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SYNTHESES AND PROPERTIES OF SOME PHOTOLABILE ß-THIOGLYCOSIDES. POTENTIAL PHOTOAFFINITY REAGENTS FOR ß-GLYCOSIDE HYDROLASES*

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Tosyloxy-3-azi-butane, -4-azi-pentane and chloroacetone were condensed with ß-D-thiosugars to yield ß-D-thioglycosides which either already had a photolabile diazirino group or they had an oxo group, which could be converted into such. All photolabile ß-thio-glycosides are excellent competitive inhibitors for their corresponding glycoside hydrolases (ß-D-galactosidase and ß-D-glucosidase). Photolysis of the glycosides in aqueous solution preferentially gave insertion products, indicating that all of the compounds were potential affinity labels. 2-Azi-propyl ß-D-thio-galactoside underwent partial photochemically induced glycoside hydrolysis.

The reversible formation of ligand-receptor complexes is the basis of many biologically important carbohydrate-protein interactions. Receptor function is important in various different proteins such as enzymes, lectins, transport proteins and antibodies. Photoaffinity labelling is an excellent method to covalently fix such ligand-receptor complexes[1]. Thus far very few carbohydrate binding proteins have been studied by photoaffinity labelling with suitable ligands, carrying sterically unobtrusive photolabile groups which also have good photochemical properties and sufficient chemical and biochemical stability[2,3,4]. Such compounds are usually difficult to synthesize because the photolabile group - preferentially a diazirino group - is directly attached to the carbon skeleton of the ligand[2-5]. For glycoside binding proteins such as glycoside hydrolases the photolabile group could be incorporated into the aglyconic moiety[2] of a pyranoside which in turn is linked to the glyconic part by an S-glycosidic bond. This would have the advantage of combining a prefabricated suitable aglycone with any 1-thio saccharide to yield an enzyme resistant photolabile thio-glycoside in the last step of the synthesis. It is advantageous to use sulfur to link the photolabile aglycone to the glycone because it allows the incorporation of the very versatile ^{35}S radioisotope into the ligand. We describe here the syntheses and photochemical properties of alkyl ß-D-thio-glycopyranosides, differing in chain length of the aglyconic portion and in glycone configuration. 1-Thio-ß-D-glucopyranose and -galactopyranose either as per-O-acetates or unprotected are easily accessible compounds[6,7]. The thiol grouping reacts with activated alcohols to give the corresponding thioglycoside in good yield. For alkylation the thiol group in 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glycopyranose p-toluenesulfonyl (tosyl) esters of the aglyconic alcohols are

*dedicated to Prof. Yu Wang on the occasion of his 80th birthday

sufficiently reactive. Whereas 4-azi-pentanol and 3-azi-butanol[8] could be activated and used as such for S-alkylation, 2-azi-1-tosyloxi-propane is too unstable to be used as an alkylating agent*. In that case 1-chloro-acetone was reacted with 1-thio-ß-D-galactose and the 2-keto-propyl 1-thio-ß-D-galactopyranoside thus formed could be converted without difficulty by the general method[8] into 2-azi-propyl 1-thio-ß-D-galactopyranoside. All azi-alkyl 1-thio-ß-D-glycopyranosides underwent rapid decay when irradiated in aqueous solution with UV-light of 350 nm (Table I) and gave the expected stable products either by intramolecular reaction to exclusively form unsaturated thioglycosides or by intermolecular insertion reaction with the solvent water to form hydroxyalkyl thioglycosides. The ratios of the two types of products (Table II) as determined by t.l.c. analysis are indicative of the affinity labelling potential of the diazirines.

Table I:
Extinction coefficient and half-life periods of the photolabile thio-sugars on irradiation

compound	ε_{348}	$t_{1/2}$ min
(2)	68	5.2
(4)	61	5.4
(7)	60	5.4
(9)	53	5.5

Table II:
Composition of irradiation products of the photolabile thio-sugars in relative** amounts (given in percent).

compound	(2)	(4)	(7)	(9)
unsaturated compounds	43	47	29	40
hydroxyalkyl-thioglycosides	51	53	51	54
free sugar	6	--	20	6

The greater the amount of intermolecular reaction that takes place, the greater the potential effectiveness of the reaction with a receptor. It is interesting to note that the formation of the alcohols remains almost constant with varying chain length between diazirino group and glycosidic sulfur, whereas formation of unsaturated thio-glycosides by intramolecular hydrogen shift increases with the chain length. A novel type of side reaction is an apparent thio-glycoside cleavage on irradiation, which yields the free sugars from 3-azi-butyl- and 2-azi-propyl thio-glycoside. Yields are highest with the latter, in which the distance between the diazirino group and the anomeric center is the shortest. No cleavage can be observed with 4-azi-pentyl thio-galactoside in which the diazirino group was furthest removed. The glyconic portion has no influence on the product rates. The mechanism of this photochemically induced thio-glycoside hydrolysis is unclear and will be elucidated in due course. It is likely that the

*Alkyldiazirines carrying a partial positive charge on a neighbouring carbon atom[9] spontaneously eliminate nitrogen.

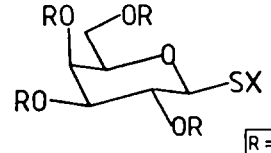
**determined by densitometry of t.l.c. plates

carbene initially formed on irradiation, or some other reactive intermediate, attacks the glycosidic sulfur atom. This could be followed by the displacement of the aglyconic portion by a solvent molecule. A strong odor in cases where the formation of the free sugar is observed supports this suggestion. The inhibition constants (Table III) determined in the usual way with the corresponding enzymes ß-D-galactosidase (ß-D-galactoside galactohydrolase, EC 3.2.1.23) from E. coli and ß-D-glucosidase (ß-D-glucoside glucohydrolase, EC 3.2.1.21) from sweet almond emulsin using nitrophenyl ß-D-glycopyranosides as substrates, demonstrate the excellent affinity of the thioglycosides for their receptors. Work is in progress to ^{35}S-label the thioglycosides and to use them as photoaffinty labels for suitable receptor proteins.

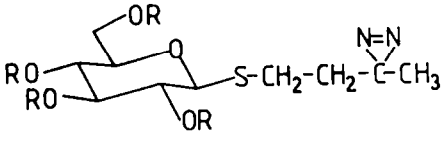
Table III: K_I values for several ß-D-glycopyranosides

	K_I mM
Isopropyl 1-thio-ß-D-galactopyranoside	0.085
(2)	0.071
(4)	0.069
(7)	0.095
(9)	1.5

Formulas



	R = Ac	R = H
X= -CH_2-CH_2-C(=N-N)-CH_3	1	2
X= -CH_2-CH_2-CH_2-C(=N-N)-CH_3	3	4
X= -CH_2-C(=O)-CH_3	5	-
X= -CH_2-C(=N-N)-CH_3	6	7



R = Ac	8
R = H	9

Experimental

Melting points were determined with a Büchi melting point apparatus and are uncorrected. Optical rotations were measured at 20° or 25° with a Perkin-Elmer 141 polarimeter. T.l.c. was performed on Silica Gel 60 F_{254} (Merck) using the solvents indicated and compounds were

visualised with 2% (v/v) concentrated sulfuric acid in methanol. Flash chromatography[10] was carried out using ICN Silica Gel, 32-63, 60A (ICN Biochemicals, D-3440 Eschwege). ^{1}H-NMR spectra were recorded with a Bruker WM 250 (250MHz) spectrometer. Solutions of the compounds were made in $CDCl_3$ (with internal Me_4Si). Elemental analyses were performed using a Perkin-Elmer 240 analyzer. UV-spectra were recorded with a ZEISS PM QII photometer. Kinetic data were determined using an Eppendorf photometer equipped with a 405nm filter. Densitograms were recorded with a Vitatron densitometer TLD 100 (Vitatron Gmbh 5 Köln). Photolyses were carried out in a Rayonet RPR 100 reactor equipped with 16 RPR 3500 Å lamps.

Enzymic reactions. ß-D-Galactosidase (ß-D-galactoside galactohydrolase, EC 3.2.1.23) from Escherichia coli was purchased from Boehringer Mannheim. Reactions were performed at 30° in 50 mM sodium potassium phosphate buffer (pH 6.8) and 1 mM magnesium chloride. The inhibition constants of (**2**), (**4**) and (**7**) were determined using an assay with 0.08 mM to 2.0 mM o-nitrophenyl ß-D-galactopyranoside (oNPGal), 0 to 0.15 mM inhibitor and 0.08 U ß-D-galactosidase. ß-D-Glucosidase (ß-D-glucoside glucohydrolase, EC 3.2.1.21) from sweet almond emulsin was purchased from Boehringer Mannheim. Reactions were performed at 30° in 50 mM sodium potassium phosphate buffer (pH 6.8). The inhibition constant of (**9**) was determined using an assay with 0.5 mM to 20 mM o-nitrophenyl ß-D-glucopyranoside (oNPG), 0 to 3 mM inhibitor and 0.08 U ß-D-glucosidase.

3-Azi-1-(2',3',4',6'-tetra-O-acetyl-ß-D-galactopyranosyl(thio))-butane (**1**). Tetra-O-acetyl-1-thio-ß-D-galaktopyranose[6] (2 g, 5.5 mmol) and 3-azi-1-(p-toluenesulfonyloxy)butane[8] (1.33 g, 6 mmol) were dissolved in methylene chloride (10 ml) and solid potassium carbonate (0.76 g, 5.5 mmol) was added. The mixture was stirred for 2 h under reflux. After filtration and evaporation the residue was subjected to flash-chromatography (cyclohexane-ethyl acetate, 2:1, R_F 0.13) to give a colourless syrup (1.98 g, 80%); $[\alpha]_D^{25}$ +0.3 (c 0.9, chloroform); ^{1}H-NMR: δ 1.07 (3H, s, CH_3); 1.68 (2H, t, H-2a, 2b; $J_{2a,3}$, $J_{2b,3}$ 7.5Hz); 2.0, 2.06, 2.07, 2.16 (3H, 4xs, 4xAc), 2.57 (2H, m, H-1a,b); 3.93 (1H, dt, H-5', $J_{5',6'a}$ 7.35 Hz $J_{5',6'b}$ 6.15 Hz); 4.10, 4.12 (1H, 2xd, H-6'a,b); 4.47 (1H, d, H-1', $J_{1',2'}$ 10.35); 5.03 (1H, dd, H-3', $J_{3',4'}$ 3.15 Hz); 5.23 (1H, t, H-2', $J_{2',3'}$ 9.75 Hz); 5.43 (1H, dd, H-4', $J_{4',5'}$ 1.3 Hz).

3-Azi-1-(ß-D-galactopyranosyl(thio))-butane (**2**). A solution of (**1**) (1 g, 2.2 mmol) in anhydrous methanol (10 ml) was stirred with methanolic 1 M sodium methoxide (0.5 ml) for 0.5 h, at which time t.l.c. (cyclohexane-ethyl acetate, 2:1) indicated the absence of (**1**). Deionisation was carried out by passing the mixture through a silica gel column (2x5 cm), using anhydrous methanol as eluent. After evaporation the residue was crystallized from ethanol-diethyl ether to give (0.55 g, 91%), mp 92-93°, $[\alpha]_D^{25}$ -29.5 (c 0.6, water). Anal. Calc. for $C_{10}H_{18}N_2O_5S$: C, 43.15; H, 6.51; N, 10.06; S, 11.49; found: C, 43.44; H, 6.56; N, 10.13; S, 11.59.

4-Azi-1-(2',3',4',6'-tetra-O-acetyl-ß-D-galactopyranosyl(thio))-pentane (**3**). Tetra-O-acetyl-1-thio-ß-D-galactopyranose[6] (1 g, 2.75 mmol) was allowed to react with 4-azi-1-(p-toluenesulfonyloxy)pentane[8] (0.7 g, 3 mmol) as described for (**1**). Flash column chromatography (cyclohexane-ethyl acetate, 2:1, R_f 0.13) gave a colourless syrup (0.94 g, 74%), $[\alpha]_D^{25}$ -26.6 (c 1.4, chloroform); ^{1}H-NMR: δ 1.01 (3H, s, CH_3); 1.49 (4H, m, H-2a,b, H-3a,b); 1.99, 2.04, 2.06, 2.12 (3H, 4xs, 4xAc); 2.66 (2H, m, H-1a,b); 3.96 (1H,dt,H-5', $J_{5',6'a}$ 7.35 Hz, $J_{5',6'b}$ 6.0 Hz); 4.12 (1H, d, H-6'a); 4.20 (1H, d, H-6'b); 4.48 (1H, d, H-1', $J_{1',2'}$ 9.75 Hz); 5.06 (1H, dd, H-3', $J_{3',4'}$ 3.15 Hz); 5.22 (1H, t, H-2', $J_{2',3'}$ 10.3 Hz); 5.43 (1H, dd, H-4', $J_{4',5'}$ 0.75 Hz).

4-Azi-1-(ß-D-galactopyranosyl(thio))-pentane (**4**). A solution of (**3**) (0.5 g, 1.1 mmol) was treated with methanolic sodium methoxide as described for compound (**2**). The product was crystallized from ethanol-diethyl ether to give (**4**) (0.3 g, 93%), mp 96-97°, $[\alpha]_D^{25}$ -30.7 (c 0.6, water). Anal. calc. for $C_{11}H_{20}N_2O_5S$: C, 45.2; H, 6.9; N, 9.58; S, 10.96; found: C, 45.19; H, 6.73; N, 9.20; S, 10.99.

2-Oxi-1-(2',3',4',6'-tetra-O-acetyl-ß-D-galactopyranosyl(thio))-propane (**5**). Tetra-O-acetyl-1-thio-ß-D-galactopyranose[6] (4 g, 11 mmol) and 1-chloro-2-propanone (1 g, 11 mmol) were dissolved in acetone (10 ml) and an aqueous solution of potassium carbonate (1.52 g, 11 mmol) was added. After 1 h at room temperature the solution was filtered and evaporated. The residue was subjected to flash chromatography (cyclohexane-ethyl acetate, 1:1, R_f 0.22) to give

a colourless syrup (2.8 g, 61%); $[\alpha]_D^{25}$ -19.5 (c 0.8, chloroform); ^{1}H-NMR: δ 1.98 (3H, s, CH_3); 2.07, 2.08, 2.18, 2.31, (3H, 4xs, 4xAc); 3.32, 3.52 (1H, 2xd, H-1a,b, $J_{1a,1b}$ 21 Hz); 3.91, (1H, dt, H-5', $J_{5',6'a}$, $J_{5',6'b}$ 6.15 Hz); 4.09 (2H, d, H-6'a,b); 4.5 (1H, d, H-1', $J_{1',2'}$ 10.35 Hz); 5.04 (1H, dd,H-3', $J_{3',4'}$ 3.15 Hz); 5.19 (1H, t, H-2', $J_{2',3'}$ 10.36 Hz); 5.42 (1H, dd, H-4', $J_{4',5'}$ 0.75 Hz).

2-Azi-1-(2',3',4',6'-tetra-O-acetyl-ß-**D**-galactopyranosyl(thio))-propane (**6**). A solution of (**5**) (2 g, 4.75 mmol) in anhydrous methanol (50 ml) and liquid ammonia (40 ml) was stirred for a 3 h period at -30° to -20°. The solution was then cooled to -50° and hydroxylamine-O-sulfonic acid (0.57 g, 5 mmol) was added. After 2 h at -30° the cooling bath was removed and the ammonia was allowed to evaporate overnight. The mixture was filtered and concentrated; no odor of ammonia could be detected. After dilution with 50 ml anhydrous methanol the solution was cooled in an ice bath and treated with triethyl amine (10 ml). Solid iodine was added in small amounts until the red colour of excess iodine persisted. The solution was concentrated, diluted with diethyl ether (100 ml) and washed with brine and aqueous sodium thiosulfate. After drying and evaporation the residue was reacetylated in pyridine-acetic anhydride (2:1, 10 ml). The crude product was subjected to flash chromatography (cyclohexane-ethyl acetate, 1:2, R_f 0.13) to give a colourless syrup (1.3 g, 63%; $[\alpha]_D^{25}$ -2.8 (c 1.4, chloroform); ^{1}H-NMR: δ 1.15 (3H, s, CH_3,); 1.98, 2.04, 2.08, 2.17 (3H, 4xs, 4xAc); 2.57 (2H, s, H-1a,b); 4.01 (2H, d, H-6'a,b); 4.04 (1H, dt, H-5', $J_{5',6'a}$, $J_{5',6'b}$ 6.15 Hz); 4.67 (1H, d, H-1', $J_{1',2'}$ 9.9 Hz); 5.1 (1H, dd, H-3', $J_{3',4'}$ 3.15 Hz); 5.21 (1H, t, H-2', $J_{2',3'}$ 10.35 Hz); 5.46 (1H, dd, H-4', $J_{4',5'}$ 0.8 Hz).

2-Azi-1-(ß-**D**-galactopyranosyl(thio))-propane (**7**). A solution of (**6**) (1 g, 2.3 mmol) was treated with methanolic sodium methoxide as described for compound (**2**). The product was subjected to flash chromatography (ethyl acetate-methanol, 5:1, R_f 0.20) to give a colourless syrup (0.55 g, 91%, $[\alpha]_D^{25}$ -40.1 (c 0.5, water); anal. calc. for $C_9H_{16}N_2O_5S$: C, 40.9; H, 6.10; N, 10.6; S, 12.13; found: C, 41.01; H, 6.16; N, 10.31; S, 12.40).

3-Azi-1-(2',3',4',6'-tetra-O-acetyl-ß-**D**-glucopyranosyl(thio))-butane (**8**). Tetra-O-acetyl-1-thio-ß-**D**-glucopyranose[7] (3 g, 8.2 mmol) and 3-Azi-1-(p-toluenesulfonyloxy) butane[8] (2.3 g, 9 mmol) were dissolved in dry methylene chloride (25 ml). After adding solid potassium carbonate (1.15 g, 8.3 mmol) the mixture was stirred under reflux overnight. The mixture was filtered and concentrated, the persisting yellow oil was purified by flash chromatography (cyclohexane-ethyl acetate 2:1, R_f 0.19) to yield a colourless syrup (2.85 g, 78%). Crystallisation from isopropanole gave 2.2 g of colourless crystals (mp 86°). $[\alpha]_D^{20}$ -43 (c 0.6, chloroform). ^{1}H-NMR: δ 1.06 (3H, s, CH_3); 1.64 (2H, t, H-2a,b); 2.01, 2.03, 2.07, 2.10, (3H, 4xs, 4xAc); 2.59 (2H, m, H-1a,b); 3.7 (1H, ddd, H-5', $J_{5',6'a}$ 2.4 Hz, $J_{5',6'b}$ 4.8 Hz); 4.13, 4.23 (2x1H, dd, H6'a,b, $J_{6',6'}$ 12.6); 4.47 (1H, d, H-1', $J_{1',2'}$ 10.2 Hz); 5.02 (1H, t, H-4', $J_{4',5'}$ 9.75 Hz); 5.06 (1H, t, H-2', $J_{2',3'}$ 9.75 Hz); 5.22 (1H, t, H-3', $J_{3',4'}$ 9.45); anal. calc. for $C_{18}H_{26}N_2O_9S$: C, 48.42; H, 5.87; N, 6.27; S, 7.18; found: C, 48.14; H, 5.88; N, 6.28; S, 7.16.

3-Azi-1-(ß-**D**-glucopyranosyl(thio))-butane (**9**). A solution of (**8**) (1.5 g, 3.36 mmol) was treated with methanolic sodium methoxide as described for compound (**2**). The product was purified by flash chromatography (ethyl acetate-methanol-water 27:2:1, R_f 0.22) to yield 0.81 g (87%) colourless syrup. $[\alpha]_D^{20}$ -52.6 (c 0.52, water).

Irradiation of the unprotected photolabile compounds. (**2**), (**4**), (**7**) and (**9**) (100 mg), dissolved in distilled water (2.5 ml), were irradiated for 1 h. T.l.c analyses (ethyl acetate-methanol-water 7:2:1) showed the following:

compound	number of products	R_f-value olefinic products	R_f-value alcoholic products	R_f-value free sugar
(**2**)	3	0.51	0.36	0.17
(**4**)	2	0.53	0.37	-
(**7**)	3	0.50	0.33	0.17
(**9**)	3	0.57	0.42	0.22

For the olefinic products a rapid bleaching of potassium permanganate solution was observed, in contrast to a slow bleaching off all sulfur containing sugars. Free sugars were identified by comparison with authentic samples.

1H-NMR studies of the acetylated products (pyridine-acetic anhydride 2:1) showed the following results:

For the unsaturated products allylic (δ 1.64-1.78) and olefinic (δ 5.36-5.88) protons were observed. The singlet signal for the methyl group (δ 1.06) and the triplet signal for the methylene group (δ 1.46) indicative of protons in neighboring positions to a diazirino group were absent. Three different doublet signals for the anomeric proton indicated the presence of three olefin isomers.

The insertion products were proven to be alcohols by observation of a multiplet signal for one proton (δ 4.9-5.13) in the range of the signals for the sugar skeleton protons and also one additional acetylic protecting group (δ 2.0-2.1). For the neighboring methyl group a doublet signal, in place of the singlet for compound (**9**), was detected. Two different doublet signals for the anomeric proton demonstrated that there were two alcohol isomeres present.

The complex ^{1}H-NMR spectra and the inseparability of the respective isomers prevent a complete interpretation of the ^{1}H-NMR data. The object of this work was to indicate the applicability of the diazirines for photoaffinity labelling and not the complete analyses of the irradiation products.

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