Pergamon

Tetrahedron Letters 41 (2000) 3879–3882

TETRAHEDRON
LETTERS

# The first, highly efficient synthesis of spacer-armed *O*-glycans on GlyCAM-1 and PSGL-1: the counter-receptors for L- and P-selectin[1]

Nobumasa Otsubo, Hideharu Ishida and Makoto Kiso *

*Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-1193, Japan*

Received 3 February 2000; revised 13 March 2000; accepted 17 March 2000

**Abstract**

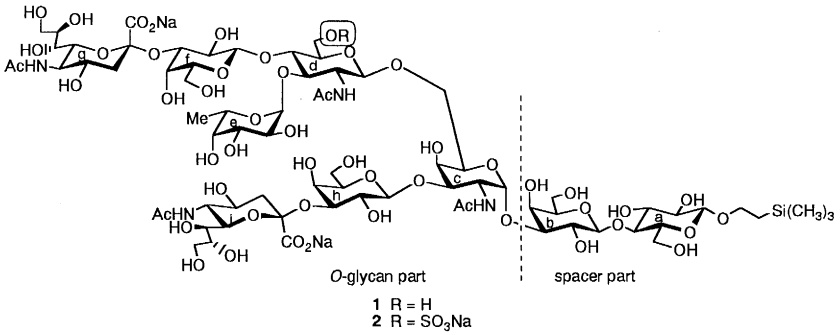
A pair of spacer-armed *O*-glycans containing sulfated/nonsulfated sialyl Le$^x$ (sLe$^x$) determinant on GlyCAM-1 and PSGL-1, the counter-receptor glycoproteins for L- and P-selectin, were synthesized for the first time in a highly efficient manner employing a simultaneous glycosylation procedure. 

*Keywords:* *O*-glycan; GlyCAM-1; PSGL-1; sialyl Le$^x$.

It has been shown[2,3] that selectin–carbohydrate interactions mediate cell adhesions involved in lymphocyte homing, inflammation, thrombosis, and cancer metastasis.[4] This has stimulated research activities not only for the endogenous carbohydrate ligands, but also their mimetics as therapeutic agents against reperfusion injury, chronic inflammatory diseases, allergy, autoimmunity, and cancer.[5] One endothelium-derived counter-receptor for L-selectin is GlyCAM-1,[6] a mucin-like glycoprotein with sulfated sLe$^x$ sequences. P-Selectin glycoprotein ligand-1 (PSGL-1),[7] a dimeric membrane mucin on leukocyte, has been suggested to be a counter-receptor for P-selectin. As a part of our studies to elucidate the structure and function of selectin ligands,[8,9] a pair of novel nonasaccharides (**1**, **2**) containing the sLe$^x$ or 6-sulfo sLe$^x$ determinant in the spacer-armed core 2 structure were constructed.

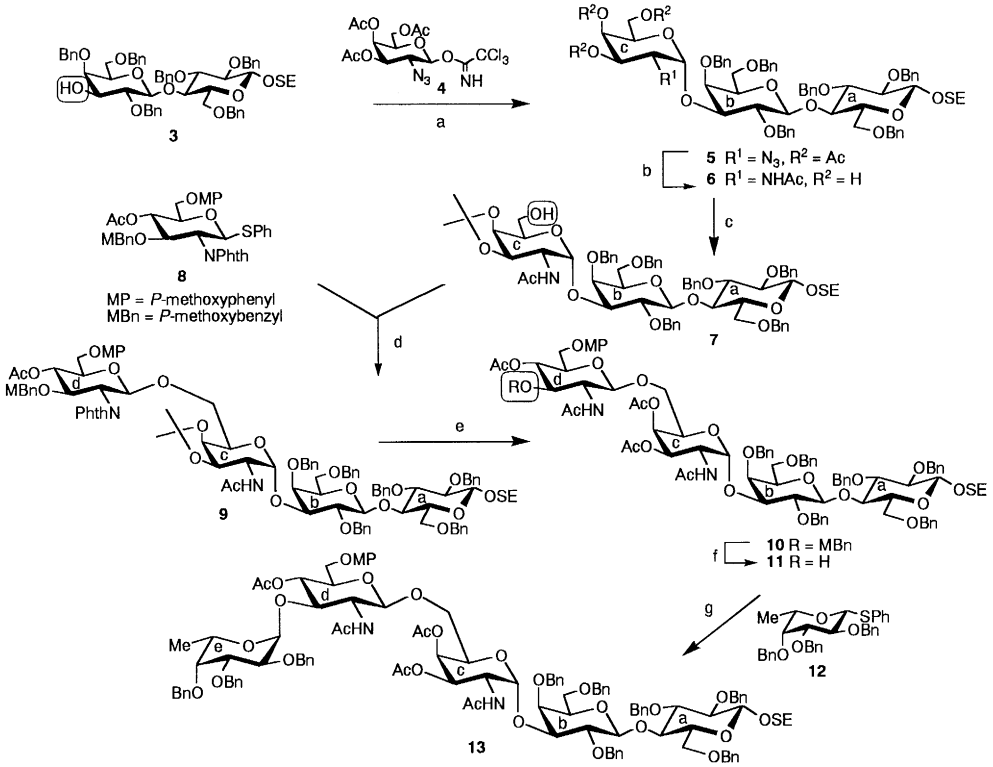
* Corresponding author. Tel: +81-58-293-2916; fax: +81-58-293-2840; e-mail: kiso@cc.gifu-u.ac.jp (M. Kiso)

0040-4039/00/$ - see front matter 
*PII:* S0040-4039(00)00492-5



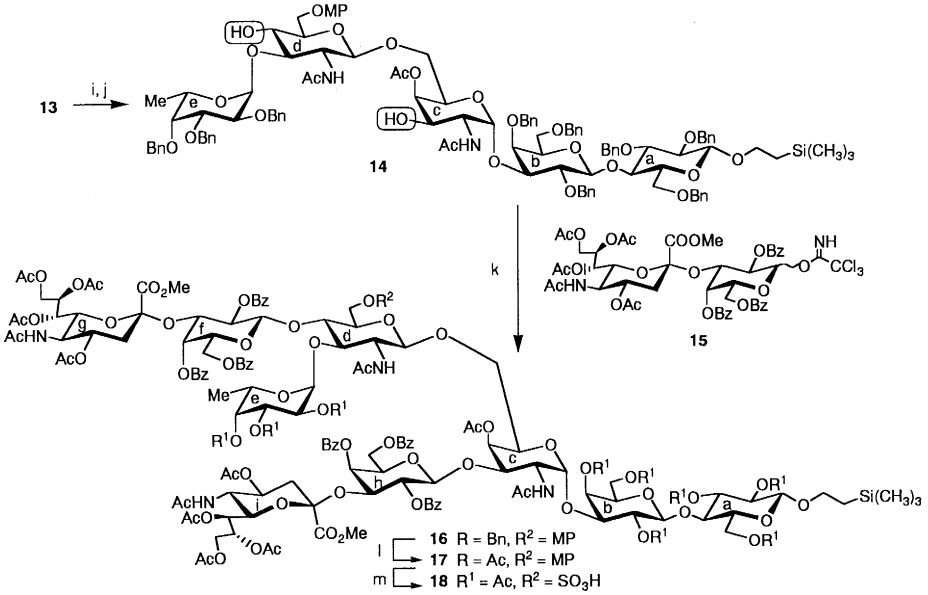

The most important problems in the synthesis of the title compounds are: (i) α-stereoselective glycosylation of the GalNAc residue with the spacer part designed for further chemical modifications, (ii) selective protection of the 3- and 6-hydroxyl groups of the GlcNAc residue that undergo fucosylation and sulfation, respectively, and (iii) efficient construction of the GlcNAcβ1→6GalNAc structure (core 6) and two sialyl-α(2→3)-Gal structures.

The TMSOTf-promoted glycosylation of **3**[10] with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl trichloroacetimidate[11] (**4**, 1.1 equiv.) in 1:1 $CH_2Cl_2$:$Et_2O$ at 0°C gave the desired α-glycoside **5** in very high yield (94%) (Scheme 1). Conversion of the azido group to an acetamido group, and subsequent 3,4-*O*-isopropylidenation of the GalNAc residue afforded **7**, that was successfully coupled with **8**, promoted[12] by NIS and TfOH, to give **9** (85%) having the core 6 structure. Removal of the isopropylidene and phthaloyl groups, and subsequent *N*-, *O*-acetylation gave **10**, from which




Scheme 1. Reagents and conditions: (a) TMSOTf, $CH_2Cl_2$–$Et_2O$, 0°C; (b) (i) $Ph_3P$, $C_6H_6$–$H_2O$, (ii) $Ac_2O$, $Et_3N$, $CH_2Cl_2$, 50°C, (iii) NaOMe, MeOH; (c) 2,2-dimethoxypropane, CSA, DMF, 70°C; (d) NIS, TfOH, $CH_2Cl_2$, −10°C; (e) (i) aq. 80% AcOH, 40°C, (ii) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, (iii) $Ac_2O$, Pyr.; (f) $SnCl_2$, TMSCl, anisole, $CH_2Cl_2$, rt; (g) NIS, TfOH, $C_6H_6$, 7°C

the *p*-methoxybenzyl (MBn) group in the GlcNAc residue was selectively cleaved by treatment with a mixture[13] of $SnCl_2$, TMSCl and anisole in $CH_2Cl_2$ to afford **11** (87%). The resulting free hydroxyl at C-3 of the GlcNAc residue in **11** was then fucosylated with **12**[14] by using the NIS/TfOH-promoted glycosylation procedure in benzene to give the desired pentasaccharide **13** (87%) (Scheme 2). This pentasaccharide was converted, by *O*-deacylation, 3,4-orthoester formation[15] in the GalNAc residue, and the opening of the orthoester with aq. 80% AcOH, into the key glycosyl acceptor **14** (93%), which was then simultaneously glycosylated with the freshly prepared sialyl-α(2→3)-galactose trichloroacetimidate donor **15**,[16] giving the desired nonasaccharide **16** (45%).




Scheme 2. Reagents and conditions: (i) MeOMe, MeOH; (j) (i) $MeC(OEt)_3$, CSA, $C_6H_6$, (ii) aq. 80% AcOH; (k) 50 mM TMSOTf, AW-300, $CH_2Cl_2$; (l) (i) $H_2$, $Pd(OH)_2$, EtOH, (ii) $Ac_2O$, Pyr.; (m) (i) CAN, $MeCN–H_2O$, (ii) $SO_3H·Pyr.$, DMF

Hydrogenolytic removal of the benzyl groups in **16** over $Pd(OH)_2$ in ethanol, followed by treatment with $Ac_2O$ in pyridine, afforded the polyacylated nonasaccharide **17** (87%). For the preparation of the GlyCAM-1 oligosaccharide **2**, the *p*-methoxyphenyl (MP) group was first cleaved by treatment[17] with ceric ammonium nitrate (CAN), and the resulting 6-OH of the GlcNAc residue was sulfated with a sulfur trioxide–pyridine complex in DMF.[14] Finally, removal of all the protective groups in **18** under the basic conditions and column chromatography on Sephadex LH-20 furnished **2** in high yield. The PSGL-1 oligosaccharide **1** was prepared by treatment of **17** with CAN, followed by *O*-deacylation and saponification of the methyl ester in NeuAc. The selected physical data of **5**–**10**, **13**, **14**, **17**, **1** and **2** are shown in Ref. 18.

In conclusion, a highly efficient synthesis of the spacer-armed *O*-glycans containing the $sLe^x$ and sulfated $sLe^x$ determinants on PSGL-1 and GlyCAM-1 has been achieved for the first time.

## Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and Japan Society for the Promotion of Science. The authors are grateful to Dr. Takao Ikami for the FABMS measurements.

## References


1. Part 114 in the series ‘Synthetic Studies on Sialoglycoconjugates’. For Part 113, see: Tanahashi, T.; Fukunaga, K.; Ozawa, Y.; Toyoda, T.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **2000**, in press.
2. McEver, R. P. *Glycoconjugate J.* **1997**, *14*, 585–591.
3. Crocker, P. R.; Feizi, T. *Curr. Opin. Struct. Biol.* **1996**, *6*, 679–691.
4. Kannagi, R. *Glycoconjugate J.* **1997**, *14*, 577–584.
5. Simanek, E. E.; McGarver, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833–862.
6. Hemmerich, S.; Leffler, H.; Rosen, S. D. *J. Biol. Chem*. **1995**, *270*, 12035–12047.
7. (a) Wilkins, P. P.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem*. **1996**, *271*, 18732–18742. (b) Leppänen, A.; Mehta, P.; Ouyand, Y.-B.; Ju, T.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem*. **1999**, *274*, 24838–24848.
8. Komba, S.; Galustian, C.; Ishida, H.; Feizi, T.; Kannagi, R.; Kiso, M. *Angew. Chem., Int. Ed. Engl*. **1999**, *38*, 1131–1133.
9. (a) Mitsuoka, C.; Ohmori, K.; Kimura, N.; Kanamori, A.; Komba, S.; Ishida, H.; Kiso, M.; Kannagi, R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1597–1602. (b) Galustian, C.; Lubineau, A.; Narvor, C.; Kiso, M.; Brown, G.; Feizi, T. *J. Biol. Chem.* **1999**, *274*, 18213–18217.
10. Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1990**, *200*, 269–285.
11. Schmidt, R. R.; Grundler, G. *Angew. Chem., Int. Ed. Engl*. **1982**, *21*, 781.
12. (a) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334. (b) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *ibid* **1990**, *31*, 4313–4316.
13. Akiyama, T.; Shima, H.; Ozaki, S. *Synlett* **1992**, 415–416.
14. Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem.* **1996**, *4*, 1833–1847.
15. Hanessian, S.; Roy, R. *Can. J. Chem.* **1985**, *63*, 163–172.
16. Hasegawa, A.; Suzuki, N.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1996**, *15*, 623–637. In the present study, the anomeric mixture (α:β=4:3) of **15** was used.
17. Nilsson, M.; Norberg, T. *J. Carbohydr. Chem.* **1990**, *9*, 1–14.
18. Selected physical data: Compound **5**: $[\alpha]_D$ +56.8° (*c* 1.6, $CHCl_3$), $^1$H NMR (400 MHz): 3.81 (dd, 1H, J=3.4, 8.6 Hz, H-2c), 5.27 (d, 1H, J=3.4 Hz, H-1c), $\nu_{max;}$ 2130 ($N_3$). Compound **6**: $[\alpha]_D$ +40° (*c* 1.0, $CHCl_3$), 1.32 (s, 3H, AcN), 4.29 (m, 1H, H-2c), 4.99 (d, 1H, J=3.6 Hz, H-1c), 5.78 (d, 1H, NH), $\nu_{max}$, 1680, 1520 (NHAc). Compound **7**: $[\alpha]_D$ +86.2° (*c* 1.0, $CHCl_3$), $^1$H NMR: 1.42, 1.46 (2s, 6H, $Me_2C$). Compound **8**: $[\alpha]_D$ +96.7° (*c* 1.2, $CHCl_3$), $^1$H NMR: 2.10 (s, 3H, AcO), 3.61, 3.79 (2s, 6H, 2MeO), 4.33 (t, 1H, J=9.9 Hz, H-2). Compound **9**: $[\alpha]_D$ +45.3° (*c* 1.6 $CHCl_3$), $^1$H NMR (500 MHz): 1.06 (s, 3H, AcN), 1.31, 1.33 (2s, 6H, $Me_2C$), 2.02 (s, 3H, AcO), 3.52, 3.75 (2s, 6H, 2MeO), 4.77 (t, 1H, J=10.6 Hz, H-4d). Compound **10**: $[\alpha]_D$ +38.3° (*c* 1.3, $CHCl_3$), $^1$H NMR: 3.71, 3.73 (2s, 6H, 2MeO), 4.84 (d, 1H, J=8.2 Hz, H-1d), 4.95 (d, 1H, J=2.3 Hz, H-4c), 4.99 (d, 1H, J=9.2 Hz, H-4d), 5.07 (d, 1H, J=3.6 Hz, H-1c), 5.47 (d, 1H, J=9.6 Hz, NH-c), 5.57 (d, 1H, J=7.3 Hz, NH-d). Compound **13**: $[\alpha]_D$ +1.9° (*c* 1.1, $CHCl_3$), $^1$H NMR: 1.00 (d, 3H, J=6.2 Hz, H-6e), 3.74 (s, 3H, MeO), 3.97 (dd, 1H, J=3.6, 9.3 Hz, H-2e). Compound **14**: $[\alpha]_D$ −1.1° (*c* 1.8, $CHCl_3$), $^1$H NMR: 1.03 (d, 3H, J=6.4 Hz, H-6e), 3.74 (s, 3H, MeO), 4.03 (dd, 1H, J=3.4, 10.3 Hz, H-2e), 4.51 (d, 1H, J=9.6 Hz, H-1d), 4.82 (d, 1H, J=2.1 Hz, H-4c), 4.87 (d, 1H, J=3.4 Hz, H-1e), 5.02 (d, 1H, J=3.6 Hz, H-1c). Compound **17**: $[\alpha]_D$ +9.9° (*c* 1.8, $CHCl_3$), $^1$H NMR: 0.97, 1.27, 1.49, 1.53, 1.74, 1.78, 1.79, 1.91, 1.93, 1.96, 2.00, 2.16, 2.18 (13s, 39H, 4AcN, 9AcO), 1.15 (d, 3H, H-6e), 1.62, 1.70 (2t, 2H, J=12.3 Hz, H-3$g_{ax}$, H-3$i_{ax}$), 2.43 (dd, $J_{vic}$=4.1 Hz, H-3$g_{eq}$, H-3$i_{eq}$), 3.68 (s, 3H, MeO), 3.77, 3.79 (2s, 6H, $2CO_2Me$), 5.43 (t, 2H, J=8.3 Hz, H-2f, H-2h). Compound **1**: $[\alpha]_D$ +1.7° (*c* 0.3, MeOH), $^1$H NMR (500 MHz, $D_2O$): 1.00 (m, 2H, TMSC$H_2CH_2$), 1.04 (d, 3H, J=5.5 Hz, H-6e), 1.77, 1.80 (2t, 2H, J=13.1 Hz, H-3$g_{ax,}$ H-3$i_{ax}$), 2.00, 2.01 (2s, 12H, 4AcN), 2.72 (dd, 2H, $J_{vic}$=4.7 Hz, H-3$g_{eq}$, H-3$i_{eq}$), 4.35 (dd, 1H, J=4.4, 11.7 Hz, H-2c), 4.47, 4.49, 4.50, 4.51, 4.56 (5d, J=7.7–8.1 Hz, five β-anomeric), 5.01 (d, 1H, J=4.0 Hz, H-1e), 5.08 (d, 1H, H-1c), FABMS $(M)^-$ 1944.7, $(M\text{-}Na+H)^-$ 1922.7, $(M\text{-}Na)^-$ 1921.7 ($C_{73}H_{122}N_4NaO_{51}Si$). Compound **2**: $[\alpha]_D$ +19.6° (*c* 0.2, MeOH:$H_2O$=1:1), $^1$H NMR (500 MHz, $D_2O$): 0.87 (m, 2H, TMSC$H_2CH_2$), 1.02 (d, 3H, J=5.5 Hz, H-6e), 1.62, 1.65 (2t, 2H, J=12.1 Hz, H-3$g_{ax}$, H-3$i_{ax}$), 1.85, 1.86 (2s, 12H, 4AcN), 2.69, 2.70 (dd, 2H, $J_{vic}$=4.3 Hz, H-3$g_{eq}$, H-3$i_{eq}$), 4.22 (dd, 1H, J=3.8, 11.8 Hz, H-2c), 4.34, 4.38, 4.40, 4.43, 4.46 (5d, 5H, J=8.0–10.3 Hz, five β-anomeric), 4.88 (d, 1H, J=3.6 Hz, H-1e), 4.95 (d, 1H, H-1c), FABMS $(M\text{-}Na)^-$ 2023.8 ($C_{73}H_{121}N_4Na_2O_{54}SSi$).