Pergamon

Tetrahedron Letters, Vol. 38, No. 40, pp. 6985-6988, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01670-5

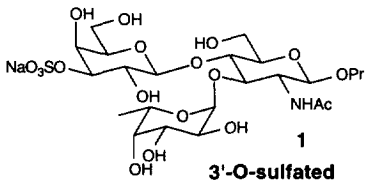
Para-Chlorobenzyl Protecting Groups As Stabilizers of the Glycosidic Linkage: Synthesis of the 3′-*O*-Sulfated Lewis X Trisaccharide

Nicola L. Pohl and Laura L. Kiessling*

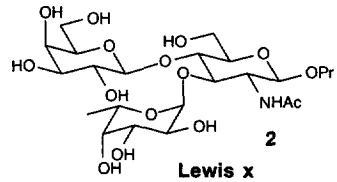
Department of Chemistry, University of Wisconsin-Madison,
Madison, Wisconsin 53706, USA

Abstract: *The 3′-O-sulfated trisaccharide Lewis x (1) and unsulfated Lewis x (2) have been synthesized using a route that highlights a more facile regioselective benzylidene ring-opening procedure and the employment of chlorobenzyl groups as a way of strengthening the acid-labile α-fucose linkage.*

Recent discoveries of biologically and medically significant carbohydrate-binding proteins have intensified efforts to develop efficient methods for carbohydrate assembly. Much of this work has focused on the development of glycosidic coupling methods and improving their yields and selectivities, yet protecting group strategies are no less important in saccharide syntheses. Their significance became acutely clear when our group desired a convergent, flexible route to the trisaccharides Lewis a (Le^a) and Lewis x (Le^x)—one that would allow the facile construction of various sulfated Lewis trisaccharides, as well as other congeners, for NMR and biological studies with the selectins E, P, and L. The importance of the selectins in the inflammatory response and the recognition that sialyl Le^a and sialyl Le^x function as selectin ligands,[1] have prompted the development of synthetic routes to relevant Le^a and Le^x derivatives.[2] The α-fucose linkage found in these trisaccharides is notoriously unstable to acid, especially in the protected stage.[3] We report an efficient approach to the synthesis of 3′-*O*-sulfated Le^x (**1**) and Le^x (**2**) that addresses this problem and introduces a useful method for regioselective benzylidene acetal ring-opening.



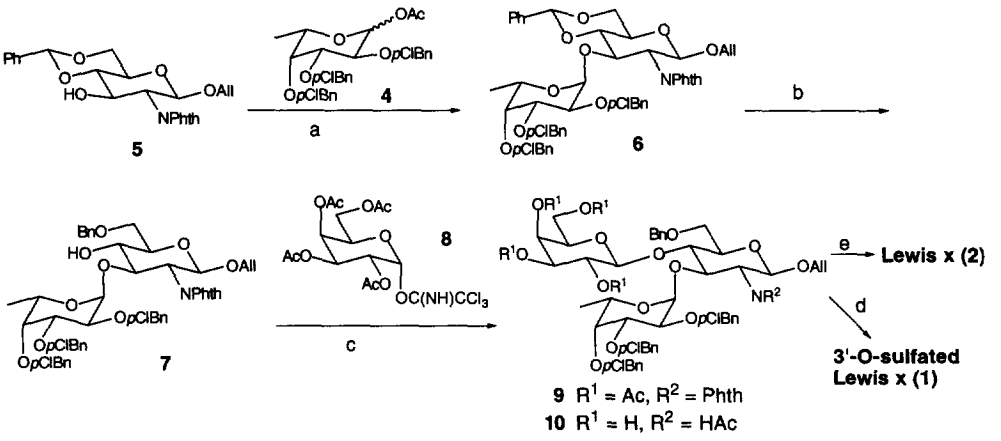
1
3'-O-sulfated Lewis x



2
Lewis x

The allyl 4,6-benzylidene-2-deoxy-2-phthalimido glucopyranoside derivative **5** (Scheme 1) was employed as a key intermediate in the synthesis. The anomeric allyl group provides a handle that can be elaborated, removed, or reduced to a propyl group to provide a single anomer of the final product. For nitrogen protection, the phthalimide

protecting group was selected; the corresponding protected *N*-acetyl glucosamine derivative was too insoluble in the nonpolar solvents required for glycosylations using trichloroacetimidate donors, and the recently introduced tetrachlorophthalimide group[4] proved unstable in the subsequent benzylidene ring-opening step. Introduction of the benzylidene acetal leaves the *C*-3 hydroxyl in **5** free for coupling to an appropriately equipped fucose residue, a transformation that was carried out with triflic acid activation to afford disaccharide **6**.

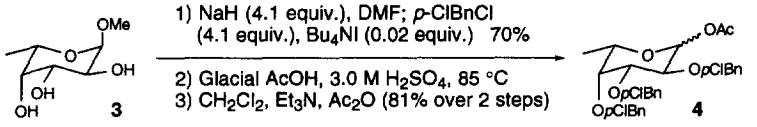


Scheme 1. *Syntheses of the 3'-O-Sulfated Lewis x and Lewis x Trisaccharides.* Conditions employed: a) 1.2 equiv. **4**, 1:5 THF/ether, 0.3 equiv. TfOH, 90%; b) 7.3 equiv. $NaCNBH_3$ in THF, 7.4 equiv. TfOH, 3 Å M.S., 81%; c) 2.0 equiv. **8**, 0.3 equiv. TfOH, ether, 85%; d) 1. ethylene diamine, nBuOH, reflux; Ac_2O, pyridine; NaOMe, MeOH, 70%, 2. Bu_2SnO (1.1 equiv.), benzene, reflux; pyridine•SO_3 (45%, 90% based on recovered starting material); 3. $Pd(OH)_2$, MeOH, H_2O, 500 psi H_2, 90%; e) $Pd(OH)_2$, MeOH, H_2O, 500 psi H_2, 90%. Pr = propyl, All = allyl, pClBn = *para*-chlorobenzyl, Phth = phthalimide, Ac = acyl, Bn = benzyl.

To release the 4-hydroxyl group for glycosylation to form the trisaccharide, the benzylidene acetal reduction was explored. The traditional method for selective ring-opening of the disaccharide benzylidene acetal, which employs $NaCNBH_3$ and etheral HCl,[5,6] led to much decomposition of the fucose-glucosamine disaccharide upon addition of etheral HCl. Because the glycosidic linkages of saccharide residues protected with electron withdrawing groups such as acetyl are more stable, we hypothesized that the use of more electron-withdrawing groups to protect the fucose should diminish some of these acid-catalyzed decomposition reactions. Previously, *para*-chlorobenzyl groups have been substituted for benzyl groups to impart greater crystallinity to compounds.[7] In our study, the chlorobenzyl groups facilitated oligosaccharide synthesis by stabilizing the glycosidic bonds because of their electron-withdrawing character.[8] The new fucose donor **4** was easily made in three steps from α-methyl-fucopyranoside **3** (Scheme 2).[9] This fucose moiety, which possesses an anomeric acetyl group, was reactive in acid-promoted glycosylation, precluding the need to use more complex or costly activating methods. As expected, the selectivity of the glycosylation was not altered by the minor change in the fucose protecting groups, and compound **6** was obtained in a yield of 90%.

While the classical method of selective benzylidene ring-opening mentioned above usually worked well with the new fucose protecting scheme, the reaction is difficult to control on small scale, and addition of acid must be constantly monitored to avoid hydrolysis of the acetal. Acids such as trifluoroacetic acid are more conveniently measured and dispensed than is gaseous HCl. Although the former has been employed in the reductive ring-

opening of the *para*-methoxybenzylidene acetal,[10] it does not promote cleavage of the benzylidene acetal in the presence of $NaCNBH_3$.[11] The stronger triflic acid, however, did facilitate the acetal reduction with the desired regioselectivity. These conditions improve the average yields for this transformation, because they are more reproducible. In addition, this reduction method is easier to implement on milligram scale and could be useful in solid phase synthesis.



Scheme 2. *Synthesis of the Fucose Monomer.*

With an effective route to compound **7**, trisaccharides **2** and **1** were generated. Glycosylation of the resulting unmasked alcohol **7** with tetraacylated galactose trichloroacetimidate **8**[12] using triflic acid activation provided the protected Lewis x trisaccharide. Removal of the phthalimide group with ethylenediamine[13] rather than hydrazine left the allyl group intact for possible further derivatizations. Subsequent *N*- and *O*-acetylation with acetic anhydride and pyridine, followed by selective *O*-deacetylation, furnished the tetraol **10**. The removal of the remaining protecting groups and the reduction of the allyl group were accomplished by catalytic hydrogenation with Pearlman's catalyst to provide the trisaccharide Lewis x (**2**) after purification by silica gel flash chromatography. Conversion of tetraol **10** to the corresponding stannylene acetal with dibutyltin oxide, followed by treatment of this intermediate with pyridine• sulfur trioxide complex in benzene[12,14] resulted in a monosulfated compound that was separated from the starting material by silica gel flash chromatography. Catalytic hydrogenation as before provided the desired 3′-*O*-sulfated Lewis x trisaccharide (**1**) after purification by silica gel flash chromatography (1:4:5, water:methanol:chloroform) and cation exchange chromatography to form the sodium salt.

In addition to facilitating the synthesis of the desired selectin ligands, the *p*-chlorobenzyl protecting groups may prove useful in other applications requiring non-participatory protecting groups with greater electronegativity than the corresponding benzyl groups. For example, the *p*-chlorobenzyl protected fucose derivative **4** can be employed in other syntheses that necessitate the introduction of this widely-occurring saccharide residue. Additionally, the use of triflic acid as an easily measurable strong acid in regioselective benzylidene acetal openings also offers promise, both for saccharide synthesis in solution and for solid phase methodologies on acid-stable resins.

Acknowledgments: This research was supported by the NIH (GM-49975) and the NSF NYI program. L.L.K. acknowledges the American Cancer Society, the Milwaukee Foundation, the Dreyfus Foundation, Zeneca Pharmaceuticals and the Sloan Foundation for support. N.L.P. acknowledges the Department of Education for a fellowship and the Marie Christine Kohler Foundation. NMR facilities were supported by grants from NSF (CHE-9208463) and NIH (S110 RRO 8389-01).

References and Notes:

1. Crocker, P. R; Feizi, T. *Curr. Opin. Struct. Biol.* **1996**, *6*, 679-691 and references therein.
2. For syntheses of Lewis x, see: Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239-9248 and references cited therein. For syntheses of 3'-*O*-sulfated Lewis x and its glucose analog, see: a) Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R. *J. Am. Chem. Soc.* **1993**, *115*, 8843-8844; b) Numomura, S.; Iida, M.; Numata, M.; Sugimoto, M.; Ogawa, T.; *Carbohydr. Res.*, **1994**, *263*, C1-C6; c) Singh, K.; Fernández-Mayoralas, A.; Martín-Lopez, M. *J. Chem. Soc., Chem. Commun.* **1994**, 775-776; d) Sanders, W. J.; Katsumoto, T. R.; Bertozzi, C. R.; Rosen, S. D.; Kiessling, L. K.; *Biochemistry*, **1996**, *35*, 14862-14867; e) Sanders, W. J.; Manning, D. D.; Koeller, K. M.; Kiessling, L. L.; *Tetrahedron*, **1997**, in press.
3. a) Ramphal, J. Y.; Zheng, Z.-L.; Perez, C.; Walker, L. E.; DeFrees, S. A.; Gaeta, F. C. A. *J. Med. Chem.* **1994**, *37*, 3459-3463; b) Kabat, E.; Baer, H.; Bezer, A. E.; Knaub, V. *J. Exp. Med.* **1948**, *88*, 43-57.
4. a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302-3303; b) Debenham, J. S.; Fraser-Reid, B. *J. Org. Chem.*, **1996**, *61*, 432-433.
5. Garegg, P. J.; Hultberg, H. *Carbohydr. Res.* **1981**, *93*, C10-C11.
6. Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97-101.
7. For examples, see the following and references therein: a) Koto, S.; Inada, S.; Morishima, N.; Zen, S. *Carbohydr. Res.* **1980**, *87*, 294-296; b) Nilsson, S.; Lönn, H.; Norberg, T. *Glycoconjugate J.* **1991**, *8*, 9-15; c) Nifant'ev, N. É.; Amochaeva, V. Y.; Shashkov, A. S.; Kochetkov, N. K. *Biorg. Khim.* **1992**, *18*, 699-715.
8. For example, acidic hydrolysis of the axial anomeric methyl group of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside at 80-85 °C required a thirty minute reaction time; heating at this temperature for an additional 20 minutes resulted in a 30% reduction in yield. Koto, S; Morishima, N.; Miyata, Y.; Zen, S. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2639-2640. This same hydrolysis reaction with the corresponding *p*-chlorobenzylated compound required one hour at 93-97 °C. Koto, S; Inada, S.; Morishima, N.; Zen, S. *Carbohydr. Res.* **1980**, *87*, 294-296. In the case of fucose, which as a 6-deoxy sugar contains one fewer electron withdrawing group than glucose, we found a less dramatic, yet still noticeable difference in the acidic hydrolyses rates between the benzylated and *p*-chlorobenzylated sugars. (Reaction 2 of Scheme 2.)
9. Data for Compound **4** (primarily the equatorial anomer): IR (NaCl, thin film) 1750, 1490, 1230, 1090 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31-7.18 (m, 12 H), 5.55 (d, J = 8.1 Hz, 1 H), 4.88 (d, J = 11.8 Hz, 1 H), 4.77-4.63 (m, 5 H), 3.89 (dd, J = 9.2, 8.3 Hz, 1 H), 3.63 (q, J = 6.4 Hz, 1 H), 3.54-3.59 (m, 2 H), 2.05 (s, 3 H), 1.22 (d, J = 6.4 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT 135) δ 169.3 (C), 136.7 (C), 136.5 (C), 133.4 (C), 133.3 (C), 129.4 (CH), 129.2 (CH), 129.0 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 94.0 (CH), 82.4 (CH), 77.9 (CH), 76.5 (CH), 74.2 (CH_2), 74.1 (CH_2), 72.2 (CH_2), 71.3 (CH), 21.0 (CH_3), 16.6 (CH_3); MS (LSIMS, 3-NBA and NaI) 601 *m/z* [M + Na, 601.1 calcd for $C_{29}H_{29}O_6Cl_3Na$].
10. Johansson, R.; Samuelsson, B. *Chem. Soc. Perkin Trans. I* **1984**, 2371-2374.
11. Triethylsilane with trifluoroacetic acid has been reported recently: DeNinno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett.* **1995**, *36*, 669-672. The combination of Et_3SiH/TfOH works in minutes rather than hours to open the acetal, yet also leads to more decomposition than the $NaCNBH_3$/TfOH mixture,which also effects rapid reductive ring opening.
12. Manning, D. D.; Bertozzi, C. R.; Pohl, N. L.; Rosen. S. D.; Kiessling, L. L. *J. Org. Chem.* **1995**, *60*, 6254-6255.
13. Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139-164.
14. Lubineau, A.; Lemoine, R. *Tetrahedron Lett.*, **1994**, *35*, 8795-8796.

(*Received in USA* 22 *July* 1997; *revised* 1 *August* 1997; *accepted* 5 *August* 1997)