Inverse electron demand Diels-Alder pyridazine elimination: synthetic tools for chemical immunology

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6

Synthetic methodology towards allylic *trans*-cyclooctene-ethers enables modification of carbohydrates: bioorthogonal manipulation of the *lac* repressor

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6.1 Introduction

Bioorthogonal bond cleavage reactions have garnered significant interest in recent years.[1–4] Amongst these new "click-to-release" reactions, the inverse electron demand Diels-Alder (IEDDA) pyridazine elimination has shown particular promise for bioorthogonal utilization.[5] The method employs a *trans*-cyclooctene (TCO) carrying an allylic substituent that upon [4 + 2] cycloaddition with a 1,2,4,5-tetrazine results in the formation of a 4,5-dihydropyridazine.[6] This 4,5-tautomer can rearrange to form two

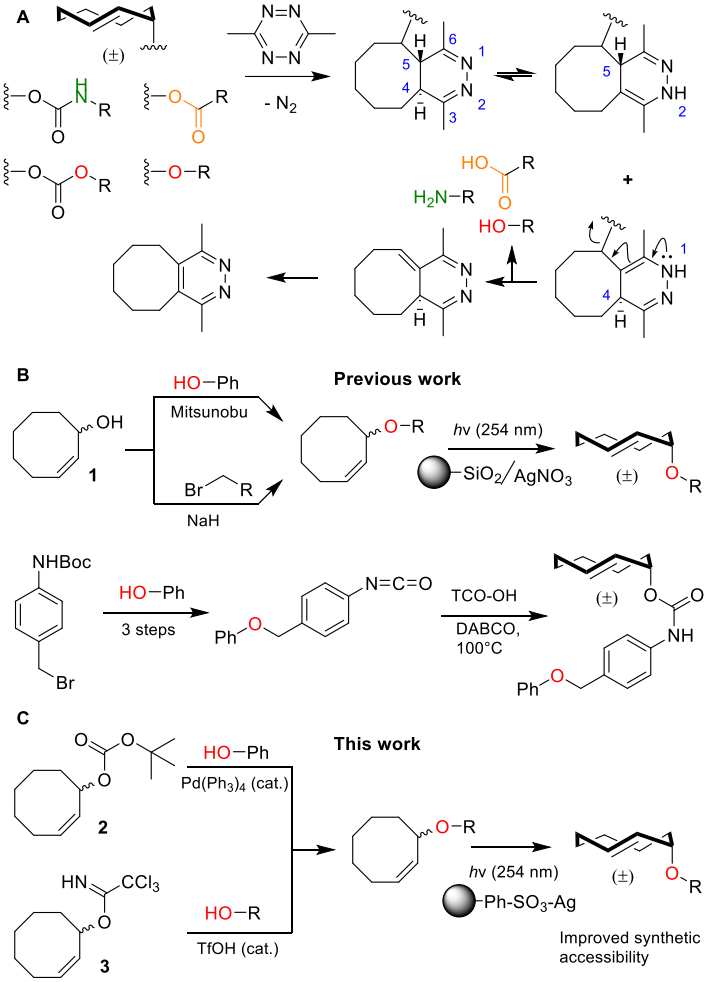


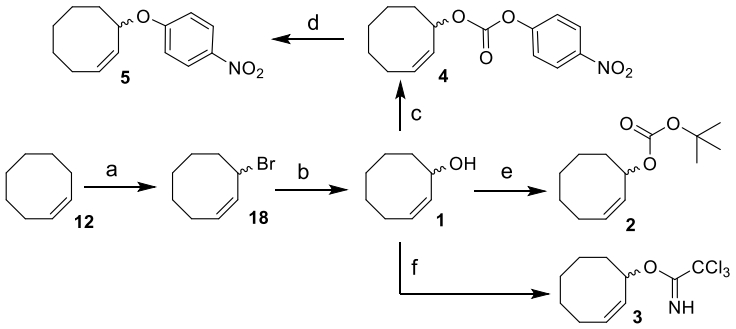
Figure 1 A) Overview of the inverse electron demand Diels-Alder (IEDDA) pyridazine elimination reaction, including the current scope of this method to decage carbamates, carbonates, ethers and esters to obtain amines (green), alcohols (red) and carboxylic acids (orange), respectively. B) Overview of the current synthetic methods to obtain allylic TCO ethers, including methods from Robillard and co-workers (top)[8] and Bernardes and co-workers (bottom).[21] C) Novel synthetic methods described in this work, including reagents **2** and **3**.

new tautomers of which the 1,4-tautomer can release the allylic payload, followed by rearomatization to the pyridazine (Figure 1A).[7,8] The excellent biocompatibility and high bimolecular reaction rate of the IEDDA pyridazine elimination[9] has given rise to a multitude of *in vitro* and *in vivo* applications such as the regulation of protein activity[10–12] and the activation of pro-drugs, in which spatiotemporal control is achieved by antibody-drug conjugates (ADCs),[13–15] nanoparticles,[16] enzymatic supramolecular self-assembly[17] or hydrogel injection.[18,19]

To date, nearly all applications for this reaction have relied on the protection of (primary) amines as TCO-carbamates (Figure 1A, green). Recently, Robillard[8] and Bernardes[20,21] showed that release of other functional groups, such as carboxylic acids (from TCO-esters) and alcohols (from TCO-carbonates or TCO-ethers), is indeed possible (Figure 1A, orange and red). Whilst TCO-esters and carbonates suffer from reduced hydrolytic stability in biological systems,[8,20,21] TCO-ethers are particularly appealing due to their high stability and surprisingly fast decaging kinetics when compared to vinyl ether analogues.[8,22–24] Unfortunately, widespread use of TCO-ethers is constricted by their challenging synthesis. Formation of the crucial *cis*-cyclooctene (CCO) ether bond, employing either Mitsunobu chemistry or nucleophilic substitution of a primary alkyl bromide with **1**, is followed by photochemical isomerization[25] to the TCO-ether and isolation of the desired axial isomer (Figure 1B, top).[8] One exception is the direct alkylation of axial TCO-OH to form a benzylic TCO-ether.[8] Additionally, Bernardes and co-workers developed a self-immolative linker in which a TCO-carbamate is connected to a benzyl ether (Figure 1B, bottom).[21] Although **1** was used as a Mitsunobu substrate with moderate success,[8] this method essentially limits the scope to phenolic nucleophiles and excludes the formation of ethers from aliphatic alcohols.[26] Furthermore, direct nucleophilic substitution with **1** is limited to primary positions,[8] in which the desired aliphatic alcohol requires an additional transformation into a leaving group. The self-immolative linker[21] requires at least four synthetic steps from the substrate, which to date has also been limited to phenols. Taken together, synthesis and utilization of TCO-ethers derived from functionalized aliphatic alcohols encountered in biological systems is currently unfeasible. To overcome this limitation, the development was undertaken of novel synthetic methods for the (regioselective) installation of TCO-ethers in biomolecules as caging moieties, based on reagents **2** and **3**, and the results these studies are presented here (Figure 1C). The methodology was applied to enable the synthesis and bioorthogonal decaging of a TCO-ether modified carbohydrate, as no TCO-protected variants of these biomolecules currently exist.

6.2 Results and discussion

A two-step procedure was envisioned in which the use of electrophilic cyclooctene reagents secured formation of a CCO-ether bond under mild conditions, followed by photochemical isomerization[25] to the desired TCO-ether. Palladium catalysis enables mild, decarboxylative conversion of allyl carbonates to allyl ethers via a reactive π-allyl cation species.[27,28] For phenols, a ring strained variant of this reaction was found feasible by transforming *para*-nitrophenyl carbonate **4** into cyclooctene ether **5** under $Pd(PPh_3)_4$ catalysis (50°C) in 92% yield (Scheme 1). Based on these initial observations, the palladium-catalyzed method for direct allylation by Grover and co-workers[29]

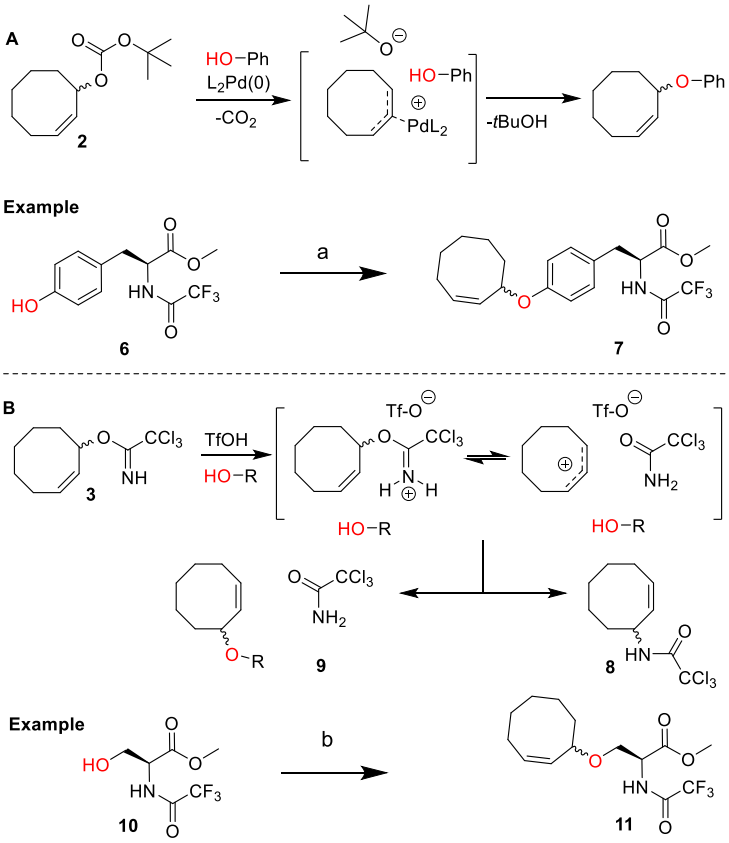


Scheme 1 Synthesis of **1**, **2**, **3**, **4** and **5** from *cis*-cyclooctene (**12**). Reagents/conditions: (a) NBS, AIBN, cyclohexane, reflux, 71%; (b) acetone, H_2O, $NaHCO_3$, reflux, 83%; (c) 4-nitrophenyl chloroformate, pyridine, DCM, 0°C to rt, 83%; (d) $Pd(PPh_3)_4$, toluene, 50°C, 92%; (e) NaHMDS, Boc-ON, THF, 0°C to rt, 81%; (f) trichloroacetonitrile, DBU, $NaHCO_3$, DCM, 0°C to rt, ~ 80% (Table S1).

would enable cyclooctene ether formation in a single step. Therefore, cyclooctene *tert*-butyl carbonate reagent **2** was designed, which decarboxylates upon coordination with a palladium catalyst (Scheme 2A). The spectator *tert*-butoxide formed in this step[29] ensures rapid deprotonation of the phenol nucleophile, which can subsequently attack the π-allyl electrophile to form the ether bond. Cyclooctene ether formation with N-trifluoroacetyl-protected L-tyrosine methyl ester **6** using reagent **2** (1.2 equivalents) under $Pd(PPh_3)_4$ catalysis (80°C) was examined instead of the previously reported Mitsunobu procedure (12% yield),[8] obtaining cyclooctene ether **7** in 80% yield (Scheme 2A).

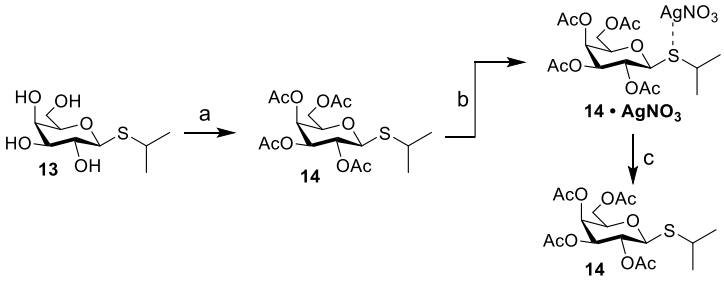
In parallel, a second reagent (**3**) was designed for the synthesis of cyclooctene ethers from aliphatic alcohols (Scheme 2B). Lewis acid triggered activation of cyclooctene trichloroimidate **3** can result in elimination and rearrangement pathways, including the formation of ionic intermediates.[30,31] Cyclooctene trichloroamide **8**, trichloroacetamide (**9**) and the desired cyclooctene ether can be formed from these reactive intermediates. N-trifluoroacetyl-protected L-serine methyl ester **10** was alkylated using 2 equivalents of **3** under triflic acid catalysis (-35°C to 0°C) to obtain **11** in 46% yield (Scheme 2B, Table S2). Furthermore, reagents **2** and **3** were both synthesized from *cis*-cyclooctene (**12**) in 3 steps (~48% yield) with a common intermediate (**1**) and a single chromatographic purification (Scheme 1, Table S1).

Carbohydrates orchestrate a diverse array of biological processes and as such, spatiotemporal control over these biological activities would be a powerful addition to the "click-to-release" toolkit. A classic example of a biological process directed by glycans is the switching of the *lac* operon.[32,33] It is a regulatory element that is used to control nutrient-dependent transcription in *E.coli*. When lactose concentration is low,



Scheme 2 A) Design of cyclooctene *tert*-butyl carbonate reagent **2** for the palladium catalyzed installation of cyclooctene ethers on phenols, including the proposed mechanism based on the work of Grover and co-workers.[29] Reagents/conditions: (a) **2**, $Pd(PPh_3)_4$, dioxane, 80°C, 80%; B) Design of cyclooctene trichloroimidate reagent **3** for the Lewis acid triggered formation of cyclooctene ethers from aliphatic alcohols. Proposed mechanism and products observed upon treating **3** with catalytic triflic acid are shown. Reagents/conditions: (b) **3**, TfOH, DCM, -35°C to 0°C, 46% (Table S2).

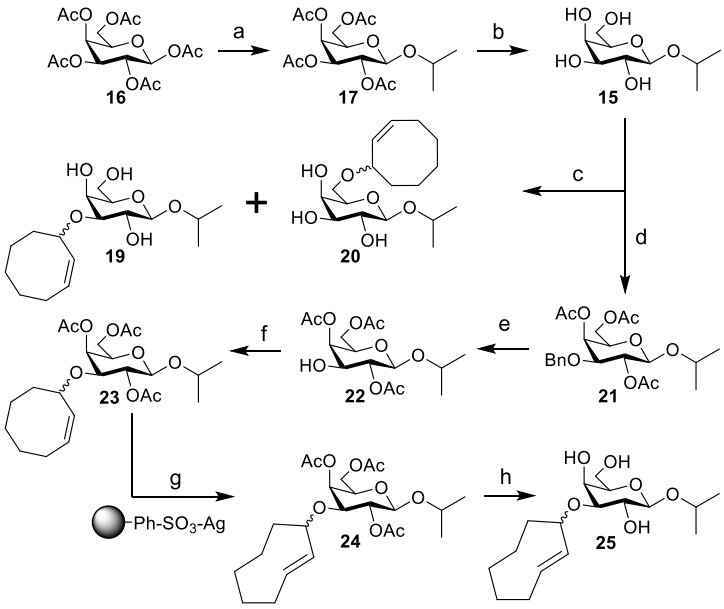
the activity of the operon is inhibited by a repressor element.[34] When the concentration of lactose increases, it will bind to the repressor, leading to a decreased affinity of the lactose-repressor complex for the operon.[35] This results in enhanced gene transcription for proteins under control of this promotor. This system has been extensively used for the expression of recombinant proteins in *E. coli* and the synthetic *lac* operon inducer isopropyl β-D-1-thiogalactopyranoside (IPTG, **13**) is a key reagent here. Control over its activity has previously been attempted using both photochemical[36] and hypoxia-triggered[37] induction strategies. IPTG (**13**) was therefore deemed an excellent model to test IEDDA pyridazine elimination on carbohydrates. Initial experiments with peracetylated IPTG (**14**) under conditions



Scheme 3 Evaluation of the fate of peracetylated IPTG (**14**) during photoisomerization conditions. Reagents/conditions: (a) Ac_2O, pyridine, rt, 100%; (b) methyl benzoate, hν (254 nm), Et_2O, heptane, rt; (c) NH_4OAc (aq), DCM, rt, 61%.

typically used for photochemical isomerization of cyclooctenes confirmed the high affinity of the thioacetal functionality towards the silver nitrate used for enrichment of the TCO-isomers upon photoisomerization (Scheme 3). Therefore, a novel inducer for the *lac* operon was designed, substituting the sulfur with oxygen to obtain an O-glycoside, isopropyl β-D-1-galactopyranoside (IPG, **15**, Scheme 4).

Starting from peracetylated β-D-galactopyranoside **16**, installation of the beta isopropyl group on the anomeric center was achieved in a one-pot, two-step procedure in 70% yield. The 'disarmed' pentaacetate was first transformed into an anomeric bromide, which was subsequently activated with stoichiometric I_2 in the presence of isopropanol to obtain **17**, according to a method reported by Field and colleagues.[38] Deacetylation gave IPG (**15**) in 90% yield. Stannylene acetal-mediated alkylation of IPG (**15**) with (Z)-3-bromocyclooct-1-ene (**18**) in the presence of CsF or TBAI as an additive at 105°C was initially investigated (Table S3). A mixture of 3-CCO-IPG (**19**, ~ 20% yield) and 6-CCO-IPG (**20**, ~ 20% yield) was obtained and purified via chromatographic separation. Decomposition of **18** at high temperature into 1,3-cyclooctadiene and hydrogen bromide[39] limited its use. Attempts to alkylate 6-TBS and 4,6-DTBS functionalized derivatives of **15** in a regioselective manner with **18** were unsuccessful (Table S4).



Scheme 4 Synthesis of IPG (**15**), 3-CCO-IPG (**19**), 6-CCO-IPG (**20**), and 3-TCO-IPG (**25**) from peracetylated β-D-galactopyranoside (**16**). Reagents/conditions: (a) i. HBr, AcOH, DCM, 0°C to rt; ii. 2-propanol, I_2, DCM, 0°C to 4°C, 70% over 2 steps; (b) NaOMe, MeOH, DCM, rt, 90%; (c) i. Bu_2SnO, toluene, 105°C; ii.**18**, CsF, toluene, 105°C, ~20% (**19**), ~ 20% (**20**) (Table S3); (d) i. Bu_2SnO, toluene, 105°C; ii. benzyl bromide, TBABr, toluene, 70°C; iii. Ac_2O, pyridine, rt, 91% over 2 steps; (e) $Pd(OH)_2/C$, H_2, EtOAc, rt, 86%; (f) **3**, TfOH, DCM, -40°C to rt, ~ 30% (Table S5); (g) methyl benzoate, h*ν* (254 nm), Et_2O/isopropanol, rt; (h) NaOMe, MeOH, rt, ~ 30% over 2 steps (Table S6).

Instead, IPG (**15**) was regioselectively benzylated using organotin chemistry followed by acetylation to obtain 2,4,6-OAc-3-OBn-IPG (**21**) in 91% yield over two steps. Hydrogenation of **21** in the presence of Pearlman's catalyst afforded 2,4,6-OAc-IPG (**22**) in 86% yield. Alkylation of **22** could be achieved in the timespan of hours at low temperature (≤ 0°C), typically employing 4 equivalents of trichloroimidate **3** and 10 mol% of triflic acid (Table S5). The crude reaction mixture was washed with aqueous NaOH (to remove **9**)[40] before purification by silica gel chromatography to obtain **23** in ≥ 30% yield. Singlet sensitized photoisomerization of **23** to **24** (Table S6) was executed with the general flow setup described by Fox and co-workers[25] using silver (I) exchanged tosic acid silica gel (TAg silica) as the stationary phase.[41] Irradiation (λ = 254 nm) of mixture of **23** and methyl benzoate in Et_2O for ± 24 h, whilst continuously circulating the reaction mixture over the stationary phase, was followed by treatment of the stationary phase with NH_3 in MeOH to obtain (partially deacetylated) **24**. Zemplén deacetylation of **24** afforded **25** after extractive desalting in ≥ 30% yield over 2 steps. This final product, 3-TCO-IPG (**25**), was exclusively obtained as the desired

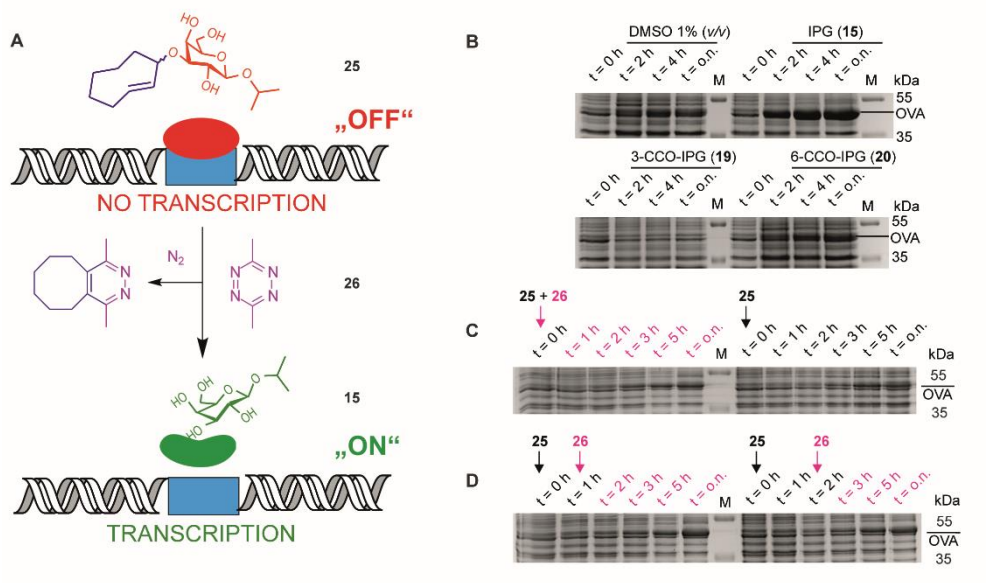


Figure 2 Recombinant expression of proteins with caged IPG variants. A) Schematic representation of the chemical control over *lac* operon activity, mediated by 3-TCO-IPG (**25**), which does not induce expression. IEDDA pyridazine elimination with 3,6-dimethyl-tetrazine (**26**) liberates IPG (**15**), which induces overexpression of the protein of interest. B) Recombinant OVA expression, comparing the effects of DMSO (negative control, 1% *v/v*), IPG (**15**, positive control, 1 mM), 3-CCO-IPG (**19**, 1 mM) and 6-CCO-IPG (**20**, 1 mM). The respective compounds were added at t = 0 h after which protein expression was followed over time. C-D) Temporal control of OVA expression via addition of tetrazine **26** (2.5 mM) in the presence of 3-TCO-IPG (**25**, 1 mM). C) Direct addition of **25** and **26** at t = 0 h (left) or addition of **25** at t = 0 h (right). D) Addition of **26** 1 h (left) or 2 h (right) after adding **25** at t = 0 h.

axial TCO isomer and its purity was optimized by carefully increasing the polarity of the photoisomerization reaction mixture with isopropanol (2 – 5%; Figure S1).

Subsequent experiments were performed to evaluate whether the TCO-modified IPG **25** could be used to control transcription (Figure 2A). For this *E. coli* was transformed to express the model protein ovalbumin (OVA) under control of the *lac* operon. Overnight cultures were inoculated in LB medium supplemented with 1% glucose to inhibit leaky expression. Next, cultures were grown in fresh LB medium (no glucose) before adding (caged) inducers (1 mM) and tetrazine (2.5 mM) in DMSO. It was first tested whether IPG (**15**) could induce expression, which it did in a comparable level to IPTG (**13**; Figure 2B and S2). Furthermore, up to 10% DMSO was tolerated for IPG (**15**) induced OVA expression (Figure S3).

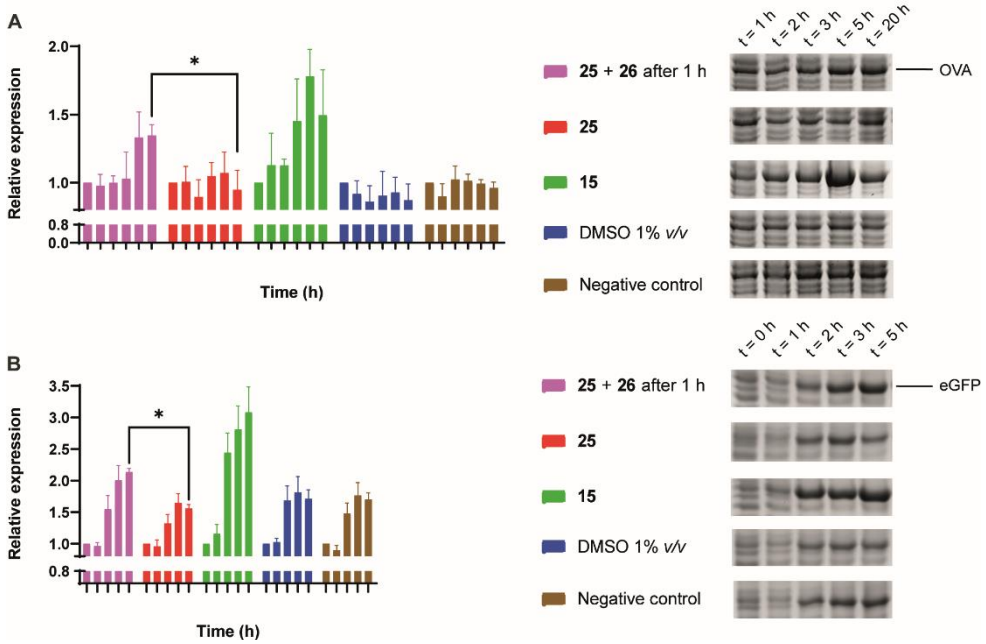


Figure 3 Replicate expression experiments for OVA (3A; N = 3) and eGFP_A206K (3B; N = 4) comparing decaging conditions (1 mM **25** at t = 0 and 2.5 mM **26** at 1 = 1 h) with addition of **25** (1 mM), **15** (1 mM), DMSO (1% *v/v*) and a negative control. Mean quantification values (Coomassie) were plotted with SD as error bars. Representative gel sections are shown to serve as an example. Relative band intensity was measured via densitometry for all experiments. For eGFP (2F), quantification was based on the Coomassie signal of the monomeric protein. *Significant difference based on an unpaired, two-tailed t-test ($P < 0.05$).

To assess the functionality of the TCO-protecting group, 3-TCO-IPG (**25**) was shown to not affect expression levels in the absence of a deprotection agent (Figure S4). Subsequently, it was analyzed whether deprotection with the prokaryote-compatible 3,6-dimethyl-tetrazine (**26**) could be used to switch on recombinant protein expression.[12,42] The presence of **26** was found to cause a minor delay on IPG (**15**) induced OVA expression without reducing overall expression levels (Figure S5).

Deprotection of 3-TCO-IPG (**25**) with **26** was studied in a time course experiment (Figure 2C-D). Simultaneous addition of both **25** and **26** at the same time did not result in an overexpression. However, a preincubation of 1 or 2 hours with **25** before addition of **26** did result in overexpression of OVA. This was particularly striking at late time points (Figure 2D). Replicate experiments (N = 3) confirmed these findings (Figure 3A). Control compounds 3-CCO-IPG (**19**) and 6-CCO-IPG (**20**) failed to induce overexpression (Figure 2B). A reduction in expression compared to the DMSO background was even observed for **19** (Figure 2B and S4).

The method was next applied to the expression of two other proteins, eGFP (Figure 3B; N = 4) and DsRed2 (Figure S6). Again, overexpression after addition of **26** was observed for both proteins based on Coomassie quantification. For eGFP, a significant overexpression was found after 5 hours (Figure 3B). Taken together, it appears this chemical method can be utilized as a general tool for temporally controlled gene expression in *E.coli*.

6.3 Conclusions

In conclusion, the synthesis of TCO-ethers using two unprecedented reagents (**2-3**) is reported, providing facile access to TCO-protected phenols and aliphatic alcohols, respectively. This methodology enables access to the modification of complex biomolecules as TCO-ethers. A carbohydrate probe, 3-TCO-IPG (**25**), made in this manner was successfully used to chemically modulate *lac* operon activity, thereby providing a first example of control over glycan activity by IEDDA pyridazine elimination. The spatiotemporal control available to this method also bodes well for other applications in which carbohydrate/receptor interactions direct biological processes.

6.4 Supporting figures

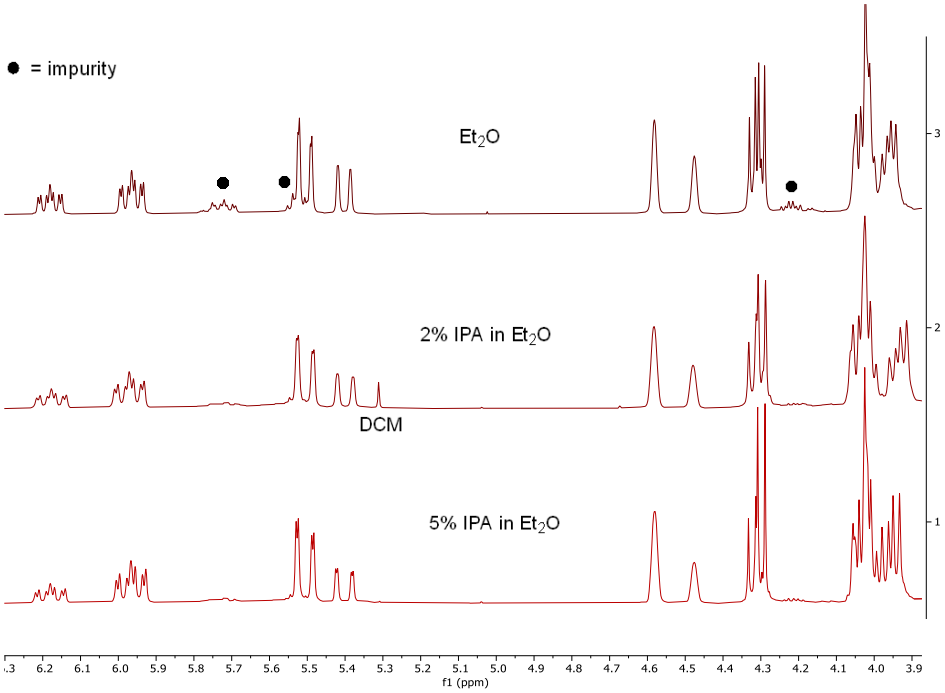


Figure S1 Partial ^{1}H NMR stack (in $CDCl_3$; 6.3 to 3.9 ppm) of 3-TCO-IPG (**25**) which was obtained as the final product after photoisomerization of **23** to **24** in the presence of different reaction solvents (Et_2O, 2% IPA in Et_2O and 5% IPA in Et_2O), followed by deacetylation of **24** to **25** in NaOMe in MeOH (0.5 M) and subsequent extractive workup. An impurity (marked with •) was encountered in **25**, which could be significantly reduced by carefully increasing the polarity of the photoisomerization reaction mixture with IPA.

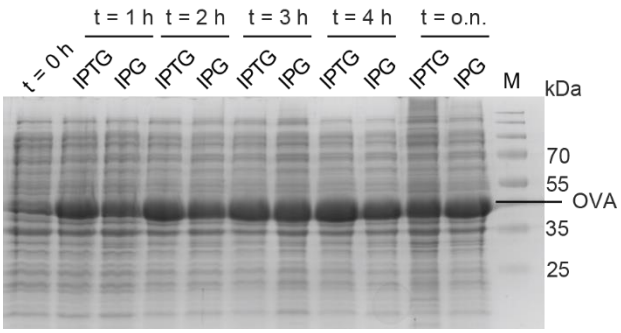


Figure S2 Overexpression of OVA with IPTG (**13**, 1 mM) and IPG (**15**, 1 mM). Samples were collected 1, 2, 3 and 4 h as well as overnight after addition of the inducer.

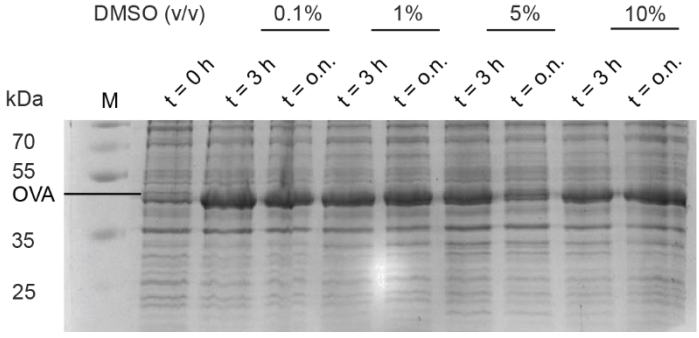


Figure S3 Effect of DMSO on IPG (**15**, 1 mM) induced ovalbumin expression levels. DMSO was used in varying volume percentages (0.1, 1, 5 and 10 % v/v) and samples were taken after 3 h and overnight.

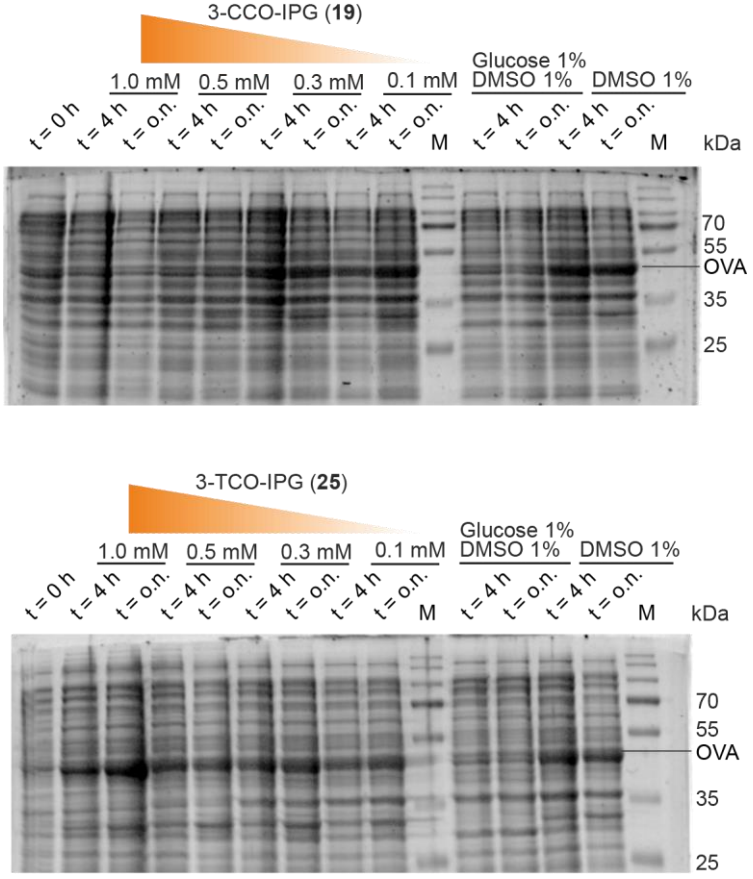


Figure S4 Inhibition of OVA expression with 3-CCO-IPG (**19**, left) at distinct concentrations (1 mM, 0.5 mM, 0.3 mM, 0.1 mM). Right: impact of 3-TCO-IPG (**25**) on OVA expression at different concentrations (1 mM, 0.5 mM, 0.3 mM, 0.1 mM). Positive control: glucose 1% (v/v) + DMSO 1% (v/v), negative control: DMSO 1% (v/v). Orange triangle indicates the decrease in concentration.

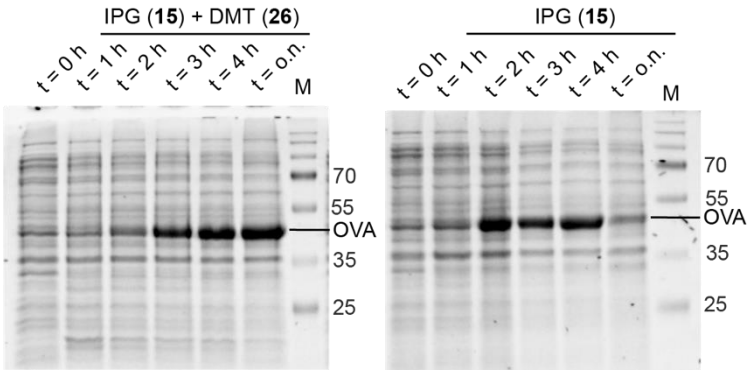


Figure S5 Left: effect of 3,6-dimethyl-tetrazine (**26**, 2.5 mM) on IPG (**15**, 1 mM) induced expression levels. Right: IPG (**15**, 1 mM) induced expression. Samples were taken at 1 - 4 h and overnight.

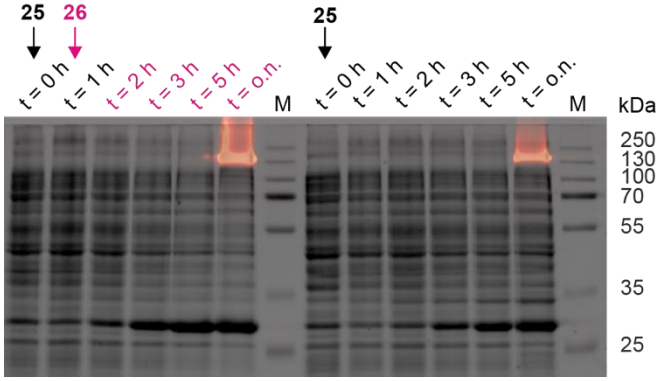
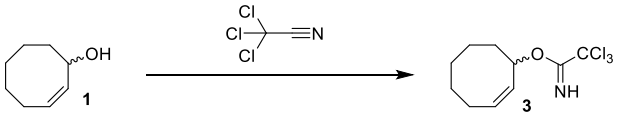


Figure S6 Temporal control of dsRed2_S4T expression via addition of tetrazine **26** (2.5 mM) after 1 h of expression in the presence of 3-TCO-IPG (**25**, 1 mM). Overlayed images of Coomassie staining and in-gel fluorescence of dsRed2 is shown. The right side represent a control (**25**) without the addition of tetrazine **26**.

6.5 Supporting Tables

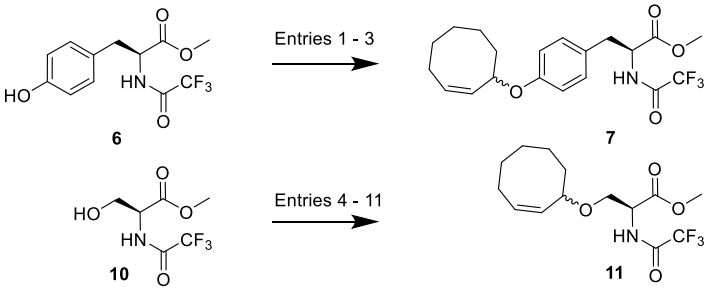
Table S1 Synthesis of Reagent **3** from cyclooctenol **1.**



	Reaction Conditions[a]							
Entry	Scale (mmol)	CCl_3CN (equiv)	Base (equiv)	Solvent (M)	Temperature (°C)	Time (h)	Purification Method[a]	Yield (%)[b]
1	5.23	2.5	DBU (2.5)	DCM (0.2)	0	1.5	Celite, Conc., Silica Gel	7
2	5.17	5.0	K_2CO_3 (5)	DCM (0.5)	0 to rt	24	-	-
2 (cont.)	-	-	DBU (0.05)	DCM (0.5)	0	2	Filter, Conc., Neutralized Silica Gel	85
3	10.08	5.0	DBU (0.05)	DCM (0.5)	0	2	Conc., Silica Gel	64
4	8.25	5.0	K_2CO_3 (5) DBU (0.05)	DCM (0.5)	0	3	Filter, Conc., Silica Gel	75
5	100	5.0	K_2CO_3 (5) DBU (0.05)	DCM (0.5)	0	4	Filter, Conc., Silica Gel	81
6	100	5.0	K_2CO_3 (5) DBU (0.05)	DCM (0.5)	0	4.5	Filter, Conc., Silica Gel	86

[a]Purification method, indicating the steps performed with the crude reaction mixture to obtain the purified reagent **3**. [b]Isolated yield. The following abbreviations were used: **Celite**: Celite was added to the reaction mixture; **Filter**: the reaction mixture was filtered (to remove K_2CO_3); **Conc.**: the reaction mixture was concentrated *in vacuo*; **Silica Gel**: the crude product was purified by silica gel chromatography; **Neutralized Silica Gel**: the crude product was purified by silica gel chromatography using neutralized silica gel (described in the experimental section).

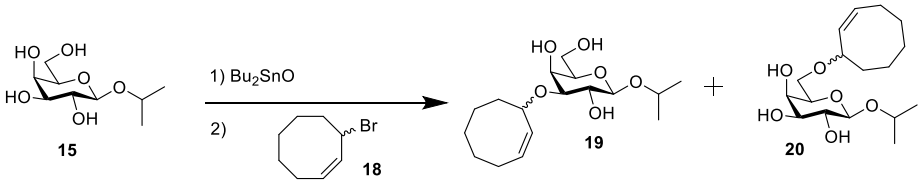
Table S2 Synthesis of cyclooctene ethers **7** and **11** using cyclooctene reagent **2** and **3**.



Reaction Conditions[a]							
Entry	Starting material (mmol)	CCO-reagent (equiv)	Additive (equiv)	Solvent (M)	Temperature (°C)	Time (h)	Product (Yield)[b]
1	**6** (0.1)	**2** (1.2)	$Pd(PPh_3)_4$ (0.05)	THF (0.1)	50	overnight	-
2	**6** (0.1)	**2** (1.2)	$Pd(PPh_3)_4$ (0.12)	Dioxane (0.1)	80	20	**7** (81%)
3	**6** (8.47)	**2** (1.2)	$Pd(PPh_3)_4$ (0.06)	Dioxane (0.1)	80	41	**7** (80%)
4	**10** (0.14)	**2** (1.4)	$Pd(PPh_3)_4$ (0.14)	Dioxane (0.1)	80	72	-
5	**10** (0.14)	**2** (1.5)	$Pd(PPh_3)_4$ (0.10)	Toluene (0.1)	105	20	-
5 (cont.)	-	-	-	Dioxane (0.1)	100	120	-
6	**10** (0.27)	**2** (1.4)	TfOH (0.1)[c] MS (4 Å)	DCM (0.1)	0	2	**11** (15%)
7	**10** (0.37)	**2** (2.0)	TfOH (0.1)[c]	DCM (0.1)	0 to rt	63	**11** (24%)
8	**10** (0.31)	**2** (2.1)	TfOH (0.1)[c]	DCM (0.1)	-50 to -30	2	**11** (45%)
9	**10** (0.34)	**2** (2.1)	TfOH (0.1)[d] MS (4Å)	DCM (0.1)	-60 to -30	overnight	**11** (26%)
10	**10** (0.31)	**2** (1.9)	TMS-OTf (0.1)[e]	DCM (0.1)	-35 to 0	4	**11** (38%)
11	**10** (0.32)	**2** (2.1)	TfOH (0.1)[e]	DCM (0.1)	-35 to 0	4	**11** (46%)

[a]**Entry 1:** Reactants (**6** + **2**) were combined in a 10 mL round-bottom flask, dissolved in THF under N_2 and degassed for 10 min by sonication. $Pd(PPh_3)_4$ was added, the container was purged with N_2 before sealing the flask and starting the reaction. **Entries 2 – 4:** Reactants (**6** or **10** + **2** + $Pd(PPh_3)_4$) were co-evaporated with anhydrous dioxane in a round-bottom flask, placed under N_2 and dissolved in anhydrous solvent. The reaction mixture was frozen at -78°C (ethanol bath) and subsequently purged with N_2 for 45 min to achieve degassing. Afterwards, the flask was sealed and the reaction was launched. **Entries 5 – 10:** Reactants (**10** + **3**) were co-evaporated with anhydrous toluene (3 x 2 mL) in a 25 mL round-bottom flask, placed under N_2 (balloon) and dissolved in anhydrous solvent. Lewis acid was added after cooling the reaction mixture in an ethanol bath. Reactions were quenched with Et_3N (2 equivalents compared to Lewis acid), impregnated with Celite Hyflo Supercel (Merck), concentrated *in vacuo* and purified by silica gel chromatography. [b]Yields denote isolated yields (%) after column chromatography. When no yield was reported (-; entries 1,4 and 5), no detectable degree of reaction took take place and the starting material (**6** or **10**) could be recovered. [c]Direct addition. [d]Addition from a freshly prepared stock solution (0.1 M in DCM) on activated molecular sieves (4Å). [e]Addition from a freshly prepared stock solution (0.1 M in DCM).

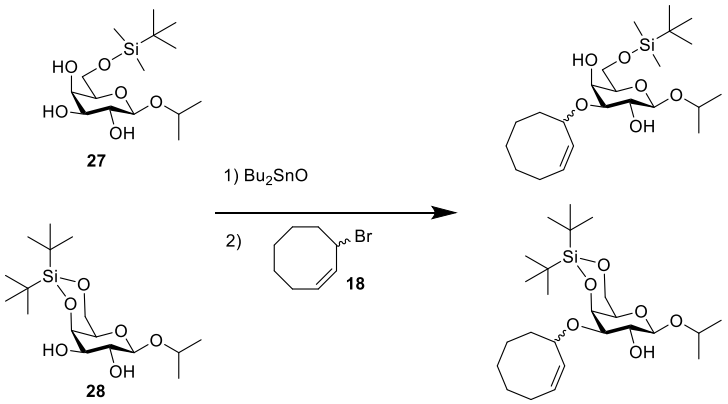
Table S3 Investigation of stannylene acetal mediated alkylation of **15** with **18**.



	Stage 1: Acetal formation[a]					Stage 2: Alkylation[b]					Yield[c]	
Entry	Scale (mmol)	Bu_2SnO (equiv)	Solvent (M)	Temp (°C)[d]	Time	**18** (equiv)	Additive (equiv)	Solvent (M)	Temp (°C)[d]	Time	**19** (%)	**20** (%)
1	0.5	1.15 eq	Toluene (0.1)	105	o.n.	1.05	-	Toluene (0.1)	105/ 90	o.n.	-	-
1 cont.	-	-	-	-	-	-	TBABr (1.05)	Toluene (0.1)	90	48 h	-	-
2	1.5	1.05 eq	Toluene (0.1)	Reflux	o.n.	2.0	CsF (2.5)	DMF (0.13)	65	48 h	-	-
3	1.0	1.2 eq	Toluene (0.1)	Reflux	o.n.	3 x 1.2	CsF (1.2)	Toluene (0.1)	reflux	48 h	23	21
4	1.15	1.2	Toluene (0.1)	105	o.n.	1.2	CsF (1.2)	Toluene (1.0)	105	o.n.	17	13
5	1.12	1.2	Toluene (0.1)	105	o.n.	2.5	CsF (2.5)	Toluene (1.0)	105	o.n.	22	18
6	1.0	1.2	Toluene (0.1)	105	o.n.	1.2	CsF (1.2) TBAI (1.2)	Toluene (1.0)	105	o.n.	16	7
7	1.0	1.2	Toluene (0.1)	105	o.n.	1.2	TBAI (1.2)	Toluene (1.0)	105	o.n.	15	7
8	1.09	1.2	Toluene (0.1)	105	o.n.	Excess	CsF (1.2)	CCO-Br (1.0)	105	o.n.	-	-
9	0.48	1.2	Toluene (0.1)	105	o.n.	Excess	CsF (1.2)	Toluene /CCO-Br, 1:1 (1.0)	105	o.n.	-	-
10	4.77	1.2	Toluene (0.1)	105	o.n.	3	CsF (1.2) DIPEA (3)	Toluene (1.0)	105	o.n.	~20	~20
11	13.53	1.2	Toluene (0.4)	105	o.n.	3	CsF(1.2) MS (3Å)	Toluene (1.0)	105	o.n.	~22	~19

[a]After dialkylstannylene acetal formation, the reaction mixture was concentrated *in vacuo* and co-evaporated 3x with anhydrous toluene. [a,b] Reactions were typically carried out in a sealed round-bottom flask (10, 25 or 50-mL) under N_2 (balloon). [c]Yields denote isolated yields after column chromatography. [d]Oil bath. **Notes for specific entries: entries 8 – 9:** Complete pyrolysis of the reaction mixture was observed; **entries 10 – 11**: Extra byproducts encountered (presumably 2-CCO-IPG), which made purification of **19** and **20** significantly more laborious compared to previous entries.

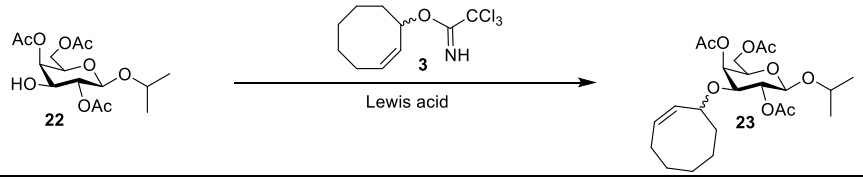
Table S4 Investigation of stannylene acetal mediated alkylation of **27** and **28** with **18**.



	Stage 1: Acetal formation[a]					Stage 2: Alkylation[b]					Yield[c]
Entry	6-TBS/ 4,6-DTBS (mmol)	Bu_2SnO (equiv)	Solvent (M)	Temp (°C)[d]	Time	**18** (equiv)	Additive (equiv)	Solvent (M)	Temp (°C)[d]	Time	3-alkyl (%)
1	**27** (0.5)	1.15 eq	Toluene (0.1)	105	o.n.	1.05	-	Toluene (0.1)	105/ 90	o.n.	-
1 cont.	-	-	-	-	-	-	TBABr (1.05)	Toluene (0.1)	90	48 h	-
2	**28** (0.5)	1.15 eq	Toluene (0.1)	105	o.n.	1.05	-	Toluene (0.1)	105/ 90	o.n.	-
2 cont	-	-	-	-	-	-	TBABr (1.05)	Toluene (0.1)	90	48 h	-
3	**27** (1.0)	1.2	Toluene (0.1)	105	o.n.	1.2	TBAI (1.2)	Toluene (1.0)	105	o.n.	-
4	**28** (1.0)	1.2	Toluene (0.1)	105	o.n.	1.2	TBAI (1.2)	Toluene (1.0)	105	o.n.	-

[a]After dialkylstannylene acetal formation, the reaction mixture was concentrated *in vacuo* and co-evaporated 3x with anhydrous toluene. [a,b] Reactions were typically carried out in a sealed round-bottom flask (10, 25 or 50-mL) under N_2 (balloon). [c]Yields denote isolated yields after column chromatography. [d]Oil bath.

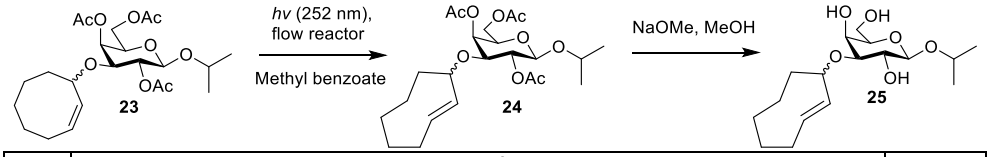
Table S5 Investigation of Lewis acid catalyzed alkylation of **22** with reagent **3**.



Reaction conditions[a]							
Entry	Scale (mmol)	reagent **2** (equiv)	Activator[b] (equiv)	Solvent (M)	Temperature (°C)	Time (h)	Yield[c] (%)
1	0.1	2.0	TfOH (0.1)	DCM (0.1)	-50 to -30	4	27
2	0.1	4.5	TfOH (0.1)	DCM (0.1)	-40 to -5	4	45
3	0.1	9.4	TfOH (0.1)	DCM (0.05)	-45 to 0	5	47
4	0.1	4.4	$BF_3 \cdot OEt_2$ (0.5)	DCM (0.1)	-30 to 0	4	42
5	1.0	4.0	TfOH (0.1)	DCM (0.1)	-50 to -5	4	40
6	1.0	4.0	TfOH (0.05)	DCM (0.05)	-30	6	20
7	1.0	4.0	TfOH (0.01)	DCM (0.1)	-40 to 0	5.5	-
Cont.	-	-	TfOH (0.02)	DCM (0.1)	0	1.5	24
8	1.0	0 -> 4.1	TfOH (0.1)	DCM (1.0 -> 0.1)	-45 to -30	22	47
9	10.1	4.0	TfOH (0.1)	DCM (0.1)	-40 to 0	6.5	29
10	21.8	3.95	TfOH (0.1)	DCM (0.1)	-30	4	34

[a]Reactants (**22** + **3**) were co-evaporated with anhydrous toluene (3x) in a round-bottom flask, placed under N_2 (balloon) and dissolved in anhydrous solvent. Lewis acid activator was added after cooling the reaction mixture in an ethanol bath. Reactions were quenched with Et_3N (2 equivalents compared to Lewis acid), diluted with Et_2O, washed with NaOH (1 M, 3x) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Crude products were purified by silica gel chromatography. **Notes for specific entries: entry 3**: precipitation of reactants was observed, which resolved back into a clear solution after warming to -10°C; **entry 8**: slow addition over 3 h of **3** in 9 mL of DCM to the reaction mixture containing **22** in 1 mL DCM and TfOH. [b]Triflic acid was added from a freshly prepared stock solution (0.1 M in DCM, entries 1-3, 5-8). At large scale, triflic acid was added directly (entries 9-10). $BF_3 \cdot OEt_2$ was added as a 0.1 M solution (commercially available, entry 4). [c]Yields denote isolated yields after column chromatography.

Table S6 Photoisomerization of **23** to **24** and subsequent deacetylation to afford **25**.



	Reaction Conditions[a]								
Entry	Scale (mmol)	Methyl benzoate (equiv)	Stationary phase (equiv)	Column fill material[b]	Solvent (M)	Flow (mL/min)	Time (h)	Yield **24**	Yield **25**
1	0.44	3	$AgNO_3 \cdot SiO_2$ (3.4)	SiO_2	50% EtOAc in heptane (4 mM)	25	47	-	-
1 cont.	0.44	3	$AgNO_3 \cdot SiO_2$ (1.3)	SiO_2	25% EtOAc in heptane (4 mM)	25	43	-	-
2	0.66	3	TAg (1.5)	Cotton	50% EtOAc in heptane (5 mM)	12.5	48	-	-
3	1.58	3 + 9	TAg (1.8)	-	25% EtOAc in heptane (13 mM)	10	48	21%	N.D.
4	0.52	3	TAg (3.0)	Cotton	Et_2O (3 mM)	10	17	30%	67%
5	2.90	3	TAg (3.0)	Cotton	Et_2O (5 mM)	30	20	Crude	40% over 2 steps
6	0.50	3	TAg (3.0)	Cotton	2% IPA in Et_2O (3 mM)	15	24	Crude	47% over 2 steps
7	0.50	10	TAg (3.0)	Cotton	10% IPA in Et_2O (3 mM)	15	90	Crude	7% over 2 steps
8	0.50	5.7	TAg (3.0)	Cotton	5% IPA in Et_2O (3 mM)	20	24	Crude	35% over 2 steps
9	0.51	10	TAg (3.0)	Cotton	5% IPA in Et_2O (3 mM)	20	48	Crude	27% over 2 steps

[a]$AgNO_3 \cdot SiO_2$ (10% wt) was prepared according to the procedure by Royzen *et al.*[25] Tosic Acid Silica (ion exchange capacity 0.60 meg/g) was subjected to ion exchange with $AgNO_3$ according to the procedure by Darko *et al.*[41] Other general considerations about the photoisomerization method can be found in the Experimental Section. When deemed necessary, a sample (~ 30 mL) of the reaction mixture was concentrated *in vacuo* and measured with ^1H NMR to evaluate the progress of the photoisomerization reaction. Afterwards, the column containing the trapped product (**24**) was washed with additional solvent (2 x reaction volume), dried over N_2 and fractionally eluted with NH_3 in MeOH (7 M). Fractions containing the partially deacetylated product (**24**) were combined and concentrated *in vacuo*. This crude product (**24**) was treated with NaOMe in MeOH (0.5 M) overnight, concentrated *in vacuo* and extractively purified to obtain **25**. [b]Material used to completely pack the column after loading of the stationary phase was complete. **Notes for specific entries: entry 1**: Leaching of Ag was observed (50% EtOAc in pentane). The crude, unreacted reaction mixture was re-used for the second part of the experiment (25% EtOAc in pentane); **entry 3**: additional methyl benzoate (9 equiv) was added after 26 h, **24** was purified with silica gel chromatography and was not reacted further**; entry 4**: **24** was purified by silica gel chromatography, **25** was purified by silica gel chromatography. Reduced yield for **entries 3 and 4** may partially be explained by loss of partially deacylated product during silica gel chromatography.

6.6 Experimental procedures - chemistry

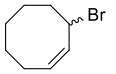
General methods: Commercially available reagents and solvents were used as received. Moisture and oxygen sensitive reactions were performed under N_2 atmosphere (balloon). DCM, toluene, THF, dioxane and Et_2O were stored over (flame-dried) 4 Å molecular sieves (8-12 mesh). Methanol and isopropanol were stored over (flame-dried) 3 Å molecular sieves. Pyridine, DIPEA and Et_3N were stored over KOH pellets. TLC analysis was performed using aluminum sheets, pre-coated with silica gel (Merck, TLC Silica gel 60 F_{254}). Compounds were visualized by UV absorption (λ = 254 nm), by spraying with either a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in H_2O, a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (10 g/L) in 10% H_2SO_4, 20% H_2SO_4 in EtOH, or phosphomolybdic acid in EtOH (150 g/L), where appropriate, followed by charring at ca. 150°C. Column chromatography was performed on Screening Devices b.v. Silica Gel (particle size 40-63 μm, pore diameter 60 Å). Celite Hyflo Supercel (Merck) was used to impregnate the reaction mixture prior to silica gel chromatography when indicated. ^1H, ^{13}C APT, ^{19}F, ^1H COSY, HSQC and HMBC spectra were recorded with a Bruker AV-400 (400/100 MHz) or AV-500 (500/125 MHz) spectrometer. Chemical shifts are reported as δ values (ppm) and were referenced to tetramethylsilane (δ = 0.00 ppm) or the residual solvent peak as internal standard. *J* couplings are reported in Hz. High resolution mass spectra were recorded by direct injection (2 μL of a 1 μM solution in H_2O/MeCN 1:1 and 0.1% formic acid) on a mass spectrometer (Q Exactive HF Hybrid Quadrupole-Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275°C) with resolution R = 240,000 at m/z 400 (mass range m/z = 160-2,000) and an external lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). The synthesis of tetrazine **26** is described in Chapter 4 and a recent publication.[43]

Preparation of neutralized silica gel: Unmodified silica gel (500 gram) was slowly dispersed into a 3 L round-bottom flask containing a stirring volume of H_2O (1.7 L). NH_4OH (28% w/w, 100 mL) was added and the alkaline suspension was stirred for 30 min. The suspension was filtered, washed with H_2O and the silica gel was dried on aluminium foil overnight at rt. The silica was transferred into a glass container and remaining traces of H_2O were removed by drying in an oven at 150°C overnight.

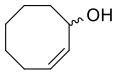
Photoisomerization methods: General guidelines were followed as described by Royzen *et al*.[25] Photochemical isomerization was performed using a Southern New England Ultraviolet Company Rayonet reactor (model RPR-100) equipped with 16 bulbs (part number RPR-2537A, λ = 254 nm). Photolysis was performed in a 187 mL or 1500 mL quartz flask (Southern New England Ultraviolet Company; part number RQV-118 or RQV-323, respectively). A HPLC pump (Jasco; model PU-2088 Plus) was used to circulate solvent through the photolysis apparatus at the indicated flow rate. An empty solid load cartridge with screw cap, frits, O-ring and end tips (4 g / 40 g; SD.0000.004 / SD.0000.040; iLOK™, Screening Devices b.v.) was manually loaded with the specified silica gel to function as the stationary phase.

Preparation of TAg silica gel: Preparation was based on the procedure described by Darko *et al*.[41] Siliabond Tosic Acid Functionalized Silica (Silicycle, product number R60530B, lot number

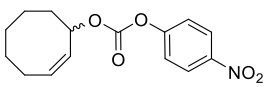
156773, particle size 40-63 μm, pore diameter 60 Å, endcapped, functional loading 0.6 mmol/g, 100 gram) was transferred to a glass silica column wrapped in aluminium foil. A solution of $AgNO_3$ (0.5 M in $MeCN/H_2O$, 9:1, 1 L) was passed over the column whilst monitoring the pH shift from acidic to neutral. The column was washed with MeOH (2 x 400 mL), acetone (2 x 400 mL) and pentane (2 x 400 mL). The TAg silica gel was dried over a dream of air and transferred to a bottle wrapped in aluminium foil for storage.



(Z)-3-bromocyclooct-1-ene (18): Synthesis was performed according to a modified procedure.[44] N-bromosuccinimide (100 g, 562 mmol, 1.0 equiv) was placed under N_2 in a 1 L round-bottom flask. Cyclohexane (400 mL), (Z)-cyclooctene (**12**, 100 mL, 770 mmol, 1.37 equiv) and AIBN (0.2 M in toluene, 2.0 mL, 0.4 mmol, 0.07 mol%) were added before connecting the flask to a reflux condenser which was subsequently purged with N_2. The mixture was refluxed (oil bath at 100°C) under N_2 for 4 h, after which the reaction mixture was allowed to cool to room temperature. The white precipitates were removed by filtration after cooling the mixture to 0°C (ice bath). The crude reaction mixture was concentrated *in vacuo* (60°C, ≤ 20 mbar) before purifying the crude product by fractional vacuum distillation to obtain **18** (75.2 g, 398 mmol, 71%, bp = 85°C at 1.3 mbar) as a colorless liquid: R_f = 0.8 (pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 – 5.73 (m, 1H), 5.65 – 5.54 (m, 1H), 5.00 – 4.89 (m, 1H), 2.30 – 2.05 (m, 3H), 2.05 – 1.92 (m, 1H), 1.76 – 1.63 (m, 2H), 1.63 – 1.47 (m, 2H), 1.45 – 1.24 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.3, 129.9, 49.0, 40.9, 29.1, 26.6, 26.2, 25.7. Spectroscopic data was in agreement with literature.[44]

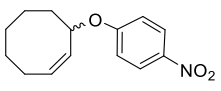


(Z)-cyclooct-2-en-1-ol (1): Synthesis was performed according to a modified procedure.[10] Cyclooctene bromide **18** (75.1 g, 397 mmol, 1.0 equiv) was dissolved in a mixture of acetone (600 mL) and H_2O (300 mL) in a 3 L round-bottom flask. $NaHCO_3$ (66.7 g, 795 mmol, 2.0 equiv) was added and the reaction mixture was stirred under reflux (oil bath at 75°C) for 4.5 h. The reaction mixture was allowed to cool to room temperature and filtered to remove excess $NaHCO_3$. The filtrate was extracted with Et_2O (3 x 500 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain **1** (41.4 g, 328 mmol, 83%) as an oil which was used in subsequent reactions without further purification: R_f = 0.3 (20% Et_2O in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (dddd, *J* = 10.3, 8.5, 7.0, 1.4 Hz, 1H), 5.52 (ddd, *J* = 10.8, 6.5, 0.8 Hz, 1H), 4.73 – 4.56 (m, 1H), 2.24 – 2.01 (m, 2H), 1.96 – 1.85 (m, 1H), 1.73 (s, 1OH), 1.69 – 1.32 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.1, 128.6, 69.4, 38.7, 29.2, 26.4, 26.0, 23.8. Spectroscopic data was in agreement with literature.[10,43,45]

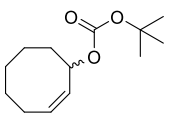


Cyclooctene carbonate 4: Cyclooctenol **1** (1.19 g, 9.45 mmol, 1.0 equiv) was dissolved in anhydrous DCM (30 mL) in a 100 mL round-bottom flask under N_2. Anhydrous pyridine (1.15 mL, 14.2 mmol, 1.5 equiv) was added and the reaction mixture was cooled to 0°C (ice bath) before adding 4-nitrophenyl chloroformate (2.29 g, 11.3 mmol, 1.2 equiv). The reaction was stirred for 48 h and allowed to warm to room temperature. The reaction mixture was diluted with H_2O (30 mL) and the aqueous layer was extracted with Et_2O (3 x 75 mL). The combined organic layers were washed with HCl (0.5 M, 2 x 100 mL), $NaHCO_3$ (satd., 2 x 100 mL) and brine (200 mL), dried over $MgSO_4$, filtered, impregnated with Celite and concentrated *in vacuo*. The impregnated crude

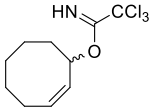
product was purified by silica gel chromatography (pentane → 3% Et_2O in pentane) to obtain **4** (2.29 g, 7.86 mmol, 83%) as a pale yellow oil: R_f = 0.6 (5% Et_2O in pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 8.33 – 8.22 (m, 2H), 7.44 – 7.34 (m, 2H), 5.78 (td, *J* = 9.3, 7.5 Hz, 1H), 5.70 – 5.53 (m, 2H), 2.31 – 2.03 (m, 3H), 1.78 – 1.47 (m, 6H), 1.47 – 1.36 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.7, 152.0, 145.4, 131.1, 129.2, 125.3 (x2), 121.9 (x2), 78.4, 34.9, 28.8, 26.5, 25.8, 23.3.



Cyclooctene ether 5: The experiment was based on a procedure for the synthesis of 1-(allyloxy)-4-nitrobenzene with palladium catalysis.[46] Cyclooctene carbonate **4** (171 mg, 0.59 mmol, 1.0 equiv) was dissolved in anhydrous toluene (2.5 mL) under N_2 in a 10 mL round-bottom flask. The reaction mixture was degassed under sonication for 10 min before adding $Pd(PPh_3)_4$ (16 mg, 14 µmol, 2.4 mol%). The reaction mixture was stirred for 90 min at 50°C (oil bath) under N_2. The reaction mixture was directly applied on a silica gel column and purified (pentane → 2% Et_2O in pentane) to obtain **5** (133 mg, 0.54 mmol, 92%) as a pale yellow oil: R_f = 0.9 (5% Et_2O in pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 – 8.03 (m, 2H), 6.98 – 6.80 (m, 2H), 5.91 – 5.72 (m, 1H), 5.45 (dd, *J* = 10.8, 7.2 Hz, 1H), 5.24 – 5.06 (m, 1H), 2.37 – 2.18 (m, 2H), 2.10 (ddt, *J* = 12.8, 8.6, 4.5 Hz, 1H), 1.85 – 1.36 (m, 8H);^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 141.2, 131.6, 131.3, 125.8 (x2), 115.4 (x2), 76.4, 35.7, 29.0, 26.8, 26.1, 23.3.



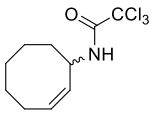
Cyclooctene reagent 2: Cyclooctenol **1** (2.28 g, 18.1 mmol, 1.0 equiv) was dissolved in anhydrous THF (40 mL) in a 250 mL round-bottom flask under N_2. The reaction mixture was cooled to 0°C (ice bath) before adding NaHMDS (40% w/w in THF, 26.9 mL, 52.4 mmol, 2.9 equiv) dropwise. The reaction mixture was stirred for 30 min at 0°C. 2-(*tert*-Butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON; 12.9 g, 52.2 mmol, 2.9 equiv) was dissolved in anhydrous THF (40 mL) in a 100 mL pear-shaped flask under N_2 and added to the reaction mixture dropwise using a double tipped needle under positive N_2 pressure. The reaction mixture was stirred overnight and allowed to warm to room temperature. The reaction was quenched by adding NH_4Cl (satd., 300 mL) and subsequently diluted with Et_2O (300 mL). The aqueous layer was extracted with Et_2O (300 mL). The combined organic layers were washed with HCl (1 M, 250 mL), $NaHCO_3$ (satd., 250 mL) and brine (250 mL), dried over $MgSO_4$, filtered, impregnated with Celite and concentrated *in vacuo*. The impregnated crude product was purified by silica gel chromatography (pentane → 0.5% Et_2O in pentane) to obtain **2** (3.30 g, 14.6 mmol, 81%) as a pale yellow oil: R_f = 0.5 (2% Et_2O in pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 5.74 – 5.62 (m, 1H), 5.53 (ddd, *J* = 10.6, 7.0, 1.2 Hz, 1H), 5.49 – 5.40 (m, 1H), 2.33 – 2.19 (m, 1H), 2.18 – 2.06 (m, 1H), 2.03 – 1.90 (m, 1H), 1.74 – 1.51 (m, 6H), 1.49 (s, 9H), 1.44 – 1.32 (m, 1H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.2, 130.7, 129.9, 82.0, 75.3, 35.1, 28.9, 27.9 (x3), 26.5, 25.9, 23.4.



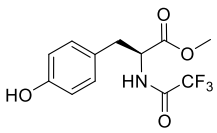
Cyclooctene reagent 3: Cyclooctenol **1** (12.64 g, 100 mmol, 1.0 equiv) was dissolved in anhydrous DCM (200 mL) in a 500 mL round-bottom flask under N_2. The reaction mixture was cooled to 0°C (ice bath) before adding K_2CO_3 (69.2 g, 501 mmol, 5.0 equiv), trichloroacetonitrile (50.2 mL, 501 mmol, 5.0 equiv) and DBU (0.755 mL, 5.01 mmol, 5.0 mol%). The suspension was stirred on ice for 4 h, filtered and concentrated *in vacuo*. The brown crude product was suspended in a small volume of toluene and

purified by silica gel chromatography (pentane → 1% Et_2O in pentane → 2% Et_2O in pentane) to obtain cyclooctene imidate **3** (21.88 g, 81 mmol, 81%) as an oil: R_f = 0.4 (2% Et_2O in pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1NH), 5.82 – 5.66 (m, 2H), 5.66 – 5.52 (m, 1H), 2.36 – 2.22 (m, 1H), 2.21 – 2.04 (m, 2H), 1.77 – 1.50 (m, 7H), 1.50 – 1.37 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 130.5, 130.2, 92.0, 77.6, 34.5, 28.8, 26.5, 25.9, 23.3. Reagent **3** was stored at -30°C under N_2 as a solid.

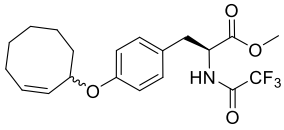
Note: Full conditions investigated for the synthesis of **3 are reported in Table S1.*



Cyclooctene amide 8: This compound was often encountered as a crude byproduct during column chromatography purifications of compounds **11** and **23**, resulting from the various reactive intermediates formed upon activation of reagent **3** with a potent Lewis acid (TfOH): ^1H NMR (400 MHz, $CDCl_3$) δ 5.83 – 5.73 (m, 2H), 5.62 – 5.53 (m, 1H), 2.33 – 2.12 (m, 2H), 2.12 – 1.98 (m, 1H), 1.77 – 1.29 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.4, 131.4, 128.6, 90.3, 78.4, 34.5, 28.7, 26.5, 25.8, 23.2.



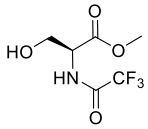
N-trifluoroacetyl-protected L-tyrosine methyl ester 6: L-tyrosine methyl ester hydrochloride (10.05 g, 43,4 mmol, 1.0 equiv) was dissolved in anhydrous DCM (80 mL) in a 250 mL round-bottom flask under N_2. The reaction mixture was cooled to 0°C (ice bath) before adding anhydrous Et_3N (6.05 mL, 43.4 mmol, 1.0 equiv). The reaction mixture was stirred for 30 min at 0°C. Subsequently, trifluoroacetic anhydride (7.35 mL, 52.1 mmol, 1.2 equiv) was added slowly to the neutralized, milky reaction mixture over 10 min. The reaction mixture was stirred and allowed to warm to room temperature. After 2 h, additional Et_3N (6.05 mL, 43.4 mmol, 1.0 equiv) was added. After 24 h reaction time, the reaction mixture was pouring in ice-cooled H_2O (100 mL). HCl (1 M, 100 mL) was added and the aqueous layer was extracted with DCM (100 mL). The combined organic layers were washed with brine (150 mL), dried over $MgSO_4$, filtered and partially concentrated *in vacuo*. The crude product was purified by crystallization in DCM to obtain **6** (5.44 g, 18.7 mmol, 43%) as white crystals: R_f = 0.3 (20% EtOAc in pentane); ^1H NMR (500 MHz, MeOD) δ 7.07 – 6.96 (m, 2H), 6.77 – 6.63 (m, 2H), 4.65 (dd, *J* = 9.9, 5.3 Hz, 1H), 3.72 (s, 3H), 3.17 (dd, *J* = 14.0, 5.3 Hz, 1H), 2.91 (dd, *J* = 14.0, 9.9 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 172.2, 158.7 (q, *J* = 37.7 Hz), 157.5, 131.2 (x2), 128.4, 117.3 (q, *J* = 286.7 Hz), 116.3 (x2), 55.8, 53.0, 36.9; ^{19}F NMR (471 MHz, MeOD) δ -76.8; HRMS: calculated for $C_{12}H_{13}F_3NO_4$ 292.07912 $[M+H]^+$; found 292.07899. Spectroscopic data was in agreement with literature.[8]



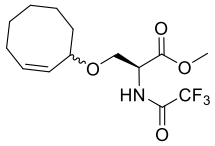
Cyclooctene ether 7: L-tyrosine methyl ester **6** (2.466 g, 8.47 mmol, 1.0 equiv) and cyclooctene *tert*-butyl carbonate reagent **2** (2.30 g, 10.2 mmol, 1.2 equiv) were combined in a 250 mL round-bottom flask, co-evaporated using anhydrous dioxane, placed under N_2 and dissolved in anhydrous dioxane (85 mL). $Pd(PPh_3)_4$ (567 mg, 0.49 mmol, 5.8 mol%) was added before freezing the reaction mixture at -78°C (ethanol bath) and subsequently purging N_2 over the frozen reaction mixture for 45 min to achieve degassing. The flask was sealed with parafilm before stirring the reaction mixture at 80°C (oil bath) for 41 h. The reaction mixture was allowed to cool to room temperature, impregnated by adding Celite and concentrated *in vacuo*. The impregnated crude product was purified by silica gel chromatography (1% EtOAc in pentane

→ 5% EtOAc in pentane) to obtain the diastereomeric mixture of cyclooctene ethers **7** (**7_A** : **7_B, ~ 1 : 1**, 2.69 g, 6.73 mmol, 80%) as a thick oil: R_f = 0.15 (5% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 – 6.88 (m, 2H), 6.83 – 6.70 (m, 2H + 1NH), 5.82 – 5.65 (m, 1H), 5.55 – 5.42 (m, 1H), 5.11 – 4.96 (m, 1H), 4.90 – 4.74 (m, 1H), 3.78 (s, 3H), 3.20 – 3.03 (m, 2H), 2.37 – 2.15 (m, 2H), 2.07 (ddt, *J* = 12.9, 8.9, 4.7 Hz, 1H), 1.79 – 1.38 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 170.6, 157.9 (x2), 156.6 (q, *J* = 36.2 Hz), 156.6 (q, *J* = 36.2 Hz), 133.0 (x2), 130.2 (x6), 126.1 (x2), 115.9 (x4), 115.7 (q, *J* = 287.8 Hz, x2), 75.3 (x2), 53.7, 53.7, 53.0, 52.9, 36.5 (x2), 35.9, 35.9, 29.2 (x2), 26.9 (x2), 26.3 (x2), 23.5 (x2); HRMS: calculated for $C_{20}H_{24}F_3NO_4Na$ 422.15496 $[M+Na]^+$; found 422.15463. Spectroscopic data was in agreement with literature.[8]

Note: No chemical shift differences were encountered on ^{1}H NMR for the two diastereoisomers of compound **7. The ^{1}H NMR signals were therefore reported as a single compound. The ^{13}C NMR reports distinct signals of the two diastereoisomers. Full conditions investigated for the synthesis of **7** are reported in Table S2.*



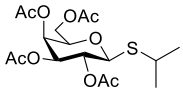
N-trifluoroacetyl-protected L-serine methyl ester 10: L-serine methyl ester hydrochloride (7.20 g, 46.3 mmol, 1.0 equiv) was dissolved in anhydrous MeOH (100 mL) in a 250 mL round-bottom flask under N_2. The reaction mixture was cooled to 0°C (ice bath) before adding anhydrous Et_3N (7.10 mL, 50.9 mmol, 1.1 equiv) dropwise. The reaction mixture was stirred for 15 min at 0°C. Ethyl trifluoroacetate (11.1 mL, 93.0 mmol, 2 equiv) was added dropwise and the reaction mixture was stirred and allowed to warm to room temperature. After 2 h, additional Et_3N (7.10 mL, 50.9 mmol, 1.1 equiv) was added. After 48 h the reaction mixture was concentrated *in vacuo*, redissolved in EtOAc (250 mL), washed with $NaHCO_3$ (satd., 200 mL), HCl (1 M, 200 mL) and brine (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (30% EtOAc in pentane → 40% EtOAc in pentane) to obtain **10** (4.74 g, 22.0 mmol, 48%) as an oil: R_f = 0.3 (30% EtOAc in pentane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 6.0 Hz, 1NH), 4.72 – 4.65 (m, 1H), 4.10 (dd, *J* = 11.5, 3.4 Hz, 1H), 3.96 (dd, *J* = 11.5, 3.3 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.7, 157.5 (q, *J* = 37.7 Hz), 115.7 (q, *J* = 288 Hz), 62.1, 54.8, 53.3; ^{19}F NMR (471 MHz, $CDCl_3$) δ -75.9. Spectroscopic data was in agreement with literature.[47]



Cyclooctene ether 11: L-serine methyl ester **10** (68.0 mg, 0.32 mmol, 1.0 equiv) and cyclooctene imidate **3** (181 mg, 0.67 mmol, 2.1 equiv) were co-evaporated with anhydrous toluene (3 x 2 mL) in a 25 mL round-bottom flask and dissolved in anhydrous DCM (3.0 mL) under N_2. The reaction mixture was cooled to -35°C (ethanol bath) before adding triflic acid (0.1 M in DCM, 0.32 mL, 32 µmol, 0.1 equiv). The reaction mixture was stirred for 4 h and gradually allowed to warm to 0°C. The reaction was quenched by adding Et_3N (8.8 µL, 63 µmol, 0.2 equiv) before adding Celite and concentrating *in vacuo*. The impregnated crude product was purified by silica gel chromatography (5% EtOAc in pentane, isocratic) to obtain the diastereomeric mixture of cyclooctene ethers **11** (**11_A** : **11_B, ~ 1 : 1**, 47.0 mg, 0.145 mmol, 46%) as an oil: R_f = 0.2 (5% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 6.7 Hz, 1NH, **11_A** + **11_B**), 5.78 – 5.65 (m, 1H, **11_A** + **11_B**), 5.43 – 5.27 (m, 1H, **11_A** + **11_B**), 4.76 – 4.65 (m, 1H, **11_A** + **11_B**), 4.28 – 4.15 (m, 1H, **11_A** + **11_B**), 4.00 (dd, *J* = 9.9, 3.0 Hz, 1H, **11_A**), 3.90 (dd, *J* = 9.9, 2.8 Hz, 1H,

11B), 3.81 (2 s, 3H, **11A** + **11B**), 3.79 (dd, *J* = 9.9, 3.1 Hz, 1H, **11B**), 3.66 (dd, *J* = 9.8, 3.1 Hz, 1H, **11A**), 2.15 – 2.04 (m, 2H, **11A** + **11B**), 1.90 – 1.78 (m, 1H, **11A** + **11B**), 1.69 – 1.30 (m, 7H, **11A** + **11B**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 169.3, 132.6, 132.5, 131.2, 131.2, 78.0, 77.7, 67.7, 67.4, 53.3 (x2), 53.1 (x2), 35.7, 35.7, 29.2, 29.1, 26.6, 26.6, 26.2 (x2), 23.6, 23.6; HRMS: calculated for $C_{14}H_{20}F_3NO_4Na$ 346.12366 $[M+Na]^+$; found 346.12350.

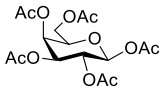
Note: the ^{13}C signals associated with the trifluoroacetate protecting group (C=O and CF_3) were not reported due to a lack of resolution in the spectrum of **11. Full conditions investigated for the synthesis of **11** are reported in Table S2.*



Peracetylated IPTG (14): Isopropyl β-D-1-thiogalactopyranoside (**13**, 477 mg, 2.0 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (6.0 mL) in a 50 mL round-bottom flask under N_2. Acetic anhydride (4.0 mL, 42.4 mmol, 21 equiv) was added and the reaction mixture was stirred for 20 h. The reaction mixture was concentrated *in vacuo*. The crude product was redissolved in Et_2O (50 mL) and washed with HCl (1 M, 3 x 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain **14** (827 mg, 2.0 mmol, 100%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 (dd, J = 3.4, 0.9 Hz, 1H), 5.22 (t, J = 10.0 Hz, 1H), 5.06 (dd, J = 10.0, 3.4 Hz, 1H), 4.58 (d, J = 10.0 Hz, 1H), 4.18 (dd, J = 11.3, 6.9 Hz, 1H), 4.10 (dd, J = 11.3, 6.4 Hz, 1H), 3.93 (td, J = 6.7, 1.0 Hz, 1H), 3.19 (hept, J = 6.8 Hz, 1H), 2.16 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.33 (d, J = 2.8 Hz, 3H), 1.31 (d, J = 2.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 170.4, 170.2, 169.6, 84.0, 74.4, 72.1, 67.6, 67.4, 61.7, 35.8, 24.1, 23.9, 21.0, 20.8, 20.8, 20.7; HRMS: calculated for $C_{17}H_{26}O_9SNa$ 429.11897 $[M+Na]^+$; found 429.11875. Spectroscopic data was in agreement with literature.[48]

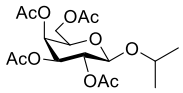
Evaluation of conditions typical for photochemical isomerization with 14: Acetylated IPTG (**14**, 413 mg, 1.02 mmol, 1 equiv) was irradiated (λ = 254 nm) for 24 h in the presence of methyl benzoate (360 mg, 2.64 mmol, 2.6 equiv) in a quartz flask containing a solution of Et_2O in heptane (1:1, 100 mL). During irradiation, the reaction mixture was continuously circulated over a silica column (4 g size, containing dry silica and 2.5 g of $AgNO_3$ impregnated silica[25](10% w/w, containing 1.47 mmol $AgNO_3$, 1.5 equiv)) at a flowrate of 25 mL/min. The column was placed in the dark and shielded with aluminium foil during the irradiation. Afterwards, the column was flushed with Et_2O in heptane (1:1, 250 mL) before drying over a stream of air. Subsequently, the contents of the column were emptied into an Erlenmeyer flask containing NH_4OH (28% w/w, 25 mL) and DCM (25 mL). The biphasic mixture was stirred for 1 h before filtration of the silica gel. The organic layer was separated and the aqueous layer was extracted with DCM (25 mL). The combined organic layers were washed with H_2O (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain **14** (252 mg, 0.62 mmol, 61%) as an oil.

*Note: Based on this experiment, we concluded that **14** has significant affinity for $AgNO_3$ (despite not forming a (trans)-cyclooctene moiety during irradiation), which would hamper the development of a TCO-caged IPTG.*



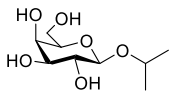
Peracetylated β-D-galactopyranoside 16: Synthesis was performed according to a modified procedure.[49] A suspension of sodium acetate (25.0 g, 305 mmol, 1.1 equiv) in acetic anhydride (350 mL, 3.71 mol, 13.4 equiv) was

stirred in a three-neck, round-bottom flask and heated towards reflux in an oil bath set at 160°C. When the suspension was fully refluxing, the flask was removed from the oil bath and D-galactose (50.0 g, 278 mmol, 1.0 equiv) was slowly added in portions to the mixture. The reaction mixture turned into a clear, yellow solution and was stirred for a further 5-10 min before pouring it into ice water (2 L). The aqueous mixture was stirred for 1 h at room temperature. DCM (600 mL) was added and the organic layer was washed with H_2O (1.5 L), $NaHCO_3$ (satd., 1.5 L), brine (1 L), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was obtained as a light yellow solid and purified by recrystallization in EtOH to obtain **16** (56.4 g, 144 mmol, 52%) as white crystals: R_f = 0.4 (30% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1H NMR (400 MHz, CDCl3) δ 5.71 (d, J = 8.3 Hz, 1H), 5.43 (dd, J = 3.4, 1.1 Hz, 1H), 5.34 (dd, J = 10.4, 8.3 Hz, 1H), 5.09 (dd, J = 10.4, 3.4 Hz, 1H), 4.21 – 4.03 (m, 3H), 2.17 (s, 3H), 2.13 (s, 3H), 2.05 (2 s, 6H), 2.00 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 170.2, 170.0, 169.5, 169.1, 92.2, 71.8, 70.9, 67.9, 66.9, 61.1, 20.9, 20.7, 20.7, 20.6; HRMS: calculated for $C_{16}H_{22}O_{11}Na$ 413.10543 $[M+Na]^+$, found 413.10521. Spectroscopic data was in agreement with literature.[49]



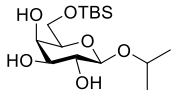
Compound 17: Peracetylated galactopyranoside **16** (50.0 g, 128 mmol, 1.0 equiv) was co-evaporated with anhydrous toluene (200 mL) in a 2 L round-bottom flask before dissolving the starting material in DCM (513 mL) under N_2. The solution was cooled to 0°C (ice bath) before adding acetic anhydride (24.2 mL, 256 mmol, 2.0 equiv) and HBr (33% w/w in AcOH, 133 mL, 769 mmol, 6.0 equiv). The reaction mixture was stirred overnight and allowed to warm to room temperature. TLC confirmed complete conversion of **16** into the corresponding anomeric bromide: R_f = 0.7 (30% EtOAc in pentane). The crude reaction mixture was concentrated *in vacuo*, placed under N_2 and redissolved in anhydrous isopropanol (640 mL) in the presence of flame-dried molecular sieves (4 Å, 75 g). The solution was cooled to 0°C (ice bath) before adding I_2 (48.7 g, 192 mmol, 1.5 equiv). The reaction mixture was stirred for 24 h at 4°C (cold room).* $Na_2S_2O_3$ (satd., 500 mL) was slowly added to quench the reaction whilst stirring. The reaction mixture was filtered, diluted with H_2O (500 mL) and subsequently extracted with EtOAc (3 x 500 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (15% EtOAc in pentane → 20% EtOAc in pentane). The beta-glycosylated product **17** (37.5 g, 96.1 mmol, 75% over 2 steps) was obtained as an oil: R_f = 0.5 (30% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 (dd, J = 3.5, 1.2 Hz, 1H), 5.18 (dd, J = 10.5, 7.9 Hz, 1H), 5.02 (dd, J = 10.5, 3.5 Hz, 1H), 4.51 (d, J = 7.9 Hz, 1H), 4.19 (dd, J = 11.2, 6.6 Hz, 1H), 4.12 (dd, J = 11.2, 6.9 Hz, 1H), 3.98 – 3.84 (m, 2H), 2.15 (s, 3H), 2.05 (2 s, 6H), 1.99 (s, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 170.5, 170.4, 169.5, 100.4, 73.4, 71.1, 70.6, 69.2, 67.2, 61.5, 23.4, 22.2, 20.9, 20.8, 20.8, 20.8; HRMS: calculated for $C_{17}H_{26}O_{10}Na$ 413.14182 $[M+Na]^+$; found 413.14146.

**Note: This reaction can also be stirred overnight and allowed to warm to room temperature, obtaining a similar yield over 2 steps at 50 mmol reaction scale.*

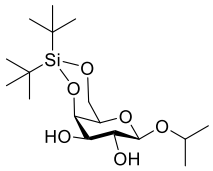


Isopropyl β-D-1-galactopyranoside (IPG; 15): Beta-galactopyranoside **17** (37.5 g, 96.1 mmol, 1 equiv) was dissolved in a mixture of anhydrous DCM (480 mL) and anhydrous MeOH (480 mL) in a 2 L round-bottom flask under

N_2. Sodium methoxide (1.04 g, 19.2 mmol, 0.2 equiv) was added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by adding Amberlyst® (H^+ form, washed 3 x with MeOH prior to usage) in small portions, gently swirling the flask and monitoring the pH until neutral. The neutralized solution was filtered and concentrated *in vacuo* to obtain IPG (**15**, 19.5 g, 87.7 mmol, 91%) as a solid: R_f = 0.4 (20% MeOH in DCM); ^{1}H NMR (400 MHz, MeOD) δ 4.33 – 4.24 (m, 1H), 4.04 (hept, *J* = 6.2 Hz, 1H), 3.84 (br s, 1H), 3.73 (d, *J* = 6.2 Hz, 2H), 3.53 – 3.42 (m, 3H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.19 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ 103.1, 76.4, 75.0, 72.5, 72.4, 70.2, 62.4, 23.8, 22.0; HRMS: calculated for $C_9H_{18}O_6Na$ 245.09956 $[M+Na]^+$; found 245.09950. **15** was redissolved in H_2O and lyophilized in small quantities for recombinant gene expression experiments.

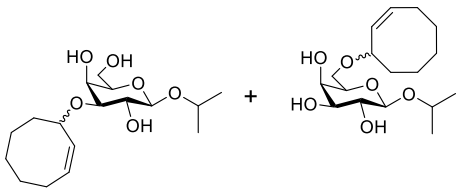


6-TBS-IPG (27): IPG (**15**, 1.117 g, 5.03 mmol, 1.0 equiv) was co-evaporated with anhydrous toluene (3 x 10 mL) in a 250 mL round-bottom flask before dissolving in anhydrous pyridine (20 mL) under N_2. The solution was cooled to 0°C before adding TBDMS-Cl (50% w/w in toluene, 2.09 mL, 6.03 mmol, 1.2 equiv). The reaction mixture was stirred and allowed to warm to room temperature. Additional TBDMS-Cl (50% w/w in toluene, 0.525 mL, 1.5 mmol, 0.3 equiv) was added after 4 and 24 h to achieve full conversion. After a total reaction time 48 h, the reaction was quenched by adding H_2O (~100 mL) and subsequently extracted with DCM (3 x 75 mL). The combined organic layers were washed with $CuSO_4$ (1 M, 50 mL), H_2O (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (50% EtOAc in pentane → 60% EtOAc in pentane) to obtain the 6-O-silylated galactopyranoside **27** (1.55 g, 4.61 mmol, 92%) as an oil: R_f = 0.1 (50% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (d, *J* = 7.5 Hz, 1H), 4.06 – 3.94 (m, 2H), 3.89 (dd, *J* = 10.4, 6.1 Hz, 1H), 3.82 (dd, *J* = 10.4, 5.5 Hz, 1H), 3.64 (dd, *J* = 9.6, 7.5 Hz, 1H), 3.57 (dd, *J* = 9.6, 3.3 Hz, 1H), 3.47 (t, *J* = 5.8 Hz, 1H), 3.29 (br s, 3OH), 1.25 (d, *J* = 6.2 Hz, 3H), 1.20 (d, *J* = 6.2 Hz, 3H), 0.89 (s, 9H), 0.08 (2 s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 101.6, 74.8, 73.9, 72.0, 71.9, 69.0, 62.6, 26.0 (x3), 23.6, 22.0, 18.4, -5.3, -5.3; HRMS: calculated for $C_{15}H_{32}O_6SiNa$ 359.18604 $[M+Na]^+$; found 359.18589.



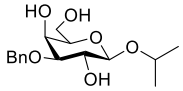
4,6-DTBS-IPG (28): IPG (**15**, 1.123 g, 5.05 mmol, 1.0 equiv) was co-evaporated with anhydrous toluene (3 x 10 mL) in a round-bottom flask before dissolving in anhydrous pyridine (20 mL) under N_2. The solution was cooled to 0°C before adding di-*tert*-butylsilanediyl bis(trifluoromethanesulfonate) (2.0 mL, 6.14 mmol, 1.2 equiv) at a rate of 0.5 mL/h (syringe pump). The reaction mixture was stirred overnight and allowed to warm to room temperature. The reaction was quenched by adding H_2O (2 mL) and subsequently diluted with EtOAc (150 mL). The mixture was washed with HCl (1 M, 3 x 75 mL), $NaHCO_3$ (satd., 100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (40% EtOAc in pentane, isocratic) to obtain the 4,6-O-silylated galactopyranoside **28** (1.31 g, 3.61 mmol, 72%) as an oil: R_f = 0.3 (50% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 – 4.34 (m, 1H), 4.31 (d, *J* = 7.6 Hz, 1H), 4.28 (dd, *J* = 12.3, 2.2 Hz, 1H), 4.22 (dd, *J* = 12.3, 1.7 Hz, 1H), 4.00 (hept, *J* = 6.2 Hz, 1H), 3.64 (dd, *J* = 9.4, 7.6 Hz, 1H), 3.52 (dd, *J* = 9.4, 3.4 Hz, 1H), 3.41 (br s, 1H), 2.68 (br s, 1OH), 2.47 (br s, 1OH), 1.24 (d, *J* = 6.2 Hz, 3H), 1.19 (d, *J* = 6.2 Hz, 3H), 1.05 (2 s, 18H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ 101.2, 73.9, 72.8, 72.3, 71.3, 71.2, 67.2, 27.6 (x3), 27.5 (x3), 23.8, 23.5, 22.0, 20.9; HRMS: calculated for $C_{17}H_{34}O_6SiNa$ 385.20169 $[M+Na]^+$; found 385.20122.

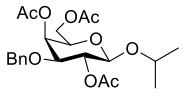


3-CCO-IPG (19) and 6-CCO-IPG (20): IPG (**15**, 248 mg, 1.12 mmol, 1.0 equiv) and dibutyltin oxide (333 mg, 1.34 mmol, 1.2 equiv) were combined in a 10 mL round-bottom flask and dissolved in anhydrous toluene (5 mL) under N_2. The reaction mixture was stirred at 105°C (oil bath) under N_2 overnight. The reaction mixture was subsequently concentrated *in vacuo*, co-evaporated with anhydrous toluene (3 x) and placed under N_2. Cyclooctene bromide **18** (528 mg, 2.79 mmol, 2.5 equiv) was dissolved in anhydrous toluene (1 mL) in a separate 10 mL pear-shaped flask under N_2. The solution containing **18** and cesium fluoride (424 mg, 2.79 mmol, 2.5 equiv) were added to the reaction mixture. The combined reaction mixture was stirred overnight at 105°C (oil bath) under N_2. The reaction mixture was diluted with EtOAc, sonicated (5 min), transferred to a 50 mL round-bottom flask, impregnated with Celite and concentrated *in vacuo*. The impregnated crude product was purified by silica gel chromatography (10% acetone in pentane → 20% acetone in pentane → 30% acetone in pentane) to obtain the regioisomers **19** (**3-CCO-IPG**; 81 mg, 0.245 mmol, 22%) and **20** (**6-CCO-IPG**; 68 mg, 0.206 mmol, 18%) separately as diastereomeric mixtures: **3-CCO-IPG (19$_A$ + 19$_B$, 0.4 : 0.6)**: R_f = 0.3 (30% acetone in pentane), 0.5 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 – 5.65 (m, 1H, **19$_A$** + **19$_B$**), 5.62 – 5.46 (m, 1H, **19$_A$** + **19$_B$**), 4.66 – 4.58 (m, 1H, **19$_A$**), 4.57 – 4.47 (m, 1H, **19$_B$**), 4.31 (d, *J* = 7.8 Hz, 1H, **19$_B$**), 4.30 (d, *J* = 7.9 Hz, 1H, **19$_A$**), 4.08 – 3.89 (m, 3H, **19$_A$** + **19$_B$**), 3.88 – 3.75 (m, 1H, **19$_A$** + **19$_B$**), 3.73 – 3.59 (m, 1H, **19$_A$** + **19$_B$**), 3.55 – 3.48 (m, 1H, **19$_A$** + **19$_B$**), 3.46 (dd, *J* = 9.5, 3.4 Hz, 1H, **19$_B$**), 3.40 (dd, *J* = 9.4, 3.4 Hz, 1H, **19$_A$**), 2.95 – 2.52 (m, 3OH, **19$_A$** + **19$_B$**), 2.15 – 2.06 (m, 2H, **19$_A$** + **19$_B$**), 2.05 – 1.96 (m, 1H, **19$_A$**), 1.96 – 1.83 (m, 1H, **19$_B$**), 1.71 – 1.29 (m, 7H, **19$_A$** + **19$_B$**), 1.27 (d, *J* = 6.2 Hz, 3H, **19$_A$** + **19$_B$**), 1.20 (d, *J* = 6.1 Hz, 3H, **19$_A$** + **19$_B$**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.4, 133.0, 131.3, 130.5, 101.5, 101.5, 79.7, 78.1, 78.1, 74.4, 74.4, 74.4, 71.7 (x2), 71.2, 70.0, 68.5, 65.9, 62.2, 62.2, 36.4, 35.9, 29.3, 29.1, 26.7, 26.7, 26.3, 26.2, 23.7, 23.7, 23.6 (x2), 21.9, 21.9; HRMS: calculated for $C_{17}H_{30}O_6Na$ 353.19346 $[M+Na]^+$; found 353.19316; **6-CCO-IPG (20$_A$ + 20$_B$, 1 : 1)**: R_f = 0.15 (30% acetone in pentane), 0.3 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 (dddd, *J* = 10.5, 8.9, 7.3, 1.3 Hz, 1H, **20$_A$ + 20$_B$**), 5.53 – 5.43 (m, 1H, **20$_A$ + 20$_B$**), 4.35 – 4.26 (m, 1H, **20$_A$ + 20$_B$**), 4.28 (d, *J* = 7.7, 1H, **20$_A$**), 4.27 (d, *J* = 7.7, 1H, **20$_B$**), 4.23 (br s, 1OH, **20$_A$** + **20$_B$**), 4.06 – 3.97 (m, 1H, **20$_A$ + 20$_B$**), 3.95 (dd, *J* = 10.7, 3.3 Hz, 1H, **20$_A$ + 20$_B$**), 3.90 – 3.81 (m, 1OH, **20$_A$** + **20$_B$**), 3.78 (dd, *J* = 9.0, 4.0 Hz, 1H, **20$_A$**), 3.72 – 3.52 (m, 3H, **20$_A$ + 20$_B$**; 1OH, **20$_A$ + 20$_B$**; 1H, **20$_B$**), 2.21 – 2.02 (m, 2H, **20$_A$ + 20$_B$**), 1.97 – 1.86 (m, 1H, **20$_A$ + 20$_B$**), 1.70 – 1.30 (m, 7H, **20$_A$ + 20$_B$**), 1.29 – 1.23 (m, 3H), 1.20 (d, *J* = 6.1 Hz, 3H, **20$_A$**), 1.19 (d, *J* = 6.1 Hz, 3H, **20$_B$**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.7, 133.7, 130.3, 130.3, 101.7, 101.7, 77.7, 77.6, 73.9 (x3), 73.6, 72.0 (x2), 71.6, 71.5, 69.4, 69.3, 68.2, 67.9, 35.9, 35.8, 29.2 (x2), 26.6 (x2), 26.3 (x2), 23.7, 23.7, 23.6, 23.6, 22.1 (x2); HRMS: calculated for $C_{17}H_{30}O_6Na$ 353.19346 $[M+Na]^+$; found 353.19312. **19** and **20** were redissolved in dioxane and lyophilized in small quantities for recombinant gene expression experiments.

Note: Full conditions investigated for the synthesis of **19 and **20** are reported in Table S3.*

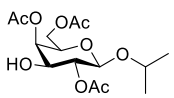


3-OBn-IPG (29): This procedure was based on the reported procedure by Geng *et al*.[50] for the regioselective benzylation of IPTG. IPG (**15**, 19.5 g, 87.7 mmol, 1.0 equiv) was co-evaporated with anhydrous toluene (3 x 100 mL) in a 1 L round-bottom flask before adding dibutyltin oxide (32.7 g, 131 mmol, 1.5 equiv) and suspending the reactants in anhydrous toluene (440 mL) under N_2. The reaction mixture was stirred overnight at 105°C (oil bath) under N_2. The reaction mixture was concentrated *in vacuo*, co-evaporated with anhydrous toluene (3 x 100 mL), placed under N_2 and redissolved in anhydrous toluene (440 mL). Tetrabutylammonium bromide (5.65 g, 17.5 mmol, 0.2 equiv) and benzyl bromide (15.6 mL, 131 mmol, 1.5 equiv) were added and the reaction mixture was stirred for 23 h at 70°C (oil bath) under N_2. The reaction mixture was allowed to cool to room temperature, concentrated *in vacuo*, redissolved in DCM (500 mL) and washed with a mixture of H_2O (500 mL) and brine (1 L). The aqueous phase was extracted with DCM (5 x 500 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (DCM → 2% MeOH in DCM) to obtain **29** (27.6 g) as a crude product (including a tetrabutylammonium derived impurity; marked in the NMR spectra) which was used in the next step without further purification: R_f = 0.5 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H), 4.75 (s, 2H), 4.30 (d, *J* = 7.8 Hz, 1H), 4.07 – 3.97 (m, 2H), 3.93 (ddd, *J* = 11.2, 6.6, 4.3 Hz, 1H), 3.85 – 3.79 (m, 1H), 3.76 (ddd, *J* = 9.7, 7.8, 2.1 Hz, 1H), 3.51 – 3.46 (m, 1H), 3.44 (dd, *J* = 9.5, 3.4 Hz, 1H), 2.78 (dd, *J* = 2.3, 1.0 Hz, 1OH), 2.49 (d, *J* = 2.2 Hz, 1OH), 2.46 (dd, *J* = 8.3, 4.4 Hz, 1OH), 1.26 (d, *J* = 6.2 Hz, 3H), 1.20 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.8, 128.7 (x2), 128.2, 128.0 (x2), 101.5, 80.2, 74.5, 72.2, 71.9, 71.2, 67.0, 62.3, 23.6, 22.0; HRMS: calculated for $C_{16}H_{24}O_6Na$ 335.14651 $[M+Na]^+$; found 335.14610.

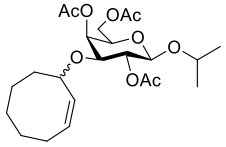


3-OBn-2,4,6-OAc-IPG (21): Crude 3-OBn-IPG (**29**, 23.6 g, max. 87.7 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (530 mL, 6.55 mol, 74.5 equiv) and acetic anhydride (350 mL, 3.71 mol, 42.2 equiv) in a 2 L round-bottom flask under N_2. The reaction mixture was stirred overnight at room temperature under N_2, concentrated *in vacuo*, redissolved in Et_2O (1 L) and washed with HCl (1 M, 3 x 500 mL). The combined aqueous layers were extracted with Et_2O (500 mL). The combined organic layers were washed with $NaHCO_3$ (satd., 500 mL) and brine (500 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain 3-OBn-2,4,6-OAc-IPG **21** (35.0 g, 79.8 mmol, 91% over 2 steps) as a solid: R_f = 0.2 (30% Et_2O in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.24 (m, 5H), 5.50 (dd, *J* = 3.4, 0.9 Hz, 1H), 5.08 (dd, *J* = 10.0, 8.1 Hz, 1H), 4.70 (d, *J* = 12.4 Hz, 1H), 4.40 (d, *J* = 12.1 Hz, 1H), 4.40 (d, *J* = 8.1 Hz, 1H), 4.16 (dd, *J* = 6.7, 0.9 Hz, 2H), 3.88 (hept, *J* = 6.2 Hz, 1H), 3.78 (td, *J* = 6.6, 1.1 Hz, 1H), 3.53 (dd, *J* = 10.0, 3.5 Hz, 1H), 2.15 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.11 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.7, 169.5, 137.7, 128.5 (x2), 127.9 (x3), 100.4, 76.8, 73.1, 71.3, 70.9, 70.9, 66.0, 62.2, 23.4, 22.2, 21.0, 21.0, 20.9; HRMS: calculated for $C_{22}H_{30}O_9Na$ 461.17820 $[M+Na]^+$; found 461.17787.

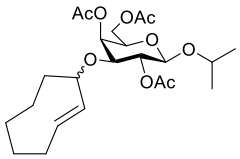
Note: precipitation of **21 may occur in the residue when filtering off the dried organic layers. If so, dilution with extra Et_2O or EtOAc ensures no product is lost.*



2,4,6-OAc-IPG (22): 3-OBn-2,4,6-OAc-IPG (**21**, 35.0 g, 79.8 mmol, 1.0 equiv) was co-evaporated with toluene (200 mL) in a 1 L round-bottom flask and subsequently dissolved in EtOAc (800 mL) under N_2. N_2 was purged through the stirring solution for 15 min (flow) before adding $Pd(OH)_2/C$ (20% w/w loading, 5.61 g, 7.99 mmol, 0.1 equiv) and purging N_2 through the stirred suspension for 45 min (flow). The reaction mixture was purged with H_2 (balloon) whilst stirring and was subsequently left to stir under H_2 (balloon) for 72 h. The reaction mixture was purged with N_2 (flow), filtered over a pad of Celite and concentrated *in vacuo* to obtain 2,4,6-OAc-IPG (**22**, 23.9 g, 68.6 mmol, 86%) as an off-white sold: R_f = 0.3 (50% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (dd, *J* = 3.6, 0.9 Hz, 1H), 4.94 (dd, *J* = 10.1, 7.9 Hz, 1H), 4.47 (d, *J* = 8.0 Hz, 1H), 4.15 (d, *J* = 6.6 Hz, 2H), 3.92 (hept, *J* = 6.2 Hz, 1H), 3.86 – 3.79 (m, 1H), 3.83 (td, *J* = 6.6, 0.9 Hz, 1H), 2.82 (d, *J* = 6.5 Hz, 1OH), 2.17 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.24 (d, *J* = 6.2 Hz, 3H), 1.16 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 171.1, 170.7, 100.0, 73.2, 73.1, 71.6, 71.0, 69.8, 62.1, 23.4, 22.2, 21.1, 21.0, 20.8; HRMS: calculated for $C_{15}H_{24}O_9Na$ 371.13125 $[M+Na]^+$; found 371.13101.



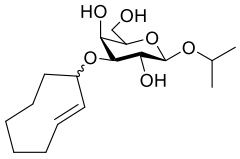
2,4,6-OAc-3-CCO-IPG (23): 2,4,6-OAc-IPG (**22**, 7.60 g, 21.8 mmol, 1.0 equiv) and cyclooctene imidate **3** (23.3 g, 86 mmol, 3.95 equiv) were co-evaporated with anhydrous toluene (3 x 150 mL) in a 1 L round-bottom flask and dissolved in anhydrous DCM (200 mL) under N_2. The reaction mixture was cooled to -40°C (ethanol bath) before adding triflic acid (0.194 mL, 2.18 mmol, 0.1 equiv). The reaction mixture was stirred for 4 h and allowed to warm to -30°C and subsequently quenched by adding Et_3N (0.608 mL, 4.36 mmol, 0.2 equiv). The neutralized reaction mixture was diluted with Et_2O (1 L), washed with NaOH (1 M, 3 x 1 L) and brine (1 L), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (10% Et_2O in pentane → 30% Et_2O in pentane) to obtain the diastereomeric mixture **23** (**23_A + 23_B, ~ 0.6 : 0.4,** 3.40 g, 7.45 mmol, 34%) as a crystalline solid: R_f = 0.35 (30% Et_2O in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 – 5.72 (m, 1H, **23_B**), 5.71 – 5.62 (m, 1H, **23_A**), 5.49 (dd, *J* = 10.7, 7.1 Hz, 1H, **23_A**), 5.38 (dd, *J* = 3.5, 0.9 Hz, 1H, **23_B**), 5.35 (dd, *J* = 3.6, 1.0 Hz, 1H, **23_A**), 5.31 (ddd, *J* = 10.7, 7.3, 1.3 Hz, 1H, **23_B**), 5.06 (dd, *J* = 10.1, 8.1 Hz, 1H, **23_A**), 5.00 (dd, *J* = 10.1, 8.1 Hz, 1H, **23_B**), 4.44 – 4.37 (m, 1H, **23_B**), 4.42 (d, *J* = 8.1 Hz, 1H, **23_A**), 4.41 (d, *J* = 8.1 Hz, 1H, **23_B**), 4.36 – 4.28 (m, 1H, **23_A**), 4.19 – 4.09 (m, 2H, **23_A + 23_B**), 3.95 – 3.84 (m, 1H, **23_A + 23_B**), 3.82 – 3.77 (m, 1H, **23_A + 23_B**), 3.58 – 3.47 (m, 1H, **23_A + 23_B**), 2.15 – 2.05 (m, 2H, **23_A + 23_B**), 2.14 (s, 3H, **23_B**), 2.12 (s, 3H, **23_A**), 2.10 (s, 3H, **23_A**), 2.09 (s, 3H, **23_B**), 2.07 (s, 3H, **23_B**), 2.06 (s, 3H, **23_A**), 1.83 – 1.74 (m, 1H, **23_A**), 1.70 – 1.27 (m, 7H, **23_A + 23_B**; 1H, **23_B**), 1.23 (d, *J* = 6.3 Hz, 3H, **23_A + 23_B**), 1.14 (d, *J* = 6.2 Hz, 3H, **23_A**), 1.13 (d, *J* = 6.0 Hz, 3H, **23_B**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8 (x2), 170.6, 170.5, 169.4, 169.4, 133.4, 133.0, 131.7, 130.1, 100.6, 100.5, 78.6, 76.5, 75.0, 74.5, 73.0, 73.0, 71.8, 71.2, 70.9, 70.7, 68.7, 66.1, 62.4, 62.4, 36.0, 35.7, 29.3, 29.3, 26.9, 26.7, 26.4, 26.3, 23.7, 23.7, 23.4, 23.4, 22.2 (x2), 21.1 (x2), 21.0, 21.0, 20.9 (x2); HRMS: calculated for $C_{23}H_{36}O_9Na$ 479.22515 $[M+Na]^+$; found 479.22483.



2,4,6-OAc-3-TCO-IPG (24): 2,4,6-OAc-3-CCO-IPG (**23**, 228 mg, 0.50 mmol, 1 equiv) was irradiated (λ = 254 nm) for 24 h in the presence of methyl benzoate (385 mg, 2.83 mmol, 5.7 equiv) in a quartz flask containing a solution of 5% isopropanol in Et_2O (150 mL). During irradiation, the reaction mixture was continuously circulated over a silica column (4 g size) containing 2.5 g of TAg silica[41] (0.6 mmol/g, containing 1.5 mmol Ag (I), 3.0 equiv) at a flowrate of 20 mL/min. The column was placed in the dark and shielded with aluminium foil during the irradiation. Afterwards, the column was flushed with 5% isopropanol in Et_2O (300 mL) before disconnecting the stationary phase from the HPLC system and drying over a stream of N_2. The column was eluted with NH_3 (7 N in MeOH) and fractions containing the product were combined and concentrated *in vacuo* to obtain the crude, partially deacetylated product **24** as an oil which was used for the next step without further purification: R_f = 0.4 (30% Et_2O in pentane).*

Note: **24 was partially deacetylated during treatment with NH_3. An analytical sample used for the NMR assignment shown below was obtained from a separate experiment (Entry 4, Table S6) in which the crude product was purified by silica gel chromatography (10% Et_2O in pentane → 30% Et_2O in pentane). This leads to loss of partially deacetylated product. Full conditions investigated for the photochemical conversion of **23** to **24** are listed in Table S6.*

Diastereomeric mixture **24** (**24_A + 24_B, ~ 0.6 : 0.4**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (ddd, *J* = 15.7, 11.2, 3.5 Hz, 1H, **24_A + 24_B**), 5.45 (d, *J* = 3.0 Hz, 1H, **24_B**), 5.42 (d, *J* = 2.9 Hz, 1H, **24_A**), 5.40 – 5.32 (m, 1H, **24_A + 24_B**), 5.14 – 5.08 (m, 1H, **24_A + 24_B**), 4.44 (d, *J* = 8.1 Hz, 1H, **24_B**), 4.42 (d, *J* = 8.1 Hz, 1H, **24_A**), 4.42 (br s, 1H, **24_B**), 4.27 (br s, 1H, **24_A**), 4.20 – 4.09 (m, 2H, **24_A + 24_B**), 3.90 (hept, *J* = 6.2 Hz, 1H, **24_A + 24_B**), 3.83 – 3.74 (m, 1H, **24_A + 24_B**), 3.68 (dd, *J* = 10.2, 3.2 Hz, 1H, **24_B**), 3.55 (dd, *J* = 9.9, 3.6 Hz, 1H, **24_A**), 2.52 – 2.38 (m, 1H, **24_A + 24_B**), 2.14 (s, 3H, **24_B**), 2.13 (s, 3H, **24_B**), 2.11 (s, 3H, **24_A**), 2.06 (2 x s, 6 H, **24_A**; 3 H, **24_B**), 2.02 – 1.73 (m, 4H, **24_A + 24_B**), 1.61 – 1.33 (m, 3H, **24_A + 24_B**), 1.24 (d, *J* = 6.2 Hz, 3H, **24_A + 24_B**), 1.14 (d, *J* = 6.2 Hz, 3H, **24_A + 24_B**), 1.00 – 0.87 (m, 1H, **24_A + 24_B**), 0.77 – 0.63 (m, 1H, **24_A + 24_B**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.6, 170.5, 170.4, 169.3, 169.2, 133.2, 132.6, 132.1, 131.6, 100.6, 100.4, 78.1, 75.6, 75.3, 74.5, 73.1, 73.0, 71.4, 71.0, 70.8, 70.7, 67.5, 65.8, 62.3, 62.1, 41.8, 40.1, 36.2, 36.0, 36.0, 35.8, 29.4, 29.3, 23.8, 23.4, 23.4, 23.3, 22.2, 22.2, 21.0, 21.0, 20.9, 20.9, 20.8 (x2).



3-TCO-IPG (25): The crude product (**24**) obtained from the photoisomerization reaction was suspended in NaOMe (0.5 M in MeOH, 5.0 mL, 2.5 mmol, 5.0 equiv) in a 50 mL round-bottom flask under N_2. The reaction mixture was stirred overnight at room temperature, concentrated *in vacuo*, resuspended in H_2O (30 mL) and extracted with DCM (5 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain the diastereomeric mixture 3-TCO-IPG **25** (**25_A** : **25_B, ~ 2 : 1**, 57 mg, 0.17 mmol, 35% over 2 steps) as a solid: R_f = 0.4 (5% MeOH in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (ddd, *J* = 16.0, 11.3, 3.8 Hz, 1H, **25_B**), 5.97 (ddd, *J* = 15.9, 11.2, 3.7 Hz, 1H, **25_A**), 5.51 (dd, *J* = 16.5, 1.9 Hz, 1H, **25_A**), 5.40 (dd, *J* = 16.5, 1.3 Hz, 1H, **25_B**), 4.58 (br s, 1H, **25_A**), 4.48 (br s, 1H, **25_B**), 4.32 (d, *J* = 8.0 Hz, 1H, **25_B**), 4.30 (d, *J* = 7.9 Hz, 1H, **25_A**), 4.08 – 4.00 (m, 2H, **25_A + 25_B**), 3.99 – 3.91 (m, 1H,

25A + **25B**), 3.86 – 3.76 (m, 1H, **25A** + **25B**), 3.75 – 3.66 (m, 1H, **25A** + **25B**), 3.56 (dd, *J* = 9.5, 3.5 Hz, 1H, **25B**), 3.54 – 3.47 (m, 1H, **25A** + **25B**), 3.43 (dd, *J* = 9.5, 3.4 Hz, 1H, **25A**), 2.79 (br s, 1OH, **25A**), 2.72 (br s, 1OH, **25B**), 2.60 – 2.43 (m, 1H + 2OH, **25A** + **25B**), 2.13 (dd, *J* = 14.8, 5.8 Hz, 1H, **25A**), 2.07 – 1.91 (m, 2H, **25A** + **25B**; 1H, **25B**), 1.91 – 1.78 (m, 1H, **25A** + **25B**), 1.74 – 1.39 (m, 3H, **25A** + **25B**), 1.27 (d, *J* = 6.3 Hz, 3H, **25A** + **25B**), 1.20 (d, *J* = 6.2 Hz, 3H, **25A** + **25B**), 1.17 – 1.05 (m, 1H, **25A** + **25B**), 0.82 – 0.69 (m, 1H, **25A** + **25B**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.2, 133.2, 132.8, 131.2, 101.6, 101.5, 79.4, 79.3, 77.9, 76.0, 74.5, 74.4, 71.9, 71.8, 71.6, 70.5, 68.4, 66.0, 62.4 (x2), 42.1, 41.5, 36.1, 36.0, 35.9, 35.8, 29.4 (x2), 23.9, 23.8, 23.6 (x2), 22.0, 21.9; HRMS: calculated for $C_{17}H_{30}O_6Na$ 353.19346 $[M+Na]^+$; found 353.19313. **25** was redissolved in dioxane and lyophilized in small quantities for recombinant gene expression experiments.

Note: deacetylation in the presence of catalytic quantities of NaOMe and/or shorter reaction times did not result in complete conversion. This instead led to a mixture of products, in which the fully deprotected product (25**) was difficult to isolate.*

6.7 Experimental procedures – molecular biology

General methods: Samples taken (corrected for OD_{600} according to the formula: (1 / OD_{600}) * 200 µL) from *E.coli* cultures were pelleted and stored at -20°C for indicated timepoints in each individual experiment described below. Samples were dissolved in a mixture of H_2O and 2x sample loading buffer (supplemented with 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, 1U/µL Benzonase in 0.125 M Tris HCl, pH 6.8; see individual experiments for deviations and amounts). Subsequently, dissolved samples were incubated for 5 min at 95°C for denaturation. 15 µL of each sample was loaded onto a 15% SDS-PAGE gel (0.75 or 1.5 mm) along with 4 µL PageRuler™ Plus Protein Marker (Thermo Scientific) unless stated otherwise and run for ~70 min at 180 V. Coomassie staining (Coomassie Brilliant Blue G-250) and in-gel fluorescence, using wavelength filters for Alexa 488 (eGFP) and Alexa 555 (dsRed2), were measured using a Chemidoc Imager (Bio-Rad). Data was subsequently processed and quantified (relative quantification using a t = 0 h band as the reference; results in Tables S7 – S14) using ImageLab software (Bio-Rad).

Lac* operon dependent overexpression protocol for ovalbumin:** The gene for ovalbumin (hereafter referred to as OVA, accession number V00383) was cloned into the pMCSG7 vector as described elsewhere[51] and transformed into the methionine auxotroph expression strain, namely *E.coli* B834(DE3) (met-aux, Genotype: F- ompT hsdSB (rB- mB-) gal dcm met(DE3), Novagen #ref 69041). The construct contained an N-terminal M<u>HHHHHH</u>SSGVDLGTENLYFG***SNA sequence for Ni-NTA purification (underlined) and a TEV-cleavage (italic bold) site. The protein was expressed from the overnight culture of a single colony. Briefly, 10 mL of this overnight culture (Ampicillin 50 µg/mL, 1% Glucose v/v, 18 h, 37°C, and 150 rpm) was used for the inoculation per 100 mL LB medium (Ampicillin 50 µg/mL, 37°C, 150 rpm). The cells were grown to an optical density at 600 nm, OD_{600}, of 0.6-1.0, washed twice (sedimented 3428 rcf, 15 min, 4°C) to remove excess glucose and resuspended with LB medium (Ampicillin 50 µg/mL) prior to the addition of the corresponding inducer (IPTG **13**, IPG **15** and its TCO caged and CCO caged derivatives).

***Lac* operon dependent overexpression protocol for dsRed and GFP:** To obtain pET16b_GFP and pET16b_DsRed2_S4T constructs, DNA fragments encoding the fluorophores were amplified by PCR. Using this PCR reaction, DsRed2 was mutated to DsRed2_S4T, to enhance the fluorescent signal.[52] GFP was derived from ATCC construct 25922.[53] The resulting fragments were ligated into the pET16b vector using the NcoI and BamHI restriction sites. All sequences were verified by Sanger sequencing (Macrogen).

primer ID	sequence 5' → 3'
T7_GFP_fwd	GGCGGCCGTCTCCCATGAGTAAAGGAGAAGAAC
T7_GFP_rev	GGCGGCGGATCCTTATTTGTATAGTTCATCC
T7_DsRed2_S4T_fwd	GGCGGCCGTCTCCCATGGCCTCCACCGAGAACG
T7_DsRed2_rev	GGCGGCCGTCTCGGATCCTTTATCTAGATCCGGTGG

fwd: forward, rev: reverse

Both constructs were transformed into B834(DE3) expression strain and the protein was expressed from the overnight culture of a single colony. 5-10 mL of this overnight culture (Ampicillin 50 μg/mL, 1% Glucose v/v, 18 h, 37°C, and 150 rpm) was used for the inoculation per 50-100 mL LB medium (Ampicillin 50 μg/mL, 37°C, 150 rpm). The cells were grown to an optical density at 600 nm, OD_{600}, of 0.6-1.0, washed twice (sedimented 3428 rcf, 15 min, 4°C) to remove excess glucose and resuspended with LB medium (Ampicillin 50 μg/mL) prior to the addition of the corresponding inducer (IPTG **13**, IPG **15** and its TCO caged and CCO caged derivatives).

Experiment 1: Induction of expression with IPTG or IPG – Figure S2: Ovalbumin was expressed as described above. For the induction, IPTG (**13**) or IPG (**15**) were used at 1 mM final concentration (stock dissolved in water 0.1 M). Samples were taken before (t = 0 h) and after (t = 1, 2, 3, 4 h and overnight) the addition of the inducer, centrifuged and pellets were dissolved in 20 μL of H_2O and 10 μL of 2x sample loading buffer. 15 μL of sample was loaded to SDS gel and analyzed as described above.

Experiment 2: Impact of DMSO on expression – Figure S3: For the induction, IPG (**15**) was used at 1 mM final concentration with varying DMSO concentrations (0.1, 1, 5 and 10% v/v). Samples were taken before (t = 0 h) and after (t = 3 h and overnight) the addition of the inducer, centrifuged and pellets were dissolved in 30 μL of H_2O and 30 μL of 2x sample loading buffer. 15 μL of sample was loaded to SDS gel and analyzed as described above.

Experiment 3A: Inhibition of OVA expression with 3-CCO-IPG – Figure S4: To check the degree of inhibition, 3-CCO-IPG (**19**) was used at distinct concentrations varying from 1, 0.5, 0.25, 0.125 mM final concentration (stock dissolved in DMSO 0.1 M). Positive (1% v/v glucose, 1% v/v DMSO) and negative controls (1% v/v DMSO) were included. Samples were taken before (t = 0 h) and after (t = 4 h and overnight) the addition of the inducer, centrifuged and pellets were dissolved in 30 μL of H_2O and 30 μL of 2x sample loading buffer. 15 μL of sample was loaded to SDS gel and analyzed as described above.

Experiment 3B: Impact of caged 3-TCO-IPG on OVA expression – Figure S4: To determine the impact of caged 3-TCO-IPG (**25**) on OVA expression levels, standard expression protocol was used. 3-TCO-IPG (**25**) was then added at distinct concentrations varying from 1, 0.5, 0.25, 0.125 mM final concentration (stock dissolved in DMSO 0.1 M). Positive (1% v/v glucose, 1% v/v DMSO) and negative controls (1% v/v DMSO) were included. Samples were taken before (t = 0 h) and after (t = 4 h and overnight) the addition of the inducer, centrifuged and pellets were dissolved in 30 μL of H_2O and 30 μL of 2x sample loading buffer. 15 μL of sample was loaded to SDS gel and analyzed as described above.

Experiment 4: Impact of tetrazine 26 on expression – Figure S5: For the induction, IPG (**15**) was used at 1 mM final concentration with 3,6-dimethyl-tetrazine (**26**) to mimic uncaging conditions (2.5 mM final concentration in DMSO). Samples were taken before (t = 0 h) and after (t = 1, 2, 3, 4 h and overnight) the addition of the inducer, centrifuged and pellets were dissolved in 30 μL of H_2O and 30 μL of 2x sample loading buffer. 15 μL of sample was loaded to SDS gel and analyzed as described above.

Experiment 5: Temporal control of ovalbumin expression via decaging of 3-TCO-IPG (25) – Figure 2C-D: For the expression, general ovalbumin expression protocol outlined in this section was utilized. Four different samples of each 10 mL were induced as follows: To all samples 3-TCO-IPG (**25**) was added in 1 mM final concentration (in DMSO). First sample was directly reacted with 3,6-dimethyl-tetrazine (DMT, **26**; 2.5 mM final concentration in DMSO), second sample after 1 h of expression and third sample after 2 h. The fourth sample served as a control not containing any DMT. Samples were taken before (t = 0 h) and after (t = 1 h, 2 h, 3 h, 5 h and overnight) adding **25** and launching the experiment, centrifuged and pellets were dissolved in 30 µL of H_2O and 30 µL of 2x sample loading buffer. 10 µL of sample was loaded to SDS gel and analyzed as described above.

Experiment 6: Comparison of inhibitory levels of 3-CCO-IPG and 6-CCO-IPG – Figure 2-B: 3-CCO-IPG (**19**) and 6-CCO-IPG (**20**) were compared with respect to their degree of inhibition on ovalbumin expression. Both caged IPGs were used at a final concentration of 1 mM (stock dissolved in DMSO 0.1 M). IPG (**15**, 1 mM) and DMSO (1% v/v) were used as positive and negative control conditions, respectively. Samples were taken before (t = 0 h) and after (t = 2, 4 h and overnight) the addition of the conditions, centrifuged and pellets were dissolved in 30 µL of H_2O and 30 µL of 2x sample loading buffer. 15 µL of sample was loaded to SDS gel and analyzed as described above.

Experiment 7: Replicate expression experiments for OVA (Figure 3A): An overnight culture of B834(DE3) containing pMSCG7_Ova was diluted 1:100 in LB medium supplemented with 50 µg/mL ampicillin and 1% glucose. Cells were grown at 37°C, 180 rpm to an OD_{600} of ~0.6-1.0 and sedimented (3428 rcf, 10 min, 4°C) before being resuspended in LB medium containing 50 µg/mL ampicillin. Cultures of 3 mL were induced with either compound **25** (1 mM), followed by the addition of **26** (2.5 mM) at t = 1 h, compound **25** (1 mM), compound **15** (1 mM) or DMSO (vehicle control; 1% *v/v*), an uninduced sample was taken along as a true negative control. Samples were taken ((0.2 / OD_{600}) x 1000 µL) before (t = 0 h) and after (t = 1 h, 2 h, 3 h, 5 h and overnight) starting the experiment, centrifuged and pellets were dissolved in 50 µL of 1*Laemmli buffer supplemented with Benzonase (0.2 U/µL). Subsequently, dissolved samples were incubated for 5 min at 90°C for denaturation and briefly centrifuged. 10 µL of each sample was resolved over a 10% SDS-PAGE (0.75 mm) along with 10 µL PageRuler™ Plus Protein Marker (Thermo Scientific) for 70 min at 180 V. Coomassie staining (Coomassie Brilliant Blue G-250) was used for protein analysis and resulted in the graph (representing N = 3) shown in Figure 2E, using t = 0 h as the reference.

Experiment 8: Replicate expression experiments for eGFP (Figure 3B): An overnight culture of B834(DE3) containing pET16b_eGFP was diluted 1:100 in LB medium supplemented with 50 µg/mL ampicillin and 1% glucose. Cells were grown at 37°C, 180 rpm to an OD_{600} of ~0.6-1.0 and sedimented (3428 rcf, 10 min, 4°C) before being resuspended in LB medium containing 50 µg/mL ampicillin. Cultures of 3 mL were induced with either compound **25** (1 mM), followed by the addition of **26** (2.5 mM) at t = 1 h, compound **25** (1 mM), compound **15** (1 mM) or DMSO (vehicle control; 1% *v/v*), an uninduced sample was taken along as a true negative control. Samples were taken ((0.4 / OD_{600}) x 1000 µL) before (t = 0 h) and after (t = 1 h, 2 h, 3 h, 5 h and overnight)

starting the experiment, centrifuged and pellets were dissolved in 100 µL of 1*Laemmli buffer (without β-mercaptoethanol) supplemented with Benzonase (0.4 U/µL). Subsequently, dissolved samples were incubated for 5 min at 37°C and briefly centrifuged. 10 µL of each sample was resolved over a 10% SDS-PAGE (0.75 mm) along with 10 µL PageRuler™ Plus Protein Marker (Thermo Scientific) for 70 min at 180 V. Coomassie staining (Coomassie Brilliant Blue G-250) was used for protein analysis) after scanning Cy2, Cy3 and Cy5 multichannel settings (532/528, 605/50 and 695/55 filters, respectively; ChemiDoc™ MP System, Bio-Rad). This resulted in the graph (representing N = 4) shown in Figure 2F, using t = 0 h as the reference.

Experiment 9: Induction of dsRED2 expression with temporal chemical control – Figure S6: dsRed2 was cloned and expressed as described above. For the induction, optimal conditions from Experiment 5 were used (addition of **25** at t = 0 h and at 1.0 mM final concentration in DMSO; addition of DMT (**26**) after 1 h and at 2.5 mM final concentration in DMSO). Samples were taken before (t = 0 h) and after (t = 1 h, 2 h, 3 h, 5 h and overnight) the addition of **25**, centrifuged and pellets were dissolved in 30 µL of H_2O and 30 µL of 2x sample loading buffer. 15 µL of sample was loaded to SDS gel (10%) and analyzed as described above. In-gel fluorescence was measured at the wavelength filter for Alexa 555 (dsRed) prior to Coomassie staining.

6.8 References

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