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Research Letter

Syntheses of 5-Thio-D-Mannose from Petrochemicals and a Disaccharide Analog Containing It

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Per-*O*-acetyl-5-thio-DL-mannose was synthesized from petrochemicals in six steps and 9% overall yield. It was then derivatized into glycosyl trichloroacetimidate and subjected to glycosidation reaction with a mannosyl acceptor to give a separatable mixture of disaccharides with 5-thio-D- and L-mannosides. This is the first synthesis of an enantiomerically pure 5-thiosugar derivative from racemic chemicals. The D-glycoside was derivatized into methyl (5-thio-α-D-mannopyranosyl)-2-*O*-α-D-mannopyranoside 6-phosphate as a potential inhibitor of a golgi α-1,2-mannosidase.

1. Introduction

5-thiosugars are aldopyranose analogs with a sulfur atom in the pyranose ring [1–3] and it has been demonstrated that oligosaccharide analogs with a 5-thiosugar at the nonreducing end are exoglycosidase-resistant [4–6]. The resistance to hydrolases is a property desired for potential drugs [7], allowing for a practical duration of potency, hence a number of 5-thiosugars have been synthesized [2] and their behaviors against glycosidases studied [8–10]. Although most of 5-thiosugars have been synthesized from natural carbohydrates in not less than 10 steps, a few trials were made for the construction of the thiopyran structure through hetero Diels-Alder reaction from petrochemicals [11–13]. With this strategy, however, 5-thiosugars are obtained necessarily in racemic form and no analogs for mammalian monosaccharides have been synthesized. One of the racemic 5-thiosugars obtained in this method is 5-thiomannuronate, a potential intermediate for the synthesis of 5-thio-D-mannose. 5-thio-D-mannose is the only 5-thiosugar that has been isolated from nature [14], awaiting studies for its biological significance. We thus report here on the synthesis of racemic 5-thiomannose (5SMan) from the product of a hetero Diels-Alder reaction reported by Prabhakaran et al. [12] and then on the glycosidation reaction of the 1-*O*-trichloroacetimidate derivative of 5SMan, which gave a diastereomeric mixture of 5SMan-containing disaccharides, and they were easily separated by column chromatography. As a result, we obtained an enantiomerically pure 5-thiosugar derivative from petrochemicals for the first time. To make the synthesis more significant and advantageous, we derivatized the disaccharide analog into the 6-*O*-phosphate derivative, 5SManα1,2Man6P, as a potential inhibitor of a golgi α-1,2-mannosidase. The native Manα1,2Man6P structure is contained in the biosynthetic intermediates of the *N*-glycans specifically expressed on lysosomal hydrolases [15]. Mannose-6-phosphates (Man6P) at the nonreducing ends of the *N*-glycans, exposed by the action of a golgi α-1,2-mannosidase toward the Manα1,2Man6P structures [16], are specifically recognized by the cargo proteins bound for lysosomes [17]. Thus, the nondigestive property of 5SMan would allow 5SManα1,2Man6P to be a potential blocking agent against the distribution of lysosomal enzymes into lysosomes, though the mannosidase is unspecific for the phosphate group.

2. Results and Discussion

Ethyl 1,4-di-*O*-acetyl-5-thiomannopyranosyluronate **3** was synthesized from 1,4-diacetocxy-1,3-butadiene **1** and ethyl thioxoacetate **2**, generated in situ from the anthracene

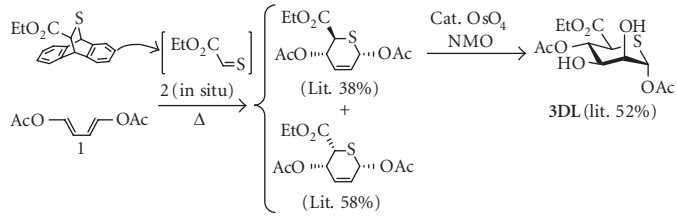
adduct, through hetero Diels-Alder and osmium oxidation reactions in a reported method [11, 12]. Prior to the reduction of the carboxylate moiety, compound **3DL** was subjected to Fischer methanolysis to give methyl (methyl 5-thiomannopyranosid)uronate **4DL** in 62%. **4DL**: R_f = 0.23 ($CHCl_3$-MeOH, 9 : 1), ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.53 (d, 1H, J = 3.6 Hz, H-1), 4.22 (t, 1H, J = 3.6 Hz, H-2), 4.16 (t, 1H, J = 10 Hz, H-4), 3.79 (s, 1H, CO_2CH_3), 3.72 (dd, 1H, J = 3.6, 10 Hz, H-3), 3.67 (t, 1H, J = 10 Hz, H-5), 3.49 (s, 3H, OCH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.6, 87.2, 71.9, 70.8, 56.5, 53.0, 43.8; ESI (M + Na)$^+$261. As attempted reduction of **4DL** with $LiAlH_4$ ended in decomposition, **4DL** was treated with $NaBH_4$, which has been occasionally successful in the reduction of sugar esters [18], to give methyl 5-thiomannopyranoside **5DL** in 77%. Spectral data of its tetraacetate were consistent with those reported for D-enantiomer [19]. Acetylation followed by acetolysis of compound **5** produced per-*O*-acetyl-5-thiomannopyranose **6DL** in 85% yield, whose NMR data coincided with those reported for its D-enantiomer [20]. We thus obtained the protected racemic 5-thiomannose **6DL** from petrochemicals in six steps and 9% overall yield. The synthesis of the corresponding 5-thio-D-mannose derivative **6D** has been achieved in ten steps from D-mannose with 14% overall yield [20], showing that the merit of this study is the fewer synthetic steps and the use of petrochemicals for raw material. Although the low yield is a downside, the most impractical aspect of this method resided in the production of racemic compounds. To minimize the demerits, we next studied the use of the racemic 5-thiomannose derivative **6DL** for the synthesis of a disaccharide analog.

Per-*O*-acetyl-5-thiomannose **6DL** was derivatized to 1-*O*-trichloroacetimidate **7DL** in the same manner as that of D-enantiomer [21] in 80% yield. The glycosidation reaction of **7DL** toward a 3-*O*-benzyl-protected mannopyranoside **8** [22] was conducted with the conditions reported for the synthesis of a similar 5-thio-D-mannose-containing disaccharide, in which the 3-*O*-benzoyl group caused the predominant production of an orthoester derivative [21]. Use of the benzyl group for 3-*O*-protection prevented the orthoester formation and methyl 2-*O*-(5-thio-α-D and L-mannopyranosyl) mannopyranoside derivatives (**9D** : **9L** = 1 : 1) were obtained in 78% yield from the racemic glycosyl donor **7DL**. The diastereomers were easily separated by silica gel column chromatography. The stereochemistry of the diastereomers was confirmed by comparison of R_f values, $[\alpha]_D{}^{25}$, and ^{1}H NMR **9D:** R_f = 0.43 (hexane-ethyl acetate, 1 : 1); $[\alpha]_D{}^{25}$ +67.7° (c 0.73, $CHCl_3$; +66.8° for the same product synthesized from D-enatiomer of **7**); ^{1}H NMR (270 MHz, $CDCl_3$)δ7.50-7.26 (m, 10H, Ph), 5.68 (s, 1H, PhCH), 5.67 (dd, 1H, J = 2.8, 4.0 Hz, H-2′), 5.45 (t, 1H, J = 10 Hz, H-4′), 5.37 (dd, 1H, J 2.8, 10 Hz, H-3′), 5.00 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1′), 4.91-4.60 (dd, 2H, J 12.2 Hz, $PhCH_2$), 4.66 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1′), 4.88 (d, 1H, J = 12.2 Hz, PhCHH), 4.66 (d, 1H, J = 2.3 Hz, H-1), 4.62 (d, 1H, J = 12.2 Hz, PhCHH), 4.30 (dd, 1H, J = 5.6, 12.2 Hz, H-6a′), 4.26 (dd, 1H, J = 4.6, 10.2 Hz, H-6a), 4.17 (t, 1H, J = 9.6 Hz, H-4), 4.10 (dd, 1H, J = 2.3, 3.0 Hz, H-2), 4.09 (dd, 1H, J = 3.6, 12.2 Hz, H-6b′), 3.97 (dd, 1H, J = 3.0, 9.6 Hz, H-3), 3.91 (t, 1H, J = 10.2 Hz, H-6b), 3.77 (ddd, 1H, J = 4.6, 9.6, 10.2, H-5), 3.45 (ddd, 1H, J = 3.6, 5.6, 10 Hz, H-5′), 3.37 (s, 3H, OCH_3), 2.12, 2.08, 2.04, 2.00 (s × 4, 12H, $COCH_3$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.5, 169.75. 169.70, 169.5, 138.4, 137.5, 128.8, 128.2, 128.1, 127.4, 127.3, 126.1, 101.5, 101.0, 83.3, 79.5, 76.0, 75.8, 73.4, 70.6, 70.3, 69.5, 68.7, 63.8, 62.1, 54.8, 39.3, 21.0, 20.7, 20.6; HR-ESMS calcd for $C_{35}H_{42}O_{14}SNa$ (M + Na)$^+$ 741.2194, found 741.2282. **9L:** R_f = 0.35 (hexane-ethyl acetate, 1:1); $[\alpha]_D{}^{25}$-72.9°(c 0.79, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ7.52-7.26 (m, 10H, Ph), 5.69 (s, 1H, PhCH), 5.45-5.40 (m, 3H, H-2′, H-3′, H-4′), 4.84(d, 1H, J = 12.2 Hz, PhCHH), 4.70 (d, 1H, J = 12.2 Hz, PhCHH), 4.69 (d, 1H, J = 3.3 Hz, H-1′), 4.68 (d, 1H, J = 1.5 Hz, H-1), 4.29 (dd, 1H , J = 1.5, 3.6 Hz, H-2), 4.25 (dd, 1H, J = 4.5, 10 Hz, H-6a), 4.13 (t, 1H, J = 9.6 Hz, H-4), 4.04 (dd, 1H, J = 4.6, 11.9 Hz, H-6a′), 3.96 (dd, 1H, J = 3.6, 9.6 Hz, H-3), 3.90 (t, 1H, J = 10 Hz, H-6b), 3.80-3.72 (m, 2H, H-5, H-5′), 3.59 (dd, 1H, J = 3.3, 11.9 Hz , H-6b′), 3.36 (s, 3H, OCH_3), 2.18, 2.01, 2.002, 1.996 (s × 4, 12H, $COCH_3$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.5, 170.2, 169.7, 169.6 (C = O), 138.2, 137.6, 128.9, 128.3, 128.1, 127.6, 126.1 (Ph), 101.5 (PhCH), 98.3 (C-1), 80.4 (C-1′), 78.8 (C-3), 74.4, 74.1, 72.9 ($PhCH_2$), 71.5, 70.2, 68.9, 68.7, 64.0, 61.3 (C-6′), 54.9 (OCH_3), 38.7 (C-5′), 21.1, 20.7, 20.65, 20.6 ($COCH_3$); HR-ESMS calcd for $C_{35}H_{42}O_{14}SNa$ (M + Na)$^+$ 741.2194, found 741.2242. with those of **9D*** synthesized from 5-thio-D-mannopyranosyl donor **7D**. This is the first synthesis of an enatiomerically pure 5-thiosugar derivative from racemic petrochemicals.

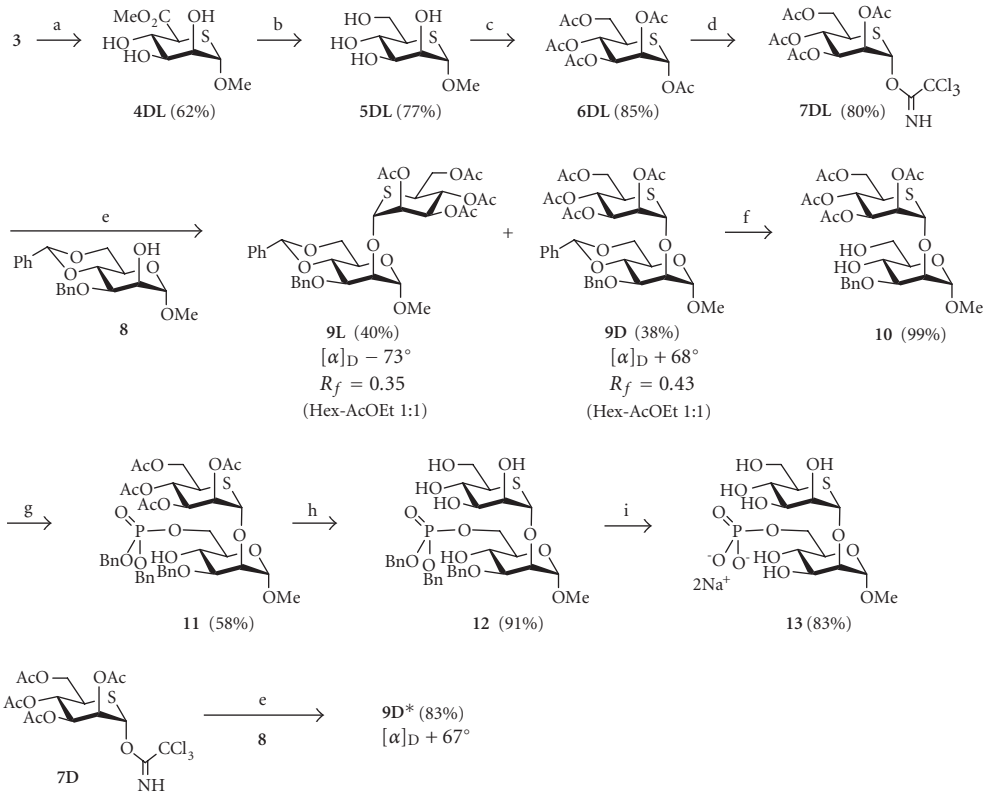
As stated in the introductory section, we modified the synthesized disaccharide **9D** into 6-*O*-phosphate. The benzylidene group of compound **9D** was deprotected by hydrolysis (99%) to give compound **10**, which was then subjected to phosphorylation conditions giving regioselectively the di-*O*-benzyl 6-*O*-phosphate **11** in 58% yield. Deacetylation (**12** in 91%) followed by debenzylation gave compound **13** in 83% yield. **13:** R_f = 0.20 (iPrOH-H_2O, 3:1); $[\alpha]_D{}^{25}$ +45.1° (c 1.0, H_2O); ^{1}H NMR (270 MHz, D_2O) δ4.86 (d, 1H, J 1.5 Hz, H-1), 4.72 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1′), 4.20 (dd, 1H, J = 2.8, 4.0 Hz, H-2′), 3.89-3.50 (m, 3H, H-2, H-6a, H-6b), 3.80 (dd, 1H, J = 3.3, 11.9 Hz, H-6a′), 3.75-3.50 (m, 6H, H-3, H-4, H-5, H-3′, H-4′, H-6b′), 3.26 (s, 3H, OCH_3), 2.98 (m, 1H, H-5′); ^{13}C NMR (67.8 MHz, D_2O) δ 100.2, 86.8, 77.7, 72.5, 72.34, 72.2, 72.0, 70.6, 70.1, 66.7, 63.3, 60.9, 55.2, 44.6; ^{31}P NMR (109.4 MHz, D_2O) δ 3.75.

3. Conclusion

We achieved the synthesis of an enantiomerically pure 5-thio-D-mannose-containing disaccharide derivative from racemic raw materials for the first time. To pursue the advantage of 5-thiosugar, we derivatized the disaccharide into 5SManα1,2Man6P, the analog of the partial structure of an intermediate oligosaccharide of the *N*-glycans on lysosomal enzymes. 5-thiopyranosides are in general



Scheme 1



Scheme 2: a. AcCl/MeOH; b. $NaBH_4/H_2O$; c. (i) Ac_2O-Py, (ii) Ac_2O, H_2SO_4; d. (i) H_2NNH_2 • AcOH/DMF, (ii) K_2CO_3, CCl_3CN; e. **8**, TESOTf/CH_2Cl_2; f. 80% AcOH; g. (i) $(BnO)_2PN(iPr)_2$, 1H-tetrazole, (ii) 30% H_2O_2; h. Et_3N-CH_3OH; i. 10% Pd-C, H_2, AcOH-EtOH.

glycosidase-resistant and the analog is a potential inhibitor of a golgi α-1,2-mannosidase that cleaves Manα1,2Man6P into Man6P.

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