Supporting information for Gatti *et al.* (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.0134757100

Supporting Text

Preparation of Thread *(E)-N,N'*- dimethyl-*bis*(2,2-diphenylethyl)-butendiamide (*E*-2).

To a stirred ice-cooled solution of *bis*-(2,2-diphenylethyl) fumaramide (1 g, 2.10 mmol) in dry tetrahydrofuran (20 ml) under nitrogen was added NaH (0.2 g, 60% dispersion in oil, excess) portion-wise under nitrogen. After the effervescence had subsided, methyl iodide (0.3 ml, excess) was added in one portion. The reaction was allowed to warm to room temperature and stirred overnight and water (20 ml) and ammonia solution (10 ml) were added drop-wise to quench the reaction. Most of the solvent was removed under reduced pressure, and the remainder was partitioned between water and CH_2Cl_2 (3 × 20 ml). The organic extracts were washed with sodium hydroxide (1 M, 20 ml) and dried over anhydrous magnesium sulfate. The filtered solution was concentrated under reduced pressure to give an oil that slowly solidified. Recrystallization from CH_2Cl_2/diisopropyl ether afforded colorless needles. Yield 0.87 g, 83%; m.p. 134-136°C; ^{1}H NMR (400 MHz, $C_2D_2Cl_4$ at 90 °C): δ= 2.79 (s, 6H, CH_3), 4.04 (d, J = 8.0 Hz, 4H, CH_2), 4.37 (t, J = 8.8 Hz, 2H, CH) 6.99 (s, 2H, CH = CH) 7.19-7.34 (m, 20H, ArH); ^{13}C NMR (100 MHz, $C_2D_2Cl_4$ at 120°C): δ= 37.5, 51.01, 55.52, 127.2, 128.9, 129.71, 132.14, 143.0, 166.70; MS (fast atom bombardment, mNBA): *m/z* (%) = 502 [$(M+H)^+$]; analysis calculated for $C_{34}H_{34}N_2O_2$ (502.65): C, 81.24; H, 6.82; N, 5.57%; found: C, 81.61; H, 6.68; N, 5.43%.

General Method for the Preparation of Benzylic Amide Macrocycle Fumaramide [2]Rotaxanes.

The threads *E*-**1-3** (1.00 mmol) and triethylamine (2.1 ml, 15.7 mmol) were dissolved in chloroform (stabilized with amylenes not ethanol, 100 ml) or, in the case of *E*-**3**, 1:9 acetonitrile/chloroform, and stirred vigorously while solutions of the diamine (1.09 g, 4 equiv.) in chloroform (45 ml) and the acid chloride (1.62 g, 4 equiv.) in

chloroform (45 ml) were simultaneously added over a period of 2 h by using motor-driven syringe pumps. After an additional 2 h the resulting suspension was filtered and concentrated under reduced pressure. The rotaxanes *E*-**4** and **5** were purified by trituration of the respective solids in dichloromethane (to remove the polar impurities - catenane, macrocycles, $Et_3HN^+Cl^-$, etc), and subsequently separating the rotaxane from unreacted thread through trituration in hot toluene. Rotaxane *E*-**6** was obtained by spontaneous crystallization from the reaction mixture as described(1). Selected data for ([2](1,7,14,20-tetraaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacosane)-((*E*)-*N*,*N*′-(dimethyl)-*bis*{2′,2′-diphenylethyl}-butendiamide)-rotaxane (*E*-**5**): yield 0.34 g (33%); m.p. 320-323°C; ^{1}H NMR (400 MHz, d_6-DMSO at 130°C): δ=2.41 (s, 6H, CH_3), 3.91 (bd, 4H, $C\underline{H}_2NCH_3$), 4.26 (bt, 2H, CH), 4.40 (bd, J = 5.4 Hz, 8H, CH_E), 5.92 (bs, 2H, CH=CH), 6.96 (s, 8H, ArH_F), 7.11-7.35 (m, 20H, ArH), 7.60 (t, J = 7.8 Hz, 2H, ArH_A), 7.78 (bt, 4H, NH_D), 8.09 (dd, J = 7.8, 1.8 Hz, ArH_B), 8.51 (bs, 2H, ArH_C); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 36.14-46.27, 44.09-44.61, 49.79, 53.10-53.9, 125.05, 127.63-129.27, 134.89, 138.18, 142.91, 165.99-166.99; fast atom bombardment-MS (*m*NBA matrix): *m/z* 1036 [$(M+H)^+$]; Analysis calculated for $C_{66}H_{62}N_6O_6$ (1035.24): C, 76.57 %; H, 6.04%; N, 8.12 %; found: C, 76.88 %; H, 6.20 %; N, 8.30 %.

Selected data for [2](1,7,14,20-tetraaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacosane)- benzyl 2-[[2-(benzyloxy)-2-oxoethyl]((*Z*)-5-(bis[2-(benzyloxy)-2-oxoethyl]amino})2,5-dioxo-3-pentenyl)amino]acetate-rotaxane (*Z*-**4**): yield 0.3 g (50%); m.p. 243-244°C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ = 3.58 (s, 4H, H_{a1}), 3.63 (s, 4H, H_{a2}), 4.36 (d, J = 5.1 Hz, 8H, H_E), 4.78 (s, 4H, H_{b1}), 4.83 (s, 2H, H_d), 4.89 (s, 4H, H_{b2}), 7.03 (s, 8H, ArH_F), 7.11-7.23 (m, 20H, ArH), 7.30 (t, J = 5.1 Hz, 4H, NH_D), 7.40 (t, J = 7.8 Hz, 2H, ArH_A), 7.60 (t, J = 1.7 Hz, 2H, ArH_C), 8.92 (dd, J = 1.7, 7.8 Hz, 4H, ArH_B); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 50.57, 50.94, 68.34, 68.79, 123.39, 128.58-129.92, 132.46, 134.36, 134.54, 131.96, 137.27, 166.23, 166.57, 167.63, 168.37; LSIMS, *m/z* = 1239 [$(rotaxane+H)^+$], 1262 [$(rotaxane+Na)^+$]. Analysis calculated for $C_{72}H_{66}N_6O_{14}$: C 69.78, H 5.37, N 6.78; found C 69.56, H 5.32, N.6.68.

Selected data for ([2](1,7,14,20-tetraaza-2,6,15,19-tetraoxo-,5,9,12,16,18,22,25-tetrabenzocyclohexacosane)-((*Z*)-*N*,*N*′-(dimethyl)-*bis*{2′,2′-diphenylethyl}-butendiamide)-rotaxane (*Z*-5): yield 0.28 g (47%); m.p. > 300 °C (decompose); ^{1}H NMR (400 MHz, $C_2D_2Cl_4$ at 130°C): δ= 2.21 (s, 6H, CH_3), 3.51 (bd, 4H, $C\underline{H}_2NCH_3$), 4.07 (bt, 2H, CH), 4.40 (bd, *J* = 5.4 Hz, 8H, CH_E), 4.92 (bs, 2H, CH = CH), 6.98 (s, 8H, ArH_F), 7.11-7.35 (m, 24H, ArH + NH_D), 7.63 (t, *J* = 7.8 Hz, 2H, ArH_A), 7.91 (bs, 2H, ArH_C), 8.13 (dd, *J* = 7.8, ArH_B); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 36.14-46.27, 44.09-44.61, 49.79, 53.10-53.9, 125.05, 127.63-129.27, 134.89, 138.18, 142.91, 165.99-166.99; fast atom bombardment-MS (*m*BNA matrix): *m/z* 1036 [$(M+H)^+$]; Analysis calculated for $C_{66}H_{62}N_6O_6$ (1035.24): C, 76.57 %; H, 6.04%; N, 8.12 %; found: C, 76.98 %; H, 6.30 %; N, 8.23 %.

Selected data for ([2](1,7,14,20-tetraaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacosane)-((*Z*)-*N*,*N*′-(dimethyl)-*bis*{2′,2′-diphenylethyl}-butendiamide)-rotaxane (*Z*-6): yield 0.27 (45%); m.p. >300 °C (decompose); ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.41 (d, 4H, $C\underline{H}_2NH$), 3.87 (t, 2H, CH), 4.38 (d, *J* = 5.4 Hz, 8H, CH_E), 5.11 (s, 2H, CH = CH), 6.83 (s, 8H, ArH_F), 6.98 (d, *J* = 7.5 Hz, 4H, ArH), 7.11-7.27 (m, 18H, ArH + NH), 7.62 (t, *J* = 7.8 Hz, 2H, ArH_A), 7.73 (t, *J* = 5.4 Hz, 4H, NH_D), 8.13 (s, 2H, ArH_C), 8.22 (d, *J*= 7.8, ArH_B); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 44.81, 44.9, 50.32, 124.8, 127.5, 128, 129.2, 129.3, 129.9, 131.2, 131.9, 134.3, 137.4, 141.8, 165.5, 166.8; MS (fast atom bombardment, mNBA): *m/z* = 1029 [$(rotaxane+Na)^+$]. Analysis calculated for $C_{64}H_{58}N_6O_6$: C 76.32, H 5.80, N 8.34; found: C 76.39, H 5.91, N 8.19.

X-Ray Crystallographic Structure Determinations.

***E*-5:** $C_{64}H_{58}N_6O_6$, *M* = 1039.22, crystal size 0.18 × 0.04 × 0.02 mm, triclinic P-1, *a* = 13.4337(13), *b* = 16.2778(16), *c* = 29.964(3) Å, α = 75.716(2), β = 87.934(2), γ = 71.880(2) °, *V* = 6028.9(10) $Å^3$, *Z* = 4, ρ_{calcd} = 1.145 $Mg•m^{-3}$; synchrotron radiation (Central Laboratory of the Research Councils Daresbury Laboratory Station 9.8, silicon monochromator, λ = 0.69290 Å), μ = 0.107 mm^{-1}, *T* = 150(2)K. 23,260 data (12,003 unique, R_{int} = 0.0466, 1.73<θ< 20.00°), were collected on a Siemens SMART CCD diffractometer by using narrow frames (0.3° in ω), and were corrected

semiempirically for absorption and incident beam decay (transmission 0.20-1.00). The structure was solved with SIR97 (2) and refined by full-matrix least-squares on F^2 values of all data (3) to give $wR = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2} = 0.2771$, conventional $R = 0.0952$ for F values of 12,003 reflections with $F_o^2 > 2\sigma F_o^2$), $S = 1.050$ for 1,480 parameters. Residual electron density extremes were 1.093 and –0.420 $Å^{-3}$.

***Z*-5:** $C_{66}H_{62}N_6O_6$, $M = 1035.22$, crystal size 0.30 × 0.14 × 0.08 mm, monoclinic, $P2_1/c$, $a = 10.5696(3)$, $b = 27.7157(9)$, $c = 10.7503(3)$ Å, $\beta = 115.2530(10)$, $V = 2848.27(15)$ $Å^3$, $Z = 2$, $\rho_{calcd} = 1.207$ Mg m^{-3}; MoK_a radiation (graphite monochromator, $\lambda = 0.71073$ Å), $\mu = 0.078$ mm^{-1}, $T = 293(2)$ K. 13,437 data (4,049 unique, $R_{int} = 0.1701$, $1.47 < \theta < 23.29°$), were collected on a Siemens SMART CCD diffractometer by using narrow frames (0.3° in ω), and were corrected semiempirically for absorption and incident beam decay (transmission 0.20-1.00). The structure was solved by direct methods and refined by full-matrix least-squares on F^2 values of all data (3) to give $wR = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2} = 0.2136$, conventional $R = 0.0811$ for F values of 4,049 reflections with $F_o^2 > 2\sigma F_o^2$), $S = 0.754$ for 361 parameters. Residual electron density extremes were 0.355 and -0.337 $Å^{-3}$. 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 nonhydrogen atoms.

Crystallographic data for ***E*-5** and ***Z*-5** (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-149672 and CCDC-149673 (***E*-5** and ***Z*-5**). Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44-1223-336-033; e-mail: teched@chemcrys.cam.ac.uk).

The Effect of Olefin Stereochemistry on Amide Bond Rotamerization in *E/Z*-4.

The energy barrier for amide bond rotamerization in tertiary amide rotaxanes increases if intercomponent hydrogen bonding occurs to stabilize the $R_2N^+ = C\text{-}O^-$

resonance contribution of the tertiary amide group (4) . In Fig. 2*a*, slow amide bond rotamerization is responsible for the magnetically distinct environments observed for H_{a1} and H_{a2} (and H_{b1}/H_{b2}). Even though the coalescence temperature for their interconversion cannot be reached in $C_2D_2Cl_4$, their exchange rate can be measured directly by Spin polarization transfer by selective inversion recovery and gives an energy barrier of 21.1 kcal•mol^{-1} at 383 K (compared to 17.2 kcal•mol^{-1} at 383 K for rotamerization in the trans thread, obtained by ^{1}H line shape analysis). In contrast, it is clear from the broadening of the H_{a1}/H_{a2} resonances in Fig. 2*b* that the same process is occurring with a lower energy barrier in the cis-rotaxane. Indeed, ^{13}C line shape analysis (^{1}H line shape analysis was not possible because the diastereotopic methylene protons in the cis thread are accidentally isochronous) experiments give the energy barrier of 20.0 kcal•mol^{-1} for *Z*-**4** at 383 K (compared to 17.4 kcal•mol^{-1} at 383 K for rotamerization of the cis thread).

1. Gatti, F. G., Leigh, D. A., Nepogodiev, S. A., Slawin, A. M. Z., Teat, S. J. & Wong, J. K. Y. (2001) *J. Am. Chem. Soc.* **123,** 5983–5989.

2. Altomare A., Burla M.C., Camalli M., Cascarano G.L., Giacovazzo C., Guagliardi A., Moliterni A.G.G., Polidori G. & Spagna R. (1999) *J. Appl. Crystallogr.* **32**, 115–119.

3. Sheldrick, G.M.Sheldrick (1994) SHELXTL *Manual* (Siemens Analytical X-Ray Instruments, Madison WI), Version 5.

4.Clegg, W., Gimenez-Saiz, C., Leigh, D. A., Murphy, A., Slawin, A. M. Z. & Teat, S. J. (1999) *J. Am. Chem. Soc.* **121**, 4124–4129.