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Molecular Recognition of Acetylaminofluorene- and Aminofluorene-modified Guanosine

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New receptors based upon a carboxamidonaphthyridine unit derivatized with the polyaromatic segments [4-(1-pyrenyl)butyroylamino] and [3-(9-anthracenyl)propanoylamino] have been synthesized and studied by ^{1}H NMR titration in their binding interaction to the guanosine-C8 adducts from the carcinogens 2-acetylaminofluorene and 2-aminofluorene. The high binding energy, in comparison with that of the non-derivatized analogues, is rationalized in terms of a bi-site interaction (hydrogen bonding and aromatic stacking). Although many factors may contribute to the strength of the host-guest complexes described herein, it appears that in some cases π-π interaction may induce a concomitant bending in the hydrogen bond system.

Keywords: Molecular-recognition, guanosine-C8 adducts, 2-acetylaminofluorene, 2-aminofluorene, carboxamidonaphthyridine

INTRODUCTION

Aromatic amines are a class of substances with carcinogenic properties that are present in multiple environmental sources, including tobacco smoke, automobile exhaust and cooked foods [1,2]. Following metabolic activation, aromatic amines are converted to electrophilic species that have the ability to bind to DNA, yielding covalent adducts [3,4]. If unrepaired, this covalent attachment of the metabolised carcinogen may cause misreplications that will ultimately lead to initiation of cancer. Since DNA adducts may be regarded as biomarkers of carcinogen exposure, much effort has been directed in recent years [5,6], towards the development of analytical techniques suitable for the detection of these species when present in DNA at levels comparable to those found *in vivo*. An approach with potential great significance is the design of abiotic receptors capable of recognising and binding selectively to specific DNA adducts.

Much of our understanding of arylamine carcinogenesis has stemmed from work conducted with 2-aminofluorene (AF) and 2-acetylaminofluorene (AAF). Following activation, both form C8-substituted deoxyguanosine derivatives as the predominant adducts *in vivo* and *in vitro* [3,4], whose conformational and mutagenic properties have been extensively studied (reviewed in Ref. [7]).

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The design of synthetic molecules capable of binding selectively to modified nucleobases and mimic enzyme-substrate interactions has attracted considerable attention in recent years [8]. Accordingly, multi-site approaches have been introduced for the molecular recognition of nucleotides, namely involving host-guest interactions by means of hydrogen bonding and aromatic stacking [8–11].

Because of the crucial role of guanine in biological processes, a number of different receptors have been designed for this nucleobase or its derivatives. Among these, carboxamidonaphthyridines, often inserted in macrocycles [9, 10] or other rigid system [11], have been widely used as abiotic base receptors. These structures are capable of forming a tight triple hydrogen-bonded complex with guanine that simulates quite efficiently the Watson-Crick base pairing interaction between guanine and cytosine.

In a previous study [12] we have used a combined experimental ^{1}H NMR and theoretical procedure as an initial approach to evaluate the relative ability of a simple naphthyridinic receptor, 7-acetylamino-2-methyl-1,8-naphthyridine (**NAPH1**), to bind to guanosine (**G**) and to *N*-(guanosin-8-yl)-2-acetylaminofluorene (**GAAF**), the guanosine adduct containing the carcinogen 2-acetylaminofluorene.

We now report the development of new carboxamidonaphthyridine-based structures, aimed at reinforcing the binding selectivity of this class of abiotic receptors towards polyarylamine-guanine adducts. While maintaining the ability to form triple hydrogen bonded complexes with the guanine moiety, the new units are further functionalized with a polyaromatic fragment, designed to provide a π-stacking interaction with the arylamine moiety of the adducts.

We describe herein the synthetic approach to receptors and substrates, and use analysis of ^{1}H NMR titration data to evaluate the binding properties of each receptor towards each of the guanosine adducts mentioned above. In addition, theoretical modelling studies are performed to assist in the elucidation of the binding complexes. Preliminary fluorescence measurements are also conducted to evaluate the extent of changes associated with the establishment of host-guest interactions between the adducts and the polyaromatic receptors.

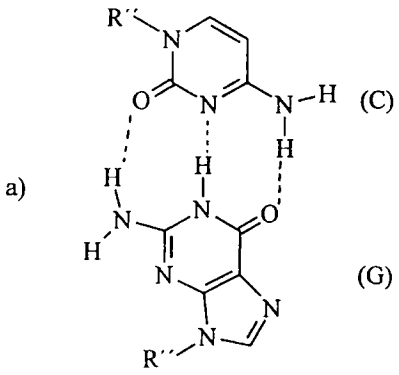
RESULTS AND DISCUSSION

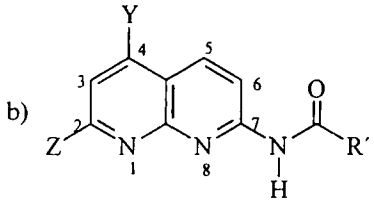
Design and Synthesis of Receptors and Substrates

In the design of receptors (Fig. 1) for guanosine-arylamine adducts, we selected the 2-carboxamido-1,8-naphthyridine structure as main building block due to its ability to generate a three hydrogen bonded complex with guanosine that simulates the Watson-Crick association between cytidine and guanosine. Furthermore, our preliminary results with **NAPH1**, both at the experimental and theoretical levels [12], indicated that such structures also have the potential to bind to guanosine-arylamine adducts.

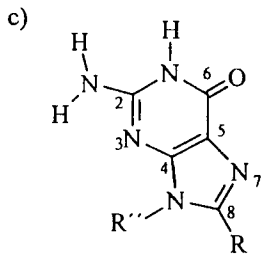
Selection of **NAPH2** as a receptor was based on computer-aided molecular modelling, which suggested that the 2-methyl substituent in **NAPH1** could produce some steric hindrance to the host-guest interaction. The bulkier receptors, **NAPH3** and **NAPH4**, were chosen to evaluate whether the incorporation of a polyaromatic segment could afford a significant π-stacking interaction with the arylamine moiety of adducts. The ultimate goal was to reinforce the host guest-interaction and thus enhance the molecular recognition of adducts.

The synthesis of **NAPH1** was described in our previous paper [12]. **NAPH2** was prepared by acid-catalysed condensation between 2,6-diaminopyridine and ethyl acetoacetate, to yield 7-amino-2-hydroxy-4-methyl-1,8-naphthyridine, which was subsequently *N*-acetylated with acetic anhydride. The *N*-acetylated product was then converted to 7-acetylamino-2-chloro-4-methyl-1,8-naphthyridine by reaction with phosphorous





	Z	Y	R′
(NAPH1)	CH_3	H	CH_3
(NAPH2)	H	CH_3	CH_3
(NAPH3)	CH_3	H	
(NAPH4)	CH_3	H	



	R
(G)	H
(GAAF)-	$R_1 = CH_3CO$
(GAF)-	$R_1 = H$

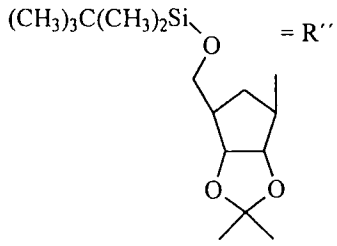


FIGURE 1 Structures of: the G : C pair (a), the receptors (hosts) [**NAPH1**, **NAPH2**, **NAPH3** and **NAPH4**] (b), and the substrates [**G**, **GAAF** and **GAF**] discussed in the text.

oxychloride. Catalytic hydrogenation [13] using 5% $Pd/CaCO_3$ afforded **NAPH2**. Similarly to **NAPH1**, both **NAPH3** and **NAPH4** were synthesized by *N*-acylation of 7-amino-2-methyl-1,8-naphthyridine. In each instance, the acylating agent was the corresponding acyl chloride, which was readily obtained from its commercially available carboxylic acid precursor using a standard procedure.

The model adducts *N*-(guanosin-8-yl)-2-acetylaminofluorene (**GAAF**) and its deacetylated analogue (**GAF**) were used in a derivatized form (Fig. 1), in order to achieve sufficient solubility in aprotic media of low dielectric constant. This requirement was essential to minimise solute-solvent interactions that might affect the assessment of host-guest interactions. The synthesis of **GAAF** has been described [12]. The non-acetylated adduct (**GAF**) was synthesised from *N*-(2′,3′-isopropylideneguanosin-8-yl)-2-acetylaminofluorene [12], through quantitative basic hydrolysis of the *N*-acetyl group in the presence of 2-mercaptoethanol [14], followed by 5′-*O*-silylation with *tert*-butyldimethylsilyl chloride. The parent nucleosides, **G** and **C**, which were needed for comparative binding studies, were obtained in a similar manner by 5′-*O*-silylation of 2′,3′-isopropylideneguanosine and 2′,3′-isopropylidenecytidine, respectively [15].

Determination of Host-guest Association Constants. General Procedure

The binding properties of the different receptors towards each guanosine derivative were studied by ^1H NMR spectroscopy. The experiment consisted of holding one component (guanosine derivative, substrate) at constant concentration and varying the concentration of the other component (receptor). The effect of complex formation was evident from the change in the chemical shifts of the labile protons involved in hydrogen bonding. The titration experiments were performed in deuterochloroform, in order to suppress solute-solvent hydrogen bonding interactions that might otherwise be present in aqueous solutions.

Significant chemical shift changes on the ^1H NMR spectra of each component were observed upon addition of the receptors, particularly for the labile protons of the solutes that might be involved in hydrogen bonding interactions. Thus, the downfield shifts of the imino (N1H) and the exocyclic amino (N^2H_2) protons of the guanosine-derived substrates (**G**, **GAF**, and **GAAF**, Tab. II) were fully consistent with the formation of a hydrogen bonding network involving those protons. We have found similar results for the same **G** protons along the titration with **C** (Tab. II), and this type of behaviour has also been reported by other authors using different guanosine derivatives in the same solvent [16–18]. In order to determine the host-guest association constants, titration curves were built in each instance by plotting the chemical shifts of the substrate imino protons against the receptor concentration (*cf.* Fig. 2 for representative examples). Data analysis was performed using a total curve-fitting algorithm implemented by Wilcox [19]. The option for continuously monitoring the substrate imino protons, as opposed to the amino protons, was driven by the fact that the latter ones suffered a gradual broadening along the titration and became totally obscured under the baseline after addition of approximately 1/2 equivalent of the receptor. By contrast, the substrate imino protons were quite sharp in the final stages of the titration (not shown), as the substrate became fully associated with the receptor.

Dimerization Studies

In order to obtain accurate host-guest association constants, dimerization interactions for each solute had to be taken into account. For that purpose, changes in the chemical shift of a suitable labile proton of each solute were monitored as a function of concomitant changes in the initial concentration of that solute. Typically,

the imino (N1H) proton of each guanosine-derived substrate and the amide NH proton of each receptor were the protons selected for this purpose. Non-linear regression analyses of the dimerization curves were then performed [19].

Figure 2 illustrates a representative titration curve of dimerization, corresponding to the **GAAF** adduct. The determination of the dimerization constants (K_{AA}) allowed the estimation of the chemical shifts at infinite dilution for the

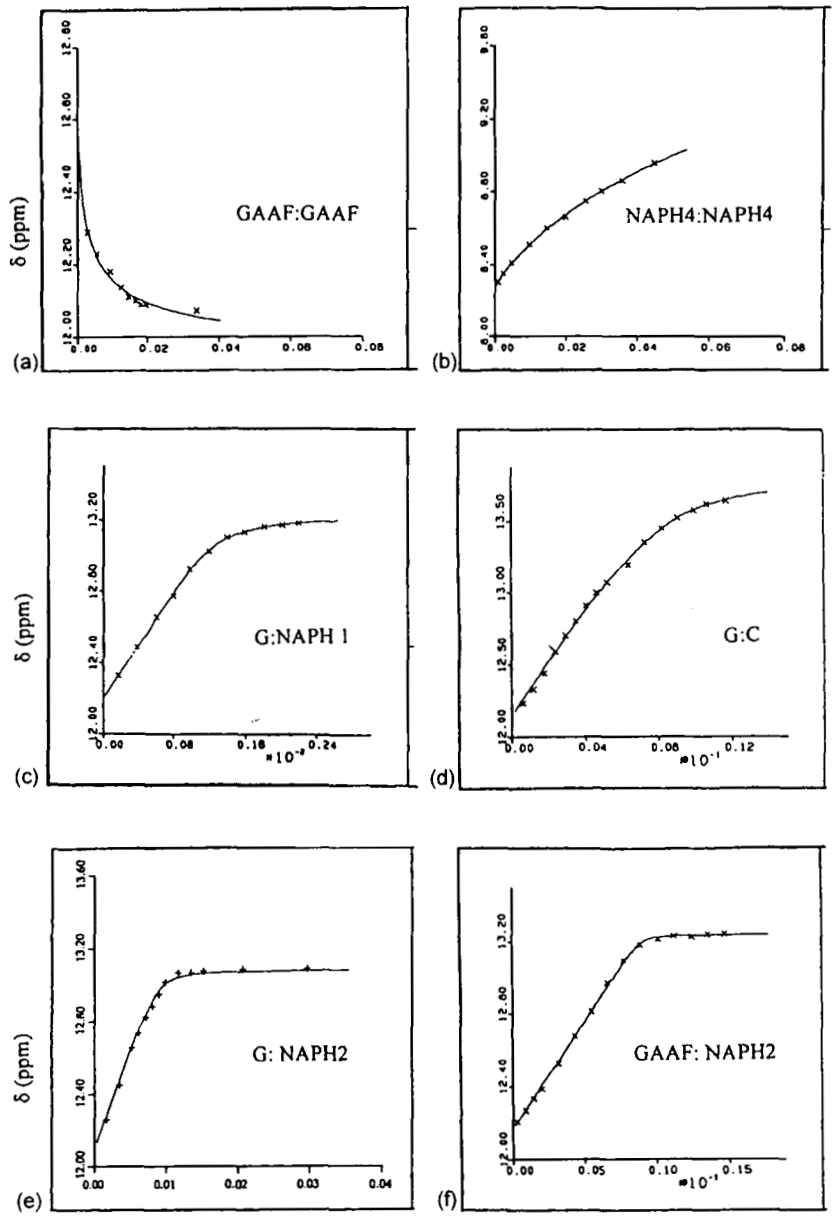


FIGURE 2 Representative plots of the ^{1}H NMR resonances of selected labile protons [the imino N1H protons of the substrates, or the amide protons of the receptor (**NAPH4**)], as a function of: the total concentration of the added receptors (host-guest studies) the total concentration of the substrate (**GAAF: GAAF**), or the total concentration of the receptor (**NAPH4: NAPH4**). Observed (×) and calculated (—) data.

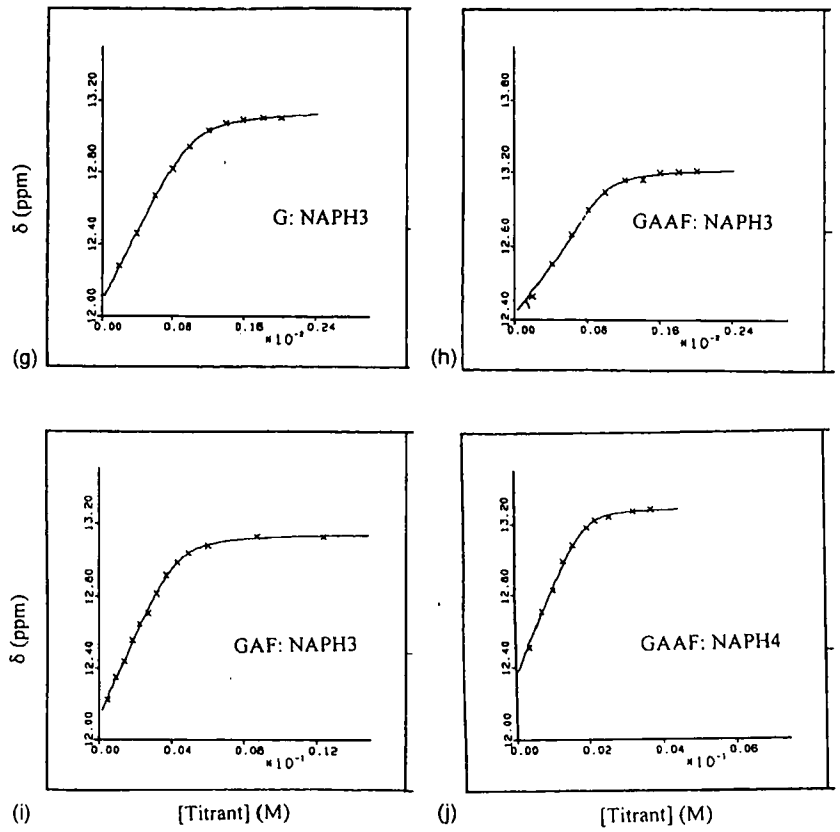


FIGURE 2 (Continued).

selected labile protons in the bound (δ_D) and unbound (δ_M) states. Thus, the mole fraction of dimerized solute could be calculated for each initial concentration and introduced in the calculations of host-guest association constants. Dimerization constants (Tab. I) were obtained for all substrates and receptors with the exception of **GAF** (*vide infra*).

Analysis of Table I indicates that the dimerization constants for the **G** and **GAAF** substrates (126 and 251 M^{-1}, respectively) are substantially high. The value found for the parent **G** is somewhat lower than that (298 M^{-1}) reported previously for 2'-deoxyguanosine [17], though within the same order of magnitude. These values are consistent with the formation of double hydrogen-bonded systems [20]. Involvement of the N1H and N^2H_2 protons of the guanine fragments in the dimerization process is conceivable, since both exhibited concentration-dependent chemical shifts (not shown).

The self-association of the **GAAF** substrate was stronger than that of **G**, as indicated by higher shifts of the imino (Tab. I) and amino protons and reflected in the approximately 2-fold higher dimerization constant (Tab. I). As observed in our previous study [12], the imino proton underwent an upfield shift upon increasing the adduct concentration, which could indicate a prevalent role for hydrophobic interactions, rather than hydrogen bonding, in the self-association of the adduct. Nonetheless, it should be noted that narrowing of the imino proton peak at the higher substrate concentrations was clearly observed (not shown). This suggests that thermal exchange between

TABLE I Binding data for dimerization: association constants (K_{AA}), maximum calculated chemical shift variations ($\Delta\delta_{max}$)[a] induced by the dimerization, and extrapolated chemical shifts of the free species (δ_{free}) for the selected labile protons[b]

A:A	K_{AA} (M^{-1})	ΔG^{295}_{AA} (kcal/mol)	$\Delta\delta_{max}$	δ_{free}[a]
			(ppm)	
G:G	126	2.8	0.238	12.370
GAAF:GAAF	251	3.2	−0.728	12.627
NAPH1:NAPH1	2	0.4	4.545	8.246
NAPH2:NAPH2	5	0.9	4.200	8.356
NAPH3:NAPH3	3	0.6	4.445	8.198
NAPH4:NAPH4	5	0.9	2.659	8.284

[a] Defined according to Ref. [19].
[b] The imino N1H protons of the substrates or the amino protons of the receptors.

free and hydrogen-bonded imino protons occurred at the lower concentrations and gradually decreased as the dimeric species became predominant.

By contrast with **G** and **GAAF**, the study of **GAF** self-association could not be performed using the same procedure. In fact, in order to use the dilution method we had to start from a concentrated solution (6×10^{-2} M). This solution, which had a deep blue colour, exhibited a very complex ^{1}H NMR spectrum with numerous broad peaks, particularly in the downfield region (not shown). This was not due to degradation of the substrate, since the ^{1}H NMR spectra of the same species in polar solvents (*e.g.*, deuteroacetone) were sufficiently resolved and exhibited all the expected signals (*vide infra*). Such behaviour is consistent with the formation of one or more associated species in deuterochloroform, with probable involvement of at least one excimer, as a result of extensive π-π stacking. Even upon successive dilutions of the parent solution, we were unable to obtain a colourless solution with a well-resolved ^{1}H NMR spectrum in deuterochloroform. Therefore, the association constants calculated for host-guest interactions involving **GAF** (Tab. II) must be regarded as lower limits.

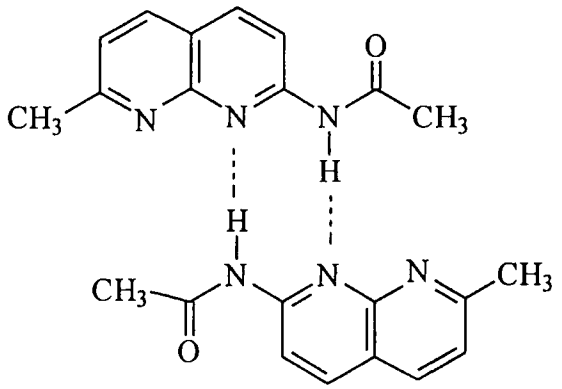
We have also conducted analyses of the dimerization behaviour for all the receptors used

TABLE II Data for binding of a series of receptors to a series of substrates: association constants (K_{AB} and ΔG^{295}_{AB}) and maximum measured ($\Delta\delta_{obser}$) and calculated ($\Delta\delta_{calc}$) chemical shift variations induced by the receptors in the imino N1H protons of the substrates

A:B	K_{AB} (M^{-1})	ΔG^{295}_{AB} (kcal/mol)	$\Delta\delta_{obser}$	$\Delta\delta_{calc}$[a]
			(ppm)	
G:C	$2.5 \pm 1.0 \times 10^3$	−4.6	1.493	1.650
G:NAPH 1	$2.8 \pm 0.8 \times 10^3$	−4.6	1.032	1.049
GAAF:NAPH1	$4.6 \pm 1.1 \times 10^3$	−4.9	1.146	0.752
GAF:NAPH1	$4.2 \pm 1.1 \times 10^3$	−4.9	1.052	1.107
G:NAPH2	$8.1 \pm 2.3 \times 10^3$	−5.3	0.998	1.017
GAAF:NAPH2	$6.5 \pm 3.5 \times 10^4$	−6.5	1.038	0.617
GAF:NAPH2	$3.8 \pm 0.5 \times 10^3$	−4.8	1.123	1.045
G:NAPH3	$2.8 \pm 0.5 \times 10^4$	−6.0	0.88	1.102
GAAF:NAPH3	$4.6 \pm 1.1 \times 10^4$	−6.3	0.950	0.602
GAF:NAPH3	$1.1 \pm 0.2 \times 10^4$	−5.5	0.85	1.032
G:NAPH4	$6.3 \pm 2.0 \times 10^3$	−5.1	0.980	1.232
GAAF:NAPH4	$3.8 \pm 1.1 \times 10^4$	−6.2	0.770	0.930
GAF:NAPH4	$1.4 \pm 0.2 \times 10^3$	−4.2	0.970	1.280

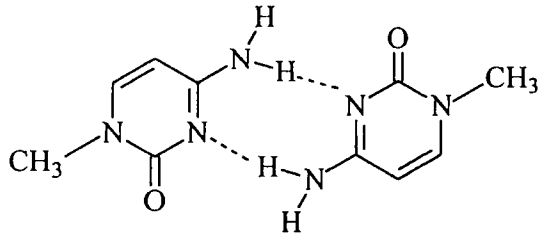
[a] Defined according to Ref. [19].

in this study, with the exception of **C**, for which there is ample information in the literature in nonaqueous solvents. Published values ($28 \pm 3\,M^{-1}$) [21] for the **C:C** interaction are 5–10 fold lower than those we obtained for **G** and **GAAF**. The dimerization of the naphthyridinic receptors was analysed on the basis of the observed changes on the chemical shifts of the amide protons (Tab. I). The results are consistent with the formation of double hydrogen-bonded aggregates (Fig. 3), although the calculated dimerization constants were found to be very low ($K = 2-5\,M^{-1}$, Tab. I). These values indicate a complexation energy (Tab. I) lower than that proposed by Schneider and co-workers [20] for similar systems (1.2 kcal/hydrogen bond) but are within the range of those reported for the dimerization of diamidopyridine derivatives ($K = 2\,M^{-1}$) [22]. Although the dimerization of the naphthyridinic receptors can be considered negligible, all the values obtained for self-association constants were included in the calculation of host-guest association constants.



NAPH1:NAPH1

(a)



C:C

b)

FIGURE 3 Structural models proposed for the self-association of **NAPH1** (a) and **C** derivatives (b).

Host-guest Interaction Studies

Upon addition of the receptors to the substrates, a downfield shift was observed in the ^1H NMR signals of the imino (N1H) protons of all substrates (**G**, **GAAF**, and **GAF**, Tab. II). These changes followed a hyperbolic variation with the receptor concentration (see Fig. 2 for representative examples) and reached the saturation plateau after addition of approximately one equivalent of receptor, which indicates the formation, in all instances, of aggregates with 1:1 host-guest stoichiometry. The amino (N^2H_2) protons of the substrates underwent a similar hyperbolic downfield shift (not shown).

Initial addition of each naphthyridinic receptor to any of the guanosine-derived substrates caused a substantial downfield shift of the amide proton signal of the receptor (*e.g.*, $\delta =$ 11.97 ppm for **NAPH1 : G** association). This was followed by a gradual upfield shift of that amide proton resonance, as the titration progressed, so that this resonance in the final stage of the titration ($\delta = 9.43$ ppm) was much closer to that of the unbound receptor ($\delta = 8.75$ ppm). While the initial downfield shift can be attributed to the establishment of a hydrogen bonding interaction with the substrate, the subsequent gradual upfield shift with increasing receptor concentration must reflect a variation in the weighted average of complexed and uncomplexed forms of the receptor along the titration.

Taken together, the ^1H NMR spectral changes observed for all the binding complexes analysed in this study, particularly in the imino and amino

protons of the guanosine-derived substrates and in the amide proton of the naphthyridinic substrates, are fully consistent with the formation of triple hydrogen-bonded systems, in which the receptors simulate the Watson-Crick base-pairing properties of cytosine. Excluding possible steric hindrance effects, all receptors should have an equal contribution to the hydrogen bonding interaction. However, since the receptors investigated had different aromatic surfaces, distinct extents of π-stacking were expected.

A comparative analysis of the binding constants calculated for the **G:C** and **G:NAPH1** complexes (Tab. II) indicates that, among the set of abiotic receptors used in this study, **NAPH1** appears to be the most adequate for strict simulation of the role of cytidine, presumably *via* a *DDA : AAD* arrangement (*D* – hydrogen bond donor atom; *A* – hydrogen bond acceptor atom). The calculated Gibbs free energies of binding (4.6 Kcal/mol) for both complexes suggest that each hydrogen bond contributed approximately 1.5 kcal/mol to the total binding energy. This is in good agreement with published results, in which an average hydrogen bond strength of 1 – 1.5 kcal/mol in chloroform has been estimated [23]. The **G:C** binding constant obtained in the present study may be slightly underestimated, because the self-aggregation of **C** was not included. However, the value calculated for the **G:C** association constant is close to the recently reported 1:1 association constant between *O*-silylated derivatives of deoxyguanosine and deoxycytidine in deuterochloroform (20,000 M^{-1}) [18]. Similar values ($1 \times 10^4\,M^{-1}$) were also found for the association constants of other derivatized G:C pairs [21]. Comparison of our experimental values to those reported in the literature should be made with caution because the use of different methodologies, mostly in the curve fitting, may affect the results. In fact substantially lower values (90 – 130 M^{-1}) have been reported for the association constant between *O*-derivatized guanosines and other similar receptors based on the 2-amino-1,8-naphthyridine molecular segment [8, 9]. In our preliminary study, we also found lower values (ca 297 and 85 M^{-1}) for the same host-guest systems (**G:C** and **G:NAPH1**, respectively) [12]. However, in that study we used a great number of broad approximations in the fitting procedure and the dimerization was not included in the host-guest calculations.

With regard to the receptor **NAPH2**, much higher constants were obtained for its association with **G** ($8.1 \times 10^3\,M^{-1}$) and **GAAF** ($6.5 \times 10^4\,M^{-1}$), (Tab. II). Furthermore, among the series of receptors studied (see Tab. II), **NAPH2** has also demonstrated the highest capability to differenciate between the normal guanosine (**G**) and the *N*-acetylated adduct (**GAAF**). The observed difference could result from many factors that govern hydrogen bond strength, including electronic and stereochemical effects. However, the effect of the methyl group in position 2 (**NAPH1**) or 4 (**NAPH2**) of the naphthyridine ring, as electron donor with potential influence on the basicity of the N^1 nitrogen, should be comparable in both isomers. Thus, stereochemical effects may account for the observed differences, since the methyl group may produce some hindrance on the closest hydrogen bond. Molecular modelling results indicate a high length (2.46 Å) for the hydrogen-bond (**NAPH1**)$N^8 \ldots$ HNH(**G**) close to the 2-methyl group, which is fully consistent with this interpretation.

The results obtained with the other naphthyridinic receptors containing polyaromatic segments (**NAPH3** and **NAPH4**) must be analysed in comparison with the homologous **NAPH1**. Both aromatic substituents, pyrenyl (**NAPH3**) and anthracenyl (**NAPH4**), caused increases in the association constants with **G** and **GAAF**. With these receptors, the binding enhancements cannot be attributed to a simple intensification of the H-bond interaction due to decreased hindrance, as happened with **NAPH2**. Although measurements were made strictly on the basis of

the hydrogen-bond interaction, that increase in binding strengths must be due to some π-π interaction, which should favour the base pairing. The existence of an aromatic interaction is also supported by the fact that, in spite of the higher values obtained for the association constant, downfield shifts of lower magnitude were obtained, thus reflecting shielding effects associated with the aromatic interaction. Both aromatic receptors demonstrated capability to discriminate between the adduct **GAAF** and the normal **G**. This feature was more pronounced in **NAPH4** (see Tab. II). However, **NAPH3** exhibited in general, higher association constants than **NAPH4**, presumably due to the presence of one additional aromatic ring.

The molecular recognition studies of the C8-guanosine-aminofluorene adduct (**GAF**) indicated association constants typically lower than those obtained with **G** and **GAAF**. This is likely to reflect the contribution of the competing self association, which could not be included in the calculation. In addition, the spatial orientation of the fluorene ring may be less adequate for the interaction with the aromatic substituents of the receptors and thus less effective than with **GAAF**. It should be noted that, compared to **GAAF**, **GAF** has a significantly smaller percentage of low energy *syn* conformers [7].

Among all the receptors studied, only **NAPH1** was able to induce some binding enhancement with **GAF**, as compared with **G**. In absolute terms, however, **NAPH3** produced a quite intense interaction with this adduct. The low chemical shift variation obtained along the titration (0.85 ppm, Tab. II) is indicative of some host-guest π-π stacking in this system.

In summary, the ^{1}H NMR titration results suggest the occurrence of cooperativity, with a two-site binding for complex formation, *via* hydrogen bonding and π-π stacking. In order to get some insight into the extent of that cooperativity, we have performed some molecular modelling studies (*vide infra*). Despite the evidence of cooperativity, it should be noted, however, that the model has some limitations. In fact, as noted above, an increase in the π-π interaction appeared to introduce some hindrance or distortion on the hydrogen bonding system.

Fluorescence Properties

In order to examine the possible fluorescence changes accompanying and following the molecular association of the adducts with the polyaromatic receptors, we studied the pyrene-

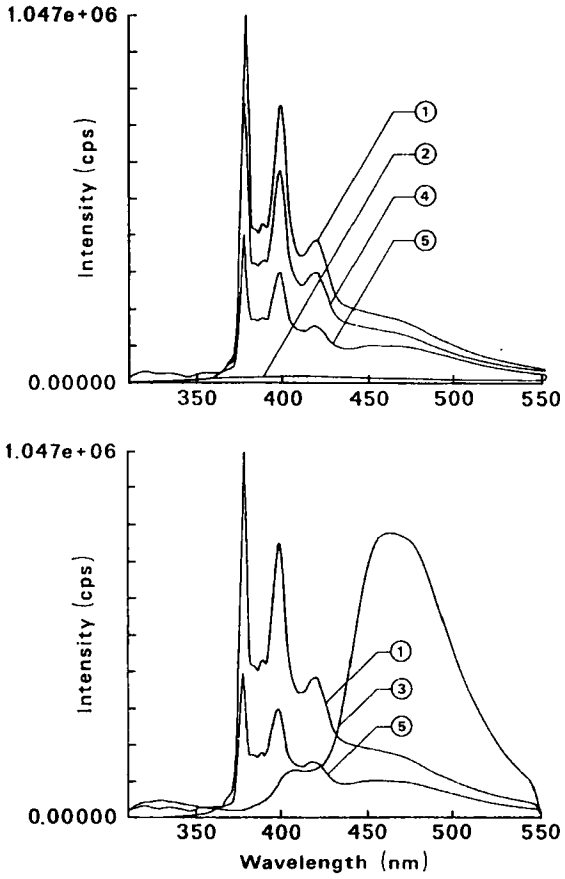


FIGURE 4 Fluorescence emission spectra (λ_{exc} = 300 nm) of: the receptor [**NAPH3**, (1)], the substrates [**G** (2) and **GAAF** (3)] and the corresponding (1:1) host-guest complexes [**NAPH3**:**G** (4) and **NAPH3**:**GAAF** (5)]. Solution concentration: 2.5×10^{-5} M.

containing receptor, **NAPH3**. Its fluorescence spectra showed the structured fluorescence emission profiles consistent with published pyrene spectra [24]: a set of fluorescence bands from 370 to 410 nm attributed to the pyrene monomer, and a weak broad band, extending from 410 to 550 nm, due to the excimer fluorescence (see Fig. 4). This was expected, since at low concentrations (3×10^{-5} M) pyrene is mainly in the monomeric form. On the other hand, the fluorescence spectrum of **GAAF** exhibited a strong broad band centred at *ca.* 472 nm, even at the lowest concentration used (6×10^{-5} M). Any bands characteristic of the fluorene segment were virtually absent [24]. This result suggests the existence of a strong excimer as a result of molecular aggregation, or an internal transfer energy band. On the other hand, upon addition of **NAPH3** to the substrate, the sudden disappearance of the 472 nm band was observed, along with some concomitant decreasing of the pyrene bands, mostly the ones corresponding to the monomer. These results are consistent with a considerable quenching of the **GAAF** exciplex by the pyrenyl receptor, although the type of intermolecular interaction involved has yet to be established. Therefore, this type of receptors containing polyaromatic substituents, and in particular the pyrene derivative, have a fluorescence behaviour of potential usefulness for application as biological probes. A detailed study of the quenching effect of **NAPH3** towards the **GAAF** adduct is underway and will be reported in due course.

Molecular Modelling

For a comparative study of the interaction between the abiotic receptors and the substrates, molecular modelling was performed by Molecular Mechanics (MM) and semi-empirical Molecular Orbital (MOPAC-AM1) methods [12, 25]. In these calculations, we first designed the molecular systems based on molecular mechanics calculations using AM1 charges. AM1 calculations were also used to get the final energy of each molecule or complex, once the total minimisation procedure revealed to be impracticable for our host-guest-systems, due to the number of atoms involved.

Molecular modelling studies of **G:NAPH1** indicated a high hydrogen-bond length on $N_8 \ldots$ HNH (2.46 Å), which was rationalized in terms of a steric hindrance attributed to the 2-methyl group of the naphthyridine unit. To overpass this problem, the isomeric receptor **NAPH2** was prepared for a comparative study. The corresponding hydrogen bond in the **G:NAPH2** complex was a little smaller (2.36 Å), which is consistent with the higher value found for the association constant (see Tab. II).

Molecular mechanics results suggested that, in addition to the hydrogen-bond interaction present in all host-guest systems studied, there is a considerable π-π-interaction, which is characterised by a nearly parallel alignment of the polyaromatic segments, namely the fluorene unit of the adducts (**GAAF** and **GAF**) and the pyrene and anthracene segments of the receptors (**NAPH3** and **NAPH4**, respectively). However the π-π-interactions may be overestimated in the calculations, as indicate by the interplanar distance between the aromatic planes ($\sim$3.8–3.9 Å) [26], which may be due to the neglecting of solvent interactions in the calculations.

Although these calculations suggest that the π-π-interactions can induce some distortion on the hydrogen-bonding systems, the effect of the two sites can be summarised in a global strengthening of the molecular interaction, as indicated by the NMR results.

CONCLUSIONS

A series of new abiotic receptors based on carboxamido-1,8-naphthyridines were devel-

oped and explored as models for a two-site type binding to aminofluorene derived C8-guanosine adducts. The high binding affinity with the guanosine derivatives stems from simultaneous hydrogen bonding, in a Watson-Crick-like base-pairing, and aromatic π-stacking. Although many factors may contribute to the strength of the host-guest complexes described herein, it appears that in some cases π-π interaction may induce some concomitant bending in the hydrogen bond system. The strength of the aromatic stacking is yet to be evaluated, and future efforts must be directed towards its quantification, in order to assess the relative contributions of the different factors.

MATERIALS AND METHODS

Instrumentation

Melting temperature were measured with a Leica Galen III hot stage apparatus and are uncorrected. Infrared (IR) spectra were recorded in a Perkin-Elmer 683 spectrophotometer. Elemental analyses were performed on a Fifons EA 1108 CHNF/O instrument. Mass spectra were obtained in a VG TRIO-2000 spectrometer. Electron impact (EI) spectra were generated at 70 eV; FAB spectra were performed with the samples dispersed in a matrix of thioglycerol or 3-nitrobenzyl alcohol.

^{1}H NMR spectra were recorded on a Varian Unity 300 spectrometer. Chemical shifts are reported in ppm (δ) from internal references {tetramethylsilane (TMS) in $CDCl_3$ solutions and sodium 3-(trimethylsilyl)-[2,2,3,3-$^2H_4$] propionate in D_2O solutions}. The following abbreviations are used: s = singlet; d = doublet; t = triplet; m = multiplet; bs = broad singlet.

Absorption spectra were obtained in a JASCO V-560 UV-Vis spectrophotometer. Fluorescence spectra were recorded with a SPEX Fluorolog F112 spectrofluorimeter. All measurements were performed at room temperature in chloroform (Merck-Uvasol). The excitation wavelength was 300 nm.

Chemicals

Analytical grade reagents were used as supplied. Whenever necessary, solvents were purified according to standard methods [27].

Syntheses

Substrates

The syntheses of the protected nucleosides, 2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl) cytidine (**C**) and 2′,3′-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosine (**G**), as well as of the adduct, *N*-[2′,3′-isopropylidene-5′-*O*-(*tert*-butylsilyl)guanosin-8-yl]-2-acetylaminofluorene (**GAAF**) have been detailed in our previous paper [12]. *N*-[2′,3′-Isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl) guanosin-8-yl]-2-aminofluorene (**GAF**) was synthesized from *N*-(2′,3′-Isopropylideneguanosin-8-yl]-2-acetylaminofluorene by a two-step procedure [12]. Specifically, the starting material (43 mg, 0.08 mmols) was quantitatively deacetylated at room temperature by hydrolysis with 1M NaOH (8 ml) containing 0.3% (v/v) of 2-mercaptoethanol [14]. After 1 h, the mixture was neutralized with 1N HCl and the product was extracted with water-saturated *n*-butanol, evaporated to dryness and used in the next step without further purification. 5′-*O*-silylation with *tert*-butyldimethylsilyl chloride, as described previously [15], was achieved in 48% yield. **^{1}H NMR** (deuteroacetone) −0.04 (6H, s, $(CH_3)_2$Si), 0.83 (9H, s, $(CH_3)_3$ Si), 1.31 (3H, s, CH_3C—O), 1.47 (3H, s, CH_3C—O), 3.83 (4H, m, H5 + H5″ + FH9), 4.15 (1H, bs, H4′), 5.11 (1H, bs, H3′), 5.59 (1H, bs, H2′), 6.13 (1H, bs, H1′), 6.83 (1H, bs, N^2H), 7.23–7.32 (2H, m, FH), 7.48 (2H, bs, FH), 7.73 (3H, bs, FH), 8.38 (1H, bs, N^2H), 8.98 (1H, bs, FNH), 11.47 (1H, bs, N1H); m/z (DCI) 645 [12%, $(M + C_2H_5)^+$], 617 [100%, $(M + 1)^+$], 331 [3%, (guanine-AF + 1)$^+$, 182 [13%, $(AFH)^+$].

Receptors

The synthesis of 7-amino-2-methyl-1,8-naphthyridine and its conversion to **NAPH1** have been described [12].

7-Amino-2-hydroxy-4-methyl-1,8-naphthyridine

Into a 500 ml flask were added 2,6-diaminopyridine (25.01 g, 0.229 mol), ethyl acetoacetate (21.4 g) and H_3PO_4 (100 ml). This mixture was heated on a water bath with vigorous stirring over a period of 3 h, upon which it got thick. The mixture was cooled, mixed with ice and water and neutralized with ammonium hydroxide. The white precipitate was filtered, washed with water and ethyl acetate and then dried in vacuum to give 12.08 g (30%) of the product; mp > 310°C, m/z (EI) 175 (M^+), ^{1}H NMR (D_2O) δ 1.69 (3H, s, CH_3), 5.72 (1H, s, Naph-H3), 6.09 (1H, d, J = 9.3 Hz, Naph-H5), 7.45 (1H, d, J = 9.3 Hz, Naph-H6).

7-Acetylamino-2-hydroxy-4-methyl-1,8-naphthyridine

To the 7-amino-2-hydroxy-4-methyl-1,8-naphthyridine (12 g, 0.069 mol) was added an excess of acetic anhydride and the mixture was refluxed for 4 h. After cooling, it was filtered and recrystallized from glacial acetic acid to give yellow crystals (11.86 g, 79%); mp > 310°C, IR (KBr, cm^{-1}) 1680 (C=O); m/z (EI) = 217 (M^+).

7-Acetylamino-2-chloro-4-methyl-1,8-naphthyridine

The 7-acetylamino-2-hydroxi-4-methyl-1,8-naphthyridine (11.5 g, 0.053 mol) was refluxed (110°C) with $POCl_3$ (100 ml) for 1 h. After cooling, the solution was poured into ice, neutralized with ammonium hydroxide and extracted with chloroform. Evaporation of the solvent and recrystallization of the solid residue from toluene gave the chlorinated derivative (8.91 g, 71%), mp = 243 – 245°C [lit. 245 – 247°C [13]; 240°C [28]], m/z (EI) 235 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.31 (3H, s, $COCH_3$), 2.69 (3H, s, CH_3), 7.25 (1H, s, Naph-H3), 8.35 (1H, d, J = 9.2 Hz, Naph-H5), 8.55 (1H, d, J = 9.2 Hz, Naph-H6), 9.14 (1H, bs, NH).

7-Acetylamino-4-methyl-1,8-naphthyridine (NAPH2)

To a solution of 7-acetylamino-2-chloro-4-methyl-1,8-naphthyridine (6 g, 0.026 mol) in 2.5% KOH-methanol (200 ml) was added 3.6 g of 5% Pd-$CaCO_3$ plus a trace of 5% Pd-C. This mixture was placed in a Parr apparatus at a pressure of 40 p.s.i. for 2 h. The mixture was filtered, the solvent was evaporated and the solid residue was extracted with dry dichloromethane. Evaporation of the solvent and recrystallization from dry ethanol gave white crystals of **NAPH2** (3.5 g, 67%); mp 198 – 200°C [lit. 202°C [13]]; ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, s, CH_3CO), 2.70 (3H, s, CH_3), 7.21 (1H, d, J = 4.5 Hz, Naph-H3), 8.38 (1H, d, J = 8.9 Hz, Naph-H5), 8.84 (1H, d, J = 8.9 Hz, Naph-H6), 8.44 (1H, d, J = 4.5 Hz, Naph-H2), 9.81 (1H, bs, NH).

4-(1-Pyrenyl)butyroyl chloride

4-(1-Pyrenyl)butyric acid (0.50 g, 1.7 mmol) and oxalyl chloride (0.22 g, 1.7 mmol) were dissolved in dry dichloromethane (50 ml). One drop of dimethylformamide was added and the solution was magnetically stirred at room temperature. After the disappearance of the starting acid (about 2 h) excess oxalyl chloride and the solvent were removed by evaporation. The obtained solid was dried in vacuum and used without further purification.

2-Methyl-7-[4-(1-pyrenyl)butyroylamino]-1,8-naphthyridine (NAPH3)

To a solution of 7-amino-2-methyl-1,8-naphthyridine (0.28 g, 1.7 mmol) and triethylamine (0.19 g, 1.9 mmol) in dry THF (30 ml) was added

dropwise a THF solution (10 ml) of freshly prepared 4-(1-pyrenyl)butyroyl chloride (0.53 g, 1.7 mmol), at room temperature and under a nitrogen atmosphere. The reaction mixture was stirred for 5 h and the solvent was removed under reduced pressure. The residue was dissolved in chloroform and washed subsequently with 1N NaOH, 3M citric acid and water to remove excess of unreacted materials. The organic layer was then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure afforded a light yellow solid, that was purified by column chromatography on silicagel with CH_2Cl_2/methanol (38/1) as the eluent. Further recrystallization from toluene gave 2-methyl-7-[4-(1-pyrenyl)butyroylamino]-1,8-naphthyridine (**NAPH3**) as yellow crystals: 301 mg (41%); mp 260–262°C.; I.R. (KBr) 1720 cm^{-1} (C=O); m/z (EI) 429 (M^+). ^1H NMR ($CDCl_3$) δ 2.37 (2 H, m, $CH_2C\underline{H}_2CH_2$), 2.68 (2 H, t, J = 6.5 Hz, $CH_2C\underline{H}_2CO$), 2.78 (3 H, s, CH_3), 3.49 (2 H, t, J = 7.5 Hz, Pyr), 7.30 (1 H, d, J = 8.1 Hz, Naph-H3), 7.89–8.17 (10 H, m, 2H Naph + 8H Pyr), 8.34 (1 H, d, J = 9.9 Hz, Pyr), 8.42 (1 H, d, J = 8.7 Hz, Naph-H6). Anal. for $C_{29}H_{23}N_3O$ Calc.: C, 81.08; H, 5.40; N, 9.79; Found: C, 80.74; H, 5.35; N, 9.74%.

9-(3-Anthracenyl)propanoyl chloride

9-Anthracenepropionic acid (0.20 g, 0.8 mmol) and oxalyl chloride (0.101 g, 0.8 mmol) were dissolved in dry dichloromethane, one drop of dry DMF was added and the reaction mixture was stirred at room temperature for 2 h. The excess of oxalylchloride and the solvent were then removed by evaporation and the solid residue was dried in vacuum, and used in the next reaction without further purification.

2-Methyl-7-[3-(9-anthracenyl)propanoyl amino]-1,8-naphthyridine (NAPH4)

The synthesis of **NAPH4** was accomplished by a procedure similar to that used for **NAPH3**. The reaction lasted for 3 h at room temperature, the workup was identical, but the product was obtained in sufficiently pure form with no need for chromatography. Recrystallization from toluene afforded yellow crystals of **NAPH4** (31%): m.p. 255–257°C; IR. (KBr) 1670 cm^{-1}:(C=O); m/z (EI) 391(M^+); ^1H NMR ($CDCl_3$) δ 2.62 (3H, s, CH_3), 2.91 (2H, t, J = 8.4 Hz, CH_2), 4.06 (2H, t, J = 8.4 Hz, CH_2), 7.19 (1H, d, J = 8.1 Hz, Naph-H3), 7.47 (4H, m, Anthr-H2, 3, 6, 7), 7.94 (1H, d, J = 8.4 Hz, Naph-5H), 8.00 (2H, d, J = 7.8 Hz, Anthr-H2, 8), 8.13 (1H, d, J = 9.0 Hz, Naph-H4), 8.25 (2H, d, J = 8.7 Hz, Anthr-H4,5), 8.36 (1H, s, Anthr-H10), 8.54 (1H, d, J = 8.7 Hz, Naph-H6), 8.86 (1H, bs, NH). Anal. Calcd. for $C_{26}H_{21}N_3O$: C, 79.24; H, 5.41; N, 10.73; Found: C, 79.47; H, 5.22; N, 10.43%.

Determination of the Association Constants

The evaluation of the host-guest association constants was undertaken by ^1H NMR spectroscopy in deuterochloroform. A general protocol was followed in which a host (nucleoside) solution (0.5 ml, 1×10^{-3} M) was titrated with a solution containing the guest (5×10^{-3} M) as well as the host at the same concentration of the titrated solution.

The concentration of the substrate (titrated solution) varied from 9×10^{-3} M (for the weaker interactions, ex. **C**:**G**) to 1×10^{-3} M (for the stronger interactions, ex. **NAPH3**:**GAAF**); the titrant solution was 5–10 times more concentrated then that of the substrate. The chemical shift of the nucleoside imino proton was monitored as a function of the guest concentration. The titration curves were analyzed by fitting with non linear regression methods, using the Hostest-II program [19] to determine association constants. These constants were used in calculating the change in free energy (ΔG).

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