Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Preparation and evaluation of in situ photocleavable mass tags with facile mass variation for matrix-free laser desorption ionization mass spectrometry

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1. Synthesis of photocleavable mass tags (PMTs)

2-(4-Bromophenyl)-4,4-dimethyl-4,5-dihydrooxazole (1). To a solution of 4-bromobenzoyl chloride (10 g, 45.6 mmol) in CH_2Cl_2 (40 mL), 2-amino-2-methylpropanol (11 mL, 114.0 mmol) in CH_2Cl_2 (10 mL) was added dropwise with stirring under N_2 at 0 °C, and the mixture was stirred at room temperature overnight. The white precipitate which formed was filtered and dissolved in H_2O and extracted with CH_2Cl_2 three times. The combined organic layer and the filtrate was washed with water and brine, and dried with anhydrous $MgSO_4$. The resulting organic layer was concentrated and the residue was purified by silica gel chromatography with hexane : EtOAc = 4 : 1 to give 12.3 g (45.3 mmol, 99 %) of 4-bromo-*N*-(1-hydroxy-2-methylpropan-2-yl)benzamide as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, 2H), 7.25 (d, 2H), 5.25 (s, 1H), 3.44 (s, 2H), 1.23 (s, 6H).

To a solution of 4-bromo-*N*-(1-hydroxy-2-methylpropan-2-yl)benzamide (9.4 g, 34 mmol) in CH_2Cl_2 (10 mL), thionyl chloride (8.6 mL, 119 mmol) was added gently in a single portion at 0 °C, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into Et_2O with vigorous stirring, and the white precipitate formed was filtered and dissolved in water. The solution was neutralized with cold 20 % NaOH and extracted with Et_2O three times and the combined Et_2O extracts were dried with $MgSO_4$. The solution was evaporated and residue was purified by silica gel chromatography with hexane : EtOAc = 4 : 1 to give 7.98 g (31.6 mmol, 92 %) of **1** as a white crystalline solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, 2H), 7.10 (d, 2H), 3.63 (s, 1H), 0.94 (s, 6H).

(4-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)phenyl)bis(4-methoxyphenyl)methanol (2). To magnesium turnings (0.31 g) under N_2 atmosphere, bromophenyl oxazoline **1** (2.7 g, 10.6 mmol) in THF (50 mL) was added with trace iodine. The mixture was refluxed for 3 h, cooled to room temperature, and DMBP in THF (20 mL) was added dropwise. The mixture was gently refluxed for 6 h, cooled to room temperature, and water (5 mL) was added with stirring. The organic phase was carefully decanted, and the remaining residue was washed several times with small portion of THF. To the combined THF aliquots, were added 5% $KHSO_4$ (50 mL) and EtOAc (50 mL). The mixture was extracted with EtOAc three times and the combined organic layer was washed with brine, dried with $MgSO_4$ and concentrated, and the residue was purified by silica gel chromatography with hexane : EtOAc = 4 : 1 to give 1.8 g (4.4 mmol, 41 %) of **2** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (d, 2H), 7.37 (d, 2H), 7.18 (d, 4H), 6.84 (d, 4H) 4.12 (s, 2H), 3.79 (s, 6H) 3.20 (s, 1H) 1.37 (s, 6H).

4-(Hydroxy-bis(4-methoxyphenyl)methyl)benzoic acid (3). The solution of **2** (1.8 g, 4.4 mmol) in 80% acetic acid (20 mL) was stirred at 60 °C for 14 h and evaporated. The residue was dissolved in 20% NaOH (in EtOH/water, v/v =1/1) and refluxed at 120 °C for 2 h, and EtOH was removed under reduced pressure. The residue was acidified to pH 4 with 6 M HCl. The solution was extracted with EtOAc three times, and the organic layer was concentrated to give 1.4 g (4 mmol, 90 %) of **3** as an orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, 2H), 7.49 (d, 2H), 7.22 (d, 4H), 6.84 (d, 4H) 3.88 (s, 6H).

2,5-Dioxopyrrolidin-1-yl 4-(hydroxybis(4-methoxyphenyl)methyl)benzoate (4). To a solution of **3** (720 mg, 2 mmol) in CH_2Cl_2 (30 mL), *N*-hydroxysuccinimide (273 mg, 2.4 mmol) was added. The mixture was cooled to 0 °C and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (368 mg, 2.4 mmol) was added, and stirred for 3 h at room temperature. The reaction mixture was washed with brine, dried with $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with hexane : EtOAc = 4 : 1 to give 532 mg (1.1 mmol, 57 %) of **4** as a yellow oil. ^{1}H NMR (500 MHz,

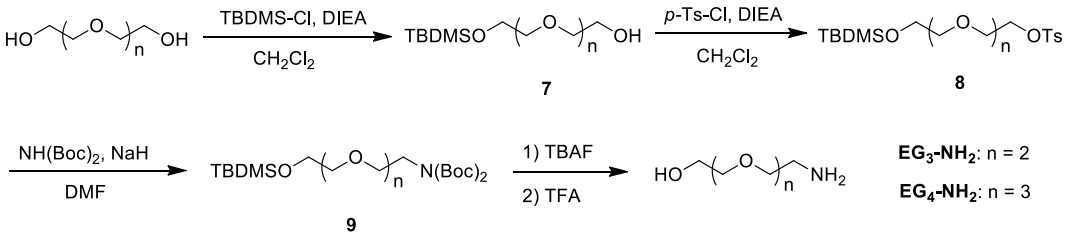
$CDCl_3$) δ 8.06 (d, 2H), 7.50 (d, 2H), 7.15 (d, 4H), 6.84 (d, 4H) 3.78 (s, 6H), 3.37 (s, 1H), 2.85 (s, 4H).

11-(((4-(((2,5-Dioxopyrrolidin-1-yl)oxy)carbonyl)phenyl)bis(4-methoxyphenyl)methyl) thio)undecanoic acid (6). The solution of **4** (532 mg, 1.1 mmol) in acetyl chloride (10 mL) was refluxed and stirred for 3 h. The mixture was cooled to 0 °C and equal volume of ether was added. After 16 h stirring, the solvent was removed under reduced pressure to give 600 mg of trityl chloride **5** as a pink crystalline solid.

To a solution of **5** (724 mg, 1.5 mmol) in CH_2Cl_2 (10 mL), 11-mercaptoundecanoic acid (327 mg, 1.5 mmol) was added slowly. The reaction mixture was stirred overnight at room temperature and concentrated to give 595 mg (0.9 mmol, 60 %) of **6** as white oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, 2H), 7.61 (d, 2H), 7.32 (d, 4H), 6.84 (d, 4H) 3.79 (s, 6H), 2.86 (s, 4H), 2.35 (m, 20H).

PMT_{Gly}. PMTs in the study were prepared by the reaction between NHS-activated ester **6** and amine-containing molecules for mass variation. As a typical example, PMT_{Gly} was prepared as follows. To a solution of **6** (250 mg, 0.37 mmol) in CH_2Cl_2 (5 mL), glycine methyl ester (468 mg, 0.7 mmol) and diisopropylethyl amine (550 μL, 4.2 mmol) were added, and the reaction mixture was stirred overnight at room temperature under N_2. The reaction mixture was washed with water, saturated NH_4Cl and brine. The organic layer was dried with $MgSO_4$ and concentrated, and the residue was purified by silica gel chromatography with EtOAc : MeOH = 4 : 1 to give 330 mg (0.5 mmol, 74 %) of **PMT_{Gly}**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, 2H), 7.40 (d, 2H), 7.22 (d, 4H), 6.74 (d, 4H) 3.72 (s, 6H), 3.59 (m, 16H), 2.27 (m, 20H).

2. Synthesis of amine-terminated oligo(ethylene glycol)



Scheme S1. Synthesis of amine-terminated oligo(ethylene glycol)

2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (7). Amine-terminated oligo(ethylene glycol) molecules, **EG_3-NH_2** and **EG_4-NH_2** were prepared by the identical synthetic route except for using tri(ethylene glycol) and tetra(ethylene glycol) as a starting material, respectively. As a typical example, **EG_4-NH_2** was prepared as follows. A mixture of tetra(ethylene glycol) (10 g, 51.5 mmol), TBDMS-Cl (5.4 g, 36.0 mmol), and DIEA (13.5 mL, 77.2 mmol) in CH_2Cl_2 (100 mL) was stirred overnight at room temperature under N_2. The reaction mixture was washed with water and NH_4Cl, and dried with $MgSO_4$. The organic layer was concentrated and the residue was purified by silica gel chromatography with hexane : EtOAc = 2 : 1 to give 3 g (9.72 mmol, 38 %) of **7** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73 (t, *J* = 4.5 Hz, 2H), 3.68 (d, 1H), 3.63 (d, 8H), 3.57 (t, *J* = 4.5 Hz, 2H), 3.53 (t, *J* = 5.5 Hz, 2H), 0.86 (s, 9H), 0.03 (s, 6H).

2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 4-methylbenzenesulfonate (8). To a solution of **7** (5.5 g, 17.9 mmol) in CH_2Cl_2 (25 mL), TsCl (6.8 g, 35.7 mmol) and DIEA (9.7 mL, 55.4 mmol) were added, and the reaction mixture was stirred overnight at room temperature under N_2. The reaction mixture was washed with water, brine and NH_4Cl, and dried with $MgSO_4$. The organic layer was concentrated and the residue was purified by silica gel chromatography with hexane : EtOAc = 6 : 1 to give 5.2 g (11.1 mmol, 63 %) of **8** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 2H), 7.28 (d, 2H), 4.09 (t, *J* = 5 Hz, 2H), 3.70 (t, *J* = 5.5 Hz, 2H), 3.61 (m, 4H), 3.55 (m, 8H), 2.36 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H).

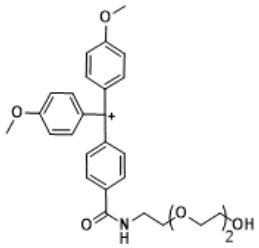
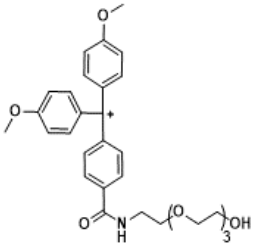
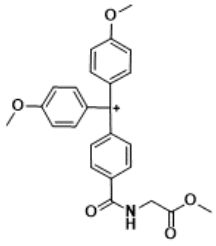
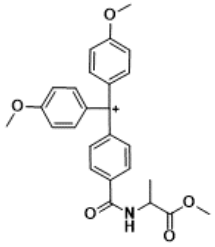
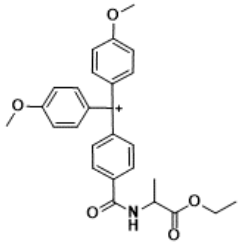
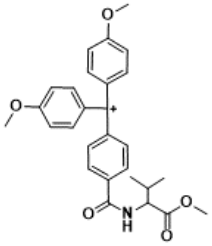
13,13,14,14-Tetramethyl-3,6,9,12-tetraoxa-13-sila-1-pentadecan-NH(Boc)$_2$ (9). A mixture of NaH (259 mg, 6.5 mmol) and $NH(Boc)_2$ (1.4 g, 6.5 mmol) in DMF (7 mL) was stirred for 2 h at room temperature. To this mixture, **8** (3 g, 6.5 mmol) was added and the reaction mixture was further stirred overnight under N_2. The reaction was quenched with water, and extracted with EtOAc three times. The combined organic layer was washed with NH_4Cl and brine, and dried with $MgSO_4$. The organic layer was concentrated and the residue was purified by silica gel chromatography with hexane : EtOAc = 2 : 1 to give 1.5 g (2.9 mmol, 45 %) of **9** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.81

(t, *J* = 7 Hz, 2H), 3.77 (t, *J* = 5.5 Hz, 2H), 3.66 (t, *J* = 8.5 Hz, 2H), 3.62 (t, *J* = 6 Hz, 2H), 3.57 (m, 8H), 1.51 (s, 18H), 0.90 (s, 9H), 0.07 (s, 6H).

EG$_4$-NH$_2$. The protected molecule **9** (1.5g, 2.9 mmol) was dissolved in THF (5 mL), followed by addition of TBAF (1.8 mL). The reaction mixture was stirred for 2 h at room temperature, concentrated, and purified by silica gel chromatography with hexane : EtOAc = 2 : 1 to give 672 mg (1.7 mmol, 59 %) of the silyl group-deprotected alcohol as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.83 (t, *J* = 6.5 Hz, 2H), 3.75 (s, 1H), 3.70 (t, *J* = 1.5 Hz, 2H), 3.69 (t, *J* = 4.5 Hz, 2H), 3.67 (t, *J* = 1.5 Hz, 2H), 3.65 (m, 8H), 1.53 (s, 18H). To a solution of the resulting silyl group-deprotected alcohol (672 mg, 1.7 mmol) in CH_2Cl_2 (5 mL), TFA (5 mL) was added, followed by further stirring for 2 h at room temperature. The solvent was concentrated to give 881 mg of **EG$_4$-NH$_2$** as a pale red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.54 (m, 16H).

3. Additional data

Table S1. Structures and molecular weights of ions from photocleaved trityl group in this study.

Ion	Structure	$[M]^+$	$[M+Na]^+$	$[M+K]^+$	Ion	Structure	M.W	$[M+Na]^+$	$[M+K]^+$
$[EG3]^+$		478.22	501.20	517.18	$[EG4]^+$		522.25	545.23	561.21
$[Gly]^+$		418.16	441.14	457.12	$[Ala1]^+$		432.18	455.16	471.14
$[Ala2]^+$		446.20	469.18	485.16	$[Val]^+$		460.21	483.19	499.17

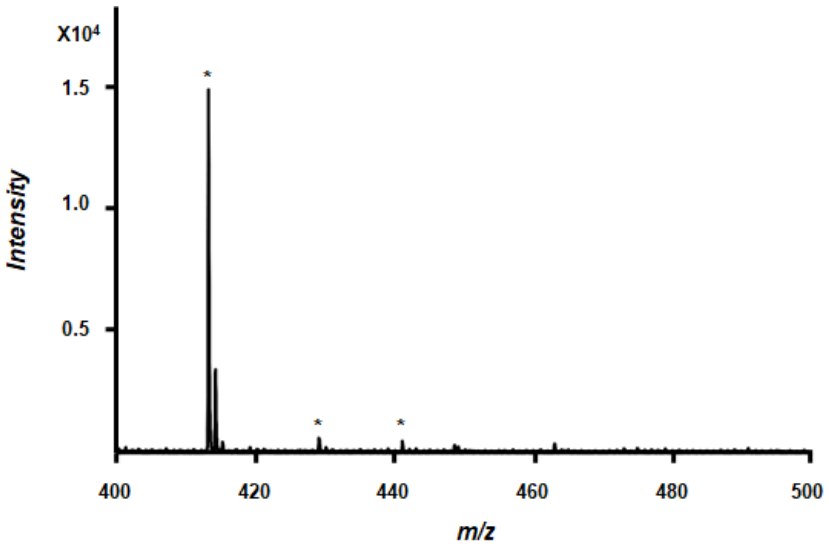


Figure S1. Mass spectrum of the magnetic particles used in this study.

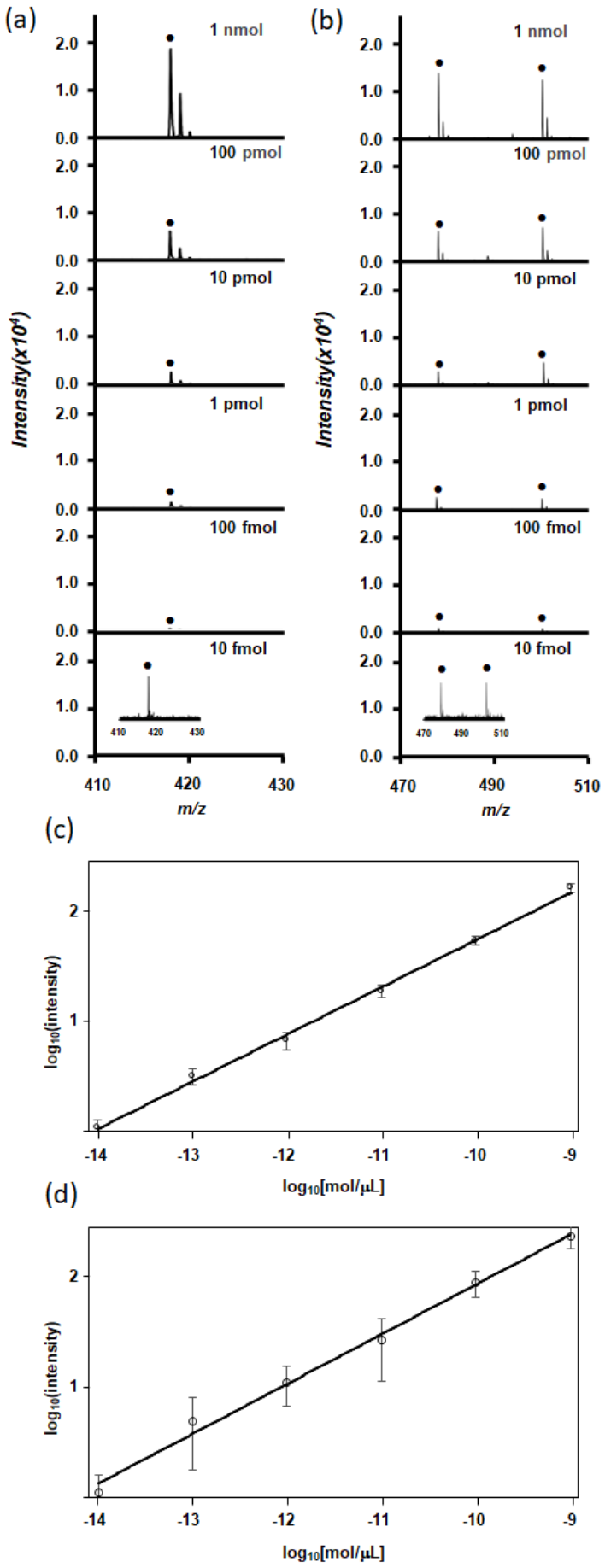
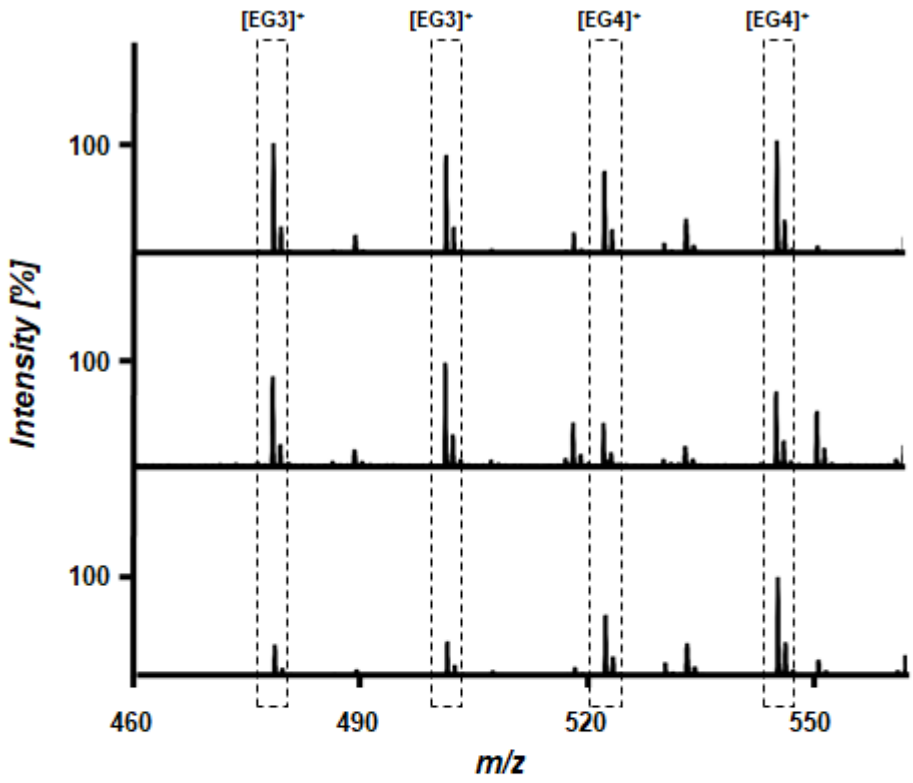


Figure S2. Sensitivity and LOD measurement of PMT_{Gly} and PMT_{EG3}. (a, b) Representative LDI MS spectra and (c, d) calibration curves ranging from 10 fmol to 1 nmol of PMT_{Gly} and PMT_{EG3}.

Table S2. Summary of relative observed mass intensity of PMT@MP mixtures at different ratios in Figure 4.

Observed / Added	Added / PMT			
	PMT_{Gly}	PMT_{Ala1}	PMT_{Ala2}	PMT_{Val}
1:1:0:0	1.00	1.15		
1:0:1:0	1.00		0.94	
0:1:1:0		1.00	1.09	
0:0:1:1			1.00	1.04
2:1:1:1	2.00	1.14	1.01	1.25
1:2:1:1	0.80	2.00	0.81	1.19
1:1:2:1	0.90	0.93	2.00	1.08
1:1:1:2	1.02	0.62	0.83	2.00



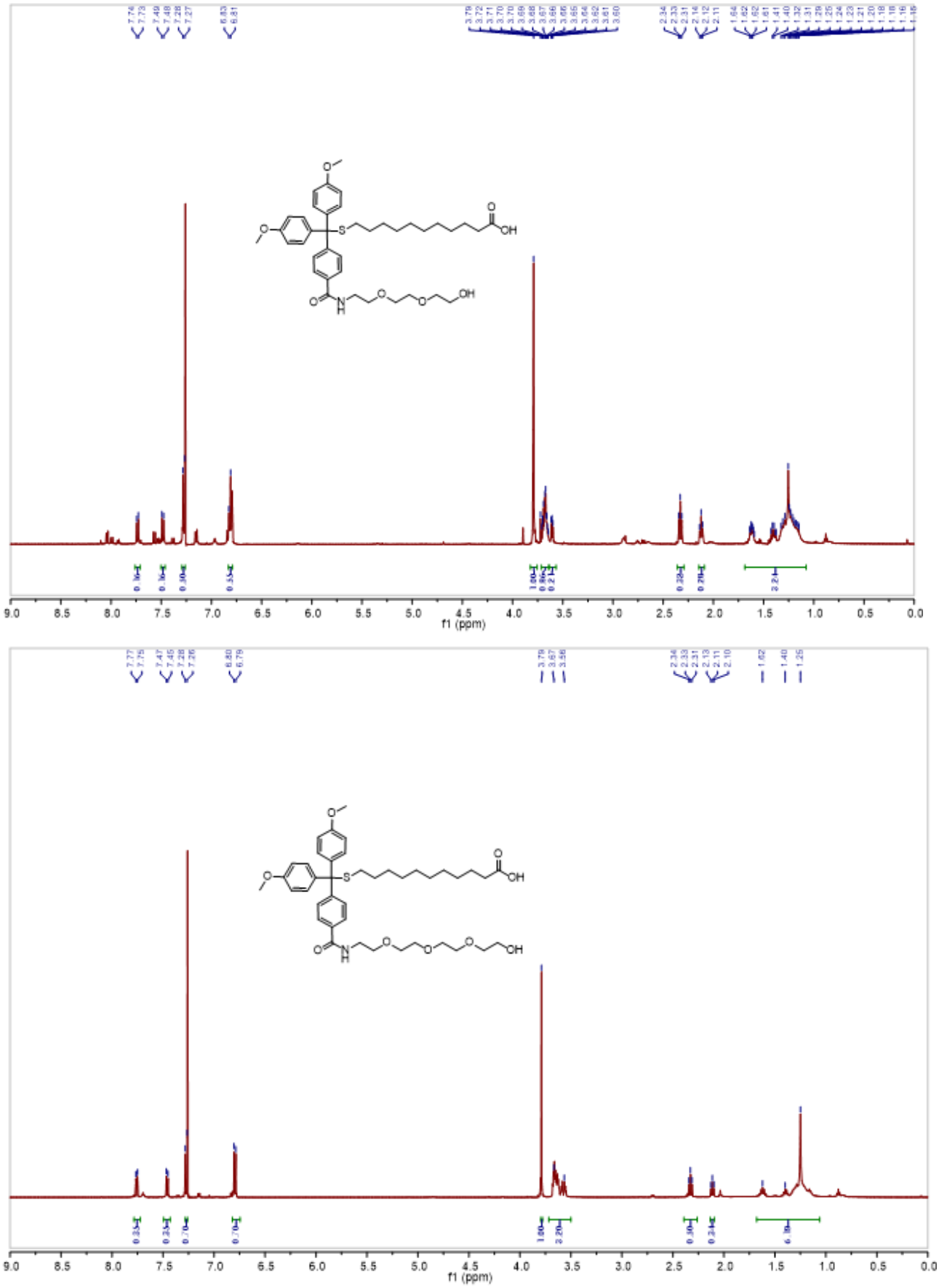
Observed / Added	Added / PMT	
	PMT_{EG3}	PMT_{EG4}
1:1	1.00	0.96
2:1	1.00	0.6
1:2	1.00	2.42

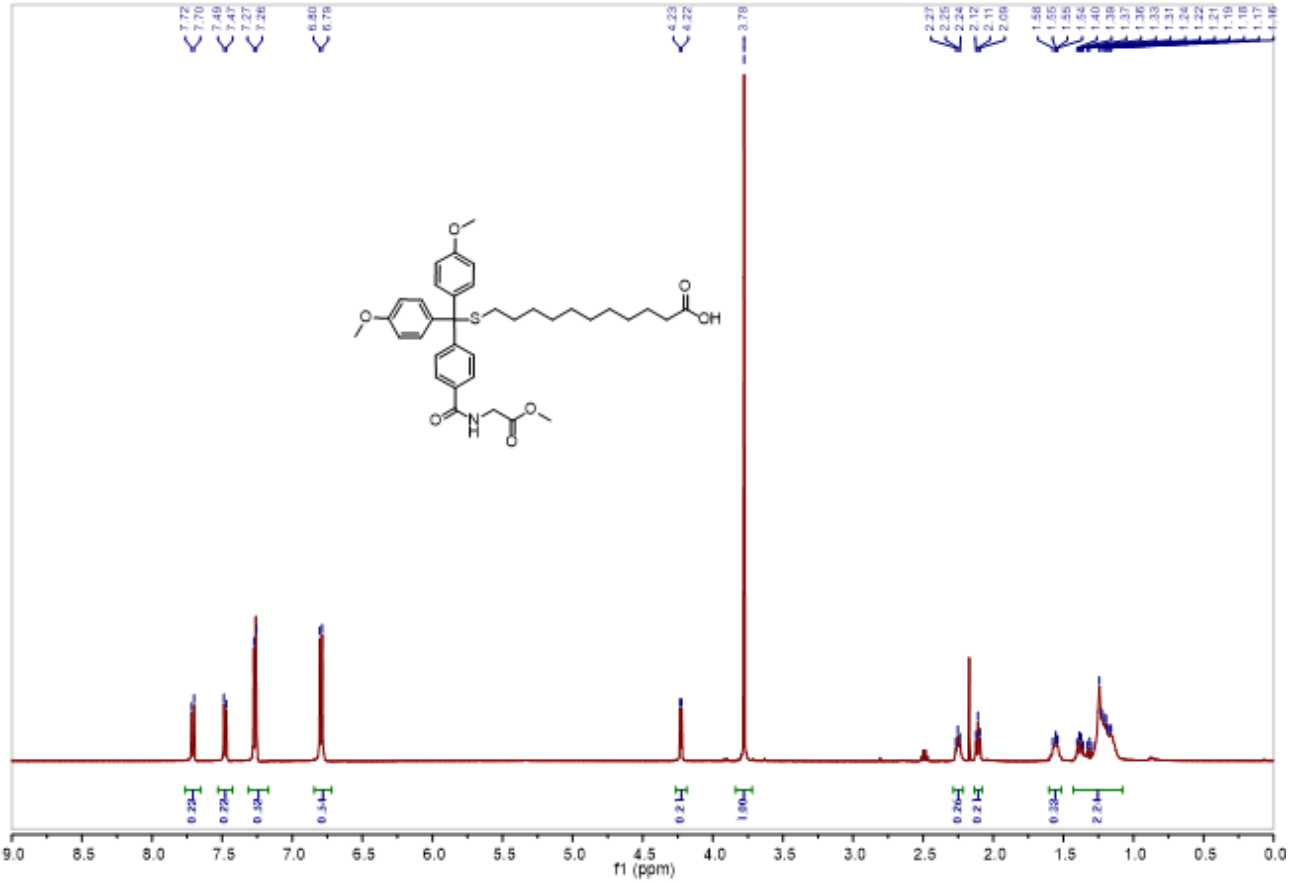
Figure S3. Representative mass spectra of mixtures of two PMT@MPs at different ratios, PMT_{EG3} : PMT_{EG4} (1:1, 2:1, 1:2) and summary of relative observed mass intensity.

Table S3. Sequence of oligonucleotides used in this study.

Oligonucleotides	Sequence (5'-3')
Loop	5'-NH_2 -C_6 -TTT TTT TTT TTT AAA GGA CCA GGC GCA ACT AAA TTC ATG GTC CCC TCT TCC CAT GAA TTT AGT TG-3'
Target (HIV)	5'-CCA TGA ATT TAG TTG CGC CTG GTC CTT TAA-3'
Hairpin1	5'-CCA TGA ATT TAG TTG AAA CCA ACA ACT AAA TTC ATG GGA AGA GGG GA-C_6-NH_2-3'
Hairpin2	5'-NH_2 -C_6 -TTG GTT TCA ACT AAA TTC ATG GTC CCC TCT TCC CAT GAA TTT AGT TG-3'

Figure S4. ^{1}H NMR data for the six PMTs used in this study.

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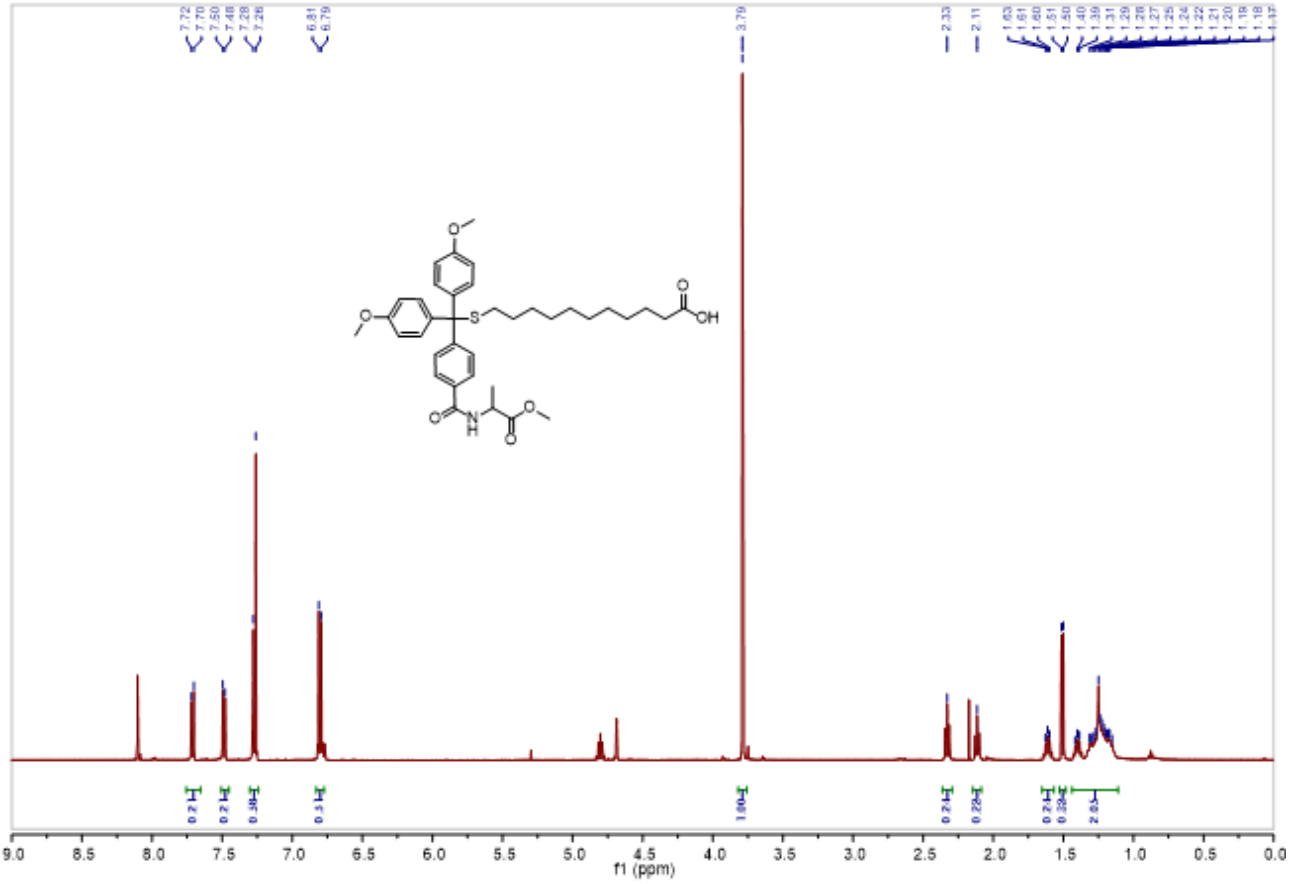
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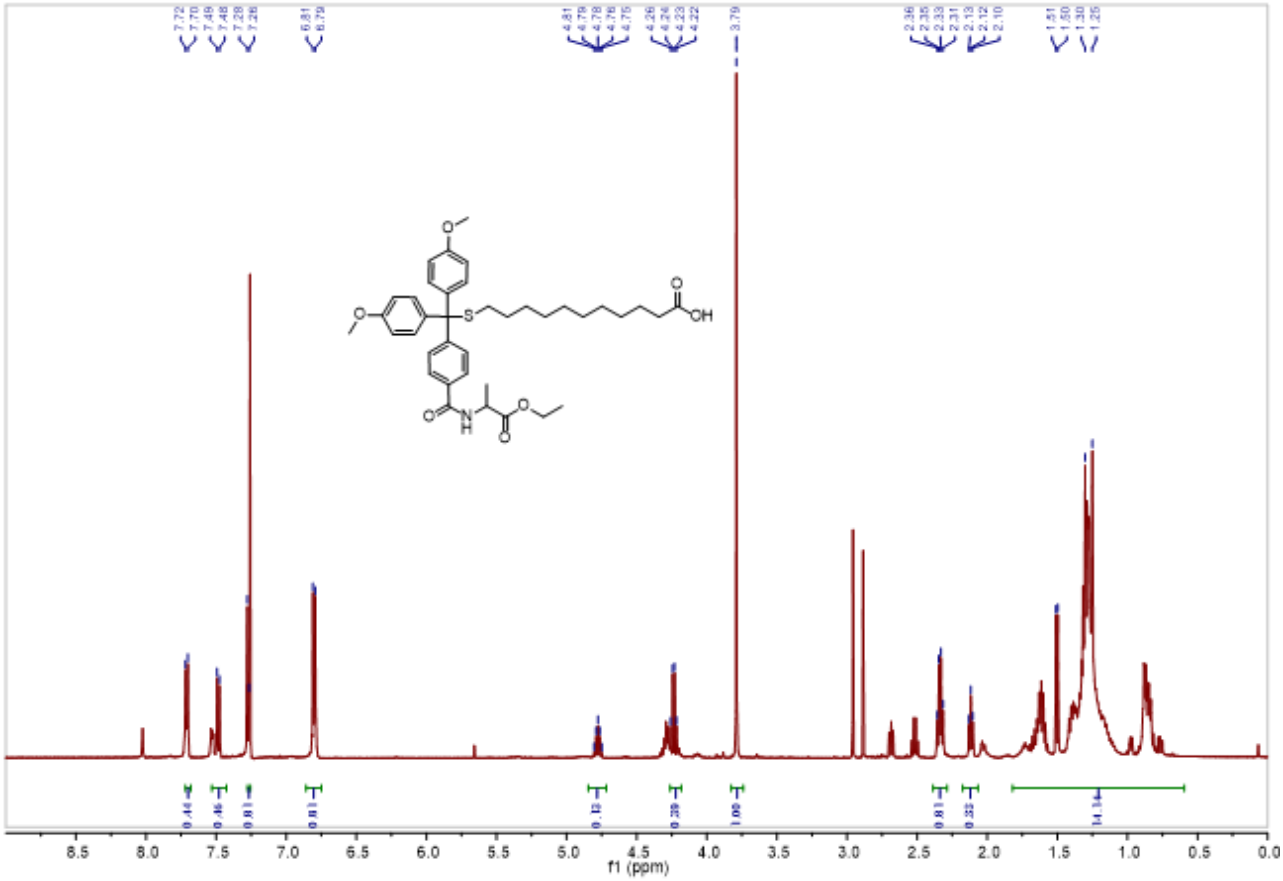
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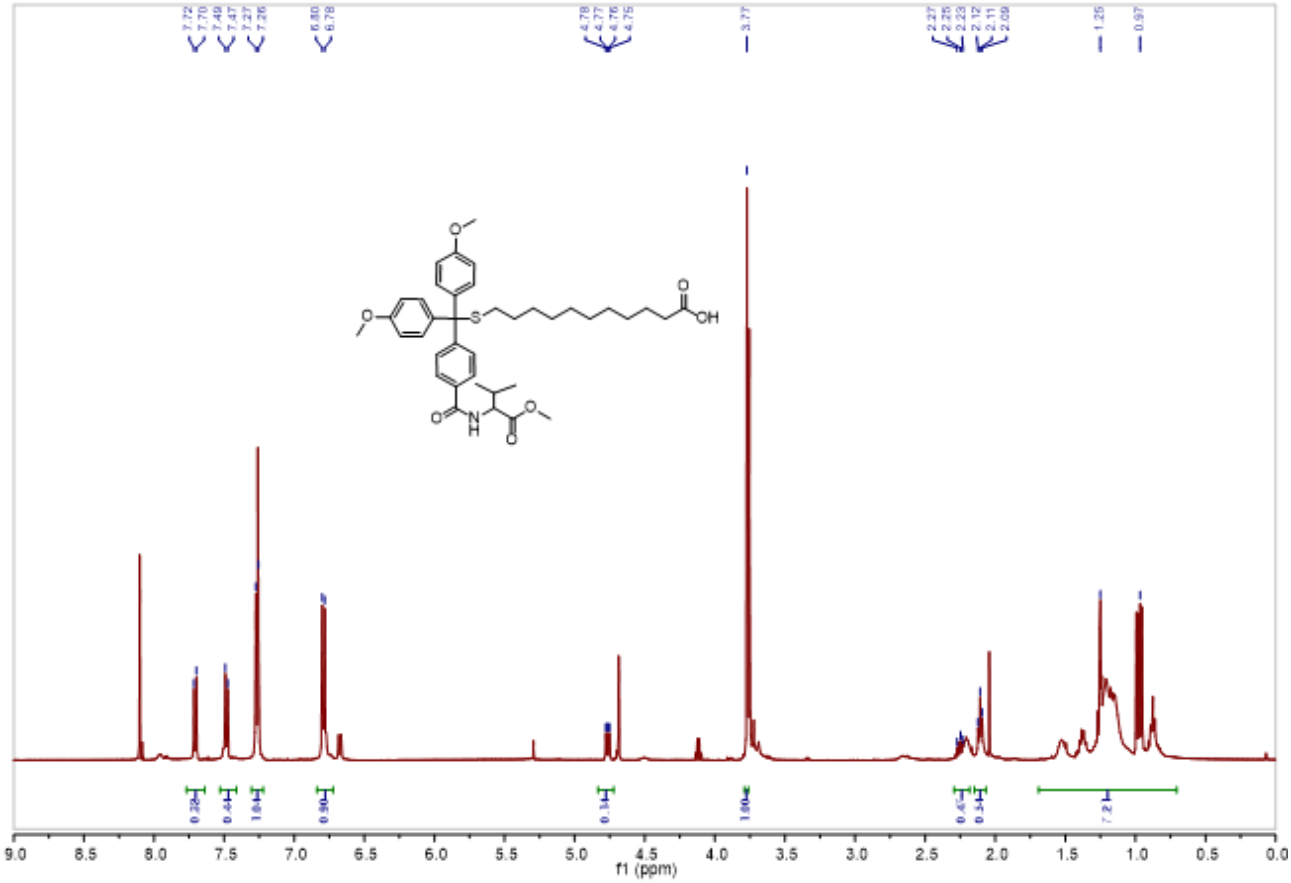
f1 (ppm)