Supplementary Notes

Supplementary Note 1

General

All NMR spectra were recorded on a Bruker AVANCE III-400 machine (9.4 T, 52 mm probe, BBFOz). HRMS measurements were conducted at the EPFL ISIC Mass Spectrometry Service using a Micro Mass QTOF Ultima from Waters Corp. HPLC analysis was performed on an Agilent Infinity 1260 HPLC system with a Waters SunFire C18 3.5 µm, 2.1 x 20 mm column. The products of the reactions were initially analyzed using an Agilent 6120 Quadrupole LC−MS spectrometer from Agilent, which was directly connected to the HPLC. Synthetic products were purified using Waters semipreparative HPLC modular system with Waters SunFire C-18 column (5 µm, 50 × 30 mm).

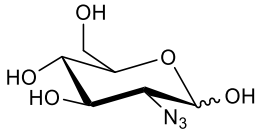
Investigation of reaction kinetics between CLP and GAz1 – GAz5

The 2nd order rate constant of reaction between CLP and GAz1-Gaz2 that resulted in release of free D-luciferin were determined by monitoring concentration of starting CLP phosphine with ^{31}P NMR (inverse ^{1}H gating)[1]. In this method stock solutions of GAz reagents, CLP, and the standard (tetraphenylphosphonium chloride) were prepared in degassed deuterated DMF (DMF-*d6*) at concentration 6 mM. The solutions were kept at -80 C before usage. In a typical experiment 350 µL of DMF-*d6* were mixed with 100 µL of each GAz and CLP stock solution, and 50 µL of the internal standard to give a final volume of 600 µL. Right after the mixture was transferred in 5 mm NMR tube which was immediately flushed with argon, sealed and placed in NMR spectrometer. ^{31}P spectra were acquired every 30 min for 24 hours. To vary the concentrations of CLP and GAz in the reaction mixture, volumes of stock solutions and DMF-*d6* were adjusted to keep the final volume constant. The peaks of starting material (-4.85 ppm) and standard (22.89 ppm) were integrated and monitored over time. The 2nd order rate constant was determined by plotting 1/[phosphine] (M^{-1}) versus time under stoichiometric conditions (n = 3).

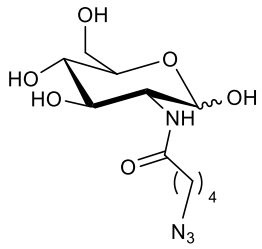
The 2nd order rate constant of reaction between CLP and GAz3-GAz5 were determined by measuring kinetics of appearance of free D-luciferin that is a product of this reaction using fluorescent readout. In this method stock solutions of D-luciferin, GAz and CLP (10 µM, 20 µM and 50 µM) compounds were prepared in PBS supplemented with 0.1 % BSA and 2 % DMF. First, calibration curve for D-luciferin fluorescence was measured in order to calculate concentration of D-luciferin in a reaction mixture at various time points (Y = 459.8X + 794.8). All the reactions equimolar amounts of CLP and GAz reagents were used. The release of D-luciferin was monitored by fluorescence (λ_{ex} = 330 nm, λ_{em} = 530 nm) over the course of 1h. The 2nd order rate constant was determined by plotting 1/[luciferin] (M^{-1}) versus time (n = 3).

Supplementary Note 2

Synthesis and characterization of BiGluc probe components.



(3*R*,4*R*,5*S*,6*R*)-3-azido-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2,4,5-triol (GAz$_1$) was purchased from Sigma-Aldrich.

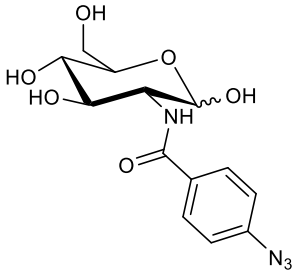


6-Azido-N-((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)hexanamide (GAz$_2$):

Glucosamine hydrochloride (158.00 mg, 0.73 mmol) was suspended in DMF (2 mL) and TEA (152 µL, 1.10 mmol) was added. The reaction mixture was cooled to 0ºC and 5-azidopentanoic acid (105.00 mg, 0.73 mmol), DCC (181.00 mg, 0.88 mmol) and HOBt (109.00 mg, 0.81 mmol) were added. The reaction was allowed to warm to room temperature and left to stir overnight. After 18 h, TLC (Chloroform: Methanol: Acetic Acid: Water 60: 20: 15: 5, CMAW) showed a formation of a non-UV active product (R_f =0.5) and a gradual disappearance of glucosamine (R_f baseline). The orange reaction mixture was concentrated *in vacuo*. Column chromatography ($CHCl_3$: CH_3OH 10:1 → CMAW) yielded the desired amide **GAz$_2$** (113.00 mg, 51%) as a slightly orange mixture of α/β isomers.

^{1}H NMR (400 MHz, CD_3OD) δ 5.10 (d, *J* = 3.4 Hz, 1H$_\alpha$), 4.58 (d, *J* = 8.3 Hz, 1H$_\beta$), 3.91 – 3.67 (m, 5H), 3.44 – 3.27 (m, 4H), 2.29 (td, *J* = 7.1, 3.7 Hz, 3H), 1.76 – 1.56 (m, 4H).

MS (ESI): calcd. m/z= 304.14 for $C_{11}H_2ON_4O_6$, found m/z = 327.1 for $[M+Na]^+$.



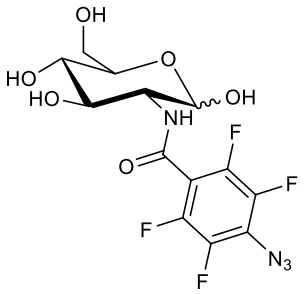
4-Azido-*N*-((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)benzamide (GAz$_3$):

To a suspension of 4-azidobenzoic acid (163 mg, 1.0 mmol) in dry DCM (5 mL) oxalyl chloride (172 µL, 2.0 mmol) was added dropwise at rt followed by addition of DMF (5 µL). The reaction mixture was stirred for 30 minutes at rt, evaporated to dryness, the residue was dissolved in dry toluene, and evaporated under reduced pressure to remove volatile acidic rests. Crude 4-azidobenzoyl chloride was dissolved in dry THF (4 mL) and added to a solution of glucosamine (324 mg, 1.5 mmol), $KHCO_3$ (350 mg, 3.5 mmol) in water (4 mL) dropwise with stirring at 0°C. The reaction mixture was stirred overnight at rt, then concentrated to remove THF, white solid formed was filtered off, washed with conc. $NaHCO_3$, water, and dried to afford 255 mg (79%) of title compound as a mixture of anomers.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.02-7.88 (m, 2H), 7.25-7.15 (m, 2H), 5.07 (d, *J* = 2.6 Hz, 0.7H, alpha-H), 4.62 (d, *J* = 8.2 Hz, 0.3H, beta-H), 3.82-3.58 (m, 3H), 3.56-3.43 (m, 2H), 3.22-3.09 (m, 2H).

The major alpha-anomer: **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 165.90, 142.58, 131.50, 129.89, 119.16, 90.85, 72.59, 71.47, 70.41, 61.58, 55.89.

HRMS (ESI-QTOF-MS): m/z calc. for $[M+Na]^+$ 347.0967, found 347.0969.



4-Azido-2,3,5,6-tetrafluoro-N-((3R,4R,5S,6R)-2,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-3-yl)benzamide (GAz$_4$):

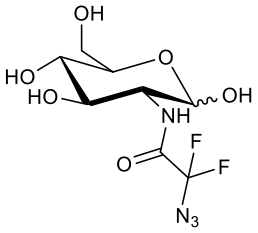
To a suspension of 4-azido-2,3,5,6-tetrafluorobenzoic acid (235 mg, 1.0 mmol) in dry DCM (5 mL) oxalyl chloride (172 µL, 2.0 mmol) was added dropwise at rt followed by addition of DMF (5 µL). The reaction mixture was stirred for 30 minutes at rt, evaporated to dryness, the residue was dissolved in dry toluene, and evaporated under reduced pressure to remove volatile acidic rests. Crude 4-azido-2,3,5,6-tetrafluorobenzoyl chloride was dissolved in dry THF (4 mL) and added to a solution of glucosamine hydrochloride (324 mg, 1.5 mmol), $KHCO_3$ (350 mg, 3.5 mmol) in water (4 mL) dropwise with stirring at 0°C. The reaction mixture was stirred overnight at rt, then concentrated to remove THF, white solid formed was filtered off, washed with conc. $NaHCO_3$, water, and dried to afford 270 mg (68%) of title compound as a mixture of anomers.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, J = 8.24 Hz, 1H), 6.59 (d, J = 4.08 Hz, 1H), 5.06 (dd, J = 3.80 Hz, 1H), 4.95 (d, J = 5.64 Hz, 1H), 4.74 (d, J = 6.24 Hz, 1H), 4.44 (dd, J = 5.78 Hz, 1H), 3.76 (m, 1H), 3.45-3.66 (m, 4H), 3.16 (m, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 157.53, 143.36 (m, J_{CF} = 248.1 Hz), 140.27 (m, J_{CF} = 248.1 Hz), 120.90 (t, $^2J_{CF}$ = 12.4 Hz), 113.24 (t, $^2J_{CF}$ = 21.5 Hz), 90.73, 72.62, 71.67, 70.53, 61.47, 55.74.

^{19}F NMR (376 MHz, DMSO-d_6) δ -144.49 (m, 2F), -154.52 (m, 2F).

HRMS (ESI-QTOF-MS): m/z calc. for $[M+Na]^+$ 419.0591, found 419.0583.



2-Azido-2,2-difluoro-N-((3R,4R,5S,6R)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)acetamide (GAz$_5$):

To a mixture of glucosamine hydrochloride (432 mg, 2.0 mmol), Na_2CO_3 (424 mg, 4.0 mmol), and dry MeOH (5 mL) was added ethyl 2-azide-2,2-difluoroacetate[2] (70% in DCM, 930 mg, ca 4 mmol) with stirring at ambient temperature. The reaction mixture was stirred overnight at the same temperature, then filtered off. The filtrate was evaporated to dryness, the residue was suspended in a mixture EtOAc + MeOH (5:1, 5 mL), filtered, and the filtrate was chromatographed on silica gel eluting with EtOAc + MeOH (25:2) to give title product as a white solid.

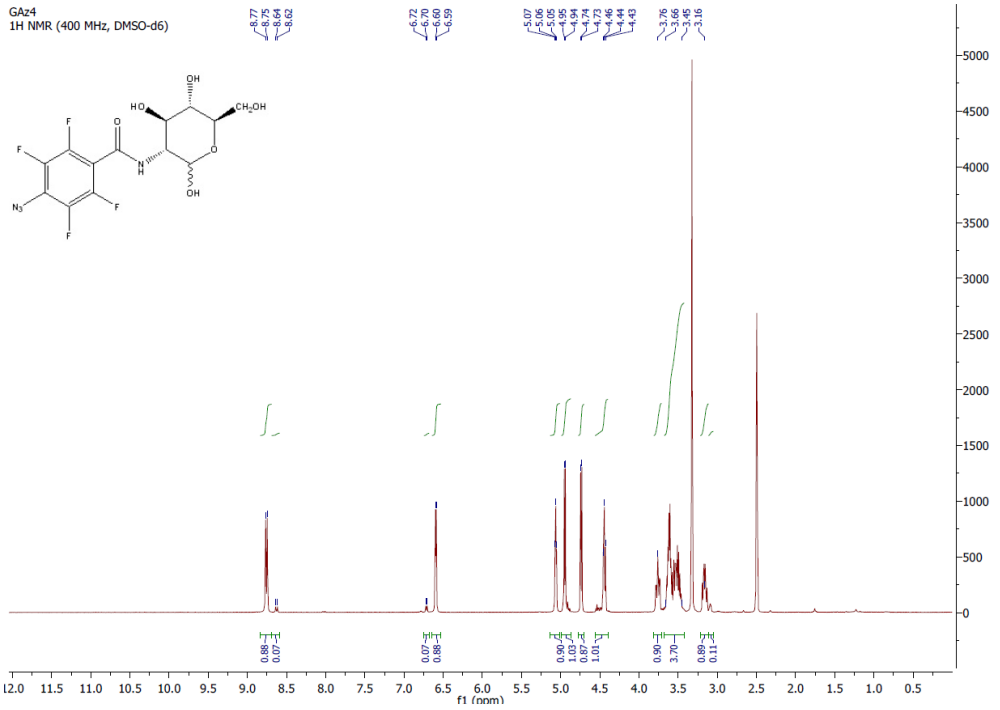
The major alpha-anomer: 1H NMR (400 MHz, DMSO-d6) δ 8.94 (d, J = 7.8 Hz, 1H), 6.64 (dd, J = 4.6, 1.2 Hz, 1H), 5.01 (dd, J = 4.9, 2.9 Hz, 2H), 4.80 (d, J = 5.8 Hz, 1H), 4.47 (dd, J = 6.4, 5.2 Hz, 1H), 3.76 – 3.66 (m, 1H), 3.66 – 3.57 (m, 3H), 3.54 – 3.45 (m, 1H), 3.20 – 3.11 (m, 1H).

The major alpha-anomer: **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.94 (t, *J* = 34.5 Hz), 115.54 (d, *J* = 267.0 Hz), 90.20, 72.62, 71.38, 69.92, 61.40, 56.02.

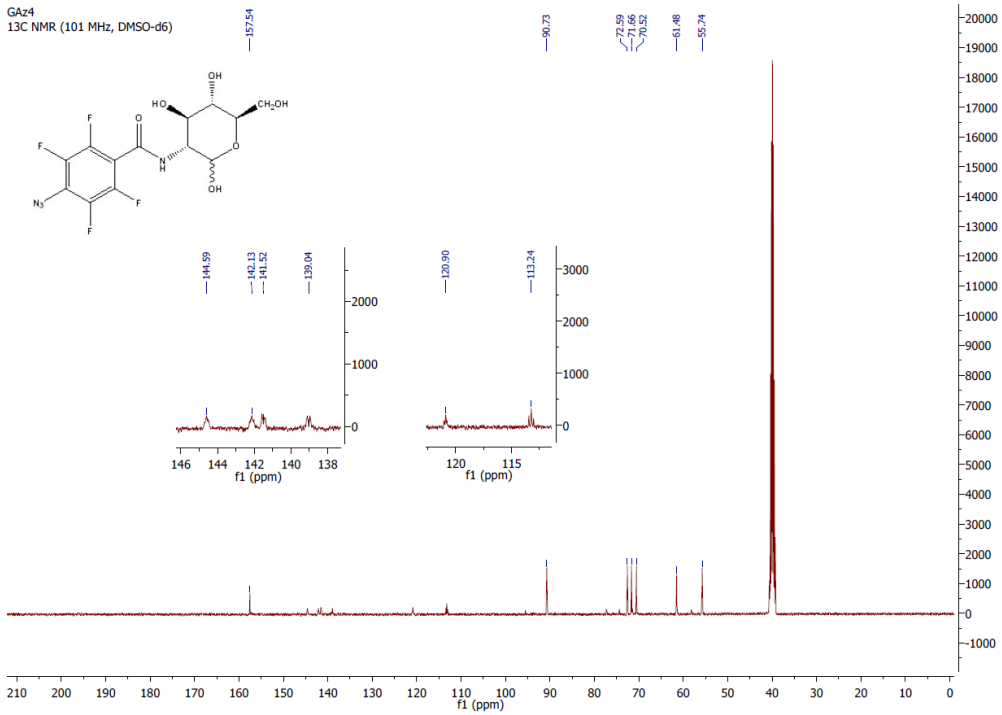
The major alpha-anomer: **^{19}F NMR** (376 MHz, DMSO-*d*6) δ -82.03 (d, *J* = 192.8 Hz), -83.12 (d, *J* = 192.9 Hz).

HPLC-MS: m/z found for $[M-H]^-$ 296.72 (ES-), for $[M-OH]^+$ 280.79, calculated M 298.07.

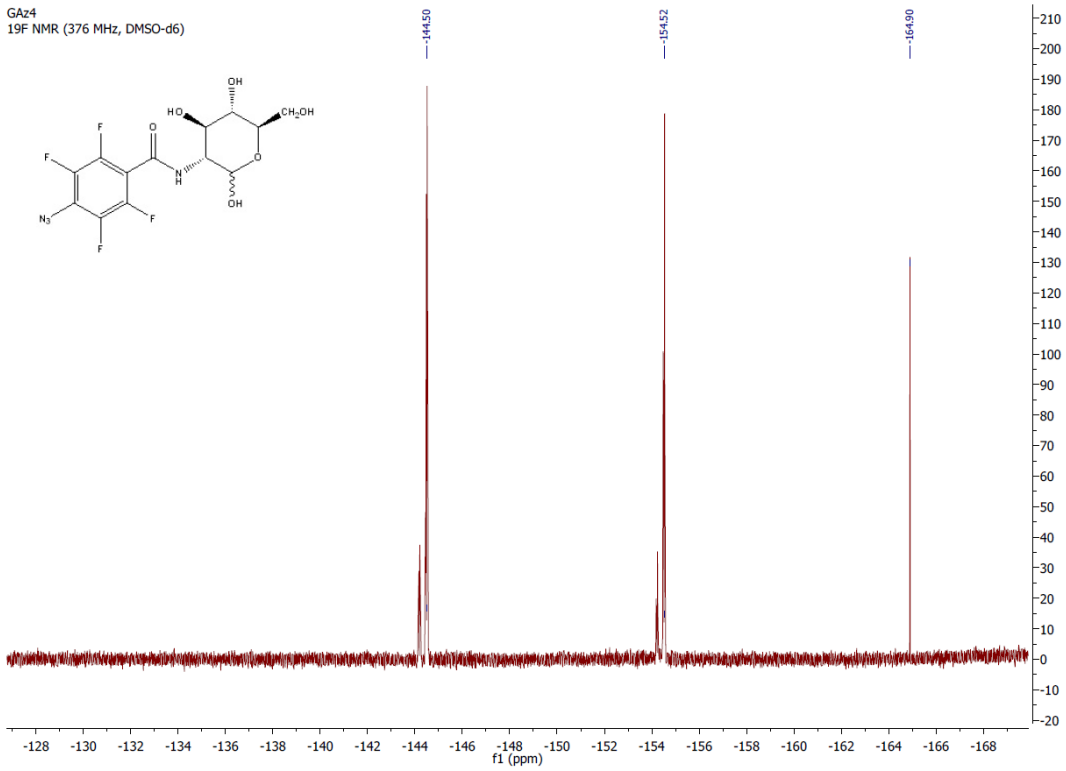
Spectrum ^{1}H-NMR of GAz4



Spectrum ^{13}C-NMR of GAz4



Spectrum ^{19}F-NMR of GAz4



((*S*)-2-(6-((2-(diphenylphosphaneyl)benzoyl)oxy)benzo[*d*]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid) (CLP):

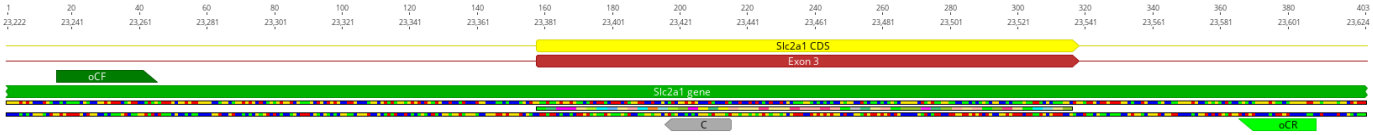
Synthesis of CLP was performed as described previously[2] with slight modifications. Briefly, in a two-neck oven-dried flask, DCC (169.60 mg, 0.82 mmol) and DMAP (4.70 mg, 0.04 mmol) were added together with 2 mL of DCM. A solution of 2-(diphenylphosphino) benzoic acid (240.00 mg, 0.78 mmol) in 6 mL of DCM was added to a flask, and the reaction was stirred for 30 minutes at RT temperature. Next, 6-hydroxybenzo[*d*]thiazole-2-carbonitrile (144.83 mg, 0.82 mmol) was added to the mixture in the flask. The reaction was stirred at RT overnight in the absence of light. The completion of the reaction was detected by the absence of 2-(diphenylphosphino) benzoic acid on TLC (DCM: CH_3OH 95:5, R_f = 0.43). The reaction mixture was filtered through paper filter, and the solvent was evaporated *in vacuo*. Crude compound was purified by column chromatography (hexane: ethyl acetate 8: 1). Fractions containing the product were detected by TLC (DCM: CH_3OH 95: 5, R_f = 0.92). Solvent was evaporated *in vacuo* to yield **2-cyanobenzo[*d*]thiazol-6-yl 2-(diphenylphosphanyl)benzoate** (207.00 mg, 79%).

To a solution of 2-cyanobenzo[*d*]thiazol-6-yl 2-(diphenylphosphaneyl)benzoate (93 mg, 0.2 mmol) in THF (2 mL) was added D-cysteine (29 mg, 0.24 mmol) followed by the addition of deionized water (1 mL) at room temperature under nitrogen with stirring. After stirring for three hours, the reaction mixture was concentrated to half of the original volume, and the product was isolated by HPLC (C18 RP column with water-MeCN gradient), to yield **CLP** (40 mg, 35%) as a yellowish powder.

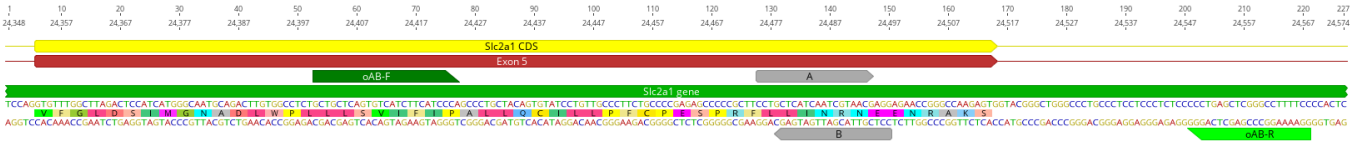
Supplementary Note 3

Sequences of anti-GLUT1 gRNA and primers used to amplify targeting regions of GLUT1 CRISPR/Cas9 plasmid.

C region (Exon 3)



AB region (Exon 5)



Exon no.	Targeting region (label)	Fwd primer name	Forward primer	Rev primer name	Reverse primer
3	C	oCF	CTCTTTTCGTCCGGAGAAACTGAACCTATG	oCR	CTTCCCCCAGAACTCCCCAGACA
5	AB	oAB-F	GCTGCTCAGTGTCATCTTCATCCCA	oAB-R	GGAAAAGGCCCGAGCTCAGGG

Exon no.	Targeting region (name)	Guide RNA	Sequences
3	C	C	ATGGGATGGGCTCTCCGTAG
5	AB	A	CCTGCTCATCAATCGTAACG
		B	CCTCGTTACGATTGATGAGC

Supplementary Note 4

Sequences of anti-GLUT1 shRNA.

Gene	RNA	Sequence	TRC number (Sigma)
SLC2A1	shGLUT1 #1	CCGGGCTGAGAACTTAACTGCTGAACTCGAGTTCAGCAGTTAAGTTCTCAGCTTTTTG	TRCN0000079328
SLC2A1	shGLUT1 #2	CCGGGTCCTATTCCATGGTTCATTGCTCGAGCAATGAACCATGGAATAGGACTTTTTG	TRCN0000311403
SLC2A1	shGLUT1 #3	CCGGGCTGAGAACTTAACTGCTGAACTCGAGTTCAGCAGTTAAGTTCTCAGCTTTTTG	TRCN0000324209
SLC2A1	shGLUT1 #4	CCGGTGAGGAGTTCTACAATCAAACCTCGAGGTTTGATTGTAGAACTCCTCATTTTTG	TRCN0000305719
SLC2A1	shGLUT1 #5	CCGGCATCCTTATTGCCCAGGTGTTCTCGAGAACACCTGGGCAATAAGGATGTTTTTG	TRCN0000079332

References:

1. Lin, F.L., Hoyt, H.M., van Halbeek, H., Bergman, R.G. & Bertozzi, C.R. Mechanistic investigation of the Staudinger ligation. *J Am Chem Soc* **127**, 2686-2695 (2005).
2. Cohen, A.S., Dubikovskaya, E.A., Rush, J.S. & Bertozzi, C.R. Real-time bioluminescence imaging of glycans on live cells. *Journal of the American Chemical Society* **132**, 8563-8565 (2010).