Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Galacto configured *N*-aminoaziridines: a new type of irreversible inhibitors of β-galactosidases

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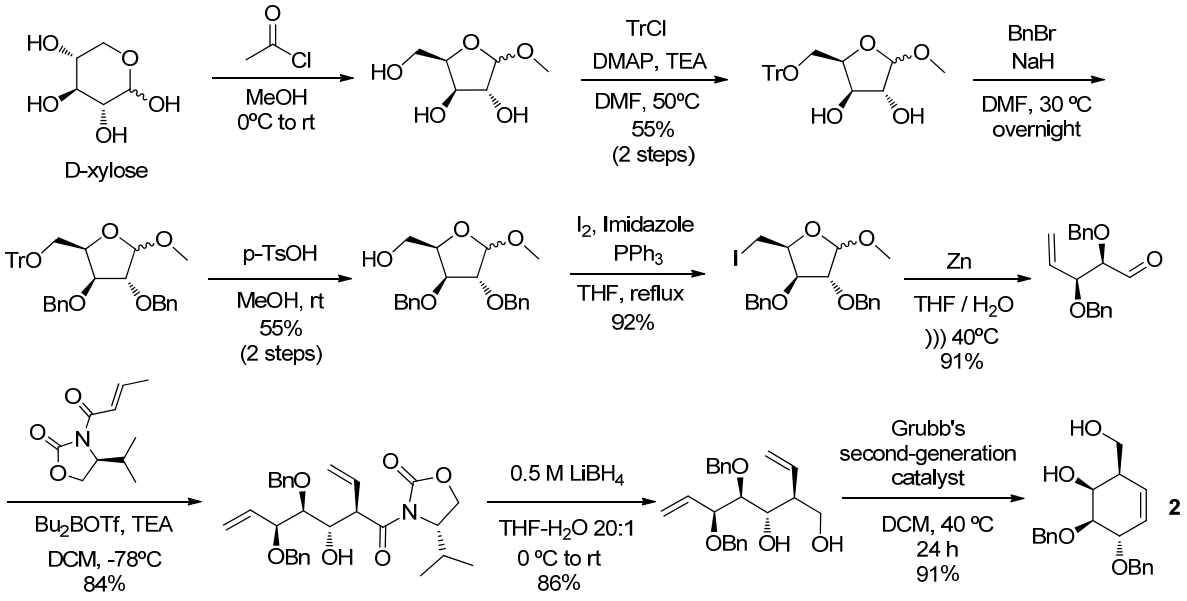
Synthesis of galacto-configured aziridine derivatives



Scheme S1. Synthesis of olefin **2** from commercial D-xylose.

Determination of purity of the synthesized compounds by HPLC

Materials and methods

To quantify the purity of the compounds assayed, different methods have been developed by Adrián Santos and Lourdes Muñozfrom the Service of Synthesis of High Added Value Molecules (SIMChem), which belongs to the Institute of Advanced Chemistry of Catalonia (IQAC) and the Spanish National Research Council (CSIC). However, the fast elution of the final compounds **1a** and **1b** due to their high polarity did not let us to detect the peaks corresponding to these products at higher retention times after a few attempts with different methods.

Solvents: Different solvents were employed in sample preparation as well as mobile phases in chromatographic analysis.

- Milli-Q water
- Acetonitrile gradient grade for HPLC 99.99%, Fischer Chemical
- Formic acid 98%, Fluka

Sample preparation: Purity of compounds has been determined. Samples were diluted in milli-Q water at 1mg/mL. An analytical balance (GR-300, from AND) was used to weight samples.

HPLC analysis

Liquid chromatography for the separation of impurities and final products was performed by means of an HPLC-DAD-ELSD Alliance from Waters (Barcelona, Spain). This chromatograph is designed as a Separation Module equipped with pump and autosampler integrated model number 2695 coupled to two detectors working in parallel, a PDA 2996 from Waters and a light scattering ELS-1000 from polymer laboratories.

Chromatographic conditions for the quantification of purity by **Method A**

Guard column	PhenomenexSecurityGuard LC-18 (4 × 3 mm ø)
Analytical column	ZORBAX **Eclipse Plus** C18 4.6x75mm; 3.5µm (S.N. USUXD01964; Agilent)
Flow	1 mL/min

ELS DETECTOR

Gas flow	1.5 L/min
Nebulization temperature	80 ºC
Evaporation temperature	90 ºC

TABLE S1. Gradient used to determine the purity of compounds by method A.

Time (min)	Water + 0.2% (v/v) formic acid (%)	MeCN + 0.2% (v/v) formic acid (%)
0.01	100	0
2.00	100	0
6.00	85	15
8.00	50	50
11.00	100	0
15.00	100	0

HPLC-MS analysis were run on a Ultimate 3000SD (Thermo Scientific Dionex) coupled to a LTQ XL ESI-ion trap. Mass spectra were recorded in negative and positive ion mode (m/z 50-1500)

Analytical Column:	ZORBAX **Eclipse Plus** C18 4.6x150mm; 3.5um (S.N. USUXC04483
Flow:	0,9 mL/min
Temperature:	30ºC

TABLE S2. Gradient used for HPLC-MS analysis.

Time (min)	Water (%)	MeCN (%)
0.01	95.0	5.0
2.00	95.0	5.0
8.00	0.0	100.0
10.00	0.0	100.0
11.00	95.0	5.0
15.00	95.0	5.0

Biological Assays

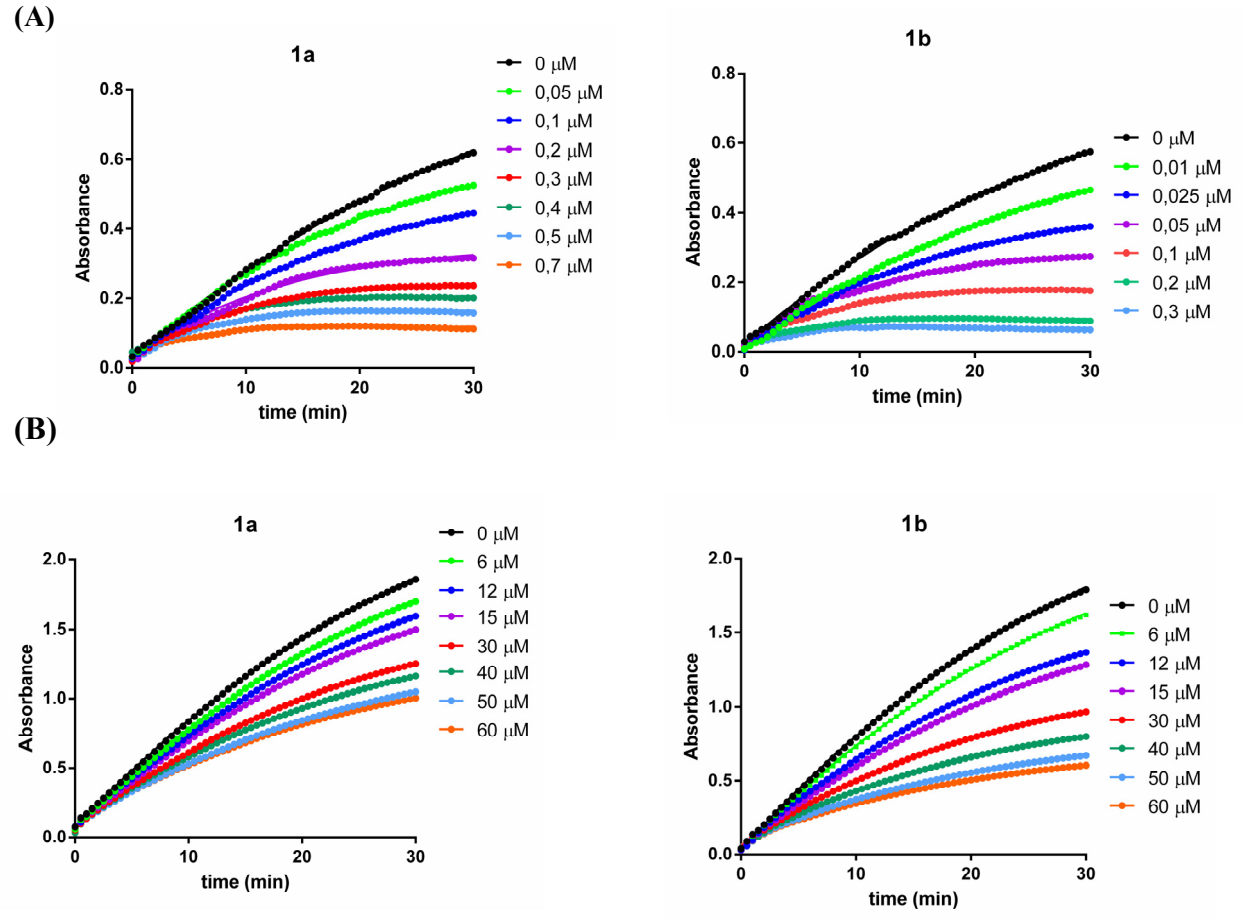
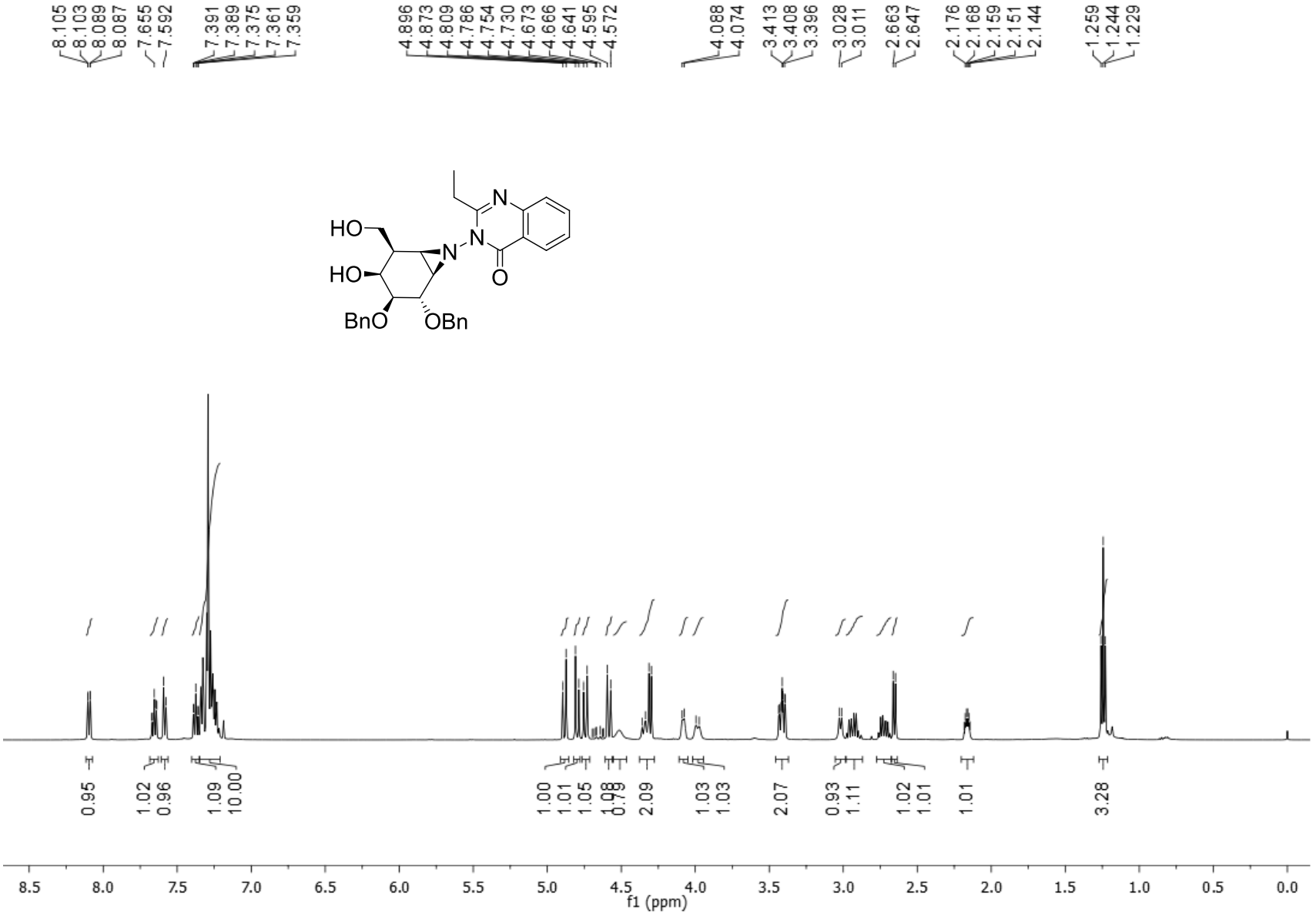
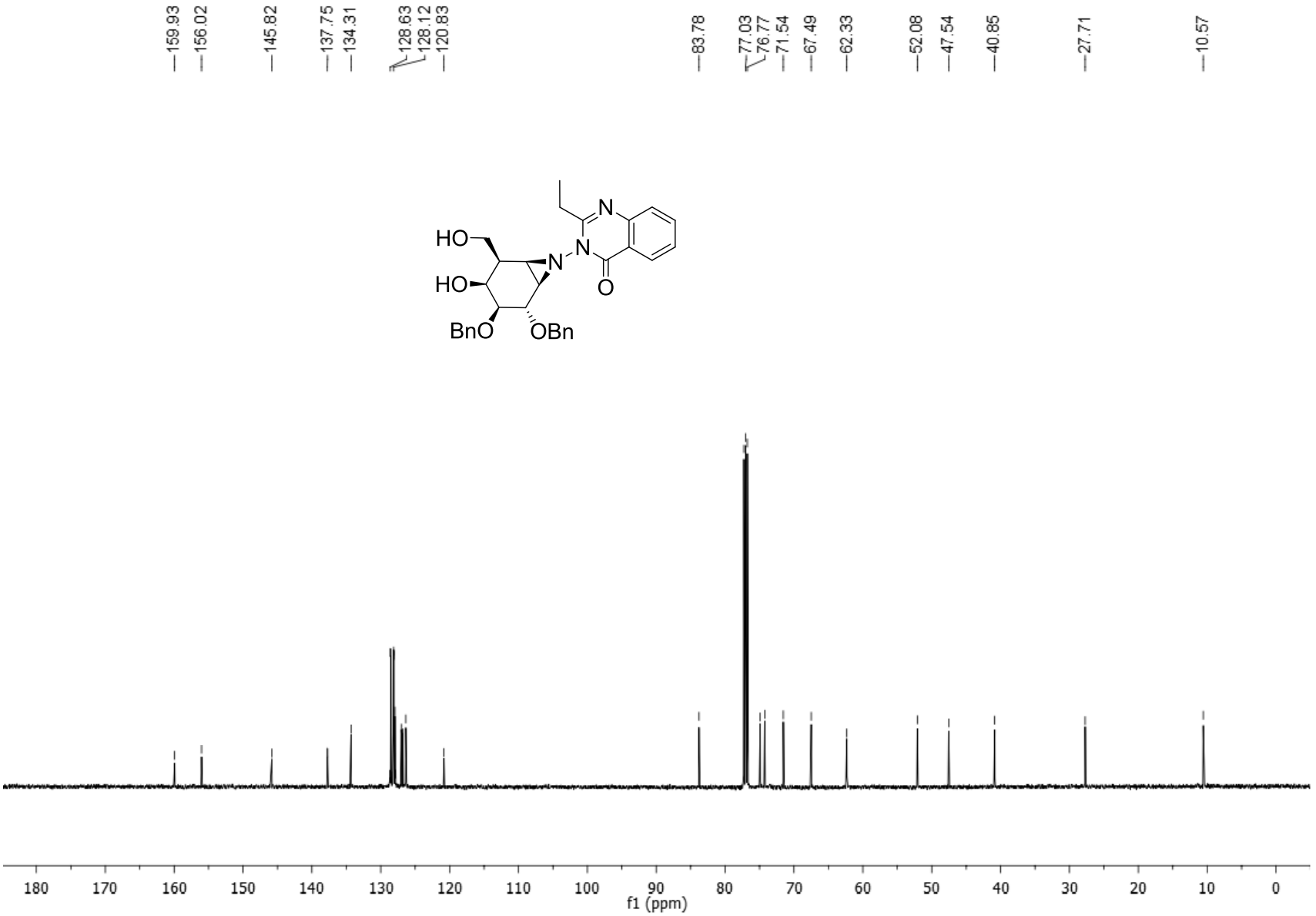


Figure S1. Progress curves for the irreversible inhibition of *Aspergillusoryzae* (A) and *Escherichia coli* (B) *β*-Galactosidases for compounds **1a** and **1b**.

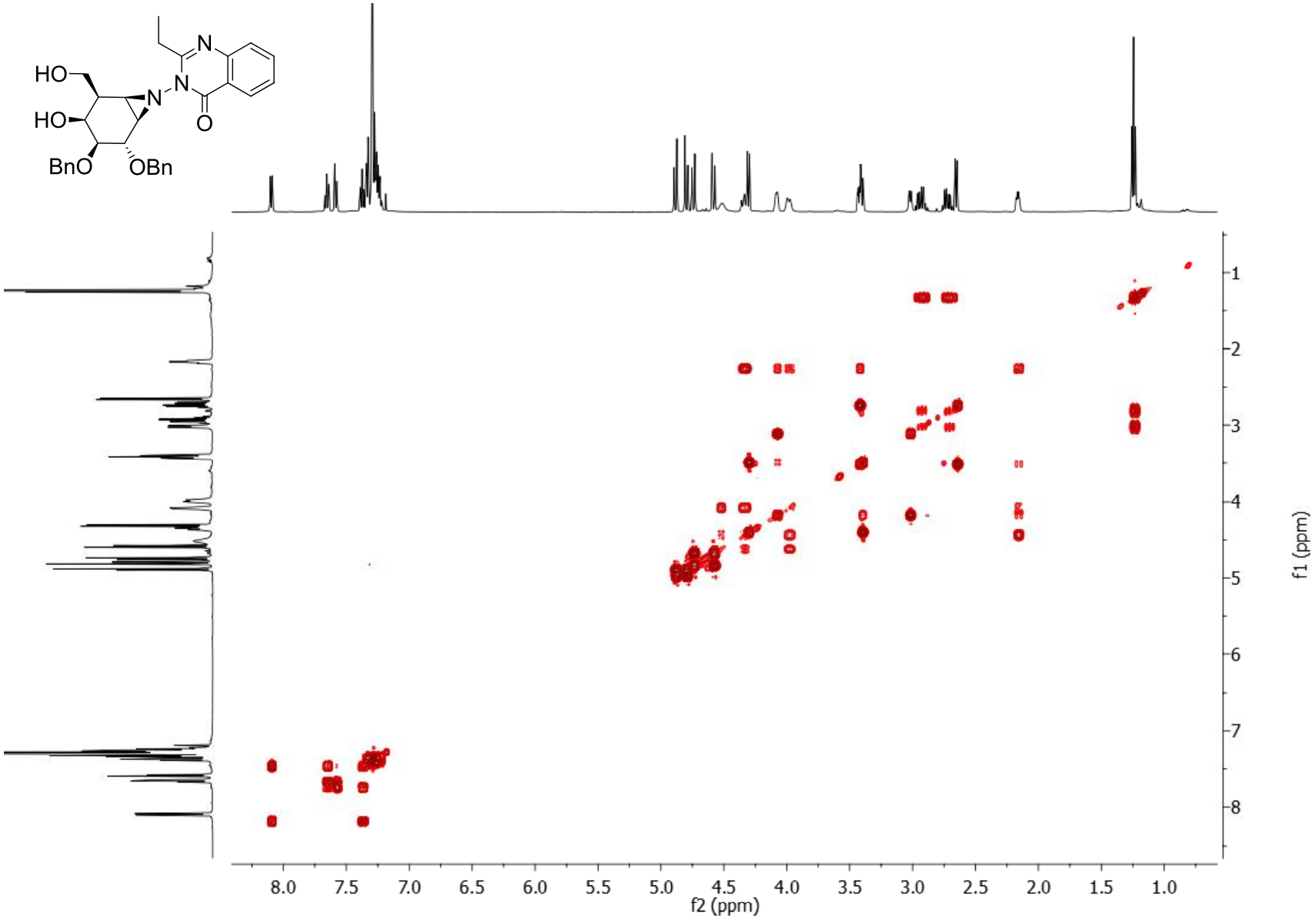
^{1}H-NMR spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)



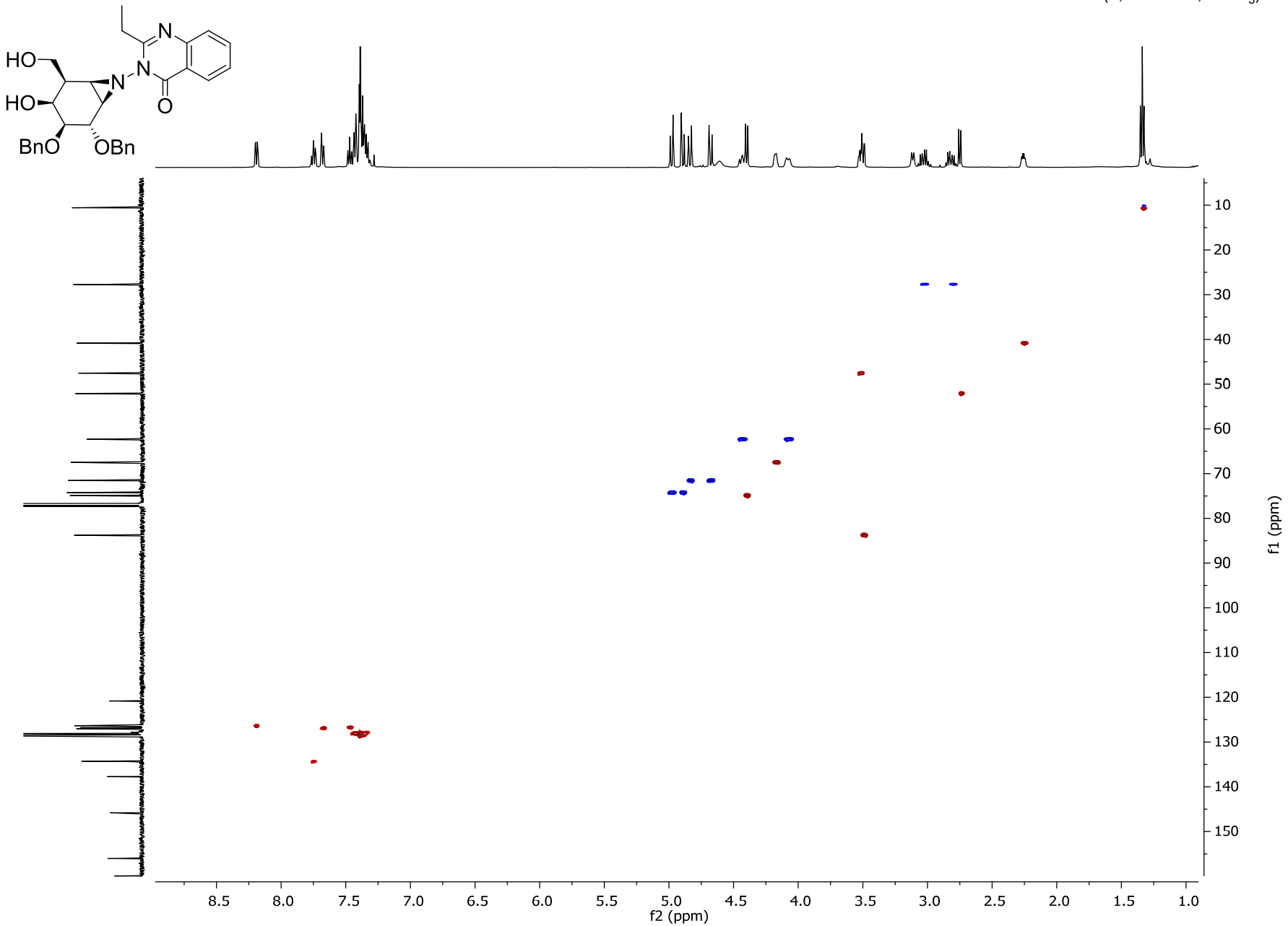
^{13}C-NMR spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 126 MHz, $CDCl_3$)



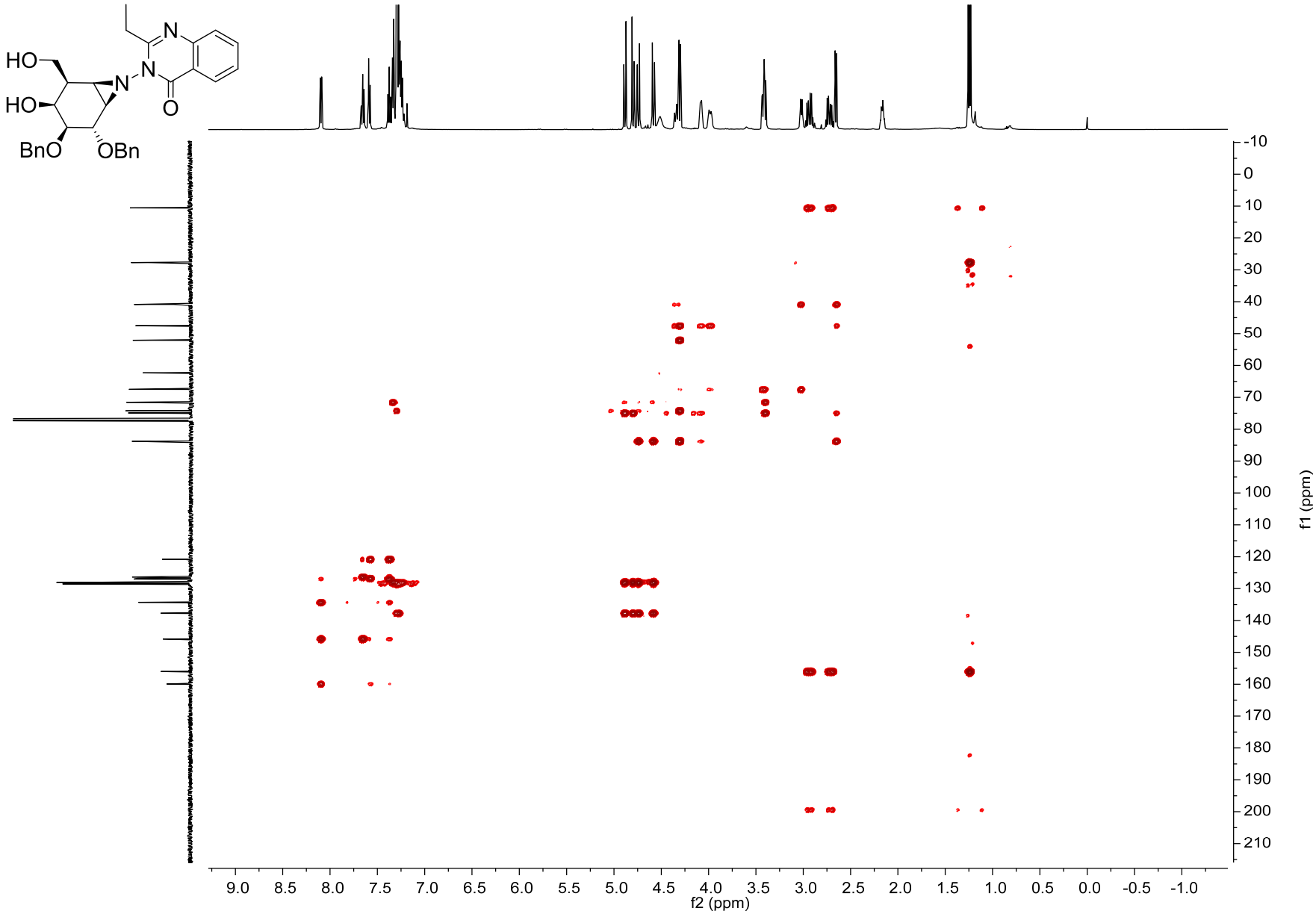
*g*COSY 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one
(**4**, 500 MHz, $CDCl_3$)



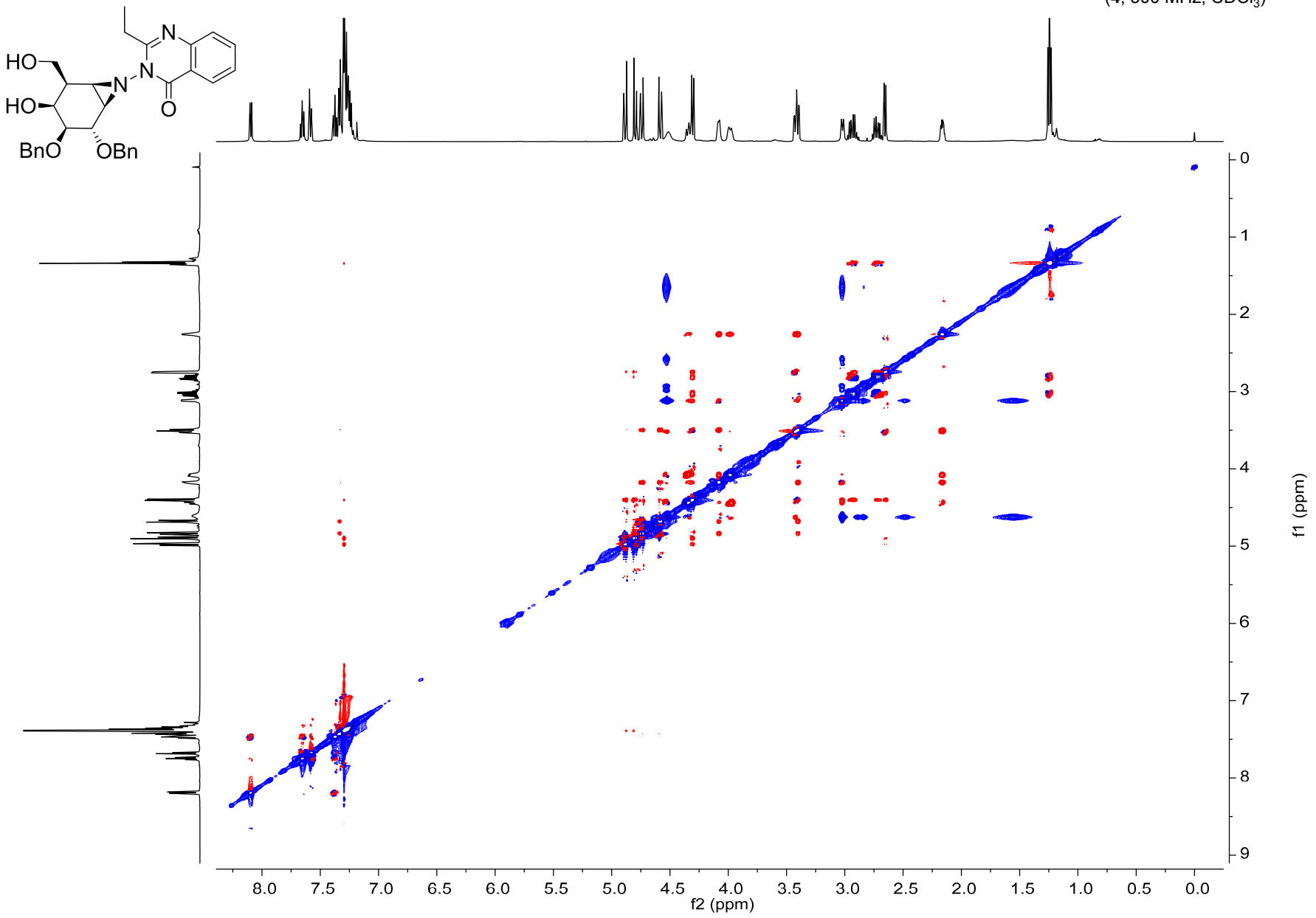
*g*HSQC spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)



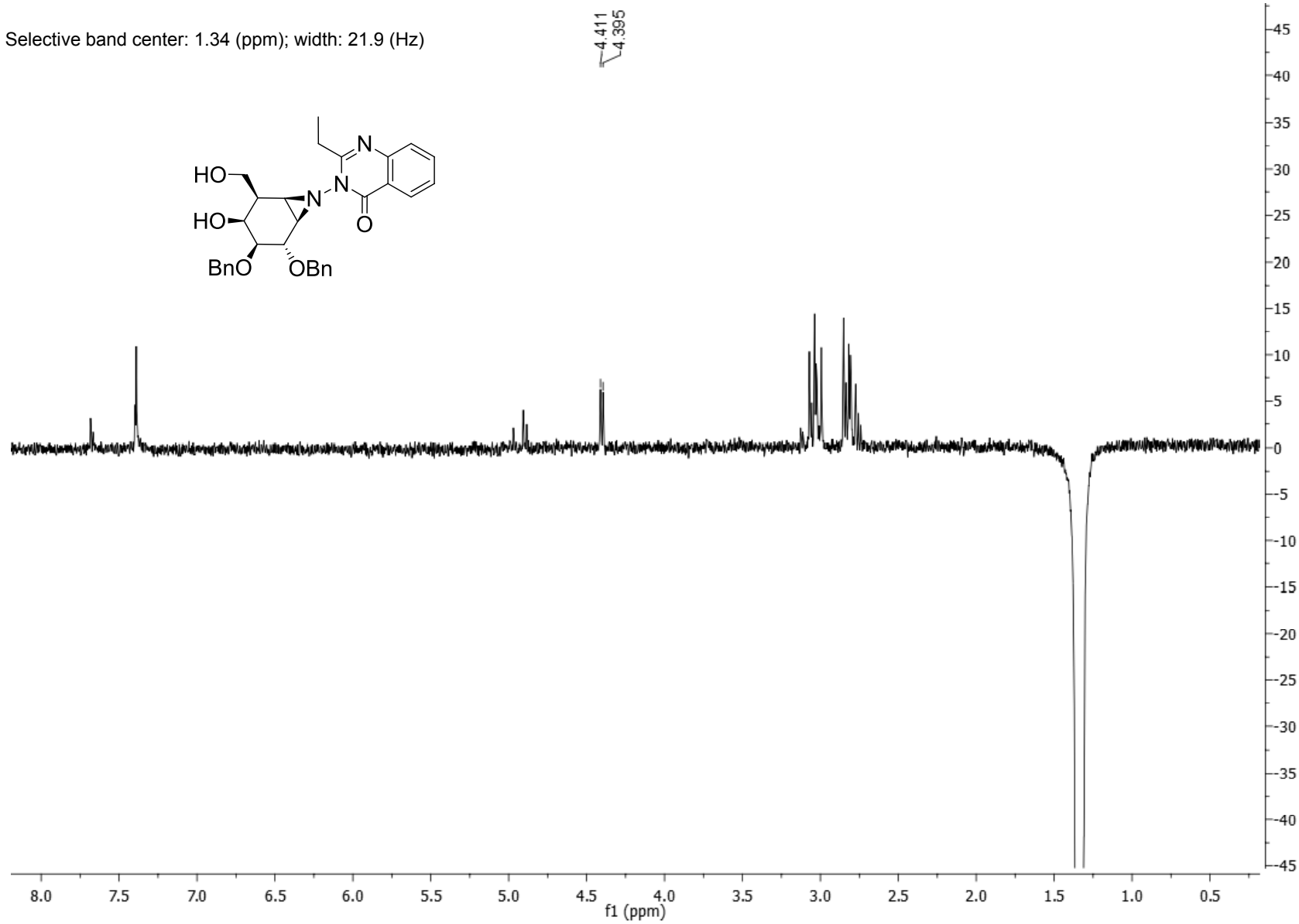
*g*HMBC spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)



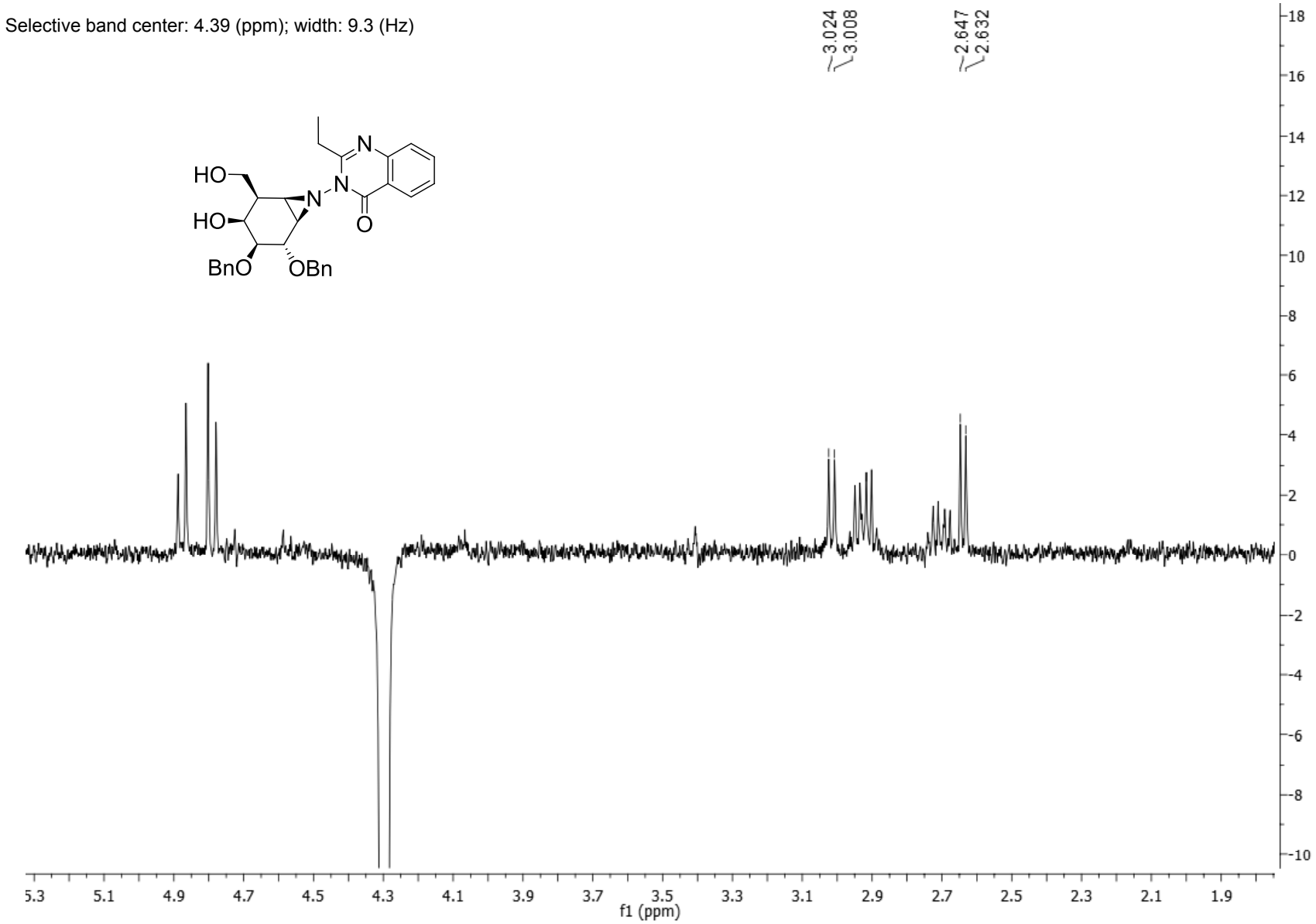
NOESY spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)



1D NOESY spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)

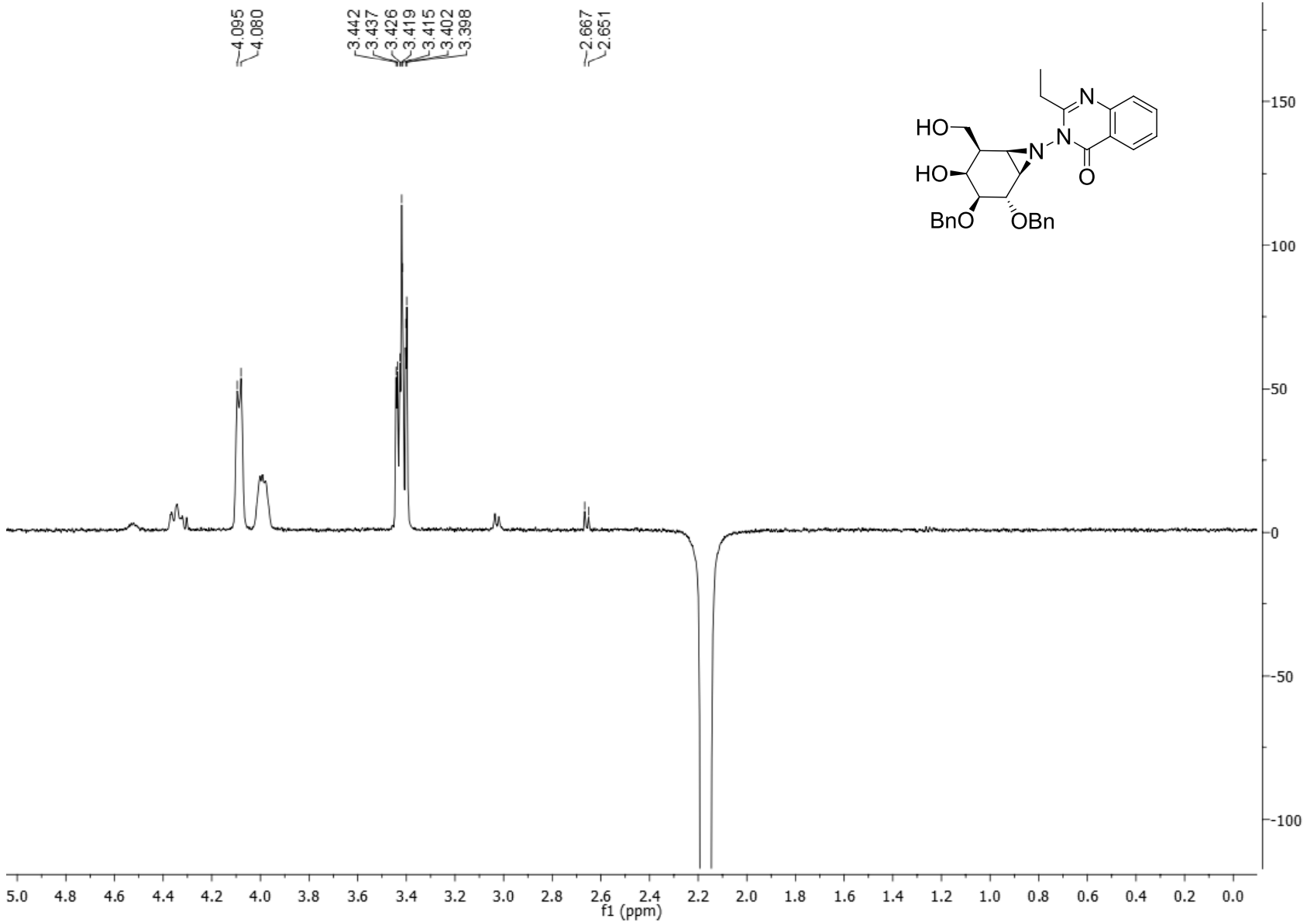


1D NOESY spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)



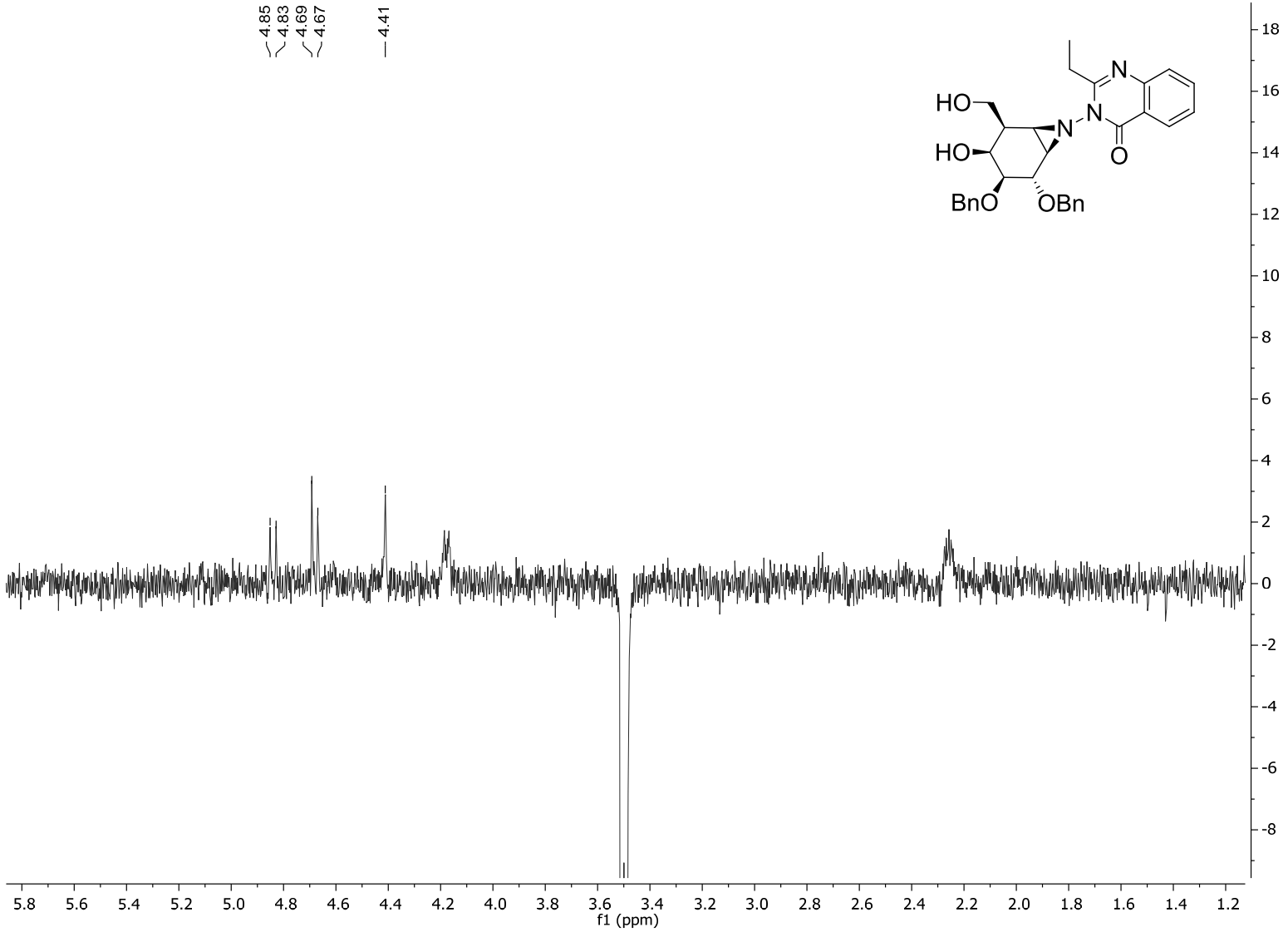
1D NOESY spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)

Selective band center: 2.26 (ppm); width: 49.4 (Hz)



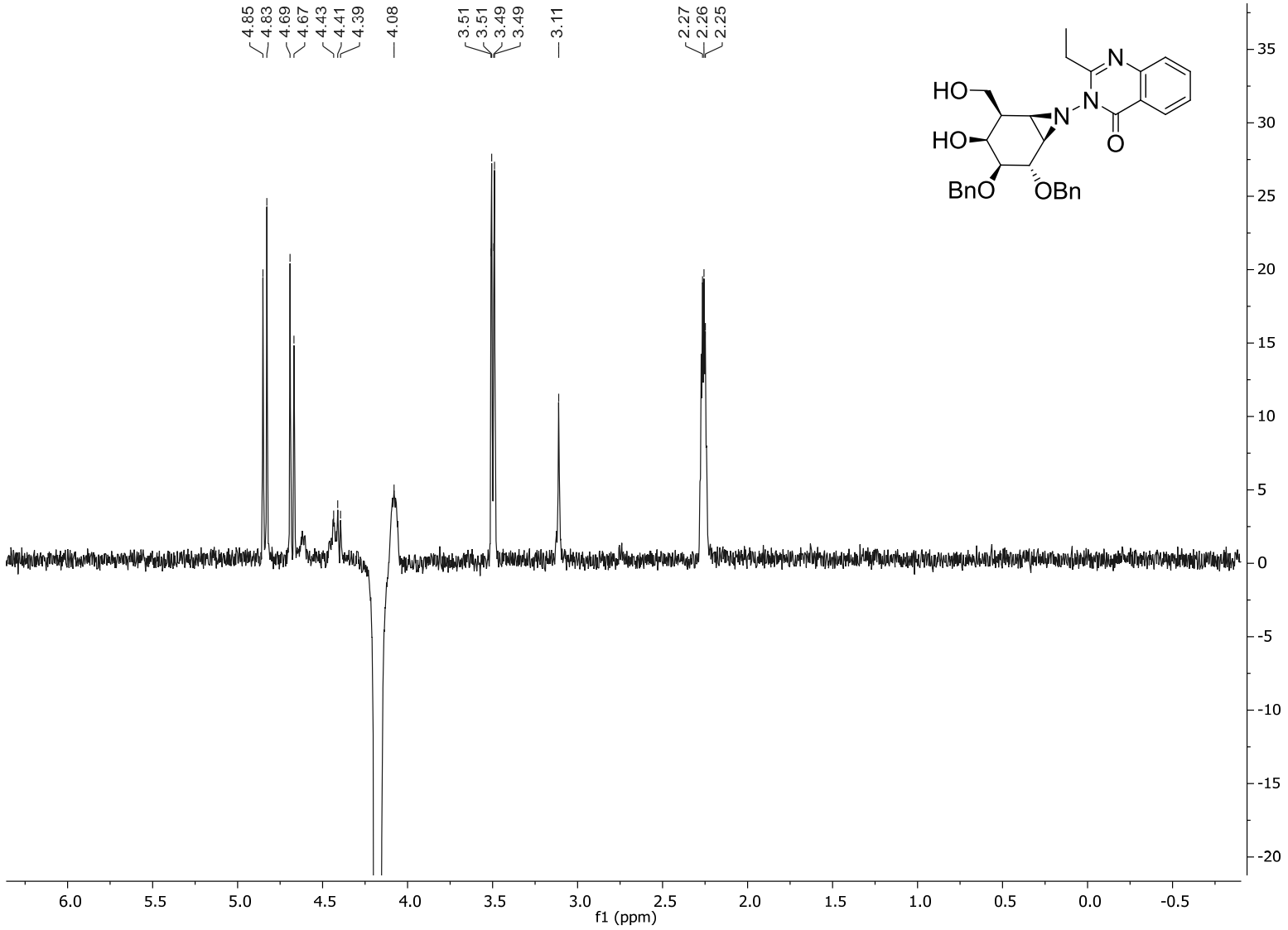
1D NOESY spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)

Selective band center: 3.49 (ppm); width: 7.3 (Hz)

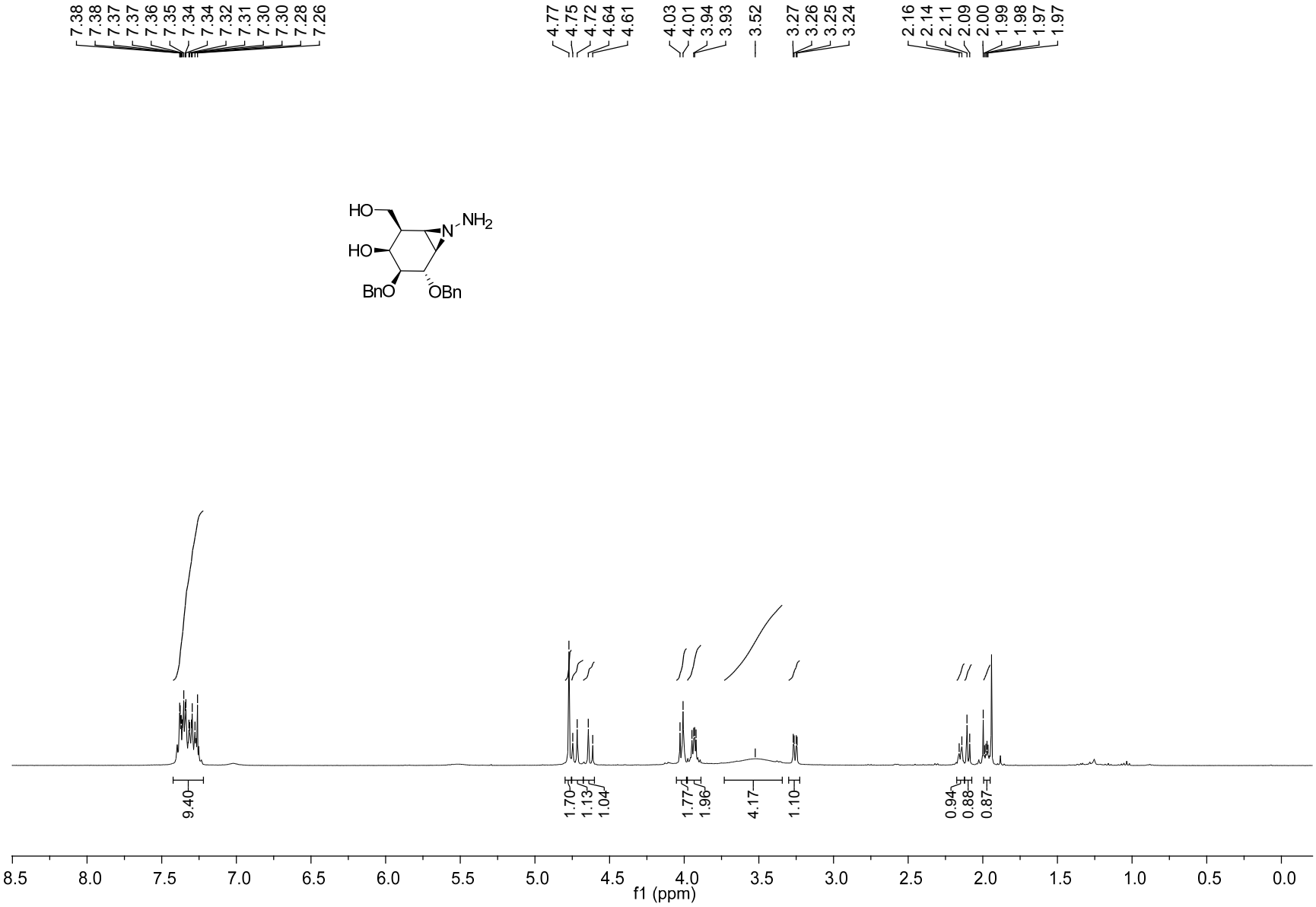


1D NOESY spectrum of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 500 MHz, $CDCl_3$)

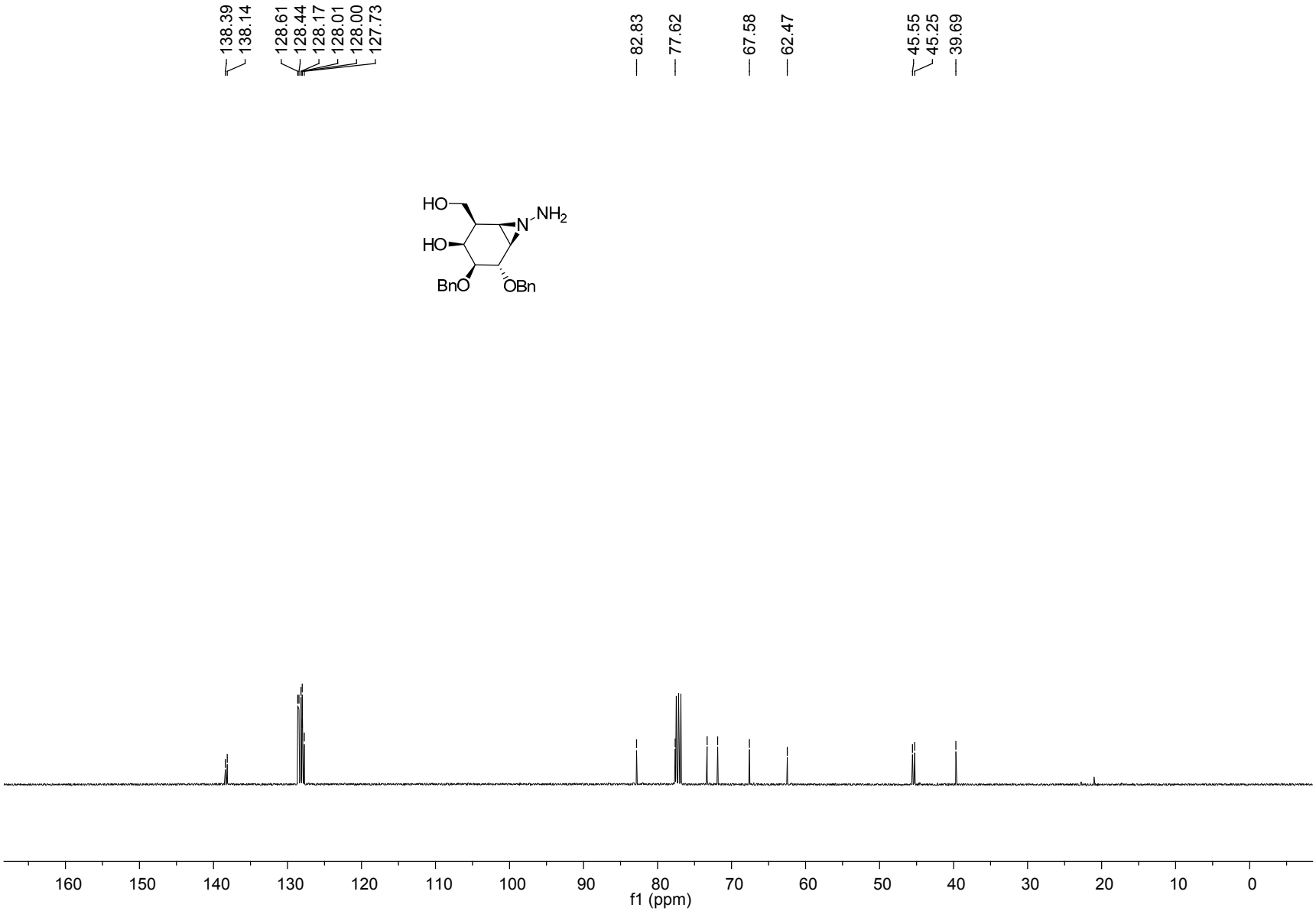
Selective band center: 4.17 (ppm); width: 21.6 (Hz)



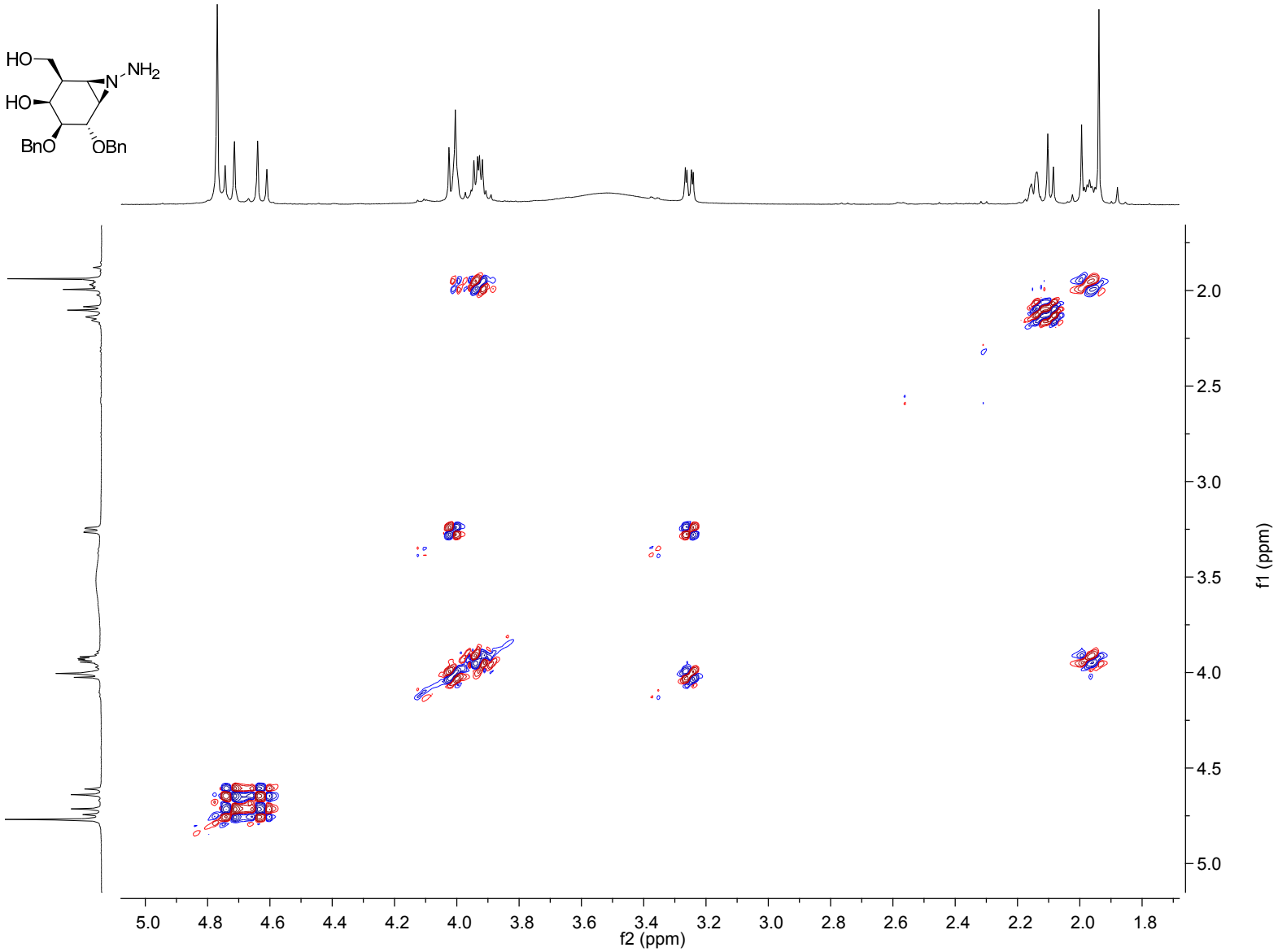
^{1}H-NMR spectrum of (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-7-amino-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-3-ol (**5**, 400 MHz, $CDCl_3$)



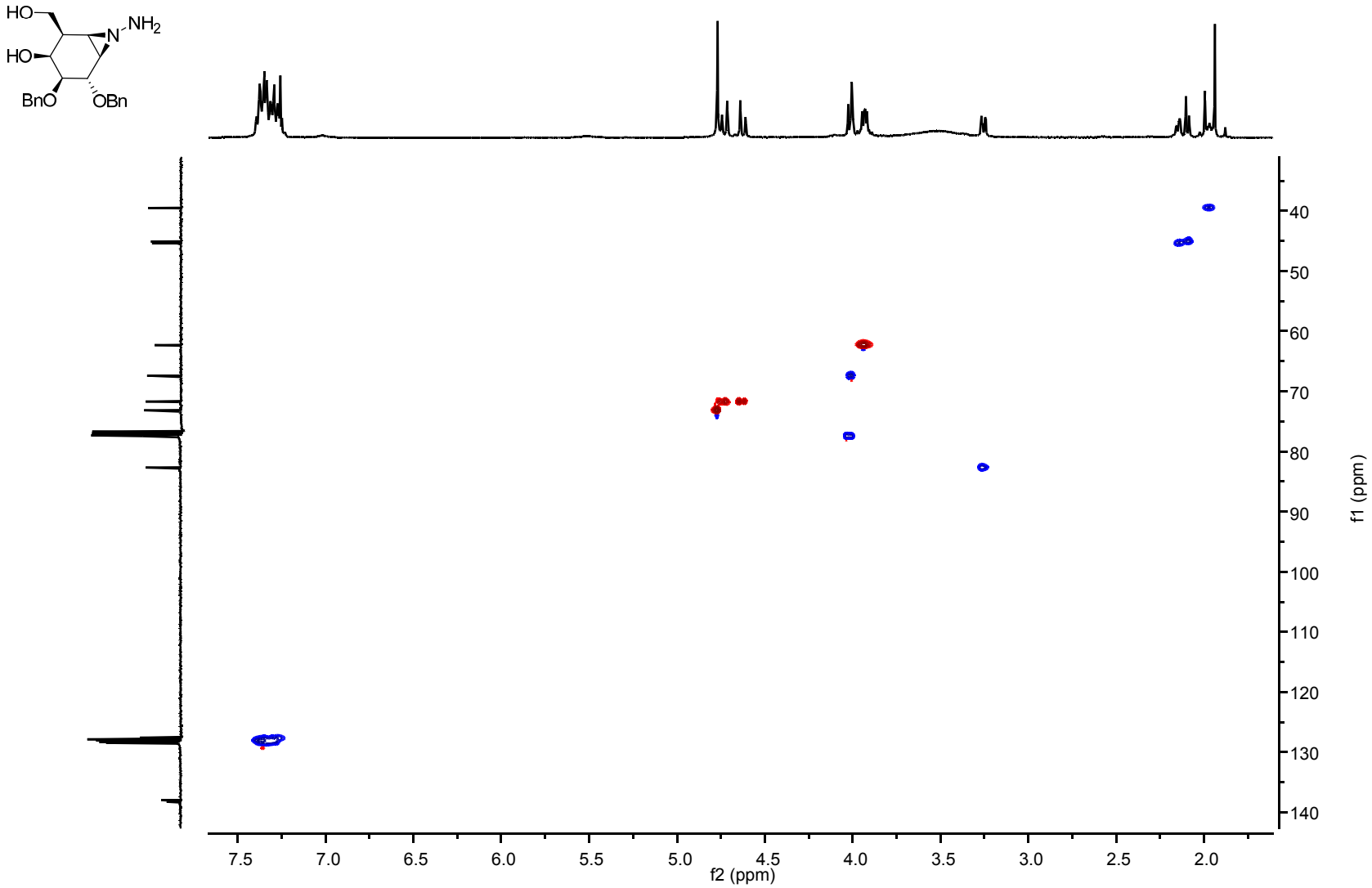
^{13}C-NMR spectrum of (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-7-amino-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-3-ol (**5**, 101 MHz, $CDCl_3$)



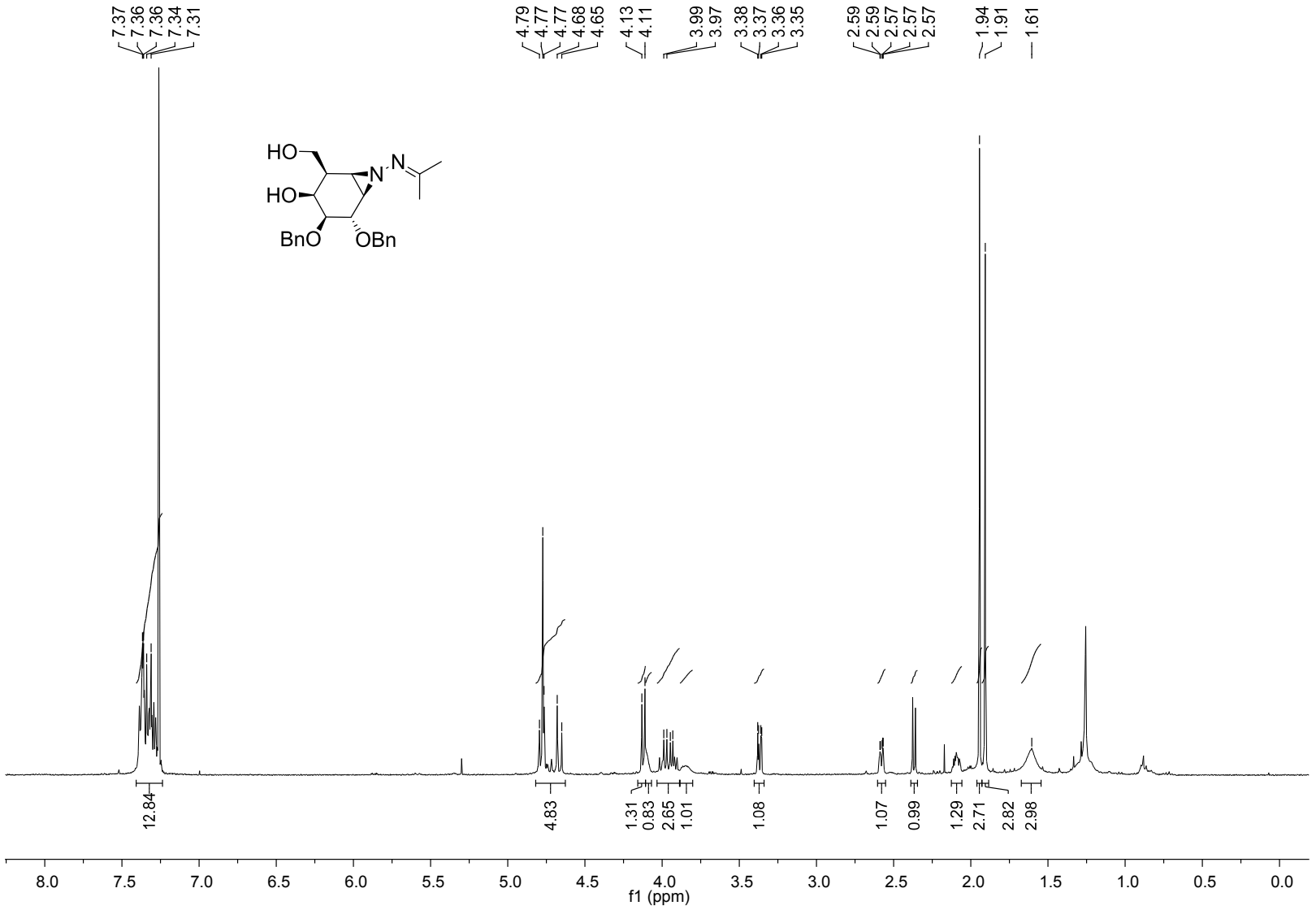
gDQCOSY (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-7-amino-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-3-ol (**5**, 400 MHz, $CDCl_3$)



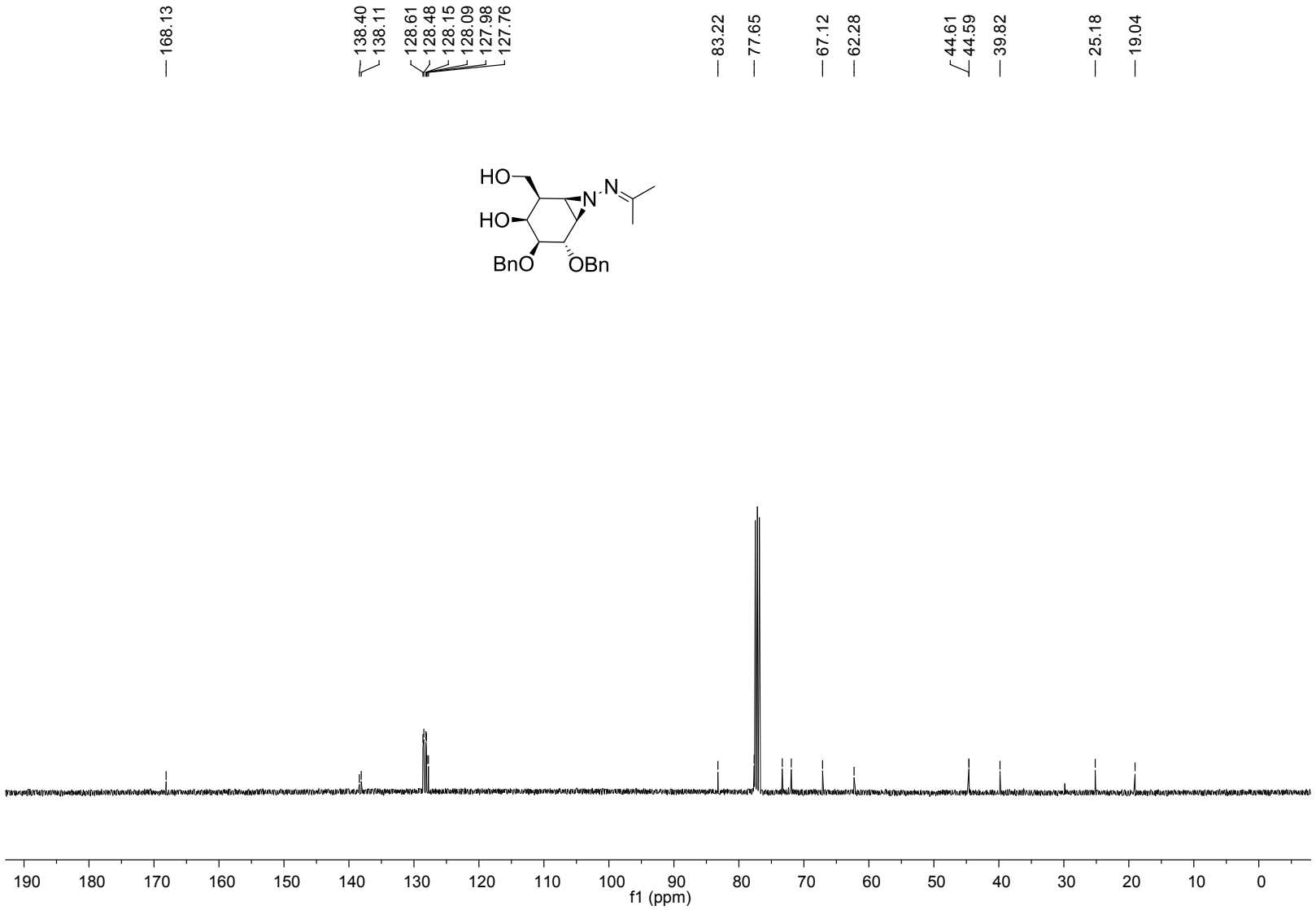
gHSQC (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-7-amino-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-3-ol (**5**, 400 MHz, $CDCl_3$)



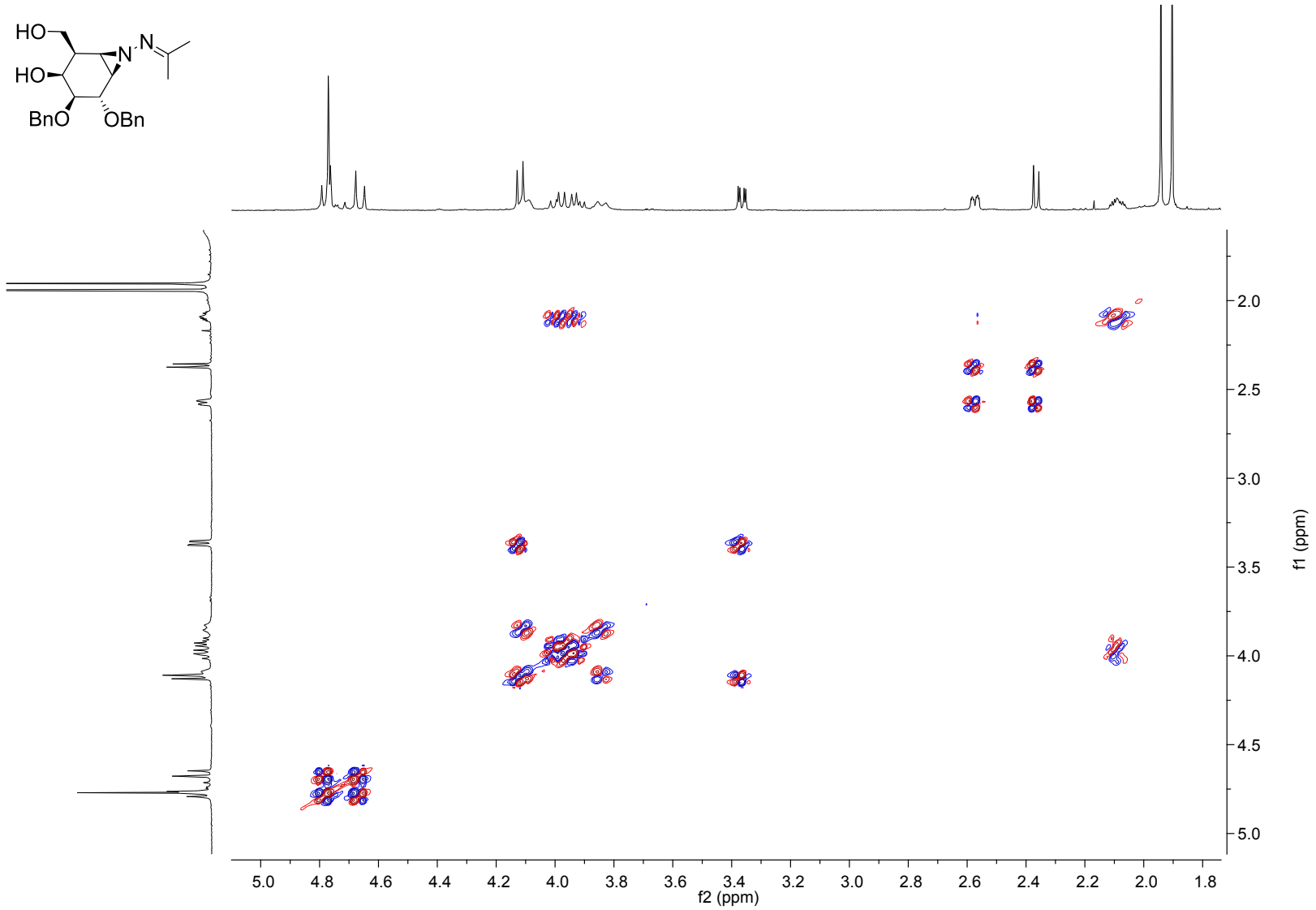
^{1}H-NMR spectrum of (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-aza bicyclo[4.1.0]heptan-3-ol (**8**, 400 MHz, $CDCl_3$)



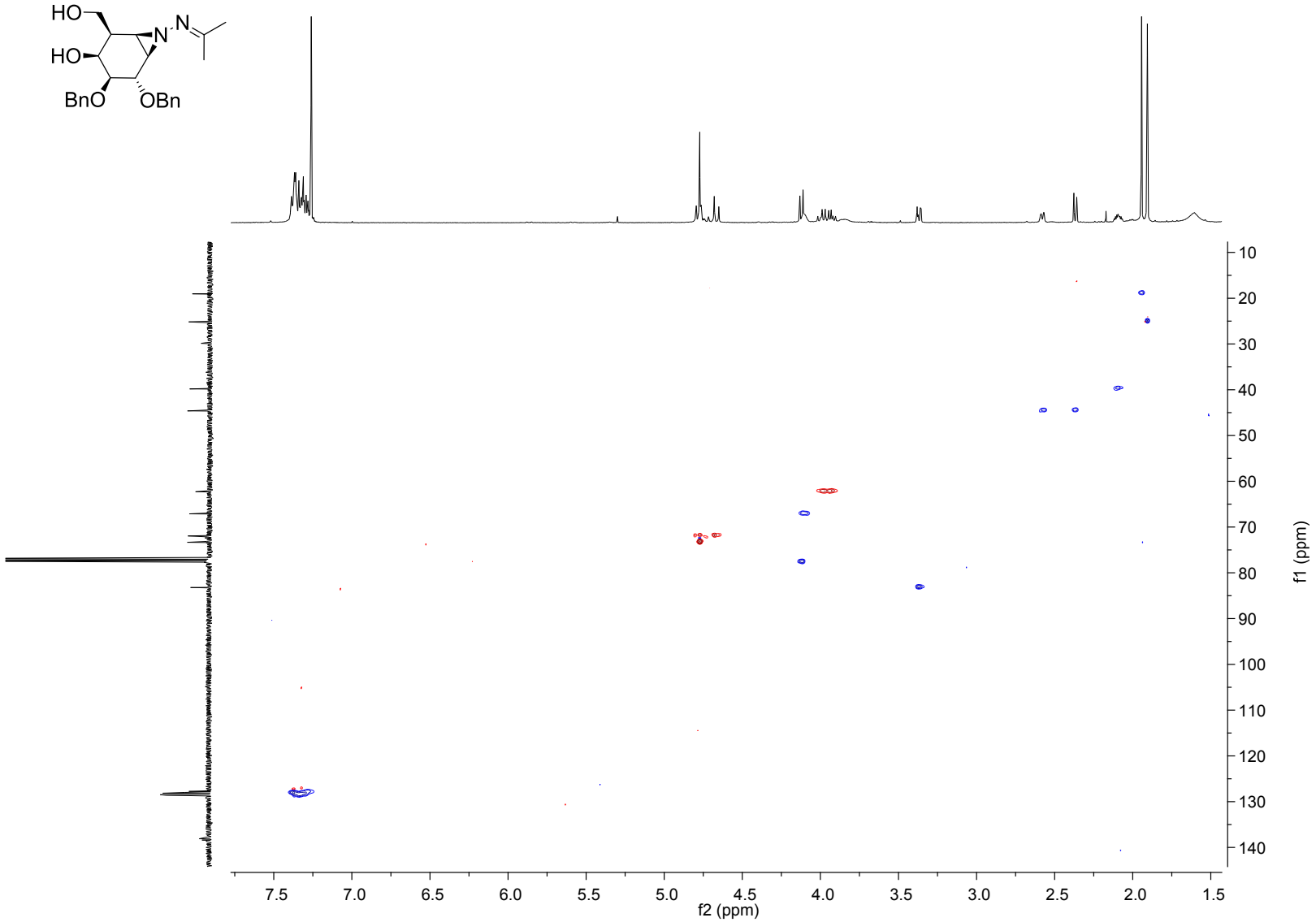
^{13}C-NMR spectrum of (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-aza bicyclo[4.1.0]heptan-3-ol (**8**, 101 MHz, $CDCl_3$)



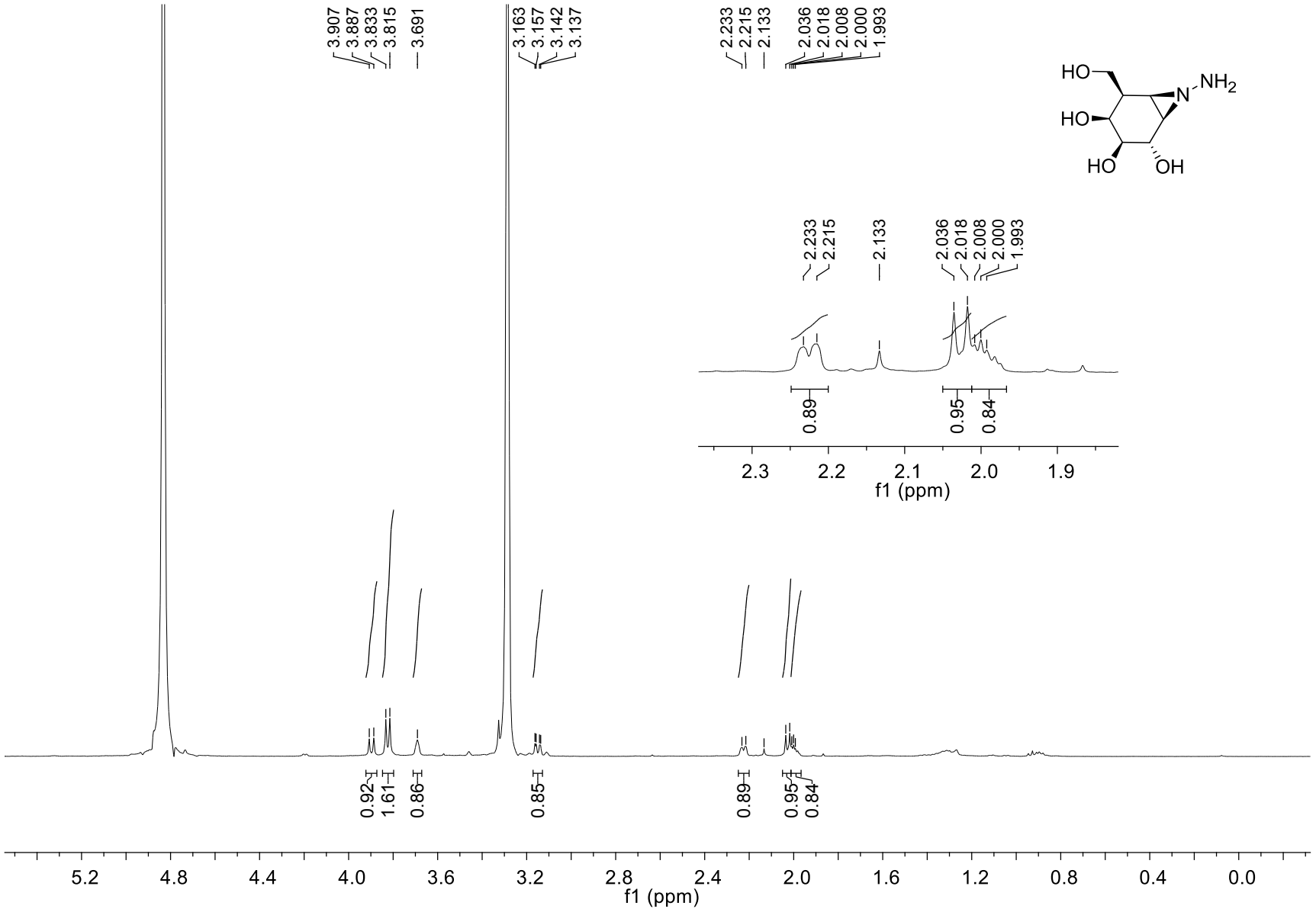
gDQCOSY (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-aza bicyclo[4.1.0]heptan-3-ol (**8**, 400 MHz, $CDCl_3$)



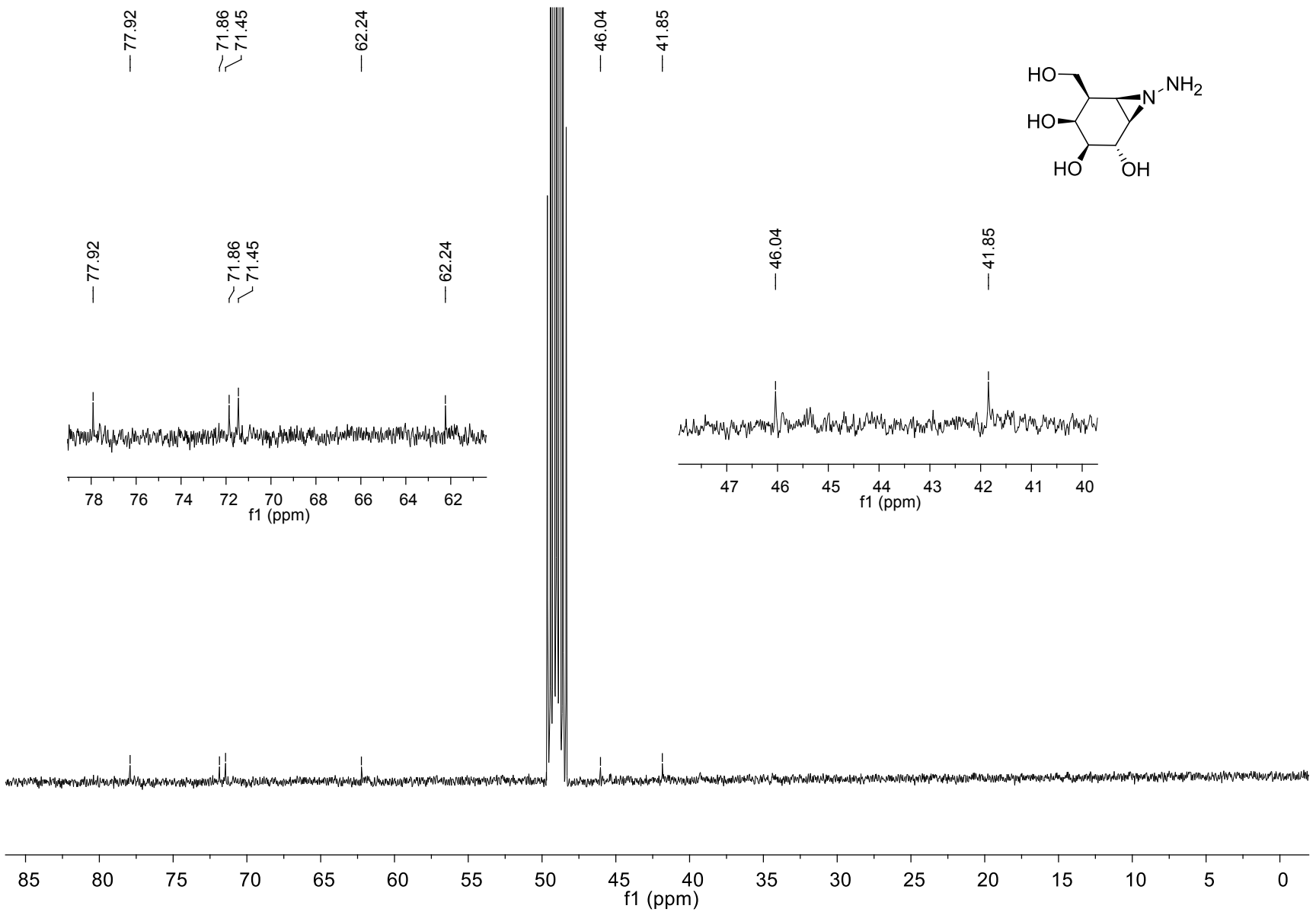
gHSQC spectrum of (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-aza bicyclo[4.1.0]heptan-3-ol (**8**, 400 MHz, $CDCl_3$)



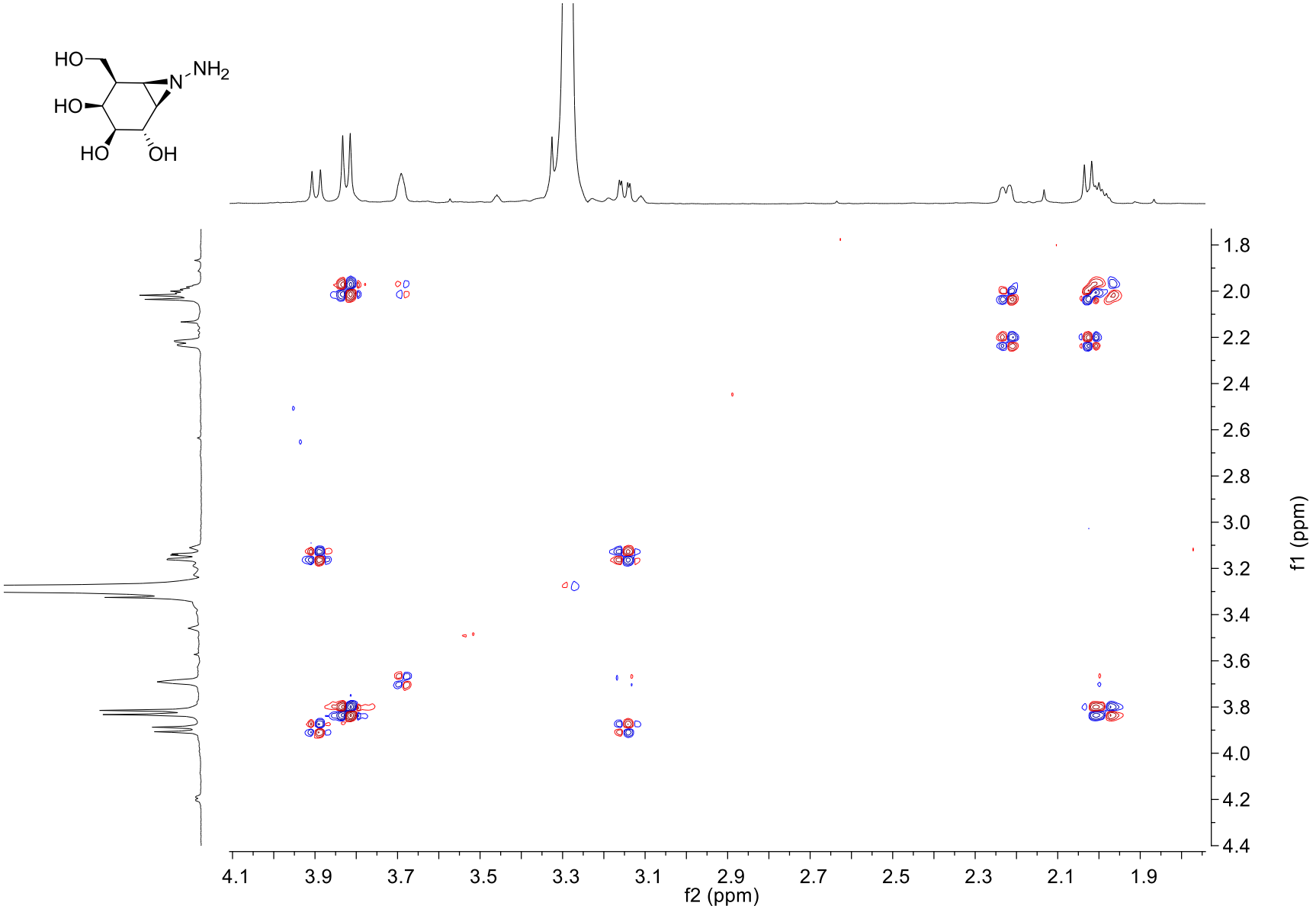
^{1}H-NMR spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1a**, 400 MHz, CD_3OD)



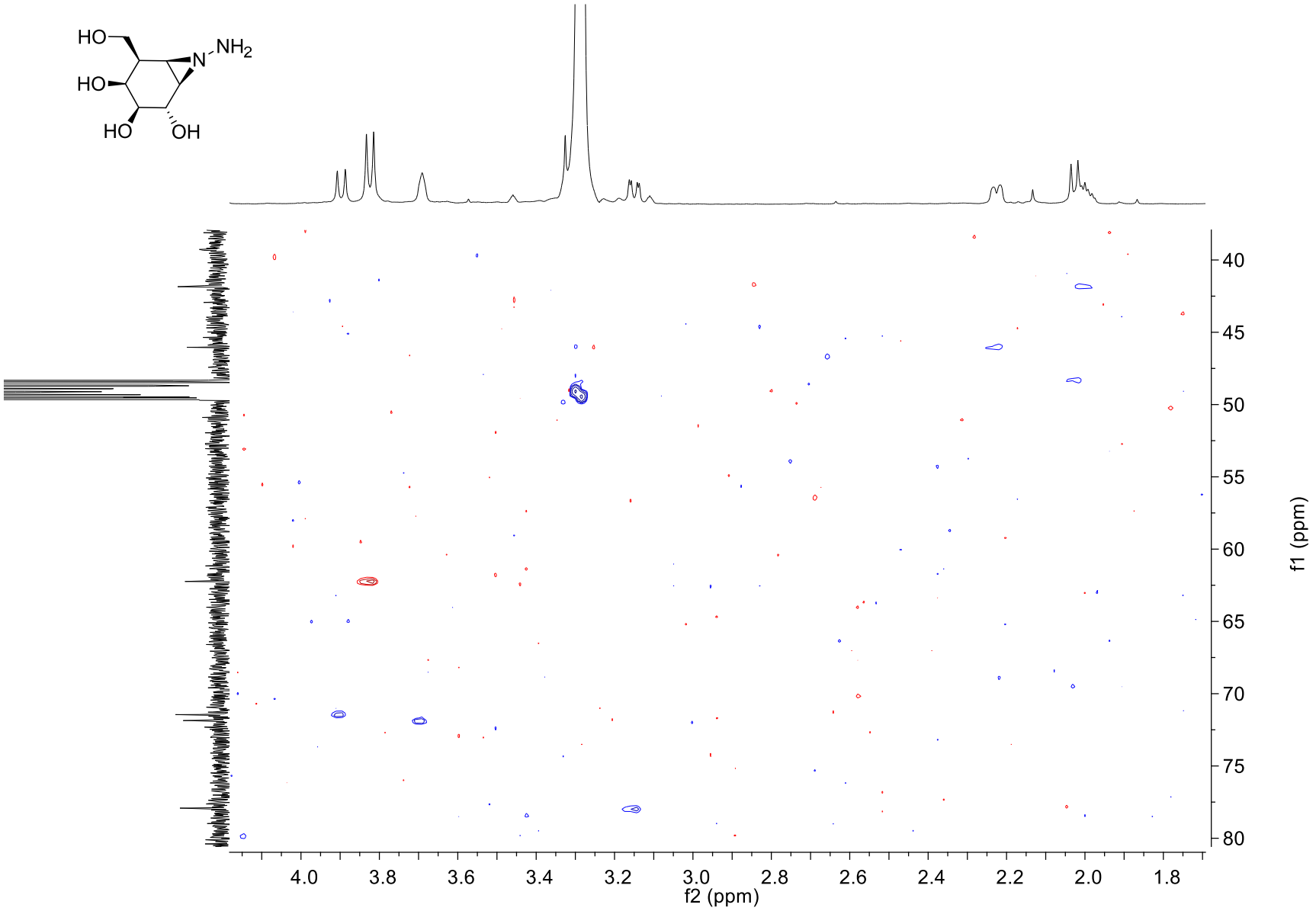
^{13}C-NMR spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1a**, 101 MHz, CD_3OD)



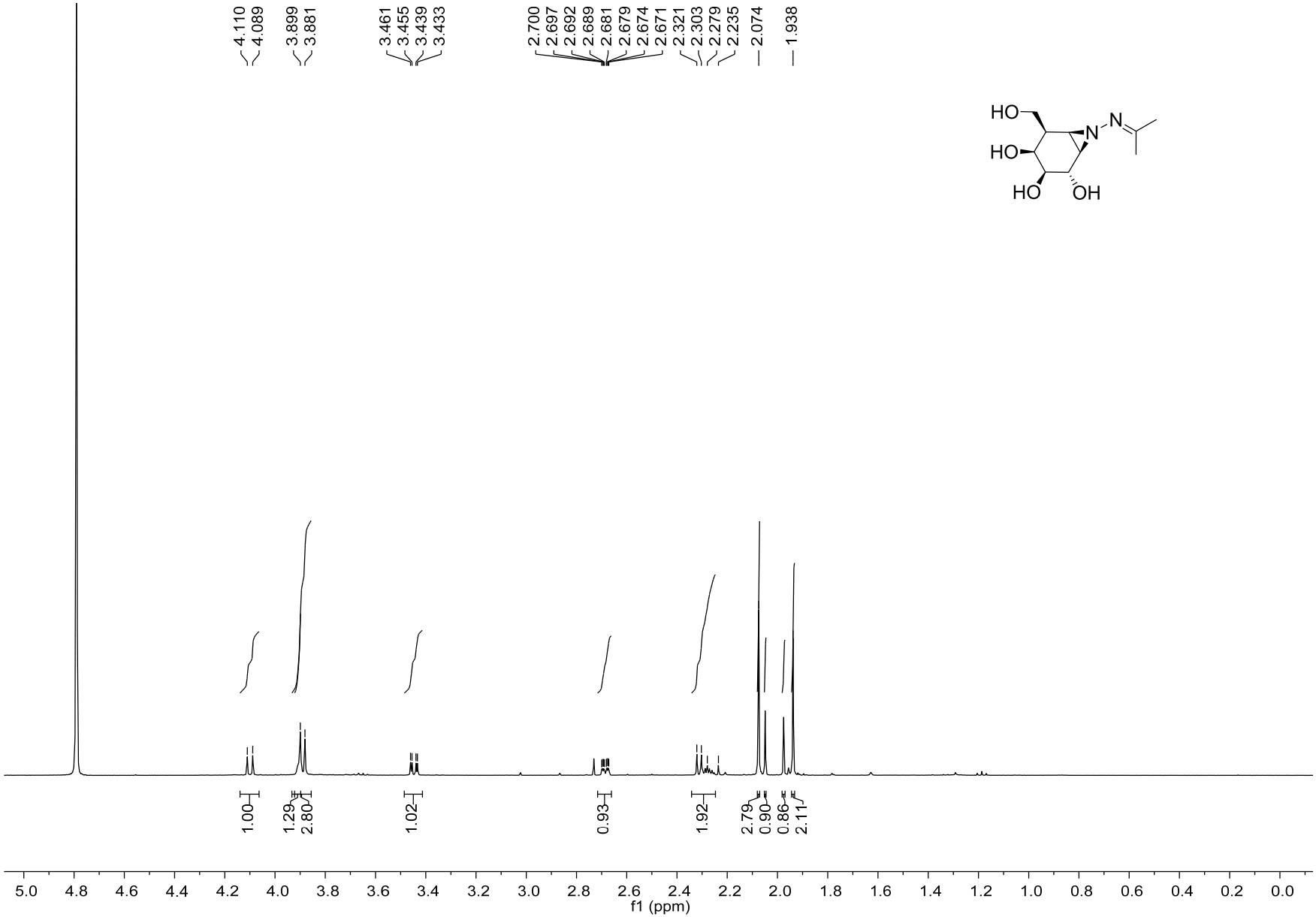
gDQCOSY (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1a**, 400 MHz, CD_3OD)



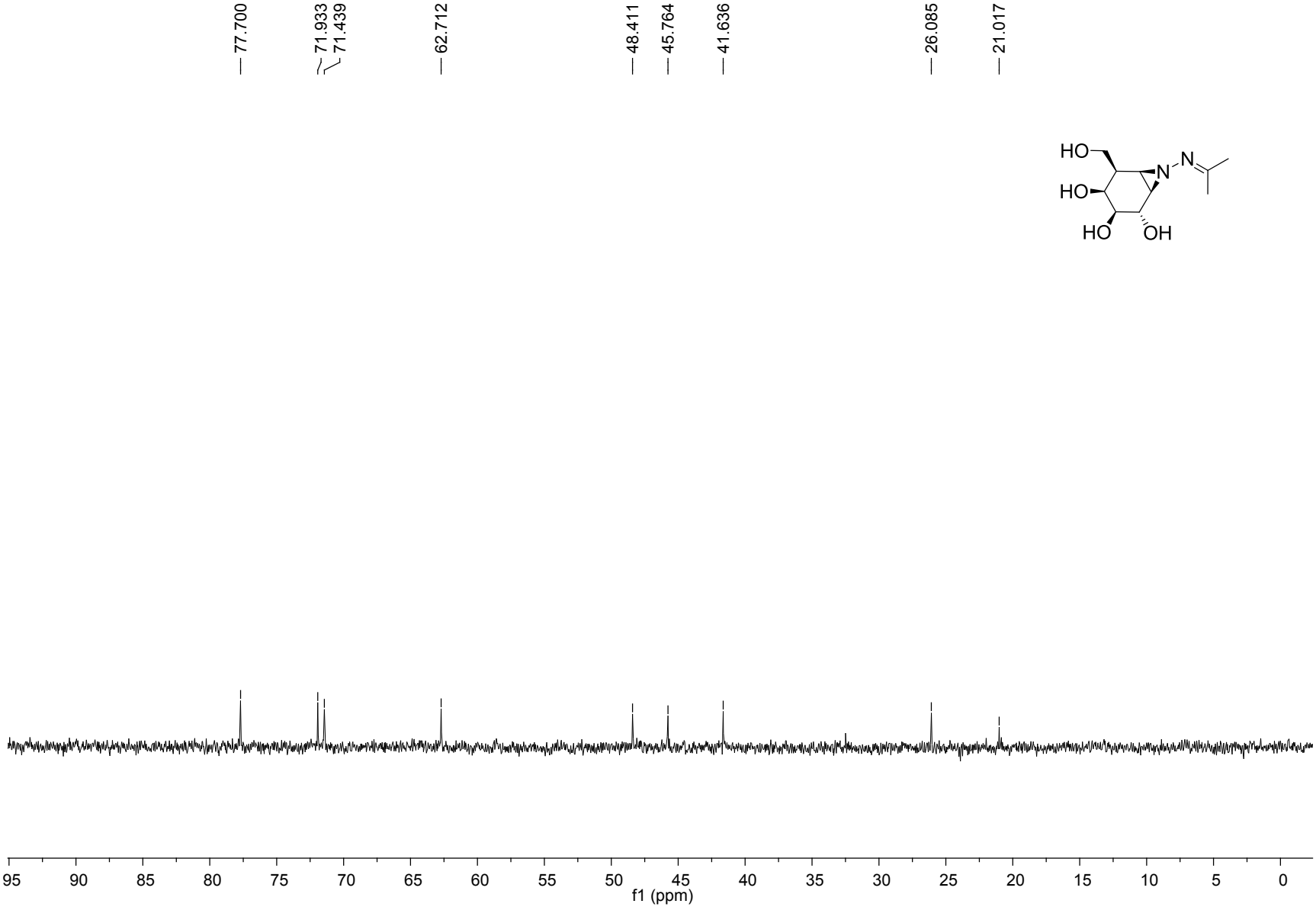
gHSQC (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1a**, 400 MHz, CD_3OD)



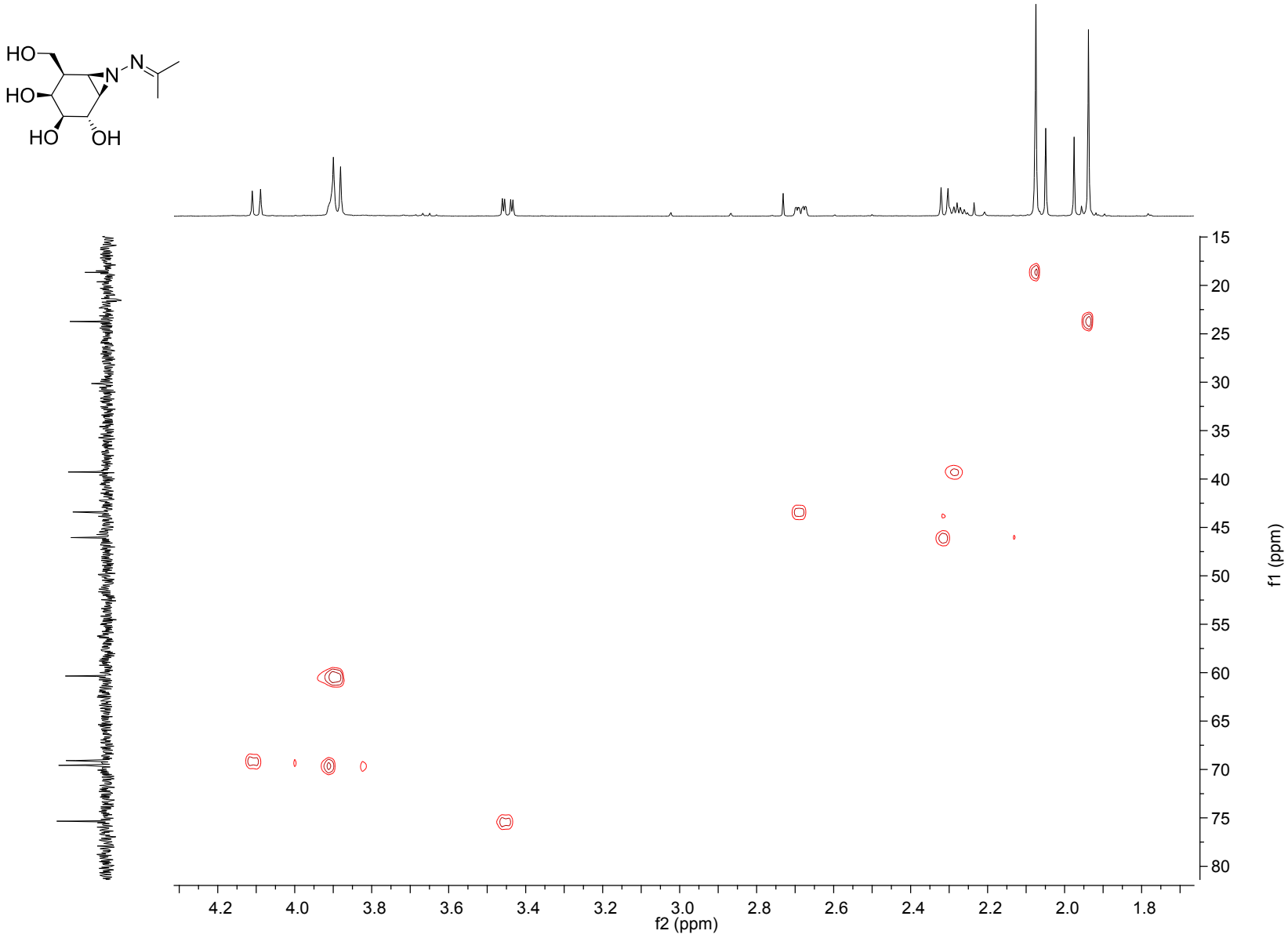
^{1}H-NMR spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1b**, 400 MHz, D_2O)



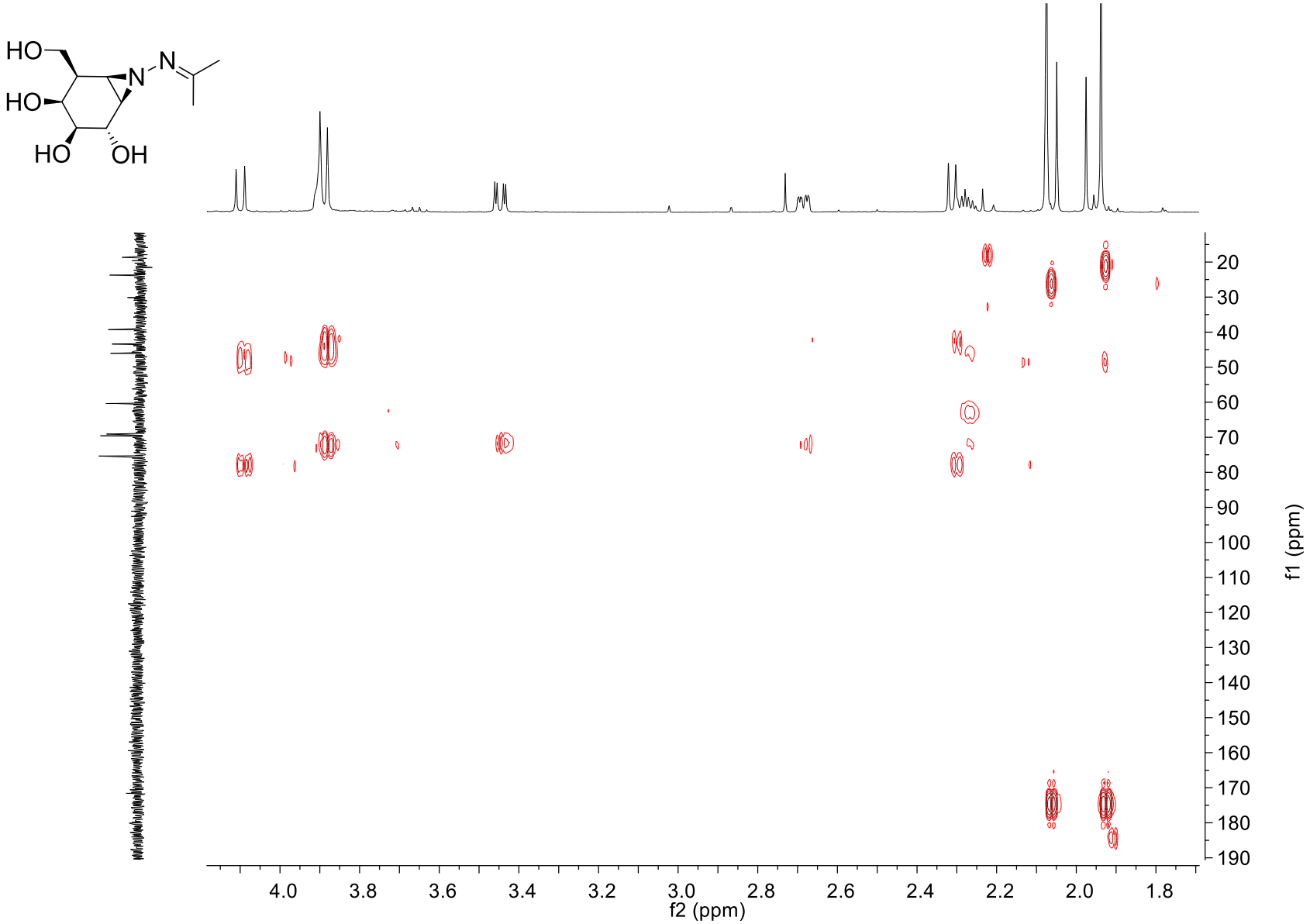
^{13}C-NMR spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1b**, 101 MHz, D_2O)



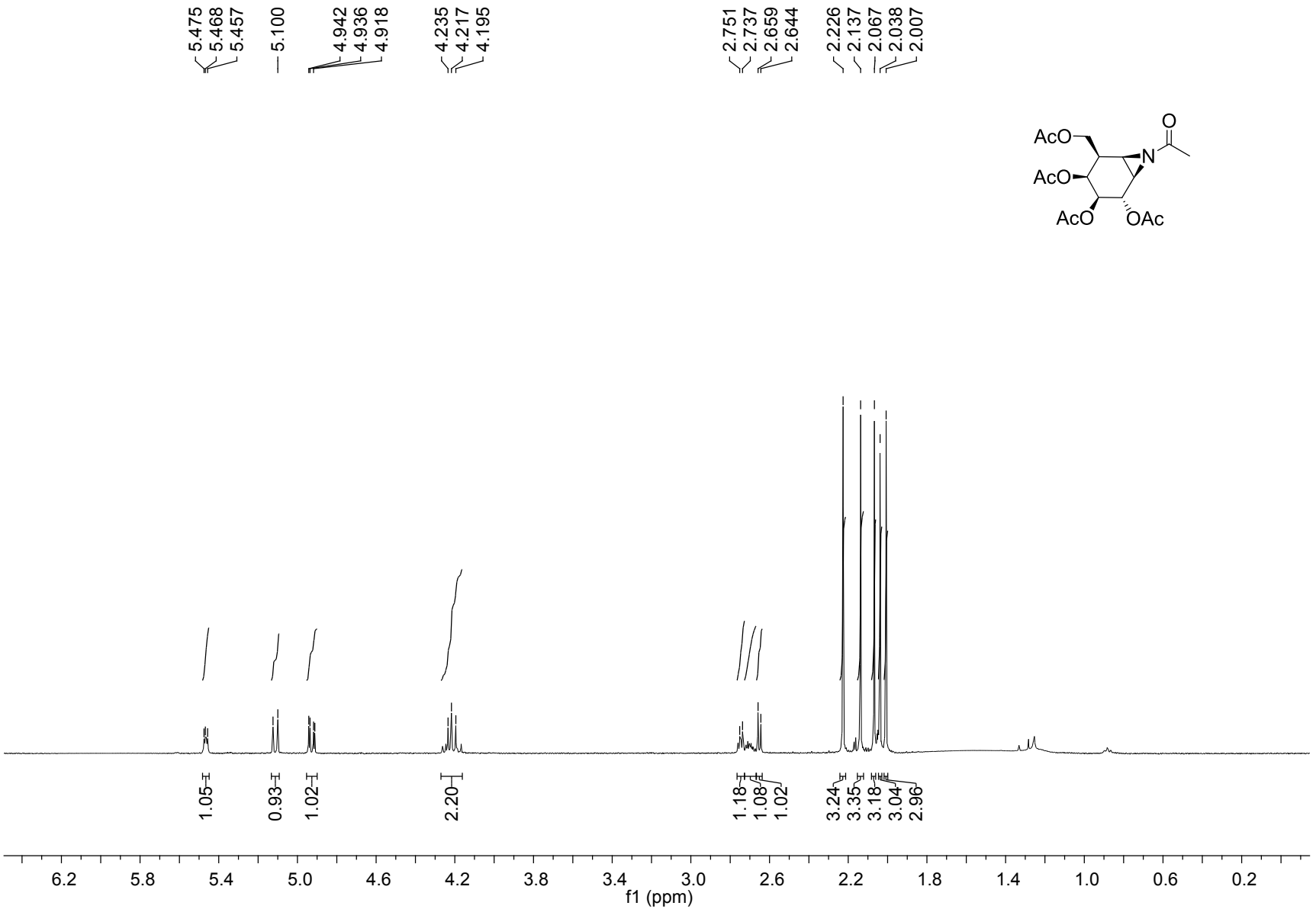
HSQC (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1b**, 500 MHz, D_2O)



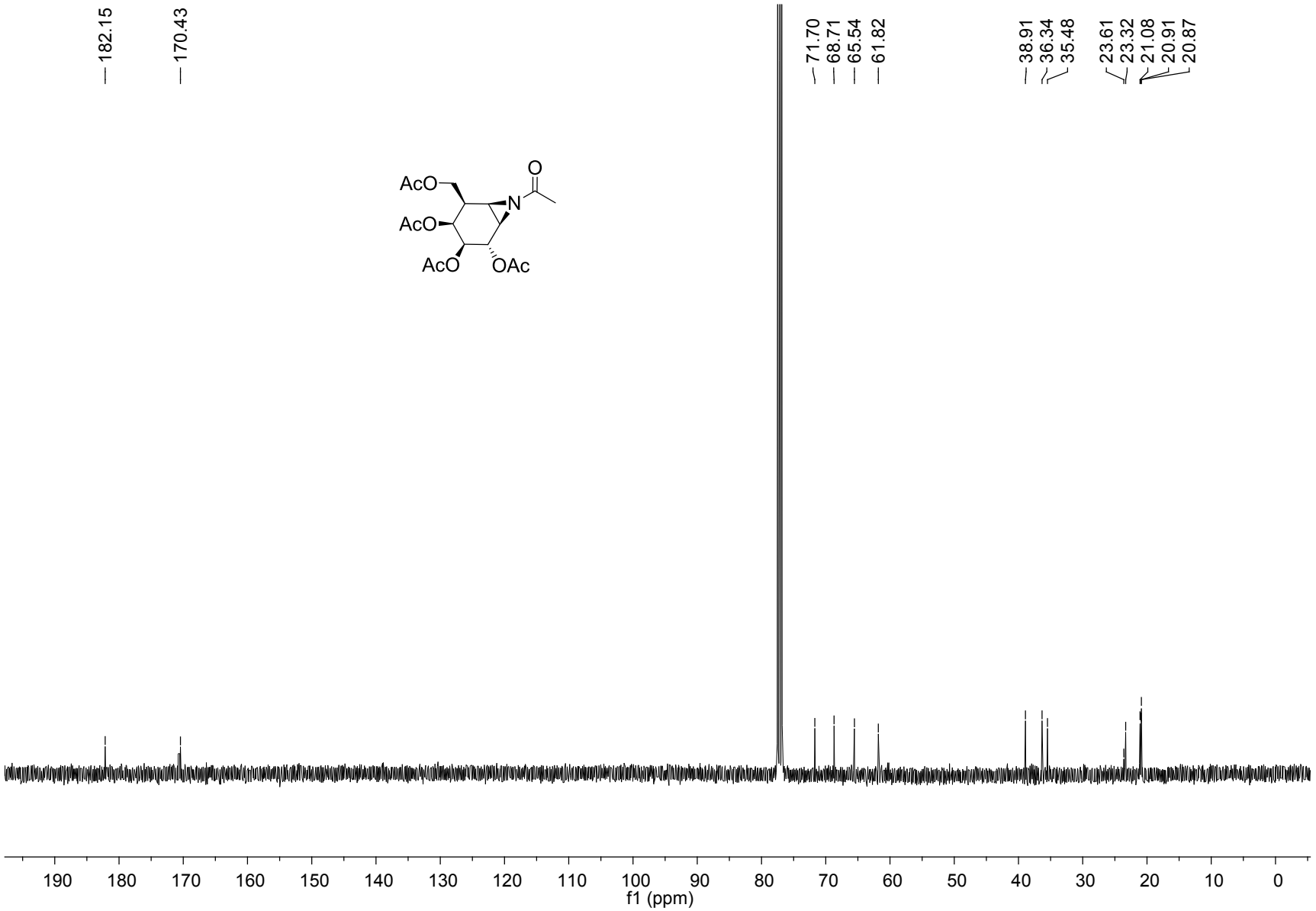
HMBC (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (**1b**, 500 MHz, D_2O)



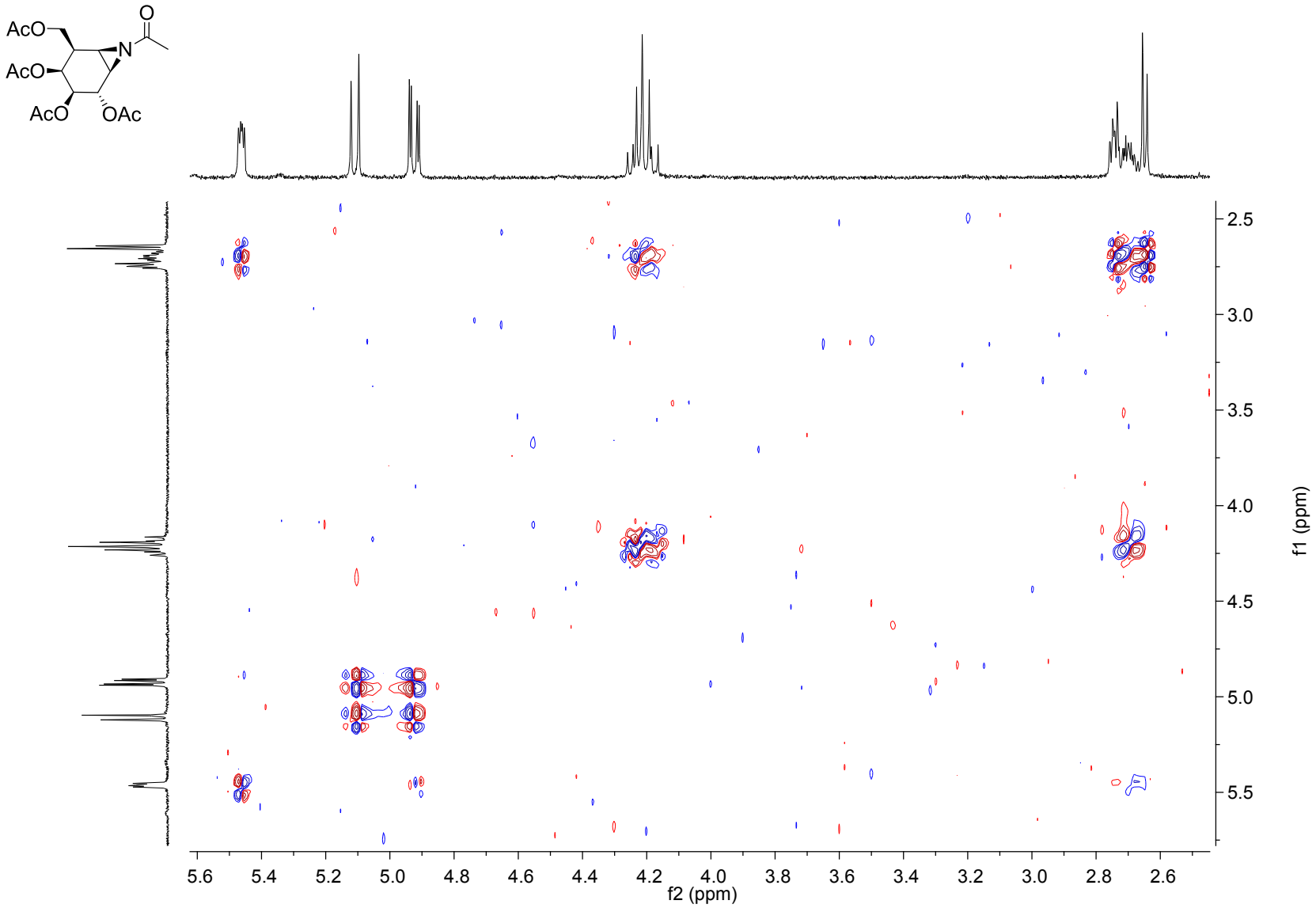
^{1}H-NMR spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(acetoxymethyl)-7-acetyl-7-azabicyclo[4.1.0]heptane-2,3,4-triyl triacetate (**7**, 400 MHz, $CDCl_3$)



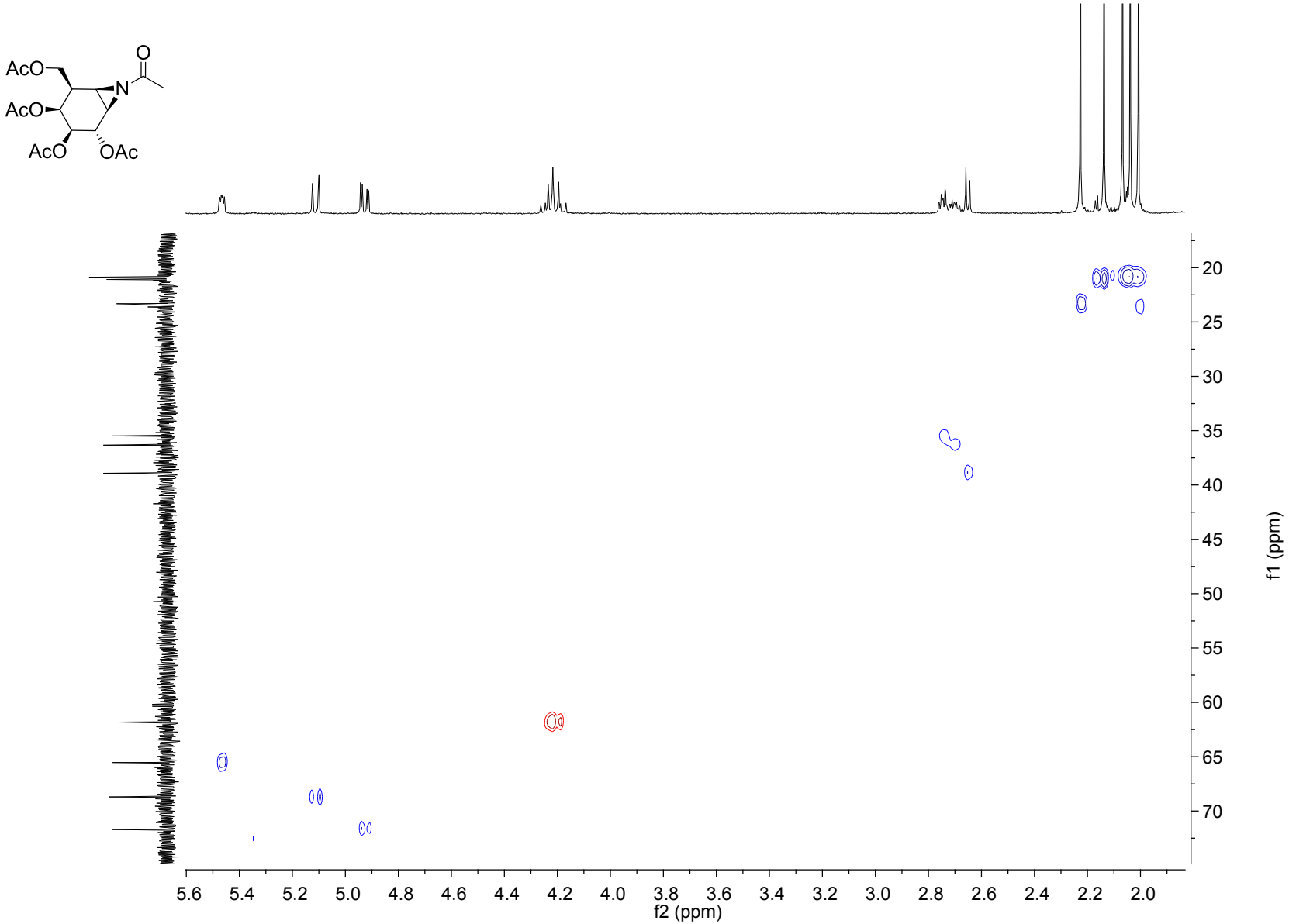
^{13}C-NMR spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(acetoxymethyl)-7-acetyl-7-azabicyclo[4.1.0]heptane-2,3,4-triyl triacetate (**7**, 101 MHz, $CDCl_3$)



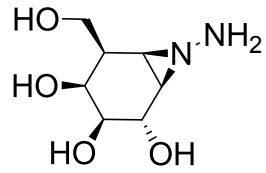
*g*DQCOSY spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(acetoxymethyl)-7-acetyl-7-azabicyclo[4.1.0]heptane-2,3,4-triyl triacetate (**7**, 400 MHz, $CDCl_3$)



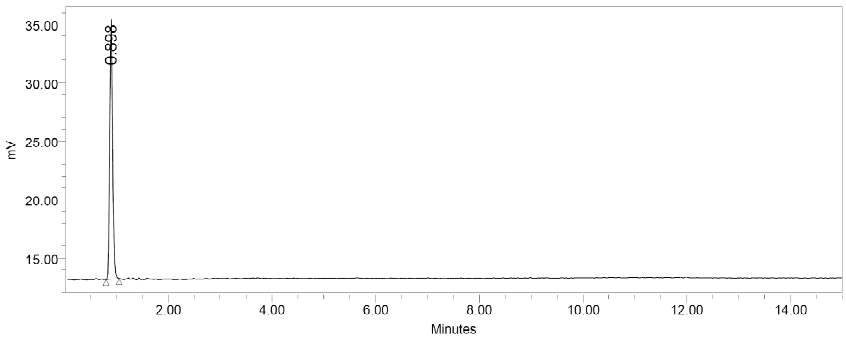
*g*HSQC spectrum of (1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(acetoxymethyl)-7-acetyl-7-azabicyclo[4.1.0]heptane-2,3,4-triyl triacetate (**7**, 400 MHz, $CDCl_3$)



HPLC chromatograms



1a



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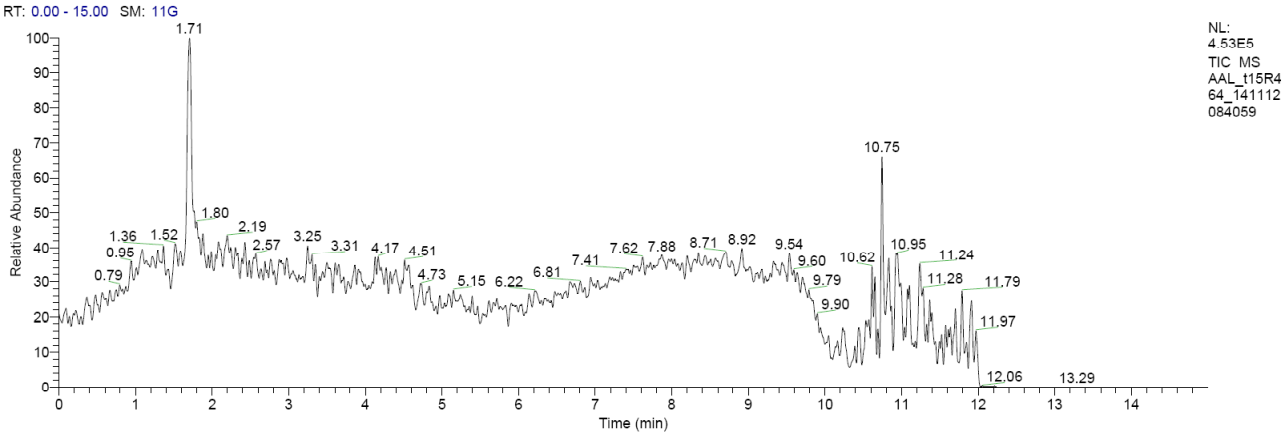
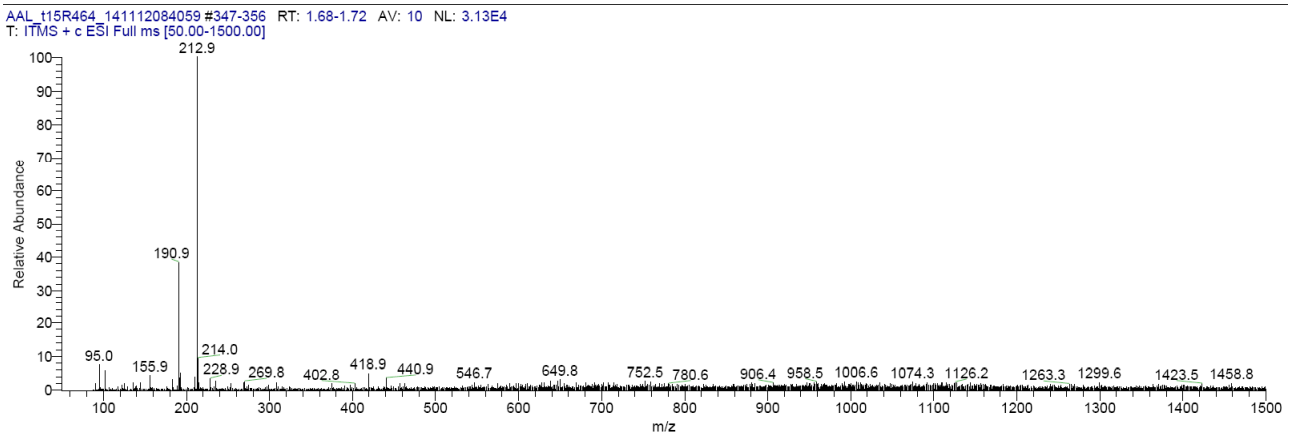
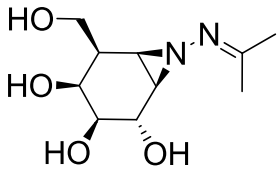
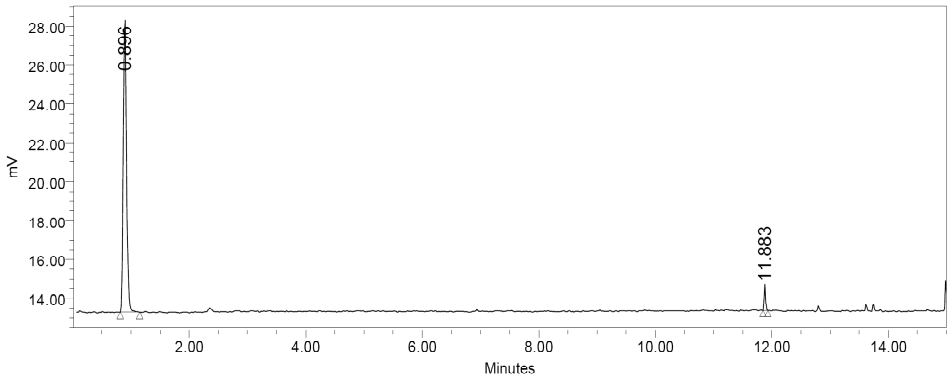






1b



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