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Multigram Synthesis of Isobutyl-β-C-galactoside as a Substitute of Isopropylthiogalactoside for Exogenous Gene Induction in Mammalian Cells

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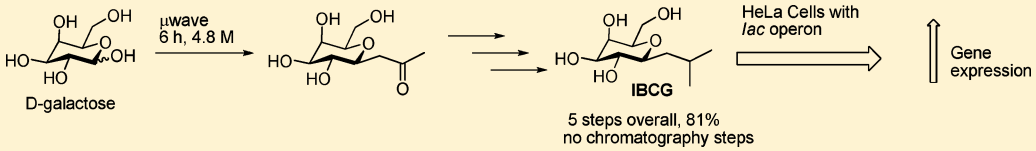
Supporting Information



ABSTRACT: Herein we report that isobutyl-β-C-galactoside (IBCG) is also a promising inducer of gene expression in mammalian cells and report a new synthetic route to the compound that should make obtaining the multigram quantities of material required for animal studies more feasible. A convenient synthesis of IBCG, an inducer of genes controlled by the *lac* operon system in bacterial cells, was achieved in 5 steps from galactose in 81% overall yield without any chromatographic separation steps. An optimized microwave-assisted reaction at high concentration was key to making the C-glycosidic linkage. A Wittig reaction on a per-O-silylated rather than per-O-acetylated or -benzylated substrate proved most effective in installing the final carbon atom.

INTRODUCTION

Genetic engineering demands a tight regulation system to control the expression of the introduced exogenous genes, and this requirement is generally achieved via inducible gene expression systems.[1−3] Among the current available inducible systems, *lac* operon-based systems are the most widely studied and used.[4,5] Isopropyl-β-D-thiogalactopyranoside (IPTG), a lactose analogue, is routinely used as an inducer of the *lac* operon in bacterial systems for *in vitro* studies to induce the expression of exogenously introduced genes.[4,6] This sugar analogue binds to *lac* repressor to activate the gene transcription machinery (Figure 1). Studies have also shown that this inducible *lac* operon/repressor system can be implemented in mammalian cells and animal systems.[7−10] Such an inducible system in mammalian cells ensures the exogenous genes are expressed only when the inducer is added and tightly regulates the gene transcription. When IPTG is used as the *lac* operon inducer in animal models, the inducer is usually dissolved in drinking water and fed to the animals. Since decomposition products resulting from IPTG have an unpleasant thiol smell, IPTG solutions have to be put into light-protected bottles and carefully monitored.[11,12] Even so, IPTG's rapid clearance and short half-life limit its usage and present a major drawback of using this popular gene induction system in animal systems.[13−15]

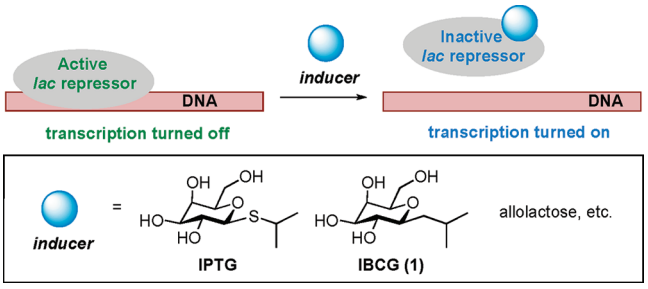


Figure 1. Lactose analogues such as allolactose, IPTG, and IBCG can serve as inducers to bind the *lac* repressor and activate gene transcription in *lac* operon regulated gene transcription.

To circumvent this stability problem, our group has reported the design and synthesis of a new *lac* inducer, isobutyl-β-C-galactoside (IBCG, **1**),[16] as a C-glycoside analogue of IPTG. Not only does this C-glycoside show at least equal gene induction ability in bacterial systems compared to that of the S-glycoside, but replacement of the S-glycosidic bond by a C-glycosidic bond renders the resulting molecule much more stable. Herein we report that IBCG is also a promising inducer of gene expression in mammalian cells and report a new synthetic route to the compound that should make obtaining the multigram quantities of material required for animal studies more feasible.

Previously, the synthesis of IBCG was achieved via either a Lewis acid promoted reaction between galactose pentaacetate and methallyltrimethylsilane or a Grignard reaction between

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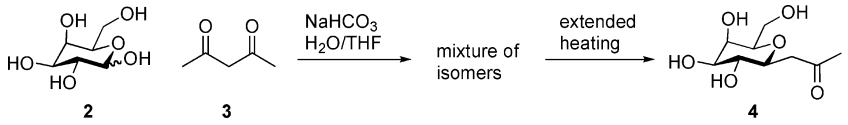
Scheme 1. Comparison of Heating Methods and Reaction Concentrations/Times for the Synthesis of β-*C*-Glycosidic Ketone 4



	Heating method	Reaction time	Concentration	Yield
Previous[28]	Conventional, 90 ℃	24 h	0.15 M	90%
Modified method	Microwave, 70 – 100 ℃	6 h	As high as 4.8 M	92%

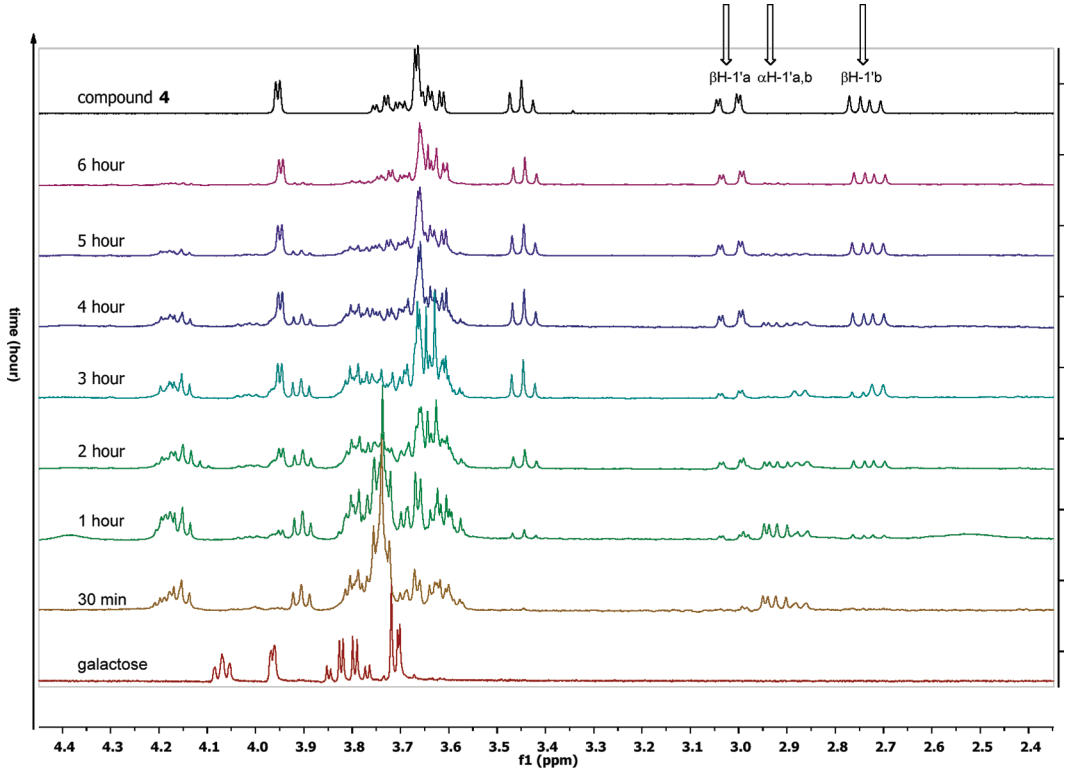


Figure 2. ^{1}H NMR spectra in D_2O of a 10.00 g reaction (2.4 M) under microwave heating conditions. Samples aliquots were removed at 30 min and 1, 2, 3, 4, 5, and 6 h.

bromoacetogalactose and excess isobutylmagnesium bromide.[16] The former method employs a large excess of a relatively expensive material (methallyltrimethylsilane, >$20/g) and shows no α/β selectivity in the *C*-glycosidic bond formation reaction. The latter method utilizes a Grignard approach, which is highly exothermic and cumbersome to scale up. Both of the methods require silica gel chromatography to purify the product. To satisfy the need for larger amounts of IBCG required for animal studies, we hoped to find a new route that could offer a high overall yield on a multigram scale without any chromatography steps.

RESULTS AND DISCUSSION

Obviously key to any successful synthesis of IBCG is the method for installation of the *C*-glycoside. Stereoselective formation of *C*-glycosidic bonds have attracted significant attentions in the recent years.[17,18] Among methods for the synthesis of β-*C*-glycosides, the one-step condensation between free sugars in aqueous solutions and 2,4-pentanedione was particularly attractive as a way to quickly make β-*C*-glycosidic ketones without prior protection of the carbohydrate hydroxyl groups.[19] A mixture of *C*-glycoside stereoisomers are formed in the initial Knoevenagel condensation; however, extended heating under basic conditions allow the equilibrium to shift to the β-*C*-glycosidic pyranose ketone (Scheme 1).[20] Many recent studies utilized this strategy as a starting point to synthesize β-*C*-glycoside analogues.[21−27] Ideally, IBCG could be obtained via a simple methylenation on the ketone by using a Wittg-type reaction. However, even though the carbonyl group in the β-*C*-glycosidic ketones obtained from natural reducing sugars should provide an excellent opportunity to perform C−C bond formation via a Wittig-type reaction to obtain other β-*C*-glycoside analogues, this possibility remains relatively unexplored. Most of the existing studies are based on aldol reactions between the β-*C*-glycosidic ketone and an aldehyde to form an α,β-unsaturated ketone. We found this apparent absence of Wittig-type reaction on β-*C*-glycosidic ketones quite intriguing and speculated that the base sensitivity

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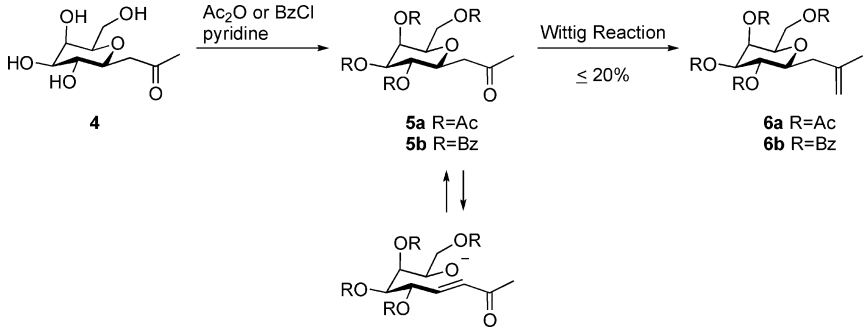
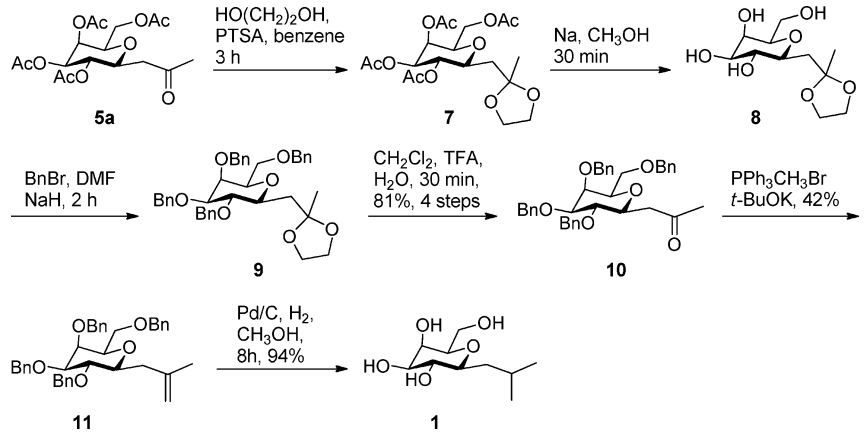
Scheme 2. Wittig Reaction on Acyl-Protected Substrates



Scheme 3. Synthesis of IBCG via a Perbenzylated Ketone



of the ketone substrates might be a contributing factor. We decided to pursue our IBCG synthesis through this route with the hope of developing an efficient method for Wittig reactions on the β-*C*-glycosidic ketones.

First, an optimized synthesis of our desired intermediate ketone 4 had to be developed. The synthesis of **4** has been reported on a 500-mg scale at 0.15 M concentrations of galactose after heating at 90 °C for 24 h.[28] Unfortunately, the long reaction time and dilute reaction conditions made the reaction less desirable for multigram syntheses. We therefore set out to probe the limits of this reaction. By careful monitoring of the reaction by ^{1}H NMR (Figure 2), we found that formation of the *C*-glycosidic bond itself was a relatively fast process; the mixture of isomers (mainly α-pyranose) was then converted to the thermodynamically more stable β-pyranose product **4** slowly upon extended heating. The characteristic peaks of β-pyranose product **4** are two doublets of doublets at δ 3.02 and 2.74 corresponding to the protons from H-1′. The disappearance of peaks corresponding to H-1′ from the α-pyranose product around δ 2.92 indicates the shift of the equilibrium has finished (Figure 2). To possibly accelerate the reaction, microwave irradiation was attempted instead of conventional heating.[29−31] We found that the *C*-glycosidic bond formation was finished under microwave irradiation in only 30 min under reflux at 70−75 °C. The top layer of the mixture containing the excess 2,4-pentanedione was then discarded, and the reaction was heated in the microwave reactor during which time the temperature of the reaction was slowly raised to 100 °C as the excess THF evaporated. After 5.5 h, ^{1}H NMR showed most of the mixture had been converted to the desired β-*C*-glycoside **4** (Figure 2). We also found out that the reaction could be run at much higher concentrations than reported. The reaction was run at a concentration from 1.2 M to as high as 4.8 M instead of the reported 0.15 M with similar results. The water was removed from the reaction mixture under reduced pressure, and then methanol/ethyl acetate was added to separate the product from extra sodium bicarbonate. The crude products still contained some sodium acetate but could be either used directly in the following reaction or purified by passing through a short silica gel plug followed by recrystallization to give the desired β-*C*-glycosidic ketone **4** in 92% yield for a 5-g scale reaction.

With a method for the larger scale synthesis of the key intermediate ketone **4** in hand, only a few steps should allow elaboration of this ketone to the final compound **1**. The synthesis of β-*C*-glycoside **4** utilizes a well-studied property that 2-carbonylalkyl-*C*-glycopyranosides tend to undergo a retro-Michael type addition initiated by the enolate formation and followed by ring opening under mild basic conditions.[32−35] However, this property that makes for a convenient synthesis of β-*C*-glycoside ketones also makes the following transformations under basic conditions troublesome. A straightforward way to do the homologation is to use the Wittig reaction.[36] No precedents for such a Wittig reaction on substrates like compound **4** have been reported. Even though the Wittig reagent is relatively basic,[37] we anticipated that there was a reasonable chance to do the desired methylenation on the base-sensitive ketone substrate if we could fine-tune the electronic properties of the pyranose ring to make the homologation, and not the enolate formation and ring-opening reaction, more favorable. The Tebbe reagent provides a less basic alternative to Wittig regents that could possibly give superior results on a base-sensitive substrate[38] but is less desirable in large-scale reactions due to its high cost. Therefore, we decided to try the

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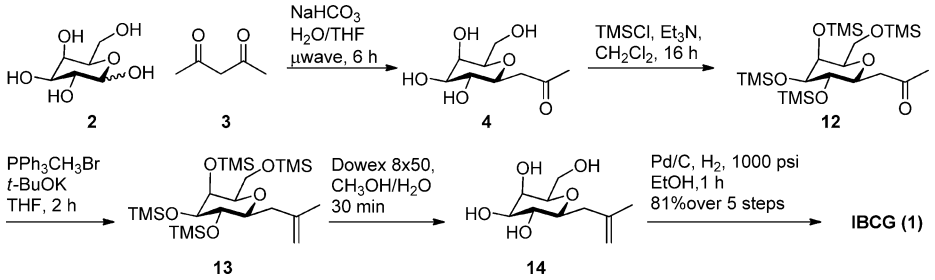
Scheme 4. Revised Synthesis of IBCG Using TMS as a Protecting Group



Wittig reaction on the per-*O*-acetylated ketone **5a**, since the per-*O*-acetylation of ketone **4** was reported to give a high yield easily[28] and provides a readily available starting point for the methylenation. The ketone **4** was per-acetylated using acetic anhydride and pyridine to give per-*O*-acetylated compound **5a**,[28] which was then subjected to a Wittig reaction (Scheme 2). However, no combination of varying base, temperature, or order of addition was found in which the desired alkene **6a** was obtained in over 20% yield. The possible side products in this reaction involved a retro-Michael addition under basic conditions, followed by possible polymerization and decomposition. The less base-sensitive benzoyl group was then installed in place of acetyl groups, but the results were worse. The protected sugar derivative **5b** was obtained in only 30% yield, and the Wittig reactions on **5b** gave the desired product in less than 5% yield. Apparently the Wittig reagent was too basic for use with these peracetylated substrates. Non-basic methylenation conditions using $TiCl_4/Mg/CH_2Cl_2$[39] on substrate **5a** were attempted, but the yield was also low (~20%). A Grignard reaction using methylmagnesium bromide on **5a** to install a methyl group also did not give satisfactory results.

The electron-withdrawing acyl groups appeared to make the retro-Michael addition more favorable, hence the side reactions and low yields. Using more electron-donating groups such as benzyl as the hydroxyl protecting groups might help. Per-*O*-benzylated compound **10** (Scheme 3) has been made previously from epimerization under basic conditions of the corresponding α-ketone,[33] which was obtained via oxymercuration followed by oxidation of α-allyl-*C*-galactoside. Benzylation of **4** seems to be a much more straightforward route to obtain **10**. However, this perbenzylation reaction turned out to be more problematic than expected. Direct benzylation of the ketone **5a** using NaH/BnBr gave complex mixtures, presumably due to ring opening under basic conditions. Similar issues were reported on the glucose ketone substrate.[40] Using NaOH/BnBr in THF with a phase transfer reagent did not give much improvement. We therefore decided to protect the ketone first, although this strategy would add undesirable additional steps to the sequence. Using ethylene glycol, PTSA, and compound **4** in a mixture of acetonitrile/benzene did not yield the ketal, possibly due to the poor solubility of the initial ketone. Interestingly, the glucose analogue could react under similar conditions.[40] The attempt at protecting ketone **4** in methanol as a dimethyl ketal also did not proceed well.

Finally the per-acetylated ketone **5a** was protected to form ketal **7** using ethylene glycol and pyridinium *p*-toluenesulfonate (PPTS) as a catalyst[40] (using TsOH as catalyst led to the decomposition of the substrate) in benzene in 3 h (Scheme 3). Deacetylation, followed by benzylation and ketal removal, gave the per-benzyl substituted ketone **10** in 81% overall yield over 4 steps (Scheme 3).

The Wittig reaction on the per-benzyl substituted ketone **10** proceeded better than on the per-acetyl substituted ketone. Using 2 equiv each of Wittig salt and *t*-BuOK, the alkene **11** was formed in 42% yield. When slightly excess base was used, the yield of **11** dropped significantly. Hydrogenation of **11** gave IBCG **1** in 94% yield. This route yields IBCG from galactose in 8 steps in 30% overall yield. However, this route is still not very satisfactory, given the low yields and long step count.

To further improve the synthesis route, further fine-tuning of the electronic effects was needed. Ideally, the Wittig substrate would be even more electron-rich than the perbenzylated substrate employed. Several examples of protected *O*-glycosides using silyl groups as temporary protecting groups have been reported.[41−45] The per-*O*-TMS protected substrates are readily prepared on large scale, and the ease of deprotection makes them very convenient to use. However, the current studies mainly focused on utilizing the improved solubility of the per-*O*-silylated substrate in organic solvents and on the Lewis acid catalyzed reactions of silyl protected hydroxyl groups. In the latter case, the silyl groups were used as a proton surrogate. Only a few studies used per-*O*-silylation as a method to change the electronic properties of the parent compound. For example, Gervay-Hague's studies of glycosyl iodides[42,46,47] showed that the per-*O*-trimethlysilyl glycosyl iodides were more reactive than per-*O*-benzyl donors in glycosylation reactions. We proposed that by per-silylation we might be able to circumvent the troublesome side reaction encountered in the Wittig reaction by making the ketone substrate more electron-rich.

The per-TMS-silylation of **4** was tested under different conditions, including using pyridine, Et_3N/DMF, and Et_3N/CH_2Cl_2. Fortunately, it was found out that using crude **4** containing large amounts of NaOAc from the condensation did not interfere with the per-silylation reaction. Since ketone **12** has good solubility in hexanes, it could be extracted from the crude mixture directly to give pure product, leaving $Et_3N{\cdot}HCl$ and NaOAc as insoluble salts. The reaction in Et_3N/CH_2Cl_2 is slower than in DMF or pyridine, but the workup process is easier. The Wittig reaction on **12** proceeded surprisingly well and gave the methylenation product **13** in high yield. After solvent removal, the crude mixture of the Wittig reaction containing **13** and triphenylphosphine oxide was redissolved in methanol/water and subjected to acidic silyl group cleavage using Dowex 50X8 (H^+ form). After filtration and solvent removal, the crude product was redissolved in water and extracted with EtOAc to remove triphenylphosphine oxide and provide an aqueous solution of alkene **14**. However, we experienced problems in the hydrogenation of this alkene. Unlike alkenes **6a** or **11**, the hydrogenation of **14** proceeded very slowly; the reaction required more than 48 h to go to

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completion under 1000 psi of H_2. We reasoned that even though the ^{1}H NMR of crude **14** seemed pure, trace amounts of phosphorus-containing compounds left due to the large amount of Wittig salt used in the methylenation reaction might be interfering with the hydrogenation reaction. Therefore, the workup procedure for the Wittig reaction was revisited. Most of the solvent was removed, and then hexane was added to remove most of the triphenylphosphine oxide by filtration. The resulting filtrate was concentrated, and the residue was dissolved in methanol/water and subjected to Dowex 50X8 to provide **14**. After EtOAc/water extraction, the hydrogenation of **14** in ethanol took less than 1 h under high pressure and could be done under atmospheric pressures of H_2 in 8 h to give **1** as a white solid. The ^{1}H NMR of crude **1** showed that it was sufficiently pure and no further purification was needed. By using this modified route, we were able to perform a larger scale synthesis of IBCG from 10.00 g of galactose **2** (55.5 mmol), and obtained 9.90 g of **1** (45.4 mmol) in 81% yield over 5 steps (95% average yield per step) without any chromatography purification (Scheme 4). The large difference in the Wittig reaction using a per-*O*-benzylated substrate versus a per-*O*-silylated substrate shows the significant affect a change in electronic properties of the substrate can make in reaction yields.

Now that a route was available to readily provide the multigram quantities of IBCG necessary to carry out animal studies, the utility of the compound in inducing gene expression in mammalian rather than bacterial cells needed to be ascertained. Mammalian cells such as HeLa cells have less diverse metabolic capabilities compared to bacterial systems, and therefore decomposition of IPTG is less of an issue on the time scale of the experiments. Such issues become problematic when whole animal experiments are envisioned. However, the permeability of IBCG compared to IPTG was unclear. In other words, would IBCG reach the site of action to induce gene expression? To test if IBCG will work as a *lac* operon inducer in mammalian cells in the induction of protein expression, assays of repressor activity utilizing the expression of fluorescent proteins in HeLa cells were preformed (Figure 3). To this end

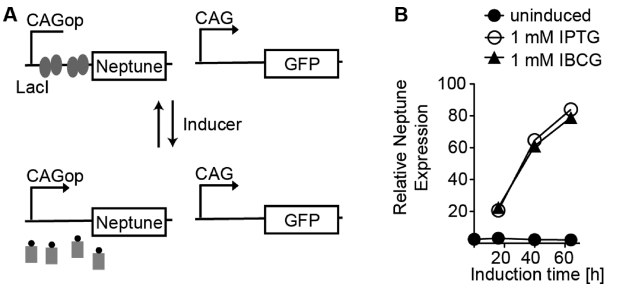

Figure 3. (A) Scheme of promoters used to evaluate the induction capabilities of IPTG and IBCG in HeLa cells. (B) Neptune expression from the CAGop promoter was normalized to GFP expression and to maximal Neptune expression in absence of LacI. The relative Neptune expression after induction with IPTG or IBCG is shown. Fluorescence intensities were determined by flow cytometry. Data shown are the average of two independent experiments.

we cloned a construct placing the far-red fluorescent protein Neptune behind the LacI-repressible Chicken −actin CMV enhancer promoter sequences (CAGop)[48] in between recognition sites for the *Sleeping Beauty* transposase.[49] As control, we used a *Sleeping Beauty* transposon containing green fluorescent protein (GFP) expressed from the original CAG promoter. HeLa cells were made transgenic for these constructs by cotransfection with a plasmid encoding SB100x,[50] an enhanced version of the *Sleeping Beauty* transposase. HeLa cells expressing a fixed ration between Neptune and GFP were purified by cell sorting. We then used SB100x-mediated transposition to make the CAGop-Neptune/CAG-GFP HeLa cells transgenic for LacI (Figure 3A). Expression of lacI resulted in an over 40-fold repression of Neptune expression, while leaving the GFP levels unaltered (data not shown). The addition of the inducers IPTG or IBCG resulted in an increase of Neptune expression over time. Our results showed that IBCG was very well tolerated by HeLa cells at 1 mM concentration and was comparable to IPTG in inducing gene expression (Figure 3B).

In summary, a convenient route for the synthesis IBCG (**1**) from galactose was developed that includes as key steps a microwave-assisted synthesis of an intermediate *β*-*C*-glycoside and a Wittig reaction on a per-*O*-silylated base-sensitive substrate. Microwave-assisted conditions could significantly reduce the reaction time for the *β*-*C*-glycoside formation and also easily allows the reaction to be run at much higher concentrations. The large yield differences of the Wittig reaction on per-acetyl-, per-benzyl-, and per-silyl-protected substrates further demonstrate the profound impact of protecting groups. TMS groups can not only be used to improve solubility of poly hydroxyl bearing compounds and react as a proton surrogate under Lewis acid promoted conditions, they can also change the electronic properties of the substrate greatly. The Wittig reaction on the per-silyl substituted *β*-*C*-glycosidic ketones provides a new and efficient way of synthesizing novel *β*-*C*-glycoside analogues. In addition, the developed route can produce material sufficiently pure for biological studies without a single chromatographic separation. Finally, IBCG has been shown capable of inducing *lac* operon promoters for induction of gene expression not only in bacterial systems but also in mammalian cells. This improved route amenable to larger scale production of IBCG coupled with the promising cell-based studies now sets the stage for testing of this system in animal studies.

EXPERIMENTAL SECTION

General Experimental Methods. Reactions were performed using flame-dried glassware under argon using anhydrous solvents unless otherwise noted. Microwave-assisted reactions were performed using a CEM Discover Microwave system. Thin layer chromatography (TLC) was performed using glass-backed silica gel plates w/UV254. Visualization of TLC plates was performed by UV light and 5% sulfuric acid/ethanol. NMR spectra were recorded on a 400 MHz for ^{1}H (100 MHz for ^{13}C) spectrometer. ^{1}H NMR and ^{13}C NMR taken in $CDCl_3$ spectra were referenced to the solvent peak at 7.260 ppm (^{1}H) and 77.0 ppm (^{13}C). Due to the severe overlap of ^{13}C signals from aromatic carbons in the range of 129−127 ppm in the ^{13}C NMR of the tetra-benzyl protected compounds **9** and **11**, only clearly discernible peaks from aromatic carbons on the benzyl groups are reported. The assignments of ^{1}H NMR peaks were made primarily from 2D ^{1}H−^{1}H COSY and edited ^{1}H−^{13}C HSQC spectra. High resolution mass spectra (HRMS, ESI mode) were obtained using a Q-TOF LC−MS.

1-C-(*β*-D-Galactopyranosyl)-propan-2-one (4). To a round-bottom flask were added D-galactopyranose **2** (4.00 g, 22.2 mmol), $NaHCO_3$ (7.40 g, 88.8 mmol), water (20 mL), and THF (10 mL). 2,4-Pentanedione **3** (4.6 mL, 44 mmol, freshly distilled) was added; the reaction started to turn light yellow. The flask was attached to a condenser and put into a microwave reactor. The temperature of the reaction was measured using the external sensor equipped in the

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microwave reactor. The reaction was heated to reflux at 70−75 °C at 80 W with stirring. After 30 min, ^{1}H NMR spectrum showed that all the galactose had been consumed, and the top layer was separated and discarded. The remaining solution was extracted with EtOAc (20 mL). Then the undissolved solid was returned to the flask, and the aqueous solution was heated at 75 °C at 80 W for another 30 min, gradually heated to 90 °C for 3 h, and then to 100 °C for another 2 h. The ^{1}H NMR spectrum indicated that the reaction was finished, and the yellow/orange reaction mixture was cooled and concentrated under reduced pressure at room temperature. Methanol/ethyl acetate (1:1) (100 mL) was added to the mixture to dissolve the product, and then the remaining salt was removed by filtration. The solution was concentrated to give a crude product of **4** containing NaOAc, which could be used directly in the following experiments. The crude product was passed through a short column using methanol/ethyl acetate 1:4 as eluting solvent, and recrystallization using methanol/ethyl acetate gave **4** as white crystals (3.21 g, 14.5 mmol). The mother liquor was concentrated and purified by silica gel column chromatography to give additional **4** (1.02 g, 4.6 mmol). ^{1}H NMR data matches data reported in the literature.[28]

The reaction was also performed on a 10.00 g scale and on a 20.00 g scale of galactose at concentrations of 2.4 and 4.8 M, respectively.

1-*C*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-propan-2-one Ethylene Ketal (7). To a solution of **5a** (500 mg, 1.30 mmol) in benzene (15 mL) were added ethylene glycol (0.145 mL, 2.60 mmol) and pyridinium *p*-toluenesulfonate (50 mg, 0.2 mmol). The reaction was heated to reflux with a Dean−Stark apparatus. After 3 h, ^{1}H NMR indicated completion of the reaction. The solvent was removed under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with $NaHCO_3$ (aq), and then dried over Na_2SO_4. Solvents were removed under reduced pressure to provide **7** (550 mg, 1.27 mmol) as a white foam that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (dd, J = 1.2, 3.3 Hz, 1H, H-4), 5.05 (t, J = 9.7 Hz, 1H, H-2), 5.02 (dt, J = 3.3, 9.7 Hz, 1H, H-3), 4.13 (dd, J = 7.2, 11.3 Hz, 1H, H-6a), 4.05 (dd, J = 6.1, 11.3 Hz, 1H, H-6b), 3.98−3.82 (m, 5H, -OCH_2CH_2O-, H-5), 3.64 (dt, J = 1.6, 9,3 Hz, 1H, H-1), 2.14 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.91 (dd, J = 8.5, 14.7 Hz, 1H, H-1′a), 1.75 (dd, J = 1.6, 14.7 Hz, 1H, H-1′b), 1.37 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 170.1, 169.9, 169.7, 108.4, 75.1, 74.0, 72.1, 68.9, 67.7, 64.4, 64.3, 61.8, 39.9, 24.5, 20.6, 20.53, 20.49, 20.42. HRMS(ESI): calcd for $C_{19}H_{28}NaO_{11}$ [M + Na]$^+$ 455.1524, found 455.1517.

1-*C*-(2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl)-propan-2-one Ethylene Ketal (9). Compound **7** (550 mg, 1.27 mmol) was dissolved in CH_3OH (15 mL), cooled to 0 °C, and treated with Na (23 mg, 1.0 mmol). After 2 h, the solvent was removed under reduced pressure to yield crude **8**. The crude product was dissolved in DMF (10 mL) and cooled to 0 °C. NaH (262 mg, 60%, 7.8 mmol) was added, and the reaction was stirred for 30 min. Benzyl bromide (0.8 mL) was added, and the reaction was stirred at room temperature for 6 h before methanol (1 mL) was added. The mixture was extracted with EtOAc, washed with water, and dried over Na_2SO_4. The solvents were removed under reduced pressure to yield **9** as a syrup that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47−7.29 (m, 20H, PhH), 5.05 (d, J = 11.2 Hz, 1H, -OCHPh), 5.01 (d, J = 11.8 Hz, 1H, -OCHPh), 4.82 (d, J = 11.8 Hz, 1H, -OCHPh), 4.74 (d, J = 11.8 Hz, 1H, -OCHPh), 4.73−4.67 (m, 2H, −CH_2Ph), 4.58 (d, J = 11.9 Hz, 1H, -OCHPh), 4.53 (d, J = 11.9 Hz, 1H, -OCHPh), 4.06 (d, J = 2.5 Hz,, H-4), 3.99−3.83 (m, 4H, -OCH_2CH_2O-), 3.75−3.65 (m, 2H, H-2, H-3), 3.64−3.59 (m, 3H, H-6a, H-6b, H-5), 3.52 (t, J = 9.0 Hz, 1H, H-1), 2.19 (d, J = 15.5 Hz, 1H,H-1′a), 1.87 (dd, J = 9.0, 15.5 Hz, 1H,H-1′b), 1.48 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 138.6, 138.4, 138.1, 128.6, 128.49, 128.45, 128.40, 128.37, 128.28, 127.98, 127.91, 127.78, 127.70, 127.67, 127.65, 127.60, 126.99, 109.4, 85.1, 78.6, 77.0, 76.87, 76.83, 73.8, 73.5, 72.4, 69.2, 65.3, 64.39, 64.35, 39.6, 24.7. HRMS(ESI): calcd for $C_{39}H_{44}NaO_7$ [M + Na]$^+$ 647.2979, found 647.2987.

1-*C*-(2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl)-propan-2-one (10). Crude benzyl ketal **9** was dissolved in CH_2Cl_2/TFA/H_2O (10:1:0.1, 10 mL total), and the reaction was stirred for 30 min. The reaction was diluted with CH_2Cl_2, washed with water and saturated $NaHCO_3$ solution, and dried over Na_2SO_4. Silica gel chromatography purification using hexanes/ethyl acetate 5:1 to 3:1 afforded the product as a white solid (675 mg, 1.16 mmol, 89.2% for 4 steps). The ^{1}H and ^{13}CNMR of **10** spectra matches previously reported data.[33]

2-(1-*C*-(2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl) methyl)-propene (11). To a flask containing PPh_3CH_3Br (830 mg, 2.32 mmol) and THF (5 mL) was added *t*BuOK (1 M in THF, 2.9 mL, 2.9 mmol) at 0 °C, and the reaction was stirred for 30 min. A solution of **10** (675 mg, 1.16 mmol) in THF (7 mL) was added dropwise into the reaction, and the mixture was stirred for 8 h. A saturated NH_4Cl solution (10 mL) was added to quench the reaction, and the reaction was extracted with EtOAc, washed with water, and dried over Na_2SO_4. Solvents were removed under reduced pressure, and silica gel column purification using hexanes/ethyl acetate 6:1 to 4:1 offered the product **11** (281 mg, 0.49 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.23 (m, 20H, PhH), 4.96 (d, J = 11.2 Hz, 1H, -OCHPh), 4.95 (d, J = 11.6 Hz, 1H, -OCHPh), 4.77 (m, 2H, C═CH_2), 4.76 (d, J = 12.9 Hz, 1H, -OCHPh), 4.68 (d, J = 12.9 Hz, 1H, -OCHPh), 4.66 (d, J = 11.2 Hz, 1H, -OCHPh), 4.64 (d, J = 11.6 Hz, 1H, -OCHPh), 4.45(ABq, J = 11.8 Hz, 2H, -OCH_2Ph), 3.99 (m, 1H, H-4), 3.67 (t, 1H, J = 8.8 Hz, H-2), 3.63(dd, 1H, J = 2.5, 8.8 Hz, H-3), 3.59−3.48 (m, 3H, H-6a, H-6b, H-5), 3.41 (dt, J = 1.6, 9.4 Hz, H-1), 2.57 (d, 1H, J = 14.6 Hz, H-1′a), 2.25 (dd, 1H, J = 9.4, 14.6 Hz, H-1′b), 1.76 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.1, 138.8, 138.5, 138.4, 138.1, 128.42, 128.36, 128.19, 128.14, 128.02, 127.85, 127.68, 127.65, 127.62, 127.55, 127.53, 112.1, 85.0, 78.9, 78.7, 77.2, 75.4, 74.4, 73.8, 73.5, 72.3, 69.2, 39.8, 23.0. HRMS(ESI): calcd for $C_{38}H_{42}NaO_5$ [M + Na]$^+$ 601.2924, found 601.2939.

Isobutyl-*C*-galactoside (1) by the benzyl ketone route. To a solution of **11** (281 mg, 0.49 mmol) in methanol was added 10% Pd/C (50 mg), and the reaction was stirred under H_2 for 8 h. The reaction was filtered and concentrated to give **1** as a hygroscopic white foam (101 mg, 0.46 mmol, 94%). The ^{1}H and ^{13}C NMR data of **1** matches the reported.[16]

1-*C*-(2,3,4,6-Tetra-*O*-trimethylsilyl-*β*-D-galactopyranosyl)-propan-2-one (12). Crude **4** (22.60 g) was synthesized from galactose (10.00 g, 55.5 mmol), $NaHCO_3$ (18.65 g, 222 mmol), and 2,4-pen-tanedione **3** (11.45 mL, 111 mmol, freshly distilled) in water (50 mL) and THF (25 mL) according to the above procedure. The crude product obtained from methanol/ethyl acetate extraction contained ca. 10.40 g NaOAc as indicated by ^{1}H NMR and used directly.

To a round-bottom flask containing crude **4** were added CH_2Cl_2 (100 mL) and Et_3N (263 mL, 1.89 mol). The reaction was cooled to 0 °C, and freshly distilled chlorotrimethylsilane (48.3 mL, 380 mmol) was added dropwise. The reaction was stirred at ambient temperature for 16 h before the solvents were removed at reduced pressure. Hexanes were added to the mixture, and the solution was filtered through Celite, washed with hexanes, and concentrated to yield crude **12** as a yellow liquid that was used without further purification.

2-(1-*C*-*β*-D-Galactopyranosyl methyl)-propene (14). To a round-bottom flask were added anhydrous PPh_3CH_3Br (29.80 g, 83.4 mmol) and *t*-BuOK (9.36 g, 83.4 mmol). The reaction was cooled to 0 °C, and THF (200 mL) was added via cannula. The resulting yellow solution was stirred at 0 °C for 5 min and warmed to room temperature in 25 min. The solution was stirred at room temperature for another 30 min and recooled to 0 °C. A solution of crude **12** in THF (30 mL) was added dropwise to the reaction over 10 min. After stirring at 0 °C for another 10 min, the ice bath was removed, and the reaction was stirred for 90 min. Acetone (5 mL) was added, and the reaction mixture was partially concentrated to a slurry, diluted with hexanes, and filtered. The filtrate was concentrated to yield crude **13** as a yellow liquid that was used without purification.

The crude product **13** was dissolved in methanol/water (120 mL/10 mL). Dowex 50X8 (H^+ form, 10.00 g) was added, and the reaction was stirred for 25 min, filtered, and concentrated under reduced pressure. The resulting residue was suspended in water (250 mL) and extracted with ethyl acetate (3 × 100 mL). The aqueous layer was concentrated to give crude **14** as a pale yellow liquid that was used

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without further purification. ^{1}H NMR (400 MHz, D_2O) δ 4.70 (d, 2H, J = 9.8 Hz, C=CH_2), 3.79 (d, 1H, J = 3.3 Hz, H-4), 3.58−3.48 (m, 2H, H-6ab), 3.48−3.41 (m, 2H, H-3, H-5), 3.31−3.26 (m, 2H, H-1, H-2), 2.44 (d, J = 15.7 Hz, 1H, H-1′a), 2.03 (dd, J = 9.0, 15.7 Hz, H-1′b), 1.61 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O) δ 143.9, 111.9, 78.5, 77.6, 73.9, 71.0, 70.0, 61.2, 39.2, 21.6. HRMS(ESI): calcd for $C_{20}H_{36}NaO_{10}$ $[2M + Na]^+$ 459.2201, found 459.2201.

Isobutyl-*C*-galactoside (1) from the per-*O*-silylated ketone. Crude **14** was dissolved in ethanol (100 mL) and a slurry of 10% Pd/C (950 mg) in water (5 mL) was added. The reaction was stirred in a Parr apparatus under H_2 at 1000 psi for 1 h. The pressure was dropped to 800 psi and then stayed stable, indicating the hydrogenation had finished. The mixture was filtered and concentrated to give **1** as a white solid (9.90 g, 45.4 mmol, 81% for 5 steps). The ^{1}H and ^{13}CNMR of **1** spectra matches previously reported data.[16]

Induction of *lac* Operon Using IBCG in HeLa cells. HeLa cells were cultured in DMEM (Dubelcco's Modified Eagle Medium) supplemented with 10% FCS (fetal calf serum) and penicillin/streptomycin at 37 °C with 5% CO_2. A detailed description of the construct cloning and generation of transgenic cells will be reported elsewhere. For the induction 1 mM IPTG (Fermentas GmbH) or 1 mM IBCG (both dissolved in water) were added. At the indicated time points HeLa cells were trypsinized and acquired on a FACSCanto II (BD Biosciences). Dead cells were excluded from the analysis by staining with 7-aminoactinomyxin (7-AAD). Data analysis was conducted with FlowJo software (Tree Star). For calculating the normalized Neptune values, mean fluorescent Neptune intensities were divided by the mean fluorescent intensities of GFP in the same cells. This value was then divided by the Neptune to GFP ratio in CAGop-Neptune/CAG-GFP HeLa cells lacking lacI and multiplied by 100.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**, **4**, **7**, **9**, **10**, **11**, and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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