DOI: 10.1002/chem.200500520

Configurational Stability of Bisindolylmaleimide Cyclophanes: From Conformers to the First Configurationally Stable, Atropisomeric Bisindolylmaleimides

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Abstract: The bisindolylmaleimides are selective protein kinase inhibitors that can adopt two limiting diastereomeric (*syn* and *anti*) conformations. The configurational stability of a range of substituted and macrocyclic bisindolylmaleimides was investigated by using appropriate techniques. With unconstrained bisindolylmaleimides, the size of the 2-indolyl substituents was found to affect configurational stability, though not sufficiently to allow atropisomeric bisindolylmaleimides to be obtained. However, with a tether between the two indole nitrogen atoms in place, the steric effect of 2-indolyl substituents was greatly exaggerated, leading to large differences in configurational stability. The rate of interconversion of the *syn* and *anti* conformers varied by over twenty orders of magnitude through substitution of a bisindolylmaleimide ring system, which was constrained within a macrocyclic ring. Indeed, the first examples of configurationally stable atropisomeric bisindolylmaleimides are reported; the half-life for epimerisation of these compounds at room temperature was estimated to be $>10^7$ years.

Keywords: atropisomerism · configurational stability · conformation analysis · macrocycles · protein kinases

Introduction

The indolocarbazole alkaloids, such as staurosporine (**1**), K252a (**2**) and rebeccamycin (**3**), are potent (typically sub-10 nM) inhibitors of a broad range of protein kinases.[1] X-ray crystal structures of protein kinase–staurosporine complexes reveal that, in each case, staurosporine is bound in an ATP-binding pocket of the kinase, and that its lactam mimics the adenine ring of ATP.[2] Despite their widespread activity, the indolocarbazoles have been useful tools in the discovery of selective kinase inhibitors. A fruitful strategy has been to disrupt the planarity of the indolocarbazole ring system to yield either bisindolylmaleimides[3] (e.g., **4**) or dianilinophthalimides[4] (e.g., **5**). The selectivity and potency of some inhibitors of therapeutically important kinases has been refined by the formation of macrocyclic analogues of **4**; for example, the bisindolylmaleimide LY333531 (**6**) selectively inhibits[5] the β isoforms of protein kinase C (PKCβ) ($IC_{50}=4.7$ nM for PKCβI and 5.9 nM for PKCβII), and the macrocyclic analogues **7** target glycogen synthase kinase-3β (GSK-3β) (with up to $IC_{50}=22$ nM).[6] PKCβ is selectively activated by elevated glucose in many vascular tissues, and the bisindolylmaleimide **6** can produce significant improvements in diabetic retinopathy, nephropathy, neuropathy and cardiac dysfunction.[7] Indeed, the bisindolylmaleimide **6** is undergoing phase III clinical trials as a therapeutic agent for preventing diabetes complications (such as diabetic retinopathy) and left ventricular hypertrophy in heart failure.[8] The mechanism of action of bisindolylmaleimides has been revealed recently in molecular detail: structures of the complexes of **10** with protein kinase A (PKA)[9] and those of **6**, **8**, **9a**, **9b** and **10** with 3-phosphoinositide-dependent protein kinase-1 (PDK-1)[10] have been determined.

Bisindolylmaleimides **11** are not planar molecules, and a 30–40° angle between the planes of the maleimide and each indole ring is typical.[11] Consequently, for simple bisindolylmaleimides **11** bearing achiral substituents R, R′ and R″, two diastereomeric (*syn* and *anti*) conformers are possible.

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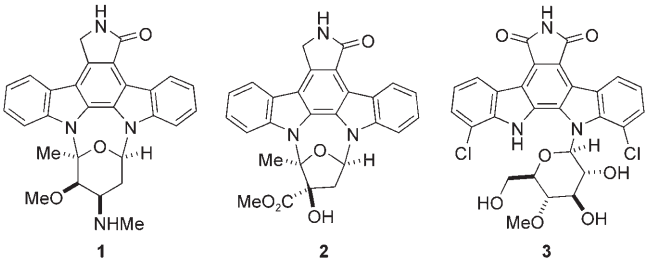
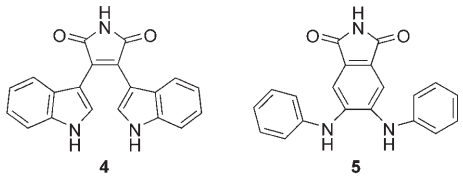
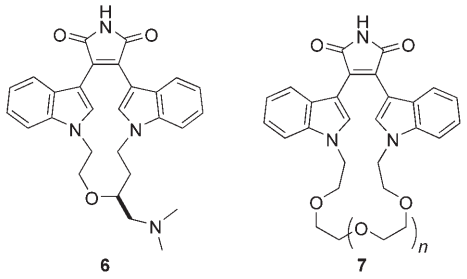
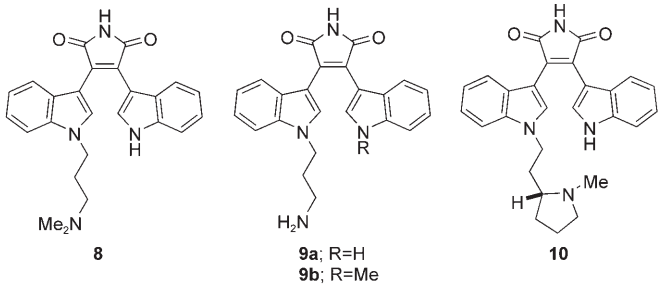


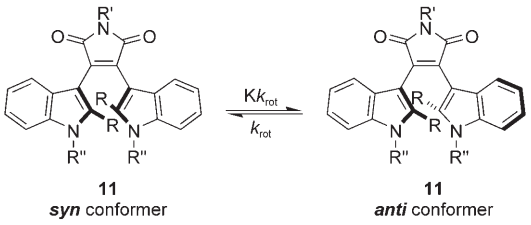




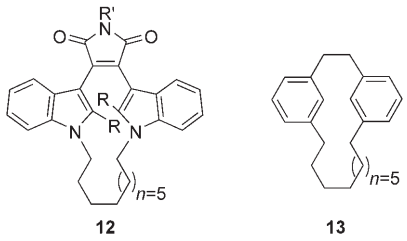


Rotation about either of the indolylmaleimide bonds results in interconversion between the conformers. We have shown that simple bisindolylmaleimides **11** with R = H and Me are not, in general, atropisomeric at ambient temperatures (the half-lives for epimerisation are much less than an arbitrary 1000 s[12]).[13]

Macrocyclic bisindolylmaleimides **12** are structurally related to [2.n]metacyclophanes (such as **13**), which may also populate two diastereomeric conformations. The configurational stability of cyclophanes is rather sensitive to substitution and the size of the macrocyclic ring: the transition state for isomerisation of a cyclophane is destabilised by steric effects, and configurational stability increases (a) as the size of the substituent(s) passed through the larger ring increases

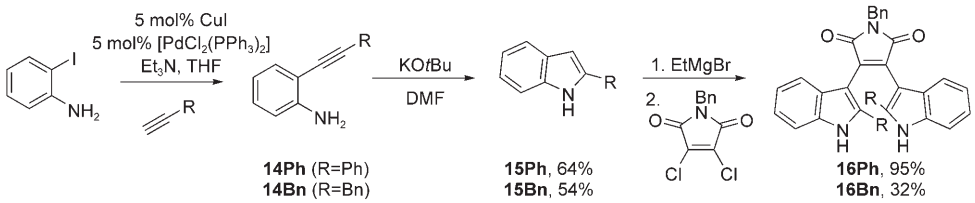


and (b) as the size of the macrocycle decreases.[14] In this paper, we present our investigation of the configurational stability of simple (**11**) and macrocyclic (**12**) bisindolylmaleimides, and we conclude that atropisomeric bisindolylmaleimides **12** may be prepared, provided that the 2-indolyl substituents, R, are sufficiently large.



Results

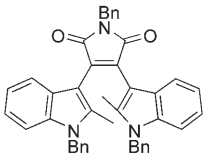
Synthesis of simple bisindolylmaleimides: The preparation of simple bisindolylmaleimides with R = H and Me has been described previously.[15] The bisindolylmaleimides **16Ph** and **16Bn** (R = Ph and Bn) were prepared in an analogous manner (Scheme 1). Hence, base-catalysed cyclisation of the 2-alkynyl anilines **14**, prepared by substitution of 2-iodo aniline, gave the indoles **15**.[16] Treatment of the indoles **15** with ethyl magnesium bromide, and reaction with *N*-benzyl 3,4-dichloromaleimide, gave the bisindolylmaleimides **16**.[15]



Scheme 1. Preparation of simple bisindolylmaleimides.

Variable temperature (VT) NMR experiments on simple bisindolylmaleimides: We investigated the interconversion of the simple bisindolylmaleimides **16Ph**, **16Bn** and **17Me** by using variable temperature 500 MHz ^{1}H NMR spectroscopy. In each case, the choice of solvent was dictated by the temperature range for which intermediate exchange was observed, and also by the solubility of each compound. The *syn* and *anti* conformers of these bisindolylmaleimides inter-

convert slowly enough at temperatures below approximately 273 K for them to give rise to two sets of discrete signals in their 500 MHz ^{1}H NMR spectra. The signals corresponding to the *syn* and *anti* conformers were assigned by careful inspection of the region of the spectrum corresponding to the benzyl substituent on the imide: the benzylic protons are enantiotopic in the *syn* conformer (H^A and $H^{A'}$) and give rise to a singlet, and are diastereotopic in the *anti* conformer (H^B and H^C) and give rise to a pair of doublets (see Figure 1). The relative populations of the *syn* and *anti* conformers were determined as a function of temperature by integration of the 500 MHz ^{1}H NMR spectrum over a range of 40 K in the slow exchange regime (see Table 1).



17Me

The barrier to interconversion between the *syn* and *anti* conformers was determined by analysis of 500 MHz ^{1}H NMR spectra recorded over a temperature range (of between 20 and 40 K) in the intermediate exchange regime (see Table 2).[17] The rate of equilibration, and hence k_{rot}, was estimated by comparison of the experimental spectra with the simulated spectra generated by gNMR analysis[18] using populations extrapolated from the slow exchange regime. Kinetic data for the isomerisation of the *anti* conformers of **16Ph**, **16Bn** and **17Me** is summarised in Table 3.

Table 1. Populations of the *syn* and *anti* conformers of the bisindolylmaleimides.

Compound	R	Solvent, T [K]	*syn* [%][a]	*anti* [%][a]	$\Delta H°$ [kJ mol^{-1}][b]	$\Delta S°$ [J mol^{-1} K^{-1}][b]	$\Delta G°$ [kJ mol^{-1}][b]
16Ph	Ph	CD_2Cl_2, 243	41	59	−7.9 ±1.0	−29±4	0.8±0.2
16Bn	Bn	CD_2Cl_2, 253	39	61	2.7±0.5	14±2	1.6±0.3
17Me	Me	[D_8]toluene, 298	65	35	–[c]	–[c]	1.4±0.2[d]
19Me (n=9)	Me	[D_6]DMSO, 300	57	43	5.0±1.5	14.2±3.0	0.8±0.1
19Me (n=10)	Me	[D_6]DMSO, 300	35	65	4.4±0.5	20±2	−1.5±0.1

[a] At temperature T, determined by integration of the 500 MHz ^{1}H NMR spectrum. [b] At 298 K, determined by extrapolation of the relative populations of the slowly exchanging conformers. [c] Not determined. [d] Determined by integration of the 500 MHz ^{1}H NMR spectrum recorded at 298 K.

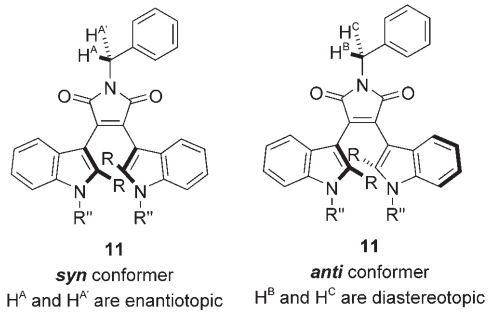


Figure 1. Enantiotopic and diastereotopic benzylic protons in the bisindolylmaleimides **11**.

Table 2. Results of VT-NMR studies on the bisindolylmaleimides.

Compound	R	Solvent	T range[a] [K]	K[b]	k_{rot} [s^{-1}][b]	$\Delta G^{\ddagger}$ [kJ mol^{-1}][c]
16Ph	Ph	CD_2Cl_2	253–283	0.71±0.10	170±30	60.3±0.5
16Bn	Bn	CD_2Cl_2	253–291	1.90±0.05	120±30	61.1±0.3
17Me	Me	[D_8]toluene	263–343	0.57±0.05	1600±200	54.8±0.3
19Me (n=9)	Me	[D_6]DMSO	323–373	0.72±0.05	0.58±0.1	74.3±0.3
19Me (n=10)	Me	[D_6]DMSO	300–373	1.8±0.2	2.0±0.3	70.3±0.3

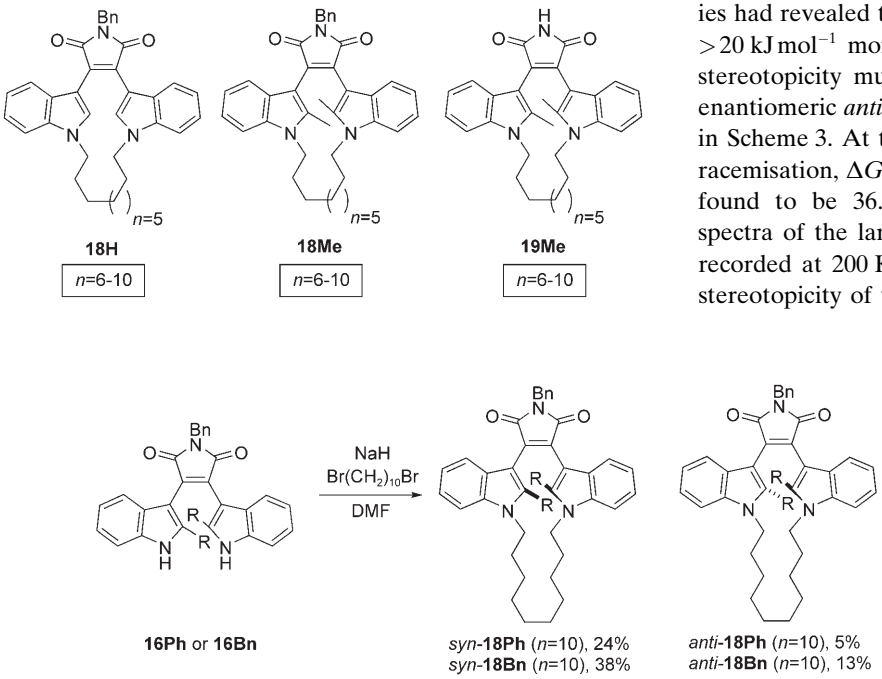
[a] Temperature range of VT-NMR experiments. [b] At 298 K. [c] For conversion of the *anti* conformer into the *syn* conformer, extrapolated to 298 K.

Synthesis of macrocyclic bisindolylmaleimides: The syntheses of the macrocyclic bisindolylmaleimides **18H**, **18Me**, **19H** and **19Me** (n=6–10) have been described previously.[15] The bisindolylmaleimides **16Ph** and **16Bn** were treated with sodium hydride in DMF, and the resulting anions were reacted with 1,10-dibromodecane to give the corresponding macrocyclic bisindolylmaleimides **18Ph** (n=10) and **18Bn** (n=10) (Scheme 2). The mac-

Table 3. Kinetic data for the epimerisation of the bisindolylmaleimides.

Compound	R	Solvent	Method	k_{rot} [s^{-1}][a]	$\Delta G^{\ddagger}$ [kJ mol^{-1}][b]	$t_{1/2}$[c]
16Ph	Ph	CD_2Cl_2	VT-NMR	170±30	60.3±0.5	4.1±0.3 ms
16Bn	Bn	CD_2Cl_2	VT-NMR	120±30	61.1±0.3	5.8±0.5 ms
17Me	Me	[D_8]toluene	VT-NMR	1600±200	54.8±0.3	0.43±0.05 ms
18H (n=6)	H	CD_2Cl_2	VT-NMR	1.1×10^{6}[e]	36.6±0.3[d]	0.3 μs[e]
18Me (n=6)	H	hexane-*i*PrOH	chiral HPLC	$<1\times10^{-3}$	>90	>10 min[f]
18Me (n=8)	H	hexane-*i*PrOH	chiral HPLC	$<1\times10^{-3}$	>90	>10 min[f]
19Me (n=9)	Me	[D_6]DMSO	VT-NMR	0.58±0.1	74.3±0.3	1.2±0.1 s
19Me (n=10)	Me	[D_6]DMSO	VT-NMR	2.9±0.3	70.3±0.3	0.23±0.03 s
18Ph (n=10)	Ph	[D_6]DMSO	NMR[g]	$<1\times10^{-17}$[e]	>160	$>10^7$ yr[e]
18Bn (n=10)	Bn	[D_6]DMSO	NMR[g]	$<1\times10^{-17}$[e]	>160	$>10^7$ yr[e]

[a] At 298 K. [b] For the conversion of the *anti* conformer into the *syn* conformer at 298 K. [c] Estimated half-life for the epimerisation of the *anti* conformer at 298 K. [d] $\Delta G^{\ddagger}$ is given at the coalescence temperature (203 K). [e] Estimated value, on the assumption that $\Delta S^{\ddagger}$ is small. [f] The enantiomeric *anti* conformers could be separated by chiral analytical HPLC. [g] Epimerisation did not occur when the *syn* and *anti* atropisomers were heated at 433 K for five days.



Scheme 2. Synthesis of atropisomeric, macrocyclic bisindolylmaleimides.

rocyclic bisindolylmaleimides were separable atropisomers whose relative configuration could be assigned by careful analysis of their 500 MHz ^{1}H NMR spectra: the benzylic protons give rise to a singlet in the *syn* atropisomer, and to a pair of doublets in the *anti* atropisomer (see Figures 1 and 2). In addition, the relative configuration of the bisindolylmaleimide *syn*-**18Ph** was determined by X-ray crystallography (Figure 3).

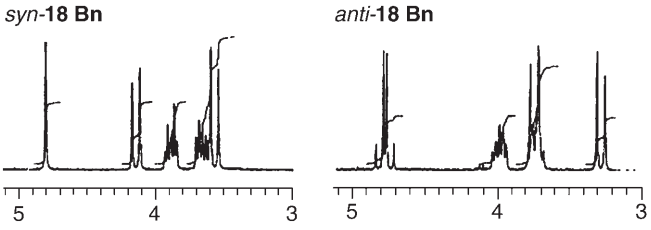


Figure 2. Partial 500 MHz ^{1}H NMR spectra of the bisindolylmaleimides *syn*- and *anti*-**18Bn**. The signal(s) corresponding to the benzylic protons on the imide nitrogen are at approximately 4.8 ppm in each case.

Investigation into the configurational stability of macrocyclic bisindolylmaleimides: As a starting point, we investigated the configurational stability of macrocyclic bisindolylmaleimides **18H**. The *syn* and *anti* conformations of the macrocycles **18H** were in fast exchange on the NMR timescale at 298 K. However, at low temperature, the region corresponding to the tether of **18H** ($n=6$) in its 500 MHz ^{1}H NMR spectrum broadened dramatically: below the coalescence temperature, 203 K, the methylene protons adjacent to indole nitrogens (NCH_AH_B) were rendered diastereotopic on the NMR timescale. Because molecular modelling studies had revealed that the *anti* conformer of **18H** ($n=6$) was >20 kJ mol^{-1} more stable that the *syn* conformer, the diastereotopicity must stem from slow interconversion of the enantiomeric *anti* conformers. This hypothesis is summarised in Scheme 3. At the coalescence temperature, the barrier to racemisation, $\Delta G^{\neq}$, of the *anti* conformer of **18H** ($n=6$) was found to be 36.6 ± 0.3 kJ mol^{-1} (see Table 3). The NMR spectra of the larger macrocycles **18H** ($n=7$–10) were also recorded at 200 K; however, for these compounds, the diastereotopicity of the protons in the tether was not revealed, suggesting that conformational interconversion in these cases was faster.

The barrier to conformational interconversion increased as the size of the 2-indolyl substituent increased. The *syn* and *anti* conformers of the macrocycles **19Me** ($n=9$ and 10) interconvert slowly enough in [D$_6$]DMSO at around, and just above, room temperature to give rise to two sets of discrete signals in their 500 MHz

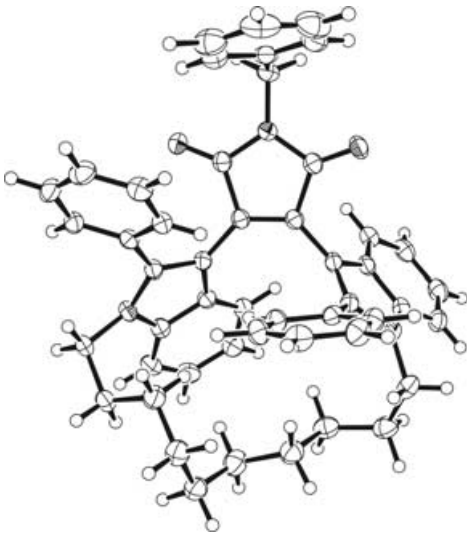
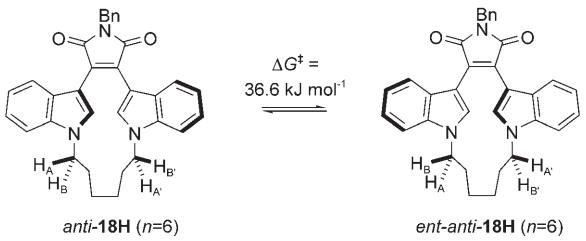


Figure 3. X-ray crystal structure of the bisindolylmaleimide *syn*-**18Ph**.



Scheme 3. Exchange of H^A and H^B by racemisation of the *anti* conformer of **18H**.

^{1}H NMR spectra (Figure 4). The *syn* and *anti* conformers were assigned, and the relative populations of the conformers were determined as a function of temperature in the slow exchange regime (see Table 1). Analysis of the NMR spectra that had been recorded over a range of higher temperatures, and were, therefore, dramatically broadened, allowed k_{rot} and $\Delta G^{\ddagger}$ to be extracted (Tables 2 and 3). The barriers, $\Delta G^{\ddagger}$, to isomerisation of the *anti* conformers of **19Me** ($n=10$) and **19Me** ($n=9$) were 70.3 ± 0.3 and 74.3 ± 0.3 kJ mol^{-1}, respectively (see Table 3).

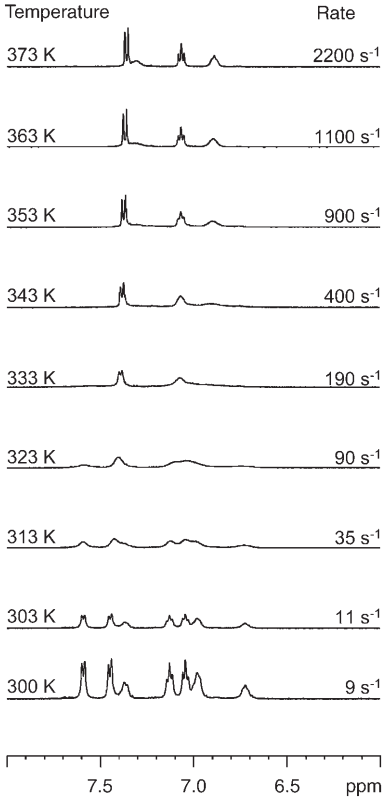


Figure 4. Variable temperature 500 MHz ^{1}H NMR spectra of the bisindolylmaleimide **19Me** ($n=10$) recorded in [D$_6$]DMSO. The rate of equilibration at each temperature, estimated by comparison of experimental and simulated spectra, is indicated.

The further reduction in the size of the macrocyclic ring had a remarkable effect on the barrier to conformational interconversion. The 500 MHz ^{1}H NMR spectra of **18Me** ($n=$ 6–8) in [D$_8$]toluene revealed that only the chiral, *anti* conformer was populated at 298 K. In this case, the barrier to *anti*→*syn* isomerisation, $\Delta G^{\ddagger}$, may be assessed indirectly by tracking the racemisation of the *anti* conformer (for which the *syn* conformer is a presumed intermediate). The benzylic protons were diastereotopic on the NMR timescale, and were used as a handle to assess the rate of racemisation. The signals corresponding to the benzylic protons did not coalesce as the sample was heated to 373 K. Interconversion between the enantiomeric *anti* conformers of **18Me** ($n=6$–8) was, therefore, sufficiently slow that its rate could not be determined by using variable temperature NMR spectroscopy. Indeed, analysis of the macrocycles **18Me** ($n=6$) and **18Me** ($n=8$) by performing chiral analytical HPLC revealed two peaks in each case, demonstrating that the half-lives of the enantiomeric *anti* conformers at 298 K were greater than the separation of the peaks (>10 min).[19] The barrier to racemisation of the *anti* conformer of **18Me** ($n=6$ and 8) was concluded to be at least 90 kJ mol^{-1}.

The arbitrary boundary[12] between conformational isomers and atropisomers was crossed by further increasing the size of the 2-indolyl substituents. Although we had been able to separate the *syn* and *anti* atropisomers of **18Ph** ($n=$ 10) and **18Bn** ($n=10$) by preparative HPLC, the effect of the larger 2-indoyl groups was remarkable. The *anti* and *syn* atropisomers of **18Ph** ($n=10$) and **18Bn** ($n=10$) were heated at 433 K for five days in [D$_6$]DMSO, and the samples were reanalysed by performing 500 MHz ^{1}H NMR spectroscopy. There was no evidence for epimerisation[20] of either of the atropisomers, indicating half-lives of at least one month at 433 K, and, hence, estimated half-lives of at least 10^7 years at 298 K. A lower limit for the barrier, $\Delta G^{\ddagger}$, to conformational isomerisation of **18Ph** ($n=10$) and **18Bn** ($n=10$) is 160 kJ mol^{-1}.

Evidence for "rocking" between unsymmetrical *syn* and *anti* conformers: Perfectly symmetrical conformers of the bisindoylmaleimides described in this paper would have identical indole rings: a C_2-symmetrical *anti* conformer has homotopic indoles, and a C_s-symmetrical *syn* conformer has enantiotopic indoles. The 500 MHz ^{1}H NMR spectra obtained for the bisindolylmaleimides **16Bn** and **19Me** ($n=$ 10) that had been cooled to around 250 K had two sets of discrete signals that corresponded to the slowly interconverting *syn* and *anti* conformers. For each conformer, there was only one set of resonances for each pair of indole rings. The conformers were either symmetrical, or were still interconverting rapidly on the NMR timescale.

Cooling the samples further resulted in the dramatic broadening of some signals: all of the indolyl signals of **16Bn**, and the indolyl signals corresponding to the *syn* conformer of **19Me** ($n=10$), were broadened. We conclude that this line broadening demonstrates that some of the conformers are, in fact, unsymmetrical; interconversion between these unsymmetrical conformers had simply been fast enough at higher temperatures (>ca. 250 K) for the indole rings to *appear* to be identical.

We have previously shown that the *syn* conformers of bisindolylmaleimides are generally unsymmetrical, a phenomenon that is also apparent in the X-ray crystal structure of *syn*-**18Ph** ($n=10$) (Figure 3). The "rocking" between unsymmetrical *syn* conformers is illustrated in Figure 5; this process does not require the 2-indolyl substituent (R=Ph) to eclipse the maleimide C=C bond, and so the associated activation barrier is rather low. The activation barrier could not be estimated because the slow exchange regime was inaccessible, and the separation of the peaks, $\delta\nu$, could not, therefore, be determined.[17,18] The effect of the "rocking" of the *syn* conformer of **18Me** ($n=10$) impacts dramatically on the

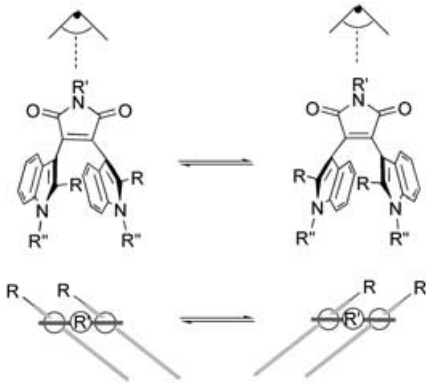
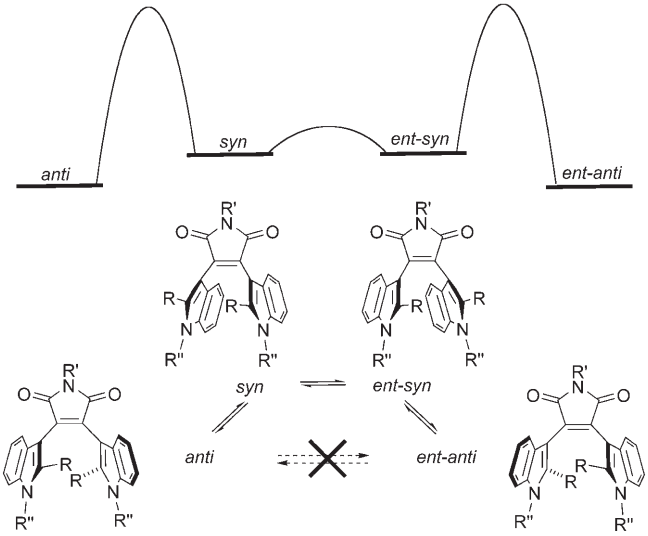


Figure 5. "Rocking" process between unsymmetrical syn conformers.

indole region of its 500 MHz ^{1}H NMR spectrum. On cooling from 243 K to 203 K, the indole rings in the *syn* conformer become inequivalent as the "rocking" process becomes comparable with the NMR timescale, and its NMR resonances broaden accordingly.

Discussion

The proposed mechanism for the racemisation of a symmetrical *anti* bisindolylmaleimide involves the stepwise rotation about the two indole–maleimide bonds (Scheme 4). Rotation about one of the inter-ring bonds of an *anti* bisindolylmaleimide gives an unsymmetrical *syn* bisindolylmaleimide that is able to equilibrate easily with the enantiomeric conformer (*ent-syn*) (see the "rocking" process described in Figure 5). The racemisation process is completed by rotation about the other inter-ring bond of the *ent-syn* conformation to give the enantiomeric *anti* bisindolylmaleimide conformer (*ent-anti*). The variable temperature NMR studies did not produce any evidence for correlated[21] rotation about the two inter-ring bonds (i.e., direct isomerisation between the enantiomeric *anti* conformers). The conformation of bisindolylmaleimides may be described in terms of the dihedral angles, θ and φ, which are defined in Figure 6. A description of the racemisation process in terms of the "traverse" of a Ramachandran-like plot is illustrated in Figure 7.



Scheme 4. A mechanism for racemisation of *anti* bisindolylmaleimides.

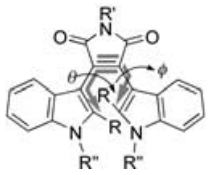


Figure 6. Definitions of the dihedral angles θ and φ.

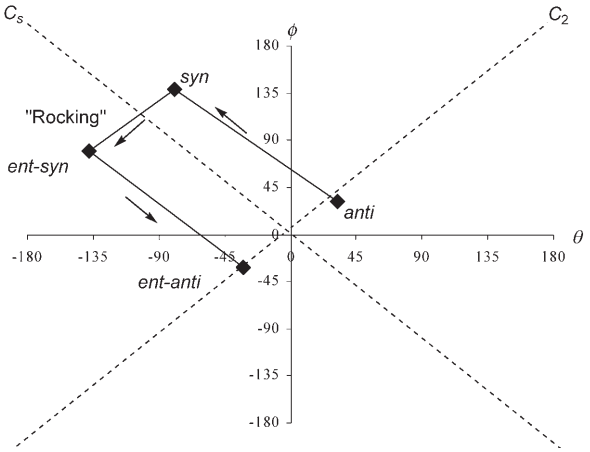
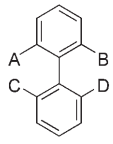


Figure 7. Traversing the Ramachandran plot during the racemisation of a symmetrical, *anti* bisindolylmaleimide.

The *anti*→*syn* isomerisation of simple bisindolylmaleimides is significantly easier than rotation around the inter-aryl bond of a similarly substituted biphenyl. The difficulty in racemisation of tetrasubstituted biphenyls **20** (with A≠B≠H and C≠D≠H) is generally sufficient to prevent resolution.[22] There is a substituent in each of the *ortho* positions flanking the [3,3′]bipyrrolyl bonds of **16Ph**, **16Bn** and **17Me** (see Figure 8, which highlights the bipyrrolyl unit), and yet, their *syn* and *anti* conformers are far from being atropisomeric.



20

The relatively low activation barrier may stem partly from the conjugation between a maleimide ring and an indole ring in the transition state, which may be more stabilising than the conjugation between two phenyl rings. However, the sizes of the substituents and the geometry of the [3,3′]bipyrrolyl ring system may also be important. The small size of the maleimide oxo group is rivalled only by a hydrogen atom (compare the length of the carbonyl moiety, 1.21 Å, with the lengths of other bonds to "small"[22] substituents: 1.39 Å for Ph–F, 1.45 Å for Ph–OH). Nevertheless, increas-

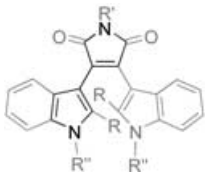


Figure 8. The [3,3′]-bipyrrolyl unit.

ing the size of the 2-indolyl R group has a remarkably small effect on the activation barriers to bond rotation: with **16Bn**, **16Ph** and **17Me**, R varies from Bn to Ph to Me, and yet, the activation barriers ($\Delta G^{\ddagger}$) vary from only 61.1 to 60.3 to 54.8 kJ mol^{-1}. The variable temperature NMR experiments were conducted in CD_2Cl_2 (for **16Bn** and **16Ph**) and in [D_8]toluene (for **17Me**). However, the rates of rotation about a single bond between sp^2 centres, for which no change in hybridisation in the transition state occurs, generally vary by less than ten-fold;[23,24] the lower barrier to epimerisation of **17Me**, though not great, is, therefore, likely to depend largely on the smaller size of the methyl substituents. Nevertheless, the impact of the size of these groups on the activation barrier is relatively small, and may stem from the internal bond angles of a bisindolylmaleimide[11] (107 and 108° in the crystal structure of **16H**), which are markedly smaller than those of a biphenyl[25] (119°). The carbon atoms that are *ortho* to the bipyrrolyl bond are, therefore, significantly further apart (2.96 and 3.30 Å[11]) than for biphenyl (2.92 Å), which may reduce steric effects in the transition state.

Tethering the bisindolylmaleimide in a macrocyclic ring—as in **18** and **19**—had a large impact on configurational stability. The presence of the tether greatly exaggerates steric effects in the transition state for conformational interconversion. Decreasing the length of the tether from ten to eight methylene groups had a large effect on configurational stability: with a indolyl 2-methyl substituent, as in **18Me** or **19Me**, the conformational half-life varied from 200 ms (with $n=10$) to >10 min (with $n=8$). More dramatically, however, the presence of a tether greatly exaggerated the effect of 2-indolyl substitution. Changing the 2-indolyl substituents from hydrogen to methyl to phenyl increased configurational stability by over twenty orders of magnitude: from conformers that interconverted on the microsecond timescale to configurationally stable atropisomers with half-lives of greater than 10^7 years!

Conclusion

With unconstrained bisindolylmaleimides, the size of the 2-indolyl substituents does affect configurational stability, but not sufficiently to allow atropisomeric bisindolylmaleimides to be obtained. However, with a tether in place, as in **18** and **19**, the steric effect of 2-indolyl substituents is greatly exaggerated, leading to large differences in configurational stability (Figure 9). The rate of interconversion of the *syn* and *anti* conformers may be varied by over twenty orders of magnitude through substitution of a bisindolylmaleimide ring system, which is constrained within a macrocyclic ring. Indeed, the macrocycles **18Ph** ($n=10$) and **18Bn** ($n=10$) are the first examples of configurationally stable atropisomeric bisindolylmaleimides.

Experimental Section

Preparative LC-MS was conducted by using a Waters 2525 binary gradient pump and detection with a Micromass ZQ mass spectrometer; an XTerra® preparative HPLC column (19×50 mm) was used. Preparative HPLC was generally conducted by using a Gilson HPLC machine and a gradient of 90→95% MeCN in H_2O over 30 min, detecting at 200 nm on a Thermohypersil 250×21.2 mm, 8 μ, Hyperprep® HS C18 column. The configurational stability of the bisindolylmaleimides was assessed by performing analytical HPLC by using an Ultron chiral column (150×4.6 mm ES-OVM). The bisindolylmaleimides **16**[15a] were prepared from the corresponding commercially available or known[16,26] indoles by the following general procedure: Ethyl magnesium bromide (300 μL of a 3 M solution in ether, 1.0 mmol) was added dropwise to a solution of the indole **15** (200 mg, 1.0 mmol) in THF (0.9 mL) and toluene (2.5 mL). The solution was heated to 50 °C for 1 h and 2,3-dichloro-*N*-benzylmaleimide (89 mg, 0.4 mmol) in toluene (1.9 mL) was added dropwise. The reaction mixture was heated to reflux for 54 h, cooled and quenched by addition of saturated aqueous ammonium chloride solution (8 mL) and stirred for 30 min. Water (30 mL) was added and the resulting precipitate was filtered, washed with water (3×10 mL) and dried under vacuum to afford the required bisindolylmaleimide.

1-Benzyl-3,4-bis-(2-phenyl-1*H*-indol-3-yl)-pyrrole-2,5-dione (16Ph): The crude precipitate was washed with water (3×10 mL) to give the bisindolylmaleimide **16Ph** (190 mg, 95%) as deep red plates. M.p. 137–139 °C; $R_f=0.30$ (1:1 diethyl ether/petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.10$ (br s, 2H; NH), 7.40–7.29 (m, 8H; indolyl 4-H, *p*-Ph, *m*-

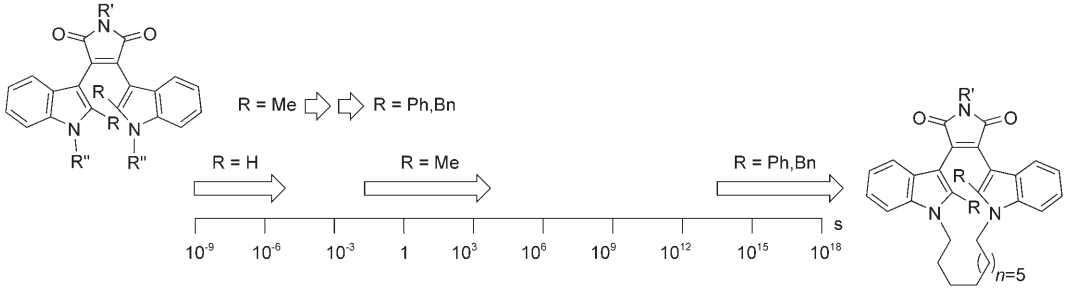


Figure 9. Typical half-lives of the *anti* conformers of bisindolylmaleimides at 298 K.

Bn, *o*-Bn), 7.18–7.08 (m, 9H; indolyl 5-H, indolyl 7-H, *p*-Bn, *m*-Ph), 6.98–6.85, (m, 6H; indolyl 6-H, *o*-Ph), 4.76 ppm (s, 2H; CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 137.8, 132.9, 128.9, 128.4, 128.3, 127.8, 123.1, 120.8, 111.3, 104.3, 42.0 ppm (eight carbon signals missing or overlapped); IR (film): $\tilde{\nu}$ = 3343, 2962, 2924, 2853, 1697 cm^{-1}; MS (ES): *m*/*z* (%): 570 (100) [M^++Na]; HRMS: *m*/*z* calcd for $C_{39}H_{28}N_3O_2$ [M^++Na]: 570.2182; found: 570.2178.

1-Benzyl-3,4-bis-(2-benzyl-1*H*-indol-3-yl)-pyrrole-2,5-dione (16Bn): The crude product was purified by conducting flash chromatography, eluting with 1:1 diethyl ether/petroleum ether and then EtOAc, to yield the bisindolylmaleimide **16Bn** (1.37 g, 32%) as red/orange prisms. M.p. 270–272 °C (from EtOAc); R_f = 0.32 (1:1 diethyl ether/petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (s, 2H; NH), 7.55 (d, *J* = 7.2 Hz, 2H; indolyl 4-H), 7.42–6.70 (m, 21H; aromatic), 4.91 (s, 2H; CH_2), 3.83 ppm (br s, 4H; 2PhCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.2, 139.2, 137.4, 137.1, 135.5, 131.7, 129.2, 128.9, 128.8, 128.7, 127.7, 126.9, 126.7, 122.2, 120.5, 110.7, 104.5, 42.0, 34.1 ppm (one carbon signal missing or overlapped); IR (film): $\tilde{\nu}$ = 3375, 3060, 1692, 1455, 1431, 1398, 741 cm^{-1}; MS (ES): *m*/*z* (%): 598.0 (100) [M^++H]; HRMS: *m*/*z* calcd for $C_{41}H_{31}N_3O_2$ [M^++H]: 598.2495; found: 598.2509.

23-Benzyl-26,27-diphenyl-6,7,8,9,10,11,12,13,14,15-decahydro-22*H*-5,25:16,21-dimethenodibenzo[*b*,*h*]pyrrolo[3,4-*e*,1,10]diazacycloicosine-22,24-dione *anti*- and *syn*-18Ph: Sodium hydride (60% dispersion in mineral oil, 32 mg, 0.8 mmol) was added portionwise to a solution of the bisindolylmaleimide (**16Ph**) (150 mg, 0.3 mmol) in DMF (12 mL) at 0 °C, and the mixture was stirred at room temperature for 15 min. 1,10-Dibromodecane (60 μL, 0.3 mmol) was added, the reaction mixture was stirred for 6 h, then quenched by the addition of water (100 mL). Ethyl acetate (150 mL) was added, the organic layers were washed with water (2× 100 mL), dried ($MgSO_4$) and evaporated under reduced pressure. Purification was achieved by conducting flash column chromatography (gradient elution 20:80→35:65 diethyl ether/petroleum ether) to yield the macrocycle *syn*-**18Ph** (45 mg, 24%) as red cubes. M.p. 304–306 °C (from DMSO); R_f = 0.2 (1:3 diethyl ether/petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.30 (m, 4H; indolyl 4-H, *p*-Ph), 7.29–7.19 (m, 9H; *m*-Ph, *o*-Bn, *m*-Bn, *p*-Bn), 7.17–7.10 (m, 4H; indolyl 5-H, indolyl 7-H), 6.92–6.86 (m, 6H; indolyl 6-H, *o*-Ph), 4.59 (s, 2H; CH_2Bn), 4.16 (dt, ^2J = 14.6, 6.9 Hz, 2H; NCH_A), 4.08 (dt, ^2J = 14.6, 6.9 Hz, 2H; NCH_B), 1.43–1.33 (m, 4H; NCH_2CH_2), 1.26–1.23 (m, 4H; $N(CH_2)_2CH_2$), 1.06–0.90 (m, 4H; $N(CH_2)_3CH_2$), 0.86–0.68 ppm (m, 4H; $N(CH_2)_4CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 141.6, 137.6, 137.4, 137.1, 136.6, 132.7, 130.9, 129.9, 127.8, 127.1, 122.5, 121.9, 120.7, 111.2, 105.8, 43.8, 41.8, 28.9, 27.4, 27.1, 25.1 ppm (two carbon signals missing or overlapped); IR (film): $\tilde{\nu}$ = 2928, 2855, 1704, 1545 cm^{-1}; MS (ES): *m*/*z* (%): 708 (100) [M^++H]; HRMS: *m*/*z* calcd for $C_{49}H_{45}N_3O_2$ [M^++H]: 708.3590; found: 708.3599.

Also obtained was the macrocycle *anti*-**18Ph** (10 mg, 5% yield); R_f = 0.3 (1:3 diethyl ether/petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.44 (d, *J* = 7.8 Hz, 2H; indolyl 4-H), 7.36 (t, *J* = 7.5 Hz, 2H; *p*-Ph), 7.35–7.27 (m, 2H; Bn), 7.24–7.22 (m, 3H; Bn), 7.12 (app t, *J* = 7.8 Hz, 2H; indolyl 7-H), 6.95–6.87 (m, 6H; H-5, *m*-Ph), 6.73–6.64 (m, 6H; indolyl 6-H, *o*-Ph), 4.85 (d, ^2J = 14.9 Hz, 1H; CH_ABn), 4.81 (d, ^2J = 14.9 Hz, 1H; CH_BBn), 3.89–3.84 (m, 2H; NCH_AH_B), 3.64–3.61 (m, 2H; NCH_AH_B), 2.19–2.14 (m, 2H; NCH_2CH_2), 2.12–1.86 (m, 2H; NCH_2CH_2), 1.64–1.04 (m, 8H; $N(CH_2)_2CH_2$, $N(CH_2)_3CH_2$), 0.88–0.65 ppm (m, 4H; $N(CH_2)_4CH_2$); IR (film): $\tilde{\nu}$ = 2928, 2855, 1704, 1545 cm^{-1}; MS (ES) *m*/*z* (%): 708 (100) [M^++H]; HRMS: *m*/*z* calcd for $C_{49}H_{45}N_3O_2$ [M^++H]: 708.3590; found: 708.3598.

Also observed was the anhydride 26,27-diphenyl-6,7,8,9,10,11,12,13,14,15-decahydro-22*H*-5,25:16,21-dimethenodibenzo[*b*,*h*]pyrrolo[3,4-*e*,1,10]diazacycloicosine-22,24-dione (12 mg, 6% yield) as yellow prisms. M.p. 265–270 °C (from $CHCl_3$); R_f = 0.90 (CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.41–7.30 (m, 4H; indolyl 4-H, *p*-Ph), 7.33 (app t, *J* = 7.6 Hz, 4H; *m*-Ph), 7.26–7.24 (m, 2H; indolyl 7-H), 7.21 (app t, *J* = 7.6 Hz, 2H; indolyl 5-H), 6.95 (app t, *J* = 7.6 Hz, 2H; indolyl 6-H), 6.90 (m, 4H; *o*-Ph), 4.19 (dt, ^2J = 14.0, 6.0 Hz, 2H; NCH_A), 4.02 (dt, ^2J = 14.0, 6.0 Hz, 2H; NCH_B), 1.64–1.49 (m, 4H; NCH_2CH_2), 1.48–1.18 (m, 4H; $N(CH_2)_2CH_2$), 1.09–0.90 (m, 4H; $N(CH_2)_3CH_2$), 0.89–0.64 ppm (m, 4H; $N(CH_2)_4CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.5, 137.0, 132.1, 130.4, 128.8, 128.5, 122.6, 121.2, 120.5, 111.1, 43.5, 29.4, 27.0, 26.8, 24.7 ppm (four carbon signals missing or overlapped); IR (film): $\tilde{\nu}$ = 2926, 2854, 1760, 1626 cm^{-1}; MS (ES) *m*/*z* (%): 641 (100) [M^++Na]; HRMS: m/z calcd for $C_{42}H_{38}N_2O_3$ [M^++Na]: 641.2780; found: 641.2808.

23-Benzyl-26,27-dibenzyl-6,7,8,9,10,11,12,13,14,15-decahydro-22*H*-5,25:16,21-dimethenodibenzo[*b*,*h*]pyrrolo[3,4-*e*,1,10]diazacycloicosine-22,24-dione *anti*- and *syn*-18Bn: By using the same general method, the bisindolylmaleimide **16Bn** (50 mg, 0.084 mmol) and 1,10-dibromodecane (19 μl, 0.084 mmol) gave a crude product that was purified by performing flash chromatography, eluting with 15:85 diethyl ether/petroleum ether, and preparative LC-MS, to give the macrocycle *syn*-**18Bn** (24 mg, 38%) as a red film. R_f = 0.36 (3:7 diethyl ether/petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59 (d, *J* = 7.8 Hz, 2H; indolyl 4-H), 7.34–7.23 (m, 11H; aromatic), 7.16 (td, *J* = 7.4, 0.9 Hz, 2H; *p*-Ph), 7.09–7.05 (m, 4H; aromatic), 6.69–6.65 (m, 4H; aromatic), 4.82 (d, *J* = 14.9 Hz, 1H; NCH_APh), 4.76 (d, *J* = 14.9 Hz, 1H; NCH_BPh), 4.02–3.97 (m, 2H; NCH_A), 3.79–3.73 (m, 2H; NCH_B), 3.76 (d, *J* = 16.9 Hz, 2H; indolyl 2-CH_AH_B), 3.30 (d, *J* = 16.9 Hz, 2H; indolyl 2-CH_AH_B), 1.74–0.82 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.6, 139.7, 138.6, 137.5, 133.1, 129.0, 128.8, 128.6, 127.8, 126.8, 126.2, 122.1, 121.0, 120.8, 110.4, 106.2, 77.6, 42.9, 42.1, 32.6, 28.1, 26.7, 26.6, 24.5 ppm (two carbon signals missing or overlapped); IR (film): $\tilde{\nu}$ = 2954, 1704, 1396 cm^{-1}; MS (ES) *m*/*z* (%): 736.3 (100) [M^++H]; HRMS: *m*/*z* calcd for $C_{51}H_{49}N_3O_2$ [M^++H]: 736.3903; found: 736.3911.

Also obtained, after further preparative HPLC, was the macrocycle *anti*-**18Bn** (*anti*, 8 mg, 13% yield) as a red film. R_f = 0.42 (3:7 diethyl ether/petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–6.87 (m, 23H; aromatic), 4.81 (s, 2H; NCH_2Ph), 4.14 (d, *J* = 16.7 Hz, 2H; indolyl 2-CH_AH_B), 3.57 (d, *J* = 16.7 Hz, 2H; indolyl 2-CH_AH_B), 3.91–3.86 (m, 2H; $2NCH_AH_B$), 3.69–3.63 (m, 2H; $2NCH_AH_B$), 1.65–0.80 ppm (m, 16H; alkyl chain); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 137.6, 137.0, 128.5, 128.4, 128.1, 127.8, 127.3, 126.7, 126.3, 122.1, 121.4, 120.5, 109.4, 77.2, 43.7, 30.9, 28.9, 26.8, 24.7, 24.3 ppm (four carbon signals missing or overlapped); IR (film): $\tilde{\nu}$ = 2926, 2855, 1701, 1419, 1395, 1347 cm^{-1}; MS (ES) *m*/*z* (%): 736.2 (100) [M^++H]; HRMS: *m*/*z* calcd for $C_{51}H_{49}N_3O_2$ [M^++H]: 736.3903; found: 736.3922.

Acknowledgements

We thank EPSRC and the University of Leeds for funding, the EPSRC National Mass Spectrometry Centre, Swansea, for mass spectrometry measurements, Stuart Warriner, Julie Fisher and Andrew Leach for helpful discussions, and Jacqueline Colley for HPLC analysis.

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Received: May 10, 2005
Published online: August 1, 2005

