A new reagent for stable thiol specific conjugation

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Competitive conjugation experiments.

N-Acetyl-L-lysine and N-acetyl-L-cysteine were allowed to incubate with the PEG *mono*-sulfone $\underline{3}$ to determine the potential for competitive amine conjugation. A discrete molecular weight PEG amine precursor was used to give the PEG-*mono*-sulfone $\underline{3}$ with a molecular weight of 2,460 g/mol. Both LC/MS and RP-HPLC were used to follow the conjugation reaction and to confirm the formation of the PEG conjugates.

Figure S1



Figure S1. Mass spectrum of discrete PEG mono-sulfone 3.



T = 20 h T = 3.5 h T = 2.5 h PEG-cysteine conjugate T = 1.5 h 4-methylbenzene sulfinic T = 0.5 h acid leaving group 5 -PEG mono-sulfone 3 T = 0.0 h 7.6 8.5 9.0 9.5 10.0 10.5 11.0 11.5 12.0 12.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 6.0 0.5 7.0 8.0 0.5

B

Figure S2. RP-HPLC at different time points for a conjugation reaction of a 1:1:1 molar mixture of discrete PEG mono-sulfone $\underline{3}$, N-acetyl-lysine and N-acetyl-cysteine detected at (A) 214 nm and (B) 280 nm.



A



Figure S3. (A) LC/MS time course for 1:1:1 molar mixture of discrete PEG *mono*-sulfone <u>3</u>, N-acetyl-lysine and N-acetyl-cysteine (diode array 200-400 nm) and (**B**) the mass spectrum for the PEG-cysteine conjugate.



Figure S4. LC/MS time course for 1:30:1 molar mixture of discrete PEG *mono*-sulfone <u>3</u>, N-acetyl-lysine and N-acetyl-cysteine (diode array 200-400nm).





Figure S5. Comparative RP-HPLC (214 nm) for (A) PEG mono-sulfone $\underline{3}$; (B) the PEG-cysteine conjugate after 20 h reaction using a 1:1:1 molar mixture of discrete PEG *mono*-sulfone $\underline{3}$, N-acetyl-lysine and N-acetyl-cysteine; (C) the partial formation of the PEG-lysine conjugate after 20 h from the reaction of 30:1 N-acetyl-lysine and discrete PEG *mono*-sulfone $\underline{3}$.



Figure S6. (A) The partial formation of the PEG-lysine conjugate after 20 h from the reaction of 30:1 N-acetyl-lysine and discrete PEG *mono*-sulfone <u>3</u>. (B) The mass spectrum of the PEG-lysine conjugate.