Table I. Comparison of Charge-Induced Chemical Shifts and Hyperfine Splittings in Aromatic Dianions and Radical Anions

Compound	Position (r)[a]	δ (dianion), ppm[b]	$\Delta\delta_r$, ppm[c]	$\lvert a_r^H\rvert$, G[d]
Anthracene (1)	1	3.36	4.61	2.74
	2	4.25	3.18	1.51
	9	1.89	6.56	5.34
Tetracene (2)	1	4.46	3.54	1.54
	2	4.85	2.65	1.16
	9	3.00	5.63	4.23
Acenaphthylene (3)	1	4.50	3.08	4.51
	2	5.04	2.46	0.45
	3	3.33	4.37	5.64
	7	4.46	2.59	3.09
Fluoranthene (4)	1	4.81	2.97	3.91
	2	5.04	2.47	0.15
	3	3.27	4.63	5.28
	7	6.89	0.95	0.08
	8	6.06	1.25	1.21
Perylene (5)	1	4.99	3.28	3.09
	2	5.93	1.52	0.46
	3	4.87	2.83	3.55

[a] See Figure 1 for numbering systems. [b] All chemical shifts were determined relative to the low-field multiplet of THF and related to internal TMS by assuming a value of δ 3.60 ppm for the solvent peak. [c] $\Delta\delta_r = \delta_r$(neutral) $- \delta_r$(dianion). For analysis of nmr of parent hydrocarbons see B. P. Dailey, S. Gordon, and N. Jonathan, *J. Chem. Phys.*, **36**, 2443 (1962), for **1** and **5**; R. H. Martin, N. Defay, F. Geerts-evrard, and S. Delavarenne, *Tetrahedron* 1073 (1964), for **2**; and M. J. S. Dewar and R. C. Fahey, *J. Amer. Chem. Soc.*, **85**, 2704 (1963), for **3**. [d] $\lvert a_r^H\rvert$ = absolute value of hyperfine splitting at position r in radical anion. Large values have negative signs, and values less than 0.5 G are probably positive. Hyperfine splitting data were taken from the following references: G. K. Fraenkel, and J. R. Bolton, *J. Chem. Phys.*, **40**, 3307 (1964), for **1**; G. K. Fraenkel, M. Kaplan, and B. G. Segal, *ibid.*, **43**, 4191 (1965), for **2** and **4**; F. Gerson, *Helv. Chim. Acta*, **49**, 1837 (1966), for **3**; and A. Carrington, F. Dravnieks, and M. C. R. Symons, *J. Chem. Soc.*, 947, (1959), for **5**.

q_r, at a trigonally hybridized position, r, in conjugated ions has been generally assumed[4] to be related by eq 1 to the charge-induced chemical shift, $\Delta\delta_r$, of a proton attached to that carbon. The constant, K, is usually taken to be approximately 10 ppm per unit

$$\Delta\delta_r = -Kq_r \qquad (1)$$

charge. The data in Table I indicate, however, that this value of K is much too low to explain the large upfield shifts observed in most of the dianions reported here. For example, the sums of charge-induced chemical shifts over all positions bearing protons for dianions **1** through **5** are 44.3, 47.3, 25.0, 24.5, and 30.5 ppm, respectively. Inasmuch as the *maximum* value of q_r in the dianions summed over all positions is -2, the *minimum* value of K which will fit these data, assuming that no carbon atom in the dianion bears a formal positive charge, ranges from 12 to 23 ppm per electron. The upper limit is approximately twice the generally accepted value obtained empirically from the nmr spectra of monocyclic polyene ions.[4a-d] Although the lower limit is in better agreement, it carries the unrealistic implication that the proton-free positions of **3** and **4** bear no negative charge. Inclusion of a term quadratic in q_r in eq 1, as has been suggested,[5] only leads to a still larger value of K. Furthermore, the probability that the dianions are present as ion triplets or higher aggregates would also be expected to have an effect on chemical shifts which is opposite to that observed.[4a,c]

Whether the apparent high sensitivity of chemical shifts to charge in the polycyclic dianions is actually the result of additional shielding effects such as anomalous paramagnetic ring current contributions or shielding from charge on adjacent atoms[4e] must await detailed theoretical analysis of the results. It is clear, however, that despite the good correlation obtained for monocyclic ions,[4] eq 1 must be used with caution in "evaluating" charge densities.

If one simply *assumes* proportionality between chemical shifts and π-charge densities in the dianions reported here, a comparison with esr hyperfine splittings in the corresponding radical anions indicates a qualitatively more uniform electron distribution in the dianions (see Table I). The effect is especially apparent at positions which have a small spin density in the radical anion but exhibit a substantial upfield shift of the proton resonance in the dianion. This observation, based on a comparison of two empirical quantities, is consistent with the apparent necessity for explicit consideration of electron repulsion effects in calculating π-electron densities in benzylic anions[4c,6] and azulene dianion.[4c]

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The Structure of Nucleocidin. III (a New Structure)

Sir:

Nucleocidin, an antitrypanosomal antibiotic, was first isolated[1a] and described[1b] in 1957. Partial structure **1** was proposed[2] based on an empirical formula of $C_{11}H_{16}N_6SO_8$ which had been derived from ir and uv spectra, elemental analysis, and chemical degradation studies. This structure was recently revised to 9-(4-O-sulfamoylpentofuranosyl)adenine[3a] ($C_{10}H_{14}N_6SO_7$) on the basis of additional chemical and spectroscopic evidence.

New spectroscopic evidence, including 100-MHz ^1H nmr, ^{19}F nmr, and mass spectral data, has now provided

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(2) C. W. Waller, J. B. Patrick, W. Fulmor, and W. E. Meyer, *J. Am. Chem. Soc.*, **79**, 1011 (1957).

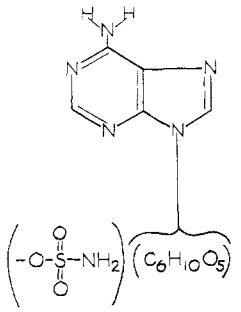
(3) (a) J. B. Patrick and W. E. Meyer, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, MEDI-024; (b) J. B. Patrick, private communication (previous analysis of the 60-MHz spectrum had suggested that hindered rotation was the cause of the doubling in the C_3-H and the C_5-methylene).

Table I

Assignment	Chemical shift,[a] pyridine-d_5	Coupling constant[b]	Chemical shift,[a] DMSO-d_6	Coupling constant[b]
$C_{1'}H$	6.99 (d)	$J_{1'2'} = 2.2$	6.25 (d)	
$C_{2'}H$	5.31 (m)	$J_{2'3'} = 6.5$ $J_{1'2'} = 2.2$	4.64 (m)	$J_{2'OH} = 5$
$C_{3'}H$	5.61 (m)	$J_{3'2'} = 6.5$ $J_{3'F} = 18.0$	4.72 (m)	
$C_{5'}H$	5.08 (d)	$J_{5'F} = J_{5''F} = 9$	4.20 (m)	$J_{5'5''} = \sim 11$ $J_{5'F} = \sim 9.6$ $J_{5''F} = \sim 12.6$
$C_{2'}OH$	6.50		5.87 (d)	$J_{2'OH} = 5$
$C_{3'}OH$	6.50		5.50 (d)	$J_{3'OH} = 7$
C_2H	8.54 (s)		8.14 (s)	
C_8H	8.54 (s)		8.30 (s)	
C_4NH_2	8.26 (s)		7.32 (s)	
$C_{5'}OSO_2NH_2$	9.5 (br)		7.63 (s)	

[a] Shifts are given in parts per million from TMS as an internal standard. [b] J values are in Hz, read from first-order splittings; d, doublet; m, multiplet; s, singlet; br, broad.

a further revised formula of $C_{10}H_{13}N_6SO_6F$ and a new proposed structure, 9-(4-fluoro-5-O-sulfamoylpentofuranosyl)adenine (**2** or a stereoisomer). This compound to our knowledge is the first fluoro sugar derivative isolated from any source and one of the very few naturally occurring fluoro compounds.[4]



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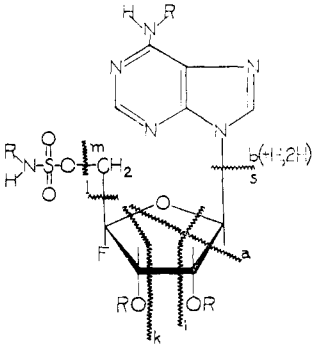

2 R = H
3 R = TMS

The nucleocidin ^1H nmr spectra at 60 and 100 MHz and the ^{19}F nmr spectrum at 56.4 MHz were obtained using dry DMSO-d_6 and pyridine-d_5 as solvents. Comparison of the 60- and 100-MHz ^1H nmr spectra (pyridine-d_5) clearly established that the additional lines in the $C_{3'}H$ and the $C_{5'}$-methylene portions of the spectrum were due to coupling constants rather than to hindered rotation.[3b] Thus, in addition to the expected H–H couplings, $C_{3'}H$ and the methylene protons at $C_{5'}$ displayed an extra splitting of 18.0 and 9.0 Hz, respectively, which were shown to be spin–spin couplings to fluorine by observation of the ^{19}F nmr spectrum. The ^{19}F nmr spectrum showed a doublet of triplets centered at +119.6 ppm (referenced to $CFCl_3$) and the doublet and triplet coupling constants agreed with the $C_{3'}H$ and the $C_{5'}$-methylene proton splittings in the ^1H nmr spectrum.

The coupling constant of 18 Hz between $C_{3'}H$ and $C_{4'}F$ is consistent with published values for vicinal H–F coupling.[5,6] The $C_{4'}F$ and the $C_{5'}$-methylene protons form an AA'X system with an apparent coupling of 9 Hz for the H–F portion of the system (pyridine-d_5). These deceptively simple patterns have been analyzed in many other spectra.[7] The observed splitting represents $^1/_2(J_{AX} + J_{A'X})$ and was measured as 9.0 Hz. In DMSO-d_6, the $C_{5'}$-methylene protons had slightly different chemical shifts so that the type of system became ABX with $J_{AB} = 11.0$ Hz, $J_{AX} \cong 9.6$ Hz, and $J_{BX} \cong 12.6$ Hz. It was not possible to check these values in the ^{19}F nmr due to excessive broadening of the resonance line. We note that $^1/_2(J_{AX} + J_{BX}) = 11.1$ Hz and does not agree with the 9.0-Hz value found in pyridine-d_5, which indicates that the H–F couplings are probably solvent dependent.[8] The solvent dependency of the $C_{3'}H$ and $C_{4'}F$ coupling could not be checked due to the unfortunate overlap of the $C_{3'}H$ and $C_{2'}H$ resonances in DMSO-d_6. However, the very dry DMSO-d_6 solution clearly revealed that there were only two hydroxyl groups in nucleocidin ($C_{2'}OH$ and $C_{3'}OH$).

The ^1H nmr spectrum of nucleocidin shows ten distinct groups of signals. All protons have been identified using the combination of 60- and 100-MHz proton spectra and homonuclear spin decoupling at 100 MHz, thus thoroughly establishing the assignments in Table I.

The mass spectrum of the tetra(trimethylsilyl) (TMS) derivative of nucleocidin provided a molecular ion at *m/e* 652 and a prominent M − 15 at *m/e* 637. Accurate mass data provided by computer analysis (Table II) confirmed the presence of fluorine in the antibiotic. Consideration of the data and structure **3** readily provides an explanation of the fragmentation pattern. Of particular interest is the fragment ion s which established both the sulfamyloxy and the fluorine as being part of the sugar moiety. In addition, the presence of the fragmentation ion m and the absence of fragment ion l is strong evidence for the presence of the sulfamyloxy at $C_{5'}$. McCloskey, *et al.*,[9] have published a report of mass spectral studies of nucleosides

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and nucleotides in which they describe a fragment ion in the mass spectrum of 5′-AMP(TMS)$_5$ analogous to m and presumably due to the loss of an unusually stable radical formed by the silylated phosphate moiety. Conversely, fragment l is a characteristic fragment in the mass spectra of TMS derivatives of furanose nucleosides but does not occur in the mass spectrum of 5′-AMP-(TMS)$_5$, nor was a fragment ion analogous to l present in our studies of the mass spectrum of nucleocidin (TMS)$_4$.

Table II

Ion	Composition	Obsd mass	Calcd mass
M^+	$C_{22}H_{45}N_6Si_4SO_6F$	652.2158	652.2182
M − CH_3	$C_{21}H_{42}N_6Si_4SO_6F$	637.1950	637.1947
m	$C_{19}H_{35}N_5Si_3O_8F$	484.2035	484.2018
s	$C_{14}H_{33}N_1Si_3SO_6F$	446.1313	446.1291
k	$C_{13}H_{23}N_5Si_2O_2$	337.1394	337.1385
i	$C_{13}H_{24}N_5Si_2O$	322.1513	322.1514
a + H	$C_9H_{14}N_5SiO$	236.0979	236.0966
b + 2H	$C_8H_{14}N_5Si$	208.0998	208.1017
b + 1H	$C_8H_{13}N_5Si$	207.0922	207.0939
b	$C_8H_{12}N_5Si$	206.0845	206.0860

Comparison between the mass spectra and the ^{1}H nmr spectra of nucleocidin and other nucleosides and nucleotides[9,10] clearly establishes the furanose nucleoside structure **2**. The downfield shift in the ^{1}H nmr spectrum of the $C_{5'}$-methylene over that of adenosine ($\Delta\delta$ = 0.6 ppm) and the presence of ion m in the mass spectrum, analogous to that found by McCloskey, *et al.*, are strong evidence for assigning the sulfamyloxy group to the $C_{5'}$ position. The assignment of the fluorine to the $C_{4'}$ position is based primarily on its approximately equal coupling to the $C_{5'}$-methylene protons and the H–F vicinal coupling to $C_{3'}$H. Additional work including heteronuclear spin decoupling is planned.

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A New Method for the Synthesis of Protected Ribooligonucleotides with 3′-Phosphate End Groups

Sir:

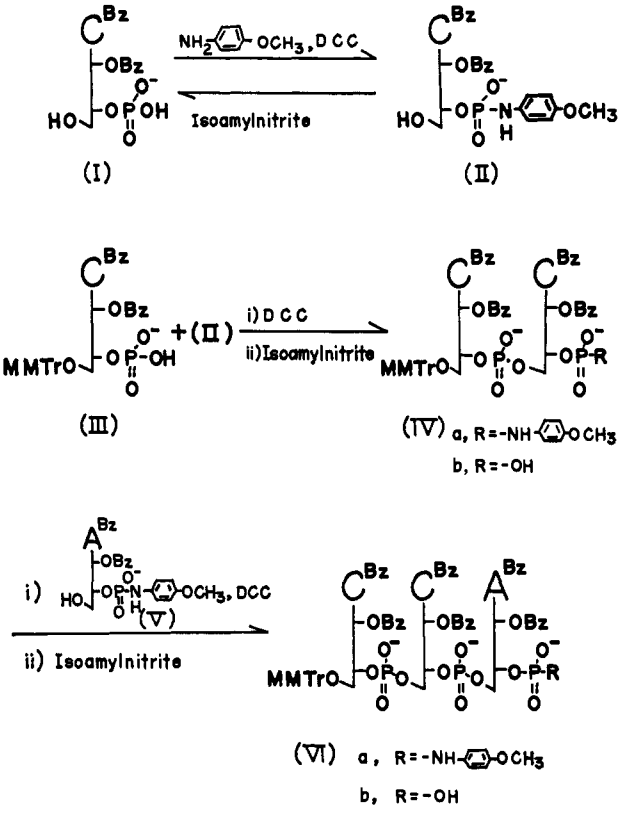
The synthesis of ribonucleotides with specific sequences is of importance for studies of the relationship between the structure and the function of nucleic acids.

In the synthesis of deoxyribopolynucleotides, stepwise condensation of preformed oligonucleotides has proved to be an advantageous method.[1] In the ribonucleotide series, however, a satisfactory procedure for the synthesis of suitably protected oligonucleotides has not been established. It is essential to use specific protection for heterocyclic rings, primary and secondary hydroxyl groups in carbohydrate moieties, and phosphomonoester groups in the synthesis of ribooligonucleotides which can be used for further condensations.

Trityl derivatives for the protection of primary hydroxyl groups and the acyl protection for heterocyclic amino groups and 2′-hydroxyl groups have been used successfully in the synthesis of triribonucleotides without phosphomonoester end groups[2] and other ribooligonucleotides.[3] With these protecting groups, acidic or alkaline treatment cannot be used for the specific removal of the protecting group on the phosphomonoester.

In this communication it has been found that aromatic amidate can be used for the protection of phosphomonoester. This group may be removed with isoamyl nitrite in a buffered solution without damaging other protecting groups on the base and the sugar moiety. Using this method a large-scale synthesis of ribooligonucleotides was performed.

Since thymidine 5′-phosphoramidate was converted by the treatment with amyl nitrite to the 5′-phosphate,[4] we have tested this reaction with 3′-phosphoranisidate of a protected nucleoside. When N,2′-O-dibenzoylcytidine 3′-phosphoro-*p*-anisidate (II), which was pre-



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