# Molecular Details of the Structure of a Psorospermin−DNA Covalent/Intercalation Complex and Associated DNA Sequence Selectivity

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*Received January 30, 1996*[⊗]

**Abstract:** Psorospermin is a DNA-reactive natural product isolated from the roots of the tropical African plant *Psorospermum febrifugum* that shows significant promise as an antileukemic agent. Incubation of this antineoplastic agent with DNA results in the production of sequence selective abasic sites on the DNA. Using high-field NMR and gel electrophoresis, the mechanism of covalent modification of DNA and the mode of interaction with DNA are determined. Psorospermin intercalates the DNA molecule, positioning the tricyclic xanthone chromophore in an orientation parallel to the adjacent base pairs. This places the epoxide in the major groove, resulting in site-directed electrophilic addition of the epoxide to N7 of guanine located to the 3′ side of the site of DNA intercalation. It is proposed in this study that the subsequent depurination of the psorospermin−*N*7-guanine adduct is the source of the previously observed *in vivo* formation of abasic sites on the DNA. Significantly, although the chemical structure and mechanism of covalent modification of DNA are very similar to those of the pluramycin class of agents, there are distinct differences in the relative reactivities and sequence selectivity between psorospermin and pluramycin-like compounds that may give rise to observed variances in biological activity. Specifically, while psorospermin is much less reactive than most of the pluramycins, it shows unique selectivity for 5′GG* sequences (the asterisk designates the site of covalent modification), which is the least reactive site for all of the pluramycins investigated thus far. On the basis of the solution NMR structure of the psorospermin−DNA covalent adduct, the underlying structural differences that give rise to this lower reactivity and different sequence specificities are proposed.

## Introduction

Psorospermin (Figure 1) is a novel cytotoxic dihydrofuranoxanthone isolated from the roots and stembark of the African plant *Psorospermum febrifugum*. The structure and complete stereochemistry of psorospermin have been established by Kupchan and co-workers[1] and Cassady and co-workers.[2] Psorospermin and related compounds show significant cytotoxicity against a variety of human tumor cell lines in tests carried out by the National Cancer Institute. On the basis of differential activity against resistant human leukemia lines and AIDS-related lymphoma, psorospermin has been selected for further development by the National Cancer Institute's Biological Evaluation Committee.[3] The suspected source for these biological activities is the compound's ability to readily react with DNA and produce DNA strand breaks, abasic sites, and protein−DNA cross-links in psorospermin-treated cells through an as yet unestablished mechanism.[4] On the basis of structural similarities with the pluramycin class of DNA-reactive agents (altromycin B and hedamycin in Figure 1), it has previously been proposed that psorospermin interacts with DNA in a manner similar to that of the pluramycin class of agents, which intercalate into the DNA helix and covalently modify guanine through epoxide-mediated electrophilic attack on N7 of guanine.[5]

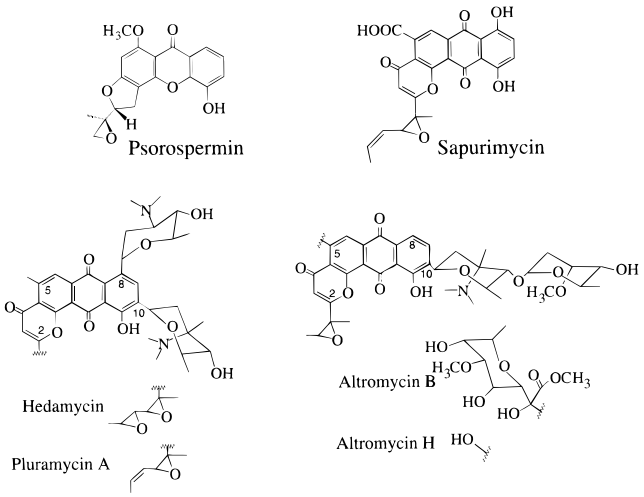


**Figure 1.** Structures of the antileukemic psorospermin and structurally analogous pluramycins: sapurimycin, hedamycin, pluramycin A, altromycin B, and altromycin H.

In the present study, we have used gel electrophoresis and high-field NMR to show that psorospermin alkylates N7 of guanine, to propose a mechanism for covalent attachment, and to determine the DNA sequence selectivity for this covalent reaction. Like the pluramycins, psorospermin intercalates the

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⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1996.

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S0002-7863(96)00319-8 CCC: $12.00 

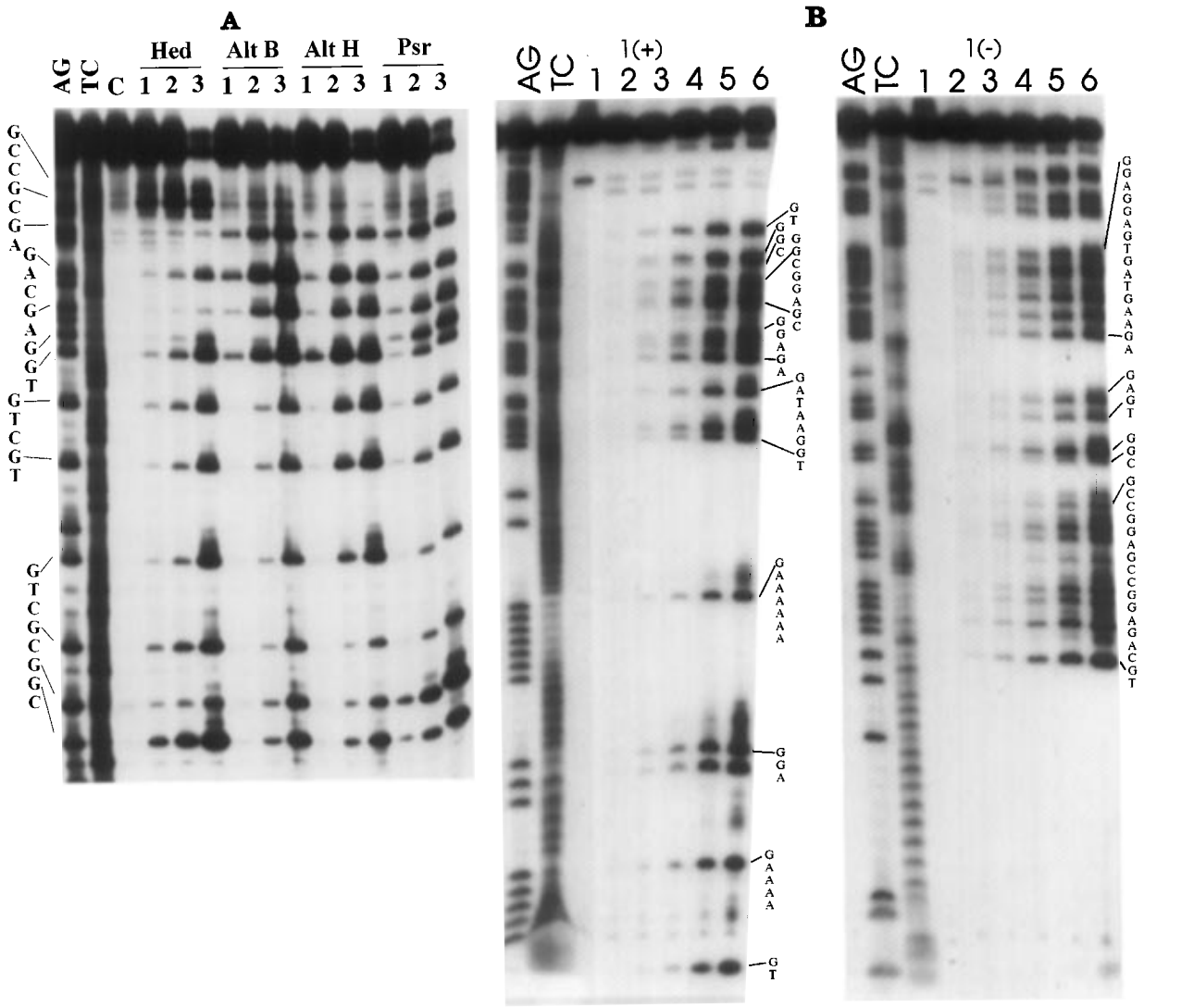


**Figure 2.** (A) Gel showing comparative strand breakage of hedamycin (Hed), altromycin B (Alt B), altromycin H (Alt H), and psorospermin (Psr). Lanes are for hedamycin, (1) 0.001 $\mu$M, (2) 0.01 $\mu$M, and (3) 0.05 $\mu$M; altromycin B, (1) 0.01 $\mu$M, (2) 0.03 $\mu$M, and (3) 0.3 $\mu$M; altromycin H, (1) 0.1 $\mu$M, (2) 1 $\mu$M, and (3) 10 $\mu$M; and psorospermin, (1) 0.1 $\mu$M, (2) 0.5 $\mu$M, and (3) 2.5 $\mu$M. (B) Gel showing concentration dependent strand breakage by psorospermin modification. Psorospermin concentrations are 0, 0.1, 1, 10, 100, and 1000 $\mu$M (lanes 1−6, respectively). On the left is the 1(+) strand and on the right is the 1(−) strand of the restriction enzyme fragment shown in Figure 3A on the opposite page. AG and TC represent purine- and pyrimidine-specific sequencing lanes.

DNA and positions the reactive epoxide in the major groove into proximity of N7 of guanine, which is located to the 3′ side of the intercalation site. NMR results indicate that covalent attachment occurs between N7 of guanine and C4′ of the epoxide on the psorospermin ligand. However, despite these similarities, the proposed precovalent mode of DNA binding is more similar to the acridine class of agents than the pluramycins. Psorospermin stacks its aromatic chromophore in an orientation parallel to the adjoining base pairs, as opposed to an orthogonal orientation characteristic of the pluramycins. In this respect, the psorospermin−DNA interaction resembles that of the quinacrine nitrogen mustard and that of the mutagen, sterigmatocystin.[6]

## Results

**Psorospermin Produces DNA Strand Breaks That Comigrate with Guanine Sequencing Lanes.** Incubation of psorospermin with 5′-end-labeled DNA, followed by heat treatment at 95 °C, produces DNA cleavage patterns on DNA sequencing gels. As seen in Figure 2A, psorospermin-modified DNA produces strand breakage patterns on polyacrylamide gels that comigrate with guanine in Maxam and Gilbert sequencing lanes. The ability of psorospermin to chemically modify guanine and thermally induce these single strand breaks in a fashion similar to that of the pluramycin agents is indicative of a depurination reaction of an alkali labile *N*7-alkylguanine adduct.[7] Although at this point it is inconclusive, the most likely mechanism of covalent modification that would produce these strand breaks at guanine would be electrophilic addition of the reactive epoxide to N7 of guanine, a mechanism much like that of the pluramycins[5] and the aflatoxins.[8]

**Reactivity and Sequence Recognition of Psorospermin**. In the strand breakage assay shown in Figure 2A, psorospermin displays significant differences in reactivity and sequence selectivity from those of the pluramycins. The most striking difference is the concentration at which psorospermin achieves reactivity with DNA equivalent to that of the pluramycins. Psorospermin is only comparable in reactivity with altromycin H, the least reactive of the pluramycins, and for equivalent reactivity is required at concentrations 10−100 times greater

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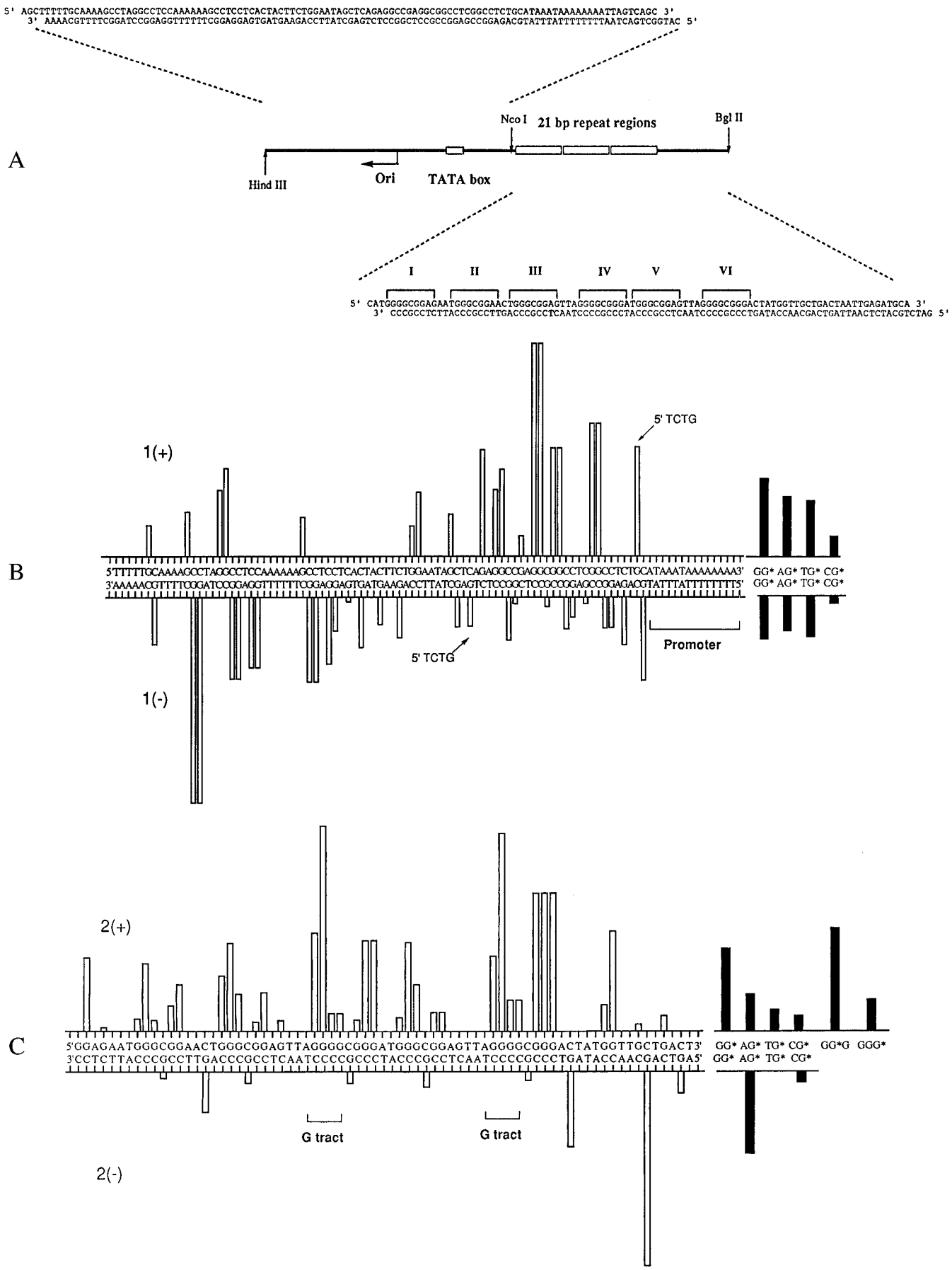


**Figure 3.** Strand breakage assays depicting reactive sites for psorospermin on the restriction enzyme fragments. Shown in (A) are the sequences of the restriction enzyme fragments containing the SV40 early promoter region used in this study. Shown in (B) and (C) are histograms depicting the relative strand breakage intensities on the restriction enzyme fragments used to compile Table 1. Histogram B corresponds to the gel shown in Figure 2B, while histogram C corresponds to strand breakage results on the other restriction enzyme fragment, included as supporting information. Inseparable bands from densitometric analysis are represented by the same height in histograms and were excluded in the calculation of average modification levels (shown as solid bars for the consensus sequences).

**Table 1.** Psorospermin High- and Medium-Reactivity Sites, the Base-Pair Compositions Flanking the Reactive Guanine, and the Consensus Sequence for Medium-Reactivity Sites, High-Reactivity Sites, and Combined High- and Medium-Reactivity Sites[a]

| high-reactivity sites | medium-reactivity sites | | | | | |
|---|---|---|---|---|---|---|
| 5′AGG*CT | 5′AGG*CC | 5′AGG*CC | 5′CGG*CC | 5′TAG*GG | 5′TAG*TG | 5′CGG*GA |
| 5′AGG*GG | 5′CAG*AG | 5′CGG*AG | 5′CTG*CA | 5′CGG*GA | 5′AGG*AG | 5′TGG*TT |
| 5′AGG*GG | 5′AGG*CG | 5′TGG*GC | 5′ATG*CA | 5′TGG*GC | 5′AGG*CT | 5′TAG*TC |
| 5′CAG*CA | 5′CGG*CC | 5′TGG*GC | 5′CAG*AG | 5′TAG*GG | 5′TGG*AG | 5′CAG*TT |

| high reactivity (4 sites) | | | | | | | medium reactivity (24 sites) | | | | | | | high and medium reactivity (28 sites) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5′ | −2 | −1 | G* | +1 | +2 | 3′ | 5′ | −2 | −1 | G* | +1 | +2 | 3′ | 5′ | −2 | −1 | G* | +1 | +2 | 3′ |
| G | 0 | 75 | 100 | 50 | 50 | | G | 0 | 63 | 100 | 29 | 37 | | G | 0 | 64 | 100 | 32 | 39 | |
| A | 75 | 25 | 0 | 0 | 25 | | A | 26 | 29 | 0 | 21 | 17 | | A | 32 | 29 | 0 | 18 | 18 | |
| T | 0 | 0 | 0 | 0 | 25 | | T | 37 | 8 | 0 | 17 | 13 | | T | 32 | 7 | 0 | 14 | 14 | |
| C | 25 | 0 | 0 | 50 | 0 | | C | 37 | 0 | 0 | 33 | 33 | | C | 36 | 0 | 0 | 36 | 29 | |
| Pu | 75 | 100 | 100 | 50 | 75 | | Pu | 26 | 92 | 100 | 50 | 54 | | Pu | 32 | 93 | 100 | 50 | 57 | |
| Py | 25 | 0 | 0 | 50 | 25 | | Py | 74 | 8 | 0 | 50 | 46 | | Py | 68 | 7 | 0 | 50 | 43 | |
| consensus | N | Pu | G* | N | N | | consensus | N | Pu | G* | N | N | | consensus | N | Pu | G* | N | N | |

[a] G* represents the covalently modified guanine. Pu signifies purine, and Py signifies pyrimidine.

**Table 2.** Comparison of the DNA Proton Chemical Shift[a] of the Bispsorospermin−[d(GATG*TACATC)]$_2$ Duplex Diadduct (2:1) with Those of the Unmodified Duplex

| base | H8/H6 | H5/5CH$_3$/H2 | H1′ | H2′/H2′ | H3′ | H4′ |
|---|---|---|---|---|---|---|
| 1G | 7.85 (0.00)[b] | | 5.58 (−0.07) | 2.67/2.51 | 4.84 (−0.02) | 4.15 (−0.07) |
| 2A | 8.30 (−0.13) | 7.95 (−0.07) | 6.27 (−0.13) | 2.90/2.74 | 5.07 (0.00) | 4.50 (−0.07) |
| 3T | 7.37 (+0.15) | **1.50 (−0.30)** | **6.05 (−0.31)** | 2.37/2.26 | 4.95 (+0.14) | **4.13 (−0.29)** |
| 4G* | **9.40 (+1.60)**[c] | | 5.94 (−0.11) | 2.79/2.74 | 4.95 (−0.18) | 4.42 (+0.07) |
| 5T | 7.20 (−0.10) | **1.45 (−0.24)** | **5.68 (−0.52)** | 2.12/2.48 | 4.87 (−0.12) | **3.17 (−0.43)** |
| 6A | 8.12 (−0.03) | | 6.08(−0.07) | 2.51/2.71 | 4.98 (−0.09) | 4.37 (0.00) |
| 7C | 7.55 (+0.14) | 4.85 (−0.10) | **5.70 (−0.34)** | 2.13/2.30 | **4.87 (+0.24)** | 4.15 (0.00) |
| 8A | **8.25 (+0.24)** | **7.52 (−0.25)** | 6.25 (−0.19) | 2.28/2.28 | 4.97 (+0.18) | 4.50 (−0.18) |
| 9T | 7.17 (−0.12) | 1.34 (−0.17) | 5.07 (−0.17) | 2.07/2.49 | 4.84 (+0.04) | **4.15 (−0.24)** |
| 10C | 7.62 (−0.15) | 5.75 (−0.18) | 6.27 (−0.17) | 2.26/2.26 | 4.56 (−0.03) | **4.01 (−0.21)** |

[a] Referenced to $H_2O$ in 99.9% $D_2O$ unless otherwise noted. [b] Chemical shift differences: (−) and (+) represent upfield and downfield shifts, respectively. Shown in bold are those greater than 0.2 ppm. [c] Exchangeable proton observed in 95% $H_2O$.

than that for the most reactive pluramycins.[9] A second major difference displayed by psorospermin is its sequence selectivity of alkylation. Analysis of strand breakage patterns on two restriction enzyme fragments (shown in Figure 3 and summarized in Table 1) reveals that psorospermin has a hierarchy of reactive sites. While there appears to be no consistent selectivity for bases to the 3′ side of the alkylated guanine, there is a distinct selectivity for the base pair to the 5′ side. For both high- and medium-reactivity sites, psorospermin shows the greatest preference for a guanine located to the 5′ side, a second preference for an adenine in the 5′ position, and only low reactivity with guanines having a pyrimidine at the same position. It is interesting that although the pluramycins also display a 5′ base pair selectivity, the sequence selectivity is significantly different from that of psorospermin. In contrast to psorospermin, pluramycins such as hedamycin and pluramycin A show the greatest reactivity with 5′(Py)G* sequences and the lowest reactivity with 5′GG* sites (the asterisk designates the site of covalent modification; Py = pyrimidine and Pu = purine).[9]

In the gel electrophoresis studies, there were two types of sequence sites of high reactivity for psorospermin that did not follow the simple consensus 5′(Pu)G* selectivity. First, from

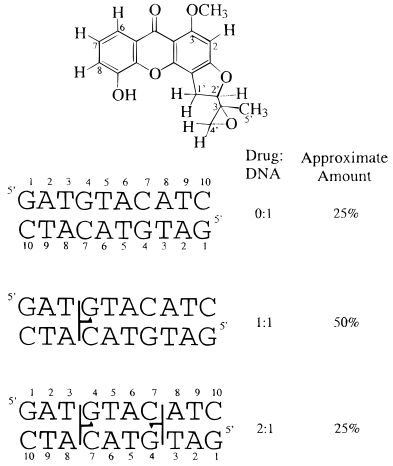


**Figure 4.** Structure and numbering scheme for psorospermin and the DNA decamer used in this study. Shown are sites of intercalation and relative amounts of each species.

both the concentration dependency (Figure 3C) and the time course kinetics experiments (data not shown), guanines located within a guanine tract demonstrated different levels of reactivity. While one would expect that each guanine following the first would be equally reactive, on the basis of the simple consensus 5′(Pu)G* selectivity, the second guanine was by far the most reactive, while each subsequent guanine to the 3′ side was less reactive than the previous one. In several sequences containing four contiguous guanines, the preference for alkylation was 5′GG*GG > 5′GGG*G > 5′GGGG*. Second, guanines located in proximity to the 3′ or 5′ side of the AT-rich promoter region had greater reactivity than guanines located distal to these regions. For example, in strand 1(+) of the restriction enzyme

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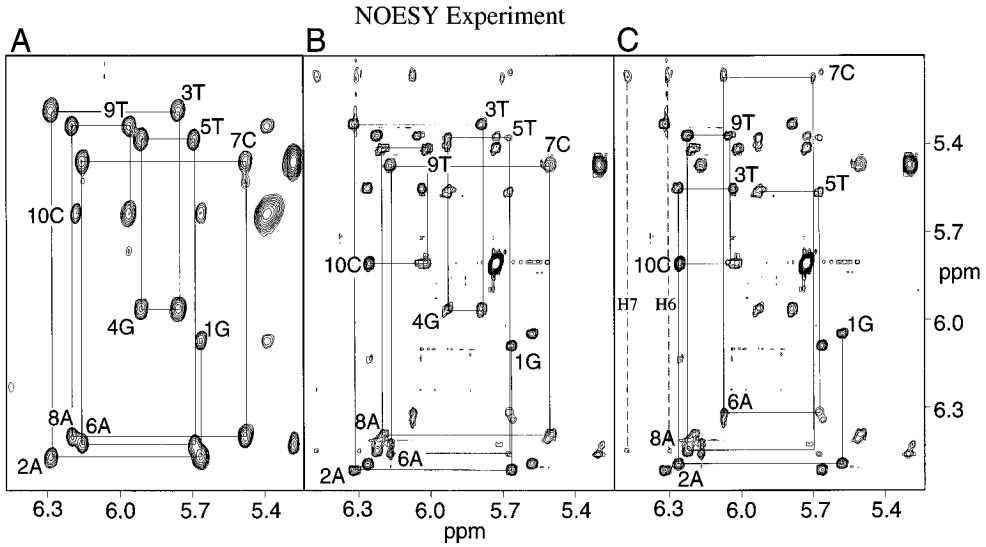


**Figure 5.** Aromatic to H1′ walks for an unreacted duplex (A), the free duplex (0:1) in a mixture with drug adduct species (B), and the psorospermin diadduct (2:1) in the reaction mixture (C).

**Table 3.** Comparison of Psorospermin Chemical Shifts in the Bispsorospermin−[d(GATGTACATC)]$_2$ Diadduct and the Free Drug

| proton assignments[a] | proton chemical shifts | | chemical shift differences[d] |
|---|---|---|---|
| | free drug[b] | 2:1 drug−DNA diadduct[c] | |
| H2 | 6.37 | 5.45 | 0.92 |
| 3′OCH$_3$ | 3.96 | 3.66 | 0.30 |
| H6 | 7.22 | 6.30 | 0.92 |
| H7 | 7.19 | 6.49 | 0.70 |
| H8 | 7.80 | 6.77 | 1.03 |
| H1′a/b | 3.30/3.49 | 2.86/3.31 | ⟨0.31⟩[e] |
| H2′ | 4.85 | 4.36 | 0.49 |
| H4′a/b | 2.73/2.98 | 4.24/5.20 | ⟨−1.87⟩[e] |
| 5′CH$_3$ | 1.43 | 1.76 | −0.33 |

[a] For numbering see Figure 4. [b] Chemical shifts in chloroform.[2] [c] Chemical shifts referenced to $H_2O$ in 99.9% $D_2O$. [d] Chemical shifts of free drug minus chemical shifts of drug in diadduct. [e] The number represents the average chemical shift difference.

fragment (see Figures 2B and 3B), the 5′TCTG* sequence adjacent to the AT-rich promoter region showed approximately 2 times higher reactivity than the same sequence located in the middle of the restriction enzyme fragment. Similar results were observed at the same junction on the opposite strand.

**Structural Interaction Made with DNA.** To study the structural interactions and determine the exact site of covalent attachment, the oligonucleotide [d(GATGTACATC)]$_2$ was prepared and reacted with psorospermin. Although psorospermin was reacted in a molar excess with the DNA, only a mixture of products corresponding to the 0:1, 1:1, and 2:1 drug−DNA adducts could be achieved in roughly a 1:2:1 ratio (see Figure 4). Due to limited amounts of available psorospermin, the sample was characterized using one-dimensional NMR experiments and two-dimensional NOESY and DQF-COSY experiments without further drug addition.[10] NMR resonances were identified for the subsets of protons corresponding to the free duplex (or 0:1 drug−DNA adduct in Figure 4) and for the 2:1 drug−DNA adduct. Resonances associated with the 1:1 drug−DNA adduct were roughly degenerate with those of the two other species; however, in some regions of the spectra, they were distinguishable from the 0:1 and 2:1 species. Similar to other guanine N7 alkylators,[5a,11] this sample degraded over several days, presumably due to depurination of the psorospermin−*N*7-guanine adduct.

**Assignment of the DNA Proton Resonances.** DNA assignments of the 10-mer duplex and the 2:1 diadduct were achieved through established methods[12] by identifying sequential NOESY connectivities between bases and creating walks leading from the 5′ end to the 3′ end of the DNA molecule; these are reported in Table 2. Shown in Figure 5A is the aromatic to H1′ walk for an unreacted duplex, in Figure 5B is the aromatic to H1′ walk for the unreacted duplex species contained in the mixture of adducts (0:1 adduct in Figure 4), and in Figure 5C is the aromatic to H1′ walk for the 2:1 diadduct species of the adduct mixture. Although the discontinuity between 7C and 8A in the aromatic to H1′ walk is partially obscured by the proximity of other cross-peaks, the aromatic to aromatic and the aromatic to H2′/H2″ walks for the drug adduct both demonstrate breaks at the 7C−8A steps due to the separation of the base pairs at this position to accommodate intercalation by the drug chromophore (unpublished results). Traversing the break in DNA connectivities are the psorospermin H6 and H7 aromatic protons, which show connectivities to both 7CH6 and 8AH8 on either side of the intercalation site (seen as vertical broken lines in Figure 5C). These results unequivocally position

**Table 4.** Summary of Intermolecular NOEs observed between Psorospermin and the 10-Mer Oligomer Duplex in the 300 ms Two-Dimensional NOESY Spectrum

| assignment[a] | 3TH1′ | 3TH3′ | 3TH2′/2″ | 3TH6 | 3TCH$_3$ | 4GH1′ | 7CH2′/2″ | 7CH5 | 7CH6 | 8AH2 | 8AH8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H2 | x | x | x | | | x | | | | | |
| H6 | | | | | | | x | | x | | x |
| H7 | | | | | | | x | x | x | | x |
| H8 | | | | | | | | x | | | |
| H1′a/b | | | | | x | | | | | | |
| H2′ | | | | | x | | | | | | |
| H4′a/b | | | | | | | | | | | |
| 5′CH$_3$ | | | x | x | x | | | | | | |
| 3OCH$_3$ | x | | | | | x | | | | x | |

[a] See Figure 4 for assignments.

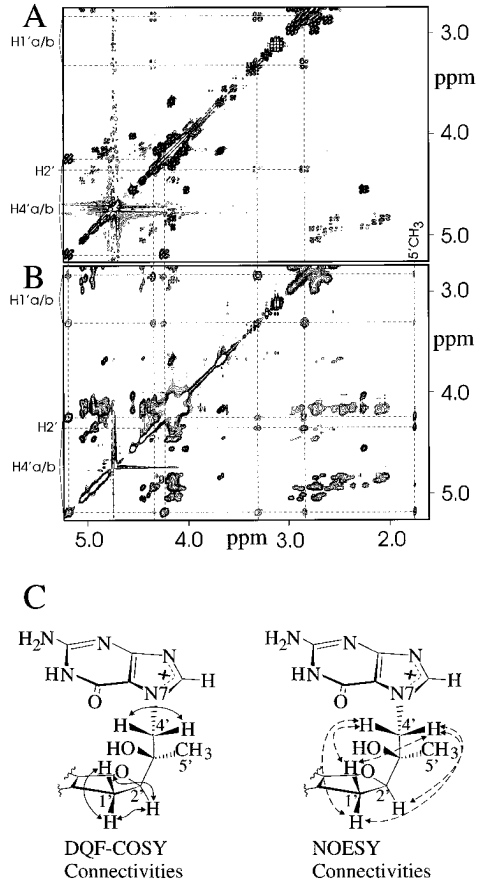


**Figure 6.** Figure showing the assignment strategy for the psorospermin proton resonances associated with the site of covalent attachment. Shown are a DQF-COSY spectrum (A), a NOESY (300 ms) spectrum (B), and a diagrammatic summary of these connectivities (C).

the drug chromophore directly to the 5′ side of the covalently modified guanine between the 3T·8A and 4G·7C base pairs.

**Assignment of Psorospermin Proton Resonances.** Psorospermin resonances were assigned using NOESY and DQF-COSY experiments and are listed in Table 3. The H6, H7, and H8 protons were identified on the basis of couplings between H7 and H8 and between H7 and H6, and the H8 proton was distinguished from the H6 proton on the basis of NOE contacts with 7CH5 and 7CH6. Positioned closer to the minor groove is the psorospermin H6 proton, which shows NOE connectivity to 7CH6. Contrasting this is the psorospermin H8 proton, which is located near the major groove and shows NOE contact with the 7CH5 proton. The psorospermin 3-methoxy signal was assigned by its chemical shift and one-dimensional resonance intensity. Identifying the psorospermin H2 proton is a strong NOE between itself and the 3-methoxy signal.

In Figure 6 is shown the assignment strategy for psorospermin protons associated with the site of covalent modification to the DNA. The H1′a and H1′b protons coupled with each other and to the H2′ proton. All three protons showed NOEs to the H4′a, H4′b, and 5′-methyl resonances. Similarly, H4′a and H4′b coupled to each other and showed NOE connectivities to the H2′ proton and the 5′-methyl. Because a single NOESY was acquired with a relatively long mixing time (300 ms), spin diffusion prevents unequivocal distinction between the H1′a and H1′b and between the H4′a and H4′b methylene protons.

**Psorospermin Covalently Modifies N7 of 4G through C4′ of the Epoxide.** On the basis of the chemical shift and the chemical nature of the DNA 4GH8 and the psorospermin H4′a and H4′b protons, we propose that N7 of guanine performs nucleophilic addition to C4′ of psorospermin to open the epoxide, leaving a residual hydroxyl on C3′ (Figure 7). The H8 aromatic proton of the drug-modified guanine (4G) is shifted to 9.4 ppm, which is approximately 1.5 ppm downfield in chemical shift of the equivalent proton in the unmodified duplex. Due to rapid exchange of this proton resonance with $D_2O$, the aromatic proton was identified and characterized in one-dimensional irradiation experiments performed in 95% $H_2O$. The 4GH8 resonance was assigned on the basis of NOE connectivities to the 4GH2′, 4GH2″, 5T methyl, and psorospermin 5′-methyl resonances. The acidic nature of this proton demonstrates the formation of the cationic adduct resulting as a consequence of alkylation of N7 of guanine (see Figure 6C). This agrees with previously reported *N*7-alkylguanine adducts,[5a,8,11] in which the H8 proton of the modified guanine has become highly exchangeable and similarly resonates between 9.5 and 10.0 ppm, significantly downfield of the equivalent proton in the unmodified duplexes. In addition, the downfield chemical shifts of psorospermin's 4′-methylene proton resonances, 2.73 and 2.98 ppm in the unreacted species compared with 4.75 and 5.25 ppm in the psorospermin−drug adduct, strongly argue that covalent attachment occurs between C4′ on the drug molecule and N7 of guanine, creating the positively charged purine adduct seen in Figure 7. Large changes in chemical shifts for protons attached two bonds away from similarly charged guanines are also experienced in both the hedamycin− and the altromycin B−(guanine N7)-DNA adducts.[5a,11]

**Interdrug−DNA Connectivities.** Summarized in Table 4

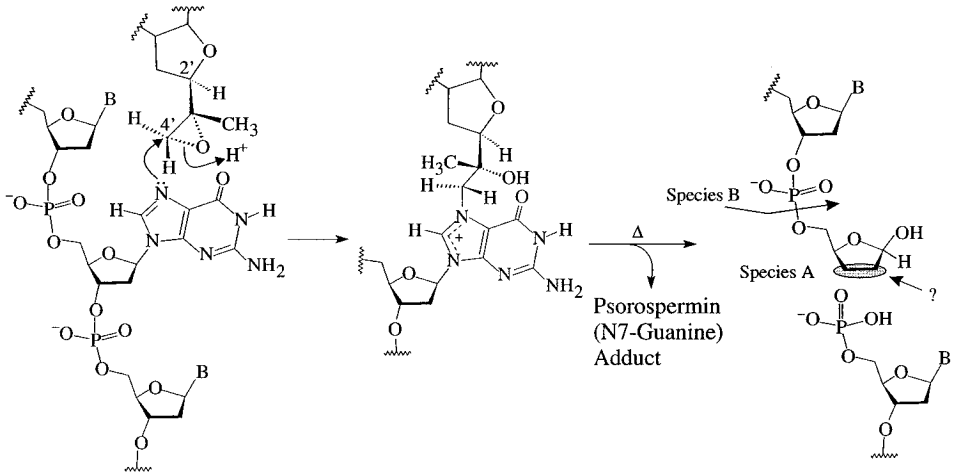


**Figure 7.** Summary of the proposed mechanism of covalent modification of DNA by psorospermin. The drug molecule intercalates the DNA molecule to position the reactive epoxide into the major groove to perform site-directed electrophilic addition on N7 of guanine. Subsequent thermal treatment induces depurination, which in turn results in strand breakage.

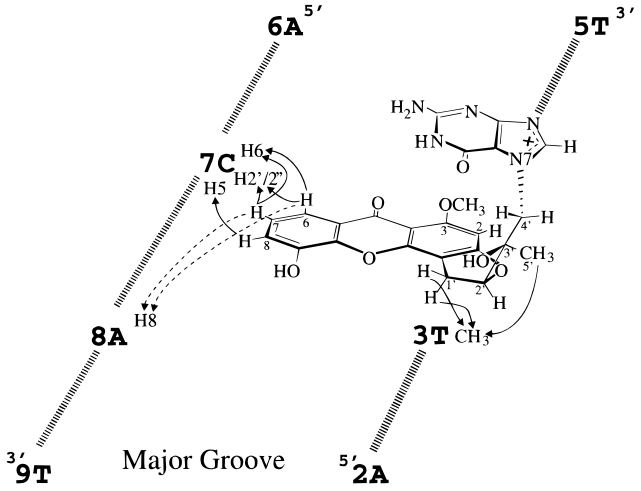


**Figure 8.** Diagrammatic summary of NOESY connectivities between psorospermin and the major groove. Intermolecular connectivities show that the long axis of the intercalating chromophore lies in a roughly parallel orientation to the adjoining base pairs.

are the NOE connectivities between the DNA oligomer and psorospermin (diagrammatically represented in Figure 8). NOEs between psorospermin and the DNA position the xanthone chromophore to the 5′ side of the modified guanine, intercalated in an orientation parallel to the adjacent 3T·8A and 4G·7C base pairs. Positioned adjacent to the nonmodified strand are the H6, H7, and H8 protons of psorospermin, which show NOE connectivities to the 7CH2′/H2″, H5, H6, and 8AH8 DNA protons. In the minor groove is the 3-methoxy of psorospermin, which shows NOE contacts to both the 3TH1′ and 4GH1′ (not shown in Figure 8). At the site of covalent attachment in the major groove, the 3T methyl shows NOE contacts to psorospermin's H1′a/b, H2′, and 5′-methyl. The 3TH6 and 3TH1′ also show NOE connectivities to the psorospermin 5′-methyl. Illustrated in Figure 9 is a molecular model depicting the interaction of psorospermin with DNA. This model was derived by docking psorospermin into the DNA on the basis of the NMR results summarized in Table 4 and Figure 8, followed by energy minimization in AMBER.[13]

## Discussion

Psorospermin interacts with DNA in a fashion very similar to that of other DNA intercalative alkylators.[5a,6,8,11] Like both the aflatoxins and the pluramycins, psorospermin intercalates into the DNA helix and can alkylate guanine located to the 3′ side of the intercalation site through nucleophilic attack by N7 of guanine on a drug resident epoxide. As seen in Figure 1, the psorospermin tetracyclic system is close in chemical structure to that of the same moiety in the pluramycins, leading one to assume similarities in drug action. However, the intercalating xanthone chromophore behaves differently from the anthraquinone chromophore of the pluramycins (compare parts A and B of Figure 10). Instead of intercalating with the axis of the tetracyclic chromophore in a perpendicular fashion between the DNA base pairs (Figure 10B), as the pluramycins and the anthracyclines do, psorospermin positions the xanthone ring structure in an orientation parallel (Figure 10A) to the adjacent base pairs. In this respect, psorospermin resembles more the alkylating acridine quinacrine and the aflatoxin precursor, sterigmatocystin (Figure 11) than the pluramycins. Quinacrine, a nitrogen mustard attached to an acridine moiety, also intercalates the DNA with the chromophore in an orientation parallel to the adjoining base pairs, but differs in that it alkylates a guanine located *two* base pairs away to the 5′ side of the intercalation site.[14] Sterigmatocystin is the most closely analogous in both chemical structure and mode of interaction with

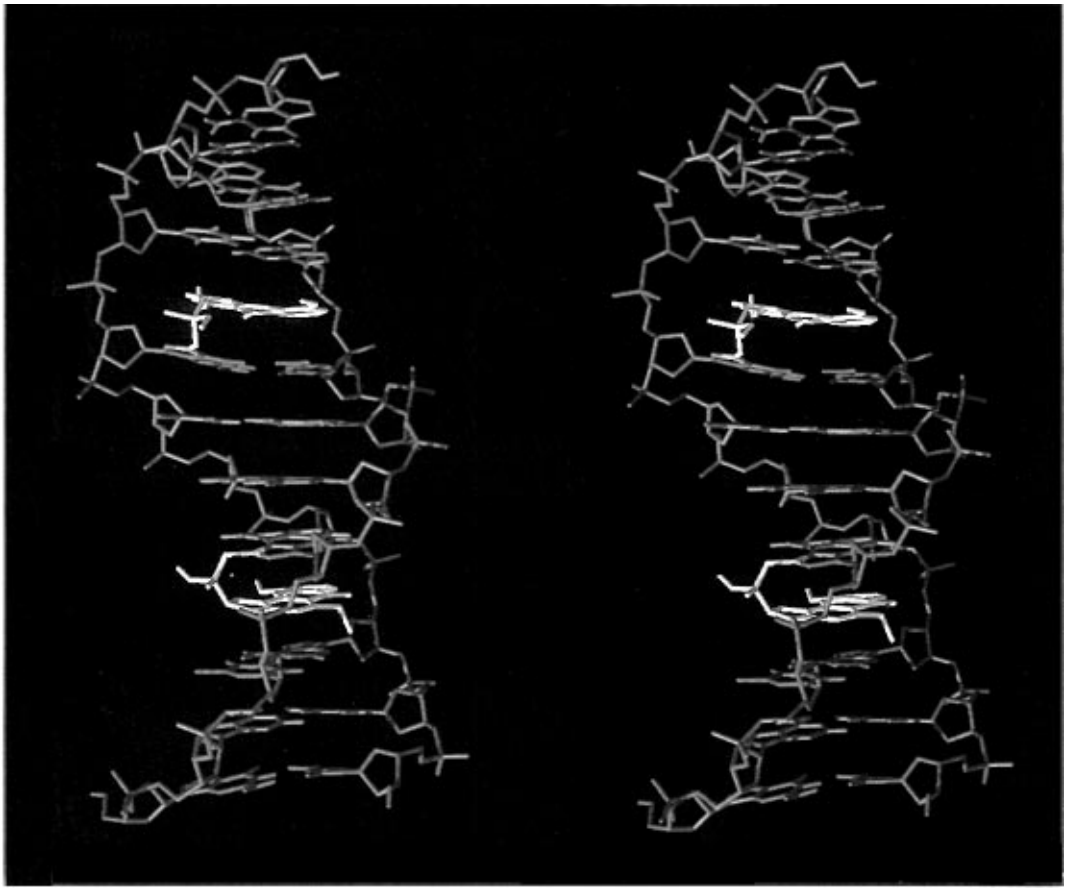

**Figure 9.** Molecular representation of the interaction of the psorospermin−DNA lesion. This model was generated using MIDAS[21] to dock psorospermin to the DNA, such that NOE connectivities were satisfied, and was then energy minimized using AMBER.[13]

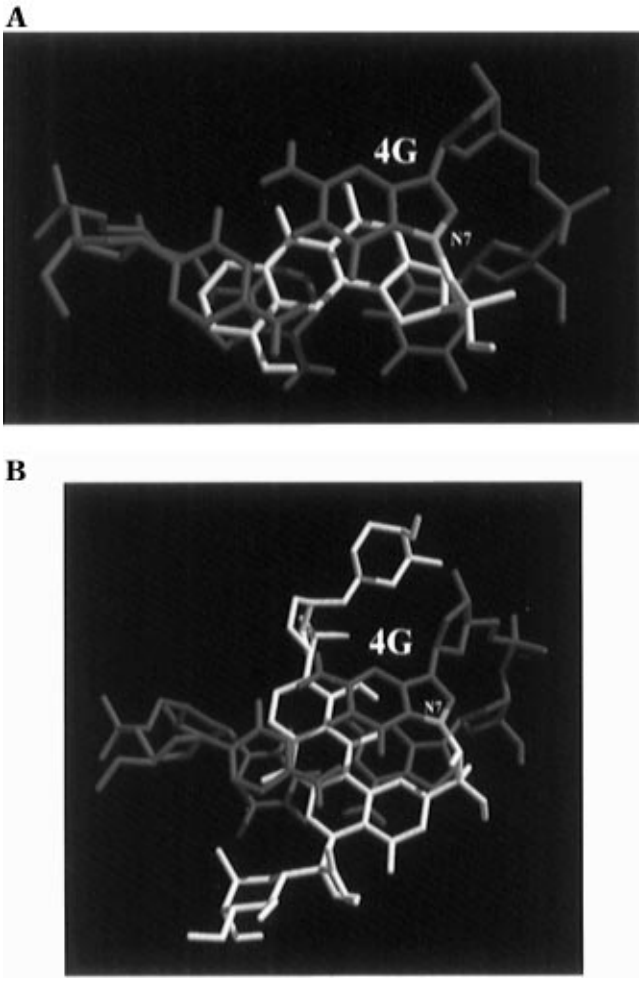


**Figure 10.** Molecular models depicting the stacking of the intercalating chromophore between the adjacent DNA base pairs. Shown in (A, top) is psorospermin, which positions the xanthone chromophore parallel to the adjacent base pairs, and shown in (B, bottom) is altromycin B, which in contrast positions its anthraquinone chromophore orthogonal to the adjacent base pairs.[11]

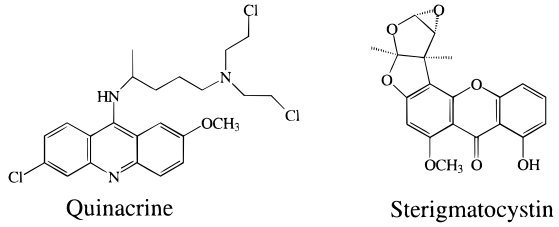


**Figure 11.** Chemical structure of the nitrogen mustard quinacrine and sterigmatocystin. The structural interaction between psorospermin and the DNA molecule used in this study resembles that of both of these compounds.

DNA. This compound also intercalates the DNA, positioning its chromophore in a parallel orientation, and it covalently modifies N7 of guanine located to the 3′ side of the intercalation site.[6b]

Because of structural similarities, it is not obvious what structural differences between sapurimycin and psorospermin (see Figure 1) dictate the orientation of the intercalating chromophore relative to the adjacent base pairs. It is possible that both agents noncovalently interact with the DNA in both orientations (i.e., parallel and perpendicular to the adjacent base pairs); however, only the orientation trapped out through covalent attachment is experimentally observed. This would argue that one role played by the carbohydrate substituents attached to the chromophore of many of the pluramycin agents would be to dictate the orientation of intercalation into the DNA to position the epoxide into proximity of N7 of guanine and thereby enhance DNA reactivity. A careful examination of the psorospermin chemical structure reveals that in order for psorospermin to also alkylate N7 of guanine located to the 3′ side of the intercalation site, the mode of precovalent binding requires that the chromophore stack *parallel* to the adjacent base pairs. Due to the stereochemistry at the 2′ position, orientation of the chromophore *perpendicular* to the adjoining base pairs would require positioning the reactive epoxide to the opposite face of the intercalating chromophore or into proximity of the base located to the 5′ side of intercalation. In retrospect it is intuitive that psorospermin would intercalate the DNA in a parallel orientation, alkylate a guanine located to the 3′ side of the intercalation site, and in doing so position the intercalating chromophore to maximize stacking interactions with the base pair to the 5′ side of the modified guanine, which is also the base pair demonstrating the greatest effect on sequence reactivity. Although the parallel orientation of the intercalating chromophore of psorospermin maximizes the stacking interactions, the kinetic acceleration of the alkylation step produced as a consequence of these noncovalent binding interactions is up to 100 times less than the optimum pluramycin alkylation rates. The absence of alkylation on the 5′ side of the intercalation, which would be a consequence of a perpendicularly oriented chromophore, suggests that only parallel stacking provides sufficient binding interactions to result in covalent reactivity.

It appears that although similar in structure, there exist differences in the interaction of psorospermin, quinacrine, and the pluramycins with DNA that are significant enough to engender each agent with different sequence selectivities for the alkylation of guanine. For example, while the pluramycins demonstrate the lowest reactivity with 5′GG* sequences,[9] this is the most reactive site of psorospermin. The sequence selectivity of psorospermin is also in contrast with quinacrine, which shows a specificity for 5′G*G(Pu) and 5′G*T(Pu) sequences.[15] While sequence selectivity can be explained for the pluramycins on the basis of interaction of attached glycosidic substituents in the DNA grooves,[9] and for quinacrine on the basis of the ability of the nitrogen mustard side chain to stretch over the intervening base pair to alkylate electrostatically favorable sites,[15] the sequence selectivity demonstrated by psorospermin appears to be dictated by more subtle interactions with the DNA molecule. It is attractive to consider the possibility that stacking interactions at a 5′(Pu)G* step position the epoxide into better proximity to N7 of guanine than a 5′-(Py)G* step (kinetic acceleration) and that this then accounts for sequence selectivity. However, as yet data that would support this hypothesis are lacking.

Significantly, similar to many of the nitrogen mustards, including quinacrine, psorospermin displays elevated reactivity in runs of guanines, compared to guanines located in other sequences. In the case of the nitrogen mustards, this was partially attributed to the enhanced nucleophilicity of N7 of guanine caused by a local increase in the electronegativity near the guanine N7 position due to neighboring bases.[15] The electronic effects of DNA bases on their neighboring base pairs have been calculated, and it has been shown that a guanine residue is able to increase the electrostatic potential of N7 of a guanine residue located either to the 3′ or the 5′ side on the same DNA strand.[16] Although the intercalating chromophore

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breaks up the run of guanines to the 5′ side of the modified guanine, a series of guanines located to the 3′ side of the reactive guanine should exhibit similar additive electrostatic effects. This would explain why guanines located to the 5′ side in runs of guanines are more reactive toward psorospermin than guanines located toward the 3′ end. Intercalating at the 5′ end would maintain the maximum number of adjacent guanines in a row to the 3′ side. The experimental observation that a hierarchy exists in runs of guanines at least up to four base pairs suggests that the molecular interactions that give rise to this sequence specificity are very subtle.

The global context can also dramatically affect the individual reactivity of specific guanines to psorospermin. For instance, the reactivity of the guanine in the 5′TCTG* sequence is dramatically enhanced by its proximity to the AT tract. Presumably, the junction between the AT tract and the 5′TCTG* sequence creates a special site, perhaps due to dynamic effects forming a partially unwound site 5′ to the alkylated guanine. An analogous effect has been noted with the TATA binding element and pluramycin. In this case, binding of TBP to the TATA elements creates a high-reactivity site for pluramycin that is located specifically to the downstream side of the TATA element.[17]

Because of the frequent occurrence of GC-rich sequences in genes associated with cell proliferation, it has been suggested that these regions could be the preferred sites of damage for GC selective agents such as the nitrogen mustards.[15] Included with these GC-rich regions are DNA elements critical for cell growth. One example of this would be the Sp1 regulatory elements shown in Figure 3A, which are involved in the control of gene expression and contain multiple, nine-base-pair GC-rich consensus sequences, 5′(T/G)GGGCGG(A/G)(G/A).[18] Last, sites of abnormally high reactivity for psorospermin may exist in regions of unusual structures (i.e., DNA repeats) at junction sites (see above) and at or around sites where proteins bind to duplex DNA to produce distortions of DNA. In this regard there are other examples of unique ternary drug−DNA−protein complexes.[17,19]

In conclusion, we have established a structural model for the psorospermin−*N*7-guanine DNA adduct and determined the optimum sequences for covalent reaction. Quite unexpectedly, we find the orientation of the intercalated moiety of psorospermin relative to the adjacent base pairs to be different from that of the structurally related pluramycins, and the sequence selectivity is also different. Our solution NMR-derived model of the psorospermin−DNA adduct permits us to rationalize the apparent orientation anomaly and the high reactivity of guanine in select sequences (e.g., G-tracts), but a firm rationale for the 5′(Pu)G* sequence selectivity is still lacking. Although simpler in structure than the pluramycins, psorospermin is of more therapeutic interest than the more complex pluramycins. The biochemical consequences of DNA adduct formation by the pluramycins and psorospermin are also different, and it will be important to identify the structural basis for these differences that lead to the more therapeutically interesting compounds.

## Experimental Procedures

**Comparison of the Reactivity and Sequence Selectivity.** A 45-mer oligonucleotide duplex containing a variety of guanines was synthesized, purified, and labeled, as described previously.[5b] The duplex was incubated with the indicated amount of drugs at 37 °C for 30 min. Reactions were stopped with the addition of excess calf thymus DNA. Strand breakage was performed in 1 M piperidine at 95 °C for 30 min. Dried DNA samples were dissolved in alkaline dye and subjected to 16% denaturing polyacrylamide gel electrophoresis. Autoradiograms were analyzed using a laser densitometer (LKB2202) coupled to a recording integrator (LKB2220).

**Preparation of Labeled Restriction Enzyme Fragments and Drug Modification.** Plasmid DNA pCAT (Promega, WI) was digested with *Nco*I, dephosporylated with calf intestinal alkaline phosphatase, and kinated with [$\gamma$-$^{32}$P]ATP. 5′-End-labeled restriction enzyme fragments were digested with *Hin*dIII/*Bgl*II to generate a unique end-labeled restriction enzyme fragment, and the desired fragment was purified on an 8% nondenaturing polyacrylamide gel. 5′-End-labeling of the complementary strand of this restriction enzyme fragment was obtained in a similar fashion, except pCAT was first digested with *Hin*dIII/*Bgl*II and then with *Nco*I after kinating with [$\gamma$-$^{32}$P]ATP. Drug modification reactions were carried out at room temperature in 10 mM Tris−HCl at pH 7.5 for 30 min using indicated drug concentrations. After the strand breakage in 100 mM piperidine at 95 °C for 15 min, modification sites were determined by 8% denaturing polyacrylamide gel electrophoresis.

**Sample Preparation and Spectroscopic Experiments.** The self-complementary d(GATGTACATC) DNA strand was synthesized on an automated DNA synthesizer (Applied Biosystems 381A) using the solid phase phosphoramidite method. The DNA synthesis was performed on a 10 $\mu$mol scale, leaving the final dimethoxytrityl group on the 5′ end of the DNA molecules. The crude sample was deprotected in concentrated $NH_4OH$ at 55 °C overnight and purified by reversed phase chromatography on a C18 column (Dynamax-300A). Purified DNA was then detritylated by dissolving in 80% acetic acid for 30 min, followed by an ether extraction to remove the acetic acid. The sample was then extensively dialyzed, and the buffer was adjusted to 10 mM $NaH_2PO_4$, 100 mM NaCl, pH 6.8.

Psorospermin samples were purified from root extracts of *Psorospermum febrifugum*, as previously detailed.[2] Drug−DNA adduct formation was achieved by mixing 1 mg of psorospermin with 6 mg of DNA, or roughly a 3:1 molar ratio in varying concentrations of dioxane ($D_8$) and $D_2O$ at 5 °C for approximately 20 h. One-dimensional NMR was used to monitor the stability of the duplex in the dioxane−water mixture to maximize solubility of psorospermin without denaturing the oligomer. Due to poor drug solubility, the reaction proceeded slowly and then stalled prior to complete reaction. Because of the scarcity of psorospermin and anticipated instability of the adduct, the incompletely reacted sample was used to collect NMR data.

All NMR experiments were performed on a Bruker AMX 500 spectrometer in 99.96% $D_2O$ or 95% $H_2O$−5% $D_2O$. Phase-sensitive, tppi two-dimensional NOESY spectra in $D_2O$ at 300 ms and a DQF-COSY experiment[10] were performed on the sample dissolved in $D_2O$. For these experiments, 1K data points were acquired in $t_1$ with a spectral width of 5000 Hz. To observe the exchangeable 4GH8 proton, one-dimensional NOE difference (1 s saturation of the 4GH8 resonance) spectra were obtained in $H_2O$ using a 1:1 echo pulse sequence[20] to suppress water.

DNA structures were created with the NUCGEN module of AMBER[13] adjusted in MIDAS[21] to form intercalation sites and to dock the psorospermin molecule. The ligand molecule was docked on the DNA according to experimentally observed NOE connectivities, and the system was minimized in AMBER to obtain the reported model.

**Acknowledgment.** We gratefully acknowledge Steve Sorey for NMR technical assistance. Research is supported in part by The University of Texas at Austin, the Public Health Service (Grants CA-49751 and GM12453-03), and the Welch Foundation. We also thank David Bishop for proofreading and editing the manuscript.

**Supporting Information Available:** Gel showing psorospermin-reactive sites on the second restriction enzyme fragment shown in Figure 3A used to determine sequence selectivity of psorospermin (1 page). Ordering information is given on any current masthead page.

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