Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Gold-Mediated Bifunctional Modification of Oligosaccharides via a Three-Component Coupling Reaction

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# SUPPORTING INFORMATION

## General Procedure

All chemicals and reagents were commercially available and used without further purification. Milli-Q® water used as reaction solvent in oligosaccharide modification and LC-MS 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. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker DPX-400 or DPX-600, Varian Unity Inova 400 NB or 500 NB spectrometer. All chemical shifts are quoted on the scale in ppm using TMS or residual solvent as the internal standard. Coupling constants (*J*) are reported in Hertz (Hz) with the following splitting abbreviations: s = singlet, br s = broad singlet, d = doublet, dd= double doublet, t = triplet and m = multiplet. Low resolution and high resolution mass spectra were obtained on an ESI source of Q-TOF 2™ mass spectrometer (Waters-Micromass, Manchester, United Kingdom) in the positive ion mode. IR spectra were reported with a Thermo Scientific Nicolet 380 FT-IR spectrometer.

## LC–MS Analysis of Bifunctional Modification of Oligosaccharides

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). 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. 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

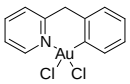
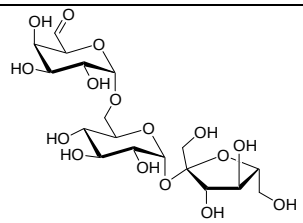
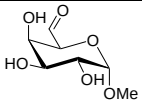
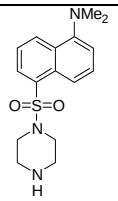
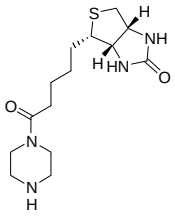
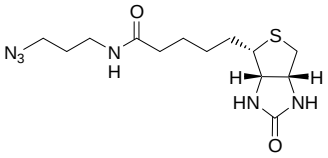
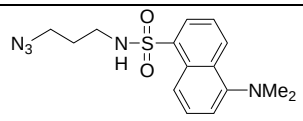
room temperature. The initial conditions for separation were 3% B in 0-3 min, followed by a linear gradient to 70% B in 4-30 min and 3% B in 31-45 min. The mass spectrometer was scanned over a *m*/*z* range of 200-2000, and the raw spectra were deconvoluted by the MassLynx 4.1 Transform Program (Waters, Manchester, UK). Desolvation and source temperatures were 150 °C and 80 °C respectively. Operating conditions optimized 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**

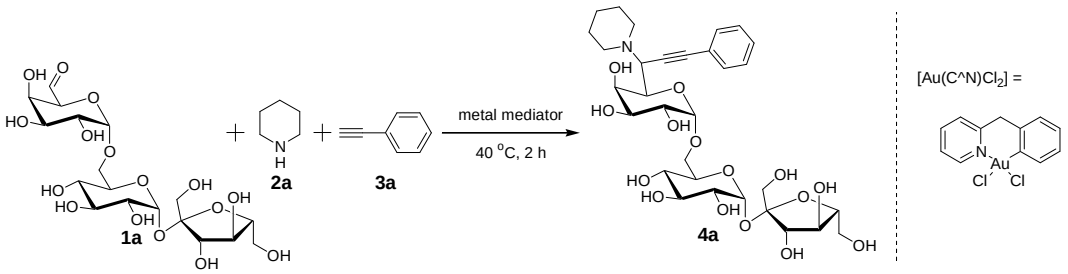
The crude reaction mixture of aldehyde-containing oligosaccharides (aldehyde) and bifunctionally modified oligosaccharides (product) was subjected to LC-MS analysis with elution time of 45 min. After data processing by MassLynx 4.1 Transform Program, aldehyde conversion at different time intervals was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

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

## Literature References

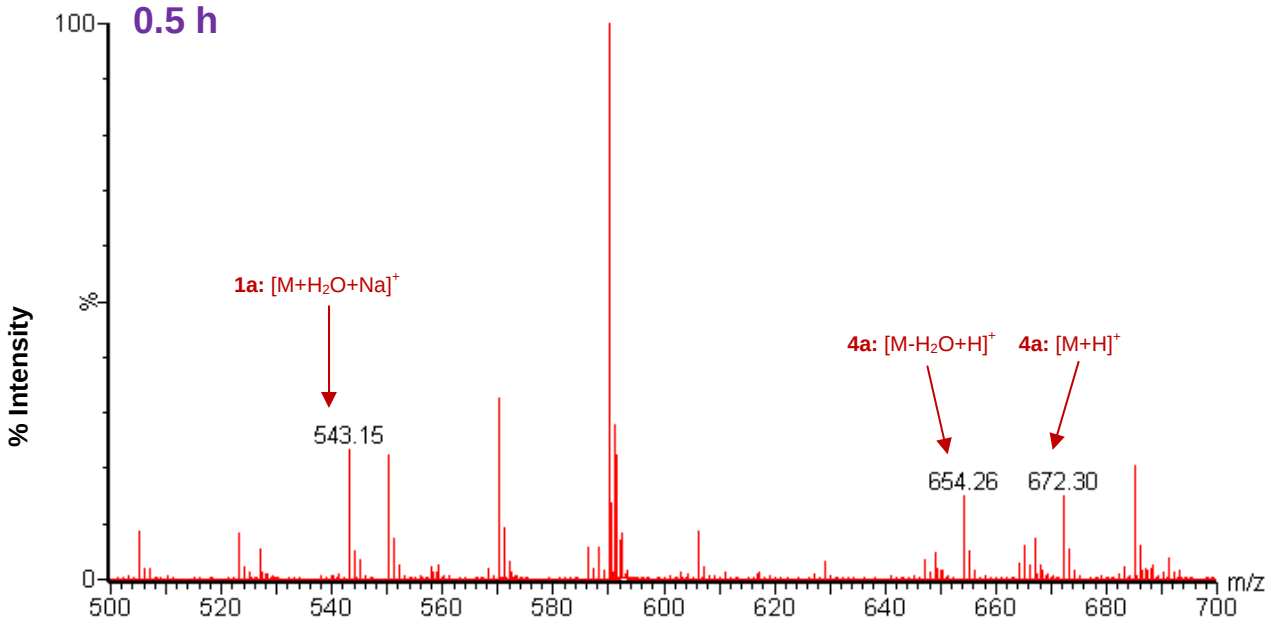
| | |
|---|---|
|  [Au(C^N)$Cl_2$] (HC^N = 2-benzylpyridine) | M. A. Cinellu, A. Zucca, S. Stoccoro, G. Minghetti, M. Manassero, M. Sansoni, *J. Chem. Soc., Dalton Trans.* 1995, **17**, 2865. |
|  D-raffinose aldehyde, **1a** | K. Parikka, M. Tenkanen, *Carbohydr. Res.* 2009, **344**, 14. |
|  Methyl alpha-D-galactopyranose aldehyde, **1c** | K. Parikka, M. Tenkanen, *Carbohydr. Res.* 2009, **344**, 14. |
|  *N*-Dansylpiperazine, **2g** | V. Sashuk, D. Schoeps, H. Plenio, *Chem. Commun.* 2009, **7**, 770. |
|  Piperazine biotinamide, **2h** | a) R. Ting, C. Harwig, U. A. D. Keller, S. McCormick, P. Austin, C. M. Overall, M. J. Adam, T. J. Ruth, D. M. Perrin, *J. Am. Chem. Soc.* 2008, **130**, 12045.<br>b) K. Susumu, B. C. Mei, H. Mattoussi, *Nat. Protoc.* 2009, **4**, 424. |
|  Biotin azide, **7a** | P. C. Lin, S. H. Ueng, M. C. Tseng, J. L. Ko, K. T. Huang, S. C. Yu, A. K. Adak, Y. J. Chen, C. C. Lin, *Angew. Chem. Int. Ed.* 2006, **118**, 4392. |
|  Dansyl azide, **7b** | W. G. Lewis, F. G. Magallon, V. V. Fokin, M. G. Finn, *J. Am. Chem. Soc.* 2004, **126**, 9152. |

**Procedure for Gold-Mediated Bifunctional Modification of D-Raffinose Aldehyde 1a**

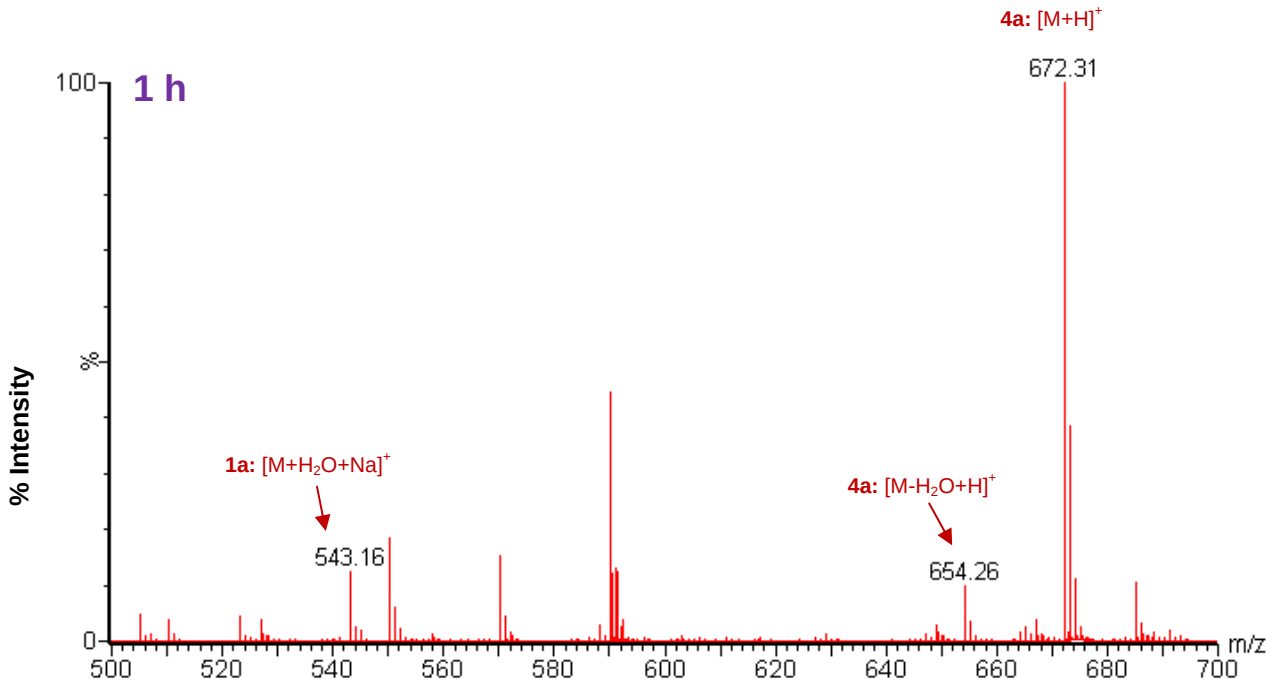



A mixture of D-raffinose aldehyde **1a** (10 µL of 100 mM in $H_2O$), piperidine **2a** (1 µL, 10 equiv.), phenylacetylene **3a** (1 µL, 10 equiv.) and $[Au(C^N)Cl_2]$ (HC^N = 2-benzylpyridine) (0.4 mg, 1 equiv.) in water (90 µL) was stirred at 40 °C. The crude reaction mixture was centrifuged. The clear liquor (2 µL) was analyzed by LC-MS for determination of the aldehyde conversion (Figure S1).

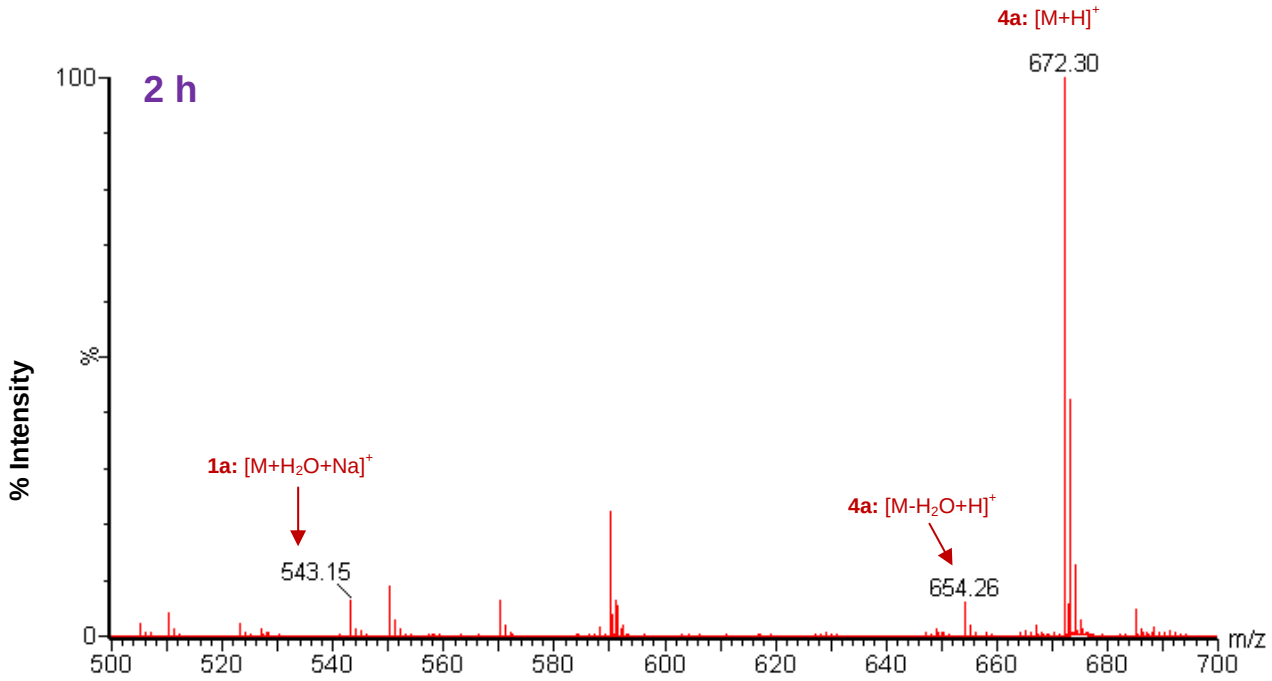
**a)**



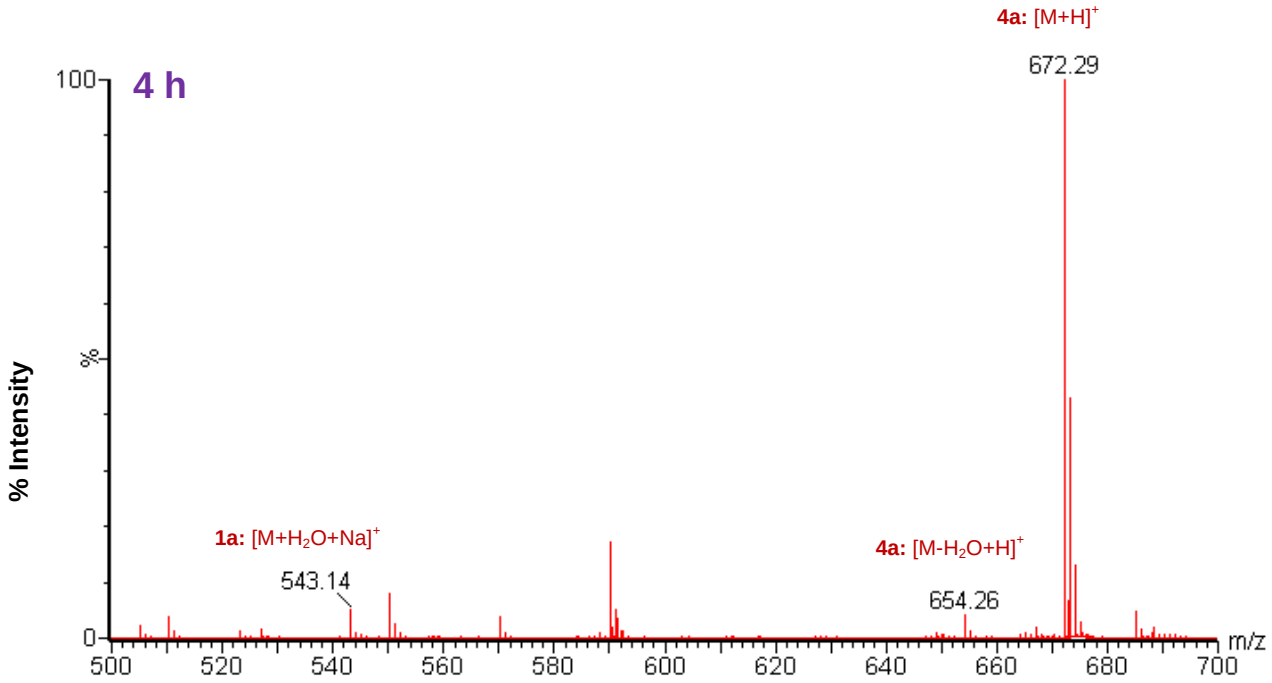
**b)**




**c)**



**d)**




**e)**

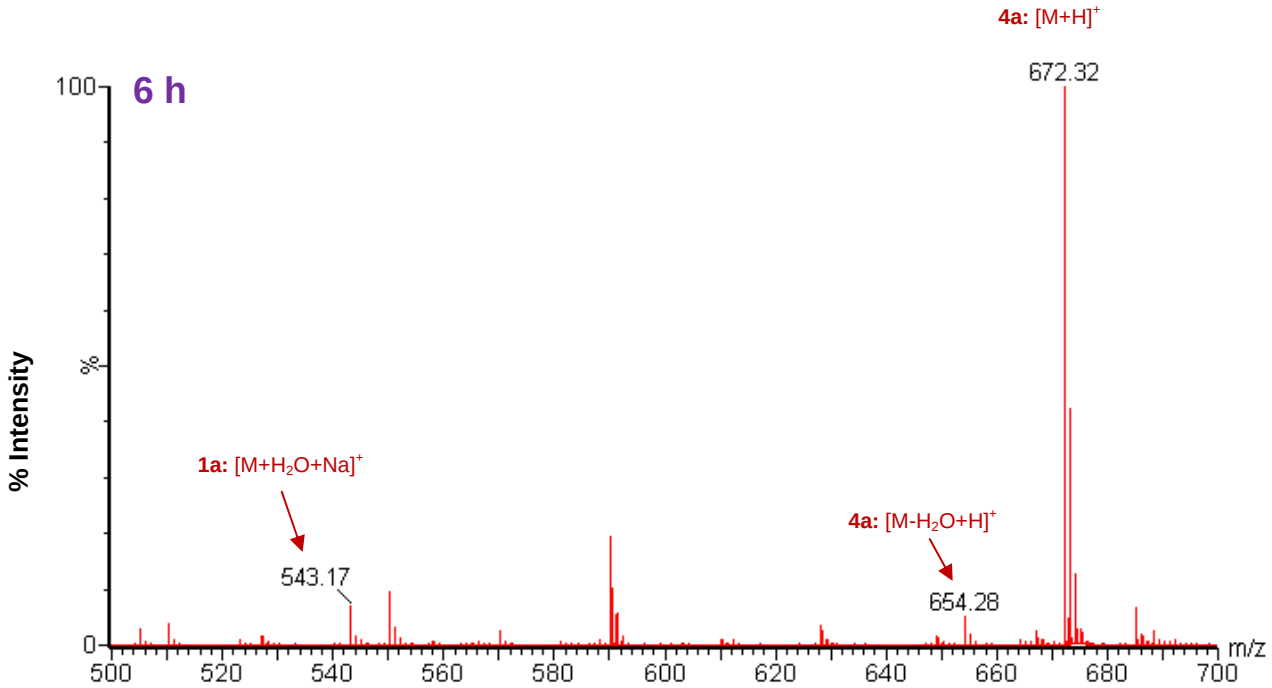



***Figure S1.*** MS spectra of **1a** (ESI source, $[M+H_2O+Na]^+$ = m/z 543) and **4a** (ESI source, $[M+H]^+$ = m/z 672 and $[M-H_2O+H]^+$ = m/z 654).

**Procedure for Synthesis of D-Stachyose Aldehyde 1b**

D-Stachyose aldehyde **1b** was generated according to the reported literature.[1] To a solution of D-stachyose (34 mg, 0.05 mmol) in water (404 μL) was added a mixture of galactose oxidase (20 μL of 54 U/mL in 50 mM PBS buffer at pH 7.1), catalase (52 μL of 21600 U/mL in 50 mM PBS buffer at pH 7.1) and horseradish peroxidase (24 μL of 188 U/mL in 50 mM PBS buffer at pH 7.1). The resulting solution was stirred at room temperature for 24 h. After the reaction, the solution was analyzed by ESI-MS (Figure S2).

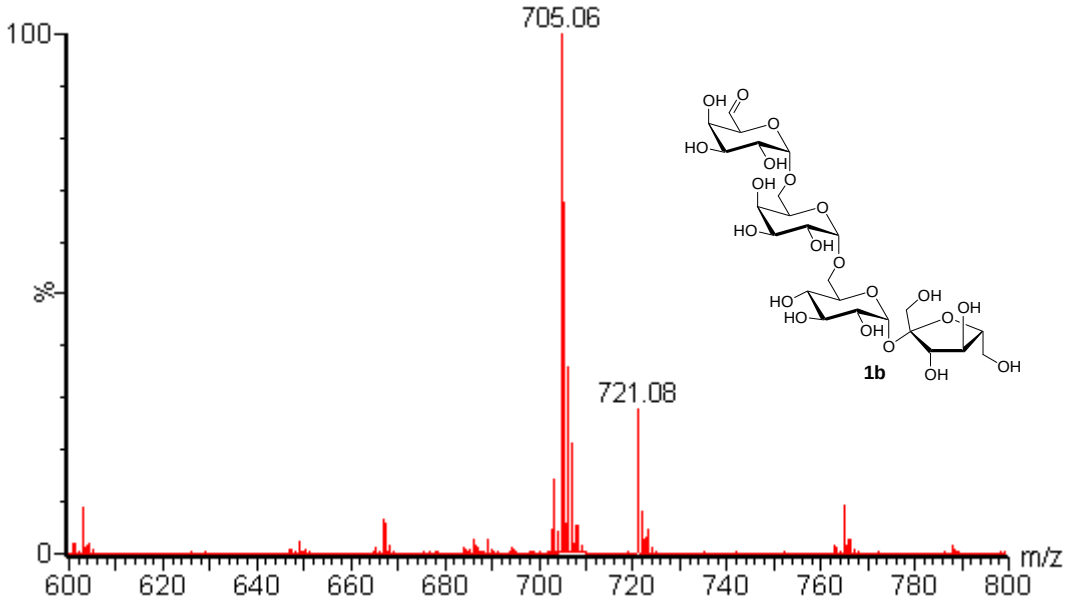
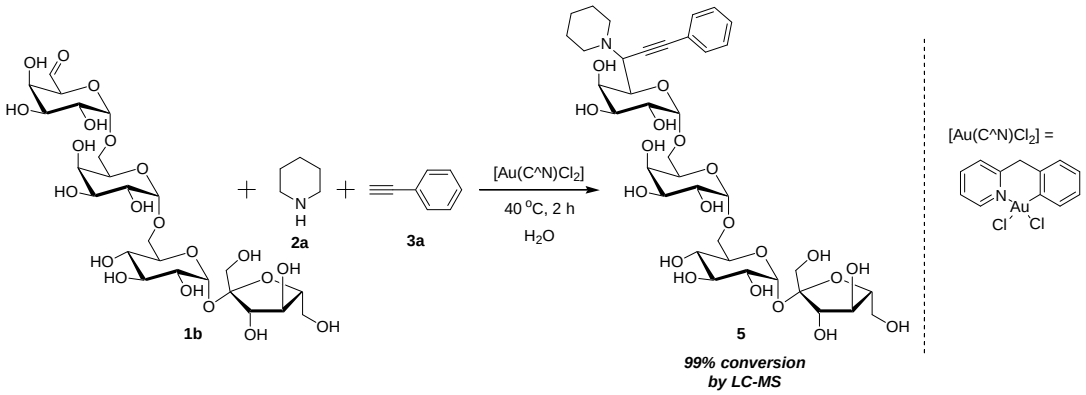



***Figure S2.*** MS spectrum of the **1b** (ESI source, $[M+H_2O+Na]^+$ = m/z 705.06, $[M+H_2O+K]^+$ = m/z 721.08).

**References**

(1) K. Parikka, M. Tenkanen, *Carbohydr. Res.* 2009, **344**, 14.

**Procedure for Gold-Mediated Bifunctional Modification of D-Stachyose Aldehyde 1b**




A mixture of D-stachyose aldehyde **1b** (10 μL of 100 mM in $H_2O$), piperidine **2a** (1 μL, 10 equiv.), phenylacetylene **3a** (1 μL, 10 equiv.) and $[Au(C^N)Cl_2]$ (HC^N = 2-benzylpyridine) (0.4 mg, 1 equiv.) in water (90 μL) were stirred at 40 °C for 2 h to give product **5**. The crude reaction mixture was centrifuged to take the clear liquor, which was analyzed by LC-MS for determination of the aldehyde conversion. The following MS spectrum of product **5** was deconvoluted from the extracted ion chromatogram (Figure S3).

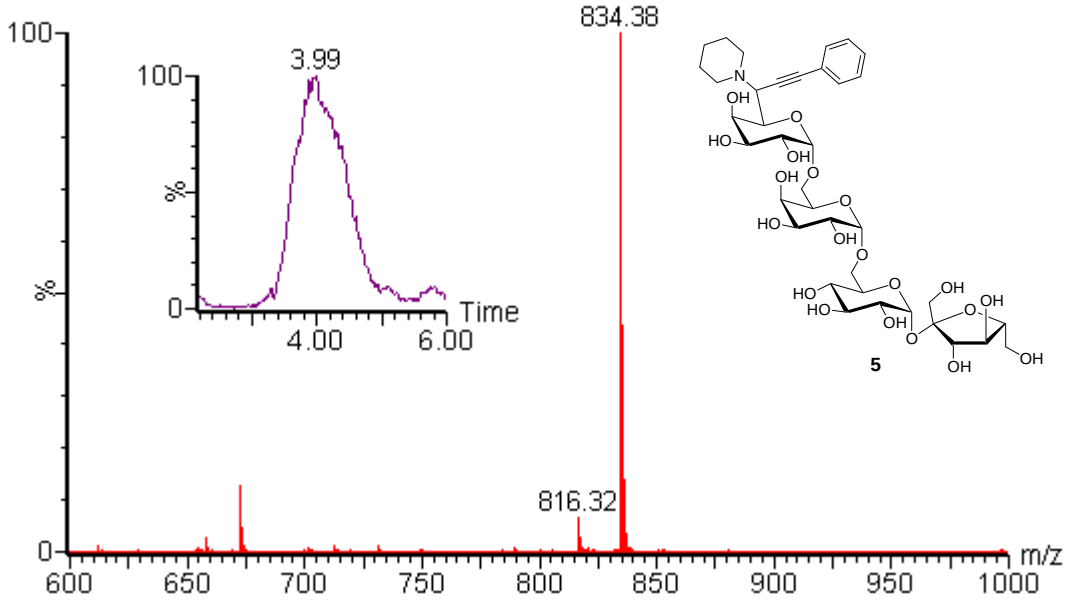



***Figure S3.*** MS spectrum of **5** (ESI source, $[M+H]^+$ = m/z 834.38, $[M-H_2O+H]^+$ = m/z 816.32) and the XIC chromatogram of **5** at t = 3.99 min (inset).

**Procedure for Time Course Experiment of Gold-Mediated Bifunctional Modification of D-Raffinose Aldehyde 1a**

A mixture of D-raffinose aldehyde **1a** (20 μL of 100 mM in $H_2O$), piperidine **2a** (1 μL, 5 equiv.), phenylacetylene **3a** (1 μL, 5 equiv.) and [Au(C^N)$Cl_2$] (HC^N = 2-benzylpyridine) (0.4 mg, 1 equiv.) in water (90 μL) was stirred at 40 $^{o}$C. The aldehyde conversion of the crude reaction mixture was then monitored by LC-MS. The clear liquor of the centrifuged reaction mixture (2 μL) was injected in 0.5, 1, 2, 4 and 6 h. The above coupling reaction of **1a** was repeated at 10, 25 and 50 equivalents of **2a** and **3a** (Figure S4).

The above coupling reaction of **1a** with **2a** and **3a** was repeated at 50 $^{o}$C (Figure S5).

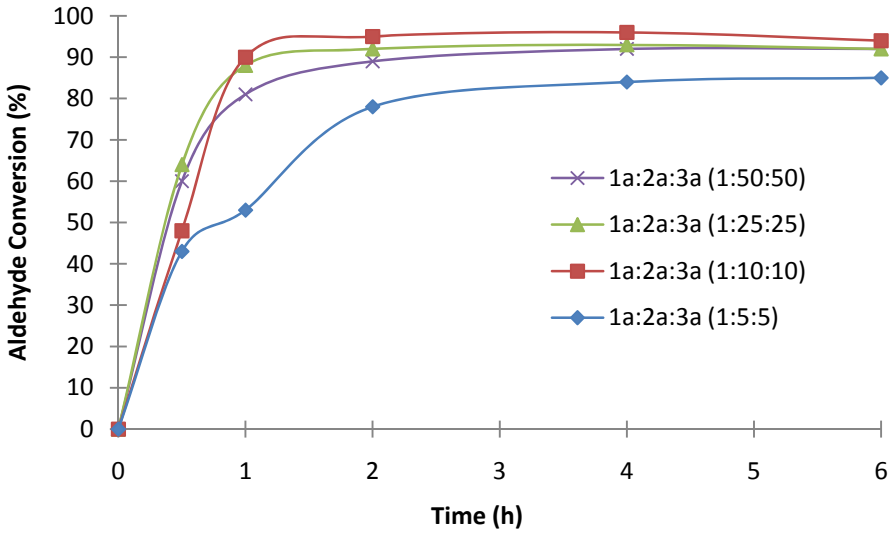



***Figure S4.*** Time course experiment of the gold-mediated bifunctional modification of D-raffinose aldehyde **1a** with piperidine **2a** and phenylacetylene **3a** at 40 °C.

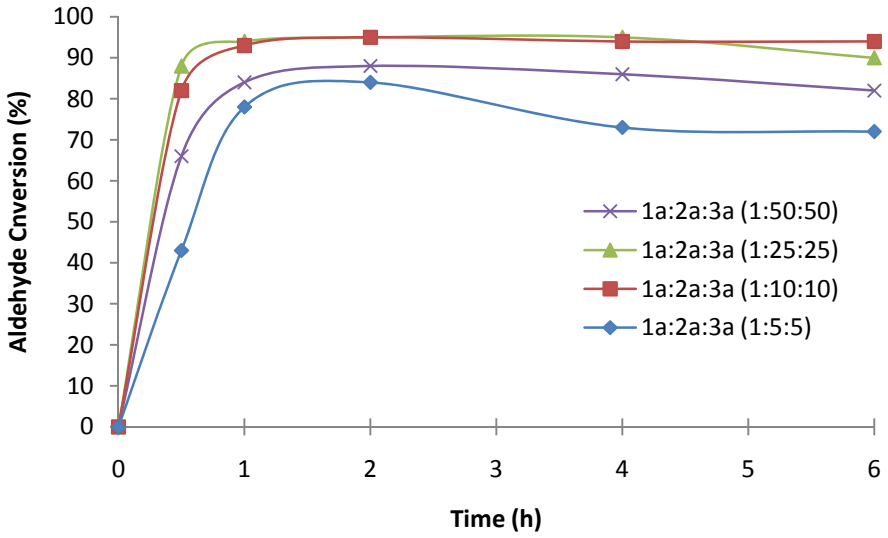
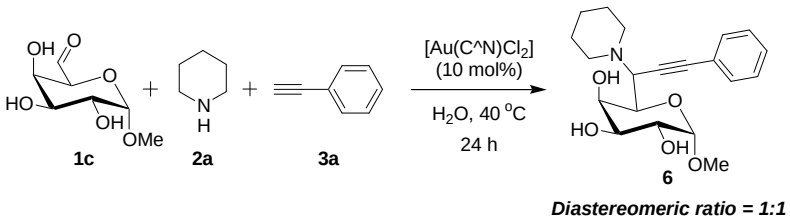



***Figure S5.*** Time course experiment of the gold-mediated bifunctional modification of D-raffinose aldehyde **1a** with piperidine **2a** and phenylacetylene **3a** at 50 °C.

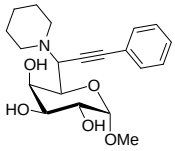
**Procedure for Gold-Mediated Bifunctional Modification of Methyl alpha-D-Galactopyranose Aldehyde 1c**




***Diastereomeric ratio = 1:1***

A mixture of methyl alpha-D-galactopyranose aldehyde **1c** (190 mg, 1 mmol), piperidine **2a** (1 mL, 10 equiv.), phenylacetylene **3a** (1 mL, 10 equiv.) and [Au(C^N)$Cl_2$] (HC^N = 2-benzylpyridine) (44 mg, 0.1 equiv.) in water (1 mL) was stirred at 40 $^{o}$C for 24 h. The reaction mixture was extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed by water (50 mL) and then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel using methanol/dichloromethane as eluent to give product **6** in 10% isolated yield (36 mg) with diastereomeric ratio 1:1.

**Characterization Data of Compound 6 (1:1 Diastereomeric Mixture)**




10% yield, pale yellow syrup; analytical TLC (silica gel 60) (8% MeOH in $CH_2Cl_2$), $R_f$ = 0.45; $^1$H NMR (500 MHz, $CD_3Cl$): $\delta$ = 7.45-7.47 (m, 4H), 7.26-7.31 (m, 6H), 5.35 (t, $J$ = 2.5 Hz, 2H), 5.05 (d, $J$ = 2.5 Hz, 2H), 4.32 (dd, $J$ = 8.0, 2.5 Hz, 2H), 4.16 (d, $J$ = 5.3 Hz, 2H), 3.72 (dd, $J$ = 8.0, 2.5 Hz, 2H), 3.55 (s, 3H, -OMe), 3.56 (s, 3H, -OMe), 2.62-2.74 (m, 4H), 2.52 (br s, 4H), 1.64-1.72 (m, 4H), 1.56-1.64 (m, 4H),

1.40-1.48 (m, 4H) ppm; $^{13}$C NMR (150 MHz, $CD_3Cl$): $\delta$ = 148.14, 148.09, 131.94, 128.43, 123.06, 103,29, 103.00, 100.39, 100.20, 87.80, 87.64, 83.82, 83.64, 72.34, 72.15, 68.27, 67.91, 61.03, 60.93, 56.94, 56.64, 51.10, 50.92, 29.86, 26.10, 24.48 ppm; IR (KBr): = 3408, 2925, 2852, 2230, 1675, 1598, 1490, 1443, 1261, 1196, 1169, 1151, 1089, 1014, 756, 691 cm$^{-1}$; MS (ESI$^+$): *m/z* (%) = 344 [M - $H_2O$ + H]$^+$; HRMS (ESI$^+$) calcd. for [$C_{20}H_{27}NO_5$ - $H_2O$ + H]$^+$ 344.1862, found 344.1861.

**Procedure for Gold-Mediated Bifunctional Modification of D-Raffinose Aldehyde Coupling with Amines 2a-2f and Alkynes 3a-3l to give 4a-4u**

A mixture of D-raffinose aldehyde (10 μL of 100 mM in $H_2O$), amines **2a-2f**, alkynes **3a-3l** and [Au(C^N)$Cl_2$] (HC^N = 2-benzylpyridine) (0.4 mg, 1 equiv.) in water (90 μL) was stirred at 40-50 $^o$C for 2-6 h. The crude reaction mixture was centrifuged to take the clear liquor, which was analyzed by LC-MS for determination of the aldehyde conversion. The following MS spectra of **4a-4u** are deconvoluted from the corresponding extracted ion chromatograms (Figure S6-S26).

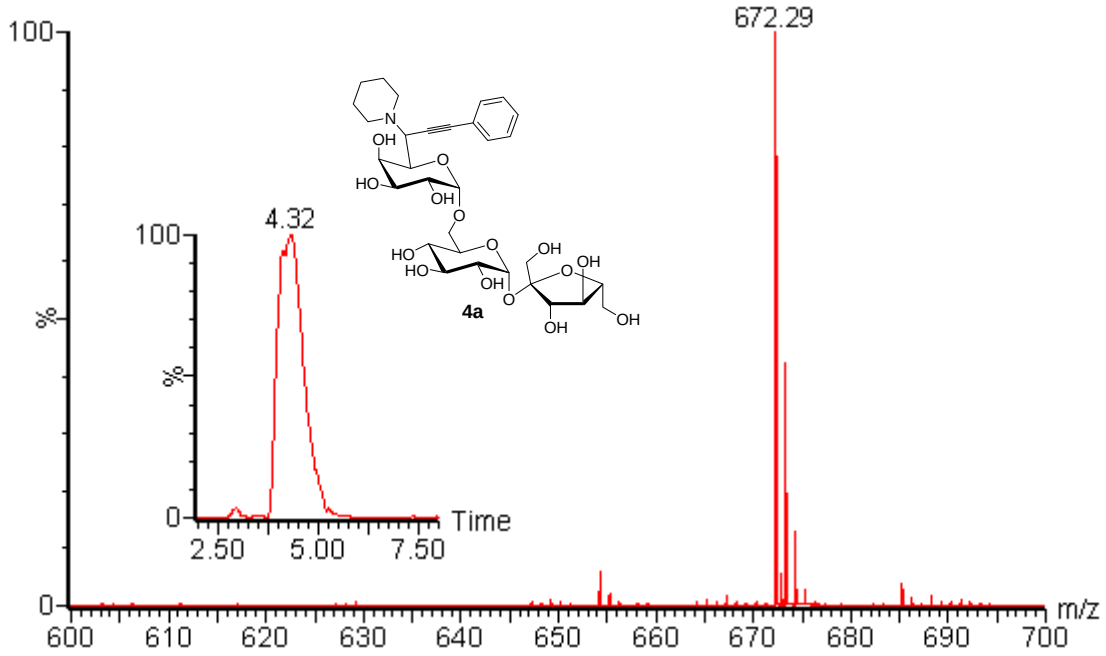



***Figure S6.*** MS spectrum of **4a** (ESI source, $[M+H]^+$ = m/z 672.29) and the XIC chromatogram of **4a** at t = 4.32 min (inset).

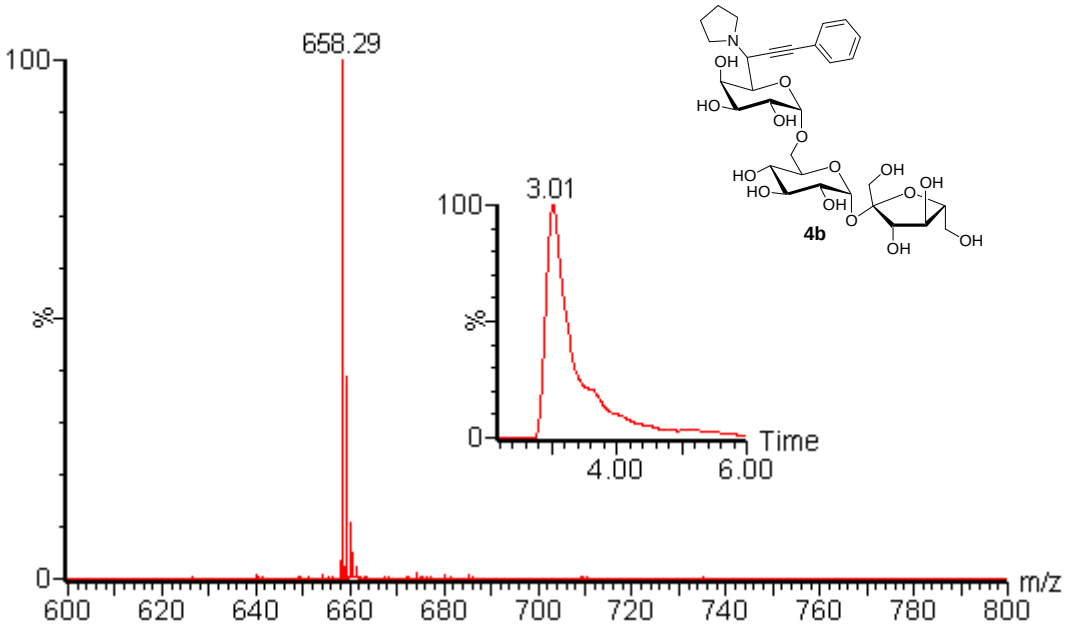



***Figure S7.*** MS spectrum of **4b** (ESI source, $[M+H]^+$ = m/z 658.29) and the XIC chromatogram of **4b** at t = 3.01 min (inset).

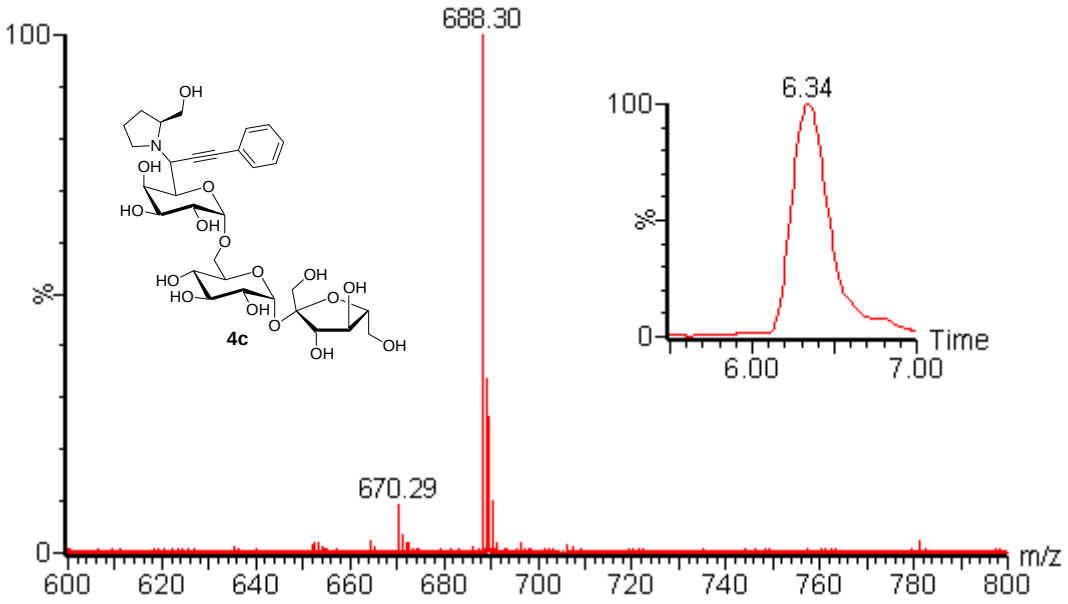



***Figure S8.*** MS spectrum of **4c** (ESI source, $[M\text{-}H_2O+H]^+$ = m/z 670.29 and $[M+H]^+$ = m/z 688.30) and the XIC chromatogram of **4c** at t = 6.34 min (inset).

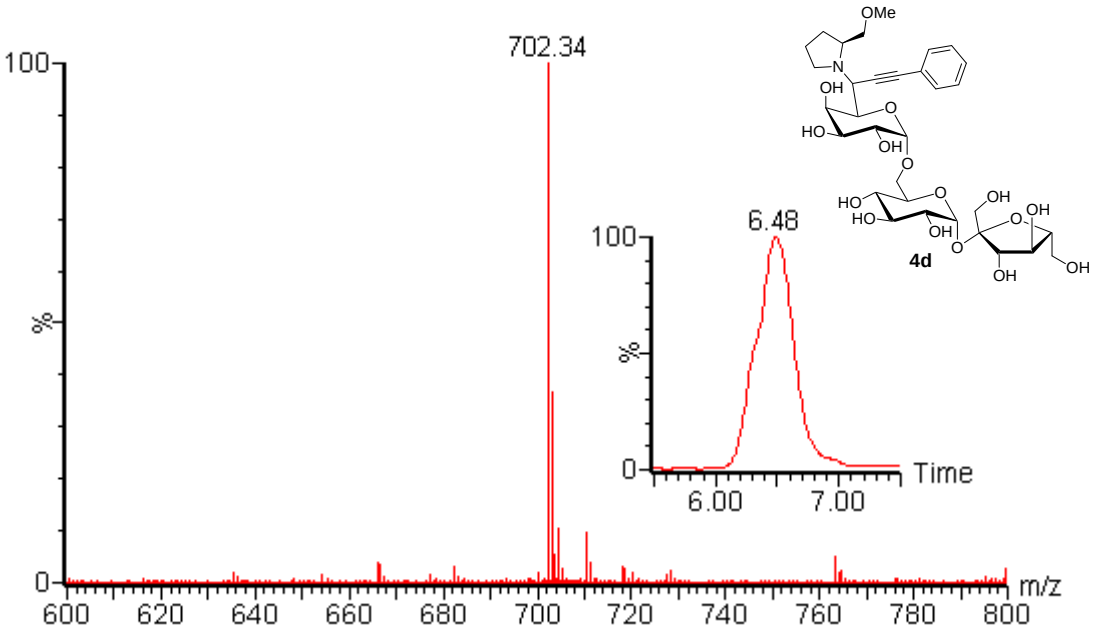



***Figure S9.*** MS spectrum of **4d** (ESI source, $[M+H]^+$ = m/z 702.34) and the XIC chromatogram of **4d** at t = 6.48 min (inset).

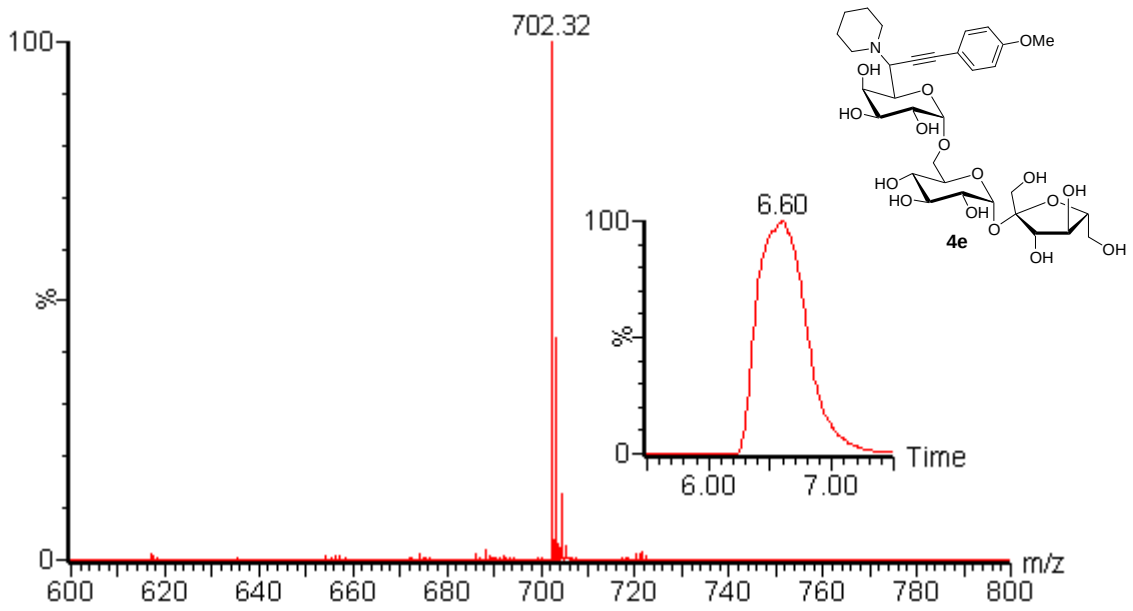



***Figure S10.*** MS spectrum of **4e** (ESI source, $[M+H]^+$ = m/z 702.32) and the XIC chromatogram of **4e** at t = 6.60 min (inset).

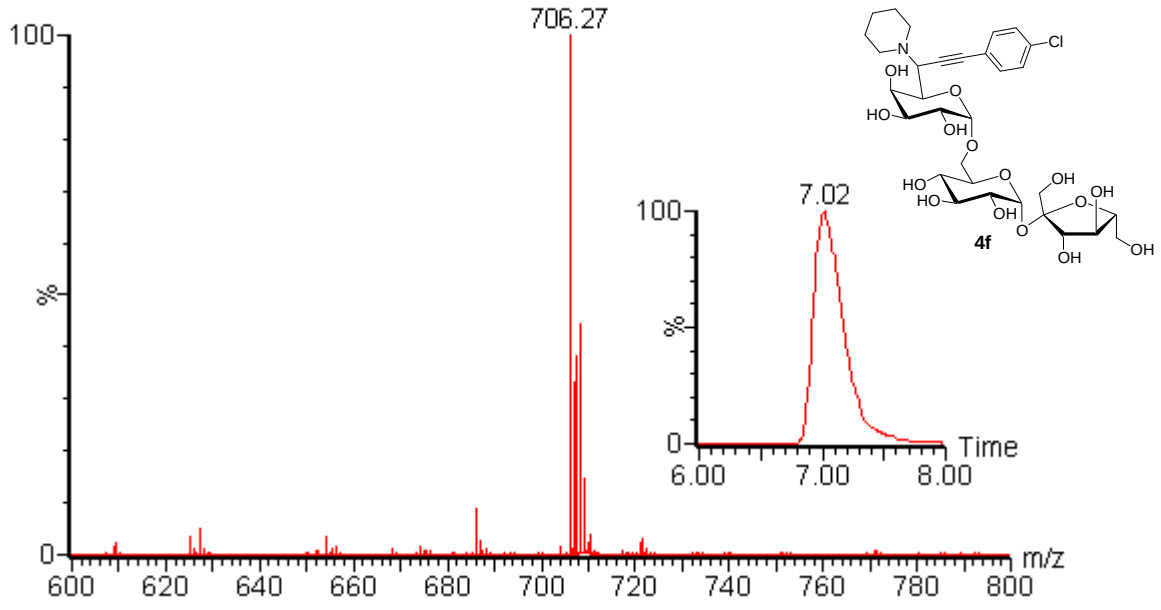



***Figure S11.*** MS spectrum of **4f** (ESI source, $[M+H]^+$ = m/z 706.27) and the XIC chromatogram of **4f** at t = 7.02 min (inset).

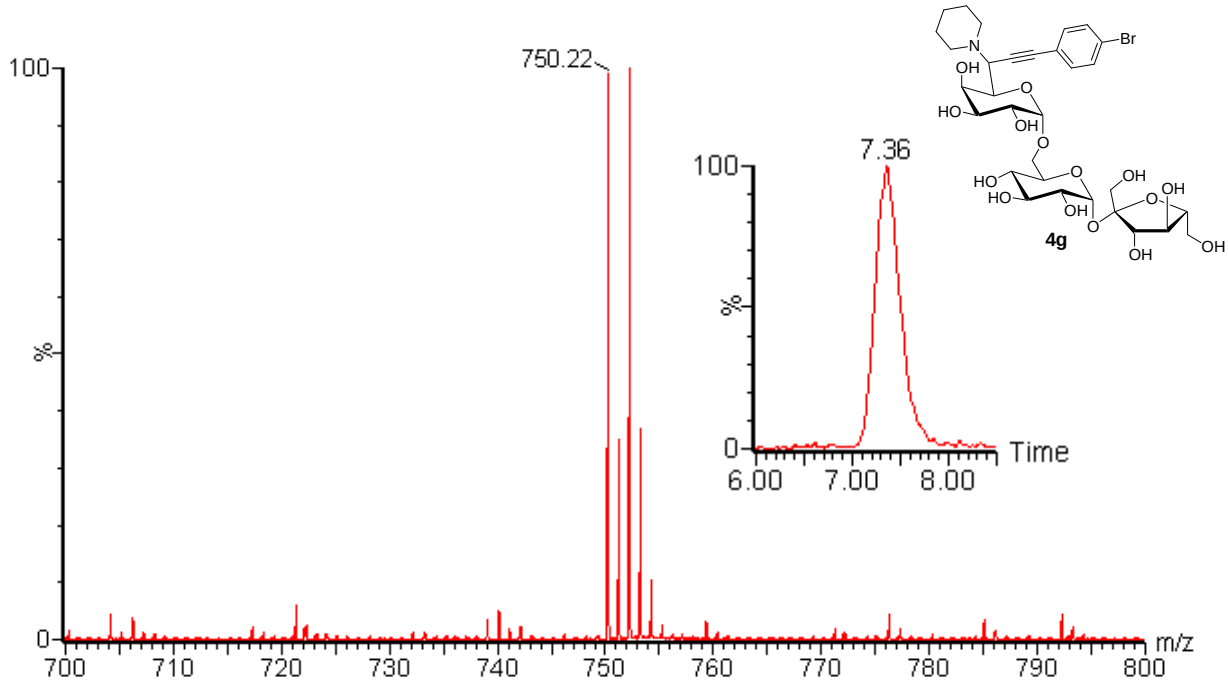



***Figure S12.*** MS spectrum of **4g** (ESI source, $[M+H]^+$ = m/z 750.22) and the XIC chromatogram of **4g** at t = 7.36 min (inset).

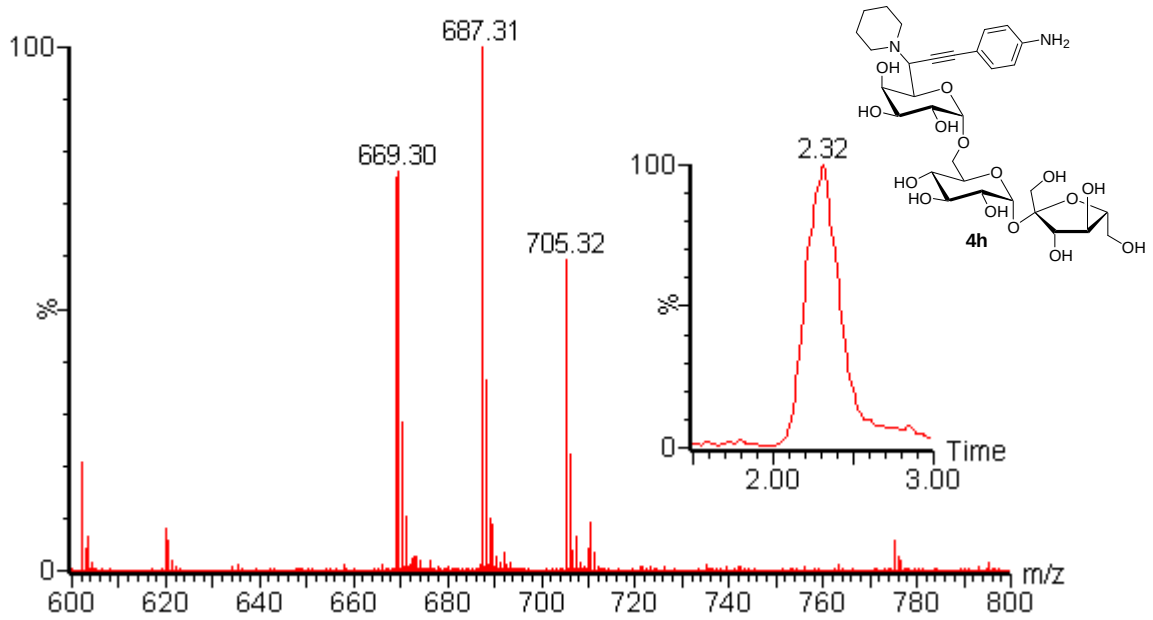



***Figure S13.*** MS spectrum of **4h** (ESI source, $[M-H_2O+H]^+$ = m/z 669.30, $[M+H]^+$ = m/z 687.31 and $[M+H_2O+H]^+$ = m/z 705.32) and the XIC chromatogram of **4h** at t = 2.32 (inset).

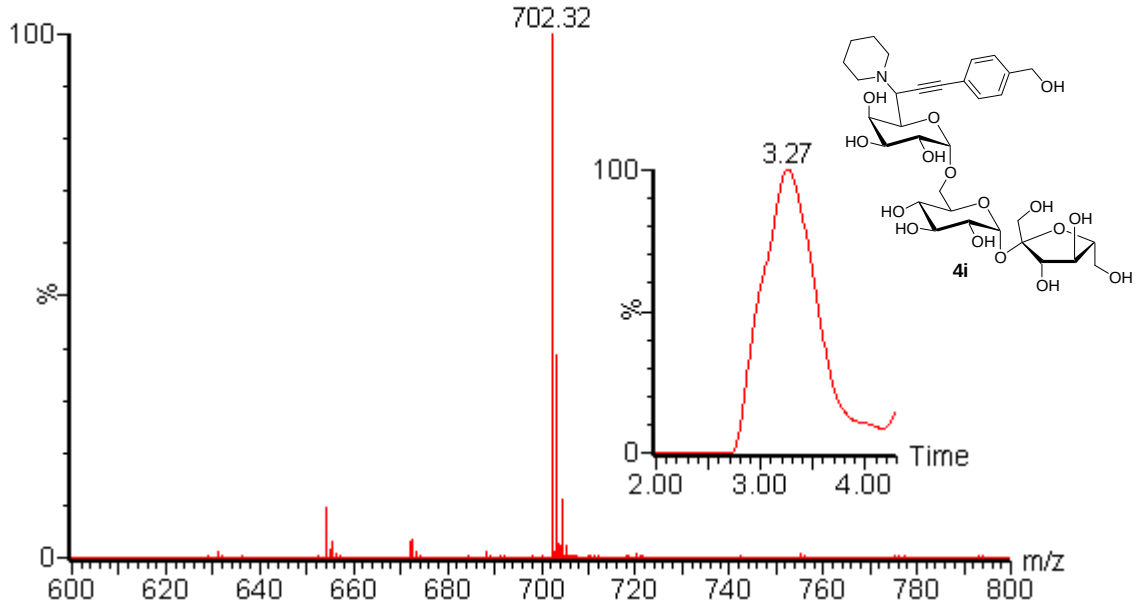



***Figure S14.*** MS spectrum of **4i** (ESI source, $[M+H]^+$ = m/z 702.32) and the XIC chromatogram of **4i** at t = 3.27 min (inset).

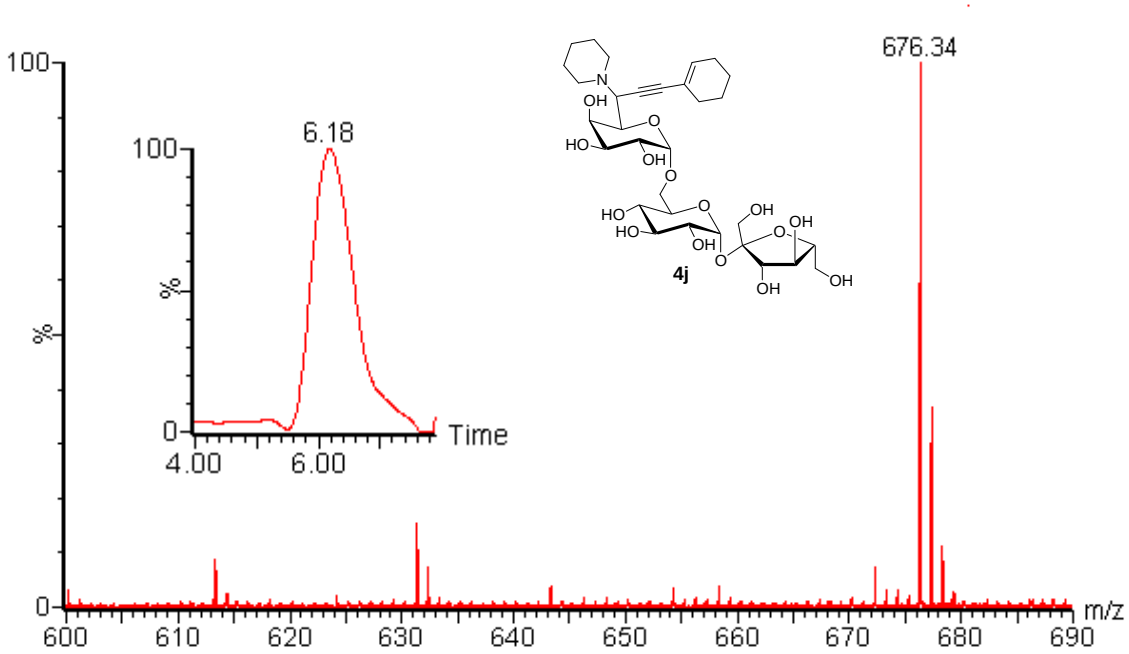



***Figure S15.*** MS spectrum of **4j** (ESI source, $[M+H]^+$ = m/z 676.34) and the XIC chromatogram of **4j** at t = 6.18 min (inset).

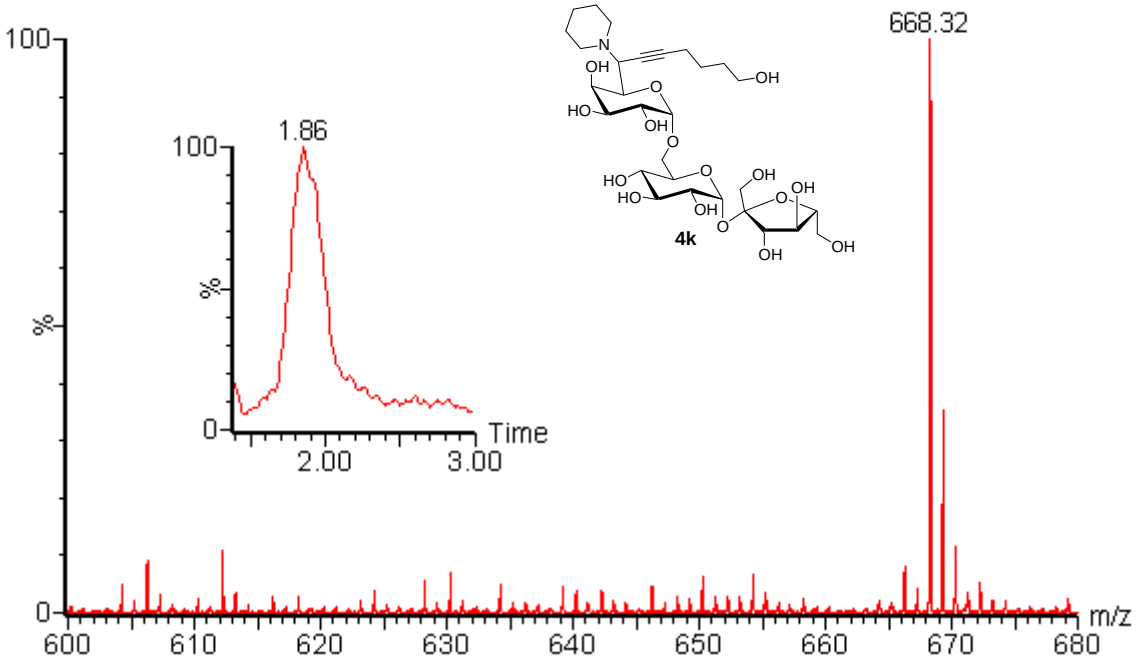



***Figure S16.*** MS spectrum of **4k** (ESI source, $[M+H]^+$ = m/z 668.32) and the XIC chromatogram of **4k** at t = 1.86 min (inset).

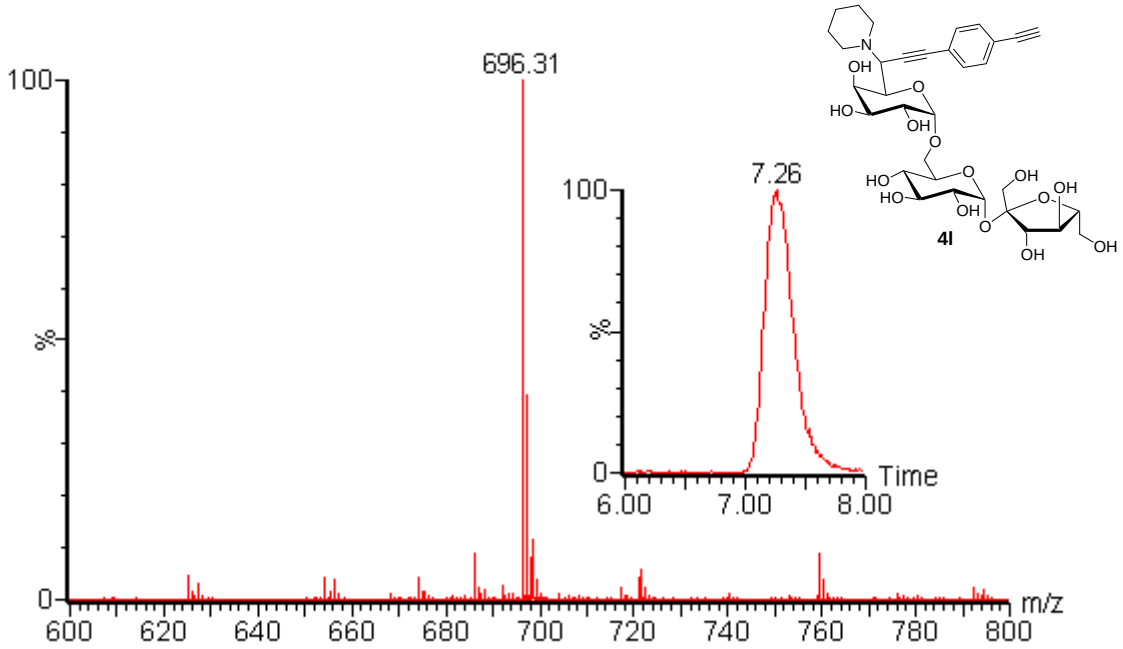



***Figure S17.*** MS spectrum of **4l** (ESI source, $[M+H]^+$ = m/z 696.31) and the XIC chromatogram of **4l** at t = 7.26 min (inset).

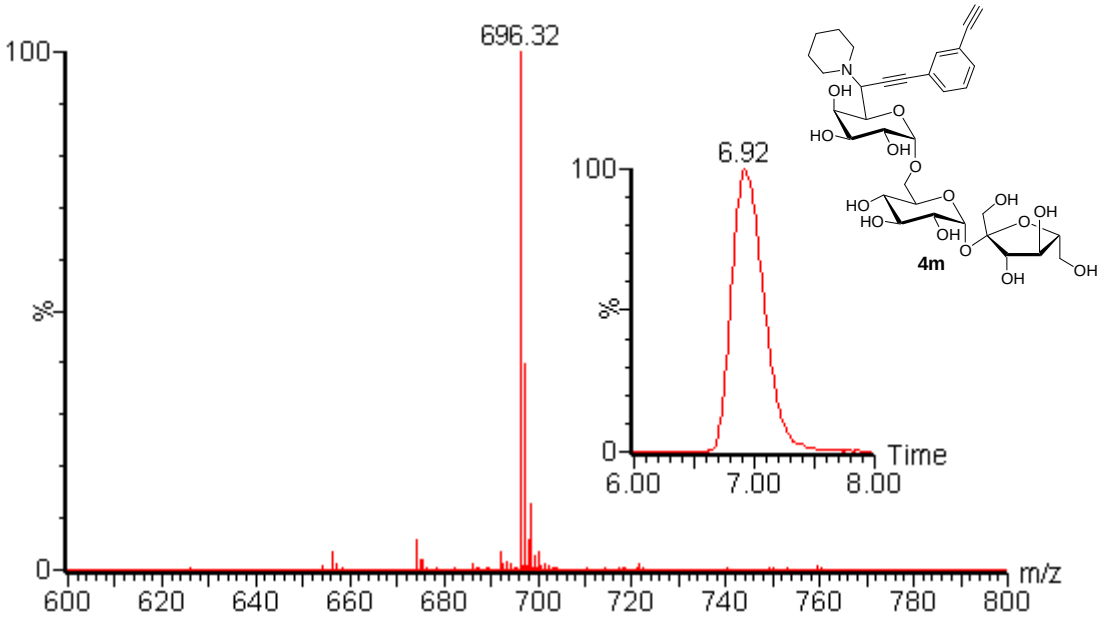



***Figure S18.*** MS spectrum of **4m** (ESI source, $[M+H]^+$ = m/z 696.32) and the XIC chromatogram of **4m** at t = 6.92 min (inset).

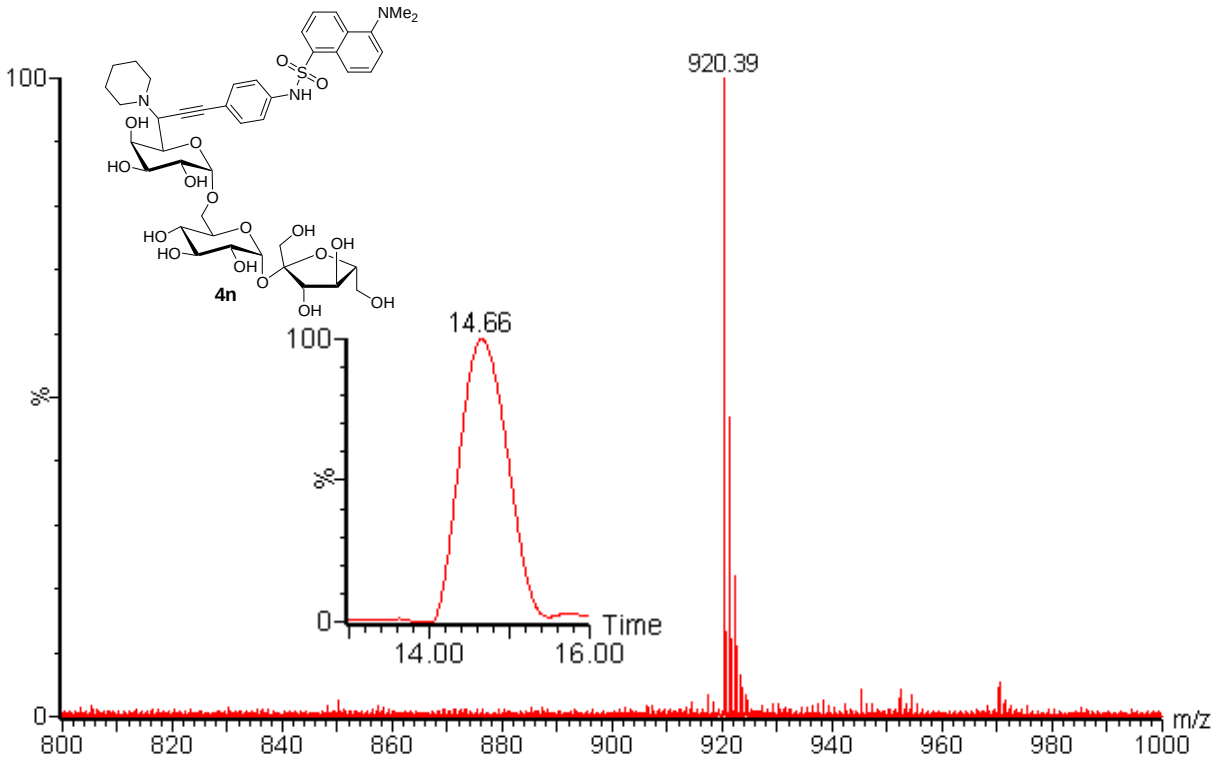



***Figure S19.*** MS spectrum of **4n** (ESI source, $[M+H]^+$ = m/z 920.39) and the XIC chromatogram of **4n** at t = 14.66 min (inset).

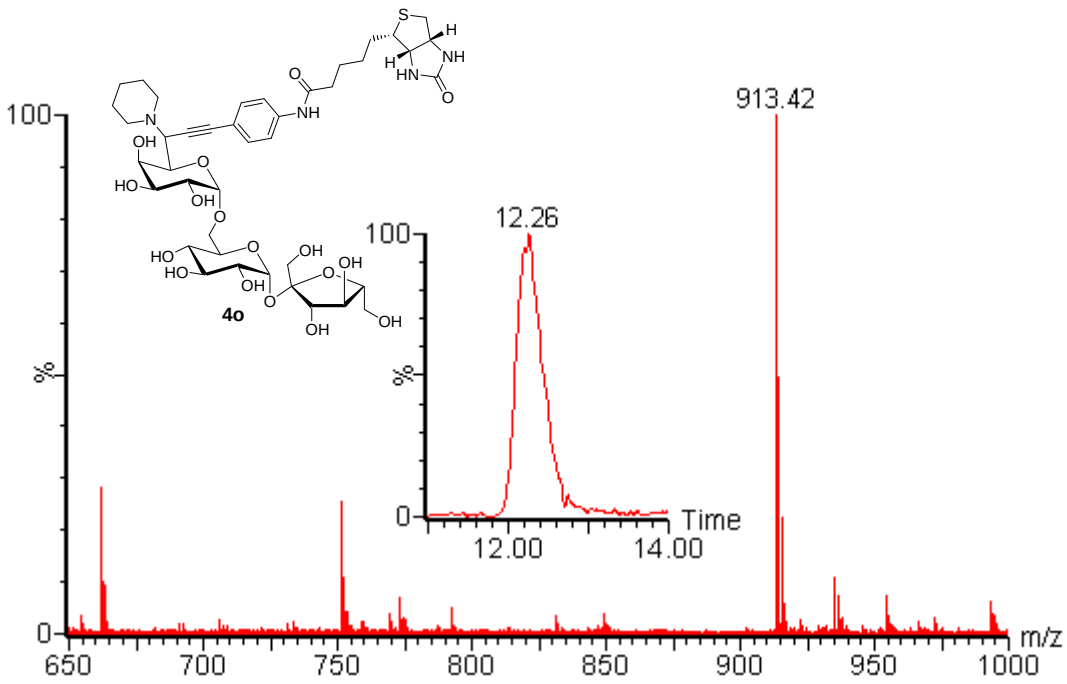



***Figure S20.*** MS spectrum of **4o** (ESI source, $[M+H]^+$ = m/z 913.42) and the XIC chromatogram of **4o** at t = 12.26 min (inset).

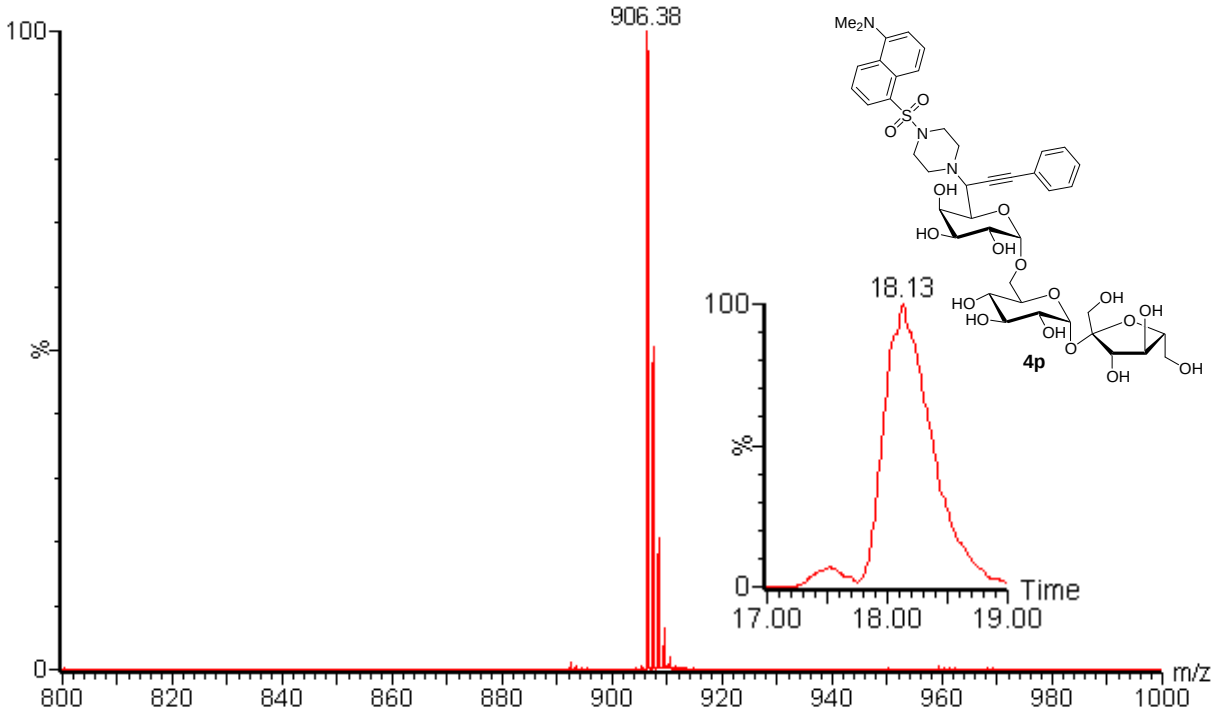



***Figure S21.*** MS spectrum of **4p** (ESI source, $[M+H]^+$ = m/z 906.38) and the XIC chromatogram of **4p** at t = 18.13 min (inset).

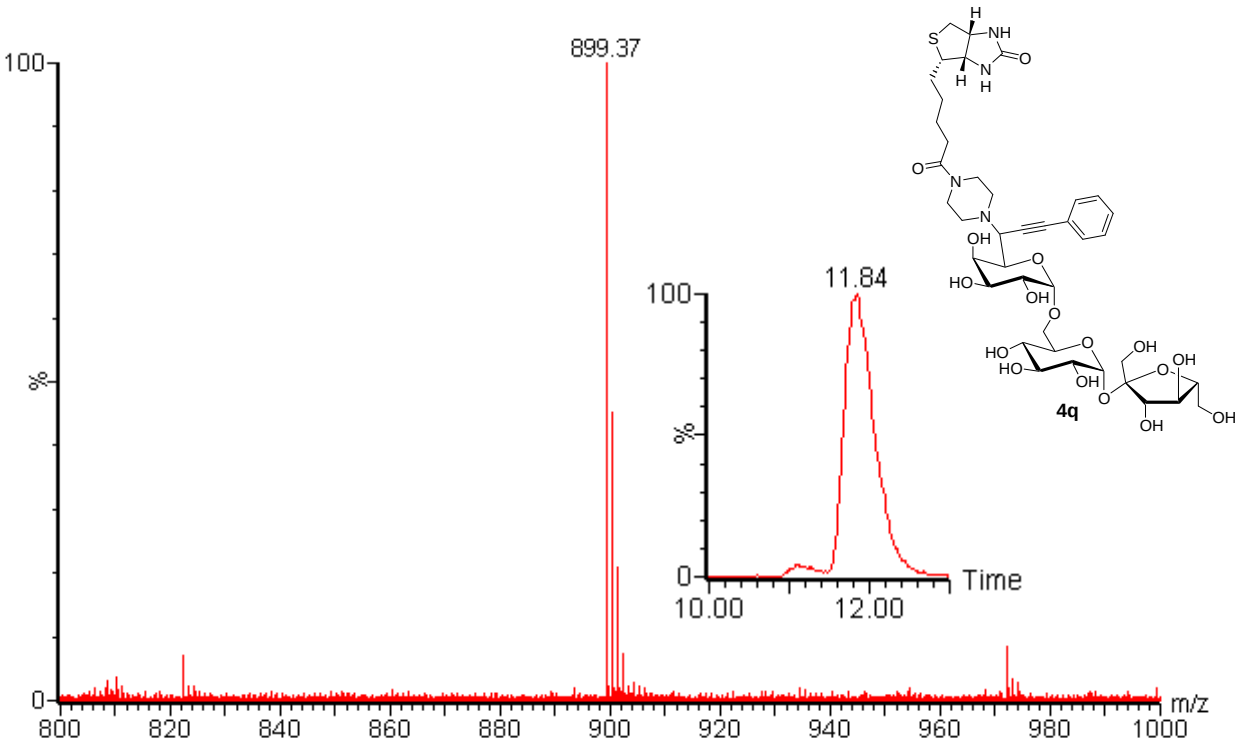



***Figure S22.*** MS spectrum of **4q** (ESI source, $[M+H]^+$ = m/z 899.37) and the XIC chromatogram of **4q** at t = 11.84 min (inset).

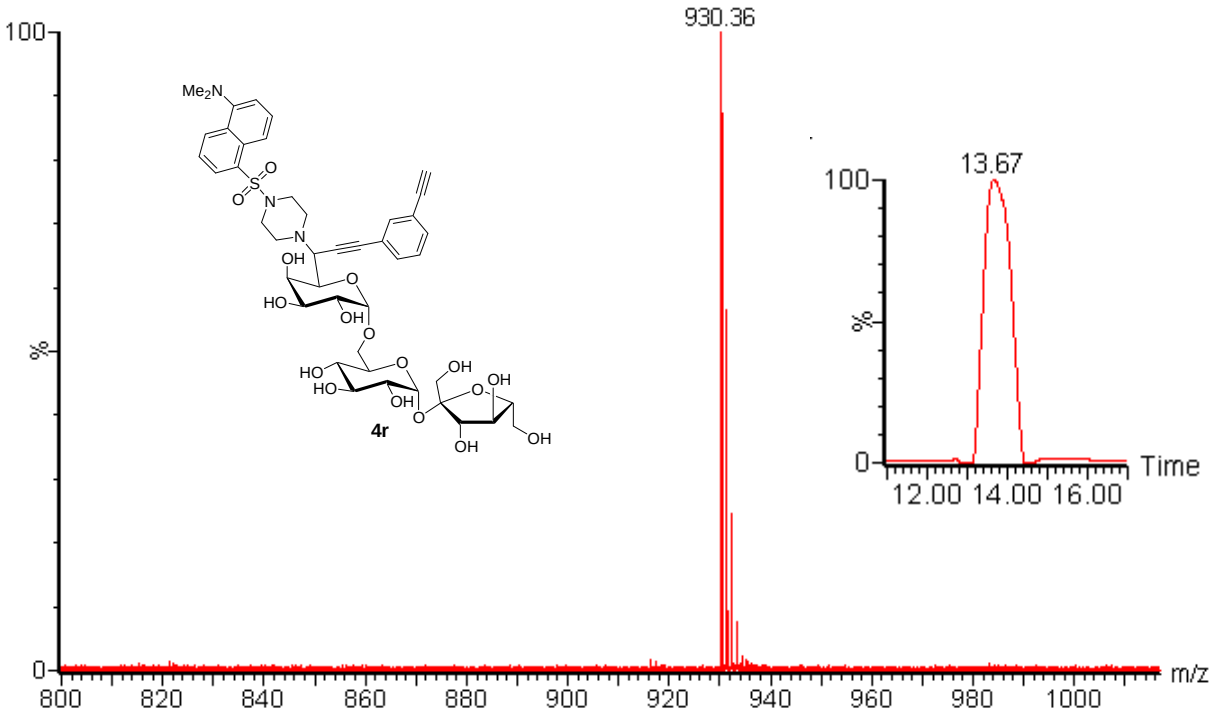



***Figure S23.*** MS spectrum of **4r** (ESI source, $[M+H]^+$ = m/z 930.36) and the XIC chromatogram of **4r** at t = 13.67 min (inset).

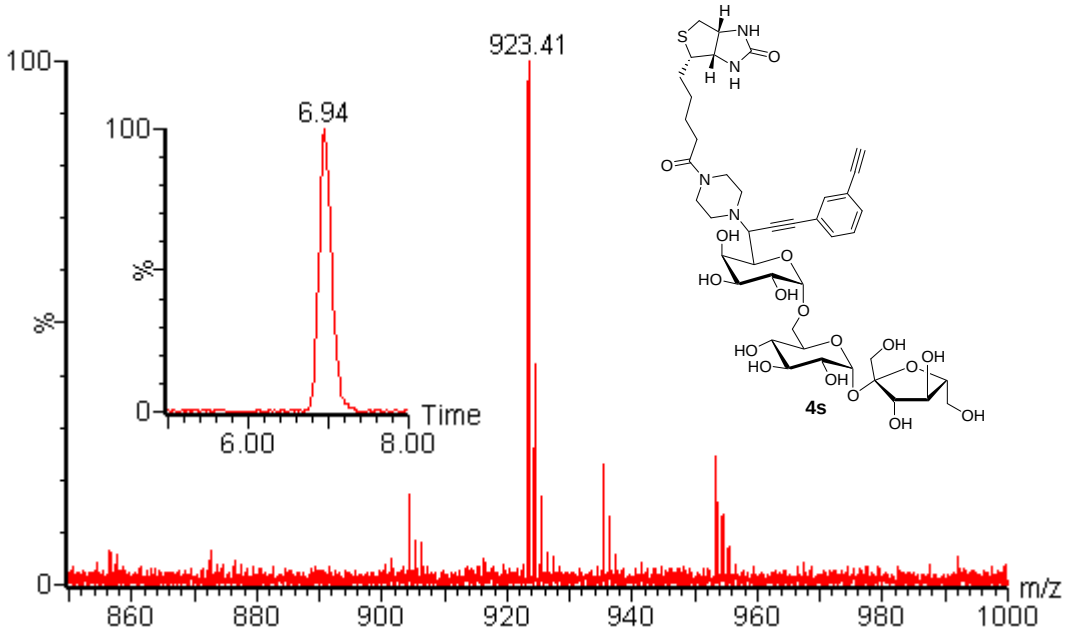



***Figure S24.*** MS spectrum of **4s** (ESI source, $[M+H]^+$ = m/z 923.41) and the XIC chromatogram of **4s** at t = 6.94 min (inset).

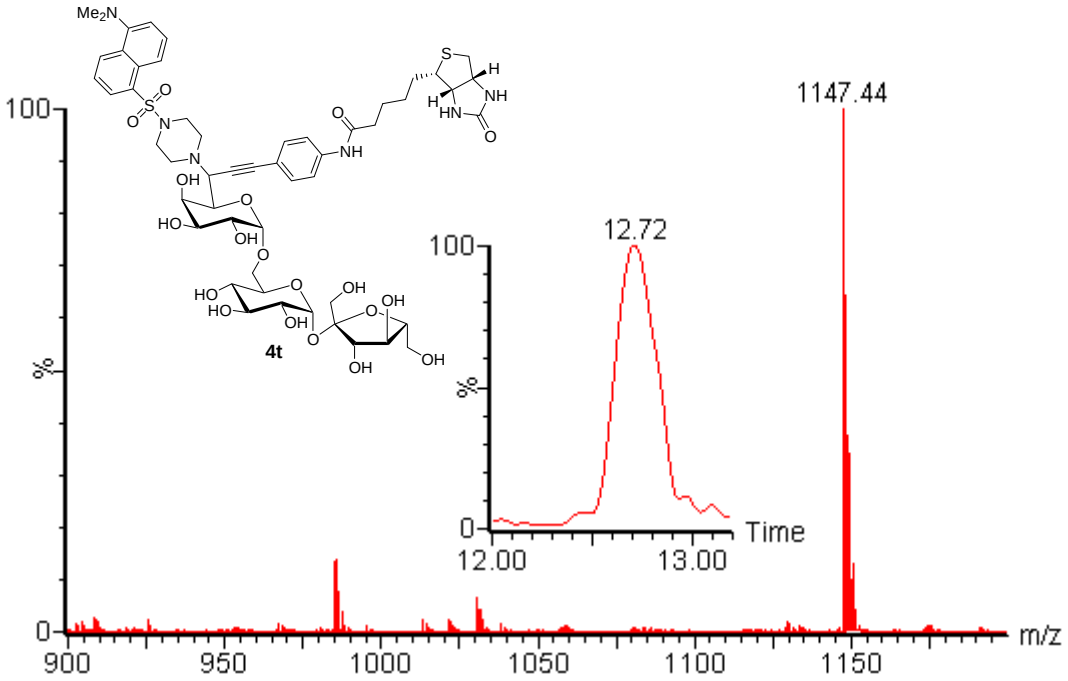



***Figure S25.*** MS spectrum of **4t** (ESI source, $[M+H]^+$ = m/z 1147.44) and the XIC chromatogram of **4t** at t = 12.72 min (inset).

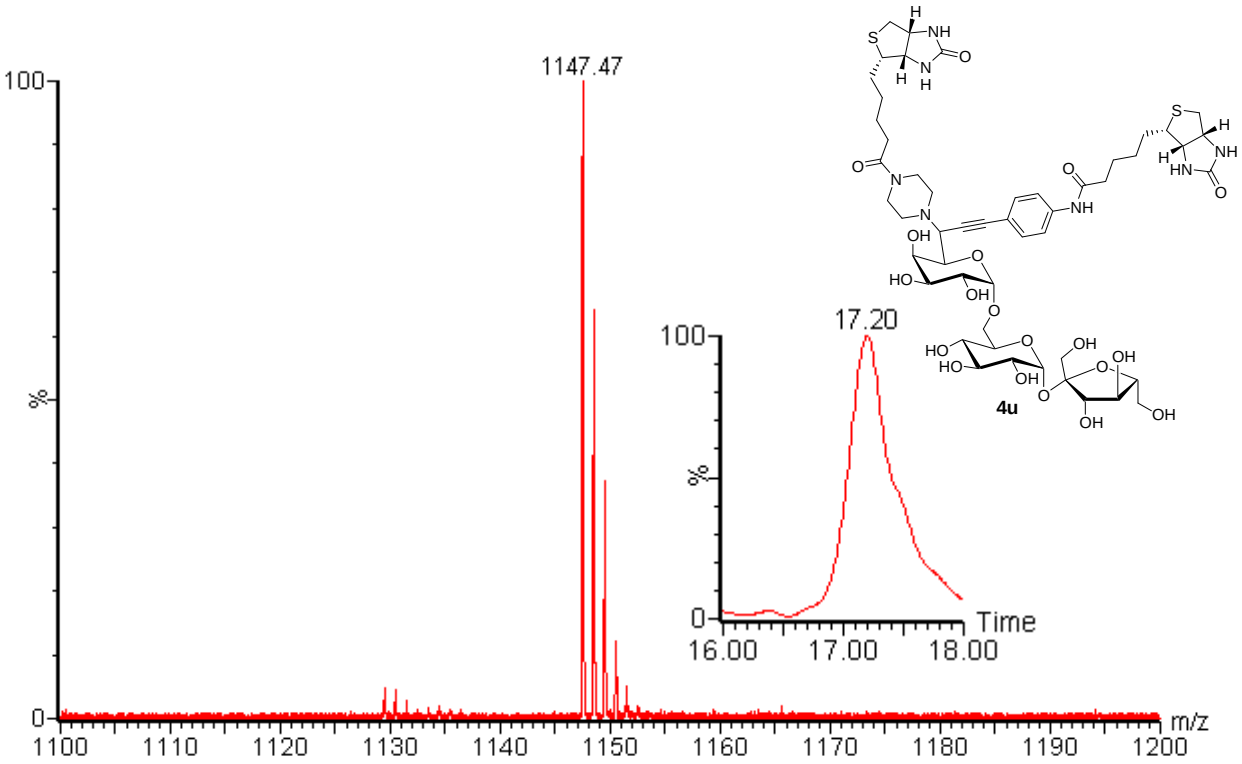



***Figure S26.*** MS spectrum of **4u** (ESI source, $[M+H]^+$ = m/z 1147.47) and the XIC chromatogram of **4u** at t = 17.2 min (inset).

**Procedure for Copper(I)-Catalyzed Sharpless Huisgen [3+2] Cycloaddition of Bifunctionally Modified D-Raffinose 4m and Organic Azides 7a-7b to give Triazole Products 8a-8b**

A clear liquor of gold-mediated bifunctionally modified D-raffinose **4m** (50 µL) after centrifugation was pippetted and mixed with $CuSO_4$ (10 µL of 1 M in $H_2O$), tris-(benzyltriazolylmethyl)amine (TBTA, 10 µL of 100 mM in DMSO / t-BuOH = 1:4), tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 10 µL of 1 M in $H_2O$) and azide **7a** or **7b** (20 µL of 100 mM in DMSO, 40 equiv.). The resulting mixture was placed at room temperature for 2 h. Then, the mixture was centrifuged. The clear liquor was analyzed by LC-MS for determination of the aldehyde conversion. MS spectra of **8a** and **8b** are deconvoluted from the corresponding extracted ion chromatograms (Figure S27-S28).

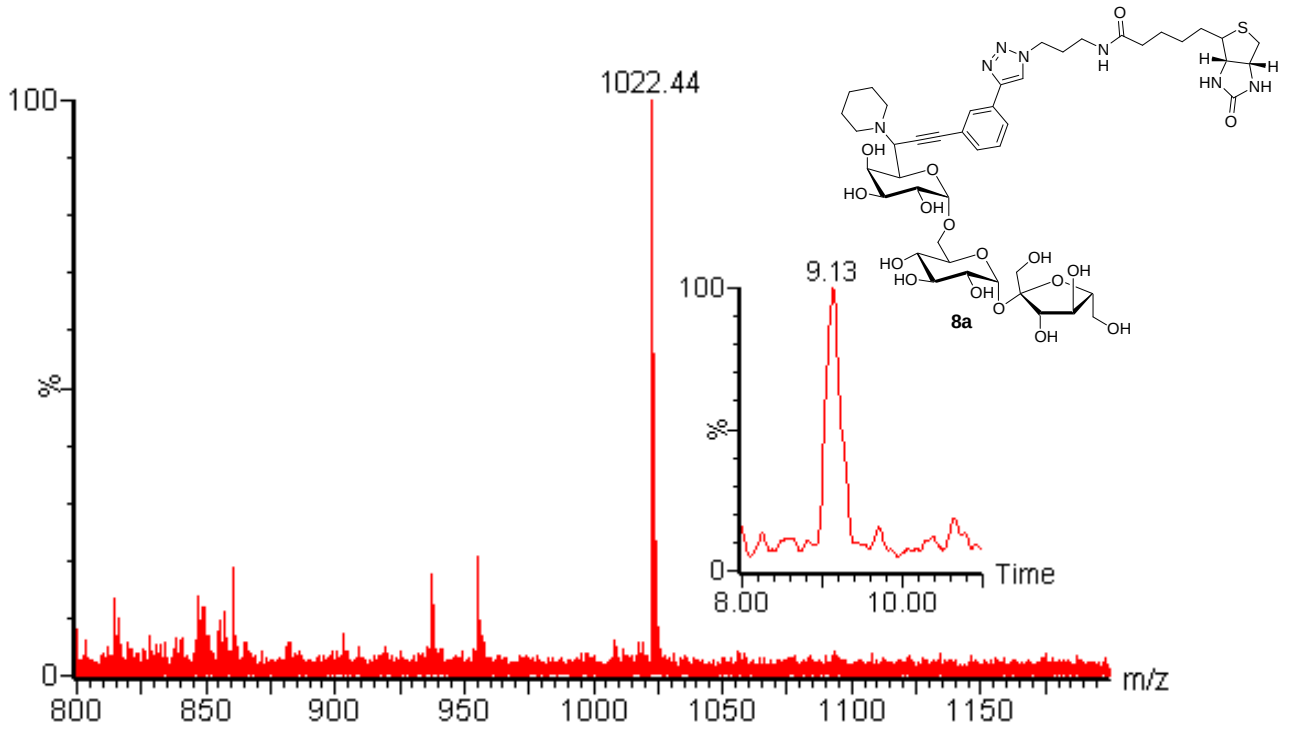



***Figure S27.*** MS spectrum of **8a** (ESI source, $[M+H]^+$ = m/z 1022.44) and the XIC chromatogram of **8a** at t = 9.13 min (inset).

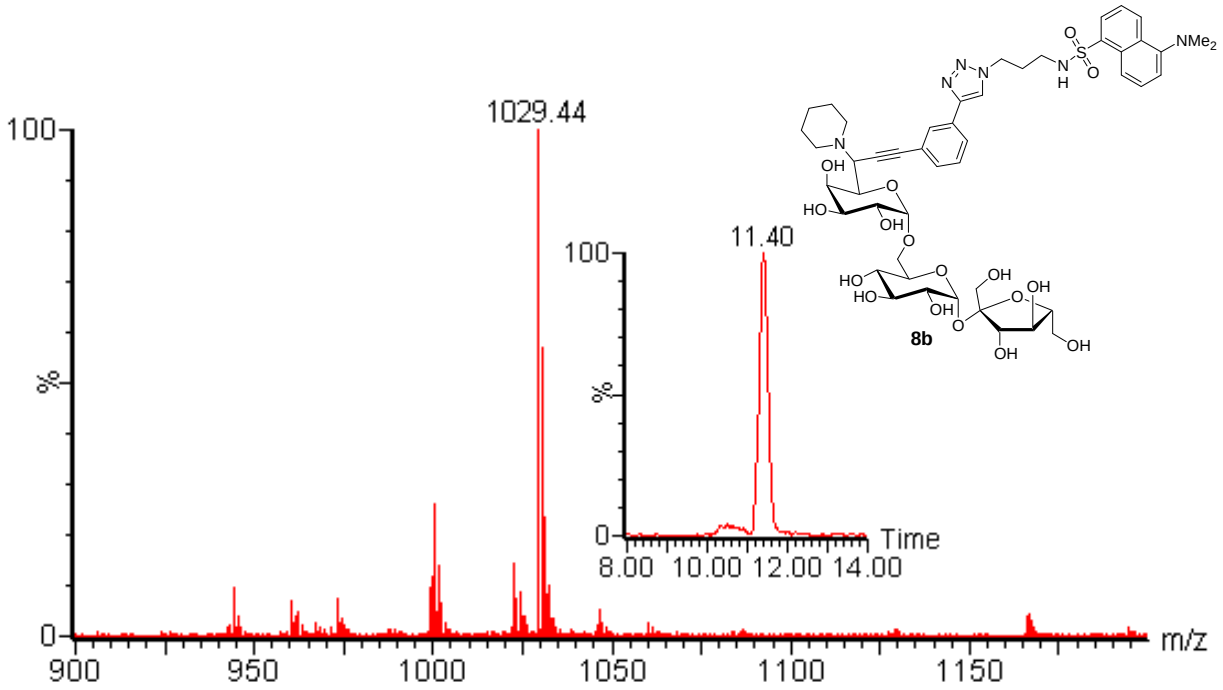
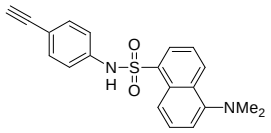



***Figure S28.*** MS spectrum of **8b** (ESI source, $[M+H]^+$ = m/z 1029.44) and the XIC chromatogram of **8b** at t = 11.4 min (inset).

**Procedure for Synthesis of Dansyl Alkyne, 3k**

A solution of dansyl chloride (135 mg, 0.5 mmol) and 4-ethynylaniline (59 mg, 0.5 mmol) in 5 mL pyridine was stirred at room temperature under nitrogen atmosphere for 30 min. The resulting mixture was washed by 2 N hydrochloric acid (20 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed by water (50 mL) and then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel using ethyl acetate/*n*-hexane as eluent to give product **3k** in 74% isolated yield.

**Charaterization Data of Dansyl Alkyne, 3k**

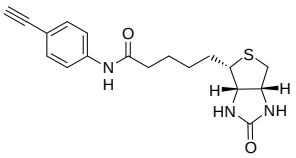


74% yield, pale green powder; analytical TLC (silica gel 60) (50% EtOAc in *n*-Hexane), $R_f$ = 0.74; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.51 (d, $J$ = 8.5 Hz, 1H), 8.33 (d, $J$ = 8.5 Hz, 1H), 8.20 (d, $J$ = 7.5 Hz, 1H), 7.56 (t, $J$ = 8.0 Hz, 1H), 7.44 (t, $J$ = 8.0 Hz, 1H), 7.23 (d, $J$ = 8.5 Hz, 2H, overlapped by peaks of -NH and $CHCl_3$), 7.20 (br s, -NH), 7.17 (d, $J$ = 7.5 Hz, 1H), 6.91 (d, $J$ = 8.5 Hz, 2H), 2.98 (s, 1H), 2.86 (s, 6H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 152.35, 137.16, 134.00, 133.21, 131.29, 130.57, 130.01, 129.68, 128.94, 123.21, 120.55, 118.68, 116.36, 115.48, 83.00, 45.52 ppm; IR (KBr): = 3266, 3239, 2994, 2942, 2837, 2781, 1606, 1586, 1574, 1504, 1314, 1162, 1147, 951, 907, 856, 789, 621 cm$^{-1}$; MS (ESI$^+$): *m/z*(%) = 351 $[M + H]^+$; HRMS (ESI$^+$) calcd. for $[C_{20}H_{18}N_2O_2S + H]^+$ 351.1167, found 351.1152.

**Procedure for Synthesis of Biotin Alkyne, 3l**

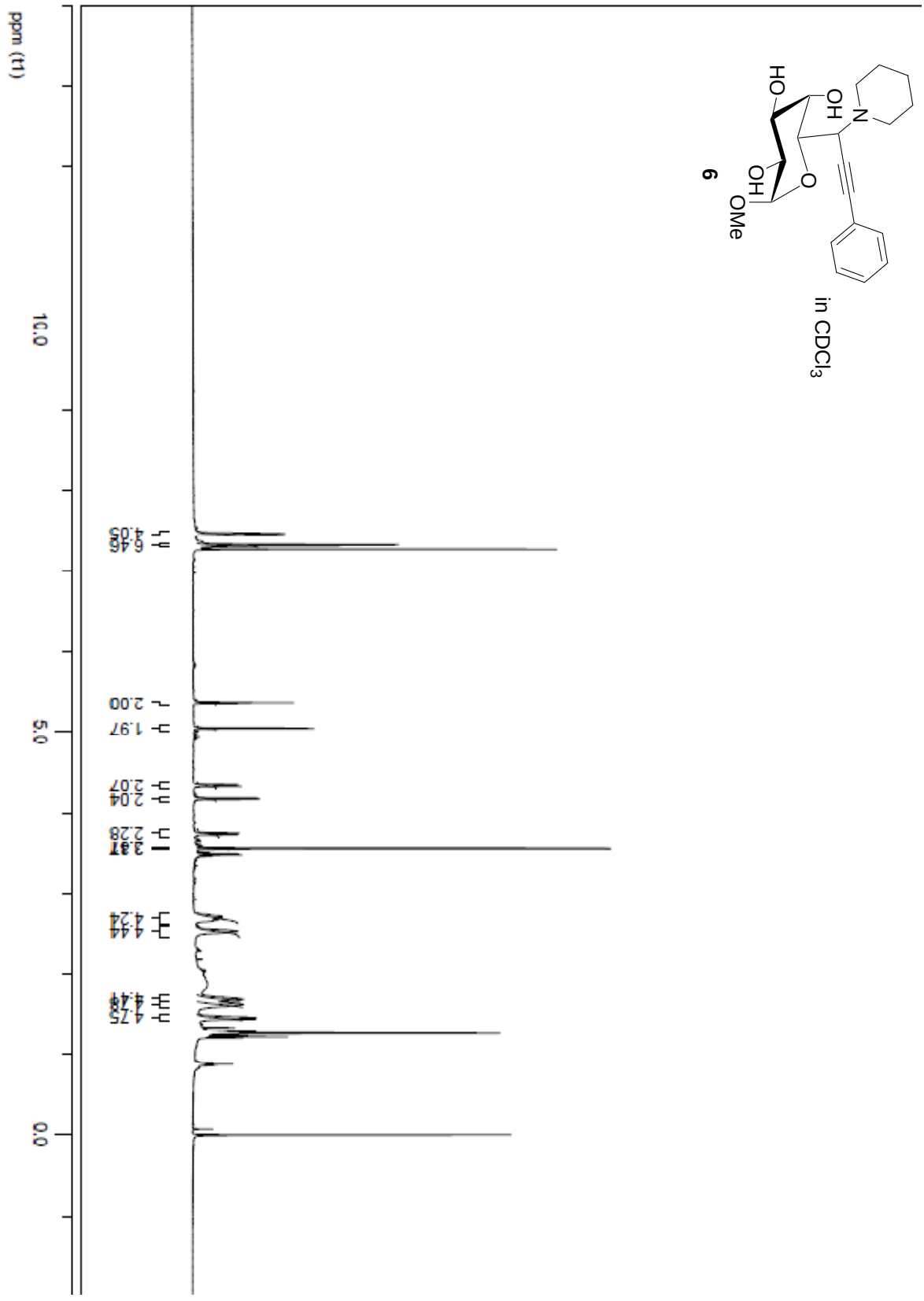
A solution of biotin (8 mg, 0.03 mmol), 4-ethynylaniline (35 mg, 0.3 mmol) and *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU, 23 mg, 0.06 mmol) in *N,N*-dimethylformamide (1 mL) and triethylamine (0.12 mL) was stirred at room temperature in darkness under nitrogen atmosphere for overnight. The combined organic layers were washed by water (50 mL) and then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel using methanol/dichloromethane as eluent give **3l** in 59% isolated yield.

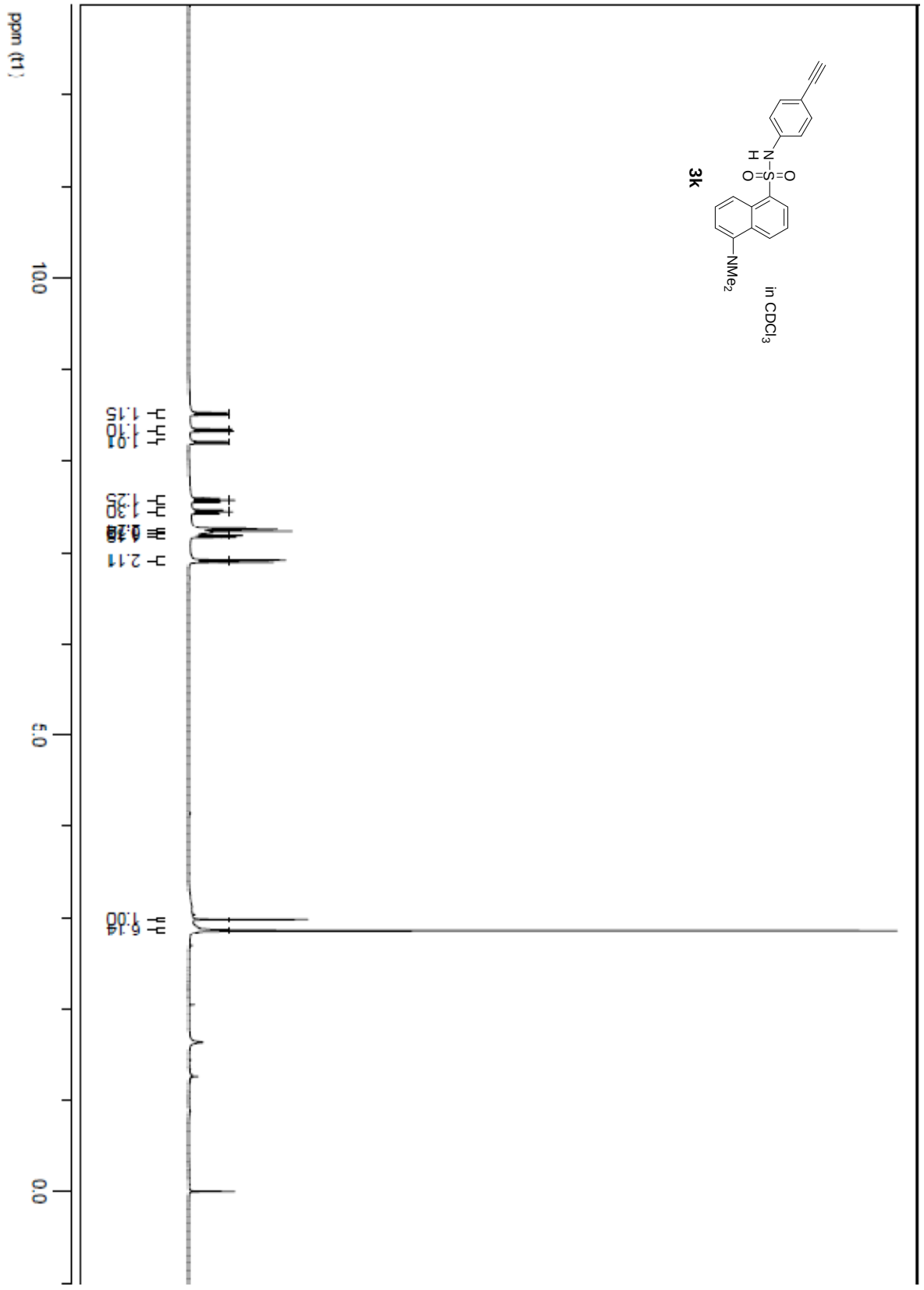
**Charaterization Data of Biotin Alkyne, 3l**

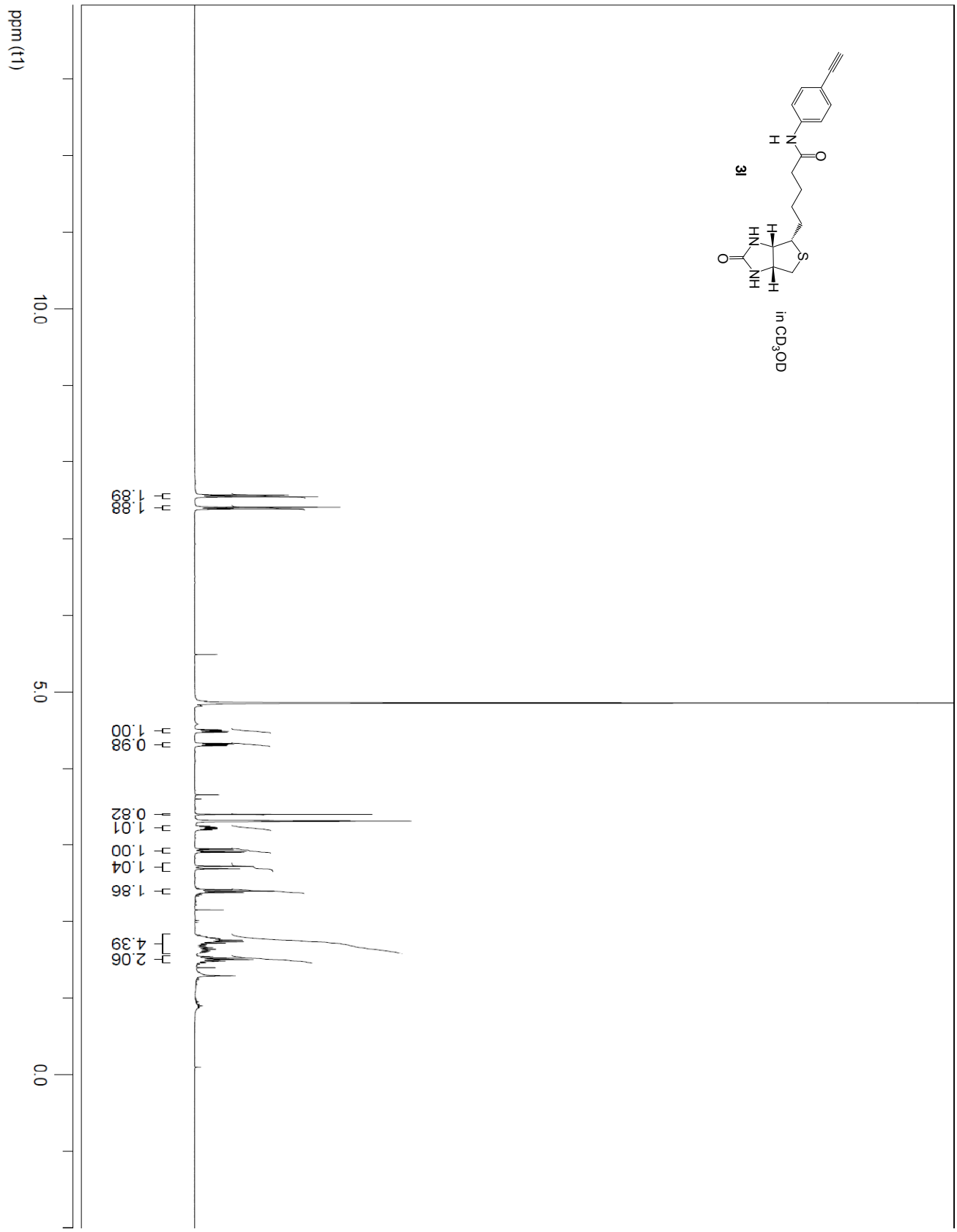


59% yield, off-white powder; analytical TLC (silica gel 60) (5% MeOH in $CH_2Cl_2$), $R_f$ = 0.56; $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ = 7.56 (d, $J$ = 8.8 Hz, 2H), 7.40 (d, $J$ = 8.8 Hz, 2H), 4.48 (dd, $J$ = 8.0, 4.5 Hz, 1H), 4.30 (dd, $J$ = 8.0, 4.5 Hz, 1H), 3.40 (s, 1H), 3.20-3.25 (m, 1H), 2.93 (dd, $J$ = 13.0, 4.5 Hz, 1H), 2.70 (d, $J$ = 13.0 Hz, 1H), 2.40 (t, $J$ = 8.0 Hz, 2H), 1.59-1.81 (m, 4H), 1.46-1.54 (m, 2H) ppm; $^{13}$C NMR (100 MHz, $CD_3OD$): $\delta$ = 173.08, 164.72, 139.02, 132.17, 119.35, 117.60, 82.84, 76.54, 61.96, 60.24, 55.53, 39.62, 36.27, 28.37, 28.10, 25.22 ppm; IR (KBr): = 3428, 3289, 2925, 2854, 2104, 1698, 1660, 1589, 1524, 1260, 839, 654 cm$^{-1}$; MS (ESI$^+$): *m/z*(%) = 344 $[M + H]^+$; HRMS (ESI$^+$) calcd. for $[C_{18}H_{21}N_3O_2S + H]^+$ 344.1433, found 344.1437.

## $^1$H NMR spectra






ppm (t1)
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5.0
0.0
1.89
1.88
0.98
1.00
0.82
1.01
1.00
1.04
1.86
4.39
2.06
3i
in CD3OD

## $^{13}$C NMR Spectra