Supplementary Information

Dissipative formation of a transient foldaxane with a fuel-like thread

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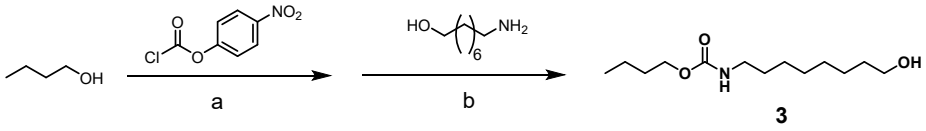
1. General Information

Reagents and materials for synthesis were purchased from Adamas-beta, Bidepharm, Innochem, or Leyan, and used without further purification unless otherwise noted. Deuterated solvents for NMR measurements were obtained from Cambridge Isotope Laboratories or Innochem. Diisopropylethylamine (DIEA) were dried over activated 4Å molecular sieves prior to use. $CDCl_3$ was used after filtration through an alumina pad. Thin Layer Chromatography (TLC) analysis was carried out using silica gel precoated on glass plates (60F-254) and observed under UV light or by dipping the plates in phosphomolybdic acid solution 20 wt. % in ethanol, followed by heating. Flash column chromatography was carried out using silica gel (200-300 mesh). NMR spectra were taken on JEOL-ECAS 400 MHz. Chemical shifts are reported in parts per million (ppm) relative to traces of the non-deuterated solvent in the corresponding deuterated solvents ($CDCl_3$: δ_H = 7.26 ppm and δ_C = 77.16 ppm). High-resolution mass spectrometry (HRMS) was recorded on a Q-TOF (ABSCIEX X500R with ESI source, and Agilent 7250 with EI source), which combines quadrupole precursor ion selection and a high-resolution accurate-mass (HR/AM) Time of Flight mass analyzer to deliver mass accuracy. Molecular modelling was performed based on the X-ray crystal structure of a foldaxane of **1** with biscarbamate guest (CCDC No. 1482295) by minimization using the Merck Molecular Force Field static (MMFFs) implemented in MacroModel version 11.9 via Maestro version 11.5 (Schrödinger).

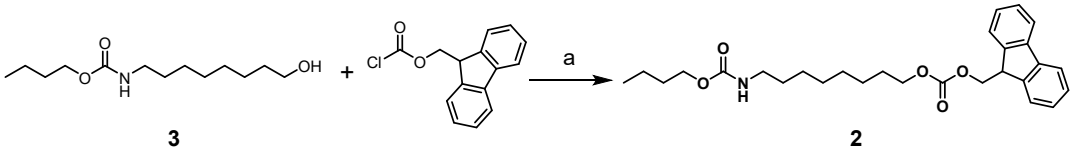
2. Synthesis and Characterization

Compound **1** was synthesized according to procedures reported by Huc et al.[1]

2.1 Synthetic schemes



Scheme S1. Synthesis of compound **3**: a) triethylamine, 4-nitrophenyl chloroformate, anhydrous CH_2Cl_2, from 0 °C to room temperature, overnight; b) 8-aminooctan-1-ol, triethylamine, anhydrous CH_2Cl_2, from 0 °C to room temperature, 12 h.



Scheme S2. Synthesis of compound **2**: a) (4-dimethylamino) pyridine, anhydrous pyridine, anhydrous CH_2Cl_2, room temperature, overnight.

2.2 Synthetic procedures

Compound 3. A solution of 1-butanol (0.16 mL, 1.72 mmol) and triethylamine (0.48 mL, 3.44 mmol, 2.0 equiv.) in dry CH_2Cl_2 (6.0 mL) was added to a solution of 4-nitrophenyl chloroformate (0.35 g, 1.72 mmol, 1.0 equiv.) in dry CH_2Cl_2 (6.0 mL) under N_2 atmosphere at 0 °C. The mixture was stirred at room

temperature overnight. A solution of 8-aminooctan-1-ol (0.16 mL, 1.72 mmol, 1.0 equiv.) and triethylamine (0.48 mL, 3.44 mmol, 2.0 equiv.) in dry CH_2Cl_2 (6.0 mL) was added to the mixture under N_2 atmosphere at 0 °C, and the mixture was stirred at room temperature for 12 h. After completion of the reaction, the solvent was washed 3 times with 1 M NaOH solution (15 mL×3), dried over $MgSO_4$, and concentrated to dryness. The crude material was purified by flash column chromatography using EtOAc /PE (1:3) as eluent to give **3** as a white solid (0.39 g, 92%). **^{1}H NMR** ($CDCl_3$, 400 MHz): 4.60 (br, 1H), 4.04 (t, J_{HH} = 6.8 Hz, 2H), 3.64 (q, J_{HH} = 6.3 Hz, 2H), 3.16 (q, J_{HH} = 6.7 Hz, 2H), 1.64-1.19 (m, 16H), 1.24 (t, J_{HH} = 1.2 Hz, 1H), 0.93 (t, J_{HH} = 7.4 Hz, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 156.98, 64.63, 62.83, 40.97, 32.73, 31.15, 30.00, 29.34, 29.25, 26.68, 25.71, 19.11, 13.79. **HRMS** (ESI): *m/z* = 468.2736 $[M+H]^+$. Calculated for $C_{28}H_{37}NO_5+H^+$: 468.2745.

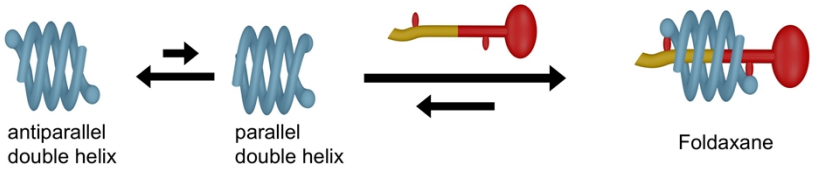
Compound 2. Under N_2 atmosphere, (9-fluorenylmethyl) chloroformate (0.61 g, 2.37 mmol, 1.5 equiv.) was added to a solution of butyl (8-hydroxyoctyl) carbamate (0.39 g, 1.58 mmol, 1.0 equiv.), dry pyridine (0.64 mL, 7.90 mmol, 5.0 equiv.) and (4-dimethylamino) pyridine (0.02 g, 0.16 mmol, 0.1 equiv.) in dry CH_2Cl_2 (8.0 mL). The resulting slurry was stirred at room temperature overnight. The solvent was washed with 1 M HCl solution (10 mL), dried over $MgSO_4$, and concentrated to dryness. The crude material was purified by flash column chromatography using EtOAc/PE (1:8) as eluent to give **2** as a colorless oil (0.64 g, 87%). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.77 (dd, J_{HH} = 1.8 Hz, J_{HH} = 7.6 Hz, 2H), 7.62 (dd, J_{HH} = 1.0 Hz, J_{HH} = 7.4 Hz, 2H), 7.41 (td, J_{HH} = 1.0 Hz, J_{HH} = 7.0 Hz, 2H), 7.32 (td, J_{HH} = 1.2 Hz, J_{HH} = 7.5 Hz, 2H), 4.60 (br, 1H), 4.40 (d, J_{HH} = 7.4 Hz, 2H), 4.27 (t, J_{HH} = 7.4 Hz, 1H), 4.17 (t, J_{HH} = 6.7 Hz, 2H), 4.05 (t, J_{HH} = 6.7 Hz, 2H), 3.16 (q, J_{HH} = 6.8 Hz, 2H), 1.74-1.27 (m, 16H) , 0.93 (t, J_{HH} = 7.4 Hz, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 156.93, 155.40, 143.51, 141.36, 127.93, 127.22, 125.24, 120.12, 69.74, 68.37, 64.66, 46.85, 41.00, 31.19, 30.06, 29.19, 28.70, 26.71, 25.69, 19.15, 13.84. **HRMS** (ESI): *m/z* = 246.2059 $[M+H]^+$. Calculated for $C_{13}H_{27}NO_3+H^+$: 246.2064.

References:

[1] S. A. Denisov, Q. Gan, X. Wang, L. Scarpantonio, Y. Ferrand, B. Kauffmann, G. Jonusauskas, I. Huc and N. D. McClenaghan, Electronic Energy Transfer Modulation in a Dynamic Foldaxane: Proof-of-Principle of a Lifetime-Based Conformation Probe, *Angew. Chem. Int. Ed.*, 2016, **55**, 1328-1333.

3. Studies of the interaction between double helix $(1)_2$ and threads

3.1 Determination of the binding constant between double helix and thread



The binding constant K_a of double helix is given by equation 1:

$$K_a = \frac{[F]}{[DH][R]} = \frac{n_F \times V_T}{n_{DH} \times n_R} \quad (1)$$

Where: [F] = foldaxane concentration; [DH] = total double helix concentration; [R] = thread concentration; n_F = number of moles of foldaxane; V_T = total volume of the sample; n_{DH} = number of moles of double helix; n_R = number of moles of thread.

Since

$$n_{DH0} = n_{DH} + n_F \quad (2)$$

$$n_{R0} = n_R + n_F \quad (3)$$

where: n_{DH0} = initial number of moles of double helix; n_{R0} = number of moles of thread added to the sample

Substituting equations (2) and (3) into (1),

$$K_a = \frac{n_F \times V_T}{(n_{DH0} - n_F) \times (n_{R0} - n_F)} \quad (4)$$

From the integration of the NMR spectrum, it is possible to obtain the fraction of bound DH host, x

$$x = \frac{n_F}{n_{DH0}} \quad (5)$$

Therefore,

$$K_a = \frac{x \times V_T}{n_{R0} - (x \times n_{DH0}) - (x \times n_{R0}) + (x^2 \times n_{DH0})} \quad (6)$$

The fraction of bound double helices x (Eq. 5) was found by integration of the region of pivaloyl group δ 0.36-0.60 ppm of the NMR spectra shown in Figure 2b to 2f. This region contains the signal for pivaloyl group protons of free double helix and foldaxane. Eq. 6 was then used to calculate K_a from each spectrum.

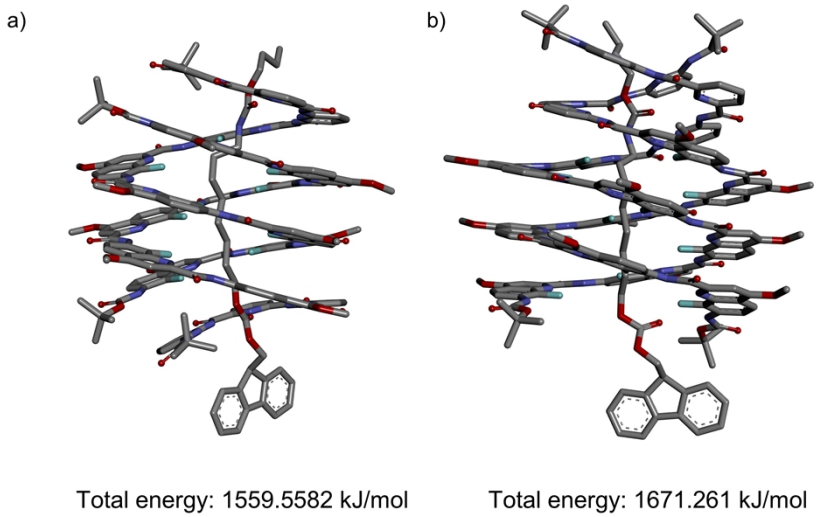


Figure S1. Energy-minimized molecular model and total energy of a) antiparallel foldaxane $(\mathbf{1})_2 \supset \mathbf{2}$ and b) parallel foldaxane $(\mathbf{1})_2 \supset \mathbf{2}$ using Merck Molecular Force Field static (MacroModel version 11.9). Isobutoxy side chains were replaced by methoxy group to simplify the calculation.

3.2 Determination of the binding constant between double helix and thread 3

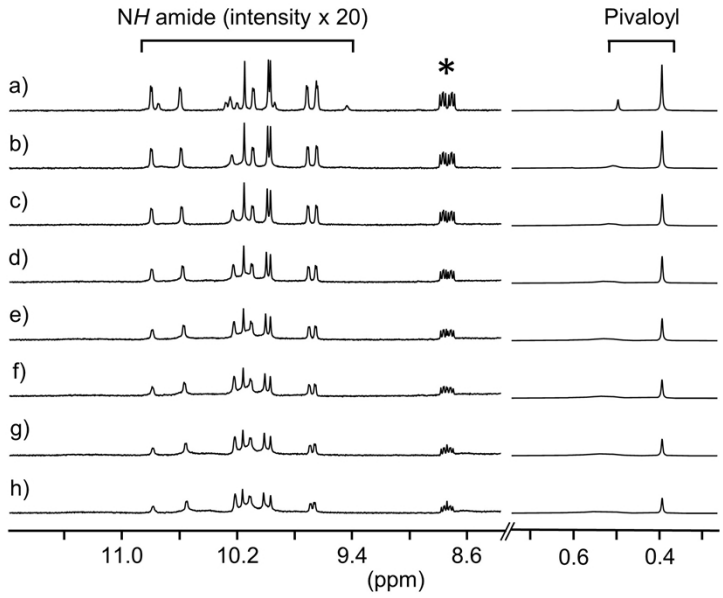


Figure S2. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of a) 2.0 mM $(\mathbf{1})_2$ and in the presence of: b) 0.5 equiv.; c) 1.0 equiv.; d) 2.0 equiv.; e) 3.0 equiv.; f) 4.0 equiv.; g) 5.0 equiv.; h) 6.0 equiv. of thread **3**. Some aromatic resonances are denoted with star.

Although fast exchange between foldaxane $(\mathbf{1})_2 \supset \mathbf{3}$ and thread **3** in the NMR timescale is observed, the

small chemical shift of proton resonance of pivaloyl group and the existence of slow parallel/antiparallel equilibrium makes it impossible to determine the binding constant between $(\mathbf{1})_2$ and thread **3** using a curve-fitting method. Alternatively, the binding constant between double helix and thread 3 can also be determined using Eq. 6 mentioned above, but the fraction of bound double helices x is determined using following equations.

The equilibrium constant between antiparallel double helix and parallel double helix of $(\mathbf{1})_2$ can be determined from the spectrum of free $(\mathbf{1})_2$.

$$K_{DH} = \frac{[DH_{anti}]}{[DH_{para}]} = \frac{[DH_{anti}]}{[DH]_0 - [DH_{anti}]} = \frac{y}{1-y} \quad (7)$$

In which

$$y = \frac{[DH_{anti}]}{[DH]_0} \quad (8)$$

Where y is the fraction of antiparallel double helix, which can be determined by integration of the region of pivaloyl group δ 0.36-0.60 ppm of the NMR spectra shown in Figure 2a.

When thread **3** is added, the fraction of bound double helices x can be determined using the following equation:

$$x = \frac{n_F}{n_{DH0}} = \frac{n_{DH0} - n_{DH-anti} - n_{DH-para}}{n_{DH0}} = \frac{[DH]_0 - [DH_{anti}] - [DH_{para}]}{[DH]_0} \quad (9)$$

where: n_{DH0} = initial number of moles of double helix; $n_{DH\text{-}anti}$ = number of moles of free antiparallel double helix; $n_{DH\text{-}para}$ = number of moles of free parallel double helix.

Substituting equations (7) into (9),

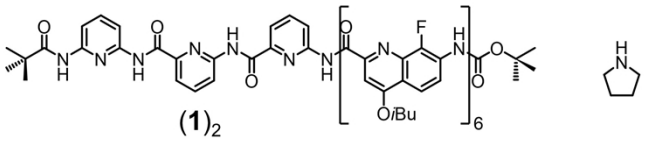
$$x = \frac{[DH]_0 - [DH_{anti}] - \frac{[DH_{anti}]}{K_{DH}}}{[DH]_0}$$

$$= 1 - \frac{[DH_{anti}]}{[DH]_0}\left(1 + \frac{1}{K_{DH}}\right)$$

$$= 1 - y'\left(1 + \frac{1}{K_{DH}}\right) \quad (10)$$

Where y' is the fraction of antiparallel double helix, which can be determined by integration of the region of pivaloyl group δ 0.36-0.60 ppm of the NMR spectra shown in Figure S2.

4. ^{1}H NMR studies of the interaction between $(1)_2$ and different secondary amines



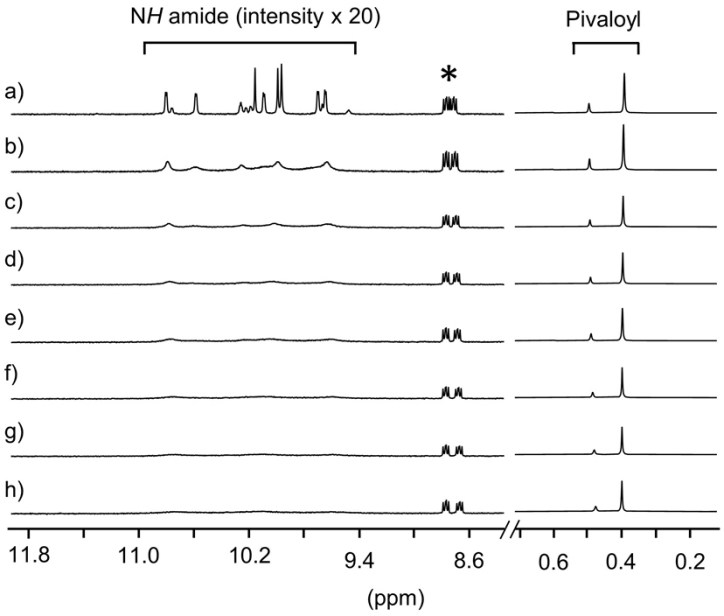
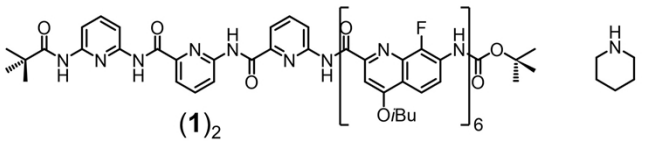


Figure S3. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of a) 2.0 mM $(\mathbf{1})_2$ and in the presence of: b) 2.0 equiv.; c) 4.0 equiv.; d) 6.0 equiv.; e) 8.0 equiv.; f) 12.0 equiv.; g) 16.0 equiv.; h) 20.0 equiv. pyrrolidine. Some aromatic resonances are denoted with star.



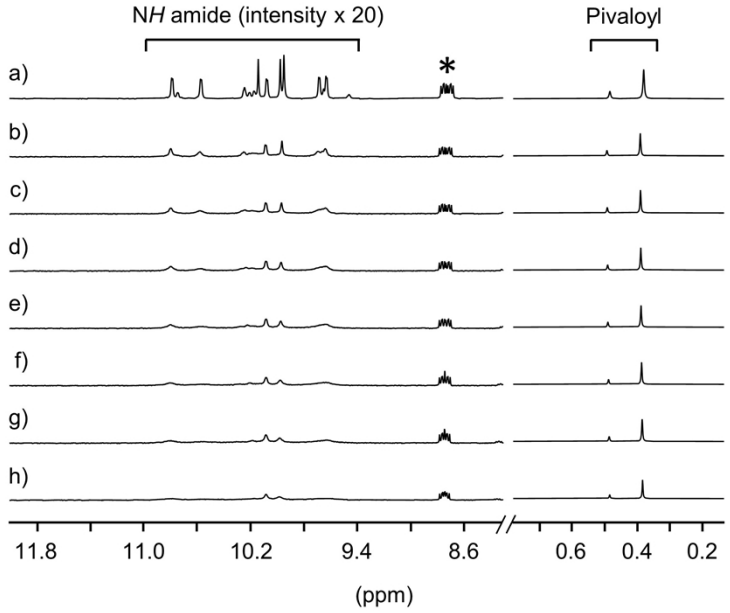
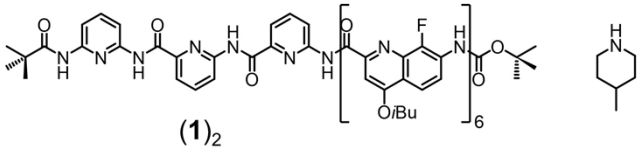


Figure S4. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of a) 2.0 mM $(\mathbf{1})_2$ and in the presence of: b) 2.0 equiv.; c) 4.0 equiv.; d) 6.0 equiv.; e) 8.0 equiv.; f) 12.0 equiv.; g) 16.0 equiv.; h) 20.0 equiv. piperidine. Some aromatic resonances are denoted with star.



$(\mathbf{1})_2$

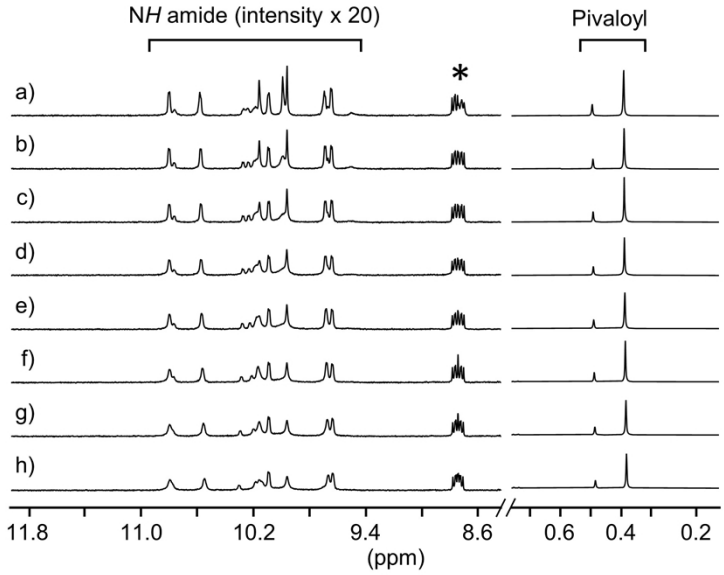


Figure S5. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of a) 2.0 mM $(\mathbf{1})_2$ and in the presence of: b) 2.0 equiv.; c) 4.0 equiv.; d) 6.0 equiv.; e) 8.0 equiv.; f) 12.0 equiv.; g) 16.0 equiv.; h) 20.0 equiv. 4-methylpiperidine. Some aromatic resonances are denoted with star.

5. Cleavage of thread 2 with different concentration of 4-methylpiperidine

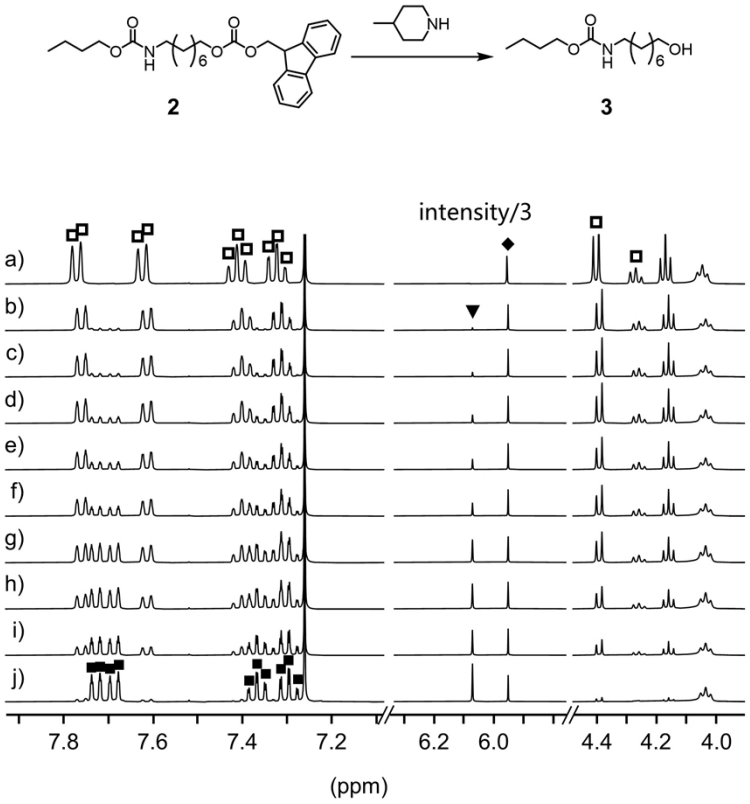


Figure S6. Extracts of ^{1}H NMR spectra (400 MHz) in$CDCl_3$ at 298K of a) 20.0 mM thread **2** alone, and in the presence of 100 mM 4-methylpiperidine after: b) 40 min; c) 70 min; d) 120 min; e) 180 min; f) 230 min; g) 400 min; h) 540 min; i) 650 min; j) 1430 min. Resonances of thread **2** and dibenzofulvene are denoted with empty and filled squares, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonance of 1,1,2,2-tetrachloroethane, which is used as an internal standard, is denoted with diamond.

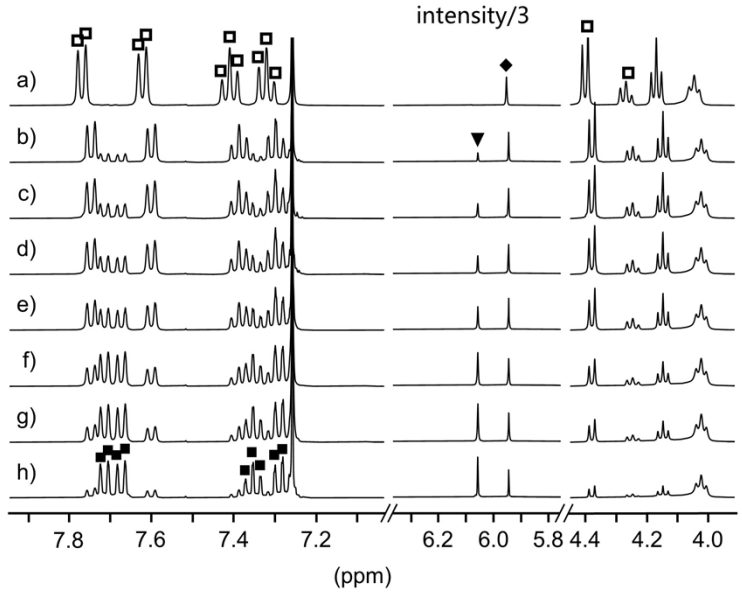


Figure S7. Extracts of ^{1}H NMR spectra (400 MHz) in$CDCl_3$ at 298K of a) 20.0 mM thread **2** alone, and in the presence of 200 mM 4-methylpiperidine after: b) 40 min; c) 70 min; d) 90 min; e) 120 min; f) 230 min; g) 270 min; h) 440 min. Resonances of thread **2** and dibenzofulvene are denoted with empty and filled squares, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonance of 1,1,2,2-tetrachloroethane, which is used as an internal standard, is denoted with diamond.

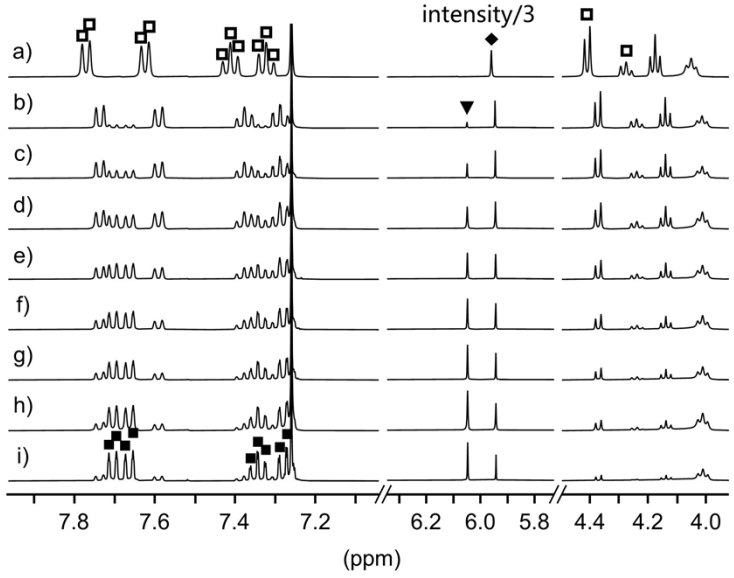


Figure S8. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of a) 20.0 mM thread **2** alone, and in the presence of 300 mM 4-methylpiperidine after: b) 9 min; c) 36 min; d) 60 min; e) 90 min; f) 120 min; g) 150 min; h) 180 min; i) 240 min. Resonances of thread **2** and dibenzofulvene are denoted with empty and filled squares, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonance of 1,1,2,2-tetrachloroethane, which is used as an internal standard, is denoted with diamond.

6. ^{1}H NMR studies of the cleavage of foldaxane with different concentrations of 4-methylpiperidine

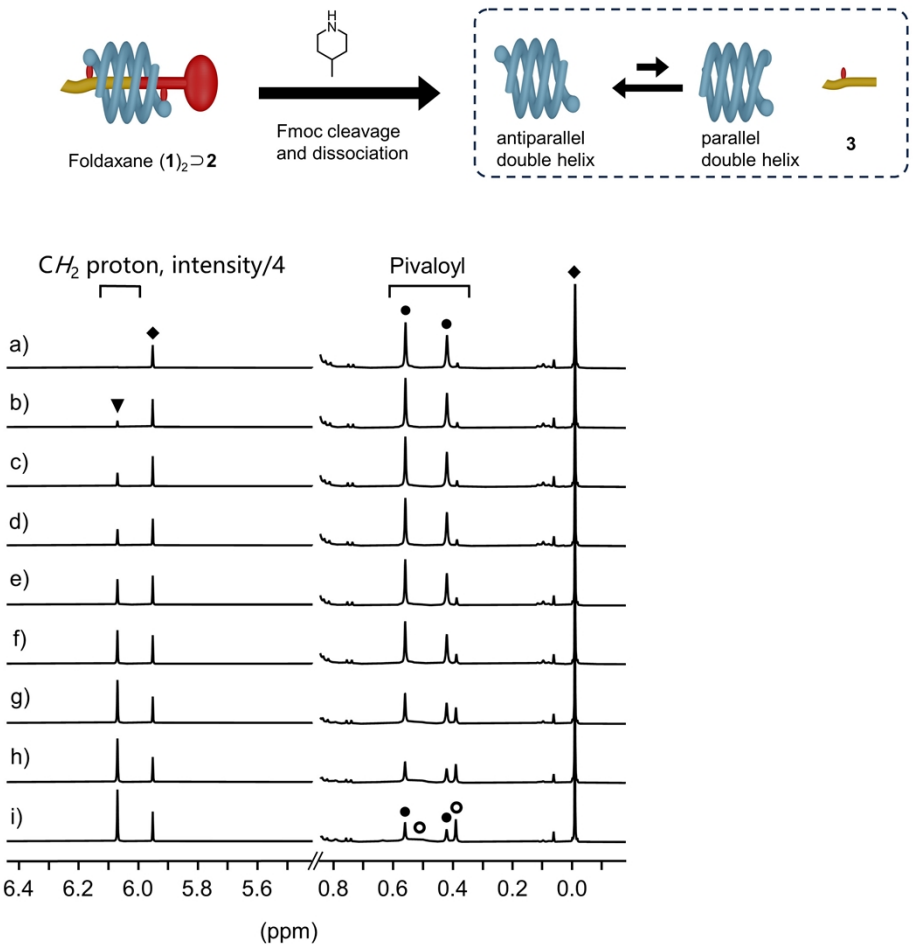


Figure S9. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of a) 2.0 mM (**1**)$_2$ and 20.0 mM thread **2** alone, and in the presence of 100 mM 4-methylpiperidine after: b) 10 min; c) 70 min; d) 170 min; e) 240 min; f) 410 min; g) 660 min; h) 1080 min; i) 1560 min. Resonances of the pivaloyl group of foldaxane (**1**)$_2$⊃**2** and free double helix (**1**)$_2$ are denoted with filled and empty circles, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonance of 1,1,2,2-tetrachloroethane and tetramethylsilane, which is used as an internal standard, is denoted with diamond.

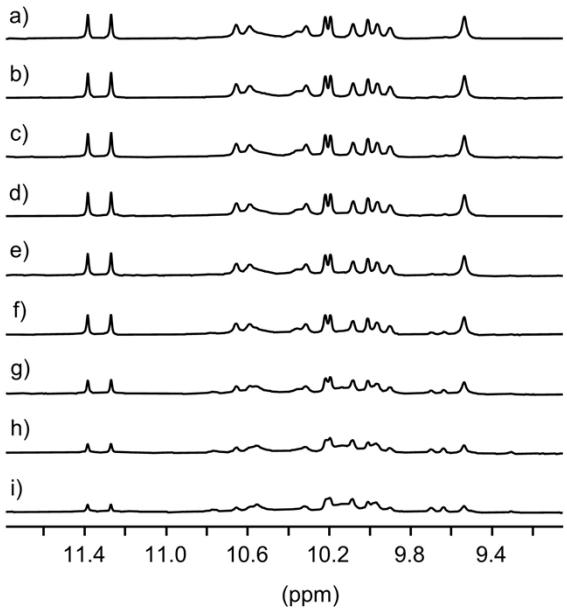


Figure S10. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K showing the amide resonances of a) foldaxane (**1**)$_2$⊃**2** formed with 2.0 mM (**1**)$_2$ and 20.0 mM thread **2**, and in the presence of 100 mM 4-methylpiperidine after: b) 10 min; c) 70 min; d) 170 min; e) 240 min; f) 410 min; g) 660 min; h) 1080 min; i) 1560 min.

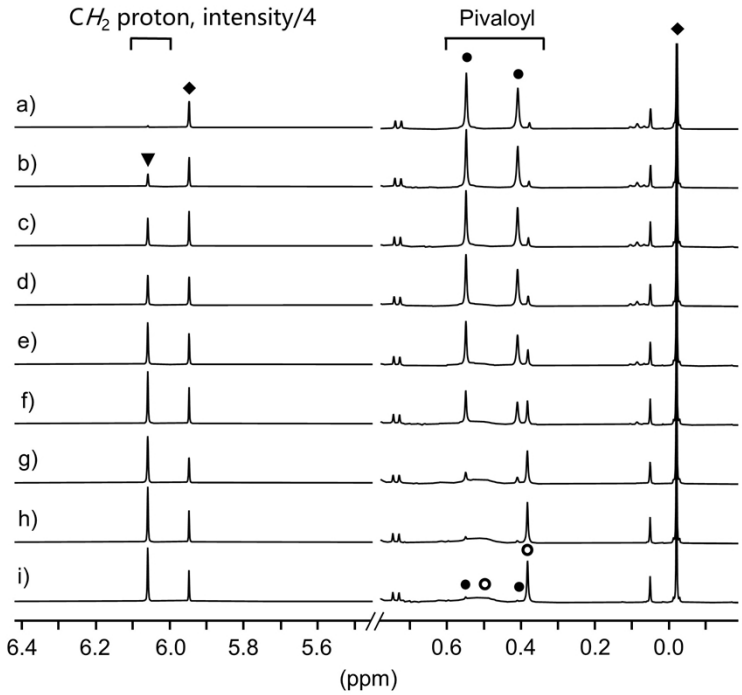


Figure S11. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of a) foldaxane (**1**)$_2$⊃**2** formed with 2.0 mM (**1**)$_2$ and 20.0 mM thread **2**, and in the presence of 200 mM 4-methylpiperidine after: b) 10 min; c) 70 min; d) 170 min; e) 240 min; f) 410 min; g) 660 min; h) 1080 min; i) 1560 min. Resonances of the pivaloyl group of foldaxane (**1**)$_2$⊃**2** and free double helix (**1**)$_2$ are denoted with filled and empty circles, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonance

of 1,1,2,2-tetrachloroethane and tetramethylsilane, which is used as an internal standard, is denoted with diamond.

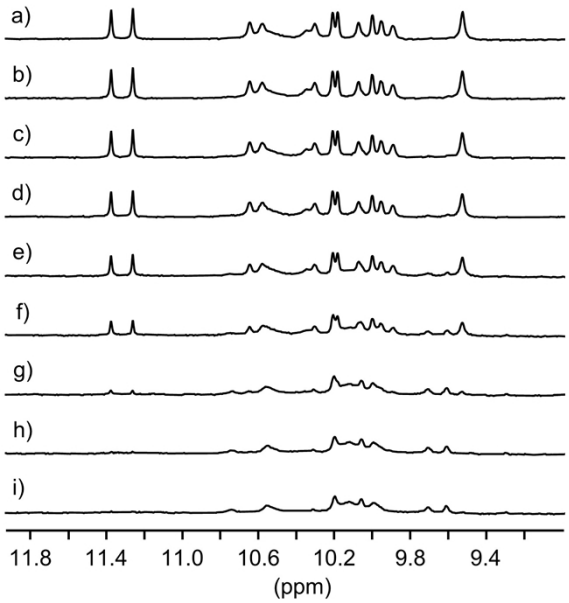


Figure S12. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K showing the amide resonances of a) foldaxane (**1**)$_2$⊃**2** formed with 2.0 mM (**1**)$_2$ and 20.0 mM thread **2**, and in the presence of 200 mM 4-methylpiperidine after: b) 10 min; c) 70 min; d) 170 min; e) 240 min; f) 410 min; g) 660 min; h) 1080 min; i) 1560 min.

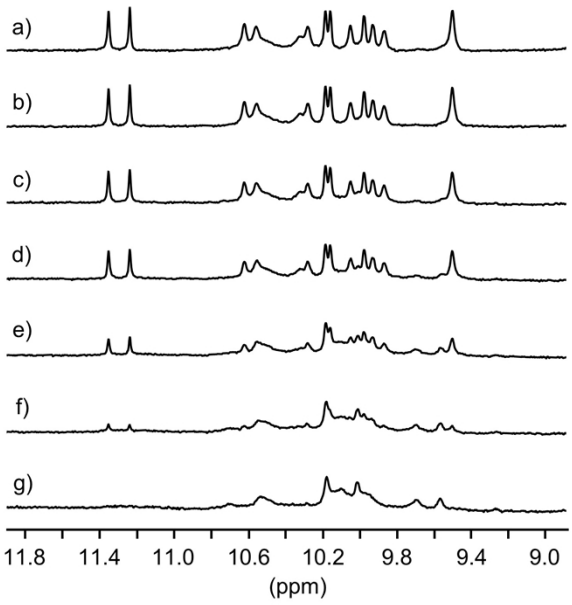


Figure S13. Extracts of ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K showing the amide resonances of a) foldaxane (**1**)$_2$⊃**2** formed with 2.0 mM (**1**)$_2$ and 20.0 mM thread **2**, and in the presence of 300 mM 4-methylpiperidine after: b) 70 min; c) 170 min; d) 240 min; e) 410 min; f) 660 min; g) 1080 min.

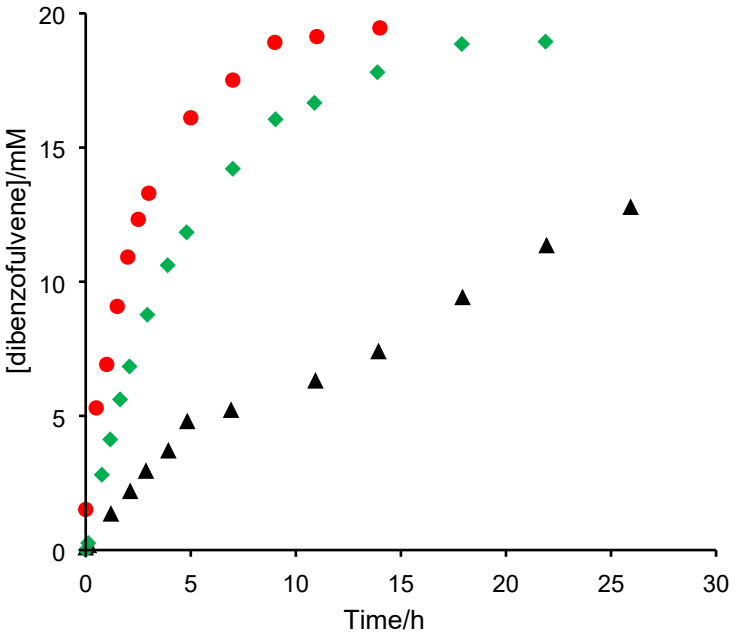


Figure S14. Time dependent concentration of dibenzofulvene **3** in a mixture of 20.0 mM **2**, 2.0 mM $(\mathbf{1})_2$ and in the presence of 300 mM (red dots), 200 mM (green diamonds), and 100 mM (black triangles) 4-methylpiperidine.

7. ^{1}H NMR studies of cycles of transient foldaxane formation

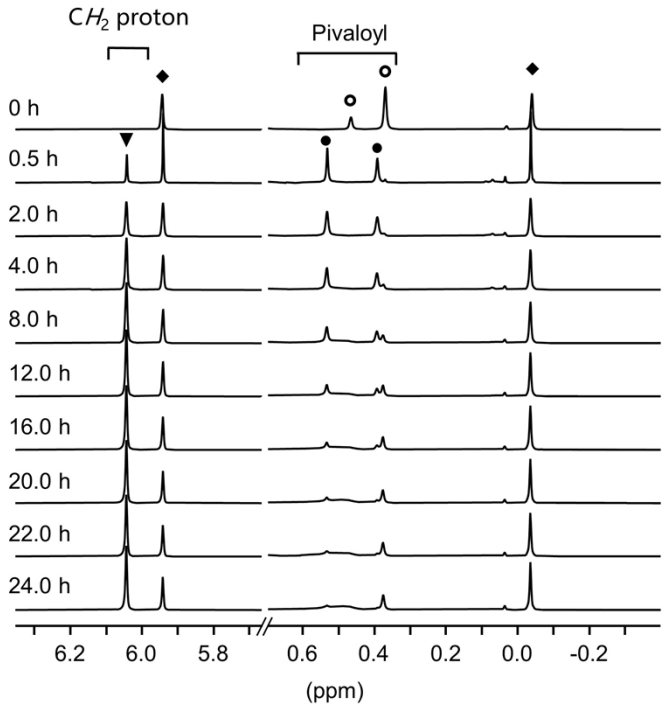


Figure S15. Extracts of time dependent ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of 2.0 mM $(\mathbf{1})_2$ and 300 mM 4-methylpiperidine before and after the first addition of 20.0 mM thread **2**. Resonances of the pivaloyl group of foldaxane $(\mathbf{1})_2\supset\mathbf{2}$ and free double helix $(\mathbf{1})_2$ are denoted with filled and empty

circles, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonances of 1,1,2,2-tetrachloroethane and tetramethylsilane, which are used as an internal standard, is denoted with diamond.

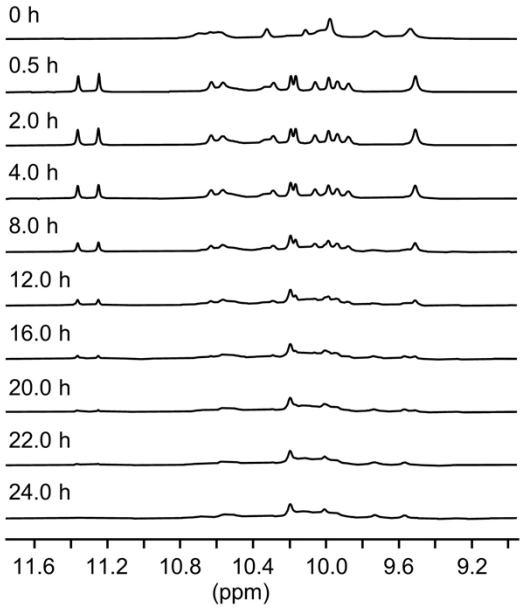


Figure S16. Extracts of time dependent ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K showing the amide resonances of 2.0 mM $(\mathbf{1})_2$ with 300 mM 4-methylpiperidine before and after the first addition of 20.0 mM thread **2**.

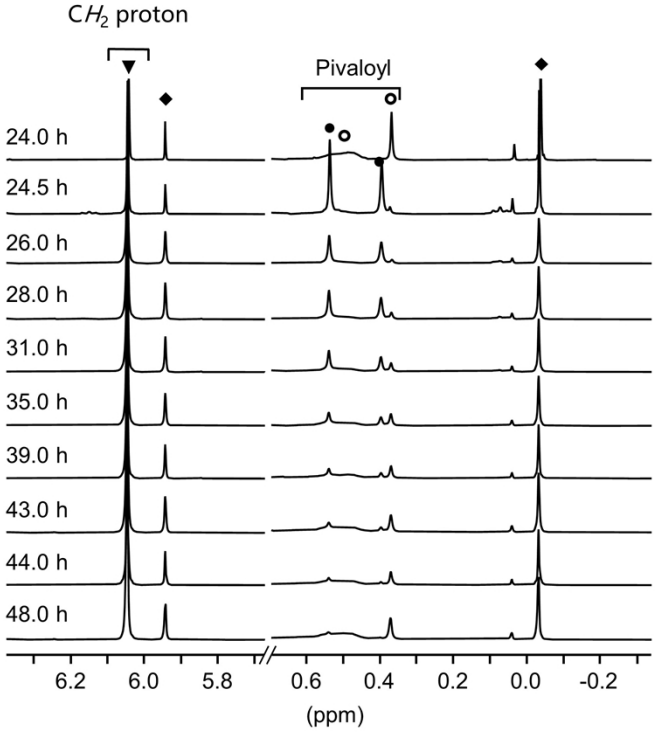


Figure S17. Extracts of time dependent ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of 2.0 mM $(\mathbf{1})_2$ and 300 mM 4-methylpiperidine before and after the second addition of 20.0 mM thread **2**. Resonances of the pivaloyl group of foldaxane $(\mathbf{1})_2\supset\mathbf{2}$ and free double helix $(\mathbf{1})_2$ are denoted with filled and empty circles, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonances of 1,1,2,2-tetrachloroethane and tetramethylsilane, which are used as an internal standard, is denoted with diamond.

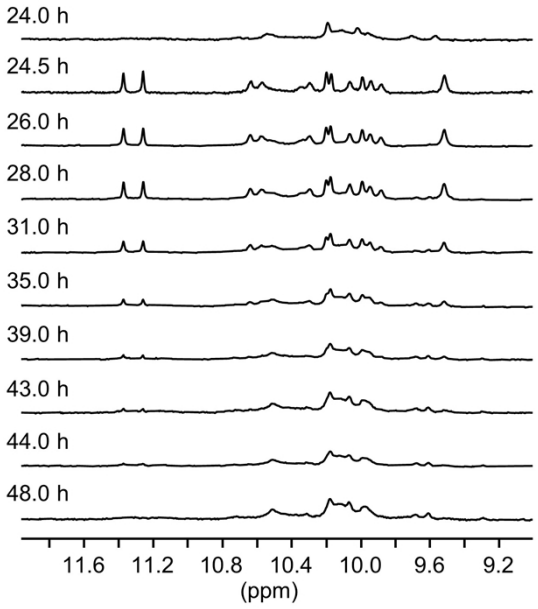


Figure S18. Extracts of time dependent ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K showing the amide resonances of 2.0 mM $(\mathbf{1})_2$ with 300 mM 4-methylpiperidine before and after the second addition of 20.0 mM thread **2**.

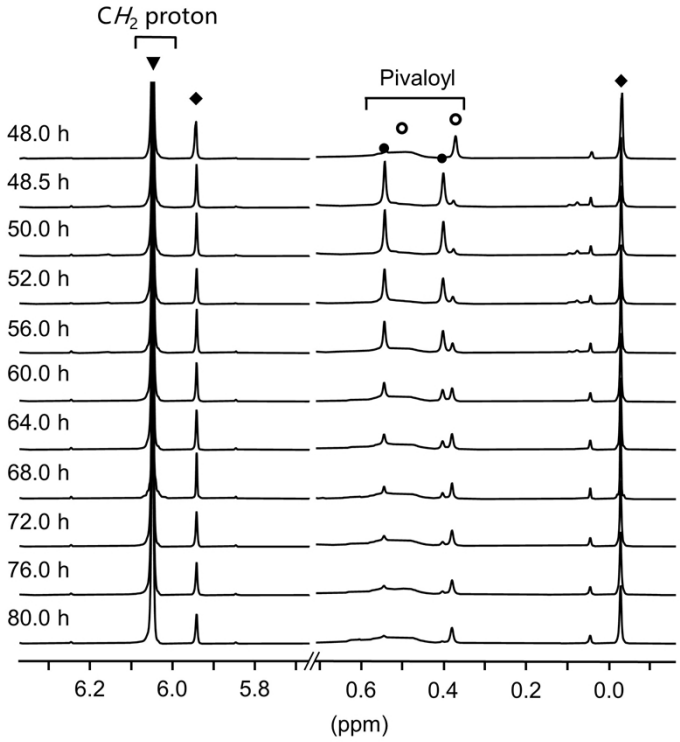


Figure S19. Extracts of time dependent ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K of 2.0 mM $(\mathbf{1})_2$ and 300 mM 4-methylpiperidine before and after the third addition of 20.0 mM thread **2**. Resonances of the pivaloyl group of foldaxane $(\mathbf{1})_2\supset\mathbf{2}$ and free double helix $(\mathbf{1})_2$ are denoted with filled and empty circles, respectively. The methylene resonance of dibenzofulvene is denoted with triangle. The resonances of 1,1,2,2-tetrachloroethane and tetramethylsilane, which are used as an internal standard, is denoted with diamond.

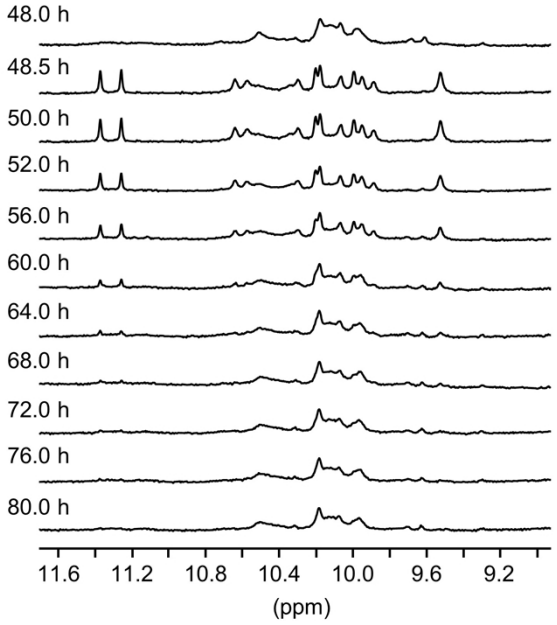


Figure S20. Extracts of time dependent ^{1}H NMR spectra (400 MHz) in $CDCl_3$ at 298K showing the amide resonances of 2.0 mM $(\mathbf{1})_2$ with 300 mM 4-methylpiperidine before and after the third addition of 20.0 mM thread **2**.

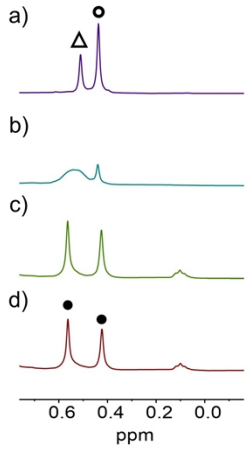


Figure S21. Extracts of ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298 K) showing the pivaloyl region of a) $(\mathbf{1})_2$ (2.0 mM), after successive addition of b) 20 mM **3**, c) 20 mM **2**, d) 20 mM **3**.

8. ^{1}H NMR and ^{13}C NMR spectra of new synthetic compounds

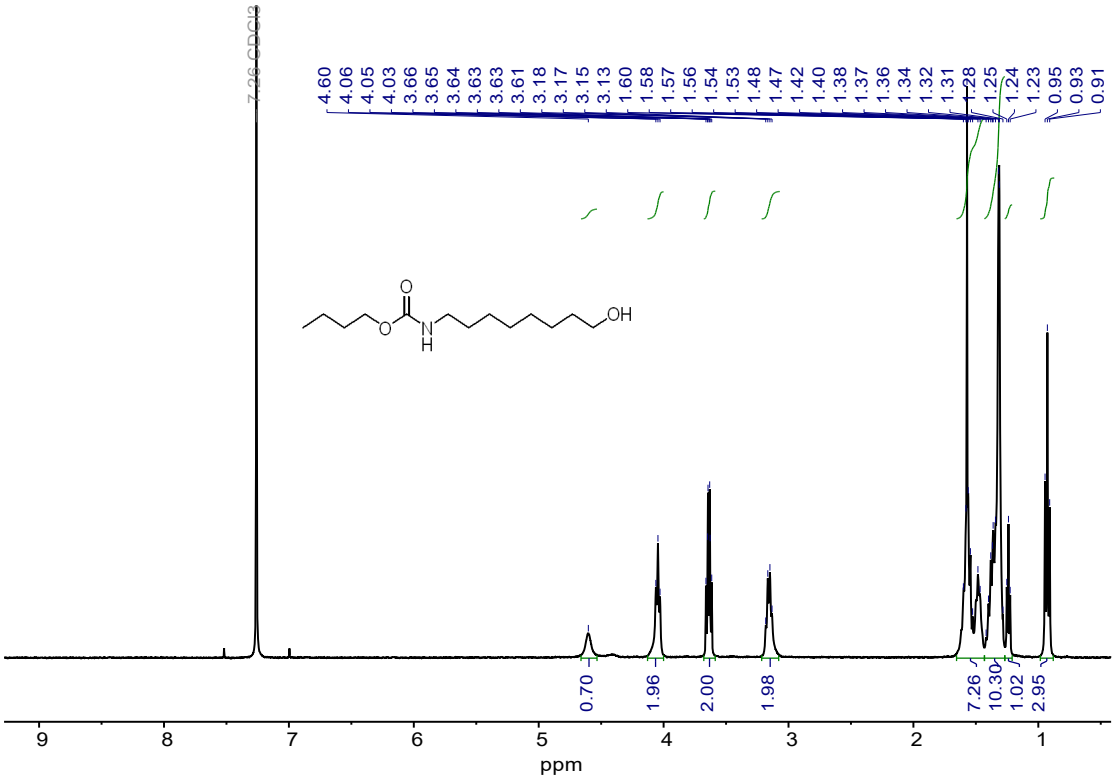


Figure S22. ^{1}H NMR spectrum (400 MHz, 298K) of compound **3** in $CDCl_3$.

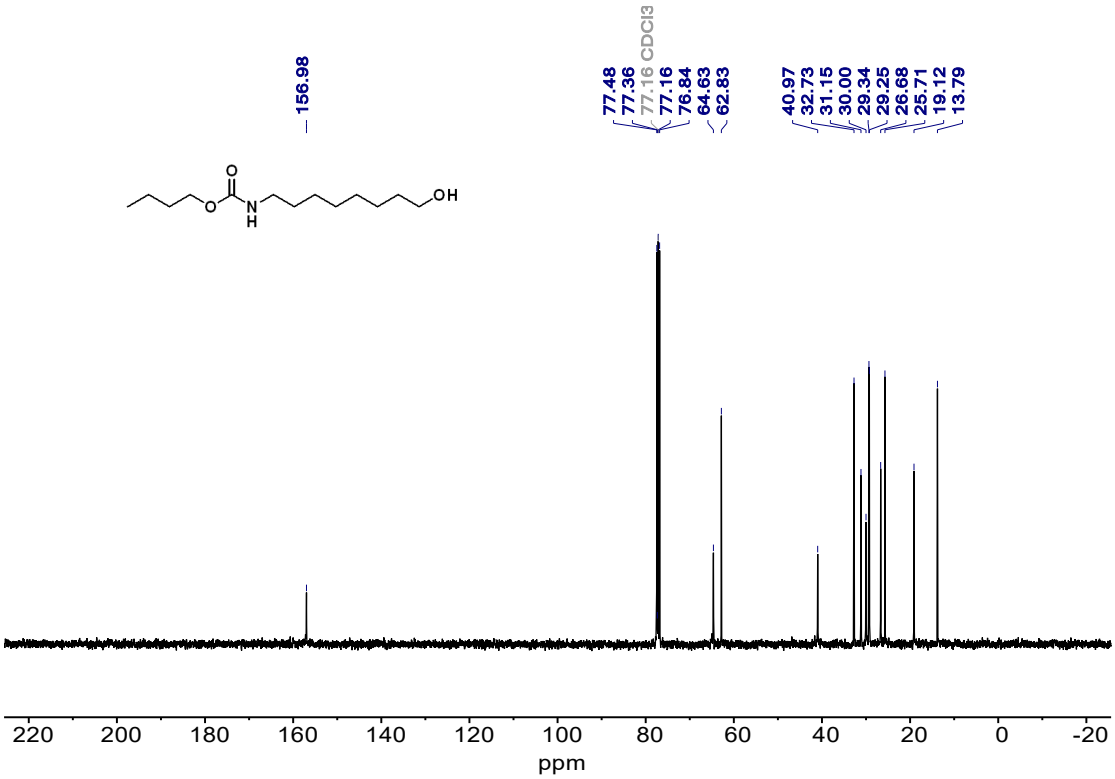


Figure S23. ^{13}C NMR spectrum (100 MHz, 298K) of compound **3** in $CDCl_3$.

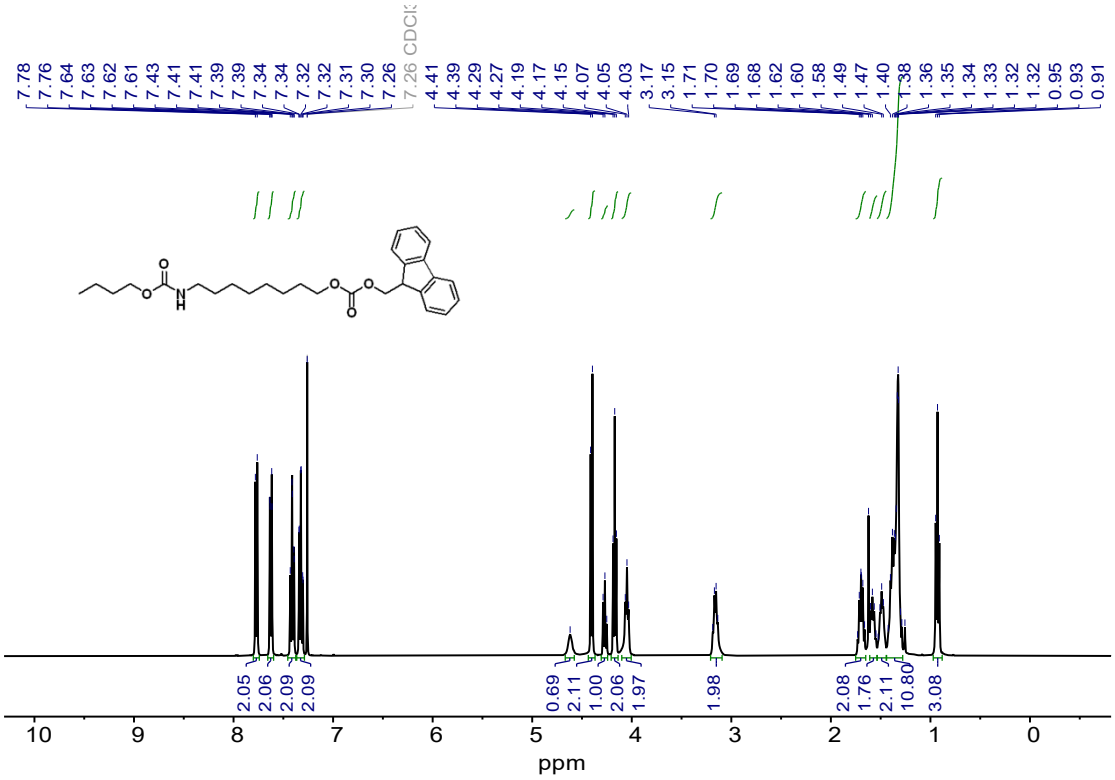


Figure S24. ^{1}H NMR spectrum (400 MHz, 298K) of compound **2** in $CDCl_3$.

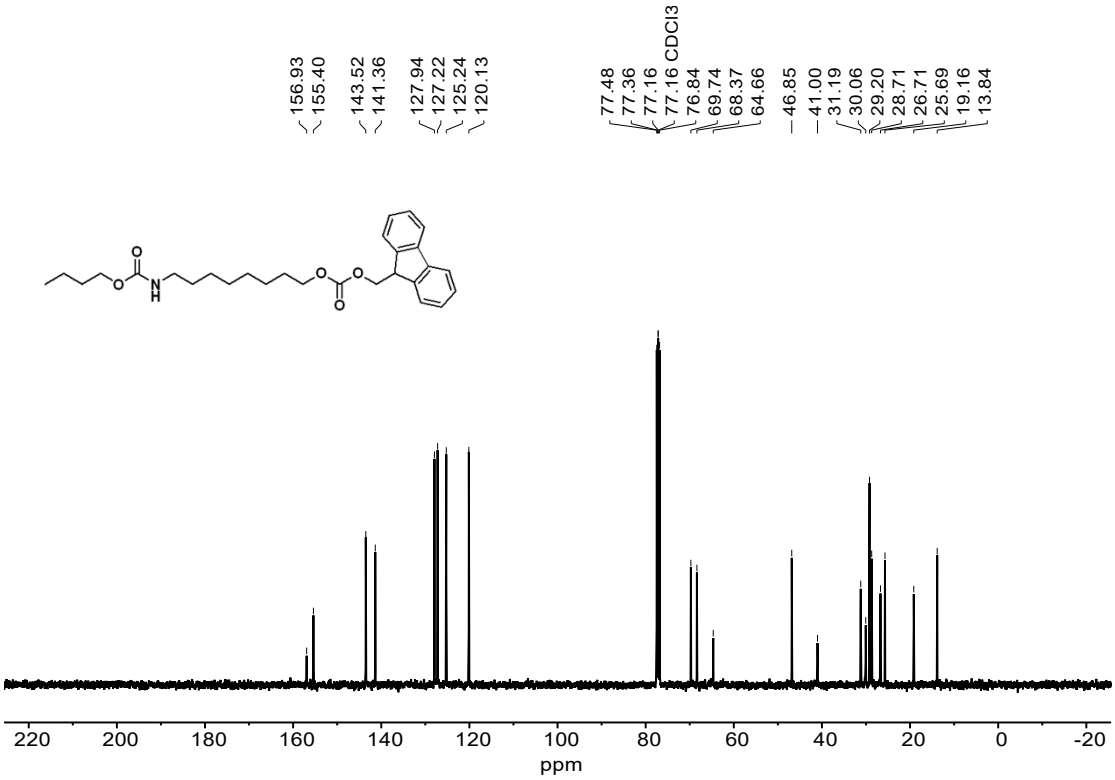


Figure S25. ^{13}C NMR spectrum (100 MHz, 298K) of compound **2** in $CDCl_3$.