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Design, Synthesis and Characterisation of Guanosine-Based Amphiphiles

Luca Simeone,[a] Domenico Milano,[a] Lorenzo De Napoli,[a] Carlo Irace,[b] Antonio Di Pascale,[b] Mariangela Boccalon,[c] Paolo Tecilla,[c] and Daniela Montesarchio*[a]

Abstract: A small library of sugar-modified guanosine derivatives has been prepared, starting from a common intermediate, fully protected on the nucleobase. Insertion of myristoyl chains and of diverse hydrophilic groups, such as an oligoethylene glycol, an amino acid or a disaccharide chain, connected through in vivo reversible ester linkages, or of a charged functional group provided different examples of amphiphilic guanosine analogues, named **G1**–**G7** herein. All of the sugar-modified derivatives were positive in the potassium picrate test, showing an ability to form G-tetrads. CD spectra demonstrated that, as dilute solutions in $CHCl_3$, distinctive G-quadruplex systems may be formed, with spatial organisations dependent upon the structural modifications. Two compounds, **G1** and **G2**, proved to be good low-molecular-weight organogelators in polar organic solvents, such as methanol, ethanol and acetonitrile. Ion transportation experiments through phospholipid bilayers were carried out to evaluate their ability to mediate H^+ transportation, with **G5** showing the highest activity within the investigated series. Moreover, **G3** and **G5** exhibited a significant cytotoxic profile against human MCF-7 cancer cells in in vitro bioassays.

Keywords: amphiphiles · antiproliferation · G-quadruplexes · ionophores · nucleosides

Introduction

Nucleolipids are hybrid molecules consisting of a lipid core covalently linked to a nucleobase, nucleoside or nucleotide. Hybrid lipid–nucleoside structures occur in eukaryotic and prokaryotic cells, showing antimicrobial, antifungal, antiviral or anti-tumour properties. Over the last two decades, several novel nucleolipids have been synthesised that combine the pharmacological potential of nucleosides or nucleotides with the aggregation properties of vesicle-forming lipids. Recently, these compounds have attracted significant attention as useful synthetic platforms in the design of artificial molecular devices and in pursuing more efficient therapeutic strategies.[1,2] In this area, nucleolipids derived from biologically active nucleosides/nucleotides can be very promising prodrugs with unique structural and biophysical properties. These features can be ascribed to their ease of self-assembly into stable liposomes in aqueous media, thus providing efficient uptake through the phospholipid bilayers by cells, and conferring protection to the drug from extracellular enzymatic degradation.[3] From an alternative perspective, increasing interest is also devoted to these compounds as gene transfection agents, particularly for DNA and siRNA in vivo delivery.[4]

Although several amphiphilic nucleolipids based on adenosine, cytosine, uridine or thymidine are described in the literature, very few examples are known for guanosine[1,5] and the potential applications of guanosine-based amphiphiles in biomedical applications and/or as self-assembling materials are almost completely unexplored. Furthermore, guanosine-based systems deserve special interest in supramolecular chemistry due to the known self-aggregation properties of guanine and guanosine derivatives. Several supramolecular architectures generated by lipophilic guanosines based on unusual structural motifs, such as G-tetrads stabilised by metal cations or, in the absence of cations, G-ribbon structures that in some cases are also organised in sheet-like two dimensional assemblies, have been described in the literature.[6–9]

Herein, the preparation of a set of amphiphilic guanosine-based derivatives by simple synthetic protocols combining different bio-inspired building blocks is described. Their structural and biological properties are investigated in pre-

[a] Dr. L. Simeone, Dr. D. Milano, Prof. L. De Napoli, Prof. D. Montesarchio
Department of Organic Chemistry and Biochemistry
University "Federico II" of Napoli
Via Cintia, 4, 80126 Napoli (Italy)
Fax: (+39) 081-674393
E-mail: daniela.montesarchio@unina.it

[b] Dr. C. Irace, Dr. A. Di Pascale
Department of Experimental Pharmacology
University "Federico II" of Napoli
Via D. Montesano, 48, 80131 Napoli (Italy)

[c] Dr. M. Boccalon, Prof. P. Tecilla
Department of Chemical Sciences, University of Trieste
Via L. Giorgieri, 34127 Trieste (Italy)

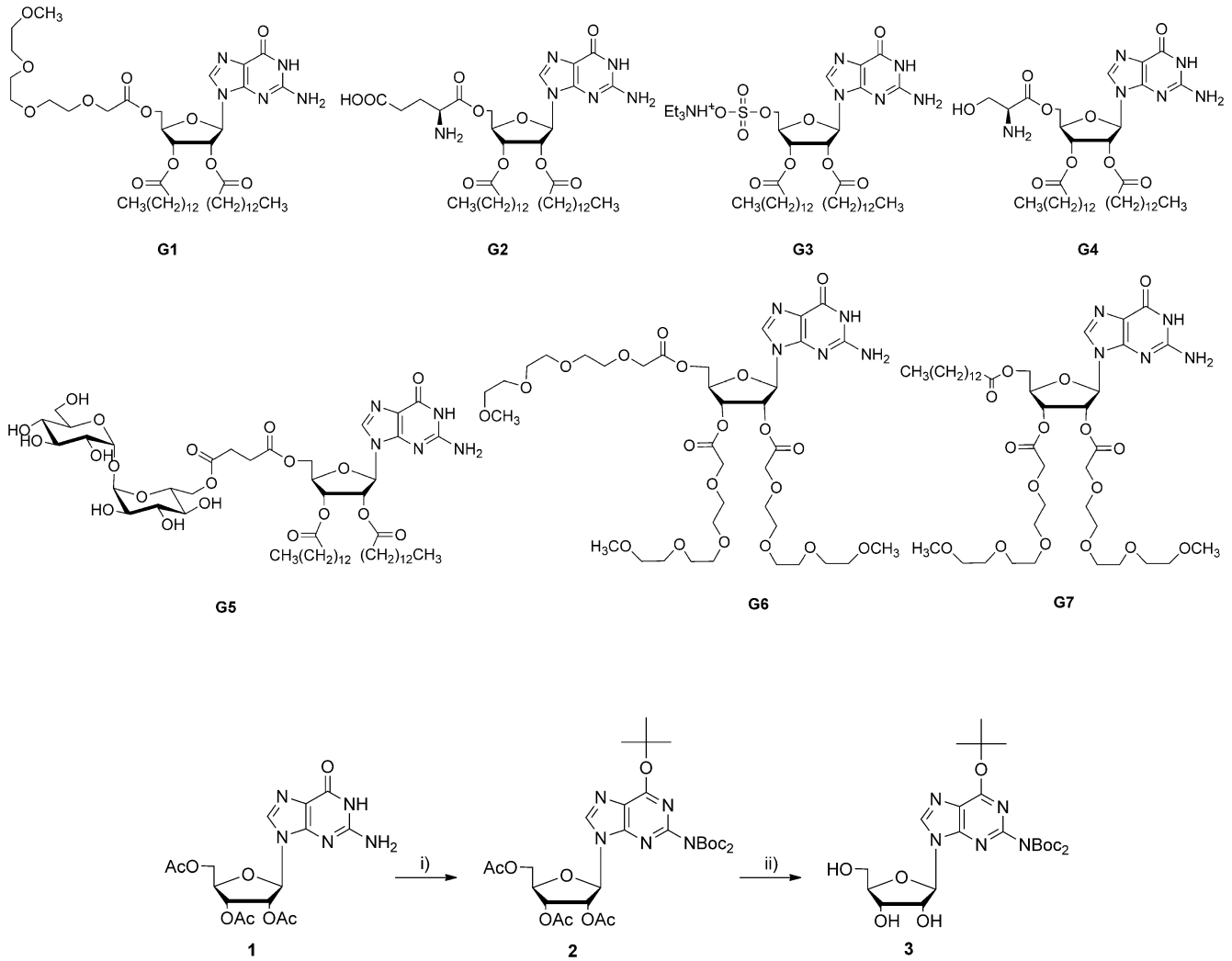
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201101827. It contains general synthetic methods, detailed molecular characterisation (^1H and ^{13}C NMR and MS data) and the synthesis of compounds **1**–**7a**–**d** and **8**–**16** and general procedures for the ionophoric activity experiments.

liminary studies aimed at identifying new tools with the potential to profitably complement the lipophilic congeners recently studied by the research groups of G. P. Spada,[6–9] J. T. Davis[9–12] and K. Araki[13–15], among others, further expanding the chemical diversity offered by nucleosides.

Results and Discussion

Synthesis of compounds G1–G7: A variety of synthetic strategies have been used to prepare nucleolipids. In most cases, the design is based on the chemical modification of the sugar functionality, thus minimising the perturbation of the nucleobase moiety and keeping their critical recognition sites unaltered. Ester functionalities are typically considered to be ideal chemical connections for nucleosidic ribose derivatisations, offering several advantages. These linkages can be obtained through straightforward and high yielding condensation reactions that do not require prior modification of the nucleoside moiety and are rapidly cleaved by cell esterases, liberating the "free" nucleosides within the cells, once transportation through the lipid bilayers has been achieved.

For the preparation of a library of amphiphilic, sugar-modified guanosine derivatives, the general design presented herein is based on the insertion of a saturated fatty acid residue, that is myristic acid, as the lipophilic chain. In the synthesised compounds, named **G1**–**G7**, this group was attached to the secondary hydroxyl groups of ribose to give bi-tailed compounds **G1**–**G5**, or to the 5′-OH group, to give monotailed derivative **G7**. Higher molecular diversity was introduced through the use of different hydrophilic groups, with polyethers (introduced in **G1**, **G6** and **G7**), charged functional groups (in **G3**), natural α-amino acids (in **G2** and **G4**) and carbohydrates (in **G5**) represented. Aiming for a simple, versatile and finely tunable synthetic method, the library of sugar-modified amphiphilic analogues was thus prepared via a common intermediate (**3**, Scheme 1) that is exhaustively protected on the nucleobase[16] and containing



Scheme 1. Synthesis of intermediate **3**: i) Boc_2O (Boc = *tert*-butoxycarbonyl), triethylamine (TEA), 4-dimethylaminopyridine (DMAP), CH_3CN, RT, 72 h, 57%; ii) NH_3, CH_3OH, 12 h, RT, quantitative.

three ribosidic OH groups available for condensation reactions with various appendages.

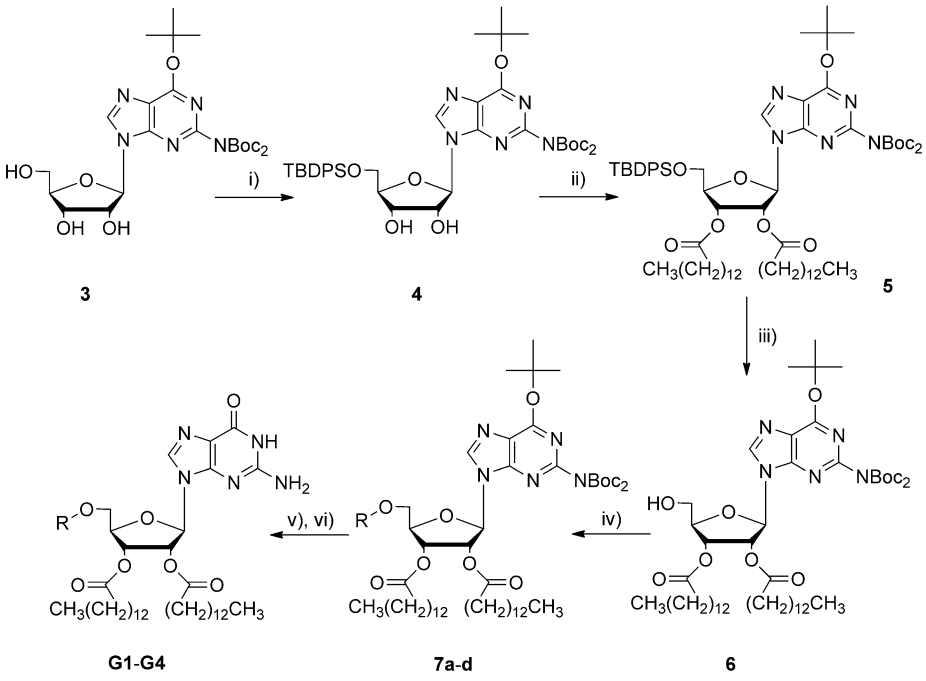
For the **G1**–**G5** series, following the approach of Grinstaff, Berthélémy and co-workers,[1] the secondary ribose OH functionalities were exploited to insert fatty acid residues, whereas the 5′-OH was derivatised with different hydrophilic groups, introduced to balance the hydrophobic contribution of the lipid chains. Compound **G7** was designed as an analogue of **G1** containing the same lipophilic and hydrophilic tails but with an inverted ratio. Uniquely, compound **G6** only contains polyether groups. In this manner, the series of compounds with 2, 1 or 0 myristic acid tails and, in turn, 1, 2 or 3 triethylene glycol groups (**G1**, **G7**, and **G6**, respectively) was generated.

The synthetic route to the target sugar-modified guanosine analogues was thus realised by starting from compound **3**, obtained in three straightforward steps from guanosine (Scheme 1). The Boc group, though seldom used for nucleobase protection in nucleoside chemistry,[17] was selected to protect the guanine moiety due to the easy installation procedure, its lipophilic character, which facilitates guanosine manipulation in organic solvents, and above all the convenient removal conditions. As a matter of fact, Boc deprotection can be achieved by mild acidic treatment (dilute trifluoroacetic acid (TFA) in anhydrous organic solvents), which is fully compatible with ester linkages and produces only volatile side products, thereby not requiring column chromatography to give the pure, deprotected products.

To obtain compound **3**, guanosine was first regioselectively protected[18] by treatment with acetic anhydride in CH_3CN and TEA. Tri-*O*-acetylated derivative **1** was then reacted with an excess of di-*tert*-butyl dicarbonate (Boc_2O) in CH_3CN in the presence of TEA and DMAP, giving fully protected nucleoside **2**. Interestingly, this compound, obtained in more acceptable yields (57%) than previously reported procedures,[17] showed a *tert*-butyl group at the O6 position. This, in analogy with a previous report on guanine protection,[16] may be attributed to spontaneous loss of CO_2 from the original N1 Boc carbamate, which has undergone an internal transposition to the O6 *tert*-butyl ether. In contrast to the previously reported case,[16] we could not isolate the tris-Boc-protected intermediate and only compound **2** was recovered after column chromatography. This derivative was then exhaustively deacetylated by treatment with methanolic ammonia, giving target compound **3** in 56% overall yield over the three steps.

The synthesis of compounds **G1**–**G5** was accomplished through two simple manipulations starting from derivative **6**, in turn obtained in three steps from intermediate **3** (Scheme 2). Reaction of **3** with TBDPSCl and imidazole in DMF provided 5′-protected nucleoside **4**, which was then condensed with myristic acid in the presence of DCC, cleanly giving 2′,3′-di-*O*-myristoyl derivative **5**. TBDPS-group removal was achieved by treatment of **5** with the $Et_3N{\cdot}3HF$ in THF, leading to target compound **6** in 70% overall yield from **3. G1** was then obtained in almost quantitative yields in two steps, involving first the coupling of 5′-OH-deprotected nucleoside **6** with monomethoxy(triethylene glycol) acetic acid, previously prepared in our laboratory,[19] by using DCC as the condensing agent, followed by an acidic treatment with TFA (10%) in CH_2Cl_2 for 2 h at RT, to achieve full nucleobase deprotection.

Hybrid nucleosides **G2** and **G4** were obtained by using a similar procedure based on the coupling of derivative **6** with commercially available Fmoc-protected α-amino acids (Fmoc-Glu(O*t*Bu)-OH and Fmoc-Ser(OTrt)-OH, respectively), and also mediated by DCC activation. The choice of Fmoc-protection in lieu of Boc-protection could, in principle, allow further derivatisation of the final compounds, for



Scheme 2. Synthesis of **G1**–**G4**: i) *tert*-butyldiphenylsilyl chloride (TBDPSCl), imidazole, DMF, RT, 2 h, 92%; ii) $CH_3(CH_2)_{12}COOH$, *N*,*N*′-dicyclohexylcarbodiimide (DCC), DMAP, 12 h, RT, 95%; iii) $Et_3N{\cdot}3HF$, THF, 48 h, RT, 80%; iv) coupling with: for **7a**: $CH_3O(CH_2CH_2O)_3CH_2COOH$ in CH_2Cl_2, DCC, RT, 1.5 h; for **7b**: Fmoc-Glu(O*t*Bu)OH (Fmoc = fluorenylmethoxycarbonyl) in CH_2Cl_2, DCC, RT, 1.5 h; for **7d**: Fmoc-Ser(OTrt)OH (Trt = trityl) in CH_2Cl_2, DCC, RT, 1.5 h; for **7c**: $SO_3{\cdot}Et_3N$, DMF, 45 min, RT, 70%; v) only for **7b** and **7d**: Fmoc removal with piperidine (10%) in DMF, RT, 20–40 min; vi) trifluoroacetic acid (TFA; 10%) in CH_2Cl_2, RT, 2 h, quantitative.

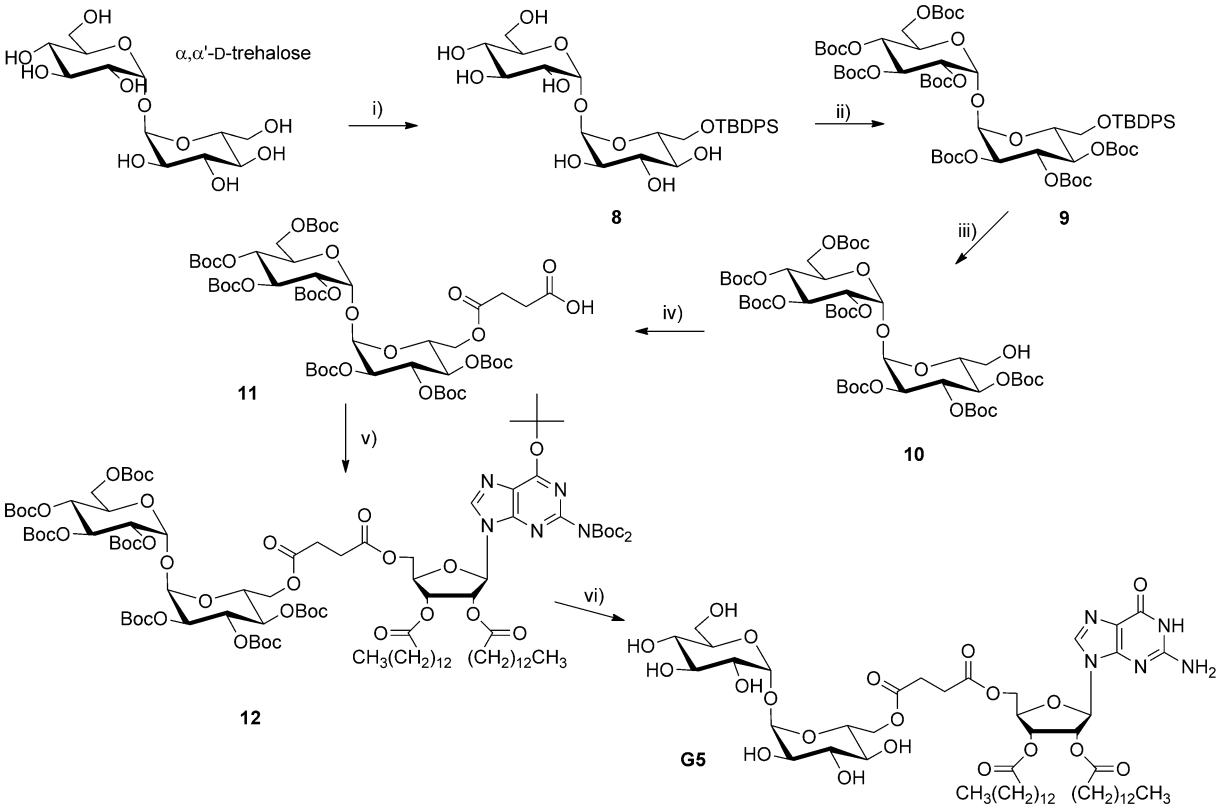
instance, by peptide elongation or attachment of additional functional groups. For both compounds, Fmoc removal, cleanly achieved by reaction with piperidine (10%) in DMF, was followed by treatment with TFA (10%) in CH_2Cl_2 for 2 h at RT, ensuring an effective one-pot deprotection of the guanine and of the side-chains of glutamic acid and serine residues, masked as *tert*-butyl ester and trityl ether groups, respectively.

Compound **G3** was prepared by reacting **6** with the commercially available complex SO_3·Et_3N in DMF, followed by TFA deprotection, carried out as described above. This gave the desired 5′-*O*-sulfate derivative in 70% yield over the two steps.

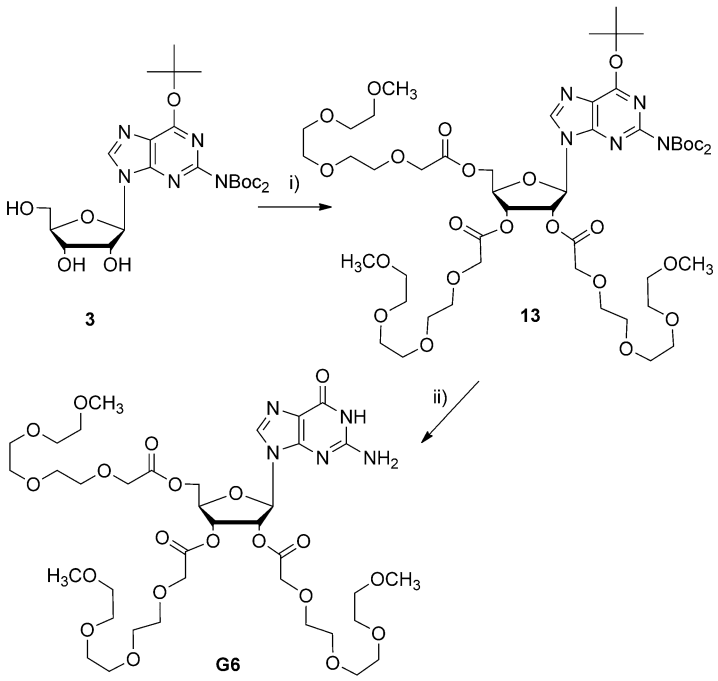
Preparation of glyco–nucleolipid hybrid **G5**, incorporating a disaccharide residue chosen to confer increased hydrophilic character to the final derivative compared to the **G1**–**G4** series, required prior synthetic elaboration of the disaccharide building block. To this end, α,α′-D-trehalose was selected on the basis of its favourable properties: this is, in fact, a readily available, symmetrical, non-reducing disaccharide that does not undergo mutarotation and shows higher chemical stability compared with other common disaccharides. The last issue is of utmost importance from a synthetic point of view considering that the overall strategy to obtain the described guanosine-containing nucleolipids is based on a final acidic treatment. The succinic group was chosen as the linker providing covalent attachment of the trehalose residue to the 5′-OH end of the guanosine scaffold. This was introduced on one primary hydroxyl of the disaccharide after it had been subjected to the following derivatisations (depicted in Scheme 3): 1) mono-silylation of the original disaccharide, carried out with TBDPSCl and imidazole in DMF at 0°C, giving **8** in 86% yield; 2) exhaustive Boc protection of the remaining seven hydroxyl groups by treatment with Boc_2O in CH_3CN in the presence of TEA and DMAP, leading to **9** in 58% yield; 3) removal of the *tert*-butyldiphenylsilyl protecting group by reaction with Et_3N·3HF in THF, providing **10** in 80% yield; 4) reaction with succinic anhydride and catalytic DMAP in THF, giving the target succinylated trehalose derivative **11** in 78% yield. This building block was finally used in the DCC-promoted coupling with nucleoside **6**, yielding target molecule **12** in 86% yield. As in the case of **G1**–**G4**, target compound **G5** was then obtained upon treatment of **12** with TFA, quantitatively giving complete sugar and nucleobase deprotection in a single step.

Derivative **G6** was obtained in two steps starting from **3**. As depicted in Scheme 4, these consisted of exhaustive derivatisation with monomethoxy(triethylene glycol) acetic acid[19] in the presence of DCC, followed by treatment of **13** with TFA.

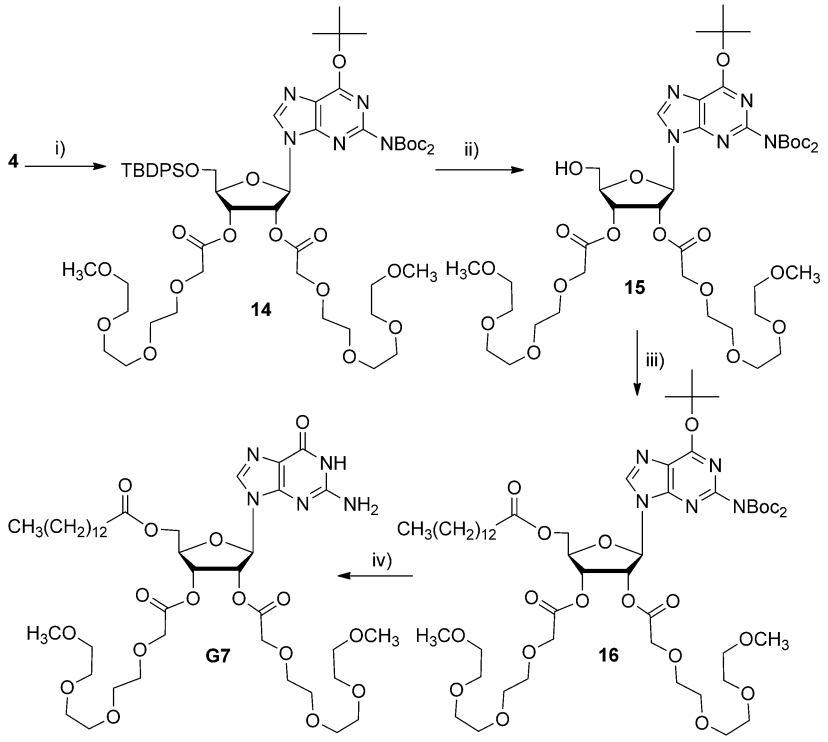
Finally, derivative **G7** was prepared from nucleoside **4** upon esterification of both secondary hydroxyl groups with monomethoxy(triethylene glycol) acetic acid (Scheme 5).[19] This lead to compound **14**, which was desilylated, coupled



Scheme 3. Synthesis of **G5**: i) TBDPSCl, imidazole, DMF, 0°C, 50 min, 86%; ii) Boc_2O, TEA, DMAP, CH_3CN, 48 h, RT, 58%; iii) Et_3N·3HF, THF, RT, 72 h, 80%; iv) succinic anhydride, DMAP, THF, RT, 24 h, 78%; v) coupling with **6**, DCC, CH_2Cl_2, RT, 3 h, 86%; vi) TFA (10%) in CH_2Cl_2, RT, 3 h, quantitative.



Scheme 4. Synthesis of **G6**: i) $CH_3O(CH_2CH_2O)_3CH_2COOH$, DCC, DMAP, CH_2Cl_2, RT, 48 h, 42%; ii) TFA (10%) in CH_2Cl_2, RT, 3 h, quantitative.



Scheme 5. Synthesis of **G7**: i) $CH_3O(CH_2CH_2O)_3CH_2COOH$, DCC, DMAP, CH_2Cl_2, RT, 1 h; ii) $Et_3N{\cdot}3HF$, THF, RT, 18 h; iii) $CH_3(CH_2)_{12}COOH$, DCC, DMAP, CH_2Cl_2, RT, 2 h; iv) TFA (10%) in CH_2Cl_2, RT, 2.5 h.

with myristic acid at the 5′-OH group, and then deprotected at the guanine moiety.

All the intermediate products synthesised were purified by silica gel column chromatography and then fully characterised by ^1H and ^{13}C NMR spectroscopy and ESI mass spectrometry. Final compounds **G1**–**G7**, obtained from the corresponding nucleobase-protected precursors by a simple treatment with TFA (10%) in CH_2Cl_2, followed by repeated co-evaporations from isopropanol until the excess TFA had disappeared completely, were identified on the basis of their NMR and ESI-MS spectra.

Characterisation of G1–G7

Gelling ability: Preliminary structural investigations have been attempted on **G1**–**G7** by means of NMR spectroscopy. In all cases, NMR analysis of the compounds dissolved in $CDCl_3$ showed dramatically broadened and very badly resolved signals in the ^1H and ^{13}C NMR spectra that prevented useful structural information from being obtained. With time, the signal broadening became more and more noticeable, particularly in the imino-proton region of the ^1H NMR spectra, suggesting the presence of growing superstructures undergoing dynamic equilibria. Upon changing the solvent from $CDCl_3$ to the less structure-inducing CD_3OD and/or varying the temperature in the range 288–328 K, only minor benefits in terms of resolution and line sharpness were observed. These results suggested a strong tendency for these compounds to form highly aggregated systems.

As a first step to determining the properties of the newly synthesised amphiphilic derivatives, we investigated their solubility and gelling ability (selected data are given in Table 1; for full characterisation, see Table S1 in the Supporting Information). A strong tendency to aggregate, attributable to the well-known ability of guanine-based systems to form a rigid network of intermolecular hydrogen bonds, has been confirmed for all of the synthesised samples, and their capacity for being organogelators evaluated by the inversion method.[20] In all cases, analysis of these systems at concentrations lower than 20 mM did not indicate any tendency to form stable gels, and higher concentrations were therefore tested.

G1 and **G2**, examined in the range 3–7% w/w (corresponding to ca. 25–70 mM), gave stable gels in polar solvents, such as methanol, ethanol and CH_3CN (pictures of the gels obtained from **G1** and **G2** are

Table 1. Solubility properties of derivatives **G1**–**G7** in selected solvents.[a]

	G1		G2		G3	G5	G6	G7
Solvent	26 mM	57 mM	57 mM	67 mM	57 mM	57 mM	57 mM	57 mM
CH_3OH	G[b] 3%	G 6%	S	G[b] 7%	S	S	S	S
ethanol	S	S	G[b] 6%	S	S	I	S	S
CH_3CN	S	G	S	S	I	I	S	I

[a] G = gel; S = homogeneous solution; I = insoluble. [b] Rapid gelification was achieved by leaving the compound at +4 °C.

shown in Figure S1 in the Supporting Information). In comparable concentration conditions (5% w/w), lipophilic guanosine derivatives based on 2′,3′-*O*-isopropylidenguanosine, carrying different *n*-alkylsilyl ethers at the 5′-position, synthesised by Araki and co-workers,[14] were found to gelate from *n*-decane, cyclohexane and *n*-hexane. Thus, the molecules we synthesised offer new examples of low-molecular-weight organogelators that may profitably complement both the known lipophilic guanosines and the hydrophilic, naturally occurring guanosine mono- and polyphosphates, given that they cover a completely different solvent polarity range that falls between highly apolar solvents and water. Analysing the obtained results from a qualitative point of view, comparison of the **G1**, **G7** and **G6** systems indicates that two long aliphatic chains are essential for gelation; on the other hand, the presence of charged (as in **G3**) or markedly hydrophilic groups (as in **G5**) appears to negatively affect the formation of stable gels. **G4** is apparently a unique case, being essentially insoluble in all of the most commonly used organic solvents, with the exception of DMSO.

CD studies: To gain useful information on the conformational behaviour of the synthesised amphiphilic compounds in organic solvents, CD spectra were acquired in the non-structuring solvent $CHCl_3$. For **G4**, which is only soluble in DMSO and therefore not directly comparable with the other systems under investigation, CD data were not acquired.

To preliminarily assess the ability of these systems to form G-tetrads, qualitative potassium picrate tests were carried out by following the procedure described for lipophilic guanosine derivatives.[21] In all cases, these colorimetric tests confirmed the ability of these compounds, dissolved in $CHCl_3$, to extract K^+ ions from aqueous solutions containing the yellow salt potassium picrate and transfer them to the organic phase. The yellow colour of the organic phase after this treatment is indicative of G-tetrad formation by the guanosine derivatives, which attract K^+ ions from aqueous solution; these cations in turn carry with them picrate anions, otherwise highly insoluble in organic solvents, to ensure electroneutrality (see Figure S2 in the Supporting Information).

Next, compounds **G1**–**G3** and **G5**–**G7** were analysed by CD spectroscopy as dilute solutions in $CHCl_3$, before and after the addition of potassium picrate. 2′,3′,5′-Tri-*O*-acetylguanosine was studied in parallel as a reference compound, having sugar modifications conferring high solubility in $CHCl_3$, but no relevant contribution to the self-assembling capability of the nucleoside; it can therefore be assumed that its overall structuring process is only guided by the guanosine moiety and not influenced by the sugar decorations. This nucleoside, found to be positive to the potassium picrate test, showed a CD spectrum with a single negative band at 259 nm, very close to the maximum exhibited in the absorbance spectrum registered in the same solvent (262 nm). This correspondence between the CD and UV spectra (see Figures S3 and S4 in the Supporting Information) points to a random distribution of the G-tetrads generated by this nucleoside in solution and thus not producing ordered three-dimensional structures with characteristic orientations of the chiral centres. Of the investigated amphiphilic derivatives, only **G3** and **G5** showed CD spectra comparable, apart from the sign, to their UV/Vis ones, in analogy with 2′,3′,5′-tri-*O*-acetylguanosine (see Figures S5 and S6 in the Supporting Information). On the contrary, **G1**, **G2**, **G6** and **G7**, all having UV/Vis profiles very similar to the reference compound (data not shown), exhibited distinctive CD spectra with two intense bands of opposite sign, diagnostic of regular G-tetrad stacking in organised and well-defined octameric complexes with C_4 or D_4 symmetry (Figures 1–4, respectively).

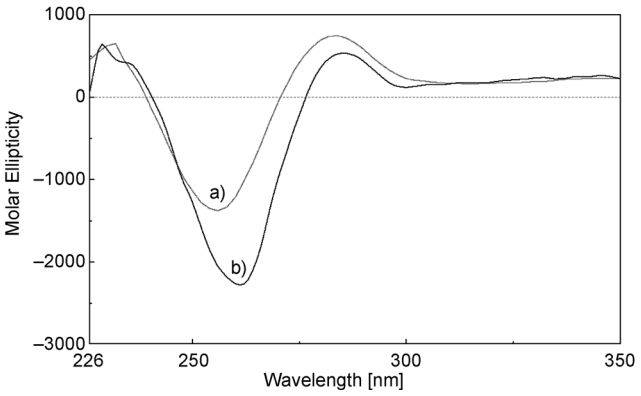


Figure 1. CD spectra of compound **G1** at 2.7 mM in $CHCl_3$; a) before addition of potassium picrate, $\lambda_{max} = 256$, 283 nm; b) after addition of potassium picrate, $\lambda_{max} = 262$, 285 nm.

Following the rationalisation introduced by A. Randazzo, G. P. Spada and co-workers,[22] the position of the opposite sign bands in the CD spectra of guanosine-based systems can provide information on the G-tetrad stacking not only in G-rich oligonucleotides, that is, in systems in which the stacked guanines within each strand are covalently linked by the sugar–phosphate oligonucleotide backbone, but also in non-covalently linked guanosine monomers. So the presence of a negative band centred at about 240 nm and a positive band at 260 nm is diagnostic of G-tetrads organised in homopolar stacking (also referred to as "H-to-T", with H and T representing the "head" and "tail" surfaces, respectively, of a G-tetrad[23] facing each other). A blueshift of 20–30 nm

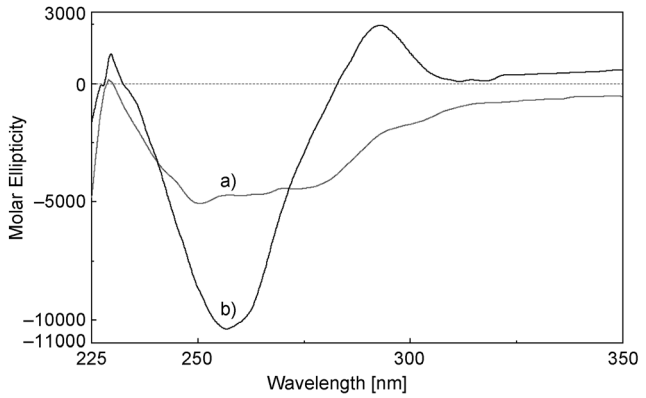


Figure 2. CD spectra of compound **G2** at 2.7 mM in $CHCl_3$; a) before addition of potassium picrate; b) after addition of potassium picrate, λ_{max} = 257, 293 nm.

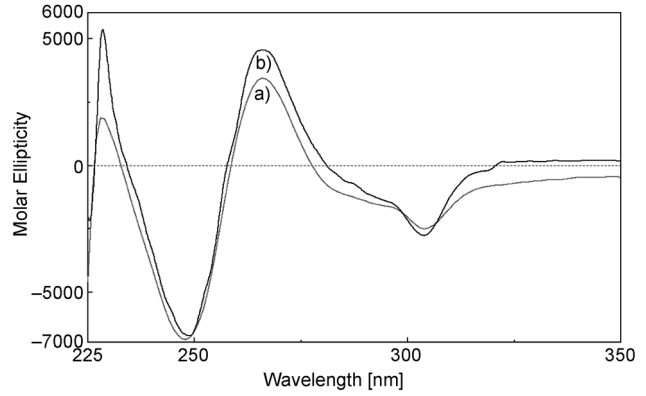


Figure 3. CD spectra of compound **G6** at 2.7 mM in $CHCl_3$; a) before addition of potassium picrate; b) after addition of potassium picrate; λ_{max} = 249, 265 nm.

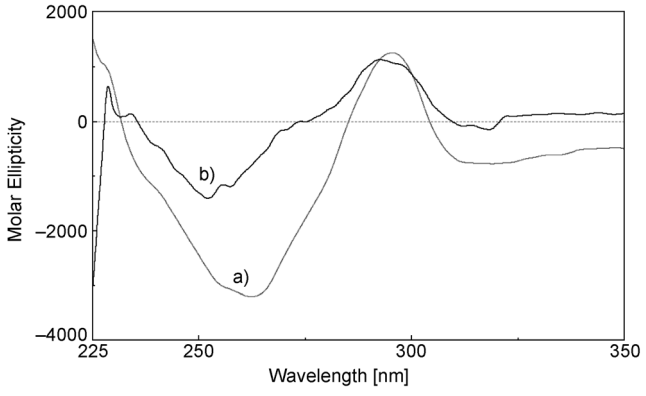


Figure 4. CD spectra of compound **G7** at 2.7 mM in $CHCl_3$; a) before addition of potassium picrate; b) after addition of potassium picrate; λ_{max} = 264, 295 nm.

of both bands is diagnostic of heteropolar, "H-to-H" or "T-to-T", stacking.

Inspection of the spectra allows us to conclude that **G6** exhibits homopolar G-tetrad stacking, whereas **G1**, **G2** and **G7** tend to generate octamers containing only "H-to-H" or "T-to-T" G-tetrad stacking. Interestingly, **G1**, **G6** and **G7** have spectra diagnostic of G-tetrad structuring even before addition of K^+ ions. Since their G-tetrad-forming ability has been previously proven by positive potassium picrate tests, these results can only be explained by assuming that these systems display an extraordinary affinity for K^+, which could be extracted from the traces present in solvents, glassware, and so forth and thus incorporated into stable complexes even before direct contact with potassium solutions. To confirm this hypothesis, after potassium picrate addition, **G1**, **G6** and **G7** were treated with an excess of the cryptand 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, which sequesters K^+ ions, and then analysed by CD spectroscopy, giving only residual CD signals (data not shown).

CD-monitored melting experiments were carried out on **G1**, **G2**, **G6** and **G7** to obtain information on the thermal stability of the stacked G-tetrad systems. For all of these compounds, under the studied conditions, a significant, nonlinear decrease in the CD signal intensity was observed on increasing the temperature, even if not in the form of well-defined S-shaped curves that refer to a single transition with a clearly identifiable melting temperature. This would be expected in the case of a single, columnar G-quadruplex structure involving all of the G-tetrads stacked in a highly hierarchical order. In all cases, curves attributable to the superimposition of multiple transitions were registered suggesting the coexistence in solution of non-homogeneous dissociating systems, probably containing a variable number of stacked G-tetrads, although essentially stable at room temperature (see Figure S7 in the Supporting Information).

Taken together, these results show that for guanosine-based self-assembly of compounds **G1**–**G3** and **G5**–**G7** in $CHCl_3$ a non-negligible role is played by the sugar functionalisation. If the ribose decorations are not able to elicit intermolecular interactions, as is the case of 2′,3′,5′-tri-*O*-acetylguanosine, no specific three-dimensional structure is observed. For **G1**, **G2**, **G6** and **G7**, the CD spectra showed the presence of regular G-tetrad stacking. However, the CD-monitored thermal denaturation curves do not support the existence of single, well-defined G-quadruplex complexes. Interestingly, of these four nucleosides, only **G6**, which exclusively carries polyether chains on the ribose moiety, is able to give homopolar stacking. On the contrary, the asymmetrically substituted guanosine derivatives carrying both lipophilic and hydrophilic groups tend to form heteropolar G-tetrad stacks. This difference in behaviour can be explained in terms of relevant contributions to the overall self-assembly given by alkyl and polyether chains, which, in contributing to self-assembly in solution, may favour a particular three-dimensional arrangement of the stacked G-tetrads. Following this interpretation, **G3** and **G5** do not show a regular G-tetrad stacking pattern in $CHCl_3$, probably because of steric hindrance of the substituents in **G5** and the presence of net negative charges in **G3** that prevent the formation of ordered aggregates.

Detailed microstructural characterisation studies on **G1**–**G7** are currently underway to determine the stability, size and shape of the corresponding superstructures, and will be reported in due course. The studies will involve a combined experimental approach by using dynamic light scattering (DLS) measurements to reveal the formation and size distribution of the aggregates, small-angle neutron scattering (SANS) measurements to provide an estimation of the thickness of the aggregates and microscope images.

Ionophoric activity: The group of J. T. Davis has recently demonstrated that potassium-promoted self-assembly of lipophilic guanosine derivatives may contribute to stabilising ion channels active in phospholipid membranes.[10–12] We therefore investigated the ionophoric activity of compounds **G1**–**G7** by using a standard base–pulse assay that reports H^+/OH^- transportation directly and cation/anion transportation indirectly. Liposomes (100 nm diameter) containing the pH-sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) were prepared in a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.0) containing NaCl (or MCl and NaX in the experiments for cation and anion selectivity, respectively; 100 mM) and, once the ionophore was added, the external pH was suddenly brought to 7.6 by addition of NaOH (or MOH in experiments for cation selectivity) and the fluorescence emission of the dye recorded. The emission maximum for HPTS is about 510 nm in both the acidic and conjugate-base forms. However, the excitation wavelength of the acidic form (403 nm) is significantly different from that of the conjugate-base form (460 nm) so that the acid/base ratio is directly reflected in the emission intensity modulation produced by alternating excitation at the two wavelengths.[24] In essence, the modulated emission signal, obtained by cycling the excitation wavelength and recording the emission at 510 nm, reports the effective pH within the liposome. An increase in HPTS fluorescence emission indicates basification of the liposome inner water pools that may be correlated to H^+/OH^- transportation and to the associated cation/anion symport or antiport.[25] The results obtained for compounds **G1**–**G7** are reported in Figure 5. Amphotericin B (AmB), a naturally occurring ionophore, was used as a positive control in these experiments.

Amphiphilic guanosine derivatives **G1**–**G4** are mostly inactive, whereas an increasing ability to discharge the pH gradient is observed for compounds **G7**, **G6** and **G5**. The most active derivative is **G5** followed by **G6**, both characterised by a large hydrophilic portion, that is, the disaccharide residue in **G5** and three monomethoxy(triethylene glycol) chains in **G6**. The activity/hydrophilicity relationship is further confirmed by the trend observed for **G1**, **G6** and **G7**, which differ in number of monomethoxy(triethylene glycol) residues: on increasing the number of polar chains, the activity increases. Similar behaviour was previously observed by us for some structurally related CyPLOS derivatives,[26] for which the activity was also strongly dependent on the number of polar ethylene glycol chains appended to a rigid macrocyclic scaffold and the activity was attributed to a destabilisation of the phospholipid bilayer caused by the insertion of polar chains.

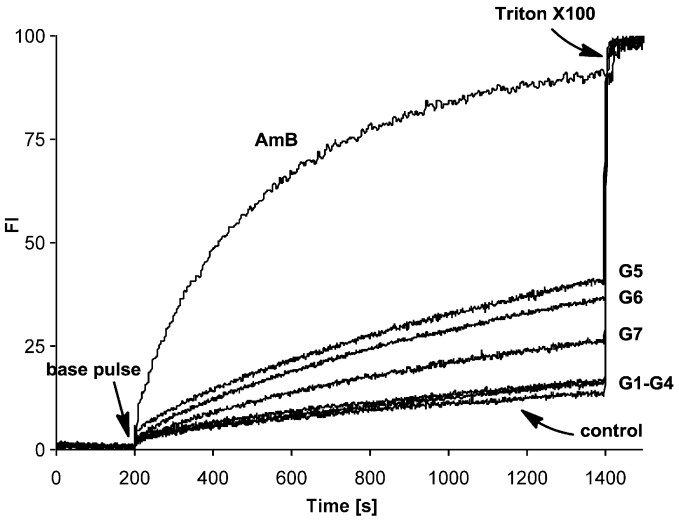


Figure 5. Normalised fluorescence change in HPTS emission (FI, λ_{ex} = 403 and 460 nm, λ_{em} = 510 nm) as a function of time after addition of the base NaOH (50 μL, 0.5 M) to EYPC/EYPG liposomes (95:5, 100 nm diameter) loaded with HPTS (0.1 mM), total lipid concentration = 0.17 mM, HEPES (25 mM), NaCl (100 mM), pH 7.0, total volume = 3 mL, 25 °C) in the presence of **G1**–**G7** derivatives (2%) and Amphotericin B (AmB; 1%). The concentration of the ionophores is given in percent with respect to the total concentration of the lipids. The control trace has been recorded in the absence of the ionophore. EYPC = egg yolk phosphatidylcholine; EYPG = egg yolk phosphatidylglycerol;

The ionophoric activity of compounds **G1**–**G7** is not influenced by the presence of K^+ ions. Experiments performed in the conditions utilised to produce Figure 5 but by using KCl instead of NaCl and KOH instead of NaOH, and therefore in the presence of a high concentration of potassium ions as the only externally added cation, show kinetic profiles for the pH discharge that are virtually superimposable on those obtained in the presence of Na^+ ions (Figure S8 in the Supporting Information). This suggests that in the phospholipid membranes the potassium ion is not able to promote the assembly of the guanosine derivatives as is observed for more concentrated solutions in chloroform. The low tendency of simple guanosine derivatives to form G-quartets in phospholipid membranes was not unexpected and parallels the findings reported by the group of J. T. Davis. Stable G-quartets have indeed only been reported for systems in which the guanosine moieties are covalently linked to a suitable scaffold[10] or inserted into derivatives in which self-assembly is mainly driven by the formation of hydrogen bonds between bis-urea[11] or bis-carbamate subunits.[12] It appears that the single interaction between potassium ions and guanosine is not strong enough to promote the self-assembling process in membranes, possibly due to the localisation of the guanosine moieties close to the surface of the bilayer where water may compete efficiently with the formation of the G-tetrads.

The hypothesis that, under the conditions of the kinetic experiments, the amphiphilic guanosine derivatives are mainly monomeric is supported by the study of the dependence of the ionophoric activity on the ionophore concentration. The kinetic profiles obtained at different concentrations of **G5** and **G6** derivatives are reported in Figure S9 in the Supporting Information. The data fit well with a first-order kinetic process and the apparent first-order rate constants were obtained by non-linear regression analysis of the fluorescence data versus time. The observed rate constants for the transportation process are reported in Figure 6. The linear trend observed is a clear indication that the active species responsible for the transportation process is monomeric.

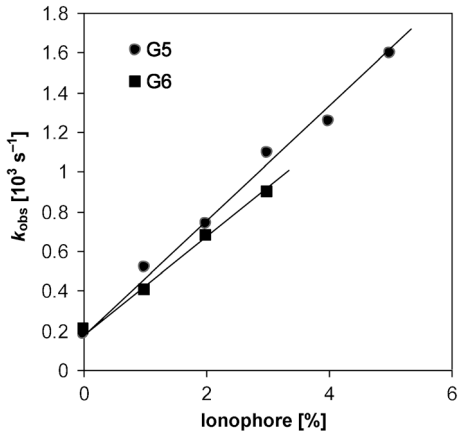


Figure 6. Dependence of k_{obs} [s^{-1}] on the concentration of ionophores **G5** and **G6**. The original kinetic profiles are reported in Figure S9 in the Supporting Information. For conditions see Figure 5.

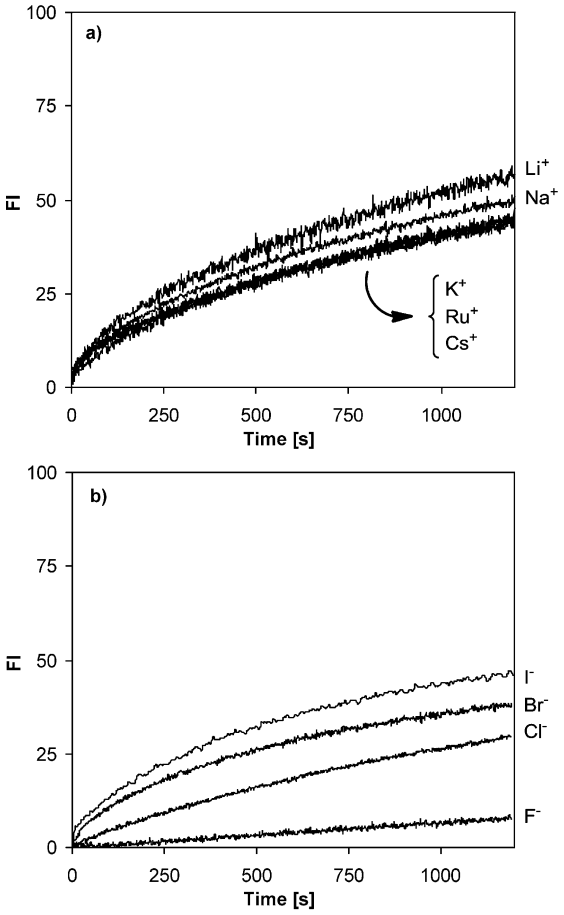


Figure 7. a) Cation selectivity for ionophore **G5** (3% concentration) by using the HPTS assay (MCl (100 mM), pH 7.0, base pulse by addition of MOH (50 μL, 0.5 M)). b) Anion selectivity for ionophore **G5** (2% concentration) by using the HPTS assay (NaX (100 mM), pH 7.0, base pulse by addition of NaOH (50 μL, 0.5 M)). The kinetics were corrected for spontaneous permeation of the different anions. Other conditions as specified in Figure 5.

To better characterise the ionophoric activity of the amphiphilic guanosine derivatives, we investigated the influence of the nature of the cation or anion present in solution on the transportation process in the case of **G5**, which is the most active derivative. The HPTS assay may indeed give indirect information on the cation and/or anion selectivity of the transportation process. The transmembrane discharge of the externally applied pH gradient, which is signalled by an increase in HPTS fluorescence emission, may derive by H^+ efflux from the liposome inner water pool or by OH^- influx from the bulk water to the inner water pool of the liposome. In any case, this ion traffic must be counterbalanced. This occurs through four processes overall: H^+/Na^+ antiport, OH^-/Cl^- antiport, H^+/Cl^- symport and Na^+/OH^- symport processes. Therefore, from a comparison of kinetic experiments performed in the presence of different cations and anions, it is possible to gain indirect evidence on their effect on the transportation process. The results obtained for the cation and anion selectivity experiments by using the group I alkali metals and the halogen anions as representative examples are reported in Figure 7a and 7b, respectively.

Inspection of Figure 7 shows that the rate of transportation does not depend a great deal on the cation present whereas it is strongly influenced by the anion and increases on going from fluoride to iodide, following the lyotropic sequence. This suggests that the transportation process is governed by the translocation of the anion and in particular limited by the cost of its dehydration.

As a final control experiment, we verified the ability of guanosine derivative **G5** to promote the leakage of calcein dye trapped inside the inner water pool of liposomes.[27] Even at a concentration of 5%, **G5** is unable to promote the leakage of calcein (Figure S10 in the Supporting Information), suggesting that the pore formed is relatively small and not large enough to allow the transit of this large anionic dye.

Taken together, the obtained results indicate that the amphiphilic guanosines **G1**–**G7** probably partition in the membrane by positioning the guanine moiety and the sugar ring close to the surface of the liposome and the appended

chains within the membrane. If the ribose decorations have a balanced amphiphilic character, as in the case of **G5** and **G6**, they destabilise the membrane, allowing the transit of small ions in a non-selective process mainly governed by the cost of dehydration of the anion. The ionophore acts in a monomeric form even in the presence of potassium ions, which appear, under the studied conditions, to be unable to promote the aggregation of the guanosine derivatives. The ionophore is too short to span the membrane and the ionophoric activity is probably related to the formation of a disordered zone in the membrane characterised by an increased permeability. Ions are therefore able to cross this disordered zone in a process that is intrinsically poorly selective and is strongly correlated to the lipophilicity of the anion.

The in vitro screening of their antiproliferative activity: Antiviral[28] and anti-cancer[29] activity has been discovered for several G-rich oligonucleotides and associated to the unusual ability of these molecules to self-assemble into G-quartet-based superstructures, which are recognised in vivo by specific proteins. On the other hand, for almost half a century, modified nucleosides with biological activities have been searched for and a great deal of biomedical interest is currently associated with nano-aggregated systems.[30] Since novel guanosine derivatives **G1**–**G7** seem to combine both the ability to form G-tetrads with the ability to generate large nano-aggregates, in vitro experiments have been carried out on a panel of cancer and non-cancer cell lines to investigate their bioactivity in a preliminary screening.

To this end, cell lines were treated for 48 h with **G1**–**G7**, at various concentrations, in a growth inhibition assay and the cytotoxicity was determined in terms of IC_{50} value (Table 2). The results show, for **G1**–**G5**, a moderate to weak selective cytotoxicity against both human MCF-7 breast adenocarcinoma and WiDr epithelial colorectal adenocarcinoma cells. In particular, **G3** and **G5** proved to be the most active compounds, exhibiting a significant antiproliferative profile against MCF-7 cells, with IC_{50} values of 22 and 17 μM, respectively. IC_{50} values within the micromolar range are generally consistent with an ability to interfere with cell viability and/or proliferation. Interestingly, in contrast to several anti-proliferative drugs, these compounds did not show unspecific in vitro cytotoxicity towards tumour and non-tumour cell lines. This might suggest specific interactions with biological targets. Detailed studies of the structure–activity relationship will be carried out in an extensive investigation aimed at developing nucleolipids with selective antineoplastic activity.

Table 2. Cytotoxicity profile of compounds **G1**–**G6** against cancer and non-cancer cell lines, IC_{50} [μM][a]. **G7** showed IC_{50} values $\geq 10^3$ μM in all cases.

Cell line[b]	G1	G2	G3	G4	G5	G6
HeLa	$>10^3$	198 ± 4	445 ± 6	130 ± 5	436 ± 9	$>10^3$
WiDr	**82 ± 6**	**90 ± 6**	135 ± 10	130 ± 11	136 ± 10	$>10^3$
MCF-7	185 ± 12	**96 ± 5**	**22 ± 4**	**46 ± 7**	**17 ± 5**	$>10^3$
C6	$>10^3$	245 ± 4	616 ± 5	152 ± 8	302 ± 5	880 ± 14
3T3-L1	$>10^3$	$>10^3$	750 ± 4	$>10^3$	$>10^3$	$>10^3$

[a] IC_{50} values are expressed as the mean ± SEM (SEM = standard error of the mean) ($n=24$) of three independent experiments. Bold values show IC_{50} values <100 μM. [b] HeLa = human cervical cancer cells; MCF-7 = human breast adenocarcinoma cells; WiDr = human epithelial colorectal adenocarcinoma cells; C6 = rat glioma cells; 3T3L1 = murine embryonic fibroblasts.

Conclusion

In this study, a general and versatile synthetic strategy has been developed to obtain a library of amphiphilic, sugar-modified analogues of guanosine, generated by very simple and high yielding manipulations starting from a common precursor, which had been fully protected on the nucleobase. Molecular diversity is ensured by insertion of different hydrophilic groups, such as amino acids, carbohydrates, oligoethers and lipophilic residues, as well as fatty acid chains. In all cases, these appendages were attached to the ribose moiety through ester linkages, obtained under mild and very effective coupling conditions and, in principle, easily cleaved inside cells by esterases.

For all synthesised compounds, qualitative potassium picrate tests confirmed the ability to extract potassium ions from aqueous solutions into $CHCl_3$ and thus to form G-tetrads. Analysis of CD spectra of dilute solutions of these derivatives in $CHCl_3$ has revealed distinctive patterns of different G-tetrad self-assemblies for **G1**, **G2**, **G6** and **G7**; for **G6**, the CD spectrum was diagnostic of homopolar stacking of the G-quartets, whereas for the other three compounds the CD bands supported the presence of G-quartets with heteropolar stacking. Two derivatives, **G1** and **G2**, show unusual gelling abilities in polar solvents, such as methanol, ethanol and acetonitrile, complementing those of lipophilic guanosine derivatives that are known to form stable organogels in highly apolar solvents. When analysed for their ion transportation abilities, **G5** was shown to be the most active compound and, as a general rule, the activity was strongly correlated with the presence of a large hydrophilic portion in the molecule. An interesting antiproliferative activity was found for **G3** and **G5** when tested on MCF-7 cancer cells, with IC_{50} values in the vicinity of 20 μM, while no cytotoxicity emerged for normal, control cells. Taken together, these data show that guanosine-based amphiphiles display a variety of unusual properties, which are largely and finely tunable, as a function of the nature and number of the ribose substituents. This renders this class of compounds of great interest for both their biological/biomedical potential and innovative applications related to the development of novel self-assembling materials.

Experimental Section

Ionophoric activity: HPTS assay: A mixture of EYPC in chloroform (225 μL, 100 mg mL^{-1}, 30 μmol) and EYPG in chloroform (60 μL,

20 mg mL^{-1}, 1.5 μmol) was evaporated with Argon flux to form a thin film and then dried under high vacuum for 3 h. This lipid cake was hydrated with HPTS solution (1.5 mL, 0.1 mM; HEPES (25 mM), NaCl (100 mM, pH 7)) for 30 min at 40 °C. The lipid suspension was submitted to five freeze–thaw cycles (−196 °C/40 °C) by using liquid nitrogen and a thermostatic bath and then extruded under nitrogen pressure (15 bar) at room temperature (10 extrusions through a polycarbonate membrane (0.1 μm)). The LUV suspension was separated from the extravesicular dye by size exclusion chromatography (SEC; stationary phase: prepacked Sephadex G-25 column; mobile phase: HEPES buffer) and diluted with HEPES buffer to give a stock solution with a lipid concentration of 5 mM (assuming 100% lipid incorporation into the liposomes). The lipid suspension (104 μL) was placed in a fluorimetric cell, diluted to 3040 μL with the same buffer solution used for the liposome preparation and kept under gentle stirring. The total lipid concentration in the fluorimetric cell was 0.17 mM. An aliquot of the ionophore solution (5–30 μL of the appropriate mother solution to obtain the desired $mol_{compound}/mol_{lipid}$ ratio) was then added to the lipid suspension and the cell was incubated at 25 °C for 30 min. HPTS emission was monitored at 510 nm and modulated by alternating excitation at 403 nm and 460 nm on a 1+1 s cycle. The concentration of the conjugate-base form is related to the emission intensity at 510 nm during the period when the dye is excited at 460 nm (E460) whereas the concentration of the protonated form is related to the emission intensity at 510 nm during the period when the dye is excited at 403 nm (E403). After incubation, the time course of fluorescence was recorded for 200 s and then NaOH (50 μL, 0.5 M) was rapidly added through an injector port and the fluorescence emission was recorded for 1200 s. Maximal changes in dye emission were obtained by final lysis of the liposomes with detergent (40 μL, 5% aqueous Triton X-100 solution). The relative intensity of E460/E403 (F) was calculated. The extent of transportation was calculated and normalised by using Equation (1), in which F^t is the relative emission intensity measured at time t, F^0 is the relative emission intensity at ionophore addition before the base pulse and F^∞ is the relative emission intensity at saturation after lysis with Triton. The apparent first-order rate constants for the transportation process were obtained by non-linear regression analysis of the fluorescence data versus time.

$$FI = \frac{(F^t - F^0)}{(F^\infty - F^0)} \times 100 \quad (1)$$

Determination of cation and anion selectivity with the HPTS assay: The vesicle suspension (104 μL stock solution, prepared as described above) was placed in a fluorimetric cell and diluted to 3040 μL with the appropriate buffer solution (HEPES (25 mM, pH 7), MCl (M = Li^+, Na^+, K^+, Rb^+ or Cs^+; 100 mM) or NaX (X = F^-, Cl^-, Br^- or I^-; 100 mM)). The total lipid concentration in the fluorimetric cell was 0.17 mM. An aliquot of the ionophore as a solution in DMSO (5–30 μL of the appropriate mother solution to obtain the desired $mol_{compound}/mol_{lipid}$ ratio) was then added to the lipid suspension and the cell was incubated at 25 °C for 30 min. HPTS emission was monitored at 510 nm and excitation wavelengths of 403 and 460 nm were used concurrently. After incubation, the time course of fluorescence was recorded for 200 s and then MOH (M = Li^+, Na^+, K^+, Rb^+ or Cs^+ depending on the cation present in the extravesicular buffer solution; 50 μL, 0.5 M;) was rapidly added through an injector port and the fluorescence emission was recorded for 1200 s. Maximal changes in dye emission were obtained by final lysis of the liposomes with detergent (40 μL, 5% aqueous Triton X-100 solution). The extent of transportation was calculated and normalised as previously described.

Cell cultures and microculture bioassays for evaluation of cell growth and proliferation: The bioactivity of compounds **G1**–**G7** was investigated by evaluation of cell viability and proliferation on a limited panel of cell lines consisting of tumour C6 rat glioma cells, HeLa human cervical cancer cells, WiDr human epithelial colorectal adenocarcinoma cells, MCF-7 human breast adenocarcinoma cells, and non-tumour 3T3L1 murine embryonal fibroblasts. Experimental details of the bioactivity studies are reported in the Supporting Information.

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[1] A. Gissot, M. Camplo, M. W. Grinstaff, P. Berthélémy, *Org. Biomol. Chem.* **2008**, *6*, 1324–1333, and references therein.
[2] H. Rosemeyer, *Chem. Biodivers.* **2005**, *2*, 977–1062.
[3] A. H. Farji, P. Wipf, *Bioorg. Med. Chem.* **2009**, *17*, 2950–2962.
[4] For recent examples, see: a) H. W. Yang, W. Jeong, E.-K. Bang, E. M. Jeon, B. H. Kim, *Org. Biomol. Chem.* **2011**, *9*, 291–296; b) C. Ceballos, S. Khiati, C. A. H. Prata, X.-X. Zhang, S. Giorgio, P. Marsal, M. W. Grinstaff, P. Berthélémy, M. Camplo, *Bioconjugate Chem.* **2010**, *21*, 1062–1069; c) S. Khiati, N. Pierre, S. Andriamanarivo, M. W. Grinstaff, N. Arazam, F. Nallet, L. Navailles, P. Berthélémy, *Bioconjugate Chem.* **2009**, *20*, 1765–1772.
[5] An example of guanosine-based amphiphiles is described in: H. R. Jung, T. K. Vu, S. K. Choi, S. M. Park, R. H. Kim, *Bull. Korean. Chem. Soc.* **2010**, *31*, 549–550. Therein, the authors produced five nucleobase-protected amphiphilic derivatives of guanosine in 17% overall yields on average. It is to be noted that the presented synthetic scheme does not allow for final guanine deprotection of the guanosine derivatives.
[6] S. Lena, S. Masiero, S. Pieraccini, G. P. Spada, *Chem. Eur. J.* **2009**, *15*, 7792–7806, and references therein.
[7] S. Lena, S. Masiero, S. Pieraccini, G. P. Spada, *Mini-Reviews in Organic Chemistry* **2008**, *5*, 262–273.
[8] G. Gottarelli, G. P. Spada, *Chem. Rec.* **2004**, *4*, 39–49.
[9] J. T. Davis, G. P. Spada, *Chem. Soc. Rev.* **2007**, *36*, 296–313.
[10] M. S. Kaucher, W. A. Harrell, J. T. Davis, *J. Am. Chem. Soc.* **2006**, *128*, 38–39.
[11] L. Ma, M. Melegari, M. Colombini, J. T. Davis, *J. Am. Chem. Soc.* **2008**, *130*, 2938–2939.
[12] L. Ma, W. A. Harrell, J. T. Davis, *Org. Lett.* **2009**, *11*, 1599–1602.
[13] K. Araki, I. Yoshikawa, *Top. Curr. Chem.* **2005**, *256*, 133–165.
[14] I. Yoshikawa, S. Yanagi, Y. Yamaji, K. Araki, *Tetrahedron* **2007**, *63*, 7474–7481.
[15] I. Yoshikawa, J. Sawayama, K. Araki, *Angew. Chem.* **2008**, *120*, 1054–1057; *Angew. Chem. Int. Ed.* **2008**, *47*, 1038–1041.
[16] R. W. Wang, B. Gold, *Org. Lett.* **2009**, *11*, 2465–2468.
[17] S. A. Sikchi, P. G. Hultin, *J. Org. Chem.* **2006**, *71*, 5888–5891.
[18] A. Matsuda, M. Shinozaki, M. Suzuki, K. Watanabe, T. Miyasaka, *Synthesis* **1986**, 385–386.
[19] L. Simeone, G. Mangiapia, C. Irace, A. Di Pascale, A. Colonna, O. Ortona, L. De Napoli, D. Montesarchio, L. Paduano, *Mol. BioSyst.* **2011**, *7*, 3075–3086.
[20] For examples, see: a) R. Iwaura, K. Yoshida, M. Masuda, K. Yase, T. Shimizu, *Chem. Mater.* **2002**, *14*, 3047–3053; b) R. Iwaura, K. Yoshida, M. Masuda, M. Ohnishi-Kameyama, M. Yoshida, T. Shimizu, *Angew. Chem.* **2003**, *115*, 1039–1042; *Angew. Chem. Int. Ed.* **2003**, *42*, 1009–1012.
[21] G. Gottarelli, S. Masiero, G. P. Spada, *J. Chem. Soc. Chem. Commun.* **1995**, 2555–2557.
[22] S. Masiero, R. Trotta, S. Pieraccini, S. De Tito, R. Perone, A. Randazzo, G. P. Spada, *Org. Biomol. Chem.* **2010**, *8*, 2683–2692.
[23] Following the classification used in Ref. [22], a G-tetrad shows the "head" surface (or H) if it is oriented with the hydrogen-bonding network going clockwise from the donor to the neighbour acceptor; "tail" (or T) is defined as the opposite, with the donor-to-acceptor Hydrogen-bonding system running anticlockwise.
[24] T. M. Fyles, H. Luong, *Org. Biomol. Chem.* **2009**, *7*, 733–738.
[25] N. Sakai, S. Matile, *J. Phys. Org. Chem.* **2006**, *19*, 452–460.
[26] a) S. Licen, C. Coppola, J. D'Onofrio, D. Montesarchio, P. Tecilla, *Org. Biomol. Chem.* **2009**, *7*, 1060–1063; b) C. Coppola, A. Paciello, G. Mangiapia, S. Licen, L. De Napoli, L. Paduano, P. Tecilla, D. Montesarchio, *Chem. Eur. J.* **2010**, *16*, 13757–13772.
[27] Y. Tsao, L. Huang, *Biochemistry* **1985**, *24*, 1092–1098.

[28] For a recent review, see: a) B. Gatto, M. Palumbo, C. Sissi, *Curr. Med. Chem.* **2009**, *16*, 1248–1265; for recent examples, see: b) G. Di Fabio, J. D'Onofrio, M. Chiapparelli, B. Hoorelbeke, D. Montesarchio, J. Balzarini, L. De Napoli, *Chem. Commun.* **2011**, *47*, 2363–2365; c) E. Pedersen, J. T. Nielsen, C. Nielsen, V. V. Filichev, *Nucleic Acids Res.* **2011**, *39*, 2470–2481.

[29] a) S. Balasubramanian, L. H. Hurley, S. Neidle, *Nat. Rev. Drug Discov.* **2011**, *10*, 261–275; b) P. J. Bates, E. W. Choi, L. V. Nayak in *Methods in Molecular Biology, Vol. 542, Gene Therapy of Cancer: Methods and Protocols*, 2nd ed. (Eds.: W. Walther, U. S. Stein), Humana Press, Totowa, **2009**, pp. 379–392; c) A. C. Girvan, Y. Teng, L. K. Casson, S. D. Thomas, S. Jueliger, M. W. Ball, J. B. Klein, W. M. Pierce, Jr., S. S. Barve, P. J. Bates, *Mol. Cancer Ther.* **2006**, *5*, 1790–1799.

[30] S. R. Meyers, F. S. Juhn, A. P. Griset, N. R. Luman, M. W. Grinstaff, *J. Am. Chem. Soc.* **2008**, *130*, 14444–14445.

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