Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

A new approach for the synthesis of *O*-glycopeptides through a combination of solid-phase glycosylation and fluorous tagging (SHGPFT)

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Supporting information:

General methods

TMSOTf was purchased from Alfa. All the other commercial materials were used without further purification as received unless otherwise noted. Dry DCM was distilled from calcium hydride. Dry THF was distilled from sodium hydride. FluoroFlash SPE cartridges (2 grams, 8 cc tube) were purchased from Fluorous Technologies, Inc. All glycosylated reactions were carried out under anhydrous conditions with freshly distilled solvents, unless otherwise noted.

Reactions were monitored by analytical thin-layer chromatography on silica gel GF254 precoated on glass plates, preparative TCL on silica gel and the silica gel for column chromatography were phased from Qingdao Haiyang Chemical and Special Silica Gel Co, Ltd. Spots were detected under UV (254 nm) and/or by staining with 10% (volume fraction) H2SO4/ethanol.

The automatic LC-MS analysis was also performed on a Thermo Finnigan LCQAdvantage mass spectrometer equipped with an Agilent HPLC system and an eluent splitter (5% eluent was split into the MS system).

High-resolution LC-MS was carried out by Agilent LC/MSD TOF using a column of Agilent ZORBAX SB-C18 (rapid resolution, 3.5 μm, 2.1 × 30 mm) at a flow of 0.40 mL/min. The solvent was MeOH/water (75:25 (v/v)), containing 5 mmol/L ammonium formate. The ion source is electrospray ionization (ESI).

MALDI mass spectra was obtained on a MALDI-TOF/TOF Analyzer 4800 Plus, Applied Biosystem, Foster City, CA, USA

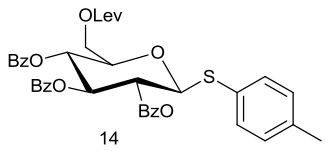
Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectroscopy were performed on Bruker Advance 400M, Varian 300 NMR and Bruker 500MHz spectrometers.Chemical shifts of ^{1}H NMR spectra are reported as in units of parts per million (ppm) downfield from $SiMe_4$ (δ 0.0) and relative to the signal of chloroform-*d* (δ = 7.260, singlet). Multiplicities were given as: s (singlet); br s (broad singlet); d (doublet); t (triplet);; dd (doublet of doublets); ddd (triple of doublets); m (multiplets) etc. The number of protons (n) for a given resonance is indicated by nH. Carbon nuclear magnetic resonance spectra (^{13}C NMR) are reported as in units of parts per million (ppm) downfield from $SiMe_4$ (δ 0.0) and relative to the signal of chloroform-*d* (δ = 77.160, triplet)

Experimental procedure

Solid-phase glycosylation. The resin and 10 equiv. glycosyl donor (relative to the loading estimated after last coupling) was dried under vacuum for 2 h and protected with Ar. Freshly distilled glycosylation solvent was added and the mixture was stirred at room temperature for 30 min. Then 20 equiv. TMSOTf was added to promote glycosylation. After quenching reaction with Et_3N, the resin was filtered and washed two times each with H_2O, 10 % DIPEA/DMF, DMF, MeOH, DCM, and Et_2O.The filteration was evaporated and the excess of the donor was purified by flash chromatography for chemical recycling.

Cleavage of aryl hydrazide linker. The resin was swollen in anhydrous DCM for 20 min and drained. A solution of NBS (3.5 equiv.) and Py (15.5 equiv.) in dry DCM was added to the resin and kept for 10 min at room temperature. The activated resin was drained and washed with dry DCM (3 times) and dry THF (2 times). After drying, it was shaken with a solution of 5% of H_2O in dry THF overnight. After filtration, the resin was washed 3 times each with DCM and distilled THF. The combined filtrate was concentrated under reduce pressure and analyzed with HPLC.

F-SPE proceudure: Firstly, The crude filtrate was concentrated, dissolved in DMF (0.1 mL) and loaded onto a cartridge with 2 g fluorous silica gel. The loaded cartridge was first washed with 25 mL of a 80%methanol/20%water mixed solution after no more detectable substance in the freshly eluant, the cartridge was then washed with 20 mL of a 100% methanol to collect the fluorous fraction. The fluorous silica gel was wash again with 20ml methanol, and stored for reusing in the next time.



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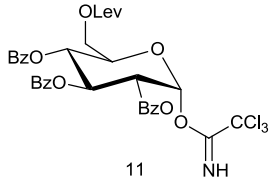
The 5(598.2mg, 1mmol) was dissolved with 10ml DCM, Lev-OH (232.0mg, 2mmol) was added and then stirred at room temperature for 5min. after that, the DCC(412.6mg, 2mmol) and DMAP(24.4mg, 0.2mmol) was added and stirring for another 16h, filtrated the insoluble substance, and the solution was removed, then the residue was purified by flash chromatography to give 6(640.3mg , 0.92mmol) as colorless oil.

$[\alpha]^{20}_D$ +10.2 (*c* 1.0, $CHCl_3$); δ_H (300MHz, $CDCl_3$) δ 7.98-7.77 (m, 6H), 7.56-7.24 (m, 11H), 7.13 (d, J=7.8Hz, 2H), 5.86 (t, J=9.6Hz, 1H), 5.49 (t, J=9.6Hz, 1H), 5.43 (t, J=9.6Hz, 1H), 4.95 (d, J=9.9Hz, 1H), 4.34 (dd, J=12.3, 3.3Hz, 1H), 4.29 (dd, J=12.3, 5.4Hz, 1H), 4.02 (ddd, J=12.3, 5.4, 3.3Hz, 1H),

2.77-2.72 (m, 2H), 2.61-2.56 (m, 2H), 2.36 (s, 3H), 2.19 (s, 3H); δ_C (125MHz, $CDCl_3$) δ 206.4, 172.3, 165.7, 165.2, 165.0, 138.7, 133.8, 133.5, 133.3, 133.2, 129.9, 129.8, 129.7, 129.2, 128.7, 128.7, 128.4, 128.4, 128.3, 127.7, 86.3, 76.1, 74.1, 70.5, 69.2, 62.9, 37.9, 29.8, 27.8, 21.2; HRMS (ESI): Calcd for $C_{39}H_{36}O_{10}NaS$ $[M+Na]^+$: 719.1921, found 719.1923.



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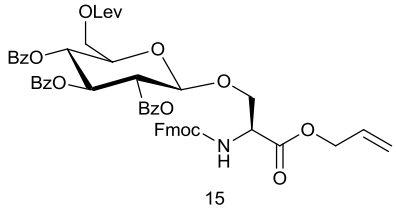
Thioglycoside 6 (505.5mg, 0.87 mmol) was dissolved in DCM/H_2O (10.4 mL, 100:1, v/v) and cooled to 0 °C. The solution was treated with NBS (156 mg, 0.88 mmol) and TMSOTf (16 μL, 0.09 mmol). The resulting mixture was stirred at room temperature and the reaction was monitored by TLC. After completion of reaction, saturated aq. $NaHCO_3$ (50 mL) was added, and the mixture was extracted with DCM (20 mL × 3). The combined organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The resulting oil was purified by flash chromatography (Petroleum ether/ethyl acetate, 5:1, v:v) on silica gel. To a solution of purified white solid in anhydrous DCM (17 mL) was added trichloroacetonitrile (521 μL, 5.20 mmol) and 1,8-dizaabicyclo [5.4.0] undec-7-ene (DBU) (17 μL, 0.11 mmol) at 0 ℃. After strirring for 4h, the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (Petroleum ether/ethyl acetate, 6:1, v:v, added 0.1 % Et_3N) on silica gel to give **3** (395.8 mg, 0.54mmol, 62% over two steps) as light yellow oil.

$[\alpha]^{20}_D$ +46.2 (*c* 3.1, $CHCl_3$); δ_H (300MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.95 (d, J=7.5Hz, 4H), 7.86 (d, J=8.1Hz, 2H), 7.54-7.26 (m, 9H), 6.82 (d, J=3.3Hz, 1H), 6.24 (t, J=9.9Hz, 1H), 5.71 (t, J=9.9Hz, 1H), 5.59 (dd, J=10.2, 3.6Hz, 1H), 4.50 (dt, J=9.9, 3.3Hz, 1H), 4.32 (d, J=3.3Hz, 2H), 2.75-2.73 (m, 2H), 2.64-2.60 (m, 2H), 2.18 (s, 3H); δ_C (125MHz, $CDCl_3$) δ 206.2, 172.2, 165.6, 165.3, 165.2, 160.5, 133.9, 133.5, 133.2, 130.1, 129.9, 129.7, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 93.1, 90.7, 70.6, 70.5, 70.1, 68.4, 62.1, 37.8, 29.8, 27.8.



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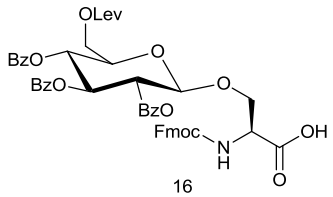
A solution of 3 (0.54mmol, 333.3mg) and Fmoc-serine-OAll (0.54mmol, 198.2mg) was azeotropic

distillated toluene twice, 4 Å ms was added, then the reaction mixture was dissolved with the dry DCM 3ml, the TMSOTf(18.1μL,0.1mmol) was dropped at -40 ℃ in the argon atmosphere and the mixture was stirred for 2.5h. Then quenched with 20μL Et_3N, after evaporation of the solution, the residue was purified by silica gel flash chromatography (Petroleum ether/ethyl acetate, 3:1,v:v) to give 7(328.7mg, 0.35mmol, 64%) as white foam.

$[\alpha]^{20}_D$ -2.0 (*c* 1.5, $CHCl_3$); δ_H (300MHz, $CDCl_3$) δ 7.93 (d, J=7.2Hz, 4H), 7.84-7.78 (m, 4H), 7.59-7.24 (m, 15H), 5.96-5.78 (m, 1H), 5.87 (t, J=9.3Hz, 1H), 5.59 (d, J=7.8Hz, 1H), 5.53 (t, J=9.9Hz, 1H), 5.46 (t, J=9.0Hz, 1H), 5.31 (d, J=16.8Hz, 1H), 5.23 (d, J=10.5Hz, 1H), 4.76 (d, J=8.1Hz, 1H), 4.62 (d, J=5.1Hz, 2H), 4.54-4.44 (m, 1H), 4.44-4.20 (m, 5H), 4.15 (t, J=6.9Hz, 1H), 4.03-3.95 (m, 1H), 3.94 (dd, J=9.9, 3.0Hz, 1H), 2.80-2.40 (m, 4H), 2.16 (s, 3H); δ_C (125MHz, $CDCl_3$) δ 206.3, 172.3, 169.0, 165.7, 165.2, 165.0, 155.8, 143.8, 143.8, 141.3, 133.5, 133.3, 131.5, 129.8, 129.8, 129.7, 129.1, 128.8, 128.7, 128.5, 128.4, 128.3, 127.8, 127.1, 125.2, 125.1, 120.0, 118.6, 101.3, 72.6, 72.2, 71.7, 69.4, 69.4, 66.9, 66.3, 62.6, 54.4, 47.2, 37.8, 29.7, 27.8; HRMS (ESI): Calcd for $C_{53}H_{49}NO_{15}Na$ $[M+Na]^+$: 962.2994, found 962.2981.



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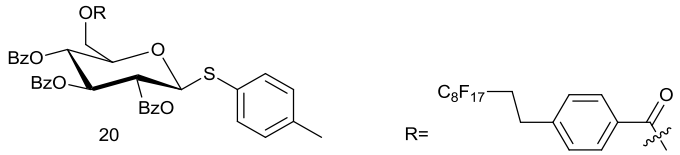
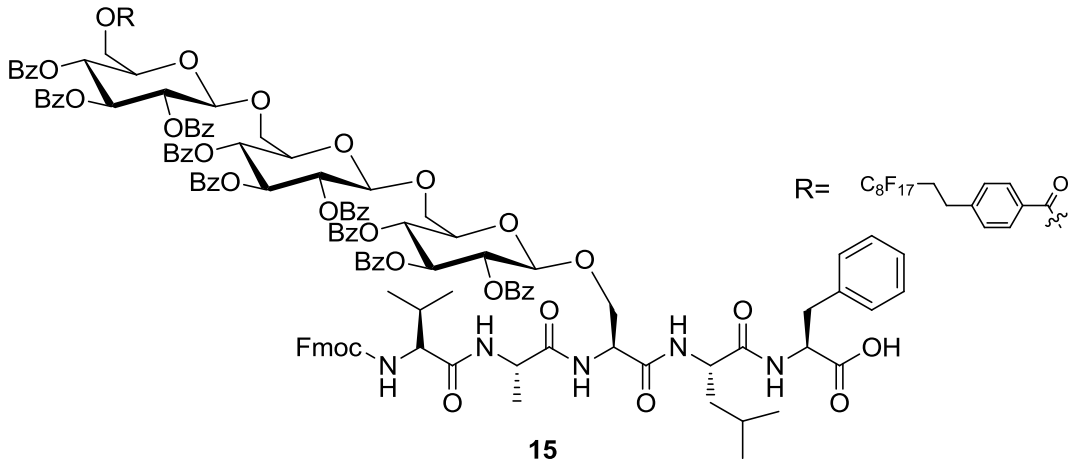
A solution of 7 (247.2mg 0.3mmol), $Pd(PPh_3)_4$ (17.5mg 0.015mmol) and morpholine (104μL 1.2mmol) in DCM (3.5ml) was stirred at room temperature for 0.5h. 50ml 2M HCl was added, then the mixture was extracted with DCM, the combined organic phase was dried over Na_2SO_4, after the filtration was evaporated, the residue was purified by chromatography to give 8 (258.9mg, 0.288mmol,96%) as colorless oil.

$[\alpha]^{20}_D$ +14.6 (*c* 1.4, $CHCl_3$); δ_H (300MHz, $CDCl_3$) 7.94-7.76 (m, 8H), 7.61-7.26 (m, 15H), 5.86 (t, J=9.6Hz, 1H), 5.76 (d, J=8.1Hz, 1H), 5.57 (t, J=9.6Hz, 1H), 5.52-5.46 (m, 1H), 4.84 (d, J=7.8Hz, 1H), 4.51-3.99 (m, 9H), 2.81-2.77 (m, 2H), 2.66-2.59 (m, 2H); δ_C (125MHz, $CDCl_3$) δ 208.2, 172.7, 171.2, 170.1, 165.7, 165.2, 156.1, 143.8, 141.3, 133.5, 133.3, 129.8, 129.1, 128.7, 128.5, 128.4, 128.3, 127.7, 127.1, 125.2, 119.9, 101.4, 72.6, 72.1, 71.8, 69.9, 69.2, 67.2, 62.3, 54.2, 47.1, 38.0, 29.8, 27.9; HRMS (ESI): Calcd for $C_{50}H_{46}NO_{15}$ $[M+H]^+$: 900.2861, found 900.2871.



A freshly preformed F-tag benzoyl chloride(350mg, 0.6mmol) was added to a solution of 13(328mg, 0.55mmol) and DMAP (12.2mg, 0.1mmol) in DCM(10ml), Et_3N(97μL, 0.7mmol) was dropped, then the reaction was stirred at reflux condition for 3h, 20ml DCM was added, the mixture was extracted with H_2O(30ml) and brine (30ml) The organic phase was dried over Na2SO4 and concentrated. Silica gel chromatography yielded 20 (567mg, 90%).

$[\alpha]^{20}_D$ +21.5 (*c* 0.9, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 8.01-7.97(m, 4H), 7.89(d, J=6.8Hz, 2H), 7.79((d, J=7.2Hz, 2H), 7.54-7.47(m, 2H), 7.42-7.36(m, 6H), 7.34-7.28(m, 4H), 7.26-7.24(m, 1H), 6.96-6.94(d,J=8Hz, 2H), 5.89(t,J=10.6Hz, 1H), 5.60(t, J=10.0Hz, 1H), 5.45(t, J=10.0Hz, 1H), 4.98(d, J=10.0Hz, 1H), 4.68(dd, J=12.0Hz, 2.8Hz, 1H), 4.47(dd, J=12.4Hz, 5.6Hz, 1H), 4.17(ddd, J=12.4Hz, 5.2Hz, 2.8Hz, 1H), 3.01(t, J=8.0Hz,2H), 2.48-2.35 (m, 2H), 2.28(s, 3H). δ_C (100 MHz, $CDCl_3$) 165.78, 165.72, 165.15, 165.03, 144.61, 144.61, 138.56, 138.56, 133.93, 133.93, 133.43, 133.31, 133.21, 130.41, 129.88, 129.84, 129.73, 129.63, 129.25, 128.78, 128.74, 128.40, 128.35, 128.32, 128.26, 127.62, 86.26, 76.24, 74.21, 70.46, 69.35, 63.08, 32.78, 32.56, 32.35, 29.70, 29.36, 29.32, 26.57, 26.52, 21.12, 19.19, 14.10, 13.72. δ_F (376 MHz, $CDCl_3$) -80.75(3F), -114.47(2F), -121.63(4F), -121.87(2F), -122.68(2F), -123.42(2F), -126.10(2F).



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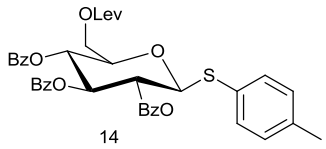
MALDI-TOF-MS (CHCA): Calcd for C139H124F17N5O34 [M]:2729.8 $[M+Na^+]^+$: Calcd for C139H124F17N5NaO34: 2752.8 found 2752.9 $[M+2Na^+-H]^+$: Calcd for C139H123F17N5Na2O34: 2774.8 found 2774.9

Copies of NMR spectra of products



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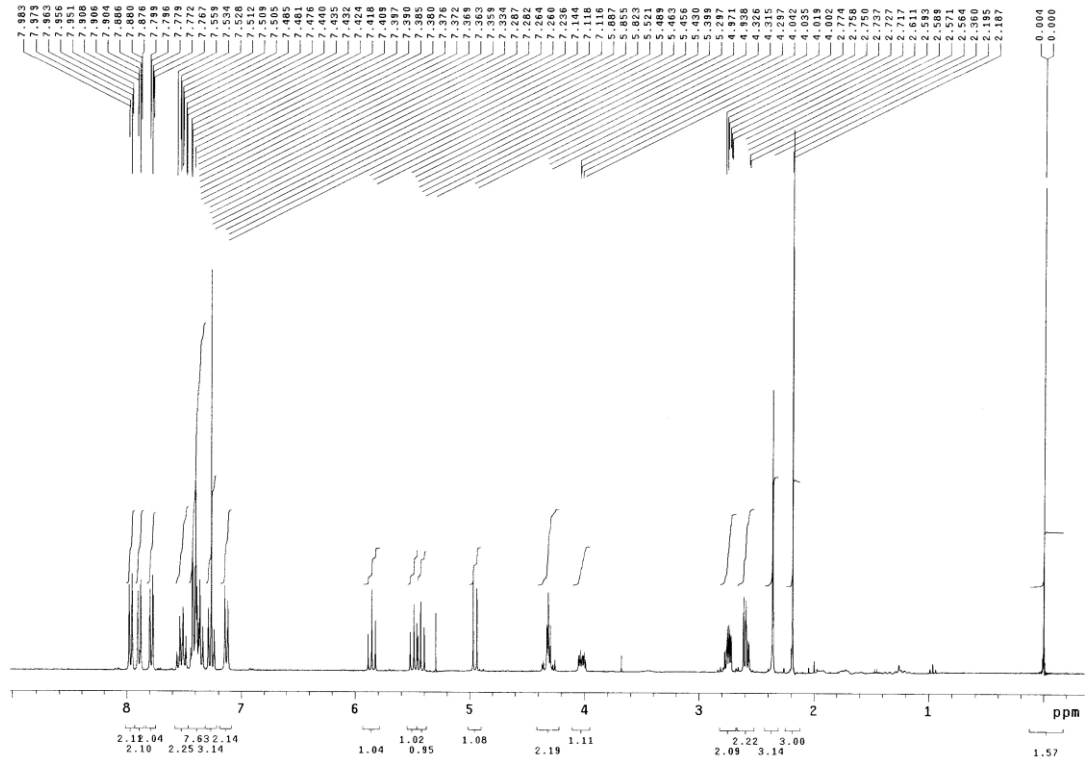

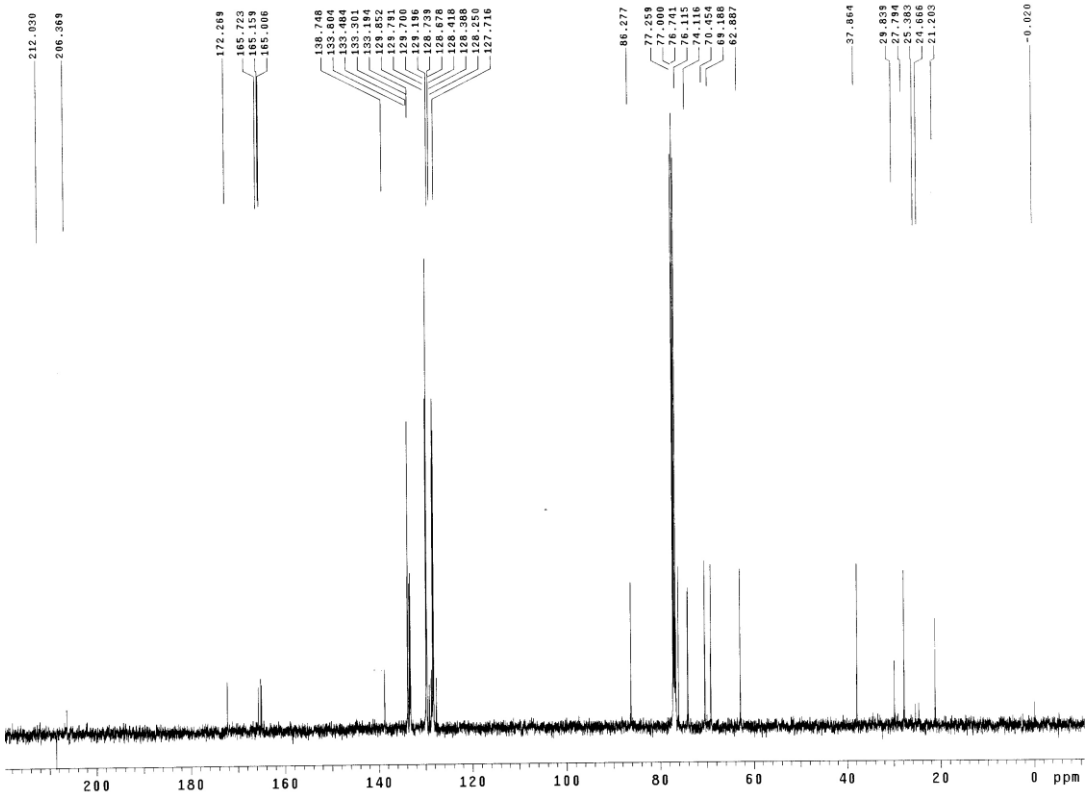

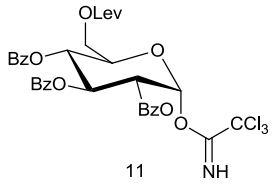

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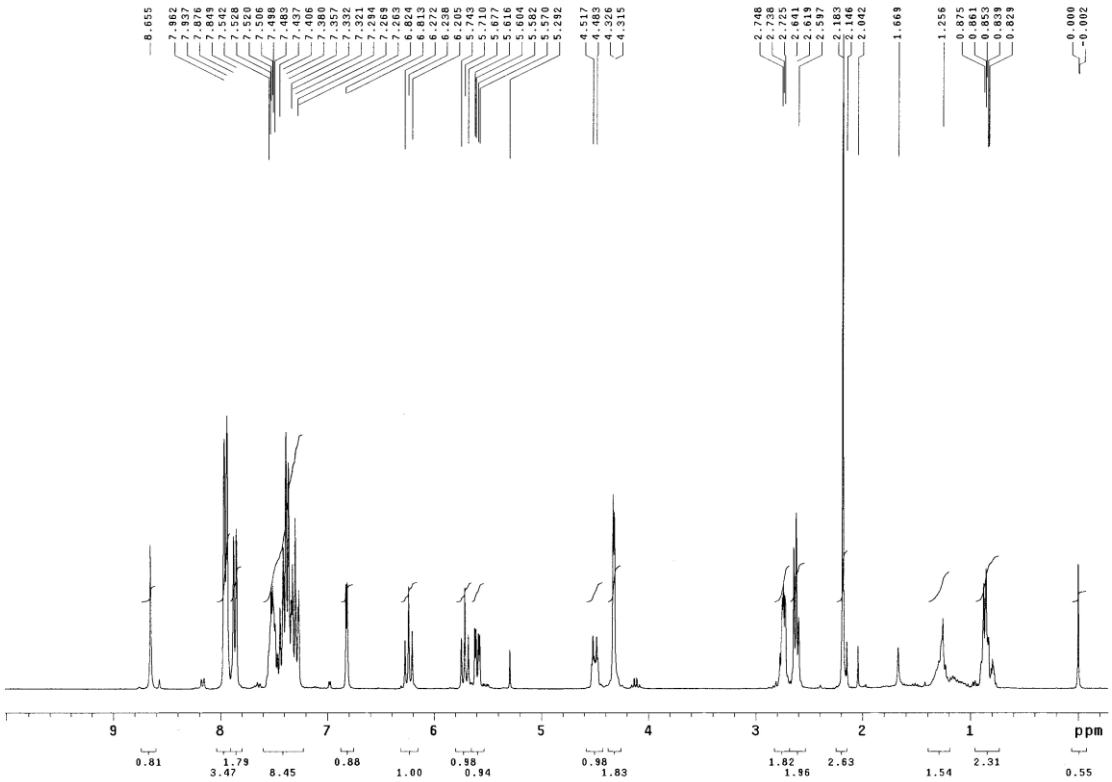

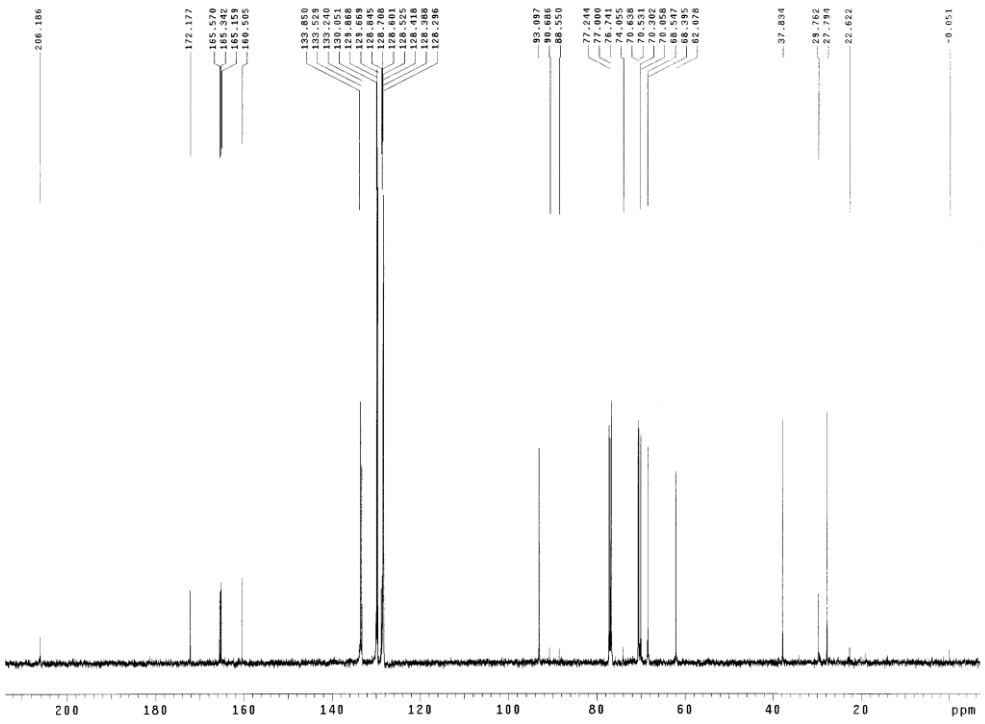

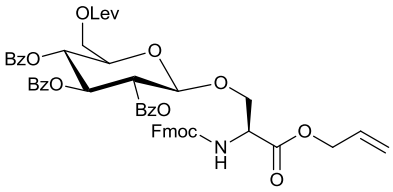

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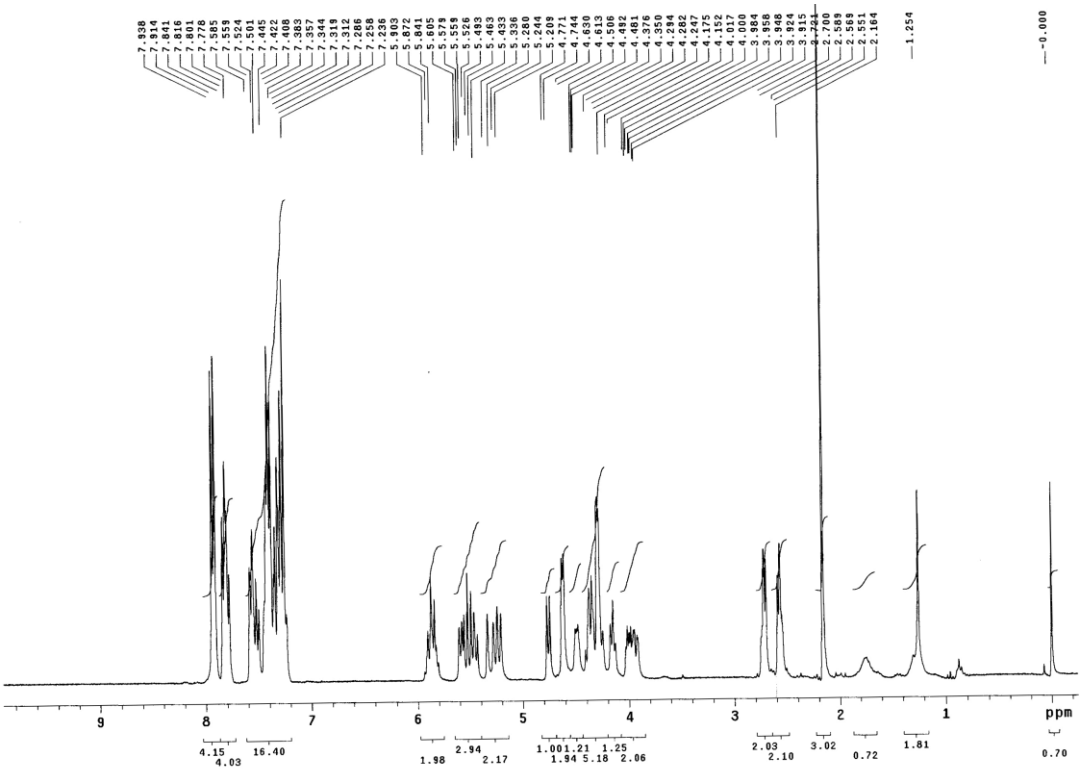

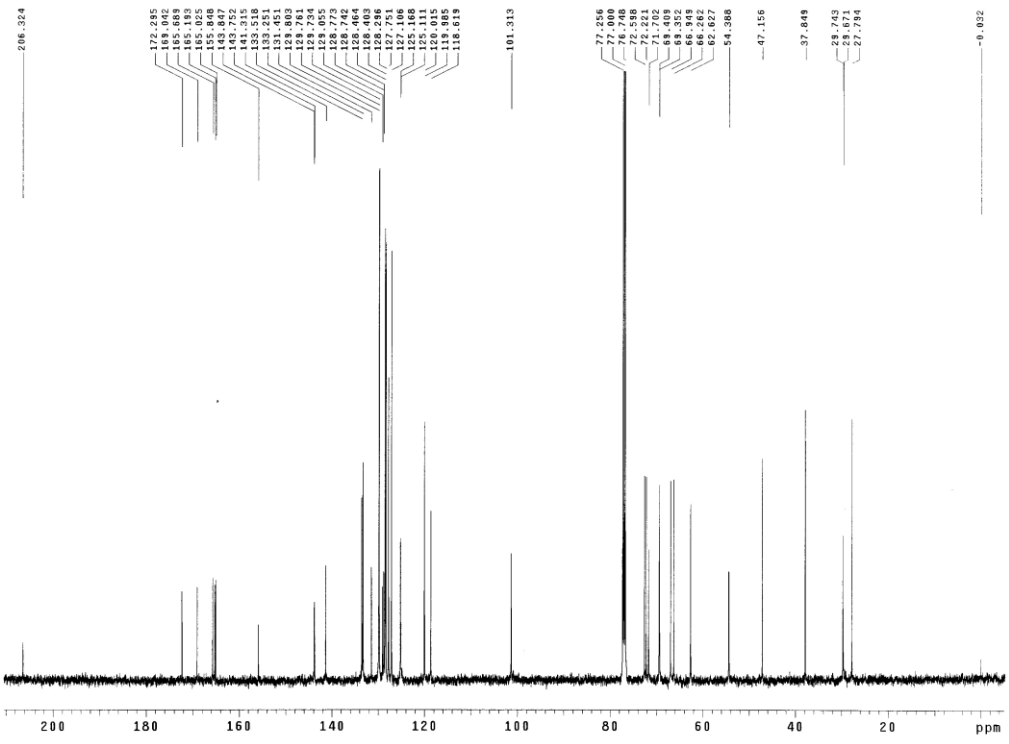

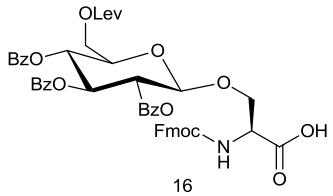

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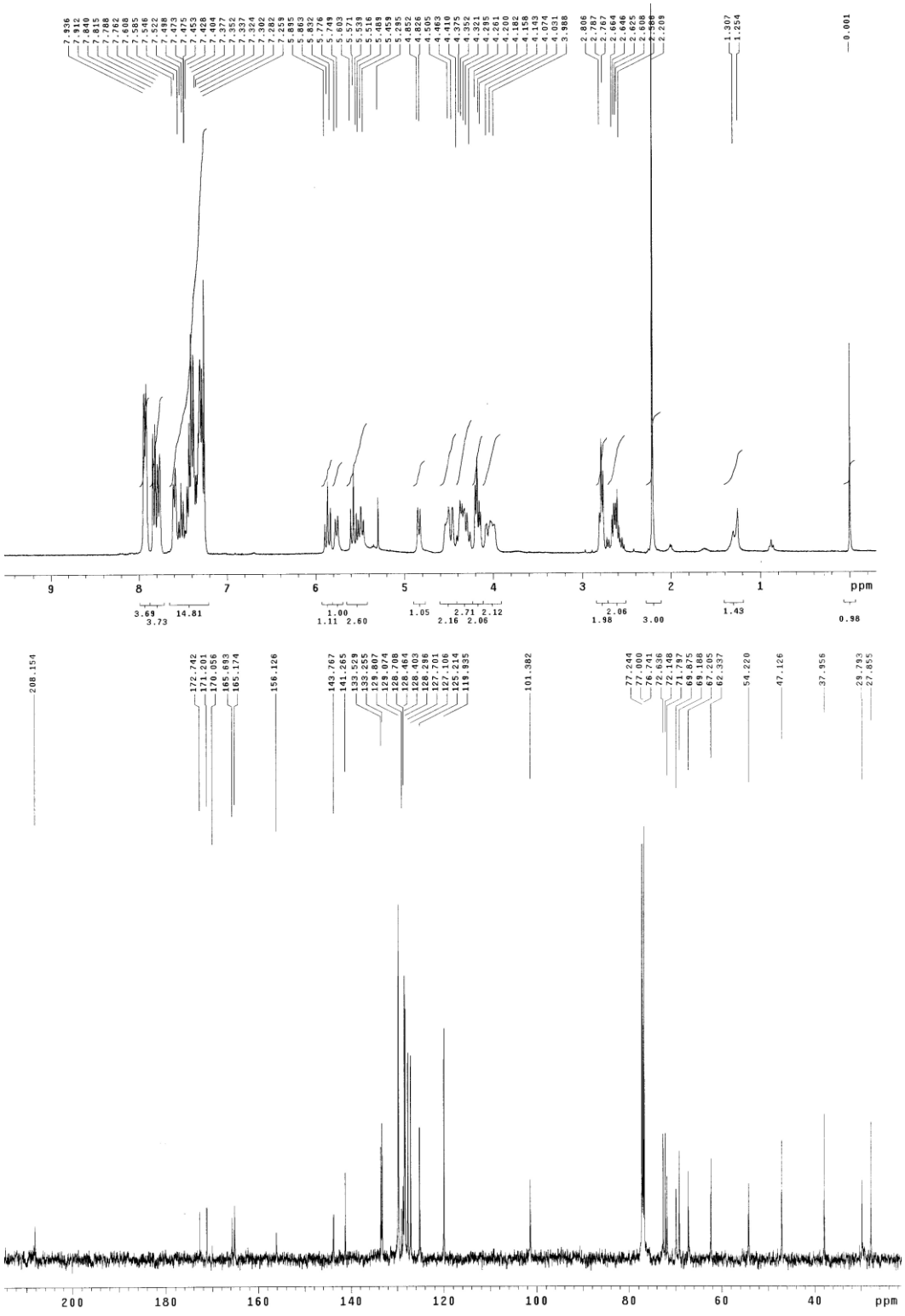

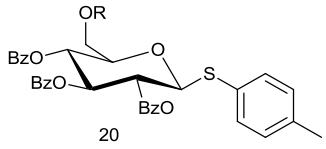

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