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A Structurally Simple Self-Immolative Reagent That Provides Three Distinct, Simultaneous Responses per Detection Event

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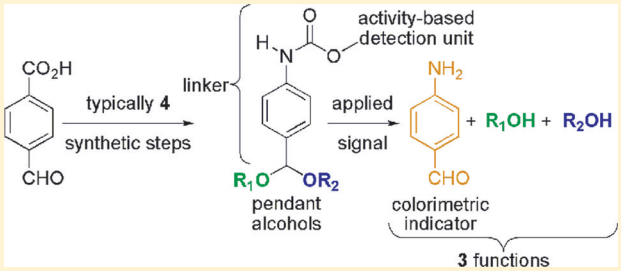
Supporting Information

ABSTRACT: A general design is presented for a stimulus-responsive small molecule that is capable of responding to a specific applied chemical or physical signal by releasing two *different* types of pendant small molecules *and* a colorimetric indicator simultaneously. A key aspect of this design is the ease with which these reagents are prepared: typically, only four synthetic steps are required. Moreover, the modular construction strategy provides access to stimuli-responsive reagents that are capable of (i) responding to a variety of applied signals and (ii) releasing a number of different small molecules that contain primary alcohols, secondary alcohols, or phenols. These stimuli-responsive reagents are stable under physiological conditions (neither hydrolysis nor thermal degradation of the reagent occurs in significant quantity), and when they are exposed to the appropriate applied signal, they release both pendant small molecules and the colorimetric indicator completely within hours. Finally, unlike other functional groups, such as carbonates, that are used to connect alcohol-bearing molecules to controlled-release reagents, the linkage described in this article increases in hydrolytic stability (rather than decreases) as the pK_a of the pendant alcohol decreases.



INTRODUCTION

Stimuli-responsive small molecules that are capable of performing more than one type of function in response to a specific applied signal[1−6] are needed for a variety of applications. These applications include certain types of therapeutics (e.g., prodrugs),[1−4,7−10] diagnostics,[2,3,11−16] "smart" packaging (e.g., for indicating and possibly preventing/slowing the spoilage of food),[17] corrosion-indicating or anticorrosion coatings,[18] and reagents for applications in the agriculture and cosmetics industries, among others.[17] However, the development and application of stimuli-responsive reagents that are capable of performing multiple functions simultaneously has been limited by the lengthy syntheses that are required to prepare these types of reagents.[1−6] This article describes a new design for stimuli-responsive reagents that are capable of releasing two different types of pendant small molecules and revealing a separate yellow indicator molecule (Figure 1), all in response to a single, specific chemical detection event. This new design should enable others to easily and quickly prepare analogous reagents for a variety of applications, including stimuli-responsive materials.[18]

Background Leading to the Design of the Controlled-Release Reagents Described Herein. The design shown in Figure 1 builds upon nearly 60 years of studies in the area of small-molecule controlled-release reagents.[1−4] This research area began with initial demonstrations of reagents that responded to a signaling molecule by releasing a single pendant molecule,[19] which led to Katzenellenbogen's revised design[20] that connects an activity-based detection unit with a pendant molecule through a linker (the general formula for this design is

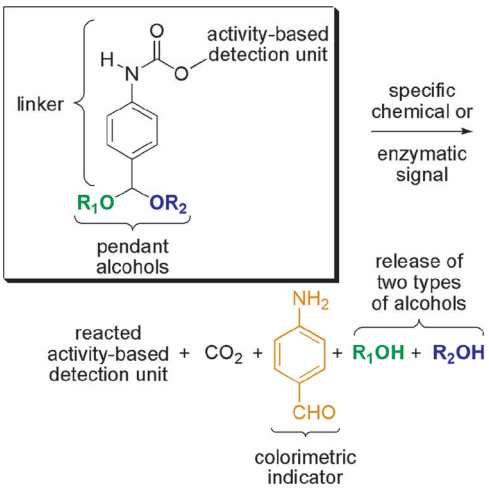


Figure 1. Design of a small-molecule controlled-release reagent that is capable of responding to a specific chemical signal by releasing two different types of alcohols and simultaneously indicating the release event by forming a yellow color. This type of controlled-release reagent is accessible in ≤4 synthetic steps.

activity-based detection unit—linker—pendant small molecule).[1,4,20−23] More recently, efforts by De Groot,[24] Shabat,[25,26] McGrath,[27] and others[1−4] have resulted in systems that use a linker to release more than one copy of a pendant small

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molecule (typically two to four copies) per detection event. The function of these latter reagents has been expanded even further, such that now they are capable of releasing more than one *type* of pendant small molecule per detection event.[5,6,28]

Despite the exceptional progress in these seminal studies,[5,6,28] access to reagents that release more than one type of pendant small molecule from a single reagent has been limited by lengthy synthetic routes, which often require 17 or more synthetic steps.[5] Herein, we provide one solution to these synthetic challenges for creating dual- and triple-release reagents that liberate alcohol-bearing small molecules.

Design of a Synthetically Accessible Stimulus-Responsive Reagent That Releases Two Different Alcohols and a Colorimetric Indicator. A number of pH-responsive acetal reagents have been prepared for the controlled release of pendant alcohols under acidic conditions via acid-catalyzed hydrolysis.[4] Our design (Figure 1) draws on acetal chemistry but differs in the mechanism of the response. We sought a reagent that is stable at neutral pH yet is capable of releasing the pendant alcohols in response to a *specific* chemical signal, such as an enzyme, through an azaquinone methide release mechanism (Figure 2), rather than through an

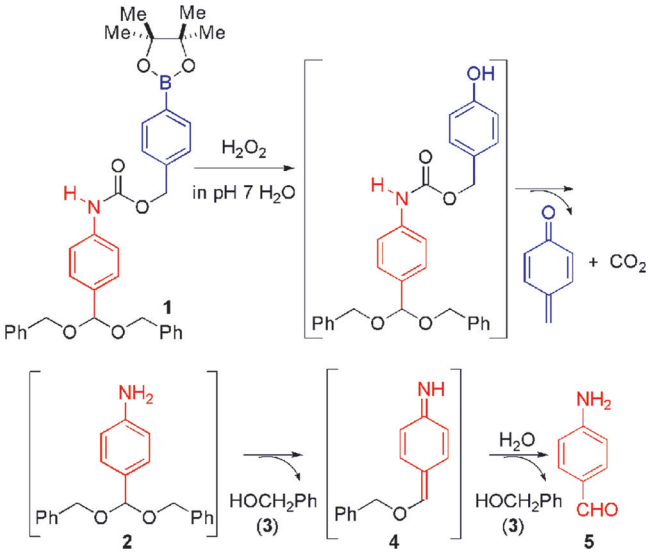


Figure 2. Proposed mechanism for the release of both equivalents of benzyl alcohol (**3**) from acetal reagent **1**. We anticipated that byproduct **5** would provide a bright yellow color as an indicator of the detection and controlled-release events.[29]

acid-catalyzed hydrolysis pathway. While the benzylic acetal shown in Figure 1 certainly is sensitive to acidic conditions, we anticipated that the reagent would be stable under the physiological conditions that would be encountered in the context of many detection scenarios.

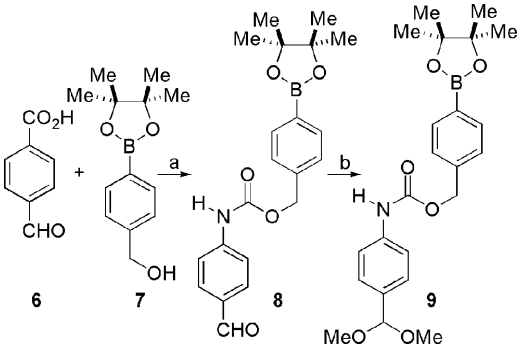
Our general design is based on the hypothesis that reaction of the activity-based detection unit with an analyte (e.g., hydrogen peroxide)[12−14] will release an aniline intermediate (**2**, Figure 2) via formation of quinone methide and loss of carbon dioxide. We reasoned that **2** would not be stable and instead would release one alcohol (e.g., **3** in Figure 2) from the benzylic acetal via formation of azaquinone methide **4**.[24] Addition of water to **4** would make an unstable hemiacetal, which would form aldehyde **5** by releasing the second pendant alcohol.

We based this design on our previous studies of an autocatalytic reagent.[29] Our expectation from these previous studies was that aldehyde byproduct **5** would provide a bright yellow color, which would serve as a direct indicator of the detection and controlled-release events.[30]

RESULTS AND DISCUSSION

Procedure for Synthesizing Model Reagent 9. We tested the efficacy of the design depicted in Figure 1 by preparing a model reagent (compound **9**, Scheme 1), which

Scheme 1. Synthesis of Model Compound 9[a]



[a]Legend: (a) DPPA, Et_3N, dioxane, 100 °C (93%); (b) TMSOMe, TMSOTf, CH_2Cl_2, 78 °C → 25 °C (95%).

was designed to release 2 equiv of methanol in response to hydrogen peroxide. As shown in Scheme 1, model compound **9** was prepared from commercially available starting materials in two synthetic steps[31] and 88% overall yield. The brevity of this synthesis is a substantial improvement over current controlled-release reagents that release two copies of the same pendant small molecule; such reagents typically require more than six steps to prepare.[32]

Stability of Model Reagent 9. Since acetals are sensitive to the pH of the solution in which they are dissolved,[33] we tested the stability of reagent **9** in 1:1 CD_3CN−buffered D_2O at various pH values and temperatures.[34] Under these conditions, less than 6% of reagent **9** (7 mM) decomposed after 6 h of exposure to 1:1 CD_3CN−buffered D_2O (2.5 mM phosphate buffer, pH 7.0) at 25 °C, as measured by integration of the acetal methine hydrogen in the ^1H NMR spectrum relative to the CHO peak in compound **8**, which was the only byproduct (other than methanol) observed in the ^1H NMR spectrum.[35] As expected, as the pH of the solution decreased (Figure 3b), the acid-catalyzed decomposition of **9** increased, and as the pH of the solution increased to pH 8 or greater, reagent **9** showed no signs of decomposition over the 6 h exposure period.

Moreover, Figure 3c reveals that moderate temperature fluctuations away from 25 °C have little effect on the nonspecific hydrolysis of reagent **9**. For example, in 1:1 CD_3CN−buffered D_2O (2.5 mM phosphate buffer, pH 8.0), reagent **9** (7 mM) shows no signs of hydrolysis (i.e., 100 ± 1% of the acetal remained) even after 6 h of heating at 35 °C. At 45 °C, only ∼3% of reagent **9** decomposed after 6 h, and significant decomposition (13 ± 2% decomposition) was observed only after reagent **9** was heated for 6 h at 55 °C.

Controlled Release of Methanol from Reagent 9 in Response to Hydrogen Peroxide. While reagent **9** is stable for many hours at neutral pH, it responds quickly and completely to a specific applied chemical signal (i.e., hydrogen peroxide). ^1H

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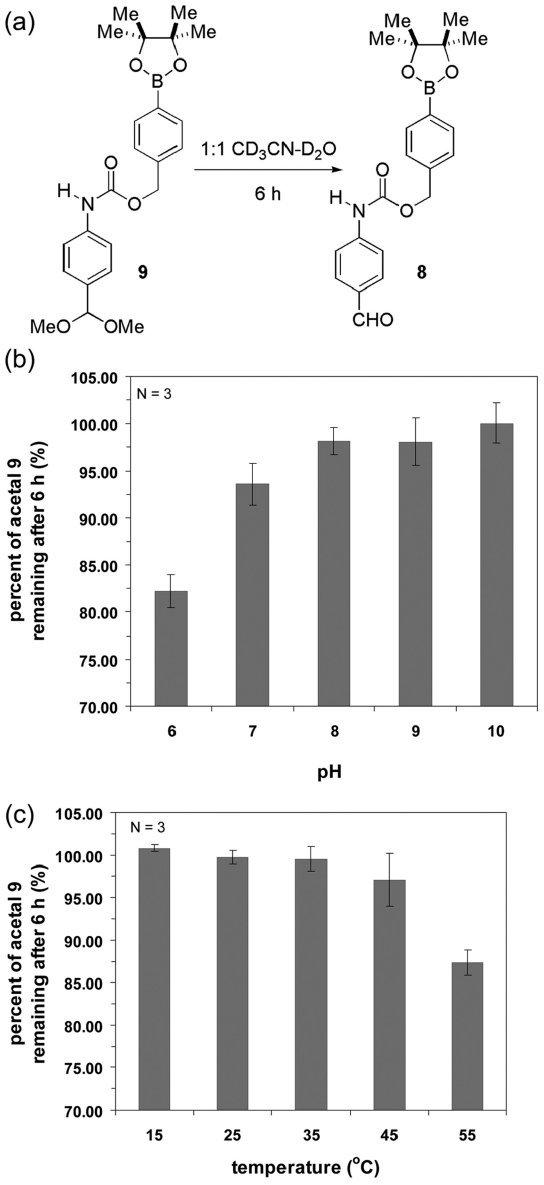

Figure 3. Measurements of the stability of model reagent **9** (7 mM) in 1:1 CD_3CN–buffered D_2O solutions ranging in pH from 6 to 10 and ranging in temperature from 15 to 55 °C. The buffer was 2.5 mM phosphate buffer for solutions with pH values of 6–8 and was 2.5 mM bicarbonate buffer for solutions with pH values of 9 and 10. (a) Aldehyde **8** and methanol were the only byproducts observed during these stability studies, and therefore, ratios of the ^{1}H NMR integration values of the aldehyde hydrogen relative to the acetal methine hydrogen were used to calculate the percent of acetal **9** remaining at the conclusion of the stability experiments. Graphs (b) and (c) reveal the percent of **9** remaining (relative to aldehyde **8**) after 6 h of exposure to the specific conditions. The experiments were performed in triplicate, and the error bars reveal the standard deviations from the average values. The experiments were conducted at 25 °C for (b) and at pH 8.0 for the experiments represented in (c).

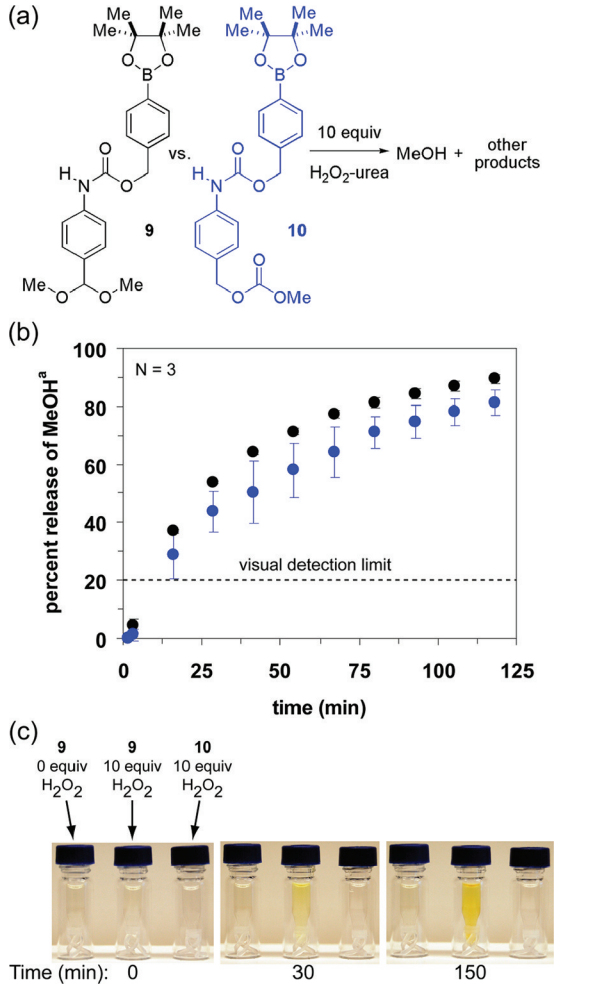

Figure 4. Rate of release of methanol from model acetal **9** and control carbonate **10**. (a) The release event was triggered in both cases by addition of 10 equiv of hydrogen peroxide–urea to either **9** or **10** (7 mM) in 1:1 CD_3CN–buffered D_2O (2.5 mM phosphate buffer, pH 8.0) at 25 °C. (b) The rate of release of methanol was monitored using ^{1}H NMR by quantifying either the disappearance of the acetal methine (**9**) or the appearance of CH_3OD (**10**) relative to 2,5-dimethylfuran (DMFu) (an internal standard).[36] The data were normalized for the number of equivalents of methanol being released. The release experiments were performed in triplicate; the data points reflect the average values (black, **9**; blue, **10**), and the error bars reveal the standard deviation from the average. The error bars for the black data points are smaller than the dots. The dotted line reflects the point above which a yellow color becomes apparent visually (see part c for example photographs) when **9** responds to hydrogen peroxide. When 20% of reagent **9** releases both equivalents of methanol, for example, the solution will have turned a distinct yellow color. (c) Visual color that appears when **9** responds to hydrogen peroxide. The color does not develop in the absence of hydrogen peroxide or for the carbonate **10**, even when **10** reacts with hydrogen peroxide. The reaction conditions are the same as described in (a). The clarity of the photographs was enhanced using Adobe Photoshop using the "Auto Levels" function.

NMR analysis shows that **9** (7 mM) releases both equivalents of methanol within ~2 h when exposed to excess hydrogen peroxide–urea (10 equiv) in 1:1 CD_3CN–buffered D_2O (2.5 mM phosphate buffer, pH 8.0) at 25 °C (Figure 4).

Because carbonate linkages are a standard strategy for appending an alcohol to a controlled-release reagent,[1–4] we prepared control compound **10** (Figure 4a) to compare the behavior of the carbonate to that of the acetal. In the presence of hydrogen peroxide, both equivalents of methanol are released slightly faster from acetal **9** than the single equivalent is released from carbonate **10**.

As expected,[29] acetal **9** also reveals a colored byproduct when hydrogen peroxide is detected (Figure 4c), thus providing a visual indication of the detection event. In contrast, the byproduct (i.e., *p*-aminobenzyl alcohol) of the reaction between **10** and hydrogen peroxide is not colored.

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Rate of Nonspecific Release of Methanol from Reagent 9. The rate of background release of methanol due to nonspecific hydrolysis of the acetal is also an important consideration when designing a controlled-release reagent. After 48 h in 1:1 CD_3CN–buffered D_2O (2.5 mM phosphate buffer, pH 8.0, 25 °C), acetal **9** (7 mM) decomposed only 2.7 ± 1.0%, which is nearly identical with the rate of nonspecific hydrolysis of carbonate **10** (7 mM) under the same conditions (2.8 ± 0.1%) (Tables S5 and S6, Supporting Information).

The rate of nonspecific hydrolysis, however, is highly dependent on the structure of the alcohol: carbonates derived from acidic alcohols (e.g., phenols) hydrolyze much more readily than carbonates derived from alcohols with pK_a values closer to 16. The same increase in sensitivity is not observed with acetals of the type depicted in Figure 1. For example, Figure 5 shows an acetal (compound **11**) that releases phenol. After 12 h in 3:1 CD_3CN–buffered D_2O (2.5 mM phosphate buffer, pH 8.0) at 25 °C, acetal **11** (4 mM) showed no signs of nonspecific hydrolysis, whereas carbonate **12** (4 mM) had hydrolyzed completely within ~4 h. These results demonstrate the versatility and stability of acetal-based controlled-release reagents and suggest that in the context of releasing phenols (or other acidic alcohols) under neutral or slightly basic conditions (i.e., typical physiological conditions), acetals may be a better choice than carbonates for linking a pendant alcohol to the parent reagent.

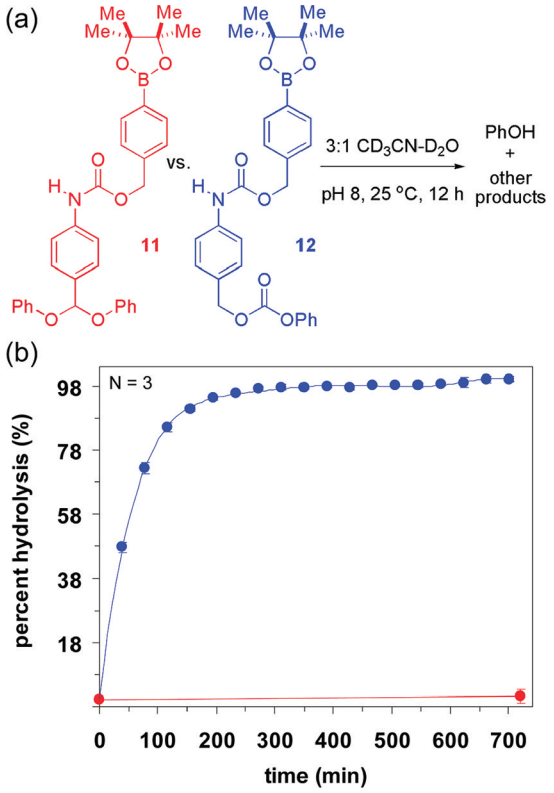

Figure 5. Rate of nonspecific hydrolysis of acetal **11** versus carbonate **12**. (a) Each compound was dissolved in 3:1 CD_3CN–buffered D_2O (2.5 mM phosphate buffer, pH 8.0) at 25 °C with 4 mM **11** or **12**. (b) The rate of nonspecific release of phenol from acetal **11** was monitored using ^{1}H NMR by integration of the acetal methine hydrogen relative to 2,5-dimethylfuran (DMFu) (an internal standard). The rate of nonspecific hydrolysis of carbonate **12** was monitored using HPLC by integration of peak areas for the hydrolysis product of **12** (the benzyl alcohol) relative to anisole (an internal standard). The only products arising from hydrolysis of **12** were the expected benzyl alcohol (i.e., the benzyl alcohol that arises after hydrolysis of the carbonate; this benzyl alcohol is compound **46** in the Supporting Information) and phenol. HPLC was used instead of ^{1}H NMR for quantifying the hydrolysis of **12** because of overlap in the ^{1}H NMR spectrum of the benzylic peaks for the carbonate (**12**) and the hydrolyzed product. The release experiments were performed in triplicate; the data points reflect the average values (red, **11**; blue, **12**), and the error bars reveal the standard deviation from the average. In most cases, the error bars are smaller than the dots.

Postulated Mechanism for the Controlled Release of Alcohols from Benzylic Acetals. We performed two experiments to explore the validity of the mechanism proposed in Figure 2. Both of these experiments used model acetal **1** because the benzyl alcohol that is released absorbs at 254 nm and is easily identifiable using LC-MS. The first experiment involved treating **1** (1.7 mM) with 10 equiv of hydrogen peroxide in 1:1 CH_3CN–buffered water (2.5 mM phosphate buffer, pH 8.0) at 25 °C, followed by LC-MS analysis of the products. The UV-active compounds identified in the LC-MS spectrum included direct products of the controlled-release reaction (benzyl alcohol **13**, phenol **14**, and aniline **15**) (Figure 6),

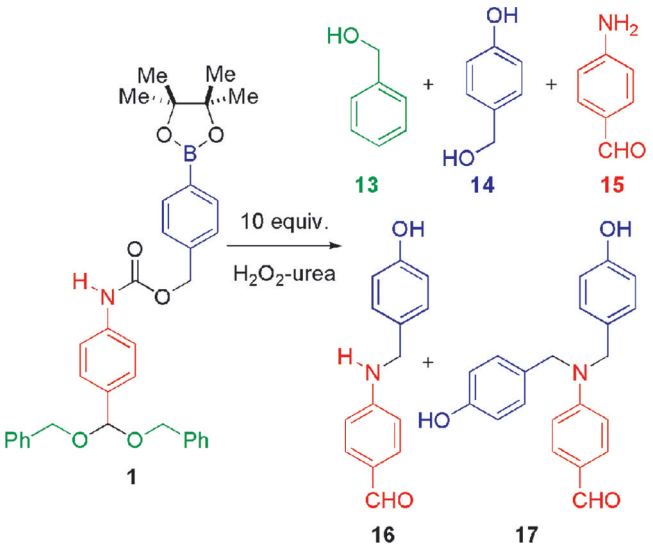

Figure 6. UV-active byproducts released upon addition of 10 equiv of hydrogen peroxide–urea to model compound **1** (1.7 mM) in 1:1 CH_3CN–buffered water (2.5 mM phosphate buffer, pH 8.0) at 25 °C. Compounds **15**–**17** are yellow, thereby providing a visual indication that hydrogen peroxide reacted with **1**.

as well as products (**16** and **17**) that arose from reaction of aniline **15** with the presumed quinone methide intermediate formed in the release process.[29] Because we found that compounds **15**–**17** are yellow,[29] this experiment also validated our strategy for releasing a colorimetric indicator for real-time monitoring of the detection event.[30]

These LC-MS results confirm the products that we expected from the proposed tandem quinone methide–azaquinone methide release mechanism (Figure 2). While we cannot say conclusively that the mechanism shown in Figure 2 is operative, a second release experiment using $H_2{}^{18}O$ water instead of $H_2{}^{16}O$ helps support the mechanistic hypothesis. This second experiment was performed using conditions analogous to those shown in Figure 6; however, when $H_2{}^{18}O$ was used instead of $H_2{}^{16}O$, the mass spectra for aldehydes **15**–**17** revealed nearly complete incorporation of ^{18}O as the aldehyde oxygen (see the Supporting Information for details). This result is consistent

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with the notion that the azaquinone methide intermediate **4** (Figure 2) is intercepted by water to form a hemiacetal that then releases the second alcohol.

Access to Diverse Activity-Based Detection Units and Acetals. Having established that the design shown in Figure 1 provides stable reagents that are capable of releasing two pendant primary alcohols, our next goal was to explore the scope of the design by evaluating the compatibility of the synthesis procedure with different activity-based detection units (which often are called triggers in related literature) and a variety of pendant alcohols.

(a). Activity-Based Detection Units. Figure 7 shows five representative controlled-release reagents, each containing a different activity-based detection unit. The chosen activity-based detection units should enable the reagents to release pendant alcohols in response to hydrogen peroxide (**9**), UV light (**19**), palladium(0) (**21**), fluoride ion (**23**), and penicillin G amidase (**25**). All reagents (with the exception of **25**; see the Supporting Information) were prepared using procedures analogous to those shown in Scheme 1 and are designed to release methanol as a model pendant alcohol. For example, exposure of **9** to hydrogen peroxide, **19** to UV light, and **23** to fluoride ion releases both equivalents of methanol from each reagent and provides the expected byproducts **15**−**17** with rates that depend on the dose of the signal and the rate of reaction between the signal and the activity-based detection unit (an example stacked plot of time-dependent LC-MS traces for **23** when exposed to fluoride is provided in Figure S2 in the Supporting Information). The uniformly high yields (82−96%) for acetal formation in the presence of these activity-based detection units highlight the mild reaction conditions used in the preparation of these highly functionalized reagents.[31]

(b). Acetals. The procedure for attaching alcohols to the controlled-release reagent is mild and effective for attaching primary alcohols (**9**, **1**, **26**, **27**), secondary alcohols (**28**, **29**), and phenols (**11**) (Figure 8). As expected, hindered alcohols such as tertiary alcohols (**30**) are not easily prepared and isolated.[37] The procedure also provides direct access to mixed acetals, such as **26**. In response to hydrogen peroxide, mixed acetal **26** releases both ethanol and benzyl alcohol, in addition to producing colorimetric byproducts **15**−**17**. Compound **26** was prepared in only two synthetic steps[38] and 62% overall yield, which is a substantial improvement over state-of-the-art controlled-release reagents that have similar levels of function.[5]

Controlled Release in Response to β-D-Glucuronidase: Release of Two Types of Fragrances and a Colorimetric Indicator. Our straightforward route to reagents that simultaneously release two different types of alcohols and a colorimetric indicator allowed us to prepare a demonstration reagent that responds to β-D-glucuronidase, which is an enzyme marker of *Escherichia coli* (*E. coli*).[39] This demonstration reagent was prepared in seven synthetic steps (Scheme 2), three of which were needed to prepare the activity-based detection unit. The demonstration reagent is capable of responding to β-D-glucuronidase in 30 mM phosphate-buffered water (pH 7.4) at 30 °C by releasing citronellol, 2-phenylethanol, and yellow indicator molecules.

In the presence of 125 units of β-D-glucuronidase, time-dependent LC-MS analysis of the reaction products reveals that reagent **36** (1.9 mM in 30 mM phosphate-buffered water (containing 18% MeOH), pH 7.4, 30 °C) proceeds through a stepwise release mechanism in which the enzyme catalyzes the hydrolysis of phenol from

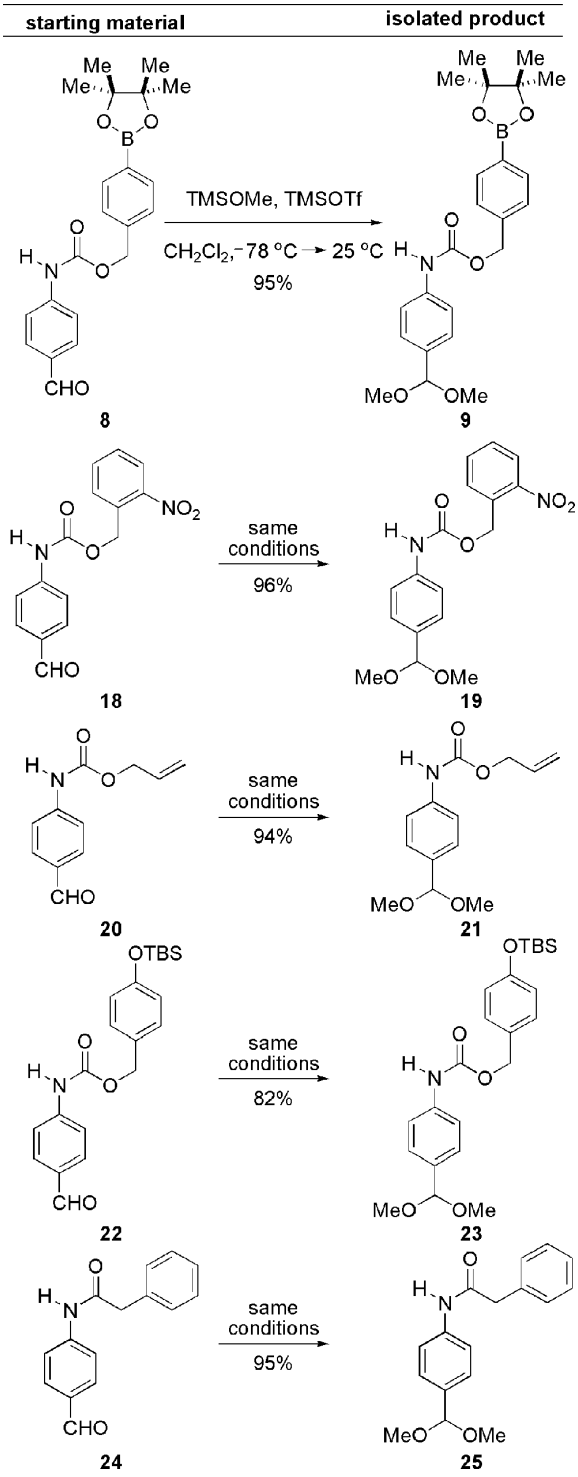

Figure 7. Synthesis of five model controlled-release reagents, each containing a different activity-based detection unit. The reagents are designed to release 2 equiv of methanol in response to the following signals: hydrogen peroxide (**9**); UV light (**19**); palladium(0) (**21**); fluoride (**23**); penicillin G amidase (**25**).

the anomeric position of the β-D-glucuronic acid unit. This first step is complete less than 20 min after exposure of **36** to β-D-glucuronidase. The amount of the phenol intermediate decreases slowly over the next 378 min until it is barely detectable by LC-MS. Peaks corresponding to citronellol (**37**), 2-phenylethanol (**38**), 4-aminobenzaldehyde (**15**), colored products **16** and **17**, and phenol **14** all increase in intensity

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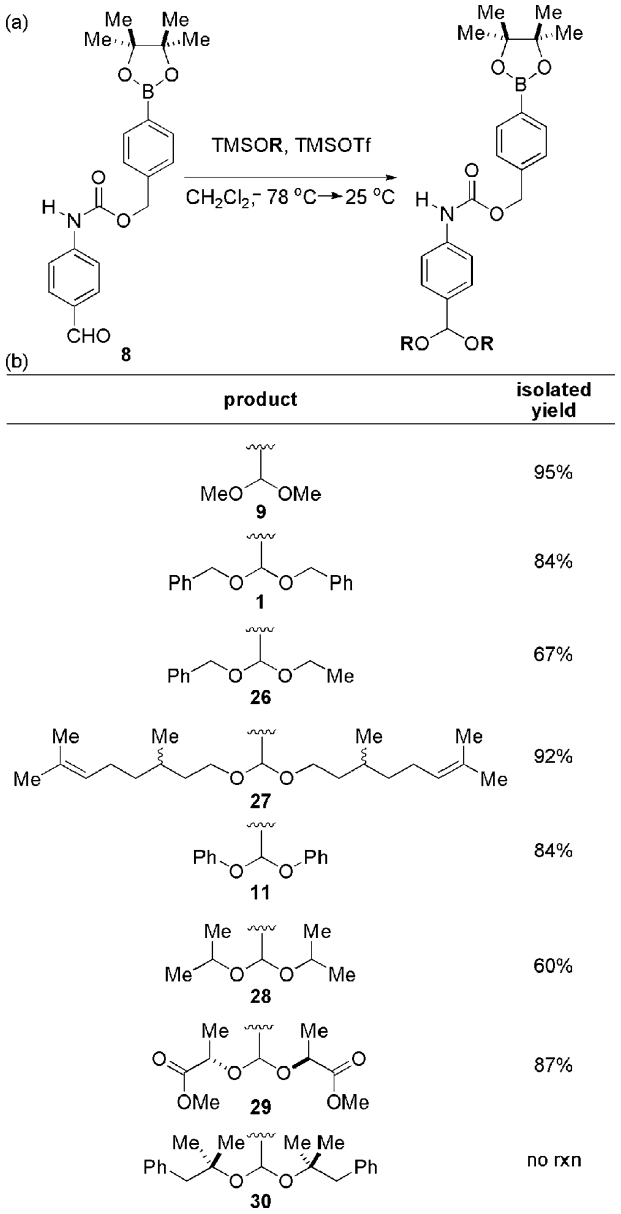

Figure 8. Synthesis of different types of acetals: (a) procedure used to prepare the acetals; (b) eight different acetals, including mixed acetal **26**.

beginning ~20 min after first exposure of **36** to β-D-glucuronidase (Figure 9a and Figure S1 (Supporting Information)), and at the end of the 378 min time period, the release of citronellol (**37**) and 2-phenylethanol (**38**) from **36** is nearly complete (Figure 9b). In the absence of β-D-glucuronidase (but under otherwise identical reaction conditions), **36** releases only 3% of citronellol (**37**) and 1% of 2-phenylethanol (**38**) after 378 min.

In addition to the analytical LC-MS results, qualitative confirmation of the detection event is provided both by the yellow color that is produced when **36** reacts with β-D-glucuronidase (Figure 9c) and by the fresh, floral smell that is emitted (see the Supporting Information for details) (citronellol is a lemon-scented compound, and 2-phenylethanol has a floral odor), which may be useful in the context of personal hygiene products.[17] In contrast, in the absence of β-D-glucuronidase, no color is observed and no scent is detected over the course of 378 min.

CONCLUSIONS

This article provides a new design for a stimulus-responsive small molecule that is capable of releasing either two copies of a pendant alcohol and a colorimetric indicator in response to an applied signal or of releasing two *different* types of alcohols and a colorimetric indicator simultaneously. The reagent is accessible typically in four or fewer synthetic steps, which is exceedingly efficient with respect to the function achieved for the number of synthetic steps required to prepare the reagent.

In addition to being efficient, the procedure for preparing these reagents is modular and thus provides access to a range of controlled-release reagents that are capable of responding to a variety of applied signals and releasing an assortment of pendant alcohols, including phenols. Extension of the system to the controlled release of phenol-based fluorescent dyes such as 7-hydroxycoumarin should provide straightforward access to selective and sensitive sensor reagents that are capable of signal amplification.

Despite the acid-sensitive acetal functionality, the reagents described herein are stable for hours under physiological conditions and are capable of responding to specific applied signals, such as the enzyme β-D-glucuronidase. This combination of ready availability, stability, and rapid and diverse response characteristics may now enable the preparation of new controlled-release reagents with multiple, diverse functions. Such reagents have the potential for use in stimuli-responsive materials,[40] including providing a possible starting point for creating new stimuli-responsive shell walls in responsive capsules.[18,41−44]

EXPERIMENTAL SECTION

General Experimental Methods. All reactions were performed in flame-dried glassware under a positive pressure of nitrogen, unless otherwise noted. Air- and moisture-sensitive liquids were transferred by syringe or cannula. Organic solutions were concentrated by rotary evaporation (1−20 mmHg; the pressure used depended on the boiling point of the solvent) at ambient temperature, unless otherwise noted. Benzyloxytrimethylsilane, ethoxytrimethylsilane, isopropoxytrimethylsilane, methoxytrimethylsilane, trimethyl(phenoxy)silane, and all other reagents were purchased commercially and were used as received unless otherwise noted. Acetonitrile, dichloromethane, 1,4-dioxane, *N*,*N*-dimethylformamide, and tetrahydrofuran were purified by the method of Pangborn et al.[45] Flash column chromatography was performed as described by Still, Kahn, and Mitre,[46] employing silica gel (60 Å pore size, 32−63 μm, standard grade, Dynamic Adsorbents). Thin-layer chromatography was carried out on Dynamic Adsorbents silica gel TLC (20 × 20 w/h, F-254, 250 μm). Deionized water was purified using a Millipore purification system (Barnstead EASYpure II UV/UF).

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 25 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to tetramethylsilane ($(CH_3)_4Si$, 0.00 ppm) or to residual protium in the solvent ($CHCl_3$, 7.27 ppm; CD_2HOD, 3.31 ppm; DHO, 4.67 ppm; CD_2HCN, 1.97 ppm).[47] Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br s = broad singlet), integration, and coupling constant (*J*) in hertz. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 25 °C. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$, δ 77.0). Photographs were obtained using a Nikon digital camera (D40). Pictures of samples indicating a colorimetric response (Figures 4c and 9c) were balanced with the "Auto Levels" function in Adobe Photoshop. LC analyses were performed using a reversed-phase phenyl-hexyl column (150 mm × 2.1 mm, 5 μm particle size). The column was equilibrated at 1:9

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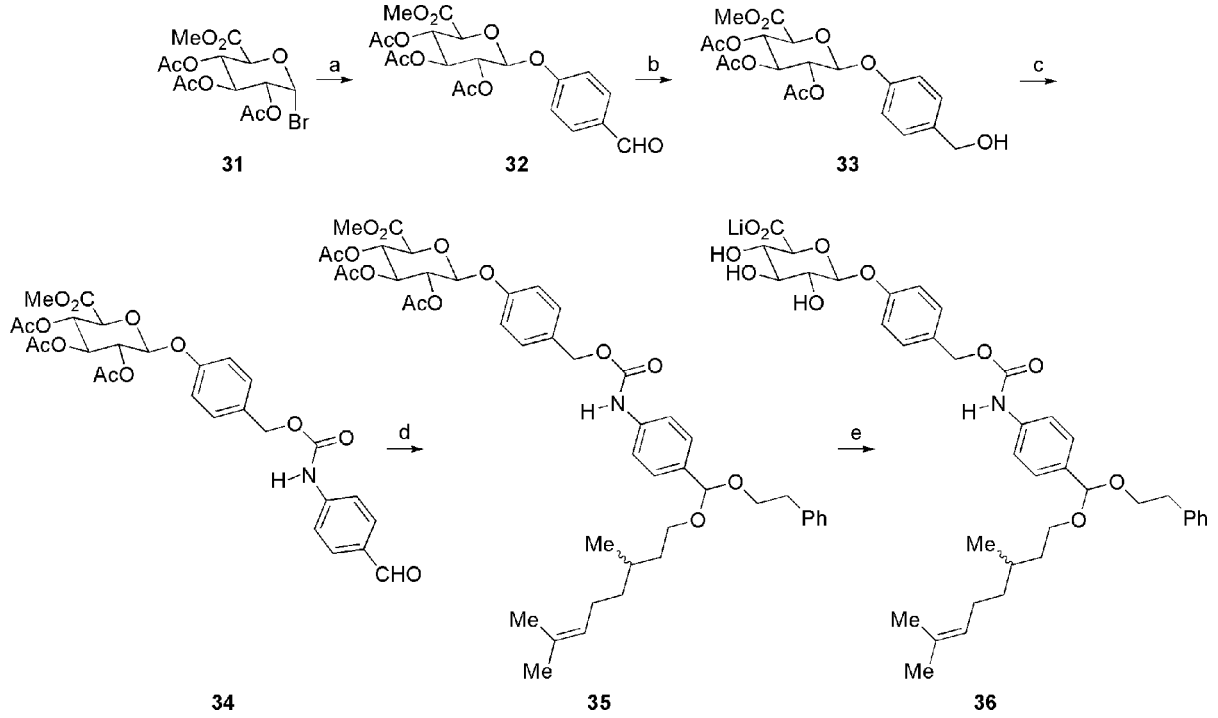
Scheme 2. Synthesis of Reagent 36, Which Releases Citronellol (37), 2-Phenylethanol (38), and a Yellow Indicator When Exposed to β-D-Glucuronidase in 30 mM Phosphate-Buffered Water (pH 7.4) at 30 °C[a]



[a]Legend: (a) 4-hydroxybenzaldehyde, Ag_2O, MeCN (52%); (b) $NaBH_4$, 1:1 CH_2Cl_2–MeOH (100%); (c) (i) 4-formylbenzoic acid, $(COCl)_2$, CH_2Cl_2, DMF, (ii) NaN_3, H_2O, acetone, (iii) 110 °C, toluene, (iv) **33**, CH_2Cl_2 (82%); (d) TMSO-citronellol, TMSO-$(CH_2)_2$Ph, molecular sieves, TMSOTf, CH_2Cl_2 (33%); (e) LiOH, H_2O, MeOH, THF, 0 °C.

acetonitrile–water at a 0.5 mL/min flow rate, and after injection of the sample, the gradient was ramped as follows: 1:3, 3 min; 1:1, 6 min, 3:1, 9 min; 9:1, 12 min; 1:9, 17 min, over a 20 min combined elution time. Tandem LC–MS analyses were performed using the same LC method with a substitution of the mobile phase to solutions of acetonitrile and water that each contained 5 mM ammonium formate. The MS (ES) parameters were as follows: gas temperature of 350 °C, drying gas flow of 10–13 L/min, nebulizer pressure of 40–60 psig, and a voltage of 3000 V.

Synthesis Procedures. *4-(tert-Butyldimethylsilyloxy)benzaldehyde (**39**).* To a round-bottom flask was added 4-hydroxybenzaldehyde (1.0 g, 8.2 mmol, 1 equiv), tetrahydrofuran (41 mL), and imidazole (1.1 g, 16 mmol, 2.0 equiv). The flask was cooled to 0 °C, and *tert*-butyldimethylsilyl chloride was added. The flask was warmed to 25 °C and was stirred for 12 h. The product mixture was concentrated by rotary evaporation. The residue was dissolved in dichloromethane and was adsorbed directly onto silica by rotary evaporation. Purification by silica gel flash column chromatography (5% ethyl acetate in hexanes, increasing to 20% ethyl acetate) afforded 4-(*tert*-butyldimethylsilyloxy)benzaldehyde (**39**; 1.8 g, 7.5 mmol, 92%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.26 (s, 6H), 1.01 (s, 9H), 6.94 (d, 2H, J = 8.9 Hz), 7.80 (d, 2H, J = 8.0 Hz), 9.90 (s, 1H). The ^{1}H NMR data are in agreement with literature values.[48]

*4-(tert-Butyldimethylsilyloxy)phenylmethanol (**40**).* To a round-bottom flask was added sequentially 4-(*tert*-butyldimethylsilyloxy)-benzaldehyde (**39**; 0.78 g, 3.2 mmol, 1 equiv), methanol (32 mL), and sodium borohydride (0.18 g, 4.9 mmol, 1.5 equiv). The reaction mixture was stirred at 25 °C for 2 h. The product mixture was diluted with saturated aqueous ammonium chloride (5 mL), and this solution was extracted using dichloromethane (3 × 20 mL). The organic extracts were combined, washed sequentially with water (1 × 20 mL) and brine (1 × 20 mL), and dried over sodium sulfate. Sodium sulfate was removed by filtration, and the solution was concentrated to dryness by rotary evaporation to afford 4-(*tert*-butyldimethylsilyloxy)-phenylmethanol (**40**; 0.49 g, 2.0 mmol, 62%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.25 (s, 6H), 1.06 (s, 9H), 4.46 (s, 2H), 6.85 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.4 Hz). The ^{1}H NMR data are in agreement with literature values.[49]

General Procedure for the Curtius Rearrangement: Procedure A. To a round-bottom flask were added sequentially 4-formylbenzoic acid (**6**; 1 equiv), 1,4-dioxane (to make a 0.5 M solution), triethylamine (1.1 equiv), diphenylphosphoryl azide (1.1 equiv), and the desired alcohol (1.1 equiv). The flask was equipped with a cold-finger reflux condenser, and the reaction mixture was heated to 105 °C. After it was stirred for 2 h at 105 °C, the product mixture was cooled to 25 °C. 1,4-Dioxane was removed by rotary evaporation, and the resulting residue was dissolved in a minimum of dichloromethane and was adsorbed directly onto silica by rotary evaporation. The product was isolated by silica gel flash column chromatography.

*(a). Allyl 4-Formylphenylcarbamate (**20**).* Reagents: 4-formylbenzoic acid (**6**; 0.50 g, 3.3 mmol, 1 equiv), 1,4-dioxane (6.6 mL), triethylamine (0.46 mL, 3.6 mmol, 1.1 equiv), diphenylphosphoryl azide (0.78 mL, 3.6 mmol, 1.1 equiv), and allyl alcohol (0.25 mL, 3.6 mmol, 1.1 equiv). The residue was purified by silica gel flash column chromatography (10% ethyl acetate in hexanes, increasing to 40% ethyl acetate) to afford allyl 4-formylphenylcarbamate (**20**; 0.54 g, 2.6 mmol, 80%) as a pale yellow solid. Mp: 116 °C. IR (cm^{-1}): 3312, 1730, 1674, 1594, 1527. ^{1}H NMR ($CDCl_3$): δ 4.69 (d, 2H, J = 5.4 Hz), 5.26 (dd, 1H, J = 0.8 Hz, J = 2.0 Hz), 5.29 (dd, 1 H, J = 1.0 Hz, J = 2.0 Hz), 5.90–6.00 (m, 1H), 7.46 (br s, 1H), 7.62 (d, 2H, J = 8.4 Hz), 7.85 (d, 2H, J = 8.0 Hz), 9.90 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 191.1, 152.7, 143.7, 131.8, 131.4, 130.4, 118.5, 117.8, 66.1. MS (TOF MS ES+, m/z): $(M + H)^+$, 206.1. HRMS (TOF MS ES+, m/z): calcd for $C_{11}H_{12}NO_3$ $(M + H)^+$ 206.0817, found 206.0799.

*(b). 2-Nitrobenzyl 4-Formylphenylcarbamate (**16**).* Reagents: 4-formylbenzoic acid (**6**; 0.50 g, 3.3 mmol, 1 equiv), 1,4-dioxane (6.6 mL), triethylamine (0.46 mL, 3.6 mmol, 1.1 equiv), diphenylphosphoryl azide (0.78 mL, 3.6 mmol, 1.1 equiv), and 2-nitrobenzyl alcohol (0.56 g, 3.6 mmol, 1.1 equiv). The residue was purified by silica gel flash column chromatography (10% ethyl acetate

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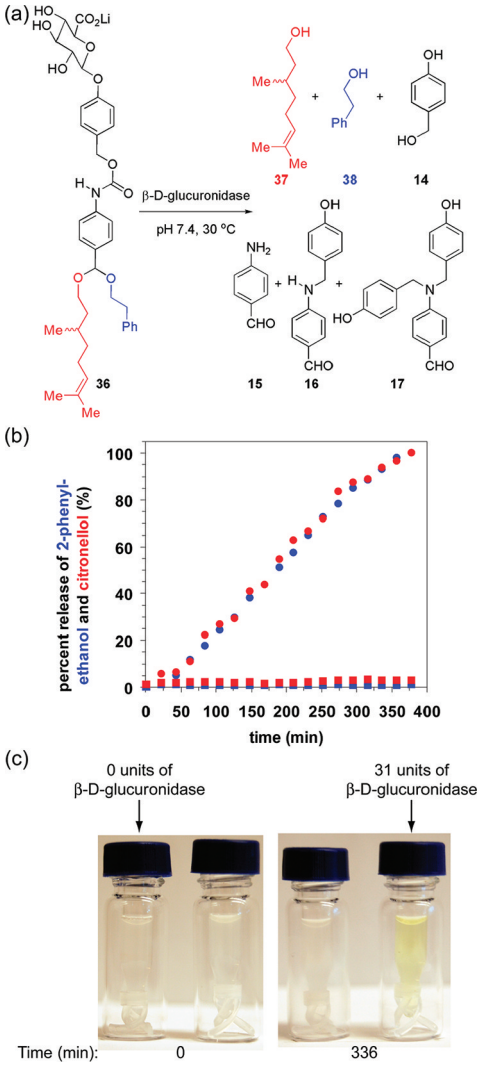

Figure 9. A stimulus-responsive small molecule that simultaneously releases 2-phenylethanol, citronellol, and yellow indicator molecules in response to *β*-D-glucuronidase under physiological conditions. (a) The stimulus-responsive reagent and the products arising from the release reaction. Products **15**–**17** provide the yellow color. (b) Rate of release of 2-phenylethanol (**38**) (blue) and citronellol (**37**) (red) when **36** (1.9 mM) is exposed to 125 units of *β*-D-glucuronidase in 4.6:1 phosphate buffer (30 mM, pH 7.4, containing 0.4% bovine serum albumin (w/v))–methanol at 30 °C. The circular data points were obtained in the presence of *β*-D-glucuronidase, and the square data points were obtained in the absence of *β*-D-glucuronidase. The percent release of citronellol and 2-phenylethanol was obtained by integration of HPLC peak areas (210 nm) of citronellol and 2-phenylethanol relative to anisole (an internal standard). No detectable change in relative peak areas occurred beyond 378 min; therefore, the final data point for each was set to 100%. (c) Time-dependent photographs of **36** in the absence (left) and presence (right) of *β*-D-glucuronidase under the same conditions as described in (b). The yellow color produced from the detection event is lighter in this figure than the color shown in Figure 4c because the concentration of the stimulus-responsive reagent (**36**) in this figure is 3.7-fold lower than the concentration of the reagent in Figure 4c. The clarity of the photographs was enhanced using the "Auto Levels" function in Adobe Photoshop.

in hexanes, increasing to 50% ethyl acetate) to afford 2-nitrobenzyl 4-formylphenylcarbamate (**16**; 0.68 g, 2.2 mmol, 69%) as a pale yellow solid. Mp: 177–179 °C. IR (cm^{-1}): 3306, 1729, 1683, 1592, 1522. ^1H NMR ($CDCl_3$): δ 5.66 (s, 2H), 7.09 (br s, 1H), 7.46–7.63 (m, 3H), 7.64–7.72 (m, 2H), 7.87 (d, 2H, J = 8.7 Hz), 8.15 (d, 1H, J = 7.9 Hz), 9.93 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 152.7, 147.4, 137.5, 133.7, 133.4, 132.4, 128.9, 128.7, 127.5, 125.0, 118.3, 102.6, 63.5. MS (MS ES+, m/z): $(M + H)^+$, 301.3. HRMS (TOF MS ES+, m/z): calcd for $C_{15}H_{13}N_2O_5$ $(M + H)^+$ 301.0824, found 301.0819.

*(c). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-Formylphenylcarbamate (**8**).* Reagents: 4-formylbenzoic acid (**6**; 1.0 g, 6.7 mmol, 1 equiv) 1,4-dioxane (13.4 mL), triethylamine (0.93 mL, 7.3 mmol, 1.1 equiv), diphenylphosphoryl azide (1.6 mL, 7.3 mmol, 1.1 equiv), and 4-(hydroxymethyl)phenylboronic acid pinacol ester (**7**; 1.7 g, 7.3 mmol, 1.1 equiv). The residue was purified by silica gel flash column chromatography (10% ethyl acetate in hexanes, increasing to 40% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate (**8**; 2.4 g, 6.2 mmol, 93%) as a pale yellow solid. Mp: 40 °C. IR (cm^{-1}): 3274, 2977, 1733, 1684, 1593, 1530. ^1H NMR ($CDCl_3$): δ 1.34 (s, 12H), 5.21 (s, 2H), 7.36 (d, 2H, J = 7.9 Hz), 7.45 (br s, 1H), 7.57 (d, 2H, J = 8.5 Hz), 7.79–7.81 (m, 4H), 9.87 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 191.9, 153.3, 144.1, 139.0, 135.5, 132.0, 131.7, 127.8, 127.6, 118.3, 84.4, 67.7, 25.3, 25.2. MS (TOF MS ES+, m/z): $(M + NH_4^+)$. HRMS (TOF MS ES+, m/z): calcd for $C_{21}H_{28}BN_2O_5$ $(M + NH_4^+)$ 399.2091, found 399.2099.

*(d). 4-(tert-Butyldimethylsilyloxy)benzyl 4-Formylphenylcarbamate (**22**).* Reagents: 4-formylbenzoic acid (**6**; 0.28 g, 1.8 mmol, 1 equiv), 1,4-dioxane (3.7 mL), triethylamine (0.26 mL, 2.0 mmol, 1.1 equiv), diphenylphosphoryl azide (0.44 mL, 2.0 mmol, 1.1 equiv), and 4-(*tert*-butyldimethylsilyloxy)phenylmethanol (**40**; 0.49, 2.0 mmol, 1.1 equiv). The residue was purified by silica gel flash column chromatography (5% ethyl acetate in hexanes, increasing to 40% ethyl acetate) to afford 4-(*tert*-butyldimethylsilyloxy)benzyl 4-formylphenylcarbamate (**22**; 0.42 g, 0.78 mmol, 42%) as a pale yellow solid. Mp: 122–123 °C. IR (cm^{-1}): 3317, 2952, 2855, 1722, 1675, 1589, 1507. ^1H NMR ($CDCl_3$): δ 0.18 (s, 6H), 0.98 (s, 9H), 5.12 (s, 2H), 6.82 (d, 2H, J = 8.4 Hz), 7.25 (d, 2H, J = 8.3 Hz), 7.57 (d, 2H, J = 8.5 Hz), 7.78 (2H, d, J = 8.6 Hz), 9.84 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 191.0, 156.1, 152.9, 143.7, 131.6, 131.3, 130.2, 128.2, 120.2, 118.0, 67.3, 25.6, 18.2, −4.4. MS (ES+, m/z): $(M + H^+)$, 386.4. HRMS (TOF MS ES+, m/z): calcd for $C_{21}H_{28}NO_4Si$ $(M + H^+)$ 386.1788, found 386.1775.

*N-(4-(Hydroxymethyl)phenyl)-2-phenylacetamide (**41**).* To a round-bottom flask was sequentially added 4-aminobenzyl alcohol (1.0 g, 8.1 mmol, 1 equiv), potassium acetate (1.6 g, 16 mmol, 2.0 equiv), and *N*,*N*-dimethylformamide (81 mL). The white suspension was cooled to −60 °C, and phenacetyl chloride (1.1 mL, 8.2 mmol, 1.0 equiv) was added dropwise to the suspension; each drop of phenacetyl chloride caused a yellow discoloration of the reaction suspension, and this color was allowed to dissipate before addition of the next drop of phenacetyl chloride. The resulting suspension was warmed to 25 °C and then was diluted with aqueous sodium hydroxide solution (20 mL, 1.0 M). The reaction solution was neutralized to pH 7 with aqueous hydrochloric acid solution (1.0 M), and the product mixture was extracted using dichloromethane (2 × 40 mL). The combined dichloromethane extracts were washed sequentially with water (2 × 40 mL) and brine (1 × 40 mL) and then were dried over sodium sulfate. The sodium sulfate was removed by filtration, and the resulting solution was concentrated by rotary evaporation. *N*-(4-(Hydroxymethyl)-phenyl)-2-phenylacetamide (**41**; 1.9 g, 7.9 mmol, 94%) was used without further purification. ^1H NMR (CD_3OD): δ 3.63 (s, 2H), 4.53 (s, 2H), 7.21–7.33 (m, 7H), 7.51–7.54 (m, 2H). The ^1H NMR data are in agreement with literature values.[50]

*N-(4-Formylphenyl)-2-phenylacetamide (**24**).* To a round-bottom flask containing dimethyl sulfoxide (0.71 mL, 10 mmol, 5.0 equiv) at 25 °C was added a solution of oxalyl chloride (0.39 mL, 4.5 mmol, 2.3 equiv) in dichloromethane (25 mL) at −78 °C. The resulting mixture was stirred for 10 min at −78 °C. To this cold reaction mixture was added a −78 °C solution of *N*-(4-(hydroxymethyl)-phenyl)-2-phenylacetamide (**41**; 0.50 g, 2.0 mmol, 1 equiv) dissolved in THF (10.0 mL). The resulting reaction solution was stirred for 15 min at −78 °C. Triethylamine (1.4 mL, 10 mmol, 5.0 equiv) was added dropwise. The reaction mixture was stirred for 15 min at −78 °C and then was warmed to 25 °C. Water (3 mL) was added to the

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product solution, followed by dichloromethane (15 mL). The layers were separated, and the organic layer was washed sequentially with water (2 × 25 mL) and brine (1 × 25 mL) and then was dried over sodium sulfate. The sodium sulfate was removed by filtration, and the filtrate was concentrated by rotary evaporation. The resulting residue was purified by silica gel flash column chromatography (20% ethyl acetate in hexanes, increasing to 60% ethyl acetate) to afford *N*-(4-formylphenyl)-2-phenylacetamide (**24**; 0.49 g, 2.0 mmol, 100%) as a yellow solid. Mp: 106 °C. IR (cm^{-1}): 3257, 3191, 2843, 2745, 1694, 1660, 1592, 1530. ^1H NMR ($CDCl_3$): δ 3.76 (s, 2H), 7.31−7.40 (m, 5H), 7.64 (d, 2H, J = 8.5 Hz), 7.80 (d, 2H, J = 8.6 Hz), 7.85 (br s, 1H), 9.89 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 191.1, 169.7, 143.4, 133.9, 132.2, 131.0, 129.4, 129.3, 127.8, 119.3, 44.8. MS (TOF MS ES+, m/z): (M + H^+), 240.1. HRMS (TOF MS ES+, m/z): calcd for $C_{15}H_{14}NO_2$ (M + H^+) 240.1025, found 240.1013.

*Methyl 1-(4-Formylphenyl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (**32**).* To a round-bottom flask was added sequentially methyl 2,3,4-tri-*O*-acetyl-D-glucopyranosyluronate bromide (**31**; 1.1 g, 2.7 mmol, 1 equiv), 4-hydroxybenzaldehyde (0.37 g, 3.05 mmol, 1.1 equiv), acetonitrile (30 mL), and silver(I) oxide (1.6 g, 6.9 mmol, 2.5 equiv). The flask was wrapped in aluminum foil, and the reaction mixture was stirred for 18 h at 25 °C. The mixture was filtered through a plug of Celite 545, with ethyl acetate as eluent (50 mL). The filtrate was collected, and the solvent was removed by rotary evaporation. The resulting residue was dissolved in ethyl acetate (75 mL), and the solution was washed with saturated aqueous sodium bicarbonate (5 × 50 mL). The organic layer was washed with brine (3 × 50 mL) and then dried over sodium sulfate. Sodium sulfate was removed by filtration, and the resulting solution was concentrated by rotary evaporation. The resulting residue was purified by silica gel flash column chromatography (25% acetone in hexanes) to afford methyl 1-(4-formylphenyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate (**32**; 0.63 g, 1.4 mmol, 52%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 9.88 (s, 1H), 7.83 (d, 2 H, J = 8.8 Hz), 7.09 (d, 2 H, J = 8.7 Hz), 5.36 (m, 3H), 5.30 (m, 3H), 4.27 (m, 1H), 3.68 (s, 3H), 2.02 (s, 9H). The ^1H NMR data are in agreement with literature values.[51]

*Methyl 1-(4-Hydroxymethylphenyl)-2,3,4-tri-O-acetyl-β-D-glucopyronuronate (**33**).* To a round-bottom flask was added methyl 1-(4-formylphenyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate (**32**; 0.59 g, 1.3 mmol, 1 equiv) and dichloromethane (14 mL), and the mixture was cooled to 0 °C. A solution of sodium borohydride (56 mg, 49 mmol, 1.1 equiv) in methanol (13.5 mL) was added via syringe, and the resulting reaction mixture was stirred at 0 °C for 30 min. The mixture was diluted with saturated aqueous ammonium chloride solution (75 mL), and the aqueous layer was extracted sequentially using dichloromethane (3 × 25 mL) and ethyl acetate (3 × 25 mL). The organic layers were combined and dried over sodium sulfate. Sodium sulfate was removed by filtration, and the solution was concentrated by rotary evaporation to afford methyl 1-(4-hydroxymethylphenyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyronuronate (**33**; 0.45 g, 1.0 mmol, 76%) as a white foam. ^1H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, 2H, J = 8.8 Hz), 6.97 (d, 2H, J = 8.7 Hz), 5.33−5.22 (m, 3H), 4.60 (s, 1H), 4.18−4.15 (m, 1H), 3.70 (s, 1H), 2.09 (s, 1H), 2.03 (d, 9H, J = 2.5 Hz). The ^1H NMR data are in agreement with literature values.[51]

General Procedure for the Curtius Rearrangement: Procedure B. To a round-bottom flask was added 4-formylbenzoic acid (**6**; 1 equiv), *N*,*N*-dimethylformamide (1 drop), and dichloromethane (to make a 0.2 M solution). The reaction mixture was cooled to 0 °C, and oxalyl chloride (2.0 equiv) was added dropwise to the reaction mixture. The mixture was stirred for 20 min at 0 °C and then was warmed to 25 °C. Dichloromethane was removed from the reaction mixture by rotary evaporation. The resulting crude acid chloride solution was dissolved in acetone (to make a 0.2 M solution), and the solution was cooled to 0 °C. To the acid chloride solution was added to a 5 °C solution of sodium azide (2.0 equiv) in water to make a 0.4 M solution. The reaction mixture was stirred until the acid chloride was consumed, as determined by thin-layer chromatography. The reaction mixture was diluted with water, and the biphasic solution was extracted twice using ethyl acetate. The combined organic layers were dried over sodium sulfate, the solids were removed by filtration, and the solution was concentrated by rotary evaporation. The resulting residue was dissolved in toluene (to make a 0.20 M solution), and the flask was affixed with a cold-finger reflux condenser. The reaction mixture was heated to 110 °C and held at that temperature until the azide was converted to the isocyanate (as determined by thin-layer chromatography). The reaction flask was cooled to 25 °C, and a solution of the desired alcohol (1.1 equiv) in dichloromethane (0.2 M) was added to the isocyanate solution. The reaction mixture was stirred at 25 °C for 12 h or until the isocyanate was consumed (as determined by thin-layer chromatography). Dichloromethane and toluene were removed from the product mixture by rotary evaporation, and the residue was dissolved in ethyl acetate. The organic solution was washed sequentially with saturated aqueous sodium bicarbonate solution (1×), water (1×), and brine (1×) and then was dried over sodium sulfate. The sodium sulfate was removed by filtration, and the solution was concentrated by rotary evaporation. The resulting residue was purified by silica gel flash column chromatography.

*(a). (2S,3S,4S,5R,6S)-Methyl 3,4,5-Triacetoxy-6-(4-((4-formylphenylcarbamoyloxy)methyl)phenoxy)-tetrahydro-2H-pyran-2-carboxylate (**34**).* Reagents: (i) 4-formylbenzoic acid (**6**; 0.25 g, 1.7 mmol, 1 equiv), oxalyl chloride (0.29 mL, 3.3 mmol, 2.0 equiv), *N*,*N*-dimethylformamide (1 drop), dichloromethane (8.4 mL), acetone (8.4 mL); (ii) sodium azide (0.22 g, 3.3 mmol, 2.0 equiv), water (8.4 mL); (iii) 1-(4-hydroxymethylphenyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyronuronate (**35**; 0.81 g, 1.8 mmol, 1.1 equiv), toluene (8.4 mL), dichloromethane (8.4 mL). The residue was purified by silica gel flash column chromatography (10% ethyl acetate in hexanes, increasing to 60% ethyl acetate) to afford (2*S*,3*S*,4*S*,5*R*,6*S*)-methyl 3,4,5-triacetoxy-6-(4-((4-formylphenylcarbamoyloxy)methyl)-phenoxy)tetrahydro-2*H*-pyran-2-carboxylate (**34**; 0.80 g, 1.4 mmol, 82%) as a white solid. Mp: 75−78 °C. IR (cm^{-1}): 3351, 2975, 1729, 1596, 1531. ^1H NMR ($CDCl_3$): δ 2.04−2.09 (m, 9H), 3.71 (s, 3H), 4.22 (d, 2H, J = 11 Hz), 5.15−5.18 (m, 3H), 5.25−5.36 (m, 3H), 6.98 (d, 2H, J = 8.0 Hz), 7.34 (d, 2H, J = 8.0 Hz), 7.45 (br s, 1H), 7.58 (d, 2H, J = 8.0 Hz), 7.81 (d, 2H, J = 8.0 Hz), 9.88 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 190.9, 170.0, 169.3, 169.1, 166.8, 156.7, 152.7, 143.5, 131.6, 131.2, 130.7, 130.1, 118.0, 117.1, 98.8, 72.6, 71.7, 71.0, 69.0, 66.8, 52.9, 20.5 (double intensity), 20.4. MS (ES+, m/z): (M + NH_4^+), 605.1. HRMS (TOF MS ES+, m/z): calcd for $C_{28}H_{33}N_2O_{13}$ (M + NH_4^+) 605.1983, found 605.1958.

General Procedure for TMSCl-Catalyzed HMDS Protection of Alcohols. To a round-bottom flask was added the desired alcohol (1 equiv) and hexamethyldisilazane (0.6 equiv). Chlorotrimethylsilane (0.05 equiv) was added dropwise via syringe. After being stirred overnight at 25 °C, the suspension was purified directly using silica gel flash column chromatography to afford the pure silyl ether.

*(3,7-Dimethyloct-6-enyloxy)trimethylsilane (**42**).* Reagents: (±)-β-citronellol (**37**; 3.0 g, 19 mmol, 1 equiv), hexamethyldisilazane (2.4 mL, 11 mmol, 0.60 equiv), chlorotrimethylsilane (0.12 mL, 0.95 mmol, 0.05 equiv). The product was purified by silica gel flash column chromatography (2% ethyl acetate in hexanes, increasing to 10% ethyl acetate) to afford (3,7-dimethyloct-6-enyloxy)trimethylsilane (**42**; 4.3 g, 18 mmol, 98%, colorless oil) as a mixture of enantiomers. IR (cm^{-1}): 2956, 2920. ^1H NMR ($CDCl_3$): δ 0.12 (s, 9H), 0.83−0.87 (m, 3H), 1.17−1.32 (m, 4H), 1.56−1.64 (m, 7H), 1.95−1.97 (m, 2H) 3.55−3.68 (m, 2H), 5.07 (t, 1H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 130.7, 124.9, 60.7, 39.8, 37.5, 29.1, 25.2, 19.2, 19.1, 17.7, −0.6. MS (TOF MS EI+, m/z): (M + H^+), 229.2; HRMS (TOF MS EI+, m/z): calcd for $C_{13}H_{29}OSi$ (M + H^+) 229.1909, found 229.1937.

*Trimethyl(phenethoxy)silane (**43**).* Reagents: 2-phenylethanol (**38**; 1.9 mL, 16 mmol, 1 equiv), hexamethyldisilazane (1.8 mL, 9.8 mmol, 0.60 equiv), chlorotrimethylsilane (0.11 mL, 0.8 mmol, 0.05 equiv). The residue was purified by silica gel flash column chromatography (2% ethyl acetate in hexanes, increasing to 10% ethyl acetate) to afford trimethyl(phenethoxy)silane (**43**; 3.0 g, 15 mmol, 94%) as a clear oil. ^1H NMR ($CDCl_3$): δ 0.20 (s, 9H), 2.96 (t, 2H, J = 7.3 Hz), 3.91 (t, 2H, J = 7.3 Hz), 7.28−7.42 (m, 5H). The ^1H NMR data are in agreement with literature values.[52]

*(S)-Methyl 2-(Trimethylsilyloxy)propanoate (**44**).* Reagents: (−)-methyl L-lactate (0.92 mL, 9.6 mmol, 1 equiv), hexamethyldisilazane

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(1.2 mL, 5.7 mmol, 0.60 equiv), chlorotrimethylsilane (61 μL, 0.48 mmol, 0.05 equiv). The residue was purified using silica gel flash column chromatography (2% ethyl acetate in hexanes, increasing to 10% ethyl acetate) to afford (*S*)-methyl 2-(trimethylsilyloxy)-propanoate (**44**; 1.4 g, 8.2 mmol, 85%) as a clear oil. IR (cm^{-1}): 2956, 1745. ^1H NMR ($CDCl_3$): δ −0.11 (s, 9H), 1.13 (d, 3H, *J* = 6.8 Hz), 3.47 (s, 3H), 4.07 (q, 1H, *J* = 6.5 Hz). ^{13}C NMR ($CDCl_3$): δ 174.2, 68.2, 51.7, 21.4, −0.2. MS (MS AP+, *m*/*z*): (M + H^+), 177.1.

*2-Methyl-1-phenyl-2-trimethylsilyloxypropane (**45**).* Reagents: 2-methyl-1-phenyl-2-propanol (0.98 mL, 6.6 mmol, 1 equiv), hexamethyldisilazane (0.84 mL, 4.0 mmol, 0.6 equiv), chlorotrimethylsilane (42 μL, 0.33 mmol, 0.05 equiv). The residue was purified using silica gel flash column chromatography (2% ethyl acetate in hexanes, increasing to 10% ethyl acetate) to afford 2-methyl-1-phenyl-2-trimethylsilyloxypropane (**45**; 1.2 g, 5.2 mmol, 79%) as a clear oil. ^1H NMR ($CDCl_3$): δ 0.35 (s, 9H), 1.48 (s, 6H), 2.98 (s, 2H), 7.44−7.53 (m, 5H). The ^1H NMR data are in agreement with literature values.[52]

General Procedure A for the TMSOTf-Catalyzed Acetal Formation. To a round-bottom flask containing flame-dried, finely ground, activated 3 Å molecular sieves (100 wt % with respect to aldehyde) was added the desired aldehyde (1 equiv), dichloromethane (to make a 0.20 M solution), and the desired silyl ether (4.0 equiv). The resulting suspension was cooled to −78 °C in an ice bath and placed under vacuum (∼1 mmHg) for 1 h at −78 °C, presumably to degas the solvent, as described in ref 11. (Note: we did not observe a decrease in the volume of the solution during this 1 h period under vacuum. We did, however, notice that the yields of the product from the reaction were substantially higher when this vacuum procedure was included in comparison to when this procedure was omitted.) The flask was filled with N_2, and trimethylsilyl trifluoromethanesulfonate (0.20 equiv) was added dropwise to the −78 °C suspension. The resulting suspension was stirred for 3−6 h at −78 °C. The resulting opaque suspension was warmed to 25 °C, and pyridine was added dropwise until a clear solution was formed. The solution was diluted with dichloromethane, and the molecular sieves were removed by filtration. The filtrate was collected and washed sequentially with saturated aqueous sodium bicarbonate solution (1×), water (1×), and brine (1×). The organic layer was dried over sodium sulfate, the sodium sulfate was removed by filtration, and the solution was concentrated by rotary evaporation. The resulting residue was purified using silica gel flash column chromatography.

*(a). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(Dimethoxymethyl)phenylcarbamate (**9**).* Reagents: 3 Å molecular sieves (0.50 g), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate (**8**; 0.50 g, 1.3 mmol, 1 equiv), dichloromethane (6.6 mL), methoxytrimethylsilane (0.72 mL, 5.2 mmol, 4.0 equiv), and trimethylsilyl trifluoromethanesulfonate (47 μL, 0.20 mmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (10% ethyl acetate in petroleum ether, increasing to 40% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(dimethoxymethyl)phenylcarbamate (**9**; 0.53 g, 1.2 mmol, 95%) as a pale yellow solid. Mp: 104 °C. IR (cm^{-1}): 3291, 2975, 1718, 1599, 1531. ^1H NMR ($CDCl_3$): δ 1.35 (s, 12H), 3.31 (s, 6H), 5.20 (s, 2H), 5.36 (s, 1H), 6.97 (br s, 1H), 7.39−7.37 (m, 6H), 7.83 (d, 2H, *J* = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 153.2, 138.9, 137.8, 134.9, 133.1, 131.2, 127.4, 127.2, 118.2, 102.7, 83.8, 66.8, 52.5, 24.7. MS (TOF MS ES+, *m*/*z*): (M + NH_4^+), 445.2. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{23}H_{34}BN_2O_6$ (M + NH_4^+) 445.2510, found 445.2527.

*(b). Allyl 4-(Dimethoxymethyl)phenylcarbamate (**21**).* Reagents: 3 Å molecular sieves (0.10 g), allyl 4-formylphenylcarbamate (**20**; 0.10 g, 0.48 mmol, 1 equiv), methoxytrimethylsilane (0.26 mL, 1.9 mmol, 4.0 equiv), dichloromethane (2.4 mL), trimethylsilyl trifluoromethanesulfonate (17 μL, 97 μmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (20% ethyl acetate in petroleum ether, increasing to 50% ethyl acetate) to afford allyl 4-(dimethoxymethyl)phenylcarbamate (**21**; 0.12 g, 0.45 mmol, 94%) as a pale yellow solid. Mp: 61 °C. IR (cm^{-1}): 3278, 2949, 1723, 1596, 1525. ^1H NMR ($CDCl_3$): δ 3.31 (s, 6H), 4.65 (d, 2H, *J* = 8.1 Hz), 5.24−5.39 (m, 3H), 5.92−6.03 (m, 1H), 6.84 (br s, 1H), 7.39 (s, 4H). ^{13}C NMR ($CDCl_3$): δ 153.6, 138.3, 133.5, 132.7, 127.9, 118.7, 103.1, 66.2, 52.9 (double intensity). MS (TOF MS ES+, *m*/*z*): (M + Na^+), 274.1. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{13}H_{17}NO_4Na$ (M + Na^+) 274.1055, found 274.1056.

*(c). 4-(tert-Butyldimethylsilyloxy)benzyl 4-(Dimethoxymethyl)-phenylcarbamate (**23**).* Reagents: 3 Å molecular sieves (0.10 g), 4-(*tert*-butyldimethylsilyloxy)benzyl 4-formylphenylcarbamate (**22**; 0.10 g, 0.27 mmol, 1 equiv), methoxytrimethylsilane (0.15 mL, 1.0 mmol, 4.0 equiv), dichloromethane (1.3 mL), trimethylsilyl trifluoromethanesulfonate (10 μL, 44 μmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (20% ethyl acetate in petroleum ether, increasing to 50% ethyl acetate) to afford 4-(*tert*-butyldimethylsilyloxy)benzyl 4-(dimethoxymethyl)phenylcarbamate (**23**; 0.12 mg, 0.22 mmol, 82%) as a pale yellow film. IR (cm^{-1}): 3332, 2980, 1734, 1607, 1531. ^1H NMR ($CDCl_3$): δ 0.19 (s, 6H), 0.99 (s, 9H), 3.30 (s, 6H), 5.08 (s, 2H), 5.35 (s, 1H), 6.74 (br s, 1H), 6.85 (d, 2H, *J* = 12 Hz), 7.26 (d, 2H, *J* = 12 Hz), 7.38 (s, 4H). ^{13}C NMR ($CDCl_3$): δ 156.3, 153.7, 138.3, 133.5, 130.5, 129.0, 127.9, 120.5, 118.6, 103.1, 67.3, 52.9, 26.0, 18.6, −4.0.

*(d). N-(4-(Dimethoxymethyl)phenyl)-2-phenylacetamide (**25**).* Reagents: 3 Å molecular sieves (0.10 g), *N*-(4-formylphenyl)-2-phenylacetamide (**24**; 0.10 g, 0.41 mmol, 1 equiv), methoxytrimethylsilane (0.23 mL, 1.7 mmol, 4.0 equiv), dichloromethane (2.1 mL), trimethylsilyl trifluoromethanesulfonate (15 μL, 83 μmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (20% ethyl acetate in petroleum ether, increasing to 50% ethyl acetate) to afford *N*-(4-(dimethoxymethyl)phenyl)-2-phenylacetamide (**25**) as a pale yellow solid (0.11 g, 0.39 mmol, 95%). Mp: 89 °C. IR (cm^{-1}): 3261, 3194, 3119, 3058, 2921, 1663, 1596, 1530. ^1H NMR ($CDCl_3$): δ 3.29 (s, 6H), 3.73 (s, 2H), 5.34 (s, 1H), 7.17 (br s, 1H), 7.26−7.43 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 169.0, 137.7, 134.3, 134.2, 129.5, 129.2, 127.7, 127.4, 119.4, 102.6, 52.5, 44.8. MS (TOF MS ES+, *m*/*z*): (M + H^+), 286.1. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{17}H_{19}NO_3$ (M + H^+): 286.1443, found 286.1437.

*(e). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(Bis-(3,8-dimethylnon-7-enyloxy)methyl)phenylcarbamate (**27**).* Reagents: 3 Å molecular sieves (0.10 g), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate (**8**; 0.10 g, 0.26 mmol, 1 equiv), dichloromethane (1.3 mL), (3,7-dimethyloct-6-enyloxy)-trimethylsilane (**42**; 0.24 g, 1.0 mmol, 4.0 equiv), and trimethylsilyl trifluoromethanesulfonate (10 μL, 0.2 mmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (10% ethyl acetate in petroleum ether, increasing to 40% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(bis(3,8-dimethylnon-7-enyloxy)methyl)phenylcarbamate (**27**; 0.18 g, 0.24 mmol, 92%) as a pale yellow film. The NMR data are complicated by diastereomers. IR (cm^{-1}): 3313, 2918, 1713, 1607, 1531. ^1H NMR ($CDCl_3$): δ 0.86−0.88 (m, 9H), 1.13−1.42 (m, 20H), 1.55−1.67 (m, 12H), 1.94−1.99 (m, 6H), 3.43−3.56 (m, 6H), 5.06−5.10 (m, 2H), 5.20 (s, 2H), 5.45 (s, 1H), 6.76 (br s, 1H), 7.35−7.40 (m, 6H), 7.82 (d, 2H, *J* = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 153.1, 138.9, 137.6, 135.0, 134.2, 131.2, 131.0, 127.4, 127.2, 124.8, 124.7, 118.2, 101.1, 83.8, 66.8, 63.4, 61.1, 39.8, 37.1, 36.7, 36.6, 29.5, 29.4, 29.1, 25.6, 25.4, 24.8, 19.5, 19.4, 17.6. MS (TOF MS ES+, *m*/*z*): (M + Na^+), 698.5. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{41}H_{62}BNO_6Na$ (M + Na^+) 698.4568, found 698.4592.

*(f). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(Bis-(benzyloxy)methyl)phenylcarbamate (**1**).* Reagents: 3 Å molecular sieves (0.10 g), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate (**8**; 0.10 g, 0.41 mmol, 1 equiv), benzyloxytrimethylsilane (0.20 mL, 1.0 mmol, 4.0 equiv), dichloromethane (1.3 mL), trimethylsilyl trifluoromethanesulfonate (12 μL, 52 μmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (10% ethyl acetate in petroleum ether, increasing to 50% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(bis(benzyloxy)methyl)phenylcarbamate (**1**; 0.13 g, 0.22 mmol, 84%) as a clear film. IR (cm^{-1}); 3311, 2978, 1714, 1607, 1528. ^1H NMR ($CDCl_3$): δ 1.34 (s, 12H), 4.58 (s, 4H), 5.21 (s, 2H), 5.73 (s, 1H), 6.76 (br s, 1H), 7.23−7.40 (m, 14H), 7.49 (d, 2H, *J* = 8.0 Hz),

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7.82 (d, 2H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$): δ 153.1, 138.9, 137.9, 134.9, 133.3, 128.4, 128.3, 127.7, 127.6, 127.5, 127.2, 126.8, 118.3, 99.9, 83.8, 66.8, 65.1, 24.8. MS (TOF MS ES+, m/z): (M + Na^+), 602.3. HRMS (TOF MS ES+, m/z): calcd for $C_{35}H_{38}BNO_6Na$ (M + Na^+) 602.2690, found 602.2722.

*(g). 2-Nitrobenzyl 4-(Dimethoxymethyl)phenylcarbamate (**19**).* Reagents: 3 Å molecular sieves (0.10 g), 2-nitrobenzyl 4-formylphenylcarbamate (**18**; 0.10 g, 0.33 mmol, 1 equiv), methoxytrimethylsilane (0.18 mL, 1.3 mmol, 4.0 equiv), dichloromethane (1.6 mL), trimethylsilyl trifluoromethanesulfonate (12 μL, 66 μmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (20% ethyl acetate in petroleum ether, increasing to 50% ethyl acetate) to afford 2-nitrobenzyl 4-(dimethoxymethyl)phenylcarbamate (**19**; 0.11 g, 0.31 mmol, 96%) as a pale yellow solid. Mp: 103 °C. IR (cm^{-1}): 3268, 3117, 2932, 2836, 1724, 1603, 1522. ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 6H), 5.36 (s, 1H), 5.60 (s, 2H), 7.02 (br s, 1H), 7.39 (s, 4H), 7.45−7.50 (m, 1H), 7.62−7.64 (m, 2H), 8.11 (d, 2H, J = 9.0 Hz). ^{13}C NMR ($CDCl_3$): δ 152.7, 147.3, 137.5, 133.7, 133.4, 132.4, 128.9, 128.7, 127.4, 125.0, 118.3, 102.6, 63.5, 52.5. MS (TOF MS ES+, m/z): (M + Na^+), 369.1. HRMS (TOF MS ES+, m/z): calcd for $C_{17}H_{18}N_2O_6Na$ (M + Na^+) 369.1063, found 369.1052.

General Procedure B for the TMSOTf-Catalyzed Acetal Formation. To a round-bottom flask containing flame-dried, finely ground, activated 3 Å molecular sieves (100 wt % with respect to the aldehyde) was added the desired aldehyde (1 equiv), dichloromethane (to make a 0.20 M solution), and the desired silyl ether (4.0 equiv). The resulting suspension was cooled to −78 °C in an ice bath and placed under vacuum (∼1 mmHg) for 1 h at −78 °C to degas the suspension. (Note: please see the comments about this step in General Procedure A.) The flask was filled with N_2, and trimethylsilyl trifluoromethanesulfonate (0.20 equiv) was added dropwise. The suspension was stirred for 1 h at −78 °C and then was warmed to 25 °C. After the mixture was stirred for 12 h at 25 °C, pyridine was added dropwise to the opaque suspension until the solution became clear. The solution was diluted with dichloromethane, and the molecular sieves were removed by filtration. The filtrate was collected and was washed sequentially with saturated aqueous sodium bicarbonate solution (1×), water (1×), and brine (1×). The organic layer was dried over sodium sulfate, the sodium sulfate was removed by filtration, and the solution was concentrated by rotary evaporation. The crude product was purified using silica gel flash column chromatography.

*(a). (2S,2′S)-Dimethyl 2,2′-((4-((4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyloxy)carbonylamino)phenyl)methylene)bis(oxy)dipropanoate (**29**).* Reagents: 3 Å molecular sieves (0.20 g), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate (**8**; 0.20 g, 0.52 mmol, 1 equiv), dichloromethane (2.3 mL), (*S*)-methyl 2-(trimethylsilyloxy)propanoate (**44**; 0.37 mL, 2.0 mmol, 4.0 equiv), and trimethylsilyl trifluoromethanesulfonate (19 μL, 0.10 mmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (20% ethyl acetate in petroleum ether, increasing to 50% ethyl acetate) to afford (2*S*,2′*S*)-dimethyl 2,2′-((4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyloxy)-carbonylamino)phenyl)methylene)bis(oxy)dipropanoate (**29**; 0.26 g, 0.45 mmol, 87%) as a pale yellow solid. IR (cm^{-1}): 3355, 2980, 1721, 1604, 1531. ^{1}H NMR ($CDCl_3$): δ 1.34 (s, 12H), 1.39 (d, 3H, J = 6.8 Hz), 1.44 (d, 2H, J = 6.8 Hz), 3.65 (s, 3H), 3.72 (s, 3H), 4.14 (q, 1H, J = 14 Hz), 4.76 (q, 1H, J = 9.4), 5.20 (s, 2H), 5.59 (s, 1H), 6.82 (br s, 1H), 7.37−7.45 (m, 6H), 7.82 (d, 2H, J = 7.9 Hz). ^{13}C NMR ($CDCl_3$): δ 174.0, 173.7, 139.3, 138.9, 135.4, 132.8, 128.2, 127.7, 118.8, 100.7, 84.2, 70.6, 69.8, 67.2, 52.3, 25.2, 19.2. MS (MS ES+, m/z): (M + NH_4^+), 589.4. HRMS (TOF MS ES+, m/z): calcd for $C_{29}H_{42}N_2O_{10}B$ (M + NH_4^+) 589.2933, found 589.2929.

*(b). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(Diphenoxymethyl)phenylcarbamate (**11**).* Reagents: 3 Å molecular sieves (0.10 g), (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate) (**8**; 0.10 g, 0.26 mmol, 1 equiv), dichloromethane (1.3 mL), trimethyl(phenoxy)silane (0.19 mL, 1.0 mmol, 4.0 equiv), and trimethylsilyl trifluoromethanesulfonate (9.3 μL, 52 μmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (5% ethyl acetate in petroleum ether, increasing to 40% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(diphenoxymethyl)phenylcarbamate (**11**; 0.12 g, 0.21 mmol, 84%) as a white solid. The ^{1}H and ^{13}C NMR spectra are complicated by rotamers. Mp: 72 °C. IR (cm^{-1}): 3326, 2976, 1732, 1605, 1526. ^{1}H NMR ($CDCl_3$): δ 1.33 (s, 12H), 5.19 (s, 2H), 6.62 (s, 1H), 6.82 (br s, 1H), 6.95−7.02 (m, 6H), 7.20−7.27 (m, 4H), 7.35−7.43 (m, 4H), 7.55 (d, 2H, J = 8.7 Hz), 7.82 (d, 2H, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 156.1, 153.1, 138.9, 138.6, 135.1, 132.4, 129.5, 129.4, 127.6, 127.4, 127.3, 122.4, 118.5, 117.5, 117.3, 100.3, 100.1, 99.8, 83.9, 66.9, 25.1, 24.8, 24.8, 24.5. Anal. Calcd: C, 71.88; N, 2.54; H, 6.21. Found: C, 71.87; N, 2.51; H, 6.23.

*(c). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(Diisopropoxymethyl)phenylcarbamate (**28**).* Reagents: 3 Å molecular sieves (0.10 g), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-benzyl 4-formylphenylcarbamate) (**8**; 0.10 g, 0.26 mmol, 1 equiv), dichloromethane (1.3 mL), isopropoxytrimethylsilane (0.19 mL, 1.0 mmol, 4.0 equiv), and trimethylsilyl trifluoromethanesulfonate (9.4 μL, 52 μmol, 0.20 equiv). The residue was purified using silica gel flash column chromatography (10% ethyl acetate in petroleum ether, increasing to 30% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(diisopropoxymethyl)phenylcarbamate (**28**; 53 mg, 0.10 mmol, 42%) as a pale yellow solid. Mp: 117 °C. IR (cm^{-1}): 3279, 2974, 1732, 1600, 1528. ^{1}H NMR ($CDCl_3$): δ 1.14−1.19 (m, 12H), 1.34 (s, 12H), 3.85−3.92 (m, 2H), 5.20 (s, 2H), 5.51 (s, 1H), 6.69 (br s, 1H), 7.34−7.42 (m, 6H), 7.82 (d, 2H, J = 7.7 Hz). ^{13}C NMR δ 153.6, 139.4, 138.0, 136.2, 135.5, 127.9, 127.7, 118.7, 99.2, 84.2, 68.2, 67.3, 25.2, 23.5, 22.9. MS (TOF MS ES+, m/z): (M + Na^+), 506.2. HRMS (TOF MS ES+, m/z): calcd for $C_{27}H_{38}BNO_6Na$ (M + Na^+) 506.2690, found 506.2690.

General Procedure for the TMSOTf-Catalyzed Mixed-Acetal Formation. To a round-bottom flask containing flame-dried, finely ground, activated 3 Å molecular sieves (100 wt % with respect to the aldehyde) was added the desired aldehyde (1 equiv), dichloromethane (to make a 0.20 M solution), and a 1:1 mixture of the desired silyl ethers (4.0 equiv total, 2.0 equiv of each silyl ether). The resulting suspension was cooled to −78 °C and was placed under vacuum (∼1 mmHg) for 1 h at −78 °C to degas the suspension. (Note: please see the comments about this step in General Procedure A.) The flask was filled with N_2, and trimethylsilyl trifluoromethanesulfonate (0.20 equiv) was added dropwise. The suspension was stirred for 1 h at −78 °C and then was warmed to 25 °C. After the mixture was stirred for 12 h at 25 °C, pyridine was added dropwise to the opaque suspension until a clear solution was formed. The solution was diluted with dichloromethane, and the molecular sieves were removed by filtration. The filtrate was collected and was washed sequentially with saturated aqueous sodium bicarbonate solution (1×), water (1×), and brine (1×). The organic solvent was dried over sodium sulfate, the sodium sulfate was removed by filtration, and the solution was concentrated by rotary evaporation. The crude product was purified using silica gel flash column chromatography.

*(a). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(Benzyloxy(ethoxy)methyl)phenylcarbamate (**26**).* Reagents: 3 Å molecular sieves (0.40 g), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate (**8**; 0.40 g, 0.95 mmol, 1 equiv), ethoxytrimethylsilane (0.29 mL, 1.9 mmol, 2.0 equiv), benzyloxytrimethylsilane (0.34 mL, 1.9 mmol, 2.0 equiv), dichloromethane (4.7 mL), and trimethylsilyl trifluoromethanesulfonate (51 μL, 0.28 mmol, 0.20 equiv). The product was purified using silica gel flash column chromatography (5% ethyl acetate in petroleum ether, increasing to 40% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(benzyloxy(ethoxy)methyl)phenylcarbamate (**26**; 0.33 g, 0.63 mmol, 67%) as a pale yellow oil. ^{1}H NMR data for compound **26** indicated a rotameric mixture, and the ^{1}H NMR spectrum taken at 60 °C showed significant coalescing of peaks. The following data were collected at 25 °C, and therefore, unassigned rotameric peaks are reported as well. IR (cm^{-1}): 3307, 2976, 1711, 1600, 1529. ^{1}H NMR ($CDCl_3$): δ 1.19−1.33 (m, 3H), 1.34 (s, 12H), 3.55−3.62 (m, 2H), 4.56 (d, 2H, J = 11 Hz), 5.20 (s, 2H), 5.60 (t, 1H, J = 40 Hz), 6.81 (s, 1H), 7.14−7.48 (m, 12H), 7.81 (d, 2H, J = 6.7 Hz). ^{13}C NMR

($CDCl_3$): δ 191.4, 153.6, 153.1, 143.9, 139.4, 138.9, 138.6, 138.4, 138.2, 135.5, 135.4, 134.3, 132.2, 131.7, 128.9, 128.7, 128.4, 128.1, 128.0, 127.9, 127.7, 118.5, 116.7, 114.5, 112.0, 100.9, 100.4, 84.4, 84.3, 67.8, 67.4, 61.4, 25.4, 25.3, 25.2, 25.1, 15.6. MS (TOF MS ES+, *m*/*z*): (M + Na^+), 540.3. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{30}H_{36}BNO_6Na$ (M + Na^+) 540.2533, found 540.2532.

*(b). (2S,3S,4S,5R,6S)-Methyl 3,4,5-Triacetoxy-6-(4-((4-((3,7-dimethyloct-6- enyloxy)(phenethoxy)methyl)phenylcarbamoyloxy)-methyl)phenoxy)tetrahydro-2H-pyran-2-carboxylate (**35**).* Reagents: 3 Å molecular sieves (0.30 g), (2*S*,3*S*,4*S*,5*R*,6*S*)-methyl 3,4,5-triacetoxy-6-(4-((4-formylphenylcarbamoyloxy)methyl)phenoxy)-tetrahydro-2*H*-pyran-2-carboxylate (**34**; 30 mg, 52 μmol, 1 equiv), (3,7-dimethyloct-6-enyloxy)trimethylsilane (**42**; 23 mg, 0.10 mmol, 2.0 equiv), trimethyl(phenethoxy)silane (**43**; 20 mg, 0.10 mmol, 2.0 equiv), dichloromethane (0.23 mL), and trimethylsilyl trifluoromethanesulfonate (1.8 μL, 0.10 mmol, 0.20 equiv). The product was purified using silica gel flash column chromatography (20% ethyl acetate in petroleum ether, increasing to 60% ethyl acetate) to afford (2*S*,3*S*,4*S*,5*R*,6*S*)-methyl 3,4,5-triacetoxy-6-(4-((4-((3,7-dimethyloct-6-enyloxy)(phenethoxy)methyl)phenylcarbamoyloxy)methyl)-phenoxy)-tetrahydro-2*H*-pyran-2-carboxylate (**35**; 15 mg, 17 μmol, 33%) as a waxy white film. The ^1H NMR and ^{13}C NMR spectra likely contain signals for rotamers, as was observed for compound **26**. The spectra also contain signals for diastereomers. IR (cm^{-1}): 2920, 1750, 1610, 1508. ^1H NMR ($CDCl_3$): δ 0.82−1.05 (m, 3H), 1.15−1.91 (m, 13H), 1.92−2.27 (m, 11H), 3.72 (s, 3H), 4.04−4.19 (m, 1H), 4.41 (d, 2H, *J* = 10 Hz), 4.57 (d, 2H, *J* = 12 Hz), 5.12−5.14 (m, 3H), 5.18−5.38 (m, 4H), 5.79−5.90 (m, 1H), 6.63 (br s, 1H), 7.00 (d, 2H, *J* = 8.0 Hz), 7.13−7.35 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 170.1, 169.3, 169.2, 166.8, 156.6, 153.2, 139.0, 137.6, 131.2, 131.1, 130.0, 128.9, 128.9, 128.2, 127.4, 126.1, 124.7, 118.1, 117.1, 101.0, 99.0, 77.2, 72.6, 71.8, 70.9, 69.0, 66.4, 65.8, 63.8, 53.0, 37.1, 36.6, 36.3, 29.5, 25.7, 25.4, 20.6, 20.5, 19.4, 17.6. MS (TOF MS ES+, *m*/*z*): (M + NH_4^+), 865.4. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{46}H_{61}N_2O_{14}$ (M + NH_4^+) 865.4123, found 865.4097.

*Lithium (2S,3S,4S,5R,6S)-6-(4-((4-((3,7-Dimethyloct-6-enyloxy)-(phenethoxy)methyl)phenylcarbamoyloxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylate (**36**).* To a round-bottom flask, cooled to 0 °C, was added (2*S*,3*S*,4*S*,5*R*,6*S*)-methyl 3,4,5-triacetoxy-6-(4-((4-((3,7-dimethyloct-6-enyloxy)(phenethoxy)-methyl)phenylcarbamoyloxy)methyl)phenoxy)tetrahydro-2*H*-pyran-2-carboxylate (**35**; 30 mg, 35 μmol, 1 equiv) and a 0 °C solution of lithium hydroxide hydrate (8.4 mg, 0.21 mmol, 6.0 equiv) in a mixture of methanol (2.6 mL), water (1.0 mL), and tetrahydrofuran (0.53 mL). The reaction mixture was stirred for 1 h at 0 °C, at which point an aliquot of the solution was removed. HPLC analysis was used to confirm complete deprotection. Methanol and tetrahydrofuran were removed from the solution by rotary evaporation, and water was removed by lyophilization to afford lithium (2*S*,3*S*,4*S*,5*R*,6*S*)-6-(4-((4-((3,7-dimethyloct-6-enyloxy)(phenethoxy)methyl)-phenylcarbamoyloxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylate (**36**) as a white solid. The product was used without further purification; therefore, a yield is not reported. The ^1H NMR and ^{13}C NMR spectra likely contain signals for rotamers, as was observed for compound **26**. The spectra also contain signals for diastereomers. Mp: 138 °C dec. IR (cm^{-1}): 3296, 2919, 2359, 2338, 1593, 1510. ^1H NMR (CD_3OD): δ 0.85−0.92 (m, 3H), 1.15−2.05 (m, 13H), 2.85 (t, 2H, *J* = 6.1 Hz), 3.2−3.75 (m, 12H), 4.92−5.13 (m, 4H), 5.40 (s, 1H), 7.04−7.30 (m, 9H), 7.30−7.40 (m, 4H). ^{13}C NMR (CD_3OD): δ 179.1, 175.1, 157.8, 154.5, 139.2, 138.9, 133.2, 130.6, 130.5, 129.3, 128.7, 127.9, 126.9, 125.8, 124.4, 117.9, 116.5, 101.4, 101.0, 76.7, 75.3, 73.4, 72.2, 67.5, 66.0, 65.9, 63.4, 63.3, 36.8, 36.7, 36.3, 35.9, 29.2, 29.1, 25.0, 24.5, 22.8, 18.7, 18.6, 16.4. MS (MS ES-, *m*/*z*): $(M - H)^-$, 706.3. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{39}H_{49}NO_{11}Li$ $(M + Li)^+$ 714.3466, found 714.3472.

*4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(Hydroxymethyl)phenylcarbamate (**46**).* To a round-bottom flask was added 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-formylphenylcarbamate (**8**; 1.3 g, 3.3 mmol, 1 equiv), dichloromethane (16 mL), and methanol (16 mL). The mixture was cooled to 0 °C, and sodium borohydride (0.16 g, 4.3 mmol, 1.3 equiv) was added. The resulting mixture was warmed to 25 °C, and after being stirred at 25 °C for 12 h, the solution mixture was diluted with saturated aqueous ammonium chloride solution (20 mL). The biphasic solution was extracted with dichloromethane (4 × 20 mL), and the combined organic layers were washed with brine (1 × 20 mL) and dried over sodium sulfate. The sodium sulfate was removed by filtration, and the solution was concentrated by rotary evaporation. The residue was purified using silica gel flash column chromatography (3% methanol in benzene, increasing to 10% methanol) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(hydroxymethyl)-phenylcarbamate (**46**; 1.0 g, 2.7 mmol, 84%) as a yellow solid. Mp: 130 °C. IR (cm^{-1}): 3454, 3236, 1726, 1604, 1537. ^1H NMR ($CDCl_3$): δ 1.33 (s, 12H), 4.60 (s, 2H), 5.19 (s, 2H), 6.86 (br s, 1H), 7.27 (d, 2H, *J* = 8.7 Hz), 7.34−7.38 (m, 4H), 7.80 (d, 2H, *J* = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 153.3, 138.9, 137.1, 136.0, 135.0, 128.2, 127.8, 127.2, 118.8, 83.8, 66.8, 64.8, 24.8. MS (TOF MS ES+, *m*/*z*): (M + NH_4^+), 401.2. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{21}H_{30}BN_2O_5$ (M + NH_4^+) 401.2248, found 401.2253.

*4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-((Phenoxycarbonyloxy)methyl)phenylcarbamate (**12**).* To a round-bottom flask was added 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(hydroxymethyl)phenylcarbamate (**46**; 0.20 g, 5.2 mmol, 1 equiv), dichloromethane (2.6 mL), *N*,*N*-diisopropylethylamine (0.23 mL, 1.3 mmol, 2.5 equiv), phenyl chloroformate (0.13 mL, 1.0 mmol, 2.0 equiv), and 4-(dimethylamino)pyridine (6.3 mg, 52 μmol, 0.10 equiv). The mixture was stirred at 25 °C for 12 h. The resulting solution was diluted with saturated aqueous ammonium chloride solution (1 × 5 mL), and the aqueous layer was extracted using dichloromethane (2 × 10 mL). The organic extracts were combined and dried over sodium sulfate. The sodium sulfate was removed by filtration, and the solution was concentrated by rotary evaporation. The residue was purified by silica gel flash column chromatography (10% ethyl acetate in hexanes, increasing to 40% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-((phenoxycarbonyloxy)methyl)phenylcarbamate (**12**; 0.24 g, 0.48 mmol, 92%) as a white solid. Mp: 88−92 °C. IR (cm^{-1}): 3352, 2976, 2359, 1727, 1600, 1532. ^1H NMR ($CDCl_3$): δ 1.37 (s, 12H), 5.22 (s, 2H), 5.23 (s, 2H), 6.88 (br s, 1H), 7.10−7.20 (m, 3H), 7.37−7.42 (m, 8H), 7.85 (d, 2H, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 153.6, 153.1, 151.0, 138.8, 138.3, 135.0, 129.8, 129.3, 127.4, 127.2, 125.9, 121.1, 120.9, 118.7, 83.8, 69.9, 66.9, 24.5. MS (MS ES+, *m*/*z*): (M + NH_4^+), 521.3. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{28}H_{34}BN_2O_7$ (M + NH_4^+): 521.2459, found 521.2465.

*4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-((Methoxycarbonyloxy)methyl)phenylcarbamate (**10**).* To a round-bottom flask was added 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-(hydroxymethyl)phenylcarbamate (**46**; 0.83 g, 2.2 mmol, 1 equiv), dichloromethane (22 mL), *N*,*N*-diisopropylethylamine (0.60 mL, 3.4 mmol, 1.5 equiv), methyl chloroformate (0.25 mL, 3.3 mmol, 1.5 equiv), and 4-(dimethylamino)pyridine (26 mg, 0.22 mmol, 0.10 equiv). The solution was stirred at 25 °C for 12 h. The resulting mixture was diluted using saturated aqueous ammonium chloride solution (20 mL), and the aqueous layer was extracted using dichloromethane (2 × 20 mL). The organic layers were combined and were dried over sodium sulfate. The sodium sulfate was removed by filtration, and the resulting solution was concentrated by rotary evaporation. The residue was purified using silica gel flash column chromatography (20% ethyl acetate in hexanes, increasing to 40% ethyl acetate) to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl 4-((methoxycarbonyloxy)methyl)phenylcarbamate (**10**; 0.24 g, 0.48 mmol, 92%) as a white solid. Mp: 96−98 °C. IR (cm^{-1}): 3350, 2972, 2358, 1714, 1610, 1532. ^1H NMR ($CDCl_3$): δ 1.34 (s, 12H), 3.76 (s, 3H), 5.08 (s, 2H), 5.16 (s, 2H), 7.19 (s, 1H), 7.27−7.40 (m, 6H), 7.79 (d, 2H, *J* = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 156.2, 153.8, 139.5, 138.7, 135.5, 130.6, 129.9, 127.8, 119.1, 84.4, 69.9, 69.8, 67.3, 55.3, 25.3. MS (TOF MS ES+, *m*/*z*): (M + NH_4^+), 459.2. HRMS (TOF MS ES+, *m*/*z*): calcd for $C_{23}H_{32}BN_2O_7$ (M + NH^{4+}) 459.2303, found 459.2294.

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General Procedure for Measuring Rates of Nonspecific Hydrolysis and Rates of Release Using ^{1}H NMR Spectroscopy. The NMR spectrometer was programmed to acquire 32 scans for each sample with a relaxation time of 2 s to ensure accurate integration values. Buffer solutions were prepared according to Gomori[53] and Sorensen,[54] with the exception that D_2O was used instead of water.

Example Procedure for Sample Preparation. Reagent **9** (3 mg) was dissolved in CD_3CN (0.49 mL), and to the resulting solution were sequentially added D_2O (0.48 mL), a 0.1 M solution of sodium phosphate or sodium bicarbonate buffer in D_2O (25 μL), and a 0.8 M solution of 2,5-dimethylfuran (an internal standard) in CD_3CN (10 μL). The vial was capped and vortexed for ~15 s. A portion (600 μL) of the solution was transferred into an NMR tube. The tube was capped and sealed with PTFE tape.

pH Stability of Model Reagent 9. The nonspecific hydrolysis of acetal **9** was monitored by ^{1}H NMR at 25 °C. The buffer solutions consisted of sodium phosphate for pH values 6.0, 7.0, and 8.0 and of sodium bicarbonate for pH 9.0 and 10.0.

A ^{1}H NMR spectrum was acquired 10 min after sample preparation and then again after 6 h. Integration of the ^{1}H NMR peaks corresponding to the aldehyde hydrogen in **8** and the acetal methine hydrogen in **9** were used to calculate the percentage of acetal **9** remaining at the conclusion of the experiment (Table S1, Supporting Information). Aldehyde **8**, acetal **9**, and methanol (arising from hydrolysis of **9**) were the only compounds in the ^{1}H NMR over the course of the experiment. Each experiment was performed in triplicate.

Thermal Stability of Model Acetal 9. The nonspecific hydrolysis of acetal **9** was monitored by ^{1}H NMR at 25 °C. The solutions were buffered using sodium phosphate, and the pH was adjusted to 8.0.

^{1}H NMR spectra were acquired immediately after sample preparation and then again after 6 h. During the 6 h time period, the samples were maintained either at 15 °C (by immersion in a water-filled refrigerated recirculator) or at 35, 45, or 55 °C (by immersion in hot water baths maintained at their respective temperatures). Integration of the ^{1}H NMR peaks corresponding to the aldehyde hydrogen in **8** and the acetal methine hydrogen in **9** were used to calculate the percentage of acetal **9** remaining at the conclusion of the experiment (Table S2, Supporting Information). Aldehyde **8**, acetal **9**, and methanol (arising from hydrolysis of **9**) were the only compounds in the ^{1}H NMR over the course of the experiment. Each experiment was performed in triplicate.

Controlled Release of Methanol from Model Reagents 9 and 10 in Response to Hydrogen Peroxide. The release of methanol from model acetal **9** and control carbonate **10** was monitored by ^{1}H NMR at 25 °C. The solutions were buffered using sodium phosphate, and the pH was adjusted to 8.0. The time intervals shown in Tables S3 and S4 (Supporting Information) are reported at the median time for each single acquisition (rather than at the start or conclusion of the acquisition).

A ^{1}H NMR spectrum was acquired to establish baseline integration values relative to 2,5-dimethylfuran (an internal standard). A solution of H_2O_2–urea in D_2O (2.9 M, 10 equiv; 23.4 μL was added to the solution of **9** and 22.6 μL was added to the solution of **10**) was added to the NMR sample. The NMR tube was agitated by inversion (3×) and returned to the spectrometer. The acquisition was started immediately and was monitored over regular preprogrammed intervals.

The release of methanol from **9** was measured by monitoring the disappearance of the acetal methine hydrogen signal in **9** relative to 2,5-dimethylfuran (Table S3). For compound **10**, the release of methanol was measured by monitoring the appearance of CH_3OD (measured relative to 2,5-dimethylfuran) (Table S4). The data were normalized for the number of equivalents of methanol released. Each experiment was performed in triplicate.

Rate of Nonspecific Release of Methanol from Reagents 9 and 10. The nonspecific hydrolysis of acetal **9** and control carbonate **10** was monitored by ^{1}H NMR at 25 °C. The solutions were buffered using sodium phosphate, and the pH was adjusted to 8.0.

^{1}H NMR spectra were acquired ~10 min after sample preparation (this time was set as t = 0 min) and then again after 6 h. Relative integration values of the ^{1}H NMR peaks corresponding to the aldehyde hydrogen in **8** and the acetal methine hydrogen in **9** were used to calculate the percentage of acetal **9** remaining at the conclusion of each experiment (Table S5, Supporting Information). Integration of the ^{1}H NMR peak corresponding to CH_3OD (relative to 2,5-dimethylfuran) was used to calculate the percentage of carbonate **10** remaining at the conclusion of each experiment (Table S6, Supporting Information). Each experiment was performed in triplicate.

Rate of Nonspecific Hydrolysis of Acetal 11 and Carbonate 12. The nonspecific hydrolysis of acetal **11** and carbonate **12** were monitored by ^{1}H NMR at 25 °C (**11**) or by HPLC (**12**) at 25 °C. The buffers were prepared using procedures identical with those described above.

^{1}H NMR spectra were acquired ~10 min after preparation of the sample and then again after 720 min. Integration of the ^{1}H NMR peak corresponding to the methine hydrogen of acetal **11** (relative to 2,5-dimethylfuran) was used to calculate the percentage of acetal **11** remaining at the conclusion of each experiment (Table S7, Supporting Information). Each experiment was performed in triplicate.

Carbonate **12** (2.0 mg) was dissolved in CD_3CN (0.74 mL), and to the resulting solution were added sequentially D_2O (225 μL), a 0.1 M solution of pH 8.0 sodium phosphate buffer (25 μL), and a 0.8 M solution of anisole in CD_3CN (10 μL). The vial was capped and vortexed for ~15 s. Samples were injected onto the HPLC at 39 min intervals, with the last injection occurring 741 min after the first injection. Integration values for the benzyl alcohol product (**46**, which arises from hydrolysis of the carbonate functionality in **12**; the peak was obtained using an absorbance detector set at 254 nm) relative to anisole (254 nm) were used to calculate the percentage of carbonate **12** remaining (Table S8, Supporting Information) at each time point. Each experiment was performed in triplicate.

Postulated Mechanism for the Controlled Release of Alcohols from Benzylic Acetals. The products arising from reaction of acetal **1** with hydrogen peroxide–urea were identified using an LC-MS. The buffers were prepared using procedures identical with those described above.

Acetal **1** (1.0 mg) was dissolved in CH_3CN (0.49 mL), and to the resulting solution were added sequentially water (0.48 mL), a 0.1 M solution of pH 8.0 sodium phosphate buffer in H_2O (25 μL), and a 0.8 M solution of anisole in CH_3CN (25 μL). The vial was capped and vortexed for ~15 s. To the vial was added a 1.73 M solution of hydrogen peroxide–urea in H_2O (10 μL), and the vial was capped and vortexed for an additional ~15 s. The mixture was allowed to stand for 3 h at 25 °C. After this period, the sample was injected into the LC-MS. The molecular weights were determined for all UV-active compounds present, and these values were compared to standards of authentic material, when possible (Table S9, Supporting Information).

Into a vial were added a 3.5 mM solution of acetal **1** in CH_3CN (49 μL), $H_2{}^{18}O$ (49 μL), a 0.1 M solution of pH 8.0 sodium phosphate buffer in H_2O (2.5 μL), and a 0.8 M solution of anisole in CH_3CN (1 μL). The vial was capped and vortexed for ~15 s. Into the vial was added a 1.73 M solution of hydrogen peroxide–urea in H_2O (1 μL), and the vial was capped and vortexed for an additional ~15 s. The mixture was allowed to stand for 3 h at 25 °C. After this period, the sample was injected into the LC-MS. The molecular weights were determined for all UV-active compounds present, and these values were compared with the retention times and m/z values found in the unlabeled H_2O study (Table S9).

Release of Two Types of Fragrances and a Colorimetric Indicator in Response to β-D-Glucuronidase: An LC-MS Study. The sample of **36** was heated and maintained at 30 °C throughout the experiment. The buffer solution was prepared according to Gomori[53] and Sorensen.[54] The solutions were buffered using sodium phosphate, and the pH was adjusted to 8.0.

Into an LC-MS vial were added a 10.8 mM solution of acetal **36** in methanol (142 μL), a 75 mM aqueous solution of sodium phosphate buffer with 1% w/v bovine serum albumin (320 μL, pH 7.4), water

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(332 μL), and a 0.8 M solution of anisole in methanol (5.0 μL). The vial was capped and vortexed for ∼15 s. A 10 μL sample was analyzed by LC-MS to establish the retention times and number of peaks prior to exposure to β-D-glucuronidase. One minute prior to the second automated injection at 19 min, a 19.23 unit/mL solution of β-D-glucuronidase (6.5 μL) was added to the vial. The vial was capped and vortexed (∼2 s). The sample was immediately injected onto the LC-MS. The peaks shown in Figure S1 (Supporting Information) were identified by comparison with the retention times and *m*/*z* values of known standards, when possible. The peak areas of 2-phenylethanol (**38**) and citronellol (**37**) were compared to the peak area of an internal standard (anisole) at 210 nm. The data are shown in Table S10 (Supporting Information).

Preparation and Analysis of 36 in the Absence of β-D-Glucuronidase. Into a LC-MS vial were added sequentially a 10.8 mM solution of acetal **36** in methanol (142 μL), a 75 mM aqueous solution sodium phosphate buffer with 1% (w/v) bovine serum albumin (320 μL, pH 7.4), water (338 μL), and a 0.8 M solution of anisole in methanol (5.0 μL). The vial was capped and vortexed for ∼15 s. The sample was injected into the LC-MS immediately. The peaks shown in Figure S1 were identified by comparison to the retention times and *m*/*z* values of known standards, when possible. The peak areas of 2-phenylethanol (**38**) and citronellol (**37**) were referenced to the peak area of an internal standard (anisole) at 210 nm. The data are shown in Table S10.

Detection of a Fragrance from Reagent 36 on Exposure to β-D-Glucuronidase. Acetal **36** was prepared both in the presence and absence of β-D-glucuronidase in a manner identical with the conditions described above. After the addition of β-D-glucuronidase to the experimental sample (and preparation of the control in the absence of β-D-glucuronidase), the samples were incubated at 30 °C. After they were heated for 380 min at 30 °C, the samples were cooled to 25 °C. The vials were wrapped in aluminum foil (to mask any differences in color), uncapped, and placed on a benchtop. Five volunteers (Kyle Schmid, Daniel Wendekier, Anthony DiLauro, Jessica Robbins, and Michael Olah) were individually and separately asked to choose a vial and describe the odor. All volunteers detected a fragrance in the experimental sample containing β-D-glucuronidase (descriptions included "citrus-like", "lemony", "rosy", and/or "floral") and detected no odor from the control sample containing no added β-D-glucuronidase.

Release of Two Types of Fragrances and a Colorimetric Indicator in Response to β-D-Glucuronidase. *Colorimetric Study.* The colorimetric response of reagent **36** in the presence and absence of β-D-glucuronidase was monitored by capturing photographs. The samples were maintained at 30 °C between photographs.

A LC-MS vial (fitted with a 250 μL glass insert) was loaded sequentially with a 5.4 mM solution of reagent **36** in a 1:1 mixture of methanol and water (71 μL), a 75 mM aqueous solution of pH 7.4 sodium phosphate buffer with 1% (w/v) bovine serum albumin (80 μL), and water (47 μL). The vial was capped and vortexed for ∼15 s. To the vial was added a 19.23 unit/μL aqueous solution of β-D-glucuronidase in 75 mM sodium phosphate with 1% (w/v) bovine serum albumin (1.6 μL). The vial was capped and vortexed for ∼15 s. Photographs were acquired over time.

Preparation of Control Sample of Reagent 36 (No Added β-D-Glucuronidase). An LC-MS vial (fitted with a 250 μL glass insert) was sequentially loaded with a 5.4 mM solution of reagent **36** in methanol (71 μL), a 75 mM aqueous solution of sodium phosphate buffer with 1% (w/v) bovine serum albumin (80 μL), and water (49 μL). The vial was capped and vortexed for ∼15 s.

■ ASSOCIATED CONTENT

Supporting Information

Tables giving experimental data and figures giving ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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