Supporting information

Practical and divergent synthesis of carbocyclic pyrazolo[3,4-d]pyrimidine nucleoside analogues

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In vitro antiparasitic assays

Antiparasitic assays were performed as described elsewhere.[24] To evaluate anti-*Leishmania* activity, *L. infantum* [MHOM/MA (BE)/67] was used with primary peritoneal mouse macrophages (PMM) as host cells. 3×104 macrophages were infected with 4.5×105 parasites per well. Compound dilutions were added after 2 h of infection. After 5 days of incubation, parasite burdens (mean number of amastigotes/macrophage) were assessed microscopically after staining with a 10% Giemsa solution. For *T. cruzi*, the Tulahuen CL2, β-galactosidase strain (nifurtimox-sensitive) was used and maintained on MRC-5 human lung fibroblasts. 4×103 MRC-5 cells were infected with 4×104 parasites per well. Parasite burdens were assessed after adding the substrate chlorophenol red β-D-galactopyranoside and spectrophotometric reading at 540 nm after 4 h incubation at 37°C. Drug susceptibility tests for *T. brucei* were performed using a resazurin assay. Susceptibility assays were performed with *T. brucei* Squib 427 or *T. b. rhodesiense* STIB-900. *T. brucei* Squib 427 was seeded at 1.5×104 parasites/well and *T. b. rhodesiense* at 4×103 parasites per well, followed by the addition of resazurin after 24 h (*T. brucei*) or 6 h (*T. b. rhodesiense*). After the addition of resazurin, plates were incubated for another 24 h followed by fluorescence detection (λ_{ex} 550 nm, λ_{em} 590 nm).

In all assays, parasite growth was compared to untreated-infected controls (100% growth) and noninfected controls (0% growth). Results were expressed as % parasite reduction at the different drug concentrations and used to calculate EC50 values from the dose-response curves.

In vitro cytotoxicity assay

MRC-5 cell cytotoxicity was evaluated as described elsewhere.[24] Briefly, 1.5×105 cells/mL cells were cultured with compound dilutions at 37°C and with 5% CO_2. After 3 days of incubation, cell viability was assessed fluorometrically after the addition of 50 µL resazurin per well. After 4 h at 37°C, fluorescence was measured. The results were expressed as a %

reduction in cell growth/viability compared to control wells and an EC_{50} value was determined.

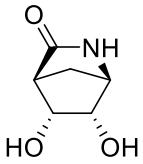
Synthetic procedures

General Information

Reactions were performed in oven dried round-bottomed flasks under an argon atmosphere sealed with rubber septa, unless otherwise stated. Reactions were magnetically stirred using teflon-coated stir bars. Reagents and solvents were purchased at the highest commercial quality and used without additional purification, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on precoated Macherey-Nagel® SIL G/UV254 plates using UV 254 nm for visualization and basic KMnO4 solution (20 g KMnO4 + 10 g K2CO3 in 1L water) as developing agent. Flash column chromatography was performed automatically using a Büchi Pure C-815 Flash system with prepacked cartridges. NMR spectra were recorded at 25 °C on a Bruker Avance 400 spectrometer. NMR spectra were referenced using residual undeuterated solvent (chloroform-*d*: ^{1}H NMR = 7.26 ppm, ^{13}C NMR = 77.16 ppm, methanol-d_4: ^{1}H NMR = 3.31 ppm, ^{13}C NMR = 49.15 ppm, dmso-d_6: ^{1}H NMR = 2.50 ppm, ^{13}C NMR = 39.51 ppm, D_2O: ^{1}H NMR = 4.75 ppm). The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal and AB = part of an AB spin system, observed scalar coupling is reported. Chemical shifts are expressed in ppm and coupling constants are given in Hertz (Hz). Weak carbon signals were assigned using HSQC/HMBC experiments. LC-MS analyses were carried out on a Waters Auto Purification System equipped with PDA and ESI-MS detection and using a Waters CORTECS C_{18} Column (4.6×100 mm, 2.7 µm) and a water/acetonitrile/formic acid linear gradient system at a flow rate of 1.44 mL/min.

(1R,4S,5R,6S)-5,6-dihydroxy-2-azabicyclo[2.2.1]heptan-3-one (8)

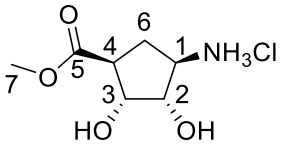
Synthesis according to literature procedure.[20] The crude was purified via flash column chromatography (1 - 7% MeOH in DCM) to yield **8** as a white solid (10.64 g, 74%).



Spectral data in accordance with literature values.[20]

Methyl-(1S,2R,3S,4R)-4-amino-2,3-dihydroxycyclopentane-1-carboxylate hydrochloride (9)

Acetyl chloride (34 ml) was carefully added to methanol (80 ml) at 0°C to generate HCl in situ. The ice bath was removed and the solution was allowed to reach room temperature (20 to 30 minutes). **8** was added and allowed to stir for 24 hours. Afterwards, the volatiles were removed in vacuo and the residue was suspended in dichloromethane (40 ml), stirred for 15 minutes, and filtered. The residue was washed with dichloromethane (20 ml), and dried. A slightly green solid **9** was obtained (11.48 g, 97%) which turned light pink over time.



^{1}H NMR (400 MHz, D_2O): δ 1.87 (dt, *J* =14, 9 Hz, 1H H6), 2.54 (dt, *J* = 14, 9 Hz, 1H H6), 3.02 (td, *J* = 9, 5 Hz, 1H, H4), 3.59 (br. quart., *J* = 8 Hz, 1H, H1), 3.76 (s, 1H, H7), 4.09 (dd, *J* = 8, 6 Hz, 1H, H2), 4.31 (t, *J* = 5 Hz, 1H, H3).

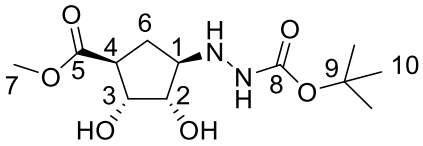
^{13}C NMR (100 MHz, D_2O): δ 27.2 (C6), 47.6 (C4), 54.5 (C1), 52.8 (C7), 72.7 (C3), 74.3 (C2), 175.4 (C5)

***2-(tert-butyl) 3,3-diethyl 1,2-oxaziridine-2,3,3-tricarboxylate* (25).**

Synthesis according to literature procedure.[22] (4.4 g, 67% over 2 steps).
Spectral data in accordance with literature values.[22]

Tert-butyl 2-((1R,2S,3R,4S)-2,3-dihydroxy-4-(methoxycarbonyl) cyclopentyl)hydrazine-1-carboxylate (12)

Synthesis according to literature procedure.[22] The crude was purified via flash column chromatography (1 - 9% MeOH in DCM) and a white solid **12** was obtained (1.86 g, 49%).



^{1}H NMR (400 MHz, chloroform-*d*): δ 1.46 (s, 9H, H10), 1.64 (dt, J = 14, 8 Hz, H6), 2.21-2.31 (m, 1H, H6'), 2.86 (td, J = 9, 5 Hz, 1H, H4), 3.39 (br. quart., J = 6 Hz, 1H, H1), 3.71 (s, 3H, H7), 3.83 (t, J = 5 Hz, 1H, H2), 4.33 (t, J = 5 Hz, 1H, H3), 6.23 (br. s, 1H, unknown). Except for one (6.23 ppm), signals corresponding to labile protons were not found.

^{13}C NMR (100 MHz, chloroform-*d*): δ 28.4 (C10), 28.7 (C6), 47.4 (C4), 52.2 (C7), 64.9 (C1), 74.1 (C3), 74.6 (C2), 78.9 (C9), 156.8 (C8), 175.6 (C5).

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{23}N_2O_6$: 291.1551; Found 291.1550.

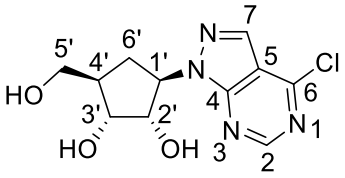
(1R,2S,3R,5R)-3-(4-chloro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (15)

12 (1.75 g, 6.68 mmol, 1.0 eq) was dissolved in dry THF (25 ml) and cooled to 0°C. LiBH4 (210 mg, 9.64 mmol, 1.4 eq) was carefully added portionwise and stirred for 5 minutes. The ice bath was removed and the mixture was stirred for 2 hours. The reaction mixture was cooled to 0°C and methanol (75 ml) was added over 1 minute, then 4N HCl in dioxane (2.33 ml) was added very carefully (gas evolution!) and stirring was continued until gas evolution ceased. Volatiles were removed in vacuo to obtain a crude white foam **11** (~1.75 g, max 6.67 mmol).

Acetyl chloride (12 ml) was carefully added to methanol (30 ml) at 0°C. After 15 minutes of stirring, the ice bath was removed and the crude **11** (1.50 g, max 5.72 mmol) was added at once. The reaction stirred for 16 hours after which the volatiles were removed in vacuo. Ethanol (20 ml) and toluene (20 ml) were added and subsequently evaporated to remove as much methanol/ethanol as possible and obtain crude **13**, which was taken in its entirety to the next step.

Crude **13** was dissolved in DMF and DIPEA was added. The mixture was cooled to 0°C and 5-(2,4-dichloropyrimidin-yl)-carbaldehyde **14** (1.01 g, max 5.71 mmol) dissolved in DMF (5 ml) was added dropwise. The reaction mixture was warmed to room temperature and after 2 to 3 hours of stirring, volatiles were evaporated. The crude was purified via flash column chromatography (1 - 9% MeOH in DCM,

then 15 - 60% acetone in DCM) to obtain **15** (875 mg, 54% over 3 steps) as a slightly yellow solid.



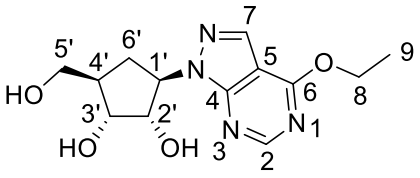
^{1}H NMR (400 MHz, methanol-d_4): δ 1.92 (ddd, *J* = 13*,* 10*,* 8 Hz, 1H, H6’), 2.22-2.33 (m, 1H, H4’), 2.39 (dt, *J* = 13*,* 9 Hz, 1H, H6’), 3.65 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 3.71 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 4.06 (dd, *J* = 6*,* 4 Hz, 1H, H3’), 4.46 (dd, *J* = 8*,* 5 Hz, 1H, H2’), 5.35 (quart, *J* = 9 Hz, 1H, H1’), 8.32 (s, 1H, H7), 8.75 (s, 1H, H2).

^{13}C NMR (100 MHz, methanol-d_4): δ 28.9 (C6’), 45.4 (C4’), 62.0 (C1’), 63.4 (C5’), 72.5 (C3’), 75.8 (C2’), 113.7 (C5), 132.0 (C7), 153.5 (C4), 154.1 (C2), 154.3 (C6).

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{11}H_{14}ClN_4O_3$: 285.0749; Found 285.0748.

(1R,2S,3R,5R)-3-(4-ethoxy-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (16)

NaH (30 mg, 3 eq, 60w% dispersion in paraffins) was slowly added to ethanol (2.5 ml) and THF (2.5 ml) and the mixture was stirred for 10 minutes. **15** (70 mg, 1 eq) was added at once. After full conversion, the reaction mixture was brought to neutral pH via careful addition of 1 M HCl and concentrated in vacuo. The crude was purified via flash column chromatography (1 - 12% MeOH in DCM) to obtain **16** (48 mg, 67%) as a white solid.



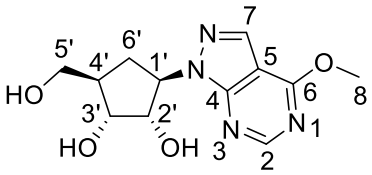
^{1}H NMR (400 MHz, methanol-d_4): δ 1.47 (t, *J* = 7 Hz, 3H, H9), 1.88 (ddd, *J* = 13*,* 10*,* 8 Hz, 1H, H6’), 2.21-2.32 (m, 1H, H4’), 2.37 (dt, *J* = 13*,* 8 Hz, 1H, H6’), 3.64 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 3.71 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 4.05 (dd, *J* = 5*,* 4 Hz, 1H, H3), 4.43 (dd, *J* = 8*,* 5 Hz, 1H, H2’), 4.64 (q, *J* = 7 Hz, 2H, H8), 5.26 (q, *J* = 9 Hz, 1H, H1’), 8.11 (s, 1H, H7), 8.49 (s, 1H, H2).

^{13}C NMR (100 MHz, methanol-d_4): δ 13.2 (C9), 29.1 (C6'), 45.5 (C4'), 61.6 (C1'), 63.1 (C8), 63.5 (C5'), 72.5 (C3'), 75.8 (C2'), 102.8 (C5), 131.1 (C7), 154.8 (C2), 154.9 (C4), 163.8 (C6).

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{19}N_4O_4$: 295.1401; Found 295.1400.

(1R,2S,3R,5R)-3-(4-methoxy-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (17)

The same procedure as compound **16** was employed using methanol instead of ethanol to obtain **17** (44 mg, 64%) as a white solid.



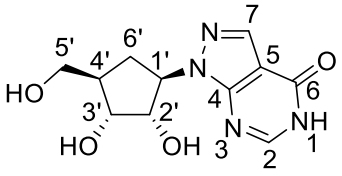
^{1}H NMR (400 MHz, methanol-d_4): δ 1.47 (t, *J* = 7 Hz, 3H, H9), 1.88 (ddd, *J* = 13, 10, 8 Hz, 1H, H6'), 2.21-2.32 (m, 1H, H4'), 2.37 (dt, *J* = 13, 8 Hz, 1H, H6'), 3.64 (dd, *J* = 11, 6 Hz, 1H, H5'), 3.71 (dd, *J* = 11, 6 Hz, 1H, H5'), 4.05 (dd, *J* = 5, 4 Hz, 1H, H3), 4.16 (s, 3H, H8), 4.43 (dd, *J* = 8, 5 Hz, 1H, H2'), 5.26 (q, *J* = 9 Hz, 1H, H1'), 8.11 (s, 1H, H7), 8.49 (s, 1H, H2).

^{13}C NMR (100 MHz, methanol-d_4): δ 29.1 (C6'), 45.5 (C4'), 53.5 (C8), 61.7 (C1'), 63.5 (C5'), 72.5 (C3'), 75.8 (C2'), 102.7 (C5), 131.0 (C7), 154.8 (C2), 154.9 (C4), 164.2 (C6).

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{17}N_4O_4$: 281.1244; Found 281.1247.

(1R,2S,3R,5R)-3-(4-hydroxy-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (18)

NaOMe (196µL of 5.4 M solution in MeOH, 1.06 mmol, 3 eq) was added to MeOH (5 ml). After addition, **15** (100 mg, 0.35 mmol, 1 eq) was added at once. After full conversion by LC-MS, volatiles were evaporated. 0.1 M aqueous NaOH (10 ml) was added to the residue and the mixture was refluxed until full conversion. All volatiles were evaporated and the crude was purified via flash column chromatography (3 - 15% MeOH in DCM) to obtain **18** (72 mg, 77% over 2 steps) as light yellow solid.



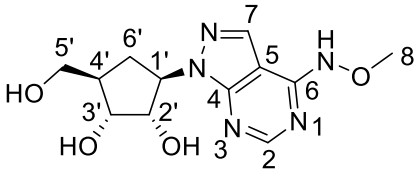
^{1}H NMR (400 MHz, methanol-d_4): δ 1.85 (ddd, *J* = 13*,* 10*,* 8 Hz, 1H, H6′), 2.19-2.29 (m, 1H, H4′), 2.37 (dt, *J* = 13*,* 8 Hz, 1H, H6′), 3.62 (dd, *J* = 11*,* 6 Hz, 1H, H5′), 3.70 (dd, *J* = 11*,* 6 Hz, 1H, H5′), 4.03 (dd, *J* = 5*,* 4 Hz, 1H, H3), 4.39 (dd, *J* = 8*,* 5 Hz, 1H, H2′), 5.18 (q, *J* = 9 Hz, 1H, H1′), 8.02 (s, 1H, H7), 8.09 (s, 1H, H2).

^{13}C NMR (100 MHz, methanol-d_4): δ 29.1 (C6′), 45.5 (C4′), 61.6 (C1′), 63.4 (C5′), 72.5 (C3′), 75.8 (C2′), 105.9 (C5), 134.4 (C2), 146.9 (C7), 152.7 (C4), 158.9 (C6).

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{11}H_{15}N_4O_4$: 267.1088; Found 267.1089.

(1S,2R,3R,5R)-3-(hydroxymethyl)-5-(4-(methoxyamino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)cyclopentane-1,2-diol (19).

O-methyl hydroxylamine hydrochloride (118 mg, 5 eq) was suspended in DMF (5 ml) after which DIPEA (294 µL, 6 eq) was added and stirred for 10 minutes. **15** (80 mg) was added at once and the mixture was heated to 60°C. After full conversion (typically 16 hours) was observed by LC-MS, volatiles were evaporated and the crude was purified by flash column chromatography (1 - 10% MeOH in DCM + ~0.2% NH4OH) to obtain **19** (75 mg, 91%) as a white solid.



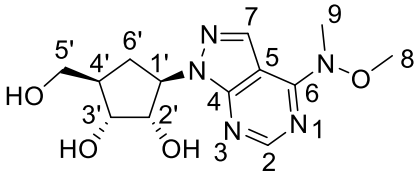
^{1}H NMR (400 MHz, methanol-d_4): δ 1.80 (ddd, *J* = 13*,* 10*,* 8 Hz, 1H, H6′), 2.16-2.26 (m, 1H, H4′), 2.30 (dt, *J* = 13*,* 8 Hz, 1H, H6′), 3.61 (dd, *J* = 11*,* 6 Hz, 1H, H5′), 3.69 (dd, *J* = 11*,* 6 Hz, 1H, H5′), 3.84 (s, 3H, H8), 4.01 (dd, *J* = 5*,* 4 Hz, 1H, H3), 4.35 (dd, *J* = 8*,* 5 Hz, 1H, H2′), 5.05 (q, *J* = 9 Hz, 1H, H1′), 7.62 (s, 1H, H7), 7.77 (s, 1H, H2).

^{13}C NMR (100 MHz, methanol-d_4): δ 29.3 (C6′), 45.3 (C4′), 60.4 (C8), 61.3 (C1′), 63.5 (C5′), 72.5 (C3′), 75.8 (C2′), 105.2 (C5), 132.0 (C2), 145.7 (C7), 149.8 (C4), 155.5 (C6).

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{18}N_5O_4$: 296.1353; Found 296.1357.

(1S,2R,3R,5R)-3-(hydroxymethyl)-5-(4-(methoxy(methyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)cyclopentane-1,2-diol (20).

The same procedure as compound **19** was employed using *N,O*-dimethyl hydroxylamine hydrochloride instead of *O*-methyl hydroxylamine hydrochloride to obtain **20 (**82 mg, 90%) as a white solid.



^{1}H NMR (400 MHz, methanol-d_4): δ 1.93 (ddd, *J* = 13*,* 10*,* 8 Hz, 1H, H6′), 2.24-2.34 (m, 1H, H4′), 2.36 (dt, *J* = 13*,* 8 Hz, 1H, H6′), 3.66 (s, 3H, H9), 3.74 (dd, *J* = 11*,* 6 Hz, 1H, H5′), 3.71 (dd, *J* = 11*,* 6 Hz, 1H, H5′), 3.91 (s, 3H, H8), 4.10 (dd, *J* = 5*,* 4 Hz, 1H, H3), 4.16 (s, 3H, H8), 4.43 (dd, *J* = 8*,* 5 Hz, 1H, H2′), 5.22 (q, *J* = 9 Hz, 1H, H1′), 8.14 (s, 1H, H7), 8.31 (s, 1H, H2).

^{13}C NMR (100 MHz, methanol-d_4): δ 28.7 (C6′), 33.8 (C9), 45.4 (C4′), 60.0 (8), 61.7 (C1′), 63.3 (C5′), 72.8 (C3′), 75.9 (C2′), 99.9 (C5), 134.3 (C7), 153.8 (C4), 154.6 (C2), 158.3 (C6).

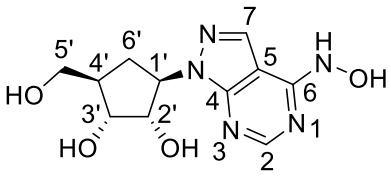
HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{20}N_5O_4$: 310.1510; Found 310.1508.

(1R,2S,3R,5R)-3-(4-(hydroxyamino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (22)

The same procedure as compound **19** was employed using *O*-benzyl hydroxylamine hydrochloride instead of *O*-methyl hydroxylamine hydrochloride to obtain **21** (125 mg, 79%) as a white solid.

21 (80 mg) was dissolved in MeOH (5 ml) after which the round-bottom flask was flushed extensively with argon. A catalytic amount of Pd/C was added and hydrogen gas was carefully bubbled through the solution for approximately 10

minutes. After 1 hour, full conversion was observed via LC-MS and argon was bubbled through the solution. The mixture was filtered over celite with extensive methanol washing steps, after which all volatiles were evaporated. The crude was purified by flash column chromatography (3 - 15% MeOH in DCM + ~0.2% NH4OH) to obtain **22** (45 mg, 57%) as a light green solid.



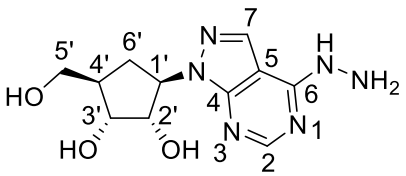
^{1}H NMR (400 MHz, methanol-d_4): δ 1.73 – 1.88 (m, 1H, H6’), 2.16-2.26 (m, 1H, H4’), 2.31 (dt, *J* = 13*,* 8 Hz, 1H, H6’), 3.62 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 3.69 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 4.02 (dd, *J* = 5*,* 4 Hz, 1H, H3), 4.35 (dd, *J* = 8*,* 5 Hz, 1H, H2’), 5.06 (q, *J* = 9 Hz, 1H, H1’), 7.50-8.21 (m, 2H, H2+H7).

^{13}C NMR (100 MHz, methanol-d_4): δ 29.3 (C6’), 45.3 (C4’), 61.3 (C1’), 63.5 (C5’), 72.5 (C3’), 75.8 (C2’), 132.1 (C2), 145.7 (C7). Quaternary 13C of purine remained undetected.

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{11}H_{16}N_5O_4$: 282.1197; Found 282.1195

(1R,2S,3R,5R)-3-(4-hydrazinyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (23)

15 (40 mg) was dissolved in THF (5 ml) and hydrazine monohydrate (1 ml) was added at once. After stirring overnight, volatiles were evaporated and the crude was purified by flash column chromatography (2 - 12% MeOH in DCM + ~0.2% NH4OH) to obtain **23** (21 mg, 54%) as a light yellow solid.



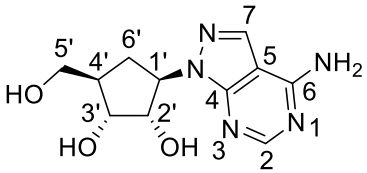
^{1}H NMR (400 MHz, methanol-d_4): δ 1.84 (ddd, *J* = 13*,* 10*,* 8 Hz, 1H, H6’), 2.19-2.30 (m, 1H, H4’), 2.34 (dt, *J* = 13*,* 8 Hz, 1H, H6’), 3.62 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 3.71 (dd, *J* = 11*,* 6 Hz, 1H, H5’), 4.04 (dd, *J* = 5*,* 4 Hz, 1H, H3), 4.40 (dd, *J* = 8*,* 5 Hz, 1H, H2’), 5.16 (q, *J* = 9 Hz, 1H, H1’), 7.82-8.68 (m, 2H, H2 + H7).

^{13}C NMR (100 MHz, methanol-d_4): δ 29.1 (C6′), 45.4 (C4′), 61.2 (C1′), 63.6 (C5′), 72.5 (C3′), 75.8 (C2′), 99.3 (C5). Quaternary 13C of purine remained undetected.

HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{11}H_{17}N_6O_3$: 281.1357; Found 281.1359.

(1R,2S,3R,5R)-3-(4-amino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (24)

15 (200 mg, 0.70 mmol) was dissolved in saturated aqueous ammonia (10 ml) and stirred at 50°C until full conversion was observed. Volatiles were evaporated and the crude was purified by flash column chromatography (2 - 12% MeOH in DCM + ~0.2% NH_4OH) to obtain **24** (67 mg, 36%) as a white solid.

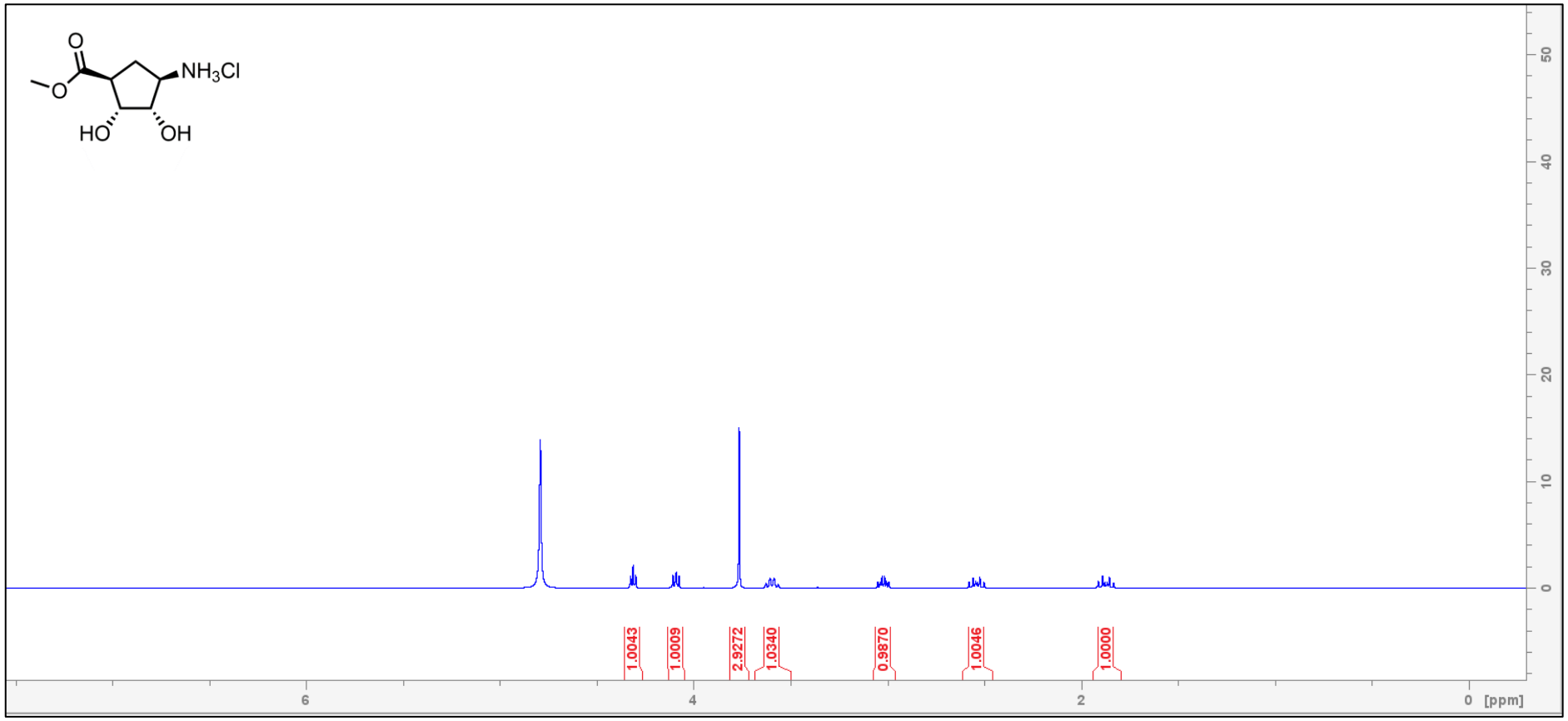


^{1}H NMR (400 MHz, D_2O): δ 1.68 (ddd, *J* = 13, 10, 8 Hz, 1H, H6′), 2.17-2.27 (m, 1H, H4′), 2.32 (dt, *J* = 13, 8 Hz, 1H, H6′), 3.60 (dd, *J* = 11, 6 Hz, 1H, H5′), 3.65 (dd, *J* = 11, 6 Hz, 1H, H5′), 4.00 (dd, *J* = 5, 4 Hz, 1H, H3), 4.35 (dd, *J* = 8, 5 Hz, 1H, H2′), 5.02 (q, *J* = 9 Hz, 1H, H1′), 8.07 (s, 1H, H7), 8.12 (s, 1H, H2).

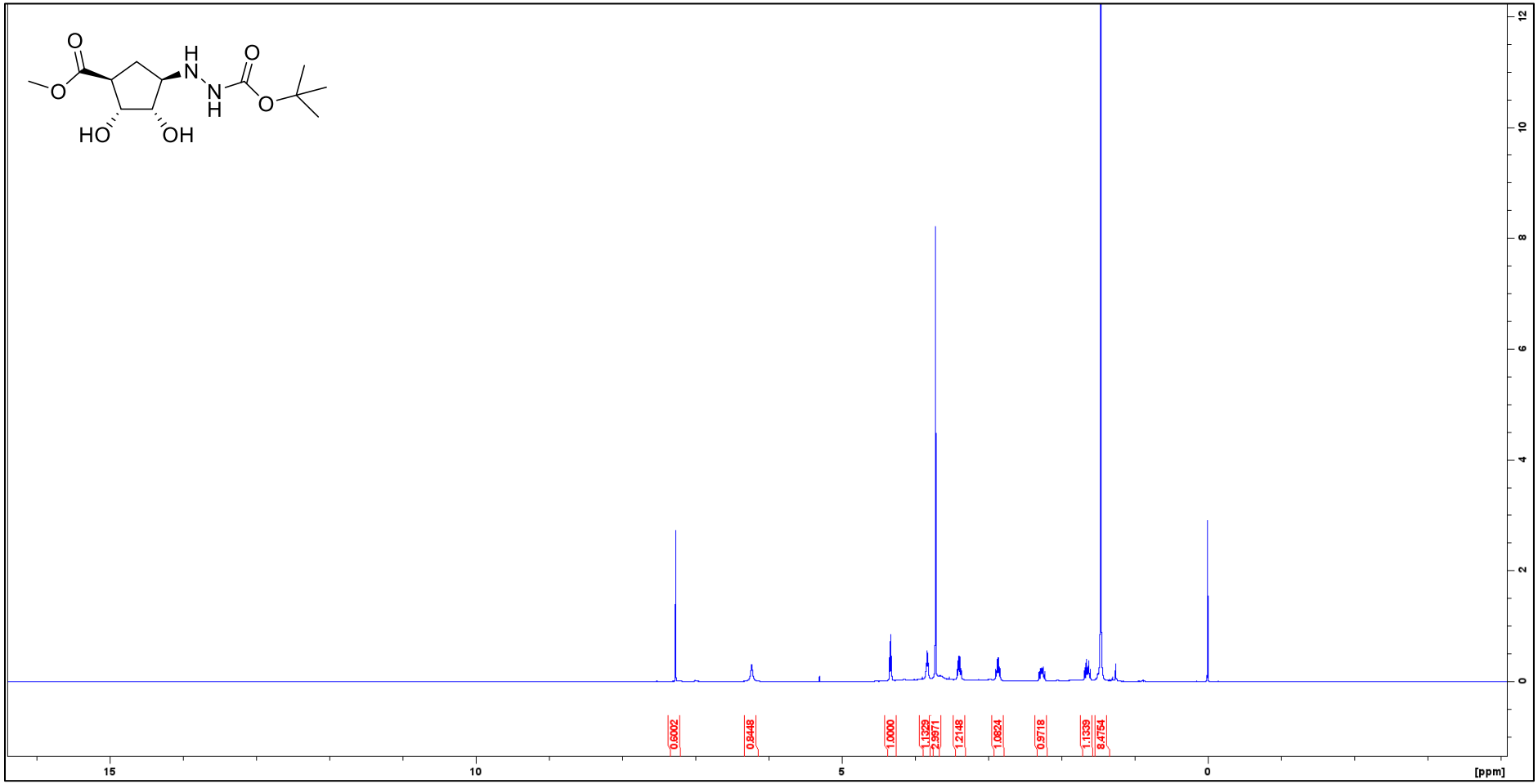
^{13}C NMR (100 MHz, D_2O): δ 28.3 (C6′), 44.6 (C4′), 60.6 (C1′), 63.3 (C5′), 71.9 (C3′), 75.3 (C2′), 99.9 (C5), 133.4 (C7), 152.6 (C4), 155.2 (C2), 157.6 (C6)

^{1}H NMR spectra

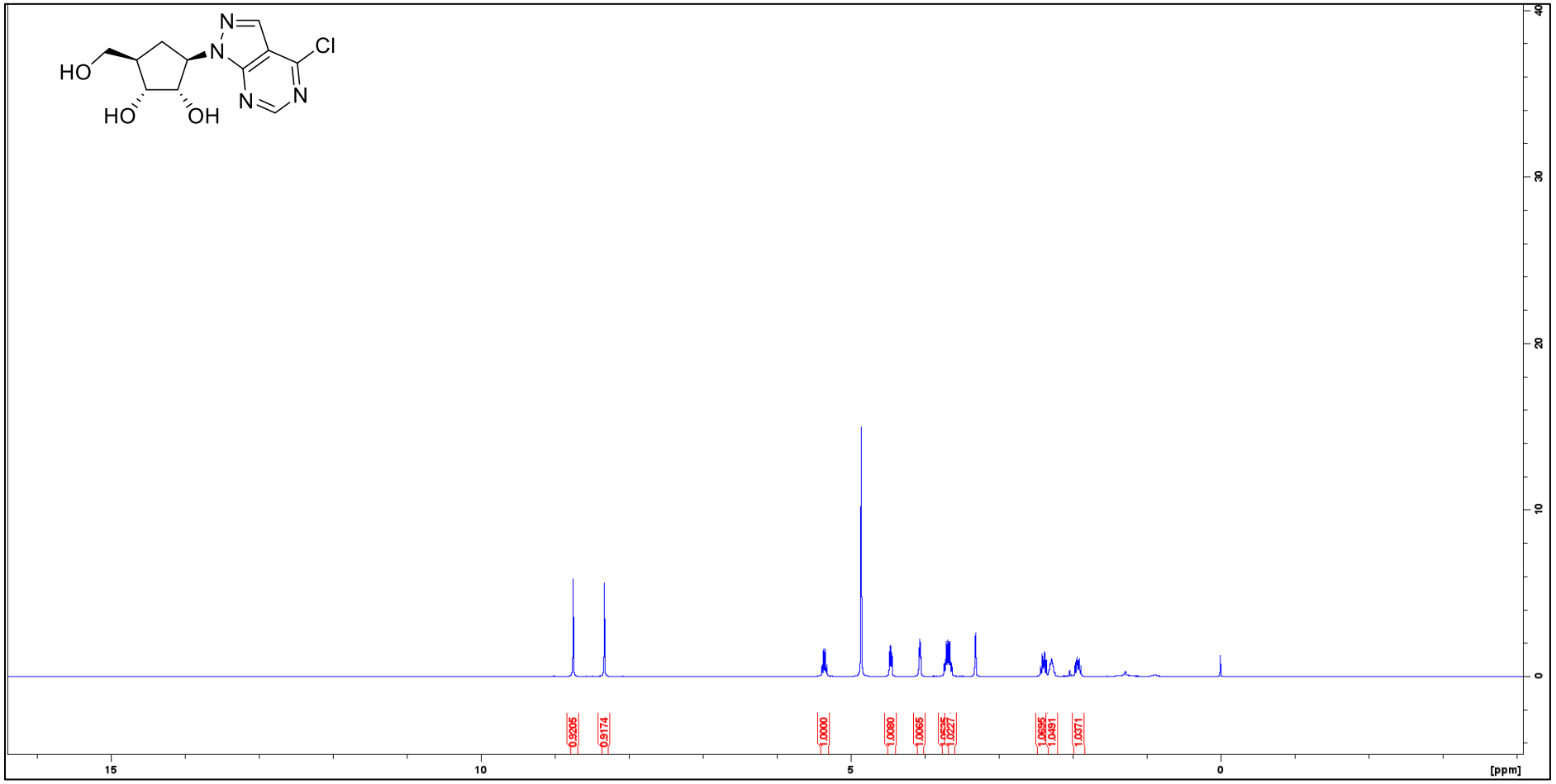
Compound **9**



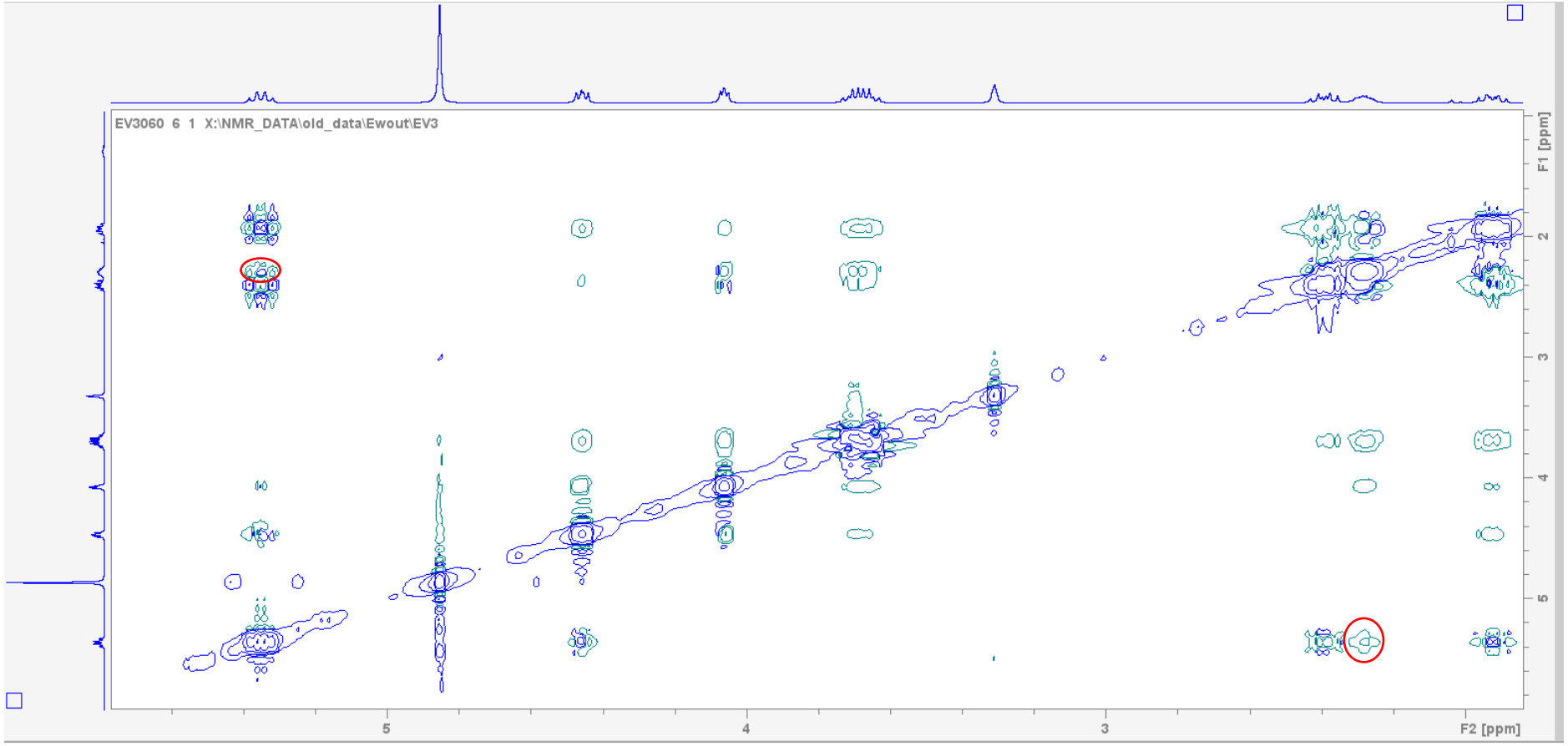
Compound **12**


O
O
H
N
N
H
O
O
HO
OH
0.6002
0.8448
1.0000
1.1922
2.9971
1.2148
1.0824
0.9718
1.1339
8.4754
15
10
5
0
[ppm]
12
10
8
6
4
2
0

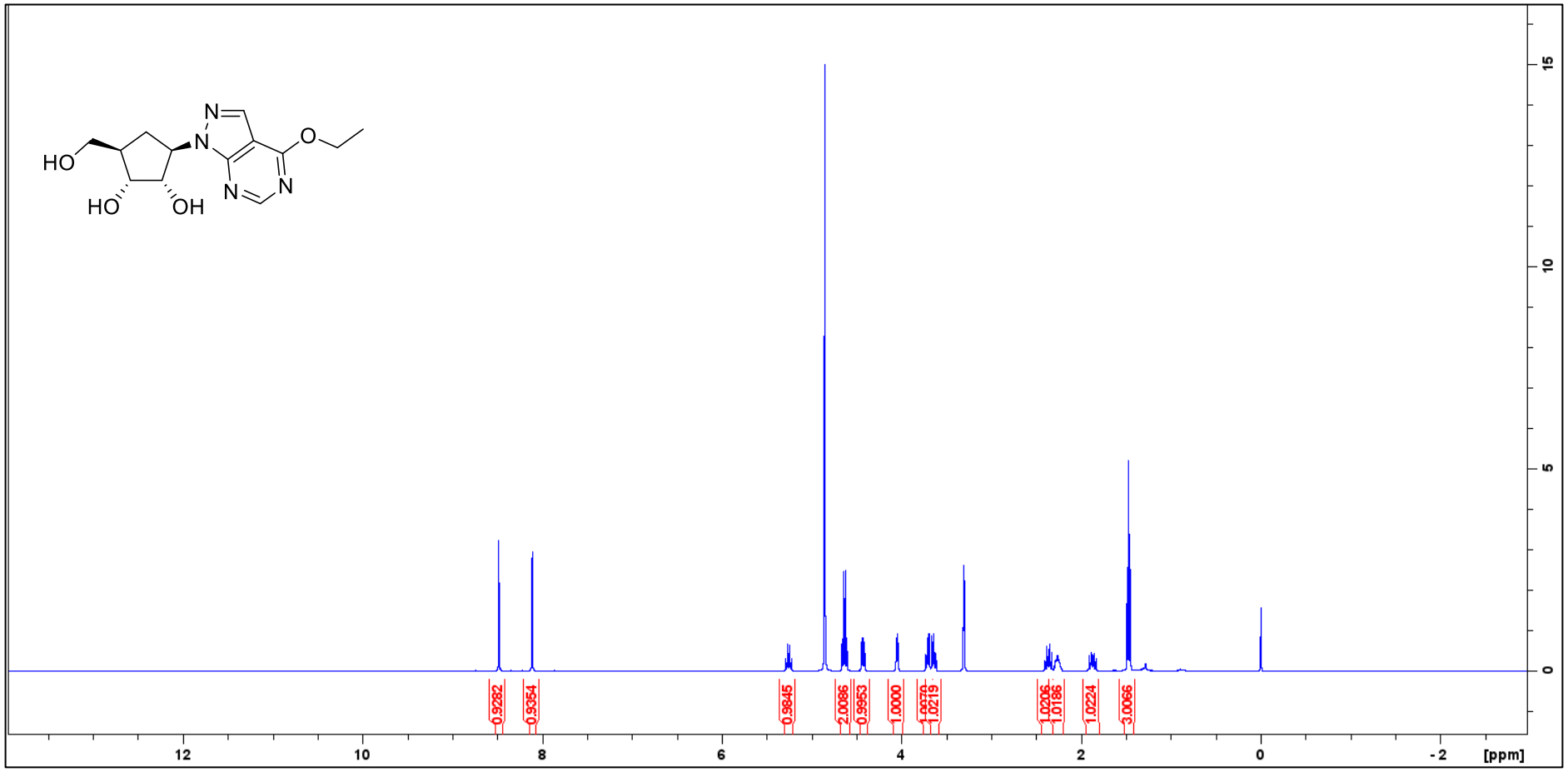
Compound **15**


HO
HO
OH
N
N
N
N
Cl
0.9205
0.9174
1.0000
1.0080
1.0065
1.0235
1.0227
1.0695
1.0491
1.0371
15
10
5
0
[ppm]
40
30
20
10
0

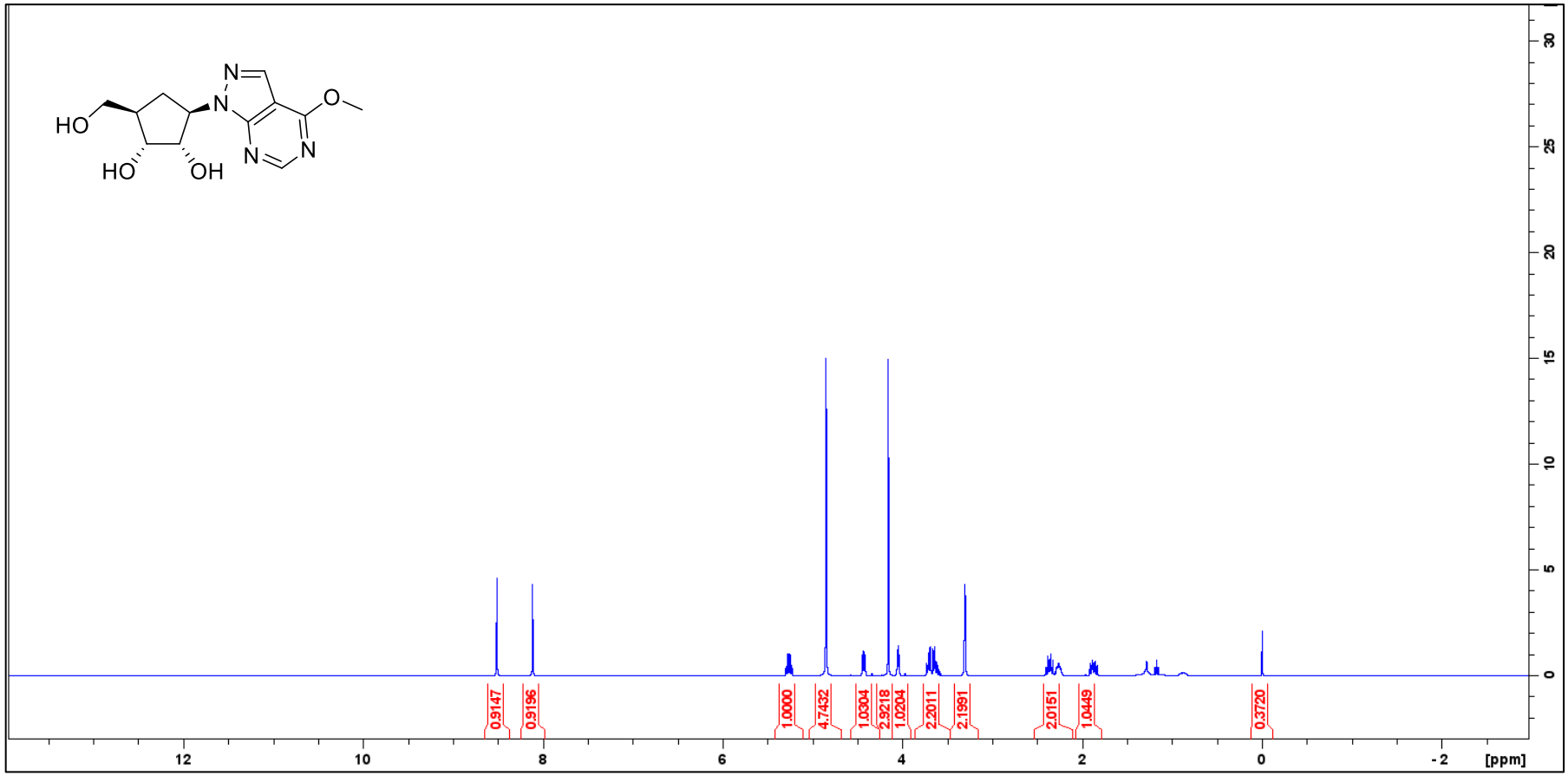
NOESY spectrum of compound **15** with cross-peak between H1’ and H4’ encircled in red, demonstrating the desired β–configuration.



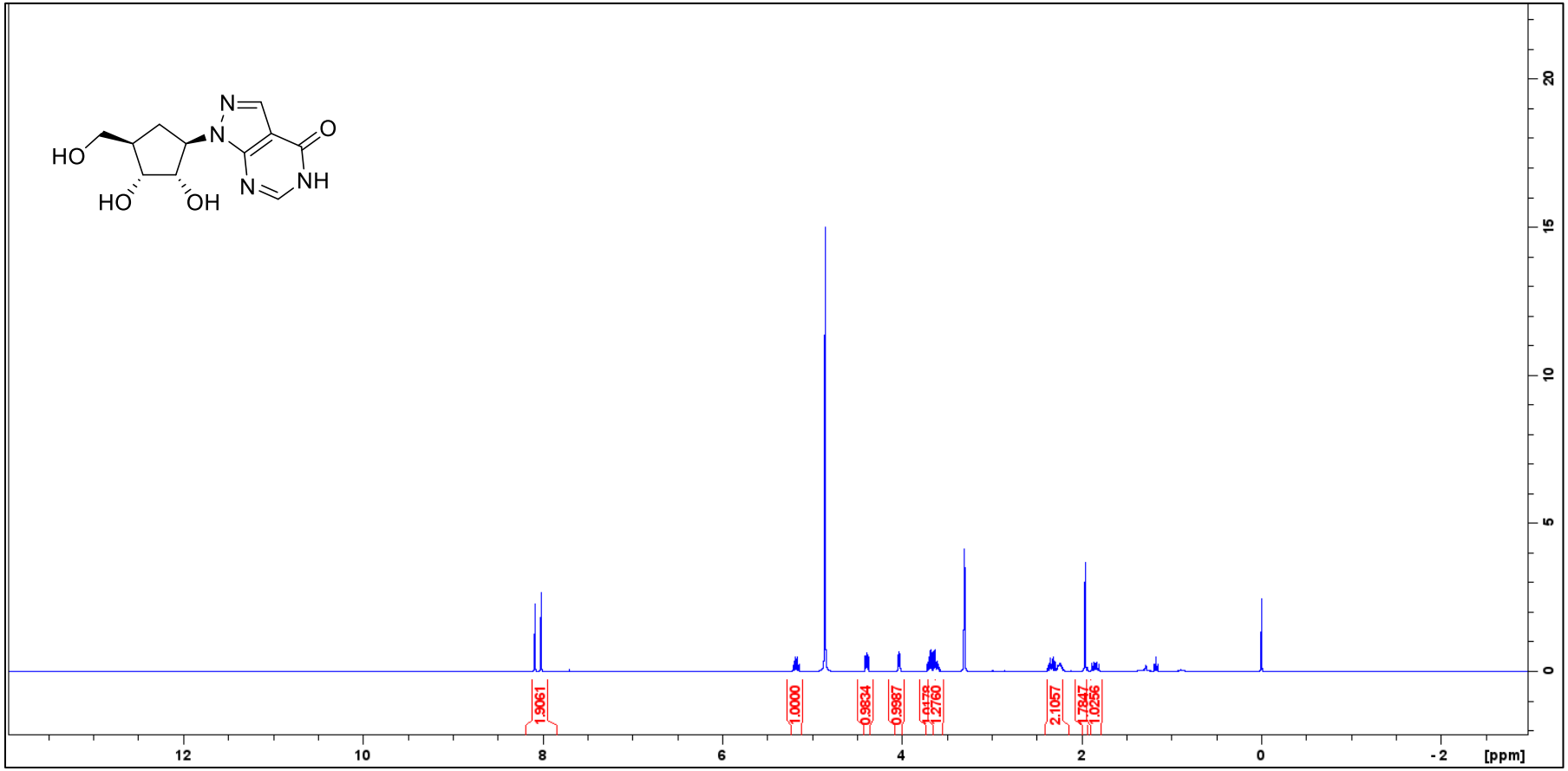
Compound **16**


HO
HO
OH
N
N
N
N
O
0.9282
0.9354
0.9845
2.0086
0.9953
1.0000
1.0219
1.0224
3.0066
12
10
8
6
4
2
0
-2
[ppm]
15
10
5
0

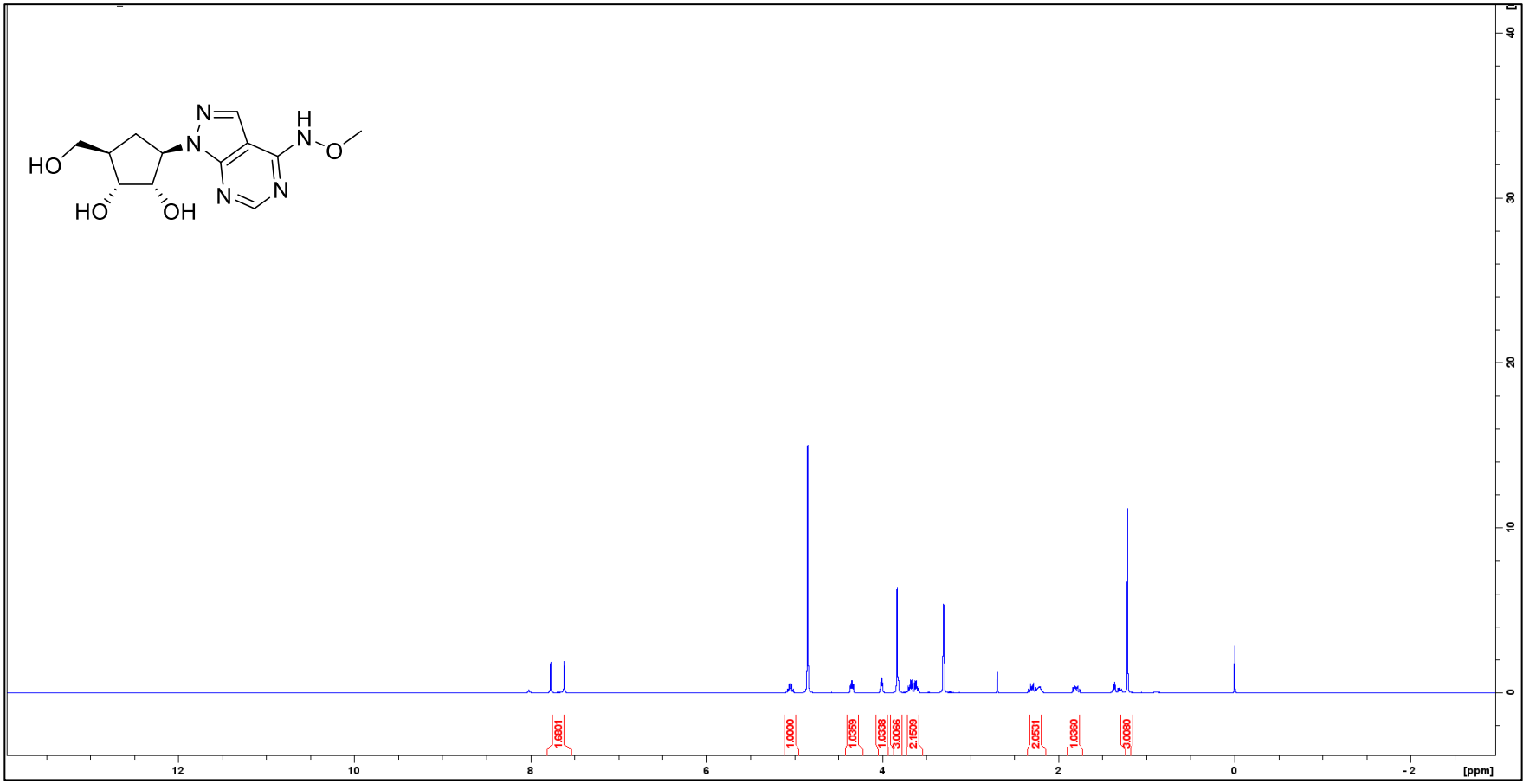
Compound **17**


HO
HO
OH
N
N
N
N
O
0.9147
0.9196
1.0000
4.7432
1.0304
2.9218
1.0204
2.2011
2.1991
2.0151
1.0449
0.3720
12
10
8
6
4
2
0
-2
[ppm]
30
25
20
15
10
5
0

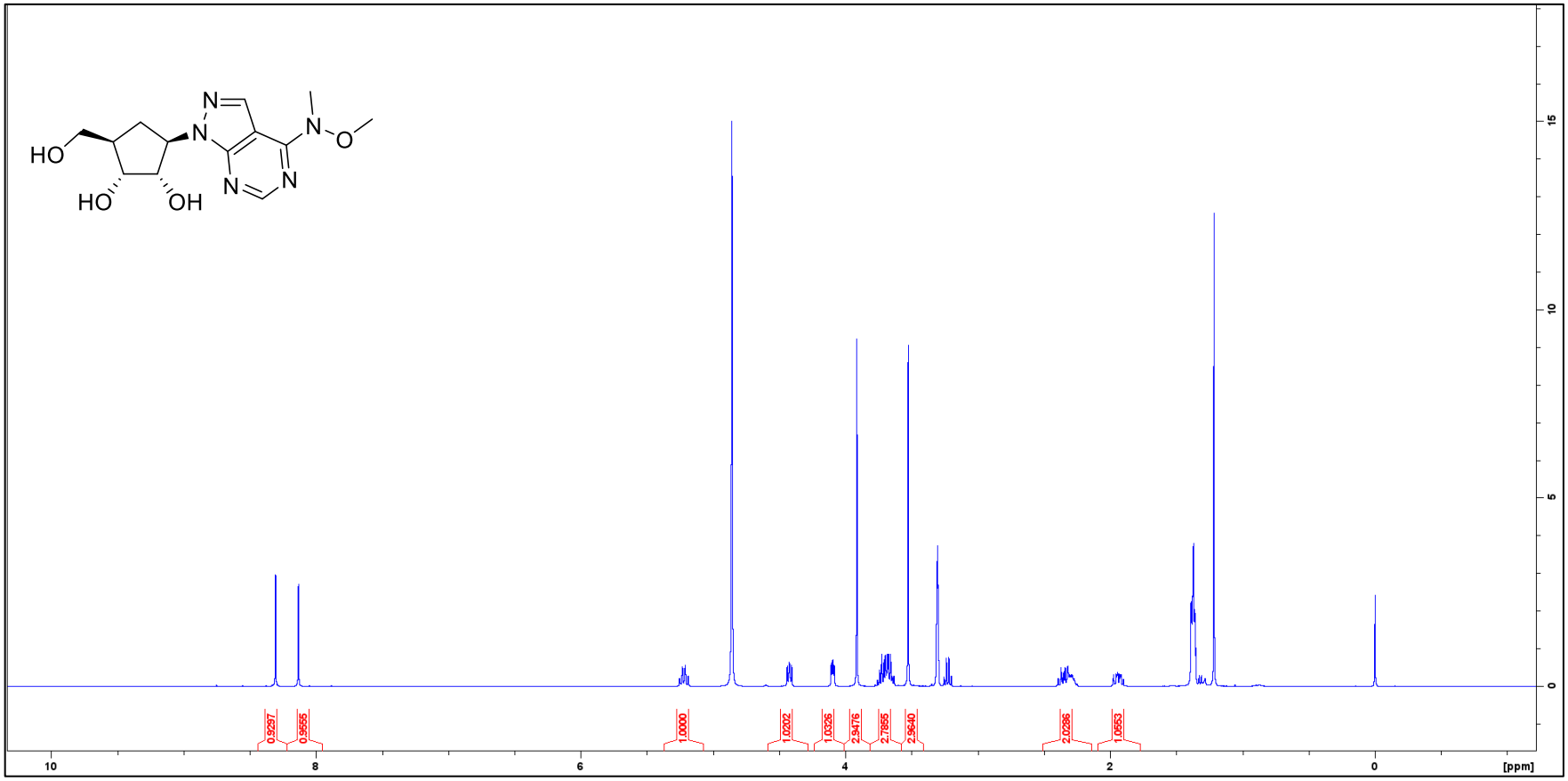
Compound **18**


HO
HO
OH
N
N
N
NH
O
1.9061
1.0000
0.9834
0.9987
1.0178
1.2760
2.1057
1.7847
1.0256
12
10
8
6
4
2
0
- 2
[ppm]
20
15
10
5
0

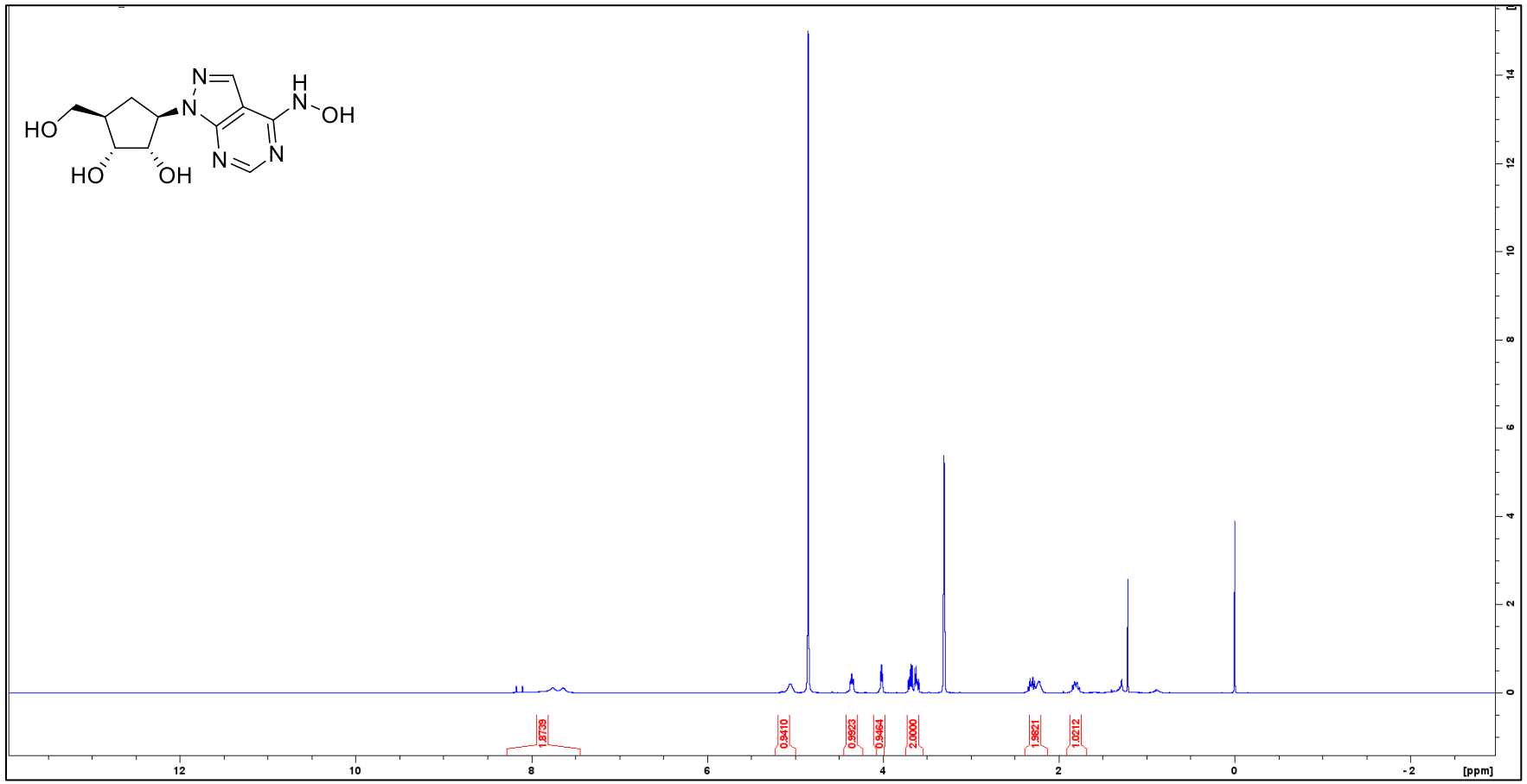
Compound **19**


HO
HO
OH
N
N
N
N
H
N
O
1.6801
1.0000
1.0369
1.0338
3.0066
2.1509
2.0531
1.0360
3.0080
12
10
8
6
4
2
0
-2
[ppm]
40
30
20
10
0

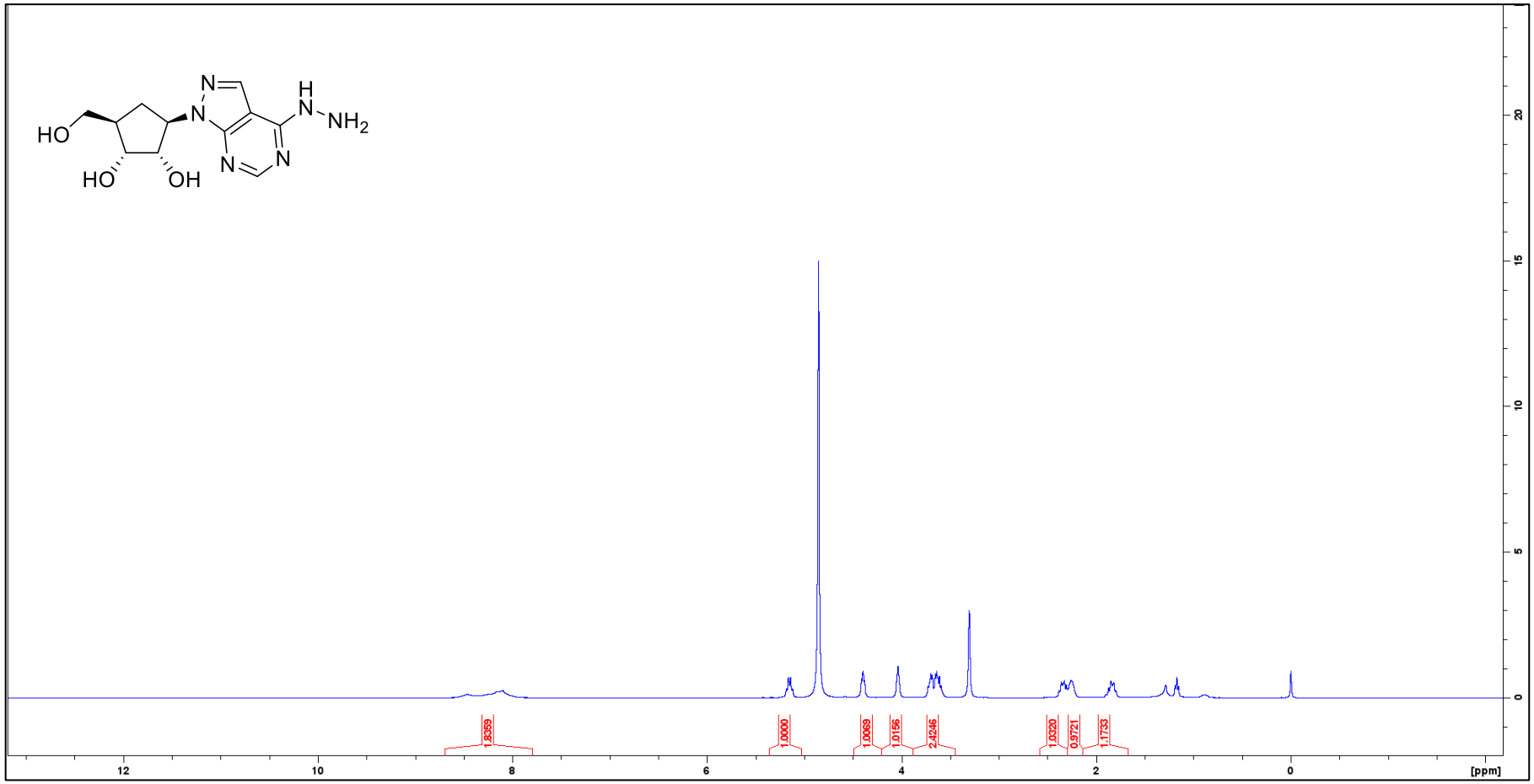
Compound **20**


HO
HO
OH
N
N
N
N
N
O
0.9297
0.9555
1.0000
1.0202
1.0326
2.9476
2.7855
2.9540
2.0286
1.0553
10
8
6
4
2
0
[ppm]
15
10
5
0

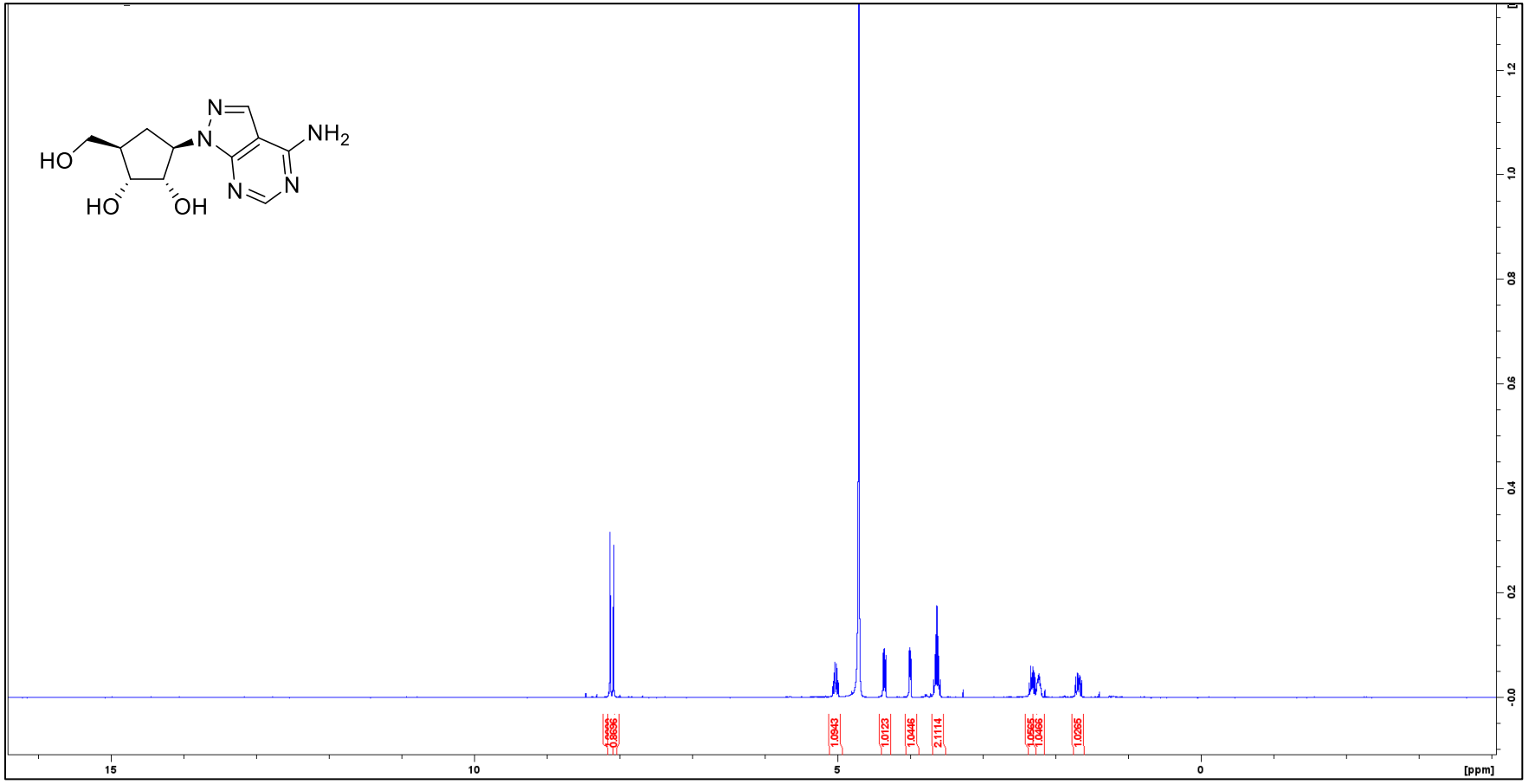
Compound **22**


HO
HO
OH
N
N
N
N
H
N
OH
1.8739
0.9410
0.9823
0.9464
2.0000
1.3821
1.0212
12
10
8
6
4
2
0
-2
[ppm]

Compound **23**

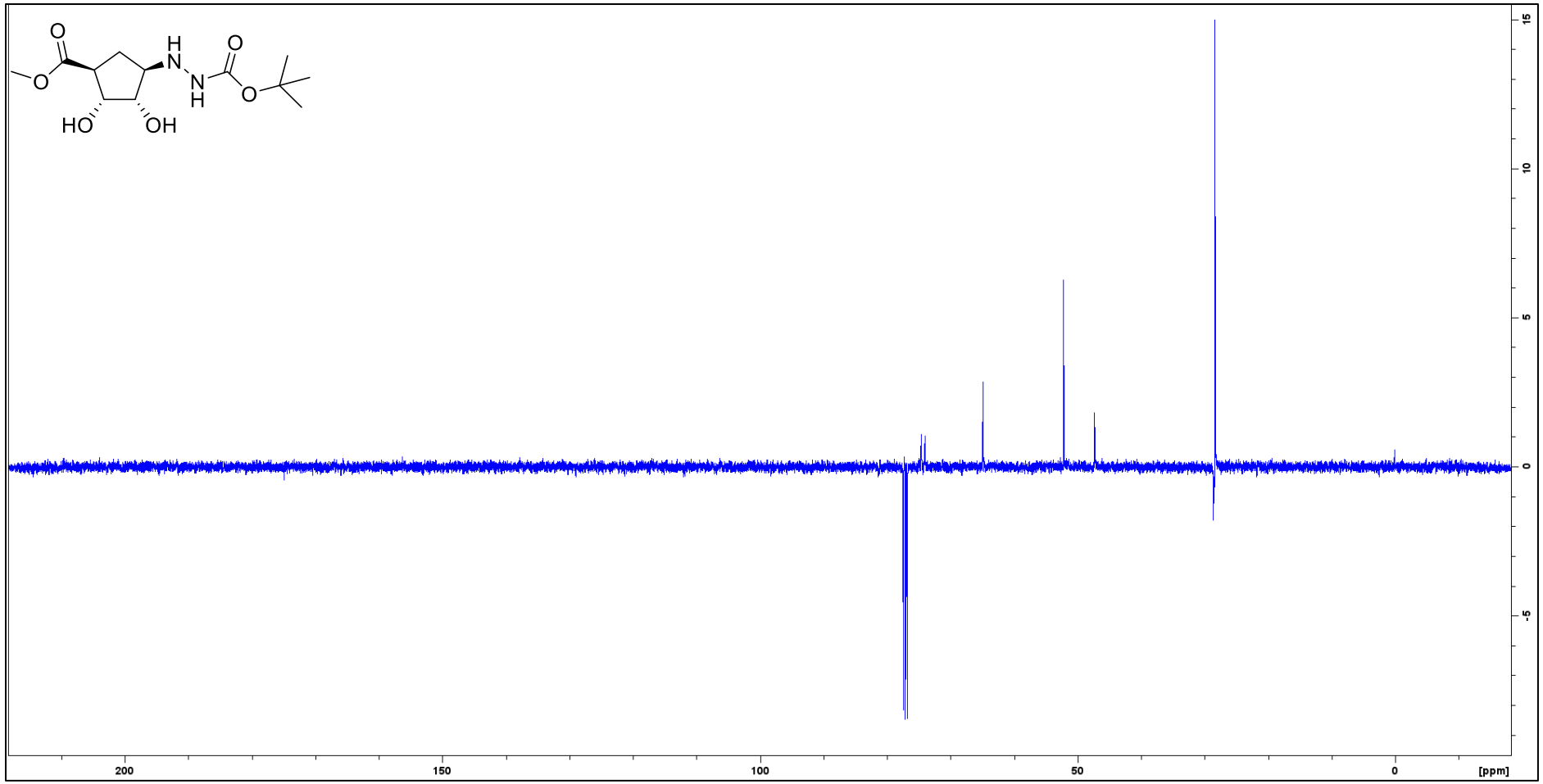

HO
HO
OH
N
N
N
N
H
N
NH2
1.8369
1.0000
1.0069
1.0156
2.4246
1.0320
0.9721
1.1733
12
10
8
6
4
2
0
[ppm]
20
15
10
5
0

Compound **24**

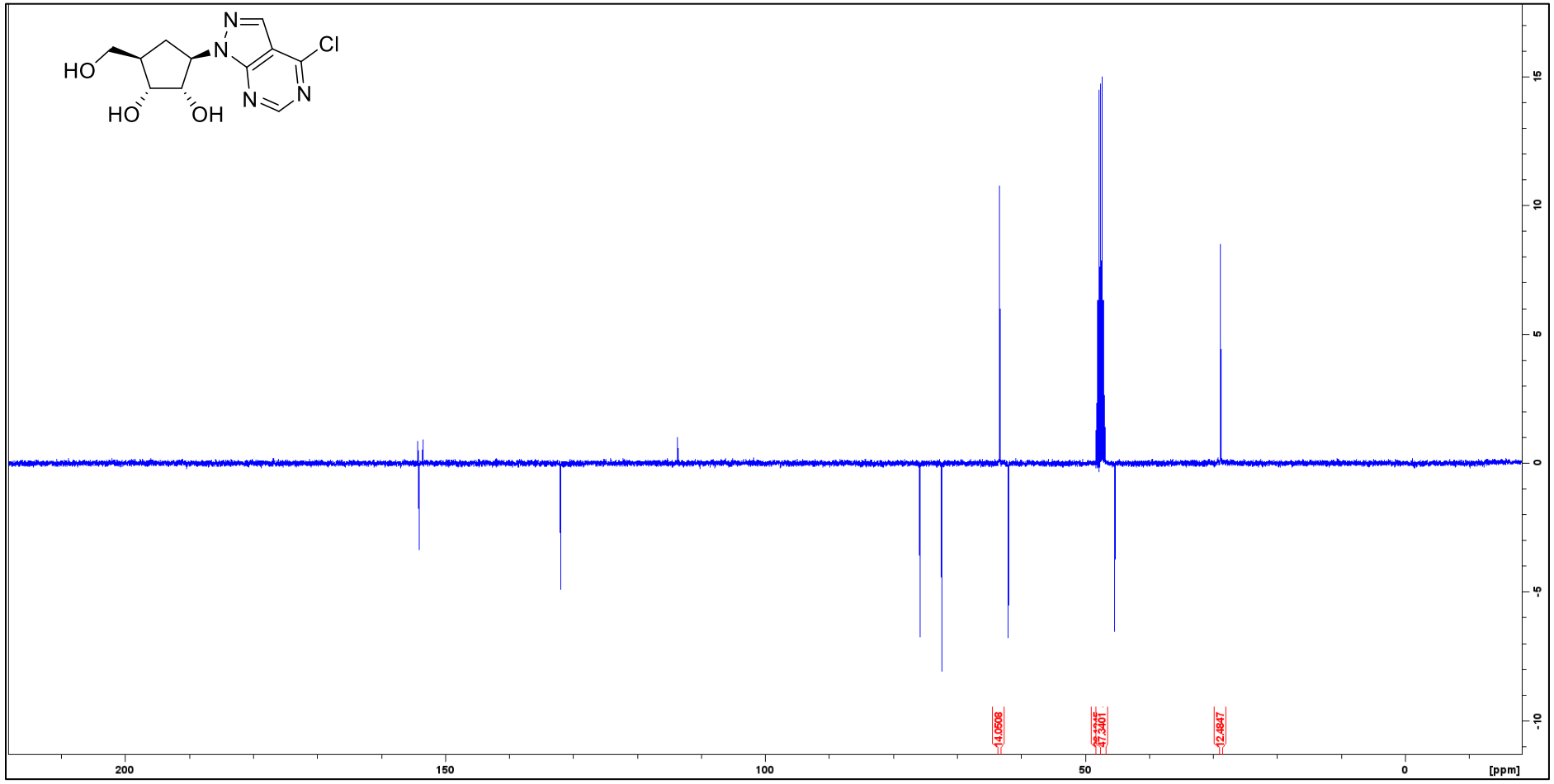


^{13}C NMR Spectra

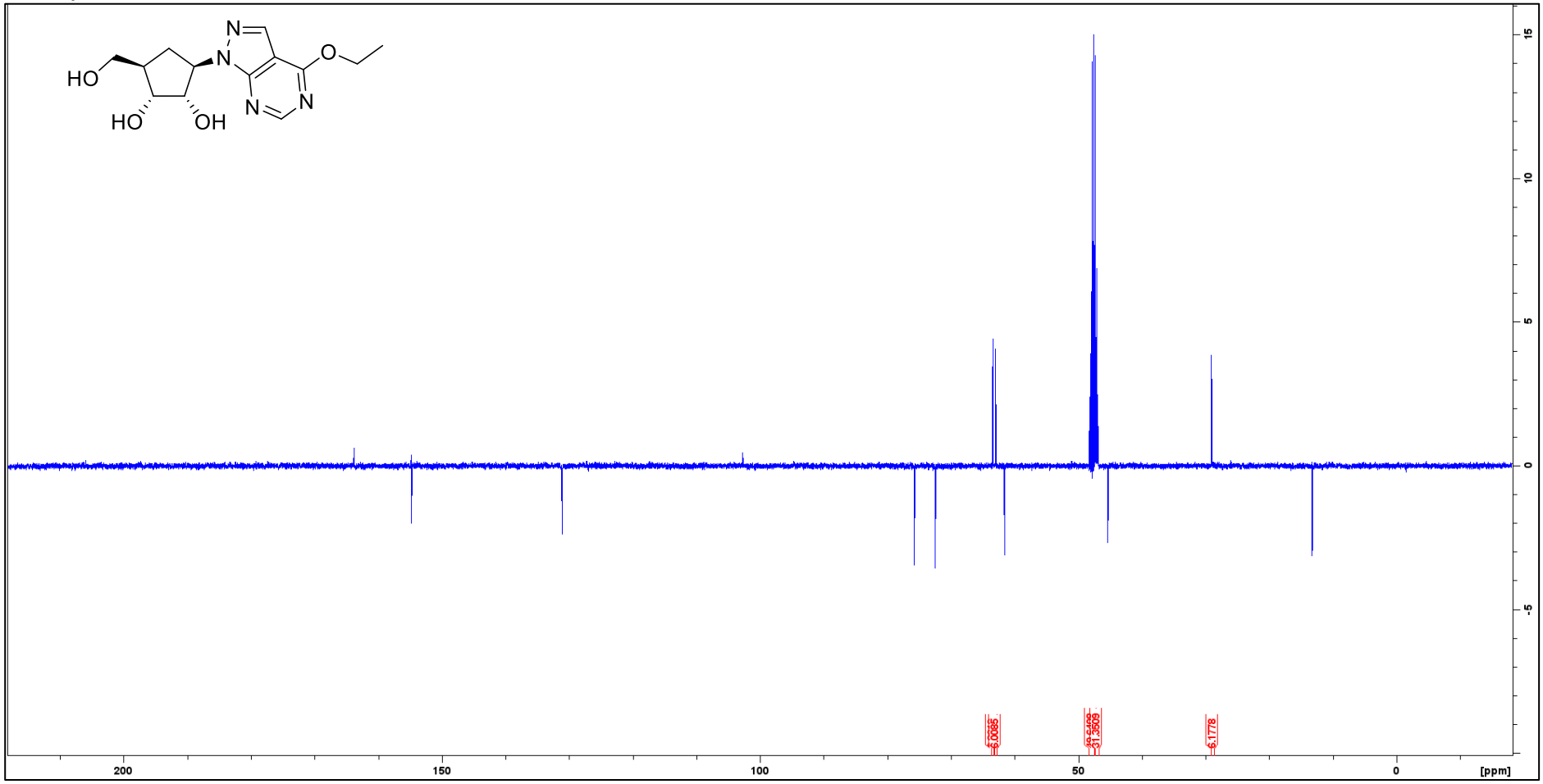
Compound **12**



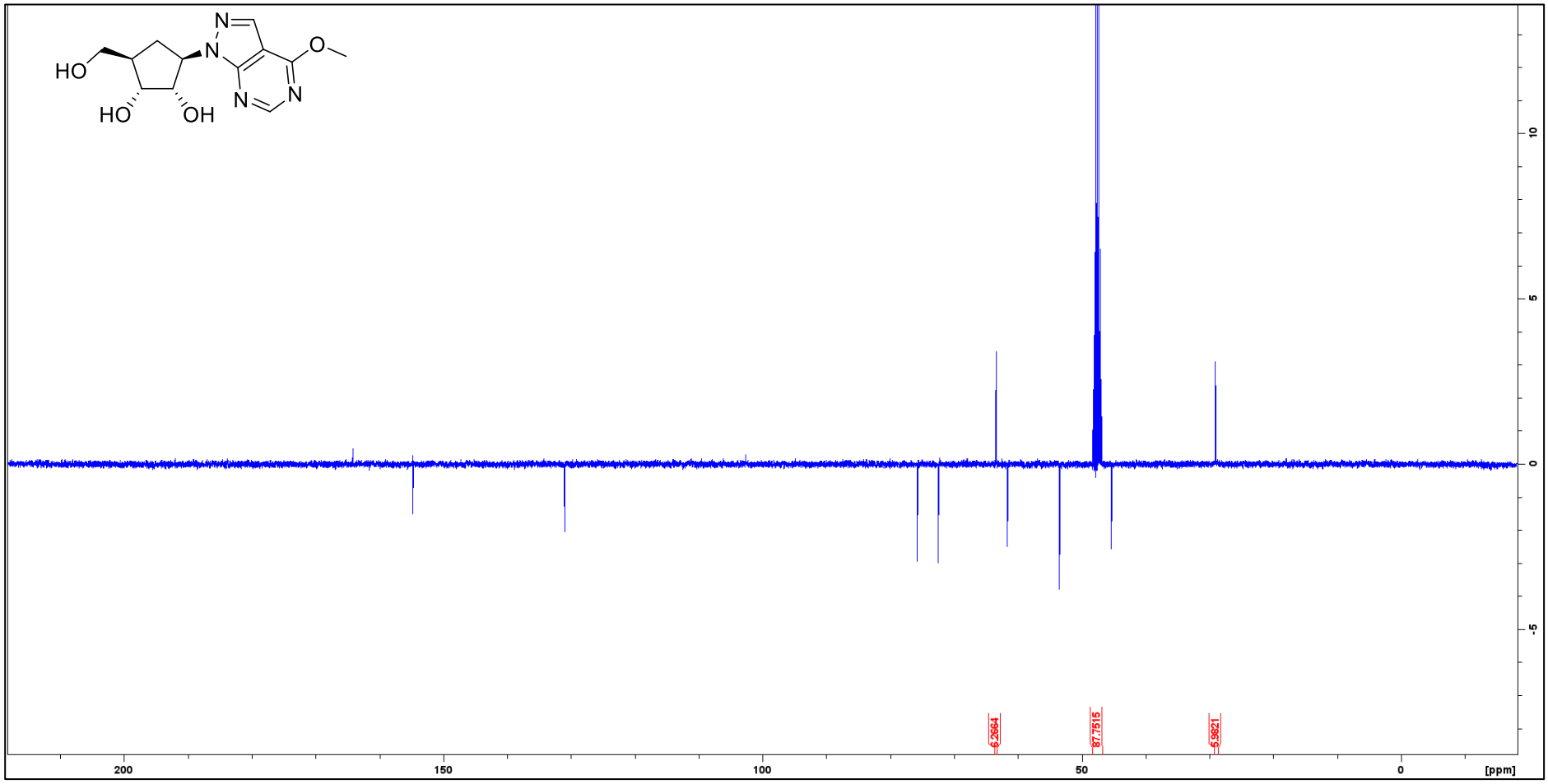
Compound **15**



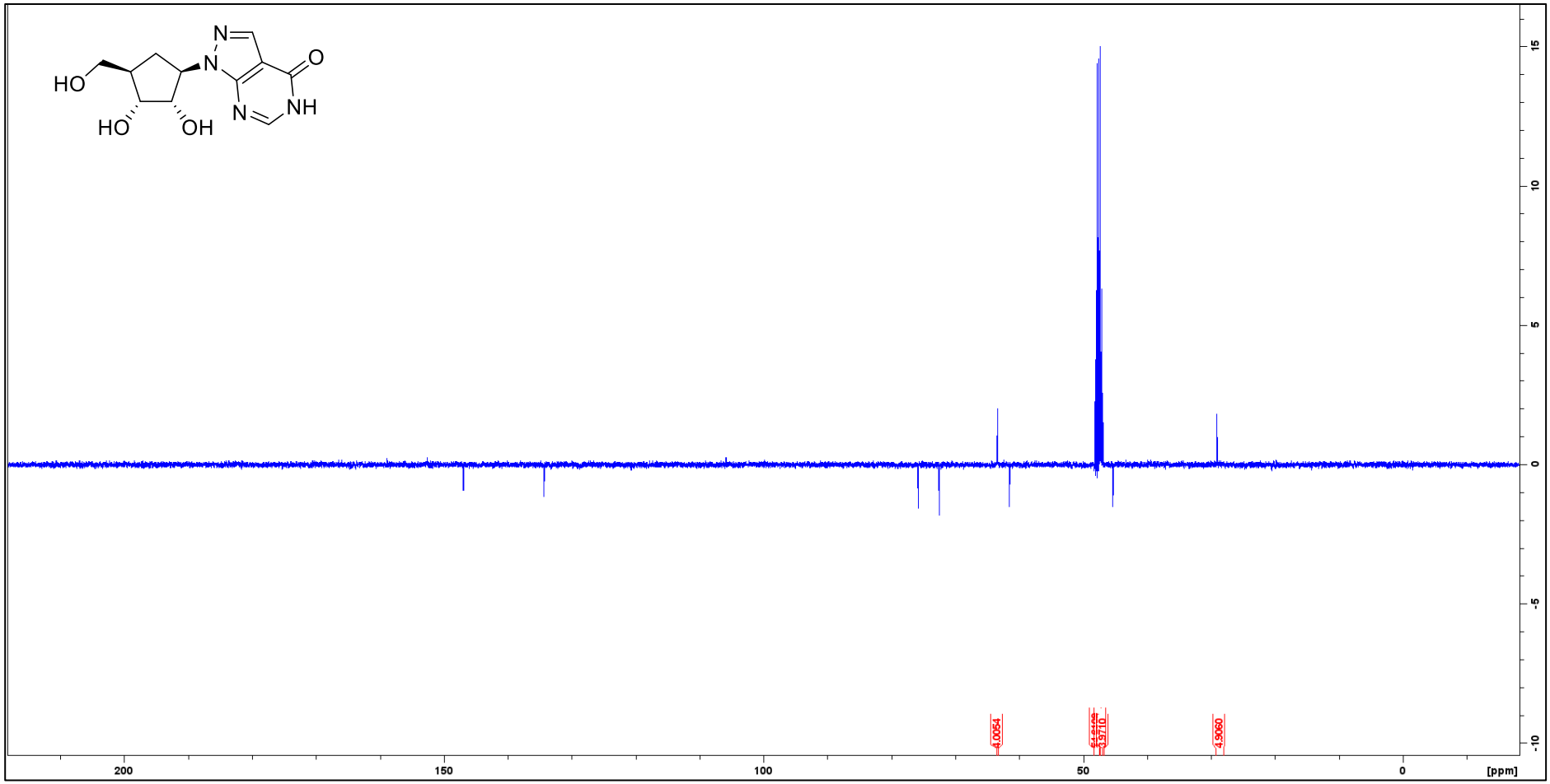
Compound **16**


HO
HO
OH
N
N
N
N
O
6.0085
41.5409
51.3609
6.1778
200
150
100
50
0
[ppm]
15
10
5
0
-5

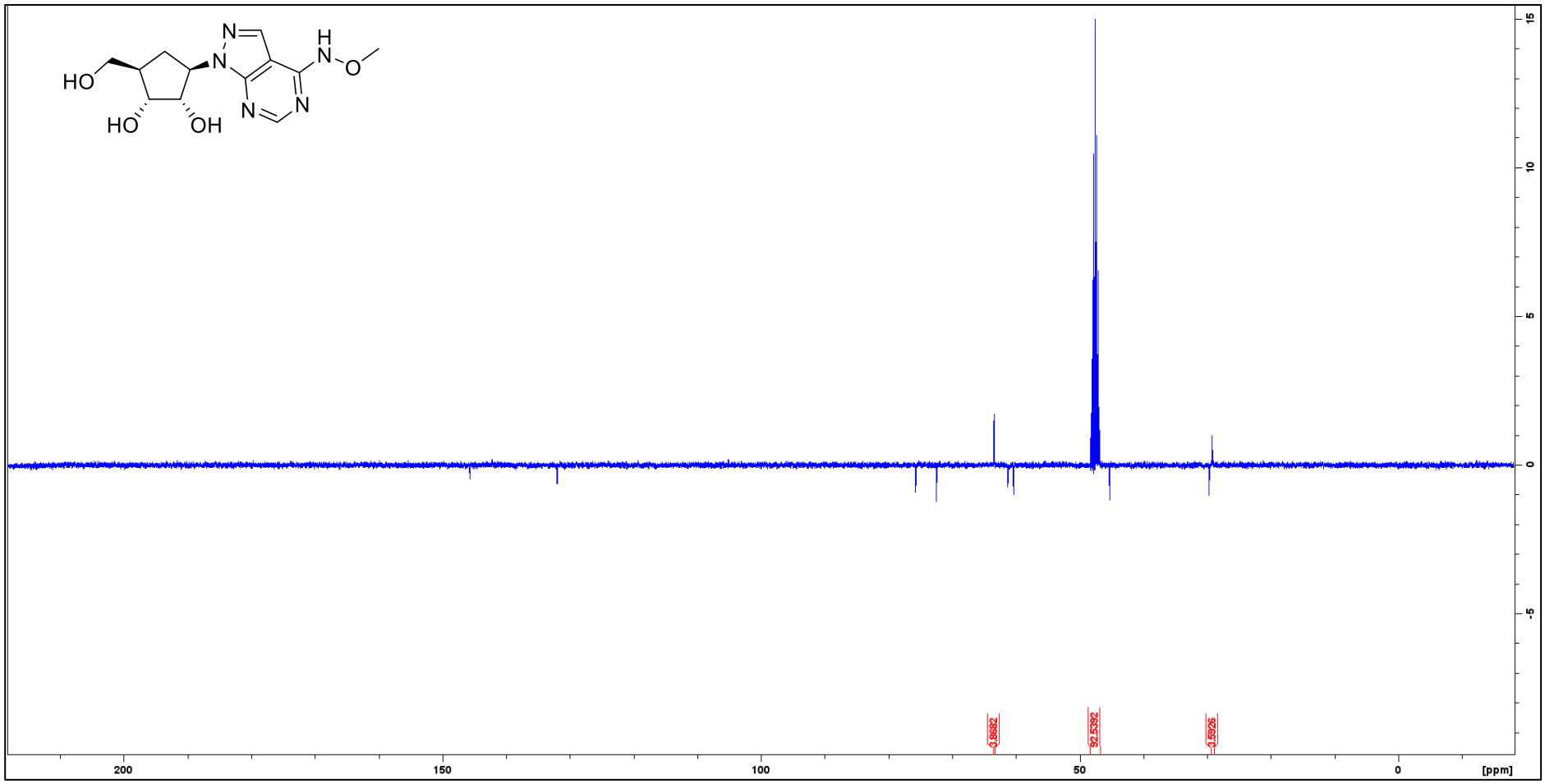
Compound **17**



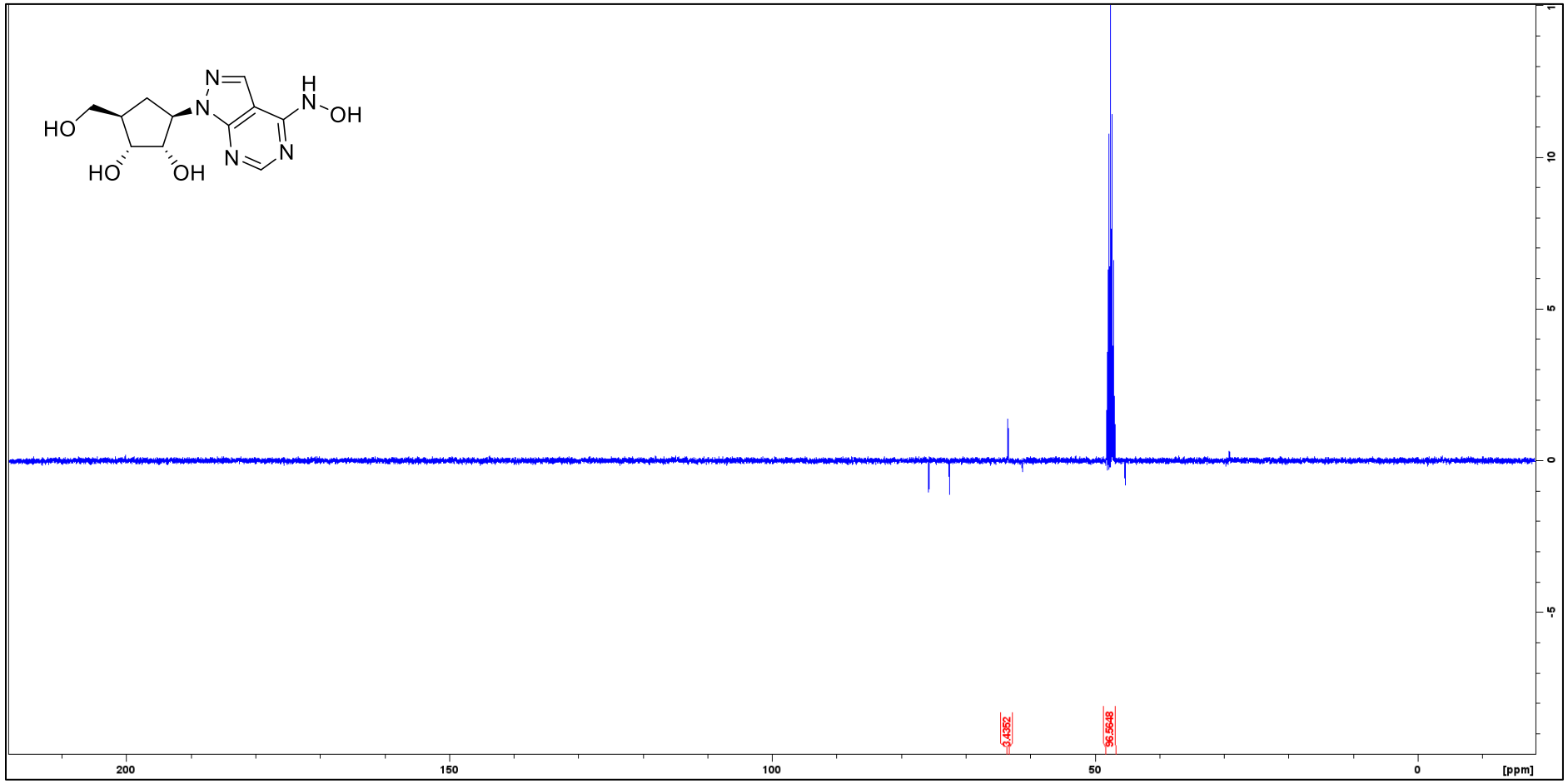
Compound **18**



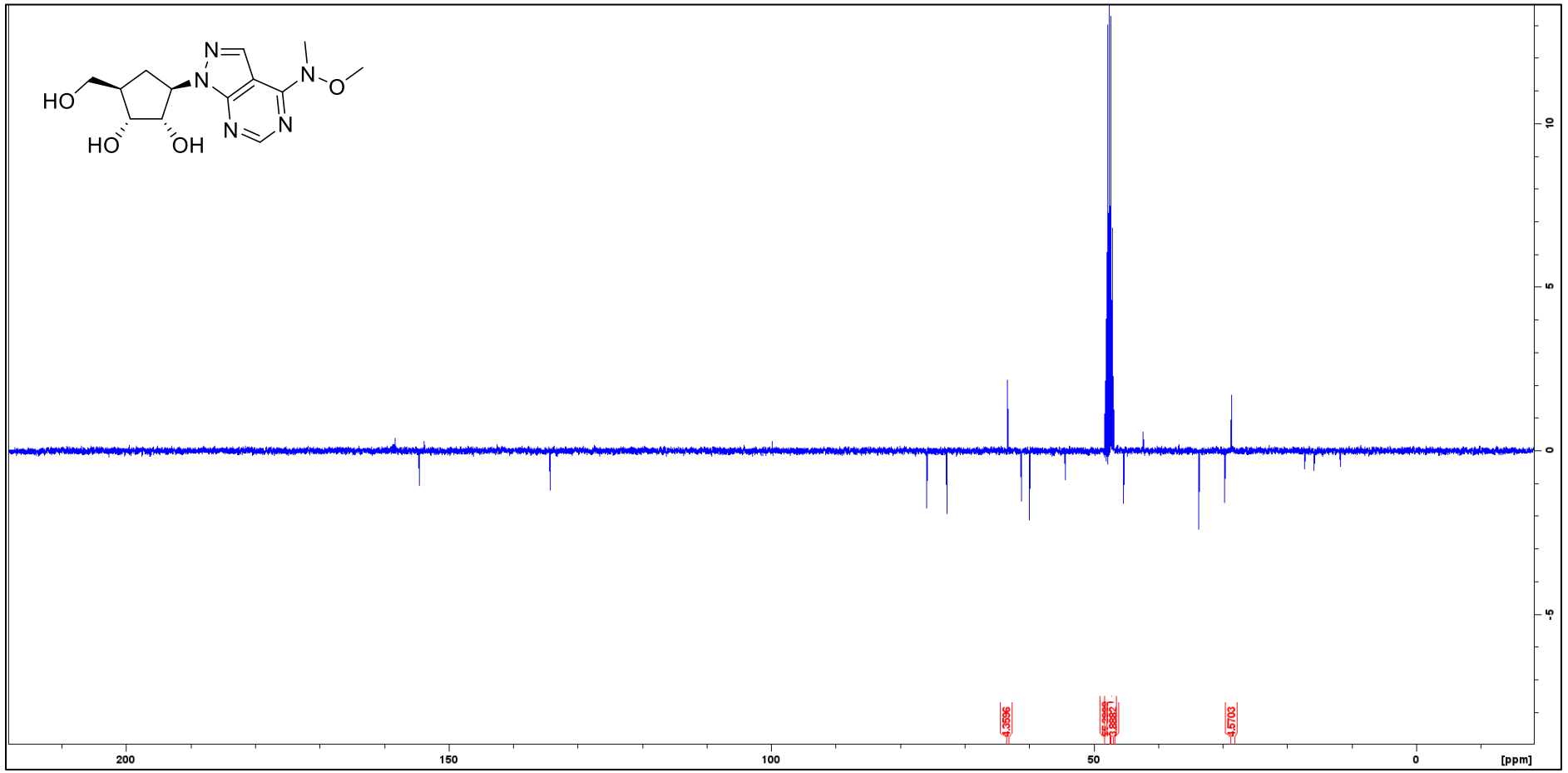
Compound **19**


HO
HO
OH
N
N
N
N
H
N
O
15
10
5
0
-5
0.8692
92.5392
0.5926
200
150
100
50
0
[ppm]

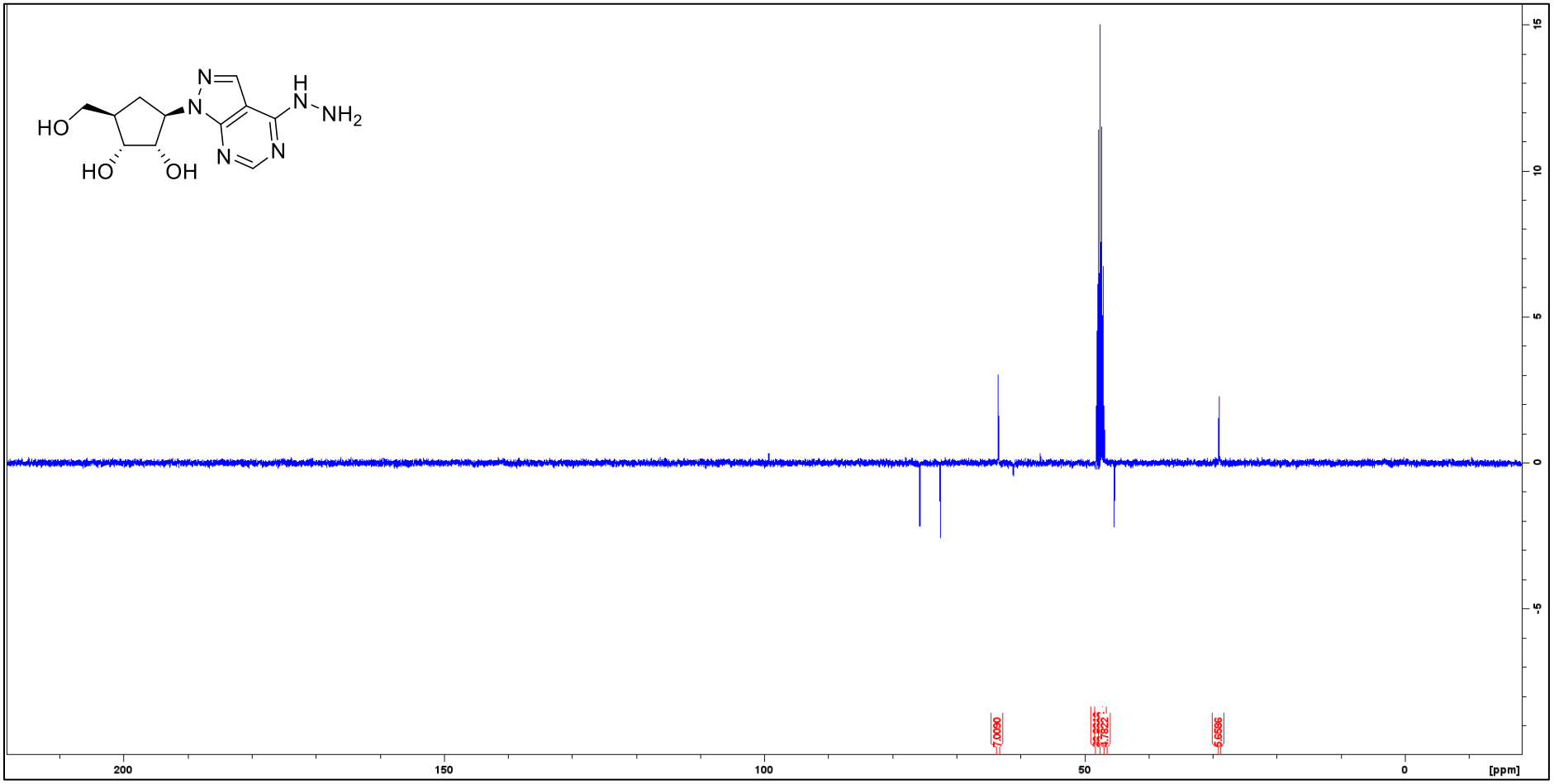
Compound **22**



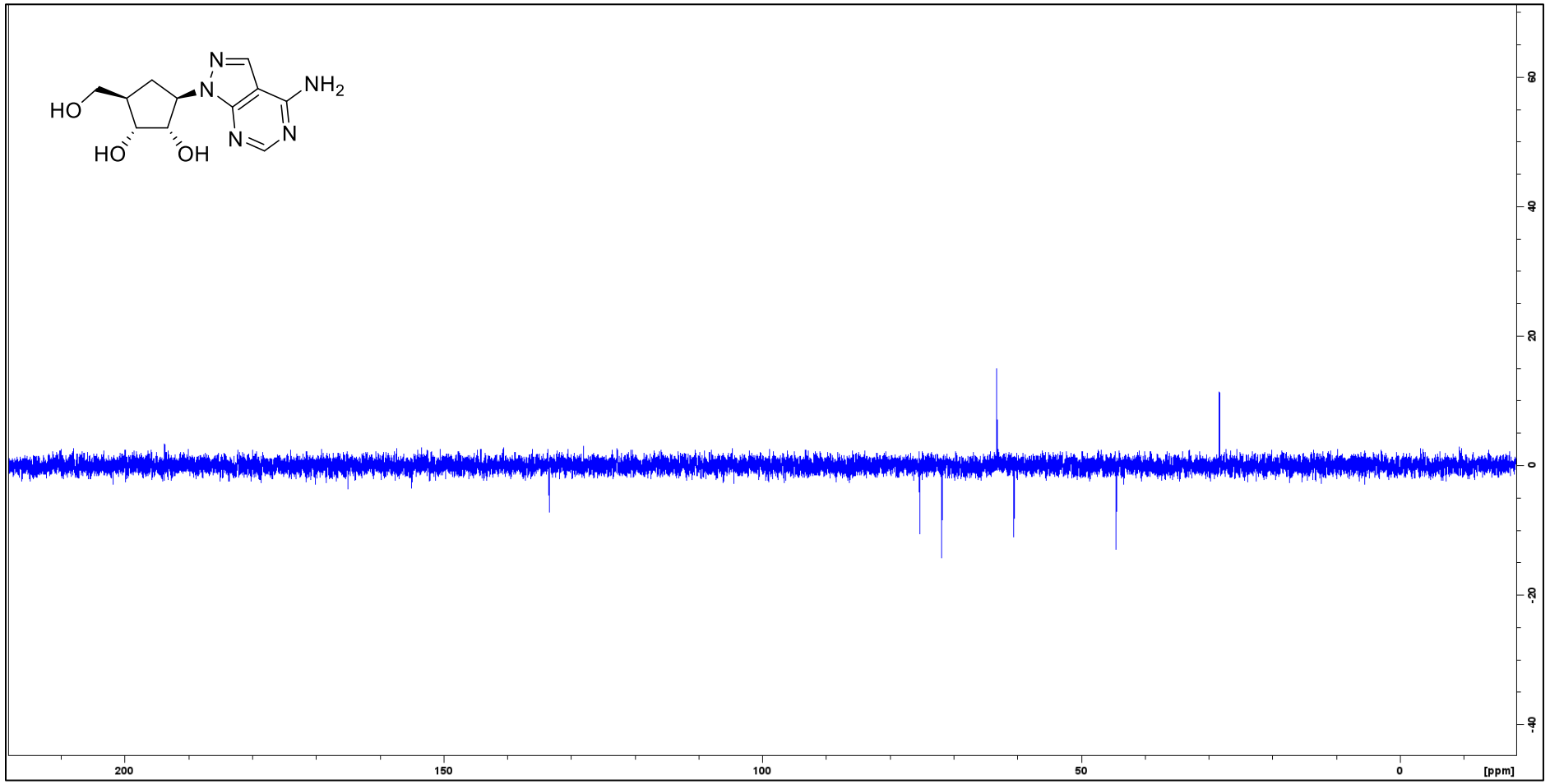
Compound **20**



Compound **23**

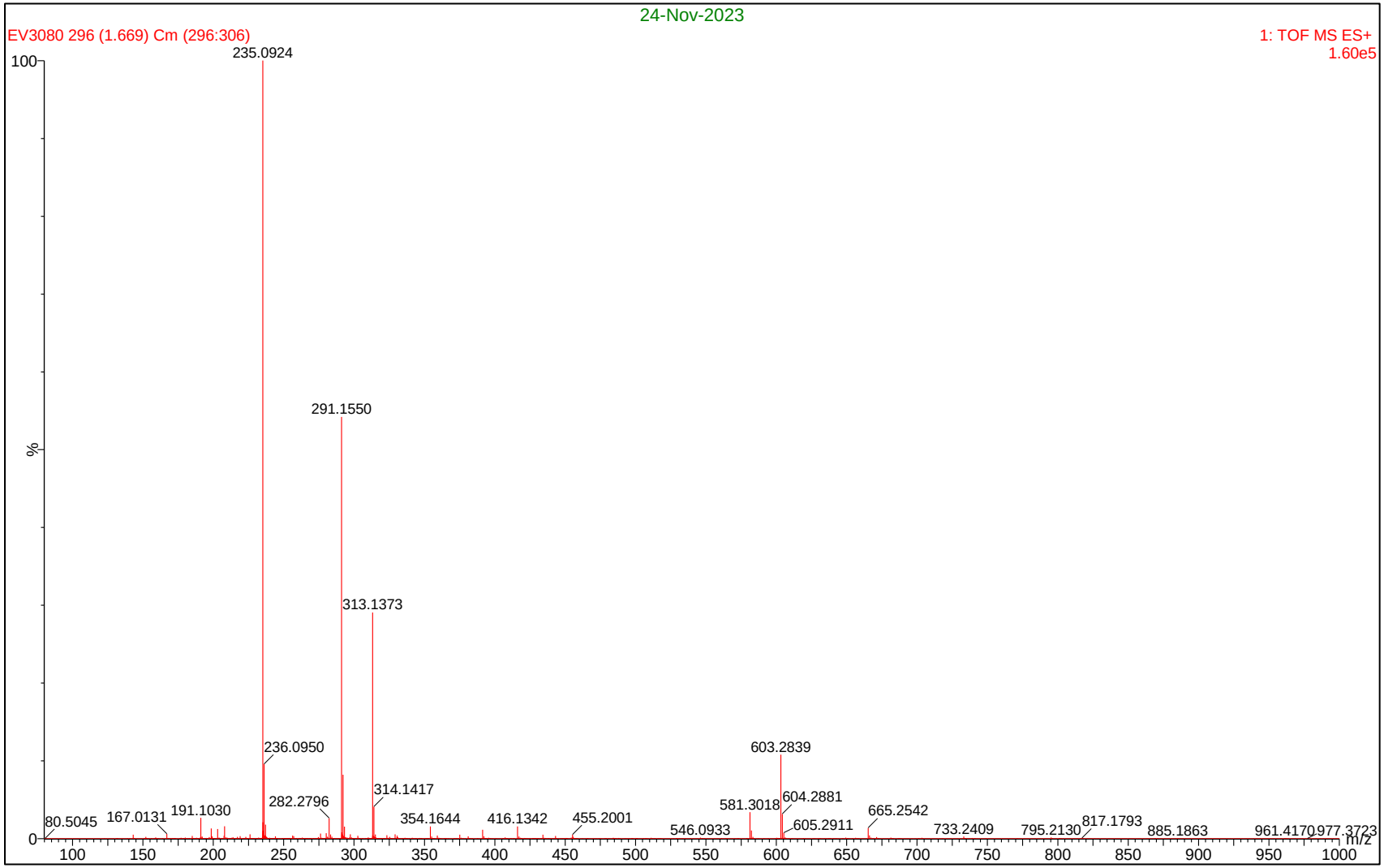

HO
HO
OH
N
N
N
N
H
N
NH2
7.0090
4.17622
5.6586
200
150
100
50
0
[ppm]
15
10
5
0
-5

Compound **24**

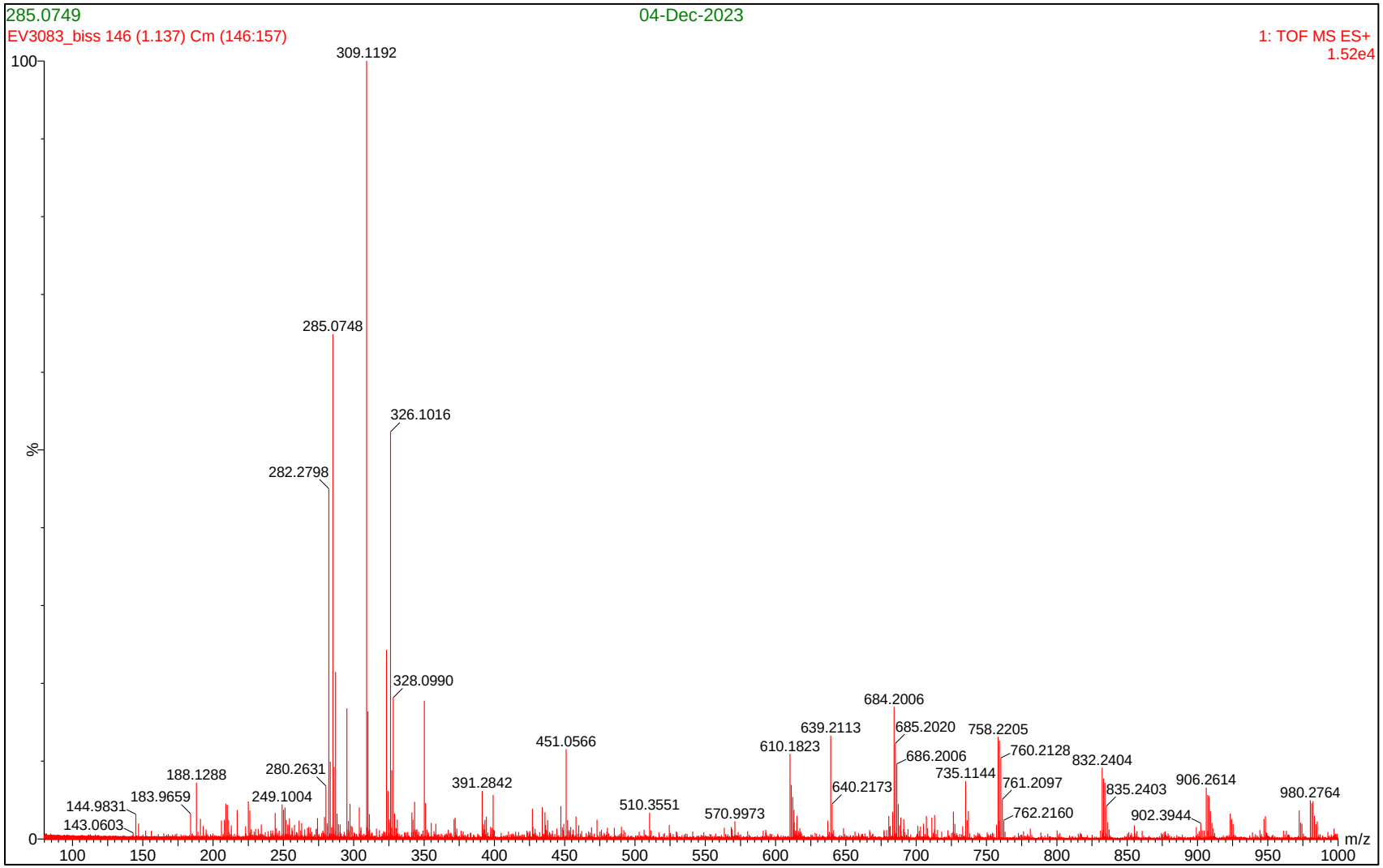


HRMS spectra

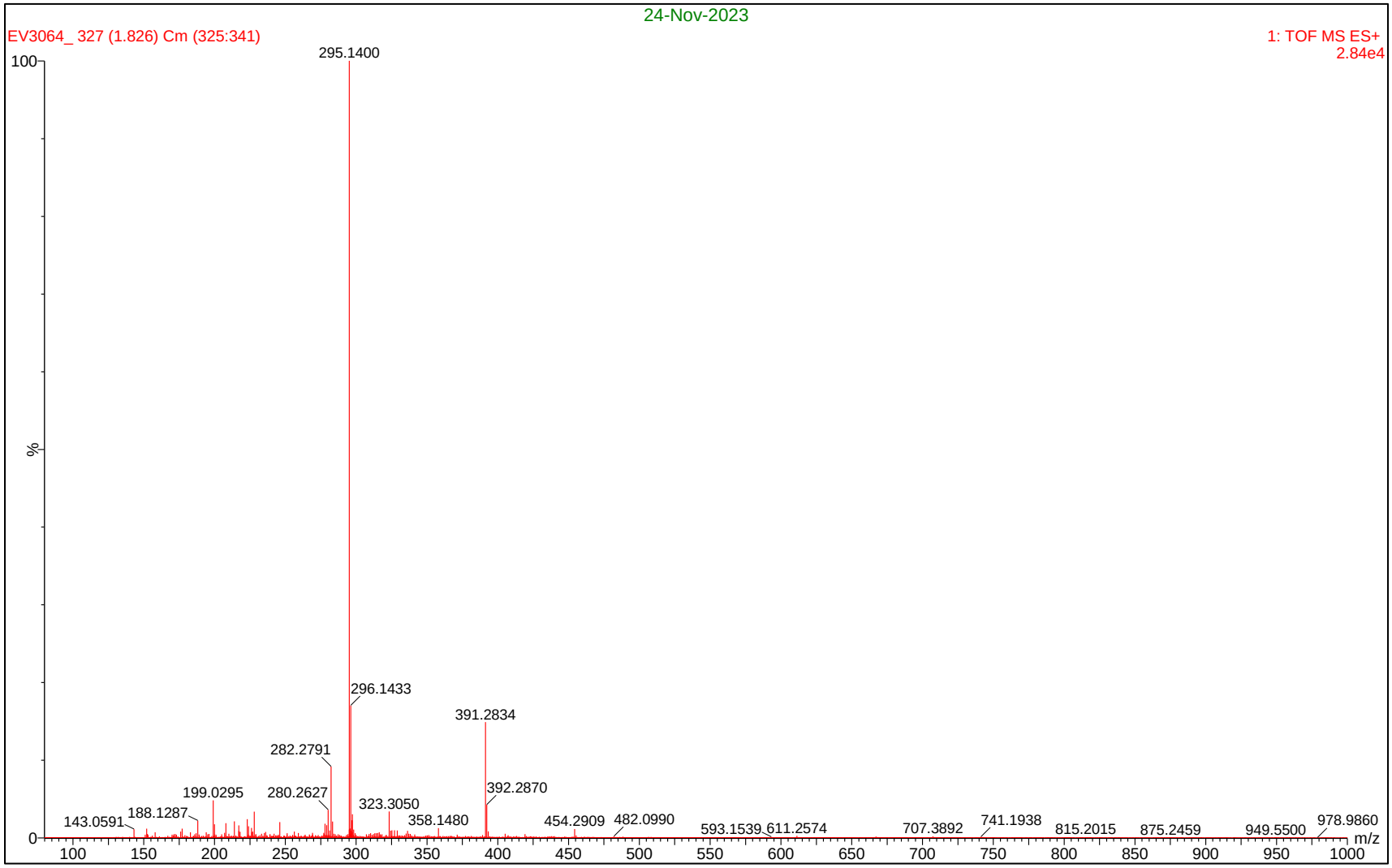
Compound **12** (11 scans)



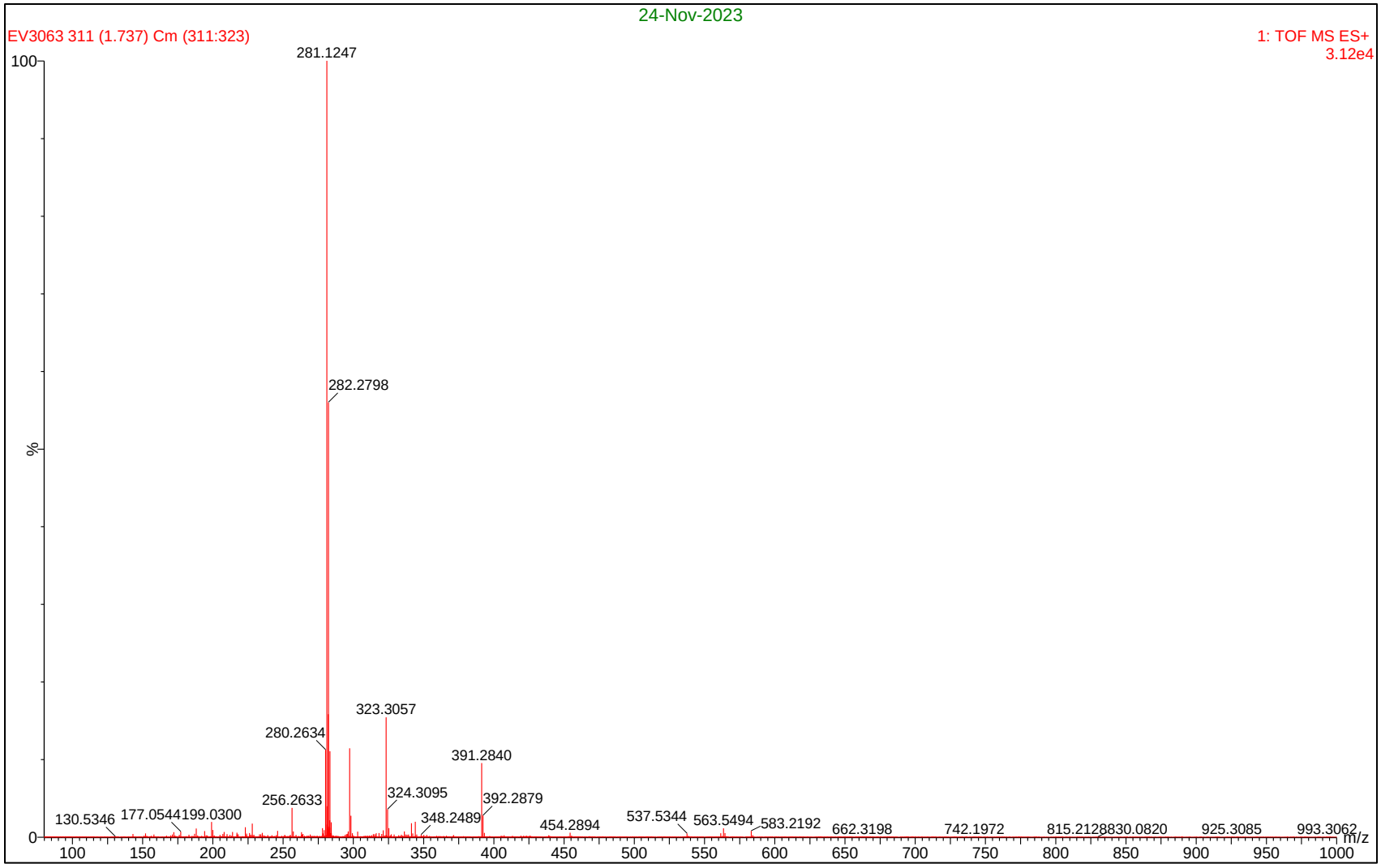
Compound **15** (12 scans)



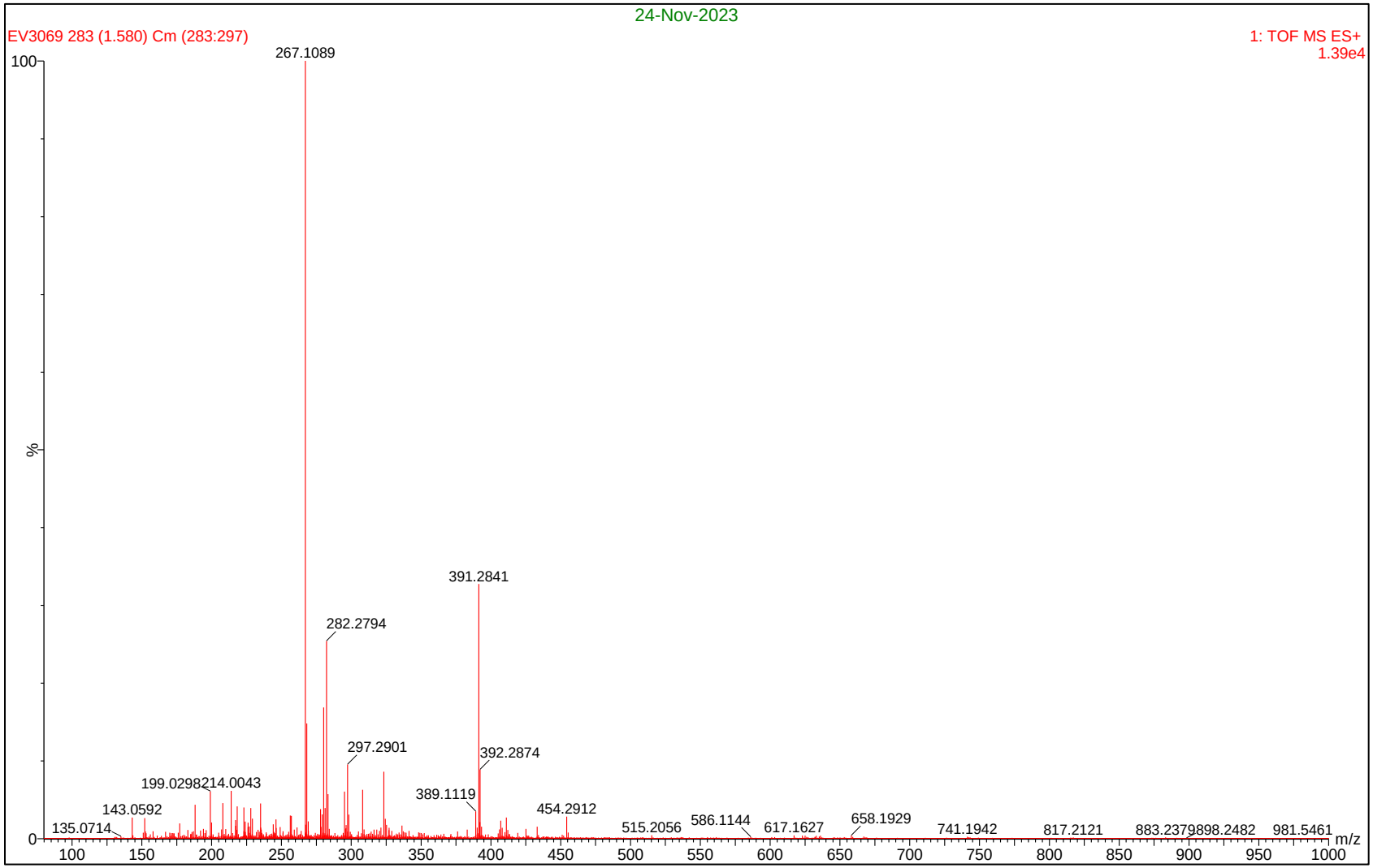
Compound **16** (17 scans)



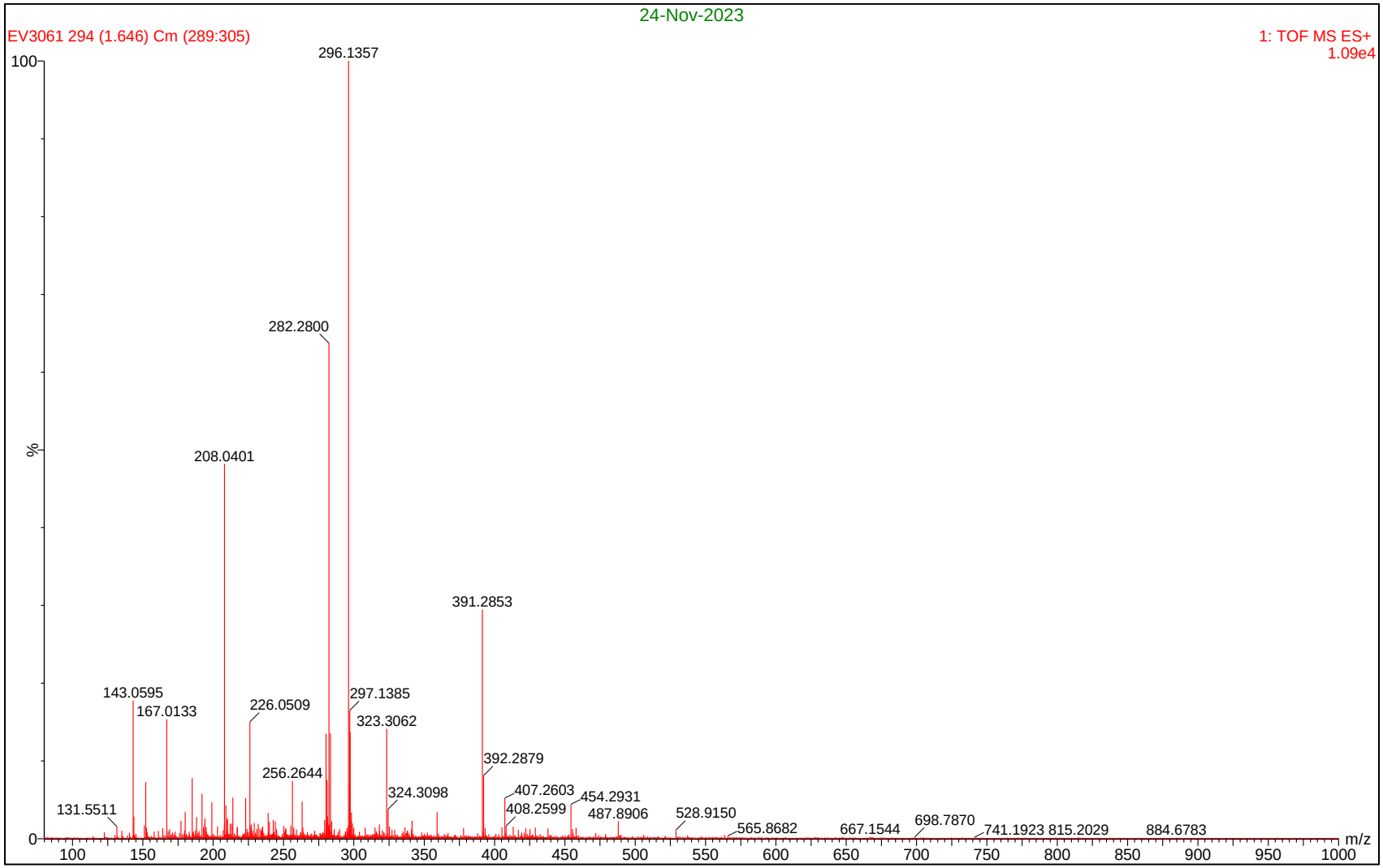
Compound **17** (13 scans)


24-Nov-2023
EV3063 311 (1.737) Cm (311:323)
1: TOF MS ES+
3.12e4
100
%
0
281.1247
282.2798
323.3057
280.2634
391.2840
324.3095
392.2879
256.2633
348.2489
130.5346
177.0544
199.0300
454.2894
537.5344
563.5494
583.2192
662.3198
742.1972
815.2128
830.0820
925.3085
993.3062
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

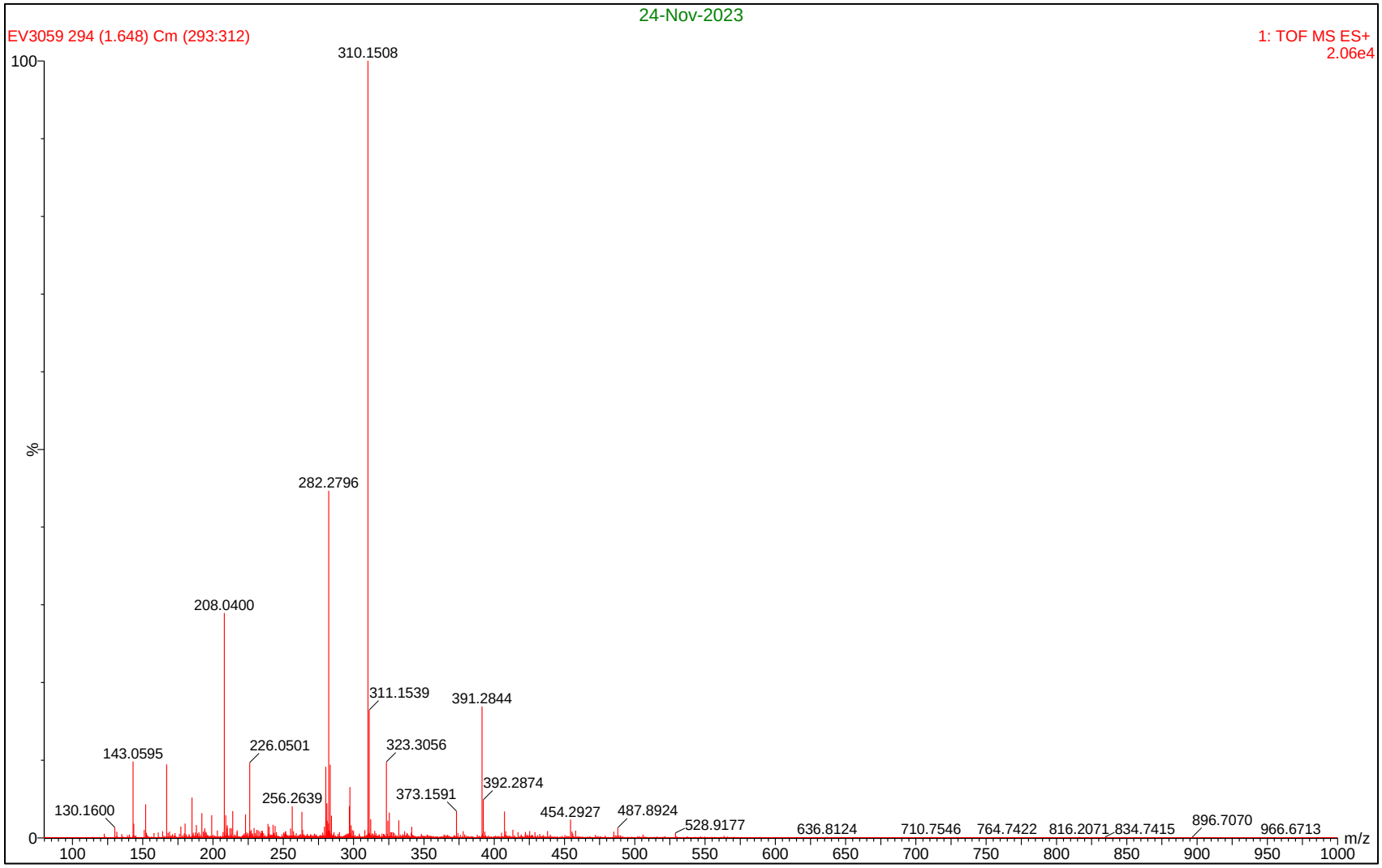
Compound **18** (15 scans)



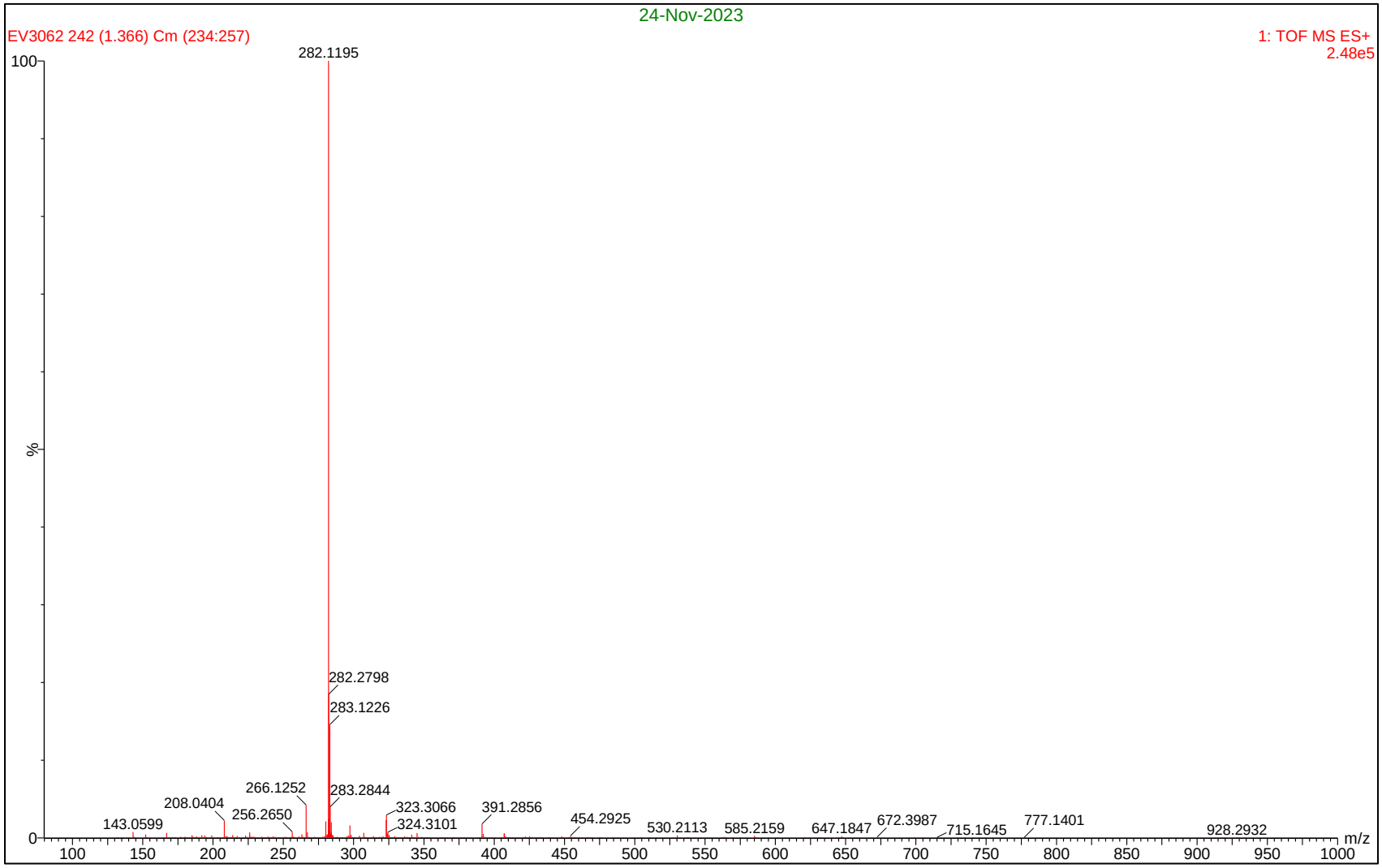
Compound **19** (17 scans)



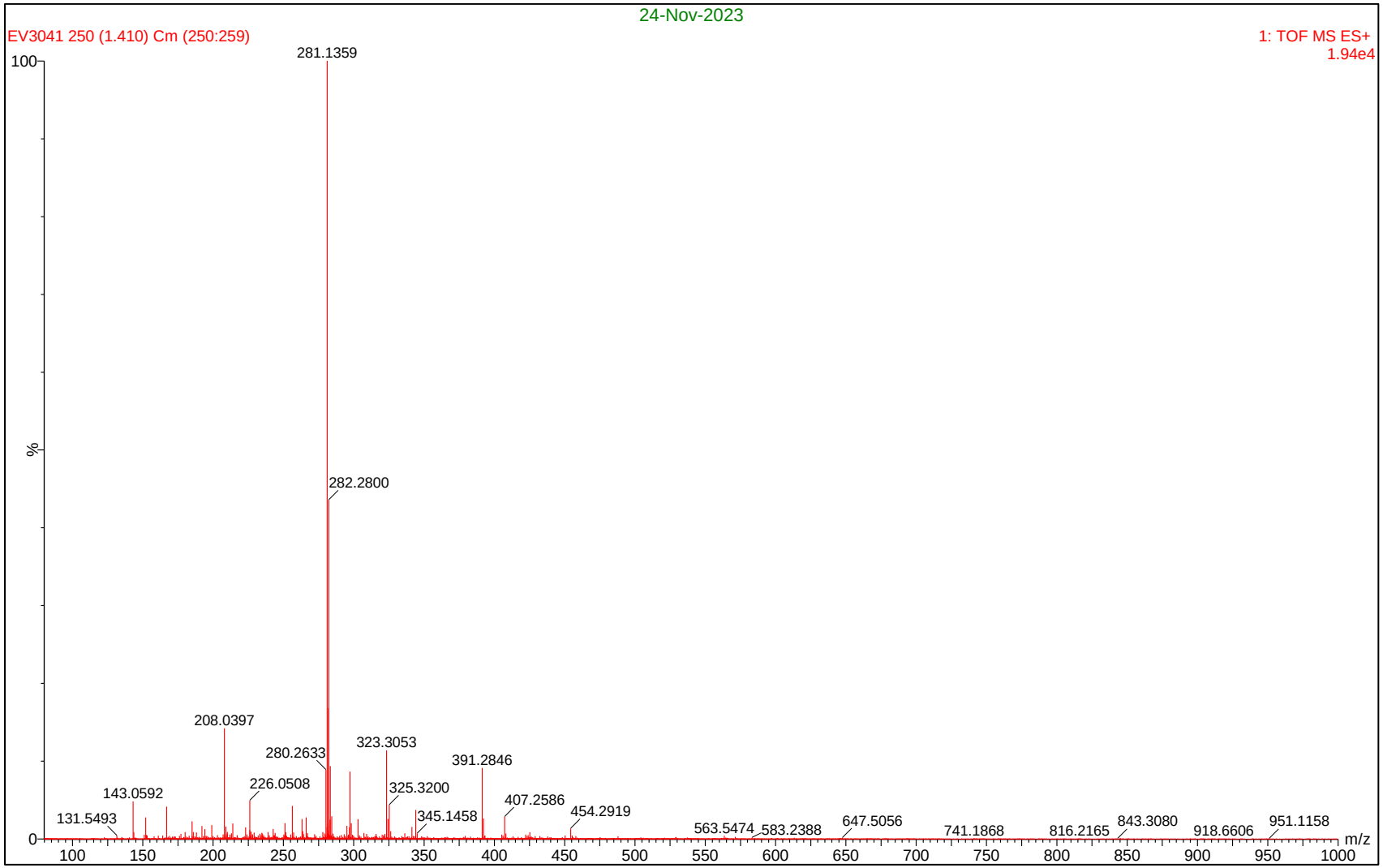
Compound **20** (20 scans)



Compound **22** (24 scans)



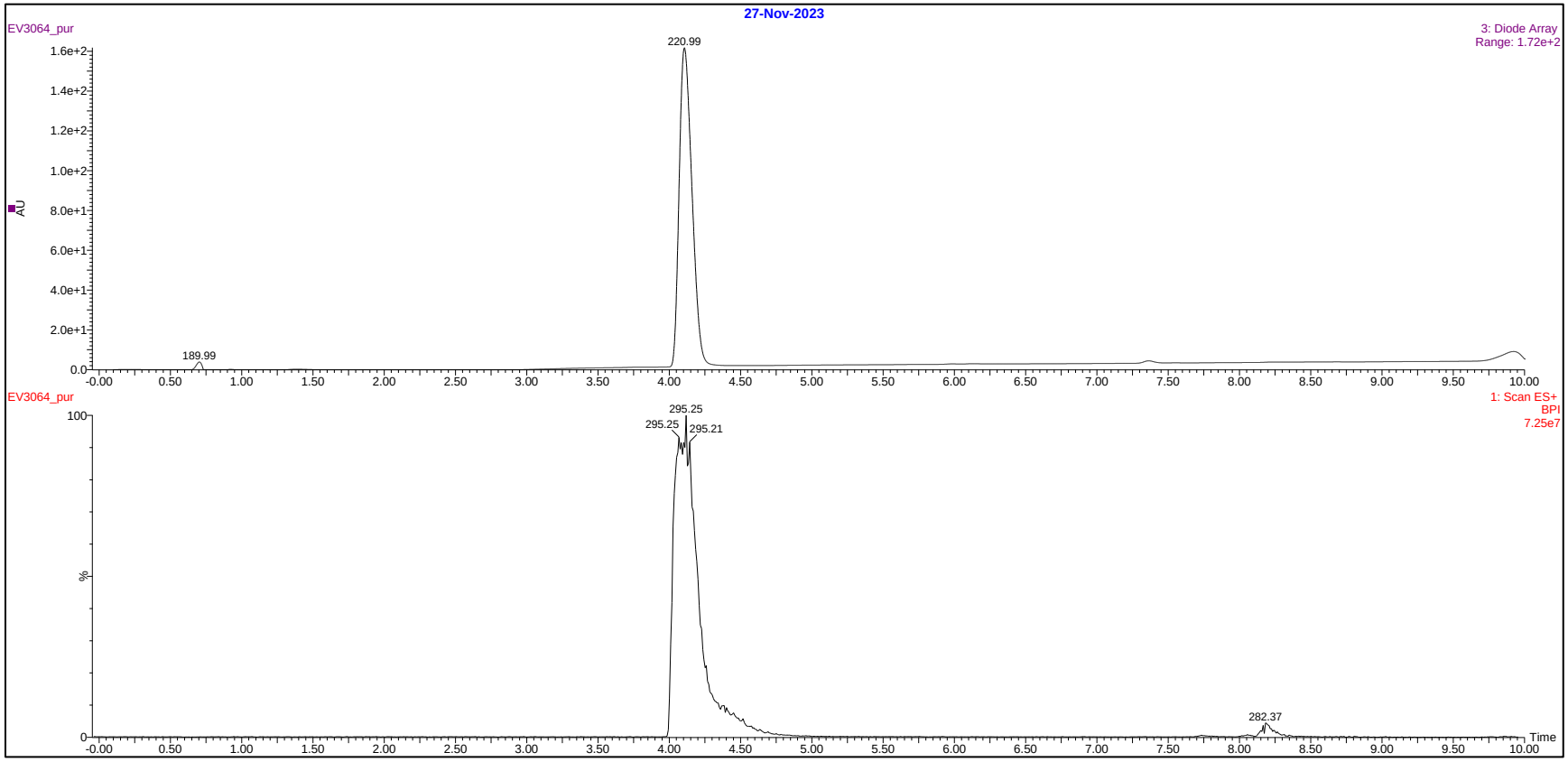
Compound **23** (10 scans)



LC-MS traces

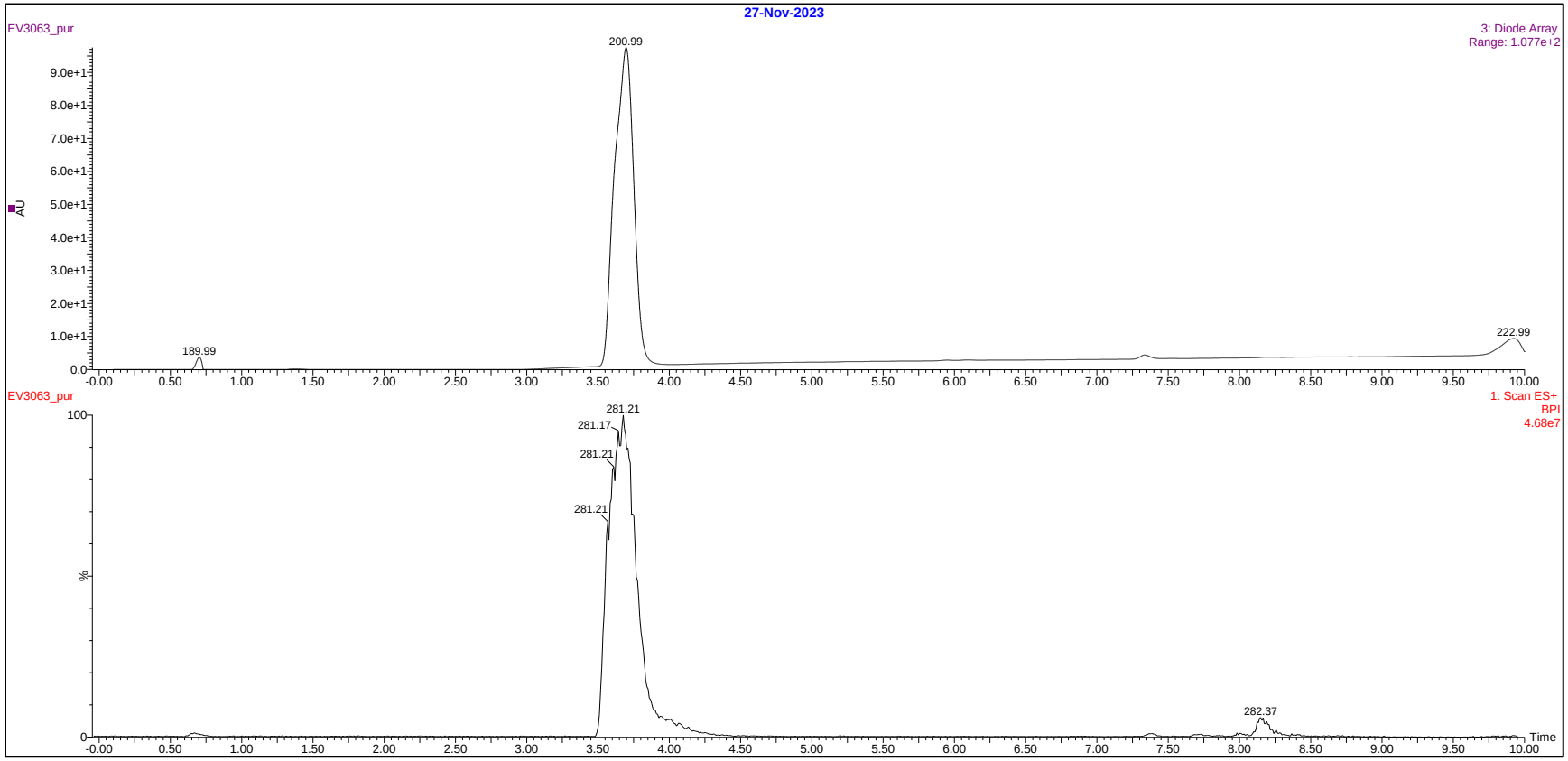
UV signals around 7.35 (mass: 485) is an artifact of the HPLC method and present in all spectra.

Compound **16**



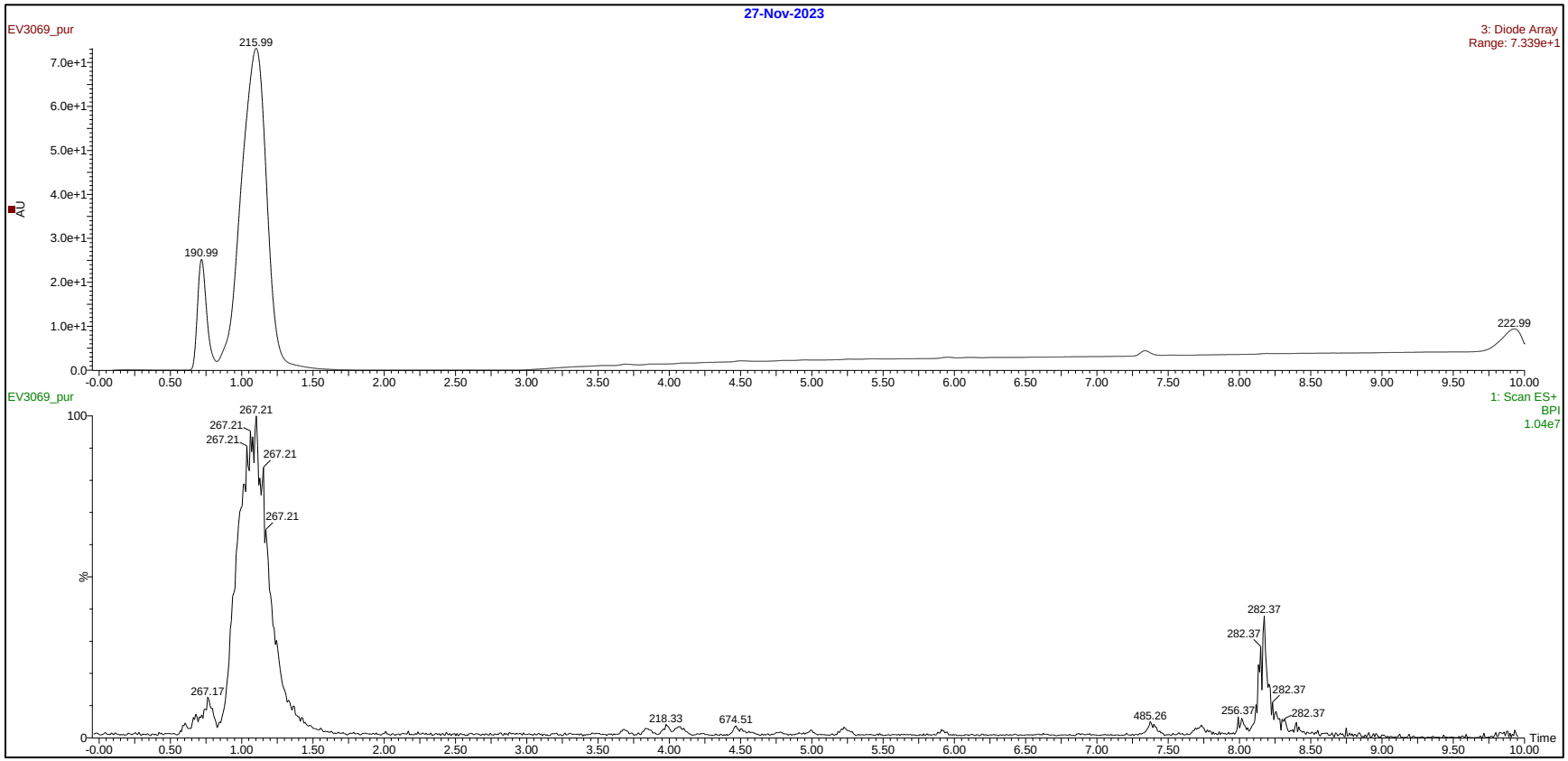
HPLC-UV trace shows no impurities

Compound **17**



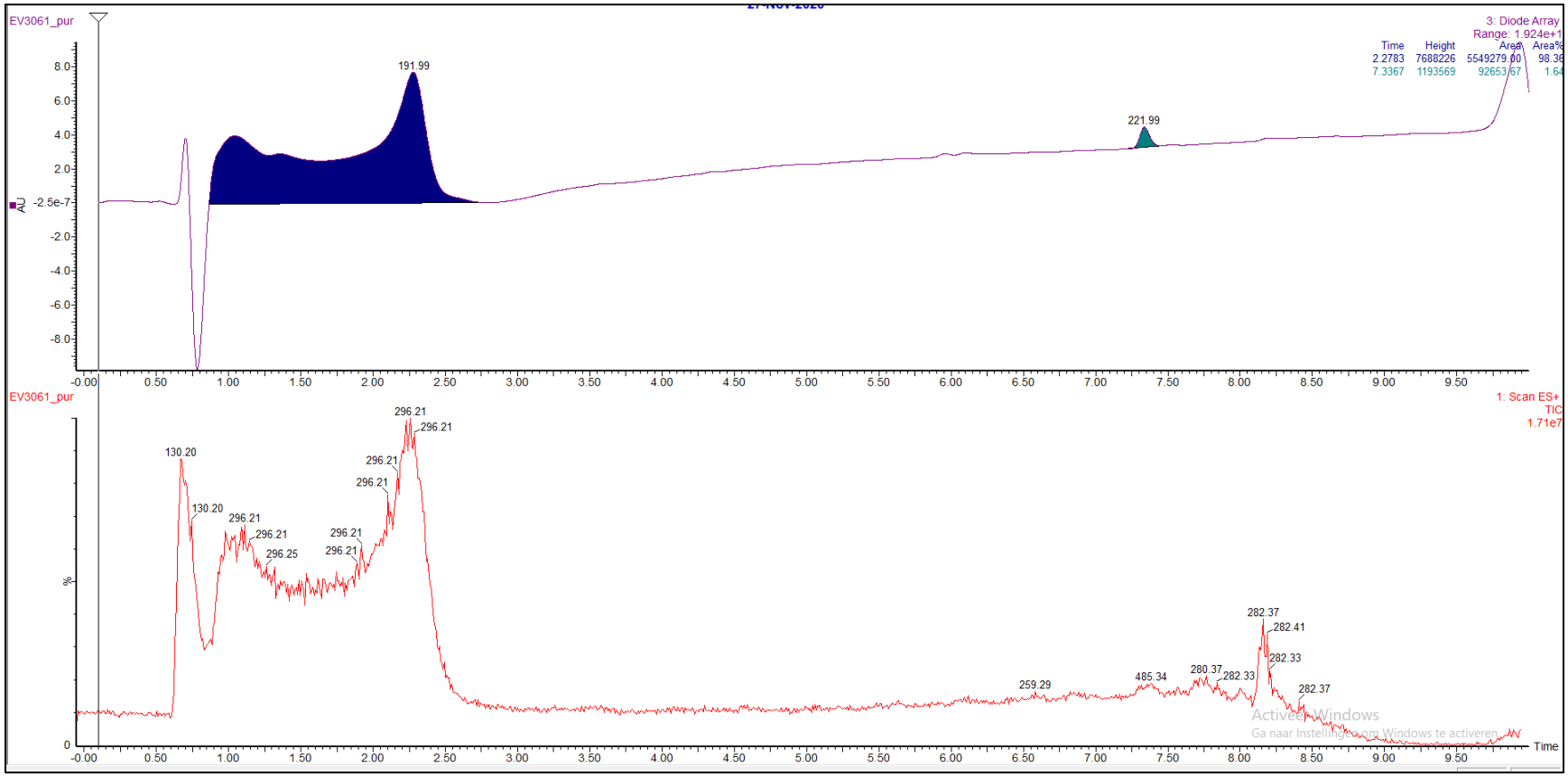
HPLC-UV trace shows no impurities

Compound **18**


27-Nov-2023
EV3069_pur
3: Diode Array
Range: 7.339e+1
215.99
190.99
222.99
AU
EV3069_pur
1: Scan ES+
BPI
1.04e7
267.21
267.17
218.33
674.51
485.26
282.37
256.37
%
Time

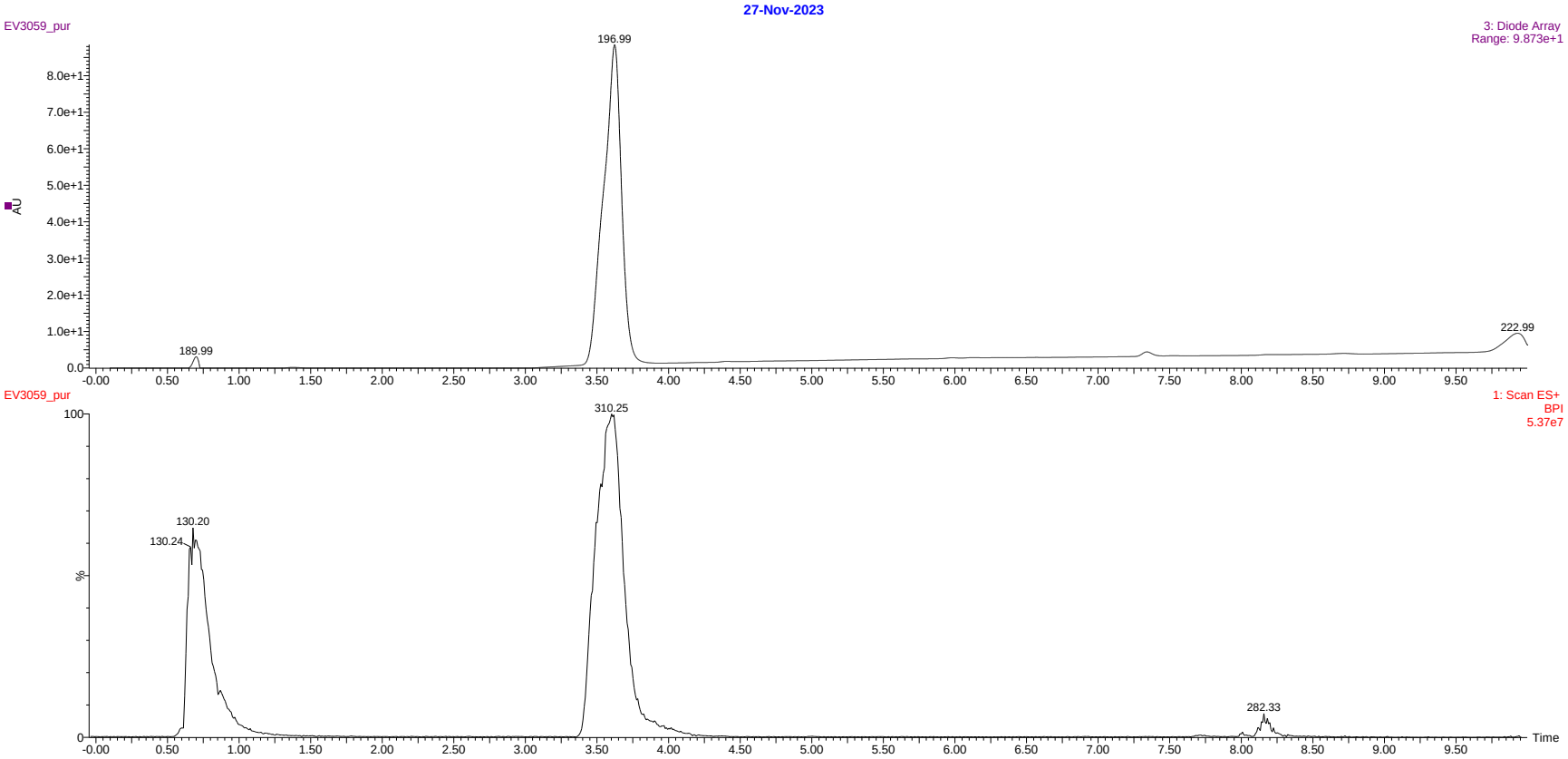
HPLC-UV trace shows no impurities

Compound **19**



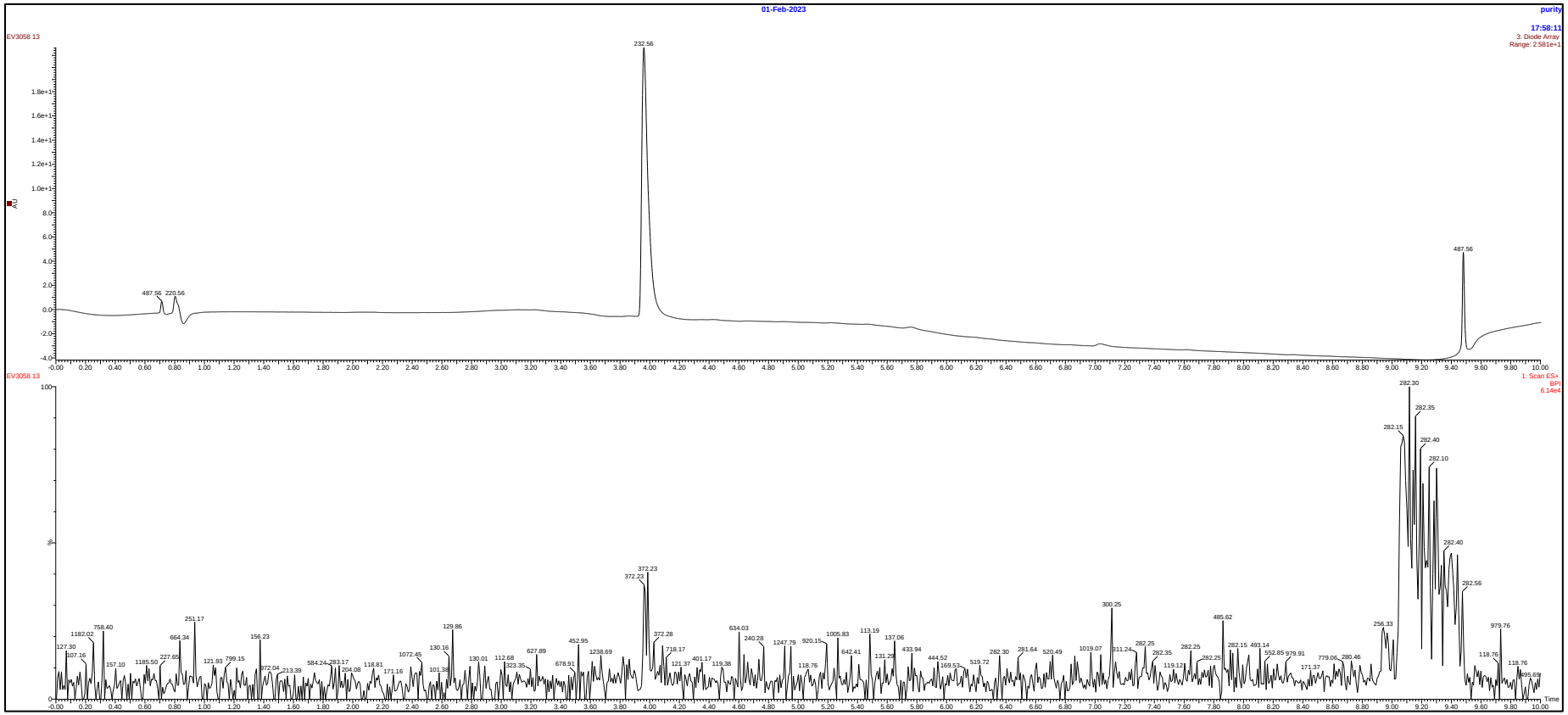
HPLC-UV trace shows >98% purity

Compound **20**



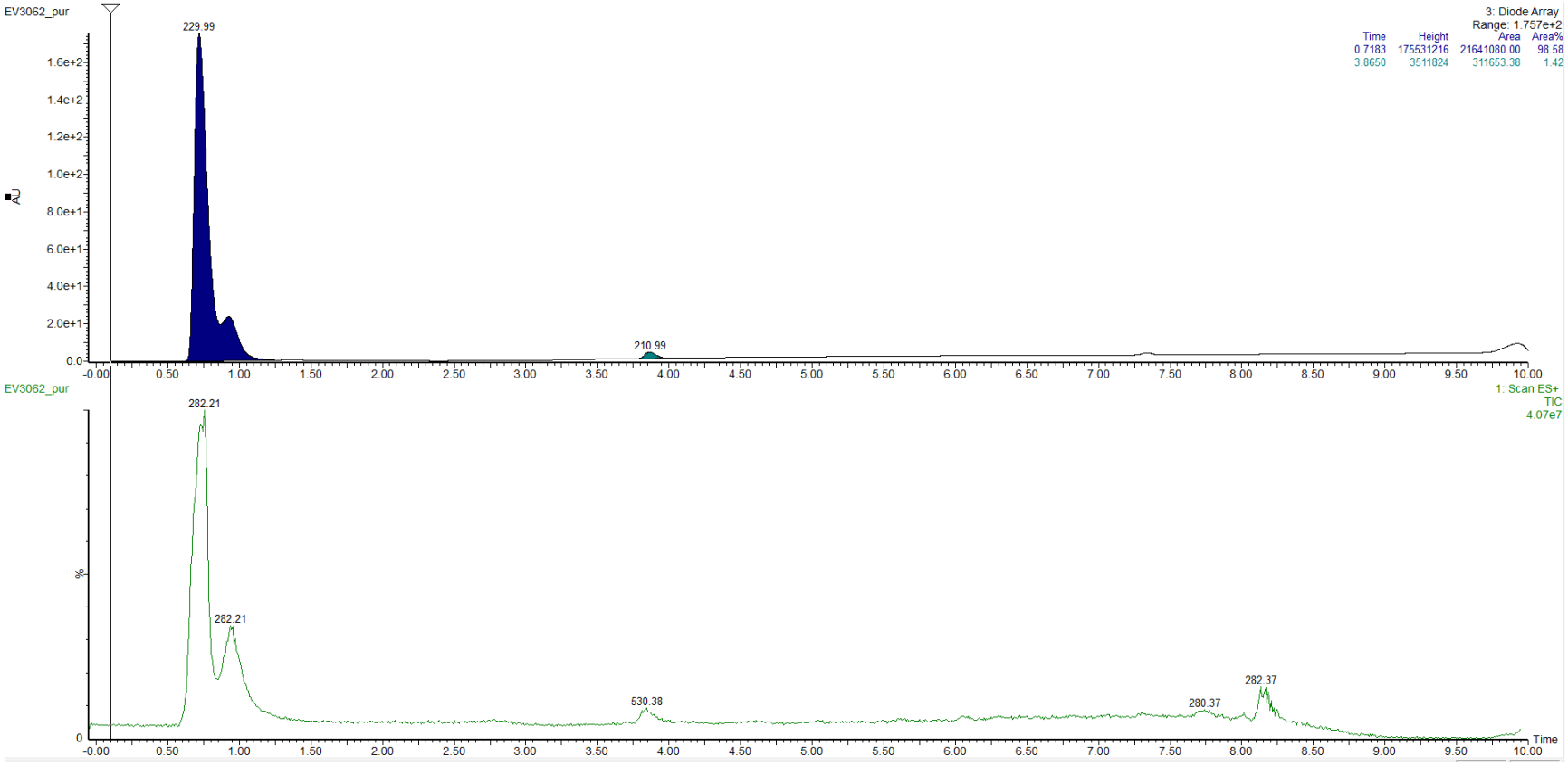
HPLC-UV trace shows no impurities

Compound **21**



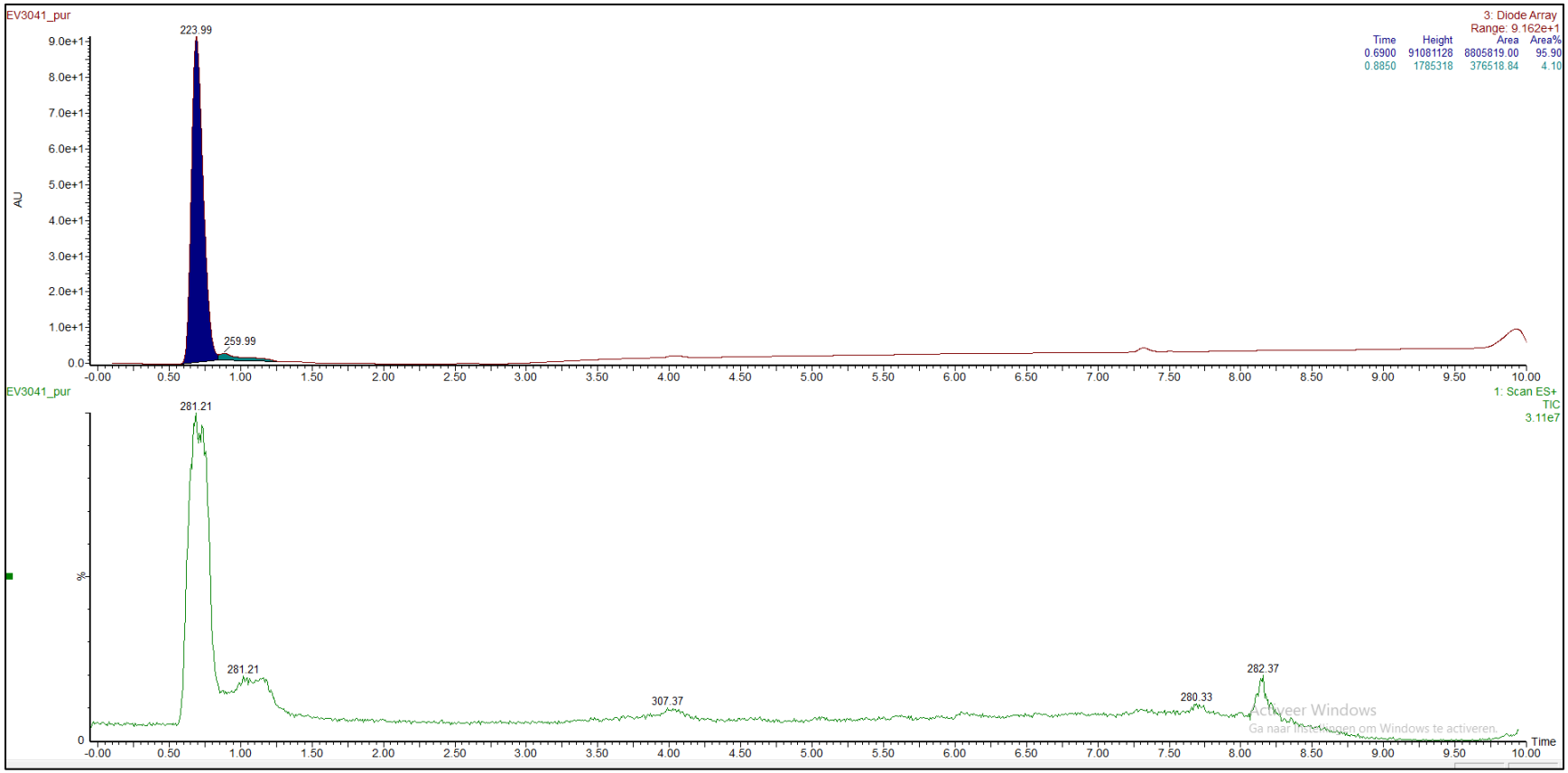
HPLC-UV trace shows no impurities

Compound **22**



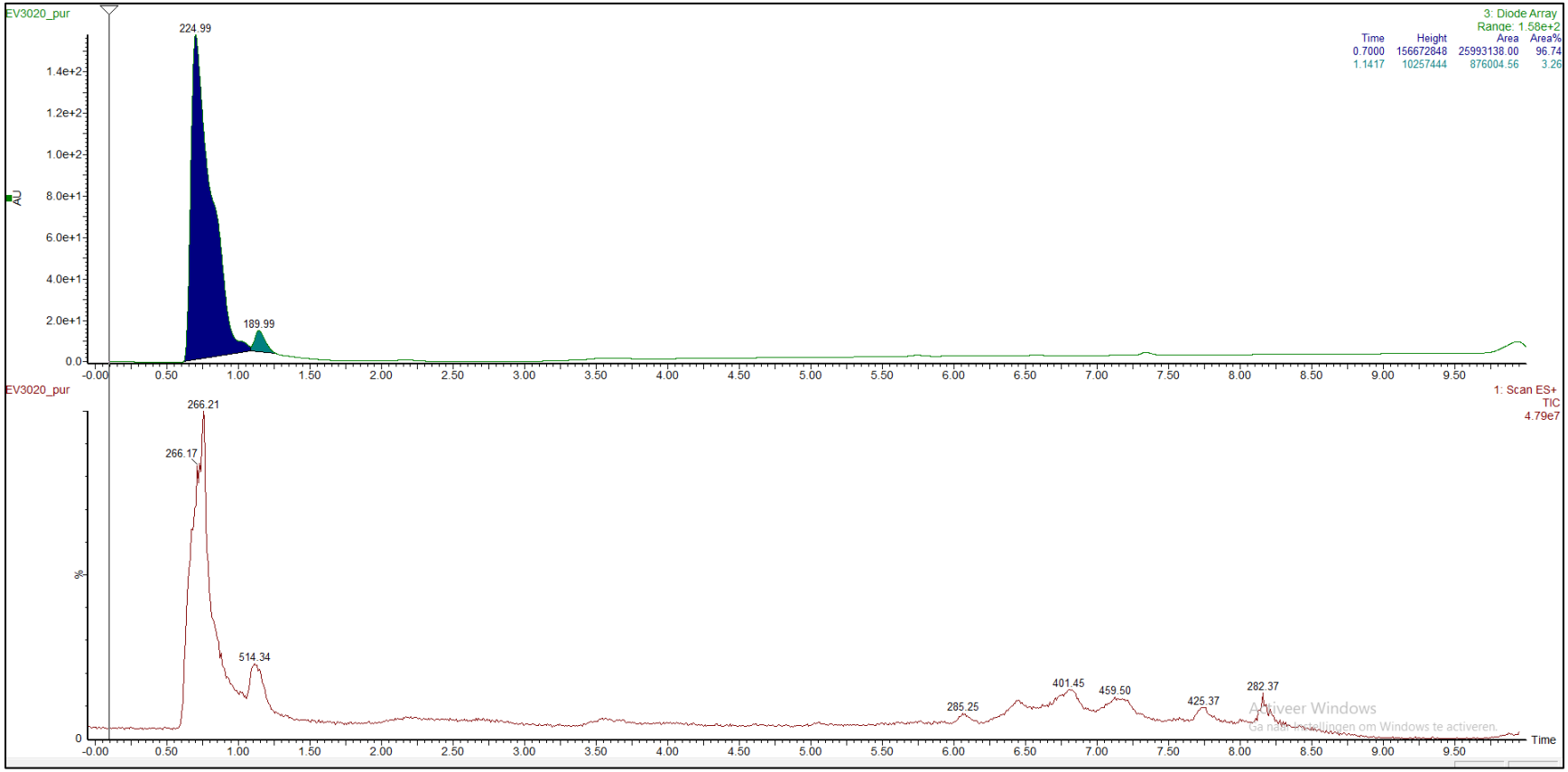
HPLC-UV trace shows >98% purity

Compound **23**



HPLC-UV trace shows >95% purity

Compound **24**



HPLC-UV trace shows >96% purity