SUPPORTING INFORMATION

A photolabile linker for the mild and selective cleavage of enriched bio-molecules from solid support

Ronald Orth and Stephan A. Sieber*

Center for integrated Protein Science Munich CIPS^{M}, Department of Chemistry and Biochemistry, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, 81377 Munich, Germany

Contents:

1) Materials

All chemicals exhibited reagent grade or better and were used without further purification. For all reactions, only commercially available solvents of highest purity, dried over molecular sieve and stored under Argon atmosphere were used. Solvents for chromatography and workup purposes were generally of reagent grade and purified before use by distillation. In all reactions, temperatures were measured externally. All experiments were carried out under argon atmosphere in flame-dried glassware. Column chromatography was performed on silica gel (0.035 – 0.070 mm, mesh 60 Å). ^{1}H NMR spectra were recorded on a 200 MHz NMR-System, 600 MHz NMR-System or a 300 MHz NMR-System and referenced to the residual proton of the deuterated solvent. ESI spectra were recorded with a LTQ FT instrument. HPLC analysis was accomplished with a C18 column 5µm (4.6x100 mm) and a PDA detector. Mobile phase (HPLC grade): A: 0.1% (*v*/*v*) TFA in H_2O; B: 0.1% TFA in Acetonitrile; Gradient: 0% B → 95% B in 25 min. Flow: 0.5 ml/min.

2) Synthesis and Methods

Solid Phase Peptide Synthesis (SPPS):

Synthesis of Fmoc-5-Ava-Lys(Mtt)-Lys(*p*-azido benzoyl)-(Rink amide)-MBHA (4)

Fmoc-Rink amide MBHA resin (50mg, 36 µmol, B_H= 0.72 mmol/g) was washed with DMF (2x) and DCM (2x) prior to Fmoc-deprotection with 20% (v/v) Piperidin/DMF for 20 min at rt. Deprotected, resin bound amine was washed with DMF (3x) and DCM (2x). Coupling of Fmoc-Lys(Mtt)-OH was achieved by using TBTU/HOBt in accordance with standard SPPS protocols. The desired, resin-bound peptide was built up by standard SPPS coupling and washing steps using the following *N*-protected building blocks: *p*-azido benzoic acid, Fmoc-Lys(Mtt)-OH and Fmoc-5-amino-valeric acid. Each coupling step was monitored by standard Kaiser assay. The exact assembly of the resin bound peptide was verified via ESI-MS. For that reason a small amount of Fmoc-protected peptide was cleaved off the resin by TFA treatment. MS (ESI) *m/z* calculated for $C_{39}H_{49}N_9O_6$ $(M+H)^+$: 740.388, found: 740.388 $(M+H)^+$.

Photolysis of Phenacyl linker (7) in solution:

Phenacyl linker (**7**) (5 nmol) was dissolved in 25 µl DMSO/ 2mM DTT in PBS (6/4). The solution was irradiated for 1 and 5 min respectively using an ordinary UV handlamp (15W, 254 nm) at a sample-lamp distance of 1 and 20 cm, respectively. Non-irradiated, dissolved Phenacyl linker (5 nmol) was used as a control. The supernatant of both solutions was analyzed via HPLC (column: XBridge™ BEH130 C18 5 µm (4.6x100 mm), linear gradient: 0 to 95% CH3CN/H_2O with 0.1% TFA in 25 min. The cleavage product was eluted at 56.31% (v/v) CH_3CN/H_2O (t_R= 14.82 min), whereas non-irradiated Phenacyl linker was eluted at 62.98% (v/v) CH_3CN/H_2O (16.573 min). To calculate the yield of cleavage product, we compared the integrals of both peaks representing non-irradiated and irradiated Phenacyl linker according to equation 1:

$$Yield\ of\ cleavage\ product\ (\%) = \frac{100 \bullet \int (irradiated\ Phenacyl\ linker)}{\int (non-irradiated\ Phenacyl\ linker)} \qquad \text{equation 1}$$

Irradiation for 5 min at 20 cm distance yielded 75 ± 11% of the cleavage product. Irradiation for 1 min at 1 cm distance yielded 89 ± 2% of the cleavage product. The experiment was carried out in triplicate. MS (ESI) *m/z* calculated for $C_{63}H_{74}N_{14}O_{10}$ $(M+formiate)^-$:1231.569, found: 1231.564 $(M+formiate)^-$.

Photolysis of Phenacyl linker (7) on avidin-beads.

The avidin-bead slurry (250 µl) was washed with PBS (3x) and suspended in PBS (700 µl) prior to addition of Phenacyl linker (**7**) (5 nmol) and Trifunctional linker (a mixture of (5)- and (6)-carboxytetramethylrhodamine isomers) (5 nmol), respectively, both dissolved in DMSO/PBS (6/4) (49 µl). The suspension was shaken for 1 h at room temperature. After washing with PBS (3x) the beads were suspended in DMSO/ 2mM DTT in PBS (6/4) (7.5 ml). Phenacyl linker containing beads were distributed in 96-well plates (30 µl per well) and irradiated for 1 min using an ordinary handlamp (15 W, 254 nm) at a sample-lamp distance of 1 cm. Trifunctional linker containing beads were incubated for 7 min at 96°C. Both supernatants were lyophilised. The remaining solids were dissolved in DMSO/H_2O (6/4) (200 µl) and analyzed via HPLC (column: XBridge™ BEH130 C18 5µm (4.6x100 mm), linear gradient: 0 to 95% CH_3CN/H_2O with 0.1% TFA in 25 min). The cleavage product was eluted at 56.31% (v/v) CH_3CN/H_2O (14.82 min), whereas Trifunctional linker was eluted at

48.90% (v/v) CH_3CN/H_2O (12.87 min) and 51.14% (v/v) CH_3CN/H_2O (13.47 min). To calculate the yield of cleavage product (see equation 1), we compared the integrals of peaks representing non-irradiated Trifunctional linker and irradiated Phenacyl linker according to equation 2:

$$Yield\ of\ cleavage\ product\ (\%) = \frac{100 \bullet \int (irradiated\ Phenacyl\ linker)}{\int (non-irradiated\ Trifunctional\ linker)} \qquad \text{equation 2}$$

Irradiation yielded 69 ± 4% relative to the Trifunctional linker cleavage product. The experiment was carried out in triplicate. MS (ESI) *m/z* calculated for $C_{63}H_{74}N_{14}O_{10}$ $(M+Cl)^-$:1221.541, found: 1221.544 $(M+Cl)^-$.

Synthesis of NHS-hexynoat.

NHS (95.2 mg, 0.83 mmol) and triethylamine (TEA, 115 µl, 0.83 mmol) were mixed in 1 ml DMF/TCM (1/9) prior to addition of hexynoyl chloride (91 µl, 0.91 mmol). The reaction mixture was stirred for 16 h at room temperature. The solution was diluted with DCM (10 ml) and extracted with 5% (m/v) NaHCO3 (1x) and H_2O (2x), dried with $MgSO_4$ and concentrated using a rotary evaporator. The NHS-hexynoat (91.4 mg, 53%) was obtained by silica gel chromatography eluted with EtOAc/DCM (3/7). R_f= 0.75; MS (ESI) *m/z* calculated for $C_{10}H_{11}NO_4$ $(M+NH_4)^+$: 227.103, found: 227.102 $(M+NH_4)^+$; ^1H NMR (DMSO, 400 MHz) δ 2.83 (s, 4H), 2.77 (t, J = 7.2 Hz, 2H), 2.34 (dt, J = 2.4 Hz, 2H), 2.00 (t, J = 2.6, 1H), 1.96 (m, 2H). ^{13}C NMR (DMSO, 100 MHz) δ 169.0 (C), 169.0 (C), 168.1 (C), 82.3 (C), 71.3 (CH), 29.6 (CH_2), 25.5 (CH_2), 25.5 (CH_2), 23.3 (CH_2), 17.7 (CH_2).

Purification of *N*-(Lysyl)hexynamide by Phenacyl linker (7)

NHS-hexynoat (32.5 nmol) and (L)-Lysine (130 nmol) were mixed in 4 µl DMSO/PBS (1/4) and incubated for 45 min at room temperature. After dilution with 12.50 µl (DMSO/tBu (3/1))/PBS (4/1) Trifunctional linker (650 nmol) or Phenacyl linker (650 nmol) were added. Further ligand (143 nmol) and CuBr (143 nmol) freshly presolved in 4 µl DMSO/tBu (3/1) were added. The reaction mixture was incubated for 35 min at room temperature. Washed avidin-bead slurry (100 µl) was suspended in 240 µl (DMSO/tBu (3/1))/PBS (6/4) and added

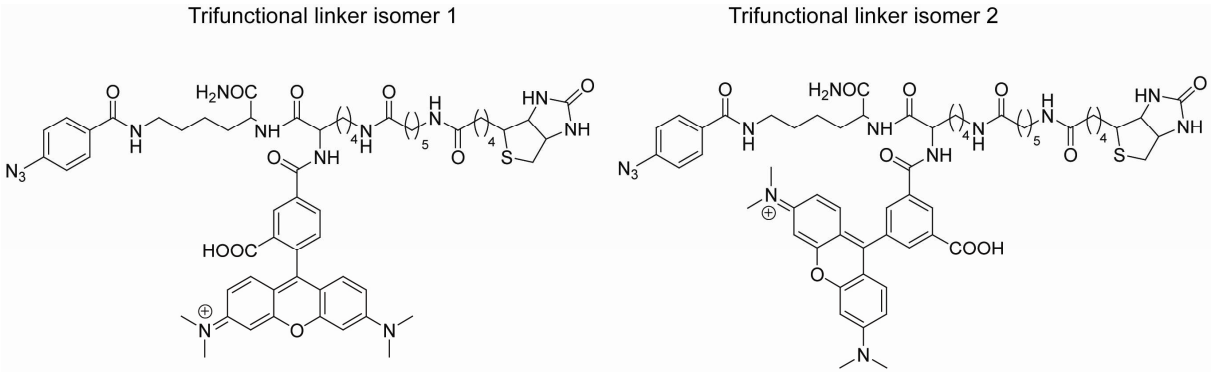
to the reaction mixture. The suspension was shaken for 45 min at room temperature. After washing with PBS (3x 1ml), photocleavage of the Phenacyl linker was performed: The beads were suspended in 200 µl DMSO/ 2mM DTT in PBS (6/4) and were irradiated for 7 min using a Xe-lamp (Thermo Oriel, Model 66921, P= 350 W-1000 W) and a yфc 2 Filter (τ >0.2 from 280 nm to 380 nm). The lamp-sample distance was 10 cm. Trifunctional linker containing beads were incubated for 7 min at 96°C. The supernatants were directly analyzed via HPLC (column: XBridge™ BEH130 C18 5µm (4.6x100 mm), linear gradient: 0 to 95% CH_3CN/H_2O with 0.1% TFA in 25 min). The photocleavage product was eluted at 51.91% (v/v) CH_3CN/H_2O (13.66 min) and 55.10% (v/v) CH_3CN/H_2O (14.50 min), whereas Trifunctional linker was eluted at 48.26% (v/v) CH_3CN/H_2O (12.70 min) and 51.11% (v/v) CH_3CN/H_2O (13.45 min).). Each peak fraction was collected and analyzed via MALDI-MS to verify its identity. The photocleavage product eluted at 51.91% (v/v) CH_3CN/H_2O (13.66 min) represents the hydroxy ketone product, whereas the photocleavage product at 55.10% (v/v) CH_3CN/H_2O (14.50 min) represents the methyl ketone product. To calculate the overall yield of cleavage product (see equation 2), we compared the integrals of peaks representing non-irradiated Trifunctional linker and irradiated Phenacyl linker. Irradiation yielded 68 ± 6% relative to the Trifunctional linker cleavage product. The experiment was carried out in triplicate. MS (MALDI) *m/z* calculated for the methyl ketone product $C_{75}H_{93}N_{16}O_{13}^{+}$ $(M)^{+}$: 1427.73, found 1426.99 $(M)^{+}$. MS (MALDI) *m/z* calculated for the hydroxy ketone product $C_{75}H_{94}N_{16}O_{14}$ $(M)^{+}$: 1443.72, found 1443.19 $(M)^{+}$.

Purification of *N*-Benzylhexynamide by Phenacyl linker (7)

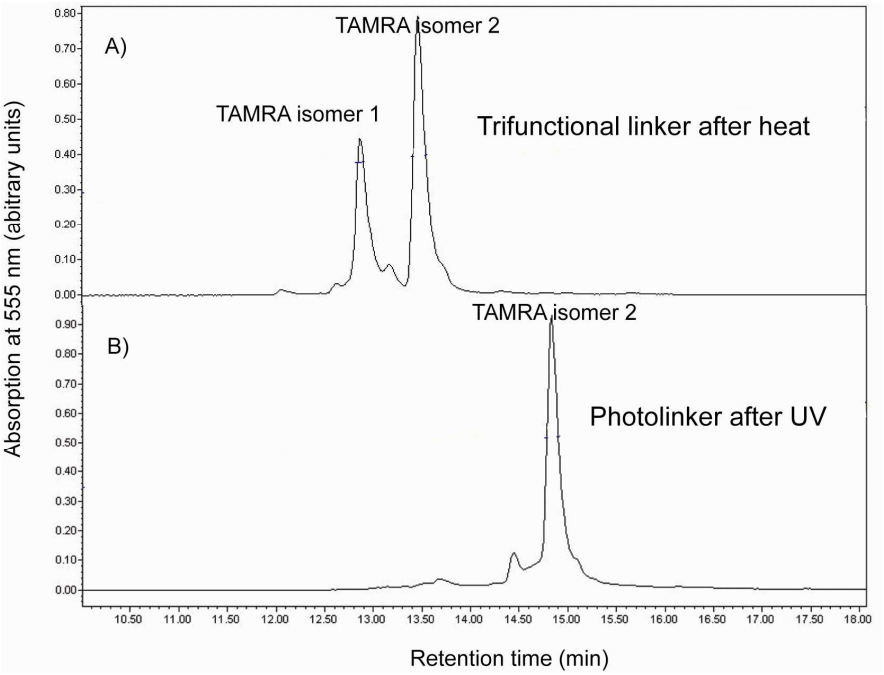
This protocol was performed as described above. Benzyl amine was used instead of L-Lysine. The photocleavage product was eluted at 60.15% (v/v) CH_3CN/H_2O (15.83 min) and 63.65% (v/v) CH_3CN/H_2O (16.75 min), whereas Trifunctional linker was eluted at 57.61% (v/v) CH_3CN/H_2O (15.16 min) and 59.89% (v/v) CH_3CN/H_2O (15.76 min).). Each peak fraction was collected and analyzed via MALDI-MS to verify its identity. The photocleavage product eluted at 60.15% (v/v) CH_3CN/H_2O (15.83 min) represents the hydroxy ketone product, whereas the photocleavage product at 63.65% (v/v) CH_3CN/H_2O (16.75 min) represents the methyl ketone product. To calculate the overall yield of cleavage product (see equation 2), we compared the integrals of peaks representing non-irradiated Trifunctional linker and irradiated Phenacyl linker. Irradiation yielded 70 ± 16% relative to the Trifunctional linker cleavage product. The experiment was carried out in triplicate. MS (MALDI) *m/z* calculated for the

methyl ketone product $C_{76}H_{90}N_{15}O_{11}^{+}$ $(M)^{+}$: 1388.69, found 1388.13 $(M)^{+}$. MS (MALDI) *m/z* calculated for the hydroxy ketone product $C_{76}H_{90}N_{15}O_{12}^{+}$ $(M)^{+}$: 1404.68, found 1404.23 $(M)^{+}$.

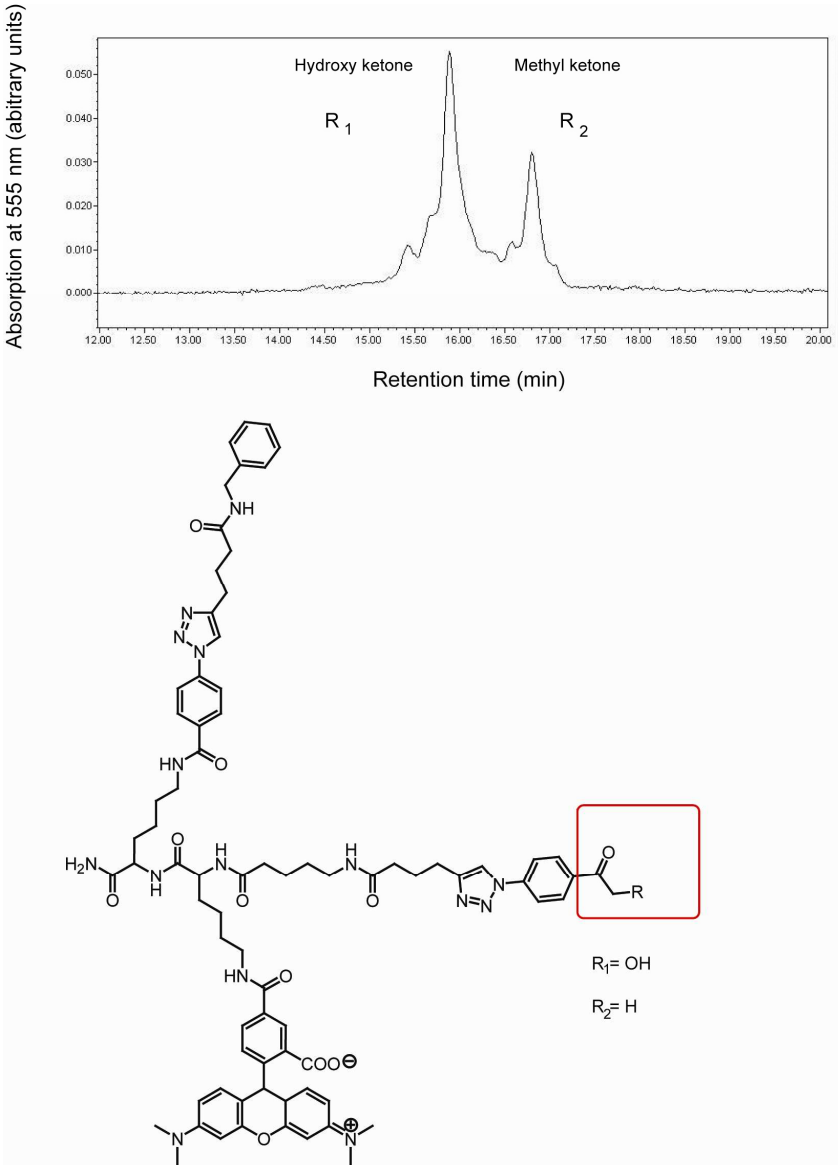
3) Supporting Figures



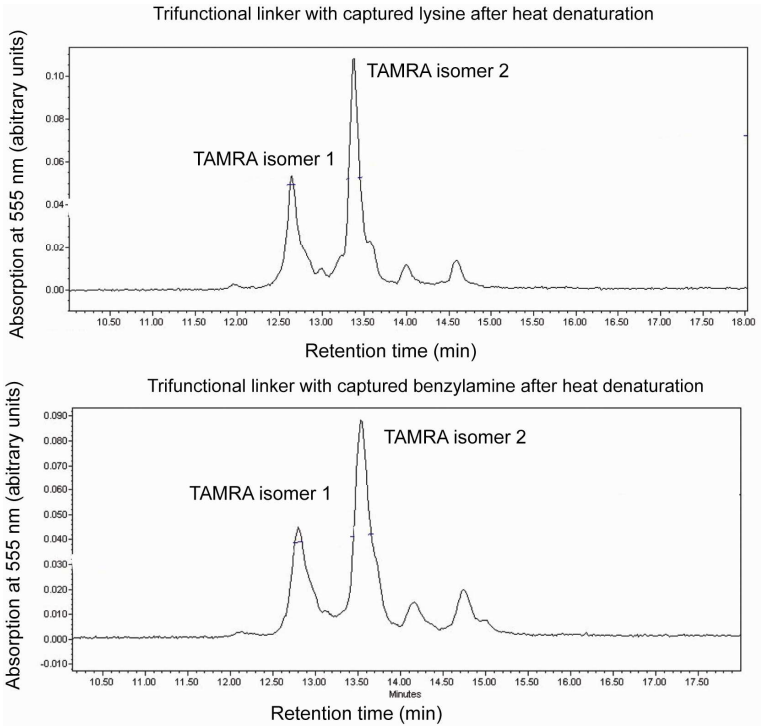
Supporting Figure 1



Supporting Figure 2

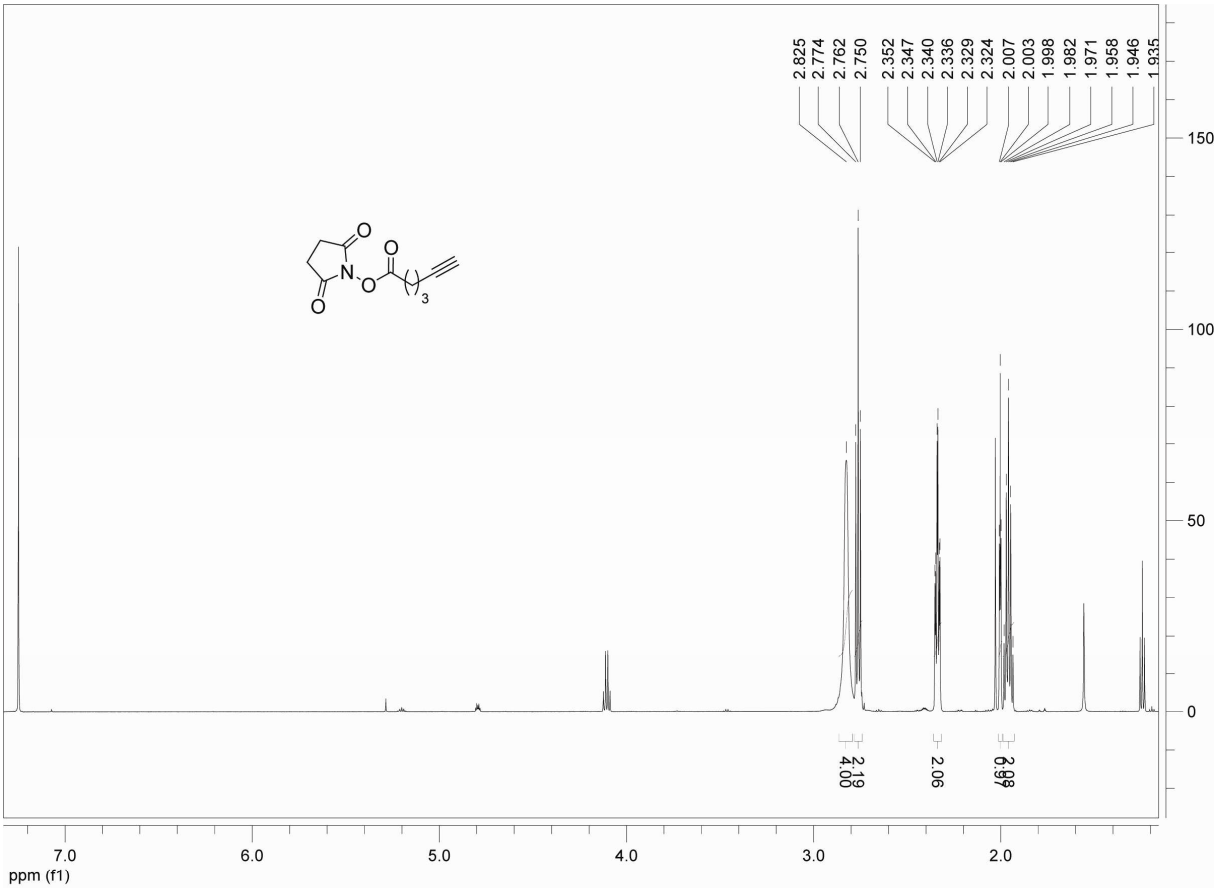


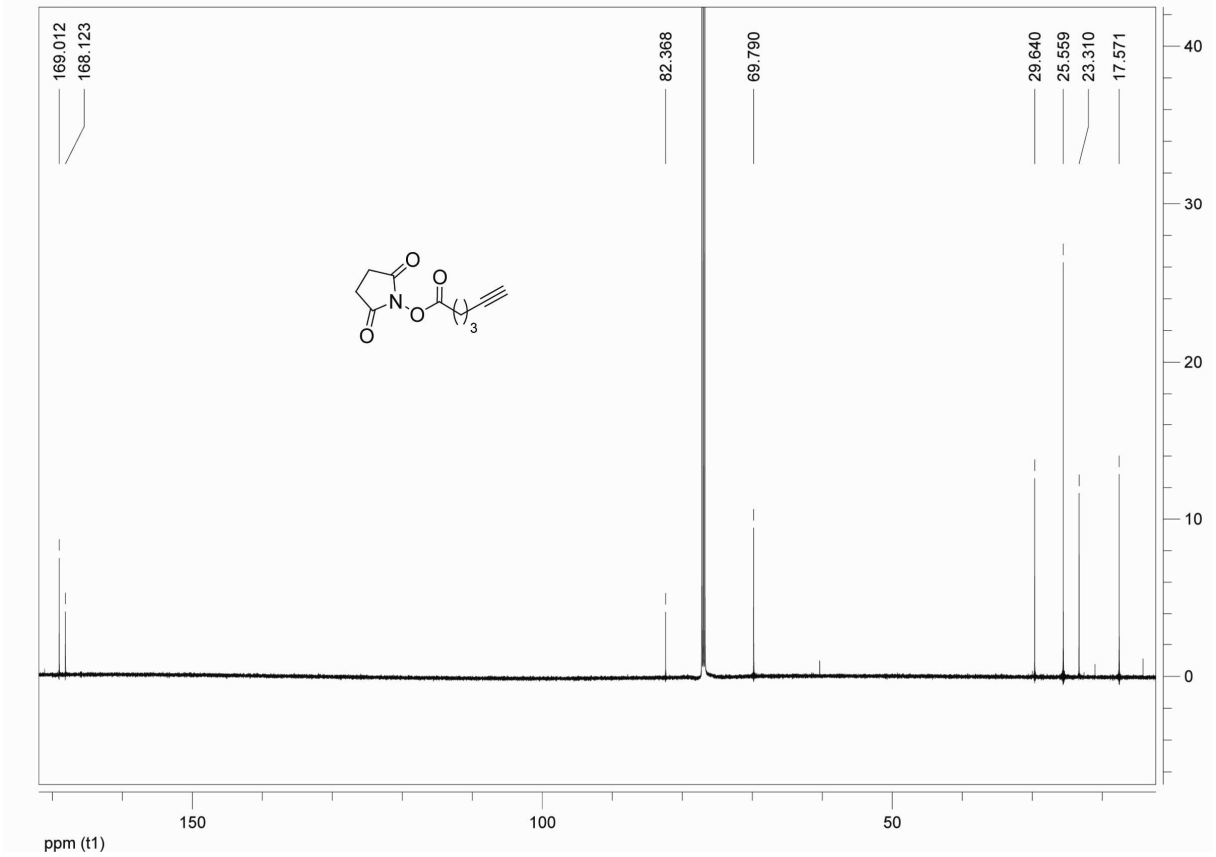
Supporting Figure 3.

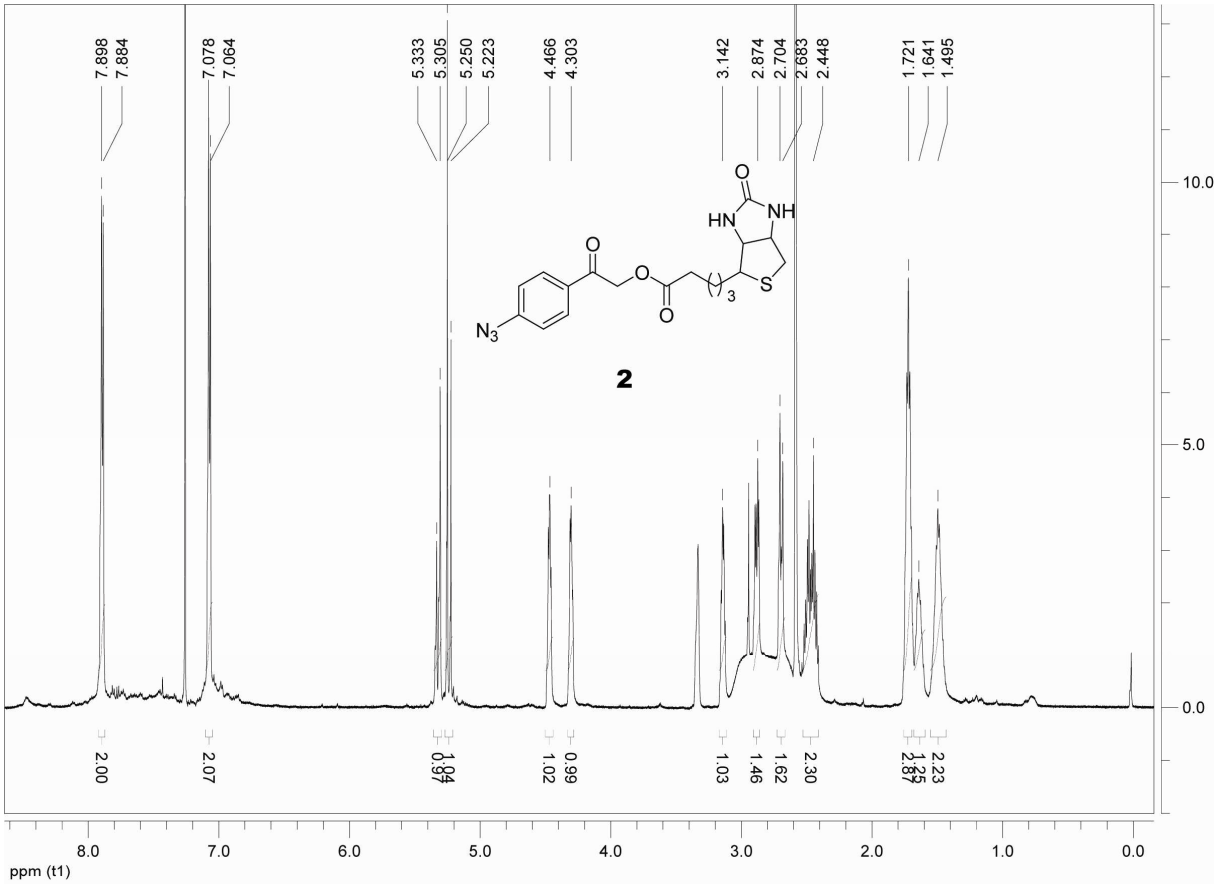


Supporting Figure 4.

4) NMR spectra:



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2.683
2.448
1.721
1.641
1.495
2.00
2.07
0.97
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1.02
0.99
1.03
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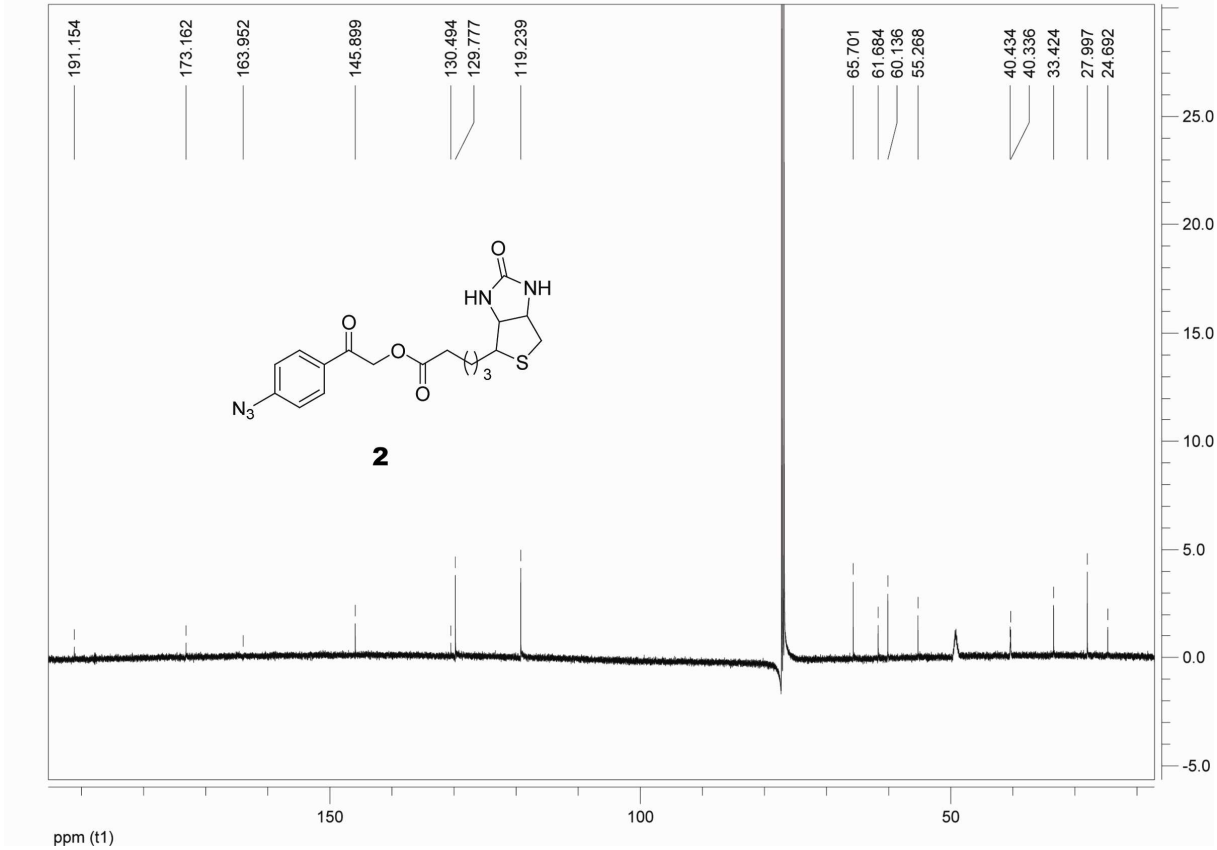
2.30
2.87
1.25
2.23
ppm (t1)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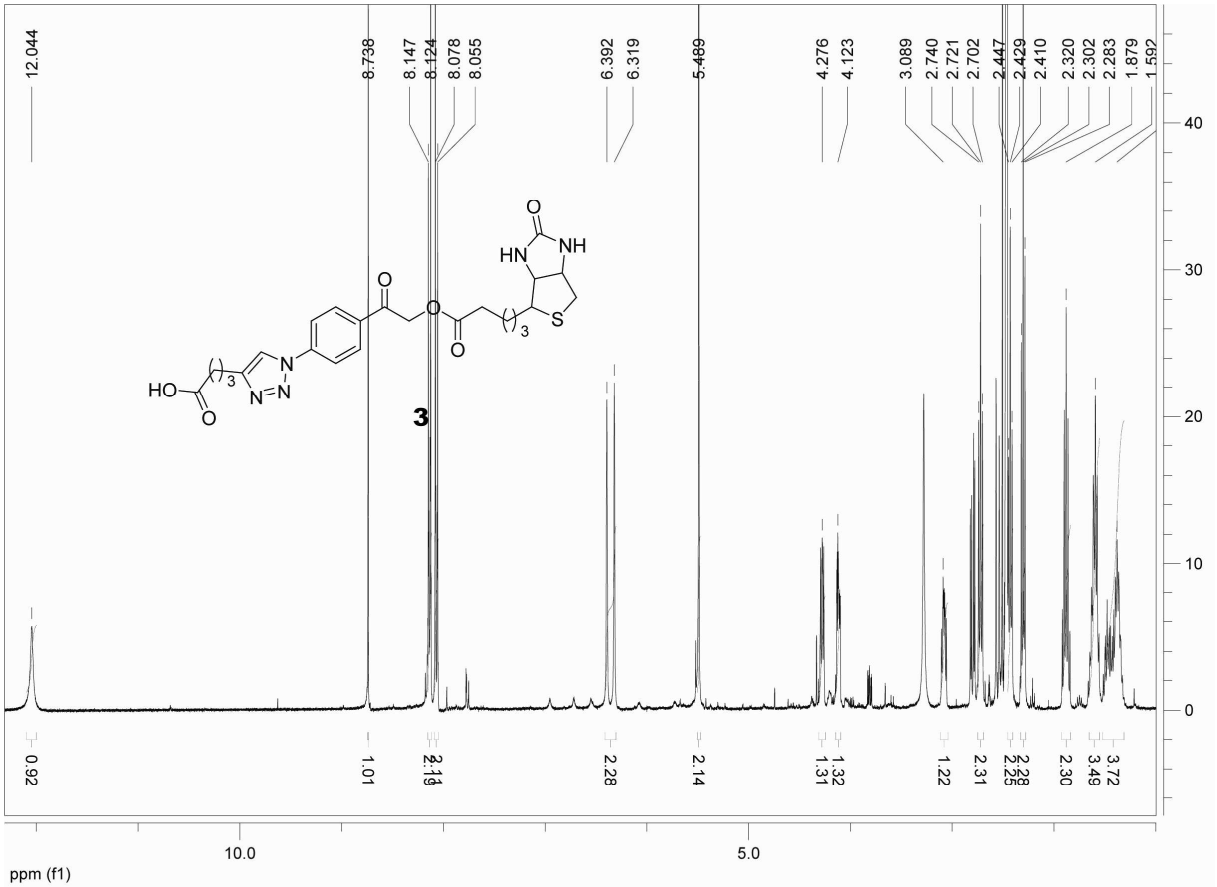
ppm (t1)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2.721
2.702
2.447
2.429
2.410
2.320
2.302
2.283
1.879
1.592
0.92
1.01
2.11
2.19
2.28
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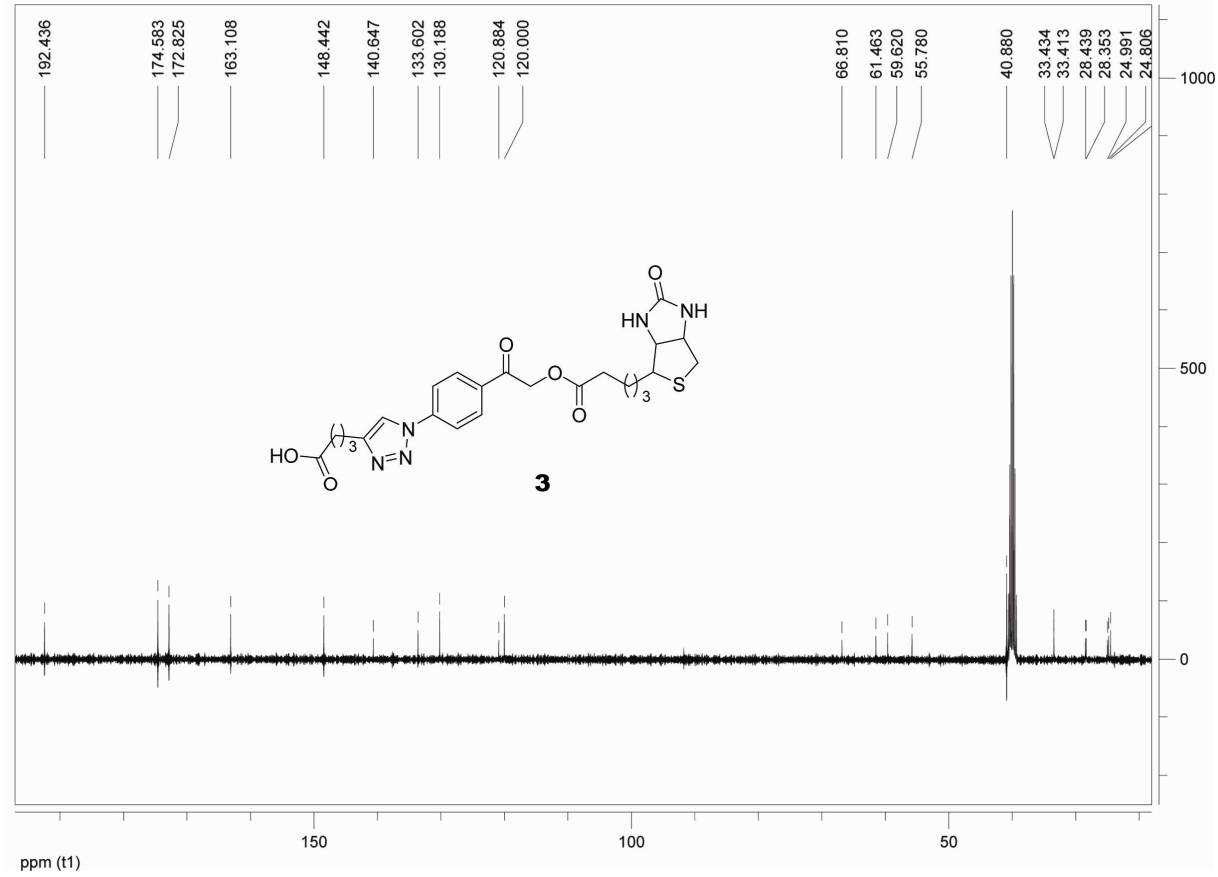
2.25
2.28
2.30
3.49
3.72
3
ppm (f1)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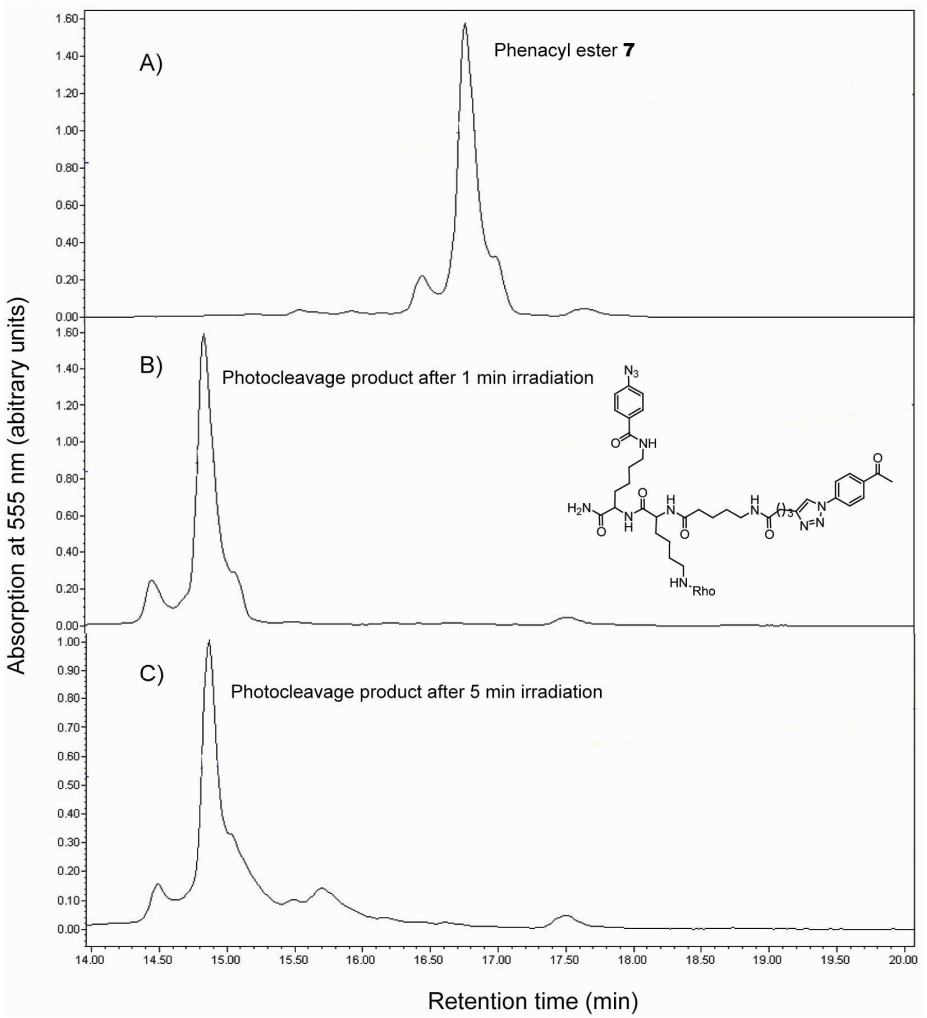
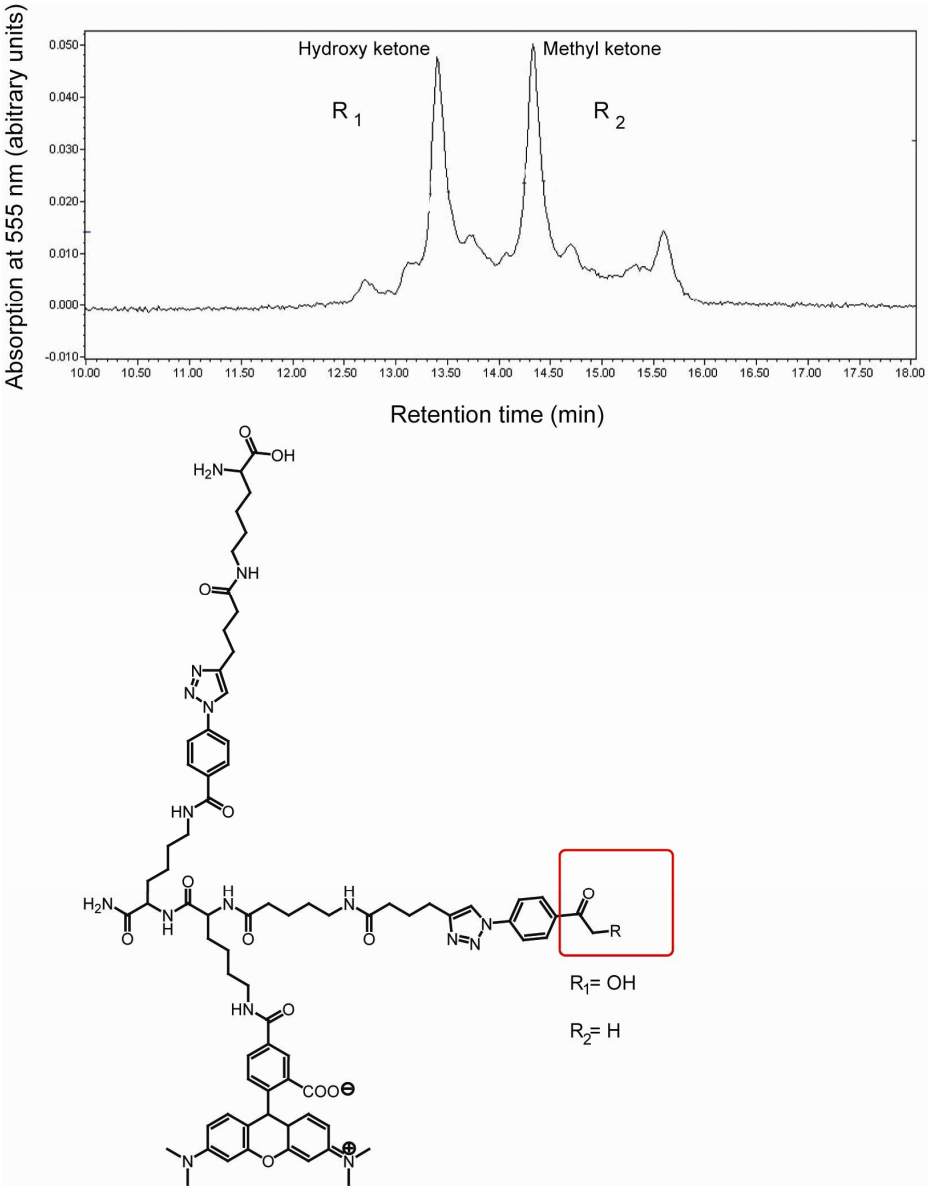
3
ppm (t1)

HPLC chromatograms:

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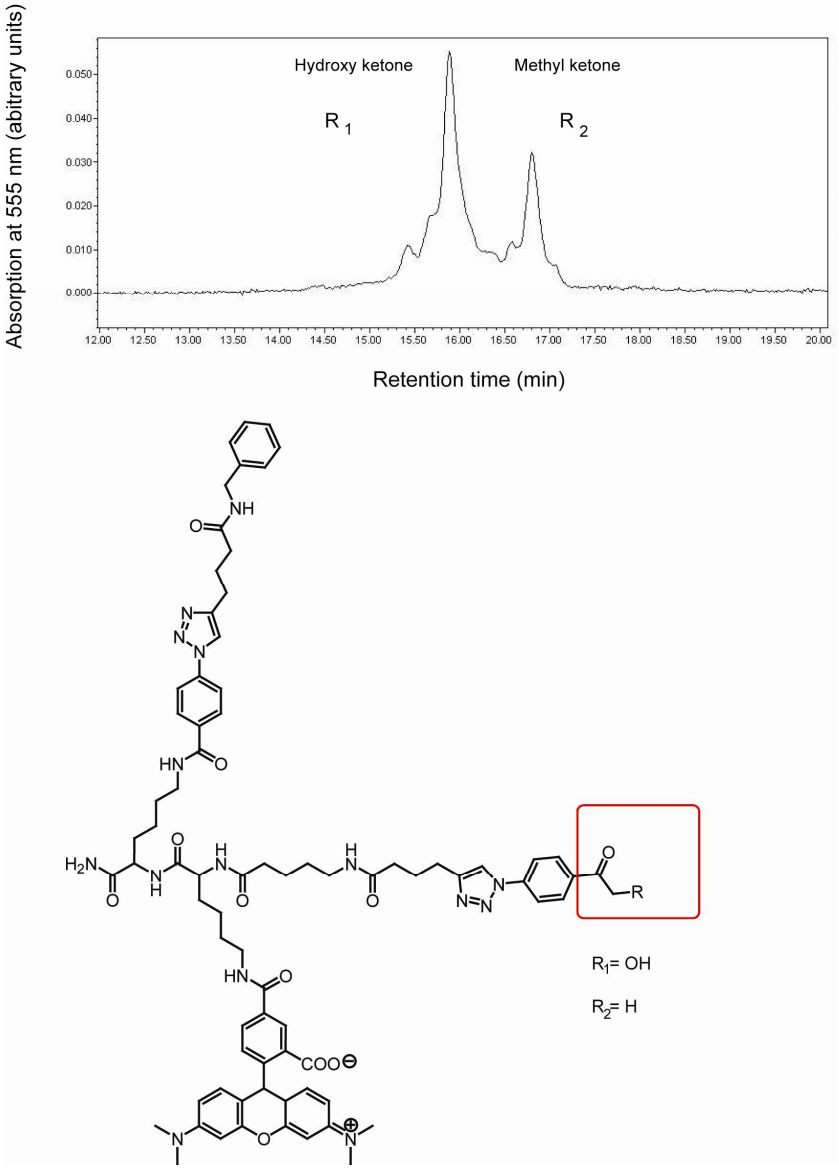
Hydroxy ketone
Methyl ketone
R 1
R 2
Retention time (min)
R1= OH
R2= H


Absorption at 555 nm (abitrary units)
Hydroxy ketone
Methyl ketone
R 1
R 2
Retention time (min)
R1= OH
R2= H