# The Second Isolable $B_{12}$-Thiolate Complex, (Pentafluorophenylthiolato)cobalamin: Synthesis and Characterization

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The synthesis, isolation, and characterization of (pentafluorophenylthiolato)cobalamin, $C_6F_5SCbl$, are reported, only the second isolable RSCbl known. The synthesis of $C_6F_5SCbl$ in 81% yield is accomplished by the dropwise addition of 0.93 equiv of $C_6F_5SH$ in MeOH under argon to 1 equiv of HOCbl·HOAc (50.7 mg) in MeOH, all at −15 °C and under darkened, red-light-only conditions. The desired $C_6F_5SCbl$ product was precipitated by the addition of cold, degassed acetone, isolated, and then characterized by FABMS and UV−visible, $^1H$ NMR, and $^{19}F$ NMR spectroscopies. Also reported is a $^1H$ NMR method for the detection of impurities in cobalamins. With the requisite, isolable RSCbl precursor to RS• and •Cbl radicals now in hand, the needed chemical precedent studies of the proposed H• abstraction step from the substrate in ribonucleoside triphosphate reductase by a RS• analogue of the protein−S• site are now possible.

## Introduction

Thiolatocobalamin complexes, RSCbl, are of considerable current interest as discrete, well-characterized anologues of the RS• plus •$Co^{II}$Cbl radical pairs that are key initial intermediates in coenzyme $B_{12}$ (adocobalamin or AdoCbl)-dependent enzymes such as ribonucleoside triphosphate reductase (RTPR).[1−4] In RTPR, a protein−S• •$Co^{II}$Cbl radical pair has recently been firmly established by Stubbe and co-workers on the basis of site-directed mutagenesis,[3] spectroscopic,[2,4] and especially their recent quantitative EPR evidence[5] for a protein−S• •$Co^{II}$Cbl radical pair separated by ca. 5−7 Å. In short, RTPR has emerged as the prototype enzyme in a class of enzymes[2,6] operating by a protein cysteinyl side-chain thiyl radical (protein−S•) mechanism.

Current interest in thiolatocobalamin complexes, RSCbl, therefore includes their use (i) as discrete chemical precursors to well-characterized RS• plus •$Co^{II}$Cbl radical pairs for use as spectroscopic models; (ii) as precursors to thiyl radicals, RS•, for chemical model studies of the proposed[7] H• abstraction step from the substrate by a protein−S• site; (iii) as complexes where the RS−Co bond dissociation energy could be established; and (iv) as a system where the information needed to understand other protein−SCoCbl products[8] can be obtained. In addition, any new, lower-temperature, controlled source of radicals (i.e., RS• in the present case) is always of both fundamental interest and practical value, for example, in free radical polymerizations or in the use of radicals in organic synthesis, as past studies of $RCoB_{12}$ complexes[9,10] and its models demonstrate.[11]

However, despite this interest and despite numerous reports of *solution* studies of RSCbls,[12] there is but one *isolable* RSCbl

(1) RTPR lead references (see elsewhere as well[2−5]): (a) Stubbe, J. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1990**, *63*, 349−419. (b) Stubbe, J. *J. Biol. Chem.* **1990**, *265*, 5329−5332. (c) Stubbe, J. *Annu. Rev. Biochem.* **1989**, *58*, 257−285. (d) Stubbe, J. *Mol. Cell. Biochem.* **1983**, *50*, 25−45. (e) Blakley, R. L. In *$B_{12}$*; Dolphin, D., Ed.; Wiley−Interscience: New York, 1982; Vol. 2, Chapter 14, pp 381−418. (f) Orme-Johnson, W. H.; Beinert, H. *J. Biol. Chem.* **1974**, *249*, 2338−2343.

(2) Licht, S.; Gerfen, G. J.; Stubbe, J. *Science* **1996**, *271*, 477−481.

(3) Booker, S.; Licht, S.; Broderick, J.; Stubbe, J. *Biochem.* **1994**, *33*, 12676−12685. Of the five catalytically active cysteines in RTPR, Cys 408 is implicated as the catalytically active protein−S• that initially abstracts H• from the substrate.

(4) Tamao, Y.; Blakley, R. L. *Biochemistry* **1973**, *12*, 24−34.

(5) Gerfen, G. J.; Licht, S.; Willems, J.-P.; Hoffman, B. M.; Stubbe, J. *J. Am. Chem. Soc.* **1996**, *118*, 8192−8197.

(6) Marsh, E. N. G. *BioEssays* **1995**, *17*, 431−441.

(7) (a) Ashley, G. W.; Harris, G.; Stubbe, J. *J. Biol. Chem.* **1986**, *261*, 3958−3964. (b) Ashley, G. W.; Stubbe, J. *Pharmacol. Ther.* **1986**, *30*, 301−329.

(8) Protein−S−CoCbl products have been suggested following inactivation of RTPR by 2′-deoxy-2′-methylenecytidine triphosphate and, apparently, a scrambling of CoCbl onto other HS−protein sites (Lawrence, C.; Stubbe, J., private communication). Hence a knowledge of bond energy and other properties of the RS−Co bonds should aid an understanding of these results as well.

(9) One example is the extensive use of R−Co(macrocycle) complexes as precursors to R• for use in organic synthesis; see refs 6−9 in ref 11b for a list of 24 lead references.

(10) Finke, R. G. In *Vitamin $B_{12}$ and $B_{12}$ Proteins*; Arigoni, D., Golding, B. T., Eds.; Wiley-VCH: New York, 1988; pp 383−402.

(11) (a) Stable or "persistent"[11b] •$Co^{II}$[porphyrin] or •$Co^{II}$[cobaloxime] metalloradical complexes are highly effective chain-transfer agents in free-radical polymerizations, lowering the MW of the polymerizations from tens of thousands, to thousands at ppm levels of the •$Co^{II}$[macrocycle] complex; see: Parshall, G. W.; Ittel, S. D. *Homogeneous Catalysis: The Applications and Chemistry of Catalysis by Soluble Transition Metal Complexes*, 2nd ed.; Wiley−Interscience: New York, 1992; pp 85−86 and references therein. (b) Daikh, B. E.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 2938−2943 and references 1a−c, 2, and 3 therein. (c) In addition, RS• are established autoxidation initiators due, in part, to their low $O_2$ sensitivity (now established to be due to the reversible reaction of RS• with $O_2$).[11e] Moreover, a RS−Co complex is likely a more reactive analogue[11f] of the disulfides, RS−SR, that have been extensively used as molecular weight control reagents in free radical polymerizations.[11g] In short, RS−Co complexes, such as those reported herein, and their ability to provide RS• and Co• at mild temperatures, are of both fundamental and practical interest. (d) Sheldon, R. A.; Kochi, J. K. *Metal Catalyzed Oxidation of Organic Compounds*; Academic Press: New York, 1981; p 27, eqs 36−38. Note that the claim, on p 27 just before eq 36, that thiyl radicals "... are inert to oxygen" is not exactly correct.[11e] (e) Evidence for the reversible reaction of RS• and $O_2$: Becker, D.; Swarts, S.; Champagne, M.; Sevilla, D. *Int. J. Radiat. Biol.* **1988**, *53*, 767 and references therein. (f) Lead refereneces to the facile $S_H2$ reactions of R−Co complexes: Finke, R. G.; Schiraldi, D. A. *J. Am. Chem. Soc.* **1983**, *105*, 7605 and see references 25a−j therein. (g) Poutsma, M. L. In *Free Radicals*; Kochi, J. K., Ed.; John Wiley: New York, 1973; Vol 2, p 136 and refs 57−59 and 140 therein.

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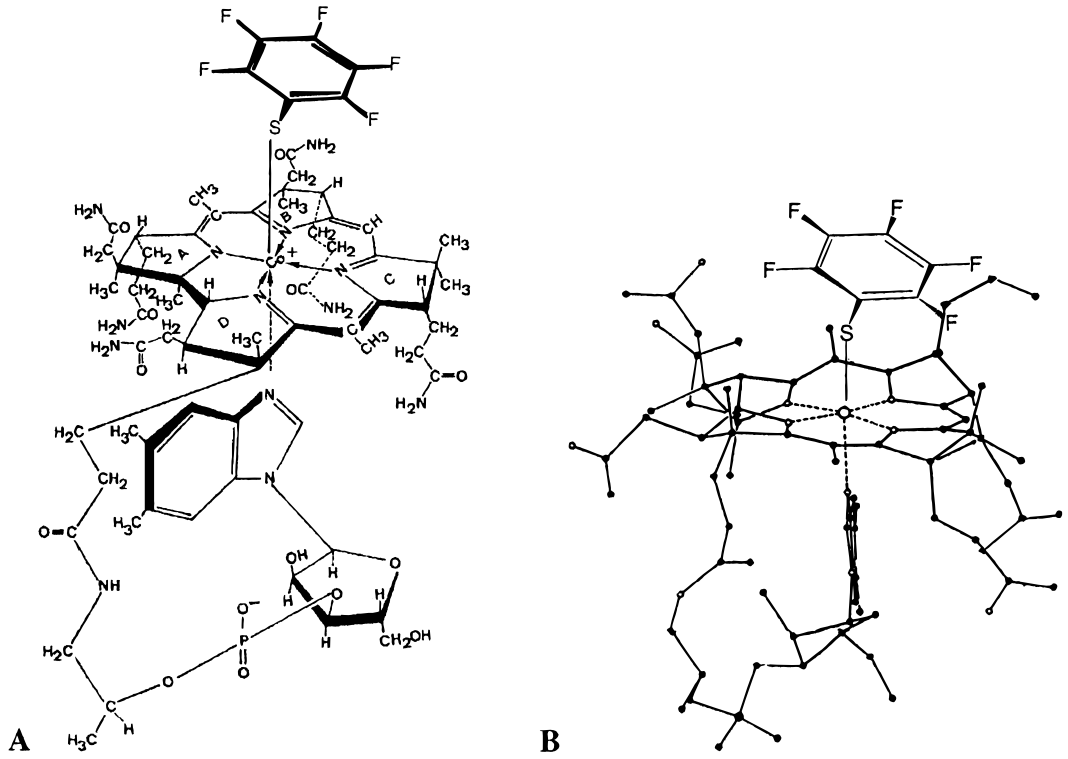



**Figure 1.** Two representations of (pentafluorophenylthiolato)cobalamin, $C_6F_5SCbl$, (A) one showing the atom types and their connectivity and (B) one emphasizing the molecule's approximate stereochemistry. See also the molecular-mechanics-based structure provided as Figure 4.

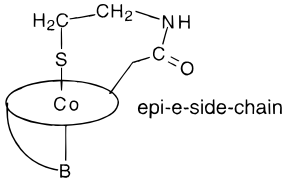
complex reported prior to the present work, glutathionylcobalamin, GluSCbl[13] (see also Hogenkamp's *intramolecular* thiolate complex of the epi-e-side chain[14,15]). This GluSCbl complex and its RS−Co bond have been characterized by EXAFS,[13a] 600 MHz NMR,[13b] and its unusual purple color and axial-ligand-sensitive[13a,16] $\gamma$ band at $\lambda_{max} = 372$ nm. It has not, however, been characterized by X-ray crystallography due to the lack of suitable single crystals that are free from disorder problems in the glutathionyl group, at least based on our own crystallographic efforts.[17] Moreover, GluSCbl is unsuitable for most of the goals (i−iv) listed above due to its facile intramolecular H• abstraction reaction.[18] There are several reports of stable RSCo(macrocycle) *$B_{12}$ model* complexes,[19−21] but in only selected cases are these expected to come even close to the properties of the more difficult to prepare, and to isolate pure, RSCbl complexes.[22]

Herein we report the synthesis, isolation, and characterization of (pentafluorophenylthiolato)cobalamin, $C_6F_5SCbl$, Figure 1. We picked this particular RS group for these initial studies because of (i) its well-precedented H• abstracting ability (i.e.,

(12) (a) Jacobsen, D. W.; Troxell, L. S.; Brown, K. L. *Biochemistry* **1984**, *23*, 2017−2025. (b) Nome, F.; Fendler, J. H. *J. Chem. Soc., Dalton Trans.* **1976**, 1212−1219. (c) Schrauzer, G. N.; Sibert, J. W. *Arch. Biochem. Biophys.* **1969**, *130*, 257−266. (d) Adler, N.; Medwick, T.; Poznanski, T. J. *J. Am. Chem. Soc.* **1966**, *88*, 5018−5020. (e) Peel, J. L. *Biochem. J.* **1963**, *88*, 296−308. (f) Hill, H. A. O.; Pratt, J. M.; Williams, R. J. P. *J. Theor. Biol.* **1962**, *3*, 423−445. (g) Pratt, J. M. *J. Chem. Soc.* **1964**, 5154−5160. (h) Hill, H. A. O.; Pratt, J. M.; Throp, R. G.; Ward, B.; Williams, R. J. P. *Biochem. J.* **1970**, *120*, 263−269. (i) Firth, R. A.; Hill, H. A. O.; Pratt, J. M.; Throp, R. G.; Williams, R. J. P. *J. Chem. Soc. A* **1969**, 381−386. (j) Cavallini, D.; Scandurra, R.; Barboni, E.; Marcucci, M. *FEBS Lett.* **1968**, *1*, 272−274. (j) Dolphin, D.; Johnson, A. W. *J. Chem. Soc.* **1965**, 2174−2181.

(13) GluSCbl references: (a) EXAFS: Scheuring, E. M.; Sagi, I.; Chance, M. R. *Biochemistry* **1994**, *33*, 6310−6315. (b) NMR: Brown, K. L.; Zou, X.; Savon, S. R.; Jacobsen, D. W. *Biochemistry* **1993**, *32*, 8421−8428. (c) Law, P. Y.; Wood, J. M. *J. Am. Chem. Soc.* **1973**, *95*, 914−919. (d) Adler, N.; Medwick, T.; Poznanski, T. J. *J. Am. Chem. Soc.* **1966**, *88*, 5018−5020. (e) Dolphin, D.; Johnson, A. W. *J. Chem. Soc.* **1965**, 2174−2181. (f) Dubnoff, J. W. *Biochem. Biophys. Res. Commun.* **1964**, *16*, 484−488.

(14) Anton, D. L.; Hogenkamp, H. P. C. *Vitamin $B_{12}$, Proceedings of the 3rd European Symposium on Vitamin $B_{12}$ and Intrinsic Factor*; Zagalak, B., Fredrich, W., Eds.; Walter de Gruyter: New York, 1979; pp 605−608.




(15) A related complex, but with a thiol bound to the c side chain, is being investigated by Professor Ken Brown and his research group: Brown, K. L.; Zou, X. *Abstracts of Papers*, 211th National Meeting of the American Chemical Society, New Orleans, LA; American Chemical Society: Washington, DC, 1996; INOR 0489.

(16) (a) Schneider, Z.; Stroinski, A. *Comprehensive $B_{12}$*; Walter de Gruyter & Co: Berlin, 1987; pp 44−92. (b) Giannotti, C. In *$B_{12}$*; Dolphin, D., Ed.; Wiley−Interscience: New York, 1982; Vol. 1, Chapter 11, pp 393−430. (c) Pratt, J. M. *Inorganic Chemistry of Vitamin $B_{12}$*; Academic Press: New York, 1972; Chapter 5, pp 46−67. (d) Hill, H. A. O.; Pratt, J. M.; Williams, R. J. P. *Chem. Br.* **1969**, 156−161. Hill, H. A. O.; Pratt, J. M.; Thorp, R. G.; Ward, B.; Williams, R. J. P. *Biochem. J.* **1970**, *120*, 263−269 (see p 264 and also Table 2 therein for an early summary of "atypical" RSCbl spectral data).

(17) Crystals of GluSCbl were grown by vapor diffusion of a saturated GluSCbl solution diluted in half by a 60% buffer solution of ammonium sulfate containing 50 mM *N*-2-hydroxyethylpiperazine *N*′-2-ethanesulfonic acid (HEPES) at pH 6. The dark red crystals grew as long rods which thickened slightly over time. A data set was collected on a single crystal (0.40 × 0.15 × 0.12 mm) at 173 K. The GluSCbl crystals are orthorhombic and belong to the space group $P2_12_12_1$ with lattice constants $a = 16.1403(4)$ Å, $b = 21.3865(2)$ Å, $c = 25.4074(7)$ Å, with $Z = 4$. Although a clear Co−S bond is apparent, only 8 of the 19 remaining non−hydrogen atoms of the glutathionyl moiety could located due, apparently, to significant apparent disorder and a lack of clear electron density in the remaining regions of the glutathione moiety (Suto, R.; Finke, R. G. Unpublished results). We are investigating this complex further, in collaboration with Professor L. Randaccio and his research group, and using synchrotron radiation.

of $C_6X_5S^\bullet$ radicals,[23] X = Cl, F) due to the increased strength of $C_6F_5S-H$ bond, the property that first brought the $C_6F_5S^-$ ligand to our attention; (ii) its anticipated stronger RS−Co bond energy;[24] (iii) its greater resistance to protonation at S (i.e., due to the electron withdrawing $C_6F_5^-$ group, $C_6F_5SH$ p$K_a$ 2.68[25] vs a typical RSH p$K_a$ of 10[26]); and, hence, (iv) because of the expectation that properties (ii) and (iii) would make $C_6F_5S-$Cbl easier to isolate.[27]

## Results and Discussion

**Five Possible Routes to RSCbl Complexes.** The general lack of stability and isolability of the RSCbl complexes reported to date caused us to take pause and carefully consider up front (a) the identifiable issues that have prevented their isolation as well as (b) all plausible alternative synthetic routes. Five possible routes to RSCbl were considered up front in this work, Figure 2, and further details are available to the interested reader as Supporting Information. The first two routes, involving $H_2OCbl^+$ (i.e., HOCbl·HX) and HOCbl in Figure 2, were chosen for initial study based on the expectation—which proved true in the studies which follow—that pH control is a major issue. Note that in the HOCbl + RSH route, second from the top in Figure 2, it is anticipated that the intimate mechanism of displacement involves a prior equilibrium, RSH + HOCbl to $RS^- + H_2OCbl^+$, and then displacement of $H_2O$, $RS^- + H_2OCbl^+ \rightarrow RSCbl + H_2O$, since $OH^-$ is such a poor leaving group.

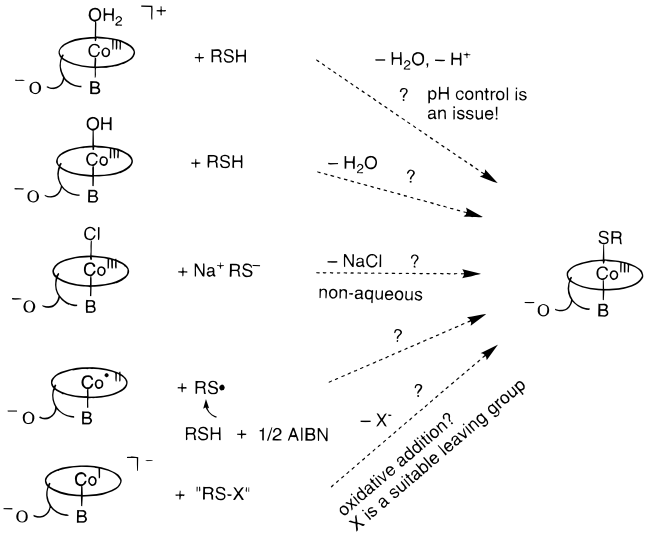



**Figure 2.** Five possible synthetic routes to RSCbl complexes.

**Development of a $^1$H NMR Method for Accessing Cobalamin Purity.** Since the usual HPLC method cannot be used to access the purity of $C_6F_5SCbl$ (as discussed later in text), as part of these studies we have developed a $^1$H NMR method to survey the purity of cobalamin complexes—a more direct and quantitative (±2−3%) way to access purity than was previously available.[28−30] Basically, the method involves surveying the $\delta$ = 5.5−8.5 ppm region where the aromatic cobalamin protons appear, specifically the three dimethylbenzimidazole protons, the C10 proton of the corrin ring, and the R1 proton of the

(18) Zhao, R.; Lind, J.; Merényi, G.; Eriksen, T. *J. Am. Chem. Soc.* **1994**, *116*, 12010−12015.
(19) Alkylthiolato(pyridinato)cobaloximes: (a) Polson, S. M.; Hansen, L.; Marzilli, L. G. *Inorg. Chem.* **1997**, *36*, 307−313. (b) Schrauzer, G. N. *Acc. Chem. Res.* **1968**, *1*, 97−105. (c) Schrauzer, G. N.; Windgassen, R. J. *J. Am. Chem. Soc.* **1967**, *89*, 3607−3612. Alkyl(thiolato)-cobaloximes: (d) Brown, K. L.; Kallen, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 1894−1901.
(20) Macrocycle = salen, salophen, $(dmg)_2$. Hennig, H.; Ritter, K. *J. Prakt. Chem.* **1995**, *337*, 125−132.
(21) Costa-type RS−Co complexes: (a) Alexander, V. *Inorg. Chim. Acta* **1989**, *163*, 143−151. (b) Pellizer, G.; Tauszik, G. R.; Costa, G. *J. Chem. Soc., Dalton Trans.* **1973**, 317−322.
(22) Elliott, C. M.; Hershenhart, E.; Finke, R. G.; Smith, B. L. *J. Am. Chem. Soc.* **1981**, *103*, 5558−5566. For example, the popular, readily prepared cobaloximes are too negative by ca. −400 mV in their $Co^{III}/Co^{II}$ redox potential as compared to cobalamins. However, they should have somewhat stronger Co−SR bonds which, depending upon the application or goal, could be a distinct advantage.
(23) $C_6X^5S^\bullet$ (X = Cl) and its ability to abstract H$^\bullet$ from unactivated alkanes are described in: Tanner, D. D.; Wada, N.; Brownlee, B. G. *Can. J. Chem.* **1973**, *51*, 1870−1879.
(24) Pauling's equation for the bond strength $D$(A−B) between elements A and B [of electronegativity $\chi_A$ and $\chi_B$ and A−A and B−B bond strengths of $D$(A−A) and $D$(B−B)] is $D$(A−B) = 1/2{$D$(A−A) + $D$(B−B)} + $a(\chi_A - \chi_B)^2$. Hence, one expects a stronger RS−Co bond for the more electronegative $C_6F_5-$ group in comparison to less electronegative aromatic and alkyl groups; see: Labinger, J. A.; Bercaw, J. E. *Organometallics* **1988**, *7*, 926−928 and references therein.
(25) Jencks, W. P.; Salvesen, K. *J. Am. Chem. Soc.* **1971**, *93*, 4433−4436.
(26) (a) See the Supporting Information, Table A. (b) Examples are 2-mercaptoethanol (p$K_a$ 9.61)[25] and cysteine (p$K_a$ 8.5); see: Kallen, R. G. *J. Am. Chem. Soc.* **1971**, *93*, 6227−6235.
(27) (a) The literature of RS−Co complexes confirms this expectation in that isolable RS−Co complexes where RS− is $C_6F_5S-$ or closely related analogues are known,[27b,c] while those of nonelectron-withdrawing RS− are uncommon. (b) Thompson, J. S.; Sorrell, T.; Marks, T. J.; Ibers, J. A. *J. Am. Chem. Soc.* **1979**, *101*, 4193. (c) Doppelt, P.; Fischer, J.; Weiss, R. *J. Am. Chem. Soc.* **1984**, *106*, 5188. Doppelt, P.; Fischer, J.; Ricard, L.; Weiss, R. *New J. Chem.* **1987**, *11*, 357. (d) Peach, M. E. *Can. J. Chem.* **1968**, *46*, 2699−2706. (e) Professor Steve Lippard and co−workers have, in completely independent studies, been able to isolate and crystallographically characterize two analogous $C_6F_5S-$Co complexes in the Co(tropocoronand) ligand system,[27e] Lippard, S. J., private communication (cited with permission). We thank Professor Lippard and his students for sharing their results prior to publication. (e) Co(tropocoronand) as a $B_{12}$ model system: Jaynes, B. S.; Doerrer, L. H.; Liu, S.; Lippard, S. J. *Inorg. Chem.* **1995**, *34*, 5735−5744; Jaynes, B. S.; Masschelein, A.; Ren, T.; Lippard, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 5589−5599.
(28) Well-known corrinoids that have been fully characterized by $^1$H NMR include adenosylcobalamin,[28a] aquacobalamin,[28b] cyanocobalamin,[28b] hydroxocobalamin,[28b] methylcobalamin,[28c,29b] dicyanocobalamin,[28d] azidocobalamin,[28b] glutathionylcobalamin,[28e] adenosylcobinamide,[28f] α-adenosylcobalamin,[28g] (adeninylpropyl)cobalamin,[28h] base-off adenosylcobalamin,[28i] neopentylcobinamide[28j] and various epi-isomers of cobalamins and cobinamides.[28j−m,29a] (a) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294. (b) Calafat, A. M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1993**, *115*, 9182−9190. (c) Brown, K. L.; Evans, D. R.; Zubkowski, J. D.; Valente, E. J. *Inorg. Chem.* **1996**, *35*, 415−423. (d) Brown, K. L.; Brooks, H. B.; Gupta, B. D.; Victor, M.; Marques, H. M.; Scooby, D. C.; Goux, W. J.; Timkovich, R. *Inorg. Chem.* **1991**, *30*, 3430−3438. (e) Brown, K. L.; Zou, X.; Savon, S. R.; Jacobsen, D. W. *Biochem.* **1993**, *32*, 8421−8428. (f) Pagano, T. G.; Yohannes, P. G.; Hay, B. P.; Scott, J. R.; Finke, R. G.; Marzilli, L. G. *J. Am. Chem. Soc.* **1989**, *111*, 1484−1491. (g) Brown, K. L.; Zou, X. *J. Am. Chem. Soc.* **1992**, *114*, 9643−9651. (h) Pagano, T. G.; Marzilli, L. G.; Flocco, M. M.; Tsai, C.; Carrell, H. L.; Glusker, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 531−542. (i) Bax, A.; Marzilli, L. G.; Summers, M. F. *J. Am. Chem. Soc.* **1987**, *109*, 566−574. (j) Brown, K. L.; Evans, D. R. *Polyhedron* **1995**, *14*, 2961−2977. (k) Brown, K. L.; Cheng, S.; Marques, H. M. *Inorg. Chem.* **1995**, *34*, 3038−3049. (l) Brown, K. L.; Cheng, S.; Zou, X.; Zubkowski, J. D.; Valente, E. J.; Knapton, L.; Marques, H. M. *Inorg. Chem.* **1997**, *36*, 3666−3675. (m) Brown, K. L.; Zou, X.; Wu, G.−Z. *Polyhedron* **1995**, *14*, 1621−1639.
(29) Examples of papers specifically using the $^1$H NMR aromatic region of cobalamins (although it is not explicitly stated how useful it is) include: (a) Brown, K. L.; Zou, X.; Evans, D. R. *Inorg. Chem.* **1994**, *33*, 5713−5720. (b) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729−1738. (c) Waddington, M. D.; Finke, R. G. *J. Am. Chem. Soc.* **1993**, *115*, 4629−4640. (d) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012−8018. (e) Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 585−592. (f) Brasch, N. E.; Hamza, M. S. A.; van Eldik, R. *Inorg. Chem.* **1997**, *36*, 3216−3222. (g) Brasch, N. E.; Müller, F.; Zahl, A.; van Eldik, R. *Inorg. Chem.* **1997**, *36*, 4891−4894.
(30) Brasch, N. E.; Finke, R. G. Manuscript in preparation.

ribose. This $^1$H NMR method finds its basis in the work of several investigators in the $B_{12}$ field,[28,29] but neither the exact details of the method nor its value has been reported previously. Further details and examples of this method will be reported elsewhere.[30]

**Synthesis and $^1$H NMR Characterization of $C_6F_5SCbl$.** Initially a synthesis from hydroxocobalamin (HOCbl) was attempted. This involved the initial deprotonation/desalting of HOCbl·HCl to give HOCbl using an Amberlite column,[31] followed by subsequent dropwise addition of 1 equiv of $C_6F_5SH$ in MeOH to 1 equiv of HOCbl in MeOH at −15 °C (eq 1).

$$\text{HOCbl} + C_6F_5SH \xrightarrow[\text{MeOH}]{-15\ ^\circ\text{C}} C_6F_5SCbl + H_2O \qquad (1)$$

(Details of the synthesis are given in the Supporting Information.) However, cobalamin impurities (≥15%, $^1$H NMR) were introduced as a result of the deprotonation/desalting process, impurities which are carried through to the product. Hence this ostensibly straightforward route to $C_6F_5SCbl$ failed to produce pure product.

The most successful synthesis utilized commercially available HOCbl·HOAc (∼11% impurity at $\delta$ = 7.25, 6.88, 6.46, 6.24, and 5.96 ppm, in $CD_3OD$, room temperature), plus the dropwise addition of 0.93 equiv of $C_6F_5SH$ in MeOH to 1 equiv of HOCbl·HOAc in MeOH at −15 °C, eq 2. Note that since the

$$\text{HOCbl·HOAc} + C_6F_5SH \xrightarrow[\text{MeOH}]{-15\ ^\circ\text{C}} C_6F_5SCbl + H_2O + \text{HOAc} \qquad (2)$$

commercially available cobalamins also contain $H_2O$ (up to 20%), an apparent slight excess of HOCbl·HOAc was used and the HOCbl·HOAc was dried in vacuo overnight prior to the synthesis. Isolation of $C_6F_5SCbl$ as a crimson solid was accomplished by concentrating the reaction solution and then adding cold acetone to induce its precipitation.

As noted above, $^1$H NMR proved crucial to determining the purity of the $C_6F_5SCbl$ product, particularly since extensive HPLC investigations failed to find conditions where $C_6F_5SCbl$ is both stable and separable from other possible cobalamin contaminants.[32] The aromatic $^1$H NMR chemical shifts of $C_6F_5SCbl$ in anaerobic $CD_3OD$ ($\delta$ = 7.17, 6.78, 6.42, 6.19(d), and 6.04 ppm at room temperature) are clearly different from those of the expected cobalamin contaminants, hydroxocobalamin, HOCbl ($\delta$ = 7.14, 6.84, 6.58, 6.16(d), and 6.11 ppm), or aquacobalamin, $H_2OCbl^+$ ($\delta$ = 7.17, 6.81, 6.64, 6.18(d), and 6.11 ppm). The $^1$H NMR spectrum of the aromatic region of $C_6F_5SCbl$ is given in Figure B, Supporting Information. The purity of the $C_6F_5SCbl$ product is 94 ± 2% by $^1$H NMR.

These syntheses are deceptively simple looking.[33] For example, when the alternative cobalamin precursor HOCbl·HCl is reacted directly with $C_6F_5SH$, cob(II)alamin ($Co^{II}Cbl$, $\lambda_{max}$ = 473 nm[34]) is produced as a side product because of the instability of $C_6F_5SCbl$ in acidic solution.[35] A modified synthesis was attempted using $LiOAc\cdot 2H_2O$ so as to avoid the formation of HCl produced during the reaction of HOCbl·HCl with $C_6F_5SH$ (i.e., to instead give HOAc and LiCl; details of the synthesis given in the Supporting Information), eq 3. In

$$\text{HOCbl·HCl} + C_6F_5SH + LiOAc\cdot 2H_2O \xrightarrow[\text{MeOH}]{-15\ ^\circ\text{C}} C_6F_5SCbl + 3H_2O + \text{HOAc} + \text{LiCl} \qquad (3)$$

this synthesis a 1.1 equiv of $C_6F_5SH$ in MeOH was added dropwise to a MeOH solution containing 1 equiv of HOCbl·HCl and 1 equiv of $LiOAc\cdot 2H_2O$. Control experiments in the absence of the cobalamin showed that both unreacted $LiOAc\cdot 2H_2O$ and product LiCl should both remain in solution under the added-acetone conditions used to precipitate the product. As desired, the $C_6F_5SCbl$ product proved to be extremely pure (≥98%) by $^1$H NMR; *however*, FABMS revealed significant (60 ± 20% by intensity) $[M + Li]^+$ and $[(M + Li) - C_6F_5S]^+$ peaks, in addition to the expected $[M + H]^+$ and $[(M + H) - C_6F_5S]^+$ peaks, due to the coprecipitation of $LiOAc\cdot 2H_2O$ or LiCl with the neutral $C_6F_5SCbl$ product. Alternatively, the $Li^+$ salts may be coprecipitating with the product via the formation of an ion-pair complex with the cobalamin (i.e., $C_6F_5SCbl\cdot(0.6 \pm 0.2)Li^+X^-$; $X = OAc^-$, $Cl^-$).

A demonstration of the sensitivity of these reactions to the exact reaction conditions is shown by the observation that no $Co^{II}Cbl$ is observed during the reaction of HOCbl·*HOAc* with $C_6F_5SH$; this suggests that the ∼0.01 M HCl causes product decomposition in MeOH, but not ∼0.01 M of the weaker acid HOAc, the acids being produced by the reaction of ∼0.01 M cobalamin (HOCbl·HCl or HOCbl·HOAc, respectively) with $C_6F_5SH$. Hence, solution pH is, as anticipated, an important consideration; the solution must not be too acidic or the product will decompose. However, the solution must also not be too alkaline, because of the known reduction of $Co^{III}Cbl$ by $RS^-$

(31) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820−4829.

(32) (a) Numerous HPLC experiments failed to find acceptable conditions for the separation of $C_6F_5SCbl$ from its possible HOCbl (·HCl or ·HOAc) or $H_2OCbl^+$ impurities. Specifically, if the product was eluted with a MeOH/aqueous phosphate buffer pH 6 mixture,[32b] and although good separation of $C_6F_5SCbl$ from $H_2OCbl^+$ was obtained, a considerable amount of $C_6F_5SCbl$ was converted to $H_2OCbl^+$ on the column and during the procedure. MeOH (100%) elutant conditions were also found to be unsatisfactory since $H_2OCbl^+$ elutes with the same retention time as $C_6F_5SCbl$, and HOCbl was found to behave irreproducibly on the column; typically the HOCbl peak moved to longer retention times and broadened considerably during the course of a series of sequential injections. One explanation for the latter results is the constantly varying pH of the column in the absence of a buffered elutant, due to the production of $H^+$ via the equilibrium $H_2OCbl^+$-(·Cl/·OAc) → HOCbl + HCl/HOAc, a reaction driven by the dilution conditions on the column and a phenomenon—deprotonation by dilution—which we have seen before in HPLC studies.[32d] (b) Aqueous phosphate buffer pH 6 was used to convert any HOCbl impurity to $H_2OCbl^+$ to allow for the easy detection of this impurity. (Note that it is not possible to convert the impurities to HOCbl via the elutant since the p$K_a$ of $H_2OCbl^+$ is ca. 8.1, but it is recommended that $C_{18}$ columns not be used near or above pH 8.5.[32c] (c) McMaster, M. C. In *HPLC: A practical user's guide*; VCH Publishers: New York, 1994. (d) Alelyunas, Y. W.; Fleming, P. E.; Finke, R. G.; Pagano, T. G.; Marzilli, L. G. *J. Am. Chem. Soc*. **1991**, *113*, 3781−3794 (see p 3792, second column, and footnote 53 therein).

(33) A referee commented on an earlier draft of this paper, which was less detailed and failed to document fully the complexities of these syntheses, that "the synthesis and isolation (of RSCbls complexes) are very straightforward...". We hope the present paper makes clearer the subtleties and complexities—which also surprised us somewhat—in the synthesis and isolation of pure examples of this little-studied subclass of cobalamins.

(34) Blaser, H.-U.; Halpern, J. *J. Am. Chem. Soc.* **1980**, *102*, 1684−1689.

(35) In *anaerobic*, aqueous pH 7.2 ± 0.1 potassium phosphate buffer (0.05 M), decomposition of $10^{-5}$ M $C_6F_5SCbl$ to $H_2OCbl^+$ occurs with $t_{1/2}$ ∼ 9 ± 1 h (3.5 half-lives of data collected). However, in pH 4.0 potassium hydrogen phthalate buffer $C_6F_5SCbl$ is hydrolyzed to $H_2OCbl^+$ with $t_{1/2}$ = 60 ± 10 min. Intriguingly, there is no change in the aromatic region of the $^1$H NMR of $C_6F_5SCbl$ over a period of 2 days in anaerobic MeOH, yet one sees ∼12% of the disulfide product, $C_6F_5S-S-C_6F_5$, by $^{19}$F NMR, indicating that 24% of Co−SR homolysis has occurred. (A control showed that the disulfide is *not* present in the starting complex.) We believe that this indicates that an insoluble (and thus invisible to NMR) cobalamin complex has been formed, the nature of which is under investigation. (b) In contrast, anaerobic, dilute (ca. $10^{-5}$ M) solutions of $C_6F_5SCbl$ are relatively stable *to light* in MeOH, a result that parallels the properies reported for solutions of cysteinylcobalamin.[12g]

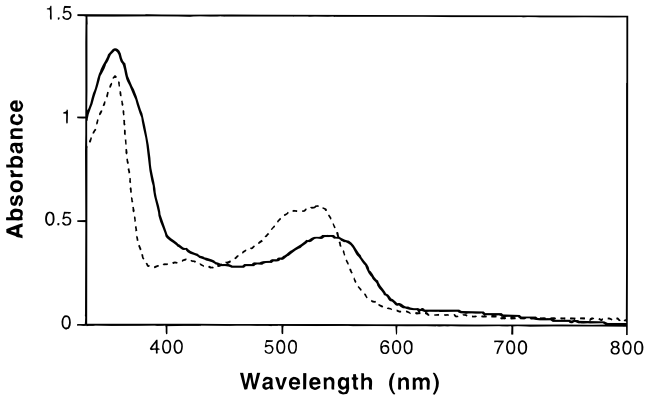



**Figure 3.** Visible spectra of $C_6F_5SCbl$ (—) and, for comparison, HOCbl (- - -) in MeOH at 25 °C. Note the shift toward longer wavelengths in the $C_6F_5SCbl$ spectrum, a shift which is characteristic of thiolatocobalamins[12b,f,j,50] (see also the discussion of this point in the main text).

to give $Co^{II}Cbl$[12a−g,36] (although $C_6F_5S^-$ would be expected to be less prone to such electron transfer compared with a typical thiol, due to the strong electron-withdrawing nature of the $C_6F_5-$ group).

The key idea used in this synthesis, then (and the subsequent syntheses of a series of other thiolatocobalamins[37]), is that the ideal solution pH needs to be ≥2 pH units below the p$K_a$ of the particular RSH (to avoid $RS^-$) but still needs to be high enough to deprotonate the monoacidic $RS(H)-Cbl^+$ to yield the desired RS−Cbl. Note here that the p$K_a$ of $RS(H)-Cbl^+$ is anticipated to be several (4−7[38]) p$K_a$ units lower than that of RSH due to its coordination to cationic $Co^{III}Cbl^+$. Temperature and solvent are two other important considerations. Repeating the reaction of HOCbl and $C_6F_5SH$ at room temperature produces up to 10% $Co^{II}Cbl$; hence the low-temperature −15 °C conditions are crucial. The choice of MeOH as solvent is also critical, since MeOH allows the low temperatures, is low boiling (and hence is readily removed), solubilizes both $B_{12}$ and most thiols of interest, and is protic so that pH control is possible.

**Further Characterization of $C_6F_5SCbl$.** Further characterization of the previously unknown $C_6F_5SCbl$ was accomplished using UV−visible, FABMS, and $^{19}F$ NMR spectroscopy. The UV−vis spectrum of $C_6F_5SCbl$ in MeOH (25 °C) is given in Figure 3, and exhibits absorption maxima at $\lambda_{max}$ ($\epsilon$, $M^{-1}$ $cm^{-1}$) of 546 nm ($8.2 \times 10^3$) and 376 nm (shoulder, $2.1 \times 10^4$), corresponding to the $\alpha/\beta$ and $\gamma$ bands observed for base-on thiolatocobalamins,[39] plus a maximum at 354 nm ($2.6 \times 10^4$). The absorption bands are shifted to longer wavelengths, resulting in a distinctive reddish-purple color, which is characteristic of *thiolato*cobalamins.[12b,f,13e,f,16] A FABMS of $C_6F_5SCbl$ in a *m*-nitrobenzyl alcohol matrix exhibits the expected protonated parent ion peak at *m*/*z* 1528.6 ($[M + H]^+$) and an informative fragmentation peak at *m*/*z* 1329.6 corresponding to the $[(M + H) - C_6F_5S]^+$ ion (Figure C, Supporting Information). The isotropic distribution pattern of the parent ion peak matches the expected computer-simulated spectrum (Figure D, Supporting Information).

The $^{19}F$ NMR spectrum of $C_6F_5SCbl$ in anaerobic $CD_3OD$ was obtained at room temperature (Figure E, Supporting Information). The $^{19}F$ chemical shifts data are listed in Table 1 along with literature $^{19}F$ chemical shifts for $C_6F_5SCH_3$ and $C_6F_5SH$ that allow one to make unequivocal peak assignments. Most importantly, $^{19}F$ NMR confirms the presence of a RS−Co bond, since there is the downfield shift accompanying the $Co^{III}$ for H substitution (i.e., when comparing $C_6F_5SCbl$ to the free thiol, $C_6F_5SH$) of primarily the fluorines which are ortho and para to S in the $C_6F_5S$ ligand, $\Delta\delta$ = 13 and 5 ppm, respectively (Figure F, Supporting Information).[40] Peak splitting arising from coupling was observed for all $^{19}F$ NMR signals; however, since the spin system is second order, coupling constants can only be obtained from simulation of the AA′M-M′X spin system. Moreover, recalling that CoCbl is chiral, $C_6F_5SCbl$ is, in the strictest terms and in the limit of slow rotation about the $S-C_6F_5$ bond, an ABMNX spin system. However, similar spin splitting patterns for $C_6F_5SCbl$ and $C_6F_5SH$ suggest that the AA′MM′X spin system and terminology are appropriate here, and rapid rotation about the $S-C_6F_5$ bond is implied. (Note that the $^{19}F$ NMR signals of $C_6F_5SCbl$ are considerably broader than $C_6F_5SH$, so that the speed of rotation about the $S-C_6F_5$ bond may be approaching the NMR time scale; NMR shimming or slower molecular tumbling of $C_6F_5SCbl$ (vs $C_6F_5SH$) were considered but were ruled out as possible sources of the broadening.)

A plausible ground-state structure of $C_6F_5SCbl$, generated by molecular mechanics calculation using Rappé's UFF force field,[41] shows that the $C_6F_5S-$ group prefers a position relatively coplanar to the corrin ring, Figure 4. Details of the UFF molecular mechanics minimizations are available in the Experimental Section.

Signals attributable to $C_6F_5S-SC_6F_5$ are also observed (2 ± 2%; Figure F, Supporting Information) after a $^{19}F$ NMR spectrum has been obtained (∼30 min at room temperature). Unfortunately, a $^{19}F$ NMR spectrum requires a reasonable number of transients to obtain an acceptable signal-to-noise ratio, which in turn makes it impossible to obtain a spectrum *immediately* after solution preparation. It is apparent that unreacted (excess) $C_6F_5SH$ shows up as $C_6F_5S-SC_6F_5$ in the

(36) Hogenkamp, H. P. C.; Bratt, G. T.; Kotchevar, A. T. *Biochemistry* **1987**, *26*, 4723−4727.

(37) CysSCbl, GluSCbl, and $C_6H_{11}SCbl$ have been synthesized; those results will be reported elsewhere along with further details of the $^1H$ NMR method developed herein, and its use to detect impurities in commercial cobalamin starting materials: Brasch, N. E.; Hsu, T.−L. C.; Doll, K. M.; Finke, R. G. *J. Inorg. Biochem.*, in press.

(38) (a) p$K_a$ of CysH = 8.5;[38e] p$K_a$ of $Cys(H)Cbl^+$ = 3.1 (i.e., 14−10.9 = 3.1).[12b] (b) p$K_a$ of $H_2O$ = 15.7; p$K_a$ of $H_2OCbl^+$ = 8.1; [38f] p$K_a$ of aquohydroxocobinamide = 10.3,[38g] (10.3[38h]). (c) p$K_a$ of the imino group of imidazole (ImH) = 14.3;[38i] p$K_a$ of imino group of $(HIm)-Cbl^+$ = 9.9,[38j] (9.6[38k]), (10.25[38l]). (d) p$K_a$ of the endocyclic imino group of histamine >14;[38m] p$K_a$ of endocyclic imino group of histaminecyanocobinamide = 11.34.[38n] (e) Kallen, R. G. *J. Am. Chem. Soc*. **1971**, *93*, 6227−6235. (f) Reenstra, W. W.; Jencks, W. P. *J. Am. Chem. Soc*. **1979**, *101*, 5780−5791. (g) Baldwin, D. A.; Betterton, E. A.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1983**, 217−223. (h) Marques, H. M.; Bradley, J. C.; Brown, K. L.; Brooks, H. *Inorg. Chim. Acta* **1993**, *209*, 161−169. (i) Hamza, M. S. A.; Pratt, J. M.; *J. Chem. Soc., Dalton Trans.* **1994**, 1367−1369. (j) Marques, H. M.; Marsh, J. H.; Mellor, J. R.; Munro, O. Q. *Inorg. Chem. Acta* **1990**, *170*, 259−269. (k) Eilbeck, W. J.; West, M. S. *J. Chem. Soc., Dalton Trans.* **1976**, 274−278. (l) Hanania, G. I. H.; Irvine, D. H. *J. Chem. Soc.* **1964**, 5694−5697. (m) Marques, H. M.; Egan, T. J.; Marsh, J. H.; Mellor, J. R.; Munro, O. Q. *Inorg. Chim. Acta* **1989**, *166*, 249−255. (n) Marques, H. M. *Inorg. Chim. Acta* **1990**, *174*, 271−273.

(39) (a) Note that the absence of the protonated, base-off form of $C_6F_5SCbl$ is confirmed by the absence of an absorption band between 440 and 480 nm[39b] characteristic of base−off cobalamins. (b) *$B_{12}$*; Dolphin, D., Ed.; Wiley−Interscience: New York, 1982; Vol. 1, p 357.

(40) The expected 2:2:1 integrals were observed in the $^{19}F$ NMR spectra of $C_6F_5SCbl$ and $C_6F_5SH$ (Figures E and F, Supporting Information).

(41) The Universal Force Field (UFF): (a) Rappé, A. K.; Casewit, C. J.; Colwell, K. S.; Goddard, W. A., III; Skiff, W. M. *J. Am. Chem. Soc.* **1992**, *114*, 10024−10035. (b) Casewit, C. J.; Colwell, K. S.; Rappé, A. K. *J. Am. Chem. Soc.* **1992**, *114*, 10035−10046. (c) Casewit, C. J.; Colwell, K. S.; Rappé, A. K. *J. Am. Chem. Soc.* **1992**, *114*, 10046−10053. (d) Rappé, A. K.; Colwell, K. S.; Casewit, C. J. *Inorg. Chem.* **1993**, *32*, 3438−3450.

**Table 1.** $^{19}$F Chemical Shifts[a,b] for $C_6F_5SCbl$, $C_6F_5SH$, and $C_6F_5SSC_6F_5$

| compound | *ortho*-F | *meta*-F | *para*-F |
|---|---|---|---|
| $C_6F_5SCbl$ (rt) | −126.4 (2F)[c] | −164.8 (2F)[d] | −156.8 (1F)[e] |
| $C_6F_5SH$ | −139.1 (2F)[c] | −164.8 (2F)[d] | −161.8 (1F)[e] |
| $C_6F_5SSC_6F_5$ | −133.5 (2F)[c] | −162.2 (2F)[d] | −150.6 (1F)[e] |
| | [lit.: −134.1 (2F)[c,f]] | [lit.: −162.9 (2F)[d,f]] | [lit.: −151.3 (2F)[e,f]] |
| $C_6F_5SCH_3$[g] | −135.8 (2F)[c] | −164.4 (2F)[d] | −157.2 (1F)[e] |

[a] Negative values refer to higher field vs the 0 ppm external reference, $CFCl_3$. (The older literature below uses the older convention in which positive numbers refer to higher fields vs $CFCl_3$; see Drago R. S. *Physical Methods for Chemists*, 2nd ed.; Saunders College: Orlando, FL, 1992; p 235.) Chemical shifts are for room-temperature data. [b] Solvent: $CD_3OD$. [c] "A" part of an AA′MM′X spin system. [d] "M" part of an AA′MM′X spin system. [e] "X" part of an AA′MM′X spin system. [f] Solvent: acetone-$d_6$; see: Peach, M. E. *Can. J. Chem.* **1968**, *46*, 2699−2706. Note that the reported coupling constants therein appear to have been obtained incorrectly (directly from the spectrum), not via simulation of the AA′MM′X spin system. [g] Dungan, C. H.; Van Wazer, J. R. *Compilation of Reported $^{19}$F Chemical Shifts;* Wiley: New York, 1970.

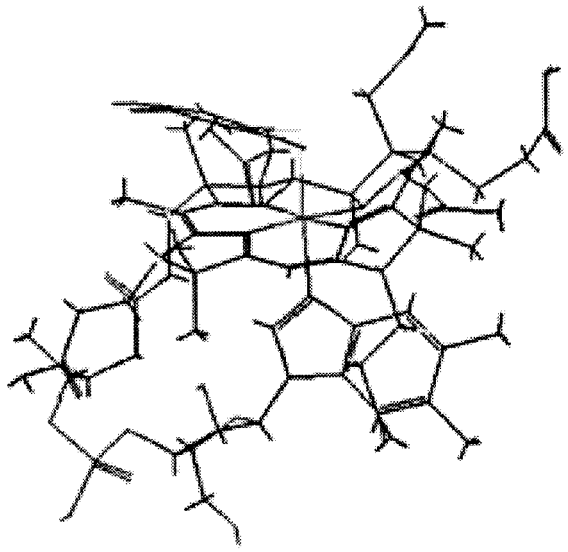


**Figure 4.** The gas-phase structure of $C_6F_5SCbl$ as predicted by UFF molecular mechanics.[41] The predicted Co−SR bond distance is ∼2.32 Å, fully in line with the generally 2.3 Å distances seen by X-ray crystallography for RS−Co(macrocycle) $B_{12}$ model and related complexes.[51] Note, however, that such gas-phase molecular mechanics structures of large complexes such as $B_{12}$ can be more readily distorted vs solid state, X-ray diffraction structures.[47] Further details of this, and of studies of other RSCbl and RCbl complexes, by UFF molecular mechanics will be reported elsewhere.[47]

product (i.e., it is also precipitated from solution with excess acetone) from the results of trials which used a slight excess of $C_6F_5SH$; hence excess $C_6F_5SH$ should be avoided. Also, $C_6F_5$-SCbl is only metastable in anaerobic $CD_3OD$, since, for example, anaerobic solutions ∼2 days old exhibit ∼10% more $C_6F_5S-SC_6F_5$ compared to those ∼0.5 h old. Interestingly, the $^1$H NMR is unchanged within this time. Bubbling $O_2$(g) through the solution for 3 min also has no effect on the spectrum; that is, no $H_2OCbl^+$ is observed, which in turn demonstrates that $Co^{II}Cbl$ is not being produced. Note that these $^{19}$F and $^1$H NMR studies reveal (a) the value and use of the $^1$H NMR detection of cobalamin impurities developed herein and (b) another example of the well-known lesson in chemistry to use as many physical handles and tools as are possible for investigating any chemical phenomenon.

**5−11% Impurities in Commercial Cobalamins.** Of broader interest to workers in cobalamin chemistry is that a closer examination of the $^1$H NMR of the HOCbl·HCl and HOCbl·HOAc revealed that the same cobalamin impurity was present in both cobalamin *starting materials* (∼5% and ∼11%, respectively; $\delta$ = 7.25, 6.88, 6.46, 6.24, and 5.96 ppm in $CD_3OD$, room temperature). This as of yet unidentified impurity is also found in the Amberlite column prepared HOCbl (∼8%). Since it is present in a higher percentage than in HOCbl·HCl, this impurity (plus the others, ca. 15% total) must have been produced as side products during the Amberlite column deprotonation/desalting process. This $\delta$ = 7.25, 6.88, 6.46, 6.24, and 5.96 ppm impurity is also present in the $C_6F_5SCbl$ product from both the HOCbl and HOCbl·HOAc syntheses; that is, *the ∼5% impurity found in the $C_6F_5SCbl$ product is an impurity carried through from the commercial starting materials*. The best synthesis developed herein, using HOCbl·HOAc, converts the ∼89% of starting material that is pure into 95% pure $C_6F_5SCbl$ product. Note, however, that ∼95% purity in an isolable RSCbl other than glutathionylcobalamin is, at present, the state of the art.

Of interest here, of course, is the identity of the impurity in the commercial HOCbl·HCl and HOCbl·HOAc. Comparison of the chemical shifts of the impurity with those of methylcobalamin, cyanocobalamin, chlorocobalamin, aquacobalamin, and hydroxocobalamin *ruled out* each of these as the possible impurity. The chemical shifts were also compared without success to the monocarboxylic acids of cyanocobalamin[42] which are known to be present in the commercially available cyanocobalamin.[42] So far we have not been able to identify the impurity, which will be necessary before rational attempts to remove it from the starting material (HOCbl·HOAc) can be undertaken. Note that since it is a common practice to isolate cobalamins via precipitation with excess acetone, it is probable that such a ∼5% impurity is present in many if not most complexes made from these commercial cobalamins and where the resultant product is not chromatographable (or has not been purified by chromatography). It is just that, until now, these levels of impurities remained generally undetected.

**Summary.** To summarize, the first two (HOCbl·HX and HOCbl) of five possible methods for the syntheses of RSCbls have been surveyed, including three variants of the HOCbl·HX method (HOCbl·HOAc, HOCbl·HCl, HOCbl·HCl+LiOAc). The results have led to an optimum synthesis for $C_6F_5SCbl$ beginning with HOCbl·HOAc and which gives 81% yields of *isolated* product, material whose purity (∼95%) is restricted only by the purity of the starting material. The presence of the desired Co−S bond in $C_6F_5SCbl$ is firmly supported by (i) the mass spectral evidence, (ii) the unusual UV−vis spectrum, which is characteristic of a crystallographically characterized[17] Co−SR bond, (iii) the $^{19}$F NMR chemical shifts, plus (iv) the lack of any other known position in $B_{12}$ (i.e., besides cobalt) where a thiolate ligand could coordinate.

Elsewhere we will report our studies on the Co−SR bond homolysis to produce clean $Co^{II}Cbl$ plus $C_6F_5^{\bullet}$ radical pairs,[43] although there are some surprises in that chemistry as well. And, now that the requisite readily prepared, isolated, and characterized $C_6F_5SCbl$ precursor is in hand, studies are in progress to exploit the anticipated superior $H^{\bullet}$ abstraction properties of the $C_6F_5S^{\bullet}$ radical[23] and other $RS^{\bullet}$ radicals in ribonucleotide reductase chemical precedent studies. Those studies will also be described elsewhere in due course.

## Experimental Section

Hydroxocobalamin hydrochloride (HOCbl·HCl, stated purity (by manufacturer's TLC method) ≥98%) and the acetate salt of hydroxocobalamin [HOCbl·HOAc, stated purity (by manufacturer's TLC method) ≥97%] were obtained from Sigma. Pentafluorothiophenol ($C_6F_5SH$, 97%) was purchased from Aldrich and was distilled before use at ∼40 °C (vapor temperature) under partial vacuum (water aspirator) and stored under argon; ≥98% pure by $^1$H NMR, $CD_3OD$, room temperature $\delta = -139.1$, −161.8, and −164.8 ppm (see Table 1 and Figure F in the Supporting Information). Deionized distilled water was purified by filtration through a Barnstead Nanopure system. Methanol was refluxed over $Mg(OMe)_2$ and always freshly distilled prior to use.

UV−vis absorption spectra (±1 nm) were recorded on a Hewlett-Packard model 8452A UV−visible diode array spectrophotometer equipped with a built-in thermoelectric Peltier cell block temperature controller operating at 25.0 ± 0.1 °C. Air-sensitive samples were prepared in Schlenk cuvettes.[44]

$^1$H NMR spectra were recorded on either a Varian Mercury-300 or Inova-300 spectrometer at room temperature and were referenced internally to 0 ppm with TMS. $^{19}$F NMR were recorded on the same instruments operating at 282.4 MHz and were referenced externally to $CFCl_3$ (0 ppm). Anaerobic $C_6F_5SCbl$ solutions were prepared in $CD_3OD$ in a Vacuum Atmospheres glovebox ($N_2$, ≤2 ppm $O_2$).

FAB mass spectrometry was performed on a VG AutoSpec double-focusing mass spectrometer operating in the positive dectection mode. Samples were dissolved in *m*-nitrobenzyl alcohol matrixes.

HPLC analysis was performed using an Alltech Versapack $C_{18}$ reversed-phase column (300 mm long × 4.1 mm ID) in conjunction with an IBM-controlled Hewlett-Packard Ti series HPLC instrument equipped with a multiwavelength detector, in-line solvent degassing unit, and a quaternary multipump. Eluting fractions were monitored by the PC-controlled Hewlett-Packard HPLC 2D ChemStation (DOS series) data processing program. HPLC solvents were filtered daily using 0.22 μm Magna Nylon membranes (Fisher) and HPLC sample solutions were filtered through a 13 mm nylon Acrodisc with 0.2 μ pore prior to injection.

**Synthesis of $C_6F_5SCbl$. (i) From HOCbl·HOAc.** All syntheses of $C_6F_5SCbl$ were performed in darkened, red light only conditions in an argon atmosphere using standard Schlenk techniques.[45] HOCbl·HOAc was dried in vacuo overnight, at room temperature prior to use. Freshly distilled MeOH was degassed by 3 pump/thaw cycles. HOCbl·HOAc (50.7 mg, $3.6 \times 10^{-5}$ mol) was dissolved in ∼2 mL of MeOH in a 50 mL sidearmed Schlenk flask containing a stir bar, and the solution was then cooled to −15 °C (dry ice/ethanol bath). A solution of freshly distilled $C_6F_5SH$ (4.5 μL, $3.4 \times 10^{-5}$ mol) in 1 mL of MeOH was added dropwise to the cobalamin solution via a 5 mL syringe with stirring. Additional MeOH (∼1 mL) was used to complete the transfer of remaining $C_6F_5SH$ into the flask containing the cobalamin solution, and the resultant solution was stirred at −15 °C for ca. 2−5 min. A ∼15 μL aliquot of the solution was withdrawn for dilution in ∼3 mL of MeOH (i.e., to $\sim 5 \times 10^{-5}$ M) to confirm that the reaction has occurred by UV−vis spectroscopy ($\lambda_{max} = 546$, 376 (shoulder), 354, and 312 nm in MeOH, 25 °C). The solution was concentrated to ∼0.3 mL under vacuum at 0 °C (ice bath) and ∼40 mL of cold acetone (−50 °C, degassed by bubbling Ar through it for an hour) were added immediately via a 20 mL syringe to yield a crimson precipitate. The solid RSCbl product was removed by filtration (Schlenk filtration apparatus), washed with three 10 mL portions of cold (∼−50 °C) degassed acetone, and dried in vacuo (0.01 Torr, overnight, rt). Yield: 45 mg (81%). $^1$H NMR aromatic signals (in $CD_3OD$, referenced to TMS, rt): $\delta$ 7.17, 6.78, 6.42, 6.19 (d), and 6.04 ppm; 94 ± 2% purity by integration (see Figure B, Supporting Information). $^{19}$F NMR signals (in $CD_3OD$, referenced to $CFCl_3$, rt): $\delta$ −126.4 (2F, *σ*-F-PhSCbl, A part of an AA′MM′X spin system), −156.8 (1F, *p*-F-PhSCbl, X part of an AA′MM′X spin system), and −164.8 (2F, *m*-F-PhSCbl, M part of an AA′MM′X spin system). UV−vis $\lambda_{max}$ ($\epsilon$, $M^{-1}$ $cm^{-1}$, in MeOH): 546 ($8.2 \times 10^3$), 376 (shoulder, $2.1 \times 10^4$), 354 ($2.6 \times 10^4$), 312 ($2.0 \times 10^4$), and 254 nm ($2.5 \times 10^4$). FABMS (in *m*-nitrobenzyl alcohol matrix) calcd molecular mass for $[C_6F_5SCbl + H^+]$, $C_{68}H_{89}O_{14}N_{13}F_5PSCo$, 1528.5; found, *m/e* 1528.6 ($[M + H]^+$) and 1329.6 ($[(M + H) - C_6F_5S]^+$) (Figures C and D of the Supporting Information). No HPLC conditions could be found where $C_6F_5SCbl$ was stable.[32] Solid $C_6F_5SCbl$ can be handled in air, but it is recommended that the compound be stored in a refrigerator under argon in the absence of light.

**(ii) From HOCbl.** See the Supporting Information.

**(iii) From HOCbl·HCl, with and without $LiOAc·2H_2O$.** See the Supporting Information.

**Synthesis of $C_6F_5S-SC_6F_5$.**[46] Bromine (0.25 g) was added dropwise to a solution of $C_6F_5SH$ (0.4 g) in 2.5 mL of acetic acid. Removal of acetic acid under vacuum resulted in a brown residue, which was then redissolved in 0.5 mL of hot ethanol. Upon cooling, white needles, $C_6F_5S-SC_6F_5$, formed; mp 50−52 °C (lit.:[46] mp 51 °C). $^{19}$F NMR ($CD_3OD$ at room temperature) $\delta$ −133.5 (2F, *o*-F-PhSCbl, A part of an AA′MM′X spin system), −150.6 (1F, *p*-F-PhSCbl, X part of an AA′MM′X spin system), −162.2 (2F, *m*-F-PhSCbl, M part of an AA′MM′X spin system); ($C_6D_6$ at rt) $\delta$ −132.1 (2F, *o*-F-PhSCbl),

(42) A putative "$B_{12}$ prime" form ("$B_{12}$′") of cyanocobalamin found at ∼0.08% levels[42a] in commercial cyanocobalamin (CNCbl) and initially thought to be an isomeric form of cyanocobalamin[42a−f] was subsequently shown to be monocarboxylic acids of cyanocobalamin.[42g] Hence, since HOCbl·HX are most likely prepared commercially from CNCbl (Sigma was unable to supply us with this information), it is possible that the impurity is one of the monocarboxylic acids of CNCbl. However, the aromatic $^1$H NMR chemical shifts of the impurities in $D_2O$ ($\delta = 7.24$, 6.83, 6.67, 6.43, 6.19, and 6.07 ppm (present in both HOCbl·HCl and HOCbl·HOAc), pD = 6.1 ± 0.2, room temperature, internally referenced to TSP) are clearly different from those of CNCbl-b−monocarboxylic acid ($\delta = 7.28$ (B7), 7.10 (B2), 6.54 (B4), 6.37 (R1), 6.10 (C10), and 6.10 (C10);[42h] in $D_2O$, pH 7.3, room temperature, referenced to TSP) and CNCbl-e−monocarboxylic acid ($\delta = 7.29$ (B7), 7.13 (B2), 6.52 (B4), 6.37 (R1), and 6.10 (C10);[42h] in $D_2O$, pH 7.3, room temperature, referenced to TSP). It has been claimed that a HOCbl′ form of HOCbl can constitute up to 15% of HOCbl stored in a refrigerator for a couple of years.[42a] Interestingly, when asked, Sigma advised us that their current batch of HOCbl·HOAc is ∼10 years old! Since it is also likely that the "HOCbl′" form of HOCbl is also actually a mixture of monocarboxylic acids of HOCbl, the $^1$H NMR chemical shifts of our impurity should be compared with these species to see if the impurity is a monocarboxylic acid of HOCbl. Unfortunately, however, the isolation and $^1$H NMR of monocarboxylic acids of HOCbl havc not been reported in the literature. Sigma informed us that they too are also concerned with the purity of their HOCbl·HOAc, and have recently investigated it for possible cyanocobalamin (1−3%) and dicyanocobinamide (undetectable amount) contaminants. (a) Katada, M.; Tyagi, S.; Rajoria, D. S.; Nath, A. In *Porphyrin Chemistry Advances*; Longo, F. R., Ed.; Ann Arbor Science: Ann Arbor, MI, 1979; pp 157−164. (b) Kohli, R. K.; Nath, A. *Inorg. Chim. Acta* **1987**, *136*, 75−79. (c) Mishra, P. K.; Gupta, R. K.; Goswami, P. C.; Venkatasubramanian, P. N.; Nath, A. *Biochim. Biophys. Acta* **1981**, *668*, 406−412. (d) Katada, M.; Tyagi, S.; Nath, A.; Petersen, R. L.; Gupta, R. K. *Biochim. Biophys. Acta* **1979**, *584*, 149−163. (e) Mishra, P. K.; Gupta, R. K.; Goswami, P. C.; Venkatasubramanian, P. N.; Nath, A. *Polyhedron* **1982**, *1*, 321−325. (f) Kohli, R. K.; Nath, A. *Biochim. Biophys. Res. Commun.* **1984**, *125*, 698−703. (g) Marzilli, L. G.; Parker, W. O., Jr.; Kohli, R. K.; Carell, H. L.; Glusker, J. P. *Inorg. Chem.* **1986**, *25*, 127−129. (h) Pagano, T. G.; Marzilli, L. G. *Biochem.* **1989**, *28*, 7213−7223.

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−148.2 (1F, *p*-F-PhSCbl), −160.0 (2F, *m*-F-PhSCbl). The above spectrum is consistent with literature (acetone-$d_6$ at 25 °C [27d]) $\delta$ −134.1 (2F, *o*-F-PhSCbl), −151.3 (1F, *p*-F-PhSCbl), and −162.9 (2F, *m*-F-PhSCbl).

**Molecular Modeling of $C_6F_5SCbl$.** All molecular modeling studies were done on an SGI Indigo-2 100 MHz computer, using the Universal Force Field (UFF) published by Rappé and co-workers.[41] To verify the validity of model structures in UFF compared to crystal structures and Marques' and Brown's modified MM2 force field for corrins, a number of controls were completed previously and will be reported in detail elsewhere.[47a] Specifically, the crystal structures used by Marques and Brown to parametrize their MM2 force field were minimized using our standard conditions listed below. The results[47] indicate that UFF performs equivalent to the modified MM2 field of Marques and Browns, at least for the particular subset of corrins modeled first elsewhere.[48]

The thiolatocobalamin structure was built into the force field by manually deleting the adenosyl ligand of a previously minimized structure of adenosylcobalamin (minimized by subjecting an imported crystal structure[49a] to 20 cycles of annealed dynamics (0.001−600.0 K with a 10 K step size), with the lowest-energy structure being further minimized under default conjugate gradient conditions. The $C_6F_5S$− ligand was then drawn in, the resulting structure was subjected to 10 cycles of annealed dynamics[49b] (0.001−600.0 K with a 10 K step size), and then the lowest-energy structure was further minimized under default conjugate gradient conditions.[49b]

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**Supporting Information Available:** Thiol p$K_a$ values (Table A); further discussion of five possible synthetic routes to RSCbl complexes; $^1$H NMR spectrum of $C_6F_5SCbl$ in anaerobic $CD_3OD$ at room temperature (Figure B); FABMS spectra (Figures C and D); $^{19}$F NMR spectrum of anaerobic $C_6F_5SCbl$ in $CD_3OD$ at room temperature (Figure E); and the $^{19}$F NMR spectrum of distilled $C_6F_5SH$ in $CD_3OD$ at room temperature (Figure F) (12 pages). Ordering information is given on any current masthead page.

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