Supporting Information for:

# Carbohydrate bis-acetal-based substrates as tunable fluorescence-quenched probes for monitoring *exo*-glycosidase activity

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## Methods

### General chemistry methods

Unless stated otherwise, chemical reactions were performed under an Argon atmosphere. Glassware was oven-dried or flame-dried under vacuum. Anhydrous solvents were used for reactions (either commercial or dried and distilled following standard procedures) and reagent grade solvents were used for workup and purification. Reactions were monitored using thin-layer chromatography (TLC) on pre-coated aluminum sheets (ALUGRAM Xtra SIL G/UV254, Macherey-Nagel). After elution, TLC plates were inspected under UV light and developed by treatment with sulfuric acid stain (10% $H_2SO_4$ in EtOH/$H_2O$), Hanessian's stain (Cerium Ammonium Molybdate), $KMnO_4$ stain or Orcinol stain. Silica gel column chromatography was performed under positive pressure using 230 – 400 Mesh silica (grade 60, Fischer Scientific). HPLC (Agilent 1100 series) was performed using Eclipse XDB-C18 columns (3.5 μm, 3.0×150 mm for analytical runs and 5.0 μm, 9.4×250 mm for semi-preparative scale purifications) using HPLC grade solvents. NMR spectra were recorded at 293 K, using either Bruker AVANCE III (400 or 500 MHz) or Bruker AVANCE II 600 MHz (TCI or QNP cryoprobes) spectrometers. Chemical shifts (ppm) were reported relative to deuterated solvents (Cambridge Isotope Laboratories Inc.) residual peaks. Abbreviations used to describe the observed peaks: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet and bs, broad singlet. Complete signal attribution was based on 1D and 2D NMR (COSY, HSQC and HMBC). High resolution mass spectra were recorded using a Bruker MicrOTOF or a Bruker maXis Impact spectrometer using positive or negative electrospray ionization (ESI).

### Fluorescence Measurements

Fluorescence kinetic experiments were performed using a Varian Cary Eclipse Spectrophotometer set for 335/493 nm excitation/emission wavelengths. Slits were set at 10 nm and the detector was set on Medium voltage. Enzymatic reactions were carried out in PBS at 37°C and were started by addition of hOGA (WT, Full-length, expressed in *E. coli.* as previously described) in a total volume of 160 μL (Starna sub-micro Fluorometer cuvette). For measurements of rates using different concentrations of substrates, a hOGA concentration of 100 nM was used (Figure 2b). For hOGA titration (Figure 2c), 25 μM of substrates were added to various concentration of hOGA.. For stopped assay, reaction was started by addition of hOGA (680 nM final) in solutions of GlcNAc-BABS in PBS. Fluorescence acquisition was paused after 1 minute and a solution of Thiamet-G in PBS (2 mM) was quickly added (final concentration of 100 μM) and data acquisition was immediately resumed. For hemiacetal breakdown rate measurements, data after Thiamet-G addition was extracted and fitted using Prism software. EDANS standard curve was established using endpoint fluorescence measurements of serial dilutions of EDANS in the relevant buffer using the same instrument parameters. Inner-Filter effect measurements were carried out in cuvette by measuring fluorescence of several concentrations of EDANS in presence of various concentrations of GlcNAc-BABS. pH stability, quenching efficiency, additional inner-filter effect experiments and lysates experiments were carried out using a Molecular Devices SpectraMax i3x plate reader. Emission/Excitation wavelengths were set at 350 and 490 nm respectively. Fluorescence measurements were performed using Costar 96-well black plates or Nunc 384-well black plates after sample preparation in 96-well mixing plates. Data were plotted and fitted using Prism software version 5.03. For cell lysates experiments, SK-N-SH cells were cultured in EMEM media supplemented with 10% FBS and Penicillin/Streptomycin antibiotics. Cells were collected, washed with PBS and lyzed using ice-cold NP-40 lysis buffer (TRIS-HCl 50 mM pH 7.4, NP-40 1%, NaCl 150 mM). After centrifugation, the supernatant was diluted in PBS. The quantity of lysate used in each measurement well (384-well plate) was equivalent to 25000 cells. Various concentration of hOGA were added in the presence of inhibitor Thiamet-G (200 μM) or vehicle (DMSO). Reactions were started by adding the substrate at a final concentration of 20 μM. This experiment was carried out in quadruplicates and the rates were determined by establishing a standard curve for EDANS fluorescence in the SK-N-SH lysate.

**Single Crystal X-ray Diffraction Structure Determinations**

Single crystals were obtained by vapor diffusion (DCM/$Et_2O$). Single crystal X-ray crystallographic analysis of compounds **4** and **5** was performed on a Bruker SMART diffractometer equipped with an APEX II CCD detector and IμSCuKα (λ = 1.54184 nm) microfocus sealed X-ray tube fitted with HELIOS multilayer optics. Colourless crystals were mounted on MiTeGen dual-thickness MicroMounts using paratone oil. The data was collected at 150(2) K via an Oxford Cryosystems cold-stream. Data was collected in a series of φ and ω scans with 0.80° image widths and 10, 20 or 30 second exposures. The crystal-to-detector distance was 40 mm. Data was processed using the Bruker APEX II software suite. All structures were solved with intrinsic phasing method,[1] and subsequent refinements were performed using ShelXL[2] within SHELXle.[3] All non-hydrogen atoms were refined anisotropically. All C–H hydrogen atoms were placed in geometrically calculated positions without further refinement. Disorder in the structure of compound **5** was modelled using PART commands in conjunction with EADP and SADI commands. All crystal structure plots were produced using ORTEP-3 (Ellipsoids at 30%).[4] CCDC numbers 1530648 and 1530649 were obtained for the two structures. A summary of the crystal data and experimental parameters for structure determinations are given in **Table S1**.

**Table S1** Crystal Data and experimental parameters

| **Compound reference** | SCD56 **(4)** | SCB118 **(5)** |
|---|---|---|
| Chemical formula | $C_{20}H_{31}NO_{13}$ | $C_{20}H_{30}BrNO_{12}$ |
| Formula Mass | 493.46 | 556.36 |
| Crystal system | Monoclinic | Orthorhombic |
| *a*/Å | 9.2809(10) | 9.1328(5) |
| *b*/Å | 24.198(3) | 22.1807(12) |
| *c*/Å | 11.1739(7) | 26.2782(14) |
| *α*/° | 90 | 90 |
| *β*/° | 104.854(4) | 90 |
| *γ*/° | 90 | 90 |
| Unit cell volume/Å$^3$ | 2425.6(4) | 5323.2(5) |
| Temperature/K | 150(2) | 150(2) |
| Space group | *P*21 | *P*212121 |
| No. of formula units per unit cell, *Z* | 4 | 8 |
| Radiation type | CuKα | CuKα |
| Absorption coefficient, $\mu$/mm$^{-1}$ | 0.980 | 2.621 |
| No. of reflections measured | 30501 | 60237 |
| No. of independent reflections | 8686 | 9403 |
| $R_{int}$ | 0.0412 | 0.0717 |
| Final $R_1$ values ($I > 2\sigma(I)$) | 0.0287 | 0.0800 |
| Final $wR(F^2)$ values ($I > 2\sigma(I)$) | 0.0726 | 0.2241 |
| Final $R_1$ values (all data) | 0.0300 | 0.0855 |
| Final $wR(F^2)$ values (all data) | 0.0736 | 0.2309 |
| Goodness of fit on $F^2$ | 1.034 | 1.147 |
| Flack parameter | 0.05(5) | 0.021(8) |

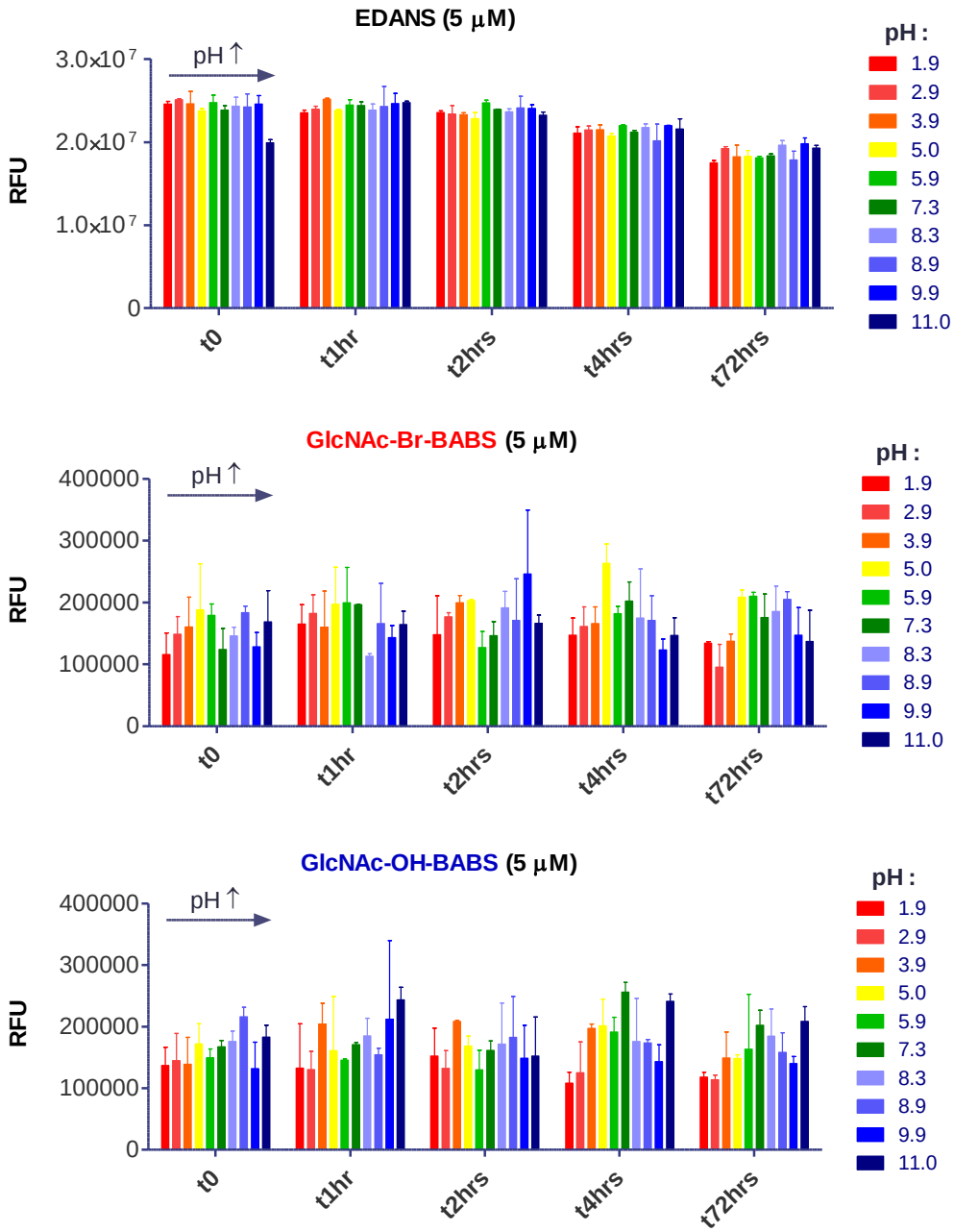



**Figure S1. GlcNAc-BABS substrates are stable over a wide range of pH.** To test the pH stability of GlcNAc-BABS, we prepared solutions at various pH by mixing 0.2 M KCl + 0.1 M HCl with 0.2 M KCl + 0.1 M KOH. Fluorescence of EDANS and GlcNAc-BABS solutions (5 μM, 45 μL) at different pH was measured at different time points in Nunc 384-well plates using a SpectraMax i3x plate reader. Data (triplicates) support the highly efficient quenching and the stability of these substrates over a wide range of pH. No significant increase of fluorescence was observed for GlcNAc-BABS even after 72 hours at pH as low a 2 and as high a 11.

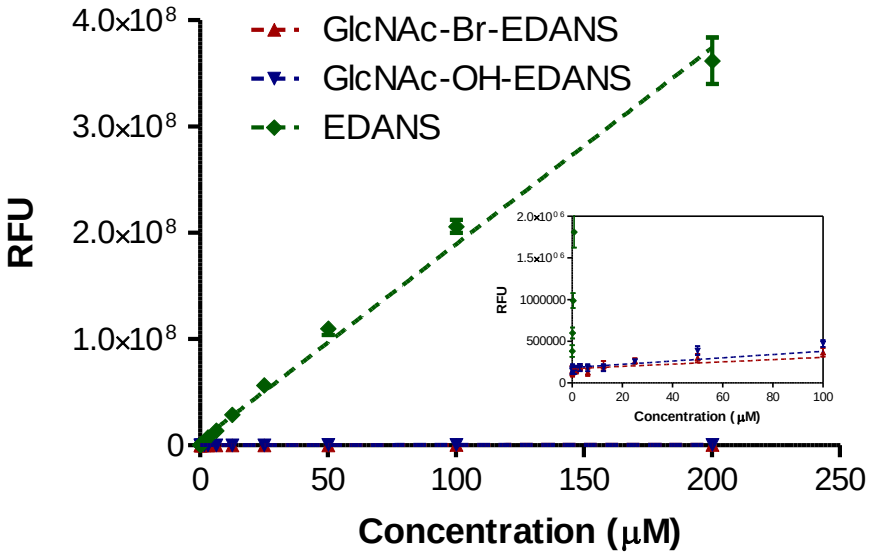



**Figure S2. GlcNAc-BABS show excellent fluorescence quenching.** Quenching efficiency was determined by measuring the residual fluorescence of different concentration of GlcNAc-BABS in PBS (45 μL) and comparing it with the fluorescence of equivalent concentrations of EDANS. Fluorescence was measured in quadruplicates (384-well plates) using a SpectraMax i3x plate reader. The quenching efficiency was measured as the ratio of slopes.

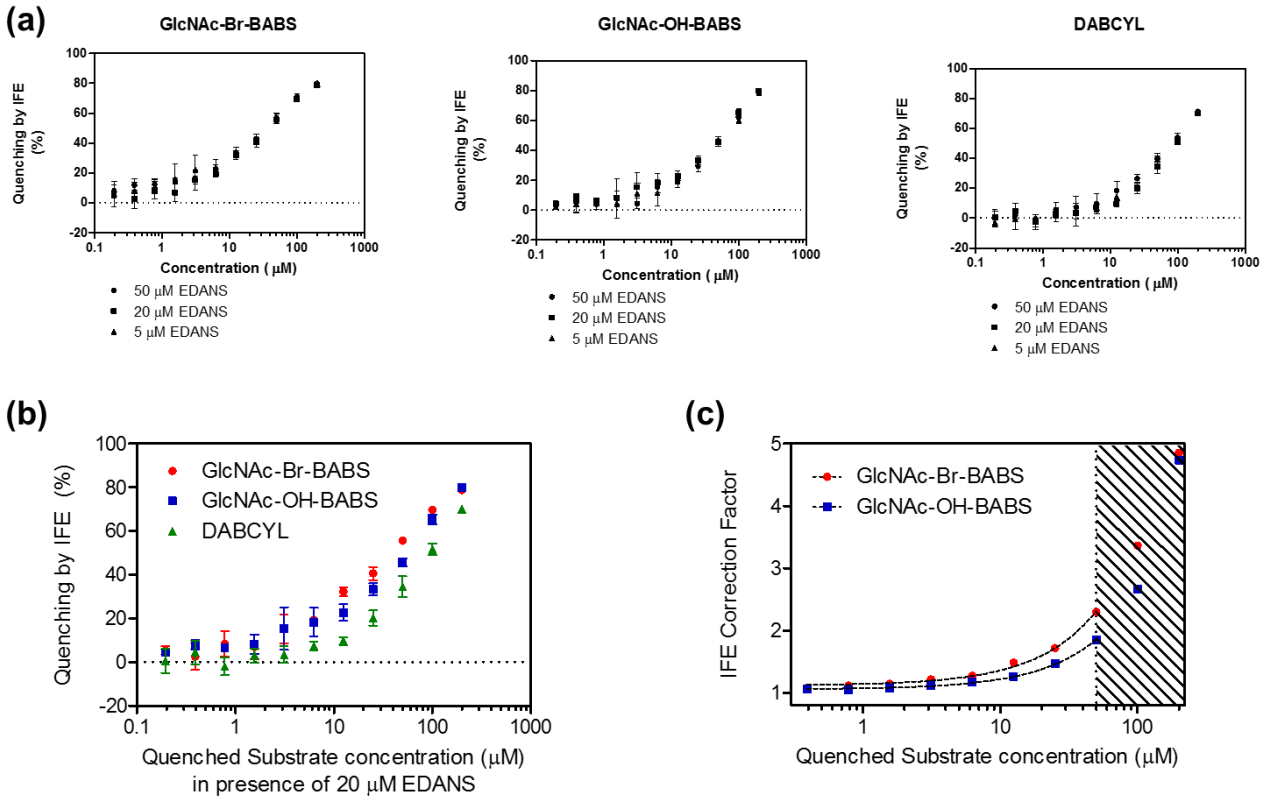



**Figure S3. GlcNAc-BABS and DABCYL quencher show similar Inner-Filter Effect (IFE).** Measurements are carried out in triplicates (PBS, V = 45 µL) using 384-well plates in a SpectraMax i3x plate reader. Error bars represent standard deviation over triplicates. (**a**) Measurement of quenching by IFE for each GlcNAc-BABS and for free DABCYL quencher using various concentration of EDANS show that quenching percentage does not depend on EDANS concentration, hence enabling the use of correction factors. (**b**) IFE Quenching of 20 µM EDANS for various concentration of GlcNAc-Br-BABS, GlcNAc-OH-BABS and DABCYL. (**c**) IFE correction factors for each concentrations of GlcNAc-BABS were derived from the average of all data in panels (a). The striped area contains concentrations that requires a correction factor higher than 2-fold (over 50 % of the EDANS signal is quenched by IFE).

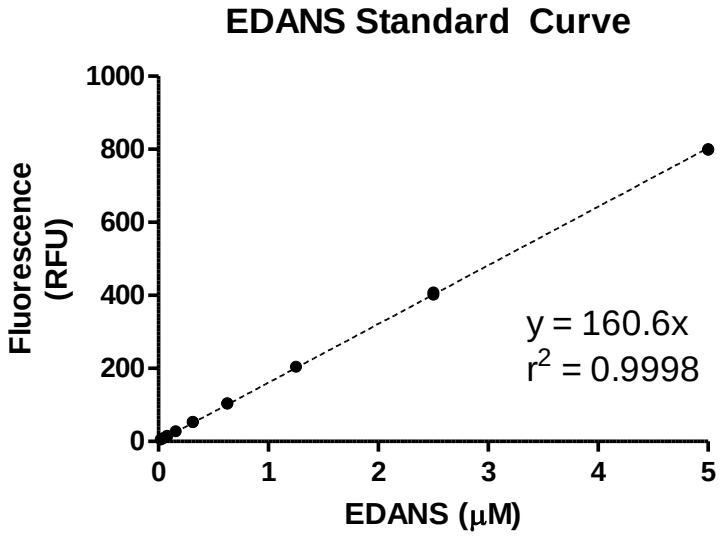



**Figure S4. Standard Curve for the EDANS fluorophore.** Fluorescence of different concentration of EDANS in PBS was measured in cuvettes (duplicates, V = 160 μL) on a Varian Cary Eclipse fluorometer. Each replicate is plotted. Linear regression through zero was used to determine a slope of 160.6 ± 0.4 RFU $\mu M^{-1}$

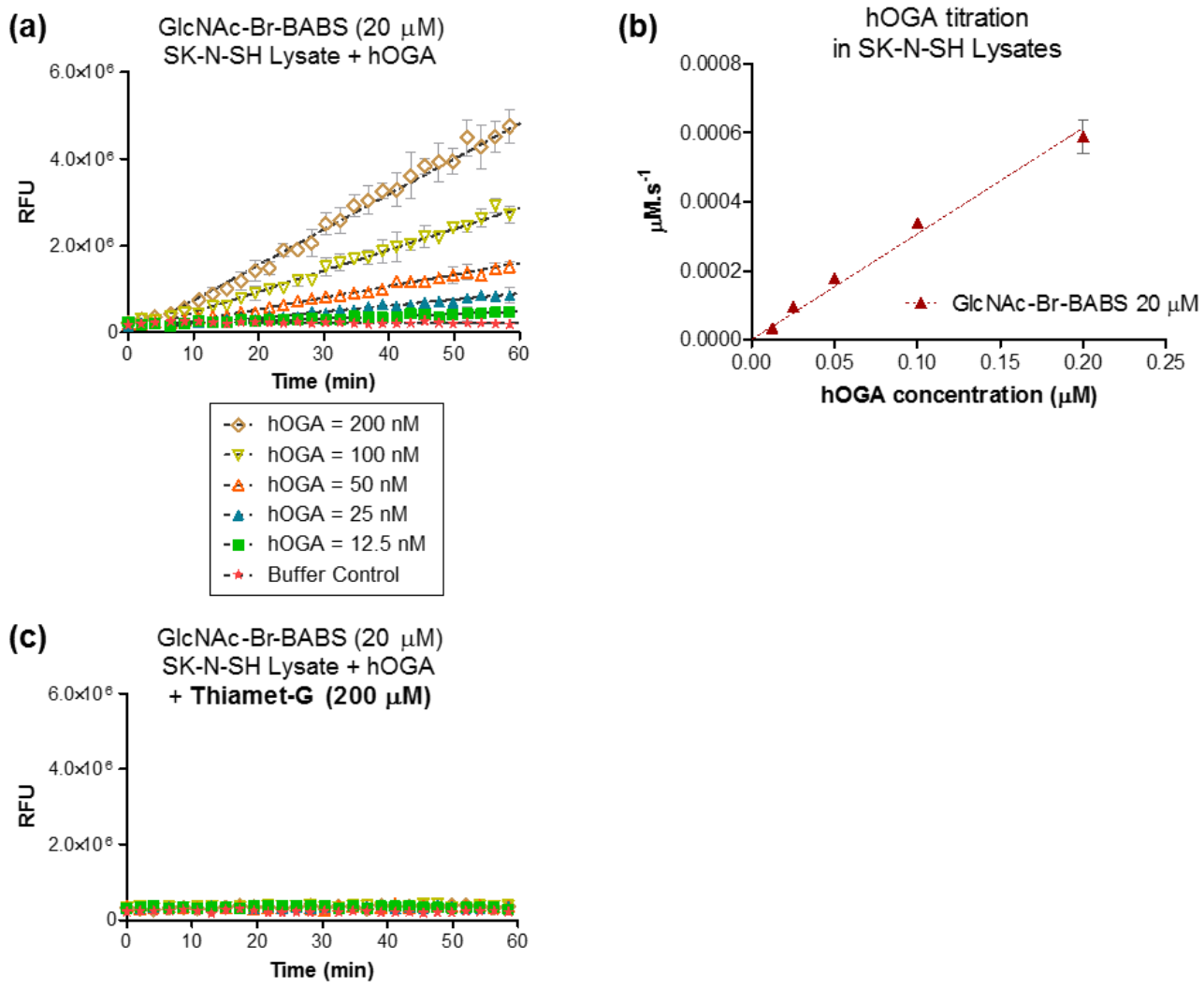
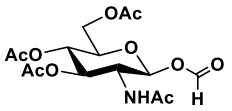



**Figure S5. GlcNAc-Br-BABS is cleaved by hOGA within cell lysates. (a)** Fluorescence was measured at 25°C in 384-well plates using 20 µM of GlcNAc-Br-BABS in SK-N-SH lysate and increasing concentrations of recombinant hOGA. Buffer control consists of PBS supplemented to lysis buffer. **(b)** Rates of GlcNAc-Br-BABS in lysates are linearly dependent on hOGA concentration and are similar to rates observed in buffer alone. These data highlight the compatibility of GlcNAc-Br-BABS with the complex milieu of a cellular extract. **(c)** Addition of the potent and selective hOGA inhibitor Thiamet-G (200 µM) completely inhibits the substrate's turnover, demonstrating the specificity of the observed signal. Error bars represent standard deviation over quadruplicates.

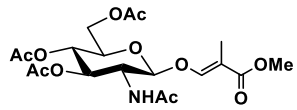
# Synthesis and compound characterization

## β-Formyl $(AcO)_3$GlcNAc (1)



A 50 mL round-bottom flask containing α-chloro-N-Acetylglucosamine (1.63 g, 4.45 mmoles) was flushed with argon. Formic acid (15 mL) was added and the suspension was cooled to 0°C using an ice bath. Under vigorous stirring, $AgNO_3$ (831 mg, 4.89 mmoles, 1.1 eq) powder was added in three portions over 5 mins. After 30 mins., the ice bath was removed and the mixture (white suspension) was stirred at r.t. for 45 mins. The crude mixture was then filtered through a plug of celite. The flask and filter were washed with $CHCl_3$ (250 mL). The filtrate was then slowly poured into 500 mL of saturated $NaHCO_3$. The organic layer was washed with saturated $NaHCO_3$ three times, then with brine. The organic layer was dried over $Na_2SO_4$ and concentrated to yield 1.136 g (66%) of crude product as a white foam. This product was pure enough for the next step but analytical samples were also obtained through crystallization (DCM:$Et_2O$). $\boldsymbol{R_f}$ = 0.20 (DCM:EtOAc, 1:1). **$^1$H NMR (500 MHz, $CDCl_3$)** $\delta$ 8.07 (s, 1H, OC(O)*H*), 5.84 (d, *J* = 8.7 Hz, 1H, H-1), 5.70 (d, *J* = 9.3 Hz, 1H, AcN*H*), 5.21 (t, *J* = 9.7 Hz, 1H, H-3), 5.14 (t, *J* = 9.7 Hz, 1H, H-4), 4.33 – 4.24 (m, 2H, H-2, H-6a), 4.16 – 4.08 (m, 1H, H-6b), 3.85 (ddd, *J* = 9.7 Hz, *J* = 4.3 Hz, *J* = 1.9 Hz, 1H, H-5), 2.09 (s, 3H, C*H*$_3$CO-6), 2.05 (s, 3H, C*H*$_3$CO-3), 2.04 (s, 3H, C*H*$_3$CO-4), 1.94 (s, 3H, C*H*$_3$CONHR).ppm. **$^{13}$C NMR (125 MHz, $CDCl_3$)** $\delta$ 171.3 ($CH_3$*C*O-3), 170.8 ($CH_3$*C*O-6), 170.4 ($CH_3$*C*O-2), 169.4 ($CH_3$*C*O-6), 92.1 (C-1), 73.2 (C-5), 72.4 (C-3), 67.8 (C-4), 61.7 (C-6), 53.2 (C-2), 23.3 (*C*$H_3$CONHR), 20.8, 20.75, 20.71 (3s, 3C, *C*$H_3$CO-(3,4,6)) ppm. **HR-ESI-MS** calcd for $C_{15}H_{22}NO_{10}$ $[M+H]^+$ 376.1238, found 376.1239; calcd for $C_{15}H_{25}N_2O_{10}$ $[M+NH_4]^+$ 393.1504, found 393.1502; calcd for $C_{15}H_{21}NNaO_{10}$ $[M+Na]^+$ 398.1058, found 398.1063.

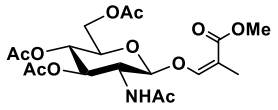
## $(AcO)_3$GlcNAc-(E)-vinylogous carbonate (2E)



***Preparation of phosphorane solution***: A 100 mL round-bottom flask containing methyl-2-bromo-propionate (4 g, 24 mmoles) was flushed with argon and 20 mL of anhydrous toluene were added. Under stirring at r.t., 5.9 mL of $PBu_3$ were added dropwise over 15 mins. The mixture was stirred overnight and then concentrated under vacuum to yield the phosphonium bromide salt. This salt can be stored at -20°C for future use. The phosphorane was generated by dissolving 6.2 g of the phosphonium salt in 75 mL of DCM and vigorously washing this organic layer with a 10 % solution of NaOH (100 mL). Drying ($Na_2SO_4$) and concentration yielded 5.40 g of the phosphorane which was kept under argon and dissolved in 32 mL of anhydrous toluene. ***Wittig reaction***: A 100 mL round-bottom flask containing the formyl glycoside **1** (1.6 g, 4.26 mmoles) was flushed with argon. Anhydrous toluene (15 mL) was added followed by a freshly prepared solution of the phosphorane in toluene (13.6 mL, 0.5 M, 1.6 eq.). The mixture was then heated to 80°C for 2 hours before TLC showed complete conversion of the starting material. The crude mixture was then concentrated under vacuum. Silica gel column chromatography ($Et_2O$:EtOAc, 8:2) yielded 1.047 g of the E-vinylogous carbonate **2E** and 511 mg of the Z-vinylogous carbonate **2Z** (82 % overall, E/Z: 2/1) as white foams. **2E:** $\boldsymbol{R_f}$ = 0.31 (DCM:EtOAc, 1:1). **$^1$H NMR (400 MHz, $CDCl_3$)** $\delta$ 7.41 (d, *J* = 1.3 Hz, 1H, OC*H*=C$^{IV}$), 5.82 (d, *J* = 8.7 Hz, 1H, AcN*H*), 5.33 (dd, *J* = 10.4 Hz, *J* = 9.4 Hz, 1H, H-3), 5.11 (t, *J* = 9.4 Hz, 1H, H-4), 5.06 (d, *J* = 8.3 Hz, 1H, H-1), 4.28 (dd, *J* = 12.4 Hz, *J* = 4.7 Hz, 1H, H-6a), 4.13 (dd, *J* = 12.4 Hz, *J* = 2.4 Hz, 1H, H-6b), 4.04 (dt, *J* = 10.4 Hz, *J* = 8.3 Hz, 1H, H-2), 3.82 (ddd, *J* = 9.4 Hz, *J* = 4.7 Hz, *J* = 2.4 Hz, 1H, H-5), 3.70 (s, 3H, OC*H*$_3$), 2.08 (s, 3H, C*H*$_3$CO-6), 2.04 (s, 3H, C*H*$_3$CO-3), 2.03 (s, 3H, C*H*$_3$CO-4), 1.94 (s, 3H, C*H*$_3$CONHR), 1.73 (d, *J* = 1.3 Hz, 3H, C*H*$_3$) ppm. **$^{13}$C NMR (100 MHz, $CDCl_3$)** $\delta$ 170.9 ($CH_3$*C*O-3), 170.8 ($CH_3$*C*O-6), 170.4 ($CH_3$*C*ONHR), 169.4 ($CH_3$*C*O-4), 168.7 (*C*(O)O$CH_3$), 153.3 (O*C*H=C$^{IV}$), 110.0 (OCH=*C*$^{IV}$), 101.0 (C-1), 72.6 (C-3), 71.7 (C-5), 68.2 (C-4), 61.9 (C-6), 54.4 (C-2), 51.6 (O*C*$H_3$), 23.4 (*C*$H_3$CONHR), 20.84, 20.79, 20.7 (3C, *C*$H_3$CO), 9.6 (*C*$H_3$) ppm. **HR-ESI-MS** calcd for $C_{19}H_{28}NO_{11}$ $[M+H]^+$

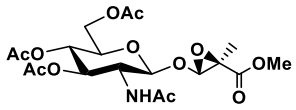
446.1657, found 446.1671; calcd for $C_{19}H_{31}N_2O_{11}$ $[M+NH_4]^+$ 463.1922, found 463.1934; calcd for $C_{19}H_{27}NNaO_{11}$ $[M+Na]^+$ 468.1476, found 468.1490; calcd for $C_{38}H_{54}N_2NaO_{22}$ $[2M+Na]^+$ 913.3060, found 913.3085

### $(AcO)_3$GlcNAc-(Z)-vinylogous carbonate (2Z)



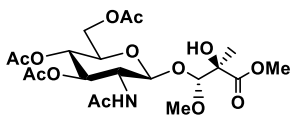
**2Z:** $R_f$ = 0.31 ($Et_2O$:EtOAc, 7:3). **$^1$H NMR (500 MHz, $CDCl_3$)** $\delta$ 7.37 (d, $J$ = 1.4 Hz, 1H, OC*H*=C$^{IV}$), 5.75 (d, $J$ = 8.9 Hz, 1H, AcN*H*), 5.29 (d, $J$ = 3.5 Hz, 1H, H-1), 5.25 (dd, $J$ = 10.7 Hz, $J$ = 9.7 Hz, 1H, H-3), 5.18 (t, $J$ = 9.7 Hz, 1H, H-4), 4.43 (ddd, $J$ = 10.7 Hz, $J$ = 8.9 Hz, $J$ = 3.5 Hz, 1H, H-2), 4.21 (dd, $J$ = 12.4 Hz, $J$ = 4.7 Hz, 1H, H-6a), 4.09 (dd, $J$ = 12.4 Hz, $J$ = 2.3 Hz, 1H, H-6b), 3.87 (ddd, $J$ = 9.7 Hz, $J$ = 4.7 Hz, $J$ = 2.3 Hz, 1H, H-5), 3.72 (s, 3H, OC$H_3$), 2.07 (s, 3H, C$H_3$CO-6), 2.04 (s, 3H, C$H_3$CO-3), 2.03 (s, 3H, C$H_3$CO-4), 1.96 (s, 3H, C$H_3$CONHR), 1.84 (d, $J$ = 1.4 Hz, 3H, C$H_3$) ppm. **$^{13}$C NMR (125 MHz, $CDCl_3$)** $\delta$ 171.6 ($CH_3CO$-3), 170.7 ($CH_3CO$-6), 170.2 ($CH_3CONHR$), 169.3 ($CH_3CO$-4), 168.3 ($C$(O)$OCH_3$), 152.3 (O*C*H=C$^{IV}$), 110.7 (OCH=*C*$^{IV}$), 98.7 (C-1), 70.7 (C-3), 69.2 (C-5), 67.6 (C-4), 61.5 (C-6), 51.9 (C-2), 51.7 (O*C*$H_3$), 23.4 (*C*$H_3$CONHR), 20.8, 20.74, 20.68 (3C, *C*$H_3$CO), 9.8 (*C*$H_3$) ppm. **LR-ESI-MS** $C_{19}H_{28}N_1O_{11}$ $[M+H]^+$ 446.2; $C_{19}H_{27}N_1NaO_{11}$ $[M+Na]^+$ 468.1

### $(AcO)_3$GlcNAc-epoxide (3)



A 50 mL round-bottom flask containing **2E** (357 mg, 0.801 mmoles) was flushed with argon. Anhydrous toluene and DCM (5+5 mL) were added and the mixture was cooled to 0°C. *m*CPBA was added in one portion (207 mg, 1.202 mmoles, 1.5 eq.) under stirring. The mixture was allowed to warm up to r.t. overnight. Completion of the reaction was monitored by running NMR on an aliquot of the crude mixture. The mixture was diluted in EtOAc and washed with $Na_2S_2O_3$ sat., $NaHCO_3$ sat., water and brine. The organic layer was dried ($Na_2SO_4$) and concentrated. Silica gel column chromatography (Hexanes:EtOAc, 3:7) provided the pure epoxide **3** (248 mg, 67 %) as a white foam. $R_f$ = 0.26 (Hex:EtOAc, 2:8). **$^1$H NMR (500 MHz, $CDCl_3$)** $\delta$ 5.67 (d, $J$ = 9.3 Hz, 1H, AcN*H*), 5.18 (t, $J$ = 10.0 Hz, 1H, H-3), 5.15 (s, 1H, OC*H*(O)C($CH_3$)$CO_2$Me), 5.10 (t, $J$ = 9.6 Hz 1H, H-4), 4.86 (d, $J$ = 8.5 Hz, 1H, H-1), 4.26 (dd, $J$ = 12.4 Hz, $J$ = 4.6 Hz, 1H, H-6a), 4.20 – 4.04 (m, 2H, H-2, H-6b), 3.82 – 3.68 (m, 4H, H-5, $CO_2CH_3$), 2.09 (s, 3H, C$H_3$CO-6), 2.03 (s, 6H, C$H_3$CO-(3,4)), 1.93 (s, 3H, C$H_3$CONHR), 1.53 (s, 3H, C$H_3$) ppm. **$^{13}$C NMR (125 MHz, $CDCl_3$)** $\delta$ 171.2 ($CH_3CO$-3), 170.8 ($CH_3CO$-6), 170.3 ($CH_3CONHR$), 169.40, 169.37 (2s, $C$(O)$OCH_3$, $CH_3CO$-4), 99.27 (C-1), 81.27 (O*C*H(O)C($CH_3$)$CO_2$Me), 72.5 (C-5), 72.2 (C-3), 68.2 (C-4), 61.9 (C-6), 59.1 (C$^{IV}$(OR)Me), 53.8 (C-2), 53.1 ($CO_2CH_3$), 23.4 (*C*$H_3$CONHR), 20.9, 20.8, 20.7 (*C*$H_3CO_2$), 12.6 (*C*$H_3$) ppm. **HR-ESI-MS** calcd for $C_{19}H_{28}N_1O_{12}$ $[M+H]^+$ 462.1606, found 462.1602; calcd for $C_{19}H_{27}N_1NaO_{12}$ $[M+Na]^+$ 484.1425, found 484.1429; calcd for $C_{19}H_{27}KN_1O_{12}$ $[M+K]^+$ 500.1165, found 500.1147.

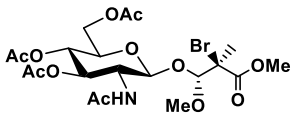
### $(AcO)_3$GlcNAc-Methyl-OH-BisAcetal (4)



A 25 mL round-bottom flask containing epoxide **3** (125 mg, 0.271 mmoles) was flushed with argon. Anhydrous methanol and DCM were added (1:1, 4 mL total). Camphorsulfonic acid (12 mg, 0.054 mmoles, 0.2 eq) was added in one portion and the mixture was stirred at r.t. After 2 hours, TLC (EtOAc) showed complete conversion of the starting material. CSA was then quenched by adding 100 μL of $Et_3N$ and the mixture was concentrated under vacuum. The crude mixture was then purified through silica gel column chromatography (EtOAc) to yield methyl-OH-bisacetal **4** (99 mg, 74 %). $R_f$ = 0.24 (EtOAc). **$^1$H NMR (500 MHz, $CDCl_3$)** $\delta$ 5.89 (d, $J$ = 8.3 Hz, 1H, NH), 5.29 (dd, $J$ = 10.4 Hz, $J$ = 9.6 Hz, 1H, H-3), 5.03 (t, $J$ = 9.6 Hz, H-4), 4.88 (d, $J$ = 8.4 Hz, 1H, H-1), 4.61 (s, 1H, OC*H*(OMe)C(OH)$CO_2$Me), 4.23 – 4.13 (m, 2H, H-6a, H-6b), 3.93 – 3.83 (m, 1H, H-2), 3.78 (s, 3H, C(O)OC$H_3$), 3.74 (ddd, $J$ = 9.6 Hz, $J$ = 5.3 Hz, $J$ = 3.1 Hz, 1H, H-5), 3.47 (s, 3H, OC$H_3$), 2.07 (s, 3H, C$H_3$CO-6), 2.03 (s, 6H,

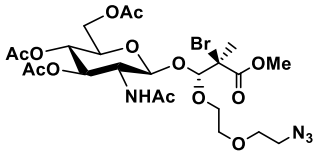
C*H*$_3$CO-(3,4)), 1.94 (s, 3H, C*H*$_3$C(O)NH-2), 1.37 (s, 3H, C*H*$_3$) ppm. **$^{13}$C NMR (125 MHz, CDCl$_3$)** $\delta$ 174.3 (*C*(O)OCH$_3$), 171.1 (CH$_3$*C*O-3), 170.8 (CH$_3$*C*(O)NH-2), 170.7 (CH$_3$*C*O-6), 169.5 (CH$_3$*C*O-4), 106.3 (O*C*H(OMe)C(OH)CO$_2$Me), 98.6 (C-1), 77.3 (C$^{IV}$(OH)MeCO$_2$Me), 72.5 (C-3), 72.1 (C-5), 68.8 (C-4), 62.5 (C-6), 57.3 (O*C*H(O*C*H$_3$)C(OH)), 55.0 (C-2), 53.1 (C(O)O*C*H$_3$), 23.5 (*C*H$_3$C(O)NH-2), 20.83, 20.80, 20.77 (3s, 3C, *C*H$_3$CO-(3,4,6), 19.6 (*C*H$_3$) ppm. **HR-ESI-MS** calcd for $C_{20}H_{32}NO_{13}$ $[M+H]^+$ 494.1868, found 494.1875; calcd for $C_{20}H_{31}NNaO_{13}$ $[M+Na]^+$ 516.1688, found 516.1690; calcd for $C_{20}H_{31}KNO_{13}$ $[M+K]^+$ 532.1427, found 532.1437.

### (AcO)$_3$GlcNAc-Methyl-Br-BisAcetal (5)




A 10 mL round-bottom flask containing vinylogous carbonate **2E** (48 mg, 0.108 mmoles) was flushed with argon. Anhydrous methanol was added (2 mL). N-bromosuccinimide (23 mg, 0.129 mmoles, 1.2 eq) was added in one portion and the mixture was stirred at r.t. After 2 hours, TLC (EtOAc) showed complete conversion of the starting material. The crude mixture was diluted in EtOAc and the organic layer was washed with 1 % $Na_2S_2O_5$, water and brine. After drying ($Na_2SO_4$) and concentration, the crude product was crystallized using DCM/Et$_2$O to afford methyl-Br-bisacetal **5** (40 mg, 66 %). $R_f$ = 0.26 (DCM:EtOAc, 1:1). **$^1$H NMR (500 MHz, CDCl$_3$)** $\delta$ 5.57 (d, *J* = 9.1 Hz, 1H, AcN*H*), 5.25 – 5.14 (m, 1H, H-3), 5.08 (t, *J* = 9.6 Hz 1H, H-4), 4.98 (s, 1H, OC*H*(OMe)R), 4.82 (d, *J* = 8.4 Hz, 1H, H-1), 4.31 – 4.09 (m, 3H, H-6a, H-6b, H-2), 3.80 (s, 3H, CO$_2$C*H*$_3$), 3.75 (ddd, *J* = 9.6 Hz, *J* = 5.5 Hz, *J* = 2.7 Hz, 1H, H-5), 3.45 (s, 3H, OC*H*$_3$), 2.05 (s, 3H, C*H*$_3$CO-6), 2.04 (s, 6H, C*H*$_3$CO-(3,4)), 1.96 (s, 3H, C*H*$_3$CONHR), 1.82 (s, 3H, C*H*$_3$) ppm. **$^{13}$C NMR (125 MHz, CDCl$_3$)** $\delta$ 171.2 (CH$_3$*C*O-3), 170.6 (CH$_3$*C*O-6), 170.2 (CH$_3$*C*ONHR), 169.6, 169.4 (2s, *C*(O)OCH$_3$, CH$_3$*C*O-4), 106.4 (O*C*H(OCH$_3$)), 101.5 (C-1), 72.5 (C-3), 72.2 (C-5), 68.6 (C-4), 62.3 (C-6), 61.2 (C$^{IV}$(Br)Me), 57.6 (O*C*H$_3$), 54.2 (C-2), 53.3 (CO$_2$*C*H$_3$), 23.7 (*C*H$_3$CONHR), 20.84, 20.81, 20.77 (*C*H$_3$CO$_2$), 19.5 (*C*H$_3$) ppm. **HR-ESI-MS** calcd for $C_{20}H_{31}BrNO_{12}$ $[M+H]^+$ 556.1024, found 556.1038; calcd for $C_{20}H_{30}BrNNaO_{12}$ $[M+Na]^+$ 578.0844, found 578.0861; calcd for $C_{20}H_{30}BrKNO_{12}$ $[M+K]^+$ 594.0583, found 594.0599.

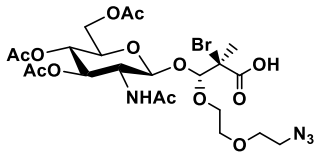
### (AcO)$_3$GlcNAc-(N$_3$-spacer)-Br-BisAcetal (6)




A 5 mL round-bottom flask containing vinylogous carbonate **2E** (200 mg, 0.449 mmoles) and 2-(2-Azidoethoxy)ethanol (1.177 g, 8.98 mmoles, 20 eq.) was flushed with argon. Anhydrous DCM was added (500 μL) followed by N-bromosuccinimide (88 mg, 0.494 mmoles, 1.1 eq). The mixture was stirred at r.t., and, after 2 hours, TLC (DCM:EtOAc, 7:3) showed complete conversion of the starting material. The crude mixture was concentrated and purified through silica gel column chromatography (Et$_2$O:EtOAc, 7:3). Because the product was still contaminated with the spacer, the pure product was obtained through precipitation using Et$_2$O:Hexanes to afford the pure Br-bisacetal **6** (168 mg, 57 %). $R_f$ = 0.16 (DCM:EtOAc, 7:3). **$^1$H NMR (400 MHz, CDCl$_3$)** $\delta$ 5.58 (d, *J* = 9.3 Hz, 1H, AcN*H*), 5.18 (dd, *J* = 10.6 Hz, *J* = 9.6 Hz, 1H, H-3), 5.15 (s, 1H, OC*H*(OR)R), 5.07 (t, *J* = 9.6 Hz, 1H, H-4), 4.81 (d, *J* = 8.4 Hz, 1H, H-1), 4.25 (dd, *J* = 12.2 Hz, *J* = 2.4 Hz, 1H, H-6a), 4.22 – 4.10 (m, 2H, H-6b, H-2), 4.00 (dt, *J* = 10.9 Hz, *J* = 4.0 Hz, 1H, ½ OC*H*$_2$CH$_2$O), 3.79 (s, 3H, OC*H*$_3$), 3.74 (ddd, *J* = 9.6 Hz, *J* = 5.3 Hz, *J* = 2.4 Hz, 1H, H-5), 3.70 – 3,63 (m, 1H, ½ OC*H*$_2$CH$_2$O), 3.61 – 3.52 (m, 4H, C*H*$_2$OC*H*$_2$), 3.33 (td, *J* = 4.7 Hz, *J* = 1.1 Hz, 2H, C*H*$_2$N$_3$), 2.06 (s, 3H, C*H*$_3$CO-6), 2.04 (s, 6H, C*H*$_3$CO-(3,4)), 1.96 (s, 3H, C*H*$_3$CONHR), 1.83 (s, 3H, C*H*$_3$) ppm. **$^{13}$C NMR (100 MHz, CDCl$_3$)** $\delta$ 171.2 (CH$_3$*C*O-3), 170.6 (CH$_3$*C*O-6), 170.2 (CH$_3$*C*ONHR), 169.44, 169.43 (2s, *C*(O)OCH$_3$, CH$_3$*C*O-4), 105.1 (O*C*H(OR)), 101.5 (C-1), 72.5 (C-3), 72.2 (C-5), 70.2, 70.1 (2s, *C*H$_2$O*C*H$_2$), 69.2 (O*C*H$_2$CH$_2$O), 68.5 (C-4), 62.2 (C-6), 61.2 (C$^{IV}$(Br)Me), 54.1 (C-2), 53.3 (O*C*H$_3$), 50.9 (*C*H$_2$N$_3$), 23.7 (*C*H$_3$CONHR), 20.82, 20.76 (2s, 3C, *C*H$_3$CO), 19.7 (*C*H$_3$) ppm. **HR-ESI-MS** calcd for $C_{23}H_{36}BrN_4O_{13}$ $[M+H]^+$ 655.1457, found 655.1452; calcd for $C_{23}H_{39}BrN_5O_{13}$ $[M+NH_4]^+$ 672.1722, found

672.1730; calcd for $C_{23}H_{35}BrN_4NaO_{13}$ $[M+Na]^+$ 677.1276, found 677.1279; calcd for $C_{23}H_{35}BrKN_4O_{13}$ $[M+K]^+$ 693.1016, found 693.1014.

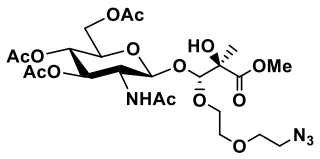
### (AcO)$_3$GlcNAc-(N$_3$-spacer)-Br-BisAcetal Carboxylic Acid (7)



A 50 mL round-bottom flask containing Br-bisacetal **6** (96 mg, 0.146 mmoles) was flushed with argon. A mixture of THF and water (1:1) was added (10 mL). LiOH•$H_2O$ was then added (61 mg, 10 eq.) in one portion and the mixture was stirred at r.t. overnight. The mixture was then diluted with methanol and the base was neutralized using Amberlite IR-120 ($H^+$-form) resin. After filtration and concentration under vacuum, the crude was dissolved under argon in 10 mL of pyridine. 4 mL of acetic anhydride were then added and the mixture was stirred at r.t. for 3 hours. The mixture was then concentrated, co-evaporated with toluene 3 times and the crude residue was then re-dissolved in THF (40 mL) and water (5 mL). After 30 minutes of stirring, the mixture was concentrated and co-evaporated with toluene. The crude product was then purified using silica gel column chromatography (1: DCM, 2: DCM + 5% methanol, 3: DCM + 10 % methanol) to afford the bisacetal free acid **7** (58 mg, 62 %). $\boldsymbol{R_f}$ = 0.18 (DCM:MeOH, 95:5). **$^1$H NMR (500 MHz, MeOD)** $\delta$ 5.31 (dd, $J$ = 10.5 Hz, $J$ = 9.5 Hz, 1H, H-3), 5.17 (s, 1H, OC*H*(OR)R), 4.99 (t, $J$ = 9.5 Hz, 1H, H-4), 4.95 (d, $J$ = 8.4 Hz, 1H, H-1), 4.30 – 4.14 (m, 2H, H-6a, H-6b), 4.11 – 4.03 (m, 1H, ½ OC$H_2$C$H_2$O), 3.98 (dd, $J$ = 10.5 Hz, $J$ = 8.5 Hz, 1H, H-2), 3.90 (ddd, $J$ = 10.1 Hz, $J$ = 4.8 Hz, $J$ = 2.6 Hz, 1H, H-5), 3.76 – 3,68 (m, 1H, ½ OC$H_2$C$H_2$O), 3.67 – 3.55 (m, 4H, C$H_2$OC$H_2$), 3.36 – 3.33 (m, 2H, C$H_2$N$_3$), 2.07 (s, 3H, C$H_3$CO-6), 2.02 (s, 3H, C$H_3$CO-4), 1.99 (s, 3H, C$H_3$CO-3), 1.93 (s, 3H, C$H_3$CONHR), 1.76 (s, 3H, C$H_3$) ppm. **$^{13}$C NMR (125 MHz, MeOD)** $\delta$ 173.6* ($CH_3$*C*ONHR, $CO_2$H), 172.2 ($CH_3$*C*O-6), 171.8 ($CH_3$*C*O-3), 171.3 ($CH_3$*C*O-4), 107.3 (O*C*H(OR)), 102.4 (C-1), 73.7 (C-3), 72.9 (C-5), 71.1, 71.0 (2s, *C*$H_2$O*C*$H_2$), 70.5 (O*C*$H_2$C$H_2$O), 70.4 (C-4), 63.3* (2C, C-6, C$^{IV}$(Br)Me), 55.4 (C-2), 51.8 (*C*$H_2$N$_3$), 23.2 (*C*$H_3$CONHR), 20.9 (*C*$H_3$), 20.7, 20.60, 20.56 (*C*$H_3$CO-(3,4,6)) ppm. **HR-ESI-MS** calcd for $C_{22}H_{34}BrN_4O_{13}$ $[M+H]^+$ 641.1300, found 641.1302; calcd for $C_{22}H_{37}BrN_5O_{13}$ $[M+NH_4]^+$ 658.1566, found 658.1565; calcd for $C_{22}H_{33}BrN_4NaO_{13}$ $[M+Na]^+$ 663.1120, found 663.1112.

**:weak signal on $^{13}$C experiment. Assignment from 2D correlations (HSQC and HMBC)*

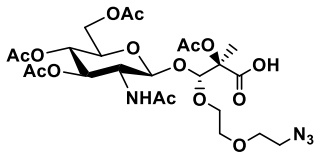
### (AcO)$_3$GlcNAc-(N$_3$-spacer)-OH-BisAcetal (8)



A 5 mL round-bottom flask containing epoxide **3** (210 mg, 0.455 mmoles) and 2-(2-Azidoethoxy)ethanol (1.192 g, 9.10 mmoles, 20 eq.) was flushed with argon. About 200 mg of flame-dried molecular sieves (4Å) and anhydrous DCM were then added (2 mL). Camphorsulfonic acid (53 mg, 0.23 mmoles, 0.5 eq.) was then added. The mixture was stirred at r.t. for 3 hours. The reaction was quenched using $Et_3N$ (100 µL) and the mixture was filtered through a plug of celite (DCM). The crude mixture was concentrated and purified through silica gel column chromatography (Hexanes:EtOAc, 6:4 until all linker has been eluted then pure EtOAc) to afford the pure OH-bisacetal **8** (178 mg, 66 %). $\boldsymbol{R_f}$ = 0.35 (EtOAc). **$^1$H NMR (500 MHz, CDCl$_3$)** $\delta$ 6.36 (d, $J$ = 9.2 Hz, 1H, AcN*H*), 5.14 (t, $J$ = 9.8 Hz, 1H, H-3), 5.04 (d, $J$ = 8.7 Hz, 1H, H-1), 4.97 (t, $J$ = 9.8 Hz, 1H, H-4), 4.87 (s, 1H, OC*H*(OR)R), 4.20 – 4.06 (m, 3H, H-6a, H-6b, H-2), 4.01 – 3.93 (m, 1H, ½ OC$H_2$C$H_2$O), 3.82 – 3.63 (m, 9H, OC$H_3$, H-5, ½ OC$H_2$C$H_2$O, C$H_2$OC$H_2$), 3.62 – 3.46 (m, 2H, C$H_2$N$_3$), 2.12 (s, 3H, C$H_3$CO-6), 2.02 (s, 3H, C$H_3$CO-4), 2.01 (s, 3H, C$H_3$CO-3), 1.93 (s, 3H, C$H_3$CONHR), 1.39 (s, 3H, C$H_3$) ppm. **$^{13}$C NMR (125 MHz, CDCl$_3$)** $\delta$ 173.8 (*C*(O)OCH$_3$), 171.0 ($CH_3$*C*O-3), 170.8 ($CH_3$*C*O-6), 170.4 ($CH_3$*C*ONHR), 169.6 (s, $CH_3$*C*O-4), 103.0 (O*C*H(OR)), 94.84 (C-1), 77.0 (C$^{IV}$(OH)Me), 73.4 (C-3), 72.2 (C-5), 70.8, 70.1 (2C, *C*$H_2$O*C*$H_2$), 69.8 (C-4), 68.5 (O*C*$H_2$C$H_2$O), 63.0 (C-6), 54.0 (C-2), 53.0 (O*C*H$_3$), 50.8 (*C*$H_2$N$_3$), 23.2 (*C*$H_3$CONHR), 21.2 (*C*$H_3$), 20.80,

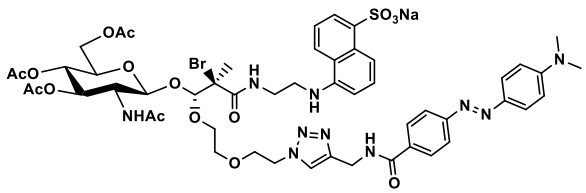
20.77 (2s, 3C, *C*$H_3$CO) ppm. **HR-ESI-MS** calcd for $C_{23}H_{37}N_4O_{14}$ $[M+H]^+$ 593.2301, found 593.2304; calcd for $C_{23}H_{40}N_5O_{14}$ $[M+NH_4]^+$ 610.2566, found 610.2572; calcd for $C_{23}H_{36}N_4NaO_{14}$ $[M+Na]^+$ 615.2120, found 615.2121.

### (AcO)$_3$GlcNAc-(N$_3$-spacer)-OAc-BisAcetal Carboxylic Acid (9)



A 50 mL round-bottom flask containing OH-bisacetal **8** (27 mg, 0.045 mmoles) was flushed with argon. A mixture of THF and water (1:1) was added (5 mL). LiOH•$H_2O$ was then added (19 mg, 10 eq.) in one portion and the mixture was stirred at r.t. overnight. The mixture was then diluted with methanol and the base was neutralized using Amberlite IR-120 ($H^+$-form) resin. After filtration and concentration under vacuum, the crude was dissolved under argon in 4 mL of pyridine. 1 mL of acetic anhydride was then added and the mixture was stirred at r.t. overnight. The mixture was then concentrated, co-evaporated with toluene 3 times and the crude residue was then re-dissolved in THF (5 mL) and water (2 mL). After 30 minutes of stirring, the mixture was concentrated and co-evaporated with toluene. The crude product was then purified using silica gel column chromatography (1: DCM, 2: DCM + 5% methanol, 3: DCM + 10 % methanol) to afford the bisacetal free acid **9** (16 mg, 57 %). $\boldsymbol{R_f}$ = 0.15 (DCM:MeOH, 90:10). **$^1$H NMR (500 MHz, MeOD)** $\delta$ 5.42 (dd, *J* = 10.4 Hz, *J* = 9.3 Hz, 1H, H-3), 5.02 – 4.96 (m, 2H, H-4, H-1), 4.89 (s, 1H, OC*H*(OR)R), 4.25 (d, J = 3.7 Hz, 2H, H-6a, H-6b), 4.00 – 3.94 (m, 1H, ½ OC$H_2$C$H_2$O), 3.88 (dt, *J* = 10.1 Hz, *J* = 3.7 Hz, 1H, H-5), 3.82 (dd, *J* = 10.4 Hz, *J* = 8.5 Hz, 1H, H-2), 3.73 – 3.67 (m, 1H, ½ OC$H_2$C$H_2$O), 3.67 – 3.58 (m, 4H, C$H_2$OC$H_2$), 3.39 – 3.34 (m, 2H, C$H_2$N$_3$), 2.10, 2.06, 2.02, 2.00 (4s, 12H, 4×C$H_3$CO), 1.90 (s, 3H, C$H_3$CONHR), 1.49 (s, 3H, C$H_3$) ppm. **$^{13}$C NMR (125 MHz, CDCl$_3$)** $\delta$ 174.1* ($CO_2$H), , 173.2* (CH$_3$*C*ONHR), 172.2, 171.8, 171.36, 171.35 (4×CH$_3$*C*O), 105.5 (O*C*H(OR)), 100.7 (C-1), 84.9* (C$^{IV}$(OAc)Me), 73.5 (C-3), 72.9 (C-5), 71.1, 71.0 (2C, *C*H$_2$O*C*H$_2$), 70.5 (C-4), 69.8 (O*C*H$_2$CH$_2$O), 63.3 (C-6), 55.9 (C-2), 51.8 (*C*H$_2$N$_3$), 23.0 (*C*H$_3$CONHR), 21.3, 20.7, 20.60, 20.57 (4×*C*H$_3$CO), 14.8 (*C*H$_3$) ppm. **HR-ESI-MS** calcd for $C_{24}H_{37}N_4O_{15}$ $[M+H]^+$ 621.2250, found 621.2243; calcd for $C_{24}H_{40}N_5O_{15}$ $[M+NH_4]^+$ 638.2515, found 638.2515; calcd for $C_{24}H_{36}N_4NaO_{15}$ $[M+Na]^+$ 643.2069, found 643.2068.
**: signal did not emerge from $^{13}$C experiment. Assignment from 2D correlations (HSQC and HMBC)*

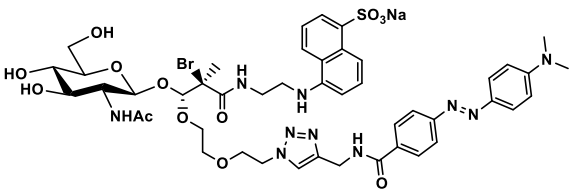
### (AcO)$_3$GlcNAc-Br-BABS (10)



A 10 mL round-bottom flask containing Br-bisacetal acid **7** (17 mg, 0.026 mmoles) and EDANS-$NH_2$ (10 mg, 0.037 mmoles, 1.4 eq.) was flushed with argon. Anhydrous DMF (2 mL) was then added, followed by DIPEA (18 µL, 0.106 mmoles, 4 eq.) and HBTU (20 mg, 0.053 mmoles, 2 eq.). The mixture was then stirred at r.t. overnight. After concentration and co-evaporation with toluene, the crude mixture was filtered over a plug of silica gel (DCM then DCM:MeOH, 9:1). This intermediate (18 mg) was transferred into a 50 mL round-bottom flask containing DABCYL-Alkyne (7.4 mg, 0.024 mmoles, 1.2 eq.). After flushing with argon, 4 mL of anhydrous DCM were added followed by DIPEA (13 µL, 0.08 mmoles, 4 eq.) and Cu(MeCN)$_4$PF$_6$ (1.5 mg, 0.004 mmoles, 0.2 eq.). The mixture was stirred at r.t. overnight. After concentration, the crude mixture was diluted in methanol and neutralized using Amberlite IR-120 ($Na^+$-form) resin. Purification through silica gel column chromatography (DCM:MeOH, 9:1) provided the acetylated GlcNAc-Br-BABS **10** (20 mg, 64 % over 2 steps). $\boldsymbol{R_f}$ = 0.43 (DCM:MeOH, 85:15). **$^1$H NMR (600 MHz, MeOD)** $\delta$ 8.15 (d, *J* = 8.6 Hz, 1H, CH-edans), 8.14 – 8.09 (m, 2H, CH-edans), 8.01 – 7.97 (m, 2H, CH-dabcyl), 7.89 – 7.82 (m, 4H, CH-dabcyl), 7.76 (bs, 1H, H-triaz), 7.38 – 7.33 (m, 2H, CH-edans), 6.86 – 6.81 (m, 2H, CH-dabcyl), 6.64 (d, *J* = 7.7 Hz, 1H, CH-edans), 5.29 (dd, *J* = 10.7 Hz, *J* = 9.2 Hz, 1H, H-3), 5.11 (s, 1H, OC*H*(OR)), 4.96 (dd, *J* = 10.2 Hz, *J* = 9.2 Hz, 1H, H-4), 4.90 – 4.87* (m, 1H, H-1), 4.64 – 4.55 (m, 2H, C$H_2$NHC(O)-dabcyl),

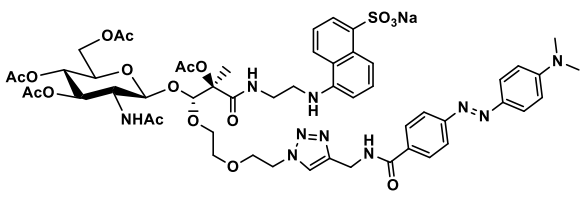
4.26 (t, *J* = 5.0 Hz, 2H, C*H*$_2$-Ntriaz), 4.14 (d, *J* = 3.7 Hz, 2H, H-6), 4.01 (dd, *J* = 10.7 Hz, *J* = 8.4 Hz, 1H, H-2), 3.85 – 3.78 (m, 2H, H-5, ½ OC*H*$_2$CH$_2$O), 3.66 – 3.60 (m, 1H, ½ C*H*$_2$CH$_2$NH-edans), 3.56 – 3.52 (m, 1H, ½ C*H*$_2$CH$_2$NH-edans), 3.51 – 3.47 (m, 1H, ½ OC*H*$_2$CH$_2$O), 3.43 (t, *J* = 5.1 Hz, 2H, C*H*$_2$CH$_2$N-triaz), 3.39 (t, *J* = 5.8 Hz, 2H, CH$_2$C*H*$_2$NH-edans), 3.20 – 3.14 (m, 2H, OCH$_2$C*H*$_2$O), 3.11 (s, 6H, N(C*H*$_3$)$_2$), 1.98, 1.98, 1.93 (3s, 9H, 3×C*H*$_3$CO), 1.92 (s, 3H, C*H*$_3$CONHR), 1.81 (s, 3H, C*H*$_3$) ppm. **$^{13}$C NMR (150 MHz, MeOD)** $\delta$ 173.5 (CH$_3$*C*ONHR), 172.1 (1s, 2C, CH$_3$*C*O, C(O)NH-edans), 171.8 (CH$_3$*C*O), 171.3 (CH$_3$*C*O), 169.5 (NHC(O)-dabcyl), 156.6 (C$^{IV}$-dabcyl), 154.7 (C$^{IV}$-dabcyl), 145.4 (C$^{IV}$-edans), 144.9 (C$^{IV}$-dabcyl), 141.8 (C$^{IV}$-edans), 135.3 (C$^{IV}$-dabcyl), 131.5 (C$^{IV}$-edans), 129.6 (CH-dabcyl), 128.7 (CH-edans), 126.8 (CH-edans), 126.5 (CH-dabcyl), 125.6 (CH-edans), 125.5 (C$^{IV}$-edans), 125.1* (CH-triaz), 123.7 (CH-edans), 123.0 (CH-dabcyl), 116.3 (CH-edans), 112.6 (CH-dabcyl), 107.0 (O*C*H(OR)), 105.1 (CH-edans), 102.2 (C-1), 73.6 (C-3), 72.8 (C-5), 70.8 (OCH$_2$*C*H$_2$O), 70.3 (C-4), 70.2 (O*C*H$_2$CH$_2$O), 70.0 (*C*H$_2$CH$_2$N-triaz), 63.5 (C$^{IV}$(OAc)Me), 63.1 (C-6), 55.3 (C-2), 51.2 (*C*H$_2$N-triaz), 44.9 (CH$_2$*C*H$_2$NH-edans), 40.4 (N(*C*H$_3$)$_2$), 40.2 (*C*H$_2$CH$_2$NH-edans), 36.2 (*C*H$_2$NH-dabcyl), 23.7 (*C*H$_3$CONHR), 21.7 (*C*H$_3$), 20.7, 20.6, 20.5 (3×CH$_3$CO) ppm. **HR-ESI-MS** calcd for C$_{52}$H$_{64}$BrN$_{10}$O$_{16}$S [M+H]$^+$ 1195.3400, found 1195.3388; calcd for C$_{52}$H$_{63}$BrN$_{10}$NaO$_{16}$S [M+Na]$^+$ 1217.3220, found 1217.3212; calcd for C$_{52}$H$_{65}$BrN$_{10}$O$_{16}$S [M+2H]$^{2+}$ 598.1736, found 598.1741; calcd for C$_{52}$H$_{63}$BrN$_{10}$Na$_2$O$_{16}$S [M+2Na]$^{2+}$ 620.1556, found 620.1560.
**: signal did not emerge from $^{13}$C experiment. Assignment from 2D correlations (HSQC and HMBC)*

## GlcNAc-Br-BABS (11)




A 10 mL round-bottom flask containing acetylated GlcNAc-Br-BABS **10** (24 mg) was flushed with argon. Anhydrous methanol (2 mL) was then added. Sodium methoxide (*ca.* 5 mg) was added and the mixture was stirred at r.t. overnight. The crude was then concentrated and dissolved in water (6 mL). One fourth of this crude was purified thanks to semi-preparative scale HPLC (C-18, H$_2$O:MeCN, 15 to 55 % MeCN gradient over 30 minutes, 2 mL.min$^{-1}$). Lyophilisation of the fractions containing the pure product provided the pure GlcNAc-Br-BABS **11** (2.0 mg) as an orange fluffy powder. **HPLC retention time** = 19.9 mins (C18 Semi-Prep, H$_2$O:MeCN, 90:10 to 45:55 over 30 minutes, 2 mL.min$^{-1}$). **$^1$H NMR (600 MHz, MeOD)** $\delta$ 8.20 – 8.10 (m, 3H, CH-edans), 8.03 – 7.96 (m, 2H, CH-dabcyl), 7.89 – 7.82 (m, 4H, CH-dabcyl), 7.77 (s, 1H, H-triaz), 7.41 – 7.33 (m, 2H, CH-edans), 6.87 – 6.80 (m, 2H, CH-dabcyl), 6.63 (d, *J* = 7.8 Hz, 1H, CH-edans), 5.10 (s, 1H, O*C*H(OR)), 4.65 (s, 2H, C*H*$_2$NHC(O)-dabcyl), 4.62 (d, *J* = 8.4 Hz, 1H, H-1), 4.25 (t, *J* = 5.1 Hz, 2H, C*H*$_2$-Ntriaz), 3.91 – 3.88 (m, 1H, ½ OC*H*$_2$CH$_2$O), 3.88 – 3.84 (m, 1H, H-6a), 3.81 (dd, *J* = 10.5 Hz, *J* = 8.4 Hz, 1H, H-2), 3.65 (dd, *J* = 11.8 Hz, *J* = 5.4 Hz, 1H, H-6b), 3.63 – 3.59 (m, 1H, ½ C*H*$_2$CH$_2$NH-edans), 3.58 – 3.53 (m, 2H, ½ OC*H*$_2$CH$_2$O, ½ C*H*$_2$CH$_2$NH-edans), 3.50 (dd, J = 10.5 Hz, J = 8.0 Hz), 1H, H-3), 3.43 (t, *J* = 4.9 Hz, 2H, C*H*$_2$CH$_2$N-triaz), 3.39 (t, *J* = 5.9 Hz, 2H, CH$_2$C*H*$_2$NH-edans), 3.36 – 3.31 (m, 2H, H-4, H-5), 3.18 – 3.12 (m, 2H, OCH$_2$C*H*$_2$O), 3.11 (s, 6H, N(C*H*$_3$)$_2$), 1.97 (s, 3H, C*H*$_3$CONHR), 1.82 (s, 3H, C*H*$_3$) ppm. **$^{13}$C NMR (150 MHz, MeOD)** $\delta$ 173.7 (CH$_3$*C*ONHR), 172.2 (C(O)NH-edans), 169.5 (NHC(O)-dabcyl), 156.5 (C$^{IV}$-dabcyl), 154.7 (C$^{IV}$-dabcyl), 146.1 (C$^{IV}$-triaz), 145.4 (C$^{IV}$-edans), 144.9 (C$^{IV}$-dabcyl), 142.1 (C$^{IV}$-edans), 135.5 (C$^{IV}$-dabcyl), 131.5 (C$^{IV}$-edans), 129.5 (CH-dabcyl), 128.6 (CH-edans), 126.7 (CH-edans), 126.4 (CH-dabcyl), 125.6 (CH-edans), 125.5 (C$^{IV}$-edans), 125.0 (CH-triaz), 123.7 (CH-edans), 123.0 (CH-dabcyl), 116.4 (CH-edans), 112.6 (CH-dabcyl), 106.6 (O*C*H(OR)), 105.0 (CH-edans), 103.0 (C-1), 78.1 (C-5), 75.5 (C-3), 72.1 (C-4), 70.9 (OCH$_2$*C*H$_2$O), 70.0 (*C*H$_2$CH$_2$N-triaz), 69.9 (O*C*H$_2$CH$_2$O), 63.7 (C$^{IV}$(Br)Me), 62.8 (C-6), 57.1 (C-2), 51.1 (*C*H$_2$N-triaz), 44.7 (CH$_2$*C*H$_2$NH-edans), 40.4 (N(*C*H$_3$)$_2$), 40.1 (*C*H$_2$CH$_2$NH-edans), 36.3 (*C*H$_2$NH-dabcyl), 23.4 (*C*H$_3$CONHR), 22.2 (*C*H$_3$) ppm. **HR-ESI-MS** calcd for C$_{46}$H$_{58}$BrN$_{10}$O$_{13}$S [M+H]$^+$ 1069.3083, found 1069.3063; calcd for C$_{46}$H$_{59}$BrN$_{10}$O$_{13}$S [M+2H]$^{2+}$ 535.1578, found 535.1576; calcd for C$_{46}$H$_{57}$BrN$_{10}$Na$_2$O$_{13}$S [M+2Na]$^{2+}$ 557.1397, found 557.1391.

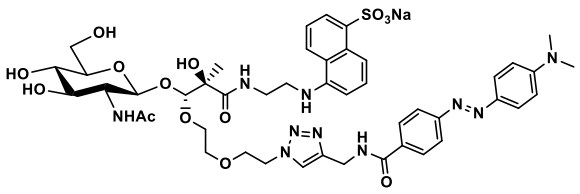
**(AcO)$_3$GlcNAc-OAc-BABS (12)**



A 50 mL round-bottom flask containing AcO-bisacetal acid **9** (43 mg, 0.069 mmoles) and EDANS-$NH_2$ (26 mg, 0.097 mmoles, 1.4 eq.) was flushed with argon. Anhydrous DMF (2 mL) was then added, followed by DIPEA (46 µL, 0.276 mmoles, 4 eq.) and HBTU (31 mg, 0.083 mmoles, 1.2 eq.). The mixture was then stirred at r.t. overnight. After filtration through a plug of celite (DCM), the crude mixture was concentrated and co-evaporated with toluene. This crude mixture was then was transferred into a 50 mL round-bottom flask containing DABCYL-Alkyne (46 mg, 0.152 mmoles, 2.2 eq.). After flushing with argon, 5 mL of anhydrous DCM were added followed by DIPEA (46 µL, 0.276 mmoles, 4 eq.) and $Cu(MeCN)_4PF_6$ (10.2 mg, 0.028 mmoles, 0.4 eq.). The mixture was stirred at r.t. overnight. After concentration, the crude mixture was diluted in methanol and neutralized using Amberlite IR-120 ($Na^+$-form) resin. Purification through silica gel column chromatography (DCM then DCM:MeOH, 95:5 then DCM:MeOH, 90:10) provided the acetylated GlcNAc-OAc-BABS **12** (66 mg, 81 % over 2 steps). $\boldsymbol{R_f}$ = 0.53 (DCM:MeOH, 90:10). **$^1$H NMR (600 MHz, MeOD)** $\delta$ 8.19 (d, *J* = 8.6 Hz, 1H, CH-edans), 8.12 (dd, *J* = 7.2 Hz, *J* = 1.2 Hz, 1H, CH-edans), 8.08 (d, *J* = 8.6 Hz, 1H, CH-edans), 8.03 – 7.98 (m, 2H, CH-dabcyl), 7.87 – 7.83 (m, 5H, H-triaz, CH-dabcyl), 7.38 – 7.33 (m, 2H, CH-edans), 6.85 (d, *J* = 9.2 Hz, 2H, CH-dabcyl), 6.63 (d, *J* = 7.6 Hz, 1H, CH-edans), 5.42 (dd, *J* = 10.6 Hz, *J* = 9.2 Hz, 1H, H-3), 4.94 (dd, *J* = 10.2 Hz, *J* = 9.2 Hz, 1H, H-4), 4.92 (d, *J* = 8.4 Hz, 1H, H-1), 4.86 (s, 1H, OC*H*(OR)), 4.65 (bs, 2H, C$H_2$NHC(O)-dabcyl), 4.33 (bs, 2H, C$H_2$-Ntriaz), 4.16 (dd, *J* = 12.3 Hz, *J* = 4.9 Hz, 1H, H-6a), 4.12 (dd, *J* = 12.3 Hz, *J* = 2.6 Hz, 1H, H-6b), 3.85 – 3.78 (m, 2H, H-2, H-5), 3.78 – 3.74 (m, 1H, ½ OC$H_2$C$H_2$O), 3.63 – 3.55 (m, 1H, ½ C$H_2$C$H_2$NH-edans), 3.55 – 3.48 (m, 1H, ½ C$H_2$C$H_2$NH-edans), 3.50 – 3.44 (m, 2H, C$H_2$C$H_2$N-triaz), 3.43 – 3.38 (m, 1H, ½ OC$H_2$C$H_2$O), 3.38 – 3.33 (m, 2H, C$H_2$C$H_2$NH-edans), 3.25 – 3.17 (m, 2H, OC$H_2$C$H_2$O), 3.12 (s, 6H, N(C$H_3$)$_2$), 2.10 (s, 3H, C$H_3$CO), 1.99 – 1.98 (m, 6H, C$H_3$CO), 1.91 (s, 3H, C$H_3$CO), 1.90 (s, 3H, C$H_3$CONHR), 1.55 (s, 3H, C$H_3$) ppm. **$^{13}$C NMR (150 MHz, MeOD)** $\delta$ 173.2 ($CH_3$*C*ONHR), 172.9 (C(O)NH-edans), 172.1 ($CH_3$*C*O), 171.8 ($CH_3$*C*O), 171.4 ($CH_3$*C*O), 171.3 ($CH_3$*C*O), 154.8 (C$^{IV}$-dabcyl), 145.3 (C$^{IV}$-edans), 144.7 (2C, C$^{IV}$-dabcyl, C$^{IV}$-triaz), 142.2 (C$^{IV}$-edans), 135.4* (C$^{IV}$-dabcyl), 131.5 (C$^{IV}$-edans), 129.6 (CH-dabcyl, CH-triaz), 128.6 (CH-edans), 126.7 (2C, CH-edans, CH-dabcyl), 125.6 (C$^{IV}$-edans), 125.3 (CH-edans), 123.7 (CH-edans), 122.9 (CH-dabcyl), 116.7 (CH-edans), 112.9 (CH-dabcyl), 105.3 (CH-edans), 104.8 (O*C*H(OR)), 100.7 (C-1), 84.6 (C$^{IV}$(OAc)Me), 73.5 (C-3), 72.8 (C-5), 70.6 (OC$H_2$*C*$H_2$O), 70.4 (C-4), 70.0 (*C*$H_2$C$H_2$N-triaz), 69.4 (O*C*$H_2$C$H_2$O), 63.0 (C-6), 55.8 (C-2), 51.3* (*C*$H_2$N-triaz), 45.1 ($CH_2$*C*$H_2$NH-edans), 40.5 (N(*C*$H_3$)$_2$), 39.8 (*C*$H_2$C$H_2$NH-edans), 36.3* (*C*$H_2$NH-dabcyl), 23.0 (*C*$H_3$CONHR), 21.4, 20.7, 20.6, 20.5 (4×$CH_3$CO), 14.5 (*C*$H_3$) ppm. **HR-ESI-MS** calcd for $C_{54}H_{67}N_{10}O_{18}S$ $[M+H]^+$ 1175.4350, found 1175.4301; calcd for $C_{54}H_{66}N_{10}NaO_{18}S$ $[M+Na]^+$ 1197.4169, found 1197.4120; calcd for $C_{54}H_{68}N_{10}O_{18}S$ $[M+2H]^{2+}$ 588.2211, found 588.2223; calcd for $C_{54}H_{66}N_{10}Na_2O_{18}S$ $[M+2Na]^{2+}$ 610.2031, found 610.2034.

**: signal did not emerge from $^{13}$C experiment. Assignment from 2D correlations (HSQC and HMBC)*

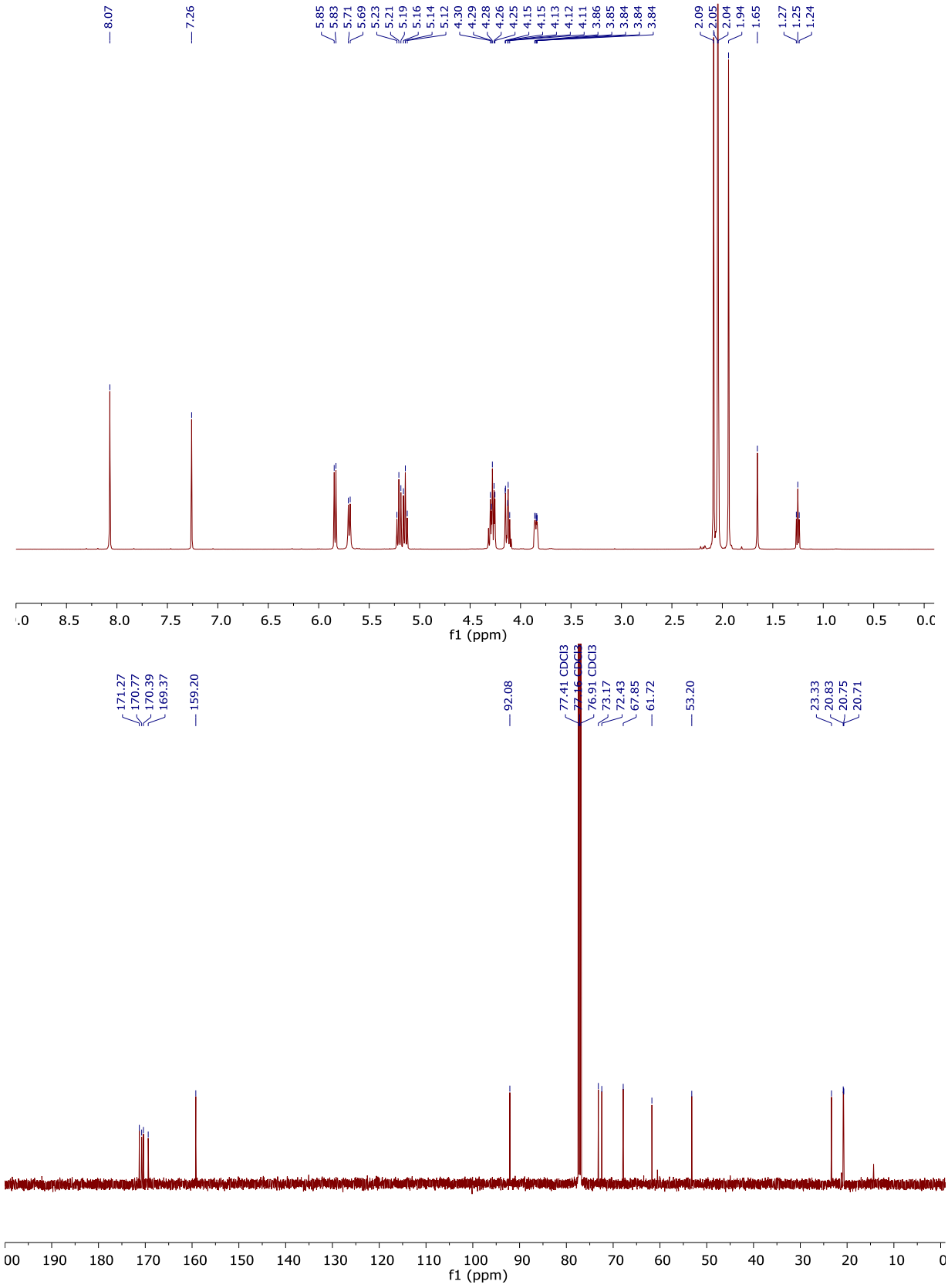
**GlcNAc-OH-BABS (13)**



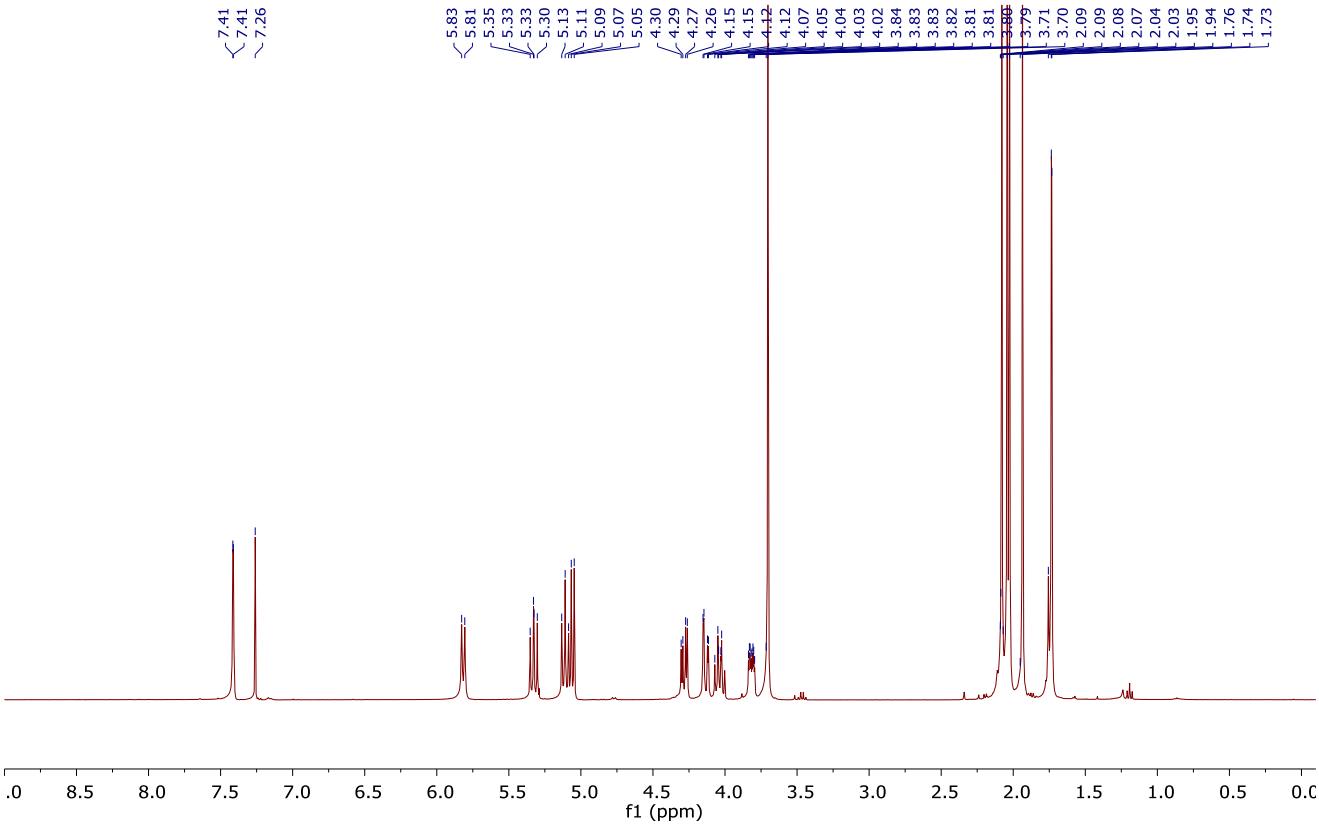
A 10 mL round-bottom flask containing acetylated GlcNAc-OAc-BABS **12** (20 mg) was flushed with argon. Anhydrous methanol (3 mL) was then added. Sodium methoxide (*ca*. 5 mg) was added and the mixture was stirred at r.t. overnight. The crude was then concentrated and dissolved in water (2 mL). This crude was purified thanks to semi-preparative scale HPLC (C-18, $H_2O$:MeCN, 15 to 55 % MeCN gradient over 30 minutes, 2 mL.min$^{-1}$). Lyophilisation of the fractions containing the pure product provided the pure GlcNAc-OH-BABS **13** (12.9 mg) as an orange fluffy powder. **HPLC**

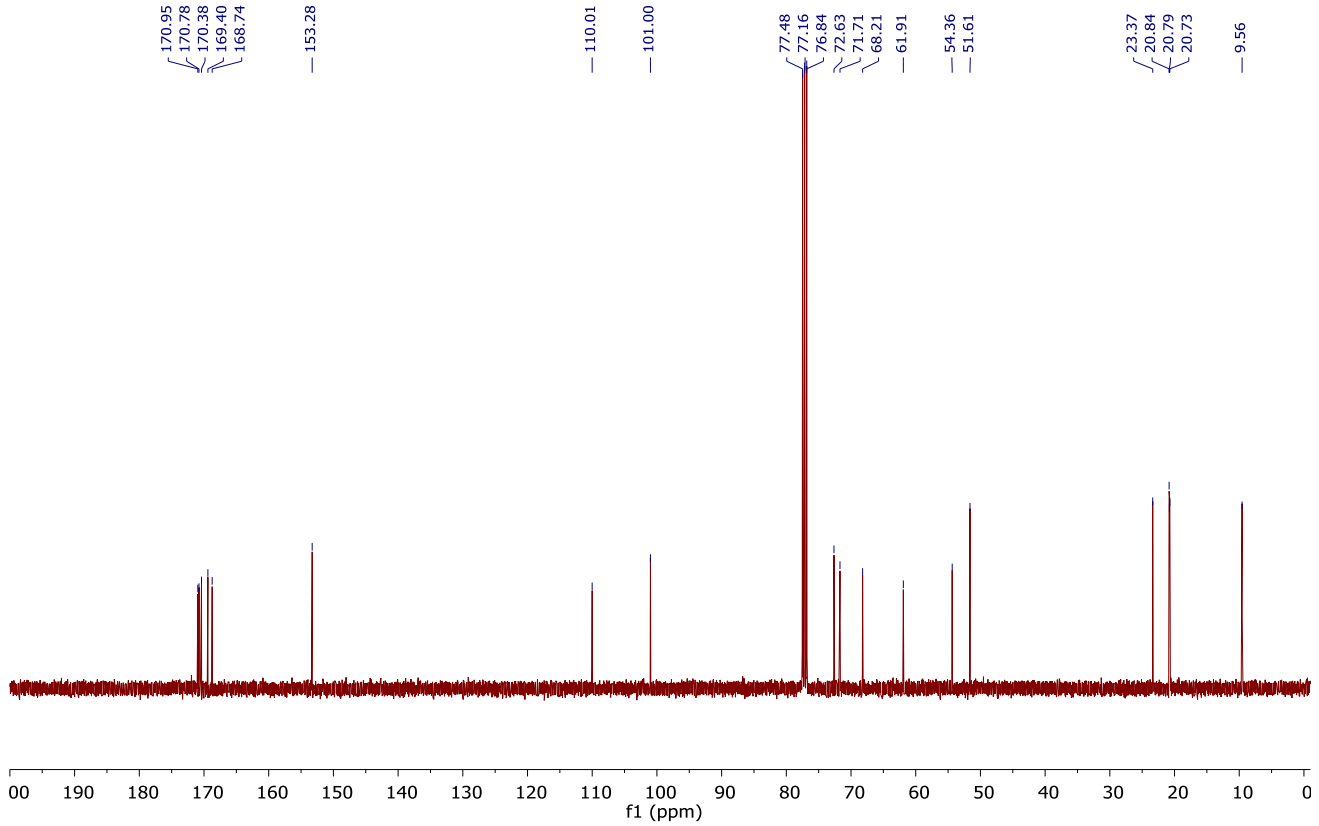
**retention time** = 18.5 mins (C18 Semi-Prep, $H_2O$:MeCN, 95:5 to 40:60 over 30 minutes, 2mL.min$^{-1}$). **$^1$H NMR (600 MHz, MeOD)** $\delta$ 8.25 – 8.16 (m, 2H, CH-edans), 8.13 (dd, *J* = 7.2 Hz, *J* = 1.2 Hz, 1H, CH-edans), 8.03 – 7.95 (m, 2H, CH-dabcyl), 7.88 (s, 1H, H-triaz), 7.87 – 7.82 (m, 4H, CH-dabcyl), 7.42 – 7.34 (m, 2H, CH-edans), 6.85 – 6.80 (m, 2H, CH-dabcyl), 6.64 (d, *J* = 7.7 Hz, 1H, CH-edans), 4.68 (s, 3H, OC*H*(OR), C*H*$_2$NHC(O)-dabcyl), 4.53 (d, *J* = 8.4 Hz, 1H, H-1), 4.44 – 4.35 (m, 2H, C*H*$_2$-Ntriaz), 3.87 – 3.80 (m, 2H, ½ OC*H*$_2$CH$_2$O, H-6a), 3.74 (dd, *J* = 10.4 Hz, *J* = 8.4 Hz, 1H, H-2), 3.72 – 3.68 (m, 1H, ½ C*H*$_2$CH$_2$NH-edans), 3.64 – 3.56 (m, 4H, H-6b, ½ OC*H*$_2$CH$_2$O, C*H*$_2$CH$_2$N-triaz), 3.49 – 3.44 (m, 1H, ½ C*H*$_2$CH$_2$NH-edans), 3.44 – 3.41 (m, 1H, H-3), 3.39 – 3.32 (m, 4H, CH$_2$C*H*$_2$NH-edans, OCH$_2$C*H*$_2$O), 3.28 – 3.25 (m, 2H, H-5, H-4), 3.10 (s, 6H, N(C*H*$_3$)$_2$), 1.97 (s, 3H, C*H*$_3$CONHR), 1.26 (s, 3H, C*H*$_3$) ppm. **$^{13}$C NMR (150 MHz, MeOD)** $\delta$176.7 (C(O)NH-edans), 174.2 (CH$_3$*C*ONHR), 169.5 (NHC(O)-dabcyl), 156.5 (C$^{IV}$-dabcyl), 154.7 (C$^{IV}$-dabcyl), 146.2 (C$^{IV}$-triaz), 145.5 (C$^{IV}$-edans), 144.8 (C$^{IV}$-dabcyl), 142.1 (C$^{IV}$-edans), 135.5 (C$^{IV}$-dabcyl), 131.5 (C$^{IV}$-edans), 129.5 (CH-dabcyl), 128.5 (CH-edans), 126.7 (CH-edans), 126.4 (CH-dabcyl), 125.6 (C$^{IV}$-edans), 125.5 (CH-edans), 125.1 (CH-triaz), 123.7 (CH-edans), 123.0 (CH-dabcyl), 116.5 (CH-edans), 112.6 (CH-dabcyl), 107.1 (O*C*H(OR)), 105.1 (CH-edans), 102.0 (C-1), 78.1 (C-5), 77.8 (C$^{IV}$(OH)Me), 75.7 (C-3), 72.1 (C-4), 71.2 (OCH$_2$*C*H$_2$O), 70.2 (*C*H$_2$CH$_2$N-triaz), 69.6 (O*C*H$_2$CH$_2$O), 62.8 (C-6), 57.5 (C-2), 51.2 (*C*H$_2$N-triaz), 45.0 (CH$_2$*C*H$_2$NH-edans), 40.4 (N(*C*H$_3$)$_2$), 39.7 (*C*H$_2$CH$_2$NH-edans), 36.3 (*C*H$_2$NH-dabcyl), 23.4 (*C*H$_3$CONHR), 22.1 (*C*H$_3$) ppm. **HR-ESI-MS** calcd for $C_{46}H_{59}N_{10}O_{14}S$ $[M+H]^+$ 1007.3927, found 1007.3911; calcd for $C_{46}H_{58}N_{10}NaO_{14}S$ $[M+Na]^+$ 1029.3747, found 1029.3724; calcd for $C_{46}H_{60}N_{10}O_{14}S$ $[M+2H]^{2+}$ 504.2000, found 504.2001; calcd for $C_{46}H_{58}N_{10}Na_2O_{14}S$ $[M+2Na]^{2+}$ 526.1819, found 526.1813.

# $^{1}H$ and $^{13}C$ NMR spectra of compound **1**

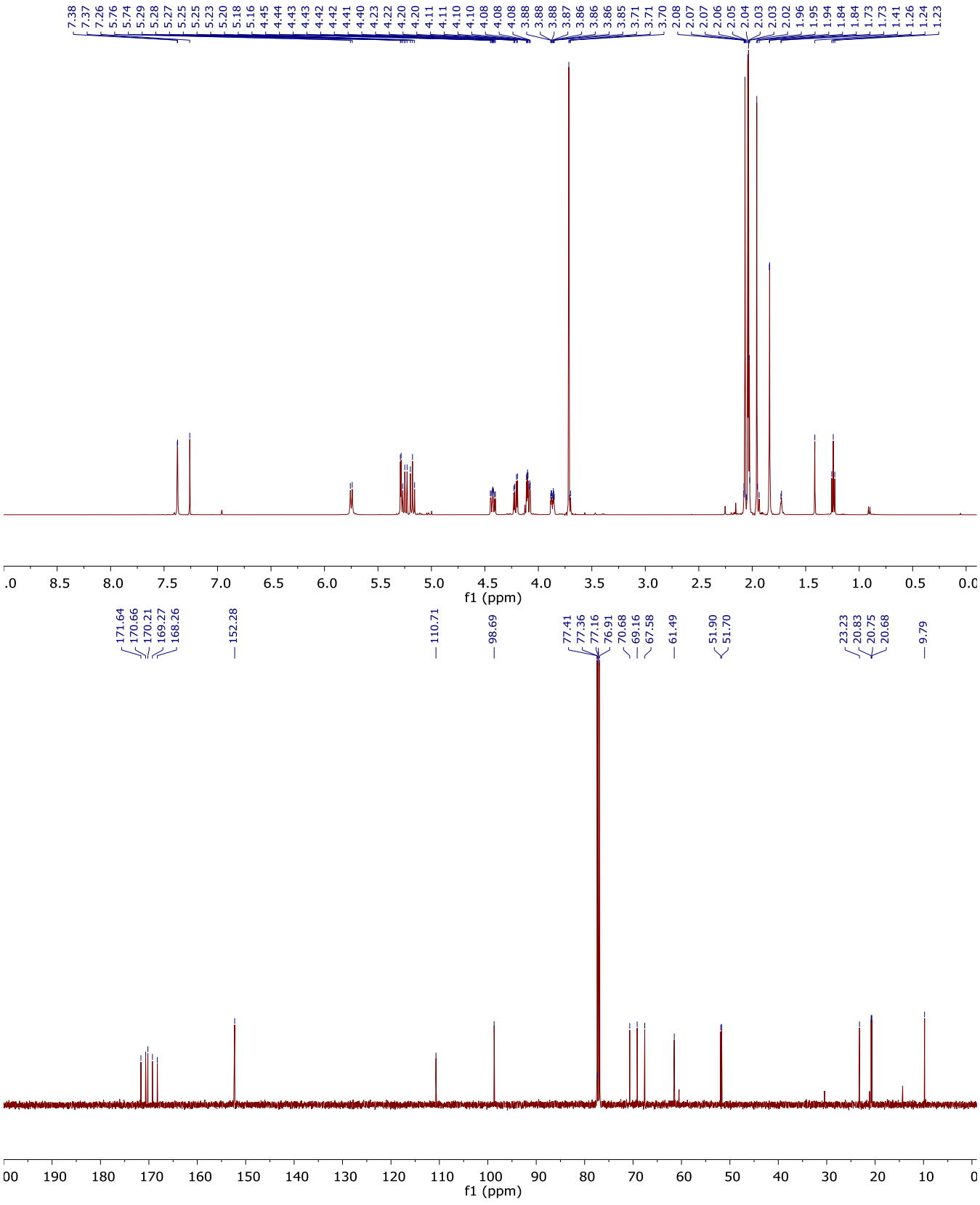


# $^{1}H$ and $^{13}C$ NMR spectra of compound **2E**

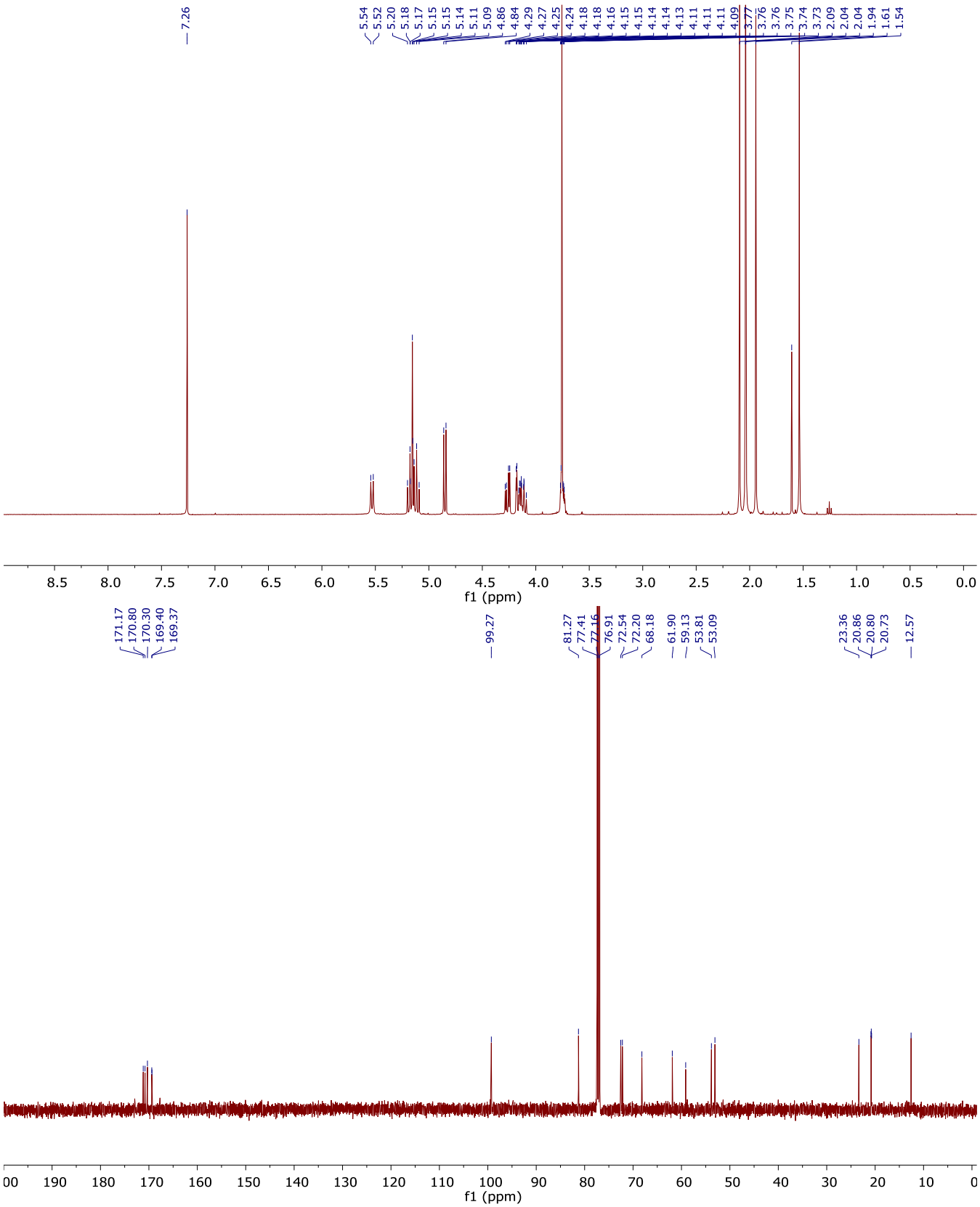


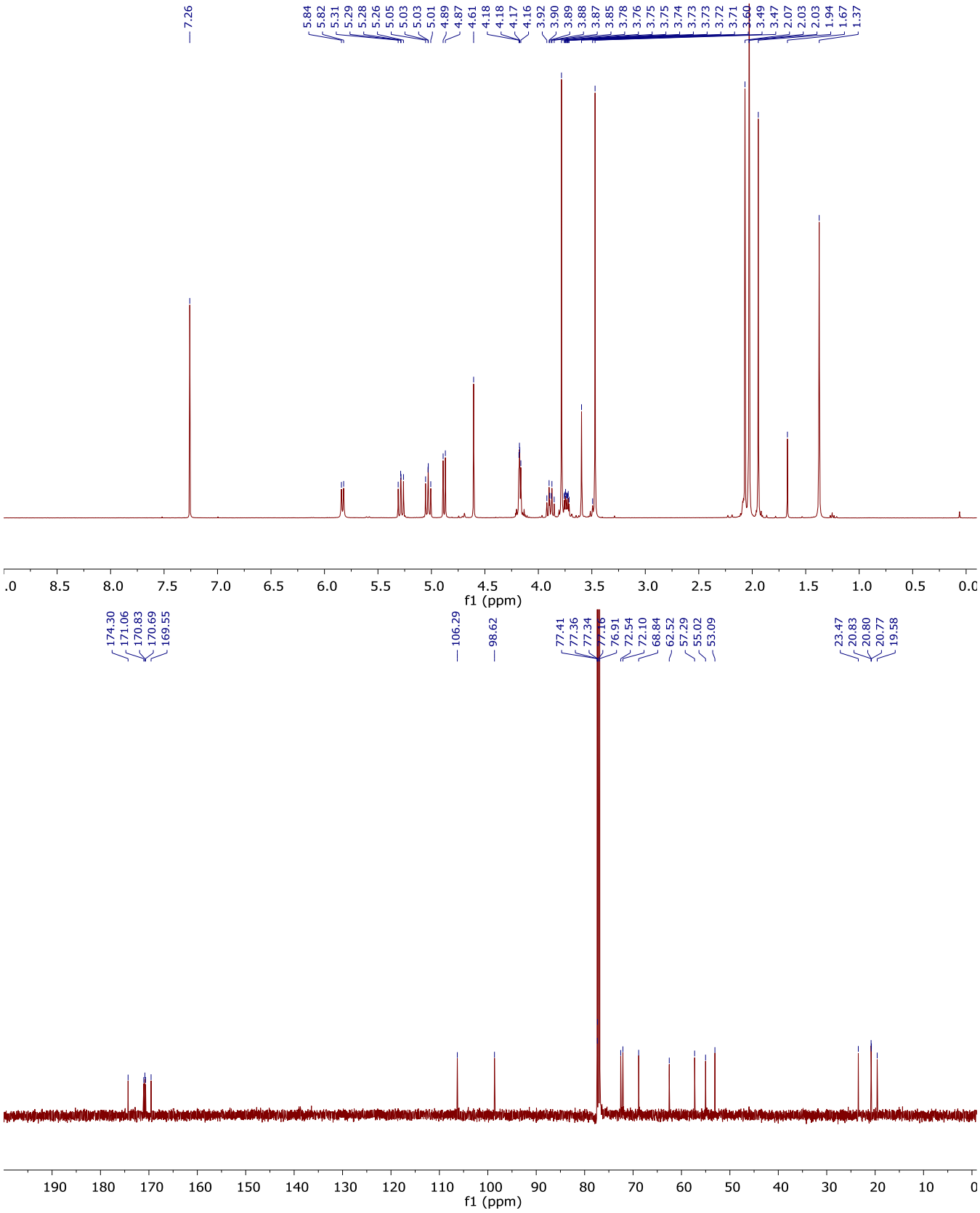
# $^{1}H$ and $^{13}C$ NMR spectra of compound **2Z**



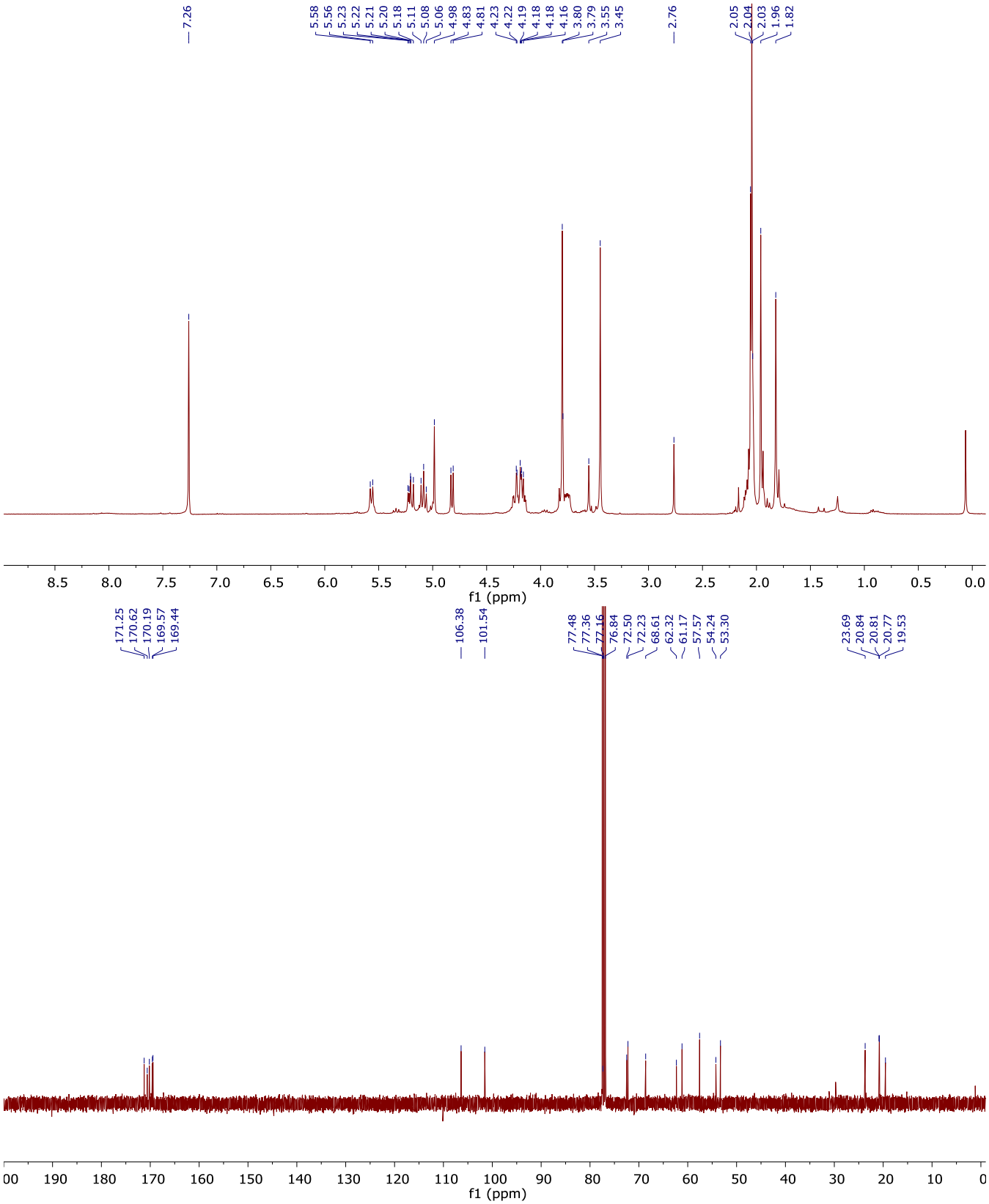
# $^{1}H$ and $^{13}C$ NMR spectra of compound **3**



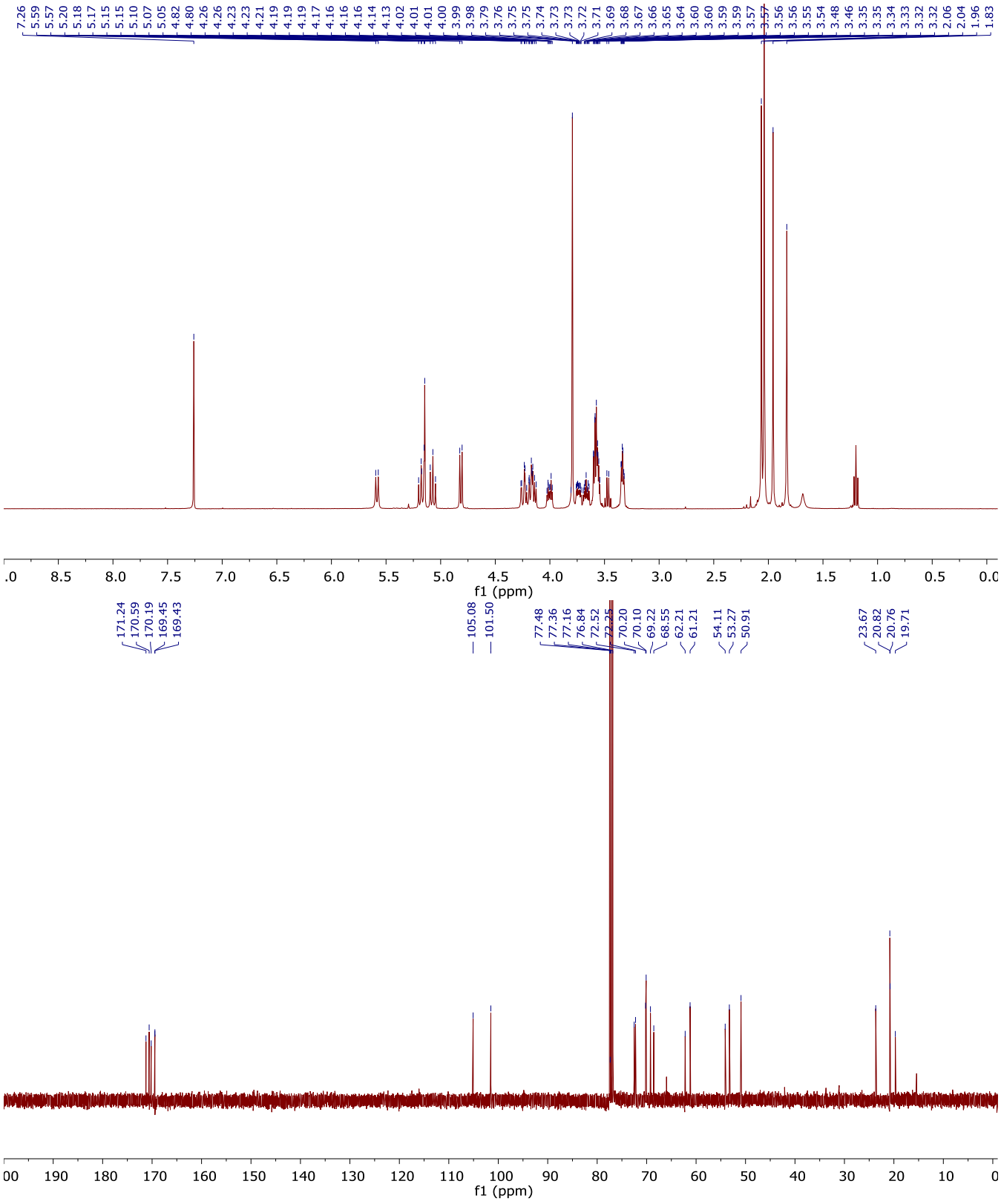
$^{1}H$ and $^{13}C$ NMR spectra of compound **4**



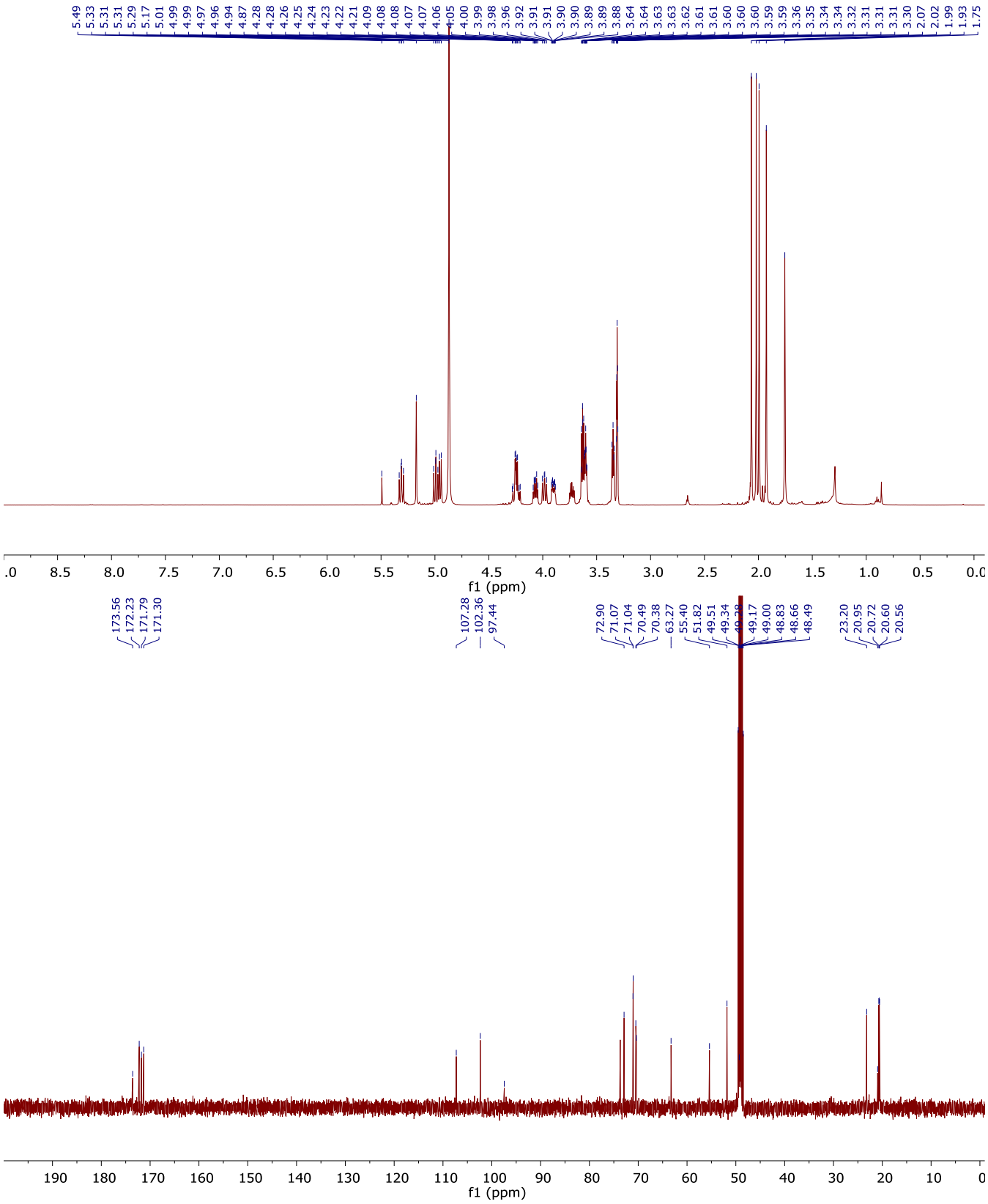
# $^{1}H$ and $^{13}C$ NMR spectra of compound **5**



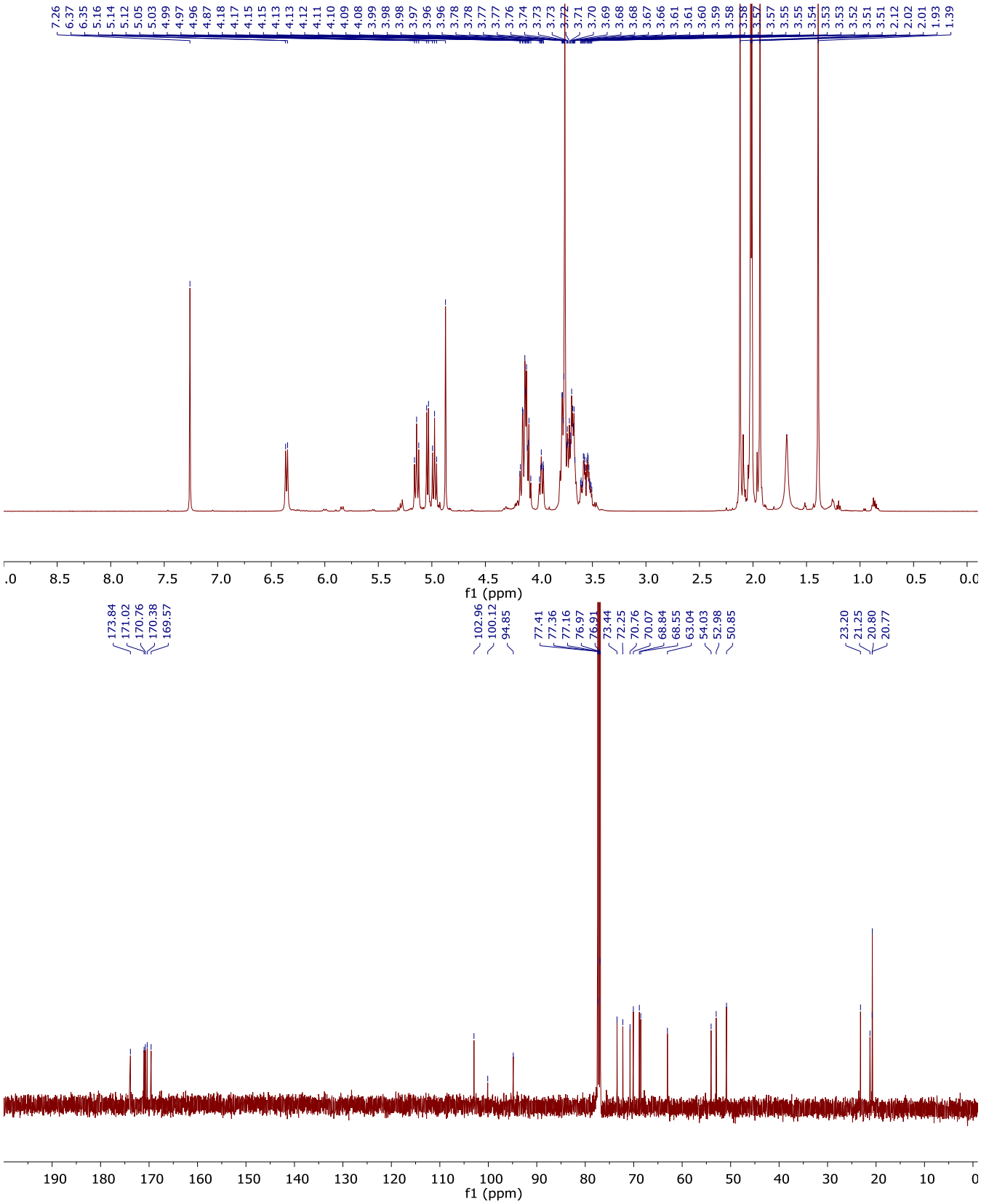
$^{1}$H and $^{13}$C NMR spectra of compound **6**



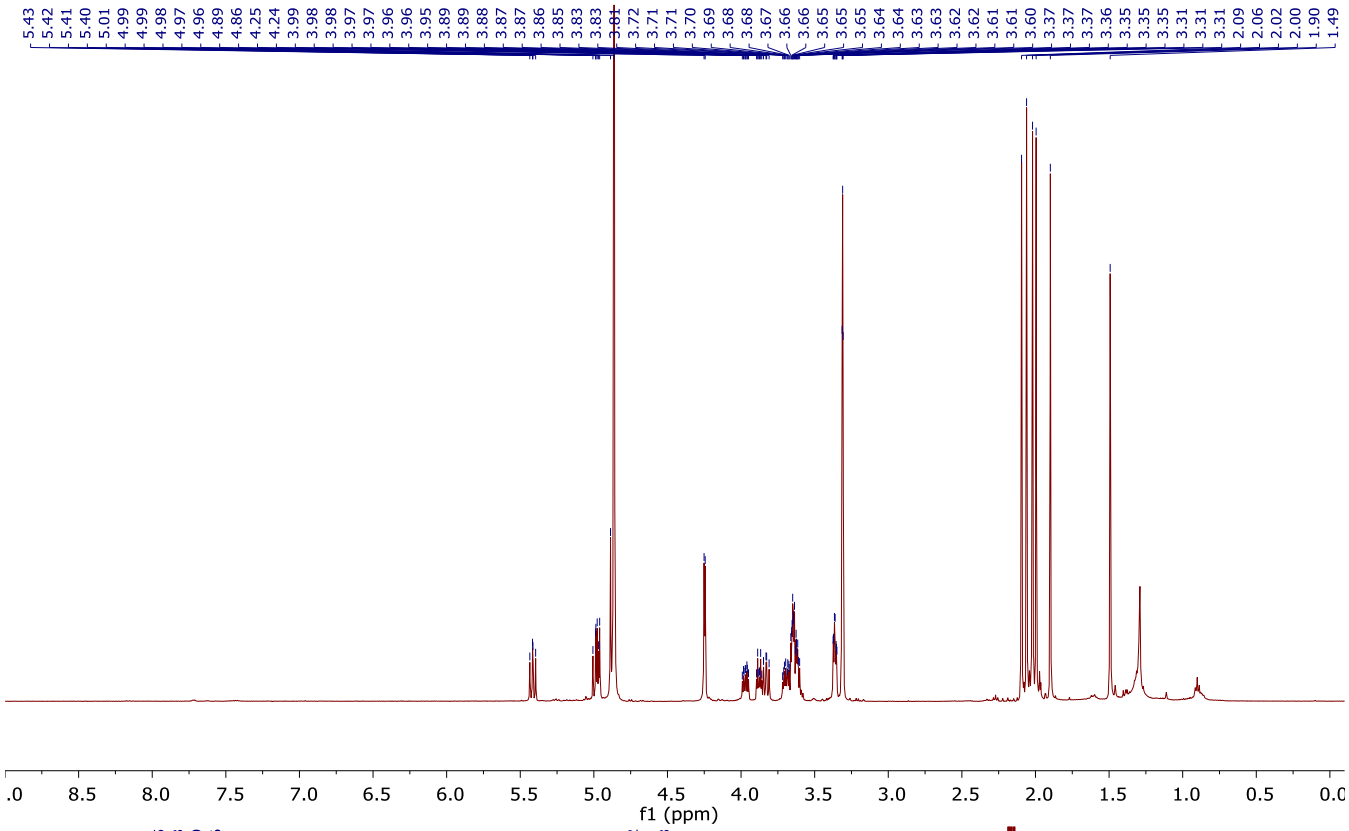
# $^{1}H$ and $^{13}C$ NMR spectra of compound **7**

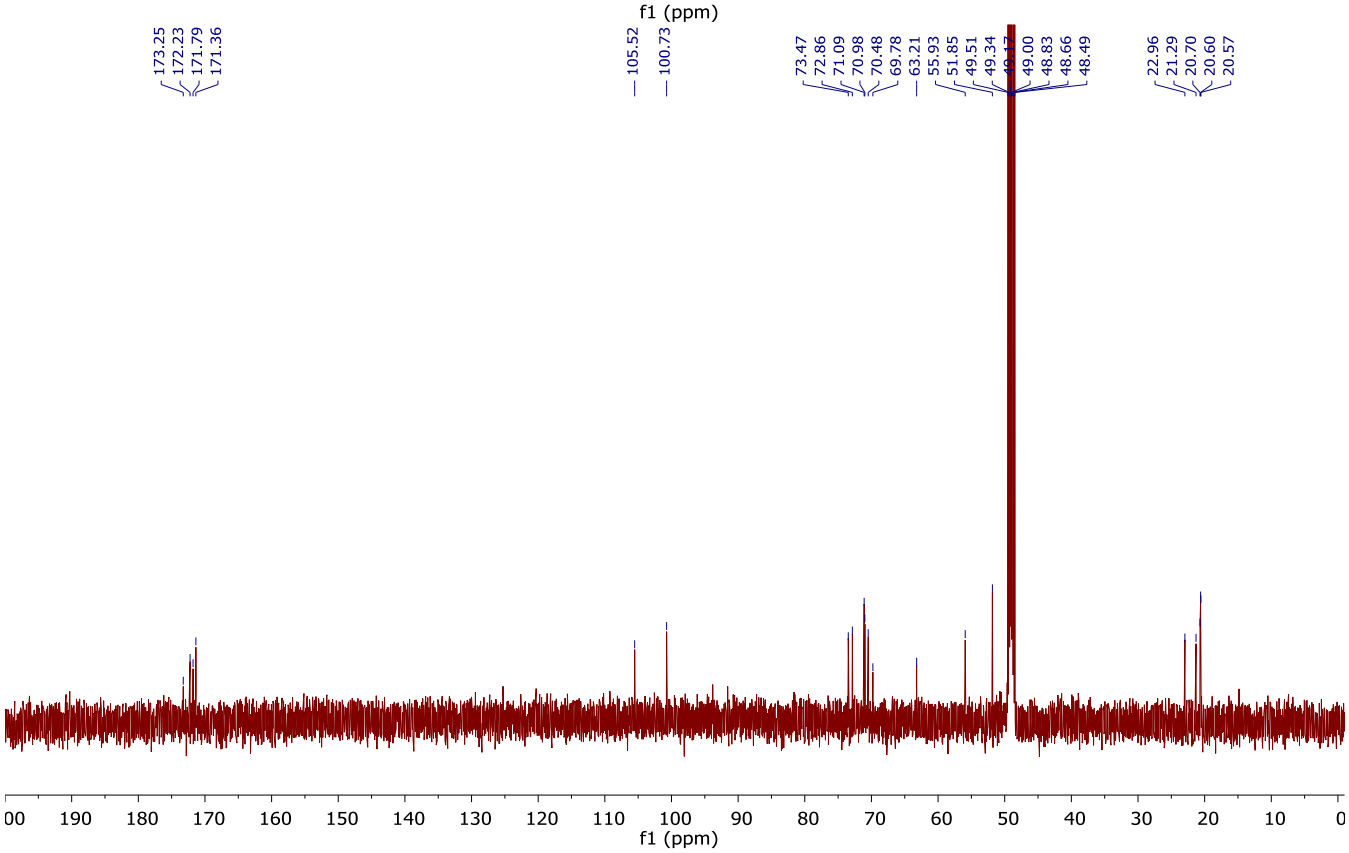


# $^{1}H$ and $^{13}C$ NMR spectra of compound **8**

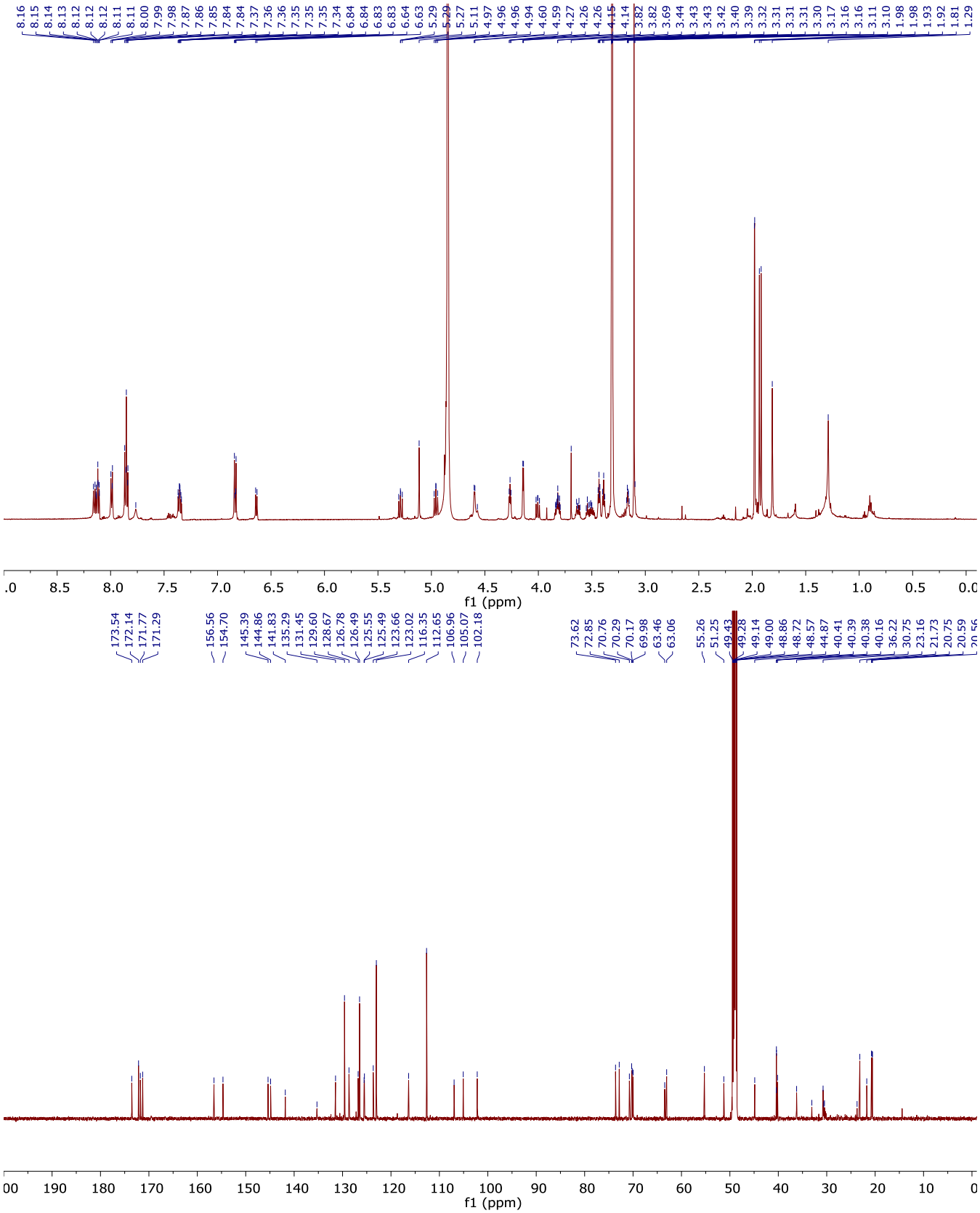


# $^{1}H$ and $^{13}C$ NMR spectra of compound **9**

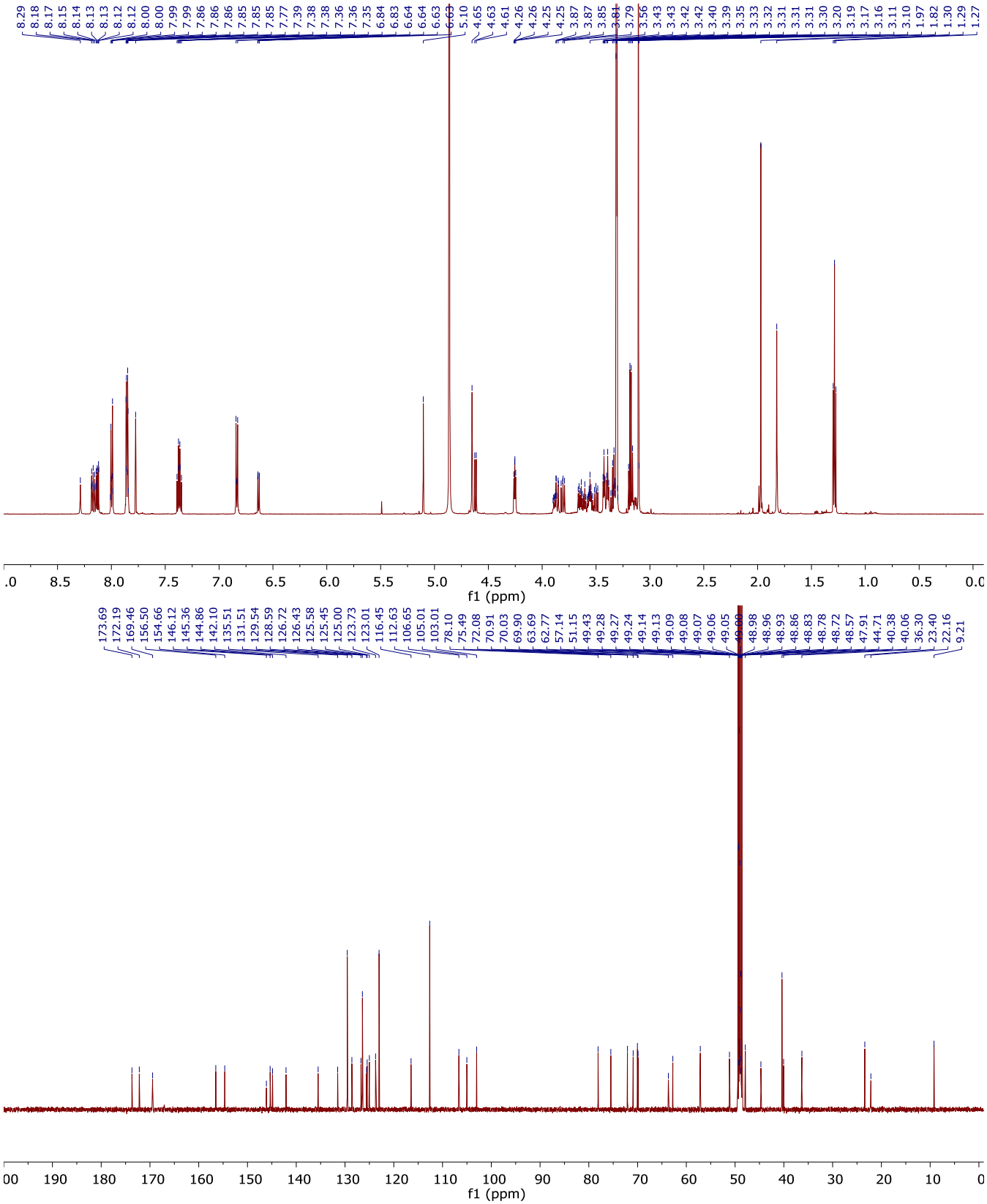


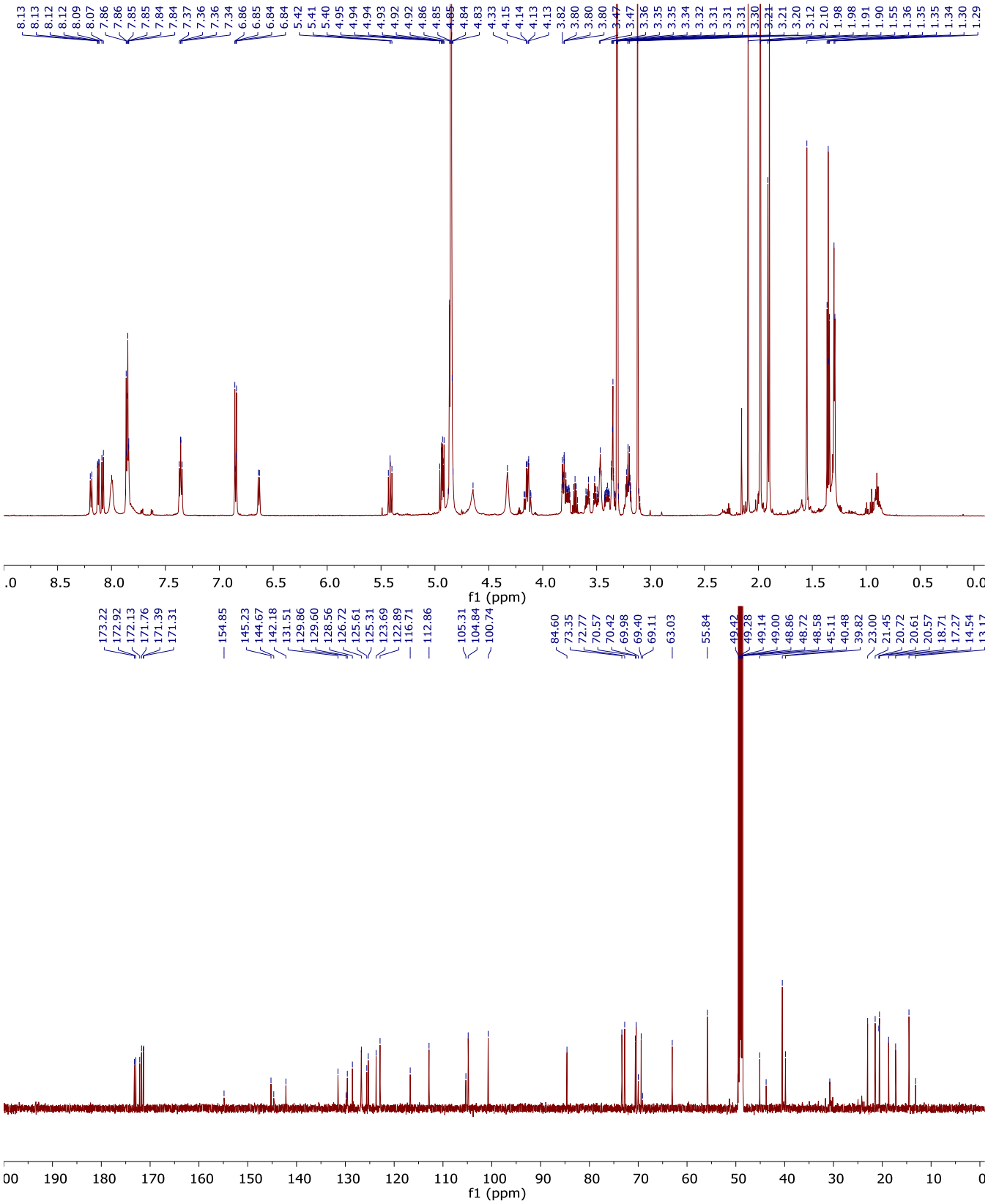
# $^{1}H$ and $^{13}C$ NMR spectra of compound **10**



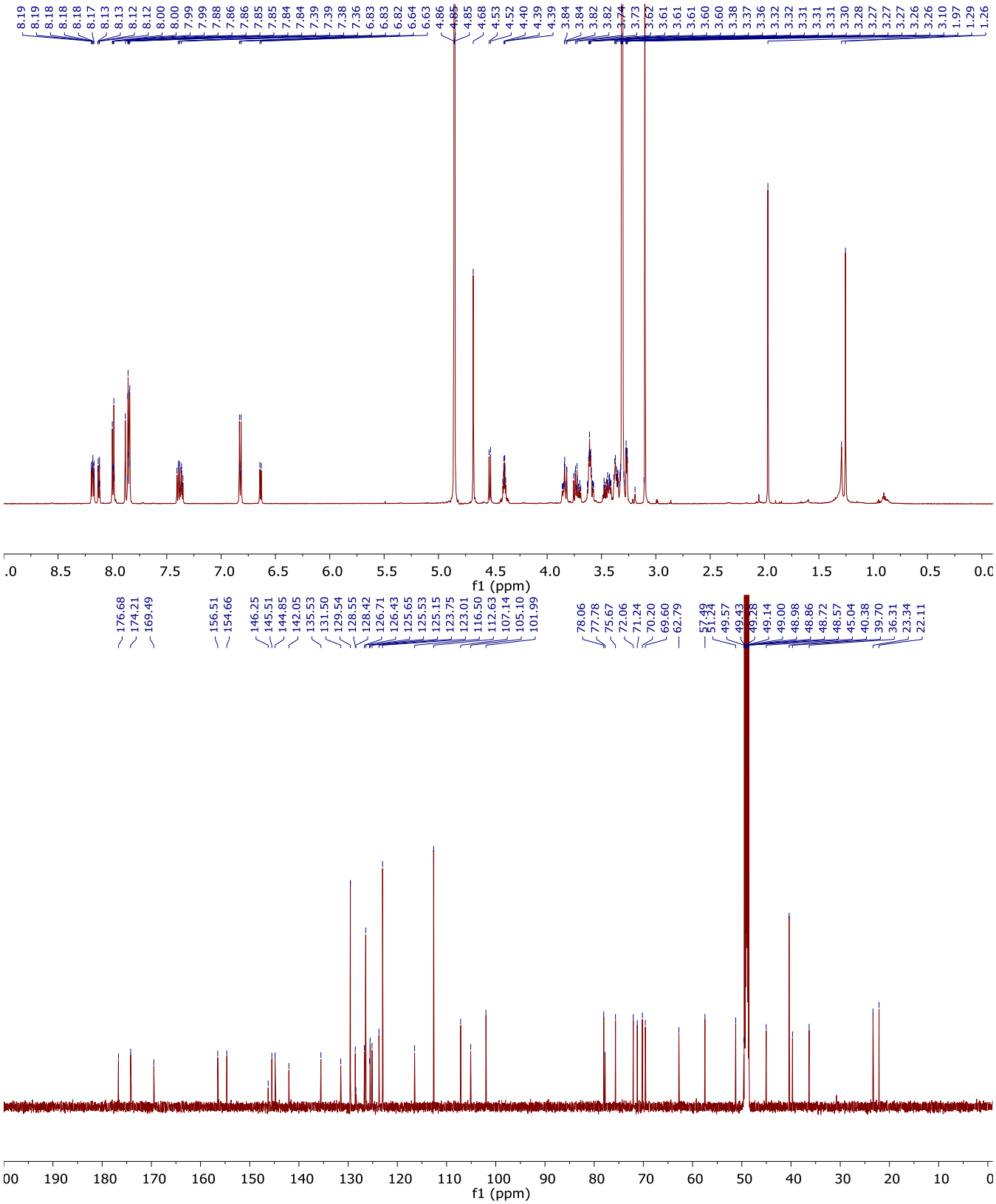
# $^{1}H$ and $^{13}C$ NMR spectra of compound **11**



# $^{1}H$ and $^{13}C$ NMR spectra of compound **12**



# $^{1}H$ and $^{13}C$ NMR spectra of compound **13**



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