

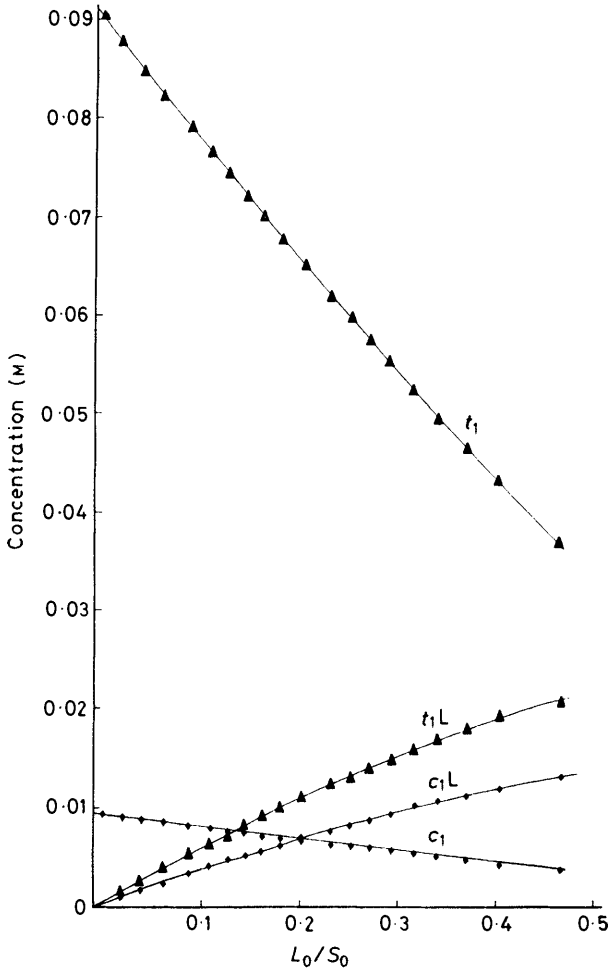


FIGURE 3 Computer calculated values of the molar concentrations of $[t_1]$, $[t_1L]$, $[c_1]$, and $[c_1L]$ *versus* experimental L_0/S_0 values in CCl_4 solution using Δ_t/Δ_c 1.8 (see Table and text). Every other point is plotted for clarity

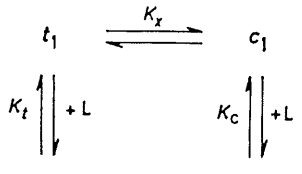
The NH proton resonances from the *cis*- and *trans*-NMF isomers were observed as one broad peak at $0 \leqslant L_0/S_0 \leqslant 0.05$ and were not observed in the region $0.05 < L_0/S_0 < 0.23$. At L_0/S_0 0.238 two separate NH peaks were found and were followed in the region $0.238 \leqslant L_0/S_0 \leqslant 0.425$. The NH peak from the *cis*-isomer was shifted by *ca.* 4.8 p.p.m. in the range $0 \leqslant L_0/S_0 \leqslant 0.425$ while the NH peak from the *trans*-isomer was shifted by 2.0 p.p.m. The NH peaks are extremely broad and quantitative evaluation of these shifts was not attempted. To our knowledge, this is the first observation of separate NH n.m.r. resonance peaks from *cis*- and *trans*-isomers of NMF.

DISCUSSION

The equilibria in the Scheme are considered as the most important for the *cis*- and *trans*-isomers of NMF when LSR is added. Since $K_c \gg K_t$ (Table), the

concentration of $[t_1]$ will decrease greatly (and $[c_1]$ slightly) so that $[t_1L]$ and $[c_1L]$ can form (Figure 3). This causes the total *cis*-isomer population, $[c_1] + [c_1L]$, to increase at the expense of the total *trans*-population, $[t_1] + [t_1L]$, as L_0/S_0 increases (Figure 2).

It is of interest, given the increasing total *cis*-population of NMF, that the *cis* lanthanide-induced shift, δ_c (Figure 1), starts to level off at L_0/S_0 *ca.* 0.2, while



SCHEME

the *trans*-shift, δ_t, does not even start to change slope until L_0/S_0 *ca.* 0.5. By reformulating (8) and (10) as (18) and (19) and noting that [L] increases as L_0/S_0 increases, it may be seen that since $K_c \gg K_t$, a much

$$\delta_c = \frac{K_tL}{1 + K_tL}\Delta_t \qquad (18)$$

$$\delta_c = \frac{K_cL}{1 + K_cL}\Delta_c \qquad (19)$$

larger value of [L] is required for $K_t[L]$ *ca.* 1 than for $K_c[L]$ *ca.* 1, and therefore δ_t will level off at a larger value of L_0/S_0 than δ_c. In fact, $K_c[L]$ is 1 at [L] 1.75×10^{-3}M, and this concentration of [L] is already present in a solution which has L_0/S_0 0.2. However, $K_t[L]$ is 1 at [L] 1.05×10^{-2}M and this concentration of [L] is not reached until $L_0/S_0 > 0.5$.

Examination of the Table reveals that the agreement factor for fitting the L_0 values to equation (14) slowly decreases as Δ_t/Δ_c increases. The problem becomes one of choosing a reasonable Δ_t/Δ_c ratio (Δ_t is an iterative parameter) which then fixes the values of the equilibrium constants. Our recent work with *NN*-dimethylformamide (DMF)–$Eu(fod)_3$–CCl_4 solutions [2] yielded values of 11.4 for Δ_t and 5.2 for Δ_c. Although there is no ***a priori*** reason for these values to be the same, it is not unlikely that the $Eu(fod)_3$ binds in a similar manner to the structurally related compounds NMF and DMF, resulting in similar values for Δ_t and Δ_c. Choosing the values of Δ_t 11.3 and Δ_c 5.0 from the Table results in values for K_c and K_t of 573 and 95.4 l mol^{-1}, respectively. The fact that $K_c \gg K_t$ is most likely due to steric effects, with the *N*-methyl group of the *trans*-isomer (1a) blocking somewhat the approach of the LSR molecule to the carbonyl oxygen binding site. The *cis* : *trans* ratio of NMF in 0.1M-D_2O solutions using $Eu(NO_3)_3$ as the shift reagent is not perturbed even at an L_0/S_0 ratio of 2.5. The Eu^{3+} ion is considerably smaller than the $Eu(fod)_3$ molecule; it seems likely, therefore, that the $Eu(fod)_3$ causes a perturbation of the *cis* : *trans* ratio *via* steric interactions.

The method presented here allows the determination of the equilibrium constants for complexation of LSR to *cis*- and *trans*-isomers of the substrate. Accurate measurements of C_t and C_c are necessary for the determination of K_t and K_c within a narrow range, and this was a likely source of significant experimental error in this work. Nevertheless, if one can measure C_t and C_c accurately, it should be possible to obtain Δ_t and Δ_c, as well as K_t and K_c, independently. A simple linear regression equation may be used with (10) to determine the value of Δ_c.[10] The results of this calculation have been included in the Table and it will be seen that Δ_c obtained from the values of Δ_t/Δ_c and Δ_t differs by *ca.* 1 p.p.m. from Δ_c obtained from (10). Furthermore, when the values calculated for $[c_1]$, $[c_1L]$, $[t_1]$, and $[t_1L]$ are summed, they range from 0.075 to 0.099 instead of being equal to 0.100M, the stoicheiometric concentration of NMF, with the largest deviation occurring at the highest value of L_0/S_0. This is attributed to errors in measurement of the $C_t : C_c$ ratio, the assumption that the concentrations have a linear dependence on L_0/S_0 (Figure 2), and perhaps a model which is too simple to describe the NMF–LSR equilibria. Although the data presented do not allow one to state unequivocally that the concentrations of 2 : 1 amide–LSR complexes are negligible, we believe that 1 : 1 complexes predominate in these solutions. In the only other detailed analysis of an amide-like system,[11] that of methyl *NN*-dimethylcarbamate [$MeOCONMe_2$–$Eu(fod)_3$–CCl_4], the equilibrium constant for the formation of 1 : 1 complexes was reported to be *ca.* 16 times as large as that for 2 : 1 complexes. Furthermore, the tendency for secondary amides to associate with each other *via* N–H • • • O=C hydrogen bonding would be expected to reduce the importance of the equilibria represented by equation (16) (the formation of 2 : 1 complexes).

The self-association of NMF through hydrogen bonding is significant in CCl_4. The equilibrium constants for dimerization and *n*-merization of *N*-methylacetamide [12] (which is predominately *trans*) are 1.5 and 23 l mol^{-1}, respectively in CCl_4, while the dimerization equilibrium constants for *cis*-lactams [13] in CCl_4 range from 110 to 470 l mol^{-1}.

Although the equations presented in the introduction to this paper were modified to include an infinite self-association model for *trans*-NMF and a cyclic dimer association model for *cis*-NMF, it was found that the fitting of the LIS data for the *N*-methyl protons was rather insensitive to the magnitudes of the hydrogen bonding equilibrium constants. Assuming, however, that K_{Hbond} (*cis*) > K_{Hbond} (*trans*), the equilibria would shift toward the formation of more *cis*-isomers, with C_c increasing at the expense of C_t. (Our calculated C_c concentrations are lower than the experimental C_c values at high L_0/S_0.) A further investigation of the competition of the LSR with another amide molecule for binding at the carbonyl oxygen site would be of interest. (Choosing a secondary amide which is exclusively a *trans*-isomer would reduce the number of parameters to be

determined.) While the LSR would be expected to reduce the degree of amide–amide self-association, it has been found that LSR increases the degree of intramolecular hydrogen bonding in two dipeptides.[14] Perhaps further study of the NH proton resonances in NMF using ^{14}N decoupling would enable the degree of hydrogen bonding to be determined. It may then be possible to extract the self-association equilibrium constants for *cis*- and *trans*-NMF from the LIS data. These constants have not yet been measured separately for *cis*- and *trans*-NMF.

In conclusion, this work has implications for conformational analysis using lanthanide shift reagents. If the molecule is rigid near the site of complexation of the LSR, the equilibrium constants for binding of the LSR to each conformation are likely to be equal. This assumption is used in virtually in all the conformational analysis being done *via* the LSR method and the LIS (Δ_i) reflects a weighted average over all possible conformations. The determination of the conformation of cyclic adenine monophosphate in solution [15] is a classic example of this approach. However, if the LSR can perturb the molecule at the site of complexation (as it does NMF and *N*-methylacetamide [2]) in such a way as to alter populations, the binding constant to each conformation is different and, if the rate of conformational change is slow on the n.m.r. time scale, the Δ_i values are obtained independently for each isomer and may be analysed as outlined in this paper. A problem which could interfere with conformational analysis *via* LSR is the following. If the LSR binds near the conformationally mobile site (for example, on the oxygen atom of alcohols) the equilibrium constants for binding to each conformer may be different. This will of course alter the conformer population. However, if the conformational change is rapid on the n.m.r. time scale, only one Δ_i will be measured for each magnetically non-equivalent nucleus (or nuclei), and the assumption is usually made that the binding constants to each conformer are equal. If the binding constants are in fact different this will lead to a determination of the structure of the complexed substrate which is not the same as that of the free substrate. It may therefore be advisable, when LSR binds to a molecule at a site which is near the conformationally mobile region, to use some independent method (for example, by showing that coupling constants do not change) to prove that the conformer populations are not altered in the presence of LSR.

EXPERIMENTAL

NMF (Eastman Organic Chemicals) was dried and fractionally distilled *in vacuo*. Spectrophotometric grade CCl_4 (Aldrich) was dried and distilled before use. Drying of the lanthanide shift reagent, $[^2H_{27}]Eu(fod)_3$ (Stohler) and preparation of the solutions have been described in detail.[16]

Fifty n.m.r. spectra were recorded, in the concentration range $0.02 \leqslant L_0/S_0 \leqslant 0.920$ with NMF remaining at 0.100M throughout (incremental dilution method [8]). Due to the difficulties in measuring peak heights or areas at high L_0 concentrations, the concentration range used initially in the calculations was $0.02 \leqslant L_0/S_0 \leqslant 0.545$. Forty measurements of the chemical shifts of the *N*-methyl, formyl, and NH protons of NMF were made at 28 °C using a Varian A-60A spectrometer. Three separate experiments were carried out to confirm the curvature in the plots at low LSR concentrations.

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