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Enzymatically triggered chromogenic cross-linking agents under physiological conditions†

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The ability to cross-link molecules upon enzymatic action under physiological conditions holds considerable promise for use in diverse life sciences applications. Here, an enzymatically triggered "click reaction" has been developed by exploiting the longstanding indigo-forming reaction from indoxyl β-glucoside. The covalent cross-linking proceeds in aqueous solution, requires the presence only of an oxidant (*e.g.*, O_2), and is readily detectable owing to the blue color of the resulting indigoid dye. To achieve facile indigoid formation in the presence of a bioconjugatable tether, diverse indoxyl β-glucosides were synthesized and studied in enzyme assays with four glucosidases including from tritosomes (derived from hepatic lysosomes) and rat liver homogenates. Altogether 36 new compounds (including 15 target indoxyl-glucosides for enzymatic studies) were prepared and fully characterized in pursuit of four essential requirements: enzyme triggering, facile subsequent indigoid dye formation, bioconjugatability, and synthetic accessibility. The 4,6-dibromo motif in a 5-alkoxy-substituted indoxyl-glucoside was a key design feature for fast and high-yielding indigoid dye formation. Two attractive molecular designs include (1) an indoxyl-glucoside linked to a bicyclo[6.1.0]nonyl (BCN) group for Cu-free click chemistry, and (2) a bis(indoxyl-glucoside). In both cases the linker between the reactive moieties is composed of two short PEG groups and a central triazine derivatized with a sulfobetaine moiety for water solubilization. Glucosidase treatment of the bis(indoxyl-glucoside) in aqueous solution gave oligomers that were characterized by absorption, dynamic light-scattering, and ^{1}H NMR spectroscopy; optical microscopy; mass spectrometry; and HPLC. Key attractions of *in situ* indigoid dye formation, beyond enzymatic triggering under physiological conditions without exogenous catalysts or reagents, are the chromogenic readout and compatibility with attachment to diverse molecules.

Introduction

Enzyme-triggered processes, where a spontaneous chemical event follows enzymatic action, are of great interest in the life sciences, particularly for diagnostic and therapeutic applications.[1–4] As a general strategy, a molecule to be released (**A**) upon action by the target enzyme is protected with a covalently attached enzyme-cleavable protecting group (**A**-PG) (Fig. 1). The molecules of **A** so-liberated can undergo myriad processes ranging from formation of dyes to assembly of nanostructures. A prevalent but less germane example in the context of the theme reported herein is enzyme–prodrug therapy, where **A**-PG constitutes a prodrug and the released molecule **A** binds to a receptor or inhibits a different enzyme.

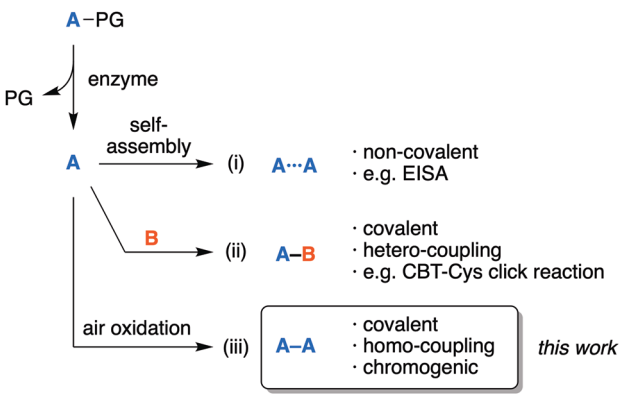

Fig. 1 Enzyme-triggered reactions of molecule **A** bearing a protecting group PG (**A**-PG). (i) Non-covalent self-assembly of **A**. (ii) Covalent hetero-coupling of **A** with acceptor **B**. (iii) Covalent homo-coupling of **A** in the presence of O_2 (this work).

The formation of dyes entails covalent – and typically intramolecular – reaction upon enzymatic removal of one or

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† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for all new compounds; single-crystal X-ray data; and further studies and data concerning indigogenic reactions. CCDC 1902488 (**18**) and 1902489 (**17**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c9nj04126e

more protecting groups.[5,6] The dye-forming reactions are members of a broad class of covalent self-assembly processes.[7] The assembly of nanostructures entails intermolecular processes that can rely on covalent or non-covalent interactions.[4] For example, in a process sometimes referred to as enzyme-instructed self-assembly (EISA),[8] a peptide cleaved by an enzyme assembles upon hydrophobic and hydrogen-bonding interactions to form a hydrogel or nanostructure [Fig. 1(i)].[9,10] In reverse, enzymatic cleavage of large structures can cause disassembly, which has been exploited to release constituent drugs as part of novel delivery processes.[10,11] Enzymes are widely used to create covalent cross-links yielding hydrogels and other soft matter; the reactions are largely done *in vitro* whereas the resulting materials are used *in vivo*,[12–15] but some processes of enzymatic cleavage followed by non-covalent self-assembly have been performed entirely *in vivo*.[10] An example of intermolecular covalent bond formation *in vivo* is provided by the cyanobenzothiazole–cysteine (CBT–Cys) click reaction,[16–18] where an enzymatically deprotected cysteine (**A**) reacts with CBT (**B**) [Fig. 1(ii)]. Structures that have been prepared using compounds bearing cysteine and CBT moieties include nanorings formed by covalent oligomerization followed by non-covalent self-assembly,[19] and nanocrystals immobilized by cross-linking.[20] Such enzyme-triggered intermolecular covalent-bond forming reactions remain rare (*versus* more known intramolecular reactions; *e.g.*, ref. 5, 6, 21 and 22); the dearth is even more surprising given extensive development of click chemistry as a potent means for bioconjugation.[23]

A far older example of enzyme-triggered covalent bond formation is the natural formation of indigo. Indoxyl-glucoside (**1**, also known as indican) upon action of a glycosidase yields indoxyl (**2**); subsequent enol–keto tautomerism affords indoline (**3**), which in the presence of air undergoes homo-coupling to give indigo (**4**) (Fig. 2).[24] Indigo is quite insoluble in water and typically precipitates upon formation in aqueous solution. This chemistry has been exploited to dye textiles dating from antiquity to the present day "blue jeans".[25,26] The homo-coupling of two molecules of **A** to afford indigo (**A–A**) occurs without an acceptor (**B**), and is irreversible owing to the oxidation process [Fig. 1(iii)]. The conversion of indoxyl-glucoside to indigo is accompanied by profound changes in the absorption spectrum, which conveniently facilitates quantitation.

The attractive features of indigo formation have led to applications far beyond textile chemistry, namely in histochemistry and enzyme assays for nearly three quarters of a century.[27,28] Indigo is very water-insoluble and precipitates at or near the site of formation, enabling histological localization of the glucosidase enzymes. Cotson and Holt studied the kinetics of indigoid-forming reactions from diverse indoxyl compounds including those bearing single bromine atoms at the 4–7 positions.[29] The bromoindoxyl compounds formed the corresponding indigoid dye 2–4 times faster than the unsubstituted indoxyl species (*e.g.*, **5** → **6**, Fig. 3). The 6,6′-dibromoindigo dates to antiquity as the regal natural pigment Tyrian purple (**7**).[30] The attractive features of indigoid dye formation thus include enzymatic triggering, chromogenicity, insoluble deposition from aqueous solution at the site of reaction, and reaction under physiological conditions. Histological and bacteriological use has been extended to include indoxyl derivatives bearing enzymatically cleavable substituents other than glucosides, including glucuronides, carboxylic esters, phosphoesters, phosphodiesters, and sulfoesters.[27] Development and use of indoxyl-glucosides in the life sciences continue unabated.[31–35] Enzymatically triggered release of substituted indoxyl compounds has been described[36–42] for molecular cross-linking and immobilization as part of a novel cancer therapy,[43] yet other than an initial set of data[39–42] obtained more than a decade ago, this promising indigoid cross-linking chemistry has remained fallow. Thus, the venerable history of indigoid chemistry and incisive use in histochemistry notwithstanding, indoxyl species have been little explored as cross-linking agents for biomolecules *in vitro* or *in vivo*.

In this paper, we describe results from lengthy studies aimed at developing indoxyl-based chromogenic covalent cross-linking agents of the general design illustrated in Fig. 4. We anticipated reasonably straightforward molecular designs but were surprised to find that the linkers we employed for attaching a bioconjugatable tether thwarted indigoid dye formation upon enzymatic cleavage of the indoxyl-glucoside. Hence, a first set of studies entailed syntheses of diverse indoxyl β-glucosides to identify the structural features compatible with facile indigoid dye formation while bearing a

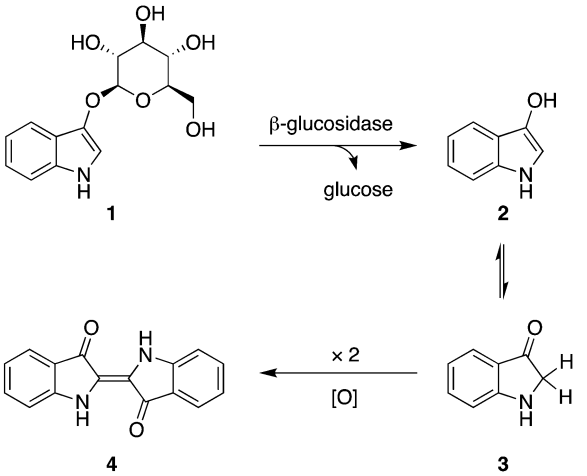


Fig. 2 Formation of indigo (**4**) from indoxyl β-glucoside (**1**) *via* indoxyl (**2**) and tautomer (**3**).

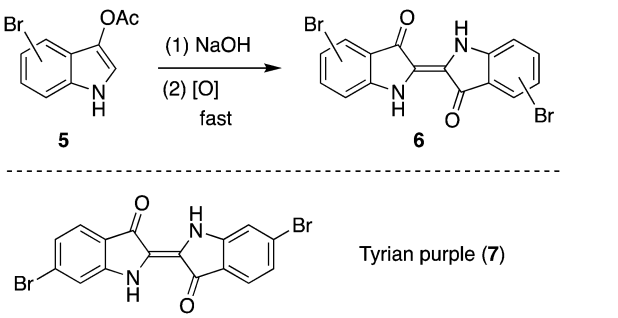


Fig. 3 Fast indigoid dye formation of bromoindoxyl compounds (top) and the famous pigment Tyrian purple (**7**).

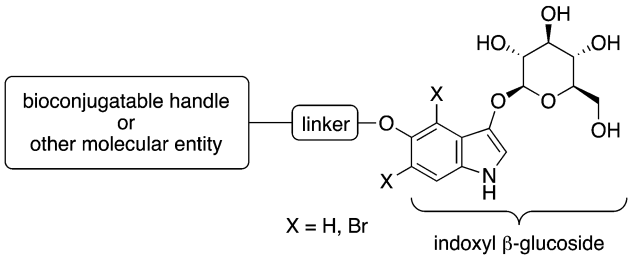


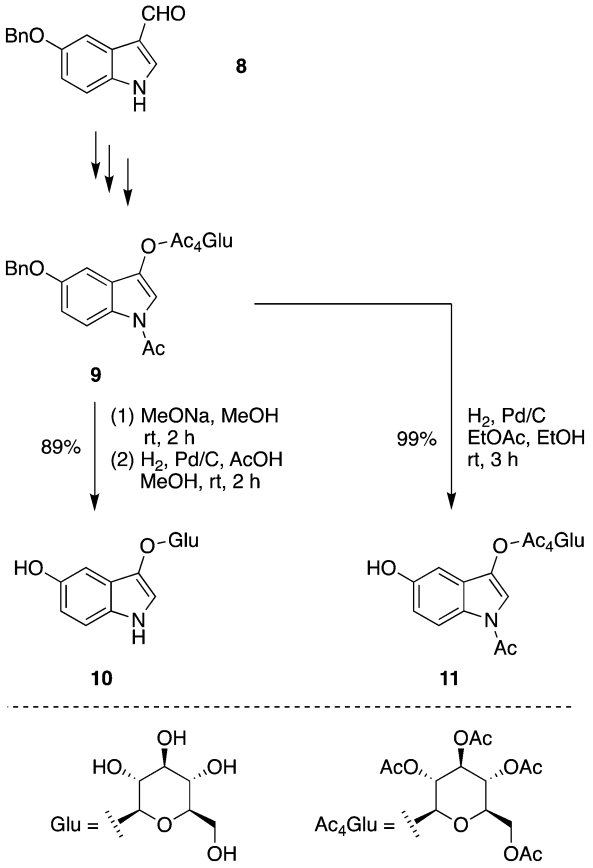
Fig. 4 General structure of indoxyl β-glucosides linked to a chemical/bioconjugatable handle.

bioconjugatable tether. Next, each structure was examined for indigoid dye formation upon treatment to several enzymatic conditions including β-glucosidases, tritosomes, and rat liver homogenates; from these studies, the 5-alkoxy-4,6-dibromoindoxyl nucleus was found to give superior results. Finally, oligomerization *via* the indigoid dye-forming reaction under physiological conditions was explored to understand the fundamental properties of this cross-linking moiety. This work establishes part of the scientific foundation for the longer-term objective wherein the enzymatically triggerable cross-linking agents yield macromolecular scaffolds for immobilization of molecular constituents *in vivo*.

Results and discussion

1. Synthesis of monomeric indoxyl species

The commercially available 5-benzyloxy-3-formylindole (**8**) provided the sole indole starting material for all new synthetic indoxyl-glucosides described herein. Compound **8** was converted in 3 steps to the fully protected 5-hydroxyindoxyl β-glucoside **9** (Scheme 1) in accord with published protocols.[39] Deprotection of the acetyl and benzyl groups of **9** provided 5-hydroxyindoxyl β-glucoside **10** in 89% yield, while debenzylation of **9** afforded acetyl-protected 5-hydroxyindoxyl β-glucoside **11** in 99% yield.

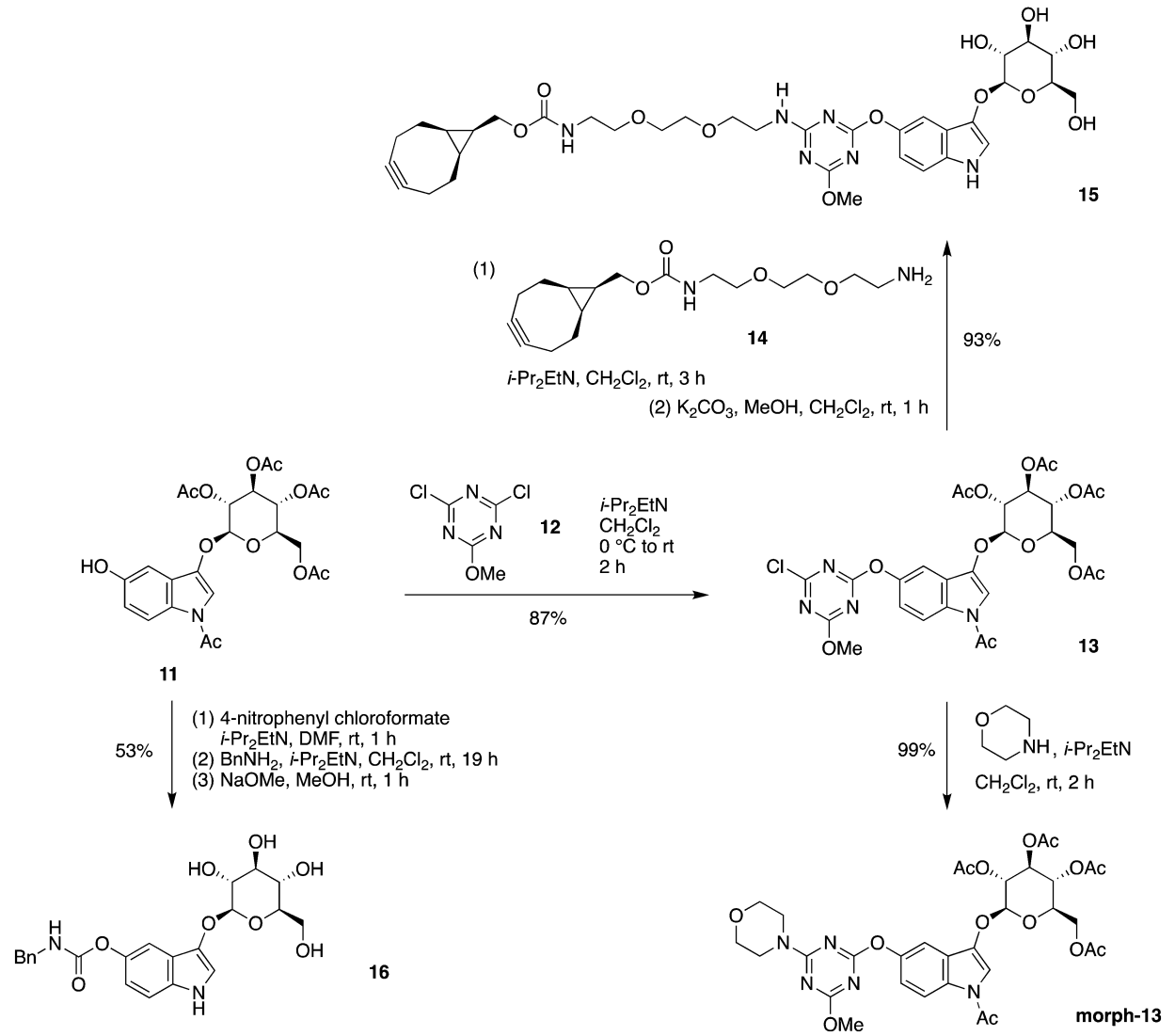


Scheme 1 Synthesis of 5-hydroxyindoxy-glucosides.

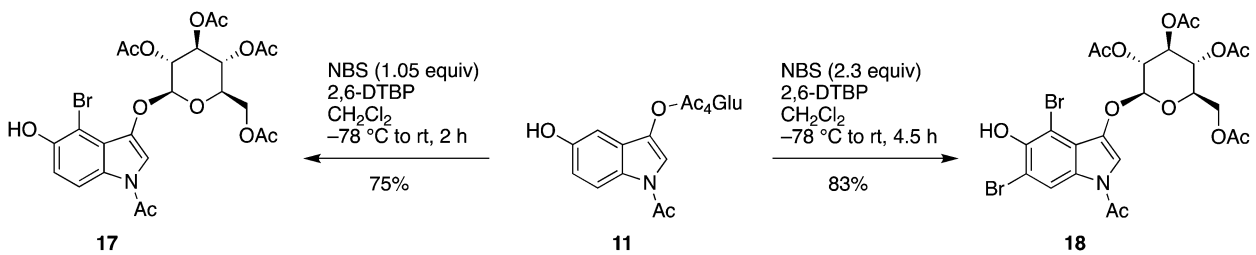
1,3,5-Triazine[44,45] and carbamate linkers were selected to derivatize the phenolic hydroxy group in **11** (Scheme 2). Thus, treatment of **11** with 2,4-dichloro-6-methoxy-1,3,5-triazine (**12**)[46] replaced one of the two chlorine atoms to form chlorotriazine **13** in 87% yield. The remaining chloride was substituted upon pilot reaction with morpholine to give **morph-13**, which retains all of the acetyl protecting groups, in 99% yield. Reaction of **13** with heterotelechelic linker **14**, which bears an amine on one terminus and a bicyclo[6.1.0]nonyl (BCN) group[47] for Cu-free click chemistry[23] on the other, was followed by acetyl removal to give the BCN-tethered indoxyl-glucoside **15** in 93% yield (Scheme 2). Treatment of **11** with *p*-nitrophenyl chloroformate afforded a carbonate intermediate, which upon reaction with benzylamine gave the carbamate. Reaction with NaOMe caused removal of the acetyl groups to give carbamate **16** in 53% yield.

In initial studies with β-glucosidase from almonds, neither **15** nor **16** afforded the corresponding indigoid species in good yield. It appeared that the alkoxy group, necessary for later bioconjugation, inhibited the indigogenic process. Thus, bromine atoms were introduced onto the indole ring to overcome the inhibitory effect of the alkoxy group (Scheme 3). Treatment of **11** with *N*-bromosuccinimide (NBS, 1.05 equiv.) afforded 4-bromo-indoxyl-glucoside **17** in 75% yield, whereas a larger quantity of NBS (2.3 equiv.) gave 4,6-dibromoindoxyl-glucoside **18** in 83% yield. The reactions were carried out in the presence of 2,6-di-*tert*-butylpyridine (2,6-DTBP), a non-nucleophilic base for proton scavenging.[48] Single-crystal X-ray structures of **17** and **18** confirmed the sugar stereochemistry and the positions of the bromine atoms (Fig. 5).

Sets of 4,6-unsubstituted, 4-bromo, and 4,6-dibromo indoxyl-glucosides wherein a linker is present at the 5-position were prepared from **10**, **11**, **17**, and **18** (Scheme 4). As indoxyl-glucosides (and the corresponding indigoid dyes) bearing 5-oxy (*e.g.*, hydroxy, alkoxy, aryloxy) substituents and bromine atoms have not been reported (although individually substituted examples are known), we compared the indigogenic reactions among these indoxyl-glucosides to investigate the effects of the bromine substituents. The triazine linker was introduced into indoxyl-glucosides **10**, **17**, and **18** *via* the successive substitution of the chlorine atoms in dichlorotriazine **12**. The reaction of **10** with triazine **12** was carried out in DMF rather than the preferred dichloromethane (used for **17** and **18**) owing to solubility limitations. Thus, 4,6-unsubstituted indoxyl-glucoside **10** was treated with **12** followed by morpholine and K_2CO_3/methanol to afford triazine derivative **19** in 59% yield. In a stepwise process, acetyl-protected 4-bromoindoxyl-glucoside **17** was reacted with **12** followed by morpholine to afford the peracetylated triazine



Scheme 2 Synthesis of 4,6-unsubstituted indoxyl compounds bearing the triazine or carbamate linker.



Scheme 3 Synthesis of 4-bromo and 4,6-dibromo substituted indoxyl-glucosides.

derivative **pre-20** (not shown) in 87% yield; subsequent treatment with K_2CO_3/methanol removed all five acetyl groups yielding the triazine **20** in 89% yield (77% overall). The reaction of acetyl-protected **18** and **12** gave 4,6-dibromoindoxyl-glucoside **21** in 67% yield.

Indoxyl-glucosides **22–24** possess a methoxycarbonyl group, which can function as an amine-reactive linker. This linker was introduced by alkylation of the 5-hydroxy group in **11**, **17**, and **18** with ethyl bromoacetate in the presence of NaH and subsequent treatment with NaOMe in MeOH. The reaction of **10** with propargyl bromide in the presence of K_2CO_3 afforded 4,6-unsubstituted indoxyl-glucoside **25** in 30% yield, which bears a propargyl group for ensuing click chemistry. Propargylation of acetyl-protected 4-bromo and 4,6-dibromo indoxyl-glucosides **17** and **18** followed by deacetylation with MeONa in MeOH provided the corresponding **26** and **27** in 84 and 89% yield, respectively.

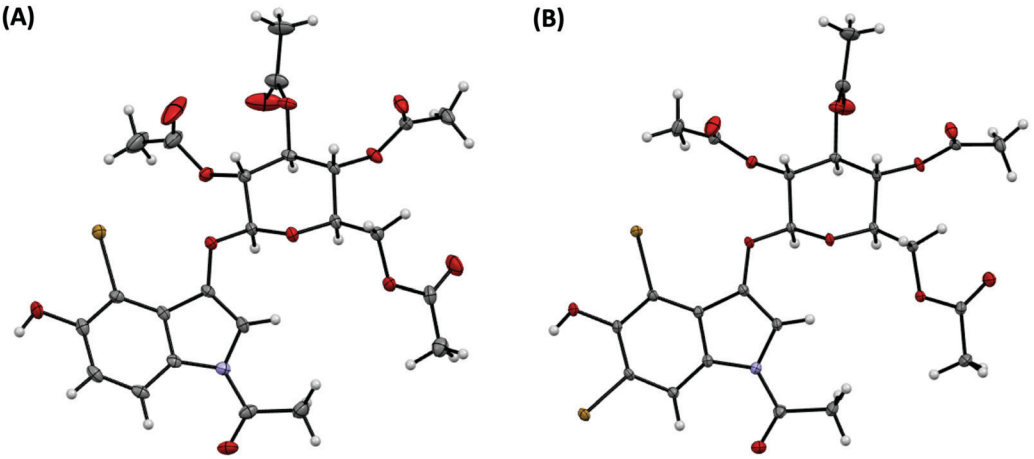
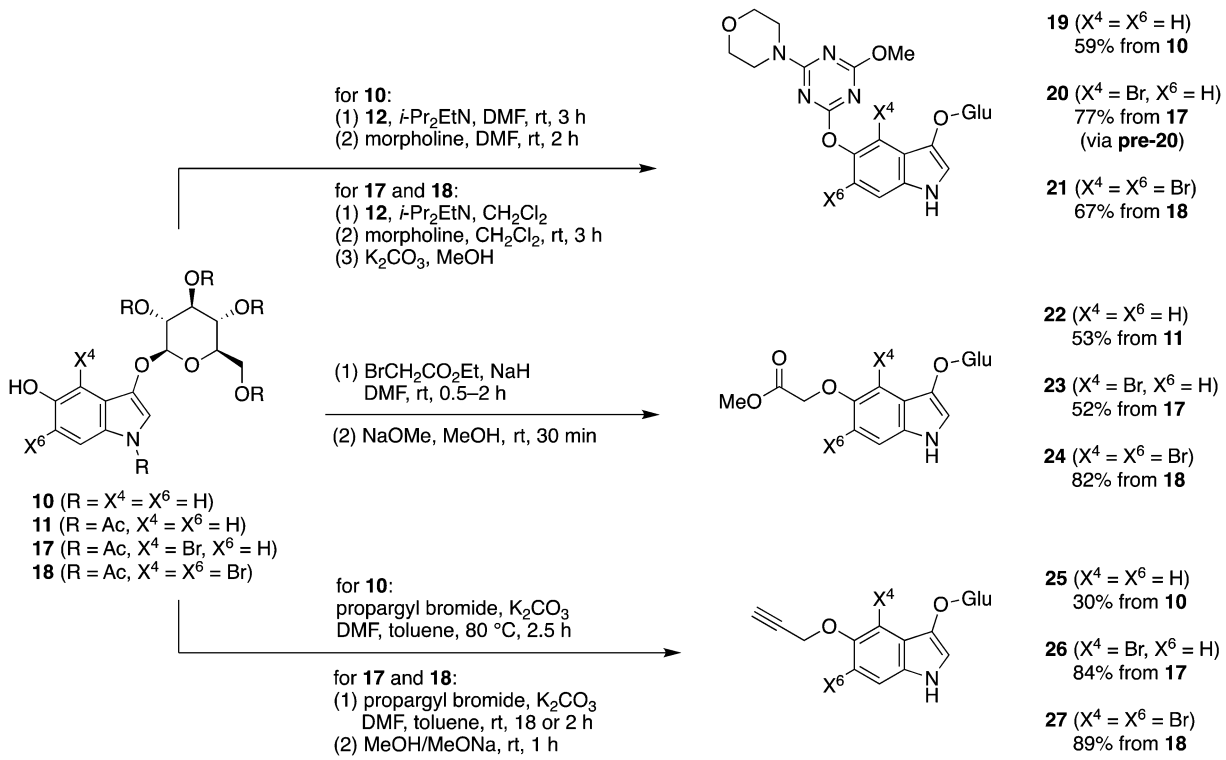


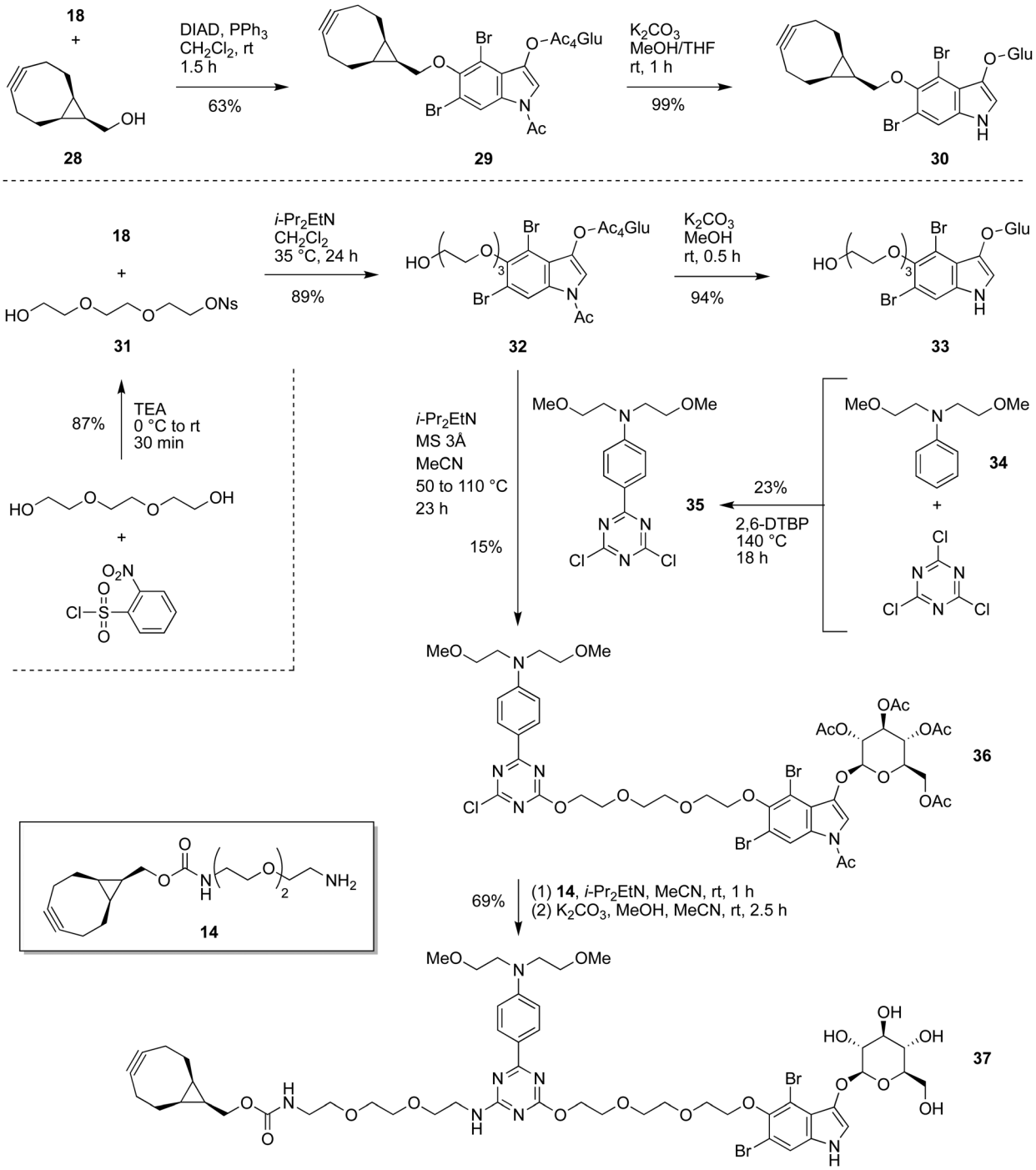
Fig. 5 ORTEP drawing of the single-crystal X-ray structures of (A) **17** and (B) **18**. All ellipsoids are contoured at the 50% probability level.



Scheme 4 Synthesis of 4,6-unsubstituted, 4-bromo, and 4,6-dibromo indoxyl-glucosides, each bearing a linker at the 5-position.

4,6-Dibromoindoxyl-glucoside **30**, which possesses the BCN group instead of the propargyl group in **27**, was prepared from **18** (Scheme 5). The Mitsunobu reaction of **18** and commercially available BCN–methanol **28** in the presence of diisopropyl diazodicarboxylate (DIAD) and Ph_3P gave **29** in 63% yield. Deacetylation of **29** with K_2CO_3/MeOH afforded **30** in 99% yield.

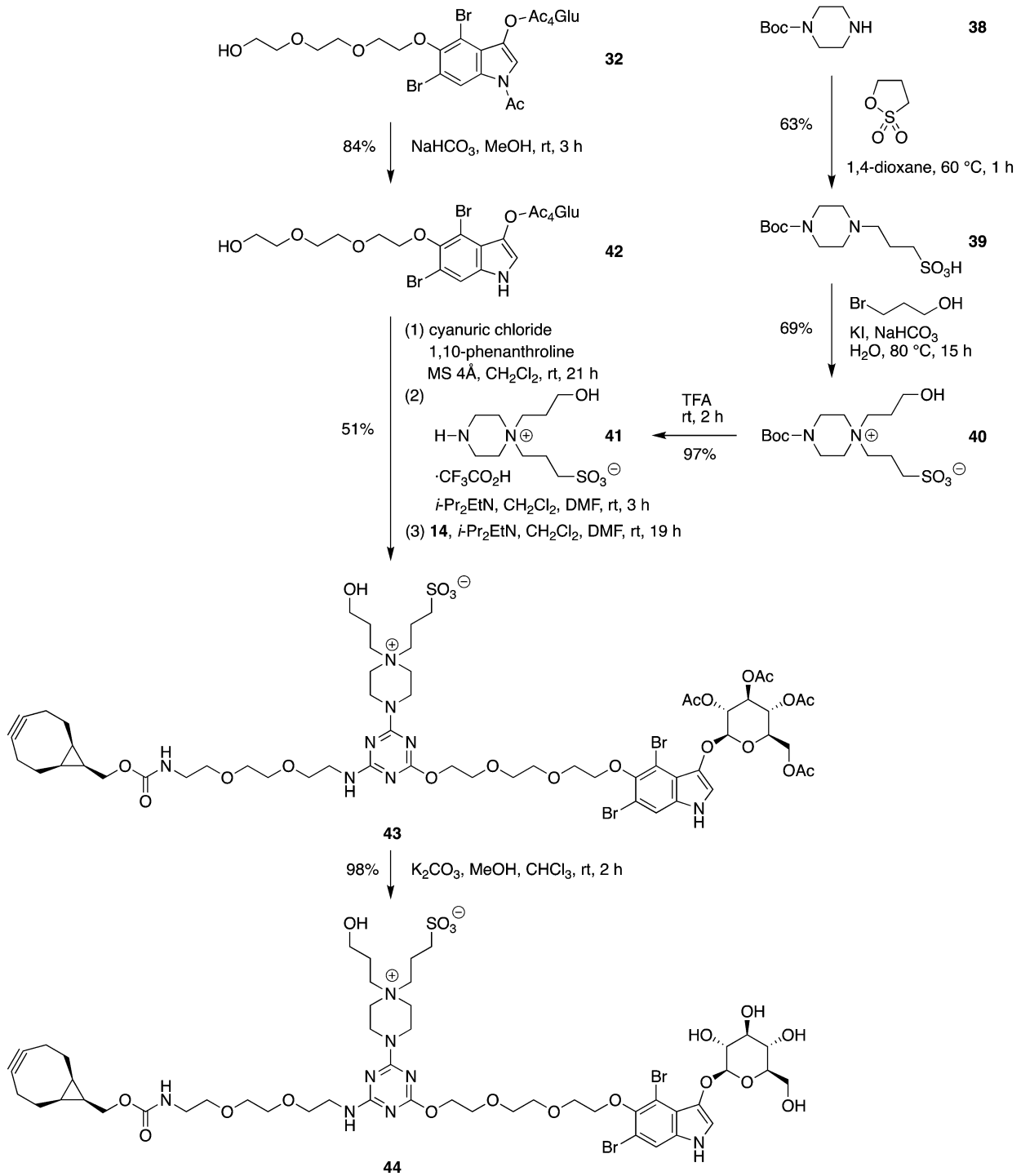
To install a long spacer, the reaction of 2-nitrobenzenesulfonyl chloride with a 20-fold excess of triethylene glycol in the presence of triethylamine afforded triethylene glycol mononosylate (**31**). Treatment of **31** with 4,6-dibromoindoxyl-glucoside **18** gave **32** bearing the triethylene glycol linker in 89% yield. The nosylate was chosen to provide a superb leaving group for reaction with the rather hindered phenol, wherein mild conditions could be employed to avoid cleavage of the base-labile *N*-acetyl protecting group and ensuing *N*-alkylation. Deprotection gave the 4,6-dibromoindoxyl-glucoside **33** in 94% yield. To further extend the triethylene glycol linker, *N*,*N*-bis(2-methoxyethyl)aniline (**34**)[49] was reacted with cyanuric chloride at elevated temperature in the presence of 2,6-DTBP, affording the *p*-anilino-substituted dichlorotriazine building block **35** in 23% yield. Here, the aniline and cyanuric chloride partners undergo respective S_EAr and S_NAr processes with each other. The reaction of **35** and indoxyl-glucoside–PEG-OH **32** gave the mono-chloro triazinyl product **36** in 15% yield. Anilinotriazines bearing amino/chloro or diamino



Scheme 5 Synthesis of 4,6-dibromoindoxyl-glucosides bearing the bicyclononyne or triethylene glycol substituent.

substituents are known to be fluorescent,[50,51] which provides a convenient marker for detection. The reaction of **36** with BCN–PEG-NH_2 (**14**) gave the 4,6-dibromoindoxyl-glucoside **37**, which contains the BCN group along with the fluorescent anilinotriazine moiety. Compound **37** exhibited limited solubility in aqueous buffers (<10 μM at room temperature) despite the presence of the glucoside and PEG units, and was deemed unsuitable for bioconjugation.

To improve the water solubility of the indoxyl-glucoside species, a sulfobetaine unit[52,53] was incorporated as a water-solubilizing group. Sulfobetaines are stable zwitterions over a wide range of pH. Synthesis of an indoxyl-glucoside bearing a sulfobetaine unit is illustrated in Scheme 6. *N*-Boc-piperazine (**38**) was treated with 1,3-propanesultone to afford **39** in 63% yield. Quaternization of the tertiary nitrogen atom in **39** with 3-bromopropanol gave Boc-protected sulfobetaine **40** in 69% yield. The Boc group was cleaved with trifluoroacetic acid (TFA) to afford piperazine-TFA salt **41** in 97% yield. Examination of **41** by ^{13}C NMR spectroscopy revealed a strong peak at δ = 163.1 ppm consistent



Scheme 6 Synthesis of a BCN-dibromoindoxyl-glucoside equipped with a water-solubilizing group.

with the carbonyl of the TFA anion; however, the expected companion quartet due to the trifluoromethyl carbon was not evident. Further examination by ^{19}F NMR spectroscopy gave a strong peak at $\delta = -76.9$ ppm, consistent with the presence of a TFA species. A series of small peaks in the ^{1}H NMR spectrum of **41** likely stem from a *tert*-butyl-related impurity present at the few percent level; still, the sample was estimated to be 95% pure and was used as is in subsequent reactions. The *N*-acetyl group of **32** was selectively deprotected with $NaHCO_3$/MeOH in 84% yield to afford **42**.

The traditional method for selective successive substitution of cyanuric chloride relies on temperature control – for example, reaction with the first nucleophile at <0 °C, the second at room temperature, and the third at ≥ 60 °C.[44,45] Here, the piperazine-TFA salt **41**, triethylene glycol-substituted indoxyl-glucoside **42**, and BCN-amine **14** were assembled at a triazine ring *via* one-flask,

successive substitution of cyanuric chloride entirely at room temperature to afford **43** in 51% yield. Rather than rely on temperature variation to control substitution, the base 1,10-phenanthroline[54] was employed to facilitate mono-substitution of cyanuric chloride with the alcohol **42**, whereas the stronger base *N*,*N*-diisopropylethylamine was used for the second and third substitutions with secondary amine **41** and primary amine **14**, respectively. Cleavage of the acetyl groups of sulfobetaine **43** provided **44** in 98% yield. Owing to the sulfobetaine unit, **44** showed superior solubility (>400 μM at room temperature) *versus* **37** in a 100 mM sodium phosphate (NaPi) buffer (pH 7.4) containing 100 mM NaCl. Purification of **43** and **44** was achieved in part by hydrophilic interaction chromatography on diol-functionalized silica, which affords intermediate polarity between bare silica and reverse phase derivatized silica.[55] In each of the triazine compounds that contain the BCN unit (**15**, **37**, **43**, **44**), examination by NMR spectroscopy revealed a number of duplicate peaks. The duplicate peaks are attributed to rotamers that arise from constrained rotation about the triazinyl carbon–nitrogen bond to which the BCN–PEG unit is attached.

With diverse indoxyl-glucoside compounds in hand, we carried out a set of studies to examine indigoid-dye formation upon enzymatic cleavage of the glucosyl unit under physiological conditions. Altogether, 14 new (**15**, **16**, **19–27**, **30**, **33**, **44**) and 2 known (**1**, **45**) synthetic indoxyl-glucosides (lacking acetyl protecting groups) were examined in an effort to identify suitable combinations of substituents to support both bioconjugation and indigoid dye formation. Ultimately, enzymes from several sources – β-glucosidase from almonds or from *Agrobacterium* sp.; tritosomes; and rat liver homogenate – were employed in corresponding buffers or media (see the Experimental section).

2. Indigogenic studies of monomeric indoxyl species

In initial studies, β-glucosidase from almonds was employed to trigger indigoid dye formation (Table 1). Thus, a mixture of this enzyme (1 unit per mL) and an indoxyl β-glucoside (0.1 μmol, 1 mM) in sodium acetate buffer (pH 5.0) containing 5% DMF was incubated at 37 °C for 16–19 h. All indigoid dye was dissolved (*vide infra*) in each case for quantitative evaluation. The parent indoxyl-glucoside (**1**) afforded indigo only in 17% yield under the reaction conditions (entry 1). By contrast, 5-bromo-4-chloroindoxyl β-glucoside (**45**, also known as X-Glu in a chromogenic assay for β-glucosides)[27] provided the corresponding indigoid dye in 74% yield (entry 2, yield calculated based on $\varepsilon = 2.00 \times 10^4\ M^{-1}\ cm^{-1}$ reported for 5,5′-dibromo-4,4′-dichloroindigo).[56] These results are consistent with Holt's report that such halo substituents on the indoxyl nucleus facilitated indigoid dye formation. The time course of the spectral changes for substrates **1** and **45** are shown in Fig. S1 (ESI†).

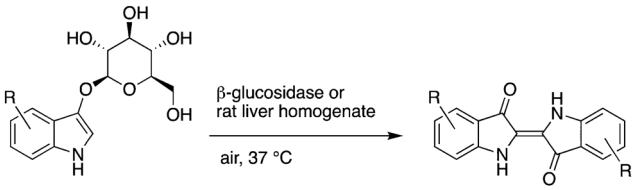
No indigoid dye was detected with **15** (entry 3), whereas **16** formed the corresponding indigoid dye, albeit in low yield (24%, entry 4). We measured the molar absorption coefficient of the parent indigo **4** in DMF/water (2 : 1) and found the value at λ_{max} near 600 nm to be $\varepsilon = 1.27 \times 10^4\ M^{-1}\ cm^{-1}$, to be compared with $\varepsilon = 1.66 \times 10^4\ M^{-1}\ cm^{-1}$ in 1,1,2,2-tetrachloroethane reported by Holt and Sadler.[56] For consistency, we have used the value in DMF/water (2 : 1) for all studies here unless noted otherwise.

Indoxyl-glucosides **19–21** bearing the triazine linker did not form the indigoid dye regardless of the presence or absence of a bromine atom (entries 5–7, respectively). In the case of 5-[(methoxycarbonyl)methoxy]indoxyl-glucosides **22–24**, the yield of indigoid dye was markedly improved as the number of bromine atoms increased (entry 8, **22**, <1%; entry 9, **23**, 68%; entry 10, **24**, 122% yield). The same trend was observed for 5-(propargyloxy)-indoxyl-glucosides **25–27** (entry 11, **25**, <5%; entry 12, **26**, 56%; entry 13, **27**, 105% yield). The calculated yields in excess of 100% stem from the value employed for the molar absorption coefficient. These results indicated a significant promoting effect of the bromine atoms on indigoid dye formation. No indigoid product was detected with BCN-indoxyl-glucoside **30** (entry 14) whereas PEG_3-indoxyl-glucoside **33** afforded indigoid dye in 52% yield (entry 15). In summary, the nature of the 5-substituent significantly affected the indigoid-forming reaction: **20**, **21**, and **30** did not afford any indigoid product regardless of the presence of bromine atoms. This may be because these substrates have low affinity for the enzyme due to the presence of the bulky triazine or BCN moiety.

The results from the glucosidase survey prompted several further experiments. First, a parallel set of studies was carried out with inclusion of several oxidants commonly employed in histochemical studies, given that the indigogenic process requires the presence of an oxidant. No substantial increase in yield was observed for the substrates shown in entries 1–15 of Table 1; the results are shown in Fig. S2 and Table S1 (ESI†). Also, the same set of substrates was examined with tritosomes (lysosomes isolated by loading with a non-ionic detergent) but the results were uniformly poor, although a low yield of indigoid dye was obtained from **16** and **19** (Table S1, ESI†). The activity of the β-glucosidase (from almonds) was affected only slightly in the presence of a non-ionic detergent (Fig. S3, ESI†). To verify that the results observed in Table 1 were reliable, a 5 mg scale reaction of **33** was carried out to isolate indigoid derivative **46**, which was obtained in 66% yield (Scheme 7). The 66% isolated yield corresponded well with the yield of 52% determined by absorption spectroscopy using the molar absorption coefficient for **46** (Table 1, entry 15).

Next, the β-glucosidase from *Agrobacterium* sp. was investigated as the trigger enzyme for indigoid dye formation. In contrast to β-glucosidase from almonds, which works chiefly under acidic conditions[57] (optimum pH 5.6),[58] β-glucosidase from *Agrobacterium* has a neutral pH optimum and maintains partial activity under acidic (pH 4–5) and basic (pH 8–9) conditions as determined by the measured rate of hydrolysis of 4-nitrophenyl β-D-glucopyranoside (Fig. S4, ESI†).[59] Given that the indigoid dye-forming reaction is reported to be faster at a basic rather than an acidic pH,[29,60] the pH effect on indigogenesis was studied with the β-glucosidase from *Agrobacterium*. The reaction was carried out using the enzyme (200 nM) and indoxyl-glucoside **33** (100 μM) in NaPi buffers (pH 4–9) containing 2% DMF at 37 °C. The progress of indigoid dye formation as a function of pH is illustrated in Fig. 6A. High to quantitative yields were attained in 3 h at pH 6–9; within

Table 1 Indigogenic reactions of diverse indoxyl-glucosides



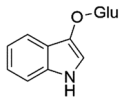
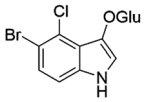
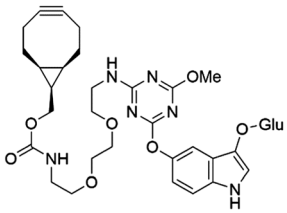
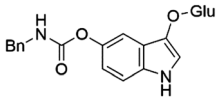
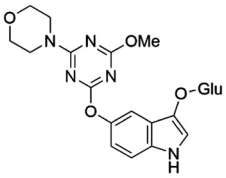
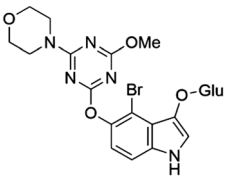
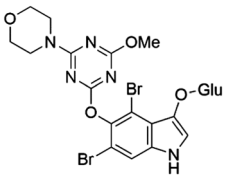
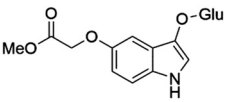
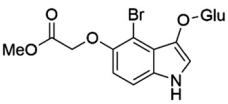
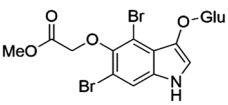
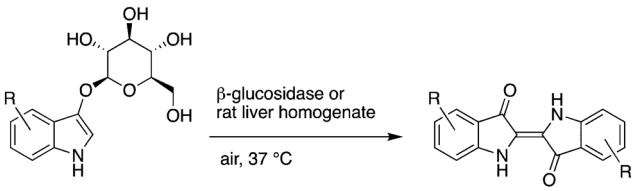
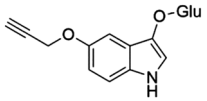
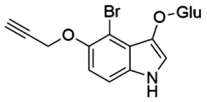
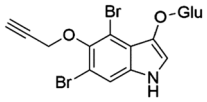
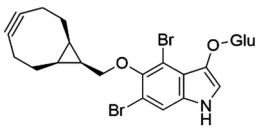
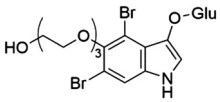
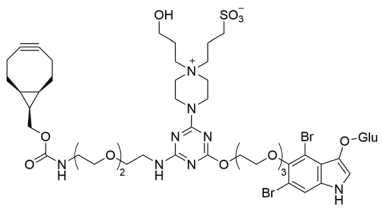
Entry	Indoxyl-glucoside	Structure	Yield[a] (%) β-Glucosidase from almonds[b]	β-Glucosidase from *Agrobacterium*[c]	Rat liver homogenate[d]
1	**1**		17	97 ± 5	10
2	**45**		74[e]	116 ± 8[e]	65
3	**15**		<1	—[f]	—[f]
4	**16**		24	—[f]	9
5	**19**		<1	46 ± 5	<5
6	**20**		<1	150 ± 3	<5
7	**21**		<1	63 ± 4 (31 ± 2)[g]	<5
8	**22**		<1	30 ± 6	<5
9	**23**		68	84 ± 4	<5
10	**24**		122 (59)[g]	209 ± 6 (102 ± 3)[g]	<5

Table 1 (*continued*)

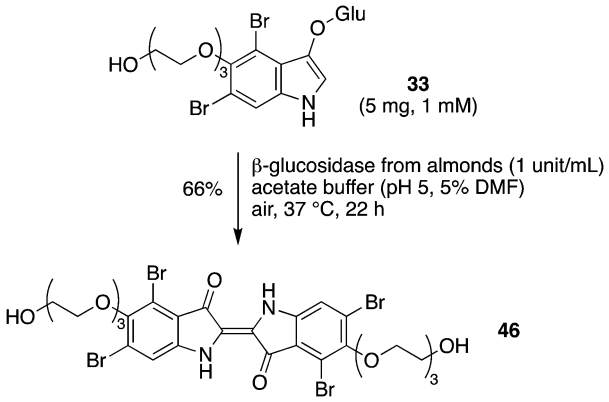


Entry	Indoxyl-glucoside	Structure	Yield[a] (%) β-Glucosidase from almonds[b]	β-Glucosidase from *Agrobacterium*[c]	Rat liver homogenate[d]
11	**25**		<5	37 ± 2	11
12	**26**		56	79 ± 1	17
13	**27**		105 (51)[g]	184 ± 3 (89 ± 2)[g]	50
14	**30**		<1	21 ± 1 (10 ± 0.4)[g]	—[f]
15	**33**		52[g]	99 ± 5[g]	<5[g] 81 ± 5[g,h]
16	**44**		—[f]	106 ± 4[g]	—[f]

[a] The yield was estimated by absorption spectroscopy with $\varepsilon = 1.27 \times 10^4$ M^{-1} cm^{-1} (DMF/H_2O = 2 : 1) measured for **4** (see the ESI) unless otherwise noted. A yield of <1% implies no color was observed by visual inspection and no absorption was observed spectroscopically. A yield of <5% implies a faint blue color was observed by visual inspection but the absorption was too weak for reliable spectroscopic determination. [b] A mixture of the indoxyl-glucoside (1 mM) and β-glucosidase from almonds (1 unit per mL) in acetate buffer (pH 5.0, sodium acetate, 50 mM, containing 5% DMF) was incubated at 37 °C for 16–19 h. [c] A mixture of the indoxyl-glucoside (100 μM) and β-glucosidase from *Agrobacterium* (200 nM) was incubated in 50 mM NaPi buffer (pH 7.0) containing 2% DMF at 37 °C for 2 h. The reaction was repeated three times. [d] The indoxyl-glucoside (1 mM) in rat liver homogenate containing 5% DMF was incubated at 37 °C for 24 h. [e] The yield was estimated from absorption spectroscopy with $\varepsilon = 2.00 \times 10^4$ M^{-1} cm^{-1} reported for 5,5′-dibromo-4,4′-dichloroindigo.[56] [f] Not conducted. [g] The yield was estimated from absorption spectroscopy with $\varepsilon = 2.6 \times 10^4$ M^{-1} cm^{-1} (DMF/H_2O = 2 : 1) measured for **46**. [h] The reaction was carried out with **33** (100 μM) and β-glucosidase from *Agrobacterium* (200 nM) plus rat liver homogenate containing 2% DMF at 37 °C for 4 h.

this range, pH 7 provided the best result. The reactions at pH 4 and 5 were also nearly complete in 3 h, although the yields were lower: 6% and 38%, respectively.

The yields of indigoid dye formation from **33** with different enzyme concentrations (200 *versus* 10 nM) at 2 h are shown in Fig. 6B (see the Experimental section and ESI† for information about buffers). Good yields (50–71%) for oxidative dimerization obtained at pH 6–8 with 10 nM enzyme suggested that enzymatic cleavage of the sugar of **33** was not the rate-limiting step in indigoid dye formation. A relatively large decrease in the yield at pH 9 with 10 nM enzyme may be attributed to the importance of the enzymatic activity under the conditions. The effect of concentration of **33** on the indigoid-forming reaction at pH 7.0 is illustrated in Fig. 6C. High yields were maintained when the concentration was >10 μM (89, 86, 85, and 90% at 56, 32, 18, and 10 μM, respectively), while lower yields were obtained at lower concentrations (62, 51, 42, and 41% at 5.6, 3.2, 1.8, and 1.0 μM, respectively). Lengthening the reaction time from 2 h to 14 h improved the yields (88, 79, and 57% at 3.2, 1.8, and 1.0 μM, respectively). The observation of substantial indigoid dye formation even at 1 μM augurs well for *in vivo* applications. Note that with 200 nM enzyme and 100 μM substrate, complete reaction requires 500 turnovers of each enzyme.



Scheme 7 Isolation of indigo **46** from a 5 mg scale reaction with **33**.

Given that reaction was still observed at 10 nM enzyme, such a modest turnover appears reasonable. In other words, the cases where incomplete reaction was observed likely were not due to limiting enzyme concentration.

With the results in hand for indoxyl-glucoside **33**, the 15 other indoxyl-glucosides shown in Table 1 (100 μM) were similarly treated with β-glucosidase from *Agrobacterium* (200 nM) in 50 mM NaPi buffer (pH 7.0) at 37 °C for 2 h. Unsubstituted indoxyl-glucoside (**1**) and the 4-chloro-5-bromo derivative **45** provided good yields (97 and 116%, entries 1 and 2, respectively). In contrast to β-glucosidase from almonds, the enzyme from *Agrobacterium* cleaved the glucoside in indoxyl-glucosides containing the triazine linker to give indigoid dye (entry 5 or 6, 46 or 150% yield). In the reactions of **22–27**, the order on the basis of yield was unsubstituted < 4-bromo < 4,6-dibromo indoxyl-glucosides as observed with β-glucosidase from almonds (entries 8–13). Indoxyl-glucoside **30** again resulted in low yield (10%, entry 14), suggesting severe steric hindrance imposed by the BCN group in the molecule. On the other hand, the indigoid dye was quantitatively formed from indoxyl-glucosides **33** and **44** (entries 15 and 16, 99 and 106% yield, respectively).

Finally, indigoid-dye formation was carried out in the presence of rat liver homogenate (Table 1, rightmost column). Good yields were obtained in the case of **45** (65%, entry 2) and **27** (50%, entry 13). Indoxyl-glucoside **33** did not form an indigoid product in rat liver homogenate (<5%, entry 15). However, when β-glucosidase from *Agrobacterium* (200 nM) was included in the presence of rat liver homogenate, the indigoid dye was obtained in 81% yield (entry 15).

3. Synthesis of dimeric indoxyl species

We sought to carry out an enzyme-triggered oligomerization using a bis(glucosyl-indoxyl) species bearing a water-solubilization moiety. A long-term goal beyond the scope of work here is to use the resulting oligomers as scaffolds for immobilization and localization of molecular constituents *in vivo*. The synthesis of the monomer for oligomerization is shown in Scheme 8. Treatment of acetyl-protected dibromoindoxyl-glucoside **32** (two molar equiv.) with cyanuric chloride resulted in substitution of two of the three chlorine atoms in the latter to give chlorotriazine **47**.

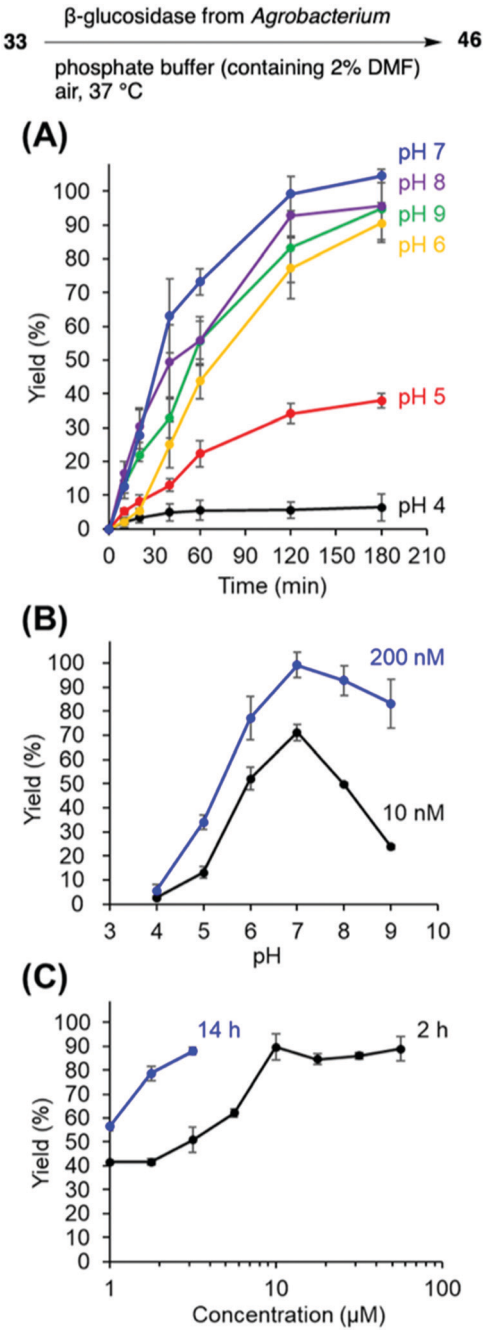


Fig. 6 Study of reaction conditions for the indigoid-forming reaction from **33** with β-glucosidase from *Agrobacterium*. (A) Effect of pH on the reaction progress [**33** (100 μM), enzyme (200 nM), 50 mM NaPi buffers, n = 3]. (B) Effect of the enzyme concentration [**33** (100 μM), 50 mM NaPi buffer (pH 7.0), 2 h, n = 3]. (C) Effect of the concentration of **33** [enzyme (200 nM), NaPi–NaCl (pH 7.0), 2 or 14 h, n = 3]. Yields were determined by absorption spectroscopy of solubilized indigoid dye.

Here, the sterically hindered strong base pempidine, which is known to facilitate nucleophilic reaction of alcohols with acid chlorides,[61] was used as the base to facilitate selective disubstitution of cyanuric chloride with the alcohol **32**. 1,2-Dichloroethane was employed as solvent instead of dichloromethane to carry out the reaction at 60 °C. Some loss of the *N*-acetyl group during

dialkoxylation of cyanuric chloride appeared responsible for purification challenges that accounted for the isolation of **47** in 53% yield.

Removal of the *N*-acetyl groups of **47** was achieved in CH_2Cl_2/methanol containing i-Pr_2EtN, a combination of solvent and hindered base employed for *N*-acetyl cleavage (in lieu of the traditional methanol containing $NaHCO_3$) owing to the poor solubility of **47** in pure methanol. Subsequent reaction with water-soluble amine **41** was performed in the presence of the hindered base 2,6-lutidine (to neutralize HCl and TFA) to afford **48** in 55% yield. Deprotection of the glucosyl *O*-acetyl groups provided the target bis(glucosyl-indoxyl) species **49** in 77% yield.

4. Oligomerization of dimeric indoxyl species

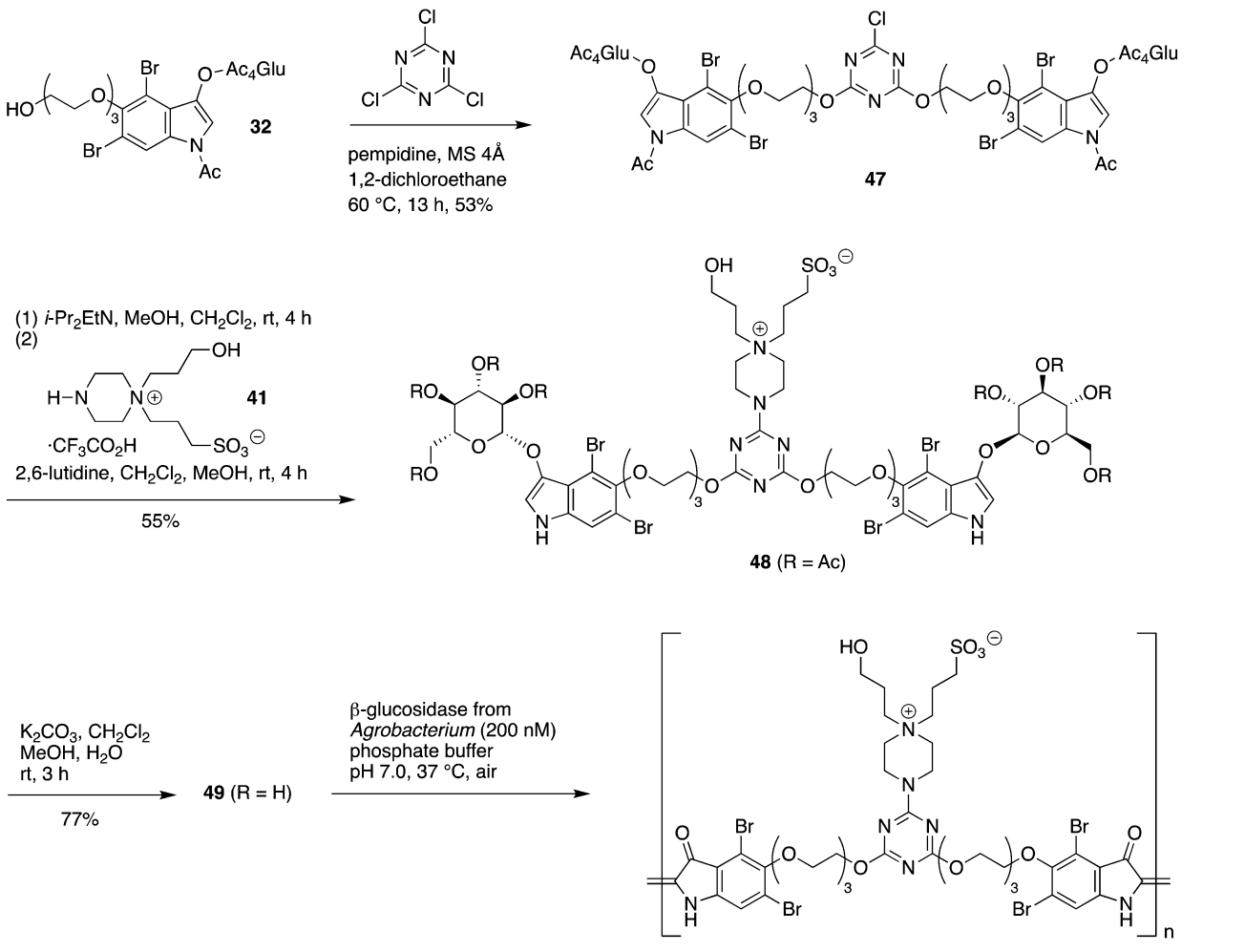
Oligomerization of **49** was carried out by treatment with β-glucosidase from *Agrobacterium* (200 nM) in NaPi buffer (pH 7.0) at 37 °C for 2–4 h (Scheme 8). Precipitation occurred during the reaction. After centrifugation, the precipitate was separated from the supernatant, washed with H_2O, and dried to afford a blue solid.

The efficacy of the indigogenic oligomerization of **49** was examined under a variety of conditions. As shown in entries 1–4 in Table 2, the yield of indigoid dye in the supernatant *versus* precipitate reversed as the concentration of **49** was decreased from 300 to 10 μM, although the total yields were in the range 17–29%. While the reactions in entries 1–4 were carried out in NaPi–NaCl (NaPi containing 50 mM NaCl), those of entries 5 and 6 (with 300 and 50 μM of **49**, respectively) were conducted in "low-salt" conditions: 10 mM NaPi containing a trace of DMF. The diminished quantity of NaCl facilitated analysis of the indigoid dye in the supernatant.

Table 2 Study of the oligomerization of bis(indoxyl-glucoside) species **49**[a]

Entry	[**49**], μM	Time, h	Yield of indigoid dye[b] (%) Supernatant	Precipitate
1[c]	300	2	6	11
2[c]	100	3	10	14
3[c]	50	2	13 ± 0.4[d]	16 ± 0.6[d]
4[c]	10	4	22	6
5[e]	300	3	13	13
6[e]	50	3	19	8

[a] The reaction was carried out at 37 °C in air with 200 nM *Agrobacterium* β-glucosidase (see Scheme 8). [b] The yield was calculated from absorption spectroscopy with $\varepsilon = 2.6 \times 10^4\ M^{-1}\ cm^{-1}$. [c] The reaction was carried out in NaPi–NaCl. [d] The reaction was repeated three times. [e] The reaction was carried out in 10 mM NaPi containing DMF (0.1–0.6%).



Scheme 8 Synthesis and oligomerization of indigogenic bis(indoxyl) derivative **49**.

The time course of the oligomerization was examined under the reaction conditions listed in entry 5 of Table 2, with 300 μM of **49**. The visible course of the reaction is shown in Fig. 7A. Noticeable changes include blue clouding and color deepening at 20 min. Centrifugation enabled isolation of the precipitate and the supernatant. Optical microscopic analysis of the precipitate suspended in H_2O showed small particles of up to several micron dimensions (Fig. 7B). Dynamic light scattering (DLS) analysis indicated that the particle size was ~680 nm (number mean, Fig. 7C). The precipitate was dissolved in DMF/DMSO (9 : 1) and is referred to as the precipitate extract. The supernatant was concentrated to dryness; the resulting residue was suspended in DMF and then filtered to remove salts. The resulting clear filtrate containing indigoid materials is referred to as the supernatant extract. The precipitate and supernatant extracts were examined by absorption spectroscopy and compared with that of **46** (dissolved in DMF) (Fig. 7D). All three samples showed a characteristic indigoid peak in the range 550–700 nm. The greater absorbance at *ca.* 300 nm in the precipitate and supernatant extracts *versus* that of **46** suggested contamination by impurities composed of indole derivatives. Size-exclusion chromatography (SEC) [DMF/DMSO (9 : 1) as eluent] using a three-column sequence[62] was applied to the precipitate extract and to the supernatant extract prepared from 300 μM of **49** (Fig. 7E). In each case, the first peak appeared at 18–20 min, and then the second peak at 38.5 min. The ratio of peak areas in the supernatant extract was 11 : 89, whereas that in the precipitate extract was 93 : 7. On the basis of a calibration

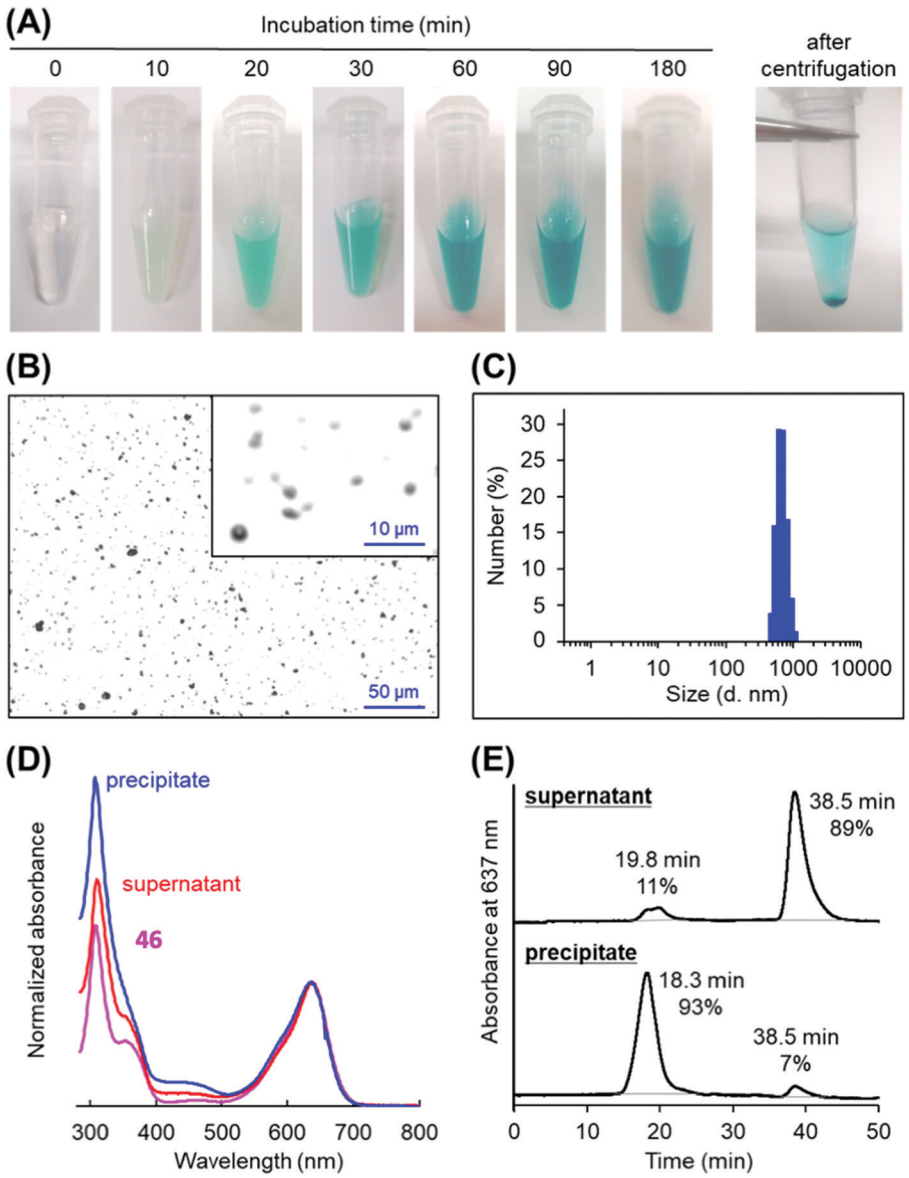


Fig. 7 (A) Time course of oligomerization with **49** under reaction conditions listed in entry 5 of Table 2. (B) Optical microscopic image (×40) of the precipitate suspended in H_2O. (C) DLS analysis of the precipitate suspended in H_2O. (D) Absorption spectra (normalized at 637 nm) of the precipitate extract in DMF/DMSO (9 : 1) (blue), the supernatant extract in DMF (red), and **46** in DMF (magenta). (E) Analytical SEC traces for the supernatant and precipitate extracts from 300 μM of **49**.

curve prepared with poly(2-vinylpyridine) standards (Fig. S5, ESI†), the molecular size for the first and second peaks was expected to be >265 and <1 kDa, respectively. The large molecular size indicated for the first peak likely implies aggregation or assembly of the oligomers. Another possible interpretation is that the chromatographic size standards employed are inappropriate for these indigoid oligomers. The chromatograms for the samples prepared with 50 μM of **49** (Fig. S6, ESI†) gave similar results but with higher purity compared with those at higher concentration. Further studies will be required to definitively gauge the size of the indigoid oligomers prepared herein.

Samples of **33**, **49**, and the precipitate derived by oligomerization of **49** were dissolved in DMSO-d_6 and examined by ^{1}H NMR spectroscopy. The spectra are shown in Fig. 8A. The lack of signals from the glucosyl group (hydroxyl protons ~5.0 ppm; the anomeric proton at 4.65 ppm) in the spectrum of the precipitate is consistent with smooth enzymatic cleavage of the sugar moiety. On the other hand, the signals in the aromatic region of the precipitate were complicated (Fig. 8B). One interpretation is that the indigoid dye exists in distinct environments and/or unknown indole derivatives other than the indigoid dye and indoxyl β-glucoside are present.

Finally, the oligomerization of **49** was carried out on a larger scale (7.87 mg) under the same "low-salt" reaction conditions as those of entry 5 in Table 2. As a result, 3.01 mg of the precipitate was obtained, which corresponds to 49% yield based on the monomer formula weight. We attempted to use mass spectrometry to gain information about the composition of the oligomeric indigoid products formed upon enzymatic treatment of **49**. Analysis of the supernatant by electrospray ionization mass spectrometry (ESI-MS) revealed negative ion peaks at m/z 1214.0 and 2428.9, consistent with monomer cyclization (n = 1) and cyclodimerization (n = 2), respectively (Fig. S7 and S8, ESI†). Analysis of the supernatant by matrix-assisted ionization mass spectrometry (MALDI-MS) revealed a progression of broad peaks extending to m/z > 10 000 with increment of m/z ~ 1210–1250 (Fig. S9, ESI†). Although the progression implies a mixture of oligomers, the observed m/z values did not match the calculated

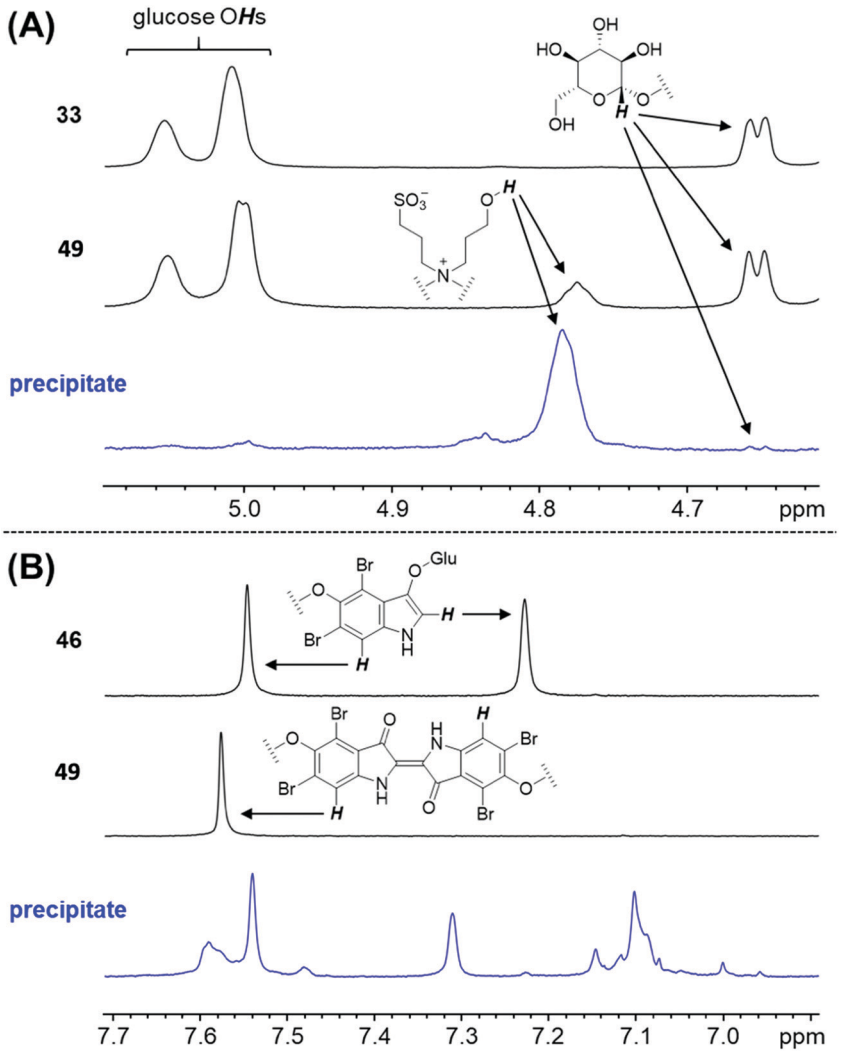


Fig. 8 Comparison of ^{1}H NMR spectra (in DMSO-d_6) of **33**, **46**, **49**, and the precipitate derived by low-salt oligomerization of **49**. (A) Spectral region (4.5–5.5 ppm) showing sugar and hydroxyl peaks. (B) Spectral region (6.9–7.7 ppm) showing aromatic protons.

values (Table S2, ESI†). Incomplete purification, decomposition by laser irradiation (especially the bromoheteroarene units), or complicated isotopic distribution caused by multiple bromine atoms may contribute to the broad peaks. Attempts to use MALDI-MS to analyze the precipitate, which was very insoluble, were unfruitful.

Outlook

The ability to carry out enzymatically triggered spontaneous cross-linking reactions under physiological conditions with no exogenous reagents or catalysts opens the door to a number of opportunities in the life sciences. Key features of the present work include the chromogenic readout and the formation of an aqueous-insoluble product. The four-fold challenge of achieving facile indigoid formation *via* enzyme triggering in the presence of a bioconjugatable tether in a synthetically accessible construct presented unexpected difficulties but after considerable development was met with the 5-alkoxy-4,6-dibromoindoxyl-glucoside structures. Altogether 36 new compounds were synthesized and characterized, including 15 target indoxyl-glucosides. Two novel molecular designs that emerged include an indoxyl-glucoside bearing a BCN group for copper-free click chemistry (**44**), and a bis(indoxyl-glucoside) (**49**); in both cases the intervening linker is composed of two short PEG groups and a central triazine derivatized with a sulfobetaine for water solubilization. Within the general designs identified, further alteration to the 4,6-dibromo-5-alkoxy aglycone moiety may include (1) replacement of the glucoside with galactosides, glucuronides or more complex polysaccharides as well as other enzyme-cleavable moieties; (2) increasing the length of the PEG spacers; (3) replacement of the BCN group (which upon click reaction yields isomers) by a functionalized cycloalkyne[63] that facilitates isomer-free generation of "clicked compounds" or other reactive handles for bioconjugation; and (4) construction of multimeric analogues of the dimeric **49**.

A nascent area of chemical biology concerns synthetic chemistry *in vivo*, for which the present indigogenic molecules would seem to be well suited. One recent thrust has been to identify oxidase enzymes that catalyze the formation of indigoids from indoles (not indoxyls) bearing diverse functional groups (aminomethyl, hydroxymethyl, carboxaldehyde, carboxylic acid).[64] A second recent thrust has been to genetically engineer the production of halogenated indoxyl-glucosides.[65] An open issue over the long term is whether the types of synthetic indoxyl species prepared herein could also be produced *via* genetic expression, thereby providing a counterpart that melds some of the attractive features of fluorescent proteins and click-chemistry processes with reactions that proceed under physiological conditions. A more immediate issue is the examination of the utility of the present structures for the covalent assembly of macromolecular scaffolds *in vivo*.

Experimental section

General methods

^{1}H NMR and ^{13}C NMR spectra were collected at room temperature in $CDCl_3$ unless noted otherwise. Chemical shifts for ^{1}H NMR spectra are reported in parts per million (δ) relative to tetramethylsilane or a solvent signal (CD_3OD, δ = 3.31 ppm). Chemical shifts for ^{13}C NMR spectra are reported in parts per million (δ), and spectra were calibrated by using solvent signals [$CDCl_3$, δ = 77.16 ppm; $(CD_3)_2SO$, δ = 39.52 ppm; CD_3OD, δ = 49.00 ppm].[66] Silica (40 μm), diol-functionalized silica (40–63 μm), and reverse phase silica (C18, 40–63 μm) were used for column chromatography. Preparative TLC separations were carried out on Merck analytical plates precoated with silica 60 F_{254}. Microscopic analysis was performed on a Zeiss Axio Imager M.2. DLS analysis was performed on a Zetasizer Nano ZS. Centrifugation was carried out at 20 000*g* at 4 °C. Absorption spectroscopy was performed in the ultraviolet-visible region (200–700 nm).

Chemicals

All solvents were reagent grade and were used as received unless noted otherwise. NBS was recrystallized from water. Na_2SO_4 was anhydrous. The CH_2Cl_2 employed in reactions was commercial anhydrous grade. Other solvents and reagents were used as received. The known compounds **9**,[39] **12**[46] and **34**[49] were prepared generally following procedures described in the literature. The ion exchange resin (DOWEX 50WX8-200) was in the protonated form and is designated DOWEX H^+.

Enzymes

β-Glucosidase from almonds (lyophilized powder, ≥2 units per mg solid) and peroxidase from horseradish were purchased from Sigma-Aldrich. β-Glucosidase from *Agrobacterium* sp. (recombinant, suspension in 3.2 M $(NH_4)_2SO_4$) was purchased from Megazyme; the concentration in solution was determined by absorption spectroscopy with $E^{0.1\%}$ = 2.20 cm^{-1} at 280 nm.[59] Tritosomes were purchased from XenoTech. Rat liver homogenate was purchased from MP Biomedicals.

Buffers

For enzymatic studies, several buffers were prepared. All buffers contain the sodium cation and were adjusted to a certain pH value with 1 decimal point before use. The chief buffers were as follows: (1) acetate buffer – prepared from sodium acetate (pH 5.0, 50 mM); (2) 50 mM NaPi buffer (pH 7.0); (3) 10 mM NaPi buffer (pH 7.0); and (4) NaPi–NaCl buffer (pH 7.0, 10 mM NaPi and 50 mM NaCl), where NaPi refers to sodium/hydrogen phosphate in aqueous solution at the indicated pH value. Other buffers were prepared for pH-variation studies. Other constituents often were present in indigogenic reactions owing to the presence of salts from the enzyme stock solutions. For example, ammonium sulfate (3.2 M) is present in the stock solution of β-glucosidase from *Agrobacterium*. Also, the first dilution of the enzyme stock solution was carried out with NaPi–NaCl buffer; thus, regardless of subsequent dilution into an NaPi buffer, residual NaCl was present. A full description of the concentrations of all reactants and buffer constituents for the various indigogenic reactions is provided in the ESI.†

Bases

A number of organic bases were used herein as part of the triazine and other substitution reactions. The pK_a value of the

conjugate acid of a given base in water is provided in parentheses as follows: 2,6-di-*tert*-butylpyridine (3.58),[48] 1,10-phenanthroline (4.96),[67] 2,6-lutidine (5.77),[48] triethylamine (10.85),[68] pempidine (1,2,2,6,6-pentamethylpiperidine, 11.25),[69] and *N*,*N*-diisopropylethylamine (11.44).[70]

5-Hydroxy-1*H*-indol-3-yl β-D-glucopyranoside (10). A suspension of **9** (917.4 mg, 1.50 mmol), having >99% stereochemical purity at the anomeric carbon, in MeOH (7.50 mL) at room temperature was treated with sodium methoxide (25 wt% solution in MeOH, 648 μL, 3.0 mmol). After 2 h, acetic acid (229 μL, 6.00 mmol) and Pd/C (10 wt%, 79.8 mg, 0.075 mmol) were added. The reaction mixture was stirred for 2 h under a H_2 atmosphere (balloon) at room temperature and then filtered through a Celite pad. The filtrate was concentrated and purified by chromatography [silica, CH_2Cl_2/MeOH (7 : 3)] to afford a pale yellow solid (417.6 mg, 89%): mp darkened >55 °C; ^{1}H NMR [400 MHz, $(CD_3)_2SO$] δ 3.09–3.29 (m, 4H), 3.42–3.53 (m, 1H), 3.65–3.76 (m, 1H), 4.47–4.55 (m, 1H), 4.59 (br s, 1H), 5.07 (br s, 1H), 5.15 (br s, 1H), 5.38 (br s, 1H), 6.58 (dd, J = 2.6, 8.6 Hz, 1H), 6.89 (d, J = 2.0 Hz, 1H), 6.98 (d, J = 2.6 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 8.68 (br s, 1H), 10.21 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 62.6, 71.5, 75.0, 78.02, 78.04, 102.5, 105.8, 112.9, 113.1, 113.5, 121.9, 130.4, 138.4, 160.0; ESI-MS obsd 334.0894, calcd 334.0897 [$(M + H)^+$, M = $C_{14}H_{17}NO_7$].

1-Acetyl-5-hydroxy-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (11). A suspension of **9** (6.911 g, 11.3 mmol) and Pd/C (10 wt%, 360.8 mg, 0.339 mmol) in ethyl acetate/EtOH (4 : 1, 113 mL) was stirred for 3 h at room temperature under a H_2 atmosphere (balloon). The reaction mixture was filtered through a Celite pad. The filtrate was concentrated and purified by chromatography [silica, CH_2Cl_2/ethyl acetate (10 : 1)] to afford a pale yellow solid (5.85 g, 99%): mp 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 2.56 (s, 3H), 3.77–3.88 (m, 1H), 4.23 (dd, J = 5.0, 12.4 Hz, 1H), 4.93–5.03 (m, 1H), 5.11–5.23 (m, 1H), 5.23–5.34 (m, 2H), 5.82–6.02 (m, 1H), 6.85–6.96 (m, 2H), 7.10 (br s, 1H), 8.22 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.63, 20.66, 20.73, 20.8, 23.7, 62.1, 68.3, 71.1, 72.4, 72.6, 101.0, 103.2, 110.9, 115.1, 117.7, 125.4, 128.3, 141.3, 153.0, 168.2, 169.58, 169.63, 170.4, 171.0; ESI-MS obsd 544.1430, calcd 544.1426 [$(M + Na)^+$, M = $C_{24}H_{27}NO_{12}$].

1-Acetyl-5-[(4-chloro-6-methoxy-1,3,5-triazin-2-yl)oxy]-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (13). A sample of i-Pr_2EtN (65.3 μL, 0.375 mmol) was added dropwise over 5 min to a suspension of **11** (130.4 mg, 0.250 mmol) and **12** (58.5 mg, 0.325 mmol) in CH_2Cl_2 (1.25 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was washed with aqueous citric acid (10%, 1 mL) followed by brine (1 mL), then dried (Na_2SO_4) and filtered. The filtrate was concentrated and purified by chromatography [silica, hexanes/ethyl acetate (2 : 3)] to afford a white solid (188.3 mg, 87%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.62 (s, 3H), 3.80–3.95 (m, 1H), 4.02 (s, 3H), 4.14–4.38 (m, 2H), 4.97–5.09 (m, 1H), 5.09–5.40 (m, 3H), 7.10–7.36 (m, 3H), 8.44 (br s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 20.6, 20.68, 20.71, 23.8, 56.3, 61.9, 68.2, 70.9, 72.37, 72.43, 100.7, 110.2, 111.1, 117.7, 119.7, 124.8, 131.4, 141.0, 147.6, 168.1, 169.2, 169.4, 170.2, 170.5, 172.5, 172.8, 173.2; ESI-MS obsd 665.1499, calcd 665.1492 [$(M + H)^+$, M = $C_{28}H_{29}ClN_4O_{13}$].

1-Acetyl-5-[(4-methoxy-6-morpholino-1,3,5-triazin-2-yl)oxy]-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (morph-13). A solution of **13** (10.0 mg, 0.015 mmol) and i-Pr_2EtN (3.4 μL, 0.020 mmol) in CH_2Cl_2 (150 μL) at room temperature was treated with morpholine (1.4 μL, 0.017 mmol). After 2 h, the reaction mixture was quenched by the addition of AcOH (0.9 μL) and directly purified by chromatography (silica, ethyl acetate) to afford a white solid (10.6 mg, 99%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.61 (s, 3H), 3.64–3.75 (m, 6H), 3.81–3.90 (m, 3H), 3.90 (s, 3H), 4.24 (dd, J = 4.5, 12.1 Hz, 1H), 4.29 (dd, J = 2.3, 12.1 Hz, 1H), 5.00–5.06 (m, 1H), 5.14–5.22 (m, 1H), 5.27–5.34 (m, 2H), 7.17 (d, J = 2.4 Hz, 1H), 7.19 (d, J = 2.4 Hz, 1H), 7.27 (s, 1H), 8.40 (br s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 20.7, 20.8, 20.9, 24.0, 44.1, 44.2, 54.8, 62.0, 66.7, 66.7, 68.3, 71.1, 72.57, 72.59, 100.8, 110.5, 110.7, 117.4, 120.8, 124.7, 131.2, 141.3, 148.6, 166.9, 168.2, 169.4, 169.5, 170.3, 170.7, 172.5, 172.7; ESI-MS obsd 716.2410, calcd 716.2410 [$(M + H)^+$, M = $C_{32}H_{37}N_5O_{14}$].

5-{[4-({1-[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl]-3-oxo-2,7,10-trioxa-4-azadodecan-12-yl}amino)-6-methoxy-1,3,5-triazin-2-yl]oxy}-1*H*-indol-3-yl β-D-glucopyranoside (15). A sample of **13** (20.3 mg, 0.0305 mmol) was added to a solution of (1*R*,8*S*,9*s*)-bicyclo-[6.1.0]non-4-yn-9-ylmethanol (10.4 mg, 0.0321 mmol) and i-Pr_2EtN (6.7 μL, 0.038 mmol) in CH_2Cl_2 (150 μL) at room temperature. After 3 h, MeOH (750 μL) and K_2CO_3 (13.3 mg, 0.096 mmol) were added. After 1 h, the reaction mixture was quenched by the addition of acetic acid (9.2 μL) and then filtered. The filtrate was concentrated and purified by chromatography [silica, CH_2Cl_2/MeOH (3 : 1)] to afford a pale yellow solid (21.0 mg, 93%): ^{1}H NMR (700 MHz, CD_3OD, ~1 : 1 mixture of rotamers) δ 0.85–0.98 (m, 2H), 1.25–1.40 (m, 1H), 1.49–1.65 (m, 2H), 2.09–2.30 (m, 6H), 3.10–3.18 (m, 1H), 3.23–3.28 (m, 1H), 3.29–3.63 (m, 14H), 3.716 (dd, J = 5.6, 11.9 Hz, 0.5H), 3.719 (dd, J = 5.7, 11.9 Hz, 0.5H), 3.87–3.93 (m, 2.5H), 3.94 (s, 1.5H), 4.10 (d, J = 8.1 Hz, 0.5H), 4.12 (d, J = 8.2 Hz, 0.5H), 4.68 (d, J = 8.1 Hz, 0.5H), 4.70 (d, J = 8.1 Hz, 0.5H), 6.67–6.73 (m, 0.5H), 6.78–6.84 (m, 0.5H), 6.87 (dd, J = 2.3, 8.7 Hz, 1H), 6.90 (dd, J = 2.3, 8.7 Hz, 1H), 7.17 (s, 1H), 7.28 (d, J = 8.7 Hz, 0.5H), 7.29 (d, J = 8.7 Hz, 0.5H), 7.47 (d, J = 2.3 Hz, 0.5H), 7.48 (d, J = 2.3 Hz, 0.5H); ^{13}C NMR (175 MHz, CD_3OD, mixture of rotamers) δ 18.9, 21.4, 21.9, 30.1, 41.2, 41.5, 41.6, 41.7, 41.9, 55.2, 55.3, 62.6, 63.70, 63.74, 70.0, 70.3, 70.8, 70.88, 70.91, 71.16, 71.22, 71.5, 75.0, 78.0, 78.16, 78.19, 99.5, 105.9, 106.0, 110.8, 111.0, 112.7, 112.9, 114.0, 114.2, 117.3, 117.5, 121.4, 121.5, 132.9, 133.0, 139.18, 139.24, 146.4, 146.6, 159.2, 159.2, 169.3, 169.5, 173.4, 173.7, 173.9, 174.1; ESI-MS obsd 743.3249, calcd 743.3247 [$(M + H)^+$, M = $C_{35}H_{46}N_6O_{12}$].

5-[((Benzylcarbamoyl)oxy)]-1*H*-indol-3-yl β-D-glucopyranoside (16). Samples of *p*-nitrophenyl chloroformate (6.0 mg, 0.029 mmol) and i-Pr_2EtN (6 μL, 0.03 mmol) were added to a solution of **11** (13.0 mg, 0.025 mmol) in CH_2Cl_2 (1 mL) at room temperature. After 1 h, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (2 mL) and stirred for 30 min at room temperature. Water (2 mL) was added, and then the

mixture was extracted with Et_2O (3 × 2 mL). The combined organic extract was washed with H_2O (2 mL), brine (2 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (1 mL). Benzylamine (3 μL, 0.03 mmol) was added to the solution at room temperature. After 20 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH (1 mL). NaOMe (25% in MeOH, 5 μL, 0.02 mmol) was added to the solution at room temperature. After 45 min, the reaction mixture was quenched by the addition of a sample of ion exchange resin (DOWEX H^+) until the pH reached near 7. The mixture was then stirred for 20 min at room temperature followed by filtration. The filtrate was concentrated under reduced pressure. Column chromatography [silica, CH_2Cl_2/MeOH (5 : 1)] afforded a colorless oil (5.9 mg, 53%): ^{1}H NMR (700 MHz, CD_3OD) δ 3.32–3.36 (m, 1H), 3.40 (t, J = 9.0 Hz, 1H), 3.43 (t, J = 9.0 Hz, 1H), 3.48 (dd, J = 9.0, 8.0 Hz, 1H), 3.71 (dd, J = 12.0, 6.0 Hz, 1H), 3.90 (dd, J = 12.0, 2.0 Hz, 1H), 4.37 (s, 2H), 4.67 (d, J = 8.0 Hz, 1H), 6.85 (dd, J = 8.0, 2.0 Hz, 1H), 7.14 (s, 1H), 7.24–7.28 (m, 2H), 7.31–7.40 (m, 4H), 7.43 (d, J = 2.0 Hz, 1H); ^{13}C NMR (175 MHz, CD_3OD) δ 45.7, 62.6, 71.5, 75.0, 78.0, 78.2, 106.0, 111.0, 112.7, 114.2, 117.5, 121.5, 128.2, 128.4, 129.6, 132.9, 139.0, 140.4, 145.4, 158.6; ESI-MS obsd 467.1419, calcd 467.1425 [$(M + Na)^+$, M = $C_{22}H_{23}N_2NaO_8$].

1-Acetyl-4-bromo-5-hydroxy-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (17). A solution of NBS in CH_2Cl_2 (100 mM, 4.20 mL) was added dropwise over 5 min to a solution of **11** (208.6 mg, 0.400 mmol) and 2,6-di-*tert*-butylpyridine (88 μL, 0.40 mmol) in CH_2Cl_2 (5.80 mL) at −78 °C. After 1.5 h, the reaction mixture was allowed to warm to room temperature with stirring for 30 min. The reaction mixture was washed with saturated aqueous $Na_2S_2O_3$ (3 mL) and brine (5 mL), then dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography [silica, hexanes/CH_2Cl_2/MeCN (2 : 1 : 1)] afforded a white solid (180.6 mg, 75%): mp 130 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.10 (s, 3H), 3.86–3.96 (m, 1H), 4.22 (dd, J = 5.4, 12.3 Hz, 1H), 4.36 (dd, J = 2.0, 12.3 Hz, 1H), 5.06 (d, J = 7.8 Hz, 1H), 5.21 (dd, J = 9.2, 9.2 Hz, 1H), 5.31 (dd, J = 9.2, 9.2 Hz, 1H), 5.40 (dd, J = 7.8, 9.2 Hz, 1H), 5.74 (s, 1H), 7.05 (d, J = 8.8 Hz, 1H), 7.22 (br s, 1H), 8.29 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8, 20.9, 21.0, 23.9, 62.1, 68.3, 71.0, 72.6, 72.8, 98.6, 100.3, 111.2, 114.7, 117.1, 122.6, 129.0, 140.5, 149.4, 167.9, 169.4, 169.5, 170.4, 170.6; ESI-MS obsd 600.0712, calcd 600.0711 [$(M + H)^+$, M = $C_{24}H_{26}BrNO_{12}$]. Suitable crystals for X-ray analysis were obtained by recrystallization from cyclohexane/$CHCl_3$.

1-Acetyl-4,6-dibromo-5-hydroxy-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (18). A solution of NBS (3.987 g, 22.4 mmol) in CH_2Cl_2 (240 mL) was added dropwise over 1 h to a solution of **11** (5.841 g, 11.2 mmol) and 2,6-di-*tert*-butylpyridine (2.47 mL, 11.2 mmol) in CH_2Cl_2 (160 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature with stirring for 3.5 h. NBS (598 mg, 3.36 mmol) was added. After 1 h, the reaction mixture was washed with aqueous $Na_2S_2O_3$ (10%, 50 mL) and brine (50 mL), then dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure. Column chromatography [silica, hexanes/ethyl acetate (1 : 1)] followed by recrystallization from CH_2Cl_2/MeOH afforded a white solid (6.32 g, 83%): mp 102–103 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 2.10 (s, 3H), 2.59 (s, 3H), 3.91 (ddd, J = 2.4, 5.2, 9.9 Hz, 1H), 4.22 (dd, J = 5.2, 12.5 Hz, 1H), 4.37 (dd, J = 2.4, 12.5 Hz, 1H), 5.05 (d, J = 7.6 Hz, 1H), 5.21 (dd, J = 9.5, 9.9 Hz, 1H), 5.31 (dd, J = 9.3, 9.5 Hz, 1H), 5.38 (dd, J = 7.6, 9.3 Hz, 1H), 6.02 (s, 1H), 7.22 (br s, 1H), 8.63 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 20.9, 21.0, 23.7, 62.1, 68.4, 71.0, 72.7, 72.8, 98.4, 100.3, 108.6, 112.0, 120.0, 122.6, 128.7, 140.2, 146.3, 167.8, 169.4, 169.5, 170.3, 170.6; ESI-MS obsd 699.9645, calcd 699.9636 [$(M + Na)^+$, M = $C_{24}H_{25}Br_2NO_{12}$]. Suitable crystals for X-ray analysis were obtained by recrystallization from cyclohexane/acetone.

5-[(4-Methoxy-6-morpholino-1,3,5-triazin-2-yl)oxy]-1*H*-indol-3-yl β-D-glucopyranoside (19). A sample of i-Pr_2EtN (13.1 μL, 0.075 mmol) was added to a solution of **10** (15.6 mg, 0.050 mmol) and **12** (9.9 mg, 0.055 mmol) in DMF (125 μL) at room temperature. After 3 h, morpholine (8.6 μL, 0.10 mmol) was added. After 2 h, the reaction mixture was passed through silica. The resulting solution was concentrated under reduced pressure. Column chromatography [silica, $CHCl_3$/MeOH (4 : 1)] afforded a white solid (15.0 mg, 59%): ^{1}H NMR (300 MHz, CD_3OD) δ 3.25–3.93 (m, 14H), 3.92 (s, 3H), 4.67 (d, J = 7.2 Hz, 1H), 6.87 (dd, J = 8.7, 1.8 Hz, 1H), 7.16 (s, 1H), 7.27 (d, J = 8.7 Hz, 1H), 7.46 (d, J = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 45.2, 55.3, 62.6, 67.5, 71.5, 75.0, 78.0, 78.2, 106.0, 110.7, 112.7, 114.1, 117.3, 121.4, 132.9, 139.2, 146.5, 167.9, 173.8, 174.0; ESI-MS obsd 506.1883, calcd 506.1880 [$(M + H)^+$, M = $C_{22}H_{27}N_5O_9$].

1-Acetyl-4-bromo-5-[(4-methoxy-6-morpholino-1,3,5-triazin-2-yl)oxy]-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (pre-20). A sample of i-Pr_2EtN (10.5 μL, 0.060 mmol) was added to a solution of **17** (24.0 mg, 0.040 mmol) and **12** (7.9 mg, 0.044 mmol) in CH_2Cl_2 (200 μL) at room temperature. After 30 min, morpholine (6.9 μL, 0.080 mmol) was added. After 3 h, the reaction mixture was quenched by the addition of acetic acid (2.2 μL) and then passed through silica (ethyl acetate as eluent). The eluent was concentrated under reduced pressure. Column chromatography [silica, hexanes/ethyl acetate (1 : 2)] afforded a white solid (27.6 mg, 87%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60–3.76 (m, 6H), 3.78–3.94 (m, 6H), 4.20 (dd, J = 5.2, 12.5 Hz, 1H), 4.37 (dd, J = 2.2, 12.5 Hz, 1H), 5.06 (d, J = 7.6 Hz, 1H), 5.19 (dd, J = 9.3, 9.3 Hz, 1H), 5.29 (dd, J = 9.3, 9.3 Hz, 1H), 5.37 (dd, J = 7.6, 9.3 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 7.30 (br s, 1H), 8.30–8.50 (m, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 20.7, 20.9, 21.0, 24.0, 44.06, 44.11, 54.8, 62.0, 66.6, 66.7, 68.3, 70.8, 72.6, 72.7, 100.3, 106.4, 112.1, 116.3, 121.5, 123.5, 132.1, 140.8, 146.2, 166.9, 168.1, 169.3, 169.5, 170.3, 170.6, 171.9, 172.6; ESI-MS obsd 816.1321, calcd 816.1334 [$(M + Na)^+$, M = $C_{32}H_{36}BrN_5O_{14}$].

4-Bromo-5-[(4-methoxy-6-morpholino-1,3,5-triazin-2-yl)oxy]-1*H*-indol-3-yl β-D-glucopyranoside (20). A suspension of **pre-20** (15.9 mg, 0.020 mmol) and K_2CO_3 (2.8 mg, 0.020 mmol) in MeOH was stirred at room temperature for 20 min. The reaction mixture was quenched by the addition of acetic acid (2.9 μL) and concentrated under reduced pressure. Column chromatography [silica, CH_2Cl_2/MeOH (1 : 2)] afforded a white solid (10.4 mg, 89%): ^{1}H NMR (300 MHz, CD_3OD) δ 3.34–4.00 (m, 17H), 4.76

(d, J = 7.8 Hz, 1H), 7.21–7.34 (m, 2H); ^{13}C NMR (175 MHz, CD_3OD) δ 45.1, 45.3, 55.29, 55.34, 62.65, 62.69, 67.5, 71.6, 75.3, 78.1, 78.3, 105.2, 106.1, 112.2, 114.7, 118.3, 119.8, 133.6, 139.2, 143.8, 167.9, 173.4, 173.8; ESI-MS obsd 584.0986, calcd 584.0987 [$(M + H)^+$, M = $C_{22}H_{26}BrN_5O_9$].

4,6-Dibromo-5-[(4-methoxy-6-morpholino-1,3,5-triazin-2-yl)oxy]-1*H*-indol-3-yl β-D-glucopyranoside (21). A sample of i-Pr_2EtN (7.3 μL, 0.042 mmol) was added to a solution of **18** (19.0 mg, 0.028 mmol) and **12** (5.54 mg, 0.031 mmol) in CH_2Cl_2 (140 μL) at 0 °C. The reaction mixture was allowed to warm to room temperature with stirring for 1 h. Morpholine (4.8 μL, 0.056 mmol) was added. After 3 h, MeOH (560 μL) and K_2CO_3 (19.3 mg, 0.14 mmol) were added. The reaction mixture was heated at 35 °C for 1 h and then allowed to cool to room temperature. The reaction mixture was quenched by the addition of acetic acid (16 μL) and filtered. The filtrate was concentrated under reduced pressure. Column chromatography [silica, CH_2Cl_2/MeOH (6 : 1)] afforded a white solid (12.5 mg, 67%): ^{1}H NMR (300 MHz, CD_3OD) δ 3.34–4.02 (m, 17H), 4.77 (d, J = 7.2 Hz, 1H), 7.30 (s, 1H), 7.56 (s, 1H); ^{13}C NMR (175 MHz, CD_3OD) δ 45.1, 45.3, 55.4, 55.5, 62.6, 67.4, 71.5, 75.2, 78.1, 78.3, 105.00, 105.02, 107.6, 111.2, 115.2, 115.7, 119.5, 133.6, 139.2, 140.6, 167.9, 172.6, 173.9; ESI-MS obsd 662.0098, calcd 662.0092 [$(M + H)^+$, M = $C_{22}H_{25}Br_2N_5O_9$].

5-(Methoxycarbonyl)methoxy-1*H*-indol-3-yl β-D-glucopyranoside (22). Ethyl bromoacetate (5.0 μL, 45 μmol) and NaH (2.0 mg, 83 μmol) were added to a solution of **11** (10.0 mg, 0.019 mmol) in DMF (1 mL) at room temperature. After 1 h, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (2 mL) and stirred for 10 min at room temperature. Water (2 mL) was added, then the mixture was extracted with Et_2O (3 × 2 mL). The combined organic extract was washed with H_2O (2 mL) and brine (2 mL), then dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH (1 mL). NaOMe (25% in MeOH, 5 μL, 0.02 mmol) was added to the solution at room temperature. After 45 min, the reaction mixture was quenched by the addition of a sample of ion exchange resin (DOWEX H^+) until the pH reached near 7. The mixture was then stirred for 20 min at room temperature followed by filtration. The filtrate was concentrated and purified by chromatography [silica, CH_2Cl_2/MeOH (5 : 1)] to afford a colorless oil (3.9 mg, 53%): ^{1}H NMR (300 MHz, CD_3OD) δ 3.34–3.56 (m, 4H), 3.72 (dd, J = 12.0, 5.0 Hz, 1H), 3.80 (s, 3H), 3.91 (dd, J = 12.0, 2.0 Hz, 1H), 4.66 (d, J = 7.5 Hz, 1H), 4.71 (s, 2H), 6.82 (dd, J = 9.0, 2.5 Hz, 1H), 7.09 (s, 1H), 7.16–7.22 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 52.5, 62.7, 67.0, 71.5, 75.1, 78.0, 78.2, 101.5, 106.0, 113.3, 113.7, 113.9, 153.1, 172.1; ESI-MS obsd 406.1109, calcd 406.1109 [$(M + Na)^+$, M = $C_{17}H_{21}NO_9$].

4-Bromo-5-(methoxycarbonyl)methoxy-1*H*-indol-3-yl β-D-glucopyranoside (23). Ethyl bromoacetate (4.0 μL, 36 μmol) and NaH (1.0 mg, 42 μmol) were added to a solution of **17** (15 mg, 0.025 mmol) in DMF (0.5 mL) at room temperature. After 1 h, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (2 mL) and stirred for 10 min at room temperature. Water (2 mL) was added, then the mixture was extracted with Et_2O (3 × 2 mL). The combined organic extract was washed with H_2O (2 mL) and brine (2 mL), then dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH (0.5 mL). NaOMe (25% in MeOH, 5 μL, 0.02 mmol) was added to the solution at room temperature. After 45 min, the reaction mixture was quenched by the addition of a sample of ion exchange resin (DOWEX H^+) until the pH reached near 7. The mixture was then stirred for 20 min at room temperature followed by filtration. The filtrate was concentrated and purified by chromatography [silica, CH_2Cl_2/MeOH (5 : 1)] afforded a colorless oil (5.4 mg, 52%): ^{1}H NMR (300 MHz, CD_3OD) δ 3.34–3.56 (m, 4H), 3.72 (dd, J = 12.0, 5.0 Hz, 1H), 3.80 (s, 3H), 3.92 (d, J = 12.0 Hz, 1H), 4.66 (s, 2H), 4.75 (d, J = 7.0 Hz, 1H), 6.91 (dd, J = 9.0, 1.0 Hz, 1H), 7.20 (dd, J = 9.0, 1.0 Hz, 1H), 7.24 (s, 2H); ^{13}C NMR (175 MHz, CD_3OD) δ 52.5, 69.7, 69.8, 71.6, 75.4, 78.3, 103.3, 105.3, 112.2, 113.9, 115.0, 120.3, 132.5, 138.9, 149.7, 171.7; ESI-MS obsd 484.0205, calcd 484.0214 [$(M + Na)^+$, M = $C_{17}H_{20}BrNO_9$].

4,6-Dibromo-5-(methoxycarbonyl)methoxy-1*H*-indol-3-yl β-D-glucopyranoside (24). Ethyl bromoacetate (3.0 μL, 27 μmol) and NaH (1.0 mg, 42 μmol) were added to a solution of **18** (12.7 mg, 0.019 mmol) in DMF (1 mL) at room temperature. After 1 h, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (2 mL) and stirred for 20 min at room temperature. Water (2 mL) was added, then the mixture was extracted with Et_2O (3 × 2 mL). The combined organic extract was washed with H_2O (2 mL) and brine (2 mL), then dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH (1 mL). NaOMe (25% in MeOH, 5 μL, 0.02 mmol) was added to the solution at room temperature. After 45 min, the reaction mixture was quenched by the addition of a sample of ion exchange resin (DOWEX H^+) until the pH reached near 7. The mixture was then stirred for 20 min at room temperature followed by filtration. The filtrate was concentrated and purified by chromatography [silica, CH_2Cl_2/MeOH (5 : 1)] to afford a colorless oil (8.3 mg, 82%): ^{1}H NMR (700 MHz, CD_3OD) δ 3.37–3.41 (m, 2H), 3.45 (t, J = 9.0 Hz, 1H), 3.53 (dd, J = 9.0, 8.0 Hz, 1H), 3.70 (dd, J = 12.0, 5.0 Hz, 1H), 3.84 (s, 3H), 3.92 (d, J = 12 Hz, 1H), 4.61 (s, 2H), 4.74 (d, J = 8.0 Hz, 1H), 7.27 (s, 1H), 7.51 (s, 1H); ^{13}C NMR (175 MHz, CD_3OD) δ 52.6, 62.7, 70.3, 71.6, 75.3, 78.2, 78.3, 105.1, 107.6, 111.6, 115.4, 116.1, 119.9, 133.2, 139.1, 145.6, 170.6; ESI-MS obsd 561.9310, calcd 561.9319 [$(M + Na)^+$, M = $C_{17}H_{19}Br_2NO_9$].

5-Propargyloxy-1*H*-indol-3-yl β-D-glucopyranoside (25). A suspension of **10** (15.6 mg, 0.050 mmol), propargyl bromide (18.6 μL, 80% in toluene, 0.125 mmol), and K_2CO_3 (17.2 mg, 0.124 mmol) in DMF (125 μL) was heated to 80 °C for 2.5 h. The reaction mixture was allowed to cool to room temperature and then passed through silica (CH_2Cl_2/MeOH = 1 : 1 as eluent). The eluent was concentrated under reduced pressure. Preparative thin layer chromatography [silica, 0.25 mm, 20 × 20 cm, $CHCl_3$/MeOH (4 : 1)] afforded a brown solid (5.3 mg, 30%): ^{1}H NMR (400 MHz, CD_3OD) δ 2.88 (t, J = 2.4 Hz, 1H), 3.32–3.54 (m, 4H), 3.73 (dd, J = 5.0, 11.8 Hz, 1H), 3.92 (dd, J = 2.2, 11.8 Hz, 1H), 4.69 (d, J = 7.6 Hz, 1H), 4.71 (d, J = 2.4 Hz, 2H), 6.79 (dd, J = 2.4, 8.8 Hz, 1H), 7.09 (s, 1H), 7.18 (d, J = 8.8 Hz, 1H), 7.28 (d, J = 2.4 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 57.6, 62.7, 71.5, 75.1,

76.1, 78.0, 78.2, 80.5, 102.2, 105.9, 113.1, 113.5, 114.1, 121.4, 131.0, 139.0, 152.8; ESI-MS obsd 372.1055, calcd 372.1054 [(M + Na)$^+$, M = $C_{17}H_{19}NO_7$].

4-Bromo-5-propargyloxy-1*H*-indol-3-yl β-D-glucopyranoside (26). Propargyl bromide (8.9 μL, 80% in toluene, 0.080 mmol) was added to a suspension of **17** (30.0 mg, 0.050 mmol) and K_2CO_3 (8.3 mg, 0.060 mmol) in DMF (0.80 mL) at room temperature. The mixture was stirred for 18 h and then treated with MeOH (0.40 mL) and NaOMe solution (30 μL, 0.5 M in MeOH, 0.015 mmol). After 1 h, the reaction mixture was quenched by the addition of acetic acid (20 μL) and concentrated under reduced pressure. Column chromatography [silica, CH_2Cl_2/MeOH = 9 : 1 to 17 : 3] afforded a pale-brown solid (18 mg, 84%): mp 146–148 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 2.90 (t, J = 2.4 Hz, 1H), 3.34–3.52 (m, 3H), 3.55 (dd, J = 8.2, 8.2 Hz, 1H), 3.65–3.75 (m, 1H), 3.92 (dd, J = 1.2, 11.8 Hz, 1H), 4.71 (d, J = 2.4 Hz, 2H), 4.74 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 7.20 (d, J = 8.8 Hz, 1H), 7.23 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 60.2, 62.7, 71.5, 75.3, 76.7, 78.0, 78.2, 80.2, 103.6, 105.2, 112.0, 114.5, 114.9, 120.2, 132.5, 138.8, 149.2; ESI-MS obsd 450.0155, calcd 450.0159 [(M + Na)$^+$, M = $C_{17}H_{19}BrNO_7$].

4,6-Dibromo-5-propargyloxy-1*H*-indol-3-yl β-D-glucopyranoside (27). Propargyl bromide (18 μL, 80% in toluene, 0.16 mmol) was added to a suspension of **18** (68 mg, 0.10 mmol) and K_2CO_3 (17 mg, 0.12 mmol) in DMF (0.80 mL) at room temperature. The mixture was stirred at room temperature for 2 h and then treated with MeOH (0.40 mL) and NaOMe solution (60 μL, 0.5 M in MeOH, 0.030 mmol). After 1 h, the reaction mixture was quenched by the addition of acetic acid (40 μL) and concentrated under reduced pressure. Column chromatography [silica, CH_2Cl_2/MeOH, 9 : 1 to 17 : 3] afforded a white solid (45 mg, 89%): mp 179–181 °C; ^{1}H NMR (700 MHz, CD_3OD) δ 2.94 (t, J = 2.6 Hz, 1H), 3.37–3.42 (m, 2H), 3.42–3.49 (m, 1H), 3.54 (dd, J = 7.9, 9.1 Hz, 1H), 3.68–3.74 (m, 1H), 3.92 (dd, J = 1.5, 11.9 Hz, 1H), 4.68 (d, J = 2.6 Hz, 2H), 4.75 (d, J = 7.8 Hz, 1H), 7.25 (s, 1H), 7.50 (s, 1H); ^{13}C NMR (175 MHz, CD_3OD) δ 61.6, 62.7, 71.6, 75.3, 76.8, 78.1, 78.3, 79.5, 105.1, 108.1, 112.3, 115.2, 115.9, 119.8, 133.1, 139.1, 145.8, ESI-MS obsd 527.9260, calcd 527.9264 [(M + Na)$^+$, M = $C_{17}H_{17}Br_2NO_7$].

1-Acetyl-5-{[(1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-yl]methoxy}-4,6-dibromo-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (29). Diisopropyl azodicarboxylate (39.4 μL, 0.20 mmol) was added to a solution of **18** (67.9 mg, 0.10 mmol), **28** (16.5 mg, 0.11 mmol), and PPh_3 (52.5 mg, 0.20 mmol) in CH_2Cl_2 (0.50 mL) at room temperature. After 1.5 h, the reaction mixture was passed through silica (ethyl acetate as eluent). The eluent was concentrated and further purified by chromatography [silica, hexanes/acetone (2 : 1) followed by hexanes/ethyl acetate (1 : 1)] to afford a white solid (51.1 mg, 63%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03–1.10 (m, 2H), 1.60–1.82 (m, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.18–2.40 (m, 6H), 2.60 (s, 3H), 3.89 (ddd, J = 2.3, 5.1, 9.7 Hz, 1H), 4.10 (d, J = 7.2 Hz, 2H), 4.20 (dd, J = 5.1, 12.5 Hz, 1H), 4.38 (dd, J = 2.3, 12.5 Hz, 1H), 5.06 (d, J = 7.6 Hz, 1H), 5.21 (dd, J = 9.2, 9.7 Hz, 1H), 5.31 (dd, J = 9.2, 9.2 Hz, 1H), 5.39 (dd, J = 7.6, 9.2 Hz, 1H), 7.25 (s, 1H), 8.70 (br s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 19.2, 20.7, 20.8, 20.9, 21.1, 21.7, 23.9, 29.5, 62.0, 68.3, 70.9, 72.0, 72.6, 72.7, 99.1, 100.3, 107.9, 112.0, 116.6, 120.4, 123.2, 131.0, 140.5, 150.1, 168.0, 169.4, 169.6, 170.4, 170.6; ESI-MS obsd 810.0761, calcd 810.0755 [(M + H)$^+$, M = $C_{34}H_{37}Br_2NO_{12}$].

5-{[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl]methoxy}-4,6-dibromo-1*H*-indol-3-yl β-D-glucopyranoside (30). K_2CO_3 (2.8 mg, 0.020 mmol) was added to a solution of **29** (16.2 mg, 0.020 mmol) in MeOH/THF (4 : 1, 200 μL) at room temperature. After 1 h, the reaction mixture was diluted with CH_2Cl_2 and passed through silica [CH_2Cl_2/MeOH (2 : 1) as an eluent] to afford a white solid (11.9 mg, 99%): ^{1}H NMR (700 MHz, CD_3OD) δ 0.97–1.06 (m, 2H), 1.65–1.77 (m, 3H), 2.14–2.21 (m, 2H), 2.21–2.34 (m, 4H), 3.37–3.44 (m, 2H), 3.44–3.52 (m, 1H), 3.55 (dd, J = 8.1, 8.9 Hz, 1H), 3.68–3.75 (m, 1H), 3.92 (d, J = 11.8 Hz, 1H), 4.08 (d, J = 7.8 Hz, 2H), 4.74 (d, J = 7.7 Hz, 1H), 7.24 (s, 1H), 7.49 (s, 1H); ^{13}C NMR (175 MHz, CD_3OD) δ 20.1, 21.7, 22.0, 30.6, 62.7, 71.5, 72.8, 75.3, 78.1, 78.3, 99.6, 105.2, 107.9, 112.5, 115.2, 116.0, 119.9, 132.8, 139.0, 146.9; ESI-MS obsd 600.0238, calcd 600.0227 [(M + H)$^+$, M = $C_{24}H_{28}Br_2NO_7$].

2-[2-(2-Hydroxyethoxy)ethoxy]ethyl 2-nitrobenzenesulfonate (31). Triethylamine (1.53 mL, 11.0 mmol) was added to a suspension of 2-nitrobenzenesulfonyl chloride (2.216 g, 10.0 mmol) in triethylene glycol (26.7 mL, 200 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature. After 30 min, the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with aqueous citric acid (10%, 100 mL) and brine (50 mL), then dried (Na_2SO_4) and filtered. The filtrate was concentrated and purified by chromatography [silica, hexanes/acetone (2 : 3)] to afford a clear pale yellow oil (2.929 g, 87%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.41 (br s, 1H), 3.54–3.59 (m, 2H), 3.59–3.66 (m, 4H), 3.66–3.75 (m, 2H), 3.76–3.83 (m, 2H), 4.38–4.47 (m, 2H), 7.74–7.79 (m, 1H), 7.79–7.85 (m, 2H), 8.13–8.20 (m, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 61.9, 68.7, 70.4, 70.9, 71.2, 72.5, 125.0, 129.9, 131.5, 132.5, 134.9, 148.4; ESI-MS obsd 336.0735, calcd 336.0748 [(M + H)$^+$, M = $C_{12}H_{17}NO_8S$].

1-Acetyl-4,6-dibromo-5-[1-hydroxy-3,6,9-trioxanon-9-yl]-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (32). A sample of i-Pr$_2$EtN (66 μL, 0.38 mmol) was added to a suspension of **18** (172.0 mg, 0.253 mmol) and **31** (110.4 mg, 0.329 mmol) in CH_2Cl_2 (253 μL) at room temperature. The reaction mixture was heated to 35 °C for 24 h and then allowed to cool to room temperature. The reaction mixture was diluted with ethyl acetate (2 mL), washed with aqueous HCl (1 M, 2 mL) and brine (2 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated and purified by chromatography (silica, ethyl acetate as an eluent) to afford a white solid (182.7 mg, 89%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.44 (br s, 1H), 2.60 (s, 3H), 3.64 (t, 2H), 3.71–3.78 (m, 4H), 3.78–3.84 (m, 2H), 3.89 (ddd, J = 2.5, 5.2, 9.9 Hz, 1H), 4.17–4.23 (m, 2H), 3.97 (t, 2H), 4.20 (dd, J = 5.2, 12.4 Hz, 1H), 4.38 (dd, J = 2.5, 12.4 Hz, 1H), 5.05 (d, J = 7.6 Hz, 1H), 5.20 (dd, J = 9.6, 9.9 Hz, 1H), 5.30 (dd, J = 9.4, 9.6 Hz, 1H), 5.38 (dd, J = 7.6, 9.4 Hz, 1H), 7.25 (s, 1H), 8.69 (br s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 20.7, 20.9, 21.1, 23.9, 61.9, 62.0, 68.3, 70.3, 70.6, 70.9, 71.0, 72.6, 72.7, 100.3, 107.7, 112.1, 116.3, 120.4, 123.2, 131.1, 140.5, 149.8, 168.0, 169.4, 169.5, 170.3, 170.6; ESI-MS obsd 832.0399, calcd 832.0422 [(M + Na)$^+$, M = $C_{30}H_{37}Br_2NO_{15}$].

4,6-Dibromo-5-[1-hydroxy-3,6,9-trioxanon-9-yl]-1*H*-indol-3-yl β-D-glucopyranoside (33). A suspension of **32** (10.2 mg, 0.013 mmol) and K_2CO_3 (0.4 mg, 0.003 mmol) in MeOH (250 μL) was stirred for 30 min at room temperature. The reaction mixture was quenched by the addition of AcOH (0.4 μL), diluted with CH_2Cl_2, and then passed through silica gel [CH_2Cl_2/MeOH (2 : 1) as eluent]. The eluent was concentrated under reduced pressure. The residue was triturated with MeOH/ethyl acetate/hexanes to afford a white solid (7.1 mg, 94%): ^{1}H NMR (700 MHz, CD_3OD) δ 7.49 (s, 1H), 7.25 (s, 1H), 4.74 (d, J = 7.7 Hz, 1H), 4.14 (t, J = 4.9 Hz, 2H), 3.95 (t, J = 4.9 Hz, 2H), 3.92 (d, J = 11.8 Hz, 1H), 3.82–3.77 (m, 2H), 3.74–3.65 (m, 5H), 3.59 (t, J = 4.8 Hz, 2H), 3.54 (dd, J = 7.8, 9.2 Hz, 1H), 3.50–3.43 (m, 1H), 3.43–3.37 (m, 2H); ^{13}C NMR (175 MHz, CD_3OD) δ 146.8, 139.0, 132.9, 119.9, 116.0, 115.2, 112.2, 107.7, 105.1, 78.3, 78.1, 75.3, 73.7, 73.6, 71.7, 71.54, 71.50, 71.3, 62.7, 62.3; HRMS (ESI-TOF) m/z: $[M + Na]^+$ calcd for $C_{20}H_{27}Br_2NNaO_{10}$ 621.9894; found 621.9891.

6-{4-[Bis(2-methoxyethyl)amino]phenyl}-2,4-dichloro-1,3,5-triazine (35). A mixture of **34** (523.2 mg, 2.50 mmol), cyanuric chloride (553.2 mg, 3.00 mmol), and 2,6-di-*tert*-butylpyridine (1.10 mL, 5.00 mmol) was heated to 140 °C for 18 h. The reaction mixture was cooled to room temperature and directly purified by column chromatography [silica, hexanes/acetone (18 : 1)] followed by recrystallization (hexanes) to afford a yellow solid (207.2 mg, 23%): mp 73–75 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 3.36 (s, 6H), 3.59 (t, J = 6.0 Hz, 4H), 3.69 (t, J = 6.0 Hz, 4H), 6.74, (d, J = 8.9 Hz, 2H), 8.30, (d, J = 8.9 Hz, 2H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 51.2, 59.2, 70.0, 111.5, 119.5, 132.4, 153.3, 171.0, 173.8; ESI-MS obsd 357.0888, calcd 357.0880 $[(M + H)^+$, M = $C_{15}H_{184}Cl_2N_4O_2]$; λ_{abs} (DMF) 369 nm; λ_{em} (DMF) 423 nm.

(10-[6-{4-[Bis(2-methoxyethyl)amino]phenyl}-4-chloro-1,3,5-triazin-2-yl]-1,4,7,10-tetraoxadec-1-yl)-1-acetyl-4,6-dibromo-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (36). A mixture of **35** (12.6 mg, 0.035 mmol), **32** (31.2 mg, 0.038 mmol), and powdered molecular sieves 3 Å (16.0 mg) in MeCN (160 μL) was treated with i-Pr_2EtN (16.7 μL, 0.096 mmol) at room temperature. The reaction mixture was heated to 50 °C for 2.5 h, 80 °C for 8 h, 90 °C for 9.5 h, and 110 °C for 3 h. After allowing to cool to room temperature, the reaction mixture was passed through a silica pad (acetone as eluent). The eluent was concentrated under reduced pressure. Column chromatography [silica, hexanes/acetone (1 : 1)] afforded a pale yellow solid (5.9 mg, 15%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.04 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.59 (s, 3H), 3.36 (s, 6H), 3.58 (t, J = 6.0 Hz, 4H), 3.67 (t, J = 6.0 Hz, 4H), 3.76–3.83 (m, 4H), 3.86–3.99 (m, 6H), 4.14–4.22 (m, 3H), 4.38 (dd, J = 2.5, 12.4 Hz, 1H), 4.63–4.68 (m, 2H), 5.05 (d, J = 7.6 Hz, 1H), 5.20 (dd, J = 9.2, 9.7 Hz, 1H), 5.30 (dd, J = 8.5, 9.2 Hz, 1H), 5.38 (dd, J = 7.6, 8.5 Hz, 1H), 6.72 (d, J = 8.8 Hz, 2H), 7.25 (s, 1H), 8.30 (d, J = 8.8 Hz, 2H), 8.68 (br s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 20.7, 20.9, 21.1, 23.9, 51.1, 59.2, 62.0, 67.8, 68.3, 69.1, 70.1, 70.3, 70.9, 71.0, 71.1, 72.6, 72.66, 72.74, 100.3, 107.7, 111.2, 112.2, 116.4, 120.4, 121.0, 123.2, 131.0, 131.8, 140.5, 149.9, 152.4, 167.9, 169.4, 169.5, 170.3, 170.6, 170.9, 171.7, 174.3; ESI-MS obsd 1130.1635, calcd 1130.1643 $[(M + H)^+$, M = $C_{45}H_{54}Br_2ClN_5O_{17}]$.

4-{(1-[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl]-3-oxo-2,7,10-trioxa-4-azadodecan-12-yl)amino}-6-{4-[bis(2-methoxyethyl)amino]phenyl}-2-{10-[1-acetyl-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-4,6-dibromo-1*H*-indol-5-yl]-1,4,7,10-tetraoxadec-1-yl}-1,3,5-triazine (37). Samples of **14** (2.4 mg, 7 μmol) and i-Pr_2EtN (1.2 μL, 7 μmol) were added to a solution of **36** in MeCN (47 μL) at room temperature. After 1 h, MeOH (188 μL) and K_2CO_3 (1.3 mg, 9 μmol) were added. After 2.5 h, the reaction mixture was diluted with CH_2Cl_2 and passed through a silica pad [CH_2Cl_2/MeOH (5 : 1) as eluent]. The eluent was concentrated under reduced pressure. Preparative TLC [silica, 0.25 mm, 20 × 20 cm, CH_2Cl_2/MeOH (15 : 1)] afforded a pale yellow solid (3.9 mg, 69%): ^{1}H NMR (700 MHz, CD_3OD, mixture of rotamers) δ 0.82–0.91 (m, 2H), 1.26–1.36 (m, 1H), 1.46–1.58 (m, 2H), 2.06–2.25 (m, 6H), 3.25–3.33 (m, 1H), 3.35 (s, 6H), 3.36–3.43 (m, 1H), 3.43–3.81 (m, 29H), 3.86–3.95 (m, 3H), 4.04–4.17 (m, 3H), 4.53–4.64 (m, 3H), 4.72–4.77 (m, 1H), 6.71–6.78 (m, 2H), 7.22–7.26 (m, 1H), 7.46 (s, 1H), 8.11–8.24 (m, 2H); ^{13}C NMR (175 MHz, CD_3OD, mixture of rotamers) δ 18.9, 21.3, 21.9, 30.1, 41.6, 41.7, 41.7, 51.9, 59.3, 62.7, 63.7, 67.1, 67.3, 70.5, 70.6, 70.7, 71.0, 71.27, 71.31, 71.4, 71.6, 71.8, 73.7, 75.3, 78.1, 78.3, 99.5, 105.1, 105.2, 107.7, 112.0, 112.3, 115.0, 115.1, 116.0, 119.9, 123.9, 124.2, 131.4, 131.6, 132.9, 139.0, 146.8, 152.5, 152.6, 159.2, 168.4, 168.7, 171.8, 172.1, 173.6, 174.0; ESI-MS obsd 1208.3363, calcd 1208.3397 $[(M + H)^+$, M = $C_{52}H_{71}Br_2N_7O_{16}]$; λ_{abs} (DMF) 341 nm; λ_{em} (DMF) 397 nm.

3-[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]propane-1-sulfonic acid (39). A sample of 1-(*tert*-butoxycarbonyl)piperazine (**38**, 2.011 g, 10.8 mmol) was added to a solution of 1,3-propane sultone (1.319 g, 10.8 mmol) in 1,4-dioxane (5.40 mL) at room temperature. The reaction mixture was heated to 60 °C for 1 h and then allowed to cool to room temperature. The precipitate was filtered and washed with ethyl acetate to afford a white solid (2.106 g, 63%): mp 210 °C (dec.); ^{1}H NMR (700 MHz, D_2O) δ 1.47 (s, 9H), 2.17–2.28 (m, 2H), 3.02 (t, J = 7.3 Hz, 2H), 3.20–3.39 (m, 2H), 2.65–3.91 (m, 6H), 4.23 (br s, 2H); ^{13}C NMR (175 MHz, D_2O) δ 20.3, 28.5, 41.5, 48.7, 52.5, 56.4, 83.8, 156.5; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.42 (s, 9H), 2.00 (tt, J = 6.8, 6.5 Hz, 2H), 2.65 (t, J = 6.5 Hz, 2H), 2.86–3.14 (m, 4H), 3.20–3.26 (m, 2H), 3.49 (d, J = 15.0 Hz, 2H), 4.03 (d, J = 15.0 Hz, 2H), 9.96 (s, 1H); ESI-MS obsd 309.1474, calcd 309.1479 $[(M + H)^+$, M = $C_{12}H_{24}N_2O_5S]$.

3-(4-(*tert*-Butoxycarbonyl)-1-(3-hydroxypropyl)piperazin-1-ium-1-yl)propane-1-sulfonate (40). 3-Bromopropanol (2.17 mL, 24 mmol) was added to a mixture of **39** (1.234 g, 4.00 mmol), $NaHCO_3$ (2.688 g, 32.0 mmol), and KI (132.8 mg, 0.80 mmol) in H_2O (1.09 mL) at room temperature. The reaction mixture was heated to 80 °C for 15 h, allowed to cool to room temperature, and washed with Et_2O (20 mL). The residue was suspended in CH_2Cl_2/MeOH (4 : 1, 25 mL) and filtered. The filtrate was concentrated and purified by chromatography [silica, CH_2Cl_2/MeOH (4 : 6)] to afford a white solid (1.011 g, 69%): ^{1}H NMR (700 MHz, CD_3OD) δ 1.48 (s, 9H), 1.95–2.03 (m, 2H), 2.14–2.24 (m, 2H), 2.90 (t, J = 6.6 Hz, 2H), 3.46–3.60 (m, 6H), 3.64–3.72 (m, 4H), 3.81 (br s, 4H); ^{13}C NMR (175 MHz, CD_3OD) δ 18.7, 25.5, 28.5, 37.8, 39.0, 48.4, 57.4, 57.7, 59.3, 82.4, 155.5, ESI-MS obsd 367.1896, calcd 367.1897 $[(M + H)^+$, M = $C_{15}H_{30}N_2O_6S]$.

3-(1-(3-Hydroxypropyl)piperazin-1-ium-1-yl)propane-1-sulfonate trifluoroacetic acid salt (41). A sample of **40** (980.2 mg, 2.67 mmol)

was dissolved in trifluoroacetic acid (1.78 mL) at room temperature. After 2 h, the reaction mixture was concentrated under reduced pressure. The residue was triturated with EtOH/Et_2O to afford a pale yellow solid (985.4 mg, 97%): ^{1}H NMR (700 MHz, CD_3OD) δ 1.95–2.10 (m, 2H), 2.15–2.30 (m, 2H), 2.97 (t, J = 6.5 Hz, 2H), 3.60–3.95 (m, 14H); ^{13}C NMR (175 MHz, CD_3OD) δ 18.9, 25.6, 38.8, 48.2, 56.4, 58.3 (br s), 58.8 (br s), 59.1, 163.1 (q, J = 34.5 Hz); ^{19}F (564 MHz, CD_3OD) δ −76.9 ppm (*versus* the reference $CFCl_3$); ESI-MS obsd 267.1371, calcd 267.1373 [$(M - CF_3CO_2H + H)^+$, M = $C_{12}H_{23}F_3N_2O_6S$].

4,6-Dibromo-5-[1-hydroxy-3,6,9-trioxanon-9-yl]-1*H*-indol-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (42). A suspension of **32** (811.4 mg, 1.00 mmol) and $NaHCO_3$ (8.4 mg, 0.10 mmol) in MeOH (5.00 mL) was stirred for 3.5 h at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was suspended in ethyl acetate. The suspension was passed through silica (ethyl acetate as eluent). The eluent was concentrated under reduced pressure to afford a white solid (650.1 mg, 84%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.04 (s, 3H), 2.05 (s, 3H), 2.096 (s, 3H), 2.103 (s, 3H), 2.50 (br s, 1H), 3.62–3.68 (m, 2H), 3.71–3.78 (m, 4H), 3.78–3.84 (m, 3H), 3.94–4.00 (m, 2H), 4.13–4.20 (m, 2H), 4.24 (dd, J = 4.8, 12.3 Hz, 1H), 4.27 (dd, J = 2.8, 12.3 Hz, 1H), 4.97 (d, J = 7.8 Hz, 1H), 5.19 (dd, J = 9.5, 9.6 Hz, 1H), 5.29 (dd, J = 9.3, 9.5 Hz, 1H), 5.37 (dd, J = 7.8, 9.6 Hz, 1H), 7.07 (d, J = 2.7 Hz, 1H), 7.45 (s, 1H), 7.94 (br s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 20.6, 20.7, 20.8, 21.1, 61.7, 61.9, 68.4, 70.2, 70.4, 70.7, 71.0, 71.9, 72.4, 72.5, 72.9, 101.0, 106.6, 112.0, 114.5, 115.2, 118.7, 131.3, 136.7, 145.9, 169.5, 169.6, 170.3, 170.7; ESI-MS obsd 768.0494, calcd 768.0497 [$(M + H)^+$, M = $C_{28}H_{35}Br_2NO_{14}$].

4-{(1-[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl]-3-oxo-2,7,10-trioxa-4-azadodecan-12-yl)amino}-6-[4-(3-hydroxypropyl)-4-(3-sulfopropyl)-piperazin-1-yl]-2-{10-[1-acetyl-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-4,6-dibromo-1*H*-indol-5-yl]-1,4,7,10-tetraoxadec-1-yl}-1,3,5-triazine (43). Cyanuric chloride (20.3 mg, 0.11 mmol) was added to a mixture of **42** (76.9 mg, 0.10 mmol), 1,10-phenanthroline (36.0 mg, 0.20 mmol), and powdered molecular sieves 4 Å (50.0 mg) in CH_2Cl_2 (0.50 mL) at room temperature. After 16 h, **41** (49.4 mg, 0.13 mmol) in DMF (0.50 mL) and i-Pr_2EtN (70 μL, 0.40 mmol) were added. After 3 h, a solution of **14** (35.7 mg, 0.11 mmol) in CH_2Cl_2 (300 μL) and a sample of i-Pr_2EtN (35 μL, 0.20 mmol) were added. After 4 h, i-Pr_2EtN (35 μL, 0.20 mmol) was added. After 15 h, the reaction mixture was diluted with CH_2Cl_2 (3 mL) and filtered. The filtrate was washed with aqueous citric acid (10%, 3 mL) and brine (3 mL), then dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure. Column chromatography [diol-functionalized silica (ethyl acetate/MeOH = 19 : 1 to CH_2Cl_2/MeOH = 5 : 1) followed by silica (CH_2Cl_2/MeOH = 5 : 1)] enabled removal of multiple byproducts and afforded a white solid (72.5 mg, 51%): ^{1}H NMR (700 MHz, $CDCl_3$, mixture of rotamers) δ 0.91 (m, 2H), 1.26–1.38 (m, 1H), 1.54 (br s, 2H), 1.80–2.00 (m, 2H), 2.01 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 2.11–2.33 (m, 9H), 2.90 (br s, 2H), 3.15–4.47 (m, 42H), 4.75 (br s, 1H), 4.92 (s, 1H), 5.16 (t, J = 9.1 Hz, 1H), 5.21–5.36 (m, 2H), 5.43 (s, 0.5H), 5.53 (s, 0.5H), 5.81 (br s, 0.5H), 6.05 (br s, 0.5H), 7.13 (s, 1H), 7.62 (s, 1H), 10.3 (s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$, mixture of rotamers) δ 17.8, 17.9, 20.1, 20.7, 20.9, 21.1, 21.5, 24.6, 29.1, 36.8, 40.5, 40.7, 40.8, 41.5, 47.4, 53.5, 56.4, 57.0, 58.3, 61.9, 62.7, 66.0, 66.1, 68.4, 69.3, 69.4, 69.7, 69.8, 70.1, 70.20, 70.23, 70.7, 70.8, 71.1, 71.9, 72.5, 73.0, 98.9, 101.1, 106.2, 111.6, 114.7, 115.7, 118.3, 131.5, 136.5, 145.6, 156.9, 165.6, 165.8, 166.7, 167.2, 169.5, 169.6, 170.2, 170.4, 170.8; ESI-MS obsd 717.1884, calcd 717.1888 [$(M + 2H)^{2+}$, M = $C_{58}H_{82}Br_2N_8O_{22}S$].

4-{(1-[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl]-3-oxo-2,7,10-trioxa-4-azadodecan-12-yl)amino}-6-[4-(3-hydroxypropyl)-4-(3-sulfopropyl)-piperazin-1-yl]-2-{10-[[β-D-glucopyranosyloxy]-4,6-dibromo-1*H*-indol-5-yl]-1,4,7,10-tetraoxadec-1-yl}-1,3,5-triazine (44). A sample of K_2CO_3 (0.3 mg, 2 μmol) was added to a solution of **43** in MeOH/CH_2Cl_2 (25 : 6, 310 μL) at room temperature. After 2 h, the reaction mixture was passed through diol-functionalized silica [CH_2Cl_2/MeOH (2 : 1) as eluent]. The eluent was concentrated under reduced pressure to afford a white solid (12.5 mg, 98%): ^{1}H NMR (700 MHz, CD_3OD, mixture of rotamers) δ 0.85–0.97 (m, 2H), 1.28–1.41 (m, 1H), 1.50–1.63 (m, 2H), 1.88–1.99 (m, 2H), 2.07–2.29 (m, 8H), 2.79–2.91 (m, 2H), 3.24–3.33 (m, 2H), 3.37–4.25 (m, 44H), 4.42–4.61 (m, 2H), 4.78 (d, J = 8.3 Hz, 1H), 7.27 (d, J = 6.8 Hz, 1H), 7.53 (s, 1H); ^{13}C NMR (175 MHz, CD_3OD, mixture of rotamers) δ 18.7, 19.0, 21.4, 21.9, 22.0, 25.5, 30.2, 37.9, 38.0, 41.5, 41.7, 48.3, 49.5, 54.8, 55.1, 57.6, 57.7, 59.3, 59.46, 59.52, 62.56, 62.62, 63.7, 67.2, 67.3, 70.6, 70.7, 71.0, 71.1, 71.3, 71.4, 71.50, 71.55, 71.85, 71.88, 73.8, 75.3, 78.2, 78.30, 78.32, 99.6, 105.1, 105.2, 107.7, 112.4, 116.2, 132.9, 139.0, 146.8, 159.2, 166.9, 167.2, 168.2, 168.6, 171.8, 172.2; ESI-MS obsd 633.1670, calcd 633.1677 [$(M + 2H)^{2+}$, M = $C_{50}H_{74}Br_2N_8O_{18}S$].

4,4′,6,6′-Tetrabromo-5,5′-bis[1-hydroxy-3,6,9-trioxanon-9-yl]-indigo (46). Samples of **33** (4.98 mg, 8.28 μmol) in DMF (414 μL), β-glucosidase in water (from almonds, 10 units/mL, 828 μL), and acetate buffer (pH 5.0, 50 mM + 5% DMF, 7038 μL) were mixed at room temperature. The reaction mixture was incubated at 37 °C under air for 22 h and then allowed to cool to room temperature. The reaction mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (20 mL). The organic layer was washed with brine (10 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. Preparative thin layer chromatography [silica, 0.25 mm, $CHCl_3$/MeOH (12 : 1)] afforded an indigo-blue solid (2.4 mg, 66%): ^{1}H NMR (700 MHz, $CDCl_3/CD_3OD$ = 9 : 1) δ 3.60–3.66 (m, 4H), 3.69–3.76 (m, 8H), 3.79–3.84 (m, 4H), 3.94–4.00 (m, 4H), 4.16–4.21 (m, 4H), 7.31 (s, 1H); ^{13}C NMR (175 MHz, $CDCl_3/CD_3OD$ = 9 : 1) δ 61.6, 70.2, 70.4, 70.9, 72.78, 72.79, 115.3, 116.0, 118.1, 122.3, 127.4, 147.9, 149.6, 185.7; ESI-MS obsd 870.8692, calcd 870.8707 [$(M + H)^+$, M = $C_{28}H_{30}Br_4N_2O_{10}$].

2,4-Bis{10-[[1-acetyl-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-4,6-dibromo-1*H*-indol-5-yl]-1,4,7,10-tetraoxadec-1-yl]}-6-chloro-1,3,5-triazine (47). Pempidine (38.1 μL, 0.21 mmol) was added to a mixture of cyanuric chloride (11.1 mg, 0.060 mmol), **32** (102.6 mg, 0.13 mmol), and powdered molecular sieves 4 Å (12.0 mg) in 1,2-dichloroethane (120 μL) at room temperature. The reaction mixture was heated to 60 °C for 13 h, allowed to cool to room temperature, and passed through a silica pad (ethyl acetate as eluent). The eluent was concentrated under

reduced pressure. The putative *N*-deacetylated byproduct showed similar chromatographic properties as the title compound. Preparative thin layer chromatography [silica, 1.0 mm, 20 × 20 cm, hexanes/acetone (6 : 4)] afforded a white solid (55.7 mg, 53%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.04 (s, 6H), 2.07 (s, 6H), 2.091 (s, 6H), 2.093 (s, 6H), 2.60 (s, 6H), 3.70–3.73 (m, 4H), 3.73–3.82 (m, 4H), 3.83–3.94 (m, 6H), 3.94–3.97 (m, 4H), 4.13–4.23 (m, 6H), 4.38 (dd, J = 1.5, 12.3 Hz, 2H), 4.55–4.62 (m, 4H), 5.05, (d, J = 7.6 Hz, 2H), 5.17–5.23 (m, 2H), 5.30 (dd, J = 9.3, 9.3 Hz, 2H), 5.34–5.41 (m, 2H), 7.25 (s, 2H), 8.68 (br s, 2H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 20.7, 20.9, 21.1, 23.8, 62.0, 68.4, 68.5, 68.9, 70.3, 70.9, 71.0, 72.6, 72.7, 100.3, 107.7, 112.2, 116.3, 120.3, 123.1, 131.0, 140.5, 149.8, 167.9, 169.4, 169.5, 170.3, 170.6, 172.5, 172.7; ESI-MS obsd 1730.0752, calcd 1730.0757 [$(M + H)^+$, M = $C_{63}H_{72}Br_4ClN_5O_{30}$].

2,4-Bis{10-[[3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-4,6-dibromo-1*H*-indol-5-yl]-1,4,7,10-tetraoxadec-1-yl]}-6-[4-(3-hydroxypropyl)-4-(3-sulfopropyl)piperazin-1-yl]-1,3,5-triazine (48). A sample of i-Pr_2EtN (19.2 μL, 0.11 mmol) was added to a solution of **47** (190.8 mg, 0.11 mmol) in CH_2Cl_2/MeOH (5 : 1, 0.84 mL) at room temperature. After 4 h, **41** (46.0 mg, 0.12 mmol) in MeOH (0.70 mL) and 2,6-lutidine (25.5 μL, 0.22 mmol) were added. After 4 h, the reaction mixture was quenched by the addition of acetic acid (12.6 μL, 0.22 mmol) and concentrated under reduced pressure. Column chromatography [silica, CH_2Cl_2/MeOH (7 : 1 to 5 : 1)] followed by trituration with H_2O afforded a pale yellow solid (114.7 mg, 55%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.78 (br s, 2H), 1.96–2.16 (m, 26H), 2.88 (br s, 2H), 3.15 (br s, 2H), 3.23 (br s, 2H), 3.36 (br s, 2H), 3.54 (br s, 4H), 3.68–3.99 (m, 22H), 4.04 (br s, 4H), 4.16–4.52 (m, 9H), 4.89 (d, J = 7.3 Hz, 2H), 5.16 (dd, J = 9.4, 9.4 Hz, 2H), 5.22–5.33 (m, 4H), 7.13 (s, 2H), 7.59 (s, 1H), 7.59 (s, 1H), 9.96 (s, 1H), 9.99 (s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 17.9, 20.7, 20.9, 21.2, 24.6, 37.0, 47.4, 56.4, 56.8, 58.0, 58.2, 61.9, 67.0, 68.4, 69.2, 70.2, 70.6, 70.7, 71.1, 71.8, 72.4, 73.0, 101.2, 0106.2, 111.6, 114.9, 115.7, 118.3, 131.4, 136.5, 145.5, 166.4, 169.6, 169.6, 170.2, 170.8, 171.6; ESI-MS obsd 1876.2076, calcd 1876.2079 [$(M + H)^+$, M = $C_{69}H_{89}Br_4N_7O_{32}S$].

2,4-Bis{10-[[3-(β-D-glucopyranosyloxy)-4,6-dibromo-1*H*-indol-5-yl]-1,4,7,10-tetraoxadec-1-yl]}-6-[4-(3-hydroxypropyl)-4-(3-sulfopropyl)piperazin-1-yl]-1,3,5-triazine (49). A sample of K_2CO_3 (0.6 mg, 4 μmol) was added to a solution of **48** (39.3 mg, 0.020 mmol) in MeOH/CH_2Cl_2 (5 : 1, 600 μL) at room temperature. After 15 min, H_2O (50 μL) was added. After 2 h, H_2O (150 μL) and K_2CO_3 (2.2 mg, 16 μmol) were added. After 1 h, reverse phase silica (320 mg) was added. The mixture was dried under reduced pressure. The residue was purified by column chromatography [reverse phase silica, H_2O to MeOH/H_2O (4 : 1)] to afford a pale yellow solid (23.9 mg, 77%): ^{1}H NMR [700 MHz, $(CD_3)_2SO$] δ 1.78–1.87 (m, 2H), 1.93–2.02 (m, 2H), 2.47–2.56 (m, 2H), 3.15 (t, J = 9.1 Hz, 2H), 3.22–3.36 (m, 6H), 3.38–3.55 (m, 11H), 3.55–3.69 (m, 11H), 3.69–3.78 (m, 6H), 3.78–3.86 (m, 4H), 3.93–4.05 (m, 6H), 4.05–4.15 (m, 2H), 4.36–4.45 (m, 4H), 4.56–4.64 (m, 2H), 4.65 (d, J = 7.6 Hz, 2H), 4.78 (br s, 1H), 4.95–5.14 (m, 6H), 7.23 (s, 2H), 7.55 (s, 2H), 10.92 (s, 2H); ^{13}C NMR [175 MHz, $(CD_3)_2SO$] δ 17.7, 24.1, 36.8, 47.3, 56.9, 57.6, 60.9, 66.4, 68.4, 69.4, 69.8, 69.9, 70.0, 72.4, 73.5, 76.8, 77.2, 99.5, 103.3, 106.1, 110.4, 113.3, 114.9, 117.9, 131.0, 137.4, 144.8, 166.4, 171.5; ESI-MS obsd 770.5642, calcd 770.5653 [$(M + 2H)^{2+}$, M = $C_{53}H_{73}Br_4N_7O_{24}S$].

Procedure for ε determination

(1) Indigo (13.1 mg, 50 μmol) was dissolved in DMF (200 mL) to prepare a 250 μM solution. An aliquot (320 μL) was withdrawn from the solution and diluted with DMF/H_2O (1680 : 1000, 2680 μL) to prepare a 40.0 μM solution. The absorption spectrum was recorded at room temperature. The average of two runs was calculated.

(2) Indigoid derivative **46** (1.6 mg, 1.8 μmol) was dissolved in $CHCl_3$/MeOH (2 : 1, 2.93 mL). An aliquot (64.0 μL) was withdrawn from the solution and concentrated under reduced pressure. The residue was dissolved in DMF/H_2O (2 : 1, 1000 μL) to prepare a 40 μM solution. The absorption spectrum was recorded at room temperature. ε_{631nm} = 2.6 × 10^4 M^{-1} cm^{-1}.

Procedures for experiments of Fig. 6

Effect of pH. A solution of **33** in DMF (2 μL, 5 mM) and a solution of β-glucosidase from *Agrobacterium* in NaPi–NaCl buffer (2 μL, 10 μM, pH 7.0) were mixed with various 50 mM sodium phosphate buffers (96 μL, pH 4.0, 5.0, 6.0, 7.0, 8.0, or 9.0). The reaction mixture was incubated at 37 °C for 10, 20, 40, 60, 90, 120, or 180 min and then diluted with DMF (200 μL) at 0 °C. After centrifugation for 1 min, the supernatant was analyzed by absorption spectroscopy. All the experiments were repeated three times.

Effect of the enzyme concentration. A solution of **33** in DMF (2 μL, 5 mM) and a solution of β-glucosidase from *Agrobacterium* in NaPi–NaCl buffer (1 μL, 1 μM, pH 7.0) were mixed with 50 mM NaPi (97 μL, pH 7.0). The reaction mixture was incubated at 37 °C for 2 h and then diluted with DMF (200 μL) at 0 °C. After centrifugation for 1 min, the supernatant was analyzed by absorption spectroscopy. All the experiments were repeated three times.

Effect of the concentration of 33. A solution containing **33** (1.00, 1.78, 3.16, 5.62, 10.0, 17.8, 31.6, or 56.2 μM) and β-glucosidase from *Agrobacterium* (200 nM) in NaPi–NaCl buffer (pH 7.0; containing 2% DMF) was incubated at 37 °C for 2 h. The total volume was 4000, 2000, 1500, 1200, 300, 300, 300, or 300 μL for the reaction at 1.00, 1.78, 3.16, 5.62, 10.0, 17.8, 31.6, or 56.2 μM, respectively. After the incubation, the reaction mixture was centrifuged for 5 min. Any precipitate was separated from the supernatant and dissolved in DMF (200–300 μL) followed by analysis by absorption spectroscopy. Additionally, the reaction with **33** at 1.00, 1.78, or 3.16 μM was carried out for a longer reaction time (14 h). All the experiments were repeated three times.

Procedures for indigogenic reactions in Table 1

Reactions with β-glucosidase from almonds. A solution of an indoxyl-glucoside in DMF (5 μL, 20 mM) and a solution of β-glucosidase from almonds in H_2O (10 μL, 10 units per mL) were mixed with acetate buffer (85 μL, pH 5.0). The reaction mixture was incubated at 37 °C for 16–19 h and then allowed to cool to room temperature. DMF (300 μL for the reactions of **15**, **16**, **1**, **19**, **22**, and **25**; or 900 μL for the reactions of **45**, **20**, **21**, **23**,

24, **26**, **27**, **30**, and **31**, respectively) was added to dissolve any indigoid precipitate. The resulting solution was analyzed by absorption spectroscopy.

Reactions with β-glucosidase from *Agrobacterium*. A solution of an indoxyl-glucoside in DMF (2 μL, 5 mM) and a solution of β-glucosidase from *Agrobacterium* in NaPi–NaCl buffer (2 μL, pH 7.0) were mixed with 50 mM NaPi buffer (96 μL, pH 7.0). The reaction mixture was incubated at 37 °C for 2 h and then centrifuged for 3 min. Any precipitate was separated from the supernatant and dissolved in DMF (200 μL). The resulting solution was analyzed by absorption spectroscopy. The experiment was repeated three times.

Reactions in rat liver homogenate. A solution of an indoxyl-glucoside in DMF (5 μL, 20 mM) was mixed with rat liver homogenate (95 μL). The reaction mixture was incubated at 37 °C for 24 h. After allowing to cool to room temperature, the reaction mixture was diluted with DMF (900 μL). The mixture was heated at 70 °C for 2 min and then centrifuged for 2 min. The supernatant was separated from any precipitate. This extraction procedure was repeated two or three times with DMF (100–500 μL). The combined supernatant (1500–1800 μL) was analyzed by absorption spectroscopy.

Reaction of 33 with β-glucosidase from *Agrobacterium* in rat liver homogenate. A DMF solution of **33** (2 μL, 5 mM) and a solution of β-glucosidase from *Agrobacterium* in NaPi–NaCl buffer (2 μL, pH 7.0) were mixed with rat liver homogenate (96 μL). The reaction mixture was incubated at 37 °C for 4 h and then centrifuged for 3 min. Any precipitate was separated from the supernatant and then suspended in DMF (200 μL). The suspension was centrifuged for 3 min. The supernatant was analyzed by absorption spectroscopy.

Oligomerization procedures

Oligomerization of 49 in NaPi–NaCl buffer. A solution of **49** (300, 100, 50, or 10 μM) and a solution of β-glucosidase from *Agrobacterium* (200 nM) in NaPi–NaCl buffer were mixed and incubated at 37 °C for 2–4 h. The total volume was 2000, 250, 300, or 2000 μL for the 300, 100, 50, or 10 μM reaction, respectively. After the incubation, the reaction mixture was centrifuged for 5 min. The supernatant was separated from any precipitate and analyzed by absorption spectroscopy. The precipitate was suspended in H_2O (the same volume as the reaction volume) and then centrifuged for 5 min. The precipitate was separated from the supernatant and dried under reduced pressure. The residue was dissolved in DMF/DMSO (4 : 1) to prepare a sample for absorption spectroscopy.

Low-salt oligomerization of 49. To minimize the NaCl content and thereby facilitate purification and analysis of the product, a solution of **49** in DMF (12 μL, 50.0 mM) and a solution of β-glucosidase from *Agrobacterium* in NaPi–NaCl buffer (40 μL, 10 μM) were mixed with 10 mM NaPi buffer (1948 μL, pH 7.0). The reaction mixture was incubated at 37 °C for 3 h and then centrifuged for 5 min. After the supernatant was removed, any precipitate was suspended in H_2O (1000 μL) and then centrifuged for 5 min. The supernatant was removed again. The precipitate was suspended in H_2O (1000 μL), from which 3 × 250 μL fractions (fraction A, B, and C) were withdrawn. Fractions A–C were individually centrifuged for 5 min, separated from the supernatant, and dried under reduced pressure to afford a blue solid in each case. The solid from Fraction A was dissolved in DMF/DMSO (9 : 1, 500 μL) to prepare a sample for absorption spectroscopy. The solid from Fraction B was dissolved in DMF/DMSO (9 : 1, 500 μL), and the resulting solution was filtered through a membrane filter (polytetrafluoroethylene, 0.45 μm) to prepare a sample ("precipitate extract") for HPLC analysis. The solid from Fraction C was suspended in H_2O (500 μL) to prepare a sample for optical microscopy, from which 40 μL was withdrawn and diluted 10-fold with H_2O to prepare a sample for DLS analysis. The combined supernatant was concentrated under reduced pressure. The residue was suspended in DMF. The suspension was filtered through cotton. The total volume of the filtrate was adjusted to 600 μL by adding DMF to prepare a solution ("supernatant extract") for analysis. An aliquot of the supernatant extract (30 μL) was diluted 10-fold with DMF to prepare a sample for absorption spectroscopy. The supernatant sample solution (200 μL) was filtered through a membrane filter (polytetrafluoroethylene, 0.45 μm) to prepare a sample for HPLC analysis. The supernatant sample solution (100 μL) was concentrated under reduced pressure to prepare a sample for MALDI-MS and ESI-MS analysis.

To prepare a sample for ^{1}H NMR analysis, the reaction was repeated on the same reaction scale. Instead of dividing the precipitate suspension in H_2O (1000 μL) into fractions A–C, the entire suspension was centrifuged for 5 min. The precipitate was separated from supernatant and dried under reduced pressure to afford a blue solid, which was dissolved in DMSO-d_6 (300 μL).

To determine a gravimetric yield of the precipitate, the reaction was carried out on a 5.10 μmol (7.87 mg) scale using 102 μL of **49** in DMF (50.0 mM). After the washing with H_2O, a blue precipitate (3.01 mg) was obtained, which corresponds to 49% based on the monomer formula weight (1215.55, $C_{41}H_{49}Br_4N_7O_{14}S$).

HPLC analysis. Analytical size-exclusion chromatography (SEC) was performed in DMF/DMSO (9 : 1, flow rate = 0.6 mL min^{-1}) at room temperature with three analytical SEC columns[62] in order (PL gel 5 μm 500 Å, 500 Å, and 1000 Å; 7.5 mm i.d. × 300 mm) using an HPLC instrument with absorption spectral detection. A calibration curve (Fig. S5, ESI†) was prepared with eight standard poly(2-vinylpyridine) samples (PSS polymer, M_W = 1110–256 000 Da, M_w/M_n = 1.04–1.24) in DMF (1 mg mL^{-1}).

Conflicts of interest

Patent applications have been filed encompassing this work for which authors HF, NM, ZW and JSL are coinventors.

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