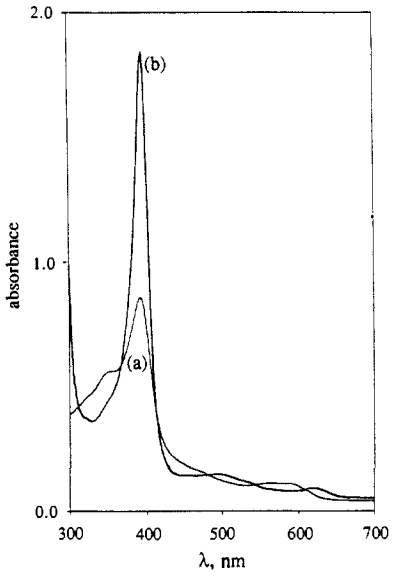

Figure 3. Visible spectra of free mesohemin (a), and the antibody-mesohemin complex (b). Both samples contained 10 μM iron(III) mesoporphyrin, 5% (v/v) DMSO, 0.5% (w/v) Triton X-100, and 95 mM Tris acetate (pH 8.0). The antibody sample contained 3 mg/mL (20 μM) protein.

10-min reaction period. Addition of a stoichiometric amount (relative to antibody binding sites) of hapten **1** completely blocked antibody catalysis. Antibody alone had no peroxidase activity. The other two antibodies specific for hapten **1** did not form mesohemin complexes with peroxidase activity.

The rate of *o*-dianisidine (**6**) oxidation was examined at several *o*-dianisidine concentrations. Catalysis by the antibody·**3** complex reaches a maximum at approximately 0.5 mM **6**, possibly due to competition for the porphyrin binding site by the aromatic substate at higher concentrations. We have not yet determined whether binding of **6** to the antibody contributes to catalysis; however, the wide range of acceptable reducing substrates in this system suggests that a specific binding site for **6** is unlikely. Hydrogen peroxide dependence was therefore investigated at the maximum, 0.5 mM **6**.

The peroxidation reaction displayed saturation kinetics with respect to hydrogen peroxide. Lineweaver–Burk plots for both free iron(III) mesoporphyrin and its antibody complex are shown in Figure 2 (lg·**3**: K_m = 24 mM, k_{cal} = 394 min^{-1}. **3**: K_m = 43 mM, k_{cal} = 166 min^{-1}). Peroxidases are among the most efficient enzymes known, with $k_{cal}/K_{m(H_2O_2)}$ values of approximately 10^7 M^{-1} s^{-1}. The corresponding value for the antibody complex is 274 M^{-1} s^{-1} compared to 64 M^{-1} s^{-1} for free mesohemin. The kinetic parameters for antibody-catalyzed peroxidation of the various reducing substrates are comparable: k_{cal}/K_m is 233 M^{-1} s^{-1} for ABTS (**7**) and 122 M^{-1} s^{-1} for pyrogallol (**4**). Peroxidation of these substrates was not catalyzed by free **3**.[12] The peroxidation of substrates **6** and **7** by a nonperoxidative heme protein, sperm whale myoglobin (Fluka), was barely detectable under identical reaction conditions.

The near-UV–visible spectrum of the mesohemin–antibody complex in reaction buffer (Figure 3) shows substantially greater absorbance in the Soret region than the free hemin, consistent with binding in a hydrophobic pocket. The antibody complex has major visible bands at 495 and 620 nm, typical of the spectrum of high-spin ferric heme proteins.[15,16] Free hemin in this case has closely spaced visible bands at 558 and 594 nm, more typical of low-spin ferric porphyrins,[16] presumably due to ligation of iron by Tris buffer. An increase in the pK_a of a coordinated water molecule upon binding to antibody (Fe^{III}–OH versus Fe^{III}–$^+OH_2$) would also explain the observed change in spin state. Although only one of the three antibodies specific for hapten **1** catalyzes mesohemin-dependent peroxidations, both of those catalyzing metal ion incorporation into porphyrin bind **3**, and the resulting complexes yield similar high-spin spectra. It will be of interest to determine whether the antibody contributes any axial ligands to the hemin iron in these complexes.

This work demonstrates that antibody complexes of natural heme cofactors can be prepared, and that these complexes can be expected to participate in many of the chemical processes that distinguish heme enzymes. In addition, eliciting antibodies to porphyrins with other *N*-alkyl groups should lead to catalysts with binding sites for both cofactor and a substrate, perhaps capable of promoting selective oxygen atom transfers.

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A New General Synthesis for Polylithium Organic Compounds

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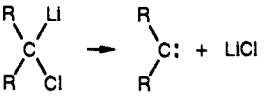
For many years it was thought by organic chemists that it was impossible to prepare polylithium organic compounds.[1] This misconception was changed by work in the early 1970s in our laboratory[2] and in the laboratory of Robert West at the University of Wisconsin.[3] It was perhaps for that reason that the reactions of organolithium reagents with chlorocarbons had not been thoroughly investigated with an eye toward synthesis of polylithium organic compounds.

It is generally believed to be impossible to employ organolithium reagents such as *n*-butyllithium or *tert*-butyllithium to polylithiate multiply halogenated organic compounds, especially if the halogens happen to be on the same carbon or on adjacent carbons. Extremely low yield reactions have been reported when halogens are on opposite ends of a long hydrocarbon chain.[4] The reason that such reaction chemistry is held to be impractical are 2-fold. Molecules having a lithium and a chlorine on the same carbon tend to undergo α lithium halide elimination producing carbenes and lithium halides. Lithium–halogen elimination also occurs



from adjacent carbons. This phenomenon is observed down to temperatures as low as −78 °C, and beyond that there have been few investigations. There is also the important competition between intermolecular coupling reactions of the lithium reagent with the chlorine-substituted species. Both types of reaction chemistry constitute "good" reasons why this reaction chemistry,

(1) Where polylithium compounds are defined as organic compounds containing two or more lithiums on the same carbon or two or more lithium on adjacent carbons. It was widely believed that having two lithiums on the same or adjacent carbon atoms would lead to a destabilization and lithium hydride elimination.
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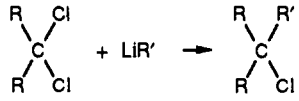

which we now report, is "unexpected". To cope with the first problem we elected to conduct the reaction at as low a temperature as possible in order to remove as much vibrational excitation as possible from the adjacent lithium and chlorine carbon bonds and minimize the possibility of elimination of lithium chloride. To minimize both reactions we selected reaction conditions with a very high concentration of *tert*-butyllithium so the reaction with the two chlorines could occur as rapidly as possible and compete with both the lithium chloride elimination reaction and the coupling reaction.

Thus, we have found at very low temperatures (−110 °C) using THF as a solvent a thermodynamic and kinetic regime where lithium–halogen exchange proceeds at a synthetic rate much more rapidly than lithium–hydrogen exchange. Temperatures are also low enough that there is a minimal problem with organolithium compounds reacting with the THF. The following new di- and trilithio compounds have been prepared:

$$Ph_2CCl_2 + 6t\text{-BuLi} \xrightarrow[THF]{-110\ ^\circ C} Ph_2CLi_2 \quad (31.0\%) \quad (1)$$

$$PhCHCl_2 + 6t\text{-BuLi} \xrightarrow[THF]{-110\ ^\circ C} PhCHLi_2 \quad (7.6\%) \quad (2)$$

$$PhCCl_3 + 9t\text{-BuLi} \xrightarrow[THF]{-110\ ^\circ C} PhCLi_3 \quad (3.2\%) \quad (3)$$

The reaction temperatures were kept as low as the solvent would allow, using a liquid nitrogen/ethanol slush with the temperature usually below −105 °C.

The reactions were quenched with d_1-ethanol and analyzed by ^1H and ^{13}C NMR on a GE GN-500 instrument and by GC/MS analysis on a Finnigan 4000 apparatus using a 30 m × 0.25 mm Heliflex bonded phase RSL-200 1.0-μm capillary column, with an injector temperature of 250 °C and an ionizer temperature of 150 °C. Percent deuterium incorporation was determined by mass spectroscopy.

Dilithiodiphenylmethane (1). Dichlorodiphenylmethane (1 mL, 5.2 mmol) was added dropwise over 5 min to a stirred solution of 21 mL of THF and 37 mL of 1.7 M *tert*-butyllithium (6:1) at −110 °C. A deep reddish-orange color became evident. Stirring was maintained for 30 min before derivatization by addition of twice as many equivalents of d_1-ethanol as *tert*-butyllithium at −110 °C. The reaction mixture was then allowed to warm to room temperature and stirred for several hours to insure complete derivatization and washed with H_2O to remove the lithium salts. The phases were then separated and the organic layers dried overnight with magnesium sulfate. The reactions were then distilled to remove solvents. Analysis of the sample gave a yield of 51.2% of diphenylmethanes, of which 60.6% was d_2-diphenylmethane, for an overall yield of 31.0% of dilithiated species **1**. MS: *m/e* (%) 167 (34.52), 168 (49.75), 169 (100), 170 (96.39), 171 (12.07). ^1H NMR (d_6-acetone): 7.14 (4), 7.20 (4), 7.23 ppm (2). ^{13}C NMR (d_6-acetone): 41.7 (5), 126.6, 129.0, 129.5, 142.0 ppm.

Dilithiophenylmethane (2). Dichlorophenylmethane (1 mL, 7.8 mmol) was added to 6 equiv of *tert*-butyllithium as above. A dark green color was visible. The reaction mixture was stirred for 2 h. The yield was determined to be 25.6% of phenylmethanes, of which 29.5% was d_2-phenylmethane, for an overall yield of 7.6% of **2**. MS: *m/e* (%) 91 (48.03), 92 (100), 93 (96.50), 94 (46.21), 95 (3.00). ^1H NMR (d_6-acetone): 2.30, 7.03 ppm (5). ^{13}C NMR (d_6-acetone): 21.4 (5), 125.4, 129.1, 130.1, 137.6 ppm.

Trilithiophenylmethane (3). Trichlorophenylmethane (1 mL, 7.1 mmol) was added to 9 equiv of *tert*-butyllithium under the conditions stated above. A deep purple color was detected. The reaction mixture was stirred for 1.75 h. The yield was determined to be 14.9% of phenylmethanes, of which 21.7% was d_3-phenylmethane, for an overall yield of 3.2% of **3**. MS: *m/e* (%) 91 (30.68), 92 (55.86), 93 (100), 94 (92.04), 95 (41.17), 96 (2.43). ^1H NMR (d_6-acetone): 7.03 ppm (5). ^{13}C NMR (d_6-acetone): 21.3 (7), 125.4, 129.2, 130.1, 137.7 ppm.

One may follow the progress of the lithium–halogen exchange reaction by quenching at various time intervals with d_1-ethanol. We have readily observed the increase of lithium substitution (lithium–halogen exchange) with time. For example, within the first 15 min after initiating reaction 1, Ph_2CDCl is obtained as a prominent hydrolysis product, whereas after 30 min no Ph_2CDCl is observed.

We believe the remaining metal–halogen exchanges to be stepwise, because upon using 1 equiv of *t*-BuLi for every halogen, the vibrant colors that are produced in these reactions disappear at around −90 °C before derivatization. Use of 3 equiv/halogen permits the polylithiated compounds to form. The excess *t*-BuLi is believed to react with the *t*-BuCl formed by the exchange reaction thus decreasing the reaction of *t*-BuCl with the newly lithiated compound.[5]

Presently the best solvent system we have found has been 4 equiv of THF/1 equiv of *t*-BuLi. This allows the *t*-BuLi to react; however, it is believed that a reaction also occurs between THF and lithium compounds at elevated temperatures.[6] This results in lower yields.

We think these experiments are the beginnings of a general synthesis for polylithium organic compounds. We expect higher yields to be forthcoming as a result of increased experience and more careful selection of conditions.

We have enough additional work in progress studying a variety of classes of multiply halogen substituted starting materials to state that this technique will be a more widely applicable synthesis. For example, we can convert hexachlorobenzene to hexalithiobenzene in over 60% yield.[7] Each reaction requires somewhat different conditions.

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Crystal Structure of *N*-Methyl-*N*-phenylretinal Iminium Perchlorate: A Structural Model for the Bacteriorhodopsin Chromophore[1]

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Bacteriorhodopsin (BR), one of a family of related pigments, is a membrane-bound polypeptide that acts as a light-driven proton pump.[2] The chromophore of BR consists of a retinal molecule covalently bound as a protonated Schiff base to the ε-amino group of lysine 216.[2] Despite the importance of this chromophore, no reliable X-ray structure of a retinal iminium salt has yet been reported.

N-Methyl-*N*-phenylretinal iminium perchlorate (**1**) was obtained by treating retinal with *N*-methylanilinium perchlorate. The phenyl substituent on **1** was selected in order to reduce the formation of various isomers and/or conformers during the

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