ORGANIC LETTERS

2003
Vol. 5, No. 21
3775−3778

Design and Synthesis of C_3-Symmetric Lewis^X Antigen

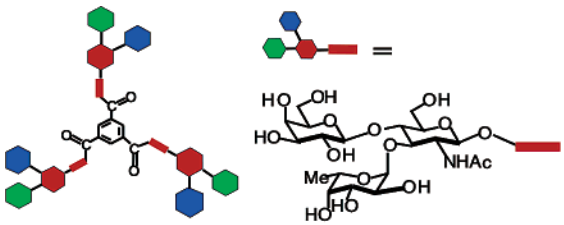
Yoshihiro Nishida,* Tomoaki Tsurumi, Kenji Sasaki, Kenta Watanabe, Hirofumi Dohi, and Kazukiyo Kobayashi*

Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan

nishida@mol.nagoya-u.ac.jp; kobayash@mol.nagoya-u.ac.jp

Received June 19, 2003 *(Revised Manuscript Received August 28, 2003)*

ABSTRACT



C_3-Symmetric Lewis^X trisaccharide

C_3-Symmetric glycoconjugates carrying three equivalent Lewis^X antigens or β-lactosides were synthesized from *p*-nitrophenyl glycosides and trimesic acid via regio- and stereocontrolled glycosylation reactions. An ^{1}H NMR study has shown that the C_3-symmetric glycoconjugates soluble in water provide useful probes to investigate the Ca^{2+}-dependent Lewis^X–Lewis^X association.

Intense interest is being directed to the design and application of multivalent glycoconjugates[1,2] carrying human oligosaccharide antigens.[3,4] Several multivalent models have already been proposed for sialyl Lewis^X and globotriaosyl antigens, in which polymers,[3,4a] dendrimers,[4c] and starfish models[4d] are widely examined. In this paper, we describe the synthesis and potential use of C_3-symmetric Lewis^X trisaccharide and β-lactoside.

Hakomori et al.[5] had previously disclosed that Lewis^X oligosaccharides bound to cell membrane lipids cause homophilic association in the presence of a calcium ion. Many studies have been directed toward this intriguing phenomenon,[6] in which Lewis^X-based dimers,[6c] liposomes,[6b] giant-liposomes,[6g] gold nanoparticles,[6e] and self-assembling monolayers[6f] have been proposed as multivalent probes.

The C_3-symmetric Lewis^X **3** (Figure 1) was designed as an alternative probe with some expectations as described below. (1) The trivalent structure will induce enhanced self-association due to multivalent effects, (2) allow a simpler analysis than nonsymmetric or dendric models due to the C_3-symmetry, and (3) show higher water solubility than usual glycolipid models because three hydrophilic Lewis^X are directed outside of the hydrophobic core. Regarding the multivalent effect, Rao, Whitesides, and co-workers[7] have evidenced in their pioneering study that a vancomycin trimer

(1) Lee, Y. C.; Lee, R. T. *Neoglycoconjugates*: *Preparation and Applications*; Academic Press: San Diego, 1994.

(2) (a) Bovin, N. V.; Gabius, H. J. *Chem. Soc. Rev.* **1995**, *24*, 413−422. (b) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 679. (c) Rye, P. D. *Biotechnology* **1997**, *14*, 155.

(3) Miyauchi, H.; Tanaka, M.; Koike, H.; Kawamura, N.; Hayashi, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 985. (b) Gordon, E. J.; Sanders, W. J.; Kiessling, L. L. *Nature* **1998**, *392*, 30.

(4) (a) Nishida, Y.; Dohi, H.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 8681. (b) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D. Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839. (c) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669.

(5) (a) Hakomori, S. *Pure Appl. Chem.* **1991**, *63*, 473. (b) Hakomori, S. *Cancer Res.* **1996**, *56*, 5309.

(6) (a) Siuadak, G.; Ichikawa, Y.; Caulfield, T. J.; Munoz, B.; Wong, C.-H. Nicolaou, K. C. *J. Am. Chem. Soc.* **1993**, *115*, 2877. (b) Geyer, A.; Gege, C.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **1999**, *38*, 1446. (c) Geyer, A.; Gege, C.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 3246. (d) Zhang, Y.; Dausse, B.; Sinay, P.; Afsahi, M.; Berthault, P.; Desvaux, H. *Carbohydr. Res.* **2000**, *324*, 231. (e) Fuente, J. M.; Barrientos, A. G.; Rojas, T. C.; Rojo, J.; Canada, J.; Fernandez, A.; Penades, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 2258. (f) Tromas, C.; Rojo, J.; Fuente, J. M.; Barrientos, A. G.; Garcia, R.; A.; Penades, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 2258. (g) Pincet, F.; Bouar, T. L.; Zhang, Y.; Esnault, J.; Mallet, J.-M.; Perez, E.; Sinay, P. *Biophys. J.* **2001**, *80*, 1354.

10.1021/ol0351293 CCC: $25.00
Published on Web 09/17/2003

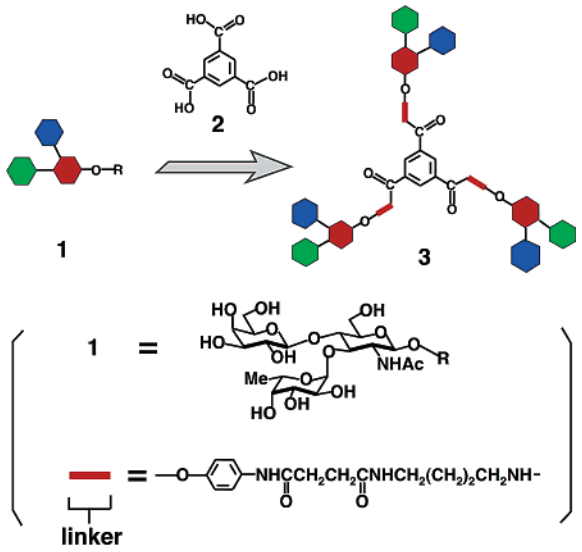
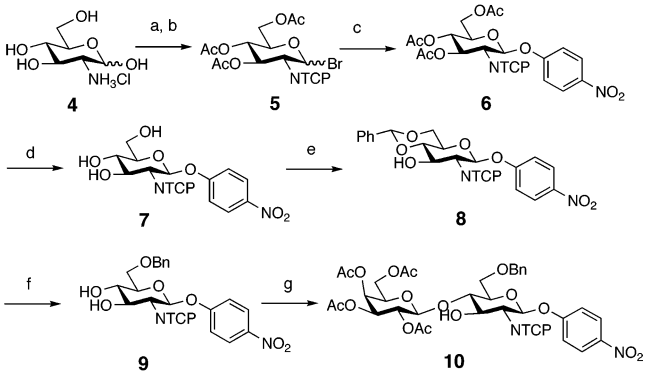


Figure 1. Conversion of an asymmetric Lewis^X trisaccharide **1** to a C_3-symmetric conjugate **3** with trimesic acid **2**. (The linker moiety shown with a red bar was required for successful conversion as cited in the text).

composed of trimesic acid **2** can induce a remarkable enhancement in the antibiotic activity. They have provided also theoretical bases to rationalize this enhancement. Thus, it can be reasonably assumed that the trimeric Lewis^X can provide a useful probe to investigate the weak Lewis^X—Lewis^X interaction.

Though there are many synthetic studies targeting the Lewis^X skeleton **1**[8] and its multivalent models,[9] there is no established way to assemble the C_n-symmetric structure. Here, choice of an appropriate aglycon (R in **1**) convertible to the C_3-symmetric structure provides a key investigative issue. In our continuous studies on the synthesis of artificial glycopolymers,[10] we have employed *p*-nitrophenyl (pNP) glycosides. In addition to the UV absorption useful for TLC detection, reduction of the pNP group affords an arylamine useful for multivalent assembly.[4a,11] Also in this work, we undertook the same strategy using pNP D-glucosaminide **7** and **9** as key intermediates (Scheme 1).

Scheme 1. Syntheses of Compounds **7** and **10**[a]

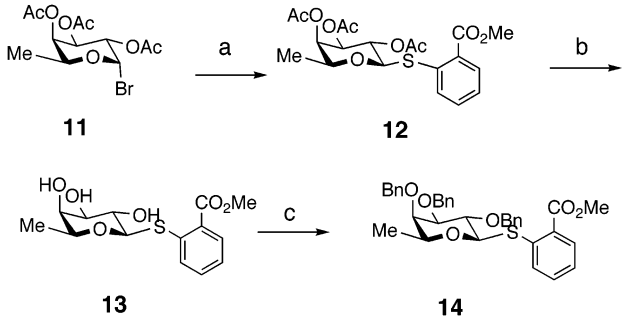


[a] Reagents: (a) (i) tetrachlorophthalic anhydride, Et_3N/MeOH, (ii) Ac_2O/Py 80%; (b) HBr−AcOH/CH_2Cl_2 58%; (c) AgOTf, *p*-nitrophenol/CH_2Cl_2 46%; (d) NaOMe/MeOH quant; (e) *p*-toluenesulfonic acid, benzaldehyde dimethyl acetal/DMF, 77%; (f) triethylsilane, trifluoroacetic acid, trifluoroacetic anhydride/CH_2Cl_2, 75%; (g) per-*O*-acetyl-α-D-galactopyranosyl trichloroacetiimidate, TMSOTf/CH_2Cl_2, 83%.

The tetrachlorophthalimide (TCP) group[12] in **7** was chosen as an N-protecting group expecting that the TCP will be removed under basic conditions without affecting the pNP *O*-glycoside linkage. Moreover, the bulky group seemed to work effectively in regioselective glycosylation reactions.[9] Compound **7** was converted to 6-*O*-benzyl derivative **9** in a conventional way. β-Galactosylation was performed using a trichloroacetiimidate method established by Schmidt et al.[13] The glycosylation of **9** was regio- and β-specific to afford pNP β-lactosaminide **10**.

For α-L-fucosylation, we applied our thio-glycosylation method using *o*-methoxycarbonyl phenyl (*o*-MCP) 1-thio-glycosides.[14] A 1-thiofucosyl donor **14** (mp = 125 °C) was prepared via an S_N2 reaction between fucosyl bromide **11** and thiosalicylate (Scheme 2). No unpleasant thiol odor was

Scheme 2 *o*-MCP 1-Thio-L-fucosyl Donor **14**[a]



[a] Reagents: (a) methyl thiosalicylate, K_2CO_3/DMF, 80%; (b) NaOMe/MoOH, 77%; (c) NaH, BnBr/DMF, 0 °C, 90%.

evolved during the preparation of **14** to allow a practical thioglycosylation methodology.

Prior to the fucosylation, the reactivity of **14** was examined with cetyl alcohol (A) and 1,2;5,6-di-*O*-isopropylidene-α-

(7) (a) Rao, J.; Lahiri, J.; Lyle, I.; Weis, R. M.; Whitesides, G. M. *Science* **1998**, *280*, 708. (b) Rao, J.; Lahiri, J.; Weis, R. M.; Whitesides, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 2698.

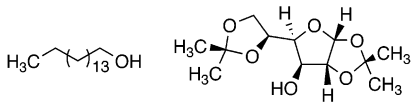
(8) (a) Jacquinet, J. C.; Sinay, P. J. *Chem. Soc., Perkin Trans 1* **1979**, 314. (b) Hindsgaul, O.; Norberg, T.; Pendu, J. Le; Lemieux, R. U. *Carbohydr. Res.* **1982**, *109*, 109. (c) Sato, S.; Itoh, Y.; Nukada, T.; Nakahara, Y.: Ogawa, T. *Carbohydr. Res.* **1987**, *167*, 197. (d) Nicolaou, K. C.; Hummel, C. W.; Iwabuchi, Y. *J. Am. Chem. Soc.* **1992**, *114*, 3126. (e) Hasegawa, A.; Ando, T.; Kameyama, A.; Kiso, M. J. *Carbohydr. Chem.* **1992**, *11*, 645. (f) Jain, R. K.; Matta, K. L. *Carbohydr. Res.* **1992**, *226*, 91. (j) Windmueller, R.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 7927. (h) Ehara, T.; Kameyama, A.; Yamada, Y.; Ishida, H.; A.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1996**, *281*, 237. (i) Ellervik, U.; Magnusson, G. *J. Org. Chem.* **1998**, *63*, 9314.

(9) (a) Esnault, J.; Mallet, J.-M.; Zhang, Y.; Sinay, P.; Bouar, T. L.; Pincet, F.; Perez, E.; *Eur. J. Org. Chem.* **2001**, 253. (b) Fuente, J. M.; Penades, S. *Tetrahedron*: *Asymmetry* **2002**, *13*, 1879.

(10) Sasaki, K.; Nishida, Y.; Tsuruimi, T.; Uzawa, H.; Kondo, H.; Kobayashi, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 4463. (b) Dohi, H.; Nishida, Y.; Furuta, Y.; Uzawa, H.; Yokoyama, S.-I.; Ito, S.; Mori, H.; Kobayashi, K. *Org. Lett.* **2002**, *4*, 355.

(11) Page, D.; Roy, R. *Glycoconjugate J.* **1997**, *14*, 345.

Table 1. Glycosylation of Cetyl Alcohol (A) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (B) with **14**



A B

entry	acceptors	solvents	*T* (°C)	time[a]	α/β[b]
1	A[c]	CH_2Cl_2	0	2	3:7
2	A[c]	CH_2Cl_2	−40	2	3:7
3	A[c]	$CH_2Cl_2 + Et_2O$	−40	24	3:7
4	B[c]	CH_2Cl_2	0	1	>9:1
5	B[c]	CH_2Cl_2	−40	2	8:2
6	B[d]	$CH_2Cl_2 + Et_2O$	−40	20	7:3

[a] Reaction time (h) until the donor was consumed out in the reaction. [b] Determined by ^1H NMR analysis (accuracy within 5%). The yield based on the amount of **14** is quantitative in every case. [c] 1.5 molar equiv of acceptor was required for the donor **14** to complete the reaction in the presence of 2 molar equiv. *N*-Iodosucciimide (NIS) and a catalytic amount of triflic acid. [d] 6 molar equiv of the acceptor (A or B) was used.

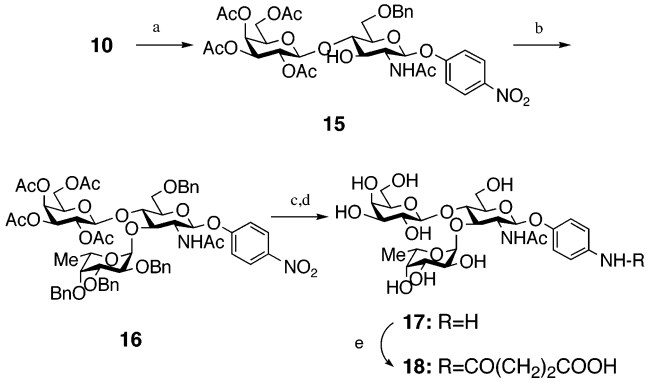
D-glucofuranose (B) as the acceptor substrates (Table 1). The reaction of **14** with the primary alcohol (A) gave β-glycoside predominantly, while it gave α-glycoside from the secondary alcohol (B). A similar tendency was observed for *o*-MCP 1-thio-D-galactosyl donors, and this was explained in terms of the participation of the *o*-carboxylate group.[14] Though the mechanism remains tentative, glycosylation reactions applying *o*-MCP 1-thio glycosides show a remarkable change in the α- and β-selectivity depending on the reactivity of the acceptors.

When the fucosyl donor **14** was applied to **10**, no product was obtained. On the other hand, an α-L-fucosyl product **16** was obtained in the reaction with **15** having an *N*-acetyl group in the vicinity of OH-3. No β-isomer was detected by the ^1H NMR analysis of the product, and every acceptor was consumed in the reaction [the yield (57%) of **16** after purification on silica gel column is not optimized]. After the removal of *O*-Ac and *O*-benzyl groups followed by the reduction of a pNP group, pAP Lewis^X **17** was obtained (Scheme 3).

In our initial design, we had assumed direct condensation between **2** and **17** to construct a rigid linker for a Lewis^X trimer. This is because the rigidity is thought to minimize the loss of entropy during a binding event and induce strong bindings.[7b] The direct condensation, however, did not proceed satisfactorily. This seemed ascribable to the low nucleophilicity of the arylamine. Thus, compound **17** was treated with succinic anhydride and diisopropylethylamine (DIPEA) and converted to **18** carrying a carboxyl group at the terminal. The core **2** was modified with 1-*N*-*tert*-butoxycarbonyl-1,4-

(12) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraiser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302.
(13) Schmidt, R. R. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
(14) (a) Dohi, H.; Nishida, Y.; Tanaka, H.; Kobayashi, K. *Synth. Lett.* **2001**, 1446. (b) Dohi, H.; Nishida, Y.; Takeda, T, H.; Kobayashi, K. *Carbohydr. Res.* **2002**, *337*, 983.

Scheme 3. Synthesis of Lewis^X Trisaccharides **17** and **18**[a]

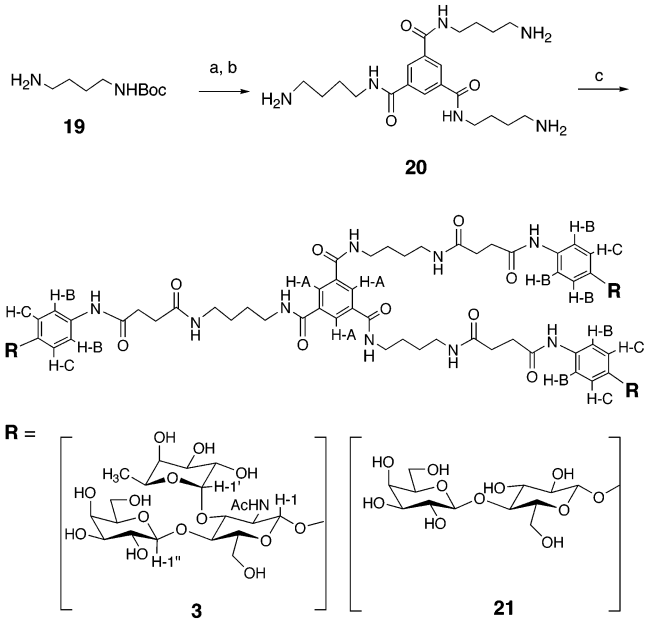


[a] Reagents: (a) (i) ethylenediamine/EtOH, 50 °C, (ii) MeOH, H_2O, Ac_2O, 62%; (b) **14**, NIS, TfOH/CH_2Cl_2, 57%; (c) NaOMe/MeOH, 91%; (d) H_2, $Pd(OH)_2$/C, MeOH; (e) succinic anhydride, DIPEA/MeOH, quant.

diaminopropane **19** to give **20** carrying three amino groups (Scheme 4). Condensation between **18** and **20** was completed nearly quantitatively with 1-hydroxybenzotriazole (HOBT) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) in the presence of DIPEA. Product **3** was identified with MALDI-TOF-MS [$(M + Na)^+ = 2603$] and ^1H NMR spectroscopy.

The above processes generated a hydrophobic amide linker with some flexibility in the conformation. However, the nature of this linker, in terms of conformation, length, and hydrophobicity, was not evaluated or optimized in the present study.

Scheme 4 C_3-Symmetric Lewis^X **3** and β-lactoside **21**[a]



[a] Reagents: (a) **2**, HOBT, EDC/DMF; (b) 4 N HCl in dioxane, quant.; (c) **18**, HOBT, EDC, DIPEA/DMF, 94%.

As a reference compound, a C_3-symmetric β-lactoside **21** was also prepared from *p*NP β-lactoside[4a,11] in the same way as described above. Though the glycoconjugates **3** and **21** provide an apparent feature like amphiphilic molecules, they show higher water solubility than glycero-glycolipids.[15] Thus, their ^{1}H NMR spectra measured in D_2O gave relatively sharp signals at room temperature (Figure 2). The spectra have

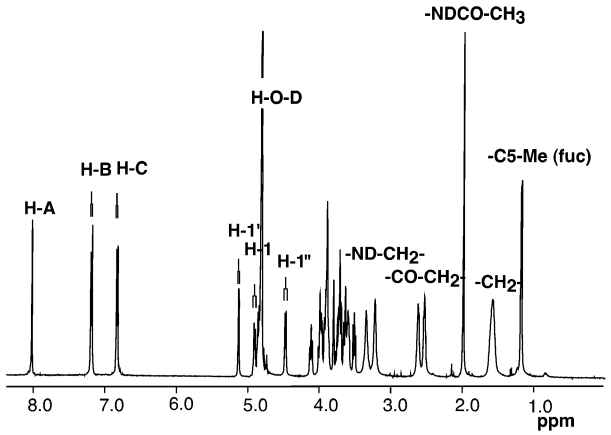


Figure 2. ^{1}H NMR spectra of **3** (500 MHz, D_2O, room temperature).

also indicated that both of the glycoconjugates hold the C_3-symmetry in the aqueous solution displaying the three sugars equivalently.

When the aqueous solution was treated with $CaCl_2$, the two conjugates turned out to behave in a different way from each other. The ^{1}H NMR signals of **3** became broader with the addition of Ca^{2+} ion between 0 and 100 mM, while those of **21** showed little broadening even at 100 mM Ca^{2+} concentration (Figure 3). The selective broadening for **3** is in agreement with the Ca^{2+}-dependent Lewis^X−Lewis^X

(15) (a) Nishida, Y.; Ohrui, H. Meguro, H.; Ishizawa, M.; Matsuda, K.; Taki, T.; Handa, S.; Yamamoto, N. *Tetrahedron Lett*. **1994**, *35*, 5465. (b) Nishida, Y.; Takamori, Y.; Ohrui, H.; Ishizuka, I.; Matsuda, K.; Kobayashi, K. *Tetrahedron Lett*. **1999**, *40*, 2371.

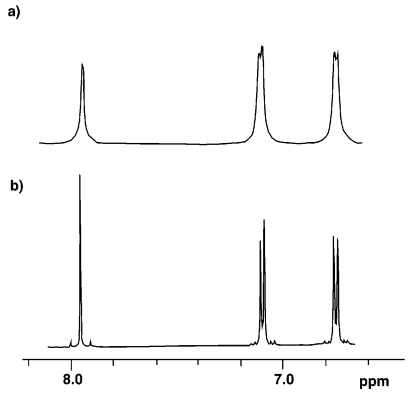


Figure 3. Effect of Ca^{2+} ion (100 mM) on ^{1}H NMR signals of Lewis^X **3** (a) and β-lactoside **21** (b) trimers (10 mM, D_2O, 500 MHz, room temperature).

association as Hakomori et al.[5] proposed. The broadening is considered to reflect the self-association that increases the apparent molecular weight of **3** in aqueous solution and accelerates ^{1}H-spin−spin relaxation.[16] These preliminary results[17] support that the C_3-symmetric conjugates can provide useful probes to investigate the Lewis^X−Lewis^X interaction. More precise analyses presently in progress will be reported elsewhere.

Acknowledgment. This study was supported in part by the 21st Century COE program "Nature-Guided Materials Processing" of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese government.

Supporting Information Available: Experiments and ^{1}H NMR data of the main products in Schemes 1−4. ^{1}H NMR and DLS profiles of compounds **3** and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0351293

(16) Bovey, F. A. *High-Resolution NMR of Macromolecules*; Academic Press: New York, 1972.

(17) A dynamic light-scattering (DLS) analysis also indicated a clear difference. In the presence of Ca^{2+} ion (5 mM), the Lewis^X trimer **3** turns to take two types of self-assembly forms with an averaged size of ca. 0.5 × 10^2 nm and larger than 2.0 × 10^2 nm, respectively.