$(NO)(PPh_3)(C{\equiv}CSnPh_3)$ (**5**) in 86% and 45% yields, respectively. The structures of **4** and **5** followed logically from their spectroscopic properties,[11] which included mass spectral parent ions and (for **5**) ^{13}C NMR chemical shifts and J_{CSn} values diagnostic of an SnC≡C linkage.[12] Hence, the isolation of **3–5** was taken as evidence for the generation of the rhenium/lithium C_2 complex $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(C{\equiv}CLi)$ (**2**)—and for the versatility of **2** as an organometallic synthon.

Next, transition metal derivatives of **2** were sought. Thus, analogous reactions were conducted with the palladium and rhodium chloride complexes *trans*-$Pd(PEt_3)_2(Cl)_2$ and *trans*-$Rh(PPh_3)_2(CO)(Cl)$ (1.1 equiv).[13] Workup gave the heterobimetallic C_2 complexes *trans*-$(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(C{\equiv}C)\text{-}Pd(PEt_3)_2(Cl)$ (**6**) and *trans*-$(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(C{\equiv}C)Rh(PPh_3)_2(CO)$ (**7**) in 66–72% yields (Scheme I).[11] These structural assignments were supported by the phosphorus coupling patterns of the C≡C ^{13}C NMR resonances.[11] In the case of **6**, two doublets of triplets were observed, consistent with a PPh_3 ligand on rhenium and two mutually trans PEt_3 ligands on palladium. Also, **7** gave a mass spectral parent ion.

In order to verify these assignments, the crystal structure of **6** was determined (supplementary material).[14] The data, which are summarized in Figure 1, show that **6** exhibits a nearly linear ReC≡CPd linkage. The Re—C and C≡C bond lengths and Re—C≡C bond angle are similar to those found earlier[15] in the methyl acetylide complex $(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)(C{\equiv}CMe)$ (2.066 (7), 1.19 (1) Å, 175.8 (7)°). These values also compare closely to those in other structurally characterized $L_nMC{\equiv}CM'L'_n$ complexes,[3b,c,6a,8] including the symmetrical dirhenium species $(CO)_5ReC{\equiv}CRe(CO)_5$ (Re—C, C≡C 2.01 (2), 1.19 (3) Å; Re—C≡C 177 (2)°).[6a] Interestingly, the ethyl groups of the two PEt_3 ligands in **6** are essentially eclipsed in the solid state. A similar feature is found in the diplatinum complex *trans,trans*-$(I)(Me_3P)_2PtC{\equiv}CPt(PMe_3)_2(I)$.[8a]

Surprisingly, low-temperature ^{31}P NMR spectra of **6** and **7** showed *two* R_3PMPR_3 resonances (**6**, THF-d_8, −60 °C: 17.2/17.0 ppm, $\Delta\nu$ 20.4 Hz; **7**, CD_2Cl_2, −80 °C: 28.5/23.1 ppm, $\Delta\nu$ 555.6 Hz). These coalesced at −42 and −26 °C, respectively, giving $\Delta G^{\ddagger}(T_c)$ of 11.7 and 10.9 ± 0.2 kcal/mol[16] for the processes that render the phosphorus nuclei equivalent. We propose that these barriers arise from steric interactions between the bulky PR_3 ligands on palladium and rhodium and the pentamethylcyclopentadienyl and PPh_3 ligands on rhenium. A simple 180° rotation about one of the three σ bonds in the Re—C≡C—M linkages would then exchange the PR_3 ligands. The observation of rotational barriers involving the termini of acetylenic compounds X—C≡C—X′ appears to be extremely rare.[17]

In summary, the lithiocarbon complex **2** is the first cleanly generated transition metal counterpart of one of the most versatile classes of building blocks in synthetic organic chemistry, acetylide ions RC≡CLi. It can be anticipated that **2** and related compounds will be useful precursors to a variety of C_2 derivatives. The chemical properties of the heterobimetallic complexes **6** and **7**, and the extension of this methodology to C_3 and C_4 complexes, are under active investigation.

Acknowledgment. We thank the DOE for support of this research.

Supplementary Material Available: Listings of general crystallographic data, atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for **4–7** (8 pages); tables of observed and calculated structure factors for **6** (18 pages). Ordering information is given on any current masthead page.

(11) Characterization of **4–7** (microanalysis, IR, and ^1H, ^{13}C, and ^{31}P NMR) is given in the supplementary material. Selected data: IR $\nu_{C\equiv C}$ (cm^{-1}, KBr) **4** 2002, **5** 1983, **6** 1944, **7** 2017 (s-ms); $^{13}C\{^1H\}$ NMR (ppm, C_6D_6, 75 MHz) **4** 131.4 (d, J_{CP} = 15.6 Hz, ReC≡), 131.1 (s, ≡CSi), **5** 138.6 (d, J_{CP} = 15.8 Hz, J_{CSn} (satellite) = 96.4 Hz, ReC≡), 119.7 (d, J_{CP} = 1.5 Hz, ≡CSn), **6** 111.7 (dt, J_{CPPh} = 15.1 Hz, J_{CPEt} = 4.3 Hz, ReC≡), 116.2 (dt, J_{CPPh} = 1.5 Hz, J_{CPEt} = 17.5 Hz, ≡CPd), **7** 134.6 (d, J_{CP} = 10.5 Hz, ReC≡), 153.4 (dt, J_{CRh} = 37.7 Hz, J_{CP} = 21.2 Hz, ≡CRh).

(12) Cauletti, C.; Furlani, C.; Sebald, A. *Gazz. Chim. Ital.* **1988**, *118*, 1.

(13) (a) Mann, F. G. *J. Chem. Soc.* **1935**, 1549. (b) Wilkinson, G. *Inorg. Synth.* **1968**, *11*, 99.

(14) Selected data from supplementary material: monoclinic, $P2_1/n$, a = 16.304 (4) Å, b = 14.861 (3) Å, c = 18.457 (6) Å, β = 101.09 (2)°, Z = 4; R = 3.1%, R_w = 3.3%.

(15) Senn, D. R.; Wong, A.; Patton, A. T.; Marsi, M.; Strouse, C. E.; Gladysz, J. A. *J. Am. Chem. Soc.* **1988**, *110*, 6096.

(16) Sandström, J. *Dynamic NMR Spectroscopy*; Academic Press: New York, 1982; Chapter 7.

(17) (a) Mew, P. K. T.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 159. (b) Since rhodium coupling is maintained above T_c in **7** ($^1J_{PRh}$ = 142.5 Hz),[11] exchange mechanisms involving PR_3 ligand dissociation can be excluded.

Chemical–Enzymatic Synthesis of 5′-Thio-*N*-acetyllactosamine: The First Disaccharide with Sulfur in the Ring of the Nonreducing Sugar

Hideya Yuasa,[†] Ole Hindsgaul,*[,†] and Monica M. Palcic[‡]

Departments of Chemistry and Food Science
University of Alberta, Edmonton
Alberta T6G 2G2, Canada

Received March 11, 1992

1-Thioglycosides, with sulfur replacing oxygen in the glycosidic linkage, have been extensively described in the literature.[1] In contrast, relatively few reports exist on the synthesis and properties of 5-thioglycosides, i.e., glycosides with sulfur replacing oxygen in the ring, and only simple alkyl glycosides have been described.[2] We report here the synthesis of a glycoside of 5′-thio-*N*-acetyllactosamine (5′S-LacNAc), the first example of this latter class of oligosaccharide.

There were several compelling reasons for initiating this work. 5′S-LacNAc is a potentially important analog of LacNAc in its own right. Since such disaccharides have not been previously prepared, their conformational properties remain unknown. Should 5′S-LacNAc serve as an acceptor for other glycosyltransferases, then analogs of more complex carbohydrate antigens, such as the sialyl-Le^x tetrasaccharide,[3] could be enzymatically prepared and used in studies on protein–carbohydrate recognition. In addition to these synthetic objectives, 5′S-LacNAc, and other 5′-thio oligosaccharides, are potentially resistant to exo- or endoglycosidases.[4] They might also either be hydrolyzed by these enzymes or they might potentially be inhibitors. Since such compounds had not been previously described, these questions could not be properly addressed.

The synthetic approach used was to prepare a 5-thiosugar nucleotide in the anticipation that such an analog would act as a donor substrate for $\beta(1\rightarrow4)$galactosyltransferase (GalT), an enzyme widely used in the combined chemical–enzymatic synthesis of oligosaccharides.[5] If this were the case, the repertoire of donor

[†] Department of Chemistry.
[‡] Department of Food Science.

(1) (a) Sinnott, M. L. *Biochem. J.* **1971**, *125*, 717. (b) Distler, J. J.; Jourdian, G. W. *J. Biol. Chem.* **1973**, *248*, 6772. (c) Reed, L. A.; Goodman, L. *Carbohydr. Res.* **1981**, *94*, 91. (d) Blanc-Muesser, M.; Defaye, J.; Driguez, H. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1885. (e) Defaye, J.; Driguez, H.; Poncet, S.; Chambert, R.; Petit-Glatron, M.-F. *Carbohydr. Res.* **1984**, *130*, 299. (f) Comtat, J.; Defaye, J.; Driguez, H.; Ohleyer, E. *Carbohydr. Res.* **1985**, *144*, 33. (g) Orgeret, C.; Seillier, E.; Gautier, C.; Defaye, J.; Driguez, H. *Carbohydr. Res.* **1992**, *224*, 29.

(2) (a) Whistler, R. L.; Van Es, T. *J. Org. Chem.* **1963**, *28*, 2303. (b) Shin, J. E. N.; Perlin, A. S. *Carbohydr. Res.* **1979**, *76*, 165. (c) Hashimoto, H.; Kawanishi, M.; Yuasa, H. *Tetrahedron Lett.* **1991**, *32*, 7087.

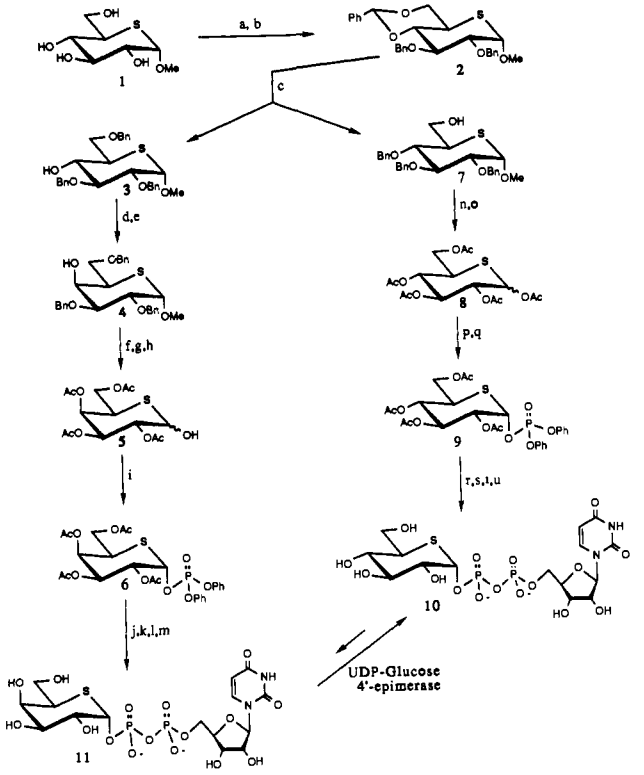
(3) (a) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130. (b) Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.

(4) (a) Shin, J. E. N.; Maradufu, A.; Marion, J.; Perlin, A. S. *Carbohydr. Res.* **1980**, *84*, 328. (b) Hashimoto, H.; Fujimori, T.; Yuasa, H. *J. Carbohydr. Chem.* **1990**, *9*, 683.

(5) (a) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365. (b) Drueckhammer, D. G.; Hennen, W. J.; Pedersen, R. L.; Barbas, C. F.; Gautheron, C. M.; Kratch, T.; Wong, C.-H. *Synthesis* **1991**, 499. (c) Palcic, M. M.; Hindsgaul, O. *Glycobiology* **1991**, *1*, 205.

0002-7863/92/1514-5891$03.00/0

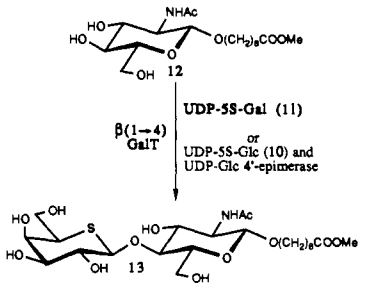
Scheme I[a]



[a] Reagents and conditions: (a) α,α-Dimethoxytoluene (1.1 equiv), *p*-TsOH (0.02 equiv), DMF, 60 °C, 1.5 h, 85%. (b) NaH (8.5 equiv), DMF, 20 °C, 4 h, then benzyl chloride (3 equiv), 20 °C, 20 h, 81%. (c) $NaBH_3CN$ (14 equiv), saturated HCl/Et_2O, THF, reflux, 3.5 h, **3** 62%, **7** 29%. (d) $(COCl)_2$ (1.5 equiv), DMSO (2.4 equiv), CH_2Cl_2, −78 °C, 15 min, then addition of **3**, −78 °C, 1.5 h, then addition of Et_3N, −78 °C, 5 min, 91%. (e) $NaBH_4$ (1.5 equiv), benzene–EtOH (1:1), 0 °C, 8 min, 88%. (f) Na–liquid NH_3, THF, −78 °C, 40 min. (g) H_2SO_4, AcOH–Ac_2O (2:5), 20 °C, 17 h, 63% from **4**. (h) H_2N-NH_2·AcOH (1.5 equiv), DMF, 50 °C, 0.5 h, 56%. (i) nBuLi (1 equiv), THF–hexane (24:1), −70 °C, 2 min, then $(PhO)_2POCl$ (5 equiv) −70 °C → −60 °C, 30 min, 60%. (j) PtO_2, H_2 (1 atm), MeOH, 20 °C, 2.5 h. (k) 1,1'-Carbonyldiimidazole (80%, 1.6 equiv), acetone, 20 °C, 18 h. (l) Uridine 5'-monophosphate (1.5 equiv), trioctylamine (1.6 equiv), DMF, 20 °C, 24 h. (m) Et_3N (17 equiv)–H_2O–MeOH (1:3:7), 20 °C, 18 h, then addition of further Et_3N (34 equiv), 20 °C, 5 h, 32% from **6**. (n) Same as f except 25 min. (o) Same as g except 63% from **7**. (p) 30% HBr–AcOH, 0 °C, 1 h. (q) $AgOPO(OPh)_2$ (1.3 equiv), benzene, reflux, 1 h, 56% from **8**. (r) Same as j except 3 h. (s) Same as k except 2.0 equiv. (t) Same as l. (u) Et_3N (44 equiv), H_2O, MeOH (3:3:7), 20 °C, 19 h, then more Et_3N (30 equiv), 20 °C, 2.5 h, 22% from **9**.

substrates for glycosyltransferases would also be significantly expanded. Both UDP-5'-thioglucose (UDP-5S-Glc, **10**) and UDP-5'-thiogalactose (UDP-5S-Gal, **11**) were chemically synthesized using established procedures as shown in Scheme I. The synthesis of **10** was modeled on earlier work of Whistler[6] and involved a Koenigs–Knorr type of phosphorylation step using silver diphenyl phosphate. In the coupling step (**9** → **10**), the anomeric phosphate group of the peracetylated glycosyl phosphate was activated using carbonyldiimidazole followed by mild deacetylation of the pyrophosphate-containing product using triethylamine–methanol–water. The synthesis of **11** involved inversion of C4 in the gluco derivative **3**. The key step was phosphorylation of the anomeric lithium alkoxide of **5** using chloro diphenyl phosphate.[7] The UDP derivative **11** was then prepared in the same manner as **10**, also on a 30-mg scale.[8]

Scheme II[a]



[a] Reaction conditions: **12** (2 mg), **11** (5 mg), GalT (1U), alkaline phosphatase (10U), $MnCl_2$ (2 mM) in 50 mM Na cacodylate (1.4 mL, pH 7.5), 37 °C, 18 h. More **11** (2 mg), GalT (1U), and alkaline phosphatase (10U) was then added, and the reaction continued for an additional 30 h. For the reaction with **10**, UDP-Glc 4'-epimerase (2U) was included.

UDP-5S-Gal (**11**) was active as a donor substrate for GalT. At room temperature, the rate of thiogalactosylation of the known acceptor **12**[9] was approximately 5% of that using normal oxygenated UDP-Gal, as judged by the rate of formation of product in TLC. Incubation of βGlcNAc-OR **12** (2 mg) with UDP-5S-Gal and GalT, under the conditions of Unverzagt et al.,[10] led to its complete conversion to 5'S-LacNAc **13**, which was isolated in quantitative yield on a C-18 SepPak.[11] Graham and Whistler[12] reported, on the basis of chromatographic evidence, that UDP-Glc 4'-epimerase could catalyze the interconversion **10** ⇌ **11**. We hereby confirm their findings, since incubation of UDP-5S-Glc (**10**) with this epimerase, in the presence of GalT, also resulted in the conversion of **12** into **13**[13] (Scheme II).

The observation that UDP-5S-Gal (**11**) is a donor substrate for GalT, as was hoped for, is itself an important observation, and a detailed study of this transfer reaction, as well as the inherent susceptibility of 5'-thiosugar nucleotides to hydrolysis, could make an important contribution to our understanding of the mechanism of glycosyl transfer. Such an investigation is, however, beyond the scope of this work.

The stability of 5'S-LacNAc to hydrolysis by only one β-galactosidase was examined using the commercial enzyme from *Escherichia coli*. Under standard assay conditions,[14] this enzyme cleaved 40% of the βGal from βGal(1→4)βGlcNAc-O-$(CH_2)_8$COOMe (LacNAc-OR) in 1 min. Under the same conditions, 28% hydrolysis of 5'S-LacNAc occurred in 120 min. Replacing the ring oxygen of LacNAc with sulfur therefore increases its stability near 200-fold to digestion by this β-galactosidase.

Acknowledgment. This work was supported by grants to both O.H. and M.M.P. from the Natural Sciences and Engineering Research Council of Canada.

(6) (a) Whistler, R. L.; Stark, J. H. *Carbohydr. Res.* **1970**, *13*, 15. (b) Graham, T. L.; Whistler, R. L. *Biochemistry* **1976**, *15*, 1189.

(7) Inage, M.; Chaki, H.; Kusumoto, S.; Shiba, T. *Chem. Lett.* **1982**, 1281.

(8) **10**. NMR (D_2O, 300 MHz) ^1H: H-5 5.97 (d, J = 8.0 Hz), H-1'' 5.40 (dd, $J_{1'',2''}$ = 2.5, $J_{1'',P}$ = 8.0 Hz). ^{13}C: 78.7 C1'' (d, $J_{C'',P}$ = 7.6 Hz), 44.3 C-5''. ^{31}P: −10.9 and −12.5 ($J_{P,P}$ = 21 Hz). **11**. ^1H: H-5 5.95 (d, J = 8.2 Hz), H-1'' 5.38 (dd, $J_{1'',2''}$ = 3.0, $J_{1'',P}$ = 7.5 Hz). ^{13}C: 79.1 C1'' (d, $J_{C'',P}$ = 7.6 Hz), 44.8 C-5''. ^{31}P: −10.8 and −12.3 ($J_{P,P}$ = 21 Hz).

(9) Palcic, M. M.; Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1987**, *159*, 315.

(10) Unverzagt, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 9308.

(11) Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49.

(12) Graham, T. L.; Whistler, R. L. *Arch. Biochem. Biophys.* **1975**, *171*, 721.

(13) **13**: ^1H NMR (D_2O, 500 MHz) H-1' 4.75 (d, $J_{1',2'}$ = 9.0 Hz), H-1 4.50 (d, $J_{1,2}$ = 8.5 Hz), H-4' 4.14 (s), H-5' 3.20 (dd, $J_{5',6a'} = J_{5',6b'}$ = 7.0 Hz); FAB MS m/z 592 (M + Na^+), 570 (M + H^+).

(14) Hydrolysis of LacNAc-OR and **13** was monitored using the Lactose Test Combination Kit (Boehringer, Mannheim). Briefly, 100 μg of substrate was incubated with *E. coli* β-galactosidase (3 U) at pH 6.6 according to the manufacturer's protocol. Released galactose was detected spectrophotometrically by oxidation using galactose-dehydrogenase with concomitant reduction of NAD. The dehydrogenase oxidized 5-thiogalactose at 13% the rate of galactose and produced the same change in absorbance at 340 nm. No hydrolysis was observed in the absence of the β-galactosidase.