Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis of a glycosidic spacer containing prodrug of a duocarmycin analogue and determination of its biological activity

Heiko J. Schuster,[a] Birgit Krewer,[a] J. Marian von Hof,[a] Kianga Schmuck,[a] Ingrid Schuberth,[a] Frauke Alves,[b] Lutz F. Tietze*[a]

[a] *Institut for Organic and Biomolecular Chemistry, Georg-August-University Göttingen, Tammannstrasse 2,37077 Göttingen (Germany); FAX: +49(0)551-399476;* [b] *Centre of Internal Medicine, Department of Haematology and Oncology of the Georg-August-University of Göttingen, Robert-Koch-Straße 40, 37075 Göttingen, Germany.*

ltietze@gwdg.de

Contents:

Synthesis of compound 14.

3-Nitro-4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-benzaldehyde (14). The acetylated galactosylbromide (1.03 g, 2.50 mmol, 1.0 equiv.), 4-hydroxy-3-nitro-benzaldehyde **13** (752 mg, 4.50 mmol, 1.8 equiv.) and $BnEt_3NCl$ (474 mg, 2.08 mmol, 0.83 equiv.) were suspended in $CHCl_3$ (11.0 mL), aqueous NaOH solution (1.25 M, 3.64 mL, 4.55 mmol, 1.8 eq.) was added and the reaction mixture heated to 70 °C. After 3.5 h at refluxing temperature the mixture was diluted with H_2O/ $CHCl_3$ (2:1, 15 mL), phases were separated and the organic layer was successively washed with cold NaOH solution (1.25 M, 2 × 5 mL) and cold brine. Filtration over cotton wool and removal of the solvents gave a yellow solid which was dissolved in absolute EtOH (~40 mL) and allowed to crystallise overnight at 4 °C. Two fractions yielded the benzaldehyde **14** as slightly yellow crystals (987 mg, 1.98 mmol, 80%). R_f = 0.46 (toluene/MeOH = 6:1); ^{1}H-NMR (300.1 MHz, $CDCl_3$): δ = 2.03, 2.09, 2.13, 2.20 (4 × s, 12 H, 4 × $COCH_3$), 4.20 (m_c, 2 H, H-5, H-6_a), 4.28 (dd, J = 12.5, 8.5 Hz, 1 H, H-6_b), 5.15 (dd, J = 10.4, 3.3 Hz, 1 H, H-3), 5.24 (d, J = 7.9 Hz, 1 H, H-1), 5.51 (dd, J = 3.3, 0.7 Hz, 1 H, H-4), 5.60 (dd, J = 10.5, 7.9 Hz, 1 H, H-2), 7.51(d, J = 8.7 Hz, 1 H, H-12), 8.09 (dd, J = 8.7, 2.1 Hz, 1 H, H-11), 8.32 (d, J = 2.2 Hz, 1 H, H-9), 9.99 (s, 1 H, ArCHO); ^{13}C-NMR (75.8 MHz, $CDCl_3$): δ = 20.5, 20.6 (4 × CO<u>C</u>H_3), 61.3 (C-6), 66.5 (C-4), 67.5 (C-2), 70.3 (C-3), 71.7(C-5), 99.9 (C-1), 118.6 (C-12), 126.8 (C-9), 131.3 (C-11), 133.2 (C-10), 141.1 (C-7), 153.4 (C-8), 169.1, 170.0, 170.2 (4 × <u>C</u>OCH_3), 188.6 (Ar<u>C</u>HO); $C_{21}H_{23}NO_{13}$ (497.41).

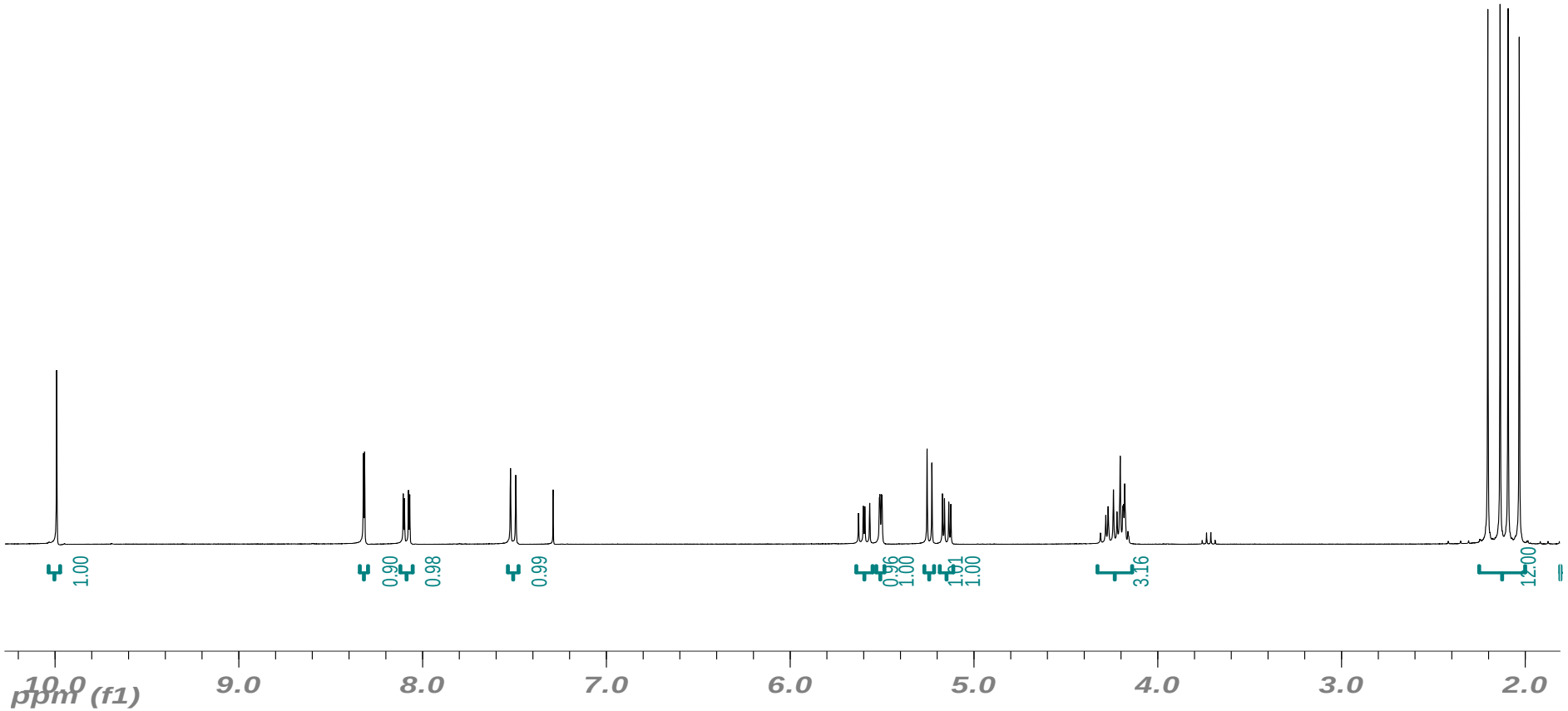

Fig. S2: ^{1}H NMR of **3-Nitro-4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-benzaldehyde (14)**.

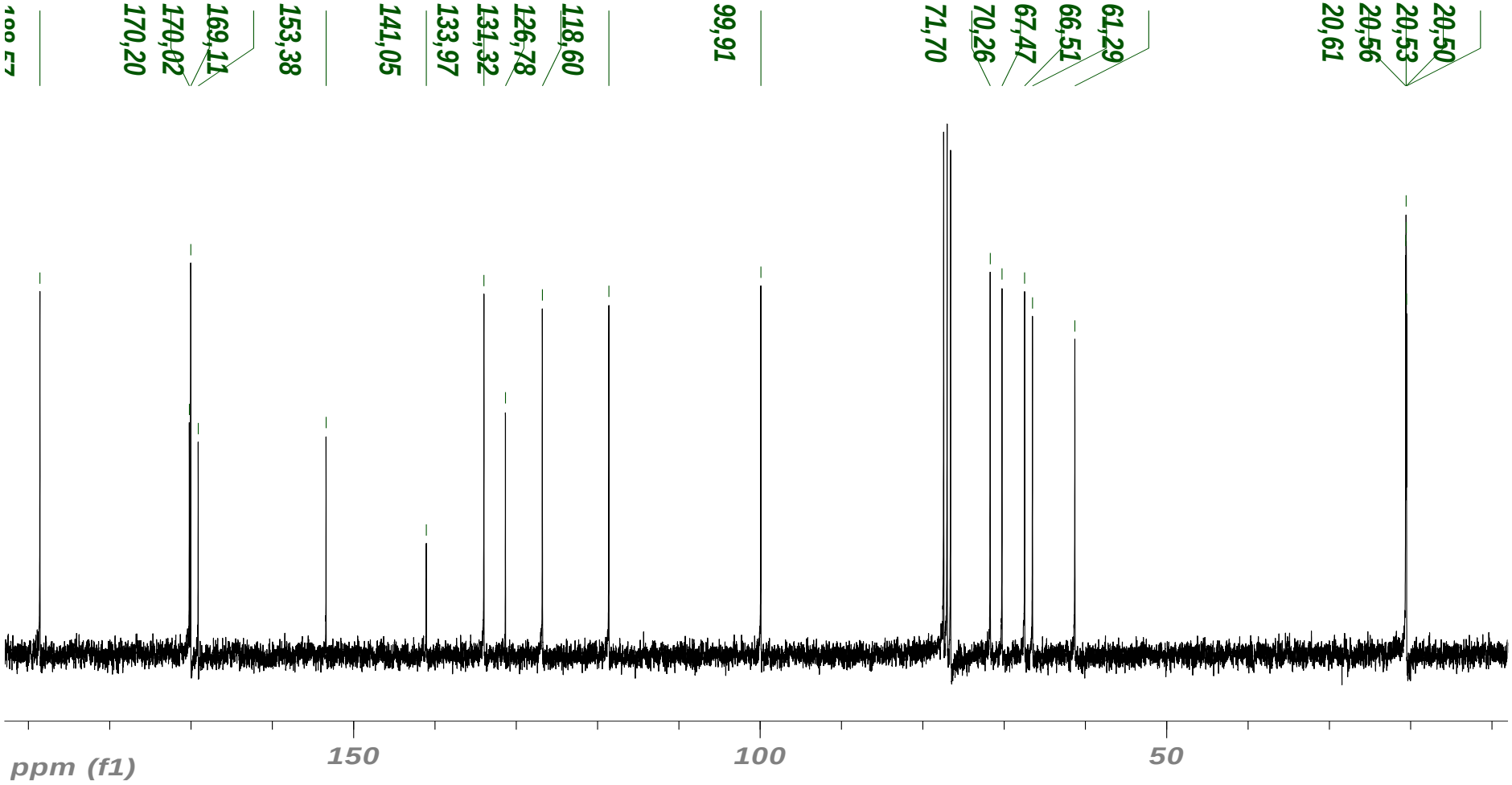

Fig. S3: ^{13}C NMR of **3-Nitro-4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-benzaldehyde (14)**.

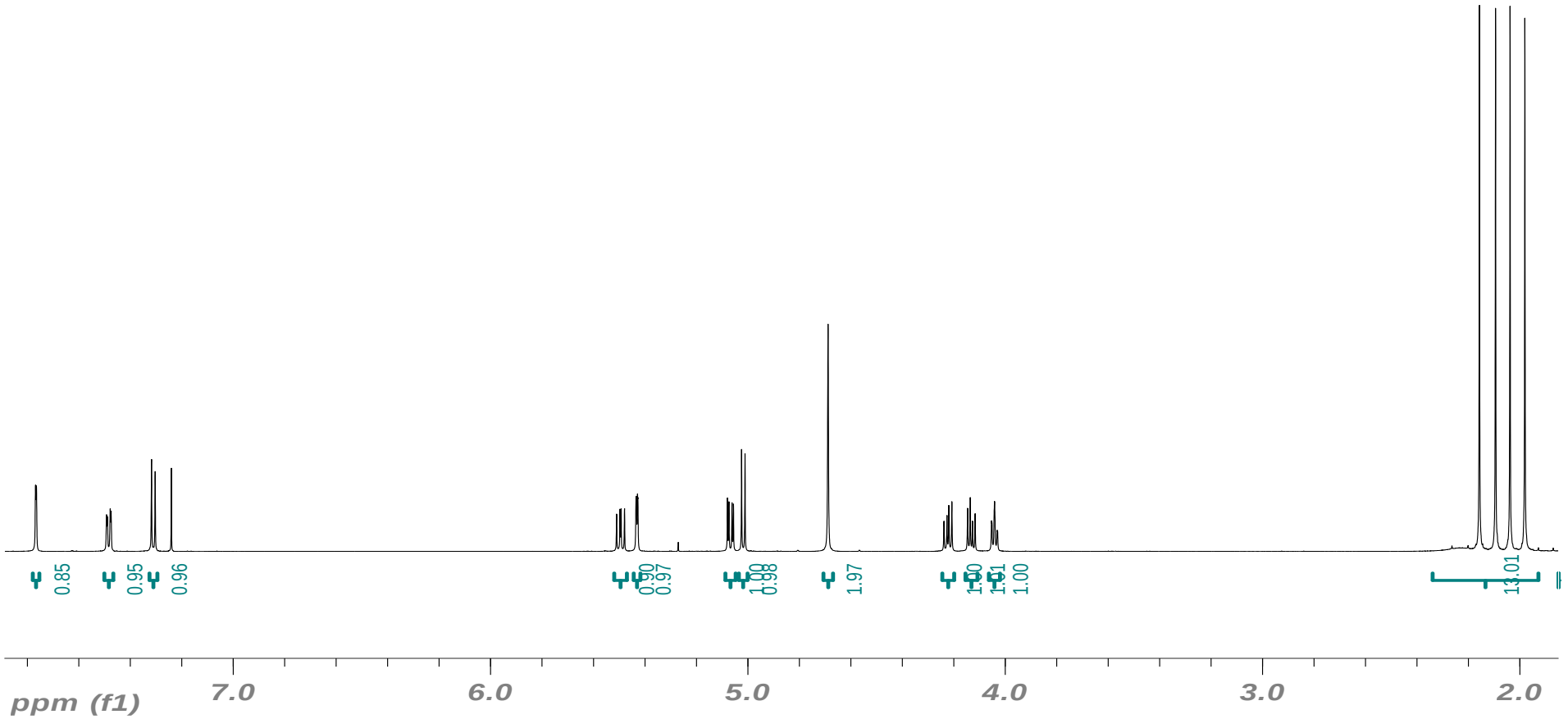

Fig. S4: ^{1}H NMR of **2,3,4,6-Tetra-*O*-acetyl-[2-nitro-4-(hydroxymethyl)phenyl]-*β*-D-galactopyranoside (15).**

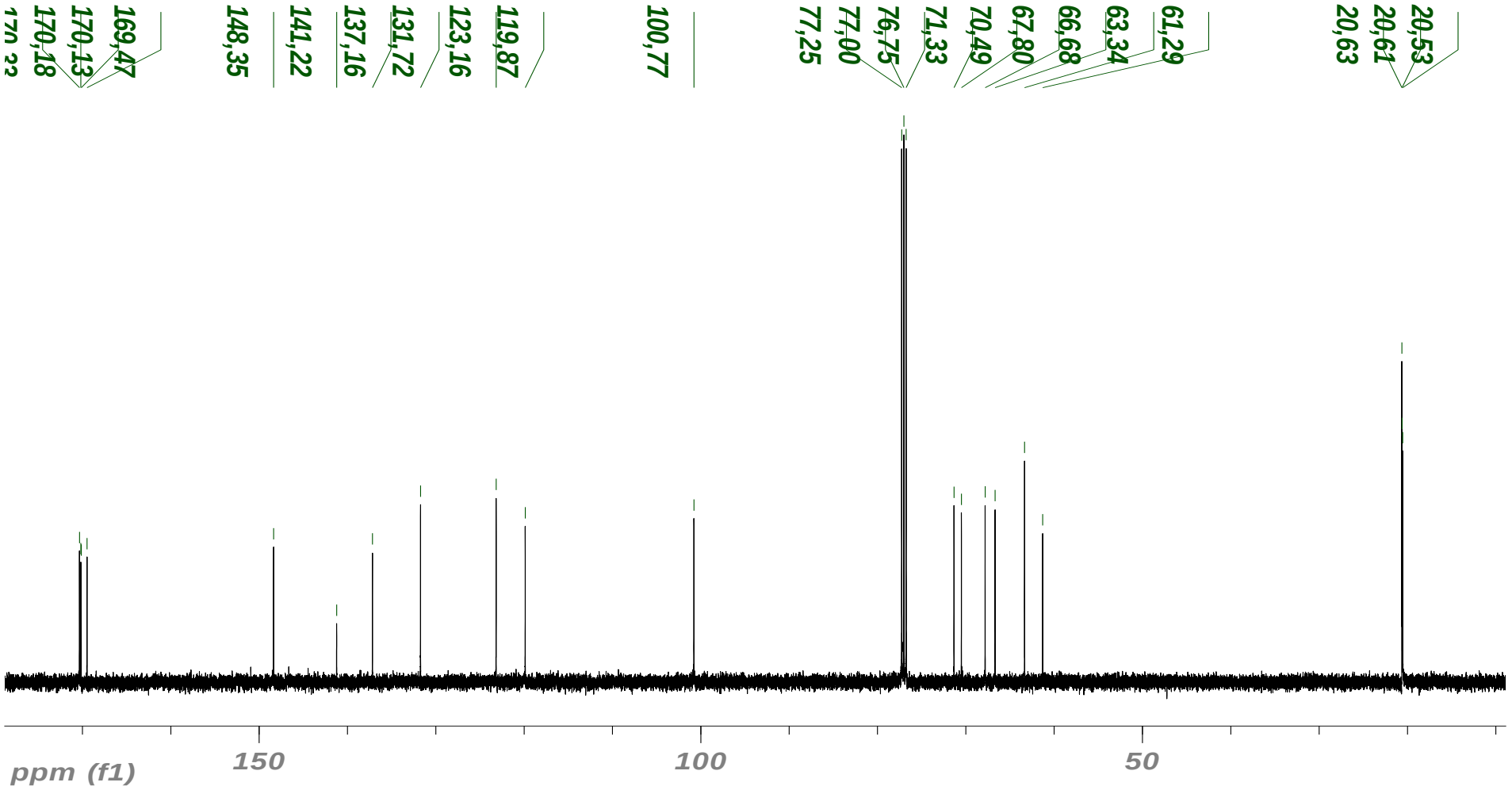

Fig. S5: ^{13}C NMR of **2,3,4,6-Tetra-*O*-acetyl-[2-nitro-4-(hydroxymethyl)phenyl]-*β*-D-galactopyranoside (15).**

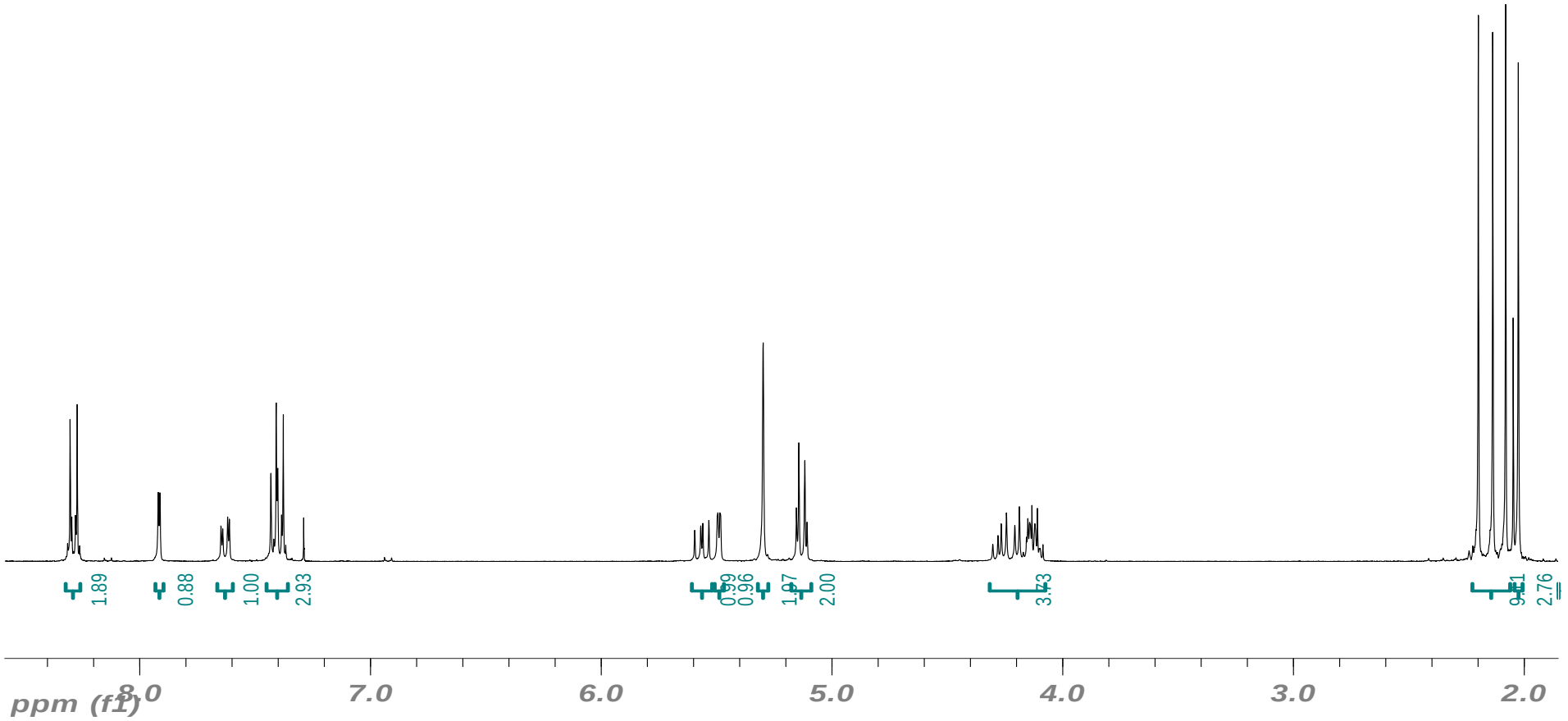

Fig. S6: ^{1}H NMR of **2,3,4,6-Tetra-*O*-acetyl-[2-nitro-4-(4-nitrophenoxycarbonyloxymethyl)phenyl]-*β*-D-galactopyranoside** (**16**).

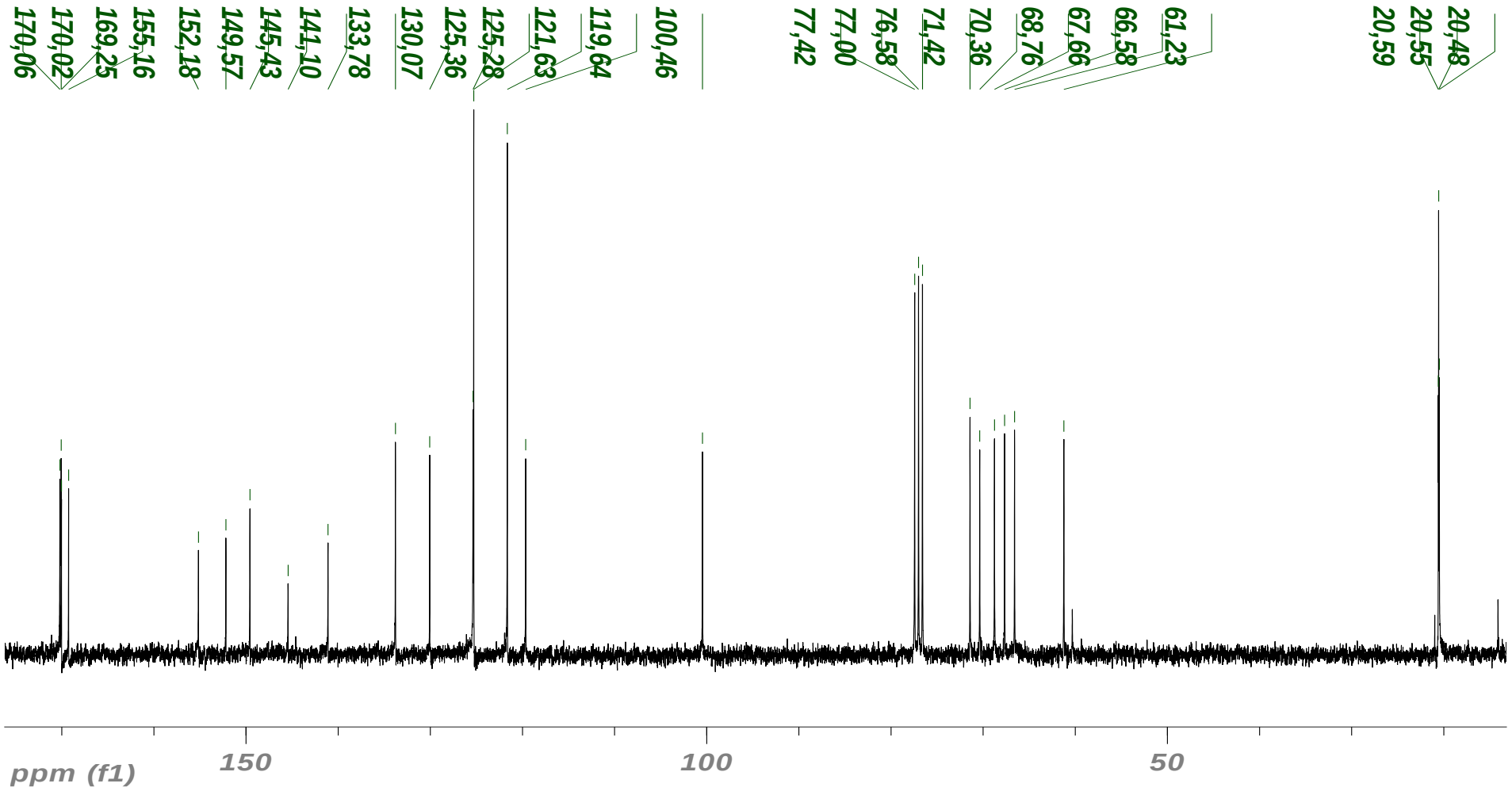

Fig. S7: ^{13}C NMR of **2,3,4,6-Tetra-*O*-acetyl-[2-nitro-4-(4-nitrophenoxycarbonyloxymethyl)phenyl]-*β*-D-galactopyranoside** (**16**).

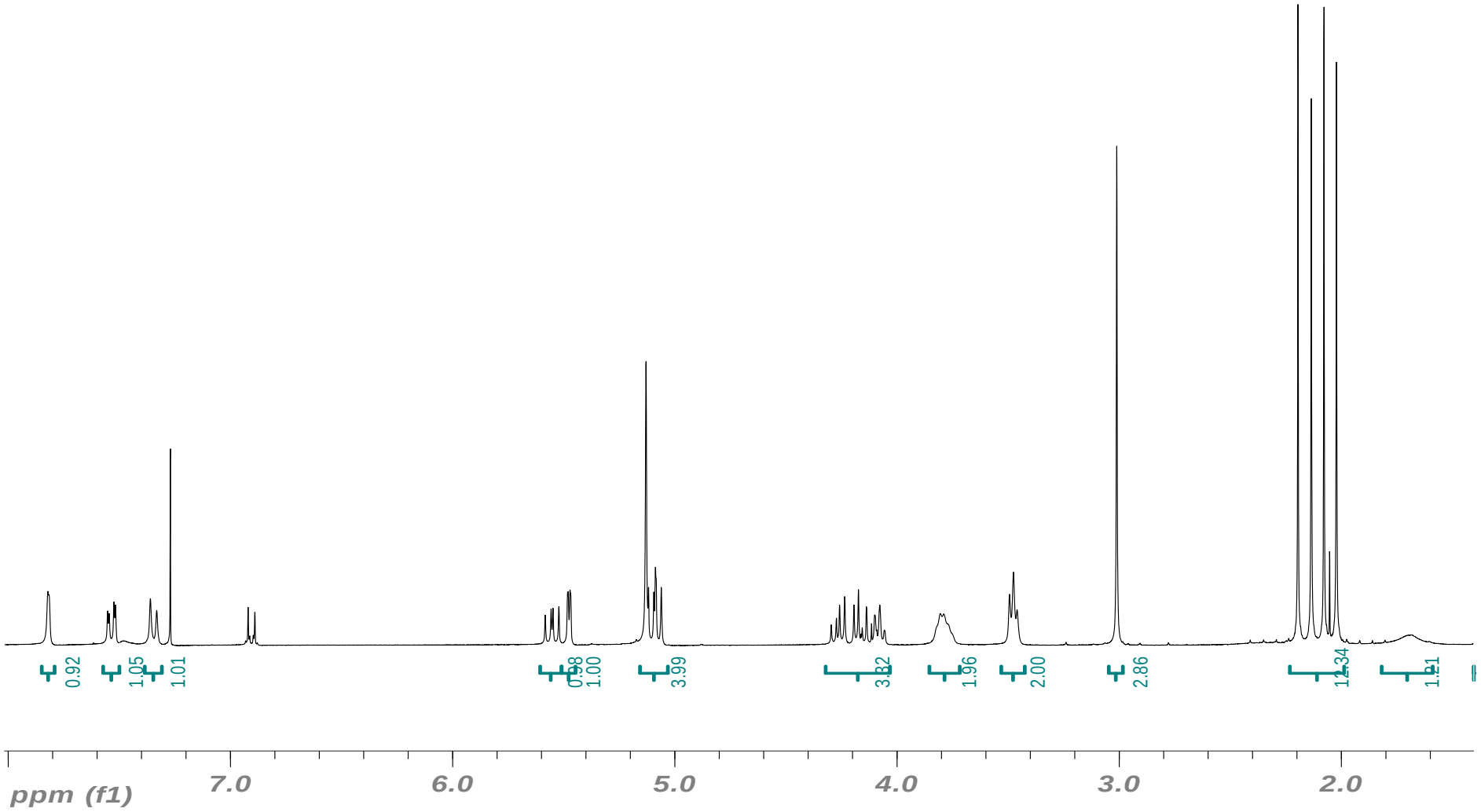

Fig. S8: ^{1}H NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-2-aminoethanol (19).**

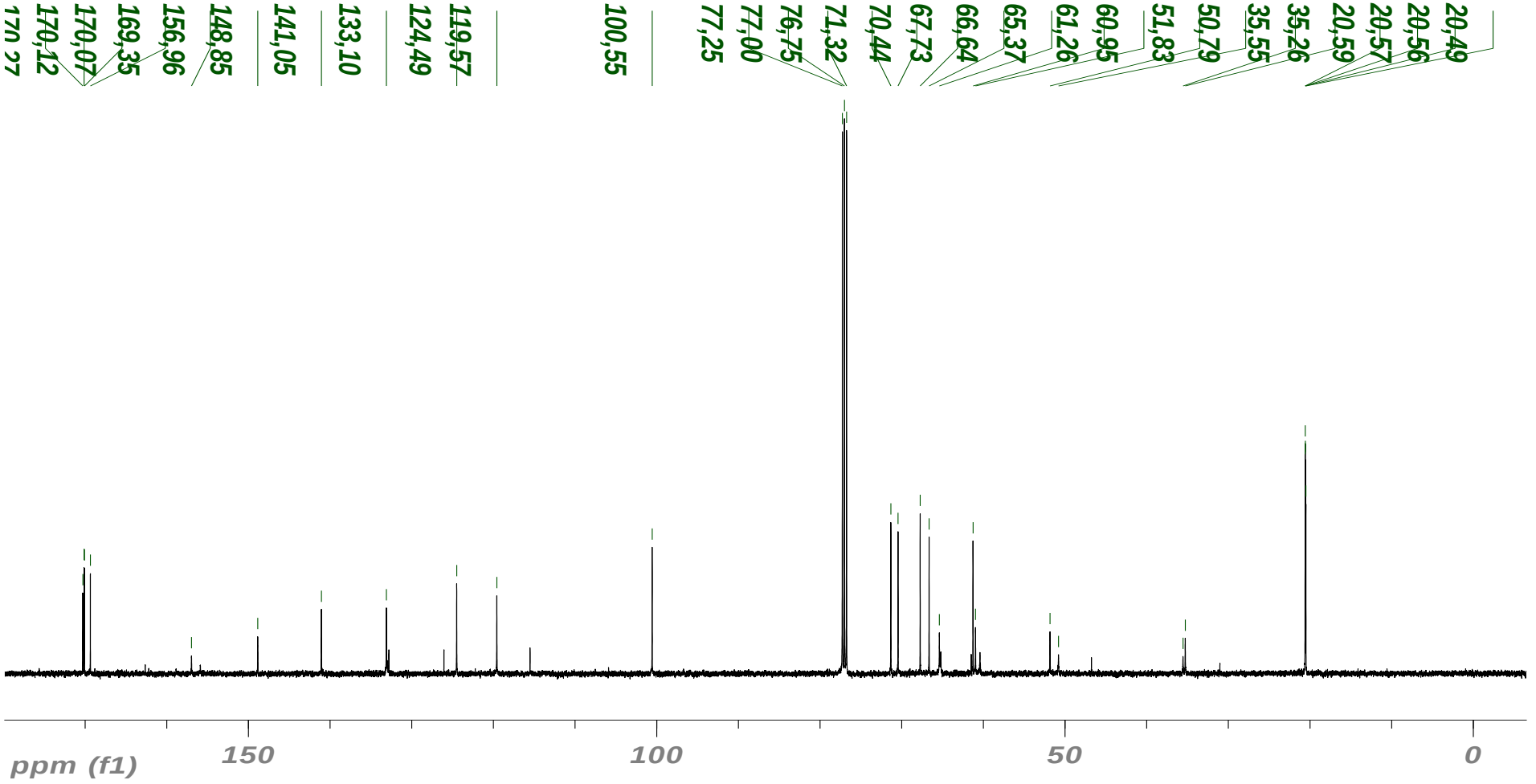

Fig. S9: ^{13}C NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-2-aminoethanol (19).**

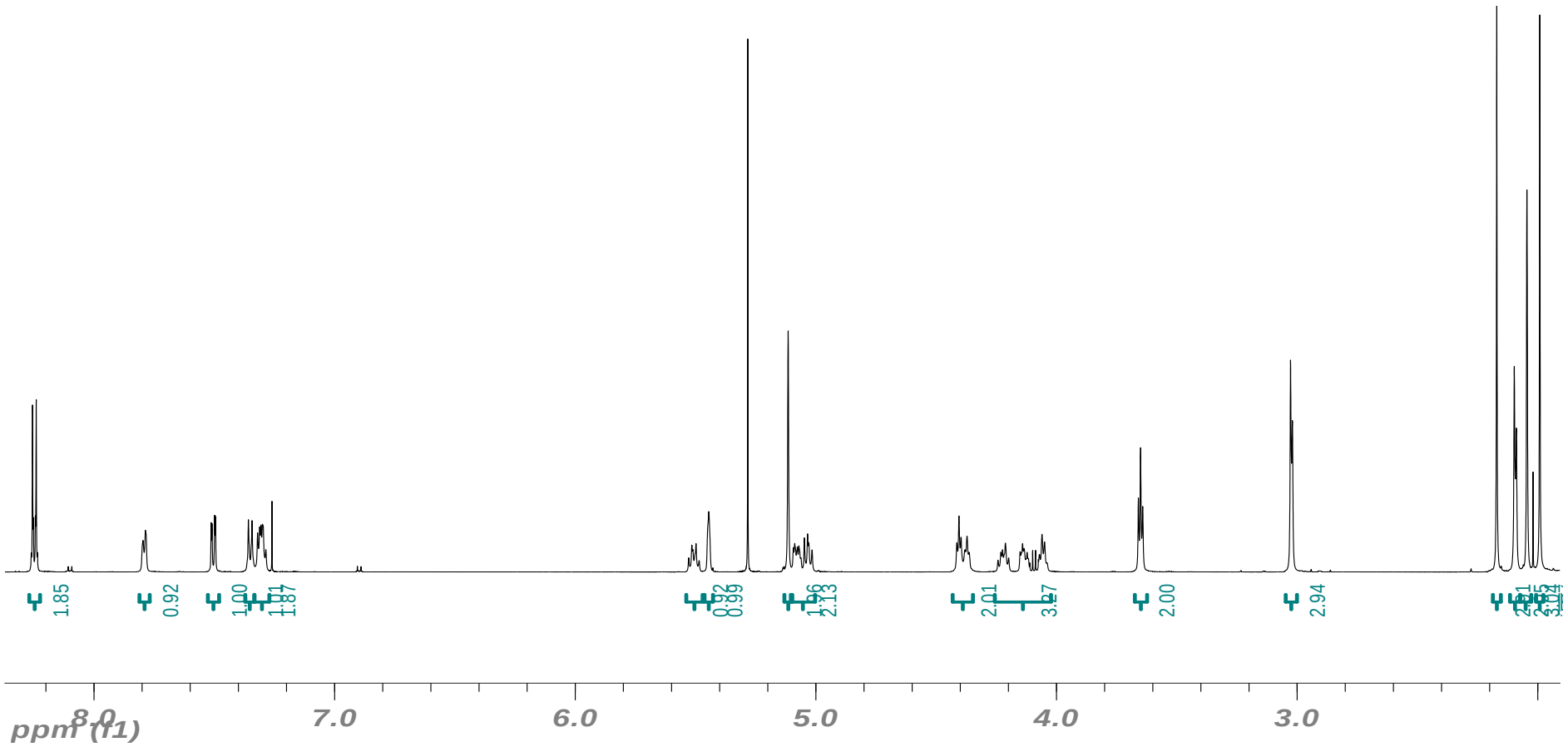

Fig. S10: ^{1}H NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-2-aminoethanol-carbonyl-4-nitrophenol (24).**

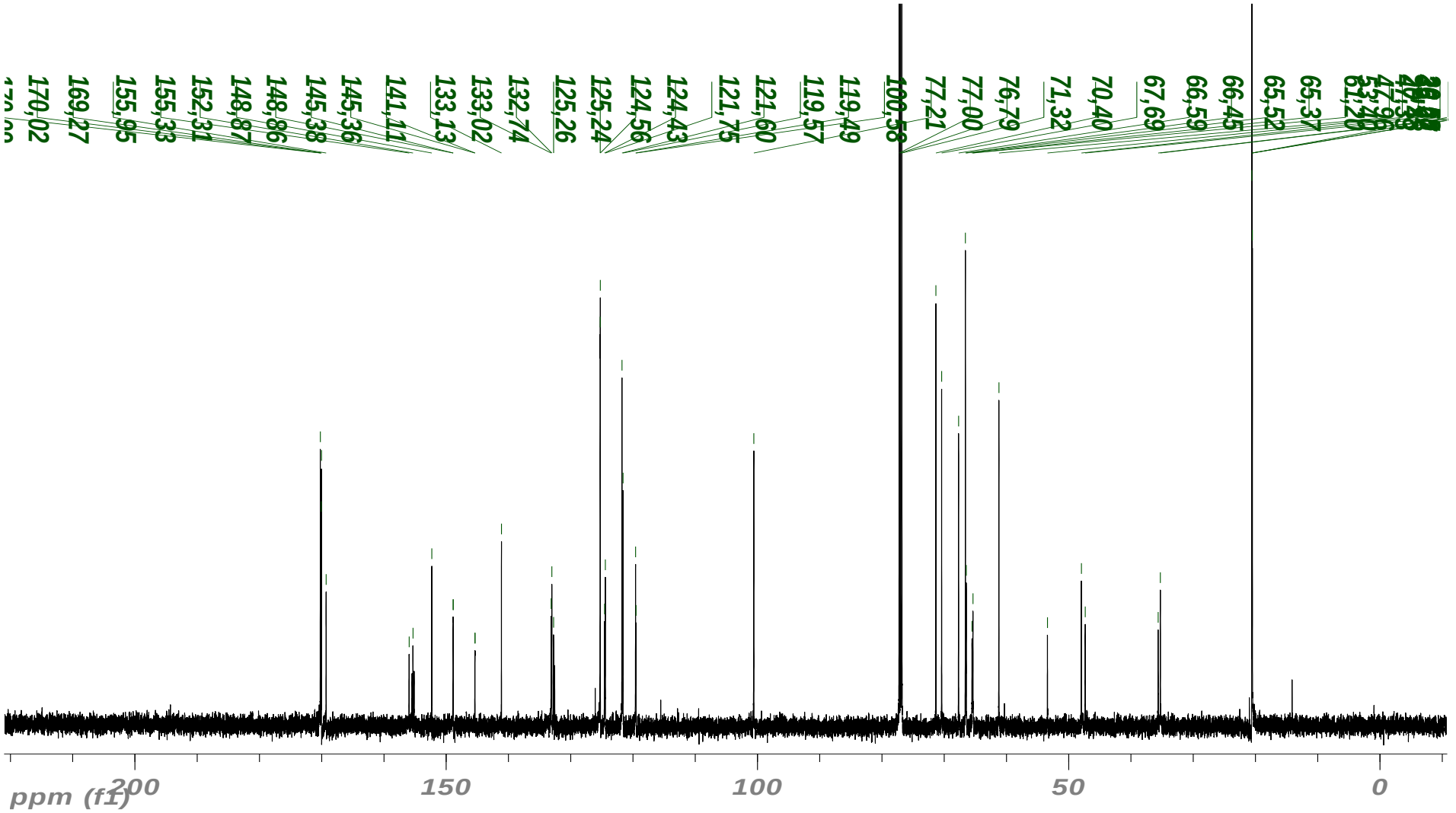

Fig. S11: ^{13}C NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-2-aminoethanol-carbonyl-4-nitrophenol (24).**

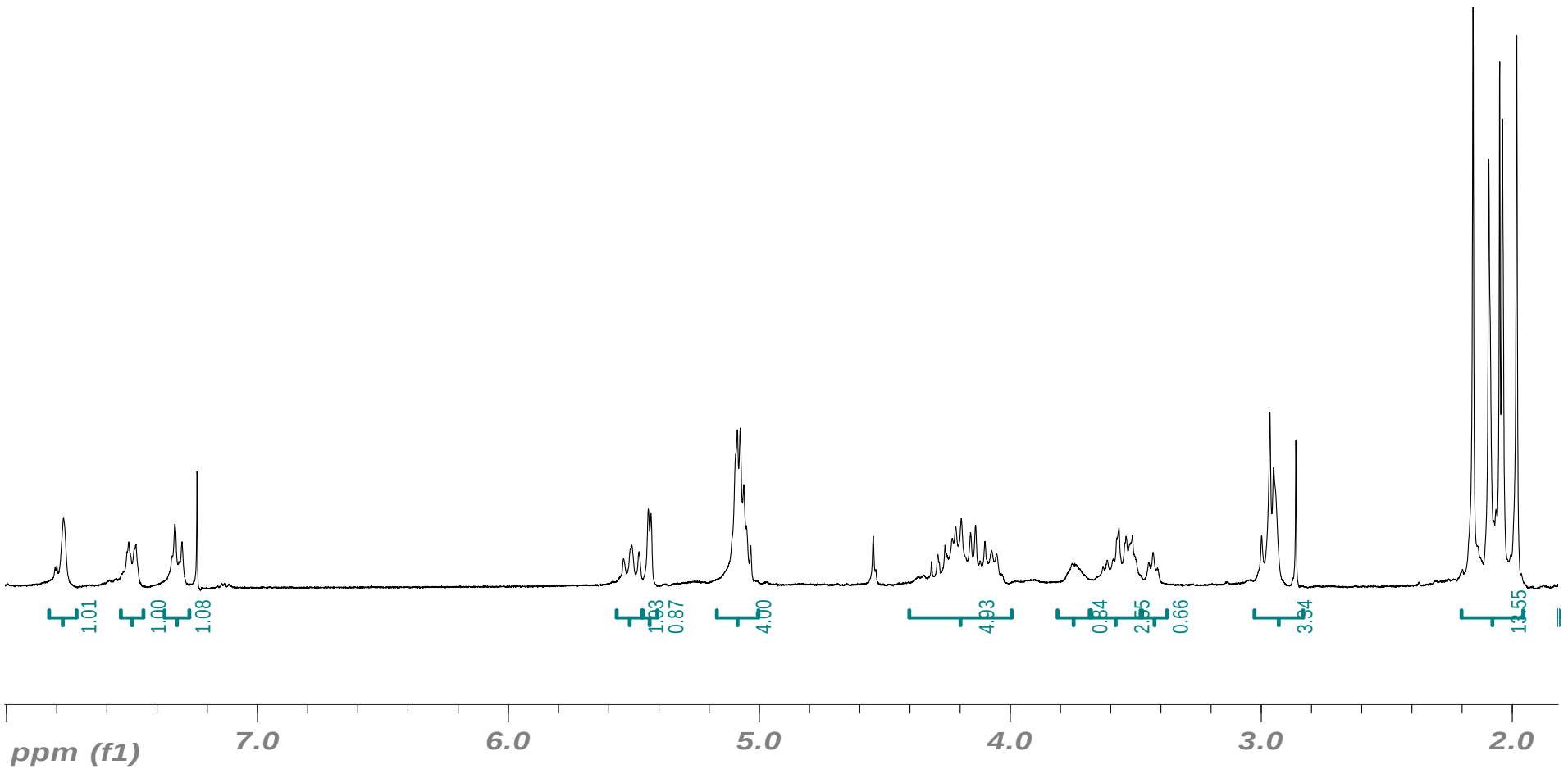

Fig. S12: ^{1}H NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-2-aminoethanol-carbonyl-chloride (22).**

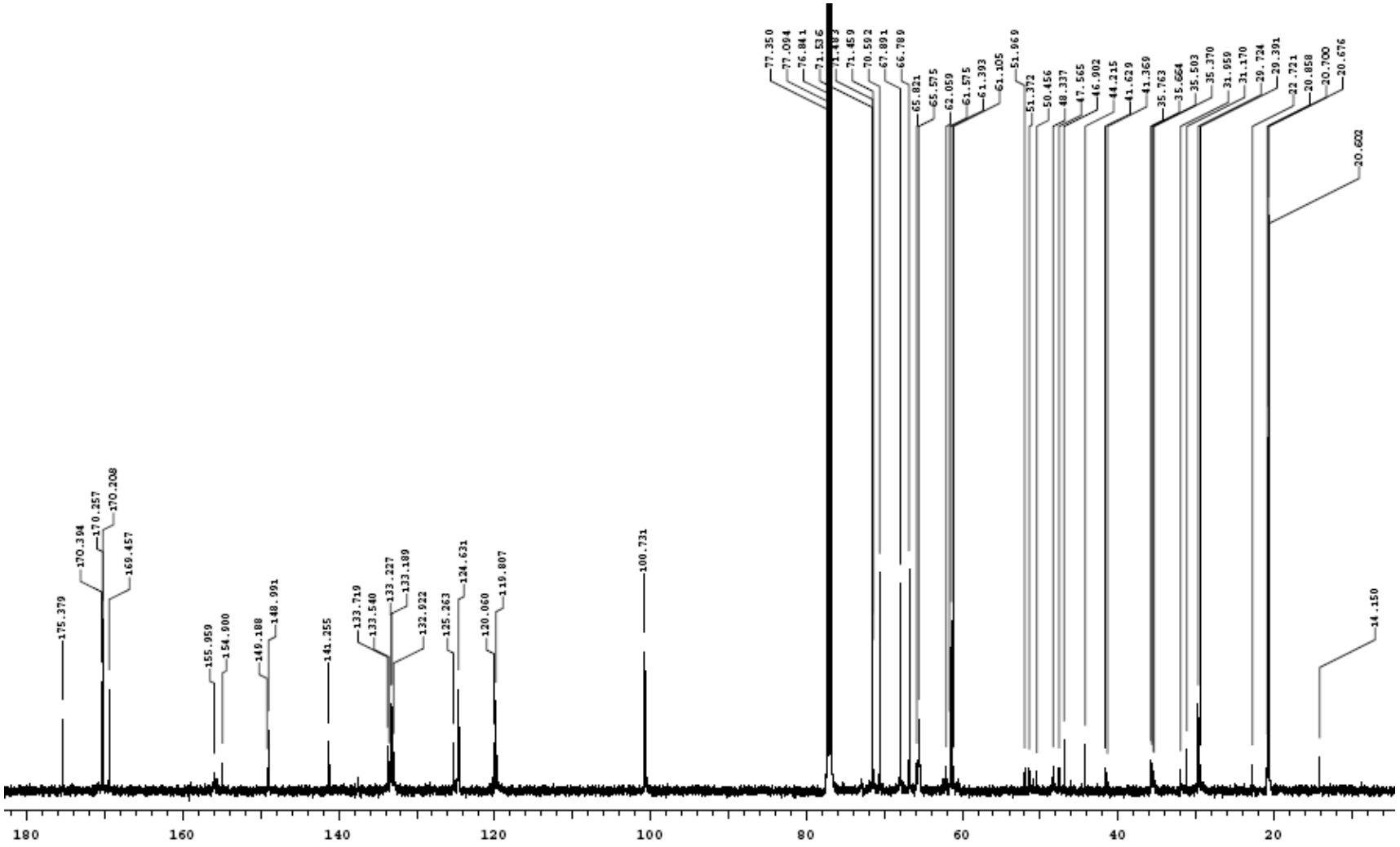

Fig. S13: ^{13}C NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-2-aminoethanol-carbonyl-chloride (22).**

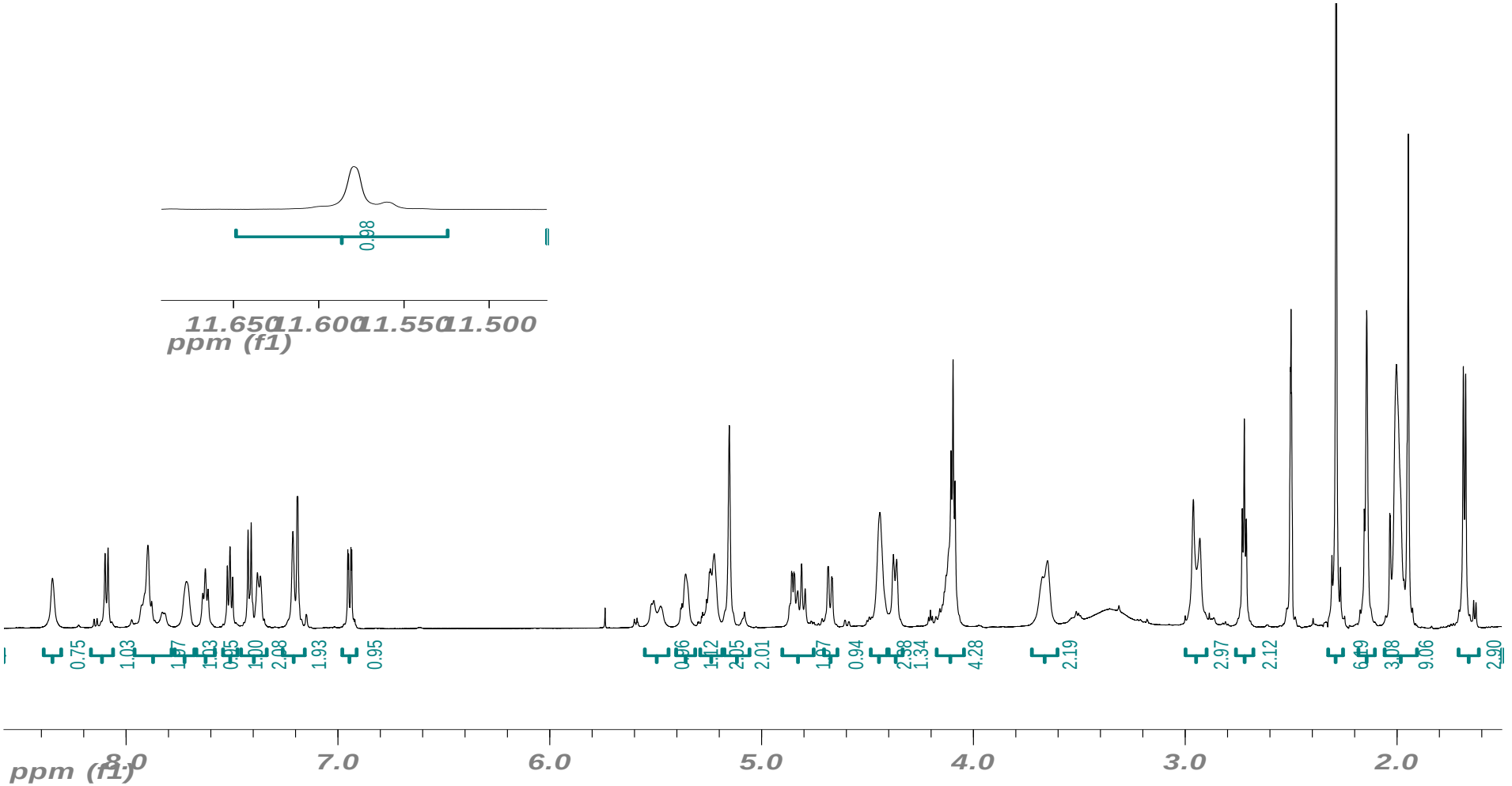

Fig. S14: ^{1}H NMR of **(+)-*N*,*N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-O-{{methyl-{(1*S*,10*R*)-1-(10-chloro-ethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)-indol-2-yl)carbonyl]-1,2-dihydro-3H-benz[*e*]indol-5-yl]}}-2-aminoethanol-carbonate ((1*S*,10*R*)-26)**.

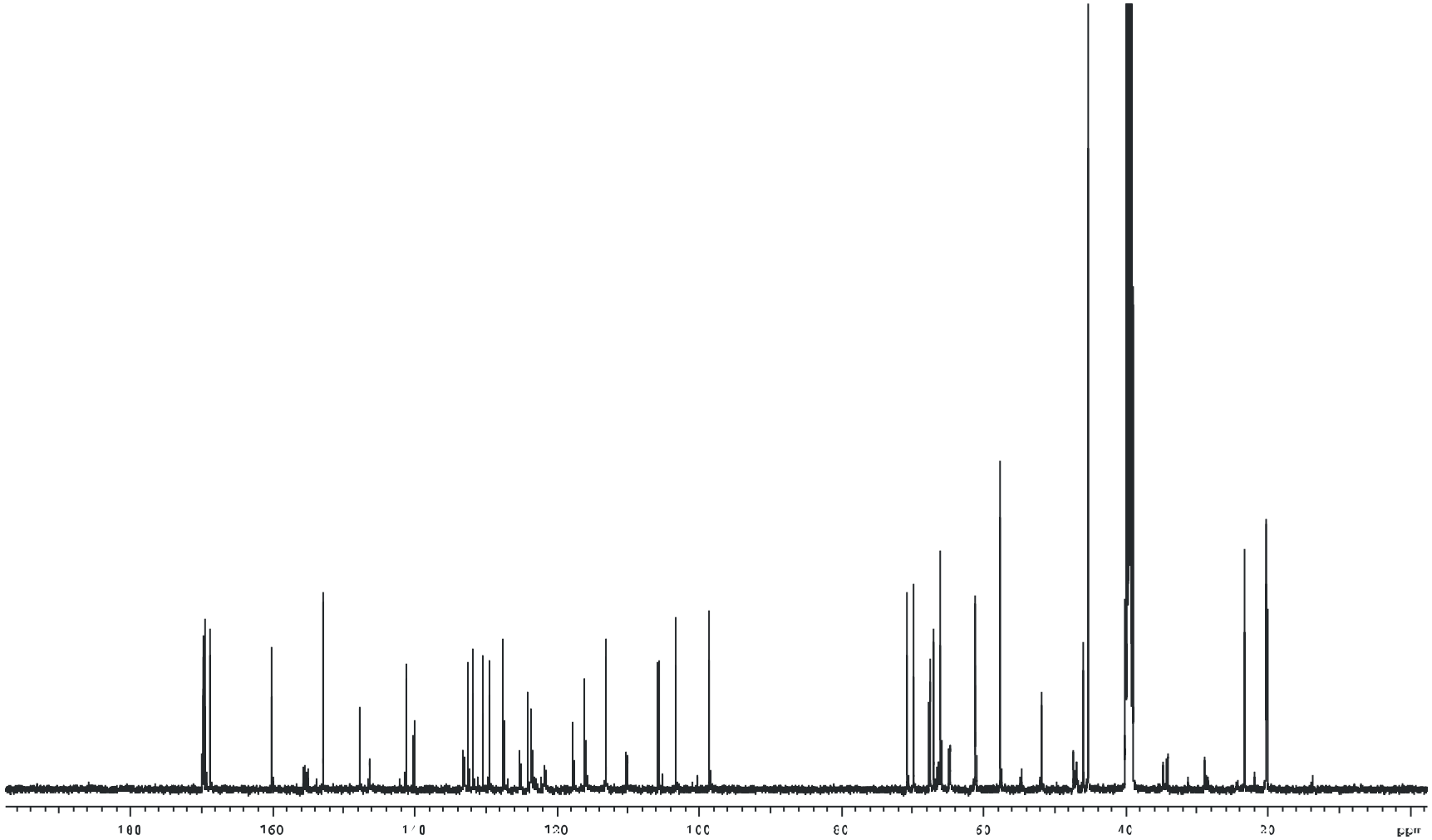

Fig. S15: ^{13}C NMR of **(+)-*N*,*N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-O-{{methyl-{(1*S*,10*R*)-1-(10-chloro-ethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)-indol-2-yl)carbonyl]-1,2-dihydro-3H-benz[*e*]indol-5-yl]}}-2-aminoethanol-carbonate ((1*S*,10*R*)-26)**.

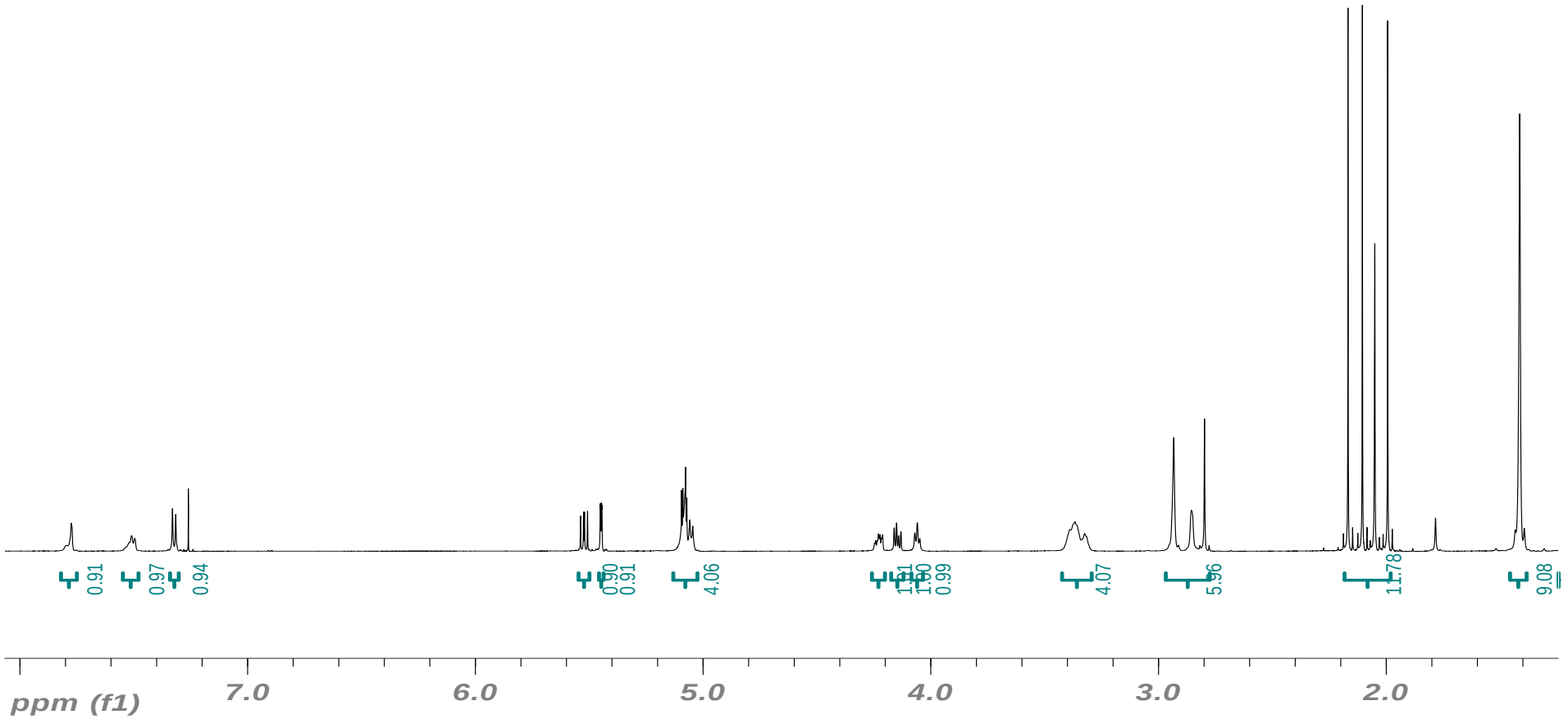

Fig. S16: ^{1}H NMR of ***N,N´*-Dimethyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-*N´*-(*tert*-butyloxycarbonyl)-ethylendiamine (20a).**

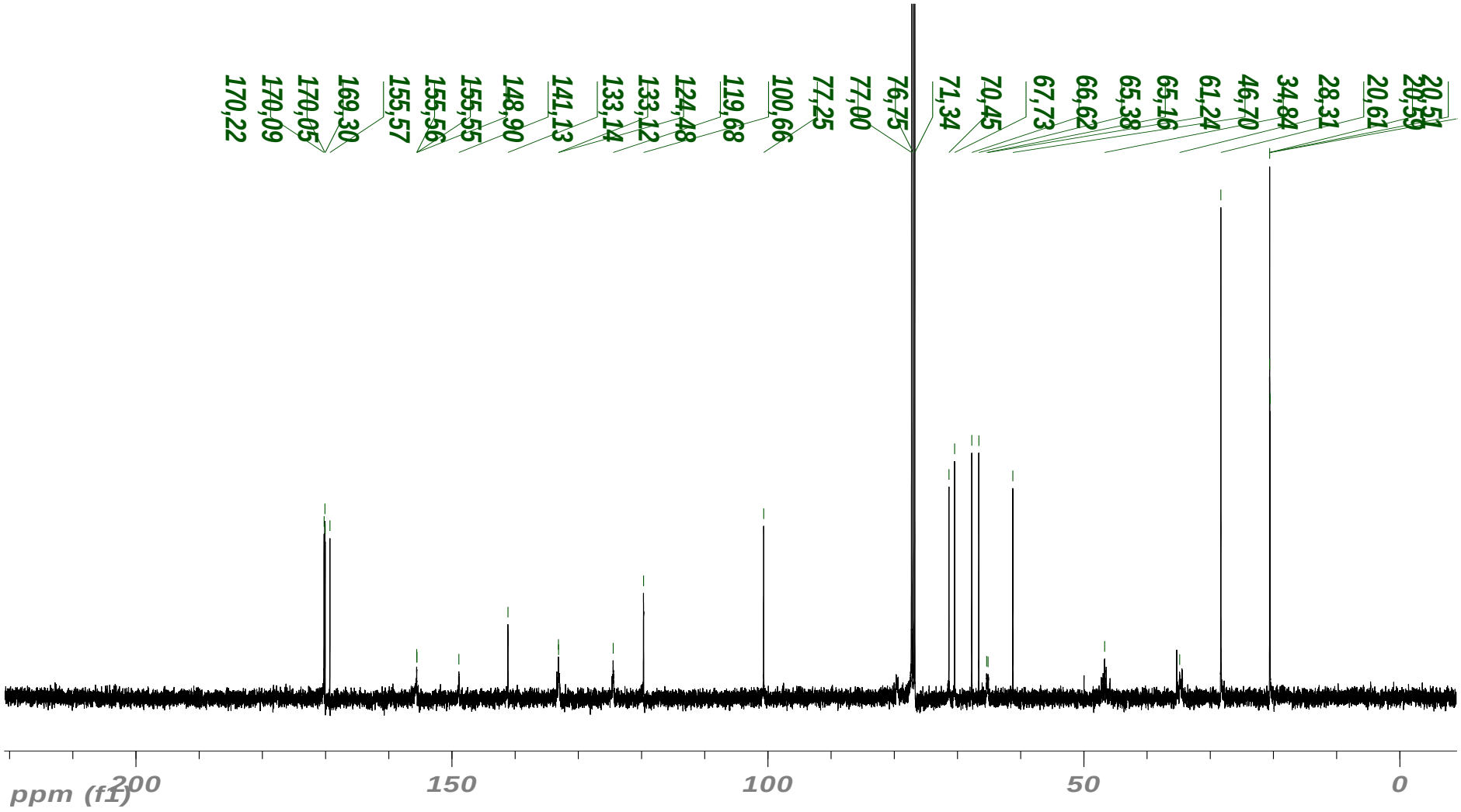

Fig. S17: ^{13}C NMR of ***N,N´*-Dimethyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-*N´*-(*tert*-butyloxycarbonyl)-ethylendiamine (20a).**

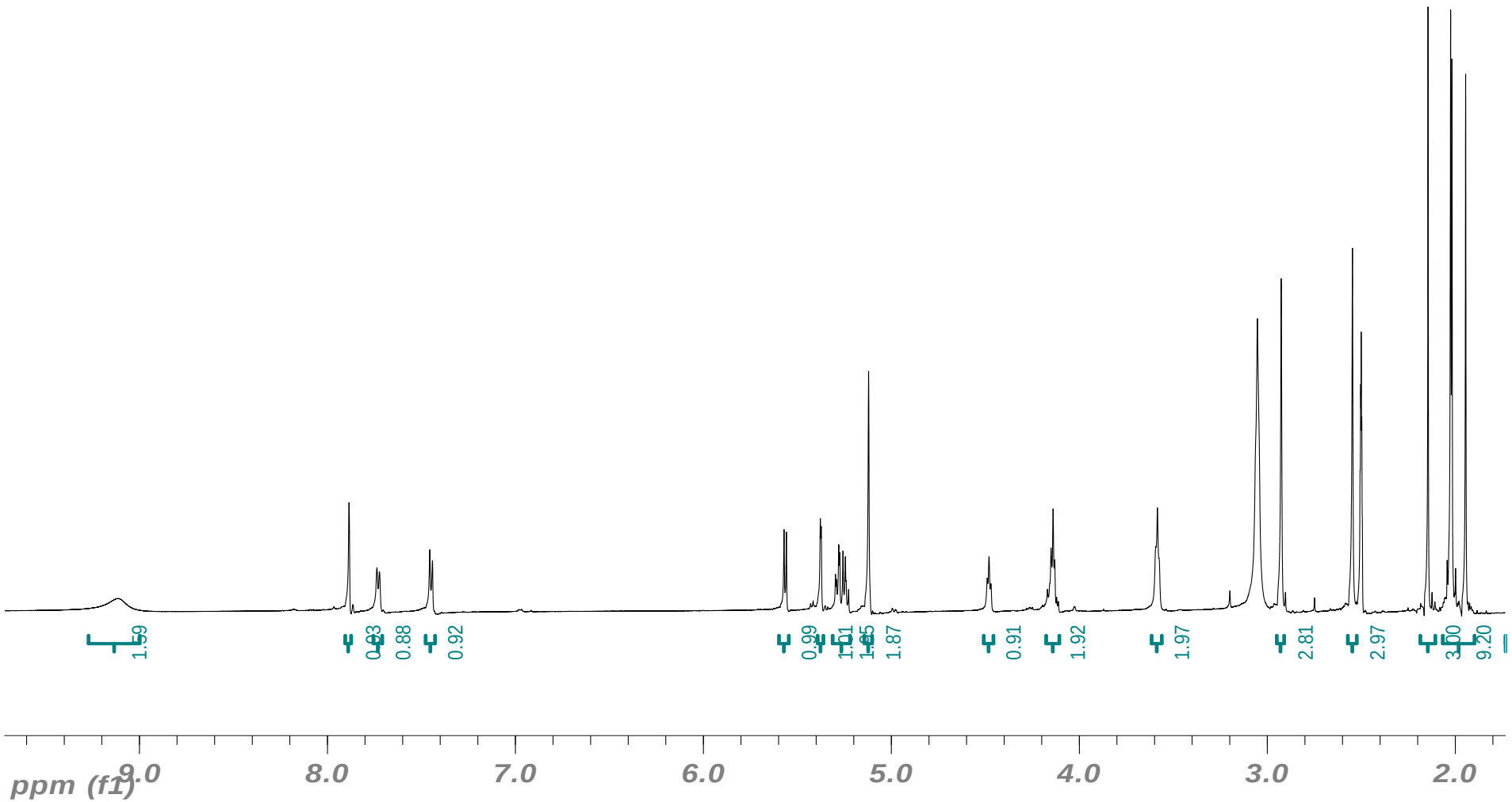

Fig. S18: ^{1}H NMR of ***N,N´*-Dimethyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-ethylendiamine-hydrochloride (20b).**

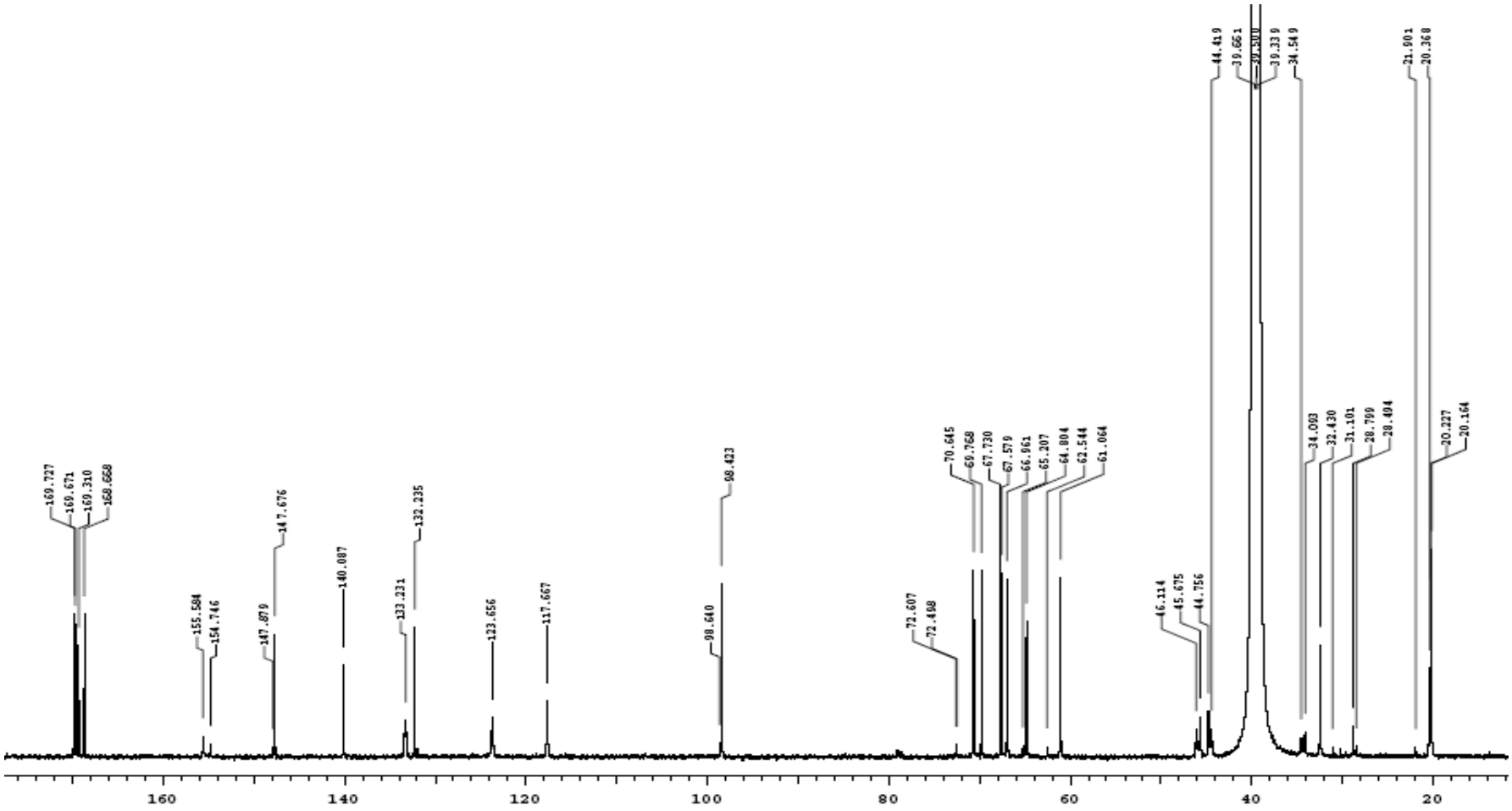

Fig. S19: ^{13}C NMR of ***N,N´*-Dimethyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-ethylendiamine-hydrochloride (20b).**

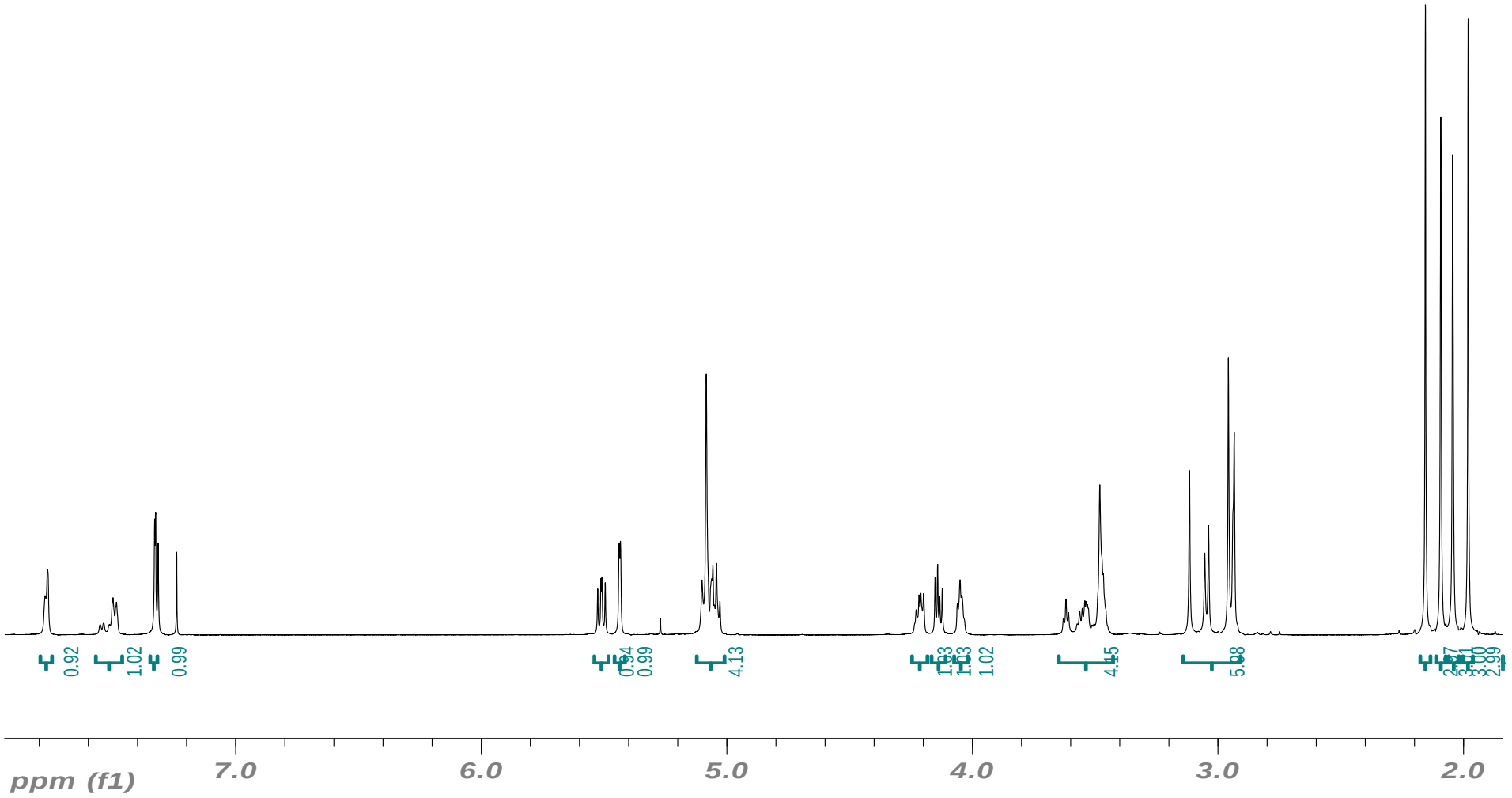

Fig. S20: ^{1}H NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-ethylendiamine-carbonyl-chloride (21)**

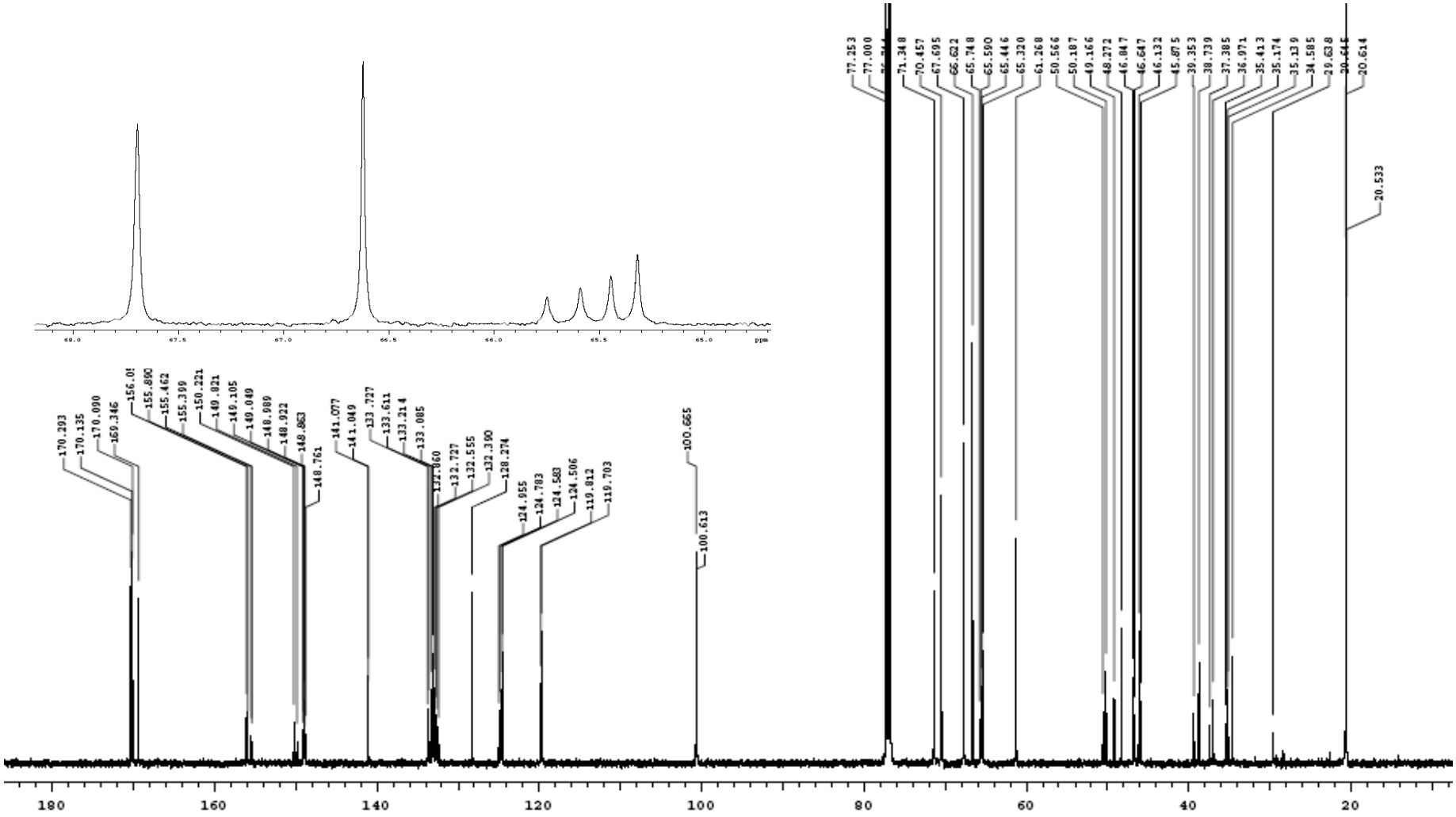

Fig. S21: ^{13}C NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-ethylendiamine-carbonyl-chloride (21)**

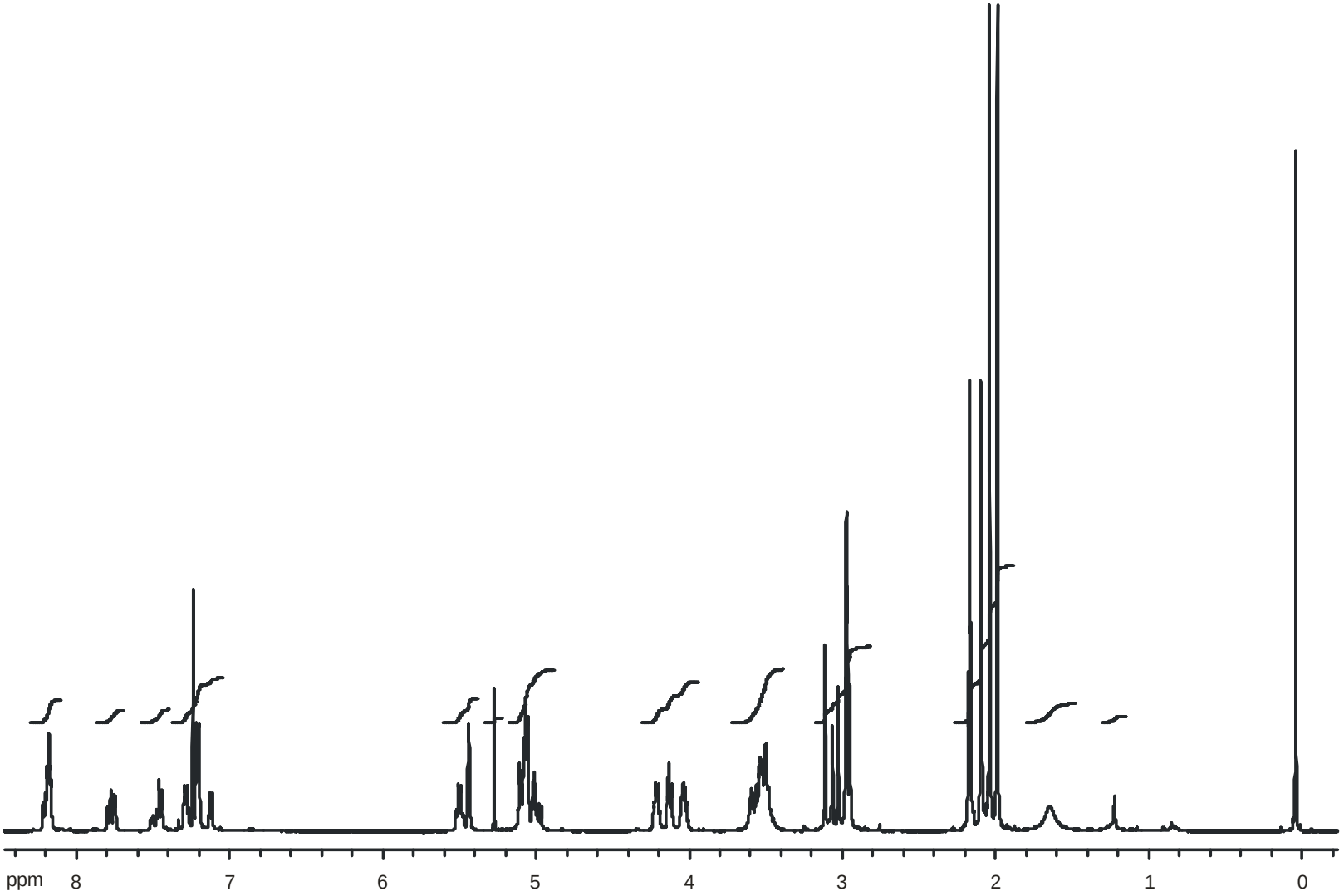

Fig. S22: ^{1}H NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-ethylendiamine-carbonyl-4-nitrophenol (23).**

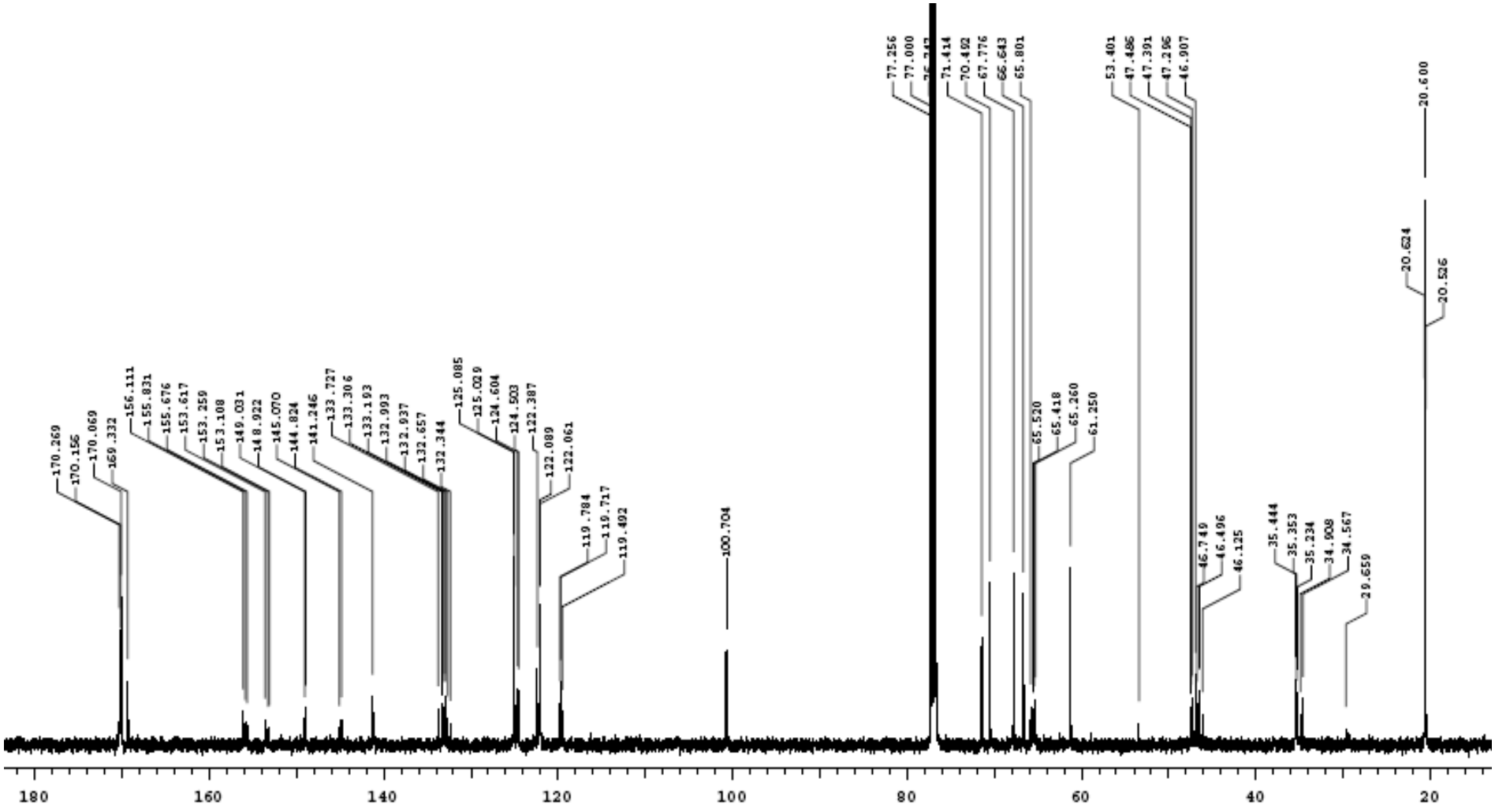

Fig. S23: ^{13}C NMR of ***N,N*-Methyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-ethylendiamine-carbonyl-4-nitrophenol (23).**

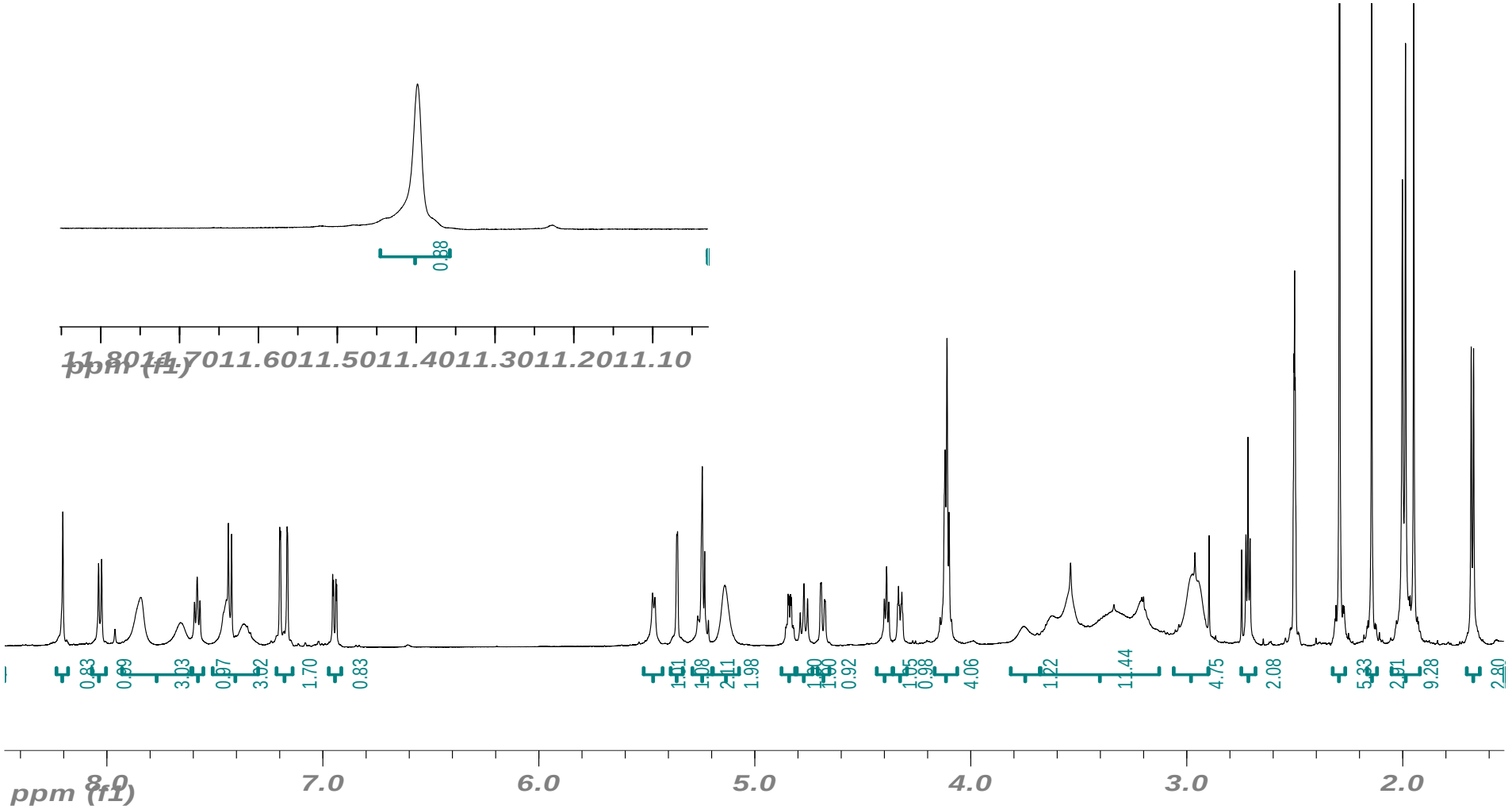

Fig. S24: ^{1}H NMR of **(+)-*N*,*N*´-Dimethyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-*O*-{{methyl-{(1*S*,10*R*)-1-(10-chloro-ethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)-indol-2-yl)carbonyl]-1,2-dihydro-3H-benz[*e*]indol-5-yl]}}-ethylendiamine carbamate ((1*S*,10*R*)-25).**

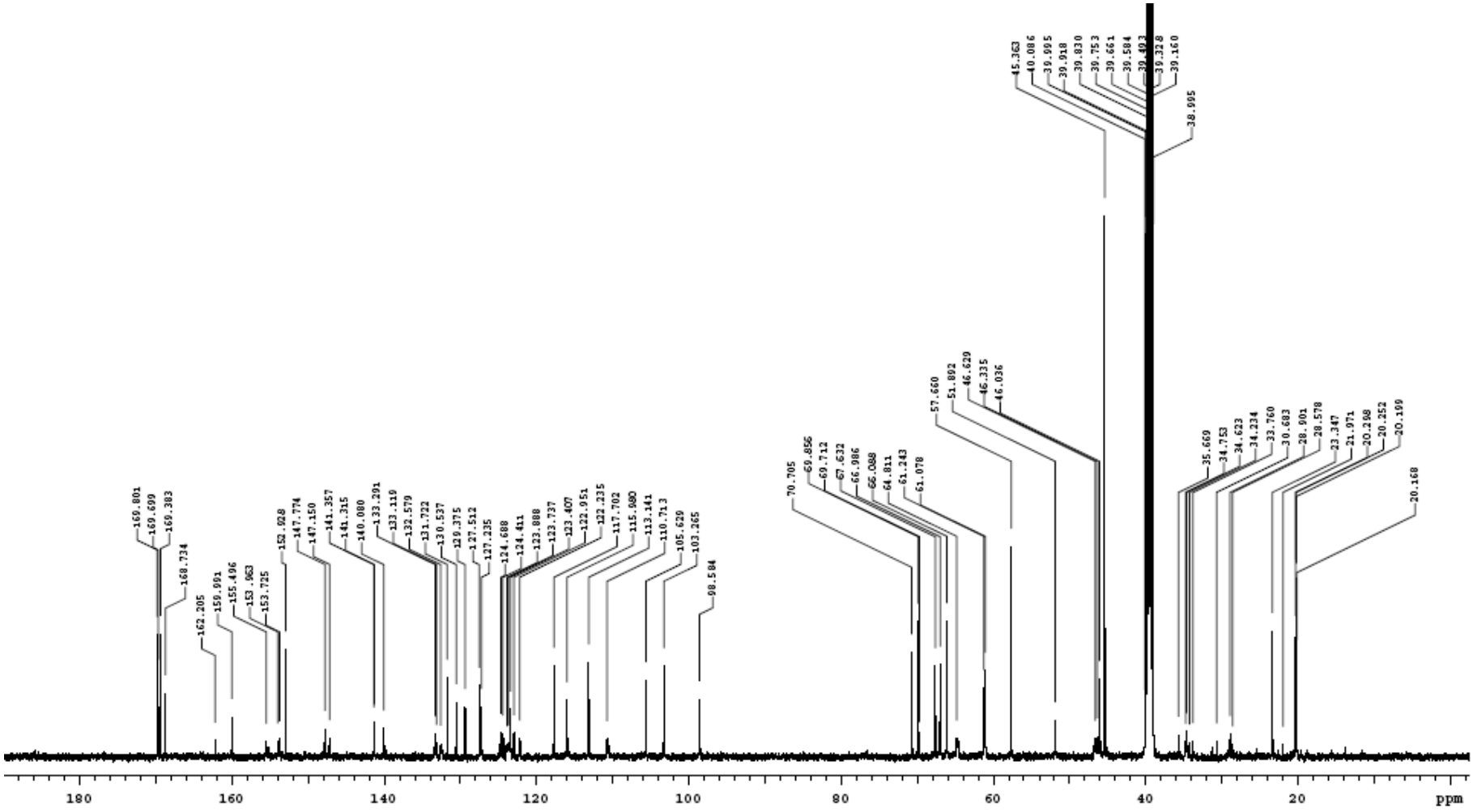

Fig. S25: ^{13}C NMR of **(+)-*N*,*N*´-Dimethyl-[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-*O*-{{methyl-{(1*S*,10*R*)-1-(10-chloro-ethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)-indol-2-yl)carbonyl]-1,2-dihydro-3H-benz[*e*]indol-5-yl]}}-ethylendiamine carbamate ((1*S*,10*R*)-25).**

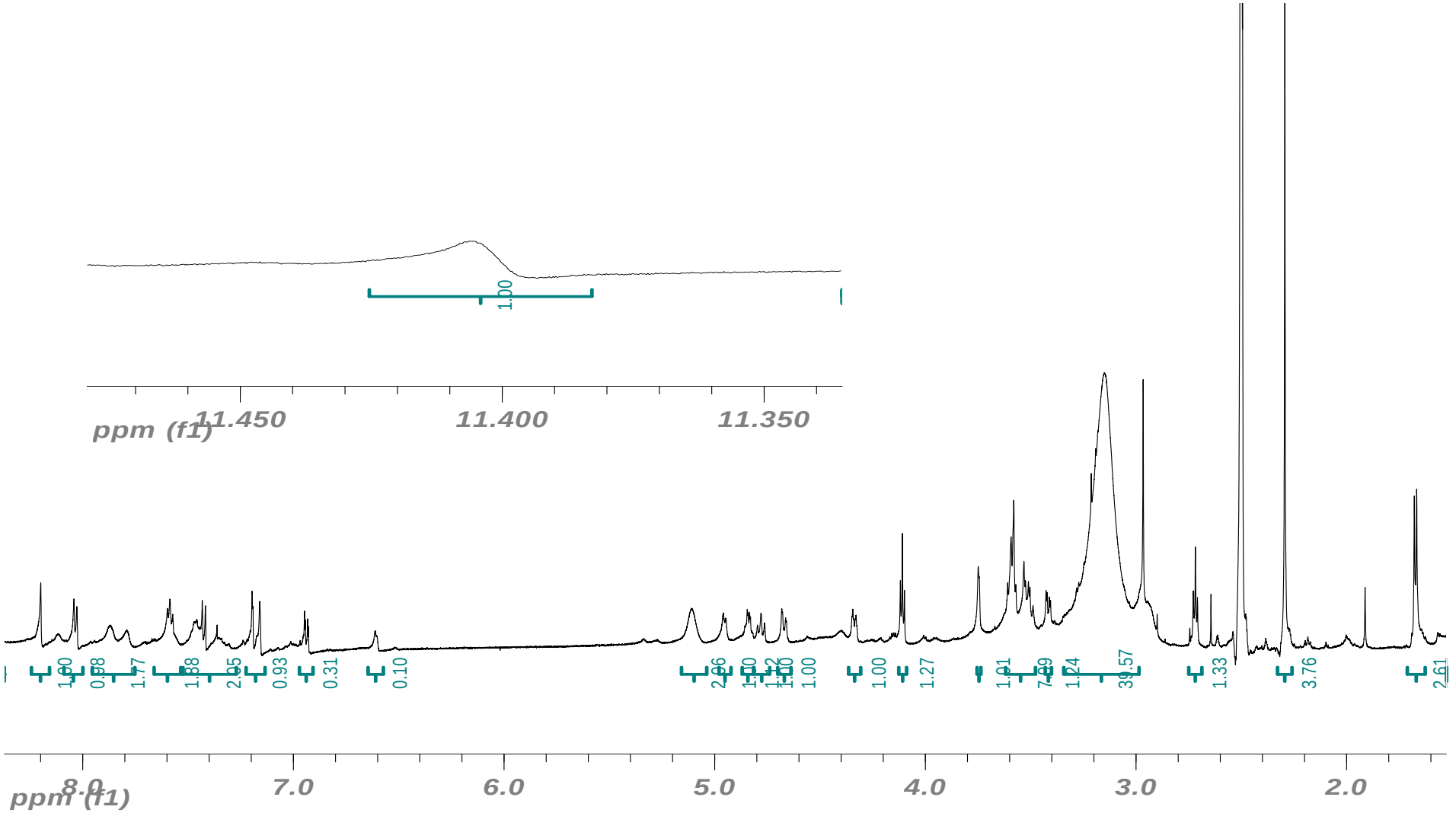

Fig. S26: ^{1}H NMR of **(+)-*N*,*N*´-Dimethyl-[4-(*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-*O*-{{methyl-{(1*S*,10*R*)-1-(10-chloro-ethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)-indol-2-yl)carbonyl]-1,2-dihydro-3H-benz[*e*]indol-5-yl]}}-ethylendiamine carbamate ((1*S*,10*R*)-11).**

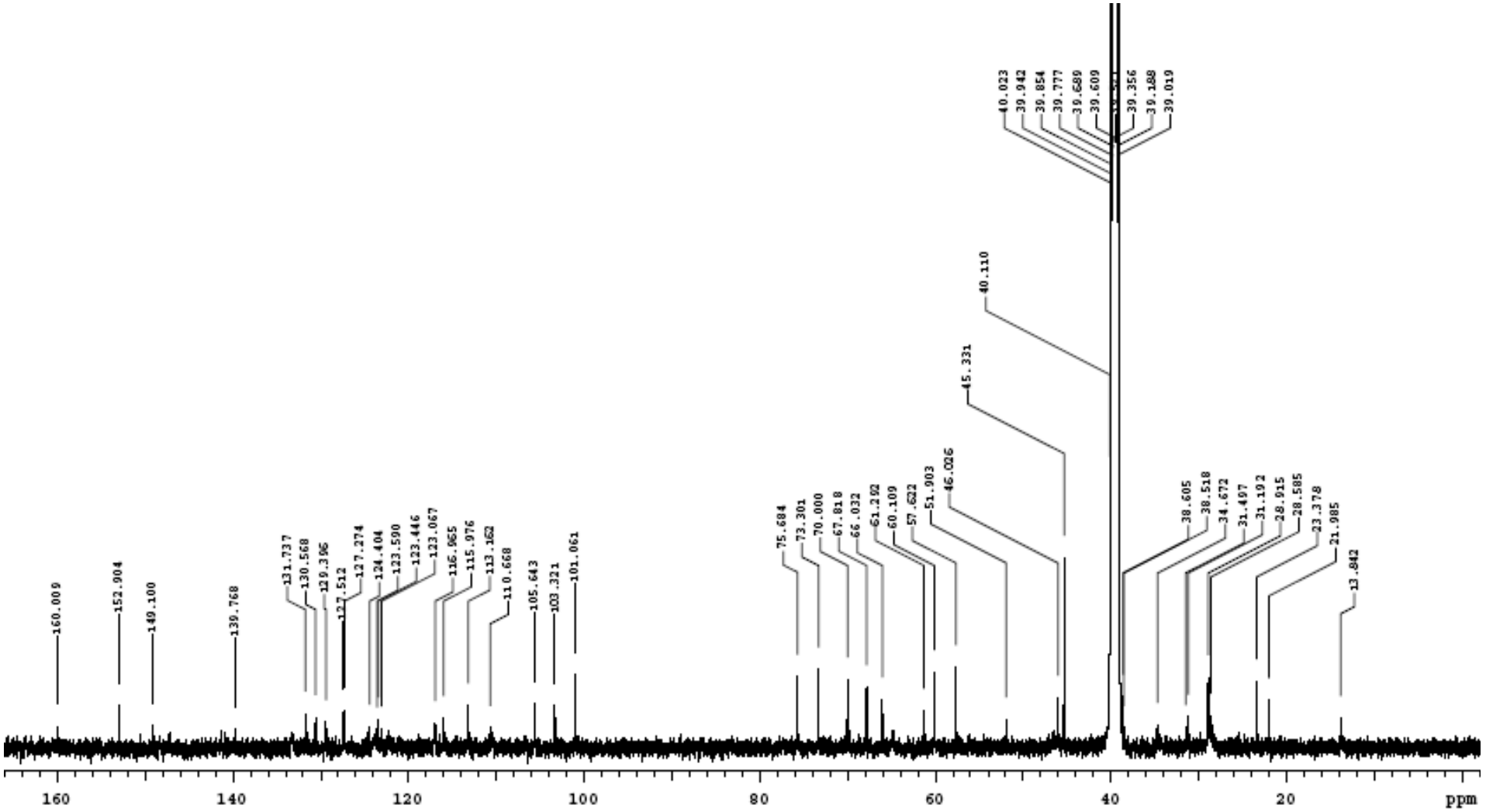

Fig. S27: ^{13}C NMR of **(+)-*N*,*N*´-Dimethyl-[4-(*β*-D-galactopyranosyl]-3-nitrobenzyl-oxycarbonyl]-*O*-{{methyl-{(1*S*,10*R*)-1-(10-chloro-ethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)-indol-2-yl)carbonyl]-1,2-dihydro-3H-benz[*e*]indol-5-yl]}}-ethylendiamine carbamate ((1*S*,10*R*)-11).**

HTCFA *in vitro* data results for the *β*-D-Galactose-diaminespacer (1*S*,10*R*)-11 on A549 cells:

without β-D-Galactosidase		with β-D-Galactosidase (4 U/mL)	
Concentration [nM]	Clone formation rate [%] pH = 7.4	Concentration [nM]	Clone formation rate [%] pH = 7.4
0	100	0	100
1.05	98.46	0.11	98.56
10.53	86.15	0.53	85.7
26.25	58.32	0.79	86.76
42.13	18.06	1.05	66.56
52.66	9.91	1.58	39.46
79.00	0.4	2.63	7.3
105.33	0.29	5.27	0.607
158.00	0.028	7.9	0.215
		10.53	0.014

The effective dosis (IC_{50}) of the prodrug (1*S*,10*R*)-**11** is 29 nM without β-D-Galactosidase and 1.3 nM in the presence of β-D-Galactosidase.

Graph of HTCFA *in vitro* results of the *β*-D-Galactose-diaminespacer (1*S*,10*R*)-11: