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N.m.r. studies of the flexibility of the glycosyl ring in thymidine and uridine nucleosides*

Nicola Hicks, Oliver W. Howarth, and David W. Hutchinson†
Chemistry Department, Warwick University, Coventry CV4 7AL (Great Britain)

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ABSTRACT

^{1}H-N.m.r. spectroscopy at various temperatures has been used to investigate the flexibility of the glycosyl ring and to calculate the percentage of N- and S-character in the most favoured conformations in solution adopted by various pyrimidine deoxyribonucleosides. The position and orientation of substituents have a definite and predictable influence on the conformation of the deoxyribose ring in these nucleosides. The deoxyribose rings in the nucleosides studied were, in general, flexible except for those of 3′-deoxy-3′-fluoro- and 3′-azido-3′-deoxy-thymidine and 2′-deoxy-2′-fluoro-5-methyl-arabinosyluracil.

INTRODUCTION

The crystal structures of a number of 2′-deoxynucleosides, *e.g.*, thymidine[1] (**1**), 3′-deoxy-3′-fluorothymidine[2] (**2**), and 3′-azido-3′-deoxythymidine[3] (AZT, **3**) have recently been determined, and the relationship between their crystal structures and their efficacies as anti-HIV agents has been discussed[4]. However, crystal structures give information on molecular conformations in the solid state, and little information can be gained concerning the flexibilities of the molecules and their shapes in solution or in an active site of an enzyme.

Recently, we have synthesised deoxyribonucleosides, using *N*-deoxyribosyltransferases from lactobacilli to catalyse the transfer of a deoxyribosyl residue or an analogue from a donor nucleoside to an acceptor base. We found[5–7] that 2′-deoxy-, 2′,3′-dideoxy-, and 2′,5′-dideoxy-nucleosides were efficient glycosyl donors for this reaction, but that 3′-substituted 2′,3′-dideoxynucleosides would not transfer their glycosyl residues to an acceptor base.

Little is known about the active sites of these enzymes, but major factors accounting for this lack of reactivity may be steric hindrance or dipolar effects that inhibit the binding of a substrate to the transferase. Another factor which may be important is the flexibility of the deoxyribose ring. The flexibility can be considered to be made up of three components: (*a*) rotation about the glycosyl bond, (*b*) rotation about C-4′–C-5′, and (*c*) pseudorotation of the deoxyribosyl ring. We have investigated the last of these components, because a substrate which adopts a rigid, unfavourable shape

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† To whom correspondence should be addressed.

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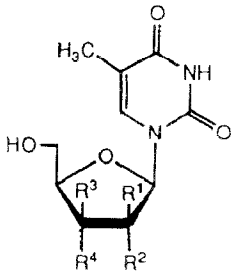
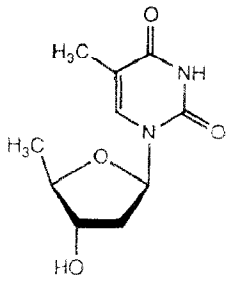
may not bind to the active site of the transferase and may be inactive as a substrate.

A number of n.m.r. studies of nucleoside conformation have been reported at constant temperature. For example, Altona and Sundaralingam demonstrated[5] that two pseudorotation ranges are most common for ribonucleosides, type N (C-2′ exo, C-3′ endo) and type S (C-2′ endo, C-3′ exo). They[6] and Davies and Danyluk[7] have described methods for calculating, from coupling constants in ^{1}H-n.m.r. spectra, the amount of N- and S-character in the favoured conformations adopted by ribo- and deoxyribonucleosides in solution. However, little work has been published on the change in conformation of the sugar residues with change in temperature, and information from variable-temperature ^{1}H-n.m.r. studies can be used to determine the flexibility of sugar rings in nucleosides. We now report the use of variable-temperature n.m.r. spectroscopy to investigate the flexibility of the deoxyribose ring in a number of deoxyribonucleosides.



1 $R^1 = R^2 = R^3 = H$, $R^4 = OH$
2 $R^1 = R^2 = R^3 = H$, $R^4 = F$
3 $R^1 = R^2 = R^3 = H$, $R^4 = N_3$
5 $R^1 = F$, $R^2 = R^3 = H$, $R^4 = OH$
7 $R^1 = R^2 = R^3 = R^4 = H$
8 $R^1 = R^2 = H$, $R^3 = R^4 = F$
10 $R^1 = R^2 = R^4 = H$, $R^3 = OH$



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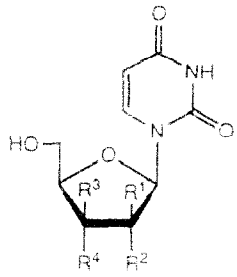

6 $R^1 = F$, $R^2 = R^3 = R^4 = H$
9 $R^1 = R^3 = H$, $R^2 = F$, $R^4 = OH$

EXPERIMENTAL

Thymidine (**1**), 3′-azido-3′-deoxythymidine (**3**), and 5′-deoxythymidine (**4**) were obtained from Sigma Chemical Co., 2′-deoxy-2′-fluoro-5-methyl-arabinosyluracil (FMAU) (**5**) was a gift from Dr. J. J. Fox (Sloan-Kettering Institute for Cancer Research, New York), 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (**6**) was a gift from Dr. J. A. Martin (Roche Products, Welwyn, U.K.), 3′-deoxy-3′-fluorothymidine[10] (**2**), 3′-deoxythymidine[11] (**7**), 3′-deoxy-3′,3′-difluorothymidine[12] (**8**), 2′-deoxy-2′-fluorouridine[13] (**9**), and 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)thymine[14] (**10**) were prepared as described.

^{1}H-N.m.r. spectra were measured on a Bruker WH400 spectrometer between 190 and 330 K. The probe temperature was calculated from the chemical shift separation of the resonances of aliphatic and hydroxyl protons of methanol[15,16]. The puckering

equilibrium of the deoxyribose rings was analysed (Table I) by the use of vicinal spin-coupling constants[8], using the formula:

$$\%S = 100 \times J_{1',2'}/(J_{1',2'} + J_{3',4'}).$$

For each compound, the differences in enthalpy and entropy between the favoured conformations adopted over the range of temperatures studied were calculated from the temperature dependence of the equilibrium constants:

$$K_{eq} = J_{1',2'}/J_{3',4'} = X_S/X_N,$$

and using a weighted least-squares fit for the van 't Hoff equation:

$$-\log K_{eq} = \Delta H/RT - \Delta S/R.$$

The coupling constants due to protons in the glycosyl moieties of the nucleosides must obey inter-relationships governed by the laws of pseudorotation and, hence, the coupling constants cannot assume random values that are independent of each other. This situation allows predictions to be made for values of $J_{H,H}$ when fluorine atoms replace hydrogen atoms in the glycosyl ring and only $J_{H,F}$ can be measured[15]. When measuring the coupling constants for fluorinated deoxyribose moieties, it was assumed, where necessary, that the corresponding $J_{H,H} = 1/2\ (J_{H,F})$. This adjustment of coupling constants is not required for the ΔH and ΔS calculations, but such a correction factor is required to estimate a value for the percentage of N- and S-character in the conformations adopted by fluorinated nucleosides.

DISCUSSION

We wished to investigate the pseudorotation of the deoxyribosyl moiety of deoxynucleosides, as this will have a bearing on the binding of these compounds to enzymes in aqueous media. The technique selected for these investigations, variable temperature ^{1}H-n.m.r. spectroscopy, requires a solvent which remains liquid over as wide a temperature range as possible. Because water is liquid over a comparatively narrow range of temperature, perdeuteromethanol is a more suitable solvent as it permits a wider range of temperatures to be studied. We find that the ^{1}H-n.m.r. spectra of solutions of our nucleosides in water and in perdeuteromethanol are very similar, suggesting that they adopt similar conformations in the two solvents and allowing comparisons to be made between results obtained in perdeuteromethanol and aqueous solutions. From the temperature dependencies of the N⇌S equilibria for compounds **1**–**10**, the enthalpy and entropy differences between the two forms were calculated (Table II).

The ^{1}H-n.m.r. spectra of 3'-deoxy-3'-fluorothymidine (**2**) in perdeuteromethanol do not vary significantly over the temperature range 208–315 K (Fig. 1). The calculated difference in enthalpy between the N- and S-conformations of **2** over this temperature range is zero. On the other hand, there is a large entropy difference (+9.1 e.u.) between the N- and S-conformations over this temperature range. This finding suggests that the conformation of the deoxyribosyl ring in **2** has >99% S-character[8], implying that **2** adopts a rigid shape with an extreme S-conformation. The n.m.r. spectra of compounds **3**–**5** also changed little over this temperature range. Calculations indicate that there are

TABLE I

Variation in coupling constants in the deoxyribosyl ring of nucleosides with temperature

Compound	*Temp. (K)*	J *(Hz)*		K_{eq}	*%S*
		H-1',2'	*H-3',4'*		
1	209	6.91[a]	2.70	2.56	74
	237	6.80[a]	3.00	2.27	69
	298	6.80[a]	3.35	2.03	67
	315	6.78[a]	3.46	1.96	67
2	208	9.69	0	[b]	>99
	259	9.60	0	[b]	>99
	278	9.37	0	[b]	>99
	297	9.25	0	[b]	>99
	315	9.13	0	[b]	>99
3	208	6.33[a]	4.49	1.41	59
	233	6.36[a]	4.70	1.35	58
	259	6.38[a]	4.79	1.33	57
	279	6.40[a]	4.84	1.32	57
	302	6.43[a]	4.85	1.33	57
	310	6.43[a]	4.80	1.34	57
4	234	6.47	3.90	1.66	62
	280	6.68	4.09	1.63	62
	296	6.69	4.16	1.61	62
	321	6.70	4.27	1.57	61
5	208	16.76[c]	4.82	1.74	64
	259	16.85[c]	4.77	1.77	64
	279	16.94	4.84	1.75	64
	300	17.03	4.85	1.76	64
	319	17.09	4.84	1.77	64
6	208	18.69[c]	5.06	1.85	65
	234	18.22[c]	5.22	1.75	64
	259	17.85[c]	5.41	1.65	62
	279	17.62[c]	5.54	1.59	61
	302	17.42[c]	5.67	1.54	61
	310	17.35	5.70	1.52	60
7	209	2.30	8.82	0.26	21
	234	2.73	8.49	0.32	24
	260	3.12	8.74	0.36	26
	276	3.26	8.70	0.37	27
	297	3.49	8.68	0.48	32
	320	3.59	7.29[d]	0.49	33
8	206	9.01	3.72[d]	4.84	83
	215	8.94	4.20[d]	4.26	81
	232	8.75	5.19[d]	3.37	77
	252	8.56	6.67[d]	2.56	72
	269	8.39	6.62[d]	2.53	72
	296	8.13	7.37[d]	2.21	69
	317	7.54	7.93[d]	1.90	66

Compound	*Temp. (K)*	J *(Hz)*		K_{eq}	*%S*
		H-1',2'	*H-3',4'*		
9	211	< 1.0	8.27	0.12	11
	235	1.19	8.09	0.15	13
	260	1.85	7.33	0.25	20
	278	2.15	7.27	0.29	23
	297	2.23	7.26	0.31	24
	307	2.34	7.23	0.32	24
10	198	1.64	3.02	0.54	35
	214	1.86	2.95	0.63	39
	231	1.99	3.04	0.66	40
	249	2.17	3.12	0.70	41
	269	2.31	3.12	0.74	43
	296	2.52	3.12	0.81	45

[a] Average value determined from unresolved peak signals. [b] Very large as $J_{3',4'} = 0$ Hz. [c] $J_{1',F}$. [d] $J_{4',F}$.

TABLE II

Enthalpy and entropy differences between N and S conformations of nucleosides

Compound	*Enthalpy difference*[a] *(kcal/mol)*	*Entropy difference*[a] *(e.u.)*
1	−0.43 (0.08)	−0.50 (0.35)
2	0.0 (0.0)	+9.13 (0.0)
3	−0.11 (0.02)	+0.16 (0.08)
4	−0.13 (0.01)	+0.495 (0.04)
5	+0.02 (0.01)	+1.18 (0.02)
6	−0.25 (0.01)	+0.029 (0.02)
7	+0.82 (0.14)	+1.17 (0.49)
8	−1.13 (0.08)	−2.38 (0.34)
9	+1.29 (0.19)	+2.04 (0.70)
10	+0.43 (0.03)	+1.03 (0.13)

[a] Standard deviations are given in parentheses.

small differences in enthalpy between the N- and S-conformations of the two compounds over this range of temperatures. However, the magnitude of the entropy differences between the N- and S-conformations increase with increasing S-character (**3**, +0.16 e.u., 59% S-character; **4**, +0.495 e.u., 62% S-character; **5**, +1.18 e.u., 64% S-character). For compounds **3** and **4**, the small entropy differences between the N- and S-conformations suggest that they have nearly equal stabilities that are unaffected by changes in temperature. On the other hand, for compound **5**, there is a comparatively large difference in entropy (1.16 e.u.) between the N- and S-conformations, which could make the deoxyribosyl ring relatively inflexible, causing the molecule to have a nearly constant percentage (65%) of S-character over the temperature range studied.

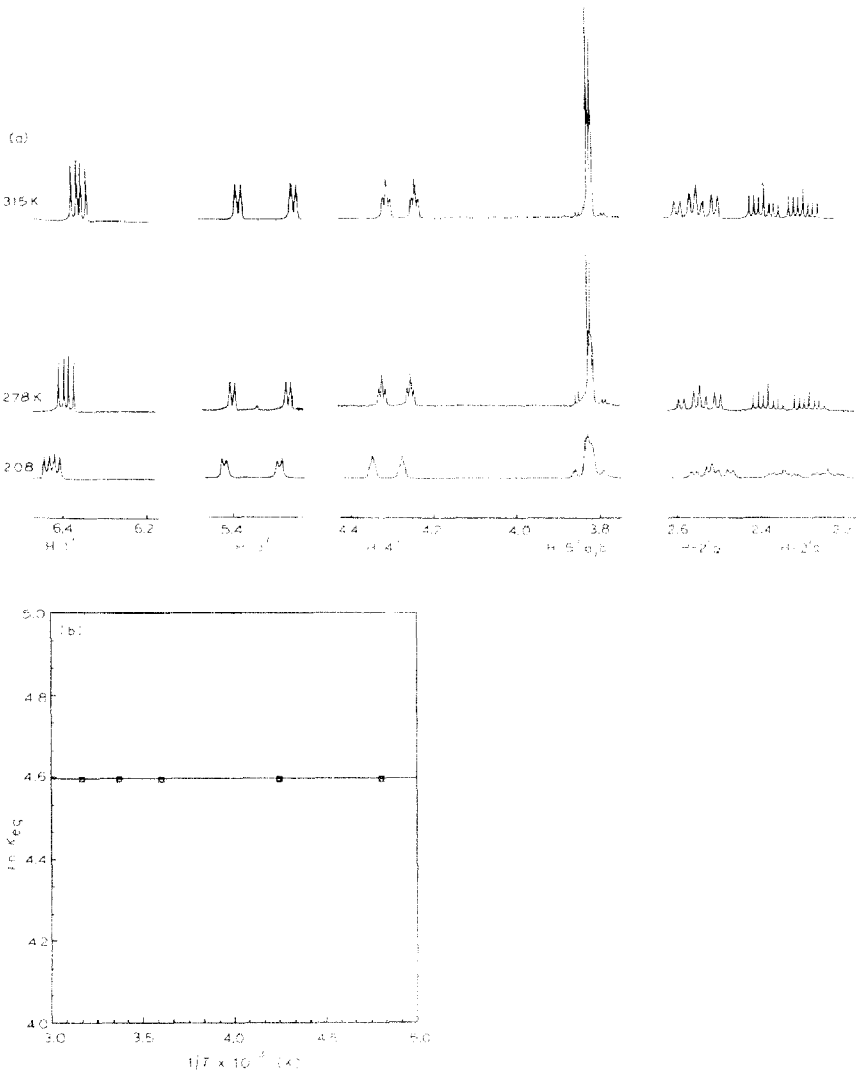

Fig. 1. (a) ^{1}H-N.m.r. spectra of 3′-deoxy-3′-fluorothymidine (**2**) measured at 208, 278, and 315 K; (b) variation of K_{eq} with temperature for **2**.

The n.m.r. spectra of other nucleosides studied changed with change in temperature (Fig. 2), indicating that the sugar rings show some conformational mobility under these conditions. The enthalpy differences for compounds **7**, **9**, and **10** are +0.82, +1.29, and +0.43 kcal/mol, respectively, indicating that, for these compounds, the N-conformation is more stable than the S-conformation. As the temperature is lowered, so the favoured N-conformations become more prevalent, indicating that the deoxyribosyl rings are flexible. In contrast, for compound **8**, there was a large negative (−1.13 kcal/mol) difference in enthalpy between the two forms, showing that the S-conformation is significantly more stable. As the temperature is reduced, the flexibility of the

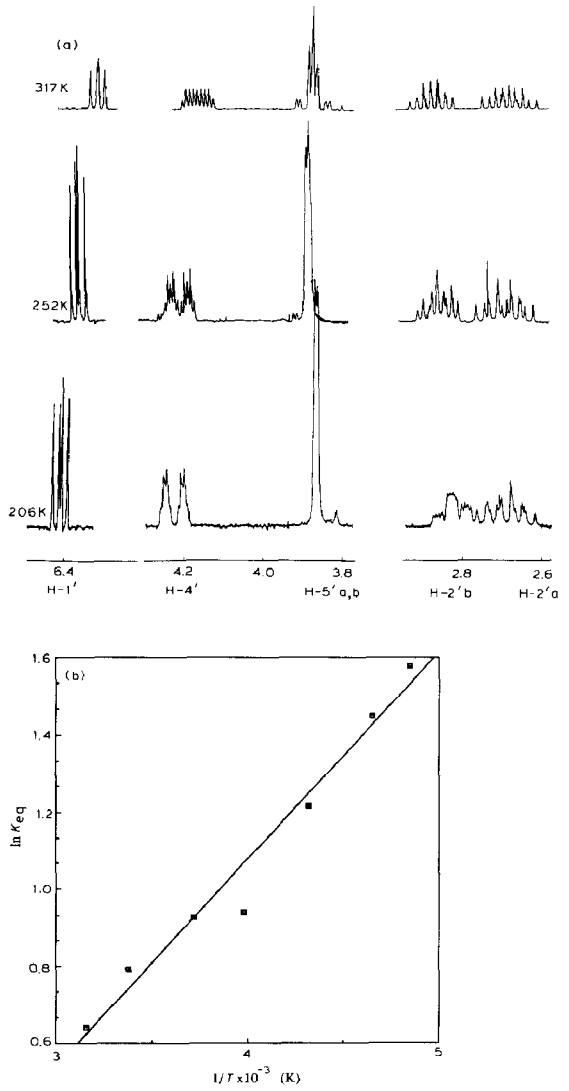

Fig. 2. (a) ^{1}H-N.m.r. spectra of 3′-deoxy-3′,3′-difluorothymidine (**8**) measured at 206, 252, and 317 K; (b) variation of K_{eq} with temperature for **8**.

deoxyribosyl ring is again shown by the increase in S-character from 69% at 296 K to 83% at 206 K. The remaining compounds show an increased percentage of the favoured S-conformation at low temperatures.

The position and orientation of substituents have a definite and predictable influence on the conformation of the deoxyribose ring. Atoms or groups on the same side of the sugar ring as C-5′ are designated "endo" and those on the opposite side of the ring to C-5′ are "exo". Those nucleoside analogues in which the 3′-substituent is "exo" favour the S-conformation, whereas those analogues with the 3′-substituent "endo" adopt N-conformations. These predictions can also be extended to the 2′-position. An

"exo" 2′-substituent appears to favour the adoption of the N-conformation, while an "endo" 2′-substituent favours the adoption of the S-conformation. In 2′,3′-disubstituted compounds, the influence of the substituents is additive. For example, 2′-fluoro "endo" and 3′-hydroxyl "exo" substitution in compound **5** enhances the amount of S-character in the conformation and renders the sugar ring almost inflexible. However, in compound **9**, the influence of the 2′-fluoro "exo" substitution appears to over-ride the effect of the 3′-hydroxyl "exo" substitution. The resulting nucleoside has the N-conformation and the sugar ring is still flexible.

Thymidine (**1**), 5′-deoxythymidine (**4**), and 3′-deoxythymidine (**7**) are active glycosyl donors with *N*-deoxyribosyltransferases from lactobacilli, and these three compounds appear to have relatively flexible sugar rings. This property may allow them to adopt the "correct" conformation at the active site of the transferase without undue input of energy. Steric and dipolar constraints may become important in the case of 3′-deoxy-3′-fluorothymidine (**2**) and AZT (**3**), making them inactive substrates for the transferases. The correlation of the shape of the sugar ring in a nucleoside in solution with antiviral activity is much more complex as several enzymic steps are involved before the nucleoside inhibits viral replication. However, it is of interest that three of the nucleosides in our study [3′-deoxy-3′-fluorothymidine (**2**), AZT (**3**), and FMAU (**5**)], which have rigid ring conformations with a predominance of S-character present at 294 K, are all active antiviral agents.

ACKNOWLEDGMENT

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REFERENCES

1 D. W. Young, P. Tollin, and H. R. Wilson, *Acta Crystallogr., Sect. B*, 25 (1969) 1423–1432.
2 P. van Roey and R. F. Schinazi, *Antiviral Chem. Chemother.*, 1 (1990) 93–98.
3 (a) P. van Roey, J. M. Salerno, W. L. Duax, C. K. Chu, M. K. Ahn, and R. F. Schinazi, *J. Am. Chem. Soc.*, 110 (1988) 2277–2282; (b) R. Parthasarathy and H. Kim, *Biochem. Biophys. Res. Commun.*, 152 (1988) 351–358; (c) I. Dyer, J. N. Low, P. Tollin, H. R. Wilson, and R. A. Howie, *Acta Crystallogr., Sect. C*, 44 (1988) 767–769; (d) A. Camerman, D. Mastropaolo, and N. Camerman, *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 8239–8242; (e) G. I. Birnbaum, J. Giziewicz, E. J. Gabe, T. S. Lin, and W. H. Prusoff, *Can. J. Chem.*, 65 (1987) 2135–2139; (f) G. V. Gurskaya, E. N. Tsapkina, N. V. Skapysova, A. A. Kraevskii, S. V. Lindeman, and Y. T. Struchkov, *Dokl. Akad. Nauk SSSR*, 291 (1986) 854–859.
4 E. W. Taylor, P. van Roey, R. F. Schinazi, and C. K. Chu, *Antiviral Chem. Chemother.*, 1 (1990) 163–173.
5 P. van Roey, J. M. Salerno, C. K. Chu, and R. F. Schinazi, *Proc. Natl. Acad. Sci. U.S.A.*, 86 (1989) 3929–3933.
6 D. Betbeder, D. W. Hutchinson, and A. O'L. Richards, *Nucleic Acids Res.*, 17 (1989) 4217–4222.
7 D. Betbeder, D. W. Hutchinson, A. O'L. Richards, D. Kinchington, and N. Mahmood, *Antiviral Chem. Chemother.*, 1 (1990) 249–253.
8 C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 95 (1973) 2333–2344.
9 D. B. Davies and S. S. Danyluk, *Biochemistry*, 13 (1974) 4417–4434.
10 P. Herdewijn, J. Balzarini, E. de Clercq, R. Pauwels, M. Baba, S. Broder, and H. Vanderhaeghe, *J. Med. Chem.*, 30 (1987) 1270–1278.
11 D. Bergstrom, E. Romo, and P. Shum, *Nucleosides Nucleotides*, 6 (1987) 53–63.
12 R. Mengel and W. Guschlbauer, *Angew. Chem. Int. Ed. Engl.*, 17 (1978) 8525.
13 A. L. van Geet, *Anal. Chem.*, 42 (1970) 679–680.

14 P. S. Raford, C. L. Fistz, and E. D. Becker, *Anal. Chem.*, 51 (1979) 2050–2051.
15 K. I. Williamson, Y. F. Li, F. H. Hall, and S. Swager, *J. Am. Chem. Soc.*, 88 (1966) 5677–5679.