ORGANIC LETTERS

2009
Vol. 11, No. 17
3918−3921

Metal-Ion-Induced Folding and Dimerization of a Glycoluril Decamer in Water

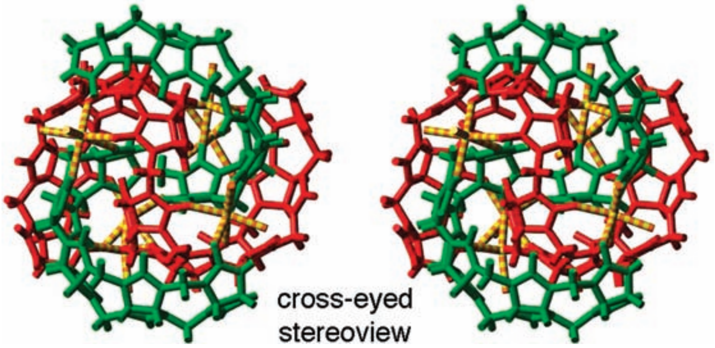
Wei-Hao Huang, Peter Y. Zavalij, and Lyle Isaacs*

Department of Chemistry and Biochemistry, University of Maryland,
College Park, Maryland 20742

LIsaacs@umd.edu

Received July 6, 2009

ABSTRACT



In a process reminiscent of RNA folding, acyclic glycoluril decamer (±)-1 undergoes double helical assembly (red/green strands) in water triggered by the neutralization of regions of high electrostatic surface potential by metal cations (Li^+, Na^+, K^+, Ca^{2+}).

The supramolecular chemistry of the cucurbituril family of molecular containers has undergone rapid expansion following the availability of cucurbit[*n*]uril (CB[*n*], $n = 5, 6, 7, 8, 10$) homologues.[1,2] These efforts have resulted in a number of practical applications including supramolecular vesicles, novel stationary phases, enzyme assays, molecular electronics, and drug delivery.[3] These applications are enabled by the remarkable binding features of CB[*n*] molecular containers toward their guests in water, which includes tight binding (K_a up to 10^{15} M^{-1}), high selectivity (K_{rel} up to 10^7), and stimuli responsiveness (e.g., pH, photochemistry, electrochemistry).[4] In this regard, CB[*n*] displays properties more

---

(1) (a) Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 4844–4870. (b) Lee, J. W.; Samal, S.; Selvapalam, N.; Kim, H.-J.; Kim, K. *Acc. Chem. Res.* **2003**, *36*, 621–630.

(2) (a) Freeman, W. A.; Mock, W. L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1981**, *103*, 7367–7368. (b) Kim, J.; Jung, I. S.; Kim, S.-Y.; Lee, E.; Kang, J.-K.; Sakamoto, S.; Yamaguchi, K.; Kim, K. *J. Am. Chem. Soc.* **2000**, *122*, 540–541. (c) Day, A. I.; Arnold, A. P.; Blanch, R. J.; Snushall, B. *J. Org. Chem.* **2001**, *66*, 8094–8100. (d) Day, A. I.; Blanch, R. J.; Arnold, A. P.; Lorenzo, S.; Lewis, G. R.; Dance, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 275–277. (e) Liu, S.; Zavalij, P. Y.; Isaacs, L. *J. Am. Chem. Soc.* **2005**, *127*, 16798–16799.

(3) (a) Lee, H.-K.; Park, K. M.; Jeon, Y. J.; Kim, D.; Oh, D. H.; Kim, H. S.; Park, C. K.; Kim, K. *J. Am. Chem. Soc.* **2005**, *127*, 5006–5007. (b) Henning, A.; Bakirci, H.; Nau, W. M. *Nat. Methods* **2007**, *4*, 629–632. (c) Wheate, N. J.; Buck, D. P.; Day, A. I.; Collins, J. G. *Dalton Trans.* **2006**, 451–458. (d) Saleh, N.; Koner, A. L.; Nau, W. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 5398–5401. (e) Miyahara, Y.; Abe, K.; Inazu, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 3020–3023. (f) Nagarajan, E. R.; Oh, D. H.; Selvapalam, N.; Ko, Y. H.; Park, K. M.; Kim, K. *Tetrahedron Lett.* **2006**, *47*, 2073–2075. (g) Eelkema, R.; Maeda, K.; Odell, B.; Anderson, H. L. *J. Am. Chem. Soc.* **2007**, *129*, 12384–12385. (h) Liu, Y.; Shi, J.; Chen, Y.; Ke, C.-F. *Angew. Chem., Int. Ed.* **2008**, *47*, 7293–7296.

(4) (a) Liu, S.; Ruspic, C.; Mukhopadhyay, P.; Chakrabarti, S.; Zavalij, P. Y.; Isaacs, L. *J. Am. Chem. Soc.* **2005**, *127*, 15959–15967. (b) Rekharsky, M. V.; Mori, T.; Yang, C.; Ko, Y. H.; Selvapalam, N.; Kim, H.; Sobransingh, D.; Kaifer, A. E.; Liu, S.; Isaacs, L.; Chen, W.; Moghaddam, S.; Gilson, M. K.; Kim, K.; Inoue, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 20737–20742. (c) Mock, W. L.; Pierpont, J. *J. Chem. Soc., Chem. Commun.* **1990**, 1509–1511. (d) Zou, D.; Andersson, S.; Zhang, R.; Sun, S.; Åkermark, B.; Sun, L. *J. Org. Chem.* **2008**, *73*, 3775–3783. (e) Wang, W.; Kaifer, A. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 7042–7046. (f) Jeon, W. S.; Kim, E.; Ko, Y. H.; Hwang, I.; Lee, J. W.; Kim, S.-Y.; Kim, H.-J.; Kim, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 87–91.

10.1021/ol901539q CCC: $40.75

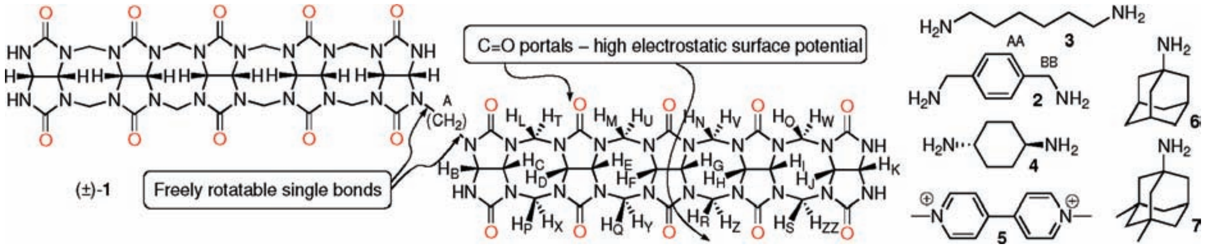


Figure 1. Structure of compounds used in this study.

typically associated with proteins than with synthetic receptors. Herein we demonstrate that members of the CB[*n*] family are truly privileged receptors that also display properties reminiscent of RNAs.

For example, RNA folding is well-known to require metal ions to stabilize the tertiary structure.[5] RNA dimerization is known to play an important role in the replication of HIV-1.[6] RNAs that sense cellular ligands (e.g., *S*-adenosylmethonine, Mg^{2+}, glucosamine-6-phosphate) resulting in a conformational change that regulates gene expression—riboswitches—have been the subject of intense investigation.[7] This paper explores the supramolecular chemistry of (±)-**1** (Figure 1), an acyclic glycoluril decamer comprising two glycoluril pentamers connected by a single CH_2 bridge, which undergoes a metal-ion-mediated folding and dimerization process that is responsive to the presence of ligands in the form of CB[*n*] guests.

Although the condensation of glycoluril (1 equiv) with formaldehyde (2 equiv) delivers CB[*n*] in high yield, reaction mixtures that are starved of formaldehyde deliver C-shaped glycoluril oligomers and *nor-seco*-CB[*n*].[8,9] From a reaction mixture comprising glycoluril (1 equiv) and formaldehyde (1.67 equiv) we isolated (±)-**1** by ion-exchange chromatography.[10] Structural elucidation was challenging. Analysis of the ESI-MS spectrum of (±)-**1** provided a molecular formula ($C_{57}H_{60}N_{40}O_{20}$) that along with the singlet (2H, 4.50 ppm) for H_A in its ^1H NMR spectrum in 20% DCl (Figure 2a) suggested the overall C_2 symmetry of (±)-**1**. Even more intriguing was the ^1H NMR spectrum recorded for solutions containing Na_2SO_4 (Figure 2c), which shows widely dispersed and sharp resonances. Of particular interest were the dramatically upfield resonances for two diastereotopic CH_2 groups at ~3.1, 5.05, and 5.25 ppm and four CH groups at 4.4, 4.7, 4.95, and 5.15 ppm. The COSY spectrum showed coupling between pairs of these upfield shifted protons, which suggested that a folding and assembly process occurred that placed these protons within the shielding region of the cavity

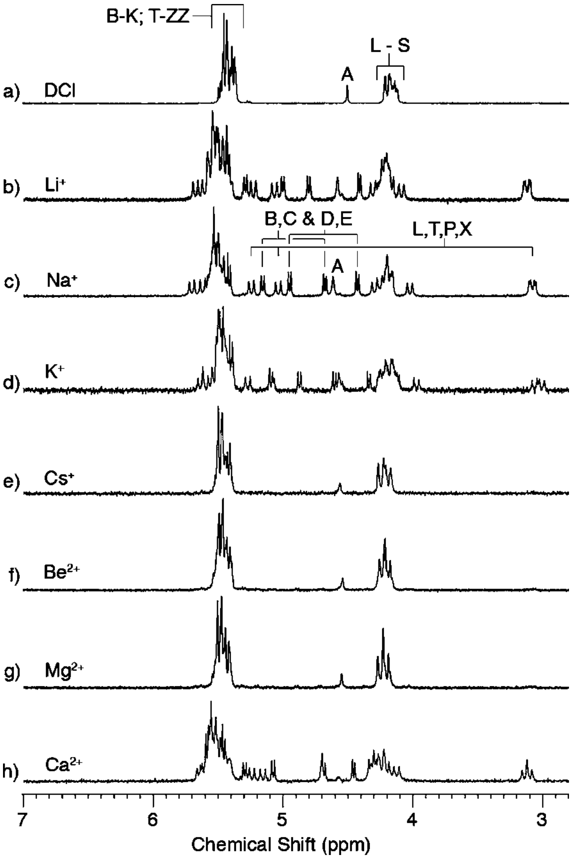


Figure 2. ^1H NMR spectra (400 MHz, 20% DCl, rt) recorded for mixtures of **1** (7.25 mM) and metal sulfate salts (100 mM): (a) no metal salt, (b) Li_2SO_4, (c) Na_2SO_4, (d) K_2SO_4, (e) Cs_2SO_4, (f) $BeSO_4$, (g) $MgSO_4$, and (h) $CaSO_4$.

---

(5) (a) Thirumalai, D.; Hyeon, C. *Biochemistry* **2005**, *44*, 4957–4970. (b) Chu, V. B.; Herschlag, D. *Curr. Opin. Struct. Biol.* **2008**, *18*, 305–314. (c) Woodson, S. A. *Curr. Opin. Chem. Biol.* **2005**, *9*, 104–109.

(6) Paillart, J.-C.; Shehu-Xhilaga, M.; Marquet, R.; Mak, J. *Nat. Rev. Microbiol.* **2004**, *2*, 461–472.

(7) (a) Breaker, R. R. *Science* **2008**, *319*, 1795–1797. (b) Montange, R. K.; Batey, R. T. *Nature* **2006**, *441*, 1172–1175. (c) Dann, C. E.; Wakeman, C. A.; Sieling, C. L.; Baker, S. C.; Irnov, I.; Winkler, W. C. *Cell* **2007**, *130*, 878–892.

(8) In IUPAC nomenclature, the prefix *nor* denotes the removal of a CH_2 group from a parent structure with formation of a new bond between the affected termini. The prefix *seco* refers to the breaking of a bond within a parent structure with addition of H atoms to the structure. Therefore, the combined prefix *nor-seco* refers to the deletion of a CH_2 group with addition of H atoms. See: Giles, P. M. *Pure Appl. Chem.* **1999**, *71*, 587–643.

(9) (a) Huang, W.-H.; Liu, S.; Zavalij, P. Y.; Isaacs, L. *J. Am. Chem. Soc.* **2006**, *128*, 14744–14745. (b) Huang, W.-H.; Zavalij, P. Y.; Isaacs, L. *Angew. Chem., Int. Ed.* **2007**, *46*, 7425–7427. (c) Huang, W.-H.; Zavalij, P. Y.; Isaacs, L. *Org. Lett.* **2008**, *10*, 2577–2580. (d) Huang, W.-H.; Zavalij, P. Y.; Isaacs, L. *J. Am. Chem. Soc.* **2008**, *130*, 8446–8454.

(10) Connection of 2 equiv of glycoluril pentamer by a single CH_2 bridge could theoretically also lead to an achiral *meso*-form. We did not observe this compound in the crude reaction mixture or during the purification of (±)-**1**.

of **1** (Supporting Information). Unfortunately, the structure could not be solved by NMR methods alone.

After some effort, we obtained X-ray quality crystals of this supramolecular structure and were able to solve its structure (Figure 3a). In this structure, two molecules of (±)-**1** of opposite handedness (red and green strands) assemble to give the heterochiral dimer (+)-**1**·(−)-**1**·(Na$^+$)$_6$. The two helices are screwed together at right angles to one another. As required on the basis of the observed upfield shifting in the ^{1}H NMR spectrum, two CH$_2$ groups (H$_L$, H$_T$, H$_P$, H$_X$) and four adjacent CH groups (H$_B$−H$_E$) define a contiguous region (highlighted in aqua) that is embraced by the opposing equivalent of **1**.[11] The van der Waals contacts between these regions provides a portion of the thermodynamic driving force for folding and assembly.[12] More critical, however, is the role played by six Na$^+$ ions (Supporting Information). Two of these Na$^+$ ions are coordinated to two adjacent ureidyl C═O groups of **1**, two adjacent ureidyl C═O groups of *ent*-**1**, and two water molecules. The other four Na$^+$ ions occur as two O-bridged disodium pairs; these disodium pairs are coordinated to three ureidyl C═O groups of **1** (one bifurcated), two adjacent ureidyl C═O groups of *ent*-**1** (one bifurcated), one bridging oxygen, and three waters.[13] In analogy to RNA folding, these Na$^+$ ions neutralize regions of high negative electrostatic potential that develop during the folding and assembly of (+)-**1**·(−)-**1**·(Na$^+$)$_6$.[14] Figure 3b shows the electrostatic potential surface (EPS) for a single strand of **1**, whereas Figure 3c shows the EPS for the dimer (+)-**1**·(−)-**1** extracted from the X-ray structure of (+)-**1**·(−)-**1**·(Na$^+$)$_6$. The labeled regions of greatly increased EPS (Figure 3b versus 3c) correspond to the Na$^+$ binding sites observed in the X-ray structure (Figure 3a). We confirmed the relevance of the C═O···Na$^+$ ion coordination observed in the crystal structure toward the solution structure by titrating a solution of (±)-**1** with Na$_2$SO$_4$ and observed a transition from free (±)-**1** to (+)-**1**·(−)-**1**·(Na$^+$)$_6$ with the addition of six Na$^+$ ions (Supporting Information) as expected. No dissociation of (+)-**1**·(−)-**1**·(Na$^+$)$_6$ was observed during dilution from 5 to 0.2 mM, which confirms the strength of this assembly.[15,16]

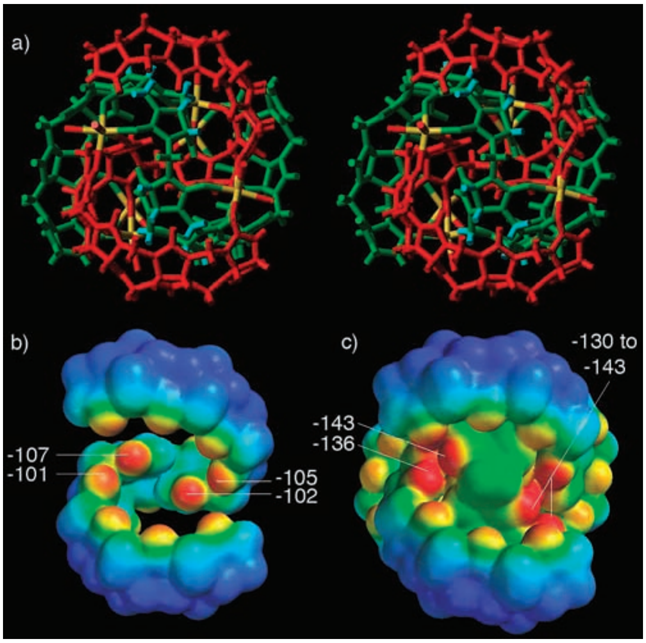


Figure 3. (a) Stereoview of (+)-**1**·(−)-**1**·(Na$^+$)$_6$ in the crystal. Color code: **1**, red; *ent*-**1**, green; Na, yellow; upfield shifted H atoms, aqua. Electrostatic potential surface plots (PM3) for (b) one strand of **1** and (c) dimer (+)-**1**·(−)-**1** excised from the crystal structure of (+)-**1**·(−)-**1**·(Na$^+$)$_6$ with Na$^+$ ions removed. The red to blue color range spans −108 to +35 kcal mol^{-1}.

Once we had determined the geometry of the (+)-**1**·(−)-**1**·(Na$^+$)$_6$ assembly and assigned its overall strength to a combination of specific ion coordination and van der Waals contacts, we decided to assess the influence of the nature of the metal ion on the assembly process. Figure 2b−e shows the ^{1}H NMR spectra recorded in the presence of an excess of Li$^+$, Na$^+$, K$^+$, and Cs$^+$. On the basis of the similar pattern of induced chemical shift changes, we are confident that smaller metal cations (Li$^+$, Na$^+$, and K$^+$) form structurally similar assemblies. In sharp contrast, the ^{1}H NMR spectrum in the presence of the larger cation Cs$^+$ (Figure 2e) is quite similar to that of free (±)-**1** (Figure 2a). The converse situation is observed for Be^{2+}, Mg^{2+}, and Ca^{2+} where assembly is only observed for the larger Ca^{2+} (Figure 2f−h). The lack of a clear trend based on cation size or charge suggests that specific coordination effects, energies of hydration, and the interplay between electrostatics and van der Waals interactions play critical roles in this system.

To further establish the linkage between the recognition properties of natural RNA (e.g., riboswitches) and those of acyclic glycoluril oligomer (±)-**1**, we decided to investigate the stimuli responsiveness of (+)-**1**·(−)-**1**·(Na$^+$)$_6$ toward the addition of cationic guests. We first investigated the ability of (±)-**1** to form complexes with **2**−**7** by ^{1}H NMR spectroscopy (Supporting Information). In all cases we observed broadening and/or upfield shifts of the resonances of the guests, which indicates cavity binding. The upfield shifts observed were more pronounced for smaller guests **2** and **3**,

---

[11] Unfortunately, we have not been able to obtain ROESY or ESI-MS results that would further corroborate the depicted dimerization process. Given the match between the number of upfield-shifted protons and the magnitude of the observed upfield shifts in solution with that predicted on the basis of the X-ray structure of (+)-**1**·(−)-**1**·(Na$^+$)$_6$ (Figure 3a), we are confident that dimerization also occurs in solution.

[12] We base this assertion on the high affinity interaction between CB[5] and CB[10] in the CB[10]·CB[5] complex.[2d]

[13] A similar disodium cluster was observed in the X-ray structure of lidded-CB[6] by Kim and co-workers. See: Jeon, Y. M.; Kim, J.; Whang, D.; Kim, K. *J. Am. Chem. Soc.* **1996**, *118*, 9790–9791.

[14] Further evidence of free rotation around the central N-CH$_2$-N bridge of free (±)-**1** comes from an examination of a second crystal structure of (±)-**1** reported in Supporting Information, which shows columnar stacks of **1** in a figure-eight type geometry.

[15] The binding of CB[6] toward Na$^+$ ions has been studied by Buschmann and co-workers (K_a = 1.7 × 10^3 M^{-1}). See: Buschmann, H.-J.; Jansen, K.; Meschke, C.; Schollmeyer, E. *J. Solution Chem.* **1998**, *27*, 135–140.

[16] The group of Fedin has extensively studied the assembly of CB[*n*] in the solid state based on ureidyl C═O···metal interactions. See: Gerasko, O. A.; Sokolov, M. N.; Fedin, V. P. *Pure Appl. Chem.* **2004**, *76*, 1633–1646.

which suggests a good fit between these guests and each of the cavities shaped by a glycoluril pentamer. Perhaps not surprisingly given the acyclic structure of (±)-**1**,[9d] none of these complexes exhibit slow exchange on the ^{1}H NMR time scale. Figure 4a shows the ^{1}H NMR spectrum recorded for (±)-**1** after addition of Na_2SO_4 to trigger assembly of (+)-**1**•(−)-**1**•$(Na^+)_6$ in D_2O. Addition of a competitive ligand for (±)-**1**, namely, **2**, triggers the disassembly of (+)-**1**•(−)-**1**•$(Na^+)_6$ (Figure 4b). Finally, we added CB[7], which is known to bind tightly to **2** ($K_a = 1.8 \times 10^9$ M^{-1}),[4a] which largely sequestered **2** as its CB[7]•**2** complex and restored the (+)-**1**•(−)-**1**•$(Na^+)_6$ complex (Figure 4c). In this manner, the folding and assembly of (±)-**1** can be seen as analogous to regulation of RNA structure in response to cellular ligands.

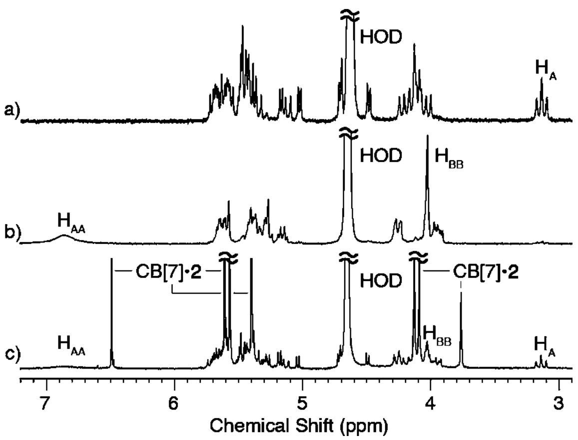


Figure 4. ^{1}H NMR recorded (400 MHz, D_2O, 100 mM Na_2SO_4) for (a) (+)-**1**•(−)-**1**•$(Na^+)_6$ (1 mM), (b) after addition of **2** (5 mM), and (c) after addition of CB[7] (5 mM).

In summary, we have reported the isolation of acyclic glycoluril decamer (±)-**1** and delineated its ability to participate in processes reminiscent of RNA structure and function. For example, (±)-**1** undergoes heterochiral folding and dimerization (e.g., (+)-**1**•(−)-**1**•$(Na^+)_6$) triggered by the addition of alkali and alkaline earth cations, which neutralize regions of high negative electrostatic potential during assembly. Triggered disassembly of (+)-**1**•(−)-**1**•$(Na^+)_6$ occurs by the addition of complexing ligands (e.g., **2**); this process can even be reversed by the addition of CB[7]. When combined with previous studies that delineated that CB[*n*] achieves affinity and selectivity characteristic of proteins,[4a,b] the RNA mimetic processes described here suggest that the CB[*n*] family is a truly privileged class of receptors for basic and applied molecular recognition in water. For example, acyclic glycoluril oligomers[9d] could be particularly powerful building blocks for the discovery of ligands for metal ions by dynamic combinatorial chemistry. Furthermore, we expect that elaborated derivatives of **1** will perform well in fluorescence sensing and imaging of metal ions in aqueous environments.[7c]

Acknowledgment. We thank the National Science Foundation (CHE-0615049 and CHE-0914745) and the University of Maryland (Wylie fellowship to W.-H.H. and GRB semester award to L.I.) for financial support.

Supporting Information Available: Synthesis and characterization data for (±)-**1**, ^{1}H NMR spectra of host•guest complexes of (±)-**1**, ^{1}H NMR titration and dilution experiments for (+)-**1**•(−)-**1**•$(Na^+)_6$, and supporting crystallographic tables and figures. Crystallographic information files for (±)-**1** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

OL901539Q