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Synthesis of blood group Forssman pentasaccharide GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ–R

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General methods

The reactions were carried out with the use of commercial reagents (Acros Organics, Belgium and Sigma-Aldrich, Germany). Anhydrous solvents were purified according to the standard procedures. Column chromatography was performed on Silica gel 60, 0.040–0.063 mm (E. Merck, Germany), gel filtration was carried out on Sephadex LH-20 (GE Healthcare, Sweden) column, elution with $CHCl_3$–MeOH (1 : 1) unless otherwise specified. Solvents were removed *in vacuo* at 30–40 ºC. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminum plates (E. Merck, Germany). Spots of compounds were visualized by dipping a TLC plate into 8% aq. H_3PO_4 and subsequent heating at >150 ºC. The Zemplen deacetylation was carried out in anhydrous MeOH by the addition of a catalytic amount of 2 M MeONa in MeOH. Na^+ ions were then removed with Dowex 50WX4 200–400 mesh (Acros Organics, USA) H^+ ion exchange resin and then the solution was concentrated *in vacuo*. Hydrogenolysis was carried out on 10% Pd/C (E. Merck, Germany) in hydrogen atmosphere. ^{1}H NMR spectra were recorded using a Bruker BioSpin GmbH 700 MHz spectrometer at 30 ºC unless otherwise specified; chemical shifts δ were referred to the peak of internal D_2O (δ 4.75), $CDCl_3$ (δ 7.27) or CD_3OD (δ 3.50); coupling constants *J* were measured in Hertz. The signals in ^{1}H NNR spectra were assigned to the corresponding protons using 2D ^{1}H–^{1}H COSY experiments. ^{13}C NMR spectra were recorded at 150 MHz. The values of optical rotation were measured on a PerkinElmer 341 digital polarimeter at 20 °C. α-D-Galactosyl bromide **1** was prepared from thioglycoside **2** according the method reported.[1] Trichloroacetimidates **3** and **4** were obtained following the known methodology.[2] Synthesis of 2-azidoethyl 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **5** Scheme 1 was described.[3]

2-Azidoethyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 6. A solution of 2-azidoethyl 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **5** (2.23 g, 4.47 mmol) in anhydrous DMF (50 ml) was cooled to 0 °C with stirring, then NaH (80% suspension in mineral oil, 804 mg, 26.8 mmol) was added portionwise,

the reaction mixture was stirred at 0 °C until gas emission was over and then benzyl bromide (3.18 ml, 26.8 mmol) was added dropwise. The mixture was stirred at rt for 12 h, quenched with methanol (5 ml) and H_2O (10 ml), diluted with $CHCl_3$ (200 ml) and washed with H_2O (3×200 ml). The organic layer was dried by filtration through cotton wool, concentrated and co-evaporated with *o*-xylene (3×100 ml). The residue was subjected to flash chromatography on silica gel with PhMe–EtOAc (15 : 1) to give the crude product (3.68 g). R_f 0.46 in *n*-C_6H_{14}–EtOAc (5 : 3). The product of benzylation was dissolved in anhydrous THF (50 ml), molecular sieves MS 4Å (~6 g) were added and the mixture was stirred at rt for 30 min. Then $NaBH_3CN$ (2.19 g, 34.8 mmol) was added, the mixture was stirred for 30 min, CH_3SO_3H (2.51 ml, 38.7 mmol) in anhydrous THF (18.8 ml) was added dropwise within 15 min at pH 2.5–3 and the mixture was stirred at rt for 1 h. Then the mixture was cooled to 0 °C, saturated aq. $NaHCO_3$ (250 ml) was added and the resulting mixture was stirred for 15 min. The solids were filtered off, the filtrate was extracted with $CHCl_3$ (3×100 ml), and the organic layer was washed with saturated aqueous $NaHCO_3$ (2×200 ml) and H_2O (200 ml), dried by filtration through cotton wool and concentrated *in vacuo*. Column chromatography on silica gel with PhMe–EtOAc (9 : 1) and subsequent crystallization from EtOAc–*n*-C_6H_{14} resulted in derivative **6** (2.51 g, 2.63 mmol, 59% for 2 steps). R_f 0.73 in *n*-C_6H_{14}–EtOAc (5 : 3), 0.38 in PhMe–EtOAc (6 : 1). ^{1}H NMR ($CDCl_3$) δ: 2.38 (d, 1H, $J_{4,OH}$ 2.7, OH-4^{II}), 3.34 (ddd ≈ br.dd, 1H, $J_{5,6a}$ 5.5, $J_{5,6b}$ 7.1, $J_{4,5}$ < 1, H-5^{II}), 3.38 (dd, 1H, $J_{2,3}$ 9.4, $J_{3,4}$ 3.2, H-3^{II}), 3.37–3.41 (m, 1H, H-5^{I}), 3.41–3.45 (m, 1H, C*H*N), 3.44 (dd, 1H, $J_{2,3}$ 9.1, $J_{1,2}$ 7.7, H-2^{I}), 3.48 (dd, 1H, $J_{6a,6b}$ 9.7, $J_{5,6a}$ 5.5, H-6a^{II}), 3.47–3.52 (m, 1H, C*H*N), 3.58 (dd ≈ t, 1H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.1, H-3^{I}), 3.60 (dd, 1H, $J_{2,3}$ 9.4, $J_{1,2}$ 7.8, H-2^{II}), 3.66 (dd, 1H, $J_{6a,6b}$ 9.7, $J_{5,6b}$ 7.1, H-6b^{II}), 3.68–3.73 (m, 1H, C*H*O), 3.71 (dd, 1H, $J_{6a,6b}$ 10.9, $J_{5,6a}$ 2.4, H-6a^{I}), 3.80 (dd, 1H, $J_{6a,6b}$ 10.9, $J_{5,6b}$ 4.5, H-6b^{I}), 3.97 (dd, 1H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.1, H-4^{I}), 4.01–4.05 (m, 2H, C*H*O, H-4^{II}), 4.40 (d, 1H, *J* 12.1, C*H*Ph), 4.40 (d, 1H, *J* 12.0, C*H*Ph), 4.41 (d, 1H, $J_{1,2}$ 7.7, H-1^{I}), 4.44 (d, 1H, $J_{1,2}$ 7.8, H-1^{II}), 4.46 (d, 1H, *J* 12.0, C*H*Ph), 4.55 (d, 1H, *J* 12.1, C*H*Ph), 4.67 (d, 1H, *J* 11.7, C*H*Ph), 4.73 (d, 2H, *J* 11.4, C*H*Ph), 4.74 (d, 1H, *J* 10.7, C*H*Ph), 4.76 (d, 1H, *J* 11.2, C*H*Ph), 4.77 (d, 1H, *J* 10.5, C*H*Ph), 4.79 (d, 1H, *J* 11.3, C*H*Ph), 4.90 (d, 1H, *J* 11.0, C*H*Ph), 4.99 (d, 1H, *J* 10.8, C*H*Ph), 7.18–7.41 (m, 30H, 6 Ph).

2-Azidoethyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 7. A mixture of lactose derivative **6** (2.0 g, 2.1 mmol), 1,1,3,3-tetramethylurea (TMU) (1.29 ml, 10.79 mmol), molecular sieves MS 4Å (~5 g) and CH_2Cl_2 (100 ml) was stirred at rt for 30 min. Then $AgOSO_2CF_3$ (1.38 g, 5.39 mmol), MS 4Å (~1 g) and freshly prepared galactopyranosyl bromide **1** (2.6 g, 5.14 mmol) in CH_2Cl_2 were added. The reaction mixture was stirred overnight in the

darkness, filtered and concentrated *in vacuo*. Column chromatography on silica gel with *n*-C_6H_{14}–EtOAc (4 : 1) gave trisaccharide **7** (2.7 g, 93%). R_f 0.40 in *n*-C_6H_{14}–EtOAc (2 : 1); $[\alpha]_D^{20}$ +32 (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz, characteristic signals) δ: 1.88, 1.94 [2 s, 2×3H, OC(O)CH_3], 3.19 (dd, 1H, $J_{2,3}$ 9.1, $J_{1,2}$ 7.7, H-2^I), 3.61 (dd ≈ t, 1H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.1, H-3^I), 3.89 (dd, 1H, $J_{2,1}$ 3.4, $J_{2,3}$ 10.7, H-2^{III}), 3.94 (dd, 1H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.1, H-4^I), 4.43 (d, 1H, $J_{1,2}$ 7.7, H-1^I), 4.48 (d, 1H, $J_{1,2}$ 7.6, H-1^{II}), 5.15 (d, 1H, $J_{1,2}$ 3.4, H-1^{III}), 5.39 (dd, 1H, $J_{4,3}$ 3.3, $J_{2,3}$ 10.7, H-3^{III}), 5.56 (dd, 1H, $J_{4,3}$ 3.3, $J_{4,5}$ 1.3, H-4^{III}), 7.15–7.35 (m, 40H, 8 Ph).

2-Trifluoroacetamidoethyl 2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 8. Trisaccharide **7** (1.0 g, 0.77 mmol) was deacetylated according to Zemplen in anhydrous MeOH (50 ml) and 2 M methanolic NaOMe (0.5 ml) at rt for 2 h, Na^+ ions were removed with Dowex H^+ ion exchange resin and the solution was concentrated *in vacuo*. The residue was dissolved in a mixture of DMF (20 ml) and H_2O (5 ml). Dithiothreitol (DTT) (595 mg, 3.85 mmol) and Et_3N (107 µl, 0.77 mmol) were then added. The reaction mixture was stirred for 2 h and concentrated. The crude product was dissolved in anhydrous MeOH (20 ml), methyl trifluoroacetate (490 µl, 3.85 mmol) and Et_3N (107 µl, 0.77 mmol) were added, the mixture was kept at rt for 3 h and concentrated *in vacuo*. Column chromatography on silica gel with PhMe–EtOAc (3 : 1) gave compound **8** (750 mg, 71%). R_f 0.35 in PhMe–EtOAc (3 : 1). ^{1}H NMR ($CDCl_3$, characteristic signals) δ: 3.47–3.57 (m, 2H, NCH_2, H-6a^{II}), 3.82–3.90 (m, 2H, OCH_2), 4.35 (d, 1H, $J_{1,2}$ 7.7, H-1^I), 4.41 (d, 1H, $J_{1,2}$ 7.6, H-1^{II}), 5.14 (d, 1H, $J_{1,2}$ 3.2, H-1^{III}), 7.15–7.35 [m, 41H, 8 Ph, N*H*C(O)CF_3].

2-Trifluoroacetamidoethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 9. To a solution of compound **8** (750 mg, 0.55 mmol) in anhydrous CH_2Cl_2 (40 ml), triethyl orthoacetate (0.20 ml, 0.69 mmol) and catalytic amount of TsOH were added. The reaction mixture was stirred at rt for 2 h and 80% aq. AcOH (0.26 ml) was then added. The mixture was kept for 30 min, quenched with pyridine (1.2 ml), concentrated and co-evaporated with PhMe (4×20 ml). The residue was purified by column chromatography on silica gel in *n*-C_6H_{14}–EtOAc–pyridine (66 : 33 : 1) to give compound **9** (617 mg, 0.44 mmol, 80%). R_f 0.50 in n-C_6H_{14}–EtOAc (1 : 1). ^{1}H NMR ($CDCl_3$, characteristic signals) δ: 1.97 [s, 3H, OC(O)CH_3], 3.47–3.55 (m, 2H, NCH_2, H-6a^{II}), 3.82–3.90 (m, 2H, OCH_2), 4.34 (d, 1H, $J_{1,2}$ 7.7, H-1^I), 4.40 (d, 1H, $J_{1,2}$ 7.6, H-1^{II}), 4.43 (m, 1H, H-3^{III}), 5.11 (d, 1H, $J_{1,2}$ 3.2, H-1^{III}), 5.38 (dd, 1H, $J_{4,3}$ 3.8, $J_{4,5}$ 1.5, H-4^{III}), 7.17–7.38 [m, 41H, 8 Ph, N*H*C(O)CF_3].

2-Trifluoroacetamidoethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→3)-4-*O*-acethyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 10. A mixture of compound **9** (273 mg, 0.194 mmol), anhydrous CH_2Cl_2 (10 ml) and molecular sieves 4Å (500 mg) was stirred for 1 h. Trimethylsilyl trifluoromethanesulfonate (TMSOTf) (43 μl of 10% solution in anhydrous CH_2Cl_2) was then added. A solution of trichloroacetimidate **3** (152 mg, 0.242 mmol) in anhydrous CH_2Cl_2 (5 ml) was added dropwise, the reaction mixture was stirred overnight, filtered, diluted with $CHCl_3$ and washed with saturated aq. $NaHCO_3$ and brine. The organic extract was dried by filtration through cotton wool and concentrated. Column chromatography on silica gel with PhMe–EtOAc (3 : 1) yielded compound **10** (258 mg, 71%). R_f 0.60 in PhMe–EtOAc (5 : 2). ^{1}H NMR ($CDCl_3$, selected signals) δ: 1.93, 1.97, 2.02, 2.12 [4 s, 4×3H, 4 OC(O)CH_3], 4.06 (d, 1H, $J_{1,2}$ 8.3, H-1^{IV}), 5.05 (d, 1H, $J_{1,2}$ 3.3, H-1^{III}), 5.09 (dd ≈ d, 1H, $J_{4,3}$ 2.5, H-4^{IV}), 5.51 (dd ≈ d, 1H, $J_{4,3}$ 3.3, H-4^{III}), 7.12–7.39 [m, 41H, 8 Ph, N*H*C(O)CF_3].

2-Trifluoroacetamidoethyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 11. Tetrasaccharide **10** (180 mg, 0.096 mmol) was dissolved in THF (20 ml) and then a solution of tetra-*n*-butylammonium fluoride hydrate (TBAF) (107 mg, 0.385 mmol) in THF (1 ml) was added. The reaction mixture was stirred at rt for 3 h, diluted with EtOAc, washed with water and brine and then concentrated. The residue was treated with Et_3N (0.5 ml) and Ac_2O (0.5 ml) and kept at rt for 2 h. The mixture was then concentrated and co-evaporated with PhMe. The crude product was subjected to column chromatography on silica gel with PhMe–EtOAc (1 : 1) to give compound **11** (151 mg, 91%). R_f 0.40 in PhMe–EtOAc (1 : 2). ^{1}H NMR ($CDCl_3$) δ: 1.48, 1.96, 2.00, 2.04, 2.14 [5 s, 5×3H, 4 OC(O)CH_3, NHC(O)CH_3], 3.20–3.23 (m, 2H, H-6a^{III}, H-6b^{III}), 3.28–3.32 (m, 2H, H-3^{II}, H-5^{II}), 3.33–3.37 (m, 2H, H-2^{I}, H-5^{I}), 3.37–3.40 (m, 1H, H-5^{IV}), 3.43–3.55 (m, 4H, NCH_2, H-4^{I}, H4^{II}), 3.62 (dd, 1H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.9, H-2^{II}), 3.68 (dd, 1H, $J_{5,6a}$ 2.0, $J_{6a,6b}$ 10.5, H-6a^{I}), 3.75 (dd, 1H, $J_{5,6b}$ 4.9, $J_{6a,6b}$ 10.5, H-6b^{I}), 3.79–3.86 (m, 2H, OCH_2), 3.87 (dd, 1H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.3, H-2^{III}), 3.94 (t, 1H, $J_{4,3}$ $J_{4,5}$ 9.3, H-3^{I}), 3.99 (dd, 1H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.4, H-3^{III}), 4.02–4.06 (m, 2H, 2×H-6), 4.07–4.17 (m, 4H, H-1^{IV}, H-2^{IV}, 2×H-6), 4.17–4.20 (m ≈ br.s, 2H, CH_2Ph), 4.24, 4.29 (2 d, 2×1H, *J* 11.9, CH_2Ph), 4.26, 4.47 (2 d, 2×1H, *J* 11.9, CH_2Ph), 4.30 (d, 1H, $J_{1,2}$ 8.0, H-1^{I}), 4.38–4.41 (m, 2H, H-1^{II}, H-5^{III}), 4.52, 4.76 (d, 2×1H, *J* 12.5, CH_2Ph), 4.56 (dd, $J_{3,2}$ 11.0, $J_{3,4}$ 3.4, H-3^{IV}), 4.59–4.65 (m, 3H, CH_2Ph, N*H*Ac), 4.64, 4.70 (2 d, 2×1H, *J* 11.1, CH_2Ph), 4.73, 4.87 (2 d, 2×1H, *J* 11.3, CH_2Ph), 4.80, 5.04 (2 d, 2×1H, *J* 11.5, CH_2Ph), 5.04 (d, 1H, $J_{1,2}$ 3.5, H-1^{III}),

5.14 (dd ≈ d, 1H, $J_{4,3}$ 3.0, H-4^{IV}), 5.53 (dd ≈ d, 1H, $J_{4,3}$ 3.5, H-4^{III}), 7.12–7.39 [m, 41H, 8 Ph, N*H*C(O)CF_3]. ^{13}C NMR ($CDCl_3$, 30 ºC) δ: 100.19 (C-1^{III}), 102.35 (C-1^{IV}), 102.72 (C-1^{II}), 103.64 (C-1^{I}).

2-Trifluoroacetamidoethyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 12. Tetrasaccharide **11** (151 mg, 0.087 mmol) was deacethylated according to Zemplen in anhydrous MeOH (6 ml) and 2 M methanolic NaOMe (40 µl) at rt for 2 h. Na^+ ions were removed with Dowex H^+ ion exchange resin and the solution was concentrated *in vacuo*. The residue (130 mg, 0.080 mmol) was dissolved in anhydrous CH_3CN (5 ml) and then benzaldehyde dimethyl acetal (20 µl, 0.13 mmol) and catalytic amount of TsOH were added. The reaction mixture was stirred for 4 h, quenched with pyridine (20 µl) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with *i*-PrOH–$CHCl_3$ (4 : 96) to yield compound **12** (87 mg, 64%). R_f 0.35 in *i*-PrOH–$CHCl_3$ (4 : 96). ^{1}H NMR [$CDCl_3$–CD_3OD (1:1), characteristic signals) δ: 1.64, 2.02, [2 s, 2×3H, OC(O)C*H*$_3$, NHC(O)C*H*$_3$], 5.07 (d, 1H, $J_{1,2}$ 3.4, H-1^{III}), 5.50 (dd ≈ d, 1H, $J_{4,3}$ 3.2, H-4^{III}), 5.51 [s, 1H, C*H* (Bd)], 7.14–7.40 [m, 46H, 9 Ph, N*H*C(O)CF_3].

2-Trifluoroacetamidoethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 13. A solution of acceptor **12** (34.3 mg, 0.02 mmol) in anhydrous Et_2O (3 ml) was stirred over molecular sieves 3Å (100 mg) at rt for 1 h. The solution was then cooled to –18 ºC and treated with TMSOTf (54 µl of 10% solution in anhydrous CH_2Cl_2), followed by addition of trichloacetimidate **4** (14.4 mg, 0.03 mmol) in anhydrous Et_2O (1 ml). The reaction mixture was stirred at –18 ºC for 20 min, allowed to warm to 4 ºC and stirred at 4 ºC overnight. Then the reaction mixture was quenched with pyridine (10 µl), diluted with $CHCl_3$ and filtered. The filtrate was concentrated and the residue was subjected to gel chromatography with $CHCl_3$–MeOH (1: 1) to give the fraction containing the target pentasaccharide. The crude product without purification was debenzylidenated with 80% aq. AcOH (20 ml) at 80°C for 2 h and acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) for 15 h. Column chromatography on silica gel with *n*-C_6H_{14}–$CHCl_3$–*i*-PrOH (7 : 2 : 1) gave compound **13** (8.4 mg, 21% for 3 steps). R_f 0.55 in *n*-C_6H_{14}–$CHCl_3$–*i*-PrOH (7 : 2 : 1). ^{1}H NMR [$CDCl_3$–CD_3OD (1:1), characteristic signals] δ: 1.62, 2.06, 1.99, 2.03, 2.05, 2.15, 2.17 [7 s, 7×3H, 6 OC(O)C*H*$_3$, NHC(O)C*H*$_3$], 4.34 (d, 1H, $J_{1,2}$ 7.8, H-1^{I}), 4.41 (d, 1H, $J_{1,2}$ 7.7, H-1^{II}), 5.11 (d, 1H,

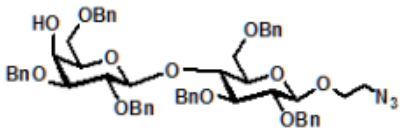
$J_{1,2}$ 3.4, H-1^{III}), 5.18 (dd, 1H, $J_{3,2}$ 11.2, $J_{3,4}$ 3.2, H-3^{V}), 5.19(d, 1H, $J_{1,2}$ 3.5, H-1^{V}), 5.37 (dd ≈ d, 1H, $J_{4,3}$ 3.2, H-4^{IV}), 5.38 (dd ≈ d, 1H, $J_{4,3}$ 3.2, H-4^{V}), 5.41 (d, 1H, $J_{NH,2}$ 7.7, N*H*Ac), 5.44 (dd ≈ d, 1H, $J_{4,3}$ 3.0, H-4^{III}), 7.12–7.38 [m, 41H, 8 Ph, N*H*C(O)CF_3].

2-Trifluoroacetamidoethyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 14. DTT (3.3 mg, 0.0200 mmol) and Et_3N (10 μl) were added to a solution of pentasaccaride **13** (8.4 mg, 0.0042 mmol) in DMF (160 μl) and H_2O (40 μl). The reaction mixture was kept for 1 h and concentrated. Anhydrous MeOH (1 ml), Ac_2O (50 μl), and Et_3N (5 μl) were then added. The mixture was kept at rt overnight, concentrated *in vacuo* and subjected to column chromatography on silica gel with *n*-C_6H_{14}–$CHCl_3$–*i*-PrOH (7 : 2 : 1) to give crude product (6.5 mg) with R_f 0.30 in *n*-C_6H_{14}–$CHCl_3$–*i*-PrOH (7 : 2 : 1), which was deacetylated according to Zemplen in anhydrous MeOH (2.0 ml) and 2 M methanolic NaOMe (20 μl) at rt for 2 h. Na^+ ions were removed with Dowex H^+ ion exchange resin and the solution was concentrated. The residue was purified by gel chromatography with $CHCl_3$–MeOH (1 : 1) and subjected to hydrogenolysis for 3 h. Then the reaction mixture was filtered and concentrated. The residue was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) at rt for 12 h, concentrated and co-evaporated with PhMe (4×0.5 ml). Column chromatography on silica gel with $CHCl_3$–*i*-PrOH (9 : 1) gave compound **14** (3.1 mg, 45% for 4 steps). R_f 0.35 in $CHCl_3$–*i*-PrOH (9 : 1). ^{1}H NMR ($CDCl_3$) *δ*: 1.97, 1.99, 2.02, 2.04, 2.05, 2.06, 2.07, 2.08, 2.09, 2.10, 2.11, 2.12, 2.13, 2.14, 2.18, 2.23 [16 s, 16×3H, 14 OC(O)C*H*$_3$, 2 NHC(O)C*H*$_3$], 3.05–3.15 (m, 1H, H-2^{V}), 3.50–3.61 (m, 2H, NC*H*$_2$) 3.62–3.67 (m, 1H, H-5^{I}), 3.76–3.82 (m, 2H, H-4^{I}, H-5), 3.83–3.87 (m, 2H, OC*H*$_2$) 3.95–4.03 (m, 3H, H-4^{II}, H-6a, H-6b), 4.05–4.24 (m, 8H, 2×H-5, 6×H-6), 4.27 (dd, 1-H, $J_{3,2}$ 10.7, $J_{3,4}$ 3.4, H-3^{III}), 4.37–4.44 (m, 2H, H-5, H-6), 4.50–4.60 (m, 4H, H-1^{I}, H-1^{II}, H-2^{IV}, H-6), 4.71–4.76 (m, 1H, H-3^{V}), 4.77 (dd, 1H, $J_{3,2}$ 10.9, $J_{3,4}$ 2.5, H-3^{II}), 4.88–4.97 (m, 4H, H-2^{I}, H-1^{III}, H-3^{IV}, H-1^{V}), 5.12 (dd, 1H, $J_{1,2}$ 7.7, $J_{2,3}$ 10.8, H-2^{II}), 5.2 (t, 1H, $J_{2,3}$ = $J_{3,4}$ 9.3, H-3^{I}), 5.23 (dd, 1H, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7, H-2^{III}), 5.28 (d, 1H, $J_{1,2}$ 8.2, H-1^{V}), 5.35 (m ≈ s, 2H, H-4^{IV}, H-4^{V}), 5.62 (dd ≈ d, 1H, $J_{3,4}$ 2.8, H-4^{III}), 6.48 (d, 1H, $J_{NH,2}$ 7.1, GalN*H*Acα), 6.59 (d, 1H, $J_{NH,2}$ 9.9, GalN*H*Acβ), 6.87–6.93 [m, 1H, NHC(O)CF_3]. ^{13}C NMR ($CDCl_3$, 30 ºC, characteristic signals) *δ*: 98.95 (C-1^{V}), 99.56 (C-1^{III}), 100.69 (C-1^{I}), 101.17 (C-1^{IV}), 101.25 (C-1^{II}).

2-Aminoethyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 15. Pentasaccharide **14** (3.1 mg, 0.002 mmol) was deacetylated under the

Zemplen conditions in anhydrous MeOH (1.0 ml), 2 M methanolic NaOMe (10 μl) at rt for 2 h and then the reaction mixture was concentrated. The crude product was dissolved in water (0.5 ml) and kept at rt for 2 h. Ion-exchange chromatography on Dowex H^+ ion exchange resin with 5% aq. ammonia gave compound **15** (1.64 mg, 91%). R_f 0.19 in EtOH–H_2O–pyridine–*n*-BuOH–AcOH (100 :10 : 10 : 10 : 2). ^{1}H NMR (D_2O, 45 °C, characteristic signals) δ: 1.97, 1.99 [2s, 2×3H, 2 OC(O)CH_3], 3.41 (t, 2H, NCH_2), 4.45 (d, 1H, $J_{1,2}$ 7.8, H-1^{I}), 4.47 (d, 1H, $J_{1,2}$ 7.9, H-1^{II}), 4.66 (d, 1H, $J_{1,2}$ 8.5, H-1^{IV}), 4.87 (d, 1H, $J_{1,2}$ 4.0, H-1^{III}), 5.00 (d, 1H, $J_{1,2}$ 3.8, H-1^{V}). ^{13}C NMR (D_2O, 35 °C, characteristic signals) δ: 22.04, 22.32 [2 OC(O)CH$_3$], 93.64 (C-1^{V}), 100.47 (C-1^{III}), 101.98 (C-1^{II}), 102.72 (C-1^{III}), 103.35 (C-1^{I}).

1. H. Lönn, *Carbohydr. Res.*, 1985, **139**, 105.
2. R. R. Schmidt and J. Michel, *Angew. Chem., Int. Ed. Engl*., 1980, **19**, 731.
3. M. A. Sablina, A. B. Tuzikov, T. V. Ovchinnikova, I. V. Mikhura and N. V. Bovin, *Russ. Chem. Bull., Int. Ed.*, 2015, **64**, 1125 (*Izv. Akad. Nauk, Ser. Khim*., 2015, 1125).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OBn
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BnO
OBn
O
N3

6

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1.00
1.03
1.99
2.02
1.01
1.01
0.99
1.01
3.04
2.01
1.00
0.99
2.00
1.00
2.02
2.01
4.02
1.00
1.00
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
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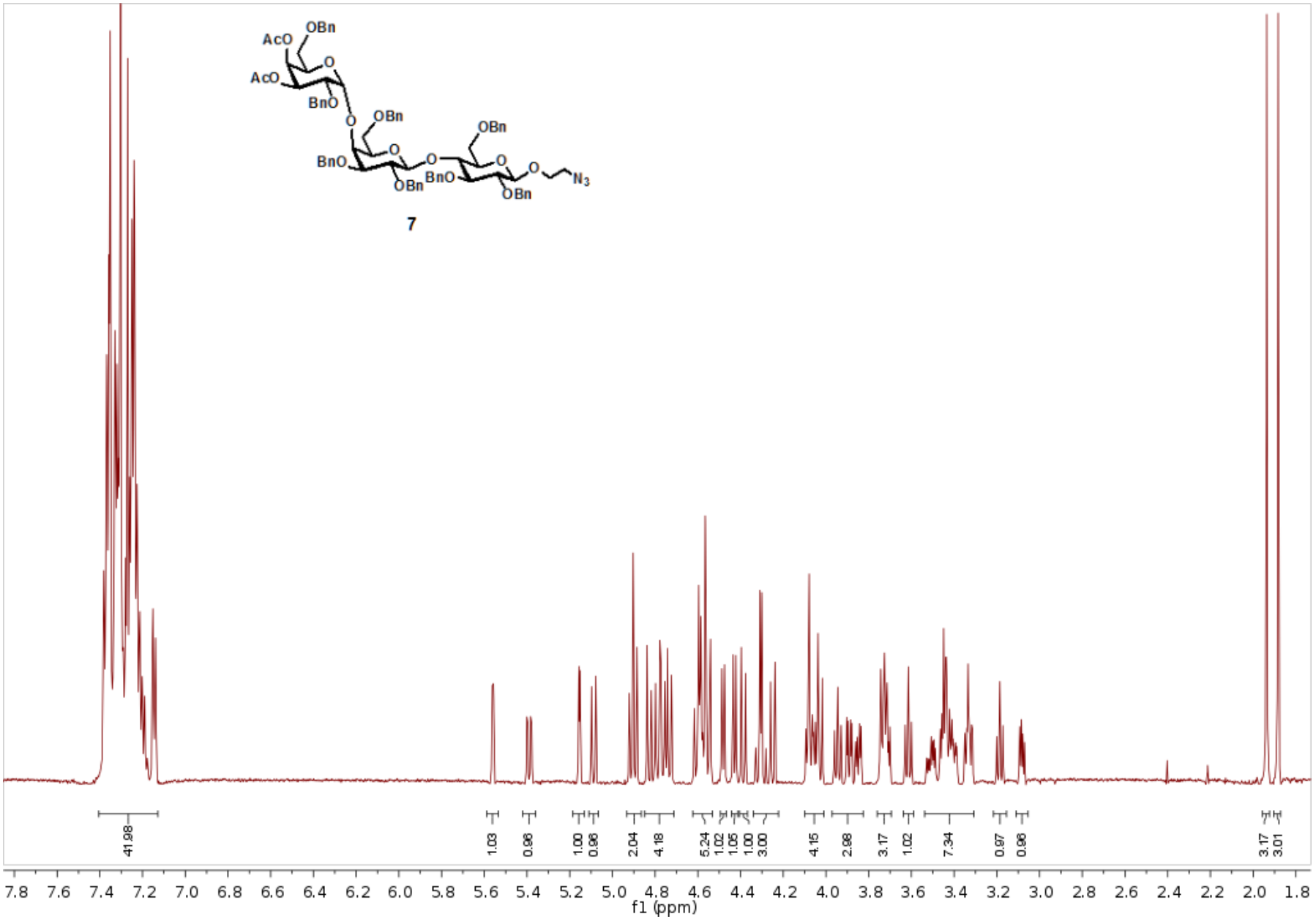
f1 (ppm)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OBn
OBn
BnO
O
O
O
N3
OBn
7
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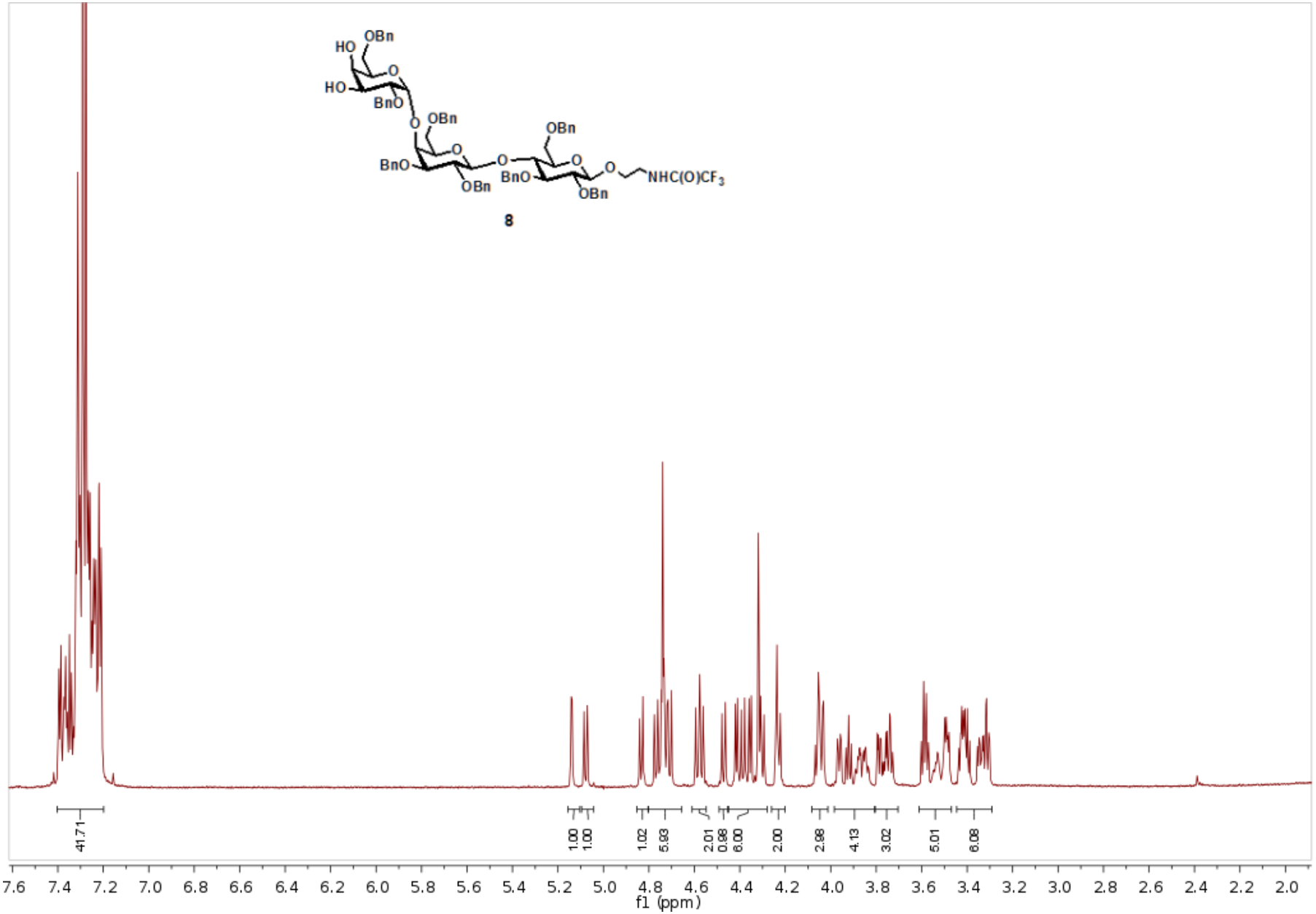
1.03
0.96
1.00
0.96
2.04
4.18
5.24
1.02
1.05
1.00
3.00
4.15
2.98
3.17
1.02
7.34
0.97
0.96
3.17
3.01
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)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OBn
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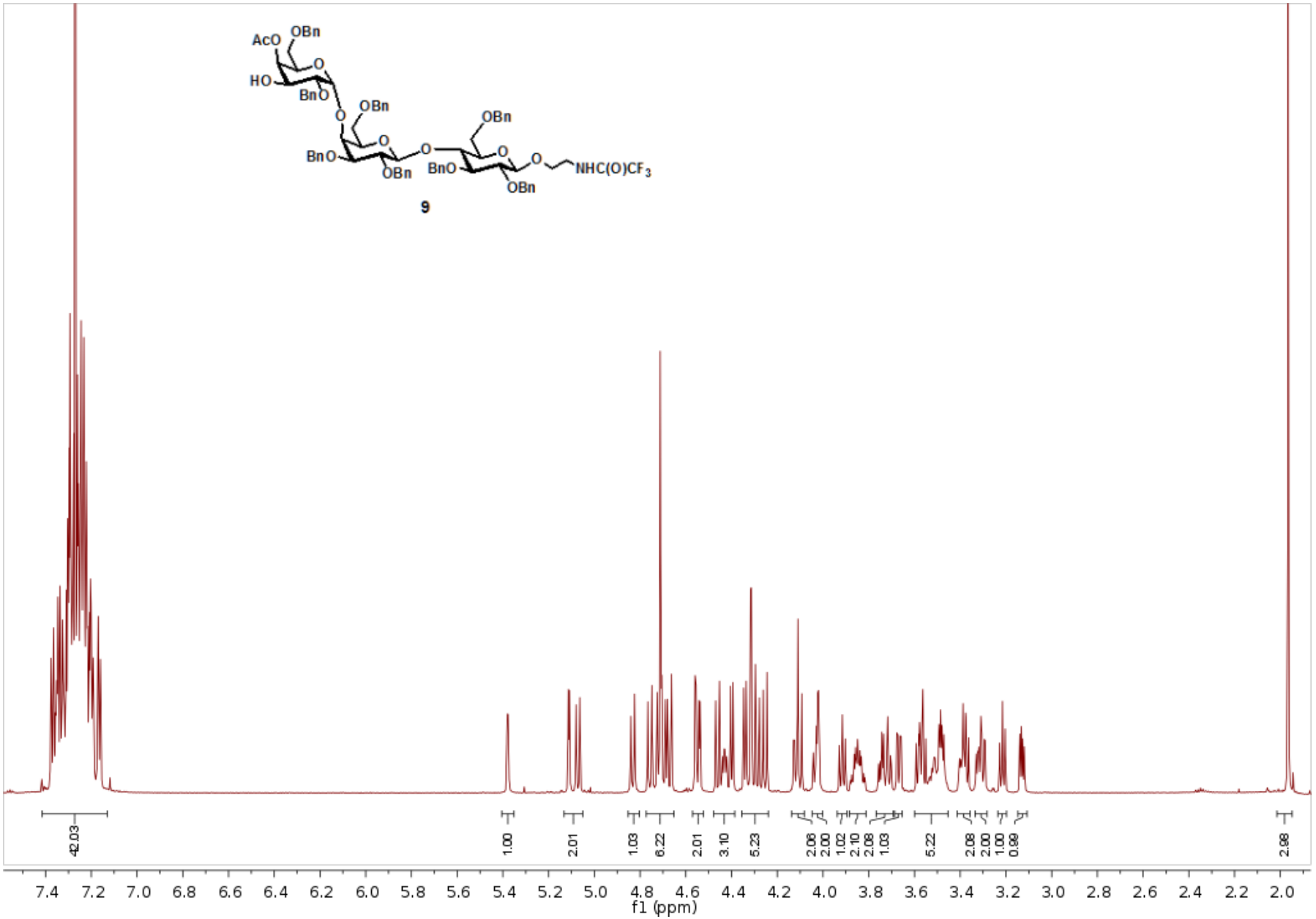
O
NHC(O)CF3
8
41.71
1.00
1.00
1.02
5.93
2.01
0.98
6.00
2.00
2.98
4.13
3.02
5.01
6.08
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)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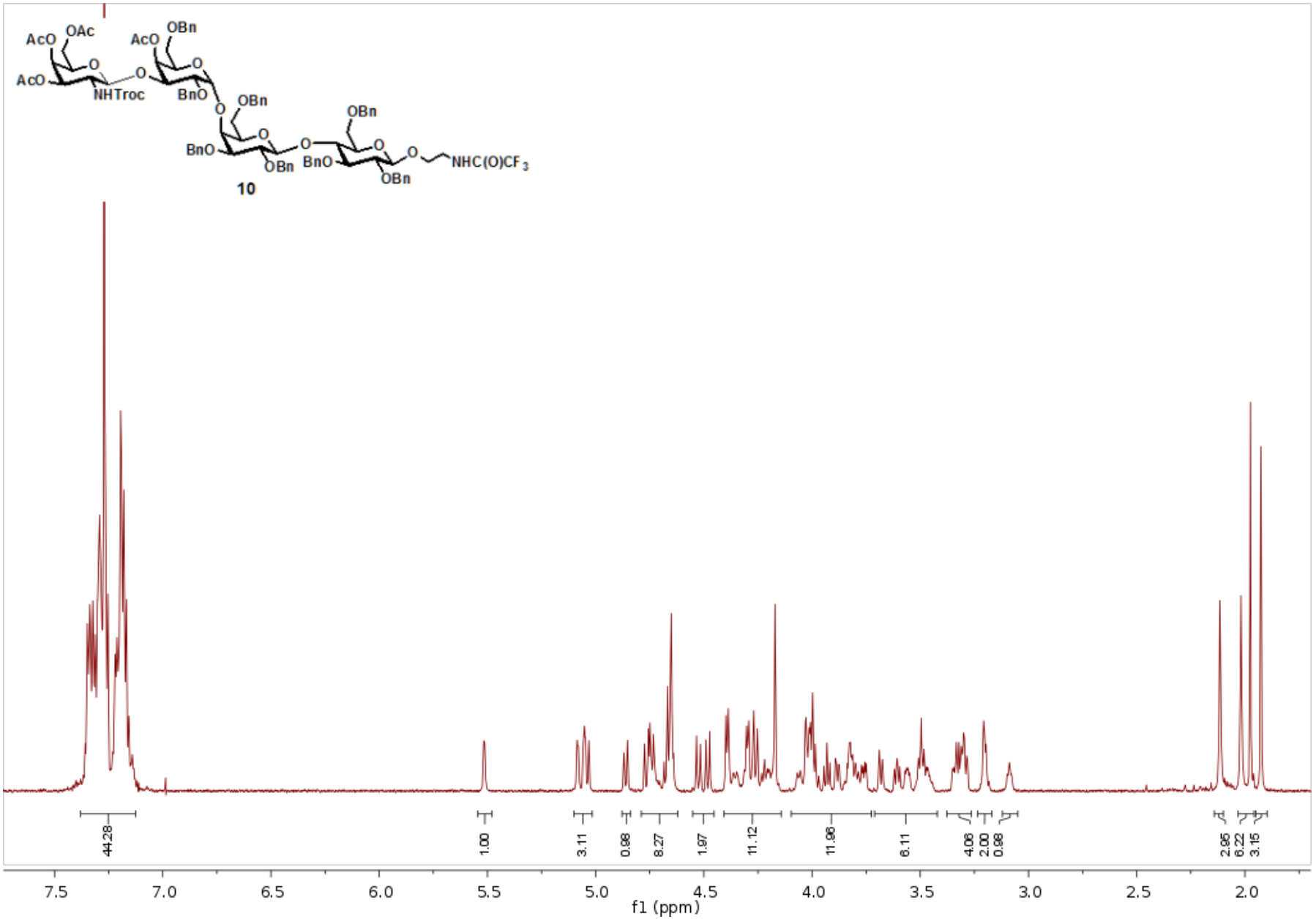
3.10
5.23
2.06
2.00
1.02
2.10
2.08
1.03
5.22
2.08
2.00
1.00
0.89
2.98
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)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AcO
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NHTroc
O
AcO
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O
BnO
O
OBn
O
BnO
OBn
O
O
BnO
OBn
O
NHC(O)CF3
OBn
10
44.28
1.00
3.11
0.98
8.27
1.97
11.12
11.96
6.11
4.08
2.00
0.88
2.95
6.22
3.15
7.5
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6.5
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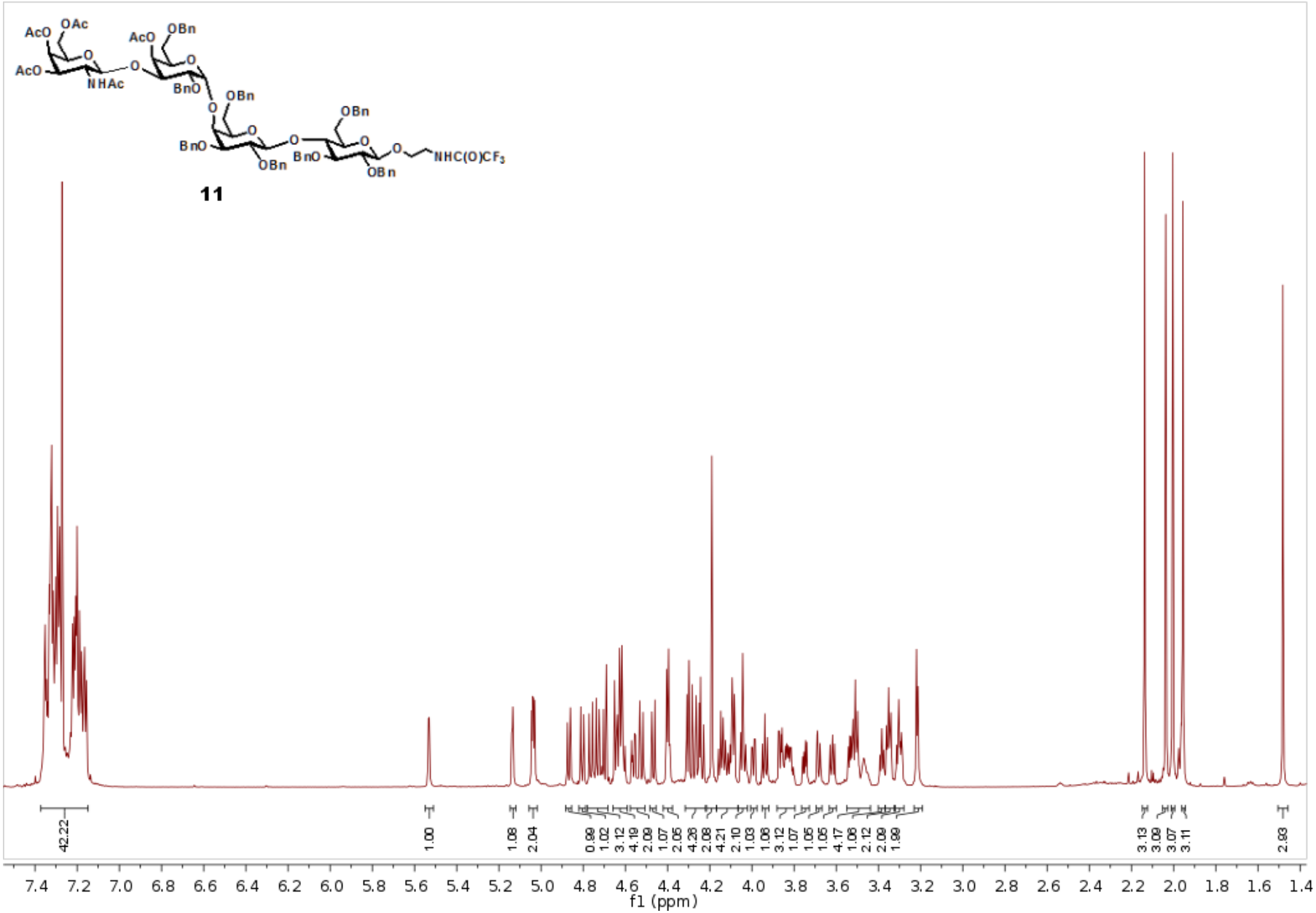
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)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BnO
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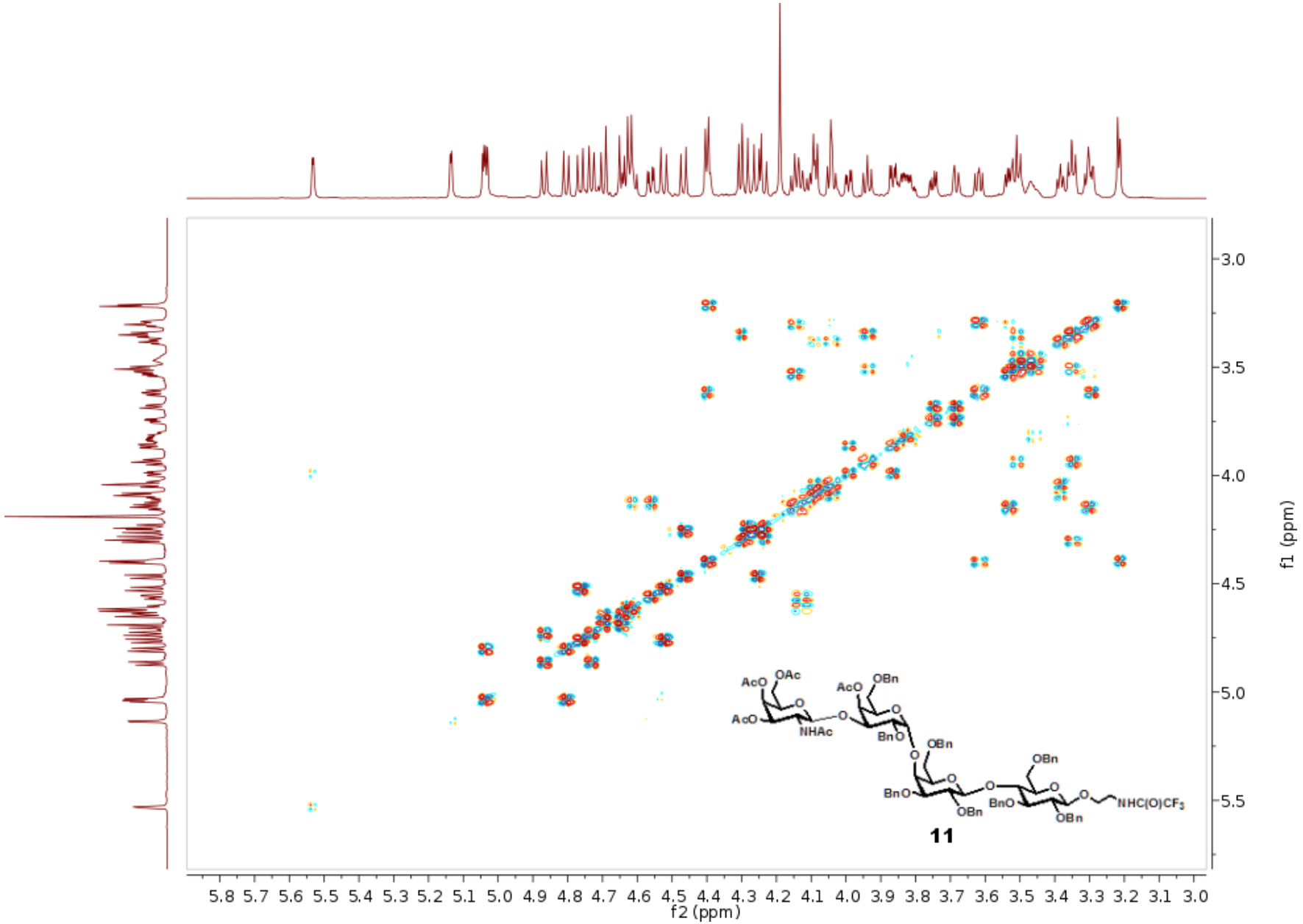
OBn
BnO
OBn
O
BnO
OBn
O
OBn
NHC(O)CF3
11
42.22
1.00
1.08
2.04
0.99
1.02
3.12
4.19
2.09
1.07
2.05
4.26
2.08
4.21
2.10
1.03
1.06
3.12
1.07
1.05
1.05
4.17
1.06
2.12
2.09
1.99
3.13
3.09
3.07
3.11
2.93
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4
f1 (ppm)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f2 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
f1 (ppm)
AcO
OAc
AcO
O
NHAc
O
AcO
OBn
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O
BnO
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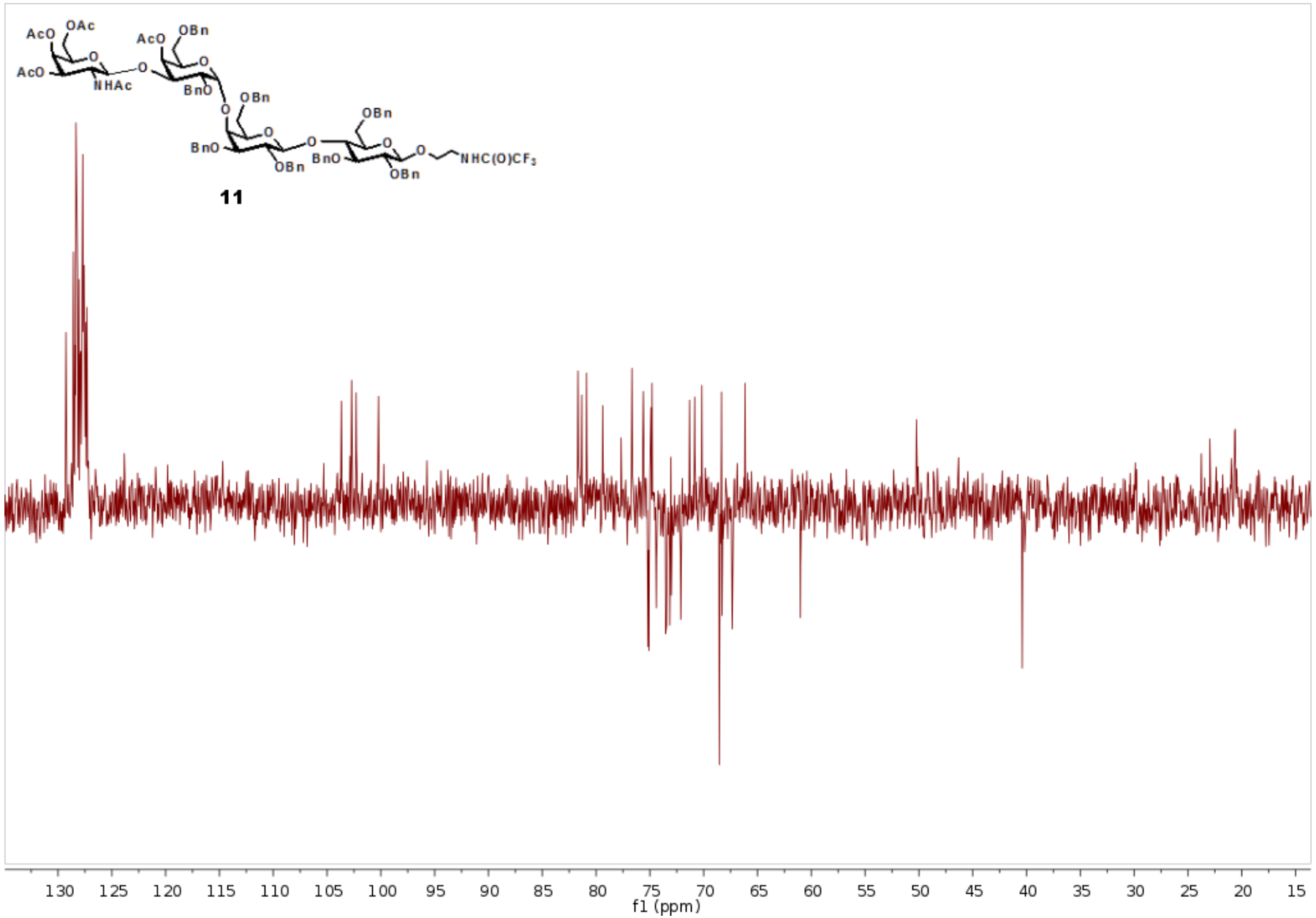
O
NHC(O)CF3
11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AcO
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O
BnO
O
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O
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OBn
O
BnO
OBn
O
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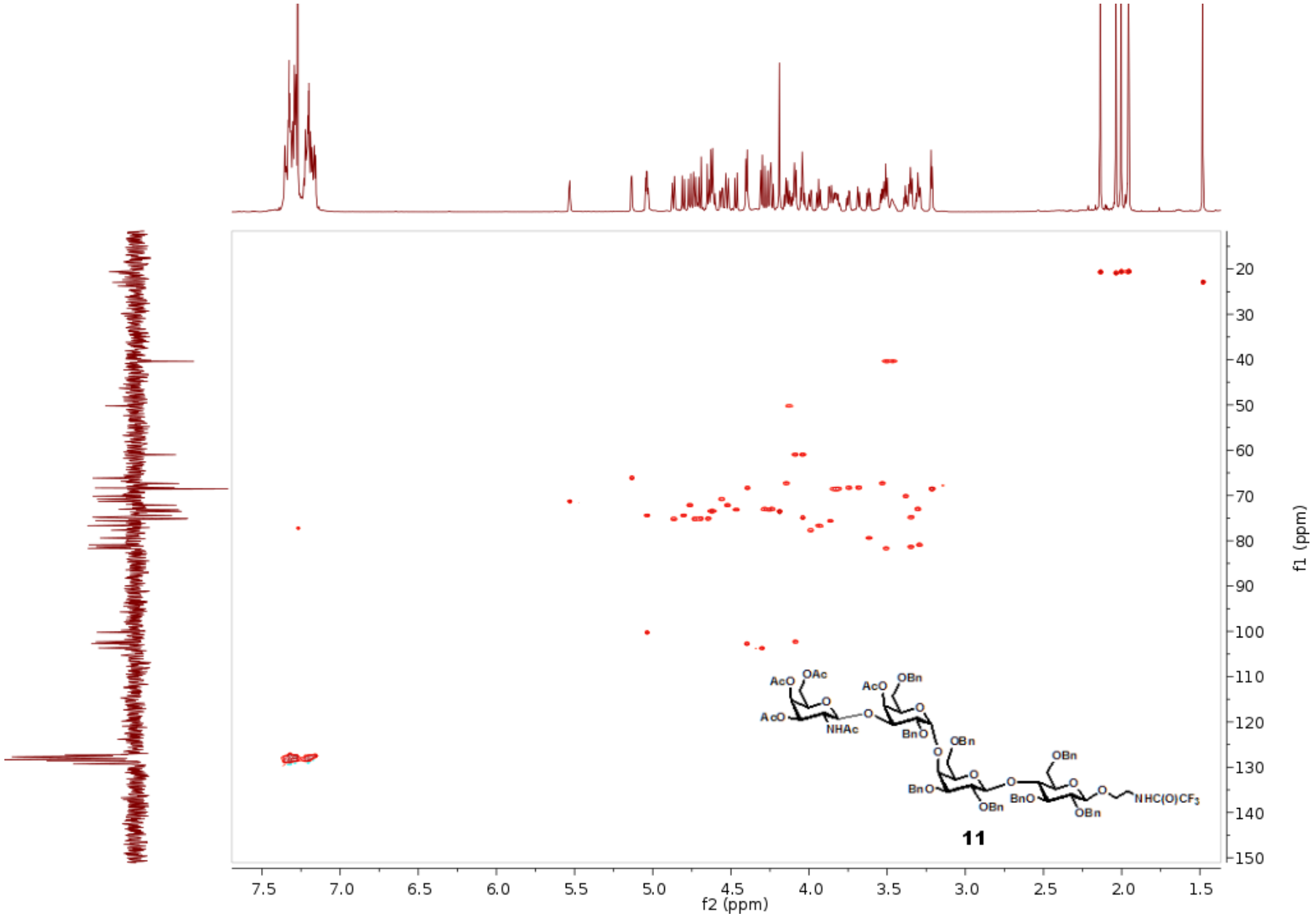
O
NHC(O)CF3
11
130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15
f1 (ppm)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5.5
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4.5
4.0
3.5
3.0
2.5
2.0
1.5
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)
AcO
OAc
AcO
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OBn
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OBn
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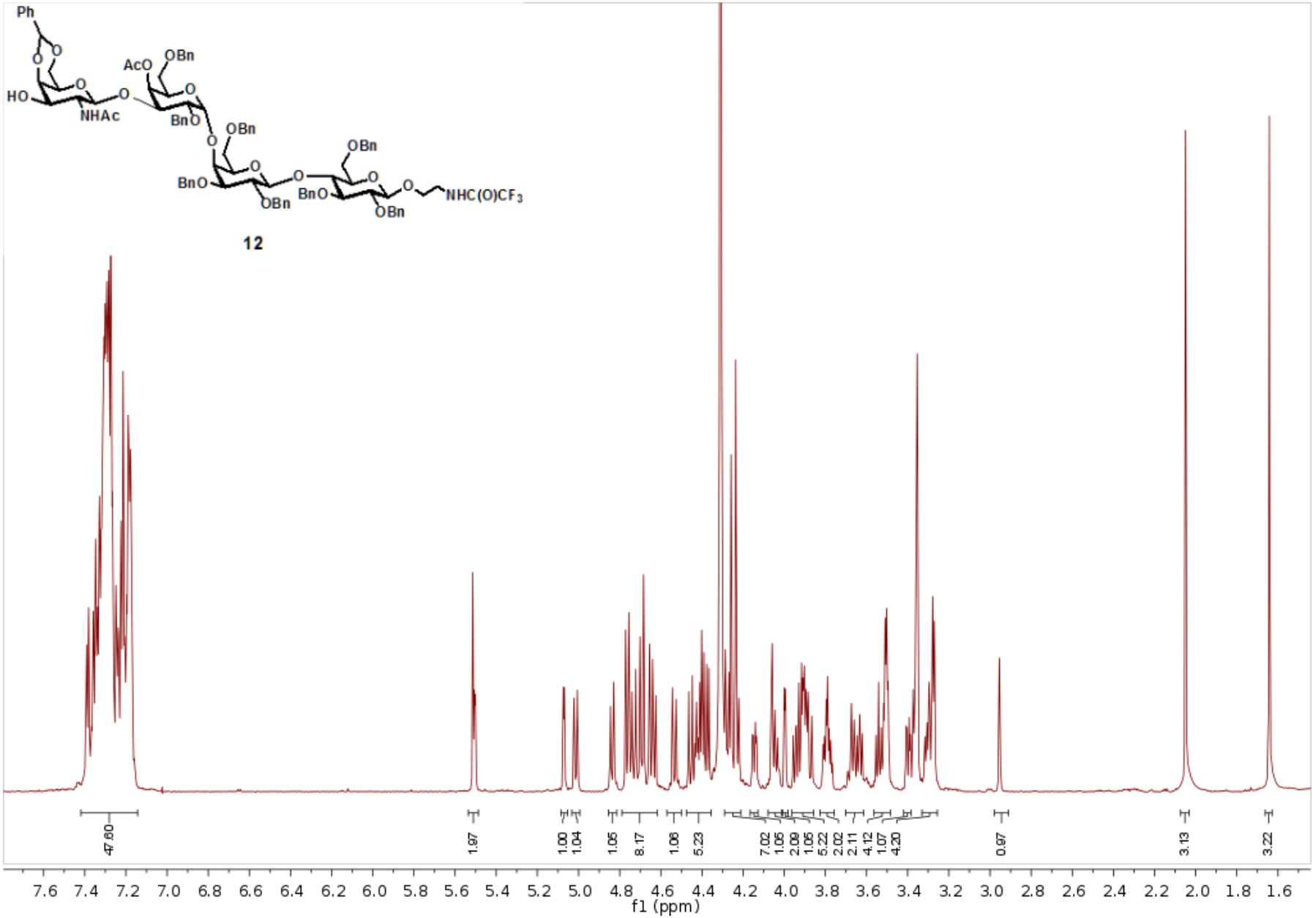
OBn
BnO
OBn
OBn
NHC(O)CF3
11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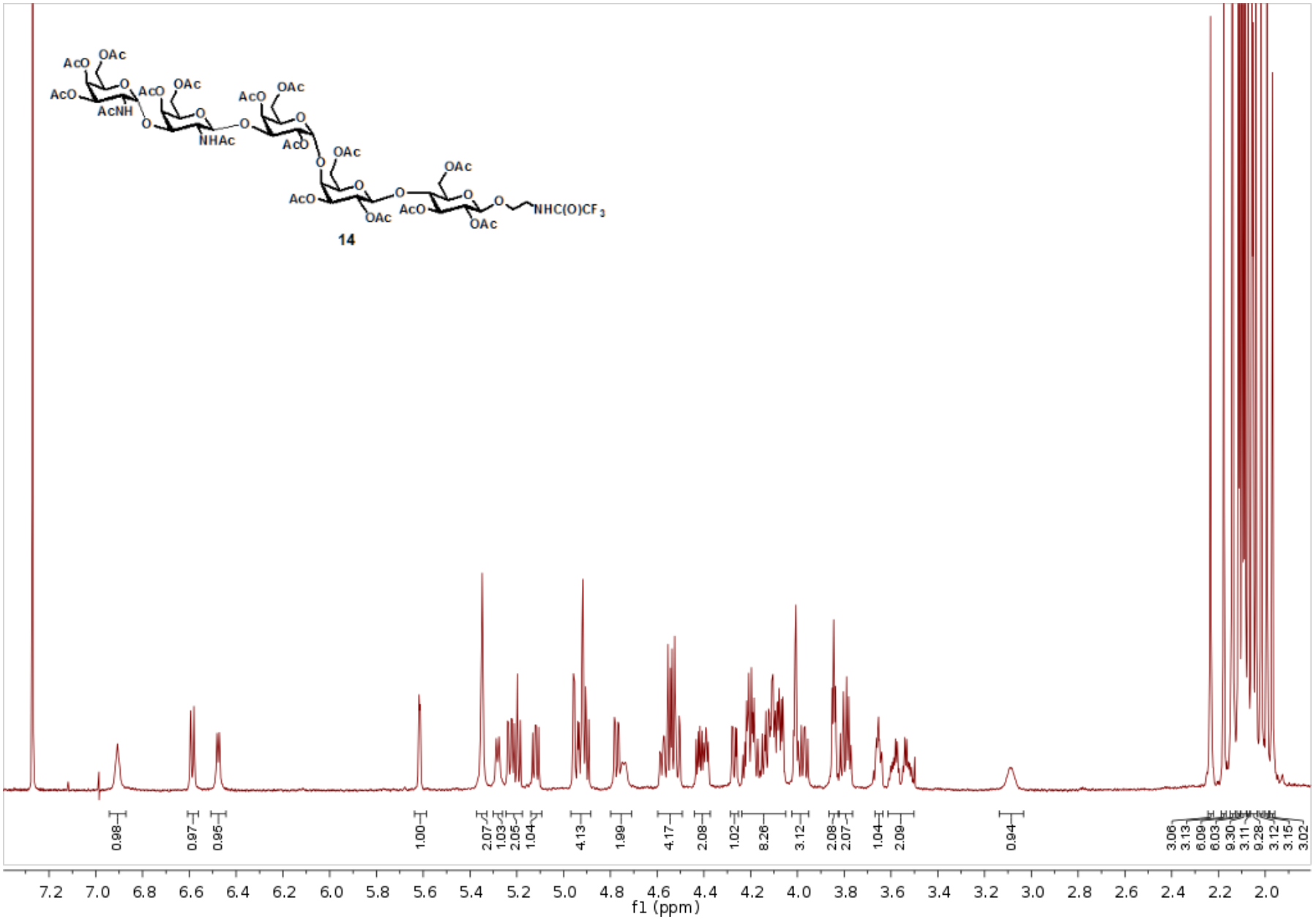
1.06
5.23
7.02
1.05
2.09
1.05
5.22
2.02
2.11
4.12
1.07
4.20
0.97
3.13
3.22
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6
f1 (ppm)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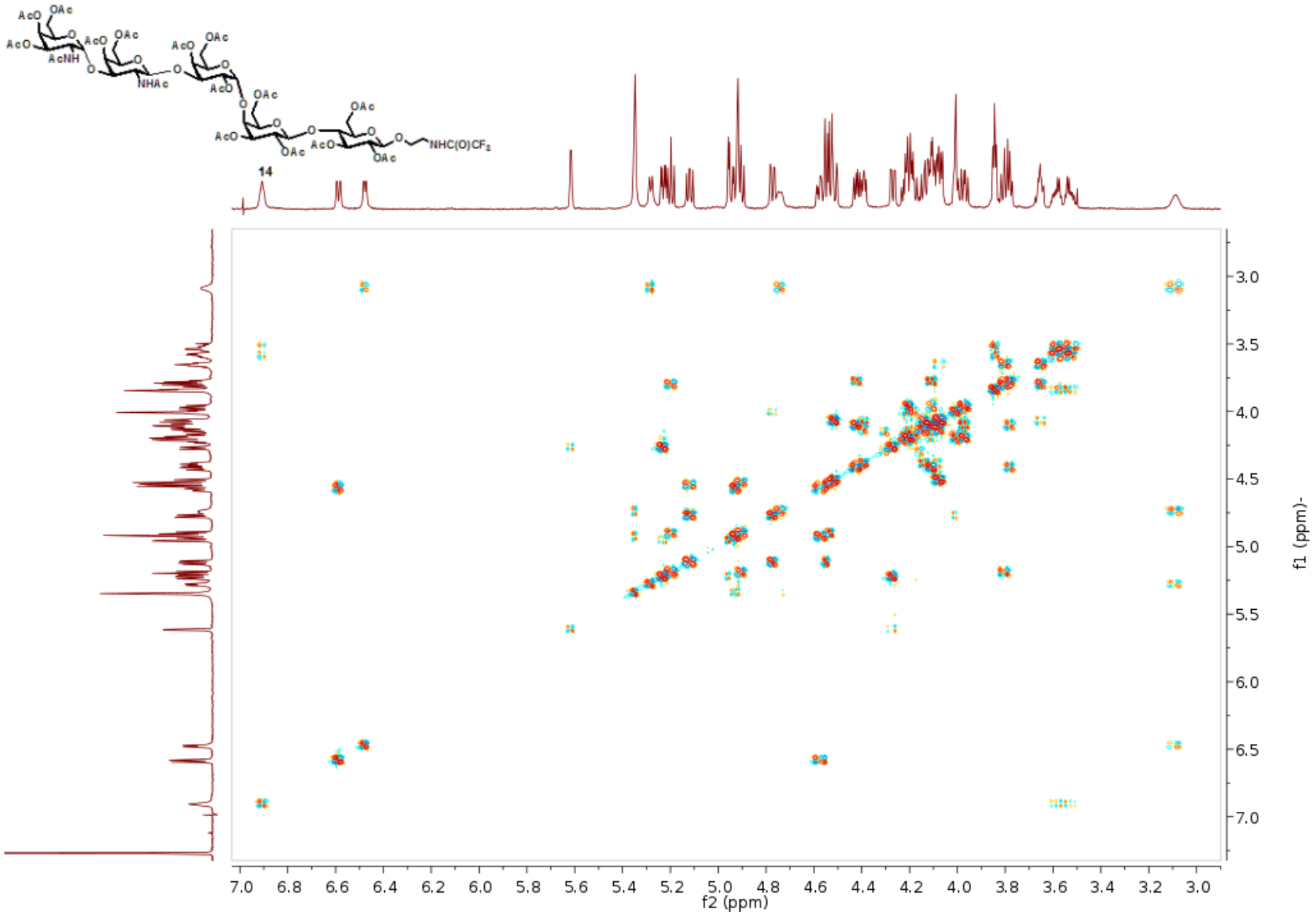
1.04
4.13
1.99
4.17
2.08
1.02
8.26
3.12
2.08
2.07
1.04
2.09
0.94
3.06
3.13
6.09
6.03
9.30
3.11
9.28
3.12
3.15
3.02
7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)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OAc
AcNH
NHAc
NHC(O)CF3
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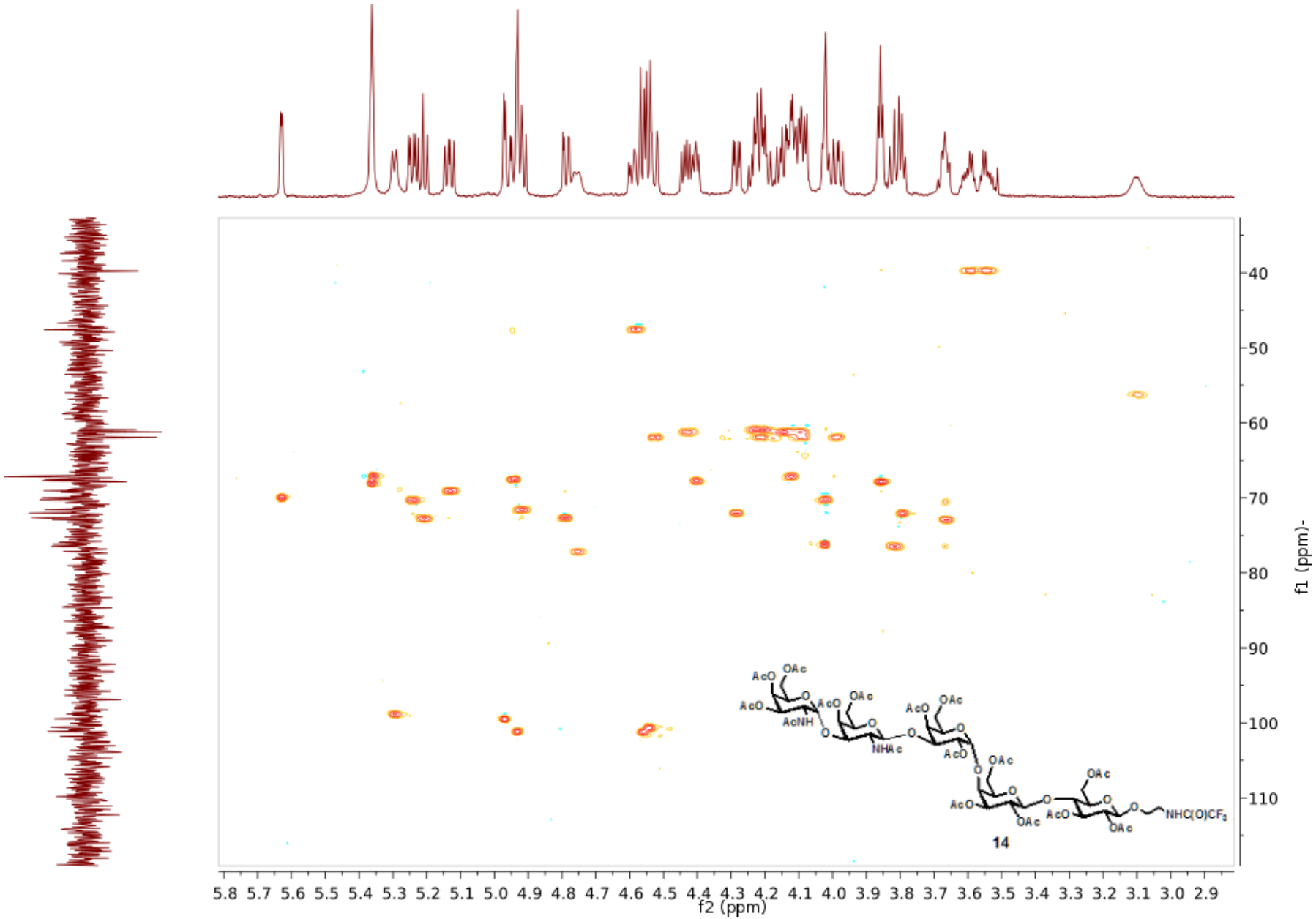
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
f2 (ppm)
f1 (ppm)-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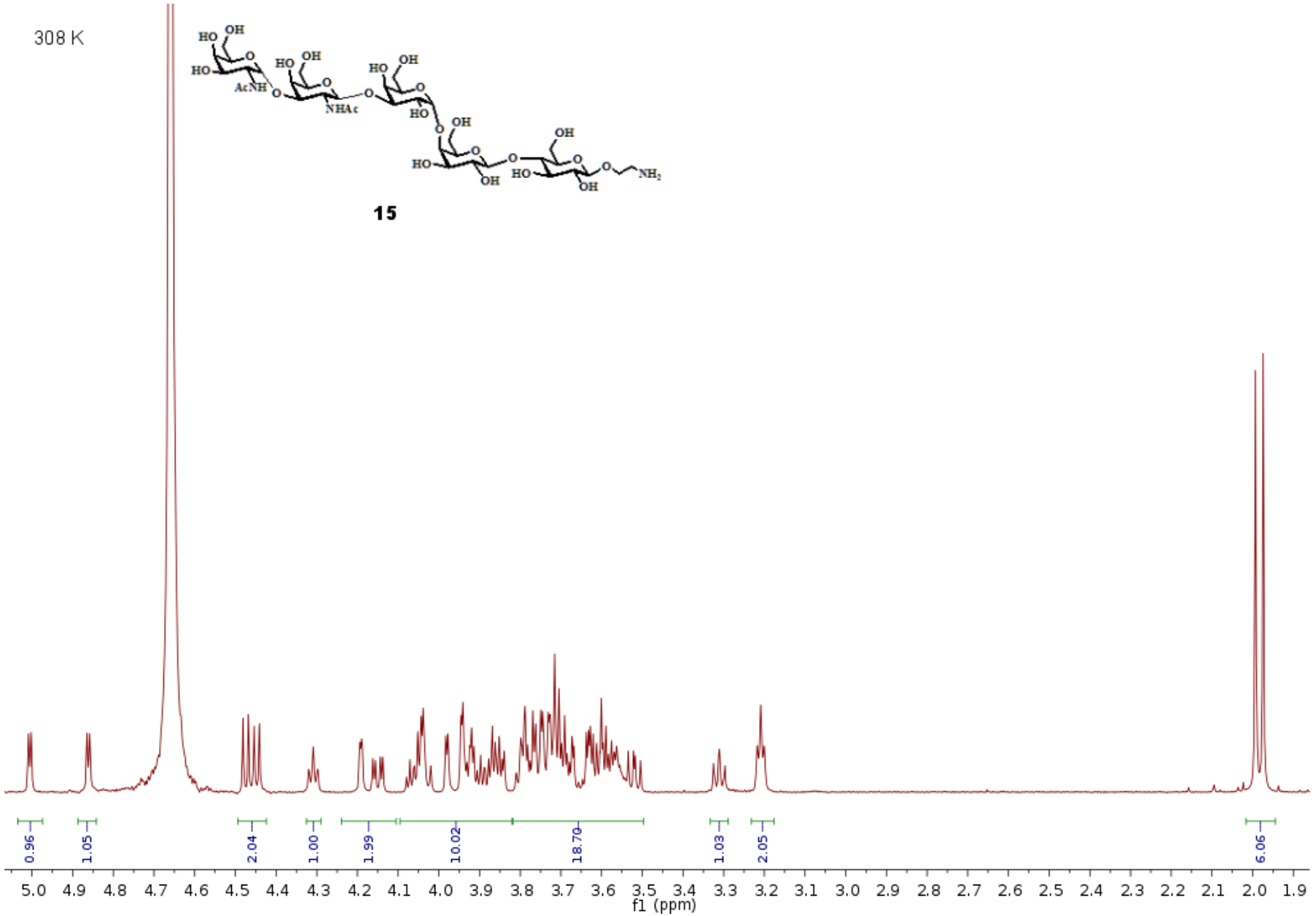
f2 (ppm)
40
50
60
70
80
90
100
110
f1 (ppm)
AcO
OAc
AcNH
NHAc
NHC(O)CF3
14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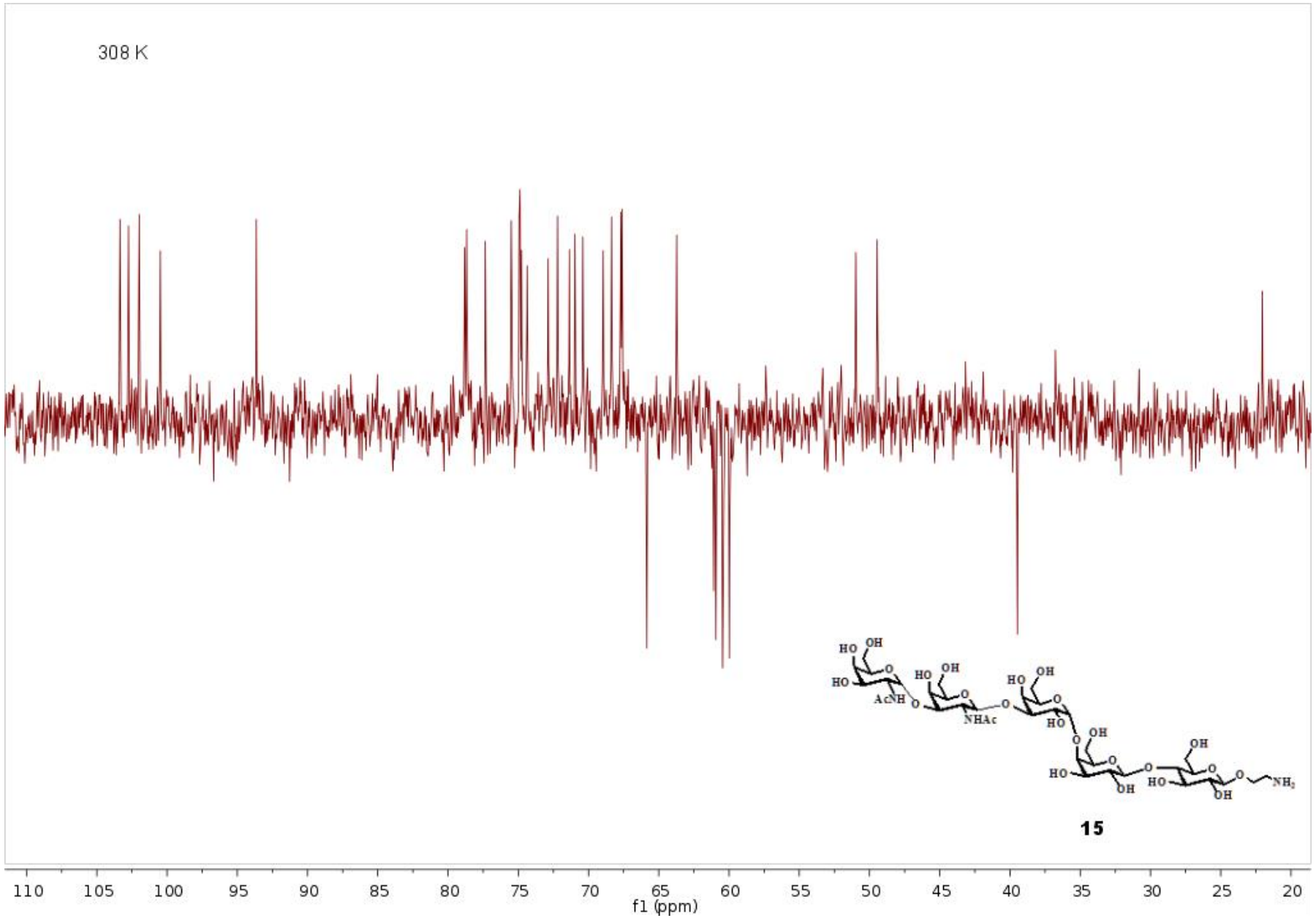
1.00
1.99
10.02
18.70
1.03
2.05
6.06
5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9
f1 (ppm)

15


308 K
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85
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75
70
65
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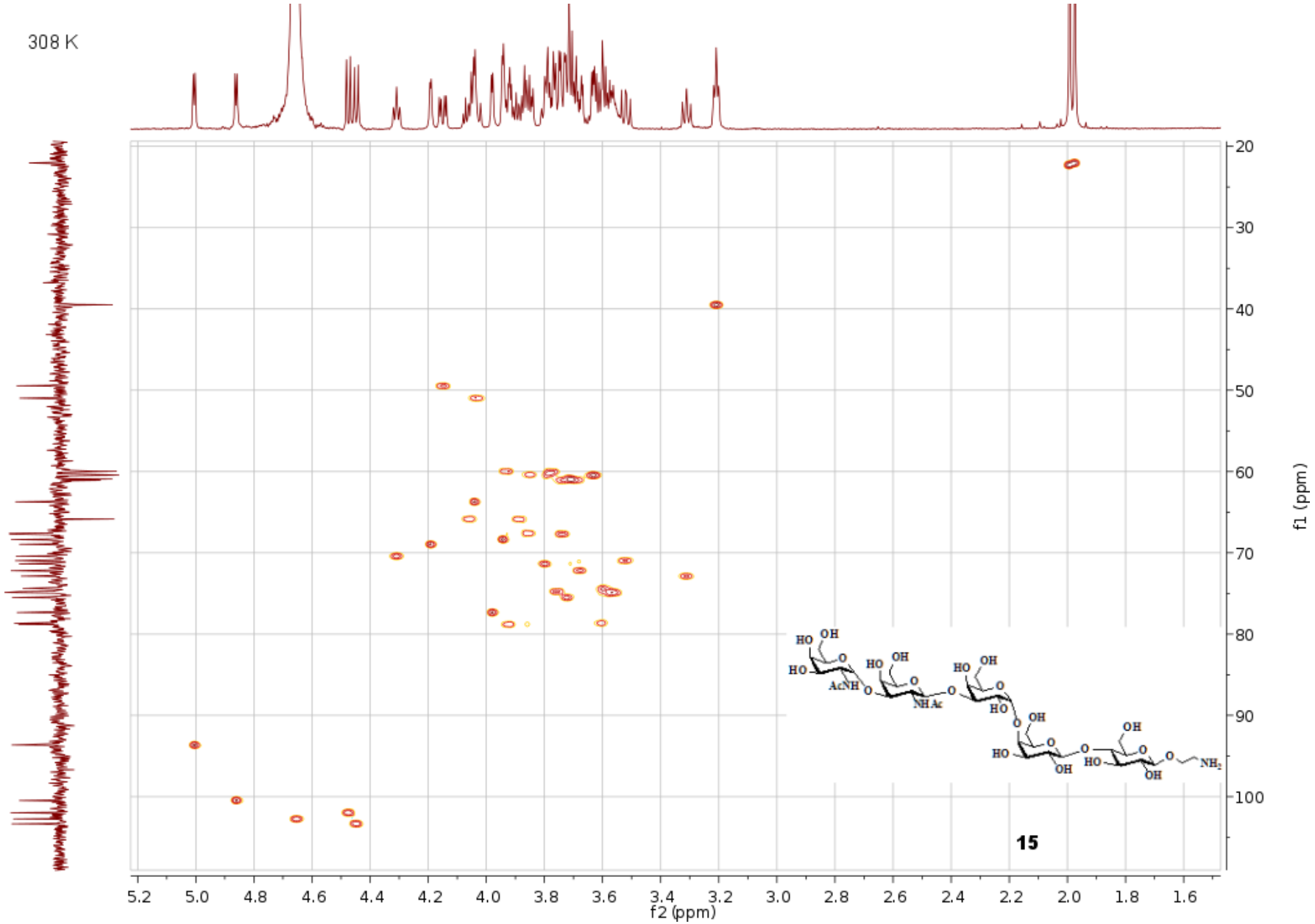
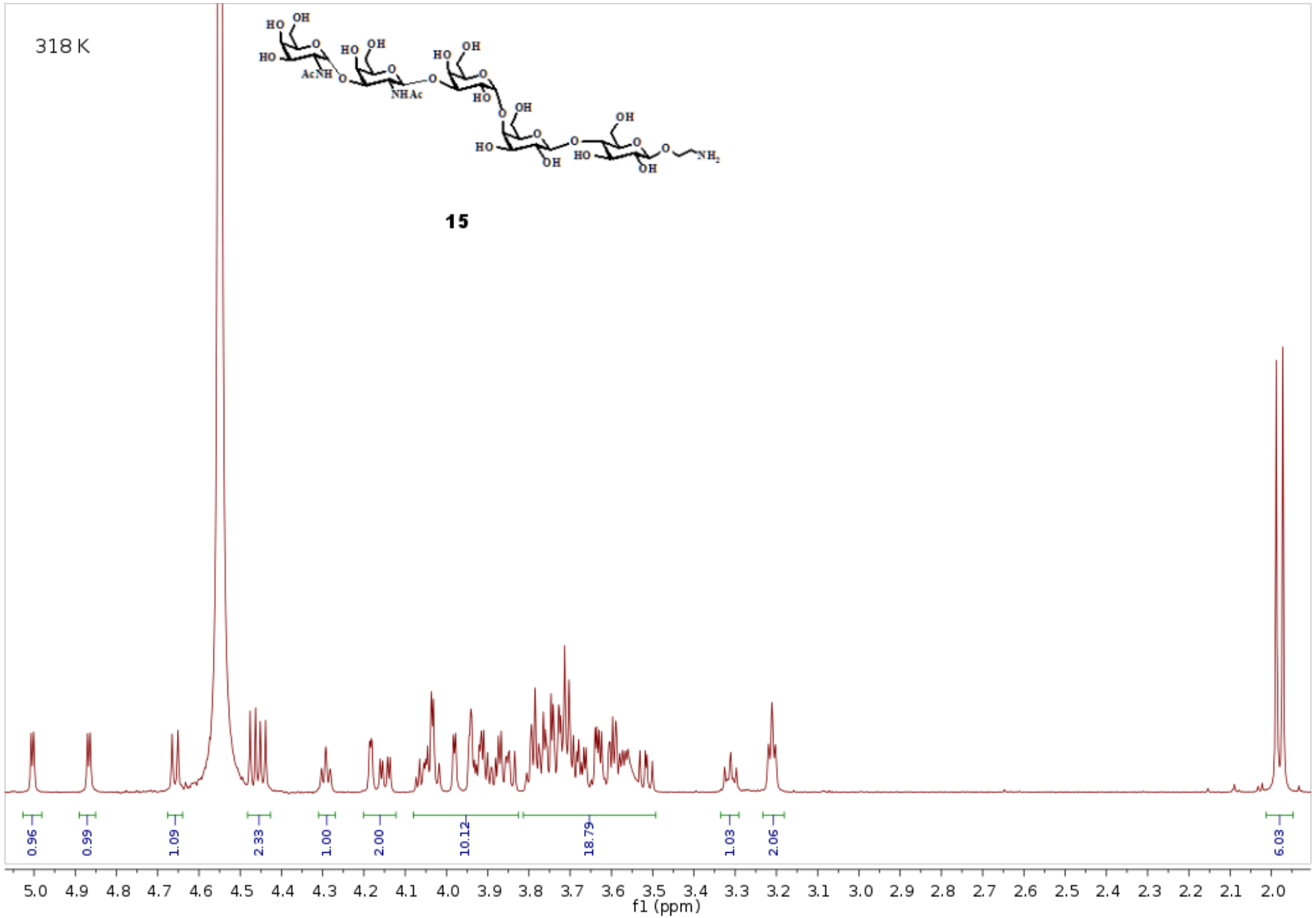
55
50
45
40
35
30
25
20
f1 (ppm)
15


308 K
f2 (ppm)
f1 (ppm)
15


318 K
15
0.96
0.99
1.09
2.33
1.00
2.00
10.12
18.79
1.03
2.06
6.03
f1 (ppm)