Supporting Information

Cluster Effect through Oligomerization of Bioactive disaccharide AMOR for pollen tube capacitation in *Torenia*

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1. General

Commercially available reagents were obtained from Tokyo Kasei, Wako Pure Chemical Industries Ltd., KANTO CHEMICAL CO., INC., Sigma-Aldrich Merck, and Nacalai tesque, and used without further purification.

The ^{1}H and $^{13}C\{^{1}H\}$ NMR were recorded on a Bruker AVANCE 600 (600 MHz for ^{1}H, 150 MHz for ^{13}C) spectrometer and a JEOL JNM-ECA500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer. Chemical shifts were reported in ppm (δ), and coupling constants were reported in Hz. ^{1}H and ^{13}C-resonances were referenced to solvent residual peaks for $CDCl_3$ (^{1}H, 7.26 ppm), D_2O (1H, 4.70 Hz), and $CDCl_3$ (^{13}C, 77.16 ppm). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, doublet of doublets (dd), doublet of triplets (dt), doublet of doublet of doublets (ddd), doublet of doublet of doublet of doublets (dddd). Spectra were processed by Bruker Top-spin (Bruker) and Delta NMR software (JEOL).

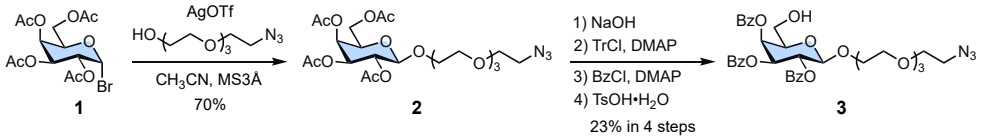
High resolution mass analyses (HRMS) were submitted to the Mass Spectrometry Laboratory at RIKEN (Wako, Japan). For crude analysis, ultra-high-performance liquid chromatography-mass spectrometry (UPLC/MS) was performed on a SHIMADZU LCMS-2020 (Shimadzu, Kyoto, Japan) equipped with a reverse phase C18 column (2.7 μm particle size, 2.1 x 100 mm), an API/ESI mass spectrometry detector, and an ultraviolet detector (Shimadzu, Kyoto, Japan). Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a Bruker Daltonics ultrafleXtreme using α-cyano-4-hydroxycinnamic acid as the matrix.

Thin-layer chromatography was performed on Merck 60 F_{254} precoated silica gel plates. Reverse phase preparative Thin-layer chromatography was performed on Merck RP-8 modified silica gel plate coated with F_{254} indicator. Column chromatography was performed on open column using silica gel (Silica Gel 60 N; 63~210 mesh or 40~50 mesh; Kanto Chemical Co., Inc.).

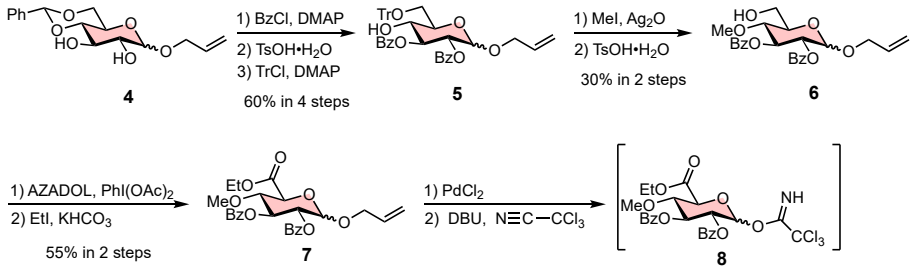
2. Synthesis schemes for AMOR oligomers

Scheme 1. Synthesis of azide-tethered AMOR with protecting groups.

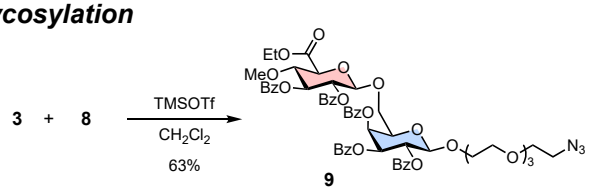
Synthesis of glycosyl acceptor



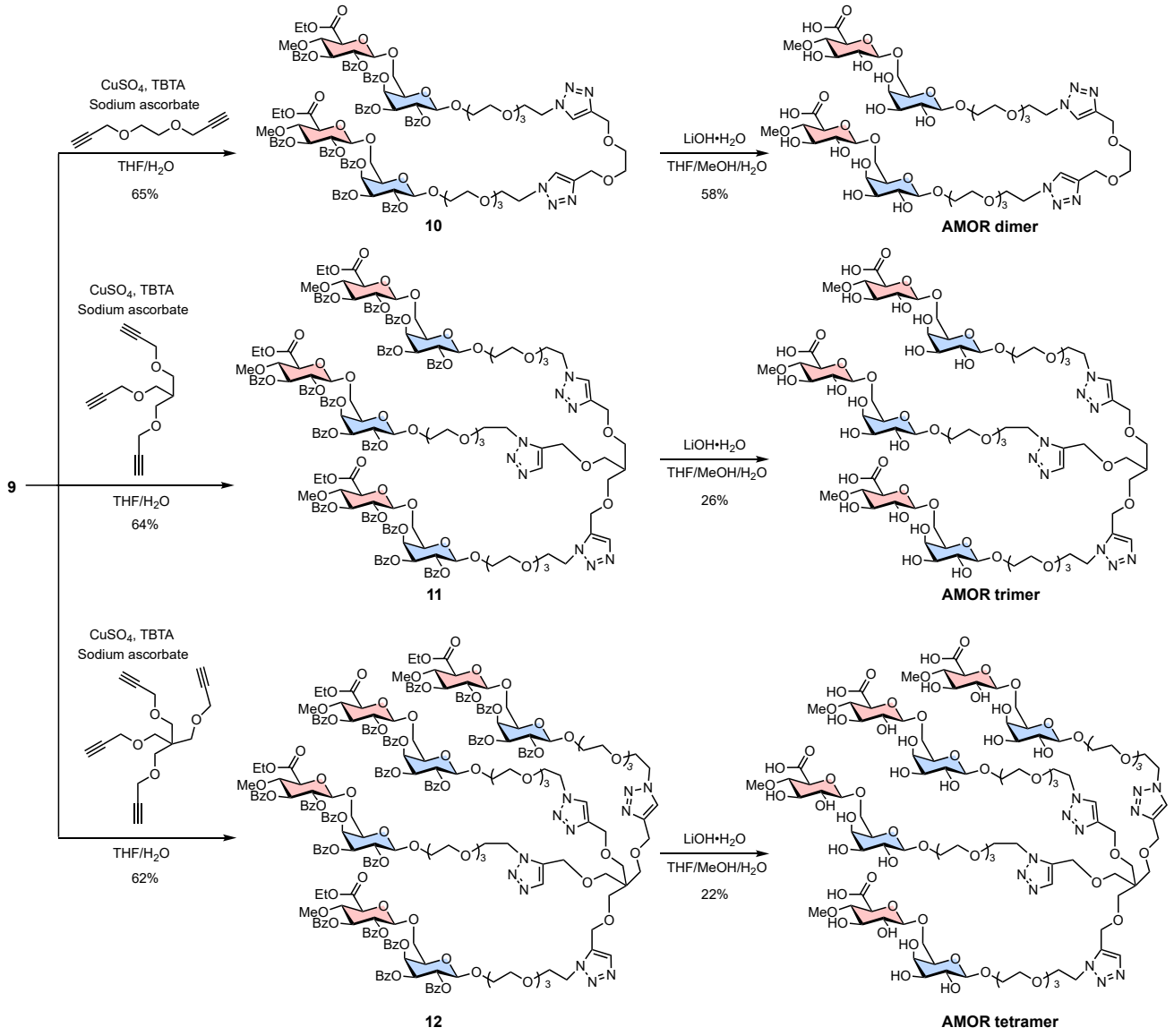
Synthesis of glycosyl donor



Glycosylation



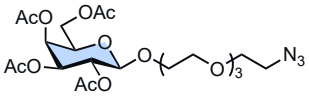
Scheme 2. Synthesis of AMOR oligomers



3. Synthesis procedures for AMOR oligomers

Compounds **4**, tetraethylene glycol mono-azide, and each alkyne linker were prepared as reported previously.[1–3]

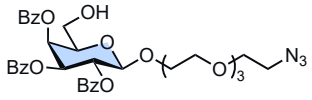
Synthesis of 2



2

AgOTf (4.18 g, 16.3 mmol) was added to a mixture of 1 (6.1 g, 14.8 mmol) and tetraethylene glycol monoazide (3.48 g, 19.8 mmol) in CH3CN (74 mL) and MS3Å at −20°C. This reaction mixture was stirred at −20°C for 90 min and then allowed to slowly warm to room temperature. The reaction mixture was stirred for 18 h at room temperature, after which the mixture was filtered through a Celite® pad and concentrated. The residue underwent column chromatography (silica gel, hexane:AcOEt = 1:0 to 50:1 to 25:1) to afford 2 as a colourless oil (6.0 g, 70%).; ^{1}H NMR (500 MHz, $CDCl_3$) δ□5.38 (d, *J* = 3.5 Hz, 1H), 5.21 (dd, *J* = 8.0, 10.5 Hz, 1H), 5.01(dd, *J* = 3.5, 10.5, 1H), 4.56 (d, *J* = 8.0 Hz, 1H), 4.17 (dd, *J* = 6.0, 10.5 Hz, 1H), 4.12 (d, *J* = 7.0, 10.5 Hz, 1H), 3.95 (dt, *J* = 4.0, 11.0 Hz, 1H), 3.91 (dd, *J* = 6.0, 7.0 Hz, 1H), 3.77–3.72 (m, 1H), 3.69–3.62 (m, 12H), 3.39 (t, *J* = 5.0 Hz, 2H), 2.15 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.4, 170.3, 169.6, 101.5, 71.0, 70.8, 70.8, 70.4, 70.2, 69.2, 68.9, 67.2, 61.4, 50.8, 20.9, 20.8, 20.7; HRMS (ESI): calculated for $[M+Na]^+$ requires *m/z* = 572.2068, found 572.2051.

Synthesis of 3



3

Step 1: Acetyl group removal

To a solution of 2 (6.00 g, 10.9 mmol) in MeOH was added NaOH (1.96 g, 49.1 mmol) at room temperature. The mixture was stirred for 90 min at room temperature, after which the pH of the reaction mixture was neutralised with AcOH. The resulting mixture was concentrated in vacuo and the residue underwent short column chromatography (silica gel, CHCl3:MeOH = 10:1 to 5:1). The roughly purified product (3.0 g) was used in the next step without further purification.

Step 2: Trityl protection of the 6-OH group

TrCl (6.58 g, 23.6 mmol) was added to a mixture of the above product (3.0 g, ~7.87 mmol) and 4-dimethylaminopyridine (DMAP; 96.1 mg, 0.787 mmol) in pyridine (26 mL) at room temperature. The reaction mixture was stirred for 16 h at room temperature, after which the reaction was quenched with an excess amount of MeOH and concentrated in vacuo. The residue underwent short column chromatography (silica gel, CHCl3:MeOH = 10:1 to 5:1). The roughly purified product was used in the next step without further purification.

Step 3: Benzoyl protection

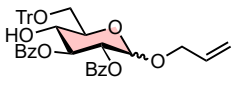
BzCl (3.2 mL, 27.5 mmol) was slowly added to a mixture of the above product and DMAP (96.1 mg, 0.787

mmol) in pyridine (31 mL) at 0°C. This reaction mixture was stirred at 0°C for 15 min and then warmed to room temperature. After stirring for 18 h at room temperature, the reaction mixture was diluted with EtOAc and washed with H2O and brine. The organic layer was dried over Na2SO4, filtered, and concentrated in vacuo. The residue was used in the next step without purification.

Step 4: Removal of trityl group

To a solution of the above product in CH2Cl2 (39 mL) and MeOH (39 mL) was added TsOH•H2O (271 mg, 1.57 mmol) at room temperature. After stirring for 3 h at room temperature, TsOH•H2O (700 mg, 4.07 mmol) was added to complete the reaction. The mixture was further stirred for 12 h, after which the reaction mixture was diluted with CH2Cl2 and washed with saturated NaHCO3 and brine. The organic layer was dried over Na2SO4, filtered, and concentrated in vacuo. The residue underwent column chromatography (silica gel, CHCl3:AcOEt = 2:1 to 1:1) to yield 3 as a white foam (1.74 g, 23% in four steps) ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (d, *J* = 7.5 Hz, 2H), 7.99 (d, *J* = 7.5 Hz, 2H), 7.81 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.49 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.39 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.25 (dd, *J* = 7.5, 7.5 Hz, 2H), 5.83 (dd, *J* = 7.9, 10.2 Hz, 1H), 5.81 (d, *J* = 3.4 Hz, 1H), 5.58 (dd, *J* = 3.4, 10.2 Hz, 1H), 4.93 (d, *J* = 7.9 Hz, 1H), 4.05–4.00 (m, 2H), 3.88–3.83 (m, 2H), 3.67–3.56 (m, 9 H), 3.52–3.45 (m, 4 H), 3.37 (t, *J* = 4.8 Hz, 2H), 2.77 (t, *J* = 6.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.9, 165.7, 165.5, 134.0, 133.5, 133.4, 130.3, 129.9, 129.6, 128.9, 128.8, 128.8, 128.6, 128.5, 101.9, 74.2, 72.0, 70.8, 70.8, 70.7, 70.2, 69.6, 69.2, 60.8, 50.8; HRMS (ESI): calculated for $[M+Na]^+$ requires *m/z* = 716.2431, found 716.2424.

Synthesis of 5



5

Step 1: Benzoyl protection

BzCl (4.7 mL, 16.3 mmol) was slowly added to a mixture of 4 (5.45 g, 17.7 mmol) and DMAP (216 mg, 1.77 mmol) in pyridine (89 mL) at 0°C. This reaction mixture was stirred at 0°C for 15 min and then warmed to room temperature. The reaction mixture was stirred for 18 h at room temperature, after which the mixture was diluted with EtOAc and washed with H2O and brine. The organic layer was dried over Na2SO4, filtered through a silica gel pad, and concentrated in vacuo. The resulting product (6.52 g) was used in the next step without further purification.

Step 2: Removal of benzylidene group

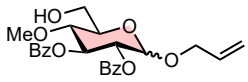
To a solution of the above product (6.52 g, ~12.6 mmol) in CH2Cl2 (67 mL) and MeOH (17 mL) was added TsOH•H2O (217 mg, 1.26 mmol) at room temperature. After stirring for 3.5 h at room temperature, TsOH•H2O (109 mg, 0.63 mmol) was added to complete the reaction. The mixture was further stirred for 1 h, after which the reaction mixture was diluted with CH2Cl2 and washed with saturated NaHCO3 and brine. The organic layer

was dried over Na2SO4, filtered, and concentrated in vacuo. The residue was used in the next step without purification

Step 3: Trityl protection of 6-OH group

TrCl (5.27 g, 18.9 mmol) was added to a mixture of the above product and DMAP (154 mg, 1.26 mmol) in pyridine (42 mL) at room temperature. After stirring for 12 h at room temperature, TrCl (4.5 g, 16.1 mmol) was added to complete the reaction. The mixture was further stirred for 12 h, after which the reaction was quenched with an excess amount of MeOH and concentrated in vacuo. The residue underwent column chromatography (silica gel, hexane:EtOAc = 6:1 to 3:1) to yield 5 as a white foam (7.08 g, 60% in three steps).; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00–7.98 (m, 4H), 7.51–7.48 (m, 8H), 7.38–7.35 (m, 4H), 7.32 (dd, *J* = 7.4, 7.4 Hz, 6H), 7.25 (t, *J* = 7.4 Hz, 3H), 5.90–5.84 (m, 1H), 5.78 (dd, *J* = 9.0, 9.0 Hz, 1H), 5.31 (dddd, *J* = 1.3, 1.4, 1.5, 15.7 Hz, 1H), 5.27–5.25 (m, 2H), 5.16 (dddd, *J* = 1.3, 1.4, 1.5, 10.3 Hz, 1H), 4.25 (dddd, *J* = 1.5, 1.5, 5.1, 13.1 Hz, 1H), 4.05 (dddd, *J* = 1.3, 1.3, 6.0, 13.1 Hz, 1H), 3.96 (ddd, *J* = 4.0, 4.8, 9.1 Hz, 1H), 3.91 (ddd, *J* = 3.9, 9.1, 9.1 Hz, 1H), 3.49 (dd, *J* = 4.0, 10.1 Hz, 1H), 3.45 (dd, *J* = 4.8, 10.1 Hz, 1H), 2.83 (d, *J* = 3.9 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.2, 166.1, 143.8, 133.7, 133.4, 133.4, 130.0, 130.0, 129.6, 129.4, 128.8, 128.5, 128.5, 128.1, 127.3, 117.7, 95.2, 87.3, 74.0, 71.6, 71.4, 70.5, 68.6, 64.0; HRMS (ESI): calculated for $[M+Na]^+$ requires *m/z* = 693.2464, found 693.2462.

Synthesis of 6



6

Step 1: Methylation

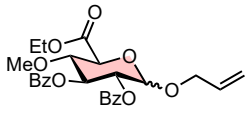
MeI (6.4 mL, 103 mmol) was added to a mixture of 5 (6.88 g, 10.3 mmol) and Ag2O (23.9 g, 103 mmol) in dimethylformamide (DMF; 52 mL) at room temperature. After stirring for 21 h at room temperature, MeI (6.4 mL, 103 mmol) and Ag2O (23.9 g, 103 mmol) were added to complete the reaction. The mixture was stirred for 18 h and then filtered through a Celite® pad. The filtrate was diluted with EtOAc and washed with H2O four times. The organic layer was dried over Na2SO4, filtered through a silica gel pad, and concentrated in vacuo. The residue was used in the next step without further purification.

Step 2: Benzylidene group removal

To a solution of the above product in CH2Cl2 (50 mL) and MeOH (50 mL) was added TsOH•H2O (710 mg, 2.06 mmol) at room temperature. The reaction mixture was stirred for 5 h at room temperature, after which the mixture was diluted with CH2Cl2 and washed with saturated NaHCO3 and brine. The organic layer was dried over Na2SO4, filtered, and concentrated in vacuo. The residue underwent column chromatography (silica gel, hexane:AcOEt = 4:1 to 2:1) to yield 6 as a colourless oil (1.36 g, 30% in two steps); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (d, *J* = 7.7 Hz, 2H), 7.97 (d, *J* = 7.7 Hz, 2H), 7.52–7.49 (m, 2H), 7.39 (dd, *J* = 7.7, 7.7 Hz, 2H), 7.37 (dd, *J* = 7.7, 7.7 Hz, 2H), 5.99 (dd, *J* = 9.6, 9.6 Hz, 1H), 5.85–5.78 (m, 1H), 5.29–5.25 (m, 2H), 5.13 (d, *J* =

10.7 Hz, 1H), 5.11 (dd, *J* = 3.5, 10.3 Hz, 1H), 4.21 (dd, *J* = 6.8, 13.2 Hz, 1H), 4.02 (dd, *J* = 4.0, 13.2 Hz, 1H), 3.93–3.84 (m, 3H), 3.67 (dd, *J* = 9.6, 9.6 Hz, 1H), 3.47 (s, 3H), 1.89 (dd, *J* = 4.3, 7.9 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.2, 165.8, 133.5, 133.4, 133.3, 130.0, 129.9, 129.8, 129.3, 128.6, 128.5, 117.8, 95.3, 77.9, 72.7, 72.3, 71.0, 68.8, 61.7, 60.7; HRMS (ESI): calculated for $[M+Na]^+$ requires *m/z* = 465.1525, found 465.1522.

Synthesis of 7



7

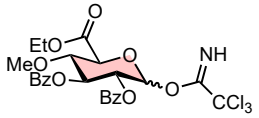
Step 1: Oxidation

AZADOL® (23.4 mg, 0.153 mmol; TCI Chemicals, Tokyo, Japan) was added to a mixture of **6** (1.35 g, 3.05 mmol) and $PhI(OAc)_2$ (2.16 g, 6.71 mmol) in CH_2Cl_2 (8 mL) and 0.1 M phosphate buffer (pH 7.0, 8 mL) at 0°C. The reaction mixture was stirred for 2 h at 0°C, after which the mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was used in the next step without purification.

Step 2: Esterification

Ethyl iodide (0.75 mL, 9.15 mmol) was added to a mixture of the above product and $KHCO_3$ (1.53 g, 51.3 mmol) in DMF (7.8 mL) at room temperature. The reaction mixture was stirred for 5 h at room temperature, after which the mixture was diluted with EtOAc and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue underwent column chromatography (silica gel, hexane:AcOEt = 4:1 to 2:1) to yield **7** as a colourless oil (820 mg, 55% in two steps); ^{1}H NMR (600 MHz, $CDCl_3$) δ□8.01 (dd, *J* = 1.0, 8.2 Hz, 2H), 7.97 (dd, *J* = 1.2, 8.2 Hz, 2H), 7.53–7.49 (m, 2H), 7.40 (dd, *J* = 8.2, 8.2 Hz, 2H), 7.37 (dd, *J* = 8.2, 8.2 Hz, 2H), 5.98 (dd, *J* = 9.7, 9.7 Hz, 1H), 5.85–5.79 (m, 1H), 5.32 (d, *J* = 3.7 Hz, 1H), 5.29 (dddd, *J* = 1.3, 1.4, 1.4, 17.1 Hz, 1H), 5.18 (dd, *J* = 3.5, 10.2 Hz, 1H), 5.15 (dddd, *J* = 1.3, 1.4, 1.4, 10.4 Hz, 1H), 4.35 (d, *J* = 9.7 Hz, 1H), 4.31 (q, *J* = 7.1 Hz, 2H), 4.27 (dddd, *J* = 1.4, 1.4, 5.0, 13.2 Hz, 1H), 4.05 (dddd, *J* = 1.3, 1.3, 6.2, 13.2 Hz, 1H), 3.85 (dd, *J* = 9.7, 9.7 Hz, 1H), 3.42 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.3, 166.0, 165.7, 133.5, 133.4, 133.2, 130.0, 129.9, 129.7, 129.2, 128.6, 128.6, 118.2, 95.7, 79.6, 72.1, 71.8, 70.3, 69.1, 62.0, 60.5, 14.3; HRMS (ESI): calculated for $[M+Na]^+$ requires *m/z* = 507.1631, found 507.1628.

Synthesis of 8



8

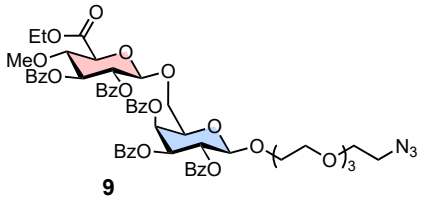
Step 1: Allyl group removal

Under an N_2 atmosphere, $PdCl_2$ (356 mg, 2.01 mmol) was added to a mixture of **7** (810 mg, 1.67 mmol) and NaOAc (342 g, 4.18 mmol) in AcOH (18 mL) and H_2O (1.8 mL) at room temperature. The reaction mixture was stirred for 48 h at room temperature, after which silica gel was added to the mixture. The volatile compounds were removed *in vacuo* and the resulting silica gel slurry underwent column chromatography (silica gel, hexane:AcOEt = 6:1 to 3:1) to yield the deprotected product (718 mg). Although this product contained small amounts of impurities, it was used in the next step without further purification.

Step 2: Trichloroacetimidation

1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU; 24 µL, 0.162 mmol) was added to a solution of the above compound (718 mg, ~1.62 mmol) and Cl_3CCN (1.6 mL, 16.2 mmol) in CH_2Cl_2 (16 mL) at 0°C. The reaction mixture was stirred at 0°C for 15 min and then warmed to room temperature. The reaction mixture was stirred again for 4 h at room temperature and then concentrated *in vacuo*. The residue underwent short column chromatography (silica gel, hexane:AcOEt = 4:1 to 2:1 + 1% Et_3N) to yield **7** as a white foam (234 mg). After column purification, the resulting product was used immediately in the next reaction due to its instability.

Synthesis of 9



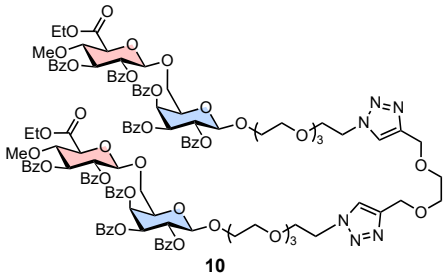
9

Under an N_2 atmosphere, TMSOTf (15 µL, 16.3 mmol) was added to a mixture of **2** (520 mg, 0.750 mmol) and **8** (234 mg, 0.419 mmol) in CH_2Cl_2 (4 mL) at −20°C. This reaction mixture was stirred at −20°C for 3 h and then allowed to slowly warm to room temperature. The reaction mixture was stirred for 6 h at room temperature and then quenched with an excess amount of Et_3N and finally concentrated. The residue underwent column chromatography (silica gel, hexane:AcOEt = 4:1 to 2:1 to 1:1) to yield **9** as a colourless foam (294 mg, 63%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (dd, *J* = 1.3, 8.3 Hz, 2H), 7.97 (dd, *J* = 1.3, 8.3 Hz, 2H), 7.94–7.92 (m, 4H), 7.74 (dd, *J* = 1.0, 8.2 Hz, 2H), 7.62–7.59 (m, 1H), 7.52–7.46 (m, 5H), 7.42–7.35 (m, 7H), 7.21 (t, *J* = 8.2 Hz, 2H), 5.80 (dd, *J* = 0.8, 3.4 Hz, 1H), 5.66 (dd, *J* = 7.9, 10.4 Hz, 1H), 5.60 (dd, *J* = 9.2, 9.2 Hz, 1H), 5.47 (dd, *J* = 3.4, 10.4 Hz, 1H), 5.38 (dd, *J* = 7.3, 9.2 Hz, 1H), 4.81 (d, *J* = 7.3 Hz, 1H), 4.72 (d, *J* = 7.9 Hz, 1H), 4.24–4.19 (m, 2H), 4.10 (ddd, *J* = 0.8, 3.4, 7.7 Hz, 1H), 4.05 (dd, *J* = 3.4, 10.9 Hz, 1H), 4.02 (d, *J* = 9.5 Hz, 1H), 3.91 (dd, *J* = 9.2, 9.5 Hz, 1H), 3.79 (dd, *J* = 7.7, 10.9 Hz, 1H), 3.65–3.63 (m, 3H), 3.59–3.58 (m, 2H), 3.51–3.45 (m, 3H), 3.40–3.30 (m, 11H), 1.27 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.1, 165.7, 165.6, 165.6, 165.3, 165.2, 133.6, 133.5, 133.4, 133.3, 133.3, 130.2, 129.9, 129.9, 129.8, 129.8, 129.6, 129.3, 129.2, 129.0, 128.7, 128.6, 128.5, 128.5, 128.4, 101.4, 101.3, 79.2, 74.4, 74.3, 73.4, 72.0, 71.8, 70.7, 70.7, 70.6, 70.3, 70.1, 69.9, 69.4, 69.0, 68.8, 62.0, 60.6, 50.8, 14.2; HRMS (ESI): calculated for $[M+Na]^+$ requires *m/z* = 1142.3746, found 1142.3733.

General click reaction procedure for the preparation of 10–12

Under an N_2 atmosphere, sodium ascorbate (80 mol %) was added to a mixture of **9** (2.1–4.2 eq.), alkyne linker (1 eq.), $CuSO_4$ (20 mol %), and Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA; 40 mol %) in tetrahydrofuran (THF) and H_2O at room temperature. This reaction mixture was stirred at room temperature for 18 h, diluted with CH_2Cl_2, and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue underwent preparative TLC (silica gel, $CHCl_3$:MeOH = 10:1) to afford **10**–**12**.

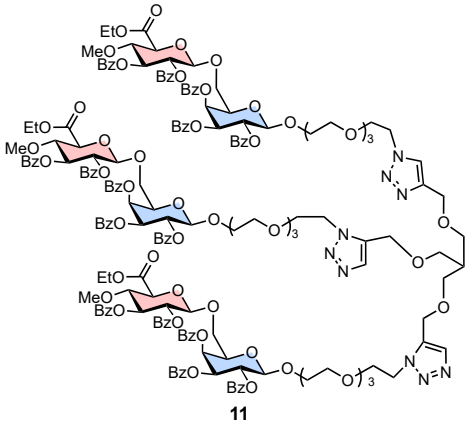
Synthesis of 10



10

The reaction was performed with **9** (34.8 mg, 31.0 μmol), bis-alkyne linker (2 mg, 14.8 μmol), $CuSO_4$ (0.5 mg, 2.96 μmol), sodium ascorbate (2.3 mg, 11.8 μmol), TBTA (3.1 mg, 5.92 μmol) in THF (1 mL) and H_2O (0.2 mL). After the above-described purification, compound **10** was afforded as a colourless oil (23.0 mg, 65%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 1.3, 8.3 Hz, 4H), 7.96 (dd, *J* = 1.3, 8.3 Hz, 4H), 7.93–7.90 (m, 8H), 7.73 (d, *J* = 1.1, 8.3 Hz, 4H), 7.70 (s, 2H), 7.61–7.58 (m, 2H), 7.52–7.44 (m, 10H), 7.41–7.32 (m, 14H), 7.21 (t, *J* = 8.3 Hz, 4H), 5.80 (dd, *J* = 0.7, 3.4 Hz, 2H), 5.66 (dd, *J* = 7.9, 10.4 Hz, 2H), 5.60 (dd, *J* = 9.2, 9.2 Hz, 2H), 5.47 (dd, *J* = 3.4, 10.4 Hz, 2H), 5.37 (dd, *J* = 7.3, 9.2 Hz, 2H), 4.81 (d, *J* = 7.3 Hz, 2H), 4.72 (d, *J* = 7.9 Hz, 2H), 4.65 (s, 4H), 4.49 (t, *J* = 5.4 Hz, 4H), 4.23–4.18 (m, 4H), 4.11 (dd, *J* = 3.4, 7.7 Hz, 2H), 4.04 (dd, *J* = 3.4, 10.9 Hz, 2H), 4.01 (d, *J* = 9.5 Hz, 2H), 3.90 (dd, *J* = 9.2, 9.5 Hz, 2H), 3.81 (t, *J* =5.4 Hz, 4H), 3.78 (dd, *J* = 7.7, 10.9 Hz, 2H), 3.68 (s, 4H), 3.66–3.63 (m, 2H), 3.52–3.50 (m, 4H), 3.48–3.42 (m, 6H), 3.40–3.32 (m, 14H), 3.28–3.26 (m, 4H), 1.26 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0. 165.6, 165.5, 165.3, 165.2, 144.9, 133.6, 133.5, 133.4, 133.3, 130.1, 129.9, 129.9, 129.8, 129.8, 129.6, 129.3, 129.3, 129.2, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 123.9, 101.4, 101.3, 79.1, 74.5, 74.3, 73.3, 72.0, 71.8, 70.7, 70.6, 70.5, 70.4, 70.2, 69.9, 69.7, 69.5, 69.3, 69.0, 68.8, 64.7, 62.0, 60.6, 50.3, 14.2; HRMS (ESI): calculated for $[M+2H]^{2+}$ requires *m/z* = 1189.9283, found 1189.9282.

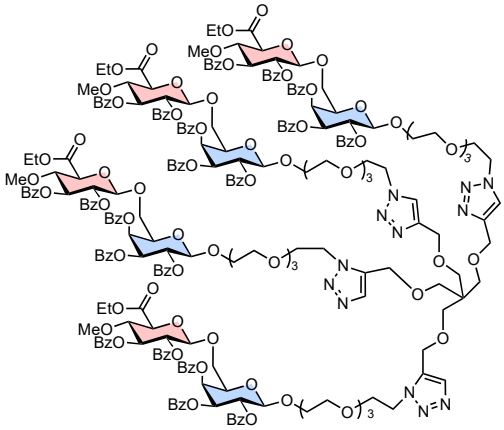
Synthesis of 11



11

The reaction was performed with **9** (35.5 mg, 31.7 μmol), tris-alkyne linker (2.2 mg, 10.6 μmol), $CuSO_4$ (0.51 mg, 3.18 μmol), sodium ascorbate (2.5 mg, 12.7 μmol), and TBTA (3.4 mg, 6.36 μmol) in THF (1 mL) and H_2O (0.2 mL). After the above-described purification, compound **11** was afforded as a colourless oil (24.3 mg, 64%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 1.3, 8.3 Hz, 6H), 7.96 (dd, *J* = 1.3, 8.3 Hz, 6H), 7.93–7.90 (m, 12H), 7.73 (dd, *J* = 1.1, 8.3 Hz, 6H), 7.67 (s, 3H), 7.61–7.58 (m, 3H), 7.52–7.44 (m, 15H), 7.41–7.32 (m, 21H), 7.21 (t, *J* = 8.3 Hz, 6H), 5.80 (dd, *J* = 0.8, 3.5 Hz, 3H), 5.66 (dd, *J* = 8.0, 10.4 Hz, 3H), 5.60 (dd, *J* = 9.2, 9.2 Hz, 3H), 5.47 (dd, *J* = 3.5, 10.4 Hz, 3H), 5.37 (dd, *J* = 7.3, 9.2 Hz, 3H), 4.81 (d, *J* = 7.3 Hz, 3H), 4.72 (d, *J* = 7.9 Hz, 3H), 4.54 (s, 6H), 4.49 (t, *J* = 5.3 Hz, 6H), 4.22–4.18 (m, 6H), 4.11 (dd, *J* = 3.4, 7.6 Hz, 3H), 4.04 (dd, *J* = 3.4, 11.0 Hz, 3H), 4.02 (d, *J* = 9.4 Hz, 3H), 3.90 (dd, *J* = 9.2, 9.4 Hz, 3H), 3.81 (t, *J* =5.3 Hz, 6H), 3.78 (dd, *J* = 7.6, 11.0 Hz, 3H), 3.66–3.63 (m, 3H), 3.54 (d, *J* = 5.9 Hz, 6H), 3.52–3.50 (m, 6H), 3.46–3.42 (m, 9H), 3.39–3.32 (m, 21H), 3.27–3.25 (m, 6H), 2.22–2.17 (m, 1H), 1.26 (t, *J* = 7.1 Hz, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.1. 165.7, 165.6, 165.3, 165.2, 145.0, 133.7, 133.5, 133.4, 133.3, 130.2, 129.9, 129.9, 129.8, 129.8, 129.6, 129.3, 129.3, 129.2, 129.0, 128.7, 128.6, 128.6, 128.5, 128.4, 123.8, 101.4, 101.3, 79.2, 74.5, 74.3, 73.3, 72.0, 71.9, 70.7, 70.5, 70.5, 70.4, 70.2, 70.0, 69.5, 69.4, 69.0, 68.9, 68.8, 64.7, 62.0, 60.6, 50.3, 40.4, 14.2; HRMS (ESI): calculated for $[M+2H]^{2+}$ requires *m/z* = 1791.1433, found 1791.1451.

Synthesis of 12



12

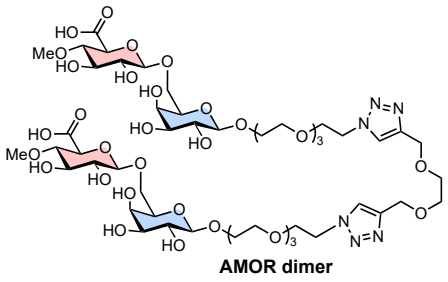
The reaction was performed with **9** (37.8 mg, 33.7 μmol), tetra-alkyne linker (2.3 mg, 8.03 μmol), $CuSO_4$ (0.51 mg, 3.21 μmol), sodium ascorbate (2.5 mg, 12.8 μmol), and TBTA (3.4 mg, 6.42 μmol) in THF (2 mL) and H_2O (0.4 mL). After the above-described purification, compound **12** was afforded as a colourless oil (23.8 mg, 62%); ^{1}H NMR (600 MHz, $CDCl_3$) ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 1.3, 8.4 Hz, 8H), 7.96 (dd, *J* = 1.3, 8.4 Hz, 8H), 7.93–7.89 (m, 16H), 7.73 (dd, *J* = 1.2, 8.4 Hz, 8H), 7.66 (s, 4H), 7.61–7.58 (m, 4H), 7.52–7.44 (m, 20H), 7.41–7.31 (m, 28H), 7.20 (t, *J* = 8.4 Hz, 8H), 5.80 (dd, *J* = 0.6, 3.6 Hz, 4H), 5.66 (dd, *J* = 8.0, 10.4 Hz, 4H), 5.60 (dd, *J* = 9.2, 9.2 Hz, 4H), 5.47 (dd, *J* = 3.5, 10.4 Hz, 4H), 5.37 (dd, *J* = 7.3, 9.2 Hz, 4H), 4.81 (d, *J* = 7.3 Hz, 4H), 4.72 (d, *J* = 7.9 Hz, 4H), 4.51 (s, 8H), 4.47 (t, *J* = 5.3 Hz, 8H), 4.22–4.18 (m, 8H), 4.11 (dd, *J* = 3.5, 7.7 Hz, 4H), 4.05 (dd, *J* = 3.5, 11.0 Hz, 4H), 4.01 (d, *J* = 9.5 Hz, 4H), 3.90 (dd, *J* = 9.2, 9.5 Hz, 4H), 3.81 (t, *J* =5.3 Hz, 8H), 3.78 (dd, *J* = 7.7, 11.0 Hz, 4H), 3.66–3.63 (m, 4H), 3.52–3.50 (m, 8H), 3.47–3.44 (m, 12H), 3.42–3.40 (m, 8H), 3.39–3.33 (m, 28H), 3.25–3.23 (m, 8H), 1.26 (t, *J* = 7.1 Hz, 12H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0. 165.6, 165.6, 165.3, 165.2, 145.0, 133.7, 133.5, 133.4, 133.3, 130.2, 129.9,

129.9, 129.8, 129.8, 129.6, 129.3, 129.3, 129.2, 129.0, 128.7, 128.6, 128.6, 128.5, 128.4, 123.8, 101.4, 101.3, 79.2, 74.5, 74.3, 73.3, 72.0, 71.8, 70.7, 70.5, 70.5, 70.4, 70.2, 69.9, 69.5, 69.4, 69.0, 68.9, 68.8, 65.0, 62.0, 60.6, 50.2, 45.4, 14.2; calculated for $[M+3H]^{3+}$ requires *m/z* = 1590.5696, found 1590.5668.

General hydrolysis reaction procedure for the preparation of multivalent AMORs

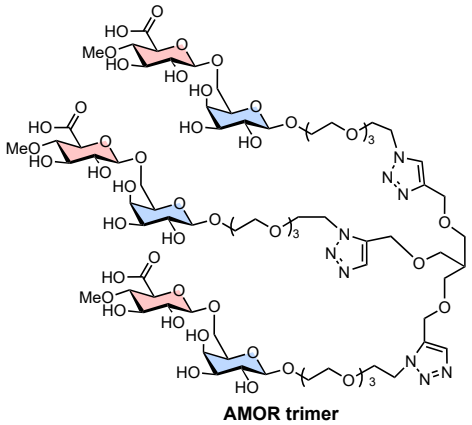
To a mixture of 10–12 (1 eq.) in THF, MeOH, and H_2O was added LiOH•H_2O (12–36 eq.). The reaction was monitored by LC–MS or MALDI-TOF MS. After completion of the reaction, the mixture was quenched with AcOH and the pH was adjusted to 4~5. The mixture was concentrated, and the residue underwent preparative TLC (C8-modified silica gel, 5% CH_3CN/H_2O) and subsequent column chromatography (Sephadex™ LH-20, MeOH) to yield multivalent AMORs. These compounds were characterised via ^{1}H NMR and mass spectroscopy.

AMOR dimer



The reaction was performed with **10** (20.0 mg, 8.90 μmol) and LiOH•H_2O (4.4 mg, 105 μmol) in THF (0.2 mL), MeOH (0.2 mL), and H_2O (0.2 mL). After the above-described purification, **AMOR dimer** was afforded as a colourless oil (6.2 mg, 58%); ^{1}H NMR (600 MHz, D_2O) δ 8.00 (s, 2H), 4.59 (s, 4H), 4.56 (t, *J* = 5.1 Hz, 4H), 4.38 (d, *J* = 7.9 Hz, 2H), 4.32 (d, *J* = 7.9 Hz, 2H), 3.97 (ddd, *J* = 4.1, 4.1, 11.7 Hz, 2H), 3.95–3.91 (m, 2H), 3.89 (t, *J* = 5.1 Hz, 4H), 3.85 (d, *J* = 3.4 Hz, 2H), 3.80–3.76 (m, 4H), 3.74–3.71 (m, 2H), 3.65–3.63 (m, 8H), 3.60 (d, *J* = 9.8 Hz, 2H), 3.58–3.55 (m, 10H), 3.54–3.51 (m, 8H), 3.46–3.42 (m, 4H), 3.39 (s, 6H), 3.26 (dd, *J* = 8.0, 9.2 Hz, 2H), 3.19 (dd, *J* = 9.5, 9.5 Hz, 2H); MS (ESI): calculated for $[M+H]^+$ requires *m/z* = 1281.5, found 1280.7.

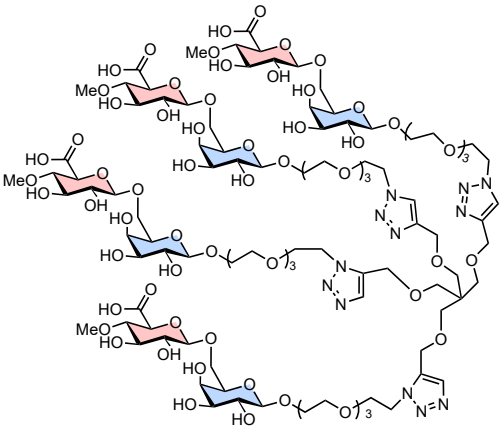
AMOR trimer



The reaction was performed with **11** (24.3 mg, 6.78 μmol) and LiOH•H_2O (5.1 mg, 122 μmol) in THF (0.3 mL), MeOH (0.3 mL) and H_2O (0.3 mL). After stirring for 6 h, LiOH•H_2O (3.0 mg, 71.4 μmol) was added to complete the reaction. The mixture was further stirred for 24 h. After the above-described purification, **AMOR trimer** was afforded as a colourless oil (3.4 mg, 26%); ^{1}H NMR (600 MHz, D_2O) δ 7.96 (s, 3H), 4.54 (t, *J* = 5.2 Hz, 6H), 4.50 (s, 6H), 4.38 (d, *J* = 7.9 Hz, 3H), 4.31 (d, *J* = 7.9 Hz, 3H), 3.97 (dt, *J* = 4.2, 11.7 Hz, 3H), 3.95–3.91 (m, 3H), 3.88 (t, *J* = 5.1 Hz, 6H), 3.86 (d, *J* = 3.5 Hz, 3H), 3.80–3.76 (m, 6H), 3.74–3.71 (m, 3H), 3.64 (t, *J* = 4.1 Hz, 6H), 3.60 (d, *J* = 9.8 Hz, 3H), 3.58–3.54 (m, 15H), 3.53–3.51 (m, 12H), 3.46–3.42 (m, 12H), 3.39 (s, 9H), 3.26 (dd, *J* = 7.9, 9.4 Hz, 3H), 3.19 (dd, *J* =

9.4, 9.4 Hz, 3H), 2.11–2.07 (m, 1H); MS (MALDI): calculated for $[M+H]^+$ requires *m/z* = 1934.8, found 1935.5.

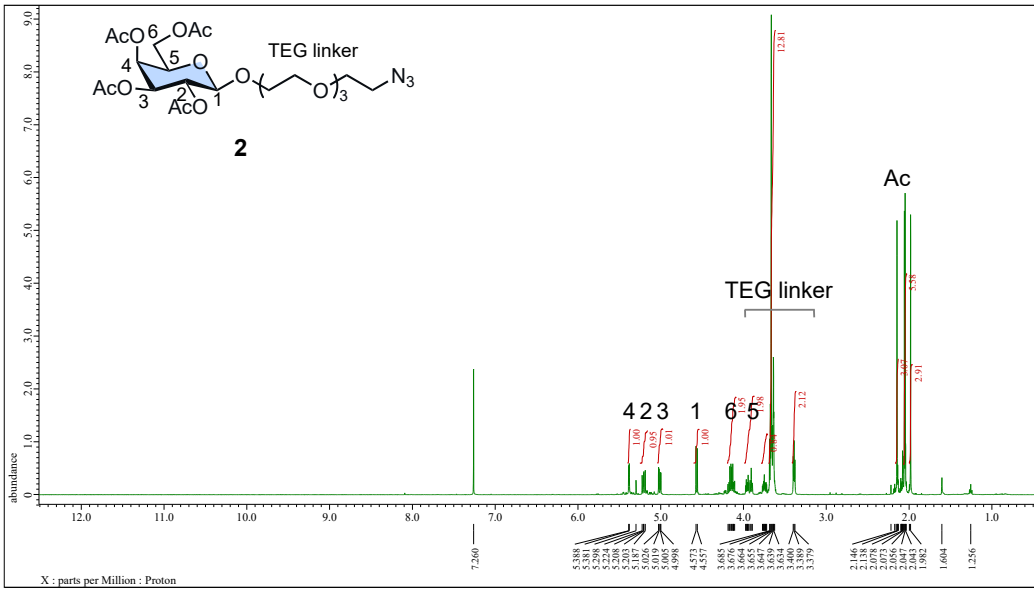
AMOR tetramer



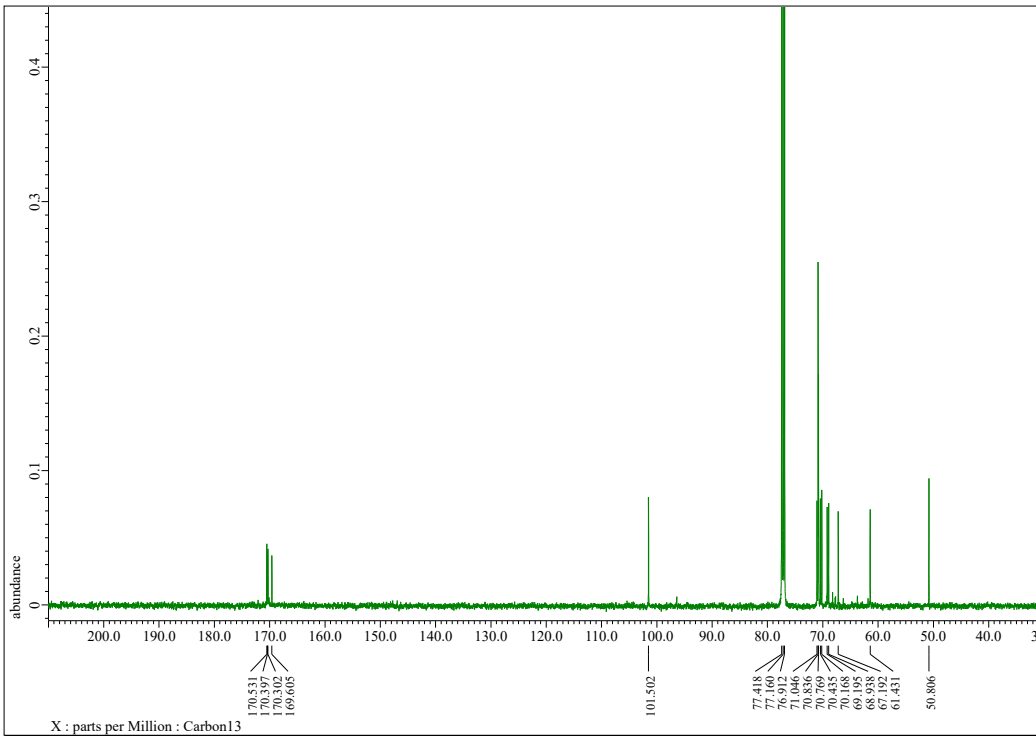
AMOR tetramer

The reaction was performed with **12** (23.8 mg, 4.99 μmol) and LiOH•H_2O (5.1 mg, 122 μmol) in THF (0.3 mL), MeOH (0.3 mL) and H_2O (0.3 mL). After stirring for 6 h, additional LiOH•H_2O (6.0 mg, 143 μmol) was added to complete the reaction. The mixture was further stirred for 48 h. After the above-described purification, **AMOR tetramer** was afforded as a colourless oil (2.8 mg, 22%); ^{1}H NMR (600 MHz, D_2O) δ 7.91 (s, 4H), 4.51 (t, *J* = 5.2 Hz, 8H), 4.44 (s, 8H), 4.38 (d, *J* = 7.9 Hz, 4H), 4.32 (d, *J* = 7.9 Hz, 4H), 3.97 (dt, *J* = 4.2, 11.8 Hz, 4H), 3.95–3.91 (m, 4H), 3.87–3.86 (m, 12H), 3.79–3.76 (m, 8H), 3.74–3.71 (m, 4H), 3.63 (d, *J* = 4.2 Hz, 8H), 3.60 (d, *J* = 9.8 Hz, 4H), 3.58–3.55 (m, 12H), 3.54–3.50 (m, 24H), 3.45–3.43 (m, 8H), 3.39 (s, 12H), 3.30 (s, 8H), 3.26 (dd, *J* = 7.9, 9.4 Hz, 4H), 3.19 (dd, *J* = 9.4, 9.4 Hz, 4H); MS (MALDI): calculated for $[M+H]^+$ requires *m/z* = 2574.0, found 2574.6.

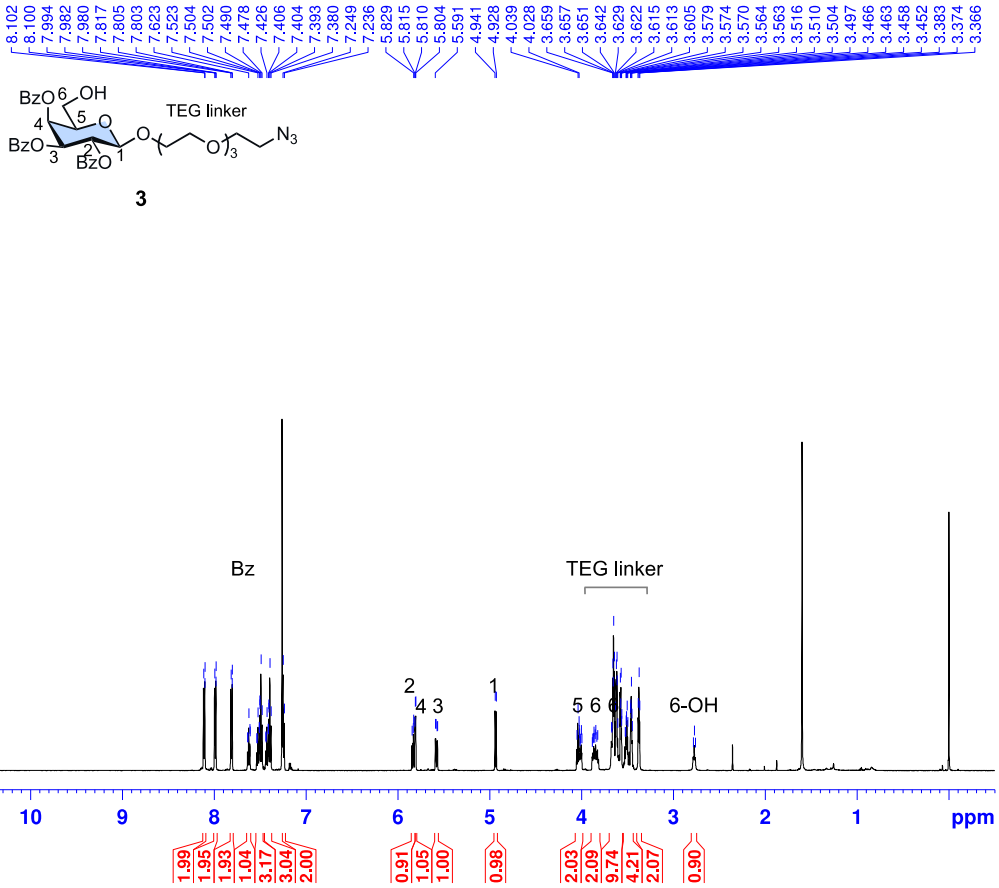
2: ^{1}H NMR (500 MHz, $CDCl_3$)



2: ^{13}C NMR (125 MHz, $CDCl_3$)



3: ^{1}H NMR (600 MHz, $CDCl_3$)

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AQ                 2.6477449 sec
RG                       362
DW                  40.400 usec
DE                   6.50 usec
TE                   298.0 K
D1                1.00000000 sec
TD0                        1

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PL1                   -1.00 dB
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SFO1           600.1337060 MHz
SI                      32768
SF             600.1300110 MHz
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SSB                        0
LB                      0.30 Hz
GB                         0
PC                      1.00
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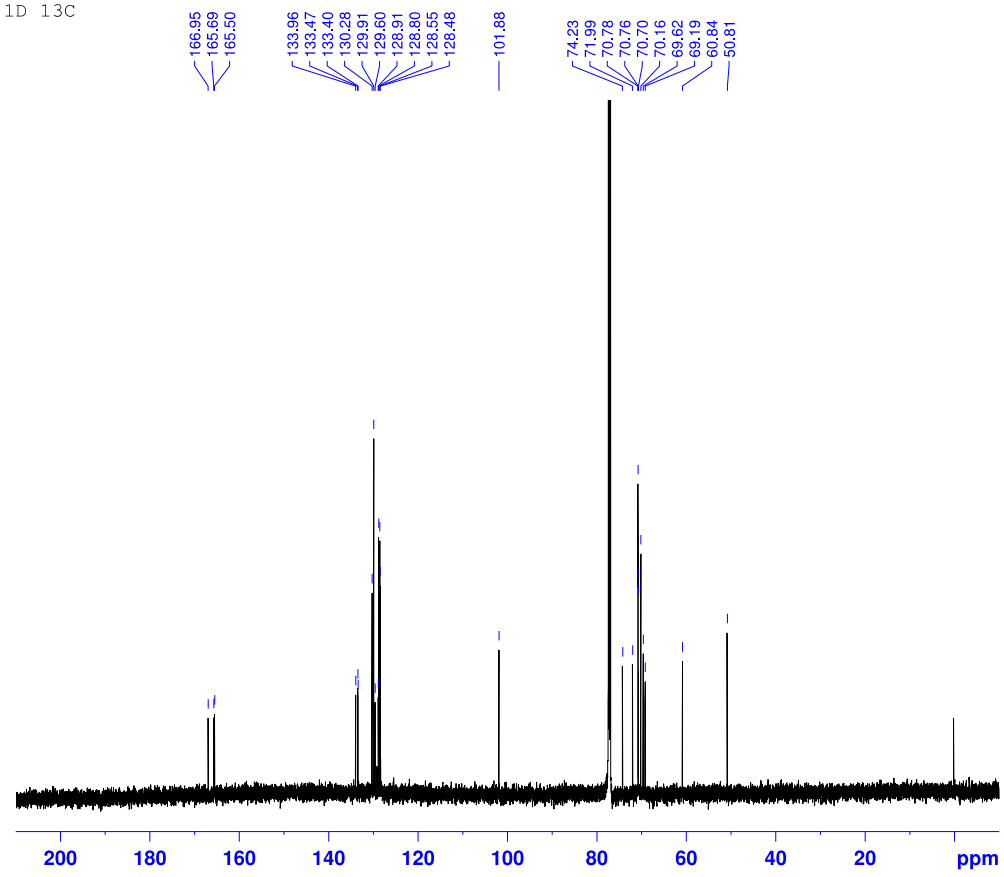
3: ^{13}C NMR (150 MHz, $CDCl_3$)

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TE                   298.0 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                        1

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P1                    15.00 usec
PL1                   -1.90 dB
PL1W          194.52256775 W
SFO1           150.9178988 MHz

======== CHANNEL f2 ========
CPDPRG2             waltz16
NUC2                      1H
PCPD2                100.00 usec
PL2                  120.00 dB
PL12                  20.96 dB
PL13                  20.96 dB
PL2W           0.00000000 W
PL12W          0.20137247 W
PL13W          0.20137247 W
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SF             150.9027868 MHz
WDW                       EM
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GB                         0
PC                      1.40
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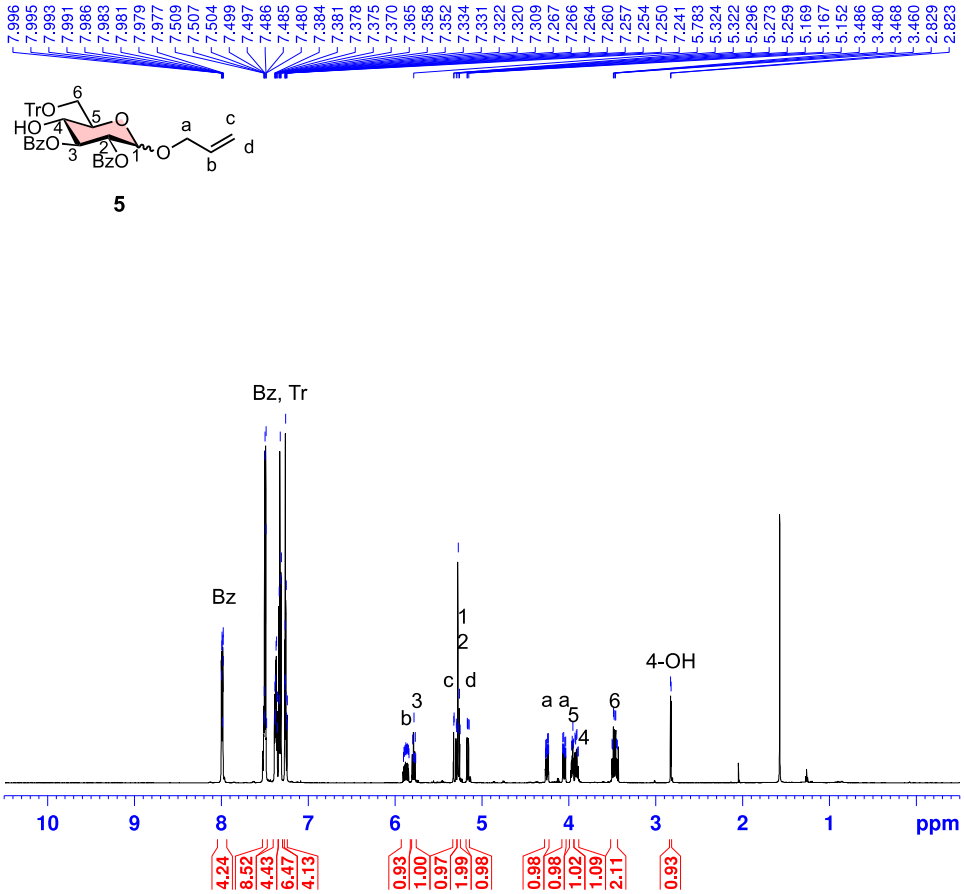
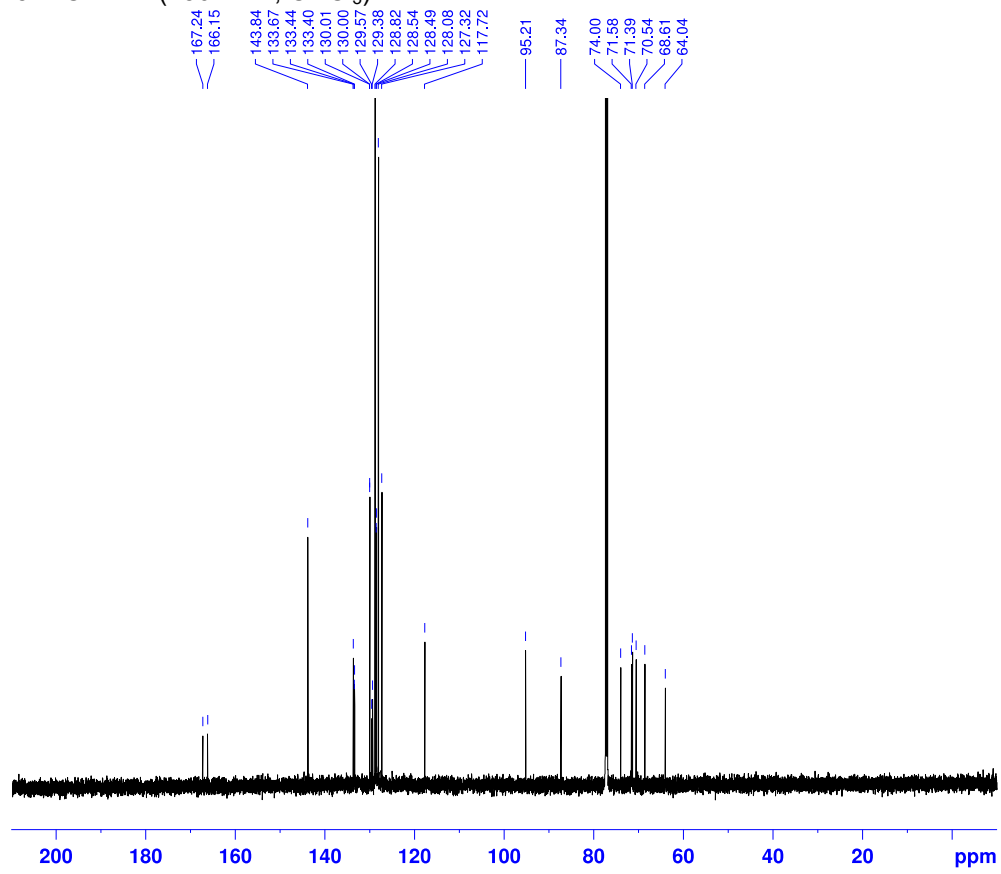
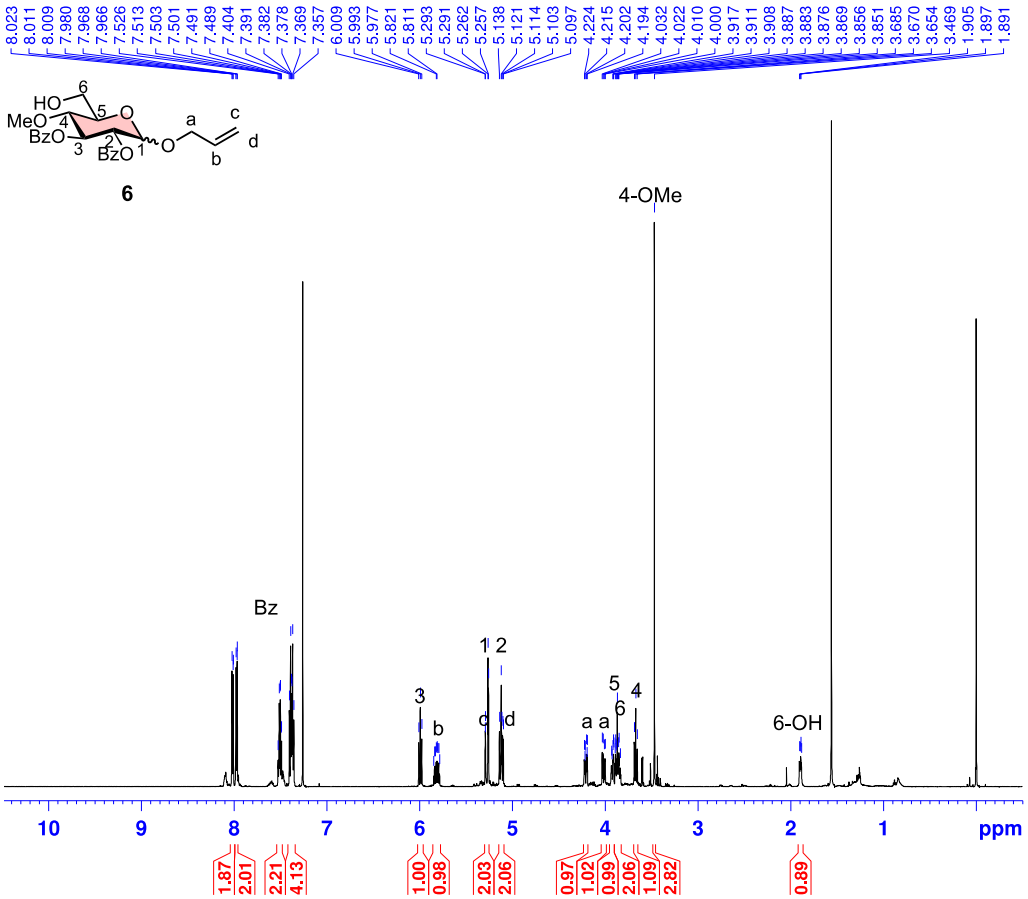
5: ^{1}H NMR (600 MHz, $CDCl_3$)



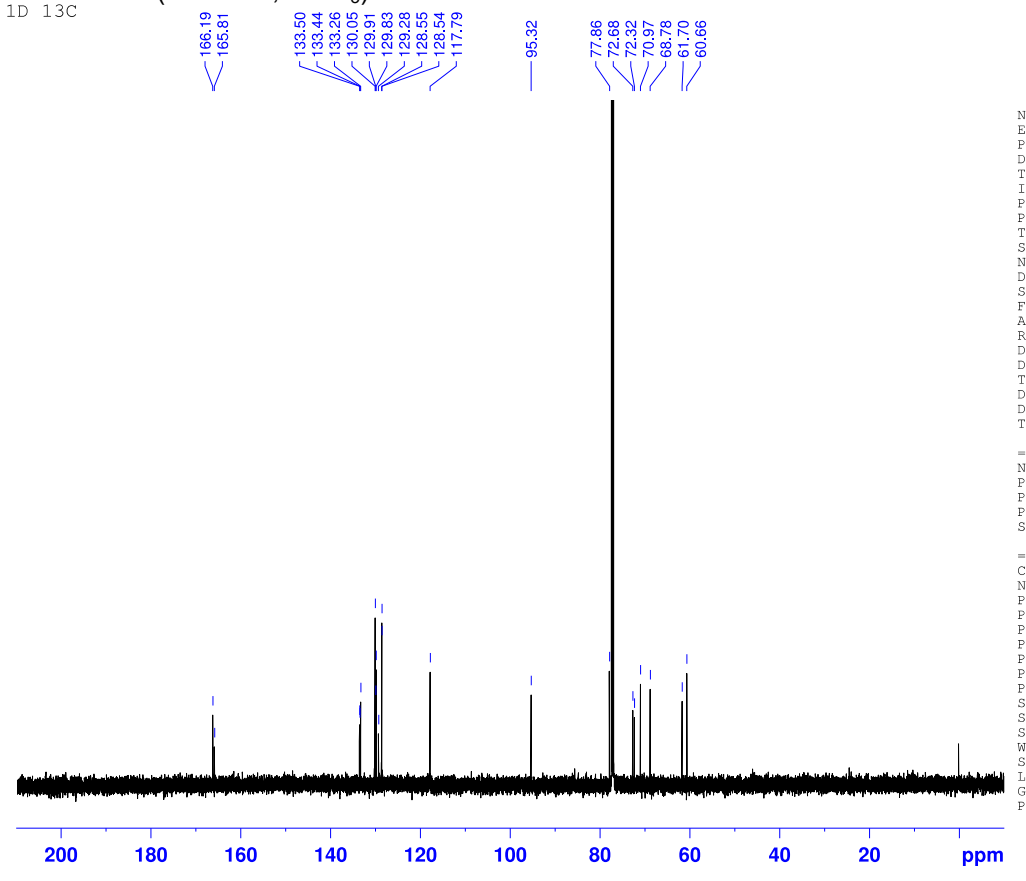
5: ^{13}C NMR (150 MHz, $CDCl_3$)



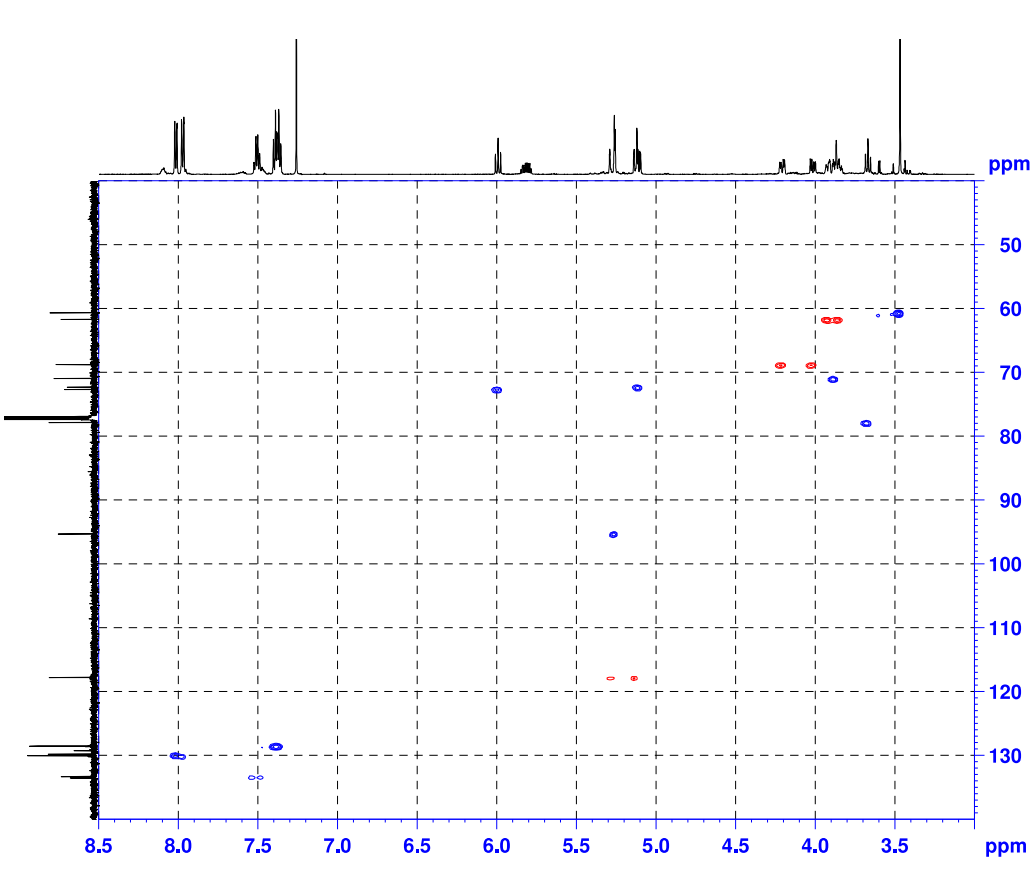
6: ^{1}H NMR (600 MHz, $CDCl_3$)



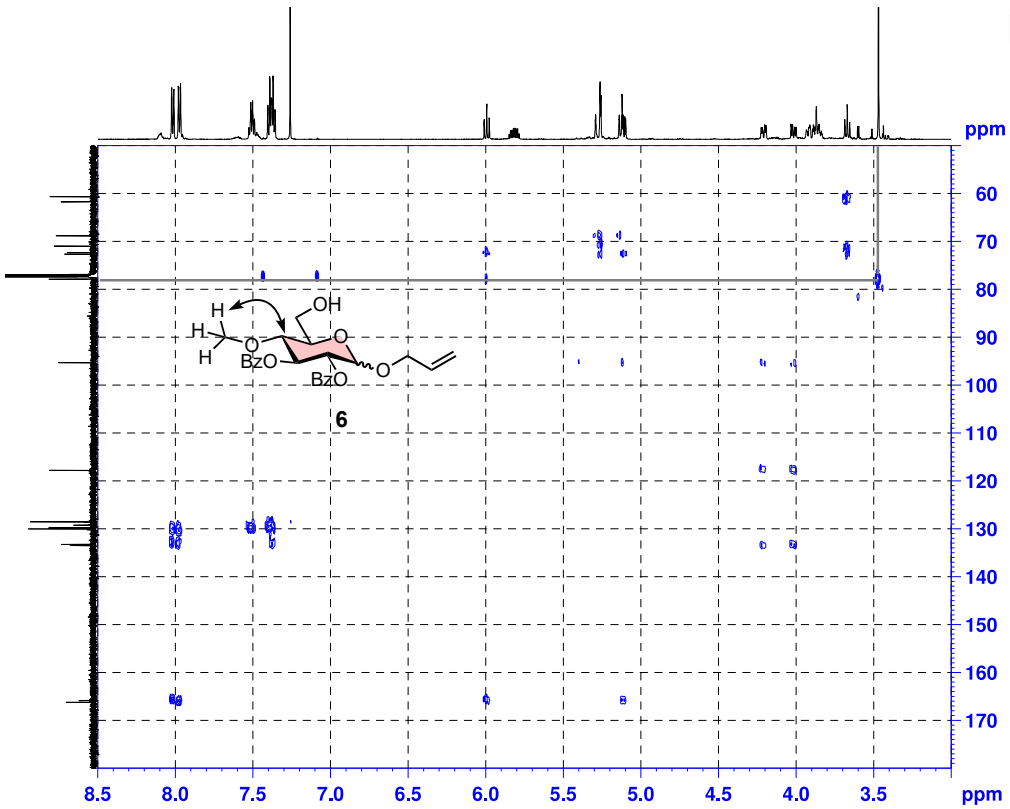
6: ^{13}C NMR (150 MHz, $CDCl_3$)



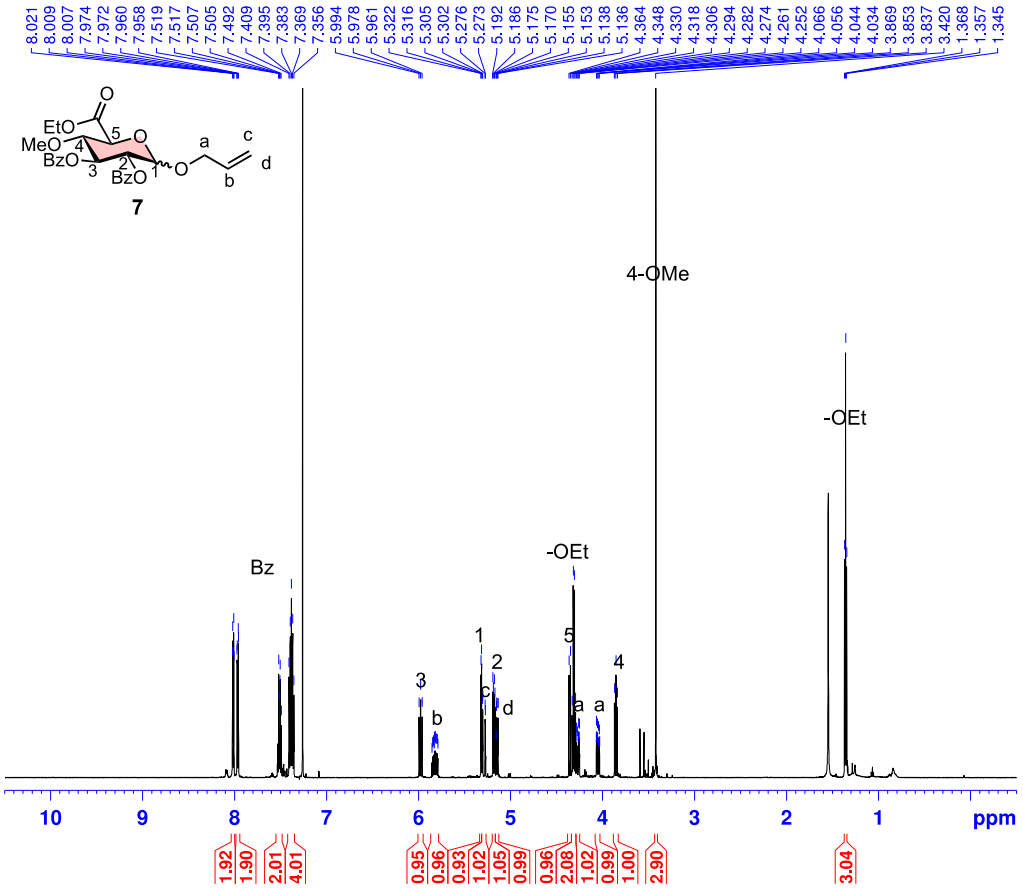
6: HSQC ($CDCl_3$)



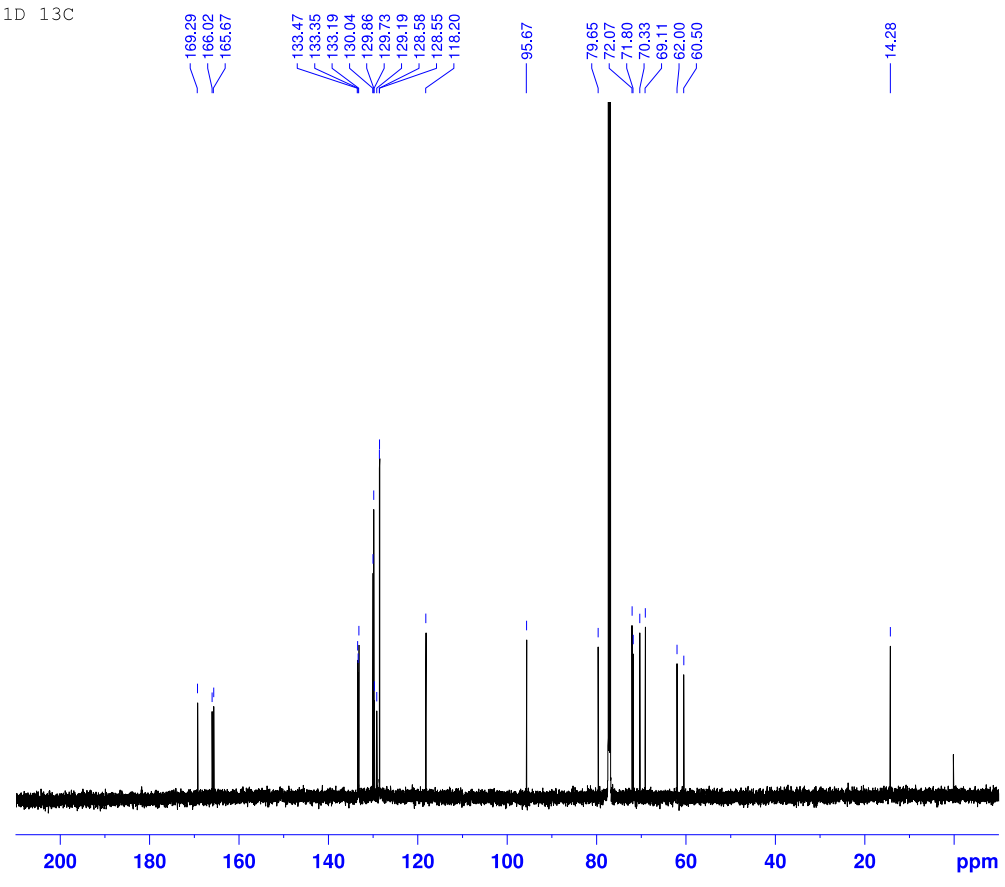
6: HMBC ($CDCl_3$)



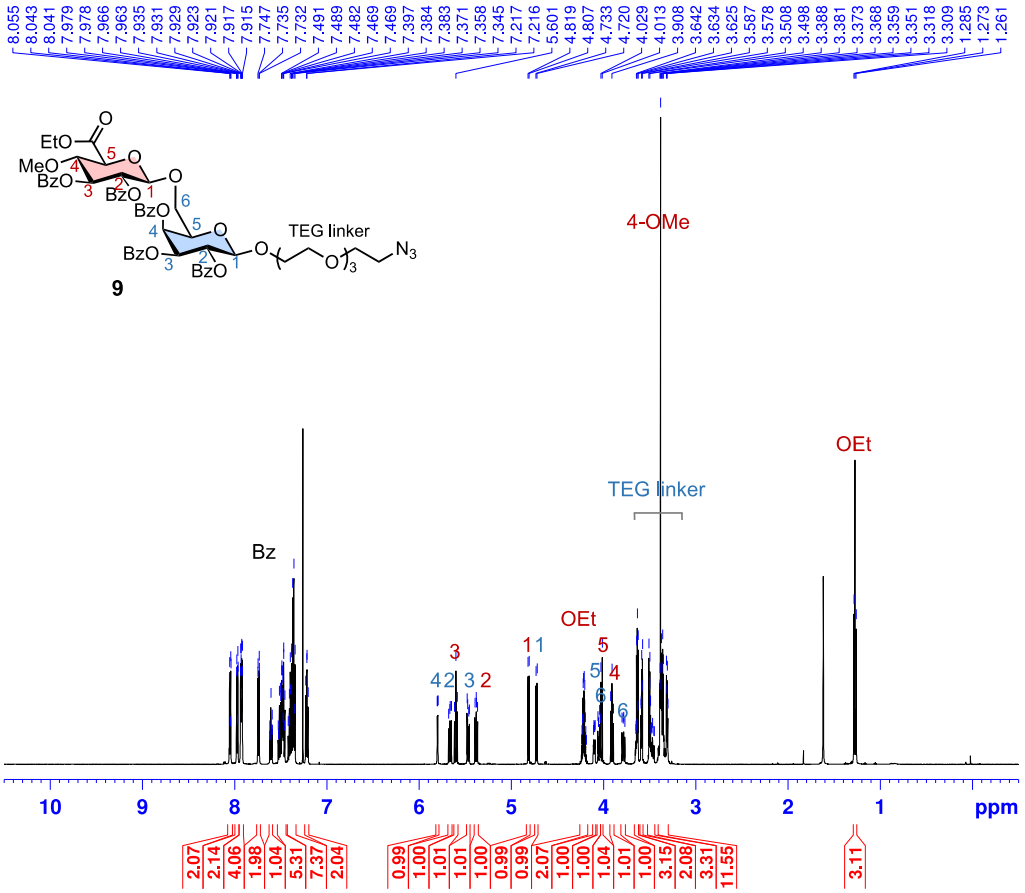
7: ^{1}H NMR (600 MHz, $CDCl_3$)



7: ^{13}C NMR (150 MHz, $CDCl_3$)

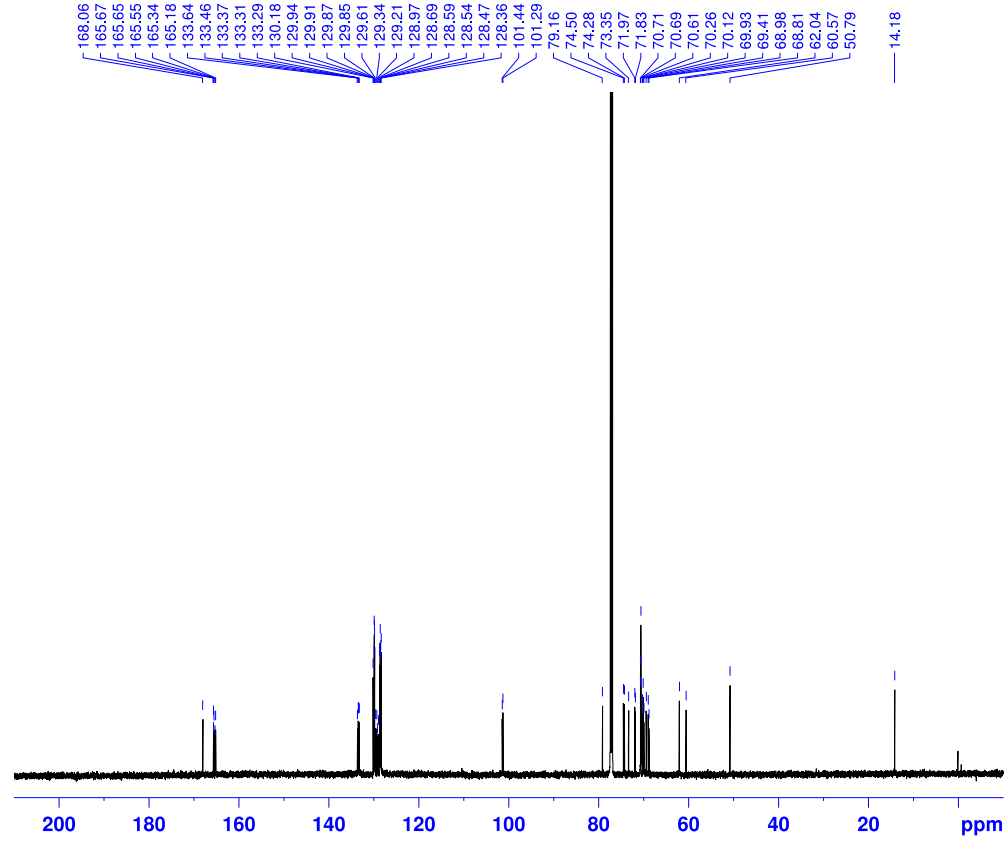


9: ^{1}H NMR (600 MHz, $CDCl_3$)

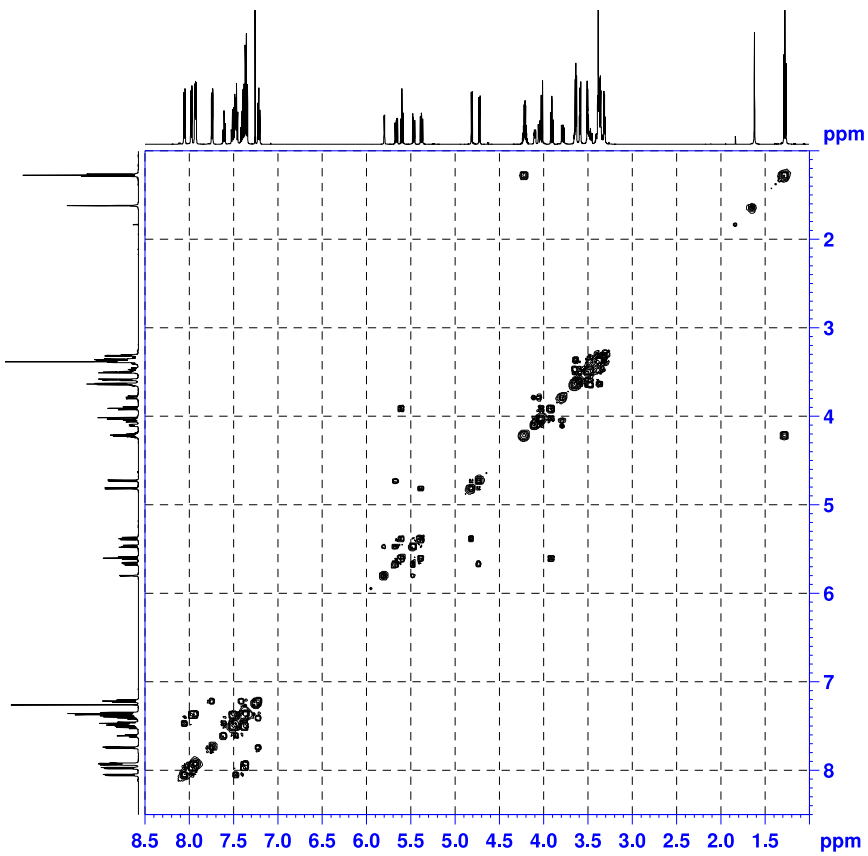


9: ^{13}C NMR (150 MHz, $CDCl_3$)

1D 13C



9: COSY ($CDCl_3$)



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D1 1.486
D13 0.000
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P0
P1
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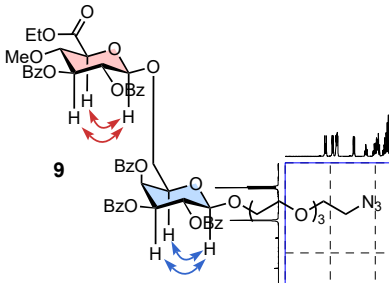
9: ROESY ($CDCl_3$)



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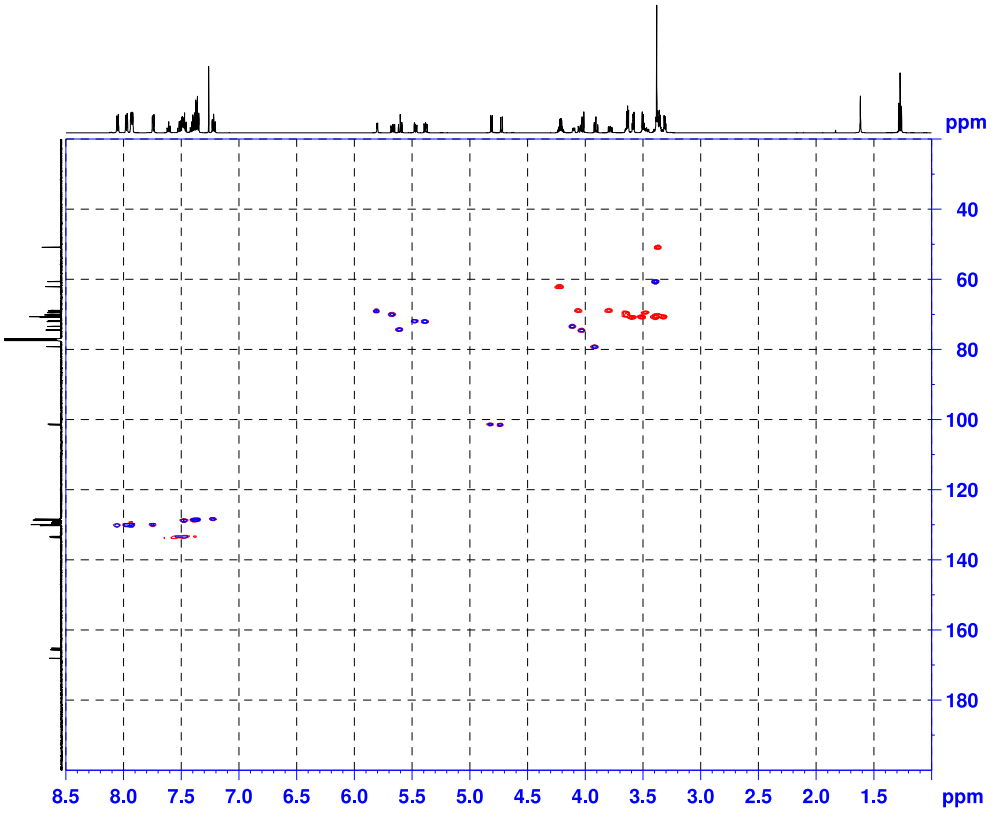
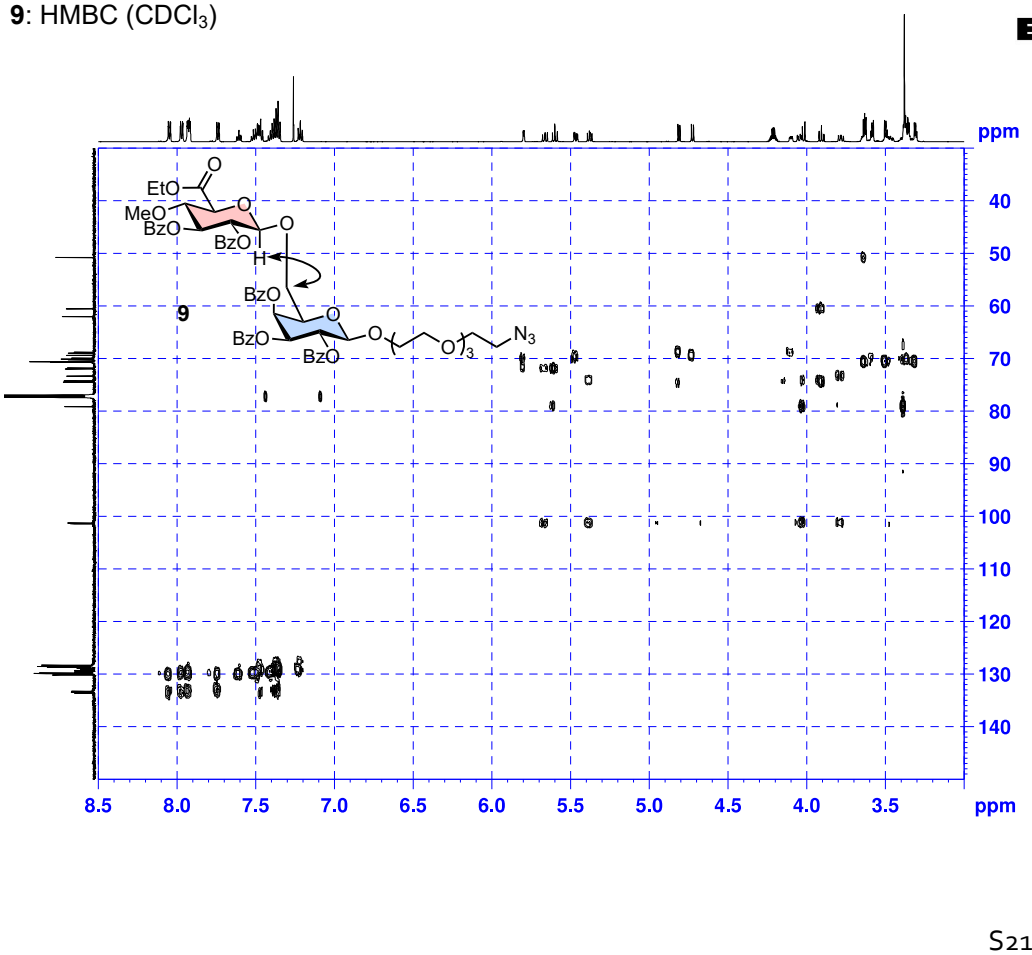
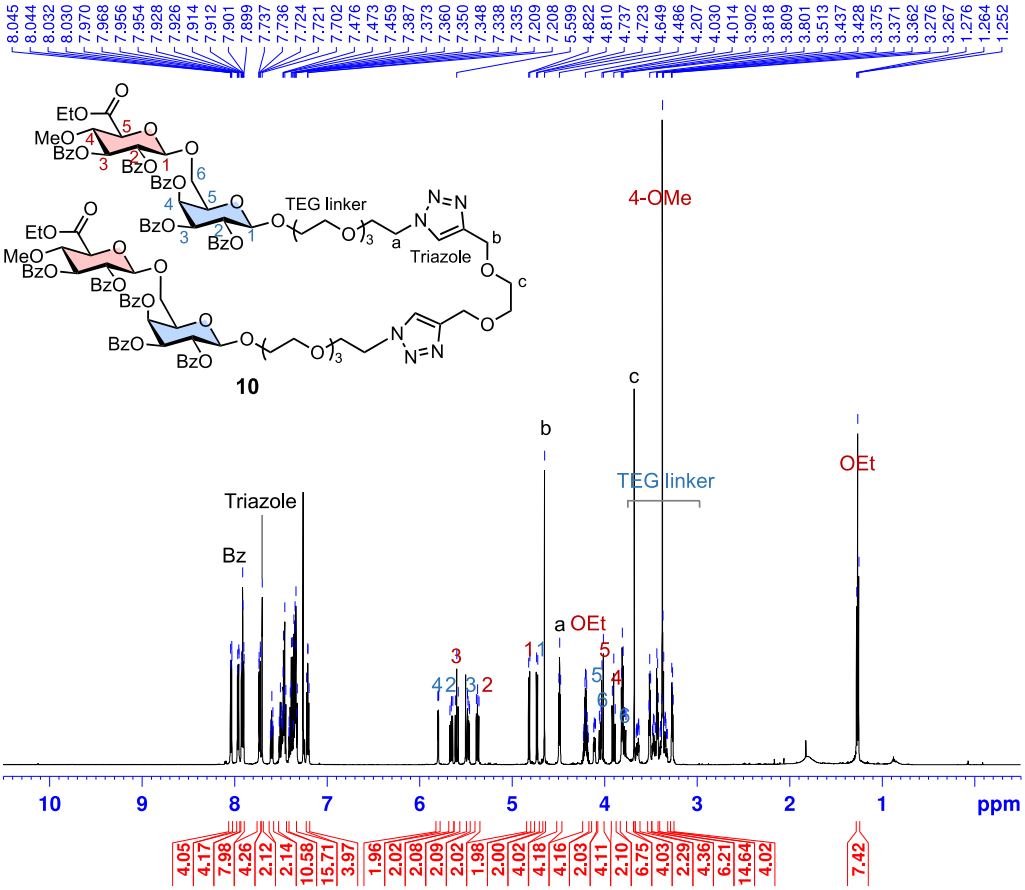
9: HSQC ($CDCl_3$)



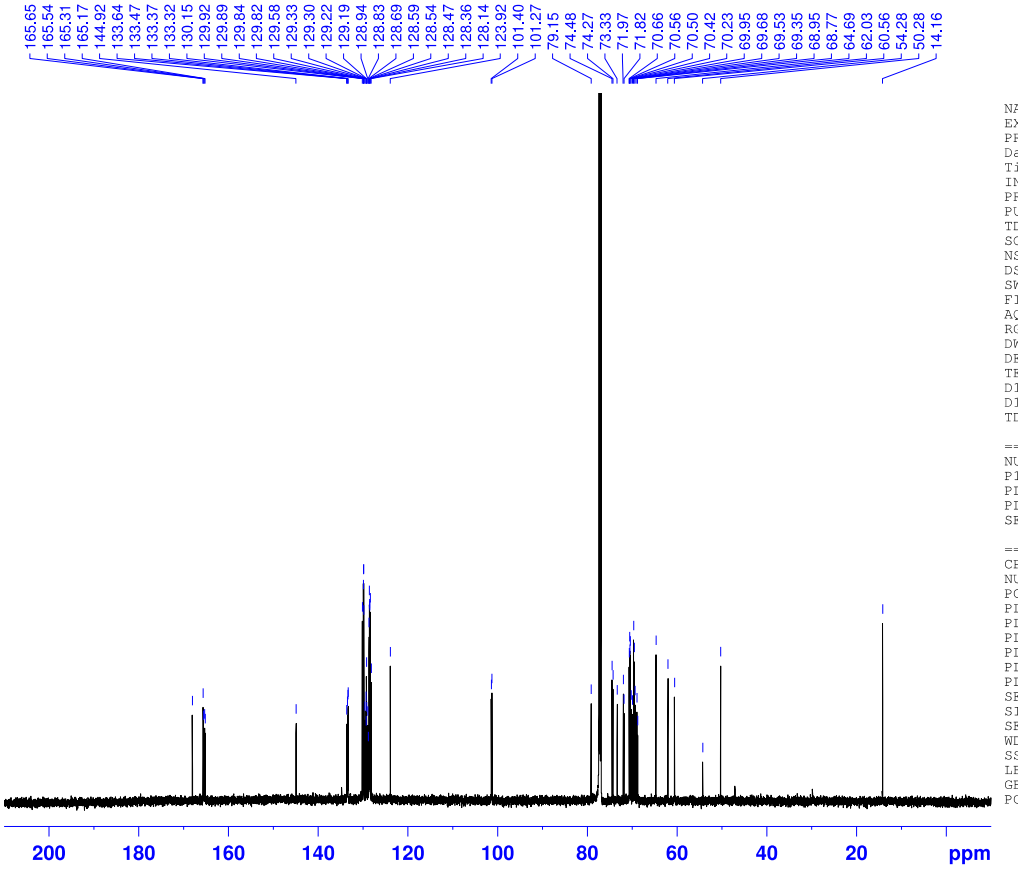
9: HMBC ($CDCl_3$)



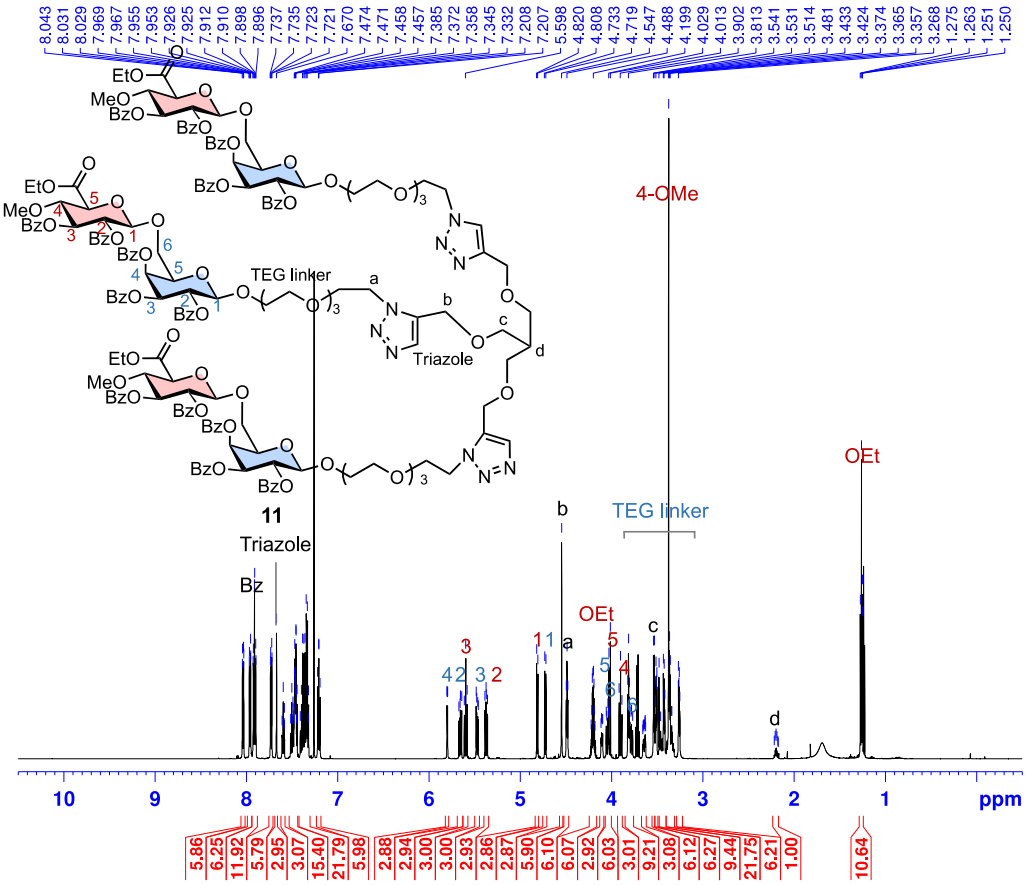
10: ^{1}H NMR (600 MHz, $CDCl_3$)



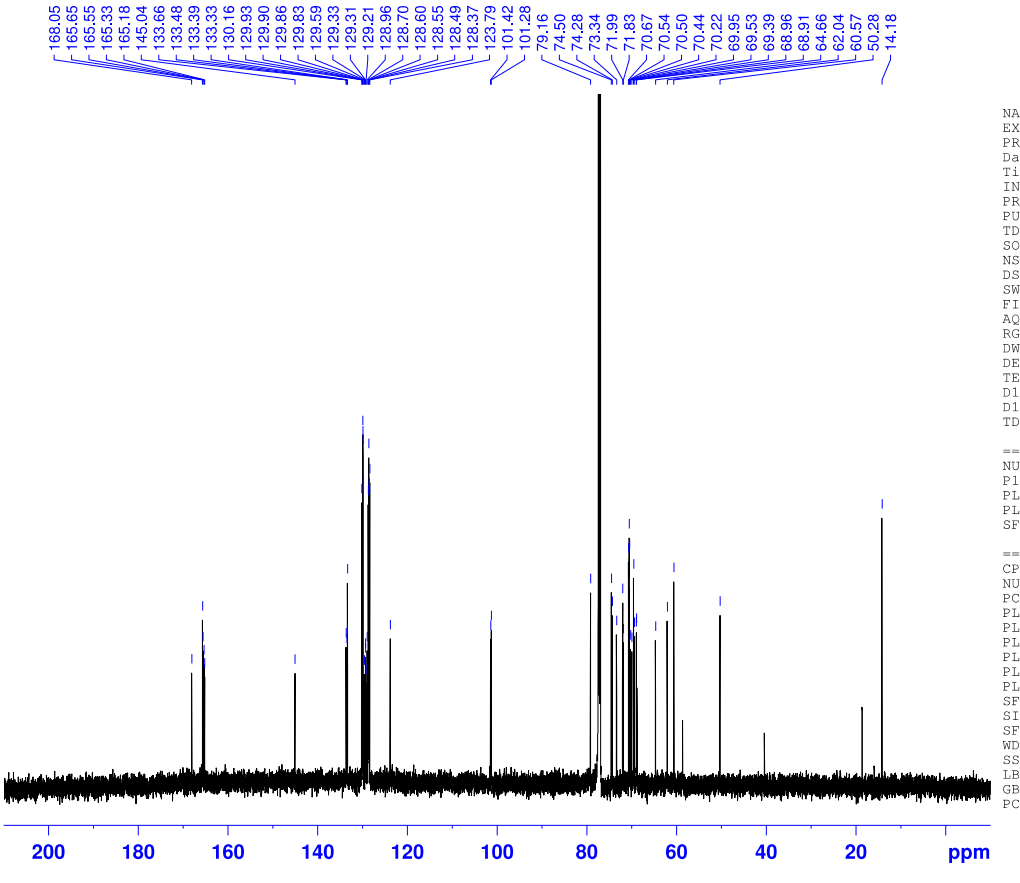
10: ^{13}C NMR (150 MHz, $CDCl_3$)



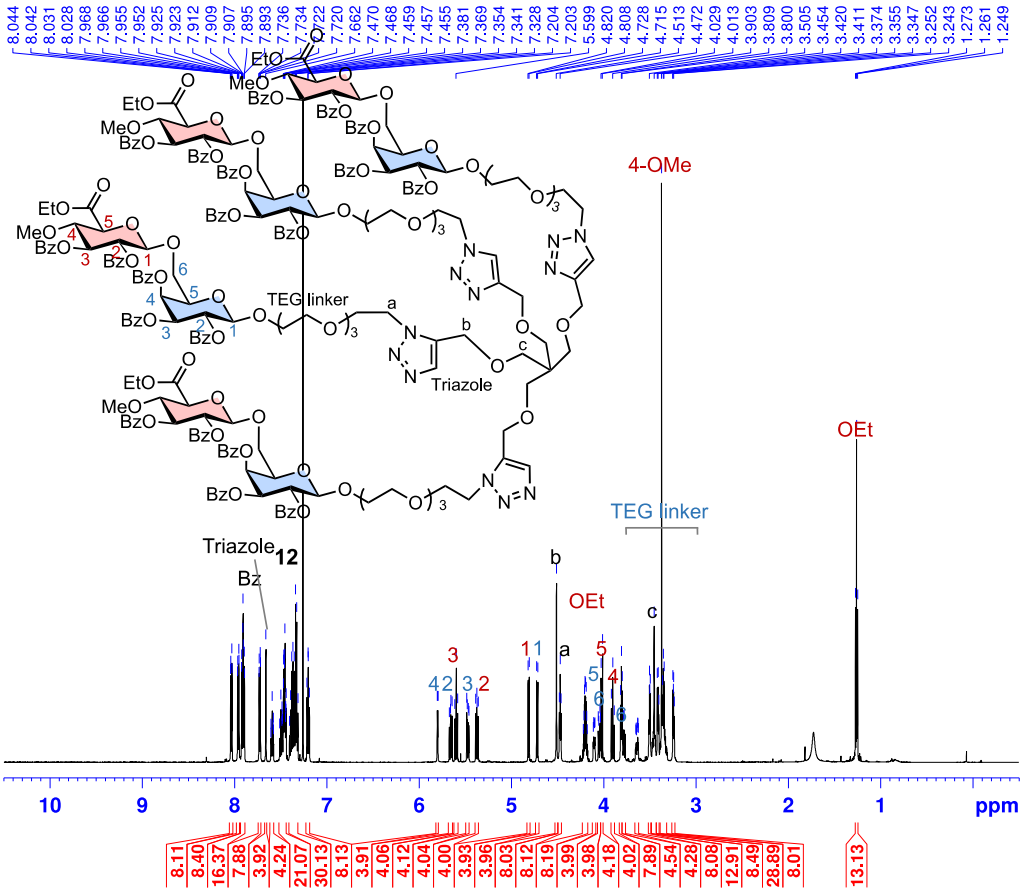
11: ^{1}H NMR (600 MHz, $CDCl_3$)



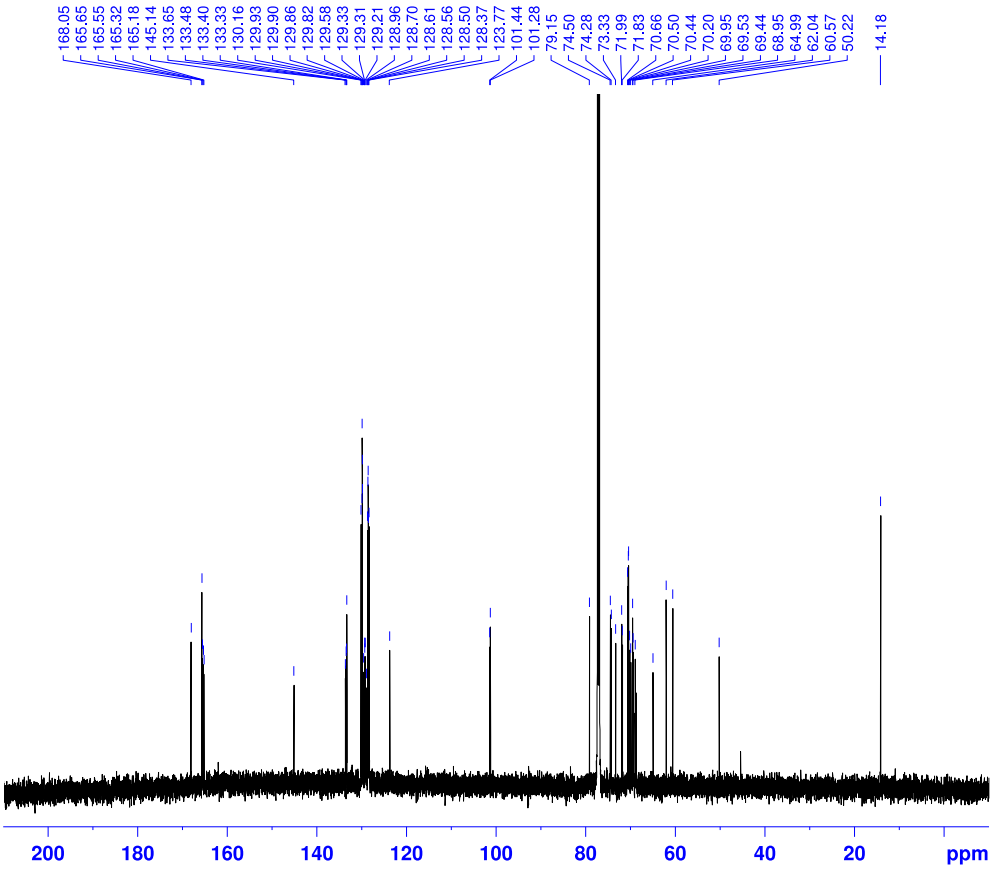
11: ^{13}C NMR (150 MHz, $CDCl_3$)



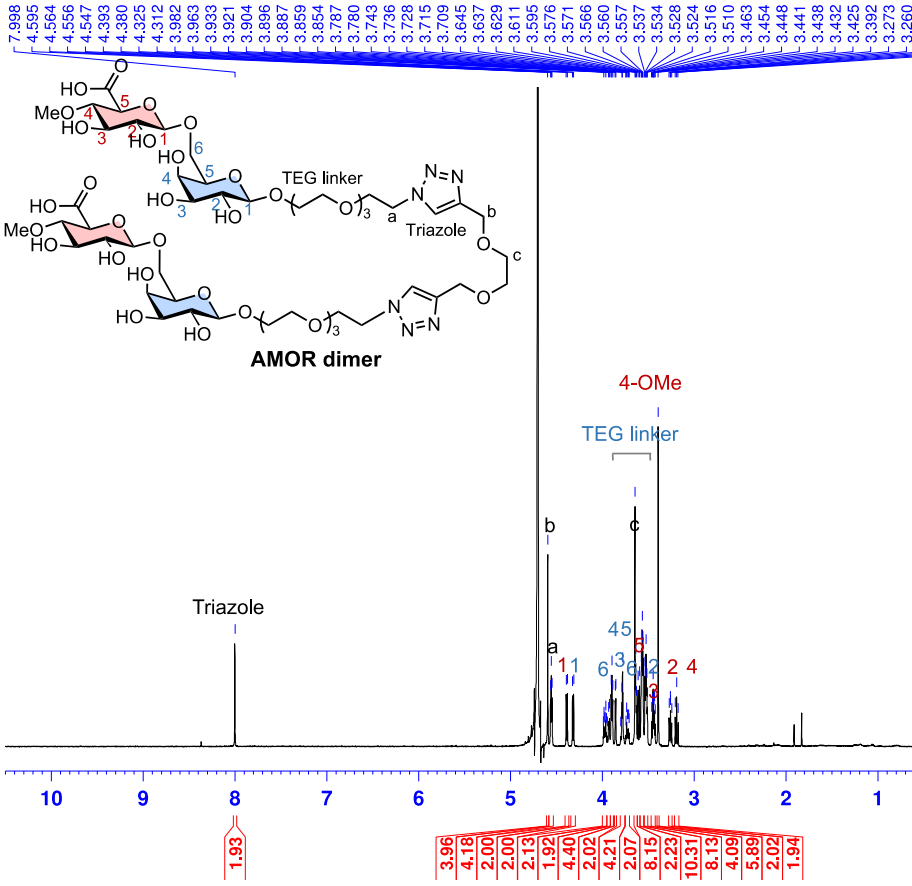
12 ^{1}H NMR (600 MHz, $CDCl_3$)



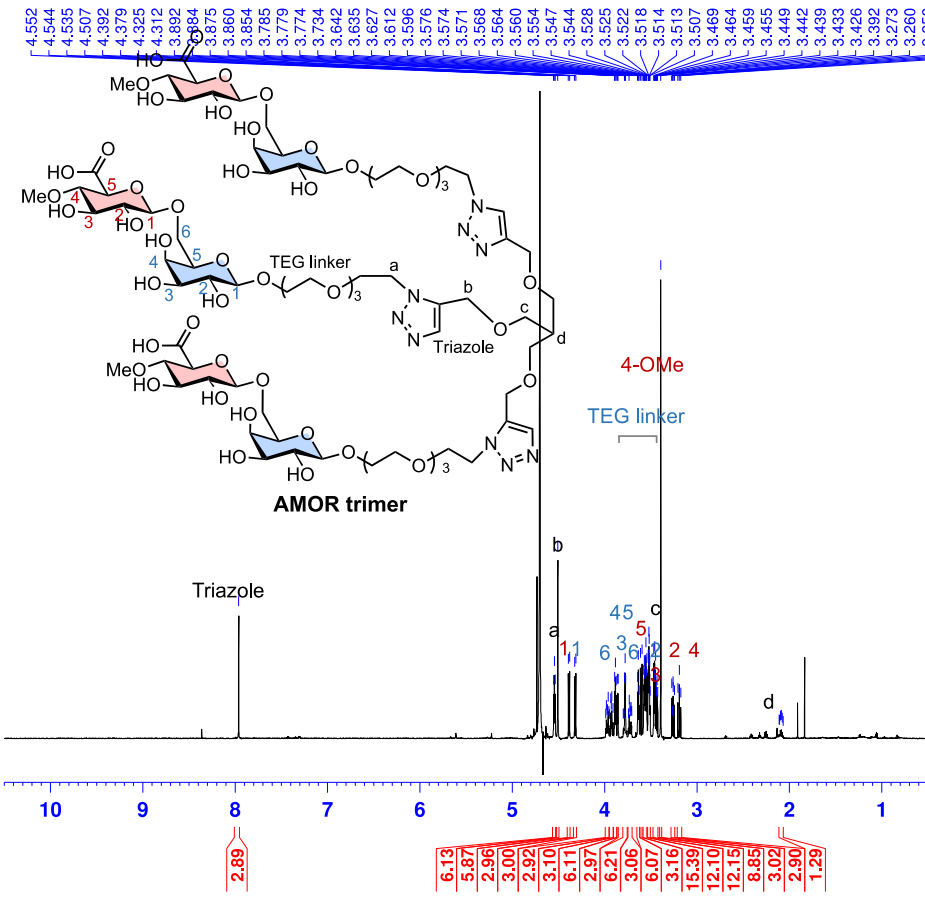
12: ^{13}C NMR (150 MHz, $CDCl_3$)



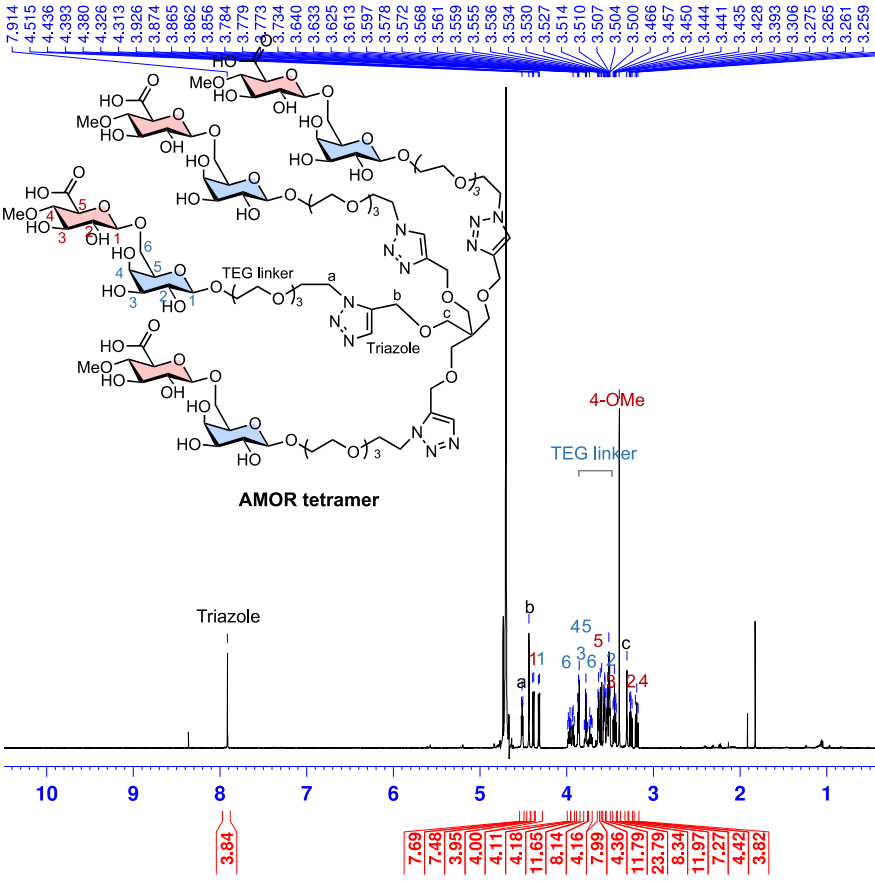
AMOR dimer: ^{1}H NMR (600 MHz, D_2O)



AMOR trimer: ^{1}H NMR (600 MHz, D_2O)



AMOR tetramer: ^{1}H NMR (600 MHz, D_2O)



4. Material and methods for AMOR bioactivity

Plant materials and growth conditions

Torenia fournieri cv. 'blue and white' (Sakata no Tane, Yokohama, Japan) plants were grown in soil at 28°C with a 16-h photoperiod (~150 μmol/m^2s^1).

AMOR activity of synthesised AMOR and AMOR oligomers

AMOR activity was measured using a previously reported AMOR assay[4,5]. The centre of an agar plate of modified Nitsch's medium in a glass-bottom dish (D210402; Matsunami Glass, Osaka, Japan) was cut into an ~18 × 22 mm^2 rectangle and the agar block was removed. In this space, growth medium [modified Nitsch's medium containing 13% polyethylene glycol 4000 (w/v) and 1% sucrose (w/v)] containing AMOR (TCI Chemicals) or AMOR oligomers with 1.5% ultra-low-gelling agarose (agarose type IX-A; Sigma) were spread to form a thin layer of agar and the cut end of a 15-mm-long hand-pollinated style was embedded before solidification. This agar plate was incubated in the dark at 28°C for 16 h. After pollen tube incubation, the H_2O-saturated silicon oil (KF-96-100CS; Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) was layered, and the pollen tubes were subjected to the guidance assay through micromanipulation. Under observation using an inverted microscope (AxioObserver Z1; Zeiss, Oberkochen, Germany), a single ovule was picked up using a glass needle produced using a glass needle puller (MCF-100; Nepa Gene, Chiba, Japan) and placed in front of the pollen tubes using a manipulator (MTK-1SH; Narishige Group, Amityville, NY, USA).

Aniline blue staining of *in vitro* and semi-*in vitro* pollen-tube growth

To observe the pollen tubes *in vitro*, pollen grains were germinated on growth medium [modified Nitsch's medium containing 13% polyethylene glycol 4000 (w/v), and 1% sucrose (w/v)] containing AMOR (TCI Chemicals) or multivalent AMORs with 1.5% ultra-low-gelling agarose (agarose type IX-A; Sigma) and incubated in the dark at 28°C for 16 h. To observe the pollen tubes semi-*in vitro*, the cut end of a 15-mm-long hand-pollinated style was placed on the growth medium containing AMOR (TCI Chemicals) or multivalent AMORs with 1.5% ultra-low-gelling agarose and incubated at dark at 28°C for 16 h. After incubation, callose plugs in pollen tubes were labelled using 0.1% aniline blue dye (in 0.1 M K_2HPO_4) and observed using an AxioObserver Z1 inverted microscope (Zeiss)[6].

5. References

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