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Note

Azido compounds as potential affinity labels for glycosidases

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Among the methods developed for the study of the active sites of enzymes, the use of substrates or substrate analogues (affinity labels) that can react irreversibly with catalytically important groups has found widespread application.

Glycosides with reactive functions in the aglycon group, such as haloacyl, haloalkyl, diazo, or epoxide groups, could be useful as affinity labels for glycosidases[1–4]. Each of the potential photo-affinity labels described here contains an azido group that can be converted into a reactive nitrene by photolysis. The nitrene should be capable of covalent bond formation with almost any amino acid residue at the active site of the glycosidases. We now report on the synthesis of some carbohydrate azide derivatives.

β-D-Xylopyranosyl azide was prepared by literature procedures[5]. The aromatic azido derivatives **3–7** (see Table I) were prepared from the acetylated nitrophenyl glycosides[6] or nitrobenzyl 1-thioglycosides[7], *via* the corresponding amines and diazonium salts. Deacetylation[8] proceeded without affecting the azide function to give compounds **8–12**.

As photo-affinity labels, the aromatic azides **8–12** have some potentially important advantages. Each compound has λ_{max} ~250 nm, so that low-energy u.v. radiation readily effects activation, thereby permitting them to be explored as affinity labels for proteins. Aliphatic azides require high-energy radiation at much shorter wavelengths, where photochemical inactivation of the enzymes is more likely to occur.

4-Azido-2-nitrophenyl glycosides[9] can be activated in solution at wavelengths above 345 nm, but the risk of spontaneous decomposition in daylight is much greater than for the azides now described. Solutions of the unsubstituted azidophenyl glycosides withstand short exposure to daylight and are stable in the dark over long periods of time.

The suitability of the azidophenyl glycosides as affinity labels for glycosidases is being evaluated.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP2 instrument and are uncorrected. Specific rotations were measured with a Perkin–Elmer Model

TABLE I

AROMATIC AZIDO β-D-GLYCOPYRANOSIDES

		Yield (%)	M.p. (degrees)	$[\alpha]_{5890}^{20}$ (degrees)	Found (%)		Formula	Calc. (%)	
					C	H		C	H
	Acetylated β-D-glycopyranosides								
3	*o*-Azidophenyl xyloside	42	70–72	−59.5	51.8	5.0	$C_{17}H_{19}N_3O_3$	51.9	4.8
4	*p*-Azidophenyl xyloside	67	118–119	−42.9	51.7	4.8	$C_{17}H_{19}N_3O_3$	51.9	4.8
5	*p*-Azidobenzyl 1-thioxyloside	22	88–90	−102.0	50.6	5.1	$C_{18}H_{21}N_3O_7S$	51.1	5.0
6	*p*-Azidophenyl glucoside	55	115–116	−17.9	51.5	5.0	$C_{20}H_{23}N_3O_{10}$	51.6	5.0
7	*p*-Azidophenyl galactoside	44	127–128	+7.0	51.4	5.0	$C_{20}H_{23}N_3O_{10}$	51.6	5.0
	β-D-Glycopyranosides								
8	*o*-Azidophenyl xyloside	62	136–137	−29.6	49.5	5.5	$C_{11}H_{13}N_3O_5$	49.4	4.9
9	*p*-Azidophenyl xyloside	64	168–170	−37.1	49.6	4.9	$C_{11}H_{13}N_3O_5$	49.4	4.9
10	*p*-Azidobenzyl 1-thioxyloside	35	120–122	−162.0	47.6	5.1	$C_{12}H_{15}N_3O_4S$	48.5	5.2
11	*p*-Azidophenyl glucoside	45	168	−68.7	48.4	5.0	$C_{12}H_{15}N_3O_6$	48.5	5.1
12	*p*-Azidophenyl galactoside	42	148–150	−52.5	48.6	5.3	$C_{12}H_{15}N_3O_6$	48.5	5.1

141 polarimeter. I.r. spectra were recorded for dispersions in Nujol with a Perkin–Elmer grating spectrometer, using CsI discs. U.v. spectra were recorded in 10mM phosphate buffer (pH 7.2), using a Beckman DKII grating spectrophotometer. The purity of the products was tested by t.l.c. on Silica Gel G (Merck) with ethyl acetate–benzene (3:7) for acetates, and acetic acid–water–ethyl acetate (1:1:3) for glycosides. Detection was effected with 5% sulphuric acid in ethanol (10 min at 120°). All final products were homogeneous on t.l.c.

β-D-*Xylopyranosyl azide* (**2**). — Sodium azide (10 g) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[10] (15 g) were added successively and with stirring to acetonitrile (18 ml, dried over calcium hydride). The mixture was refluxed for 1.5 h and, after cooling and filtration, was concentrated *in vacuo*. The resulting syrup crystallized from isopropyl ether–methanol (at 4°). After two further recrystallizations from methanol, 2,3,4-tri-*O*-acetyl-*β*-D-xylopyranosyl azide (**1**; 5.4 g, 37%) was obtained having m.p. 84.1–84.6°, $[\alpha]_D^{21}$ −83° (*c* 1, chloroform); lit.[11] m.p. 87.5°, $[\alpha]_D^{16}$ −79.3°; ν_{max}^{CsI} 2120 (N_3), 1750 cm^{-1} (C=O).

Conventional[8] deacetylation of **1**, followed by crystallization of the product from pentyl alcohol–light petroleum at −18° and two recrystallizations from pentyl alcohol, gave **2** (0.5 g, 43%), m.p. 109–110°, $[\alpha]_D^{21}$ −59° (*c* 1, methanol); ν_{max}^{CsI} 2120 (N_3), and broad OH-band above 3000 cm^{-1}; λ_{max} 270 nm. (Found: C, 34.3; H, 5.4. $C_5H_9N_3O_4$ calc.: C, 34.3; H, 5.1%).

Preparation of aromatic azido derivatives. — The acetylated amines were obtained by catalytic hydrogenation[12] over palladium of the corresponding nitrophenyl[6] or nitrobenzyl[7] glycosides, and the products were diazotised immediately. Attempts to crystallize the amines usually lowered the yield considerably. The hydrogenation mixture was filtered over Celite and concentrated *in vacuo*, and the syrupy residue was dissolved in cold (4°) 3.6M hydrochloric acid (5–7 equiv.). Cold, 20% aqueous sodium nitrite (1 equiv.) was added dropwise with stirring and cooling in ice–water. After reaction for 0.5 h, cold, 25% aqueous sodium azide (1 equiv.) was added with further cooling. It was important, at this stage, to keep the precipitating azido compounds suspended by vigorous agitation. After stirring for another hour at room temperature, no more diazo compound could be detected (alkaline *β*-naphthol). The precipitated azido derivatives were collected and washed with water until acid-free. Compounds **3**–**7** were crystallized and recrystallized from ethanol (−18°). Deacetylations were effected conventionally[8], and the resulting glycosides (**8**–**12**) were crystallized from methanol or ethanol. Physical constants and analytical data are recorded in Table I. The i.r. spectra contained the characteristic stretching band for azido compounds (ν_{max}^{CsI} 2120 cm^{-1}). The u.v. spectra showed λ_{max} at 252 and 282 nm for the *p*-azidophenyl derivatives, 238 and 249 nm for the *o*-azidophenyl xyloside, and 256 and 290 nm for the *p*-azidobenzyl 1-thioxyloside.

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