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Synthesis of methyl α- and β-N-dansyl-D-galactosaminides, probes for the combining sites of N-acetyl-D-galactosamine-specific lectins*

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The synthesis of the methyl α- and β-*N*-dansyl-D-galactosaminides is described using methyl α,β-2-azido-2-deoxy-D-galactopyranoside as starting material. This was reduced to the corresponding methyl α,β-2-amino-2-deoxy-D-galactopyranoside and then treated with dansyl chloride to yield a mixture of methyl α,β-*N*-dansyl-D-galactosaminides which was separated into individual anomeric forms by flash chromatography on silica gel. Methyl α-*N*-dansyl-D-galactosaminide was used as a fluorescent indicator ligand in continuous substitution titrations to determine the association constants of nonchromophoric carbohydrates with the *N*-acetyl-D-galactosamine specific lectin from *Erythrina corallodendron.*

Keywords: *N*-Acetylgalactosamine, lectins, dansyl derivatives, spectrofluorimetry, association constants

Abbreviations: ECorL, *Erythrina corallodendron* lectin; MeαGalNDns, methyl 2-deoxy-2-(5-dimethylamino-1-naphthalenesulfamido)-α-D-galactopyranoside; MeβGalNDns, methyl 2-deoxy-2-(5-dimethylamino-1-naphthalenesulfamido)-β-D-galactopyranoside.

Dansyl (5-dimethylamino-1-naphthalenesulfonyl) derivatives of mono- and oligosaccharides are valuable probes for the combining sites of carbohydrate specific proteins, since their fluorescence increases markedly upon binding to such proteins. Thus, dansyl-substituted glycosides of oligosaccharides derived from nigeran have been used in studies of antibodies [1], *N*-dansyl-D-glucosamine in studies of hexokinase [2] and *N*-dansyl-D-galactosamine in studies of the binding sites of several lectins specific for D-galactose and *N*-acetyl-D-galactosamine [3–5]. The latter probe has been prepared only on a milligram scale and has not been fully characterized. Moreover, the fact that it is a mixture of α and β anomers can limit its usefulness.

Here we describe the synthesis of the α and β anomers of methyl *N*-dansyl-D-galactosaminide. As starting material we used the α,β mixture of methyl 2-azido-2-deoxy-D-galactopyranosides (**1**) [6]. Reduction yielded the corresponding α,β mixture of methyl 2-amino-2-deoxy-D-galactopyranosides (**2**), which was treated with dansyl chloride. The desired products, methyl 2-deoxy-2-(5-dimethylamino-1-naphthalenesulfamido)-α-D-galactopyranoside (MeαGalNDns) (**3**) and methyl 2-deoxy-2-(5-dimethylamino-1-naphthalenesulfamido)-β-D-galactopyranoside (MeβGalNDns) (**4**) were fractionated by flash chromatography on silica gel. We also describe the use of MeαGalNDns as a fluorescent indicator ligand to determine the binding constants of simple carbohydrates with *Erythrina corallodendron* lectin (ECorL), the 3-dimensional structure of which, as a complex with lactose, has been solved [7]. A preliminary report on the synthetic work has been presented [8].

Materials and methods

All reagents were of the highest quality available. Melting points were determined with a Fisher-Johns apparatus, and are uncorrected. Microanalyses were performed at the Hebrew University of Jerusalem. ^{1}H-NMR spectra were recorded in ^2H_2O, unless otherwise specified, with a Bruker-WH-270 spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants in Hz. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Fast atom bombardment

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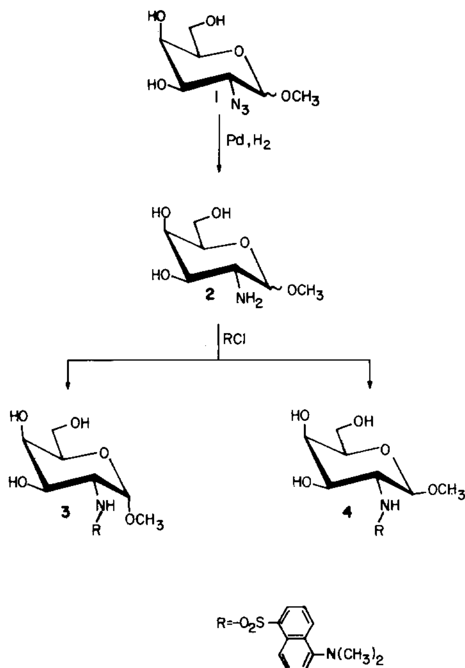

mass spectrometry was analysed in a Finnigan TSQ-70 mass spectrometer by the Mass Spectrometry Center at the Technion, Haifa. UV measurements were made with a Uvicon 810 spectrophotometer.

Fluorescence spectra were taken with an Aminco SPF-500 instrument and are uncorrected. Fluorescence titrations [9, 10] were made with a fluorimeter constructed in the laboratory using a chopped light source (stabilized 150 W halogen lamp), lock-in phase detection, excitation at 365 nm and emission at wavelengths larger than 450 nm (Schott KV 450 filter).

ECorL was prepared by affinity chromatography on lactose-Sepharose [11]. The concentration of the lectin was determined spectrophotometrically at 280 nm. The optical factor of 1.53 mg^{-1} cm^2 at 280 nm and M_r of 29,000 were used to calculate the molarity of the lectin solutions. The titration procedures and data analysis using the necessary concentrations at equilibrium have been described by De Boeck *et al.* [9, 10].

Results and discussion

Synthesis

Methyl 2-azido-2-deoxy-D-galactopyranoside (**1**) [6] (1.0 g, 4.29 mmol, α,β mixture at a 1:2 ratio as judged by its ^{1}H-NMR spectrum, in 30 ml anhydrous methanol) was hydrogenolysed over 10% Pd–H_2 (0.2 g) for 18 h at room temperature, and the suspension was filtered. The filtrate was evaporated *in vacuo* to give, after purification by flash chromatography [12] in chloroform–methanol, 1:1 by vol, syrupy **2** (0.6 g) R_F 0.51 in chloroform–methanol–ammonia, 5:4:1 by vol, which was used for the next step without further characterization.

To an aqueous solution of **2** (0.5 g, 2.42 mmol in 15 ml 0.2 M $NaHCO_3$, pH 8.5) was added dansyl chloride (Pierce Chemicals, USA; 1.3 g, 4.82 mmol in 5 ml acetone), and the mixture was stirred for 3 h at room temperature in the dark. The reaction was followed by thin layer chromatography (chloroform–methanol, 9:1 by vol); in this system, the starting material had an R_F of 0.00; when the reaction was complete, the mixture of two products, R_F 0.32 and 0.18, was poured onto ice and extracted several times with ethyl acetate. The organic layer was washed (water), dried ($MgSO_4$) and evaporated under reduced pressure. The solid product (0.7 g) was fractionated by flash chromatography, using chloroform–methanol, 9:1 by vol, as eluant. Two fractions were eluted and crystallized, each from water and ethyl acetate–petroleum ether, to give 0.3 g of the α anomer **3** (28.3% yield, R_F 0.32 in chloroform–methanol, 9:1 by vol, and 0.82 in dichloromethane–methanol, 7:3 by vol) and 0.14 g of the β anomer **4** (13.2% yield, R_F 0.18 in chloroform–methanol, 9:1 by vol).

Data for compound **3**: m.p. 194–196 °C; $[\alpha]_D^{21}$ −57.2 (*c* 1, methanol); $\varepsilon_{330} = 4.77 \times 10^3$. ^{1}H-NMR ($C^2H_3O^2H$): δ 8.55 (d, 1H, naphthyl); 8.30 (d, 2H, naphthyl); 7.68–7.50 (m, 2H, naphthyl); 7.21 (d, 1H, naphthyl); 3.94 (d, 1H, $J_{3,4}$ 2.73 Hz, H-4); 3.70 (d, 1H, $J_{1,2}$ 1.86 Hz, H-1a); 3.52 (dd, 1H, H-2); 2.88 (s, 6H, CH_3); 2.63 (s, 3H, OCH_3).
Analytical data. Calc. for $C_{19}H_{26}N_2O_7S$: C, 53.51; H, 6.14; N, 6.57; S, 7.52. Found: C, 53.55; H, 6.295; N, 6.5; S, 7.2.

Data for compound **4**: m.p. 129–131 °C; $[\alpha]_D^{21}$ −120 (*c* 1, methanol); $\varepsilon_{330} = 4.75 \times 10^3$; ^{1}H-NMR: δ 8.28–8.12 (m, 3H, naphthyl); 7.51–7.43 (m, 2H, naphthyl); 7.04 (d, 1H, naphthyl); 3.90 (d, 1H, $J_{3,4}$ 2.8 Hz, H-4); 3.82 (d, 1H, $J_{1,2}$ 8.33 Hz, H-1β); 3.64 (m, 3H); 3.46 (t, 1H, H-5); 3.18 (t, 1H, $J_{1,2} = J_{2,3} = 8.33$ Hz, H-2); 2.60 (s, 6H, CH_3); 2.13 (s, 3H, OCH_3).
Analytical data. Calc. for $C_{19}H_{26}N_2O_7S$: C, 53.51; H, 6.14; N, 6.57; S, 7.52. Found: C, 53.48; H, 6.44; N, 6.23; S, 7.79.

The molecular weight of the products was confirmed by fast atom bombardment mass spectrometry: for compound **3**, $[M + H]^+$ at *m/z* 427.1 and $[M - H]^-$ at *m/z* 425.6; for compound **4**, $[M + H]^+$ at *m/z* 427.0 and $[M - H]^-$ at *m/z* 425.6. The ultraviolet absorption spectra were similar, with a slight difference in the absorption maxima (328.2 nm for MeαGalNDns and 325.7 nm for MeβGalNDns) that was confirmed by the excitation spectra of free MeαGalNDns and MeβGalNDns. The concentration of these compounds was determined spectrophotometrically at their absorption maximum using the value $\varepsilon = 4.8 \times 10^3\ M^{-1}$ [3], which is almost identical with the experimentally determined one.

Fluorescence measurements

The fluorescence intensity of MeαGalNDns and MeβGalNDns was enhanced in each case by more than three-fold upon binding to ECorL; data for the α-glycoside are shown in Fig. 1a. There is also a blue shift in the fluorescence emission maximum from about 550 nm to

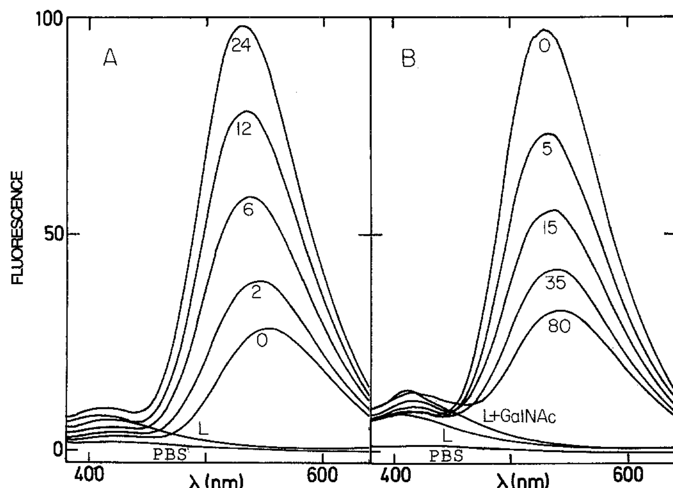

Figure 1. (a) Increase in fluorescence intensity and blue shift of the fluorescence emission maximum of MeαGalNDns (2.2 mM, 0.4 cm × 1 cm × 4.5 cm cuvette, 820 μl phosphate buffered saline, 22.5 °C, spectrum labelled 0) upon addition of ECorL (310 μM) in successive aliquots resulting in totally added volumes of 2, 6, 12 and 24 μl. (b) Effect on the fluorescence emission spectrum of a mixture (844 μl) of 2.16 μM MeαGalNDns and 8.8 μM ECorL (spectrum 0) upon addition of *N*-acetyl-D-galactosamine (210 mM) in final total volumes of 5, 15, 35 and 80 μl. The top curve (0) in panel b corresponds to the top curve (24) in panel a. Excitation was at 365 nm. PBS, buffer alone; L, lectin (8.8 μM) in buffer; L + GalNAc, lectin (8.04 μM) and GalNAc (13.8 mM) in buffer.

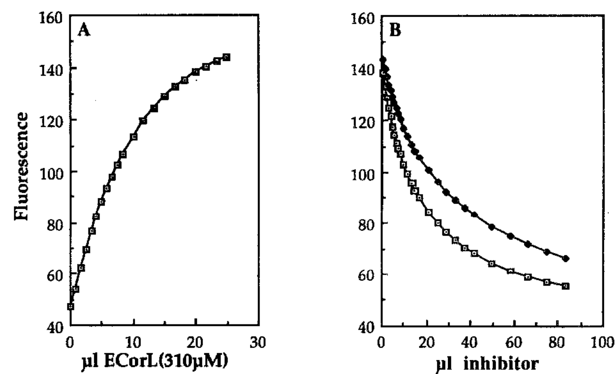

Figure 2. (a) Direct fluorimetric titration of MeαGalNDns (0.7 cm × 0.7 cm × 4.5 cm cuvette, 22.5 °C, 821 μl; 2.2 μM) with ECorL (310 μM). (b) Displacement titration of MeαGalNDns from its complex with the lectin by 9.9 mM *N*-acetyllactosamine (◆) or 210 mM *N*-acetyl-D-galactosamine (□). The first data point of the substitution titration corresponds to the last data point of the direct titration. Measurements of fluorescence were at 525 nm.

530 nm, as shown for MeαGalNDns (Fig. 1a). These fluorescent changes were carbohydrate specific, since they could be almost completely reversed upon addition of inhibitory sugars (Fig. 1b). Similar results were obtained with Meβ GalNDns (data not shown).

Association constants for the fluorescent ligands were determined from direct titration data with ECorL (Fig. 2a), according to [4]. MeαGalNDns and MeβGalNDns were found to have association constants of $4.6 \times 10^5\ \mathrm{M}^{-1}$ and $1.4 \times 10^5\ \mathrm{M}^{-1}$, respectively.

Binding of nonfluorescent, inhibitory carbohydrates was studied by monitoring their ability to displace MeαGalNDns from its complex with ECorL in substitution titrations (Fig. 2). MeαGalNDns was chosen as the fluorescent indicator ligand for substitution titration. Association constants for methyl α-D-galactopyranoside, methyl β-D-galactopyranoside, *N*-acetyl-D-galactosamine, lactose and *N*-acetyllactosamine were determined from displacement titration data giving values of $7.3 \times 10^2\ \mathrm{M}^{-1}$, $3.5 \times 10^2\ \mathrm{M}^{-1}$, $9.3 \times 10^2\ \mathrm{M}^{-1}$, $1.15 \times 10^3\ \mathrm{M}^{-1}$ and $7.1 \times 10^3\ \mathrm{M}^{-1}$, respectively.

Based upon the K_a values, MeαGalNDns binds to the lectin three times better than MeβGalNDns, and methyl α-D-galactopyranoside binds twice as well as methyl β-D-galactopyranoside. The lectin, therefore, has a slight preference for α-anomers.

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