Organic & Biomolecular Chemistry

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 2903

Enzyme-mediated nutrient release: glucose-precursor activation by β-galactosidase to induce bacterial growth

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Received 9th December 2012,
Accepted 24th February 2013

DOI: 10.1039/c3ob27385g

www.rsc.org/obc

Bacteria will gain an advantage if they are able to metabolize nutrients that are inaccessible for other bacteria. To demonstrate this principle, we developed a simple model system, which mimics how bacteria exploit natural carbon sources. A masked glucose precursor that is activated by β-galactosidase was used as a carbon source for bacterial growth in a glucose-deficient medium. No bacterial growth was observed in the presence of control substances in which β-galactosidase mediated cleavage did not lead to glucose release. This study represents a proof-of-principle example in which a bacterium can grow in a nutrient-free medium by inducible, enzyme-mediated nutrient release from a precursor.

Introduction

Bacteria will gain an advantage if they are able to adapt to changing environmental conditions. For instance, an increase in the frequency of antibiotic resistance in bacteria due to random mutations or exchange of genetic material between different species of bacteria has been observed for most major classes of antibiotics used to treat a wide variety of respiratory illnesses, skin disorders, and sexually transmitted diseases.[1] Resistance genes as well as genes encoding for enzymes required for metabolizing nutrients are organized in operons. This regulated, clustered expression ensures that these proteins are not produced constitutively, but that expression is only induced if needed.

Bacteria preferentially exploit carbon sources that are most readily accessible and the expression of enzymes to metabolize secondary carbon sources is usually suppressed.[2] The presence of glucose in the culture medium prevents bacteria from the use of other carbon sources.[3] However, if only secondary carbon sources are available, the enzymes needed for metabolizing these carbon sources become activated to allow for bacterial growth in the absence of glucose.

A well-known gene cluster is the *lac* operon that regulates the transport and metabolism of lactose in *E. coli*. It consists of three genes encoding for the enzymes β-galactosidase (β-gal), lactose permease and thiogalactoside transacetylase. The *lac* operon only becomes activated in the absence of glucose and in the presence of lactose or non-metabolizable lactose metabolites.

Here, we disclose a system utilizing the process by which bacteria exploit natural carbon sources. A novel glucose release-system that is based on a masked glucose precursor can be cleaved by the enzyme β-galactosidase. *E. coli* strains expressing β-gal release glucose from the masked precursor and utilize this non-natural precursor to grow. However, a β-gal cleavage site was required for bacterial growth to underscore the specificity of the system described here.

The system described here may not just allow for release of nutrients, but also may be a basis for the design of novel antibiotic release systems to combat bacterial pathogens. To specifically target resistant bacteria, antibiotics will be released from a masked precursor by the action of resistance proteins.

Results and discussion

The inducible expression of operon-encoded resistance proteins or catabolic enzymes can be exploited to release active components from inactive precursors. In this proof-of-principle study, we focused on the enzyme-catalyzed release of a missing vital nutrient from an available precursor to provide bacteria expressing the respective enzyme with an evolutionary advantage. We employed an *E. coli* strain expressing the

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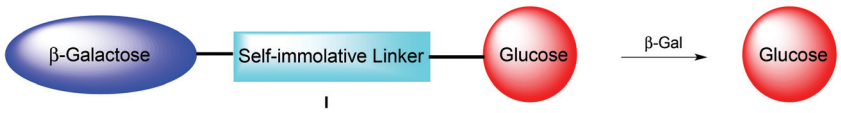

Fig. 1 Masked precursor of glucose activated by β-gal to release free glucose.

lactose-metabolizing enzyme β-galactosidase (β-gal) that grows fast in the presence of the carbon source glucose. Instead of glucose we nourished the *E. coli* with a masked precursor of glucose that can be activated by β-gal or, alternatively, with a control compound that did not allow for β-gal mediated glucose release.

The β-gal activated masked precursor of glucose was designed based on examples of β-gal activated prodrugs (Fig. 1).[4] A β-galactose triggering group is attached to glucose through a self-immolative linker to form precursor type **I**. The precursor is designed to release the glucose moiety upon cleavage of the head β-galactose from the linker.

Self-immolative linkers that "sacrifice" themselves in order to implement their essential function are widely used for drug delivery systems[5–8] and diagnostic applications.[9,10] We have demonstrated the self-immolative disassembly in several molecular systems such as dendrimers[11–15] and polymers[16,17] and shown that such linkers can efficiently disassemble under physiological conditions.

Design and synthesis of a β-gal activated precursor of glucose

The design of the glucose release system relies on a self-immolative aminal-carbamate linkage we developed earlier (Fig. 2).[18] The β-gal activated precursor of **1** is composed of a glucose molecule attached through a self-immolative linker to β-galactose – a substrate for β-gal. Upon incubation of **1** with β-gal, phenol **1a** is obtained and immediately undergoes 1,6-elimination to release aminal intermediate **1b**. The latter is further disassembled through elimination of imminium-methylene to release a free glucose molecule.

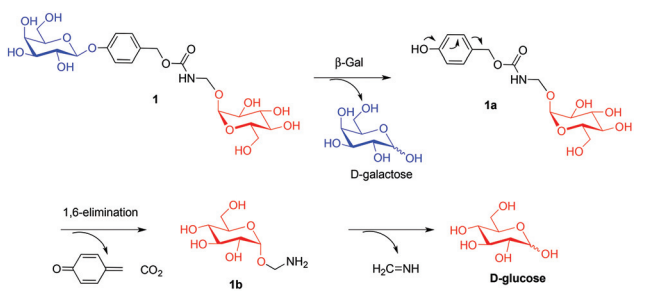

Fig. 2 β-Gal activated glucose precursor to release free glucose.

Compound **1** was synthesized commencing with a Curtius rearrangement of acyl azide **2a** to generate an isocyanate intermediate that was reacted *in situ* with benzylalcohol derivative **2** to afford carbamate **2b** (Fig. 3). Cleavage of the acetate esters using catalytic amounts of potassium carbonate in methanol afforded compound **1**.

As a control compound, we synthesized a masked glucose precursor that cannot undergo activation with β-gal (Fig. 4). Curtius rearrangement of acylazide **2a** generated an isocyanate, which was trapped *in situ* with benzylalcohol to give carbamate **3a**. The latter was subjected to mild basic conditions to afford glucose precursor **3**.

Evaluation of β-gal-mediated glucose release

To determine whether β-gal would release sufficient quantities of glucose from the masked precursor **1** to promote bacterial growth, the β-gal expressing *E. coli* strain BL21(DE3)[19] was grown in the presence of either 0.5 g l^{-1} or 1.0 g l^{-1} glucose or in the presence of the glucose-releasing **1**. The concentration of **1** was normalized such that complete glucose release would correspond to 0.5 g l^{-1} or 1.0 g l^{-1} glucose, respectively. Indeed, substantial bacterial growth was observed in the presence of **1** as the only rapidly metabolizable carbon source (Fig. 5). Bacterial growth was reduced when compared to growth in the presence of glucose indicating that glucose release from the precursor was incomplete. However, bacterial growth in the presence of **1** was markedly increased compared to control compound **3** that does not allow for β-gal-mediated glucose release. This finding indicates that glucose release from compound **1** is indeed specifically induced by β-gal cleavage. Interestingly, β-gal mediated cleavage of control compound **3** releases one D-galactose residue that also represents a carbon source, which can lead to *E. coli* growth. Yet, the respective *gal* operon encoding the galactose-metabolizing enzymes needs to be induced before galactose metabolization can start.[20] Thus, though our system is potentially leaky with respect to D-galactose release from **3**, we did not observe substantial bacterial growth at least during short incubation times up to overnight. This finding indicates that bacterial growth

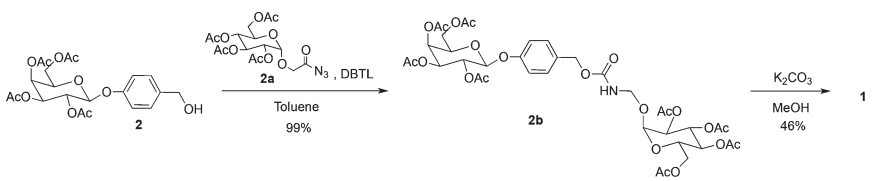

Fig. 3 Chemical synthesis of β-gal activated glucose precursor **1**.

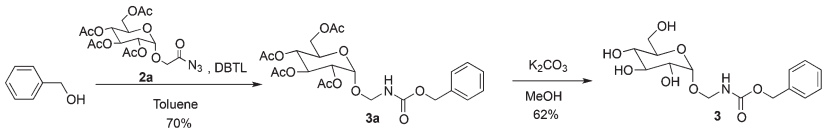

Fig. 4 Chemical synthesis of non-activated glucose precursor **3**.

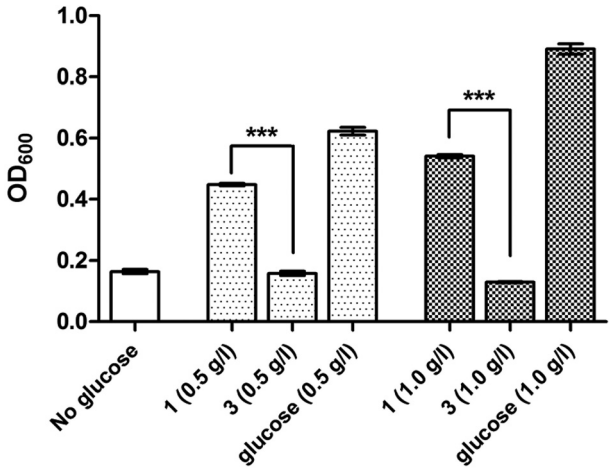

Fig. 5 Bacterial growth of *E. coli* strain BL21(DE3) in an MDG medium containing either glucose, or equivalent concentrations of **1** or the negative control compound **3**. The β-gal expressing *E. coli* strain BL21(DE3) was grown in the presence of glucose, compound **1** or **3** as the only carbon source. While β-gal-mediated glucose release from compound **1** led to substantial bacterial growth, no bacterial growth was observed in the presence of control compound **3**. Data are presented as mean ± SD. Data are representative of two independent experiments.

was indeed specifically mediated by β-gal-mediated glucose release from the masked glucose precursor **1**. It should be noted that in addition to the glucose precursor **1** or control compound **3** the MDG minimal medium also contains vitamins and amino acids that could in theory be exploited as secondary carbon sources by *E. coli*. However, no bacterial growth was observed in minimal medium in the absence of glucose (data not shown) demonstrating that glucose supplementation was essential for bacterial growth.

To further confirm the specificity of the system, we determined the growth rate of an *E. coli* strain lacking β-gal expression (XL-1 blue) in the presence of the masked glucose precursor **1**. As expected, no bacterial growth was observed (data not shown). Though this *E. coli* strain might considerably differ in its growth rate, the experiment provides further evidence for the specificity of the glucose-release system.

Measurement of β-gal expression using a new NIR fluorescent probe

To detect β-gal expression by the *E. coli* strain BL21(DE3), we synthesized a new β-gal probe based on the recently developed near infrared (NIR) fluorescent dye QCy7 (Fig. 6).[21] Probe **4** is composed of a β-galactose substrate attached to the NIR dye through a short self-immolative linker. Cleavage of β-galactose by β-gal generates intermediate **4a**, which further disassembles through 1,6-elimination to release phenolate **4b**. The latter can undergo internal charge transfer to form the NIR fluorescent dye SulfoQCy7.

The synthesis of probe **4** commenced with the bromination of benzylalcohol **2** by carbon tetrabromide and triphenyl phosphine generating benzylbromide derivative **4c** (Fig. 7). Williamson etherification of **4c** with commercially available 4-hydroxy isophthalaldehyde gave ether derivative **4d**. Condensation of dialdehyde **4d** and indolium **5** followed by cleavage of the acetate esters by a catalytic amount of potassium carbonate afforded probe **4**.

β-Gal expression can be up-regulated by lactose or lactose metabolites such as isopropyl-β-D-1-thiogalactopyranoside (IPTG).[19] Since β-gal is only expressed at marginal levels in the *E. coli* strain BL21(DE3), bacteria were grown in the presence of lactose (Fig. 8) or IPTG (data not shown) to induce β-gal expression. To analyze whether β-gal was present in the bacteria or secreted into the medium, bacterial lysates were prepared and β-gal expression was measured in lysates as well as the culture supernatant by adding probe **4**. Indeed, fluorescence measurements indicated only a basal, but detectable

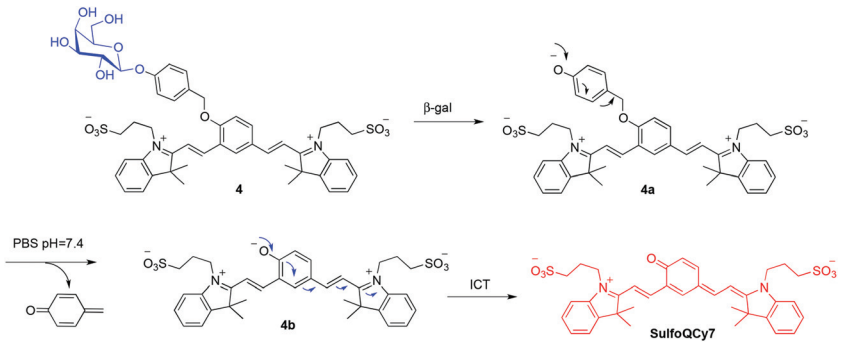

Fig. 6 Mechanism β-gal activated probe **4** to release the NIR fluorescent dye SulfoQCy7.

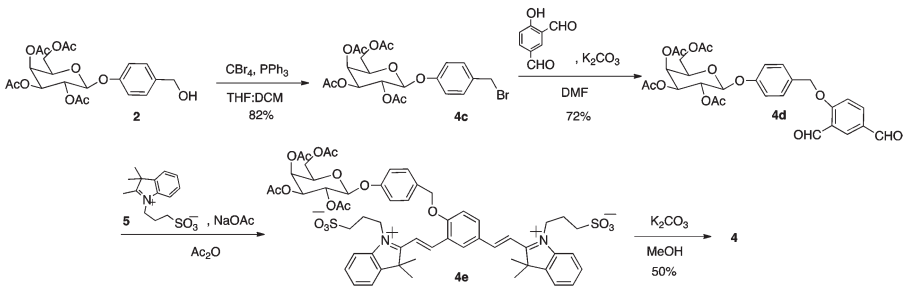

Fig. 7 Chemical synthesis of a β-gal fluorescent probe.

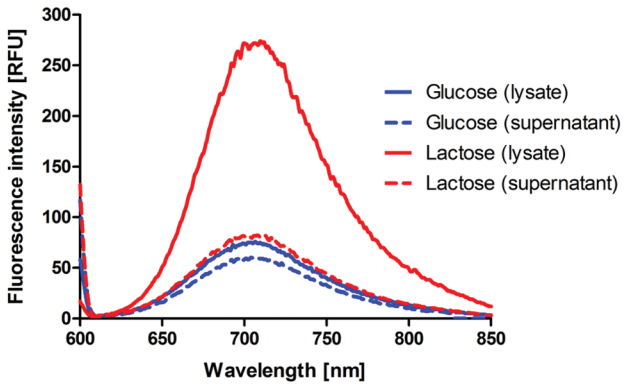

Fig. 8 Fluorescence measurement of β-gal expression by *E. coli* strain BL21 (DE3) using probe **4**. The β-gal expressing *E. coli* strain BL21(DE3) was grown in an MDG medium with 0.5 g l^{-1} glucose, or with 0.5 g l^{-1} lactose to further enhance β-gal expression. Bacteria were disrupted and β-gal expression was measured either in bacterial lysate or supernatant by adding 50 μM of probe **4**. Samples were excited at 590 nm and conversion into SulfoQCy7 was measured by recording the fluorescence spectrum between 600 nm and 850 nm. Only marginal expression of β-gal was observed in bacterial lysate (blue curve, solid) and supernatant (blue curve, dashed) when bacteria were grown in the presence of glucose. When bacteria were grown in lactose as the only carbon source, only a slightly increased β-gal level was detected in the supernatant (red curve, dashed). However, a markedly increased β-gal expression could be measured in the bacterial lysate (red curve, solid). The diagram is representative of two independent experiments.

expression of β-gal both in bacterial lysates and in the supernatant when bacteria were grown in the presence of glucose as the only metabolizable carbon source (Fig. 8). Notably, β-gal expression was sufficient to promote bacterial growth in the presence of compound **1** (ref. Fig. 5). Lactose led to an increased β-gal expression, which was mainly detected in the bacterial lysate indicating that β-gal is only marginally released into the supernatant.

To further demonstrate that probe **4** is indeed valuable for measuring β-gal expression, the *E. coli* strain BL21(DE3) was again grown in either glucose- or lactose-containing medium and β-gal was visualized by confocal microscopy (Fig. 9). No β-gal expression was detected in fixed bacteria that had been grown in the presence of glucose suggesting that the limit of detection in the confocal microscopy measurement was higher compared to spectrometric fluorescence measurement (ref. Fig. 8). However, lactose again induced substantial β-gal expression thus confirming the results obtained in the fluorescence measurement assay. This finding further demonstrates the utility of probe **4** to detect β-gal expression.

Conclusions

In summary, we have designed and synthesized a masked glucose precursor that can be activated by β-galactosidase. This glucose precursor is sufficient for bacterial growth in a glucose-deficient medium. Glucose release was specifically mediated by β-gal as demonstrated by a control compound where glucose release was impossible. A new β-gal-activated NIR fluorescent probe was employed to detect overexpression of β-gal by confocal fluorescence microscopy. This proof-of-principle study demonstrates that bacteria can accomplish an evolutionary advantage. By adapting the teachings of this study, we are currently designing an antibiotic release system for activation by bacterial resistance proteins. Antibiotic release from the precursor and "induced suicide" would only occur in the presence of the resistance protein as a selective tool to fight the bacterial defense mechanism.

Materials and methods

Synthesis

General methods. All reactions requiring anhydrous conditions were performed under an argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin layer chromatography (TLC): silica gel plates Merck 60 F_{254}: compounds were visualized by irradiation with UV light. Flash chromatography (FC): silica gel Merck 60 (particle size 0.040–0.063 mm), eluent given in parentheses. High pressure liquid chromatography (HPLC): C18 5u, 250 × 4.6 mm, eluent given in parentheses. Preparative HPLC: C18 5u, 250 × 21 mm, eluent given in parentheses. ^{1}H-NMR spectra were measured using a Bruker Avance operated at 400 MHz as mentioned. ^{13}C-NMR spectra were measured using a Bruker Avance operated at 100 MHz as mentioned. The chemical shifts are expressed in δ relative to TMS (δ = 0 ppm) and coupling constants J in Hz. The spectra were recorded in $CDCl_3$ as a solvent at room temperature

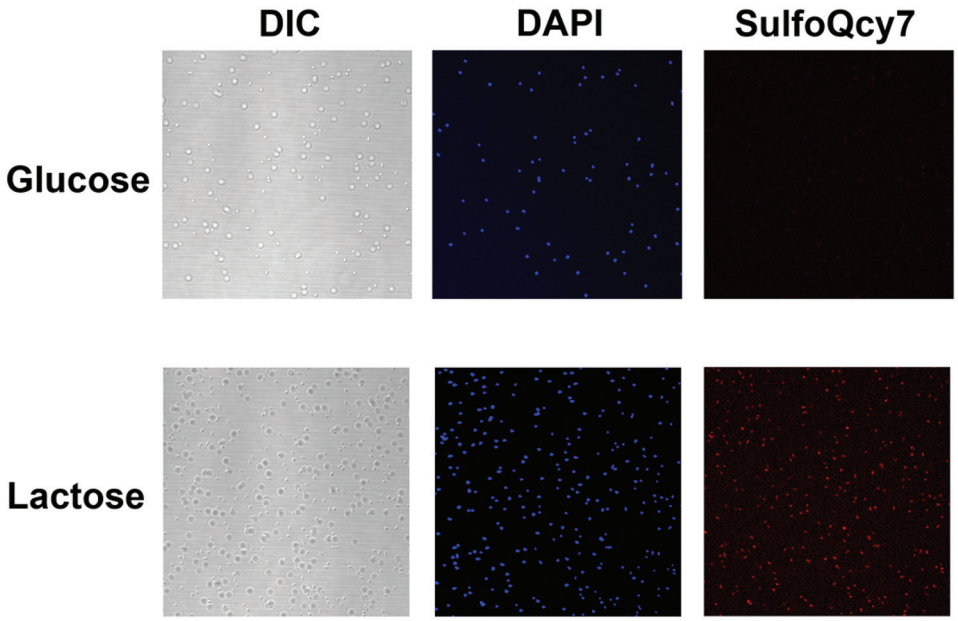

Fig. 9 Confocal fluorescence microscopy measurement of β-gal expression by *E. coli* strain BL21(DE3) using probe **4**. The β-gal expressing *E. coli* strain BL21(DE3) was grown in an MDG medium with 1.0 g l^{-1} glucose, or with 1.0 g l^{-1} lactose to enhance β-galactosidase expression. Cells were pelleted and fixed before 200 μM of probe **4** was added for β-gal detection. In addition, DAPI was used for staining bacterial DNA. The fluorescence of the cells was visualized by confocal microscopy. While no β-gal expression was detected in fixed bacteria grown in the presence of glucose, lactose induced a substantial β-gal expression as measured with probe **4**. The experiment was repeated twice with similar results.

unless stated otherwise. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich.

Compound 2b. Compound **2**[22] (150 mg, 0.33 mmol) was dissolved in 4 ml dry toluene. Compound **2a**[18] (240 mg, 0.55 mmol) was added, followed by a catalytic amount of DBTL. The reaction mixture was heated to 80 °C, stirred for 30 min under an Ar atmosphere, and monitored by TLC dyed with CAM (EtOAc–Hex 60 : 40). After completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (THF–Hex 40 : 60) to give compound **2b** (293 mg, quan) as a viscous clear liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (2H, d, J = 7.9 Hz), 7.12 (2H, d, J = 7.9 Hz), 5.86–5.84 (1H, m), 5.63–5.56 (2H, m), 5.40 (1H, d, J = 3.8 Hz), 5.26–5.17 (5H, m), 5.15 (2H, s), 4.97 (1H, dd, J = 10.0, 3.4 Hz), 4.37–4.19 (6H, m), 2.39 (3H, s), 2.35 (3H, s), 2.19 (6H, s), 2.17–2.14 (12H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.44, 171.14, 171.13, 171.03, 170.86, 170.36, 170.15, 158.92, 152.25, 136.49, 131.55, 130.81, 129.22, 128.98, 126.26, 117.72, 100.23, 95.22, 71.74, 71.53, 71.23, 70.68, 69.32, 69.11, 68.40, 67.57, 67.46, 62.61, 62.02, 23.43, 21.92, 21.41, 21.36. MS (ESI): m/z calc. for $C_{37}H_{47}NO_{22}$: 857.3; found: 880.3 $[M + Na]^+$. HPLC grad. 10–90% ACN in water 20 min: 17.9 min, λ = 220 nm.

Compound 1. Compound **2b** (270 mg, 0.31 mmol) was dissolved in 15 ml MeOH. K_2CO_3 (174 mg, 1.2 mmol) was added to the suspension and the reaction mixture stirred at room temperature for 2 h and monitored by RP-HPLC (grad. 10% to 90% ACN in water, 20 min). After completion, the reaction mixture was concentrated under reduced pressure and diluted with MeOH–H_2O–AcOH (1 : 1 : 0.1). The crude product was purified by preparative RP-HPLC (grad. 0–20% ACN in water, 20 min) to give probe **1** (74 mg, 46%) as a white solid.

^{1}H NMR (400 MHz, MeOD): δ = 7.30 (2H, d, J = 8.4 Hz), 7.08 (2H, d, J = 8.4 Hz), 5.05 (2H, bs), 4.95–4.78 (5H, m), 4.70 (1H, d, J = 8.6 Hz), 3.90 (1H, d, J = 4.5 Hz), 3.81–3.57 (9H, m). ^{13}C NMR (100 MHz, MeOD): δ = 162.94, 162.55, 162.48, 162.34, 162.16, 158.41, 158.28, 131.05, 129.96, 128.71, 117.03, 102.12, 97.41, 77.39, 76.28, 74.29, 74.13, 73.34, 72.58, 71.56, 70.94, 70.09, 69.55, 66.84, 61.82, 61.73. MS (ESI): m/z calc. for $C_{21}H_{31}NO_{14}$: 521.2; found: 544.2 $[M + Na]^+$, HRMS: m/z calc. 544.1642; found 544.1643. HPLC grad. 0–20% ACN in water 20 min: 14.5 min, λ = 220 nm.

Compound 3a. Compound **2a** (80 mg, 0.18 mmol) was dissolved in 4 ml of dry toluene. Commercially available benzylalcohol (25.9 mg, 0.24 mmol) was added, followed by a catalytic amount of DBTL. The reaction mixture was heated to 80 °C, stirred for 30 min under an Ar atmosphere and the progress was monitored by TLC (EtOAc–Hex 40 : 60). After completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc–Hex 30 : 70) to give compound **3a** (67 mg, 70%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.33 (5H, m), 5.54 (1H, t, J = 9.8 Hz), 5.19 (2H, d, J = 3.1 Hz), 5.16 (2H, s), 5.04 (1H, t, J = 9.8 Hz), 4.88 (1H, dd, J = 10.2, 3.1), 4.31–4.01 (4H, m), 2.05 (3H, s), 2.03 (3H, s), 2.01 (3H, s), 2.05 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.37, 170.77, 170.30, 156.83, 136.68, 129.24, 128.94, 128.56, 96.48, 71.59, 71.15, 70.64, 69.03, 68.27, 67.75, 62.48, 23.56, 21.29, 21.21, 21.17. MS (ESI): m/z calc. for

$C_{23}H_{29}BNO_{12}$: 511.48; found: 534.2 $[M + Na]^+$. HPLC grad. 10–90% ACN in water 20 min: 9.4 min, λ = 260 nm.

Compound 3. Compound **3a** (50 mg, 0.10 mmol) was dissolved in 5 ml MeOH. A catalytic amount of K_2CO_3 was added to the suspension and the reaction mixture stirred at room temperature for 60 min, and the progress was monitored by RP-HPLC (grad. 10% to 90% ACN in water, 20 min). After completion, the reaction mixture was diluted with 2.5 ml MeOH, 2.5 ml water and 0.5 ml AcOH. The crude product mixture was purified by preparative RP-HPLC (grad. 10% to 90% ACN in water, 40 min) to give compound **3** (32 mg, 62%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$+ drop of MeOD): δ = 7.30–7.25 (5H, m), 5.05 (2H, s), 5.01 (2H, d, J = 3.1 Hz), 4.78 (1H, t, J = 8.6 Hz), 4.67 (1H, t, J = 6.3 Hz), 3.74–3.59 (4H, m), 3.51 (1H, dd, J = 10.2, 3.1), 3.43 (1H, t, J = 9.2). ^{13}C NMR (100 MHz, MeOD): δ = 171.85, 158.13, 137.32, 128.91, 128.32, 128.25, 97.68, 74.35, 73.39, 72.65, 70.89, 70.31, 67.13, 61.85. MS (ESI): m/z calc. for $C_{15}H_{21}NO_8$: 343.13; found: 366.1 $[M + Na]^+$. HPLC grad. 10–90% ACN in water 20 min: 5.0 min, λ = 260 nm.

Compound 4c. Compound **2** (130 mg, 0.28 mmol) was dissolved in 4 ml DCM–THF 1 : 1. PPh_3 (173 mg, 0.66 mmol) was added and after 3 minutes CBr_4 (218 mg, 0.66 mmol) was added. The reaction mixture was stirred for 10 minutes at room temperature under an Ar atmosphere and the progress monitored by TLC (EtOAc–Hex 40 : 60). After completion, the reaction mixture was evaporated under reduced pressure. The crude product was filtered through a small silica gel column (EtOAc–Hex 40 : 60) and taken for the next step without further purification.

Compound 4d. Crude **4c** (82 mg, 0.16 mmol) was dissolved in 1 ml dry DMF and commercially available 4-hydroxy-isophthalaldehyde (23.8 mg, 0.16 mmol) was added and the solution stirred at room temperature overnight under an Ar atmosphere and the progress was monitored by TLC (EtOAc–Hex 30 : 70). After completion, the reaction mixture was diluted with EtOAc, and was washed with saturated ammonium chloride solution. The organic layer was separated, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc–Hex 30 : 70) to give compound **4d** (54 mg, 63%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.53 (1H, s), 9.97 (1H, s), 8.37 (1H, d, J = 2.2 Hz), 8.13 (1H, dd, J = 8.7, 2.2 Hz), 7.40 (2H, d, J = 8.6 Hz), 7.22 (2H, d, J = 8.7 Hz), 7.07 (2H, d, J = 8.6 Hz), 5.50 (1H, dd, J = 10.5, 2.5 Hz), 5.48 (1H, d, J = 2.6 Hz), 5.26 (2H, s), 5.13 (1H, dd, J = 10.5, 3.4 Hz), 5.09 (1H, d, J = 7.9 Hz), 4.27–4.15 (2H, m), 4.10 (1H, t, J = 6.6 Hz), 2.21 (3H, s), 2.09 (3H, s), 2.08 (3H, s), 2.04 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 190.88, 189.22, 171.09, 170.98, 170.87, 170.13, 165.61, 157.84, 136.32, 132.78, 130.59, 130.51, 129.93, 125.82, 117.97, 114.28, 100.18, 71.81, 71.48, 71.36, 69.27, 67.51, 62.00, 21.49, 21.42, 21.34. MS (ESI): m/z calc. for $C_{29}H_{30}O_{12}$: 570.5; found: 609.2 $[M + K]^+$.

Compound 4e. A mixture of compound **4d** (54 mg, 0.1 mmol), NaOAc (17.2 mg, 0.21 mmol) and compound **5**[21] (59 mg, 0.21 mmol) was dissolved in 1 ml Ac_2O. The reaction mixture was stirred for 60 minutes at 80 °C under an Ar atmosphere and the progress was monitored by RP-HPLC (grad. 10%–90 ACN in water, 20 min). After completion, the reaction mixture was concentrated by evaporation under reduced pressure and the crude product was taken to the next step without further purification. HPLC grad. 10–90% ACN in water 20 min: 15.1 min, λ = 426 nm.

Compound 4. The acetate derivative **4e** was dissolved in 10 ml MeOH. K_2CO_3 (cat. amount) was added to the suspension and the reaction mixture stirred at room temperature for 60 min and the progress was monitored by RP-HPLC (grad. 10%–90 ACN in water, 20 min). After completion, the reaction mixture was diluted with 3 ml MeOH, 3 ml H_2O, 600 µl AcOH, and purified by preparative RP-HPLC (grad. 10%–90 ACN in water, 20 min) to give compound **4** (44 mg, 51%) as an orange solid.

^{1}H NMR (400 MHz, MeOD): δ = 9.16 (1H, s), 8.68 (1H, d, J = 16.4 Hz), 8.55 (1H, d J = 16.2 Hz), 8.35 (1H, dd, J = 8.6, 2.2 Hz), 8.13 (1H, d, J = 16.4 Hz), 8.04 (1H, d, J = 16.2 Hz), 7.93–7.49 (11H, m), 7.23 (2H, d, J = 8.6 Hz), 5.37 (2H, s), 4.98–4.95 (4H, m), 3.97–3.56 (7H, m), 3.14–2.86 (4H, m), 2.52–2.22 (4H, m), 2.14 (6H, s), 1.91 (6H, s). ^{13}C NMR (410 MHz, MeOD): δ = 183.47, 183.22, 163.58, 158.98, 154.54, 148.92, 144.70, 144.63, 141.44, 138.98, 133.61, 130.53, 130.36, 130.27, 130.02, 128.96, 128.38, 124.81, 123.44, 117.43, 115.69, 115.40, 114.86, 114.50, 102.05, 76.42, 74.19, 72.11, 71.64, 69.60, 61.87, 53.26, 46.35, 45.99, 30.04, 26.46, 26.22, 24.88, 23.56, 22.09. MS (ESI): m/z calc. for $C_{49}H_{56}N_2O_{13}S_2^-$: 945.1; found: 967.3 $[M + Na]^+$. HPLC grad. 10–90% ACN in water 20 min: 10.6 min, λ = 426 nm.

Minimal medium

The medium used was based on a minimal medium for growing freezer stocks and working cultures (MDG).[23] The medium was prepared freshly by mixing autoclaved stocks of salts as 20× (0.5 M) $Na_2HPO_4{\cdot}7H_2O$ (Sigma-Aldrich), 20× (0.5 M) KH_2PO_4 (VWR), 20× (1 M) NH_4Cl (Serva), 20× (0.1 M) Na_2SO_4 (Merck) and 500× (1 M) $MgSO_4{\cdot}7H_2O$ (Fluka). A 1000× trace metal solution was added, consisting of 50 µM $FeCl_3{\cdot}6H_2O$ (Sigma), 20 µM $CaCl_2$, 10 µM $MnCl_2{\cdot}4H_2O$ (Alfa-Aesar), 10 µM $ZnSO_4{\cdot}7H_2O$ (Santa Cruz Biotechnology), 2 µM $CoCl_2{\cdot}6H_2O$ (Santa Cruz), 2 µM $CuCl_2{\cdot}2H_2O$ (Sigma Aldrich), 2 µM $NiCl_2{\cdot}6H_2O$ (Merck), 2 µM $Na_2MoO_4{\cdot}2H_2O$ (Merck), 2 µM $Na_2SeO_3{\cdot}5H_2O$ (AlfaAesar) and 2 µM H_3BO_3 (Santa Cruz). A 100× BME vitamin solution (Sigma-Aldrich) was added, consisting of 0.1 mg ml^{-1} D-biotin, 0.1 mg ml^{-1} choline chloride, 0.1 mg ml^{-1} folic acid, 0.2 mg ml^{-1} myo-inositol, 0.1 mg ml^{-1} niacinamide, 0.1 mg ml^{-1} D-pantothenic acid·1/2Ca, 0.1 mg ml^{-1} pyridoxal·HCl, 0.1 mg ml^{-1} pyridoxine·HCl, 0.01 mg ml^{-1} riboflavin, 0.1 mg ml^{-1} thiamine·HCl. A 100× non-essential amino acid solution (Invitrogen) was added, consisting of 890 µg ml^{-1} alanine, 1500 µg ml^{-1} asparagine·H_2O, 1330 µg ml^{-1} aspartic acid, 1470 µg ml^{-1} glutamic acid, 750 µg ml^{-1} glycine, 1150 µg ml^{-1} proline, and 1050 µg ml^{-1} serine.

As a metabolizable carbon source, the MDG medium was supplemented by adding a stock of 1 M glucose (Calbiochem) or stocks of the different compounds (10 mM) to the respective final concentration. Since the compounds were dissolved in dimethyl sulfoxide (DMSO), equivalent amounts of DMSO were added to the medium as a control.

Bacterial growth

β-Gal expressing *Escherichia coli* strain BL21 (DE3) (Invitrogen) was maintained as glycerol stocks. Preliminary cultures were grown in LB medium (Roth). Bacteria were grown in 50 ml sterile glass flasks or 15 ml sterile plastic tubes (Greiner) in a Multitron incubator (Infors) at 37 °C, shaking at 300 rpm. LB agar plates were inoculated overnight, single colonies were then picked and grown to log phase in LB medium. An aliquot of 500 μl of the LB liquid culture was taken and washed two times in a minimal medium. To adapt the bacteria to the minimal medium, the aliquot was transferred into a 20 ml MDG medium with glucose added as a single carbon source at a final concentration of 1 g l^{-1}. Bacteria were grown overnight and were then directly used for the experiments.

For the bacterial growth assays, 2 ml of MDG medium were supplemented with glucose at final concentrations of 0.5 g l^{-1} or 1.0 g l^{-1} or with the respective compounds (**1** or control compound **3**). To assess glucose release-dependent bacterial growth, concentrations of the compounds were normalized to equivalent glucose concentrations as in a glucose-containing medium. Subsequently, bacteria taken from the overnight preculture were inoculated 1 : 200. Bacterial growth was detected by measuring the absorbance at a wavelength of 600 nm with a spectrophotometer (Ultrospec 6300pro, Amersham). Each measurement was performed in triplicate.

Fluorescence measurements

A 2 ml bacterial culture taken from a pre-culture was incubated overnight in an MDG medium with 0.5 g l^{-1} D(+)-glucose (Calbiochem) or with 0.5 g l^{-1} D(+)-lactose (Fluka), respectively. Bacteria were disrupted by treating them on ice with a Branson-sonifier (B12) for 5 s and were then incubated with 50 μM of probe **4** (stock at 50 mM in DMSO). To prevent degradation of the disrupted bacteria, 1 mM PMSF (Roth) was added. Bacteria were incubated for 2 h following the bacterial growth protocol, pelleted afterwards at 10 000 g in an Eppendorf centrifuge and the supernatant was sterile filtered.

To analyze the β-galactosidase-mediated conversion of **4** into SulfoQCy7, samples of bacterial lysate or the supernatant were excited at 590 nm and the fluorescence spectrum between 600 nm and 850 nm was recorded on a Fluorescence spectrometer (LS 55, Perkin Elmer). A serial dilution made from a 140 U ml^{-1} stock solution of β-galactosidase (Sigma-Aldrich) in an MDG medium was used as a positive control.

Confocal laser scanning microscopy (LSM)

An overnight *E. coli* BL21 (DE3) culture was incubated in an MDG medium containing 1 g l^{-1} glucose or 1 g l^{-1} lactose, respectively, as described. Cells were pelleted and resuspended in 4% PFA (Merck) dissolved in PBS buffer, pH 7.2. Fixation was carried out at RT for 30 min before washing the cells three times and resuspending them in 5 mM HEPES buffer, pH 7.2. The following compounds were then added to the final concentrations: DAPI (Invitrogen) to 1 μM, probe **4** to 200 μM, EDTA (pH 7.2) to 4 mM. Cells were incubated at RT for 30 min and were washed two times in HEPES buffer. The fluorescence of the cells was visualized by confocal microscopy *vs*. differential interference contrast (DIC) on an LSM 700 (Carl Zeiss) following the instructions for using the software Zen 2009.

Statistical analysis

Statistical analyses were performed with the unpaired Student *t* test. All statistical analyses were performed with the Prism software (GraphPad).

Abbreviations

AcOH	Acetic acid
Ac_2O	Acetic anhydride
K_2CO_3	Potassium carbonate
DBTL	Dibutyltin dilaurate
DCM	Dichloromethane
DMF	*N*,*N*′-Dimethylformamide
EtOAc	Ethylacetate
Hex	*n*-Hexanes
MeOH	Methanol
THF	Tetrahydrofuran
CAM	Ceric ammonium molybdate

Acknowledgements

We thank Nele Reeg and Georgi Tadeus for their assistance with bacterial growth assays as part of their internships. D.S. thanks the Israel Science Foundation (ISF) and the German-Israeli Foundation (GIF) for financial support. P.H.S. and B.L. thank the Max Planck Society for the very generous support. Financial support by the German Federal Ministry of Education and Research (to B.L.) is also gratefully acknowledged.

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