Using Hydrogen Bonding to Control Carbamate C–N Rotamer Equilibria

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In chloroform solution, the syn/anti rotamer ratios for N-(2-pyridyl)carbamates, 3, and N-phenylcarbamates, 4, are close to 0.05. Addition of the double hydrogen bonding acetic acid moderately stabilizes the syn rotamer of 4, but has no measurable effect on the syn/anti ratio for 3. Conversely, the hydrogen bon donor–acceptor-donor triad in 2,6-bis(octylamido)pyridine, 1, strongly stabilizes the syn rotamer of 3, but has no effect on the syn/anti ratio for 4. The Ks for syn-3:1 is 10−6−104 times higher than the Ks for anti-3:1. This implies that the alkoxy oxygen in anti-3 is a much poorer hydrogen bond acceptor than the carbonyl oxygen in syn-3, most likely because of a combination of steric and electrostatic factors.

Introduction

Rotational changes about individual bonds in a protein backbone can sometimes induce large changes in protein tertiary structure and consequently protein function. In general, the secondary amide linkages in proteins have a strong preference for the anti C–N rotamer. A recent survey of 399 protein structures found only 0.03% of the peptide bonds had adopted a syn C–N conformation. With tertiary amides, the syn/anti rotamer equilibrium constant is closer to unity, and protein derivatives in particular readily undergo amide bond rotation at physiological temperatures. In fact, peptidyl-prolyl isomerases (rotamases) are enzymes that specifically catalyze proline amide rotation.

The carbamate group also exists as syn and anti rotamers, with the anti rotamer favored by 1.0–1.5 kcal/mol for steric and electrostatic reasons (Figure 1). As with tertiary amides, the more-balanced rotamer equilibria and low activation energies mean that carbamates can act as conformational switches in molecular devices. A relevant example is the recent ion channel work of Woolley and co-workers. They used a carbamate linker to connect different ammonium groups to the entrance of the channel-forming peptide, gramicidin. Cation flux through the channel was found to be controlled by the thermal syn/anti isomerization of the carbamate linker. The ion channel model proposed by Woolley suggests that a compound with the ability to change the carbamate syn/anti ratio may be a chemical regulator of ion channel flux.

Figure 1. Carbamate rotamers.

Figure 2. Stabilization of syn-carbamate by hydrogen bonding with acetic acid.

But what sort of molecule could be used to alter the carbamate C–N rotamer equilibrium?

In 1996, Nudelman and co-workers reported that the syn rotamer of a carbamate group can be stabilized by hydrogen bonding with a carboxylic acid (Figure 2). In particular, they showed that the syn/anti rotamer ratios for N-alkyl carbamates (i.e., R' = alkyl in Figure 1) in CDCl3 solution could be increased from their normal values of around 0.1 to 0.5 by the addition of large amounts of acetic acid. We were intrigued by this discovery, as it coincides with our interest in conformational switches. Consequently, we set out to design a more effective hydrogen bonding system that selectively perturbs the syn/anti rotamer equilibrium of a target carbamate group.

We chose to evaluate the abilities of acetic acid and 2,6-bis(octylamido)pyridine, 1, to perturb the syn/anti

\[
\begin{align*}
C_6H_{15} & \quad N & \quad C_6H_{15} \\
1 \text{ R} &= H \\
2 \text{ R} &= CH_3
\end{align*}
\]

\[
\begin{align*}
\text{R} & \quad \text{O} & \quad \text{N} & \quad \text{b R} = \text{cholesterol} \\
3 \text{ a R} &= \text{ferbetyl} \\
4 \text{ a R} &= \text{ferbetyl}
\end{align*}
\]

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Spectroscopy. In general, the most diagnostic peak was the tert NH peak, which increased in intensity upon addition of acetic acid at low temperature. The 1H spectrum of a mixture of tert-butyl carbamates 3a and 4a in CDCl3 at 25 °C showed the tert NH peak at 10.60 ppm. In all cases, the tert NH peak was not observed at 4 °C. However, the tert NH peak increased in intensity upon addition of acetic acid at low temperature.

Figure 3. Association of 1 with the syn rotamer of 3 is favored over association with the anti rotamer.

Figure 4. Disfavored association of 1 with the syn rotamer of 4.

_ratio_ carbamates 3 and 4. We hypothesized that the syn rotamer of an N-(2-pyridyl)carbamate, 3a, with its acceptor-donor-acceptor (ADA) triad, would be preferentially stabilized by forming three hydrogen bonds with the DAD array in 1 (Figure 3). Moreover, triad 1 should stabilize the syn rotamer of 3 much better than the syn rotamer of tert-butyl N-phenylcarbamate, 4 (Figure 4).

**Results**

Compounds 1–4 were prepared in straightforward fashion using standard procedures. The carbamate syn/anti rotamer ratios were determined by 1H NMR spectroscopy. In general, the most diagnostic peak was the carbamate NH signal. In a preliminary study, solutions of tert-butyl carbamates 3a and 4a in CDCl3 were titrated with 1H acetic acid at 25 °C. In all cases, the syn NH peaks were not observed so the titrations were repeated at lower temperatures. In the absence of acetic acid, the syn rotamers of 3a and 4a were not observed until the temperature was lowered to −20 °C. The anti to syn equilibrium constants, K_{s/a}, at this temperature were both 0.05. In the case of phenyl carbamate 4a, the signals corresponding to the syn rotamer increased in intensity upon addition of acetic acid at low temperature. The 1H spectrum of a mixture of 4a (6 mM) and acetic acid (120 mM) in CDCl3 at −20 °C showed the anti NH at δ 6.58 ppm and the syn NH at δ 8.43 ppm, with a syn/anti rotamer ratio of 0.12. Addition of much larger amounts of acetic acid increased the syn/anti ratio to 0.4. The 1H spectrum of 2-pyridyl carbamate 3a (6 mM) and acetic acid (120 mM) in CDCl3 at −20 °C showed only one set of peaks corresponding to the anti rotamer, but the anti NH signal had moved substantially from its δ 6.5 to 6.7 ppm upon cooling but no peak was observed for the syn NH. On the other hand, 1 was found to significantly stabilize the syn rotamer of 3a. A sample of 3a (6 mM) and 1 (60 mM) in CDCl3 was examined by 1H NMR at −10, −20, −30, and −40 °C. In addition, mixtures of 3a/1 at ratios of 1:1, 1:5, 1:10, and 1:20 were examined at −20 °C. The chemical shifts of both the anti and syn NH signals for 3a were listed in Tables 1 and 2, along with the apparent anti to syn equilibrium constants, K_{s/a}. The chemical shift data from Table 2 is also presented as a graph in Figure 5. The anti NH signal chemical shift is more sensitive to changes in temperature and relative amount of 1 than is the syn NH signal. K_{s/a} for a sample containing [3a] = 6 mM

**Table 1. Temperature Dependence of NH Chemical Shifts and K_{s/a} for 3a in the Presence of 1**

<table>
<thead>
<tr>
<th>T, °C</th>
<th>δ anti NH, ppm</th>
<th>δ syn NH, ppm</th>
<th>K_{s/a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>−10</td>
<td>8.64</td>
<td>10.51</td>
<td>0.82</td>
</tr>
<tr>
<td>−20</td>
<td>8.96</td>
<td>10.58</td>
<td>0.96</td>
</tr>
<tr>
<td>−30</td>
<td>9.32</td>
<td>10.64</td>
<td>1.38</td>
</tr>
<tr>
<td>−40</td>
<td>9.68</td>
<td>10.70</td>
<td>1.78</td>
</tr>
</tbody>
</table>

a [3a] = 6.0 mM and [1] = 60 mM in CDCl3; K_{s/a} is the anti to syn equilibrium constant.

**Table 2. NH Chemical Shifts and K_{s/a} for Different Ratios of 3a:1**

<table>
<thead>
<tr>
<th>3a:1</th>
<th>δ anti NH, ppm</th>
<th>δ syn NH, ppm</th>
<th>K_{s/a}</th>
<th>ΔG, kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:0</td>
<td>8.64</td>
<td>8.64b</td>
<td>0.05</td>
<td>+1.52</td>
</tr>
<tr>
<td>1:1</td>
<td>8.71</td>
<td>10.36</td>
<td>0.18</td>
<td>+0.87</td>
</tr>
<tr>
<td>1:5</td>
<td>8.83</td>
<td>10.56</td>
<td>0.59</td>
<td>+0.27</td>
</tr>
<tr>
<td>1:10</td>
<td>8.96</td>
<td>10.58</td>
<td>0.96</td>
<td>+0.02</td>
</tr>
<tr>
<td>1:20</td>
<td>9.26</td>
<td>10.60</td>
<td>1.50</td>
<td>−0.21</td>
</tr>
</tbody>
</table>

a In CDCl3 at −20 °C; [3a] ranged from 9.80 mM to 7.14 mM over the course of the titration; K_{s/a} is the anti to syn equilibrium constant. b See ref 10.
For this system provides values of $\Delta H^\circ = -3.2$ kcal/mol and $\Delta S^\circ = -12.8$ cal/mol K. The $^{13}$C NMR spectrum of a mixture of 3a (100 mM) and 1 (1.0 M) in CDCl$_3$ at $-20$ °C shows two sets of signals for 3a. The best resolved pair correspond to the quaternary carbon of the tert-butoxy group at $\delta$ 81.2 (anti) and 84.0 (syn) ppm.

$^3$H and $^{13}$C NMR spectra of mixtures of 3a and N-methylated control 2, at a ratio of 1:10 in CDCl$_3$ at $-20$ °C exhibit only one set of signals for 3a. The chemical shifts closely match those for 3a on its own (the peak for the quaternary carbon of the tert-butoxy group is at $\delta$ 81.0 ppm). In other words, 2 does not stabilize the syn rotamer of 3a.

The rotamer equilibria for cholesteryl derivatives 3b and 4b are very similar to the tert-butyl analogues described above. The $^1$H NMR spectrum of a mixture of phenyl carbamate 4b (10 mM) and 1 (100 mM) at $-20$ °C shows no evidence for the syn rotamer, whereas spectra of a mixture of 2-pyridyl carbamate 3b (10 mM) and bis(amide)pyridine 1 (100 mM) at $-20$, $-30$, and $-40$ °C closely match those for the 3a/1 system. The anti NH signal moves significantly downfield, and $K_{s/a}$ increases as the temperature is decreased from $-20$ to $-40$ °C (Figure 6).

**Discussion**

At low temperatures, the syn/anti rotamer ratio, $K_{s/a}$, for N-alkyl carboxamides (i.e., $R' =$ alkyl in Figure 1) in CDCl$_3$ solution is $<0.1$. For the N-aromatic carboxamides 3 and 4, $K_{s/a} < 0.05$, indicating that their syn rotamers are particularly unfavored. A likely explanation is the steric hindrance shown in Figure 7. Addition of acetic acid to a solution of phenyl carbamate 4a in CDCl$_3$ at $-20$ °C stabilizes the syn rotamer of 4a and raises the syn/anti rotamer ratio from 0.05 to 0.4. This observation is consistent with the work of Nudelman and co-workers and is attributed to the acetic acid stabilizing the syn rotamer by hydrogen bonding (Figure 2). Acetic acid has no observable effect on the syn/anti rotamer ratio for 2-pyridyl carbamates 3a. In this case, the carboxylic acid prefers to donate a hydrogen bond to the more basic pyridyl nitrogen and form the complex shown in Figure 8. The association constant for this complex must be reasonably high because the carbamate NH signal moves substantially from its chemical shift of $\delta$ 8.63 ppm in the absence of acetic acid to $\delta$ 10.00 ppm.

In CDCl$_3$ solution the triad 1 strongly stabilizes the syn rotamer of 3a and raises the syn/anti rotamer ratio from 0.05 to 1.5 (Table 2). The failure of the N-methylated control 2 to affect the syn/anti ratio is strong evidence that the stabilization provided by 1 is due to its hydrogen bonding properties. This stabilization increases as the temperature is lowered (Table 1). A van't Hoff plot of the temperature dependence of the apparent anti to syn equilibrium constant, $K_{s/a}$ for 3a in the presence of 1 provides $\Delta H^\circ = -3.2$ kcal/mol and $\Delta S^\circ = -12.8$ cal/mol K. Assuming $\Delta H^\circ = +1.5$ kcal/mol for the anti to syn isomerization in the absence of 1 means that under these conditions the syn-3a:1 complex is 4.7 kcal/mol (3.2 + 1.5) more stable than the anti-3a:1 complex.

This difference in stabilities is confirmed by the different sensitivities of the syn and anti NH chemical shifts to temperature and concentration changes. The chemical shift of the syn NH signal for 3a (or 3b) (6 mM) in the presence of 1 (60 mM) hardly moves as the temperature is lowered (Table 1 and Figure 6), indicating that syn-3 is saturated with 1 at these concentrations. This is confirmed by the titration curve shown in Figure 5 which shows that less than 5 mol equiv of 1 is needed to saturate syn-3a. The anti NH chemical shift, however, moves significantly downfield as the temperature is decreased (Table 1), or as the relative amount of 1 is increased (Figure 5), indicating that anti-3 (or anti-3b) is far from saturation. The titration curves in Figure 5 clearly show that the association of 1 with syn-3a is much stronger than with anti-3a. A fully quantitative treatment of the titration data would involve fitting the binding curves to the scheme shown in Figure 3. However, the errors would be very large due to the many independent variables and the small number of data points. Instead, a semiquantitative analysis was conducted. We determined an overall association constant of $4 \times 10^2$ M$^{-1}$ by titrating 1 with 3a in CDCl$_3$ at $-20$ °C$^{11}$ and assumed that it was approximately equal to $K_s$ for the syn-3a:1 equilibrium.$^{12}$ The anti-3a:1 titration curve was then fitted to a 1:1 binding model using

(11) The chemical shift of the NH signal for bis(amide) 1 (2 mM) was determined as a function of different amounts of 3a (0.2–10 mM), and the resulting curve fitted to a 1:1 binding model.$^{13}$ The homodimerization of acylated 2-amino-pyridines such as 3a is known to be sufficiently weak ($K_s \sim 2$ M$^{-1}$) that it can be ignored for this calculation.

standard regression methods, which resulted in a $K_a$ of $\sim 0.1 \text{ M}^{-1}$. While the uncertainties associated with the van't Hoff and titration analyses are probably quite large, they both agree that the $K_a$ for syn-$3a$.1 is $10^{11}$--$10^{14}$ times higher than the $K_a$ for anti-$3a$.1. This implies that the tert-butyloxy oxygen in anti-$3a$.1 is a much poorer hydrogen bond acceptor than the carbonyl oxygen in syn-$3a$.3, most likely because of a combination of steric and electrostatic factors (Figure 3).

As expected, the cholesterol derivative, $3b$, behaves much like the tert-butyl analogue except the values for $K_{sa}$ are slightly lower (compare Figure 6 with Table 1). Cholesterol carboxamides are used as building blocks for a range of supramolecular assemblies such as DNA-containing lipoplexes, liquid crystals, gels, and Langmuir–Blodgett films. In each case the macroscopic properties of the assembly are controlled by apparently subtle changes in the shape of the cholesteryl component. The next goal of this research is to prepare liquid crystalline films that incorporate cholesterol carboxamides such as $3b$ and to determine if the films’ optical properties change when exposed to compounds such as bisamide $1$.17

Conclusions

In CDCl$_3$ solution, the double hydrogen bonding acetic acid moderately stabilizes the syn rotamer of phenyl carbamate $4$ (Figure 2) but has no measurable effect on the syn/anti ratio for 2-pyridyl carbamate $3$ (Figure 8). Conversely, the donor–acceptor-donor triad $1$ strongly stabilizes the syn rotamer of $3$ (Figure 3), but has no effect on the syn/anti ratio for $4$, presumably because of steric hindrance to the formation a hydrogen bonded complex (Figure 4).

Experimental Section

The low-temperature NMR studies were conducted on a Varian 500 MHz instrument. The probe temperatures were measured with a calibrated, digital thermocouple which is accurate to $\pm 0.5$ °C. Fresh bottles of CDCl$_3$ (Aldrich) were used to prepare the NMR samples.

2,6-Bis{octylamido}pyridine, 1. 2,6-Diamino.pyridine (0.5 g, 4.58 mmol) was dissolved in ethyl acetate and added to an aqueous solution of NaOH (5.7 M, 2 mL). The reaction flask was cooled to 0 °C, and octanoyl chloride (2 mL, 11.5 mmol) was added dropwise. The reaction mixture was stirred for several hours and then washed five times with 0.2 M NaOH. The organic layer was dried over MgSO$_4$ and the solvent was evaporated. The product was recrystallized from chloroform/hexane to give a white solid. Yield: 30%. mp 99–100 °C (lit. 92–93 °C)$.^{10}$ R$_t$ (methylene chloride) = 0.59; $^{1}$H NMR (300 MHz, CDCl$_3$) _δ_ 1.52 (s, 9 H), 6.95 (t, 1 H, _J_ = 6.7 Hz), 7.68 (t, 1 H, _J_ = 8.0 Hz), 7.98 (d, 1 H, _J_ = 5.5 Hz), 8.30 (d, 1 H, _J_ = 7.5 Hz), 8.72 (s, 1 H); $^{13}$C NMR (125 MHz, CDCl$_3$) _δ_ 28.5, 80.8, 112.6, 118.1, 137.8, 147.7, 152.9, 153.2; FAB MS m/z 195 (MH$^+$); FAB HRMS (calcld for C$_{24}$H$_{23}$NO$_2$: 372.1547, found 372.1535).

2,6-Butyl (2-Pyridyldi)carbamate, 3a. Di tert-butylicarbonate (2.4 g, 11 mmol) was added to 2-aminopyridine (940 mg, 10 mmol) in tert-butyl alcohol. The mixture was stirred overnight at 30–40 °C. The solvent was evaporated, and the residue was filtered through silica gel with methylene chloride as the eluant. The product was a white solid (with THF as the solvent, the major product was N,N′-bis(2-pyridyl)urea, as described in the literature$^{10}$). Yield: 60%. mp 93–94 °C (lit. 92–93 °C).$^{10}$ R$_t$ (methylene chloride) = 0.59; $^{1}$H NMR (300 MHz, CDCl$_3$) _δ_ 1.52 (s, 9 H), 6.95 (t, 1 H, _J_ = 6.7 Hz), 7.68 (t, 1 H, _J_ = 8.0 Hz), 7.98 (d, 1 H, _J_ = 5.5 Hz), 8.30 (d, 1 H, _J_ = 7.5 Hz), 8.72 (s, 1 H); $^{13}$C NMR (125 MHz, CDCl$_3$) _δ_ 28.5, 80.8, 112.6, 118.1, 137.8, 147.7, 152.9, 153.2; FAB MS m/z 195 (MH$^+$); FAB HRMS (calcld for C$_{24}$H$_{23}$NO$_2$: 372.1547, found 372.1535).

Cholesteryl N(2-Pyridyl)carbamate, 3b. 2-Aminopyridine (0.5 g, 5.31 mmol) was added to solution of cholesteryl chlorofluoride (2.38 g, 5.31 mmol) and K$_2$CO$_3$ (3.76 g, 26.5 mmol) in dry THF (22 mL). The reaction was allowed to reflux for 3 h before the solvent was evaporated. The residue was recrystallized from 1,1-nitromethane/methylene chloride. Yield: 32%; mp 212–214 °C. R$_t$ (ethyl acetate) = 0.75; $^{1}$H NMR (300 MHz, CDCl$_3$) _δ_ 0.68 (s, 6 H), 0.86 (d, 6 H, _J_ = 7.8 Hz), 0.92 (d, 3 H, _J_ = 6.6 Hz), 0.93–2.43 (m, 22 H), 1.04 (s, 6 H), 4.64 (m, 1 H, _J_ = 5.6 Hz), 5.41 (d, 1 H, _J_ = 5.4 Hz), 6.97 (t, 1 H, _J_ = 6.2 Hz), 7.49 (s, 1 H), 7.67 (t, 1 H, _J_ = 7.1 Hz), 7.95 (d, 1 H, _J_ = 8.7 Hz), 8.24 (d, 1 H, _J_ = 7.3 Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) _δ_ 28.5, 36.4, 36.8, 36.4, 30.6, 37.2, 38.7, 39.7, 40.0, 42.6, 50.3, 56.4, 56.9, 75.3, 81.0, 112.6, 118.4, 188.6, 123.0, 138.4, 138.6, 139.8, 147.9, 152.9, 153.2; FAB MS m/z 507 (M$^+$); FAB HRMS (calcld for C$_{46}$H$_{44}$N$_2$O$_7$: 726.3031, found 726.2972). Anal. Calcd for C$_{46}$H$_{44}$N$_2$O$_7$: C, 67.71; H, 4.99; N, 7.47.

Cholesteryl N(2-Pyridyl)carbamate, 4b. 2-Aniline (0.5 g, 5.36 mmol) was added to solution of cholesteryl chlorofluoride (2.40 g, 5.36 mmol) and K$_2$CO$_3$ (3.80 g, 26.8 mmol) in dry THF.
The reaction was allowed to reflux for 3 h before the solvent was evaporated. The residue was recrystallized from 1:1 hexane/methylene chloride. Yield: 78%. mp 169–171 °C. 

\( \text{Rf (ethyl acetate)} \) 0.80. \( \text{\( ^1\H\) NMR (300 MHz, CDCl}_3 \)} \& 0.68 (s, 6 H), 0.87 (d, 6 H, \( J = 7.8 \ \text{Hz} \)), 0.85–2.47 (m, 22 H), 0.92 (d, 3 H, \( J = 6.6 \ \text{Hz} \)), 1.03 (s, 6 H), 4.61 (m, 1 H, \( J = 5.5 \ \text{Hz} \)), 5.40 (d, 1 H, \( J = 5.4 \ \text{Hz} \)), 6.53 (s, 1 H), 7.05 (t, 1 H, \( J = 7.2 \ \text{Hz} \)), 7.30 (t, 2 H, \( J = 8.0 \ \text{Hz} \)), 7.38 (d, 2 H, \( J = 7.8 \ \text{Hz} \)); \( \text{\( ^1\C\) NMR (75 MHz, CDCl}_3 \)} \& 12.1, 18.9, 19.6, 21.3, 22.8, 23.0, 24.1, 24.5, 28.2, 28.3, 28.4, 32.1, 36.0, 36.4, 36.8, 37.2, 38.7, 39.7, 40.0, 42.5, 50.2, 56.4, 56.9, 75.1, 118.8, 123.0, 123.4, 129.2, 138.3, 139.8, 153.3; \( \text{FAB MS m/z} \) 504 (M – 1)+. Anal. Calcd for C\(_{34}\)H\(_{51}\)NO\(_2\): C, 80.74; H, 10.16; N, 2.77. Found: C, 81.00; H, 10.09; N, 2.71.

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