1, which migrated like d(CGCGAATTCGCG)$_2$.

The modified DNAs were also characterized by their susceptibility to enzymatic digestion. The d(CGCGAATTCGCG)$_2$ dodecamer contains an *Eco*RI recognition site (GAATTC), and since this enzyme only cleaves double-stranded DNA, hairpin **3** should not be a substrate. Indeed, [^{32}P] end-labeled **3** was not cleaved by *Eco*RI endonuclease. However, both **1** and **2** were cut as efficiently as d(CGCGAATTCGCG)$_2$, indicating that these two molecules exist in duplex form. Next, we examined the conformational integrity of the modified DNAs. On a denaturing gel, **2** and **3** migrated as a dodecamer and as a hairpin, respectively, whereas reduced duplex **1** migrated like d(CGCGAATTCGCG)$_2$ (Figure 2B). In thermal denaturation experiments, **2** displayed a sharp melting transition at 94 °C in contrast to the biphasic curve obtained with the parent dodecamer.[13] Upon heating from 10 to 99 °C, the absorbance of **3** rose linearly to just over 3% of the initial optical density value.

To summarize, disulfide crosslinking was employed to trap a thermally induced DNA hairpin. These results suggest that the d(CGCGAATTCGCG)$_2$ premelting intermediate is also a hairpin structure and demonstrate the utility of our modification in the synthesis of both ground-state and non-ground-state DNA conformations. The ease with which this crosslink is introduced coupled with the finding that it does not inhibit the action of kinases or endonucleases should make these disulfide-crosslinked DNAs useful in studies of nucleic acid structure and function.

Supplementary Material Available: Procedures for the synthesis and characterization of the cross-linked oligomers (4 pages). Ordering information is given on any current masthead page.

(12) The ^{1}H NMR spectrum of **3** shows three G/C base pairs which is consistent with the proposed structure.

(13) Bis-crosslinked **2** is similar to DNA dumbbells; see, for example: Erie, D. A.; Jones, R. A.; Olson, W. K.; Sinha, N. K.; Breslauer, K. J. *Biochemistry* **1989**, *28*, 268-273. Ashley, G. W.; Kushlan, D. M. *Biochemistry* **1991**, *30*, 2927-2933.

Intramolecular Coupling of Two Radical Centers through 1,1-Diphenylethylene Chromophores. Isomeric Dinitroxides Vinylogous to Trimethylenemethane, Tetramethyleneethane, and Pentamethylenepropane

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Our motive for the syntheses and analyses of isomeric vinylidenebis(radical X-substituted benzenes) **1** has two facets. As an extension of our efforts to design and construct new organic polymers in high-spin ground states, we became interested in knowing whether polymers **2** would be high-spin and how strong the electron spins in X could couple each other in **2**.[1] For this purpose, studies of dimeric prototypes should be very instructive. On the other hand, *p,p'*-, *m,p'*-, and *m,m'*-**1** (X = CH_2•) are related in connectivity to trimethylenemethane (**3**) tetramethyleneethane (**4**), and pentamethylenepropane (**5**), respectively. Since the ground spin states of **4** and **5** are still controversial, in contrast to the well-established triplet of **3**,[2] we thought the delineation

(1) Iwamura, H. *Adv. Phys. Org. Chem.* **1990**, *26*, 179. Fujii, A.; Ishida, T.; Koga, N.; Iwamura, H. *Macromolecules* **1991**, *24*, 1077.

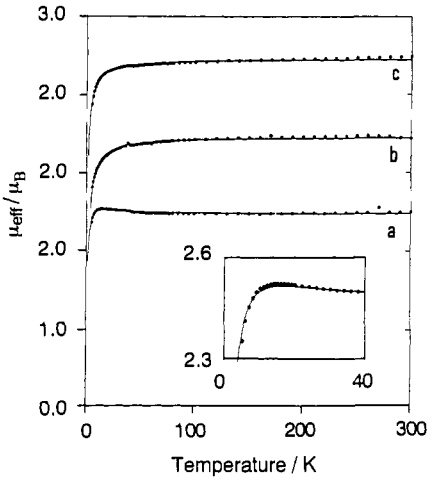
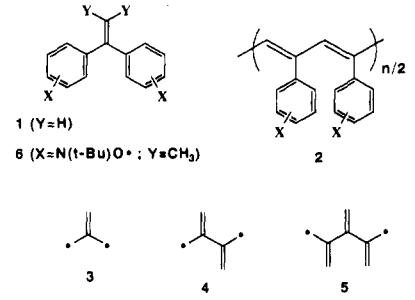


Figure 1. Plots of μ_{eff}/μ_B vs temperature for (a) *p,p'*-, (b) *m,p'*-, and (c) *m,m'*-**6**.

of the manner in which the electron spins couple in isomeric **1** might shed light on the discussion of the ground-state spins of **4** and **5**.



The experimental work has been carried out on the isomeric vinylidene derivatives **6** with additional steric protection.[3] The corresponding dibromo compounds were lithiated with 4 equiv of *tert*-butyllithium and allowed to react with 2 equiv of 2-nitroso-2-methylpropane to give the hydroxyamines, which were then treated with Ag_2O to give **6**. Purification was accomplished by chromatography on silica gel to give analytically pure samples.[4]

EPR spectra of **6** in toluene at room temperature consisted of five unperturbed lines ($|J| > |a_N|$ = 11.2 G). The dipolar interaction between the nitroxides was unexpectedly small as revealed by their EPR spectra in frozen matrices: spectral widths as a measure of $2D$ were less than 90 G, but $\Delta m_s = 2$ transitions for triplet species were clearly observed at $g = 4$. The signals were too small to study the temperature dependence of their intensities.

The magnetic susceptibility was measured for powder samples of **6** on a Quantum Design SQUID susceptometer in the temperature range 5-300 K. The results are expressed in terms of μ_{eff}/μ_B vs temperature plots in Figure 1. The μ_{eff} values approaching 2.45 μ_B at room temperature are indicative of the near degeneracy of the singlet and triplet states for the three isomers. The plots were analyzed in terms of a Bleaney-Bowers equation

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(3) A preliminary study shows that *p,p'*-dinitroxide **1** (X = *N*(*t*-Bu)O•) readily undergoes polymerization to form an insoluble solid as soon as the solvent is removed: Ishida, T. Ph.D. Dissertation, University of Tokyo, Tokyo, Japan, 1991.

(4) The empirical factors, F, introduced to correct slight reductions in the effective magnetic moment of the samples made for SQUID measurements due to the presence of nondiradical impurities were 0.97, 0.88, and 0.94 for *p,p'*-, *m,p'*-, and *m,m'*-**6**, respectively.

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Table I. Energy Gaps between the Singlet and Triplet States in Isomeric Dinitroxides 6

6	$\Delta E_{S-T}/cm^{-1}$ [a]	θ/K	GS from VB	GS from MO
p,p'	10.6	-2.0	S = 1	nondisjointed
m,p'	-3.4	-2.0	S = 0	disjointed
m,m'	-1.8	-2.1	S = 1	doubly disjointed

[a] The energy gap between the two states: $\Delta E_{S-T} = 2J$. +/- signs represent triplet/singlet ground states, respectively.

with a Weiss field and purity factor F[4] (eq 1) and refined by a SALS program[5] to give the results summarized in Table I.

$$\chi = F\frac{2Ng^2\mu_B^2}{k(T-\theta)[3+\exp(-2J/kT)]} \quad (1)$$

The measurement of the absolute μ_{eff} values and their temperature dependence over wide temperature ranges makes it possible to conclude that, whereas both dipole-dipole and exchange couplings between the two nitroxide radicals in 6 are rather weak, the p,p' isomer has a triplet and the m,p' and m,m' isomers have singlet ground states. The exchange coupling in 2 (X = p-N(t-Bu)O·) may not be very strong but should be ferromagnetic. The ground states of 4 and 5 are suggested to be singlet. The coupling between the radical molecules in neat solid samples is always weakly antiferromagnetic, as revealed by the small negative θ values.

p,p'-6 is nondisjointed and is predicted by molecular orbital theory (MO)[2c,d] to have a triplet ground state and the largest magnitude of ΔE_{S-T}; the disjointed m,p'-6 is predicted to have a singlet ground state and a small magnitude of ΔE_{S-T}. The m,m' isomer is classified as a "doubly disjointed" system in the sense that the carbons with substantial positive density are separated by three carbons, and therefore the isomer is predicted to have a singlet ground state with the smallest magnitude gap of all. The results in Table I are in line with these predictions. However, formal application of a topology/valence bond theory (VB)[6] would have predicted a ferromagnetic interaction between the m,m' spins ($S = (n^* - n)/2 = (9-7)/2 = 1$).[7]

The observed small absolute values of ΔE_{S-T} are annoying, as semiempirical calculations on sterically unprotected diradicals 1 (X = NHO·, N:) usually give ΔE_{S-T} on the order of 1 kcal/mol.[8] MM2 calculations give an estimate of the propellar-type torsion of the phenyl rings out of the vinylidene and dimethylvinylidene planes as 40° and 54°, respectively. It has been shown that, in phenyl nitroxides, the electron spins are much more localized in the nitroxide moieties than in the hydrocarbon radicals.[9] These two factors appear to attenuate the topological effect of the radical centers on the mode of the exchange coupling. Studies that would amplify the trend found in this study are necessary on a series of sterically unbiased systems with larger spin polarization on the phenyl rings.[3] Such studies are in progress.[10]

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Supplementary Material Available: Full experimental description of isomeric dinitroxides 6 including their EPR spectra in solid toluene solutions and figurative presentations of the VB theory predicting the ground-state spin $S = (9-7)/2 = 1$ in m,m'-6 and the MO theory showing singly disjointed m,p'-6 and doubly disjointed m,m'-6 (3 pages). Ordering information is given on any current masthead page.

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Synthesis and Structural Analysis Using 2-D NMR of Sialyl Lewis X (SLe^x) and Lewis X (Le^x) Oligosaccharides: Ligands Related to E-Selectin [ELAM-1] Binding

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The sialyl Lewis X (SLe^x) determinant (NeuAc-α-2,3-Gal-β-1,4-[Fuc-α-1,3]-GlcNAc), compound 1, is a ligand for E-selectin (endothelial leucocyte adhesion molecule 1, or ELAM-1), a member of the selectin family of cell adhesion molecules.[4-7] Interactions between E-selectin and leucocyte-bound SLe^x or closely related oligosaccharides are thought to be important early events in the inflammation process.[8,9] Binding analysis has shown that the sialic acid (NeuAc) and the fucose (Fuc) moieties are essential for high affinity. The related desialylated trisaccharide Le^x (Gal-β-1,4-[Fuc-α-1,3]-GlcNAc), for example, is not a high-affinity ligand for E-selectin.[4-7] In this communication, we describe the syntheses of SLe^x 1 and the β-O-allyl glycoside of Le^x 2 using a cloned fucosyltransferase and their complete NMR spectral assignments including ROESY and NOESY experiments in order to investigae the conformation of these compounds in solution.

The synthesis of β-O-allyl Le^x, compound 2, starts with the construction of the β-O-allyl-N-acetyllactosamine derivative 5 (Scheme I).[10] The glycosyl acceptor, β-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl) trichloroacetimidate 3[11] was treated with the β-O-allyl glycoside of a selectively protected GlcNAc derivative (compound 4) using boron trifluoride etherate ($BF_3 \cdot OEt_2$) as a catalyst to give, after deprotection, compound 5.[12,13]

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(2) Cytel Corporation, 3525 John Hopkins Court, San Diego, CA 92121.
(3) Roche Products Ltd., 40 Broadwater Road, P.O. Box 8, Welwyn Garden City, Hertfordshire, A17 3AY UK.
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