ORGANIC LETTERS

2003
Vol. 5, No. 15
2627−2630

Novel Guanosine–Cytidine Dinucleoside that Self-Assembles into a Trimeric Supramolecule

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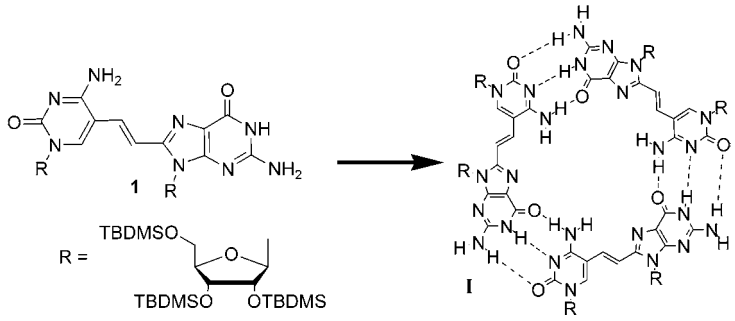
Received May 6, 2003

ABSTRACT



Synthesis and assembly studies of a guanosine–cytidine dinucleoside 1 that self-assembles into a trimeric supramolecule (I) are presented. Dinucleoside 1 was obtained by utilizing two consecutive palladium-catalyzed cross-coupling reactions. Ensemble I was analyzed by ESI-MS, NMR spectroscopies, size exclusion chromatography (SEC), and vapor pressure osmometry (VPO).

Engineering molecules that utilize noncovalent interactions such as hydrogen bonds, to form organized self-assembled structures, including linear, cyclic, and three-dimensional arrays, provides a timely challenge within the generalized field of supramolecular chemistry.[1] Part of the appeal of this area derives from the fact that noncovalent assemblies display unique properties such as reversible binding behavior that are otherwise unattainable by traditional covalent synthesis. They can also provide interesting models for various biological processes, including recognition, transport, and replication. Our group and others have focused on a biomimetic approach to self-assembly, i.e., modifying naturally occurring nucleoside analogues to form novel self-assembled structures.[2] Recently, we have shown that synthetic guanine analogues that bear a pendant aryl group off of the purine base can form tetrameric G-quartet structures without any stabilizing cations.[2a] Furthermore, we have previously shown that artificial dinucleosides containing rigid guanosine−cytidine subunits can be prepared and that these assemble to form cohesive dimers.[2b,c] Such guanosine−cytidine (DDA·AAD)

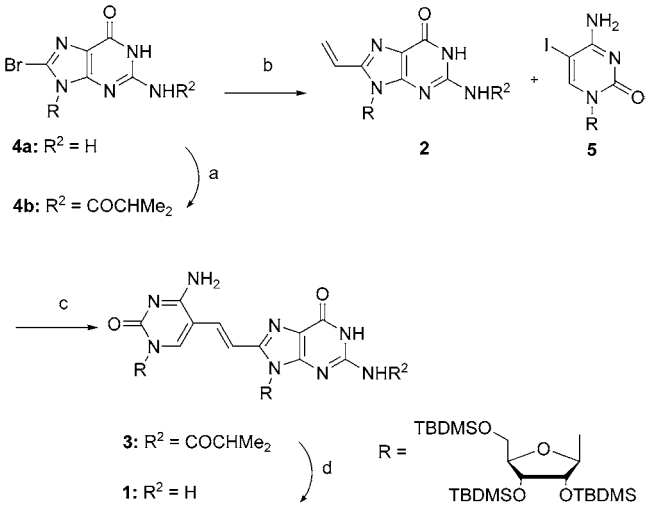
(1) For reviews, see: (a) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312−1319. (b) Lehn, J.-M. *Polym. Int.* **2002**, *51*, 825−839. (c) Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2383−2426. (d) Conn, M. M.; Rebek, J., Jr. *Chem. Rev.* **1997**, *97*, 1647−1668. (e) Fredericks, J. R.; Hamilton, A. D. *In Comprehensive Supramolecular Chemistry*; Atwood, J. L., Ed.; Pergamon: New York, 1996; Vol. 9, pp 565−594. (f) Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071−4097. For representative cyclic oligomers of particular interest, see: (g) Yang, J.; Fan, E.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 5314−5315. (h) Zafar, A.; Geib, S. J.; Hamuro, Y.; Hamilton, A. D. *New. J. Chem.* **1998**, 137−141. (i) Davis, J. T.; Tirumala, S. K.; Marlow, A. L. *J. Am. Chem. Soc.* **1997**, *119*, 5271−5272. (j) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060−4067. (k) Zimmerman, S. C.; Duerr, B. F. *J. Org. Chem.* **1992**, *57*, 2215−2217. (l) Sontjens, S. H. M.; Sijbesma, R. P.; van Genderen, M. H. P.; Meijer, E. W. *Macromolecules* **2001**, *34*, 3815−3818. (m) Mascal, M.; Hext, N. M.; Warmuth, R.; Moore, M. H.; Turkenburg, J. P. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2204−2206. (n) Boucher, E.; Simard, M.; Wuest, J. D. *J. Org. Chem.* **1995**, *60*, 1408−1412. (o) Zerkowski, J. A.; Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 5473−5475.

10.1021/ol034765y CCC: $25.00
Published on Web 06/27/2003

couples are attractive "building blocks" because they associate in only one well-defined orientation and are more stable than their two-point adenine−thymine counterparts. However, such systems are hard to make and manipulate. They are, for instance, only sparingly soluble in organic solvents. They are also highly sensitive toward many reaction conditions. Thus, considerable effort has been devoted to developing synthetic analogues of the natural GC hydrogen bonding motif and using them to assemble ingenious structures, including Janus-type molecules, self-assembling dendrimers, and helical rosette nanotubes.[3] However, such motifs have not been prepared using the natural nucleic acid bases ("nucleobases"). Here, we report the synthesis and assembly studies of a novel guanosine−cytidine dinucleoside **1** that aggregates via Watson−Crick hydrogen bonds to form a trimeric supramolecule (**I**).

The synthesis of **1** was achieved by employing two consecutive palladium-catalyzed cross-coupling reactions as shown in Scheme 1. The *tert*-butyldimethylsilyl (TBDMS)-protected 8-bromoguanosine **4a**[4] was converted to **4b** under standard protection protocols.[5] Compound **4b** was then subjected to cross-coupling with tributyl(vinyl)tin under Stille conditions. The vinyl derivative **2** was, in turn, subjected to a Herrmann palladacycle-assisted Heck olefination (palladium(II) acetate and tri(*o*-tolyl)phosphine)[6] with the appropriately protected 5-iodocytidine **5**.[7] This afforded the *trans*-guanosine−cytidine derivative **3**, which was then converted to **1** by stirring in a methanolic solution of ammonia for 24 h.

Scheme 1. Synthesis of Guanosine−Cytidine Dinucleoside **1**[a]



[a] Reaction conditions: (a) isobutyryl chloride, pyridine, 60%; (b) $Pd(PPh_3)_4$, tributyl(vinyl)tin, PhMe, 100 °C, 75%; (c) $Pd(OAc)_2$, tri(*o*-tolyl)phosphine, triethylamine, 90 °C, 77%; (d) NH_3, MeOH, CH_2Cl_2, 62%.

Initial indications of self-aggregation came as compound **1** was being purified. The dinucleoside possesses seven possible polar hydrogen bonding sites per molecule, which should make **1** insoluble in strongly apolar solvents. However, dinucleoside **1** is completely soluble in hexanes, whereas compound **3** (which has the exocyclic amino group of guanosine protected) is not. This observation not only provides an important, albeit qualitative, "hint" that self-assembly is taking place, it also suggests the notion that an ordered supramolecule is being formed. This is because aggregation into, e.g., tapelike polymers would necessarily expose vacant hydrogen bonding sites and lead to formation of an insoluble precipitate.[8] Independent of such considerations, to purify **1**, rather polar conditions had to be employed, i.e., column chromatography over silica gel utilizing 10% methanol in methylene chloride as the eluent. While not established rigorously, it is considered likely that methanol (a known H-bond disruptor) serves to break the aggregates, exposing the polar monomers.

Further evidence for self-assembly came from ^{1}H NMR spectroscopic studies carried out at room temperature in $CDCl_3$. In particular, the NH signal of the guanosine imino proton (**NH-1**) (Figure 1), exchangeable with CD_3OD, is seen to resonate at 13.82 ppm. This large downfield shift (about

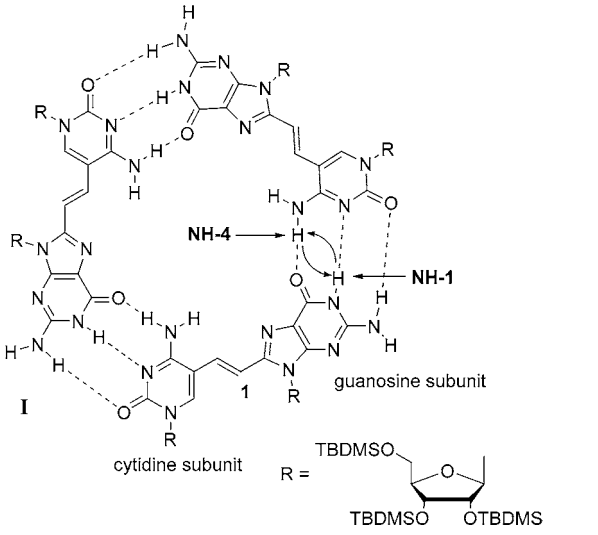


Figure 1. Self-assembly of **1** into trimeric species **I**.

(2) (a) Sessler, J. L.; Sathiosatham, M.; Doerr, K.; Lynch, V.; Abboud, K. A. *Angew. Chem., Int. Ed.* **2000**, *19*, 1300−1303. (b) Sessler, J. L.; Wang, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1726−1729. (c) Sessler, J. L.; Wang, R. *J. Org. Chem.* **1998**, *63*, 4079−4091. (d) Schall, O. F.; Gokel, G. W. *J. Am. Chem. Soc.* **1994**, *116*, 6089−6100. (e) Chen, H. C.; Ogo, S.; Fish, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 4993−5001. (f) Mezzina, E.; Mariana, P.; Itri, R.; Masiero, S.; Pieraccini, S.; Spada, G. S.; Spinozzi, F.; Davis. J. T.; Gottarelli, G. *Chem. Eur. J.* **2001**, *7*, 389−395. (g) Drain, C. M.; Fischer, R.; Nolen, E. G.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, 243−245.

(3) (a) Marsh, A.; Silvestri, M.; Lehn, J.-M. *Chem. Commun.* **1996**, 1527−1528. (b) Mascal, M.; Hext, N. M.; Warmuth, R.; Arnall-Culliford, J. R.; Moore, M. H.; Turkenburg, J. P. *J. Org. Chem.* **1999**, *64*, 8479−8484. (c) Ma, Y.; Kolotuchin, S. V.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2002**, *124*, 13757−13769. (d) Fenniri, H.; Mathivanan, P.; Vidale, K. L.; Sherman, D. M.; Hallenga, K.; Wood, K. V.; Stowell, J. G. *J. Am. Chem. Soc.* **2001**, *123*, 3854−3855. (e) Fenniri, H.; Deng, B.; Ribbe, A. E. *J. Am. Chem. Soc.* **2002**, *124*, 11064.

2 ppm downfield compared to the imino proton signals of reference compounds **4a**, **4b**, and **2**) is additionally insensitive to concentration in apolar solvents such as $CDCl_3$, a finding that is consistent with the guanosine subunit participating in strong hydrogen bonding interactions.

Yet additional support for the proposed self-assembly came from $^{15}N-^{1}H$ heteronuclear multiple quantum coherence (HMQC) experiments. Among other things, these showed that the exocyclic NH amino protons on the cytidine subunit are inequivalent: ^{1}H resonances at 9.86 and 5.68 ppm correspond to protons on the same nitrogen, implying slow $C-NH_2$ bond rotation on the NMR time scale. Such findings are consistent with the hypothesis that the cytidine subunit also participates in a hydrogen bonding process. Two-dimensional NOESY experiments revealed that the **NH-4** signal (at 9.86 ppm) does indeed correspond to the cytidine amino proton involved in the presumed hydrogen bonding interactions. Finally, two-dimensional NOESY experiments show strong cross-peaks between the guanosine imino proton (**NH-1**) and the cytidine amino proton (**NH-4**), indicating a close spatial arrangement between these two protons (cf. Figure 1 curved arrows and Figure 2). This is consistent with

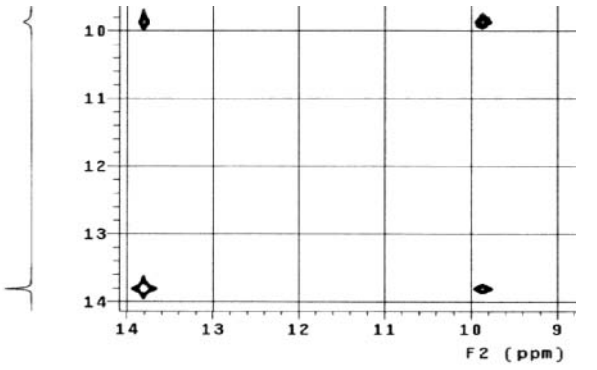


Figure 2. Portion of the two-dimensional NOESY spectrum of **1** (500 MHz, $CDCl_3$) showing strong cross-coupling of protons (**NH-1**) on guanosine at 13.82 ppm and (**NH-4**) on cytidine at 9.86 ppm.

the Watson−Crick base-pair between (as opposed to within) individual monomers of **1**.

Although NMR spectroscopic and solubility data fully support the conclusion that dinucleoside **1** forms aggregates through Watson−Crick hydrogen bonding interactions it does not provide insight into the size of the resulting species. Thus, an ESI-MS analysis of **1** was performed to elucidate the size of the self-assembling aggregates.[9] Due to mass range limitations, the only ascribable aggregates observed are those for doubly charged trimeric species, both with and without alkali metal adducts (ions corresponding to $[3\cdot\mathbf{1} + 2H]^{2+}$, $[3\cdot\mathbf{1} + H + Na]^{2+}$, $[3\cdot\mathbf{1} + H + K]^{2+}$, $[3\cdot\mathbf{1} + 2Na]^{2+}$, $[3\cdot\mathbf{1} + 2K]^{2+}$, $[3\cdot\mathbf{1} + Na + K]^{2+}$, and $[3\cdot\mathbf{1} + Na + MeOH]^{2+}$) as seen in Figure 3.

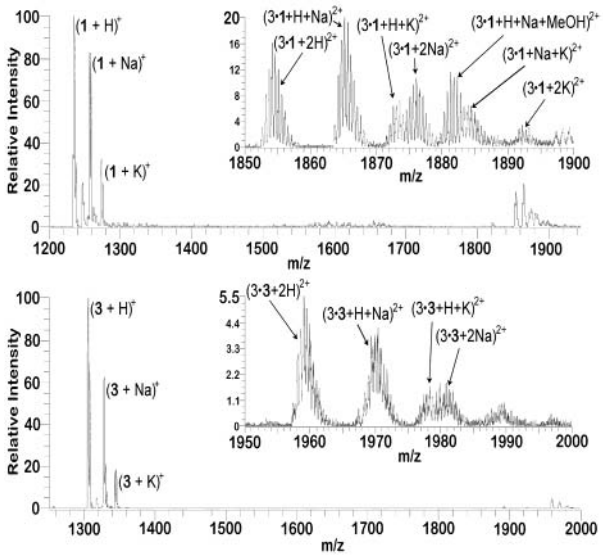


Figure 3. ESI mass spectra of **1** (top) and **3** (bottom), each at 50 μM in 10% $CHCl_3$/MeOH, demonstrating the presence of doubly charged trimer ions. Insets are higher resolution ZoomScans of the trimer ions.

No significant ions corresponding to multiply charged dimeric or higher order aggregates were observed.[10] The range of trimeric adducts present in the mass spectrum for **1** can be rationalized by the formation of a cyclic trimer (**I**), creating a cavity capable of binding both sodium and potassium that are present as contaminants. Although spectra of the control system **3** also show a few trimeric species, $[3\cdot\mathbf{3} + 2H]^{2+}$, $[3\cdot\mathbf{3} + H + Na]^{2+}$, $[3\cdot\mathbf{3} + 2\ Na]^{2+}$, and $[3\cdot\mathbf{3} + H + K]^{2+}$, the much lower intensities of these latter peaks relative to the monomer leads us to suggest that these ions probably result from nonspecific gas-phase interactions.[11] These experiments, however, clearly demonstrate that a trimeric species of **1** is present in the gas phase.

Size exclusion chromatography (SEC) retention studies were performed to verify the aggregate state in solution.[12] SEC traces of guanosine−cytidine derivative **1** in THF at millimolar concentrations exhibit one peak with a retention time of 17.30 min. On the basis of comparisons to polystyrene standards, this corresponds to a mean molecular weight (M_n) of 3740 daltons (which is close to the calculated value

(4) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron* **1997**, *53*, 3035−3045.
(5) Sessler, J. L.; Wang, B.; Harriman, A. *J. Am. Chem. Soc.* **1995**, *117*, 704−714.
(6) Herrmann, W. A.; Brossmer, C.; Ofele, K.; Reisinger, C.-P.; Priermeier, T.; Beller, M.; Fischer, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1844−1849.
(7) Bobek, M.; Kavai, I.; Sharma, R. A.; Grill, S.; Dutschman, G.; Cheng, Y.-C. *J. Med. Chem.* **1987**, *30*, 2154−2157.
(8) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 905−916.
(9) For review in the utilization of mass spectrometry for characterization of supramolecular aggregates, see: Schalley, C. A. *Mass. Spectrom. Rev.* **2001**, *20*, 253−309.
(10) Nonspecific higher order aggregegation in ESI may generate small ions between 1600 and 1700 TH in the top spectrum of Figure 3.

for a trimeric species of 3704 daltons). At these concentrations, the peak is sharp, as would be expected for a well-defined structure (see Figure 4, inset).

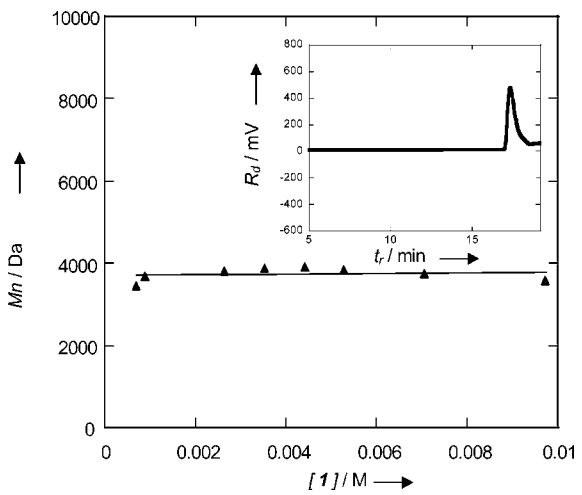


Figure 4. Change in the SEC-derived molecular weight (M_n) as a function of injected concentration. Inset: Sample SEC trace of **1** with an initial concentration of 3.53 mM in THF (t_r, retention time; R_d, detector response).

Although considered unlikely on the basis of the above observation, it is to be noted that if tapelike polymers were being formed, the SEC traces of **1** at higher concentrations would be expected to reveal a dramatic increase in effective molecular weight due to the resulting increased degree of polymerization.[13] However, in our case, concentration-independent SEC traces are seen over the 0.001 < [**1**] < 0.01 M concentration range in THF, all of which correspond to a constant molecular weight of around 3740 daltons. We thus take this as prima facie evidence for the formation of a discrete, self-assembled, cyclic trimer,[3c] specifically the one represented by supramolecular structure **I**.

Although structure **I** dominates at higher concentrations, it must be noted that we could effect the deaggregation of the trimer under high dilution conditions. Specifically, as the 0.001−0.01 M initial concentration is decreased to the micromolar level, a broad peak with a large tail is visible, indicative of deaggregation on the SEC column.[1g] Furthermore, the M_n for this species is around 2200, consistent with a species that contains, at least in part, uncomplexed monomers and dimeric aggregates.

Vapor pressure osmometry studies of **1** at 30 °C in dichloromethane over the concentration range 0.002−0.01 M gave a mean molecular weight for the aggregate of 3881 (when sucrose octaacetate was used as the standard), which is slightly higher than the calculated value for the trimer (3704). This minor mismatch most likely reflects the notoriously poor precision of this method, although it could indicate the additional presence of some higher order aggregates such as, e.g., a cyclic or acyclic tetramer. Importantly, VPO analysis of **3** revealed an M_n of 1393, which is consistent with the presence of a monomer only.

This work demonstrates the possibility of using a biomimetic approach to assemble discrete trimeric, cyclic supramolecules. Such structures are potentially useful in the construction of self-assembled dendrimers and other nanostructures. Further studies of incorporating such dinucleosides into ribo- and deoxyribonucleic acids to form new materials are currently in progress.

Acknowledgment. This work was supported by the Robert A. Welch Foundation (Grant F-1018 to J.L.S and Grant F-1155 to J.S.B.). We thank Dr. Steve Sorey at the University of Texas NMR facility for his assistance and Joe Reczek for helpful discussions.

Supporting Information Available: Complete synthetic experimental, ^{1}H NMR two-dimensional NOESY, ^{15}N−^{1}H NMR HMQC, SEC studies, VPO analysis, and ESI-MS studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Control **3** was specifically chosen because, by protecting the exocyclic amine with an isobutyrylamide group, there is the possibility of forming an intramolecular hydrogen bond with the carbonyl moeity of the amide and the guanosine imino proton (**NH-1**). In this conformation, the protecting group not only eliminates the availability of two of the three Watson−Crick H-bonding sites of guanosine but also introduces steric crowding. Thus, it is unlikely that **3** will form Watson−Crick H-bonds in this conformation. However, if the intramolecluar H-bond is disrupted, then control compound **3** could form Watson−Crick H-bonds (using the amide NH as an alternative to the regular amino NH of guanosine) and hence possibly form a cyclic trimer. However, all the solution-phase studies are consistent with **3** being present as a monomer. In addition, it has been previously shown that the use of this protecting group serves to block Watson−Crick hydrogen bonding interactions (see: Sessler, J. L.; Sathiosatham, M.; Brown, C. T.; Rhodes, T. A.; Wiederrecht, G. *J. Am. Chem. Soc.* **2001**, *123*, 3655−3660). Thus, the trimeric species observed in the mass spectra most likely reflects nonspecific gas-phase interactions, rather than a discrete ensemble such as **I**.

(12) (a) Zimmerman, S. C.; Zeng, F.; Reichert, D. E. C.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095−1098. (b) Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. *Acc. Chem. Res.* **1995**, *28*, 37−44.

(13) Michelson, U.; Hunter, C. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 764−767.