Electronic Supplementary Material (ESI) for Chemical Communications

Multifunctional bioconjugation by Mortia-Baylis-Hillman reaction in aqueous medium

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Supporting Information

General Experimental Section.

All reagents were commercially available and used without further purification. Milli-Q® water used as reaction solvent in oligosaccharide modification and LC-MS analysis was deionised using a Milli-Q® Gradient A10 system (Millipore, Billerica, USA). Flash column chromatography was performed using silica gel 60 (230-400 mesh ASTM) with ethyl acetate/n-hexane or methanol/dichloromethane as eluent. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity Inova 400 NB and 500 NB spectrometers. For ^{1}H NMR (500 MHz), tetramethylsilane (TMS) served as internal standard (δ = 0 ppm) and data are reported as follows: chemical shift (in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (in Hz), and integration. Mass spectra were measured using a Q-TOF 2 ™ mass spectrometer with a ESI source (Waters-Micromass, Manchester, United Kingdom). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was accomplished on a Mini-protean Tetra Cell (Bio-Rad, USA).

LC–MS Analysis of Oligosaccharides and Peptides.

Mass spectrometry analysis was performed using the ESI source of Q-TOF 2 ™ (Waters-Micromass, Manchester, United Kingdom) in the positive ion mode. Mobile-phase A was made of 0.5% formic acid in Milli-Q® water. The CapLC® system (Waters, Manchester, United Kingdom) was equipped with a Poroshell 300SB-C18 column (1.0 mm ID × 75 mm, 5µm) with ZORBAX Poroshell guard column (1.0 mm ID × 17 mm, 5 µm) (Agilent-Technologies Inc., Wilmington, United States of America). Mobile-phase B was made of 0.5% formic acid in acetonitrile. 2 µl of sample was injected with a flow rate of 40 µl/min at room temperature. The grandient program was set to 3% B for 0-3 min, followed by a linear gradient to 70%

B in 4-30 min and 3% B in 31-45 min. The mass spectrometer was monitored over a *m*/*z* range of 200-2000, and the raw spectra were deconvoluted by the MassLynx 4.1 Transform Program (Waters, Manchester, United Kingdom). Desolvation and source temperatures were 150 °C and 80 °C, respectively. Operating conditions for the detection of reaction mixture were the following: capillary voltage 3 kV, sample cone voltage 30 V, extraction voltage 4 V and collision cell voltage 10 eV.

Calculation of Aldehyde Conversion

The crude reaction mixture of aldehyde-containing oligosaccharides (aldehyde) and modified oligosaccharides (product) was subjected to LC-MS analysis with elution time of 45 min. After data processing by MassLynx 4.1 Transform Program, aldehyde conversion at different time intervals was determined by measuring the relative peak intensities of aldehyde and product in the mass spectrum as follows:

$$\textit{Aldehyde Conversion (\%)} = \left(1 - \frac{\textit{Relative Peak Intensity of Aldehyde}}{\textit{Relative Peak Intensities of Aldehyde and Product}}\right) \text{X } 100\%$$

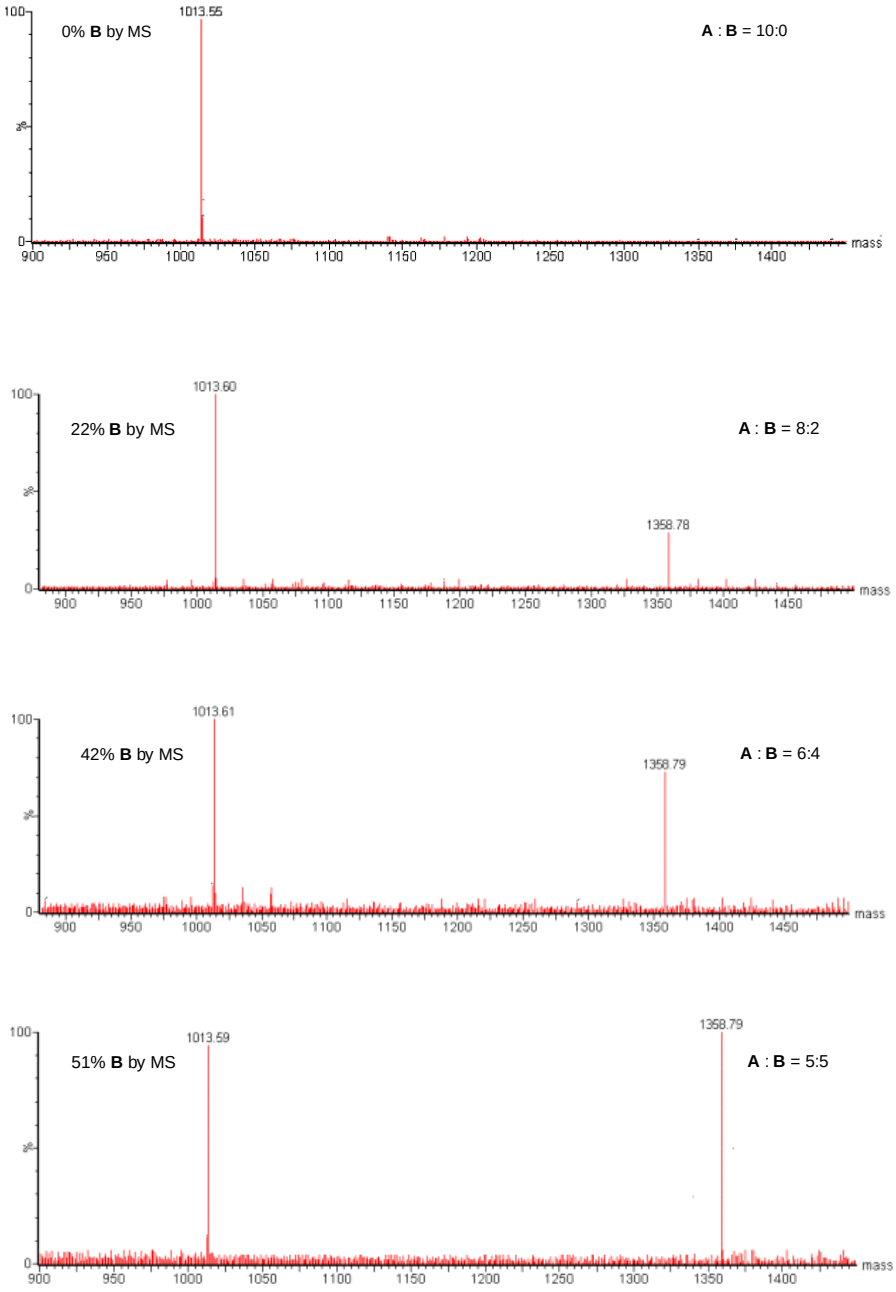
Calculation of Peptide Conversion

The crude reaction mixture of cysteine-containing peptide STSSSCNLSK (unmodified peptide) and modified peptide (product) was subjected to LC-MS analysis with elution time of 45 min. After data processing by MassLynx 4.1 Transform Program, peptide conversion at different time intervals was determined by measuring the relative peak intensities of peptide and product in the mass spectrum as follows:

$$\textit{Peptide Conversion (\%)} = \left(1 - \frac{\textit{Relative Peak Intensity of Unmodified Peptide}}{\textit{Relative Peak Intensities of Unmodified Peptide and Product}}\right) \text{X } 100\%$$

We have studied the ionization tendency of the unmodified and modified peptides in ESI. Different ratios of unmodified peptide (**A**, MW = 1013) and modified peptide (**B**, MW = 1358) (**A**:**B** = 10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10) were prepared and subjected to ESI-MS analysis. The percentage of **B** were found to be 0%, 22%, 42%,

51%, 60%, 80% and 100% by ESI-MS. The ESI-MS spectra were shown below. A graph of percentage of **B** by MS against the percentage of **B** present in the solution was plotted ($R^2 = 0.9993$). Thus, the unmodified and modified peptides were found to have *similar* ionization tendency in ESI.



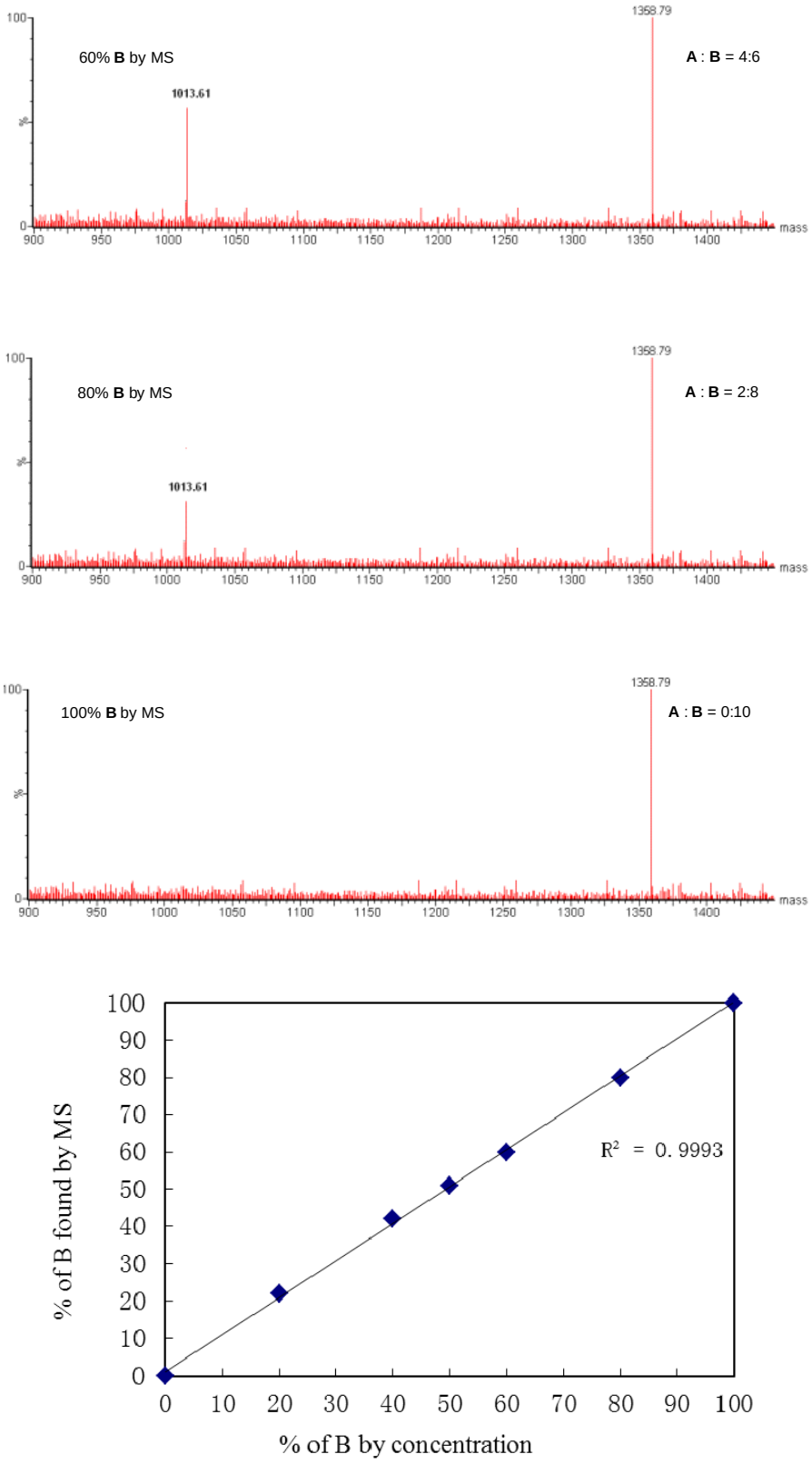
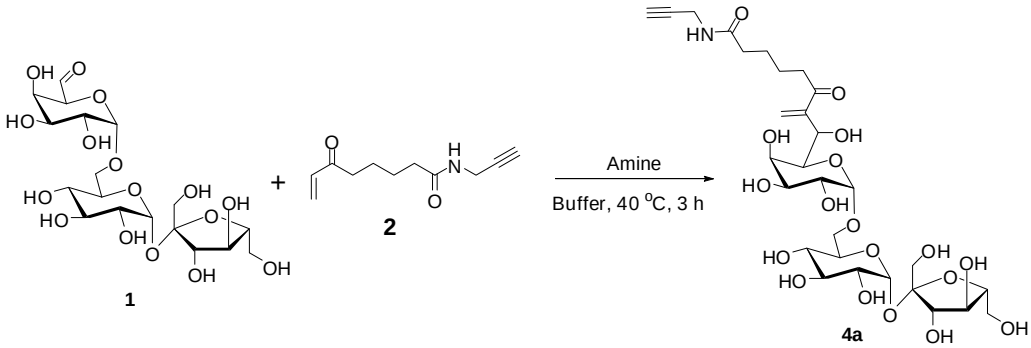


Figure S1 MS spectra unmodified peptide **A** (ESI source, $[M+H]^+$ = m/z 1013) and modified peptide **B** (ESI source, $[M+H]^+$ = m/z 1358).

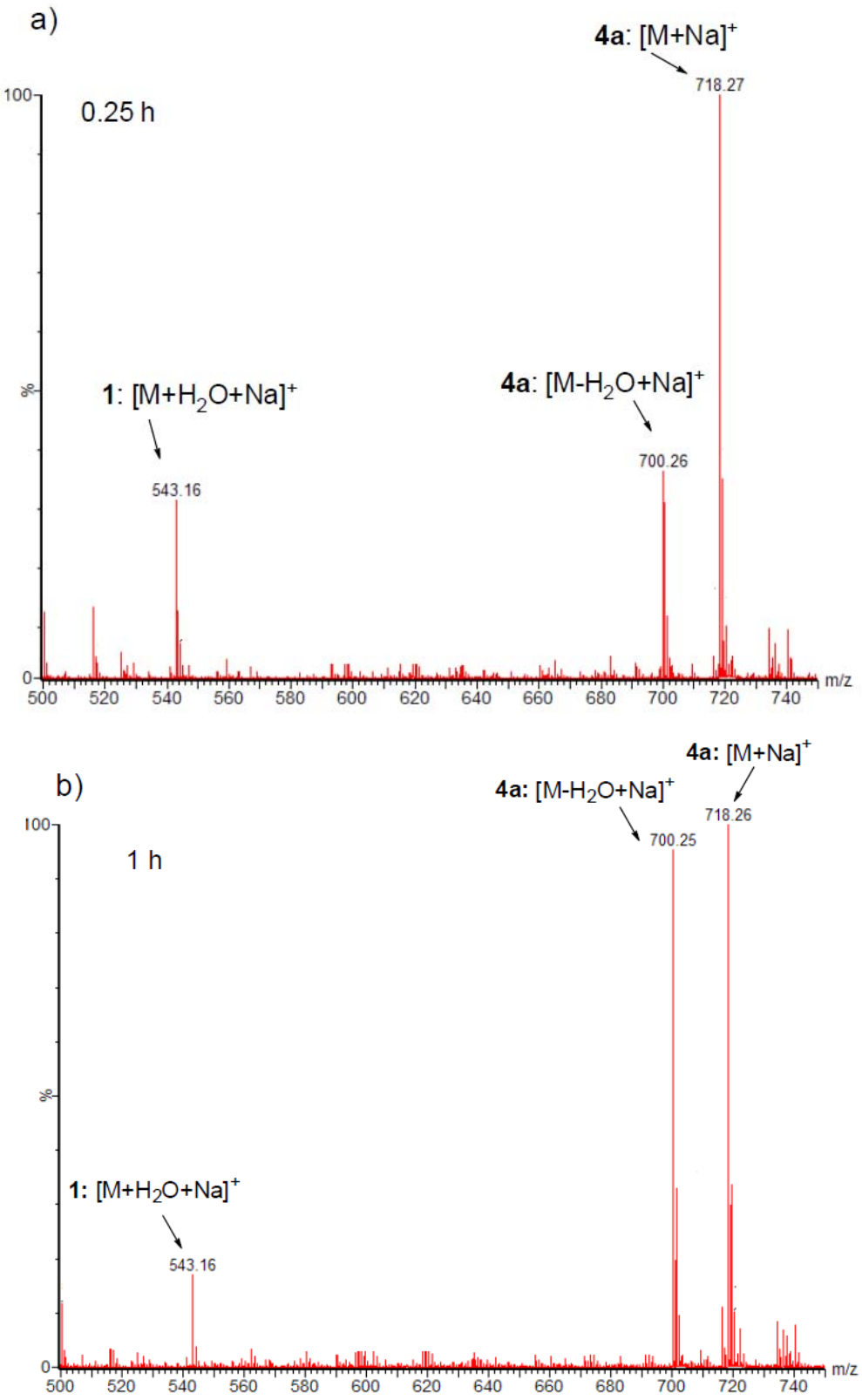
Table S1 Studies on reaction conditions of MBH reaction of D-raffinose aldehyde **1** with vinyl ketone **2**.[a]



Entry	Amine (equivalent)	Buffer (pH)	Conversion (%)[b]
1	DABCO (1)	5.1	70
2	-	5.1	0
3	DABCO (1)	6.2	75
4	-	6.2	0
5	***DABCO** (1)*	***7.4***	***88***
6	DABCO (0.5)	7.4	78
7	-	7.4	0
8	DABCO (1)	8.0	96
9	-	8.0	10
10[c]	DABCO (1)	9.3	97
11[c]	-	9.3	65
12[d]	DABCO (1)	7.4	86
13[e]	DABCO (1)	7.4	90
14	DMAP (1)	7.4	88
15	Quinuclidine (1)	7.4	86
16	3-Quinuclidinol (1)	7.4	85
17	DBU (1)	7.4	60
18	Imidazole (1)	7.4	30

[a] Reaction conditions: D-raffinose aldehyde **1** (10 mM), **2** (3 equiv.), amine (1 equiv.), PBS buffer solution (50 mM), 40 °C, 3 h. [b] Conversion was determined by LC-MS analysis of the crude reaction mxiture. [c] $NaHCO_3$ solution (100 mM). [d] Reaction performed at 25 °C. [e] D-Raffinose aldehyde **1** (1 mM).

Procedure for Time Course Experiment of MBH Reaction of D-Raffinose Aldheyde 1 with Vinyl Ketone 2. A mixture of D-raffinose aldehyde **1** (20 μL of 100 mM in H_2O) and vinyl ketone **2** (1 equiv., 20 μL of 100 mM in DMSO) in the presence of DABCO (1 equiv., 20 μL of 100 mM in H_2O) in $NaHCO_3$ buffer (140 μL, pH 9.3) was kept at 40 °C. The aldehyde conversion of the crude reaction mixture was monitored by LC-MS. The clear liquor of the centrifuged reaction mixture was injected in 15 min, 1 h, 2 h, 3 h and 4 h. The above coupling reactions of **1** were repeated using 3 and 5 equivalents of **2**.



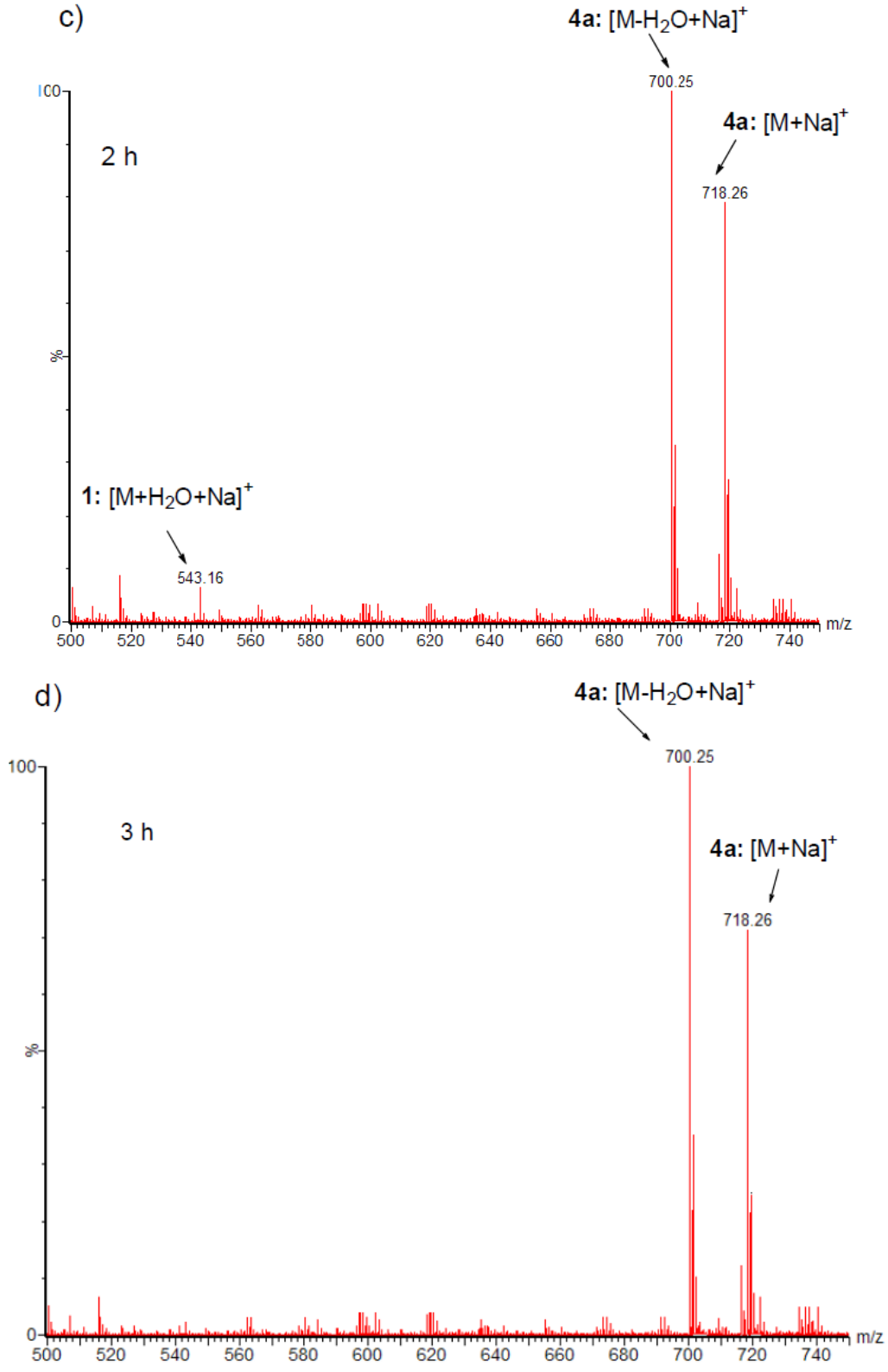
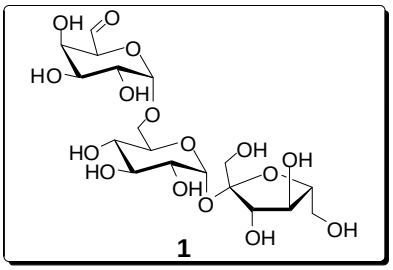


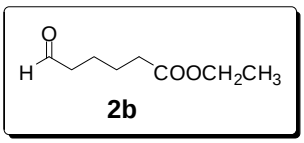
Figure S2 MS spectra of **1** (ESI source, $[M+H_2O+Na]^+$ = m/z 543.16) and **4a** (ESI source, $[M-H_2O+Na]^+$ = m/z 700.25, $[M+Na]^+$ = m/z 718.26).

Literature References of Known Compounds

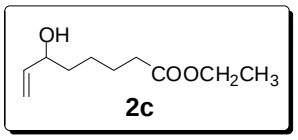


1

K. Parikka and M. Tenkanen, *Carbohydr. Res.,* 2009, **344**, 14-20.



2b



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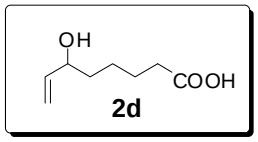
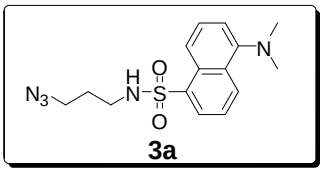


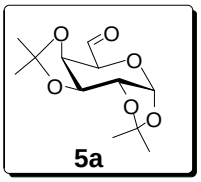
2d

B. M. Trost, and T. R. Verhoeven, *J. Am. Soc. Chem.,* 1980, **102**, 4743-4763.



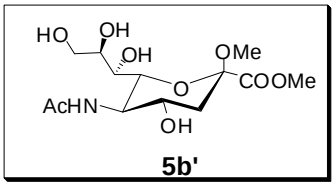
3a

a) W. G. Lewis, F. G. Magallon, V. V. Fokin and M. G. Finn, *J. Am. Soc. Chem.,* 2004, **126**, 9152-9153; b) D. D. Diaz, K. Rajagopal, E. Strable, J. Schneider and M. G. Finn, *J. Am. Soc. Chem.,* 2006, **128**, 6056-6057.



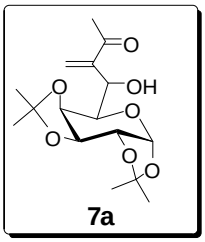
5a

A. W. Mazur and G. D. Hiler, II, *J. Org. Chem.,* 1997, **62**, 4471-4475.



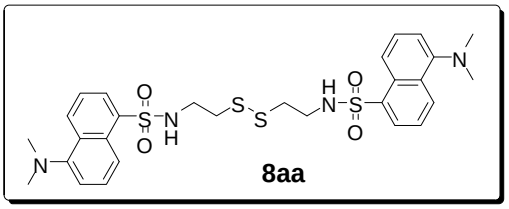
5b'

T. Q. Gregar and J. Gervay, *J. Org. Chem.,* 2004, **69**, 1001-1009.



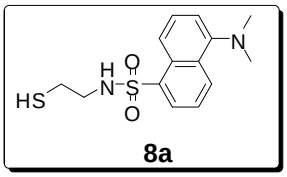
7a

A. Manjuvani and V. Kannan and P. R. Krishna, *Tetrahedron: Asymmetry,* 2005, **16**, 2691-2703.



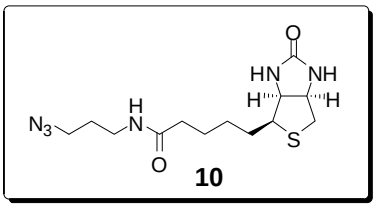
8aa

C. Robinson, R. F. Hartman and S. D. Rose, *Bioorg. Chem.,* 2008, **36**, 265-270.



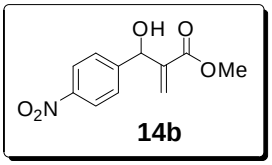
8a

K. Kuroda, G. A. Caputo and W. F. DeGrado, *Chem. –Eur. J.*, 2009, **15**, 1123-1133.



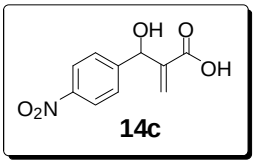
10

A. J. Lampkins, E. J. O'Neil and B. D. Smith, *J. Org. Chem*., 2008, **73**, 6053-6058.



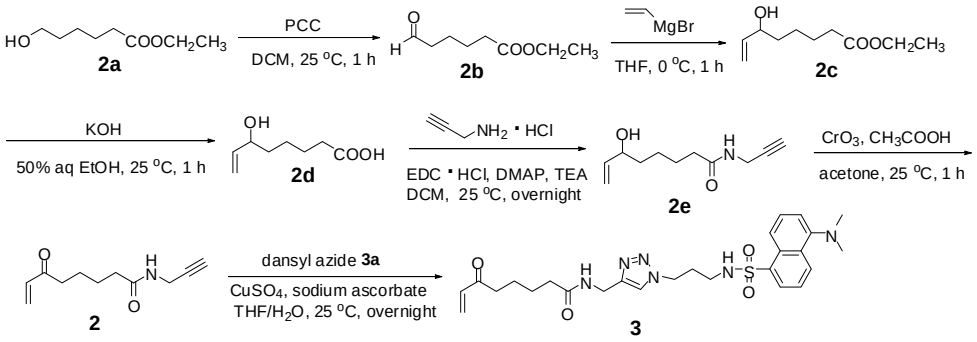
14b

Y. Guo, G. Shao. L. Li, W. Wu, R. Li, J. Li, J. Song, L. Qiu, M. Prashad, and F. Y. Kwong, *Adv. Synth. Catal*., 2010, **352**, 1539-1553.



14c

T. Bosanac and C. S. Wilcox, *Chem. Commun.,* 2001, 1618-1619.



Scheme S1 Synthesis of vinyl ketones **2** and **3**.

Compound 2e. To a solution of 6-hydroxyl-7-octenoic acid **2d** (0.173 g, 0.9 mmol), propargylamine hydrochloride (0.086 g, 0.9 mmol), EDC·HCl (0.179 g, 0.9 mmol) and DMAP (0.030 g, 0.2 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (1 mL). After stirring at room temperature for overnight, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 5% HCl aqueous solution (3×10 mL), $NaHCO_3$ saturated aqueous solution (3×10 mL), and brine (3×10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent was concentrated. The residue was purified by flash column chromatography (50% EtOAc in hexane) to give **2e** as a colorless oil (0.108 g, 62% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.05 (br, 1H), 5.80-5.87 (m, 1H), 5.20 (dt, J = 17.0, 1.5 Hz, 1H), 5.07 (dt, J = 10.5, 1.0 Hz, 1H), 4.09 (br, 1H), 4.02 (dd, J = 5.5, 3.0 Hz, 2H), 2.18-2.22 (m, 2H), 2.13 (d, J = 4.0 Hz, 1H), 1.39-1.66 (m, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 141.4, 114.7, 80.0, 72.9, 71.6, 36.7, 36.3, 29.3, 25.5, 25.1 ppm; ESI-MS m/z 196 $[M+H]^+$; HRMS (ESI) for $C_{11}H_{18}NO_2$ $[M+H]^+$ calcd: 196.1120, found: 196.1118.

Compound 2. Finely ground chromium (VI) oxide (0.121 g, 1.2 mmol) was suspended in acetone (5 mL) containing acetic acid (0.35 mL, 6.1 mmol). The mixture was stirred at room temperature for 30 min. Then, cooled to 0 °C and a solution of **2e** (0.118 g, 0.6 mmol) in acetone (1 mL) was slowly added to the above mixture. After stirring at room temperature for 1 h, the mixture was filtered over silica gel, eluting with dichloromethane. The eluent was concentrated and purified by flash column chromatography (30% EtOAc in hexane) to give **2** as a white solid (66.1 mg, 51%

yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.36 (dd, J = 18.0, 10.5 Hz, 1H), 6.24 (dd, J = 18.0, 1.0 Hz, 1H), 5.91 (br, 1H), 5.85 (dd, J = 10.5, 1.0 Hz, 1H), 4.07 (dd, J = 5.5, 2.5 Hz, 2H), 2.63-2.66 (m, 2H), 2.23-2.25 (m, 3H), 1.66-1.70 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.8, 172.4, 136.7, 128.4, 79.8, 71.7, 39.3, 36.3, 29.3, 25.4, 23.5 ppm; ESI-MS m/z 216 $[M+Na]^+$; HRMS (ESI) for $C_{11}H_{15}NO_2Na$ $[M+Na]^+$ calcd: 216.1000, found: 216.0998.

Compound 3. To a solution of compound **2** (0.0512 g, 0.27 mmol) in THF/H_2O (1:1, 5 mL) was added dansyl azide **3a** (0.089 g, 0.27 mmol), sodium L-ascorbate (0.053 g, 0.27 mmol) and $CuSO_4$ (0.013 g, 0.08 mmol). The reaction mixture was stirred at room temperature for overnight and added water (5 mL). The mixture was extracted with ethyl acetate (3×10 mL), dried over anhydrous $NaSO_4$, filtered, and concentrated under reduced pressure to give a residue, and then purified by flash column chromatography (5% MeOH in DCM) to give **3** as a yellow oil (0.113 g, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 8.5 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 8.18 (d, J = 6.0 Hz, 1H), 7.45-7.52 (m, 3H), 7.15 (d, J = 7.5 Hz, 1H), 6.83 (br, 1H), 6.44 (br, 1H), 6.28 (dd, J = 17.5, 10.5 Hz, 1H), 6.15 (d, J = 17.0 Hz, 1H), 5.77 (d, J = 10.5 Hz, 1H), 4.46 (d, J = 5.0 Hz, 2H), 4.32 (t, J = 6.5 Hz, 2H), 2.91 (q, J = 6.0 Hz, 2H), 2.86 (s, 6H), 2.54 (t, J = 7.0 Hz, 2H), 2.18 (t, J = 7.0 Hz, 2H), 2.01-2.06 (m, 2H), 1.54-1.62 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.0, 173.2, 152.3, 145.1, 136.7, 134.9, 130.7, 130.1, 129.7, 129.6, 128.5, 128.4, 123.4, 123.1, 118.9, 115.5, 47.6, 45.6, 40.3, 39.3, 36.3, 35.0, 30.3, 25.3, 23.5 ppm; ESI-MS m/z 527 $[M+H]^+$; HRMS (ESI) for $C_{26}H_{35}N_6O_4S$ $[M+H]^+$ calcd: 527.2441, found: 527.2430.

Procedure for MBH Reaction of D-Raffinose Aldeheyde 1 with Vinyl Ketone 2 to give 4a. A mixture of unprotected D-raffinose aldehyde **1** (10 mM, 10 μL of 100 mM in H_2O) and vinyl ketone **2** (30 mM, 10 μL of 300 mM in DMSO) in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) (10 mM, 10 μL of 100 mM in H_2O) in PBS buffer (pH 7.4, 70 μL) was kept at 40 °C for 3 h. The crude reaction mixture was analyzed by LC-MS for determination of the aldehyde conversion.

Procedure for Modification of D-Raffinose Aldeheyde 1 with Fluorescent Vinyl Ketone 3 to give 4b. A solution of unprotected D-raffinose aldehyde **1** (10 mM, 10 µL of 100 mM in H_2O) and fluorescent vinyl ketone **3** (10 mM, 10 µL of 100 mM in DMSO, 1 equiv.) in the presence of DABCO (10 mM, 10 µL of 100 mM in H_2O, 1 equiv.) in $NaHCO_3$ buffer (100 mM, pH 9.3, 70 µL) was kept at 40 °C for 6 h. The clear liquor of the centrifuged reaction mixture was analyzed by LC-MS for determination of the aldehyde conversion.

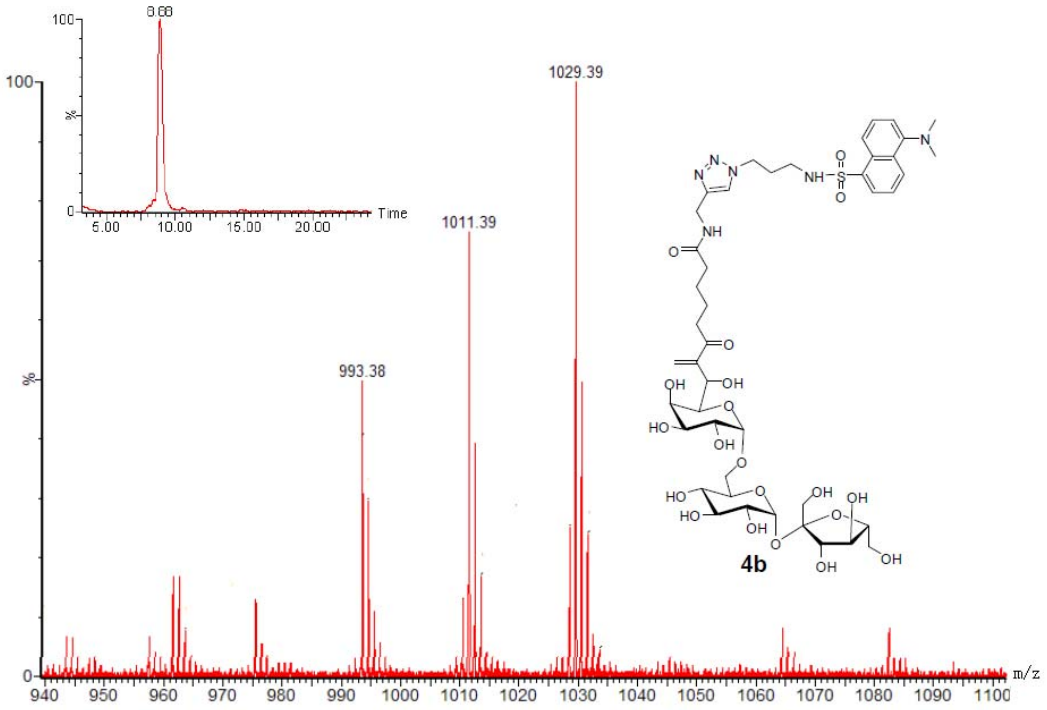
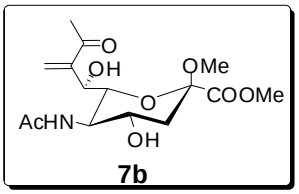


Figure S3 MS spectrum of **4b** (ESI source, $[M\text{-}2H_2O+H]^+$ = m/z 993.37, $[M\text{-}H_2O+H]^+$ = m/z 1011.39, $[M+H]^+$ = m/z 1029.39) and the XIC chromatogram of **4b** at = 8.8 min (inset).

Compound 5b. To a solution of compound **5b'** (0.168 g, 0.5 mmol) in MeOH/H_2O (1:1, 5 mL) was added $NaIO_4$ (0.266 g, 1.25 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h. After filtration of the mixture, the filtrate was concentrated in vacuo to give the residue used for the next step without further purification.

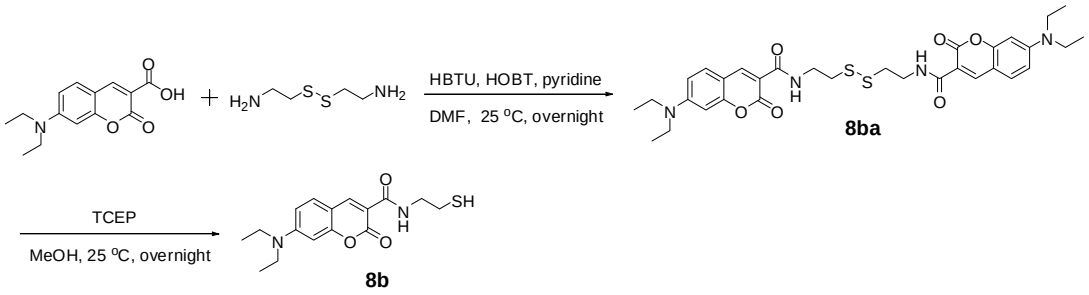
General Procedure for the Synthesis of 7a-7b from Monosaccharide Aldehydes 5a and 5b with Methyl Vinyl Ketone 6. To a solution of aldehyde **5a** or **5b** (1 mmol) in 1,4-dioxane/H_2O (1:1, 5 mL) were added DABCO (1 mmol) and methyl vinyl

ketone **6** (3 mmol). The reaction mixture was stirred at room temperature for overnight and added water (5 mL). The mixture was extracted with ethyl acetate (3×10 mL), dried over anhydrous $NaSO_4$, filtered, and concentrated under reduced pressure to give a residue, and then purified by flash column chromatography.



7b

Compound 7b. White solid (0.126 g, yield: 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.34 (br, 1H), 6.29 (br, 1H), 4.69 (br, 2H), 3.85-3.90 (m, 1H), 3.72 (s, 3H), 3.60-3.65 (m, 1H), 3.46 (dd, J = 10.0, 1.0Hz, 1H), 3.27 (s, 3H), 2.69 (dd, J = 12.5, 4.5 Hz, 1H), 2.41 (s, 3H), 2.10 (s, 3H), 1.75-1.82 (m, 1H), 1.26 (t, J = 7.5 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.4, 174.1, 168.8, 146.9, 127.3, 99.2, 74.1, 68.1, 67.0, 53.8, 52.5, 51.7, 40.5, 26.5, 23.3 ppm; ESI-MS m/z 368 $[M+Na]^+$; HRMS (ESI) for $C_{15}H_{23}NO_8Na$ $[M+Na]^+$ calcd: 368.1321, found: 368.1319.



Scheme S2 Synthesis of Thio-Based Coumarin **8b**

Compound 8ba. 7-(Diethylamino)-coumarin-3-carboxylic acid (0.138 g, 0.5 mmol), cystamine dihydrochloride (0.068 g, 0.3 mmol), HBTU (0.130, 0.3 mmol), and HOBt (0.068 g, 05mmol) were dissolved in dry DMF (5 mL) and added pyridine (0.2 mL). After stirring at room temperature for 24 h, the mixture was diluted with CH_2Cl_2 (10 mL) and washed with 5% HCl aqueous solution (15 mL), saturated $NaHCO_3$ aqueous solution (15 mL), and brine (20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (50% EtOAc in hexane) to give **8ba** as a yellow soild (0.149

g, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (br, 2H), 8.71 (s, 2H), 7.44 (d, J = 9.0 Hz, 2H), 6.66 (dd, J = 8.5, 2.0 Hz, 2H), 6.51 (d, J = 2.5 Hz, 2H). 3.79 (q, J = 13.0, 6.5 Hz, 4H), 3.45 (q, J = 7.0 Hz, 8H), 2.97 (t, J = 6.5 Hz, 4H), 1.26 (t, J = 7.5 Hz, 12H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.6, 162.9, 157.9, 152.8, 148.4, 131.4, 110.5, 110.12, 108.6, 96.8, 45.3, 38.9, 38.0, 12.7 ppm; ESI-MS m/z 639 $[M+H]^+$; HRMS (ESI) for $C_{32}H_{39}N_4O_6S_2$ $[M+H]^+$ calcd: 639.2311, found: 639.2314.

Compound 8b. Tris-(2-carboxyethyl)phosphine (TCEP) (0.030 g, 0.1 mmol) was added to a solution of **8ba** (0.032 g, 0.05 mmol) in methanol (1 mL), and followed by addition of water (0.1 mL). The mixture was stirred at room temperature for overnight. Then the mixture was diluted with CH_2Cl_2 (20 mL) and washed with brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (50% EtOAc in hexane) to give **8b** as a yellow solid (13 mg, 75% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.07 (br, 1H), 8.69 (s, 1H), 7.42 (d, J = 8.5 Hz, 1H), 6.64 (dd, J = 9.0, 2.0 Hz, 1H), 6.49 (d, J = 2.5 Hz, 1H). 3.62 (q, J = 6.5 Hz, 2H), 3.45 (q, J = 7.0 Hz, 4H), 2.75 (q, J = 7.0 Hz, 2H), 1.23 (t, J = 7.5 Hz, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 163.0, 157.9, 152.8, 148.4, 131.4, 110.3, 110.2, 108.6, 96.8, 45.3, 43.1,24.7, 12.6 ppm; ESI-MS m/z 321 $[M+H]^+$; HRMS (ESI) for $C_{16}H_{20}N_2O_3S$ $[M+H]^+$ calcd: 321.2311, found: 321.2314.

Procedure for Bifunctional Modification of Alkyne-bearing D-Raffinose 4a with Thiol-based Dansyl 8a and Coumarin 8b Followed by Click Reaction with Biotin Azide 10 to give 11a-11b. To a solution of alkyne-modified D-raffinose **4a** (10 mM in PBS buffer, pH 8.0, 100 μL) was added thiol-based dansyl **8a** and coumarin **8b** (2 μL of 500 mM stock solution in DMSO). The reaction mixture was kept at 40 °C for 2 h. The crude reaction mixture was centrifuged. The clear liquor was taken out for determination of aldehyde conversion by LC-MS. Without further purification, the crude reaction mixture of **9a** and **9b**-modified D-Raffinose (90 μL) containing an alkyne moiety were further modified with biotin azide **10**. To the above solution (90

µL) were added biotin azide **10** (2.6 µL of 500 mM stock solution in DMSO), sodium ascorbate 1.3 µL of 1 M stock solution in H_2O) and $CuSO_4$ (1.3 µL of 1 M stock solution in H_2O). The reaction mixture was kept at 25 °C for 12 h. The crude reaction mixture was centrifuged. The clear liquor was taken out for determination of aldehyde conversion by LC-MS.

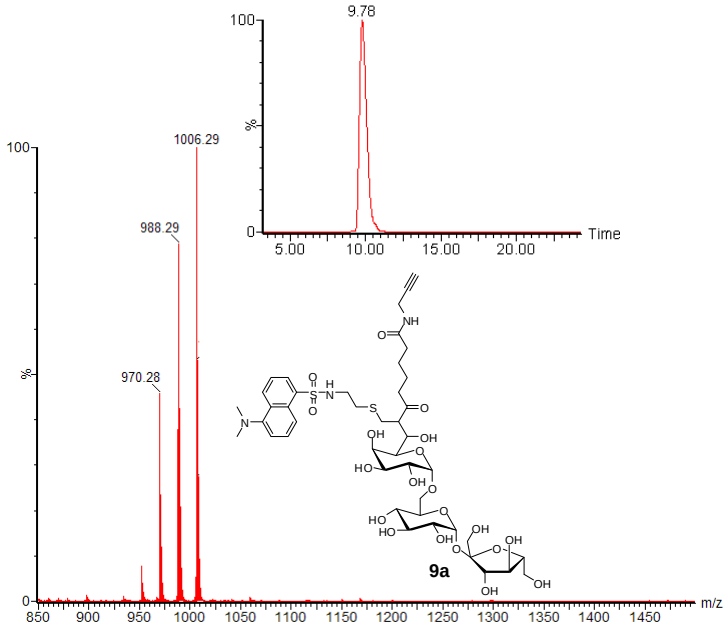


Figure S4 MS spectrum of **9a** (ESI source, $[M-2H_2O+H]^+$ = m/z 970.28, $[M-H_2O+H]^+$ = m/z 988.29, $[M+H]^+$ = m/z 1006.29) and the XIC chromatogram of **9a** at = 9.8 min (inset).

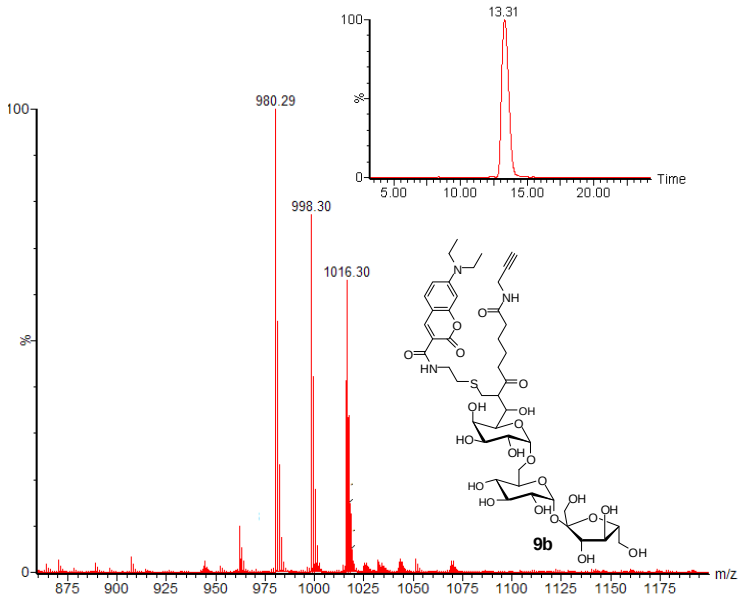


Figure S5 MS spectrum of **9b** (ESI source, $[M-2H_2O+H]^+$ = m/z 980.29, $[M-H_2O+H]^+$ = m/z 998.30, $[M+H]^+$ = m/z 1016.30) and the XIC chromatogram of **9b** at = 13.3 min (inset).

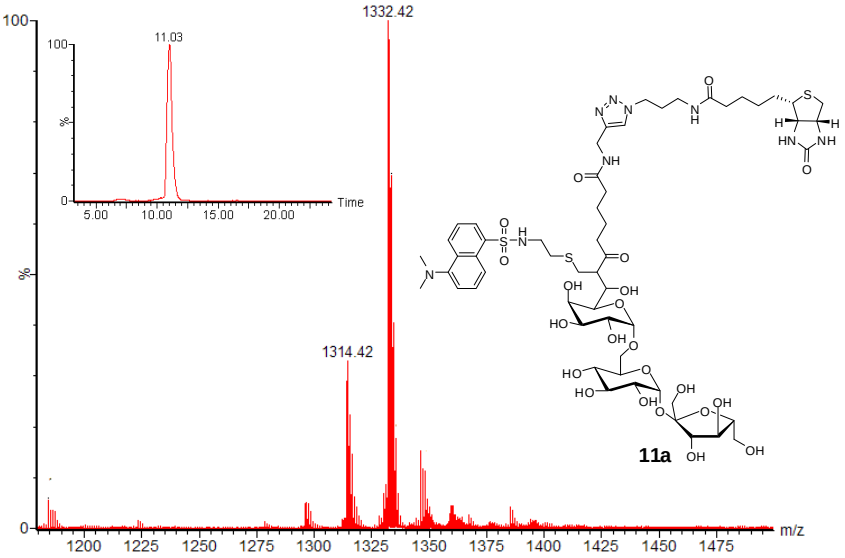


Figure S6 MS spectrum of **11a** (ESI source, $[M\text{-}H_2O+H]^+$ = m/z 1314.42, $[M+H]^+$ = m/z 1332.42) and the XIC chromatogram of **11a** at = 11.0 min (inset).

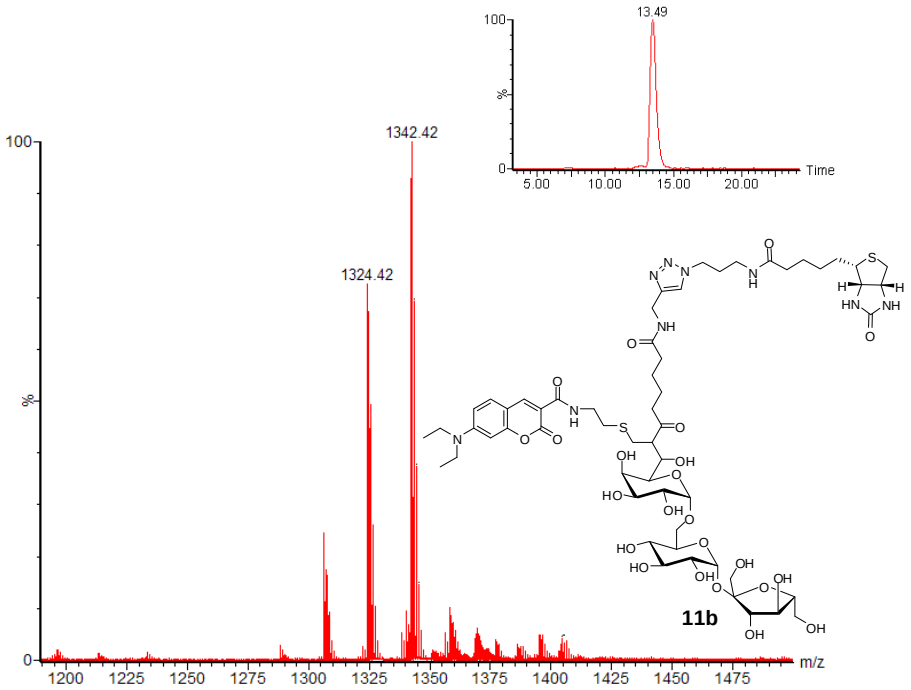


Figure S7 MS spectrum of **11b** (ESI source, $[M\text{-}2H_2O+H]^+$ = m/z 1306.20, $[M\text{-}H_2O+H]^+$ = m/z 1324.42, $[M+H]^+$ = m/z 1342.42) and the XIC chromatogram of **11b** at = 13.5 min (inset).

Procedure for Modification of Cysteine-Containing Peptide STSSSCNLSK 12 with β-hydroxyl-α-methylene-carbonyl-bearing MBH Adducts 7a and 7b. STSSSCNLSK **12** (10 μL, 1 mM in H_2O), β-hydroxyl-α-methylene-carbonyl-bearing MBH Adducts **7a** or **7b** (5 μL, 10 mM in DMSO) and PBS buffer (85 μL, pH 8.0) were mixed in a 1.5 mL Eppendorf tube. The reaction mixture was kept at 40 °C for 2 h. and then cysteine-modified peptides were analyzed by LC-MS/MS.

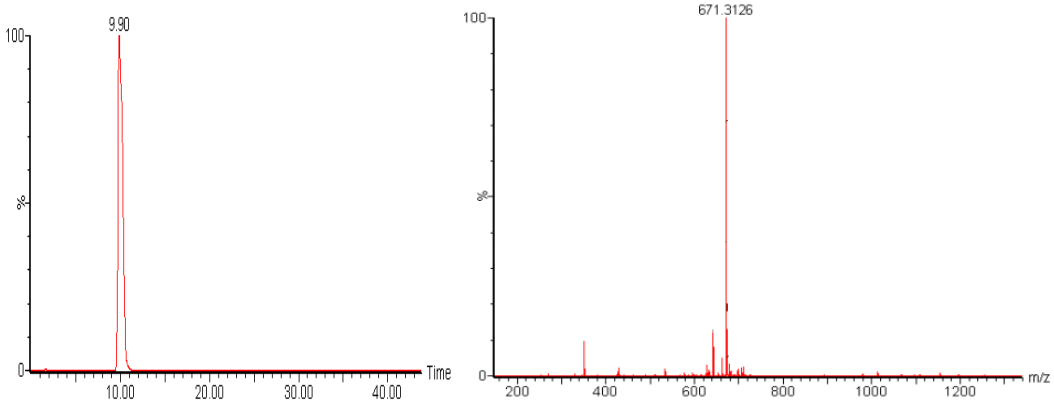


Figure S8 LC-MS spectra of **7a**-modified STSSSCNLSK (ESI source, doubly charge ion m/z = 671.31) and the XIC chromatogram of **7a**-modified STSSSCNLSK at = 9.9 min (left).

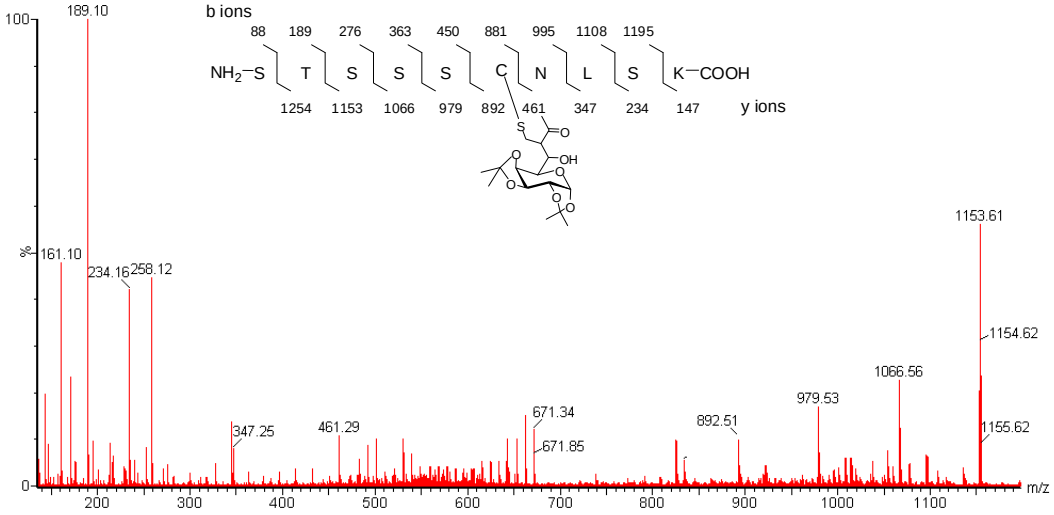


Figure S9 LC-MS/MS spectrum of **7a**-modified STSSSCNLSK (ESI source, doubly charge ion m/z = 671.3).

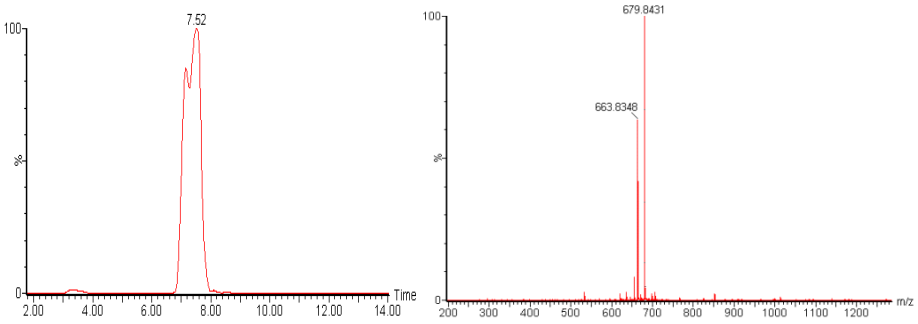


Figure S10 LC-MS spectra of **7b**-modified STSSSCNLSK (ESI source, doubly charge ion m/z = 679.8, $[M\text{-}CH_3OH+2H]^{2+}$ = m/z 663.8) and the XIC chromatogram of **7b**-modified STSSSCNLSK at = 7.5 min (left).

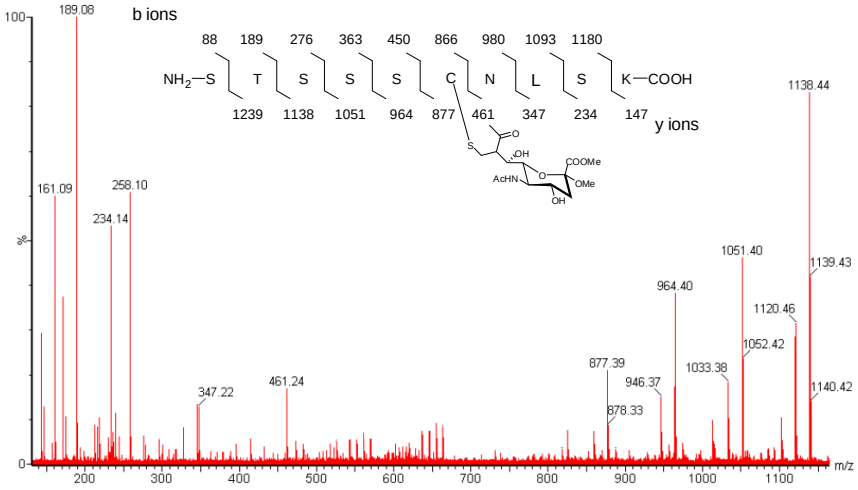


Figure S11a LC-MS/MS spectrum of **7b**-modified STSSSCNLSK (ESI source, doubly charge ion m/z = 663.8).

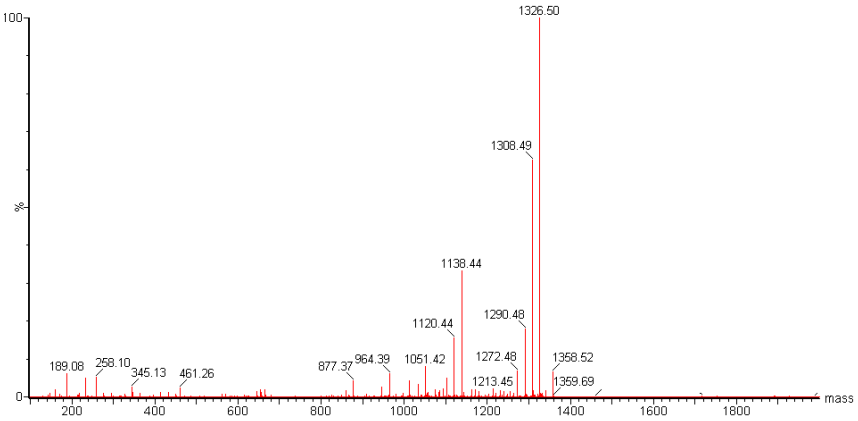
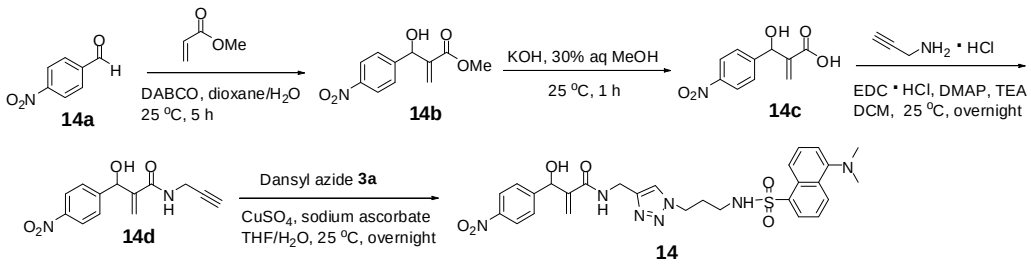


Figure S11b LC-MS/MS spectrum of **7b**-modified STSSSCNLSK (ESI source, doubly charge ion m/z = 679.8).



Scheme S4 Synthesis of **14**

Compound 14d. To a solution of **14c** (0.205 g, 0.9 mmol), propargylamine hydrochloride (0.093 g, 1.0 mmol), EDC·HCl (0.193 g, 1.0 mmol) and DMAP (0.024 g, 0.2 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (1 mL). After stirring at room temperature for overnight, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 5% HCl aqueous solution (3×10 mL), $NaHCO_3$ saturated aqueous solution (3×10 mL), and brine (3×10 mL). The oragnic phase was dried over anhydrous $MgSO_4$, filtered and the solvent was concentrated. The residue was purified by flash column chromatography (50% EtOAc in hexane) to give **14d** as a white solid (0.140 g, 60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (d, J = 7.0 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 6.41 (br, 1H), 5.90 (s, 1H), 5.62 (d, J = 6.0 Hz, 1H), 5.56 (s, 1H), 4.12 (d, J = 5.5 Hz, 1H), 4.00-4.02 (m, 2H), 2.14 (t, J = 5.0 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 148.4, 147.7, 144.2, 127.2, 123.9, 122.2, 78.9, 74.4, 72.3, 29.5 ppm; ESI-MS m/z 283 $[M+Na]^+$; HRMS (ESI) for $C_{13}H_{12}N_2O_4Na$ $[M+Na]^+$ calcd: 283.0695, found: 283.0688.

Compound 14. To a solution of compound **14d** (0.06 g, 0.2 mmol) in THF/H_2O (1:1, 5 mL) were added dansyl azide **3a** (0.05 g, 0.15 mmol), sodium L-ascorbate (0.053 g, 0.27 mmol) and $CuSO_4$ (0.013 g, 0.08 mmol). The reaction mixture was stirred at room temperature for overnight and added water (5 mL). The mixture was extracted with ethyl acetate (3×10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue, and then purified by flash column chromatography (5% MeOH in DCM) to give **14** as a yellow oil (75.8 mg, 85% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, J = 8.5 Hz, 1H), 8.22 (d, J = 9.0

Hz, 1H), 8.14 (d, J = 7.5 Hz, 1H), 8.04 (d, J = 8.5 Hz, 2H), 7.67 (br, 1H), 7.46-7.49 (m, 2H), 7.41-7.43 (m, 2H), 7.12 (d, J = 7.5 Hz, 1H), 6.37 (s, 1H), 5.93 (s, 1H), 5.58 (s, 1H), 5.41 (s, 1H), 5.24 (s, 1H), 4.37-4.47 (m, 2H), 4.27 (t, J = 6.5 Hz, 2H), 2.86 (s, 8H), 1.95-2.00 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 152.3, 149.0, 147.4, 144.4, 144.3, 134.5, 130.9, 130.1, 129.7, 128.7, 127.4, 123.6, 123.4, 123.3, 122.6, 118.7, 115.5, 73.9, 47.6, 45.6, 45.0, 40.3, 30.1 ppm; ESI-MS m/z 594 $[M+H]^+$; HRMS (ESI) for $C_{28}H_{32}N_7O_6S$ $[M+H]^+$ calcd: 594.2129, found: 594.2135.

Procedure for Modification of Bovine Serum Albumin (BSA) with 14. A solution of BSA (10 μL of 0.5 mM in H_2O), **14** (5 μL of 50 mM in DMSO), DABCO (5 μL of 50 mM in H_2O) and PBS (80 μL, 50 mM, pH 8.1) were mixed in a 1.0 mL Eppendorf tube. In the case of lysozyme, a solution of lysozyme (5 μL of 1.0 mM in H_2O), **14** (5 μL of 50 mM in DMSO), DABCO (5 μL of 50 mM in H_2O) and PBS (85 μL, 50 mM, pH 8.1) were mixed in a 1.5 mL Eppendorf tube. The reaction mixture was kept at 37 ^oC for 5 h. The protein solution was spin filtered three times by Ultrafree-MC microcentrifuge filter (nominal MW limit of 10,000 Da) for 20 min at 4,000 rpm to remove the excess reagent. The cysteine-modified BSA was subjected to SDS-PAGE and ESI-MS ananlysis.

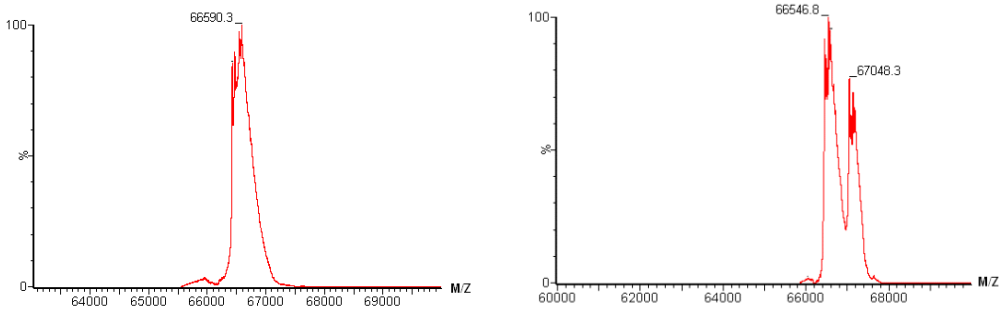


Figure S12 ESI-MS spectra of BSA (left) and product mixture with **14**-modified BSA (right).

Procedure for SDS-PAGE Analysis. The **14**-modified BSA (10 μL) was mixed with 2X loading buffer (10 μL) in a 1.5 mL Eppendrof tube and boiled for 10 min. The boiled solution (10 μL) was analyzed by 12% SDS-PAGE and fluorescence visualization and finally stained with Coomassie blue.

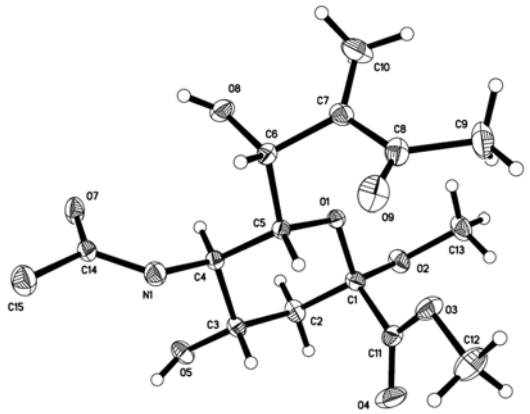


Figure S13. Crystal structure of **7b**

Table S2. Crystal data and structure refinement for BCLGL1 (22 Feb 2011).

Identification code	lgl1
Empirical formula	$C_{15}H_{23}NO_8 .H_2O$
Formula weight	363.36
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P2(1)2(1)2(1)
Unit cell dimensions	a = 7.0942(2) Å α= 90°.
	b = 10.4907(3) Å β = 90°.
	c = 24.0613(6) Å γ = 90°.
Volume	1790.72(8) $Å^3$
Z	4
Density (calculated)	1.348 Mg/m^3
Absorption coefficient	0.112 mm^{-1}
F(000)	776
Crystal size	0.60 x 0.60 x 0.50 mm^3
Theta range for data collection	2.58 to 27.56°.
Index ranges	-9<=h<=9, -13<=k<=13, -31<=l<=29
Reflections collected	20702
Independent reflections	4117 [R(int) = 0.0313]
Completeness to theta = 27.56°	99.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7456 and 0.8833

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4117 / 0 / 234
Goodness-of-fit on F^2	1.005
Final R indices [I>2sigma(I)]	R1 = 0.0398, wR2 = 0.1080
R indices (all data)	R1 = 0.0501, wR2 = 0.1161
Absolute structure parameter	-0.4(10)
Largest diff. peak and hole	0.238 and -0.234 e.$Å^{-3}$

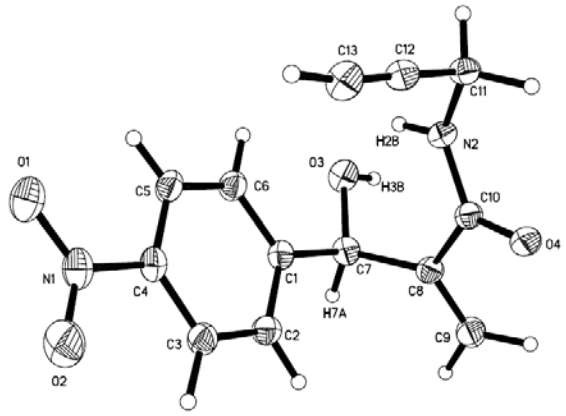


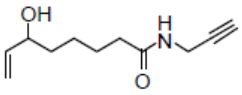
Figure S14. Crystal structure of **14d**

Table S3. Crystal data and structure refinement for BCLGL32 (27 Oct 2011)

Identification code	lgl32
Empirical formula	C_{13} H_{12} N_2 O_4
Formula weight	260.25
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)/n
Unit cell dimensions	a = 8.5310(4) Å α= 90°.
	b = 8.9258(3) Å β= 103.000(3)°.
	c = 17.4253(6) Å γ = 90°.
Volume	1292.86(9) $Å^3$
Z	4
Density (calculated)	1.337 Mg/m^3
Absorption coefficient	0.101 mm^{-1}
F(000)	544
Crystal size	0.36 x 0.30 x 0.22 mm^3
Theta range for data collection	2.47 to 27.61°.
Index ranges	-11<=h<=11, -11<=k<=11, -22<=l<=22
Reflections collected	20473
Independent reflections	2963 [R(int) = 0.0679]
Completeness to theta = 27.61°	98.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7456 and 0.5528
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2963 / 0 / 221

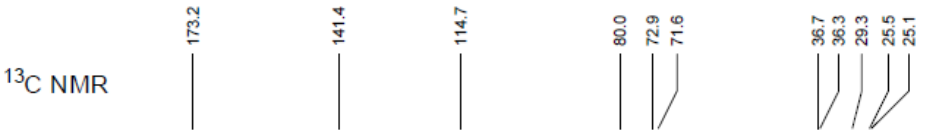
Goodness-of-fit on F^2	1.003
Final R indices [I>2sigma(I)]	R1 = 0.0628, wR2 = 0.1478
R indices (all data)	R1 = 0.1474, wR2 = 0.2349
Extinction coefficient	0.052(3)
Largest diff. peak and hole	0.258 and -0.224 e.$Å^{-3}$

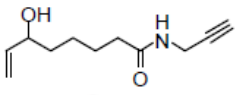
^{1}H NMR



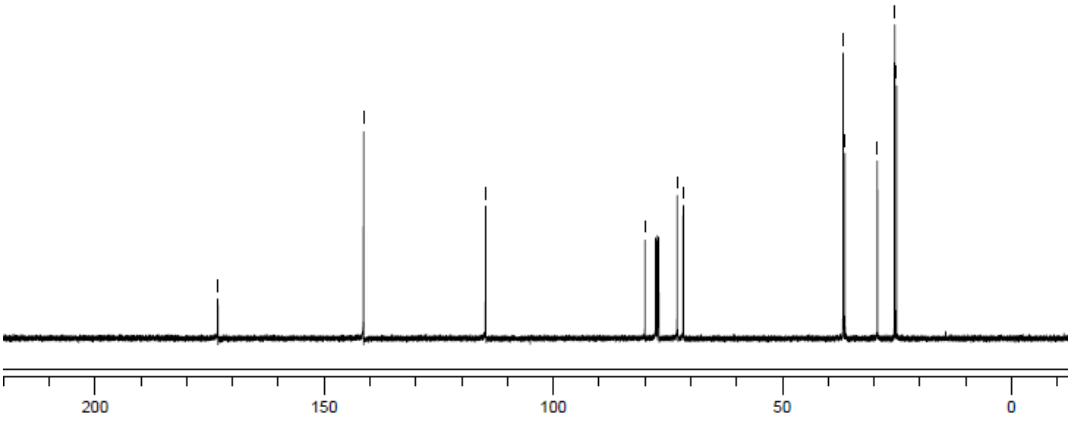
2e

^{13}C NMR

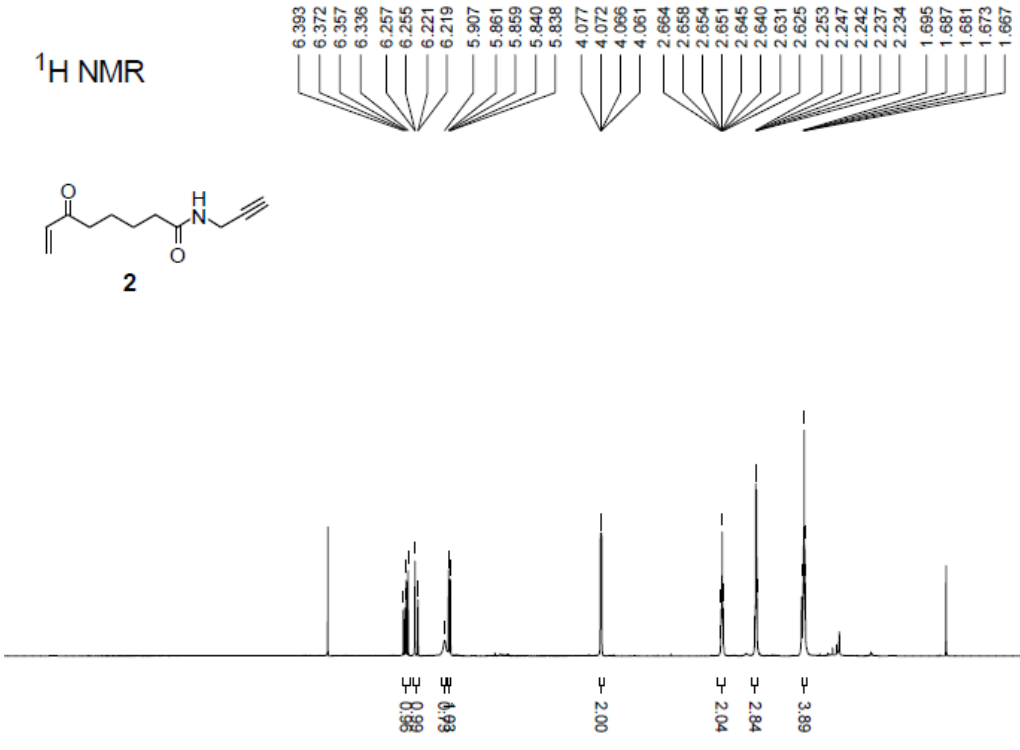




2e

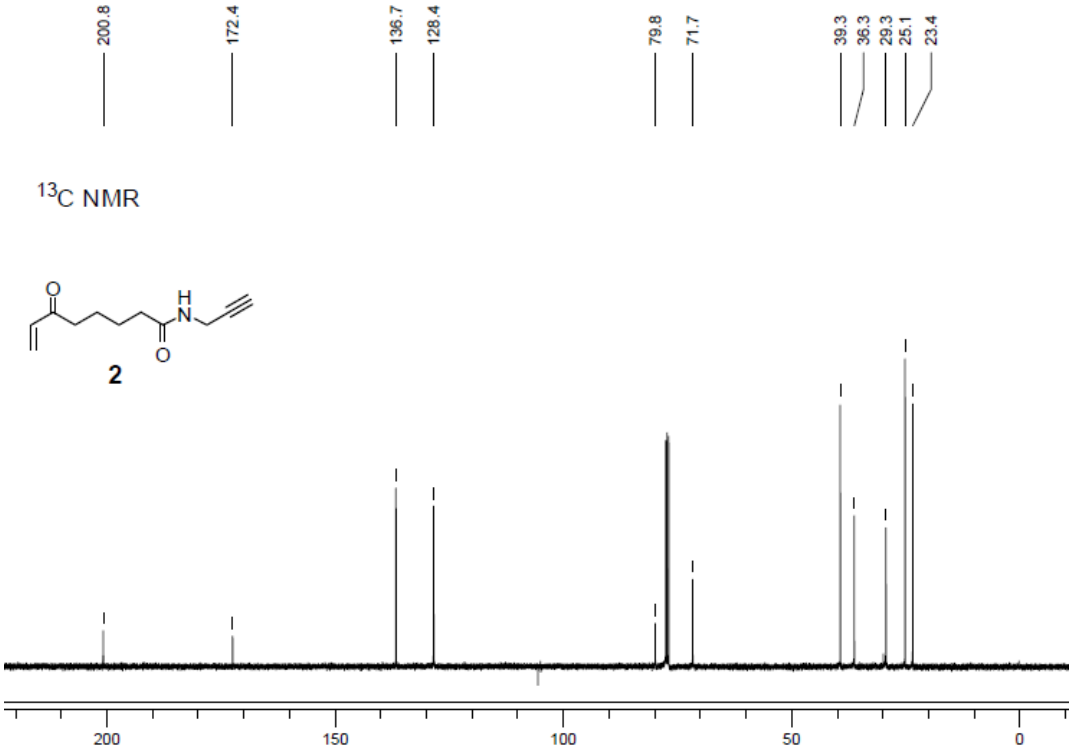


^{1}H NMR

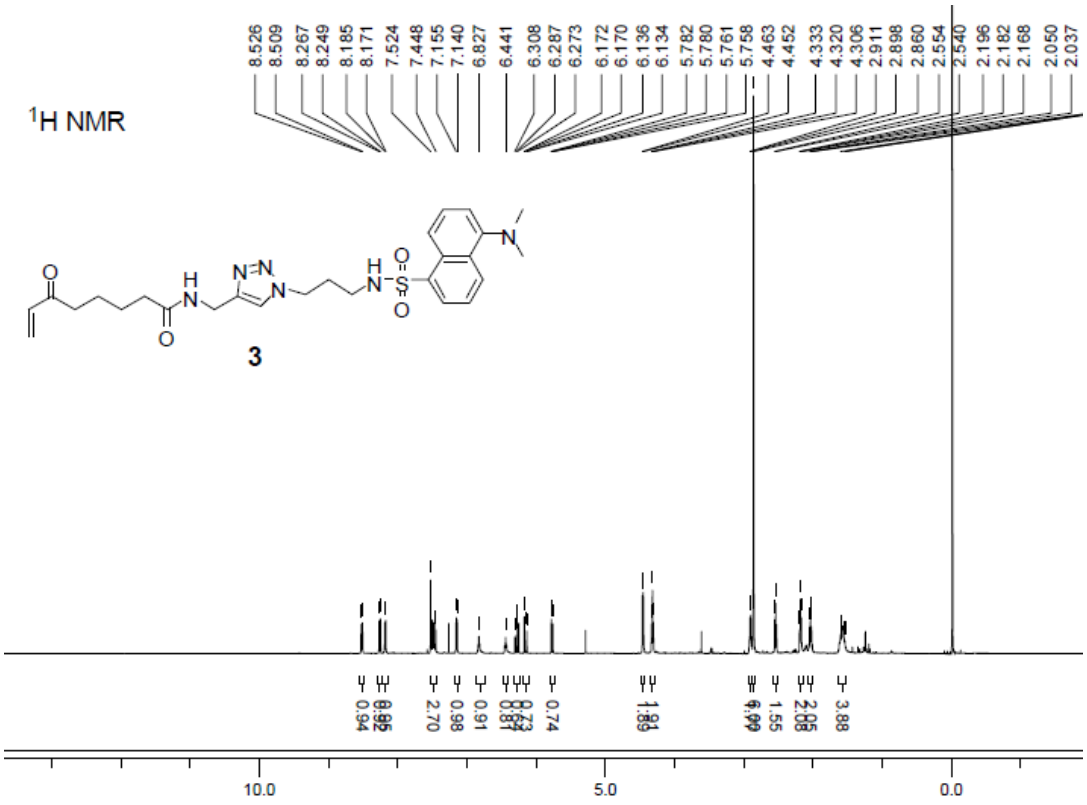


2

^{13}C NMR



2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8.249
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8.171
7.524
7.448
7.155
7.140
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6.441
6.308
6.287
6.273
6.172
6.170
6.136
6.134
5.782
5.780
5.761
5.758
4.463
4.452
4.333
4.320
4.306
2.911
2.898
2.860
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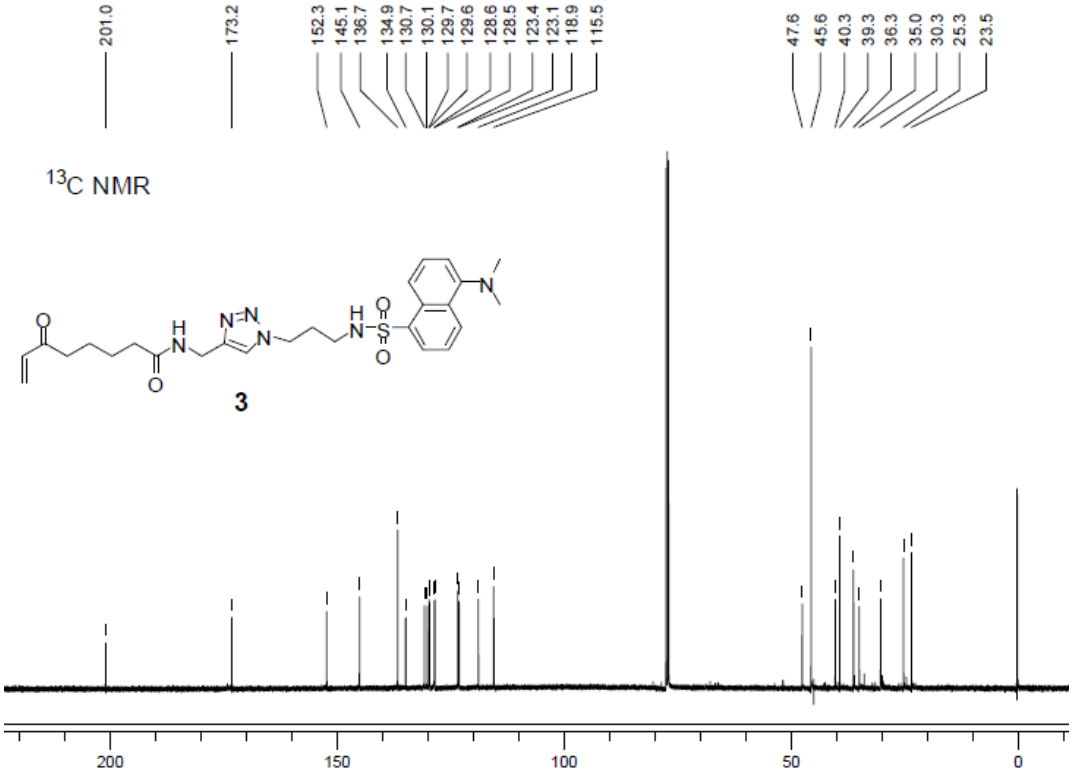
2.540
2.196
2.182
2.168
2.050
2.037
3
10.0
5.0
0.0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152.3
145.1
136.7
134.9
130.7
130.1
129.7
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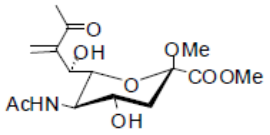
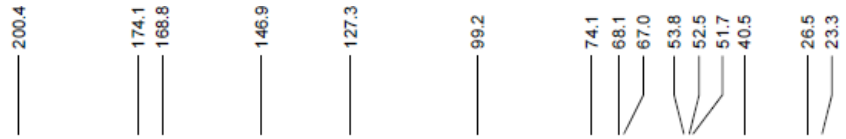
128.5
123.4
123.1
118.9
115.5
47.6
45.6
40.3
39.3
36.3
35.0
30.3
25.3
23.5
3
200
150
100
50
0

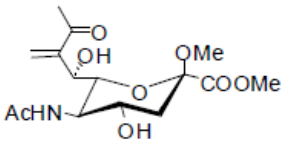
^{1}H NMR



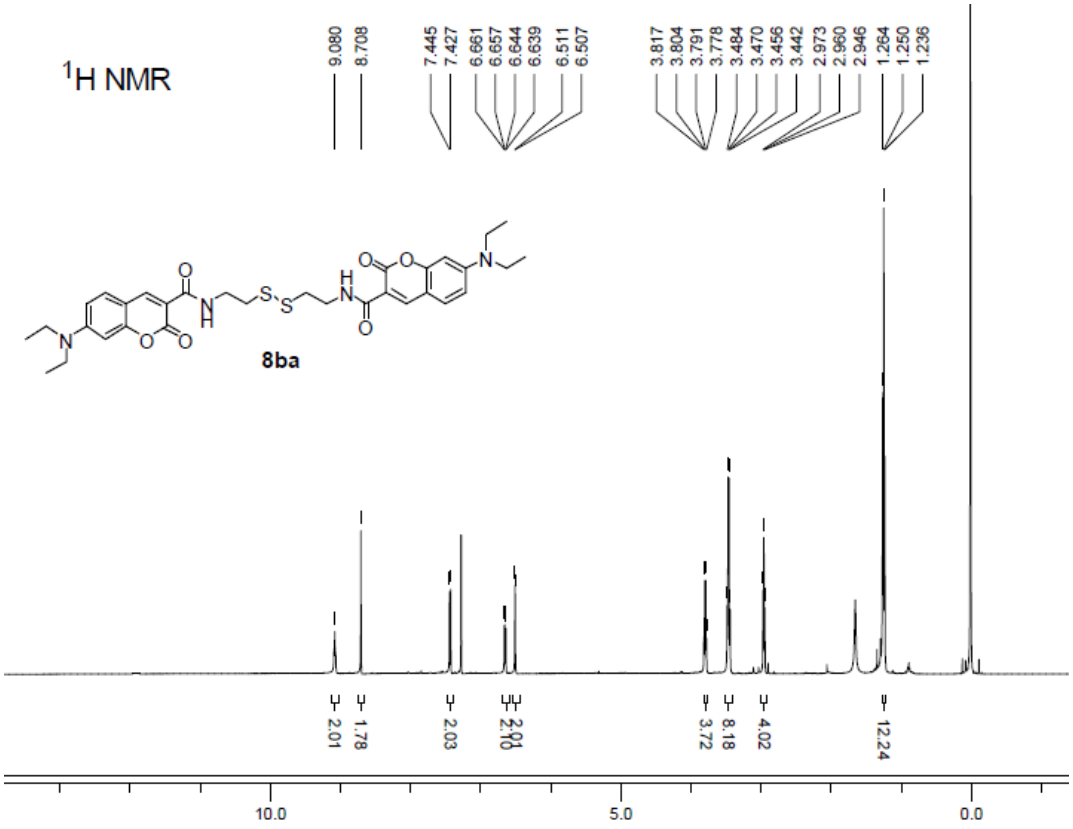
7b

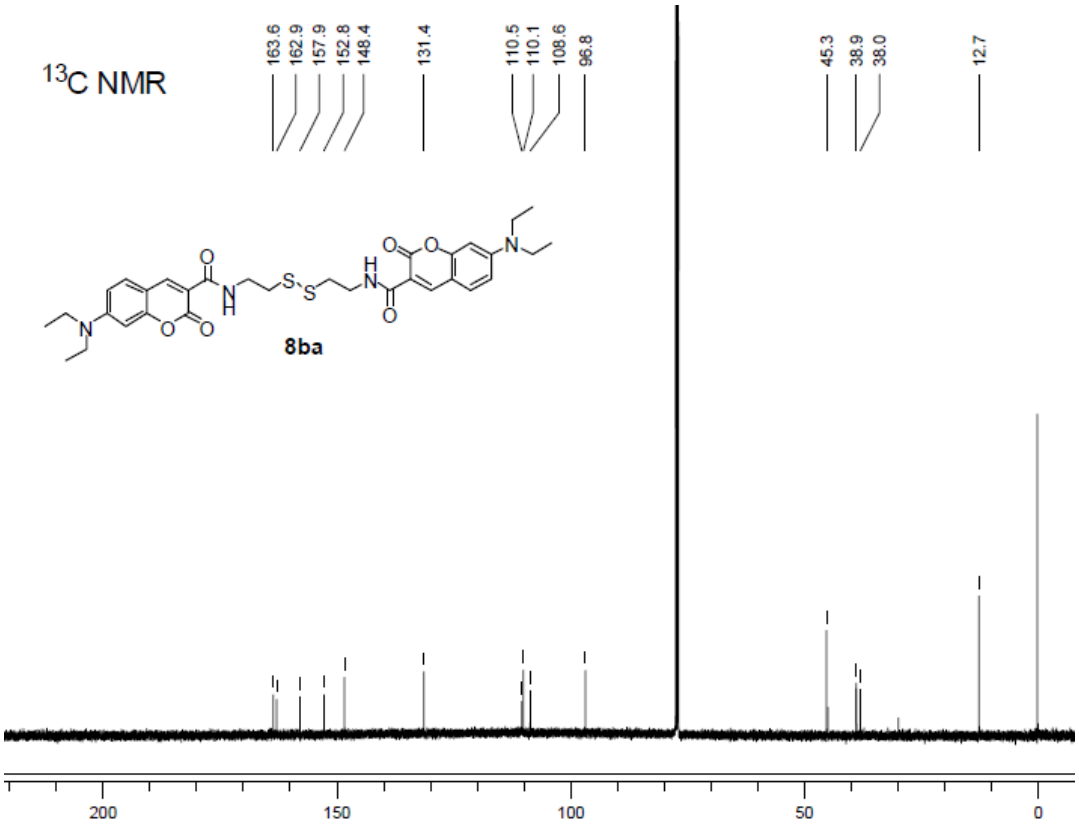


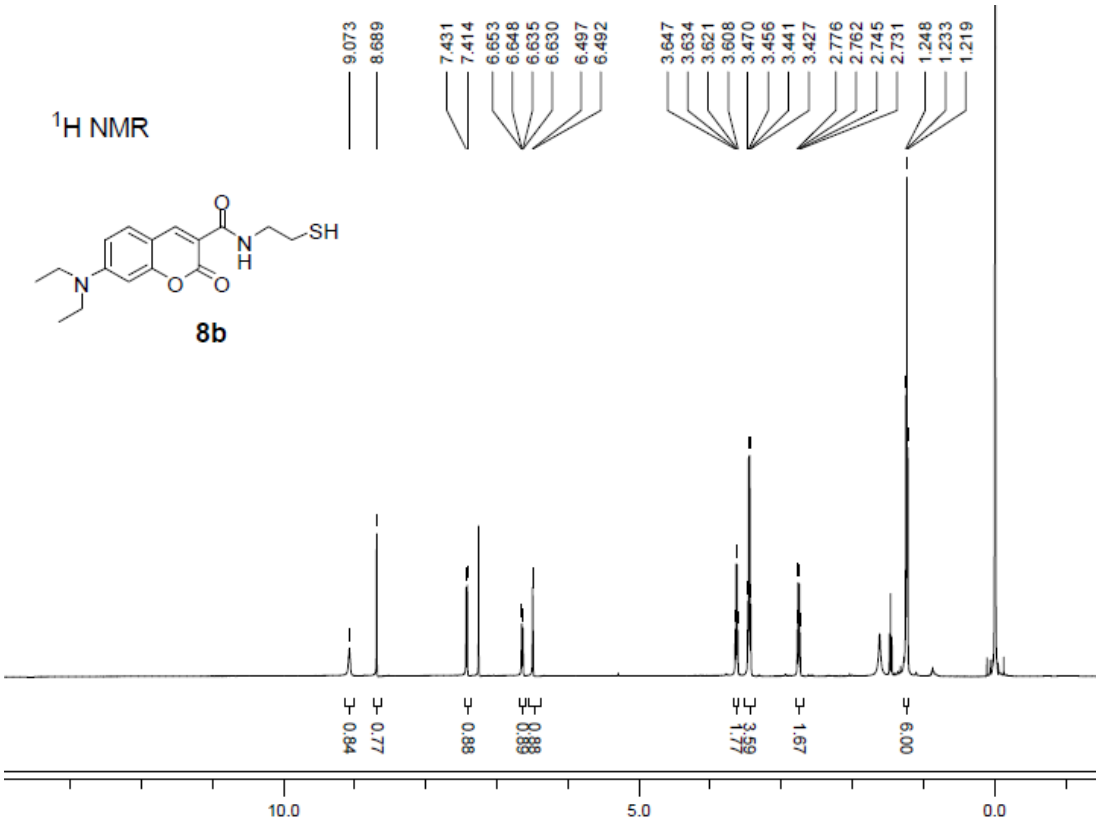
^{13}C NMR

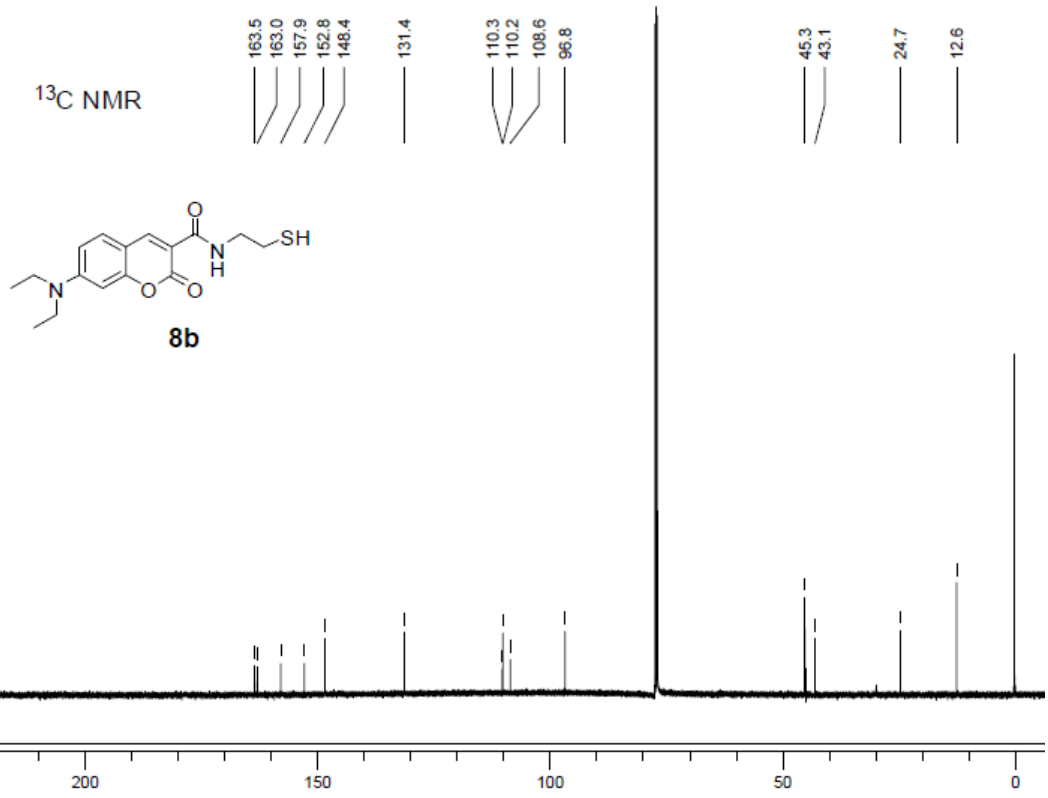


7b


^{1}H NMR
9.080
8.708
7.445
7.427
6.661
6.657
6.644
6.639
6.511
6.507
3.817
3.804
3.791
3.778
3.484
3.470
3.456
3.442
2.973
2.960
2.946
1.264
1.250
1.236
8ba
2.01
1.78
2.03
2.01
3.72
8.18
4.02
12.24
10.0
5.0
0.0

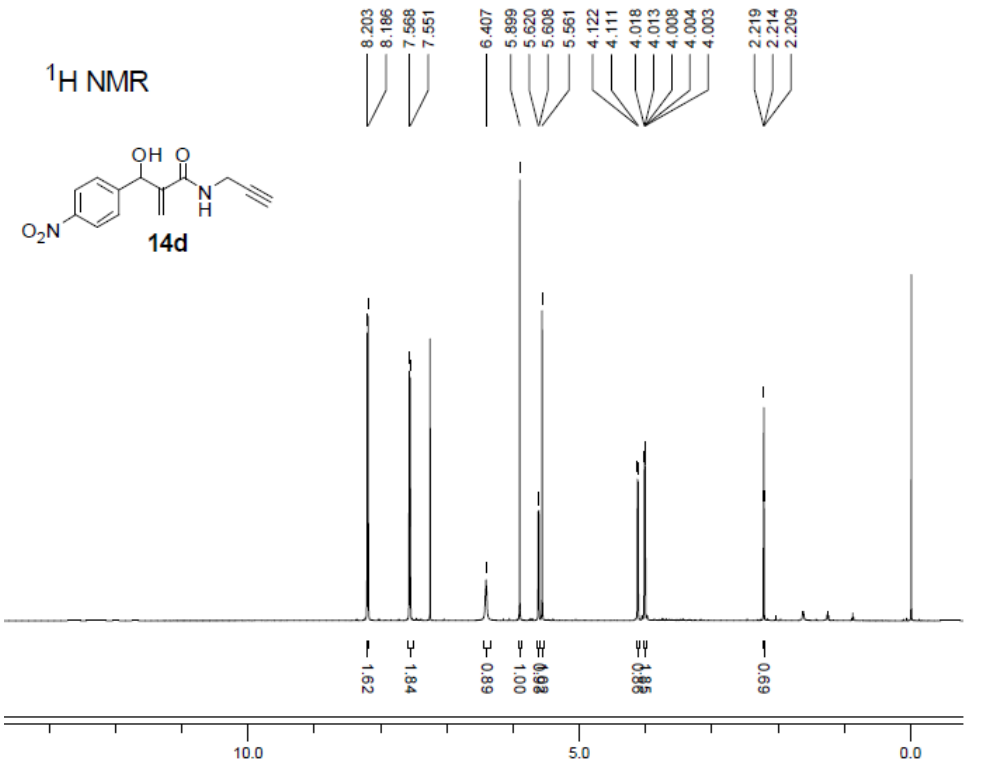

^{13}C NMR
163.6
162.9
157.9
152.8
148.4
131.4
110.5
110.1
108.6
96.8
45.3
38.9
38.0
12.7
8ba
200
150
100
50
0

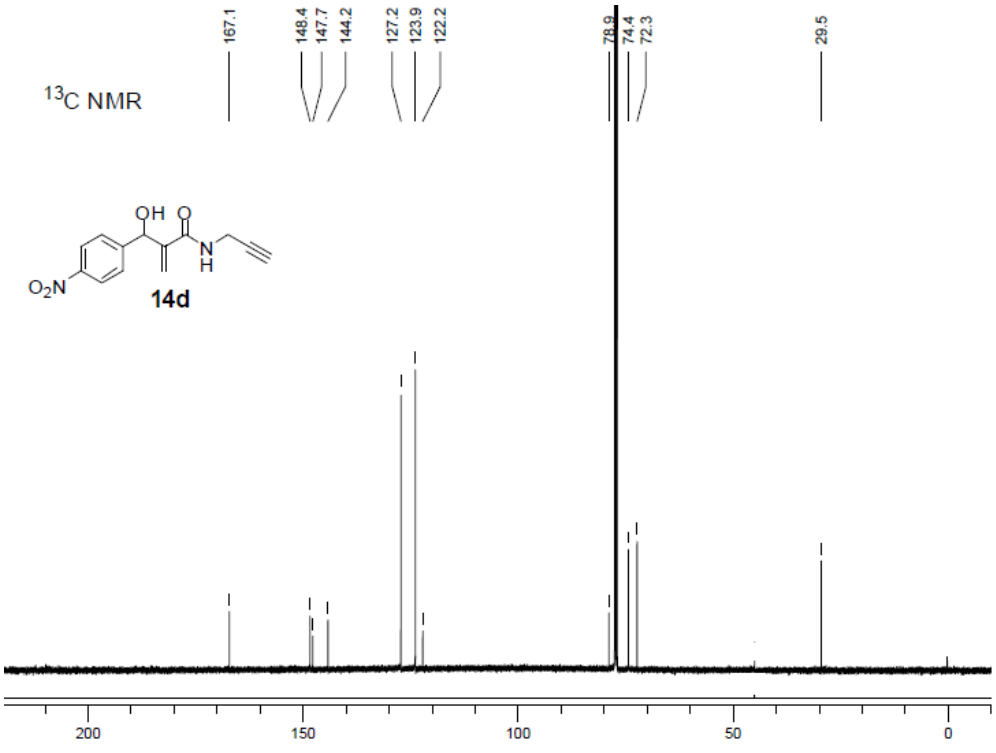

^{1}H NMR
9.073
8.689
7.431
7.414
6.653
6.648
6.635
6.630
6.497
6.492
3.647
3.634
3.621
3.608
3.470
3.456
3.441
3.427
2.776
2.762
2.745
2.731
1.248
1.233
1.219
0.84
0.77
0.88
0.89
0.88
3.59
1.77
1.67
6.00
10.0
5.0
0.0

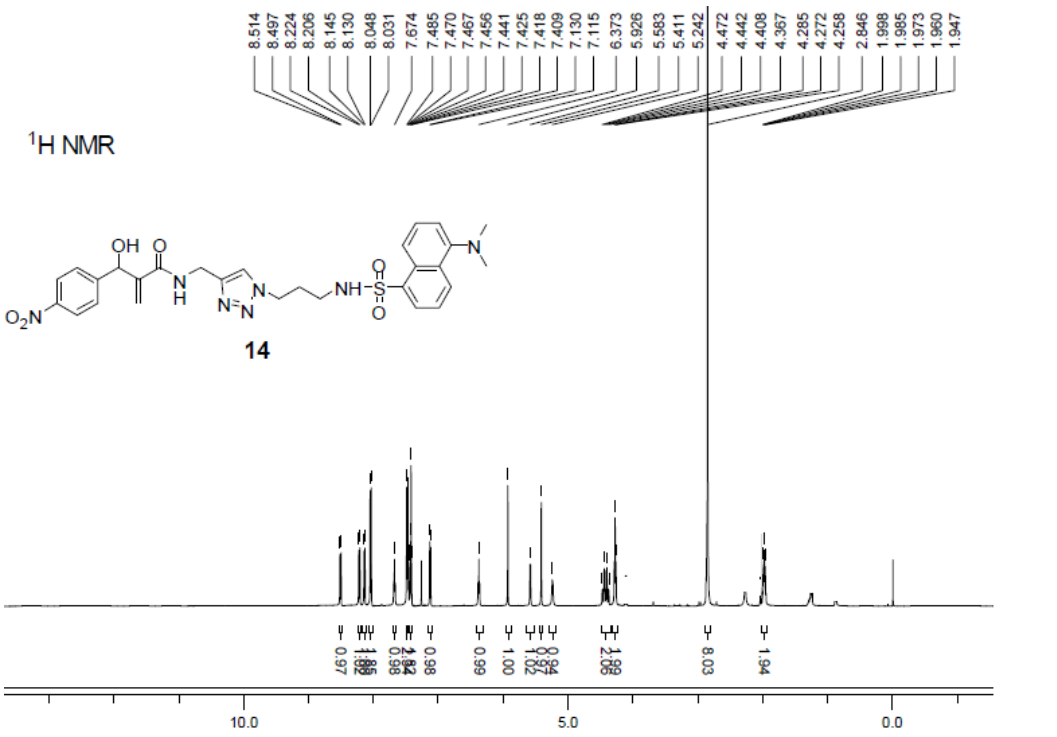

^{13}C NMR
163.5
163.0
157.9
152.8
148.4
131.4
110.3
110.2
108.6
96.8
45.3
43.1
24.7
12.6
200
150
100
50
0

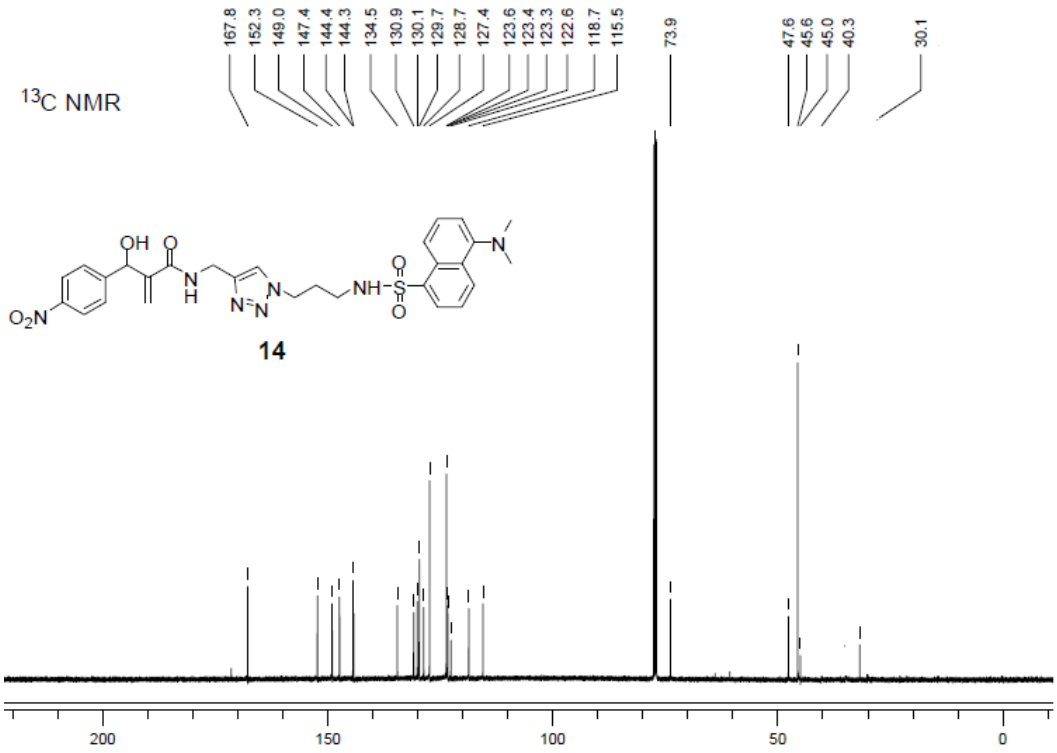
SH
N
H
O
N
O
O

8b


^{1}H NMR
8.203
8.186
7.568
7.551
6.407
5.899
5.620
5.608
5.561
4.122
4.111
4.018
4.013
4.008
4.004
4.003
2.219
2.214
2.209
OH
O
N
H
O_2N
14d
1.62
1.84
0.89
1.00
0.99
0.69
10.0
5.0
0.0


^{13}C NMR
167.1
148.4
147.7
144.2
127.2
123.9
122.2
78.9
74.4
72.3
29.5
OH
O
N
H
O_2N
14d
200
150
100
50
0


^{1}H NMR
8.514
8.497
8.224
8.206
8.145
8.130
8.048
8.031
7.674
7.485
7.470
7.467
7.456
7.441
7.425
7.418
7.409
7.130
7.115
6.373
5.926
5.583
5.411
5.242
4.472
4.442
4.408
4.367
4.285
4.272
4.258
2.846
1.998
1.985
1.973
1.960
1.947
OH
O
N
H
N
N
NH
S
O
O
O_2N
N
14
0.97
0.98
0.99
1.00
2.06
8.03
1.94
10.0
5.0
0.0


^{13}C NMR
167.8
152.3
149.0
147.4
144.4
144.3
134.5
130.9
130.1
129.7
128.7
127.4
123.6
123.4
123.3
122.6
118.7
115.5
73.9
47.6
45.6
45.0
40.3
30.1
OH
O
N
H
N
N
NH
S
O
O
O_2N
N
14
200
150
100
50
0