Supporting Information

for

One-Pot Multienzyme-Catalyzed Assembly of Pentasaccharide Repeating Units from Group B *Streptococcus* Capsular Polysaccharides and Their Related Oligosaccharide Derivatives

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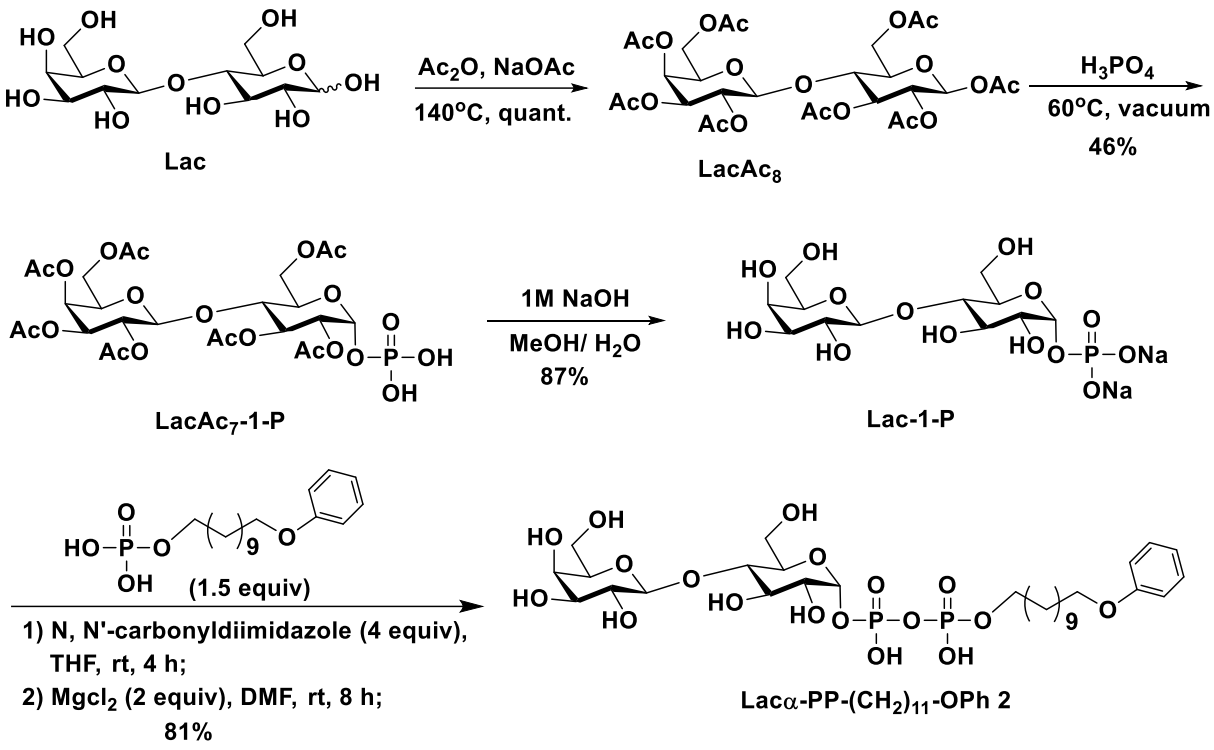


Scheme S1. Chemical synthesis of Lacα-PP-$(CH_2)_{11}$-OPh **2**.

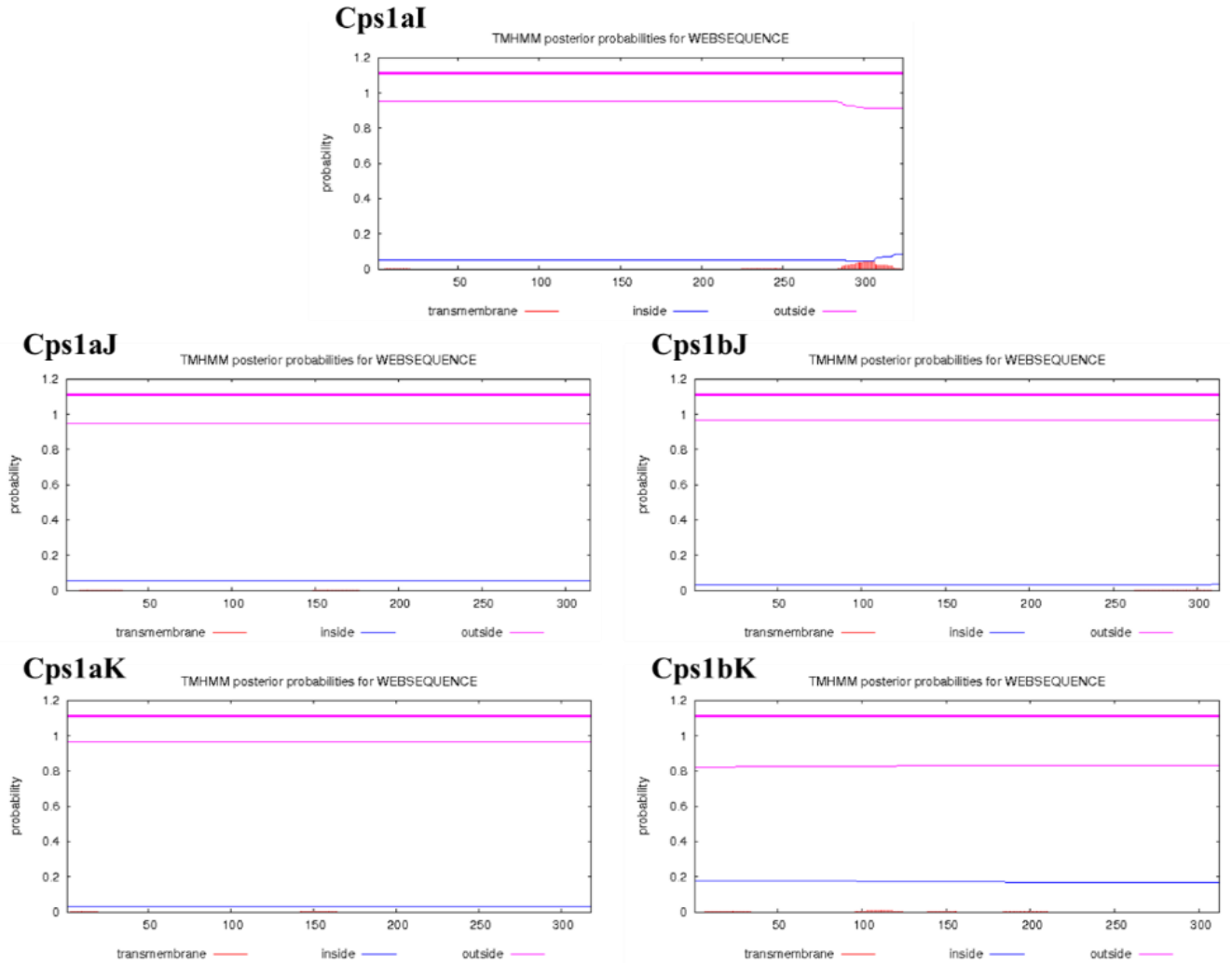


Figure S1. Prediction of transmembrane helices in five target glycosyltransferases by TMHMM Server v. 2.0 software.

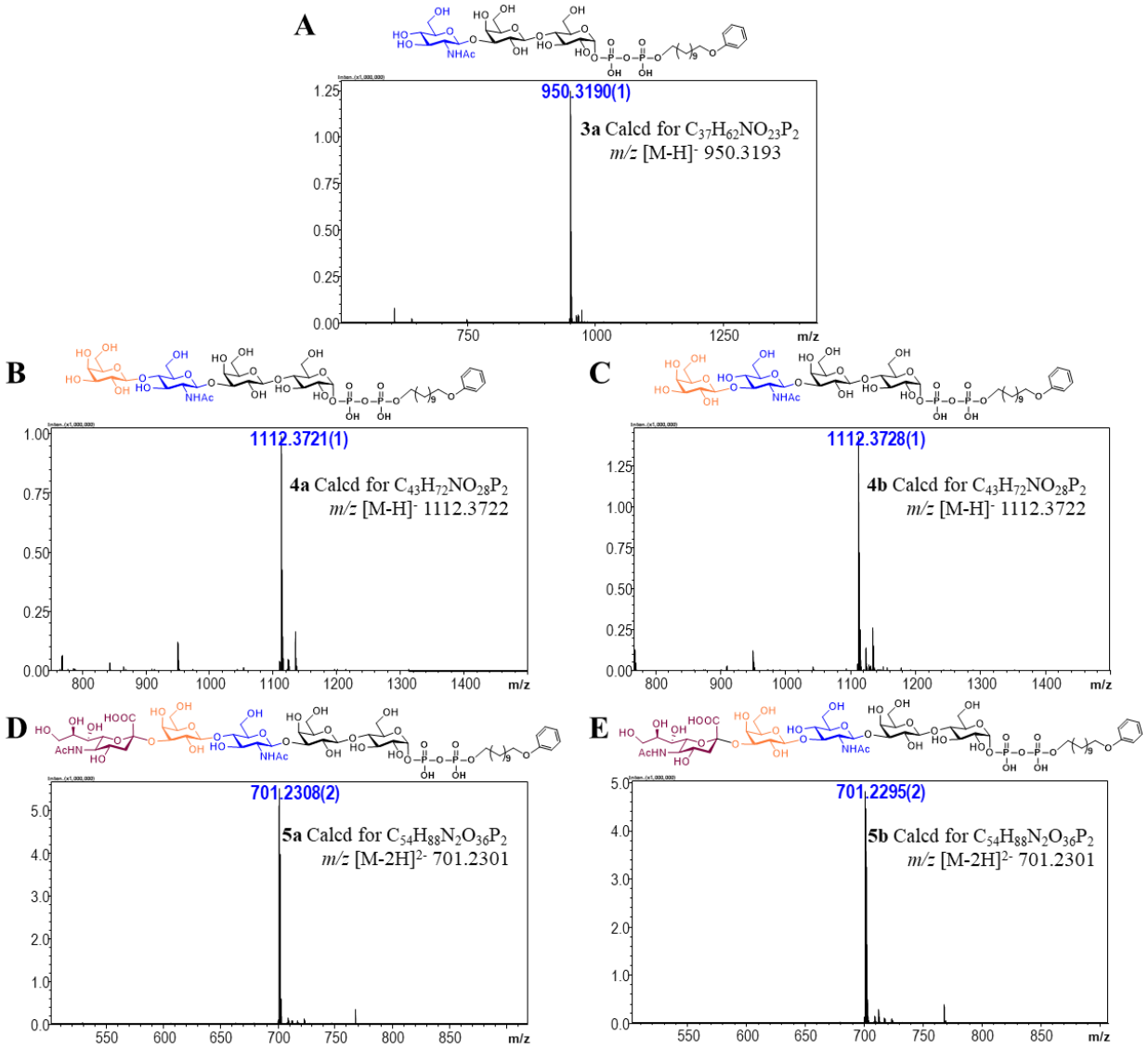


Figure S2. ESI-HRMS analysis of reaction products catalyzed by Cps1aI (A), Cps1aJ (B), Cps1bJ (C), Cps1aK (D) andCps1bK (E), respectively.

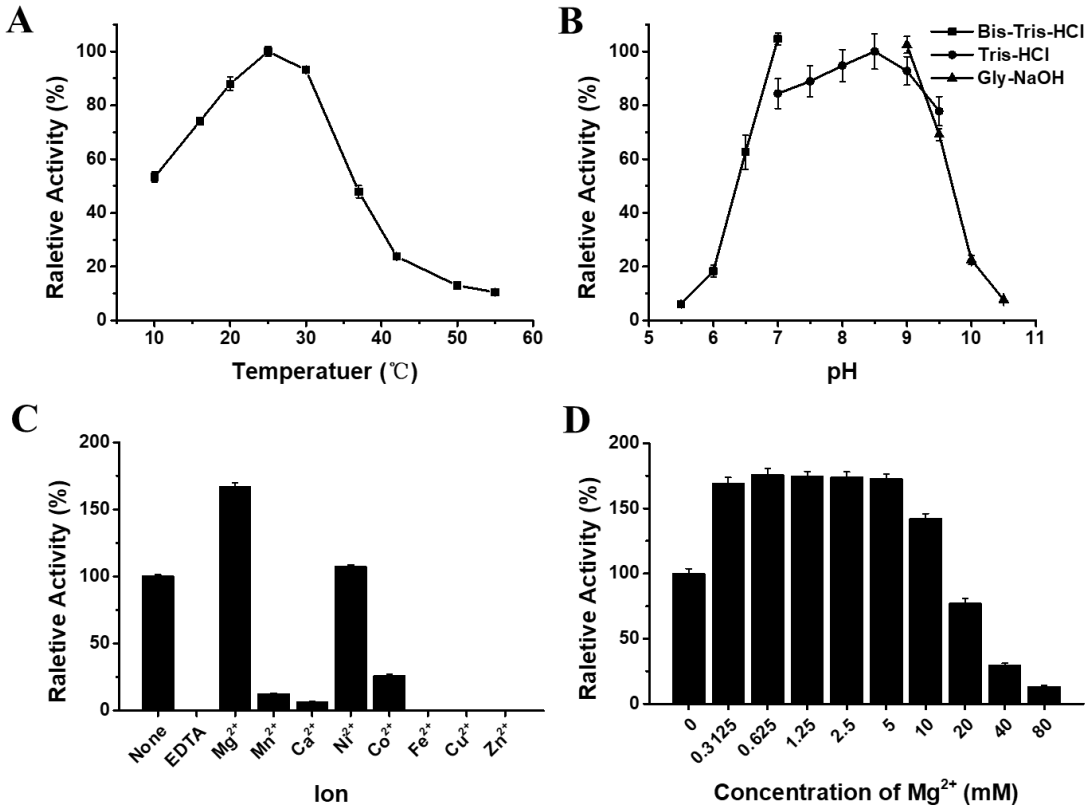


Figure S3. The influences of temperature (A), pH (B), ion (C) and concentration of Mg^{2+} (D) on the relative enzymatic activities of N-acetylglucosaminyltransferase Cps1aI to catalyze the reaction of Lacα-PP-$(CH_2)_{11}$-OPh **2** with UDP-GlcNAc. Each error bar represents the standard deviation of three experiments.

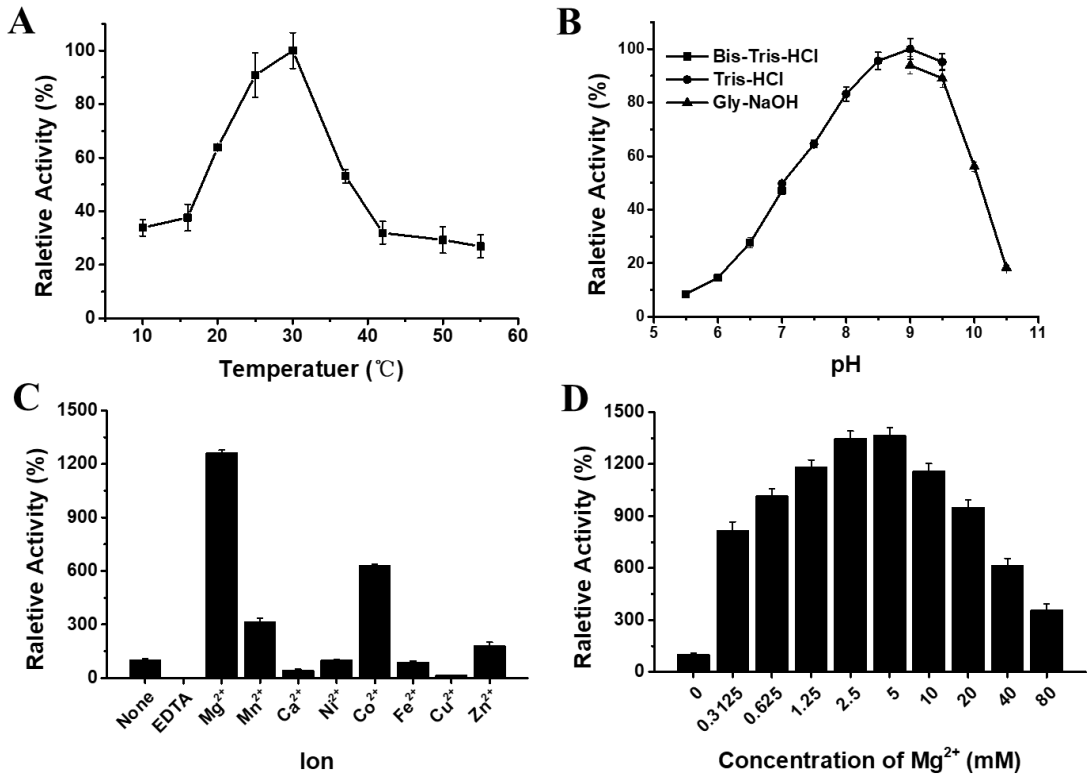


Figure S4. The influences of temperature (A), pH (B), ion (C) and concentration of Mg^{2+} (D) on the relative enzymatic activities of β-1,4-galactosyltransferase Cps1aJ to catalyze the reaction of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** with UDP-Gal. Each error bar represents the standard deviation of three experiments.

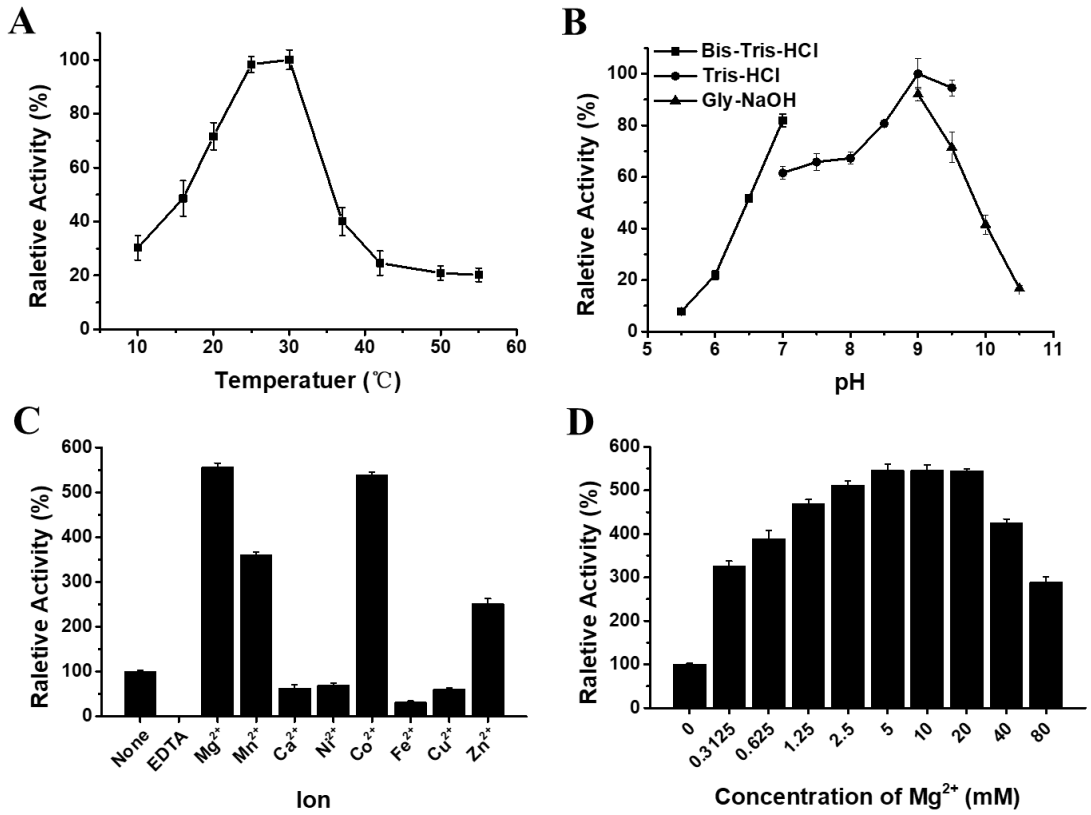


Figure S5. The influences of temperature (A), pH (B), ion (C) and concentration of Mg^{2+} (D) on the relative enzymatic activities of β-1,3-galactosyltransferase Cps1bJ to catalyze the reaction of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** with UDP-Gal. Each error bar represents the standard deviation of three experiments.

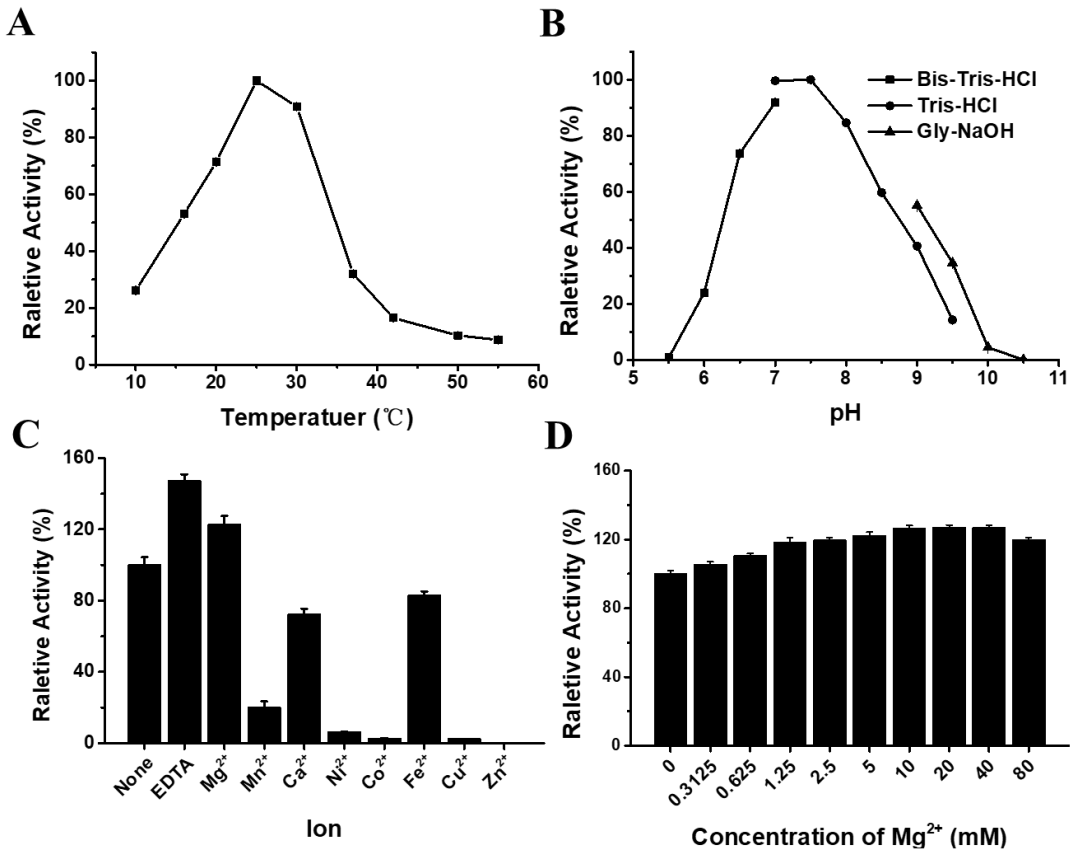
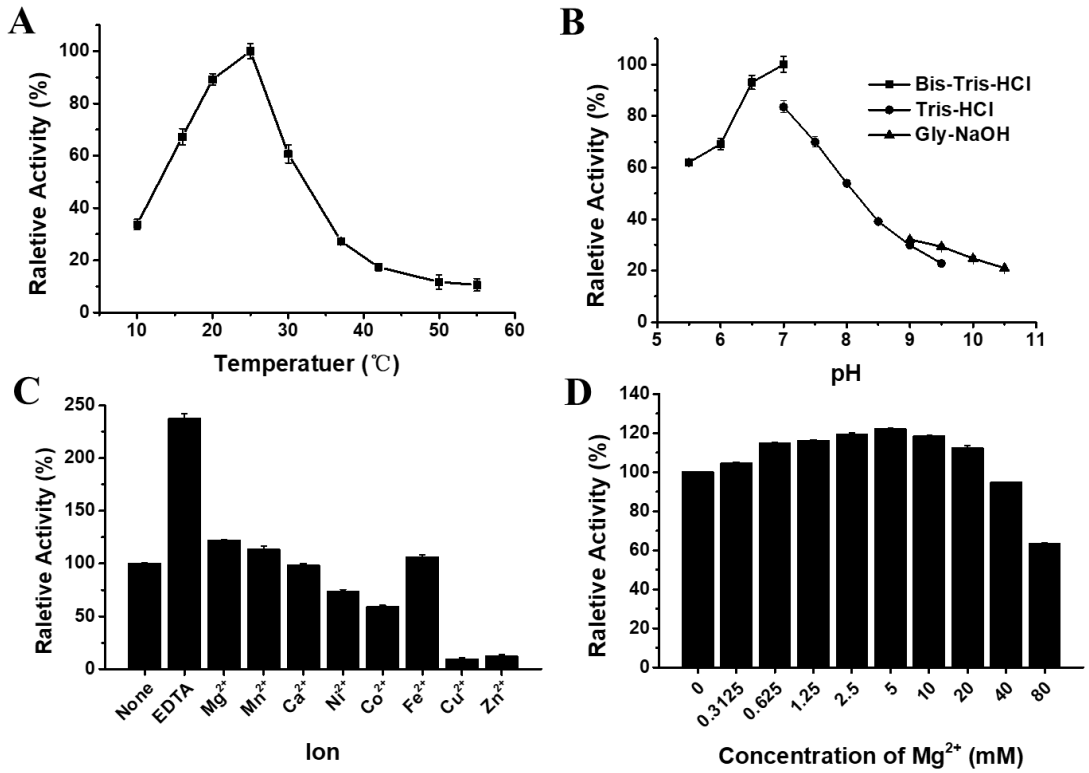


Figure S6. The influences of temperature (A), pH (B), ion (C) and concentration of Mg^{2+} (D) on the relative enzymatic activities of α-2,3-sialyltransferase Cps1aK to catalyze the reaction of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** with CMP-NeuNAc. Each error bar represents the standard deviation of three experiments.



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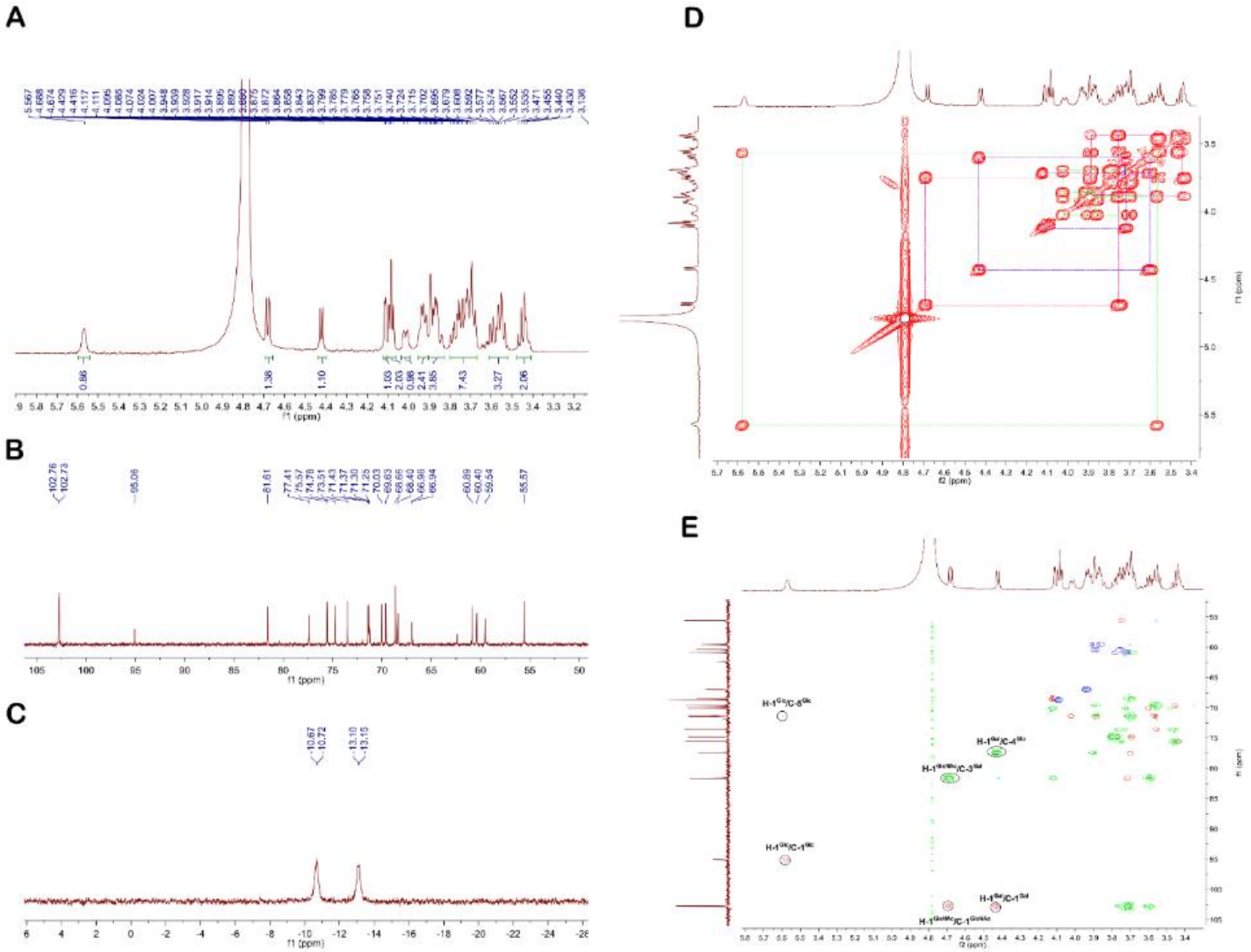
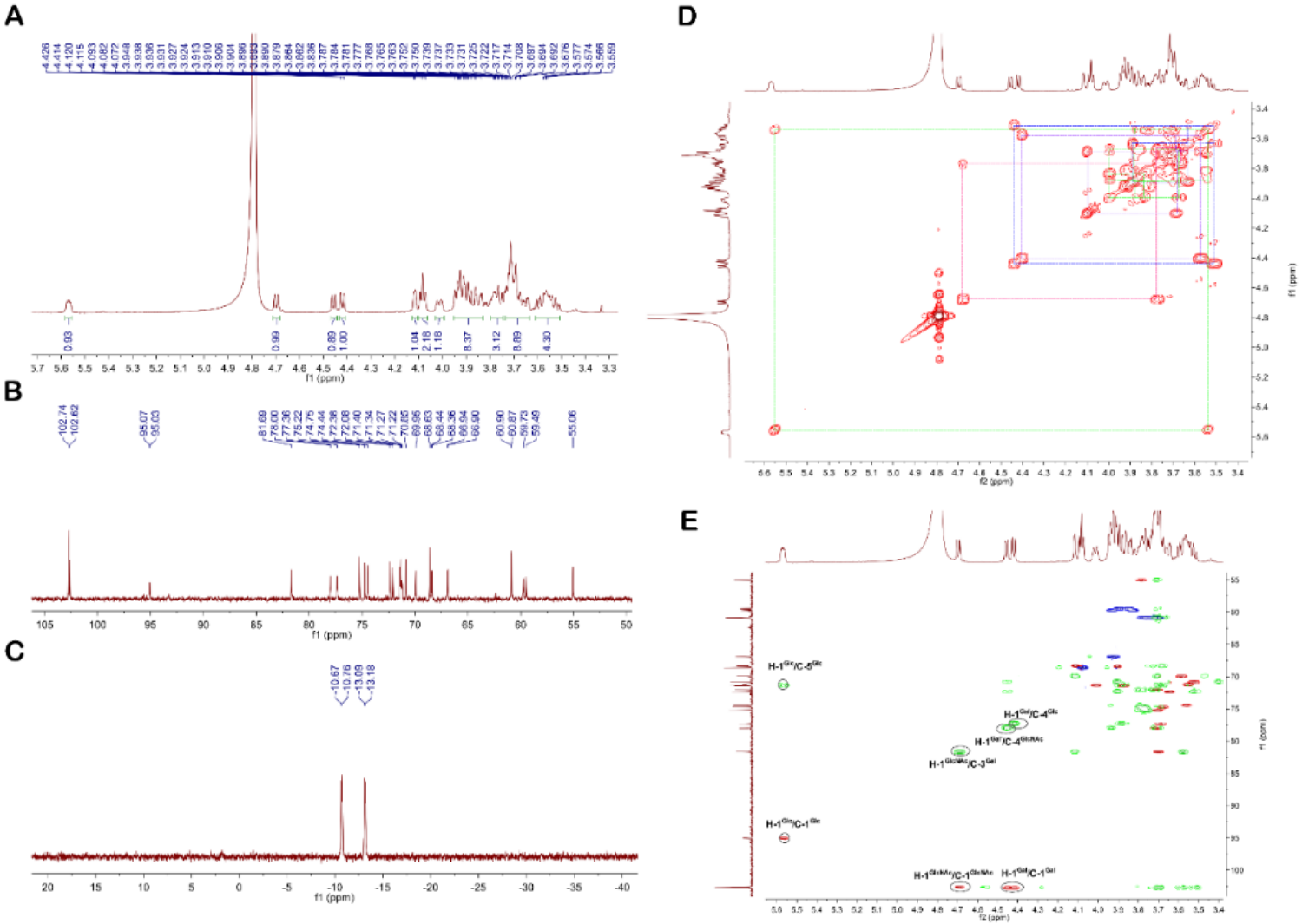


Figure S8. Structural charaterization of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** via NMR analysis. Selected ^{1}H NMR (A), ^{13}C NMR (B), ^{31}P NMR (C), gCOSY (D) and combined gHSQC (red-blue singals) and gHMBC (green singals) spectra of trisaccharide **3a**.



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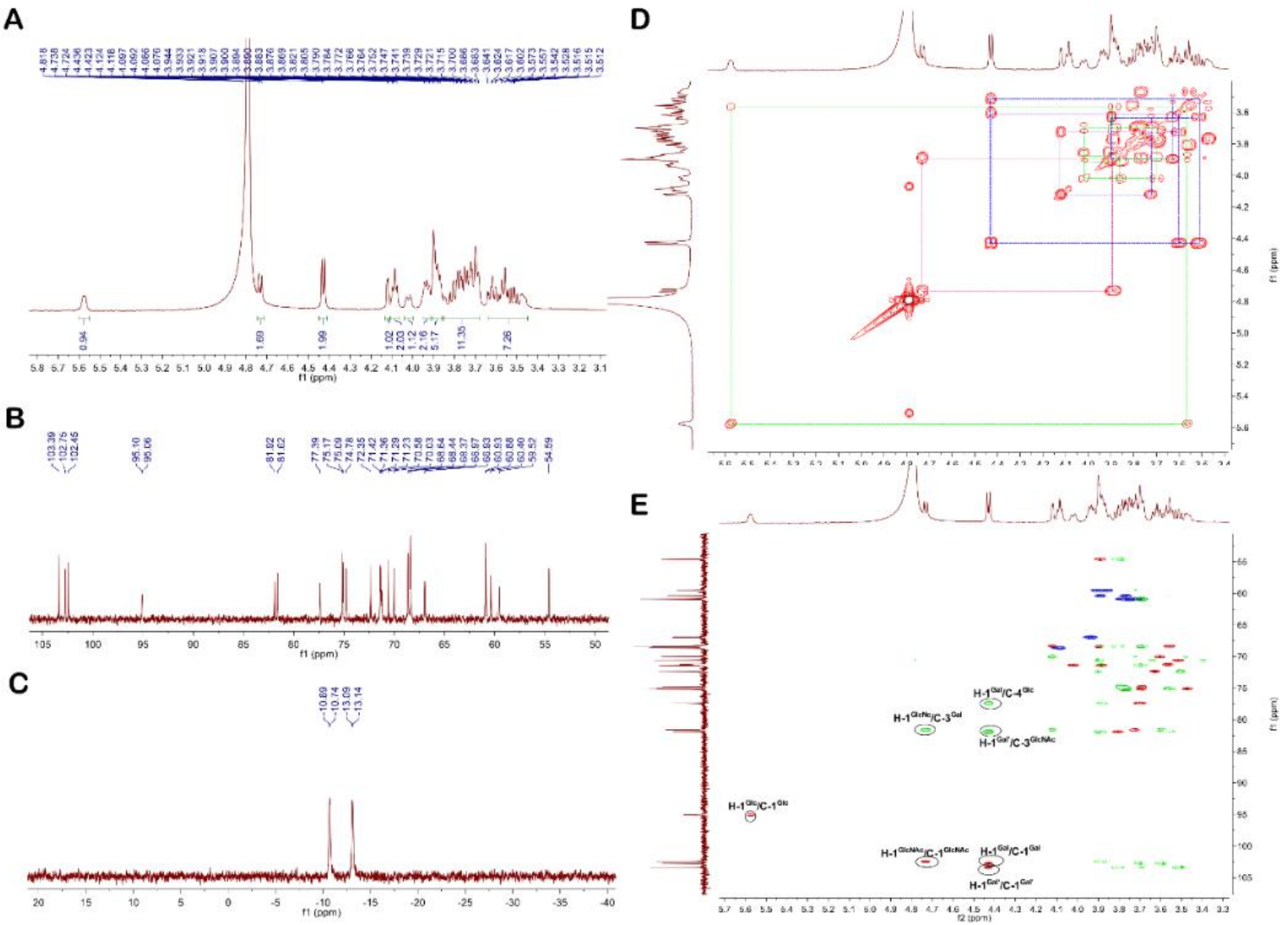

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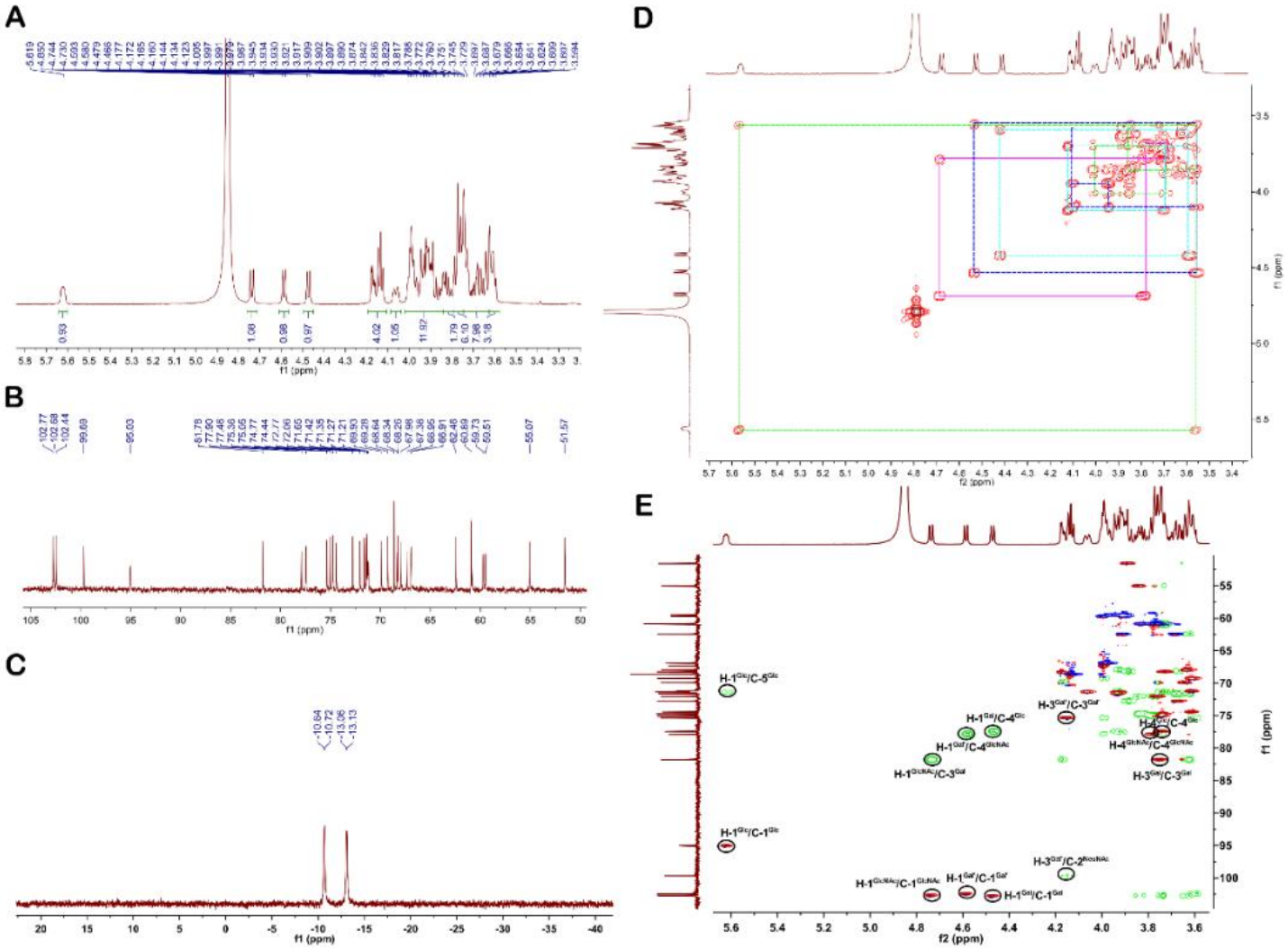

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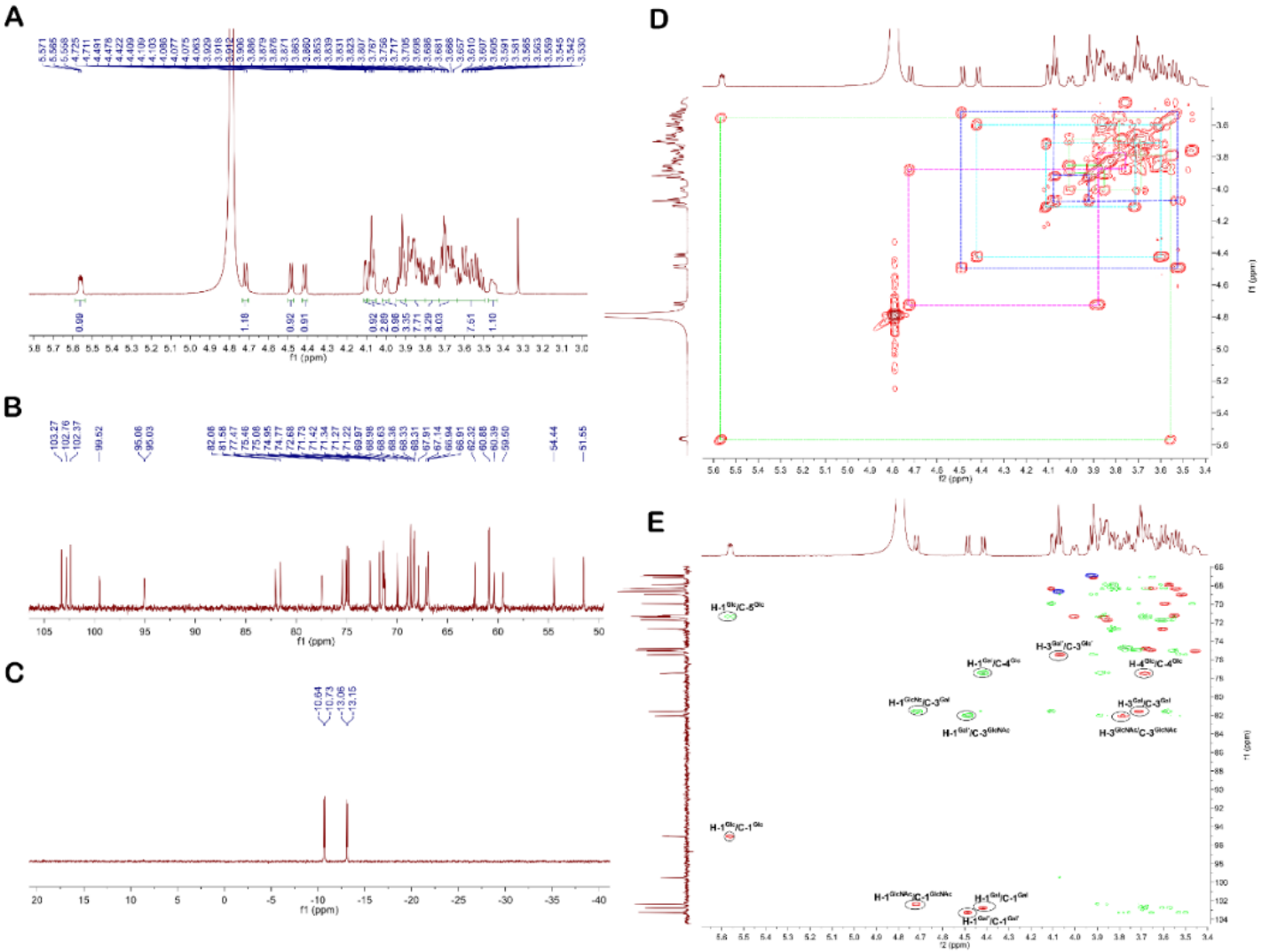

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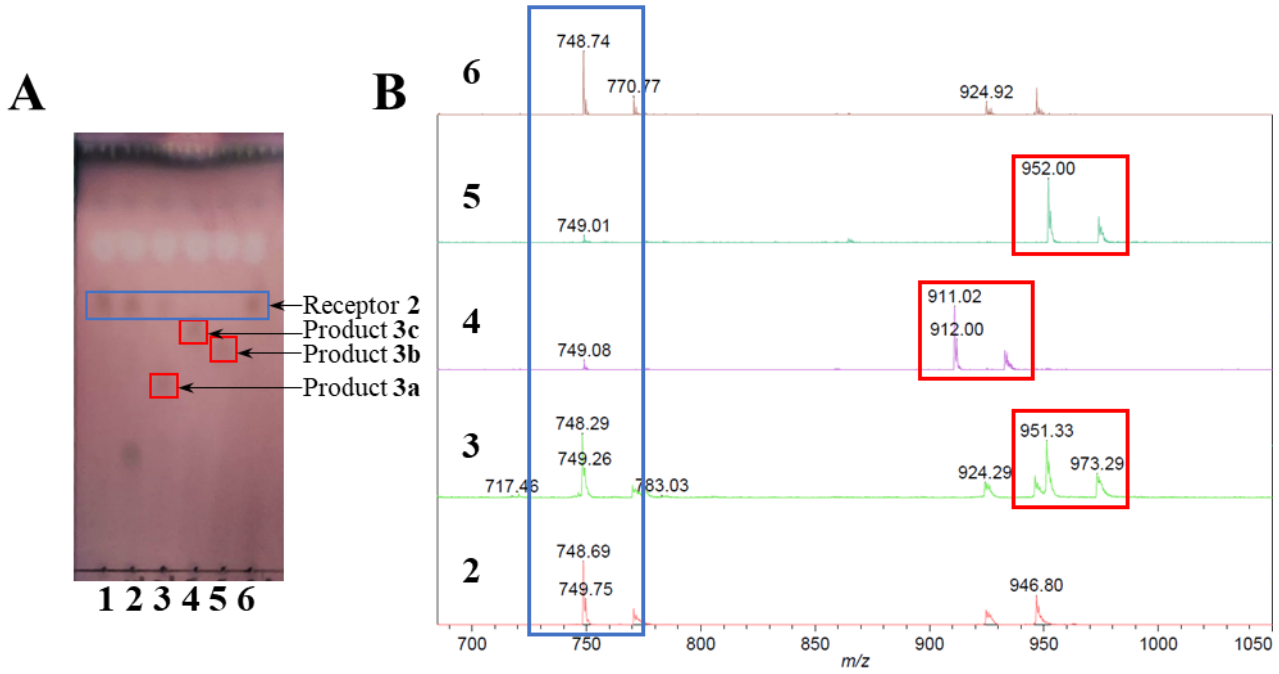
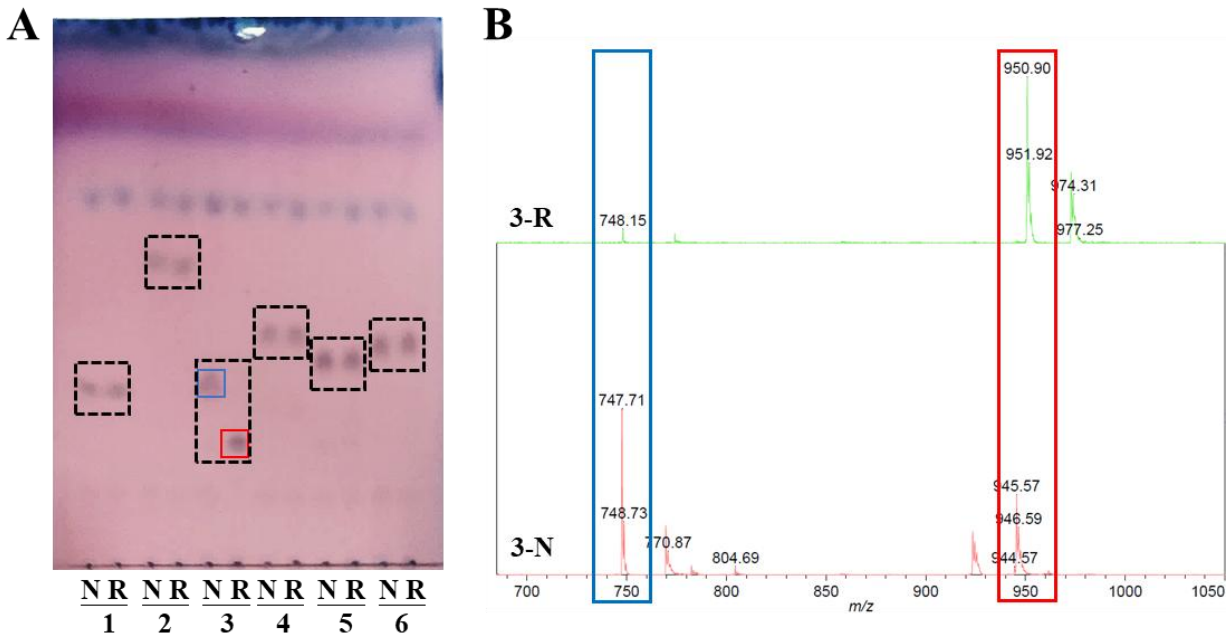


Figure S13. TLC (A) and MALDI-TOF-MS (B) traces of donor substrates recognition of Cps1aI. ***1***, negative control reaction with heat-deactivated Cps1aI; ***2-6***, enzyme reactions of Lacα-PP-$(CH_2)_{11}$-OPh **2** with UDP-Gal, UDP-GalNAc, UDP-Glc, UDP-GlcNAc and UDP-GlcAcatalyzed by Cps1aI. The signal peaks of Lacα-PP-$(CH_2)_{11}$-OPh **2** and trisaccharide products in the MALDI-TOF-MS spectra are shown in blue and red solid boxes, respectively (MALDI-TOF-MS error range ±1 Da).



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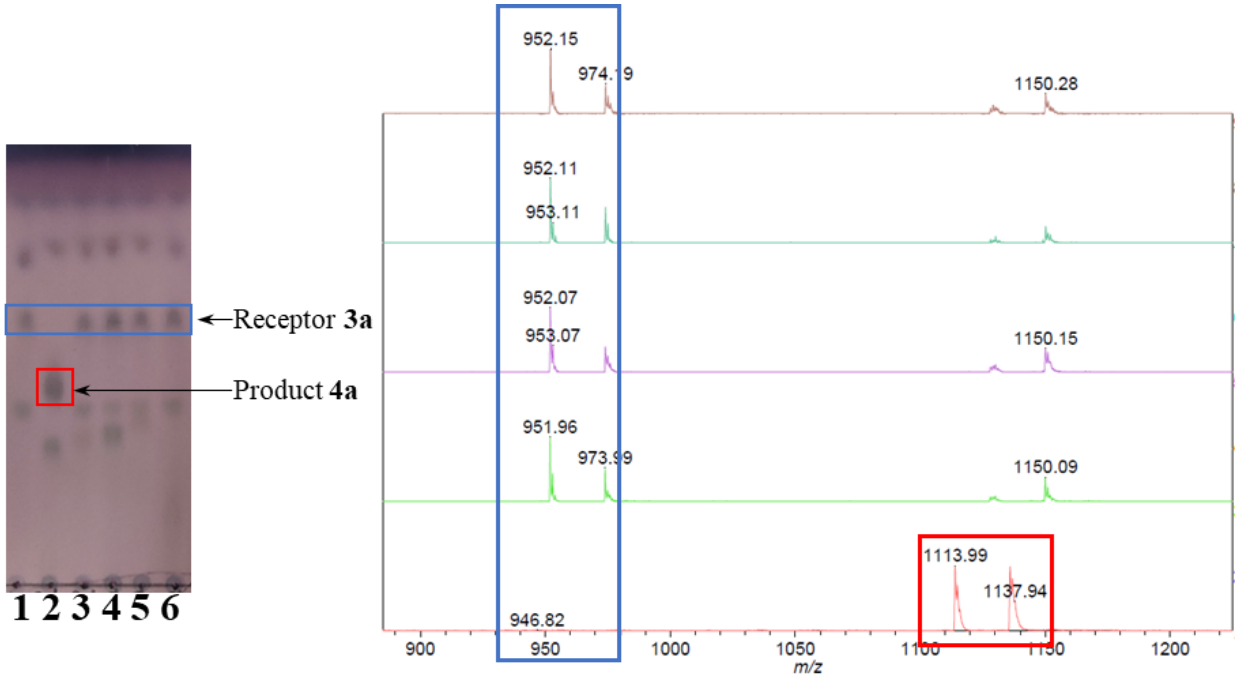
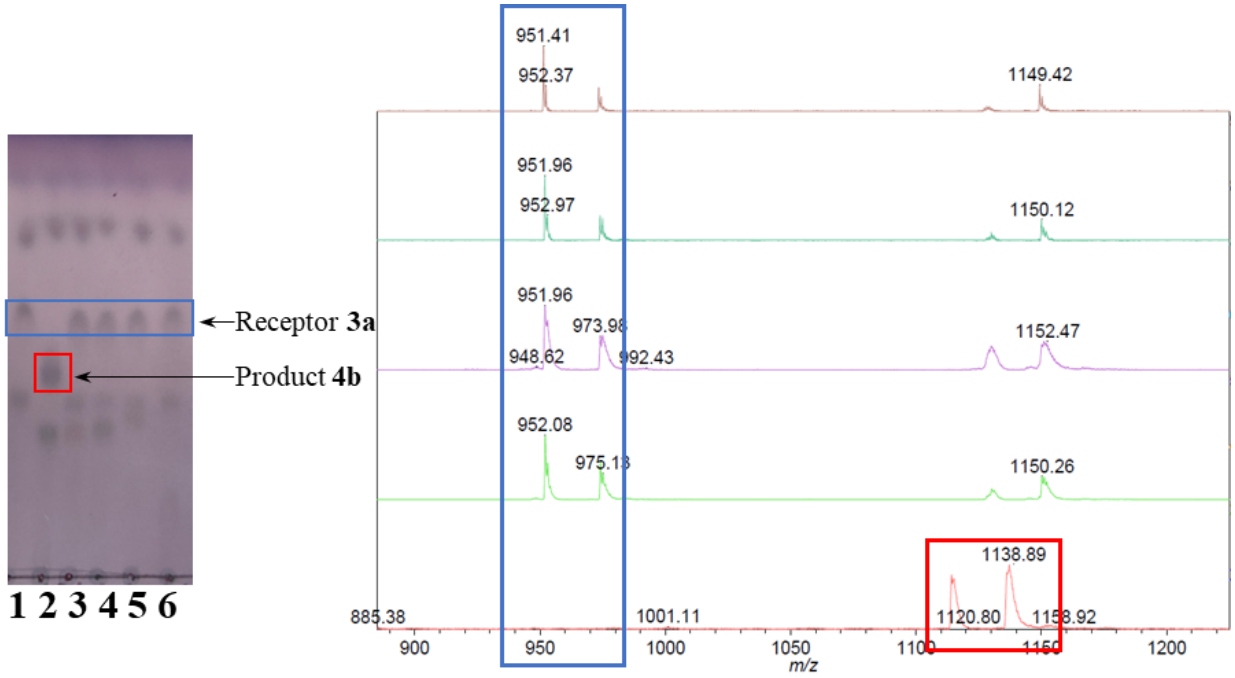


Figure S15. TLC (A) and MALDI-TOF-MS (B) traces of donor substrates recognition of Cps1aJ. ***1***, negative control reaction with heat-deactivated Cps1aJ; ***2-6***, enzyme reactions of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** with UDP-Gal, UDP-GalNAc, UDP-Glc, UDP-GlcNAc and UDP-GlcA catalyzed by Cps1aJ. Positive results are marked with red dotted boxes in TLC analysis; the signal peaks of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** and tetrasaccharide products in the MALDI-TOF-MS spectra are shown in blue and red solid boxes, respectively (MALDI-TOF-MS error range ±1 Da).



FigureS16. TLC (A) and MALDI-TOF-MS (B) traces of donor substrates recognition of Cps1bJ. ***1***, negative control reaction with heat-deactivated Cps1bJ; ***2-6***, enzyme reactions of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** with UDP-Gal, UDP-GalNAc, UDP-Glc, UDP-GlcNAc and UDP-GlcA catalyzed by Cps1bJ. Positive results are marked with red dotted boxes in TLC analysis; the signal peaks of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** and tetrasaccharide products in the MALDI-TOF-MS spectra are shown in blue and red solid boxes, respectively (MALDI-TOF-MS error range ±1 Da)

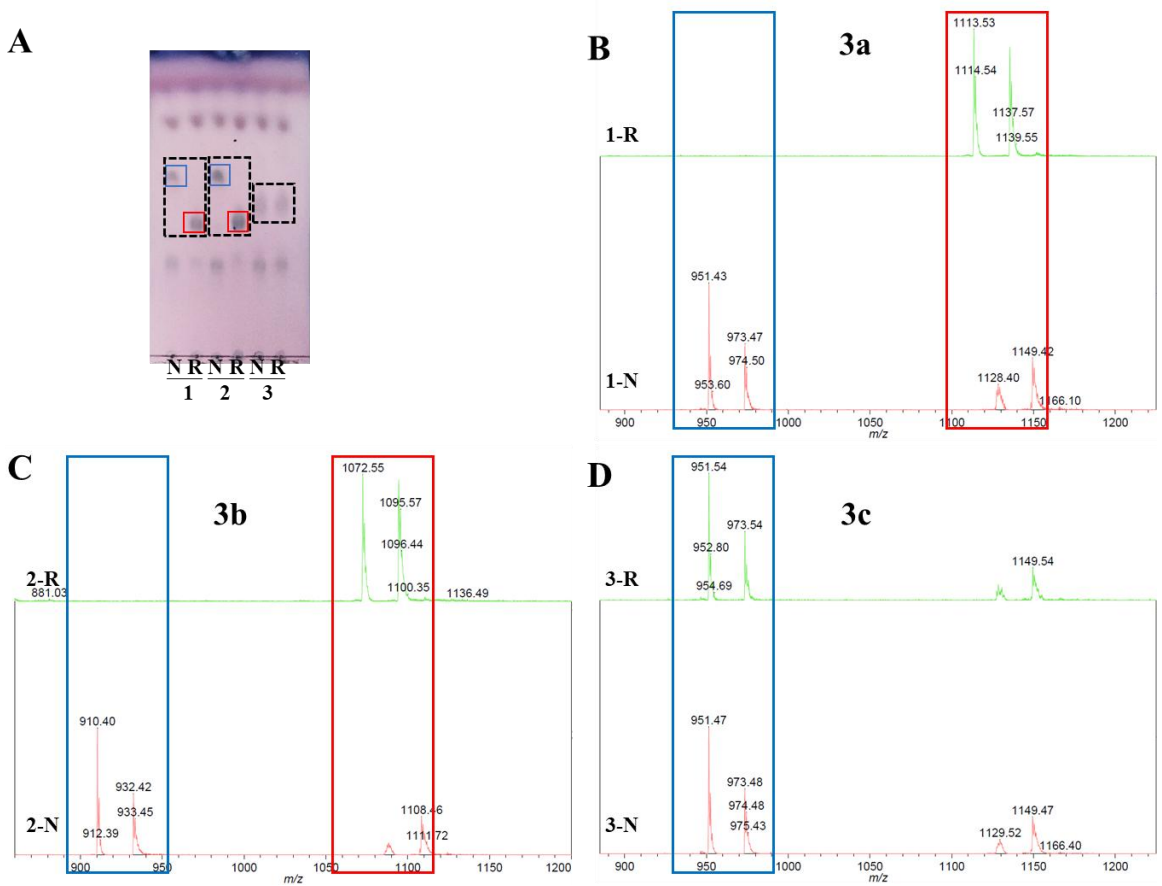
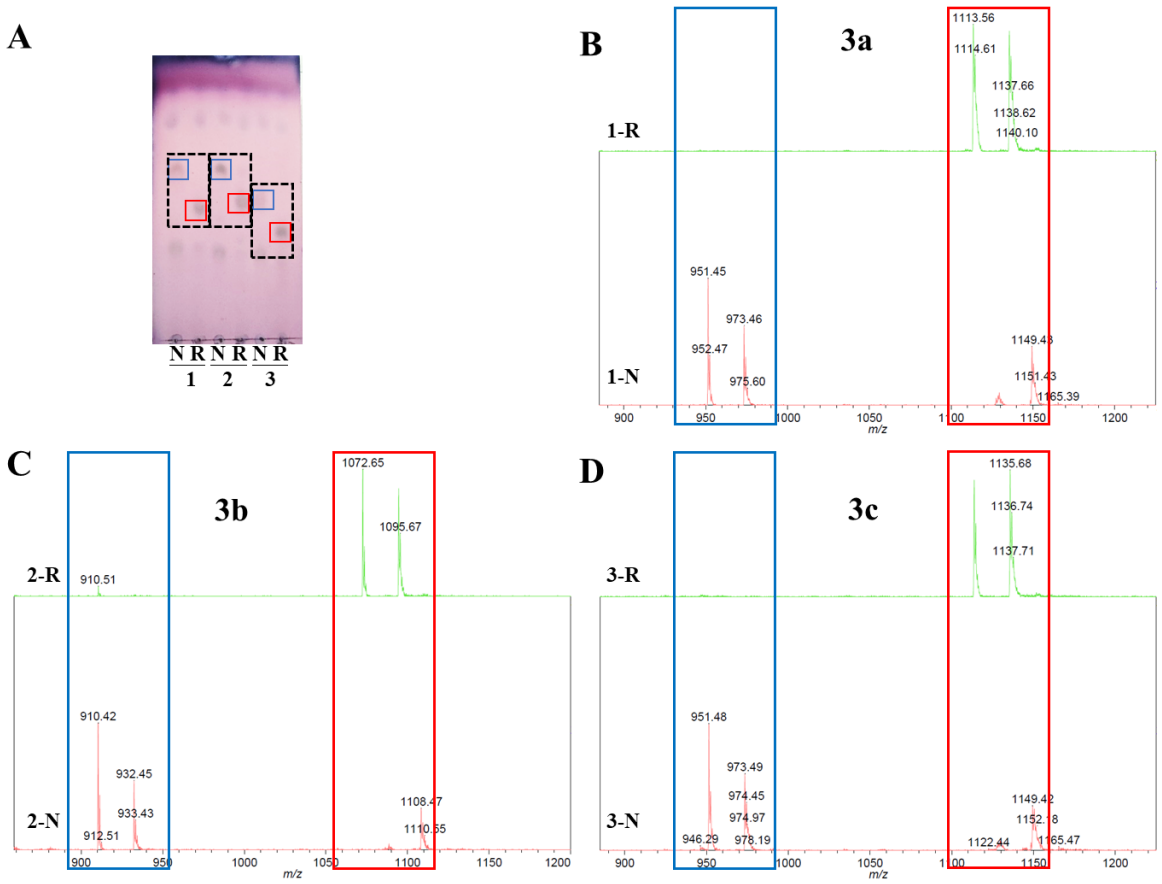


Figure S17. TLC (A) and MALDI-TOF-MS (B-D) traces of receptor substrates recognition of Cps1aJ. ***N***, negative control reaction with heat-deactivated Cps1aJ; ***R***, enzyme reactions of Cps1aJ. ***1-3***, enzyme reactions of UDP-Gal with GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a**, Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** and GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** catalyzed by Cps1aJ. Positive results are marked with red dotted boxes in TLC analysis; the signal peaks of trisaccharide receptor substrates and tetrasaccharide products in the MALDI-TOF-MS spectra are shown in blue and red solid boxes, respectively (MALDI-TOF-MS error range ±1 Da)



FigureS18. TLC (A) and MALDI-TOF-MS (B-D) traces of receptor substrates recognition of Cps1bJ. ***N***, negative control reaction with heat-deactivated Cps1bJ; ***R***, enzyme reactions of Cps1bJ. ***1-3***, enzyme reactions of UDP-Gal with GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a**, Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** and GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** catalyzed by Cps1bJ. Positive results are marked with red dotted boxes in TLC analysis; the signal peaks of trisaccharide receptor substrates and tetrasaccharide products in the MALDI-TOF-MS spectra are shown in blue and red solid boxes, respectively. (MALDI-TOF-MS error range ±1 Da)

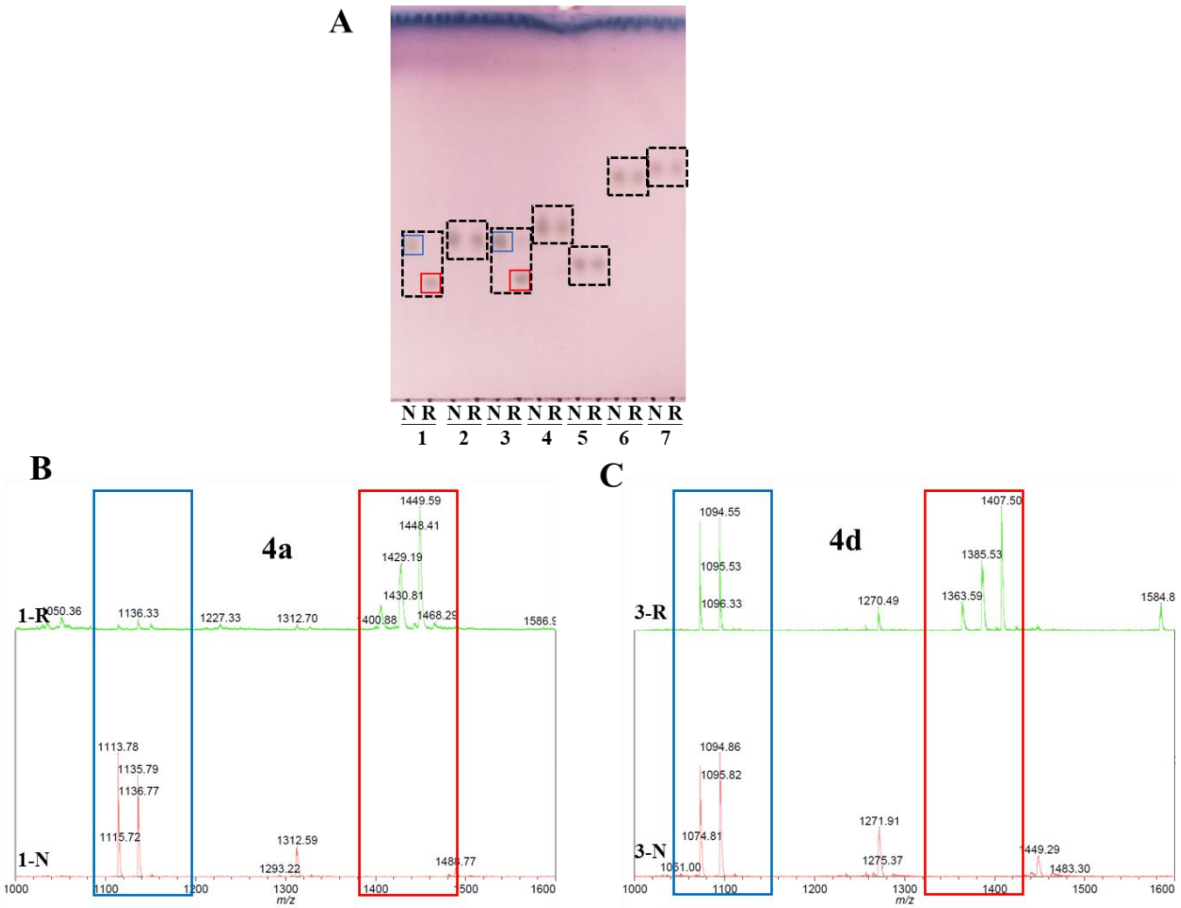


Figure S19. TLC (A) and MALDI-TOF-MS (B-C) traces of receptor substrates recognition of Cps1aK. ***N***, negative control reaction with heat-deactivated Cps1aK; ***R***, enzyme reactions of Cps1aK. ***1-7***, enzyme reactions of CMP-NeuNAc with Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a**, Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b**, Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d**, Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e**, Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c**, Galβ-1,4-Rhaβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh, and Lacα-PP-$(CH_2)_{11}$-OPh **2** catalyzed by Cps1aK. Positive results are marked with red dotted boxes in TLC analysis; the signal peaks of tetrasaccharide receptor substrates and pentasaccharide products in the MALDI-TOF-MS spectra are shown in blue and red solid boxes, respectively. (MALDI-TOF-MS error range ±1 Da).

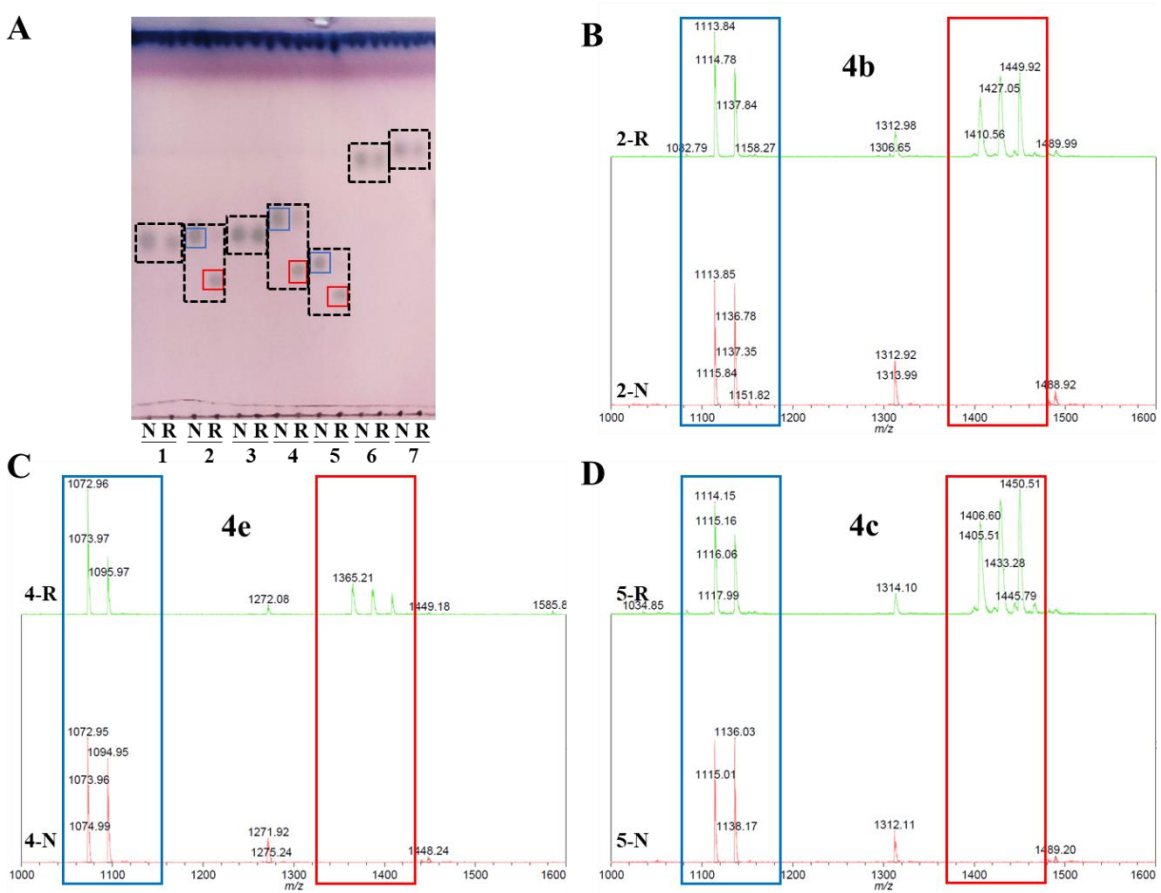


Figure S20. TLC (A) and MALDI-TOF-MS (B-D) traces of receptor substrates recognition of Cps1bK. ***N***, negative control reaction with heat-deactivated Cps1bK; ***R***, enzyme reactions of Cps1bK. ***1-7***, enzyme reactions of CMP-NeuNAc with Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a**, Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b**, Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d**, Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e**, Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c**, Galβ-1,4-Rhaβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh, and Lacα-PP-$(CH_2)_{11}$-OPh **2** catalyzed by Cps1bK. Positive results are marked with red dotted boxes in TLC analysis; the signal peaks of tetrasaccharide receptor substrates and pentasaccharide products in the MALDI-TOF-MS spectra are shown in blue and red solid boxes, respectively. (MALDI-TOF-MS error range ±1 Da).

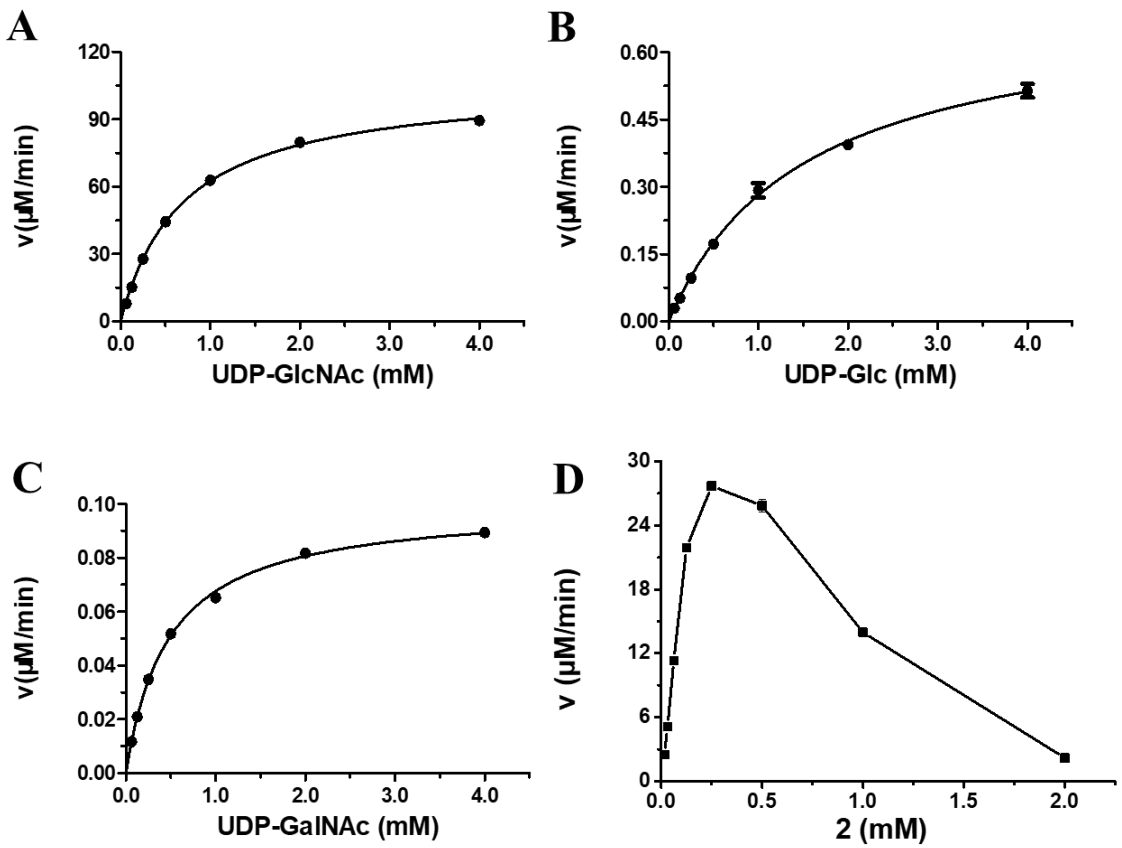


Figure S21. Enzyme kinetics studies of Cps1aI for UDP-GlcNAc (A), UDP-Glc (B) and UDP-GalNAc (C) and the influences of concentration of receptor substrate Lacα-PP-$(CH_2)_{11}$-OPh **2** (D) on the relative enzymatic activities of Cps1aI. Each error bar represents the standard deviation of three experiments.

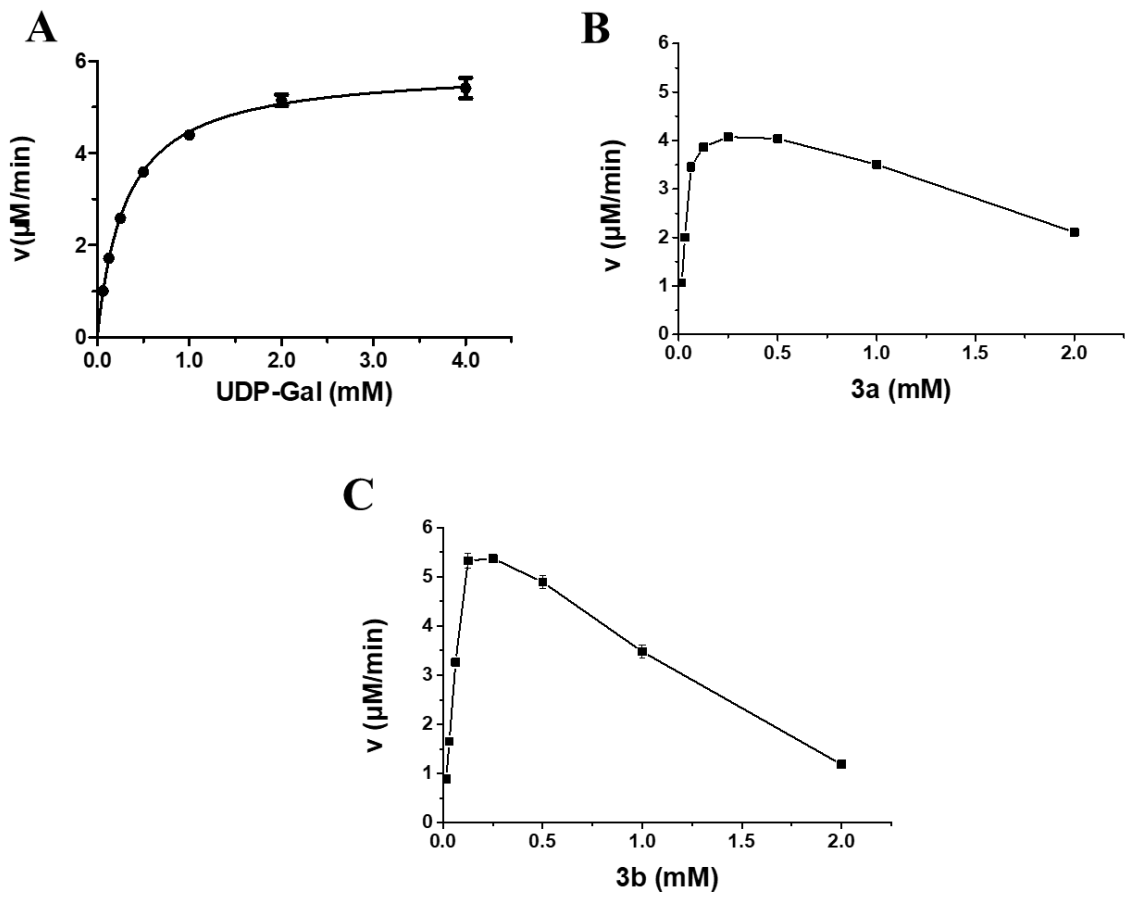


Figure S22. Enzyme kinetics studies of Cps1aJ for UDP-Gal (A) and the influences of concentration of trisaccharide receptor substrate **3a** (B) and **3b** (C) on the relative enzymatic activities of Cps1aJ. Each error bar represents the standard deviation of three experiments.

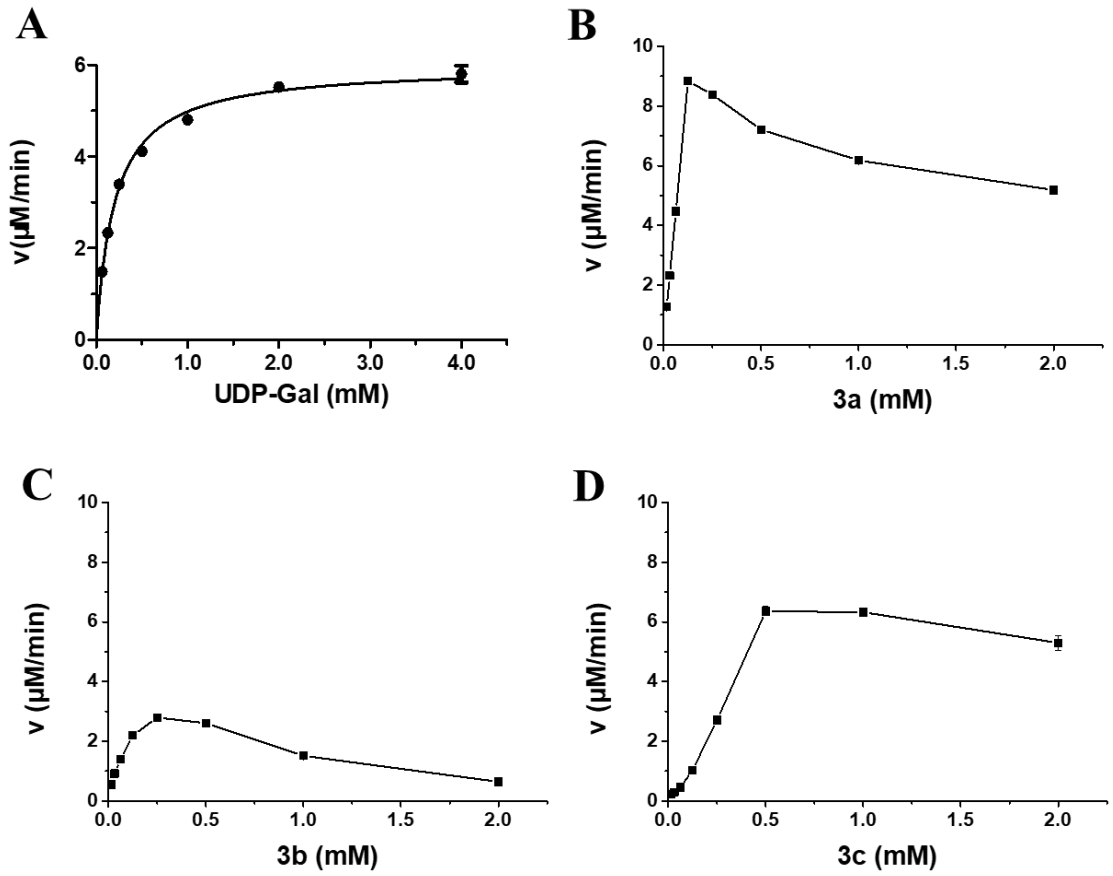


Figure S23. Enzyme kinetics studies of Cps1bJ for UDP-Gal (A) and the influences of concentration of trisaccharide receptor substrate **3a** (B), **3b** (C) and **3c** (D) on the relative enzymatic activities of Cps1bJ. Each error bar represents the standard deviation of three experiments.

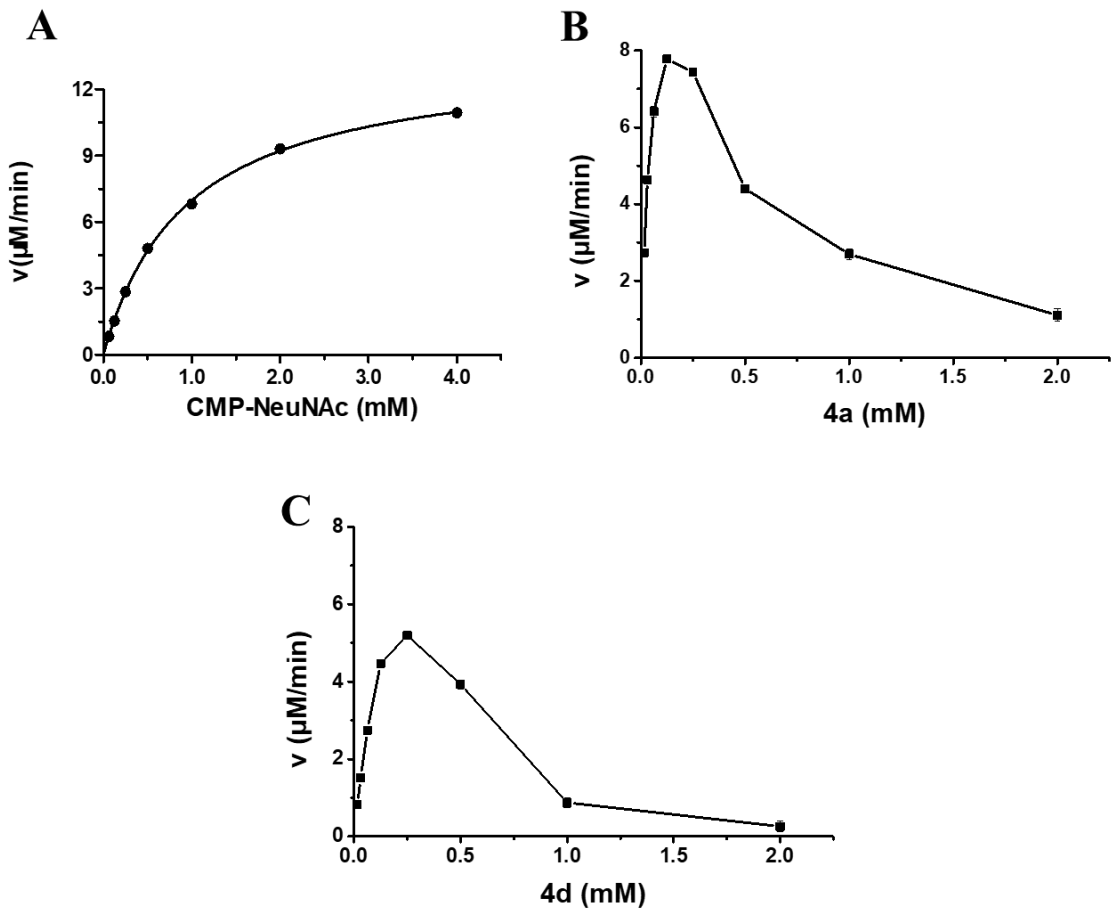


Figure S24. Enzyme kinetics studies of Cps1aK for CMP-NeuNAc (A) and the influences of concentration of tetrasaccharide receptor substrates **4a** (B) and **4d** (C) on the relative enzymatic activities of Cps1aK. Each error bar represents the standard deviation of three experiments.

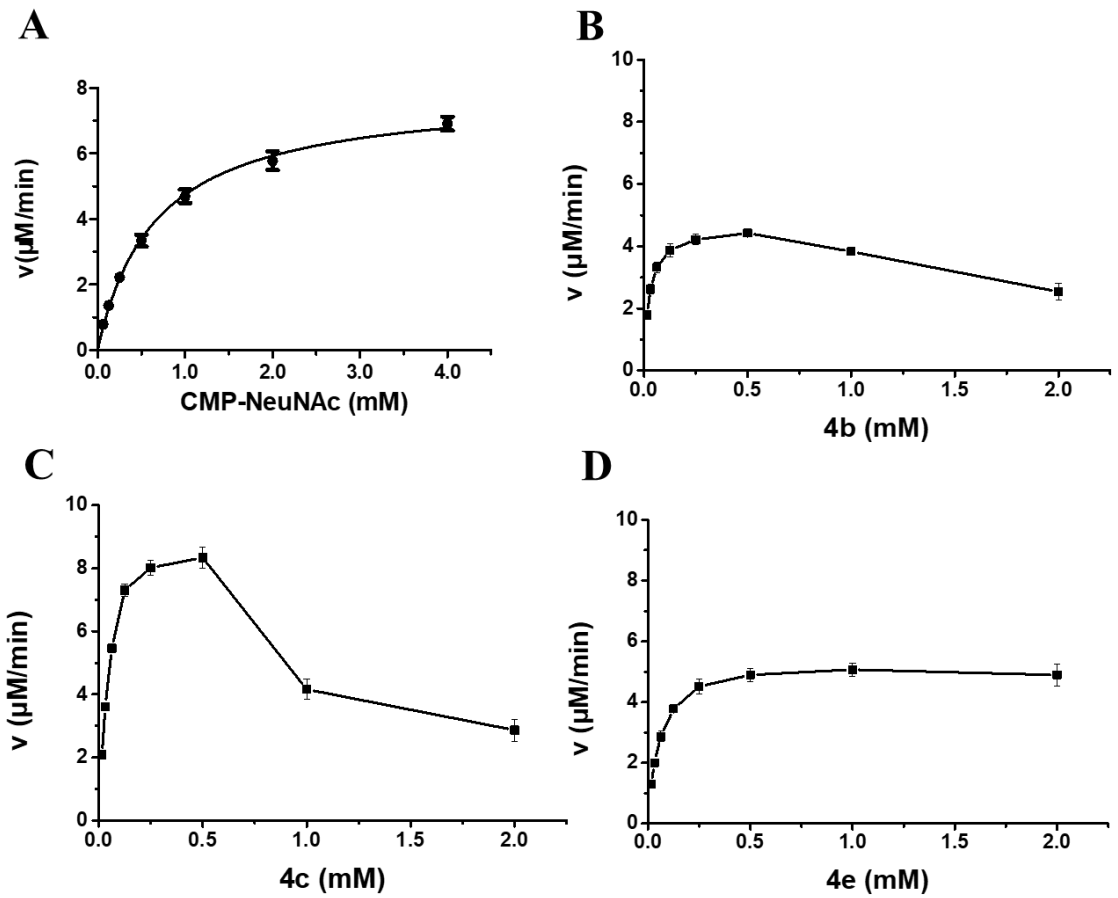


Figure S25. Enzyme kinetics studies of Cps1bK for CMP-NeuNAc (A) and the influences of concentration of tetrasaccharide receptor substrates **4b** (B), **4c** (C) and **4e** (D) on the relative enzymatic activities of Cps1bK. Each error bar represents the standard deviation of three experiments.

II. Experientials Section

Materials. *Escherichia coli* Top10 and BL21(DE3) were used as hosts for transformation and expression, respectively. Both strains were grown in Luria-Bertani (LB) medium supplemented with 100 μg/mL ampicillin at 37 °C. The restriction enzymes were obtained from NEB. The T4 DNA Ligase was from TransGen Company. Yeast extract, agar and tryptone were purchased from OXOID. The C18-SepPak column was from Waters. Other reagents were obtained from Sangon Biotech. Chemicals and solvents were from Sigma-Aldrich.

Preparation of sugar nucleotides. UDP-Glc was prepared using thymidylyltransferase Cps23FL according to the reported method.[1] UDP-GlcNAc, UDP-Gal, and CMP-NeuNAc were prepared using fusion enzymes GalK-USP-ET64, GlmU-NahK-ET64, and NanA-CSS-ET64, respectively, according to the reported protocols.[2]

Cloning, overexpression and purification of Cps1aI, Cps1aJ, Cps1aK, Cps1bJ and Cps1bK. DNA sequences of complete *cpsIaI*, *cpsIaJ, cpsIaK, cpsIbJ* and *cpsIaK* genes of GBS Ia and Ib were derived from the GenBank (CP000114.1, *cps1aI*: 1225745-1226719, 975 bp, *cps1aJ*: 1224764-1225711, 948 bp and *cps1aK*: 1223724-1224680, 957 bp; AB050723.1, *cps1bJ*: 5008-5949, 942 bp and *cps1bK*: 5942-6880, 939 bp) and synthesized by Sangon Biotech. The amplified fragments were digested with the appropriate restriction endonucleases (*Sma* I and *Xho* I for *cps1aI*, *cps1aJ* and *cps1aK*, *Bam*H I and *Xho* I for *cps1bJ* and *cps1bK*), cloned in plasmid pGEX-4T-1, transferred in *E. coli* BL21(DE3) for protein overexpression. Plasmid DNA sequencing was performed by Sangon Biotech. *E. coli* BL21 (DE3) harboring the recombinant plasmids were allowed to grow until OD_{600} reached 0.6-0.8 in Luria-Bertani (LB) medium supplemented with 100 μg/mL ampicillin (37 °C, 200 rpm). After induction by 0.3 mmol/L isopropyl-1-thio-β-D-galactopyranoside (IPTG) for another 20 h (16 °C, 110 rpm), cells were harvested by centrifugation (8,000 rpm, 10 min, 4 °C). The harvested cell pellets which expressed target proteins were re-suspended in phosphate-buffered saline (PBS, pH 7.4), disrupted by sonication and then cleared by centrifugation (12,000 rpm, 30 min, 4 °C). The resultant supernatants of Cps1aI, Cps1aJ, Cps1aK, Cps1bJ and Cps1bK were subjected to a Glutathione Sepharose 4 Fast Flow column (GE Healthcare) which was pre-equilibrated with PBS buffer (pH 7.4). The GST-tagged proteins were eluted with the same buffer containing 10 mmol/L glutathione reductase (GSH) (pH 7.4). The purified fractions were pooled, concentrated and then desalted using an Amicon Ultra 30-kDa centrifugal filter (Millipore). Finally, the purified protein was re-suspended in PBS buffer (pH 7.4) containing 20% glycerol (*v/v*) and stored at -80 °C. The purity and homogeneity of five proteins were identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and their concentrations were determined using the Thermo Scientific™ NanoDrop One calibrated with the extinction coefficient predicted by ExPASy (http://web.expasy.org/protparam/).

Preliminary identification of enzyme activities of Cps1aI, Cps1aJ, Cps1aK, Cps1bJ and Cps1bK. The enzymatic activities of purified Cps1aI, Cps1aJ, Cps1aK, Cps1bJ, and Cps1bK were preliminarily examined in the Tris-HCl buffer (50 mmol/L, pH 7.5) containing 1.2 mmol/L nucleotide sugar (UDP-GlcNAc for Cps1aI, UDP-Gal for Cps1aJ and Cps1bJ, or CMP-NeuNAc for Cps1aK and Cps1bK), 1.0 mmol/L acceptor substrate (Lacα-PP-$(CH_2)_{11}$-OPh **2** for Cps1aI, GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** for

Cps1aJ and Cps1bJ, Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** for Cps1aK or Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** for Cps1bK), 5 mmol/L $MgCl_2$, and 200 μg/mL purified enzyme in a total volume of 20 μL. The reaction was incubated at 25 °C using heat-deactivated corresponding enzyme as the negative control, and then detected at 2 h for Cps1aI, 4 h for Cps1aJ and Cps1bJ, or 1 h for Cps1aK and Cps1bK, respectively, by thin-layer chromatography (TLC) (EtOAc:CH_3OH:H_2O:HOAc = 6:3:2:0.5 (*v/v/v/v*)) or matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) analysis.

Biochemical characterization of β-1,3-N-acetylglucosaminyltransferase Cps1aI. The reaction system for the detail characterization of purified Cps1aI contained 1 mmol/L UDP-GlcNAc, 1 mmol/L Lacα-PP-$(CH_2)_{11}$-OPh **2** and 24 μg/mL of purified enzyme in 50 mmol/L buffer system. Reactions were allowed to proceed for 10 min, terminated by boiling at 100 °C for 30 s, and finally cleared by centrifugation (12,000 rpm, 10 min). Then, the supernatant was analyzed with HPLC (DionexCarboPac™ PA-100 column, 4 × 250 mm, 0-1 mol/L ammonium acetate buffer eluent). The reaction was monitored by generation of the byproduct UDP, which had the strong UV absorption at 260 nm and could be easily quantitated by HPLC.

Investigating the influences of temperatures, pH values and metal cations on the activity of Cps1aI. For determination of temperature effect, the reactions were carried out at different temperatures (10, 16, 20, 25, 30, 37, 42, 50, 55 °C) in 50 mmol/L Tris-HCl buffer (pH 8.5). For determination of pH effect on enzymatic activity, pH values were varied from 5.5 to 10.5 with three different buffer systems at 25 °C. The buffer systems were as follows: Bis-Tris-HCl (50 mmol/L, pH 5.5, 6.0, 6.5, and 7.0), Tris-HCl (50 mmol/L, pH 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5), and Gly-NaOH (50 mmol/L, pH 9.0, 9.5, 10.0 and 10.5). The effects of various metal ions on enzyme activity were assessed in the presence of the following metal salts including 5 mmol/L ethylenediamine tetraacetic acid (EDTA), $MgCl_2$, $MnCl_2$, $CaCl_2$, $NiSO_4$, $CoSO_4$, $FeSO_4$, $CuSO_4$ and $ZnSO_4$ in Tris-HCl buffer (pH 8.5) at 25 °C. To obtain the optimized Mg^{2+} concentration, the enzymatic reactions were carried out under varied concentrations of Mg^{2+} (0.3125-80 mM). Negative controls were performed in parallel under the same conditions using heat-deactivated Cps1aI. Relative activity concluded from pH and temperature test was defined as the relative value to the maximum enzyme activity, and the effect of metal ions on enzyme activity was determined using the activity measured without adding ions as the reference value.

Substrate specificity study of Cps1aI. For acceptor substrate specificity study, Lacα-PP-$(CH_2)_{11}$-OPh **2**, Lac, Lac-O$(CH_2)_2N_3$, Rhaβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh[3], Galα-1,3-Glcα-PP-$(CH_2)_{11}$-OPh[4] and Glcα-1,3-Glcα-PP-$(CH_2)_{11}$-OPh[4] were examined with UDP-GlcNAc as nucleotide donor, respectively. For donor substrate specificity study, UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc and UDP-GlcA were examined with Lacα-PP-$(CH_2)_{11}$-OPh **2** as acceptor substrate, respectively. The reaction was performed in the optimized condition for Cps1aI.

Enzyme kinetics of Cps1aI. The enzymatic reactions were carried out under above-optimized conditions, i.e. in Tris-HCl buffer (50 mmol/L, pH 8.5) containing 12 μg/mL of Cps1aI and 5 mmol/L $MgCl_2$ with varied concentrations of UDP-sugar (UDP-GlcNAc, UDP-Glc or UDP-GalNAc) and Lacα-PP-$(CH_2)_{11}$-OPh **2** at 25 °C. Then, reactions were performed for 10 min using saturated UDP-GlcNAc

(2 mmol/L) and varied concentrations of Lacα-PP-$(CH_2)_{11}$-OPh **2** (0.015625-2.0 mmol/L) or using Lacα-PP-$(CH_2)_{11}$-O-Ph **2** (1 mmol/L) and varied concentrations of UDP-sugar (0.0625-4.0 mmol/L). The results obtained were used to calculate the initial reaction velocities and to determine the Michaelis constant (K_m and k_{cat}) values using the GraphPad Prism 6.04 program.

Biochemical characterization of β-1,4-galactosyltransferase Cps1aJ and β-1,3-galactosyltransferase Cps1bJ
The reaction system for the detail characterization of purified Cps1aJ and Cps1bJ contained 1 mmol/L UDP-Gal, 1 mmol/L GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** and 100 μg/mL of purified enzyme in 50 mmol/L buffer system. Reactions and detected methods followed those as mentioned above.

Investigating the influences of temperatures, pH values and metal cations on the activity of Cps1aJ and Cps1bJ. For determination of temperature effect, the reactions were carried out at different temperatures (10, 16, 20, 25, 30, 37, 42, 50, 55 °C) in 50 mmol/L Tris-HCl buffer (pH 9.0). For determination of pH effect on enzymatic activity, pH values were varied from 5.5 to 10.5 with the foregoing three different buffer systems at 30 °C. The effects of various metal ions on enzyme activity and the optimized Mg^{2+} concentrations were assessed in Tris-HCl buffer (pH 9.0) at 30 °C. Negative controls were performed in parallel under the same conditions using heat-deactivated Cps1aJ or Cps1bJ. Relative activity concluded from pH and temperature test was defined as the relative value to the maximum enzyme activity, and the effect of metal ions on enzyme activity was determined using the activity measured without adding ions as the reference value.

Substrate specificity study of Cps1aJ and Cps1bJ. For acceptor substrate specificity study of the two galactosyltransferases, GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a**, Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** and GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** were examined with UDP-Gal as nucleotide donor, respectively. For donor substrate specificity study, UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc and UDP-GlcA were examined with GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** as acceptor substrate, respectively. The reactions were performed in the optimized condition for Cps1aJ or Cps1bJ.

Enzyme kinetics of Cps1aJ and Cps1bJ. The enzymatic reactions were carried out under above-optimized conditions, i.e. in Tris-HCl buffer (50 mmol/L, pH 9.0) containing 50 μg/mL of Cps1aJ or Cps1bJ and 5 mmol/L $MgCl_2$ with varied concentrations of UDP-Gal and acceptor substrate (GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a**, Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b**, and GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c**) at 30 °C. Then, reactions were performed for 10 min using saturated UDP-Gal (2 mmol/L) and varied concentrations of receptor substrates (0.015625-2.0 mmol/L) or using GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** (1 mmol/L) and varied concentrations of UDP-Gal (0.0625-4.0 mmol/L). The results obtained were used to calculate the initial reaction velocities and determine the Michaelis constant (K_m and k_{cat}) values using the GraphPad Prism 6.04 program.

Biochemical characterization of α-2,3-sialyltransferases Cps1aK and Cps1bK. The reaction system for the detail characterization of purified Cps1aK and Cps1bK contained 1 mmol/L CMP-NeuNAc, 1 mmol/L Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh

4a for Cps1aK (20 μg/mL) or Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** for Cps1bK (200 μg/mL) in 50 mmol/L buffer system. Reactions were allowed to proceed for 10 min, terminated by boiling at 100 °C for 30 s, and finally cleared by centrifugation (12,000 rpm, 10 min). Then, the supernatants were analyzed with HPLC to quantitate formation of pentasaccharide NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** for Cps1aK or NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** for Cps1bK that had the strong UV absorption at 211 nm (C18 reverse phase column, 4.6 × 250 mm, 10-100% methanol in water containing 10 mmol/L NH_4HCO_3 as gradient eluent).

Investigating the influences of temperatures, pH values and metal cations on the activity of Cps1aK and Cps1bK. For determination of temperature effect, the reactions were carried out at different temperatures in 50 mmol/L Tris-HCl buffer (pH 7.0). For determination of pH effect on enzymatic activity, pH values were varied from 5.5 to 10.5 with the aforementioned three different buffer systems at 25 °C. The effects of various metal ions on enzyme activity and the optimized Mg^{2+} concentrations were assessed in Tris-HCl buffer (pH 7.0) at 25 °C. Negative controls were performed in parallel under the same conditions using heat-deactivated Cps1aK or Cps1bK. Relative activity concluded from pH and temperature test was defined as the relative value to the maximum enzyme activity, and the effect of metal ions on enzyme activity was determined using the activity measured without adding ions as the reference value.

Acceptor substrate specificity study of Cps1aK and Cps1bK. For acceptor substrate specificity study of purified enzymes, Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a**, Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b**, Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c**, Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d**, Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e**, Galβ-1,4-Rhaβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh[3] and Lacα-PP-$(CH_2)_{11}$-OPh **2** were examined with CMP-NeuNAc as nucleotide donor, respectively.

Enzyme kinetics of Cps1aK and Cps1bK. The enzymatic reactions were carried out under above-optimized conditions, i.e. in Tris-HCl buffer (50 mmol/L, pH 7.0) containing 10 μg/mL of Cps1aK or 100 μg/mL of Cps1bK with varied concentrations of CMP-NeuNAc and acceptor substrate (**4a** and **4d** for Cps1aK, or **4c**, **4d**, and **4e** for Cps1bK) at 25 °C. Then, reactions were performed for 10 min using saturated CMP-NeuNAc (2 mmol/L) and varied concentrations of acceptor substrates (0.015625-2.0 mmol/L) or using corresponding acceptor substrate (1 mmol/L) and varied concentrations of CMP-NeuNAc (0.0625-4.0 mmol/L). The results obtained were used to calculate the initial reaction velocities and determine the Michaelis constant (K_m and k_{cat}) values using the GraphPad Prism 6.04 program.

Chemical synthesis of Lacα-PP-$(CH_2)_{11}$-OPh 2.

Acetylation. A solution of Ac_2O (30 mL) and NaOAc (5 g) was heated up to 140°C (reflux), and lactose (5 g) was added in portion. The resulting mixture was stirred at 140°C for 1 h and poured into ice water (100 mL). The mixed solution was extracted with EtOAc (100 mL), washed by $NaHCO_3$ (aq) and NaCl (aq), dried over Na_2SO_4, filtered and concentrated. The yellow-oil product was recrystallized from MeOH/CH_2Cl_2 (550mL, v/v 10:1) to give 5.5 g white solid peracetylated lactose ($LacAc_8$).

Phosphorylation.[5,6] Crystalline phosphoric acid (3 g) was dried *in vacuo* over phosphorous pentoxide for 12 h. The peracetylated lactose (3.4 g) was added and the mixture was heated at 60°C *in vacuo*. After 2 h, heating was ceased and the resulting dark black mixture was dissolved in anhydrous tetrahydrofuran (THF) (25 mL). The solution was cooled to 0°C and concentrated ammonium hydroxide (2.5 mL) was added until ~pH 7. The precipitate of ammonium phosphate was filtered off and washed with THF (100 mL). The combined filtrates were evaporated to give a syrupy residue that was purified by flash column chromatography (EtOAc 100% to EtOAc/MeOH 50/50). The peracetylated lactose-1-phosphate (LacAc7-1-P, 1.7 g) was obtained as a white solid.

Deacetylation. Sodium hydroxide (0.1 mol/L) was slowly dripped into MeOH (50 mL) containing LacAc7-1-P (1.0 g). The reaction was stirred at room temperature until white turbidity appeared, then 50 mL water and 1M sodium hydroxide was added to the reaction solution until ~pH 7. Fractions containing crude products in methanol and water mixed solvent were collected and concentrated, and the resultant supernatant was performed by gel filtration with Bio-Gel P2 and resultant product was collected and then lyophilized. The product lactose-1-phosphate disodium salt (Lac-1-P, 550 mg) was obtained as white powder.

$MgCl_2$-catalyzed diphosphate bond formation.[3,7] Amixture of 11-phenoxyundecyl dihydrogen phosphate (550 mg, 1.6 mmol) and *N, N′*-carbonyldiimidazole (1.0 g, 6.4 mmol) in anhydrous THF (5 mL) was stirred at room temperature for 4 h, and then dry methanol (0.5 mL) was added. The resulting solution was stirred for another 1 h, and then concentrated to yield the crude 11-phenoxyundecyl dihydrogen phosphorimidazolide which was directly used for next step. The above generated product was added to a vigorously stirred suspension of Lac-1-P (500 mg, 1.05 mmol) and $MgCl_2$ (250 mg, 2.6 mmol) in *N, N*-dimethylformamide (DMF) (5.0 mL), and the resulting reaction mixture was then stirred for 8 h at room temperature, at which time TLC analysis (EtOAc:CH_3OH:H_2O:HOAc = 8:3:2:0.5 (*v/v/v/v*)) indicated the completion of reaction. Then, the reaction mixture was filtered and loaded on a Sephadex LH-20 column for purification using methanol as an eluent. Fractions containing the desired product were collected, concentrated, and further purified on a silica gel flash column chromatography using 10:3:2 EtOAc-CH_3OH-H_2O as eluents. The desired product Lacα-PP-$(CH_2)_{11}$-OPh **1** (650 mg, 81%) was obtained as white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O): δ 7.36–7.32 (m, 2H, Ph), 7.02–6.67 (m, 3H, Ph), 5.50 (dd, J = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.41 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.08–4.02 (m, 2H, -CH_2CH_2OPh), 3.99 (br d, J = 10.2, 1H, H-5^{Glc}), 3.91 (q, J = 6.6 Hz, 2H, -OCH_2CH_2-), 3.89–3.81 (m, 4H, H-3^{Glc}, H-6a,b^{Glc}, H-4^{Gal}), 3.78 (dd, J = 11.4, 7.8 Hz, 1H, H-6a^{Gal}), 3.73–3.65 (m, 3H, H-4^{Glc}, H-5,6a^{Gal}), 3.62 (dd, J = 10.2, 3.6 Hz, 1H, H-3^{Gal}), 3.56–3.50 (m, 2H, H-2^{Glc}, H-2^{Gal}), 1.77–1.69 (m, 2H, -CH_2CH_2-), 1.64–1.57 (m, 2H, -CH_2CH_2-), 1.44–1.37 (m, 2H, -CH_2CH_2-), 1.35–1.25 (m, 12H, -CH_2CH_2-); ^{13}C NMR (150 MHz, D_2O): δ 158.0, 129.7 (2C), 121.3, 114.8 (2C), 102.8 (C-1^{Gal}), 95.0 (d, $J_{C,P}$ = 6.0 Hz, C-1^{Glc}), 77.5 (C-4^{Glc}), 75.2 (C-5^{Gal}), 72.4 (C-3^{Gal}), 71.4 (C-3^{Glc}), 71.3 (C-5^{Glc}), 71.2 (d, $J_{C,P}$ = 7.5 Hz, C-2^{Glc}), 70.8 (C-2^{Gal}), 68.6 (-OCH_2CH_2OPh), 68.5 (C-4^{Gal}), 66.9 (d, $J_{C,P}$ = 6.0 Hz, -OCH_2CH_2-), 60.9 (C-6^{Gal}), 59.5 (C-6^{Glc}), 29.7 (d, $J_{C,P}$ = 7.5 Hz, -OCH$_2CH_2$-), 28.5 (3C), 28.4, 28.3, 28.2 25.0, 24.8 (8C, -OCH$_2CH_2$(CH_2)$_8$$CH_2$OPh); ^{31}P NMR (243 MHz, D_2O): δ -10.70 (d, J = 19.4 Hz), -13.13 (d, J = 19.4 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for $C_{29}H_{50}O_{18}P_2$ 747.2400 [M - H]$^-$; Found 747.2404.

Enzymatic synthesis of trisaccharides 3a, 3b and 3c. Milligram-scale production of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a**, Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** and GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** were performed in a 10 mL

reaction system containing 2.4 mmol/L UDP-sugar (UDP-GlcNAc、UDP-Glc or UDP-GalNAc), 2 mmol/L Lacα-PP-$(CH_2)_{11}$-OPh **2**, 5 mmol/L $MgCl_2$ and 50 μg/mL purified Cps1aI in 50 mmol/L Tris-HCl buffer (pH 8.5). The solutions were incubated at 25 °C for 2 h, 5 h or 12 h until the substrate **2** was completely converted to trisaccharide product as monitored by TLC analysis, and then quenched by boiling for 30 s. The mixtures were vortexed and centrifuged to remove the formed precipitate and the resultant supernatants were freeze-dried, re-suspended with methanol (5 mL) and filtered to remove the precipitation again. The filtrates were loaded on a semi-preparative HPLC (C18 reverse phase column: 10 × 250 mm, 10-100% methanol in water containing 10 mmol/L NH_4HCO_3 as gradient eluent) in batches for purification. The fractions containing trisaccharide product were pooled and concentrated to produce **3a** (16.5 mg, 87%), **3b** (14.6 mg, 80%) and **3c** (15.8 mg, 83%) as white solid.

3a: ^{1}H NMR (600 MHz, D_2O): δ 7.37 (t, *J* = 7.8 Hz, 2H, Ph), 7.06-7.01 (m, 3H, Ph), 5.57 (br s, 1H, H-1^{Glc}), 4.70 (d, *J* = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.42 (d, *J* = 7.8 Hz, 1H, H-1^{Gal}), 4.11 (d, *J* = 3.6 Hz, 1H, H-4^{Gal}), 4.09 (t, *J* = 6.0 Hz, 2H, -CH_2CH_2OPh), 4.01 (br d, *J* = 10.2 Hz, 1H, H-5^{Glc}), 3.96–3.90 (m, 2H, -OCH_2CH_2-), 3.90–3.83 (m, 4H, H-3^{Glc}, H-6a,b^{Glc}, H-6a^{GlcNAc}), 3.81–3.66 (m, 7H, H-4^{Glc}, H-3^{Gal}, H-5^{Gal}, H-6a,b^{Gal}, H-2^{GlcNAc}, H-6b^{GlcNAc}), 3.62–3.52 (m, 3H, H-2^{Glc}, H-2^{Gal}, H-3^{GlcNAc}), 3.32–3.27 (m, 2H, H-4,5^{GlcNAc}), 2.02 (s, 3H, CH_3CO), 1.78–1.72 (m, 2H, -CH_2CH_2-), 1.66–1.59 (m, 2H, -CH_2CH_2-), 1.46–1.40 (m, 2H, -CH_2CH_2-), 1.37–1.26 (m, 12H, -CH_2CH_2-); ^{13}C NMR (150 MHz, D_2O): δ 174.9 (CH_3CO), 158.0, 129.8 (2C), 121.3, 114.9 (2C), 102.8 (C-1^{Gal}), 102.7 (C-1^{GlcNAc}), 95.07 (d, $J_{C,P}$ = 6.0 Hz, C-1^{Glc}), 81.6 (C-3^{Gal}), 77.4 (C-4^{Glc}), 75.6 (C-5^{GlcNAc}), 74.8 (C-5^{Gal}), 73.5 (C-3^{GlcNAc}), 71.43 (C-3^{Glc}), 71.37 (C-5^{Glc}), 71.28 (d, $J_{C,P}$ = 7.5 Hz, C-2^{Glc}), 70.0 (C-2^{Gal}), 69.6 (C-4^{GlcNAc}), 68.7 (-OCH_2CH_2OPh), 68.4 (C-4^{Gal}), 66.9 (d, $J_{C,P}$ = 6.0 Hz, -OCH_2CH_2-), 60.9 (C-6^{Gal}), 60.4 (C-6^{GlcNAc}), 59.5 (C-6^{Glc}), 55.6 (C-2^{GlcNAc}), 29.7 (d, $J_{C,P}$ = 6.0 Hz, -OCH$_2$$CH_2$-), 28.57, 28.54, 28.52, 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -OCH_2CH_2(CH_2)$_8$$CH_2$OPh), 22.1 ($CH_3$CO); ^{31}P NMR (243 MHz, D_2O): δ -10.69 (d, *J* = 12.1 Hz), -13.12 (d, *J* = 12.1 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for $C_{37}H_{62}NO_{23}P_2$ 950.3193 [M - H]$^-$; Found 950.3190.

3b: ^{1}H NMR (600 MHz, D_2O): δ 7.35 (t, *J* = 7.8 Hz, 2H, Ph), 7.03–6.99 (m, 3H, Ph), 5.56 (br s, 1H, H-1^{Glc}), 4.63 (d, *J* = 7.8 Hz, 1H, H-1$^{Glc'}$), 4.47 (d, *J* = 7.8 Hz, 1H, H-1^{Gal}), 4.14 (d, *J* = 3.2 Hz, 1H, H-4^{Gal}), 4.06 (t, *J* = 6.6 Hz, 2H, -CH_2CH_2OPh), 3.99 (br d, *J* = 10.2 Hz, 1H, H-5^{Glc}), 3.94–3.89 (m, 2H, -OCH_2CH_2-), 3.89–3.81 (m, 4H, H-3^{Glc}, H-6a,b^{Glc}, H-6a$^{Glc'}$), 3.80–3.74 (m, 2H, H-3^{Gal}, H-6a^{Gal}), 3.73–3.66 (m, 5H, H-4^{Glc}, H-2,5,6b^{Gal}, H-6b$^{Glc'}$), 3.54 (br d, *J* = 9.6 Hz, 1H, H-2^{Glc}), 3.47 (t, *J* = 9.0 Hz, 1H, H-3$^{Glc'}$), 3.43–3.36 (m, 2H, H-4,5$^{Glc'}$), 3.20–3.16 (m, 1H, H-2$^{Glc'}$), 1.77–1.71 (m, 2H, -CH_2CH_2-), 1.64–1.57 (m, 2H, -CH_2CH_2-), 1.45–1.37 (m, 2H, -CH_2CH_2-), 1.36–1.24 (m, 12H, -CH_2CH_2-); ^{13}C NMR (150 MHz, D_2O): δ 158.0, 129.8 (2C), 121.3, 114.8 (2C), 103.6 (C-1$^{Glc'}$), 102.4 (C-1^{Gal}), 95.1 (d, *J* = 6.0 Hz, C-1^{Glc}), 81.8 (C-3^{Gal}), 77.3 (C-4^{Glc}), 75.6 (C-5$^{Glc'}$), 75.4 (C-3$^{Glc'}$), 74.8 (C-5^{Gal}), 73.1 (C-2$^{Glc'}$), 71.4 (C-3^{Glc}), 71.32 (C-5^{Glc}), 71.27 (d, $J_{C,P}$ = 7.5 Hz, C-2^{Glc}), 70.0 (C-2^{Gal}), 69.3 (C-4$^{Glc'}$), 68.6 (-OCH_2CH_2OPh), 68.2(C-4^{Gal}), 66.9 (d, *J* = 6.0 Hz, -OCH_2CH_2-), 60.9 (C-6^{Gal}), 60.3 (C-6$^{Glc'}$), 59.5 (C-6^{Glc}), 29.7 (d, *J* = 7.5 Hz, -OCH$_2$$CH_2$-), 28.6, 28.5 (2C), 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -OCH_2CH_2(CH_2)$_8$$CH_2$OPh); ^{31}P NMR (243 MHz, D_2O): δ- 10.68 (d, *J* = 21.8 Hz), -13.11 (d, *J* = 21.8 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for $C_{35}H_{59}O_{23}P_2$ 909.2928 [M - H]$^-$; Found 909.2934.

3c: ^{1}H NMR (600 MHz, D_2O): δ 7.30 (t, *J* = 7.8 Hz, 2H, Ph), 6.99–6.94 (m, 3H, Ph), 5.50 (dd, *J* = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.54 (dd, *J* = 8.4, 1H, H-1^{GalNAc}), 4.35 (dd, *J* = 7.8, 1H, H-1^{Gal}), 4.06 (d, *J* = 3.0 Hz, 1H, H-4^{Gal}), 4.01 (t, *J* = 6.6 Hz, 2H, -CH_2CH_2OPh), 3.95 (br d, *J* = 10.2 Hz, 1H, H-5^{Glc}), 3.90–3.76 (m, 7H, H-3^{Glc}, H-6a,b^{Glc}, H-2,4^{GalNAc}, -OCH_2CH_2-), 3.74–3.60 (m, 8H, H-4^{Glc}, H-3,5^{Gal}, H-6a,b^{Gal},

H-3^{GalNAc}, H-6a,b^{GalNAc}), 3.60-3.56 (m, 1H, H-5^{GalNAc}), 3.54–3.48 (m, 2H, H-2^{Glc}, H-2^{Gal}), 1.96 (s, 3H, CH_3CO), 1.70–1.65 (m, 2H, -CH_2CH$_2$-), 1.60–1.52 (m, 2H, -CH$_2$CH_2-), 1.40–1.32 (m, 2H, -CH_2CH$_2$-), 1.31–1.19 (m, 12H, -CH$_2$CH_2-); ^{13}C NMR (150 MHz, D$_2$O): δ 175.0 (CH$_3$*C*O), 157.9, 129.7 (2C), 121.2, 114.7 (2C), 103.2 (C-1^{GalNAc}), 102.7 (C-1^{Gal}), 95.0 (d, *J* = 6.4 Hz, C-1^{Glc}), 81.3 (C-3^{Gal}), 77.2 (C-4^{Glc}), 74.8 (C-5^{GalNAc}), 74.7 (C-5^{Gal}), 71.3 (C-3^{Glc}), 71.3 (C-5^{Glc}), 71.2 (d, *J* = 9.0 Hz, C-2^{Glc}), 70.6 (C-3^{GalNAc}), 70.0 (C-2^{Gal}), 68.53 (-OCH_2CH$_2$OPh), 68.45 (C-4^{Gal}), 67.6 (C-4^{GalNAc}), 66.9 (d, *J* = 6.0 Hz, -O*C*H$_2$CH$_2$-), 60.89 (C-6^{Gal}), 60.85 (C-6^{GalNAc}), 59.4 (C-6^{Glc}), 52.4 (C-2^{GalNAc}), 29.6 (d, *J* = 6.0 Hz, -OCH$_2$*C*H$_2$-), 28.52, 28.49, 28.48, 28.32, 28.25, 28.15, 25.0, 24.7 (8C, -OCH$_2$CH$_2$(*C*H$_2$)$_8$CH$_2$OPh), 22.1 (*C*H$_3$CO); ^{31}P NMR (243 MHz, D$_2$O): δ -10.76 (d, *J* = 19.4 Hz), -13.17 (d, *J* = 19.4 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for $C_{37}H_{62}NO_{23}P_2$ 950.3193 [M - H]$^-$; Found 950.3194.

Enzymatic synthesis of tetrasccharides 4a, 4b, 4c, 4d and 4e

Procedure A. Milligram-scale production systems of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **4a** and Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **4d** were 2 mL reaction mixture of 50 mmol/L Tris-HCl buffer (pH 8.5) containing 2.4 mmol/L UDP-Gal, 2 mmol/L **3a** or **3b**, 5 mM MgCl$_2$ and 200 μg/mL Cps1aJ. Milligram-scale production systems of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **4b**, Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **4c** and Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **4e** were 2 mL reaction mixture of 50 mmol/L Tris-HCl buffer (pH 8.5) containing 2.4 mmol/L UDP-Gal, 2 mmol/L **3a**, **3b**, or **3c**, 5 mM MgCl$_2$ and 200 μg/mL Cps1bJ. All of the reaction systems were incubated at 30 °C for 12 h, at which time TLC indicated the complete consumption of acceptor substrate, and then quenched by boiling for 30 s. After purification protocol as mentioned above, the fractions containing desired product were collected, then concentrated and lyophilized to afford **4a** (3.6 mg, 81%), **4d** (3.4 mg, 79%), **4b** (3.5 mg, 79%), **4c** (3.2 mg, 72%) and **4e** (3.3 mg, 77%) as white powders.

Procedure B. The one-pot two-enzyme synthesis of **4a**, **4b**, **4c**, **4d** and **4e** was achieved with Cps1aI and Cps1aJ or Cps1bJ using Lacα-PP-(CH$_2$)$_{11}$-OPh **2** as the starting acceptor substrate. A 5 mL reaction mixture of 50 mmol/L Tris-HCl buffer (pH 8.5) containing 2.4 mmol/L UDP-GlcNAc/UDP-Glc, 2.4 mmol/L UDP-Gal, 2 mmol/L **2**, 5 mmol/L MgCl$_2$, 50 μg/mL Cps1aI and 200 mg/mL Cps1aJ to producing **4a**/**4d**. A 5 mL reaction mixture of 50 mmol/L Tris-HCl buffer (pH 8.5) containing 2.4 mmol/L UDP-GlcNAc, UDP-Glc or UDP-GalNAc, 2.4 mmol/L UDP-Gal, 2 mmol/L **2**, 5 mmol/L MgCl$_2$, 50 μg/mL Cps1aI and 200 mg/mL Cps1bJ to producing **4b**/**4c**/**4e**. All of the reaction systems were incubated at 25 ℃ for 12 h, at which time TLC indicated the complete consumption of acceptor substrate, and then quenched by boiling for 30 s. After purification protocol as mentioned above, the fractions containing desired product were collected, then concentrated and lyophilized to afford **4a** (8.9 mg, 80%), **4d** (8.3 mg, 77%), **4b** (9.2 mg, 84%), **4c** (7.8 mg, 70%) and **4e** (7.9 mg, 74%) as white powders.

4a: ^{1}H NMR (600 MHz, D$_2$O): δ 7.37 (t, *J* = 7.8 Hz, 2H), 7.06–7.00 (m, 3H), 5.57 (dd, *J* = 6.6, 3.0 Hz, 1H, H-1^{Glc}), 4.69 (d, *J* = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.46 (d, *J* = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.40 (d, *J* = 7.2 Hz, 1H, H-1^{Gal}), 4.12 (d, *J* = 3.0 Hz, 1H, H-4^{Gal}), 4.08 (t, *J* = 6.4 Hz, 2H, -CH$_2$CH_2OPh), 4.01 (br d, *J* = 10.2 Hz, 1H, H-5^{Glc}), 3.96–3.81 (m, 8H, H-3^{Glc}, H-6a,b^{Glc}, H-4$^{Gal'}$, H-6a,b^{GlcNAc}, -OCH_2CH$_2$-), 3.81–3.62 (m, 12H, H-4^{Glc}, H-3,5^{Gal}, H-6a,b^{Gal}, H-2,4,5^{GlcNAc}, H-3,5$^{Gal'}$, H-6a,b$^{Gal'}$), 3.61–3.50 (m, 4H, H-2^{Glc}, H-2^{Gal}, H-3^{GlcNAc}, H-2$^{Gal'}$), 2.02 (s, 3H, CH_3CO), 1.79–1.72 (m, 2H, -CH_2CH$_2$-), 1.66–1.59 (m, 2H, -CH$_2$CH_2-), 1.46–1.40 (m, 2H, -CH_2CH$_2$-), 1.36–1.25 (m, 12H, -CH$_2$CH_2-); ^{13}C NMR (150 MHz, D$_2$O): δ 174.8 (CH$_3$*C*O), 158.0, 129.8 (2C), 121.3, 114.8 (2C), 102.7 (2C, C-1^{Gal}, C-1$^{Gal'}$), 102.6 (C-

1^{GlcNAc}), 95.0 (d, J = 6.0 Hz, C-1^{Glc}), 81.7 (C-3^{Gal}), 78.0 (C-4^{GlcNAc}), 77.4 (C-4^{Glc}), 75.2 (C-5^{GlcNAc}), 74.7 (C-5^{Gal}), 74.4 (C-3^{GlcNAc}), 72.4 (C-3$^{Gal'}$), 72.1 (C-5$^{Gal'}$), 71.4 (C-3^{Glc}), 71.34 (C-5^{Glc}), 71.25 (d, J = 7.5 Hz, C-2^{Glc}), 70.8 (C-2$^{Gal'}$), 69.9 (C-2^{Gal}), 68.6 (-OCH_2CH$_2$OPh), 68.44 (C-4$^{Gal'}$), 68.36 (C-4^{Gal}), 66.9 (d, J = 6.0 Hz, -OCH_2CH$_2$-), 60.9 (C-6^{Gal}), 60.87 (C-6$^{Gal'}$), 59.7 (C-6^{GlcNAc}), 59.5 (C-6^{Glc}), 55.1(C-2^{GlcNAc}), 29.7 (d, J = 7.5 Hz, -OCH$_2$CH_2-), 28.5, 28.5 (2C), 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -OCH$_2$CH$_2$(CH_2)$_8$CH$_2$OPh), 22.1 (CH_3CO); ^{31}P NMR (243 MHz, D$_2$O): δ -10.70 (d, J = 21.8 Hz), -13.13 (d, J = 21.8 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for $C_{43}H_{72}NO_{28}P_2$ 1112.3722 [M - H]$^-$; Found 1112.3721.

4b: ^{1}H NMR (600 MHz, D$_2$O): δ 7.37 (t, J = 7.8 Hz, 2H, Ph), 7.06–7.01 (m, 3H, Ph), 5.57 (br s, 1H, H-1^{Glc}), 4.73 (d, J = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.43 (d, J = 7.8 Hz, 2H, H-1^{Gal}, H-1$^{Gal'}$), 4.12 (d, J = 3.6 Hz, 1H, H-4^{Gal}), 4.09 (t, J = 6.6 Hz, 2H, -CH$_2$CH_2OPh), 4.02 (br d, J = 10.8 Hz, 1H, H-5^{Glc}), 3.97–3.83 (m, 8H, H-3^{Glc}, H-6a,b^{Glc}, H-2,6a^{GlcNAc}, H-4$^{Gal'}$, -OCH_2CH$_2$-), 3.83–3.67 (m, 10H, H-4^{Glc}, H-3,5^{Gal}, H-6a,b^{Gal}, H-3,6b^{GlcNAc}, H-5$^{Gal'}$, H-6a,b$^{Gal'}$), 3.65–3.45 (m, 6H, H-2^{Glc}, H-2^{Gal}, H-4,5^{GlcNAc}, H-2,3$^{Gal'}$), 2.02 (s, 3H, CH_3CO), 1.79–1.73 (m, 2H, -CH_2CH$_2$-), 1.67–1.60 (m, 2H, -CH$_2$CH_2-), 1.47–1.41 (m, 2H, -CH_2CH$_2$-), 1.39–1.26 (m, 12H, -CH$_2$CH_2-); ^{13}C NMR (150 MHz, D$_2$O): δ 174.9 (CH$_3$CO), 158.0, 129.8 (2C), 121.3, 114.9 (2C), 103.4 (C-1$^{Gal'}$), 102.7 (C-1^{Gal}), 102.4 (C-1^{GlcNAc}), 95.1 (d, J = 6.0 Hz, C-1^{Glc}), 81.9 (C-3^{GlcNAc}), 81.6 (C-3^{Gal}), 77.4 (C-4^{Glc}), 75.2 (C-5$^{Gal'}$), 75.1 (C-5^{GlcNAc}), 74.8 (C-5^{Gal}), 72.3 (C-3$^{Gal'}$), 71.42 (C-3^{Glc}), 71.36 (C-5^{Glc}), 71.3 (d, J = 9.0 Hz, C-2^{Glc}), 70.6 (C-2$^{Gal'}$), 70.0(C-2^{Gal}), 68.6 (-OCH_2CH$_2$OPh), 68.43 (C-4$^{Gal'}$), 68.37 (2C, C-4^{Gal}, C-4^{GlcNAc}), 66.9 (d, J = 6.0 Hz, -OCH_2CH$_2$-), 60.97 (C-6^{Gal}), 60.93 (C-6$^{Gal'}$), 60.4 (C-6^{GlcNAc}), 59.5 (C-6^{Glc}), 54.6 (C-2^{GlcNAc}), 29.7 (d, J = 7.5 Hz, -OCH$_2$CH_2-), 28.5 (3C), 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -OCH$_2$CH$_2$(CH_2)$_8$CH$_2$OPh), 22.1 (CH_3CO); ^{31}P NMR (243 MHz, D$_2$O): δ -10.70 (d, J = 12.1 Hz), -13.12 (d, J = 12.1 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for $C_{43}H_{72}NO_{28}P_2$ 1112.3722 [M - H]$^-$; Found 1112.3728.

4c: ^{1}H NMR (600 MHz, D$_2$O): δ 7.30 (t, J = 7.8 Hz, 2H, Ph), 7.00–6.93 (m, 3H, Ph), 5.50 (dd, J = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.60 (dd, J = 8.4, 1H, H-1^{GalNAc}), 4.38–4.34 (m, 2H, H-1^{Gal}, H-1$^{Gal'}$), 4.10 (d, J = 3.6 Hz, 1H, H-4^{GalNAc}), 4.05 (d, J = 3.0 Hz, 1H, H-4^{Gal}), 4.01 (t, J = 6.0 Hz, 2H, -CH$_2$CH_2OPh), 4.00–3.92 (m, 2H, H-5^{Glc}, H-2^{GalNAc}), 3.90–3.75 (m, 7H, H-3^{Glc}, H-6a,b^{Glc}, H-3^{GalNAc}, H-4$^{Gal'}$, -OCH_2CH$_2$-), 3.74–3.59 (m, 10H, H-4^{Glc}, H-3,5^{Gal}, H-6a,b^{Gal}, H-6a,b^{GalNAc}, H-5$^{Gal'}$, H-6a,b$^{Gal'}$), 3.59–3.47 (m, 4H, H-2^{Glc}, H-2^{Gal}, H-5^{GalNAc}, H-3$^{Gal'}$), 3.44 (t, J = 9.0 Hz, 1H, H-2$^{Gal'}$), 1.95 (s, 3H, CH_3CO), 1.72–1.66 (m, 2H, -CH_2CH$_2$-), 1.60–1.52 (m, 2H, -CH$_2$CH_2-), 1.40–1.33 (m, 2H, -CH_2CH$_2$-), 1.31-1.19 (m, 12H, -CH$_2$CH_2-); ^{13}C NMR (150 MHz, D$_2$O): δ 175.0 (CH$_3$CO), 157.9, 129.7 (2C), 121.2, 114.7 (2C), 104.7 (C-1$^{Gal'}$), 102.9 (C-1^{GalNAc}), 102.7 (C-1^{Gal}), 95.0 (d, J = 6.4 Hz, C-1^{Glc}), 81.2 (C-3^{Gal}), 79.3 (C-3^{GalNAc}), 77.2 (C-4^{Glc}), 74.8 (C-5^{GalNAc}), 74.7 (C-5^{Gal}), 74.5 (C-5$^{Gal'}$), 72.2 (C-3$^{Gal'}$), 71.32 (C-3^{Glc}), 71.29 (C-5^{Glc}), 71.2 (d, J = 7.5 Hz, C-2^{Glc}), 70.4 (C-2$^{Gal'}$), 70.0 (C-2^{Gal}), 68.5 (-OCH_2CH$_2$OPh), 68.44 (C-4^{Gal}), 68.38 (C-4$^{Gal'}$), 67.8 (C-4^{GalNAc}), 66.9 (d, J = 6.0 Hz, -OCH_2CH$_2$-), 60.8 (2C, C-6^{Gal}, C-6^{GalNAc}), 60.7 (C-6$^{Gal'}$), 59.4 (C-6^{Glc}), 51.2 (C-2^{GalNAc}), 29.64 (d, J = 7.5 Hz, -OCH$_2$CH_2-), 28.5 (3C), 28.3, 28.2, 28.1, 24.9, 24.7 (8C, -OCH$_2$CH$_2$(CH_2)$_8$CH$_2$OPh), 22.1 (CH_3CO); ^{31}P NMR (243 MHz, D$_2$O): δ -10.78 (d, J = 19.4 Hz), -13.18 (d, J = 19.4 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for $C_{43}H_{72}NO_{28}P_2$ 1112.3722 [M - H]$^-$; Found 1112.3728.

4d: ^{1}H NMR (600 MHz, D$_2$O): δ 7.34 (t, J = 7.8 Hz, 2H, Ph), 7.03-6.96 (m, 3H, Ph), 5.55 (dd, J = 6.6, 3.0 Hz, 1H, H-1^{Glc}), 4.65 (d, J = 7.8 Hz, 1H, H-1$^{Glc'}$), 4.46 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.40 (d, J = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.13 (d, J = 2.4 Hz, 1H, H-4^{Gal}), 4.06 (t, J = 6.6 Hz, 2H, -CH$_2$CH_2OPh), 3.99 (br d, J = 10.2 Hz, 1H, H-5^{Glc}), 3.94–3.81 (m, 7H, H-3^{Glc}, H-6a,b^{Glc}, H-6a$^{Glc'}$, H-4$^{Gal'}$, -OCH_2CH$_2$-), 3.80–3.74 (m, 4H, H-3,6a^{Gal}, H-6b$^{Glc'}$, H-6a$^{Gal'}$), 3.74–3.59 (m, 9H, H-4^{Glc}, H-2,5,6b^{Gal}, H-3,4$^{Glc'}$, H-3,5,6b$^{Gal'}$), 3.56–3.47 (m, 3H, H-2^{Glc}, H-5$^{Glc'}$, H-2$^{Gal'}$), 3.38 (t, J = 8.4 Hz, 1H, H-2$^{Glc'}$), 1.77–1.69 (m, 2H, -CH_2CH$_2$-), 1.64–1.57 (m, 2H, -CH$_2$CH_2-), 1.44–1.37 (m, 2H, -

C*H*$_2$CH$_2$-), 1.35–1.23 (m, 12H, -CH$_2$C*H*$_2$-); ^{13}C NMR (150 MHz, D$_2$O): δ 158.0, 129.8 (2C), 121.3, 114.8 (2C), 103.5 (C-1$^{Glc'}$), 102.8 (C-1$^{Gal'}$), 102.4 (C-1^{Gal}), 95.1 (d, *J* = 6.0 Hz, C-1^{Glc}), 81.9 (C-3^{Gal}), 77.9 (C-4$^{Glc'}$), 77.3 (C-4^{Glc}), 75.2 (C-5$^{Gal'}$), 74.8 (C-5^{Gal}), 74.5 (C-5$^{Glc'}$), 74.0 (C-3$^{Glc'}$), 72.9 (C-2$^{Glc'}$), 72.4 (C-3$^{Gal'}$), 71.4 (C-3^{Glc}), 71.31 (C-5^{Glc}), 71.27 (d, *J* = 9.0 Hz, C-2^{Glc}), 70.8 (C-2$^{Gal'}$), 70.0 (C-2^{Gal}), 68.6 (-OC*H*$_2$CH$_2$OPh), 68.4 (C-4$^{Gal'}$), 68.2 (C-4^{Gal}), 66.9 (d, *J* = 6.0 Hz, -O*C*H$_2$CH$_2$-), 60.9 (2C, C-6^{Gal}, C-6$^{Gal'}$), 59.7 (C-6$^{Glc'}$), 59.5 (C-6^{Glc}), 29.70 (d, *J* = 7.5 Hz, -OCH$_2$*C*H$_2$-), 28.6, 28.5 (2C), 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -OCH$_2$CH$_2$(*C*H$_2$)$_8$CH$_2$OPh); ^{31}P NMR (243 MHz, D$_2$O): -10.74 (d, *J* = 21.8 Hz), -13.18 (d, *J* = 21.8 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for C$_{41}$H$_{69}$O$_{28}$P$_2$ 1071.3456 [M - H]$^-$; Found 1071.3450.

4e: ^{1}H NMR (600 MHz, D$_2$O): δ 7.33 (t, *J* = 7.8 Hz, 2H, Ph), 7.02-6.96 (m, 3H, Ph), 5.53 (dd, *J* = 6.6, 3.6 Hz, 1H, H-1^{Glc}), 4.65 (d, *J* = 7.8 Hz, 1H, H-1$^{Glc'}$), 4.62 (d, *J* = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.45 (d, *J* = 7.8 Hz, 1H, H-1^{Gal}), 4.11 (d, *J* = 2.4 Hz, 1H, H-4^{Gal}), 4.04 (t, *J* = 6.4 Hz, 2H, -CH$_2$C*H*$_2$OPh), 3.97 (br d, *J* = 10.2 Hz, 1H, H-5^{Glc}), 3.92–3.80 (m, 7H, H-3^{Glc}, H-6a,b^{Glc}, H-6a$^{Glc'}$, H-4$^{Gal'}$, -OC*H*$_2$CH$_2$-), 3.79–3.63 (m, 11H, H-4^{Glc}, H-2,3,5^{Gal}, H-6a,6b^{Gal}, H-3,6b$^{Glc'}$, H-5$^{Gal'}$, H-6a,6b$^{Gal'}$), 3.61 (dd, *J* = 9.6, 3.0 Hz, 1H, H-3$^{Gal'}$), 3.55–3.45 (m, 4H, H-2^{Glc}, H-2,4$^{Glc'}$, H-2$^{Gal'}$), 3.44-3.39 (m, 1H, H-5$^{Glc'}$), 1.75–1.68 (m, 2H, -C*H*$_2$CH$_2$-), 1.62–1.55 (m, 2H, -CH$_2$C*H*$_2$-), 1.43–1.35 (m, 2H, -C*H*$_2$CH$_2$-), 1.34–1.21 (m, 12H, -CH$_2$C*H*$_2$-); ^{13}C NMR (150 MHz, D$_2$O): δ 157.9, 129.7 (2C), 121.2, 114.7 (2C), 103.4 (C-1$^{Glc'}$), 103.1 (C-1$^{Gal'}$), 102.3 (C-1^{Gal}), 95.0 (d, *J* = 6.0 Hz, C-1^{Glc}), 83.8 (C-3$^{Glc'}$), 81.8 (C-3^{Gal}), 77.1 (C-4^{Glc}), 75.1 (2C, C-5$^{Glc'}$, C-5$^{Gal'}$), 74.8 (C-5^{Gal}), 72.9 (C-2$^{Glc'}$), 72.4 (C-3$^{Gal'}$), 71.3 (C-3^{Glc}), 71.3 (C-5^{Glc}), 71.2 (d, *J* = 9.0 Hz, C-2^{Glc}), 71.0 (C-2$^{Gal'}$), 69.9 (C-2^{Gal}), 68.5 (-OC*H*$_2$CH$_2$OPh), 68.4 (C-4$^{Gal'}$), 68.1 (C-4^{Gal}), 67.8 (C-4$^{Glc'}$), 66.8 (d, *J* = 6.0 Hz, -O*C*H$_2$CH$_2$-), 60.9 (C-6^{Gal}), 60.8 (C-6$^{Gal'}$), 60.2 (C-6$^{Glc'}$), 59.4 (C-6^{Glc}), 29.63 (d, *J* = 6.0 Hz, -OCH$_2$*C*H$_2$-), 28.50, 28.49 (2C), 28.3, 28.2, 28.1, 24.9, 24.7 (8C, -OCH$_2$CH$_2$(*C*H$_2$)$_8$CH$_2$OPh); ^{31}P NMR (243 MHz, D$_2$O): δ -10.74 (d, *J* = 21.8 Hz), -13.17 (d, *J* = 21.8 Hz); ESI-(-)-TOF HRMS *m/z*: Calcd for C$_{41}$H$_{69}$O$_{28}$P$_2$ 1071.3456 [M - H]$^-$; Found 1071.3446.

Enzymatic synthesis of pentasaccharides 5a, 5b, 5c, 5d, and 5e

Procedure A. Milligram-scale production systems of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **5a** and NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **5d** were 2 mL reaction mixture of 50 mmol/L Tris-HCl buffer (pH 7.0) containing 3 mmol/L CMP-NeuNAc, 2 mmol/L **4a** or **4d** and 40 μg/mL Cps1aK. Milligram-scale production systems of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **5b**, NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **1c**, and NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-(CH$_2$)$_{11}$-OPh **1e** were 2 mL reaction mixture of 50 mmol/L Tris-HCl buffer (pH 7.0) containing 3 mmol/L CMP-NeuNAc, 2 mmol/L **4b, 4c**, or **4e** and 400 μg/mL Cps1bJ. All of the reaction systems were incubated at 25 °C for 2 h, at which time TLC indicated the complete consumption of acceptor substrate, and then quenched by boiling for 30 s. After purification protocol as mentioned above, the fractions containing desired product were collected, then concentrated and lyophilized to afford **5a** (4.8 mg, 86%), **5d** (4.5 mg, 81%), **5b** (4.6 mg, 82%), **5c** (4.5 mg, 80%) and **5e** (4.6 mg, 84%) as white powders.

Procedure B. The one-pot three-enzyme synthesis of **5a**, **5b**, **5c**, **5d** and **5e** was carried out with Cps1aI, Cps1aJ/Cps1bJ and Cps1aK/Cps1bK enzymes using Lacα-PP-(CH$_2$)$_{11}$-OPh **2** as the starting acceptor substrate. A 5 mL reaction mixture of 50 mmol/L Tris-HCl buffer (pH 7.5) containing 2.4 mmol/L UDP-GlcNAc or UDP-Glc, 2.4 mmol/L UDP-Gal, 3 mM CMP-NeuNAc, 2 mmol/L **2**, 5 mmol/L MgCl$_2$, 50 μg/mL Cps1aI, 200 mg/mL Cps1aJ and 40 μg/mL Cps1aK to produce **5a**/**5d**. A 5 mL reaction mixture of 50 mmol/L

Tris-HCl buffer (pH 7.5) containing 2.4 mmol/L UDP-GlcNAc, UDP-GalNAc, or UDP-Glc, 2.4 mmol/L UDP-Gal, 3 mM CMP-NeuNAc, 2 mmol/L **2**, 5 mmol/L $MgCl_2$, 50 μg/mL Cps1aI, 200 mg/mL Cps1bJ and 400 μg/mL Cps1bK to producing **5b**/**5c**/**5e**. All of the reaction systems were incubated at 25 °C for 2 h, at which time TLC indicated the complete consumption of acceptor substrate, and then quenched by boiling for 30 s. After purification protocol as mentioned above, the fractions containing desired product were collected, then concentrated and lyophilized to afford **5a** (11.6 mg, 83%), **5d** (10.9 mg, 80%), **5b** (11.2 mg, 80%), **5c** (11.0 mg, 78%), and **5e** (10.5 mg, 77%) as white powders.

5a: ^{1}H NMR (600 MHz, D_2O): δ 7.36 (t, J = 7.8 Hz, 2H, Ph), 7.05–7.00 (m, 3H, Ph), 5.56 (dd, J = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.68 (d, J = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.53 (d, J = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.41 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.12 (d, J = 3.2 Hz, 1H, H-4^{Gal}), 4.10 (dd, J = 9.6, 3.0 Hz, 1H, H-3$^{Gal'}$), 4.07 (t, J = 6.6 Hz, 2H, -CH_2CH_2OPh), 4.00 (br d, J = 10.2 Hz, 1H, H-5^{Glc}), 3.96–3.81 (m, 11H, H-3^{Glc}, H-6a,b^{Glc}, H-6a,b^{GlcNAc}, H-4$^{Gal'}$, H-5,8,9a^{NeuNAc}, -OCH_2CH_2-), 3.80–3.65 (m, 12H, H-4^{Glc}, H-3,5^{Gal}, H-6a,b^{Gal}, H-2,3,4^{GlcNAc}, H-5$^{Gal'}$, H-6a,b$^{Gal'}$, H-4^{NeuNAc}), 3.65–3.53 (m, 7H, H-2^{Glc}, H-2^{Gal}, H-5^{GlcNAc}, H-2$^{Gal'}$, H-6,7,9b^{NeuNAc}), 2.74 (dd, J = 12.0, 4.2 Hz, 1H, H-3$_{eq}^{NeuNAc}$), 2.01 (s, 6H, CH_3CO), 1.81–1.72 (m, 3H, H-3$_{ax}^{NeuNAc}$, -CH_2CH_2-), 1.65–1.59 (m, 2H, -CH_2CH_2-), 1.46–1.39 (m, 2H, -CH_2CH_2-), 1.37–1.24 (m, 12H, -CH_2CH_2-); ^{13}C NMR (150 MHz, D_2O): δ 174.9 (CH_3CO), 174.8 (CH_3CO), 173.8 (C-1^{NeuNAc}), 158.0, 129.8 (2C), 121.3, 114.9 (2C), 102.8 (C-1^{Gal}), 102.7 (C-1^{GlcNAc}), 102.5 (C-1$^{Gal'}$), 99.7 (C-2^{NeuNAc}), 95.1 (d, J = 6.0 Hz, C-1^{Glc}), 81.8 (C-3^{Gal}), 77.9 (C-4^{GlcNAc}), 77.5 (C-4^{Glc}), 75.4 (C-3$^{Gal'}$), 75.1 (C-5$^{Gal'}$), 74.8 (C-5^{Gal}), 74.5 (C-5^{GlcNAc}), 72.8 (C-6^{NeuNAc}), 72.08 (C-3^{GlcNAc}), 71.7 (C-8^{NeuNAc}), 71.44 (C-3^{Glc}), 71.37 (C-5^{Glc}), 71.26 (d, J = 9.0 Hz, C-2^{Glc}), 69.9 (C-2^{Gal}), 69.3 (C-2$^{Gal'}$), 68.7 (-OCH_2CH_2OPh), 68.4 (C-4^{Gal}), 68.3 (C-4^{NeuNAc}), 68 (C-7^{NeuNAc}), 67.4 (C-4$^{Gal'}$), 66.9 (d, J = 6.0 Hz, -OCH_2CH_2-), 62.5(C-9^{NeuNAc}), 60.93 (C-6^{Gal}), 60.91 (C-6$^{Gal'}$), 59.7 (C-6^{GlcNAc}), 59.5 (C-6^{Glc}), 55.1 (C-2^{GlcNAc}), 51.6 (C-5^{NeuNAc}), 39.5 (C-3^{NeuNAc}), 29.72 (d, J = 6.0 Hz, -OCH_2CH_2-), 28.6, 28.5(2C), 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -$OCH_2CH_2(CH_2)_8CH_2$OPh), 22.1 (CH_3CO), 21.9 (CH_3CO); ^{31}P NMR (243 MHz, D_2O): δ -10.68 (d, J = 17.0 Hz), -13.09 (d, J = 17.0 Hz); ESI-(-)-TOF HRMS *m/z*: [M - 2H]$^{2-}$ Calcd for $C_{54}H_{88}N_2O_{36}P_2$ 701.2301; Found 701.2308.

5b: ^{1}H NMR (600 MHz, D_2O): δ 7.36 (t, J = 7.8 Hz, 2H, Ph), 7.05–7.00 (m, 3H, Ph), 5.56 (dd, J = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.72 (d, J = 8.4 Hz, 1H, H-1^{GlcNAc}), 4.48 (d, J = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.41 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.11 (d, J = 3.2 Hz, 1H, H-4^{Gal}), 4.09–4.05 (m, 3H, H-3$^{Gal'}$, -CH_2CH_2OPh), 4.00 (br d, J = 10.2 Hz, 1H, H-5^{Glc}), 3.95–3.90 (m, 3H, H-4$^{Gal'}$, -OCH_2CH_2-), 3.90–3.80 (m, 8H, H-3^{Glc}, H-6a,b^{Glc}, H-2,6a^{GlcNAc}, H-5,8,9a^{NeuNAc}), 3.80-3.73 (m, 3H, H-6a^{Gal}, H-3,6b^{GlcNAc}), 3.73–3.64 (m, 8H, H-4^{Glc}, H-3,5,6b^{Gal}, H-5$^{Gal'}$, H-6a,b$^{Gal'}$, H-4^{NeuNAc}), 3.64–3.49 (m, 7H, H-2^{Glc}, H-2^{Gal}, H-4^{GlcNAc}, H-2$^{Gal'}$, H-6,7,9b^{NeuNAc}), 3.47–3.43 (m, 1H, H-5^{GlcNAc}), 2.73 (dd, J = 12.6, 4.8 Hz, 1H, H-3$_{eq}^{NeuNAc}$), 2.01 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.79–1.72 (m, 3H, H-3$_{ax}^{NeuNAc}$, -CH_2CH_2-), 1.65–1.59 (m, 2H, -CH_2CH_2-), 1.46–1.40 (m, 2H, -CH_2CH_2-), 1.36–1.26 (m, 12H, -CH_2CH_2-); ^{13}C NMR (150 MHz, D_2O): δ 174.8 (2C, CH_3CO), 173.8 (C-1^{NeuNAc}), 158.0, 129.8 (2C), 121.3, 114.9 (2C), 103.3 (C-1$^{Gal'}$), 102.8 (C-1^{Gal}), 102.4 (C-1^{GlcNAc}), 99.5 (C-2^{NeuNAc}), 95.0 (d, J = 4.5 Hz, C-1^{Glc}), 82.1 (C-3^{GlcNAc}), 81.6 (C-3^{Gal}), 77.5 (C-4^{Glc}), 75.5 (C-3$^{Gal'}$), 75.1 (C-5^{GlcNAc}), 74.9 (C-5$^{Gal'}$), 74.8 (C-5^{Gal}), 72.7 (C-6^{NeuNAc}), 71.7 (C-8^{NeuNAc}), 71.4 (C-3^{Glc}), 71.3 (C-5^{Glc}), 71.24 (d, J = 7.5 Hz, C-2^{Glc}), 69.97 (C-2^{Gal}), 68.98 (C-2$^{Gal'}$), 68.6 (-OCH_2CH_2OPh), 68.4 (C-4^{GlcNAc}), 68.33 (C-4^{Gal}), 68.31 (C-4^{NeuNAc}), 67.9 (C-7^{NeuNAc}), 67.1 (C-4$^{Gal'}$), 66.9 (d, J = 4.5 Hz, -OCH_2CH_2-), 62.3 (C-9^{NeuNAc}), 60.90 (C-6^{Gal}), 60.87 (C-6$^{Gal'}$), 60.4 (C-6^{GlcNAc}), 59.5 (C-6^{Glc}), 54.4 (C-2^{GlcNAc}), 51.6 (C-5^{NeuNAc}), 39.6 (C-3^{NeuNAc}), 29.7 (d, J = 7.5 Hz, -OCH_2CH_2-), 28.5, 28.5 (2C), 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -$OCH_2CH_2(CH_2)_8CH_2$OPh), 22.2 (CH_3CO), 21.9 (CH_3CO); ^{31}P NMR

(243 MHz, D_2O): δ -10.67 (d, J = 21.8 Hz), -13.10 (d, J = 21.8 Hz); ESI-(-)-TOF HRMS *m/z*: $[M - 2H]^{2-}$ Calcd for $C_{54}H_{88}N_2O_{36}P_2$ 701.2301; Found 701.2295.

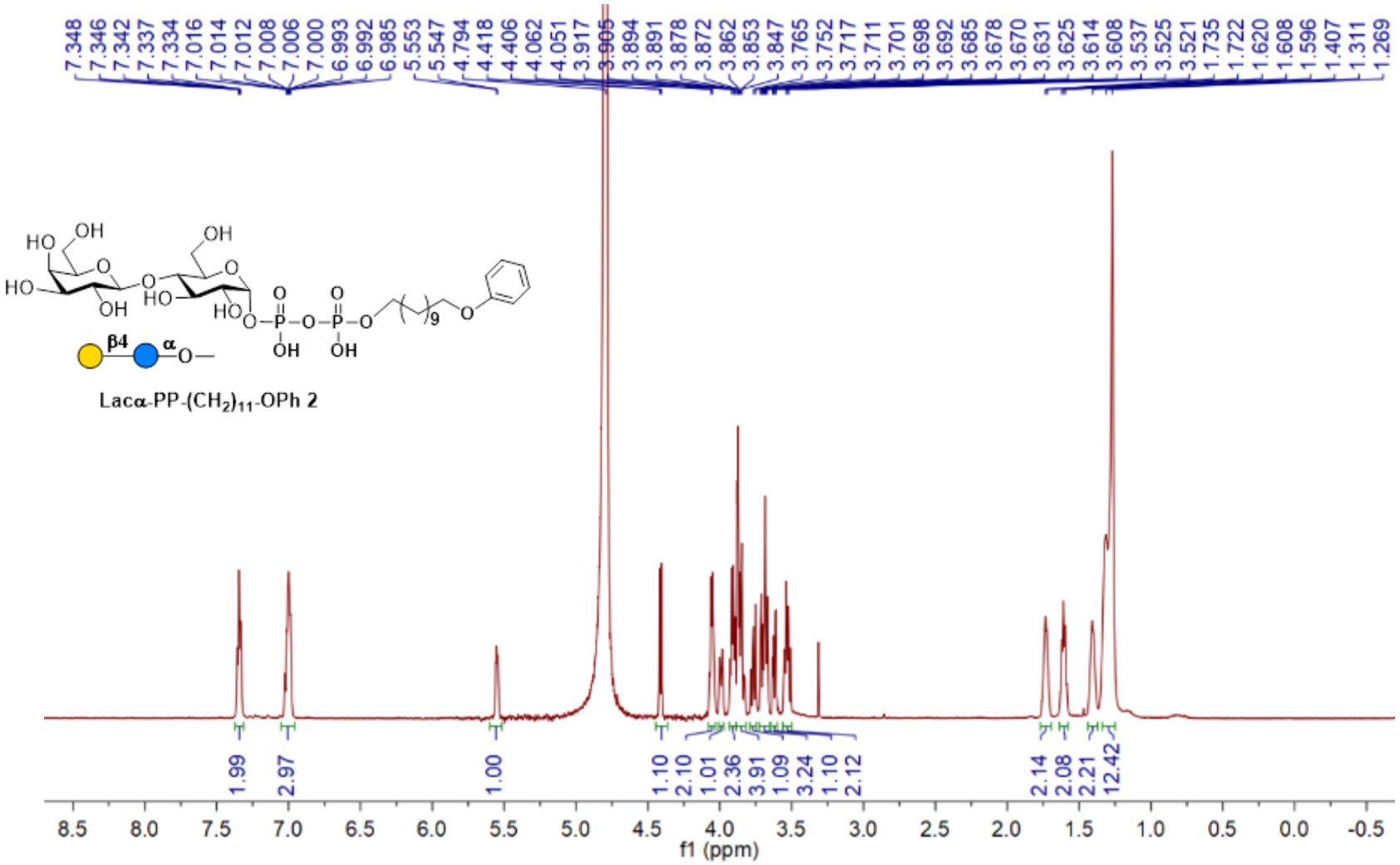
5c: ^{1}H NMR (600 MHz, D_2O): δ 7.35 (t, J = 7.8 Hz, 2H, Ph), 7.03–6.98 (m, 3H, Ph), 5.55 (dd, J = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.65 (d, J = 8.4 Hz, 1H, H-1^{GalNAc}), 4.48 (d, J = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.40 (d, J = 7.8 Hz, 1H, H-1^{Gal}), 4.14 (d, J = 1.8 Hz, 1H, H-4^{GalNAc}), 4.09 (d, J = 2.4 Hz, 1H, H-4^{Gal}), 4.08–4.01 (m, 4H, H-2^{GlcNAc}, H-3$^{Gal'}$, -CH_2CH_2OPh), 3.99 (br d, J = 10.2 Hz, 1H, H-5^{Glc}), 3.94–3.78 (m, 10H, H-3^{Glc}, H-6a,b^{Glc}, H-3^{GalNAc}, H-4$^{Gal'}$, H-5,8,9a^{NeuNAc}, -OCH_2CH$_2$-), 3.78–3.61 (m, 11H, H-4^{Glc}, H-3,5^{Gal}, H-6a,b^{Gal}, H-6a,b^{GalNAc}, H-5$^{Gal'}$, H-6a,b$^{Gal'}$, H-4^{NeuNAc}), 3.61–3.47 (m, 7H, H-2^{Glc}, H-2^{Gal}, H-5^{GalNAc}, H-2$^{Gal'}$, H-6,7,9b^{NeuNAc}), 2.71 (dd, J = 12.0, 4.2 Hz, 1H, H-3$_{eq}^{NeuNAc}$), 1.99 (s, 6H, CH_3CO), 1.78-1.70 (m, 3H, H-3$_{ax}^{NeuNAc}$, -CH_2CH$_2$-), 1.64–1.57 (m, 2H, -CH_2CH_2-), 1.44–1.37 (m, 2H, -CH_2CH$_2$-), 1.35–1.23 (m, 12H, -CH_2CH_2-); ^{13}C NMR (150 MHz, D_2O): δ 175.0 (CH_3CO), 174.8 (CH_3CO), 173.9 (C-1^{NeuNAc}), 158.0, 129.8 (2C), 121.3, 114.8 (2C), 104.46 (C-1$^{Gal'}$), 102.9 (C-1^{GlcNAc}), 102.8 (C-1^{Gal}), 99.5 (C-2^{NeuNAc}), 95.1 (d, J = 7.5 Hz, C-1^{Glc}), 81.3 (C-3^{Gal}), 79.6 (C-3^{GalNAc}), 77.4 (C-4^{Glc}), 75.4 (C-3$^{Gal'}$), 74.8 (C-5^{GalNAc}), 74.6 (C-5^{Gal}), 74.5 (C-5$^{Gal'}$), 72.7 (C-6^{NeuNAc}), 71.7 (C-8^{NeuNAc}), 71.4 (C-3^{Glc}), 71.3 (C-5^{Glc}), 71.2 (d, J = 7.5 Hz, C-2^{Glc}), 70.0 (C-2^{Gal}), 68.9 (C-2$^{Gal'}$), 68.6 (-OCH_2CH$_2$OPh), 68.5 (C-4^{Gal}), 68.3 (C-4^{NeuNAc}), 67.9 (C-7^{NeuNAc}), 67.7 (C-4^{GalNAc}), 67.2 (C-4$^{Gal'}$), 66.9 (d, J = 6.0 Hz, -OCH$_2$CH$_2$-), 62.3 (C-9^{NeuNAc}), 60.9 (2C, C-6^{Gal}, C-6^{GalNAc}), 60.8 (C-6$^{Gal'}$), 59.5 (C-6^{Glc}), 51.5 (C-5^{NeuNAc}), 51.2 (C-2^{GalNAc}), 39.6 (C-3^{NeuNAc}), 29.7 (d, J = 7.5 Hz, -OCH$_2$$CH_2$-), 28.54, 28.51, 28.50, 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -OCH$_2$CH$_2$(CH$_2$)$_8$CH$_2$OPh), 22.2 (CH$_3$CO), 21.9 (CH$_3$CO); ^{31}P NMR (243 MHz, D_2O): δ -10.73 (d, J = 19.4 Hz), -13.16 (d, J = 19.4 Hz); ESI-(-)-TOF HRMS *m/z*: $[M - 2H]^{2-}$ Calcd for $C_{54}H_{88}N_2O_{36}P_2$ 701.2301; Found 701.2291.

5d: ^{1}H NMR (600 MHz, D_2O): δ 7.32 (t, J = 7.8 Hz, 2H, Ph), 7.01–6.95 (m, 3H, Ph), 5.52 (dd, J = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.62 (d, J = 8.4 Hz, 1H, H-1$^{Glc'}$), 4.45 (d, J = 7.2 Hz, 1H, H-1$^{Gal'}$), 4.45 (d, J = 7.2 Hz, 1H, H-1^{Gal}), 4.12 (d, J = 3.0 Hz, 1H, H-4^{Gal}), 4.05 (dd, J = 9.0, 2.4 Hz, 1H, H-3$^{Gal'}$), 4.03 (t, J = 6.6 Hz, 2H, -CH_2CH_2OPh), 3.96 (br d, J = 10.2 Hz, 1H, H-5^{Glc}), 3.92–3.71 (m, 13H, H-3^{Glc}, H-6a,b^{Glc}, H-3,6a^{Gal}, H-6a,b^{Glc}, H-4$^{Gal'}$, H-5,8,9a^{NeuNAc}, -OCH_2CH$_2$-), 3.70–3.54 (m, 9H, H-4^{Glc}, H-2,5,6b^{Gal}, H-3$^{Glc'}$, H-5$^{Gal'}$, H-6a,b$^{Gal'}$, H-4^{NeuNAc}), 3.54–3.48 (m, 7H, H-2^{Glc}, H-4,5$^{Glc'}$, H-2$^{Gal'}$, H-6,7,9b^{NeuNAc}), 3.34 (t, J = 9.0 Hz, 1H, H-2$^{Glc'}$), 2.69 (dd, J = 12.0, 4.2 Hz, 1H, H-3$_{eq}^{NeuNAc}$), 1.96 (s, 3H, CH_3CO), 1.76–1.68 (m, 3H, H-3$_{ax}^{NeuNAc}$, -CH_2CH$_2$-), 1.61–1.55 (m, 2H, -CH_2CH_2-), 1.41–1.35 (m, 2H, -CH_2CH$_2$-), 1.33–1.20 (m, 12H, -CH_2CH_2-); ^{13}C NMR (150 MHz, D_2O): δ 174.9 (CH_3CO), 173.8 (C-1^{NeuNAc}), 158.0, 129.8 (2C), 121.3, 114.8 (2C), 103.5 (C-1$^{Glc'}$), 102.5 (C-1$^{Gal'}$), 102.4 (C-1^{Gal}), 99.7 (C-2^{NeuNAc}), 95.1 (d, J = 4.5 Hz, C-1^{Glc}), 82.0 (C-3^{Gal}), 77.8 (C-4$^{Glc'}$), 77.3 (C-4^{Glc}), 75.4 (C-3$^{Gal'}$), 75.0 (C-5$^{Gal'}$), 74.8 (C-5^{Gal}), 74.5 (C-5$^{Glc'}$), 73.9 (C-3$^{Glc'}$), 72.8 (C-2$^{Glc'}$), 72.7 (C-6^{NeuNAc}), 71.6 (C-8^{NeuNAc}), 71.4 (C-3^{Glc}), 71.3 (C-5^{Glc}), 71.2 (d, J = 9.0 Hz, C-2^{Glc}), 69.9 (C-2^{Gal}), 69.2 (C-2$^{Gal'}$), 68.6 (-OCH_2CH$_2$OPh), 68.3 (C-4^{NeuNAc}), 68.1 (C-4^{Gal}), 67.9 (C-7^{NeuNAc}), 67.3 (C-4$^{Gal'}$), 66.9 (d, J = 6.0 Hz, -OCH$_2$CH$_2$-), 62.4 (C-9^{NeuNAc}), 60.9 (2C, C-6^{Gal}, C-6$^{Gal'}$), 59.7 (C-6$^{Glc'}$), 59.5 (C-6^{Glc}), 51.6 (C-5^{NeuNAc}), 39.5 (C-3^{NeuNAc}), 29.7 (d, J = 7.5 Hz, -OCH$_2$$CH_2$-), 28.56, 28.53 (2C), 28.4, 28.3, 28.2, 25.0, 24.8 (8C, -OCH$_2$CH$_2$(CH$_2$)$_8$CH$_2$OPh), 21.9 (CH$_3$CO); ^{31}P NMR (243 MHz, D_2O): δ -10.70 (d, J = 19.4 Hz), -13.14 (d, J = 19.4 Hz); ESI-(-)-TOF HRMS *m/z*: $[M - 2H]^{2-}$ Calcd for $C_{52}H_{85}NO_{36}P_2$ 680.7169; Found 680.7170.

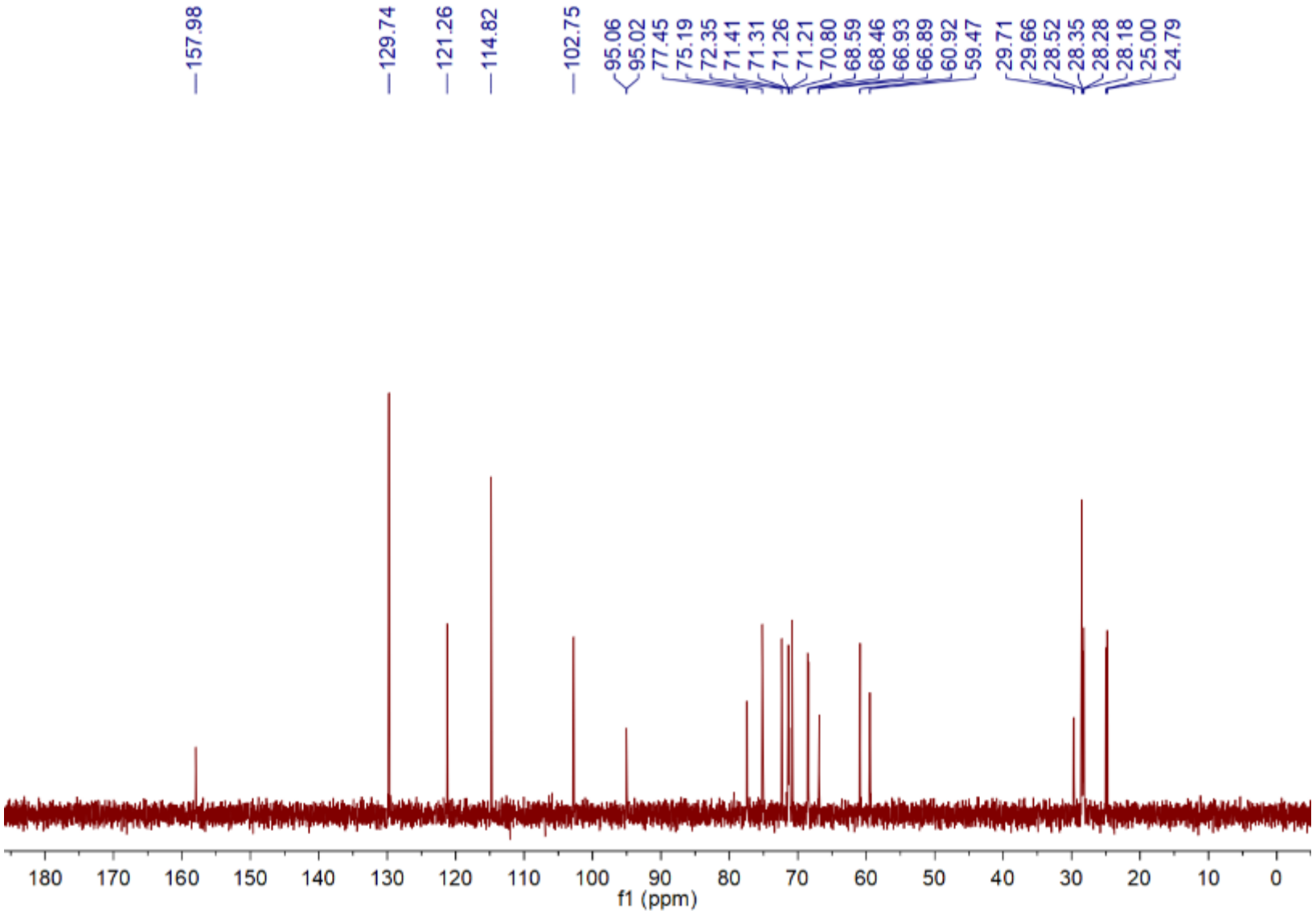
5e: ^{1}H NMR (600 MHz, D_2O): δ 7.31 (t, J = 7.8 Hz, 2H, Ph), 7.00–6.95 (m, 3H, Ph), 5.52 (dd, J = 7.2, 3.6 Hz, 1H, H-1^{Glc}), 4.68 (d, J = 7.8 Hz, 1H, H-1$^{Gal'}$), 4.63 (d, J = 7.8 Hz, 1H, H-1$^{Glc'}$), 4.43 (t, J = 7.8 Hz, 1H, H-1^{Gal}), 4.10 (d, J = 3.2 Hz, 1H, H-4^{Gal}), 4.06 (dd, J = 10.2, 3.0 Hz, 1H, H-3$^{Gal'}$), 4.03 (t, J = 6.6 Hz, 2H, -CH_2CH_2OPh), 3.96 (br d, J = 10.2 Hz, 1H, H-5^{Glc}), 3.91–3.77 (m, 10H, H-3^{Glc}, H-6a,b^{Glc}, H-6a$^{Glc'}$, H-4$^{Gal'}$, H-5,8,9a^{NeuNAc}, -OCH_2CH$_2$-), 3.76–3.59 (m, 12H, H-4^{Glc}, H-2,3,5^{Gal}, H-6a,6b^{Gal}, H-3,6b$^{Glc'}$, H-5$^{Gal'}$, H-6a,6b$^{Gal'}$,

H-4^{NEuNAc}), 3.59–3.44 (m, 7H, H-2^{Glc}, H-2,4$^{Glc'}$, H-2$^{Gal'}$, H-6,7,9b^{NeuNAc}), 3.42–3.33 (m, 1H, H-5$^{Glc'}$), 2.69 (dd, J = 12.0, 4.2 Hz, 1H, H-3$_{eq}$^{NeuNAc}), 1.96 (s, 3H, CH_3CO), 1.77–1.67 (m, 3H, H-3$_{ax}$^{NeuNAc}, -CH_2CH$_2$-), 1.61–1.54 (m, 2H, -CH$_2$CH_2-), 1.41–1.34 (m, 2H, -CH_2CH$_2$-), 1.33-1.21 (m, 12H, -CH$_2$CH_2-); ^{13}C NMR (150 MHz, D$_2$O): δ 174.8 (CH$_3$*C*O), 173.8 (C-1^{NeuNAc}), 157.9, 129.7 (2C), 121.2, 114.8 (2C), 103.3 (C-1$^{Glc'}$), 102.7 (C-1$^{Gal'}$), 102.3 (C-1^{Gal}), 99.6 (C-2^{NeuNAc}), 95.0 (d, J = 6.0 Hz, C-1^{Glc}), 83.8 (C-3$^{Glc'}$), 81.8 (C-3^{Gal}), 77.2 (C-4^{Glc}), 75.4 (C-3$^{Gal'}$), 75.1 (C-5$^{Glc'}$), 74.9 (C-5$^{Gal'}$), 74.8 (C-5^{Gal}), 72.8 (C-2$^{Glc'}$), 72.7 (C-6^{NeuNAc}), 71.7 (C-8^{NeuNAc}), 71.4 (C-3^{Glc}), 71.3 (C-5^{Glc}), 71.2 (d, J = 9.0 Hz, C-2^{Glc}), 69.9 (C-2^{Gal}), 69.5 (C-2$^{Gal'}$), 68.6 (-OCH_2CH$_2$OPh), 68.25 (C-4^{NeuNAc}), 68.16 (C-4^{Gal}), 67.9 (C-7^{NeuNAc}), 67.8 (C-4$^{Glc'}$), 67.3 (C-4$^{Gal'}$), 66.9 (d, J = 6.0 Hz, -O*C*H$_2$CH$_2$-), 62.4 (C-9^{NeuNAc}), 60.88 (C-6^{Gal}), 60.85 (C-6$^{Gal'}$), 60.3 (C-6$^{Glc'}$), 59.4 (C-6^{Glc}), 51.5 (C-5^{NeuNAc}), 39.4 (C-3^{NeuNAc}), 29.6 (d, J = 7.5 Hz, -OCH$_2$*C*H$_2$-), 28.52, 28.50, 28.48, 28.32, 28.26, 28.2, 25.0, 24.8 (8C, -OCH$_2$CH$_2$(*C*H$_2$)$_8$CH$_2$OPh), 21.9 (*C*H$_3$CO); ^{31}P NMR (243 MHz, D$_2$O): δ -10.71 (d, J = 21.8 Hz), -13.14 (d, J = 21.8 Hz); ESI-(-)-TOF HRMS *m/z*: [M - 2H]$^{2-}$ Calcd for $C_{52}H_{85}NO_{36}P_2$ 680.7169; Found 680.7163.

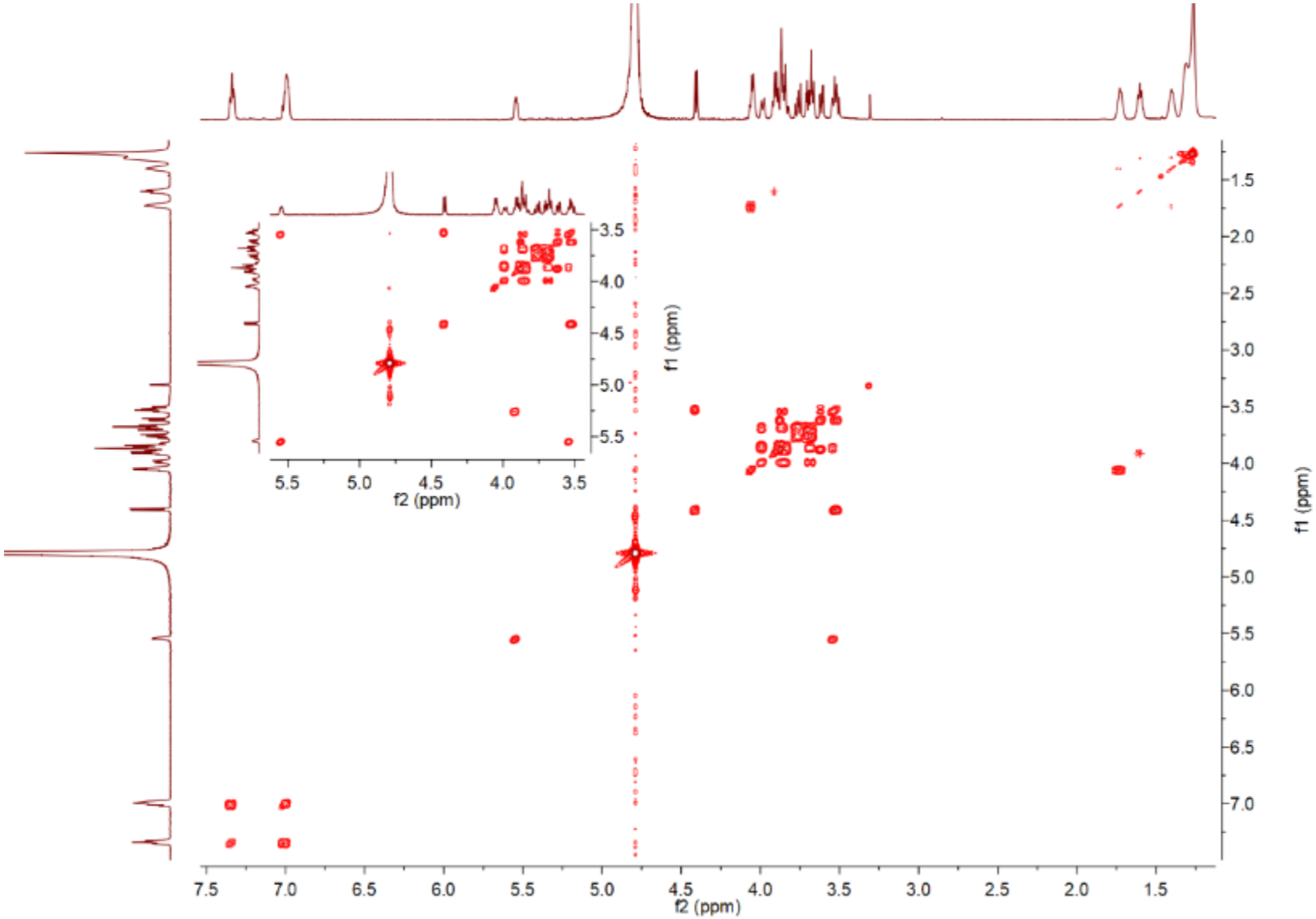
II. NMR and ESI-HRMS spectra



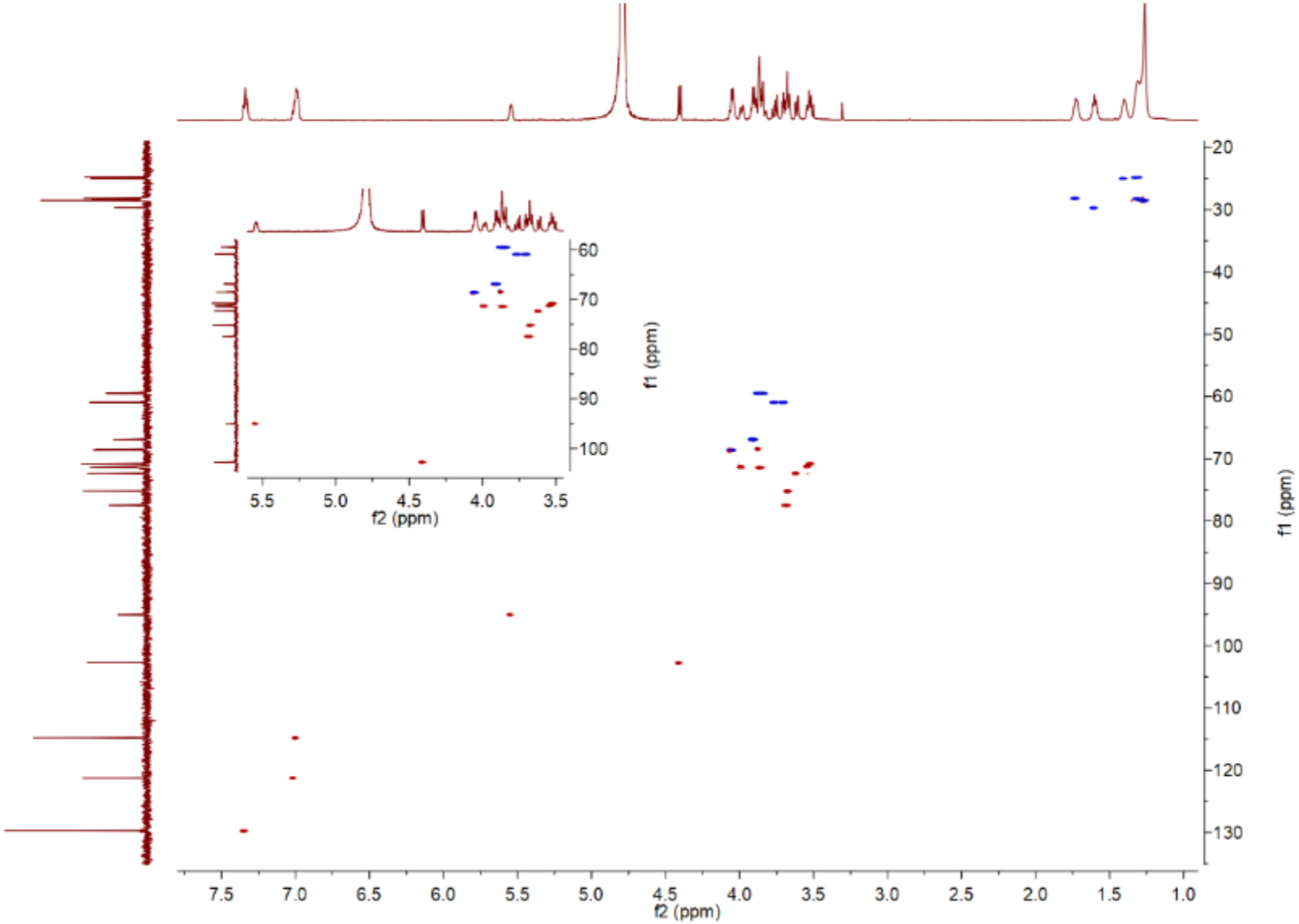
^{1}H NMR spectrum of Lacα-PP-$(CH_2)_{11}$-OPh **2** (D_2O, 600 MHz, 25 °C)



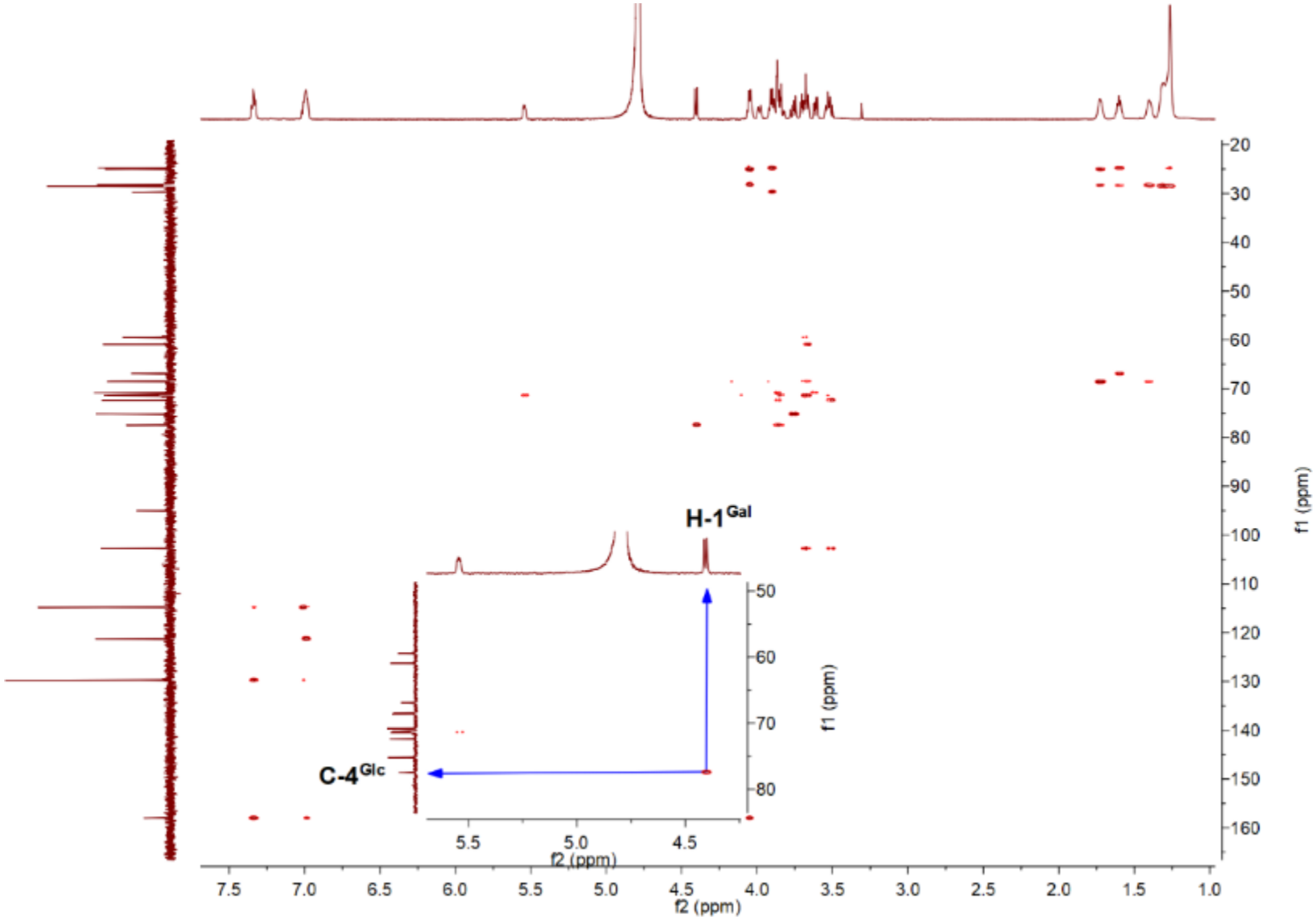
^{13}C NMR spectrum of Lacα-PP-$(CH_2)_{11}$-OPh **2** (D_2O, 150 MHz, 25 °C)



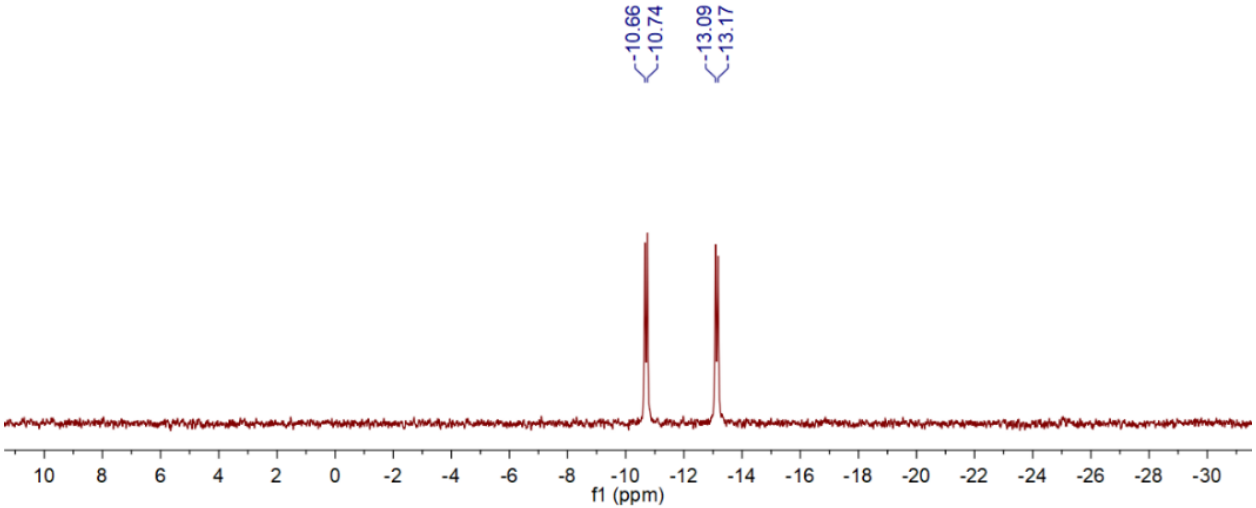
COSY spectrum of Lacα-PP-$(CH_2)_{11}$-OPh **2** (D_2O, 600/600 MHz, 25 °C)



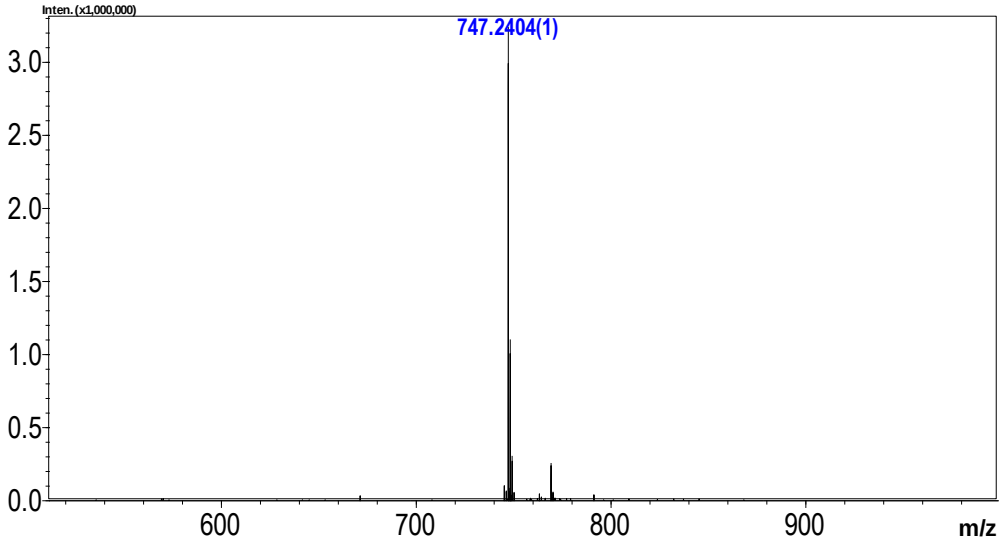
HSQC spectrum of Lacα-PP-$(CH_2)_{11}$-OPh **2** (D_2O, 600/150 MHz, 25 °C)



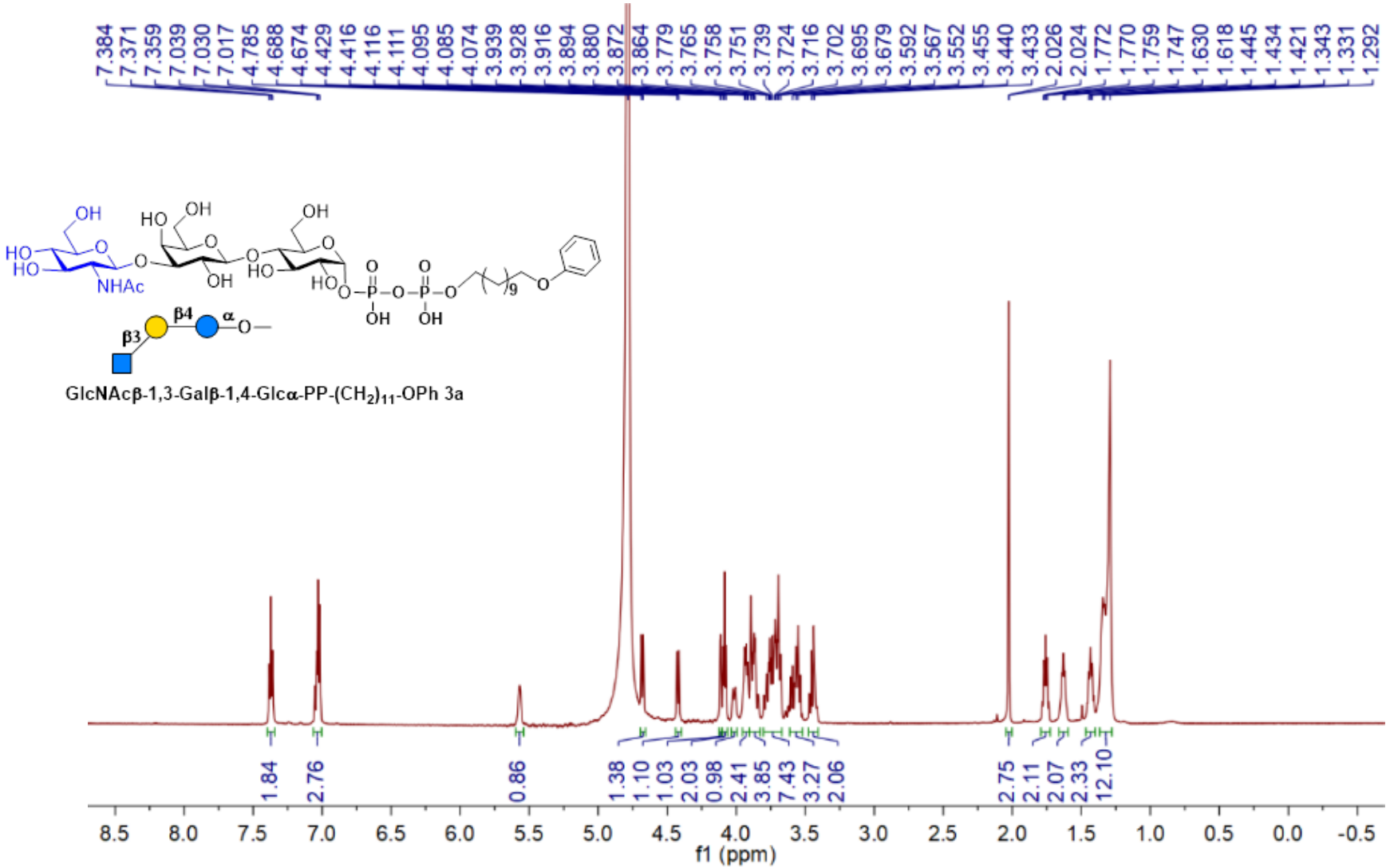
HMBC spectrum of Lacα-PP-$(CH_2)_{11}$-OPh **2** (D_2O, 600/150 MHz, 25 °C)



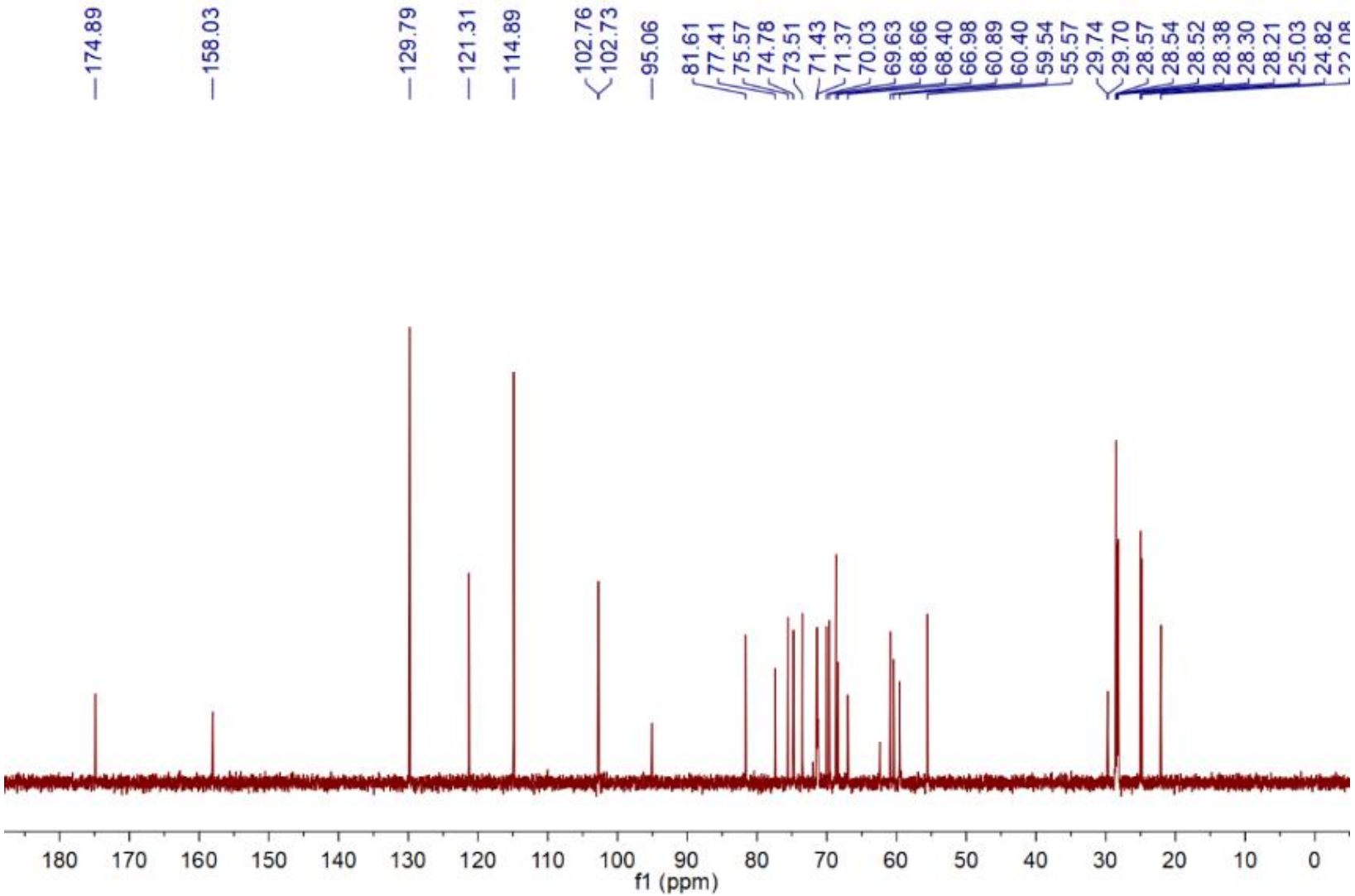
^{31}P NMR spectrum of Lacα-PP-$(CH_2)_{11}$-OPh **2** (D_2O, 243 MHz, 25 °C)



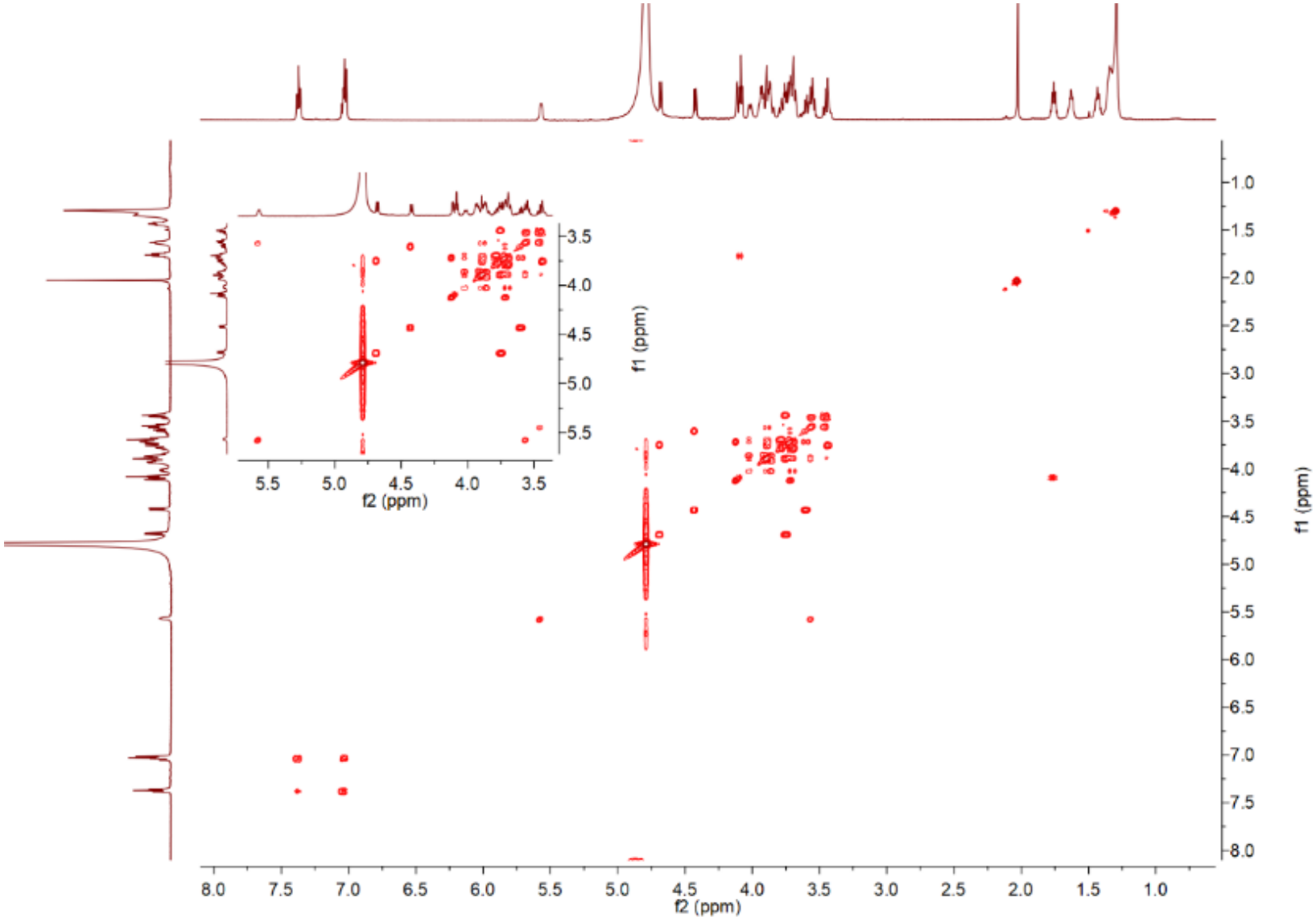
ESI-(-)-TOF HRMS of Lacα-PP-$(CH_2)_{11}$-OPh **2**



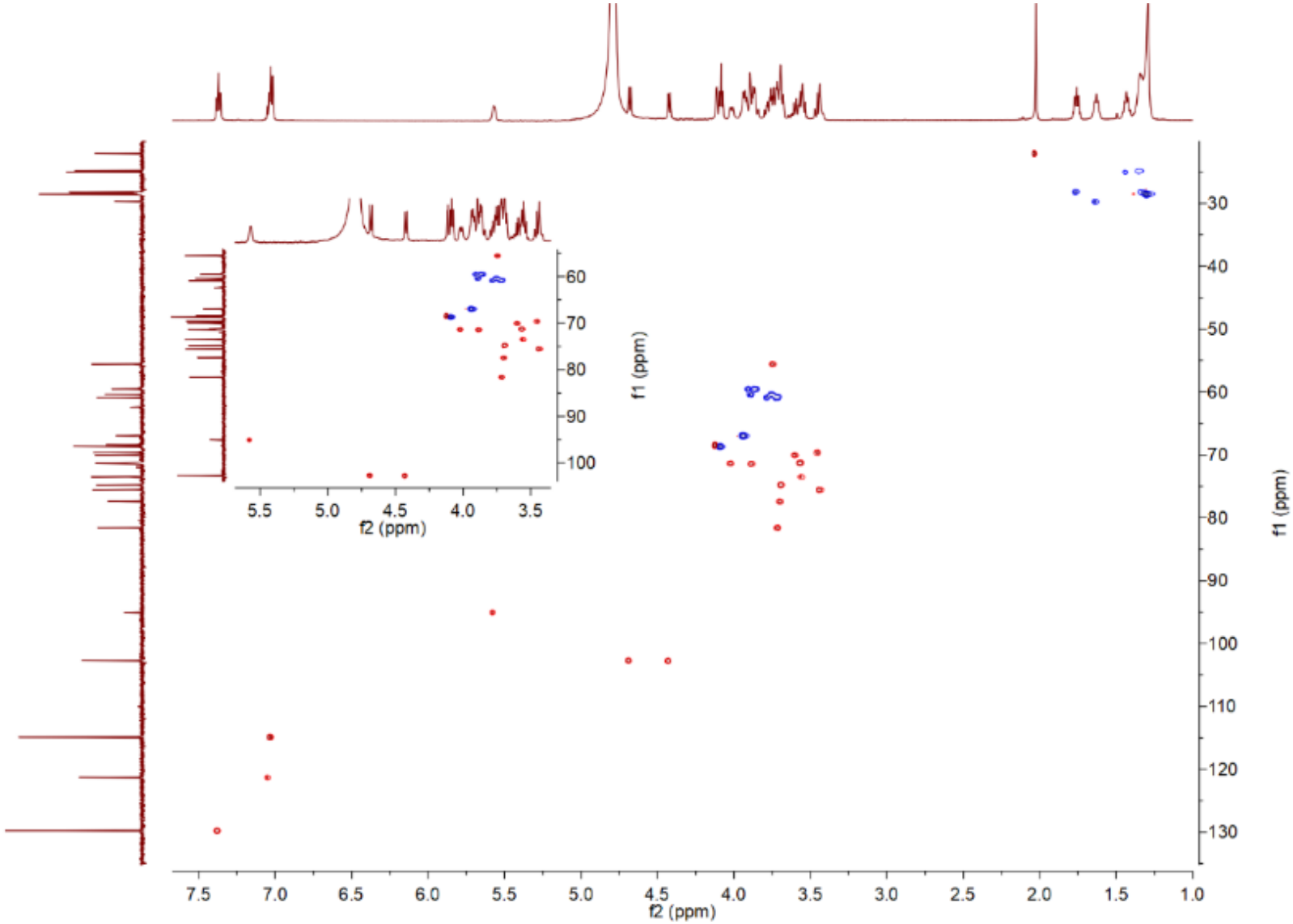
^{1}H NMR spectrum of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** (D_2O, 600 MHz, 25 °C)



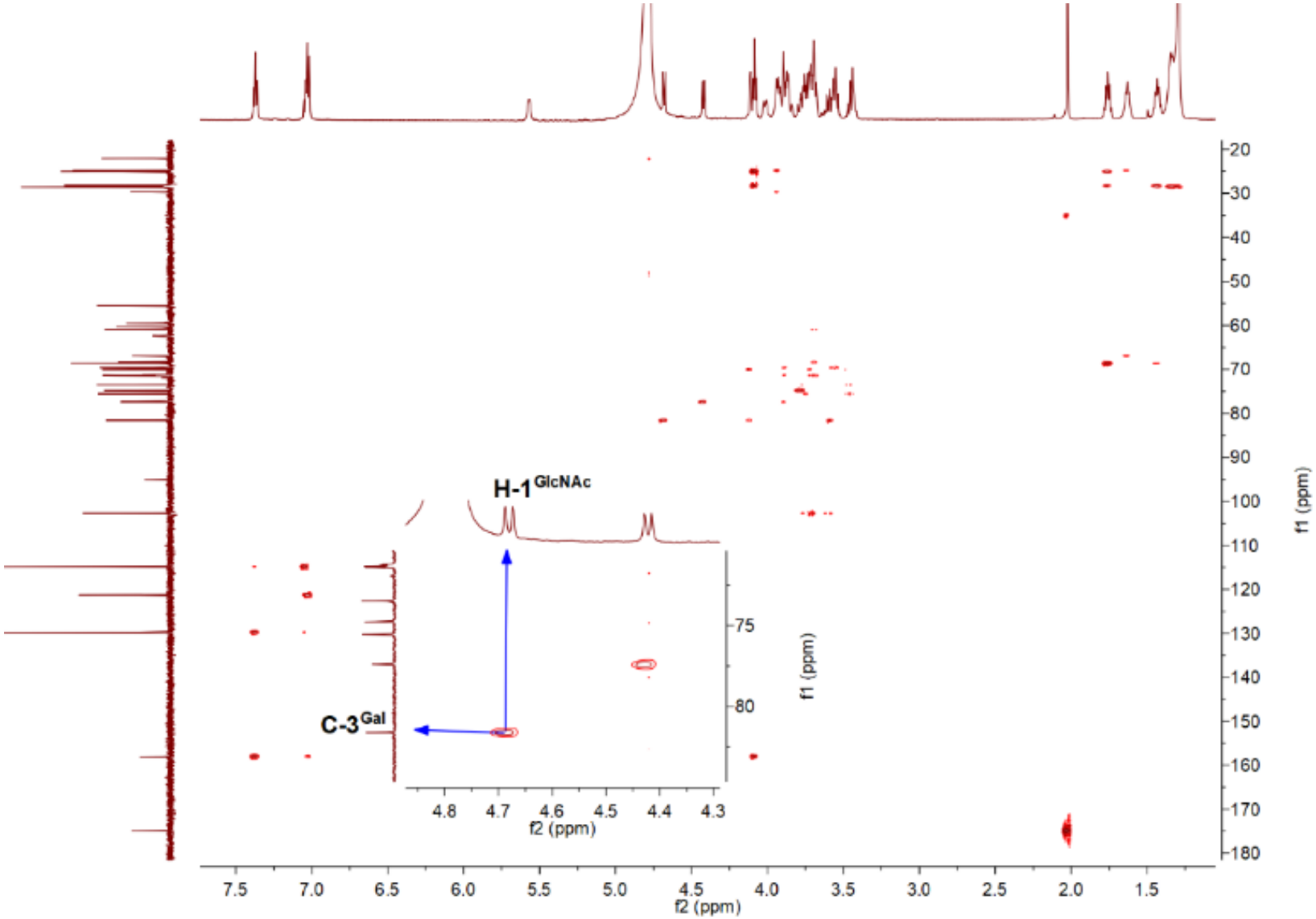
^{13}C NMR spectrum of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** (D_2O, 150 MHz, 25 °C)



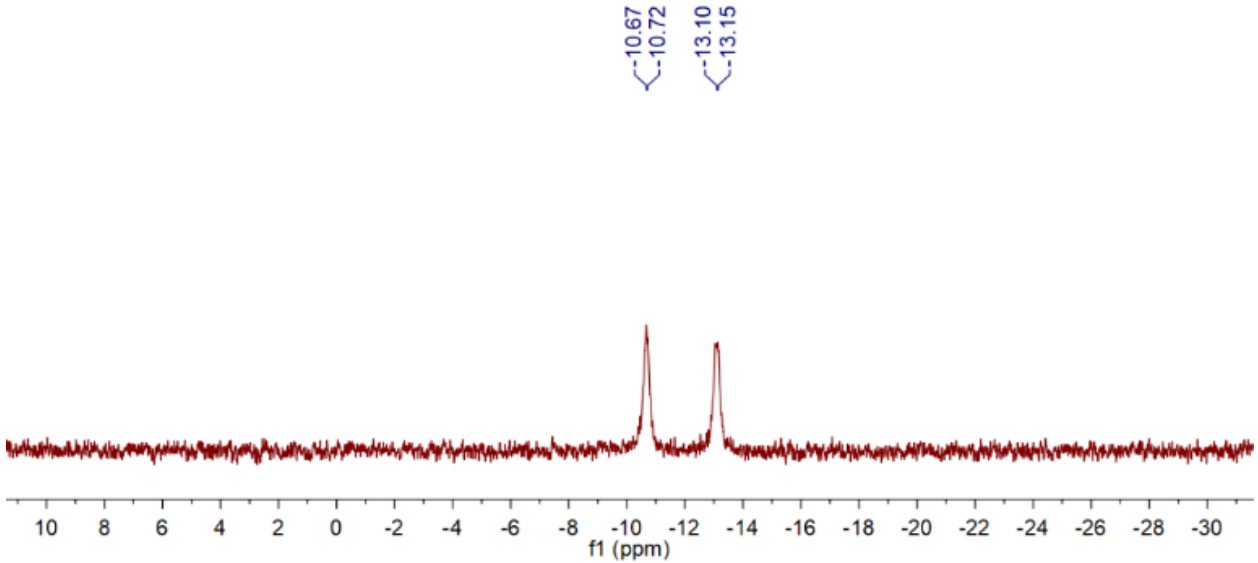
COSY spectrum of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** (D_2O, 600/600 MHz, 25 °C)



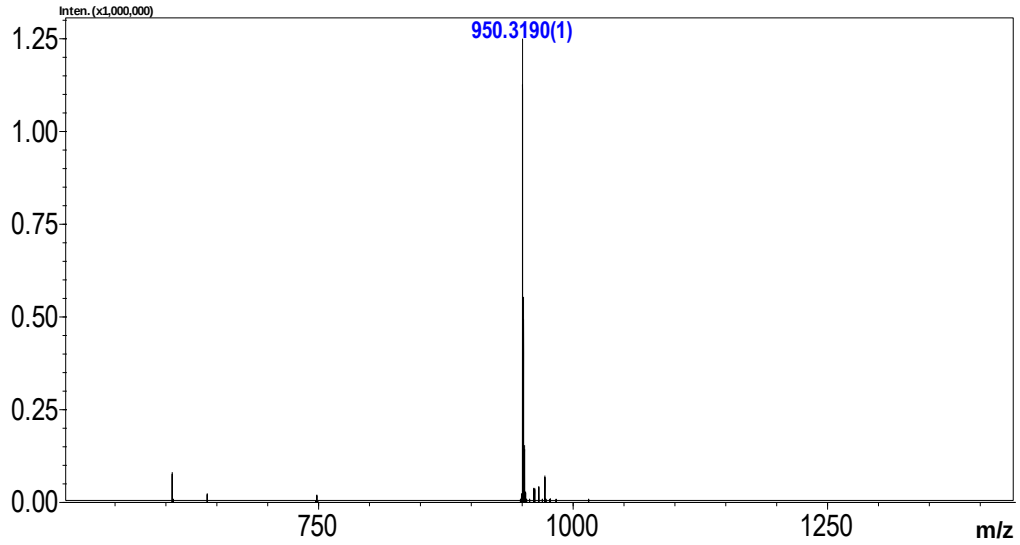
HSQC spectrum of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** (D_2O, 600/150 MHz, 25 °C)



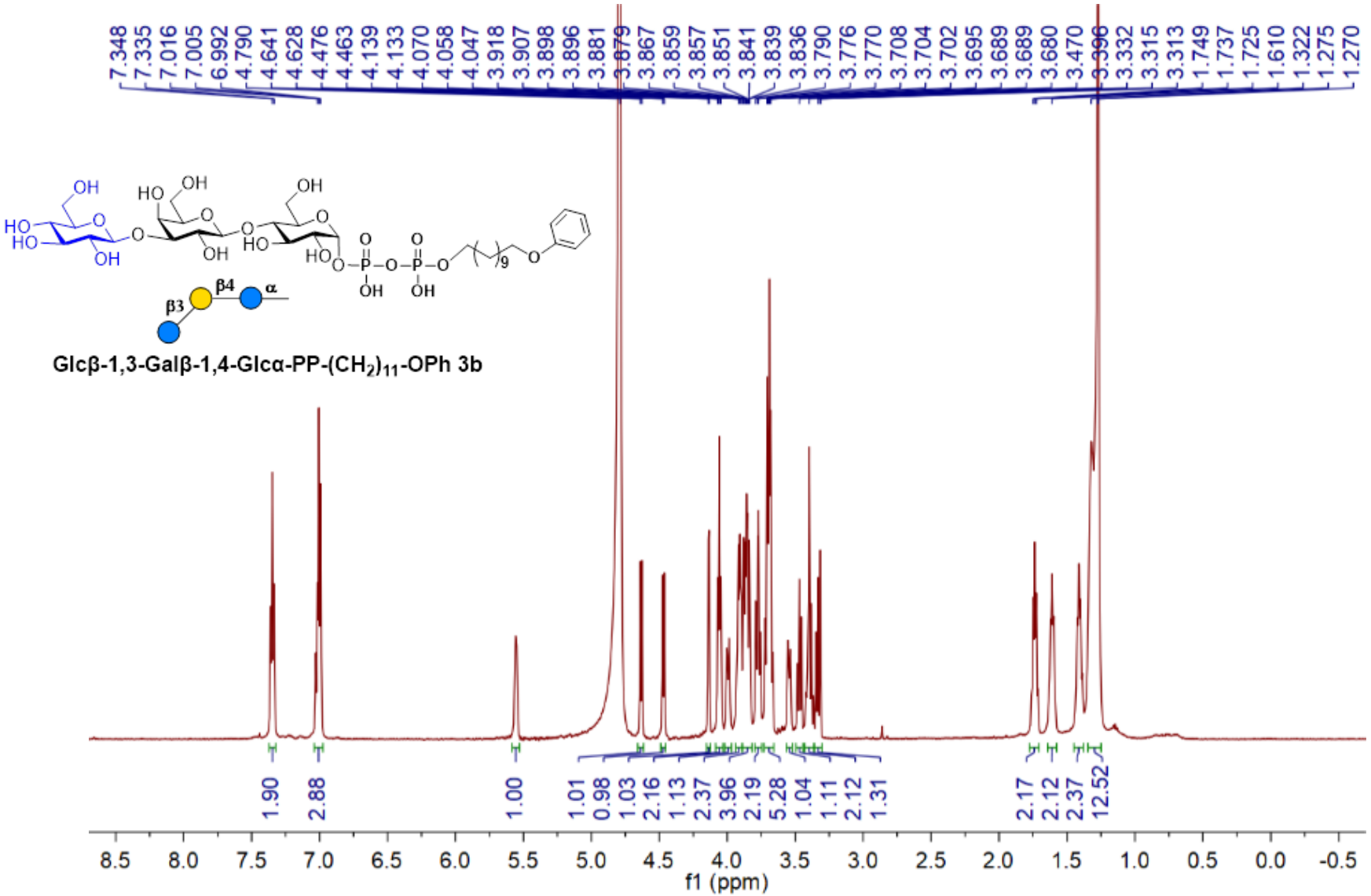
HMBC spectrum of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** (D_2O, 600/150 MHz, 25 °C)



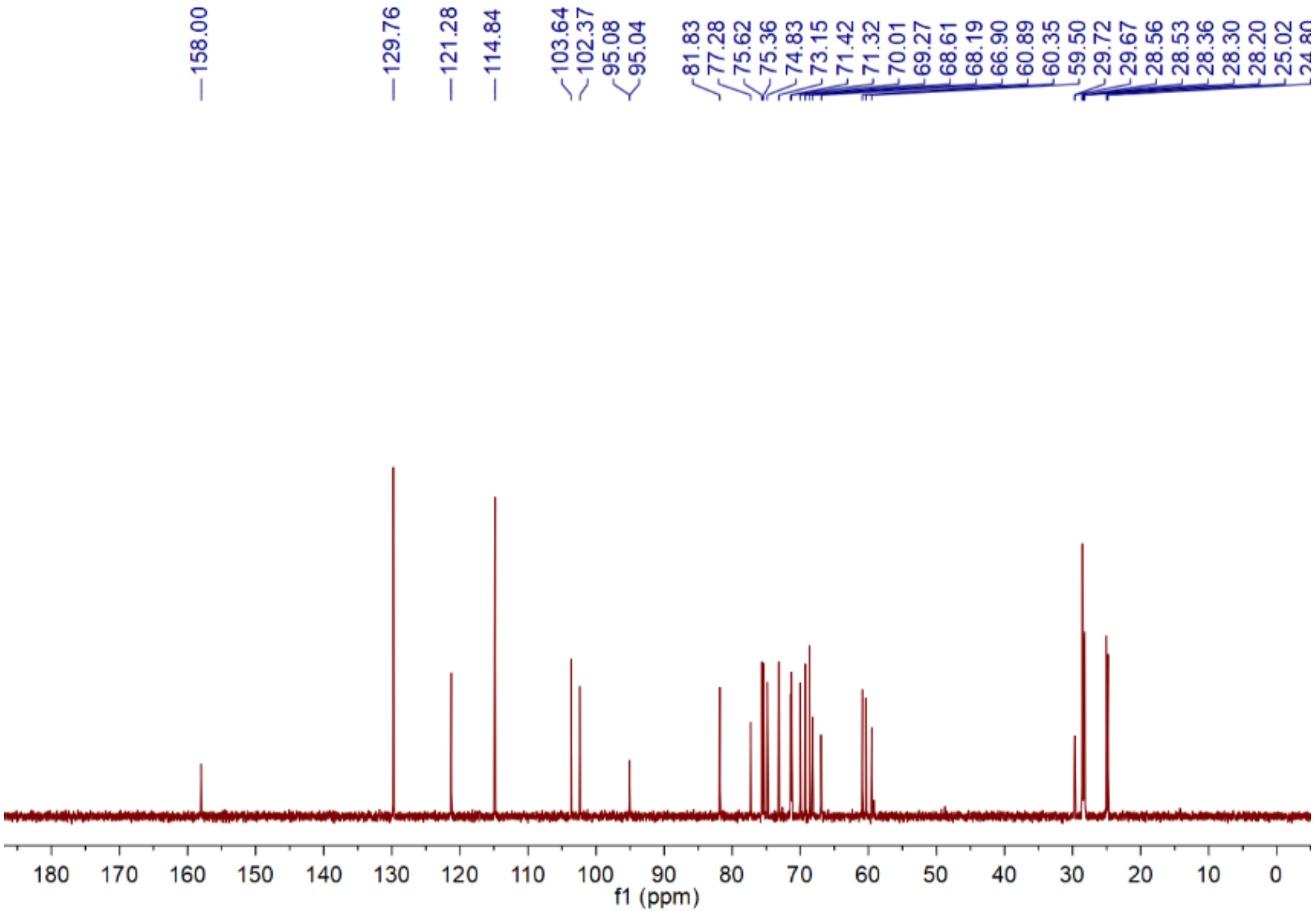
^{31}P NMR spectrum of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a** (D_2O, 243 MHz, 25 °C)



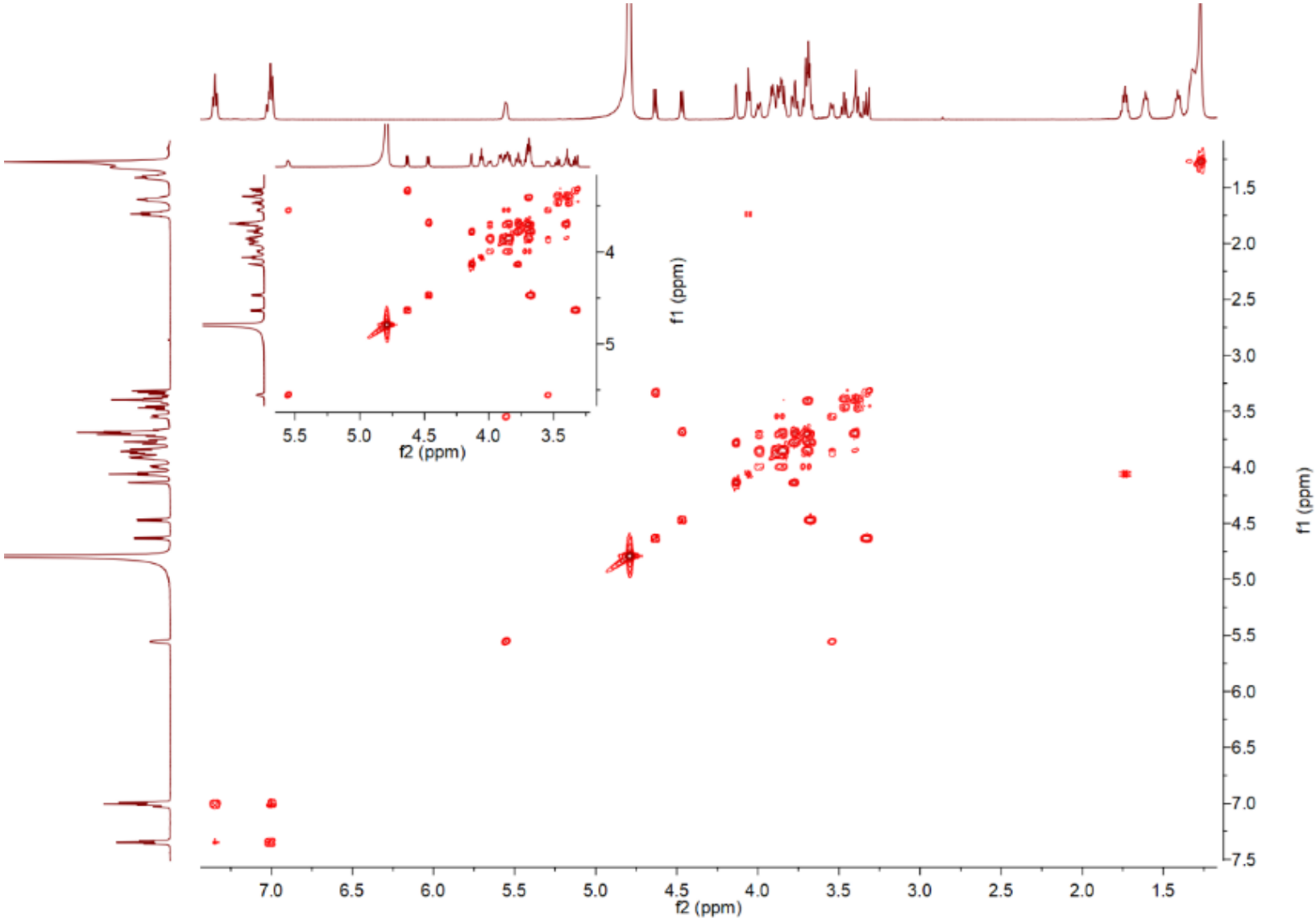
ESI-(-)-TOF HRMS of GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3a**



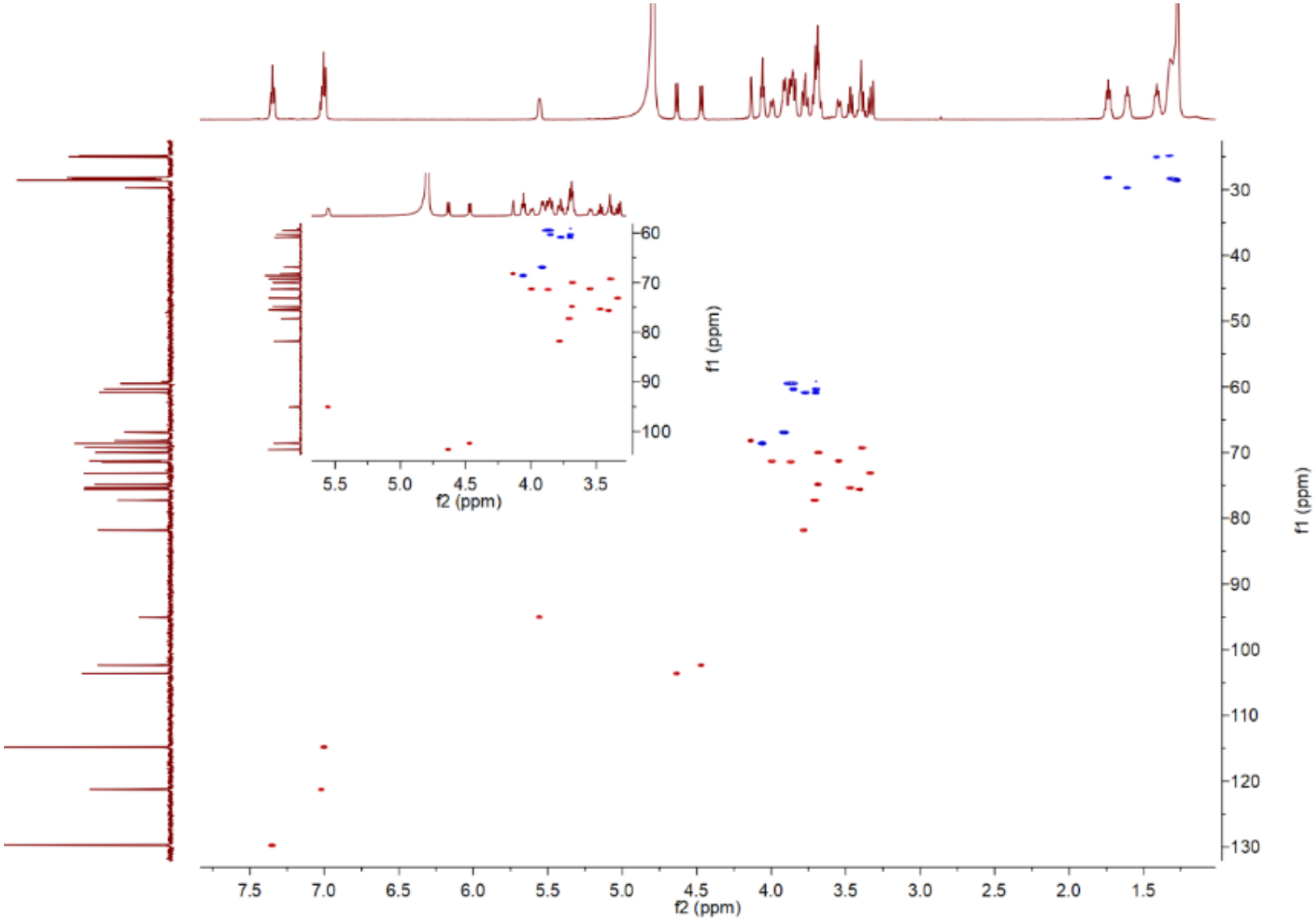
^{1}H NMR spectrum of Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** (D_2O, 600 MHz, 25 °C)



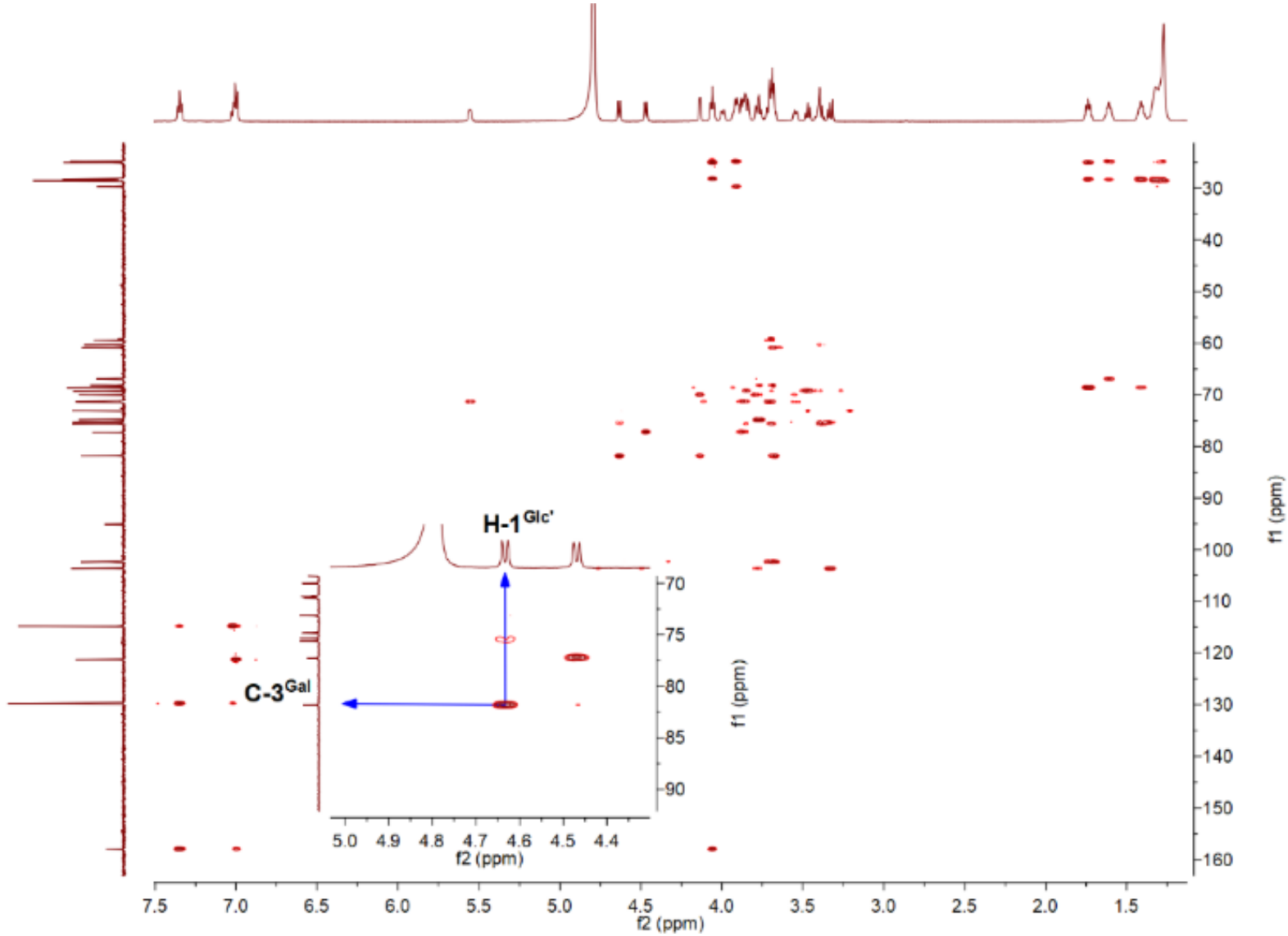
^{13}C NMR spectrum of Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** (D_2O, 150 MHz, 25 °C)



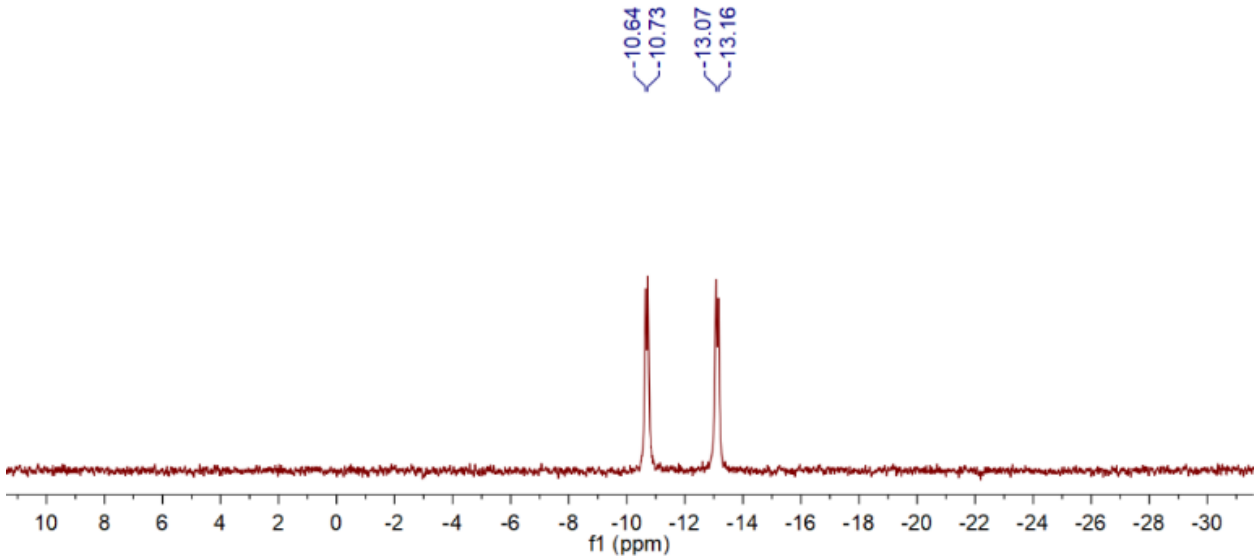
COSY spectrum of Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** (D_2O, 600/600 MHz, 25 °C)



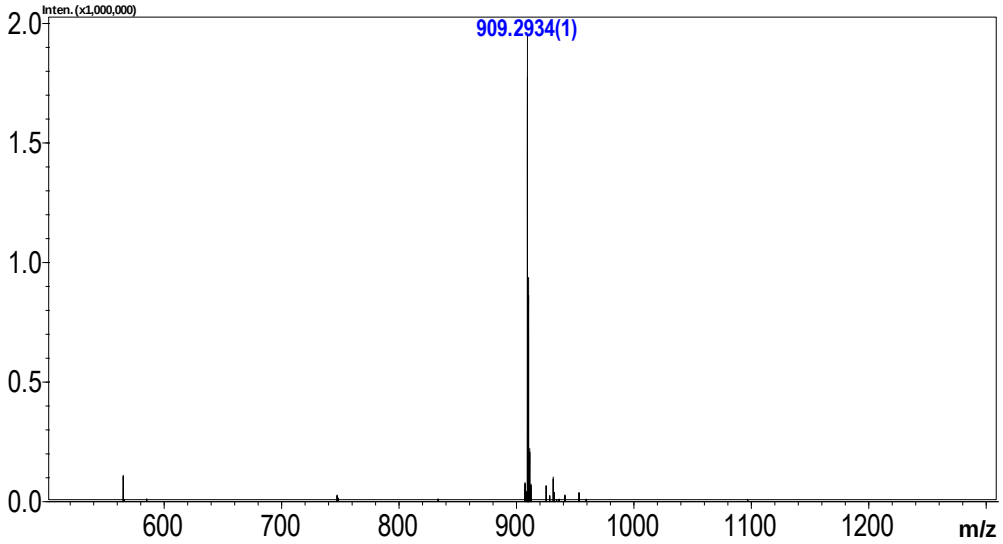
HSQC spectrum of Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** (D_2O, 600/150 MHz, 25 °C)



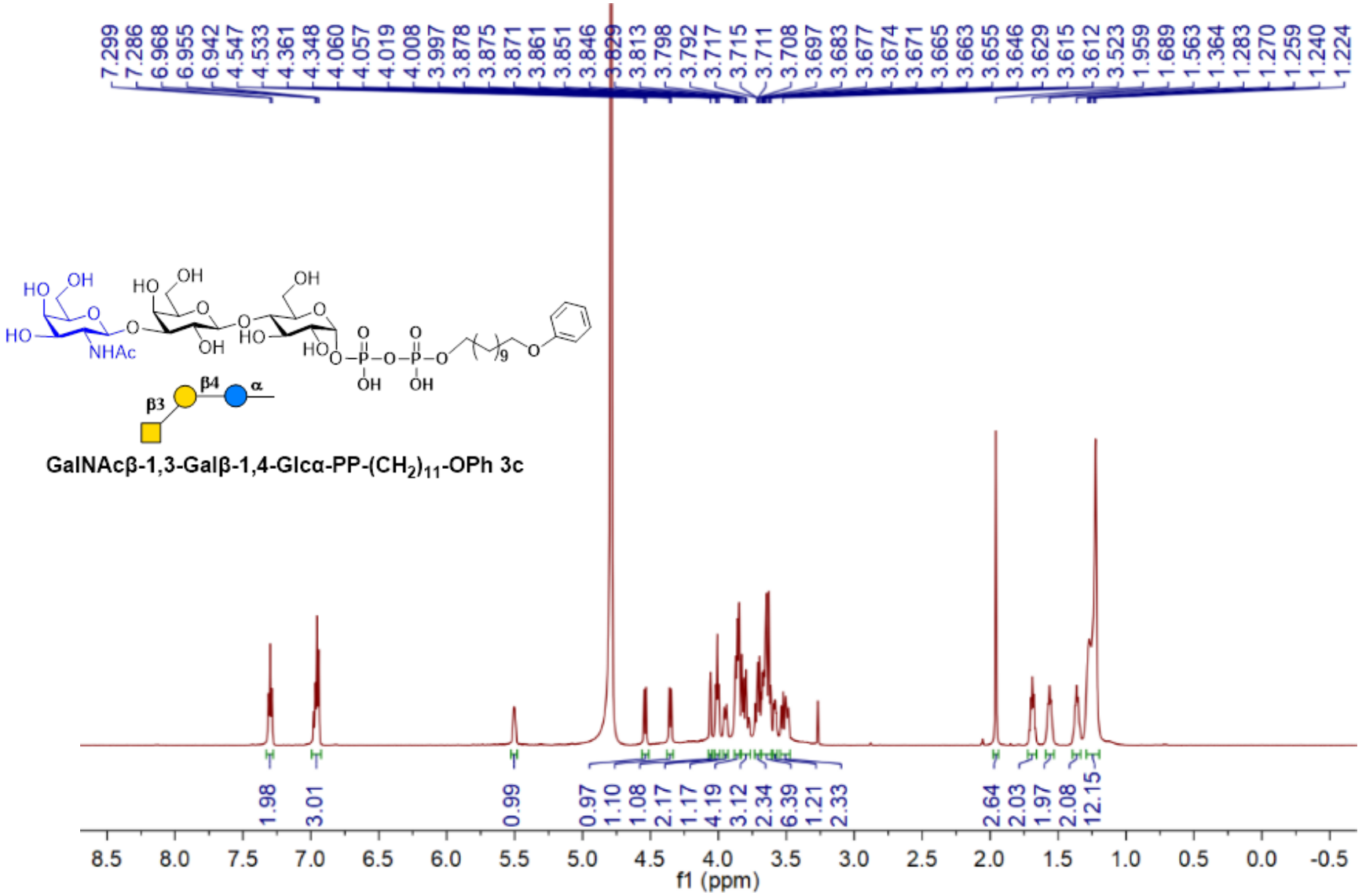
HMBC spectrum of Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** (D_2O, 600/150 MHz, 25 °C)



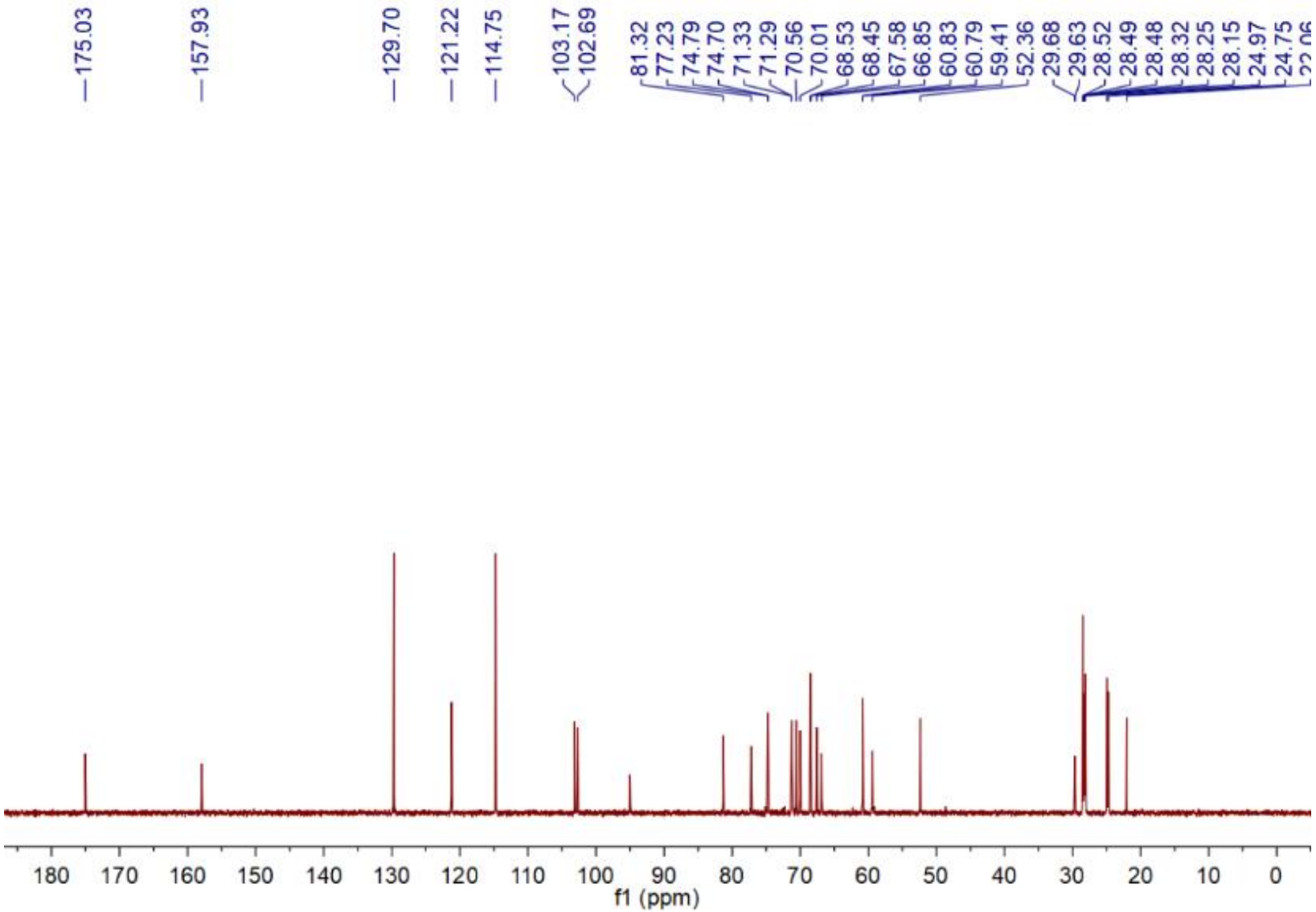
^{31}P NMR spectrum of Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b** (D_2O, 243 MHz, 25 °C)



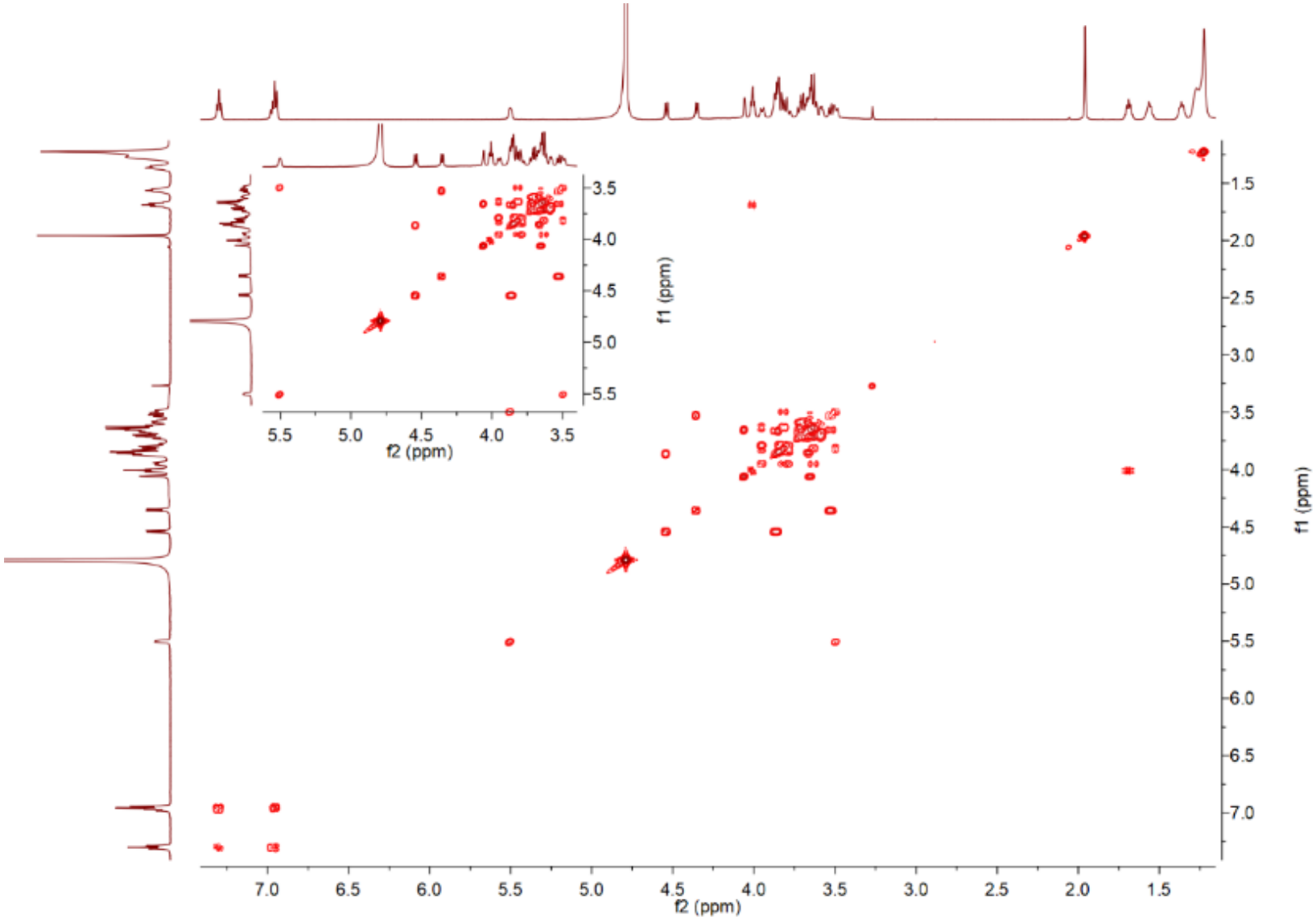
ESI-(-)-TOF HRMS of Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3b**



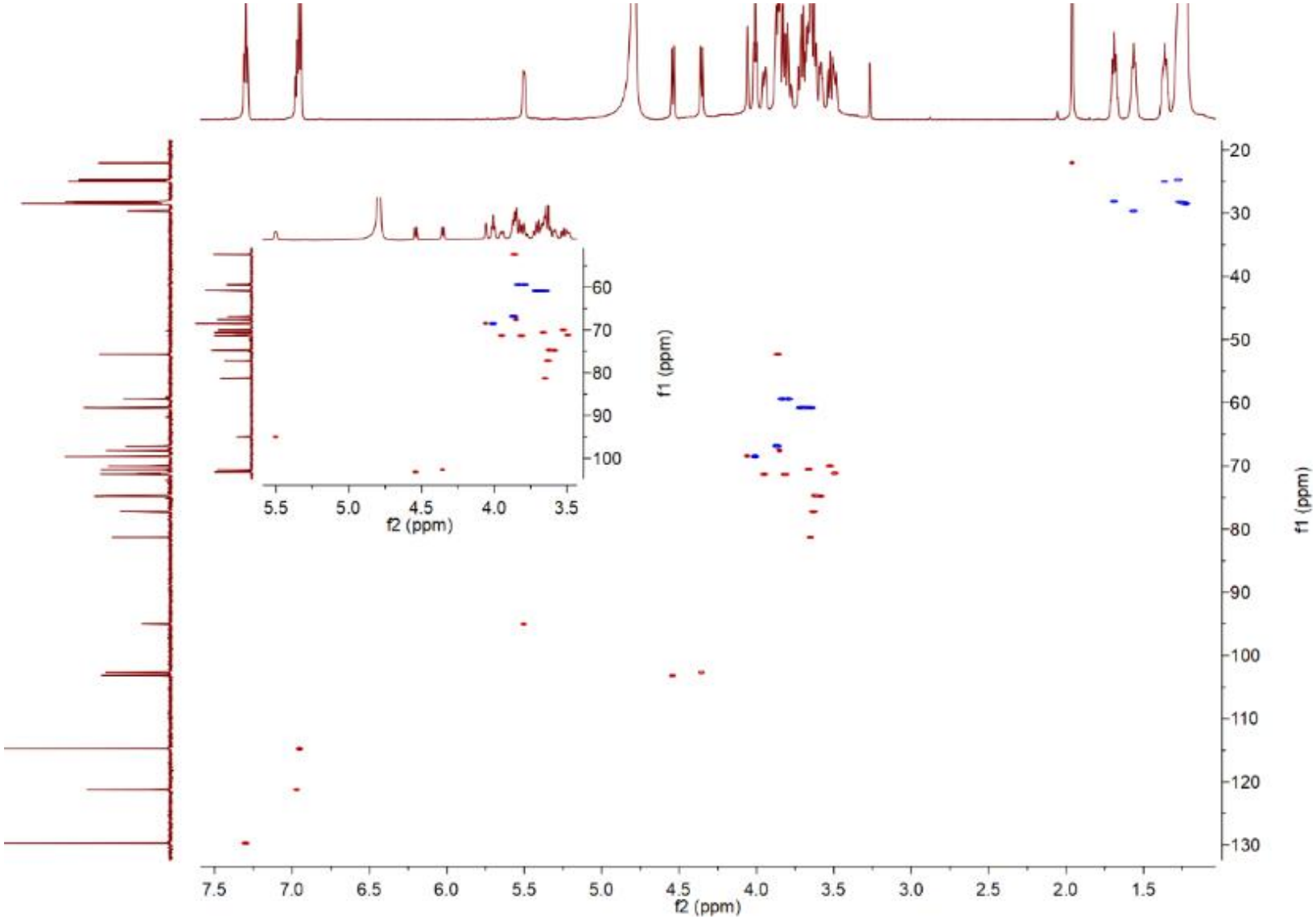
^{1}H NMR spectrum of GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** (D_2O, 600 MHz, 25 °C)



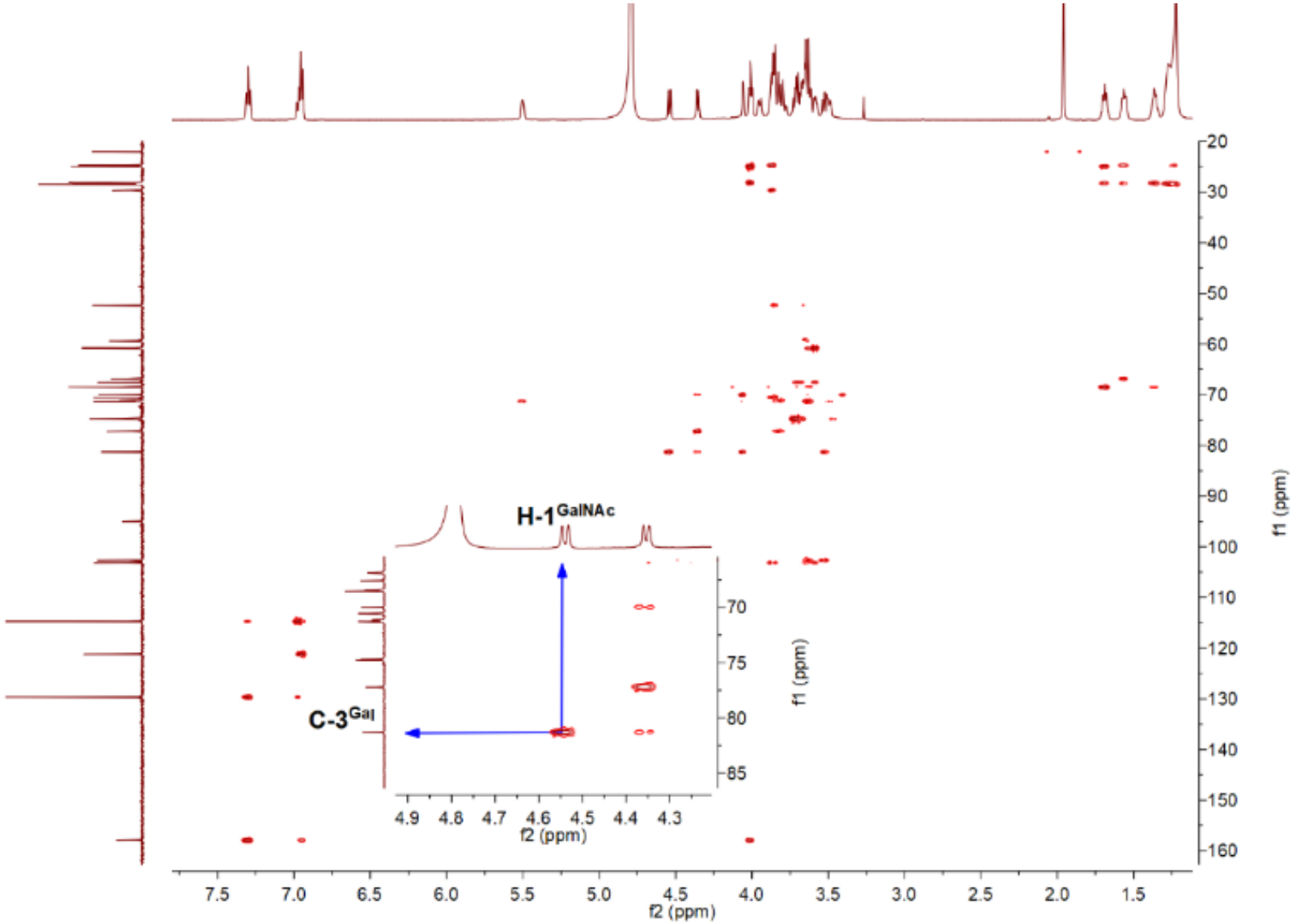
^{13}C NMR spectrum of GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** (D_2O, 150 MHz, 25 °C)



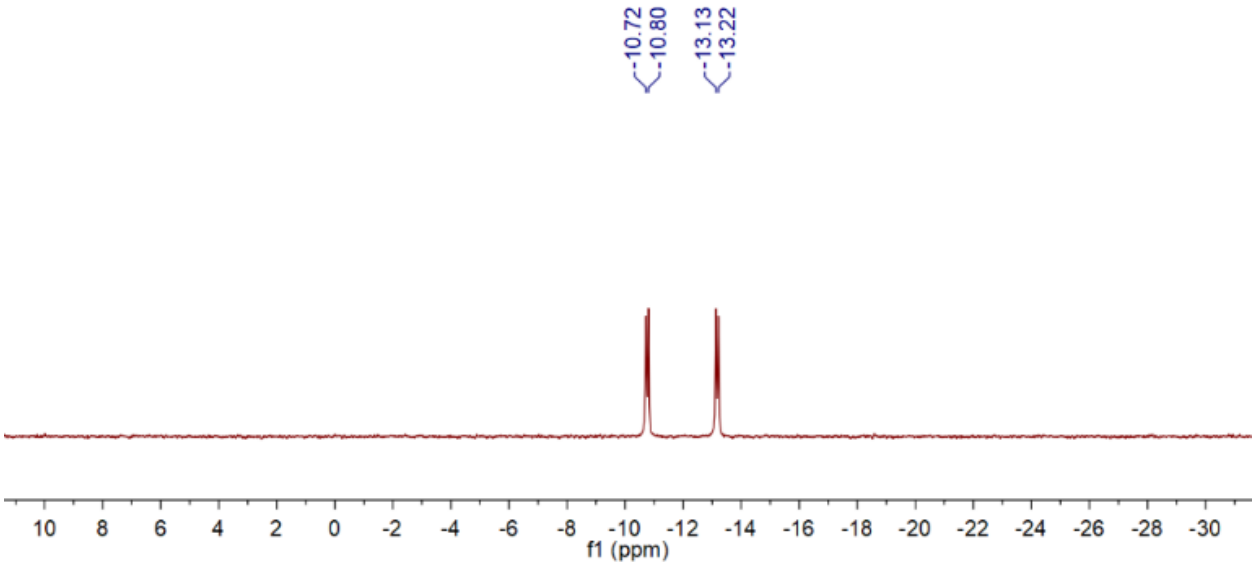
COSY spectrum of GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** (D_2O, 600/600 MHz, 25 °C)



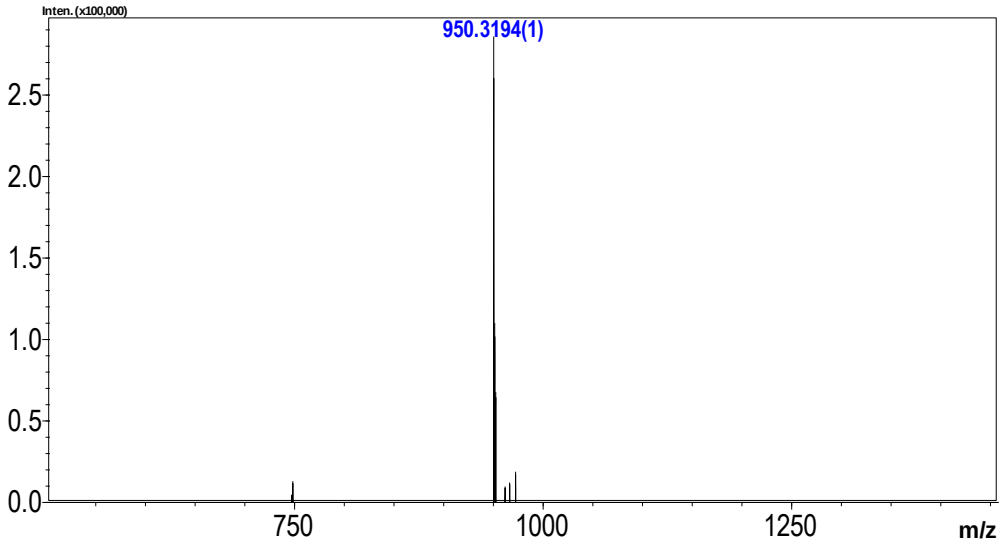
HSQC spectrum of GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** (D_2O, 600/150 MHz, 25 °C)



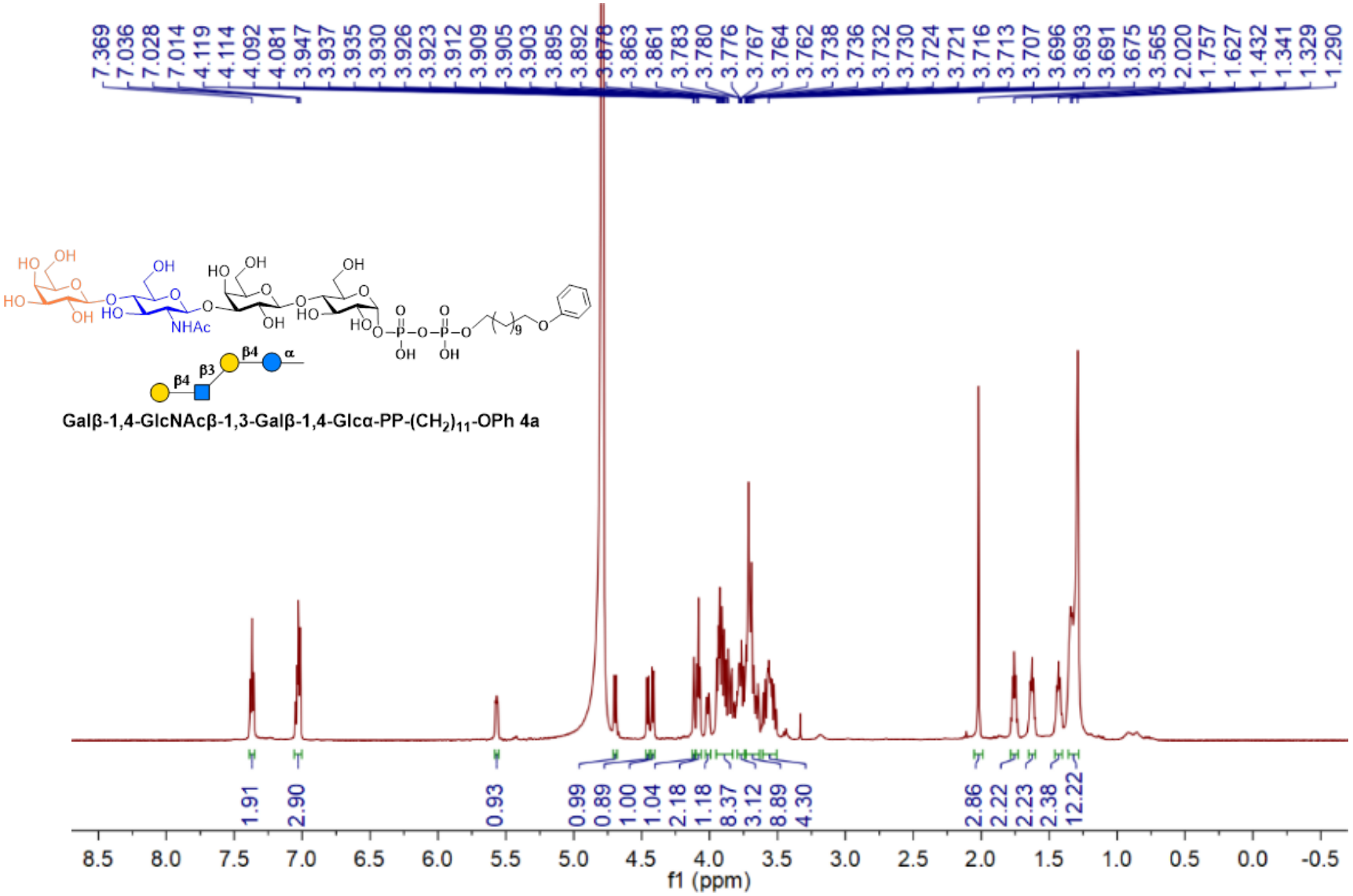
HMBC spectrum of GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** (D_2O, 600/150 MHz, 25 °C)



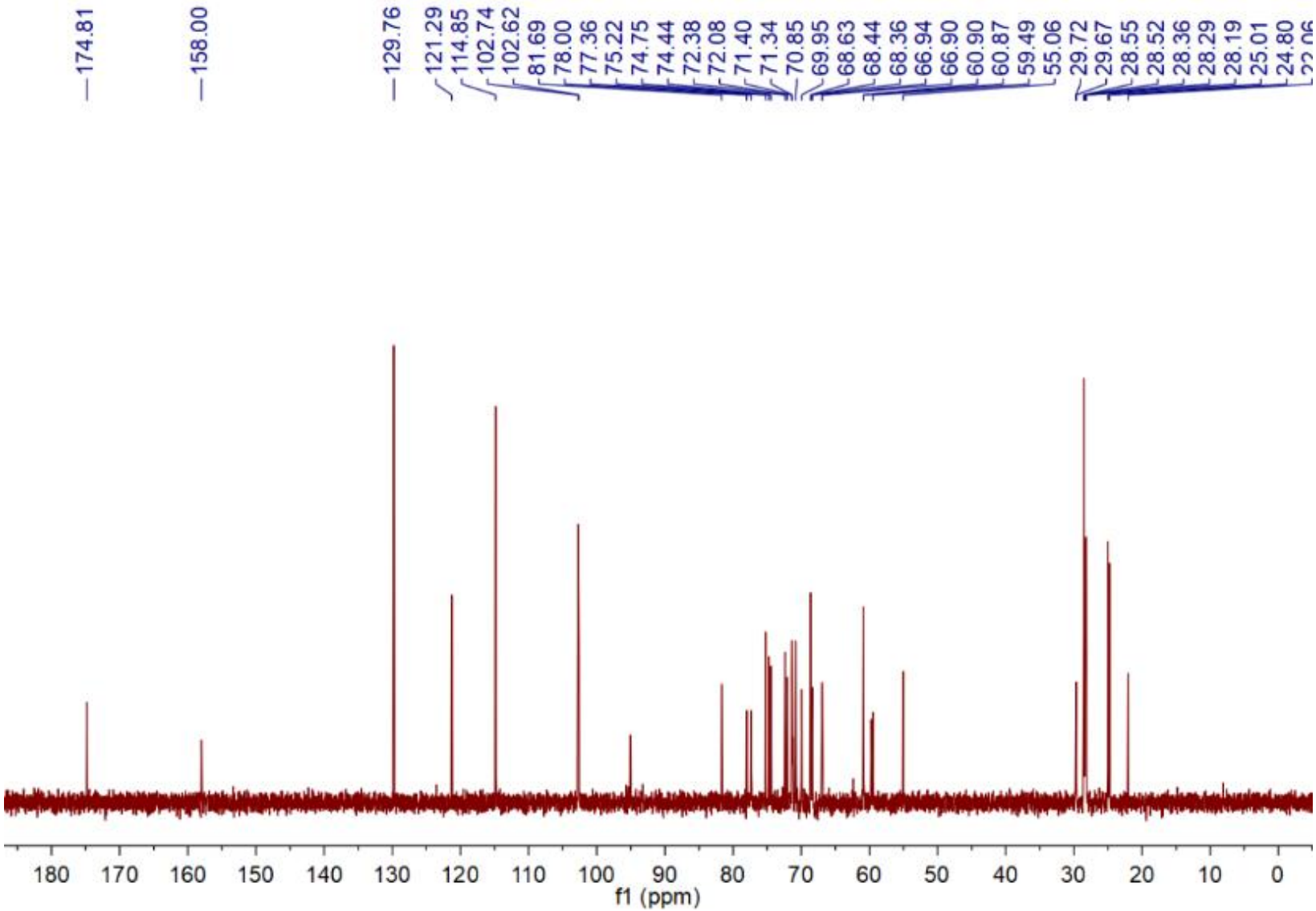
^{31}P NMR spectrum of GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c** (D_2O, 243 MHz, 25 °C)



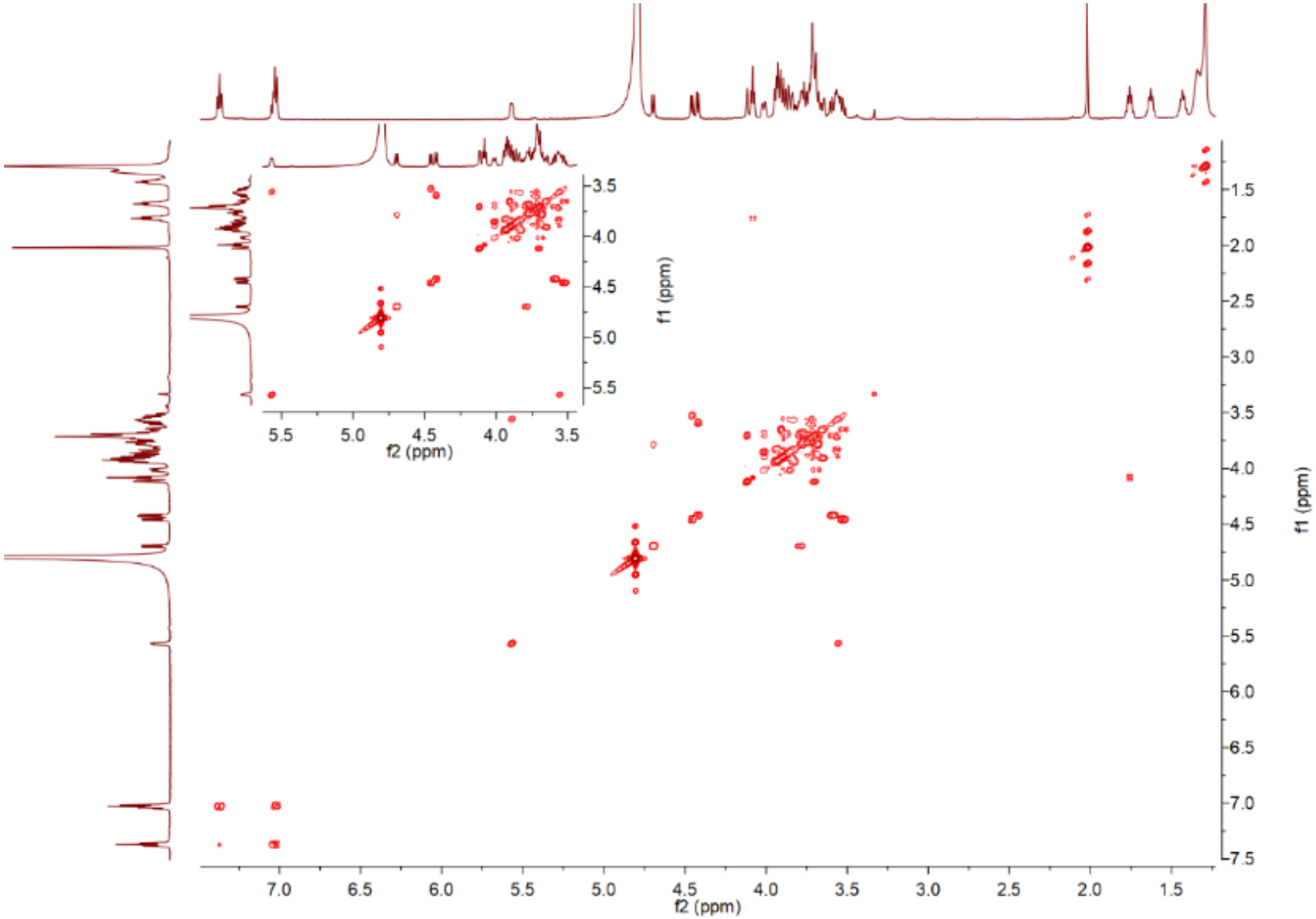
ESI-(-)-TOF HRMS of GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **3c**



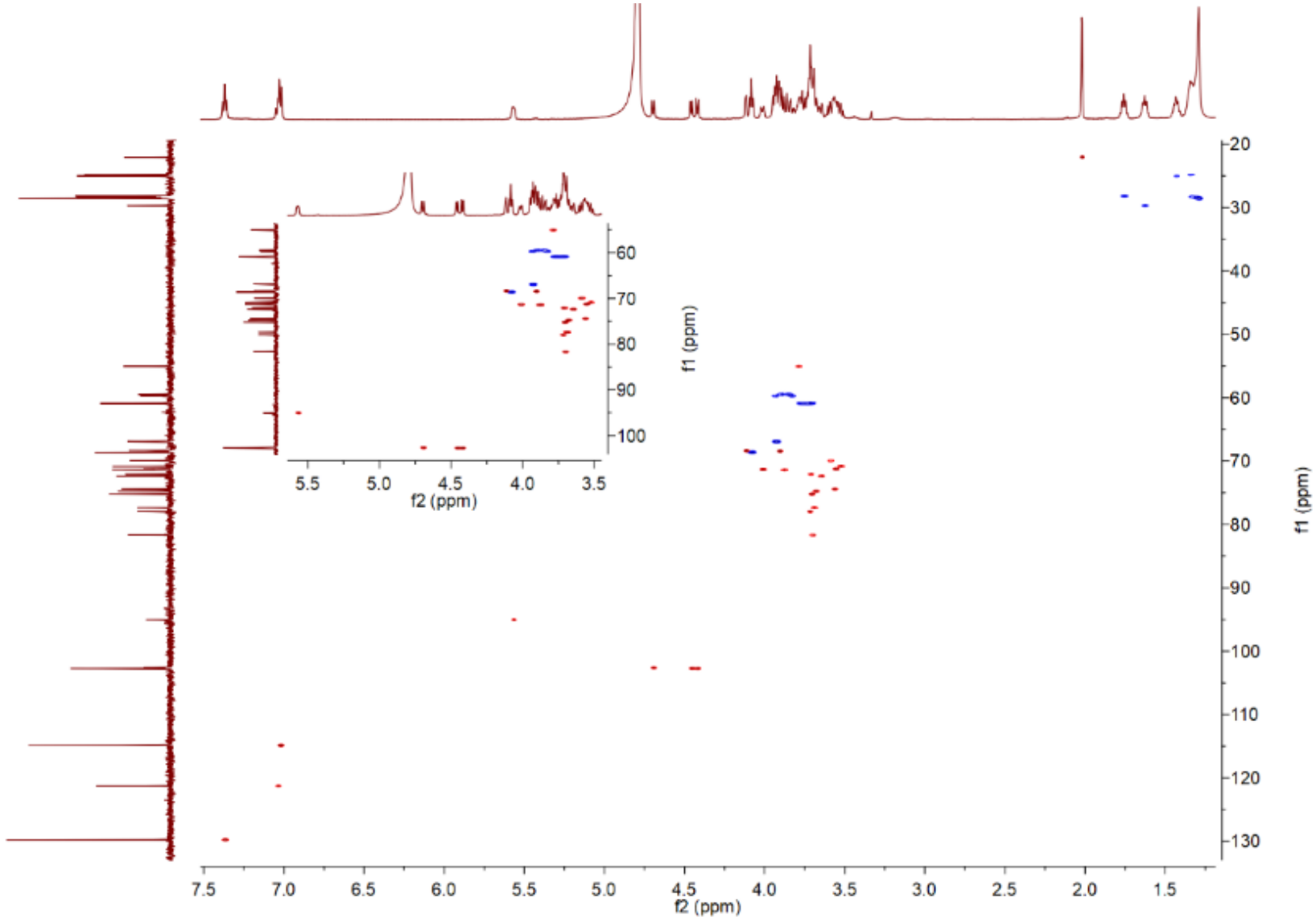
^{1}H NMR spectrum of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** (D_2O, 600 MHz, 25 °C)



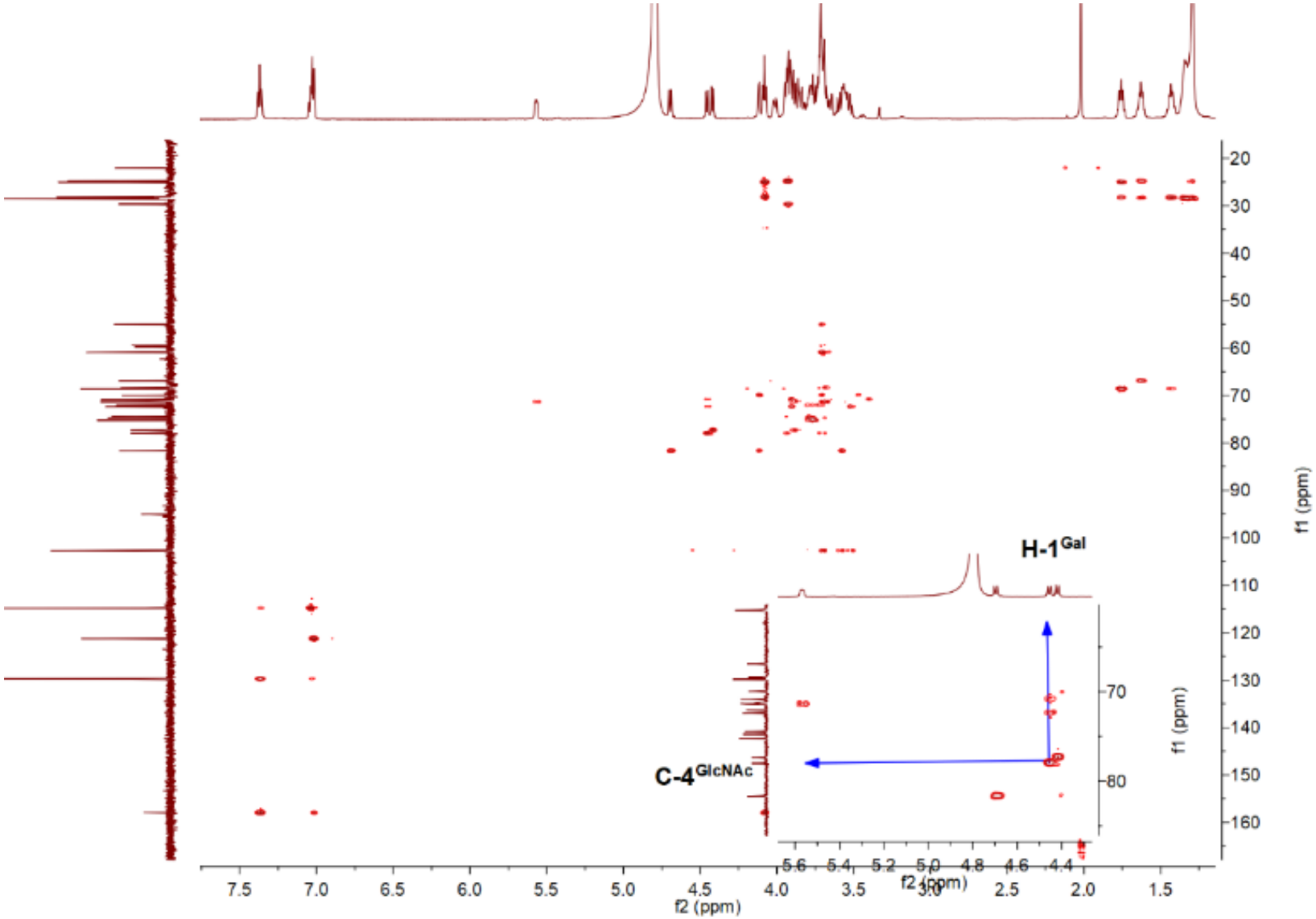
^{13}C NMR spectrum of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** (D_2O, 150 MHz, 25 °C)



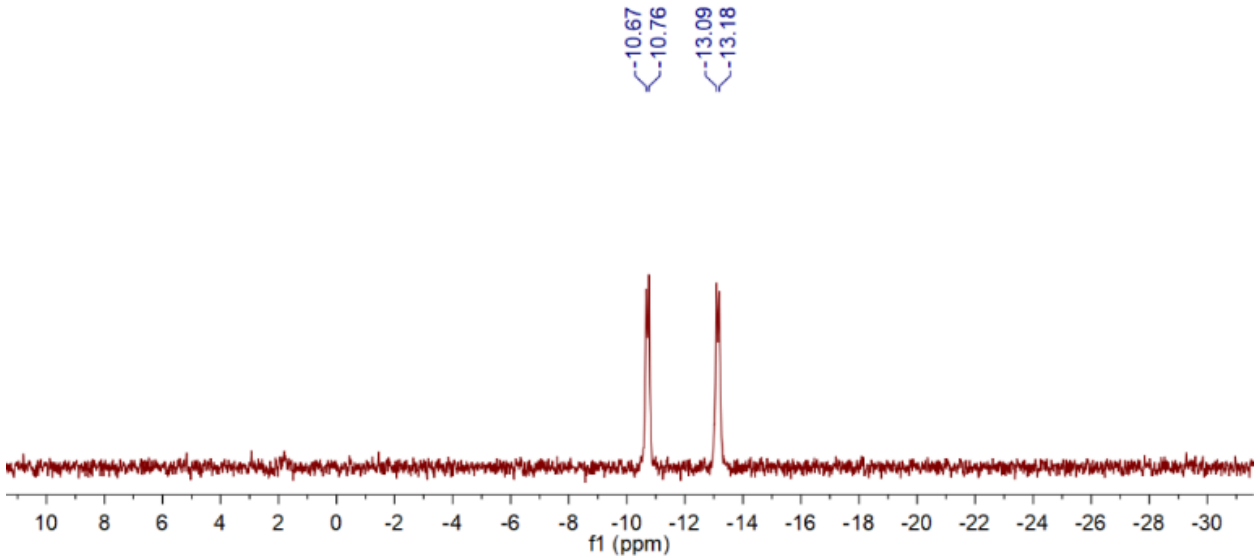
COSY spectrum of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** (D_2O, 600/600 MHz, 25 °C)



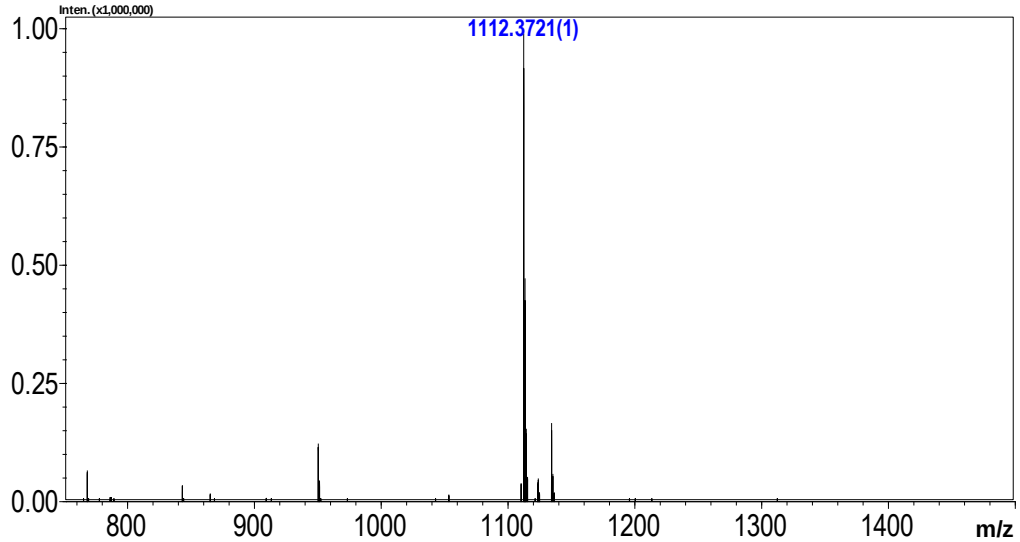
HSQC spectrum of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** (D_2O, 600/150 MHz, 25 °C)



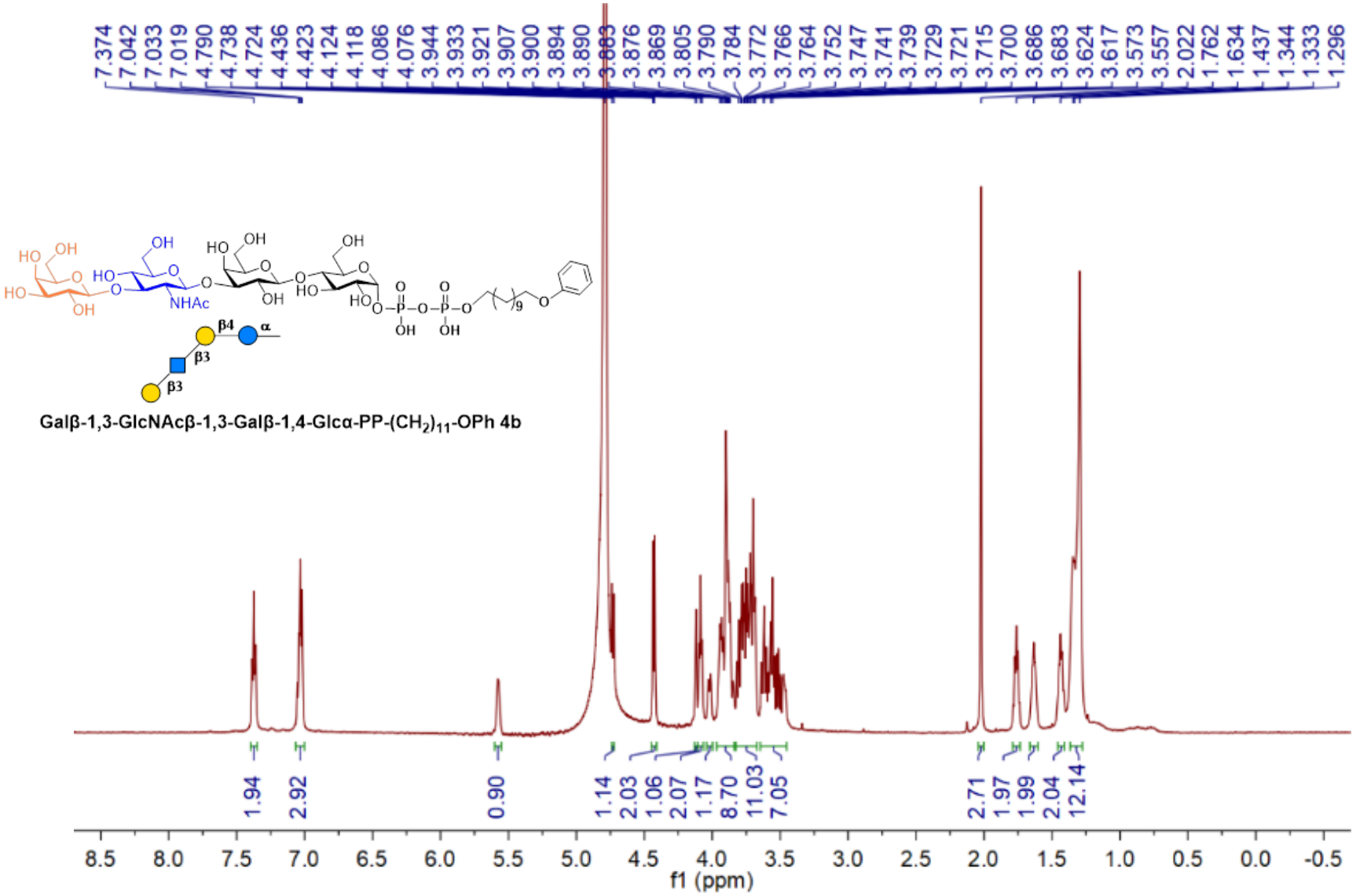
HMBC spectrum of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** (D_2O, 600/150 MHz, 25 °C)



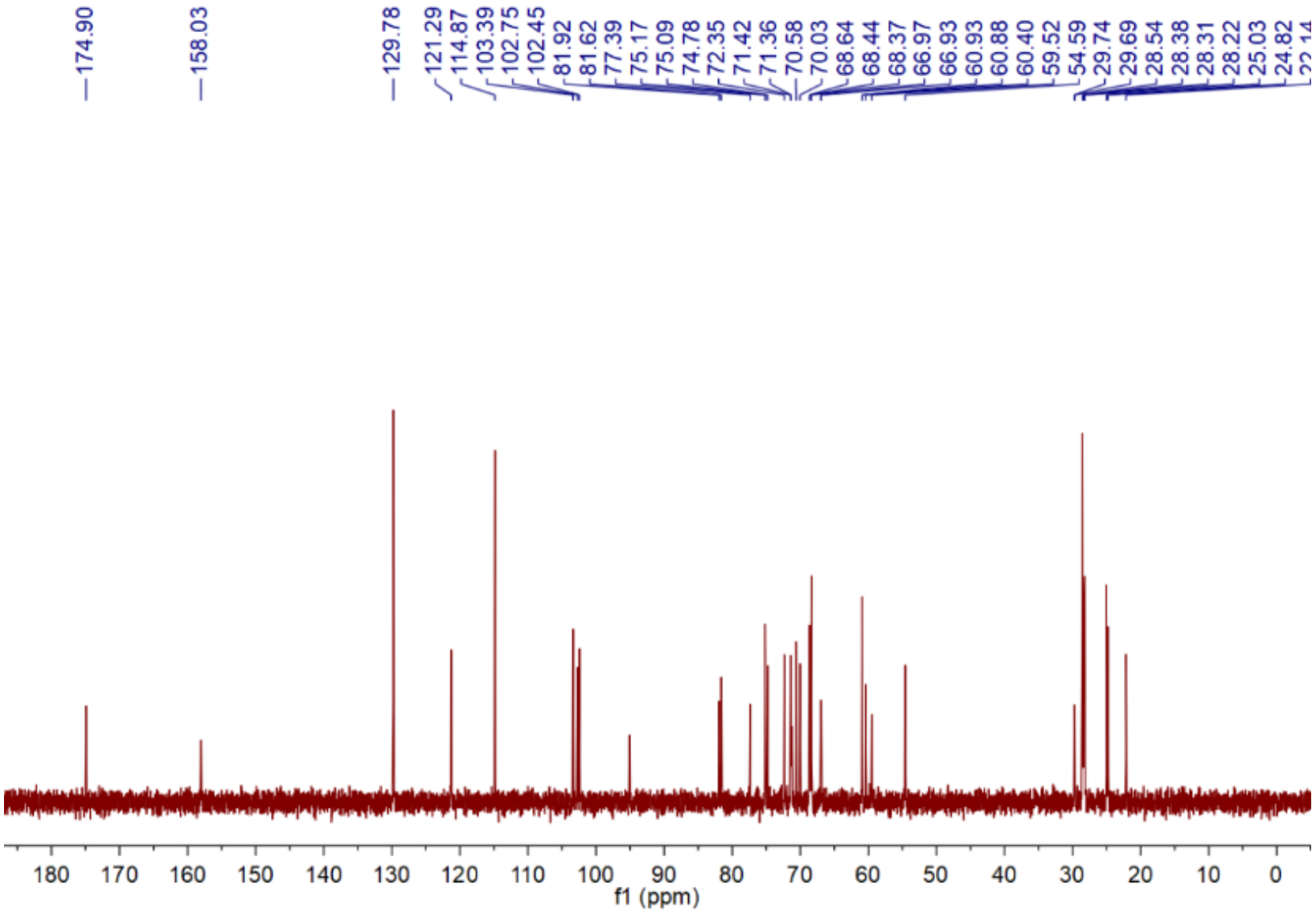
^{31}P NMR spectrum of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a** (D_2O, 243 MHz, 25 °C)



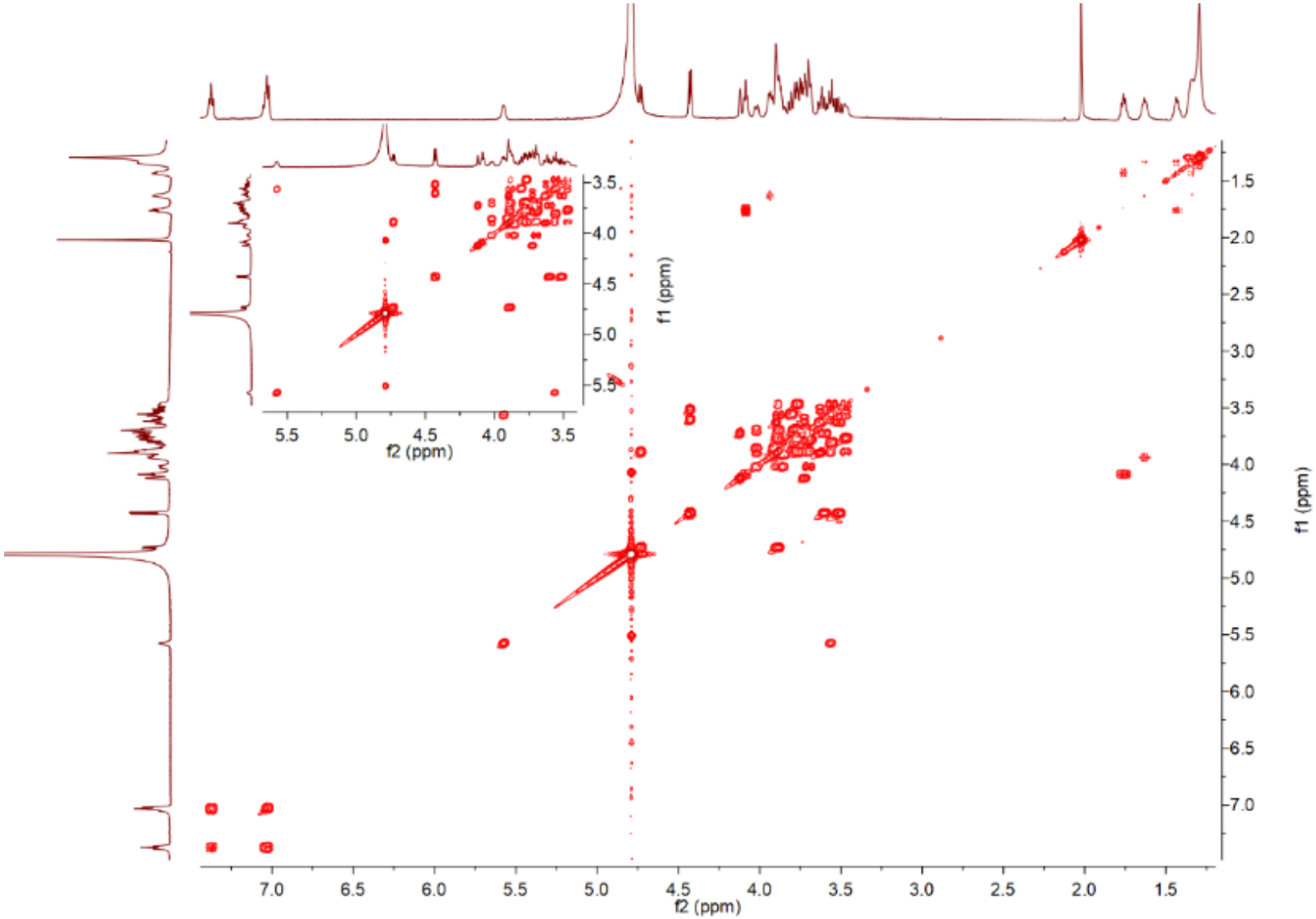
ESI-(-)-TOF HRMS of Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4a**



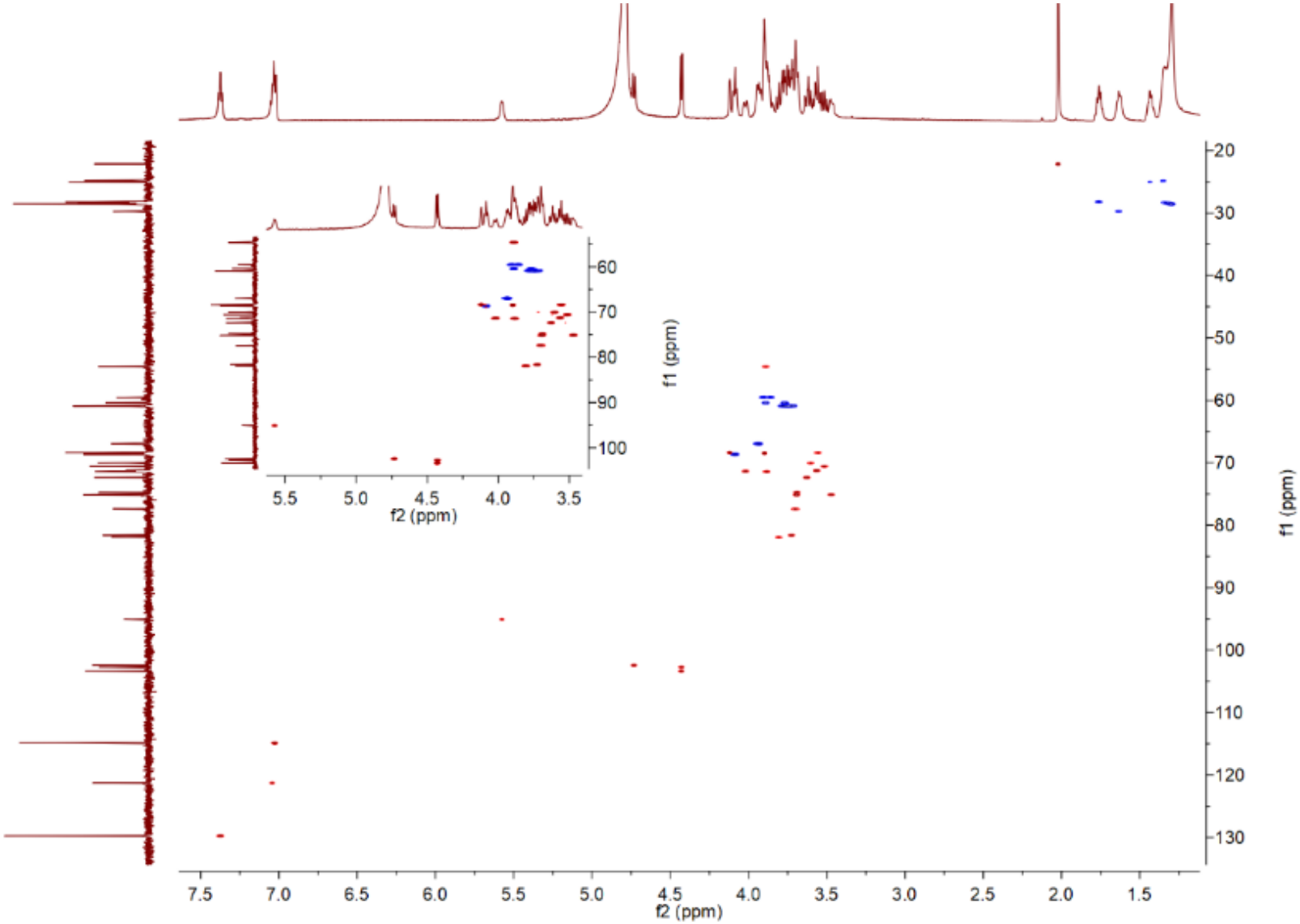
^{1}H NMR spectrum of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** (D_2O, 600 MHz, 25 °C)



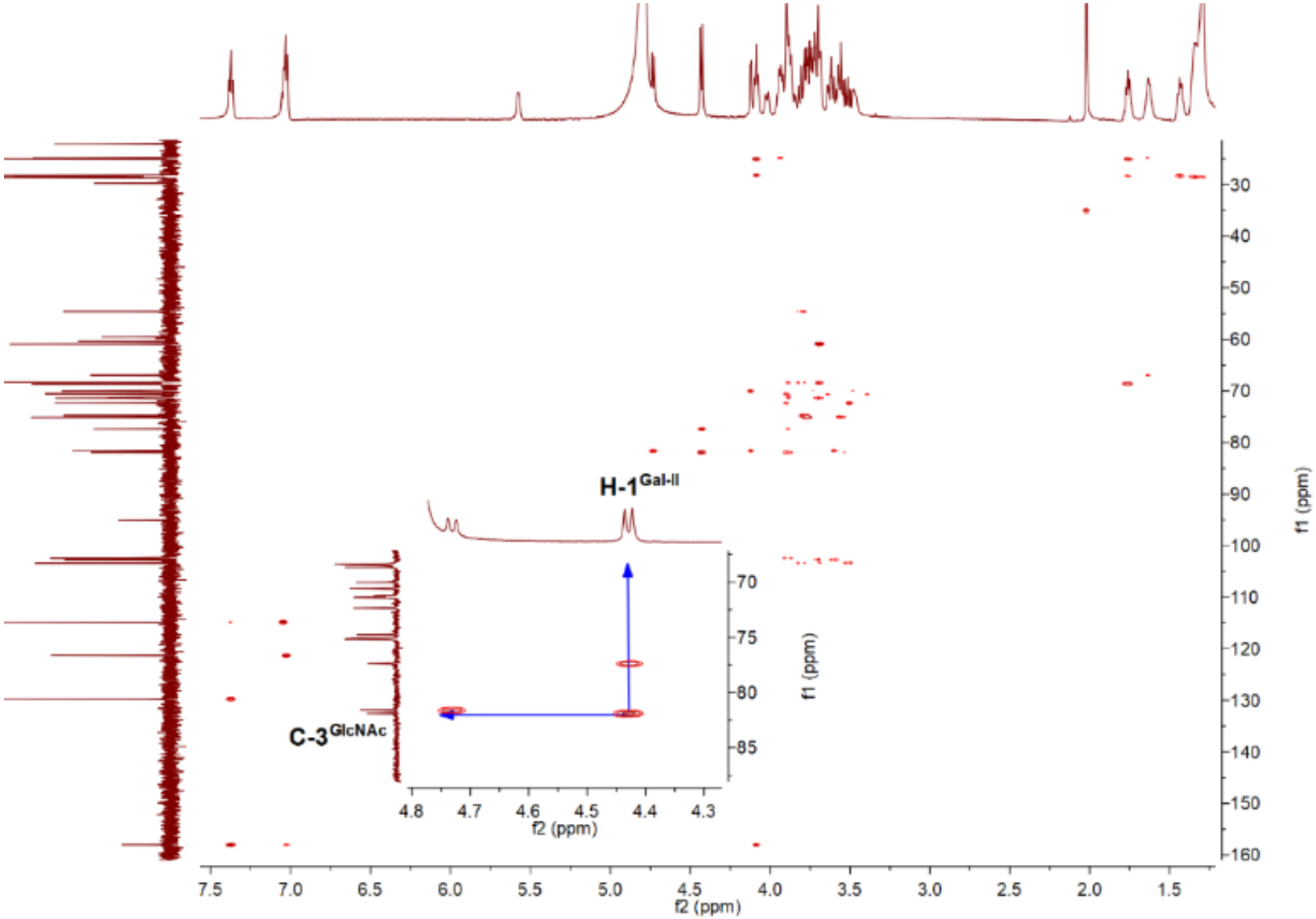
^{13}C NMR spectrum of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** (D_2O, 150 MHz, 25 °C)



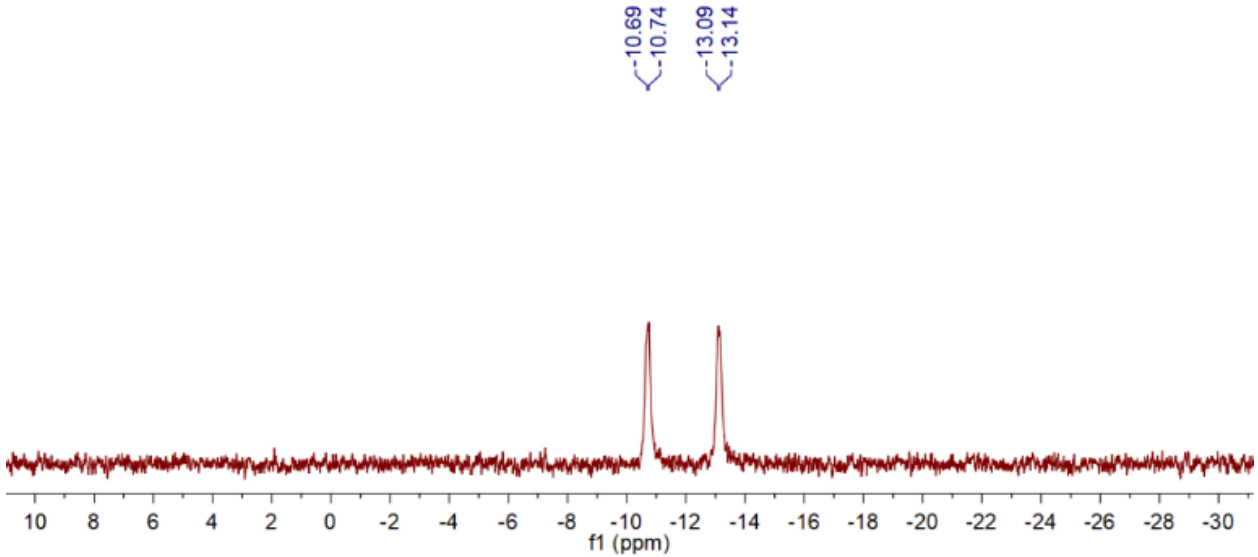
COSY spectrum of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** (D_2O, 600/600 MHz, 25 °C)



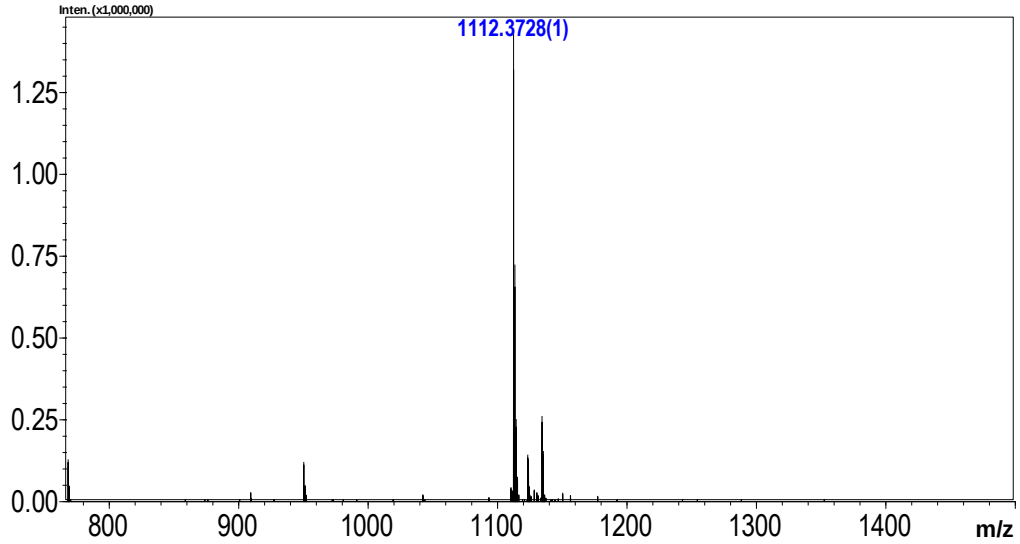
HSQC spectrum of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** (D_2O, 600/150 MHz, 25 °C)



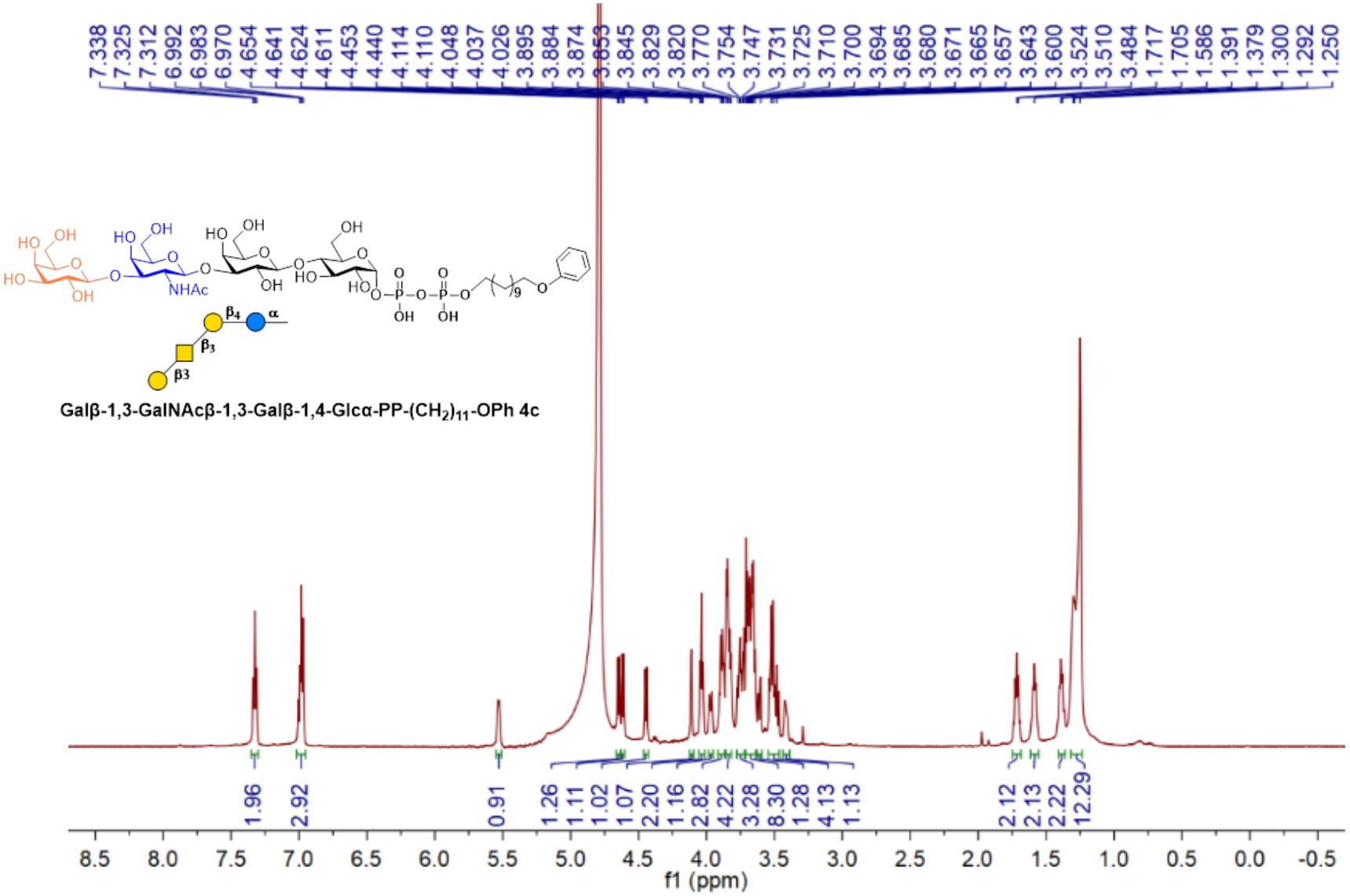
HMBC spectrum of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** (D_2O, 600/150 MHz, 25 °C)



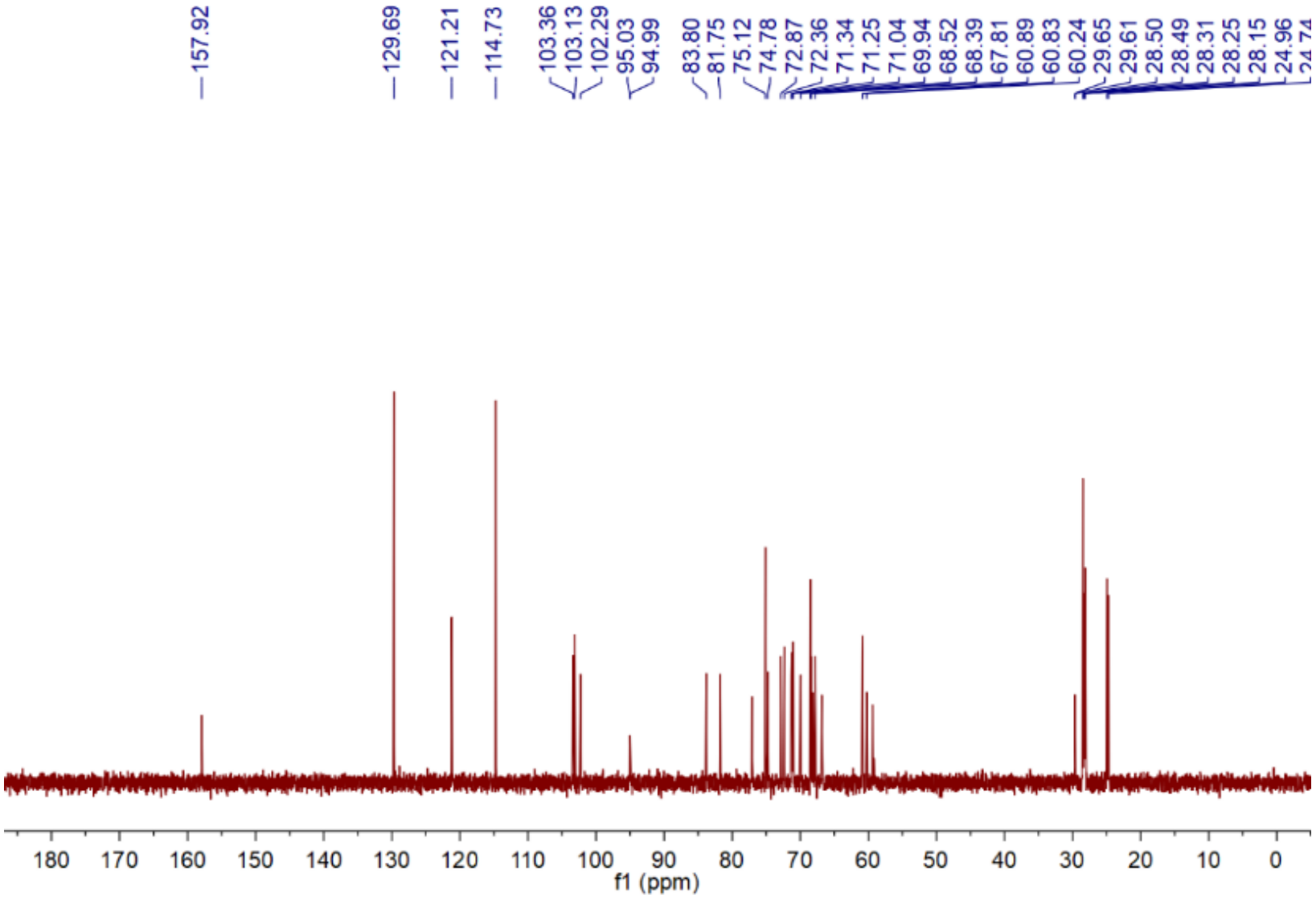
^{31}P NMR spectrum of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b** (D_2O, 243 MHz, 25 °C)



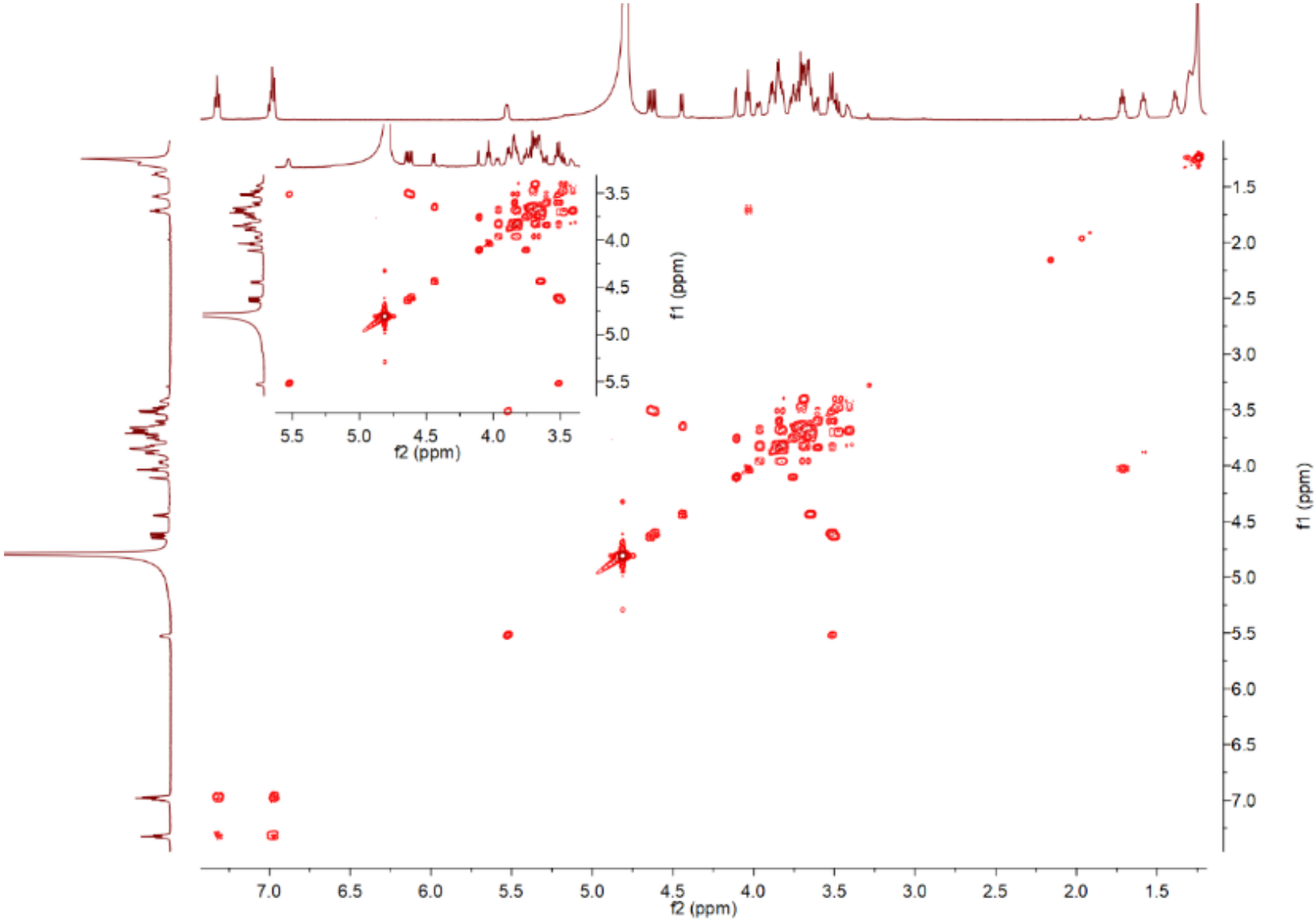
ESI-(-)-TOF HRMS of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4b**



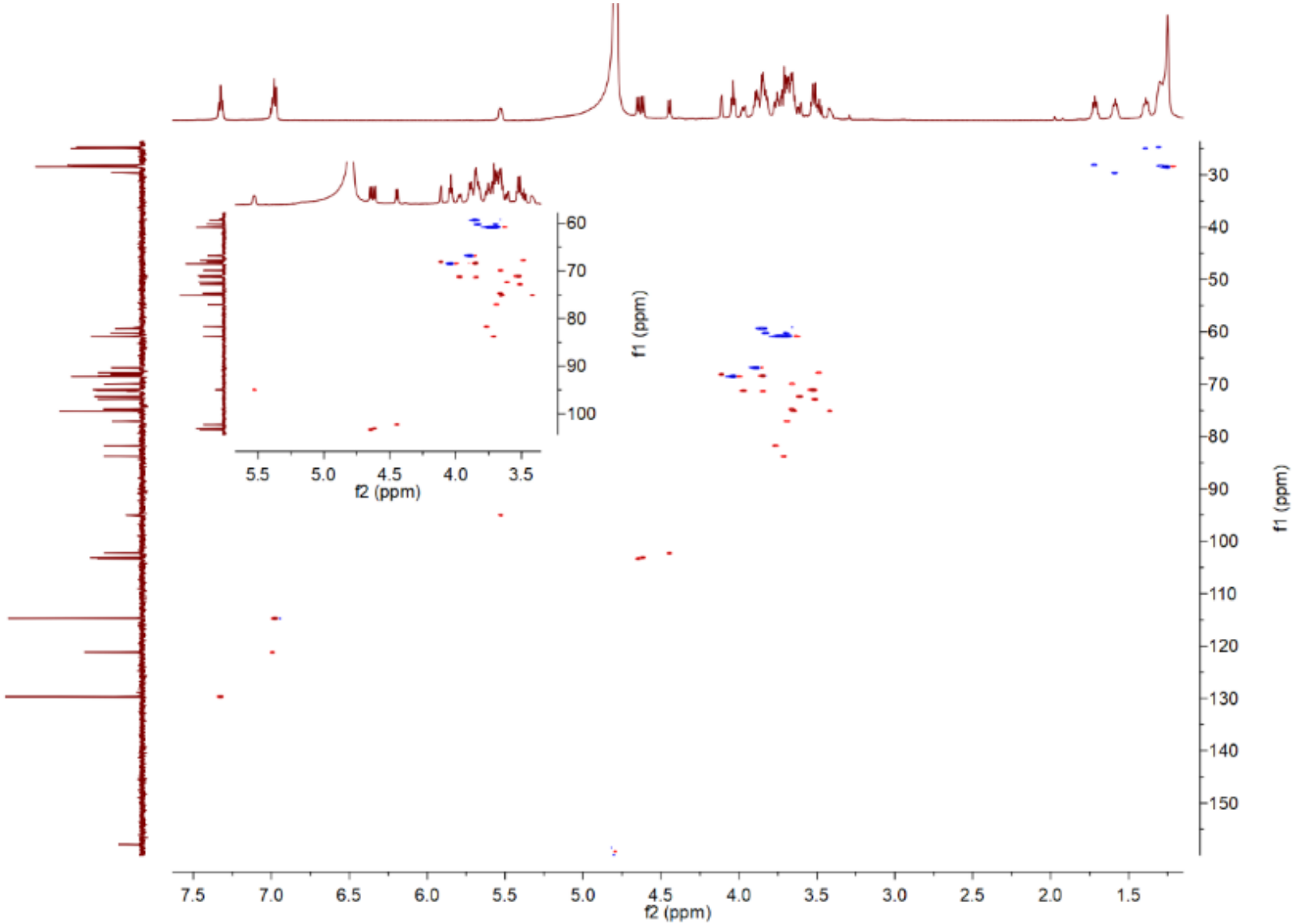
^{1}H NMR spectrum of Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c** (D_2O, 600 MHz, 25 °C)



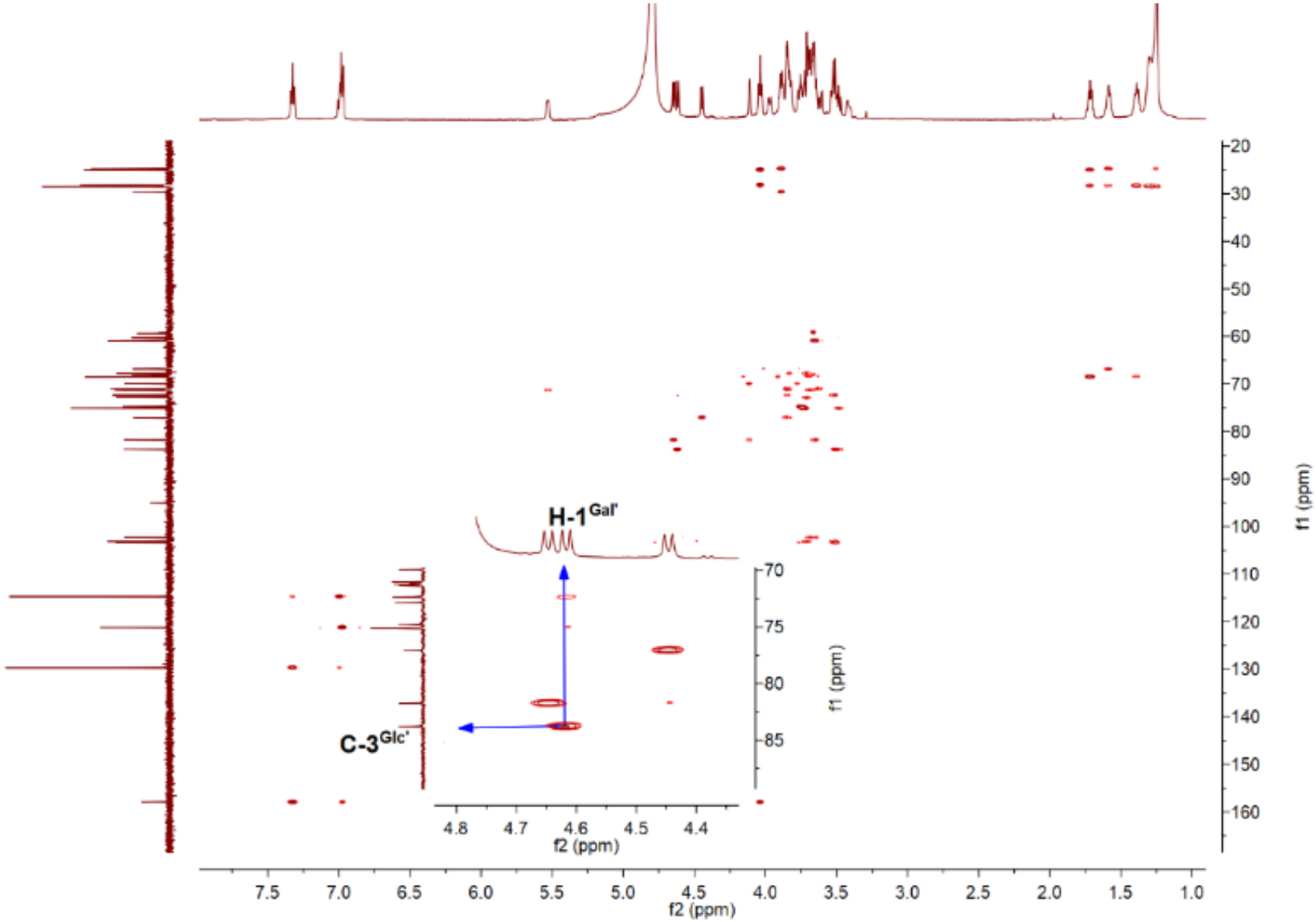
^{13}C NMR spectrum of Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c** (D_2O, 150 MHz, 25 °C)



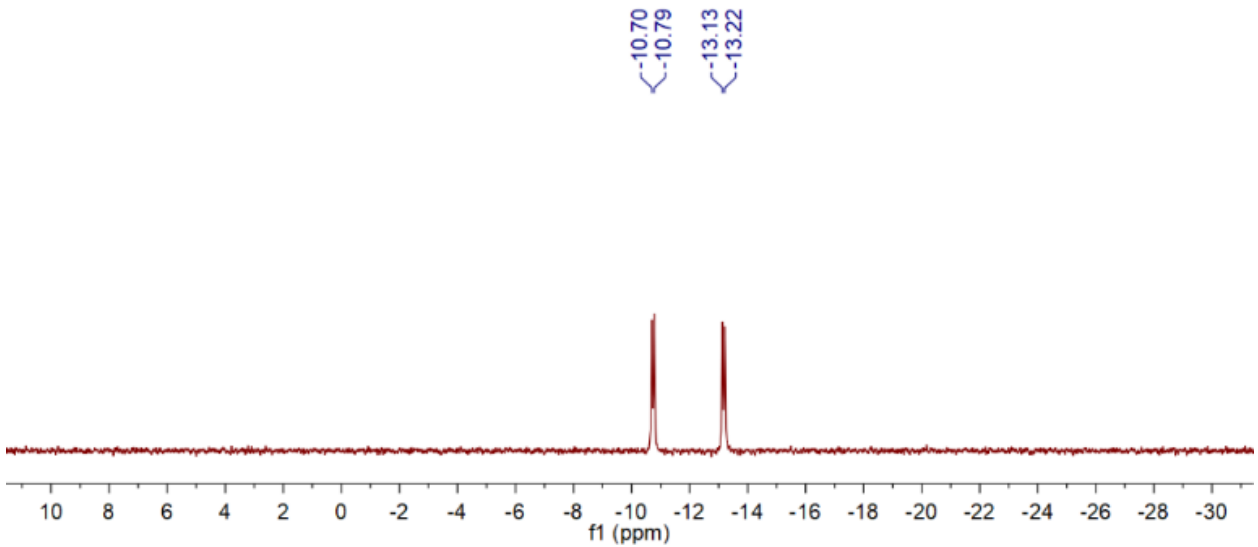
COSY spectrum of Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c** (D_2O, 600/600 MHz, 25 °C)



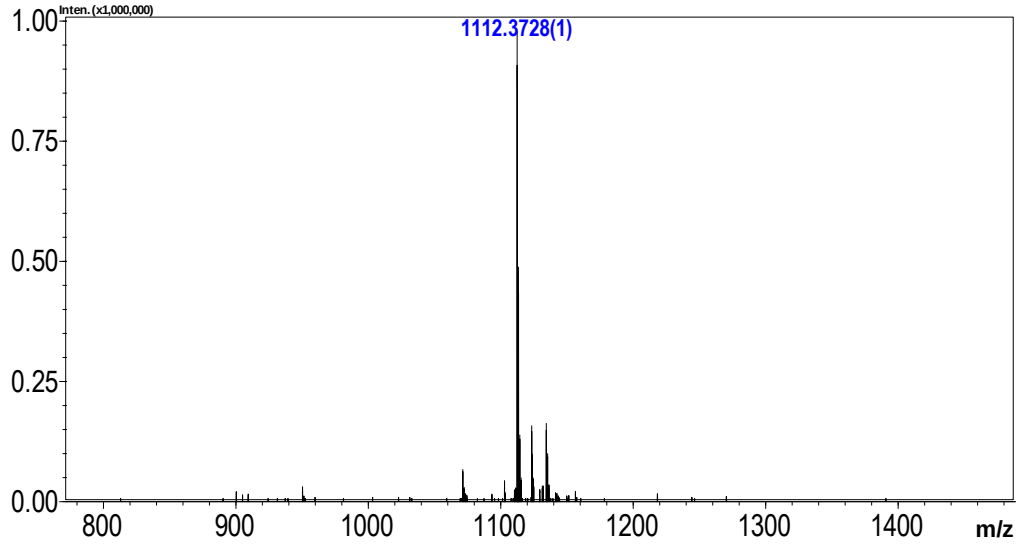
HSQC spectrum of Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c** (D_2O, 600/150 MHz, 25 °C)



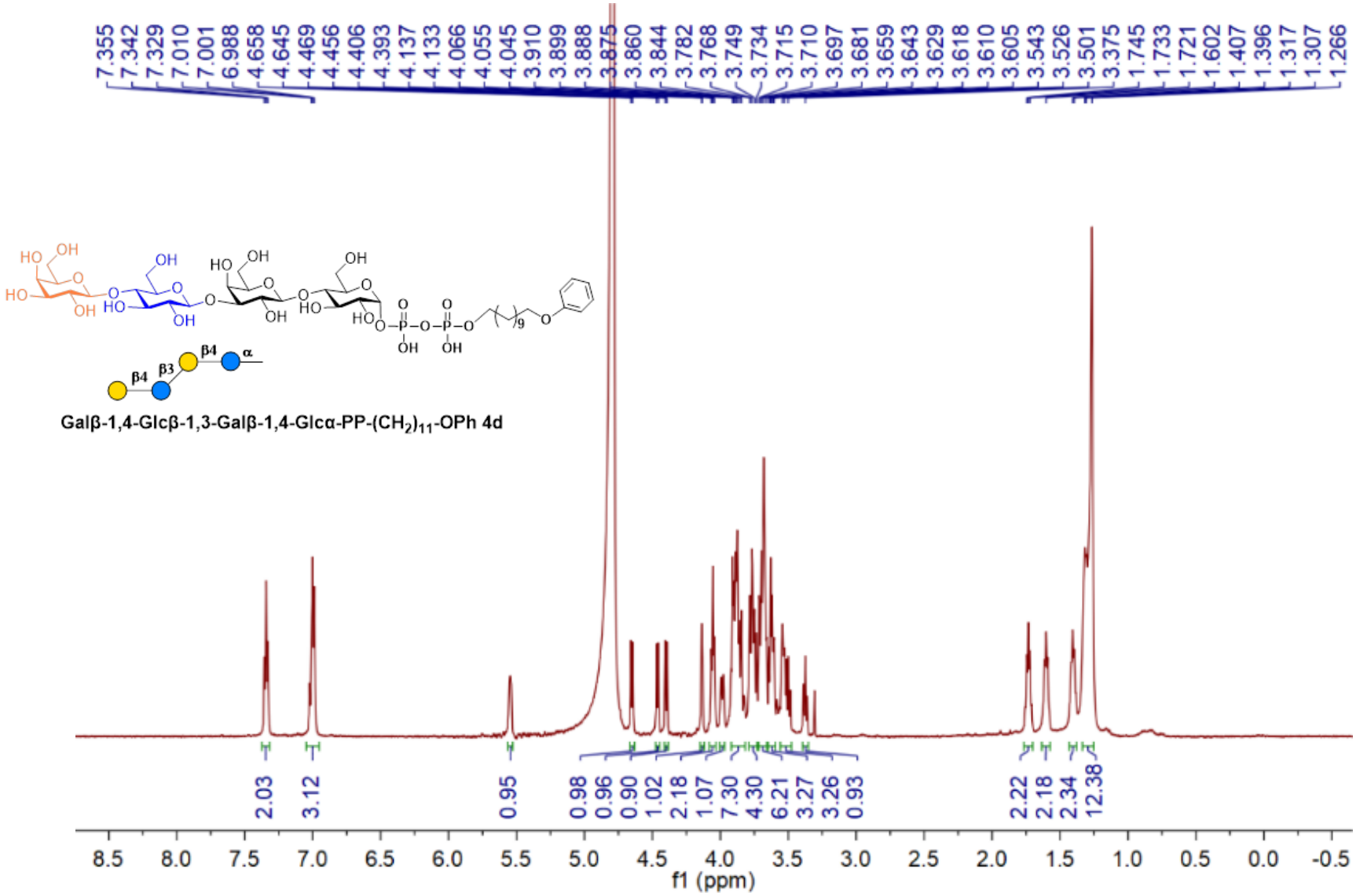
HMBC spectrum of Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c** (D_2O, 600/150 MHz, 25 °C)



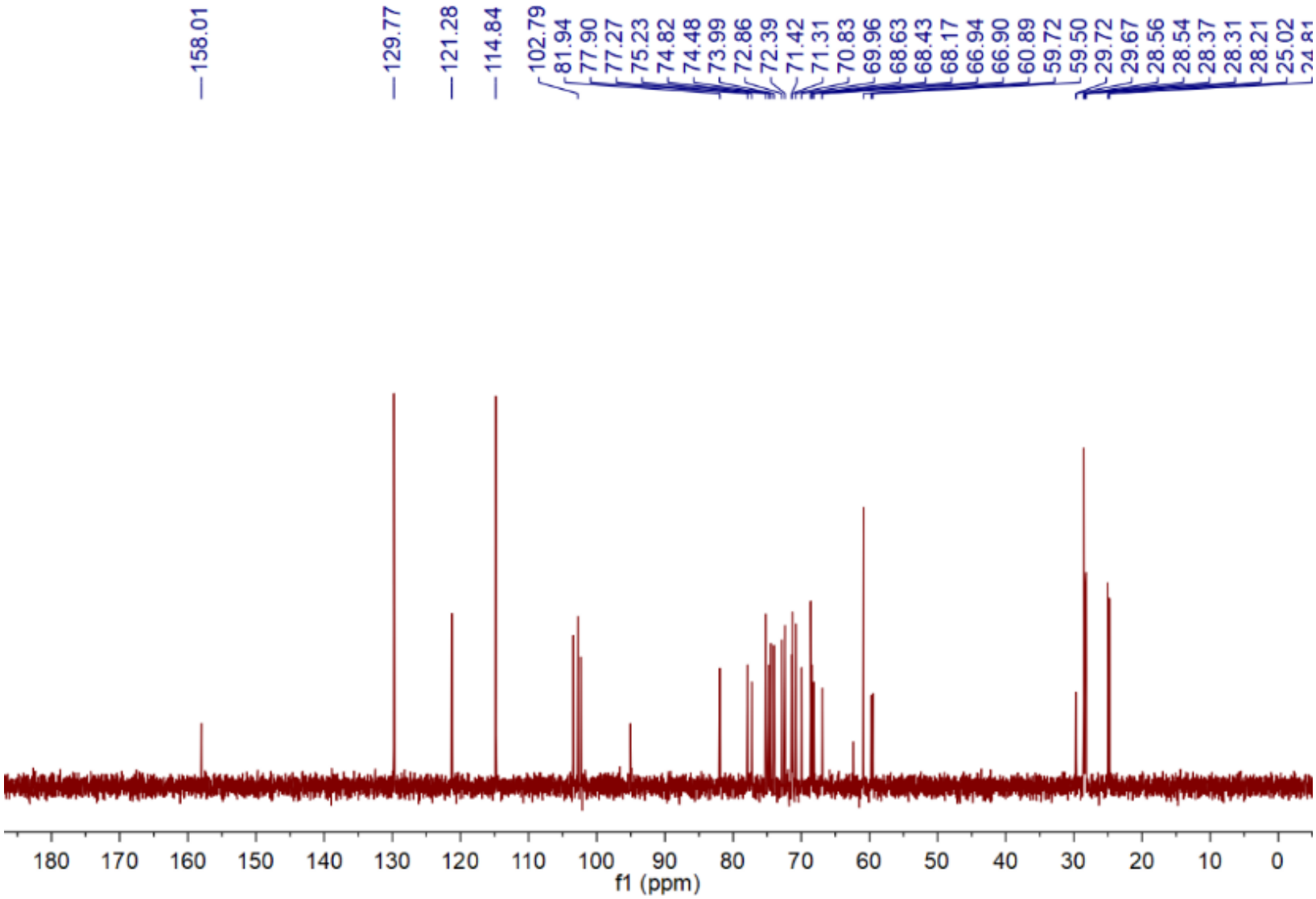
^{31}P NMR spectrum of Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c** (D_2O, 243 MHz, 25 °C)



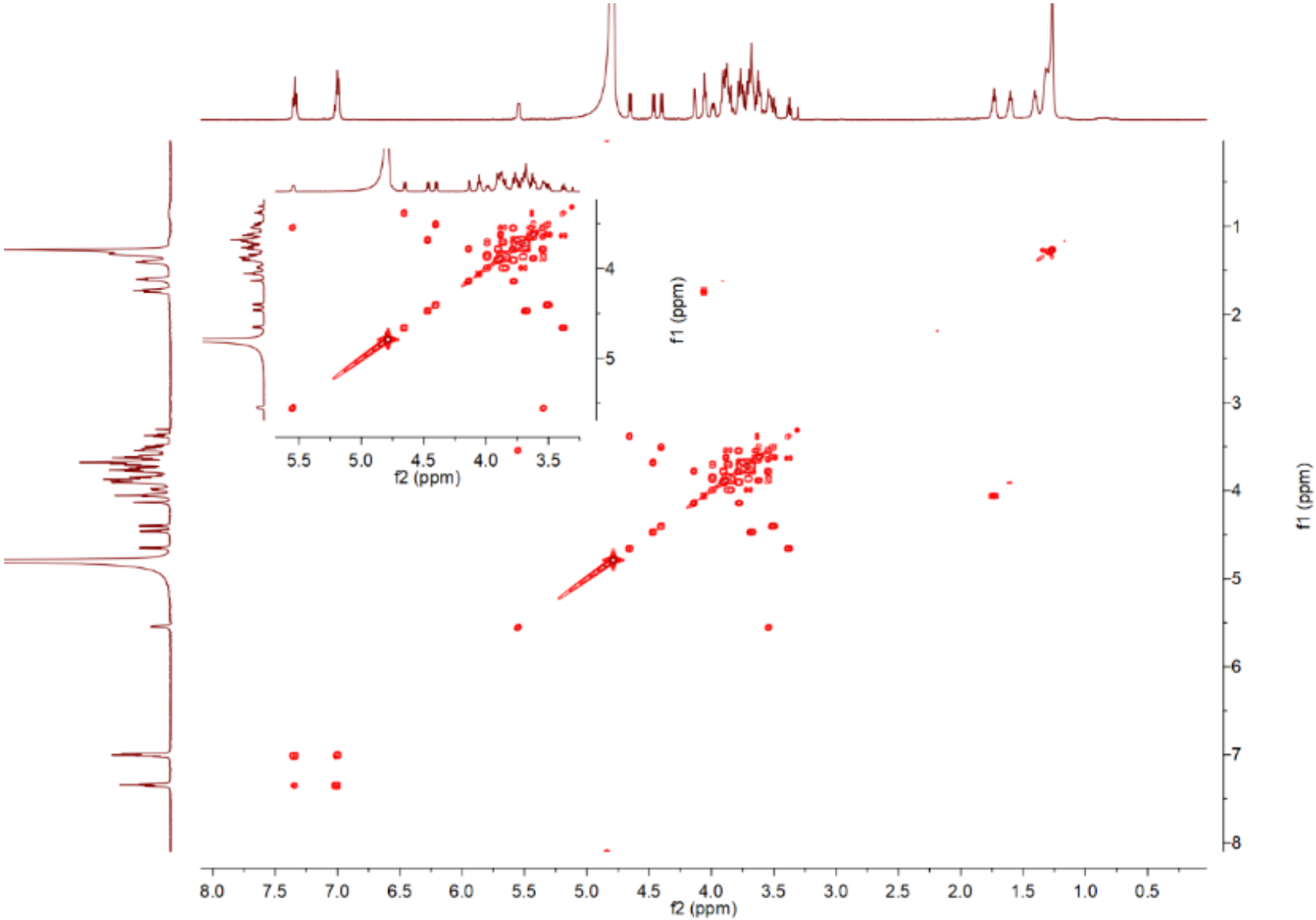
ESI-(-)-TOF HRMS of Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4c**



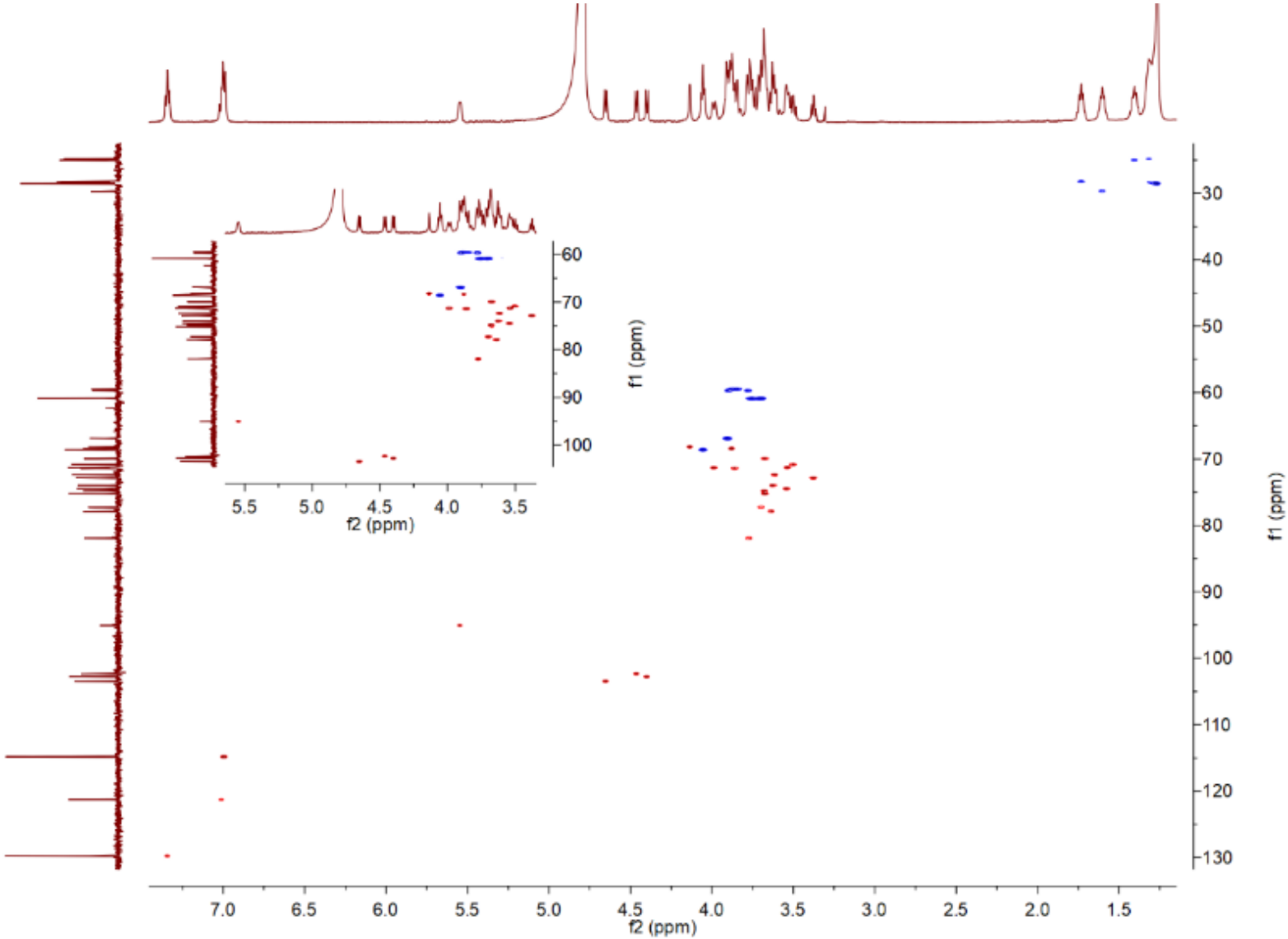
^{1}H NMR spectrum of Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d** (D_2O, 600 MHz, 25 °C)



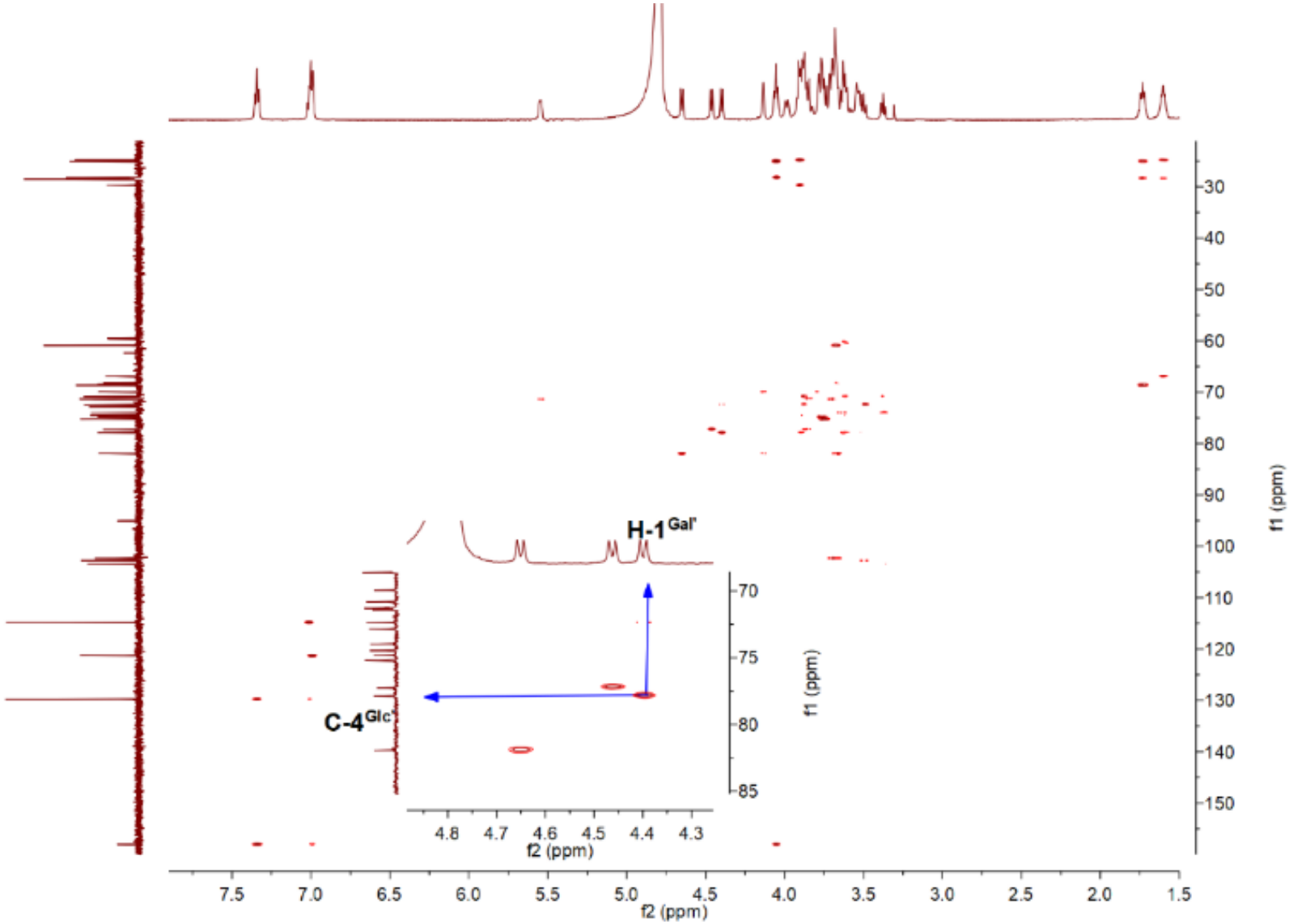
^{13}C NMR spectrum of Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d** (D_2O, 150 MHz, 25 °C)



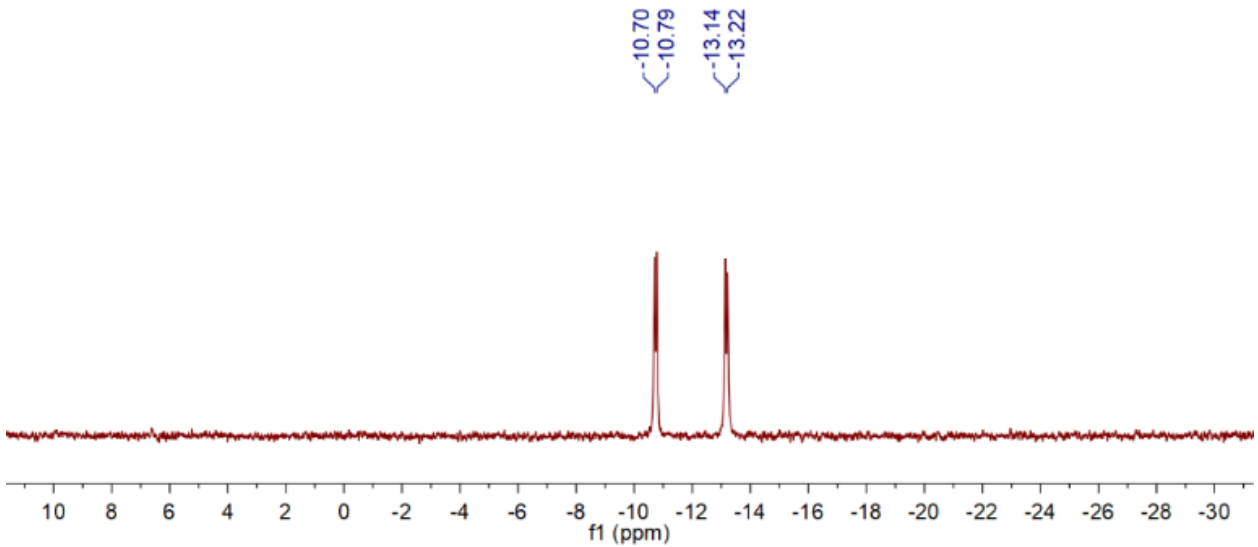
COSY spectrum of Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d** (D_2O, 600/600 MHz, 25 °C)



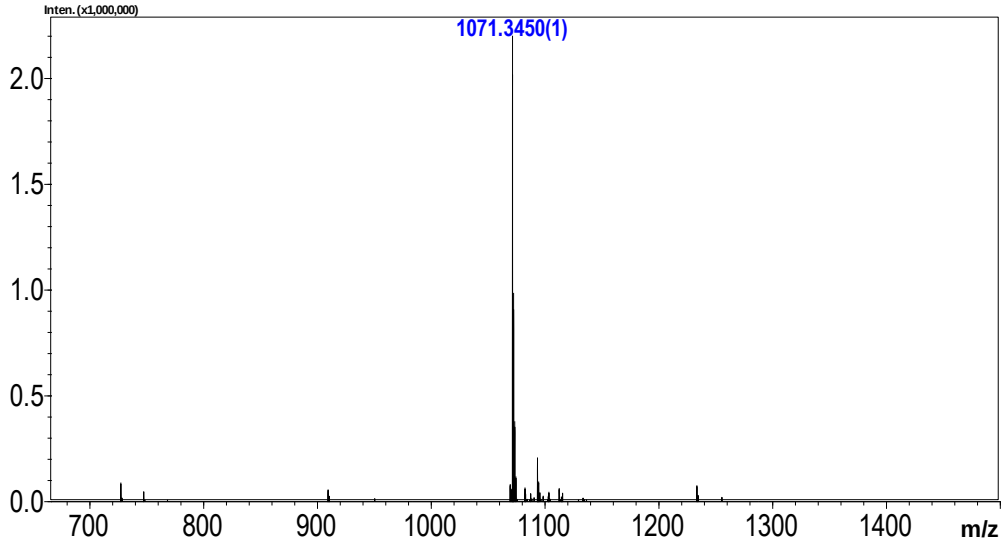
HSQC spectrum of Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d** (D_2O, 600/150 MHz, 25 °C)



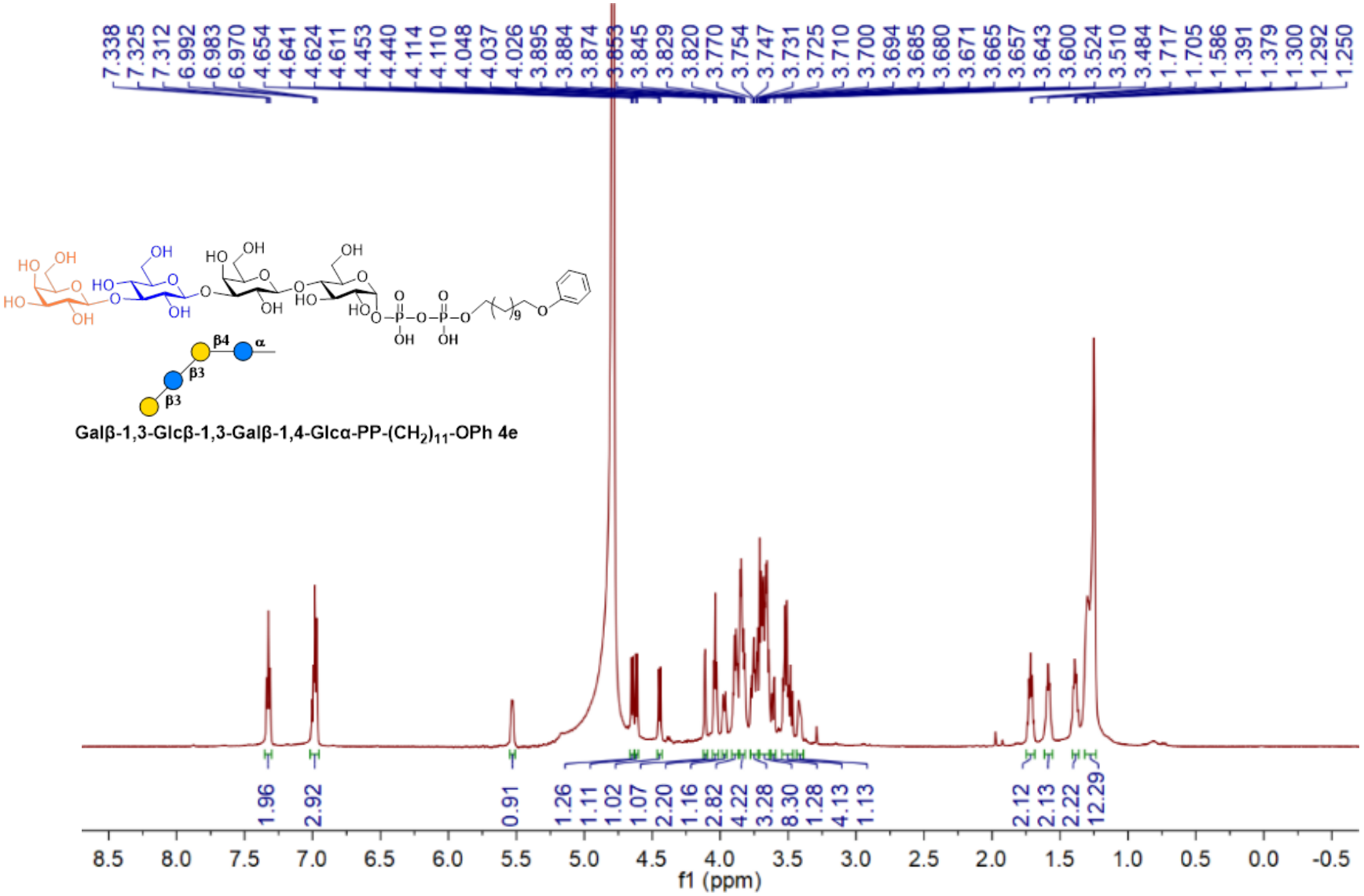
HMBC spectrum of Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d** (D_2O, 600/150 MHz, 25 °C)



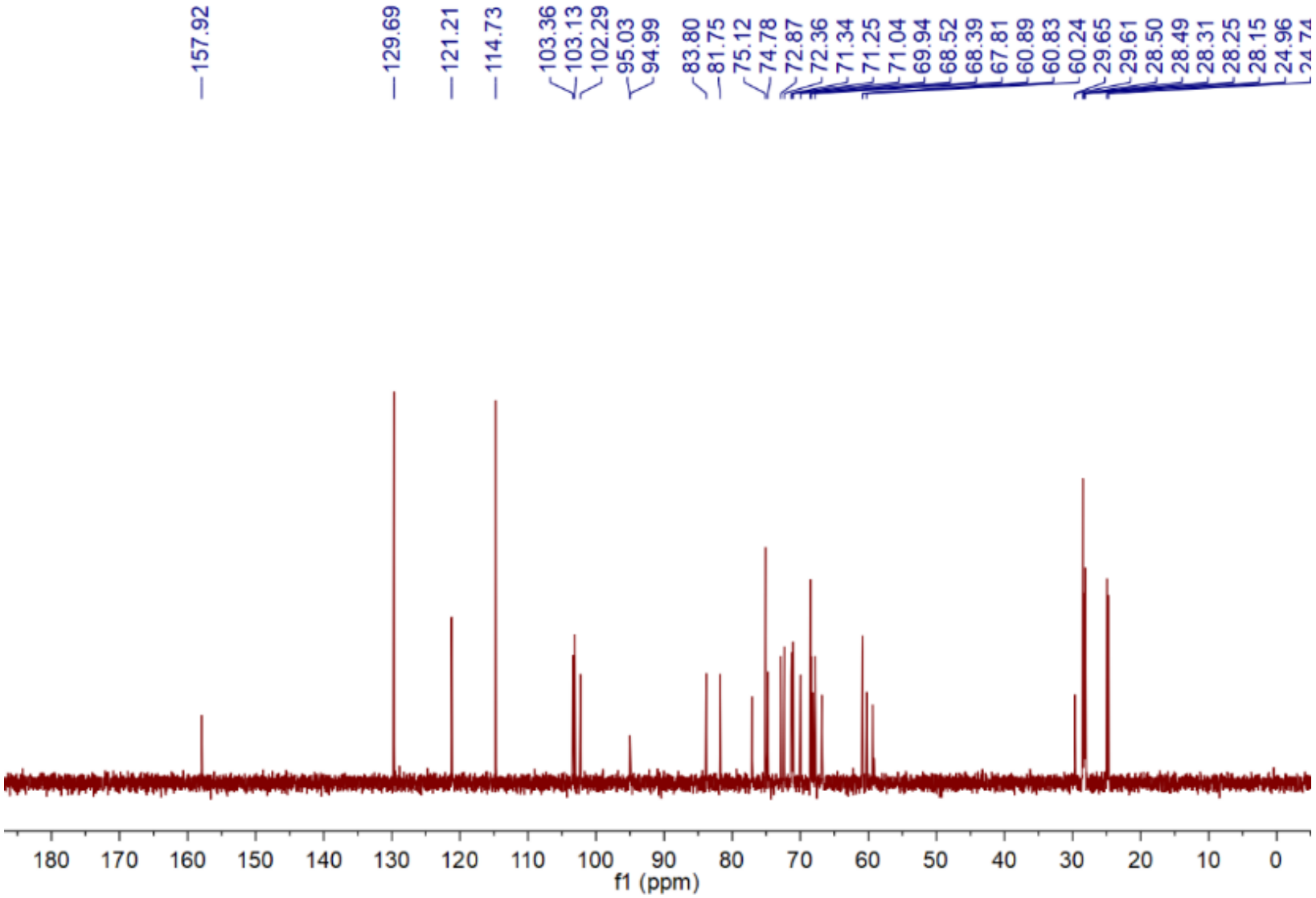
^{31}P NMR spectrum of Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d** (D_2O, 243 MHz, 25 °C)



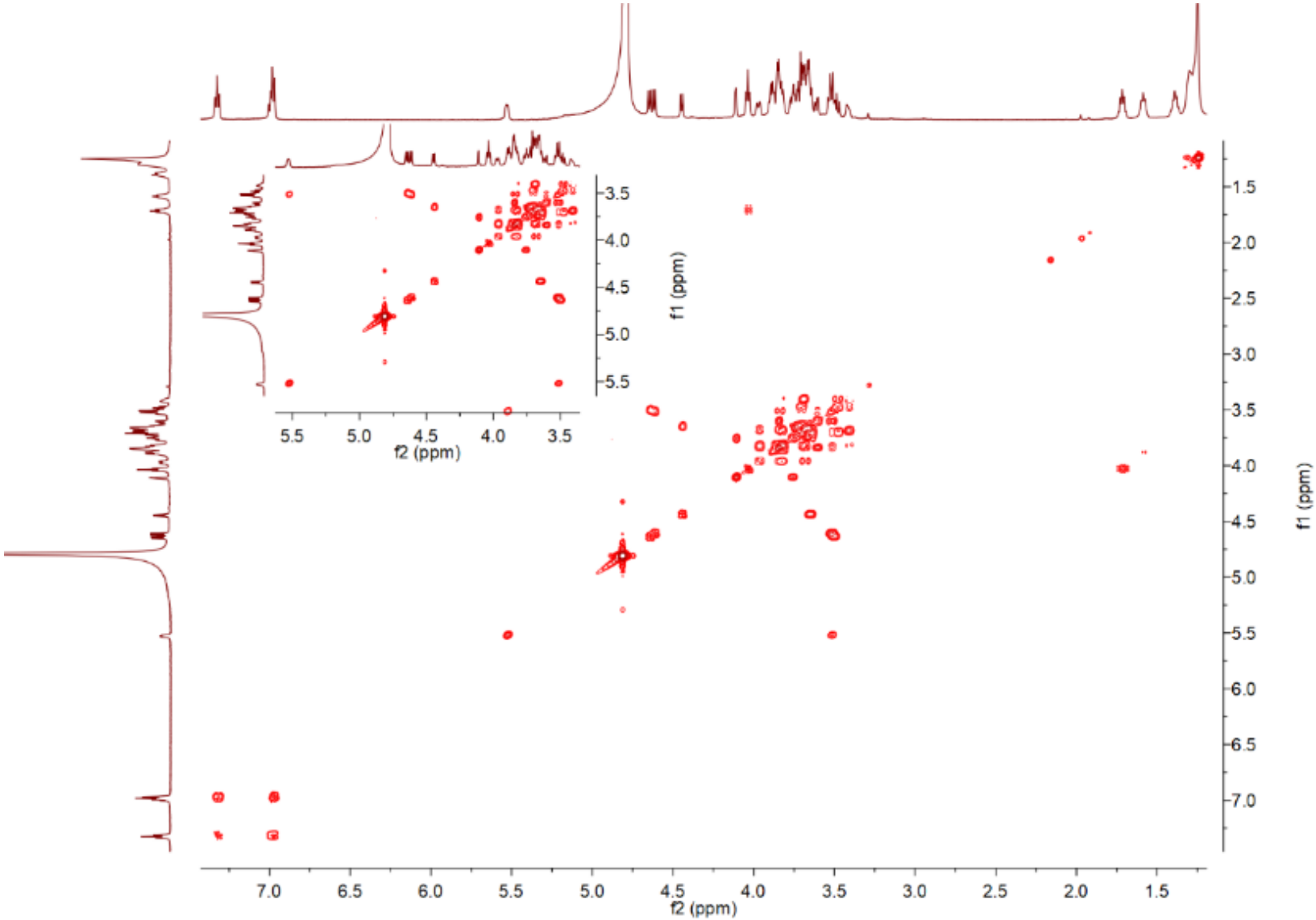
ESI-(-)-TOF HRMS of Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4d**



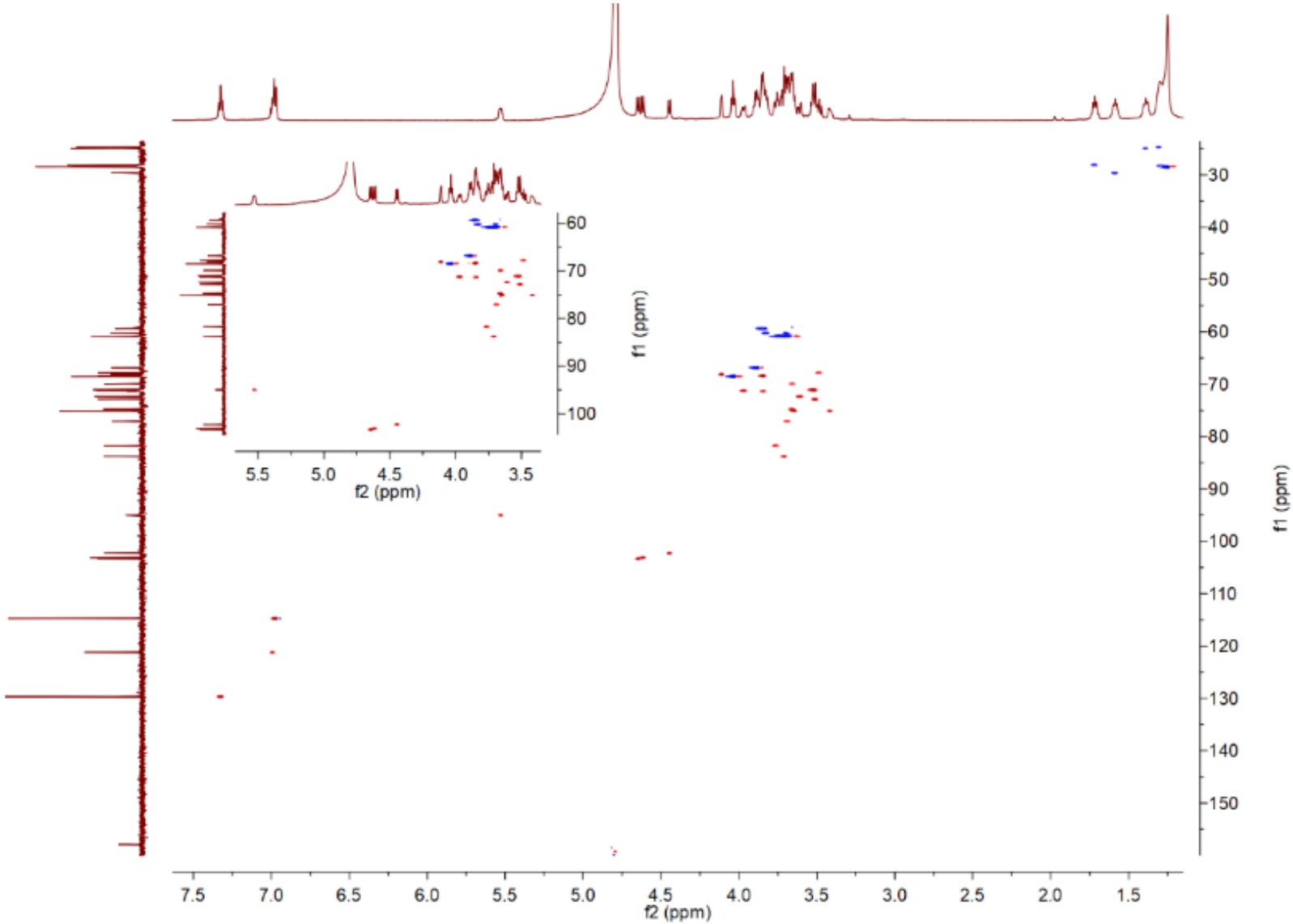
^{1}H NMR spectrum of Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e** (D_2O, 600 MHz, 25 °C)



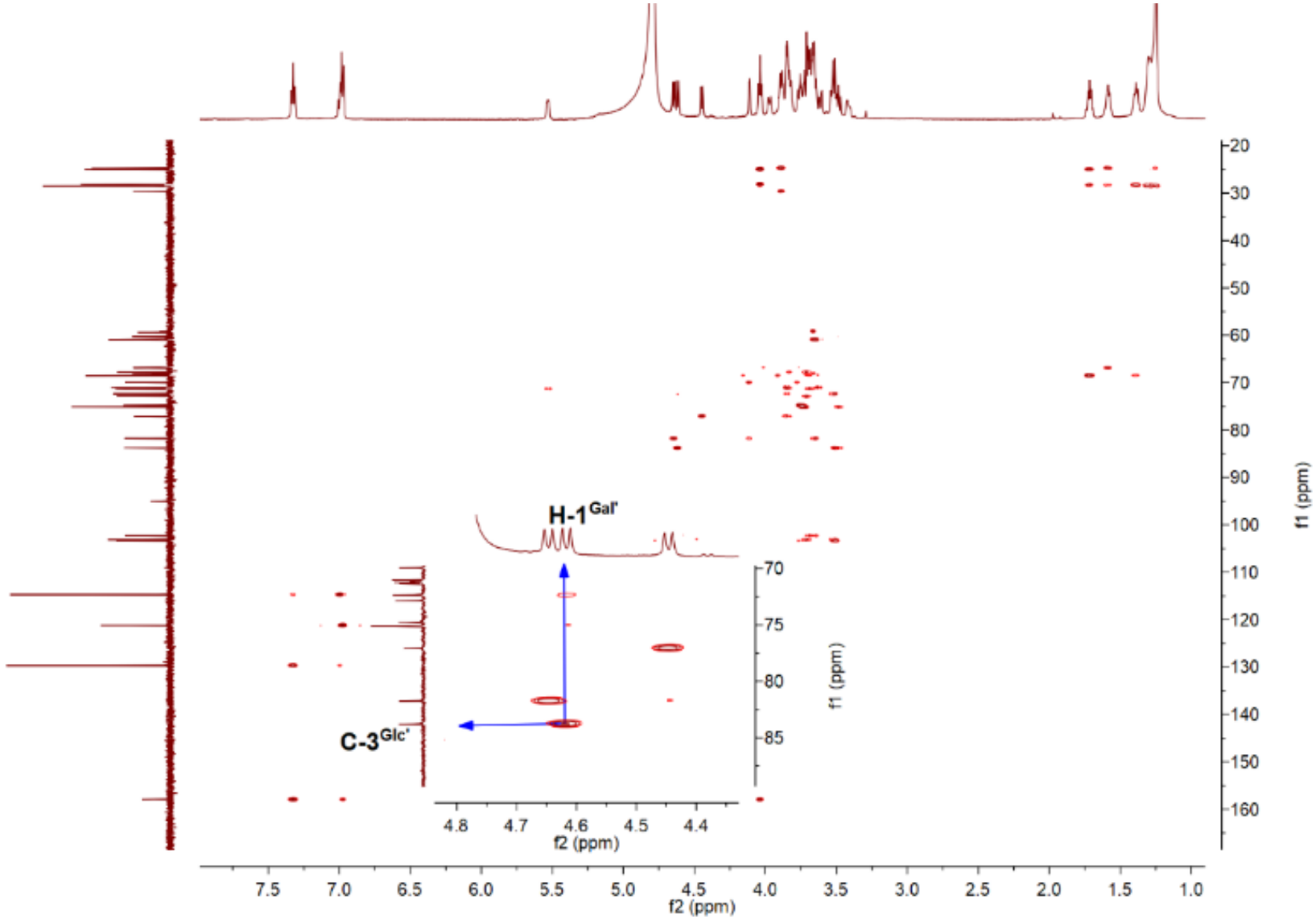
^{13}C NMR spectrum of Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e** (D_2O, 150 MHz, 25 °C)



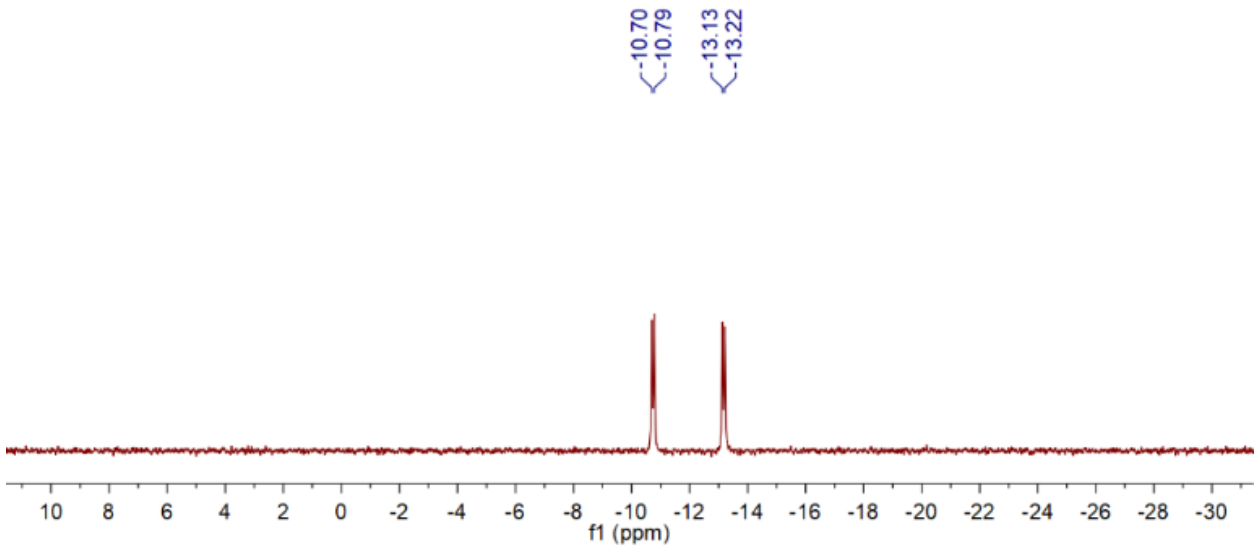
COSY spectrum of Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e** (D_2O, 600/600 MHz, 25 °C)



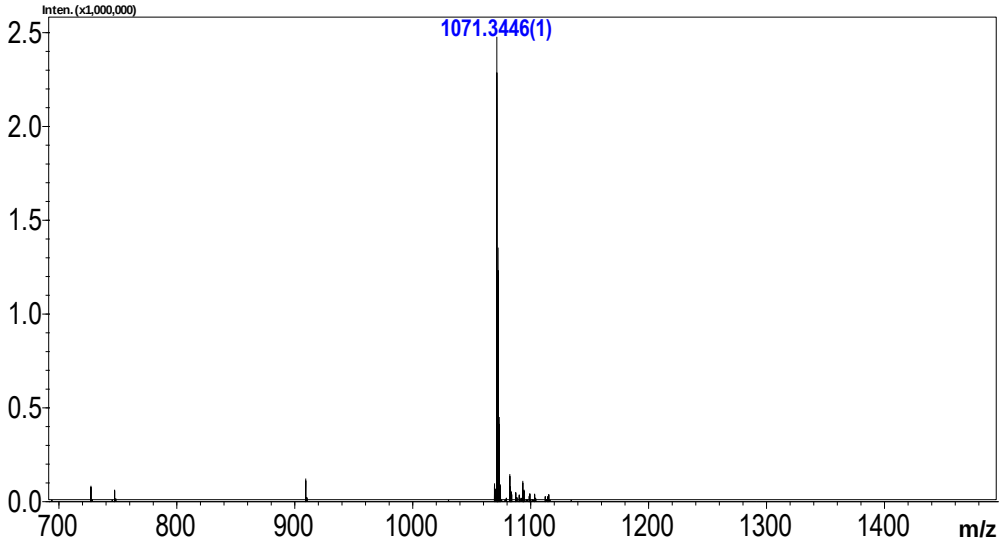
HSQC spectrum of Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e** (D_2O, 600/150 MHz, 25 °C)



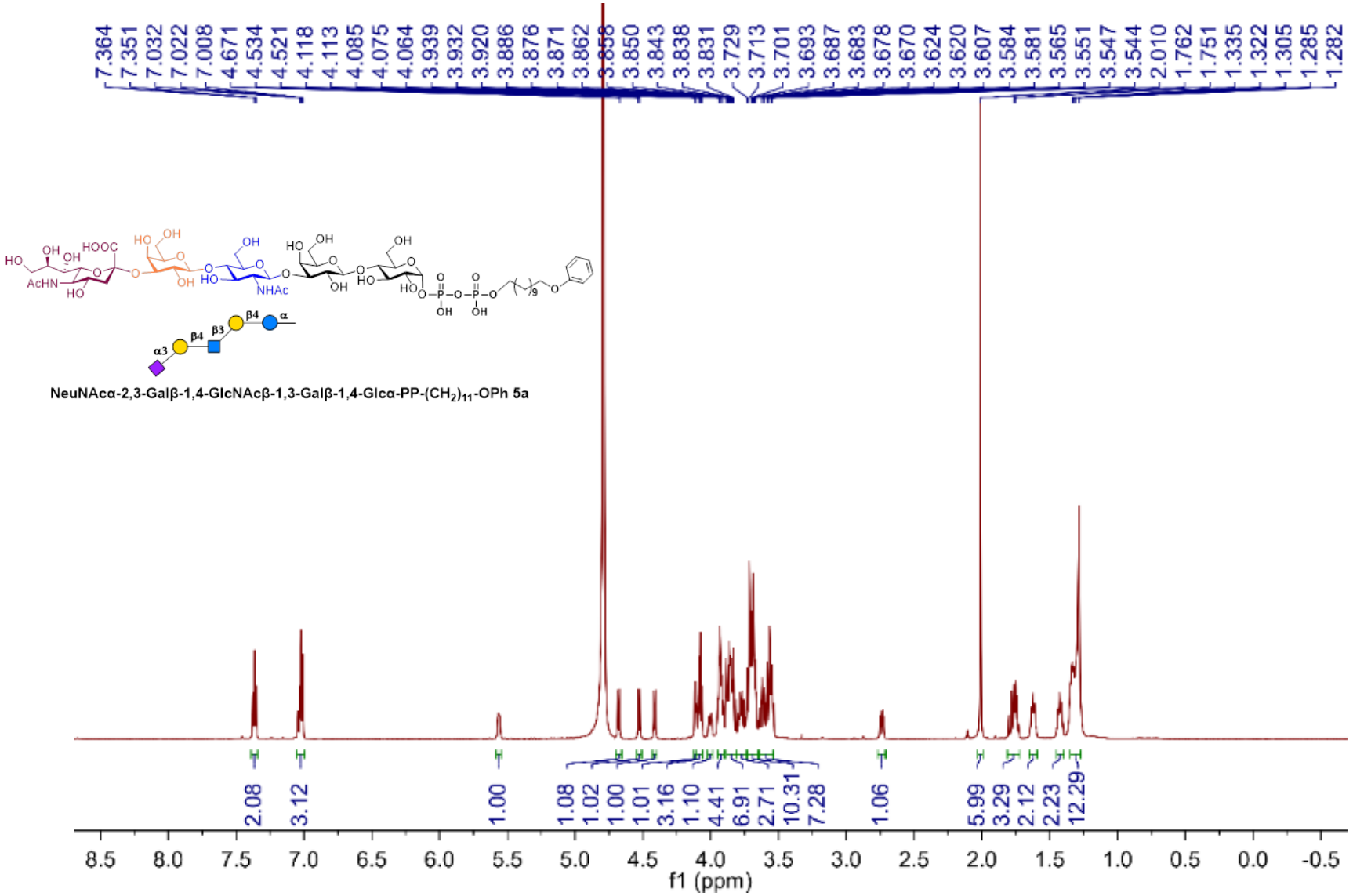
HMBC spectrum of Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e** (D_2O, 600/150 MHz, 25 °C)



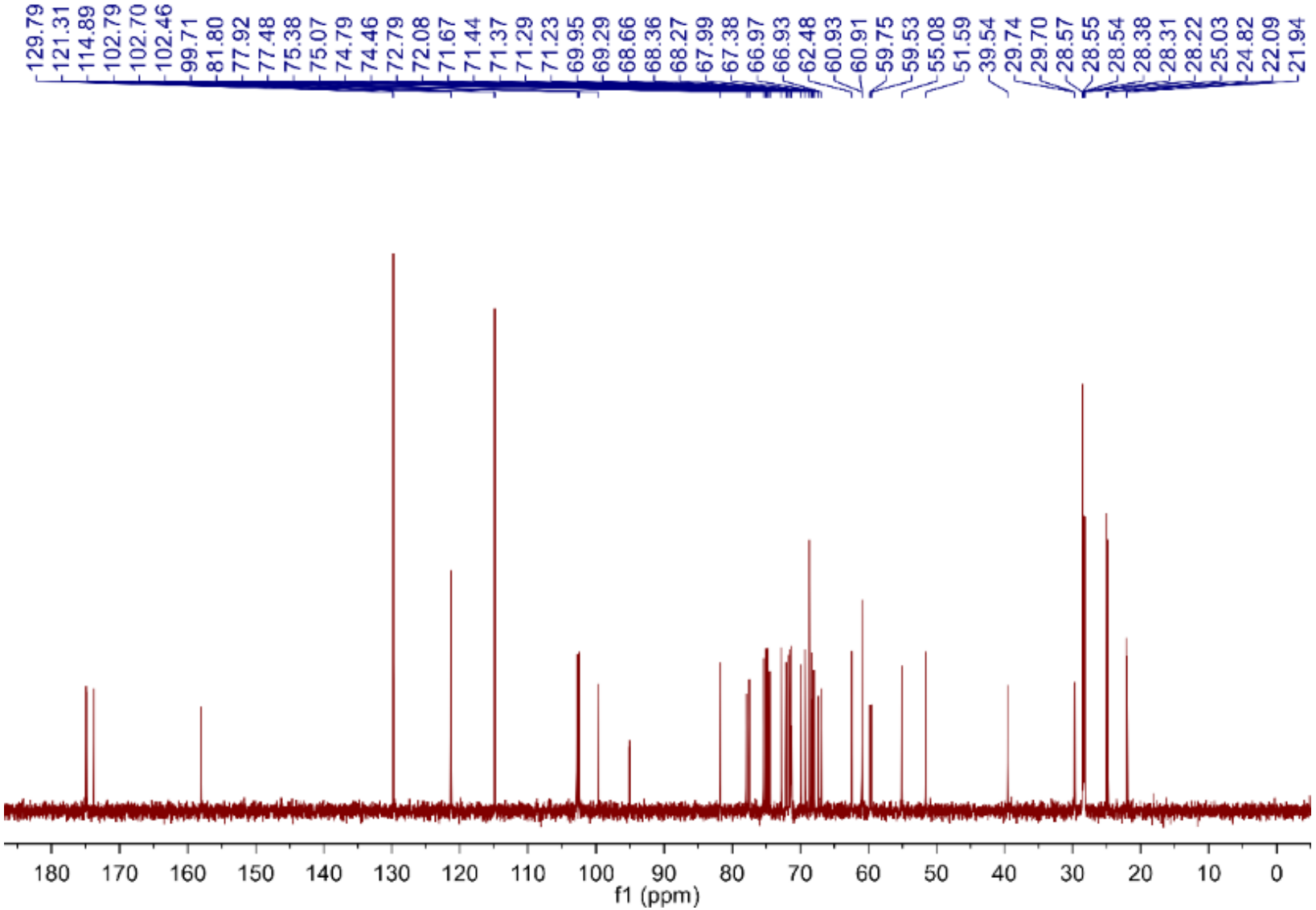
^{31}P NMR spectrum of Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 243 MHz, 25 °C)



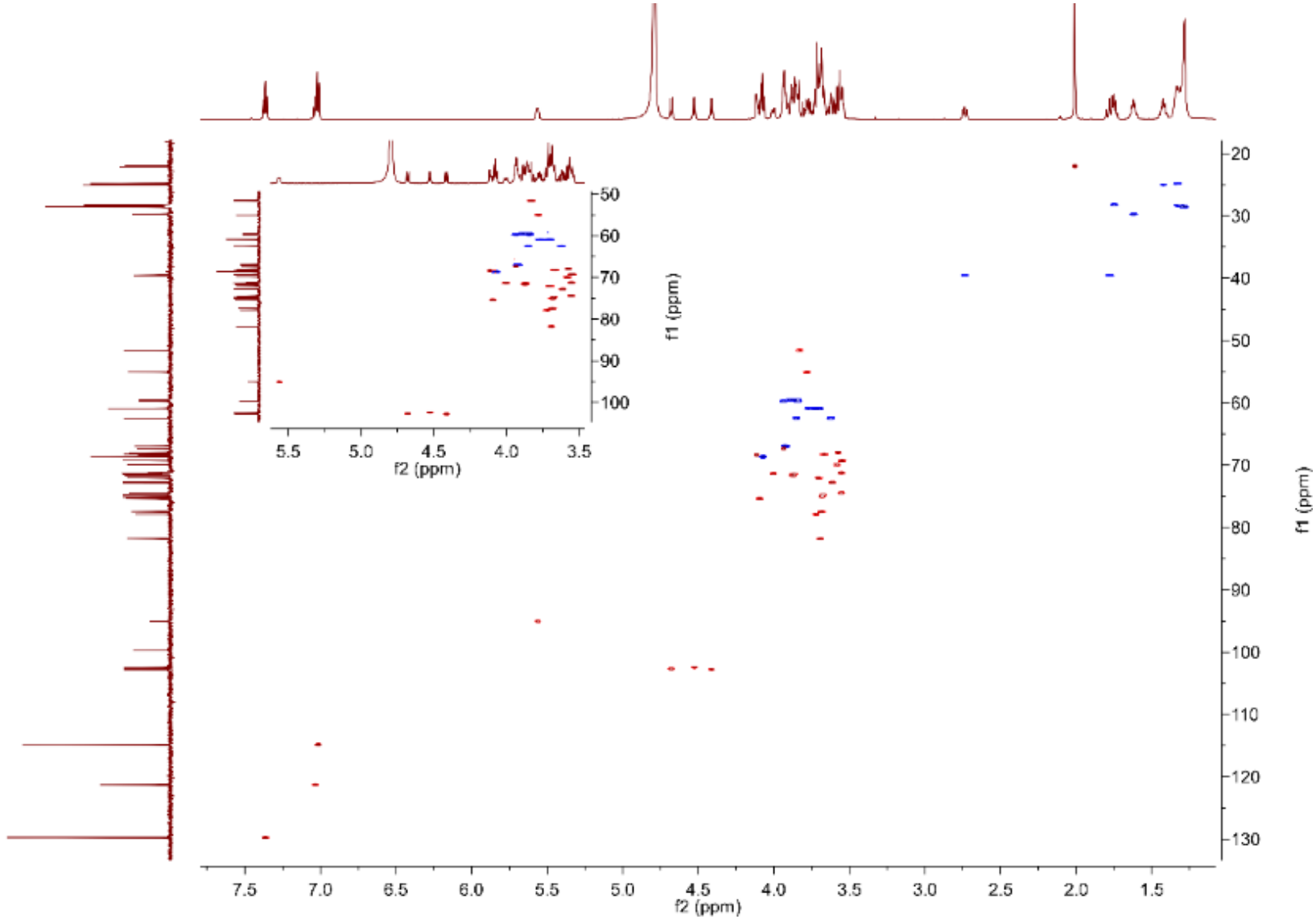
ESI-(-)-TOF HRMS of Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **4e**



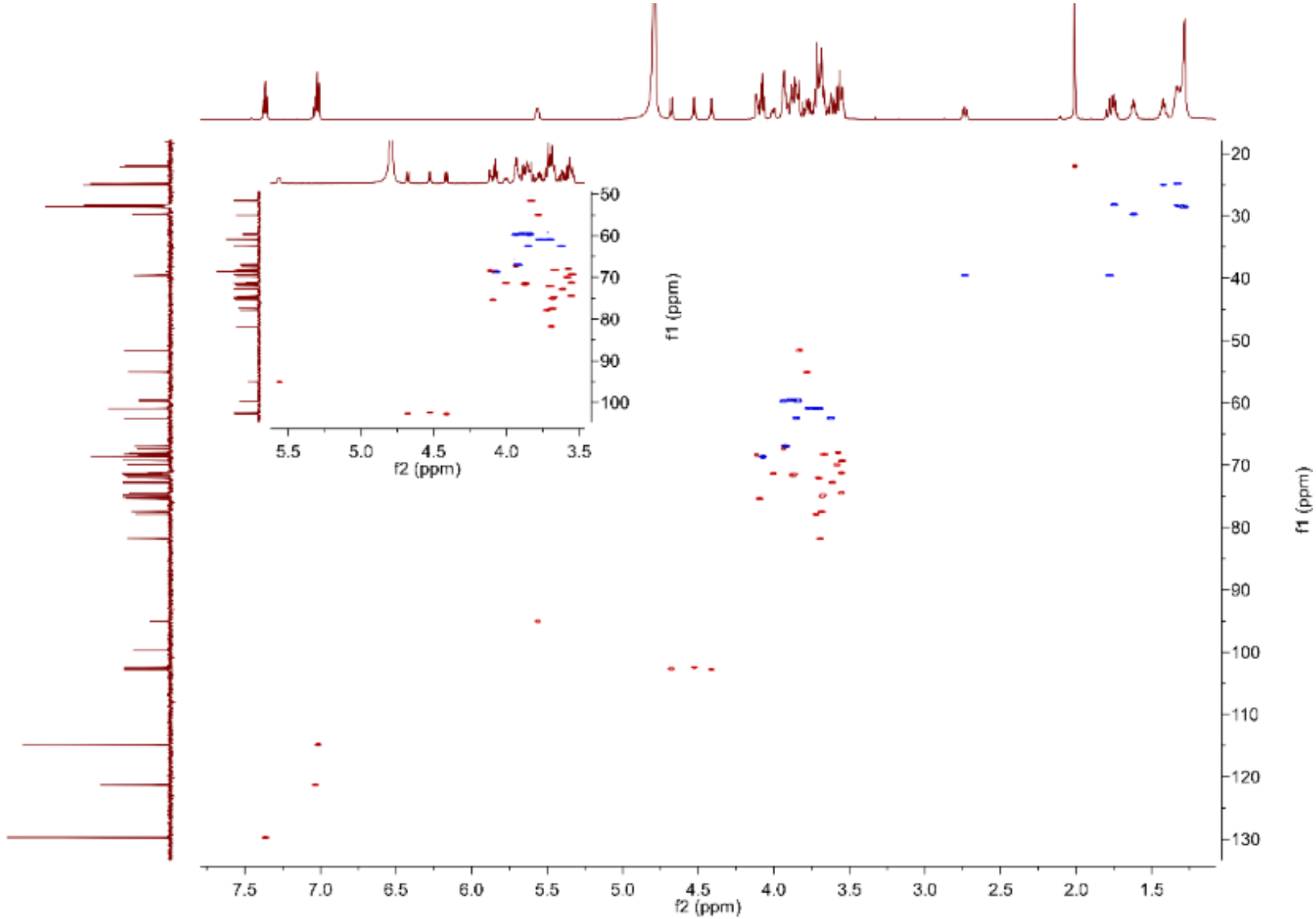
^{1}H NMR spectrum of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** (D_2O, 600 MHz, 25 °C)



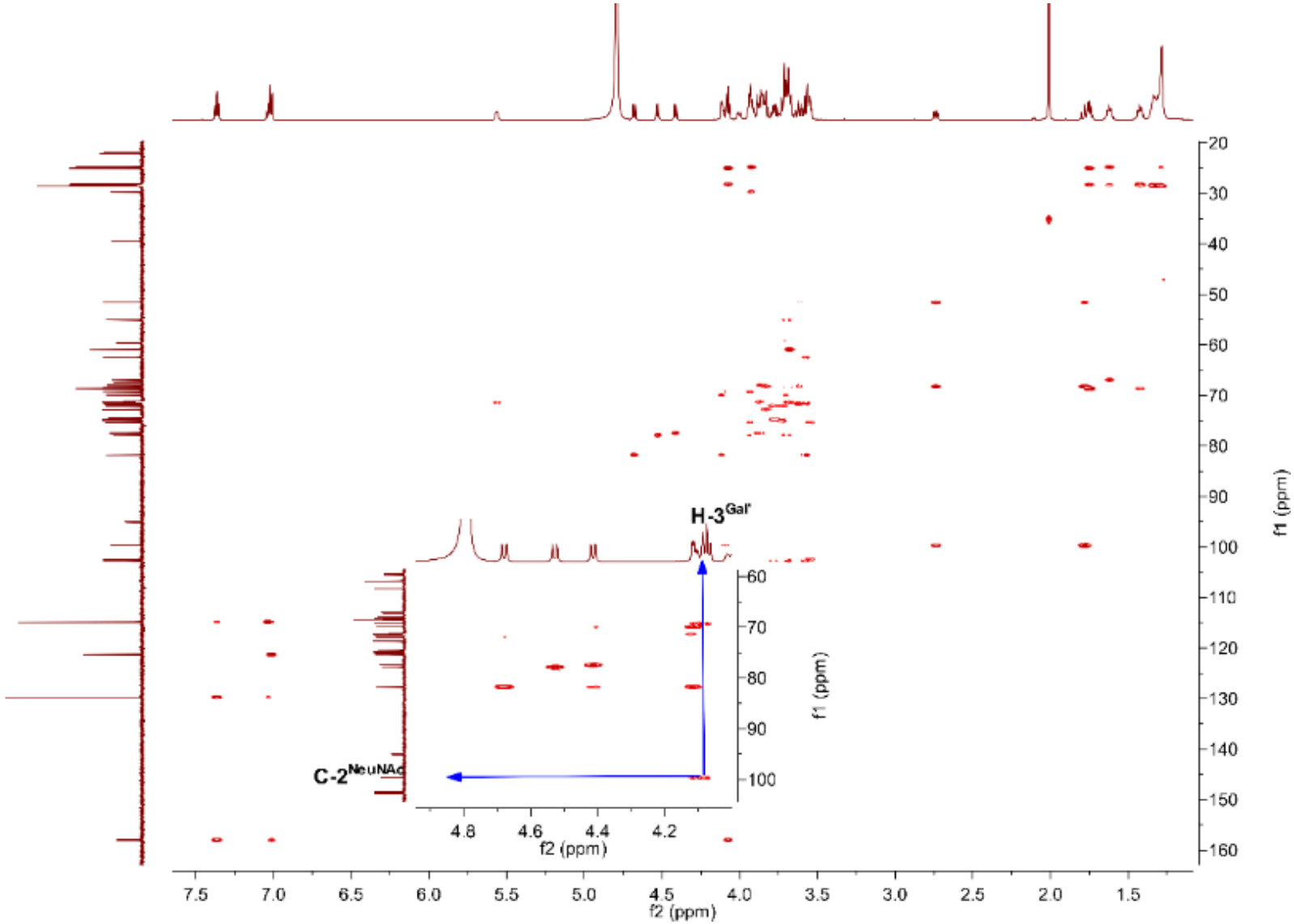
^{13}C NMR spectrum of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** (D_2O, 150 MHz, 25 °C)



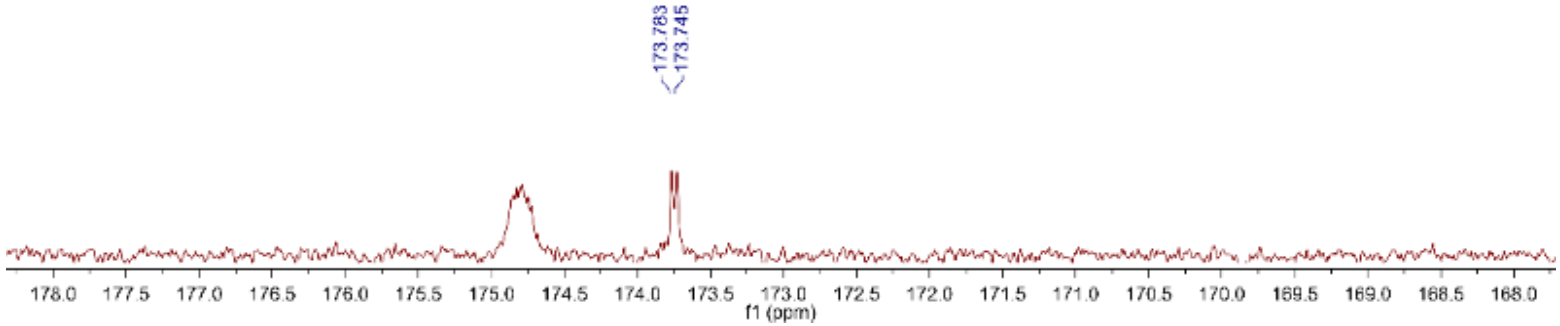
COSY spectrum of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** (D_2O, 600/600 MHz, 25 °C)



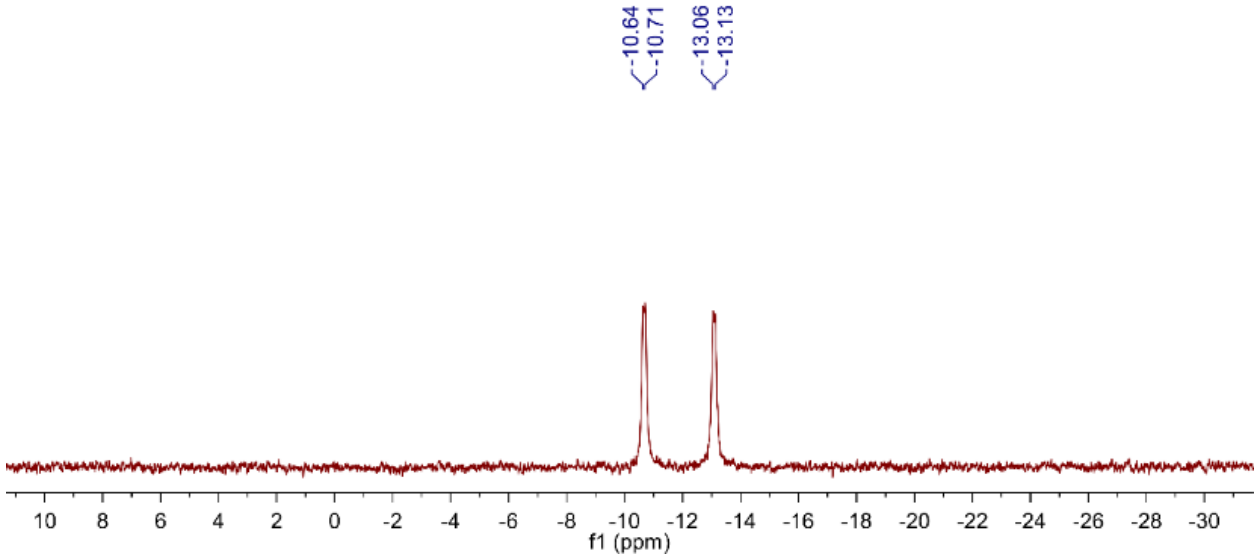
HSQC spectrum of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** (D_2O, 600/150 MHz, 25 °C)



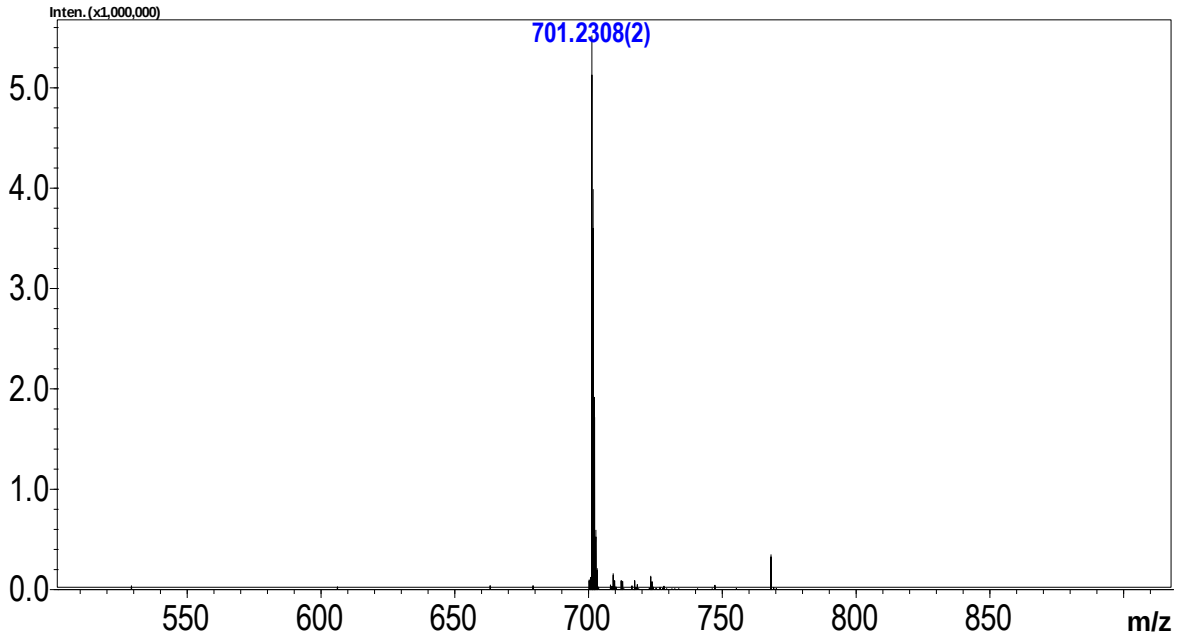
HMBC spectrum of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** (D_2O, 600/150 MHz, 25 °C)



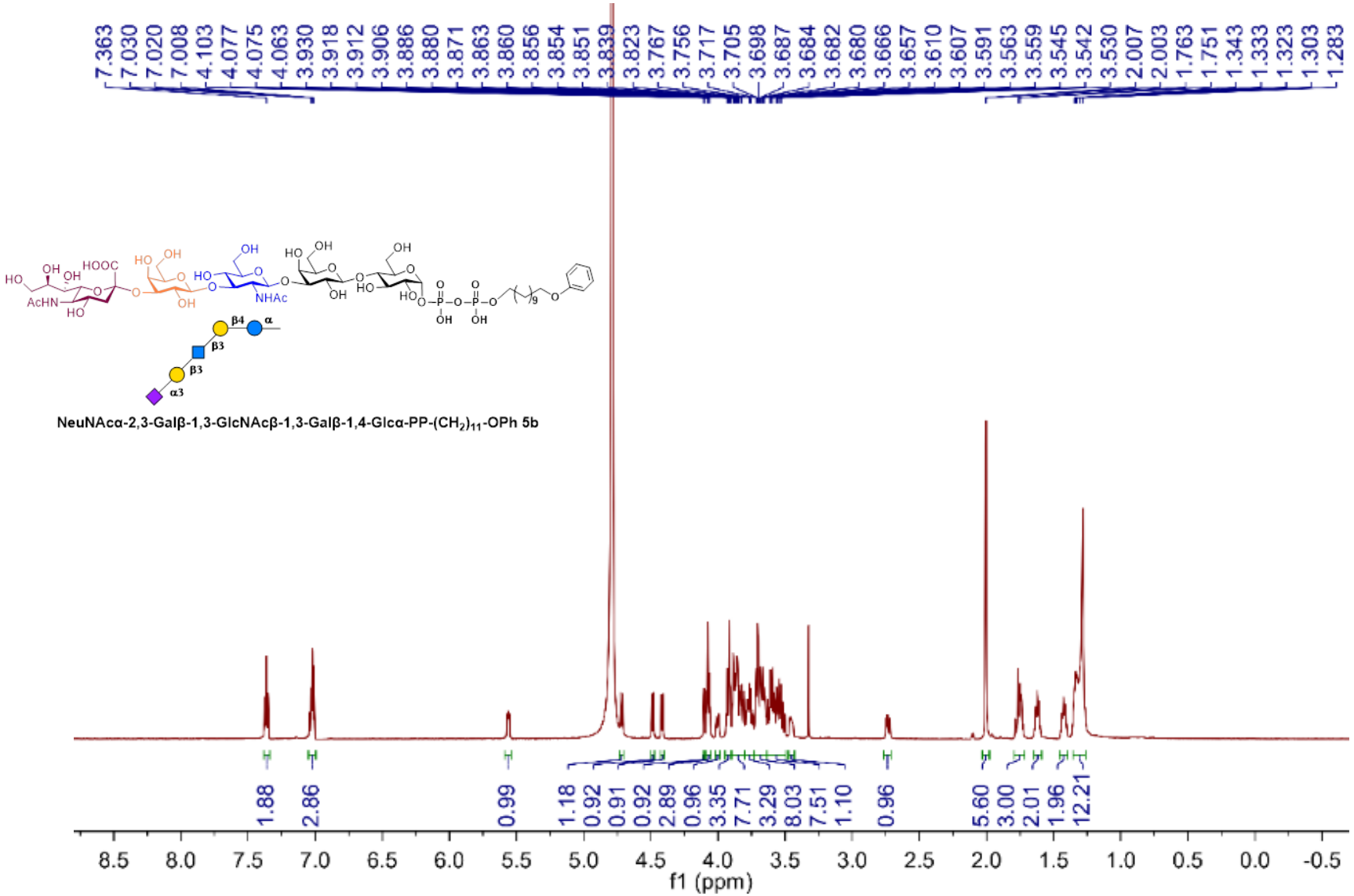
Selected non-decoupling ^{13}C NMR spectrum of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** (D_2O, 150 MHz, 25 °C)



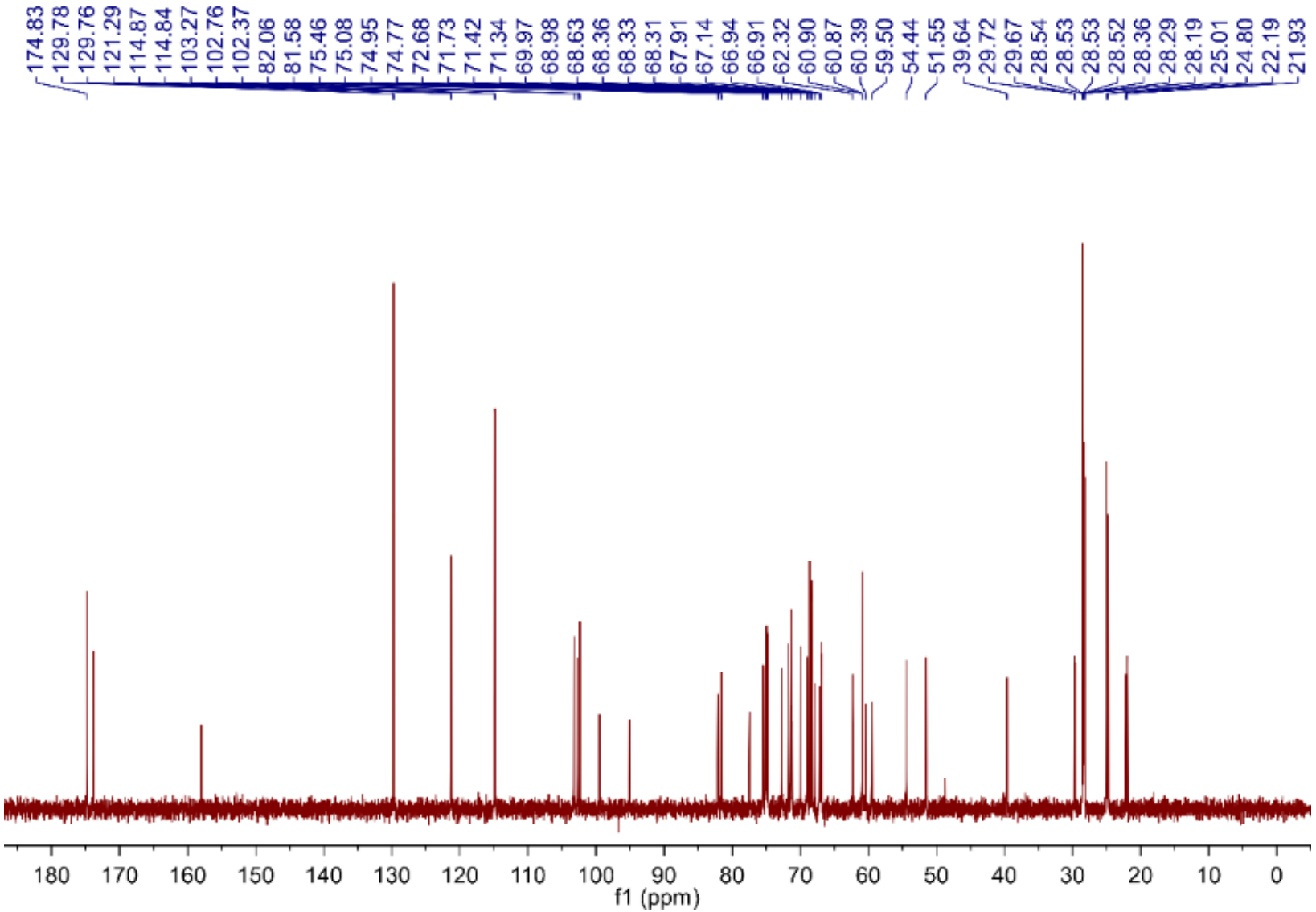
^{31}P NMR spectrum of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a** (D_2O, 243 MHz, 25 °C)



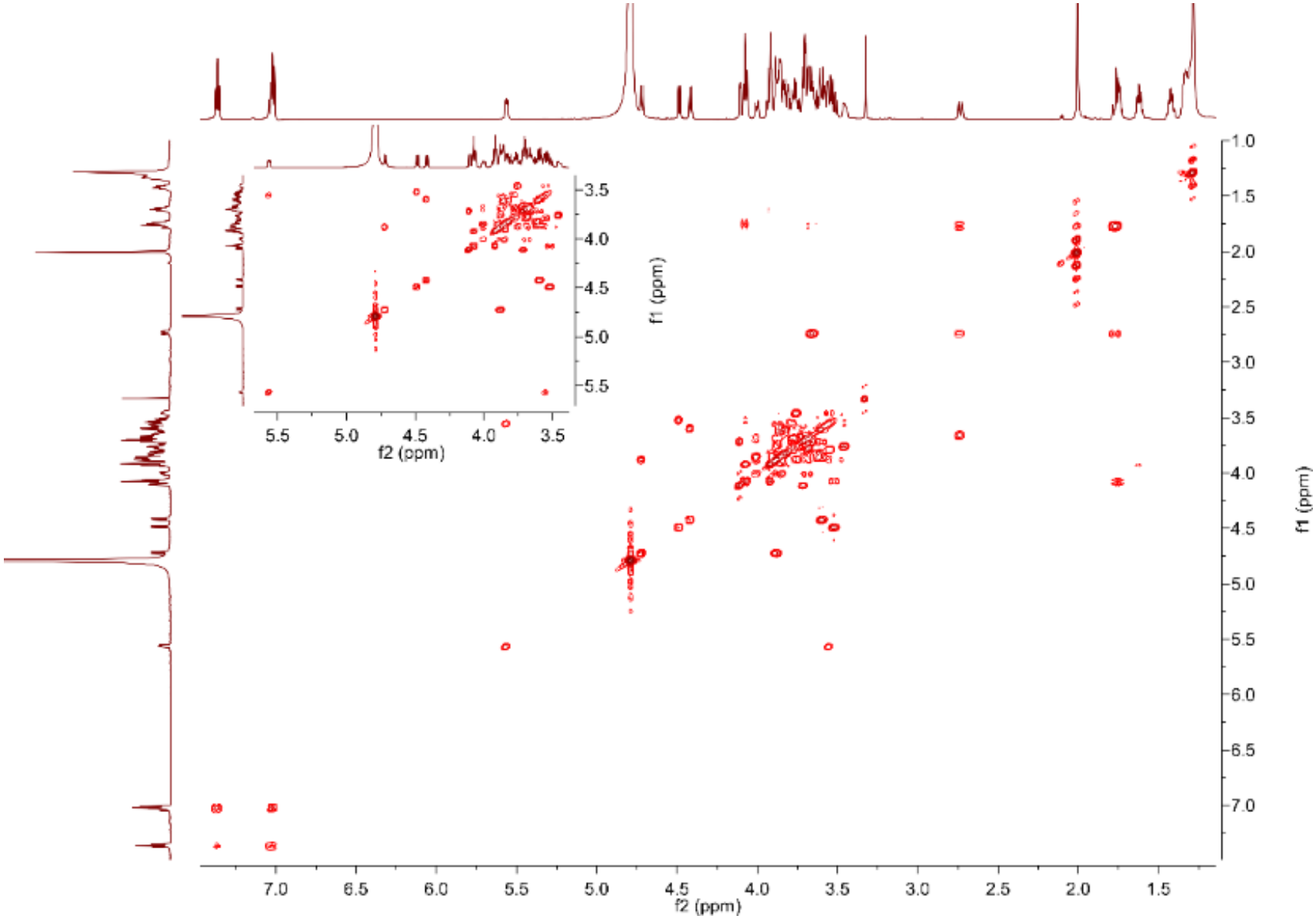
ESI-(-)-TOF HRMS of NeuNAcα-2,3-Galβ-1,4-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5a**



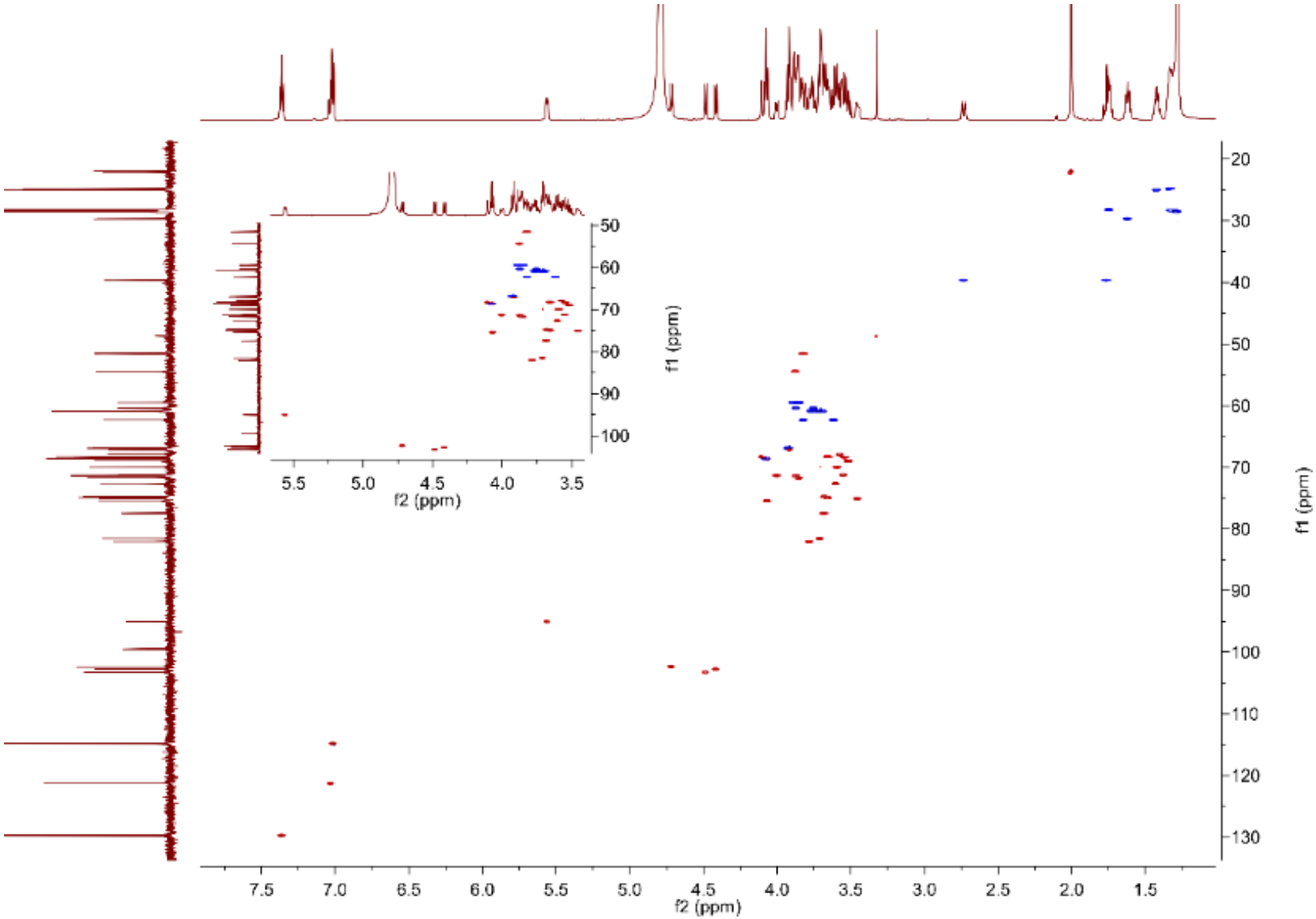
^{1}H NMR spectrum of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 600 MHz, 25 °C)



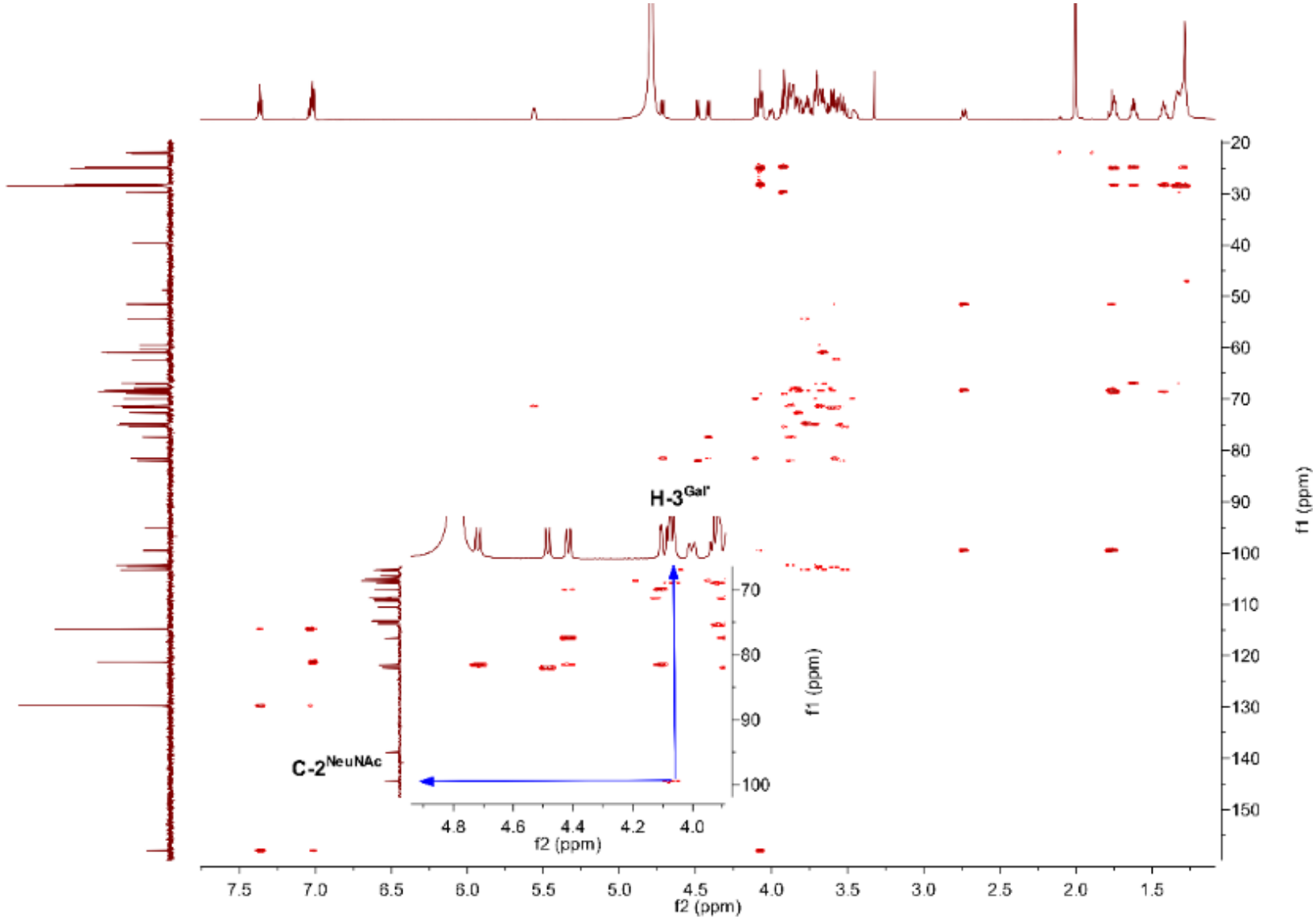
^{13}C NMR spectrum of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 150 MHz, 25 °C)



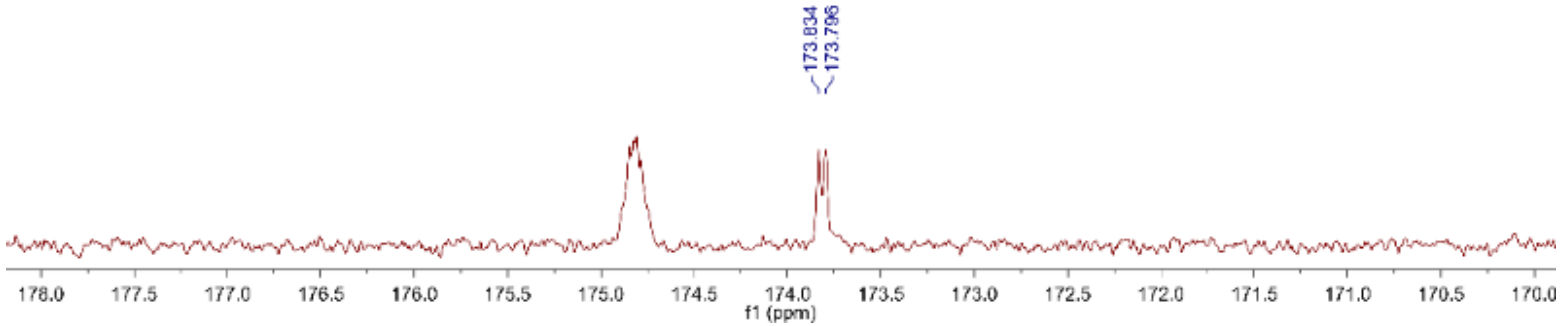
COSY spectrum of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 600/600 MHz, 25 °C)



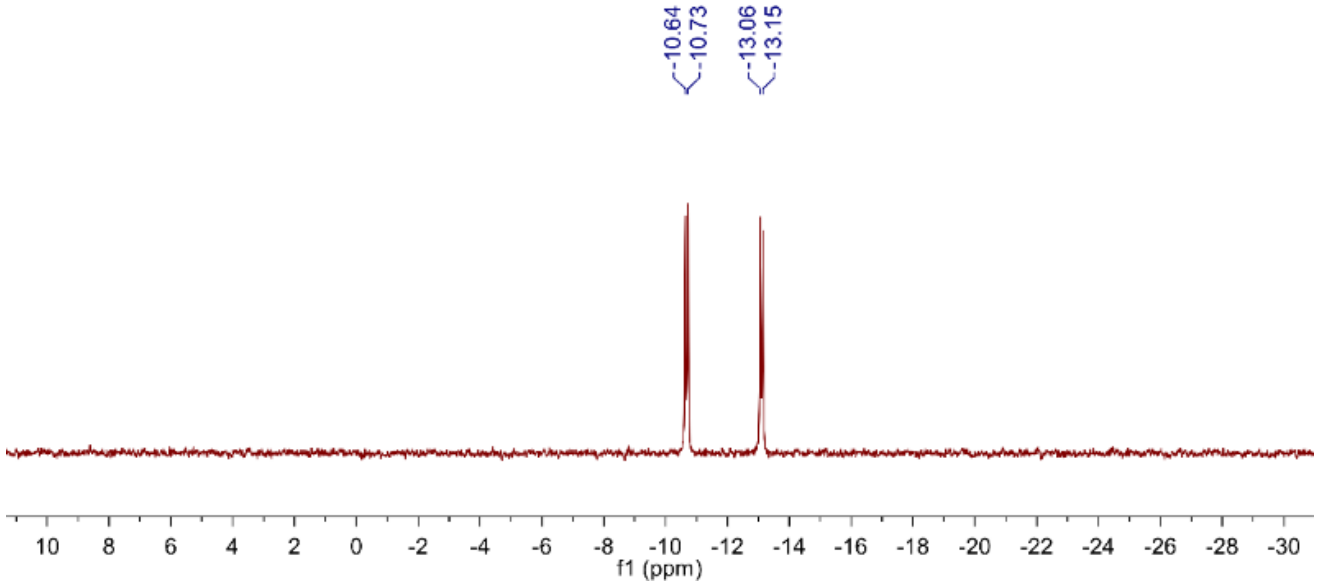
HSQC spectrum of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 600/150 MHz, 25 °C)



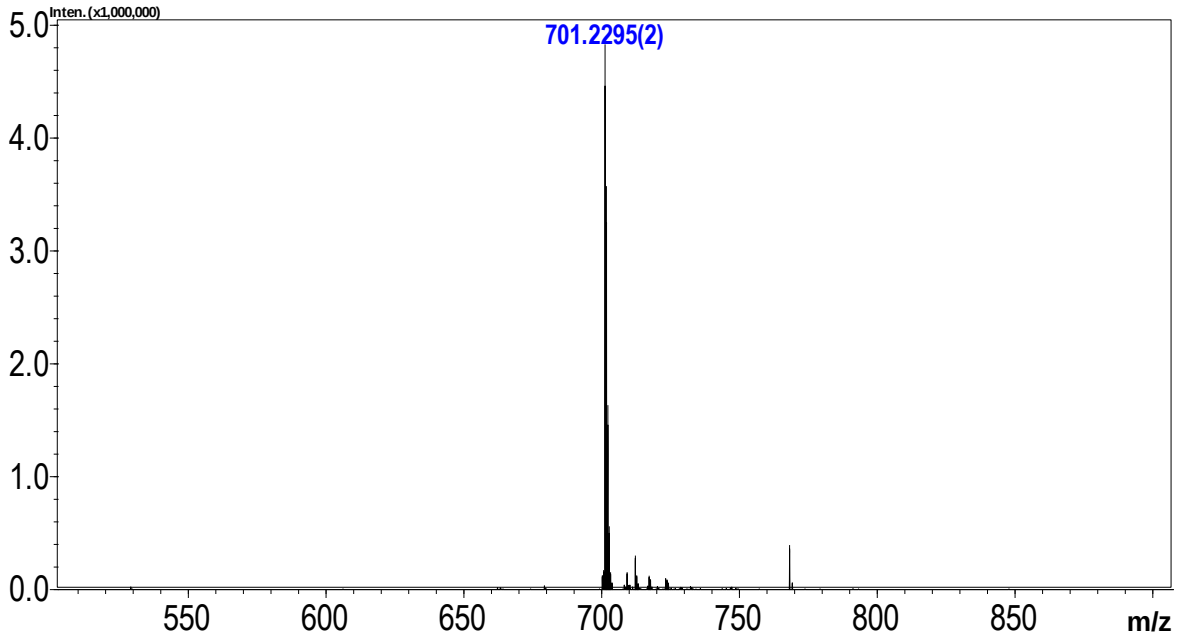
HMBC spectrum of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 600/150 MHz, 25 °C)



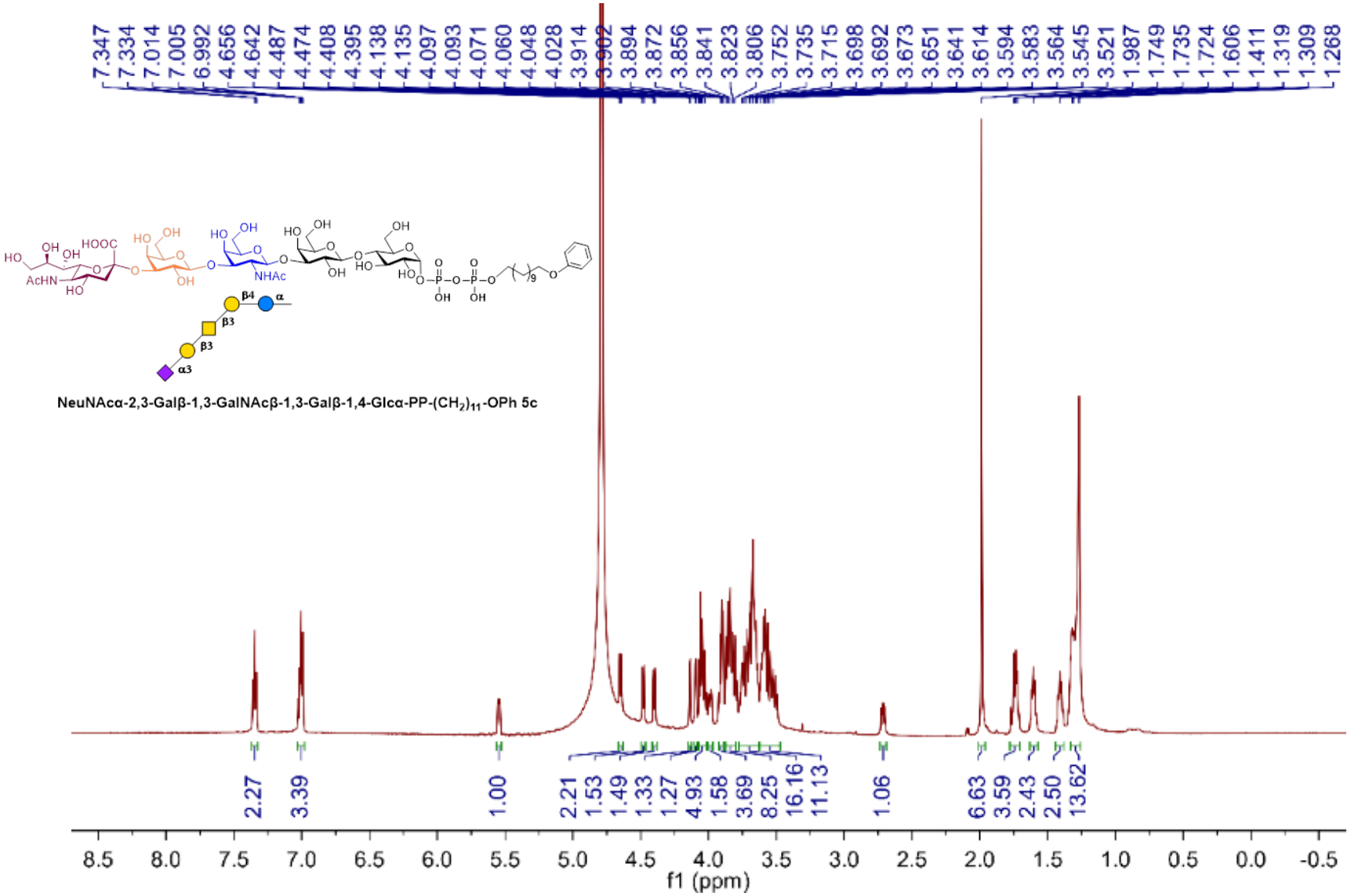
Selected non-decoupling ^{13}C NMR spectrum of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 150 MHz, 25 °C)



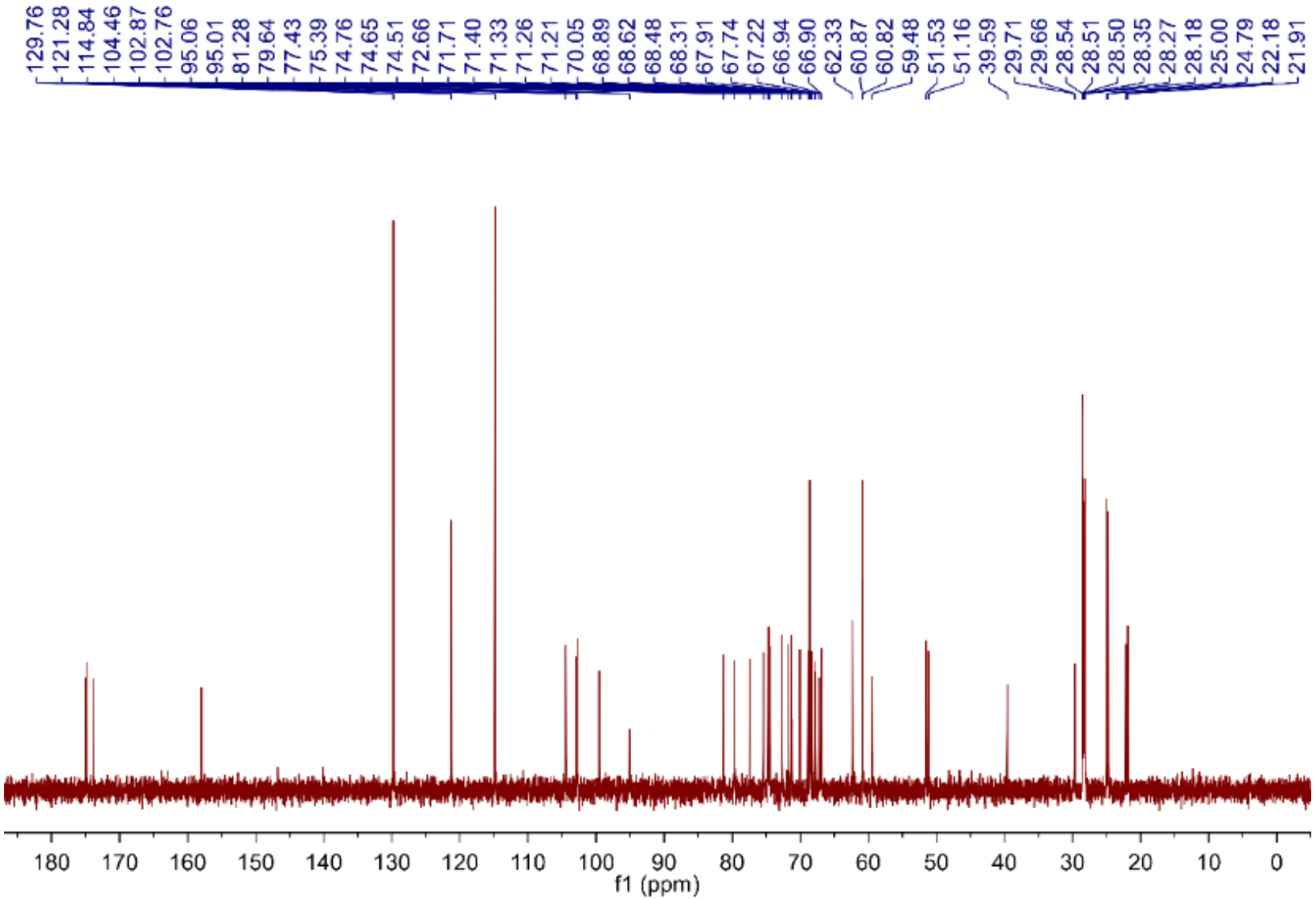
^{31}P NMR spectrum of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b** (D_2O, 243 MHz, 25 °C)



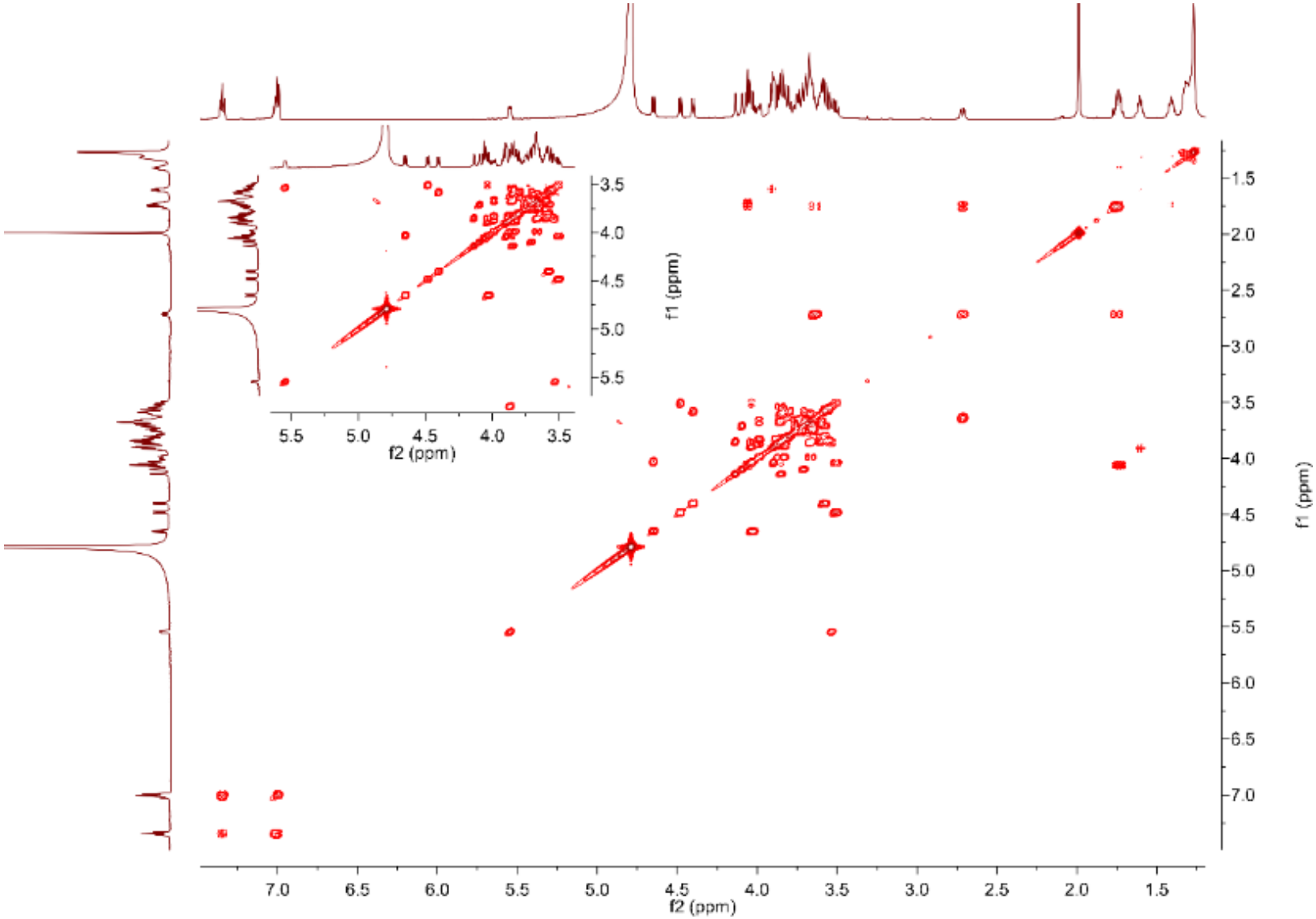
ESI-(-)-TOF HRMS of NeuNAcα-2,3-Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5b**



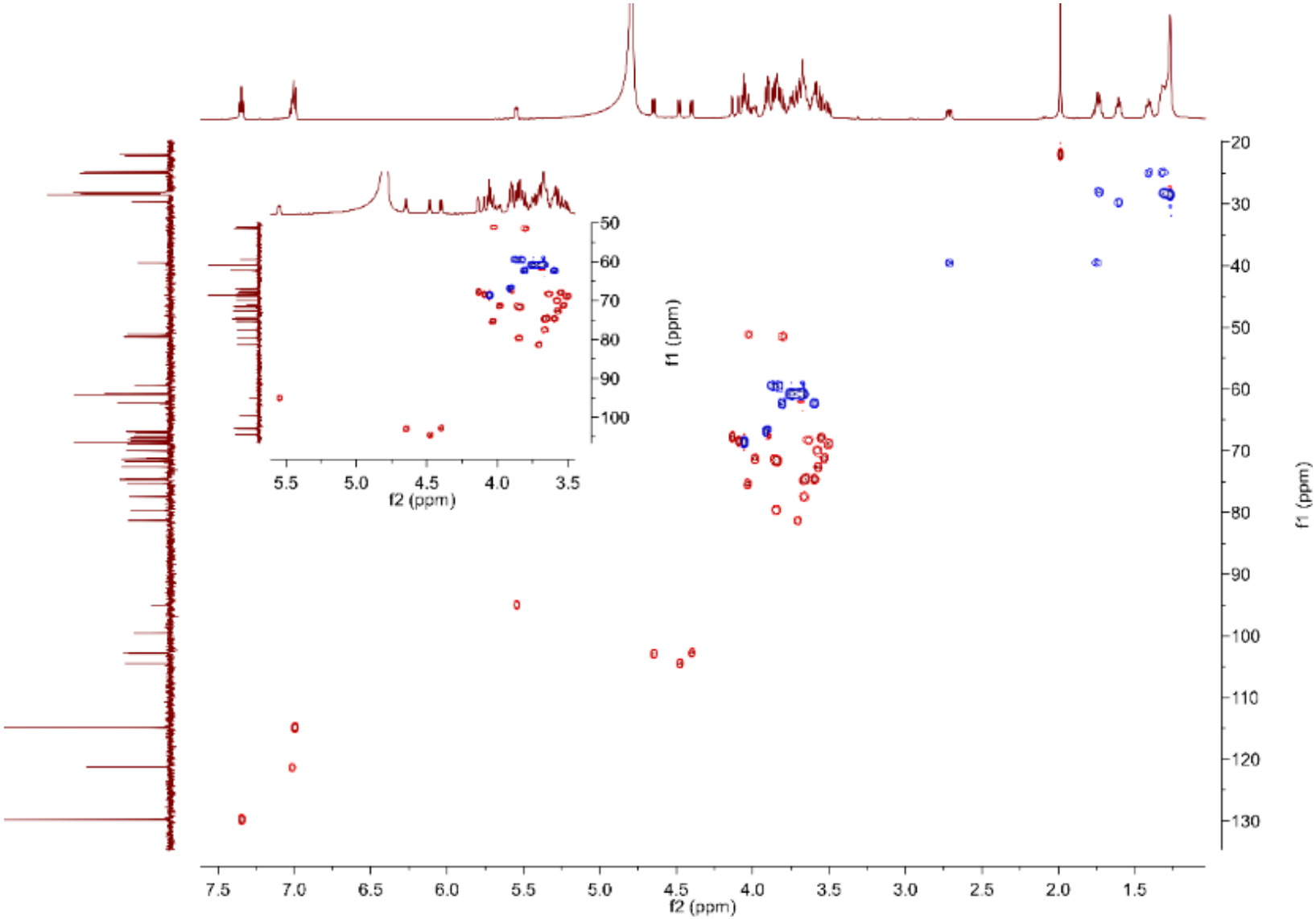
^{1}H NMR spectrum of NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5c** (D_2O, 600 MHz, 25 °C)



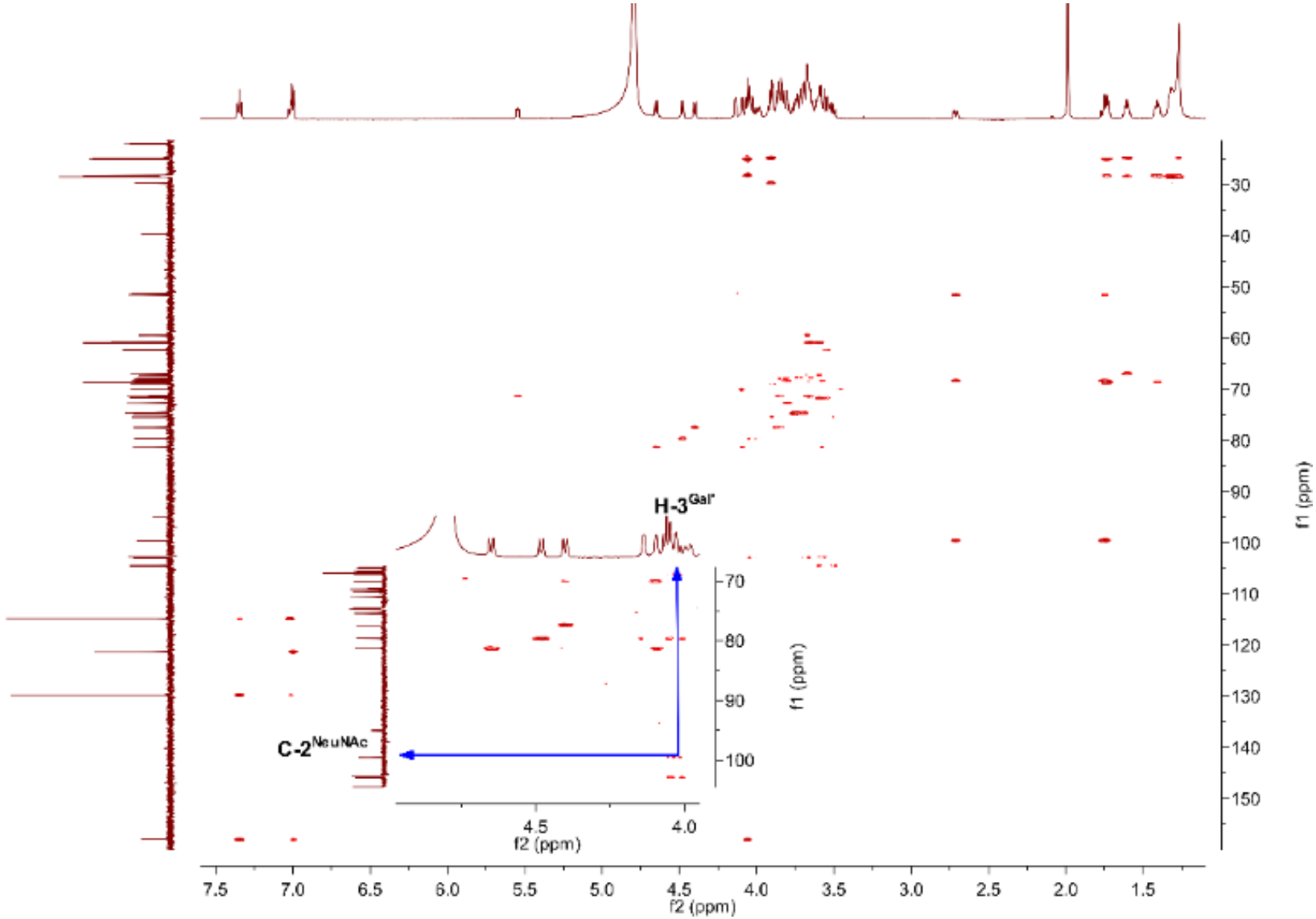
^{13}C NMR spectrum of NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5c** (D_2O, 150 MHz, 25 °C)



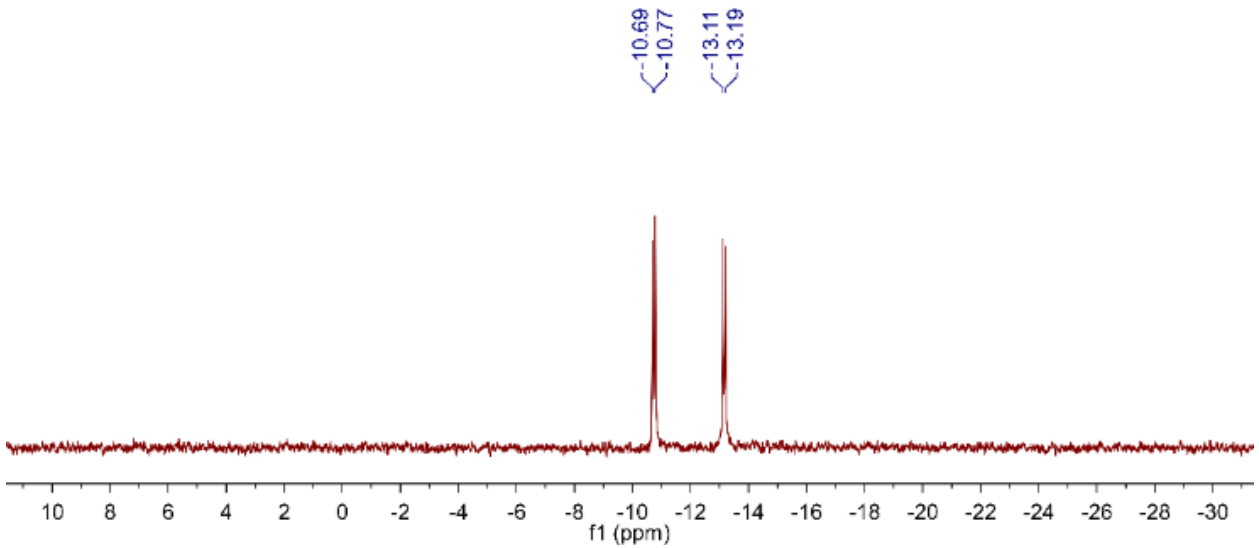
COSY spectrum of NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5c** (D_2O, 600/600 MHz, 25 °C)



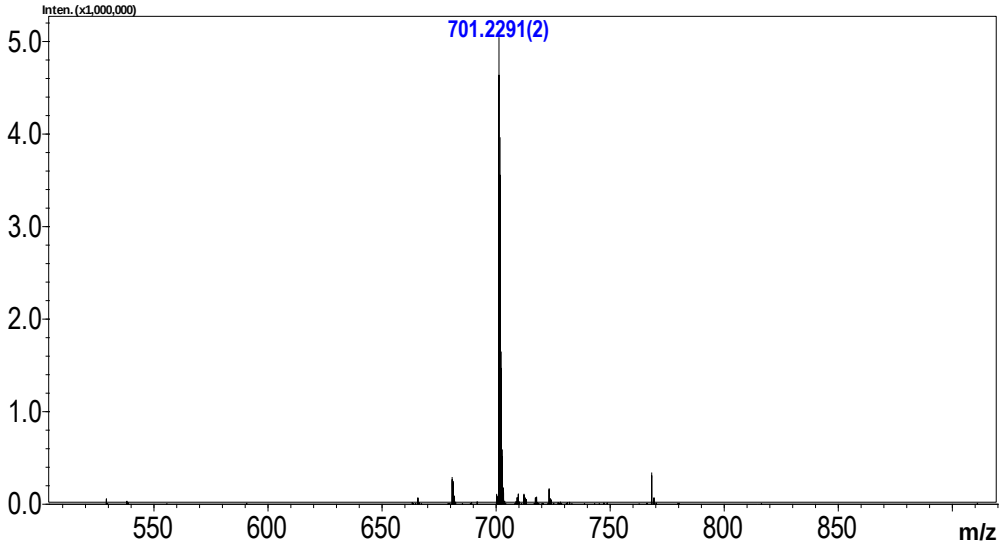
HSQC spectrum of NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5c** (D_2O, 600/150 MHz, 25 °C)



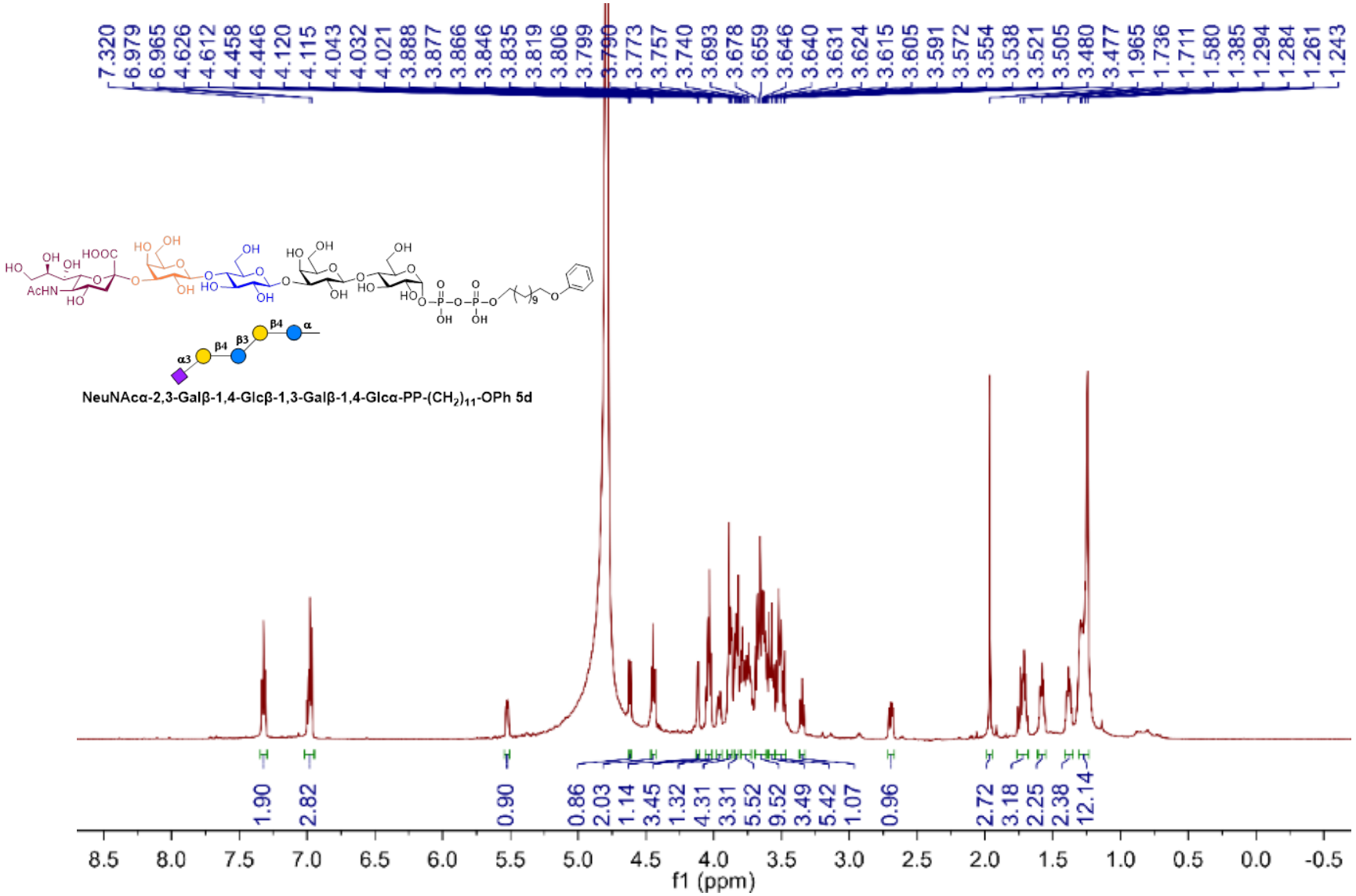
HMBC spectrum of NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5c** (D_2O, 600/150 MHz, 25 °C)



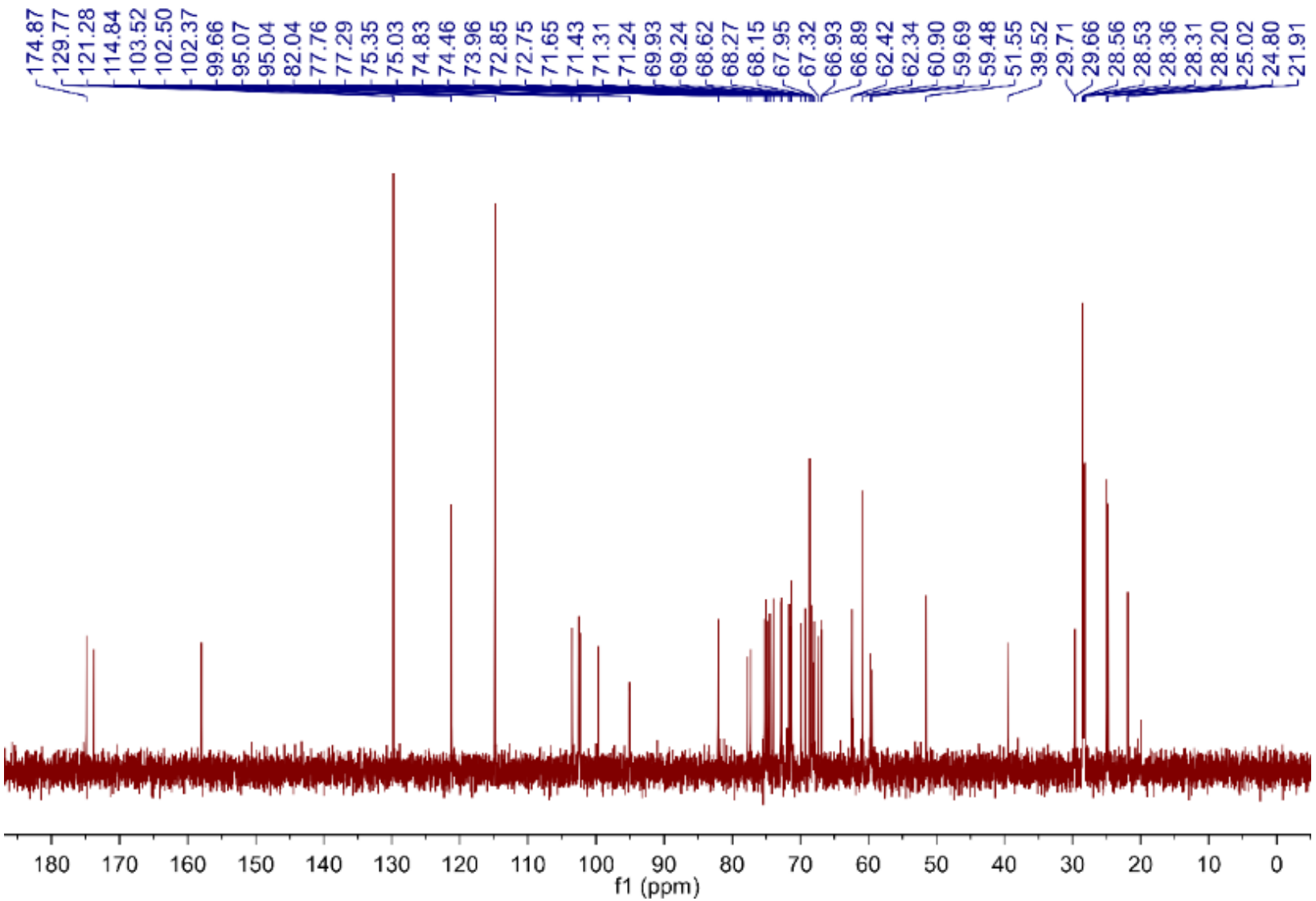
^{31}P NMR spectrum of NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5c** (D_2O, 243 MHz, 25 °C)



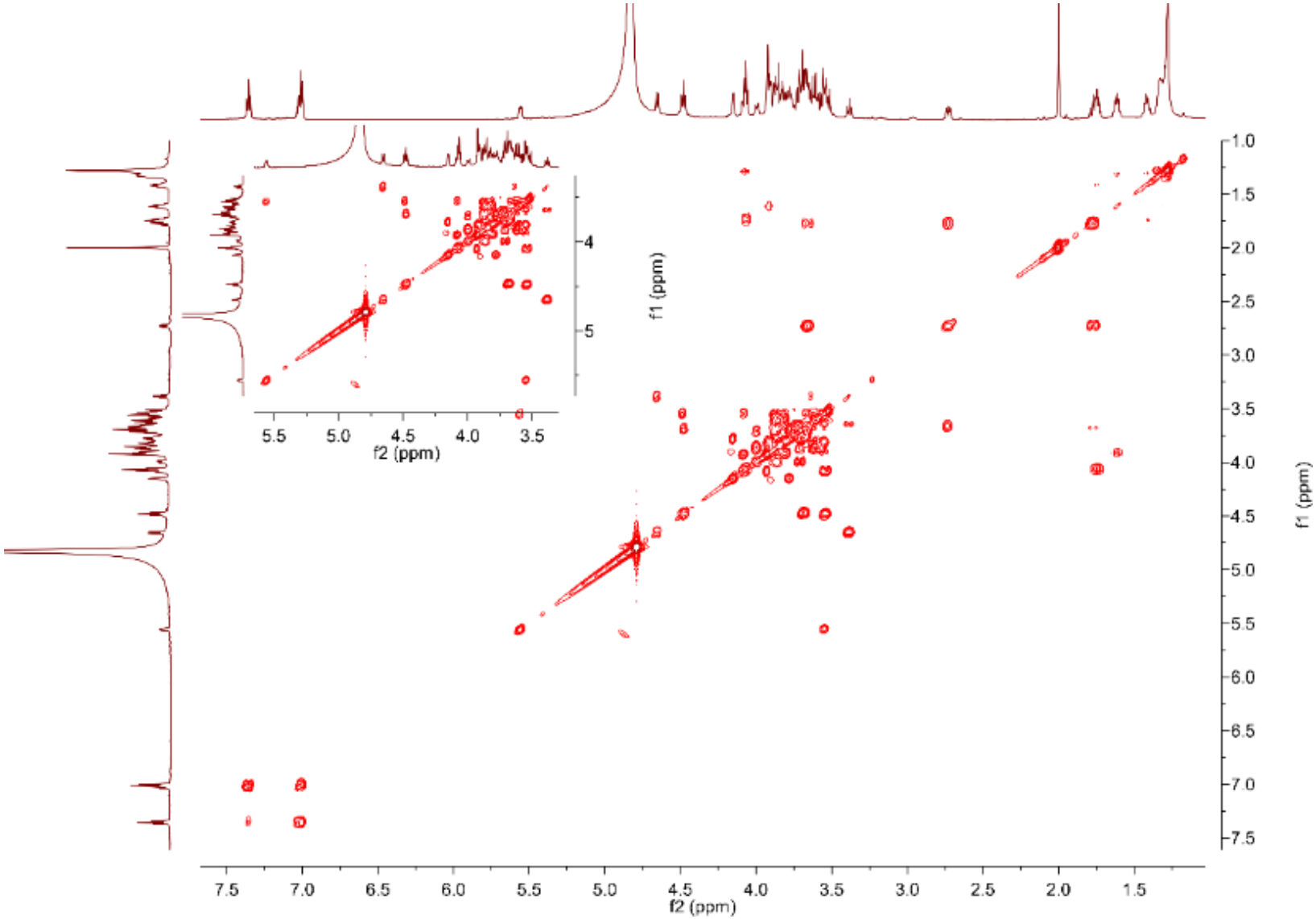
ESI-(-)-TOF HRMS of NeuNAcα-2,3-Galβ-1,3-GalNAcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5c**



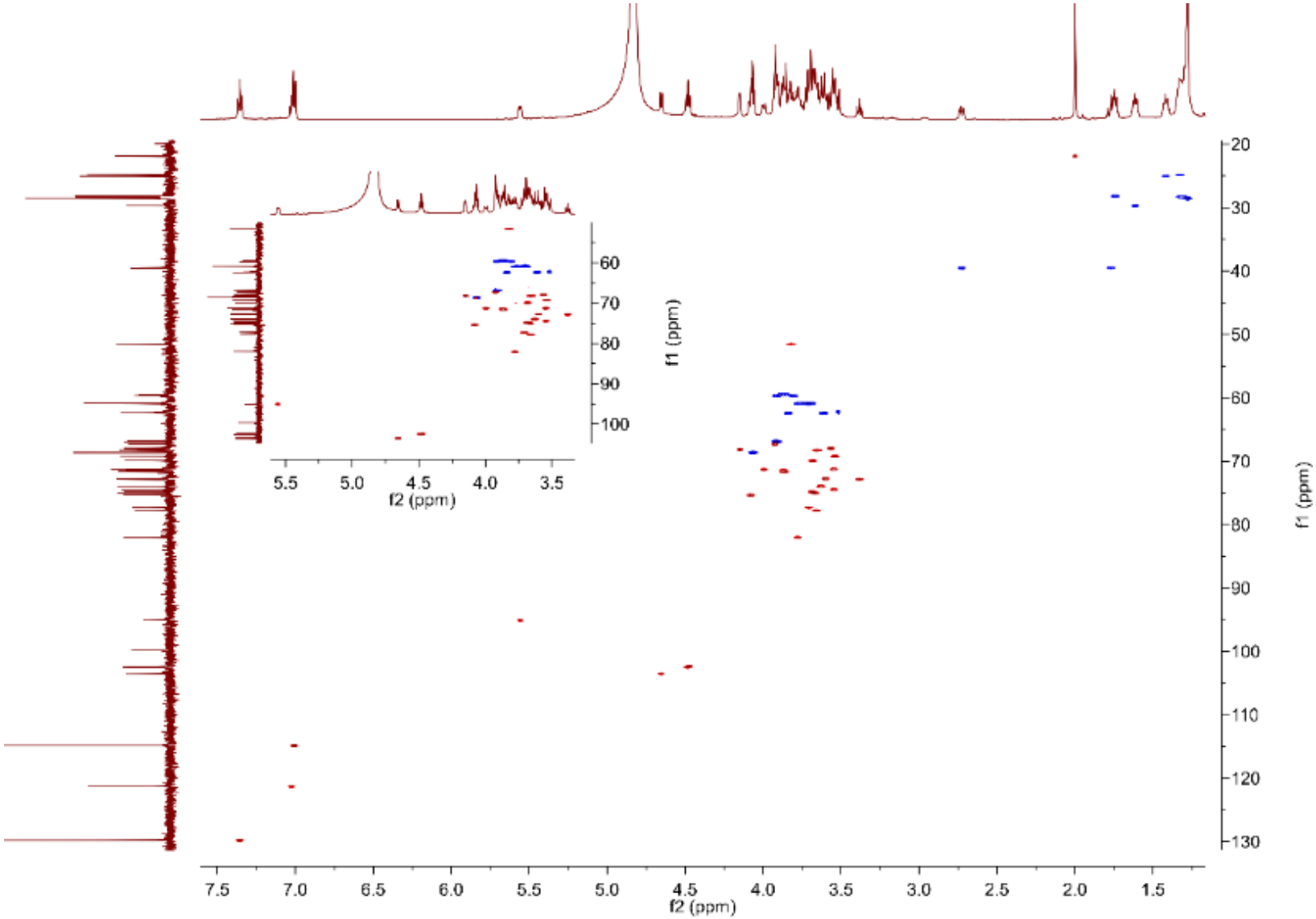
^{1}H NMR spectrum of NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5d** (D_2O, 600 MHz, 25 °C)



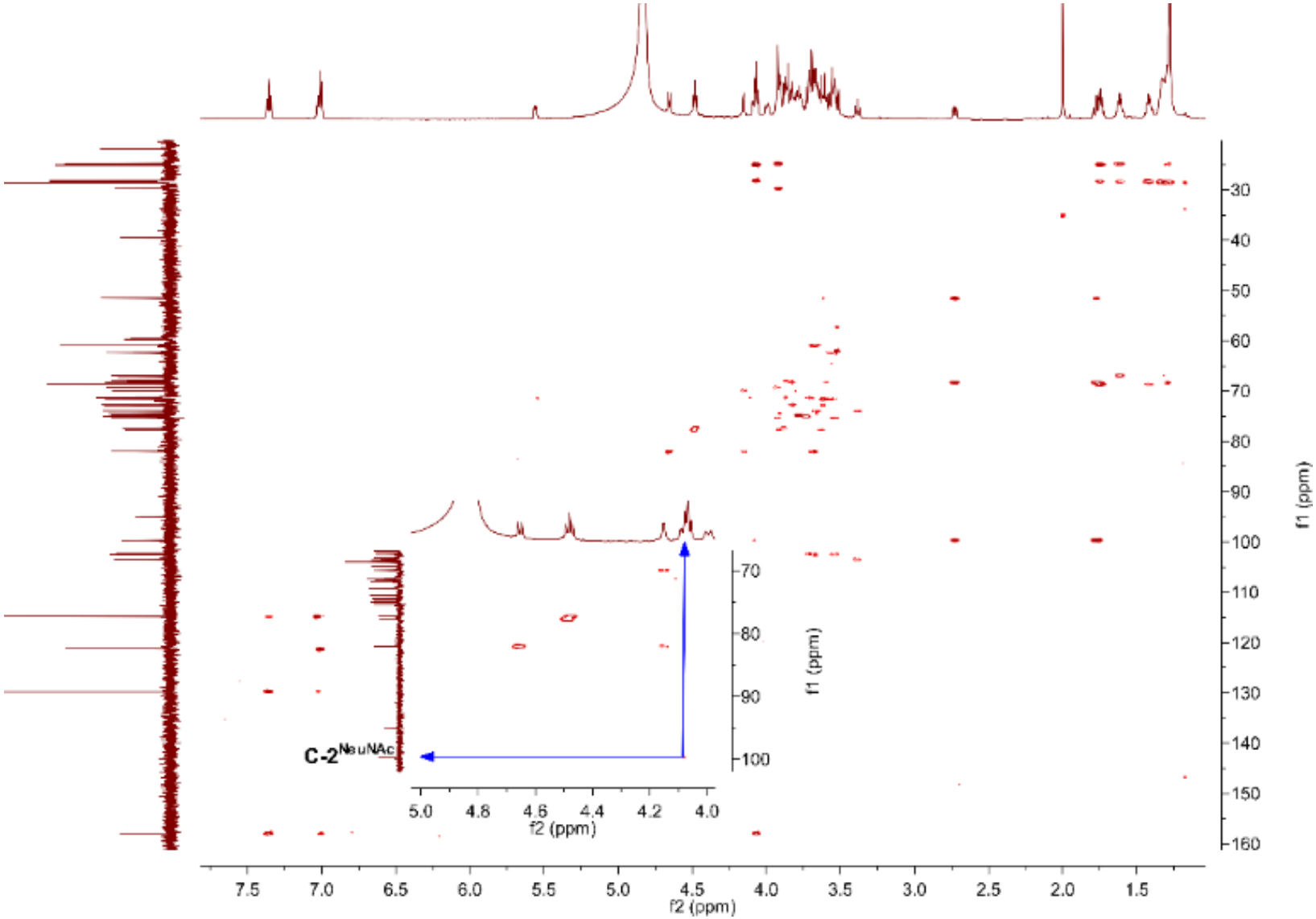
^{13}C NMR spectrum of NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5d** (D_2O, 150 MHz, 25 °C)



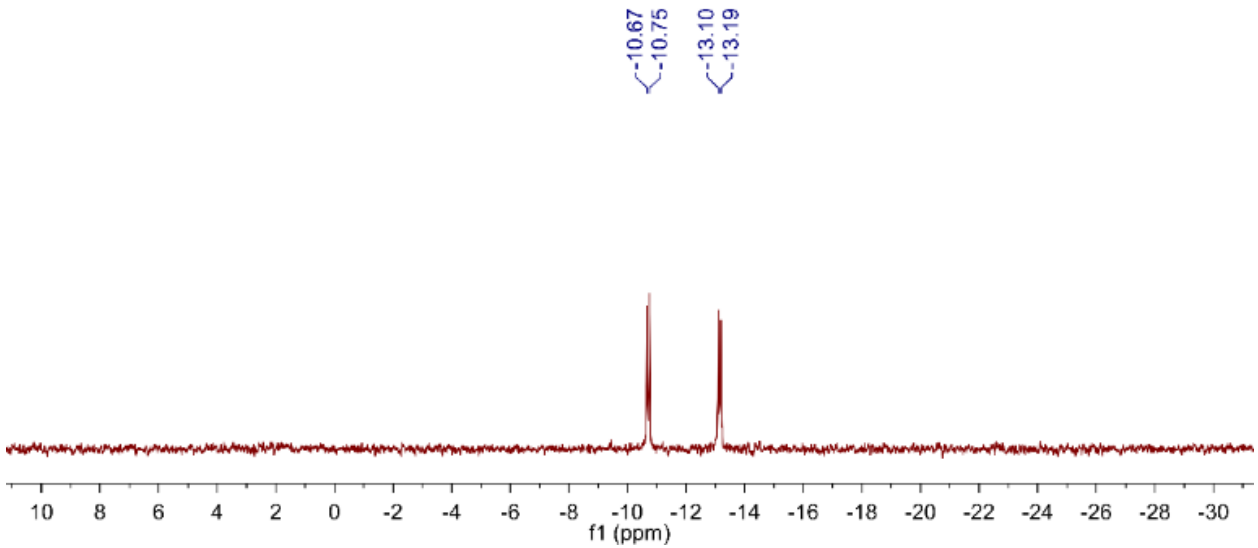
COSY spectrum of NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5d** (D_2O, 600/600 MHz, 25 °C)



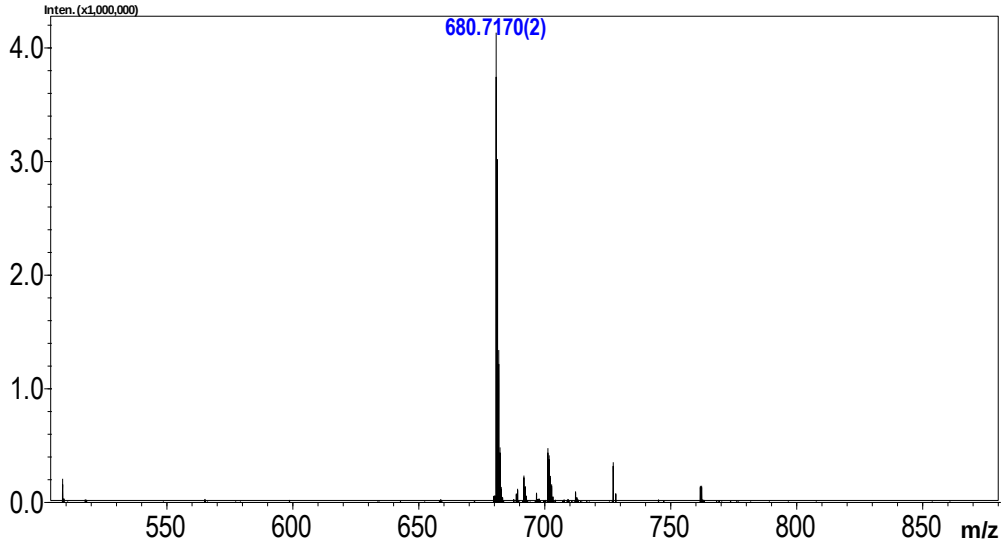
HSQC spectrum of NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5d** (D_2O, 600/150 MHz, 25 °C)



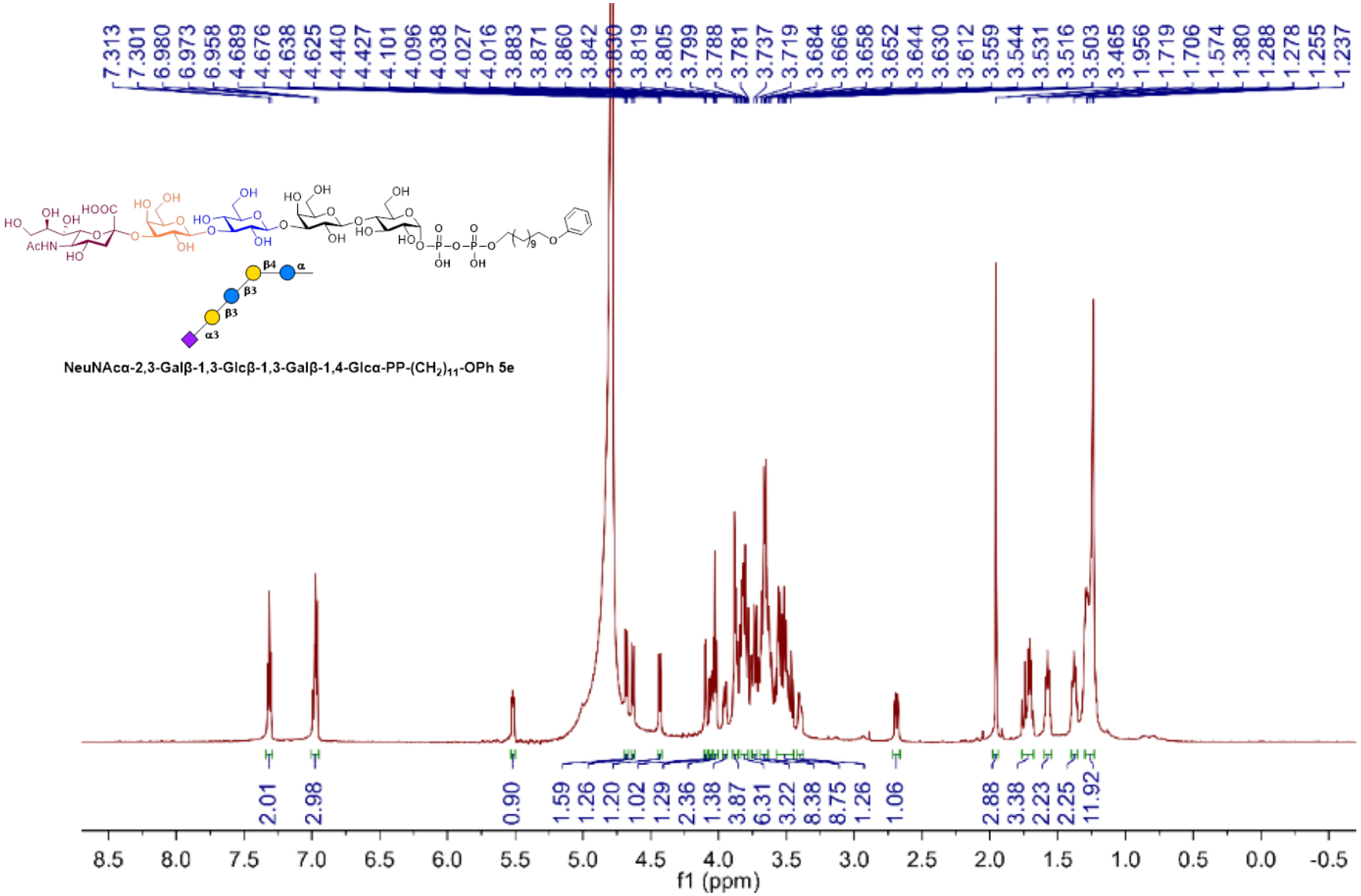
HMBC spectrum of NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5d** (D_2O, 600/150 MHz, 25 °C)



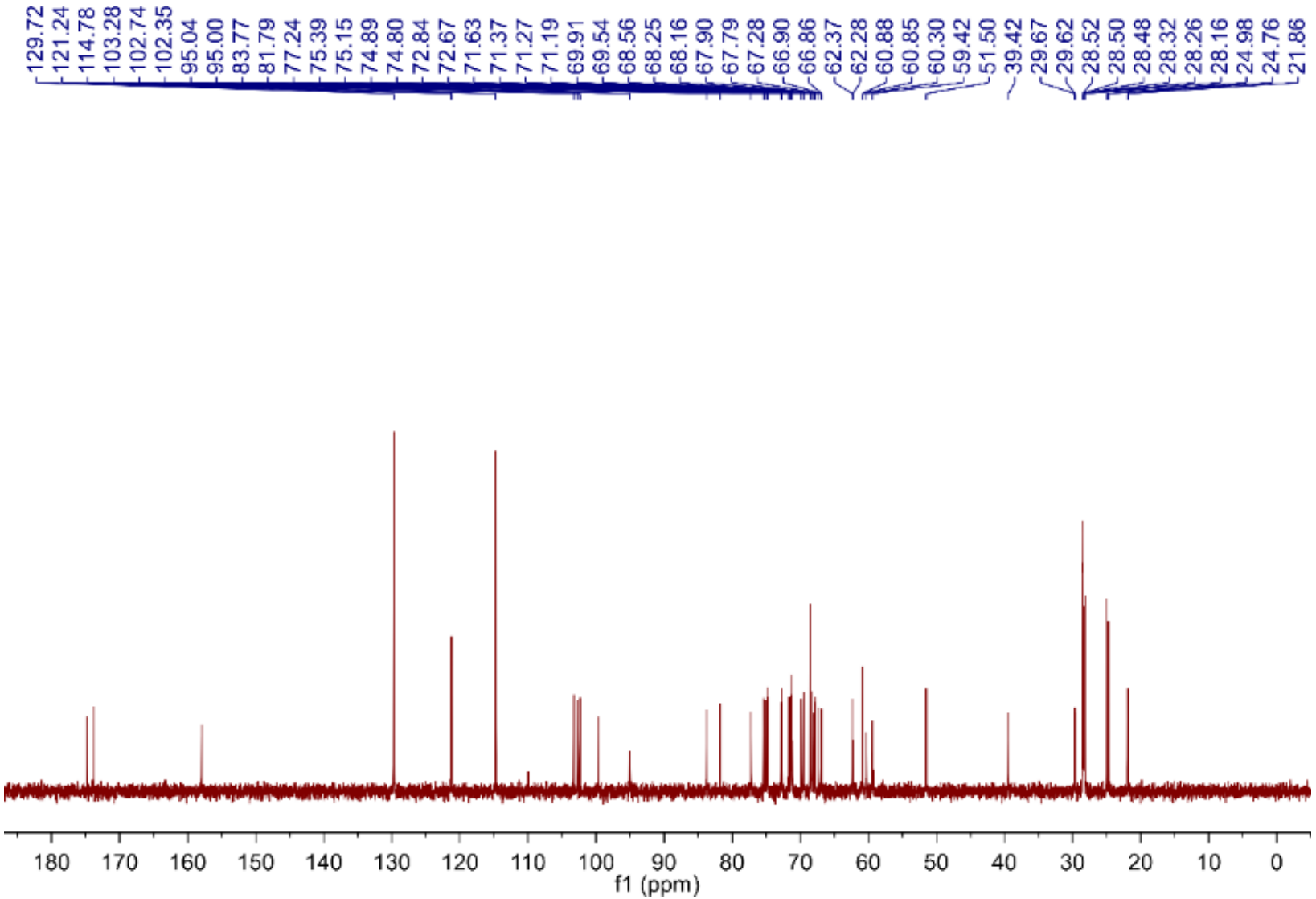
^{31}P NMR spectrum of NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5d** (D_2O, 243 MHz, 25 °C)



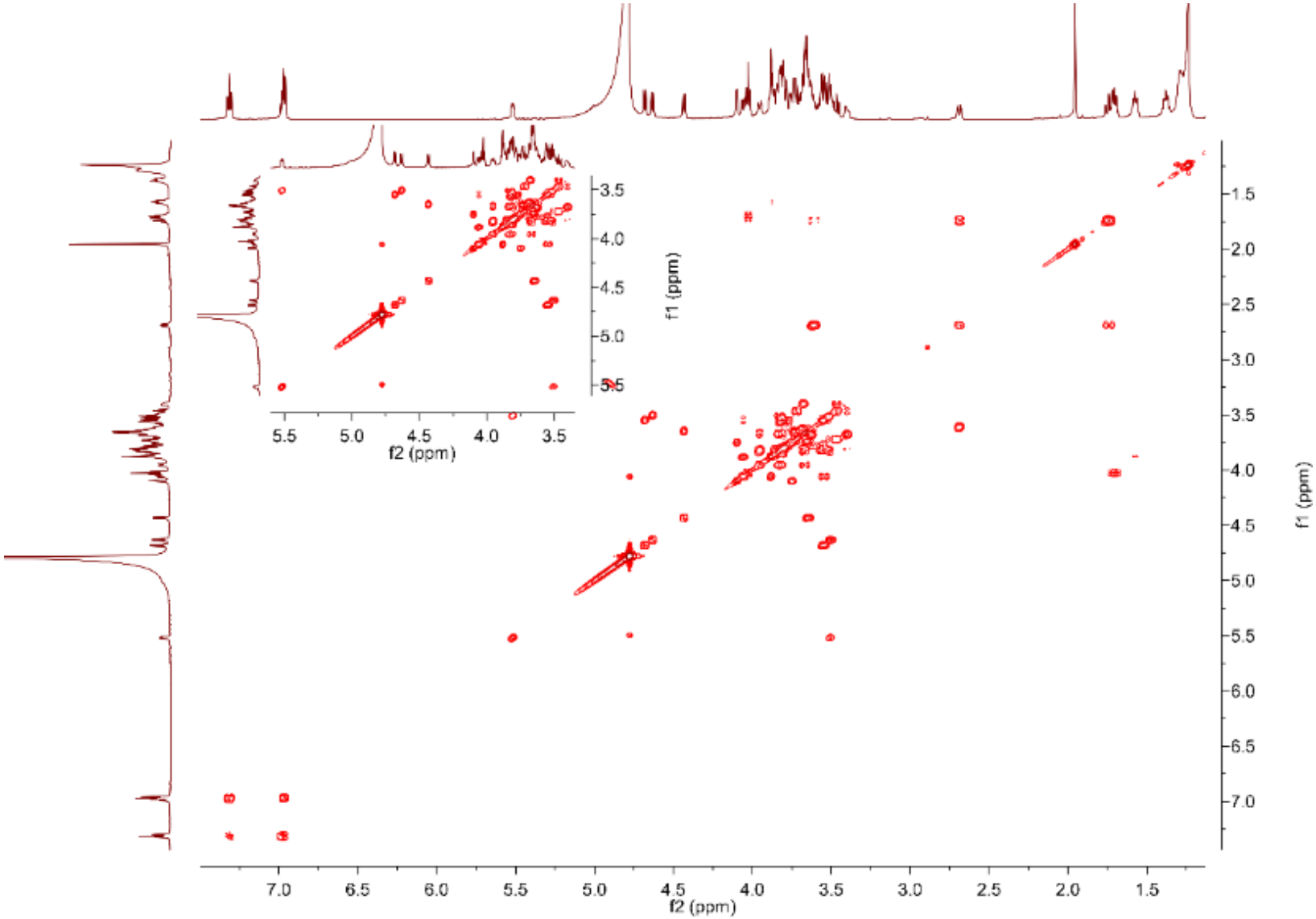
ESI-(-)-TOF HRMS of NeuNAcα-2,3-Galβ-1,4-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5d**



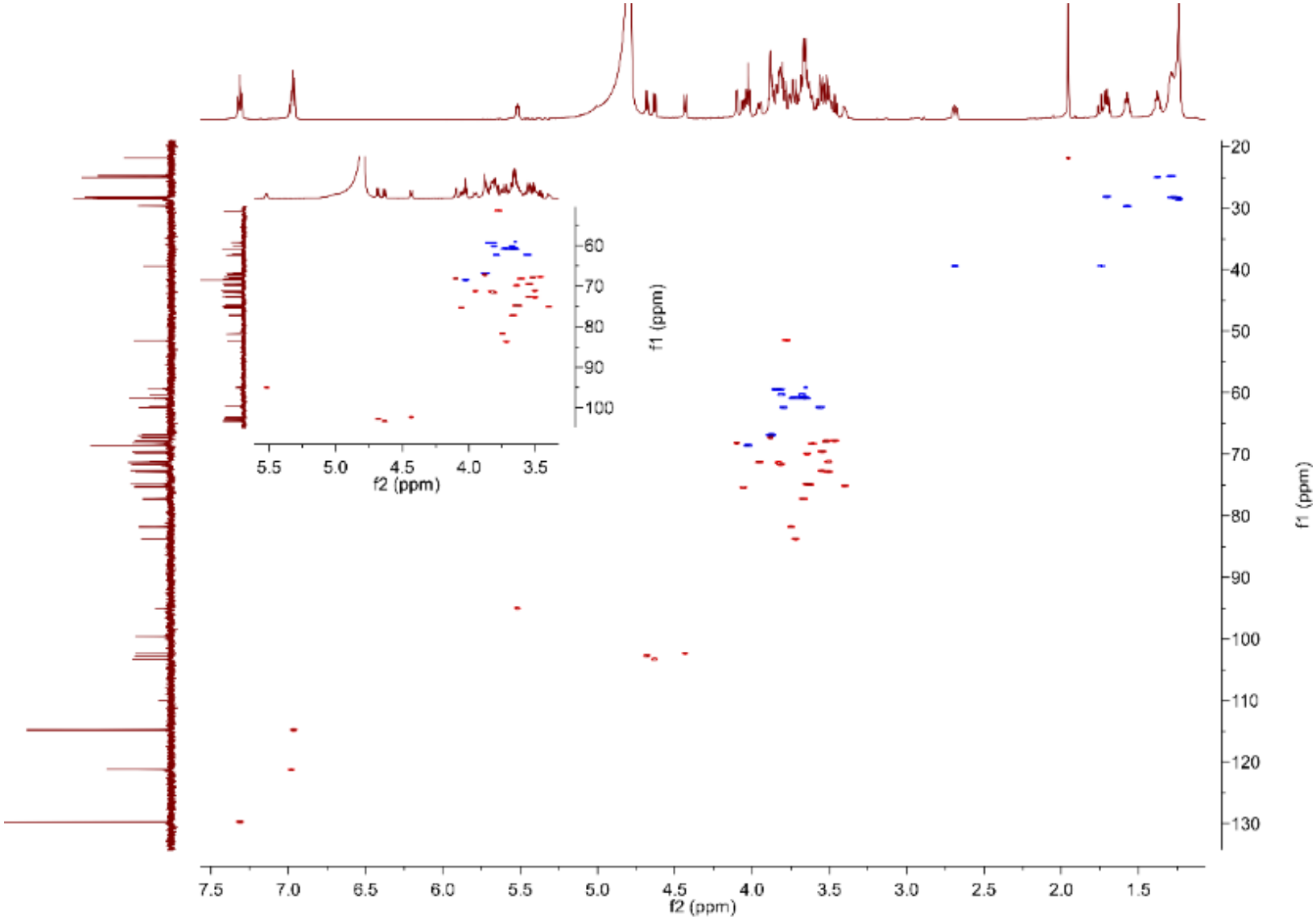
^{1}H NMR spectrum of NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5e** (D_2O, 600 MHz, 25 °C)



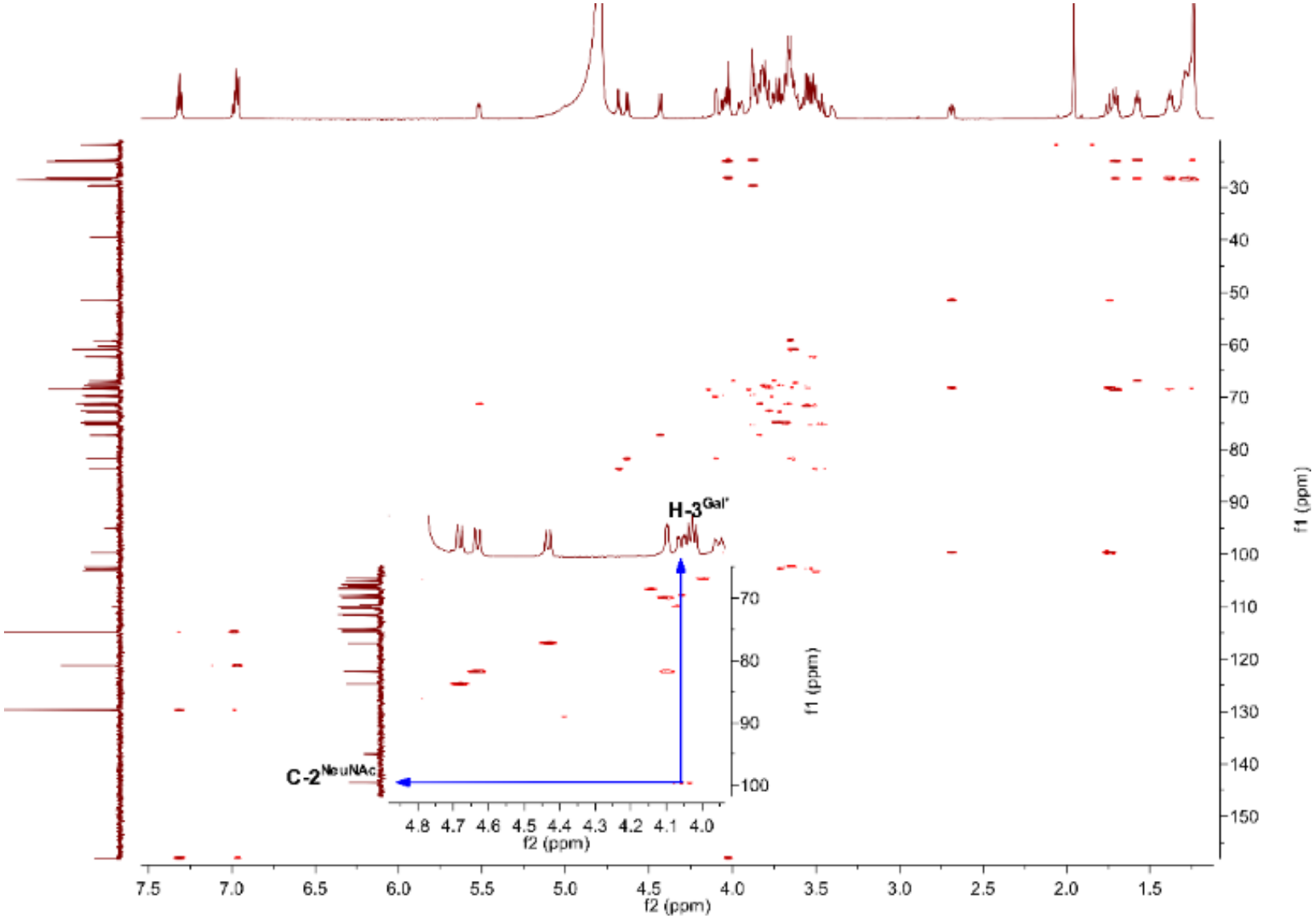
^{13}C NMR spectrum of NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5e** (D_2O, 150 MHz, 25 °C)



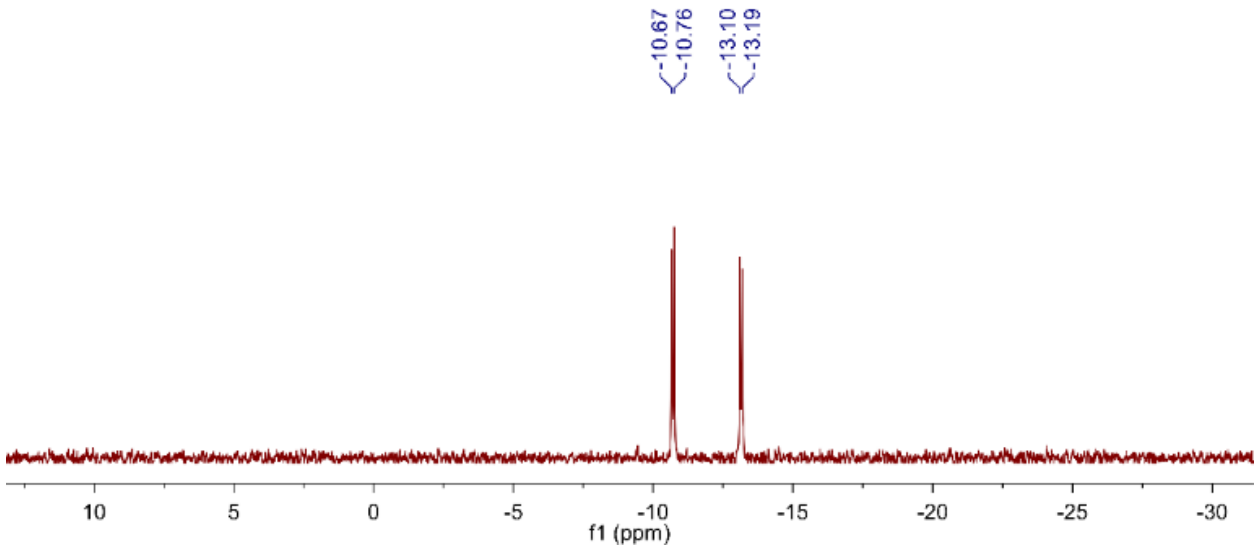
COSY spectrum of NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5e** (D_2O, 600/600 MHz, 25 °C)



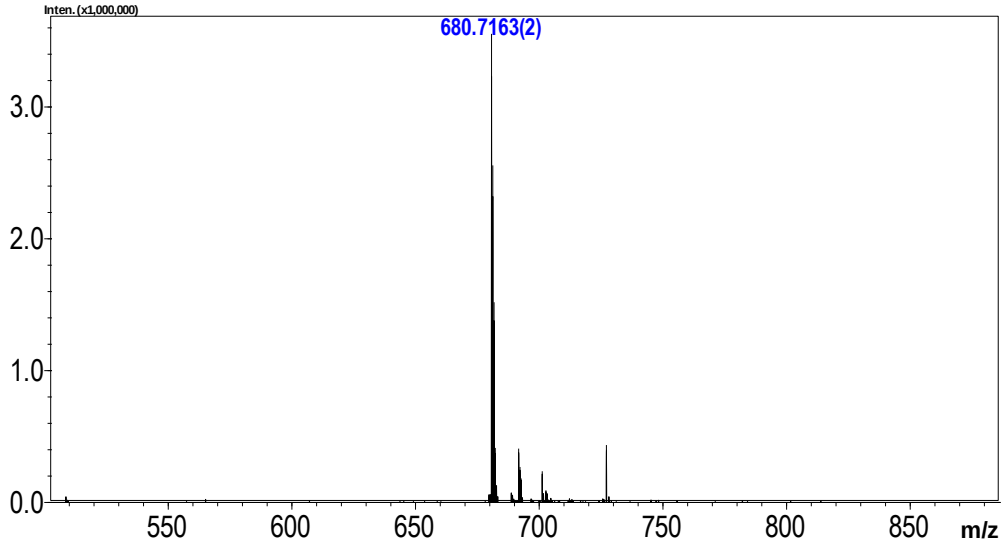
HSQC spectrum of NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5e** (D_2O, 600/150 MHz, 25 °C)



HMBC spectrum of NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **5e** (D_2O, 600/150 MHz, 25 °C)



^{31}P NMR spectrum of NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **1e** (D_2O, 243 MHz, 25 °C)



ESI-(-)-TOF HRMS of NeuNAcα-2,3-Galβ-1,3-Glcβ-1,3-Galβ-1,4-Glcα-PP-$(CH_2)_{11}$-OPh **1e**

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