The Design of Inhibitors of Protein Kinase C; The Solution Conformation of Staurosporine

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NMR spectra in several solvents show that the conformation of the tetrahydropyran ring in staurosporine changes from a chair form in the free base to a boat conformation on protonation, mainly owing to the increased solvation requirement of $-NH_2Me^+$ *vs.* –NHMe; implications for the bioactive conformation are discussed.

Staurosporine **1**, a natural product first isolated from *Streptomyces staurosporeus*,[1] is a potent inhibitor of several protein kinases, notably protein kinase C (PKC),[2] where it appears to bind strongly to the catalytic subunit.[3] A series of bisindolylmaleimide inhibitors (*e.g.* **2**) of PKC has been designed from the staurosporine structural lead in our laboratories.[4] A key design goal was to access the putative staurosporine amine binding-site on the enzyme from a bisindolylmaleimide, based on a hypothetical common binding mode of the two types of inhibitor. Consideration of the possible spatial relationships of the staurosporine amine group to its aglycone moiety was therefore necessary, especially in the design of conformationally restricted amines. To this end the solution conformation of the tetrahydropyran ring of staurosporine was examined by NMR analysis, in both the free base and protonated forms.

Previous NMR studies[1,5,6] have shown that in the free base, the expected chair conformation **1A** of the pyran ring seen in the crystal structure is maintained. Thus vicinal couplings for proton $1'_\alpha2'_\alpha$, $1'_\alpha2'_\beta$, $2'_\beta3'_\alpha$, $2'_\alpha3'_\alpha$ and $3'_\alpha4'_\alpha$ (see Table 1) are those expected for a slightly distorted chair conformation, with dihedral angles close to those seen in the crystal.[7] NOE difference experiments used to assign the three methyl

Table 1 Chemical shifts and coupling constants for staurosporine[a,b]

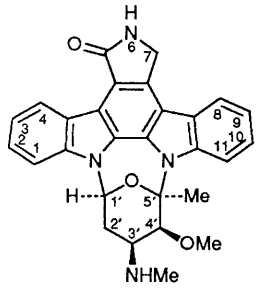
A Free Base

	δ			J/Hz	
Proton	$CDCl_3$	CD_3OD	Protons	$CDCl_3$	CD_3OD
1'α	6.57	6.67	1'α2'α	5.8	6.6
2'α	2.39	2.56	1'α2'β	1.3	1.9
2'β	2.76	2.64	2'α2'β	14.8	14.4
3'α	3.36	3.33	2'α3'α	3.6	4.4
4'α	3.89	4.07	2'β3'α	3.6	5.9
NMe	1.67	1.77	3'α4'α	3.6	3.1
OMe	3.42	3.19	—	—	—
CMe	2.37	2.40	—	—	—

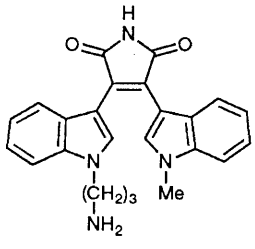
B Protonated form

	δ			J/Hz	
Proton	CD_3OD–D^+	D_3O^+	$CDCl_3$–D^+	Protons	CD_3OD–D^+	D_3O^+
1'α	6.80	6.15	6.79	1'α2'α	9.4	9.4
2'α	3.31	3.27	3.22	1'α2'β	3.0	1.8
2'β	2.28	1.92	2.63	2'α2'β	12.8	13.5
3'α	4.05	4.03	4.14	2'α3'α	7.0	7.4
4'α	4.36	4.25	4.42	2'β3'α	11.6	11.2
NMe	2.83[c]	2.67	2.79	3'α4'α	1.9	1.6
OMe	2.34[c]	1.96	2.50	—	—	—
CMe	2.57[c]	2.87	2.61	—	—	—

[a] Chemical shifts for other protons not included. They are consistent with those previously reported. [b] *Ca.* 0.5 mg of staurosporine (Fluka) was dissolved in 0.4 ml of each solvent. NMR spectra were obtained on the Bruker AM-400 spectrometer using standard operating conditions. [c] Assigned by NOE difference experiments: NMe···NOEs to H_3' and H_4'; OMe···NOE to H_4'; CMe···NOEs to H_4', H_3' and H_{11} (δ 8.0).



1



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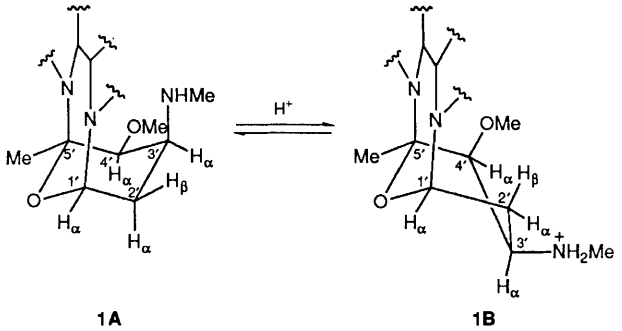

1A 1B

groups[5,6] confirm that the *N*-methyl is in an axial position, its abnormally high field chemical shift (*e.g.* δ 1.67 in $CDCl_3$) reflecting the ring current effect of the adjacent polycyclic aromatic group.

Protonation of the amino group with DCl (for deuteriomethanol and deuteriated water) or [$^2H_1$]trifluoroacetic acid-(TFA) (for the deuteriochloroform solution) led to substantial changes in the NMR spectrum. Assignments were carried out using 2D COSY and 1D NOE difference experiments (see Table 1 for data). In CD_3OD–DCl, proton $1'_\alpha$ has coupling constants of 9.4 and 3.0 Hz to $2'_\alpha$ and $2'_\beta$ respectively. In turn proton $2'_\beta$ has a large coupling to $3'_\alpha$ (11.6 Hz) whereas $J_{2'\alpha3'\alpha}$ is 7.0 Hz, and $J_{3'\alpha4'\alpha}$ is small (1.7 Hz). These values can only be accommodated by a boat conformation **1B** with the protonated NHMe group equatorial. To confirm this the dramatic changes in the chemical shift of the NMe and OMe groups (a downfield shift of 1.16 ppm for the NMe and an *upfield* shift of 1.08 ppm for the OMe) reflect both ring current and charge effects in the boat conformation relative to the chair. Similar chemical shift changes are observed for D_2O–DCl and for $CDCl_3$–TFA solutions but line broadening precluded the accurate measurement of coupling constants in the latter solvent.

Normally in cyclohexane derivatives the boat conformation is estimated to be *ca.* 6 kcal mol^{-1} (1 cal = 4.184 J) higher in energy than the chair[8] and a similar conformational behaviour is seen for tetrahydropyran.[9] The twist boat conformation, which is *ca.* 5 kcal mol^{-1} less stable than the chair, is not possible in the tetrahydropyran ring of staurosporine owing to the fixed diaxial bonds holding the polycyclic aromatic rings. Calculations of gas-phase enthalpies of chair and boat forms of protonated staurosporine by both molecular mechanics (MOLOC)[10] and semiempirical methods (MOPAC v.5 with AM1 and PM3 Hamiltonians)[11] show the chair form to be clearly favoured. The enthalpy differences obtained were: 6 kcal mol^{-1} (MOLOC), 14 kcal mol^{-1} (PM3) and 19 kcal-mol^{-1} (AM1). Entropic contributions to the free energy (arising from the increased rotational freedom of the methylamino group in the boat, possibly offset by decreased rotational freedom of the methoxy group) should be comparatively small.[12]

The conformational change in solution is therefore due to solvation effects which override the strain effects apparent in the calculated enthalpies of the isolated molecules. A qualitatively similar effect is seen in the conformational behaviour of aminocyclohexanes where protonation increases the prefer-

ence for the less sterically demanding equatorial orientation by 0.7 kcal mol^{-1}.[12] Both effects can be explained by the drive to surround the protonated group by solvent, lowering the free energy by H-bonding or dielectric effects, which are both stronger in the case of charged species. This is equivalent to the common but less accurate description of such effects as an increase in the effective size of a charged group. The magnitude of the present effect appears to be much greater than in the aminocyclohexane case and reflects the large steric influence of the diaxial polyaromatic moiety.

The strain energy of the boat conformation is reflected in the unusually low pK_a of 5.3 for the amine in water.

Coupling constants for the protonated species suggest a conformer ratio of at least 95:5 (corresponding to a free energy difference of 1.8 kcal mol^{-1}) in favour of the boat. This difference is not, however, sufficient to rule out binding of the chair form to the enzyme. Also, in view of the low pK_a, the possibility of free base binding cannot be discounted. Both conformers must therefore be considered in the design of staurosporine analogues for PKC inhibition.

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