DNA-catalyzed glycosylation using aryl glycoside donors

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Oligonucleotides, peptides, sugars, and DNA-anchored conjugates

Oligonucleotides and peptides. DNA oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA) or prepared by solid-phase synthesis on an ABI 394 instrument using reagents from Glen Research. All oligonucleotides were purified by 7 M urea denaturing PAGE with running buffer 1× TBE (89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3) as described previously.[1] Peptides were synthesized on the solid phase as described.[2]

Sugars. 2-Chloro-4-nitrophenyl β-D-glucuronide, the immediate precursor to DNA-anchored glycosyl donor conjugate **1a**, and 4-nitrophenyl α-D-glucuronide, the immediate precursor to DNA-anchored glycosyl donor conjugate **2b**, were synthesized as described in the final section of this ESI. 4-Nitrophenyl β-D-glucuronide, the immediate precursor to DNA-anchored glycosyl donor conjugate **1b**, was obtained from Chem-Impex (cat. no. 21811). Phenyl β-D-glucuronide, the immediate precursor to DNA-anchored glycosyl donor conjugate **1c**, was obtained from Carbosynth (cat. no. MP04835).

Preparation of DNA-anchored peptide conjugates. DNA-anchored peptide conjugates were synthesized by disulfide formation between a DNA HEG-tethered 3′-thiol and the N-terminal cysteine side chain of the peptide. The experimental procedure is provided in our recent report.[3]

Preparation of DNA-anchored glycosyl donor conjugates. The 5′-NH_2-DNA was prepared with a C_6 tether between the 5′-phosphate and the amino group. An aqueous 20 μL sample containing 2 nmol of 5′-NH_2-DNA, 100 mM MOPS pH 7.0, 30 mM *O*-arylglucuronic acid (from a 300 mM stock in DMF), and 450 mM DMT-MM[4] was incubated at room temperature for 24 h. The reaction was diluted to 800 μL with water, filtered, and purified by HPLC [Shimdazu Prominence instrument; Phenomenex Gemini-NX C_{18} column, 5 μm, 10 × 250 mm; gradient of 15% solvent A (20 mM triethylammonium acetate in 50% acetonitrile/50% water, pH 7.0) and 85% solvent B (20 mM triethylammonium acetate in water, pH 7.0) at 0 min to 30% solvent A and 70% solvent B at 45 min with flow rate of 3.5 mL/min]. Fractions were pooled, dried in a SpeedVac, and redissolved in water. See Table S2 for MALDI mass spectrometry analysis.

oligonucleotide purpose	oligonucleotide sequence
Selections with DNA 3′-OH or DNA-HEG-CAAYAA + **1a** (ArO-β-D-Glc-DNA)	
DNA 3′-OH substrate	GGATAATACGACTCACTAT
DNA-HEG-CAAYAA substrate	GGATAATACGACTCACTAT–HEG-CAAYAA
DNA-anchored glycosyl donor substrate	ArO-β-D-Glc–GAAGAGATGGCGACTTCG [a]
forward primer for selection	CGAAGTCGCCATCTCTTC
reverse primer for selection	$(AAC)_4$XCCATCAGGATCAGCT
random pool for selection	CGAAGTCGCCATCTCTTC-N_{40}-ATAGTGAGTCGTATTAAGCTGATCCTGATGG
splint for ligation step during selection [b]	ATAGTGAGTCGTATTATCCTCCATCAGGATCAGCTTAATACGACTCACTAT
Reselection of 11GV112 with DNA 3′-OH + **1a** (ArO-β-D-Glc-DNA)	
DNA 3′-OH substrate	GGATCTAGCACATCCCTAT
DNA-anchored glycosyl donor substrate	ArO-β-D-Glc–GAAACTTTTTAGATTTCG [c]
forward primer for selection	CGAAATCTAAAAAGTTTC
reverse primer for selection	$(AAC)_4$XCCATCAGGATCAGCT
partially randomized pool for selection [d]	CGAAATCTAAAAAGTTTC-N_{40}-ATAGGGATGTGCTAGAAGCTGATCCTGATGG
splint for ligation step during selection [b]	ATAGGGATGTGCTAGATCCTCCATCAGGATCAGCTTCTAGCACATCCCTAT
Selection with DNA 3′-OH + **2b** (ArO-α-D-Glc-DNA)	
DNA 3′-OH substrate	GGATCCTGGATACAAATAT
DNA-anchored glycosyl donor substrate	ArO-α-D-Glc–GAACAGGTTTATACTTCG [c]
forward primer for selection	CGAAGTATAAACCTGTTC
reverse primer for selection	$(AAC)_4$XCCATCAGGATCAGCT
random pool for selection	CGAAGTATAAACCTGTTC-N_{40}-ATATTTGTATCCAGGAAGCTGATCCTGATGG
splint for ligation step during selection [b]	ATATTTGTATCCAGGATCCTCCATCAGGATCAGCTTCCTGGATACAAATAT

Table S1. Oligonucleotide sequences used in this report. All sequences are written 5′ to 3′. In the reverse PCR primer for selection, X denotes the HEG spacer to stop Taq polymerase.

[a] The tabulated 18 nt sequence was used for deoxyribozyme assays. For the selection process itself, the 36 nt sequence that additionally included $(AAC)_6$ at its 3′-terminus was used in odd-numbered rounds, to enable a larger PAGE shift during the selection step. This was done to avoid survival of aberrantly migrating products that consistently migrate at the same gel position.

[b] The underlined T in the splint was absent in the round 1 ligation step because the DNA pool in this step was prepared by solid-phase synthesis and therefore did not have an untemplated A nucleotide at its 3′-end. The underlined T was included in all other selection rounds to account for the untemplated A nucleotide that is added at the 3′-end of each PCR product by Taq polymerase.

[c] The tabulated 18 nt sequence was used for deoxyribozyme assays. For the selection process itself, the 36 nt sequence that additionally included $(AAC)_6$ at its 3′-terminus was used in all selection rounds.

[d] The partially randomized pool was prepared such that each nucleotide of the initially random (N_{40}) region is the parent nucleotide with 75% probability and one of the other three nucleotides with 25% probability.

MALDI mass spectrometry data

substrate or product identity	$[M+H]^+$ calcd.	$[M+H]^+$ found	error, % (found – calcd.)
1a glycosyl donor substrate [a]	6099.8	6100.1	+0.005
1b glycosyl donor substrate [a]	6065.8	6066.2	+0.007
1b glycosyl donor substrate [b]	5990.8	5990.7	–0.002
1c glycosyl donor substrate [a]	6020.8	6021.4	+0.010
2b glycosyl donor substrate [a]	6065.8	6066.1	+0.005
2b glycosyl donor substrate [b]	5990.8	5991.1	+0.005
1a glycosylation product from 11GV112	11722.8	11716.7	–0.05

Table S2. MALDI mass spectrometry data. Each glycosyl donor substrate was synthesized as described in "Preparation of DNA-anchored glycosyl donor conjugates". The glycosylation product was synthesized using the single-turnover assay procedure in conditions *A* for 24 h, scaled up as follows: 50 µL sample volume, 200 pmol glycosyl acceptor substrate, 250 pmol of deoxyribozyme, and 300 pmol of DNA-glycosyl donor substrate. The sample was purified by 20% PAGE, desalted with Millipore C_{18} ZipTip, and analyzed on a Bruker UltrafleXtreme MALDI-TOF mass spectrometer with matrix 3-hydroxypicolinic acid in positive ion mode at the UIUC School of Chemical Sciences Mass Spectrometry Laboratory.

[a] DNA sequence 5′-GAAGAGATGGCGACTTCG-3′.

[b] DNA sequence 5′-GAACAGGTTTATACTTCG-3′.

In vitro selection procedure

The key selection step of each round is shown in Fig. 2, and full nucleotide details are depicted in Fig. S1. All oligonucleotide sequences are listed in Table S1.

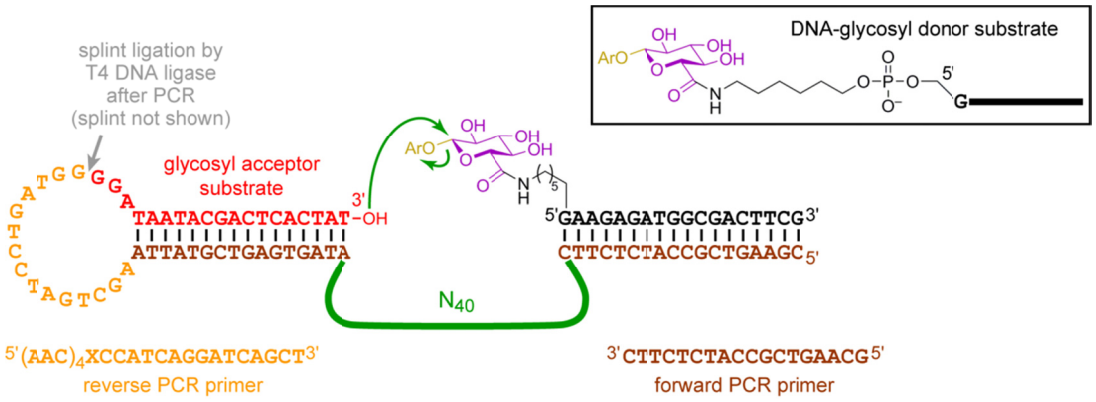


Figure S1. Details of the selection step of *in vitro* selection with the DNA oligonucleotide glycosyl acceptor. The specific sequences shown here were used in the first selection experiment, with the 2-chloro-4-nitrophenyl β-D-Glc glycosyl donor **1a**. See Table S1 for oligonucleotides used in all selection experiments. The 5′-NH_2-DNA was prepared with a C_6 tether between the 5′-phosphate and the amino group.

Procedure for ligation step in round 1. A 25 µL sample containing 600 pmol of DNA pool, 750 pmol of DNA splint, and 900 pmol of 5′-phosphorylated glycosyl acceptor substrate (DNA 3′-OH or DNA-HEG-CAAYAA) was annealed in 5 mM Tris, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. To this solution was added 3 µL of 10× T4 DNA ligase buffer (Fermentas) and 2 µL of 5 U/µL T4 DNA ligase (Fermentas). 10× T4 DNA ligase buffer that lacks DTT (400 mM Tris, pH 7.8, 100 mM $MgCl_2$, and 5 mM ATP) was used with the disulfide-linked oligonucleotide-peptide conjugate. The sample was incubated at 37 °C for 12 h and purified by 8% PAGE.

Procedure for ligation step in subsequent rounds. A 17 μL sample containing the PCR-amplified DNA pool (~5–10 pmol), 30 pmol of DNA splint, and 50 pmol of 5′-phosphorylated glycosyl acceptor substrate (DNA 3′-OH or DNA-HEG-CAAYAA) was annealed in 5 mM Tris, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. To this solution was added 2 μL of 10× T4 DNA ligase buffer (Fermentas) and 1 μL of 1 U/μL T4 DNA ligase (Fermentas). 10× T4 DNA ligase buffer that lacks DTT (400 mM Tris, pH 7.8, 100 mM $MgCl_2$, and 5 mM ATP) was used with the disulfide-linked oligonucleotide-peptide conjugate. The sample was incubated at 37 °C for 12 h and purified by 8% PAGE.

Procedure for selection step in round 1. Each selection experiment was initiated with 200 pmol of the ligated pool. A 20 μL sample containing 200 pmol of ligated pool and 300 pmol of DNA-glycosyl donor substrate was annealed in (conditions *A*) 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA or (conditions *B*) 5 mM CHES, pH 9.0, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. The selection reaction was initiated by bringing the sample to 40 μL total volume containing (conditions *A*) 70 mM HEPES, pH 7.5, 40 mM $MgCl_2$, 20 mM $MnCl_2$, 1 mM $ZnCl_2$, and 150 mM NaCl or (conditions *B*) 50 mM CHES, pH 9.0, 40 mM $MgCl_2$, and 150 mM NaCl. The Mn^{2+} was added from a 10× stock solution containing 200 mM $MnCl_2$. The Zn^{2+} was added from a 10× stock solution containing 10 mM $ZnCl_2$, 20 mM HNO_3, and 200 mM HEPES at pH 7.5; this stock solution was freshly prepared from a 100× stock of 100 mM $ZnCl_2$ in 200 mM HNO_3. The metal ion stocks were added last to the final sample. The sample was incubated at 37 °C for 14 h.

Procedure for selection step in subsequent rounds. A 10 μL sample containing the ligated pool and 30 pmol of DNA-glycosyl donor substrate was annealed in (conditions *A*) 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA or (conditions *B*) 5 mM CHES, pH 9.0, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. The selection reaction was initiated by bringing the sample to 20 μL total volume containing (conditions *A*) 70 mM HEPES, pH 7.5, 40 mM $MgCl_2$, 20 mM $MnCl_2$, 1 mM $ZnCl_2$, and 150 mM NaCl or (conditions *B*) 50 mM CHES, pH 9.0, 40 mM $MgCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h.

Procedure for PCR. In each selection round, two PCR reactions were performed, 10-cycle PCR followed by 30-cycle PCR. First, a 100 μL sample was prepared containing the PAGE-purified selection product, 200 pmol of forward primer, 50 pmol of reverse primer, 20 nmol of each dNTP, 10 μL of 10× Taq polymerase buffer [1× = 20 mM Tris-HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, and 0.1% Triton X-100], and Taq polymerase. This sample was cycled 10 times according to the following PCR program: 94 °C for 2 min, 10× (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. Taq polymerase was removed by phenol/chloroform extraction. Second, a 50 μL sample was prepared containing 1 μL of the 10-cycle PCR product, 100 pmol of forward primer, 25 pmol of reverse primer, 10 nmol of each dNTP, 20 μCi of α-^{32}P-dCTP (800 Ci/mmol), 5 μL of 10× Taq polymerase buffer, and Taq polymerase. This sample was cycled 30 times according to the following PCR program: 94 °C for 2 min, 30× (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. Samples were separated by 8% PAGE.

Cloning and screening of individual deoxyribozymes. The PCR primers used for cloning were the forward primer used in selection and the reverse primer as 5′-`TAATTAATTAATTACCCATCAGGATCAGCT`-3′. The 10-cycle PCR product from the appropriate selection round was diluted 10^3-fold. A 50 μL sample was prepared containing 1 μL of the diluted 10-cycle PCR product from the appropriate selection round, 25 pmol of forward cloning primer, 25 pmol of reverse cloning primer, 10 nmol of each dNTP, 5 μL of 10× Taq polymerase buffer, and Taq polymerase. This sample was cycled 30 times according to the following PCR program: 94 °C for 2 min, 30× (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. The sample was separated by 1% agarose gel and extracted using a GeneJET Gel Extraction Kit (Fermentas). The extracted product was quantified by absorbance (A_{260}) and diluted to 4–8 ng/μL. A 1 μL portion of the diluted PCR product was inserted into the pCR2.1-TOPO vector using a TOPO TA cloning kit (Life Technologies). Individual *E. coli* colonies harboring plasmids with inserts were identified by blue-white screening and grown in LB/amp media. Miniprep DNA was prepared using a GeneJET Plasmid Miniprep Kit (Fermentas) and screened for properly sized inserts by EcoRI digestion and agarose gel analysis.

Before sequencing, assays of individual deoxyribozyme clones were performed with PAGE-purified DNA strands prepared by PCR from the miniprep DNA, using the single-turnover assay procedure described below.

Single-turnover deoxyribozyme assay procedure

Single-turnover deoxyribozyme assay. The glycosyl acceptor substrate (DNA 3′-OH or DNA-HEG-CAAYAA) was 5′-^{32}P-radiolabeled using γ-^{32}P-ATP and T4 polynucleotide kinase (Fermentas), using 10× kinase buffer (500 mM Tris, pH 7.6, 100 mM $MgCl_2$, 1 mM spermidine, and 50 mM DTT); DTT was omitted from the buffer for the disulfide-linked oligonucleotide-peptide conjugate. A 10 μL sample containing 0.2 pmol of 5′-^{32}P radiolabeled glycosyl acceptor substrate, 10 pmol of deoxyribozyme, and 30 pmol of DNA-glycosyl donor substrate were annealed in (for conditions *A*) 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA or (for conditions *B*) 5 mM CHES, pH 9.0, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice 5 min. The DNA-catalyzed reaction was initiated by bringing the sample to 20 μL total volume containing (conditions *A*) 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$ and 150 mM NaCl or (conditions *B*) 50 mM CHES, pH 9.0, 40 mM $MgCl_2$, and 150 mM NaCl. For assays of 16MJ132 and 16MJ101, similar k_{obs} and yield were observed with 0.2–0.5 mM Zn^{2+}, whereas the yield was lower at or above 0.6 mM Zn^{2+}; in the assays of Fig. 4 and related experiments, 0.4 mM Zn^{2+} was used. The sample was incubated at 37 °C. At appropriate time points, 2 μL aliquots were quenched with 5 μL of stop solution (80% formamide, 1× TBE [89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3], 50 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol). Samples were separated by 20% PAGE and quantified using a Phosphorimager. Values of k_{obs} were obtained by fitting the yield versus time data directly to first-order kinetics; i.e., yield = $Y\bullet(1 - e^{-kt})$, where $k = k_{obs}$ and Y is the final yield.

Selection progressions

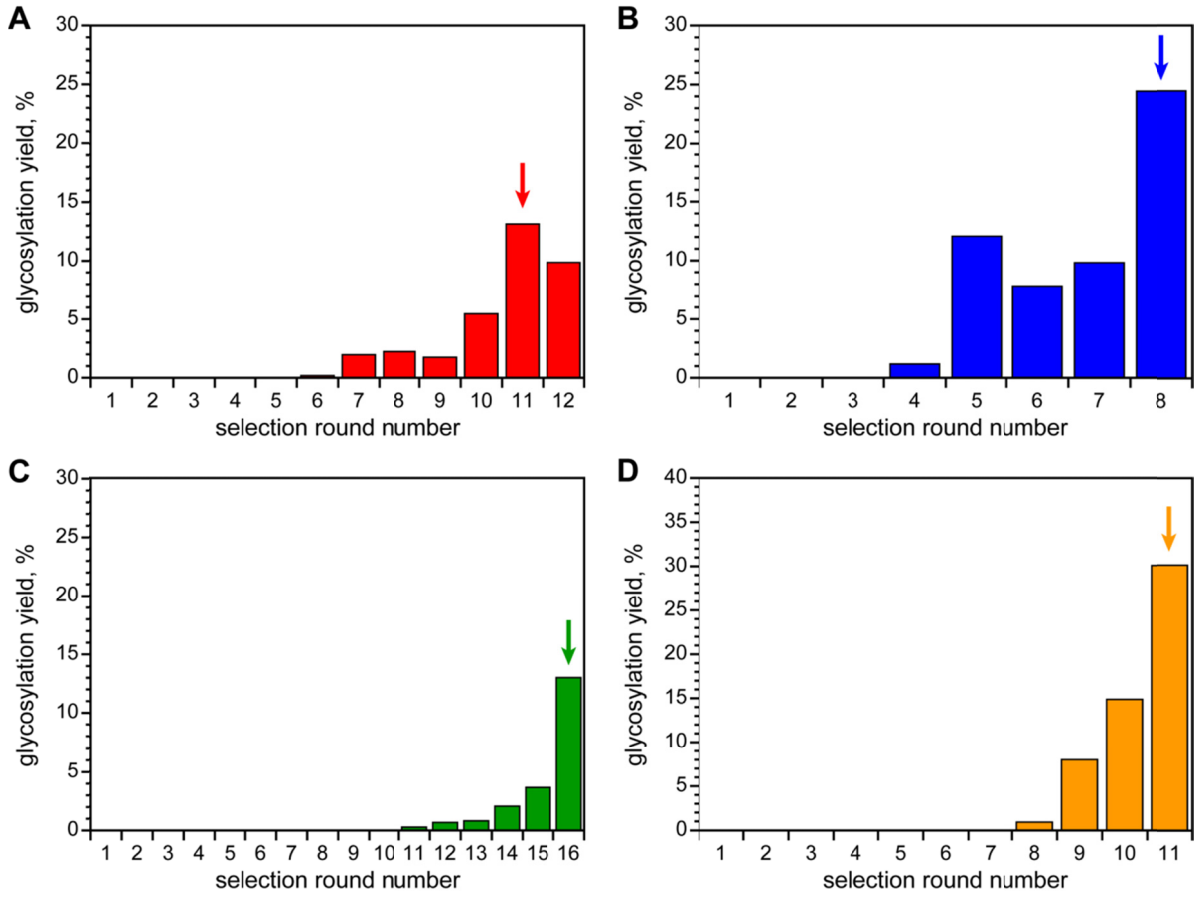


Figure S2. Progressions of the *in vitro* selection experiments. The arrows marks the cloned rounds. (A) Selection with DNA 3′-OH as glycosyl acceptor and 2-chloro-4-nitrophenyl β-D-Glc **1a** as glycosyl donor. (B) Reselection of 11GV112 from the **1a** selection. (C) Selection with DNA 3′-OH as glycosyl acceptor and 4-nitrophenyl α-D-Glc **2b** as glycosyl donor. (D) Selection with the DNA-anchored Tyr peptide substrate (Figure S4) as glycosyl acceptor and 2-chloro-4-nitrophenyl β-D-Glc **1a** as glycosyl donor.

Sequences of individual deoxyribozymes

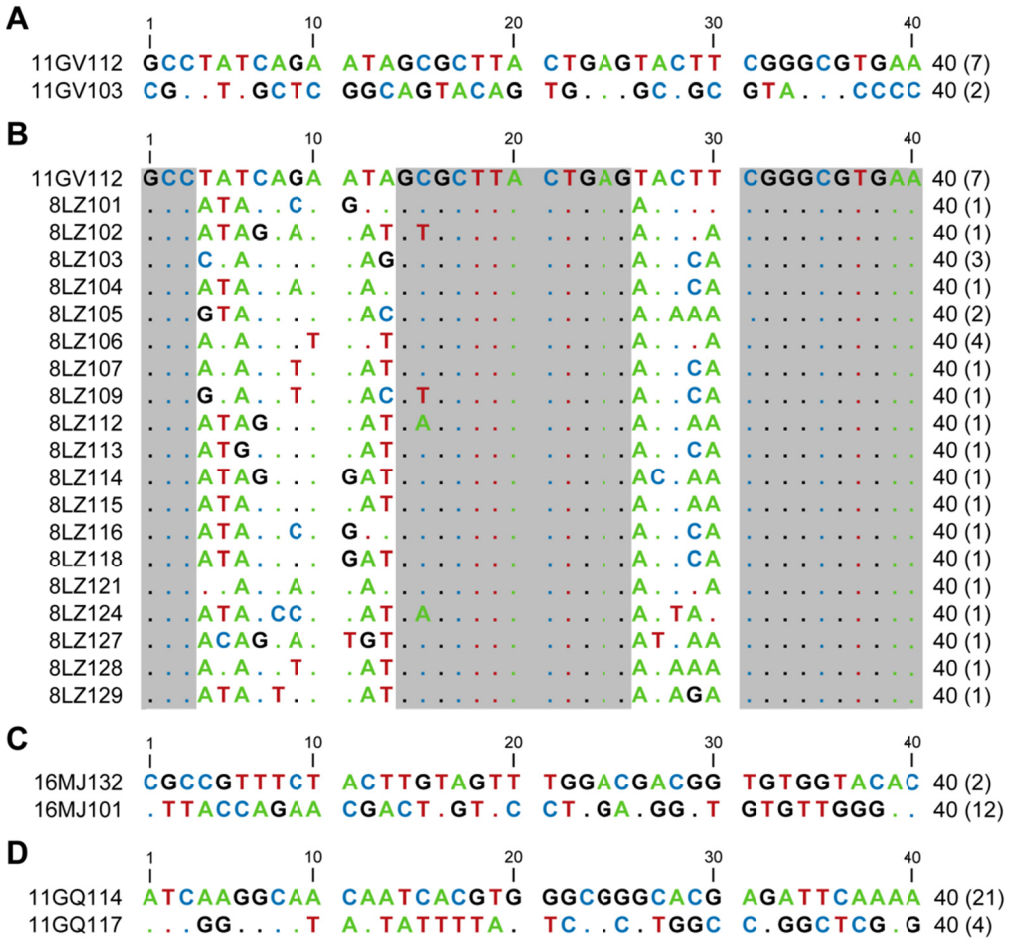


Figure S3. Sequences of the initially random regions of the new deoxyribozymes. Each alignment shows only the 40 nucleotides of the initially random (N_{40}) region as determined through the selection process. Grey boxes denote conserved sequence elements. A dot denotes conservation, i.e., the same nucleotide as in the uppermost sequence; a dash denotes a gap. In each set of sequences, next to the sequence length in nucleotides on the far right is shown (in parentheses) the number of times that sequence was found during cloning. (A) Deoxyribozymes identified by selection with DNA 3′-OH as glycosyl acceptor and 2-chloro-4-nitrophenyl β-D-Glc **1a** as glycosyl donor. These deoxyribozymes were used as 5′-CGAAGTCGCCATCTCTTC-N_{40}-ATAGTGAGTCGTATTA-3′. (B) Deoxyribozymes identified by reselection of 11GV112 from the **1a** selection. These deoxyribozymes were used as 5′-CGAAATCTAAAAAGTTTC-N_{40}-ATAGGGATGTGCTAGA-3′. (C) Deoxyribozymes identified by selection with DNA 3′-OH as glycosyl acceptor and 4-nitrophenyl α-D-Glc **2b** as glycosyl donor. These deoxyribozymes were used as 5′-CGAAGTATAAACCTGTTC-N_{40}-ATATTTGTATCCAGGA-3′. In 16MJ101, the underlined A in the 3′-binding arm was found to be mutated to C; this mutation was strictly required for activity. (D) Deoxyribozymes identified by selection with the DNA-anchored Tyr peptide substrate (Figure S4) as glycosyl acceptor and 2-chloro-4-nitrophenyl β-D-Glc **1a** as glycosyl donor. These deoxyribozymes were used as 5′-CGAAGTCGCCATCTCTTC-N_{40}-ATAGTGAGTCGTATTA-3′.

In vitro selection experiment with peptide substrate

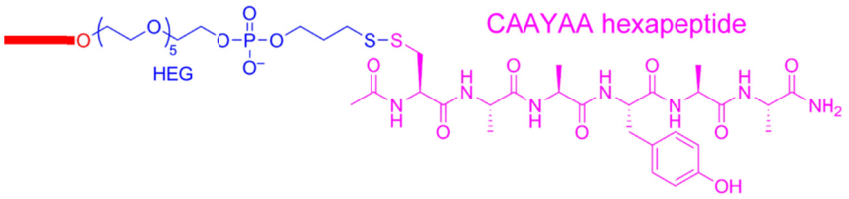


Figure S4. Glycosyl acceptor substrate structure for *in vitro* selection experiment with a peptide substrate. The sequence of the DNA anchor oligonucleotide is the same as shown in Figure S1. HEG = hexa(ethylene glycol). The substrate was prepared as described.[3]

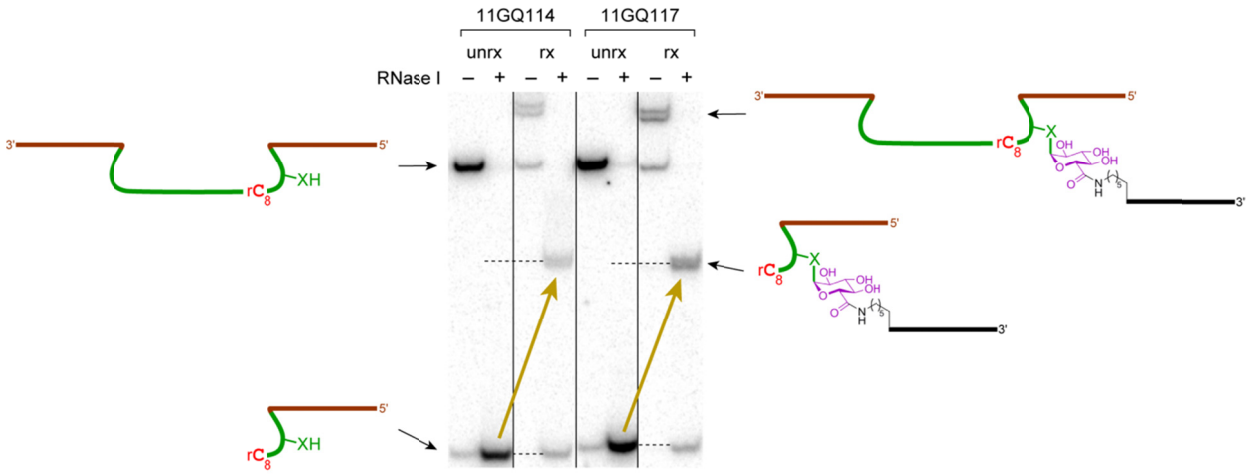


Figure S5. For 11GQ114 and 11GQ117, establishing reaction of a DNA nucleobase functional group within or very near to the 5′-end of the N_{40} region as the nucleophile that attacks the glycosyl donor. These two deoxyribozymes were identified from the selection experiment that used the DNA-anchored CAAYAA hexapeptide as the glycosyl acceptor. Each deoxyribozyme was synthesized with a ribonucleotide monomer at position C8 of the initially random N_{40} region (see Figure S3D for full sequences). The branched product formed from each 5′-^{32}P-radiolabeled deoxyribozyme with the DNA-anchored glycosyl donor **1a** (in the presence of the peptide-free DNA oligonucleotide glycosyl acceptor, which did not react) was PAGE-purified. The product from both deoxyribozymes is reproducibly observed as a closely spaced pair of bands, suggesting two nearby reaction sites. Lanes marked "unrx" are for unreacted deoxyribozymes; lanes marked "rx" are for reaction products. Each sample was treated with RNase I to cleave after the single embedded rC8. After RNase I treatment, the rC8 reaction product migrates on 20% PAGE much higher than the corresponding cleavage standard (gold arrows in figure), revealing attachment of the DNA-glycosyl donor strand at a site to the 5′-side of C8 (at undetermined functional group designated "XH"). Approximately 5–8% of ribonucleotide cleavage is observed even without RNase I treatment (formation of lower band in each "unrx –" lane). Also, the PAGE-purified reaction product undergoes ~30–40% of spontaneous reversal of glycosylation reaction (formation of lower band in each "rx –" lane), and the original-size deoxyribozyme created by this reversal is then cleaved by RNase I ("rx + lanes"). Analogous experiments with rA4 or rA5 (11GQ114) and rG4 or rG5 (11GQ117) led to no reaction products; these ribo modifications inhibit deoxyribozyme catalysis. Analogous experiments with rA1 (11GQ114 and 11GQ117) led to reaction products, but these products were not cleaved by RNase I, suggesting that the modification site is very close to nucleotide A1.

Assessing stability of UDP-GlcNAc under various incubation conditions

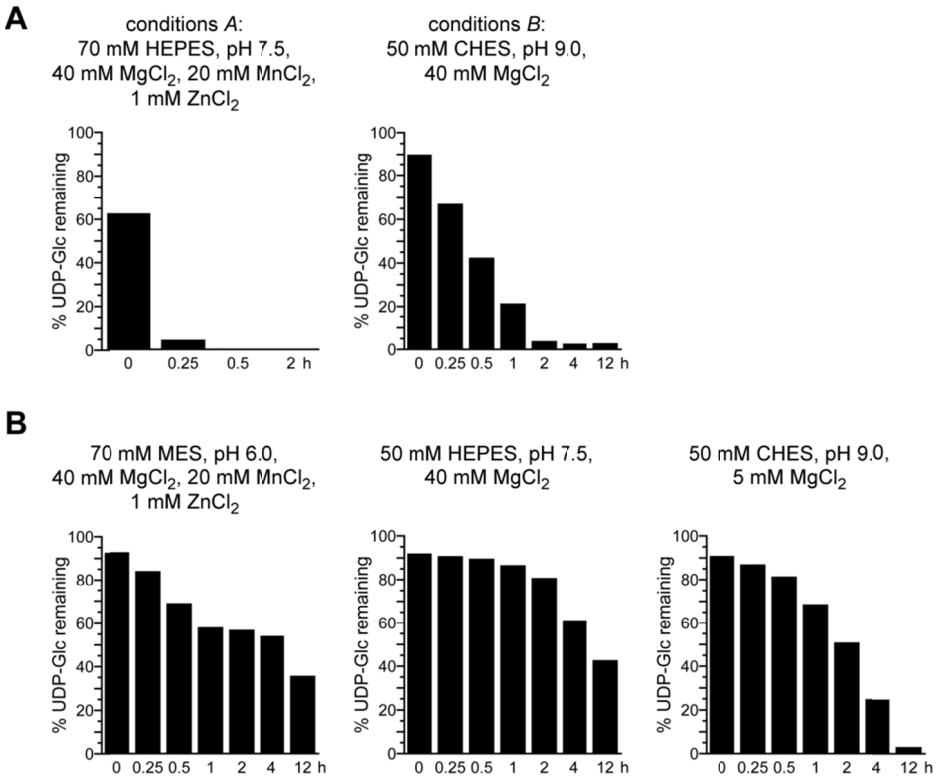


Figure S6. Assessing stability of DNA-anchored UDP-Glc under various conditions. (A) Instability of DNA-anchored UDP-Glc under selection conditions *A* and *B*. (B) Validation of three milder conditions for selection with DNA-anchored UDP-Glc. All conditions additionally included 150 mM NaCl and were at 37 °C. Data for "t = 0 h" represents 30 s incubation. From the data in panel B, the three indicated conditions were used for selection, with incubation times of 2 h (or 12 h for round 1 of selections at pH 6.0 and 7.5), but no activity was observed.

Synthesis of *O*-arylglycoside compounds

Reagents were commercial grade and used without purification unless otherwise indicated. Dry CH_3CN was obtained from Acros Acroseal bottles. Thin-layer chromatography (TLC) was performed on silica gel plates pre-coated with fluorescent indicator with visualization by UV light (254 nm). Flash column chromatography was performed with silica gel (230-400 mesh). NMR spectra were recorded on a Varian Unity instrument. The chemical shifts in parts per million (δ) are reported downfield from TMS or DSS (0 ppm) and referenced to the residual proton signal of the deuterated solvent, as follows: $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm), or D_2O (4.79 ppm) for ^1H NMR spectra; $CDCl_3$ (77.2 ppm) for ^{13}C NMR spectra. Apparent multiplicities of ^1H NMR peaks are reported as s (singlet), d (doublet), or m (multiplet and overlapping spin systems), along with values for apparent coupling constants (*J*, Hz). Mass spectrometry data were obtained at the UIUC School of Chemical Sciences Mass Spectrometry Laboratory using a Waters Quattro II instrument (LR-ESI).

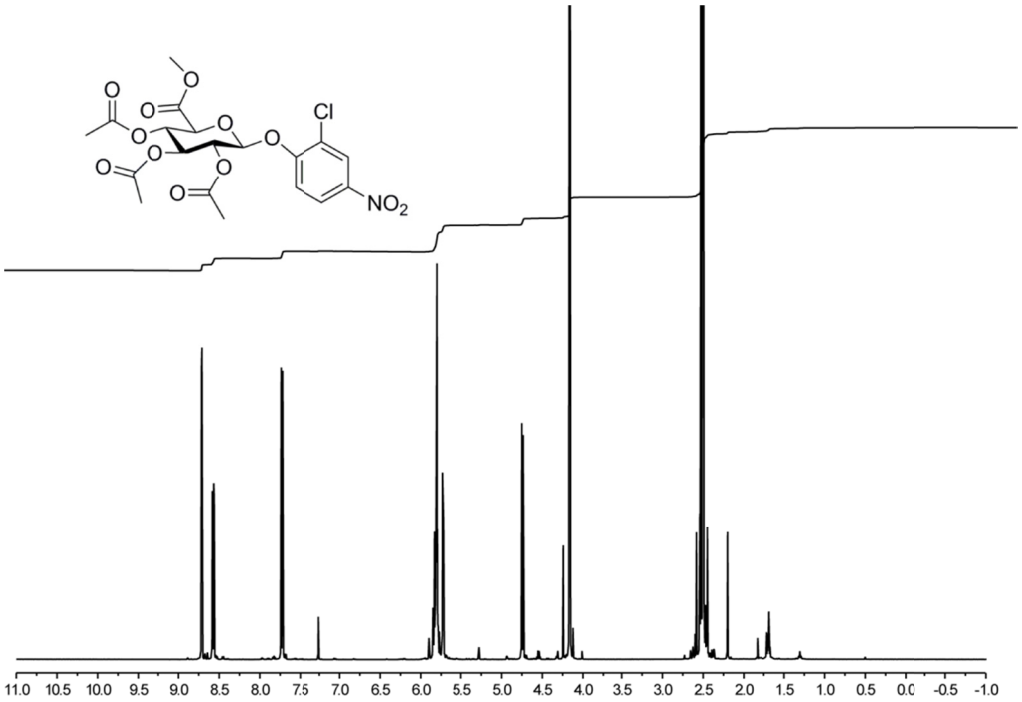
2-Chloro-4-nitrophenyl 2,3,4-tri-*O*-acetyl-β-D-glucuronide methyl ester (see ref. 5 for analogous procedure)

In a flame-dried 25 mL round-bottom flask under argon, 1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucuronide methyl ester (105 mg, 0.264 mmol, 1.0 equiv; Carbosynth, cat. no. MB04460) was dissolved in 2.8 mL of anhydrous CH_3CN. 2-Chloro-4-nitrophenol (49 mg, 0.282 mmol, 1.1 equiv) and Ag_2O (151 mg, 0.652 mmol, 2.5 equiv) were added, the flask was covered with aluminum foil, and the mixture was stirred at room temperature for 24 h, at which point the reaction was complete as assessed by TLC. The mixture was filtered through Celite, and the yellow filtrate was concentrated on a rotary evaporator. The resulting light brown oil was dissolved in 30 mL of ethyl acetate, washed with saturated $NaHCO_3$ (3 × 25 mL) and saturated NaCl (3 × 25 mL), dried over $MgSO_4$, and concentrated on a rotary evaporator. The light brown solid was purified by flash column chromatography, eluting with 3:1 hexanes:ethyl acetate to 1:1 hexanes:ethyl acetate, to provide a white solid (113 mg, 0.231 mmol, 87% yield).

TLC: R_f = 0.69 (1:1 hexanes:ethyl acetate).

^1H NMR: (500 MHz, $CDCl_3$) δ 8.30 (d, *J* = 2.7 Hz, 1H), 8.15 (dd, *J* = 9.1 Hz, 2.7 Hz, 1H), 7.31 (d, *J* = 9.1 Hz, 1H), 5.43-5.36 (m, 3H), 5.32-5.30 (m, 1H), 4.32 (d, *J* = 8.6 Hz, 1H), 3.75 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H) ppm.

^{13}C NMR: (125 MHz, $CDCl_3$) δ 170.0, 169.4, 169.1, 166.7, 143.4, 139.6, 126.3, 125.0, 123.8, 116.7, 99.0, 72.8, 71.0, 71.0, 70.3, 68.7, 53.2, 20.7, 20.62, 20.59 ppm.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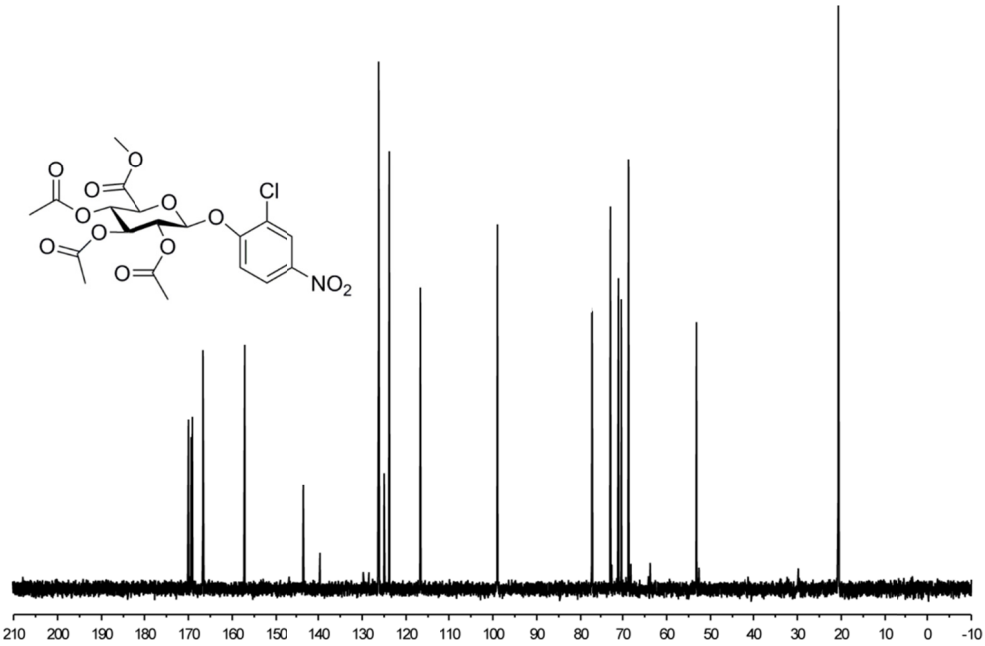
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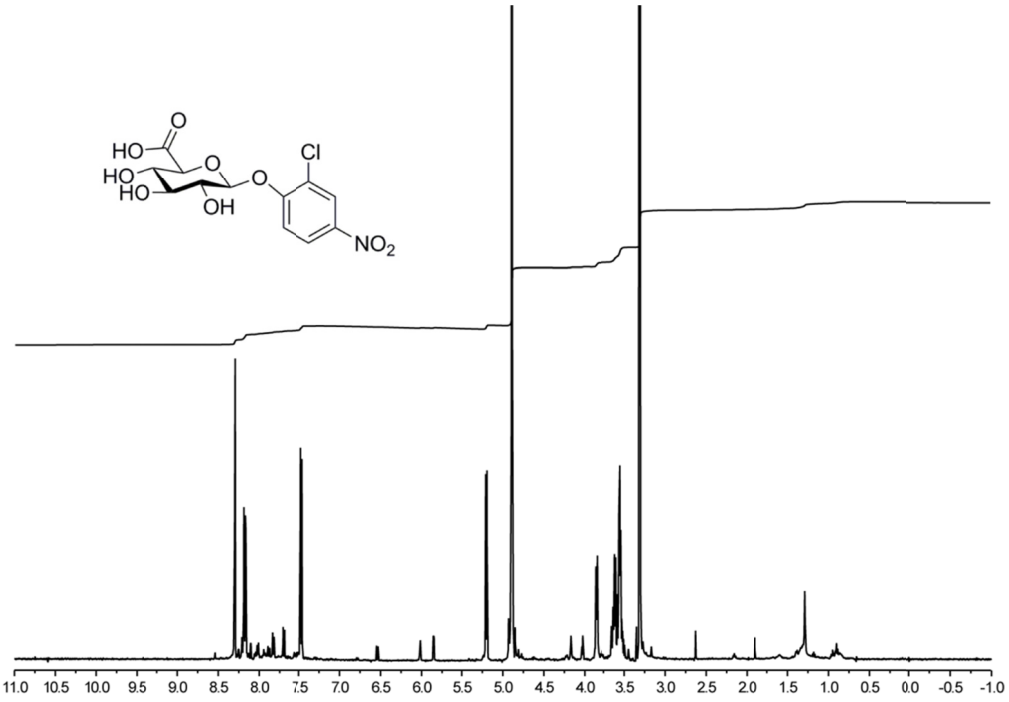
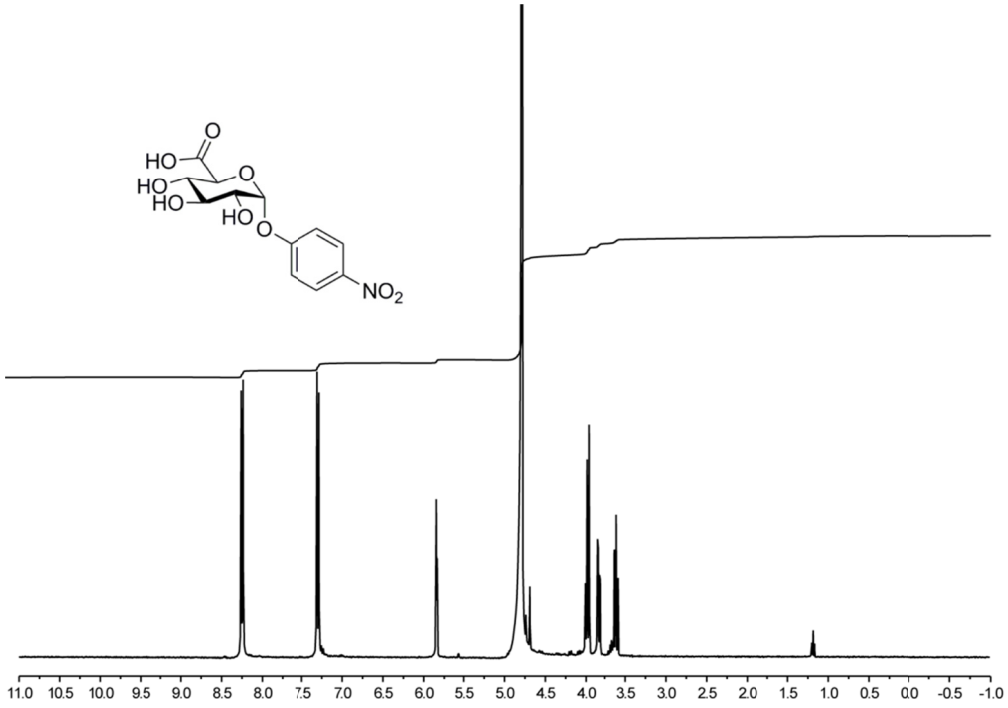
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

2-Chloro-4-nitrophenyl β-D-glucuronide (immediate precursor to **1a**; see ref. 6 for analogous procedure) 2-Chloro-4-nitrophenyl 2,3,4-tri-*O*-acetyl-β-D-glucuronide methyl ester (100 mg, 0.204 mmol) was dissolved in 10 mL of CH_3OH. To this solution, 10 mL of 200 mM NaOH was added, and the yellow solution was stirred at room temperature for 3 h. The solution was neutralized with small portions of Amberlite IR-120 H^+ form resin (monitored with pH paper), filtered through a cotton plug, and concentrated on a rotary evaporator. The yellow residue was dissolved in 3 mL of CH_3OH and passed through a silica plug, eluting with 20% CH_3OH in CH_2Cl_2. The fractions containing the compound were concentrated on a rotary evaporator. The resulting residue was dissolved in 3 mL of ethanol, cooled on ice, and crystallized upon addition of 30 mL of cold hexanes. The free-floating solid was collected by vacuum filtration on a fritted funnel and washed with cold hexanes. The product was eluted through the fritted funnel with CH_3OH, which was concentrated on a rotary evaporator, providing the product as a yellow solid (9 mg, 0.026 mmol, 13% yield).
^{1}H NMR: (500 MHz, CD_3OD) δ 8.30 (d, J = 2.8 Hz, 1H), 8.18 (dd, J = 9.2, 2.8 Hz, 1H), 7.47 (d, J = 9.2 Hz, 1H), 5.20 (d, J = 7.6 Hz, 1H), 3.86 (d, J = 9.1 Hz, 1H), 3.65-3.53 (m, 3H) ppm.
MS: m/z calcd. for $C_{12}H_{11}ClNO_9$ $[M–H]^-$ 348.7; found 348.1.



4-Nitrophenyl α-D-glucuronide (immediate precursor to **2b**; see ref. 7 for analogous procedure)
4-Nitrophenyl α-D-glucoside (106 mg, 0.35 mmol, 1 equiv; Research Products International Corp., cat. no. N82080) was added to 5.3 mL of 100 mM $NaHCO_3/Na_2CO_3$ buffer, pH 10.7, in a 25 mL round-bottomed flask, and the mixture was stirred until most of the solid dissolved. NaBr (8 mg, 0.078 mmol, 0.22 equiv) and TEMPO (0.9 mg, 0.006 mmol, 0.02 equiv) were added, and the yellow solution was cooled to 0 °C in an ice-water bath. 1.22 mL of 5% aqueous NaOCl was added, and the solution was allowed to return to room temperature with stirring. After 3 h, another 1.22 mL of 5% aqueous NaOCl and 0.9 mg of TEMPO were added, and the solution was stirred at room temperature for 3 d. The reaction was quenched by addition of 4.5 mL MeOH, and the solution was stirred for 1 h at room temperature. The solution was concentrated on a rotary evaporator to approximately 2 mL. The resulting solution was purified by eluting through a Waters Sep-Pak Plus C_{18} Cartridge (0.5 mL loading volume, eluted with 5 mL of 2% aqueous CH_3OH at 1 mL/min flow rate, followed by 5 mL of CH_3OH). The appropriate fractions as assessed by silica gel TLC were concentrated on a SpeedVac. The resulting light yellow solid was triturated with 3 × 5 mL of EtOH and dried on a SpeedVac, providing a light yellow powder (141 mg) that was used without further purification.
^{1}H NMR: (500 MHz, D_2O) δ 8.24 (dd, J = 9.4, 0.8 Hz, 2H), 7.29 (dd, J = 9.3, 0.8 Hz, 2H), 5.83 (d, J = 3.6 Hz, 1H), 4.01-3.94 (m, 2H), 3.83 (dd, J = 9.9, 3.7 Hz, 1H), 3.64-3.57 (m, 1H) ppm.
MS: *m*/*z* calcd. for $C_{12}H_{13}NaNO_9$ $[M+Na]^+$ 338.2; found 338.2.



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