Synthesis of a C-Disaccharide Analog of the Thomsen-Friedenreich (T) Epitope

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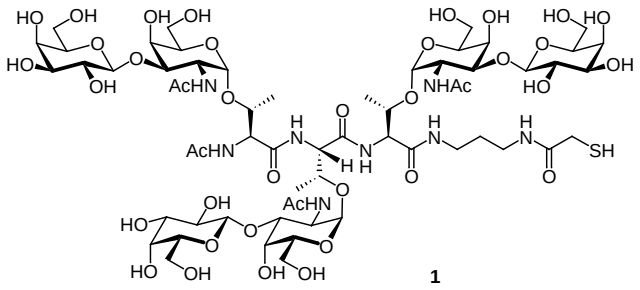
Abstract: The 2,3,4,6-tetra-O-[(*tert*-butyl)dimethylsilyl]-C-β-D-galactopyranosylformaldehyde (2,6-anhydro-1,3,4,6-tetra-O-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptose, **2**) was condensed with isolevoglucosenone (**3**) in the presenc e of Et_2AlI to give 1,6-anhydro-3-C-{(1*R*)-2,6-anhydro-3,4,5,7-tetra-O-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-C-yl}-2,3-dideoxy-β-D-*glycero*-hex-2-enopyranos-4-ulose (**8**). Diastereoselective conjugate addition of BnONHBn to **8**, followed by diastereoselective ketone reduction with $LiBH_4$ and treatment with Me_3SiSPh/ZnI_2 provided phenyl 3-C-{(1*R*)-2,6-anhydro-3,4,5,7-tetra-O[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-C-yl}-2-[(*N*-benzoxyl-*N*-benzyl)amino]-2,3-dideoxy-1-thio-β-D-*galacto*-hexopyranoside (**14**).

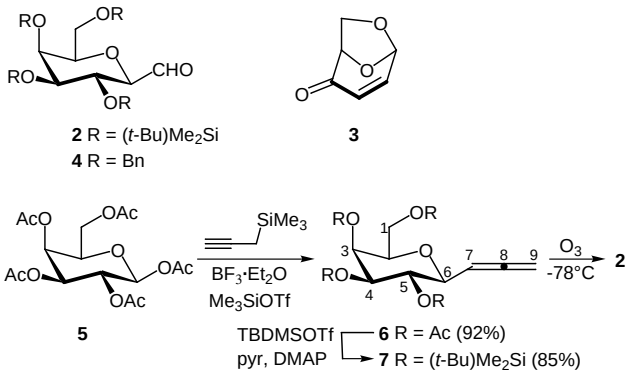
Key words: Baylis-Hillman reaction with Et_2AlI, C-disaccharide, epitope T, C-β-D-galactopyranosylformaldehyde, isolevoglucosenone, non-hydrolyzable epitope analog

Malignancy is often associated with profound alterations in cell surface bound carbohydrate components of glycoconjugates.[1] Such structural changes are due to incomplete glycosylation or novel glycosylation by tumor cells. Among the tumor associated carbohydrate antigens the Thomsen-Friedenreich antigen (T antigen) is found in carcinoma-associated mucins; it is a disaccharide, Galβ1→3GalNAcα→O linked to serine or threonine. The T antigens have been prepared and their immunogenicity in conjugate vaccines has been confirmed.[2] The clustered antigen motifs such as **1** prepared by Danishefsky and coworkers[3] have demonstrated the potential for antitumor vaccines[4] based on T antigen conjugates.



1

Disaccharide conjugates are relatively short-lived in the blood stream because of their hydrolysis catalyzed by ubiquitous glycosidases. Disaccharide mimetics such as C-linked disaccharide analogs offer an improved stability towards hydrolysis as required for a disaccharide-based vaccine. We report here our efforts toward the synthesis of a C-glycoside analog of epitope T disaccharide. The synthesis relies on our recently developed methodology for the synthesis of C(1→3)-linked disaccharides based on a Baylis-Hillmann type of condensation[5] between the D-galactose derived carbaldehyde **2** and isolevoglucosenone (**3**) induced with a dialkyl aluminum salt.



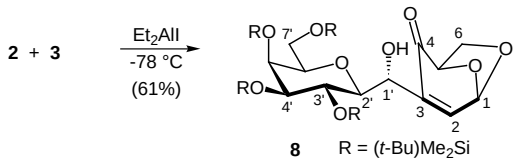
Scheme 1

The required C-β-D-galactopyranosylformaldehyde **2** was prepared following a procedure similar to that described by Bednarski and co-workers[6] for the preparation of **4**, starting from D-galactose pentaacetate (**5**).[7] Trimethylsilyl triflate (2 equivalents), then $BF_3{\cdot}OEt_2$ (3 equivalents) were added dropwise to a solution of **5** and propargyl trimethylsilane (3 equivalents) in MeCN at 0 °C. After 2 h at 0 °C acidic work-up (1 N HCl) and flash chromatography on silica gel gave **6** in 92% yield. Zemplén methanolysis (MeONa/MeOH) of **6** followed by silylation with (*t*-Bu)$Me_2SiOSO_2CF_3$/pyridine and 4-dimethylaminopyridine afforded **7** in 85% yield.[8] Ozonolysis (2% O_3 in O_2, CH_2Cl_2, −78 °C) provided aldehyde **2** (Scheme 1), an unstable compound that must be used directly in the next operation (Scheme 2).

A 1 M solution of Et_2AlI in toluene was added to a 1:1.5 mixture of crude **2** and isolevoglucosenone (**3**)[9] in CH_2Cl_2 at −78 °C. After 2 h at −78 °C and acidic work-up, enone **8** was obtained as the major product of condensation in 61% yield.[10] The diastereoselectivity of the reaction was not established unambiguously. Nevertheless, by analogy with all the other cases of aldol condensations involving aluminum enolates derived from isolevoglucosenone (Zimmerman-Traxler model,[11] steric factors), the

hydroxymethano linker must have the (*R*) configuration.[5] The ^{1}H NMR spectrum of **8** confirmed the configuration of the C-glycosidic linkage, with ^3J(H-2',H-3') = 7.0 Hz and ^3J(H-3',H-4') = 8.6 Hz.

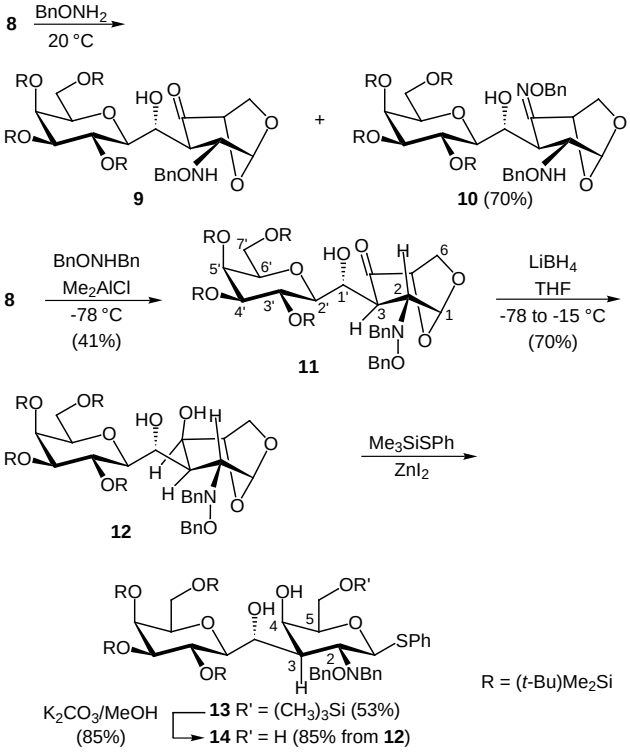
When a neat mixture of enone **8** and O-benzyl hydroxylamine was stirred at 20 °C, oxime **10** was obtained and isolated in 70% yield. Control experiments indicated that the initial conjugate addition of $BnONH_2$ to **8** giving adduct **9** is somewhat faster than the oxime formation, although the latter reaction could not be avoided (Scheme 3). We thus looked for a nitrogen nucleophilic reagent that would not undergo 1,2-addition to enone **8** or to its product of 1,4-addition. We found that N,O-dibenzyl hydroxylamine adds to enone **8** between −78 °C and −15 °C in the presence of one equivalent of Me_2AlCl. This gave rise to pure **11**, the product of conjugate addition, in 41% yield.[12] Attempts to reduce the carbonyl group of **11** with $NaBH_4$ induced fast 1,4-elimination of BnONHBn. Using $(i\text{-Bu})_2AlH$ led to decomposition. Finally, ketone **11** was reduced with $LiBH_4$ in THF into the desired D-galactosamine derivative **12** with high diastereoselectivity and good yield (70%). The structure of **12** was deduced from its ^{1}H NMR and 2D ^{1}H-NOESY spectra. It was confirmed by the structures of the derivatives **13** and **14** (see below).



Scheme 2

Since our C-disaccharide analog of the antigen T sugar must be conjugated with serine and threonine we converted the anhydro moiety of **12** into a more flexible glycoside donor.[13] With this goal in mind we obtained a single diastereomer of the phenyl thiogalactopyranoside **13**, on treatment with Me_3SiSPh and ZnI_2,[14] in 53% yield after column chromatography on silica gel. This compound was not stable in the presence of water, losing its trimethylsilyl moiety rapidly at room temperature. It was thus treated with MeOH in the presence of K_2CO_3 to provide triol **14** in 85% yield. The latter product was obtained in 85% yield directly from **12** when omitting the purification of the intermediate product **13**. The ^{1}H NMR spectra of **13** and **14** confirmed their structures.[15] In particular the β-C-D-galactopyranosyl configuration of the 2-aminohexose moiety was established by the vicinal coupling constants ^3J(H-1,H-2) = 9.5 Hz, ^3J(H-2,H-3) = 11.5 Hz, ^3J(H-3,H-4) and ^3J(H-4,H-5) <3 Hz and by the 2D-NOESY ^{1}H NMR spectrum of **14**.

This letter describes a short and stereoselective approach to a partially protected C-disaccharide analog of the oligosaccharide portion of the T-antigen. Our work establishes another strategy for the preparation of C(1→3) disaccharides that are C-glycosides of aminosugars.[16]



Scheme 3

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(8) Data for **7**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.27 (*q*, 1H, ^4J(H-7,H-9a) = ^4J(H-7,H-9b) = ^3J(H-6,H-7) = 7.0 Hz, H-7), 4.80 (ddd, 1H, ^2J = 10.8 Hz, ^4J(H-7,H-9a) = 6.7 Hz, ^5J(H-6,H-9a) = 1.9 Hz, H-9a), 4.76 (ddd, 1H, ^2J = 10.8 Hz, ^4J(H-7,H-9b) = 6.7 Hz, ^5J(H-6,H-9b) = 1.9 Hz, H-9b), 4.42 (d, 1H, ^3J(H-5,H-6) = 7.0 Hz, H-6), 4.22 (m, 1H, H-3), 4.13 (m, 1H, H-5), 3.91 (m, 1H, H-4), 3.74 (m, 2H, H-1), 3.63 (m, 1H, H-2), 0.93, 0.92, 0.91, 0.89 (4s, 36H, 4 *t*-Bu), 0.10, 0.095, 0.09, 0.085, 0.08, 0.07, 0.05, 0.04 (8s, 24H, 4 Me_2Si).
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(10) Data for **8**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.39 (dd, 1H, ^3J(H-1,H-2) = 3.5 Hz, ^4J(H-2,H-1') = 1.0 Hz, H-2); 5.83 (d, 1H, ^3J(H-1,H-2) = 3.5 Hz, H-1), 4.75 (dd, 1H, ^3J(H-5,H-6) = 6.4 Hz, ^3J(H-5,H-6) = 1.3 Hz, H-5), 4.45 (dd, 1H, ^3J(H-3',H-4') = 8.6 Hz, ^3J(H-2',H-3') = 7.0 Hz, H-3'), 4.26 (d, 1H, ^2J = 12.4 Hz, H-7'a), 4.23 (ddd, 1H, ^3J(H-1',H-2') = 7.0 Hz, ^3J(H-1',OH) = 6.7 Hz, ^4J(H-2,H-1') = 1.0 Hz, H-1'), 4.06 (dd, 1H, ^2J = 8.3 Hz, ^3J(H-5, H_{exo}-6) = 6.4 Hz, H_{exo}-6), 3.99 (br.d, 1H, ^3J(H-3',H-4') = 8.6 Hz, ^3J(H-4',H-5') <2 Hz, H-4'), 3.96 (br.d, 1H, ^3J(H-5',H-6') = 4.1 Hz, H-5'), 3.91 (t, 1H, ^3J(H-1',H-2') = ^3J(H-2',H-3') = 7.0 Hz, H-2'), 3.80 (dd, 1H, ^3J(H-5',H-6') = 4.1 Hz, ^3J(H-6',H-7'b) = 1.9 Hz, H-6'), 3.75 (dd, 1H, ^2J = 12.4 Hz, ^3J(H-6', H-7'b) = 1.9 Hz, H-7'b), 3.65 (dd, 1H, ^2J = 8.3 Hz, ^3J(H-5,H_{endo}-6) = 1.3 Hz, H_{endo}-6), 2.94 (d, 1H, ^3J(H-1',OH) = 6.7 Hz, OH), 0.94, 0.93, 0.91, 0.90 (4s, 36H, 4 *t*-Bu), 0.14, 0.12, 0.10, 0.09, 0.08, 0.07, 0.06, 0.05 (8s, 24H, 4 Me_2Si). Anal. Calcd for $C_{37}H_{74}O_9Si_4$: C 57.32, H 9.62; found: C 57.37, H 9.58.
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(12) Data for **11**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.47-7.41 (m, 2H), 7.36-7.30 (m, 3H), 7.28-7.25 (m, 3H), 7.21-7.14 (m, 2H), 6.12 (s, 1H, H-1), 4.50 (dd, 1H, ^3J(H-5,H-6) = 5.6 Hz, ^4J(H-3,H-5) = 0.9 Hz, H-5), 4.43 & 4.18 (2d, 2H, ^2J = 10.2 Hz, CH_2(BnO)), 4.26 (dd, 1H, ^3J(H-1', H-2') = 7.0 Hz, ^3J(H-3,H-1') = 2.5 Hz, H-1'), 4.24 (d, 1H, ^3J(H-2,H-3) = 11.0 Hz, H-2), 4.22 & 3.92 (2d, 2H, ^2J = 13.0 Hz, H_2C(7')), 4.18 (dd, 1H, ^3J(H-3',H-4') = 11.9 Hz, ^3J(H-2',H-3') = 9.2 Hz, H-3'), 3.98 (br.d, 1H, ^3J(H-5',H-6') = 3.9 Hz, ^3J(H-4',H-5') <2 Hz, H-5'), 3.87 (ddd, 1H, ^3J(H-2',H-3') = 9.2 Hz, ^3J(H-1',H-2') = 7.0 Hz, ^4J(H-2',H-4') = 1.7 Hz, H-2'), 3.82 (d, 1H, ^3J(H-5',H-6') = 3.9 Hz, H-6'), 3.81 (d, 1H, ^2J = 7.6 Hz, H_{endo}-6), 3.75 (dd, 1H, ^2J = 7.6 Hz, ^3J(H-5,H_{exo}-6) = 5.8 Hz, H_{exo}-6), 3.72 (dd, 1H, ^3J(H-3',H-4') = 11.9 Hz, ^4J(H-2',H-4') = 1.7 Hz, H-4'), 3.64 & 3.27 (2m, 2H, CH_2(BnN)), 2.64 (dd, 1H, ^3J(H-2,H-3) = 11.0 Hz, ^3J(H-1',H-3) = 2.5 Hz, H-3), 0.97, 0.97, 0.91, 0.88 (4s, 36H, 4 *t*-Bu), 0.15, 0.14, 0.14, 0.12, 0.08, 0.07, 0.015, 0.01 (8s, 24H, 4 Me_2Si); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_c 214.9, 136.7, 130.1, 129.3, 128.2, 100.3, 79.4, 77.3, 73.3, 70.2, 67.3, 67.0, 66.5, 65.2, 58.5, 58.4, 46.1, 26.0, 18.4, 18.3, 18.1, 18.0, -4.4, -4.5, -4.6, -4.8, -4.9, -5.0, -5.3, -5.3, -5.5.
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(15) Data for **14**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.66- 7.57 (m, 4H), 7.42-7.30 (m, 6H), 7.25-7.20 (m, 3H), 7.02-6.96 (m, 2H), 4.90 (d, 1H, ^3J(H-1,H-2) = 9.5 Hz, H-1), 4.47 & 4.04 (2d, 2H, ^2J = 9.5 Hz, CH_2(BnN)), 4.46 (m, 1H, H-4), 4.44 & 4.22 (2d, 2H, ^2J = 8.5 Hz, CH_2(BnO)), 4.27 (dd, 1H, ^3J(H-5',H-6') = 7.5 Hz, ^3J(H-6',H-7'b) = 2.2 Hz, H-6'), 4.26 (m, 1H, H-3'), 4.13 (m, 1H, H-2'), 4.09 (m, 1H, H-2), 4.03 (m, 1H, H-1'), 3.97 (dd, 1H, ^2J = 12.0 Hz, ^3J(H-5,Ha-6) = 6.5 Hz, Ha-6), 3.91 (d, 1H, ^3J(H-5',H-6') = 7.5 Hz, H-5'), 3.86 (d, 1H, ^2J = 4.2 Hz, Ha-7'), 3.82 (dd, 1H, ^3J(H-3',H-4') = 12.5 Hz, ^4J(H-2',H-4') = 1.5 Hz, H-4'), 3.78 (dd, 1H, ^2J = 4.2 Hz, ^3J(H-6',Hb-7') = 2.2 Hz, Hb-7'), 3.74 (dd, 1H, ^2J = 12.0 Hz, ^3J(H-5,Hb-6) = 2.5 Hz, Hb-6), 3.39 (br. dd, 1H, ^3J(H-5,Ha-6) = 6.5 Hz, ^3J(H-5,Hb-6) = 2.5 Hz, H-5), 2.42 (br. d, 1H, ^3J(H-2,H-3) = 11.5 Hz, H-3), 0.93 & 0.90 (2s, 36H, 4 *t*-Bu), 0.11-0.05 (m, 24H, 4 Me_2Si); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_c 137.9, 132.0, 130.0, 129.6, 128.8, 128.4, 128.3, 128.1, 127.4, 127.2, 85.8, 80.0, 73.2, 70.4, 69.1, 67.8, 67.3, 65.9, 63.6, 60.3, 58.6, 41.6, 26.1, 26.0, 25.9, 25.9, 25.7, 18.4, 18.1, 18.0, 17.8, -4.5, -4.5, -4.6, -4.8, -4.9, -5.0, -5.4; CI-MS (NH_3) m/z 1100 (13, $[M+H]^+$), 1099 (76, $M^{\bullet+}$), 991 (11, $[M+H\text{-PhS}]^{+\bullet}$), 990 (11, $[M\text{-PhS}]^+$), 91 (100).
(16) For the synthesis of α-D-Man*p* (1→3)CH_2-D-GalNAc and α-D-Man*p* (1→3)CH_2-D-TalNAc, see: Pasquarello, C.; Picasso, S.; Demange, R.; Malissard, M.; Berger, E. G.; Vogel, P. *J. Org. Chem.* **2000**, *65*, 4251.

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