ORGANIC LETTERS

2004
Vol. 6, No. 23
4163−4166

# Synthesis and Lectin Binding Ability of Glycosamino Acid–Calixarenes Exposing GlcNAc Clusters

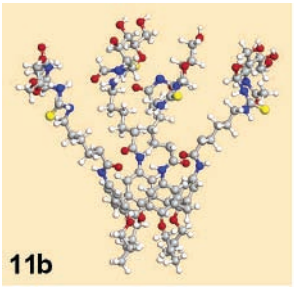
**Grazia M. L. Consoli,* Francesca Cunsolo, Corrada Geraci,* and Valentina Sgarlata**

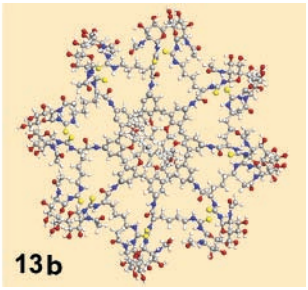
*Istituto di Chimica Biomolecolare, Sezione di Catania CNR, Via del Santuario 110, I-95028 Valverde (CT), Italy*

*corrada.geraci@icb.cnr.it*

**Received July 22, 2004** *(Revised Manuscript Received September 21, 2004)*

## ABSTRACT




**Novel calix[4 or 8]arene-based glycoconjugates exposing terminal *N*-acetyl-D-glucosamine clusters have been synthesized using amino acid–calixarenes as building blocks. The obtained glycosamino acid–calixarenes 9b–14b have lectin-binding ability and amplified inhibitory effects on erythrocyte agglutination induced by wheat germ (*Triticum vulgaris*) agglutinin (WGA). The inhibitory ability is dependent on the presence of the spacer and on the shape and rigidity of the calixarene skeleton.**

Recent advances in glycobiology have shown that carbohydrate−carbohydrate and carbohydrate−protein interactions are involved in important biological events.[1] The affinity and specificity of these interactions strongly depend on polyvalency.[2] It is known that multiple copies of carbohydrate ligands interact in natural systems with multiple complementary sites on the receptor, resulting in a stronger and more specific carbohydrate−receptor binding (cluster effect).[3]

Understanding multivalent binding in detail represents a challenge of great interest in bio-organic chemistry because the manipulation of carbohydrate−protein interaction could pave the way for therapeutic and diagnostic applications.[4]

A wide variety of simplified carbohydrate model systems have been developed to investigate the significance of the polyvalency in carbohydrate−protein interactions.[5] Multivalent presentation of carbohydrate groups on small molecular template, cyclodextrin, dendrimer, dendron, and calixarene scaffolds gave rise to low valency systems (4−20 carbohydrate groups) while higher valency systems were obtained using proteins and polymers as platforms.[6] Studies carried out on several of these polyvalent model systems

(1) *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinay, P., Eds.; Wiley-VCH: Weinheim, 2000.

(2) (a) Mammen, M.; Choi, S.-K., Whitesides G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754. (b) Mortell, K. H.; Weatherman, R. V.; Kiessling, L. L. *J. Am. Chem. Soc.* **1996**, *118*, 2297. (c) Horan, N.; Yan, L.; Isobe, H.; Whitesides, G. M.; Kahne, D. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, 11782.

(3) (a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321. (b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555.

(4) (a) Mourez, M.; Kane, R. S.; Mogridge, J.; Metallo, S.; Deschatelets, P.; Sellman, B. R.; Withesides, G. M.; Collier, R. J. *Nat. Biotechnol.* **2001**, *19*, 958. (b) Merritt, E. A.; Zhang, Z.; Pickens, J. C.; Ahn, M.; Hol, W. G.; Fan, E. *J. Am. Chem. Soc.* **2002**, *124*, 8818. (c) Danishefsky, S. J.; Allen, J. R. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 836. (d) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Limg, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669. (e) Walker, F.; Vicens, Q.; Westhof, E. *Curr. Opin. Chem. Biol.* **1999**, *3*, 694. (f) Sears, P.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2300.

(5) (a) Fulton, D. A.; Stoddart, J. F. *Org. Lett.* **2000**, *2*, 1113. (b) Cairon, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 1615. (c) Lindhorst, T. K.; Dubeer, M.; Krallmann-Wenzel, U.; Ehlers, S.; Kieburg, C. *Eur. J. Org. Chem.* **2000**, 2027.

10.1021/ol0485767 CCC: $27.50 
**Published on Web 10/13/2004**

indicated that the nature of the scaffold, the length of the tether between scaffold and carbohydrate moieties, and the carbohydrate density determine the success or failure of polyvalent carbohydrate−protein interactions.[7]

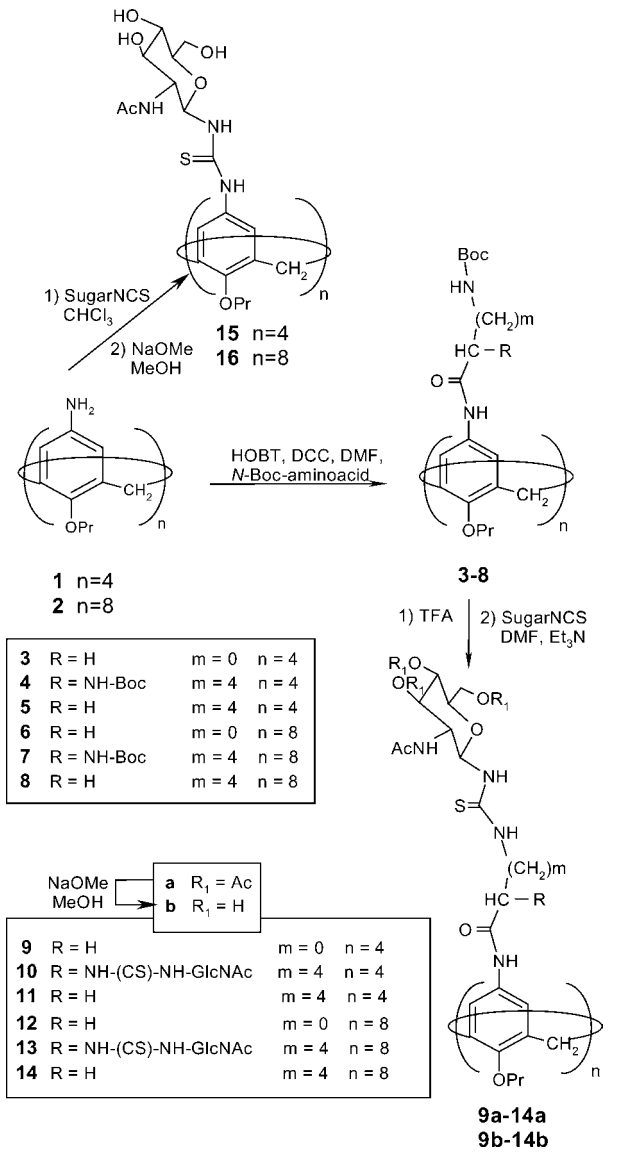
To contribute to these studies, we synthesized novel calixarene-based glycoconjugates bearing an amino acid spacer between the terminal glycoside moiety and the calixarene skeleton[8] (glycosamino acid−calixarenes).

To investigate the influence of shape and mobility of the calixarene scaffold in carbohydrate-protein interaction, we selected as platforms two compounds, the *cone*-blocked tetraminocalix[4]arene **1**[9] and the conformationally mobile octaminocalix[8]arene **2**.[10] Their functionalization with appropriate amino acid spacers allowed manipulation of the density and mobility of the terminal sugar groups. In this work, we focused our attention on the synthesis and lectin-binding ability of glycocalixarenes exposing multiple copies of *N*-acetyl-D-glucosamine (GlcNAc), since this carbohydrate group is widely present in natural glycoproteins. In addition, GlcNAc−receptor interactions are involved in important physiological and pathological processes regarding transcription, translation, nuclear transport, cell signaling, and cell migration.[11]

Treatment of compounds **1** and **2** with *N*-Boc-amino acids (L-glycine, L-lysine, and 6-aminohexanoic acid) in the presence of DCC and HOBT in DMF as the solvent afforded calix[4 or 8]arene derivatives **3**−**8** in good yields (40−78%, Scheme 1).

Their *N*-deprotection with TFA provided the glycosyl acceptor amino groups that were directly reacted, in saline form, with GlcNAc isothiocyanate[12] as glycosyl donor. Glycosyl derivatives **9a**−**14a** were thus obtained (50−95% yield), each bearing the carbohydrate unit linked by a $\beta$-thioureido bridge[13] to the amino acid moiety.

**Scheme 1**




All the synthesized compounds were characterized by NMR and ESI-MS spectroscopy, which in every case proved the exhaustive functionalization of the calixarene scaffold.

The presence of an AX system for $ArCH_2Ar$ groups in the proton spectra of calix[4]arene derivatives **9a**−**11a** confirmed the *cone* conformation. The corresponding glycosamino acid calix[8]arene derivatives **12a**−**14a** showed a broad singlet for $ArCH_2Ar$ groups indicative of their conformational mobility.

The synthetic procedure shown in Scheme 1 was successfully extended to other sugar-amino acid couples. Thus, glycine− and lysine−calix[4 or 8]arenes bearing terminal $\beta$-thioureido-D-galactose or -L-fucose groups were also obtained (70−85% yield).[14]

(6) (a) Houseman, B. T.; Mrksich, M. *Top. Curr. Chem.* **2002**, *218*, 1 and references therein. For glycocalixarenes and glycocyclodextrins, see: (b) Roy, R.; Kim, J. M. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 369. (c) Fulton, D. A.; Stoddart, J. F. *Bioconjugate Chem.* **2001**, *12*, 655. For glycodendrimers, see: (d) Roy, R.; Baek, M.-G. *Rev. Mol. Biotechnol.* **2002**, *90*, 291. (e) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742.

(7) (a) Baisch, G.; Ohrlein, R. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1812. (b) Miyauchi, H.; Yuri, M.; Tanaka, M.; Kawamura, N.; Hayashi, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 989. (c) Wittmann, V.; Takayama, S.; Gong, S. W.; Wetiz-Schmidt, G.; Wong, C.-H. *J. Org. Chem.* **1998**, *63*, 5137. (d) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessiling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922. (e) Woller, E. K.; Cloninger, M. J. *Org. Lett.* **2002**, *4*, 7. (f) Autar, R.; Khan, A. S.; Schad, M.; Hacker, J.; Liskamp, R. M. J.; Pieters, R. J. *ChemBioChem* **2003**, *4*, 1317.

(8) (a) Gutsche, C. D. *Calixarenes*; Royal Society of Chemistry: Cambridge, 1989. (b) Gutsche, C. D. *Calixarenes Revisited*; Royal Society of Chemistry: Cambridge, 1998. (c) *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens J., Eds.; Kluwer: Dordrecht, 2001.

(9) Verboom, W.; Durie, A.; Egberink, R. J. M.; Asfari, Z.; Reinhoudt, D. N. *J. Org. Chem.* **1992**, *57*, 1313.

(10) Consoli, G. M. L.; Cunsolo, F.; Geraci, C.; Mecca, T.; Neri, P. *Tetrahedron Lett.* **2003**, *44*, 1313.

(11) (a) Hart, G. W. *Annu. Rev. Biochem.* **1997**, *66*, 315. (b) Hart, G. W.; Kreppel, L. K.; Corner, F. I.; Arnold, C. S.; Snow, D. M.; Ye, Z.; Cheng, X.; DellaManna, D.; Caine, D. S.; Earles, B. J.; Akimoto, Y.; Cole, R. N.; Hayes, B. K. *Glycobiology* **1996**, *6*, 711. (c) Corner, F. I.; Hart, G. W. *J. Biol. Chem.* **2000**, *275*, 29179.

(12) D-GlcNAc isothiocyanate in $\beta$-form was synthesized by the solution method reported in: Camarasa, M. J.; Fernández-Resa, P.; García-López, M. T.; De Las Heras, F. G.; Méndez-Castrillón, P. P.; San Felix, A. *Synthesis* **1999**, 509.

(13) For other $\beta$-thioureidoglycocalixarenes, see: (a) Saitz-Barria, C.; Torres-Pinedo, A.; Santoyo-Gonzáles, F. *Synlett* **1999**, 1891. (b) Casnati, A.; Sansone, F.; Ungaro, R. *Acc. Chem. Res.* **2003**, *36*, 246. (c) Sansone, F.; Chierici, E.; Casnati, A.; Ungaro, R. *Org. Biomol. Chem.* **2003**, *1*, 1802, and ref 10.

Compounds **9a**−**14a** were de-*O*-acetylated under conventional Zemplen conditions to give the corresponding water-soluble hydroxy derivatives **9b**−**14b** in almost quantitative yield (Scheme 1). The water solubility ranges from $3.5 \times 10^{-5}$ M to $2.9 \times 10^{-4}$ M, **9b** and **10b** being the least and the most soluble compounds, respectively.

Interaction of glycocalixarenes **9b**−**14b**, **15**, and **16**[10] with wheat germ agglutinin (WGA), a lectin known to be *N*-acetyl-$\beta$-D-glucosamine specific,[15] was studied by turbidimetric[16] and hemagglutination inhibition tests.

Direct binding of these compounds to WGA was evidenced by the rapid formation of a turbid suspension. To demonstrate the direct involvement of the carbohydrate moieties in the lectin binding, D-GlcNAc was added to the mixtures containing lectin−glycocalixarene aggregates. A large molar excess (251−580 times) of the inhibitor sugar was required to disrupt the cross-linking interaction up to give a clear homogeneous solution (Figure 1).[17] The addition

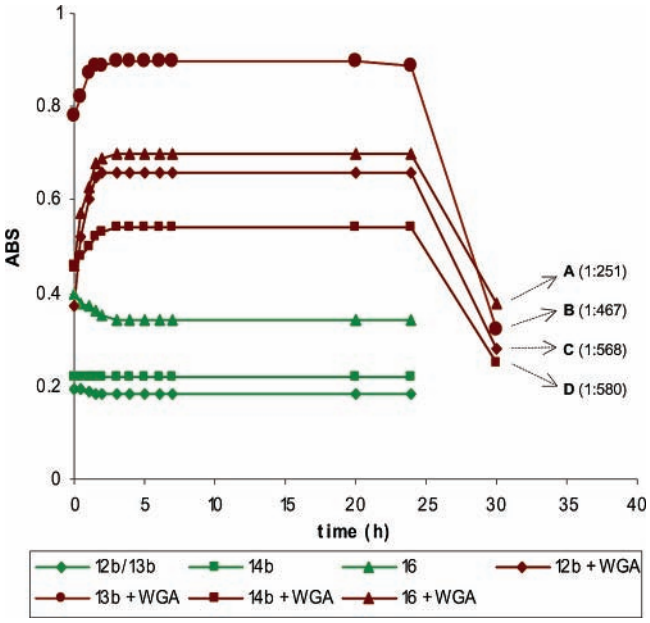



**Figure 1.** Turbidimetric analyses: absorbance of compounds **12b**−**14b** and **16** (green signals) increases after addition of WGA (brown signals). A−D points indicate the glycocalixarene-WGA cross-linking disruption by addition of monomeric GlcNAc. In parentheses is reported the molar excess of sugar inhibitor required to obtain clear homogeneous solutions.

of the same amounts of D-galactose to the turbid suspensions did not give rise to an absorbance decrease, thus confirming the specific involvement of D-GlcNAc units in the lectin interaction. Compounds **9b**−**14b**, **15**, and **16** showed no interaction with PNA lectin, specific for D-galactose, thus demonstrating their lectin recognition specificity.

The biomimetic properties of **9b**−**14b**, **15**, and **16** were estimated from their ability to inhibit WGA-dependent hemagglutination of a 2% suspension of human erythrocytes. The results, summarized in Table 1, indicate that the multivalent calixarene derivatives all possess enhanced lectin affinity with respect to the monomeric GlcNAc.

**Table 1.** Inihibitory Effects on the WGA-Induced Hemagglutination by Monomeric D-GlcNAc and by Ligands **9b**−**14b**, **15**, and **16**

| compd | MIC (mM)[a] | rel potency[b] |
|---|---|---|
| D-GlcNAc | 200 | 1 |
| **9b** | 0.20 | 250 |
| **10b** | 1.20 | 21 |
| **11b** | 0.16 | 312 |
| **15** | 0.58 | 86 |
| **12b** | 0.50 | 50 |
| **13b** | 0.69 | 18 |
| **14b** | 0.40 | 62 |
| **16** | 0.62 | 40 |

[a] MIC: minimum inhibitory concentration. [b] Value is on a per-hapten basis.

The amplification of lectin−sugar interaction is ascribable to clustering effect, which was observed also with other macromolecular ligands such as glycol−chitin,[18] cyclodextrins,[19] and glycopolymers[18] having terminal *N*-acetyl-D-glucosamine residues. In particular, the best result was obtained with ligand **11b** (MIC = 0.16 mM), which was found 312-fold more potent than D-GlcNAc (MIC = 200 mM) used as control. The results in Table 1 show that introduction of an amino acid spacer between the carbohydrate moieties and the calixarene platform positively influences the sugar−lectin binding. In fact, derivatives **9b**, **11b**, **12b**, and **14b**, all bearing linear spacers, have an inhibitory potency higher than the analogous derivatives without spacers (**15**, **16**), likely due to the higher flexibility of the carbohydrate arms. Nevertheless, increase of the linear spacer length from glycine to 6-aminohexanoic acid caused only a slight enhancement of the inhibitory potency.

In contrast, lectin-binding ability is more sensitive to the scaffold structure. In fact, compounds **9b**−**11b** and **15** built on a rigid *cone*-blocked calix[4]arene skeleton have a higher inhibitory ability than corresponding compound **12b**−**14b** and **16** built on a conformationally mobile calix[8]arene skeleton. We suggest that the all-*syn* stereochemistry of the sugar moieties in glycocalix[4]arene derivatives could be more favorable for cooperative interactions.

(14) Unpublished results.

(15) (a) Lis, H.; Sharon, N. In *The Biochemistry of Plants: A Comprensive Treatise, Protein and Nucleic Acids*; Marcus, A., Ed.; Academic Press: New York, 1981; Vol. 6, p 371. (b) Sallas, F.; Nishimura, S.-I. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2091. For review: (c) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803.

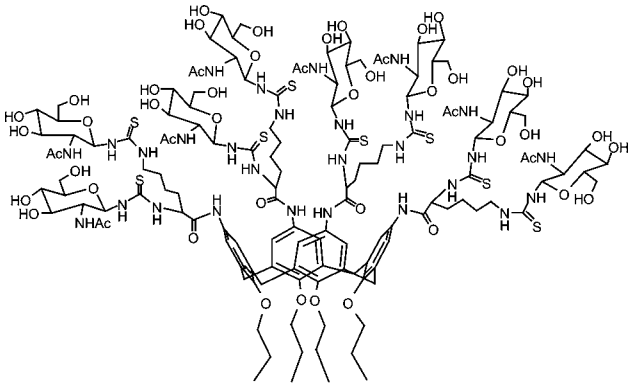
(16) (a) Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391. (b) Fujimoto, T.; Shimizu, C.; Hayashida, O.; Aoyama, Y. *J. Am. Chem. Soc.* **1998**, *120*, 601.

(17) Disruption of the cross-linking between WGA and calix[4]arene derivatives **9b**−**11b** and **15** required a higher than 400-fold molar amount of D-GLcNAc.

(18) (a) Nishimura, S.-I.; Furuike, T.; Matsuoka, K.; Maruyama, K.; Nagata, K.; Kurita, K.; Nishi, N.; Tokura, S. *Macromolecules* **1994**, *27*, 4876. (b) Okada, M. *Prog. Polym. Sci.* **2001**, *26*, 67.

(19) Furuike, T.; Seiichi, A.; Nishimura, S.-I. *Tetrahedron* **2000**, *56*, 9909.

Branched compounds **10b** and **13b** bearing 8 and 16 sugar groups, respectively, displayed lower inhibitory ability than all the other glycocalixarenes, thus suggesting that the sugar moieties are less exhibited on the surface and consequently less available to form cross-linking with the lectin. This behavior is in agreement with observations reported about other polyvalent systems in which higher valency sterically prevent the cross-linking due to an improper spacing or geometry of the carbohydrate presented on the scaffold surface.[20]



**10b**

The amphiphilic nature of the glycosamino acid−calixarenes suggested that they could give rise to supramolecular aggregates similar to those reported by Aoyama for glycoresorcinarenes.[21] This was confirmed by preliminary dynamic light scattering analysis on compound **13b**, which showed the formation of aggregates at 0.89 mM concentration and at pH 7.2 in phosphate-buffered saline.[22] Therefore, aggregation states in addition to conformational features have to be taken into account in lectin−glycocalixarene cross-linking formation. More in-depth studies are in progress to investigate the supramolecular aggregation of **9b**−**14b** in solution and the nature of their aggregates with WGA.

In conclusion, novel GlcNAc-exposing amino acid calixarenes have been efficiently synthesized. Their specific lectin-binding ability and amplified lectin affinity have been shown as well as the effects of the scaffold mobility and nature spacer.

Compounds here reported are novel "nanostructures"[23] useful to study recognition phenomena involving surface GlcNAc and open perspectives toward their application as drug delivery systems or inhibitors of GlcNAc-receptor binding involved in pathological events.[24] Finally, since GlcNAc is a known substrate in glycosylation of natural glycoproteins, the compounds here reported appear interesting building blocks for the construction of more complex calixarene-based glycoconjugates.

**Acknowledgment.** We thank Prof. P. Neri for helpful discussions and Prof. M. Piattelli for a critical reading of the manuscript. Thanks are also due to Dr. Norberto Micali (IPCF - Sez. di Messina, CNR) for light scattering measurements and Mr. A. Renda (ICB - Sez. di Catania, CNR) for ESI-MS spectra.

**Supporting Information Available:** Experimental procedures, and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0485767

(20) (a) Lee, Y. C.; Lee, R. T. *Bioconjugate Chem.* **1997**, 8, 762. (b) Andre, S.; Ortega, P. J. C.; Perez, M. A.; Roy, R.; Gabius, H. J. *Glycobiology* **1999**, *9*, 1253. (c) Cairo, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessiling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 1615.

(21) (a) Hayashida, O.; Kato, M.; Akagi, K.; Aoyama, Y. *J. Am. Chem. Soc.* **1999**, *121*, 11597. (b) Hayashida, O.; Mizuki, K.; Akagi, K.; Matsuo, A.; Kanamori, T.; Nakai, T.; Sando, S.; Aoyama, Y. *J. Am. Chem. Soc.* **2003**, *125*, 594. (c) Aoyama, Y. *Chem. Eur. J.* **2004**, *10*, 588.

(22) 0.01 M phosphate with [NaCl] = 0.5 M, [$MnCl_2$] = 0.1 mM, and [$CaCl_2$] = 0.1 mM.

(23) For example, compounds **12b** and **13b** in the "open" unfolded conformation built on the typical pleated loop structure have an estimated molecular dimension of 4.1 and 5.0 nm, respectively.

(24) (a) Wassler, M. J.; Shur, B. D. *J. Cell Sci.* **2000**, *113*, 237. (b) Shur, B. D.; Evans, S. C.; Lu, Q. *Glycoconjugate J.* **1998**, *15*, 537. (c) Houseman, B. T.; Mrksich, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 782.