

Supporting Information

for

Acylsulfonamide safety-catch linker: promise and limitations for solid-phase oligosaccharide synthesis

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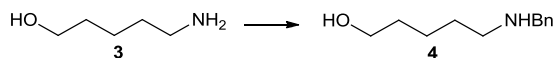
[‡]Equally contributing authors

Experimental section

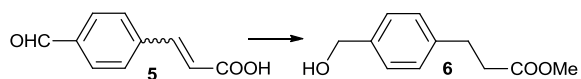
General information for chemical synthesis: All chemicals used were reagent grade and were used as supplied, except where noted. All reactions were performed in oven-dried glassware under an inert atmosphere, unless noted otherwise. Reagent grade *N,N*-dimethylformamide (DMF) was dried over activated molecular sieves prior to use. Dichloromethane (CH₂Cl₂), toluene and tetrahydrofuran (THF) were purified by a Cycle-Tainer Solvent Delivery System, unless noted otherwise. Analytical thin-layer

chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or by dipping the plate in a cerium sulfate ammonium molybdate (CAM) solution. Flash column chromatography was carried out by using forced flow of the indicated solvent on Fluka Kieselgel 60 (230–400 mesh). All automated glycosylations were performed on an automated oligosaccharide synthesizer prototype. LC–MS chromatograms were recorded on an Agilent 1100 Series spectrometer. Loading determination of functionalized resins was obtained by using a Shimadzu UV-MINI-1240 UV spectrometer. ^1H , ^{13}C NMR spectra were recorded on a Varian Mercury 400 (400 MHz) spectrometer in CDCl_3 with chemical shifts referenced to internal standards CDCl_3 (7.26 ppm, ^1H ; 77.0 ppm, ^{13}C), unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet for ^1H NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. High-resolution mass spectral (HRMS) analyses were performed by the MS-service in the Department of Organic Chemistry at the Free University Berlin. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer.

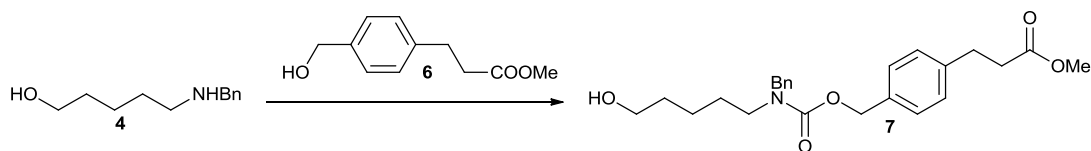
Synthesis of acylsulfonamide safety-catch linker



***N*-Benzyl-5-aminopentanol (4).** A solution of 5-aminopentanol **3** (18.8 g, 180 mmol) in CH_2Cl_2 (800 mL) was treated with PhCHO (20.4 mL, 200 mmol) and Na_2SO_4 (102.5 g, 1.8 mol) at room temperature, stirred for 18 h, filtered, and concentrated. The crude imine was solubilized in EtOH (110 mL) and treated with NaBH_4 (10.4 g, 273 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 h, then at room temperature for 4 h, quenched with water (60 mL), and concentrated. The residue was solubilized in EtOAc (600 mL) and washed with 5% aqueous NaHCO_3 solution (2×250 mL). The aqueous layers were re-extracted once with EtOAc and the combined organic phases were dried over MgSO_4 , filtered, and concentrated. After distillation of the impurity (BnOH) (140 °C, 1 mbar), *N*-benzyl-5-aminopentanol (**4**) [1] (28.9 g, 82%) was left as a colorless oil, which was applied directly to the next reaction.

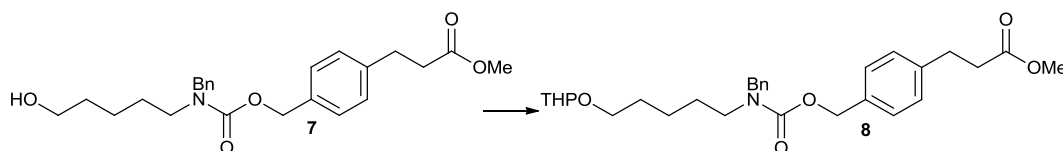


Methyl 3-(4-(hydroxymethyl)phenyl)propanoate (6). A mixture of 4-formylcinnamic acid (**5**) (4.00 g, 16.0 mmol) and Pd/C (500 mg, 10% Pd) in ethanol (170 mL) and DIPEA (5 mL) was stirred at room temperature for 6 h under a H₂ atmosphere. After filtration and evaporation, the crude product was dissolved in MeOH (160 mL), and 2 mL H₂SO₄ was added. The mixture was stirred overnight, followed by neutralization with 2 N NaOH solution and subsequent evaporation. The crude ester was purified by flash chromatography (*n*-hexane/ethyl acetate 3:1 → 2:1) to afford **6** [**2**] (3.8 g, 88%) as a white solid. *R*_f 0.43 (cyclohexane/EtOAc 1:1). ¹H NMR (CDCl₃, 400 MHz): δ 2.18 (s, 1H), 2.58 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.63 (s, 3H), 4.59 (s, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H); ¹³C NMR (CDCl₃, 100 MHz): δ 30.5, 35.6, 51.6, 64.9, 127.2, 128.3, 138.9, 139.8, 173.3; IR (CHCl₃): 3368, 2924, 1738, 1438, 1366, 1198, 1015, 817 cm⁻¹; HRMS–ESI (*m/z*): [M + Na]⁺ calcd for C₁₁H₁₄O₃, 217.0841; found, 217.0832.



Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoate (7). A solution of **6** (4.0 g, 20.5 mmol) in acetonitrile (110 mL) was treated with triethylamine (8.7 mL, 62 mmol) at room temperature then cooled to 0 °C. *N,N'*-Disuccinimidyl carbonate (DSC, 8.0 g, 31 mmol) was added. The solution was then warmed to 18 °C over 1 h, diluted with ethyl acetate (400 mL) and washed with saturated aqueous NaHCO₃ solution. The resultant aqueous layer was re-extracted once with ethyl acetate. The combined organic phases were dried over MgSO₄, filtered and concentrated in vacuo to afford the mixed carbonate, which was used without further purification. A solution of the crude carbonate in CH₂Cl₂ (45 mL) was added to a solution of **4** (8.0 g, 42 mmol) and triethylamine (7.3 mL, 52 mmol) in CH₂Cl₂ (110 mL) at 0 °C. The solution was stirred

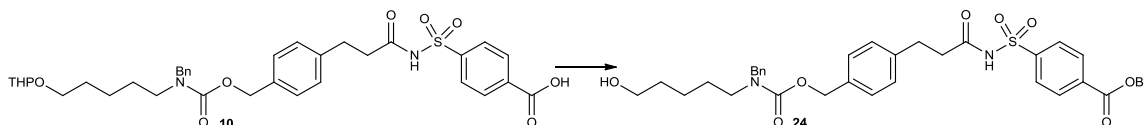
for 50 min at room temperature, diluted with CH₂Cl₂ and washed with saturated aqueous NaHCO₃ solution. The aqueous layer was re-extracted twice with CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and concentrated. Flash column chromatography on silica gel (cyclohexane/EtOAc 2:1 → 1:1) afforded **7** (7.4 g, 85%) as a colorless oil. *R*_f 0.27 (cyclohexane/EtOAc 1:1); ¹H NMR (CDCl₃, 400 MHz): δ 1.17–1.32 (m, 3H), 1.43–1.53 (m, 4H), 2.61 (br, 2H), 2.93 (br, 2H), 3.19–3.26 (m, 2H), 3.45–3.62 (m, 2H), 3.65 (s, 3H), 4.48 (d, *J* = 6.8 Hz, 2H), 5.12 (d, *J* = 11.2 Hz, 2H), 7.15–7.37 (m, 9H); ¹³C NMR (CDCl₃, 100 MHz): δ 22.8, 27.3, 27.8, 30.5, 32.2, 35.4, 46.0, 46.9, 50.0, 50.4, 51.5, 62.4, 66.9, 127.7, 128.0, 128.4, 128.6, 134.6, 134.7, 137.8, 140.2, 173.2; IR (CHCl₃): 3439, 3030, 2935, 2863, 2386, 1735, 1517, 1496, 1429, 1362, 1226, 1072 cm⁻¹; HRMS–ESI (*m/z*): [M + Na]⁺ calcd for C₂₄H₃₁NO₅Na, 436.2100; found, 436.2092.



Methyl 3-(4-((benzyl(5-*O*-tetrahydropyranyloxy)pentyl)carbamoyloxy)methyl)phenyl)propanoate (8**).** To a mixture of **7** (6.8 g, 16.4 mmol) and 3,4-dihydro-2*H*-pyran (7.0 mL, 82.2 mmol, 5 equiv) in anhydrous CH₂Cl₂ (50 mL), pyridinium *p*-toluenesulfonate (2.1 g, 8.2 mmol, 0.5 equiv) was added and the solution was stirred overnight. Next, saturated NaHCO₃ solution was added and the resultant organic layer dried over MgSO₄, filtered and concentrated. Flash column chromatography on silica gel (cyclohexane/EtOAc 4:1 → 3:1) afforded **8** (7.1 g, 86%) as a colorless oil. *R*_f 0.66 (cyclohexane/EtOAc 1:1); ¹H NMR (CDCl₃, 400 MHz): δ 1.13–1.30 (m, 3H), 1.41–1.52 (m, 8H), 1.57–1.79 (m, 3H), 2.55 (br, 2H), 2.86 (br, 2H), 3.12–3.26 (m, 3H), 3.38–3.43 (m, 1H), 3.59 (s, 3H), 3.74–3.79 (m, 1H), 4.44 (t, *J* = 12.4 Hz, 2H), 5.06 (d, *J* = 10.6 Hz, 2H), 7.08–7.21 (m, 9H); ¹³C NMR (CDCl₃, 100 MHz): δ 19.7, 23.5, 25.5, 29.4, 30.6, 30.8, 35.6, 51.6, 62.4, 66.9, 67.0, 67.3, 77.2, 98.9, 127.2, 127.8, 128.1, 128.2, 128.4, 128.5, 137.9, 140.2, 173.2; IR (CHCl₃): 2939, 2865, 1737, 1723, 1517, 1496, 1454, 1227, 1034 cm⁻¹; HRMS–ESI (*m/z*): [M + Na]⁺ calcd for C₂₉H₃₉NO₆Na, 520.2675; found, 520.2673.

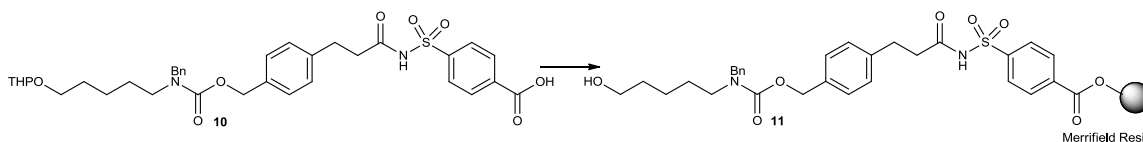
3-(4-((Benzyl(5-*O*-tetrahydropyranyloxy)pentyl)carbamoyloxy)methyl)phenyl)propanoic acid (9**).** To a solution of **8** (5.1 g, 10.2 mmol) in THF/water (60 mL, 4:1), LiOH·H₂O (1.23 g, 30.7 mmol, 3 equiv) was added. The solution was stirred under reflux for 2 h and then cooled to room temperature. The solution was adjusted to pH 5–6 by using IR-120 (H⁺). The mixture was then filtered and concentrated. Flash column chromatography on silica gel (DCM/MeOH 150:1 → 100:1) afforded **9** (4.8 g, 95%) as a light-yellow oil. *R*_f 0.33 (DCM/MeOH 50:1); ¹H NMR (CDCl₃, 400 MHz): δ 1.26–1.33 (m, 2H), 1.49–1.62 (m, 7H), 1.67–1.84 (m, 2H), 2.55 (br, 2H), 2.67 (br, 2H), 2.96 (d, *J* = 4.3 Hz, 2H), 3.20–3.40 (m, 3H), 3.48–3.53 (m, 1H), 3.64–3.68 (m, 1H), 3.82–3.87 (m, 1H), 4.50 (d, *J* = 8.8 Hz, 2H), 4.57 (t, *J* = 6.5 Hz, 1H), 5.14 (d, *J* = 10.0 Hz, 2H), 7.17–7.29 (m, 9H); ¹³C NMR (CDCl₃, 100 MHz): δ 19.5, 22.8, 25.4, 27.3, 27.8, 29.3, 30.3, 30.6, 35.4, 46.0, 47.0, 50.1, 50.4, 62.3, 67.0, 67.3, 98.7, 127.2, 127.7, 128.1, 128.4, 134.7, 137.8, 140.0, 156.2, 156.7, 177.3, 177.6; IR (CHCl₃): 3029, 2939, 2864, 1732, 1517, 1496, 1474, 1419, 1355, 1226, 1184, 1086, 1074, 1021, 972 cm⁻¹; HRMS–ESI (*m/z*): [*M* + Na]⁺ calcd for C₂₈H₃₇NO₆Na, 506.2519; found, 506.2512.

chromatography on silica gel (DCM/MeOH/HOAc 150:1:0.05 \rightarrow 100:1:0.05) afforded **10** (2.9 g, 60%) as a colorless yellow oil. R_f 0.20 (DCM/MeOH 50:1); ^1H NMR (CDCl_3 , 400 MHz): δ 1.22–1.31 (m, 3H), 1.49–1.58 (m, 8H), 1.69–1.85 (m, 2H), 2.57 (br, 2H), 2.84 (d, J = 6.9 Hz, 2H), 3.20–3.35 (m, 3H), 3.52–3.69 (m, 3H), 3.84–3.92 (m, 1H), 4.47–4.58 (m, 3H), 5.07 (t, J = 16.9 Hz, 2H), 6.94–7.07 (m, 3H), 7.15–7.29 (m, 6H), 7.99–8.05 (m, 2H), 8.12–8.16 (m, 2H); ^{13}C NMR (CDCl_3 , 100 MHz): δ 19.4, 22.6, 23.2, 23.3, 25.3, 27.7, 29.2, 29.3, 29.8, 30.6, 31.7, 37.7, 46.2, 47.0, 50.1, 50.4, 62.3, 62.4, 62.5, 67.1, 67.2, 67.4, 98.9, 127.1, 127.3, 127.5, 128.0, 128.2, 128.5, 130.4, 134.3, 134.6, 137.4, 137.5, 139.5, 139.7, 134.2, 156.5, 157.0, 168.0, 170.7; IR (CHCl_3): 2939, 2865, 1672, 1454, 1354, 1172, 1021, 862 cm^{-1} ; HRMS–ESI (m/z): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{35}\text{H}_{42}\text{N}_2\text{O}_9\text{SNa}$, 689.2509; found, 689.2497.

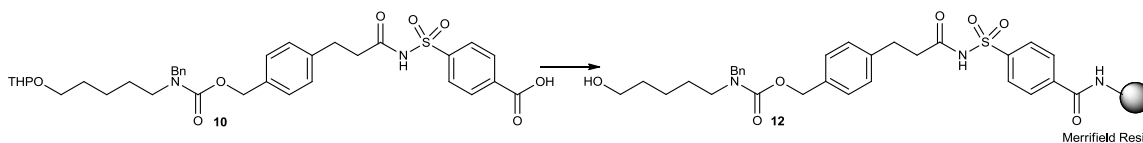


Benzyl 4-(N-(3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propion-yl)sulfamoyl)benzoate (24). To a solution of DCC (46 mg, 0.22 mmol) and DMAP (6 mg, 0.05 mmol) in anhydrous CH_2Cl_2 (5 mL), **10** (286 mg, 0.43 mmol) was added with benzyl alcohol (0.18 mL, 1.72 mmol) at room temperature. The mixture was stirred for 8 h and filtered. The filtrate was evaporated to afford the crude product that was dissolved in MeOH (5 mL). p -TsOH $\cdot\text{H}_2\text{O}$ (42 mg, 0.22 mmol) was added to the solution, which was then stirred for another 6 h, and concentrated. Flash column chromatography on silica gel (cyclohexane/EtOAc 2:1 \rightarrow 1:1) afforded **24** (158 mg, 55%) as a colorless oil over two steps. R_f 0.49 (cyclohexane/EtOAc 1:2); ^1H NMR (CDCl_3 , 400 MHz): δ 0.75–0.90 (m, 2H), 1.09–1.19 (m, 5H), 1.34–1.45 (m, 5H), 2.42 (t, J = 7.5 Hz, 2H), 2.73 (br, 1H), 3.10–3.19 (m, 2H), 3.35–3.52 (m, 2H), 4.40 (d, J = 12.6 Hz, 2H), 4.93–5.06 (m, 2H), 6.90–7.37 (m, 14H), 7.94 (dd, J = 1.8, 6.9 Hz, 2H), 8.08 (d, J = 8.4 Hz, 2H); ^{13}C NMR (CDCl_3 , 100 MHz): δ 14.1, 22.6, 27.2, 29.6, 32.1, 35.8, 37.9, 46.0, 46.7, 50.0, 50.4, 62.6, 67.1, 67.4, 127.1, 127.3, 127.6, 128.0, 128.2, 128.3, 128.5, 128.6, 130.3, 134.5, 135.3, 137.6, 142.7, 156.4, 156.8, 164.8, 170.4, 170.6; IR (CHCl_3): 3032, 2931, 2859,

1723, 1673, 1578, 1496, 1453, 1429, 1352, 1171, 1017, 958 cm^{-1} ; HRMS–ESI (m/z): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_8\text{SNa}$, 695.2403; found, 695.2408.

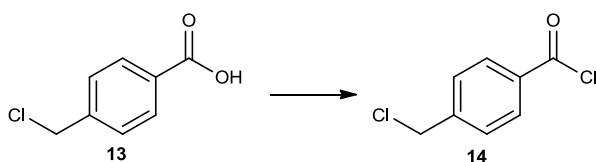


Linker-functionalized Merrifield chlorine resin 11. Merrifield chlorine resin (300 mg) was swollen by overnight incubation in CH_2Cl_2 with gentle rotation on a rotavap. The solvent was drained and a solution of **10** (1.5 equiv) in DMF (2 mL) was added to the resin followed by Cs_2CO_3 (2.0 equiv) and then TBAI (1.5 equiv). The solution was incubated at 60 °C overnight while rotating on the rotavap. The coupling reaction was repeated once. Subsequently, the resin was washed successively with DMF/Water (1:1), DMF, MeOH, CH_2Cl_2 , MeOH, CH_2Cl_2 and then allowed to swell in CH_2Cl_2 for 1 h. The fully-swollen resin was placed in a flask with DMF (2 mL) and CsOAc (2.0 equiv) was added. The solution was allowed to rotate at 60 °C on the rotavap overnight. The capping reaction was repeated once. The capped, functionalized resin was washed successively with DMF/Water (1:1), DMF, MeOH, CH_2Cl_2 , MeOH, CH_2Cl_2 . Afterwards, the resin was incubated and shaken overnight in a 30 mM solution of $p\text{TsOH} \cdot \text{H}_2\text{O}$ in MeOH. This THP removal reaction was repeated twice. The solution was then drained and the resin washed successively with CH_2Cl_2 , MeOH and CH_2Cl_2 prior to drying in high vacuum.

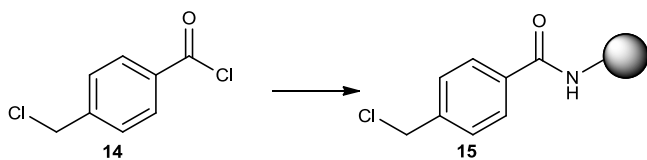


Linker-functionalized Merrifield amino resin 12. The Merrifield amino resin (300 mg) was swollen by overnight incubation in CH_2Cl_2 . The solvent was drained and a solution of **10** (1.5 equiv) in DMF (2 mL) was added to the resin followed by addition of HOBT (6 equiv) and DIC (6 equiv). The resin solution was shaken overnight at room temperature. Subsequently, the coupling reaction was repeated once. The resin was then washed

successively with CH₂Cl₂, MeOH and CH₂Cl₂, and then capped with a solution of CH₂Cl₂/pyridine/Ac₂O (2:1:1). The capping reaction was carried out one more time. The resin was then washed successively with CH₂Cl₂, MeOH, and CH₂Cl₂, and then with a 0.03 M solution of PTSA monohydrate in CH₂Cl₂ to remove pyridine. The linker-functionalized resin was then shaken overnight in a 30 mM solution of *p*TsOH·H₂O in CH₂Cl₂. This THP removal reaction was repeated twice. The solution was drained and the resin was washed successively with CH₂Cl₂, MeOH and CH₂Cl₂ prior to drying under a vacuum.

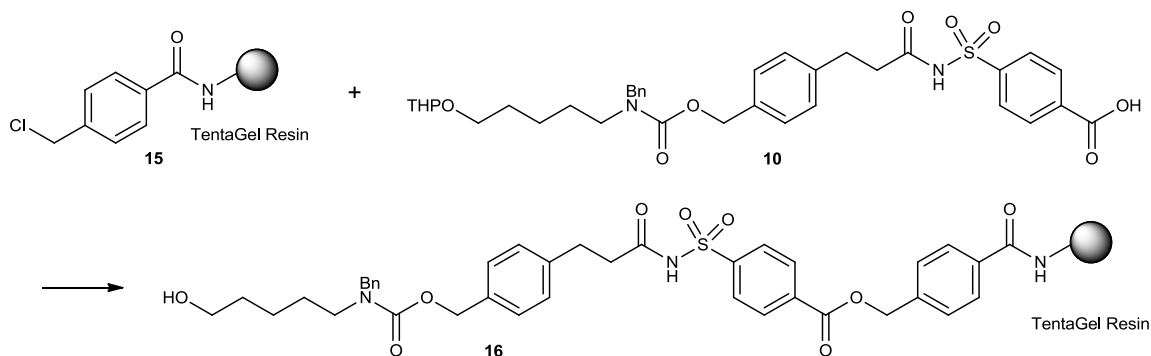


(Chloromethyl)benzoyl chloride (14). To a flask containing (chloromethyl)benzoic acid **13** (2.00 g, 11.76 mmol), oxalyl chloride (5 mL) was added under the control of a bubbler. Next, a drop of DMF was carefully added and the reaction was stirred at room temperature overnight. The solvents were evaporated to give 2.20 g (99%) of pure **14** [3] as a syrup. ¹H NMR (400 MHz, CDCl₃): δ 8.12 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 4.63 (s, 2H).



Spacer-functionalized Merrifield amino resin 15. The TentaGel amino resin (300 mg) was swollen by incubating in CH₂Cl₂ overnight. The solvent was drained and a solution of **14** (2.0 equiv) in CH₂Cl₂/pyridine (2 mL, 3:2) was added. The resin was shaken overnight at room temperature, and then the coupling reaction was carried out one more time. The spacer-functionalized resin was washed with CH₂Cl₂, MeOH and CH₂Cl₂, and then was capped with CH₂Cl₂/pyridine/Ac₂O (2:1:1). The capping reaction was repeated once. The functionalized resin was then shaken overnight. Next a series of washes was

performed with CH₂Cl₂, MeOH, CH₂Cl₂, and then a 30 mM solution of *p*-TsOH·H₂O in CH₂Cl₂ to remove pyridine, prior to drying in high vacuum



Linker-functionalized Merrifield amino resin 16. Functionalized TentaGel resin **15** (300 mg) was swollen by overnight incubation in CH₂Cl₂ with gentle rotation on a rotovap. The solvent was drained and a solution of **10** (1.5 equiv) in DMF (2 mL) was added to the resin followed by Cs₂CO₃ (2.0 equiv) and TBAI (1.5 equiv). The solution was incubated at 60 °C overnight in the rotovap, after which the coupling reaction was repeated once. Next, the resin was washed successively with DMF/water (1:1), DMF, MeOH, CH₂Cl₂, MeOH, CH₂Cl₂, and then allowed to swell in CH₂Cl₂ for 1 h. The fully-swollen resin was placed in a flask with DMF, and CsOAc (2.0 equiv) was added. The solution was rotated at 60 °C in the rotavap overnight. The capping reaction was repeated once. The functionalized resin was then washed successively with DMF/water (1:1), DMF, MeOH, CH₂Cl₂, MeOH, and CH₂Cl₂. The resin was then incubated overnight with shaking in a 30 mM solution of *p*-TsOH·H₂O in CH₂Cl₂. After this, the THP removal reaction was repeated twice. The solution was drained and the resin was washed successively with CH₂Cl₂, MeOH and CH₂Cl₂ prior to drying in high vacuum.

General method to determine the loading of functionalized resins **11**, **12** and **16**.

By using a method adapted from the work of Gude and co-workers [4], dried functionalized resin (20–30 mg) was placed in a syringe equipped with a frit (5 mL syringe). CH₂Cl₂ (2–3 mL) was added to the syringe for 1 h to swell the resin, and then

drained. Next, 1 mL of CH₂Cl₂, 0.1 mL of pyridine, 100 mg of FmocCl, was added and the syringe was shaken for at least 6 h. The solution was drained and the resin was washed with CH₂Cl₂ (three times), MeOH (three times), then alternating washes of CH₂Cl₂ and MeOH, each three times. The resin was again swollen in CH₂Cl₂ and drained. A 2% (v/v) DBU in DMF solution (2 mL) was added to the syringe, which was shaken for at least 1 h. Finally, the solution was drained from the resin into a vial and retained.

For each solution obtained from **11**, **12** and **16**, 0.160 mL was added to a 10 mL graduated flask and diluted to 10 mL with acetonitrile. To calculate the loading of each resin, UV absorption at 304 nm was obtained, by using 2% (v/v) DBU in DMF solution (0.160 mL) diluted to 10 mL with acetonitrile as a blank control. The loading was calculated according to the following formula:

$$\text{Loading [mmol/g]} = \frac{A_{304} \times 16.4}{\text{g resin}}$$

In this way at 304 nm loading was determined to be:

Functionalized resin **11** = 0.566 mmol/g of resin

Functionalized resin **12** = 0.249 mmol/g of resin

Functionalized resin **16** = 0.433 mmol/g of resin

General procedures for automated syntheses

Thioglycoside glycosylation - activation with NIS/triflic acid:

The chosen functionalized resin was loaded into the reaction vessel of the synthesizer. The temperature of the reaction vessel was adjusted to 25 °C and the resin was treated with successive washes of DMF, THF, 0.2 M acetic acid in CH₂Cl₂ and CH₂Cl₂ (six times, each wash with 2 mL for 25 s). For glycosylation, a solution of thioglycoside building blocks (**17** or **19**; 3 equiv or 1.1 equiv in 1 mL CH₂Cl₂) was delivered to the reaction vessel followed by the addition of 1 mL of activation solution (170 mM NIS and 17 mM triflic acid in dioxane/CH₂Cl₂ 1:1). After 15 min, the glycosylation solution was drained and the resin was washed once with 2 mL dioxane, followed by washes with 0.2 M acetic

acid in CH₂Cl₂ and CH₂Cl₂ successively (six times, each wash with 2 mL for 25 s). This glycosylation reaction was repeated twice.

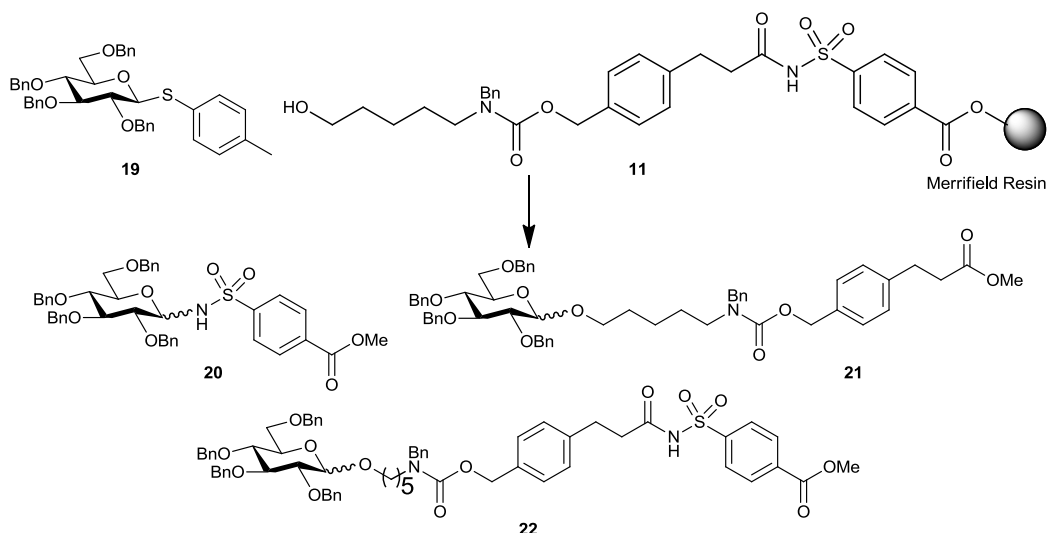
Thioglycoside glycosylation - activation with DMTST:

The chosen functionalized resin was loaded into the reaction vessel of the synthesizer. The temperature of the reaction vessel was adjusted to 25 °C and the resin was washed with DMF, THF, 10% acetone in CH₂Cl₂ and CH₂Cl₂ successively (six times, each wash with 2 mL for 25 s). For glycosylation reactions the temperature was adjusted to –15 °C and a solution of thioglycoside building blocks (**17** or **19**; 3 equiv in 1 mL CH₂Cl₂) was delivered to the reaction vessel. The reaction was initiated by the addition of 1 mL of 181 mM DMTST solution (181 mM Me₂S₂ and 181 mM MeOTf in CH₂Cl₂). After 3 h the glycosylation solution was drained and the resin was washed with CH₂Cl₂ (six times, each wash with 2 mL for 25 s). This glycosylation reaction was repeated twice.

Trichloroacetimidate glycosylation - activation with TMSOTf:

The chosen functionalized resin was loaded into the reaction vessel of the synthesizer. The temperature of the reaction vessel was adjusted to 25 °C and the resin was washed with DMF, THF, 10% acetone in CH₂Cl₂ and CH₂Cl₂ successively (six times each with 2 mL for 25 s). The resin was washed with 2 mL of 17 mM triflic acid in CH₂Cl₂, and CH₂Cl₂ one time each, and the temperature was adjusted to –15 °C. For glycosylation, a solution of glycosyl trichloroacetimidate building block **18** (3 equiv in 0.75 mL CH₂Cl₂) was delivered to the reaction vessel followed by the addition of 0.25 mL of 50 mM TMSOTf in CH₂Cl₂. After 30 min the glycosylation solution was drained and the resin was washed with CH₂Cl₂ (six times with 2 mL for 25 s). The acidic prewash with triflic acid and the glycosylation reaction was repeated twice.

Example of an experimental procedure



Automated glycosylation to safety-catch linker:

Functionalized resin **11** (50 mg, loading: 0.566 mmol/g, 28.3 μ mol) was loaded into the reaction vessel of the synthesizer. The temperature of the reaction vessel was adjusted to 25 $^{\circ}$ C and the resin was treated with successive washes of DMF, THF, 0.2 M acetic acid in CH_2Cl_2 and CH_2Cl_2 (six times, each wash with 2 mL for 25 s) and one time with activation solution (170 mM NIS and 17 mM triflic acid in dioxane/ CH_2Cl_2 1:1; 2 mL for 25 s) and CH_2Cl_2 (2 mL for 25 s). For glycosylation, a solution of building block **19** (55 mg, 3 equiv, 84.9 μ mol) was delivered to the reaction vessel followed by the addition of 1 mL of activation solution (170 mM NIS and 17 mM triflic acid in dioxane/ CH_2Cl_2 1:1). After 15 min, the glycosylation solution was drained and the resin was washed once with 2 mL dioxane, followed by washes CH_2Cl_2 (six times, each wash with 2 mL for 25 s) and one time with activation solution (170 mM NIS and 17 mM triflic acid in dioxane/ CH_2Cl_2 1:1; 2 mL for 25 s) and CH_2Cl_2 (2 mL for 25 s). This glycosylation reaction was repeated twice and the resin was finally washed with CH_2Cl_2 (six times with 2 mL for 25 s).

For the Zemplén cleavage the resin was removed from the reaction vessel of the synthesizer and was put into a syringe equipped with a filter frit. The solid support was swollen in CH_2Cl_2 and a NaOMe solution (0.25 M in MeOH, 0.5 mL) was added. The reaction was shaken at room temperature. After 10 h, the solution was collected and the resin was washed with CH_2Cl_2 and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1 (six times, each wash with 2 mL).

The solutions were combined and neutralized with IR-120 (H⁺) and the solvents were removed in vacuo.

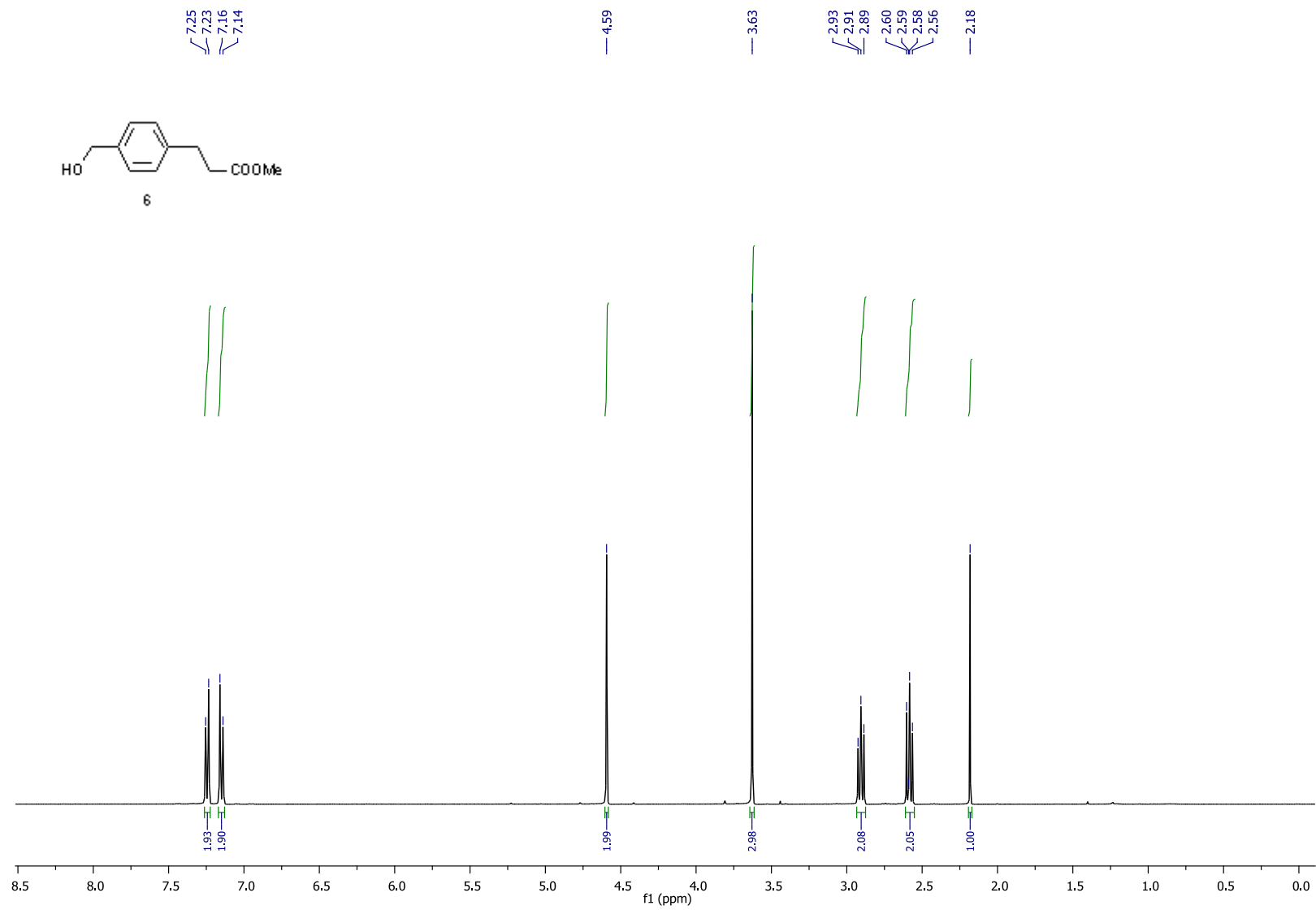
RPHPLC–MS analysis:

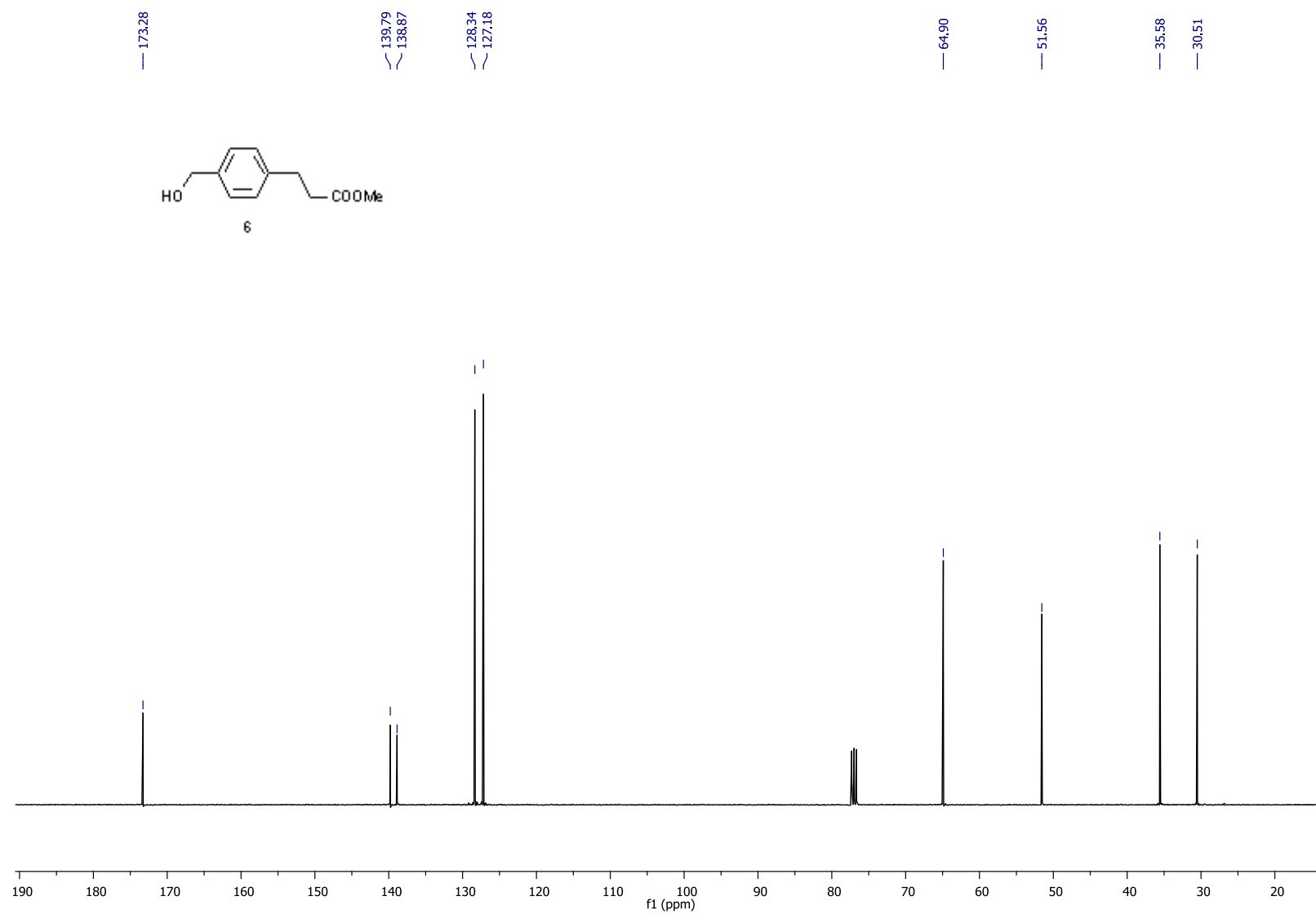
The crude product mixture was analyzed by RPHPLC–MS (column: Nucleosil C4 (120 mm × 5 mm); detection: UV detection at 209, 230 and 254 nm; eluents: water (+ 0.1% formic acid) and acetonitrile (+ 0.1% formic acid); gradient: isocratic at 10% for 5 min → 10% → 80% acetonitrile in 30 min → 95% acetonitrile in 10 min; for chromatogram and mass spectra, please see below).

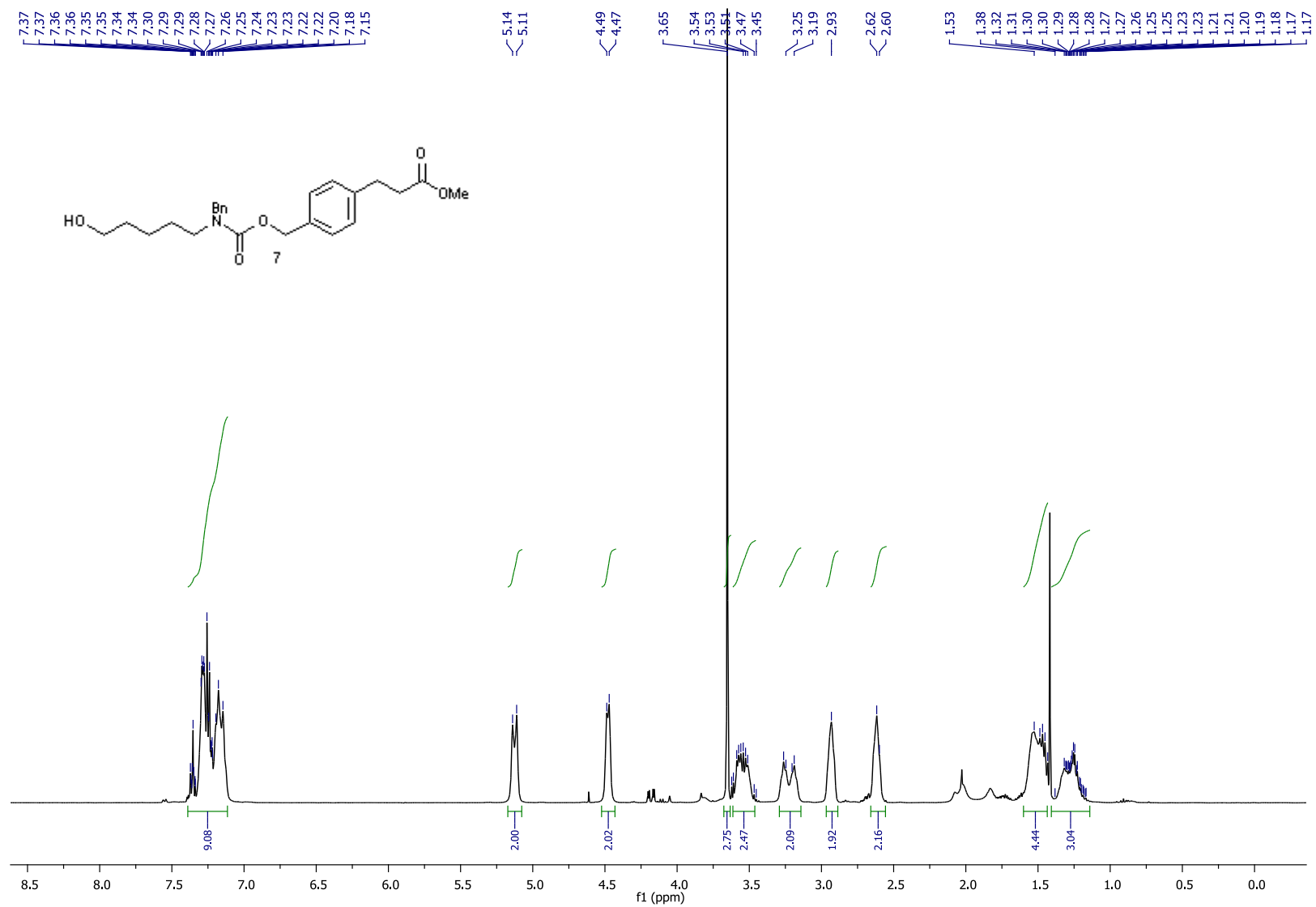
compound	retention time [min]	m/z	
		calcd for	obsd
20	26.4	C ₄₂ H ₄₃ NO ₉ S [M+NH ₄] ⁺ 755.3	755.2
21	31.2	C ₅₈ H ₆₅ NO ₁₀ [M+NH ₄] ⁺ 953.5	953.3
22	30.2	C ₆₅ H ₇₀ N ₂ O ₁₃ S [M+NH ₄] ⁺ 1136.5	1136.3

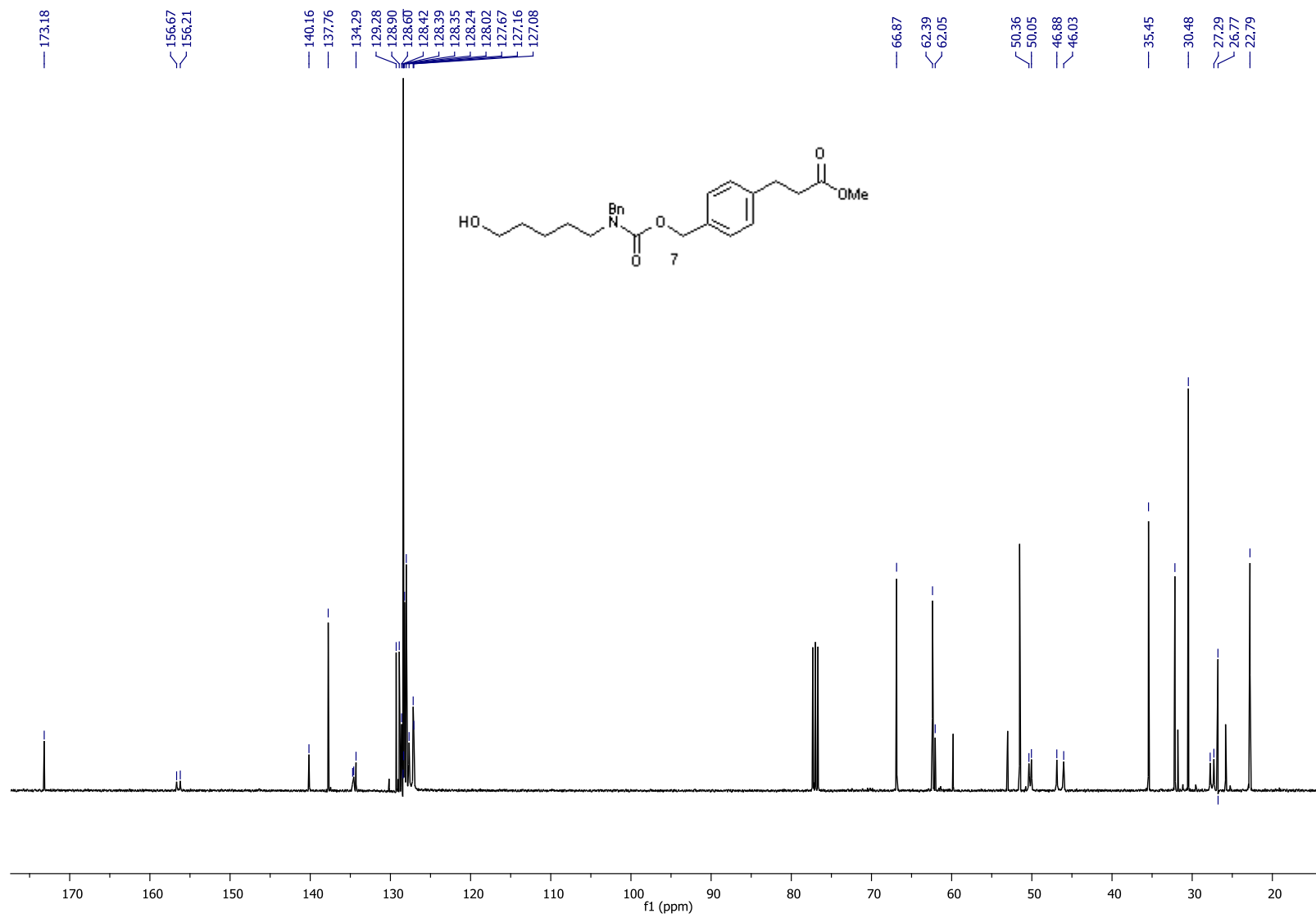
References

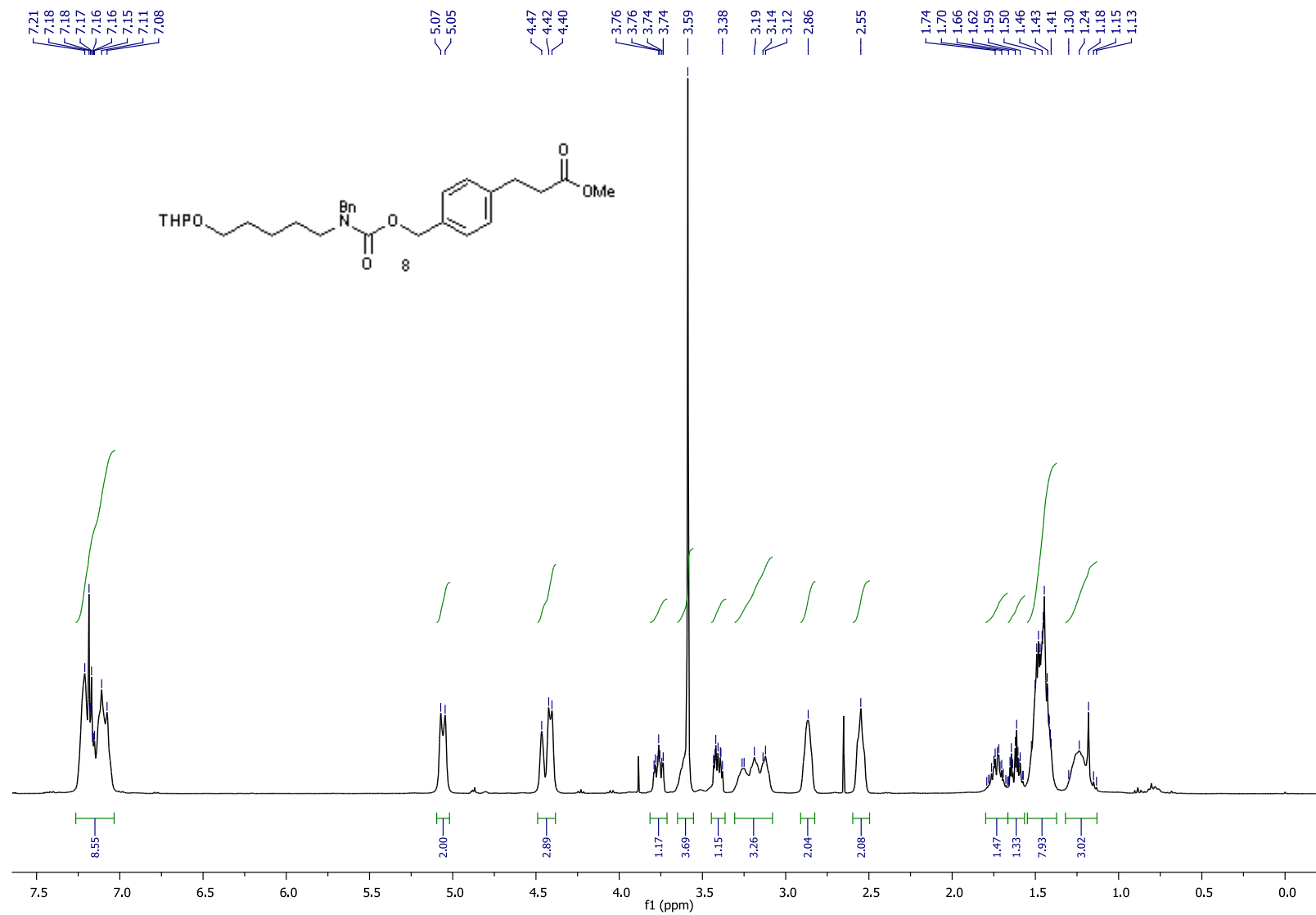
1. Scriabine, I. *Bull. Soc. Chim. Fr.* 1947, 454.
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3. Norris, J. F.; Ware, V. W. *J. Am. Chem. Soc.* **1939**, 61, 1418.
4. M. Gude, M.; Ryf, J.; White, P. D. *Lett. Pept. Sci.*, **2002**, 9, 203.

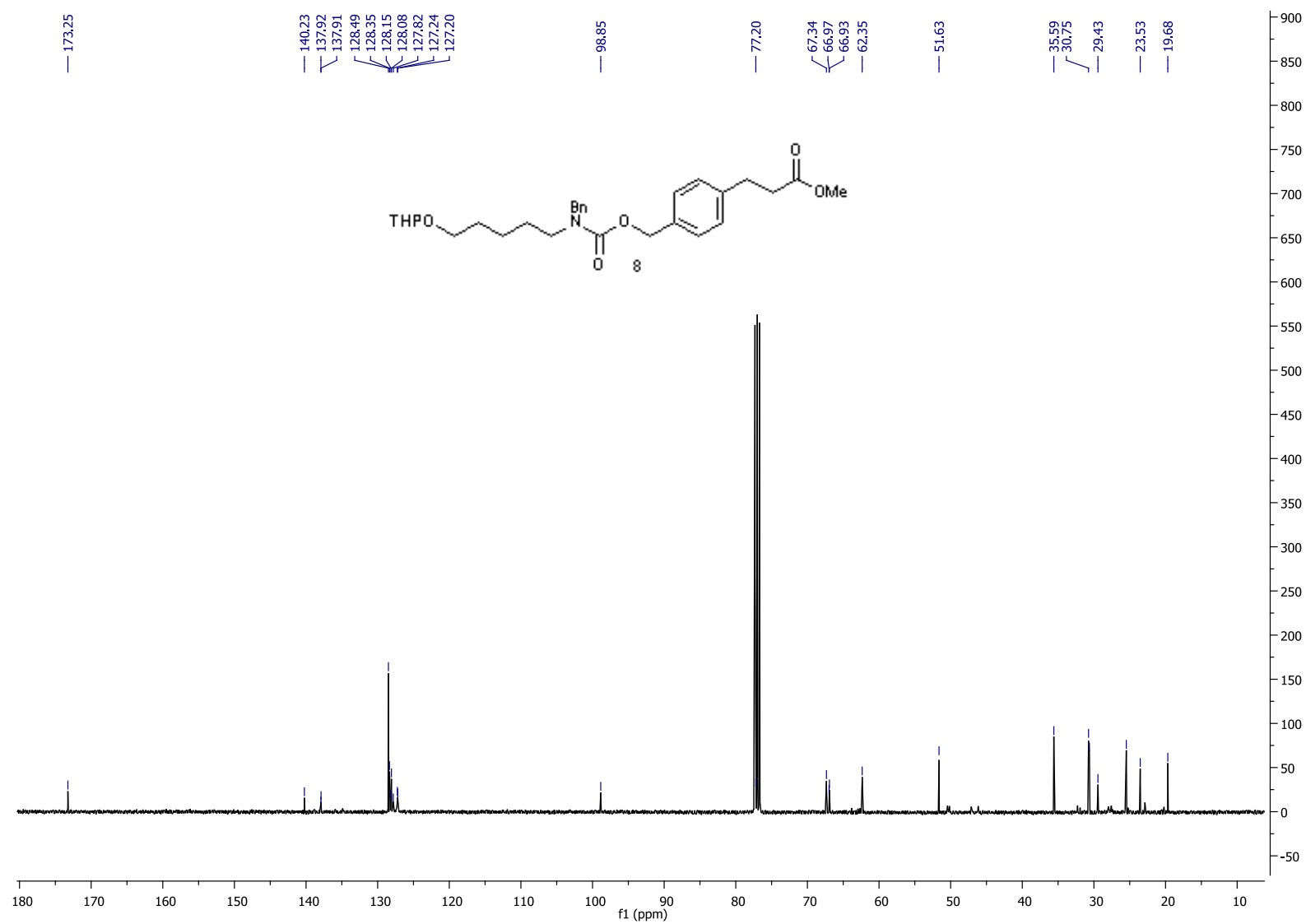


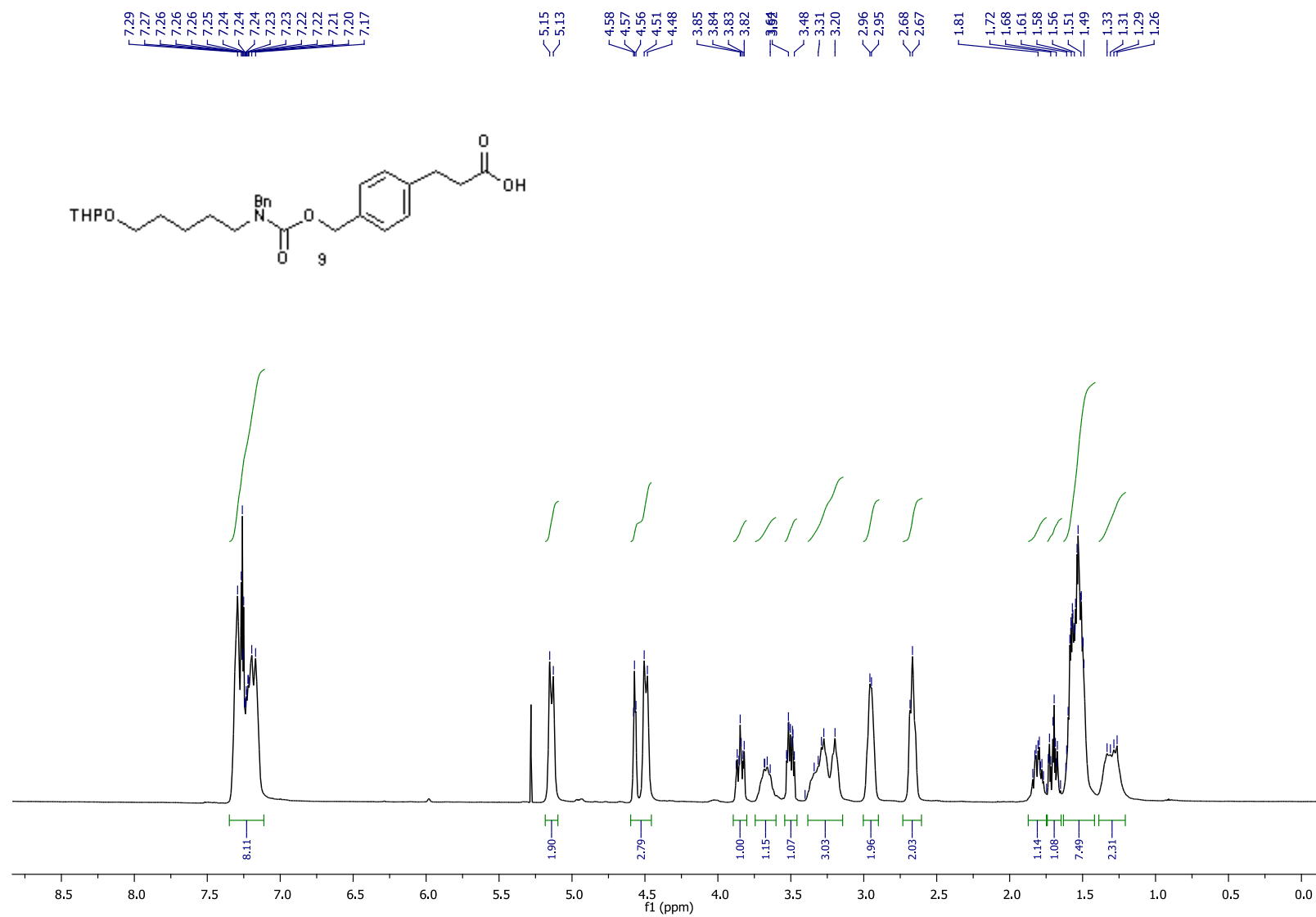


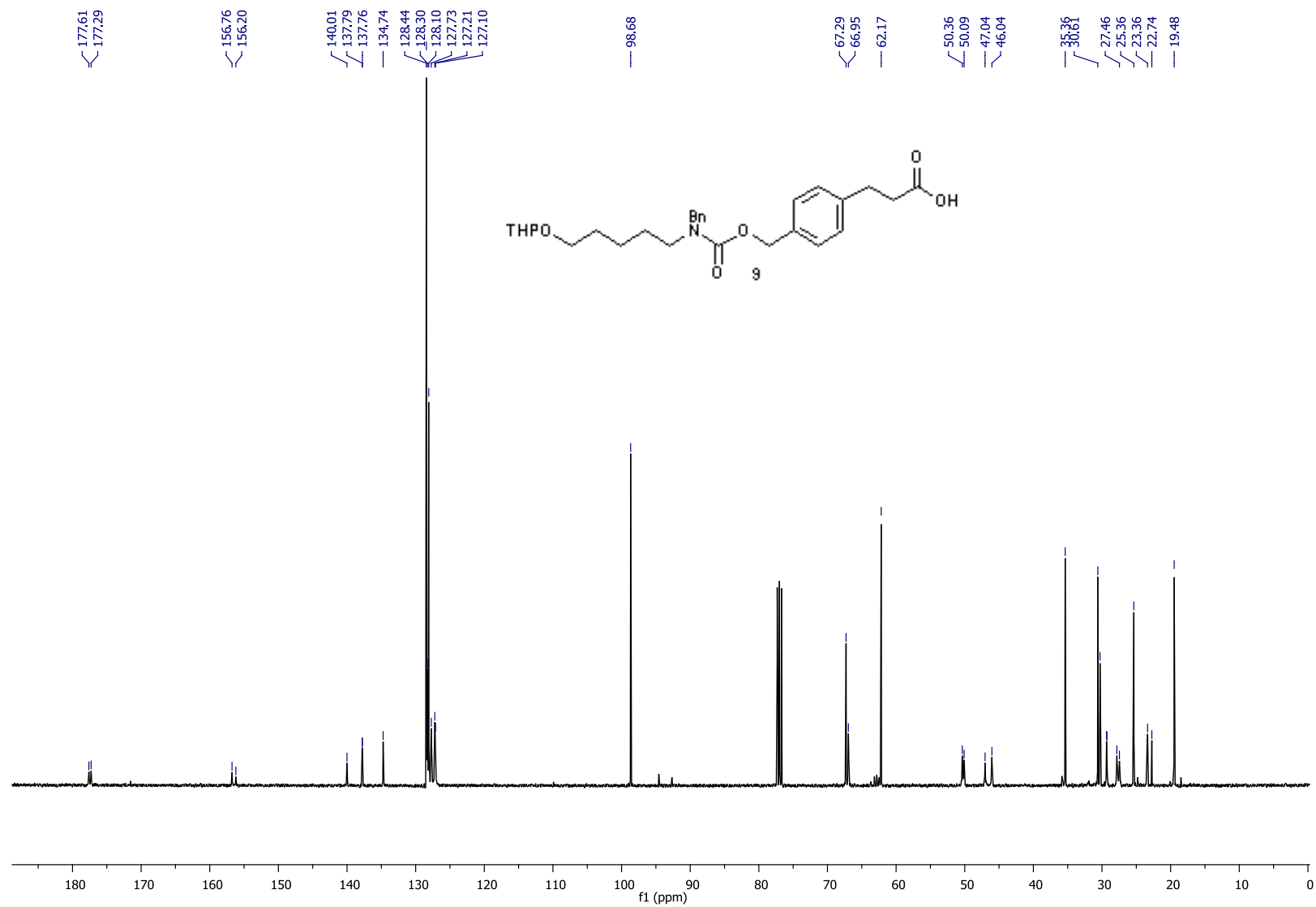


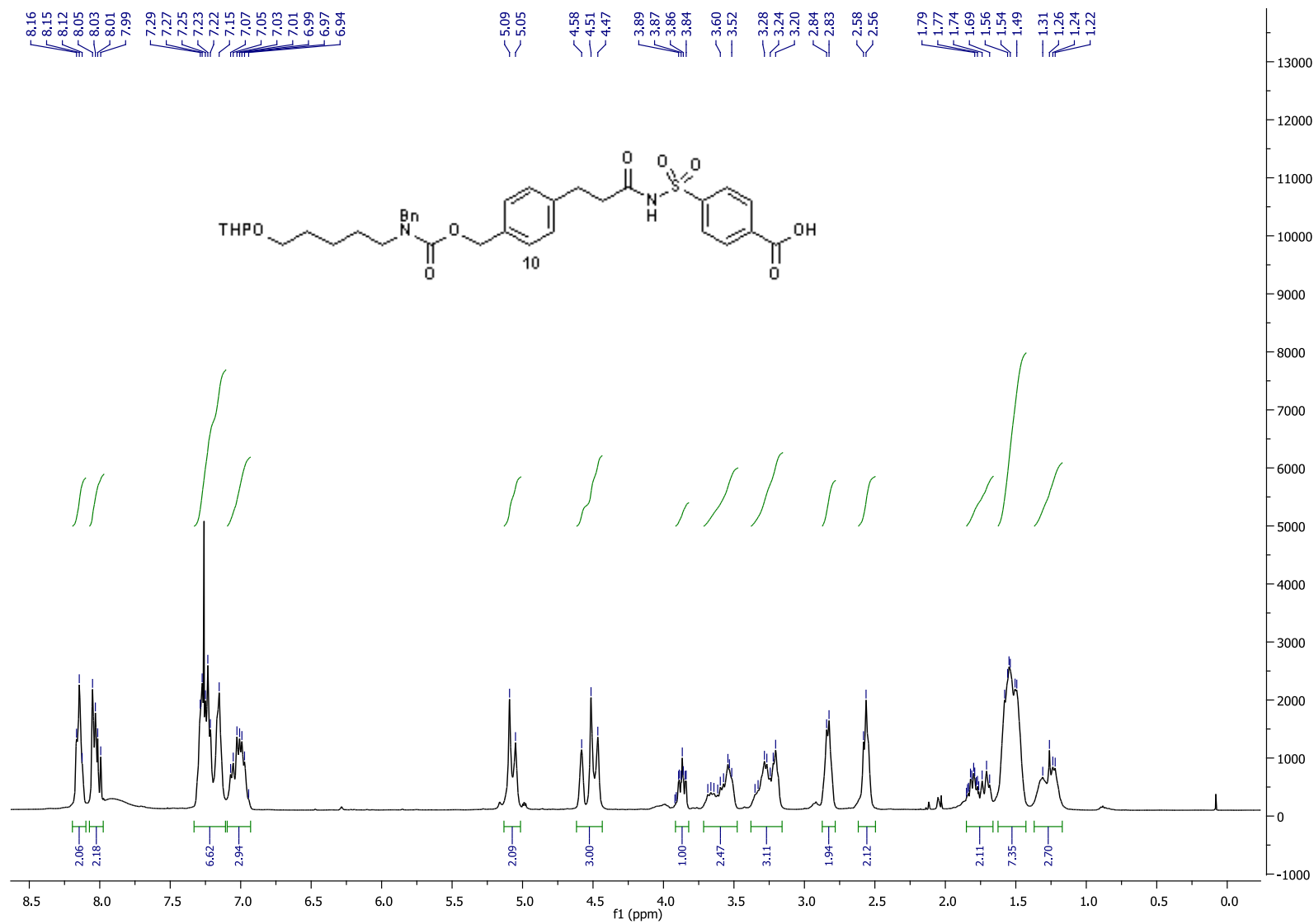


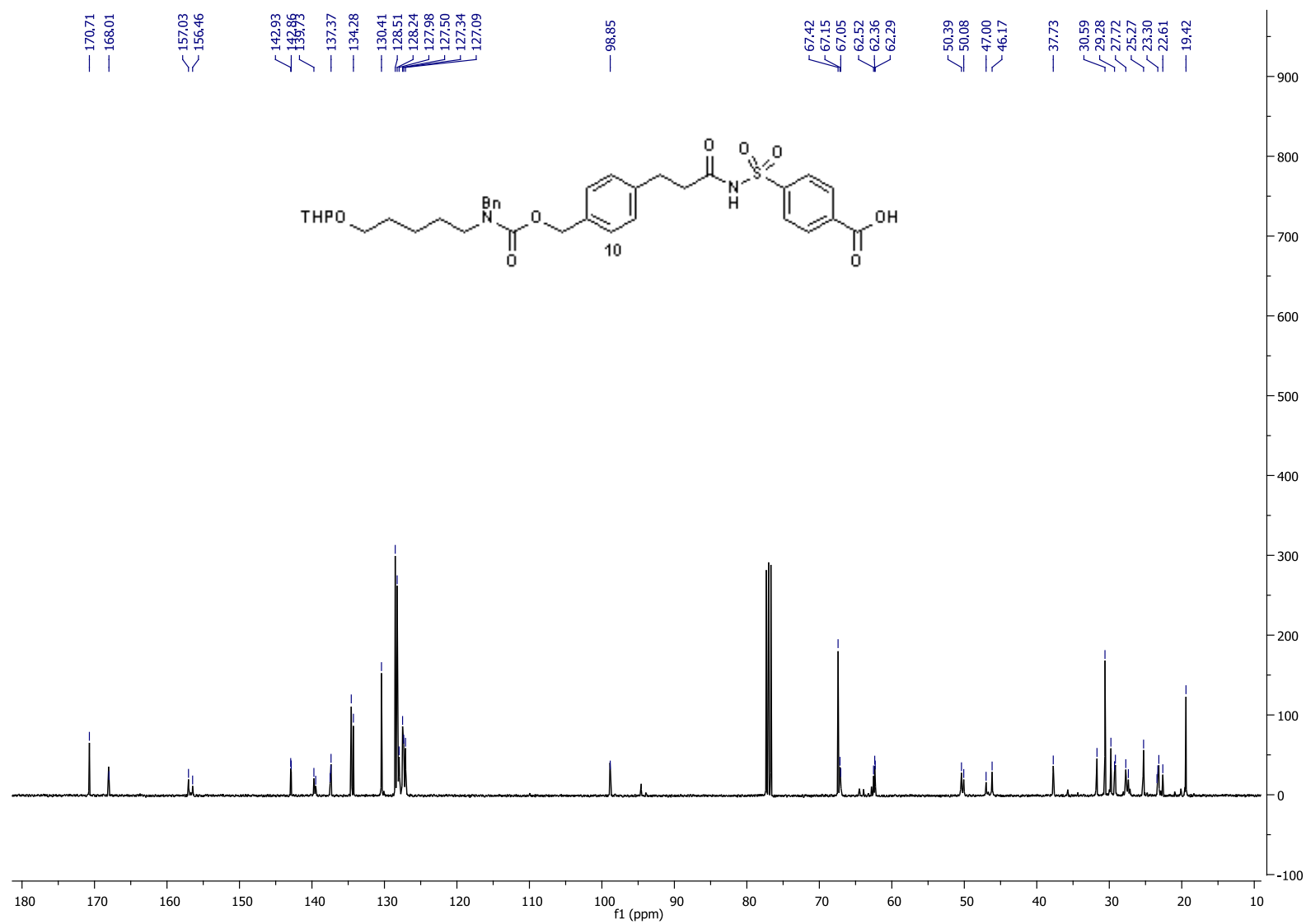


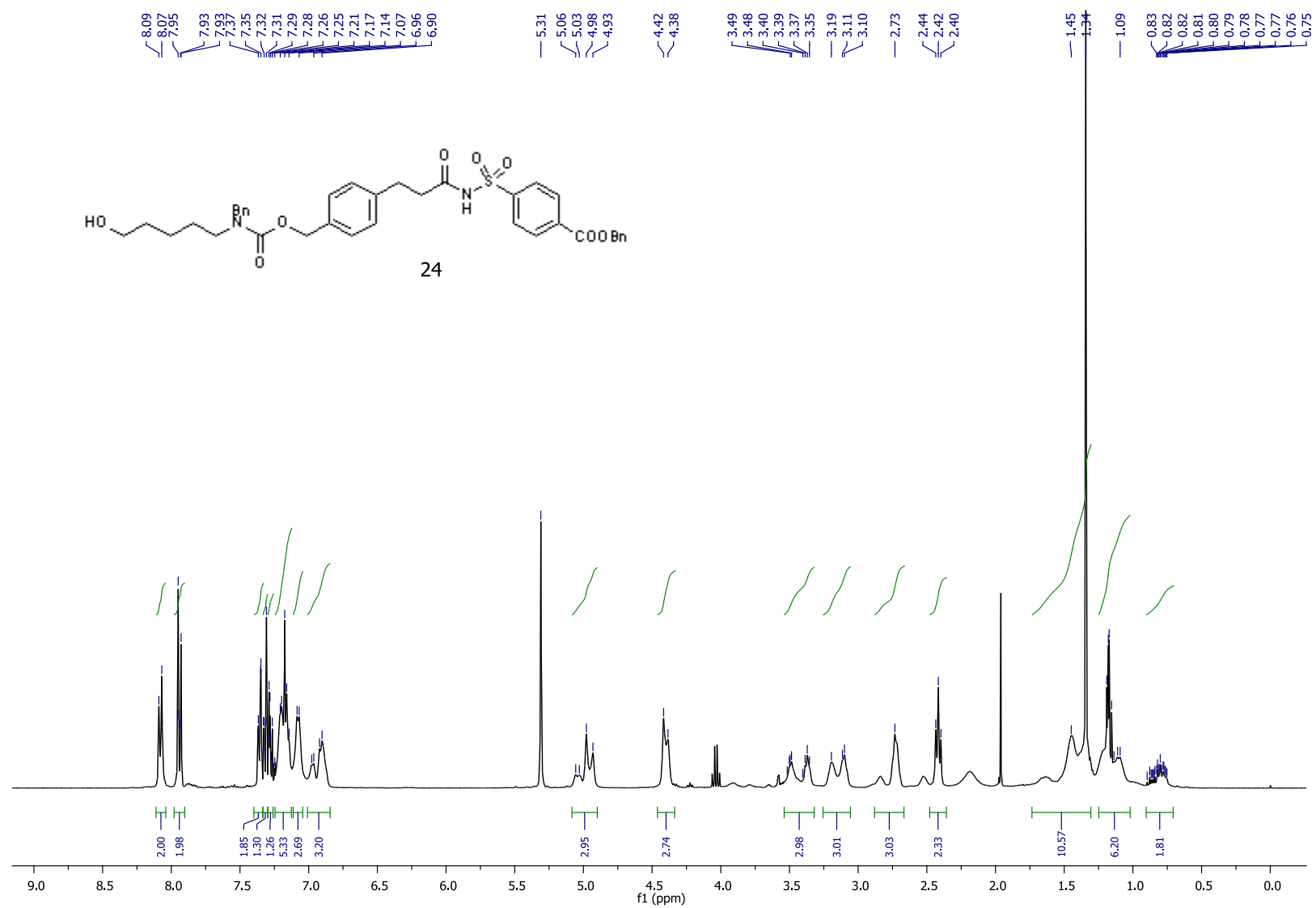


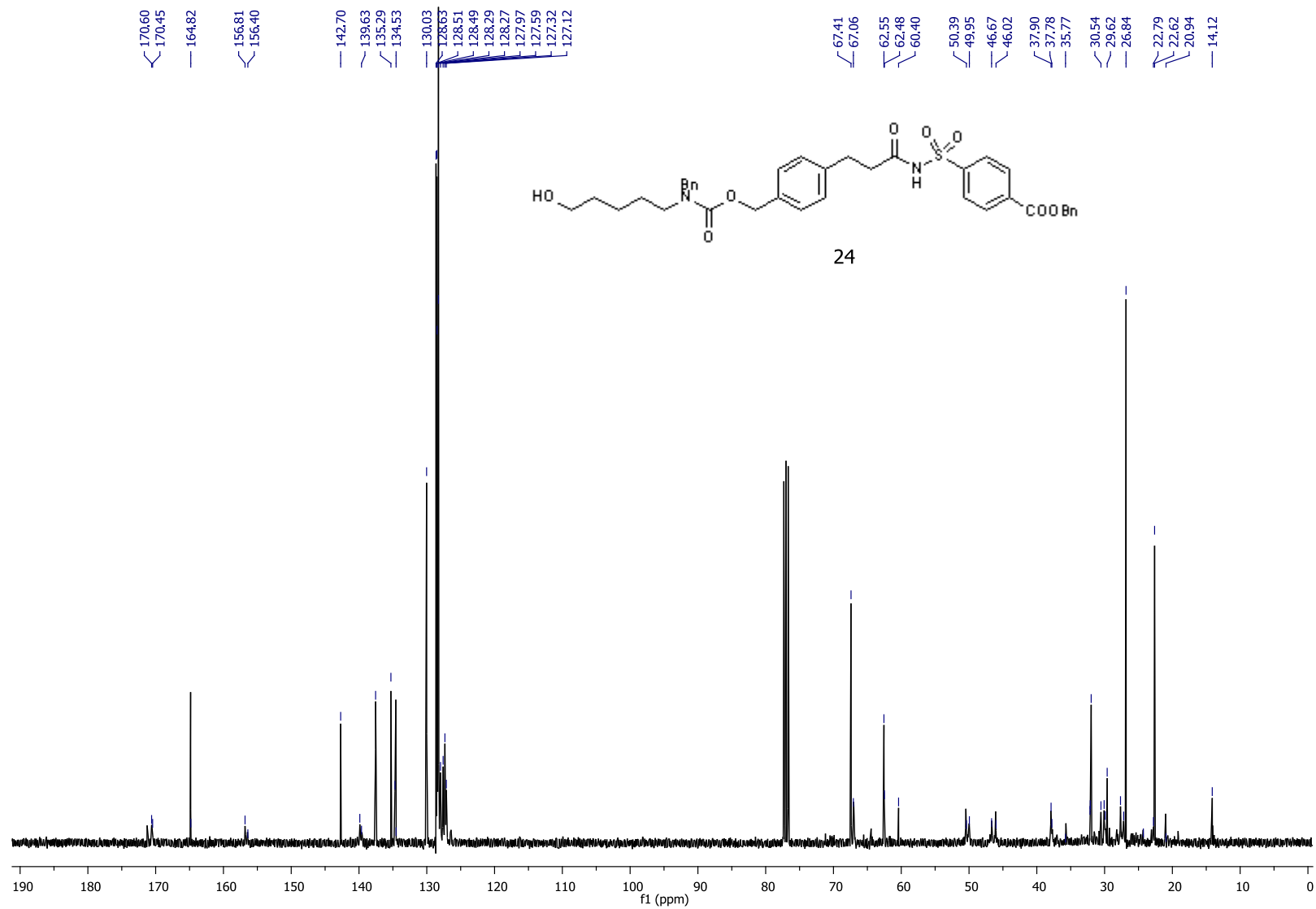


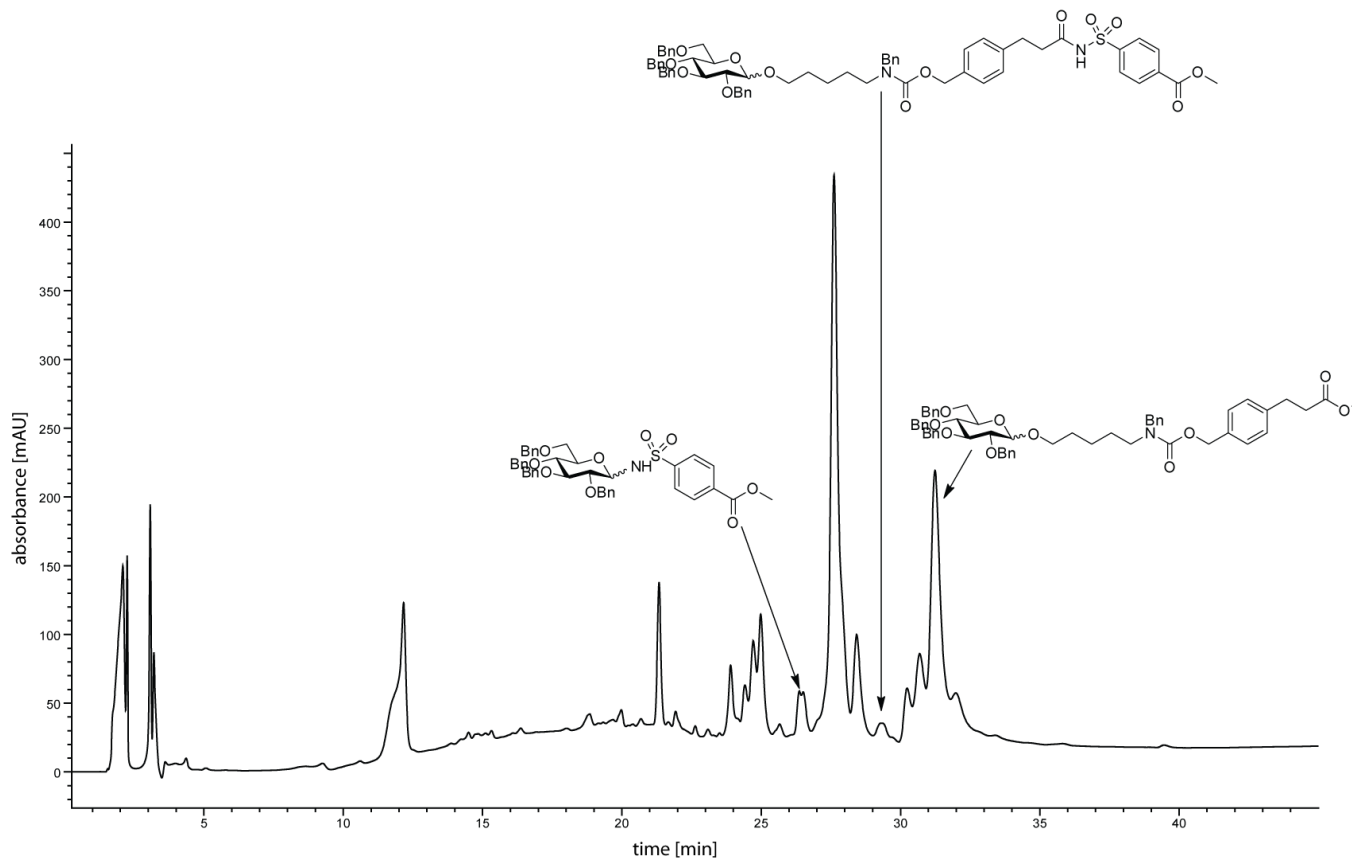




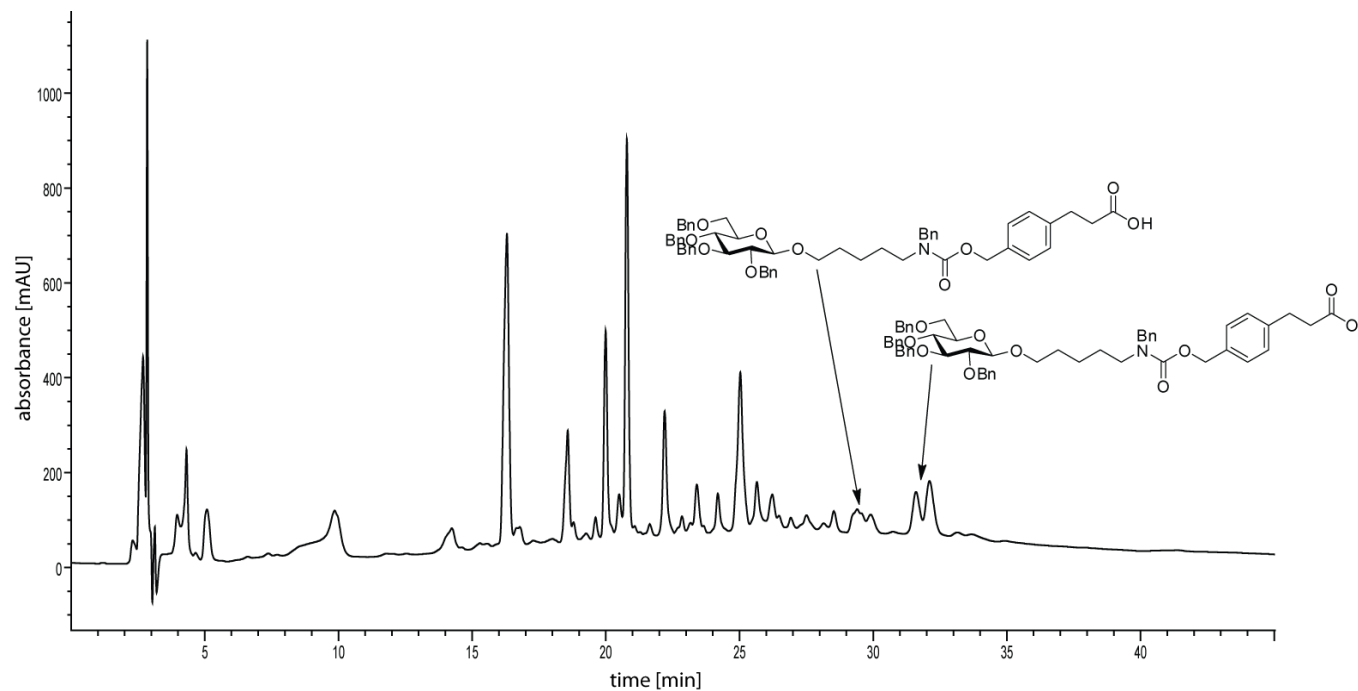




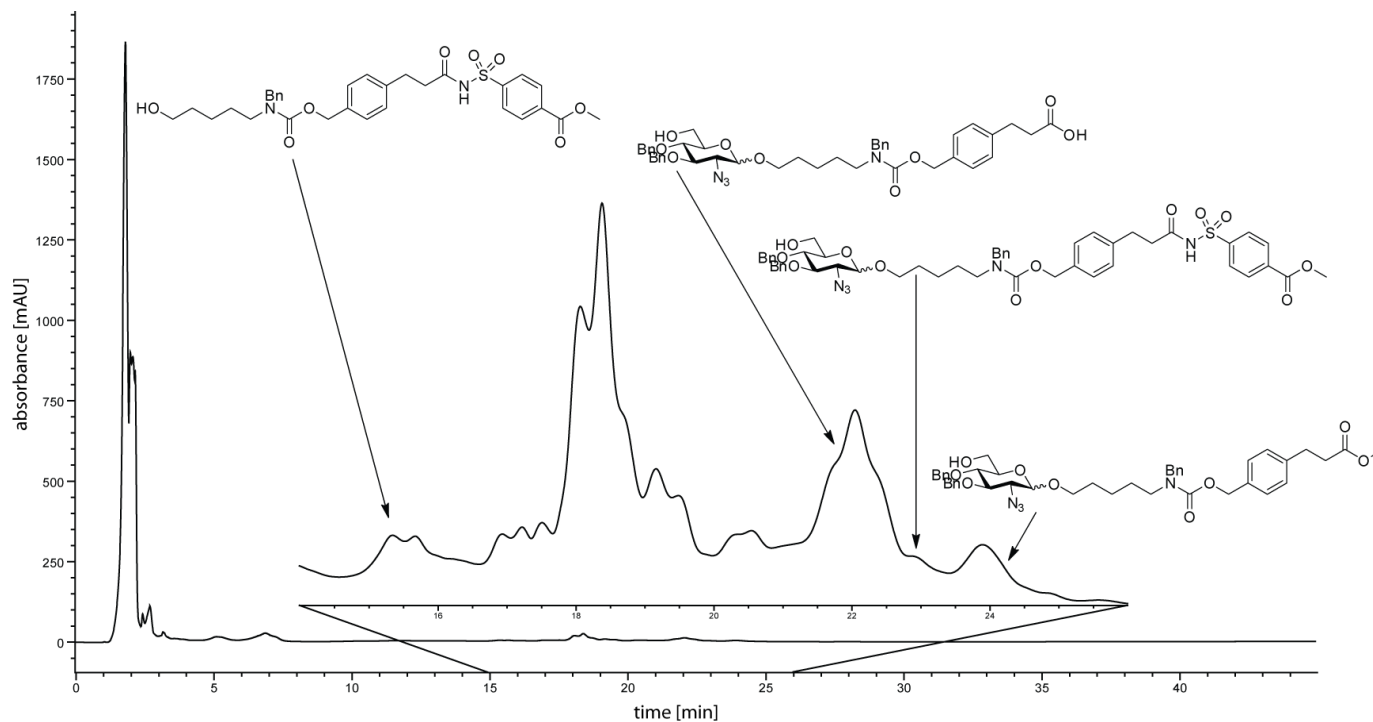




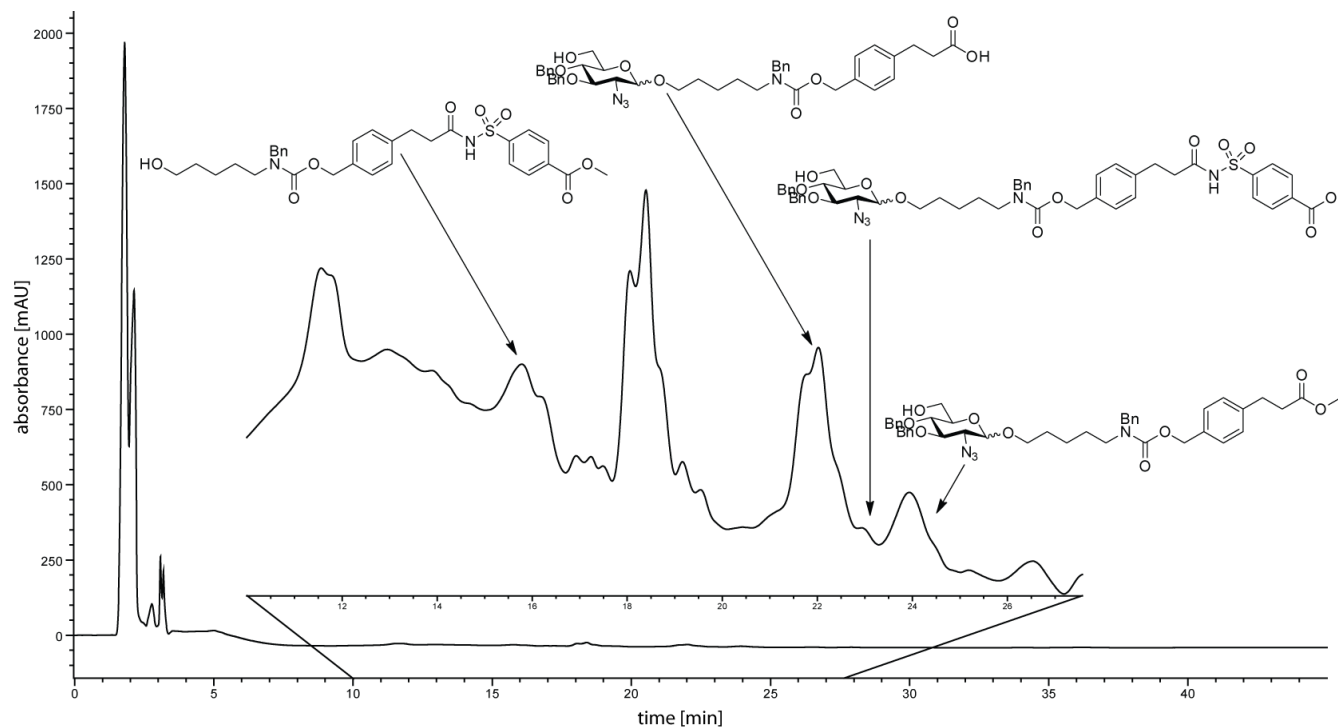
LC–MS analysis: Glycosylation was performed with **19** (3×3 equiv) and **11** under NIS/TfOH activation and previous washing step with activator solution followed by Zemplén-cleavage reaction; conditions for RP chromatography: column: Nucleosil C4 (120 mm \times 5 mm); detection: UV detection at 230 nm; eluents: water (+ 0.1% formic acid) and acetonitrile (+ 0.1% formic acid); gradient: isocratic at 10% for 5 min \rightarrow 10% \rightarrow 80% acetonitrile in 30 min \rightarrow 95% acetonitrile in 10 min.



LC–MS analysis: Glycosylation was performed with **19** (3×3 equiv) and **12** under NIS/TfOH activation and previous washing step with activator solution followed by Zemplén-cleavage reaction; conditions for RP chromatography: column: Nucleosil C4 (120 mm \times 5 mm); detection: UV detection at 210 nm; eluents: water (+ 0.1% formic acid) and acetonitrile (+ 0.1% formic acid); gradient: isocratic at 10% for 5 min \rightarrow 10% \rightarrow 80% acetonitrile in 30 min \rightarrow 95% acetonitrile in 10 min.

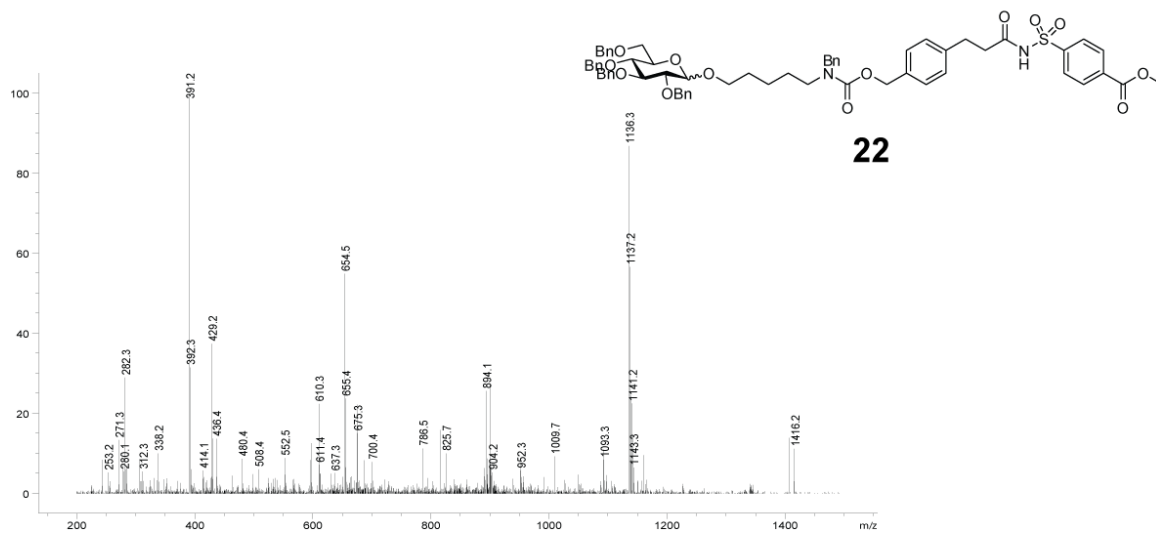
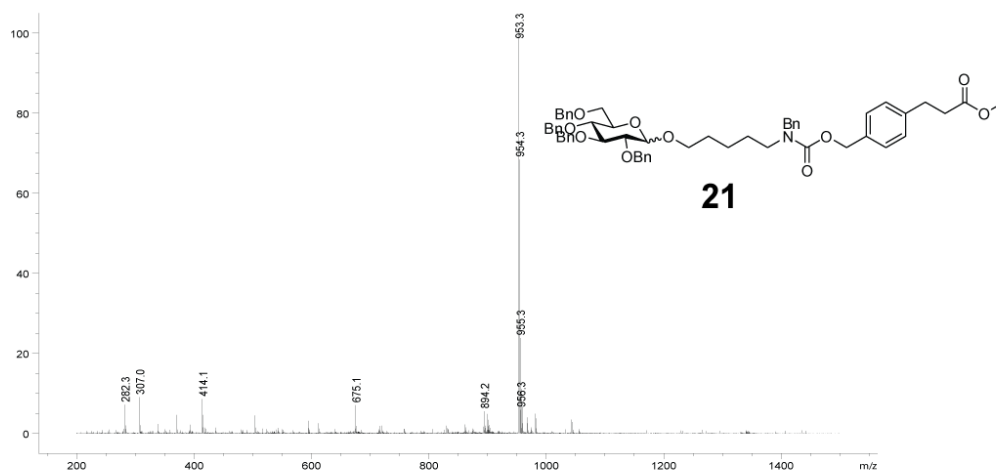
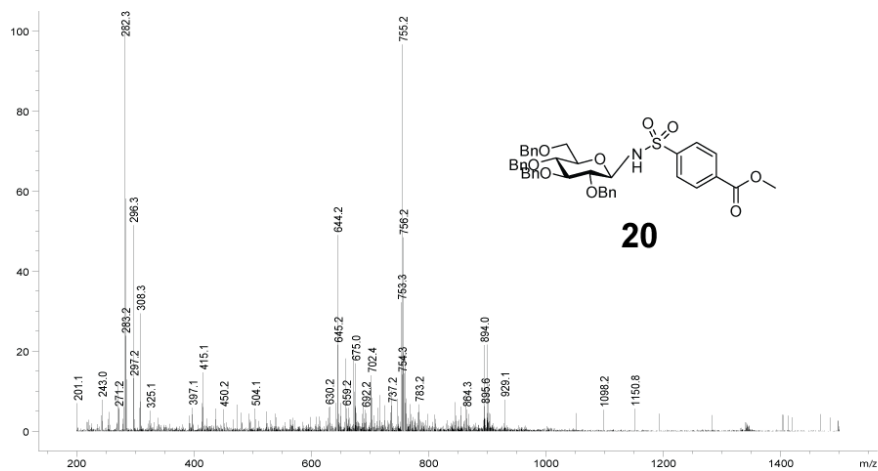


LC–MS analysis: Glycosylation was performed with **17** (3×1.1 equiv) and **11** under NIS/TfOH activation without previous washing step with activator solution followed by Zemplén-cleavage reaction; conditions for RP chromatography: column: Nucleosil C4 (120 mm \times 5 mm); detection: UV detection at 230 nm; eluents: water (+ 0.1% formic acid) and acetonitrile (+ 0.1% formic acid); gradient: isocratic at 10% for 5 min \rightarrow 10% \rightarrow 80% acetonitrile in 30 min \rightarrow 95% acetonitrile in 10 min.



LC–MS analysis: Glycosylation was performed with **17** (3×1.1 equiv) and **11** under NIS/TfOH activation and previous washing step with activator solution followed by Zemplén-cleavage reaction; conditions for RP chromatography: column: Nucleosil C4 (120 mm \times 5 mm); detection: UV detection at 230 nm; eluents: water (+ 0.1% formic acid) and acetonitrile (+ 0.1% formic acid); gradient: isocratic at 10% for 5 min \rightarrow 10% \rightarrow 80% acetonitrile in 30 min \rightarrow 95% acetonitrile in 10 min.

The peaks in the chromatograms are polystyrene (Merrifield resin)-derived compounds with strong UV absorbance. See also: K. Tanaka, Y. Fujii, H. Tokimoto, Y. Mori, S.-i. Tanaka, G.-m. Bao, E. R. O. Siwu, A. Nakayabu and K. Fukase, *Chem. Asian J.*, 2009, **4**, 574. and the related supporting information.



MS spectra: compounds 20–22 (taken from RPHPLC–MS analysis).