in the presence of hydroquinone at 25 °C for 24 h cyclized to 14 via the biradical 13 in 50% yield.[23]

Thus these results demonstrate that our synthetic analogues 4 and 5 undergo transannular ring closure to produce 12 and 14, respectively, in the same manner as naturally occurring NCS-chr and esperamicin-calichemicin. Moreover MM2 calculations are useful in designing the synthetic intermediate 6 to construct the highly strained bicyclo[7.3.0] enediyne 2.

(23) 1,4-Hydroquinone was added as the radical quencher. We could not detect the benzenoid adduct 15 in the absence of hydroquinone.

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Supplementary Material Available: Compound characterization data, experimental procedures, discussion of conformational analysis, and copies of spectra (20 pages). Ordering information is given on any current masthead page.

Conformation of DNA-Bound Spermidine by Double ^{13}C Labeling

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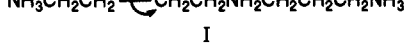
Summary: Double-^{13}C-labeling experiments show conclusively that the central bond of the C_4 unit in spermidine adopts an anti configuration when bound to various types of DNA. Double-^{13}C-labeling is a powerful though laborious method of securing conformational information on biomolecules in aqueous media.

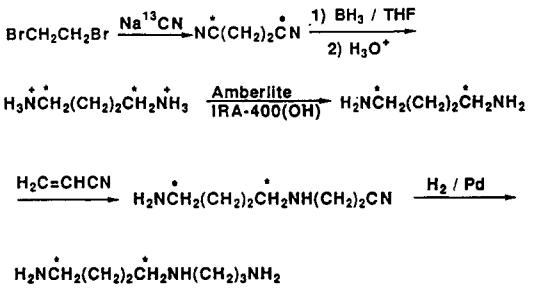
Several years ago we began studying the conformations of flexible molecules by double-^{13}C-labeling.[1] The method is based on the long-range NMR coupling ($^3J_{cc}$) in acyclic compounds that possess two ^{13}C atoms spaced four atoms apart (*C-C-C-C*). These couplings respond to the dihedral relationship between the labeled carbons in a typical Karplus fashion.[2] Although compounds with two ^{13}C atoms can be tedious to synthesize, they provide anti-gauche ratios at explicit sites, information that is difficult to obtain by other means. The method has already been applied to hydrocarbon chains dissolved in diverse solvents, to succinic acid derivatives at varying pH, to an enzyme-inhibitor complex, and to surfactant tails embedded in micellar aggregates.[1] We describe herein the rotamer population of double-^{13}C-labeled spermidine (I) bound to DNA. The results foreshadow applications to macromolecular chemistry in general.



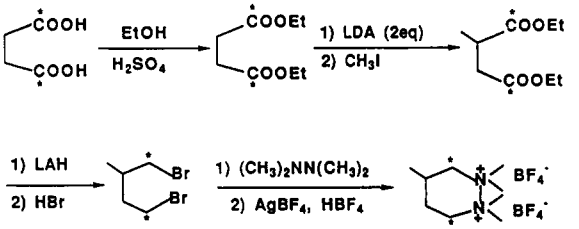
I

Spermidine belongs to a group of naturally occurring polyamines known to complex with DNA.[3] Interest in such compounds stems from their surge in concentration just prior to the synthesis phase of cellular proliferation.[4]

Scheme I



Scheme II



It has been suggested that potential regulatory functions of the polyamines may involve electrostatic association to the DNA phosphates as well as specific binding to certain DNA sequences.[5] At present, however, only limited information is available on the role of spermidine in modulating gene expression related to cell growth.

A crystal structure of spermine, $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, complexed with B-DNA shows that the tetramine is stretched across the major groove while interacting with phosphates and a guanine base.[6] The central bond of the spermine C_4 unit is gauche. On the other hand, an X-ray structure of a spermine complex with A-DNA reveals an anti central bond.[5] X-ray pictures of spermidine/DNA complexes are unavailable. Previously reported NMR chemical shift data suggested tentatively that spermidine, associated with 5'-AMP, adopts a gauche conformation within its C_4 unit.[7,8] We establish below that

(1) Menger, F. M.; Carnahan, D. W. *J. Am. Chem. Soc.* **1986**, *108*, 1297. Menger, F. M.; Dulany, M. A.; Carnahan, D. W.; Lee, L. H. *J. Am. Chem. Soc.* **1987**, *109*, 6899. Menger, F. M.; Lee, L. H. *Tetrahedron Lett.* **1988**, *29*, 757. Menger, F. M.; D'Angelo, L. L. *J. Am. Chem. Soc.* **1988**, *110*, 8241.

(2) Marshall, J. L. *Carbon-Carbon and Carbon-Proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis*; Verlag Chemie: Deerfield Beach, FL, 1983. Barfield, M.; Burfitt, I.; Doddrell, D. *J. Am. Chem. Soc.* **1975**, *97*, 2631.

(3) Ganem, B. *Acc. Chem. Res.* **1982**, *15*, 290. Goldemberg, S. H., Algranati, I. D., Eds. *The Biology and Chemistry of Polyamines*; IRL Press: Oxford, 1990.

(4) Tabor, C. W.; Tabor, H. *Ann. Rev. Biochem.* **1984**, *53*, 749.

(5) Jain, S.; Zon, G.; Sundaralingam, M. *Biochemistry* **1989**, *28*, 2360.

(6) Drew, H. R.; Dickerson, R. E. *J. Mol. Biol.* **1981**, *151*, 535.

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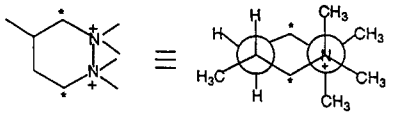
I prefers an anti configuration when bound to several nucleic acids.

Double-^{13}C-labeled spermidine (Scheme I) at pD = 2–7, where it is fully protonated, gave $^3J_{cc}$ = 5.9 Hz.[9] We assume, partly on the basis of MACROMODEL calculations,[10] that 5.9 Hz reflects an average value heavily weighted toward the anti conformation. In order to obtain $^3J_{cc}$ for a pure gauche relationship, we synthesized the double-^{13}C-labeled heterocycle II (Scheme II) in which a ring structure enforces a gauche disposition upon the isotopic carbons. Compound II provided $^3J_{cc}$ (gauche) = 4.3 Hz,



II

a value that probably represents an upper limit for gauche interactions in acyclic compounds where there exists only a single "coupling route" between carbons.[11] The minimum 1.6-Hz difference between anti and gauche allowed for easy NMR discrimination between the two states.

In a typical experiment, 0.2 mM 98% isotopically labeled spermidine was mixed with a 25-fold base-pair excess of DNA (0.1 M phosphate buffer in D_2O, pD = 7.0, 0.1 M NaCl, 25 °C).[12] A low concentration of I was required to avoid precipitate formation, while large amounts of DNA were needed to bind all the spermidine and assure that free spermidine contributed nothing to the couplings. Total binding of I to DNA was demonstrated by observing a 0.7-ppm upfield shift of the labeled carbons in the presence of excess DNA. A plot of chemical shift vs [DNA] reached "saturation" (i.e. no further change) at only a 5-fold base-pair excess of calf thymus DNA.[13] NMR spectra were recorded on a 500-MHz instrument (3600-Hz spectral width and 15 000 aquisitions). Use of a 32-phase INADEQUATE pulse sequence[14] suppressed all signals except the two doublets from the labeled spermidine.

Six forms of DNA, given here with their classifications and excess over I, were investigated:[15] calf thymus (random, 53×); poly(dA)·poly(dT) (duplex, 30×); poly(dA)·poly(dT)$_{12-18}$ and poly(dC)·poly(dG)$_{12-18}$ (template primers, 12×); poly(dA-dT)·poly(dA-dT), and poly(dG-dC)·poly(dG-dC) (alternating copolymers, 20×). Calf thymus (5–45 °C) and other DNA's (25 °C) all gave observed coupling constants of 6.0 ± 0.1 Hz. This establishes conclusively that, as in free solution, the 4-carbon segment of spermidine prefers to be predominantly anti when associated with the nucleic acids. Note that our experiments cannot, of course, differentiate between (a) an anti spermidine fixed rigidly to a discrete DNA binding site and (b) a mobile and flexible spermidine whose configuration averaged over many binding sites is heavily weighted toward anti.[16] Although spermidine may dance and cartwheel across the DNA surface, it certainly does *not* do so while contorted in a gauche conformation. In this regard, the behavior differs from the spermine/B-DNA complex where multiple negative charges stabilize the gauche form.

Docking calculations[17] were carried out on spermidine in the major and minor grooves of the oligonucleotide, poly(dG-dC)$_{10}$. It was found that spermidine prefers the minor groove by 6 kcal/mol and that, in agreement with the NMR data, the triamine adopts therein an anti configuration. Our computations have a measure of uncertainty owing, in part, to their neglect of solvent. Double-^{13}C-labeling, on the other hand, pertains to aqueous media where biomolecules normally reside. Only the need to synthesize the dilabeled compounds, admittedly an arduous task, tarnishes an otherwise simple and informative method applicable to a variety of biochemical problems.

Acknowledgment. This work was supported by the National Institutes of Health.

(7) Bunce, S.; Knong, E. S. W. *Biophys. Chem.* **1978**, *8*, 357.
(8) The tentative conclusion of Bunce and Knong[7] disagrees with an anti conformation evident from our coupling data on mixtures of I and a 55-fold excess of 5′-AMP.
(9) In organic solvents ($CDCl_3$) and aqueous base (pD = 13), $^3J_{CC}$ for the unprotonated triamine falls between 4.0 and 4.4 Hz. Molecular mechanics calculations show that this corresponds to about 31% gauche and 69% anti.
(10) W. Clark Still, Columbia University.
(11) Both $^3J_{cc}$ (anti) = 5.9 Hz and $^3J_{cc}$ (gauche) = 4.3 Hz are about 2.3 Hz larger than those observed and calculated[1,2] for simple hydrocarbons; we presume the difference arises from an inductive effect of the cationic nitrogens.
(12) Spermidine is tricationic under these conditions. See pK_a values in Kimberly, M. M.; Goldstein, J. H. *Anal. Chem.* **1981**, *53*, 789.
(13) The DNA/spermidine association constant is reported to be 10^6 M^{-1}. Rubin, R. L. *J. Bacteriol.* **1977**, *129*, 916. Assuming this value, it can be calculated that under our conditions the spermidine is >99% bound to DNA.
(14) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* **1980**, *102*, 4849.
(15) Nucleic acids were purchased from Pharmacia.
(16) Wemmer, D. E.; Srivenugopal, K. S.; Reid, B. R.; Morris, D. R. *J. Mol. Biol.* **1985**, *185*, 457. These authors discuss the uncertainty with regard to spermidine/DNA complexes. Relatively sharp spermidine line widths in our case suggest substantial mobility.
(17) The united-atom AMBER force field as implemented in the MACROMODEL program[10] was used in the calculations (rms set to 0.2 kcal mol^{-1} $Å^{-1}$). Calculations were performed with the aid of an Evans and Sutherland PS 390 workstation interfaced to a Micro-Vax II.

A Tritiated Anion Radical

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Summary: The first tritiated anion radical (that of monotritiated [8]annulene) has been prepared and its EPR spectrum recorded.

Since its original observation, ^{3}H NMR is routinely studied without ^{1}H interference by selective tuning of the probe to the Larmor frequency of ^{3}H.[1-2] However, EPR spectroscopists have not been able to make use of tritiated

(1) (a) Prestwich, G. D. *Chemtracts: Org. Chem.* **1988**, *2*, 182. (b) Williams, P. G. *Fusion Technol.* **1988** *14*(2, Pt. 2A), 840. (c) Saljoughian, M.; Williams, P. G. *J. Org. Chem.* **1987**, *52*, 3481.

0022-3263/91/1956-3468$02.50/0