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Molecular recognition of guanosine and 2-acetylaminofluorene-modified guanosine. A comparative study

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Abbreviations

AAF, 2-acetylaminofluorene
Ac, acetyl
AF, 2-aminofluorene
C, 2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)cytidine
DCI, desorptive chemical ionization
dG-C8-AAF, *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene
dG-C8-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene
F, fluorene
G, 2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosine
G-AAF, *N*-[2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosin-8-yl]-2-acetylaminofluorene
MD, molecular dynamics
MM, molecular mechanics
NAPH, 7-acetylamino-2-methyl-1,8-naphthyridine
TMS, tetramethylsilane

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The ability of an abiotic receptor, 7-acetylamino-2-methyl-1,8-naphthyridine, to bind to guanosine was analysed by a combination of NMR determinations and molecular modeling studies. The results indicate that this receptor simulates the base-pairing properties of cytidine in its Watson-Crick interaction with guanosine. Binding of the same receptor to *N*-(guanosin-8-yl)-2-acetylaminofluorene, the guanosine adduct containing the carcinogen 2-acetylaminofluorene, was found to occur in a similar manner. The calculated binding energies show that the molecular recognition of the adduct is lower than that of the unmodified guanosine. The theoretical studies suggest that the predominance of an abnormal low energy *syn* conformation for the adduct is the main structural feature accounting for the observed decrease of the host-guest interaction.

INTRODUCTION

In recent years, there has been a growing interest in the design of synthetic receptors capable of recognizing and binding biological molecules. The modeling of enzyme activities and the understanding of the structural features behind the ability of proteins to recognize specific nucleotide sequences have been major goals of these studies (1-4).

Selective hydrogen bonds play a crucial role in the mutual recognition of complementary bases in DNA, and are ultimately responsible for ensuring the fidelity of the replication and transcription processes. Therefore, much of the work aimed at the molecular recognition of nucleoside bases has been focused on the search for receptors that simulate the Watson-Crick interactions prevailing in DNA. One such group consists of derivatives of 7-amino-1,8-naphthyridine, which serve as receptors for guanosine (2,3).

Deoxyguanosine is the primary site of DNA damage caused by exposure to carcinogenic arylamines and arylamides. Following metabolic activation, these carcinogens bind to DNA yielding C8-substituted deoxyguanosine derivatives as the major covalent adducts (5,6). Since adduct formation may affect subsequent events, such as replication, repair and mutation induction, it is regarded as a critical step in the carcinogenic process.

Much of our understanding of the mechanisms of arylamine carcinogenesis and mutagenesis has resulted from work conducted with 2-aminofluorene (AF) and its *N*-acetyl derivative, 2-acetylaminofluorene (AAF) (5,6). In the last two decades, spectroscopic and theoretical methods have been used to elucidate the prevalent conforma-

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tions of DNA containing *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF) or its 2-acetylaminofluorene (dG-C8-AAF) analogue (7-11, and references cited therein). More recently, the advent of modern biochemical techniques has allowed the study of the mutation profiles associated with these adducts (12, and references cited therein). The picture emerging from these studies indicates that, in contrast to the non-acetylated adduct, dG-C8-AAF exists preferentially in an abnormal *syn* conformation about the glycosyl bond. As a result, the AAF-modified guanosine is displaced and becomes exposed to the outside medium, while the AAF fragment stacks with the neighboring bases. This conformational change may explain the ability of dG-C8-AAF to induce frameshift mutations.

In view of the biological damage induced by arylamine-DNA adducts, the development of abiotic receptors capable of recognizing and binding selectively to these lesions could have great significance. For instance, receptors with the appropriate binding properties could be derivatized to contain substituents that would allow the detection of very low levels of DNA adducts, comparable to those found after *in vivo* exposure to carcinogens, by sensitive techniques such as electron microscopy. Although a similar rationale underlies the use of antibodies (13), an approach based on the establishment of host-guest hydrogen-bonding interactions would permit the location of adducts in a particular conformation (e.g., those with an exposed base-pairing region), along a piece of DNA. As part of a program aimed at elucidating the hydrogen-bonding abilities of arylamine-DNA adducts, we selected *N*-(guanosin-8-yl)-2-acetylaminofluorene as starting point. The selection was made on the basis of simplicity of adduct synthesis (*vide infra*) and on considerations that the preferred conformation of the AAF-modified guanosine, by disrupting the G:C base pair, will increase the ability of the base to bind with external receptors.

In the present work, we describe a combined experimental (^{1}H NMR) and theoretical study of the association between an abiotic receptor, 7-acetylamino-2-methyl-1,8-naphthyridine, and *O*-derivatized forms of both the guanosine-AAF adduct and non-modified guanosine. The structures of the resulting complexes are elucidated, and the binding energies calculated in order to gain insight on the factors controlling the hydrogen-bonding ability of both the receptor and the adduct.

RESULTS AND DISCUSSION

Design and Synthesis of Receptors and Substrates. The selection of 7-acetylamino-2-methyl-1,8-napthhthyridine (NAPH) as a receptor for guanosine-arylamine adducts was based both on reported data for the association between 1,8-naphthyridine derivatives and guanosine (2,3) and on our computer-based molecular modeling experiments. Receptors possessing the 1,8-naphthyridine structure and a suitably located amine (amide) substituent can generate three hydrogen bonds with guanosine (Figure 1a) that are reminiscent of the natural Watson-Crick association between guanosine and cytidine (Figure 1b). Since NAPH has the advantage of being readily obtainable from commercially accessible materials (14), we performed molecular modeling studies of this potential receptor (*vide infra*) in order to predict if it could bind satisfactorily to guanosine derivatives. The results indicated that a methyl substituent in the C-2 position of the naphtyridine ring would not produce any significant hindrance to the host-guest interactions; therefore, NAPH was chosen to conduct the study. The synthesis of this receptor involved an easy two-step procedure, consisting of ring closure condensation of 2,6-diaminopyridine with 3-ketobutanal dimethylacetal in acidic medium (14), followed by standard acetylation of the free amino group.

N-(Guanosin-8-yl)-2-acetylaminofluorene was selected as the model adduct for the reasons stated above; however, some additional requirements had to be considered. An aprotic solvent of low dielectric constant was needed to decrease or suppress solute-solvent interactions that otherwise might compete with the host-guest association. Spectroscopic studies conducted with nucleosides and nucleoside derivatives at the monomer level (15-25) have indicated consistently that chloroform is an appropriate solvent to optimize the pairing of comple-

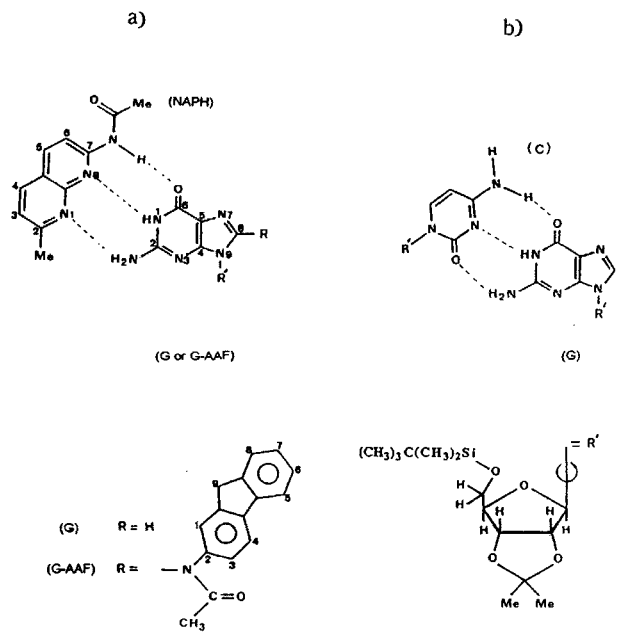

Figure 1 Structures of the substrates (guests) [G, G-AAF] and receptors (hosts) [NAPH, C] discussed in the text, with indication of the hydrogen bonds involved in complex formation.

mentary bases. By contrast, water has been reported to favour stacking interactions (26,27). Therefore, deuterochloroform was selected to conduct the ^{1}H NMR binding study.

In order to achieve sufficient solubility in chloroform and prevent the involvement of the sugar hydroxyl groups in hydrogen bonding, a derivatized form of the guanosine-AAF adduct was used. Thus, room temperature reaction between commercially available 2′,3′-isopropylideneguanosine and *N*-acetoxy-*N*-acetyl-2-aminofluorene (*N*-acetoxy-AAF) in aqueous ethanol (28) yielded *N*-(2′,3′-isopropylideneguanosin-8-yl)-2-acetylaminofluorene. Further derivatization of the 5′-hydroxyl with the lipophilic *tert*-butyldimethylsilyl group (29) then afforded *N*-[2′,3′-isopropylidene-5′-(*O*-*tert*-butyldimethylsilyl)guanosin-8-yl]-2-acetylaminofluorene (G-AAF). The parent 2′,3′-isopropylideneguanosine, as well as its complementary 2′,3′-isopropylidene-cytidine, were derivatized in a similar manner to yield 2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosine (G) and 2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)cytidine (C), respectively. These protected nucleosides were used in parallel G:NAPH and G:C comparative binding studies.

^{1}H NMR Binding Studies

General Procedure. The binding constants of the host-guest (receptor-substrate) systems were determined by ^{1}H NMR spectroscopy through "reverse" titration of the nucleoside guest (G or G-AAF) with the host (NAPH). The experiment consisted of holding the concentration of the guest at a constant value (9 mM), while the concentration of the host was gradually increased (~0.5-15 mM), until no further changes were apparent. The standard G:C association, which has been previously studied in the same solvent (21-25), was analysed in a similar manner. This allowed for a direct comparison between the relative stabilities of the complexes containing NAPH and that of the normal G:C base pair.

Host-guest interactions. Compared with the spectra of the pure hosts and guests, significant chemical shift changes were observed upon mixing for the labile protons of each set of two components. By contrast, the remaining protons were virtually unaffected. Thus, addition of NAPH caused an initial downfield shift of the NAPH amide proton and gradual downfield shifts of the imino (N_1H) and exocyclic amino (N^2H_2) protons of both G and G-AAF, as illustrated in Figure 2 for the G:NAPH interaction. These specific shifts presumably resulted from changes in the chemical environment caused by hydrogen bonding. It is noteworthy that downfield shifts of similar magnitude were detected for the same G protons upon titration with C (Table 1). G and C have been shown to associate in a Watson-Crick manner in chloroform (21-23); therefore, our results indicate that NAPH and either G or G-AAF also form host-guest complexes in chloroform, through involvement of the labile protons in a triple hydrogen-bond association that resembles the natural G:C interaction.

Labile protons of the nucleoside substrates. The imino (N_1H) protons of both G (Figure 2) and G-AAF (not shown), which resonated in the farthest downfield region of the spectrum, suffered a slight broadening during the initial stages of the titration. This was followed by a gradual sharpening, as the NAPH receptor became the major component of the mixture. Such behaviour is consistent with initial thermal exchange between non-bonded (substrate) and hydrogen-bonded (complex) imino protons, which virtually ceased as the substrate became fully associated with the receptor. The downfield shifts of the imino protons followed a hyperbolic behaviour upon addition of the NAPH receptor and reached ~1.1 ppm on saturation, both for the G:NAPH and the G-AAF:NAPH mixtures. A somewhat more intense downfield shift of the same proton (~1.5 ppm) was obtained, upon saturation, for the G:C complex (Table 1).

In contrast with the imino protons, the amino (N^2H_2) protons of G (Figure 2) and G-AAF suffered a continuous broadening along the titration and became totally obscured under the baseline after addition of ~1/2 equivalent of the NAPH receptor. A similar behaviour was observed for the same protons in the G:C mixture. Upon formation of one hydrogen bond with the receptor, the two protons of the amino group should be in different chemical environments and give rise to separate resonances in the spectrum. However, rotation about the guanosine C2-N bond is known to occur at a sufficient rate to cause severe broadening of the guanosine amino protons, frequently precluding their observation (30). For G:C mixtures in chloroform, it has been demonstrated that such rotation takes place within the base-paired state, while the analogous rotation about the cytidine C4-N bond is more hindered and requires base-pair disruption (24).

Similarly to those of the imino protons, the downfield shifts observed for the G and G-AAF amino protons appeared to vary hyperbolically with the concentration of the receptor (not shown). However, their broadening restricted the number of measurable data points and prevented the clear definition of a saturation plateau.

Amide (NHAc) proton of the NAPH receptor. Initial addition of the NAPH receptor to the substrate caused a rather substantial downfield shift of the amide proton. For example, this proton resonated ~3 ppm downfield from that of the pure NAPH when the mixtures contained a 10-30 fold molar excess of the substrate (G or G-AAF). As the receptor concentration increased, we observed a gradual shift of the same proton in the opposite direction. By the end of the titration, the NAPH receptor was present in a 1.5-2 fold molar excess and the

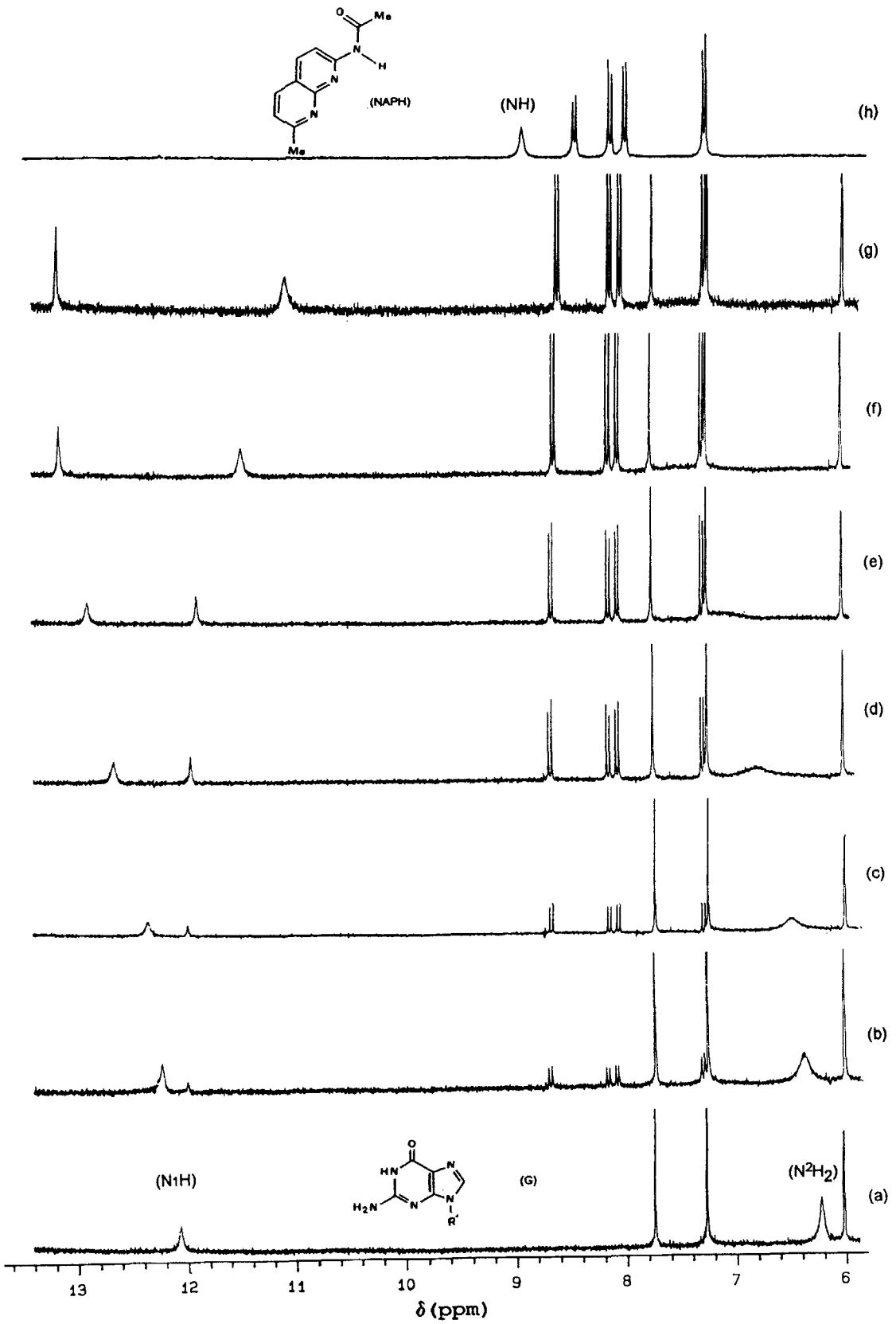

Figure 2 Representative 300-MHz ^{1}H NMR spectra showing the effect of varying the concentration of the receptor (NAPH) on the imino (N_1H) and amino (N^2H_2) protons of the substrate (G) and the amide (NH) proton of the receptor. The spectra were recorded in $CDCl_3$ at 295 K, with solutions containing G (9.0 mM) and, respectively, (a) 0.0, (b) 0.6, (c) 1.2, (d) 2.3, (e) 7.5, (f) 11.5, and (g) 13.4 mM of the receptor. The spectrum of the receptor (h) is shown at 22.4 mM.

Table 1 Chemical shift variations ($\Delta\delta$) observed for the imino (N_1H) and amino (N^2H_2) protons of the substrates as a result of host-guest interactions.

	$\Delta\ \delta$ (ppm)[a]				
Complex	N_1H *18:1 Mixture*	N_1H *1:1 Mixture*	N_1H *Saturation*	N_1H *Extrapolated*[b]	N^2H_2 *18:1 Mixture*
G:NAPH	0.173	1.039	1.127	1.43	0.159
G-AAF:NAPH	0.057	0.915	1.146	2.58	0.100
G:C	0.127	0.920	1.493	2.93	0.053

[a]$\Delta\ \delta = \delta$ (substrate in the mixture) - δ ("free" substrate).
[b]Calculated for the saturation plateau by extrapolation using the "best fit" curve.

amide proton had already shifted ~1 ppm upfield in each mixture (G:NAPH and G-AAF:NAPH). This is well apparent in Figure 2 for the G:NAPH complex. A similar upfield shift of the hydrogen-bonded cytidine amino proton was observed for the G:C mixture while G remained in excess; however, in agreement with previous findings (21,24), the two cytidine amino protons were exchange-broadened and became undetected as the concentration of C increased (not shown).

In order to establish if self-association of the NAPH receptor, possibly through π-stacking of the aromatic rings (31), contributed to the observed variation of the amide proton resonances, solutions of pure NAPH were used to measure the chemical shift of that proton as a function of concentration. Since the experiments did not reveal significant changes in the resonances of any of the NAPH protons, it appears that NAPH self-association is negligible, at least within the range of concentrations that were used in the titration studies. Therefore, the occurrence of gradual upfield shifts of the amide proton upon addition of the receptor can be ascribed to time-averaged contributions of complexed (hydrogen-bonded) and free protons, the fractions of which varied along the titration.

Binding constants. Each titration curve (binding isotherm) was obtained by plotting the chemical shift variations of the substrate imino (N_1H) proton as a function of the receptor total concentration. The rationale for monitoring this proton was based upon three main reasons: *(i)* it was clearly involved in the host-guest associations; *(ii)* it was present, with similar environments, in all the complexes that were analysed; and *(iii)* it could be followed easily throughout the titrations, until saturation conditions were reached. A representative titration curve, corresponding to the G:NAPH complex, is shown in Figure 3; the discrete points represent experimental data and the curve is the calculated best fit.

For each host-guest system, the binding constants (K_{bind}) were derived from analysis of the corresponding titration curves through a non-linear curve-fitting procedure, assuming 1:1 equilibrium processes (32, 33). The results and the corresponding Gibbs free energies for complex formation are shown in Table 2.

G:NAPH complex. A comparative analysis of the binding constants calculated for the G:NAPH and G:C complexes (297 M^{-1} and 85 M^{-1}, respectively) indicates that the abiotic NAPH receptor is adequate to simulate the natural molecular recognition of guanosine by cytidine. Since both constants were obtained with satisfactory accuracies (± 8%), the results further suggest that, under the conditions of this study, NAPH has a slightly better capability to associate with G than does C itself. The reasons for this difference are not totally clear. The possibility of steric hindrance introduced by the presence of the bulky 5′-*O*-(*tert*-butyldimethylsilyl) group in the sugar fragment of C, which was absent from NAPH, was not supported by molecular modeling studies (*vide infra*). *O*-Derivatized forms of C are known to dimerize in non-aqueous solvents (21). Since the titration of G was conducted by adding aliquots of a concentrated solution of C, where self-aggregation might have occurred, the G:C binding constant may have been slightly underestimated.

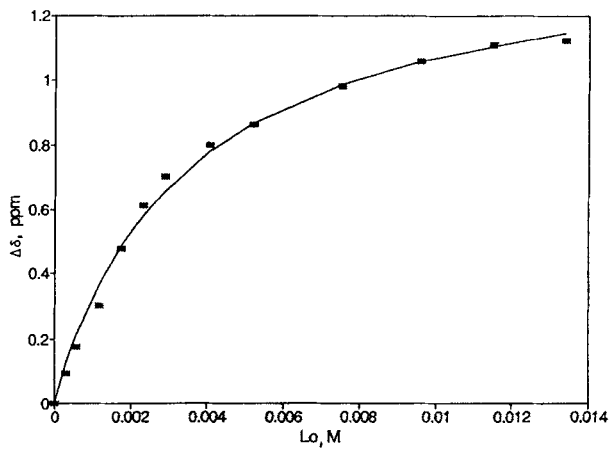

Figure 3 Plot of the chemical shift variations ($\Delta\delta$) for the imino proton (N_1H) of G (0.009 M) as a function of the total concentration (L_0) of the NAPH receptor (0-0.015 M).
$\Delta\delta = \delta$ (N_1H) - δ_0(N_1H); δ_0 was measured in the absence of receptor.
(—) Calculated best fit; (▪) experimental data points.

Table 2 Binding constants (K_{bind}) and Gibbs free energies (ΔG^{295}) determined by ^1H NMR titrations.

Substrate	*Receptor*	K_{bind} *(M^{-1})*	ΔG^{295}*(kcal/mol)*
G	NAPH	297 ± 22	−3.4
G-AAF	NAPH	56 ± 13	−2.4
G	C	85 ± 7	−2.6
G-AAF	G-AAF	5	−0.9

Nonetheless, the Gibbs free energies of binding were roughly in the same range (~3 kcal/mol) for the two complexes, suggesting that each hydrogen bond contributed approximately 1 kcal/mol to the total binding energy. This is in good agreement with published estimates of the average hydrogen bond strength (1-1.5 kcal/mol) in chloroform (4). Furthermore, binding constants of approximately 90-130 M^{-1} in chloroform have been reported for the association between *O*-derivatized guanosines and other simple receptors based on 2-amino-1,8-naphthyridine (2,3).

Previous studies have indicated that G self-associates through hydrogen-bonding in nonaqueous solvents, forming dimers and more complex aggregates (21, 22, and references cited therein). Since G was the species present at constant concentration throughout the titrations, we attempted to quantify this interaction but detected no measurable chemical shift changes upon dilution of a parent G solution. Therefore, the self-association of G appears negligible in the range of concentrations used in this study and is unlikely to have caused significant errors in the calculated binding constants for the host-guest complexes.

Interestingly, a much higher constant (~20,000 M^{-1}) has been reported recently, on the basis of ^1H NMR measurements, for the 1:1 association between *O*-silylated derivatives of deoxyguanosine and deoxycytidine in deuterochloroform (25). This number was calculated with a different curve fitting program and represents approximately a two-fold difference in the calculated free energies of G:C binding compared to the present work. Even assuming that our samples might have absorbed some water, the resulting decrease in the measured binding constants would not have accounted for such discrepancy (see 34). It remains to be established if the use of dissimilar calculation methodologies could have caused a difference of such magnitude in the binding constant.

G-AAF:NAPH complex. As indicated in Table 2, a lower binding constant (56 M^{-1}) was obtained for the association between the AAF adduct and the NAPH receptor. The estimated accuracy of the calculation (±20%) was worse than the ±8% found for the complexes involving G; however, this was not unexpected since the best accuracies are normally obtained with strong binding complexes, for which the magnitudes of the chemical shift variations tend to be much larger than the uncertainties in peak locations. Nonetheless, accuracies within ±15% are quite common when complex formation is studied by NMR (35).

In contrast to the observations with G (*vide supra*), a small but measurable self-association was detected with the G-AAF adduct. This was indicated by a slight upfield shift of the imino (N_1H) proton upon increasing the adduct concentration ($\Delta\delta_{max}$ = 0.22 ppm). The fact that the G-AAF imino proton was shifted upfield suggests that hydrophobic interactions, rather than hydrogen bonds, play a role in the self-association of the adduct. Despite being too weak to cause detectable changes in the chemical shifts of the aromatic protons, these interactions are likely to involve π-stacking with the polyaromatic aminofluorene residue. Assuming a dimerization process, a linear graphical method, based on an approach by Spurr and Byers (32, 36), indicated a self-association constant (K_{dim}) of 5 M^{-1} for the adduct (Figure 4). Although the association capability of the adduct appeared to be low within the range of concentrations used in the host-guest study, it may explain the relatively low accuracy of the G-AAF:NAPH binding constant. Thus, the value (56 M^{-1}) of this binding constant should be regarded as a minimum value. Since K_{dim} was only 10% of the calculated K_{bind}, the curve-fitting procedure was not corrected to account for self-aggregation of the adduct.

Despite the higher uncertainty in the binding strength of the G-AAF:NAPH complex, as compared to its G:NAPH analogue, it seems clear that the AAF adduct has a lower affinity for the abiotic NAPH receptor than the parent G. The decrease in the Gibbs free energy of association for the G-AAF:NAPH complex (~1 kcal/mol, Table 2) suggests that one less hydrogen bond may be involved in the binding. Molecular simulation studies (*vide infra*) were performed to test this supposition.

Molecular Modeling

The theoretical modeling studies were initiated with a conformational analysis intended to locate the global minimum energy conformers of the substrates (G and G-AAF) and receptors (C and NAPH) that would serve as starting points for the molecular recognition studies. For this purpose, **M**olecular **D**ynamics/**M**olecular **M**echanics

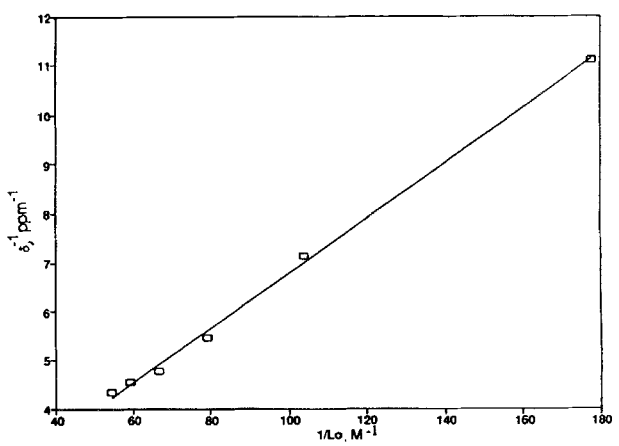

Figure 4 Double-reciprocal plot for the dimerization of the adduct, G-AAF. The study was conducted in $CDCl_3$ at 295 K; δ is the observed chemical shift for the imino proton (N_1H) and L_0 is the total concentration of the substrate.
(—) Calculated best fit; (□) experimental data points.

(**MD/MM**) methods were used, with further refinement by AM1 methods. For the sake of simplicity, the calculations were conducted using trimethylsilyl, instead of *tert*-butyldimethylsiyl, as the protecting group for the 5′-hydroxyls of the substrates and the C receptor. These molecules will be designated as G′, G′-AAF and C′ in the following paragraphs.

Adduct conformation. As expected, the molecular modeling studies confirmed the remarkable conformational change associated with G-AAF adduct formation that is well documented in the literature (9, 12, and references cited therein). This alteration is illustrated by a comparison of the backbone glycosylic torsion angles (χ=$C_4N_9C'_1O'$) that were found for the lowest energy conformers of G′ and G′-AAF. Thus, while G′ showed an unequivocal preference for the normal *anti* conformation (χ~207°), the AAF adduct tended to adopt an abnormal *syn* conformation (χ = 27°). This trend was also observed in the lowest energy conformers of the corresponding binding complexes (Figure 5). Specifically, torsion angles of 221 and 233°, both in the *anti* domain, were found for G′ in the G′:C′ and G′:NAPH complexes, respectively. By contrast, χ was calculated to be in the *syn* domain (13°) for the AAF-modified guanosine in G′-AAF:NAPH.

Simulation of molecular recognition interactions. The modeling of host-guest interactions with MM methods depends primarily upon electrostatic interactions, which are accounted for *via* the equation $E_{elec} = \Sigma\, q_i q_j / \varepsilon\, r_{ij}$, summed over all pairs of i and j atoms in host and guest, where q_i and q_j are the charges on individual atoms within each pair and r_{ij} is the distance between them. The assignment of atomic charges in this summation is difficult because the charges cannot be calculated directly from quantum mechanics and the established ways of partitioning the wave function lead to different values (37). A further complexity is that these charges will vary with conformation (38). Therefore, one specific set of charges, no matter how correctly calculated, will not necessarily be applicable for calculations on flexible molecules. An additional complexity associated with calculations on host-guest systems is that the atomic charges are likely to be affected by the formation of the host-guest interactions through hydrogen bonds.

In view of the difficulty in establishing the correct charges, which impinges directly upon the calculations, a combination of MM, MD and quantum mechanics was used for the calculation of the host-guest structures and the binding energies. Thus, we have taken the global minimum structures for each receptor and substrate and used the molecular graphics system INSIGHT (39) to dock the substrate to a satisfactory distance (2.2 Å) from the receptor, with the adjacent rings of receptor and substrate in coplanar positions. For each receptor:substrate pair, this structure was then minimized by MM and subsequently subjected to MD for 200 ps, with structures saved at 1 ps intervals. These structures were then reminimized by MM and the lowest energy conformer was submitted to a quantum mechanics calculation with geometry optimization, using the AM1 hamiltonian *via* MOPAC 5.0, since the AM1 method has proved to be successful in the modeling of hydrogen bonds (40).

A summary of structural and energy data found for the three binding complexes (G′:NAPH, G′-AAF:NAPH, and G′:C′) is presented in Table 3; for simplicity, only the data corresponding to global minimum energies are listed. Figure 5 illustrates a diagram of the lowest energy conformations obtained for these binding complexes, with an indication of the lengths of the hydrogen bonds involved in complex formation. Interestingly, a comparison between the global minimum energy conformers calculated for the binding complexes and the uncomplexed forms (not shown), indicated that the complexation processes did not affect the predominant glycosyl bond conformations of the nucleoside derivatives, which remained *anti* for G′ and C′ and *syn* for the G′-AAF adduct.

The theoretical studies support the main conclusions drawn from the experimental NMR studies (*vide supra*). As indicated in Table 3, the G′-AAF adduct has a lower capability of being recognized by the abiotic NAPH receptor than does the parent guanosine. The observed decrease in the binding energy (ΔE_{bind} = 1.3 kcal/mol) is consistent with the decrease in the angle between the guanosine and naphthyridine planes (Table 3) and seems to have resulted from the weakening, or even disruption, of one hydrogen bond. In fact, the bond involving the exocyclic amino protons of the guanosyl fragment of the adduct (Figure 5) was found to be longer (2.62 Å) than the upper limit (2.5 Å) usually assumed for effective hydrogen bonds.

The calculations also suggest that the weakening of one hydrogen bond in the G′-AAF:NAPH complex is accompanied by a slight strengthening of the two remaining hydrogen bonds. The observations that *(i)* the G-AAF imino proton was more deshielded than that of G upon saturation, and *(ii)* the initial addition of the NAPH receptor deshielded the amino protons of G to a greater extent than those of the adduct (Table 1), provide experimental data to support the theoretical predictions.

An alternative model of host-guest association, in which the guanosine O^6 and N_1H would form two hydrogen bonds with the acetyl residue of the NAPH receptor, can be excluded on the basis of both the experimental and the theoretical results. In fact, the observation of downfield shifts for the guanosine amino protons of G and G-AAF upon addition of the receptor indicates that these protons are involved in hydrogen bonding. In addition, the binding energy calculated using this model was the same for both the G′:NAPH and G′-AAF:NAPH

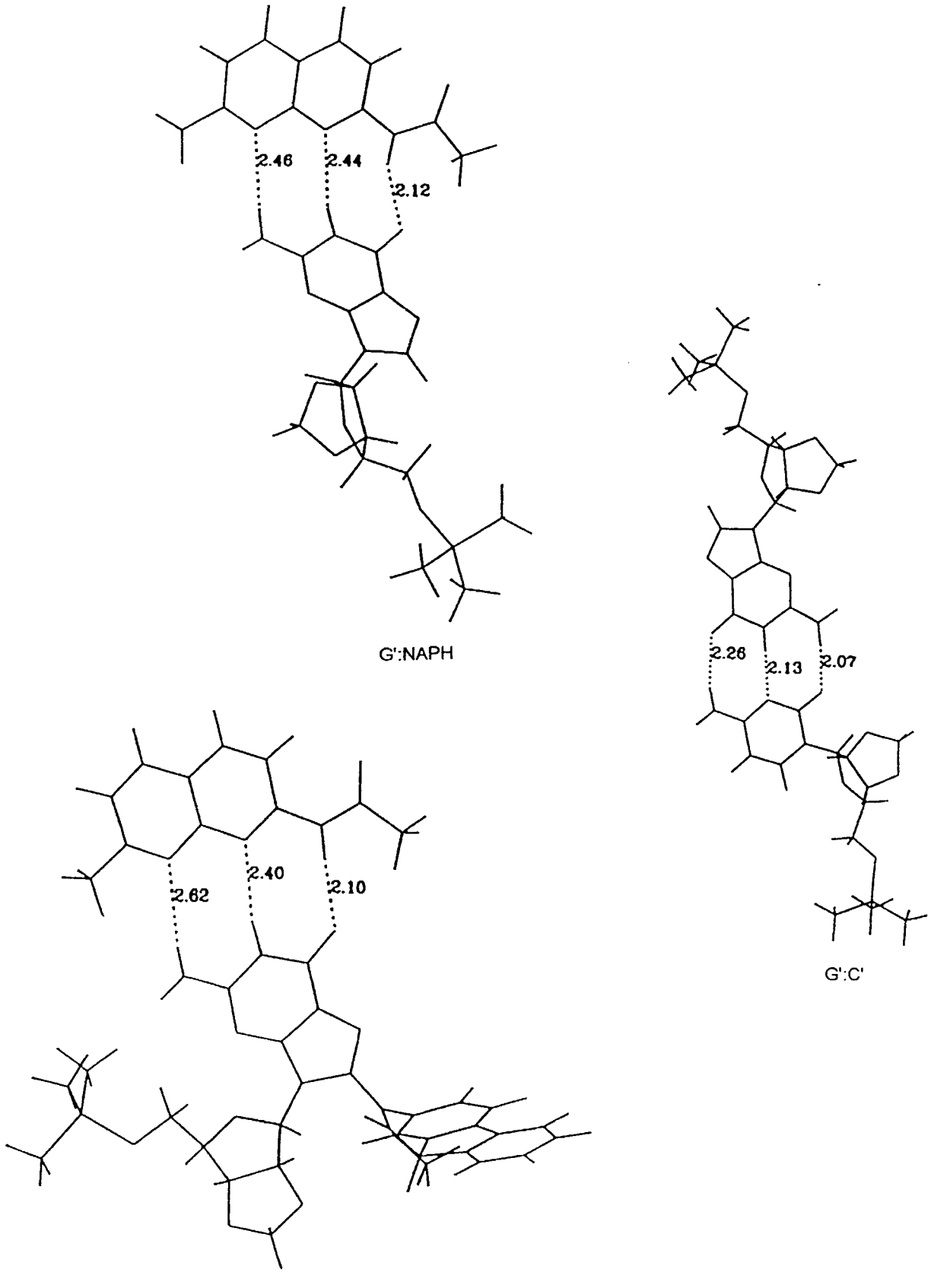

Figure 5 Diagrams of the most stable conformations for the binding complexes, calculated by MD/MM techniques followed by AM1 refinement. Hydrogen bonds are shown as dashed lines, with indication of the corresponding bond lengths (Å).

complexes (−11.7 ± 1 kcal/mol) and lower than the binding energies shown in Table 3.

Since the polyaromatic AAF substituent was found to be distant from the weakened hydrogen bond (Figure 5), the conformational change associated with adduct formation appears to be the main factor responsible for the lower molecular recognition of the adduct. In fact, the AM1 method revealed negligible changes, upon adduction, in the charges of the guanosine atoms involved in hydrogen bonding (1-2%, Table 3). This suggests that electronic effects induced by the presence of the AAF fragment do not play a role in the process. On the other hand, a comparison of the low energy conformers shown in Figure 5 suggests that rotation about the glycosyl bond from the *anti* (G′) to the *syn* (G′-AAF) conformation places the bulky 5′-*O*-*tert*-butyldimethysilyl group within a close vicinity of the 2-methyl substituent of the NAPH receptor, thus creating an unstabilizing steric interaction.

Interestingly, the theoretical studies confirmed that the NAPH receptor has a somewhat better ability to bind to guanosine derivatives than does cytidine. This contrasts with the indication that the paired bases in the G′:C′ complex were closer to coplanarity, with the hydrogen bonds being shorter, and the hydrogen bond angles being closer to linearity (Table 3). The existence of a slight stacking effect between the naphthyridine and guanosine moieties may be the factor contributing to the increased binding ability of the NAPH receptor.

Conclusions

Using a combination of NMR determinations and molecular modeling studies, this work has shown that the new receptor, 7-acetylamino-2-methyl-1,8-naphthyridine (NAPH), has the ability to simulate the base-pairing properties of cytidine in its Watson-Crick interaction with guanosine. The same receptor binds to the guanosine adduct with the carcinogen 2-acetylaminofluorene, *N*-(guanosin-8-yl)-2-acetylaminofluorene (G-AAF), in a similar manner. The calculated binding energies indicate that NAPH associates more strongly with guanosine than with G-AAF. Theoretical simulations suggest that the preference of the adduct for an abnormal *syn* conformation about the glycosyl bond is the main structural feature accounting for the decrease in the molecular recognition of this species. However, since the base pairing region of an adducted *syn* guanosine in DNA will be exposed to the outside medium, while the non-modified guanosines will be paired with cytidines, the results suggest that receptors based on the naphthyridine structure might be useful for locating AAF-DNA adducts along a DNA molecule.

EXPERIMENTAL PROCEDURES

Instrumentation. Melting temperatures were obtained with a Köfler hot-stage apparatus and are uncorrected. Microanalyses were conducted in a Perkin-Elmer 240 instrument. IR spectra were recorded in a Perkin-Elmer 683 spectrophotometer and UV spectra in a Beckman DU-40 UV/vis spectrophotometer. ^{1}H NMR spectra were performed on a Varian Unity 300 spectrometer.

Chemicals. 2′,3′-Isopropylidenecytidine hydrochloride and 2′,3′-isopropylideneguanosine were purchased from Sigma Chemical Co., St. Louis, MO, USA, and were used as received. Silica gel was obtained from E. Merck, Darmstadt, Germany, and Sephadex LH-20 from Pharmacia/PL Biochemicals, Piscataway, NJ, USA. All other reagents were purchased from Aldrich, either through Aldrich Chemical Co., Milwaukee, WI, USA or Sigma-Aldrich Química, S.A., Madrid, Spain. Solvents were purified by standard methods prior to being used.

Syntheses

Protected Nucleosides. 2′,3′-Isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)cytidine (C) and 2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosine (G) have been described (22). Both were prepared from the corresponding 2′,3′-isopropylidene precursors by an adaptation of the procedure of Ogilvie *et al.* (29). Specifically, solid *tert*-butyldimethylsilyl chloride (2-3 equivalents) was

Table 3 Binding energies (E_{bind}) and structural parameters for the host-guest interactions calculated by theoretical simulations (MD/MM+AM1).

Complex	E_{bind}[a] *(kcal/mol)*	*Hydrogen bond lengths (Å)*	*Hydrogen bond angles (A-H..B, °)*	$\phi_S \wedge \phi_R$ (°)[b]	*Guanine atomic charges*
G′:NAPH	−14.26	2.12 (NH..O)	150.1 (NH..O)	16	-0.389 (O^6)
		2.44 (N..HN)	146.5 (N..HN)		-0.345 (N_1)
		2.46 (N..HNH)	164.2 (N..HNH)		-0.346 (N^2)
G′-AAF:NAPH	−12.96	2.10 (NH..O)	151.5 (NH..O)	38	-0.385 (O^6)
		2.40 (N..HN)	169.8 (N..HN)		-0.343 (N_1)
		2.62 (N..HNH)	163.1 (N..HNH)		-0.360 (N^2)
G′:C′	−11.03	2.07 (NH..O)	173.0 (NH..O)	8	-0.393 (O^6)
		2.13 (N..HN)	168.4 (N..HN)		-0.357 (N_1)
		2.26(N..HNH)	167.3 (N..HNH)		-0.350 (N^2)

[a] $E_{bind} = E_{complex} - (E_{receptor} + E_{substrate})$
[b] Mean angle between the naphthyridine/cytidine (R) and the guanosine/AAF-guanosine (S) planes

added to a solution of the 2′,3′-isopropylidene-nucleoside (0.4 mmol) in dry pyridine (1 ml) and the mixture was stirred at room temperature for 3-4 h, until thin layer chromatography (silica gel, chloroform/methanol, 5/1) indicated the absence of the starting nucleoside. The crude reaction mixture was then loaded on a silica gel column prepacked in chloroform, the pyridine was eluted with chloroform, and the product was recovered with chloroform/methanol (10/1). The silylated nucleosides were isolated after recrystallization from chloroform/*n*-hexane.

C: 72% yield; mp 130-132°C; UV ($CHCl_3$) λ_{max} 244, 281 nm; ^{1}H NMR ($CDCl_3$, 10 mM solution) δ 0.08 [6H, s, ($C\underline{H}_3$)$_2$Si], 0.88 [9H, s, ($C\underline{H}_3$)$_3$C], 1.36 (3H, s, $C\underline{H}_3$C-O), 1.59 (3H, s, $C\underline{H}_3$C-O), 3.79 (1H, dd, *J*=-11.6 Hz, *J*′=3.1 Hz, H5″), 3.93 (1H, d, *J*=-11.6 Hz, H5′), 4.37 (1H, m, H4′), 4.65 (1H, dd, *J*=6.1 Hz, *J*′ = 2.4 Hz, H3′), 4.73 (1H, dd, *J*=6.1, *J*′=2.4 Hz, H2′), 5.92 (1H, d, *J*=2.4 Hz, H1′), 6.27 (1H, d, *J*=7.3 Hz, H5), 7.82 (1H, d, *J*=7.3 Hz, H6).

G: 69% yield; mp 298-300°C (dec); UV ($CHCl_3$) λ_{max} 258 nm; ^{1}H NMR ($CDCl_3$, 17 mM solution) δ 0.02 [6H, s, ($C\underline{H}_3$)$_2$Si], 0.85 [9H, s, ($C\underline{H}_3$)$_3$C], 1.40 (3H, s, ($C\underline{H}_3$C-O), 1.61 (3H, s, $C\underline{H}_3$C-O), 3.77 (1H, dd, *J*=-11.0 Hz, *J*′=3.7 Hz, H5″), 3.87 (1H, d, *J*=-11.0 Hz, H5′), 4.36 (1H, bs, H4′), 4.92 (1H, d, *J*=3.7 Hz, H3′), 5.18 (1H, bs, H2′), 6.00 (1H, bs, H1′), 6.45 (2H, bs, $N^2\underline{H}_2$), 7.94 (1H, s, H8), 12.08 (1H, bs, $N_1\underline{H}$).

N-(2′,3′-Isopropylideneguanosin-8-yl)-2-acetylaminofluorene. The synthesis of the C8-guanosine adduct was performed by reaction of 2′,3′-isopropylideneguanosine with *N*-acetoxy-*N*-acetyl-2-aminofluorene (*N*-acetoxy-AAF). For this purpose, *N*-hydroxy-2-aminofluorene was prepared by reduction of 2-nitrofluorene with Pd/C and hydrazine hydrate (41) and then quantitatively acetylated with excess acetic anhydride to yield *N*-acetoxy-AAF (mp 108-110°C, lit [28]110°C).

In a typical modification reaction, *N*-acetoxy-AAF (50 mg) in absolute ethanol (10 ml) was added to a solution of 2′,3′-isopropylideneguanosine (60 mg) in 2 mM sodium citrate, pH 7.2 (20 ml). The mixture was vigorously stirred at room temperature for 24 h and the solvent was then evaporated. The crude mixture was resuspended in water (20 ml) and the degradation products of *N*-acetoxy-AAF were removed with diethyl ether (3 × 1 volume). The adduct was then extracted from the aqueous phase with *n*-butanol (3 × 1 volume), the butanol was evaporated, and the residue was dissolved in 50% aqueous methanol and chromatographed on Sephadex LH-20, using a water/methanol gradient. The pure adduct (48 mg, 49%) eluted with 75% methanol and was used without further purification. λ_{max} 277.5, 303 (sh) [A277.5/A303=2.1] nm; ^{1}H NMR (CD_3OD, 3.7 mM solution) δ 1.32 (3H, s, $C\underline{H}_3$C-O), 1.54 (3H, s, $C\underline{H}_3$C-O), 2.15 (3H, s, $C\underline{H}_3$C=O), 3.72-3.80 (2H, m, H5′+H5″), 3.92 (2H, s, F$\underline{H}$9), 4.27 (1H, bs, H4′), 5.14 (1H, bs, H3′), 5.41 (1H, bs, H2′), 5.92 (1H, bs, H1′), 7.30-7.39 (2H, m, F$\underline{H}$6+F$\underline{H}$7), 7.45-7.60 (3H, m, F$\underline{H}$1+F$\underline{H}$3+F$\underline{H}$8), 7.81-7.88 (2H, m, F$\underline{H}$4+F$\underline{H}$5).

N-[2′,3′-Isopropylidene-5′-O-(tert-butyldimethylsilyl) guanosin-8-yl]-2-acetylaminofluorene (G-AAF). *N*-(2′,3′-Isopropylideneguanosin-8-yl)-2-acetylaminofluorene was silylated as described for the non-modified nucleosides and recovered in 46% yield after silica gel chromatography (chloroform-methanol, 25/1) and recrystallization from chloroform/*n*-hexane.

^{1}H NMR ($CDCl_3$, 10 mM solution) δ -0.07 [6H, s, ($C\underline{H}_3$)$_2$Si], 0.78 [9H, s, ($C\underline{H}_3$)$_3$C], 1.34 (3H, s, $C\underline{H}_3$C-O), 1.53 (3H, s, $C\underline{H}_3$C-O), 2.16 (3H, s, $C\underline{H}_3$C=O), 3.72-3.81 (1H, m, H5′+H5″), 3.89 (2H, s, F$\underline{H}$9), 4.26 (1H, bs, H4′), 4.90 (1H, bs, H3′), 5.53 (1H, bs, H2′), 5.90 (1H, bs, H1′), 6.18 (2H, bs, $N^2\underline{H}_2$), 7.31-7.40 (3H, m, F$\underline{H}$3+F$\underline{H}$6+F$\underline{H}$7), 7.53 (1H, d, *J*=6.7 Hz, F$\underline{H}$8), 7.65 (1H, s, F$\underline{H}$1), 7.75-7.79 (2H, m, F$\underline{H}$4+F$\underline{H}$5); 12.17 (1H, bs, $N_1\underline{H}$); m/z (DCI) 687 [18%, $(M+C_2H_5)^+$], 659 [100%, $(M+H)^+$], 373 [16%, (guanine-C8-AAF+1)$^+$], 224 [36%, $(AAFH)^+$], 167 [12%, $(F+1)^+$].

7-Amino-2-methyl-1,8-naphthyridine. 2,6-Diaminopyridine (3.27 g) and 3-ketobutanal dimethyl acetal (3.86 g) were heated at 90 °C in H_3PO_4 (30 ml) for 3 h. The mixture was cooled, neutralized, and extracted several times with chloroform. The organic extracts were combined, washed with water, dried with anhydrous Na_2SO_4, and concentrated under vacuum. A crude precipitate was obtained upon addition of petroleum ether. Recrystallization from toluene yielded 7-amino-2-methyl-1,8-naphthyridine (2.72 g, 57%): mp 189-191 °C {lit[14]175-185 °C for the crude material and 217-218 °C upon chromatography and recrystallization}; ^{1}H NMR ($CDCl_3$) δ 2.69 (3H, s, $C\underline{H}_3$), 4.99 (2 H, bs, $N\underline{H}_2$), 6.71 (1 H, d, *J* = 8.4 Hz, H3), 7.08 (1H, d, *J* = 8.1 Hz, H6), 7.82 (2 H, d, *J* = 8.4 Hz, H4+H5).

7-Acetylamino-2-methyl-1,8-naphthyridine (NAPH). 7-Amino-2-methyl-1,8-naphthyridine (2.54 g) was refluxed in acetic anhydride (50 ml) for 40 min and upon cooling a yellow precipitate was obtained. Recrystallization from methylene chloride/toluene (3/2) gave yellow needles of NAPH (2.21 g, 69%): mp 277-278 °C; IR (KBr), 1700 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, s, $C\underline{H}_3$C=O), 2.76 (3H, s, $C\underline{H}_3$), 7.29 (1H, d, *J* = 8.1 Hz, H3), 8.02 (1H, d, *J* = 8.1 Hz, H4), 8.15 (1H, d, *J* = 8.4 Hz, H5), 8.47 (1H, d, *J* = 8.4 Hz, H6), 8.75 (1H, bs, NH); Anal. for $C_{11}H_{11}N_3O$ Calc. : C, 65.67; H, 5.47; N, 20.90; Found C, 65.47; H, 5.50; N, 20.76.

^{1}H NMR studies. ^{1}H NMR studies were conducted at 22 °C. Except where noted otherwise, the samples were dissolved in deuterochloroform and the chemical shifts were measured relative to internal tetramethylsilane (TMS). The spectra were acquired using 16K data points, over a 6000-Hz spectral width. Whenever neces-

sary, protons were assigned on the basis of homonuclear decoupling experiments. The association constants (binding constants, K_{bind}) for the host-guest systems were determined by fitting of titration curves. These curves were obtained upon monitoring the chemical shift of the imino proton of the guest nucleoside (substrate) as a function of the host concentration. Solutions of the substrate, held at 9 mM, were titrated with aliquots of a concentrated solution of the receptor (host), to reach host concentrations in the range 0.5-15 mM. The titration curves were analysed, and the binding constants calculated with the usual algorithm for 1:1 equilibrium binding processes, either through standard nonlinear regression methods or by linearization with the double-reciprocal plot of the binding data (32, 33).

Theoretical studies. Conformational analyses of receptors, substrates, and complexes were conducted using the INSIGHT/DISCOVER package (39). Molecular dynamics (MD) was used to generate different conformations and molecular mechanics (MM) was used for refinement. The temperature was 1000 K and the time-step was 1fs. Two hundred structures were saved at intervals of 100 steps into a trajectory file. Each structure was then minimized using MM, with the default CVFF force field, together with template charges and distance-dependent dielectric constants to simulate the solvent. The global minima calculated for simple and complex molecules were subsequently minimized by the AM1 (MOPAC) semi-empirical method included in the software package.

The binding energy (E_{bind}) of a complex represents the difference between the energy of the complex and the sum of the individual energies of the substrate and the receptor.

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