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Chemical Synthesis of a Core 2 Branched Pentasaccharide Containing a Carboxylate Group

Jie Xia, James L. Alderfer and Khushi L. Matta*

Molecular & Cellular Biophysics, Roswell Park Cancer Institute, Em & Carlton Streets, Buffalo, NY 14263, USA

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Abstract—Design and synthesis of a carboxylate-containing pentasaccahride **1** with the Galβ(1–4) (Fucα1–3)GlcNAcβ(1–6){3-[1-carboxymethyl]-Galβ(1–3)}GalNAcα–OMe sequence, which is obtained through regioselective coupling of the 6-OH of a novel acceptor **9** with Lewis^x donor **10** catalyzed by NIS-TfOH are described.

In the last decade we have witnessed tremendous efforts in the syntheses of potential selectin ligands.[1] However, most of these studies have been centered on the syntheses of SLe^x type structures. In our laboratory, we have clearly shown that the NeuAcα2–3Galβ(1–3)GalNAc sequence of core 2, GlcNAcβ(1–6)[Galβ(1–3)]GalNAcα–, branched structure, as found in *O*-linked glycoprotein, plays a part in binding with L-, E-, and P-selectins.[2] Recently, Cummings and co-workers[3] have synthesized a series of sulfoglycopeptides and have shown that a glycopeptide compound having Galβ(1–3)GalNAc along with SLe^x at the C6 position of GalNAcα was active. Moreover, studies of mice deficient in core 2 branching enzymes support the role of core 2 glycans in binding with L- and P-selectins.[4] In our continued interest in the synthesis of core 2 branched structures containing sulfate, fucose and sialic acid,[5] we report the design and synthesis of the title compound.

The synthesis of compound **1** was accomplished as illustrated in Schemes 1 and 2. Monosaccharide acceptor **3** was treated with 2,3,4,6-tetra-*O*-acetylgalactosyl bromide **4** in the presence of $Hg(CN)_2$[6] to afford disaccharide **5** in good yield (65%). *O*-deacetylation of disaccharide **5** in MeOH–CH_2Cl_2 with 1 M sodium methoxide at room temperature provided compound **6** in high yield (94%). Tin acetal [7] prepared in situ from disaccharide **6** could be selectively alkylated with methyl bromoacetate in the presence of tetrabutylammonium iodide at a reasonable temperature (60–65 °C), giving compound **7** in acceptable yield (59%) in two steps. Complete chloroacetylation of compound **7** with chloroacetic anhydride using $NaHCO_3$ as a base in dry DMF afforded compound **8**. Removal of the 4,6-*O*-benzylidene group from compound **8** gave the desired disaccharide acceptor **9** in high yield (94%).

Because of the much higher reactivity of the primary hydroxyl group, glycosylation of 6-HO of acceptor **9** with trisaccharide donor **10** [8] provided compound **11** as the only glycosylation product under controlled reaction conditions (NIS-TfOH promoted system).[9] The β(1–6) linkage of compound **11** was confirmed by observation of a strong NOE cross peak between H^c-1 of sugar residue **c** and H^a-6a, H^a-6b of sugar residue **a** in the 2D ROESY spectrum. β-Configuration of sugar residue **c** was confirmed by the presentation of a large coupling constant ($^3J_{1c,2c} = 7.8$ Hz). Compound **11** was then *O*-dechloroacetylated in the presence of thiourea and 2,6-lutidine to give compound **12**. Regioselective sulfation of OH-6 of galactose residue **b** in compound **12** was achieved by treatment with SO_3·pyridine complex in dry pyridine at low temperature to give compound **13** in good yield (85%). The systematic removal of protecting groups in **13** was accomplished in four steps to give target molecule **1**. Thus, removal of benzyl groups from compound **13** was achieved by treatment with Pd–C (10%) under hydrogen atmosphere at room temperature, resulting in compound **14**. Compound **14** was then treated with Ac_2O–pyridine in the presence of DMAP, providing compound **15**. Removal of methyl and the phthalimido groups from **15** was executed through a one-pot, three-step procedure, by treatment of compound **15** with a larger excess of anhydrous LiI in dry pyridine at refluxing temperature, followed by treatment with hydrazine hydrate in ethanol, then acetylation with acetic anhydride–pyridine (1:1) in the presence of a cat-

*Corresponding author. Tel.: +1-716-845-2397; fax: +1-716-845-3458; e-mail: klmatta@yahoo.com

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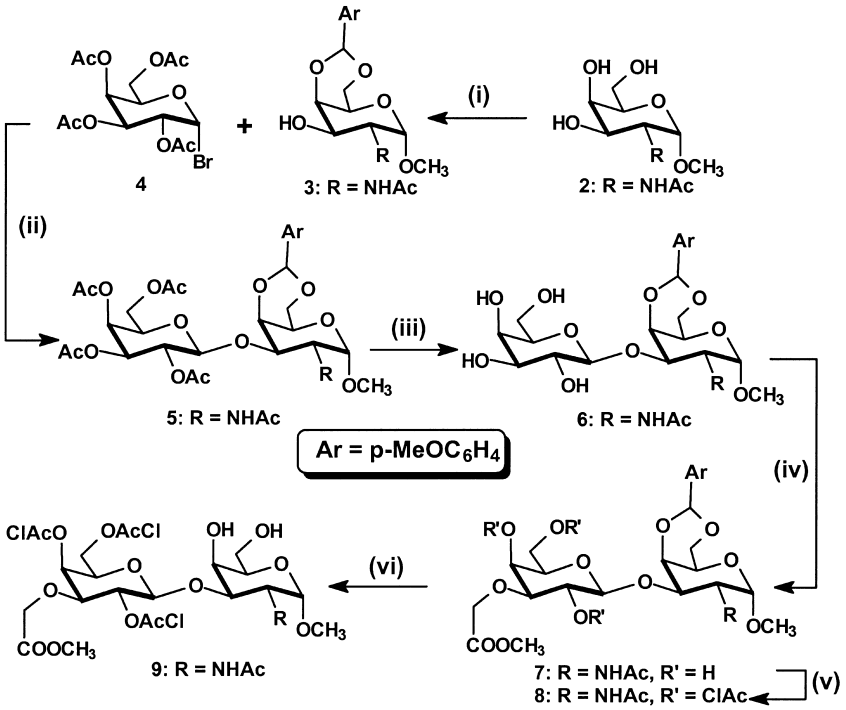
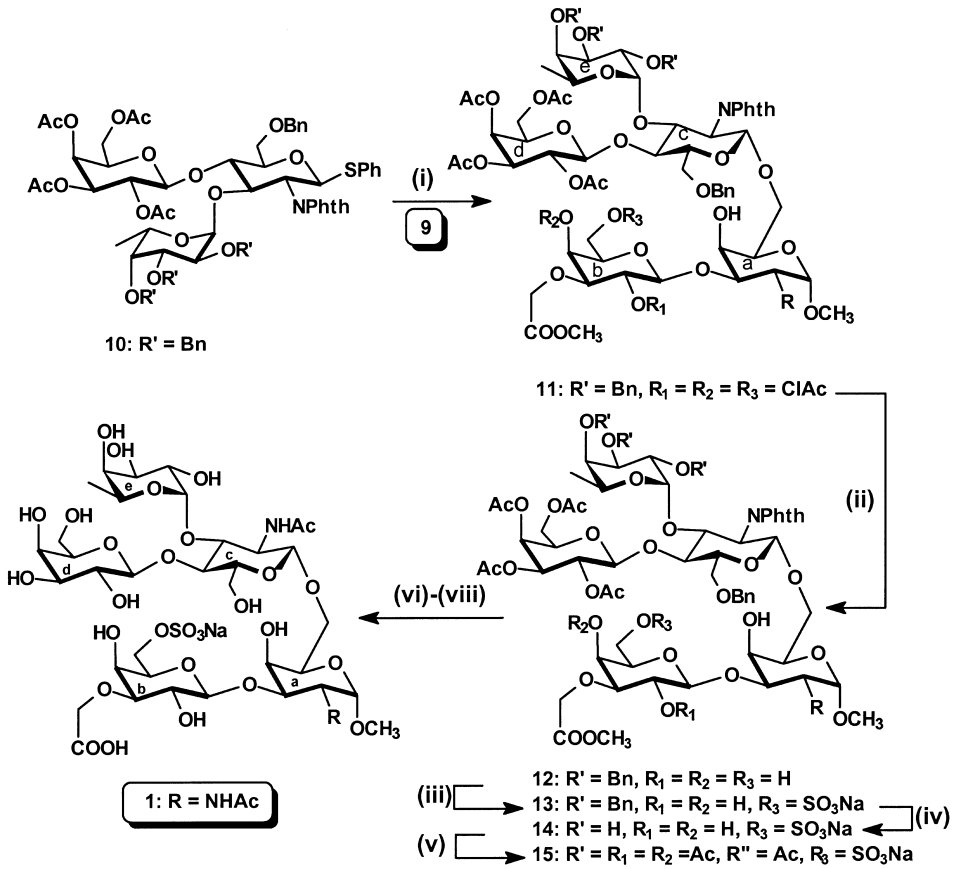


Scheme 1. (i) p-MeOPhCH$(OCH_3)_2$, PTS, CH_3CN, rt, 12 h, 75%; (ii) **4** (1.5 equiv), $Hg(CN)_2$, benzene:CH_3NO_2 (1:1), 40–45 °C, 12 h, 65%; (iii) CH_3ONa–CH_3OH (1 M), CH_2Cl_2:CH_3OH (1:1), pH 10.0, rt, 2 h, 94%; (iv) (a) *n*-Bu_2SnO/CH_3OH, refluxing, 3–4 h; (b) *n*-Bu_4NI–$BrCH_2COOCH_3$, 60–65 °C, 48 h, two steps, 57%; (v) $(ClCH_2CO)_2O$ (12 equiv)/$NaHCO_3$(12 equiv)–DMF, rt, 12 h, 65%; (vi) HOAc (60%), 60–65 °C, 1 h, 94%.



Scheme 2. (i) NIS-TfOH, CH_2Cl_2, 4A-MS, −65 to −60 °C, 2 h, 45%; (ii) thiourea-2,6-lutidine, CH_2Cl_2, refluxing, 12 h, 85%; (iii) SO_3–pyridine, pyridine, 0 °C, 6–9 h, 85%; (iv) Pd–C (10%), H_2, 9 h, 86%; (v) Ac_2O:pyridine (1:1) DMAP, rt, 12 h, 75%; (vi) LiI-pyridine, 125–130 °C, 6 h; (vii) CH_3OH–$NH_2NH_2{\cdot}H_2O$ (5:1), 80–85% °C, 4–5 h, then, Ac_2O:pyridine (1:1), DMAP, rt, 12 h; (viii) 1 M CH_3ONa–CH_3OH (cat), CH_3OH–H_2O, rt, 24 h, 35% in three steps.

alytic amount of DMAP. The above acetylated compound was then treated with a catalytic amount of 1 M sodium methoxide–methanol solution at room temperature overnight to afford target molecule **1**. The structure and purity of **1** was fully confirmed by TLC, NMR, MS and FAB.[10]

Our earlier studies show that core 2 branched structures having 6-*O*-sulfate at galactose in the Galβ(1–3)GalNAc arm bind with selectins (L- and P-Selectins). Our preliminary binding study of compound **1** indicated that this molecule can bind with these two selectins (L- and P-). Detailed inhibition studies will be reported elsewhere.

In summary, we describe a convergent route for the synthesis of a core 2 branched pentasaccharide containing a carboxylate group.

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10. Selected physical data for compound **1**: ^{1}H NMR (D_2O, 600 MHz) δ 5.18 (d, 1H, $J_{1,2}=3.6$ Hz, H^e-1), 4.83 (dd, 1H, H^e-5), 4.84–4.83 (d, 1H, $J_{1,2}=4.0$ Hz, H^a-1), 4.56 (d, $J_{1,2}=8.0$ Hz, H^c-1), 4.50 (d, 1H, $J_{1,2}=7.8$ Hz, H^d-1), 4.46 (d, 1H, $J_{1,2}=7.6$ Hz, H^b-1), 4.32 (dd, 1H, $J=2.8$ Hz, H^a-4), 4.20 (dd, 2H, CH_2COO), 4.16–3.90 (m, 13H, H^a-5, H^c-4, H^b-4, H^c-2), 3.85–3.47 (m, 12H, H^e-4, H^a-6a, H^d-3, H^b-3, H^c-5, H^d-2, H^b-2), 3.38 (s, 3H, OCH_3), 2.14 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.21 (d, 3H, $J=6.4$ Hz, CH_3^e); ^{13}C NMR (D_2O, 100.6 MHz) δ 170.78 (C=O), 170.00 (C=O), 169.68 (C=O), 103.31, 100.72, 100.07, 97.42, 96.90, 89.83, 80.73, 76.09, 74.24, 73.90, 73.67, 72.40, 71.44, 71.29, 70.78, 68.25, 68.15, 67.99, 67.54, 67.37, 67.18, 66.11, 65.45, 64.04, 63.22, 63.14, 60.18, 57.26, 56.20, 51.01, 48.63, 48.30, 47.40, 39.99, 23.15 (Ac), 23.00 (Ac), 14.50 (CH_3); FABMS (*m*/*z*) (positive ion model) calcd for $C_{37}H_{60}O_{30}N_2$-SNa: 1090.5 $[M]^+$; found 1091.8 $[M+1]^+$.