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 isoforms of protein kinase C (PKCb).2 The bisindolylmaleimide 3 selectively inhibits the β isoforms 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 selectively inhibits the \( \beta \) isoforms of protein kinase C (PKC\( \beta \)).

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 \( ^1H \) 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 \( ^1H \) NMR spectrum of 5a recorded at low temperature, the protons in its six-atom tether (e.g. NCH\( _2 \)H\( _3 \)) were revealed to be diastereotropic. 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 its anti conformer; 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\(^{-1} \) at 373 K, were not broadened, suggesting that racemisation of 5a was rather more difficult. Indeed, analysis of the cyclophanes 6a–e (n = 6–8), recorded in \( d_6 \)-toluene at 373 K, were not broadened, suggesting that racemisation of 6a–e was rather more difficult. Indeed, analysis of the cyclophanes 6a (n = 6) and 6e (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](image-url)
are generally resolvable, it is, perhaps, surprising that the Table 2 Barriers to interconversion between conformers of the cyclophanes group can be rivalled by only a hydrogen atom (compare the length interconversion between limiting conformers (2)).


5. C. P. Budzelaar, gNMR, ver. 5.0, University of Nijmegen, The Netherlands.


min), 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.

There is a substituent in each of the ortho positions flanking each [3,3']bipyrrrol bond of 7 (the bipyrrrol unit is shown in black); given that tetrasubstituted biphenyls 8 (A ≠ B ≠ C ≠ D ≠ H) are generally resolvable, 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 bipyrrrol bond are further apart (2.96 and 3.30 Å) than for biphenyl 8 (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; 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, the activation energy for interconversion between limiting conformers (anti and syn conformers in this case) is critically dependent on the length of the tether. The half lives, tₜ, 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


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