Total Synthesis of Sulfated Le^x and Le^a-Type Oligosaccharide Selectin Ligands

K. C. Nicolaou,* N. J. Bockovich, and D. R. Carcanague

Department of Chemistry
The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, California 92037
Department of Chemistry
University of California, San Diego
9500 Gilman Drive
La Jolla, California 92093

Received June 4, 1993

The recognition of the role of the selectins in the recruitment of leukocytes to inflammation sites via vascular adhesion and rolling led to extensive studies in both chemistry and biology.[1] Following the initial identification of sialyl Le^x-type molecules as ligands as E-selectin,[2] a recent report[3] disclosed the isolation of a mixture of two sulfated tetrasaccharides [**1**, sulfated Le^x, and **2**, sulfated Le^a (Figure 1)] from an ovarian cystadenoma glycoprotein which exhibited E-selectin binding properties comparable to those of the sialylated compound (Sialyl Le^x). Due to the importance of these ligands to adhesion processes and their extreme scarcity, their synthesis was deemed important.[4] In this communication we report the first total syntheses of both **1** and **2** and their truncated analogs **3**[5] and **4** (Figure 1).

Compounds **1**–**4** were constructed from key intermediates **5**–**10**[6] (Figure 1) by stereoselective transformations as described below. The synthesis of the sulfated Le^x-type tetrasaccharide **1** is summarized in Scheme I. Thus, the glycosyl donor **10** was coupled with the glycosyl acceptor **5** under standard Mukaiyama conditions ($AgClO_4$–$SnCl_2$)[7] to form, selectively,[8] the β-linked glycoside **11** in 90% yield. Treatment of **11** with $MeNHNH_2$ in refluxing ethanol resulted in removal of both the acetate and the phthalimide groups, leading to the corresponding amino alcohol (**12**), which was acetylated to give the amide **13** (80% overall yield). Desilylation of **13** using fluoride ion led to hydroxy compound **14** (95%), which was coupled with the galactosyl fluoride **6**, furnishing trisaccharide **15** (75% yield) as a single stereoisomer.[8] Selective removal of the allyl protecting group from **15** [$H_2Ru(PPh_3)_4$, then acid hydrolysis] gave the hydroxy compound **16** (81%), which was coupled with the fucosyl fluoride derivative **7**[9] ($AgClO_4$–$SnCl_2$) to give, stereoselectively, tetrasaccharide **17** (85%) with the desired α-fucose anomeric linkage. Reaction of **17** with thiourea led to selective removal of the chloroacetyl group to afford **18** (81%), which was converted to the sulfated compound **19**, in 95% yield, by exposure to the SO_3·NMe_3 complex in anhydrous pyridine. Finally, deacetylation of **19** followed by hydrogenolysis gave the targeted sulfated Le^x tetrasaccharide **1** in 80% overall yield.

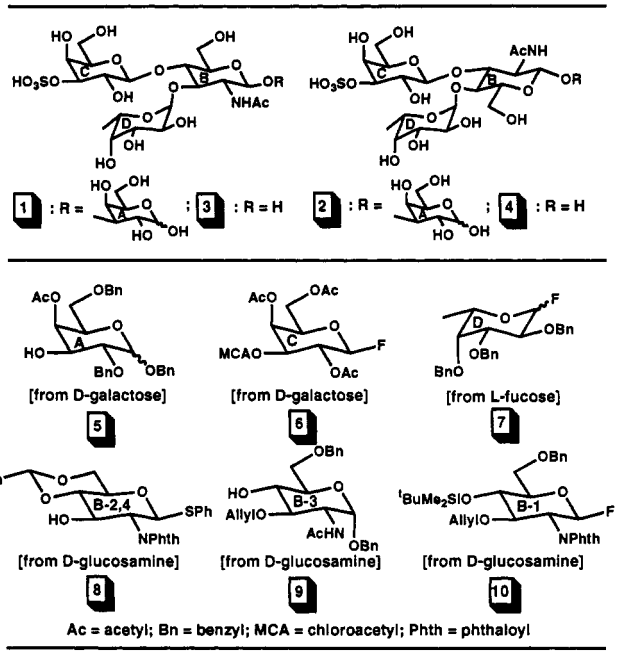


Figure 1. Sulfated Le^x (**1**, **3**) and Le^a (**2**, **4**) target molecules and key intermediates (**5**–**10**) for their chemical synthesis.

The synthesis of the sulfated derivative **3** lacking the galactose unit at the reducing end was accomplished as depicted in Scheme II using the carbohydrate units **6**, **7**, and **9**[4d] and chemistry similar to that described above.

However, for the synthesis of the sulfated Le^a-type compounds **2** and **4**, a different strategy had to be developed due to unexpected glycosidation problems. Scheme III summarizes the successful routes to **2** and **4**. Thus, coupling of carbohydrate units **6** and **8** under Mukaiyama–Suzuki conditions (Cp_2HfCl_2–$AgOTf$)[10] in the presence of 2,6-di-*tert*-butyl-4-methylpyridine led, stereoselectively,[8] to the β-glycoside **25** in 63% yield. Regioselective opening of the benzylidene ring by treatment with $NaCNBH_3$–HCl gave the secondary alcohol **26** in 76% yield. Coupling of **26** with fucosyl fluoride **7**[9] led to the trisaccharide **27** (95%, α-anomer), which was converted via a DAST–NBS reaction[11] to the glycosyl fluoride **28** in 80% yield. Fluoride **28** served as a common precursor to both **2** and **4**. For the synthesis of the tetrasaccharide **2**, the sequence involved coupling of **28** with the galactose derivative **5** (Cp_2HfCl_2–$AgOTf$) leading, stereoselectively,[8] to compound **29** (58%). The chloroacetate moiety was removed from **29**, and the sulfate group was attached in its place (SO_3·NMe_3), furnishing **31** via **30** (40% overall). Removal of both the phthalimide and acetate groups from **31** by treatment with NH_2NH_2·H_2O at 100 °C was followed by acetylation of the

(1) (a) Lasky, L. A. *Science* **1992**, *258*, 964. (b) Borman, S. *Chem. Eng. News* **1992**, *70*(49), 25. (c) Travis, J. *Science* **1993**, *260*, 906.

(2) (a) Bevilacqua, M. P.; Stengelin, S.; Gimbrone, M. A.; Seed, B. *Science* **1989**, *243*, 1160. (b) Lowe, J. B.; Stoolman, L. M.; Nair, R. P.; Larsen, R. D.; Berhend, T. L.; Marks, R. M. *Cell* **1990**, *63*, 475. (c) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130. (d) Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132. (e) Tiemeyer, M.; Swiedler, S. J.; Ishihara, M.; Moreland, M.; Schweingruber, H.; Hirtzer, P.; Brandley, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1138. (f) Springer, T. A.; Lasky, A. *Nature* **1991**, *349*, 196.

(3) Yuen, C.-T.; Lawson, A. M.; Chai, W.; Larkin, M.; Stoll, M. S.; Stuart, A. C.; Sullivan, F. X.; Ahern, T. J.; Feizi, T. *Biochemistry* **1992**, *31*, 9126.

(4) For synthetic studies in the area of sialyl Le^x compounds, see: (a) Palcic, M. M.; Venot, A.; Ratcliffe, R. M.; Hindsgaul, O. *Carbohydr. Res.* **1989**, *190*, 1. (b) Dumas, D. P.; Ichikawa, Y.; Wong, C.-H.; Lowe, J. B.; Nair, R. P. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 425. (c) Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *209*, C1. (d) Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J. *J. Chem. Soc., Chem. Commun.* **1991**, 870. (e) Nicolaou, K. C.; Hummel, C. W.; Iwabuchi, Y. *J. Am. Chem. Soc.* **1992**, *114*, 3126. (f) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Oriyama, T.; Griffith, D. A.; Wong, C.-H.; Dumas, D. P. *J. Am. Chem. Soc.* **1992**, *114*, 8329. (g) Danishefsky, S. J.; Koseki, K.; Griffith, D. A.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Oriyama, T. *J. Am. Chem. Soc.* **1992**, *114*, 8331. (h) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.

(5) It is interesting to note that compound **3** had been synthesized prior to the disclosure of the natural products **1** and **2**: Chandrasekaran, E. V.; Jain, R. K.; Matta, K. L. *J. Biol. Chem.* **1992**, *267*, 23806.

(6) For the synthesis of these intermediates, see the supplementary material and refs 4d and 9.

(7) Mukaiyama, T.; Muria, Y.; Shoda, S. *Chem. Lett.* **1981**, 431.

(8) Neighboring-group participation in this glycoside bond forming reaction is presumed to be responsible for this stereoselectivity.

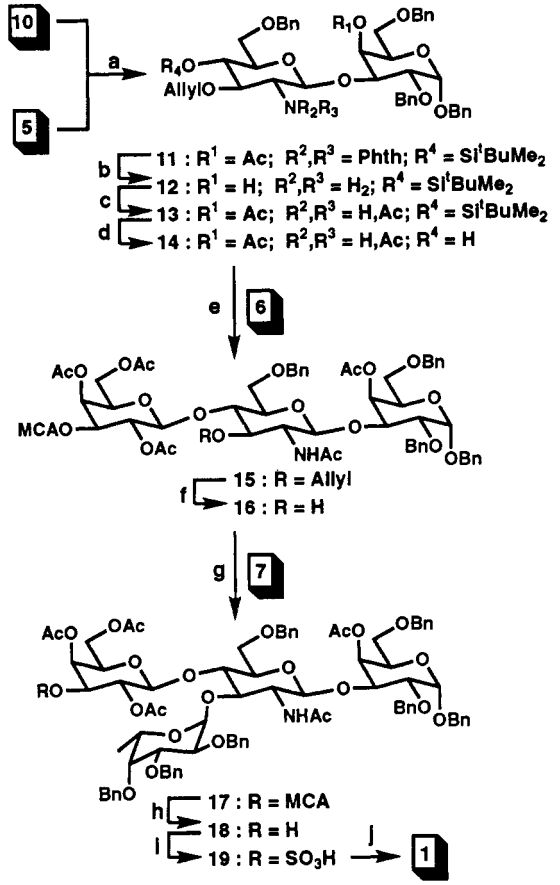
(9) Nicolaou, K. C.; Caulfield, T. J.; Kataoka, H.; Stylianides, N. A. *J. Am. Chem. Soc.* **1990**, *112*, 3693.

(10) Matsumoto, T.; Maeta, H.; Suzuki, K.; Tsuchihashi, G. *Tetrahedron Lett.* **1988**, *29*, 3567.

(11) Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Chem. Soc.* **1984**, *106*, 4189.

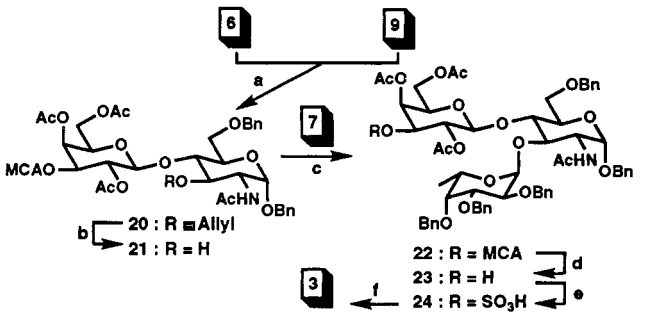
0002-7863/93/1515-8843$04.00/0

Scheme I. Total Synthesis of Sulfated Le^x Tetrasaccharide **1**[a]



[a] Reagents and conditions: (a) 2.0 equiv of **5**, 3.0 equiv of $AgClO_4$, 3.0 equiv of $SnCl_2$, 4-Å molecular sieves, CH_2Cl_2, 0 → 25 °C, 4 h, 90%; (b) methyl hydrazine–EtOH (1:1), 95 °C, 48 h; (c) excess Ac_2O, excess Et_3N, DMAP (cat.), CH_2Cl_2, 25 °C, 4 h, 80% for two steps; (d) 2.0 equiv of Bu_4NF, THF, 25 °C, 1 h, 95%; (e) 2.0 equiv of **6**, 3.0 equiv of $AgClO_4$, 3.0 equiv of $SnCl_2$, 4-Å molecular sieves, CH_2Cl_2, 0 → 25 °C, 5 h, 75%; (f) (i) $H_2Ru(PPh_3)_4$ (cat.), EtOH, 95 °C, 4 h, (ii) *p*-TsOH (cat.), MeOH, 25 °C, 1 h, 81%; (g) 2.0 equiv of **7**, 3.0 equiv of $AgClO_4$, 3.0 equiv of $SnCl_2$, 4-Å molecular sieves, Et_2O, –20 → 0 °C, 4 h, 85%; (h) 5.0 equiv of thiourea, 2.0 equiv of 2,6-lutidine, EtOH, 65 °C, 5 h, 81%; (i) 20 equiv of SO_3·NMe_3, pyridine, 25 °C, 24 h, 95%; (j) (i) 2.0 equiv of NaOMe, MeOH, 45 °C, 5 h (ii) H_2, $Pd(OH)_2$, MeOH–H_2O (2:1), 48 h, 80%.

Scheme II. Total Synthesis of Sulfated Le^x Trisaccharide **3**[a]

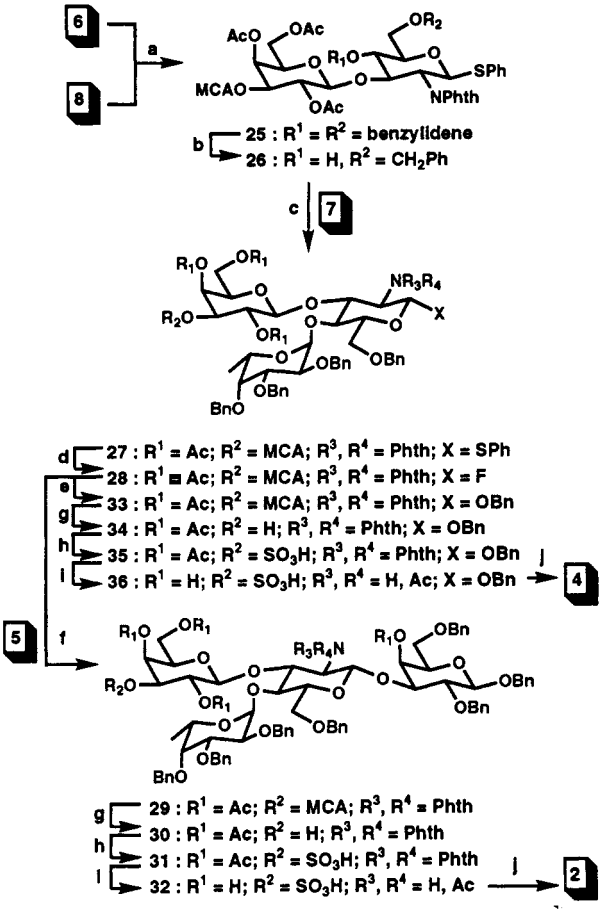


[a] Reagents and conditions: (a) 2.0 equiv of **6**, 3.0 equiv of $AgClO_4$, 3.0 equiv of $SnCl_2$, 4 Å molecular sieves, CH_2Cl_2, 0 → 25 °C, 3 h, 81%; (b) (i) $H_2Ru(PPh_3)_4$ (cat.), EtOH, 80 °C, 1 h, (ii) *p*-TsOH (cat.), MeOH–CH_2Cl_2 (4:1), 25 °C, 2 h, 82%; (c) 2.0 equiv of **7**, 3.0 equiv of $AgClO_4$, 3.0 equiv of $SnCl_2$, 4-Å molecular sieves, Et_2O–THF (3:1), –15 → 0 °C, 3 h, 85%; (d) 5.0 equiv of thiourea, 2.0 equiv of 2,6-lutidine, EtOH–CH_2Cl_2 (1:1), 65 °C, 5 h, 90%; (e) 20 equiv of SO_3·NMe_3, pyridine, 25 °C, 24 h, 86%; (f) (i) 2.0 equiv of NaOMe, MeOH, 25 °C, 4 h, (ii) H_2, $Pd(OH)_2$, MeOH, 25 °C, 7 days, 74%.

generated amino group to give the amide **32** in 73% overall yield. Final deprotection to generate the naturally occurring compound **2** was achieved by hydrogenolysis (95% yield).

The synthesis of the trisaccharide **4** proceeded by glycosylation

Scheme III. Total Synthesis of Sulfated Le^a Ligands **2** and **4**[a]



[a] Reagents and conditions: (a) 4.0 equiv of **6**, 5.0 equiv of AgOTf, 5.0 equiv of Cp_2HfCl_2, 1.0 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, 4-Å molecular sieves, CH_2Cl_2, 0 → 25 °C, 6 h, 63%; (b) 10.0 equiv of $NaCNBH_3$, ethereal HCl, 3-Å molecular sieves, THF, 0 °C, 30 m, 76%; (c) 3.0 equiv of **7**, 4.0 equiv of $AgClO_4$, 4.0 equiv of $SnCl_2$, 4-Å molecular sieves, Et_2O–THF (5:1), –10 → 0 °C, 1 h, 95%; (d) 3.0 equiv of DAST, 1.25 equiv of NBS, CH_2Cl_2, –78 → –20 °C, 2 h, 80%; (e) 8.0 equiv of benzyl alcohol, 5.0 equiv of Cp_2HfCl_2, 5.0 equiv of AgOTf, 4-Å molecular sieves, CH_2Cl_2, 0 → 25 °C, 18 h, 95%; (f) 3.0 equiv of **5**, 3.0 equiv of Cp_2HfCl_2, 3.0 equiv of AgOTf, 4-Å molecular sieves, CH_2Cl_2, 0 → 25 °C, 4 h, 58%; (g) 5.0 equiv of thiourea, 2.5 equiv of 2,6-lutidine, EtOH–CH_2Cl_2 (1:1), 65 °C, 12 h **30**, 79%, **34**, 89%; (h) 20 equiv of SO_3·NMe_3, pyridine, 25 °C, 24 h, **31**, 50%, **35**, 76%; (i) (i) hydrazine hydrate–EtOH (1:1), 100 °C, 3 h, (ii) excess of Ac_2O, excess of Et_3N, MeOH, 25 °C, 10 min, **32**, 73%, **36**, 50%; (j) H_2, $Pd(OH)_2$, MeOH–H_2O (2:1), 25 °C, 48 h, **2**, 95%, **4**, 82%.

of benzyl alcohol with fluoride **28** leading to compound **33** (95%), which was converted to **4** as described above for **2** (Scheme III).

The described chemistry renders the natural sulfo oligosaccharides **1** and **2**, as well as their simpler Le^x and Le^a sulfate analogs **3** and **4**, available in pure form for extensive biological investigations. Further studies envisioned in this field may expand the library of biological tools and provide leads for therapeutic agents in the area of inflammation and related conditions.

Acknowledgment. Stimulating discussions with Dr. Ten Feizi, Clinical Research Centre, Glycoconjugates Section, England, regarding the chemistry and biology of the title compounds are thankfully acknowledged. We also thank Drs. Dee Hua Huang and Gary Siuzdak for the NMR and mass spectroscopy work, respectively. This work was financially supported by the National Institutes of Health and the Scripps Research Institute. D.R.C. thanks the National Institutes of Health for a postdoctoral fellowship, 1992–1993.

Supplementary Material Available: Schemes for the synthesis of compounds **5**, **6**, **8**, and **10** and listings of selected physical data for compounds **11**, **15**, **18**, **21**, **22**, **25**, **27**, **29**, **1–4** (14 pages). Ordering information is given on any current masthead page.