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SYNTHESIS OF PHOTOAFFINITY LABELING DERIVATIVES OF D-GLUCOSE AND D-GALACTOSE

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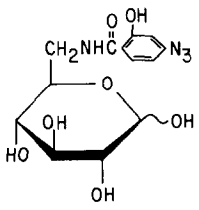
ABSTRACT

Two new photoaffinity reagents having photoreactive groups attached to C-6 of D-galactose have been prepared. 6-*N*-(4-Azido-2-hydroxybenzoyl)-D-glucopyranosylamine and 6-*N*-(4-azido-2-hydroxybenzoyl)-D-galactopyranosylamine were synthesized by acylation of the protected amino sugars with 4-azidosalicoyl chloride or by treating the amine with 4-azidosalicylic acid. Subsequent iodination of the aromatic ring yields the diiodo derivative. Deprotection yields the sugar derivatized at C-6 by the diiodinated photoaffinity reagent. Photoaffinity reagents having high specific activity may be prepared by this procedure.

INTRODUCTION

Our interest in the mechanism of sugar transport in human erythrocytes has led to synthesis of a series of alkyl glucosides containing photolabile side-chains[1]. In view of the proposed asymmetry of binding of the membrane surfaces[2] it was of interest to extend our studies to include sugars having photolabile groups attached to C-6 of the framework. To this end we have devised syntheses of 6-(4-azido-2-hydroxybenzamido)-6-deoxy-D-glucopyranose(**1**) and 6-(4-azido-2-hydroxybenzamido)-6-deoxy-D-galactopyranose (**2**). The role of the azidophenyl ring in these compounds was twofold: to serve as the locus of the photolabile azide function and



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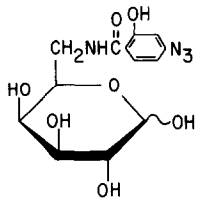

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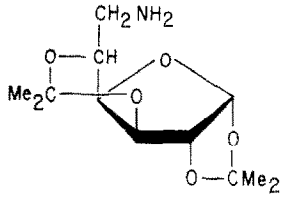
to serve as a site for incorporation of radioactive iodine (^{125}I). This label would allow convenient assay of the metabolites in physiological studies

RESULTS AND DISCUSSION

The key synthetic step involved acylation of the protected amines **3** (ref. 3) and **4** (ref. 4) with either 4-azidosalicoyl chloride (Method A) or with 4-azidosalicylic acid–*N*,*N*′-dicyclohexylcarbodiimide (Method B)[6]

Method A. — Treatment of 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (**3**) with 4-azidosalicoyl chloride, in the presence of triethylamine in dichloromethane, gave 6-(4-azido-2-hydroxybenzamido)-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranosyle (**5**). Similarly, treatment of 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**6**). The structures of **5** and **6** are supported by elemental analyses, mass spectra (molecular ion at *m/z* 420; 100%); n.m.r. spectra (24 protons attributable to different groups), and i.r. spectra (presence of azide and carbonyl absorption).



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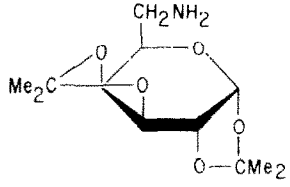

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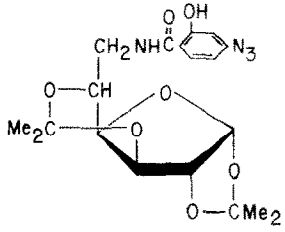

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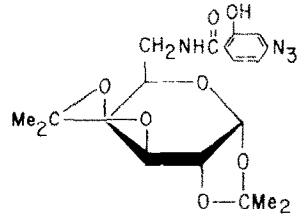

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Method B. — Compounds **5** and **6** could also be prepared by treating the amino sugars **3** and **4*** with 4-azidosalicylic acid[5] in the presence of *N*,*N*′-dicyclohexylcarbodiimide[6] in dichloromethane. The condensation products (**5**) obtained by either method, A or B, were identical in every respect. However, compound **6** obtained by method A melted at 101–103° whereas the product obtained by method B melted at 150–151°. On the basis of following evidence it was concluded that compound **6** exists in two dimorphic forms. Both forms gave acceptable elemental analyses consistent with the molecular formula $C_{19}H_{24}N_4O_7$ and had superposable solution-phase n.m.r. spectra. Although the i.r. spectra (Nujol) for

*Compounds **3** and **4** by the borohydride reduction method except that longer reaction times were required, generally 35–40 h

both compounds showed absorption bands attributable to NH, N_3, and C=O groupings, they differed in the fingerprint region.

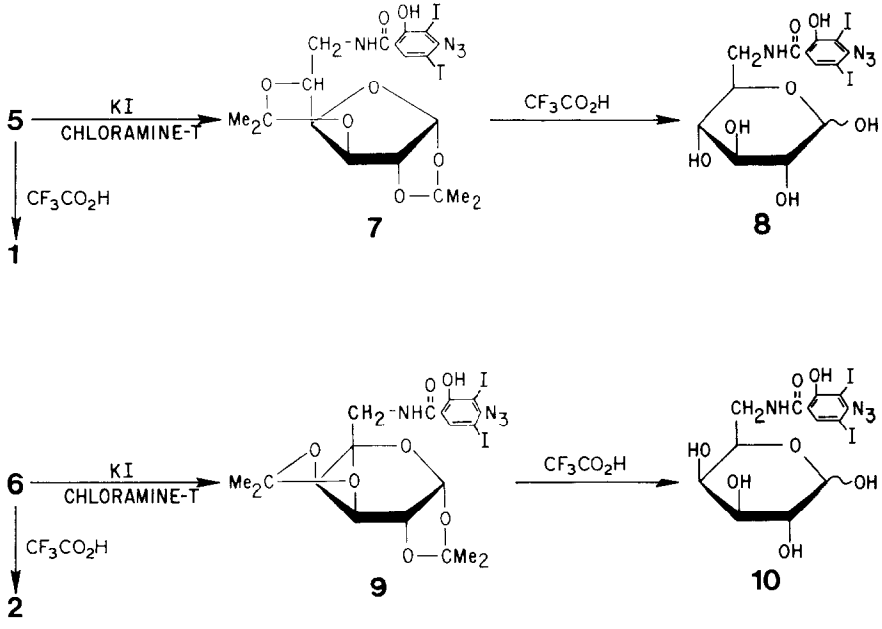
The mixed melting-point of the two dimorphic forms was at 150–151°, namely the m.p. of the higher-melting, dimorph. The low-melting form was converted into the high-melting one when crystals of the high-melting form were added to a solution of the low-melting form during crystallization, or when the low-melting form was melted and allowed to solidify. The i.r. spectrum of the low-melting form, after conversion into the high-melting form, was superposable on the i.r. spectrum of the high-melting form originally obtained by Method B.

Method B gave higher yields of **5** and **6** as compared with Method A, was a simpler procedure, and gave a more-pure product.

The mild conditions developed by Hunter and Greenwood[7] for incorporation of radioactive iodine into surface proteins were used to iodinate compounds **5** and **6**. Under carefully controlled conditions, these compounds reacted with potassium iodide in the presence of chloramine-T to give the diiodo derivatives **7** and **8**. Consistent with these structures are the mass spectra, which showed molecular ions at *m/z* 672 and the n.m.r. spectra which showed one-proton singlets in the aromatic regions. The deblocked sugars **8** and **10** were obtained by hydrolysis of the acetals with trifluoroacetic acid. Similarly **5** and **6** were hydrolyzed to give **1** and **2**.

When 5 mCi of radioactive ^{125}I was used for idination in the presence of unlabeled potassium iodide, 16×10^6 c.p.m. of radioactivity was incorporated into the deprotected compound.

One of the most powerful features of the photoaffinity-labeling technique is its ability, upon light activation, to generate an intermediate of such high reactivity that covalent attachment to a ligand binding-site may be effected, even when there is no nucleophilic reactive group at the site (For a review, see ref. 8). For this



reason the compounds prepared may be of interest to investigators in other fields and are likely to react with a wide variety of receptors. It is probable, for instance, that the photoactivated glucose and galactose compounds described will react with the active sites of disaccharidases, glucosylating enzymes, and enzymes involved in sugar metabolism, many of which possess high specificity towards these sugars. Another application may be the interaction with membrane-surface receptors, such as the D-galactose-specific recognition system of mammalian liver[9,10] or glycoprotein-specific, lectin-like receptor, as well as with plant lectins, all of which may be investigated by photoaffinity-labeling techniques. We have applied these compounds as inhibitors of sugar transport in erythrocytes. Preliminary binding-studies to ghost preparations will be reported separately.

EXPERIMENTAL

Spectra. — Nuclear magnetic resonance data were obtained with a Varian Model T-60 spectrometer for solutions in $CDCl_3$ or Me_2SO-d_6, with tetramethylsilane as internal standard. I.r. spectra were recorded with a Perkin–Elmer Model 137 spectrometer. The mass spectra were determined with a 5985 Hewlett–Packard GC/MS mass spectrometer at 70 eV. Microanalyses were performed by Robertson Laboratories, Florham Park, New Jersey.

4-Azidosalicylic acid. — This compound was prepared by a slight modification of method described in the literature[5]. A solution of sodium nitrite (26 g, 0.38 mol) in water (150 mL) was added to a cooled suspension of 4-aminosalicylic acid (40 g, 0.261 mol) in 500 mL of conc. HCl and water (600 mL) at 0–5°. After stirring for 15 min, a small amount of urea was added to decompose the excess of nitrous acid, and the mixture was filtered through Celite. To the cooled filtrate was added a solution of sodium azide (24.4 g, 0.38 mol) in water (150 mL) at 0–5°. After stirring for 1 h at 0° the solid 4-azidosalicylic acid was filtered off. Crystallization from methanol gave 28 g (59%) of light-colored needles, m.p. 193–195° (lit.[6] m.p. 193–195°).

4-Azidosalicoyl chloride. — Oxaloyl chloride (6.5 g, 0.05 mol) was added to 4-azidosalicylic acid (3.6 g, 0.02 mol) in benzene (250 mL) and boiled gently for 4 h under reflux. The solvent was removed by distillation under diminished pressure. The residual solid was repeatedly dissolved in benzene and solvent evaporated to remove traces of oxaloyl chloride. The product was crystallized from petroleum ether to yield 3.2 g (80%) of a solid; ν_{max}^{Nujol} 2110 (N_3) and 1695 cm^{-1} (CO). The 4-azidosalicoyl chloride thus obtained was used without further characterization.

6-(4-Azido-2-hydroxybenzamido)-6-deoxy-1,2:3,5-di-O-isopropylidene-α-D-glucofuranose (**5**). — *Method A.* A solution of 4-azidosalicoyl chloride (2 g, 0.01 mol) in dichloromethane (150 mL) was added dropwise to a stirred solution of compound **3** (2.5 g, 10 m) and triethylamine (6 g, 60 mmol) in dichloromethane (50 mL) at 0°. After the addition, stirring was continued and the mixture was allowed to warm to room temperature during ~1 h. The solvent was removed on a rotary

evaporated under diminished pressure. The residue was dissolved in ether and washed successively with cold water, dilute hydrochloric acid, 10% sodium hydrogencarbonate solution, and water. The solid product remaining after removal of ether was recrystallized from methanol as colorless needles; yield 2.6 g (62%); m.p. 157–159°; ν_{max}^{Nujol} 3350 (NH), 2100 (N_3), and 1633 cm^{-1} (CO); n.m.r. ($CDCl_3$): δ 1.4 (s, 3 CH_3), 1.5 (s, CH_3), 3.7 (M, 3 H), 4.26 (s, 2 H), 4.6 (d, $J_{1,2}$ 4.5 Hz, H-2), 6.05 (d, $J_{1,2}$ Hz, H-1), 6.65 (m, ArH-3,5, NH), 7.4 (d, $J_{5,6}$ 8 Hz; ArH-6), and 12.5 (broad, OH).

Anal. Calc. for $C_{19}H_{24}N_4O_7$: C, 54.28; H, 5.75; N, 13.33. Found: C, 54.10; H, 5.58; N, 13.22.

Method B. A solution of *N,N'*-dicyclohexylcarbodiimide (4.4 g, 21.3 mmol) in dichloromethane (150 mL) was added dropwise to a cooled solution of **3** (5.2 g, 0.02 mol) and 4-azidosalicylic acid (3.6 g, 0.02 mol) in dichloromethane)200 mL) at 5–10°. After the addition, the mixture was stirred at room temperature overnight. The *N,N'*-dicyclohexylurea that separated was removed by filtration. Removal of the solvent gave a solid that was recrystallized from methanol as colorless needles; yield 7 g (83%); m.p. 156–158°.

Anal. Calc. for $C_{19}H_{24}N_4O_7$: C, 54.28; H, 5.75; N, 13.33. Found: C, 54.54; H, 5.69; N, 13.35.

The product obtained by either Method A or Method B had identical i.r. and n.m.r. spectra and had the same R_F value in t.l.c. (silica gel, 3:2 ether–hexane).

*6-(4-Azido-2-hydroxybenzamido)-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-*D*-galactopyranose* (**6**). — *Method A.* Reaction of 4-azidosalicoyl chloride with compound **4** by Method A gave solid product (**6**) that was recrystallized from ether–petroleum ether as needles; yield 2.6 g (62%) m.p. 101–103° remelting at 149–150°; ν_{max}^{Nujol} 3300 (NH), 2130 (N_3), and 1630 cm^{-1} (CO); n.m.r. ($CDCl_3$): δ 1.30 (s, CH_3), 1.35 (s, CH_3), 4.25 (m, 6 H), 4.25 (d, $J_{1,2}$ 4 Hz, H-2), 5.65 (d, $J_{1,2}$ 4 Hz, H-1), 6.65 (m, ArH-3,5, NH), 7.36 (d, $J_{5,6}$ 8 Hz, ArH-6), and 13.48 (s, OH); *m/z* 420 (mol. ion, base peak), 405 (42%, $M^+ - CH_3$), and 392 (50%, $M^{\cdot+} - N_2$).

Anal. Calc. for $C_{19}H_{24}N_4O_7$: C, 54.28; H, 5.75; N, 13.33. Found: C, 54.33; H, 6.0; N, 13.04.

Method B. Reaction of **4**, with 4-azidosalicyclic acid and *N,N'*-dicyclohexylcarbodiimide according to Method B gave the high-melting dimorph of **6** which,, on recrystallization from ether–petroleum ether (yield 85%), melted at 149–151°; ν_{max}^{Nujol} 3265 (NH), 2130 (N_3), and 1630 cm^{-1} (CO).

Anal. Calc. for $C_{19}H_{24}N_4O_7$: C, 54.28; H, 5.71; N, 13.33. Found: C, 54.26; H, 5.94; N, 13.10.

*6-(4-Azido-2-hydroxy-3,5-diiodobenzamido)-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-*D*-glucofuranose* (**7**). — A solution of compound **5** (210 mg, 50 μmol) and potassium iodide (400 mg, 0.24 mmol) in *N,N*-dimethylformamide (5 mL) was cooled to 0°. A solution of 700 mg of chloramine-T in *N,N*-dimethylformamide (5 mL) and acetic acid (1 mL) was added. The mixture was kept overnight at 5° and poured into ice–water. The solid that separated after a few min was

filtered off, washed repeatedly with cold water and 100 mL 5% sodium thiosulfate solution, and finally with cold water until the filtrate was neutral. The product was crystallized from ether–petroleum ether or methanol–water and gave fine needles; yield 310 mg (92%); m.p. 95–97°; ν_{max}^{Nujol} 3400 (broad, NH), 2120 (N_3), and 1650 cm^{-1} (CO); n.m.r. ($CDCl_3$); δ 1.38 (s, CH_3), 1.42 (s, 2 CH_3), 1.52 (s, CH_3), 3.65 (m, 3 H), 4.30 (m, 2 H), 4.61 (d, $J_{1,2}$ 4 Hz, H-2), 6.1 (d, $J_{1,2}$ 4 Hz, H-1), 6.76 (broad, NH), 7.86 (s, ArH-6), and 13.4 (s, OH); *m/z* 672 (M^+) and 388 ($M^+ - I_2 - N_2 - H_2$).

Anal. Calc. for $C_{19}H_{22}N_4O_7I_2$: C, 33.92; H, 3.27; N, 8.33; I, 37.79. Found: C, 33.26; H, 3.29; N, 8.33; I, 37.80.

*6-(4-Azido-2-hydroxy-3,5-diiodobenzamido)-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-*D*-galactopyranose* (**9**). — This compound was obtained in 95% yield by iodination of compound **6** as needles from methanol–water; m.p. 90–92°; ν_{max}^{Nujol} 3290 (NH), 2110 (N_3), and 1660 cm^{-1} (CO); n.m.r. ($CDCl_3$): δ 1.33 (s, CH_3), 1.36 (s, CH_3), 1.46 (s, CH_3), 1.50 (s, CH_3), 4.3 (m, 6 H), 4.35 (d, $J_{1,2}$ 4 Hz, H-2), 5.5 (d, $J_{1,2}$ 4 Hz, H-1), 6.9 (s, NH), 7.7 (s, ArH-6), and 13.59 (s, OH); 672 (M^+), 517 ($M^+ - I - N_2$) and 388 ($M^+ - I_2 - N_2 - H_2$).

Anal. Calc. for $C_{19}H_{22}N_4O_7I_2$: C, 33.92; H, 3.27; N, 8.33; I, 37.79. Found: C, 34.68; H, 3.46; N, 7.98; I, 37.40.

*6-(4-Azido-2-hydroxy-3,5-diiodobenzamido)-6-deoxy-*D*-glucopyranose* (**8**). — A mixture of compound **7** (180 mg, 0.27 mmol), and trifluoroacetic acid (3 mL) was stirred for 4 h at room temperature under nitrogen. The trifluoroacetic acid was removed by distillation under diminished pressure. The residue was stirred in water and the water evaporated off. Repeated trituration with water and evaporation gave a solid that was filtered off, washed with water, and dried in a vacuum desiccator. Crystallization from acetonitrile or methanol gave cream-colored needles, yield 150 mg (95%); m.p. 197–200°; ν_{max}^{Nujol} 3400, 3200 (OH), and 2100 cm^{-1} (N_3).

Anal. Calc. for $C_{13}H_{14}N_4O_7I_2$: C, 26.35; H, 2.35; N, 9.46; I, 42.91. Found: C, 26.62; H, 2.41; N, 9.64; I, 43.05.

*6-(4-Azido-2-hydroxy-3,5-diiodobenzamido)-6-deoxy-*D*-galactopyranose* (**10**). — This compound was obtained from compound **9** in 95% yield. Crystallization from methanol gave needles, m.p. 165–167° (decomp.); ν_{max}^{Nujol} 3300 (broad, OH) and 2100 cm^{-1} (N_3).

Anal. Calc. for $C_{13}H_{14}N_4O_7I_2$: C, 26.35; H, 2.36; N, 9.46; I, 42.91. Found: C, 26.30; H, 2.59; N, 9.23; I, 42.45.

*6-(4-Azido-2-hydroxybenzamido)-6-deoxy-*D*-glucopyranose* (**1**). — This compound was obtained from compound **3** in 96% yield. The product was crystallized from methanol–benzene, m.p. 200° (decomp.); ν_{max}^{Nujol} 3300, 3200 (OH), 2100 (N_3), and 1640 cm^{-1} (CO); *m/z* 340 (M^+), 322 ($M^+ - 2\ H_2O$) and 304 ($M^+ - 2\ H_2O$).

Anal. Calc. for $C_{13}H_{16}N_4O_7$: C, 45.88; H, 4.70; N, 16.47. Found: C, 45.98; H, 4.95; N, 16.55.

*6-(4-Azido-2-hydroxybenzamido)-6-deoxy-*D*-galactopyranose* (**2**). — This compound was obtained from compound **4** in 96% yield. Crystallization from ethyl acetate gave a colorless, amorphous solid, m.p. 182–184° (decomp.); ν_{max}^{Nujol} 3400, 3200 (broad, OH), and 2110 cm^{-1} (N_3).

Anal. Calc. for $C_{13}H_{16}N_4C_7$: C, 45.88; H, 4.70; N, 16.47. Found: C, 45.93; H, 4.75; N, 16.16.

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