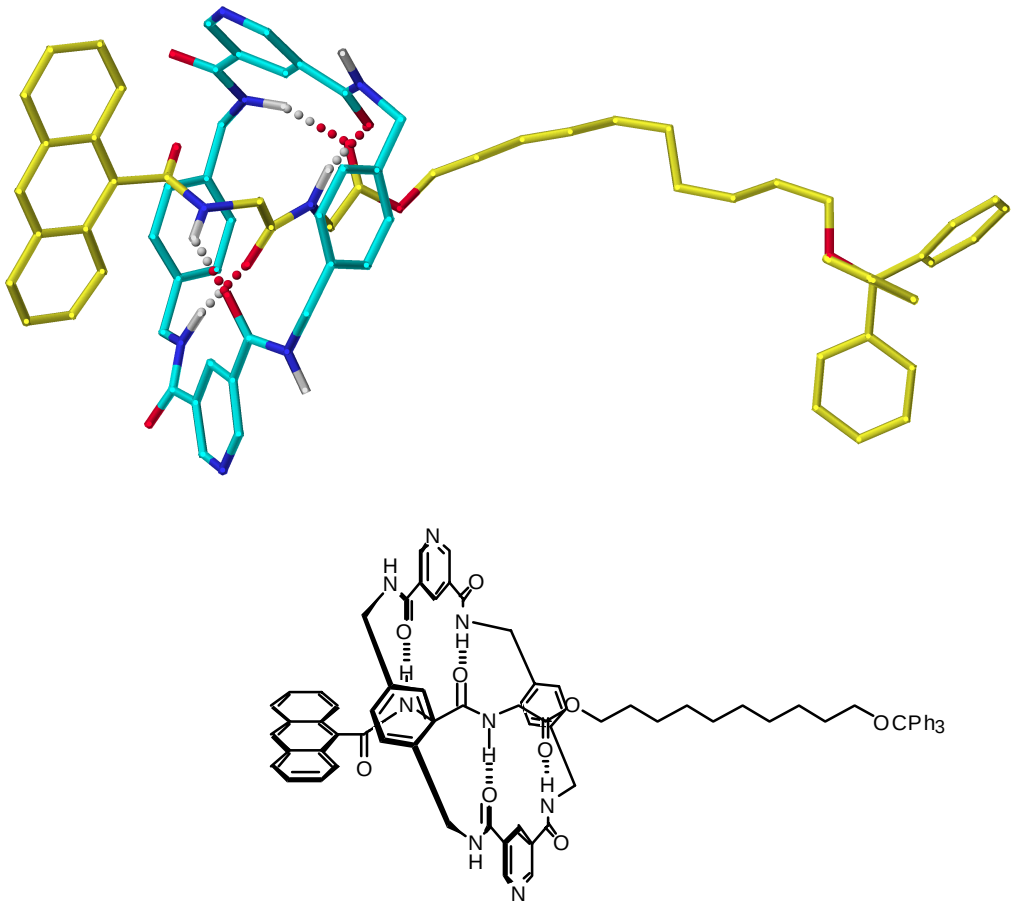
Supporting Information



X-ray Crystallographic Structure Determinations. :

$C_{78}H_{76}N_8O_9$, M=1269.47, crystal size 0.1×0.02×0.005mm, Monoclinic P2(1)/n, a=11.3523(3), b=35.6055(6), c=16.1772(3) Å, β=95.3420(10)°, V=6510.5(2)Å^3, Z=4, ρ_{calcd}=1.295 g cm^{-3}; MoK$_a$ radiation (graphite monochromator, λ=0.68740 Å), μ=0.086 mm^{-1}, T=160(2)K. 36465 data (13893 unique, R_{int}=0.1993, 1.11<θ<26.95°), were collected on a Siemens SMART CCD diffractometer using narrow frames (0.3° in ω), and were corrected semi-empirically for absorption and incident beam decay (transmission 0.16234-1.000). The structure was solved by direct methods and refined by full-matrix least-squares on F^2 values of all data (G.M.Sheldrick, SHELXTL manual, Siemens Analytical *X*-ray Instruments, Madison WI, USA, 1994, version 5) to give $wR=\{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$=0.3941, conventional R=0.2156 for F values of 13893

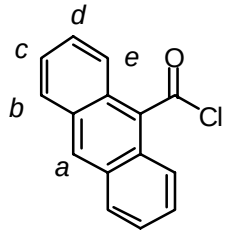
reflections with $F_o^2 > 2\sigma(F_o^2)$, S=1.312 for 881 parameters. Residual electron density extremes were 0.510 and -0.387 $Å^{-3}$. Amide hydrogen atoms were refined isotropically subject to a distance constraint N-H = 0.98 Å, with the remainder constrained; anisotropic displacement parameters were used for all non-hydrogen atoms.

Crystallographic data for (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-159773. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: Int. code + (1223)336-033; e-mail: teched@chemcrys.cam.ac.uk).

Synthesis of Anthracene-9-carbamide glycylglycine [2]rotaxane.

The thread (**2**) used to carry out the rotaxane formation reaction was prepared in a simple five step synthetic route and then used to prepare rotaxane **1** that was used in the studies described in this communication.

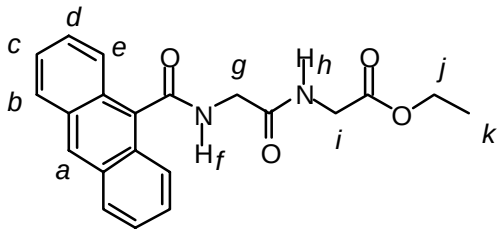
Anthracene-9-carbonyl chloride. 5 g of anthracene-9-carboxylic acid was suspended in 40 ml of dry toluene and 5 ml of oxalyl chloride was added. A few drops of dimethylformamide were added to the reaction mixture which was then heated to 50°C until a clear yellow solution was obtained. The solvent was evaporated under vacuum and the residue recrystallised from hexane to afford the title compound in 80% yield.



^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 7.55-7.45 (m, 4H, ArH_{cd}), 7.94 (d, 2H, ArH_b, J = 8.0 Hz), 8.09 (d, 2H, ArH_e J =8.0 Hz), 8.49 (s, 1H , ArH_a). FAB-MS (*m*NBA matrix): m/z 241 (M+H).

***N*-(9-Anthroyl) glycylglycine ethyl ester.** To a stirring solution of glycylglycine ethyl ester hydrochloride (3.92 g, 20 mmol) and triethylamine (4.04 g, 40mmol) in 150 ml of anhydrous chloroform was added, in small portions, 4.82 g (20mmol) of anthracene-9-carbonyl chloride. The solution was stirred for an hour and evaporated to dryness. The

residue was recrystallised from an ethanol /water mixture to afford colourless crystals of the title compound (5.48 g) in 75% yield.

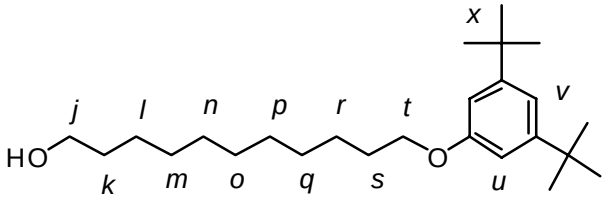


^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm)= 1.28 (t, 3H, $COOCH_2C\underline{H}_3{}_k$, *J*=7.0 Hz), 3.95 (d, 2H, $COCH_2{}_iNH$, *J* = 5.5 Hz), 4.16 (q, 2H, $COOC\underline{H}_2{}_jCH_3$, *J* = 7.0 Hz), 4.33 (d, 2H, $COC\underline{H}_2{}_gNH$, *J* =5.5 Hz), 6.95 (t, 1H, NH_h, *J*=5.5 Hz), 7.08 (t ,1H, NH_f, *J*=5.5 Hz), 7.55-7.45 (m, 4H, $ArH_{c,d}$), 7.94 (d, 2H, Ar H_b, *J* = 8.0 Hz), 8.09 (d, 2H, ArH_e *J* =8.0 Hz), 8.49 (s, 1H , ArH_a)

^{13}C(100Hz): δ(ppm) = 14.5, 41.7, 43.8, 61.9, 127.3, 128.4, 129.0, 131.2, 131.3, 169.3, 169.9, 170.6

FAB-MS (mNBA matrix) m/z 365 (M-CH_3) anal. calcd for $C_{22}H_{23}N_2O_4$:
C 69.64, H 6.11, N 16.87; found C 69.7, H 6.2, N 16.7.

1-(3,5-ditertbutylphenoxy)-undecan-11-ol. 3,5-Di-t-butylphenol (2.06g, 10 mmol), 11-bromo-1-undecanol (2.51g, 10mmol) and potassium carbonate (1.66, 12mmol) were refluxed in acetonitrile for 5 days under a nitrogen atmosphere. The reaction mixture was cooled, filtered and the acetonitrile evaporated under reduced pressure to afford a colourless liquid. This was purified by column chromatography (silica gel, 1-2% methanol in dichloromethane) to give the title compound in 80% yield as a pale yellow oil.

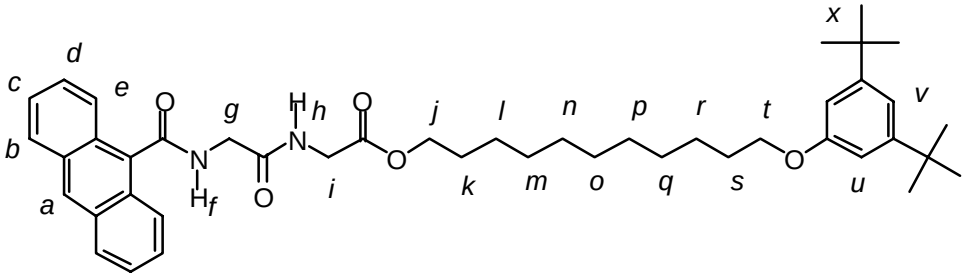


^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm)= 1.31 (m, 32H, $(CH_2)_{lmnopqr}$, $C(CH_x3)_3$, 1.6 (m, 2H, $OCH_2C\underline{H}_2{}_s$), 1.75 (q, 2H, $OCH_2C\underline{H}_2{}_k$, *J*= 7.0 Hz), 3.57 (t, 2H, $OHC\underline{H}_2$j, *J* = 7.0 Hz), 4.00 (t, 2H, $OCH_2{}_t$, *J* = 7.0 Hz), 6.70 (d, 2H, ArH_u, *J* = 1.5Hz), 6.95(t, 1H, ArH_v,*J*=1.5Hz).

^{13}C NMR (100 MHz,$CDCl_3$): δ(ppm)= 29.85, 29.86, 29.92, 29.95, 29.98, 30.03, 31.83, 33.18, 35.39, 63.41, 68.21, 109.26, 115.20, 152.51, 159.09

FAB-MS (*m* NBA matrix) 379 (M+H).

Anthroylglycylglycine thread (2). 2.75 g of 1-(3,5-ditertbutyl phenoxy)-undecan-11-ol (7.36 mmol), 2.68g (7.36mmol) of *N*-(9-Anthroyl) glycylglycine ethyl ester and 60mg of bis(dibutylchlorotin)oxide were refluxed together with stirring in dry toluene (80 ml) under nitrogen for 1 hour until all 9-anthroyl glycylglycine ethyl ester had dissolved. Then, the toluene was distilled off over a period of two hours. Further portions of toluene and bis(dibutylchlorotin) oxide were added and the distillation repeated until the reaction was complete by tlc. The remaining residue was taken up in hexane. The sticky colourless solid obtained was then filtered and washed with diisopropyl ether to afford compound **2** in 80% yield.

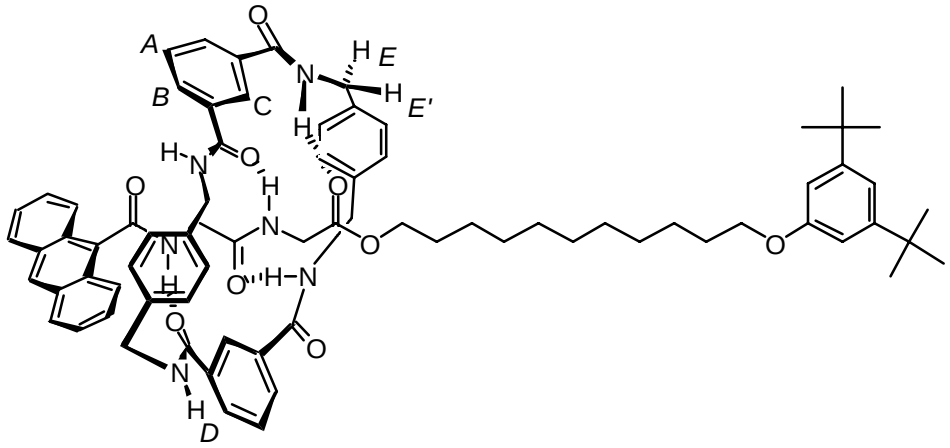


^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.40 (m, 32H, $CH_{2\ mnopqr}$, CCH_{3_x}) 1.48 (m, 2H, CH_{2_l}), 1.69 (p, 2H, $OCH_2C\underline{H}_{2_s}$, *J* =7.0 Hz) 1.79 (p, 2H, $OCH_2C\underline{H}_{2_k}$, *J* =7.0 Hz), 3.98 (t, 2H, Ar-OCH_{2_t}, *J* = 7.0 Hz), 4.15 (d, 2H, $CONHC\underline{H}_{i}{}_2CO$, *J* = 5.5 Hz), 4.21(t, 2H, OCH_{2_j}, *J* = 7.0 Hz), 4.44 (d, 2H, $NHC\underline{H}_{g}{}_2CO$, *J* = 5.5 Hz), 6.67 (t, 1H, NH_h, *J*=5.5 Hz), 6.78 (d, 2H, ArH_u, *J*=1.5 Hz), 6.88 (t, 1H, NH_f, *J*=5.5 Hz), 7.03 (t, 1H, ArH_v, *J* =1.5 Hz), 7.45 (m, 4H, ArH_{cd}), 8.04 (d, 2H, ArH_a, *J* = 8.0 Hz), 8.15 (d, 2H, ArH_e, *J* = 8.0 Hz), 8.50 (s, 1H, ArH_a).

^{1}H NMR (400 MHz, d6-DMSO): δ(ppm)= 1.23-1.40 (m, 32H, $CH_{2\ lmnopqr}$, CH_{3_x}), 1.65 (m, 2H, $OCH_2C\underline{H}_2$), 1.70 (m, 2H, $OCH_2C\underline{H}_2$), 3.95 (t, 2H, OCH_{2_t}, J= 7.0 Hz), 4.01 (d, 2H, $CONHC\underline{H}_{2_i}$, *J*=5.5 Hz), 4.09 (t, 2H, OCH_{2_j}, *J*=7.0 Hz), 4.14 (d, 2H, $CONHC\underline{H}_{2_g}$, *J*= 5.5 Hz), 6.70 (d, 2H, ArH_u, *J*=1.5 Hz), 6.95 (t, 1H, ArH_v, *J*=1.5Hz) 7.55 (m, 4H, ArH_{cd}), 8.12 (m, 2H, ArH_e), 8.26 (m, 2H, ArH_b), 8.53 (t, 1H, NH_h, *J*= 5.5Hz), 8.66 (s, 1H, ArH_a), 9.06 (t, 1H, NH_f, *J* =5.5 Hz)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) =26.17, 26.51, 28.92, 29.54, 29.78, 29.81, 29.88, 31.81, 35.32, 41.86, 43.98, 66.20, 68.27, 109.34, 115.18, 125.46, 125.90, 127.27, 128.63, 128.85, 128.97, 131.22, 131.49, 152.55, 152.50, 159.20, 168.98, 169.90, 170.52.
FAB-MS (mNBA matrix) 695 [MH], anal. cald. for C_{44} H_{58} N_2 O_5 C 76.05, H 8.41,N 4.03 found C 75.90 H 8.45 N 3.99

Anthroylglycylglycine isophthaloyl macrocycle [2]rotaxane (1). Separate solutions of p-xylylenediamine (2.04 g 15mmol) plus triethylamine(2.7g, 30 mmol) and of isophthaloyl dichloride (3.030 g, 15 mmol) were both made up to 40 ml of amylene stabilised chloroform. Both solutions were added dropwise, simultaneously (via syringe pump) to a stirred solution of 0.520 g (0.75 mmol) of **2,** over a 3 hour period. The reaction mixture was filtered through a pad of celite and the liquid evaporated to dryness under reduced pressure. The residue was dried under vacuum and purified by column chromatography (silica gel, 1-2% methanol in dichloromethane as eluent) to afford 0.200 g of compound **1** (20% yield).



^{1}H NMR ($CDCl_3$): δ (ppm) 1.27(m, 30 H, $CH_{2\ lmnopqr}$, CCH_{3X}), 1.49 (m, 2H, $OCH_2C\underline{H}_{2s}$), 1.80 (m, 2H, $OCH_2C\underline{H}_{2k}$), 3.33 (d, 2H, $CONHCH_{2i}$, *J*=5.5Hz), 3.70 (d, 2H, $CONHCH_{2g}$, *J*=5.5Hz), 3.97 (t, 2H, $OCH2_t$, *J*=7.0Hz), 3.98 (t, 2H, OCH_{2j}, *J*=7.0 Hz, 4.35, 4.55 (dd, 8H, Ar $CH_{2EE'}$, *J*=5.5Hz), 5.1 (t, 1H, NH_f, *J*=5.5Hz), 6.78 (d, 2H, ArH_u, *J*=1.5Hz), 6.99 (s, 8H, ArH_F), 7.03 (d, 2H, ArH_v, *J*=1.5 Hz), 7.26 (m, 4H, $ArH_{e,h}$), 7.42 (t, 2H, $ArH_{c,d}$ *J*=8.0Hz), 7.63 (t, 4H, NH_D, *J*=5.0Hz, 2H ArH_A), 8.01 (d, 2H, ArH_b, *J*=8.0Hz), 8.13 (d, 4H, ArH_B , *J*=8.0Hz), 8.26 (s, 2H, ArH_C), 8.52 (s, 1H, ArH_a).

^{1}H NMR (d_6-DMSO): δ(ppm)= 0.34-0.47 (m(b), 8H, CH_{nmop}), 0.62 (m, 2H, CH_{2q}), 0.81-0.90 (m, 4H, $CH_{2l,r}$), 1.26 (m, 20H, CCH_{3x}, CH_{2s}), 1.34 (m, 2H, CH_{2k}), 3.53 (t, 2H, OCH_{2t}, *J*=7.0Hz), 3.70 (t, 2H, OCH_{2j}, *J*=7.0Hz), 3.83 (d, 2H, $CONHCH_{2i}$, *J*=5.5Hz), 4.09 (d, 2H, $CONHCH_{2g}$, *J*=5.5Hz), 4.29-4.37 (dd, 8H, Ar $CH_{EE'}$, *J*=5.Hz), 6.69 (d, 2H, ArH_u , *J*=1.5 Hz), 6.93(t, 1H, ArH_v, *J*=1.5 Hz), 7.17 (s, 8H, ArH_F), 7.49 (t, 2H, ArH_A, *J*=8.0 Hz), 7.51(m, 4H, $ArH_{c,d}$), 7.88 (d , 2H, ArH_B, *J*=8.0 Hz), 8.01(s, 2H, ArH_C,), 8.10 (m, 2H, ArH_b), 8.23 (m, 2H, ArH_b), 8.39(t, 1H, NH_h, *J*=5.5 Hz), 8.64 (s, 1H, ArH_a), 8.81(t, 4H, NH_D, *J*=5.5Hz), 9.02(t, 1H, NH_f, *J*=5.5Hz).

^{13}C NMR ($CDCl_3$): δ (ppm)= 26.19, 28.86, 29.58, 29.81, 29.90, 30.08, 31.83, 35.34, 41.62, 42.65, 45.12, 66.54, 68.231, 109.28, 115.20, 124.79, 125.69, 126.04, 127.15, 128.30, 129.19, 129.61, 131.15, 131.29, 136.62, 137.58, 148.94, 152.51, 159.11, 165.05, 169.00, 169.9, 172.45, 173.29.

FAB-MS (mNBA matrix) [MH],1258 anal. calcd for C_{78} H_{92} N_6 O_9, C 74.49,H 7.37, N 6.68 found C74.6, H 7.4, N 6.5

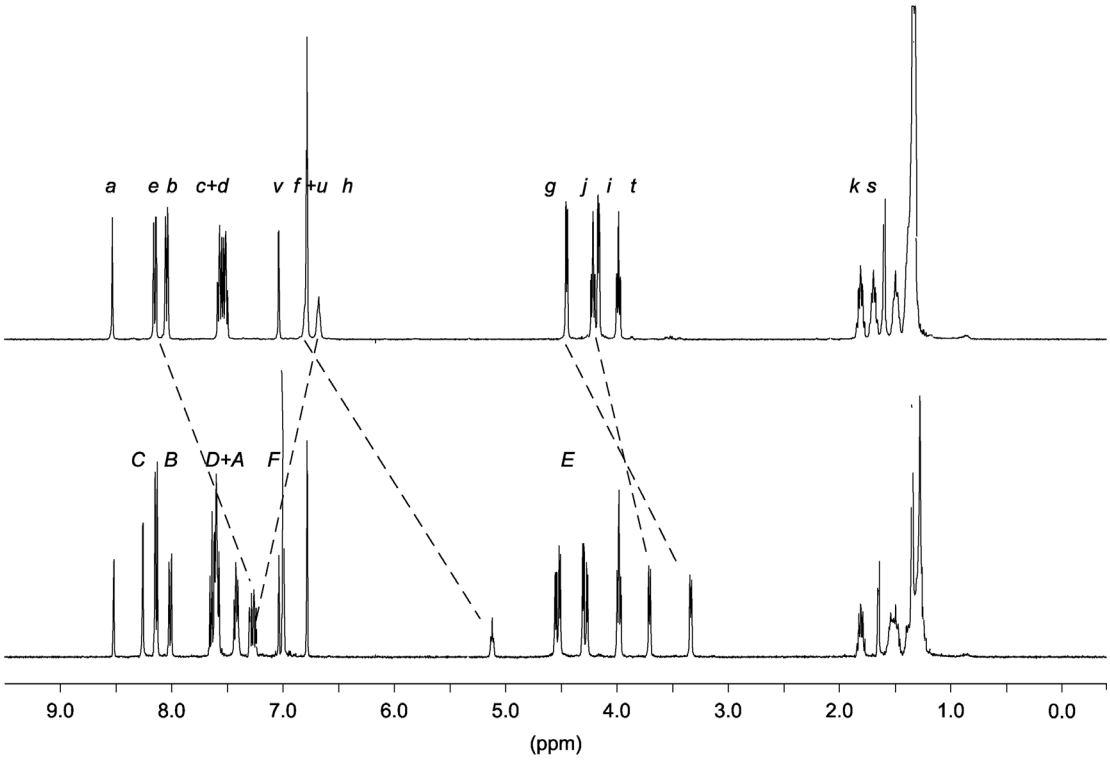


Figure 1. ^1H NMR of thread **2** (top) and rotaxane **1** (bottom) in $CDCl_3$ (298K)

The assignments of the two glycine methylene groups and two non-macrocycle amide protons in rotaxane **1** and thread **2** were made by ^1H gradient ROE experiments (GOESY – an accurate type of nOe experiment) of both glycine methylene groups (Figure 2). The individual amide protons were assigned by their $^3J_{HH}$ coupling to the methylene groups detected in COSY experiments.

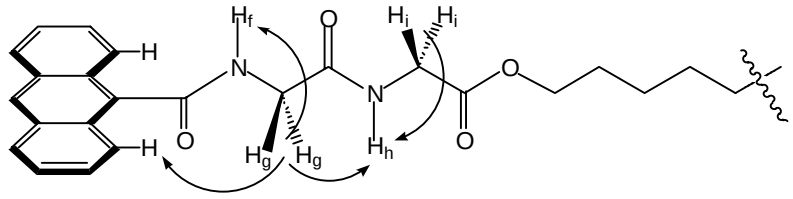


Figure 2. Intra-thread through-space close contacts in anthracene rotaxane **1** and thread **2** in (298K, 400 MHz) from ^{1}H NMR gradient ROE (GOESY) experiments. The rotaxane **1** also shows NOE contacts between the CH_2 groups g and i and the "inner" H-atoms of the macrocycle (C, D, F).

Following these assignments, the room temperature ^{1}H NMR spectrum of rotaxane **1** in $CDCl_3$ (Figure 1) provides a picture of the major way in which the macrocycle interacts with the peptide portion of the thread.

First, the resonances of the protons H_g and H_i in **1** are shifted to higher fields with respect to the same signals in **2**. This is similar to other glycylglycine rotaxanes (see refs 4, 5 and Clegg, W.; Gimenez-Saiz, C.; Leigh, D. A.; Murphy, A.; Slawin, A. M. Z.; Teat, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 4124-4129). From comparison with shielding effects in peptide rotaxanes where the positioning of the macrocycle is well established, the magnitude of the shift for the H_i methylene group suggests that the macrocycle spends a significant amount of time over the *C*-terminus glycine residue.

The two non-macrocycle NH protons are subjected to two different possible effects: deshielding as a consequence of H-bonding to the carbonyl groups of the macrocycle and shielding from the phenyl rings of the macrocycle. For NH_h in **1** we see a net deshielding of $\Delta\delta$=+0.59 ppm suggesting that the gly-gly amide group acts as a strong H-bond donor. The NH_f is shifted upfield ($\Delta\delta$= -1.78 ppm) which is consistent with the encapsulation of the amide group and heavy and efficient shielding from the macrocycle xylylene residues.

Together these results show clearly that the dominant structure (or structures) of **1** present in $CDCl_3$ solution has the macrocycle positioned over the peptide units. There may be several different H-bonding motifs present between macrocycle and thread but the anthracenoyl amide does not seem to be effective as an H-bond donor. This is consistent with preliminary molecular dynamics simulations, which indicate that hydrogen bonds predominantly involve the two carbonyls of the gly-gly fragment, and NH_h. It appears that

the anthracenoyl amide group is not easily accessible due to the presence of the bulky anthracene unit. The X-ray structure of a compound very similar to **1** (the rotaxane with an exopyridyl macrocycle shown on p.1 of this Supporting Information) shows that the dihedral angle between the anthracene chromophore and the carbonyl group in the rotaxane structure is close to 70°. Another feature found in the ^{1}H NMR spectra of **1** and **2**, is that the H_e protons in the anthracene stopper experience a shielding effect due to the proximity of the macrocycle. This is, again, consistent with an almost perpendicular arrangement of the anthracene unit and the corresponding carbonyl group. Interestingly, the MD simulations reproduce this feature, and show that hydrogen bonding to the anthracene amide strongly increases when the dihedral angle between the anthracene and the amide is constrained to small values.

Low temperature ^{1}H NMR experiments did not yield further useful information regarding the structure of **1** for reasons of its poor solubility at low temperatures.

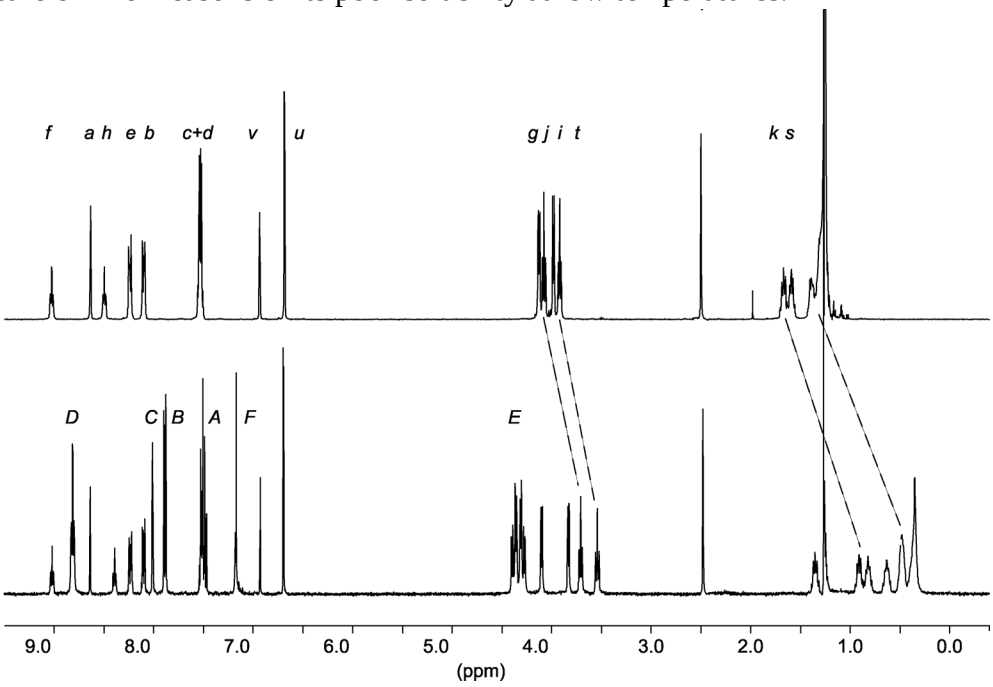


Figure 3. ^{1}H NMR of thread **2** (top) and rotaxane **1** (bottom) in d_6-DMSO (298K).

In d_6-DMSO (Figure 3), a hydrogen bond disrupting solvent, the H-bond pattern between thread and macrocycle is broken and the macrocycle moves to the aliphatic chain through a polarophobic effect. In this case, no significant shifts are observed for H_g and H_i and the NH resonances. However, the resonances for the aliphatic chain protons are shifted upfield because of the presence of the macrocycle.