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Caged Compounds of a 2-Deoxyglucose: Facile Synthesis and Their Photoreactivity

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Abstract: An *o*-nitrobenzyl and an *o*-nitrophenethyl derivatized 2-deoxyglucose (caged 2-deoxyglucoses) were synthesized from 3,4,6-tri-*O*-acetyl-D-glucal in only two steps in moderate to good yields as isomeric mixtures, which were irradiated at 350 nm to afford a 2-deoxyglucose. Decomposition of the *o*-nitrophenethyl derivative upon photolysis proceeded more efficiently than that of the *o*-nitrobenzyl derivative.

Photoinduced release of biologically important molecules from chemically modified bioactive compounds has been finding its extensive application for investigating biological and medical events. Caged compounds, photoactivatable precursor of bioactive compounds, are powerful tools in this context.[1-3] Caged compounds of several kinds of bioactive molecules such as ATP, cAMP, peptides, nucleosides, and neurotransmitters have been synthesized and applied to biological systems. Although more and more attention has been paid to sugars and neoglycoconjugates as an important molecule for cell recognition or adhesion,[4] not so many photolabile sugars were developed so far.[5-8] A 2-deoxyglucose has been used as a metabolic inhibitor for a glucose, and recently, it was reported to be concerned with the induction of apoptosis in U937 cells.[9] In the course of our study to develop caged compounds that control apoptosis in immunocyte,[10-13] we planed to synthesize caged compounds of a 2-deoxyglucose. In this paper, we describe the synthesis and photochemical reactivity of caged compounds of a 2-deoxyglucose.

The synthesis of an *o*-nitrobenzyl and an *o*-nitrophenethyl derivatized 2-deoxyglucose is accomplished only by two steps using 3,4,6-tri-*O*-acetyl-D-glucal **1** as a starting material, which is depicted in Scheme 1. Glycosylation of **1** with an *o*-nitrobenzyl or an *o*-nitrophenethyl alcohol in the presence of triphenylphosphine hydrobromide,[14] followed by deacetylation with sodium methoxide afforded target molecules **3a** and **3b** in more than 80 and 50% yield, respectively, over two steps. Facile availability of starting materials as well as a short and simple synthetic route for obtaining the target molecules is an important factors for biological application, and the present synthetic strategy for **3** satisfies these criteria.

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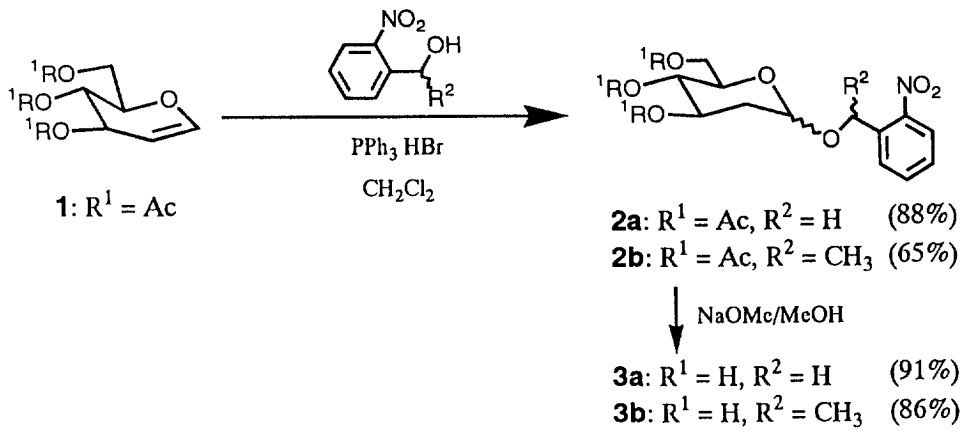

Scheme 1. Synthesis of caged 2-deoxyglucoses **3**.

The structure of the target molecules was confirmed by their ^1H and ^{13}C NMR as well as H-H and C-H COSY spectra. The *o*-nitrobenzyl derivatized 2-deoxyglucose **3a** was obtained as a mixture of anomeric isomers, and chromatographic separation of them was quite difficult. Each isomer was identified by the $^1J_{CH}$ of the anomeric position;[15] which were 168 and 164 Hz for α- and β-isomer, respectively. The ratio of anomeric isomers (α/β of **3a**) was found to be 7/3 which was estimated by integration of the corresponding protons of both anomers. The *o*-nitrophenethyl derivative **3b** similarly prepared was also obtained as a mixture of four isomers. Judging from ^{13}C NMR spectra of **3b**, the ratio of α/β was approximately 3/1, and each anomer has an equal amount of diastereomers.

The absorption maximum of a methanol solution of **3a** and **3b** was 259 nm (ε 5740) and 257 nm (ε 4250), respectively. Because the UV-B may give serious damage to biosystem, irradiation of **3** was carried out at 350 nm where the molar absorptivities (ε) in methanol were 280 for **3a** and 310 for **3b**. A 500 μM solution of **3** was prepared in 10φ Pyrex tube and irradiated within a Rayonet photochemical reactor (RPR 3500 Å x 4). Time-dependent decrease of **3a** and **3b** monitored by HPLC is shown in Figure 1, in which 50% of starting materials were decomposed after 3 and 1 min irradiation for **3a** and **3b**, respectively. Interestingly, photo-decomposition of both **3a** and **3b** proceeded more efficiently in phosphate-buffered saline (PBS) at the beginning of this reaction, and only about 20 seconds irradiation was enough to decompose 50% of **3b**.

In order to confirm release of 2-deoxyglucose upon irradiation, a 50 mM methanol-d_4 solution of **3a** was prepared in a 5φ NMR tube and irradiated. The ^1H NMR spectra taken during photolysis showed that the intensity of signals due to **3a** was decreased with a concomitant increase in the signals derived from a released 2-deoxyglucose as shown in Figure 2. Release of a 2-deoxyglucose was also confirmed by the anisaldehyde-positive spot of TLC which was clearly monitored more than 35 min of irradiation. Judging from the observation that there was no anisaldehyde-positive spot of TLC other than those of **3a** and a 2-deoxyglucose, **3a** releases mainly a target molecule, 2-deoxyglucose. We could also observe a similarly efficient release of a 2-deoxyglucose from the irradiated **3b** using both ^1H-NMR and TLC analysis.

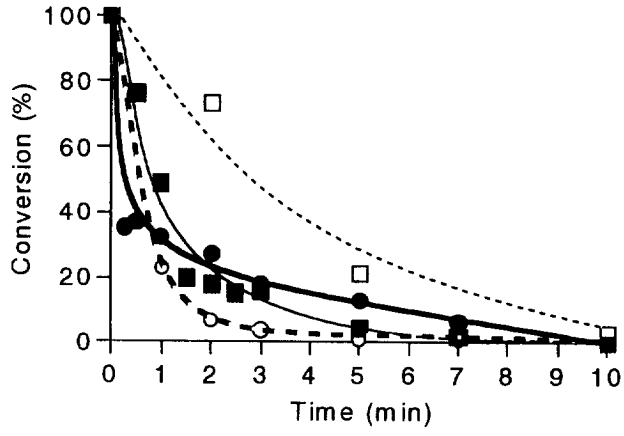

Figure 1. The decrease of **3** during photolysis in methanol or PBS was estimated by HPLC analysis. □ : **3a** in MeOH(···), ○ : **3a** in PBS(···), ■ : **3b** in MeOH(—), ● : **3b** in PBS(—).

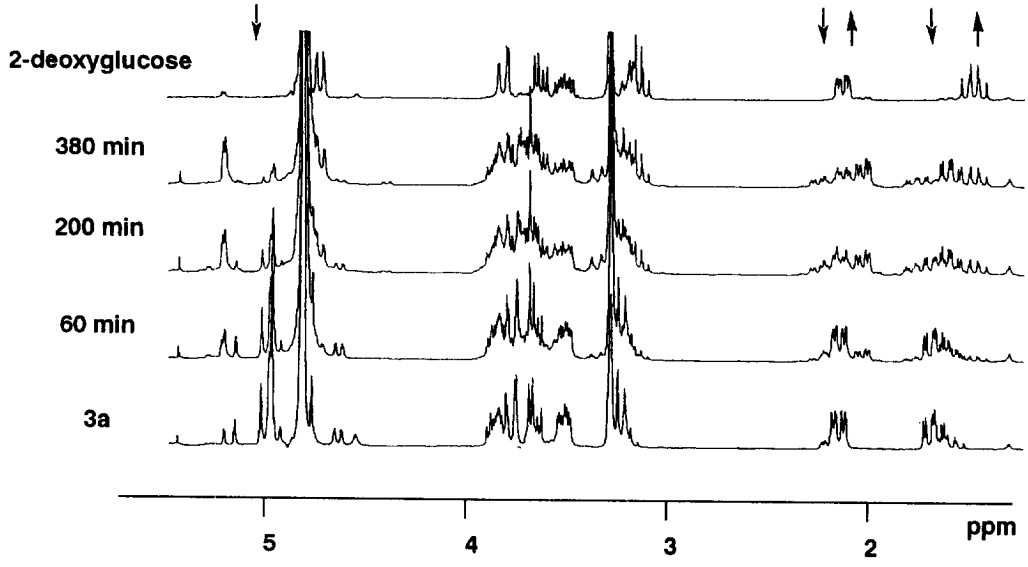

Figure 2. Time-dependent change in the ^{1}H NMR spectra of a solution of the caged 2-deoxyglucose **3a** in methanol-d_4 at 0, 60, 200, and 380 min of irradiation. The ^{1}H NMR spectrum of 2-deoxyglucose is also shown. Decrease and increase of the signals due to C2 protons and benzyl protons of **3a** as well as C2 protons of a 2-deoxyglucose was indicated by arrows.

High water solubility and stability in a physiological salt solution are also required for the application of caged compounds to biological system. Estimated from UV-vis spectra of a saturated solution, **3a** and **3b** dissolve into PBS up to 2 M and 0.8 M, respectively. HPLC analysis showed that more than 90% of **3a** and **3b** was remained intact after 1 week of standing in a PBS solution (500 μM) at room temperature in the dark. Moreover, no anisaldehyde-positive spots of TLC other than **3a** were observed even after a solution of **3a** in PBS was standing 60 days in the dark.

Thus, photoreactivity, stability and solubility in PBS of these compounds are found to be favorable for biological application. Quantitative analysis of the generation of a 2-deoxyglucose during photolysis of **3** is currently under investigation. We are also attempting to assay the efficiency of these caged compounds as phototrigers of a 2-deoxyglucose using 2-deoxyglucose sensitive cell lines.

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