

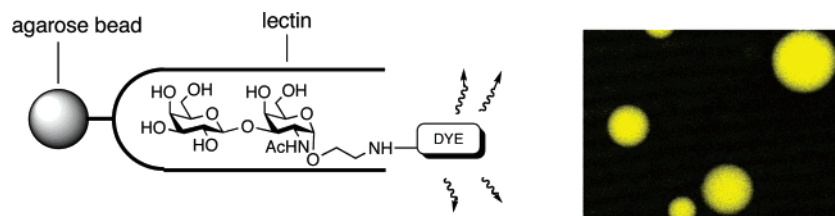
## Investigation of Nonspecific Effects of Different Dyes in the Screening of Labeled Carbohydrates against Immobilized Proteins

Meenakshi Dowlut, Dennis G. Hall,\* and Ole Hindsgaul\*,†

Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada

dennis.hall@ualberta.ca; hindsgaul@crc.dk

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Carbohydrates play an important role in life processes, and combinatorial chemistry can provide useful sources of thousands of synthetic carbohydrates as potential ligands for biological receptors. To accelerate the detection of positive hits arising from specific interactions between a carbohydrate and a protein, the use of fluorescent dyes can serve as a reliable detecting tool. A study of labeled carbohydrates to lectins conjugated to a solid-support shows that succinimidyl 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoate (NBD-X) dye provides by far the lowest level of nonspecific interaction with immobilized protein. This observation is in stark contrast with the commonly used labeling reagents constituted of charged and aromatic groups, for instance, FITC and TAMRA dyes.

## Introduction

Protein binding to cell-surface carbohydrates plays an important role in many biological processes that would encompass immune response, cancer cell metastasis, inflammation, and fertilization.<sup>1</sup> Many parasitic, bacterial, and viral pathogens recognize and adhere to a host's cell surface through protein–carbohydrate interactions. Because of the roles of protein–carbohydrate interactions<sup>2</sup> in human disease, carbohydrate-based therapeutics is an intense area of research. Consequently, there has been growing interest in the development of carbohydrate and glycopeptide libraries.<sup>3</sup> The use of biphasic assays based on the binding of soluble, tagged

ligands to an immobilized protein target is a popular approach. However, the problem of nonspecific interactions between the labeled ligand and the solid support or the receptor often makes the screening process troublesome, especially with fluorescently labeled substrates that have charged or hydrophobic functional groups. Yet, fluorescence labeling of carbohydrates is still an attractive recourse in the visualization of oligosaccharides<sup>4</sup> during a purification<sup>5</sup> process or the monitoring of a substrate for an enzyme.<sup>6</sup>

Fluorescent labels can prove to be useful tools as they have specific wavelengths of emission and they enable the monitoring of extremely low concentrations of the chemical species. This approach avoids the recourse to hazardous radioactive tags, another tool used in biological screening studies.

† Current address: Department of Chemistry, Calsberg Laboratory, Gamle Calsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark.

(1) (a) Fukuda, M.; Hindsgaul, O. *Molecular Glycobiology*; Oxford University Press: New York, 1994. (b) Dwek, D. *Chem. Rev.* **1996**, *96*, 683–720. (c) Hakomori, S.; Zhang, Y. *Chem. Biol.* **1997**, *4*, 97–104. (d) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674. (e) Rudd, P. M.; Elliott, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. *Science* **2001**, *291*, 2370–2376. (f) Alper, J. *Science* **2003**, *301*, 159–160. (g) Sharon, N.; Lis, H. *Glycobiology* **2004**, *14*, 53R–62R. (h) Cobb, B. A.; Kasper, D. L. *Eur. J. Immunol.* **2005**, *35*, 352–356.

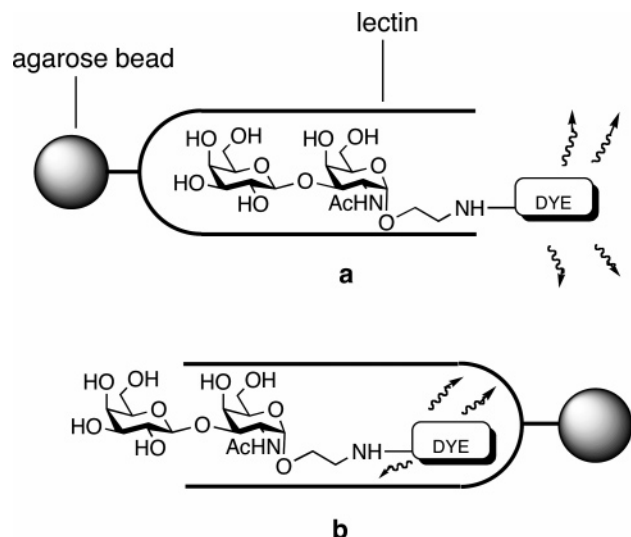
(2) (a) Liener, I. E.; Sharon, N.; Goldstein, I. J. *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Academic Press: New York, 1986. (b) Itzkowitz, S. H.; Bloom, E. J.; Kokal, W. A.; Modin, G.; Hakomori, S.; Kim, Y. S. *Cancer* **1990**, *66*, 1960–1966. (c) Hiraizumi, S.; Takasaki, S.; Ohuchi, N.; Harada, Y.; Nose, M.; Mori, S.; Kobata, A. *Jpn. J. Cancer Res.* **1992**, *83*, 1063–1072.

(3) (a) Haase, W. C.; Seeberger, P. H. *Curr. Org. Chem.* **2000**, *4*, 481–511. (b) Ramström, O.; Lehn, J. M. *ChemBioChem* **2000**, *1*, 41–48. (c) Seeberger, P. H.; Haase, W. C. *Chem. Rev.* **2000**, *100*, 4349–4393. (d) Arya, P.; Barkley, A.; Randell, K. D. *J. Comb. Chem.* **2002**, *4*, 193–198. (e) Lockhoff, O.; Frappa, I. *Comb. Chem. High Throughput Screening* **2002**, *5*, 361–372.

(4) (a) Hase, S.; Ikenaka, T.; Matsushima, Y. *J. Biochem.* **1981**, *90*, 1275–1279. (b) Hu G. F. *J. Chromatogr. A* **1995**, *705*, 89–103.

(5) (a) Jackson, P. *Methods Enzymol.* **1994**, *230*, 250–265. (b) Rudd, P. M.; Dwek, R. A. *Curr. Opin. Biotechnol.* **1997**, *8*, 488–497. (c) Anumula, K. R.; Du, P. *Anal. Biochem.* **1999**, *275*, 236–242.

(6) Shibaev, V. N.; Veselovsky, V. V.; Lozanova, A. V.; Maltsev, S. D.; Danilov, L. L.; Forsee, W. T.; Xing, J.; Cheong, H. C.; Jedrzejewski, M. *J. Bioorg. Med. Chem. Lett.* **2000**, *10*, 189–192.



**FIGURE 1.** (a) Favorable interactions between the agarose-supported PNA lectin and labeled disaccharide. (b) Unfavorable "nonspecific" interactions between the agarose-supported PNA lectin and labeled disaccharides **5a–c**.

Unfortunately, most organic dyes contain several aromatic rings that can induce weak "nonspecific" interactions with the aromatic units of the protein receptor. Consequently, positive hits are hard to distinguish in a definite way from negative ones, especially at high concentrations of the fluorescent species.

To avoid this type of nonspecific interaction, a suitable labeling reagent would be a small molecule with a minimum of intrinsic charges. Such a labeling reagent would also eliminate the need for using unusual assay conditions such as high salt and detergent-containing buffers that help reduce problems of nonspecificity. Upon inspecting potential new dyes recently developed for noncarbohydrate ligands, we anticipated that succinimidyl 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoate (NBD-X) could be an ideal labeling reagent. Unfortunately, studies comparing labeling dyes are rare. Herein, we describe a semiquantitative study comparing NBD-labeled carbohydrates<sup>7</sup> with carbohydrates labeled with other commonly used dyes.<sup>8</sup> This model study (Figures 1 and 2) can be of use not only to the field of carbohydrate chemistry but also in the screening of other molecules.

## Results and Discussion

**Design and Synthesis.** Our biphasic screening model consisted of two different lectins immobilized on agarose beads, namely PNA lectin (*Arachis hypogaea*) and Con A lectin (*Canavalia ensiformis*).<sup>9</sup> These lectins were chosen because of their commercial availability, low cost,

and strong binding affinities<sup>10,11</sup> (with a range of  $10^7$ – $10^8$  M<sup>-1</sup>) to saccharides. For instance, PNA lectin<sup>12</sup> binds to the T-antigen with  $K_a = 1 \times 10^7$  M<sup>-1</sup>, while Con A lectin<sup>11</sup> has a strong binding affinity to glycopeptides containing 3,6-di-*O*- $\alpha$ -D-mannopyranosyl- $\alpha$ -D-mannopyranoside ( $K_a = 2.4 \times 10^7$  M<sup>-1</sup>). It should be kept in mind, however, that these binding constants are for soluble oligosaccharides and that the binding constants of the same oligosaccharide structures linked to agarose beads may be substantially different due to both the linking arms used and to possible steric interference from the solid support. The solid support chosen for this study is beaded agarose, which is compatible with water, the ideal biologically relevant solvent for reactions involving deprotected carbohydrates. Thus, results with agarose can serve as a useful guide for other water-compatible supports such as Tentagel resins or aqueous biphasic assays such as ELISA.

Therefore, on the basis of the values of binding constants found in the literature, the chosen ligand for PNA lectin would be 2-aminoethyl ( $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\alpha$ -D-galactopyranoside (**5**) having the core structure of the T-antigen,<sup>13</sup> with an aminoethyl group to attach the fluorescent label. 2-Aminoethyl 3,6-di-*O*- $\alpha$ -D-mannopyranosyl- $\alpha$ -D-mannopyranoside (**10**) is the ligand of choice for Con A.

The ligand for the agarose–PNA conjugate was synthesized as follows (Scheme 1). The acetimidate groups were introduced at the anomeric and 3 positions of the glycoside by reaction with DBU and trichloroacetone.<sup>14,15</sup> Glycosylation with 2-azidoethanol<sup>16</sup> under Lewis acid catalysis gave only the  $\alpha$  anomer. This unusual and unexpected  $\alpha$ -selectivity was discovered while developing a facile synthesis of  $\alpha$ -GalNAc-serine<sup>15</sup> and was attributed to intramolecular neighboring group participation of the benzylidene oxygen atoms, which would prevent attack by the alcohol from the  $\beta$  face. The reaction is very sensitive to the protecting-group patterns, but it works well in this case. This operation was followed by the removal of the acetimidate group, in a subsequent step, to yield the donor **2**, which underwent a glycosylation reaction<sup>17</sup> with tetra-*O*-acetyl galactopyranosyl bromide **3** in a nitromethane/toluene mixture (1:1) at 70 °C, affording **4** with a yield of 61%. After removal of the benzylidene group and acetate groups, the final disaccharide product **5** was obtained.

We then proceeded to the synthesis of ligand **10** for Con A lectin (Scheme 2). The peracetylated mannose was converted into the bromide to serve as the acceptor in

(9) The abbreviations used are: PNA, peanut agglutinin; Con A, concanavalin A; GlcNAc, *N*-acetylglucosamine; PBS, saline phosphate buffer.

(10) It was reported by Baenziger's group that glycopeptides with association constants in the range of  $(4.5\text{--}25) \times 10^6$  M<sup>-1</sup> are retained by Concanavalin A-sepharose and those with association constants in the range of  $(0.3\text{--}4.0) \times 10^6$  M<sup>-1</sup> are not retained.

(11) Baenziger, J. U.; Fiete, D. *J. Biol. Chem.* **1979**, *254*, 2400–2407.

(12) Neurohr, K. J.; Young, N. M.; Mantsch, H. H. *J. Biol. Chem.* **1980**, *255*, 9205–9209.

(13) T-Antigen is the disaccharide  $\beta$ -Gal-(1 $\rightarrow$ 3)- $\alpha$ -D-Gal-NAc, which is O-linked to serine and threonine.

(14) Yule, J. E.; Wong, T. C.; Gandhi, S. S.; Qiu, D.; Riopel, M. A.; Koganty, R. R. *Tetrahedron Lett.* **1995**, *36*, 6839–6842.

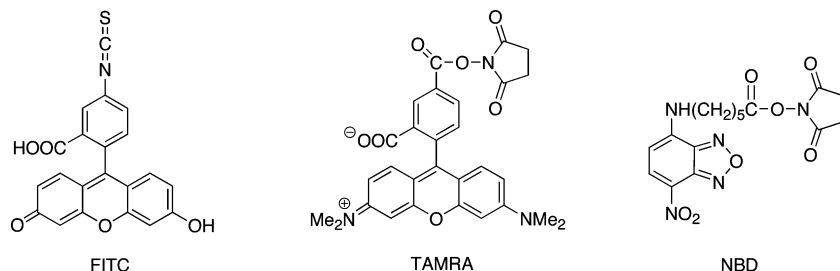
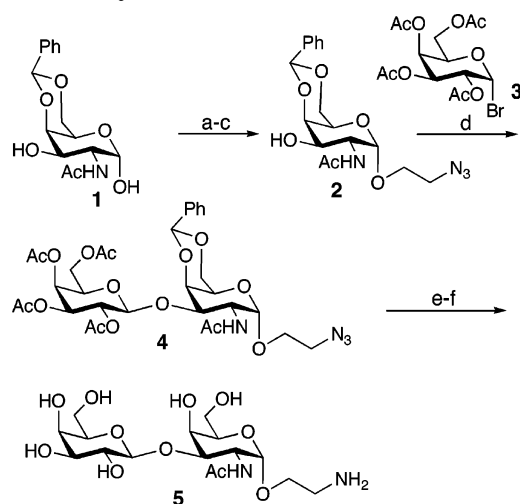
(15) Qiu, D.; Koganty, R. R. *Tetrahedron Lett.* **1997**, *38*, 961–964.

(16) (a) Chernyak, A. Y.; Sharma, G. V. M.; Kononov, L. O.; Krishna, P. R.; Levinsky, A. B.; Kochetkov, N. K. *Carbohydr. Res.* **1992**, *223*, 303–309. (b) Forster, M. O.; Fierz, H. E. *J. Chem. Soc.* **1908**, *93*, 1174–1179.

(17) Ratcliffe, R. M.; Baker, D. A.; Lemieux, R. U. *Carbohydr. Res.* **1981**, *93*, 35–41.

(7) (a) Chen, J.; Profit, A. A.; Prestwich, G. D. *J. Org. Chem.* **1996**, *61*, 6305–6312. (b) Honda, S.; Okeda, J.; Iwanaga, H.; Kawakami, S.; Taga, A.; Suzuki, S.; Imai, K. *Anal. Biochem.* **2000**, *286*, 99–111. (c) Sugimoto T.; Wada, Y.; Yamamura, S.; Ueda, M. *Tetrahedron* **2001**, *57*, 9817–9825. (d) Suzuki, S.; Honda, S. *Electrophoresis* **2003**, *24*, 3577–3582.

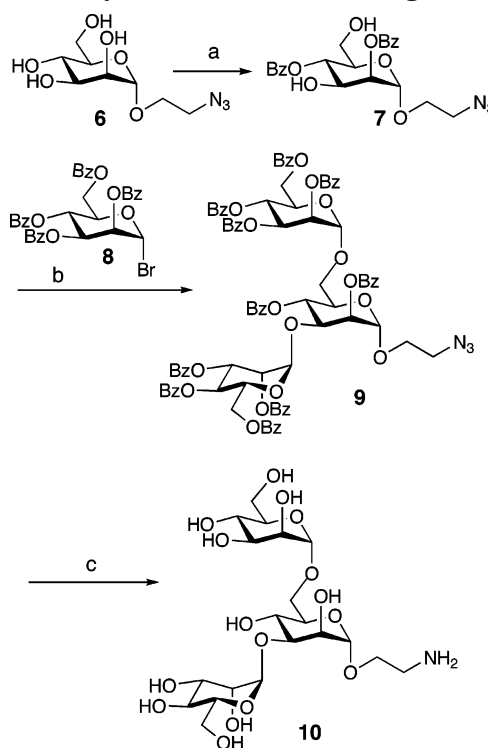
(8) (a) Zhang, Y.; Le X.; Dovichi, N. J.; Compston, C. A.; Palcic, M. M.; Diedrich, P.; Hindsgaul, O. *Anal. Biochem.* **1995**, *227*, 368–376. (b) Stoll, M. S.; Feizi, T.; Loveless, R. W.; Chai, W.; Lawson, A. M.; Yuen, C. T. *Eur. J. Biochem.* **2000**, *267*, 1795–1804. (c) Drummond, K. J.; Yates, E. A.; Turnbull, J. E. *Proteomics* **2001**, *1*, 304–310. (d) Arnosti, C. *J. Chromatogr. B* **2003**, *793*, 181–191.

**FIGURE 2.** Dye reagents used as labels for model carbohydrate molecules.**SCHEME 1. Synthesis of Disaccharide 5<sup>a</sup>**

<sup>a</sup> Reagents and conditions: (a)  $\text{Cl}_3\text{CCN}$ , DBU, DCM,  $-20\text{ }^\circ\text{C}$ , 1 h, 90%; (b)  $\text{HOCH}_2\text{CH}_2\text{N}_3$ ,  $\text{BF}_3\cdot\text{Et}_2\text{O}$ , molecular sieves (3 Å),  $-20\text{--}0\text{ }^\circ\text{C}$ , 20 min, 61%; (c) 80% AcOH, rt, 45 min, 95%; (d)  $\text{Hg}(\text{CN})_2$ , molecular sieves (4 Å),  $\text{CH}_3\text{NO}_2/\text{toluene}$  (1:1),  $70\text{ }^\circ\text{C}$ , 4–6 h, 92%; (e) 80% AcOH,  $50\text{ }^\circ\text{C}$ , 2 h, 67%; (f) (i) NaOMe, MeOH, rt, 30 min, (ii)  $\text{H}_2/\text{Pd}$ , quant.

the reaction with 2-azidoethanol and silver triflate.<sup>18,19</sup> The removal of the acetyl groups afforded **6**, which was then converted to the corresponding diortho ester<sup>20</sup> that could then be selectively opened under acidic conditions. This reaction produced **7**, the donor for the subsequent glycosylation reaction with 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-mannopyranosyl bromide **8**. The trisaccharide **9**, with a 3,6 linkage,<sup>21</sup> was subsequently obtained in a relatively good yield of 67%. The latter was then debenzoylated and hydrogenated over 10% Pd–C as catalyst to afford trisaccharide **10**, the ligand for Con A. The synthesis of the labeled carbohydrate molecules<sup>8a</sup> was performed by standard thiourea or amide bond formation under basic aqueous conditions (Scheme 3).

To investigate whether fluorescence is due solely to the specific binding between the dye conjugates of **5** and **10** with the lectin, both negative and positive experiments were planned. In the case of the PNA lectin, no binding should take place with the labeled trisaccharides of **10** (Con A ligand); hence, the latter was the negative control, whereas for Con A lectin, the conjugated disaccharides of **5** (PNA ligand) were used in the negative control assays.

**SCHEME 2. Synthesis of the Con A Ligand 10<sup>a</sup>**

<sup>a</sup> Reagents and conditions: (a)  $\text{C}_6\text{H}_5\text{C}(\text{OCH}_2\text{CH}_3)_3$ , CSA, TFA, rt, 2 h, 49%; (b)  $\text{AgOTf}$ , DCM,  $0\text{ }^\circ\text{C}$ , 1 h, 67%; (c) (i) NaOMe, MeOH, rt, 6 h, (ii)  $\text{H}_2/\text{Pd}$ , rt, 24 h, 89%.

To investigate the lowest concentration of the labeled carbohydrate for fluorescence to be detected in the confocal microscope, the lectins supported on agarose beads were incubated in different concentrations ( $1 \times 10^{-5}$  to  $1 \times 10^{-4}$  M) of the labeled saccharides in both the positive and negative controls. After the beads were washed with a fixed volume of buffer solution to remove any unbound conjugated carbohydrate, the average intensity of a lectin bead in the sample was taken from a plot of pixel intensity versus diameter of the bead (Figure 3). This was repeated for three different beads of the same size (200–250  $\mu\text{m}$ ) in the sample, thus giving an average of the bead intensity in the sample (Tables 1 and 2).

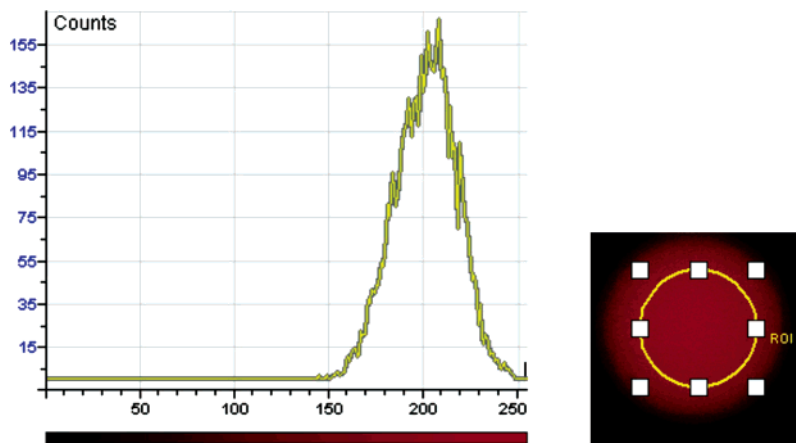
Due to the different intensities of emission and to avoid the problem of saturation of the pictures at higher fluorescence, different settings had to be chosen for the differently labeled glycosides bound to the agarose-supported lectins. The tables show the comparison of the positive and negative controls of the three dyes for both PNA and Con A lectins. As shown with its high level of background fluorescence in the negative controls,

(18) Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, C13–C16.

(19) Garegg, P. J.; Norberg, T. *Acta Chem. Scand., Ser. B* **1979**, *33*, 116–118.

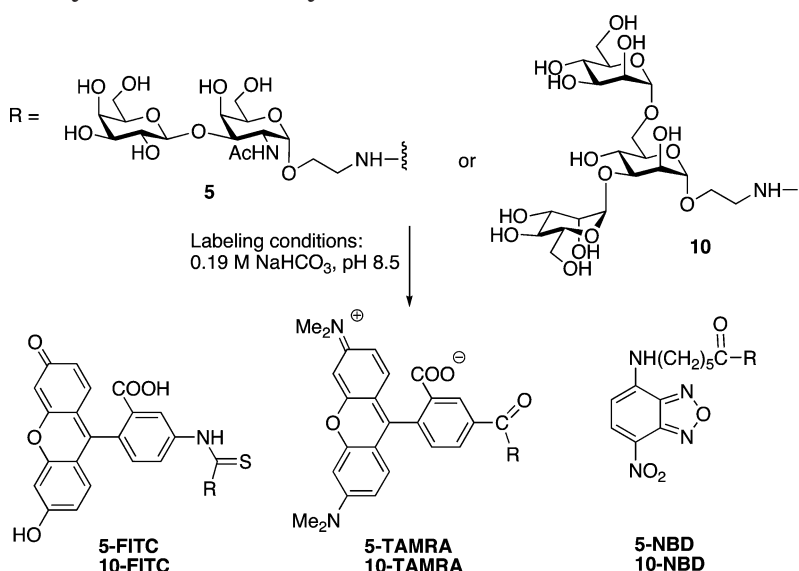
(20) Oscarson, S.; Tidén, A. K. *Carbohydr. Res.* **1993**, *247*, 323–328.

(21) The 3,6 linkage and the  $\alpha$ -anomers were confirmed by HMQC, HMBC, and APT analysis. See the Supporting Information.



**FIGURE 3.** Average pixel intensity of 8-bit images (0–255) versus the distance ( $\mu\text{m}$ ) across the bead (the example shown is a Con A-agarose bead incubated with  $10^{-4}$  M of the labeled trisaccharide **10-TAMRA**). See the Experimental Section for details on the interpretation of these graphs with respect to the data of Tables 1 and 2.

### SCHEME 3. Synthesis of Dye-Labeled Carbohydrates



**TABLE 1.** Average Intensities of the Agarose-Supported PNA Beads Treated with Different Concentrations of Labeled Saccharides 5 and 10

concn of labeled saccharides ( $10^{-5}$ M)	pixel intensity of beads					
	FITC		TAMRA		NBD	
	+ control <b>5-FITC</b>	– control <b>10-FITC</b>	+ control <b>5-TAMRA</b>	– control <b>10-TAMRA</b>	+ control <b>5-NBD</b>	– control <b>10-NBD</b>
10	250	45	236	81	106	0
5	48	0	105	79	13	0
1	10	0	57	75	8	0

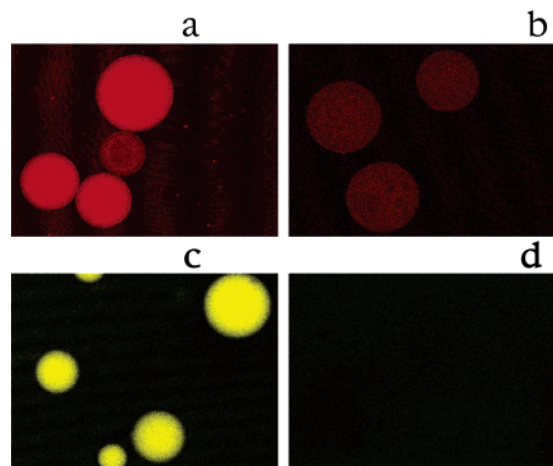
**TABLE 2.** Average Intensities of the Agarose-Supported Con A Beads Treated with Different Concentrations of Labeled Saccharides 5 and 10

concn of labeled saccharides ( $10^{-5}$ M)	pixel intensity of beads					
	FITC		TAMRA		NBD	
	+ control <b>10-FITC</b>	– control <b>5-FITC</b>	+ control <b>10-TAMRA</b>	– control <b>5-TAMRA</b>	+ control <b>10-NBD</b>	– control <b>5-NBD</b>
10	222	30	204	152	175	0
5	129	23	104	139	46	0
1	116	0	98	43	22	0

TAMRA displayed the greatest level of nonspecific interactions. However, compared to FITC and TAMRA

dyes, NBD-labeled carbohydrates showed by far the lowest level of nonspecific interactions as demonstrated





**FIGURE 4.** Fluorescence of the PNA-agarose beads treated with (a) + control in  $1.0 \times 10^{-5}$  M TAMRA-labeled disaccharide **5-TAMRA** (b) – control in  $1.0 \times 10^{-5}$  M TAMRA-labeled trisaccharide **10-TAMRA** (c) + control in  $1.0 \times 10^{-5}$  M NBD-labeled disaccharide **5-NBD** (d) – control in  $1.0 \times 10^{-5}$  M NBD-labeled trisaccharide **10-NBD**.

**TABLE 3. Qualitative Study of Binding Strength of Lectins toward NBD-Labeled Saccharides**

time taken to wash beads (s)	volume of buffer (mL)	lectins (pixel intensity of bead)	
		PNA	ConA
15	0.25	163	209
45	0.5	55	169
105	1.0	22	96
165	1.0	0	47
225	1.0	0	24
285	1.0	0	14
345	1.0	0	5
405	1.0	0	0

by the absence of fluorescence in the negative controls for both lectins (Figure 4).

### Binding Studies

To confirm the usefulness of the NBD dye for monitoring interactions between carbohydrates and proteins, dye-labeled glycosides ( $10 \times 10^{-5}$  M) were incubated with the agarose-supported lectins PNA and Con A. The beads became bright fluorescent yellow. The beads were then washed with equal volumes of buffer (PBS for PNA lectin and Tris buffer for Con A lectin) after equal intervals of times until the yellow color was completely washed out. For the PNA lectin incubated with the NBD-labeled disaccharide, 165 s was required for complete washing compared to 405 s (Table 3) in the case of the Con A lectin incubated with the NBD-labeled trisaccharide. This result agrees with the above-mentioned literature values of binding constants reported for the different lectins.

### Conclusions

Hydrophobic and other non-specific interactions often occur between lectins and dye molecules on labeled glycosides, which make specific interactions hard to ascertain. The predominantly large and polyaromatic labeling dyes traditionally used are rendered impractical

because of the difficulty in distinguishing positive hits from negative hits especially at high concentrations of the labeled ligands. This study confirms that NBD-X dye is an ideal labeling tool in the screening or monitoring of the binding interactions between promising ligands and immobilized protein receptors. Our studies suggest that NBD-labeled libraries of synthetic carbohydrates and other small molecules could be screened in a reliable fashion against bead-supported and surface-immobilized proteins with minimal interference from nonspecific dye interactions.

### Experimental Section

**General Procedure for Conjugating<sup>8a</sup> the Dye Molecules to the Saccharide.** To a stirring solution (pH = 8.5) of the saccharide in 0.185 M  $\text{NaHCO}_3$  (0.5 mL), the labeling agent in anhydrous DMF (0.5 mL) was added. Stirring was continued for 4–6 h at rt. The solvent was evaporated, and the crude material purified using reverse-phase chromatography on a C-18 column. Lyophilization of the required fractions gave the dye-labeled saccharides as fluffy solids.

**Fluorescein Conjugate of 2-Aminoethyl ( $\beta$ -D-Galactopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\alpha$ -D-galactopyranoside (5-FITC).** With the disaccharide **5** (20 mg, 0.47 mmol) and fluorescein isothiocyanate isomer I (4.2 mg, 0.011 mmol), the conjugated disaccharide (8.8 mg, 0.011 mmol) was obtained in quantitative yield as a yellow fluffy solid:  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.71 (br s, 1H, Ar), 7.55 (br d, 1H, Ar,  $J$  = 6 Hz), 7.38 (d, 1H, Ar,  $J$  = 8.5 Hz), 7.30–7.24 (m, 2H, ArH), 6.97 (d, 1H, Ar,  $J$  = 2 Hz), 6.70–6.64 (m, Ar, 3H), 4.96 (d, 1H, H-1,  $J_{1,2}$  = 3.0 Hz), 4.40–4.30 (m, 2H), 4.23 (br s, 1H), 4.04–3.30 (m, 14H), 2.30 (s, 3H,  $\text{COCH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ )  $\delta$  180.9, 174.9, 174.2, 167.5, 160.6, 159.2, 131.8, 130.3, 126.0, 125.2, 123.3, 112.8, 105.2, 103.9, 97.7, 78.1, 75.2, 72.8, 71.1, 70.8, 69.8, 69.0, 68.8, 66.6, 61.1, 48.8, 44.9, 22.5; HR-ESMS calcd for  $[\text{M} - \text{H}]^-$ ,  $\text{C}_{37}\text{H}_{40}\text{N}_3\text{O}_{16}\text{S}$ , 814.2135, found 814.2138.

Characterization data for compounds **5-TAMRA**, **5-NBD**, **10-FITC**, **10-TAMRA**, and **10-NBD** are found in the Supporting Information.

**General Procedure for Preparing On-Bead Assays.** A solution of the gel solution (20  $\mu\text{L}$ ) was placed in a polypropylene vessel, followed by 250  $\mu\text{L}$  of the saccharide solution ( $10 \times 10^{-5}$  M,  $5 \times 10^{-5}$  M,  $1 \times 10^{-5}$  M) made in the required buffer. The mixture was vortexed for 4 h at rt. The resin was then filtered and rinsed with the buffer solution (1 mL) for 30 s. Then, a volume (250  $\mu\text{L}$ ) of the buffer solution was added to the polypropylene vessel containing the lectin-agarose beads, which was vortexed for 15 s. The gel solution (10  $\mu\text{L}$ ) was then placed on the glass slide and covered for viewing under the microscope. Different filters were used for the dyes when measuring the intensities of the fluorescent beads. The values of Tables 1 and 2 are independent of the examples shown in Figure 3. They are average pixel intensities calculated by the confocal microscope's computer software.

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**Supporting Information Available:** Full experimental details and characterization of new compounds along with NMR spectral reproductions. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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