Tetrahedron Letters 49 (2008) 5593–5596

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis and lectin-binding activity of luminescent silica particles peripherally functionalized with lactose

Ken Hatano *, Tetsuya Yamazaki, Koji Yoshino, Naoto Ohyama, Tetsuo Koyama, Koji Matsuoka, Daiyo Terunuma

Division of Material Science, Graduate School of Science and Technology, Saitama University, 255 Shimo-Ohkubo, Sakura-ku, Saitama 338-8570, Japan

ARTICLE INFO

Article history:
Received 2 July 2008
Accepted 4 July 2008
Available online 9 July 2008

Keywords:
Carbohydrate
Glycocluster
Rubpy
Silica particles
Lectin-binding
Luminescent scaffold
Silane coupling agent

ABSTRACT

A novel O-protected lactose (Galβ1→4Glcβ1–) derivative bearing trimethoxysilyl group at the aglycon was developed as a silane coupling agent. Reaction of the coupling agent with tris(2,2′-bipyridine)ruthenium (II) dichloride (Rubpy) doped silica particle gave a Rubpy-doped silica particle peripherally functionalized with O-protected lactose derivative. De-O-protection of the particle with aqueous ammonia provided lactose-coating Rubpy-doped silica particles, combining luminophor encapsulated in silica matrix and carbohydrate having lectin-recognition ability. Specific adhesion of fluorescein isothiocyanate-labeled peanut agglutinin (FITC-PNA) to the lactose-coating Rubpy-doped silica particles was confirmed by fluorescence microscopic analysis.

Glycoconjugates, such as glycoprotein and glycolipids, are generally located on cell surfaces and play a key role in the process of cell adhesion with proteins of pathogens; that is, the early stage of cell adhesion involves carbohydrate-mediated specific recognition of pathogens. It is known that the clustering effect of carbohydrates enhances individual interaction between carbohydrates and proteins.[1] This effect has been applied for the molecular design of artificial inhibitors of pathogens such as toxins, bacteria and viruses, and several forms of glycoclusters have been developed.[2] We previously reported the syntheses of some glycoclusters[3] in which carbosilane dendrimers were employed as carbohydrate scaffolds, and we revealed the biological activities of some of these glycoclusters.[3e,g,h,4] We have been interested in the synthesis of luminescent glycoclusters because of their high potentiality for biomarkers of a variety of lectins and pathogens, and we recently reported the first synthesis and the unique optical properties of a luminescent glycocluster possessing a silole-core carbosilane dendrimer as a luminescent scaffold.[5]

On the other hand, silica particles are widely used in not only industrial applications but also fundamental research. Preparations and applications of monodisperse silica particles[6] and silica coating of other inorganic colloids[7] have been investigated in detail. Immobilizations of antibodies,[8] enzymes,[9] catalysts,[10] and magnetic substances[11] on a silica surface has attracted considerable attention in both clinical and chemical biology from the viewpoint of biocompatible silica particles. However, there have been few reports on the synthesis and application of carbohydrated silica particles.[12] In the course of our studies on glycoclusters, we became interested in a glycocluster in which luminescent silica particles are employed as a carbohydrate scaffold. Here, we report the synthesis of lactose-conjugated silica particles containing Rubpy as a luminophor and their lectin-binding activity.

Synthesis of Rubpy-doped silica particles **1** was carried out by the water-in-oil microemulsion method described previously.[13] The synthesized Rubpy-doped particles **1** were uniform in shape with an average diameter of about 500 nm (Fig. 1A).[14] The commonly used protocol for immobilization of functional compounds such as carbohydrate involves a surface modification of silica particles to combine the compound and silica particles. In this work, we used a new approach to carbohydrate-coating silica particles by means of a novel carbohydrated silane coupling agent. This procedure takes advantage of a simple approach to conjugate silica particles and carbohydrates.

The key intermediate **3a** was readily prepared in quantitative yield by hydrosilylation of a 1-*O*-pentenyl lactoside **2a** with trichlorosilane using $H_2PtCl_6{\cdot}6H_2O$ as a catalyst and subsequent methanolysis in the presence of a small amount of pyridine (Scheme 1). The ^1H NMR spectrum of **3a** showed a characteristic signal based on $Si(OCH_3)_3$ at 3.55 ppm.[15] However, the trimethoxysilyl derivative **3a** was slightly moisture-sensitive and slowly underwent intermolecular substitution leading to oligo- and

* Corresponding author. Tel./fax: +81 48 858 3535.
E-mail address: khatano@fms.saitama-u.ac.jp (K. Hatano).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.07.034

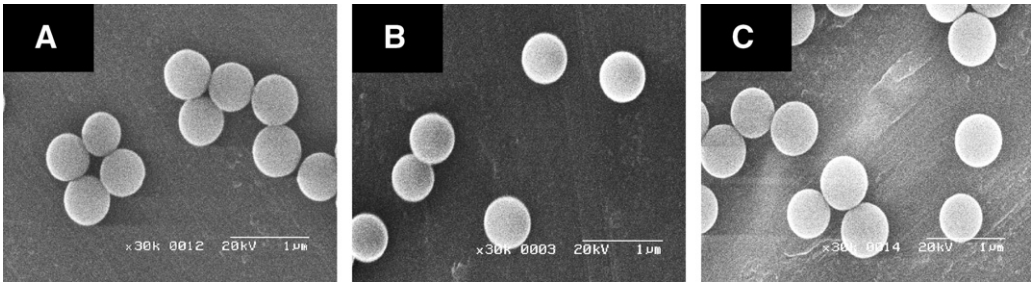
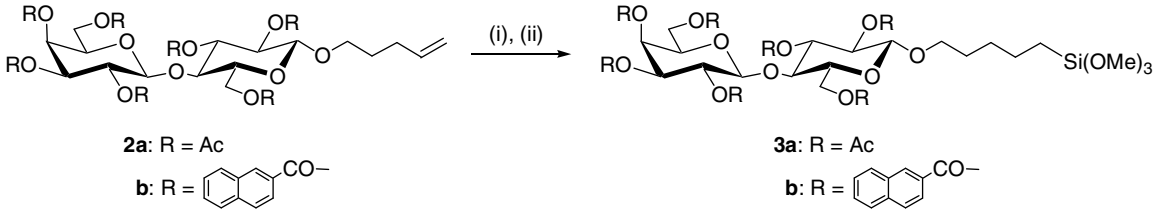


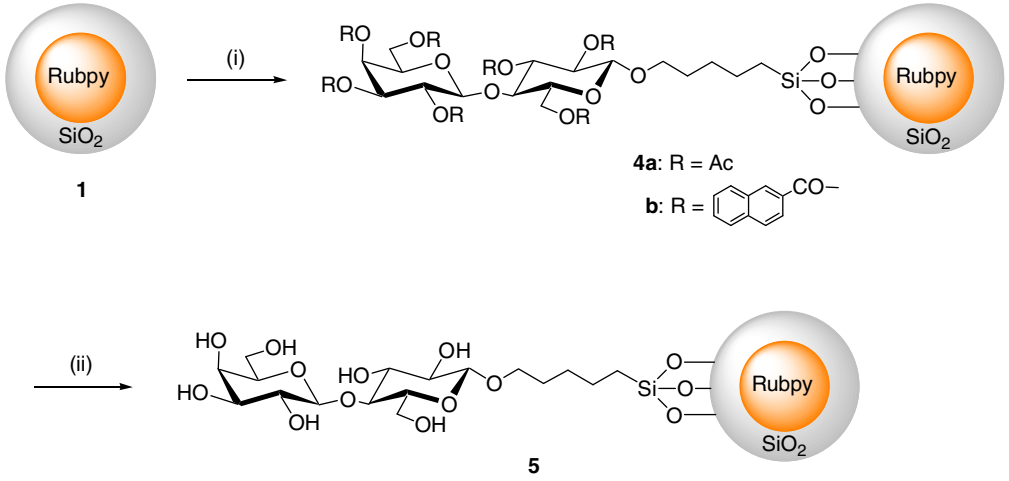
Figure 1. SEM mages of (A) Rubpy-doped silica particles **1**, (B) silica particles functionalized with acetyl-protected lactose derivative **4a** and (C) lactose-functionalized silica particles **5**.



Scheme 1. Reagents and conditions: (i) $HSiCl_3$, Speir cat., THF, rt→50 °C; (ii) MeOH, pyridine, THF.

polysiloxanes. Therefore, synthesized **3a** was immediately immobilized onto the Rubpy-doped silica particles **1** by a standard *sol–gel* process (see Scheme 2).

Reaction of **1** (1.000 g) with 10 wt% of the lactose derivative **3a** (0.12 mmol) in toluene at ambient temperature for 12 h followed by heating the reaction mixture at 80 °C for 3 h afforded 938 mg



Scheme 2. Reagents and conditions: (i) trimethoxysilylated lactose derivative **3**, toluene, rt→80 °C; (ii) NH_4OH, MeOH, rt.

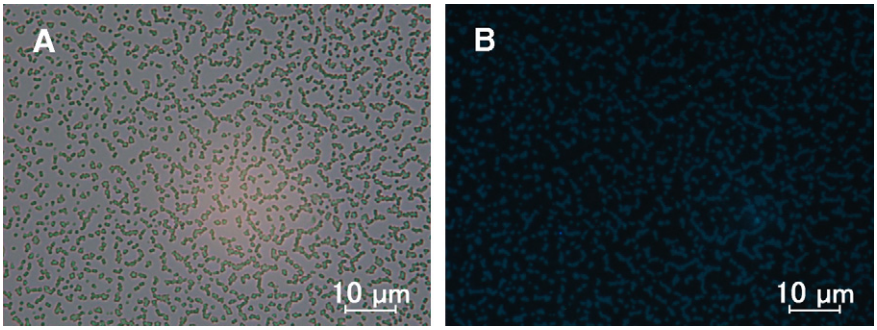


Figure 2. Fluorescence microscopy images of silica particles **4b** at 500 times magnification under transmitted light (A) and through a *Nikon* UV-2A filter set with 355/50-nm excitation and >420 nm emission (B).

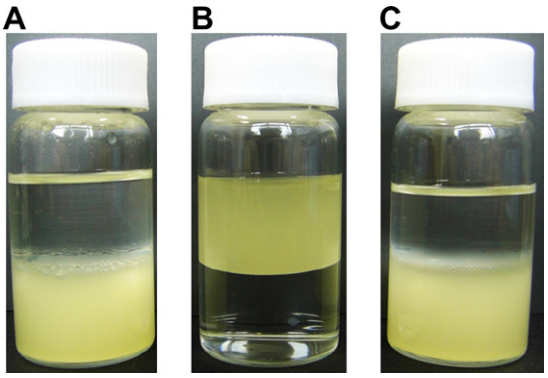


Figure 3. Dispersion of particles in two different immiscible liquids (ethyl acetate/water). (A) Rubpy-doped silica particles **1**, (B) silica particles functionalized with acetyl-protected lactose derivative **4a** and (C) lactose-functionalized silica particles **5**.

of **4a**, which was purified by centrifugation and washing away physically adsorbed lactose derivatives. The IR spectrum of the particles **4a** showed vibrations corresponding to C=O of the acetyl group and Si–O of silica particles at 1753 and 1096 cm^{-1}, respectively.[16] Thus, acetyl-protected lactose must be immobilized on the Rubpy-doped silica particles **1**. However, there is no obvious difference between scanning electron microscopy (SEM) images of the synthesized particles **4a** and the parent particles **1** (Fig. 1A and B). In order to shed light on the immobilization of lactose derivative, therefore, analogous silica particles **4b** in which the naphthoyl moiety emits blue light by appropriate photoexcitation were synthesized.[16] Fluorescence microscopy images of **4b** are shown in Figure 2. When **4b** was exposed to 330–380 nm UV light, blue luminescence attributed to the naphthoyl group was detected from all particles (Fig. 2B). The observed blue luminescence indicates that naphthoyl protected lactose is infallibly immobilized on the silica particles **4b**. Consequently, the surfaces of the particles **4a** are presumably functionalized with an acetyl-protected lactose derivative despite the fact that SEM images of the particles and the parent silica particles **1** are similar.

De-O-acetylation of particles **4a** (500 mg) with aqueous ammonia in methanol yielded the corresponding lactose-coating particles **5** (430 mg) after centrifugation and washing with ethyl acetate and water. The structure of **5** was confirmed by an IR spectrum, in which the peak at 1753 cm^{-1} due to carbonyl group of **4a** disappeared.[17] All Rubpy-doped silica particles obtained were analyzed by SEM (Fig. 1). Morphological analysis showed that all silica particles were spherical and uniform in size. The diameter of both silica particles **4a** and **5** was ca. 500 nm, comparable to the size of the parent particles **1**. Solvent affinity tests of all silica particles prepared were carried out in two different immiscible liquids

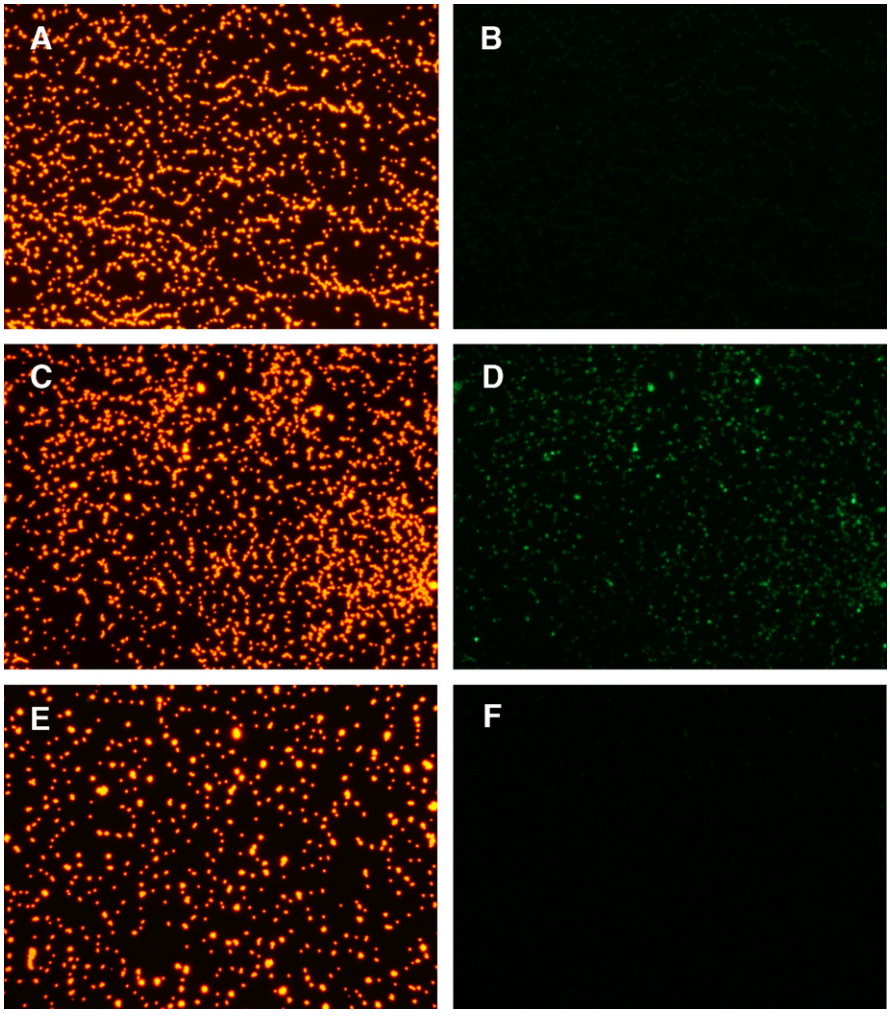


Figure 4. Fluorescence microscopy images of silica particles **1** (A and B) and **5** (C and D) treated with FITC-PNA, and **5** (E and F) treated with FITC-ConA at 500 times magnification. (A, C and E) *Nikon* B-2A filter set with 470/40-nm excitation and >520 nm emission was used for the images. (B, D and F) Semrock BrightLine® GFP-3035B filter set with 472/30-nm excitation and 520/35-nm emission was used for the images.

(water/ethyl acetate). Interestingly, silica particles **1** and **5** possessing free hydroxyl groups at terminal positions were dispersed in the aqueous layer (Fig. 3A and C), while particles **4a**, in which all the hydroxyl groups were protected by the acetyl group, were dispersed in the organic layer (Fig. 3B). Thus, the terminal functional group of silica particles strongly affects the solvent affinity. It should be noted that lactose could be immobilized easily onto silica particles by the standard *sol–gel* process utilizing trimethoxysilylated lactose derivative **3** and subsequent de-O-acetylation with aqueous ammonia.

Fluorescence methods have been used extensively to study the specific adhesion of lectin with glycoclusters. To demonstrate lectin-binding activity of the lactose-functionalized silica particles **5**, we next investigated binding with FITC-PNA, which efficiently adheres to oligosaccharides bearing a terminal galactose moiety by carbohydrate–protein affinity.[18] The silica particles **5** (20 mg) were treated with FITC-PNA (0.5 mL, 0.137 μM) and then washed with Hepes buffer and acetone in a Pasteur pipette. Analogous treatments of the parent particles **1** with FITC-PNA, and of the lactose-functionalized silica particles **5** with FITC-labeled concanavalin A (FITC-ConA), mannose/glucose-binding lectin, were carried out for the comparison. Fluorescence microscopy images of the resultant particles are shown in Figure 4. Bright orange fluorescence from Rubpy doped in the silica particles was detected from all types of particles; however, green fluorescence attributed to FITC was observed only from silica particles **5** treated with FITC-PNA, clearly indicating that FITC-PNA adheres only to silica particles of which surfaces are functionalized with lactose. Rubpy-doped silica particles peripherally functionalized with carbohydrate such as **5** are potentially useful for identification and labeling of a target lectin by means of the clustering effect of carbohydrates and fluorescence from Rubpy. Further investigations of Rubpy-doped silica particles functionalized with bioactive oligosaccharides and their applications to labeling of pathogens are currently in progress.

Acknowledgments

This work was supported by grants from the Japan Science and Technology Agency (Research for Promoting Technological Seeds) and Ministry of Health, Labour, and Welfare of Japan (Health and Labour Science Research Grant for Research on Advanced Medical Technology; 14-N-015).

Supplementary data

Supplementary data (the experimental and analytical data for new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.07.034.

References and notes

1. (a) Lee, Y. C.; Townsend, M. R.; Hardy, M. R.; Lönngren, J.; Arnarp, J.; Haraldsson, M.; Lönn, H. *J. Biol. Chem.* **1983**, *258*, 199–202; (b) Lee, Y. C. *FASEB J.* **1992**, *6*, 3193–3200.
2. See following reviews and references cited therein (a) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578; (b) Andre, S.; Liu, B.; Gabius, H.-J.; Roy, R. *Org. Biomol. Chem.* **2003**, *1*, 3909–3916; (c) Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291–310; (d) Schengrund, C.-L. *Biochem. Pharmacol.* **2003**, *65*, 699–707.
3. (a) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839–7842; (b) Matsuoka, K.; Kurosawa, H.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Carbohydr. Res.* **2000**, *329*, 765–772; (c) Matsuoka, K.; Oka, H.; Koyama, T.; Esumi, Y.; Terunuma, D. *Tetrahedron Lett.* **2001**, *42*, 3327–3330; (d) Matsuoka, K.; Ohtawa, T.; Hinou, H.; Koyama, T.; Esumi, Y.; Nishimura, S.-I.; Hatano, K.; Terunuma, D. *Tetrahedron Lett.* **2003**, *44*, 3617–3620; (e) Mori, T.; Hatano, K.; Matsuoka, K.; Esumi, Y.; Toone, E. J.; Terunuma, D. *Tetrahedron* **2005**, *61*, 2751–2760; (f) Yamada, A.; Hatano, K.; Koyama, T.; Matsuoka, K.; Esumi, Y.; Terunuma, D. *Carbohydr. Res.* **2006**, *341*, 467–473; (g) Yamada, A.; Hatano, K.; Koyama, T.; Matsuoka, K.; Takahashi, N.; Hidari, K. I. P. J.; Suzuki, T.; Suzuki, Y.; Terunuma, D. *Bioorg. Med. Chem.* **2007**, *15*, 1606–1614; (h) Sakamoto, J.-I.; Koyama, T.; Miyamoto, D.; Yingsakmongkon, S.; Hidari, K. I. P. J.; Jampangern, W.; Suzuki, T.; Suzuki, Y.; Esumi, Y.; Hatano, K.; Terunuma, D.; Matsuoka, K. *Bioorg. Med. Chem. Lett* **2007**, *17*, 717–721.
4. (a) Nishikawa, K.; Matsuoka, K.; Kita, E.; Okabe, N.; Mizuguchi, M.; Hino, K.; Miyazawa, S.; Yamasaki, C.; Aoki, J.; Takashima, S.; Yamakawa, Y.; Nishijima, M.; Terunuma, D.; Kuzuhara, H.; Natori, T. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7669–7674; (b) Nishikawa, K.; Matsuoka, K.; Watanabe, M.; Igai, K.; Hino, K.; Hatano, K.; Yamada, A.; Abe, N.; Terunuma, D.; Kuzuhara, H.; Natori, Y. *J. Infect. Dis.* **2005**, *191*, 2097–2105.
5. Hatano, K.; Aizawa, H.; Yokota, H.; Yamada, A.; Esumi, Y.; Koshino, H.; Koyama, T.; Matsuoka, K.; Terunuma, D. *Tetrahedron Lett.* **2007**, *48*, 4365–4368.
6. Stöber, W.; Fink, A.; Bohn, E. *J. Colloid Interface Sci.* **1968**, *26*, 62–69.
7. For example: (a) Fu, W.; Yang, H.; Bala, H.; Liu, S.; Li, M.; Zou, G. *Mater. Chem. Phys.* **2006**, *100*, 246–250; (b) Zhu, M.; Qian, G.; Wang, Z.; Wang, M. *Mater. Chem. Phys.* **2006**, *100*, 333–336; (c) Aslam, M.; Fu, L.; Li, S.; Dravid, V. P. *J. Colloid Interface Sci.* **2005**, *290*, 444–449; (d) Liu, S.; Zhang, Z.; Wang, Y.; Wang, F.; Han, M.-Y. *Talanta* **2005**, *67*, 456–461.
8. (a) Hun, X.; Zhang, Z. *Biosens. Bioelectron.* **2007**, *22*, 2743–2748; (b) Gong, J.-L.; Liang, Y.; Huang, Y.; Chen, J.-W.; Jiang, J.-H.; Shen, G.-L.; Yu, R.-Q. *Biosens. Bioelectron.* **2007**, *22*, 1501–1507; (c) Hun, X.; Zhang, Z. *Food Chem.* **2007**, *105*, 1623–1629.
9. Qhobosheane, M.; Santra, S.; Zhang, P.; Tan, W. *Analyst* **2001**, *126*, 1274–1278.
10. (a) Faria, E. A.; Ramalho, H. F.; Marques, J. S.; Suarez, P. A. Z.; Prado, A. G. S. *Appl. Catal. A: Gen.* **2008**, *338*, 72–78; (b) Cámera, R.; Rimada, R.; Romanelli, G.; Autino, J. C.; Vázquez, P. *Catal. Today* **2008**, *133-135*, 822–827.
11. (a) Claesson, E. M.; Mehendale, N. C.; Gebbink, R. J. M. K.; Koten,G.-V.; Philipse, A. P. *J. Magn. Magn. Mater.* **2007**, *311*, 41–45; (b) Ocaña, M.; Andrés-Vergés, M.; Pozas, R.; Serna, C. J. *J. Colloid Interface Sci.* **2006**, *294*, 355–361.
12. Glucose-, maltose- and β-CD-conjugated silica particles were synthesized by click chemistry and its application to separation materials for hydrophilic interaction liquid chromatography were demonstrated. Guo, Z.; Lei, A.; Zhang, Y.; Xu, Q.; Xue, X.; Zhang, F.; Liang, X. *Chem. Commun.* **2007**, 2491–2493; Synthesis of galactose-functionalized silica particles and their application to identification of live liver cancer cells in a mixed cell system have been reported. Peng, J.; Wang, K.; Tan, W.; He, X.; He, C.; Wu, P.; Liu, F. *Talanta* **2007**, *71*, 833–840.
13. Lian, W.; Litherland, S. A.; Badrane, H.; Tan, W.; Wu, d.; Baker, H. V.; Guling, P. A.; Lim, D. V.; Jin, S. *Anal. Biochem.* **2004**, *334*, 135–144.
14. The size of silica particles **1** could be regulated by control of stirring speed and concentration of the solution. We have so far been able to synthesize of monodisperse silica particles **1** with sizes ranging from 150 to 700 nm. In this study, lager silica particles **1** were utilized for the fluorescence microscopic analysis of lectin binding
15. *Compound* **3a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.34 (s, 1H, H-4′), 5.17 (t, 1H, J = 9.2 Hz, H-3), 5.09 (dd, 1H, $J_{1',2'}$ = 8.4 Hz, $J_{2',3'}$ = 9.6 Hz, H-2′), 4.93–5.02 (d, 1H, $J_{2',3'}$ = 10.4 Hz, H-3′), 4.86 (t, 1H, J = 8.6 Hz, H-2), 4.42–4.47 (m, 3H, H-1, H-1′, H-6b), 4.05-4.15 (m, 3H, H-6′b, H-6′a, H-6a), 3.72-3.88 (m, 1H, H-5′, OCH_2*a*), 3.55 (s, 9H, Si–O–CH_3), 3.44–3.50 (m, 1H, OCH_2*b*), 1.96, 2.03, 2.05, 2.11, 2.14 (s × 7, 21H, O*Ac*), 1.53–1.66 (m, 4H, CH_2CH$_2$CH$_2$Si, OCH$_2$CH_2), 1.32–1.41 (m, 2H, CH_2CH$_2$Si), 0.59–0.63 (m, 2H, Si–CH_2).
16. *Compound* **4a**: IR (KBr) 3636 (w, sh), 3449 (w, br), 3206 (w, br), 2990 (w, sh), 1753 (m) 1213 (m, sh), 1096 (s), 469 (m) cm^{-1}; **4b**: IR (KBr): 3648 (w, sh), 3483 (w, br), 3073 (w, sh), 2963 (w, sh), 1734 (w) 1194 (m, sh), 1101 (s), 473 (m) cm^{-1}.
17. *Compound* **5**: IR (KBr) 3364 (m, br), 3233 (m, br), 2947 (w), 1088 (s), 463 (m) cm^{-1}.
18. Lotan, R.; Skutelsky, E.; Danon, D.; Sharon, N. *J. Biol. Chem.* **1975**, *250*, 8518–8523.