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Molecular Labels for the analysis of Amines and Diols by Spray Based Ionization

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Supplemental Information

Contents

Methylimidazolium mass label data

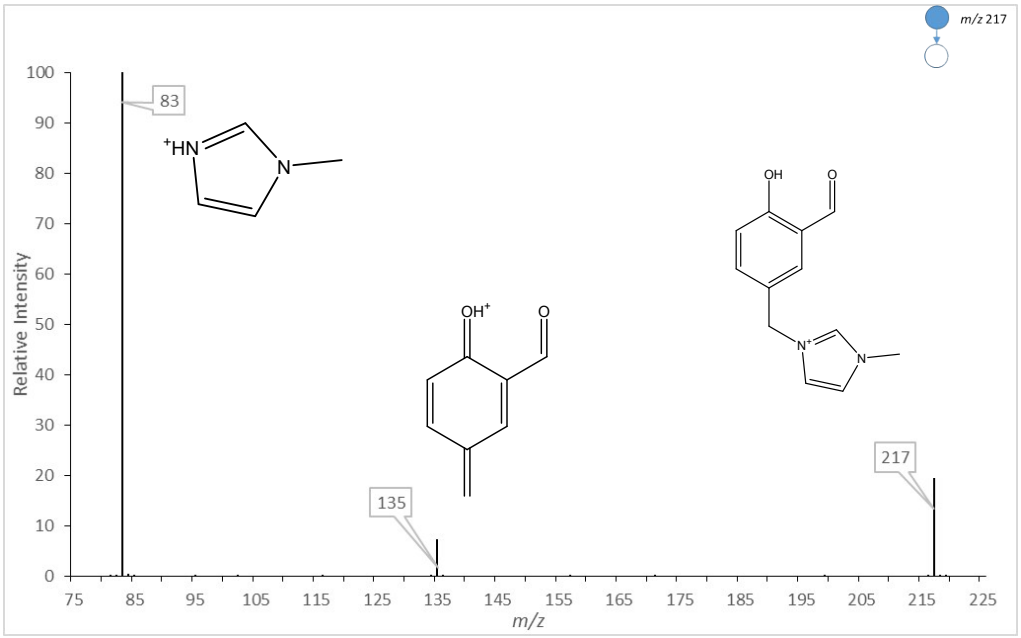

Figure S1a: *product ion MS/MS spectrum of the methylimidazolium mass label*

Orbitrap data: m/z 217.09963 ($C_{12}H_{13}O_2N_2$, -2.415ppm), m/z 135.04376 ($C_8H_7O_2$, -2.192 ppm), m/z 83.06024 ($C_4H_7N_2$, -1.623ppm)

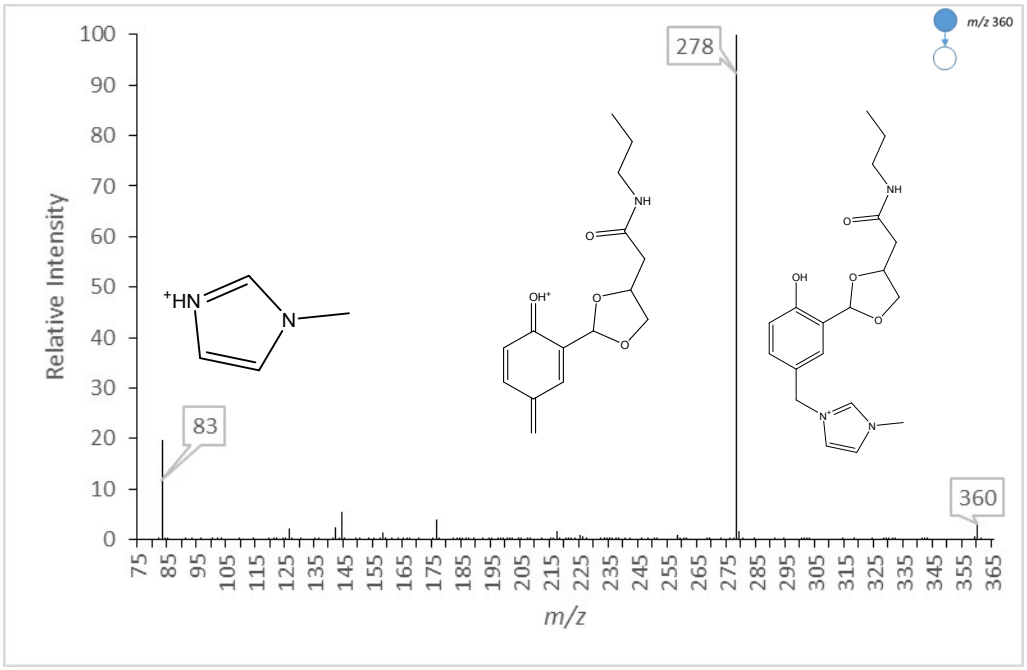

Figure S1b: *product ion MS/MS data of acetalated methylimidazolium mass label*

Orbitrap data: m/z 278.13803 ($C_{15}H_{20}O_4N$, -2.354 ppm), 83.06023 ($C_4H_7N_2$, -1.734 ppm)

p-Methoxyphenylimidazolium mass label data

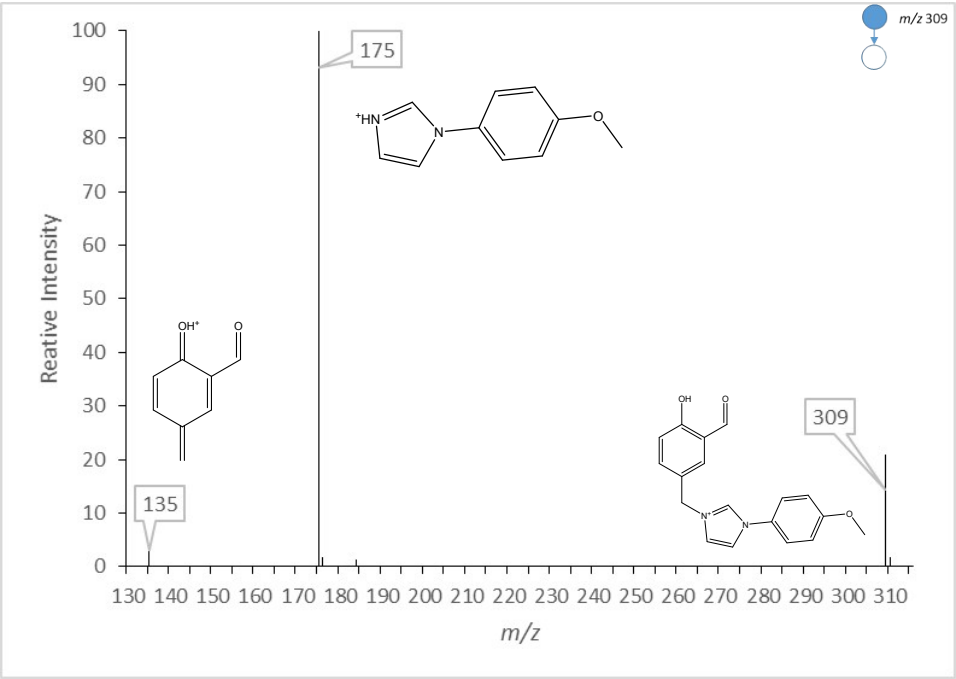

Figure S2a: *product ion MS/MS spectrum of p-methoxyphenylimixazolium mass label*

Orbitrap data: 309.12299 ($C_{18}H_{17}O_3N_2$, -1/226 ppm), m/z 175.08649 ($C_{10}H_{11}ON_2$, -0.568 ppm), m/z 135.04397 ($C_8H_7O_2$, -0.637 ppm)

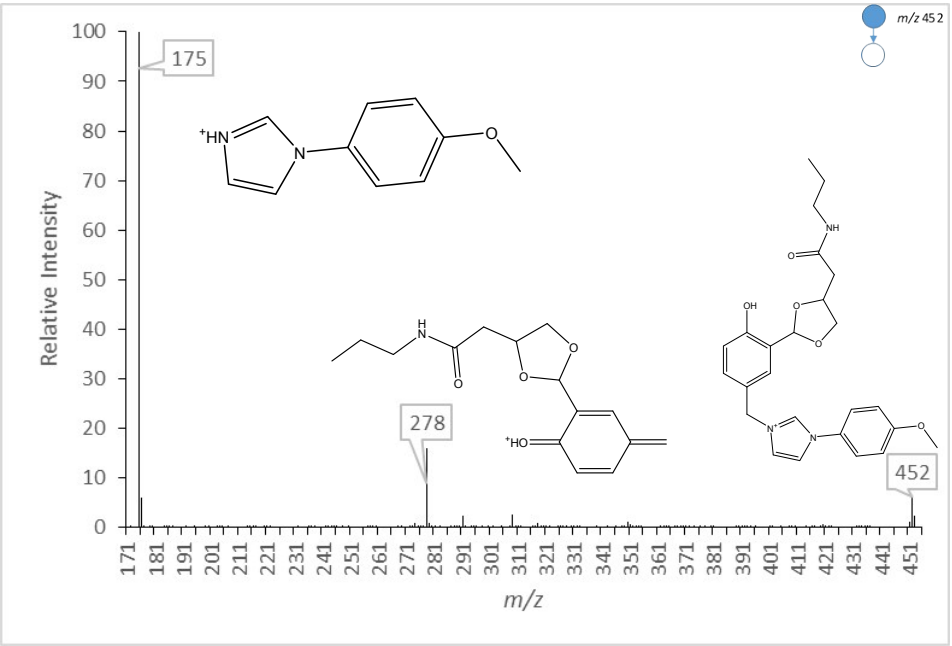

Figure S2b: *product ion MS/MS of acetalated p-methoxyphenylimidazolium mass label*

Orbitrap data: m/z 278.13826 ($C_{15}H_{20}O_4N$, -1.527 ppm), m/z 175.08637 ($C_{10}H_{11}ON_2$, -1.254 ppm)

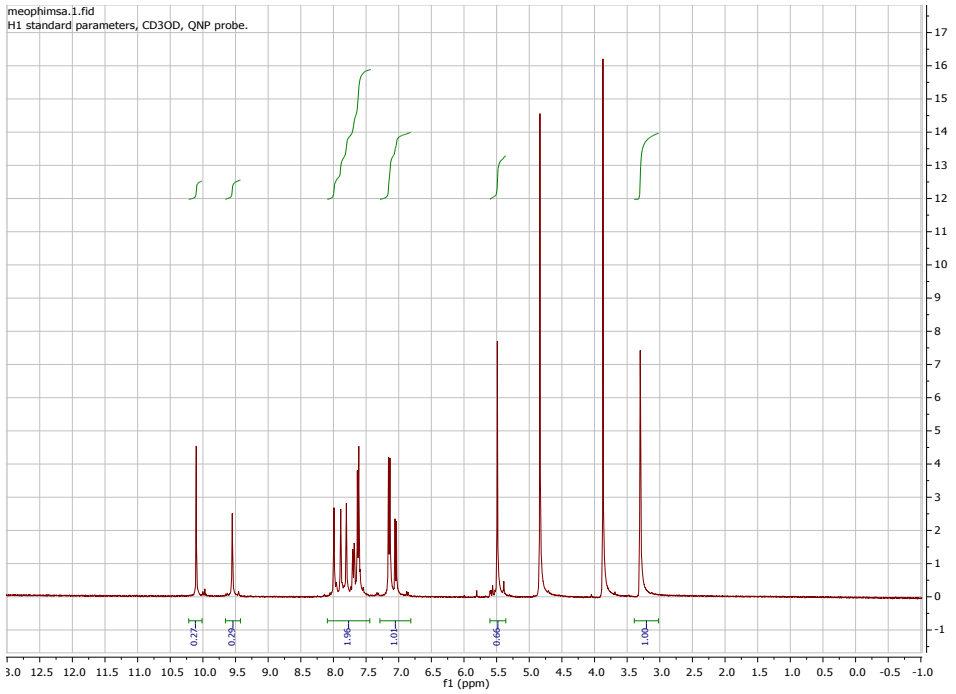

Figure S2c: *^{1}H NMR spectrum of the p-methoxyphenylimidazolium mass label*

Pyridinium mass label data

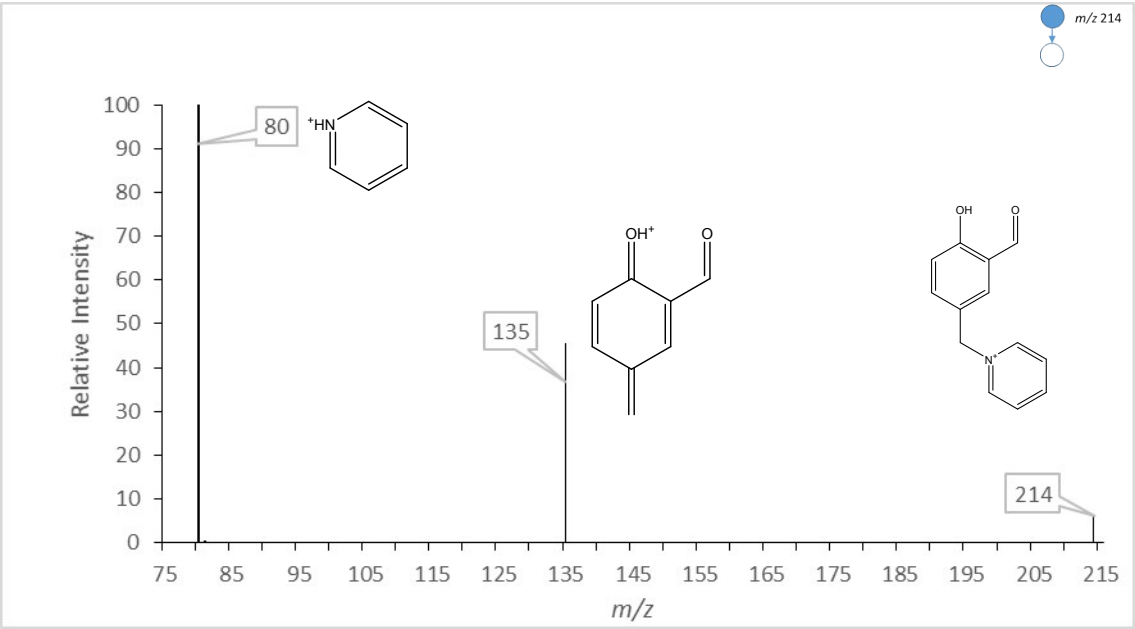

Figure S3: *product ion MS/MS spectrum of pyridinium mass label (used as internal standard)*

Orbitrap data: m/z 214.08595 ($C_{13}H_{12}O_2N$, -1.425 ppm), m/z 135.04390 ($C_8H_7O_2$, -1.155 ppm), m/z 80.04943 (C_5H_6N, -0.572 ppm)

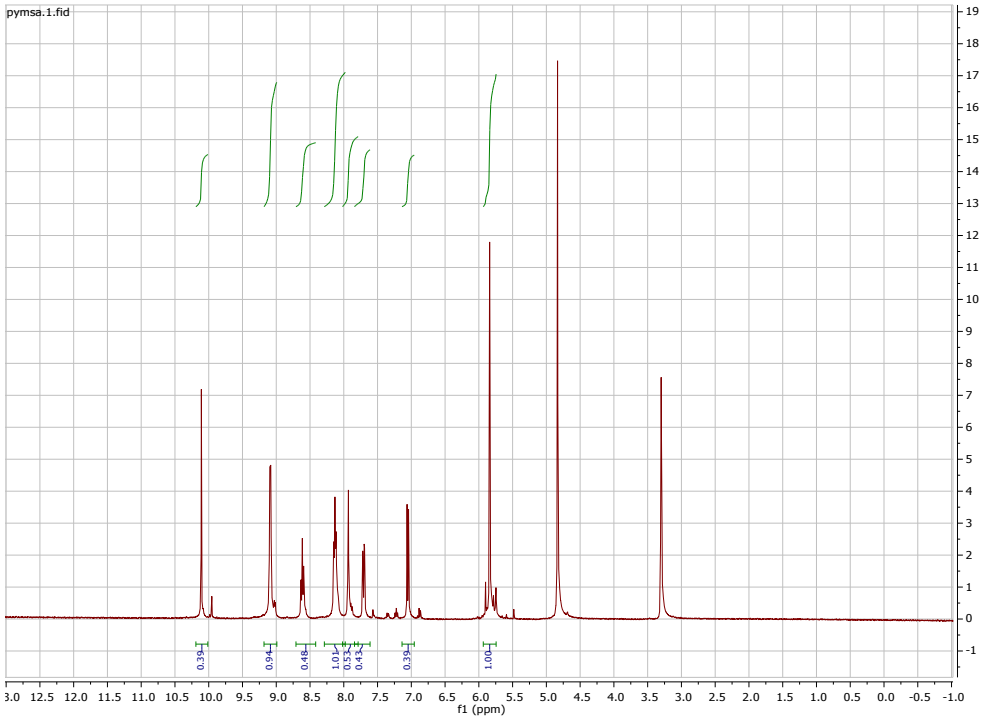

Figure S3b: *^{1}H NMR spectrum of the pyridinium mass label from methanol-d_4*

N(n-Butylimidazolium) Mass Label Data

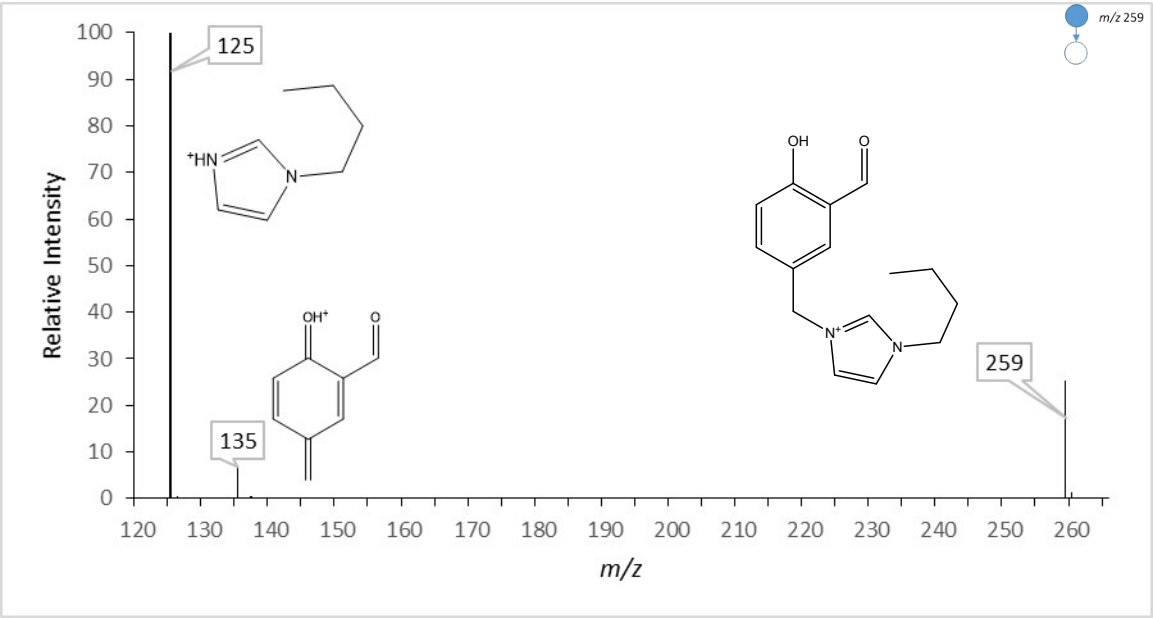

Figure S4a: *product ion MS/MS spectrum of butylimidazolium mass label*

Orbitrap data: m/z 259.14379 ($C_{15}H_{19}O_2N_2$, -1.213 ppm), m/z 135.04394 ($C_8H_7O_2$, -0.859 ppm), m/z 125.10723 ($C_7H_{13}N_2$, -0.759 ppm)s

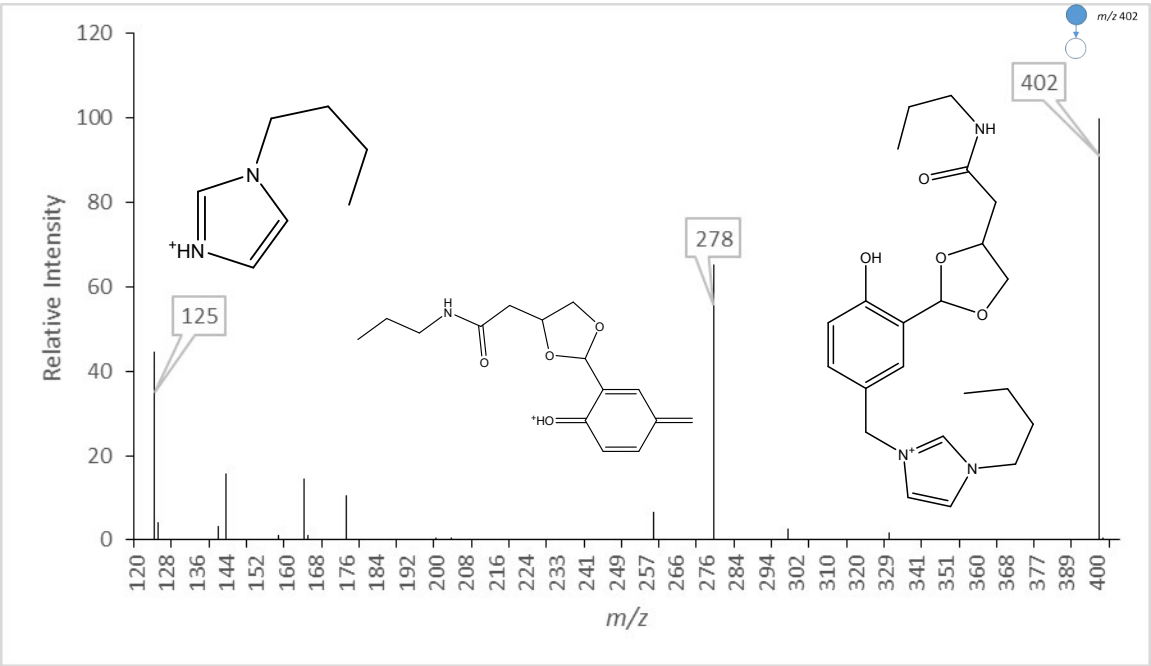

Figure S4b: *product ion MS/MS of acetalated butylimidazolium mass label*

Orbitrap data: m/z 402.23809 ($C_{22}H_{32}O_4N_3$, -1.598 ppm), m/z 278.13829 ($C_{15}H_{20}O_4N$, -1.419 ppm), m/z 125.10714 ($C_7H_{13}N_2$, -1.479 ppm)

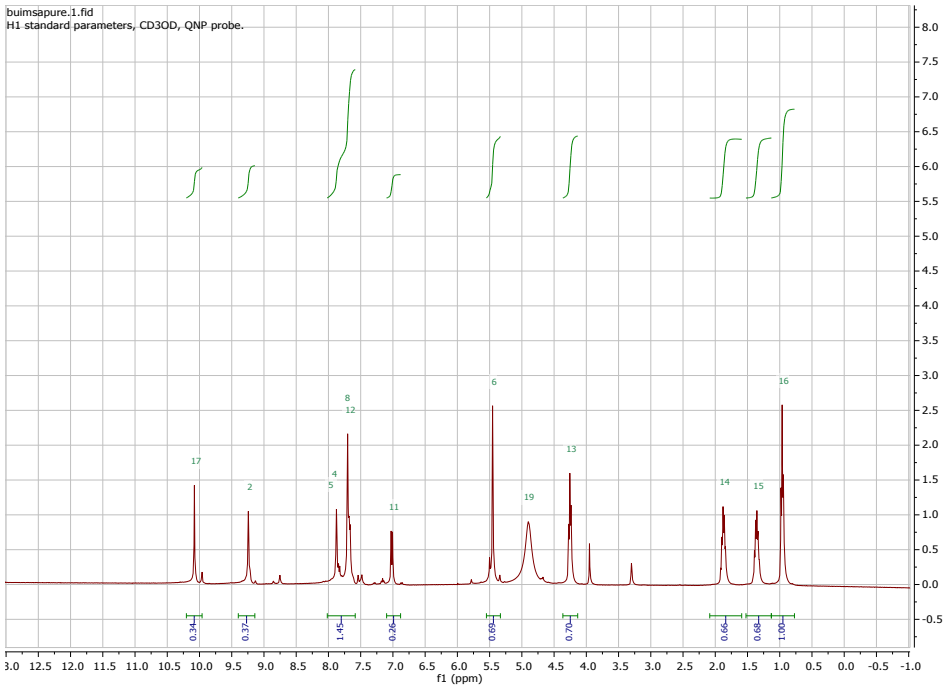

Figure S4c: *^{1}H NMR spectrum of the butylimidazolium mass label*

Data for 3,4-dihydroxy-N-propylbutyramide

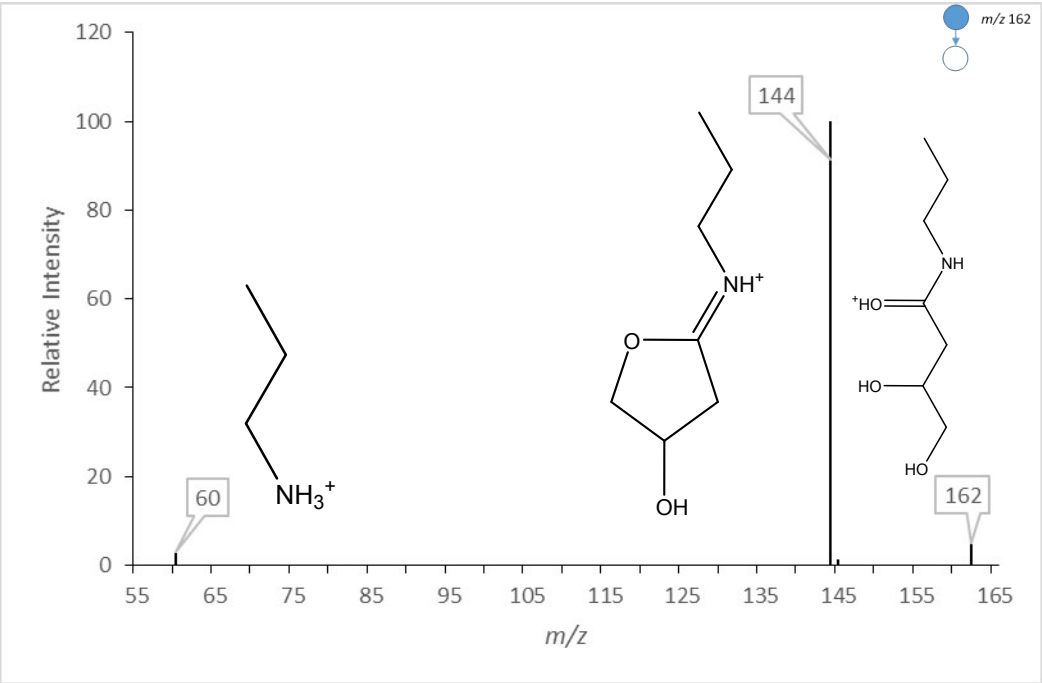

Figure S5a: *product ion MS/MS of 3,4-dihydroxy-N-propylbutyramide*

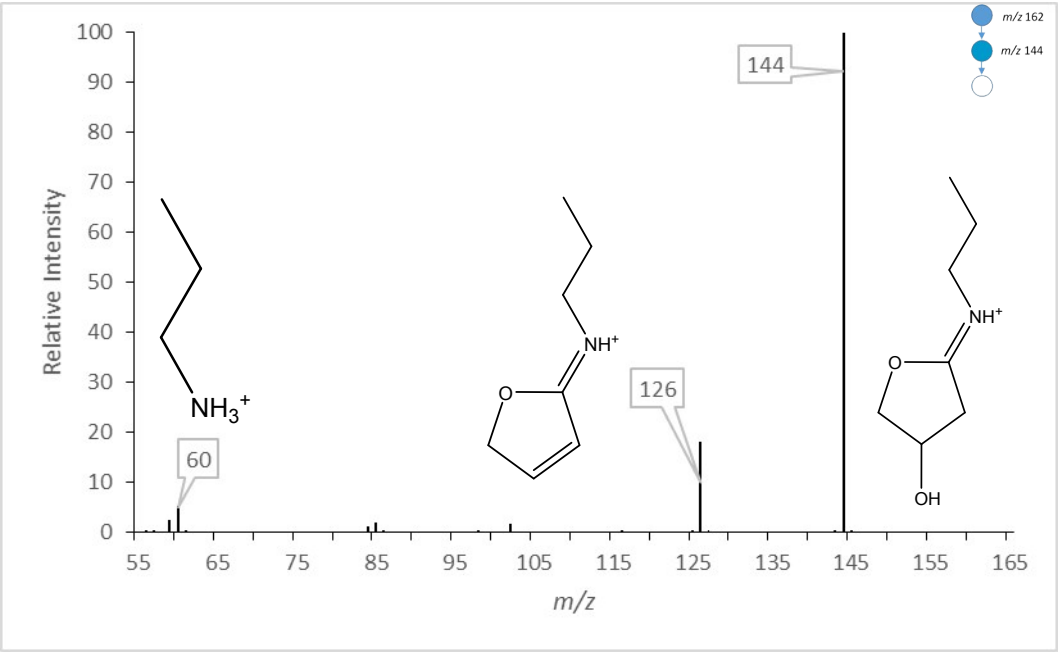

Figure S5b: *product ion MS/MS/MS of the major fragment of 3,4-dihydroxy-N-propylbutyramide*

Hydrolysis study of the acetal linkage over time in PBS buffer

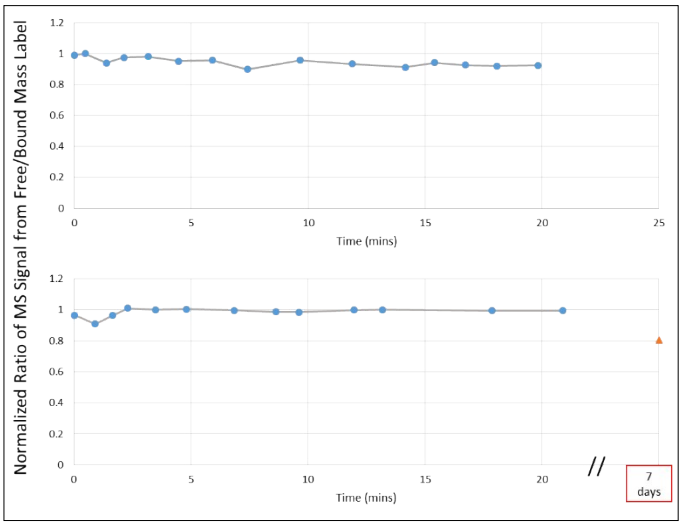

Figure S6: *Plots of the change in the ratio of the signals corresponding to the acetylated and free mass label where the acetal was formed between 3, 4-dihydroxy-N-propylbutyramide (top) and ethylene glycol (bottom). Triangle = after storage for 7 days at 2°C in 1:1 MeOH:PBS buffer.*

A change of 10 % in the ratio of free/bound mass label in Figure S6 would correspond to a 5% release of mass label because the signal for the free mass label increases linearly with the decrease in the signal of the bound material.

Hydrolysis study of the imine linkage over time in PBS buffer

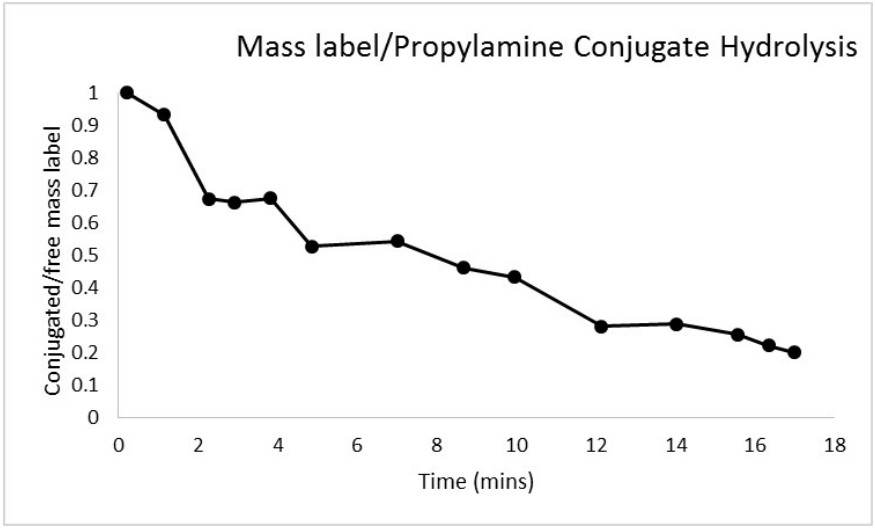

Figure S7a: *the hydrolysis of the propylamine conjugate imine over time in 1:1 PBS:methanol*

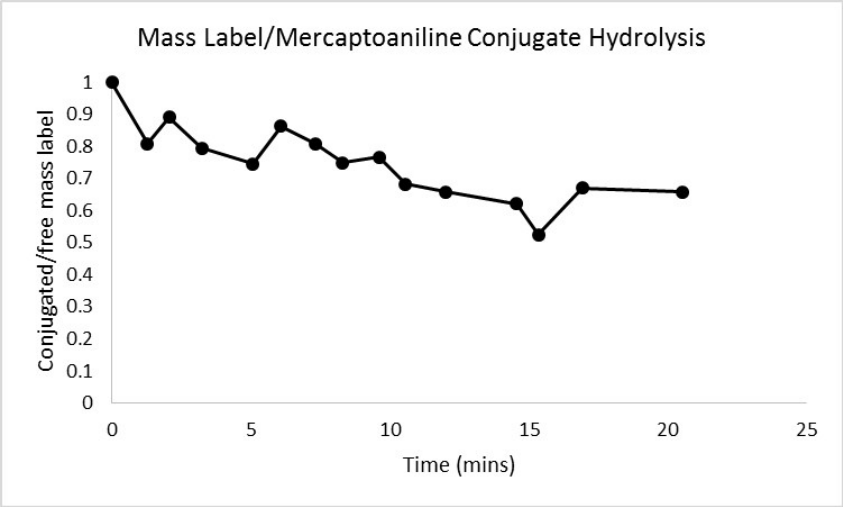

Figure S7b: *the hydrolysis of the mercaptoaniline conjugate imine over time in 1:1 PBS:Methanol*

Mass spectra of the conjugation and release of the methylimidazolium mass label from imines

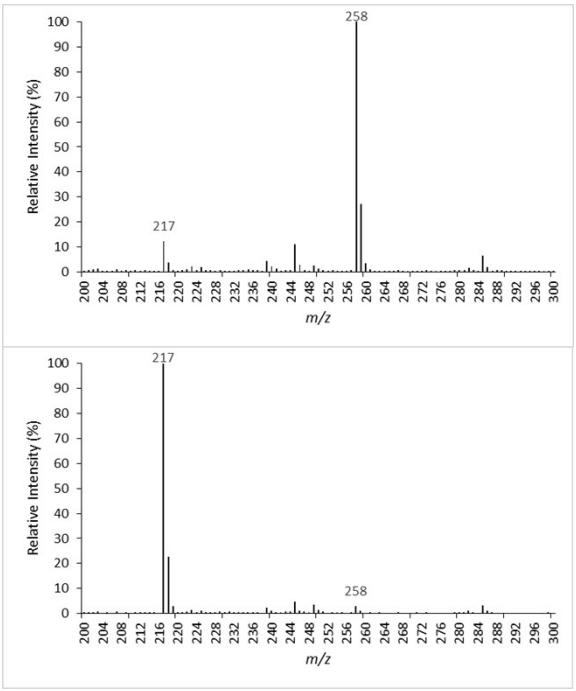

Figure S8: *the mass label conjugated to propylamine (m/z 258) analyzed from methanol (top), The mass label released (m/z 217) from the propylamine conjugate imine analyzed from methanol:water, 1% formic acid (bottom)*

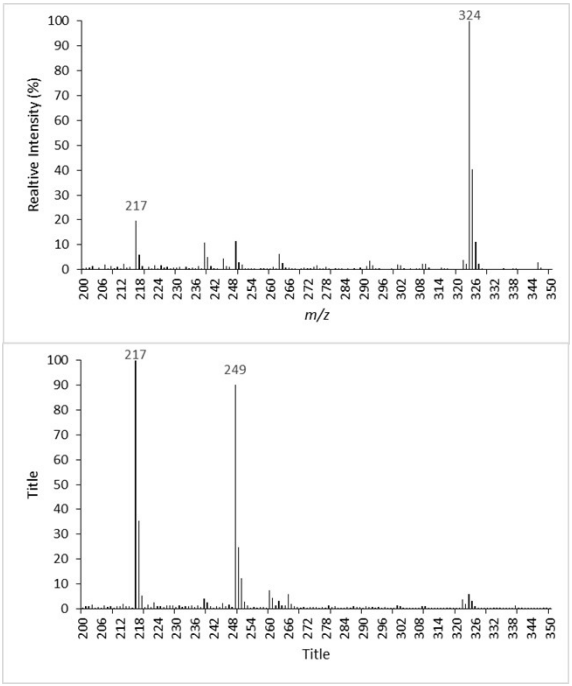

Figure S8b: *The mass label conjugated to mercaptoaniline (m/z 324), analyzed from neat methanol (top), The mass label released (m/z 217) from the mercaptoanilinie conjugate imine as well as the resulting methanol hemi-acetal (m/z 249) analyzed from methanol:water, 1% formic acid (bottom)*

Note: the formation of the methanol hemi-acetal seems to be catalyzed from the imine. The hydrolysis of the imine appears to be competitive between the formation of the aldehyde to eject the amine and the incorporation of a molecule of methanol to form the same. This is understandable given that the imine was exposed to an abundance of methanol during both conjugation and release.

Discussion of reaction yield approximation

The approximation of the yield of the acetalization reaction of the methylimidazolium mass label with 3,4-dihydroxy-N-propylbutyramide was done by comparing the relative intensities of the signal related to the product of the reaction to the signal corresponding to the starting material. No internal standard was used in the traditional sense. The experiment was conducted in this way due to the difficulty of isolation of mass labels and their conjugates from DMF and is assumed to be qualitative.

In order for this approximation to be valid, the ionization efficiency of both species must essentially be the same. A secondary necessary assumption is that there is no competitive reaction or degradation of the starting material. Due to the nature of the ions (imidazolium), it is assumed that they do have essentially the same ionization efficiencies. Due to the nature of the reaction, it is assumed that no degradation occurs and competitive reaction of the starting material is unlikely so the ratio of the signals in the mass spectrum should provide a reasonable approximation of reaction yield.

The reason that the experiment was conducted in this manner was due to the difficulty of isolation of either the product or starting material from DMF. This problem is negated in the case(s) where nanoparticles are involved in the reaction because the product (derivitized SiNPs) is isolated by centrifugation.

Note on the quantitation of the release of labels from nanoparticles

The total mass of nanoparticles that contributed to the release of the material was less than the initially weighed amount because some mass was inevitably lost during the washing steps. The mass of the nanoparticles was 3 mg (measured as 3.3 mg on a scale with uncertainty ca. 0.1 mg). This was established after washing the remaining nanoparticles with acetone and allowing them to dry in the open air whereupon they were weighed. It is possible that there was a small amount of excess water weight in the measurement.

In the main text, the inefficiency of washing the nanoparticles is discussed. Two mass spectra are provided below to illustrate this discussion. In these spectra, *m/z* 217 corresponds to the mass label and *m/z* 80 is its fragment (protonated imidazole). *m/z* 214 is the pyridinium analogue of the mass label which was used as an internal standard and its fragment ion of interest is *m/z* 83 (protonated pyridine).

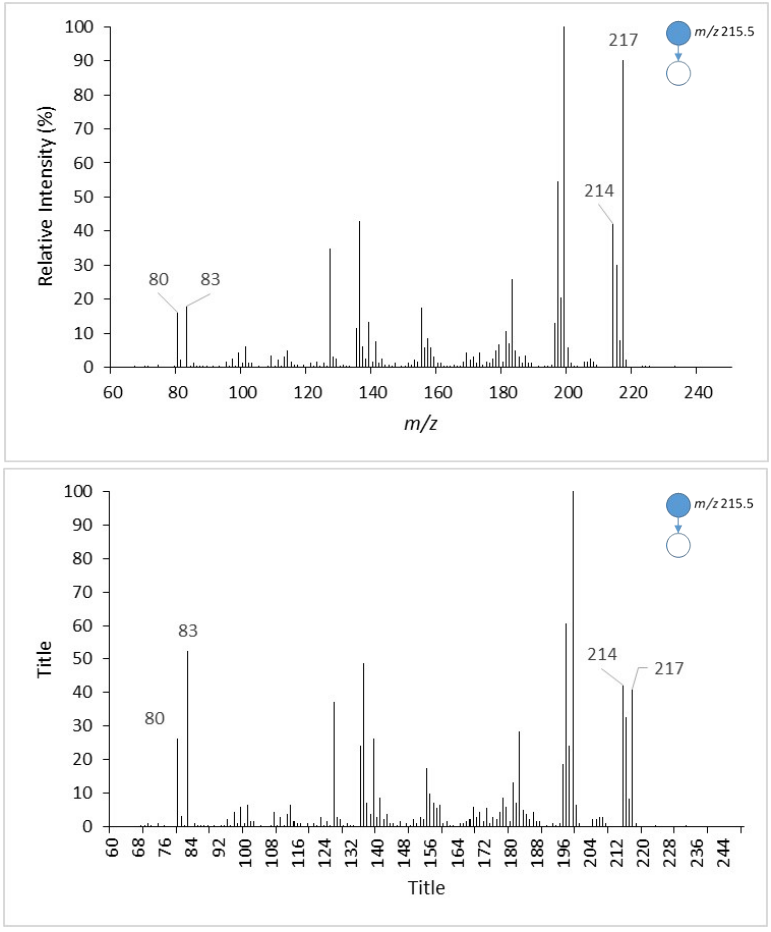

Figure S9*: Top- CID product ion mass spectrum (isolation window over 4 mass units centered on m/z 215.5) of the supernatant of the final wash where the supernatant was diluted 100x before internal standard addition. Bottom- CID product ion mass spectrum (isolation window over 4 mass units centered on m/z 215.5) of the solution into which mass labels were released from NPs where the solution was diluted 10,000x before internal standard addition*