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Total synthesis of a sialylated and sulfated oligosaccharide from O-linked glycoproteins

Jie Xia, Conrad F. Piskorz, James L. Alderfer, Robert D. Locke and Khushi L. Matta *

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

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Abstract

The total synthesis of a sialylated and sulfated oligosaccharide **1** representative of a structure occurring in respiratory mucins has been accomplished. Our strategy depends upon the employment of 2-naphthymethyl (NAP) protection for hydroxyl functions. Choice of the well-defined sialyl donor **15** was made because of its enhanced reactivity over the parent compound **14** for glycosylation.

Glycoproteins and glycolipids are major components of the outer surface of mammalian cells. The hydrophilic oligosaccharide head groups of these glycoproteins and glycolipids are recognized as playing a significant role in many important biological processes, including fertilization, immune defense, parasitic infection, cell growth, cell–cell adhesion, and inflamation.[1] There is tremendous interest in structural studies of the sulfated carbohydrate chains of O-linked mucinous glycoproteins, such as CF respiratory mucin,[2a] colonic tumor associated glycoproteins[2b] and the natural ligands for selectin.[2c] The chemical synthesis of well-defined oligosaccharides which occur as a part of these mucinous glycoproteins is receiving increased attention.[3]

Consideration of the different reactivities of the sugar hydroxyl groups, in combination with detailed structural information available from two-dimensional homonuclear (^{1}H–^{1}H) (DQF-COSY, ROESY, TOCSY) and heteronuclear (^{13}C–^{1}H) (HMQC, or *g*-HSQC and HMBC) NMR correlation experiments, has afforded the development of a new strategy of regioselective and stereoselective glycosylation through the employment of unprotected or partially-protected acceptors.[4] Advancement of this new approach may overcome the traditional tedious multi-step protection–deprotection schemes and offer a much shorter and easier route to complex biologically active oligosaccharide molecules. Herein, we describe the total synthesis of complex oligosaccharide **1**.

Treatment of compound **4**[5] with bis(tributyltin)oxide[6] in refluxing toluene followed with naphthylmethyl bromide in the presence of tetrabutylammonium iodide gave **5** in 69% yield. Regioselective glycosylation of the 4-hydroxyl group of diol **5** with 2,3,4,6-tetra-*O*-acetyl-β-galactopyranosyl fluoride **6**[7] was successfully achieved using $SnCl_2$–AgOTf[8] as a catalyst. Apparently, the presence of a 6-naphthyl methyl group at HO-6 enhanced the reactivity of the relatively inactive HO-4 of diol **5** and disaccharide

* Corresponding author.

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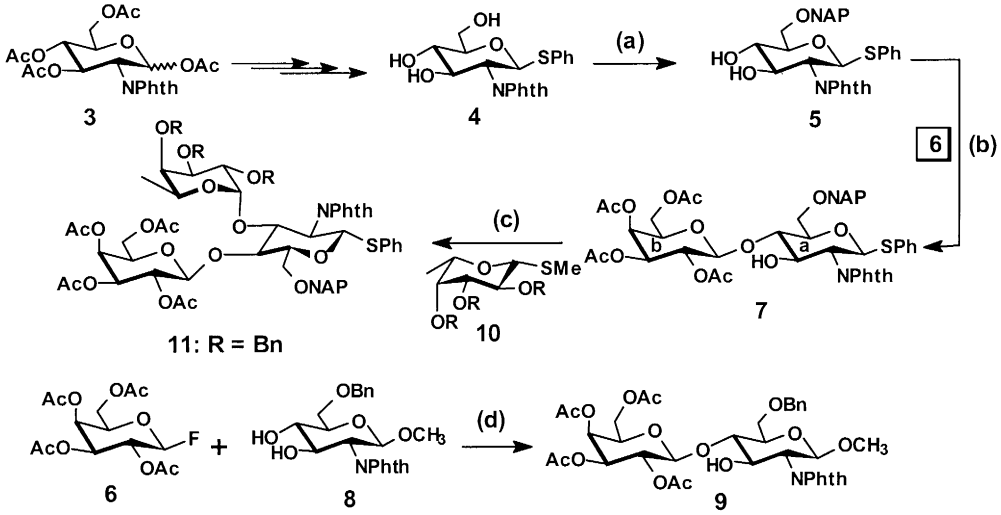
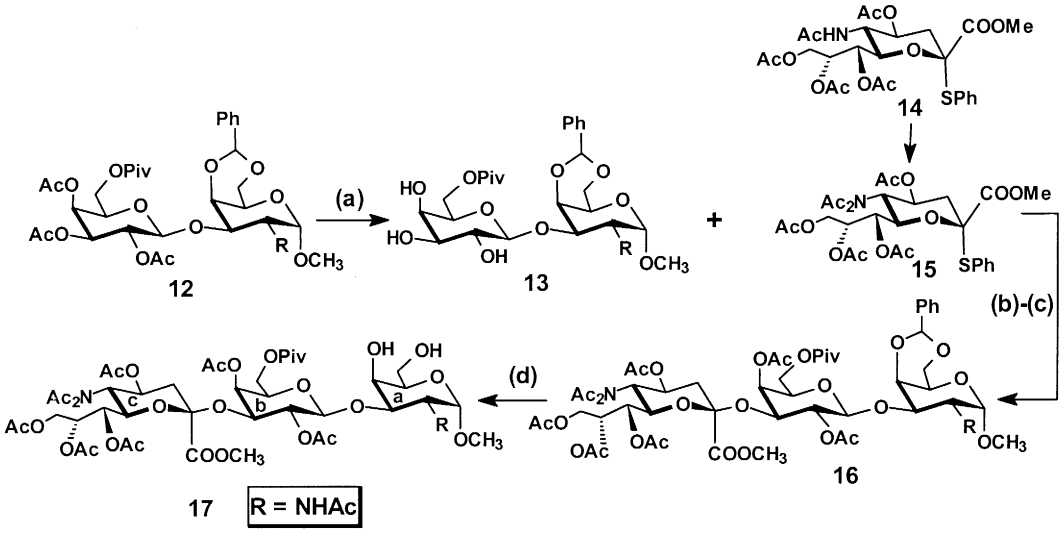
7 was obtained in 75% yield. A strong NOE cross peak between H^b-1 and H^a-4 of disaccharide **7** was indicative of a β(1→4) interglycoside linkage. A similar glycosylation of acceptor **8** with donor **6** also afforded the β(1→4) linked disaccharide **9**, the structure of which was established by a combination of 2D NMR (DQF-COSY and ROESY) and X-ray structural analysis.[9] Disaccharide **7** was further fucosylated with the known methyl tri-*O*-benzyl-thio-β-L-fucoside **10**[10] under catalysis by $CuBr_2$–*n*-Bu_4NBr to give **11** in excellent yield (88%) (Scheme 1).



Scheme 1. (a) *n*-Bu_2SnO–benzene, refluxing, 4 h, Bu_4NBr, NAPBr, 80–85°C, 48 h, 69%; (b) **6**, $SnCl_2$–AgOTf, CH_2Cl_2–toluene, 4A-MS, −15 to 0°C, 12 h, 75%; (c) $CuBr_2$–*n*-Bu_4NBr, $ClCH_2CH_2Cl$:DMF (5:1), 4A-MS, rt, N_2, 16–24 h, 87%; (d) $SnCl_2$–AgOTf/CH_2Cl_2–toluene, 4A-MS, −15 to 0°C, 12 h, 73%

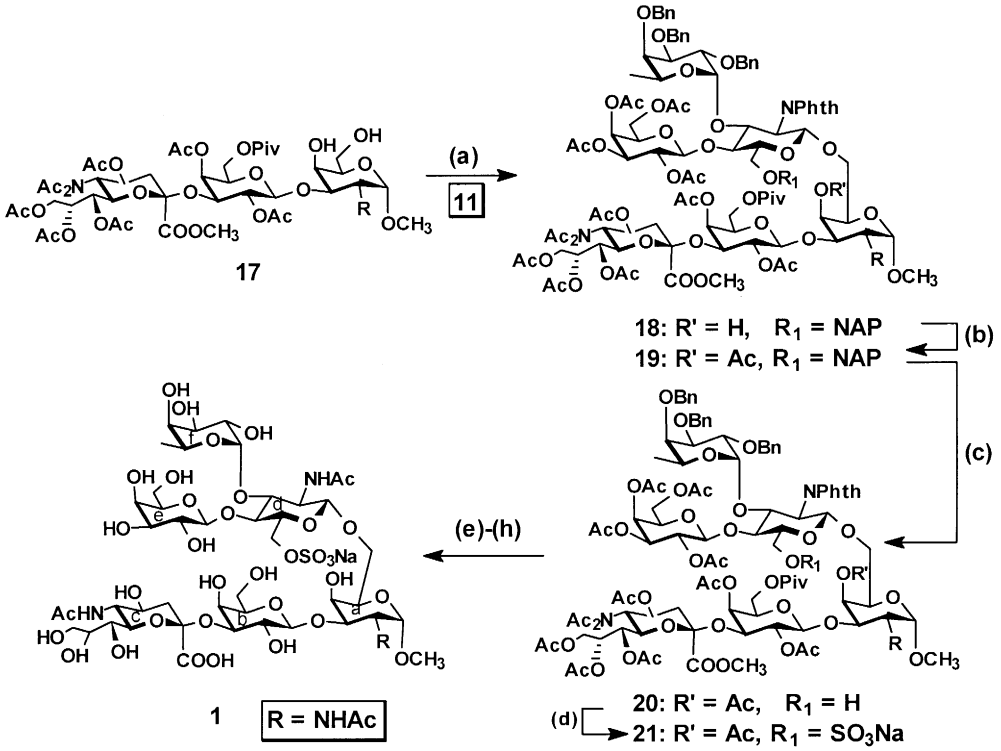
The preparation of **17** was performed as outlined in Scheme 2. Selective removal of the *O*-acetyl groups of **12**[11] in the presence of 6-*O*-pivaloyl was successfully achieved through treatment with a sodium methoxide solution at −20 to −15°C giving **13** in an excellent yield (81%). We were then able to achieve the regioselective and stereoselective α-sialylation of acceptor **13** with new sialyl donor **15** (β-



Scheme 2. (a) 1 M CH_3ONa–CH_3OH/CH_3OH–CH_2Cl_2, −20 to −15°C, 20 min, 81%; (b) NIS–TfOH/CH_2Cl_2–CH_3CN (1:1) −45 to −40°C, 12 h, 45%; (c) Ac_2O:pyridine (1:1), rt, 12 h; (d) 60% HOAc, 60–65°C, 2 h, 75%

configuration was determined by X-ray structural analysis)[9] which was prepared according to literature protocol.[12] The (2→3) linkage of **16** was confirmed by observation of a weak NOE cross peak between H-3 of the galactose residue and H-3a of the sialic acid residue.[13a] An α-configuration of the glycoside was deduced from an observation of the chemical shifts of H-4 (5.56–5.47pm), H-7 (5.25 ppm) and a large coupling constant of $J_{=7,8}$ (7–8 Hz) in the sialic acid residue.[13b,13c]

Compound **16** was treated with 60% HOAc to give **17** in 75% yield. Target oligosaccharide **1** was prepared according to Scheme 3. Regioselective glycosylation of HO-6 of **17** with **11** was performed under controlled reaction conditions resulting in β(1→6)-linked **18** in 79% yield. The presence of a β(1→6) linkage in oligosaccharide **18** was confirmed by observation of NOE cross peaks between H-6a and H-6b of the *N*-acetyl-galactose residue and H-1 of NPhth-protected glucose residue. Compound **18** was treated with pyridine:Ac_2O (1:1) and a catalytic amount of DMAP to give **19** in 85% yield. Removal of 2-naphthymethyl (NAP) protection from **19** was effected by treatment with DDQ.[14] Conversion of **20** into **21** was obtained by treatment with SO_3·pyridine in dry pyridine. Compound **21** was converted into **1** in four steps: (a) removal of benzyl with Pd–C (10%) under a H_2 atmosphere; (b) removal of methyl from the carboxyl group with lithium iodide[3b] in refluxing pyridine under a N_2 atmosphere; (c) removal of the *N*-phthalimido group with methanol:NH_2–NH_2·xH_2O (5:1), followed by pyridine:Ac_2O (1:1) in the presence of catalytic amounts of DMAP; (d) *O*-deacetylation with 1 M sodium methoxide in a methanol–water solution at room temperature. The structure and purity of **1** was established by two-dimensional homonuclear correlation experiments (^{1}H–^{1}H DQF-COSY, ROESY, TOCSY), ^{13}C NMR experiments and FABMS spectroscopy.[15]



Scheme 3. (a) NIS–TfOH/CH_2Cl_2, 4A-MS, −65 to −60°C, 2 h, 79%; (b) Ac_2O–pyridine, DMAP, rt, 85%; (c) DDQ/CH_2Cl_2:CH_3OH (4:1), rt, 12 h, 80%; (d) SO_3·pyridine/pyridine, 0–5°C, 6 h, 86%, then Na^+–resin, rt, 4 h; (e) LiI–pyridine, 120–125°C, 6–8 h; (f) NH_2–NH_2·H_2O:CH_3OH (1:5), 80–85°C, then Ac_2O:pyridine (1:1), rt, 12 h; (g) 1 M CH_3ONa–CH_3OH, rt, 12 h; (h) Pd–C (10%)/CH_3OH:HOAc (1:1), H_2, 12 h, 35% in four steps

In conclusion, we have described an efficient route towards the total synthesis of complex sialylated and sulfated oligosaccharide. Our strategy takes advantage of the electron-rich NAP protecting group, which can be readily removed with DDQ, and the sialyl donor **15**.

Acknowledgements

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References

1. (a) Varki, A. *Glycobiology* **1993**, *3*, 97; (b) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res*. **1995**, *28*, 321; (c) Dwek, R. *Chem. Rev*. **1996**, *96*, 683; (d) Ganem, B. *Acc. Chem. Res*. **1996**, *29*, 340; (e) Shimanel, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev*. **1998**, *98*, 833; (f) Wong, C.-H. *Acc. Chem. Res*. **1999**, *32*, 376.
2. (a) Lo-Guidice, J.-M.; Wieruszeski, J.-M.; Lemoine, J.; Verbert, A.; Roussel, P.; Lamblin, G. *J. Biol. Chem.* **1994**, *269*, 18 794; (b) Capon, C.; Wieruszeski, J.-M.; Lemoine, J.; Byrd, J. C.; Leffler, H.; Kim, Y. S. *J. Biol. Chem.* **1997**, *272*, 31957; (c) Hemmerich, S.; Leffler, H.; Rosen, S. D. *J. Biol. Chem.* **1995**, *270*, 12035.
3. (a) Chandrasekaran, E. V.; Jain, R. K.; Matta, K. L. *Glycoconj. J.* **1992**, *9*, 23 806; (b) Nicokaou, K. C.; Bockovich, N. J.; Carcanague, D. R. *J. Am. Chem. Soc.* **1993**, *115*, 8843; (c) Tsuboi, S.; Isogai, Y.; Hada, N.; King, J. K.; Hindsgaul, O.; Fukuda, M. *J. Biol. Chem.* **1996**, *271*, 27213.
4. (a) Marra, A.; Sinay, P. *Carbohydr. Res*. **1990**, *195*, 303; (b) Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res*. **1991**, *212*, 277; (c) Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc*. **1992**, *114*, 8748; (c) Jain, R. K.; Vig, R.; Rampal, R.; Chandrasekaran, E. V.; Matta, K. L. *J. Am. Chem. Soc.* **1994**, *116*, 12 123; (d) Wilstermann, M.; Konow, L. O.; Nilsson, U.; Ray, A. K.; Magnusson, G. *J. Am. Chem. Soc*. **1995**, *117*, 4742; (e) Ding, Y.; Labbe, J.; Kanie, O.; Hindsgaul, O. *Bioorg. Med. Chem.*, **1996**, *4*, 683; (f) Sames, D.; Chen, X.-T.; Danishefsky, S. J. *Nature* **1997**, *389*, 587; (g) Gan, Z.; Cao, S.; Wu, Q.; Roy, R. *J. Carbohydr. Res*. **1999**, *18*, 755; (h) Schwarz, J. B.; Kuduk, S. D.; Chen, X.-T.; Sames, D.; Glunz, P. W.; Danishefsky, S. J. *J. Am. Chem. Soc*. **1999**, *121*, 2662.
5. Sato, S.; Ito, Y.; Nukada, T.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res*. **1987**, *167*, 197.
6. David, S.; Hanessian, S. *Tetrahedron* **1985**, 41, 643.
7. Hall, L. D.; Manville, J. F.; Bhacca, N. S. *Can. J. Chem*. **1969**, *47*, 1.
8. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett*. **1981**, 431.
9. Detailed X-ray structural analysis of compounds **9** and **15** will be published elsewhere.
10. Jain, R. K.; Matta, K. L. *Carbohydr. Res*. **1990**, *208*, 51.
11. Jain, R. K.; Piskorz, C. F.; Chandrasekaran, E. V.; Matta, K. L. *Carbohydr. Res*. **1995**, *271*, 247.
12. (a) Demchenko, A.; Boons, G. J. *Tetrahedron Lett*. **1998**, *39*, 3065; (b) Demchenko, A.; Boons, G. J. *Chem. Eur. J*. **1999**, *5*, 1278.
13. (a) Scheffler, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W.-T.; Weisemann, R.; Peters, T. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 1841; (b) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Griffith, D. A.; Oriyama, T.; Marsden, S. P. *J. Am. Chem. Soc.* **1995**, *117*, 1940; (c) Ehara, T.; Kameyama, A.; Yamada, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1996**, *281*, 237.
14. Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169.
15. Selected physical properties of compound **1**: ^{1}H NMR (D_2O, 600 MHz) δ 5.12 (d, 1H, $J_{1,2}$=3.6 Hz, H^f-1), 4.85–4.81 (dd, 1H, H^f-5), 4.78–4.77 (d, 1H, $J_{1,2}$=3.0 Hz, H^a-1), 4.61–4.59 (d, 1H, $J_{1,2}$=8.4 Hz, H^d-1), 4.56–4.55 (d, 1H, $J_{1,2}$=7.8 Hz, H^e-1), 4.54–4.53 (d, 1H, $J_{1,2}$=7.8 Hz, H^b-1), 4.39 (s, 1H, H^e-4), 4.32–4.30 (m, 1H, H^a-2), 4.21– 4.15 (s, 3H, H^d-6b, H^d-6a, H^a-4), 4.10–4.00 (m, 5H, H^a-5, H^b-3, H^a-6b, H^d-4, H^a-3), 3.95–3.56 (m, 25H, H^d-2, H^f-3, H^d-3, H^a-6a, H^c-6, H^f-4, H^c-5, H^c-4, H^f-2, H^e-3), 3.57–3.48 (m, 2H, H^e-2, H^b-2), 3.37 (s, 3H, OCH_3), 2.78–2.75 (dd, 1H, *J*=3.0 Hz, *J*=11.4 Hz, H^c-3e), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.82–1.80 (t, 1H, J_{gem}=12.0 Hz, H^c-3a), 1.20 (d, 3H, *J*=7.6 Hz, $CH^f{}_3$); ^{13}C NMR (D_2O, 100.6 MHz) δ 173.3 (C=O), 173.1 (C=O), 173.0 (C=O), 172.9 (C=O), 103.4, 100.5, 100.4, 98.7, 97.5, 97.0, 76.2, 74.6, 73.9, 73.7, 73.6, 72.2, 71.9, 71.7, 71.4, 70.9, 70.7, 69.9, 69.6, 68.2, 68.1, 68.0, 67.7, 67.4, 67.6, 67.0, 66.7, 66.3, 65.6, 64.9, 62.2, 60.3, 59.8, 54.6, 53.9, 50.6, 47.5, 38.6 (CH_2), 21.2 (Ac), 21.0 (Ac), 21.0 (Ac), 14.1 ($CH_3{}^f$); FABMS (positive ion mode) (*m/z*) calcd for $C_{46}H_{76}O_{36}N_3SNa_2$ (M^++Na): 1324.5; found: 1324.9 (M^++Na).