Pergamon

Tetrahedron, Vol. 53, No. 51, pp. 17425-17440, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)10191-0

Conformationally Restricted Diamines as Spacers for Parallel β-sheet Formation

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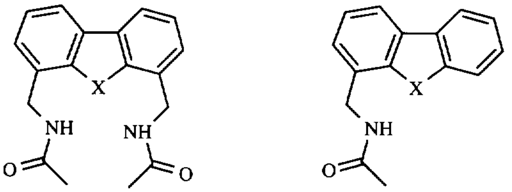
Abstract: The dibenzofuran-based diamide 4,6-bis(N-acyl-aminomethyl)dibenzofuran is shown by ^1H NMR and IR to have favourable geometry for intramolecular hydrogen bonding. The corresponding dibenzothiophene-based diamide 4,6-bis(N-acyl-aminomethyl)dibenzothiophene does not fulfill the geometric requirements for intramolecular hydrogen bonding. The intramolecularly hydrogen bonded state of the dibenzofuran-based diamide **1a** is found to be 1±0.5 kcal/mol more favoured enthalpically and 4±2 cal/(K·mol) disfavoured entropically than the non-hydrogen bonded state. © 1997 Elsevier Science Ltd.

The three dimensional structure of molecules, both small organic molecules and large biological macromolecules, is of vital importance for their reactivity, selectivity and function. It is desirable to be able to design and construct molecules with a predetermined geometry and through the geometry be able to influence the reactivity and selectivity. Numerous attempts to control the secondary structure of peptides through the inclusion of non-natural amino acids with rigid geometry designed to nucleate α-helix or β-sheet in the peptide chain have been accomplished[1]. β-Sheet structure can be induced in different ways; by the aid of a template like Kemps diacylaminoepindolidione derivative[2], by a β-turn mimic enforcing chain reversal[3,4] or by attaching peptide chains to a molecular scaffold with the required geometric parameters to induce β-sheet[5]. Several β-turn mimics and β-sheet nucleators have been designed, synthesised and tested for the nucleation of anti-parallel β-sheets[4], but only a very few have been designed for the induction of parallel β-sheets[6].

Non-natural amino acids able to effect β-sheet nucleation when introduced into a polypeptide should be of great interest in the development and modification of bioactive peptides. The folding of proteins into their native three-dimensional structure is a field of large current interest and debate[7]. Several theories exists, one claiming that this folding process is initiated by the nucleation of secondary structure elements[8]. A β-sheet nucleator with known thermodynamic properties (ΔH and ΔS for generating the first H-bond in the β-sheet) could be a model system to study physico-chemical properties of the protein folding process. β-Turn mimics can also be used in the construction of templates which in turn induces tertiary structure into attached peptide chains (Mutter templates; TASP)[9].

Design of a successful β-sheet nucleator implies the fulfillment of certain geometric and thermodynamic requirements. The nucleator should be rigid so that the distance and angle between the attachment points for peptide chains to the nucleator are fixed and well-defined, and close to the values found in β-sheets in proteins. It is an absolute necessary, but not sufficient, requirement that the nucleator generates the first hydrogen bond in the growing β-sheet[4d]. The nucleation of β-sheet structure from random coil structure for a polypeptide chain represents a decrease in conformational entropy; the nucleator should be designed to compensate this entropy cost of chain reversal.

We have designed and synthesised two diamines based on the rigid skeletons of dibenzofuran and dibenzothiophene. Molecular modelling indicates that the dibenzofuran analog; 4,6-bis(aminomethyl)dibenzofuran, could be expected to meet the geometrical requirements set for a β-sheet nucleator. The corresponding dibenzothiophene analog was not expected to fulfill the geometrical requirements and was included as a negative control in the study. In this work we have investigated the precence of intramolecular hydrogen bonding in the diamides **1a** and **1b** (see Figure 1), to probe to what extent the designed spacer molecules fulfills the geometrical requirements set for a β-sheet nucleator. The study also probes the thermodynamic properties of diamide **1a** by estimation of the equilibrium constant for hydrogen bonding, and the enthalpy and entropy for hydrogen bonding.



1a : X = O
1b : X = S

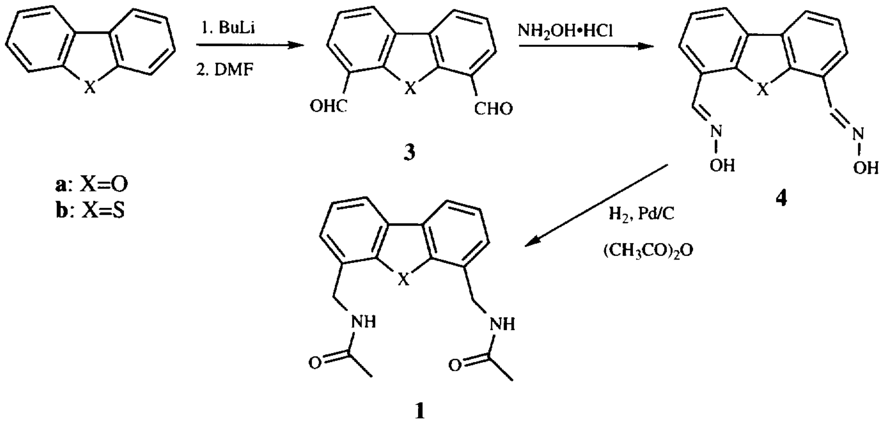
2a : X = O
2b : X = S

Figure 1. Diamides **1a** and **1b**, and monoamides **2a** and **2b**.

RESULTS AND DISCUSSION

Preparation of diamides

The dibenzofuran-based diamide, 4,6-bis(N-acyl-aminomethyl)dibenzofuran, was prepared according to Scheme 1. Dilithiation with *n*-buthyllithium and quenching of the dianion with dimethylformamide yielded 4,6-dibenzofurandicarbaldehyde. Treatment with hydroxylamine furnished the dioxime, which was reduced to 4,6-bis(aminomethyl)dibenzofuran by catalytic hydrogenation. Catalytic hydrogenation of the dioxime in the presence of acetic anhydride yielded 4,6-bis(N-acyl-aminomethyl)dibenzofuran.



Scheme 1

The dilithiation of dibenzothiophene is known to be nontrivial[10]; both regioselectivity and yield is much lower than in the corresponding reaction for dibenzofuran. Attempted dilithiation of dibenzothiophene following the general procedure resulted in a mixture of products containing only about 30 % of the desired 4,6-dialdehyde. Eight parallel reactions where the reflux time was varied from four to thirtytwo hours revealed no systematic dependence of amount of 4,6-dialdehyde with reaction time. The reactions resulted in variable mixtures of products of which the five most abundant were isolated and characterized. The product mixtures contained unreacted dibenzothiophene (DBT) (15 %; average relative amount over eight parallels), 4-DBT-carbaldehyde (29 %), 4,6-DBT-dicarbaldehyde (29 %), 1-DBT-carbaldehyde (10 %), 1,9-DBT-lactone (10*H*-9-Oxa-4-thia-cyclohepta[*def*]fluoren-8-one) (9 %) and 1- or 4-(1-hydroxyethyl)-DBT (6 %). The lactone is presumably formed from 1,9-dilithio-DBT, which is stabilized as a double lithium bridged structure[11], via an intramolecular Canizzario-like reaction of the 1,9-bis(N,N′-dimethylaminomethylalkoxide)-DBT followed by cyclisation to the lactone.

The lithiation of DBT was then studied further using the more reactive lithiation reagent *sec*-buthyllithium with hexane as solvent and variation of temperature and reaction time. This study revealed that lithiation of DBT with *sec*-buthyllitium and short reaction time results in highly improved yield and regioselectivity. Thus, treating DBT with 3 equivalents of both TMEDA and *sec*-buthyllithium, and heating to 60 °C for 30 minutes before addition of 5 equivalents of DMF at -78 °C yielded about 70 % isolated 4,6-DBT-dicarbaldehyde. When this work was finished, another group reported a similar improved procedure for 4,6-dilithiation of DBT with *n*-buthyllithium and TMEDA[12].

Diamide **1b**, 4,6-bis(N-acyl-aminomethyl)dibenzothiophene, was then prepared from the dialdehyde in an analogous manner to the dibenzofuran derivative.

Preparation of monoamides

In the following IR- and NMR-studies, reference values from compounds with no possibility to form intramolecular hydrogen bonds were needed, and for this purpose monoamides **2a**, 4-(N-acyl-aminomethyl)dibenzofuran, and **2b**, 4-(N-acyl-aminomethyl)dibenzothiophene (see Figure 1), were prepared. Monoamides **2a** and **2b** were prepared starting from dibenzofuran and dibenzothiophene following the same procedure as for the dibenzofuran-based diamide **1a**, but using only 1.1 equivalents of *n*-buthyllithium.

IR-studies

The N-H stretch region of the IR spectra for compounds **1** and **2** are shown in Figure 2, and the IR streching frequencies for the amide N-H bonds are compiled in Table 1. All four compounds show amide N-H strech absorptions at 3450 cm^{-1} as expected for a free N-H group[13]. Compound **1a** shows an additional broader absorption at 3365 cm^{-1} which is typical for a hydrogen bonded N-H group[13]. At a concentration of 1 mM, intermolecular interactions can be excluded[14] and these results therfore strongly suggests the existence of an intramolecular hydrogen bond in **1a**. *A priori,* two possible hydrogen bonding conformers of diamide **1a** can be envisioned; between an amide hydrogen and a carbonyl oxygen resulting in a 12-membered ring and between an amide hydrogen and the dibenzofuran oxygen resulting in a 6-membered ring[4d]. The possibility of an intramolecular hydrogen bond existing between the amide hydrogen and the dibenzofuran oxygen should also be present in monoamide **2a**. Since the IR-spectrum of monoamide **2a** only show absorbtion for a free amide hydrogen, this possibility can be excluded and it should therefore also be excluded for diamide **1a**.

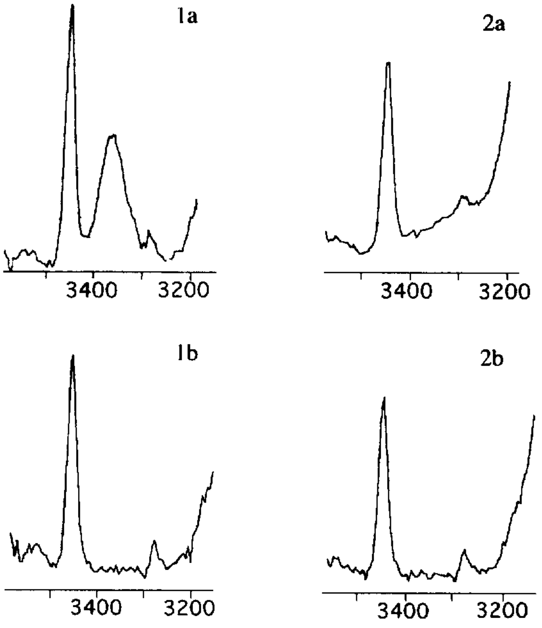


Figure 2. N-H stretch region of the IR spectra for 1 mM Solutions of Amides **1** and **2** in $CHCl_3$.

Table 1. IR Absorbtions and Molar Extinction Coefficients, ε, for 1 mM Solutions of Amides **1** and **2** in $CHCl_3$.

Compound	Absorbtion (cm^{-1})	ε
1a	3450	160
	3365	
1b	3450	224
2a	3450	106
2b	3450	122

The ratio of hydrogen bonded versus non-hydrogen bonded conformers of diamide **1a** is related to the intensity of the respective NH streching absorbtion bands, and the percentage of hydrogen bonding can be calculated provided the extinction coefficient for the free amide NH absorbtion signal is known:

$$\% \text{ hydrogen bonding} = 100(1 - \varepsilon_{obs}/2\varepsilon_0) \qquad (1)$$

where ε_{obs} is the observed extinction coefficient and ε_0 is the extinction coefficient of the free amide proton[15]. The extinction coefficient from the amide NH absorption of monoamide **2a** should be a reasonable estimate for ε_0

for the free amide proton of diamide **1a**. (ε_0 is multiplied by two in Eq. 1 to compensate that monoamide **2a** holds only one amide proton while diamide **1a** holds two.) Table 1 contain molar extinction coefficients for amides **1** and **2**, and using these values a hydrogen bonding estimate of 25 % for diamide **1a** is calculated, meaning that 25 % of the amide protons or 50 % of the diamide **1a** molecules at a given time participates in hydrogen bonding. An estimate for the level of uncertainty in this calculation can be deduced from the extinction coefficients of amide NH absorbtions of amides **1b** and **2b**. The relation is expected to be $\varepsilon_{1b}=2\varepsilon_{2b}$ and was found to be 1.8, implying an uncertainty of 10 %.

NMR-studies

Insight into hydrogen bonding in amides can also be achieved by ^{1}H NMR from the chemical shift of the amide hydrogen and the variation of the chemical shift of the amide hydrogen with temperature. Participation in hydrogen bonding moves the amide proton signal toward lower field[16]. Monoamides **2** were prepared to obtain reference values for non-hydrogen bonding amide protons. Large differences in resonance value between amide protons in corresponding mono- and diamides would indicate intramolecular hydrogen bonding in the diamide. Amide-amide intermolecular interactions could be excluded by performing the NMR experiments at 1 mM concentrations[15]. Table 2 contains the δNH values of diamides **1** and monoamides **2** at 294 K.

Table 2. Chemical Shift Values and Temperature Dependences of Amide Protons for 1 mM solutions of Amides **1** and **2** in $CDCl_3$.

Compound	δNH [a] ppm	Δ(δNH)/ΔT ppm/K
1a	6.583	-0.0043 [b]
1b	5.881	-0.0020 [c]
		-0.0036 [d]
		-0.0024 [e]
2a	6.006	-0.0023 [b]
2b	5.815	-0.0018 [b]

[a] At 294 K, [b] 213-333 K, [c] 293-333 K, [d] 253-283 K, [e] 213-243 K

The difference in δNH for dibenzofuran-based amides **1a** and **2a** is 0.577 ppm, whilst the difference between dibenzothiophene-based diamides **1b** and **2b** is substansially smaller; 0.066 ppm. The large difference in δNH value between amides **1a** and **2a** further supports the results from IR studies, namely the existence of an intramolecular hydrogen bond in diamide **1a**. Diamide **1b**, on the other hand, show no sign of such interaction.

Figure 3 shows a plot of temperature *vs.* chemical shift for 1 mM solutions of amides **1** and **2** in $CDCl_3$, and Table 2 contains the corresponding Δδ/ΔT values. Both monoamides **2** reveal linear relations between temperature and chemical shift of the amide proton with correlation coeffisients of 0.998 for **2a** and 0.997 for **2b**. The slope of the temperature dependence were -0.0024 ppm/K and -0.0018 ppm/K for monoamide **2a** and **2b** respectively, in accordance with the value for amide NH protons of small peptides exposed to solvent chloroform found by Stevens et al[17]. Diamide **1a** also shows a linear temperature dependence, with a slope of -0.0042 ppm/K, twice the value of monoamide **2a** and outside the range found by Stevens et al, but relatively

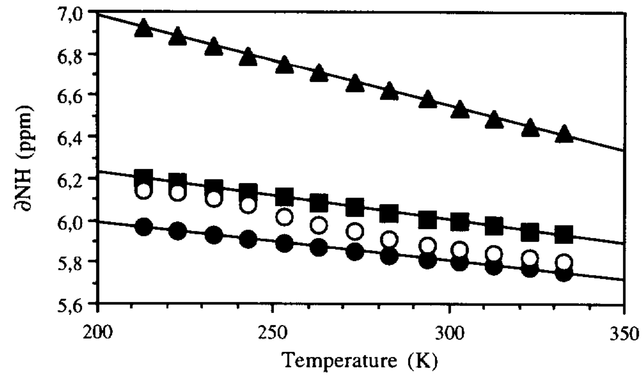


Figure 3. Chemical shift of amide protons as a function of temperature for 1 mM solutions of amides **1** and **2** in $CDCl_3$. Diamide **1a**: ▲; monoamide **2a**: ■; diamide **1b**: o; monoamide **2b**: ●.

small compared to highest values found in other similar studies[18]. According to previously accepted rules, small $\Delta\delta/\Delta T$ values should be interpreted as either amide protons completely free of hydrogen bonding or amide protons completely locked in hydrogen bonding[17,19]. It is apparent from the IR streching frequencies for the amide N-H bond and the chemical shift for the amide proton that both these possibilities can be excluded for amide **1a**. Gellman et al[18a] have shown that amide protons displaying small $\Delta\delta/\Delta T$ values can be equilibrating between hydrogen bonded and non-hydrogen bonded conformations when the difference in enthalpy between these conformations is small. Diamide **1a** has available degenerate hydrogen bonding conformations of equal energy as indicated in Figure 4. Only one amide proton signal can be detected in the ^{1}H NMR spectrum of **1a** in the temperature interval between 212 and 333 K, and the equilibrium between non-hydrogen bonded and hydrogen bonded conformations must therefore be rapid at the NMR time scale. Rapid equilibrium between hydrogen bonding and non-hydrogen bonding conformers at all measured temperatures is an indication of relatively low enthalpic gain by generating the hydrogen bond. The value of $\Delta\delta/\Delta T$ also depend on the limiting chemical shift values for non-hydrogen bonded and fully hydrogen bonded amide protons (see below); a small difference between these values will also result in a small value of $\Delta\delta/\Delta T$. Comparison of $\Delta\delta/\Delta T$ values from different compounds is thus only valid provided the interval set up by δ_n and δ_b are of comparable sizes because the size of $\Delta\delta/\Delta T$ is dependent on this interval.

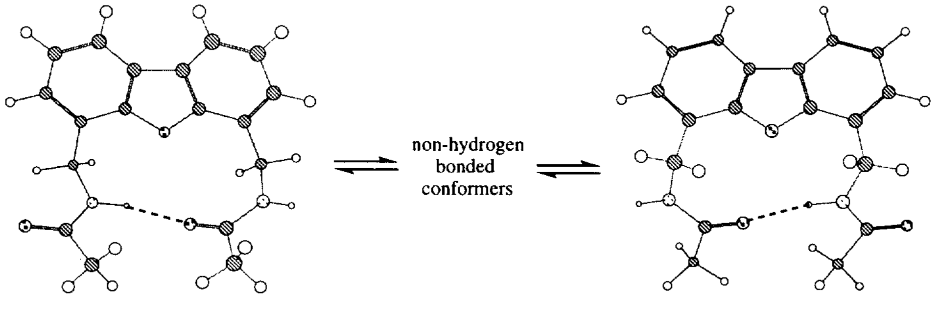


Figure 4. Equilibrium between degenerate hydrogen bonding conformers of diamide **1a**.

In contrast to amides **1a** and **2**, diamide **1b** shows a non-linear variation of the temperature dependence of the amide proton chemical shift over the full temperature range. This non-linearity is indicative of conformational heterogeneity[20]. Closer inspection shows however linear correlation in three discrete temperature intervals. Between 294-333 K and 213-243 K the slopes were -0.0024 ppm/K and -0.0020 ppm/K respectively; temperature coefficients of similar magnitude as monoamides **2**. The temperature interval from 253 K to 284 K gave a slope of -0.0036 ppm/K, possibly indicating a conformational transition in this temperature interval, not necessarily involving hydrogen bond formation; the increase in chemical shift in this transition is very small compared to what would be expected for the "freezing out" of a completely hydrogen bonded conformer.

The enthalpic gain and entropic cost in generating intramolecular hydrogen bonding in diamide **1a** can be calculated via a Van´t Hoff plot provided the equilibrium constant K_{eq} for hydrogen bonding is known at different temperatures[18a,21]. The equilibrium constant is given by the expression

$$K_{eq} = (\delta_{obs}-\delta_n)/(\delta_b-\delta_{obs}) \qquad (2)$$

where δ_{obs} is the observed chemical shift of the amide proton, δ_n is the chemical shift for the non-hydrogen bonded conformers and δ_b is the chemical shift for the fully hydrogen bonded state[22]. Amide-proton chemical shift values from monoamide **2a** in $CDCl_3$ should provide a good estimate for δ_n for diamide **1a** at all temperatures. A δNH value of 8.45 ppm is found for monoamide **2a** dissolved in DMF-d_7, and this value is chosen as an estimate for δ_b for **2a**. This value of 8.45 ppm corresponds well with the limiting chemical shift value of concentrated N-methylacetamide of 8.4 ppm found by Gellman[18a]. However, in diamide **1a** complete hydrogen bonding can only involve one of the two amide protons at a time. The estimate for δ_b in **1a** should therefore be an average of non-hydrogen bonding and fully hydrogen bonding amide proton shift values; $\delta_{b,\mathbf{1a}} = (\delta_{n,\mathbf{2a}} + \delta_{b,\mathbf{2a}})/2$, which gives the value 7.23 ppm for $\delta_{b,\mathbf{1a}}$. From the IR extinction coefficients at room temperature a hydrogen bonding percentage of 50 was calculated, implying an equilibrium constant of 1. Calculating K_{eq} at 294 K from Eq.2 using the values chosen for $\delta_{b,\mathbf{1a}}$ and δ_n results in a value of 0.89, indicating that the chosen values are reasonable. Estimating the temperature dependence of $\delta_{b,\mathbf{1a}}$ as an average of the temperature dependence of **2a** in DMF-d_7 ($\delta_{b,\mathbf{2a}}$) and in $CHCl_3$ ($\delta_{n,\mathbf{2a}}$) gives a value of -0.0041 ppm/K for $\Delta\delta_{b,\mathbf{1a}}/\Delta T$. Solvent-exposed amide-protons in strongly hydrogen bonding solvents are expected to show large $\Delta\delta/\Delta T$ values[21], whereas amide-protons locked in an intramolecular hydrogen bond are expected to show small $\Delta\delta/\Delta T$ values[17,19] . Here $\Delta\delta_{b,\mathbf{1a}}/\Delta T$ is ment to be an estimate for an intramolecularly locked hydrogen bond, and the temperature dependence derived from **2a** in DMF-d_7 should therefor be regarded as a highest estimate for $\Delta\delta_{b,\mathbf{1a}}/\Delta T$. A smallest estimate of the temperature dependence would be zero. A more reasonable estimate might be a value of -0.0023 ppm/K; the same temperature dependence as the non-hydrogen bonded amide proton. Table 3 lists values for $\Delta H°$ and $\Delta S°$ for the equilibrium between non-hydrogen bonded and hydrogen bonded conformers of diamide **1a** calculated from Van´t Hoff plots generated using the three different values for $\Delta\delta_{b,\mathbf{1a}}/\Delta T$. Figure 5 shows the Van´t Hoff plot generated using $\Delta\delta_{b,\mathbf{1a}}/\Delta T$=-0.0023 ppm/K. Considering the highest and lowest values in Table 3 as extremes indicates that the hydrogen bonded state of diamide **1a** is 1±0.5 kcal/mol more favoured enthalpically and 4±2 cal/K·mol disfavoured entropically than the non-hydrogen bonding state.

Table 3. ΔH° and ΔS° for Hydrogen Bonding in **1a** Calculated from Van't Hoff Plots Generated Using Three Different Values for $\Delta\delta_{b,1a}/\Delta T$.

$\Delta\delta_{b,1a}/\Delta T$ ppm/K	ΔH° kcal/mol	ΔS° cal/K·mol	R^2
0	-1.6	-5.8	0.9962
-0.0023	-0.9	-3.5	0.9796
-0.0041	-0.5	-2.0	0.9547

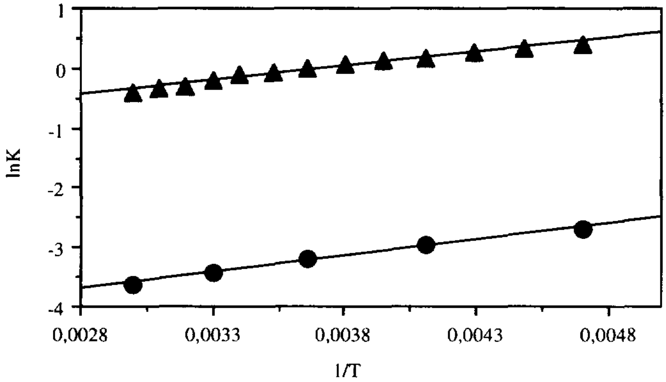
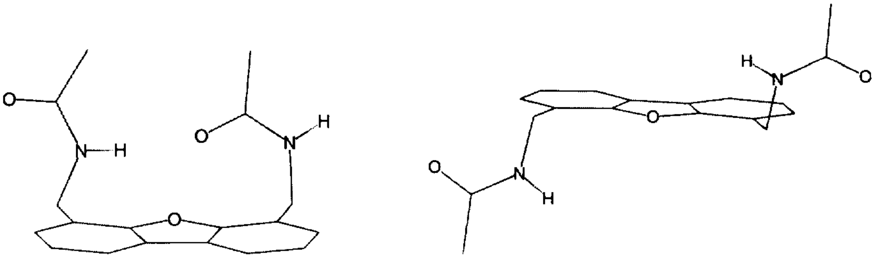


Figure 5. Van't Hoff plots for diamide **1a**; ▲: data from variable temperature NMR chemical shift of amide protons, plot generated using $\Delta\delta_{b,1a}/\Delta T$=-0.0023 ppm/K; ●: data from MC/SD simulations, hydrogen bonded populations calculated during 10 ns with AMBER* force field and GB/SA $CHCl_3$.

Computer simulations

Conformational searching of diamide **1a** using the AMBER* force field in the MacroModel[23] software finds hydrogen bonded conformer **I** (see Figure 6) as the global minimum. Inspection of this conformer of **1a** reveals a H···O distance of 1.99 Å, close to optimum value found from crystallographic data[24]. Bond angles in this structure are found to be 166° for the N-H···O angle and 149° for the H···O=C angle, both values in good accordance with values calculated by Peters&Peters[25]. The next higher energy conformer, conformer **II** (see Figure 6), is a C_2 symmetric conformer with the aminomethyl sidechains pointing in opposite directions without hydrogen bonding. The energy difference between the global minimum and the lowest energy non-hydrogen bonding conformer is 0.4 kcal/mol, which is in reasonable agreement with the value of ΔH° for hydrogen bonding in **1a** determined by NMR. Conformational searching of diamide **1b** finds 11 conformers within the lowest 3 kcal/mol, and none of these have intramolecular hydrogen bonding. The energy difference between the two lowest energy conformers is 0.02 kcal/mol; the lowest in energy of these has a conformation similar to conformer **II** of **1a**. The next higher energy conformer has both aminomethyl sidechains pointing to the same face of the aromatic ring (C_s symmetric); and is similar to the third lowest conformer of **1a**.



Conformer I Conformer **II**

Figure 6. Conformers **I** and **II** from conformational search of diamide **1a**.

An estimation of the population of hydrogen bonded conformations of **1a** was performed running 10 ns Monte Carlo/Stocastic dynamics simulations at 300 K and the GB/SA model for chloroform. Setting the criteria for hydrogen bonding as[26] H···O distance <2.5 Å, N-H···O bond angle >120° and H···O=C angle >90° results in a population of hydrogen bonded conformations of mere 3 %. This value is clearly not in accordance with results form IR- and NMR-studies. The discrepancy may partly be explained by lack of good quality parameters[27]. The temperature dependence of the equilibrium constant for hydrogen bonding can be estimated by performing simulations at variable temperatures. Figure 5 shows the Van't Hoff plot generated from simulation data. The calculated value for $\Delta H°$ for hydrogen bonding of -1.1 kcal/mol from this plot is in good agreement with the experimental value. The calculated entropy change of -10 cal/mol·K is clearly an overestimate, but still in reasonable agreement with the experimental value.

Conclusion

The dibenzofuran-based diamide 4,6-bis(N-acyl-aminomethyl)dibenzofuran satisfies the first neccesary requirement for a parallel β-sheet nucleator by generating the first hydrogen bond in the growing β-sheet as probed by the diamide **1a**. No intramolecular hydrogen bonding in diamide **1b** could be detected by IR or NMR, as expected from molecular modelling studies. The intramolecularly hydrogen bonded state of diamide **1a** is 1±0.5 kcal/mol more favoured enthalpically and 4±2 cal/(K·mol) disfavoured entropically than the non-hydrogen bonded state. The maximum enthalpic gain from formation of a geometrically optimal hydrogen bond is estimated to 4 kcal/mol[28,29]. Compared to this value, the enthalpic gain from hydrogen bond generation in **1a** is only moderate. Despite the small enthalpic gain by hydrogen bonding, the equilibrium constant for hydrogen bonding is still close to unity at room temperature. The explanation for this is the small conformational entropic cost in the formation of the hydrogen bond in the rigid spacer. The rigid framework of the dibenzofuran system thus seems to eliminate most of the entropic cost connected with locking the amide N-H bond and carbonyl bond in a suitable geometry for hydrogen bonding. The thermodynamic parameters for the hydrogen bond in diamide **1a** are very similar to the enthalpy and entropy for the hydrogen bond in β-turn forming model Pro-X dipeptides in CH_2Cl_2[30]. On this background the dibenzofuran-based diamine can be expected to induce parallel β-sheet formation as effectively as a Pro-X turn can induce an anti-parallel β-sheet. Whether the dibenzofuran-based spacer is sufficient to induce parallel β-sheet will be adressed in further studies.

EXPERIMENTAL

IR experiments. All IR spectra were recorded at room temperature on a Shimadzu IR-470 spectometer. All samples were recorded as 1 mM solutions in $CHCl_3$ in KBr cells with path length 1.75 mm. Before sample preparation, $CHCl_3$ was passed through a coloumn of alumina to remove water and ethanol. Solvent subtraction did not give complete cancelling of solvent signals, but the interesting frequency interval for observation of amide N-H stretching was free of disturbing solvent signals.

NMR experiments. All NMR spectra were recorded on a Jeol Eclipse 400 MHz spectrometer. For variable temperature recordings, 10 mm sample tubes were used. The samples were prepared as 1 mM solutions in $CDCl_3$, and $CDCl_3$ was used as reference at all temperatures. $CDCl_3$ was purchased from Merck in 10 ml sealed ampoules, containing max. 0.005% water. The ampoule was opened under argon atmosphere and transferred directly into dry NMR tubes containing the weighed amount of amide. All samples/solutions contained 2-3 equivalents of water, roughly equal to the maximum amount in the purchased solvent. ^1H NMR spectra were recorded from 213 K to 333 K at 10 degree intervals. The same procedure was followed at each temperature: stabilization of temperature, shimming and recording of 256 scans.

Molecular modeling. Molecular modeling was performed using MacroModel v 5.0 software[22] on Silicon Graphics INDY and O2 workstations. All calculations were performed with the AMBER* force field using chloroform GB/SA treatment. To find low energy conformations for compounds **1a** and **2a**, 4000-8000 step Monte Carlo conformational searches were performed. Conformations were minimised with Polak-Ribiere conjugated gradient until the gradient was below 0.05 kJ/Å. The dynamics calculations were performed using mixed model Monte Carlo/ stochastic dynamics with a time step of 1.0 fs, 10 ns total simulation time, random torsional rotations between ±30° and ±180° applied to four bonds and a 1:1 ratio of SD to MC steps. The population of hydrogen bonding conformers was estimated by counting all conformers with a $H\cdots O$ distance $<$ 2.5 Å, a $N\text{-}H\cdots O$ angle $> 120°$ and a $H\cdots O{=}C$ angle $> 90°$ [27]. Confirmation of convergence was based on the following: i) A fluctuation in cumulative average potential energy ≤ 0.1 kJ/mol after half of the total simulation time. ii) Simulations starting from substantially different conformations resulting in the same hydrogen bonding population to within 0.3 %. iii) Similar distribution of torsional angles for symmetric torsional angle and enantiomeric conformations.

Preparation of amides 1 and 2

General remarks. Diethyl ether used in lithiation reactions was first dried over $MgSO_4$ for some days, then refluxed with $LiAlH_4$ and distilled directly into the reaction flask. Hexane was dried over calsium chloride overnight, refluxed with calsium hydride and distilled directly into the reaction flask. All equipment used in lithiation reactions was dried either in a dessicator under reduced pressure or in an oven at 108 °C. Dibenzofuran and dibenzothiophene was dried in a dessicator under reduced pressure over night. DMF was dried over 3Å molsieves for at least 72 hours before destillation under reduced pressure, and stored over 4Å molsieve under inert atmosphere. TMEDA was dried over 4Å molsieves. Melting points were obtained on a Büchi 535 apparatus in open capillaries.

*4,6-dibenzofurandicarbaldehyde (**3a**)*

To a solution of dibenzofuran (2.52 g, 0.015 moles) and TMEDA (5.23 g, 0.045 moles) in 100 ml dry diethyl ether under argon atmosphere, was added 28 ml (0.045 moles) of a 1.6 M solution of *n*-butyllitium in hexane. After refluxing for 16 hours, DMF (3.29 g, 45 mmoles) was added during 5 minutes under cooling on ice, and the mixture stirred at room temperature for 24 hours. The mixture was then poured into 150 ml cold 1M HCl and the phases separated. The acidic water phase was extracted several times with dichloromethane, totally about 250 ml. The combined organic phases was washed with 1M HCl, water and brine, and dried over $MgSO_4$. After filtration and evaporation of solvent resulted a yellowish solid (3.03 g), which according to GC analyses consisted of about 87 % 4,6-dibenzofurandicarbaldehyde. The crude product was crystallised from 2-propanol yielding 1.45 g of **3a**. A small portion was further purified by flash chromatography on a silica coloumn eluting with heptane/ethyl acetate 4:1 yielding a pale yellow solid; mp 222.5-224.7 °C (dec.); ^1H NMR (DMSO-d_6): δ 7.69 (t, J=7.7 Hz, 2H), 8.09 (d, J=7.7, 2H), 8.60 (d, J=7.7, 2H), 10.53 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 121.78, 124.71, 128.33, 129.36, 155.60, 189.03; MS (EI): m/z 224 (100%) M, 223 (93%), 196 (9%), 168 (10%), 139 (68%).

*4,6-dibenzofurandicarbaldehyde dioxime (**4a**)*

To 0.67 g (3 mmoles) of 4,6-dibenzofurandicarbaldehyde was added ethanol (13.5 ml), pyridine (3 ml) and 2.70 g (39 mmoles) hydroxylamine hydrochloride, and the mixture was refluxed for 1.5 hours. Most of the solvent was removed by evaporation before thorough washing with water. The resulting product was left open in the hood until no smell of pyridine could be detected, yielding 0.66 g (87 %) of a pale green powder which, as judged by NMR, contained only very small amounts of impurities. The crude product was used in the next step without further purification; ^1H NMR (DMSO-d_6): δ 7.47 (t, J=7.7 Hz, 2H), 7.83 (d, J=7.7 Hz, 2H), 8.21 (d, J=7.7 Hz, 2H), 8.61 (s, 2H), 11.66 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 117.6, 122.0, 123.6, 124.0, 124.5, 142.9, 152.8; MS (FAB): m/z 255 (90%) M+1, 239 (100%) M+1-16, 224 (33%), 133 (72%); MS (EI): m/z 254 (21%) M, 236 (35%), 218 (100%), 209 (13%), 193 (57%), 164 (57%).

*4,6-bis(N-acyl-aminomethyl)dibenzofuran (**1a**)*

To a solution of 0.15 g (0.6 mmoles) of 4,6-dibenzofurandicarbaldehyde dioxime in 30 ml acetic acid and 10 ml acetic anhydride under inert atmosphere was added 0.066 g of 10 % activated palladium on charcoal, and hydrogen supplied at atmospheric pressure. After 36 hours, the catalyst was removed by filtration through a layer of Celite and acetic acid and acetic anhydride removed by evaporation under reduced pressure. Isolation by addition of water, filtration and crystallisation from ethanol yielded 4,6-bis(N-acyl-aminomethyl)dibenzofuran as a greyish solid; mp 237.2-239.8 °C (dec.); ^1H NMR ($CDCl_3$): δ 2.07 (s, 6H), 4.81 (d, J=6.4 Hz, 4H), 6.55 (broad s, 1H), 7.30 (m, 2H), 7.40 (d, J=6.3 Hz, 2H), 7.87 (dd, J=7.8 and 1.5 Hz, 2H); MS (FAB): m/z 311 (100 %) M+1, 252 (98 %).

4,6-dibenzothiophenedicarbaldehyde (3b)

1. Dilithiation with n-BuLi. Attempted dilithiation of dibenzothiophene following the same procedure as for dibenzofuran resulted in a mixture of products. After separation on silica coloumns with heptane/ethyl acetate as eluent, five major products were isolated: 1-dibenzothiophenecarbaldehyde, 4-dibenzothiophenecarbaldehyde, 4,6-dibenzothiophenedicarbaldehyde, 10*H*-9-oxa-4-thiacyclohepta[*def*]fluoren-8-one, and 1- or 4-(1-hydroxyethyl)dibenzothiophene.

1-dibenzothiophenecarbaldehyde. Pale yellow solid; mp 95.3-97.0 °C; ^{1}H NMR (CDCl$_3$): δ 7.53 (m, 2H), 7.61 (dd, J=7.3 and 7.8 Hz, 1H), 7.91 (dd, J=7.1 and 2.2 Hz, 1H), 7.98 (dd, J=7.3 and 1.0 Hz, 1H), 8.10 (dd, J=7.8 and 1.0 Hz, 1H), 8.97 (dd, J=7.1 and 2.2 Hz, 1H), 10.71 (s, 1H); ^{13}C NMR (CDCl$_3$): δ 122.75, 124.80, 125.71, 127.21, 127.58, 128.50, 130.41, 133.94, 134.47, 134.56, 140.25, 141.39, 191.88; MS (EI): m/z 212 (80 %) M, 184 (100%), 183 (23 %), 152 (9%), 139 (35%).

4-dibenzothiophenecarbaldehyde. ^{1}H and ^{13}C NMR data were in accordance with litterature data[31]; MS (EI): m/z 212 (100 %) M, 184 (72%), 183 (36 %), 152 (8%), 139 (45%).

10H-9-Oxa-4-thia-cyclohepta[def]fluoren-8-one. Yellow solid; mp 179.4-182.2 °C (dec.); ^{1}H NMR (CDCl$_3$): δ 5.44 (s, 2H), 7.40 (d, J=7.3 Hz, 1H), 7.57 (dd, J=7.8 and 7.3 Hz, 1H), 7.65 (t, J=7.8 Hz, 1H), 7.92 (d, J=7.8 Hz, 1H), 8.14 (dd, J=7.8 and 1.0 Hz, 1H), 8.43 (dd, J=7.8 and 1.0 Hz, 1H); ^{13}C NMR (CDCl$_3$): δ 68.91, 122.33, 123.02, 126.55, 126.77, 127.74, 128.07, 131.51, 132.94, 133.52, 134.73, 139.54, 140.27, 168.07; MS (EI): m/z 240 (100 %) M, 211 (90 %), 195 (11 %), 183 (42 %), 152 (23 %), 139 (29 %).

4- or 1-(1-hydroxyethyl)dibenzothiophene: (tentatively assigned). Colorless oil. ^{1}H NMR (CDCl$_3$): δ 1.66 (d, J=6.6 Hz, 3H), 2.10 (broad s, 1H), 5.26 (q, J=6.6 Hz, 1H), 7.43-7.53 (m, 4H), 7.86 (m, 1H), 8.08 (dd, J=7.5 and 1.3 Hz, 1H), 8.15 (m, 1H); ^{13}C NMR (CDCl$_3$): δ 23.29, 70.01, 120.76, 121.68, 122.76, 122.92, 124.47, 124.94, 126.90, 135.56, 136.35, 136.39, 139.54, 140.08; MS (EI): m/z 228 (33 %) M, 213 (30 %), 210 (100 %), 197 (3 %), 185 (56 %), 184 (36 %), 165 (9 %), 152 (6 %), 139 (19 %).

2. Dilithiation with sec-BuLi. To a solution of dibenzothiophene (0.92 g, 5 mmoles) and TMEDA (1.74 g, 15 mmoles) in 50 ml hexane at -78 °C under argon atmosphere was added 15 mmoles (11.5 ml) of *sec*-butyllitium (circa 1.3 M solution in cyclohexane). The colour of the reaction mixture changed from near colourless to sharp yellow when *sec*-butyllitium was added. The reaction mixture was heated at 60 °C for 30 minutes, then cooled to -78 °C again, and 1.83 g (25 mmoles) DMF was added. After stirring at room temperature overnight, the mixture was poured into cold 1 M HCl, and ethyl acetate was added. The two phases were separated, and the water phase extracted with several portions of ethyl acetate. The combined organic phases was washed with water and brine. Solid material still present in the organic phase was removed by filtering, washed thoroughly with water on a Büchner-funnel and dried in a desiccator to yield 0.50 g of a pale yellow solid/powder. The combined organic phases was dried over MgSO$_4$. After filtration and evaporation of solvent, the resulting sticky yellow solid was washed with several portions of diethyl ether to remove by-products and then with water, and dried in a desiccator to yield 0.34 g of a pale yellow solid/powder. Both portions of solid material consisted of 4,6-

dibenzothiophenedicarbaldehyde with only minor amounts of impurities as judged by NMR. The crude product amounted to about 70 % yield, and was used in the next step without further purification; ^{1}H and ^{13}C NMR data was in accordance with litterature[12]; MS (EI): m/z 240 (100%) M, 212 (45%), 184 (62%), 139 (50%).

*4,6-dibenzothiophenedicarbaldehyde dioxime (**4b**).*

To 0.3 g (1.25 mmoles) of 4,6-dibenzothiophenedicarbaldehyde was added 5.5 ml ethanol, 1.1 ml pyridine and 1.13 g (16 mmoles) of hydroxylamine hydrochloride, and the resulting mixture was refluxed for 1.5 hours. After cooling solid material was removed by filtration and solvent removed by evaporation. The then resulting pale green solid was washed thoroughly with water in a Büchner-funnel and left open in the hood to remove traces of pyridine. After several days, including two in a desiccator under reduced pressure, a faint smell of pyridine could still be detected. The content of pyridine was less than 0.2 equivalents as judged by NMR. The crude product contained no other impurities, and was used in the next step without further purification. The yield of 4,6-dibenzothiophenedicarbaldehyde dioxime was 86% according to the ^{1}H NMR spectrum. ^{1}H NMR (DMSO-d_6): δ 7.62 (m, 2H), 7.72 (m, 2H), 8.49 (m, 2H), 8.55 (d, J=2.0 Hz, 2H), 11.68 (d, J=2.0 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 122.8, 125.0, 127.8, 129.3, 135.5, 136.1, 149.0; MS (FAB): m/z 271 (100 %) M+1, 255 (73%) M+1-16, 240 (15%); MS (EI): m/z 270 (10%) M, 252 (18%), 236 (29%), 234 (100%), 209 (16%), 164 (19%).

*4,6-bis(N-acyl-aminomethyl)dibenzothiophene (**1b**).*

To a solution of 4,6-dibenzothiophenedicarbaldehyde dioxime (0.05 g, 0.18 mmoles) in a 3:1 mixture of acetic acid and acetic anhydride was added 0.02 g of 10% palladium on charcoal under inert atmosphere. Hydrogen was supplied at atmospheric pressure. After 24 hours the reaction was complete according to TLC. The catalyst was removed by filtration trough a layer of Celite, and the solvent removed by evaporation under reduced pressure. The resulting pale yellow solid was washed with water to remove traces of acetic acid and acetic anhydride, dried in a desiccator and crystallised from ethanol. This yielded 53 % of pure diamide **1b** as white crystals; mp 272.5-273.6 °C (dec.); ^{1}H NMR (1 mM in CDCl$_3$): δ 2.09 (s, 6H), 4.74 (d, J=5.4 Hz, 4H), 5.88 (broad s, 1H), 7.47 (m, 4H), 8.10 (dd, J=7.8 and 1.5 Hz, 2H); MS (FAB): m/z 327 (71%) M+1, 268 (100%).

*4-(N-acyl-aminomethyl)dibenzofuran (**2a**).*

4-Dibenzofurancarbaldehyde was prepared from dibenzofuran following the same procedure as for 4,6-dibenzofurandicarbaldehyde, but using only 1.1 equivalents of *n*-buthyllithium and without addition of TMEDA. The ^{1}H NMR spectrum of 4-dibenzofurancarbaldehyde was in accordance with litterature data[32]. 4-Dibenzofurancarbaldehyde oxime was prerared following the same procedure as for 4,6-dibenzofurandicarbaldehyde dioxime. ^{1}H NMR (CDCl$_3$ + CD$_3$OD 10-20 %) : δ 7.37 (m, 2H), 7.50 (m, 1H), 7.64 (m, 1H), 7.75 (m, 1H), 7.97 (m, 2H), 8.65 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 112.32, 118.16, 121.89, 122.39, 123.73, 123.91, 123.95, 124.94, 125.82, 128.50, 144.13, 153.03, 156.07. The monoamide **2a** was prepared in an analogous manner to diamide **1a** and isolated as a beige solid; mp 155.8-157.2 °C (dec.); ^{1}H NMR (CDCl$_3$) : δ 2.04 (s, 3H), 4.81 (d, J= 5.9 Hz, 2H), 6.04 (s, broad, 1H), 7.31 (m, 1H), 7.36 (m, 1H), 7.42 (m, 1H), 7.48 (m, 1H), 7.59 (m, 1H), 7.88 (m, 1H), 7.95 (m, 1H); ^{13}C NMR (CDCl$_3$): δ 23.44, 38.80, 111.82, 120.13, 120.89, 122.08, 123.03, 123.11, 124.26, 124.40, 127.10, 127.39, 154.33, 156.10, 170.07; MS (FAB) : m/z 240 (25%) M+1, 181 (100%).

4-(N-acyl-aminomethyl)dibenzothiophene (2b).

4-Dibenzothiophenecarbaldehyde was prepared from dibenzothiophene following the same procedure as for 4,6-dibenzofurandicarbaldehyde, but using only 1.1 equivalents of *n*-buthyllithium and without addition of TMEDA. 4-Dibenzothiophene-carbaldehyd oxime was prerared following the same procedure as for 4,6-dibenzofurandicarbaldehyde dioxime. The monoamide **2b** was prepared in an analogous manner to diamide **1a** and isolated as a beige solid; mp 181.7-182.4 °C (dec.); ^{1}H NMR ($CDCl_3$) : δ 2.08 (s, 3H), 4.73 (d, J=5.9 Hz, 2H), 5,83 (s, broad, 1H), 7.42 (m, 1H), 7.48 (m, 3H), 7.88 (m, 1H), 8.11 (d, J=7.3 Hz, 1H), 8.17 (m, 1H); ^{13}C NMR ($CDCl_3$) : δ 23.45, 43.17, 121.09, 121.86, 122.95, 124.69, 125.01, 126.39, 127.06, 132.16, 135.71, 136.35, 138.44, 139.15, 170.17; MS (FAB) : m/z 256 (16%) M+1, 197 (100%).

ACKNOWLEDGMENTS

The authors thank The Norwegian Research Council for financial support.

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14. No hydrogen bonding could be detected for **1b**, **2a** or **2b** at this concentration. It should therefore be a valid assumption that **1a** is also free of intermolecular hydrogen bonding. In addition it seems from the litterature (refs. 4d, 15a, 17, 18a) that amide concentrations around 1 mM are generally found to be free of intermolecular hydrogen bonding.

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(*Received in UK* 27 *May* 1997; *revised* 15 *October* 1997; *accepted* 16 *October* 1997)