n-Pentenyl Glycosyl Orthoesters as Versatile Intermediates in Oligosaccharide Synthesis. The Proteoglycan Linkage Region[1]

John G. Allen[†,2] and Bert Fraser-Reid*[,‡]

Department of Chemistry
Paul M. Gross Chemical Laboratories
Duke University, Durham, North Carolina 27708
Natural Products and Glycotechnology Research Institute, Inc.
4118 Swarthmore Road, Durham, North Carolina 27707

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Proteoglycans[3] are biologically ubiquitous highly glycosylated glycoprotein conjugates with widely varying roles in structure[4] (as in cartilage and tendons), lubrication[5] (as in synovial fluid and "Wharton's jelly" of the placenta), blood anticoagulation,[6] and light transmission through the cornea.[7] Not surprisingly, aberrations in proteoglycan metabolism can therefore have serious consequences, leading to diseases such as rheumatoid arthritis and cystic fibrosis.[8]

In view of their implication in such disabling disorders, interest in proteoglycan biosynthesis has been heightened.[9] Some recent findings can be interpreted with the aid of the schematic construct **1** (Figure 1). Several different proteoglycans have in common a highly conserved[7] tetrasaccharide **linkage region** joining a glycosaminoglycan (GAG) group to a core protein through entities **Y** and **Z**. Unit **Y** is either serine or threonine, the xylose−serine bond being unique in that it does not occur in other mammalian glycoconjugates. Unit **Z** is GlcNAc or GalNAc,[10] a critical differentiation representing the point at which the biosynthetic routes to glucosaminoglycans (such as heparin) and galactosaminoglycans (such as dermatan and chondroitin sulfates) diverge.[11] Recent studies by Esko[11] and others[12] have identified the core protein as a regulatory factor in this biosynthetic outcome. A tetraglycosylserine corresponding to the linkage region, and suitably protected as in **2**, is therefore of interest, since the serine moiety allows for specific elaboration of the core protein in either direction. In this communication we describe a synthesis[13] of the required tetrasaccharide and its direct coupling to give **2** that draws heavily upon the chemistry of *n*-pentenyl donors.[14]

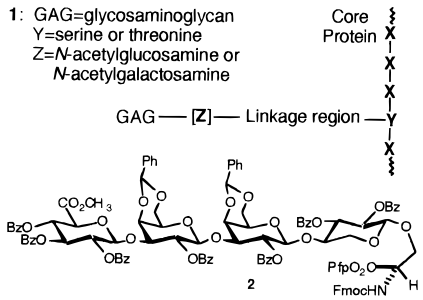
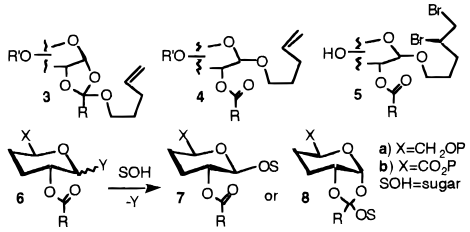


Figure 1. Schematic representation of a proteoglycan (**1**) and synthetic linkage region construct (**2**).

Scheme 1



Recent studies in our laboratory have shown that *n*-pentenyl orthoesters can be valuable synthetic intermediates, stemming from their ability to undergo rearrangement to *n*-pentenyl glycosides (NPGs) under mild conditions, thereby enabling a given precursor to serve as a donor (e.g., **3** or **4**; Scheme 1) or an acceptor (e.g., **5**).[15] Orthoester formation during coupling reactions, on the other hand, is an unwelcome occurrence common to glycosyl donors of uronic acids.[16] Thus, whereas 2-*O*-acyl hexoses such as **6a** react routinely to give 1,2-*trans*-products such as **7**, the uronate counterpart **6b** may give an orthoester **8** as the major product under similar conditions. Accordingly, our studies were prompted, in part, by challenges facing the glucuronate component of the target **2**.

Retrosynthesis leads to the coupling partners **9**−**12** shown in Scheme 2 and reflects insights we have gained from exploratory experiments. Thus, the uronate retron **9** was equipped with a trichloroacetimidate activating group so as to permit orthogonal coupling to the NPG **10c**.[17] Often orthoester formation is obviated by use of a pivaloyl protecting group at O2;[16f,18] however, we chose benzoyl[19] for the case at hand to ensure β-selectivity, as well as easier removal in the future. A convergent route bringing together the GlcUA-Gal and Gal-Xyl disaccharides would save steps and allow the galactoside residue **10** to serve twice. Furthermore, the labile xylose−serine bond would be formed last.

† Duke University.
‡ Natural Products and Glycotechnology (NPG) Research Institute, Inc.

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(2) From the Ph.D. Dissertation of J. G. A., Duke University, May 1998.

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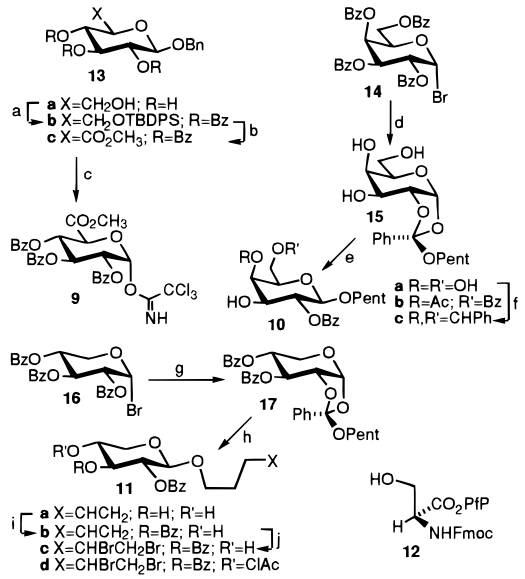
Scheme 2[a]



[a] (a) TBDPSCl and DMAP in pyridine for 48 h, then BzCl (92%). (b) (i) HF-pyridine; (ii) Jones oxidation, then CH_2N_2 (86%, three steps). (c) (i) Pd−C, H_2; (ii) Cl_3CCN, DBU, 0 °C, 20 min (80%, two steps). (d) (i) Bu_4NI, lutidine, room temperature (rt), 24 h; (ii) NaOMe, 0 °C, 4 h. (e) TESOTf, PentOH, rt, 4 h (41%, three steps). (f) $PhCH(OMe)_2$, CSA (94%). (g) PentOH, lutidine, rt, 10 d (87%). (h) (i) NaOMe, 0 °C, 3 h (74%); (ii) PentOH, CSA (80%). (i) (i) 1.05 equiv of TBSOTf, lutidine, CH_2Cl_2, −78 °C, 1 h (83%); (ii) BzCl, pyridine, CH_2Cl_2; (iii) TBAF, THF, 0 °C (80%, two steps). (j) Et_4NBr, Br_2 (90%). Pent = pent-4-enyl.

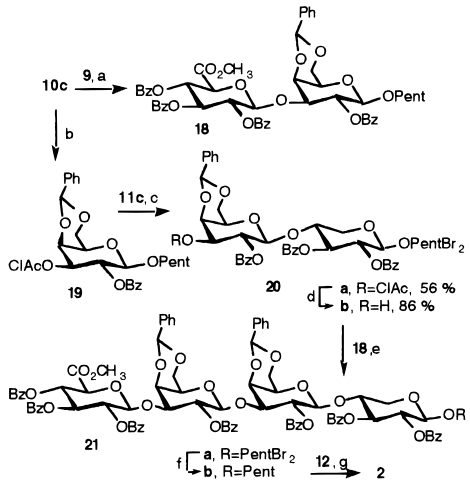
Synthesis of trichloroacetimidate **9** from commercially available benzyl *β*-D-glucoside (**13a**) was achieved by the high-yielding route shown in Scheme 2, featuring an efficient (92%) one-pot silylation−acylation procedure,[20] to obtain C6 differentiated **13b**, and thence methyl glucuronate **13c** and **9** (69% for five steps).

With respect to the galactoside acceptor, our preliminary work had shown that a triester **10b** was so deactivated that it reacted to give orthoester products only.[21] We hoped that replacing two of the esters with a benzylidene group would enhance reactivity. Accordingly, the *n*-pentenyl orthoester **15** was prepared routinely from galactosyl bromide **14**, and rearrangement in the presence of excess pentenyl alcohol in order to obviate self-condensation afforded **10a** in 41% yield over three steps. The desired benzylidene derivative **10c** was obtained routinely in 94% yield.

The xylose retron **11c** required differentiation at O4. An *n*-pentenyl orthoester, in this case **17**, again proved valuable in transforming xylosyl bromide **16** to the diol **11a**. It transpired that subtle differentiation between the C3 and C4 hydroxyl groups of **11a** was best accomplished by relying on steric factors. Thus, monosilylation yielded **11b** as the major regioisomer (8:1), and standard operations led to the 2,3-di-*O*-benzoyl xyloside **11c**. Dibromination[14] then provided the sidetracked dibromopentanyl acceptor **11c** (31% over five steps).

Increased reactivity of benzylidene-protected galactoside acceptor **10c**, in contrast to the result using acceptor **10b**, was revealed in the coupling with **9**. As is shown in Scheme 3, a short reaction time at 0 °C, to minimize orthoester formation and acid-catalyzed decomposition of the benzylidene group, led overwhelmingly to the desired product **18**, ready to serve directly as the disaccharide donor.

Scheme 3[a]



[a] (a) 1.2 equiv of **9**, TESOTf, CH_2Cl_2, 0 °C, 15 min (89%, 3:1 mixture of **18** and corresponding orthoester). (b) ClAcCl, pyridine (89%). (c) 1.27 equiv of **11c**, NIS, TESOTf, −20 °C, 1.25 h (56%). (d) CS_2, H_4N_2, *i*-Pr_2EtN, rt, 20 min (86%). (e) 1.8 equiv of **18**, NIS, TESOTf, 0 °C, 15 min (52%). (f) NaI, MEK, 50 °C, 12 h (83%). (g) 2 equiv of **12**, NIS, TESOTf, 0 °C, 15 min (35%). $PentBr_2$ = 4,5-dibromopentanyl.

The second galactoside synthon **19** was obtained from **10c** by chloroacetylation. Coupling with sidetracked NPG **11c** then afforded disaccharide **20a**.[22] Standard dechloroacetylation of **20a** with thiourea in hot ethanol caused decomposition; however, the use of hydrazine dithiocarbonate, as pioneered by van Boeckel,[23] succeeded in providing **20b** in 86% yield after only 20 min at room temperature.

NIS-TESOTf-promoted coupling of disaccharides **18** and **20b** proceeded smoothly at 0 °C in 15 min, giving tetrasaccharide **21a** in the respectable yield of 52%. Mild reductive debromination, effected by warming with excess sodium iodide in methyl-ethyl ketone,[24] restored *n*-pentenyl activation to **21b** (80% yield).[25]

The direct[26] coupling of tetrasaccharide donor **21b** with serine acceptor **12**[27] was carried out at 0 °C in 15 min to give the desired material **2** in 35% yield. By this short and efficient route, ∼100 mg of the target material **2** was prepared. Incorporation of this synthetic probe into peptides for biological investigations is underway and will be reported in due course.

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Supporting Information Available: Text giving experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Please see Supporting Information for unpublished work.

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