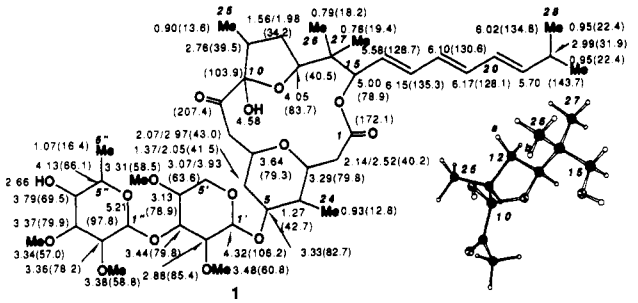

Figure 1. Structure and NMR assignment of polycavernoside A (**1**) and the stereo structure of a cyclohemiketal model corresponding to C8–C15 of **1** deduced from MM2. ^{1}H NMR chemical shifts and ^{13}C NMR chemical shifts (in parentheses) are those in CD_3CN.

MHz)[4] between H12a/Me26, H13/Me27, H15/Me26, and H16/Me27. The connectivities of C1/C2 and C8/C9 were further supported by the chemical shifts of H_2-2 (δ 2.14, 2.52) and H_2-8 (δ 2.07, 2.97) typical for methylenes α to carbonyl. The deuterium-exchangeable signals at δ 4.58 and 2.66 in the ^{1}H NMR spectrum (CD_3CN) were assigned to 10-OH and 4″-OH, respectively, on the basis of the cross peaks due to $^2J_{CH}$ between C10/10-OH and C4″/4″-OH in HMBC spectra. The connectivities of two remaining quaternary carbons, C9 and C10, were deduced from the NOE between H8b/H11.[4] The adjacent carbonyl (C9) caused a significant downfield shift of 10-OH (δ 4.58) by an anisotropic effect and formation of a hydrogen bond. The ether linkage between C3/C7 was evident from the NOE between H3/H7. The remaining hemiketal carbon (C10) and an oxycarbon (C13) were linked to form a tetrahydrofuran ring; $^3J_{HH}$ of H11–H13 agreed with those expected from MM2 energy calculations.[5,6]

The glycosidic residue, *O*-2,3-di-*O*-methylfucopyranosyl-(1″–3′)-*O*-2,4-di-*O*-methylxylopyranosyl-(1′–5), was deduced from the cross peaks in HMBC spectra due to $^3J_{CH}$ between C2′/OMe2′, C4′/OMe4′, C2″/OMe2″, C3″/OMe3″, C5/H1′, and C3′/H1″, those in NOESYs (400 and 600 MHz) due to NOEs between H1′/H3′, H1′/H5a′, H5/H1′, and H3′/H1″, and from $^3J_{HH}$ of H1′–H5′ and H1″–H6″. The positive FABMS supported this struture by showing prominent fragment ions (*m/z* 651, 633, 491, and 473) corresponding to sequential loss of each residue.

The above results led to **1** as the planar structure of polycavernoside A and allowed assignment of all ^{1}H and ^{13}C signals (Figure 1). The carbon backbone of **1**, a 3,5,7,13,15-pentahydroxy-9,10-dioxotricosanoic acid, is a new molecular entity. A smaller macrocycle, a trioxatridecane, is reminescent of the aplysiatoxins, which contain trioxadodecane.[7] The similarity of observed symptoms in experimental animals and human patients supports the belief that **1** and **2** caused the intoxication.[8] Algal toxicity likely was much higher in April than in June, as indicated by a rapid decrease of toxicity in samples collected afterward. Although the unique molecular structure of the aglycone offers no hint, the methylated fucose of **1** suggests its algal origin. The sudden and transient occurrence of the toxins in the alga remains unexplained, but may provide a clue to previous outbreaks of fatal food poisoning caused by two other *Gracilaria*, *G. chorda*[9] and *G. verrucosa*.[10,11]

Acknowledgment. We thank Drs. M. Murata, Tohoku University, and M. Isobe, Nagoya University, for discussions; S. Matsunaga, Tokyo University, for measuring 600-MHz NOESY; and P. J. Scheuer, University of Hawaii, for reading the manuscript. This work was supported by a grant from the Ministry of Education, Science, and Culture, Japan.

Supplementary Material Available: Table of ^{13}C and ^{1}H NMR assignments and ^{1}H and ^{13}C NMR, 2D HOHAHA, ^{1}H–^{1}H COSY, HMQC, HMBC, 2D *J*, ROESY, and NOESY spectra of **1** (14 pages). Ordering information is given on any current masthead page.

(4) NOESY spectra were recorded at 270 MHz (200 ms) at 27 °C in CD_3CN (positive and negative NOEs), at 400 MHz (200 ms) at −27 °C, and at 600 MHz (700 ms) at 27 °C in C_5D_5N (all negative NOEs). The ROESY spectrum was recorded at 400 MHz (200 ms) at 27 °C in CD_3CN. NOE difference spectra were measured at 400 MHz at −27 °C in C_5D_5N.

(5) MM2 energy calculations done on a cyclohemiketal model (Figure 1) constructed on the basis of the observed NOE (H8b/H11), $^3J_{H11/H\text{-}12a}$ (11.6 Hz), and $^3J_{H\text{-}12a/H13}$ (11.6 Hz) led to dihedral angles of H11/H12a, 165.7°; H11/H12b, 44.6°; H12a/H13, 161.0°; and H12b/H13, 38.2° for a stable conformer. Coupling constants of H11/H12a, H11/H12b, H12a/H13, and H12b/H13, calculated by the modified Karplus equation,[6] were 12.1, 5.6, 11.4, and 4.1, respectively, and agreed with the observed values (11.6, 6.7, 11.6, and 5.1 Hz).

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Addition of Azides to C_{60}: Synthesis of Azafulleroids

M. Prato,† Q. Chan Li, and F. Wudl*

Institute for Polymers and Organic Solids
Department of Chemistry and Physics
University of California at Santa Barbara
Santa Barbara, California 93106

V. Lucchini

Dipartimento di Scienze Ambientali
Universita di Venezia, Dorsoduro 2137
30133 Venezia, Italy

Received October 5, 1991

Although macroscopic amounts of buckminsterfullerene C_{60}[1] are becoming increasingly accessible through easier and more economic methods of synthesis,[2] the functionalization of fullerenes is not yet a trivial task due to the multifunctionality of C_{60} which usually results in the formation of numerous inseparable products.[3] We have recently demonstrated that stable, pure fulleroids can be obtained by allowing C_{60} to react with substituted diazomethanes.[4] In this communication, we report our preliminary results on the reaction of C_{60} with organic azides[5] which provides an excellent method for the preparation of "azafulleroids".

Refluxing an equimolar solution of C_{60} and [(trimethylsilyl)-ethoxy]methyl azide ($SEMN_3$) (**1a**) in chlorobenzene overnight produced two major products (24% and 30%, respectively, based on C_{60} conversion), which were purified by column chromatography (silica gel, mixtures of hexanes/toluene). A more polar compound (**A**, see structures below) was relatively stable at room temperature, but was transformed to a less polar product when heated in refluxing chlorobenzene for a few hours or for a few

† On leave from CMRO-CNR and Department of Organic Chemistry, University of Padova, Italy.

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(5) A very preliminary account of C_{60}'s reactivity toward *p*-nitrophenyl azide has appeared in ref 3.

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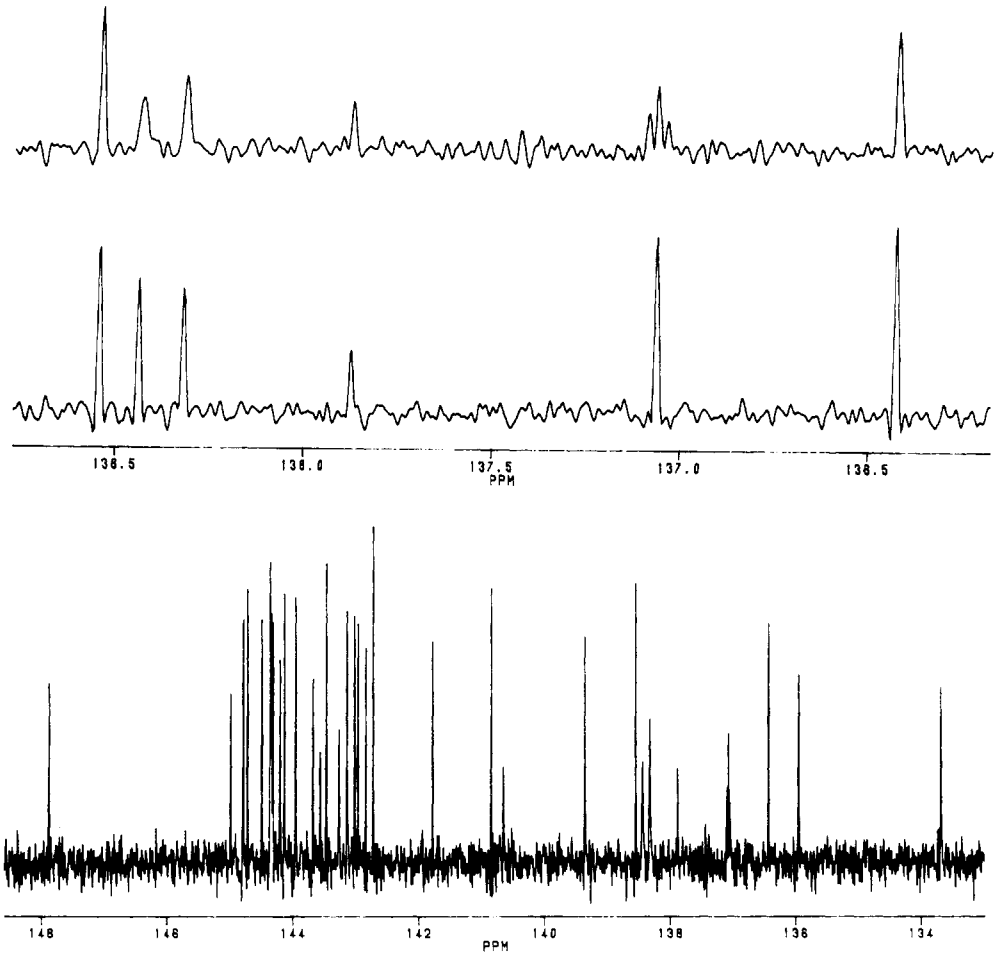
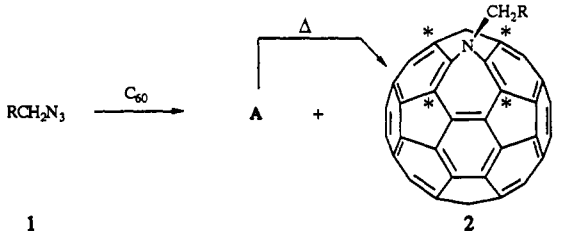


Figure 1. Fulleroid ("aromatic") region of the ^{13}C NMR spectrum of **2a** (bottom). The expanded section (136.4–138.6 ppm) is a comparison of the 50% ^{15}N-labeled sample (top) with the unlabeled sample. Note the "triplet" at 137.06 ppm in the labeled section.

minutes in the solid state at 180 °C.

Although the same reactivity with formation of two products could be observed with the substituted benzyl azides **1b–d**, in this communication we emphasize the SEM azide reaction. The stable product was assigned the azafulleroid structure **2a** on the basis of a combination of ^{13}C and ^{15}N NMR spectroscopy (see below).



1 **2**

a, R = $OCH_2CH_2SiMe_3$

b, R = C_6H_5

c, R = 4-C_6H_4-OMe

d, R = 4-C_6H_4-Br

The azafulleroids (**2a–d**) exhibit the typical electrochemical properties and electronic spectroscopy of fulleroids. Compounds **2** show four reversible reduction waves in their cyclic voltammograms (**2a**: E1, −275; E2, −854; E3, −1404; E4, −1898 mV. C_{60}: E1, −238; E2, −838; E3, −1418; E4, −1921 mV vs Ag/AgCl/3 M NaCl. Pt working and counter electrodes; see supplementary material for **2b–d**), and their UV–vis spectra are very similar to that of C_{60}. The FAB-MS of compounds **2** show the typical M^+ cluster with loss of the alkyl or benzyl moiety to give $[C_{60}N]^+$ and C_{60}^+. The ^1H NMR spectrum of the alkyl substituent is very similar to that of the starting azides with a small downfield shift due to the influence of the carbon sphere.[6] The ^{13}C NMR ($CDCl_3$, TMS) of **2a** exhibits four aliphatic carbons at 83.25, 66.84, 18.17, and −1.19 ppm as well as 32 peaks in the aromatic region between 148 and 133 ppm (Figure 1). Of the 32, 28 signals have a relative intensity of 2, and 4 signals have a relative intensity of 1. The total integrated area for the aromatic region sums up to 60 carbons. Because all of the fullerene carbons are in the sp^2 region of the spectrum, compounds **2** *must possess the open annulene structure*, rather than the aziridine structure. Symmetry arguments support the following possibilities: (i) a 6,5 junction on the fullerene with free pyramidal inversion at nitrogen; (ii) a 6,5 junction on the fullerene with a "frozen", single N-invertomer; and (iii) a 6,6 junction on the fullerene with a "frozen" N-invertomer.

In the case of 1,6-methylimino[10]annulene,[7] the methyl group freely inverts down to −80 °C. Also, the ^1H spectrum of compound **2a** does not show, down to −65 °C, any hint of "freezing". Hence, the observed fast inversion at N at ambient temperature supports hypothesis i.

In order to obtain further information on the structure of azafulleroids, ^{15}N-labeled $SEMN_3$ was prepared from SEMCl and a $Na^{15}NN_2$. Because the labeling on the azide is 50% at N-1 and 50% at N-3, addition to fullerene and loss of nitrogen yield 50% labeling at the azafulleroid nitrogen. ^{15}N NMR spectroscopy gave one peak at 73.92 ppm (referenced to external liquid ammonia). The aromatic ^{13}C resonance at 137.06 ppm (relative intensity of 2) is split by ^{15}N–^{13}C coupling with J = 5.0 Hz (Figure 1). The methylene resonance at 83.25 ppm is similarly split (J = 7.6 Hz) and also subject to a slight isotope shift (δ 83.23). These

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are the resonances of the carbon atoms directly bonded to nitrogen. The two aromatic resonances at δ 138.43 and 138.32 (each with an integration of 2) are broadened and are attributable to the starred 5- and 6-membered-ring carbon atoms (see **2**, structure). Efforts to clarify the structure of **A**[8] are underway.

We have shown above that azafulleroids and not fullerene aziridines are formed as the ultimate product of addition of a number of organic azides to C_{60}. A combination of ^{15}N and ^{13}C NMR was employed to elucidate the structure. The azafulleroids are more electronegative than their carbon analogs but not as electronegative as unsubstituted C_{60}, as determined by cyclic voltammetry.

Acknowledgment. We thank the National Science Foundation for support through Grants DMR-88-20933, DMR-91-11097, and CHE-89-08323.

Supplementary Material Available: Spectroscopic data (IR, UV–vis, FAB-MS, and ^1H NMR) for azide **1a** and azafulleroids **2a–d**, ^{13}C NMR data for **2a**, and a table of cyclic voltammetry data (4 pages). Ordering information is given on any current masthead page.

(8) The ^{13}C NMR spectrum shows a resonance as low as 159.8 ppm coupled to ^{15}N which is clearly incompatible with a triazoline, the structure which was expected from the normal mode of 1,3-dipolar addition of an alkyl azide with C_{60}.

Sequence-Specific DNA Binding by a Geometrically Constrained Peptide Dimer

Takashi Morii,*,† Masatoshi Simomura, Shinji Morimoto, and Isao Saito

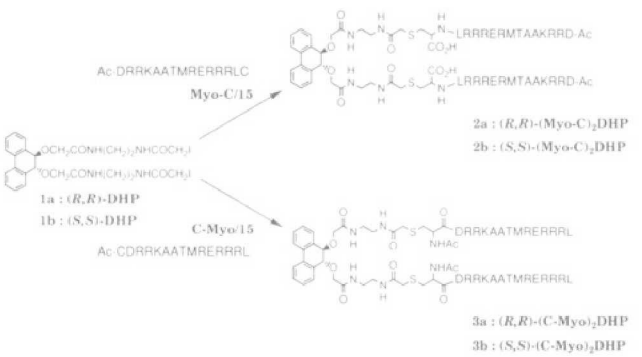
Department of Synthetic Chemistry
Faculty of Engineering, Kyoto University
Sakyo-ku, Kyoto 606, Japan
Received August 24, 1992

Recent structural and functional analyses of eukaryotic transcriptional regulatory proteins have indicated that the sequence-specific DNA recognition activities lie in the structural motifs containing a relatively small number of amino acid residues, such as helix–turn–helix,[1–3] leucine zipper,[4–6] at least three types of zinc fingers,[7,8] and helix–loop–helix (HLH).[9,10] At the same time, these studies have revealed a common feature of the sequence-specific DNA-binding proteins. That is, many DNA-binding proteins bind DNA as dimers. In the native dimeric proteins, the chemical structure of the dimerization motif determines the geometry of each monomer subunit. This constrained positioning of the DNA-binding regions would facilitate the direct interaction between amino acid residues of the protein and the nucleic acid base pairs.

We describe a system to represent such constrained arrangements of DNA-binding motifs by using enantiomeric and C_2-symmetric templates derived from 9,10-dihydrophenanthrene-9,10-diol ((*R*,*R*)-DHP and (*S*,*S*)-DHP). In the simplest case, there are four constraints possible for dimeric peptide motifs, right-handed or left-handed arrangement of each peptide, and two orientations for the polarity of the peptides (an N-terminus to N-terminus or C-terminus to C-terminus arrangement). Four differently constrained dimeric peptides are synthesized by using oligopeptides derived from the HLH protein, MyoD. It is shown that a dimer with right-handed and C-terminus to C-terminus arrangements of the peptide binds specifically to the native MyoD binding site.

The chiral templates (*R*,*R*)- and (*S*,*S*)-DHP were synthesized from enantiomers of *trans*-9,10-dihydrophenanthrene-9,10-diol with well-defined absolute configurations at C-9 and C-10.[11] The (9*R*,10*R*) isomer was used to achieve the right-handed geometry

Scheme I



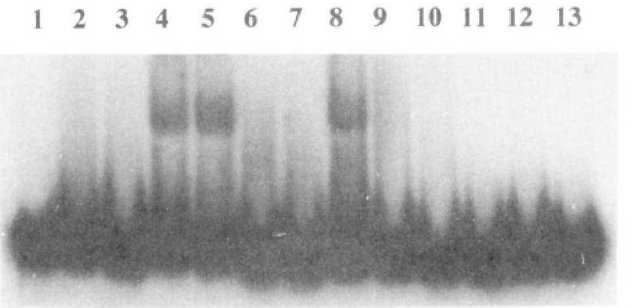


Figure 1. DNA binding of dimeric peptide models to the oligonucleotide containing the MyoD binding sequence. Gel mobility shift assays (14) indicate that (*R*,*R*)-(Myo-C)$_2$DHP shows an affinity to MCK probe. Lane 1, no peptides; lanes 2, 6, and 10, 2.5 μM (*R*,*R*)-(C-Myo)$_2$DHP; lanes 3, 7, and 11, 2.5 μM (*S*,*S*)-(C-Myo)$_2$DHP; lanes 4, 8, and 12, 2.5 μM (*R*,*R*)-(Myo-C)$_2$DHP; lanes 5, 9, and 13, 2.5 μM (*S*,*S*)-(Myo-C)$_2$DHP. The concentration of the dimeric peptide was determined with an extinction coefficient at 270 nm (ϵ_{270}) of 17 000 M^{-1} cm^{-1}. Nonspecific competitor DNA (calf thymus DNA) was added to the binding mixture such that the final concentration is 20 (lanes 1–5) or 40 μM (lanes 6–9). A specific competitor (non-radiolabeled MCK25) was added to the binding mixture to a final concentration of 500 nM (duplex) (lanes 10–13). Binding solutions contained in 10 μL: 20 mM Tris (pH 7.6), 25 mM NaCl, 10 000 cpm (~0.01 pmol) 5′-^{32}P-labeled MCK25 (double stranded), and 2.5 μM dimeric peptides when present. Non-radiolabeled duplex MCK25 was added where indicated. The binding mixtures were incubated at 4 °C for 30 min. After addition of 1 μL of 40% glycerol, the mixtures were loaded onto 10% PAGE gel (29:1 acrylamide/bisacrylamide), run in TAE buffer (7 mM Tris, 3 mM sodium acetate, and 1 mM EDTA) at 4 °C, and analyzed by autoradiography.

* Author to whom correspondence should be addressed.
† Present address: Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan.

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