

# Synthesis of diazo ketone solid supports and their application towards the enrichment of phosphorylated peptides

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## Supplemental Information

### Figure #1.

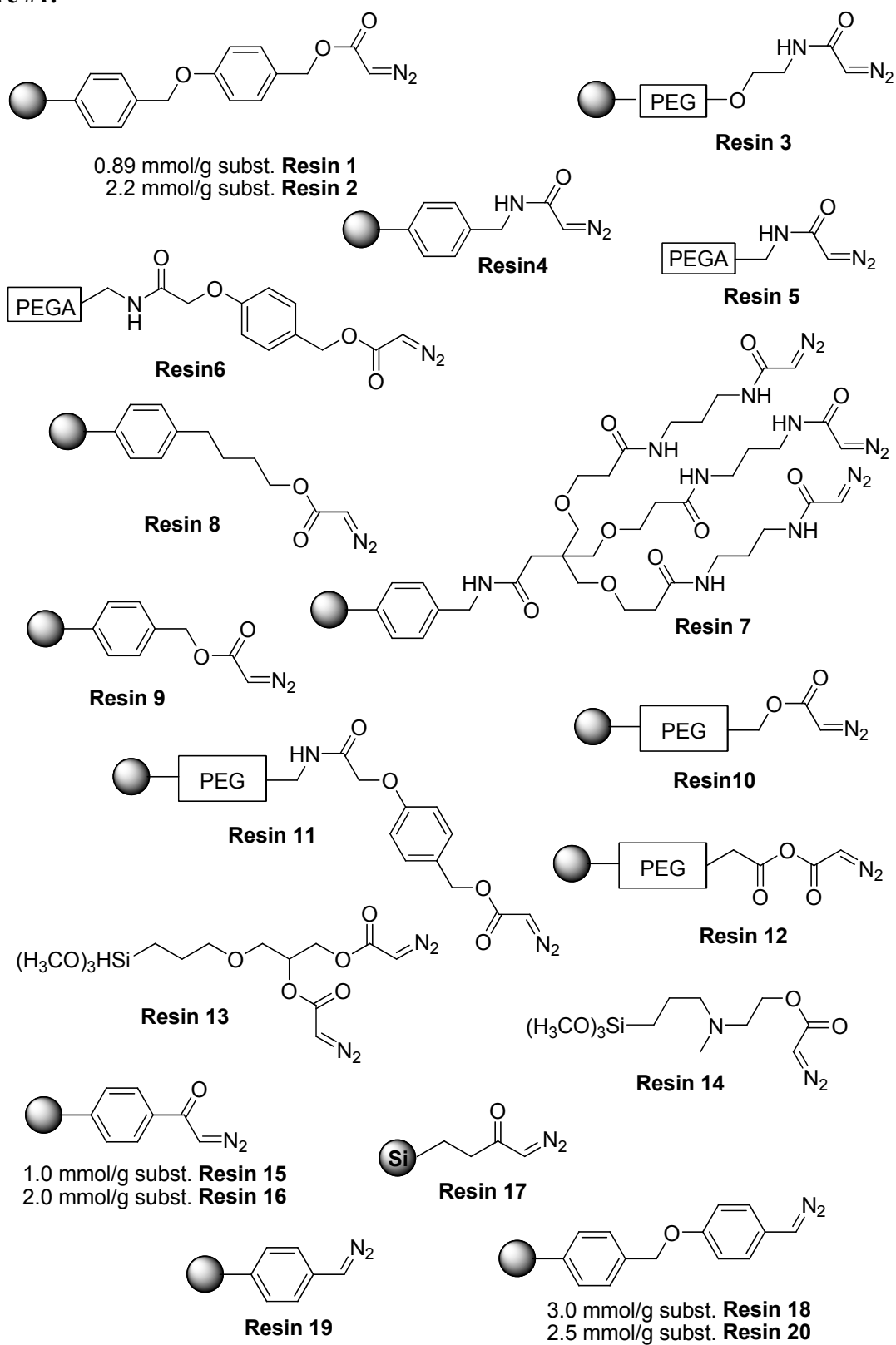


Figure #1. Structures of the synthesized diazo-functionalized solid supports.

**Table #1-A.**

<b>Resin/Silica</b>	<b>Substitution</b>	<b>Mesh size</b>
<b>1</b>	0.89 mmol/g	100-200 mesh
<b>2</b>	2.2 mmol/g	100-200 mesh
<b>3</b>	0.3 mmol/g	90 $\mu\text{m}$
<b>4</b>	1.3 mmol/g	35-40 mesh
<b>5</b>	0.4 mmol/g	130 $\mu\text{m}$
<b>6</b>	0.4 mmol/g	50-100 mesh
<b>7</b>	0.28 mmol/g	100-200 mesh
<b>8</b>	3.5 mmol/g	200-400 mesh
<b>9</b>	0.98 mmol/g	100-200 mesh
<b>10</b>	0.28 mmol/g	130 $\mu\text{m}$
<b>11</b>	0.27 mmol/g	90 $\mu\text{m}$
<b>12</b>	0.30 mmol/g	130 $\mu\text{m}$
<b>13</b>	N/A	N/A
<b>14</b>	N/A	N/A
<b>15</b>	1.0 mmol/g	100-200 mesh
<b>16</b>	2.0 mmol/g	100-200 mesh
<b>17</b>	1.6 mmol/g	40-63 $\mu\text{m}$
<b>18</b>	3.0 mmol/g	50-100 mesh
<b>19</b>	0.9 mmol/g	100-200 mesh
<b>20</b>	2.5 mmol/g	200-400 mesh

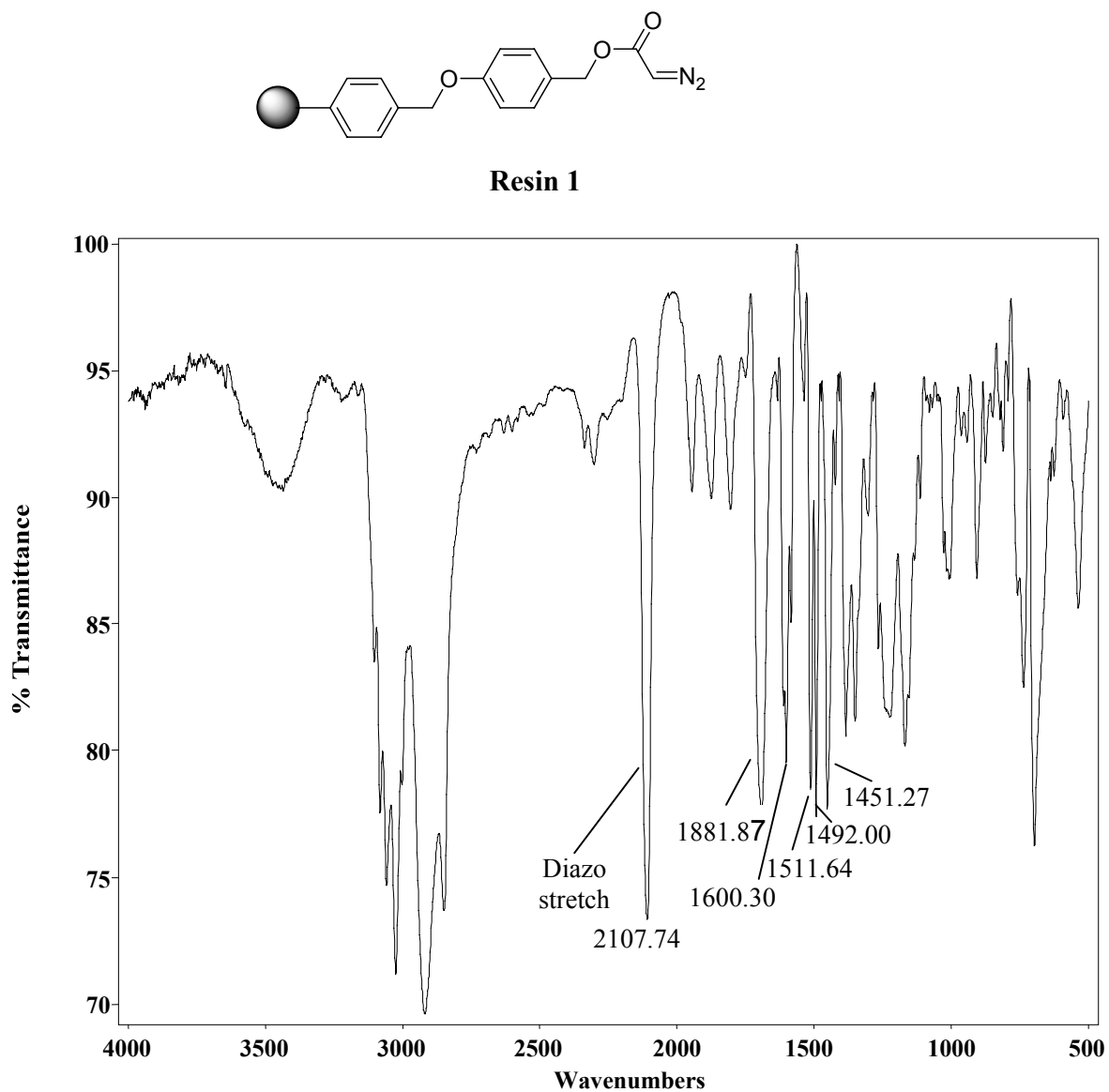
**Table #1-A. Level of substitution and mesh size of each polymer prior to diazotization.**

**Table #1-B.**

<b>Mesh Size</b>	<b>Micrometer equivalent</b>
50	297
100	149
200	74
400	37

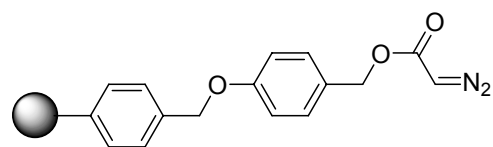
**Table #1-B. Conversion of mesh size to micrometers.**

Figure #2-A.

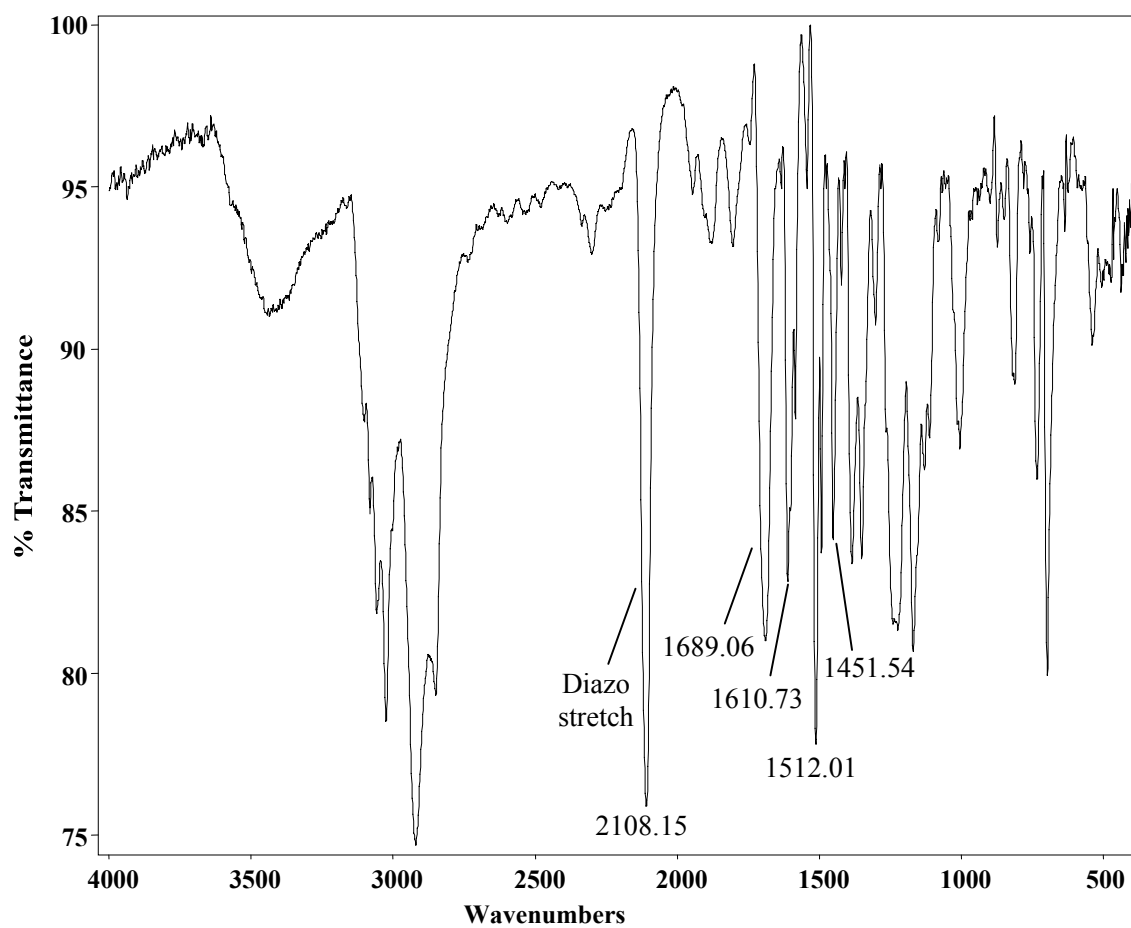


A. IR spectrum of  $\alpha$ -diazo ester wang resin (0.89 mmol/g loading).

Figure #2-B.

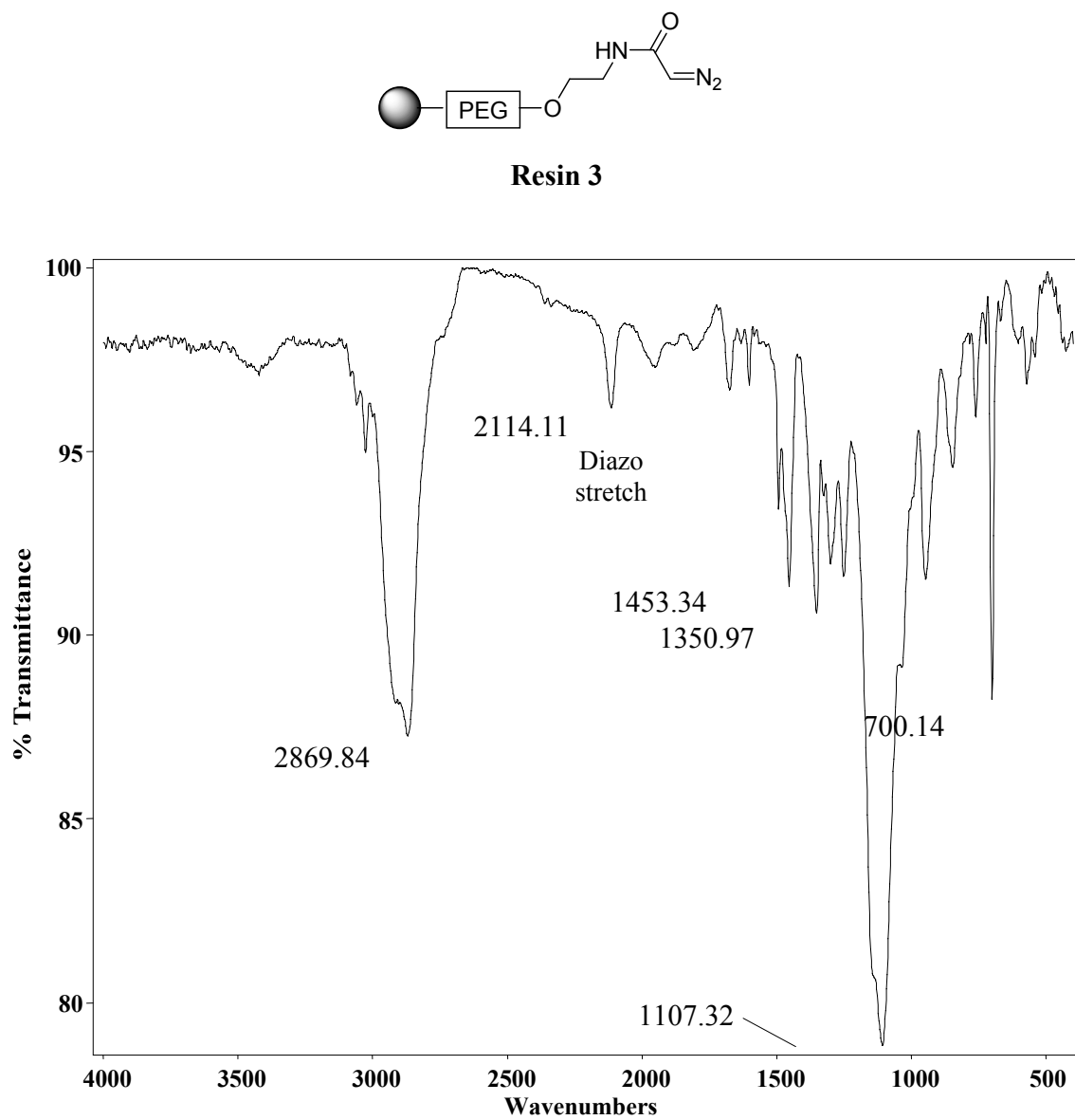


Resin 2



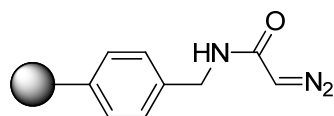
B. IR spectrum of  $\alpha$ -diazo ester wang resin (2.2 mmol/g loading).

Figure #2-C.

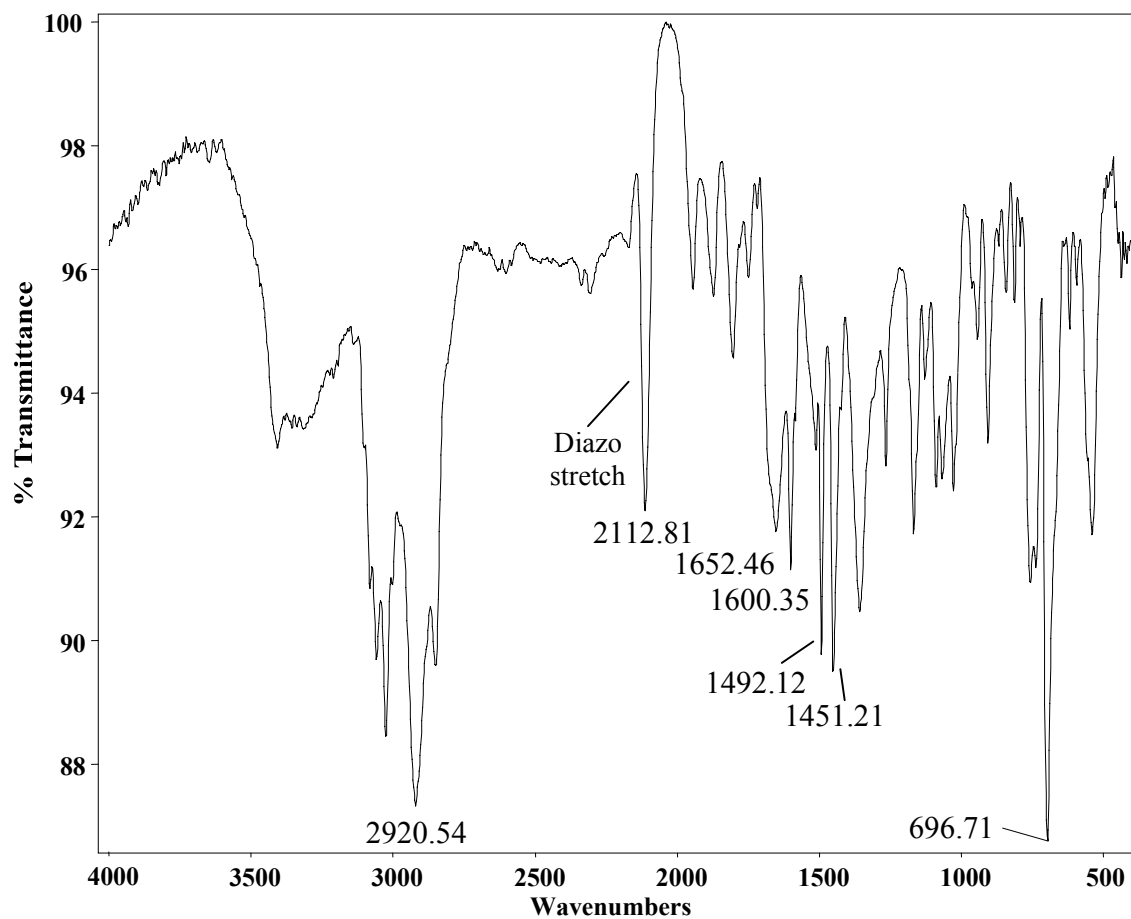


C. IR spectrum of diazo amide tentagel.

Figure #2-D.

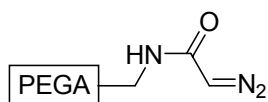


Resin 4

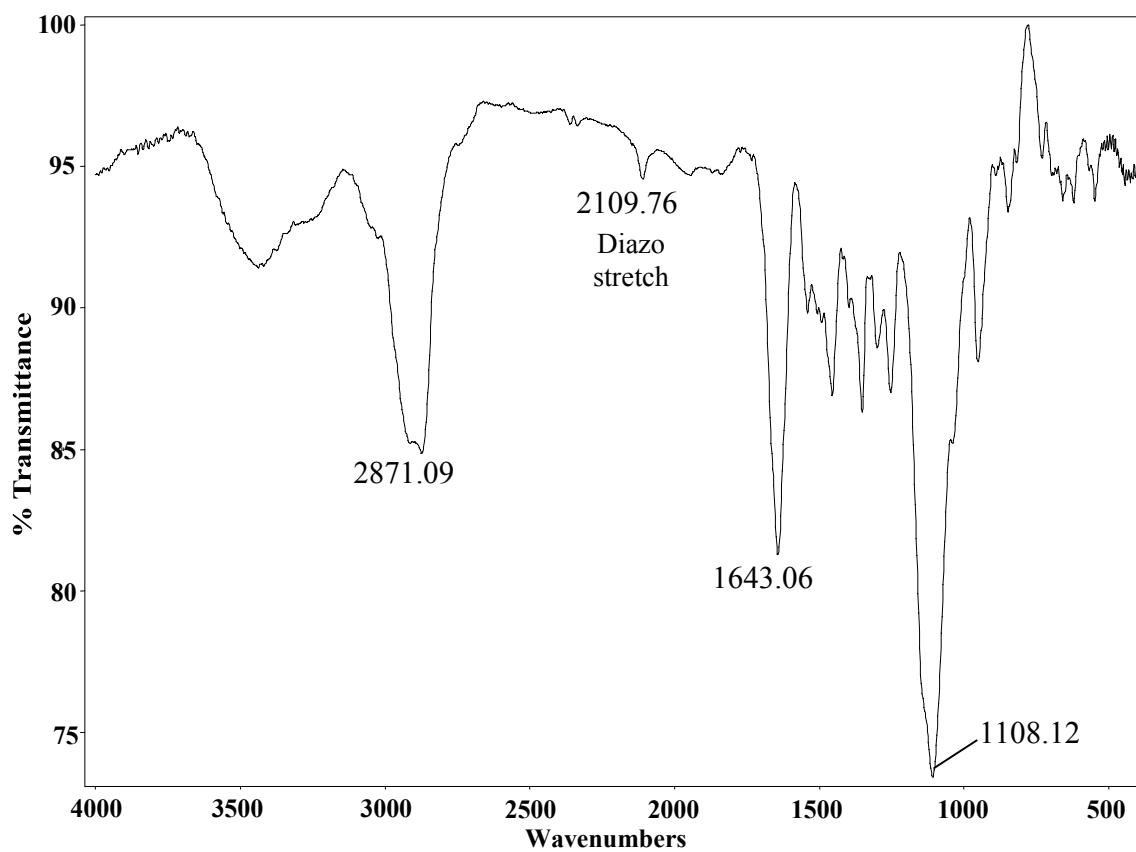


D. IR spectrum of diazo amide megabead.

Figure #2-E.



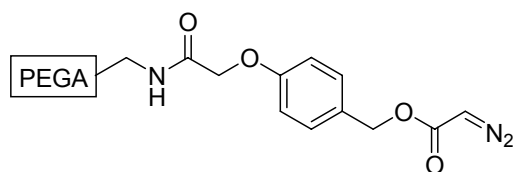
Resin 5



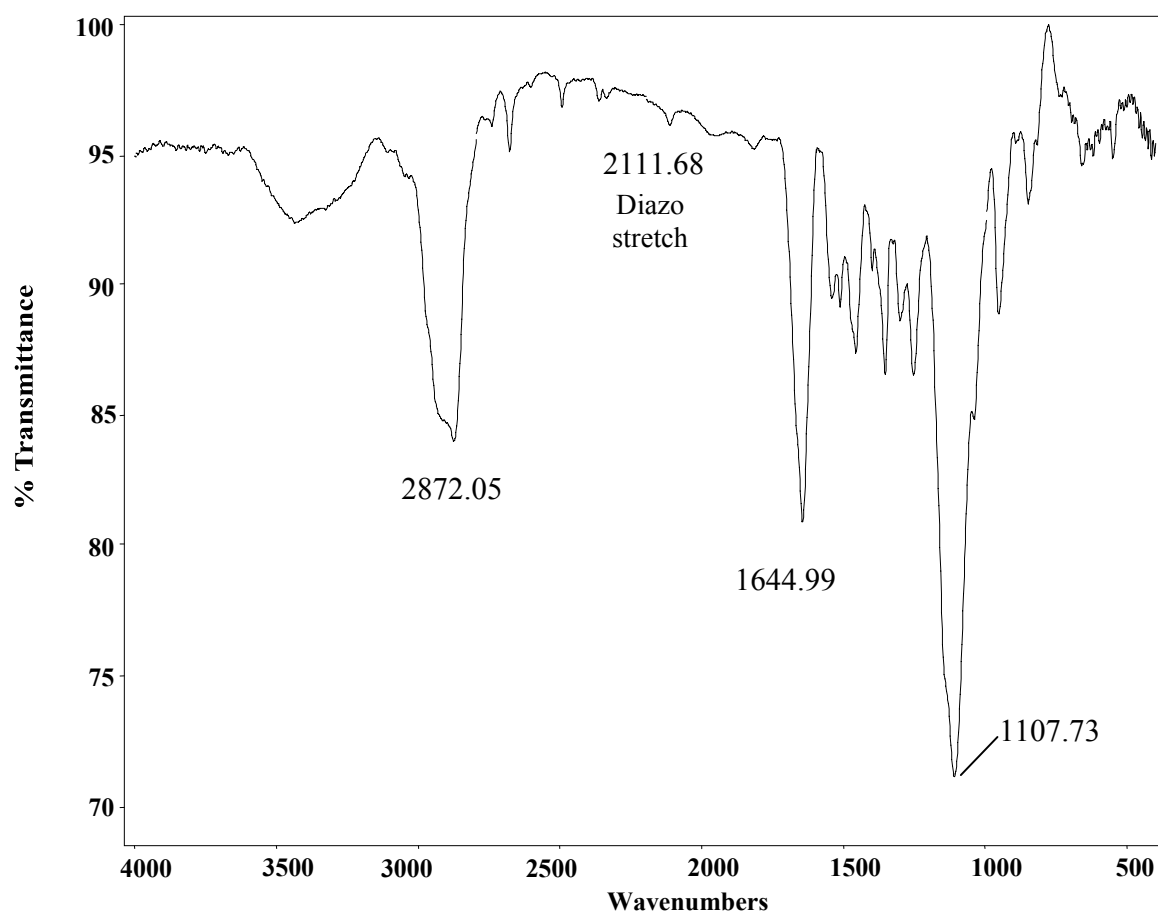
E. IR spectrum of PEGA diazo amide resin.



Figure #2-F.

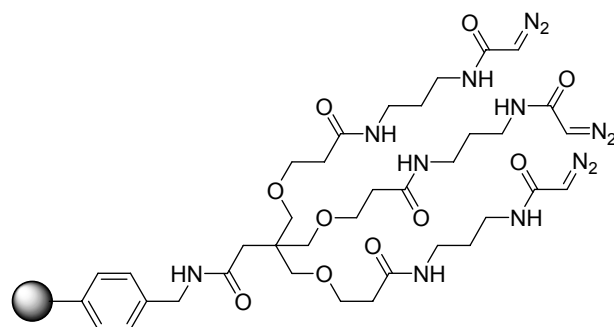


Resin 6

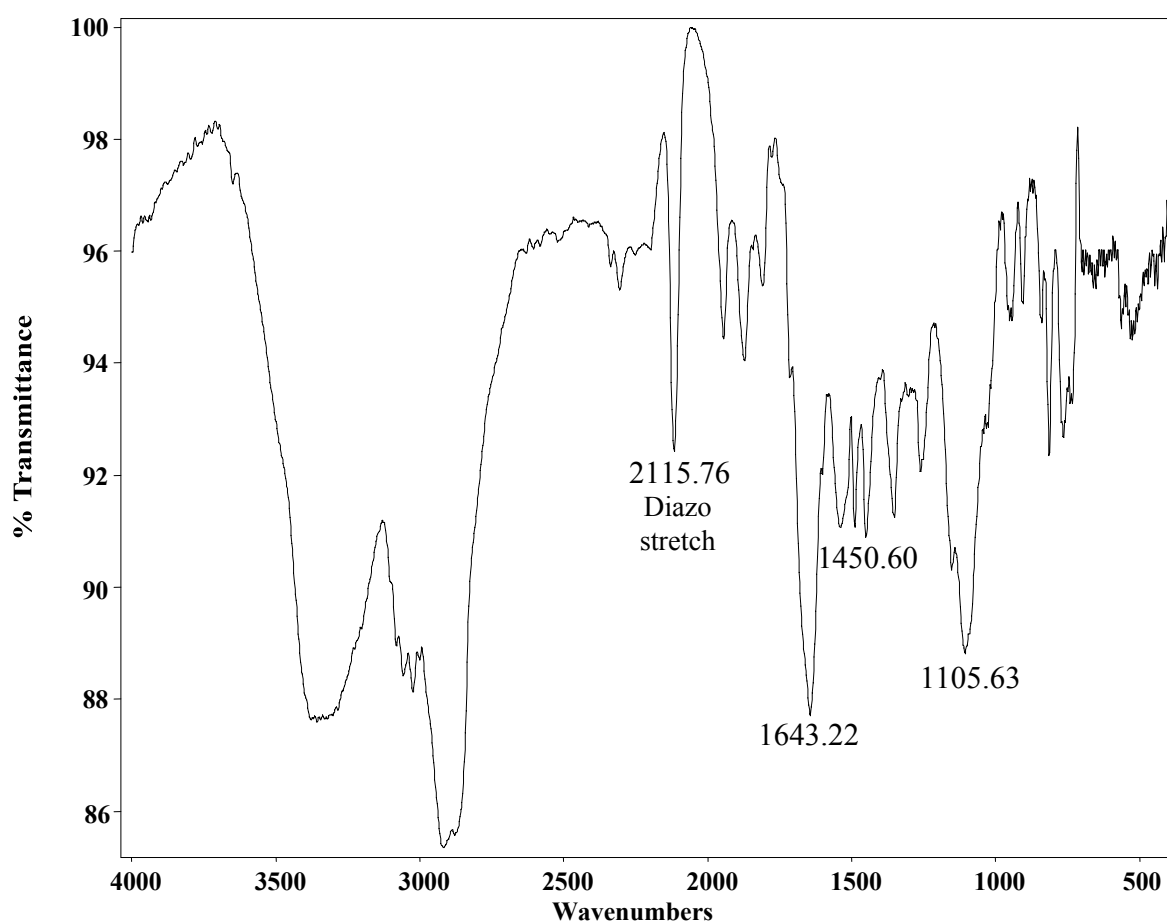


F. IR spectrum of PEGA HMPA diazo acetate resin.

Figure #2-G.

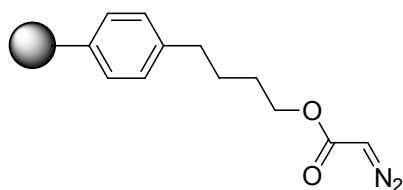


Resin 7

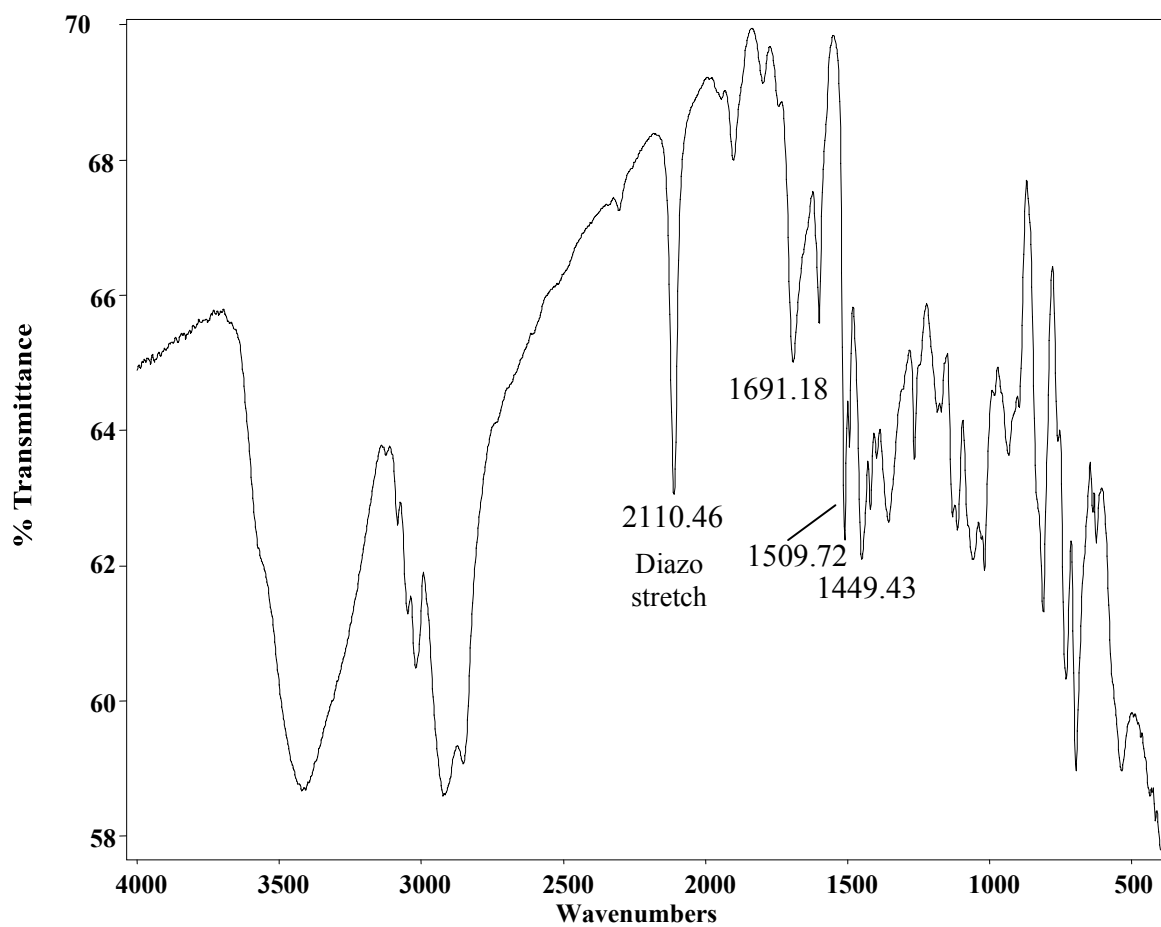


G. IR spectrum of diazo amide dendrimer.

Figure #2-H.

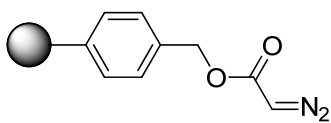


Resin 8

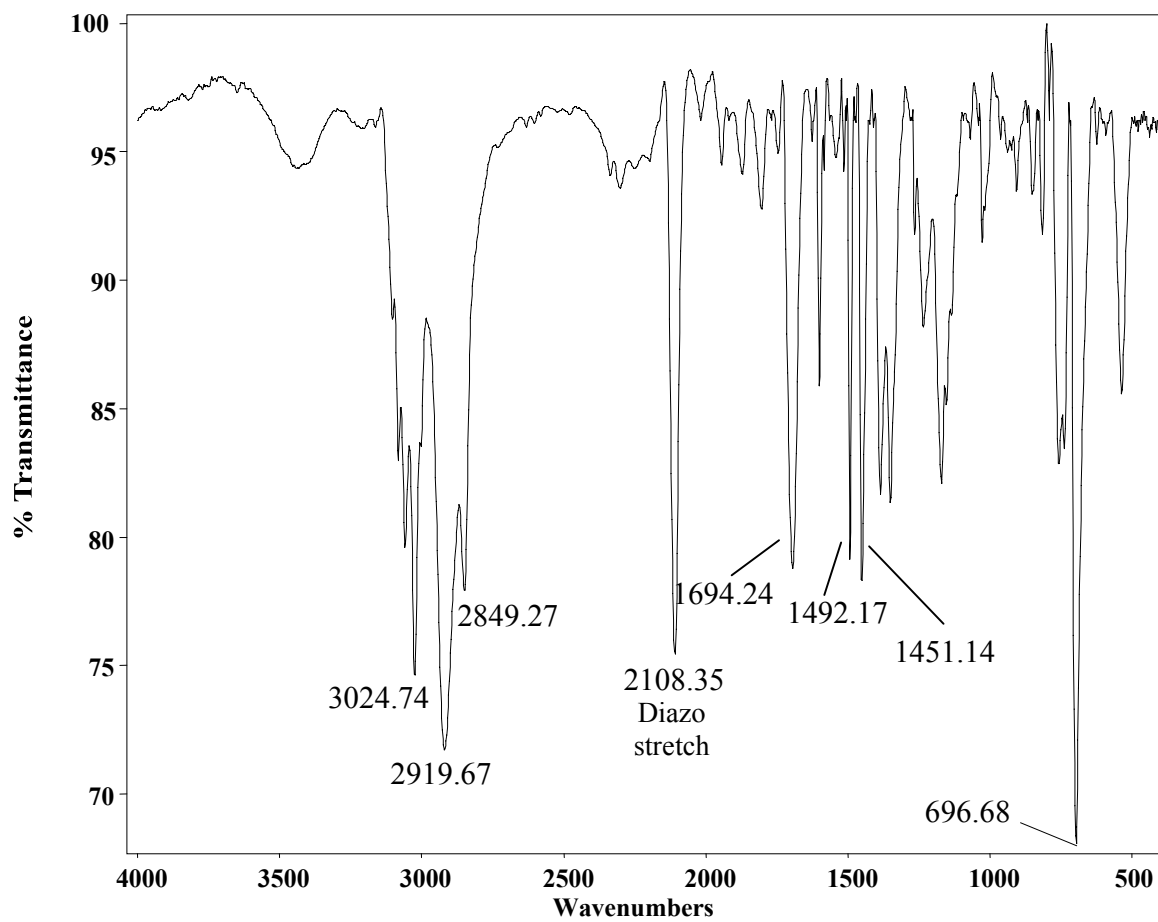


H. IR spectrum of 4-butyl diazo acetate polystyrene.

Figure #2-I.

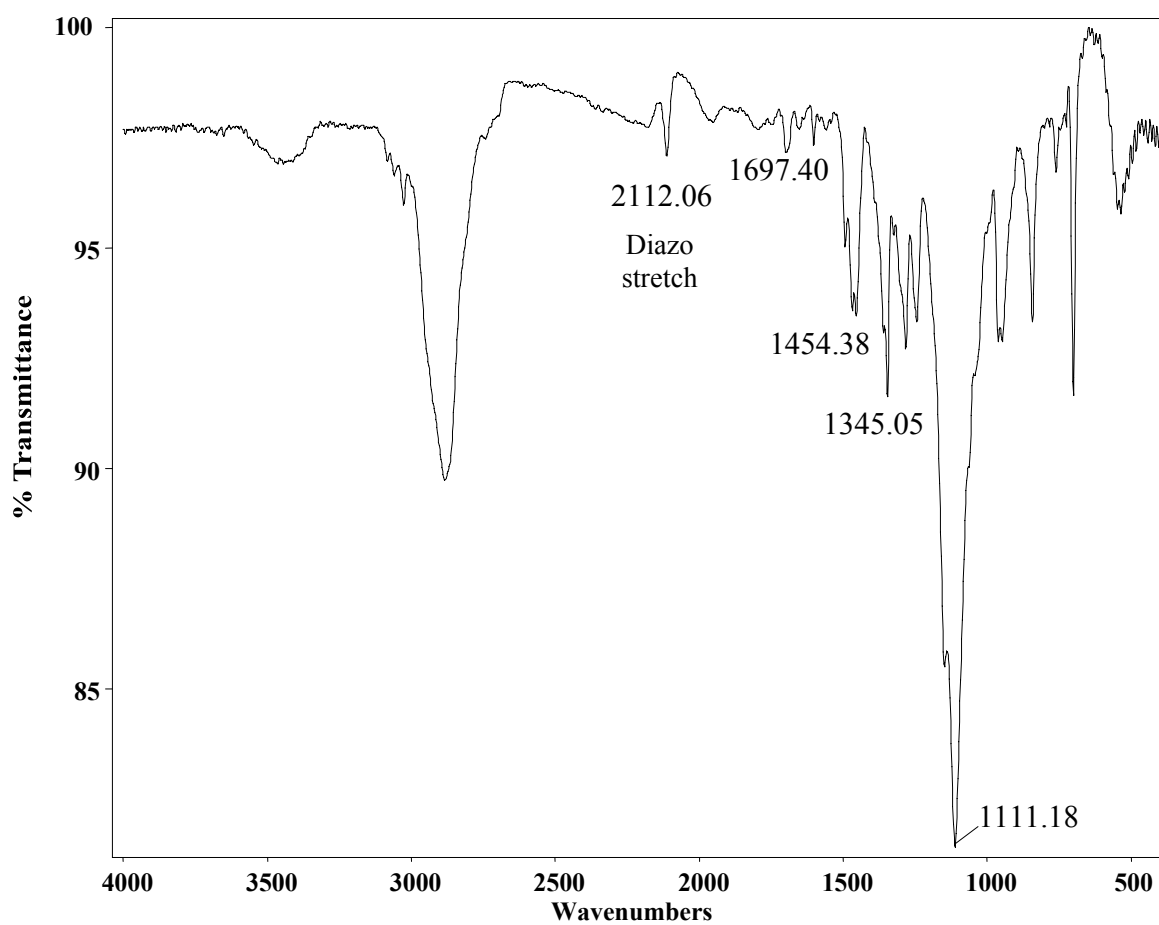


Resin 9



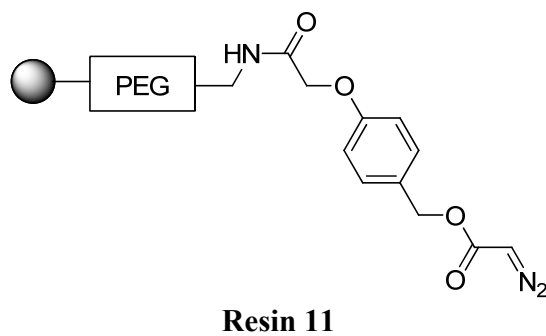
I. IR spectrum of diazo acetate polystyrene.

Figure #2-J.

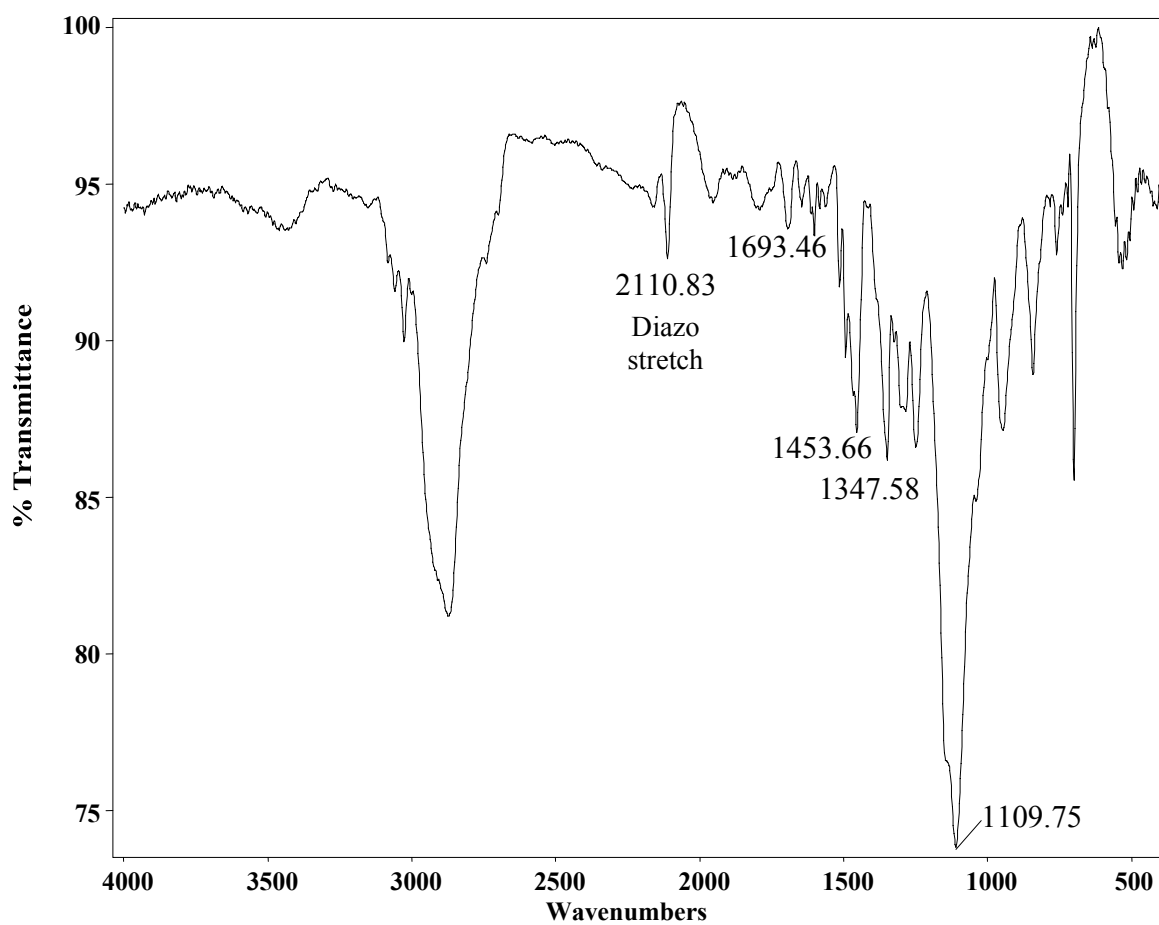


J. IR spectrum of NovaSyn diazo acetate resin.

Figure #2-K.

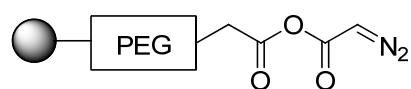


Resin 11

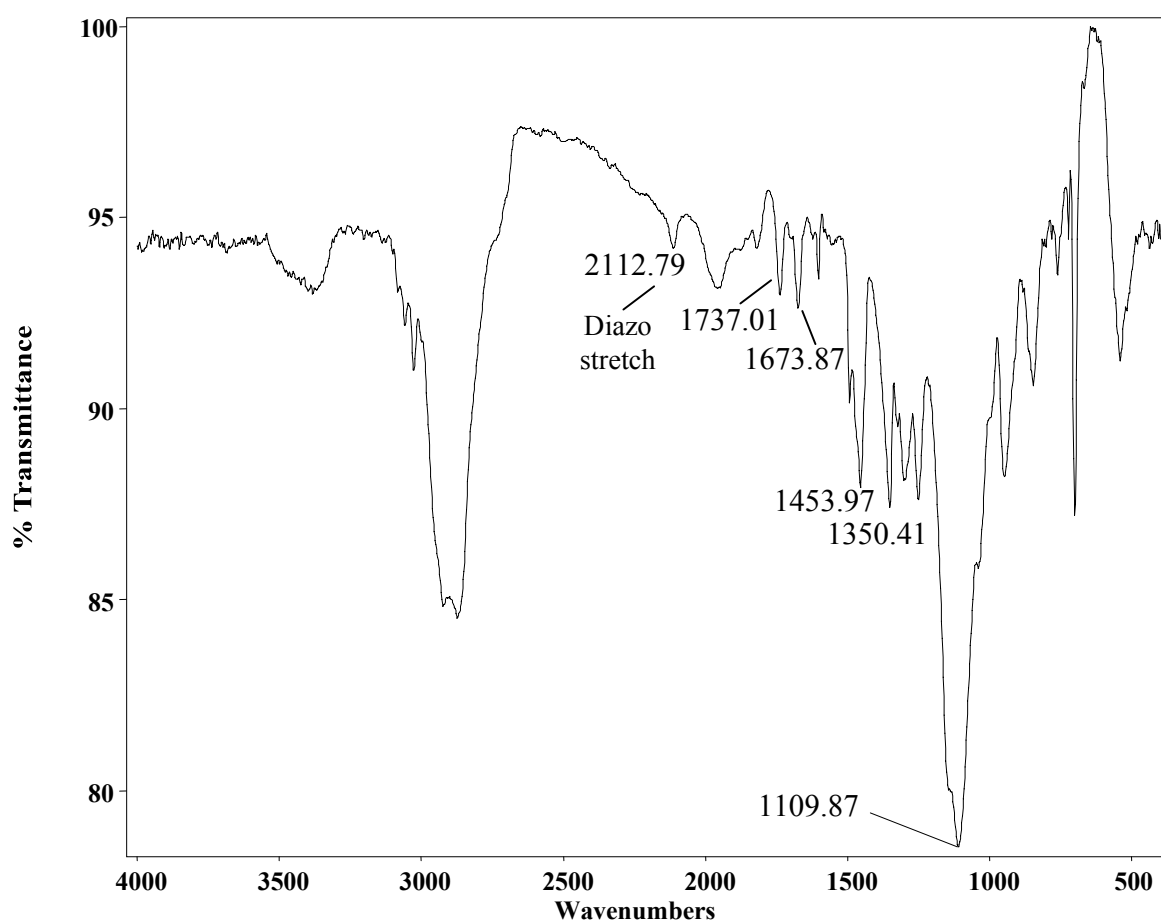


K. IR spectrum of NovaSyn diazo acetate TG HMP resin.

Figure #2-L.

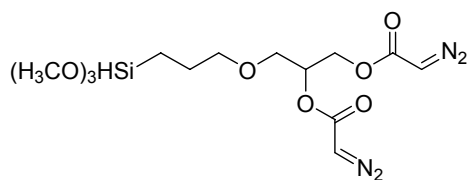


Resin 12

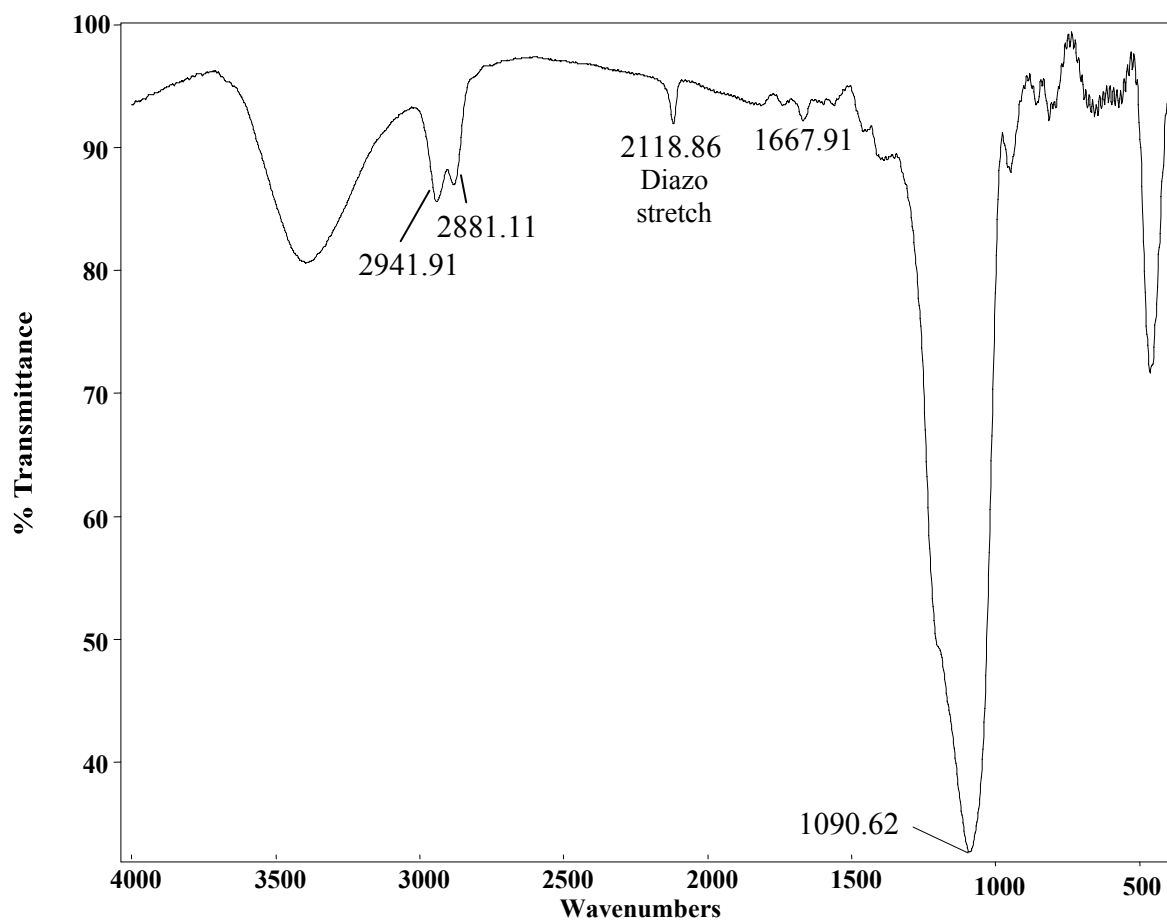


L. IR spectrum of NovaSyn diazo anhydride resin.

Figure #2-M.



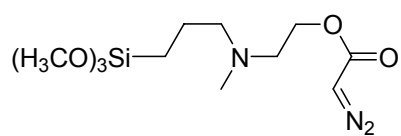
Resin 13



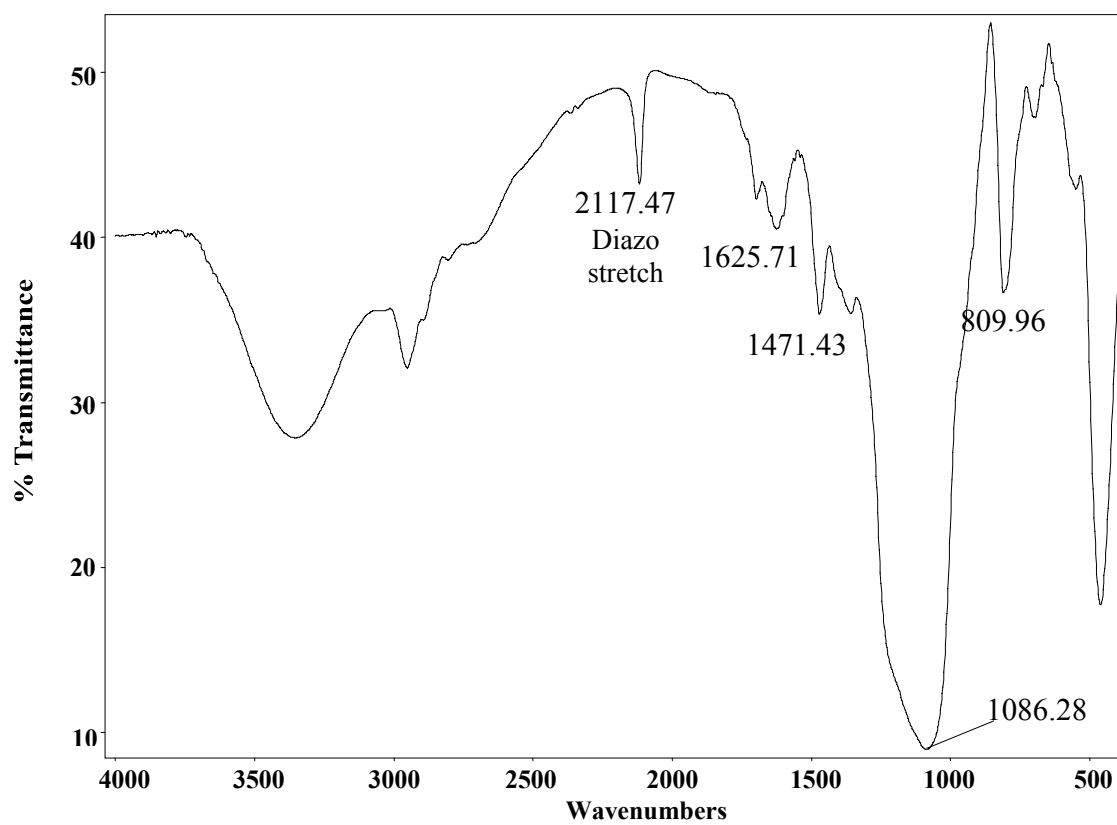
M. IR spectrum of vicinal diazo acetate silica.



Figure #2-N.

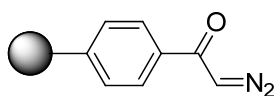


Resin 14

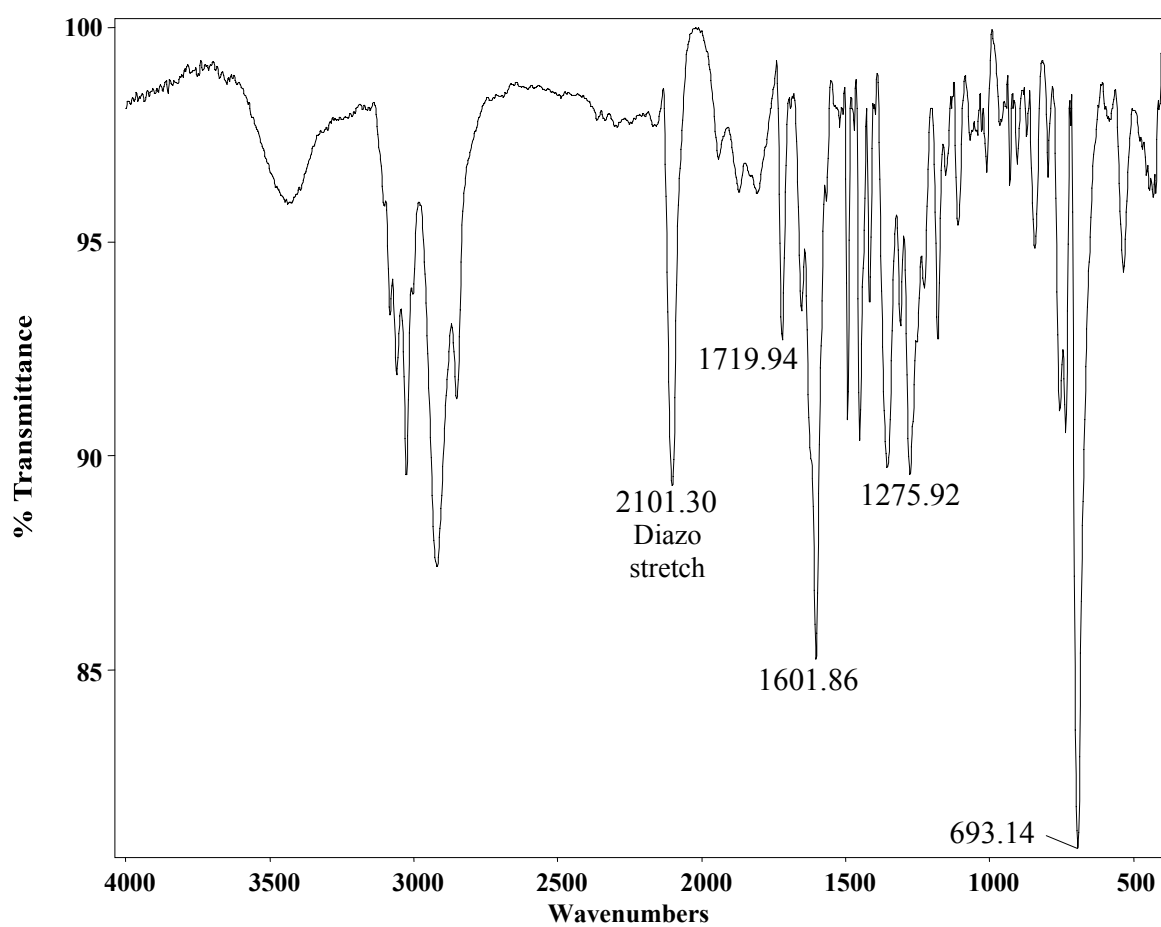


N. IR spectrum of NHNMPTS diazo amide silica.

Figure #2-O.

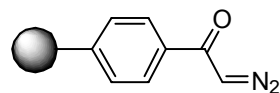


Resin 15

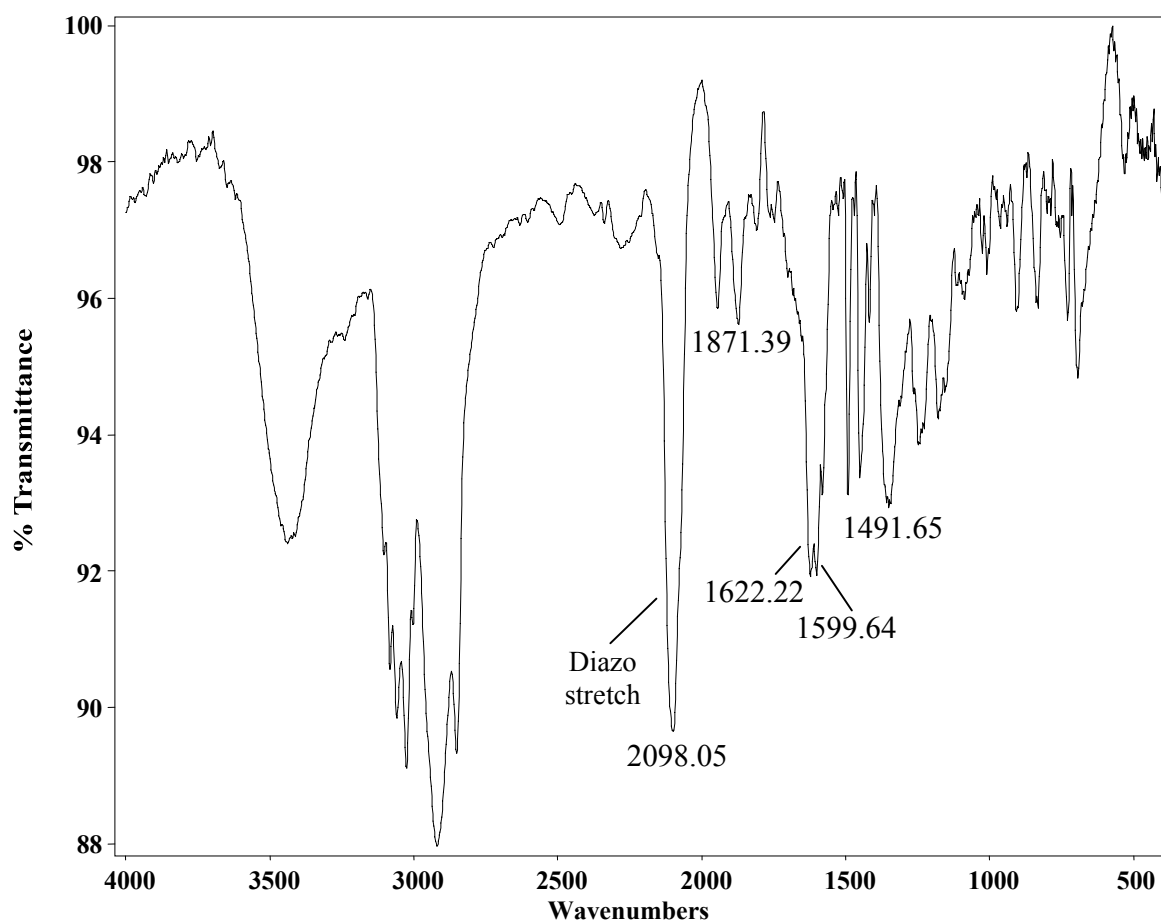


O. IR spectrum of the diazo ketone polystyrene (1.0 mmol/g loading).

Figure #2-P.

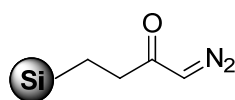


Resin 16

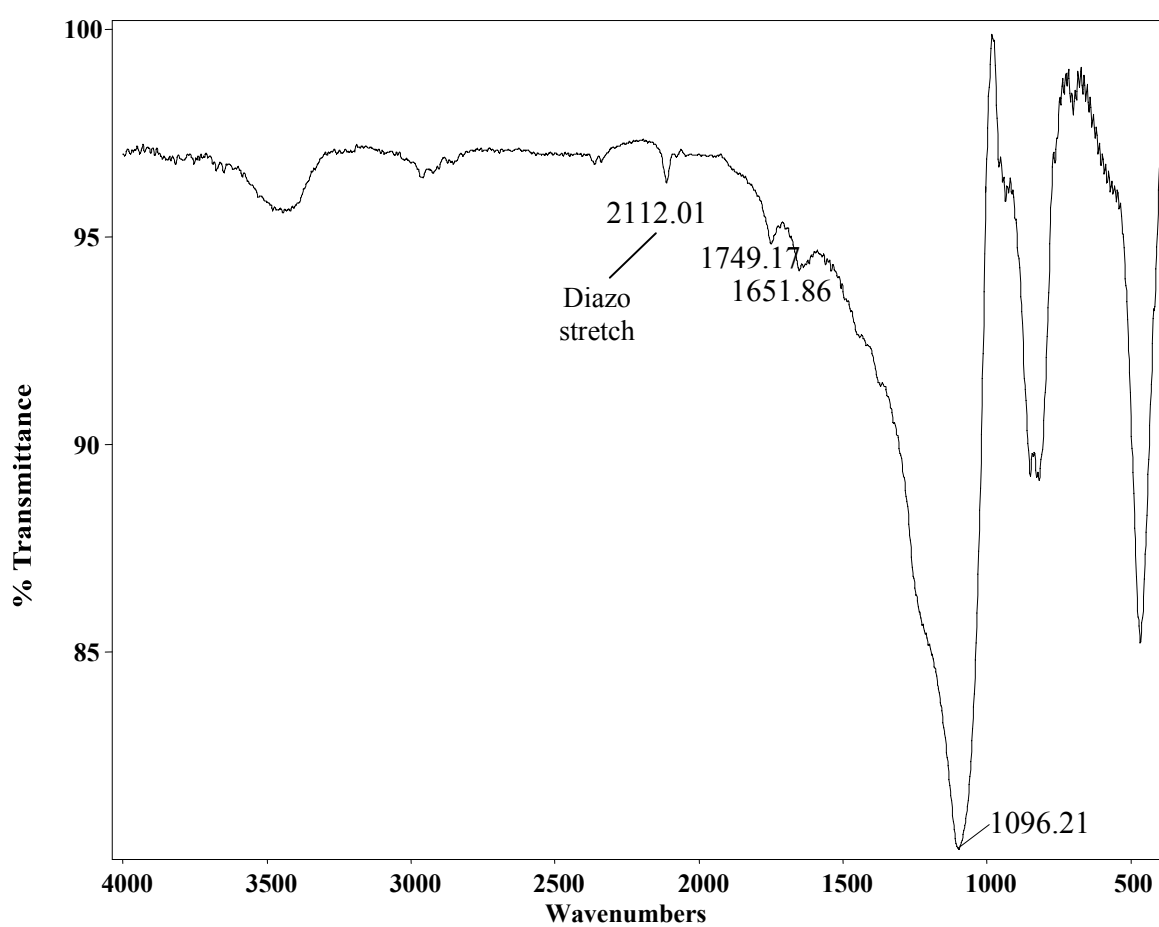


P. IR spectrum of the diazo ketone polystyrene (2.0 mmol/g loading).

Figure #2-Q.

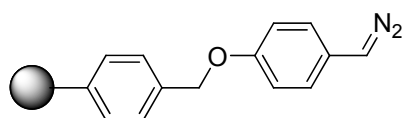


Resin 17

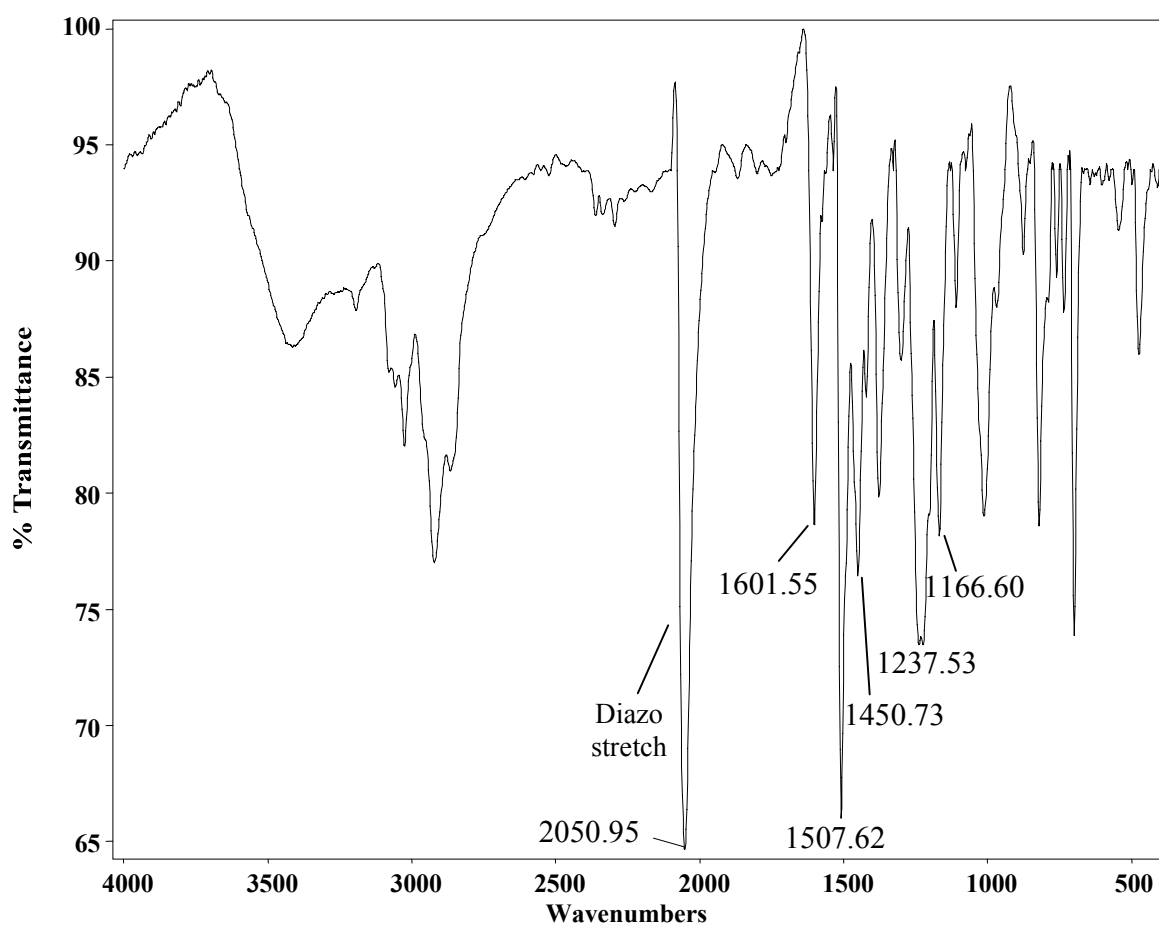


Q. IR spectrum of diazo ketone silica (1.6 mmol/g loading).

Figure #2-R.

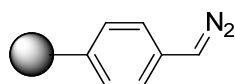


Resin 18

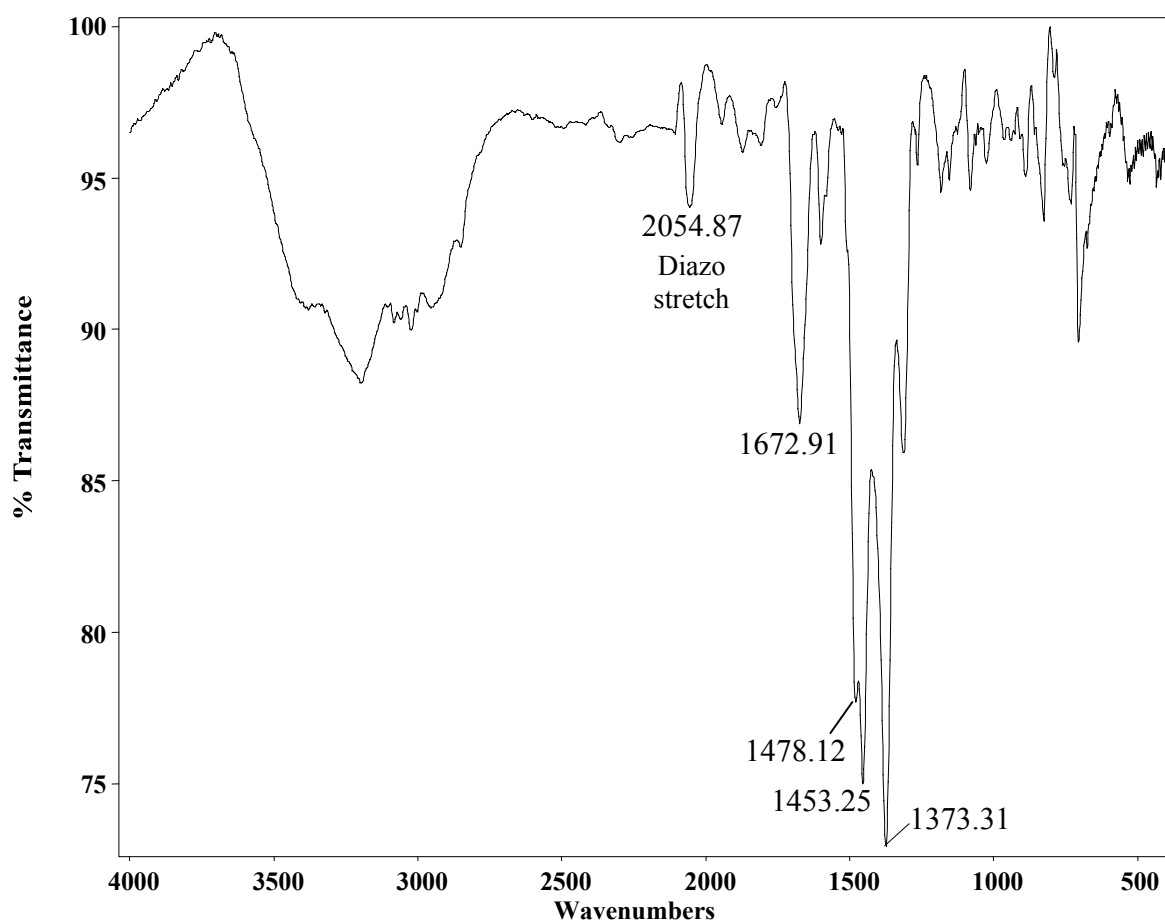


R. IR spectrum of benzyloxy phenyl diazo methane polystyrene (StratoSpheres, 50-100 mesh).

Figure #2-S.

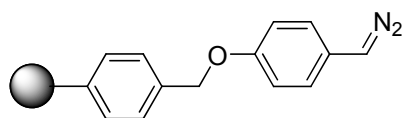


Resin 19

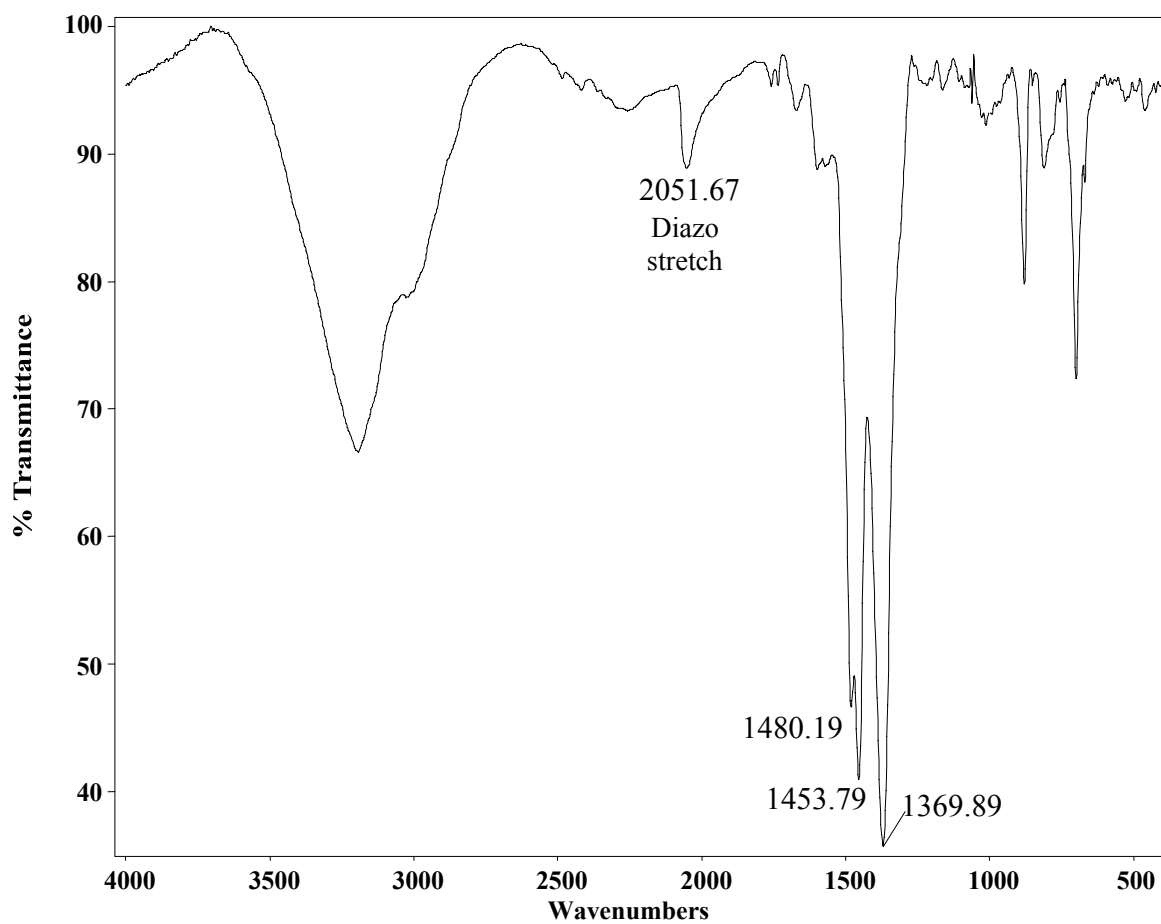


S. IR spectrum phenyl diazo methane polystyrene (100-200 mesh).

Figure #2-T.

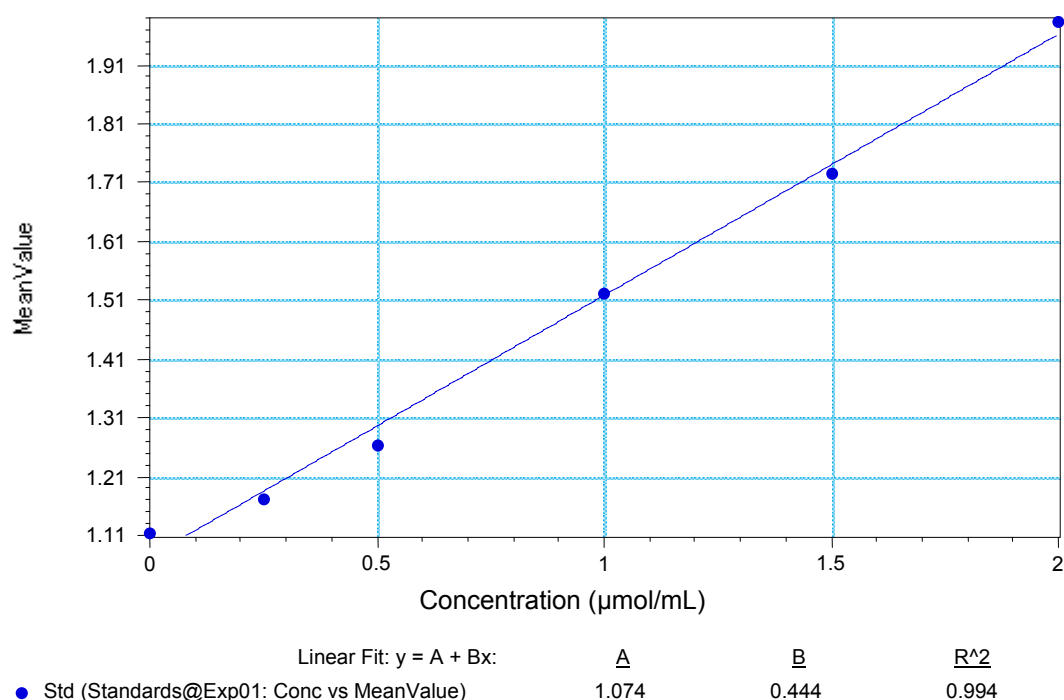


Resin 20



T. IR spectrum of benzyloxy phenyl diazo methane polystyrene (200-400 mesh).

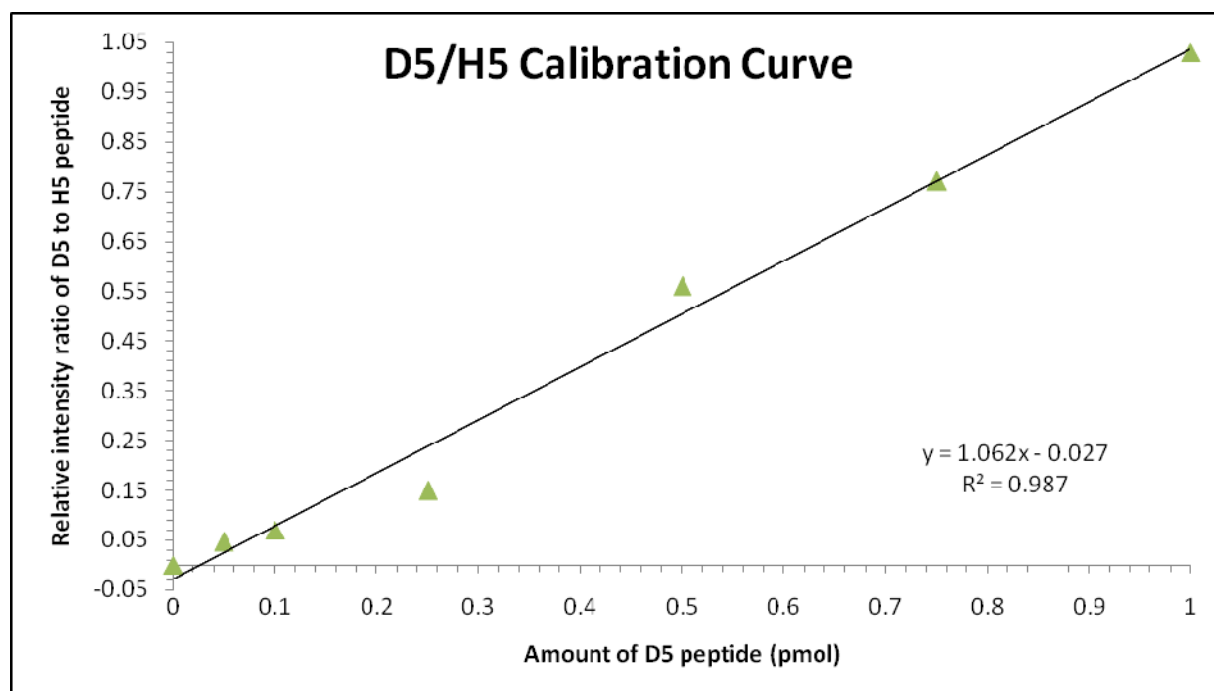
**Supplementary Figure #1: A-T** IR spectra of diazo-functionalized solid supports. Diagnostic IR stretch at 2105-2150  $\text{cm}^{-1}$  corresponding to diazo moiety. All synthesized materials shown were assessed for their enrichment capabilities. The diazo ketone polystyrene yielded the best results.



### Supplementary Figure #2: Fmoc-glycine standard curve

Six concentrations of Fmoc glycine were subjected to Fmoc liberation in the presence of base (DBU). The amount of liberated Fmoc was measured in absorbance at a wavelength of 304 nm. Subsequent analysis to quantify the amount of liberated Fmoc present was determined by the above standard curve and its corresponding linear equation.





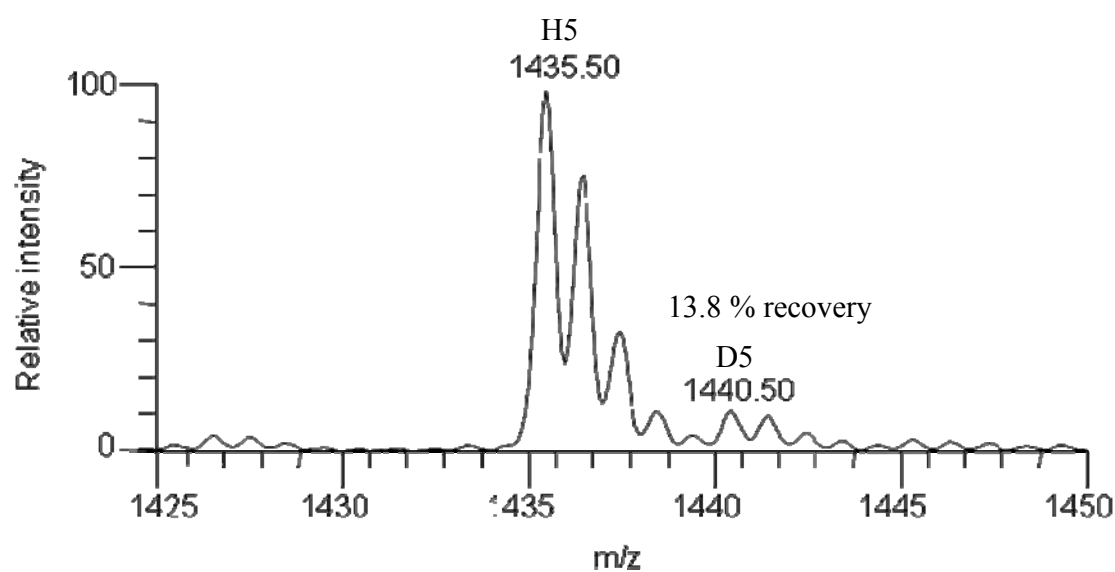
### Supplementary Figure #3: H5/D5 synthetic phosphopeptide standard curve

Various amounts of the synthetic labeled D5 peptide (50, 100, 250, 500, 750, 1000 fmol) were spotted with 1000 fmol of H5 peptide and the calculated ratio between the two peptides were plotted against the actual relative abundance in the spectra. The standard curve was then used to determine the amount of D5 present within the enrichment sample by comparing the relative abundance of H5/D5 in the sample.

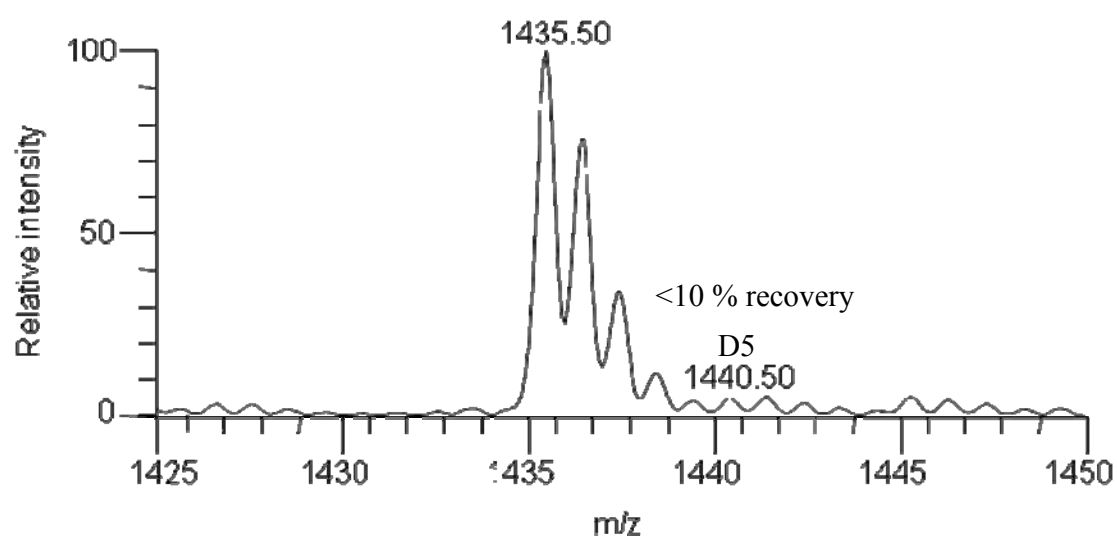
### Supplementary Figures #4A-W: H5/D5 synthetic phosphopeptide recovery spectra

The enrichment of the D5 peptide was tested in duplicate for each of the 20 different diazo functionalized materials and prior to analysis the enrichment samples were co-spotted with 1000 fmol of the H5; theoretically corresponding to 100% recovery. The relative abundance ratio was calculated and the actual amount of D5 peptide present was determined using the aforementioned standard curve.

Figure #4-A.

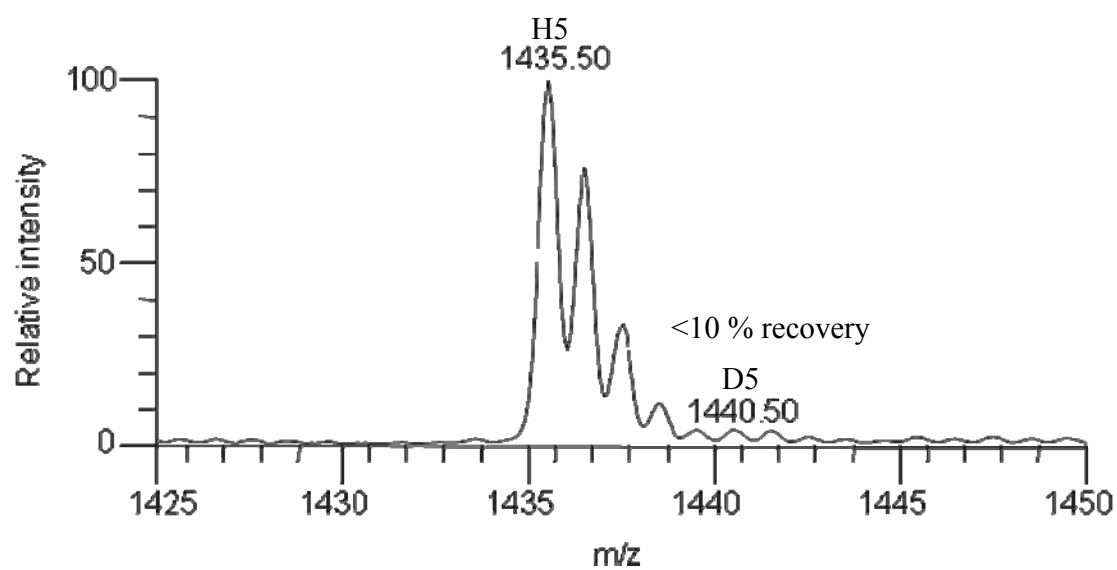


H5

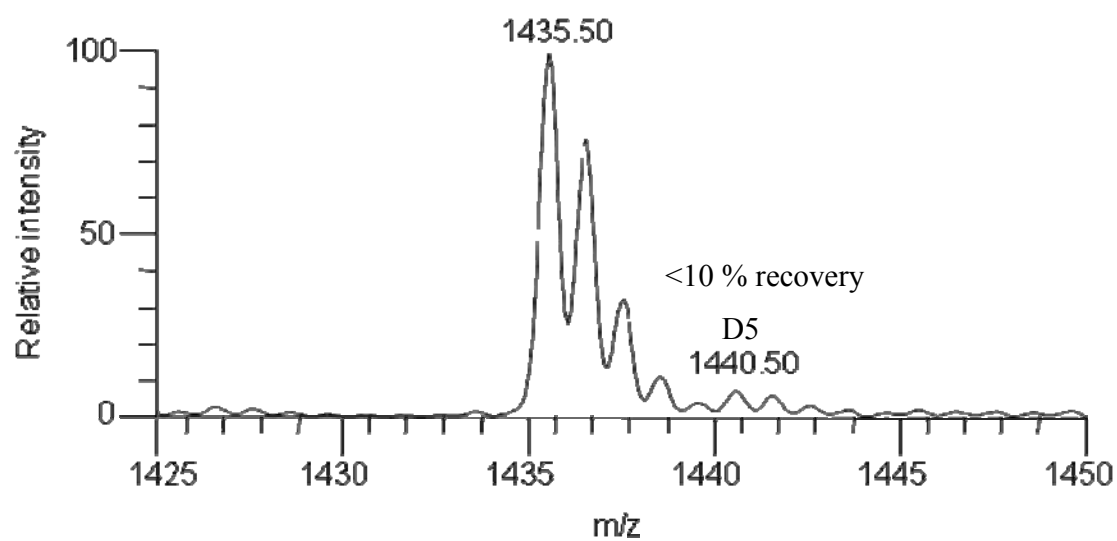


A. Mass spectra of D5 enrichment using diazo acetate Wang resin (**Resin 1**)

**Figure #4-B.**

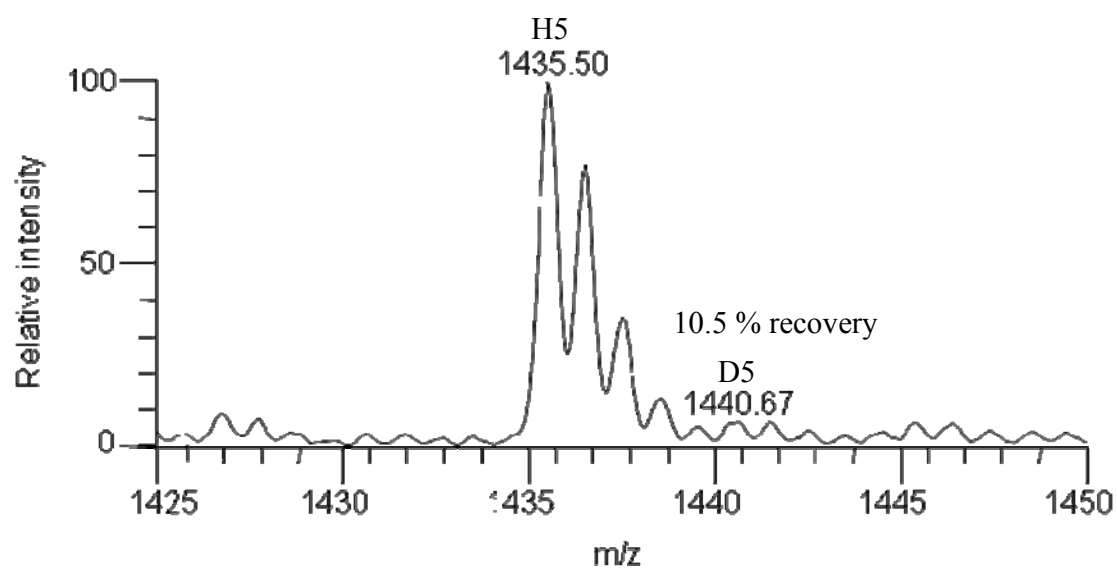


H5

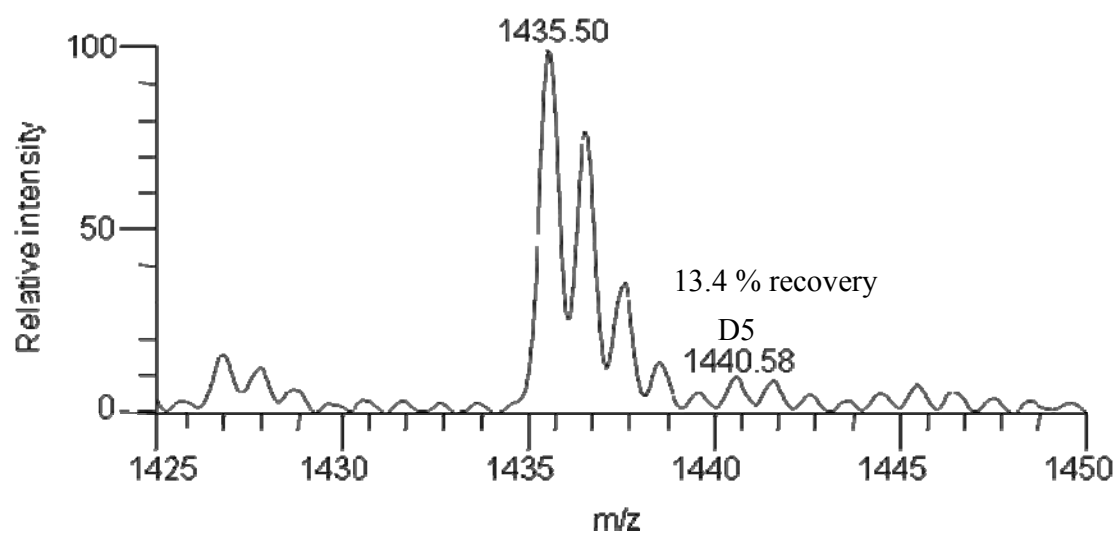


B. Mass spectra of D5 enrichment using diazo acetate Wang resin (**Resin 2**)

Figure #4-C.

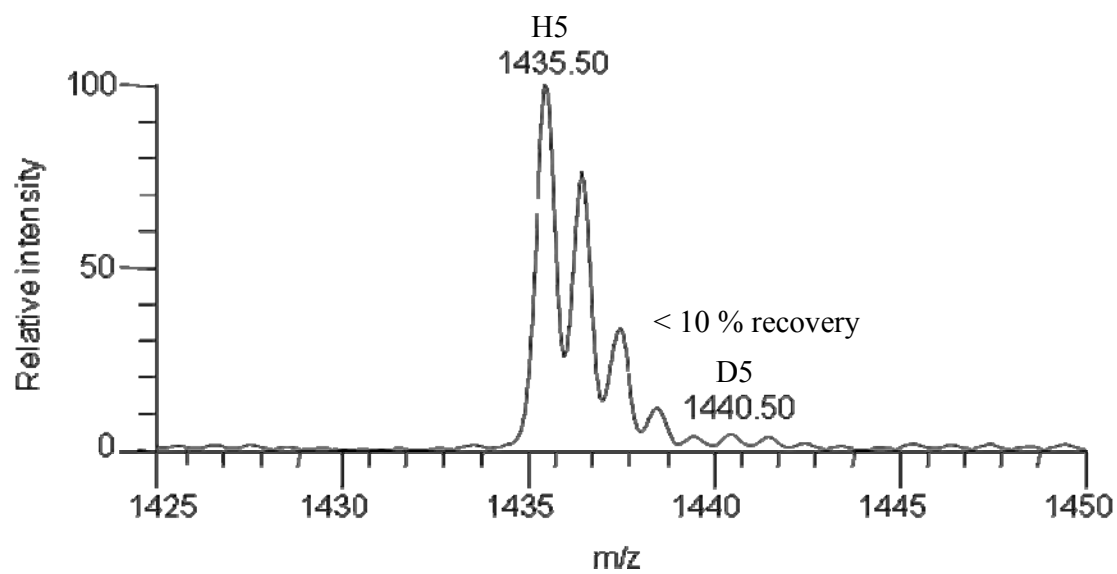


H5

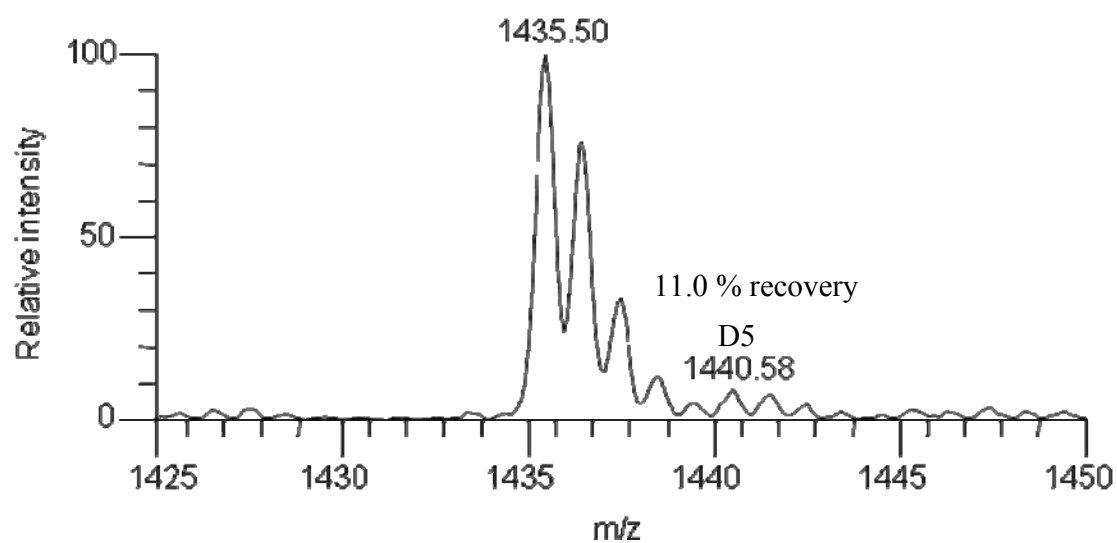


C. Mass spectra of D5 enrichment using diazo amide tentagel resin (**Resin 3**)

Figure #4-D.

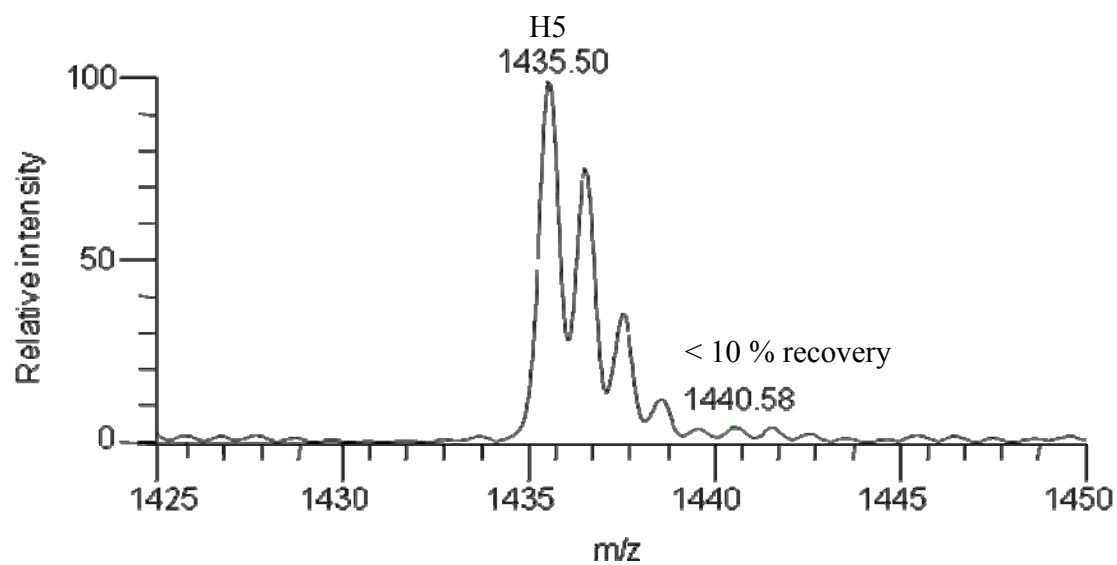


H5

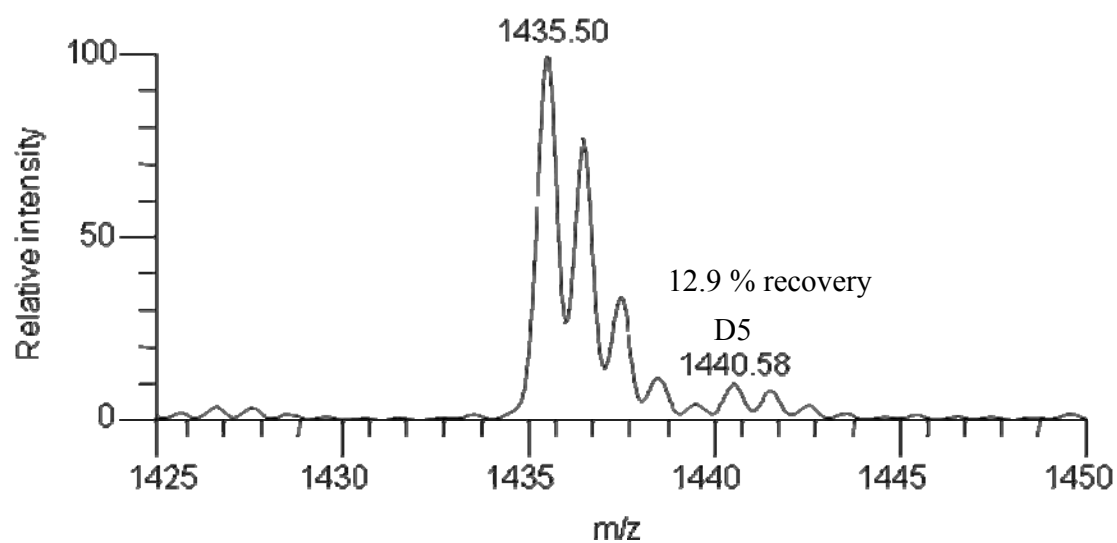


D. Mass spectra of D5 enrichment using diazo amide megabead resin (**Resin 4**)

Figure #4-E.

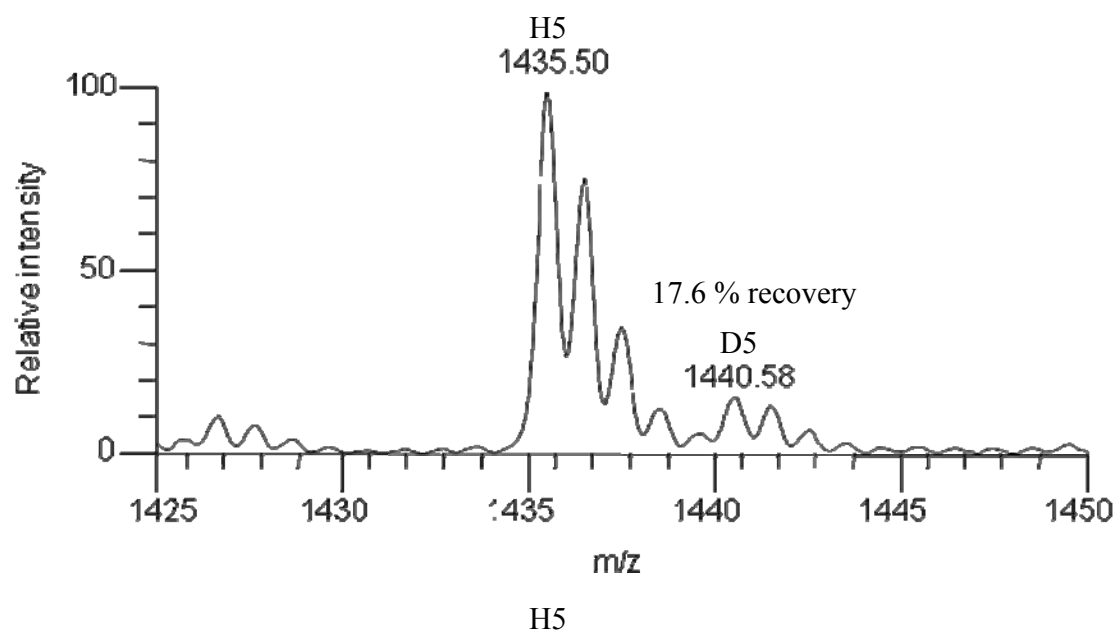


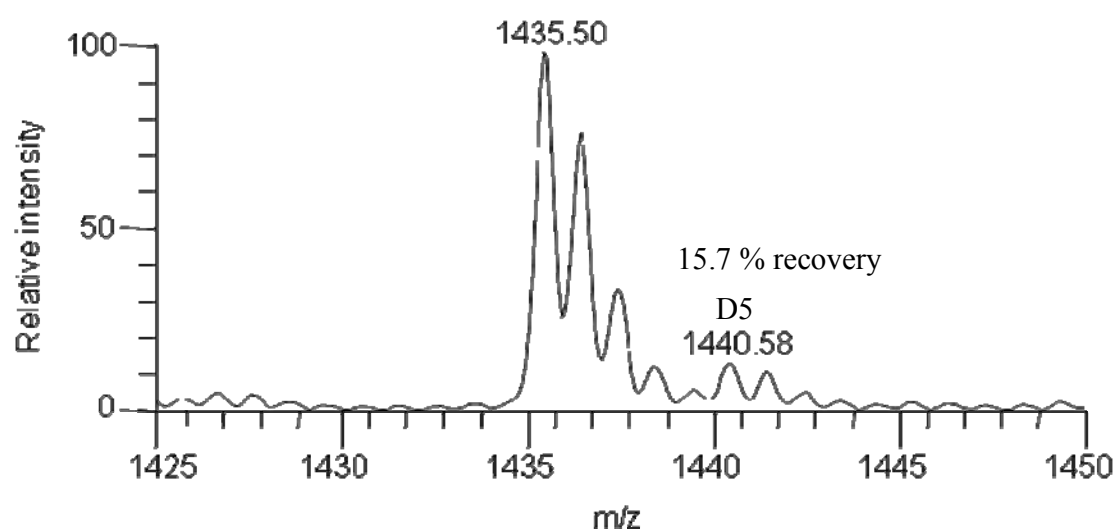
H5



E. Mass spectra of D5 enrichment using diazo amide PEGA resin (**Resin 5**)

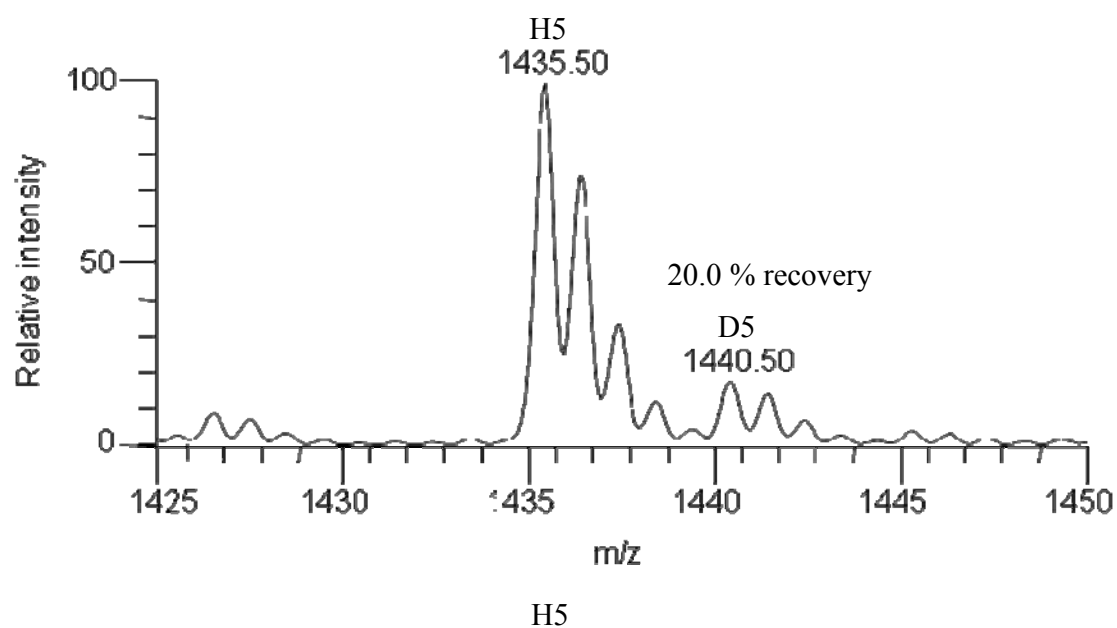
Figure #4-F.



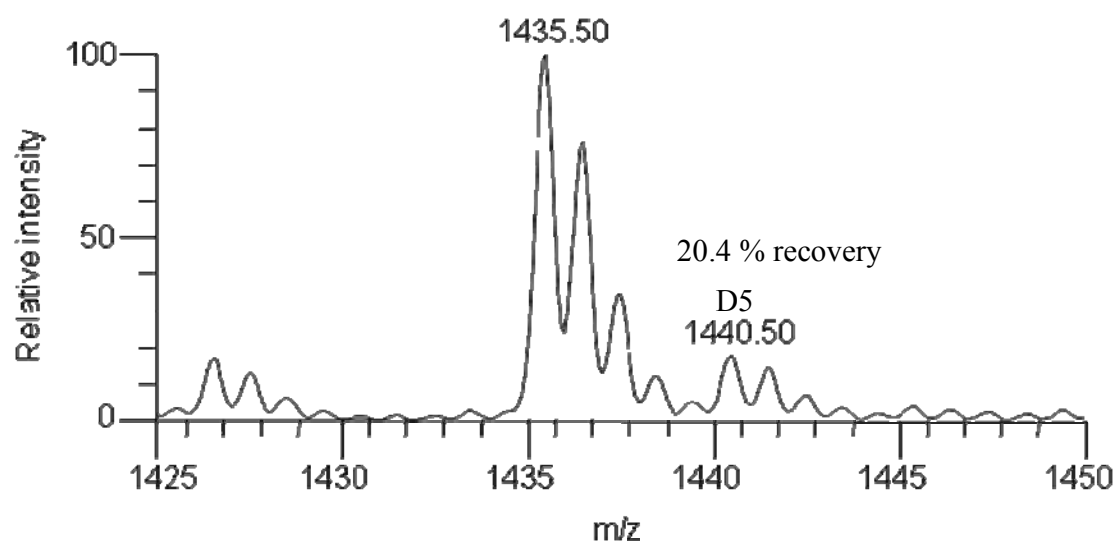


F. Mass spectra of D5 enrichment using diazo acetate PEGA HMPA resin (**Resin 6**)

Figure #4-G.

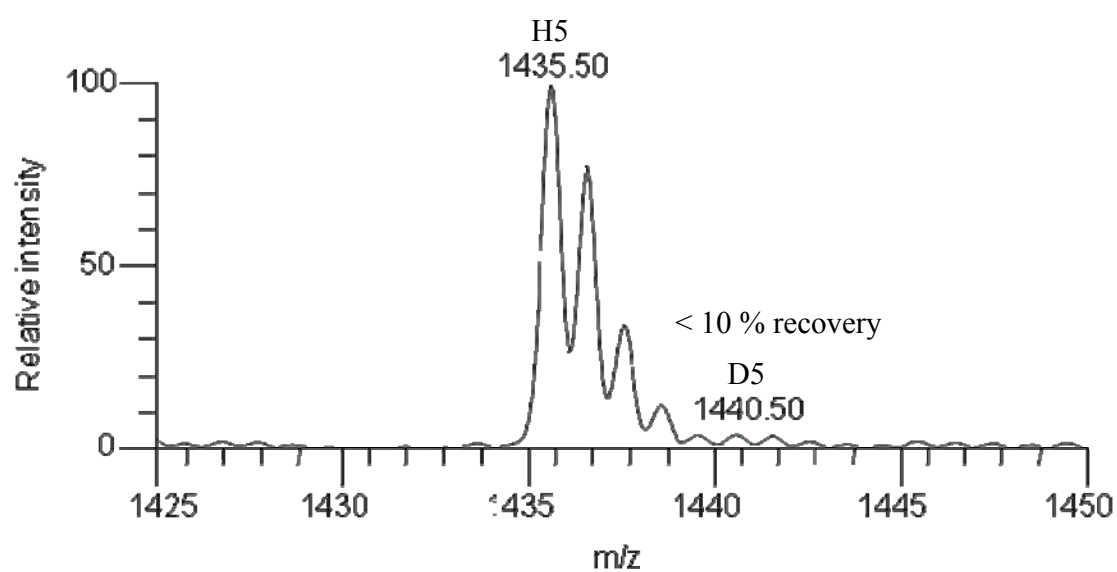


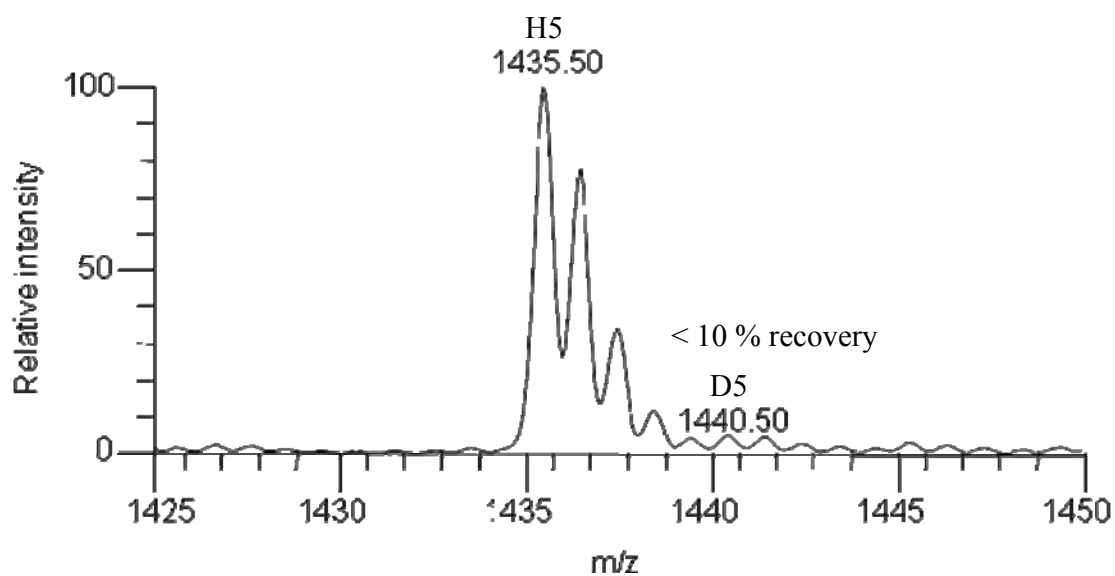




G. Mass spectra of D5 enrichment using diazo amide dendrimer resin (**Resin 7**)

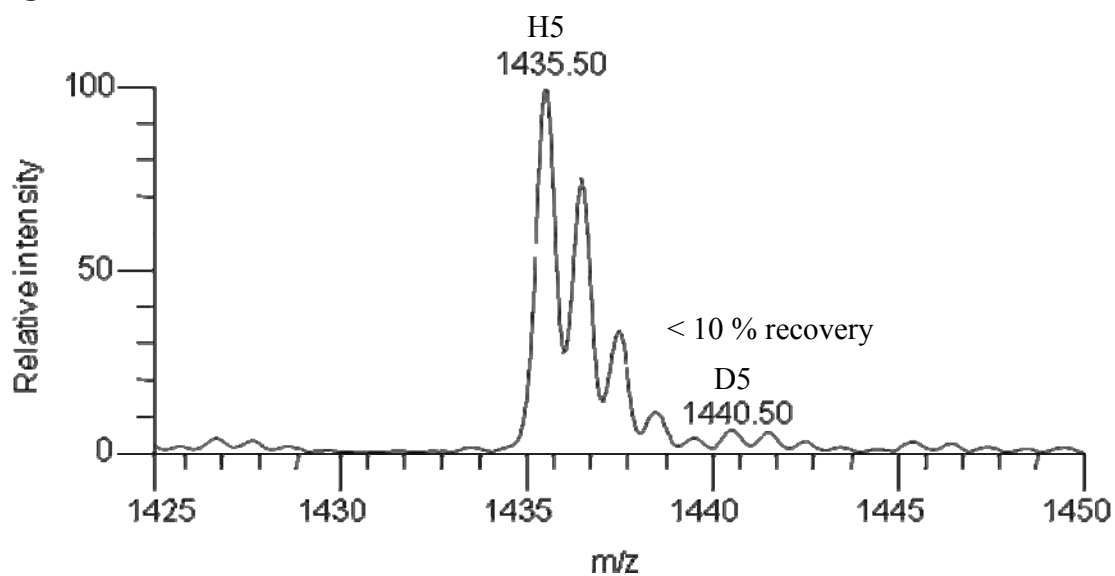
Figure #4-H.



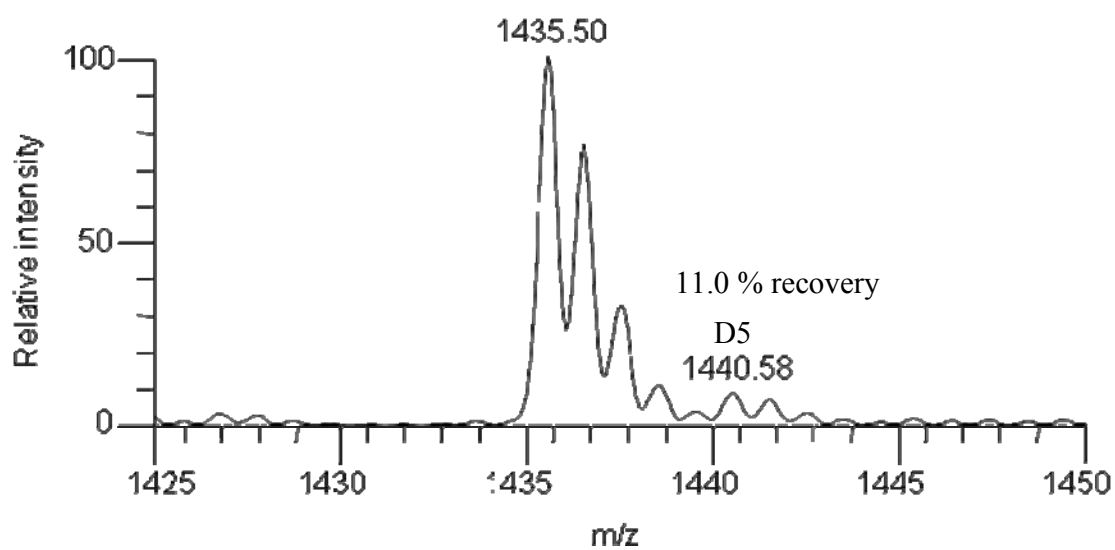


H. Mass spectra of D5 enrichment using 4-diazo acetate polystyrene (**Resin 8**)

Figure #4-I.

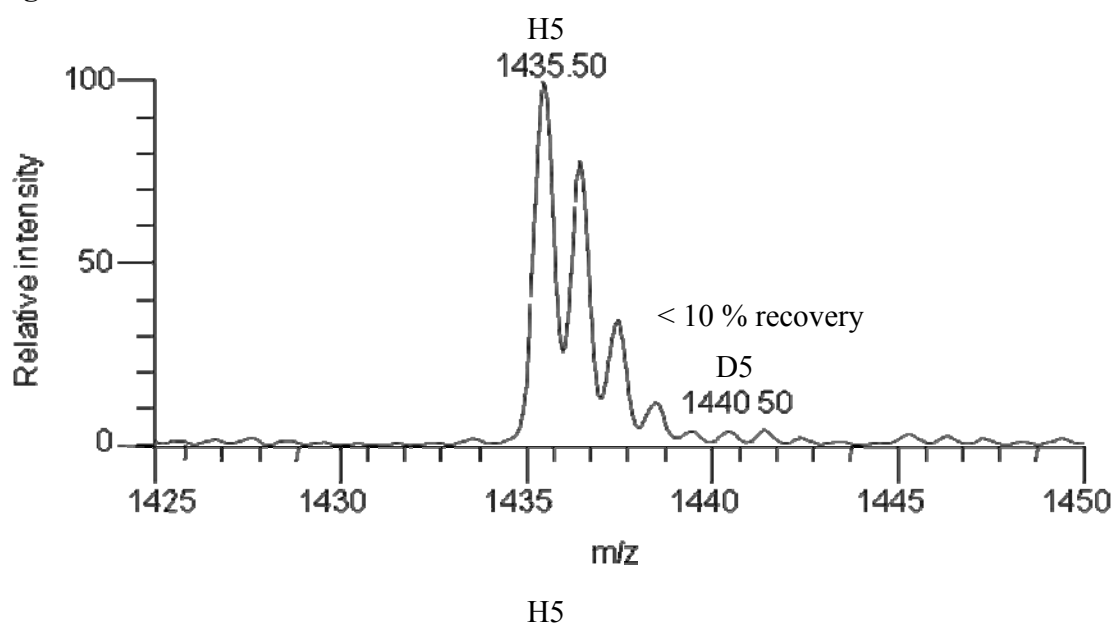


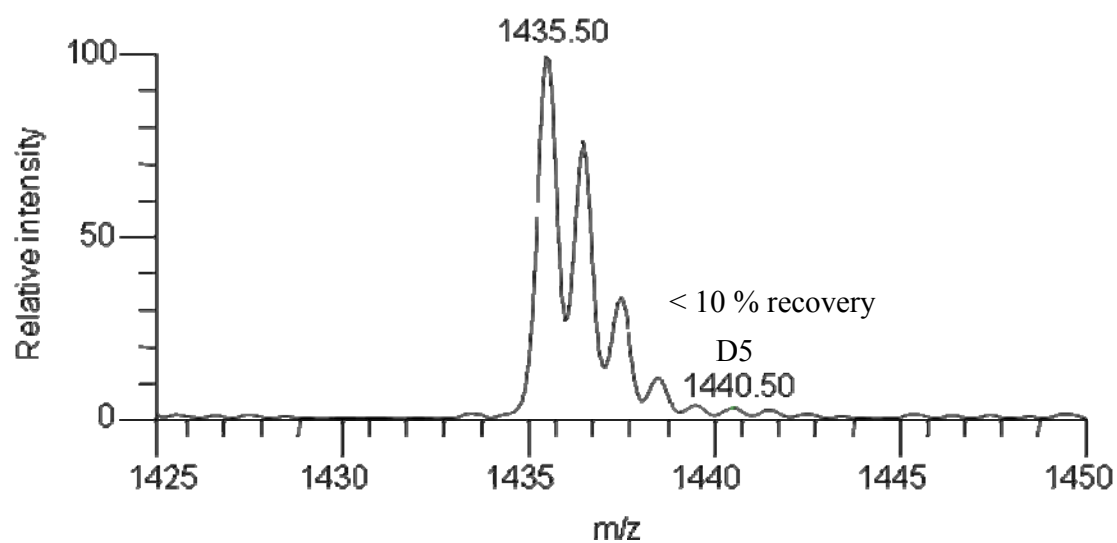
H5



I. Mass spectra of D5 enrichment using diazo acetate polystyrene (**Resin 9**)

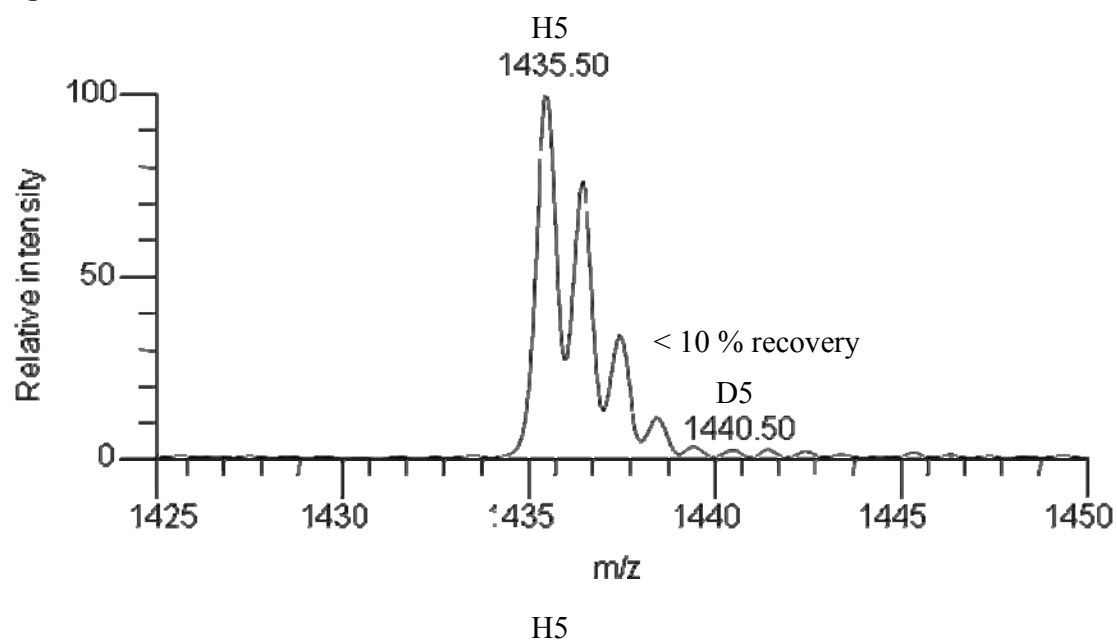
Figure #4-J.

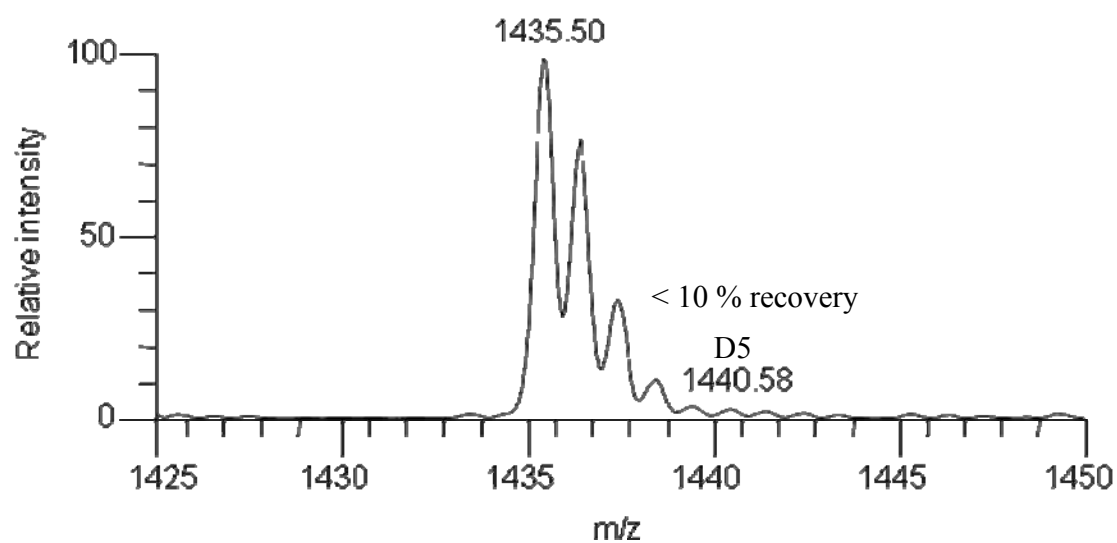




J. Mass spectra of D5 enrichment using NovaSyn diazo acetate resin (**Resin 10**)

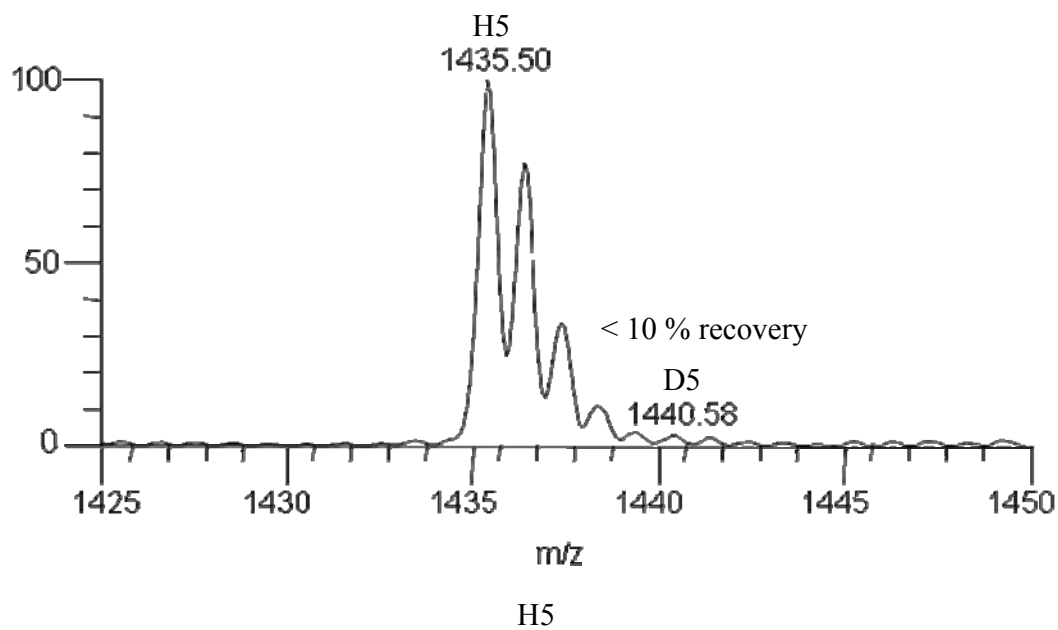
Figure #4-K.

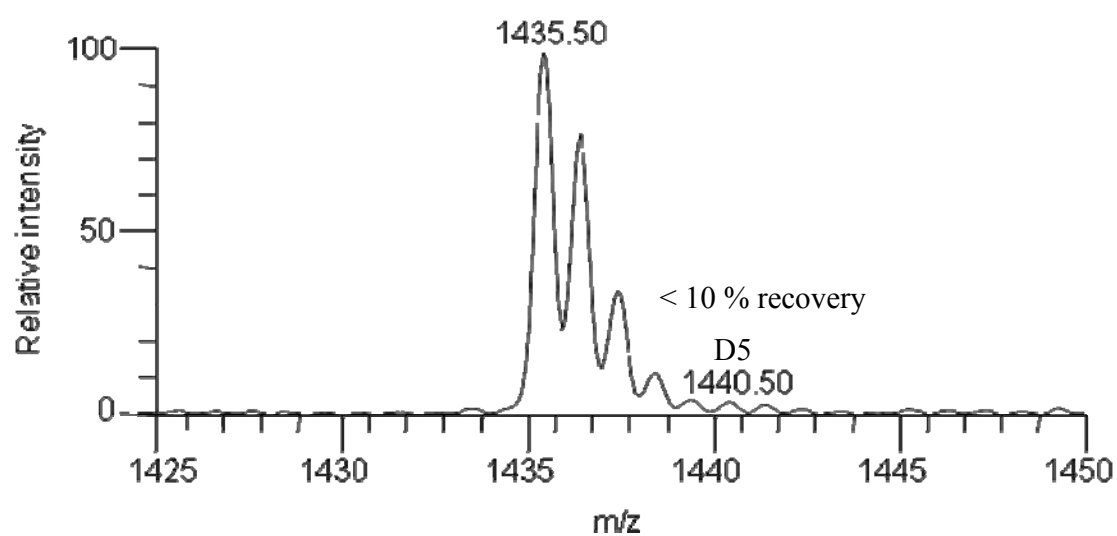




K. Mass spectra of D5 enrichment using NovaSyn TG HMP diazo acetate resin (**Resin 11**)

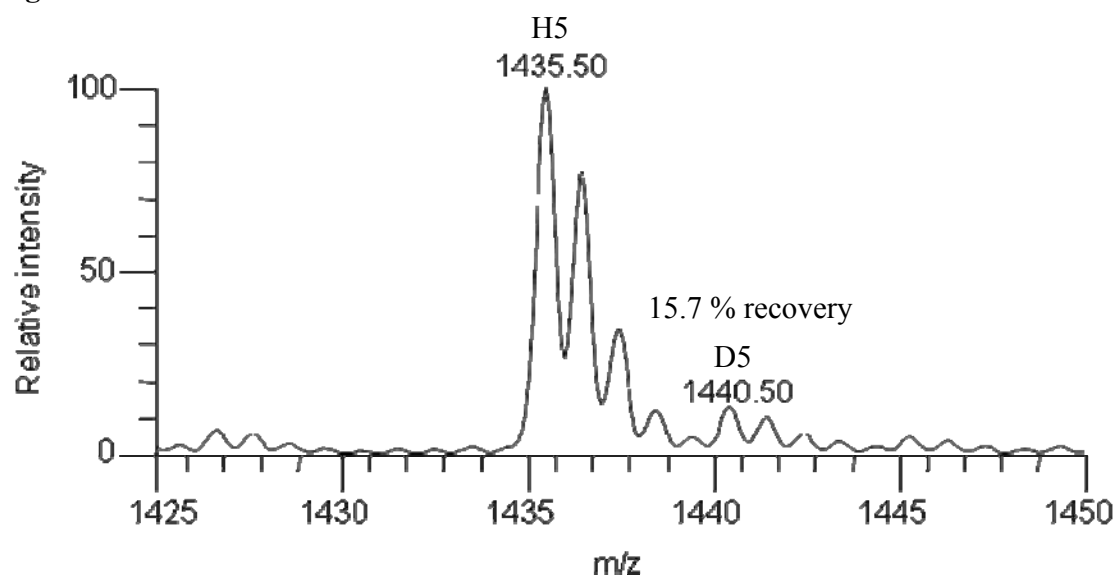
Figure #4-L.



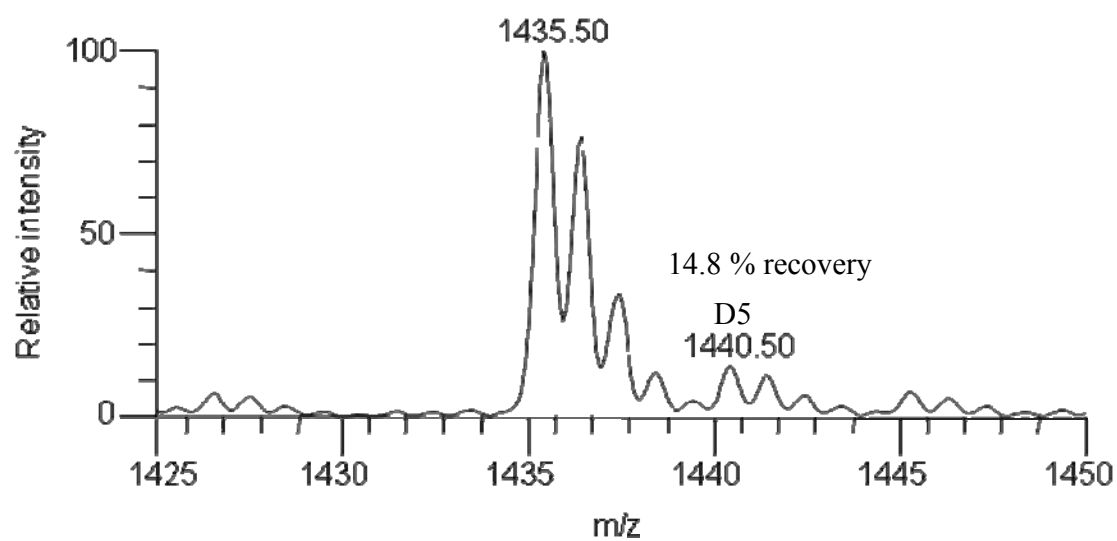


L. Mass spectra of D5 enrichment using NovaSyn diazo anhydride resin (**Resin 12**)

Figure #4-M.

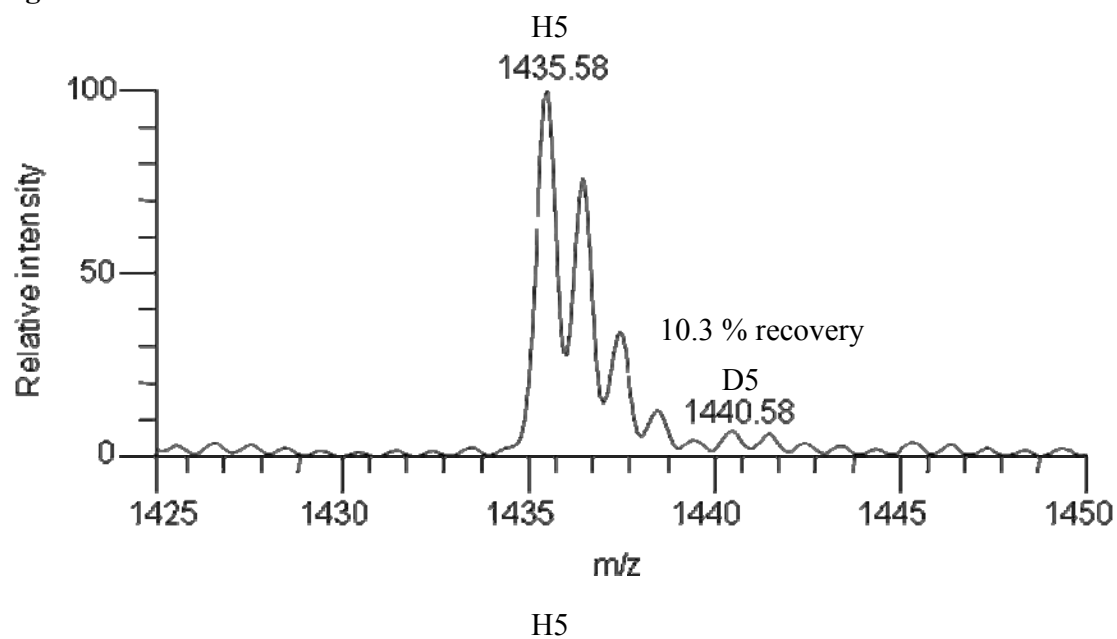


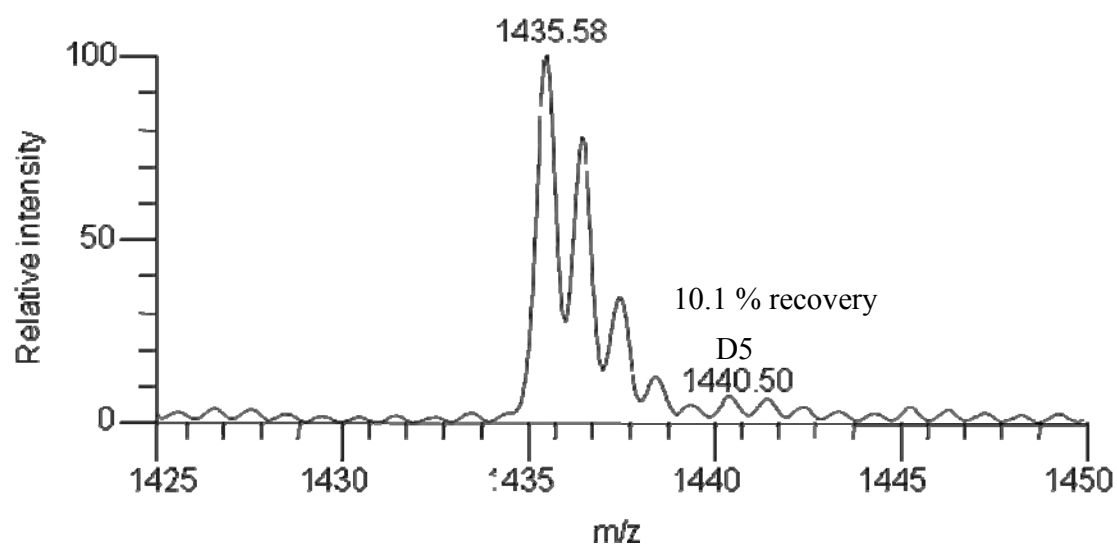
H5



M. Mass spectra of D5 enrichment using vicinal diazo acetate silica (**Resin 13**)

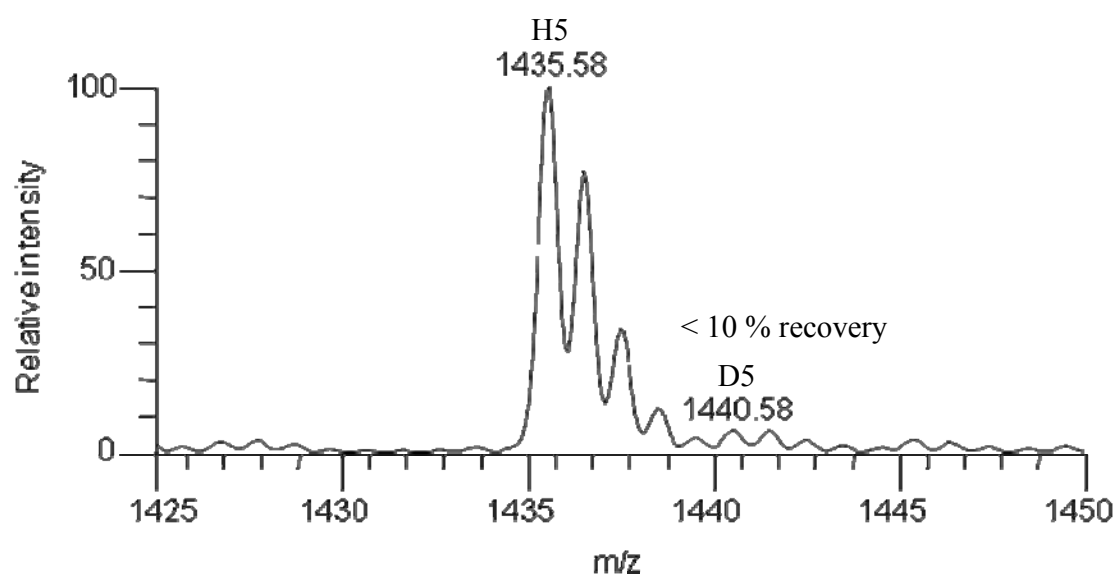
Figure #4-N.



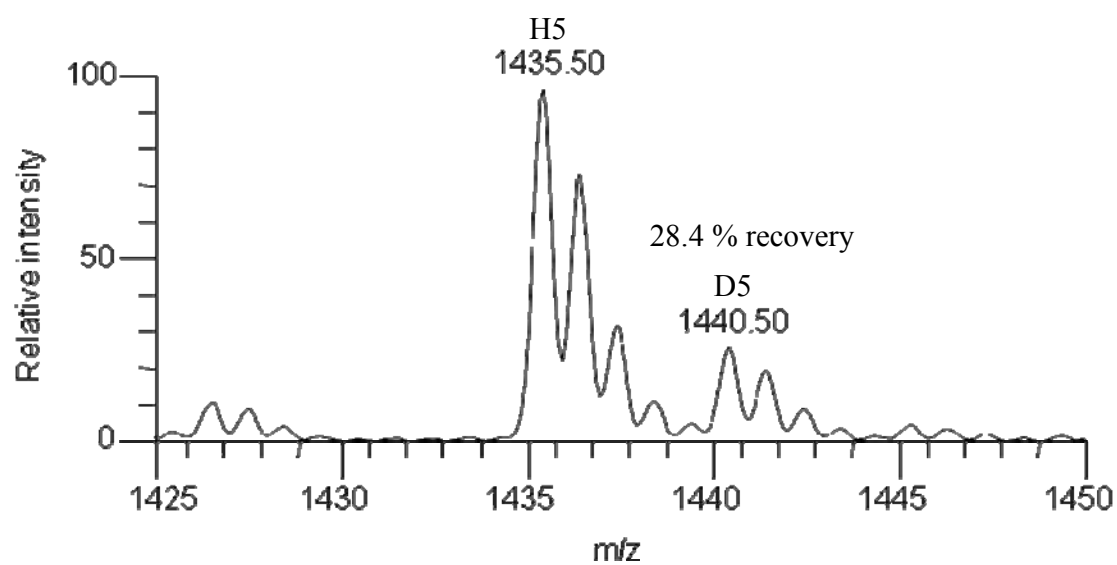


N. Mass spectra of D5 enrichment using diazo amide silica (**Resin 14**)

Figure #4-O.



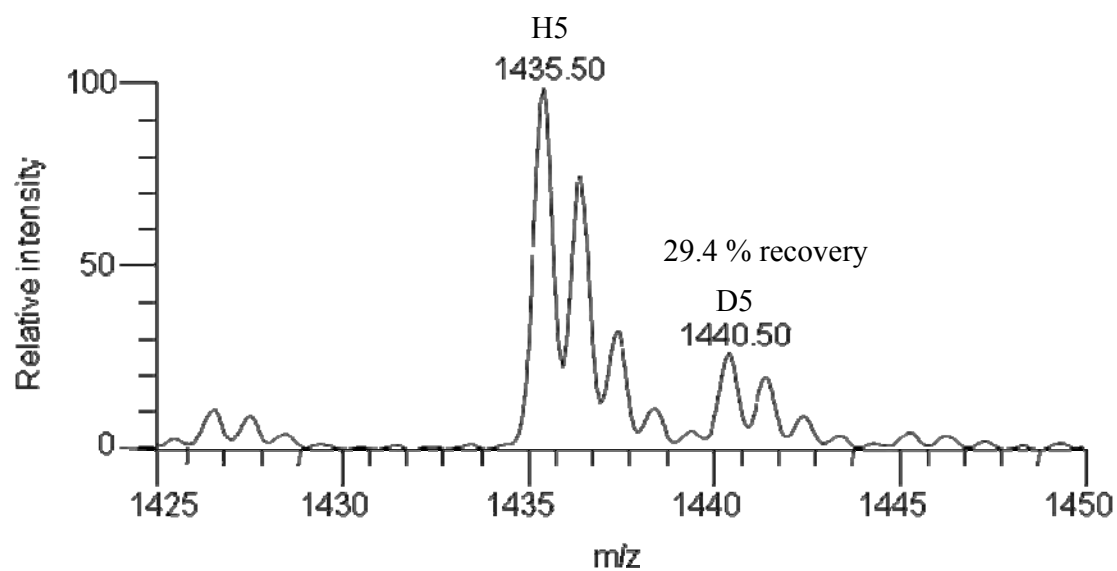
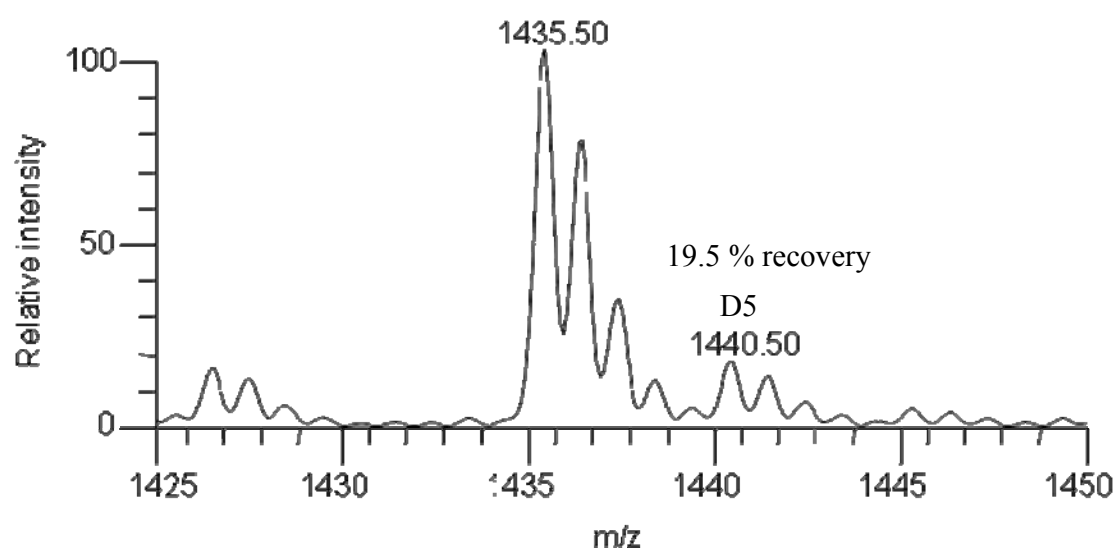




O. Mass spectra of D5 enrichment using diazo ketone resin (**Resin 15**)

**Figure #4-P.**

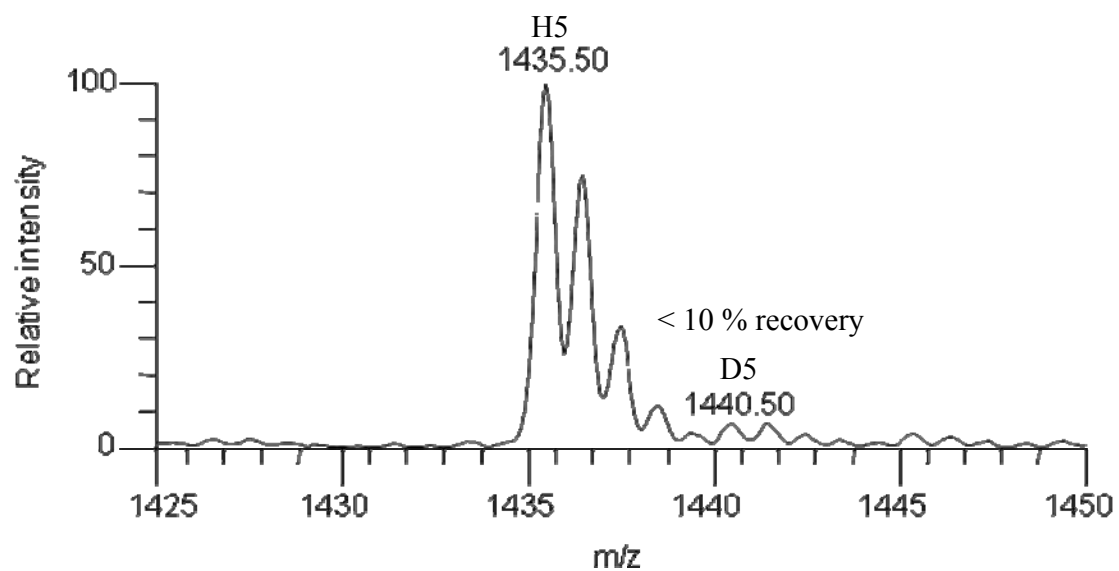
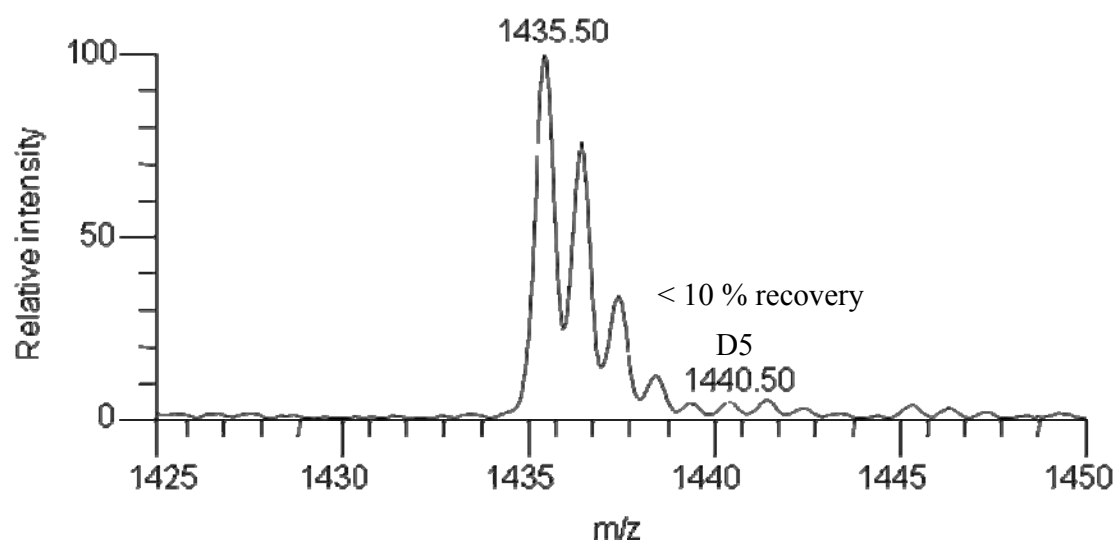
H5



P. Mass spectra of D5 enrichment using diazo ketone resin (**Resin 16**)

**Figure #4-Q.**

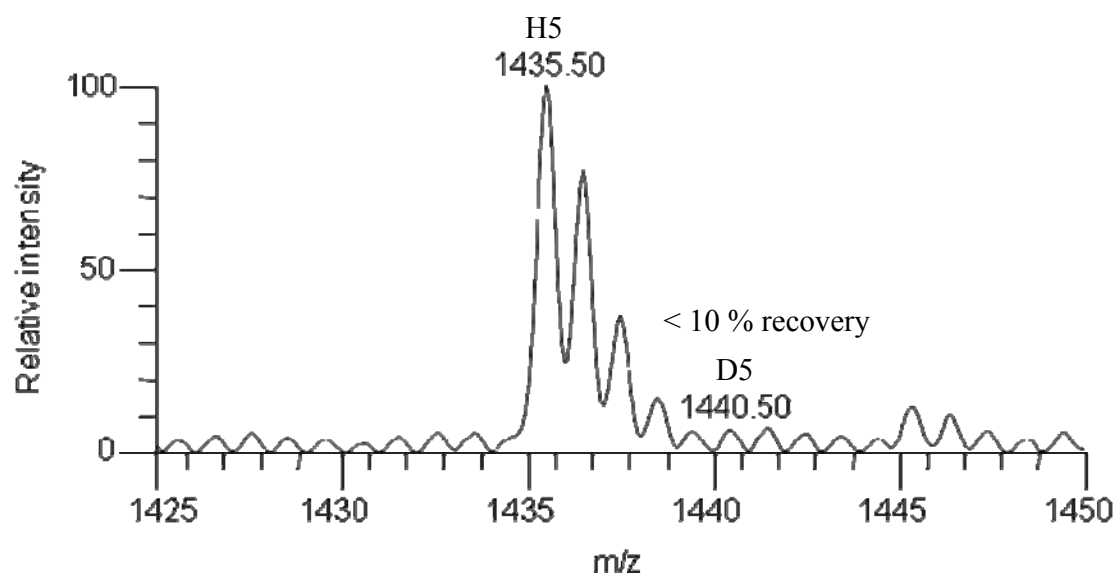
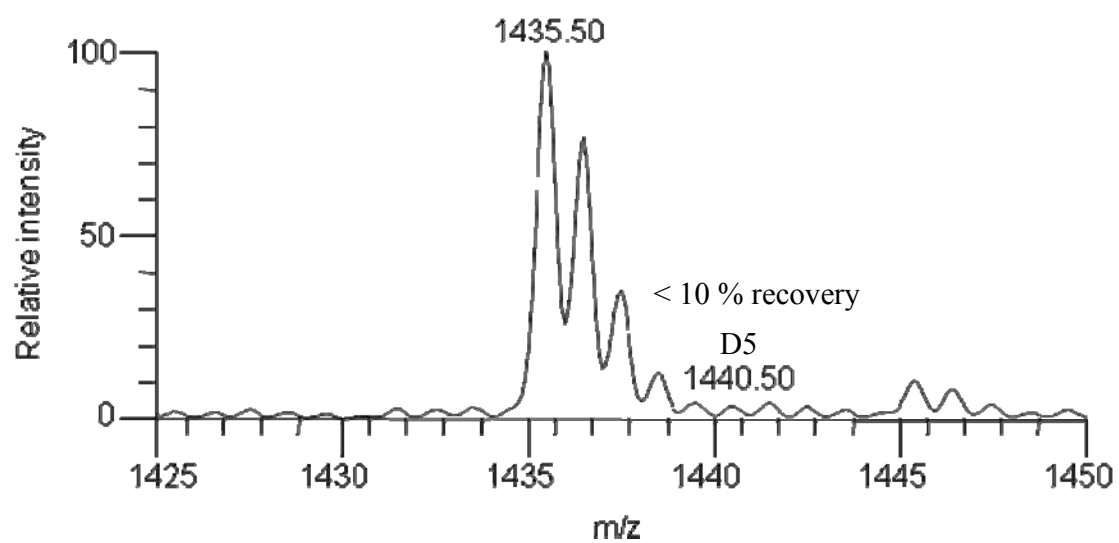
H5



Q. Mass spectra of D5 enrichment using diazo ketone silica (**Resin 17**)

Figure #4-R.

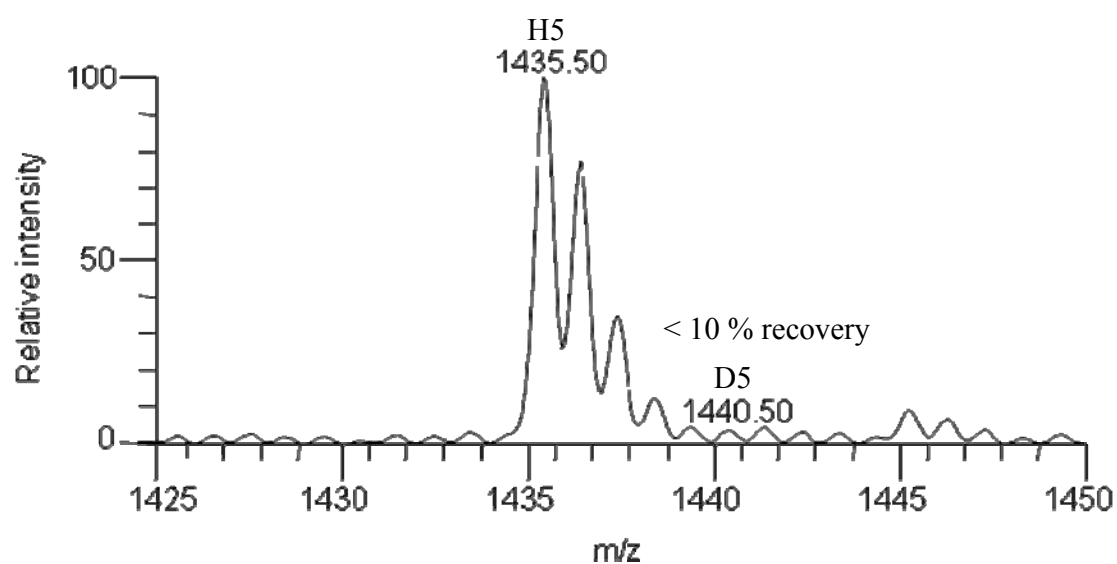
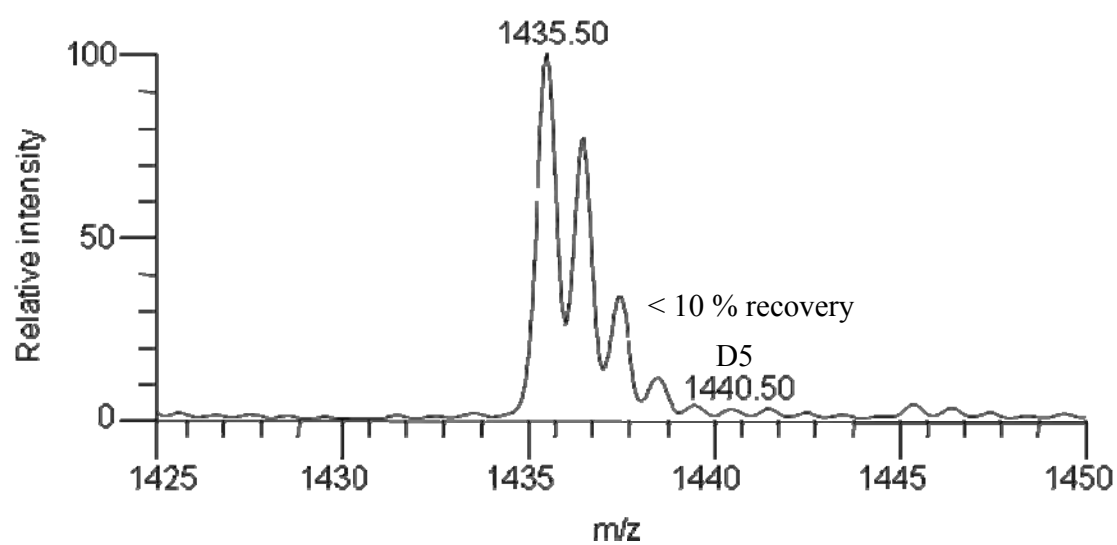
H5



R. Mass spectra of D5 enrichment using StratoSpheres phenyl diazo methane (**Resin 18**)

Figure #4-S.

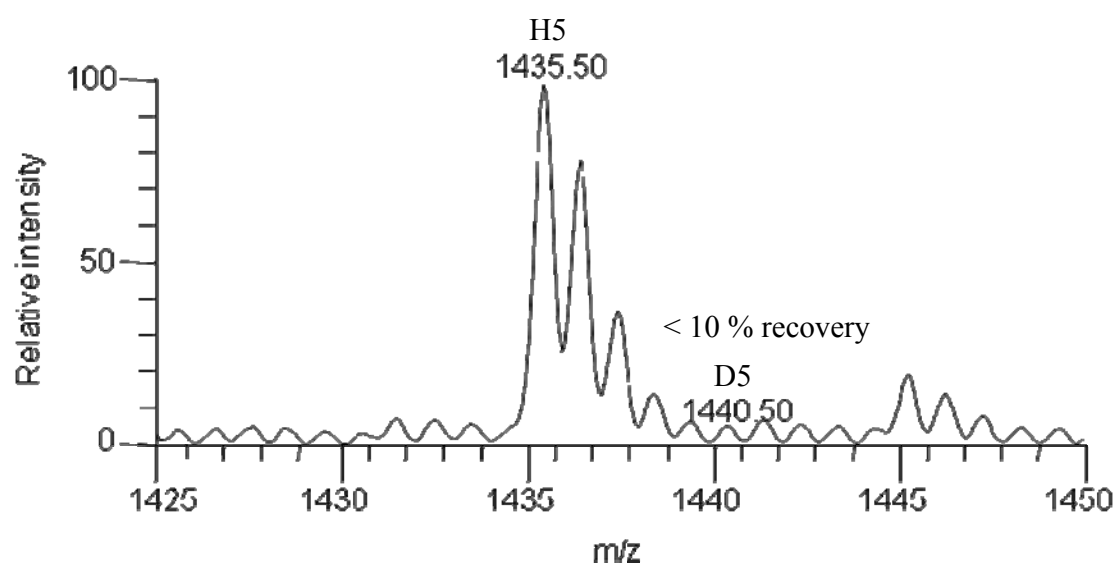
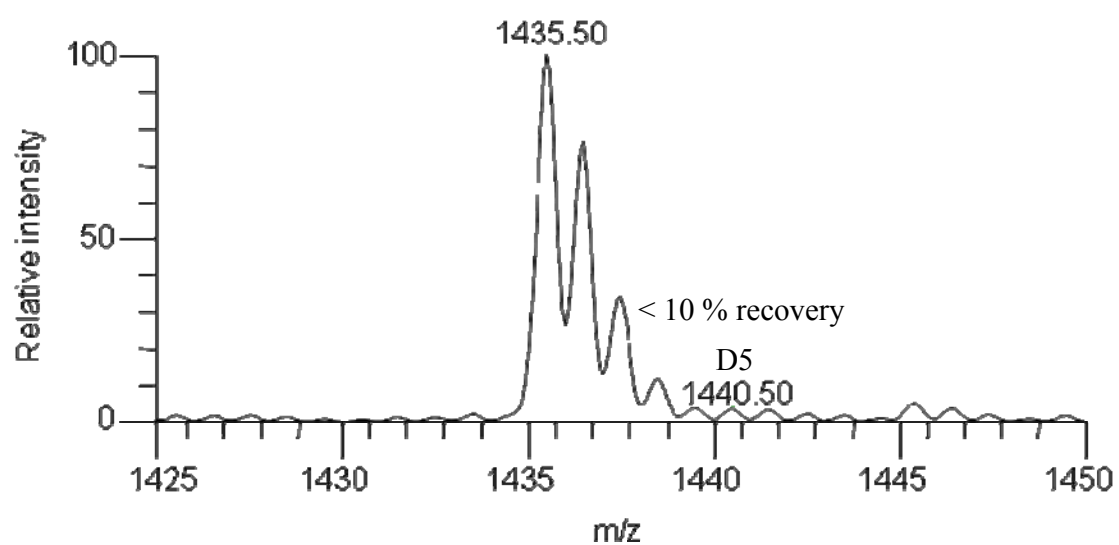
H5



S. Mass spectra of D5 enrichment using phenyl diazo methane polystyrene (**Resin 19**)

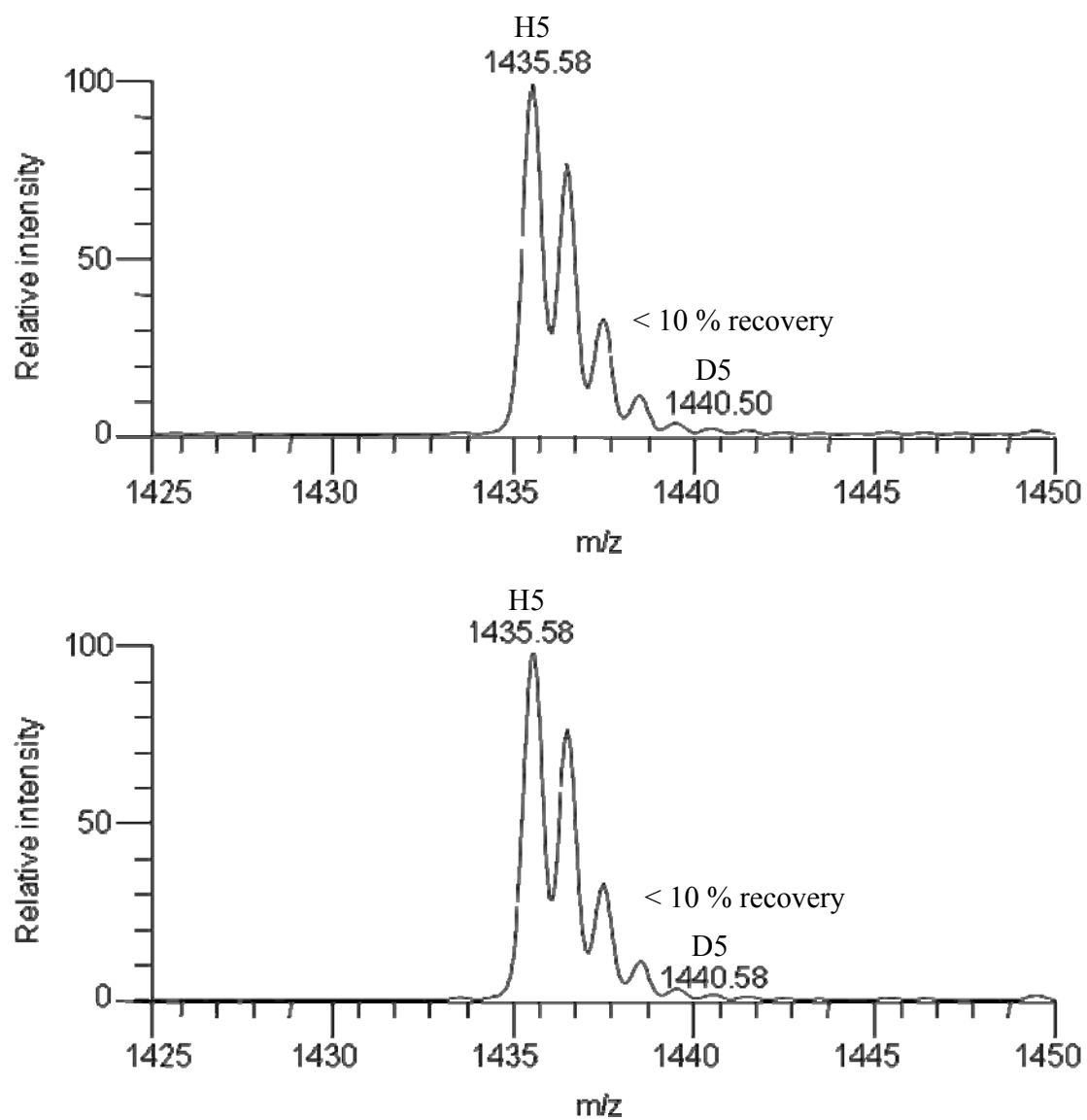
Figure #4-T.

H5



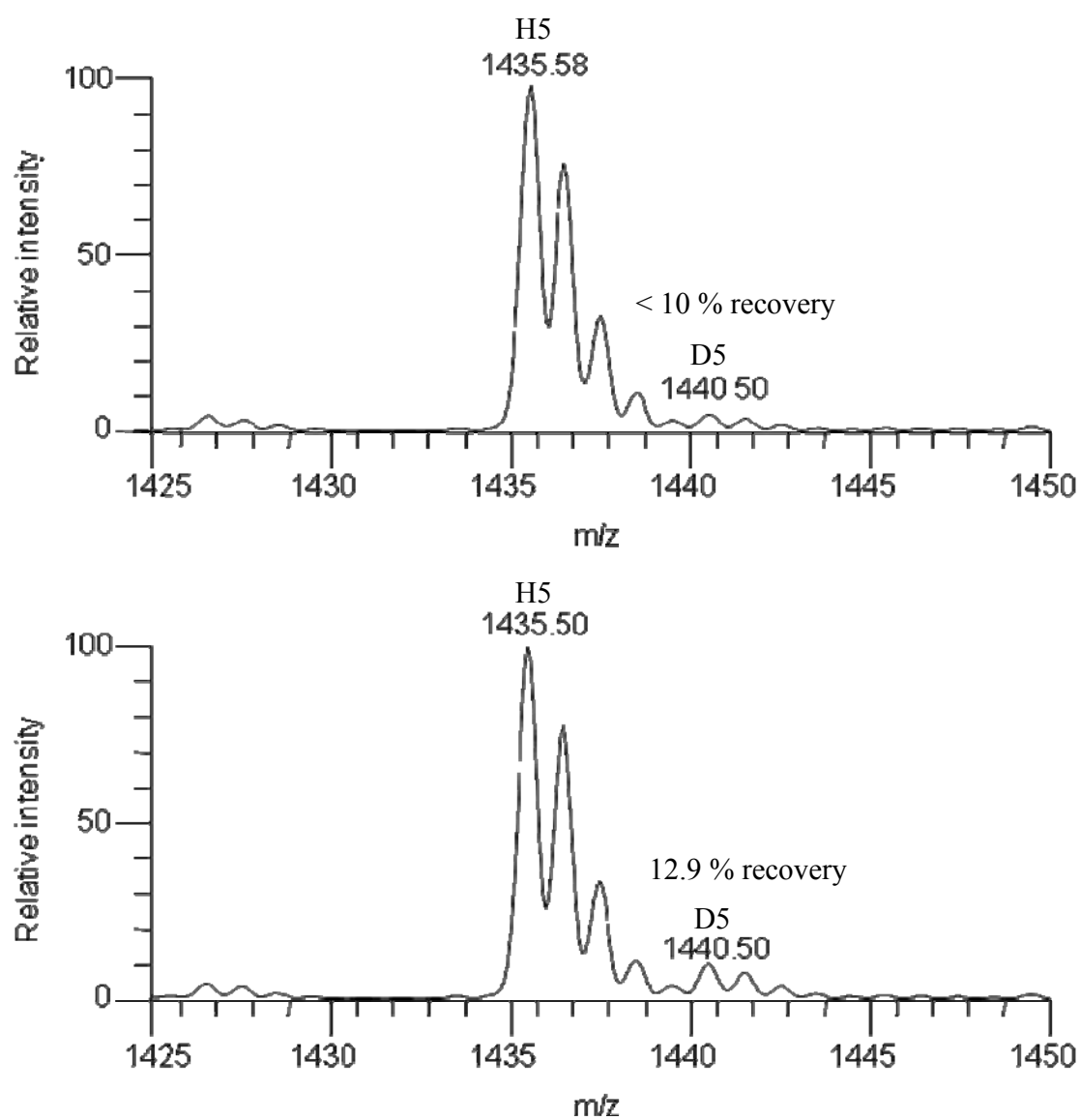
T. Mass spectra of D5 enrichment using phenyl diazo methane polystyrene (**Resin 20**)

Figure #4-U.



U. Mass spectra of D5 enrichment using Millipore IMAC Ziptips

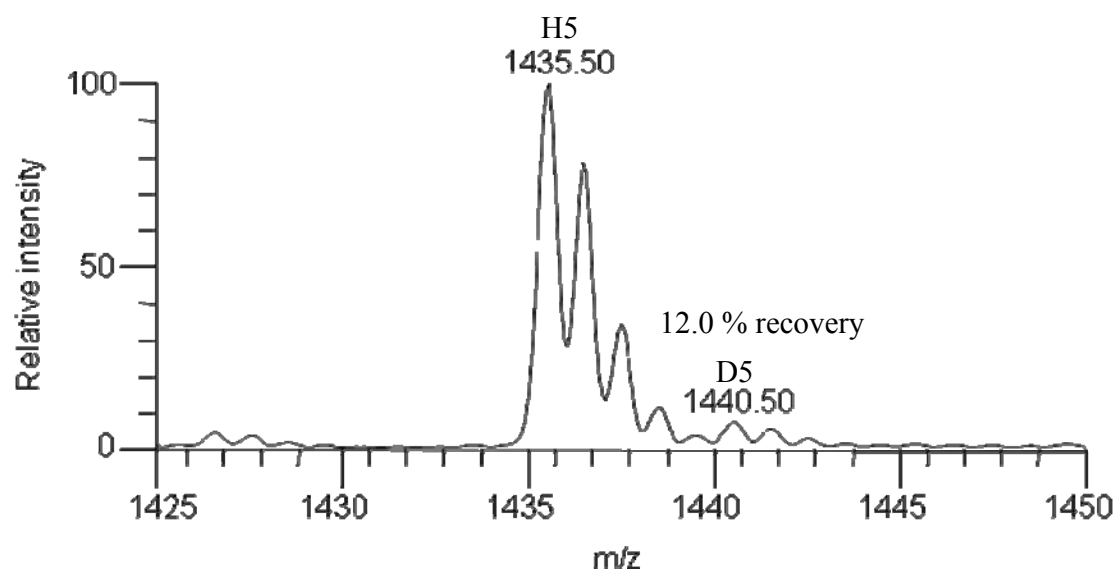
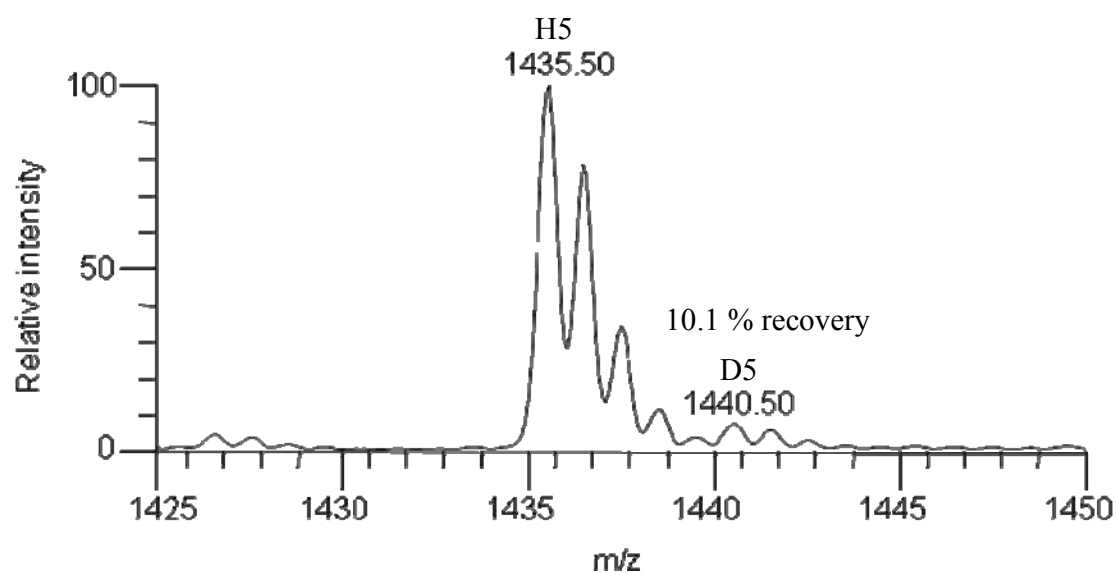
Figure #4-V.



V. Mass spectra of D5 enrichment using Glygen TiO<sub>2</sub> NuTips

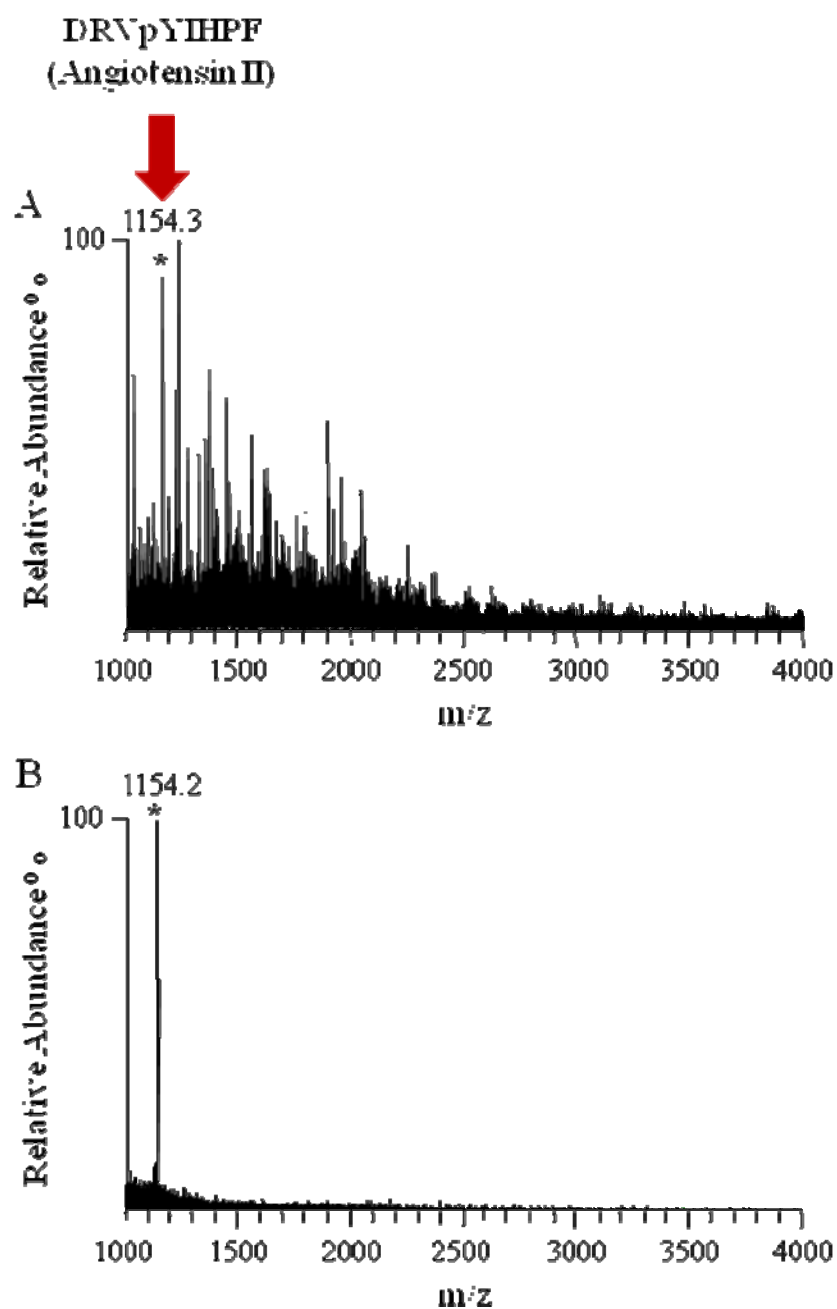
Figure #4-W.





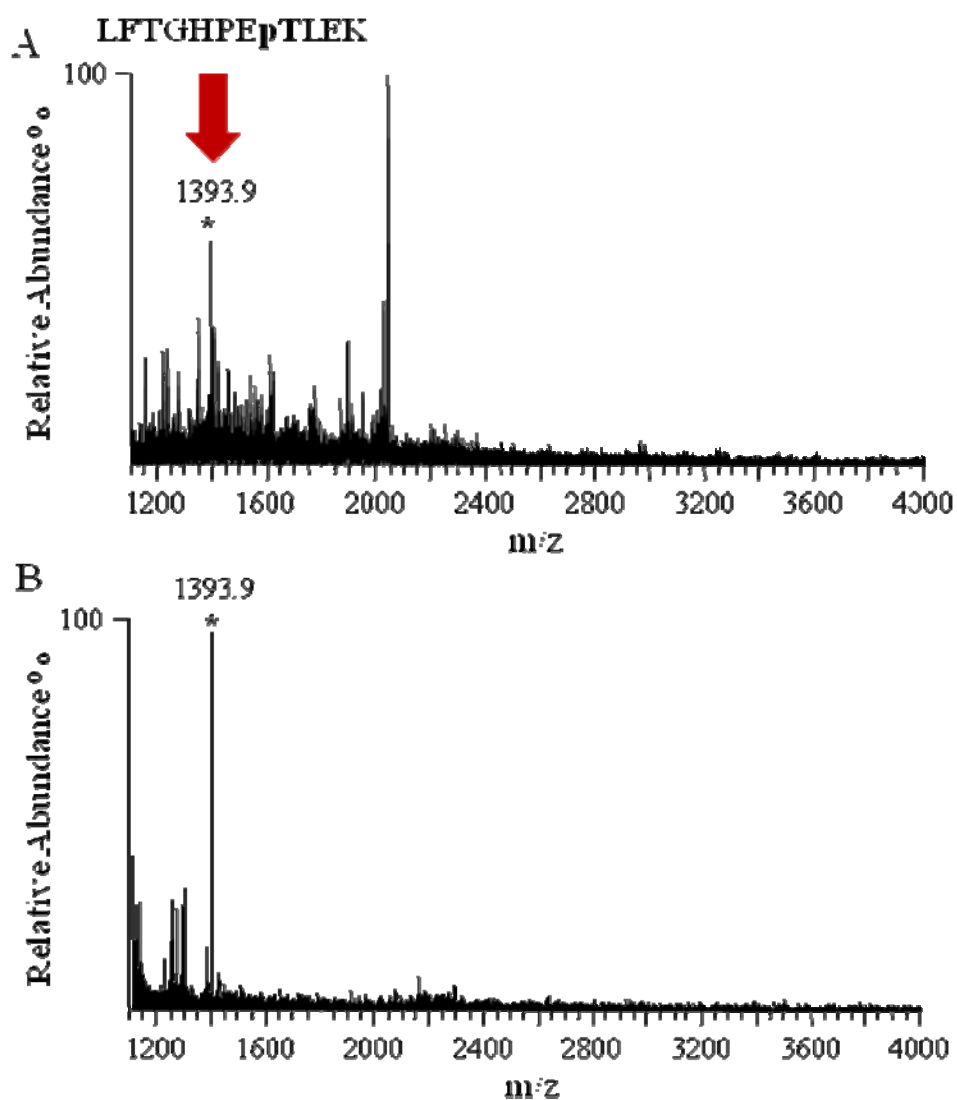
W. Mass spectra of D5 enrichment using Glycogen ZrO<sub>2</sub> NuTips

**Supplementary Figure #5: Angiotensin II (phosphotyrosine) enrichment using diazo ketone resin**



**Supplemental Figure 5:** A) Spectra of angiotensin II (1 picomole) spiked into a three non-phosphorylated protein digest (0.1  $\mu\text{g}$  of each). B) Spectra of an aliquot (25 picomole) of the enrichment of angiotensin II (DRVpYIHPF). 100 picomole of Ang II was spiked into a digest containing 10  $\mu\text{g}$  each of three unphosphorylated proteins and exposed to the diazo ketone **16** solid support for enrichment. This indicates the success of the solid-phase technique for phosphotyrosine peptides.

**Supplementary Figure #6: Synthetic phosphothreonine peptide enrichment using diazo ketone resin**



**Supplemental Figure 6:** A) Spectra of LFTGHPEpTLEK. (1 picomole) spiked into a three non-phosphorylated protein digest (0.1  $\mu\text{g}$  of each). B) Spectra of an aliquot (15 picomole) of the enrichment of synthetic peptide, LFTGHPEpTLEK. 75 picomole of the synthetic peptide was spiked into a digest containing 7.5  $\mu\text{g}$  each of three unphosphorylated proteins and exposed to the diazo ketone **16** solid support for enrichment. This indicates the success of the solid-phase technique for phosphothreonine peptides.