J|A|C|S
COMMUNICATIONS

Published on Web 06/30/2005

Dendrimer-like PEO Glycopolymers Exhibit Anti-Inflammatory Properties

Shyam M. Rele,* Wanxing Cui, Lianchun Wang, Sijian Hou, Ginger Barr-Zarse, Daniel Tatton, Yves Gnanou, Jeffrey D. Esko, and Elliot L. Chaikof*

Departments of Surgery and Biomedical Engineering, Emory University, and School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, Georgia 30322

Received February 24, 2005; E-mail: echaiko@emory.edu; smrele@emory.edu

Selectin-induced leukocyte rolling on endothelial surfaces is an essential step in mediating events leading to inflammatory and cell-mediated immune responses.[1] Characteristically, the adhesion cascade is facilitated by the interaction of selectins with O-glycosylated protein ligands that present sulfated derivatives of the tetrasaccharide sialyl Lewis *x* (Neu5Acα3Galβ4(Fucα3)GlcNAc-, sLe^x). Significant effort has been directed toward generating sLe^x mimetics in the form of small molecules, polymers, liposomes, and protein conjugates, as competitive inhibitors of selectin-mediated binding events.[2] However, relatively weak affinity, susceptibility to hydrolytic cleavage, potential antigenicity, and short circulating half-life, in addition to the absence of a convenient synthetic route are acknowledged limitations of sLe^x-derivatized bioconjugates. Thus, motivation exists to develop simpler therapeutic oligosaccharide analogues as selectin-binding antagonists, which exhibit multiple and cooperative receptor binding properties.

The simultaneous presentation of saccharide epitopes on an appropriate macromolecular scaffold creates a multivalent display that amplifies the affinity of glycoside-mediated receptor targeting.[3,4] In this regard, well-defined branched poly(ethylene oxide) (PEO) polymers provide singularly useful scaffolds for in vivo blockade of selectin binding due to their defined molecular architecture, hydrophilicity, and availability of multiple surface reactive sites.[5] Moreover, the branched polymer structure also provides a mechanism for controlling accessibility, mobility, density, and supramolecular organization of pendant sugar epitopes, as additional elements that may facilitate the design of optimal selectin-binding antagonists with defined circulating half-life. Herein, we report a simple strategy for the synthesis of 1st and 2nd generation dendrimer-like PEO glycopolymers bearing sulfated β-lactose as potential L-selectin inhibitors. Most current glycoconjugation strategies describe the use of an aliphatic or aromatic aglycone linker that carries an amine, thiol, thiourea, acid, or active ester as the reactive moiety. Significantly, by using imidated lactose donors and a Schmidt glycosidation coupling protocol, protecting group manipulations were minimized in the process of glycopolymer synthesis.

Well-defined three ($M_n \sim 5200$ mu) and four arm ($M_n \sim 5100$ mu) PEO stars (1st generation) carrying hydroxy end groups were synthesized by anionic polymerization using "core-first" methodology, as previously reported.[6a,b] In addition, a dendrimer-like 2nd generation PEO star polymer (12 arm) was synthesized based on a phosphazene core. This polymer consisted of a 1st generation of six PEO arms, produced by an "arm-first" strategy onto the phosphazene core, followed by a 2nd generation of 12 hydroxy-terminated PEO branches polymerized directly onto the original six arm core ($M_n \sim 52$ kD).[6c] β-Lactose octaacetate was selectively brominated at the anomeric center and subsequently activated to the imidate donor (**A**).

Glycosylations of hydroxy-terminated PEO star and dendrimer-like polymers by trichloroacetimidate glycosidation methodology,

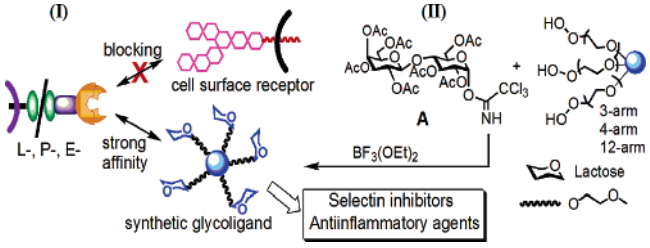

Figure 1. (I) Selectin blockade using polyvalent glycoconjugates. (II) Glycosidation of terminal hydroxyls using lactose imidate (**A**).

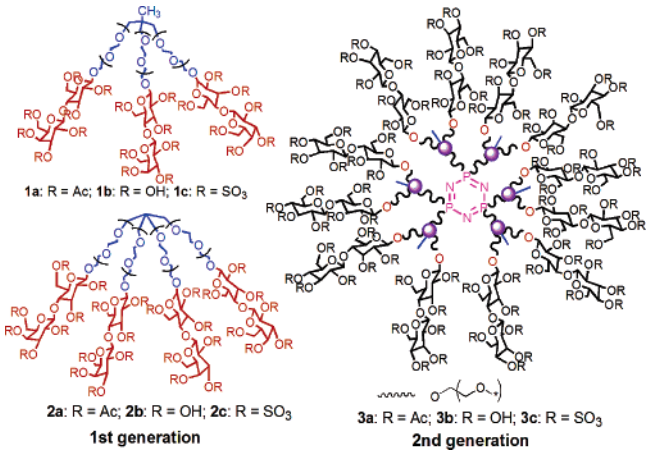

Figure 2. Saccharide-functionalized PEO star and "dendrimer-like" polymers as selectin ligands.

using $BF_3 \cdot OEt_2$ as a Lewis acid activator resulted in the covalent attachment of acetyl-protected lactose residues in high yield. Zemplen conditions for deacetylation (NaOMe/MeOH) followed by lactose sulfation using an excess of SO_3·trimethylamine complex furnished target PEO glycoclusters carrying terminal sulfated oligosaccharides **1c**, **2c**, and **3c**. The efficiency, homogeneity, and degree of ligand (lactose) loading on the PEO polymers were estimated by ^{1}H NMR spectroscopy, as well as by mass estimates obtained by MALDI-TOF and laser light scattering. Moreover, FTIR and SDS−PAGE analysis provided additional evidence of sulfated lactose units (see Supporting Information).

Specifically, the relative intensities of the anomeric H:Ac:CH_3 signal ratio of 6:20.9:3 for the three arm (**1a**) derivative and an integration ratio of 7.95:27.97:8 for the four arm glycocluster **2a** indicated complete glycosylation of the hydroxyl groups on the parent PEO precursor. The increase in molecular weight was further corroborated using MALDI-TOF (**1a**: 6899 mu, **2a**: 7524 mu) and LLS measurements, confirming quantitative functionalization. Likewise, an NMR integration ratio of 3.9:13.7:3, as well as MALDI-TOF, demonstrated a high degree of lactose conjugation (>95%) onto the dendritic PEO scaffold of the 12-arm, 2nd generation, branched compound. Subsequent deprotection followed

10.1021/ja0511974 CCC: $30.25

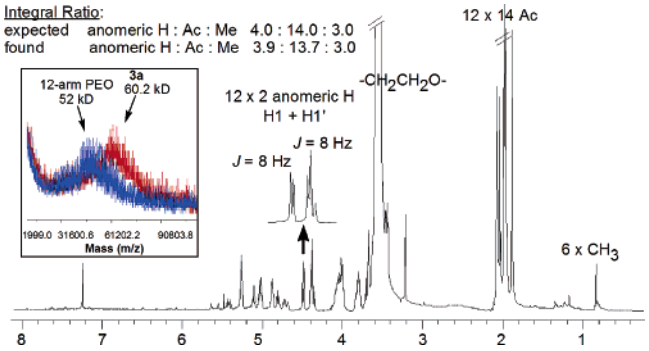

Figure 3. ^{1}H NMR (600 MHz) spectrum of **3a**. Inset shows the MALDI-TOF spectra of the original hydroxyl-terminated polymer and **3a**.

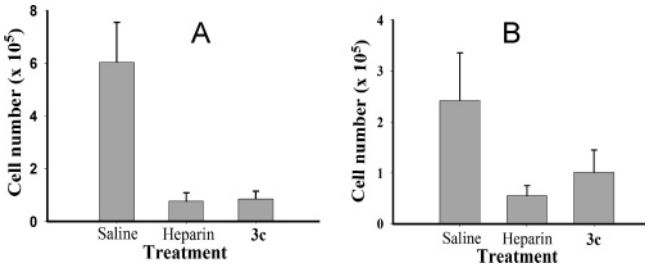

Figure 4. Neutrophil (A) and macrophage (B) content in thioglycollate-induced peritoneal inflammation ($n = 6$). **1c**/**2c** did not exhibit in vivo activity. Each bar represents the average value ± SD.

by sulfation produced a highly charged sulfated glycodendron **3c** (observed SELDI-TOF: 61.8 kD, expected value ~62 kD).

Recent investigations have demonstrated that heparin exhibits anti-inflammatory properties by mediating blockade of L- and P-selectins via sulfate-dependent interactions.[7] Moreover, in vitro observations have illustrated that sulfated esters promote selectin binding when appropriately oriented on a lactose core. For example, a sulfated lactose derivative (6,6′-disulfo lactose), lacking fucose and sialic acid residues, was superior to sLe^x as an in vitro inhibitor of L-selectin binding to GlyCAM-1.[8] Since selectin−glycoligand binding is greatly amplified through multivalent presentation of oligosaccharide determinants, we explored the capacity of sulfated branched star and dendrimer-like glycopolymers to limit inflammatory responses in vivo via presumed selectin-dependent blockade. Acute inflammation was induced in a mouse model by thioglycollate injection into the peritoneal cavity. Potency was valence-dependent with **1c**/**2c** exhibiting little activity, while **3c** (0.5 mg/mouse IV) dramatically reduced neutrophil and macrophage recruitment by 86 and 60%, respectively (Figure 4, $p < 0.05$). Although heparin inhibited inflammatory cell recruitment to a similar degree, concurrent anticoagulant effects limit heparin's clinical applicability. In contrast, **3c** does not exhibit anti-thrombin activity (data not shown). The ability of **3c** to inhibit the adhesion of U937 cells to immobilized selectins was examined to determine whether the observed in vivo effect was mediated by presumed selectin blockade. **3c** selectively blocked the adhesion of U937 cells to L-selectin in a dose-dependent manner ($IC_{50} = 2.4$ nM), but did not exhibit anti-P-selectin or E-selectin activity (Figure 5). Therefore, **3c** is among the most potent L-selectin inhibitors yet reported.[2a,8−10] Significantly, precedence exists for increased biological activity as a consequence of ligand presentation by dendrimeric scaffolds when compared to linear counterparts.[2b] Despite significant anti-L-selectin activity, the observed in vivo activity was surprising since it has been recently reported that heparin's anti-inflammatory activity in vivo is critically dependent on its ability to inhibit *both* L- and P-selectin mediated inflammatory cell

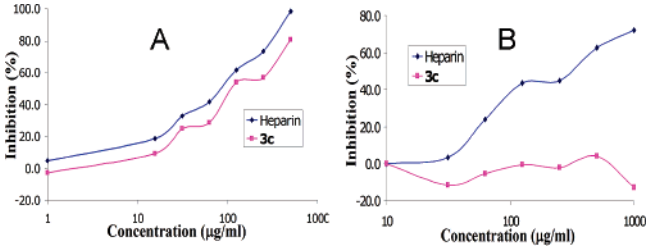

Figure 5. U937 cell adhesion to immobilized (A) L-selectin, (B) P-selectin. Neither heparin nor **3c** exhibited inhibitory activity toward E-selectin. Inhibition was not observed in the absence of test compound or heparin. Data represent means of at least $n = 3$, SD < 10%).

adhesion.[7] Indeed, a compound acting solely as a selective inhibitor of L-selectin is not anticipated to block in vivo leukocyte infiltration so completely. Thus, it is likely that **3c**, like heparin, blocks chemokine binding to the endothelium, which would further limit leukocyte extravasation.

In summary, a new class of high molecular weight polysulfated PEO dendrimer-like glycopolymer has been synthesized by a combination of arm-first and core-first methodologies followed by trichloroacetimidate glycosidation as a facile bioconjugation strategy. This is the first report to describe the synthesis and biological evaluation of complex branched PEO heparinoid mimics, which provides an easily accessible route to carbohydrate-based compounds with anti-inflammatory activity in vivo.

Acknowledgment. This work was supported by NIH Grants HL57345 to J.D.E. and HL60464 to E.L.C.

Supporting Information Available: Detailed synthetic procedures MALDI-TOF, SDS−PAGE, and NMR analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Ulbrich, H.; Eriksson, E.; Lindbom, L. *Trends Pharmacol. Sci*. **2003**, *24*, 640−647. (b) Ley, K. *Trends Mol. Med*. **2003**, *9*, 263−268. (c) Hopfner, M.; Alban, S.; Schumacher, G.; Rothe, U.; Bendas, G. *J. Pharm. Phamacol.* **2003**, *55*, 697−706. (d) Lowe, J. B.; Ward, P. A. *J. Clin. Invest.* **1997**, *99*, 822−826.

(2) (a) Sanders, W. J.; Gordon, E. J.; Oren, D.; Beck, P. J.; Ronen, A.; Kiessling, L. L. *J. Biol. Chem.* **1999**, *274*, 5271−5278. (b) Reuter, J. et al. *Bioconjugate Chem.* **1999**, *10*, 271−278. (c) Lin, C.-C.; Kimura, T.; Wu, S.-H.; Weitz-Schmidt, G.; Wong, C.-H. *Biorg. Med. Chem. Lett.* **1996**, *6*, 2755−2760. (d) Spevak, W.; Foxall, C.; Charych, D.; Dasgupta, F.; Nagy, J. O. *J. Med. Chem.* **1996**, *39*, 1018−1020. (e) DeFrees, S. A.; Phillips, L.; Guo, L.; Zalipsky, S. *J. Am. Chem. Soc.* **1996**, *118*, 6101−6104. (f) Manning, D. D.; Strong, L.; Hu, X.; Beck, P.; Kiessling, L. L. *Tetrahedron* **1997**, *53*, 11937−11952. (g) Thoma, G.; Duthaler, R. O.; Magnani, J. L.; Patton, J. T. *J. Am. Chem. Soc.* **2001**, *123*, 10113−10114. (h) Barbaro, J. *Curr. Org. Chem*. **2004**, *8*, 883−902.

(3) (a) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578. (b) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794.

(4) (a) Aoyama, Y. *Chem.—Eur. J*. **2004**, *10*, 588−593. (b) Casnati, A.; Sansone, F.; Ungaro, R. *Acc. Chem. Res.* **2003**, *36*, 246−254. (c) Shaunak, S. et al. *Nat. Biotechnol*. **2004**, *22*, 977−984.

(5) (a) Frey, H.; Haag, R. *Rev. Mol. Biotechnol*. **2002**, *90*, 257−267. (b) Zalipsky, S. *Adv. Drug Delivery Rev*. **1995**, *16*, 157−182.

(6) (a) Hou, S.; Chaikof, E.; Taton, D.; Gnanou, Y. *Macromolecules* **2003**, *36*, 3874−3881. (b) Angot, S.; Taton, D.; Gnanou, Y. *Macromolecules* **2000**, *33*, 5418−5424. (c) Hou, S.; Taton, D.; Saule, M.; Logan, J.; Chaikof, E.; Gnanou, Y. *Polymer* **2003**, *44*, 5067−5074.

(7) Wang, L.; Brown, J.; Varki, A.; Esko, J. *J. Clin. Invest.* **2002**, *110*, 127−136.

(8) (a) Bruehl, R.; Bertozzi, C.; Rosen, S. *J. Biol. Chem*. **2000**, *275*, 32642−32648. (b) Rosen, D. *Am. J. Pathol.* **1999**, *155*, 1013−1020. (c) Galustian, C.; Lubineau, A.; le Narvor, C.; Kiso, M.; Brown, G.; Feizi, T. *J. Biol. Chem*. **1999**, *274*, 18213−18217.

(9) (a) Roy, R.; Park, W. C.; Zanini, D.; Foxall, C.; Srivastava, O. P. *Carbohydr. Lett*. **1997**, *2*, 259. (b) Mowery, P.; Yang, Z.-Q.; Gordon, E.; Beck, D.; Spencer, A.; Alon, R.; Kiessling, L. *Chem. Biol*. **2004**, *11*, 725−732.

(10) Xie, X.; Rivier, A.-S.; Zakrzewicz, A.; Bernimoulin, M.; Zeng, X.-L.; Wessel, H. P.; Schapira, M.; Spertini, O. *J. Biol. Chem*. **2000**, *275*, 34818−34825.

JA0511974