An ESI-MS and NMR Study of the Self-Assembly of Guanosine Derivatives

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The self-assembly of guanosine (G) derivatives in the presence of alkali-metal ions gives octameric or polymeric aggregates composed of stacked G quartets. This process is studied for some lipophilic G derivatives by means of ESI-MS. The ESI-MS results are discussed in the light of complementary information obtained from NMR and SANS (small-angle neutron scattering) studies. ESI-MS gives an excellent picture of the self-assembly process and gives new information on the effect of different cations and anions on the dimensions of the assembled species, information that could not have been obtained with SANS and NMR alone.

1. Introduction. – Among the nucleobases, guanine (G) has the special ability to form self-assembled tetrameric species. The current explanation of this property relies on the presence in guanine of two H-bond donor (NH(1), NH(2)) and two acceptor (O(6), N(7)) groups that are located approximately 90° relative to each other (*Fig. 1*). Although guanine tetramers (G quartets) constitute the basic structure of fibres [1], gels [2], liquid-crystalline phases [3], and biologically important systems [4], to our knowledge, only in a single case has an isolated G quartet been unequivocally observed [5].

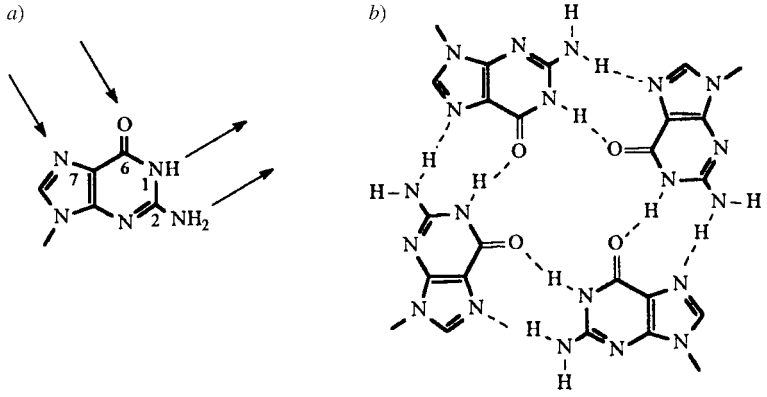


Fig. 1. a) *A guanine base and* b) *a G quartet.*

The effect of alkali-metal ions on the self-assembly processes has largely been studied in solution [2][6][7] and, more recently, in the solid state by high-resolution single-crystal X-ray [8]. Alkali-metal ions are located between the quartets and are likely to be coordinated by eight O-atoms of two superimposed quartets (*Fig. 2*). The

cations constitute a sort of cement that holds the complex structure together. The stabilization of the assembled species is in the order: $K^+ > Na^+$, $Rb^+ \gg Cs^+$, Li^+. This selectivity profile appears to correspond to fitting the ion size with the size of the octamer's central cavity [6][8].

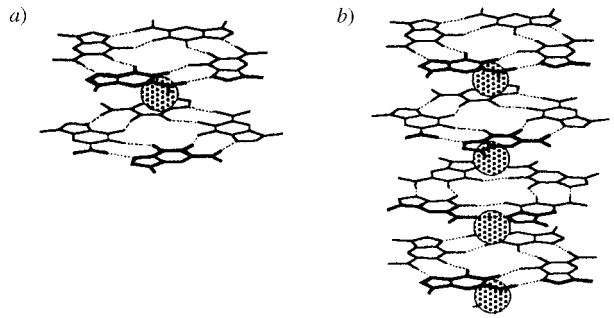
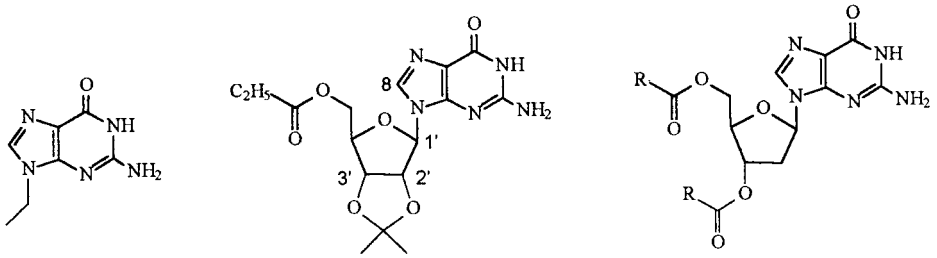


Fig. 2. a) *The octameric* $[G_8M]^+$ *and* b) *polymeric* $[G_4M]_n^{n+}$ *structures formed by guanine derivative G and alkali-metal ions M*. M is represented as a sphere.

Recently, we have synthesized and studied some lipophilic G derivatives. These compounds act as self-assembled ionophores and are able to transfer alkali-metal ions from H_2O into organic solvents [9–13]. Furthermore, they are able to discriminate chiral anions [11] and to give lyotropic phases in hydrocarbon solvents [13]. According to the relative concentration of the lipophilic derivatives and alkali-metal ions, and considering the molecular structure of the derivatives, octamers or polymers are formed, with the ions being masked by the lipophilic G quartets.

This being the state of the art, we were puzzled by a recent study on the self-assembly of 9-ethylguanine **1** as mediated by alkali-metal ions carried out by means of only ESI-MS [14]. In this work, the major species observed were G quartets containing alkali-metal ions, while no experimental data or comments on octameric or polymeric species were reported. Furthermore, the small Li^+ cation was reported to have an unexpectedly high stabilizing effect on G-quartet formation.

We report here a similar ESI-MS study carried out on derivative **1** and compounds **2** and **3**. Unlike 9-ethylguanine, the assembled species of derivative **4**, an analog of **3** with



1 **2** **3** R=C_2H_5

4 R=C_9H_{19}

longer alkyl chains, has been established unequivocally in solution by means of independent techniques (NMR, SANS) [9][10][13], while new NMR data for compound **2** are presented in this work. The significance of ESI-MS data for describing the self-assembly in solution will be discussed in light of results obtained with these independent and complementary solution techniques. This investigation represents a contribution towards establishing the use and limitations of ESI-MS in the field of supramolecular chemistry [15]. In the present case, the ESI-MS data are in excellent agreement with those obtained from NMR and have the further advantage of giving quantitative details on the effects of anions and cations.

2. Results. – 2.1. *ESI-MS Measurements.* 2.1.1. *9-Ethylguanine* (**1**). The spectra shown in *Fig. 3* were obtained for equimolar solutions (10^{-3} M) of 9-ethylguanine **1** with alkali-metal perchlorates in H_2O/MeOH 9:1. We report the spectra obtained for equimolar solutions since they gave the best spectral results. Compound **1** has a pronounced selectivity for Na^+ over K^+ according to these ESI-MS experiments[1]). In both Na^+ and K^+ solutions, the base peak corresponds to the molecular ion charged by the alkali-metal ion $[\mathbf{1}M]^+$. The dimeric $[\mathbf{1}_2M]^+$ and tetrameric $[\mathbf{1}_4M]^+$ species have peaks of lower intensities with respect to the base peak. In the case of addition of K^+ salt, traces of Na^+ are revealed in the mass spectrum. Noteworthy is the singly charged peak at 1471.8 (inset *Fig. 3,a*), a peak about 1200 times less intense than the base peak, revealing the presence of the octameric species $[\mathbf{1}_8K]^+$. The octamer $[\mathbf{1}_8K]^+$ exists even in this competitive solvent mixture that would inhibit the formation of H-bonds between the guanine molecules. Measurements carried out at lower desolvation temperatures did not show substantial changes in ion intensities. No octameric species with Na^+ was observed.

2.1.2. *2′,3′*-O-*Isopropylidene-5′*-O-*propanoylguanosine* (**2**). The samples examined by ESI-MS contained 8 mol of G derivative **2** per mol of the alkali-metal salt $LiClO_4$, $NaClO_4$, or $KClO_4$, in $CHCl_3$ (10^{-3} M) and were obtained by solid-liquid extraction, unless otherwise stated. This molar ratio was chosen to obtain the octameric aggregates already characterized by NMR. The 1:4 ratio was used either to promote the formation of a polymeric aggregate or to approach the tetrameric species observed for compound **1**.

The spectrum of the solution of **2** with $KClO_4$ (*Fig. 4,a*) is characterized by the presence of only one intense peak corresponding to the octameric species $[\mathbf{2}_8K]^+$. Also the spectrum of the solution of **2** with $NaClO_4$ (*Fig. 4,b*) shows only one peak, related to the species $[\mathbf{2}_8Na]^+$. No monomeric or tetrameric adducts of **2** bound to Na^+ or K^+ are present. The spectrum (not shown) of a solution of **2** with $LiClO_4$ in $CHCl_3$ shows only the species $[\mathbf{2}_8Na]^+$, originating from background Na^+. Similar measurements were also carried out with the picrate salts, and the mass spectra are substantially identical with the exception of sodium picrate (NaPic). In this case, a weak signal corresponding to the hexadecameric species $[\mathbf{2}_{16}Na_3]^{3+}$ is observed in addition to the base peak of the octameric species. Again, no signal corresponding to the tetrameric species $[\mathbf{2}_4Na]^+$ is detected. To exclude the presence of tetrameric species, the spectra

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[1]) The use of a 1 mM solution of **1** eluted on HPLC with a 0.1 mM salt solution in H_2O/MeOH 9:1 has been reported in [14].

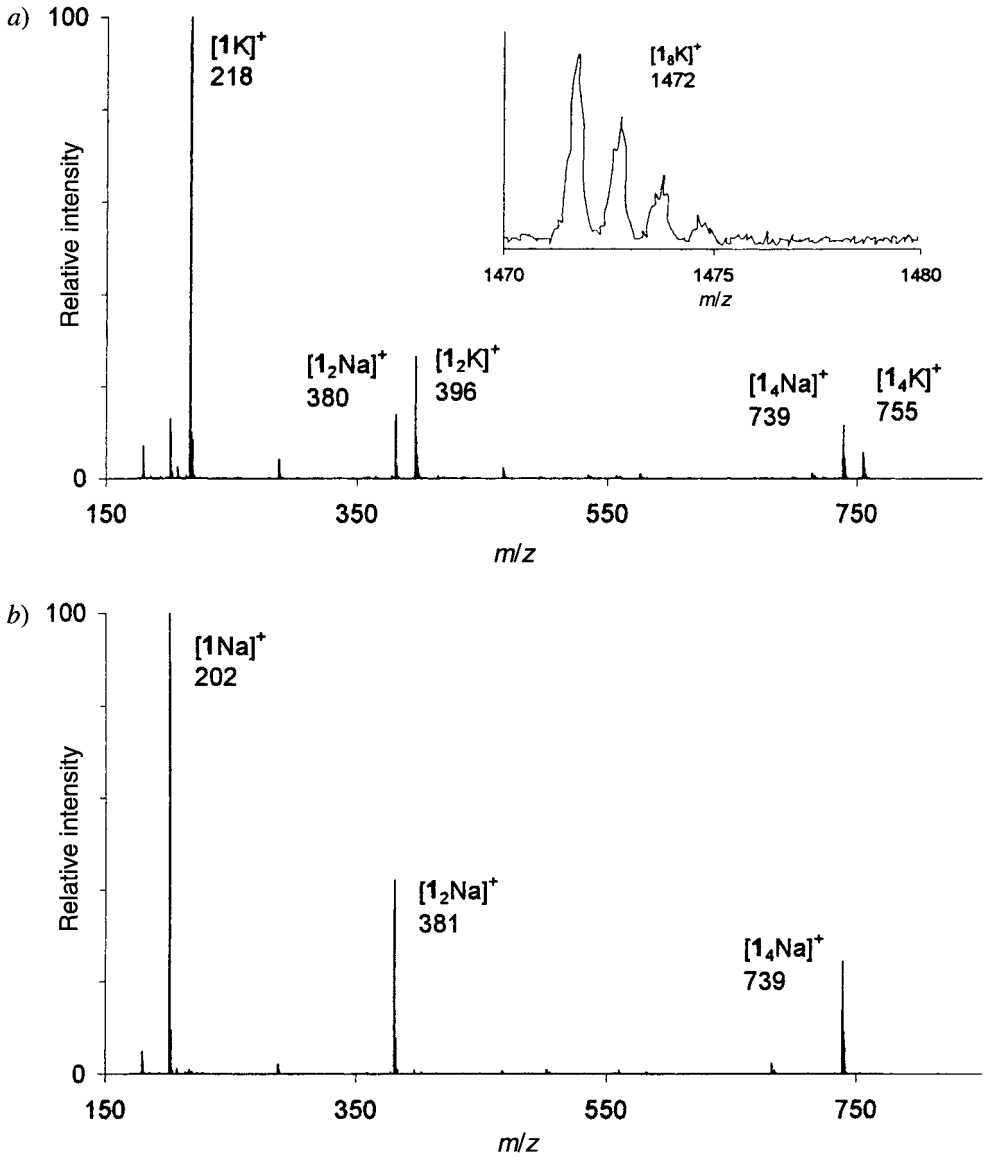

Fig. 3. *ESI-MS of* **1** *in the presence of an equimolar amount of* a) $KClO_4$ *and* b) $NaClO_4$ *in* $H_2O/MeOH$ *9:1*

were recorded for solutions containing 4 mol of **2** per mol of perchlorate salt to favor the formation of a possible tetramer, as for compound **1**. In the case of $KClO_4$, the spectrum is identical to that of *Fig. 4,a*. As to $NaClO_4$, the peak corresponding to the octamer $[\mathbf{2}_8Na]^+$ is still present although it is a very weak signal. The base peak corresponds to the species $[\mathbf{2}_8Na_2]^{2+}$, and a peak corresponding to the larger aggregate $[\mathbf{2}_{16}Na_3]^{3+}$ appears. The peak for the tetramer $[\mathbf{2}_4Na]^+$ is not detected. With $LiClO_4$, the

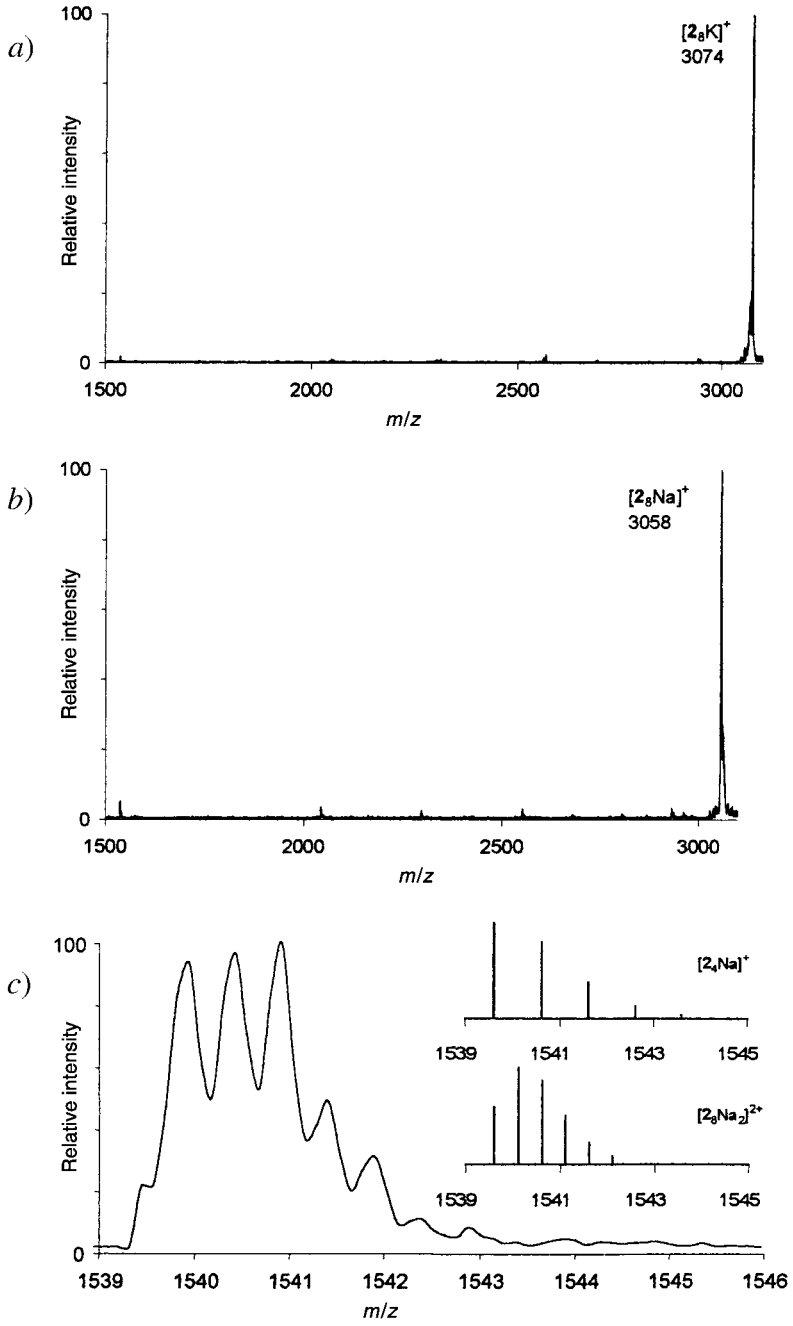

Fig. 4. *ESI-MS of* **2** *in the presence of* a) $KClO_4$ *and* b) $NaClO_4$ *in* $CHCl_3$, *and* c) *with* $NaClO_4$ *in* $H_2O/MeOH$ *9:1* (**2** salt molar ratio 8:1). The insets show the calculated isotope-peak-intensity patterns for $[\mathbf{2}_8Na_2]^{2+}$ and $[\mathbf{2}_4Na]^+$.

base peak is still $[\mathbf{2}_8Na]^+$, and the tetramer $[\mathbf{2}_4Li]^+$ is not observed. The spectra of **2** were run also with picrate salts in the 1:4 ratio. For the K^+ salt, no changes are observed with respect to those in the 1:8 case: only the species $[\mathbf{2}_8K]^+$ is detected. For the Na^+ salt, several oligomeric species appear. However, after washing the organic solution with water, only the octamer is detected in the ESI-MS (the same is also true for NMR, see NMR measurements).

For the sake of comparison of **2** with compound **1**, the MS of a solution containing 8 mol of **2** per mol of $NaClO_4$ salt in H_2O/MeOH 9:1, the same solvent as that used for compound **1**, was recorded (*Fig. 4,c*). The base peaks of the spectrum correspond to the monomer $[\mathbf{2}Na]^+$ at *m*/*z* 402 and the dimer $[\mathbf{2}_2Na]^+$ at *m*/*z* 781. At *m*/*z* 1540, a doubly-charged peak, approximately 50 times weaker than the monomer peak, was observed corresponding most probably to $[\mathbf{2}_8Na_2]^{2+}$. Although the peak separation indicates a doubly charged species, the isotope peak intensity pattern does not coincide with the calculated isotope model for $[\mathbf{2}_8Na_2]^{2+}$. This could possibly be caused by the presence of some tetramer $[\mathbf{2}_4Na]^+$. Also peaks corresponding to $[\mathbf{2}_{16}Na_3]^{3+}$ and $[\mathbf{2}_{12}Na_2]^{2+}$ of similar intensity are detected. The peak corresponding to $[\mathbf{2}_8Na]^+$ (*m*/*z* 781) is *ca.* 15 times less intense than the peak at *m*/*z* 1540.

2.1.3. *2′-Deoxy-3′,5′-O-dipropanoylguanosine* (**3**). The samples examined by ESI-MS contained 8 mol of the deoxyguanosine derivative **3** per mol of the alkali-ion salt, either sodium or potassium picrate (NaPic or KPic), in $CHCl_3$ unless otherwise stated. The picrate salt was used to have a better comparison of the MS data with the NMR [9][10][13] and SANS [13] data obtained for derivative **4** with alkali-metal picrates.

Regarding the MS obtained for the solution containing **3** and K^+ (*Fig. 5,a*) the peak at *m*/*z* 3074 is attributed to the octameric $[\mathbf{3}_8K]^+$ species. This octamer is also detected as a doubly-charged protonated species $[\mathbf{3}_8KH]^{2+}$ at *m*/*z* 1537. The peak at *m*/*z* 2315 is identified as the dodecameric species $[\mathbf{3}_{12}K_2]^{2+}$. Also, the hexadecameric species $[\mathbf{3}_{16}K_3]^{3+}$ is observed at *m*/*z* 2062. The peaks at *m*/*z* 2568 and 2694 are most probably attributable to the species $[\mathbf{3}_{20}K_3]^{3+}$ and $[\mathbf{3}_{28}K_4]^{4+}$. These signals were unexpected, as they lack one metal ion corresponding to the expected stoichiometry for the polymers. Application of a cone voltage greater than 40 V led to the disappearance of these two peaks, but also resulted in the appearance of new peaks due to fragmentation. Five-fold dilution of the solution of **3** with K^+ gave a spectrum of lower intensity, where the peaks due to the polymeric species lacking the ion are missing. The mass spectrum was also recorded for a solution of 8 mol of derivative **3** per mol of KI. Our earlier NMR study established that these conditions favored the formation of the octamer over oligomers [10]; one can observe indeed an increase of the octamer peak in the ESI-MS. In both spectra, the peaks of lower intensity adjacent to the principal peaks at *m*/*z* 2062, 2315, 2568, 2694, and 3074 are identified as polymeric species containing either both K^+ and Na^+ or only Na^+.

With regard to the solution of **3** and the Na^+ salt (*Fig. 5,b*), similar conclusions can be drawn. The major peak corresponds to the dodecameric species at *m*/*z* 2299. The peak of the octameric species at *m*/*z* 3058 is much less intense with respect to the dodecamer. Finally, the peaks probably belonging to the hexadecameric species $[\mathbf{3}_{16}Na_3]^{3+}$ and the species $[\mathbf{3}_{20}Na_3]^{3+}$ at *m*/*z* 2046 and 2552, respectively, are of significant intensity. Again, the latter signal at *m*/*z* 2552 was unexpected as the corresponding species lacks one metal ion corresponding to the expected stoichiometry

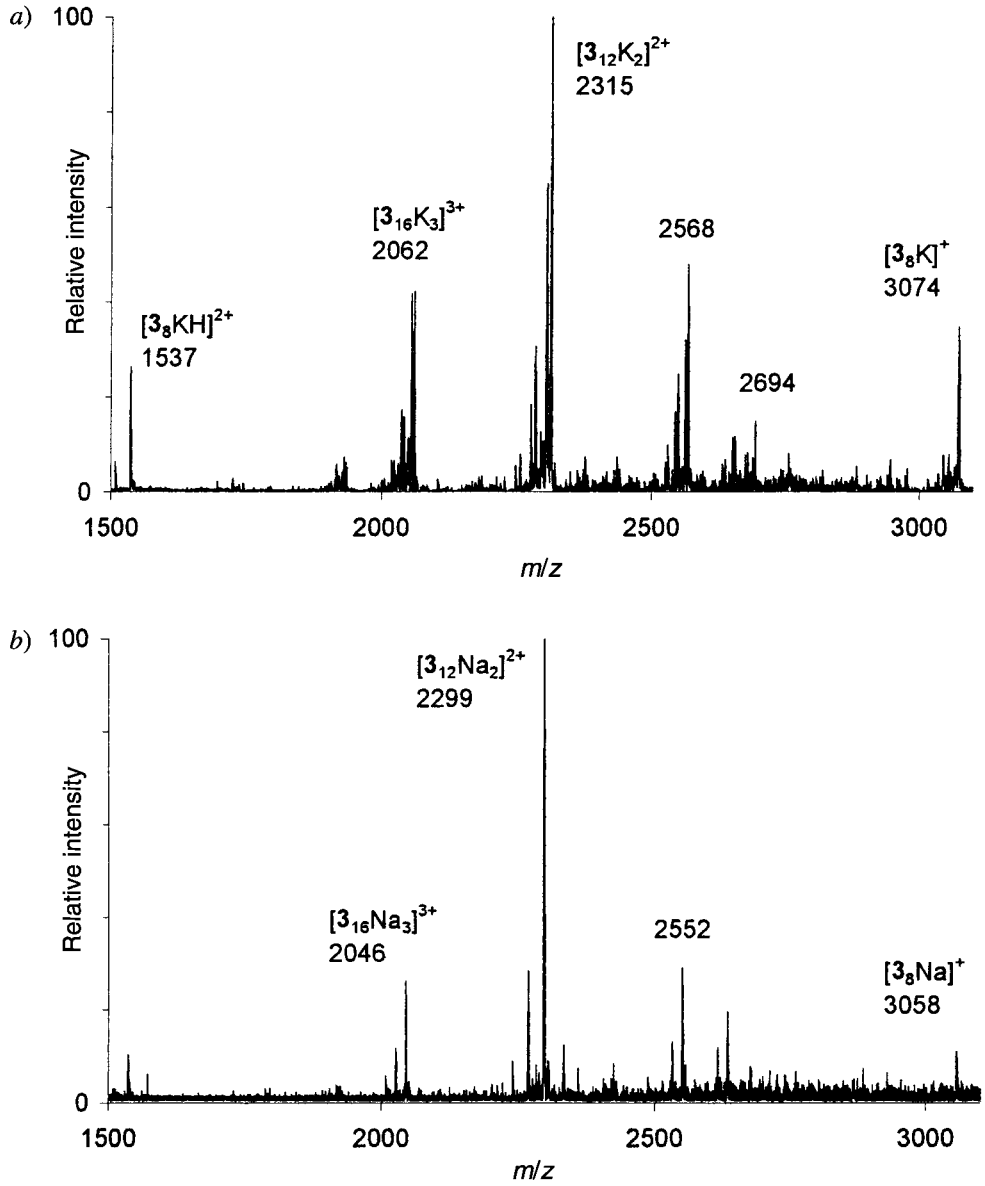

Fig. 5. *ESI-MS of* **3** *in the presence of* a) *KPic and* b) *NaPic* (**3**/salt molar ratio 8:1) in $CHCl_3$.

of the polymers. Enhancement of the cone voltage led to the disappearance of the *m*/*z* 2552 peak, but also resulted in the appearance of new peaks due to fragmentation. Also the spectrum of a solution of 16 mol of **3** per mol of Na^+ was recorded, since low ion content was found to favor the formation of the octamer [9]: the relative intensity of the octamer peak is slightly higher than in *Fig. 5*, *b*.

2.2. *NMR Measurements.* For derivative **4**, the stoichiometry and the geometry of the self-assembled aggregates obtained from NMR data have already been reported [9][12][13]. We repeated the ^{1}H-NMR measurements for derivative **3** in the same concentration range as that used for ESI-MS: no relevant changes with respect to the data obtained for **4** at higher concentration were observed. For the isopropylidene derivative **2**, the ^{1}H-NMR spectra in $CDCl_3$, after extraction of NaPic and Kpic, are shown in *Fig. 6*. The spectra of the complexes of **2** differ from those of the complexes of the deoxy derivatives, where doubling of the NMR lines was observed [9]. In the present case, no doubling is observed, while a shielding of some signals and a substantial line sharpening takes place upon complexation: the ^{1}H-NMR spectrum indicates a different geometry for the aggregates formed by **2**. The stoichiometry of these complexes was deduced by integration of the picrate and the H–C(1′) signals. For Kpic, a 1:8 ratio was obtained, regardless of the amount of KPic added, indicating exclusive formation of octamers (*Fig. 6*,*c*). In the case of NaPic, a solution prepared with the ratio 1:8 of NaPic/**2** gave a spectrum (*Fig. 6*,*b*) analogous to the one described above for the K^+ octamer; when the solution was prepared with a ratio 1:4 of NaPic/**2**, a 1:4 stoichiometry was obtained (*Fig. 6*,*a*), indicating the major presence of polymeric species[2]).

3. Discussion. – The principal problem in using ESI-MS in supramolecular chemistry is to determine whether the peaks present in the spectra correspond to species present in solution, or whether these peaks are the result of processes occurring in the mass spectrometer [15]. Once this problem has been solved, ESI-MS can give new information on the supramolecular chemistry of the system. In the present paper, we have focused on the comparison between ESI-MS and NMR spectroscopy. This comparison allows one to estimate whether some ESI-MS signals are important for describing the self-assembly in solution; furthermore, new quantitative data on the assembled species formed as a function of added cations and anions are obtained.

For isopropylidene derivative **2**, the interpretation is relatively simple. In the case of 8 mol of **2** per mol of $NaClO_4^+$ or $KClO_4^+$, only a single signal is present corresponding to the respective octamers. For the 4:1 molar ratio of nucleoside/$NaClO_4$, oligomeric species larger than $[\mathbf{2}_8Na]^+$ are also present[3]). In the presence of Li^+, only the Na^+-containing octamer can be detected, indicating that Li^+ does not fit well into the octameric structure. Furthermore, no Li^+-containing tetramers were detected.

In the case of KPic and NaPic, we can directly compare ESI-MS with NMR data for the binding of metal cations by compound **2**. With KPic only, the octamer is

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[2]) In liquid-liquid extraction starting with a ratio of 1 mol of NaPic per 4 mol of derivative **2** (or after washing the polymer-containing organic phase with H_2O), the main species is the octamer. This seems reasonable considering the solubility of the salts in H_2O and the lower stability of the polymer with respect to the octamer.

[3]) The 2′,3′-*O*-isopropylidene derivatives of this nucleoside series are particularly avid towards cations, and they even complex Na^+ ions present only as contaminants in solvents or in the silica gel used for chromatographic purifications.

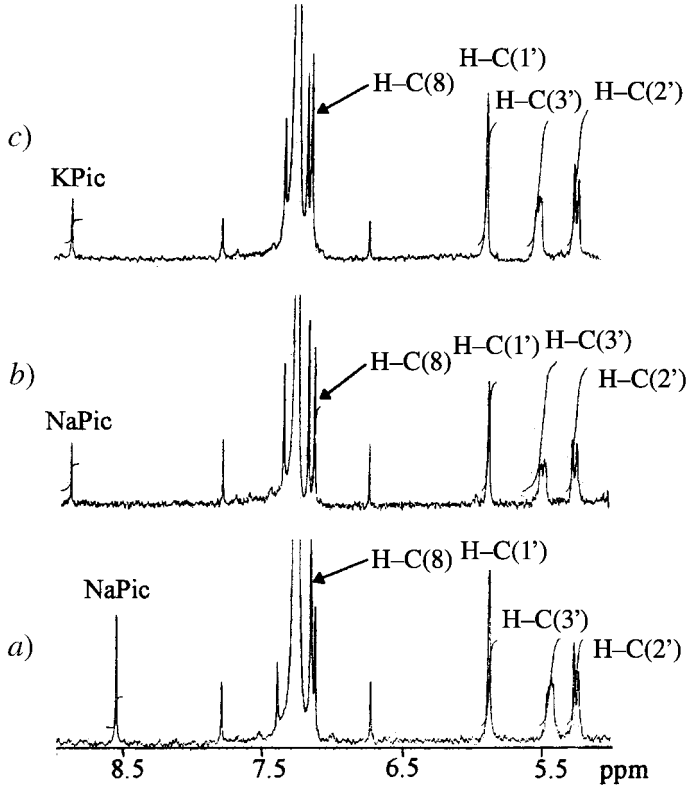

Fig. 6. *^{1}H-NMR Spectra of* **2** (1.12 mM) *in $CDCl_3$ in the presence of* a) *NaPic in the ratio* **2***/NaPic 4 : 1,* b) *NaPic in the ratio* **2***/NaPic* 8 : 1, *and* c) *Kpic in the ratio* **2***/KPic* 8 : 1. The spectra were recorded at the concentration used for ESI-MS measurements. At this concentration, the $CHCl_3$ signal and his side bands are evident.

observed with both techniques. With NaPic, the species present range from the octamer to higher polymeric aggregates according to the experimental conditions. These experiments indicate that both the Na^+ cation and the picrate anion favor polymerization of **2** with respect to K^+ and ClO_4^-, which both promote formation of the octamer.

All these data fit perfectly with the information on the assembled G species available from previous work. In particular, Li^+ does not promote at all the formation of the octamer, while K^+ favors formation of significant octameric species. Furthermore, the picrate anion seems to favor polymeric species (see the discussion on derivative **3**).

The spectra of diester derivative **3**, although displaying more complex ESI-MS patterns, still fit well with the data obtained by SANS and NMR [9][10][13] on the related decanoate nucleoside **4**. With these NMR and SANS techniques, both the octameric and polymeric assemblies have been completely characterized in terms of both stoichiometry and geometry [10][13]. These techniques show that, in $CDCl_3$ solution, the amounts of octamer and polymeric derivatives depend on the molar ratio

between nucleoside and alkali-metal ions, and also on the anion type[4]). In the presence of KPic (*Fig. 5,a*) or KI (guanosine/salt molar ratio 8:1), the octamer can be easily detected together with the oligomers composed of 3 G quartets and 2 K^+ ions and 4 G quartets and 3 K^+ ions. In the case of KI, the signal of the octamer is enhanced with respect to the KPic salt, as expected from NMR measurements [10][5]). The increase in the hexadecamer/octamer ratio in the presence of picrate is in full agreement with the data on derivative **2** and the crystal structure of the similar nucleoside 5′-*O*-[(*tert*-butyl)dimethylsilyl]-2′,3′-*O*-isopropylideneguanosine where the picrate anions connect two octamers through H-bonds, and are likely to favor higher polymeric species [8b]. The signals at *m*/*z* 2568 and 2694 seem to correspond to 5 G quartets with 3 K^+ and 7 G quartets with 4 K^+ ions, respectively, and do not correspond to any species detected in solution so far with different techniques (see *Sect. 2*). We, therefore, suppose that these species with signals at *m*/*z* 2568 and 2694 are probably formed in the spectrometer during the desolvation phase of the ESI process and are without chemical significance in solution.

In the presence of NaPic (guanosine/salt molar ratio 8:1), the signal of the octamer is very small with respect to those of the oligomers (*Fig. 5,b*), and increases slightly when the amount of nucleoside **3** is enhanced[6]). This different ability of Na^+ to promote the formation of oligomers larger than the octamer has not been investigated before with different techniques, but seems very likely in the light of the present ESI-MS and NMR results.

In the case of 9-ethylguanine (**1**), no self-assembly experiments with independent techniques are available. Only with K^+ do our mass spectra show formation of the octamer. The major signals for the quartets (with K^+ or Na^+) and also for dimers and monomers with Na^+ or K^+ dominate (in [14], the spectra are very similar, but no octamer was reported). These results are not unexpected considering the compulsory use of H_2O as the solvent for the measurements on **1** and the very low stability of any aggregates in this competing solvent. In the light of all the results reported above, we think that the isolated G quartets containing alkali-metal ions do not reflect the situation in solution, but are rather the result of events occurring during ionization in the mass spectrometer.

In conclusion, ESI-MS spectra of lipophilic guanosine derivatives are in excellent agreement with data obtained with independent NMR and SANS techniques. Together, these data give a more precise description of the oligomeric aggregates and of the effect of ions on this oligomerization. In particular, Na^+ and picrate favor the polymerization process, while K^+, ClO_4^-, and I^- favor the formation of the octamer. This discrimination could allow the construction of selected polymeric structures by assembling the preformed K^+-containing octamers with NaPic. These present results on noncovalent aggregates of limited stability provide a further demonstration of the value of ESI-MS techniques for studying self-assembly processes in non aqueous solutions. If

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4) SANS Measurements show that, for 8 mol of nucleoside per mol of KPic, also oligomers are present, even when the octamer is the most abundant species [13].

5) The intensity ratio of $[\mathbf{3}_8K]^+/[\mathbf{3}_{16}K_3]^{3+}$ increases from 0.875 in the presence of the picrate anion to 5.6 in the case of the iodide anion.

6) The intensity ratio of $[\mathbf{3}_8Na]^+/[\mathbf{3}_{16}Na_3]^{3+}$ increases from 0.43 to 0.54, when the guanosine/salt molar ratio is enhanced from 8:1 to 16:1.

used *cum grano salis*, ESI-MS can give detailed information on the process, complementary to that obtained with NMR, scattering, and osmometric techniques.

Experimental Part

9-Ethylguanosine (**1**) is a commercial product from *Sigma* and was used without further purification.

2′,3′-O-*Isopropylidene-5′*-O-*propanoylguanosine* (**2**). *2′,3′*-O-*Isopropylideneguanosine* (*Sigma*; 1 mmol) was dried over P_2O_5 *in vacuo* for 2 h and then suspended in MeCN (15 ml). Redistilled Et_3N (1.3 mmol), *N*,*N*-dimethylpyridin-4-amine (DMAP; 0.16 mmol), and propanoic anhydride (1.2 mmol) were added, and the resulting mixture was stirred overnight at r.t. MeOH (0.5 ml) was then added, and stirring was continued for 20 min. The mixture was filtered and the precipitate washed several times with small portions of MeCN and *Millipore* H_2O: anal. pure **2** (50% yield). ^{1}H-NMR (200 MHz, (D_6) DMSO): 0.96 (*t*, *Me*CH_2); 1.32 (*s*, Me); 1.51 (*s*, Me); 2.29 (*q*, Me*C*H_2); 4.05–4.16 (*m*, H–C(4′), 2 H–C(5′)); 5.13 (*dd*, H–C(3′)); 5.24 (*dd*, H–C(2′)); 6.01 (*d*, H–C(1′)); 6.53 (br *s*, NH_2); 7.85 (*s*, H–C(8)); 10.70 (br. *s*, NH). ESI-MS ($CHCl_3$/MeOH): 380.05 (100, [**2** + H]$^+$).

2′-Deoxy-3′,5′-O-*dipropanylguanosine* (**3**). For 2 h, 2′-deoxyguanosine monohydrate (*Fluka*; 1 mmol) was dried over P_2O_5 *in vacuo* and then suspended in MeCN (20 ml). Redistilled Et_3N (2.2 mmol), DMAP (0.25 mmol), and propanoic anhydride (2.2 mmol) were added, and the resulting mixture was stirred at r.t. for 5 h. MeOH (0.5 ml) was then added, and stirring was continued for 20 min. The mixture was filtered, the precipitate washed several times with small portions of MeCN and *Millipore* H_2O, and finally crystallized from abs. EtOH: **3** (56% yield). ^{1}H-NMR (200 MHz, (D_6) DMSO): 0.95–1.10 (*m*, 2 *Me*CH_2); 2.22–2.45 (*m*, 2 MeCH_2, 1 H–C(2′)); 2.81–3.00 (*m*, H–C(2′)); 4.12–4.36 (*m*, 2 H–C(5′), H–C(4′)); 5.33 (*m*, H–C(3′)); 6.13 (*dd*, H–C(1′)); 6.5 (br. *s*, NH_2); 7.91 (*s*, H–C(8)); 10.64 (br. *s*, NH). ESI-MS ($CHCl_3$/MeOH): 380.01 (100, [**3** + H]$^+$).

ESI-MS Experiments. A *ZMD Micromass* single quadrupole mass spectrometer operating at 4000 *m*/*z* was used. A *Hamilton* syringe driven by a *Harvard* pump was used for direct injection of the sample into the mass spectrometer, at a rate of 10 µl/min. A capillary voltage of 3 kV and a cone voltage of 30 V were applied for the experiments with **1** dissolved in H_2O; a desolvation temp. of 150° was used. Changing the temp. from 100 to 200° did not change significantly the absolute intensity of the peaks nor did it affect the relative intensity of the peaks. In the case of the spectra obtained for **2** and **3**, the capillary and cone voltages were set to 3.8 kV and 40 V, resp. The same desolvation temp. was used. Applying higher cone voltages gave spectra with the same principal peaks, and in all cases, peaks corresponding to fragmentation began to appear. In the interval 120–160°, no temp. effects were observed on peak intensity, and no fragmentation occurred. Below 120°, the peak intensity decreased, and above 160°, some fragmentation started to occur. The charge of the species corresponding to an observed ion were deduced directly from the spacing of the isotope peaks for masses lower than *m*/*z* 2000. In the case of polymers of the K^+-containing solutions of **3**, the charge was deduced from the space between the principal peak and the minor adjacent peaks at the left. These minor peaks are due to species containing not only K^+ ions but also one or more Na^+ ions instead of K^+. The space between these peaks corresponds to a half, a third, *etc.* of 16, the mass difference between K^+ and Na^+. The conclusions drawn for the K^+ solns. were then extended also to the Na^+ solns. For all experiments, 10^{-3} M solutions of **1**–**3** were used. All solns. were prepared by solid-liquid extraction of the salt with the guanosine-derivative soln. It was not possible to register the mass spectra of **1** in the presence of NaPic or KPic in 100% $CHCl_3$ or $CHCl_3$/MeCN (1:1), due to the insolubility of **1**.

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