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Towards configurationally stable bisindolylmaleimide cyclophanes: potential tools for investigating protein kinase function

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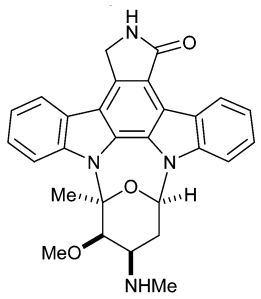
The effect of macrocycle size and substitution on the configurational stability of some bisindolylmaleimide cyclophanes was determined.

Staurosporine, **1**, is a potent broad spectrum inhibitor of many protein kinases.[1] However, despite its potency, its lack of specificity limits its value as a tool for studying kinase function. Nonetheless, staurosporine has been a useful lead for the discovery of selective kinase inhibitors. For example, some bisindolylmaleimides, in which the planarity of the indolocarbazole ring system has been broken, are potent and selective inhibitors of particular kinases:[2] the bisindolylmaleimide[3] **2** selectively inhibits the β isoforms of protein kinase C (PKCβ) and can produce significant improvements in diabetic retinopathy, nephropathy, neuropathy and cardiac dysfunction.[4]



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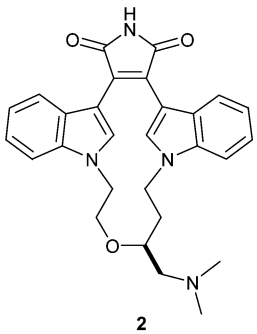

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Using procedures which were amenable to automation, we prepared a series of [2.*n*]metacyclophanes **5** and **6** in which the length of the tether, *n*, and the substituent, R, were varied (see Table 1). The effect of R and *n* on the barrier to interconversion between the limiting diastereomeric (*syn* and *anti*) conformers was investigated (Fig. 1).

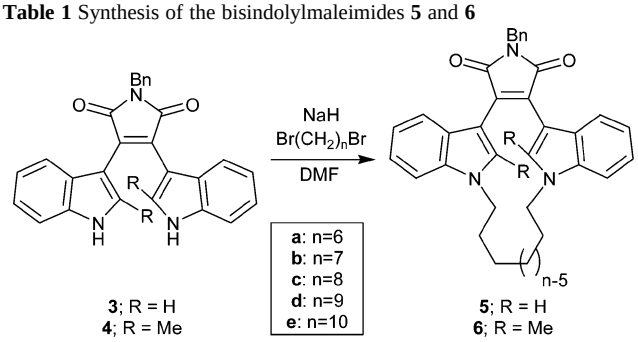
The 500 MHz ^{1}H NMR spectra of the bisindolylmaleimides **5** (R = H) revealed that their conformations were in fast exchange on the NMR timescale at 298 K. However, in the ^{1}H NMR spectrum of **5a** recorded at low temperature, the protons in its six-atom tether (*e.g.* NCH_AH_B) were revealed to be diastereotopic. Molecular modelling studies using density functional theory (B3LYP/6-31G*) revealed that the *syn* conformer of **5a** was > 20 kJ mol^{-1} less stable than that its *anti* confomer; the diastereotopicity must stem, therefore, from slow interconversion between the two enantiomeric *anti* conformers. The barrier to racemisation of **5a** was 36.6 kJ mol^{-1} in CD_2Cl_2 at 203 K (Table 2). The NMR spectra of **5b–e** were also recorded at 200 K; however, the diastereotopicity of the protons in the tether was not revealed, suggesting that interconversion was faster in these cases. In each case, we propose that the *syn* conformer is an intermediate in the racemisation of the *anti* conformer.

In contrast, the 500 MHz ^{1}H NMR spectra of the macrocycles **6** were in slow exchange at room temperature. The ratios of the *syn* and *anti* conformers were determined as a function of temperature by careful analysis of the NMR spectra: the benzylic protons are enantiotopic in the *syn* conformer and diastereotopic in the *anti* conformer. Shorter tethers selectively destabilised the *syn* conformer: for the macrocycles **6**, the ratios of *anti* and *syn* conformers were 64 : 36 (*n* = 10), 42 : 58 (*n* = 9) and > 99 : < 1 (*n* = 6–8).

Above room temperature, the NMR spectra of **6d** (*n* = 9) and **6e** (*n* = 10) broadened dramatically. The rate of exchange between the *anti* and *syn* conformers could be determined as a function of temperature by comparison of experimental and simulated spectra.† The barriers to interconversion between the conformers showed that macrocycle size had an important effect on the configurational stability of the cyclophanes **6**: at 298 K, the barriers for isomerisation of the *anti* confomers were 66.6 kJ mol^{-1} (for **6e**, *n* = 10) and 70.8 kJ mol^{-1} (for **6d**, *n* = 9) (Table 2).

The ^{1}H NMR spectra of **6a–c** (*n* = 6–8), recorded in d_8-toluene at 373 K, were not broadened, suggesting that racemisation‡ of **6a–c** was rather more difficult. Indeed, analysis of the cyclophanes **6a** (*n* = 6) and **6c** (*n* = 8) by chiral analytical HPLC at 298 K revealed two peaks, demonstrating that the half-lives of the enantiomeric *anti* conformers were greater than the separation of the peaks (10

Table 1 Synthesis of the bisindolylmaleimides **5** and **6**



3; R = H
4; R = Me

5; R = H
6; R = Me

Entry	Starting material	*n*	R	Product	Yield (%)
1	**3**	6	H	**5a**	69
2	**3**	7	H	**5b**	73
3	**3**	8	H	**5c**	62
4	**3**	9	H	**5d**	74
5	**3**	10	H	**5e**	71
6	**4**	6	Me	**6a**	44
7	**4**	7	Me	**6b**	76
8	**4**	8	Me	**6c**	62
9	**4**	9	Me	**6d**	53
10	**4**	10	Me	**6e**	63

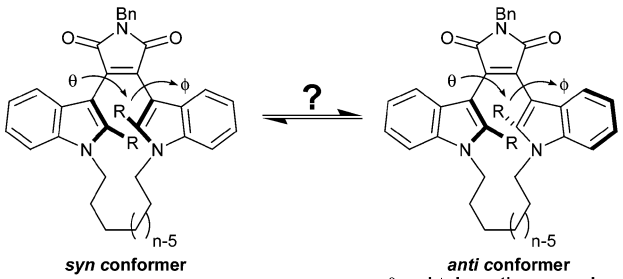


Fig. 1 Limiting conformations of bisindolylmaleimides.

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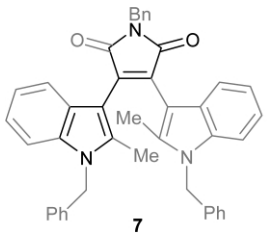
Table 2 Barriers to interconversion between conformers of the cyclophanes **5** and **6**

	n	R	Equilibration between…	Ratio at 298 K	$\Delta G°^{a}$/kJ mol^{-1}	$\Delta G_f^{‡a}$/kJ mol^{-1}	$\Delta G_b^{‡a}$/kJ mol^{-1}	$t_{\frac{1}{2}}^{a,b}$
5a	6	H	***anti*** ⇌ *ent*-***anti***	50 : 50[c,d]	0	36.6[e]	36.6[e]	< 0.3 μs[f]
6a	6	Me	***anti*** ⇌ *ent*-***anti***	50 : 50[c,g]	0	> 90	> 90	> 10 min[h]
6c	8	Me	***anti*** ⇌ *ent*-***anti***	50 : 50[c,g]	0	> 90	> 90	> 10 min[h]
6d	9	Me	***anti*** ⇌ ***syn***	42 : 58[i]	−0.82	70.8[j]	71.7[j]	290 ms
					$\Delta H°$ = −5.1 kJ mol^{-1}	$\Delta H_f^{‡}$ = 68.9 kJ mol^{-1}	$\Delta H_b^{‡}$ = 74.0 kJ mol^{-1}	
					$\Delta S°$ = −14.1 J mol^{-1} K^{-1}	$\Delta S_f^{‡}$ = −6.5 J mol^{-1} K^{-1}	$\Delta S_b^{‡}$ = 7.6 J mol^{-1} K^{-1}	
6e	10	Me	***anti*** ⇌ ***syn***	64 : 36[i]	1.5	66.6[j]	65.1[j]	50 ms
					$\Delta H°$ = −4.4 kJ mol^{-1}	$\Delta H_f^{‡}$ = 66.7 kJ mol^{-1}	$\Delta H_b^{‡}$ = 71.1 kJ mol^{-1}	
					$\Delta S°$ = −20.0 J mol^{-1} K^{-1}	$\Delta S_f^{‡}$ = 0.2 J mol^{-1} K^{-1}	$\Delta S_b^{‡}$ = 20.2 J mol^{-1} K^{-1}	
7	—	Me	***anti*** ⇌ ***syn***	35 : 65[i]	−1.5	51.5[j]	53.0[j]	< 0.12 ms

[a] At 298 K. [b] Of the *anti* conformer. [c] Symmetry demands a 50 : 50 ratio of enantiomeric *anti* conformers. [d] Molecular modelling (B3LYP/6-31G*) suggests that the *syn* conformer is at least 20 kJ mol^{-1} less stable. [e] Determined by variable temperature NMR in CD_2Cl_2. $\Delta G^{‡}$ is given at the coalesence temperature (203 K). [f] On the assumption that $\Delta S^{‡}$ is small. [g] Integration of the 500 MHz ^{1}H NMR spectrum revealed a > 99 : < 1 mixture of *anti* and *syn* conformers. [h] The enantiomeric *anti* conformers were resolved by chiral analytical HPLC. [i] Ratio of the *anti* and *syn* conformers determined by integration of the 500 MHz ^{1}H NMR spectrum. [j] Determined by comparison of simulated spectra, generated using gNMR,[5] with experimental spectra recorded at several temperatures in d_8-toluene.

min),[6] and, hence, that the barrier to racemisation was at least 90 kJ mol^{-1}.

The tether had a smaller effect on the configurational stability of the cyclophanes **6** than did the indolyl 2-methyl groups. The barrier to isomerisation of the *anti* conformer of the bisindolylmaleimide **7**, in which the tether had been removed, was 51.5 kJ mol^{-1}; in contrast, the barrier to racemisation of **5a**, in which R = H, was just 36.6 kJ mol^{-1}.



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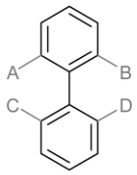

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There is a substituent in each of the *ortho* positions flanking each [3,3′]bipyrrolyl bond of **7** (the bipyrrolyl unit is shown in black); given that tetrasubstituted biphenyls **8** (A ≠ B ≠ H; C ≠ D ≠ H) are generally resolvable,[7] it is, perhaps, surprising that the conformers of bisindolylmaleimides such as **7** are not atropisomers. However, the internal bond angles of a biphenyl[8] (119°) are markedly wider than those of a bisindolylmaleimide[9] (107° and 108° in the crystal structure of **3**), so the carbons which are *ortho* to the bipyrrolyl bond are further apart (2.96 and 3.30 Å[8]) than for biphenyl[7] (2.92 Å). In addition, small size of the maleimide oxo group can be rivalled by only a hydrogen atom (compare the length of its carbonyl bond, 1.21 Å, with bonds to other "small" substituents:[7] 1.39 Å for Ph–F, 1.45 Å for Ph–OH). Furthermore, conjugation between a maleimide and an indole in the transition state is far more stabilising than conjugation between two phenyl rings.

In summary, the addition of indolyl 2-methyl substituents to bisindolylmaleimides such as **3** is not sufficient for configurational stability. The effect of indolyl 2-methyls may be exaggerated by the presence of a tether between the nitrogen atoms of the indoles (→**6**). Like other metacyclophanes,[10] the activation energy for interconversion between limiting conformers (*anti* and *syn* confomers in this case) is critically dependent on the length of the tether. The half lifes, $t_{\frac{1}{2}}$, of the *anti* conformers of the [2.*n*] metacyclophanes **6** increase from 50 ms (with *n* = 10) to 290 ms (with *n* = 9) to greater than 10 min (with *n* ≤ 8).

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Notes and references

† gNMR was used to produce simulated spectra based on populations of the *syn* and *anti* conformers extrapolated from the slow exchange regime.
‡ The cyclophanes **6a–c** exclusively (> 95 : 5) populated the chiral (*anti*) conformer.

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