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Synthesis of Isobutyl-*C*-galactoside (IBCG) as an Isopropylthiogalactoside (IPTG) Substitute for Increased Induction of Protein Expression

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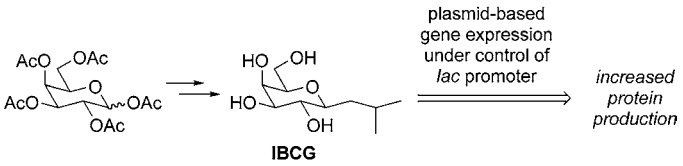
ABSTRACT



IBCG

Addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to bacterial cultures is often used to induce expression of plasmid-based genes for the production of recombinant proteins under control of the lac promoter, but a simple method to circumvent the inherent instability of this compound has not been addressed experimentally. Herein we report the first synthesis of isobutyl-*C*-galactoside (IBCG), the *C*-glycoside analogue of IPTG, and show that IBCG is superior to IPTG in inducing protein expression over long induction times.

Addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to bacterial cultures is a long-standing way to induce expression of plasmid-based genes for the production of recombinant proteins under the control of the *lac* promoter, but is not without its share of problems.[1] The *S*-glycoside analogue binds to the *lac* repressor in *Escherichia coli*, thereby preventing binding of the repressor protein to DNA and blocking gene transcription (Figure 1).[2] From the standpoint of experimental practicality, IPTG and its solutions should be stored below room temperature to prevent decomposition over time. In addition, multiple additions of IPTG are often necessary for longer induction times as the compound degrades under culture conditions. A more stable version of IPTG would be desirable to circumvent these issues and provide greater control of protein expression, especially over long periods of induction. Herein we report the first synthesis of the *C*-glycoside analogue of IPTG and show that it cannot only serve as a functional replacement of IPTG in inducing expression of proteins under control of the *lac* promoter, but it actually appears to be superior to IPTG for long induction times.

Replacement of the C−O anomeric linkage in naturally occurring *O*-glycosides with a C−S or C−C bond is known to engender hydrolytic stability, providing derivatives capable of adopting similar conformations and hence retaining

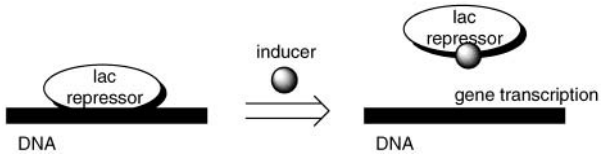


Figure 1. Small molecules such as IPTG, allolactose, and IBCG can bind to the lac repressor to allow gene transcription machinery access to DNA for the production of proteins.

(1) For example, see: (a) Sanden, A. M.; Prytz, I.; Tubulekas, I.; Forberg, C.; Le, H.; Hektor, A.; Neubauer, P.; Pragai, Z.; Harwood, C.; Ward, A.; Picon, A.; Teixeira de Mattos, J.; Postma, P.; Farewell, A.; Nystrom, T.; Reeh, S.; Pedersen, S.; Larsson, G. *Biotechnol. Bioeng.* **2003**, *81*, 158−166. (b) Boos, W.; Schaedel, P.; Wallenfels, K. *Eur. J. Biochem.* **1967**, *1*, 382−394. (c) Burstein, C. *Bull. Soc. Chim. Biol.* **1965**, *47*, 1901−1904. (d) Ariatti, M.; Gupthar, A. S.; Zemlicka, J. *Med. Sci. Res.* **1993**, *21*, 769−770.

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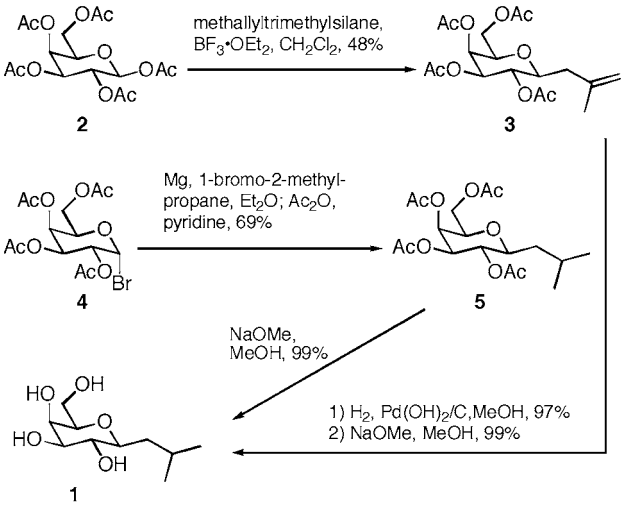
Scheme 1. Synthesis of IBCG[a]



function.[3] However, the *S*-glycosidic linkage suffers from its susceptibility to both chemical and enzymatic oxidation and cleavage reactions. The *S*-glycoside IPTG serves as an analogue of allolactose, which is formed from lactose in the bacterial cell. Apparently, only the galactose portion of the larger carbohydrate is necessary to disrupt the repressor protein/DNA binding interaction. Substitution of the 6-OH of galactose with methyl or hydrogen, for instance, destroys its induction capabilities.[1b] In contrast, the sulfur of the galactosidic bond is not implicated in a particular binding interaction that would be destroyed in the absence of lone pairs as seen by inspection of the recent X-ray structure of the *E. coli lac* repressor bound to IPTG.[4] Therefore, substitution of the anomeric atom appeared feasible provided the increased conformational mobility of the carbon side chain did not interfere with binding or significantly diminish cell permeability.

Synthetic routes to IPTG[5] do not lend themselves directly to the synthesis of the *C*-glycoside. Fortunately, recent advancements in the synthesis of these types of saccharide structures provide many practical options. Two synthetic routes to the isobutyl-*C*-galactoside (IBCG, **1**) analogue were pursued in parallel (Scheme 1). In the first, treatment of galactose pentaacetate **2** with methallyltrimethylsilane in the presence of boron trifluoride etherate[6] gave a mixture of α and β anomers, which were separated by silica gel column chromatography to provide the β-anomer **3** in 48% yield. While this scheme did provide the desired compound after hydrogenation of the alkene and Zemplen deacylation, separation of the anomeric mixture was tedious. Alternatively, an approach relying on a Grignard reaction previously applied to glucose derivatives[7] was developed. Commercially available bromoacetogalactose[8] **4** was treated with excess isobutylmagnesium bromide to provide exclusively the desired β-anomer of the *C*-linked glycoside. A proton−proton COSY NMR spectrum[9] confirmed the anomeric configuration of the isolated product by evidence of the 9 Hz coupling between protons 1 and 2 of the galactose ring. Deprotection of the acetyl groups with sodium methoxide resulted in the desired IBCG.[9] The latter route easily allowed gram-scale synthesis of the analogue.

To compare the ability of the *C*-glycoside to take the place of IPTG in the induction of protein expression, assays of promoter activity that rely on production of a fluorescent protein were pursued.[10−13] Reporters for gene activity in bacteria that rely on green fluorescent protein (GFP) and its variants have become very popular as they have been validated by direct comparison to traditional reporter assays that use either chloroamphenicol acetyltransferase[10] or β-galactosidase.[11] For bacterial studies, the enhanced green fluorescent protein[12] (EGFP) is particularly useful as it is not toxic to *E. coli* like the wild-type GFP itself and the protein folds and autocatalyzes formation of its fluorophore with a half-life of less than 45 min rather than hours. Therefore, the gene for EGFP (BD Biosciences) was ligated into a pET vector plasmid, using NcoI and EcoRI to have control of EGFP protein expression with a *lac* promoter system and to have low background protein expression levels. This plasmid was transformed into *E. coli* BL21 DE(3) cells for protein expression studies. The cells were grown to an optical density of 0.7 (at 600 nm) and then protein production was induced with either IBCG or IPTG. The fluorescence emission at 507 nm after excitation at 488 nm, normalized for the number of cells, was plotted as a function of time (Figure 2) as previously reported.[13] As expected, in the absence of IBCG and IPTG, the culture showed no activity at the *lac* promoter. In contrast, EGFP fluorescence was considerably larger among induced cells in the presence of IBCG or IPTG. After induction times of greater than 4 h, the IBCG induced cells show even greater fluorescence than the IPTG induced cells. This surprising result suggests that the *C*-glycoside analogue may act as a superior promoter or that it is more stable in the culture conditions over time.

Clearly, the more stable *C*-glycoside analogue IBCG serves as a surrogate for the commonly used inducer of

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(9) See Supporting Information.

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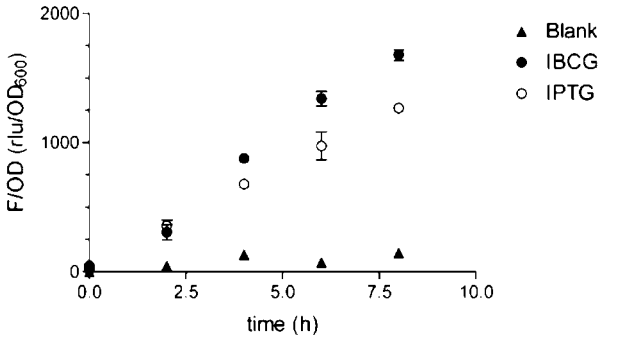

Figure 2. Comparison of the expression of the enhanced green fluorescent protein under *lac* promoter control in BL21 DE(3) cells over time by the increase of fluorescence emission (at 507 nm after excitation at 488 nm) corrected for cell densities: blank, uninduced cells; IPTG, addition of 5.78 mM final concentration of IPTG; and IBCG, addition of 5.78 mM final concentration of IBCG. Experiments were run in duplicate. Rlu = relative light units.

protein expression IPTG and shows advantages at long induction times. In addition, solutions of the analogue do not need to be made as frozen aliquots, but can be autoclaved in water and stored at room temperature for ready addition of the inducer to cell cultures. The latter property is especially valuable for small-scale culture induction and for cases in which a more precise concentration of inducer is desirable. These results and the earlier X-ray structural work are both consistent with the supposition that the anomeric linkage of the natural inducer allolactose does not form any critical binding interactions with the *lac* repressor protein. In addition, the *C*-glycoside retains cell permeability. This work suggests that, in studies on biological systems dependent on *O*-glycosides, lone pairs in the glycosidic linkage are not necessarily required and the *C*-glycosides may offer distinct advantages beyond stability over *S*-glycosides in mimicking carbohydrate activities and functions.

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Supporting Information Available: Experimental procedures and copies of ^{1}H, ^{13}C, and $^{1}H-^{1}H$ COSY NMR spectra for the synthesis of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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