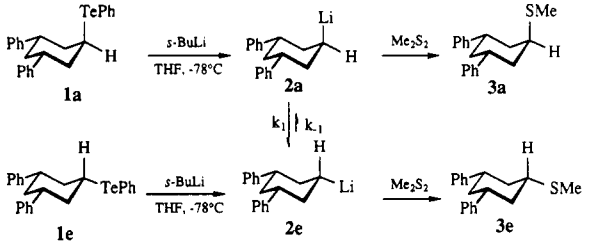
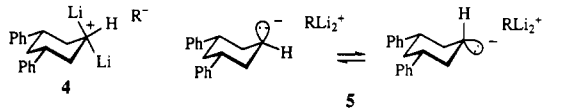


a preponderance of equatorial lithium reagent **2e** and equilibrated to the same 8/92 mixture of **2a** and **2e** (see Figure 1). These results represent the first example of a non-cyclopropyl hydrocarbon alkyllithium reagent with useful configurational stability on the laboratory time scale in THF or any ether solvent.

The rate of equilibration was accelerated by lithium iodide and was strongly dependent on the total organolithium concentration.[12b] Reasonable pseudo-first-order kinetics were obtained when the concentration of *sec*-butyllithium was optimum (36 mM, $t_{1/2}$ = 9 min), as shown in Figure 1. At 96 mM, $t_{1/2}$ was 3 min, at 24 mM, approximately 18 min, but considerable scatter was observed at both the higher and lower concentrations, the former because the rate was too fast, and the latter because the concentration of RLi was too low for accurate work.

A reasonable interpretation of this concentration dependence is that the inversion process involves an aggregation state higher than that present in THF. If **2**, like *sec*-butyllithium,[15] is largely monomeric in THF, then the inversion may involve a dimer (this fits our rate data best) or a higher aggregate. The concentration dependence of primary Grignard reagent inversion has been rationalized by invoking aggregate formation.[16] An aggregate may provide mechanistic opportunities for moving a cation from one face of a carbanion to the other not available to a monomer, for example, by formation of a rapidly inverting free carbanion **5** or a triple ion intermediate such as **4** with a symmetrically dilithiated planar carbanion,[17] or by related pathways as explored theoretically for methyllithium dimer.[18]



We have tested the aggregation hypothesis. A complexing agent that effectively prevents dimerization might enhance the configurational stability of **2a** by preventing aggregate-based lithium exchange. The tridentate ligand *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDTA) is uniquely suited for the purpose: it is a strong complexing agent[1c,19] and is capable of occupying the three free coordination sites of a monomeric organolithium reagent, thus inhibiting dimerization.[15,20]

The cleavage of the tellurides **1a** and **1e** was repeated in the presence of PMDTA (1/3/15 ratio of telluride, *sec*-butyllithium, and PMDTA), with the results shown in Figure 1. The isomerization rate was reduced by a factor of 20, with a half-life for equilibration longer than 2 h. The equilibration constant was not significantly affected by the presence of PMDTA (a ratio of 7/93 **3a**/**3e** was obtained at −45 °C). Furthermore, the rate of isomerization was unaffected by an 8-fold increase in the concentration of *sec*-butyllithium, provided that at least a 5-fold excess of PMDTA over lithium was maintained.[21] Under optimum conditions at −78 °C with short reaction times, **1a** gave 94% axial sulfide **3a**, and **1e** gave 98% equatorial sulfide **3e**. In contrast, the rate of epimerization increased when run in ether/THF mixtures compared to pure THF (most lithium reagents form higher aggregates in ether than in THF). The bidentate complexing agent tetramethylethylenediamine (TMEDA, 5 equiv per RLi) produced no significant change in the rate of isomerization of **2a**.[22]

We believe that this is the first example in which configurational isomerization of a lithium reagent was slowed by increase in the strength of lithium coordination.[23] However, the configurational isomerization of neohexylmagnesium chloride[16a] and 3-cyclohexenylmagnesium bromide is slower in diglyme and THF than in ether.[16a] We speculate that there may be two "islands" of relatively high configurational stability for alkyllithium reagents as solvent polarity is changed. One is in hydrocarbon solvents in which the charge separation needed for C–Li bond reorganization is energetically costly, and a second is in more polar solvents containing predominantly monomeric species where aggregate-assisted isomerization is inhibited, as proposed in the current study. One might anticipate that further increases in solvent polarity could again facilitate isomerization by stabilizing separated ion pairs, the mechanism usually invoked for polar-solvent-accelerated isomerizations.[2b,c,6a,c,12]

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Reduction and Electrochemistry of C_{60} in Liquid Ammonia

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We report here the reduction of C_{60} slurries by solvated electrons (e_s^-) generated electrochemically in liquid ammonia and the cyclic voltammetry (CV) of the C_{60}^- solution produced. Waves corresponding to both soluble and insoluble forms of C_{60}^{n-} were observed, with n spanning the range 0–6 (depending on the supporting electrolyte used). C_{60} in solvents in which C_{60} and its reduced products are soluble, e.g., CH_2Cl_2, benzene, and toluene, showed up to five CV waves,[1-4] and by use of mixed solvents at low temperature, the presence of six CV redox waves for $1e^-$

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Table I. Peak Potentials in Liquid Ammonia and Comparison to Solution Data[a]

		ammonia/KI[b]		ammonia/LiI		MeCN[c]	toluene/MeCN[d]
wave	species	E_{pa} (V)	E_{pc} (V)	E_{pa} (V)	E_{pc} (V)	E_{pc} (V)	$E_{1/2}$ (V)
I	(0,−1)	−1.04	−1.10 (f + s)	−1.06	−1.21 (f + s)	−1.17	−0.98
II	(−1,−2)	−1.56	−1.60 (s)	−1.52	−1.60 (s)	−1.39	−1.37
III	(−2,−3)	−2.00	−2.03 (s)	−1.85	−1.98 (f + s)	−1.88	−1.87
IV	(−3,−4)	−2.37	−2.40 (s)	−2.29	−2.39 (f + s)	−2.24	−2.35
V	(−4,−5)	−2.43	−2.58 (f + s)	−2.68	−2.78 (f + s)		−2.85
VI	(−5,−6)	−3.03	−3.08 (f + s)				−3.26

[a] All potentials are referenced to the Fc/Fc^+ couple. In ammonia, the potentials were referenced to the potential for solvated electron generation (at 2 mA/cm^2) and then referenced to the Fc/Fc^+ couple.[15,16] Surface wave, f; solution wave, s. [b] See Figure 1. [c] Reference 7. [d] Reference 5a.

reduction steps up to C_{60}^{6-} was demonstrated,[5] showing complete filling of the three degenerate molecular orbitals of C_{60}.[6] We previously showed[7,8] that C_{60} was insoluble in MeCN and that redox processes of films of C_{60}, involving doping of the films with electrolyte cation, M^+ [M^+ = tetra-*n*-butylammonium (TBA^+), Li^+, K^+], could be observed. Studies of film mass changes with the quartz crystal microbalance (QCM)[9,10] have indicated higher solubility of the reduced forms in MeCN, with the C_{60}^{3-} form soluble in TBA^+ solutions. Indeed, slurries of C_{60} could be reduced electrochemically in MeCN to the −3 form, which could then be oxidized to insoluble films on the electrode.[10] We now describe experiments in liquid NH_3 at −70 °C, where C_{60} slurries are dissolved by reaction with e_s^-.

C_{60} is insoluble and chemically inert in liquid NH_3.[11] A mass spectrum of a C_{60} sample which had been exposed to liquid NH_3 at −60 °C for 5 h followed by removal of the NH_3 at room temperature showed only one peak (m/z = 720), and the visible spectrum of the same sample in a toluene solution was identical to that of untreated C_{60}. However, the C_{60}^- form is soluble in a NH_3/KI solution. After 1 equiv of e_s^- was generated to reduce the C_{60} slurry, a red-brown solution appeared with no C_{60} particles remaining in the cell. In a typical experiment, 14 mg of C_{60} in 10 mL of NH_3 was reduced by 1 equiv of e_s^-, generated at a constant current at a Pt mesh electrode, to form a 1.9 mM C_{60}^- solution, which was then studied by CV.[14] CV scans in this solution toward negative potentials performed at a 1-mm-diameter Pt disk electrode, starting at the rest potential, E_r [ca. −1.37 V vs Fc/Fc^+ (Fc = ferrocene)], at a scan rate, v, of 20 mV/s, produced a series of reduction waves. If the scan was reversed after wave IV (reduction to C_{60}^{4-}), the CV scan toward positive potentials showed three stepwise oxidations to soluble −3, −2, and −1 forms of C_{60} (Figure 1A). The peak potential splittings (ΔE_p) for waves II, III, and IV were 40 mV (RT/F at −70 °C), and i_p for each peak was proportional to $v^{1/2}$. Thus C_{60}^-, C_{60}^{2-}, C_{60}^{3-}, and C_{60}^{4-} are all soluble and the corresponding CV waves are reversible. If the CV scan is continued beyond wave V, a film of the K salt of C_{60}^{5-} forms and the corresponding oxidation on reversal resembles a stripping peak to form dissolved C_{60}^{4-} (Figure 1B). However, two additional small peaks appear, together with the oxidation waves of dissolved C_{60}^{4-} and C_{60}^{3-}. In this case, these waves have the characteristics of surface CV waves and probably represent the oxidation of some undissolved C_{60}^{4-} and C_{60}^{3-} forms. When the scan continues to more negative potentials, a wave corresponding to reduction of the insoluble C_{60}^{5-} salt to an insoluble −6 form (based on the i_p–v behavior) occurs (Figure 1C). A scan from the C_{60}^- E_r toward positive potentials produces an anodic wave corresponding to the oxidation of C_{60}^- to C_{60} (wave I), and on reversal, the reduction of C_{60} (present in the form of both film and dissolved species) occurs at −1.10 V.

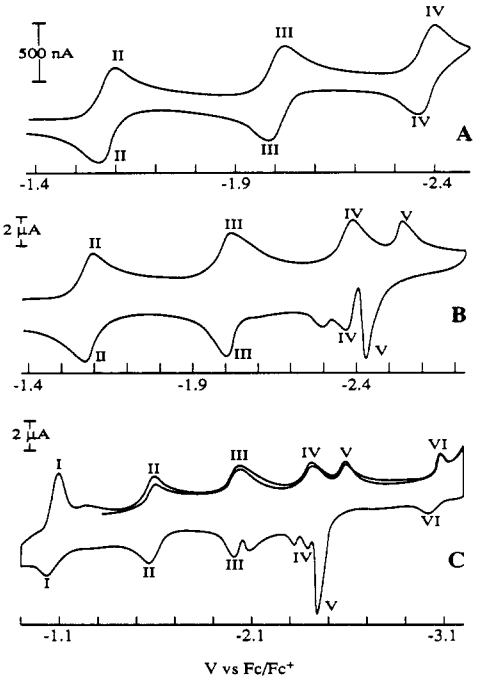

Figure 1. Cyclic voltammograms of a 1.9 mM C_{60}^- solution prepared after generating 1 equiv of e_s^- in liquid ammonia/KI (0.1 M) at −70 °C, when (A) the potential was scanned at v = 20 mV/s and reversed beyond wave IV; (B) the potential was scanned at 500 mV/s and reversed beyond wave V; and (C) the whole potential range of the solution was scanned at v = 500 mV/s. The working electrode was a 1-mm-diameter Pt disk electrode, and the concentration of KI was 0.1 M.

Coulometric titration with e_s^- was carried out to confirm the formation of the C_{60}^{6-} species. In such an experiment, a known excess of e_s^- was generated at constant current and the initially red-brown solution became a suspension of black particles (the K salt of C_{60}^{6-}). A back-titration was then conducted by holding the potential constant at −3.14 V, just beyond wave VI, where e_s^- is oxidized. The end point was determined by measuring the rest potential of the microelectrode until it reached a potential of −3.14 V. The net amount of e_s^- consumed corresponded to 5.81 mol of e_s^-/mol of C_{60}^{6-}. A similar experiment was conducted in NH_3/(TBA)CF_3SO_3 solution, where the TBA^+ salts of all forms (C_{60}^{n-}, n = 1–6) are insoluble. Generation of excess e_s^- followed by back-titration yielded 6.19 e_s^- per C_{60}.

If the supporting electrolyte was a Li salt, the CV of the C_{60}^- solution was very different. A cathodic CV scan from the E_r at ca. −1.51 V showed only four reductions, suggesting that only C_{60}^{5-}

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was produced before generation of e_s^-. On the basis of the CV behavior for each reduction, the only soluble species were the −1 and −2 ions; all other anions were either insoluble or partially soluble. Note that in the LiI CV, all of the reduction waves were about equally spaced in potential, while wave V in Figure 1B is shifted to a less negative potential, probably because of the precipitation of K_5C_{60} from soluble C_{60}^{4-}. The potentials of the different waves in both NH_3/LiI and NH_3/KI solutions and a comparison to those in other solvents are given in Table I.[14]

These results support the strong dependence of the redox chemistry of C_{60} on both solvent and cation of the supporting electrolyte and demonstrate that electrochemical reduction to the −6 state can be accomplished in liquid NH_3 in the presence of K^+. Electrolysis of C_{60} slurries by e_s^- generated electrochemically is quantitative and provides a useful new approach in the preparation of C_{60} compounds and films.

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Ligand-Promoted Dimerization of Oligonucleotides Binding Cooperatively to DNA

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In biological systems, specific protein–DNA interactions are often modulated by ligands as in the regulation of gene expression.[1] Many transcription factors are active as dimers, but inactive as monomers. In at least one case, dimerization is regulated by an additional protein cofactor.[2] We recently described the design of a triple helical complex comprising two oligonucleotides which bind adjacent sites on DNA cooperatively through dimerization.[3] If dimerization could be controlled by additional ligands, such an artificial nucleic acid system would be analogous to the naturally occurring inducible protein systems. We now report that the stability of the dimerization domain can be enhanced by a small molecule, echinomycin (E). The strengths of ligand-promoted control of oligonucleotides binding cooperatively to DNA are quantitated by affinity cleaving.

Our design begins with a Y-shaped structure consisting of two oligonucleotides (Figure 1).[3] Each of these oligonucleotides contains a recognition domain (11 or 15 bases) and a dimerization domain (5 bases) separated by a one-base linker.[3] Site-specific DNA recognition is achieved through specific Hoogsteen hydrogen bonding and local triple helix formation (T·AT and C+GC triplets)[4] while dimerization occurs through Watson–Crick hydrogen bonding. The stability of the Watson–Crick dimerization domain can be controlled both by length and/or by the addition of sequence-specific DNA-binding peptides or proteins. In order to minimize dimerization in the absence of ligand, a 5-bp mini-helix was used (Figure 1). To obtain ligand-mediated control of dimerization, a specific 4-bp recognition sequence for the DNA-binding drug echinomycin (E) was incorporated into the dimerization domain. Echinomycin (E), a bis-intercalating minor

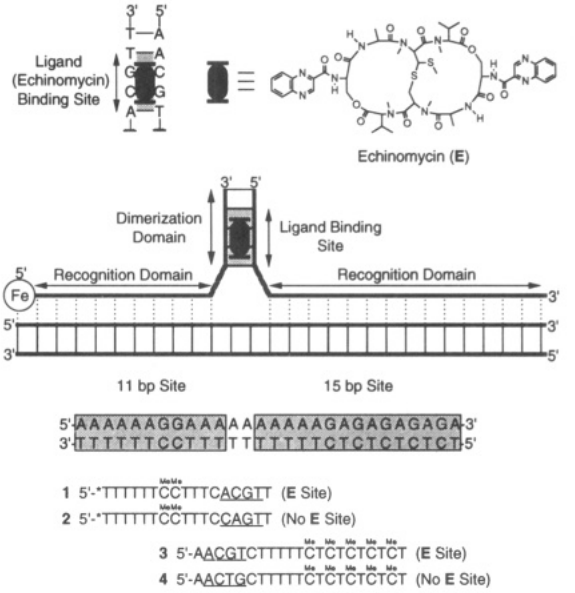

Figure 1. Schematic representation of a Y-shaped nucleic acid complex composed of two triple helix forming oligonucleotides which dimerize through formation of a small segment of Watson–Crick double helical DNA. Contained within the mini-helix is a high-affinity site for the DNA-binding molecule, echinomycin (E).

groove-binding molecule, is known to bind sequence specifically to the 4-bp site 5′-ACGT-3′ and stabilize duplex DNA formation at micromolar concentrations (Figure 1).[5–8] Echinomycin (E) binding should therefore augment the stability of the 5-bp dimerization domain and increase the affinity of the resulting dimeric oligonucleotide complex for its specific DNA target site.

Four oligonucleotides, **1**–**4**, were synthesized to test this design (Figure 1). Oligonucleotides **1** and **3** contain the echinomycin recognition sequence 5′-ACGT-3′ in the dimerization domain while **2** and **4** do not. The modified base thymine–EDTA (T*) was incorporated at the 5′-termini of oligonucleotides **1** and **2**, each targeted to the 11-bp site, to allow analysis of site-specific binding by the affinity cleaving method.[4a,9] The binding affinities of oligonucleotide pairs **1**,**3** and **2**,**4** in the presence and absence of echinomycin (E) were measured.

Affinity cleavage experiments were performed on a ^{32}P-end-labeled restriction fragment (852 bp) containing the adjacent 11- and 15-bp target sites (Figure 2).[3] Reaction of oligonucleotide–EDTA·Fe **1** (100 nM) with the target DNA (pH 7.0, 37 °C) alone or in the presence of echinomycin (E) results in little cleavage (Figure 2, lanes 2 and 3). A reaction of **1** in the presence of **3** (1.0 μM)[10] affords a modest increase in cleavage, revealing that some cooperativity occurs even with a 5-bp dimerization domain (lane 4). Upon addition of echinomycin (E) (50 μM) to the reaction containing oligonucleotides **1** and **3**, a dramatic increase in cleavage occurs (lane 5). This demonstrates that the small ligand E significantly enhances the affinity of oligonucleotides **1** and **3** for its site. Only minimal cleavage is obtained in the reaction containing **2**, **4**, and E (lane 10), demonstrating that this result depends on a sequence-specific ligand-binding site within the dimerization domain. For comparison, significant cleavage is obtained in a reaction containing **2** and **4** when the concentration of **2** is raised by a factor of 10 from 100 nM to 1.0 μM (lane 11). This demonstrates that oligonucleotide **2** binds

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