Supporting Information

Stereocontrolled synthesis of polyhydroxylated bicyclic azetidines as new class of iminosugars

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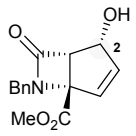
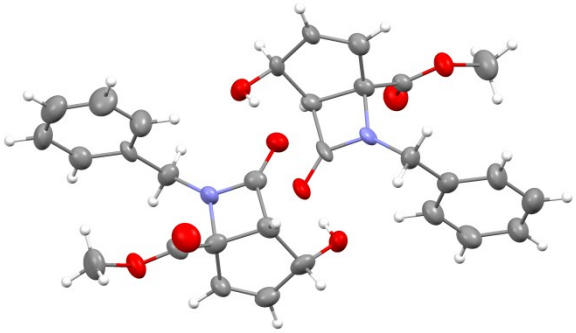
Structure of 7a determined by X-ray diffraction



7a



Formula: $C_{15}H_{15}N_1O_4$

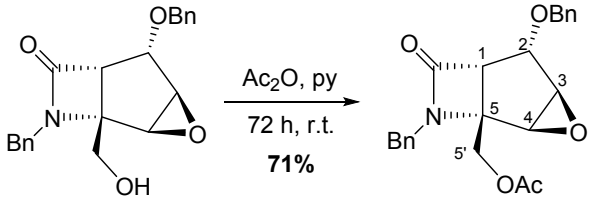
Unit Cell Parameters: a 6.2246(2) b 26.4216(12) c 8.1454(4) P21

Thermal ellipsoids at 50% probability, hydrogen atoms drawn as spheres of 0.15Å radius for clarity

The crystal structure has been deposited at the Cambridge Crystallographic Data Centre (CCDC 1832560). The data is available free of charge at www.ccdc.cam.uk/conts/retrieving.html

Synthesis of epoxy acetate 21' for NOESY analysis

In order to assess the stereochemistry of the epoxidation, acetate **21'** was obtained by esterification of compound **21**:



21 **21'**

To a solution of epoxide **21** (8.0 mg, 0.02 mmol, 1 equiv) in Ac_2O (0.20 mL, 2 mmol, 100 equiv) was added pyridine (0.20 mL, 2.4 mmol, 120 equiv). The solution was stirred at r.t. for 72 h. Water (3 mL) was added slowly at 0 °C. The aqueous phase was extracted with CH_2Cl_2 (3 x 4 mL). The combined organic layers were then washed with aqueous 2M HCl (2 x 2 mL) and saturated aqueous $NaHCO_3$ (2 mL), dried over Na_2SO_4, filtered and concentrated giving acetate **21'** (6 mg, 0.015 mmol, 71 %) with analytical purity as yellowish oil.

R_f 0.19 (petroleum ether/ethyl acetate, 2:1).

IR (film) 2918, 1752, 1736, 1246, 1230, 1096, 1037 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.46-7.42 (m, 2H, H-Ph), 7.40-7.28 (m, 8H, H-Ph), 4.98 (d, *J*= 11.3 Hz, 1H, $PhCH_2O$), 4.74 (d, *J*= 15.3 Hz, 1H, $PhCH_2N$), 4.53 (d, *J*= 11.3 Hz, 1H, $PhCH_2O$), 4.52 (d, *J*= 11.6 Hz, 1H, CH_2OAc), 4.23-4.11 (m, 3H, $PhCH_2N$, H-2, CH_2OAc), 3.65 (d, *J*= 7.4 Hz, 1H, H-1), 3.49 (d, *J*= 2.1 Hz, 1H, H-3), 3.06 (d, *J*= 2.0 Hz, 1H, H-4), 2.05 (s, 3H, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.2 (C=O), 163.6 (C-7), 137.6 (Cq-Ar), 136.6 (Cq-Ar), 129.0 (CH-Ar), 128.7 (CH-Ar), 128.4 (2 CH-Ar), 128.2 (CH-Ar), 128.1 (CH-Ar), 75.0 (C-2), 73.0 ($PhCH_2O$), 68.3 (C-5), 62.2 (CH_2OAc), 61.6 (C-1), 60.4 (C-3), 57.2 (C-4), 44.7 ($PhCH_2N$), 20.7 (CH_3).

HRMS (ESI) *m/z* 394.163 ($[M+H]^+$, calcd. for $C_{23}H_{24}NO_5$: 394.165).

NOE interactions between H-4 and $PhCH_2N$ (see figure 22) probe that alkene epoxidation proceeded from the least hindered convex face.

Inhibition assays protocol & results

The glycosidases α-Galactosidase (from green coffee beans), β-Galactosidase (from *E. coli*), α-Glucosidase (from *Saccharomyces cerevisiae),* β-Glucosidase (from almonds), α-mannosidase (from Jack Bean) were purchased from Sigma Aldrich and their corresponding *p*-nitrophenyl α- or β-glycopyranoside were purchased from Fluorochem.

General procedure for inhibition assay

Inhibitory potencies were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against their respective *p*-nitrophenyl α- or β- glycopyranoside in the presence (at 1 mM) and absence of inhibitor. Kinetics were performed in appropriate buffer and were started by enzyme addition for a total well volume of 100 µL. After 15 to 40 min incubation at given temperature, the reaction was quenched by addition of 100 µL of 1 M Na_2CO_3. The absorbance of the resulting solutions were determined at 405 nm. For sake of convenience and consistency, 100 mM mother solutions were prepared in DMSO and 10 mM solutions with 10% DMSO were prepared in the proper buffer and used directly for a final DMSO concentration of 1% in all wells. Previously, the stability of the enzymes in presence of various concentrations of DMSO was controlled and the enzyme activity was unaffected.

Specific conditions for each enzyme are the following ones: α-glucosidase (0.067 M phosphate buffer, pH 6.8, 37 °C, K_m = 0.5 mM), β-glucosidase (0.1 M acetate buffer, pH 5, 37 °C, K_m = 3.9 mM), α-galactosidase (0.1 M phosphate buffer, pH 6.5, 26 °C K_m = 1 mM), β-galactosidase (0.05 M phosphate buffer with 1 mM $MgCl_2$, pH 7.3, 30 °C, K_m = 0.15 mM), α-mannosidase (0.2 M acetate buffer, pH 5, 25 °C, K_m = 2.0 mM)

Inhibition assays

Enzyme	**20a**	**20b**	**27**	**28**	Galafold™	1-deoxygulonojirimycin
α-Glucosidase (*Saccharomyces cerevisiae)*	31 ± 7	46 ±13	32 ± 10	66 ± 4	unknown	unknown
β-Glucosidase (almonds)	27 ± 14	28 ± 6	8 ± 5	25 ± 3	K_i = 540 µM[1]	< 50[2]
α-Galactosidase (green coffee beans)	46 ± 9	21 ± 2	19 ± 3	-16 ± 9	K_i = 0.013 µM[3] K_i = 0.0016 µM[1]	IC_{50} = 160µM[2] IC_{50} = 400 µM[4]
β-Galactosidase (*E. coli*)	-8 ± 0.5	-15 ± 2	n.a.	-16 ± 3	K_i = 13 µM[3], K_i = 12.5 µM[1]	unknown
α-mannosidase (Jack Bean)	n.a.	n.a.	14 ± 5	n.a.	< 50[2]	< 50[2]

Table 1. Inhibitory activity of compounds **20**, **27**, **28**, Galafold and 1-deoxygulonojirimycin. Percentage (%) of inhibition at 1 mM of inhibitor except noted otherwise. n.a. : no activity.

[1] G. Legler, S. Pohl, *Carbohydr. Res.* **1986**, *155*, 119–129.

[2] A. Kato, N. Kato, E. Kano, I. Adachi, K. Ikeda, L. Yu, T. Okamoto, Y. Banba, H. Ouchi, H. Takahata, *et al.*, *J. Med. Chem.* **2005**, *48*, 2036–2044.

[3] G. Schitter, E. Scheucher, A. J. Steiner, A. E. Stütz, M. Thonhofer, C. A. Tarling, S. G. Withers, J. Wicki, K. Fantur, E. Paschke, *et al.*, *Beilstein J. Org. Chem.* **2010**, *6*, 21.

[4] N. Asano, S. Ishii, H. Kizu, K. Ikeda, K. Yasuda, A. Kato, O. R. Martin, J. Fan, *Eur. J. Biochem.* **2000**, *267*, 4179–4186.

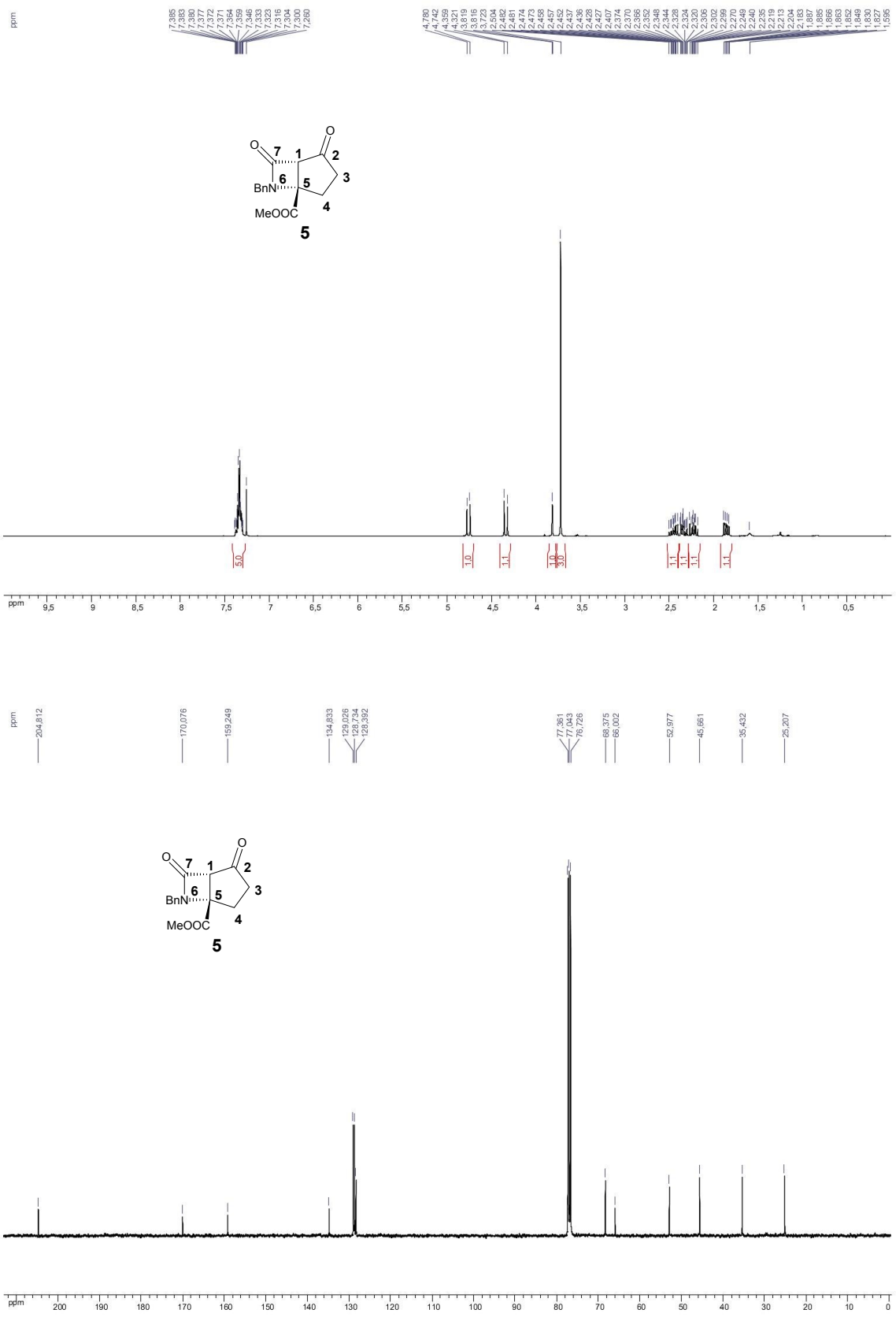


Fig 1. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100MHz, $CDCl_3$) spectra of compound **5**.

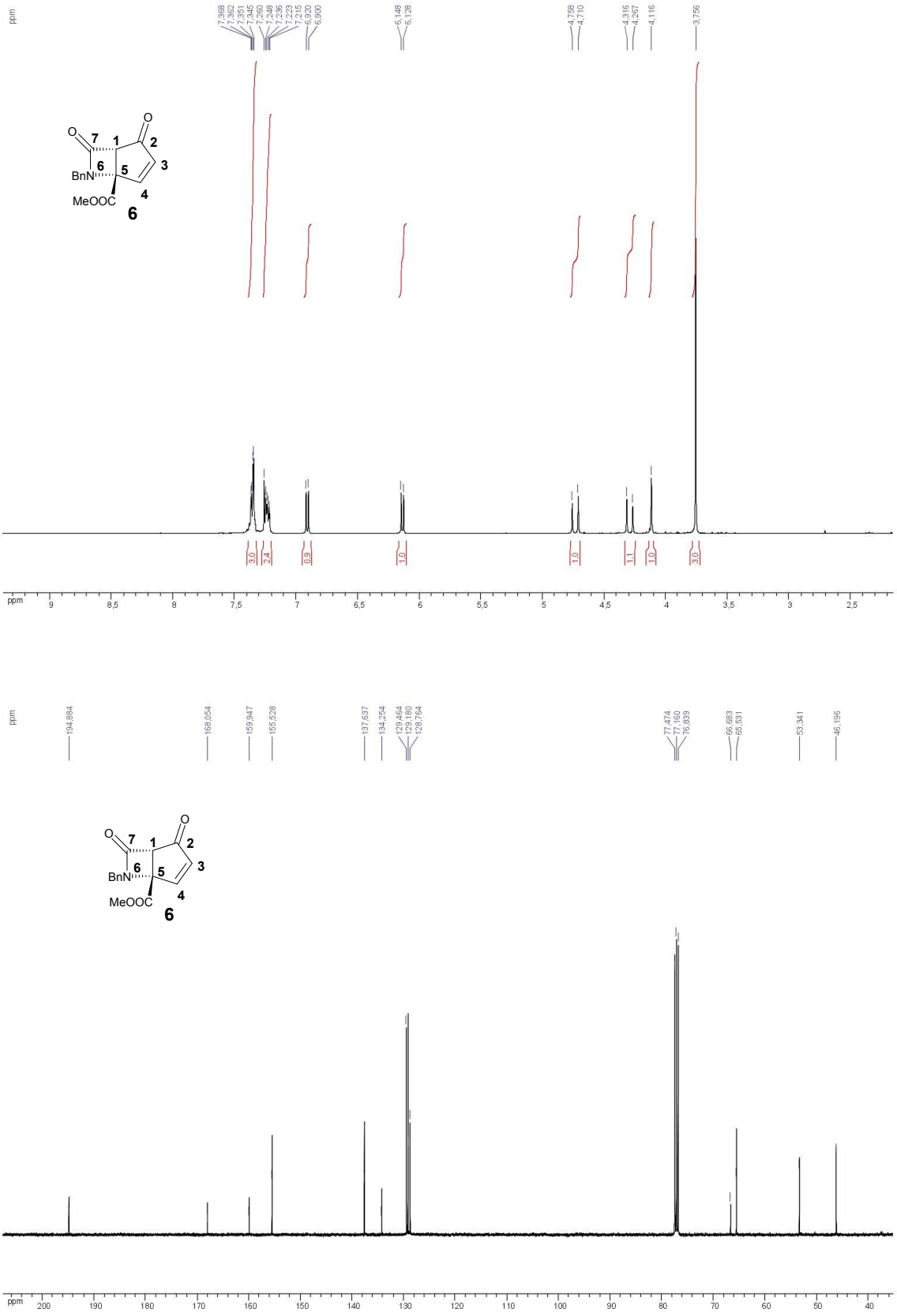


Fig 2. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **6**.

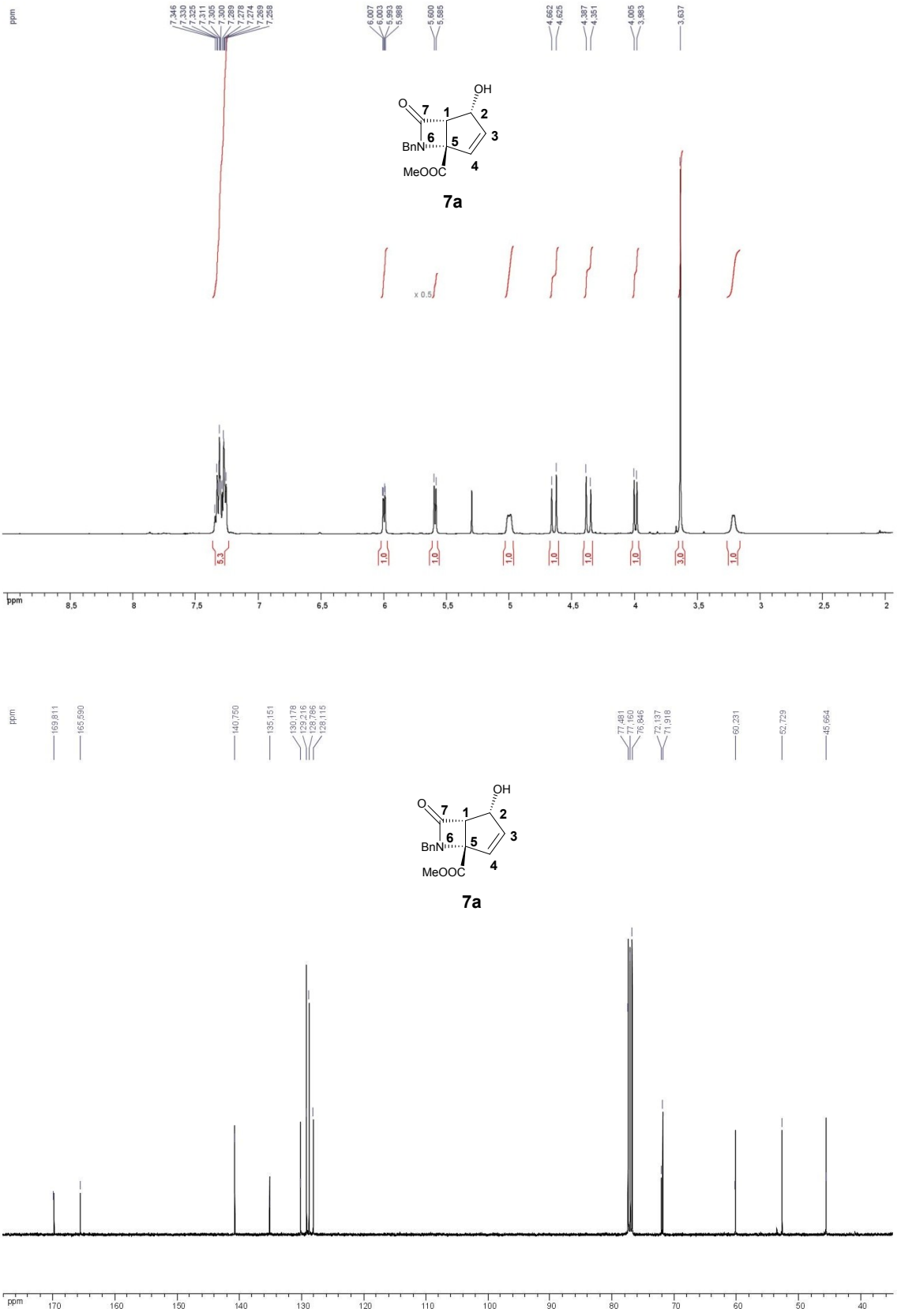


Fig 3. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **7a**.

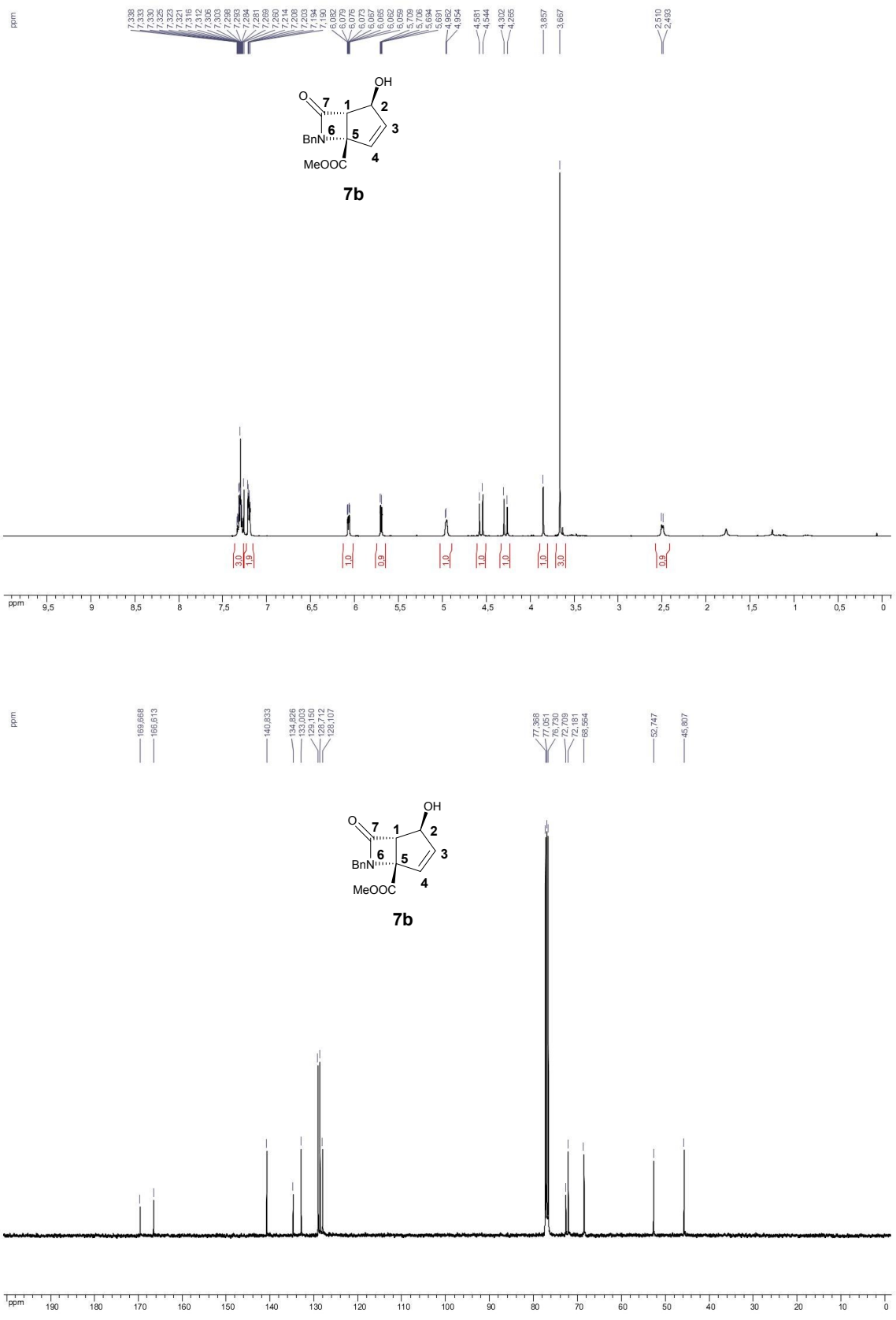


Fig 4. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **7b**.

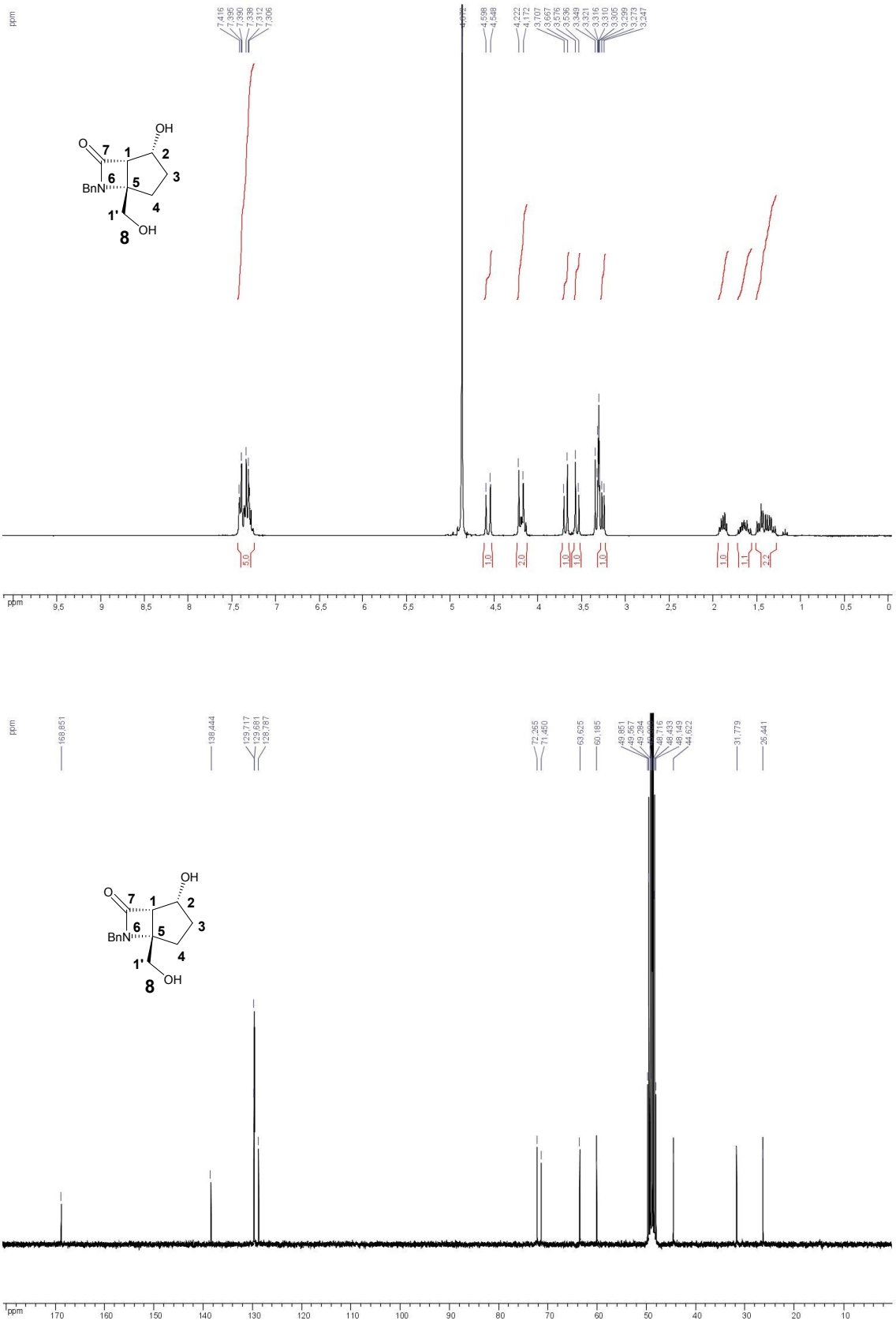


Fig 5. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C (75 MHz, CD_3OD) spectra of compound **8**.

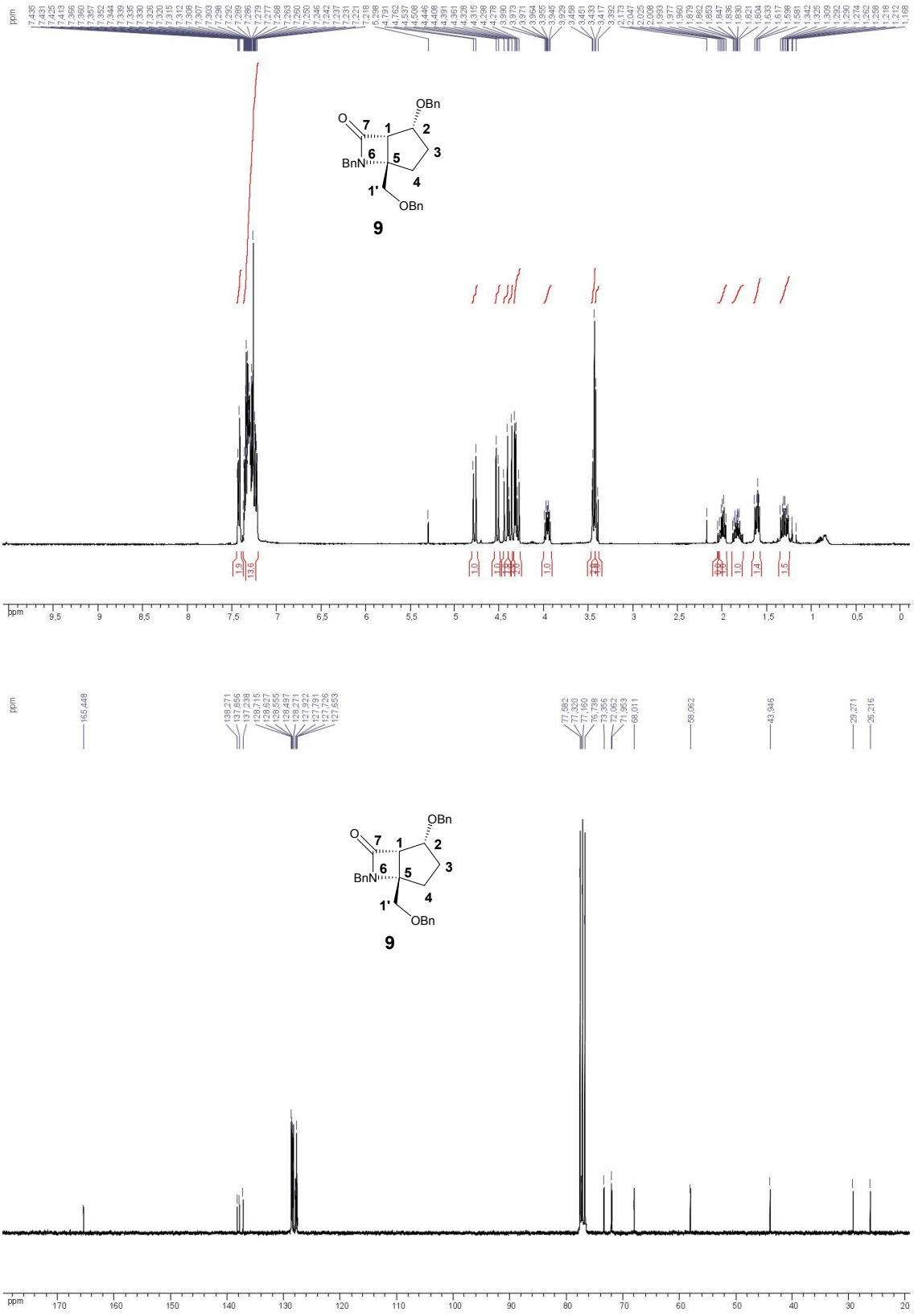


Fig 6. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C (75 MHz, CD_3OD) spectra of compound **9**.

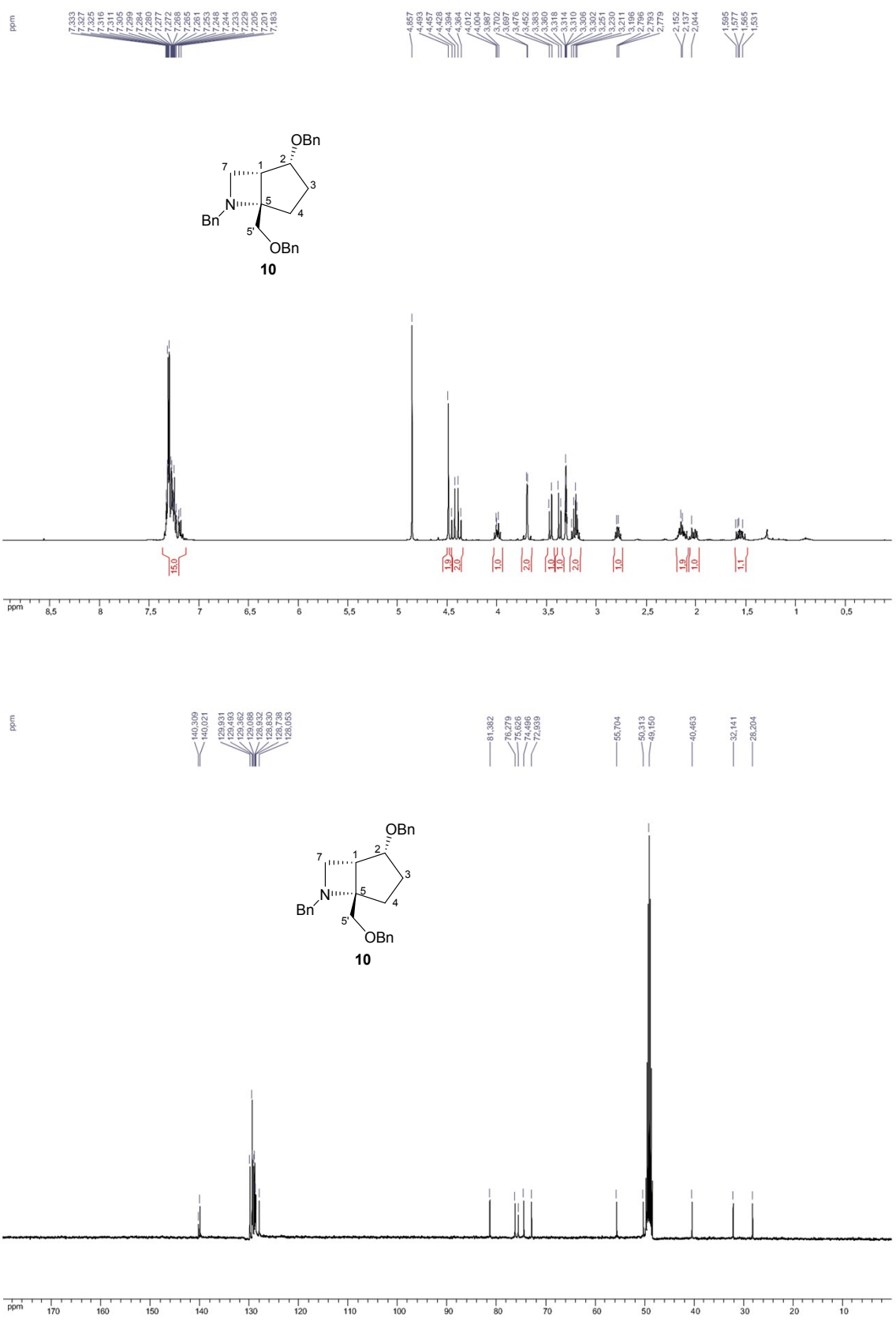


Fig 7. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) spectra of compound **10**.

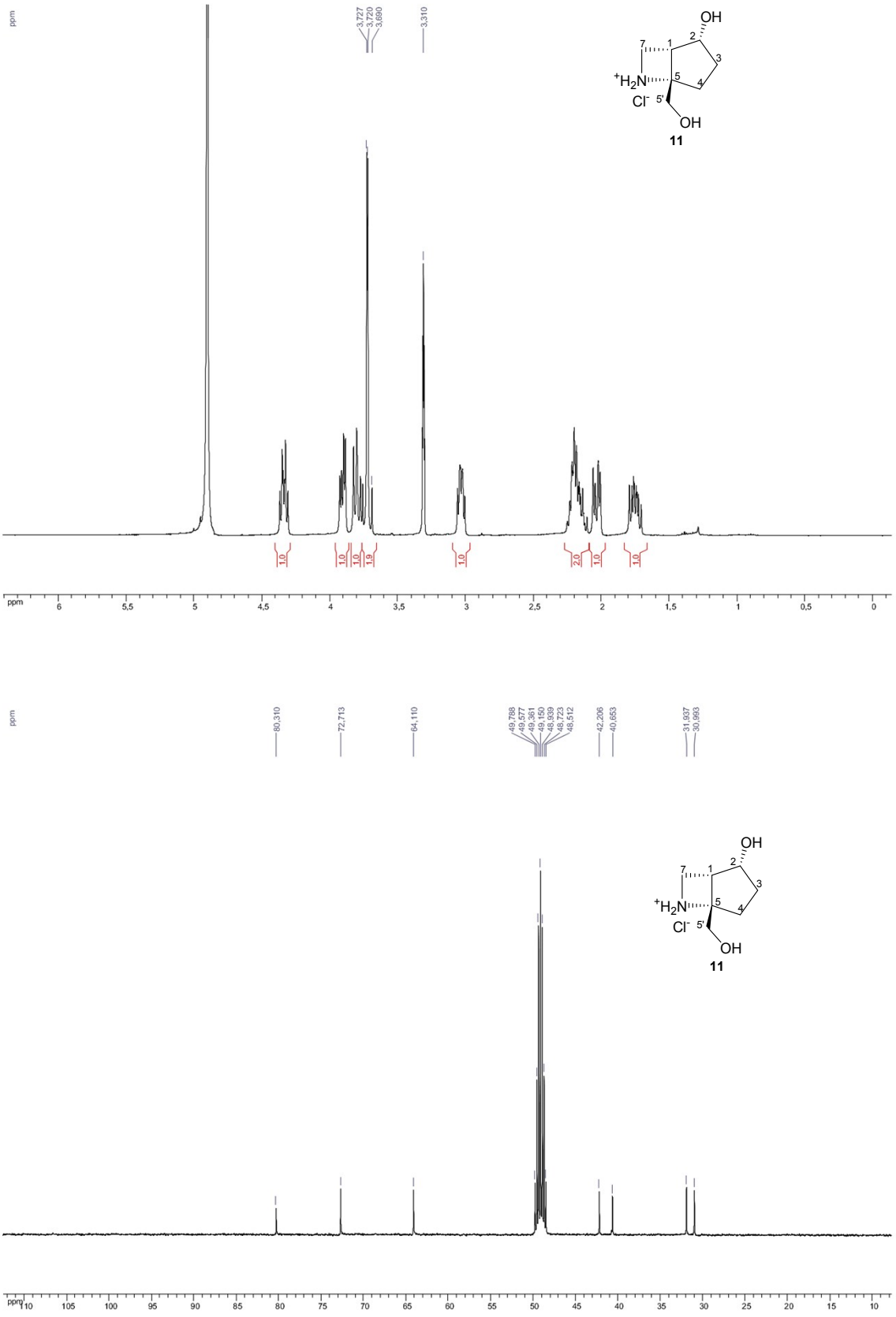


Fig 8. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) spectra of compound **11**.

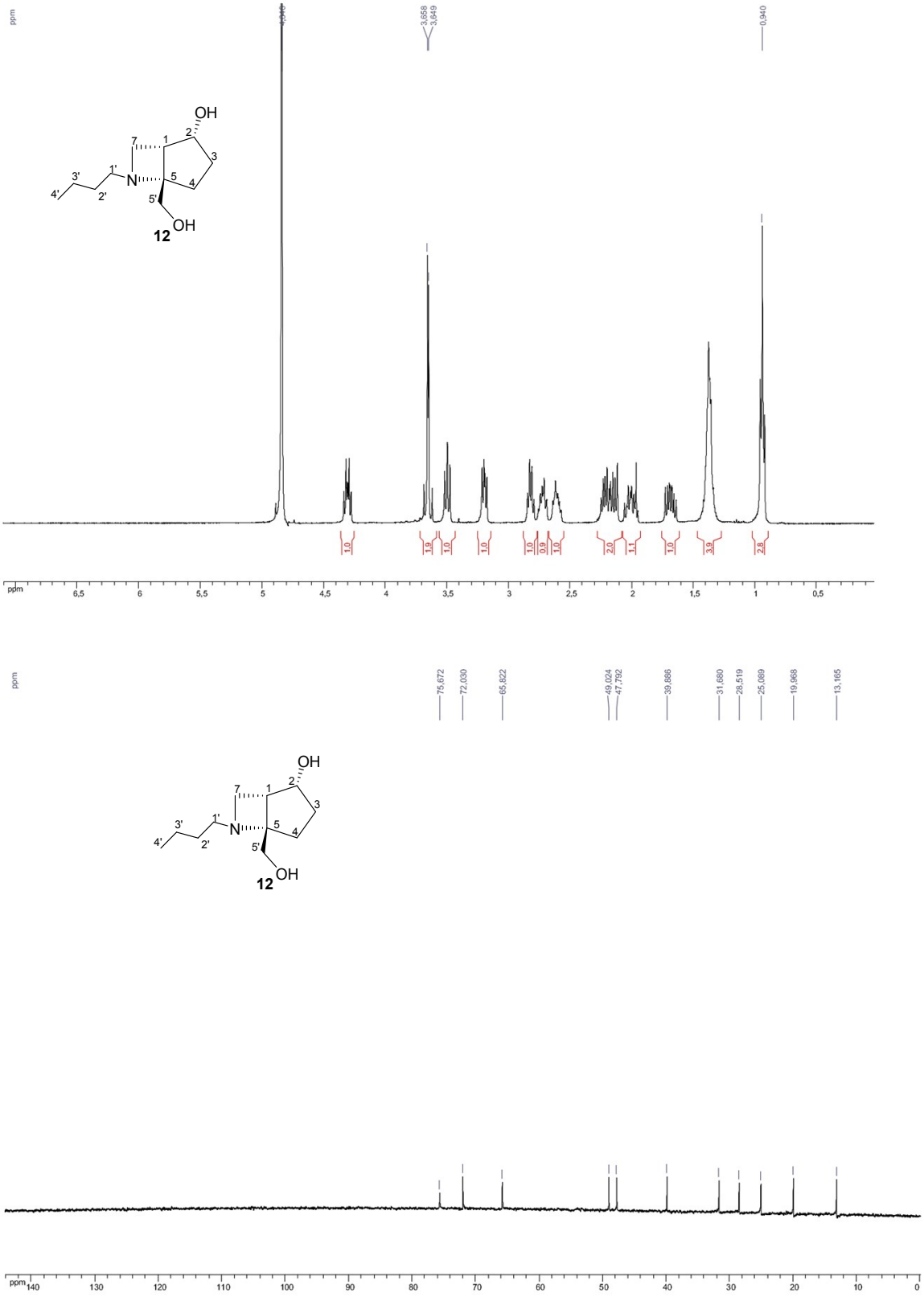


Fig 9. ^{1}H NMR (400 MHz, D_2O) and ^{13}C (100 MHz, D_2O) spectra of compound **12**.

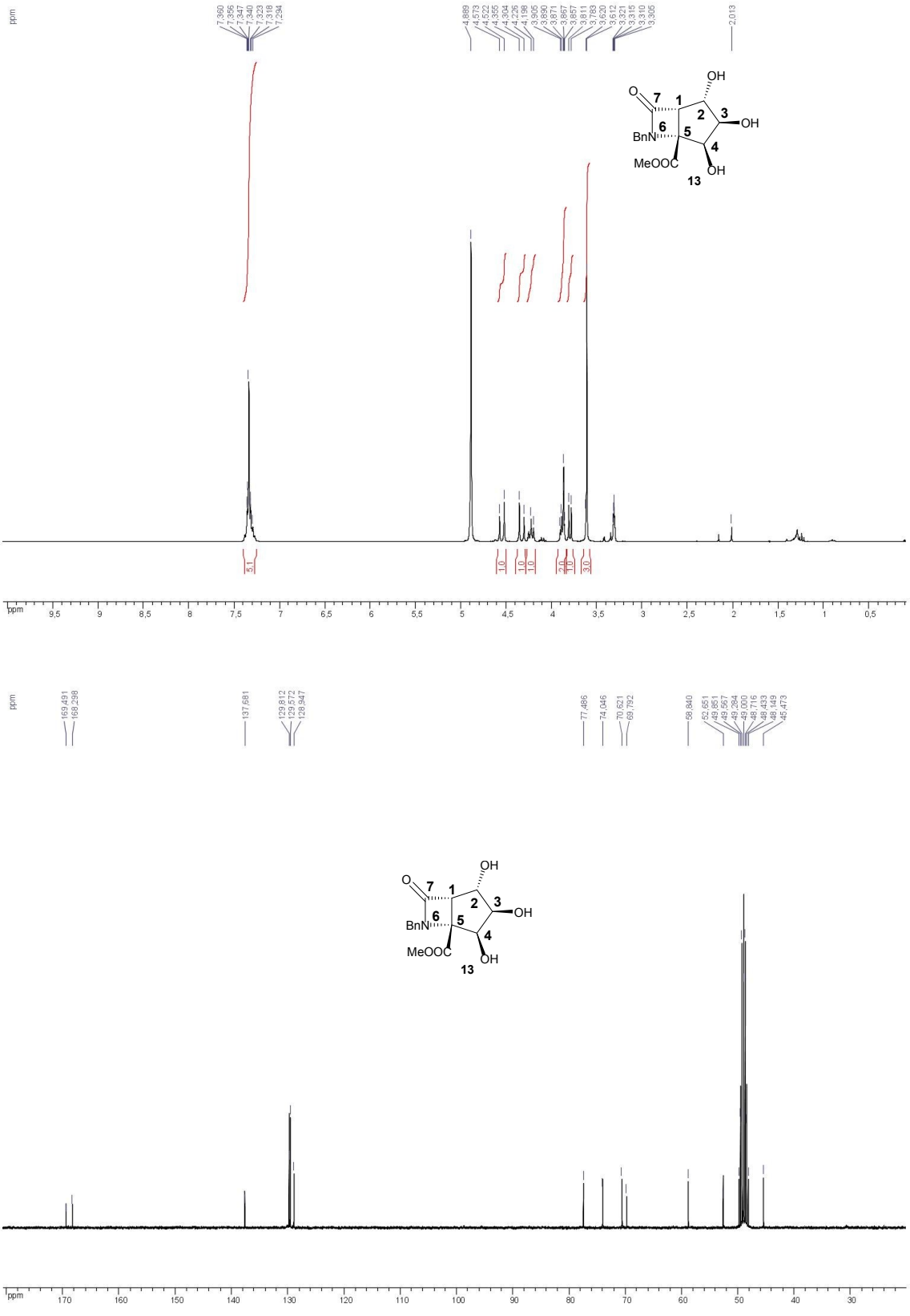


Fig 10. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C (75MHz, CD_3OD) spectra of compound **13**.

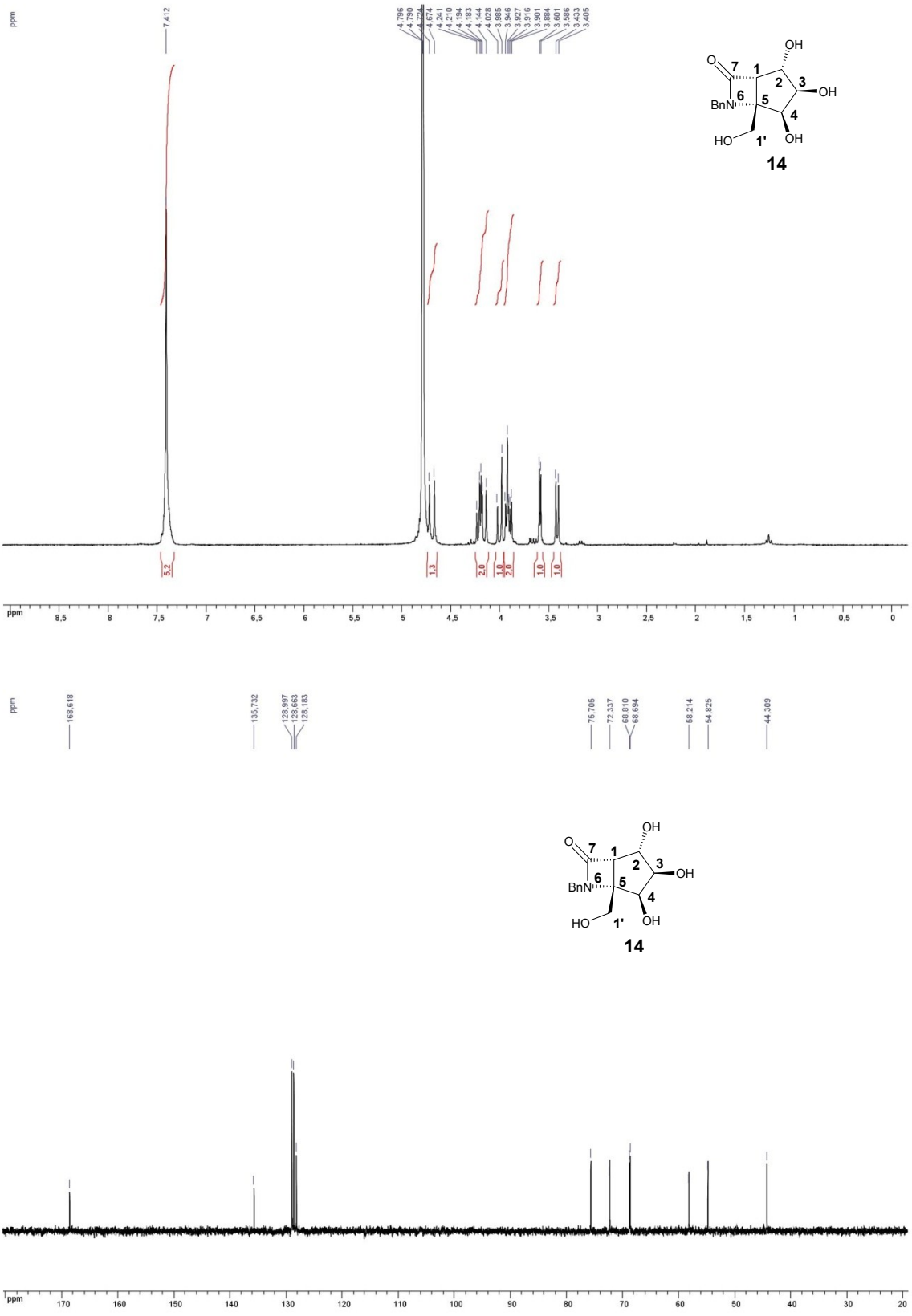


Fig 11. ^{1}H NMR (300 MHz, D_2O) and ^{13}C (75MHz, D_2O) spectra of compound **14**.

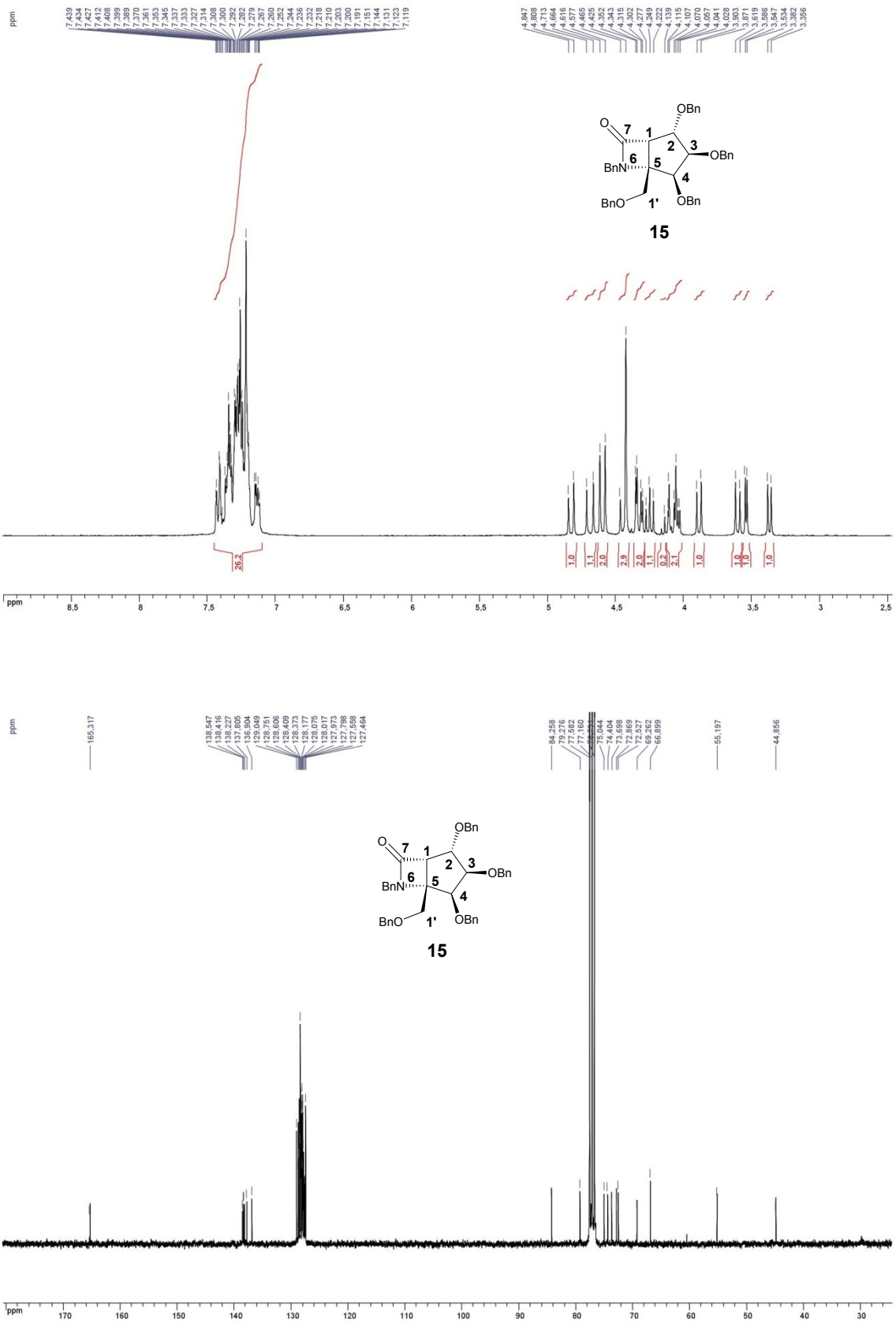


Fig 12. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C (75 MHz, $CDCl_3$) spectra of compound **15**.

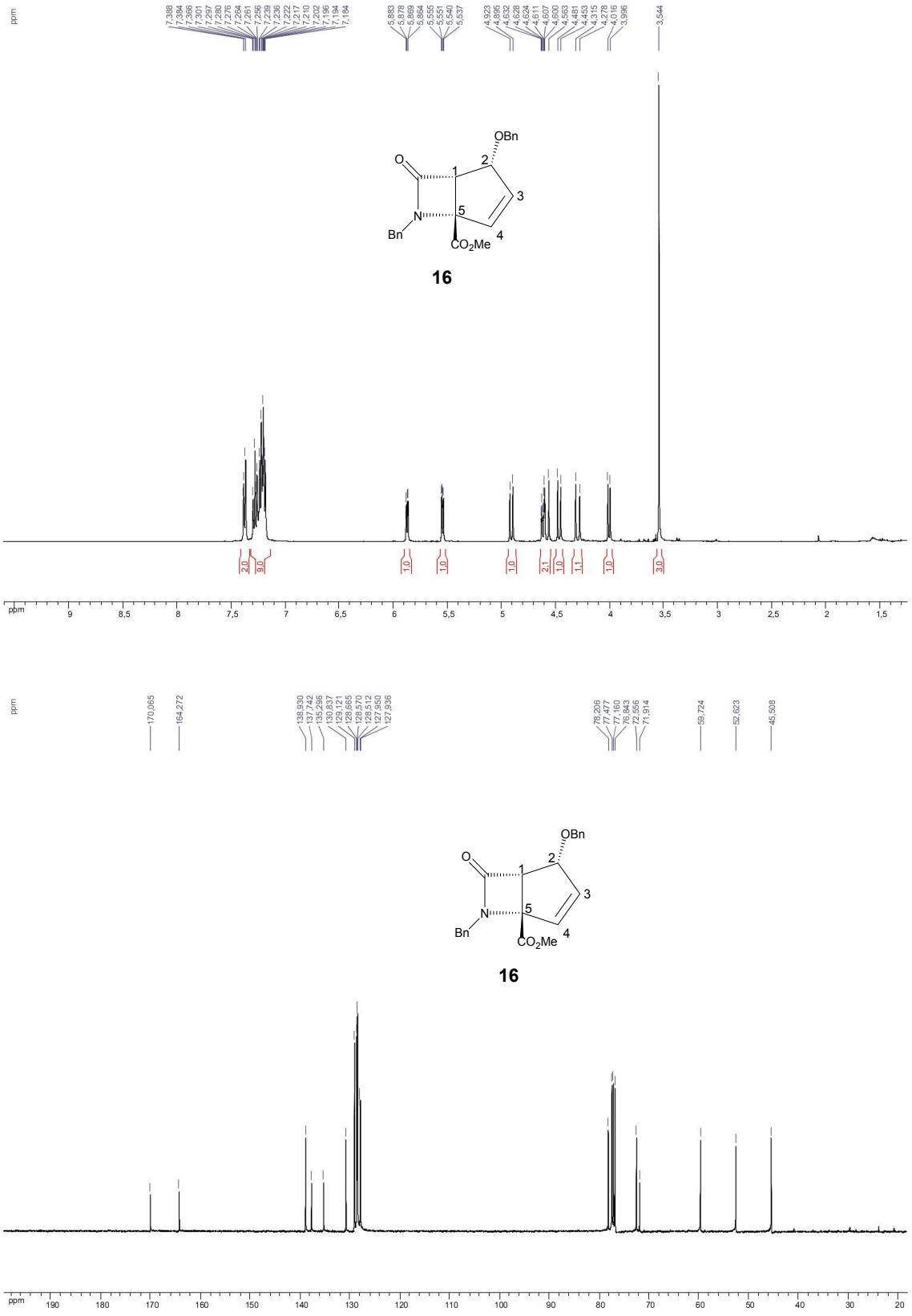


Fig 13. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **16**.

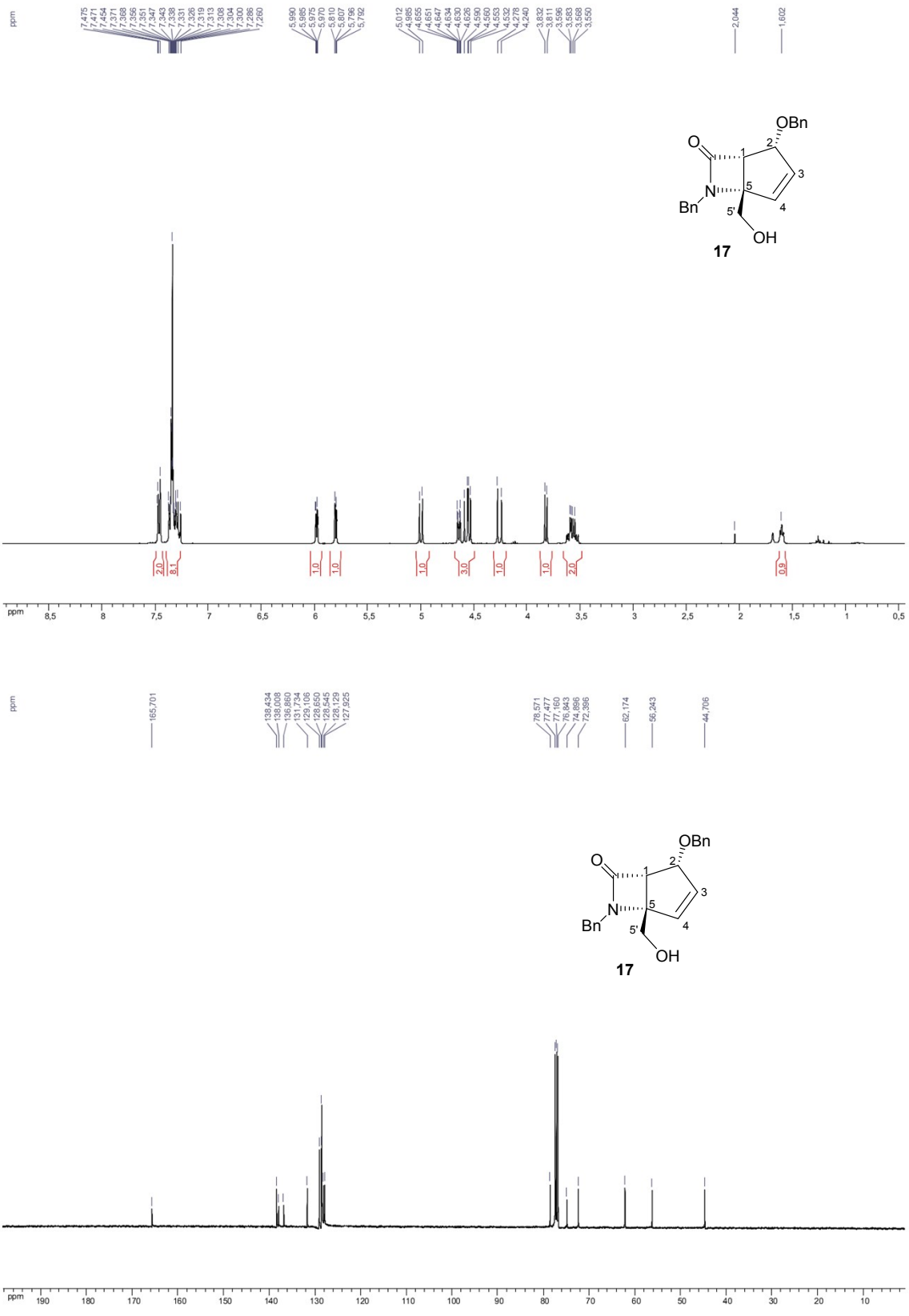


Fig 14. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **17**.

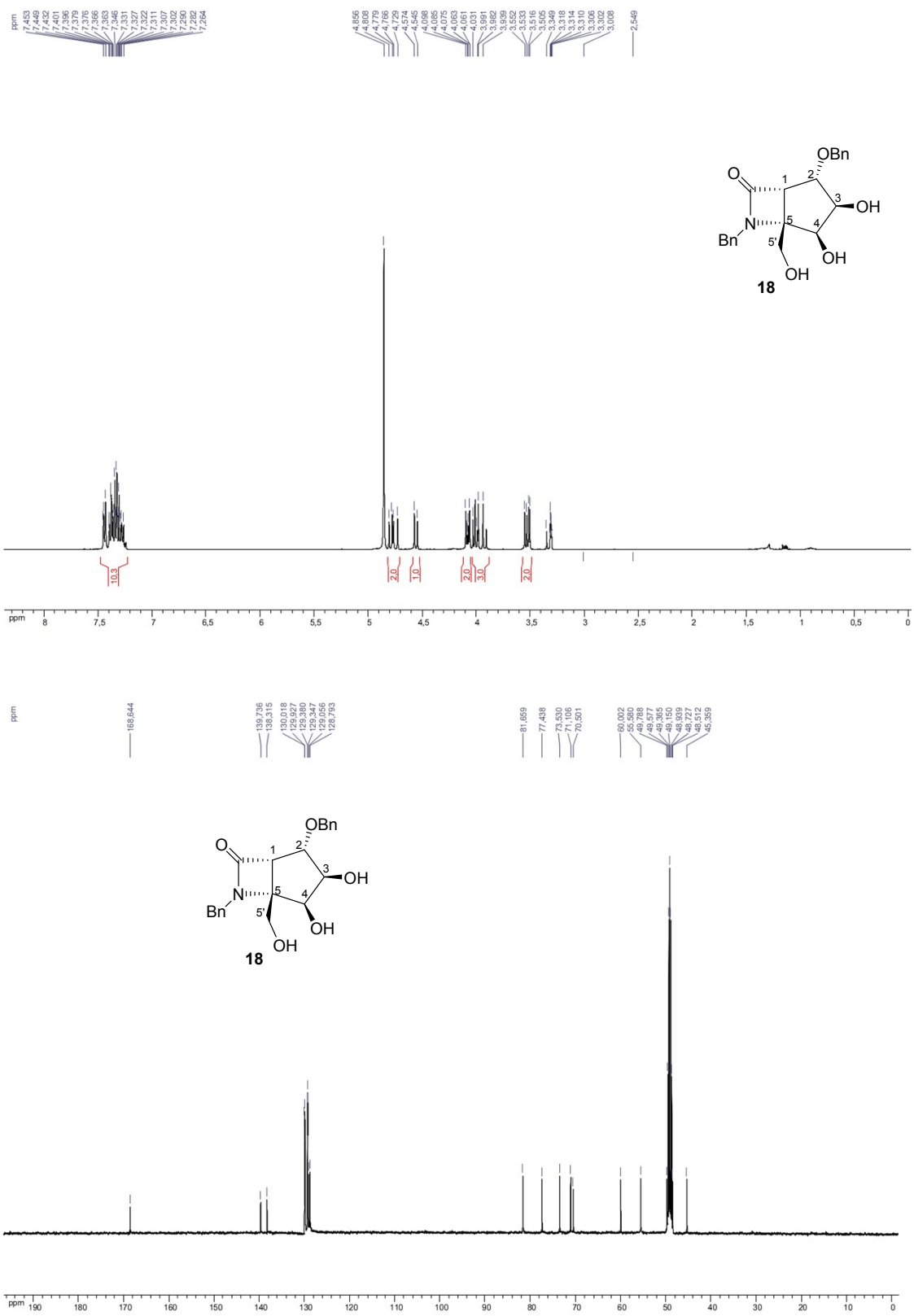


Fig 15. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) spectra of compound **18**.

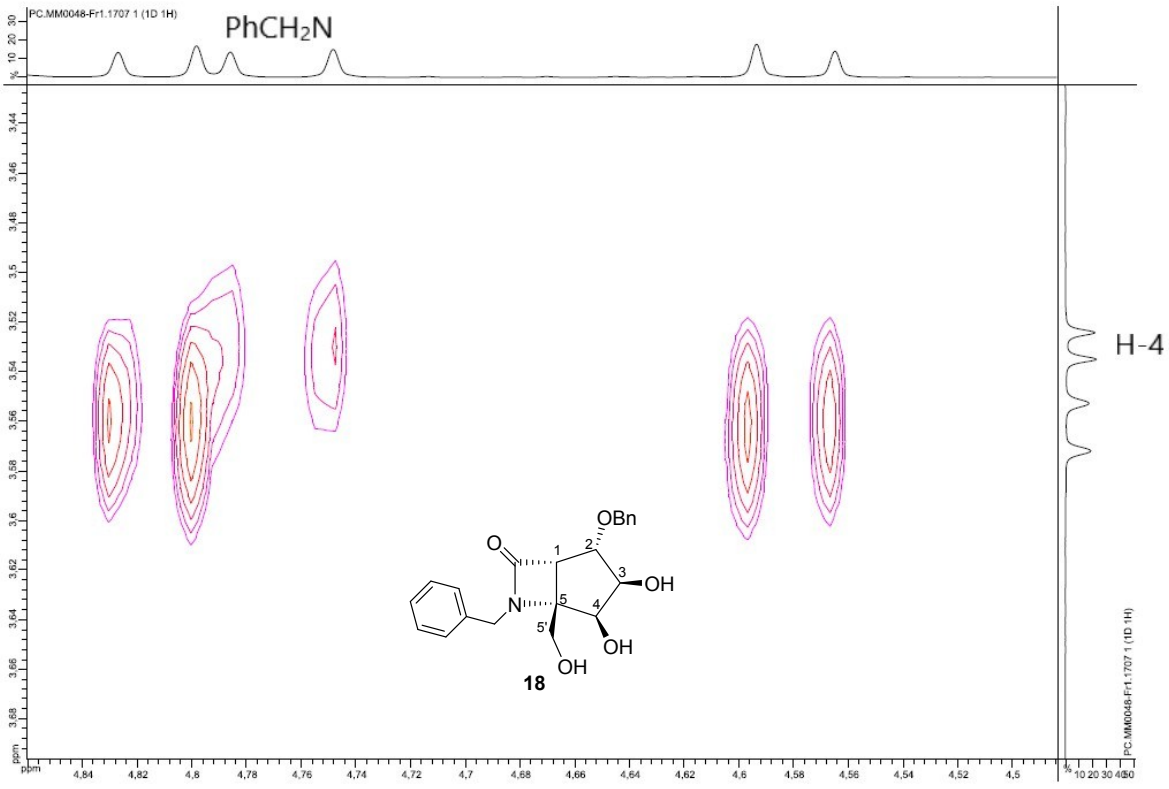


Fig 15. 2D NOESY NMR spectrum of compound of compound **18**.

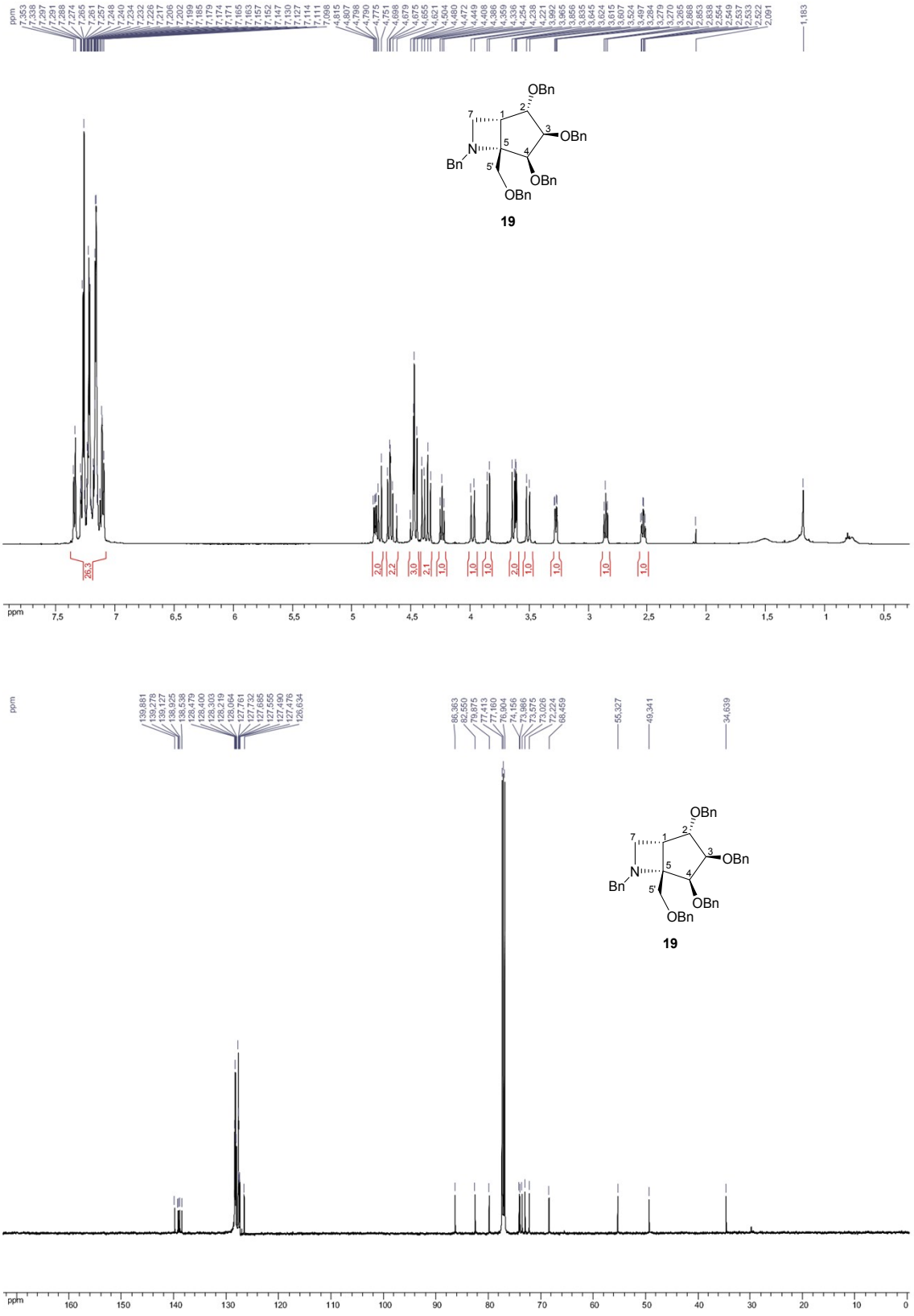


Fig 16. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) spectra of compound **19**.

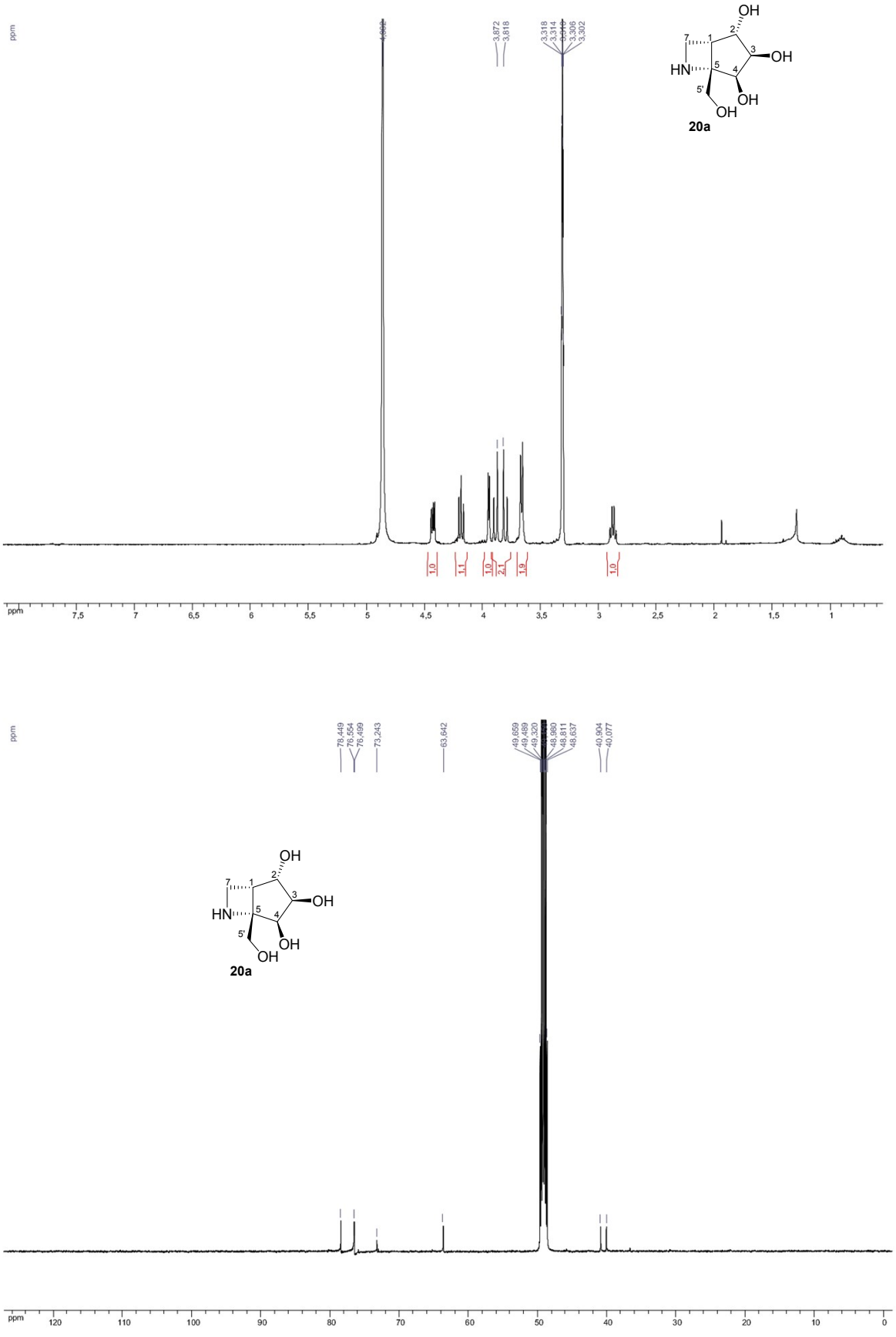


Fig 17. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) spectra of compound **20a**.

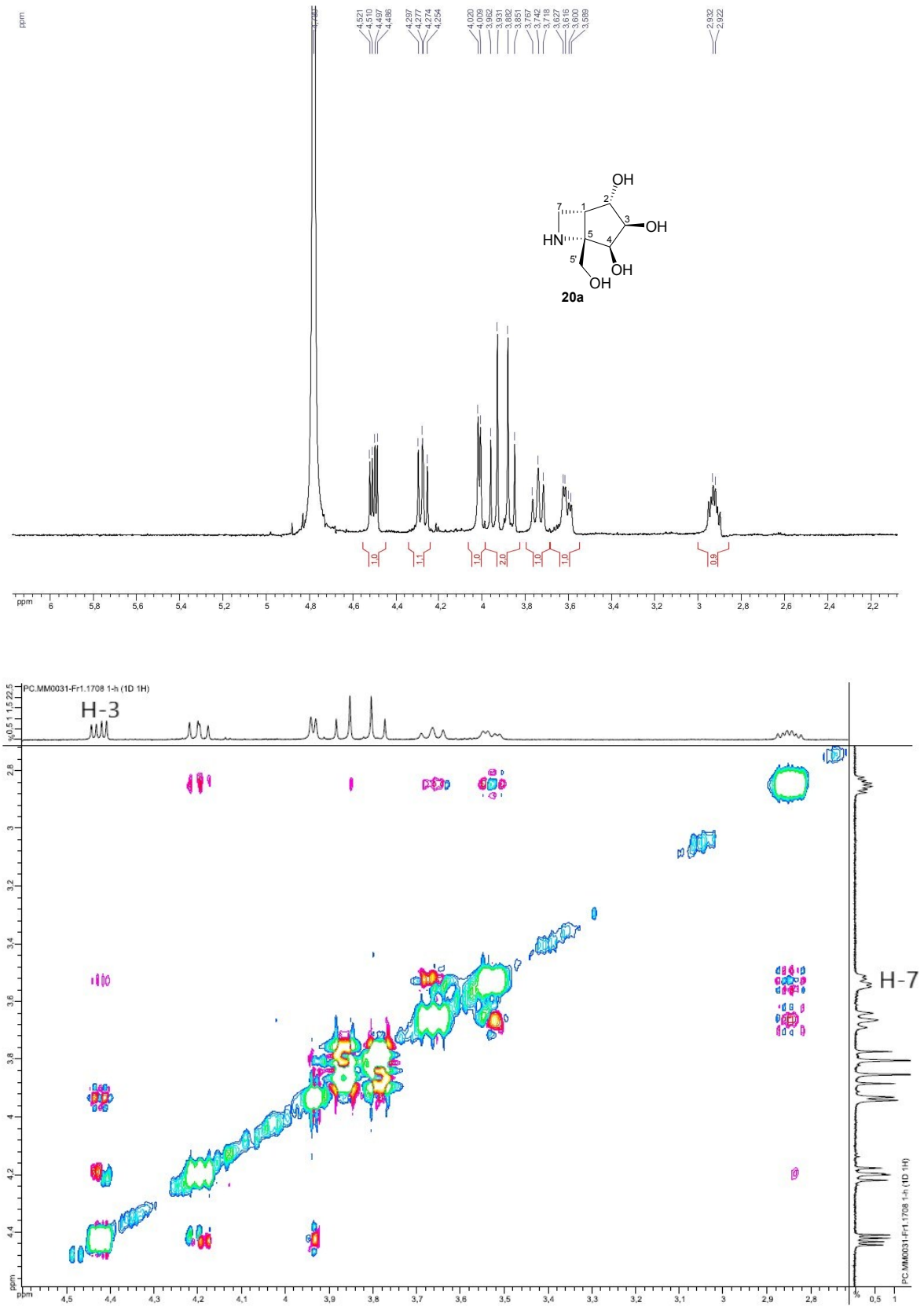


Fig 18. ^{1}H NMR (400 MHz, D_2O) and 2D NOESY NMR spectra of compound **20a**.

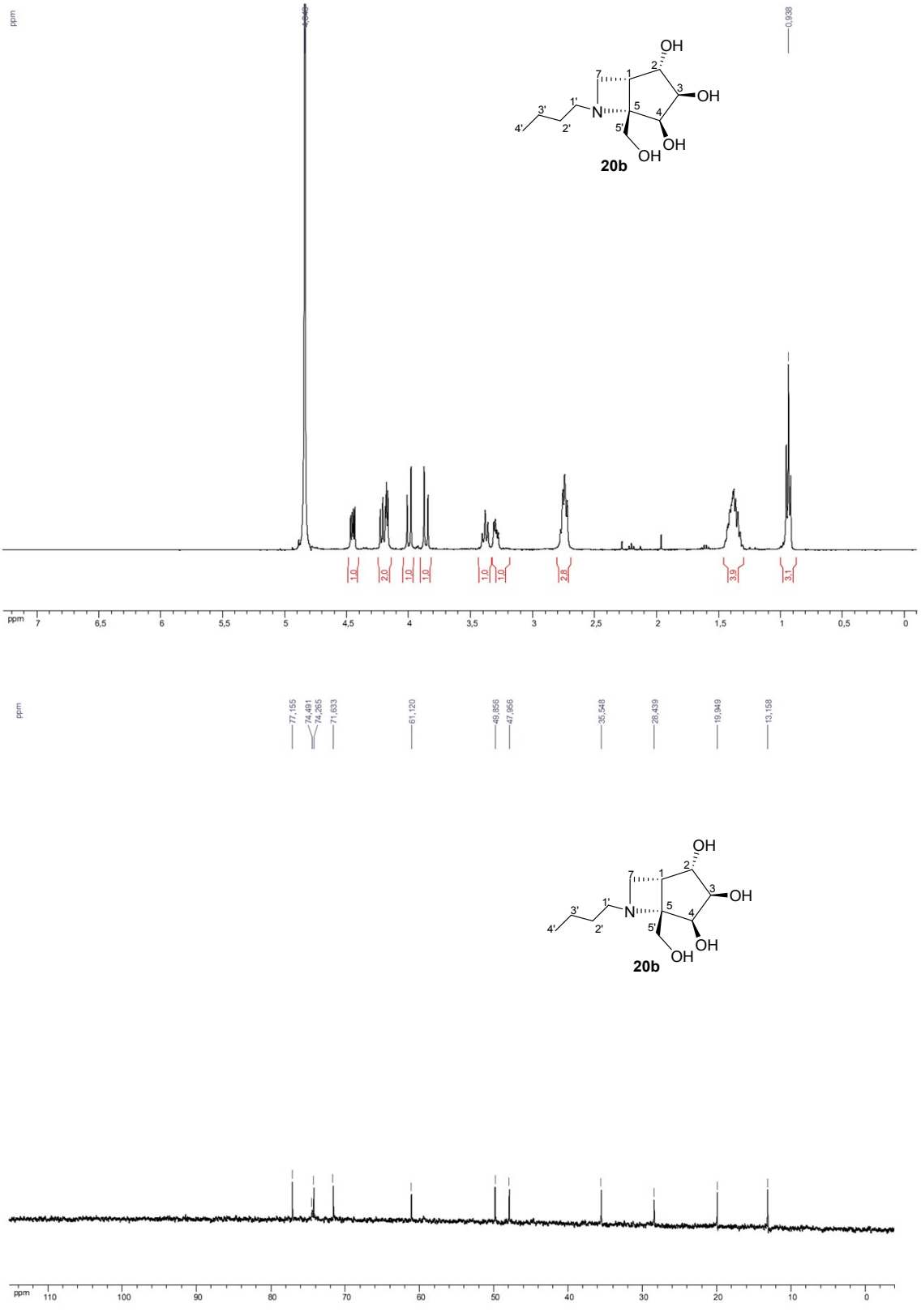


Fig 19. ^{1}H NMR (400 MHz, D_2O) and ^{13}C (100 MHz, D_2O) spectra of compound **20b**.

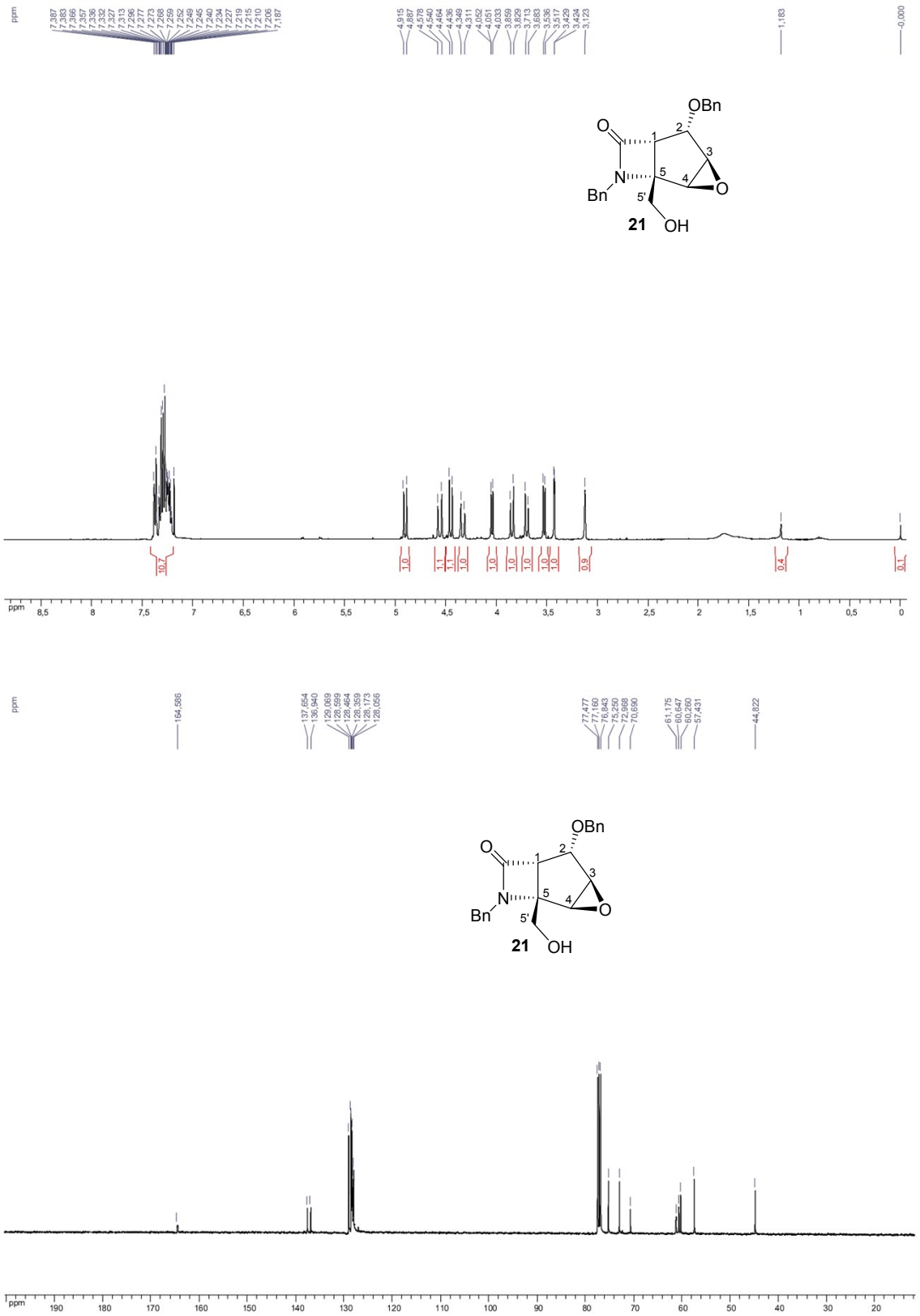


Fig 20. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **21**

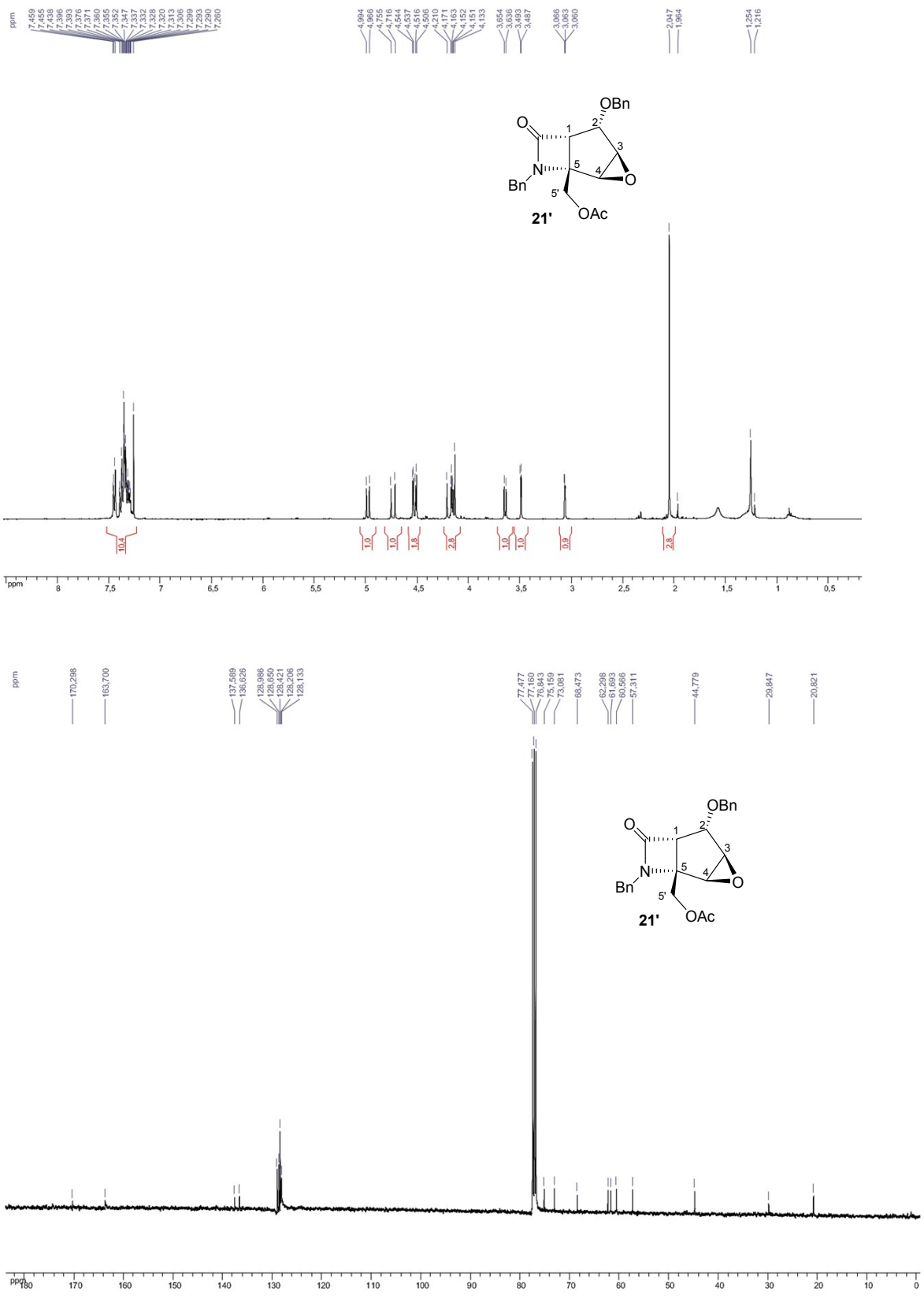


Fig 21. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **21'**.

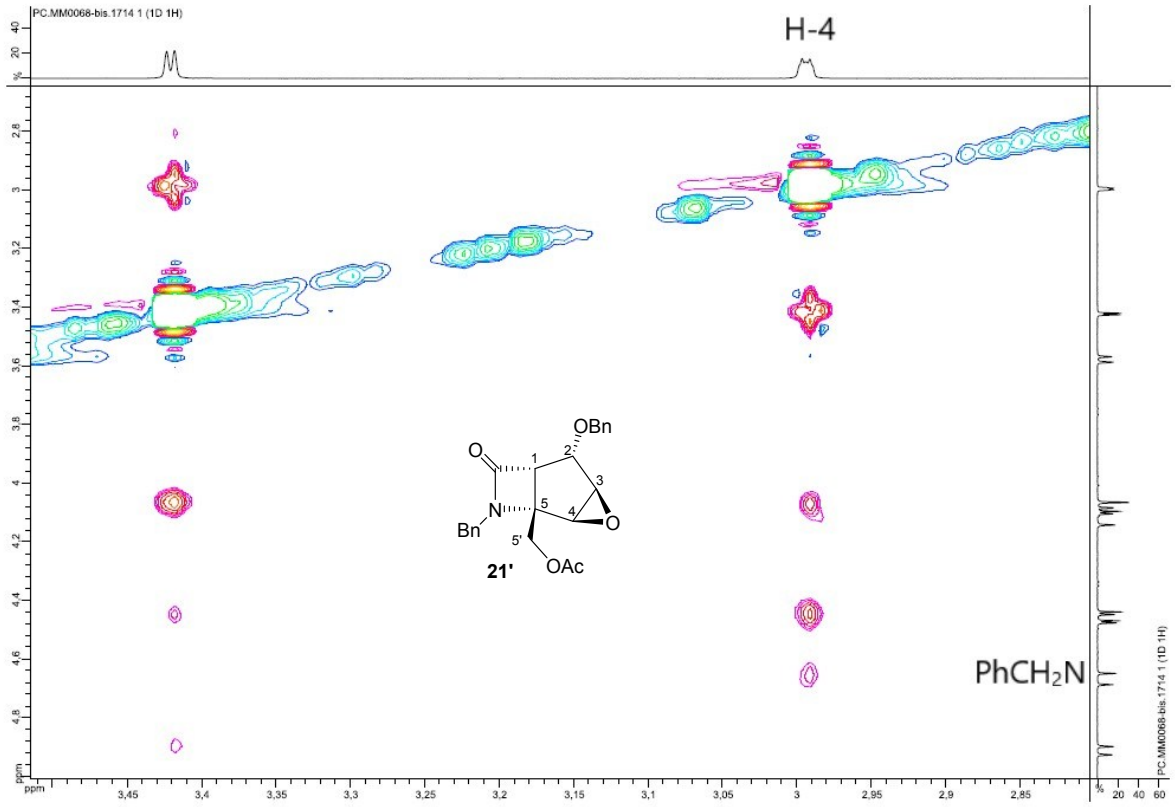


Fig 22. 2D NOESY NMR spectrum of compound **21'**.

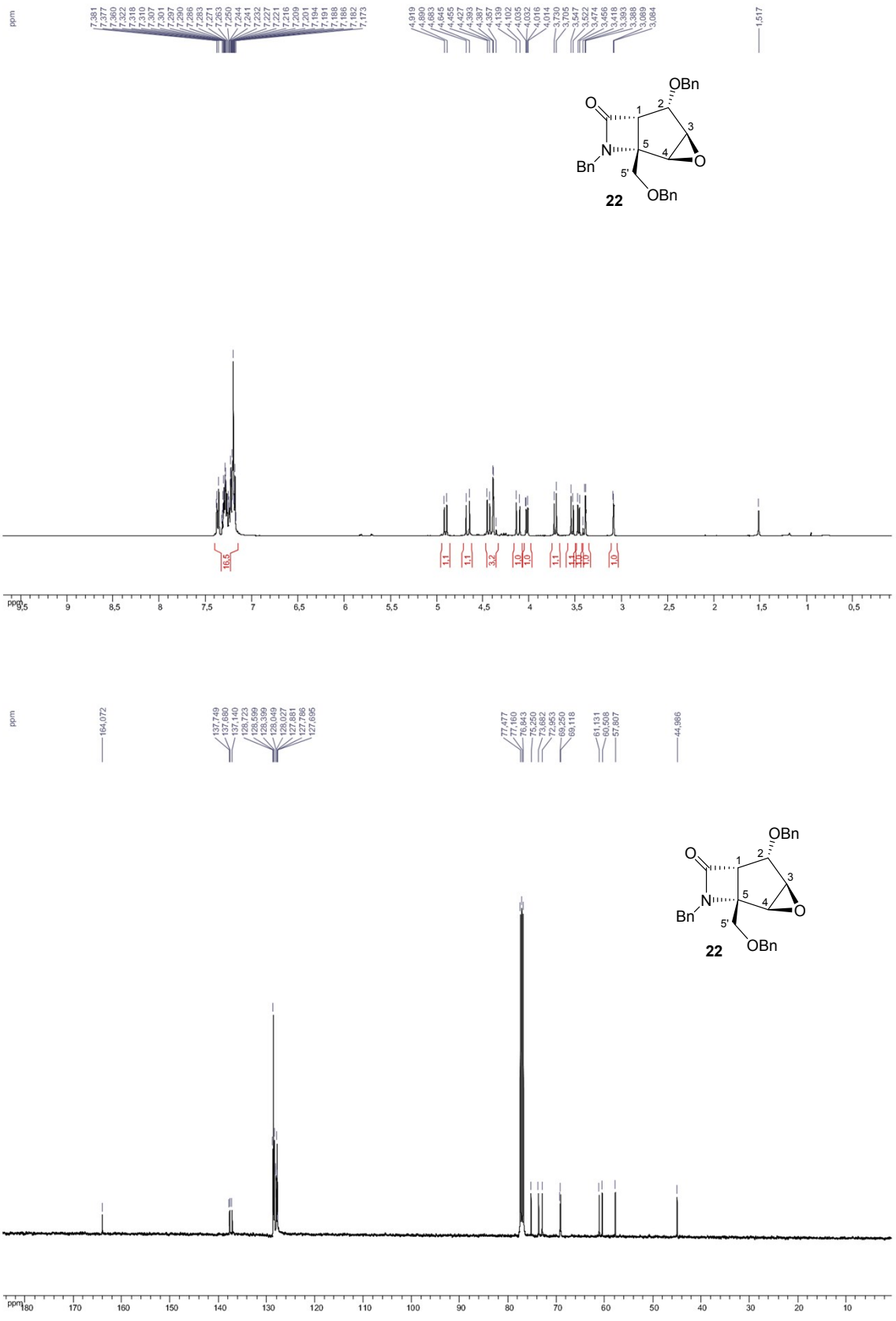


Fig 23. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **22**.

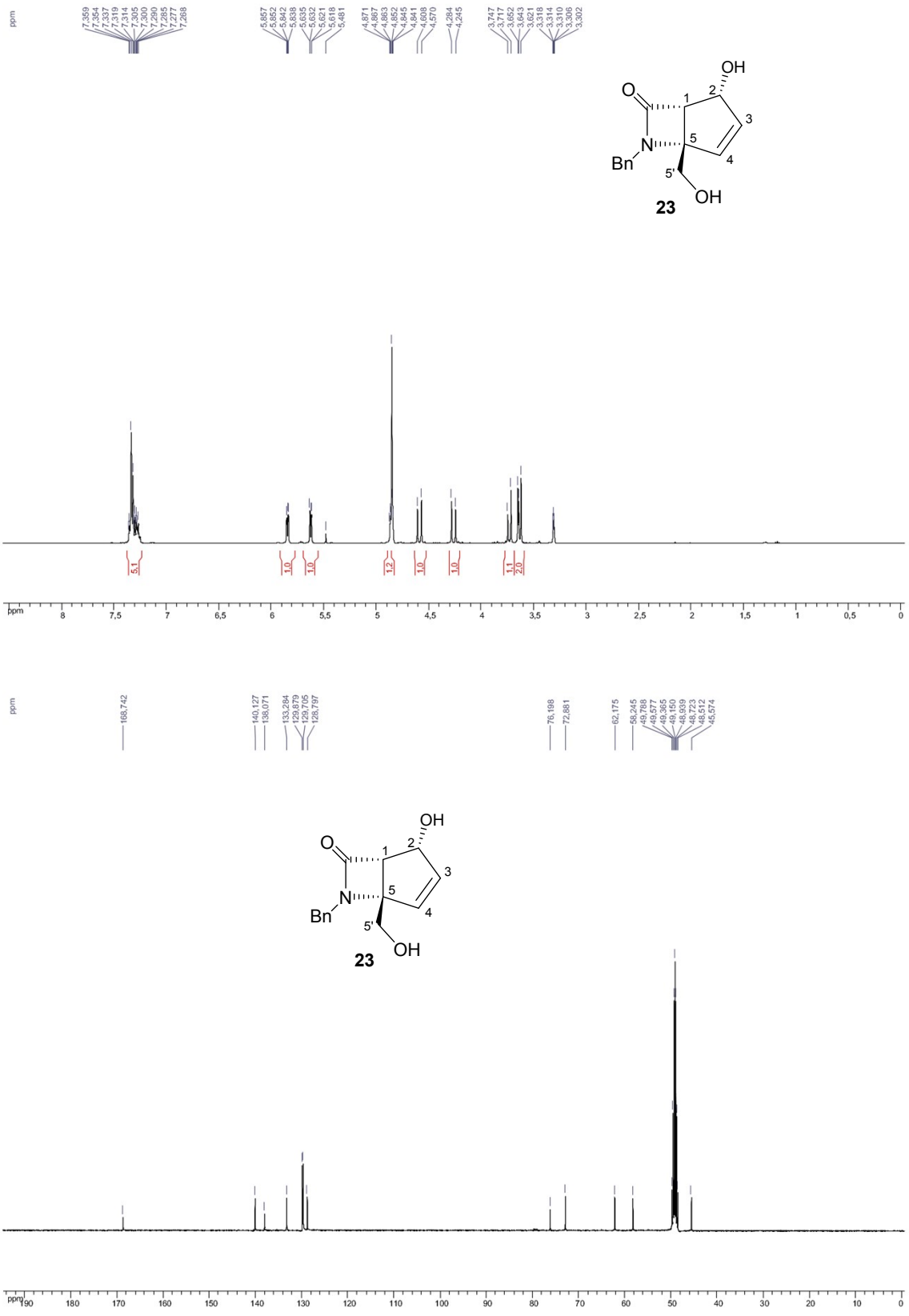


Fig 24. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) spectra of compound **23**.

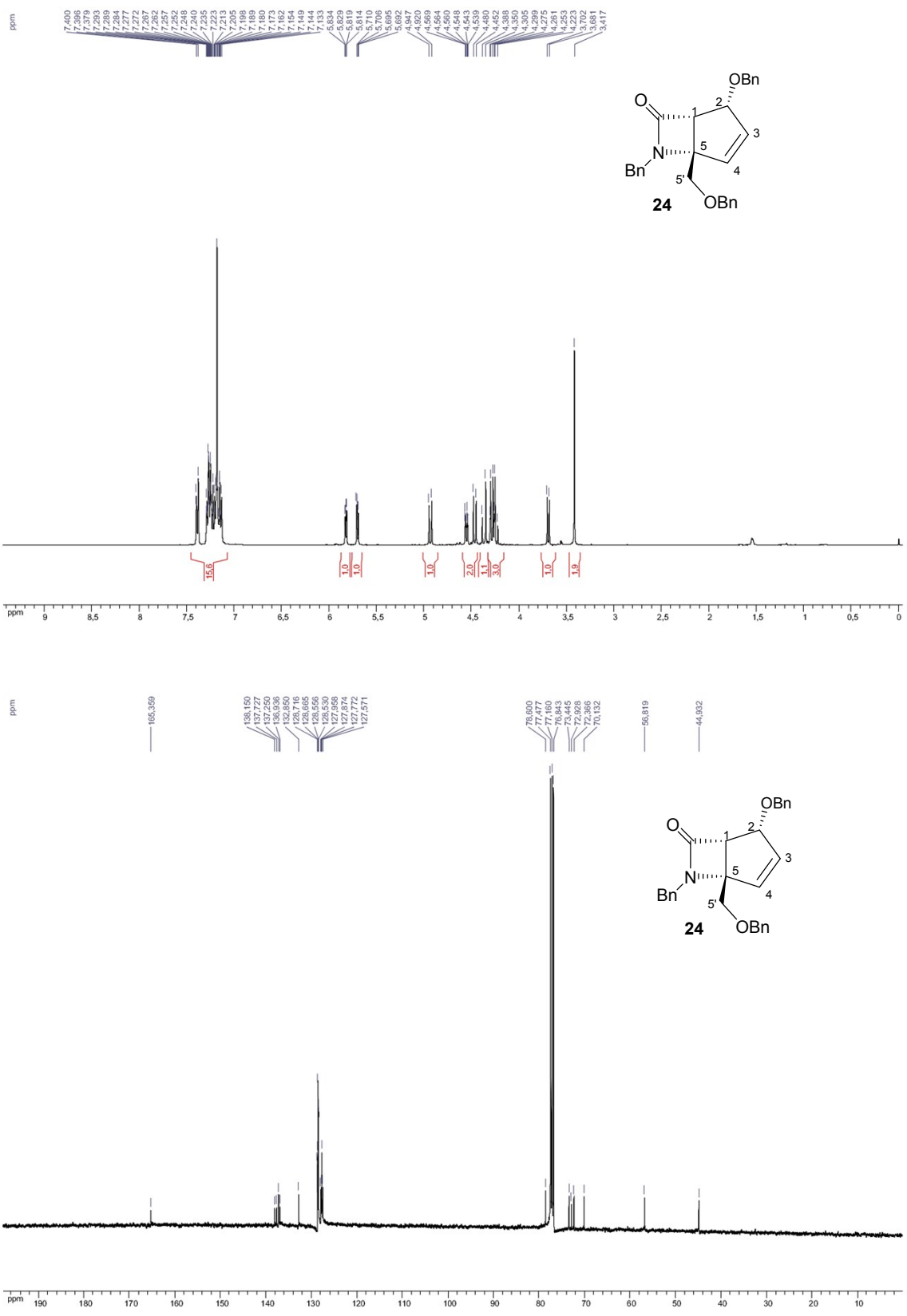


Fig 25. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **24**.

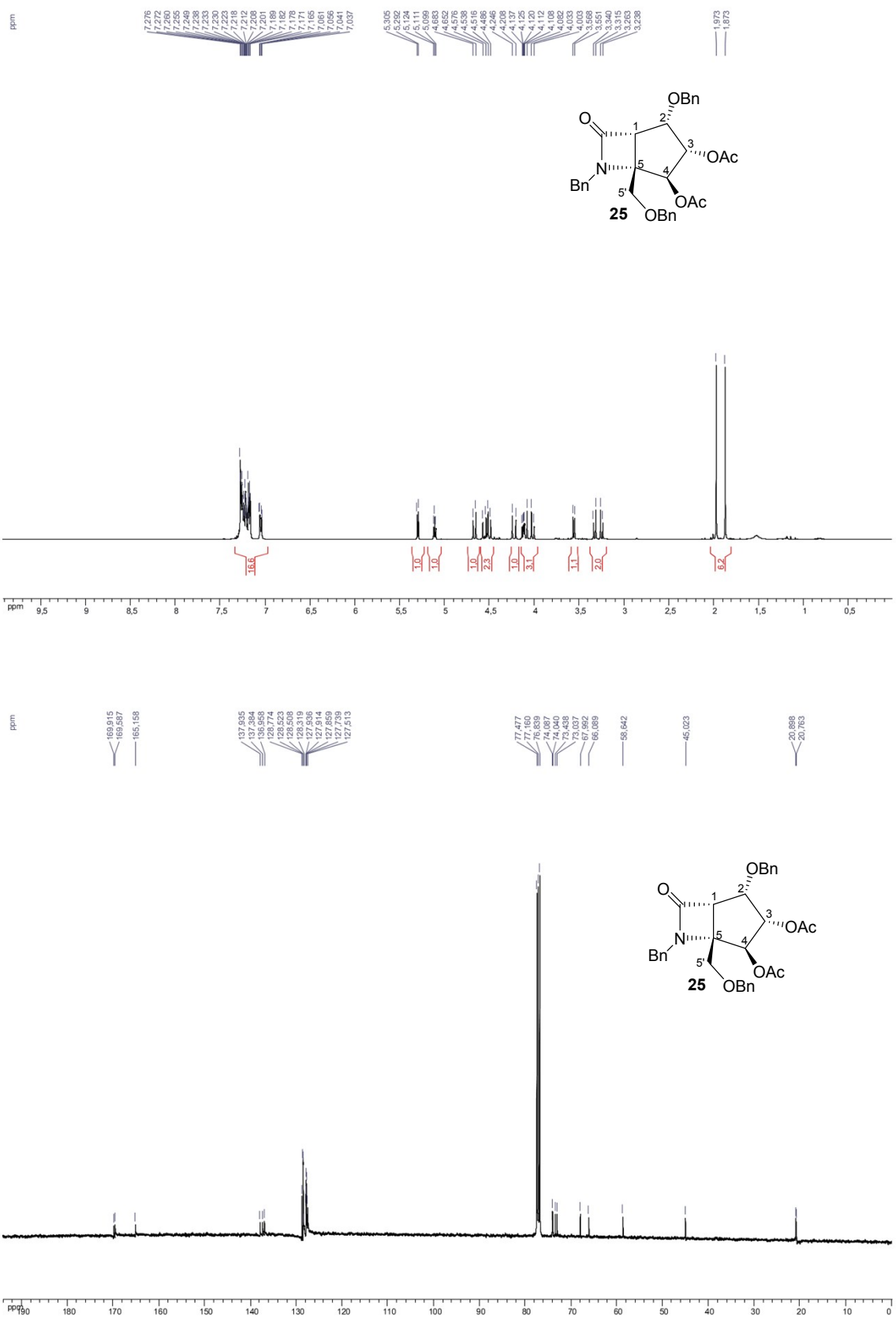


Fig 26. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) spectra of compound **25**.

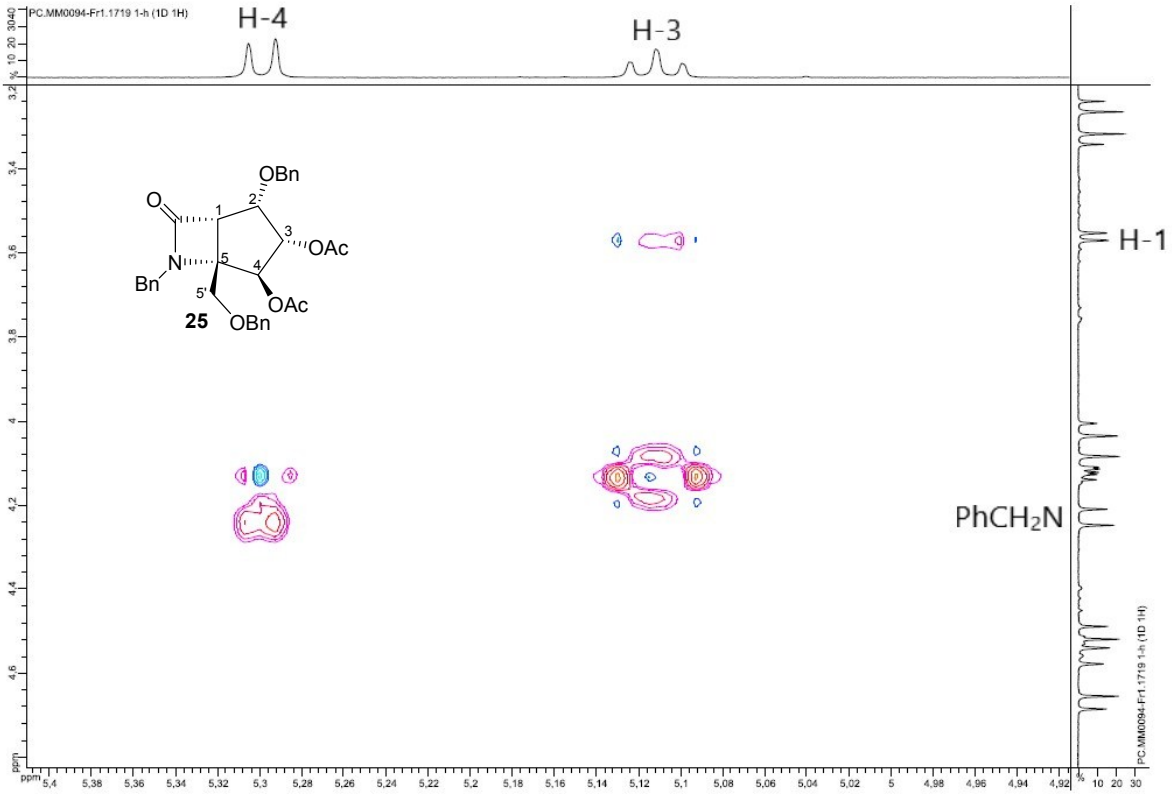


Fig 22. 2D NOESY NMR spectrum of compound **25.**

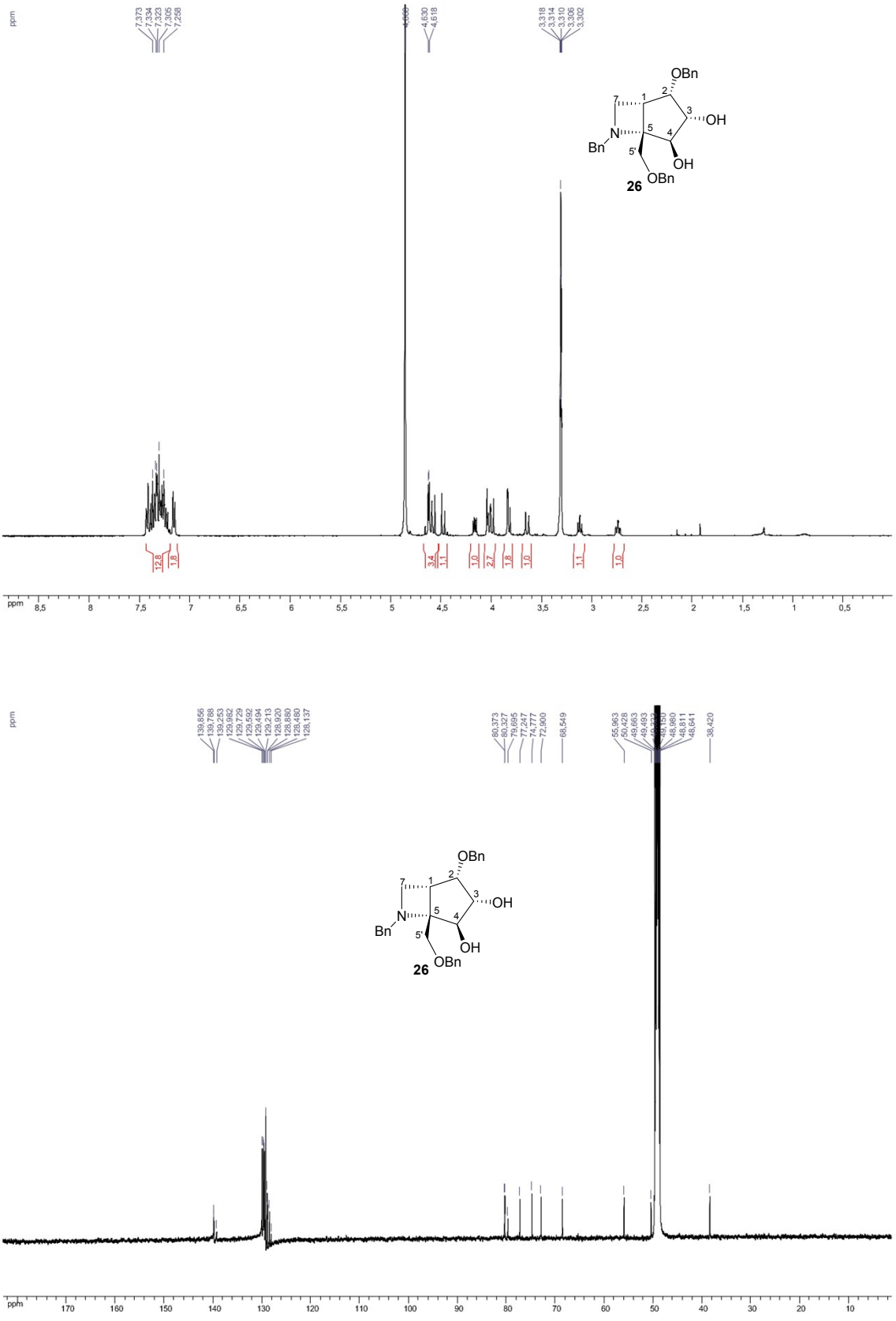


Fig 23. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) spectra of compound **26**.

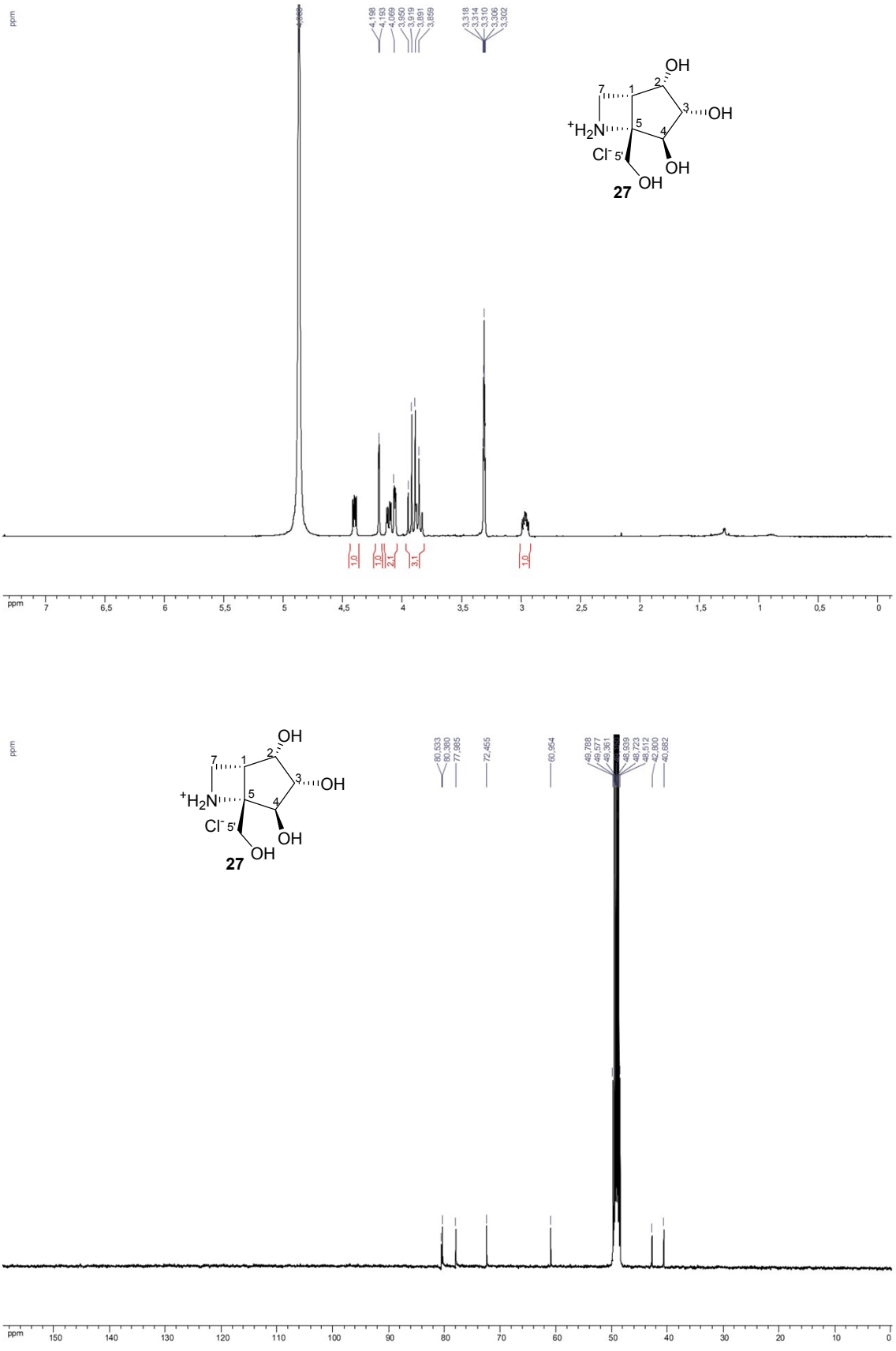


Fig 24. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) spectra of compound **27**.

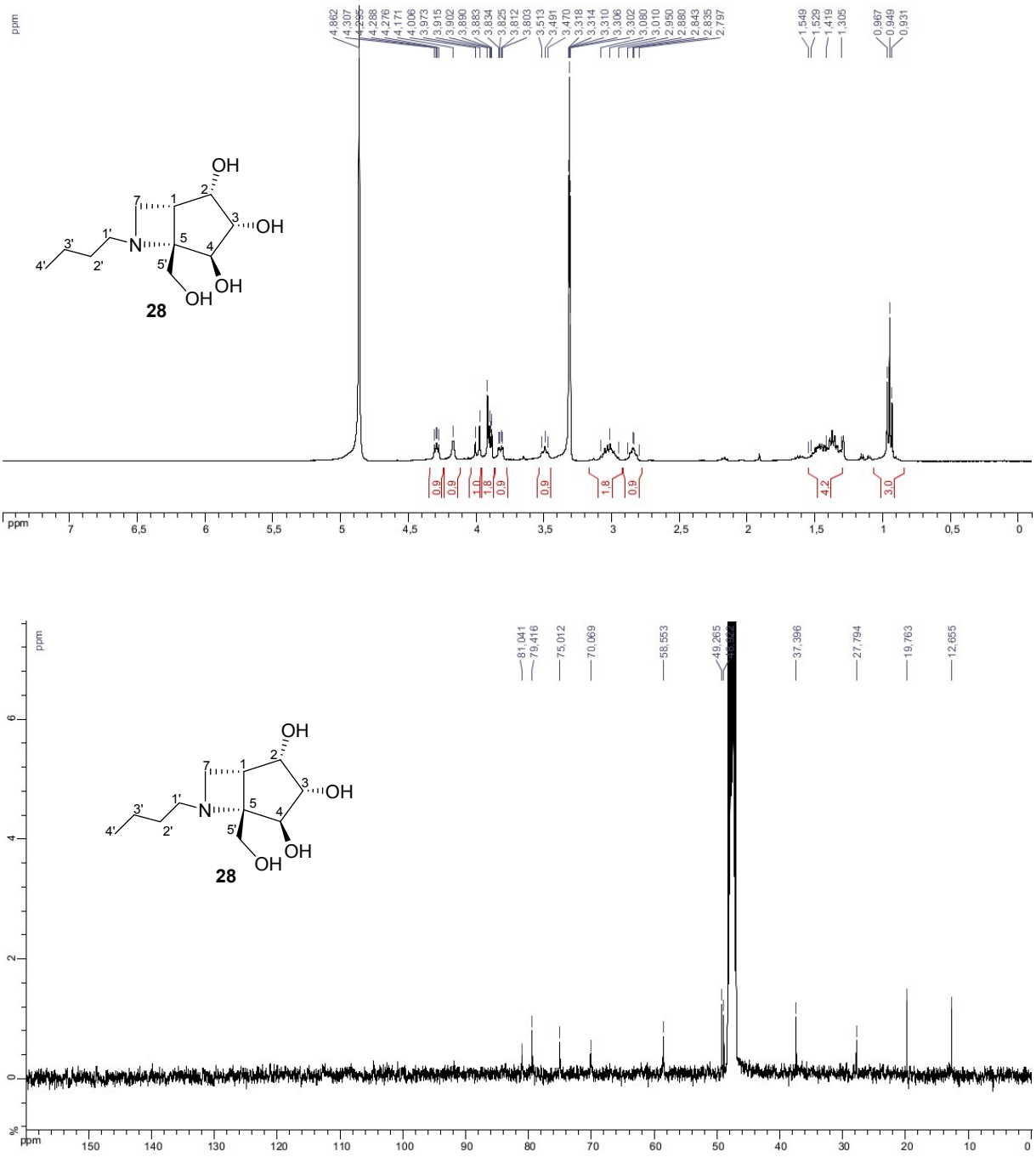


Fig 25. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) spectra of compound **28**.