Electronic Supplementary Information

Imidazolium-Tagged Glycan Probes For Non-Covalent Labeling Of Live Cells

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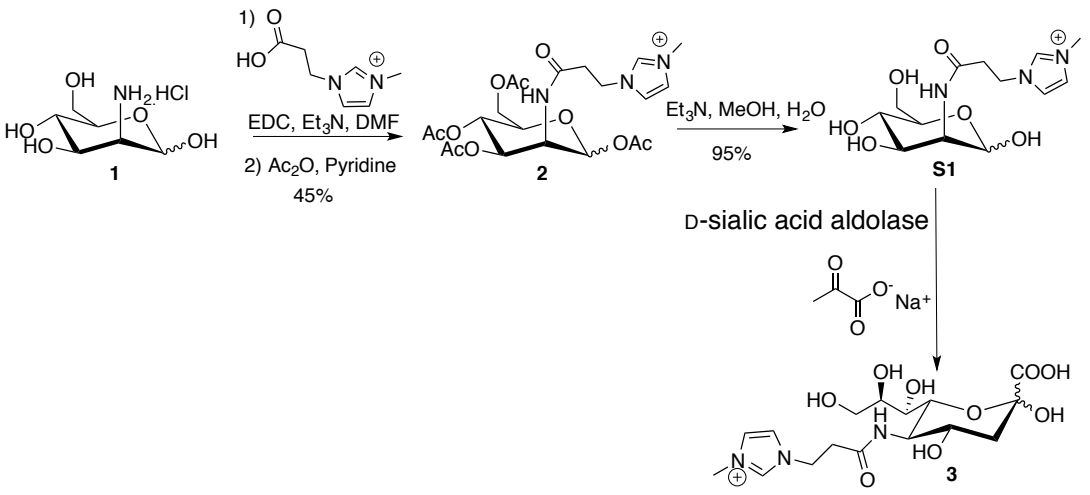
Content:

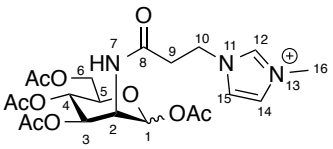
Experimental Procedures

General. Chemicals were purchased and used without further purification. Dry solvents were obtained by distillation using standard procedures, or by passage through a column of anhydrous alumina using equipment from Anhydrous Engineering (University of Bristol) based on the Grubbs' design. Reactions requiring anhydrous conditions were performed under N_2; glassware and needles were either flame dried immediately prior to use, or placed in an oven (150 °C) for at least 2 h and allowed to cool in a desiccators or under reduced pressure.Liquid reagents, solutions or solvents were added *via* syringe through rubber septa; solid reagents were added *via* Schlenk type adapters. Teflon rings were used between the joints of the condensers and round bottom flasks. Reactions were monitored by TLC on Kieselgel 60 F254 (Merck), with UV light (254 nm) detection and by charring with 10% sulfuric acid in ethanol. Flash column chromatography was performed using silica gel [Merck, 230−400 mesh (40−63 μm)]. Extracts were concentrated *in vacuo* using both a Büchi rotary evaporator (bath temperatures up to 40 °C) at a pressure of 15 mmHg (diaphragm pump) or 0.1 mmHg (oil pump), as appropriate, and a high vacuum line at room temperature. Water soluble compounds were freeze dried on a Lytotrap Plus (LTE Scientific LTD). ^{1}H NMR and ^{13}C NMR spectra were measured in the solvent stated at 400, 500 or 600 MHz. Chemical shifts are quoted in parts per million from residual solvent peak ($CDCl_3$: ^{1}H - 7.26 ppm and ^{13}C - 77.16 ppm) and coupling constants (*J*) given in Hertz. Multiplicities are abbreviated as: b (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or combinations thereof. Dynamic Light Scattering (DLS) and Zeta analysis are carried out using Malvern Instruments, Nano_S90 Red Laser Model ZEN1690 for DLS and Nano-Z ZEN 2600 for Zeta potential.

Chemical Synthesis

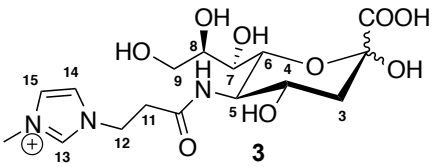


Scheme S1. Synthesis of ITag-glycan probes **2** and **3**.



2

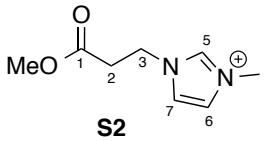
To a flask containing D-mannosamine hydrochloride (300 mg, 1.39 mmol) and N-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (533 mg, 2.78 mmol) in dry DMF (5.3 mL) a solution of 1-(2-carboxyethyl)-3-methyl-1*H*-imidazol-3-ium chloride (400 g, 2.09 mmol) in DMF (1 mL) was added. After 10 min the reaction was cooled to 0 ºC and NEt_3 (2.8 mL, 20.0 mmol) was added. The reaction was then stirred for 24h at room temperature until the starting material was consumed. The reaction mixture was concentrated under vacuum and the crude was redissolved in DMF (7 mL) followed by the addition of pyridine (1.4 mL) and acetic anhydride (1.3 ml). The reaction mixture was stirred at room temperature for 8 h. Once the starting material was consumed, the solvents were evaporated under vacuum. The product purified by column chromatography (1 to 20% MeOH in DCM) to afford **2** as 4:3 mixture of anomers (310 mg, 46%). ^{1}H NMR (500 MHz, d_2o) δ: 8.75 (s, H-12), 8.74 (s, H-12), 7.49 (appt, *J* = 2 Hz, H-14), 7.48 (appt, *J* = 2 Hz, H-14), 7.45 (appt, *J* = 2 Hz, H-15), 7.43 (appt, *J* = 2 Hz, H-15), 5.94 (d, *J* = 2.5 Hz, H-1), 5.86 (d, *J* = 1.5 Hz, H-1), 5.30 (dd, *J* = 4.5, 10.0 Hz, H-3), 5.20 (dd, *J* = 4.0, 9.0 Hz, H-3), 5.12 (appt, *J* = 10.0 Hz, H-4), 5.06 (appt, *J* = 9.5 Hz, H-4), 4.69 (dd, *J* = 2.0, 4.0 Hz, H-2), 4.54 (dd, *J* = 2.0, 4.5 Hz, H-2), 4.49-4.41 (m, H-10), 4.35-4.31 (m, H-6), 4.26 (ddd, *J* = 2.5, 5.0, 10.0 Hz, H-5), 4.15 (dd, *J* = 2.5, 12.5 Hz, H-6), 4.09-4.05 (m,H-6, H-5), 3.85 (s, NCH_3), 3.84 (s, NCH_3), 3.06-2.94 (m, H-9), 2.16 (s, CH_3), 2.08 (s, CH_3), 2.07 (s, CH_3), 2.06 (s, CH_3), 1.92 (s, CH_3), 1.91 (s, CH_3). ^{13}C NMR (126 MHz, d_2o) δ = 173.65, 172.92, 172.89, 172.72, 172.70, 172.24, 171.65 (C=O), 136.30 (C-12), 123.89, 123.84, 122.23, 122.21 (C-14, C-15), 91.76 (C-1), 90.74 (C-1), 72.42 (C-5), 71.14 (C-3), 69.86 (C-3), 69.69 (C-5), 65.55 (C-4), 65.44 (C-4), 62.28 (C-6), 62.27 (C-6), 49.35 (C-2), 49.30 (C-2), 35.65 (CH_3), 35.88 (C-9), 35.20 (C-9), 20.31 (CH_3), 20.25 (CH_3), 20.23 (CH_3), 20.18 (CH_3). HR-MS for $C_{21}H_{30}N_3O_{10}^+$ (*m/z*): (M^+) Calculated: 484.1926; found 484.1928.



3

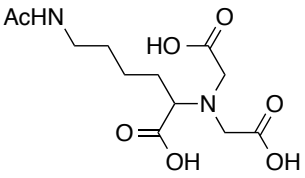
Compound **2** (10 mg, 0.021 mmol) was dissolved in a mixture of triethylamine/methanol/water (1:8:1, 2 ml). The reaction was stirred for 12 h until disappearance of the starting material. 200 µl of aqueous HCl (1M) was added and the solvents were evaporated under vacuum. The product **S1** was used without further purification in the next step (8.1 mg, 95%). ^{1}H NMR (500 MHz, d_2o) δ: 8.65 (s, H-12), 7.45-7.36 (m, H-14, H-15), 4.96 (d, *J* = 1.9 Hz, H-1), 4.49-4.43 (m, H-10), 4.39-4.22 (m, H-2), 4.00-

3.96 (m, H-3), 3.83 (s, NCH_3), 3.82 (s, NCH), 3.79-3.63 (m, 2 X H-6, H-4), 3.48-3.40 (m, H-5), 3.38-3.30 (m, H-5), 2.95-2.82 (m, H-9, H-9). ^{13}C NMR (126 MHz, d_2o) δ = 173.27, 172.34 (C=O), 136.04 (C-12), 121.96, 123.55 (C-14, C-15), 92.60 (C-1), 71.88 (C-4), 68.22 (C-3), 66.62 (C-5), 60.46 (C-6), 53.51 (C-2), 52.85 (C-2), 45.33 (C-10), 35.58 (CH_3), 35.42 (C-9). HR-MS for $C_{13}H_{22}N_3O_6^+$ (*m/z*): (M^+) Calculated: 316.1509; found 316.1512. A solution of aldolase (8.5 mg, 140 µL PBS) was added to a solution of compound **S1** (2.2 mg, 140 µL PBS). A solution of sodium pyruvate (7.7 mg, 100 µL PBS) and dithiothreitol (0.3 mg, 30 µL PBS) were added. The reaction mixture was shaken for 80 h at 37 ºC. After that time the reaction mixture was heated at 90 ºC for 5 min, centrifuged at 5000 rpm for 10 min, and the supernatant was freeze-dryed. The reaction mixture was purified by reverse phase HPLC (C18 with TMS endcapping, 100Å, water/methanol gradient from 5% to 20% over 20 min) to yield **3** (0.6 mg). ^{1}H NMR (500 MHz, D_2O) δ 8.61 (s, H-13), 7.38 (bs, H-14/15), 7.32 (bs, H-14/15), 4.39 (appt, J = 6.0 Hz, H-12), 3.84-3.48 (m, H-7, H-4, H-5, CH_3, H-9, H-8), 3.46 (dd, J = 6.5, 11.0 Hz, H-9), 3.25-3.20 (m, H-6), 2.81 (appt, J = 6.0 Hz, H-11), 2.43 (dd, J = 12.5, 2.5 Hz, H-3a), 1.68 (appt, J = 12.5 Hz, H-3b). ^{13}C NMR (126 MHz, D_2O) δ 172.18 (C=O), 135.99 (C-13), 123.41, 121.98 (C-14, C-15), 70.42 (C-8), 70.08 (C-7), 69.00 (C-6), 67.27 (C-4), 63.54 (C- 9), 52.09 (C-5), 45.28 (C-12), 39.56 (C-3), 35.70 (NCH_3), 35.67 (C-11). HR-MS: $C_{16}H_{26}N_3O_9^+$ (m/z): (M^+) Calculated: 404.1664; found 404.1663



S2

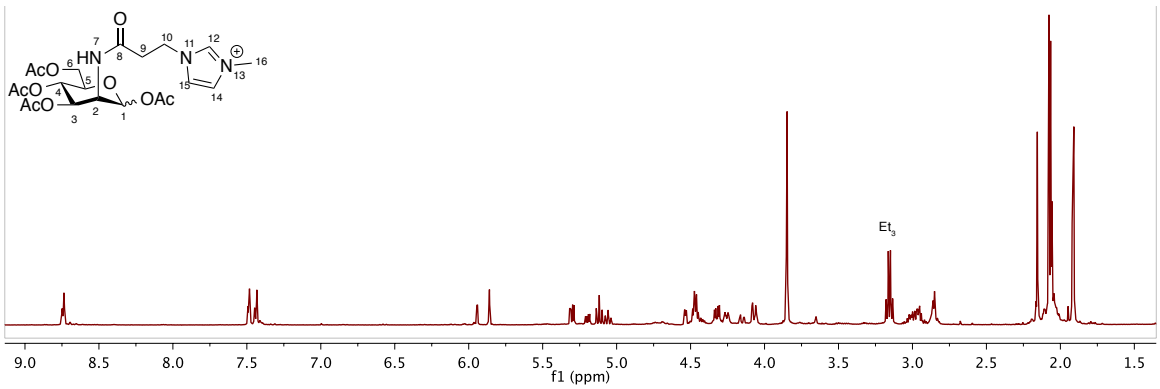
1-(2-carboxyethyl)-3-methyl-1H-imidazol-3-ium bromide[1] (150 mg, 0.79 mmol) was dissolved in dry methanol (16 mL). Trifluoroacetic acid (300 µL, 4.74 mmol) was added and the reaction mixture was refluxed for 12 h until the starting material was consumed. The solvents were evaporated under vacuum and the reaction crude was purified on a silica gel column (DCM/MeOH 9:1) to afford compound **S2** (89 mg, 83%). MS for $C_8H_{13}N_2O_2^+$ (*m/z*): (M^+) Calculated: 169.0972; found 169.0974. ^{1}H NMR (400 MHz, cd3od) δ 9.13 (s, 1H, H-5), 7.76, 7.65 (s, 1H, 1H, H-6, H-7), 4.58 (t, J = 6.5 Hz, 2H, H-3), 4.00 (s, 3H, NCH_3), 3.72 (s, 3H, OCH_3), 3.08 (t, J = 6.4 Hz, 2H, H-2). ^{13}C NMR (101 MHz, cd3od) δ 171.26 (C=O), 136.85 (C-5), 122.36, 123.42 (C-6, C-7), 51.45 (OCH_3), 44.86 (C-3), 35.31 (NCH_3), 33.62 (C-2).

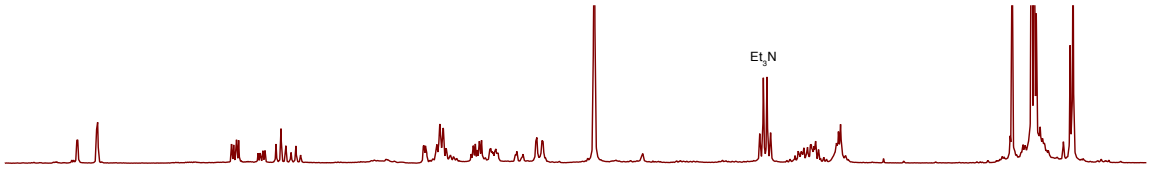


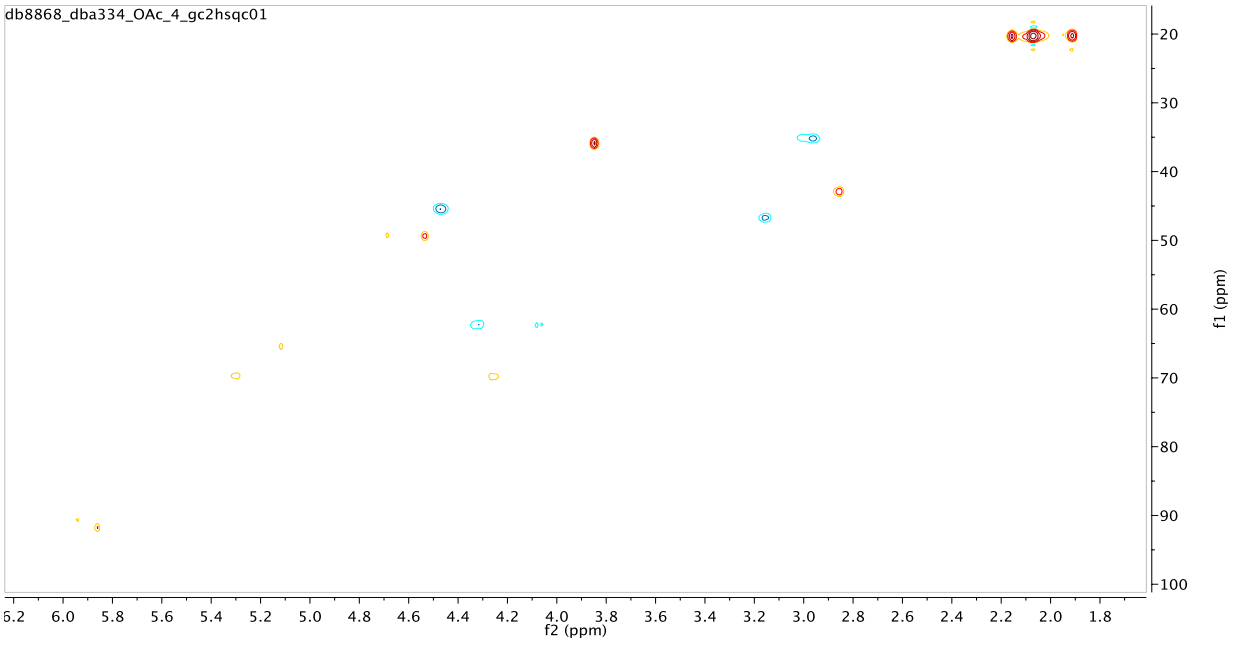
S3

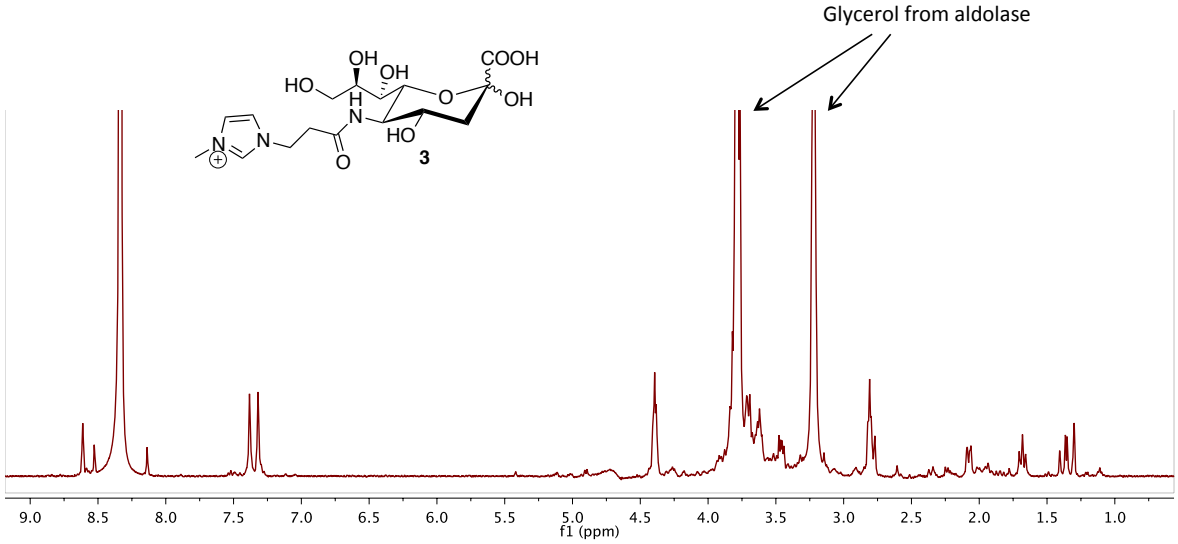
Nε-acetyl-Nα,Nα-bis(carboxymethyl)lysine **S3** was synthesised following literature procedures.[2]

NMR spectra








Glycerol from aldolase
OH
OH
COOH
HO
H
N
O
OH
HO
N
N
O
3
9.0
8.5
8.0
7.5
7.0
6.5
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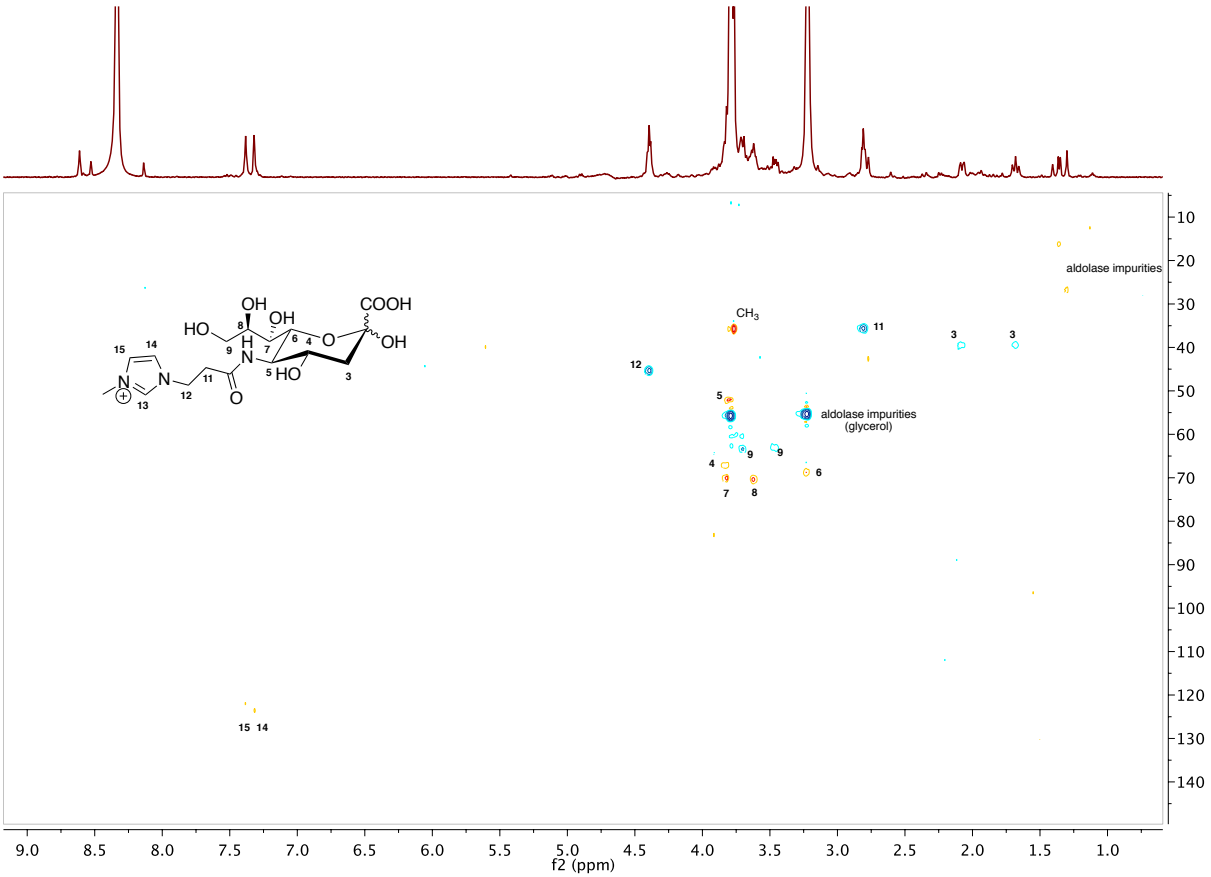
2.0
1.5
1.0
f1 (ppm)


aldolase impurities
CH3
aldolase impurities
(glycerol)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
9.0
8.5
8.0
7.5
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6.5
6.0
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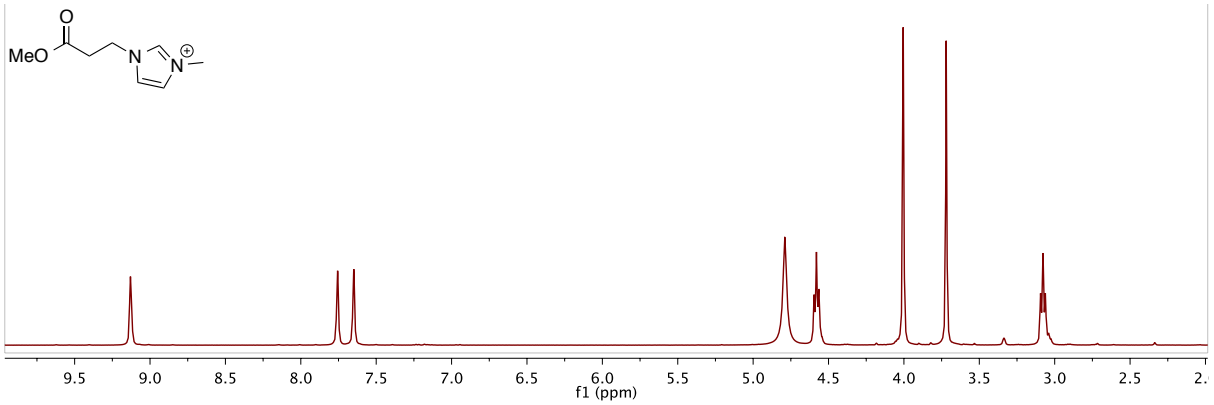
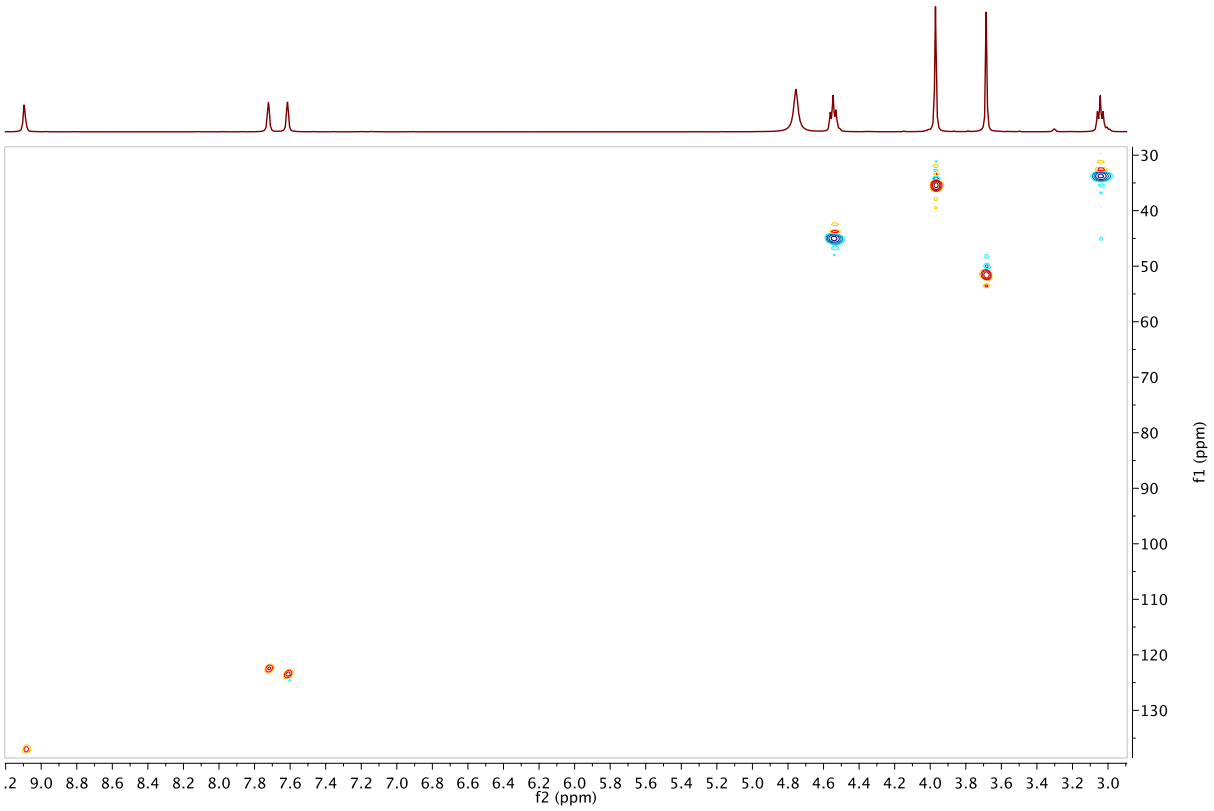
1.5
1.0
f2 (ppm)


MeO
O
N
N
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5
f1 (ppm)


30 40 50 60 70 80 90 100 110 120 130
f1 (ppm)
.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0
f2 (ppm)

NMR titration experiments

NMR titrations using a model ILs 3-methyl-1-imidazol-3-ium-propionic acid[3] with Ni^{2+}:NTA or 3-methyl-1-imidazol-3-ium-propionate **S2** with a nitriloacetic acid (NTA **S3**) showed that the ITags can interact with the Ni^{2+}:NTA and NTA labels.

A) NMR titrations using model IL 3-methyl-1-imidazol-3-ium-propionic acid[3] with Ni^{2+}:NTA in PBS show that an interaction with the Ni^{2+}:NTA labels in the presence of high salt content is possible. However, Ni^{2+} is paramagnetic and as such spectra run in the presence of Ni^{2+} showed very broad signals, and although slight shifts in the spectra can be observed as the Ni^{2+}:NTA is titrated in, quantification is not possible.

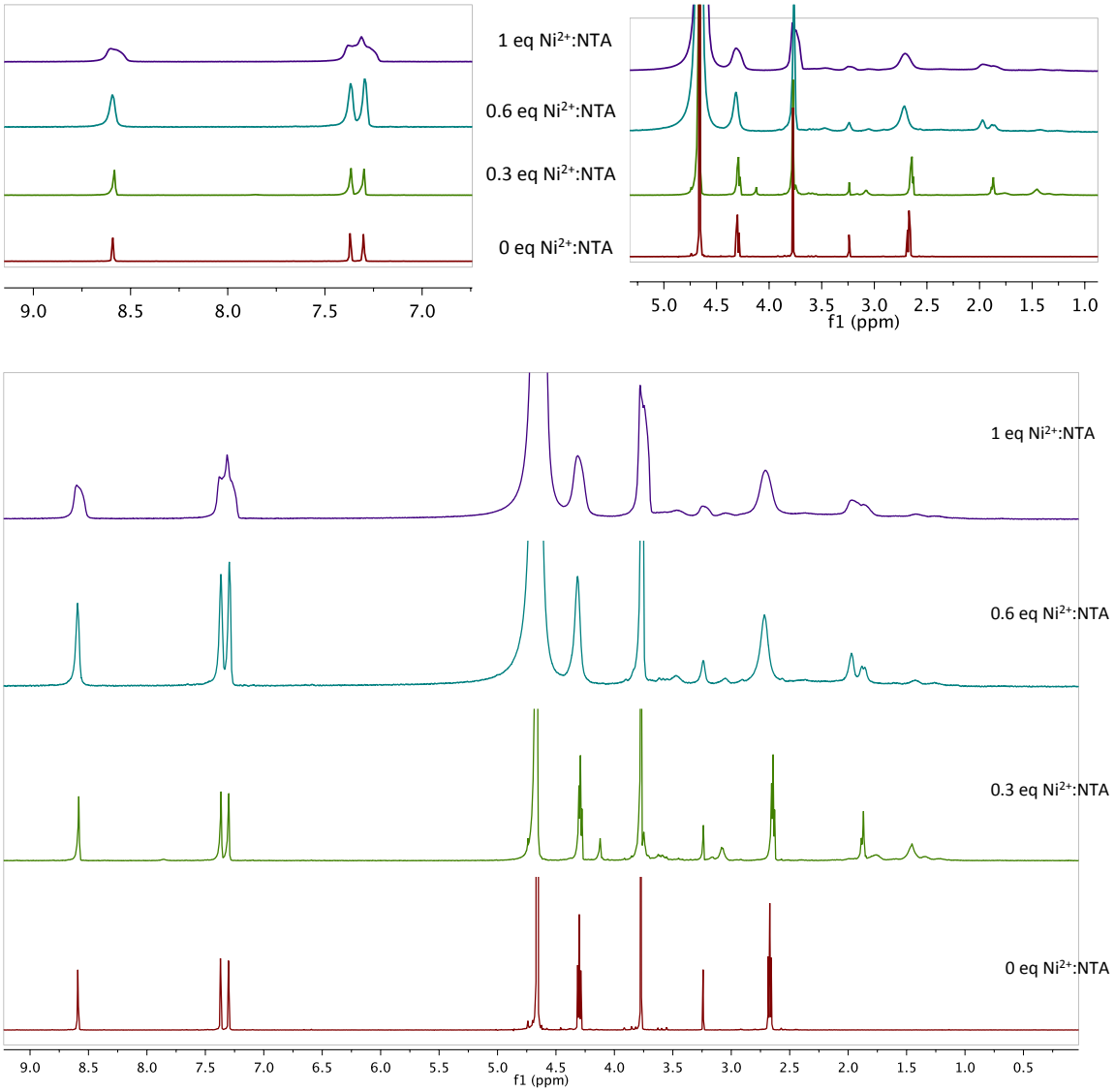
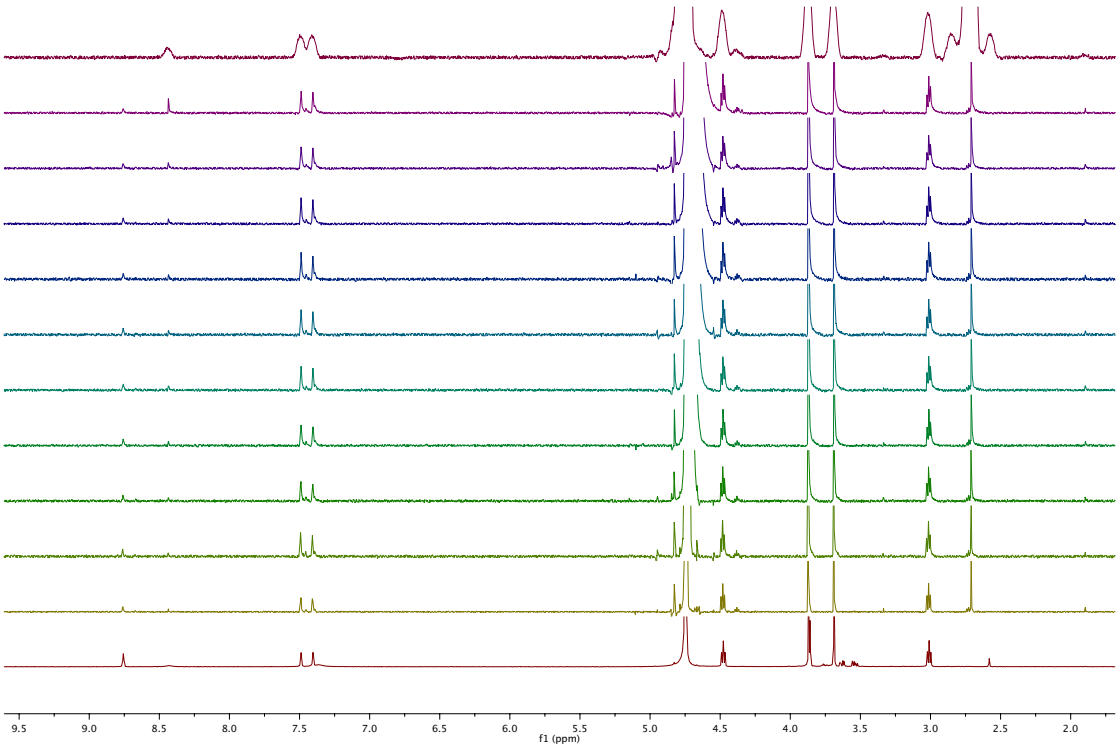


Figure S2A NMR experiment of model 3-methyl-1-imidazol-3-ium-propionic acid IL with Ni^{2+}:NTA in PBS.

B) NMR titrations using model IL 3-methyl-1-imidazol-3-ium-propionate **S2** with a nitriloacetic acid (NTA **S3**) derivative in PBS also showed that the ITags can interact with the NTA labels even in the presence of high salt content.

^{1}H NMR titration experiments were performed at 500 MHz on a Varian 500B spectrometer. Binding studies were performed with a 2 mM solution of **S2** in PBS (D_2O). Solutions of **S3** were prepared in PBS (D_2O). Aliquots of **S3** solutions (from 0 to 0.3 M) were added to **S2** solution (DMSO as internal standard) and the ^{1}H NMR spectra were recorded. Integration values for the peak corresponding to the shifted signal for H-5 of **S2** were fitted to a Michaelis-Menten model using GraphPad Prism software, yielding a binding constant of K = 22 ± 9 mM. Spectra and fitting curve are given in Fig. S3



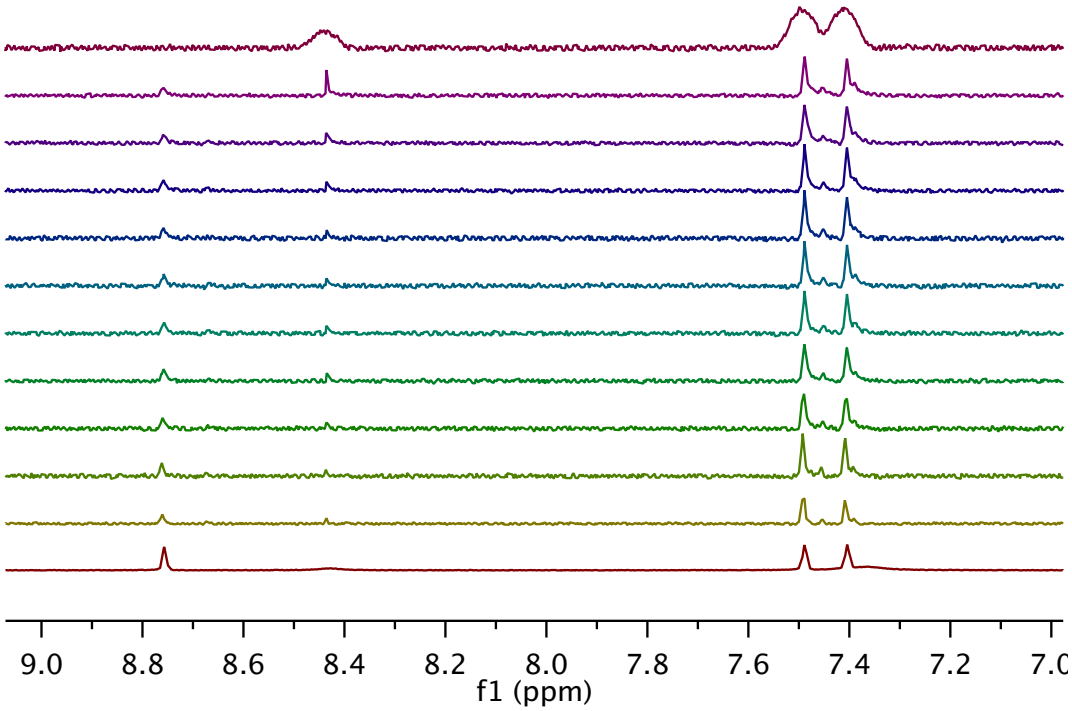


Figure S2B NMR experiment of model imidazolium IL **S2** with **S3** in PBS and expansion.

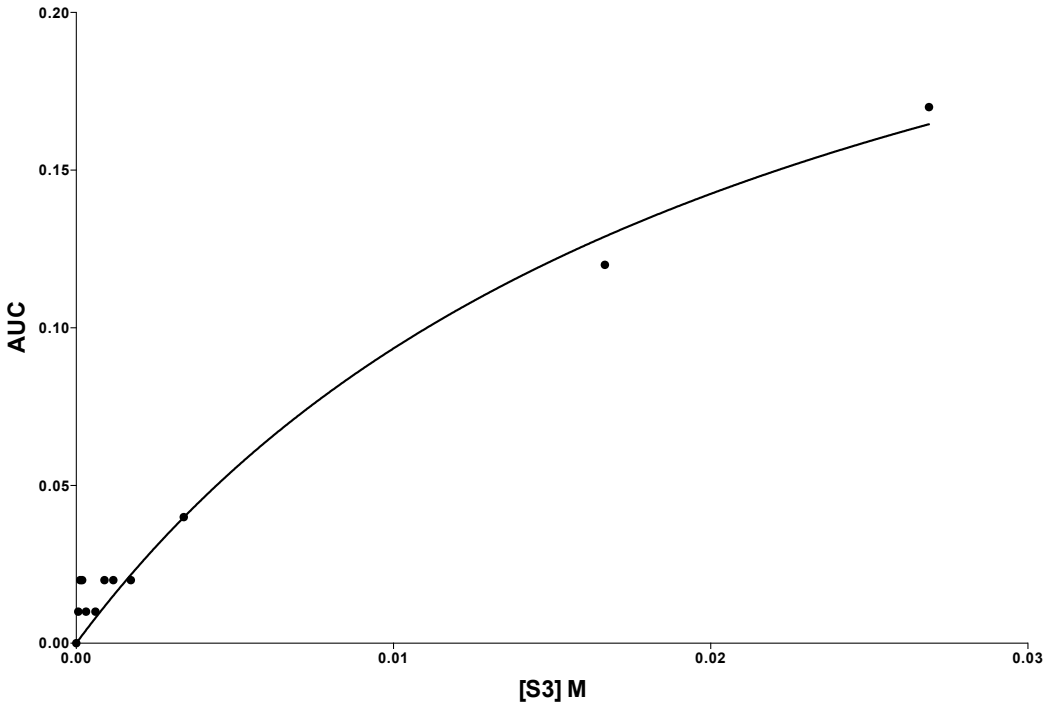


Figure S3. Binding curve obtained by non-linear fitting. AUC (Integration value for H-5 signal from **S2**) *vs* concentration of titrated NTA **S3**.

Cell culture protocols

Araki Sasaki (AS) - SV40-immortalized human corneal epithelial cell line was grown in Dulbecco's Minimal Essential Medium (MEM) ; *HeLa - Human cervical carcinoma cell line (*HeLa) in Minimal Essential Medium (MEM); *Jurkat - Human leukemic T cell lymphoblast cell line was grown in suspension in* Advance RPMI 1640; *MCF7 - Human breast adenocarcinoma cell line* in either MEM or RPMI 1640 for direct comparisons with MDA, while *MDA-MB-231 - Human breast adenocarcinoma cell line* was grown in Dulbecco's MEM with GlutaMAX - high glucose (4.5 g/L D-glucose), and *E12 a mucin-producing clone of HT29 (*HT29-MTX-E12) was grown in Dulbecco's MEM with 4.5 g/L D-glucose.

All growth media were supplemented with antibiotic-antimycotic (Anti-Anti) and 10 % fetal bovine serum (FBS); all cell culture media and additives were purchased from Invitrogen, Life Technologies. Confluent cultures were detached from the surface using trypsin (Tryp LE Express) and plated at $2*10^4$ cells/well in either petri dishes (Mat-Tek 35 mm, with 14mm glass microwell) for imaging, or 96-well plates for other tests. All cell culture media and additives were purchased from Invitrogen, Life Technologies.

Confocal microscopy

All images were acquired on a Leica SP5 confocal system equipped with a Leica DMI 6000 inverted microscope. The images were analysed using Volocity software (PerkinElmer). NucBlue™ Live Cell Stain (Hoechst 33342) for nuclei was purchased from Life Technologies. The marker was used in line with manufacturer's protocols.

Toxicity assays

The influence of Man-ITag **2** on cell metabolism was assessed using AlamarBlue (Life Technologies), a cytosolic substrate for reductive metabolism (resazurin to resorufin) whose fluorescence spectrum changes on reduction by cytosolic enzymes. To allow for any differences in cell numbers in each well, AB fluorescence was expressed per unit protein (BCA Pierce, ThermoScientific). The metabolic competence of cells after 72 hours exposure to **2** is expressed as percentages of control (Figure S4). It only the highest concetrations of **2** (1 – 5mM) that caused marked decreases in metabolic competency. These concentrations were not used in further experiments.

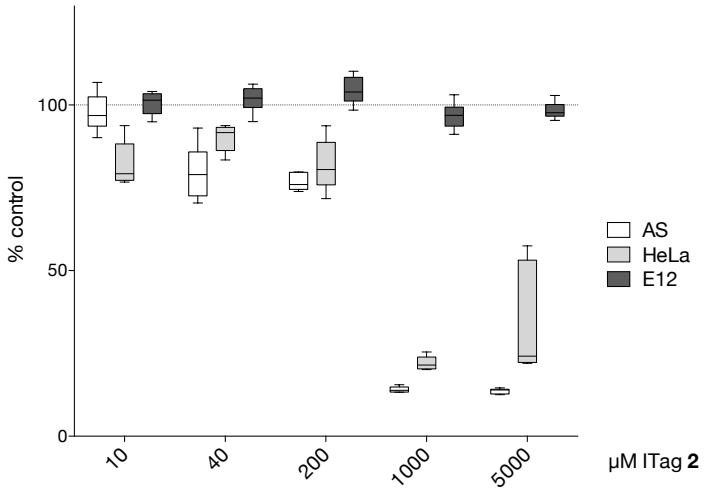


Figure S4. Metabolic competence of cell lines after 72 h exposure to **2** (Ac_4ManN-Itag), expressed as % of control (100%). Cells shown: AS – Araki Sasaki human corneal epithelial cells, HeLa –human cervical cancer cell line; E12 – mucin secreting intestinal cells HT29-MTX-E12

Any changes in cell survival after exposure to **2**, were quantified by measuring calcein fluorescence (Figure S5). The fuorescence, retained within live cells, results from activity of esterases on (nonfluorescent) calcein AM (Molecular Probes). There were no statistically significant changes in cell numbers following 3 days of exposure to **2**.

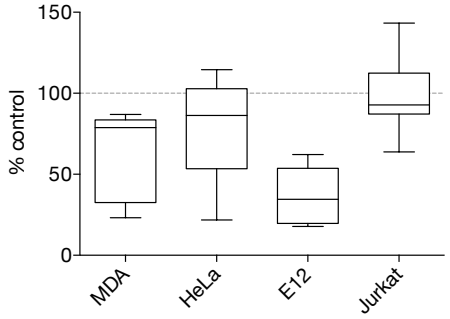


Figure S5. Cell numbers, assessed by CalceinAM fluorescence, after 72 h exposure to **2** (2 µM Ac_4ManN-ITag), expressed as % of control (100%). Medians of treated cells are not different from controls (Kruskal–Wallis multiple comparisons).

Zeta Potential measurements

Zeta potential analyses were carried out with a Malvern Instruments Nano-Z ZEN 2600 in 0.1 M phosphate buffer. Cells were either untreated (0), or treated with 0.2 or 2 μM Ac_4ManITag (**2**) for 3 days. No significant changes in zeta potential were detected (ANOVA) after treatment with **2**.

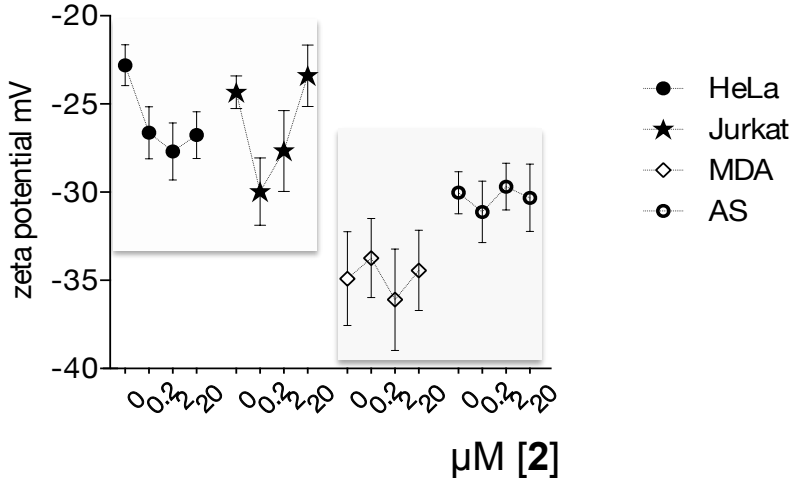


Figure S6. ***Zeta potential measurements:*** Three patterns of change in surface charge can be identified, though differences are not statistically significant in any of the cell lines investigated: a steep decrease at 0.2 μM ITag **2** followed by a plateau (HeLa) or more positive surface (Jurkat), or no change at all as in MDA and AS.

Incubation and detection of cell surface labelled sialic acids

Jurkat T-lymphocytes (Jurkat) and breast cancer cell line MDA-MB-231 (MDA), colon cancer cell line HT29-MTX-E12 (E12), cervical cancer cells HeLa and Araki Sasaki SV40-immortalized human corneal epithelium (AS) were incubated with 0-200 μM of **2** for 72 hours. Labeled glycans were visualized with NTA-Atto 550. Biotinylated sialic acid-binding lectins *Maackia Amurensis* II (MAA) or *Sambucus Nigra Agglutinin* (SNA), both from Vector Laboratories, were detected usind DyLight labeled neutravidin (Life Technologies).

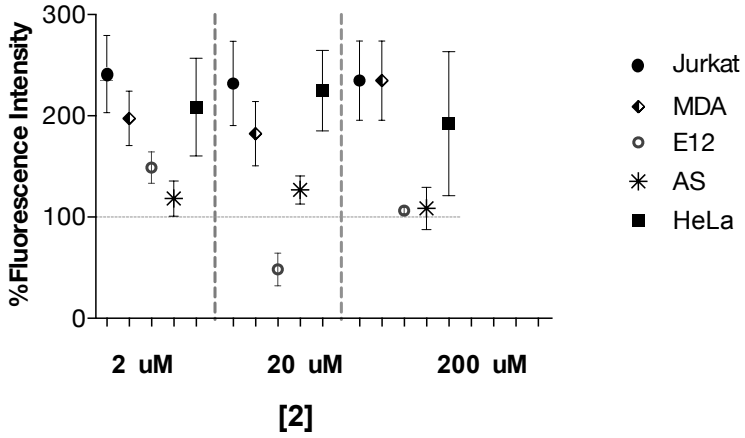


Figure S7A. Ni^{2+}:NTA-ATTO labeling of the cell surface after incubation with **2** (2-200 μM shown) in complete cell growth medium for 72 h. Fluorescence of label is expressed as % of control (100%). Increased amounts of **2** from 0.2 to 200 μM did not necessarily cause a significant increase in cell surface fluorescence (Fig. 3).

Sialidase cell incubation.

Sialidase (neuraminidase) from *Arthrobacter ureafaciens* (Sigma, Poole UK) was used to assess the effect of sialic acid removal on NTA-ATTO binding to the cell surface after 72 hour incubation with Man-ITag **2** (2 μM). After a 72 h incubation with **2** (or medium as control) cells ($2x10^4$) were treated with 3μU/well in 50 μL reaction buffer (Sigma), pH 6 for 1.5h at 37 ^oC. The cells were washed three times in PBS pH7.4 to dilute the enzyme out, and incubated with NTA-ATTO 550 (λ_{ex} 554nm/λ_{em} 576nm) for 1.5h in tissue culture medium as appropriate for the cell line. After further 3 rinses with PBS NTA-ATTO fluorescence was quantified in each well. (Figure 7b)

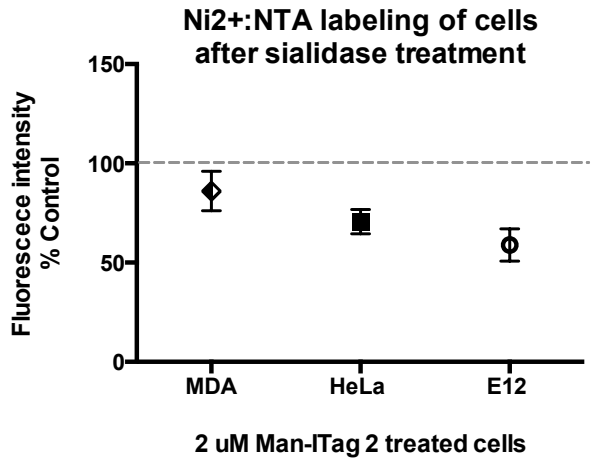


Figure S7B. Ni^{2+}:NTA-ATTO labeling of the cell surface of MDA, HeLa and E12 that have been incubated with **2** (2 μM) in complete cell growth medium for 72 h, followed by treatment with sialidase to remove cell surface sialic acids. Fluorescence of label is expressed as % of control (100%), which is defined as Ni^{2+}:NTA-ATTO labelled cells that had been treated with Man-ITag **2** (2 μM). Cells that had been expose to the sialidase, show a decrease in fluorescence, which suggest that: a) sialidase is able to hydrolise Neu5N-ITag moieties and b) further supports the hypothesis that NTA is able to bind to the ITag groups present on the sialic acids.

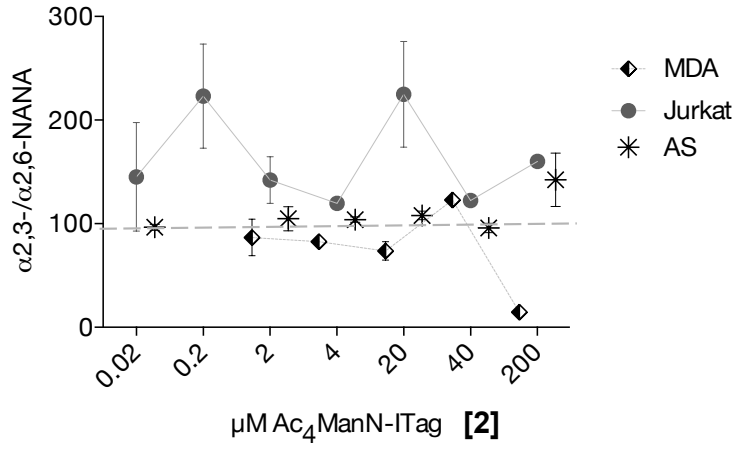


Figure S8. Influence of **2** on sialic acid expression: alteration of ratios of α2,3-/α2,6-sialic acids respective to untreated controls (100). Note that in Jurkat α2,3-sialic acids are enhanced under the influence of **2**, in AS ratios are conserved, while in MDA there is a decrease in the representation of α2,3-sialic acids at the highest concentration of **2** only.

Cell adhesion and aggregation

Adhesion to fibronectin-coated wells

Jurkat, E12, Hela (not shown) and MDA cells were exposed to concentrations of $ManAc_4$-ITag **2** between 0.2 and 20 µM for 72 h, and then allowed to adhere to a fibronctin-coated plate for 2h at 37°C in RPMI supplemented with 2% FBS. After removal of supernatant and washing, cell numbers were assessed by calcein fluorescence. The expression of ITag on the surface of adherent cells and cells in solution was assessed by Calcein Am and NTA-ATTO 550 fluorescence, respectively.

Figure S9 presents the number of cells that ahered to the fibronectin plate and of those that remained floating in the medium. An effect of ITag treatment was to increase, but not significantly except at 2 µM, the proportion of non-adherent cells.

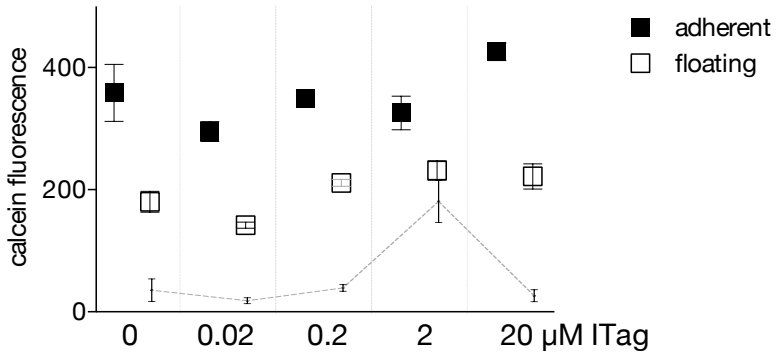


Figure S9. Effect of $ManAc_4$ITag **2** concentration on the number of Jurkat cells adhering to fibronectin as detected by Calcein AM. The dotted line represents the ratio of non-adherent to adherent cells, to highlight that this ratio is constant, except for at 2 µM ITag, when the proportion of floating cells is significantly increased (ANOVA, Tukey post hoc, $p<0.001$).

No significant differences in adherence to fibronectin are observed for MDA or E12 cells exposed to 20µM of **2** (figure S10 and S11), or in the NTA-ATTO fluorescence of adhering and floating cells (Figure S10). (control vs treatment groups unpaired t test, $p<0.05$)

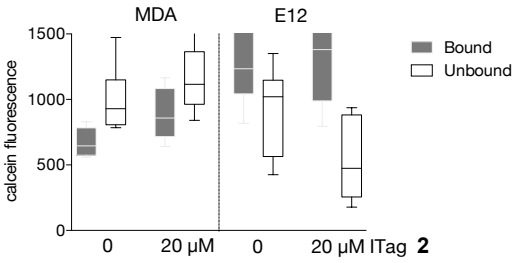


Figure S10. Effect of $ManAc_4$ITag **2** concentration on the number of MDA or E12 cells adhering to fibronectin as detected by Calcein AM.

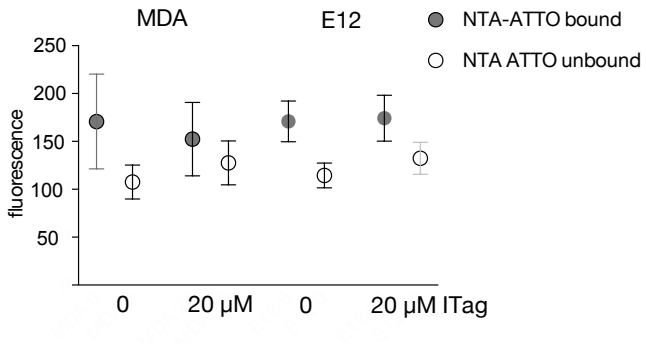
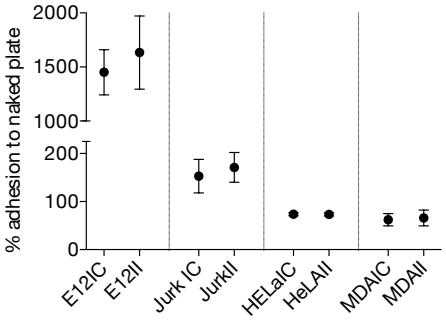


Figure S11. Expression of ITag at the cell surface of adherent and non-adeherent cells as detected by labelling with Ni^{2+}NTA-ATTO 550 (NTA).

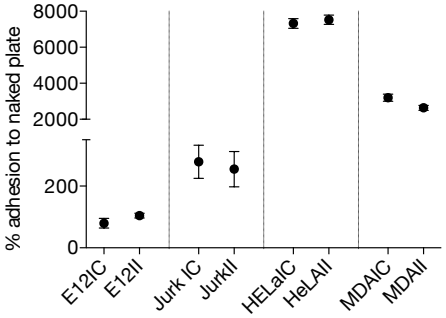
Cell aggregation

Effects on cell-cell aggregation were assessed quantifying the adherence of MDA, Jurkat, HeLa and E12 cells, that had been pretreated with **2** at 20 µM to monolayers of control or treated cells, using adherence of naked wells as controls. HeLa, MDA, E12 and Jurkat which were untreated or pretreated with 2 µM ManITag **2** were plated in 96-well plates at 10^5 cells/well (for adherent cultures) or set up in 25 cm^2 T-flasks and stored for 3 days at 37 °C. Cells then allowed to adhere to monolayers of same or different cells for 2h at 37°C in RPMI supplemented with 2% FBS. After removal of supernatant and washing, cell numbers were assessed by calcein fluorescence.

A



B



C

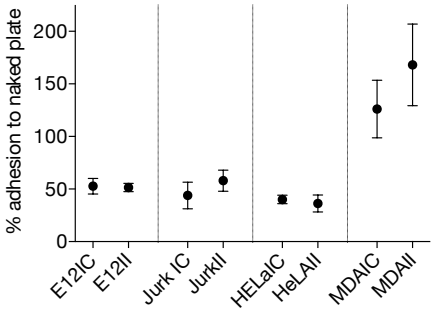


Figure S12. Cell-cell interactions after treatment with $ManAc_4ITag$ **2**. Responses depend on the two cell lines interacting. Cells were allowed to adhere to plated MDA (A); HeLa (B); and E12 (C) for 2h at 37°C in growth medium supplemented with 2% FBS. Cells were treated with 20 μM $ManAc_4ITag$ **2** (**I**) for 72h before the experiment, or untreated (**C**). Cell aggregation with the monolayer was monitored by loading them with calcein for 30 minutes prior to plating. Combinations of plated and calcein-labelled cells were explored: monolayers untreated and ITag-treated cells (**IC**); ITag treated monolayers and ITag treated cells (**II**). All results were normalized to ITag-treated cells binding to the naked wells.

Figure S12 shows that ITag treatment had little or no effect on adherence of cells to confluent mononlayers of same or different cell types.

Purification of ITag labelled glycoproteins:

Cell lysates were dyalised for 24 h at 4 °C and then freeze dried. The samples were redissolved in 500 µl of a 1:1 solution of PBS 0.02M and NaCl 1M (buffer A) and purified using a HitrapTM Q HP column.

Method A: The column was conditioned and washed with buffer A, the sample was eluted using 1:1:1 solution of PBS (0.02M), NaCl (1M), and EDTA (0.05M).

Method B: The column was loaded with ni using A solution of $NiSO_4$ (0.1M). After washing the column with miliQ water, the column was equilibrated and washed with buffer A. The sample was eluted using a gradient of imidazole (0.02 to 0.5 M in buffer A). The buffer of the fractions collected was then exchanged to miliQ water and aliquots of each fraction were subjected to electrophoresis on 4-12% NuPAGE gels and blotting and imaged as above. After electroblotting on polyvinylidene fluoride, the blots were incubated with SNA lectin and imaged.

Alternatively, the fractions produced after purification were incubated with mercaptoacetic acid coated QDs[3] (5 µL, 24 mg/mL) for 20 min before running the electrophoresis.

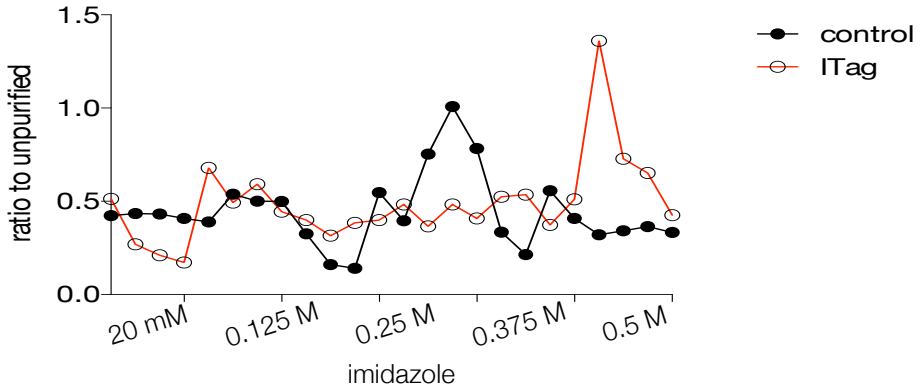


Figure S13. SNA stain purification profile (shown as a ratio to unpurified) of untreated (control) and ITag treated MDA cells (2 µM of **2**) glycoproteins eluted with different concentrations of imidazole.

References

(1) Forbes, D. C.; Patrawala, S. A.; Tran, K. L. T. *Organometallics* **2006**, *25*, 2693.

(2) Bonomi, R.; Scrimin, P.; Mancin, F. *Org Biomol Chem* **2010**, *8*, 2622.

(3) Benito-Alifonso, D.; Tremel, S.; Hou, B.; Lockyear, H.; Mantell, J.; Fermin, D. J.; Verkade, P.; Berry, M.; Galan, M. C. *Angew Chem Int Edit* **2014**, *53*, 810.