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The Use of a Peptidic Scaffold for the Formation of Stable Guanine Tetrads: Control of a H-bonded Pattern in Water

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Controlling the formation of hydrogen bonds is crucial for the development of supramolecular architectures. In this context, biological systems are the source of inspiration of supramolecular research for designing two- and three-dimensional nanostructures of increasing complexity. Among all biological macromolecules, DNA is certainly the most promising candidate. Indeed, DNA-based materials are relevant examples of structures for which the self-assembly of hydrogen-bonded nucleobases is used to create highly ordered and complex architectures.[1] In particular, guanine derivatives are known to self-assemble through hydrogen bonding into various discrete architectures, including dimers, ribbons, and quartets.[2] The G-quartet formed by the coplanar arrangement of four Hoogsteen-paired guanine bases represents a relevant model for studies of molecular self-assembly and noncovalent synthesis. Stacked guanine tetrads are found in particular DNA secondary structures, such as G-quadruplex DNA, which is hypothesized to have consequences in several biological processes.[3] Furthermore, guanine-based structures are of interest for applications in molecular electronics.[4]

One challenging goal is to organize small molecules, such as the guanine building blocks, into well-defined assemblies. Self-assembly of guanines has been mainly exploited by using lipophilic systems to create smart supramolecular architectures in organic solvents. Indeed, water is a highly competitive solvent that disfavors hydrogen bonds between small molecules. Cations also play an important role in the structuring of assemblies based on the G-quartet motif.[5] For these reasons, self-assembly of guanines in water are mainly carried out in high concentrations of guanines and salts. Rivera and co-workers have recently studied the self-assembly of a 2′-deoxyguanosine derivative, which was modified at the C8 position by a *m*-acetylphenyl group and at the 2′-position by a dimethylamino moiety. At neutral pH, the positively charged ammonium groups render the molecule soluble in water. Interestingly, they demonstrated that the molecule is able to self-assemble in water as a result of the formation of a hexadecamer.[6] They attributed the enhanced stability to a combination of factors, including the formation of extra H bonds and increased π-stacking interactions. Nevertheless, the studies were performed by using 1 M KCl, which emphasizes the difficulties of creating hydrogen bonds between small molecules in water.

For stabilization of only one G-quartet tetrad, the use of a scaffold has been proposed. Pioneered by Davis and coworkers, guanosine moieties were anchored to a calixarene to constrain the nucleobases in the assembled conformation.[7] More recently Sherman and Nikan reported the use of a cavitand scaffold and showed a higher and cation-independent stability due to the pre-organization of the guanine units.[8] However, due to the hydrophobic nature of the scaffold, the studies were carried out in organic solvent.[9] Herein, we report on a new system that is able to form a stable G-tetrad in water without the addition of any cations. The method is based on the use of a cyclodecapeptide scaffold introduced by Mutter for the design of template-assembled synthetic proteins.[10] This scaffold has been used recently for the efficient formation of G-quadruplexes.[11]

Compounds **3** and **4** have been designed and synthesized by Huisgen 1,3-dipolar cycloaddition (Scheme 1). Interestingly, the resulting triazole linkers are shown to actively participate in the structuring of the G-quartet, as described below.[12] Compound **3** was prepared from the cyclodecapeptide scaffold **1**, which was functionalized with alkyne moieties by incorporating propargylglycine-modified amino acids during the solid-phase peptide synthesis. The coupling with 5′-azido-2′,3′-*O*-isopropylideneguanosine (**2**)[13] was achieved by 1,3-dipolar cycloaddition, which was catalyzed by Cu^{I}, affording the desired compound in 79% yield after purification. The solubility of **3** in water was too low for NMR studies and the isopropylidene residues were therefore removed under acidic conditions (TFA/H_2O, 50:50, v/v) to obtain **4** in 69% yield. Compound **4** was found to be fully soluble in water (see the Supporting Information).

^{1}H NMR analysis of starting-unit **2** was carried out in DMSO (Figure 1a) and revealed a well-resolved spectrum with the chemical shift of the imino proton (NH) at $\delta =$ 10.69 ppm, which is the classical value found for free guanine in solution. ^{1}H NMR analysis was also performed in water with guanosine, because **2** is not soluble under these

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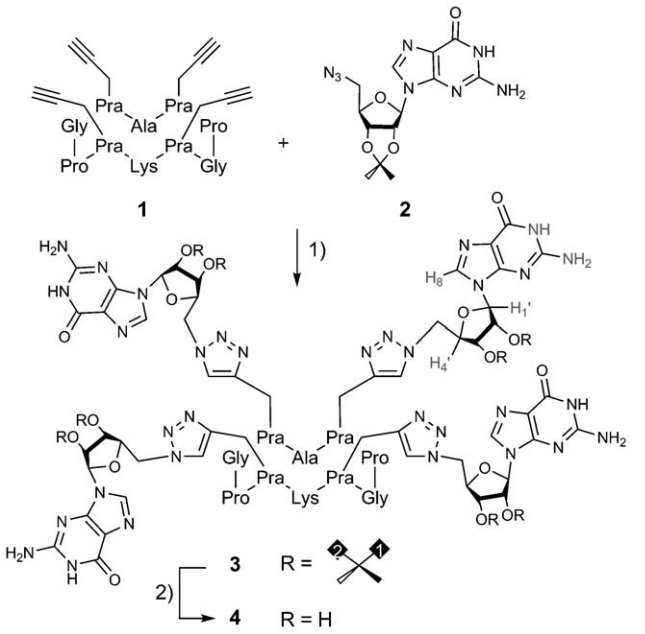

Scheme 1. Synthesis of compounds **3** and **4**. 1) Cu^0, phosphate-buffered saline (PBS)/DMSO/*t*BuOH (50:40:10; 10 mM PBS, 0.3 M NaCl, pH 8.0), 55 °C; 2) trifluoroacetic acid (TFA)/H_2O (50:50). Pra is the L-propargylglycine-modified amino acid.

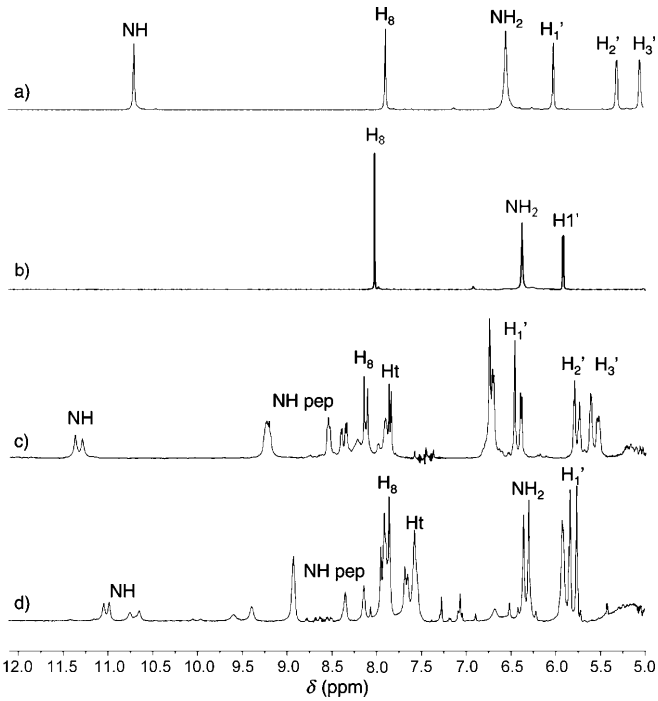

Figure 1. 500 MHz ^{1}H NMR spectra of a) compound **2** in [D_6]DMSO at RT; b) guanosine in H_2O/D_2O (90:10 v/v) at 10 °C; c) compound **3** in H_2O/CD_3CN (50:50 v/v) at 10 °C, and d) compound **4** in H_2O/D_2O (90:10 v/v) at 10 °C.

condition. As anticipated, the imino proton is not observed (Figure 1 b) due to the rapid exchange with the solvent, thus demonstrating the absence of structuring of guanosine units in water. For compounds **3** and **4**, NMR analyses were carried out in a mixture of H_2O/CD_3CN and in water, respectively. Prior to NMR-sample preparation, the compounds were thoroughly desalted by size-exclusion chromatography (see the Supporting Information). Interestingly, the imino proton is detected and resonates at $\delta = 11.33$ ppm for **3**, whereas a duplication of the imino proton signal, at $\delta =$ 11.01 and 10.70 ppm, is observed for **4** (Figure 1 d). The observation of imino signals in water as well as the higher chemical-shift values in comparison with **2** indicate the existence of hydrogen bonds and suggest that the guanine bases are organized. Furthermore, ^{1}H NMR analysis was carried out in H_2O/CD_3CN by varying the temperature from −10 to 50 °C to access the stability of the structured system. The disappearance of the signal corresponding to imino protons was observed when the temperature reached 30 °C (see also thermal denaturation studies by CD analysis). All these data demonstrates that the scaffold allows efficient formation of a stable, structured guanosine motif in an aqueous solution. A detailed NMR conformational study by using TOCSY, double-quantum-filter (DQF)-COSY, and NOESY techniques was then performed (see the Supporting Information).

2D-NOESY experiments, which were used for the full assignment of protons chemical shifts of compound **3** (see Figure 11–14 and Table 1 in the Supporting Information), showed a number of correlations between the protons of the guanosines moieties and the protons of the peptidic scaffold. In particular, strong correlations between the H8 and H1′ for **3** are observed; this is indicative of a *syn* conformation along the glycosidic bond (Figure 2).[14] This conformation is known to prevent the formation of structures, such as guanine ribbons,[15] and thus validates the formation of the guanine quartet under these conditions. Note that the imino proton signal is slightly split. Two NOE correlations, between H8 and H4′ for two guanosine residues and between NH_{Pra} (amido proton of the peptidic scaffold, see the Supporting Information for the nomenclature) and H4′ for two

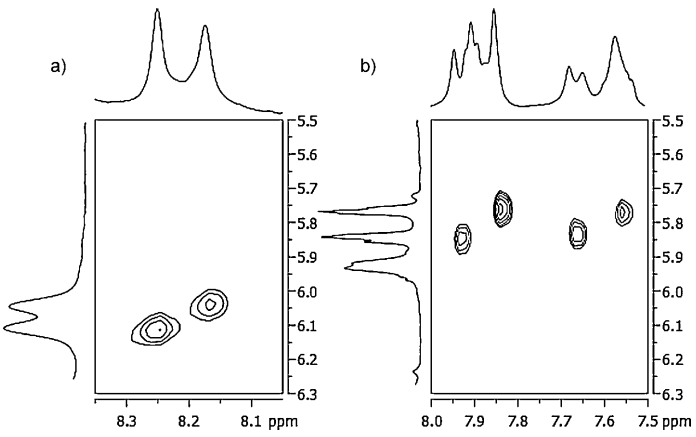

Figure 2. Parts of the NOESY spectra showing the correlations between the H8 and H1′ protons for a) **3** in H_2O/CD_3CN (50:50 v/v) at −10 °C and b) **4** in H_2O/D_2O (90:10 v/v) at 10 °C.

other guanosine residues, are observed. This evidences that the four guanosines are nonequivalent; two of them are organized in the plan of the peptide (i.e., are close to the peptidic backbone that produces the above-mentioned NOE correlation) and two of them are outside of the peptidic backbone structure. This causes the diastereotopy of the imino protons of the G-quartet and thus explains the split of the imino signal in the ^{1}H NMR spectrum.

Molecular modeling by using the different NOE constraints (see the Supporting Information) was therefore performed and the so-obtained structure was found quite rigid (Figure 3a,b). It is also remarkable that the triazole linkers

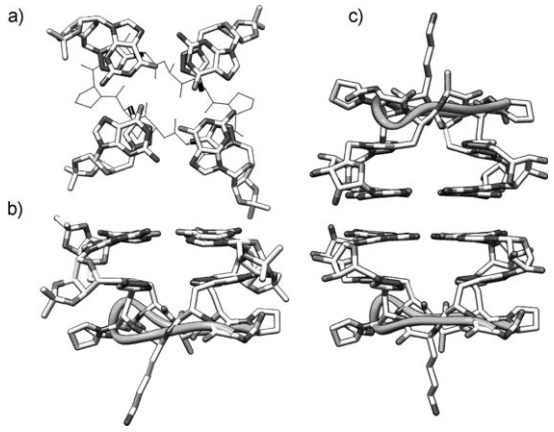

Figure 3. Molecular modeling using NOE constraints: a) Top view (the peptidic scaffold is shown as a thinner structure to improve clarity), b) side view of monomer **3**, and c) side view of the proposed dimer **4**, by using NOE constraints from **3**. The gray ribbon indicates the peptidic backbone of the scaffold.

participate in the structuring of the compound; the aromatic part of the guanosine is stacked on a tetrad formed by the four triazole residues. Indeed, the distance between the G-quartet and the triazole tetrad has been estimated to 3.6 Å, which is similar to the distance between two G-quartets in DNA quadruplexes.[16]

Cations, such as K^+ or NH_4^+, are known to contribute to the stability of G-quadruplex structures.[5] Thus, ^{1}H NMR studies were also carried out in presence of these cations. No significant changes of the spectra could be observed upon addition of NH_4Cl. In particular, imino protons resonate at the same chemical shift as in the absence of cations (see the Supporting Information). Upon addition of KCl, a slightly different chemical shift is observed for the imino protons; this indicates an interaction of K^+ cations with the guanines tetrads. Such behavior can be also observed for G-quartets stabilized in long G-wire tracks[17a] and for G-quadruplex motifs prepared in 40% polyethyleneglycol-crowding medium.[17b]

Compound **4** displays a more complex ^{1}H NMR spectrum in which most of the signals are split (Figure 1d). 2D experiments suggest a mixture of two products, as evidenced by the NOE correlations between NH and Hα of the peptidic scaffold (data not shown). In particular, the appearance of four signals corresponding to the H8 and H1′ correlations in comparison with **3** is consistent with the co-existence of two products (Figure 2b). This latter observation is also indicative of a *syn* conformation along the glycosidic bond for both products. To differentiate the two co-existing products in solution, diffusion experiments (DOSY) were performed (see the Supporting Information). This method has been used recently for determining the molecular weight of unimolecular quadruplexes and the stoichiometry of nucleic-acid quadruplex folds.[18]

Two main products were distinguished and their hydrodynamic radii (R_h) were estimated (see the Supporting Information).[19] The ratio R_{h1}/R_{h2} was around 1.7, which indicates the co-existence of two products in solution, one being approximately twice as big as the other one. Thus compound **4** was proposed to form a dimer in solution. The imino region of the ^{1}H NMR spectrum of compound **4** can be assigned as follows: the two peaks at $\delta = 11.01$ ppm correspond to the imino protons of the monomer and the two peaks at $\delta = 10.70$ ppm to the imino protons of the dimer. The small difference should result from the shielding of the imino proton by the two stacked G-tetrads in the dimer. Because a single set of signals is observed for the dimer, the two G-quartets are homotopic and the dimer displays a D_4 symmetry.

Molecular modeling of dimer **4** was thus performed using the observations described above. NOE correlations between H8 and H4′, and between NH_{Pra} and H4′ are no longer observed in the NOESY spectrum of compound **4** (data not shown), suggesting a more flexible structure. These constraints were thus removed from molecular-modeling study. A head-to-head dimer was proposed and the so-obtained, minimized structure is depicted in Figure 3c.[20] The G-quartet motifs are stabilized by the formation of the dimer through the stacking of two tetrads. The distance between the two G-quartets is similar to stacked tetrads found in G-quadruplexes (around 3.5 Å). However, we were unable to observe NOE correlations between the two moieties.

To verify the structure and evaluate the stability of the different architectures, circular-dichroism (CD) analyses were performed. This technique has been widely employed to study biological structures, notably DNA G-quadruplexes.[21] Spectra were recorded in the same conditions as for the NMR studies. Note also that the additional peptidic scaffold and triazole chromophores could contribute to the observed CD bands.[22] The spectrum of compound **3** displays a minimum at $\lambda = 230$ nm, corresponding mainly to the β-sheet conformation adopted by the cyclodecapeptide, and a maximum at 281 nm with a shoulder at 265 nm (Figure 4a, solid line), which are in agreement with a guanine tetrad. Compound **4** shows a rather different spectrum. At 10 °C, in pure water, the CD spectrum of **4** displays a minimum at 240 nm and two maxima at 260 and 288.5 nm, respectively (Figure 4b, solid line). The global shape of the CD spectrum is consistent with stacked tetrads.[21] Thermal denaturation was then carried out to investigate the stability of both ar-

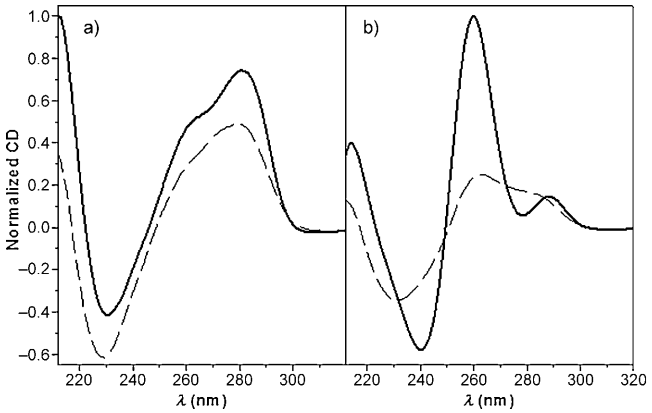

Figure 4. CD spectra of a) compound **3** in H_2O/CH_3CN (50:50 v/v) and b) compound **4** in H_2O. The spectra were recorded at 10 (solid line) and 90 °C (dashed line).

chitectures. The CD spectra were found to be quite similar for compound **3** at the different temperatures (Figure 4a depicts the CD spectra at 10 and 90 °C). A transition could be observed during a melting experiment and the thermal denaturation of the so-formed tetrad was evaluated at 29 °C. These results are consistent with the different NMR observations (see above). On the contrary, dramatic changes were observed for compound **4**. Indeed, the intensity of the signal at 260 nm strongly decreases upon heating to 90 °C. At high temperatures the spectrum becomes quite similar to the one of compound **3**.[23] This is again in agreement with the formation of a dimer for compound **4**, which on heating dissociates to the monomer. Note that the head-to-head dimer structure is proposed although CD data is more consistent with a head-to-tail dimer.[22] However, in this case, the presence of the triazole moiety might contribute to the CD signal.[22,24]

In summary, we have designed and synthesized two original architectures based on a scaffold assistance to form G-quartets in water without the addition of cations.[25] In addition to the entropically driven formation of the G-quartet, the strategy takes advantage of the triazole linkers for further stabilization of the G-quartet motif. We are currently interested in studying the behavior of such architectures in presence of monovalent and divalent cations. Moreover, preliminary studies have shown that the use of compound **3** in the presence of free guanosine monophosphate (GMP) allows the nucleation of G-quartet aggregates. The designed architectures display a free amino moiety on the lower face of the cyclodecapeptide scaffold; this moiety can be used for the functionalization of a surface and the growth control of quartet fibers on surfaces. These approaches may allow studies and understanding of complex phenomena, such as the self-assembly of nucleobases in aqueous media.

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Keywords: cyclodecapeptide · G-quartets · hydrogen bonds · scaffolds · supramolecular chemistry

[1] a) S. Sivakova, S. J. Rowan, *Chem. Soc. Rev.* **2005**, *34*, 9–21; b) J. L. Sessler, C. M. Lawrence, J. Jayawickramarajah, *Chem. Soc. Rev.* **2007**, *36*, 314–325; c) U. Feldkamp, B. Saccà, C. M. Niemeyer, *Angew. Chem.* **2009**, *121*, 6110–6114; *Angew. Chem. Int. Ed.* **2009**, *48*, 5996–6000, and references therein.

[2] a) J. T. Davis, *Angew. Chem.* **2004**, *116*, 684–716; *Angew. Chem. Int. Ed.* **2004**, *43*, 668–698; b) J. T. Davis, G. P. Spada, *Chem. Soc. Rev.* **2007**, *36*, 296–313; c) A. Likhitsup, R. J. Deeth, S. Ottob, A. Marsh, *Org. Biomol. Chem.* **2009**, *7*, 2093–2103.

[3] a) S. Neidle, *FEBS J.* **2010**, *277*, 1118–1125; b) A. De Cian, L. Lacroix, C. Douarre, N. Temine-Smaali, C. Trentesaux, J.-F. Riou, J.-L. Mergny, *Biochimie* **2008**, *90*, 131–155; c) L. Oganesian, J. Karlseder, *J. Cell Sci.* **2009**, *122*, 4013–4025.

[4] R. Rinaldi, G. Maruccio, A. Biasco, V. Arima, R. Cingolani, T. Giorgi, S. Masiero, G. P. Spada, G. Gottarelli, *Nanotechnology* **2002**, *13*, 398–403.

[5] a) G. Wu, A. Wong, Z. Gan, J. T. Davis, *J. Am. Chem. Soc.* **2003**, *125*, 7182–7183; b) X. Shi, K. M. Mullaugh, J. C. Fettinger, Y. Jiang, S. A. Hofstadler, J. T. Davis, *J. Am. Chem. Soc.* **2003**, *125*, 10830–10841; c) A. Ciesielski, S. Lena, S. Masiero, G. P. Spada, P. Samori, *Angew. Chem.* **2010**, *122*, 2007–2010; *Angew. Chem. Int. Ed.* **2010**, *49*, 1963–1966.

[6] a) J. E. Betancourt, J. M. Rivera, *J. Am. Chem. Soc.* **2009**, *131*, 16666–16668; b) M. Garcıa-Arriaga, G. Hobley, J. M. Rivera, *J. Am. Chem. Soc.* **2008**, *130*, 10492–10493.

[7] F. W. Kotch, V. Sidorov, Y. F. Lam, K. J. Kayser, H. Li, M. S. Kaucher, J. T. Davis, *J. Am. Chem. Soc.* **2003**, *125*, 15140–15150.

[8] a) M. Nikan, J. C. Sherman, *Angew. Chem.* **2008**, *120*, 4978–4980; *Angew. Chem. Int. Ed.* **2008**, *47*, 4900–4902; b) M. Nikan, J. C. Sherman, *J. Org. Chem.* **2009**, *74*, 5211–5218.

[9] NMR studies should be carried out at a low temperature (−40 °C) in $CDCl_3$, in which the guanine-linked cavitand is able to form a G-quartet. On the other hand, no self-assembly could be observed in DMSO.

[10] M. Mutter, P. Dumy, P. Garrouste, C. Lehman, M. Mathieu, C. Peggion, S. Peluso, A. Razaname, G. Tuchscherer, *Angew. Chem.* **1996**, *108*, 1588–1591; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1482–1485.

[11] a) P. Murat, D. Cressend, N. Spinelli, A. Van der Heyden, P. Labbé, P. Dumy, E. Defrancq, *ChemBioChem* **2008**, *9*, 2588–2591; b) P. Murat, R. Bonnet, A. Van der Heyden, N. Spinelli, P. Labbé, D. Monchaud, M.-P. Teulade-Fichou, P. Dumy, E. Defrancq, *Chem. Eur. J.* **2010**, *16*, 6106–6114.

[12] A system with a more flexible linker between the scaffold and the guanine units has been prepared. Unfortunately, we were unable to observe the formation of a G-quartet in water (see the Supporting Information for the structure of this compound). This emphasizes the unique role of the triazole linkers in the structure of the G-quartet.

[13] M. G. Stout, M. J. Robins, R. K. Olsen, R. K. Robins, *J. Med. Chem.* **1969**, *12*, 658–662.

[14] D. J. Patel, S. A. Kozlowski, A. Nordheim, A. Rich, *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 1413–1417.

[15] J. L. Sessler, M. Sathiosatham, K. Doerr, V. V. Lynch, K. A. Abboud, *Angew. Chem.* **2000**, *112*, 1356–1359; *Angew. Chem. Int. Ed.* **2000**, *39*, 1300–1303.

[16] a) S. L. Forman, J. C. Fettinger, S. Pieraccini, G. Gottarelli, J. T. Davis, *J. Am. Chem. Soc.* **2000**, *122*, 4060–4067; b) X. Liu, I. C. M. Kwan, S. Wang, G. Wu, *Org. Lett.* **2006**, *8*, 3685–3688.
[17] a) N. Borovok, T. Molotsky, J. Ghabboun, D. Porath, A. Kotlyar, *Anal. Biochem.* **2008**, *374*, 71–78; b) Z.-Y Kan, Y. Yao, P. Wang, X.-H Li, Y. Hao, Z. Tan, *Angew. Chem.* **2006**, *118*, 1659–1662; *Angew. Chem. Int. Ed.* **2006**, *45*, 1629–1632.
[18] a) A. Ambrus, D. Yang, *Anal. Biochem.* **2007**, *367*, 56–67; b) P. Groves, M. Webba da Silva, *Chem. Eur. J.* **2010**, *16*, 6451–6453.
[19] D. K. Wilkins, S. B. Grimshaw, V. Receveur, C. M. Dobson, J. A. Jones, L. J. Smith, *Biochemistry* **1999**, *38*, 16424–16431.
[20] Due to a mixture of two products (i.e., the monomer and the dimer), the complete structural determination of compound **4** was not possible. Some crucial NOE correlations, such as H8 and H1′ correlations, were observed. Thus, the dimer model was built by using the same NOE constraints as for **3**, except for the mentioned correlations. Some minimization steps (using VA09A conjugate gradient and steepest descent) were performed to place the two tetrads in a head-to-head position allowing a D_4 symmetry for the system.
[21] S. Masiero, R. Trotta, S. Pieraccini, S. De Tito, R. Perone, A. Randazzo, G. P. Spada, *Org. Biomol. Chem.* **2010**, *8*, 2683–2692.
[22] Further discussions on additional chromophores can be found in Sherman's works (see ref. [8]).
[23] The slight differences could be attributed to the ribose moiety. In fact, in compound **3** the ribose pucker is constrained due to the isopropylidene on the 2′,3′-hydroxyl groups, whereas in compound **4**, the sugar ring is more flexible.
[24] The example of the head-to-head dimer shown by A. Randazzo and co-workers (see ref. [22]) displays, in CD spectroscopy, two minima at 240 and 290 nm, respectively, and a maximum at 260 nm. In our case we observed a minimum at 240 nm and a maximum at 260 nm. The absence of minimum at 290 nm can be attributed to the presence of the triazole moiety, which absorbs light in this wavelength range.
[25] Note that during synthesis of the compounds, cations-containing buffers were used. Thus, it is reasonable to think that one cation is still present in the centre of the G-quartet, as previously reported.

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