structure and chemical reactivity of **1** and related isolable carbenes.

Acknowledgment. We are indebted to F. Davidson for his work on the NMR spectroscopy and Dr. D. A. Dixon for helpful discussions.

Supplementary Material Available: A complete description of the X-ray crystallographic determinations of **1** and 1,3-di-1-adamantylimidazolium tetraphenylborate, including tables of fractional coordinates, isotropic and anisotropic thermal parameters, bond distances, and bond angles (17 pages); tables of structure factor amplitudes for **1** and 1,3-di-1-adamantylimidazolium tetraphenylborate (16 pages). Ordering information is given on any current masthead page.

Nuclear Magnetic Resonance of Hydroxyl and Amido Protons of Oligosaccharides in Aqueous Solution: Evidence for a Strong Intramolecular Hydrogen Bond in Sialic Acid Residues

Leszek Poppe and Herman van Halbeek*

Complex Carbohydrate Research Center and Department of Biochemistry
The University of Georgia, Athens, Georgia 30602

Received August 20, 1990

NMR studies of exchangeable protons of carbohydrates in aqueous solution are severely hampered by fast chemical exchange in conjunction with short relaxation times. Ten years after the first report[1a] of hydroxyl (OH) proton resonances for carbohydrates in aqueous solution at different pH values, studying OH protons of oligosaccharides in H_2O at or near ambient temperature to extract three-dimensional structural information from their resonances is still far from routine.[1b] A number of investigators have used DMSO as the solvent to circumvent chemical exchange of OH and NH protons with residual water.[2a-h] In this communication we present a new approach to the NMR study of OH and NH protons in aqueous solution. The OH protons and their spatial neighbors are observed in pre-steady-state NOE experiments[3] with careful water suppression. This approach largely overcomes the problem of fast exchange. We demonstrate that the OH proton at C8 of sialic acid (the terminal monosaccharide residue in many biologically active glycoprotein and glycolipid carbohydrate chains) is involved in a strong, specific intramolecular hydrogen bond that is independent of the type of linkage in which the sialic acid is involved.

The oligosaccharides studied are NeuAcα(2→3)Galβ(1→4)Glc (3′-sialyllactose) (**1**) and NeuAcα(2→6)Galβ(1→4)Glc (6′-sialyllactose) (**2**). They were dissolved in 85% H_2O/15% $(CD_3)_2CO$ and analyzed in 5-mm NMR tubes at 261 K with a Bruker AM 500 spectrometer. Chemical shifts are referenced to internal DSS, by setting the chemical shift of the ^1H signal of residual $(CD_3)CO(CD_2H)$ to δ 2.204 ppm. The pulse sequences used in the H_2O-suppressed one-dimensional (1D) TOCSY[4a,b] (total correlation spectroscopy, also known as homonuclear Hartmann-Hahn spectroscopy), ROESY[5a,b] (rotating-frame NOESY), and NOESY[6] (nuclear Overhauser enhancement spectroscopy) experiments are shown in Figure 1. Spectra of compounds **1** and **2** are shown in Figures 2 and 3, respectively. The signals of the nonexchangeable (CH) protons of **1** and **2** were assigned by means of 1D TOCSY[4b] in D_2O solution. The assignments for **1** were in agreement with those in the literature.[10,11] The complete assignment of the protons of **2** will be published elsewhere. The spectra of **1** and **2** (see Figures 2a and 3a) show NH and OH signals at δ 8-9 ppm and 5.6-7.6 ppm, respectively. The NH protons exchange with solvent protons at rates <0.1 s^{-1}; thus, they are readily observed as relatively narrow lined doublets. However, the exchange rates of OH protons with the solvent are greater than the HO-CH coupling constants, even at 261 K; consequently, any observable OH signals are broad, which makes their assignment by tracing J connectivities impossible.

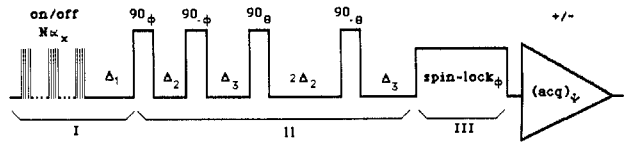


Figure 1. Pulse sequence for 1D ^1H NMR experiments in aqueous solution with H_2O suppression utilizing the 1-1 echo technique[7] (stage II), followed by a short trim pulse. Normal 1D spectra were obtained without stage I and with a short trim pulse (about 3 ms) in stage III. Stage I consisted of a DANTE pulse train[8] which was used for selective inversion (180° pulse) in TOCSY and ROESY experiments, or for presaturation in the NOE experiments. The delay Δ_1 is the mixing time for 1D pre-steady-state NOE experiments.[3,6] For other types of experiments, Δ_1 was set just long enough to allow for a change of decoupler power. In 1D ROESY[5a,b] experiments, stage III consisted of a long spin-lock pulse or a train of short pulses;[9] for 1D TOCSY[4a,b] experiments, stage III consisted of the MLEV-17 pulse sequence sandwiched between two trim pulses. The phases of pulses and receiver were as follows: ϕ = 4x, 4($-x$), 4y, 4($-y$), θ = x, y, $-x$, $-y$, $-x$, $-y$, x, y, y, $-x$, $-y$, x, $-y$, x, y, $-x$, ψ = x, $-x$, x, $-x$, $-x$, x, $-x$, x, y, $-y$, y, $-y$, $-y$, y, $-y$, y. The first pulse in the MLEV-17 sequence had a phase ϕ + 90°.

The subspectrum of the sialic acid residue of **1**, including its NH signal, was obtained by 1D TOCSY upon selective inversion of the NeuAc H3eq signal (Figure 2b). The H3ax, H4, H5, NH, and H6 signals are readily assigned from their multiplet structure and J signature. Figure 2c shows a 1D ROESY spectrum of **1** recorded with irradiation of the NeuAc NH proton. Several spatial connectivities to the NeuAc ring protons identified in trace 2b are clearly observable; the strongest NOE is visible between NH and H6. Traces d and e in Figure 2 show 1D NOE difference spectra recorded with irradiation of OH protons at δ 6.23 and 6.44 ppm. The strong NOE interaction between the proton at δ 6.23 and the ring proton H6 (trace d) is reminiscent of that observed for G_{M1} in DMSO solution;[2h] it identifies[13] the proton that

(1) (a) Symons, M. R. C.; Benbow, J. A.; Harvey, J. M. *Carbohydr. Res.* **1980**, *83*, 9-20. (b) Adams, B.; Lerner, L. *Abstracts of Papers*, 199th National Meeting of the American Chemical Society, Boston, MA; American Chemical Society: Washington, DC, 1990; CARB 93.

(2) (a) Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Tetrahedron* **1966**, *22*, 3061-3083. (b) Lemieux, R. U.; Bock, K. *Jpn. J. Antibiot. Suppl.* **1984**, *32*, 163-177. (c) Christofides, J. C.; Davies, D. B.; Martin, J. A.; Rathbone, E. B. *J. Am. Chem. Soc.* **1986**, *108*, 5738-5743. (d) Scott, J. E.; Heatley, F.; Hull, W. E. *Biochem. J.* **1984**, *220*, 197-205. (e) Dabrowski, J.; Poppe, L. *J. Am. Chem. Soc.* **1989**, *111*, 1510-1511. (f) Poppe, L.; Dabrowski, J.; Von der Lieth, C. W.; Koike, K.; Ogawa, T. *Eur. J. Biochem.* **1990**, *189*, 313-315. (g) Poppe, L.; Von der Lieth, C. W.; Dabrowski, J. *J. Am. Chem. Soc.* **1990**, *112*, 7762-7771. (h) Acquotti, D.; Poppe, L.; Dabrowski, J.; Von der Lieth, C. W.; Sonnino, S.; Tettamanti, G. *J. Am. Chem. Soc.* **1990**, *112*, 7772-7778.

(3) Gronenborn, A. M.; Clore, G. M. *Prog. NMR Spectrosc.* **1985**, *17*, 1-32.

(4) (a) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521-528. (b) Bax, A.; Davis, D. G.; Sarkar, S. K. *J. Magn. Reson.* **1985**, *63*, 230-234.

(5) (a) Bothner-By, A.; Stephens, R. L.; Lee, J. M.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811-813. (b) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207-213.

(6) Williamson, M. P.; Neuhaus, D. *J. Magn. Reson.* **1987**, *72*, 369-375.

(7) Sklenar, V.; Bax, A. *J. Magn. Reson.* **1987**, *74*, 469-479.

(8) Morris, G. A.; Freeman, R. *J. Magn. Reson.* **1978**, *29*, 433-462.

(9) Bax, A. *J. Magn. Reson.* **1988**, *77*, 134-147.

(10) Lerner, L.; Bax, A. *Carbohydr. Res.* **1987**, *166*, 35-56.

(11) Breg, J.; Kroon-Batenburg, L. M. J.; Strecker, G.; Montreuil, J.; Vliegenthart, J. F. G. *Eur. J. Biochem.* **1989**, *178*, 727-739.

(12) Kuroda, Y.; Wada, A.; Yamazaki, T.; Nagayama, K. *J. Magn. Reson.* **1989**, *84*, 604-610.

0002-7863/91/1513-363$02.50/0

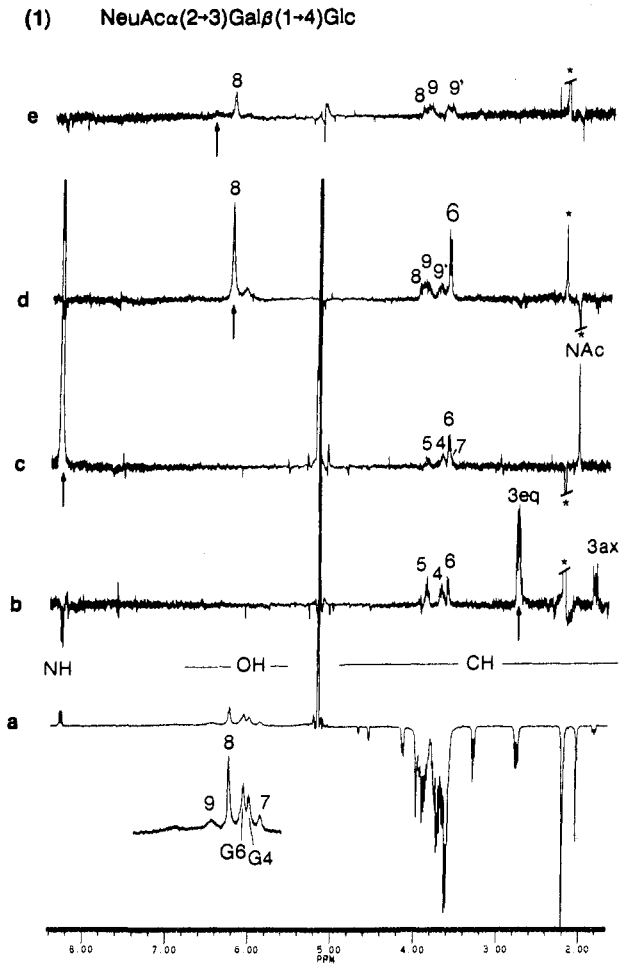


Figure 2. ^{1}H NMR spectra of trisaccharide **1** (5 mM, pH 7.7). Assignments refer to NeuAc protons, unless otherwise indicated (G stands for Gal). (a) 1D spectrum, $\Delta_2 = 0.25$ ms, $\Delta_3 = 0.33$ ms, 128 scans. (b) 1D TOCSY spectrum obtained with selective inversion of the NeuAc H3eq resonance by an 11-ms DANTE pulse, with mixing time 70 ms, $\Delta_2 = 0.19$ ms, $\Delta_3 = 0.30$ ms, 160 scans. (c) 1D ROESY spectrum with selective inversion of the NeuAc NH resonance, 150-ms mixing time, $\Delta_2 = 0.19$ ms, $\Delta_3 = 0.30$ ms, 640 scans. (d) 1D pre-steady-state NOE spectrum with preirradiation of NeuAc OH8 for 0.9 s, $\Delta_2 = 0.25$ ms, $\Delta_3 = 0.33$ ms, 640 scans. (e) 1D pre-steady-state NOE spectrum with preirradiation of NeuAc OH9 for 0.9 s, $\Delta_2 = 0.25$ ms, $\Delta_3 = 0.35$ ms, 416 scans. The relaxation delay was 1.1 s, and the acquisition time was 1.36 s for all spectra. The time-domain spectra were multiplied by an exponential function with line broadening of 0.5 Hz. The intensity of the residual solvent peak in difference spectra was reduced by postacquisition data manipulation to remove the zero-frequency component, thereby introducing a −180° first-order phase shift over the spectral width while slightly attenuating signals close to the H_2O resonance.[12] The artifacts marked by an asterisk are due to poor subtraction or spurious irradiation by the DANTE pulse.

resonates at δ 6.23 as NeuAc OH8. The chemical shift of the OH8 signal showed a remarkably small temperature coefficient (~0.001 ppm/K), and no line broadening occurred for OH8 upon increasing the pH value above 7. These observations indicate that the OH8 proton is involved in an intramolecular hydrogen bond. For G_{MI} in DMSO, this strong intramolecular hydrogen bond was experimentally verified to be between OH8 and either the NeuAc ring oxygen or the NeuAc carboxyl group, or both.[2h] The broad signal at δ 6.44 ppm was identified as NeuAc OH9, since presaturation gave dipolar connectivities to both NeuAc C9 methylene protons and also either a dipolar or exchange connectivity with the OH8 proton (Figure 2, trace e). A series of pre-steady-state NOE experiments with different presaturation times (spectra not shown) allowed the resonances at δ 6.05, 6.00, and 5.87 ppm to be assigned to protons Gal OH6, Gal OH4, and NeuAc OH7, respectively. The remaining OH protons could not be assigned unambiguously.

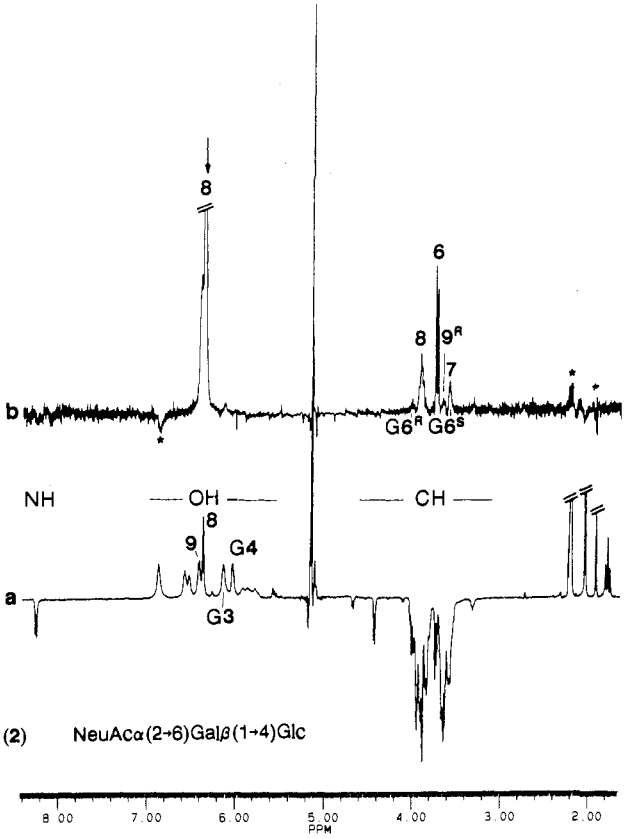


Figure 3. ^{1}H NMR spectra of trisaccharide **2** (5 mM, pH 7.4). Both spectra were recorded with $\Delta_2 = 0.4$ ms and $\Delta_3 = 0.4$ ms, with pulse sequences described in Figure 1. Assignments refer to NeuAc protons, unless otherwise indicated (G stands for Gal). (a) 1D spectrum, 128 scans. (b) Pre-steady-state NOE difference spectrum with preirradiation of NeuAc OH8 for 0.9 s; 640 scans. Both time-domain spectra were multiplied by a 0.5-Hz line-broadening exponential function. The intensity of the residual solvent peak in difference spectra was reduced by postacquisition data manipulation[12] (see caption to Figure 2). The artifacts marked by an asterisk are due to poor subtraction or spurious irradiation by the DANTE pulse.

The ^{1}H NMR spectrum of compound **2** is shown in Figure 3a. As for **1**, the NeuAc OH8 signal has the smallest exchange contribution to the line width and the smallest temperature variation of chemical shift (~0.001 ppm/K) among the OH signals. Trace b in Figure 3 shows a 1D pre-steady-state NOE difference spectrum obtained upon presaturation of OH8; the strong contact to NeuAc H6 is clearly visible.[13] These observations indicate that the same intramolecular hydrogen bonding as observed for **1** is also present in the α(2→6)-linked sialic acid of **2**, implying that the hydrogen bond is independent of the type of linkage in which the sialic acid is involved. In addition, proton OH8 gives NOE contacts to $H6^R$ and/or $H6^S$ of the adjacent Gal residue,[14] a valuable source of distance information since no in-

(13) Independent evidence for the assignment of the sharp OH signal at δ 6.23 ppm (for **1**) and δ 6.39 ppm (for **2**) to NeuAc OH8 was obtained from ^{13}C NMR spectra of **1** and **2** dissolved in H_2O, D_2O, and H_2O/D_2O (50/50, v/v). The ^{13}C spectra were recorded at ambient temperature, and isotope effects on the ^{13}C chemical shifts were traced according to the SIMPLE procedure (Christofides, J. C.; Davies, D. B. *J. Am. Chem. Soc.* **1983**, *105*, 5099–5105). Among the signals of hydroxyl-bearing carbons, only that of NeuAc C8 at δ 74.4 ppm (for **1**) and 74.3 ppm (for **2**) experienced a significant line broadening. The only OH signal observable in the ^{1}H spectra at ambient temperature is that of NeuAc OH8.

(14) The signals of protons Gal $H6^S$ and NeuAc $H9^R$ overlap (see Figure 3b). In addition, there is efficient magnetization transfer between the two methylene protons Gal $H6^R$ and $H6^S$. Therefore, it is not possible to define the interresidual NOE contact between NeuAc OH8 and the Gal C6 protons more precisely.

terglycosidic NOE contacts were found for this linkage in D_2O solution.[11]

The presented material proves the existence of a strong intramolecular hydrogen bond involving the OH8 proton of sialic acid residues in aqueous solution, analogous to that found in DMSO solution.[2h] This hydrogen bond is probably the main source of rigidity of the glycerol side chain in sialic acids that has been reported for various sialyl oligosaccharides in different solvents.[15a-e] Thus, we have shown that exchangeable protons of carbohydrates in H_2O can be analyzed by NMR spectroscopy, and that some OH protons serve as long-range sensors in conformational analysis of carbohydrates. Studying OH protons may yield unique information about hydrogen bonding and other three-dimensional structural features such as interproton distances that will add a new dimension to structural studies of complex carbohydrates in aqueous solution.

Acknowledgment. This research was supported by NIH Grants P41-RR-05351 and P01-AI-27135.

(15) (a) Brown, E. B.; Brey, W. S.; Weltner, W. *Biochim. Biophys. Acta* **1975**, *399*, 124-130. (b) Christian, R.; Schultz, G.; Brandstetter, H. H.; Zbiral, E. *Carbohydr. Res.* **1987**, *162*, 1-11. (c) Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8273-8279. (d) Poppe, L.; Dabrowski, J.; Von der Lieth, C. W.; Numata, M.; Ogawa, T. *Eur. J. Biochem.* **1989**, *180*, 337-342. (e) Sabesan, S.; Bock, K.; Lemieux, R. U. *Can. J. Chem.* **1984**, *62*, 1034-1045.

Redox-Switched Molecular Aggregates: The First Example of Vesicle Formation from Hydrophobic Ferrocene Derivatives

Julio C. Medina, Isabelle Gay, Zhihong Chen, Luis Echegoyen,* and George W. Gokel*

Department of Chemistry, University of Miami Coral Gables, Florida 33124

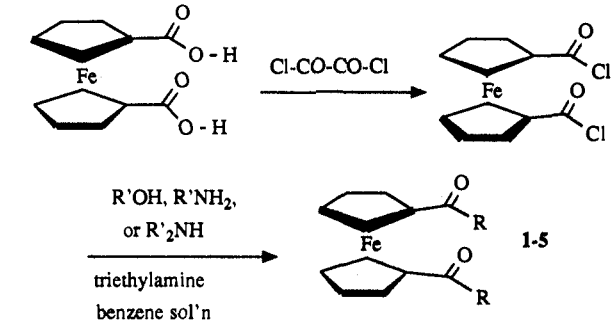
Received May 2, 1990
Revised Manuscript Received October 15, 1990

Ferrocene is a remarkable organometallic compound in many respects. Although containing an integral metal, formally in the Fe(II) state, it dissolves readily in hydrocarbon solvents such as hexane and must be considered extremely nonpolar. Our interest in redox-switched systems[1] led us to consider the ferrocene system as a neutral-cation redox pair that is an alternative to the nitrobenzene and anthraquinone neutral-anion redox pairs we have studied extensively. We are not the first to recognize the potential of ferrocene for its use either in switchable complexation or in molecular aggregation. Hall and co-workers,[2] Beer and co-workers,[3] and Saji and co-workers[4] have made several cation complexing agents based on compounds containing one or more ferrocene nuclei. Saji[5] has recently demonstrated that certain ferricinium salts aggregate into micelles. We report here a novel class of ferrocenyl steroids and present the first evidence for aggregation of these systems and particularly for vesicle formation controlled by redox-switched ferrocene amphiphiles.

Synthesis of the ferrocenyl amphiphiles was accomplished by two approaches shown herein as Schemes I and II. Compounds **1-5** were prepared by heating, under reflux (6 h), commercially available (Aldrich) 1,1'-ferrocenedicarboxylic acid with oxalyl chloride and a catalytic amount (1.1%) of pyridine and then treating the dichloride (52%, mp 92-94 °C) with the alcohol or amine in benzene solution containing triethylamine. The ester and amide derivatives were obtained according to Scheme I as follows: **1**, R = $OC_{17}H_{35}$, 69%; **2**, R = *O*-cholestanyl, 27%, **3**, R = *O*-cholesteryl, 45%; **4**, R = $NHC_{18}H_{37}$, 64%; and **5**, R = $N(C_{18}H_{37})_2$, 77%.

Compound **6** was prepared by treating [(dimethylamino)-methyl]ferrocene with dihydrocholesterol (cholestanol) in the presence of methyl iodide and acetone[6] as shown in Scheme II. Methyl iodide induces the Menschutkin reaction[7] followed by carbocation formation and alcoholysis to give the product shown in 7% yield. The syntheses and physical properties of compounds **1-6** (including **4**, previously reported[8]) are summarized in Table

Scheme I



Scheme II

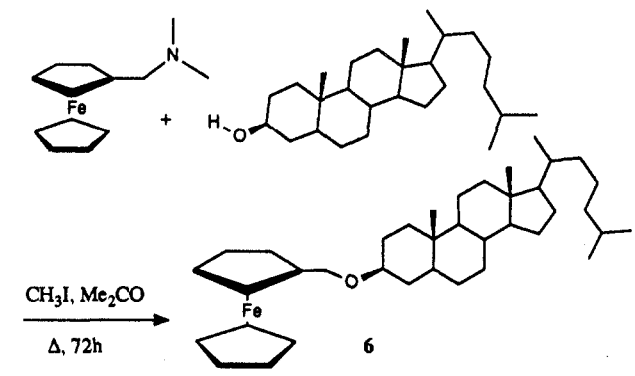


Table I. Ferrocenyl-Derived Amphiphile Precursors[a]

compd no.	substituent site	substituent identity	yield, %	$[\alpha]_D$[b]	mp, °C	$E^\circ_{1/2}$,[c] mV
1	1,1'	$COOC_{17}H_{35}$	69		65-67	972
2	1,1'	COO-dihydrocholesteryl	27	24.4	287-291	941
3	1,1'	COO-cholesteryl	45	-0.44	267-269	945
4	1,1'	$CONHC_{18}H_{37}$[d]	64		105-107	806
5	1,1'	$CON(C_{18}H_{37})_2$	77		32-34	711
6	1	CH_2O-dihydrocholesteryl	7	11.8	134-136	509

[a] All structures are new compounds and had combustion analyses within ±0.4% of theory for C and H as well as IR, NMR, and mass spectra (see supplementary material) in accord with their assigned structures. [b] In CH_2Cl_2, c = 1-4. [c] Determined vs Ag/AgCl in CH_2Cl_2. [d] Reference 8.

* Address correspondence concerning design and synthesis to G.W.G. and concerning aggregate formation and characterization to L.E.

(1) (a) Kaifer, A.; Echegoyen, L.; Gustowski, D.; Goli, D. M.; Gokel, G. W. *J. Am. Chem. Soc.* **1983**, *105*, 7168. (b) Gustowski, D. A.; Echegoyen, L.; Goli, D. M.; Kaifer, A.; Schultz, R. A.; Gokel, G. W. *J. Am. Chem. Soc.* **1984**, *106*, 1633. (c) Gustowski, D. A.; Gatto, V. J.; Kaifer, A.; Echegoyen, L.; Godt, R. E.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1984**, 923. (d) Kaifer, A.; Gustowski, D. A.; Echegoyen, L.; Gatto, V. J.; Schultz, R. A.; Cleary, T. P.; Morgan, C. R.; Rios, A. M.; Gokel, G. W. *J. Am. Chem. Soc.* **1985**, *107*, 1958. (e) Delgado, M.; Echegoyen, L.; Gatto, V. J.; Gustowski, D. A.; Gokel, G. W. *J. Am. Chem. Soc.* **1986**, *108*, 4135. (f) Echegoyen, L.; Gustowski, D. A.; Gatto, V. J.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1986**, 220. (g) Echeverria, L.; Delgado, M.; Gatto, V. J.; Gokel, G. W.; Echegoyen, L. *J. Am. Chem. Soc.* **1986**, *108*, 6825. (h) Echegoyen, L. E.; Yoo, H. K.; Gatto, V. J.; Gokel, G. W.; Echegoyen, L. *J. Am. Chem. Soc.* **1989**, *111*, 2440.

(2) Hall, C. D.; Sharpe, N. W.; Danks, I. P.; Sang, Y. P. *J. Chem. Soc., Chem. Commun.* **1989**, 417.

(3) (a) Beer, P. D.; Bush, C. D.; Hamor, T. A. *J. Organomet. Chem.* **1988**, *339*, 133. (b) Beer, P. D.; Sikanyika, H.; Blackburn, C.; McAleer, J. F. *J. Orgnanomet. Chem.* **1988**, *350*, C15. (c) Beer, P. D.; Blackburn, C.; McAleer, J. F.; Sikanyika, H. *Inorg. Chem.* **1990**, *29*, 378.

(4) Saji, T.; Kinoshita, I. *J. Chem. Soc., Chem. Commun.* **1986**, 716.

(5) Saji, T.; Hoshino, K.; Aoyagui, S. *J. Chem. Soc., Chem. Commun.* **1985**, 865.

(6) Gokel, G. W.; Marquarding, D.; Ugi, I. K. *J. Org. Chem.* **1972**, *37*, 3052.

(7) Takayama, C.; Fujita, T.; Nakajima, M. *J. Org. Chem.* **1979**, *44*, 2871.

0002-7863/91/1513-365$02.50/0