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STRUCTURE-ACTIVITY RELATIONSHIP STUDY ON N-GLYCOSYL MOIETIES THROUGH MODEL BUILDING OF DNA AND ELLIPTICINE N-GLYCOSIDE COMPLEX

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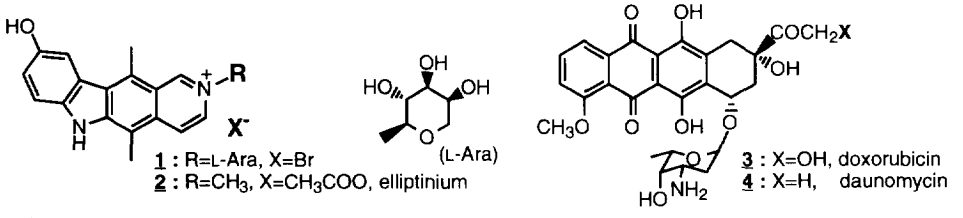
Abstract: Ellipticine N-glycoside derivatives have been synthesized to elucidate the role of the N-glycosyl moiety. Model-building study of the DNA-ellipticine N-glycoside complex suggested a major groove binding of the sugar moiety and roles of the hydroxyl groups at C-2', -3', and -4'. Copyright © 1996 Elsevier Science Ltd

Since topoisomerase I and II play an important role in cell proliferation, these enzymes have been targets for anticancer agents, forming a ternary complex with DNA and the enzymes. Recently crystal structures of topoisomerase I and II have been uncovered[1,2] and a mode of action of these enzymes on DNA has been proposed although structure of DNA-enzyme complex or DNA-drug-enzyme ternary complex has not yet been solved.

Ellipticine N-glycosides, which showed potent antitumor activity,[3] have been established as inhibitors of the topoisomerase II, since the complexes of those derivatives and DNA[4] interfered with the action by the enzyme.[5] The ternary complex formation of topoisomerase II, DNA and anticancer agents such as elliptinium (**2**) and doxorubicin (**3**), have also been suggested.[6]



1 : R=L-Ara, X=Br
2 : R=CH_3, X=CH_3COO, elliptinium
3 : X=OH, doxorubicin
4 : X=H, daunomycin

Figure 1. Chemical Structures of the Antitumor Derivatives

2-(α-L-Arabinopyranosyl)-9-hydroxyellipticinium bromide (**1**), one of the most potent antitumor ellipticine N-glycosides against various experimental tumors, has a characteristic glycosidic moiety at N-2. In order to investigate the role of the sugar moiety, we have prepared its mono-O-methyl arabinopyranosyl derivatives (**5**, **6**, and **7**), focusing on the role of the hydroxyl groups.

The arabinopyranosyl moiety has three hydroxyl groups at C-2', C-3', and C-4'. Firstly, 2'-O-methyl derivative **5** was synthesized starting from the compound **1**. Protection of the 3'- and 4'- hydroxyl groups by isopropylidene group and the following methoxymethylation of the phenolic hydroxyl group at C-9 gave the mono-hydroxy derivative which was then methylated by treatment of CH_2N_2-BF_3•OEt_2.[7] Deprotection of the groups at C-3', C-4', and C-9, afforded the 2'-mono-O-methylated derivative **5**. Secondly, 3'-mono-O-methyl derivative **6** was prepared by condensation of 9-acetoxyellipticine (**8**) with 2,4-di-O-benzoyl-3-O-methyl-α-L-

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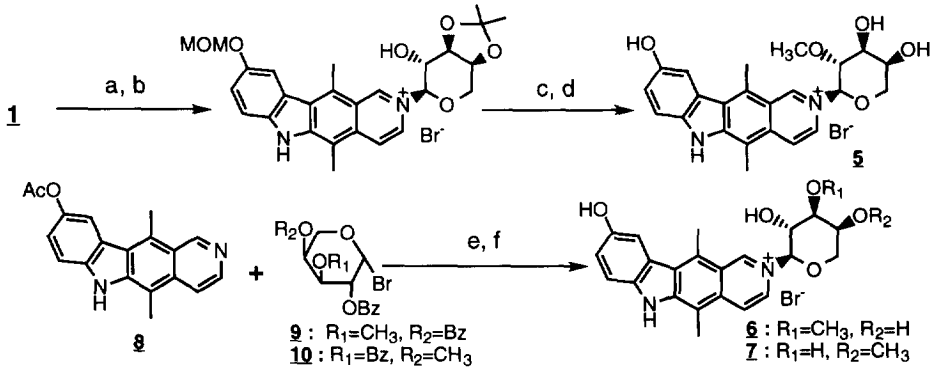
arabinopyranosyl bromide (**9**)[8] in the presence of $CdCO_3$ in CH_3NO_2,[9] and following deprotection of the O-acyl groups. 4'-Mono-O-methyl derivative **7** was prepared in the same way from 2,3-di-O-benzoyl-4-O-methyl-α-L-arabinopyranosyl bromide (**10**).[8]

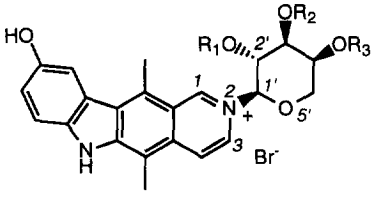


Scheme 1. Preparation of O-methyl Derivatives

a) 2,2-dimethoxypropane / p-TosOH ; b) CH_2OCH_2Cl / $iPrNEt_2$; c) CH_2N_2 / $BF_3{\cdot}OEt_2$ / 0℃ ; d) 90%AcOH / 0.05%HBr / 90℃ ; e) $CdCO_3$ / CH_3NO_2 / reflux ; f) NaOMe / MeOH.

Antitumor activity of those O-methyl derivatives are shown in Table **1**. The result implies an importance of all hydroxyl groups in the sugar moiety. In particular, C-2' hydroxyl group is of much significance for the activity and thus would have a considerable influence on the molecular recognition in DNA-drug and/or the ternary complex formation.

Table 1. Antitumor Activity of Ellipticine N-Glycoside Derivatives against L1210 in Mice[a]



Compd No.	R_1	R_2	R_3	T/C(%)[b]	Activity Score[d]
1	H	H	H	>960[c]	++
5	CH_3	H	H	123	-
6	H	CH_3	H	169	+
7	H	H	CH_3	167	+

a) L1210 cells (10^5) were implanted into the abdominal cavity of the BDF_1 mice and each compound (30mg/kg) was injected intraperitoneally for 5 consecutive days. ; b) Average percentage of life time in 6 mice, treated mice / control. ; c) Survival mice over the period of 80 days were appeared. ; d) According to the criteria of NCI, T/C >125% is regarded to be active.

Hence, complex structure models have been constructed in order to investigate the role of the hydroxyl groups, using $G_1C_2G_3C_4G_5C_6$ hexamer as a double helical B-form DNA model.[10] Four initial models of the complex were built in which the sugar moiety resides at either major or minor groove and ellipticine moiety intercalates between G_3 and C_4 by two modes as shown in Figure **2**. A parallel mode (left in Fig. **2**) follows the mode of ellipticine(**2**),[11] while the other is a vertical mode (right) as found in the crystal structure of the daunomycin(**4**)-DNA complex.[12] The initial water-solvated complex models, unwinding the base pair in proper degrees (20° for the parallel mode[13,14] and 8° for the vertical mode[11]), were optimized using AMBER force field[15,16] implemented in program MACROMODEL.[17] The N-glycosyl derivatives took the vertical mode, starting from both of the intercalating modes. This preference may be largely due to unfavoured sterical repulsions between DNA-phosphate group and the sugar moiety in the parallel intercalation.

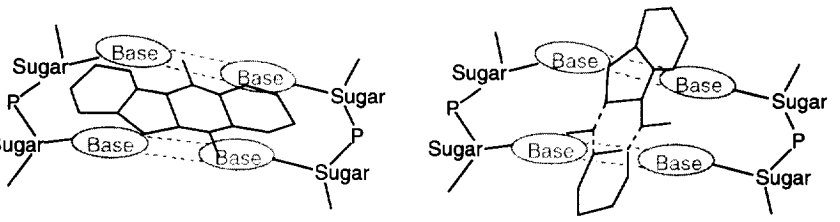


Figure 2. Two possible intercalation modes of the ellipticine. Parallel mode (left) and vertical mode(right).

Through conformational search on the torsion angle of the glycosidic bond (C1-N2-C1'-O5') in the complex models, two energy minima were found at -125.8° and 76.1° for the arabinose residue in the minor groove binding (structures **A** and **B** in Table **2**) and the other two energy minima at -118.5° and 94.2° for major groove binding (structures **C** and **D**). The conformation of the sugar moiety in the most stable structure **C** is consistent with the solution structure of **1** obtained by NMR experiment which indicated the presence of NOE between C-1 and C-1' protons in DMSO.[18]

Table 2. Torsion Angle and Minimized Energy of Ellipticine Glycoside - DNA Complex

Complex Structure	Torsion Angle of Glycosidic Bond (degree) Initial Low-Energy Structure[b]	Minimized Structure[c]	Minimized Energy (kcal / mol)
A (minor)[a]	-120	-125.8	-258.3
B (minor)	90	76.1	-255.2
C (major)	-110	-118.5	-268.8
D (major)	100	94.2	-251.7

a) The groove of sugar location. ; b) Initial 36 conformations were generated by rotating the torsion angle of glycosidic bond (1C-2N-1'C-5'O) with 10 degrees interval in minor or major groove binding. ; c) Minimized structures were obtained from the four low-energy conformers.

Daunosamine moiety in the crystal structure of the DNA-daunomycin complex resides at the minor groove. However, the lowest-energy structure of the present complex model indicated the N-glycosyl moiety preferentially binds at major groove. Since the arabinopyranosyl moiety is connected directly to the intercalating moiety (hydroxyellipticine), this sugar moiety would prefer major groove to minor one due to sterical repulsion at the narrow minor groove. Figure **3** shows the optimized-complex structure **C** in which the C-2' hydroxyl group rests in the position, forming hydrogen bonds[19] with C4'-amino group of cytidine and C6'-carbonyl of guanine, while the C-3' and -4' hydroxyl groups locate outbound at the surface.

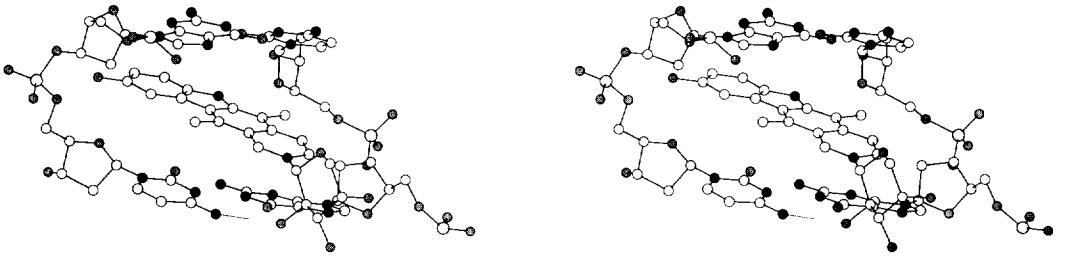


Figure 3. Stereoview of the Complex Structure **C**

Only the ellipticine-N-glycoside **1** and base-pairs stacking the guest molecule are shown and hydrogen atoms are omitted for clarity. Each atom is described as follows (C : open circle; N : closed; O, P : shaded). Dotted lines show plausible hydrogen bonds between C-2' hydroxyl group and base pair.

Complex models of DNA and the O-methylated N-glycosyl derivatives (**5**, **6**, and **7**) were constructed from the complex model **C**, substituting 2'-, 3'-, or 4'- hydroxyl group with methoxyl group, respectively.[20] The glycosidic torsion angles and averaged difference of the ellipticine and arabinose moieties of the energy-minimized structures from the complex structure **C** are listed in Table **3**.[21] The 2'-O-methylated sugar moiety of the compound **5** showed a remarkable rotation of the glycosidic bond and a consequent shift of the position of each moiety, while the other two derivatives **6** and **7** could stay at the initial position. This difference accounts for the significance of the C-2' hydroxyl group for the activity. Thus, the C-2' hydroxyl group would have a role for binding to DNA, whereas the C-3' and C-4' hydroxyl groups would be involved in the recognition by the topoisomerase II.

Table 3. Conformational Difference of the Methylated Ellipticine Glycoside in the DNA Complex Models

Compd No.	Substitution[a] R_1	R_2	R_3	Torsion Angle[c] (degree)	Difference[d] (degree)	Distance of Displacement (Å)[b] Ellip.	Sugar
1	H	H	H	-118.5	-	-	-
5	CH_3	H	H	-89.2	+29.3	0.99	1.67
6	H	CH_3	H	-117.3	+1.2	0.29	0.29
7	H	H	CH_3	-115.0	+3.5	0.25	0.23

a) Structure is depicted in the figure of Table 1. ; b) Average value of atomic deviation of each moiety. ; c) C1-N2-C1'-O5'. ; d) Difference of the torsion angle of each derivative from compound **1**.

References and Notes

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18. Conformation search of ellipticine-N-glycoside **1** alone, gave similar energy-minimum conformations having the torsion angle of the glycosidic bond at -122.8° and 62.7°, respectively. NOE experiment of compound **1** indicated the presence of only one conformation at the former torsion angle in DMSO-d_6, whereas the both conformations were observed in D_2O (*i.e.*, NOEs between C_1 and C_1', and C_3 and C_1'.
19. A-T base pair has the same hydrogen bond at the major groove.
20. All the DNA atoms were fixed for the minimization of the complex structure of the methylated ellipticine derivatives with the DNA.
21. Complex models of DNA and O-methylated derivatives for the other conformations (**A**, **B**, and **D**) were also examined. A similar structural change of the 2'-O-methylated derivative was obtained from the conformation **A**. However, no significant conformational change was observed in the other remaining models.

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