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Toward Supramolecular Ion Channels Formed by Oligonucleotide Analogs: Hydrophobic Guanine Dimers

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Abstract In this model study toward supramolecular channels formed by oligonucleotide analogs, we describe the synthesis of hydrophobic guanine dimers **1** - **4**. We found that the solubility of guanine dimers **1** - **4** is significantly reduced compared to hydrophobic (iso)guanosine monomers and independent of the nature of substituents at the exocyclic amine. This indicates that the (deoxy)ribose is important to form soluble, ionophoric G-quartets. © 1998 Elsevier Science Ltd. All rights reserved.

We have recently shown that rigid-rod molecules represent a promising and versatile new class of nonpeptide ion channels.[1-3] For instance, the organization of oligo(*p*-phenylene)s in lipid bilayers is governed by their length with respect to the thickness of the hydrophobic part of the membrane, while activity,[1] selectivity,[2] and incorporation into lipid bilayers[3] are regulated by the nature of the substituents attached to the rigid-rod scaffold. As most nonpeptide ion channel models,[4-7] substituted oligo(*p*-phenylene)s act as monomers or as unstable, poorly organized suprastructures with uncertain spectroscopic characteristics and intermolecular interactions.[2,8] In sharp contrast, peptide-based ion channel models tend to form highly ordered assemblies which mimic structural aspects of neuronal channel proteins.[9] To self-assemble membrane-spanning rigid-rod molecules into a well-organized, "barrel-stave"-type suprastructure, we focused our attention on potential substituents which are known to form planar, cyclic complexes and, when attached to oligo(*p*-phenylene)s, may afford rationally designed, supramolecular nonpeptide channel models as outlined in Figure 1A.

Guanine was selected for this model study because differently substituted guanosines,[10] isoguanosines,[11] folates,[12] as well as telomeric DNA oligonucleotides[13] or thrombin inhibiting DNA aptamers[14] all self-assemble spontaneously into cyclic arrays. Using 3',5'-didecanoyl-2'-deoxyguanosine, Gottarelli and coworkers recently found ionophoric, Hoogsteen-bonded G-quartets in $CHCl_3$;[10] Davis' group made similar observations with hydrophobic isoguanosines.[11] However, to use G-quartets for rigid-rod shaped, supramolecular channel models, the significance of the substituents at N^2 and N^9 for the self-assembly of guanine derivatives into cyclic arrays needed clarification. Thus, the biphenyl-linked guanine dimers **1** - **4** (Figure 1B), which simulate the situation with oligo(*p*-phenylene)s (Figure 1A) on a less complex level and carry N^2-substituents with increasing size and hydrophobicity, were synthesized and studied.

The syntheses of **1** - **4** are shown in Scheme 1. The purinylacetic acid **5** was prepared from chloropurine **6** according to a recently reported two step procedure.[15] Diazotization of amine **5** with *tert*-butyl nitrite in the presence of 60% HF/pyridine gave fluoropurine **7**. The N^2-substituted guanines **8** - **10** were obtained through

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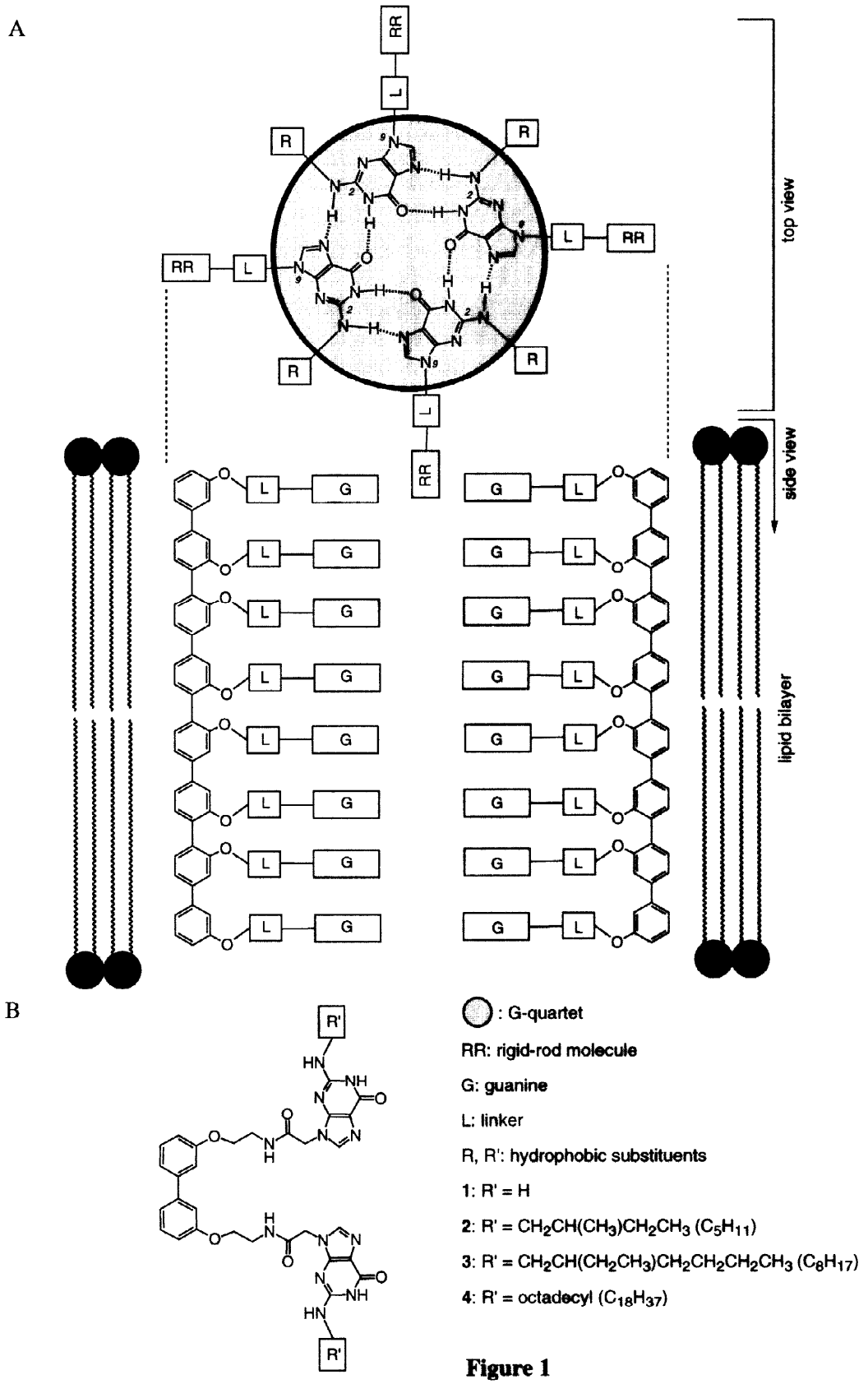
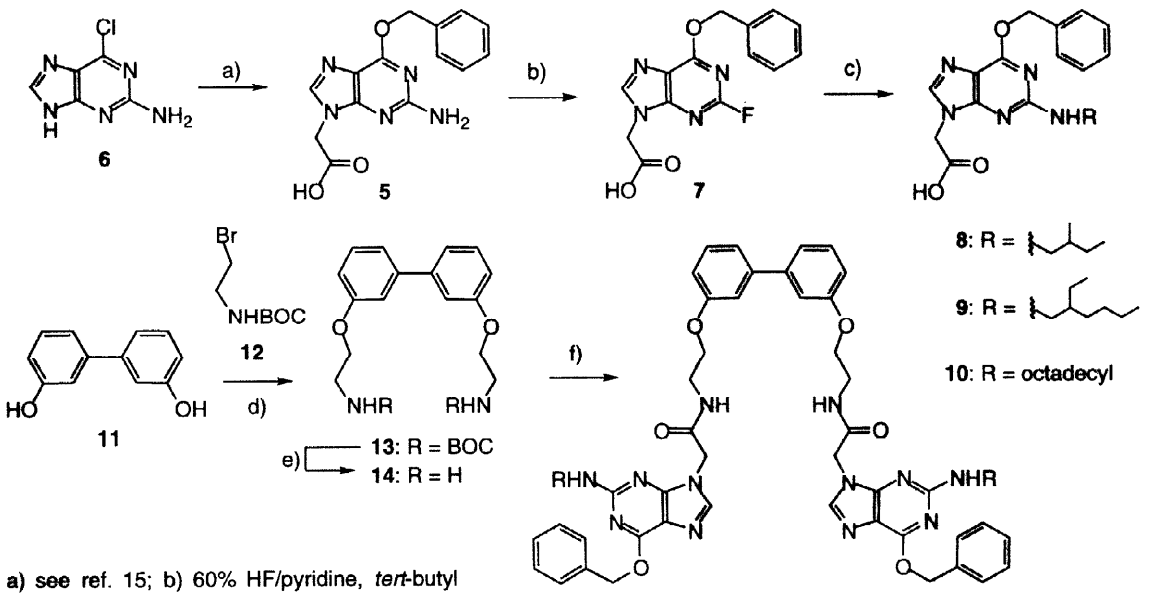

A
RR
L
R
top view
side view
G
lipid bilayer
B
R'
: G-quartet
RR: rigid-rod molecule
G: guanine
L: linker
R, R': hydrophobic substituents
1: R' = H
2: R' = CH2CH(CH3)CH2CH3 (C5H11)
3: R' = CH2CH(CH2CH3)CH2CH2CH2CH3 (C8H17)
4: R' = octadecyl (C18H37)

Figure 1

reaction of **7** with the corresponding amines.[16] The biphenyl linker was synthesized from biphenol **11**, which is readily prepared from the corresponding bianisole by treatment with BBr_3. BOC-protected bromoethylamine **12** was reacted with biphenol **11**, and deprotection of the resulting ether **13** gave diamine **14**. Coupling of the acids **5**, **8** - **10** with diamine **14** afforded the diamides **15** - **18**, and acid-catalyzed deprotection yielded the guanine dimers **1** - **4**.[17]

The guanine dimers **1** - **4** (Fig. 1B) were practically insoluble in most common solvents. Namely, the solubilities were around 1 mM in DMSO and below 34 µM in H_2O, MeOH, EtOH, octanol, $CHCl_3$, CH_2Cl_2, TFE, THF, and hexanes in the presence and absence of dry potassium picrate. Compared to hydrophobic guanosine[10] and isoguanosine[11] monomers, the solubility of **1** - **4** in $CHCl_3$ is more than 500-times reduced. Standard methods to study ionophoric properties (e.g., Cram's picrate extraction) and suprastructures in nonpolar solvents were thus not applicable. A drastic increase in turbidity upon addition of 40 µl **1** - **4** in DMSO (1 mM) to 2 ml of a suspension of uniformly sized EYPC-SUVs (small unilamellar vesicles composed of fresh egg yolk phosphatidylcholine, 100 mM HEPES, 100 mM KCl or NaCl, pH 7.1) indicated poor incorporation into EYPC-bilayers.

Scheme 1



a) see ref. 15; b) 60% HF/pyridine, *tert*-butyl nitrite, -42°, 55%; c) NH_2R, MeOH, rt, **8**: 61%, **9**: 51%, **10**: 37%; d) Cs_2CO_3, DMF, 100°, 68%; e) TFA, CH_2Cl_2, 89%; f) **5**, **8**, **9**, or **10**, EDC, DMAP, DMF, rt, **15**: 37%, **16**: 44%, **17**: 51%, **18**: 29%; g) TFA, quant.

In summary, the solubility of hydrophobic guanine dimers **1** - **4** is more than 500-times lower than that of hydrophobic (iso)guanosine monomers and independent of size and hydrophobicity of substituents at the exocyclic amine. The plausible but not definite conclusion is that the (deoxy)ribose subunit is essential to form soluble, hydrophobic G-quartets. Vigorous studies with guanosines are ongoing.

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17. All products gave satisfactory spectroscopic data. For example: **3:** ^{1}H NMR (300 MHz, DMSO-d_6, 1 mM) δ 10.29 (s, 2H, *fast* exchange with D_2O), 8.49 (t, 2H, J = 6.02 Hz, *fast* exchange with D_2O), 7.63 (s, 2H), 7.33 (t, 2H, J = 8.0 Hz), 7.21 (br. d, 2H, J = 8.0 Hz), 7.15 (br. s, 2H), 6.92 (br. d, 2H, J = 8.0 Hz), 6.26 (br. t, 2H, *fast* exchange with D_2O), 4.66 (s, 4H), 4.06 (t, 4H, J = 5.49 Hz), 3.48-3.46 (m, 4H), 3.15-3.12 (m, 4H), 1.38-1.17 (m, 18H), 0.79, 0.78 (2t, 12H, J = 7.6 Hz). FAB-HRMS: calc. for $C_{46}H_{63}N_{12}O_6$: 879.5012. Found: 879.4993. **1**: FAB-HRMS: calc. for $C_{30}H_{31}N_{12}O_6$: 655.2513. Found: 655.2490. **2**: FAB-HRMS: calc. for $C_{40}H_{51}N_{12}O_6$: 795.4088. Found: 795.4055. **4**: FAB-HRMS: calc. for $C_{66}H_{103}N_{12}O_6$: 1159.8105. Found: 1159.8124.