Carbohydrate Research, 63 (1978) 99–103

5-(DIMETHYLAMINO)-1-NAPHTHALENESULFONYL (DANSYL) DERIVATIVES OF SACCHARIDES

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(Received August 19th, 1977; accepted for publication, September 1st, 1977)

ABSTRACT

6′-*O*-Dansylgentiobiose, 6-*O*-(6-*O*-dansyl-β-D-galactopyranosyl)-D-galactose, and methyl 6-*O*-dansyl-β-D-galactopyranoside have been prepared.

INTRODUCTION

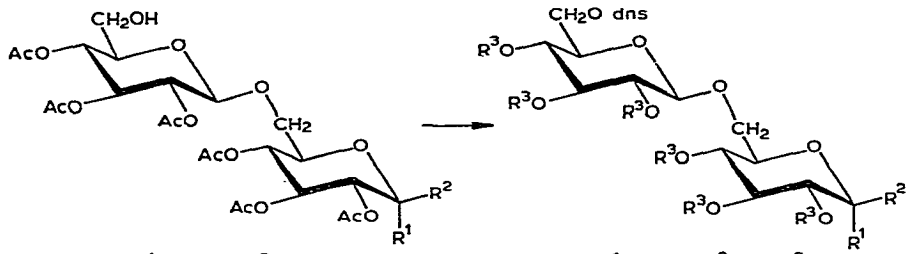
A large number of immunoglobulins having specificity for carbohydrate antigens show ligand-induced, fluorescence changes[1] (LIFC) that offer a very sensitive probe for studying the interaction of haptens and immunoglobulins quantitatively. Proteins not showing LIFC would not, however, be open to this kind of study, unless the ligand itself could be made fluorescent. Binding of the ligand to the proteins may then cause a change (or shift) in the fluorescence observed. Thus, it is useful to have available carbohydrate haptens bearing a fluorescent group. We have therefore prepared a number of 5-(dimethylamino)-1-naphthalenesulfonic esters of saccharides.

There is a report in the literature, by Biddle and Pardhan[2], on some *N*-dansyl derivatives of aminated polysaccharides. These workers prepared a carbonyl derivative of the polysaccharide by oxidation with potassium dichromate, converted it into the oxime by treatment with hydroxylamine, and obtained an amino derivative by subsequent reduction; the amine was then treated with dansyl chloride. J. D. Reeves *et al.*[3] prepared the *N*-dansyl derivative of 2-aminoethyl 1-thio-β-D-galactoside. Rather than derivatize saccharides by routes similar to these, we were interested in the direct reaction of dansyl chloride with hydroxyl groups. Wada and co-workers reported the direct dansylation of the 2-hydroxyl group in some D-ribonucleotides[4], but found D-ribose itself unreactive under the conditions used. We now report the synthesis of some dansyl derivatives of saccharides by a simple and direct method.

RESULTS AND DISCUSSION

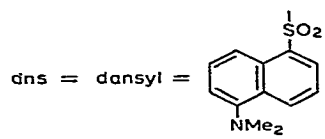
We have developed a way to derivatize saccharides directly to yield dansyl derivatives, namely, reaction of the substrate with dansyl chloride in pyridine. This procedure is selective for primary hydroxyl groups.

6′-*O*-Tritylgentiobiose heptaacetate (1 mol) was detritylated with one mol of hydrogen bromide[5] to yield two fractions; pure 1,2,3,4,2′,3′,4′-hepta-*O*-acetyl-α-gentiobiose (**1**), and the impure β anomer (**2**). Treatment of the separate fractions with dansyl chloride in pyridine yielded the corresponding, crystalline 6′-*O*-dansyl-gentiobiose α- and β-heptaacetates (**3** and **4**). Deacetylation of **4** gave crystalline 6′-*O*-dansylgentiobiose (**5**).

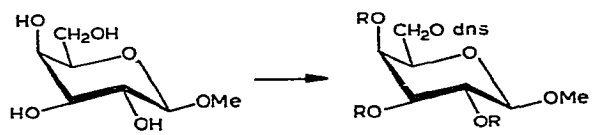


1 R^1 = OAc, R^2 = H
2 R^1 = H, R^2 = OAc

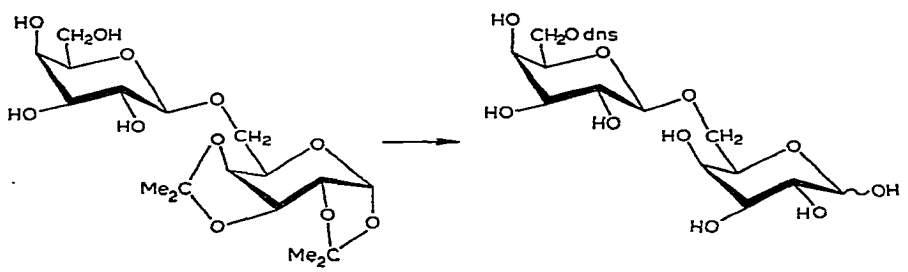
3 R^1 = OAc, R^2 = H, R^3 = Ac
4 R^1 = H, R^2 = OAc, R^3 = Ac
5 R^1, R^2 = H, OH, R^3 = H



dns = dansyl =



6 R = H
7 R = Ac



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Next we wished to ascertain whether a saccharide could be directly dansylated at the primary hydroxyl group without prior protection of the other hydroxyl groups. Methyl β-D-galactopyranoside (1 mol) was treated with one mol of dansyl chloride in pyridine at low temperature to produce a mixture consisting mostly of mono- (together with some di-) dansyl derivative. The mono-*O*-dansyl derivative (**6**) was amorphous, but periodate oxidation showed it to be the 6-*O*-substituted compound. It yielded a crystalline triacetate (**7**). Thus, it appears that dansylation is reasonably

selective for primary hydroxyl groups under the conditions specified, and saccharides undergo selective derivatization without prior protection of the other potentially available hydroxyl groups. This was confirmed by the preparation of 6-*O*-(6-*O*-dansyl-β-D-galactopyranosyl)-D-galactose (**8**), a compound that could be obtained by selective dansylation of 6-*O*-β-D-galactopyranosyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[6] followed by removal of the isopropylidene groups with aqueous trifluoroacetic acid.

EXPERIMENTAL

General methods. — The progress of all reactions was monitored by t.l.c. on silica gel GF (250 μm, Analtech Inc.), and components were detected by u.v. light, or charring by hot, 10% sulfuric acid, or both. Products were separated on precoated plates (20 × 20 cm) of silica gel 60 F-254 (2 mm; Merck, Darmstadt, Germany), or silica gel GF (1 mm, Analtech Inc.). Specific rotations were measured with a Perkin–Elmer 141 polarimeter, and n.m.r. spectra were recorded with a Varian HA-100 instrument at 100 MHz.

Dansyl chloride [5-(dimethylamino)-1-naphthalenesulfonyl chloride] was obtained from Aldrich Chemical Co. It was purified by extraction with warm hexane; the residue was removed, and the solution yielded material having m.p. 66–69°. All reactions were protected from light.

*1,2,3,4,2',3',4'-Hepta-*O*-acetyl-6'-*O*-dansyl-α- and -β-gentiobiose* (**3** and **4**). — Detritylation of an anomeric mixture of 6'-*O*-tritylgentiobiose heptaacetate (1.8 g) with hydrogen bromide[5] in acetic acid yielded a product that crystallized in needles from dichloromethane overnight. The anomeric mixture of products **1** and **2** (0.728 g) was filtered off, and washed with 4:1 hexane–dichloromethane; it had m.p. 120–175°, $[\alpha]_D^{20}$ +4° (*c* 1, chloroform). A sample dried overnight in a vacuum oven at 100° had m.p. 177–185°. Extraction of the concentrated mother liquor with warm dichloromethane yielded a small amount (68 mg) of pure 1,2,3,4,2',3',4'-hepta-*O*-acetyl-α-gentiobiose (**1**). Recrystallization from hot, aqueous ethanol gave clusters of prismatic needles; m.p. 172–174°, $[\alpha]_D^{20}$ +58.7° (*c* 0.8, chloroform). Comparison (t.l.c.) of **1** with the mixture showed no appreciable difference in mobilities in various systems.

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: 48.76; H, 5.80.

Compound **1** (22 mg) in pyridine (200 mL) was stirred with crushed molecular sieve (12 mg) and dansyl chloride (28 mg) for 5 h. The slurry was mixed with dichloromethane, and the suspension filtered to remove the molecular sieve, which was then washed with dichloromethane. The filtrate was evaporated, the residue was extracted with acetone, and the extract was evaporated, to give a crystalline mass. Recrystallization from acetone–water yielded yellow needles of **3** (19 mg, 63.3% yield). After a second recrystallization, from hot methanol, **3** had m.p. 171–173°, $[\alpha]_D^{20}$ +51.2° (*c* 0.67, chloroform). In a second experiment, using no molecular sieve, the results were the same. In this case, a few drops of water were added at the completion of the reaction, to dissolve the pyridinium chloride and promote crystallization.

Anal. Calc. for $C_{38}H_{47}NO_{20}S$: C, 52.47; H, 5.45; N, 1.61. Found: C, 52.64; H, 5.43; N, 1.48.

A portion (370 mg) of the mixture of **1** and **2** dissolved in pyridine (3 mL) was treated with dansyl chloride (470 mg) overnight at room temperature. The resulting solution was chilled in an ice bath, and diluted with water, yielding a crystalline product that was collected by filtration, and washed successively with cold water and methanol (yield 375 mg). After two recrystallizations from hot methanol (fine, yellowish needles), the β anomer **4** (163 mg) had m.p. 186–188° and $[\alpha]_D^{20}$ +10.5° (*c* 1.0, chloroform).

Anal. Calc. for $C_{38}H_{47}NO_{20}S$: C, 52.47; H, 5.45; N, 1.61; S, 3.69. Found: C, 52.52; H, 5.74; N, 1.50; S, 3.57.

Compounds **3** and **4** cochromatographed (t.l.c.) in several different systems. Comparative n.m.r. studies showed the equatorial, anomeric proton of **3** farther downfield (δ 6.32), as expected, with $J_{1,2}$ 3.6 Hz. The anomeric-proton signal (δ 5.68) of **4** showed a larger value of $J_{1,2}$ (7.6 Hz) for the *trans*-diaxial protons on C-1 and C-2.

6'-O-Dansylgentiobiose (**5**). — Compound **4** (90 mg) in methanol (3 mL) was treated with 0.1M NaOMe (0.8 mL) for 2.5 h at room temperature, and the mixture kept overnight in a refrigerator. T.l.c. then showed that deacetylation had proceeded without decomposition. The product crystallized from 95% ethanol (27 mg; 45.4% yield). Recrystallization from ethanol gave the monohydrate of **5** (yellowish needles, m.p. 145–150°). Periodate oxidation of a sample (1 mg) of **5** by the method of Avigad[7] resulted in the consumption of 5.6 mol of oxidant per mol. By this method, sucrose (as a standard) required 3.3 mol per mol.

Anal. Calc. for $C_{24}H_{33}NO_{13}S \cdot H_2O$: C, 48.56; H, 5.94; N, 2.36; S, 5.40. Found: C, 48.84; H, 6.00; N, 2.31; S, 5.25. Melted cautiously in high vacuum at 150°, Calc. for $C_{24}H_{33}NO_{13}S$: C, 50.08; H, 5.78; N, 2.43. Found: C, 50.38; H, 5.97; N, 2.55.

Methyl 6-O-dansyl-β-D-galactopyranoside (**6**). — To a stirred, chilled solution of methyl β-D-galactopyranoside (1 g) in pyridine (10 mL) containing molecular sieve (0.5 g) was added a solution of dansyl chloride (1.4 g) in acetone (6 mL), dropwise, during 30 min. After 2 h, the mixture was cooled, and kept overnight in a refrigerator. The molecular sieve was removed by filtration, and washed with pyridine, and the filtrate and wash were combined and concentrated *in vacuo* to a thin syrup. Dilution of the syrup with water yielded a gum; the water was decanted, the gum was dissolved in dichloromethane, the solution was dried with sodium sulfate, the suspension was filtered, and the filtrate was evaporated *in vacuo* to a yellowish foam (1.5 g). Two major, highly fluorescent, yellow bands were isolated when the product was separated on five preparative-t.l.c. plates (2 mm thickness) in 8:1 dichloromethane–methanol. Based on the n.m.r. spectrum, the more-mobile didansylate (0.1 g) showed a 4:1 ratio of -NMe_2 to -OMe protons, whereas the monodansylate **6** (0.7 g) showed a 2:1 ratio. Compound **6** had $[\alpha]_D^{20}$ +8.9° (*c* 1.0, methanol), and consumed 2.05 mol of periodate[8] per mol, yielding 1.09 mol of formic acid (3 and 5 h).

Methyl 2,3,4-tri-O-acetyl-6-O-dansyl-β-D-galactopyranoside (**7**). — A portion

(110 mg) of **6** was rechromatographed, and then acetylated overnight with acetic anhydride and pyridine. The gummy product was dissolved in warm ethanol, and crystallized readily in the cold. Recrystallization from chloroform–hexane yielded yellowish, hexagonal prisms; 120 mg (84.2% yield), m.p. 141–142.5°, $[\alpha]_D^{20}$ −1.3° (*c* 1.0, chloroform).

Anal. Calc. for $C_{25}H_{31}NO_{11}S$: C, 54.24; H, 5.65; N, 2.53; S, 5.79. Found: C, 54.30; H, 5.59; N, 2.44; S, 5.49.

The triacetate **7** was completely deacetylated in methanol by a catalytic amount of sodium methoxide after 4 h in the cold, but some decomposition was noted by t.l.c.

6-O-(6-O-Dansyl-β-D-galactopyranosyl)-D-galactopyranose (**8**). — A solution of 6-*O*-β-D-galactopyranosyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[6] (25 mg) in pyridine (0.4 mL), containing crushed molecular sieve (20 mg), was chilled to 5°, and dansyl chloride (20 mg) was added in portions while stirring. After 2 h, more dansylating reagent (50 mg) was added; after a total reaction-time of 3.5 h, the mixture was filtered to remove the molecular sieve, which was successively washed thoroughly with methanol and acetone. The filtrate and wash were combined, diluted with water, and evaporated *in vacuo* to a residue which was extracted with methanol. The extract was concentrated, and resolved on one preparative plate (1 mm) in 5:1 dichloromethane–methanol, to yield the monodansyl derivative (20 mg); $[\alpha]_D^{20}$ −50.6° (*c* 1.5, chloroform). As the dansyl derivative is insoluble in water, the periodate oxidation, according to the Fleury–Lange method[8], was conducted in an aqueous solution containing ~30% of methanol. The compound consumed 1.8 mol of oxidant per mol and produced 0.8 mol of formic acid.

Anal. Calc. for $C_{30}H_{41}NO_{13}S$: C, 54.95; H, 6.30; N, 2.14. Found: C, 55.15; H, 6.71; N, 2.02.

The amorphous dansylate (50 mg) was treated for 5 min at 25° with trifluoroacetic acid containing 10% of water (1.7 mL) to cleave the two isopropylidene groups. The solution was evaporated *in vacuo*, and 3 portions of ether were added to, and evaporated from, the mixture. The resulting product was purified by chromatography (1 mm plate), using 4:1 ethyl acetate–methanol. Compound **8** (30 mg) was obtained as a yellowish, hydrated powder, $[\alpha]_D^{20}$ +6.6° (*c* 0.6, methanol).

Anal. Calc. for $C_{24}H_{33}NO_{13}S \cdot H_2O$: C, 48.56; H, 5.94; N, 2.37; S, 5.40. Found: C, 48.35; H, 6.02; N, 2.39; S, 5.11.

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