2'- AND 3'-SUBSTITUTED SANGIVAMYCINS: CONFORMATIONAL RESTRICTION BY THE *GAUCHE* EFFECT

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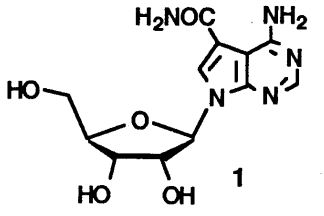
Conformational restrictions of sangivamycin (**1**) could be achieved by the use of the *gauche* effect of the substituents on the ribofuranose moiety. The conformational deviations obtained by this method were found to nicely correlate with the inhibitory activity of PKC.

KEYWORDS protein kinase C inhibitor; molecular design; conformational restriction; gauche effect; NMR analysis; NOE

In the process of developing an inhibitor of protein kinase C (PKC), we have studied the active conformation of sangivamycin (**1**), a rather selective inhibitor of PKC, by designing its conformationally restricted derivatives. In the previous paper, we reported that the introduction of the methyl group into the β-face of the ribofuranose ring could induce the deviation of the conformer population and that the population of the proposed active conformation (*anti* C3'-*endo* **1a**) nicely correlates with the inhibitory activity of PKC.[2)] However, since the introduction of the methyl group slightly weakened the inhibition of PKC, we designed molecules to exhibit this active conformation predominantly by another type of conformational restriction and to have stronger inhibitory activity. Herein we wish to report our molecular design by using the *gauche* effect[3)] as a conformational lock.



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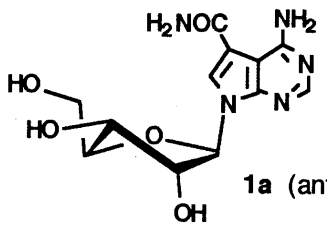
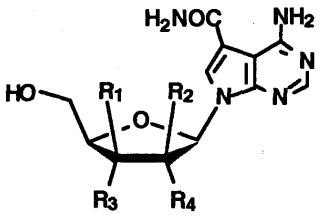


1a (anti C3'-endo)

Uesugi and Guschlbauer have independently reported that the population of the C3'-*endo* conformer increases linearly with the electronegativity of the 2'-substituent in 2'-substituted uridine and adenosine derivatives.[4,5)] This correlation suggests that a more electronegative substituent favors an axial orientation on ribofuranose moiety as the *gauche* effect.[6)] Along this line, Guschlbauer has extended this empirical rule to *arabino*- and *xylo*- nucleoside and has predicted their preferred conformations to be C3'-*endo* and C2'-*endo* respectively. In the case of *arabino*-uridine this prediction has been verified.[7)] However, further systematic studies are necessary for exploring the conformational properties of 2'- and 3'-substituted nucleoside and clarifying their relations with the biological activities. We describe a study of this *gauche* effect using sangivamycin (**1**) as a substrate.

Table I. Summary of ^{1}H NMR and X-Ray Studies; Estimation of the Conformer Population and PKC Inhibitory Ability of Sangivamycin and Its Derivatives



	R_1	R_2	R_3	R_4
1	H	H	OH	OH
2	H	H	H	OH
3	OH	H	H	OH
4	F	H	H	OH

	R_1	R_2	R_3	R_4
5	H	H	OH	H
6	H	OH	OH	H
7	H	H	F	OH

	1	**2**	**3**	**4**	**5**	**6**	**7**
NOE of 1'-H (%) [a,b]	5	4	3	2	3	1	5
2'-H (%)	5	5	3	6	2	-	6
3'-H (%)	2	3	-	-	1	1	0
$J_{1',2'}$ (Hz) [b,d]	5.9	2.0	2.3	2.6	7.6	(4.6)	7.9
$J_{3',4'}$ (Hz) [b,d]	3.9	8.3	(4.0)	(3.0)	2.3	4.2[c]	< 1
X-ray structure [e]	*anti* C3'-*endo*	*anti* C3'-*endo*	*anti* C2'-*endo*	-	*anti* C2'-*endo*	*anti* C3'-*endo*	-
anti (%)	60	70	80	80	80	90	60
syn (%)	40	30	20	20	20	10	40
C2'-*endo* (%)	60	20	20	30	80	60	80
C3'-*endo* (%)	40	80	80	70	20	40	20
Inhibition of PKC [f]	10	14	11	11	5	2	8

a) On irradiation of 8-H (purine numbering). b) Measured in d_6-DMSO (270 MHz except c). c) 400 MHz. d) Values in parentheses are coupling constants $J_{1',2'\alpha,4'}$ or $J_{3'\alpha,4'}$. e) The detailed conformation is shown in note12. f) Values are represented as the relative inhibitory activity of PKC [1/ IC_{50}(sangivamycin) = 10].

Applying this empirical rule for the conformational restriction of sangivamycin, we designed the compounds **2** - **7**. 3'-Deoxysangivamycin (**2**),[8] *xylo*-sangivamycin (**3**) and 3'-deoxy-3'-fluoro-*xylo*-sangivamycin (**4**) were designed to exhibit C3'-*endo* conformation predominantly and thus expected to have stronger inhibitory activity of PKC. On the other hand, 2'-deoxysangivamycin (**5**),[8] *arabino*-sangivamycin (**6**)[8b] and 3'-deoxy-3'-fluoro-sangivamycin (**7**) were predicted to prefer C2'-*endo* conformation by considering the *gauche* effect.

Sangivamycin derivatives thus designed were prepared in the conventional manner[10] and their conformational properties were analyzed by ^{1}H NMR[11] and X-ray diffraction studies.[12] Although it is difficult to evaluate the *anti*/*syn* ratios quantitatively on the basis of simple NOE experiments,[11] it is possible to calculate the conformational deviation caused by this molecular modification semi-quantitatively. Judging from the NOE experiments, the compounds prefer *anti* conformation (60 to 90 %), as shown in Table I. On the other hand, the C2'-*endo*/C3'-*endo* ratios were found to vary largely as predicted by the *gauche* effect. The predominance of C3'-*endo* conformation in **2** - **4** (the left side of the table) is a characteristic feature compared with sangivamycin. Similarly, the compounds **5** and **7** were found to exhibit C2'-*endo* conformation predominantly by ^{1}H NMR analysis (the right side of the table). One exception is the C2'-*endo*/C3'-*endo* ratio of *arabino*-sangivamycin (**6**), which was found to be almost the same as that of sangivamycin itself. Hruska has reported that *arabino*-adenosine slightly preferred the C3'-*endo* conformation in DMSO, and discussed the repulsive interaction between 2'- and 5'-hydroxyl groups.[13] The X-ray structure of **6**, which is consistent with the X-ray structure of *arabino*-adenosine,[14] supports this repulsive interaction. The solid-state conformations of *xylo*-sangivamycin (**3**), which was found to exhibit *anti* C2'-*endo* conformation predominantly in solution, is slightly confusing at this stage. However, the conformational properties of the other compounds, especially those of fluoro substituted sangivamycins, were nicely predicted by the *gauche* effect (**4** *vs.* **7** in Table I).

The inhibition of PKC by these compounds *in vitro* is shown in Table I. It is difficult to generalize these results simply, but the compounds having slightly stronger inhibitory activity were found to exhibit C3'-*endo* conformation predominantly, except for **7**. The inhibitory activity correlates also with the presence of 2'-hydroxyl group (**1** - **4** and **7**). However, considering the very weak cytotoxicity of 2'-deoxysangivamycin (*ca.* 10^4 times weaker than sangivamycin) reported by Townsend,[8a] the disappearance of the hydroxyl group is not so important for PKC inhibition in our study. Unfortunately we could not obtain clear results, but we think the structure-activity relationship observed here might better explain by the population of the active conformation of these compounds.

In conclusion, we were able to design the conformationally restricted sangivamycin derivatives by considering the *gauche* effect. The compounds which were designed to exhibit the desired conformation have stronger inhibitory activity than the others, as expected. Generally structural modifications in relation to biological activities are undertaken by considering the alternation of the chemical properties primarily, but we think that it is important to evaluate the conformational deviation derived from the structural modifications.

REFERENCES AND NOTES

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3) The term *gauche* effect in this paper denotes the effect that directs torsion angles of groups X-C-C-Y (X, Y electronegative substituents like OH, F) into *gauche* and tends to avoid *antiperiplanar*. For example, O4'-C1'-C2'-O2' and O4'-C4'-C3'-O3' are such groupings in a ribose. See reference 6.

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10) Sangivamycin derivatives modified at the 3' position (**2**, **3**, **4** and **7**) were prepared by coupling the base moiety with appropriately substituted sugars and successive functional group manipulation. 2'-Deoxy-sangivamycin (**5**) and *arabino*-sangivamycin (**6**) were prepared according to the literature methods.[9]

11) Crystallographic data has been deposited with the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England. The selected conformational data on the crystalline structures (sugar puckering and χ): **1** ^{3}E, -122°. **2** $^3T_4$, -124°. **3** $_1T^2$, -116°. **5** $^2T_1$, -110°. **6** ^{3}E, -117°.

12) The calculation of C2'-*endo*/C3'-*endo* conformation ratio: W. Saenger, "Principles of Nucleic Acid Structure," Springer-Verlag, New York, 1984, chapter 3.5. The calibration between NOE and *syn*/*anti* ratio: H. Rosemeyer, G. Tóth, B. Golamkiewicz, Z. Kazimierczuk, W. Bourgeois, U. Kretschmer, H-P. Muth, and F. Seela, *J. Org. Chem.*, **55**, 5785 (1990).

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(Received December 15, 1992)