Regioselective Deacetylation and Glycosylation in the Synthesis of the Sialyl Lewis X Tetrasaccharide, a Key Component of the Recognition Site of PSGL-1

Maciej Pudelko,* Danuta Kowalczyk, Horst Kunz

Institut für Organische Chemie, Johannes-Gutenberg Universität Mainz, Duesbergweg 10–14, 55128 Mainz, Germany
Fax +49(6131)3922338; E-mail: pudelko@uni-mainz.de

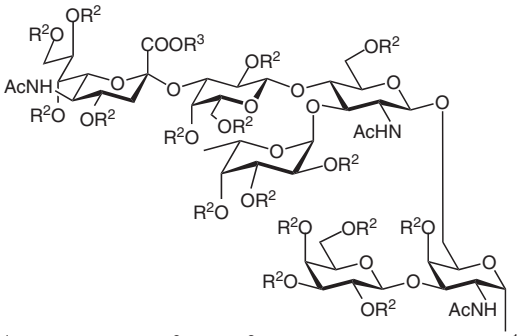
Received 26 September 2010

Abstract: A high-yielding regioselective deprotection of three out of five hydroxy groups of a Lewis X trisaccharide, which proceeds under very mild basic conditions, and a regio- and stereoselective sialylation reaction enable an efficient access to the sialyl Lewis X tetrasaccharide.

Key words: glycosylation, regioselective deacetylation, solid-phase synthesis, glycopeptides, sialyl Lewis X

Murine P-selectin expressed by activated platelets and endothelial cells exhibits the highest ligand affinity and specificity of all selectins. It is thought to bind to the N-terminus of murine PSGL-1, which has a different amino acid sequence from the human PSGL-1.[1] Recent studies showed that for a sufficient binding of murine PSGL-1 to P-selectin, the presence of sialyl Lewis X at Thr-17 and the *O*-sulfation of Tyr-13 in glycopeptide **2** (Figure 1) are crucial. Only then does the tethering and rolling of cells expressing murine PSGL-1 to P-selectin occur.[2] This suggests that relatively short sulfated glycopeptides are well suited for the inhibition of the P-selectin/PSGL-1 interaction, which is an important step during the inflammatory response and during the metastasis of tumor cells.

Herein, we report the regioselective de-*O*-acetylation of a Lewis X trisaccharide **12** which is a crucial intermediate in the preparation of the sialyl Lewis X tetrasaccharide **15** and of the oxazoline donor **5** derived from it. In our laboratories, the chemical synthesis of different recognition domains of PSGL-1[3] was previously developed, which has afforded various glycopeptides and glycopeptide mimics that served as selectin ligands containing sialyl Lewis X.[4] Some of them contained the sialyl Lewis X-core2-threonine building block **1**, which undoubtedly constitutes the most demanding target in the synthesis of the PSGL-1 glycopeptide **2**. According to a novel retrosynthetic analysis (Scheme 1), the hexasaccharide construct **1** should be accessible from a partially deprotected T antigen-threonine conjugate **4**, which is a known intermediate in the synthesis of tumor-associated antigens.[5,6] It is coupled to the sialyl Lewis X oxazoline derivative **5**. The oxazoline functionality should enable the facile stereoselective formation of the β-glycosidic bond between donor **5** and acceptor **4**.



1 R^1 = Fmoc-**Thr**-OH, R^2 = Ac, R^3 = Me

2 R^1 = Ac-Phe-Glu-Asp-Pro-Asp-Tyr(13)-Thr(14)-Tyr(15)-Asn(17)-**Thr**-Asp-Pro-OH
$R^2 = R^3 = H$; OSO_3^- at Tyr(13) and Tyr(15)

Figure 1

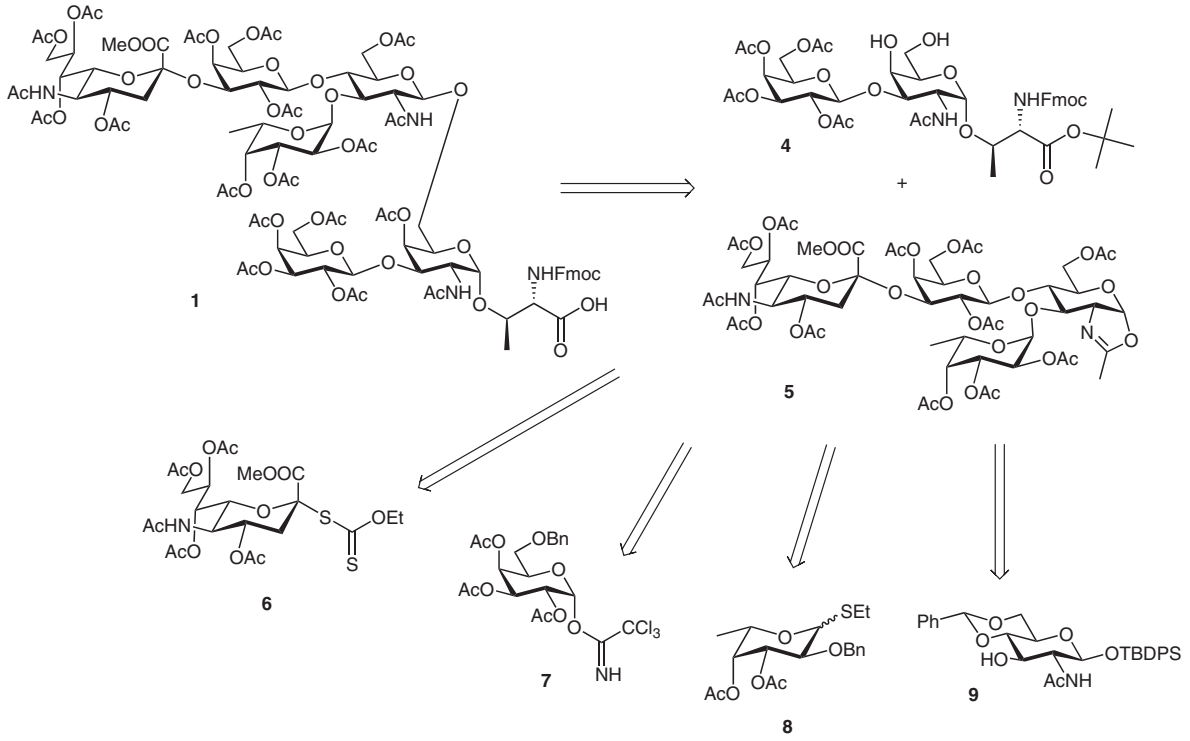
In addition, the natural acetamido functionality in the glucosamine unit should be re-established. This transformation is considered feasible when copper(II) salts are used as mild Lewis acids[7] for activating the oxazoline ring during glycosylation.

The axial 4-OH group in **4** usually is of low reactivity, and is not expected to compete with the primary hydroxy group in position 6.

The protected *N*-acetylglucosamine building block **9** equipped with the *tert*-butyldiphenylsilyl group[8] at the anomeric oxygen, which is stable under acidic and basic conditions, was used as the starting material for the construction of the sialyl Lewis X tetrasaccharide **15** (Scheme 2). Compound **9** was glycosylated with the thiofucoside donor **8**[9] bearing acetyl protecting groups in positions 3 and 4 under adapted in situ anomerization conditions[10] and gave the disaccharide **10** in 58% yield. In order to stereoselectively obtain the α-fucoside structure the benzyl ether in position 2 had to be installed in the fucoside donor **8**.

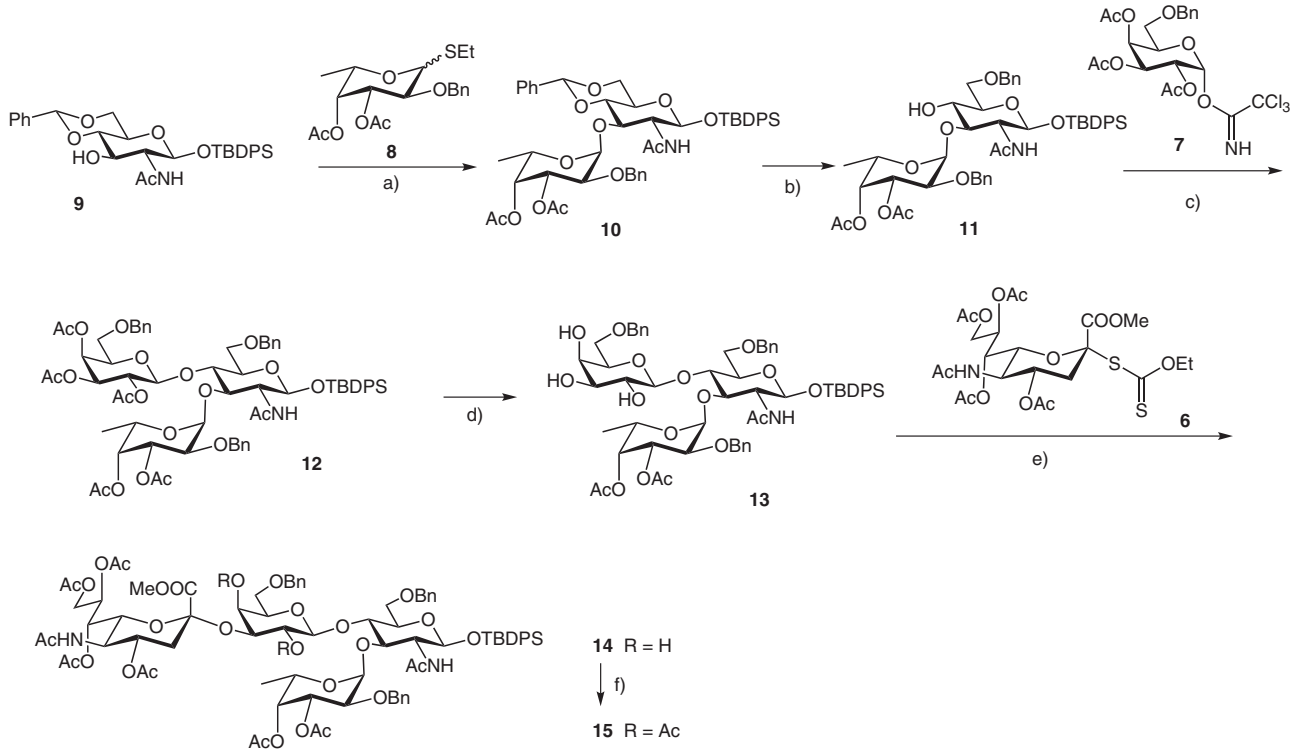
Acetyl protecting group at the α-fucoside moiety should decrease the sensitivity of this glycosidic bond towards acidic conditions as it is well established that *O*-benzyl protecting groups on the fucose moiety are responsible for the high acid-sensitivity of the fucoside bond.[11] The new protecting group strategy concerning the fucose portion was different from the previous one.[3] The intention was to circumvent the exchange of protecting groups in order to make the saccharide part suitable for the acidic conditions

SYNLETT 2010, No. 20, pp 3023–3026
Advanced online publication: 17.11.2010
DOI: 10.1055/s-0030-1259054; Art ID: G27410ST



Scheme 1

of solid-phase glycopeptide synthesis. Trifluoromethanesulfonic acid/triethylsilane-promoted regioselective acetal ring-opening[12] at –78 °C furnished the disaccharide **11** with a free 4-OH position at glucosamine unit in 81% yield. Acceptor **11** was then reacted with the galactosyl trichloroacetimidate **7** in a TMSOTf-promoted glycosylation according to Schmidt,[13] and gave the Lewis X trisaccharide **12** in 81% yield. In a crucial step the three *O*-



Scheme 2 *Reagents and conditions*: (a) **8** (1.1 equiv), $CuBr_2$ (1.37 equiv), NBu_4Br (1.37 equiv), 4Å MS, DMF–CH_2Cl_2 (1:1), r.t., 2 d, 58% yield; (b) TfOH (2.9 equiv), Et_3SiH (1.5 equiv), 4Å MS, –78 °C, 4.5 h, CH_2Cl_2, 81% yield; (c) **7** (1.8 equiv), TMSOTf (0.23 equiv), CH_2Cl_2, – 50 °C slowly to r.t., 17 h, 81% yield; (d) cat. NaOMe–MeOH, r.t., pH 7.5, 94% yield; (e) **6** (2.32 equiv), AgOTf (2.33 equiv), MeSBr (2.33 equiv), 4Å MS, MeCN–CH_2Cl_2 (5:1), –50 °C → –28 °C, 17 h, 76% yield; (f) Ac_2O–Py, DMAP (0.9 equiv), r.t., 90% yield.

acetyl groups of the galactose unit of Lewis X trisaccharide **12** were selectively cleaved by very mild transesterification with a freshly prepared solution of catalytic sodium methoxide in methanol. Interestingly, when the pH value did not exceed 7.5 (wet pH indicator), it was possible to obtain **13** as a single regioisomer in 94% yield, with unaffected *O*-acetyl groups on the fucose moiety after neutralization with the acidic ion-exchanger Amberlyst 15. The NMR spectrum of **13** displays the typical downfield shifts of protons H3 and H4 on the fucose moiety that bear *O*-acetyl protecting groups. However, when the same reaction was performed at pH 8.5–9.0, the completely deacetylated Lewis X trisaccharide was obtained after 20 hours at room temperature. We presume that the methoxide ion in catalytic amounts initially attacks the acetyl group on the most readily accessible 3-position of the galactose unit. Subsequently, intramolecular acetyl transfer occurs which leads to the removal of the *O*-acetyl groups from the adjacent 2-OH and 4-OH positions in the galactose. It appears that this acetyl migration does not apply to the two *O*-acetyl groups on the fucose moiety.

Taking advantage of this highly regioselective deacetylation of **12**, the reaction of **13** with the sialyl donor **6**[14] according to previously published methodology[6,15] using AgOTf/Me–S–Br promoting system stereo- and regioselectively gave the tetrasaccharide **14** in 76% yield, thereby exploiting the nitrile effect.[16] Acetylation of the 2-OH and 4-OH groups of the galactose unit gave the sialyl Lewis X **15** in 90% yield. It is worth noting that the anomeric TBDPS group remained unaffected during all these conversions towards **15** (Scheme 2).

In conclusion, we have efficiently synthesized the sialyl Lewis X tetrasaccharide **15** using an altered protective group strategy concerning the glucosamine acceptor **9** and the fucose donor **8**. The key step in this synthesis is the regioselective deacetylation of three out of five hydroxy groups of a Lewis X trisaccharide **12** using very mild transesterification protocol,[17] in which a strict control of the pH was a pivotal feature. It is also worth noting, that the glycosylation of typically unreactive 4-hydroxy group of the disaccharide unit **11** with the galactosyl donor **7** proceeds with high yield. Further work towards the tetrasaccharide oxazoline **5** and the hexasaccharide **1** representing the building block for solid-phase synthesis of glycopeptide **2** is currently in progress.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. Included are experimental procedures and spectroscopic characterization data for compounds **11**, **12**, **14**, and **15**.

Acknowledgment

M.P. is grateful for the stipend provided by the Alexander von Humboldt Foundation. Support from the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

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(17) **Typical Procedure for Regioselective O-Deacetylation of the Trisaccharide 12**: Trisaccharide **12** (2.11 g, 1.69 mmol) was dissolved in MeOH (150 mL) and freshly prepared NaOMe solution in MeOH was added carefully until pH 7.5 was obtained. Reaction was performed at r.t. and monitored by TLC every 30 min. After completion of the reaction (4 h 40 min), the mixture was neutralized with Amberlyst 15 to pH 6–6.5. The resin was removed by filtration, washed with MeOH, and the filtrate was concentrated under vacuum to give the crude **13** (1.78 g, 94% yield) as a white amorphous material. Trisaccharide **13** can be additionally purified on silica gel with EtOAc as eluent (R_f 0.35 in EtOAc). Spectroscopic data for **13**: $[\alpha]_{24D}$ –26.6 (c = 2.00, MeOH). ^{1}H NMR (COSY, CD_3OD): δ = 7.66–7.72 (4 H, m, H_{ar}), 7.19–7.39 (21 H, m, H_{ar}), 5.30–5.34 (2 H, m, H1-Fuc {5.33}, H3-Fuc {5.32}), 5.22 (1 H, d, H4-Fuc, $J_{3,4}$ = 2.6 Hz), 5.04–5.06 (1 H, m, H5-Fuc), 4.29–4.80 (8 H, m, CH_2Bn-2a {4.78}, CH_2-Bn-1a {4.66}, H1-GlcNAc {4.60}, CH_2Bn-1b {4.58}, CH_2Bn-2b {4.45}, CH_2Bn-3a {4.44}, H1-Gal {4.42}, CH_2Bn-3b {4.30}), 4.06 (1 H, t*, H4-GlcNAc, $J_{3,4} \cong J_{4,5} \cong$ 9.3 Hz), 3.92–3.96 (1 H, m, H3-GlcNAc), 3.77–3.86 (5 H, m, H6a,b-Gal {3.85}, H6a-GlcNAc {3.84}, H2-Fuc {3.82}, H4-Gal {3.78}), 3.40–3.47 (3 H, m, H2-Gal {3.46}, H5-Gal {3.43}, H6b-GlcNAc {3.42}), 3.30–3.35 (2 H, m, H3-Gal {3.33}, H2-GlcNAc {3.31}), 3.07–3.10 (1 H, m, H5-GlcNAc), 2.03, 1.94, 1.80 (9 H, 3 × s, MeAc), 1.05 (9 H, s, Me-*t*-Bu), 1.04 [3 H, d, Me(6)-Fuc, $J_{5,6}$ = 6.5 Hz]. ^{13}C NMR (BB, HSQC, 100.6 MHz, CD_3OD): δ = 173.18, 172.48,

171.56 (C=O), 140.18, 139.78 (C_{ipso}-Ar), 137.17, 137.09 ($C_{o,m,p}$-Ar), 134.78, 134.18, 130.97 (C_{ipso}-Ar), 130.79, 129.35, 129.18, 129.06, 128.71, 128.65, 128.49, 128.43 ($C_{o,m,p}$-Ar), 103.41 (C1-Gal), 98.04 (C1-Fuc), 97.77 (C1-GlcNAc), 76.68 (C5-GlcNAc), 75.45 (C3-GlcNAc), 75.05 (C5-Gal), 75.02 (C2-Fuc), 74.82 (C3-Gal), 74.75 (C4-GlcNAc), 74.44 (CH_2Bn-1), 74.29 (CH_2Bn-3), 73.56 (C4-Fuc), 73.39 (CH_2Bn-2), 72.86 (C2-Gal), 71.25 (C3-Fuc), 70.34 (C6-Gal), 69.77 (C4-Gal), 68.87 (C6-GlcNAc), 65.53 (C5-Fuc), 48.80 (C2-GlcNAc), 27.41 (Me-*t*-Bu), 23.69, 20.99, 19.98 (MeAc), 16.33 [Me(6)-Fuc]. MS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{61}H_{75}NO_{17}SiNa$: 1144.47; found: 1144.48. HRMS (ESI–TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{61}H_{75}NO_{17}SiNa$: 1144.4702; found: 1144.4690.