quadrupole coupling constant (QCC_{eff}) of 166 ± 2 kHz is constant between RH levels 0%–90%, indicating that there is no significant change in the amplitude of the motion.

The RNA results were compared to those of the analogous DNA dodecamer ([d(CG*CG*A*A*TTCG*CG*)]$_2$).[3,4] First, the ^{2}H T_1 values of RNA and DNA which are significantly different at the low hydration levels converge with increasing hydration (Figure 2). Second, the horn-to-horn splittings and line shapes of RNA are similar to those of DNA at all hydration levels up to 88% RH, indicating that their amplitudes of internal motions are similar. The differences in ^{2}H T_1 values between DNA and RNA at 0%, 66%, and 80% RH, given the similar line shapes, are consistent with a difference in rates despite the uncertainty in amplitudes (12.5 ± 2.0° assuming a two-site jump[12]). The percent differences in rates are calculated to be 200 ± 61% at 0% RH, 120 ± 65% at 66% RH, and 151 ± 94% at 80% RH. It is important to note that as the hydration level increases the internal rate decreases, assuming the motion is in the fast regime. The ^{2}H T_1 values, correspondingly, becomes less sensitive to changes in rate. Therefore, at higher hydration levels (88% and 90% RH), although the DNA and RNA line shapes are still similar, the uncertainty in the ^{2}H T_1 values precludes any knowledge of rate convergence or divergence.

Given the rates and amplitudes of motion in DNA and RNA from solid-state data, we can attempt to explain the ^{1}H T_1 differences observed in solution. The experimental RNA/DNA nonselective ^{1}H T_1 ratio equals 1.7 ± 0.2[1] for the purine H8 nuclei. The calculated ^{1}H T_1 ratio based on proton density using idealized coordinates[13,14] for A-form RNA and B-form DNA tumbling in solution[15,16] without internal motion is 1.2–1.3 and therefore falls short of the experimental range. The calculated ^{1}H T_1 ratio including internal motion parameters obtained at 80% RH (two-site jump: amplitude = 12.5 ± 2.0°, $^{RNA}\tau_c$ = 14 ± 3 ps, $^{DNA}\tau_c$ = 33 ± 7 ps) is 1.5–1.6 and is within the experimental bounds, implying that motion is required to account for the RNA/DNA ^{1}H T_1 ratio. However, calculated ^{1}H T_1 values fail to successfully reproduce the experimental values, e.g., $^{calcd}T_1$(DNA) = 3.24 ± 0.04 s and $^{exptl}T_1$(DNA) = 1.9 ± 0.3 s → %Δ(DNA) = 68% (%Δ(RNA) = 56%). There are several possible contributions to the ^{1}H T_1 value that were ignored. For example, differential $J_0(0)$ contamination of the nonselective proton H8 T_1 values between DNA and RNA could not be quantitated and could either raise or lower the calculated ^{1}H T_1 ratio. This exercise highlights the difficulty of extracting accurate dynamic information from high-resolution ^{1}H T_1 data of biological macromolecules. In spite of the inability to account for the nonselective ^{1}H T_1 differences in solution, solid-state experiments allow us to successfully extract dynamical information and to rigorously conclude that the amplitudes of DNA and RNA purines are similar at 0%, 66%, 80%, and 88% RH and that their internal rates of motion are different at 0%, 66%, and 80% RH.

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Registry No. r(CGCGAAUUCGCG), 118681-51-9; d(CGCGAATTCGCG), 77889-82-8.

(1) Wang, A. C.; Kim, S.-G.; Flynn, P. F.; Chou, S.-H.; Orban, J.; Reid, B. R. *Biochemistry* **1992**, *31*, 3941–3946.
(2) Tsang, P.; Vold, R. R.; Vold, R. L.; Kearns, D. R. 28th Experimental NMR Conference, 1987.
(3) Kintanar, A.; Huang, W.-C.; Schindele, D. C.; Wemmer, D. E.; Drobny, G. P. *Biochemistry* **1980**, *28*, 282–293.
(4) Huang, W.-C. Ph.D. Thesis, University of Washington, Seattle, WA, 1989.
(5) Alam, T. M.; Drobny, G. P. *Biochemistry* **1990**, *29*, 3422–3430.
(6) Bendel, P.; Murphy-Boesch, J.; James, T. L. *Biochim. Biophys. Acta* **1983**, *759*, 205–213.
(7) Brandes, R.; Vold, R. R.; Vold, R. L.; Kearns, D. R. *Biochemistry* **1986**, *25*, 7744–7751.
(8) Shindo, H.; Hiyama, Y.; Roy, S.; Cohen, J. S.; Torchia, D. A. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1631–1640.
(9) Brandes, R.; Vold, R. R.; Kearns, D. R. *J. Mol. Biol.* **1988**, *202*, 321–332.
(10) de Fontaine, D. L.; Ross, D. K.; Ternai, B. *J. Magn. Reson.* **1975**, *18*, 276–281.
(11) Taylor, J. R. *An Introduction to Error Analysis*; University Science Books: Mill Valley, CA, 1982.
(12) Lipari, G.; Szabo, A. *Biochemistry* **1981**, *20*, 6250–6256.
(13) Arnott, S.; Hukins, D. W. L. *Biochem. Biophys. Res. Commun.* **1972**, *47*, 1504–1509.
(14) Arnott, S.; Hukins, D. W. L. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 1392–1399.
(15) Tirado, M. M.; de la Torre, J. G. *J. Chem. Phys.* **1980**, *73*, 1986–1993.
(16) Eimer, W.; Williamson, J. R.; Boxer, S. G.; Pecora, R. *Biochemistry* **1990**, *29*, 799–811.

Deuterium Quadrupole Echo NMR Spectra and Spin–Lattice Relaxation of Synthetic Polyribonucleotides

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The nuclear spin relaxation behavior and internal motion of solid DNA fibers, films, and powders have been the subject of several studies in recent years.[1-8] So far similar NMR studies of solid polyribonucleotides have not been performed, although Bendel et al.[9] reported limited T_1 data for solid poly(I) and poly(I)·poly(C). In addition, Drobny et al.[10] have recently conducted deuterium relaxation studies of an *oligo*ribonucleotide. Here we report the results of deuterium T_1 measurements on synthetic polyribonucleotides deuterated in the purine 8-position. A partial account of these results was presented elsewhere.[11]

Sodium salts of the single-stranded polyribonucleotides pA, pI, pG, pU, and pC were obtained from Pharmacia P-L Biochemicals. Deuteration of the 8-position of pA, pI, and pG was achieved by heating the polyribonucleotides to 65 °C for about a week at pH 4–6 in a D_2O solution containing NaCl (0.05 M), Tris (0.01 M), Na_4EDTA (0.005 M), and the appropriate amount of DCl, followed by re-exchange of ND and OD by repeated lyophilization and redissolution in H_2O. The integrity of the single-stranded material was checked by polyacrylamide gel (7%) electrophoresis, and the concentration was determined by UV spectroscopy. Equivalent amounts of complementary single-stranded material were then added to allow formation of the duplexes poly(A)·poly(U), poly(I)·poly(C), and poly(G)·poly(C), henceforth referred to simply as AU, IC, and GC. It is particularly important to use accurate equivalents for poly(A) and poly(U), which are known to form triple-stranded UAU readily.[12] Duplex formation was verified by observation of the known hypochromic shifts in the UV spectrum, after which the duplex solutions were dialyzed for 8–24 h against distilled water and lyophilized to give fluffy, white powders. Polyacrylamide gel electrophoresis was used to determine the size distribution of the RNA samples; the average length was found to be 100 base pairs, with some material present in the range ~30–200 base pairs. A ca. 10% (w/w) extra sodium ion content was established from atomic absorption spectrophotometry.

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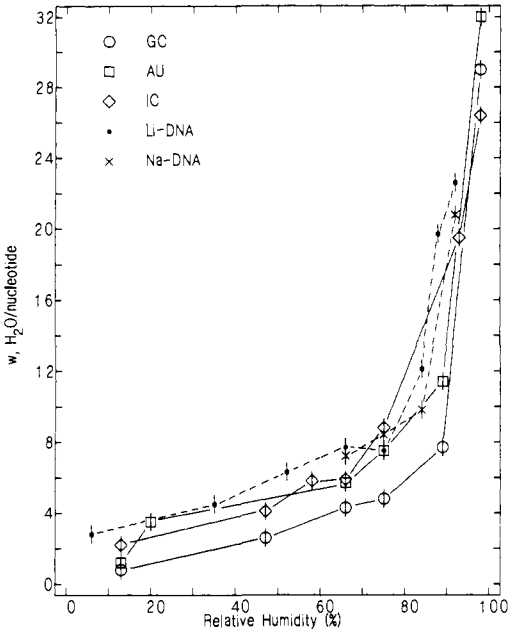

Figure 1. Water content, w, of solid polynucleotides samples determined gravimetrically (see text) as a function of relative humidity. The DNA data are from ref 6.

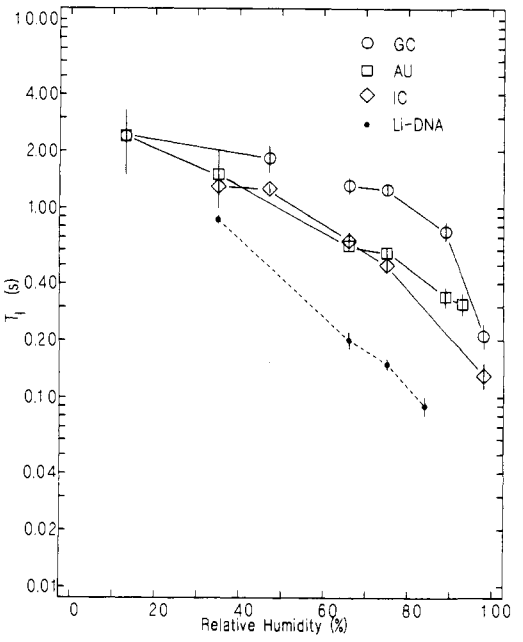

Figure 2. Spin-lattice relaxation times T_1 (26 °C, 38.4 MHz) of the purine 8-deuteron in poly(A)·poly(U), poly(I)·poly(C), and poly(G)·poly(C) as a function of relative humidity. T_1 data for calf thymus Li-DNA[6] are included for comparison. Not included in this graph are results obtained at 76.8 MHz for AU; $T_1 = 1.3 \pm 0.2$ s at 66% RH and $T_1 = 1.0 \pm 0.1$ s at 75% RH, both substantially longer than T_1 at 38.4 MHz. The quadrupole echo spectra were obtained with 90° pulse widths of 1.85 μs and a quadrupole echo pulse spacing of 40 μs. The T_1 values were determined from the growth of magnetization at the perpendicular edges of powder patterns recorded at a series of recycling delays.

The water content of each type of duplex was determined by desiccating a sample of known weight (~150 mg) at 0.1 Torr and 100–110 °C. Individual NMR samples were equilibrated at 25 °C over saturated salt solutions[13] at the desired relative humidities (%RH). The water content was determined by monitoring the weight gain of each sample, corrected for original water content as determined by the dehydration procedure described above. In Figure 1 the water content, w, defined as moles of H_2O per nucleotide, is plotted as a function of relative humidity. Also shown in Figure 1 are comparable results obtained previously[6] for Li- and Na-DNA. The data illustrate the slightly lower propensity of RNA to take up water, with GC hydrating less readily than AU or IC. AU and IC appear to absorb ca. 1 mol and GC ca. 3 mol of water *less* than DNA[6] at and below 75% RH. It should be noted that systematic errors in these measurements are hard to eliminate: not only is it difficult to remove the last traces of water, but dry nucleic acids take up water readily during the weighing operations. Nevertheless, the results are in concert with hydration studies of nucleic acid solutions,[14,15] which show that RNA binds less water than DNA and poly(G)·poly(C) less than poly(A)·poly(T).

Deuterium quadrupole echo spectra of the duplexes (80–130 mg, 5 mm o.d., 8000–20000 scans) were obtained at 26 °C and 38.4 MHz on a homebuilt pulsed NMR spectrometer. As for DNA, the RNA deuterium NMR spectra were observed to be classical "Pake patterns" with quadrupolar splittings that changed only slightly (<2 kHz) below 90% RH. "Rigid" values of the deuterium quadrupole coupling parameters, $e^2qQ/h = 176 \pm 1$ kHz, $\eta = 0.06 \pm 0.01$ and $e^2qQ/h = 178 \pm 1$ kHz, $\eta = 0.05 \pm 0.01$, respectively, were determined from AU and IC samples dried over P_2O_5 for several days at room temperature. They are comparable to those observed for dry calf thymus DNA samples.[6]

T_1 values of the Na salts of the three synthetic RNA duplexes were measured at 38.4 MHz. The results are plotted in Figure 2 as a function of relative humidity together with data obtained previously[6] for the Li salt of calf thymus DNA at the same Larmor frequency. It is clear that T_1 for RNA drops with increasing humidity, but the drop is less pronounced than for either Li- or Na-DNA.[3,6,7] Figure 2 also shows that at intermediate humidities T_1 of GC is longer than T_1 of either AU or IC. As noted in the caption to Figure 2, the T_1 value for AU at 76.8 MHz is substantially longer than that at 38.4 MHz, indicating that the motion responsible for relaxation is on the slow side of the T_1 minimum.

It is clear from this and previous relaxation studies[1-9] that internal motion increases and T_1 drops with hydration of solid nucleic acids. The present T_1 data make RNA appear more rigid than DNA, a difference that is also reflected in poly(I)·poly(C) ^{31}P NMR spectra,[16] which retain their full width at higher humidities than ^{31}P spectra of calf thymus DNA.[3] However, the hydration data in Figure 1 indicate that the smaller intermediate drop in T_1 observed for RNA is due not to RNA being inherently more rigid than DNA, but rather due to RNA absorbing less water than DNA at the same relative humidity. The longer T_1 value observed for GC than for AU and IC similarly correlates with the lower tendency of GC to take up water.

It remains to be determined if the internal motions in solid RNA differ from those in DNA. Low signal-to-noise ratios of our present NMR spectra have so far prevented a more thorough

(1) DiVerdi, J. A.; Opella, S. J. *J. Mol. Biol.* **1981**, *149*, 307.
(2) Fujiwara, T.; Shindo, H. *Biochemistry* **1985**, *24*, 896.
(3) Mai, M. T.; Wemmer, D. E.; Jardetzky, O. *J. Am. Chem. Soc.* **1983**, *105*, 7149.
(4) DiVerdi, J. A.; Opella, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 1761.
(5) Vold, R. R.; Brandes, R.; Tsang, P.; Kearns, D. R.; Vold, R. L.; Rupprecht, A. *J. Am. Chem. Soc.* **1986**, *108*, 302.
(6) Brandes, R.; Vold, R. R.; Vold, R. L.; Kearns, D. R. *Biochemistry* **1986**, *25*, 7744.
(7) Shindo, H.; Hiyama, Y.; Roy, S.; Cohen, J. S.; Torchia, D. A. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1631.
(8) Brandes, R.; Vold, R. R.; Kearns, D. R.; Rupprecht, A. *Biochemistry* **1990**, *29*, 1717.
(9) Bendel, P.; Murphy-Boesch, J.; James, T. L. *Biochim. Biophys. Acta* **1983**, *759*, 205.
(10) Wang, A. C.; Kennedy, M. A.; Reid, B. R.; Drobny, G. P., preceding communication in this issue.
(11) Tsang, P.; Vold, R. R.; Vold, R. L.; Kearns, D. R. 28th Experimental NMR Spectroscopy Conference, Asilomar, CA, 1987.
(12) Massoulie, J. *Eur. J. Biochem.* **1968**, *3*, 428.
(13) Weast, R. C. In *Handbook of Chemistry and Physics*, 52nd ed.; The Chemical Rubber Company: Cleveland, OH, 1971; p D-214.
(14) Marky, L. A.; Kupke, D. W. *Biochemistry* **1989**, *28*, 9982.
(15) Marky, L. A. Personal communication.
(16) Tsang, P.; Vold, R. R. Unpublished data.

analysis, but the amplitude and degree of uniaxiality of libration can in principle be determined from the orientation dependence of T_1 and from the observed *effective* quadrupole coupling parameters as was done for calf thymus DNA.[6,17] It would be particularly worthwhile to monitor the onset of large angle slow motion with increasing hydration of both polynucleotides via spin-alignment echo decays[18] or hole-burning experiments.[19,20]

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Registry No. Poly(A)·poly(u), 24936-38-7; poly(I)·poly(C), 24939-03-5; poly(G)·poly(C), 25280-45-9.

(17) Brandes, R.; Vold, R. R.; Kearns, D. R.; Rupprecht, A. *J. Mol. Biol.* **1988**, *202*, 321.
(18) Spiess, H. W. *J. Chem. Phys.* **1980**, *72*, 6755.
(19) Kuhns, P. L.; Conradi, M. S. *J. Chem. Phys.* **1982**, *77*, 1771.
(20) Lin, T.-H.; Vold, R. R. *J. Phys. Chem.* **1991**, *95*, 9032.

Synthesis, Structure, and Characterization of a Mixed-Valence [Ni(II)Ni(III)] Thiolate Dimer

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Stable Ni(III) thiolate compounds as models for the Ni(III)–cysteine center in hydrogenase enzymes continue to be of interest.[1] Attention has been drawn to the unusual properties of the nickel-containing hydrogenase enzymes.[2] In particular, these hydrogenase enzymes contain a stable Ni(III) center that exhibits a Ni(III)/Ni(II) redox couple between −0.390 and −0.640 V vs SCE.[2] Spectroscopic evidence suggests that the coordination environment about the nickel contains three or four sulfur ligands in a distorted octahedral or square pyramidal array.[3] In the quest for reasonable models for the nickel center in hydrogenases, it seemed that the ligand, $[P(o\text{-}C_6H_4S)_3]^{3-}$,[4-6] might provide a suitable coordination environment for the nickel. DeVries and Davison have shown that $[P(o\text{-}C_6H_4S)_3]^{3-}$ coordinates to Tc(III) to provide five- or six-coordinate complexes of the formulation $[Tc[P(o\text{-}C_6H_4S)_3](CNR)_n]$ (n = 1 or 2).[5] We report that the reaction of $[P(o\text{-}C_6H_4S)_3]^{3-}$ with Ni(II) yields a dimeric product, $[Ni_2\{P(o\text{-}C_6H_4S)_3\}_2]^{2-}$, that can be oxidized by two consecutive one-electron processes to give the stable [Ni(III)Ni(II)] and [Ni(III)Ni(III)] species, respectively.

(1) (a) Fox, S.; Wang, Y.; Millar, M. *J. Am. Chem. Soc.* **1990**, *112*, 3218. (b) Krüger, H. J.; Holm, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 2955. (c) Sellman, D.; Fünfgelder, S.; Pöhlmann, G.; Knoch, F.; Moll, M. *Inorg. Chem.* **1990**, *29*, 4772. (d) Kumar, M.; Day, R. O.; Colpas, G. J.; Maroney, M. J. *J. Am. Chem. Soc.* **1989**, *111*, 5974.
(2) (a) Lancaster, J. R., Jr., Ed. *The Bioinorganic Chemistry of Nickel*; VCH Publishers, Inc.: New York, 1988. (b) Hausinger, R. P. *Microbiol Rev.* **1987**, *51*, 22. (c) Cammack, R. *Adv. Inorg. Chem.* **1988**, *32*, 297.
(3) (a) Lindahl, P. A.; Kojima, N.; Hausinger, R. P.; Fox, J. A.; Teo, B. K.; Walsh, C. T.; Orme-Johnson, W. H. *J. Am. Chem. Soc.* **1984**, *106*, 3062. (b) Scott, R. A.; Wallin, S. A.; Czechowski, M.; DerVartanian, D. V.; LeGall, J.; Peck, H. D., Jr.; Moura, I. *J. Am. Chem. Soc.* **1984**, *106*, 6864.
(4) Abbreviations: $P(o\text{-}C_6H_4SH)_3$ = tris(2-mercaptophenyl)phosphine; $P(o\text{-}C_6H_4SH)_2(Ph)$ = bis(2-mercaptophenyl)phenylphosphine; $2\text{-}PhC_6H_4SH$ = 2-phenylbenzenethiol.
(5) (a) de Vries, N.; Davison, A.; Jones, A. G. *Inorg. Chim. Acta* **1989**, *165*, 9. (b) de Vries, N.; Cook, J.; Jones, A. G.; Davison, A. *Inorg. Chem.* **1991**, *30*, 2662.
(6) Block, E.; Ofori-Okai, G.; Zubieta, J. *J. Am. Chem. Soc.* **1989**, *111*, 2327.

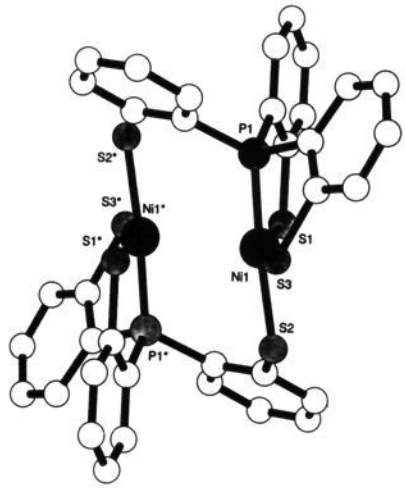

Figure 1. Structural diagram of $[Ni_2\{P(o\text{-}C_6H_4S)_3\}_2]^{2-}$. Selected bond distances (Å) and angles (deg): Ni1–S1 2.174 (4), Ni1–S2 2.233 (4), Ni1–S3 2.219 (5), Ni1–P1 2.109 (4); P1–Ni1–S1 89.2 (2), P1–Ni1–S2 171.8 (2), P1–Ni1–S3 84.6 (2), S1–Ni1–S2 96.7 (2), S1–Ni1–S3 156.0 (2), S2–Ni1–S3 87.8 (2).

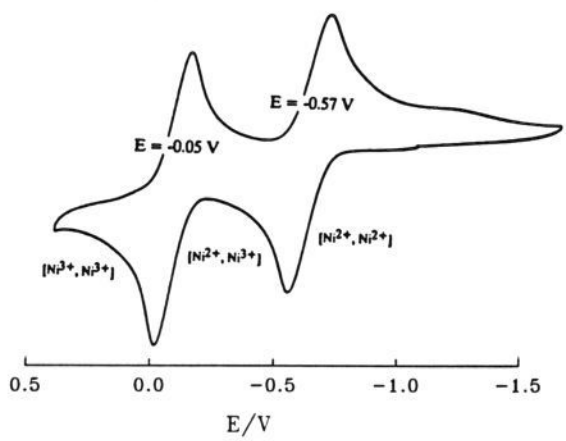

Figure 2. Cyclic voltammetric response (200 mV/s) of $[Pr_4N]_2[Ni_2\{P(o\text{-}C_6H_4S)_3\}_2]$ (**1**) in DMF with the indicated peak potentials vs SCE.

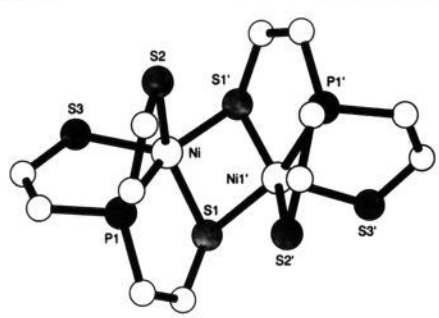

Figure 3. Structural diagram of the core of the $[Ni_2\{P(o\text{-}C_6H_4S)_3\}_2]^-$ anion (parts of the phenyl rings have been omitted for clarity). Selected bond distances (Å) and angles (deg): Ni1–Ni1′ 2.501 (2), Ni1–S1 2.251 (2), Ni1–S2 2.238 (2), Ni1–S3 2.373 (2), Ni1–P1 2.114 (2), Ni1–S1′ 2.260 (2); S1–Ni1–S1′ 82.13 (9), S1–Ni1–S2 153.71 (8), S1–Ni1–S3 105.49 (8), S1–Ni1–P1 88.17 (7), S1′–Ni1–P1 163.78 (8), S2–Ni1–S3 99.62 (8), S2–Ni1–P1 84.09 (7), S3–Ni1–P1 90.07 (7), Ni1–S1–Ni1′ 67.32 (7).

The reaction of $[P(o\text{-}C_6H_4SLi)_3]$ with 1 equiv of $Ni(acac)_2$ and $[(n\text{-}Bu)_4N]Br$ in methanol gives $[(n\text{-}Bu)_4N]_2[Ni_2\{P(o\text{-}C_6H_4S)_3\}_2]$ (**1**) in 72% yield. The X-ray structure of **1** is shown in Figure 1.[7] The dianion, $[Ni_2\{P(o\text{-}C_6H_4S)_3\}_2]^{2-}$, which has crystallographic

(7) Crystal data for **1**·$2CH_3OH$: $Ni_2S_6P_2O_2N_2C_{70}H_{104}$, monoclinic, $P2_1/c$, a = 11.777 (5) Å, b = 19.421 (7) Å, c = 16.911 (5) Å, β = 99.95 (4)°, V = 3810 (5) Å^3, Z = 2. A total of 1809 unique reflections with $I > 3\sigma(I)$ were refined to R = 0.065 and R_w = 0.065.

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