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

Open-chain thiamine analogues as potent inhibitors of thiamine pyrophosphate (TPP)-dependent enzymes

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Supplementary Information (SI)

Enzyme assays

Evaluation of the inhibitory activity of compounds against Porcine PDH E1 *in vitro*

Porcine PDH E1 was purchased from Sigma. Porcine PDH E1 activity was determined by monitoring 2,6-dichlorophenolindophenol (DCPIP) reduction at 600 nm using a microplate reader (CLARIOstar) and conducted as described[2] with some modifications. The percentage inhibition of compounds against porcine PDH E1 was assayed at specified concentrations. The reaction buffer (50 mM KH_2PO_4 and 1 mM $MgCl_2$, pH 7) contained thiamine pyrophosphate (TPP) at specified concentrations, 0.25 mM 2,6-dichlorophenolindophenol (DCPIP), and 2 mg/mL porcine PDH E1. The reaction mixture was preincubated at 37 °C for 30 min, then reaction was initiated by adding pyruvate to a final concentration of 50 mM. To determine the half-maximal inhibitory concentration (IC_{50}) the TPP concentration was set at 10 µM, and inhibitor concentration was varied. Specific activity was calculated using the molar extinction coefficient of DCPIP, 21 mM^{-1} cm^{-1}.[3] The enzyme IC_{50} values were calculated from non-linear regression curve fitting using GraphPad Prism. The compound affinity (K_I) values were calculated by comparison to the K_M value of TPP; $K_{M(TPP)}$ was found to be 0.05 µM which is consistent with the value previously reported.[4]

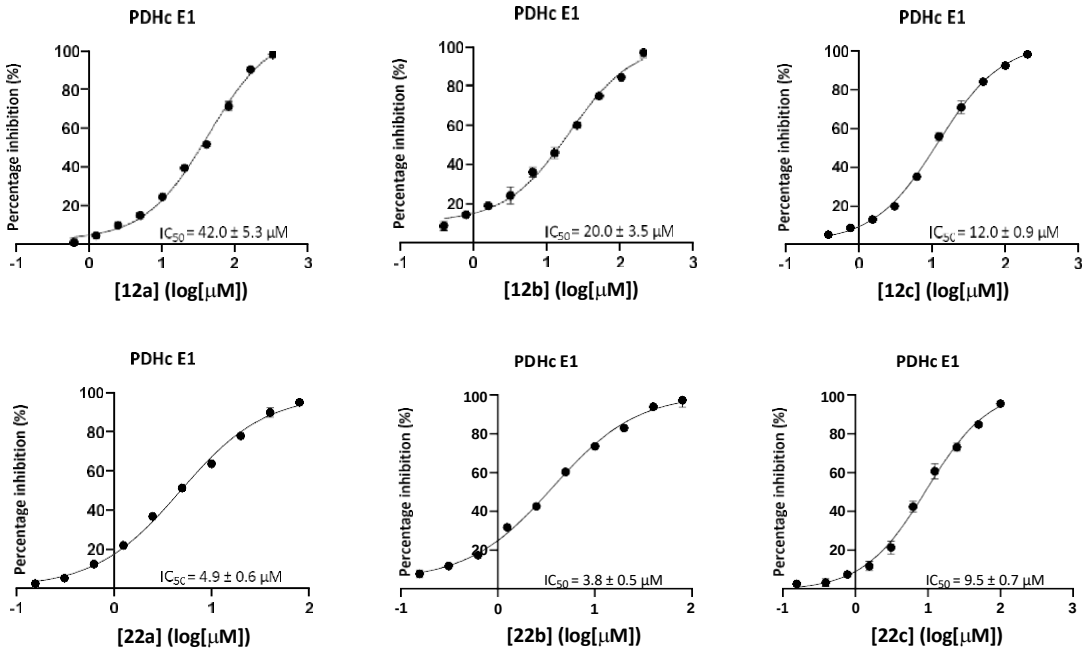


Figure S1. Measurement of porcine PDH E1 IC_{50} values at [TPP] = 10 µM. Measurements were made in triplicate. Where the error bars are not visible, they are smaller than the symbols. Best-fit nonlinear regression curves are shown.

***S. cerevisiae* PDC inhibitory activity assay.** *S. cerevisiae* PDC was purchased from Sigma. Its activity was determined by monitoring DCPIP reduction at 600 nm using a microplate reader (CLARIOstar) and conducted as described above with some modifications. The percentage inhibition of compounds was assayed at the specified final concentration. The reaction buffer (50 mM KH_2PO_4 and 1 mM $MgCl_2$, pH 7) contained 200 µM TPP, 0.27 mM DCPIP, and 0.15 mg/mL *S. cerevisiae* PDC. The reaction mixture was preincubated at 37 °C for 60 min, then reaction was initiated by adding pyruvate to a final concentration of 70 mM. Specific activity was calculated using the molar extinction coefficient of DCPIP, 21 mM^{-1} cm^{-1}.[3]

***E. coli* OGDH E1 inhibitory activity assay.** *E. coli* OGDH E1 was from our previous work[5] and had been donated by R. Frank. Its activity was determined by monitoring DCPIP reduction at 600 nm using a microplate reader (CLARIOstar) and conducted as described[5]with some modifications. The percentage inhibition of compounds was assayed at the specified final concentration. The reaction buffer (50 mM KH_2PO_4 and 2 mM $MgCl_2$, pH 7) contained 50 µM TPP, 0.5 mM DCPIP, and 6.7 mg/mL *E. coli* OGDH E1. The reaction mixture was preincubated at 37 °C for 60 min, then reaction was initiated by adding α-ketoglutarate to a final concentration of 10 mM. Specific activity was calculated using the molar extinction coefficient of DCPIP, 21 mM^{-1} cm^{-1}.[3]

***A. viridans* PO inhibitory activity assay.** *A. viridans* PO and horseradish peroxidase were purchased from Sigma. *A. viridans* PO activity was determined by monitoring appearance of quinoneimine dye at 550 nm using a microplate reader (CLARIOstar) and conducted as described[6] with some modifications. The percentage inhibition of compounds was assayed at the specified final concentration. The reaction buffer (50 mM KH_2PO_4 and 10 mM $MgCl_2$, pH 5.9) contained 50 µM TPP, 10 µM flavin adenine dinucleotide (FAD), 0.15% 4-aminoantipyrine, 0.3% N-ethyl-N-(2-hydroxy-3- sulfopropyl)-m-toluidine (EHSPT), 50 µg/mL horseradish peroxidase and 0.35 U/mL *A. viridans* PO. The reaction mixture was preincubated at 37 °C for 30 min, then reaction was initiated by adding pyruvate to a final concentration of 50 mM. 1 unit of PO activity is defined as 1 µmol of hydrogen peroxide produced per minute.

***Z. mobilis* PDC inhibitory activity assay.** *Z. mobilis* PDC was expressed and purified following a reported method.[7] *Z. mobilis* PDC activity was determined by monitoring reduced nicotinamide adenine dinucleotide (NADH) consumption at 340 nm using a microplate reader (CLARIOstar) and conducted as described[7] with some modifications. The percentage inhibition of compounds was assayed at the specified final concentration. The reaction buffer (50 mM MES-KOH and 5 mM $MgCl_2$, pH 6.5) contained 50 µM TPP, 150 µM NADH, 10 U/mL alcohol dehydrogenase (ADH) and 0.5 µM of active sites of *Z. mobilis* PDC. The reaction mixture was preincubated at 37 °C for 60 min, then reaction was initiated by adding pyruvate to a final concentration of 10 mM.

Table S1. Summary of inhibitory activity of hydroxamate 17b on TPP-dependent enzymes.

	Inhibition (%)[a]			
[TPP]:[inhibitor]	***S. cerevisiae* PDC[b]**	***Z. mobilis* PDC[c]**	***E. coli* OGDH E1[c]**	***A. viridans* PO[c]**
1:1	<15	48 ± 4	27 ± 5	<15
1:5	<15	71 ± 6	60 ± 5	<15

[a] Data are the means of measurements in three technical replicates. [b] [TPP] = 200 µM, [**17b**] = 200 µM (1:1) and 1000 µM (1:5). [c] [TPP] = 50 µM, [**17b**] = 50 µM (1:1) and 250 µM (1:5).

Computational docking

Docking of TPP, compounds were executed using CCDC GOLD docking program with PDB: 6CFO for human PDH E1. The ligand (adduct of TPP with acetyl phosphinate) that forms part of chain A was removed and the resulting cavity was selected as the binding site. Our molecules were generated using Mercury. GA runs were set at 50 and efficiency of docking calculations was set to "Very Flexible" (200%). No early termination was permitted. CHEMPLP and GoldScore were the docking scoring and rescoring respectively.[1] For all other GOLD-specific docking options the default settings were used. Interactions between docked compounds and protein models are shown using CCDC GOLD. Goldscores were used to select best binding poses as GOLD has been optimised for the prediction of the best ligand binding poses; many factors are ignored or approximated when calculating a docking score so it does not reflect binding affinities.

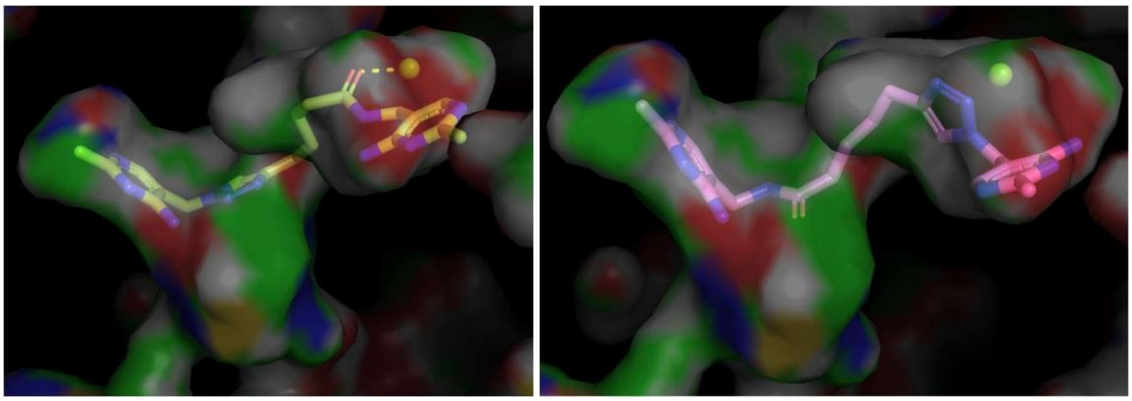


Figure S2. The two possible binding modes of the longest bis-pyrimidine 12c in the TPP pocket of human PDH E1. The expected binding mode in which the pyrimidine-CH_2-triazole motif occupies the binding region of TPP's pyrimidine-CH_2-thiazolium (left, Goldscore 47.5) and the alternative binding mode in which the pyrimidine-CH_2-amide motif occupies the binding region instead (right, Goldscore 40.5). In both cases, there is a pyrimidine moiety extending deeper into the pyrophosphate pocket. The Goldscores suggest that the left-hand pose, with the triazole in the thiazolium pocket and amide in the pyrophosphate pocket, is slightly more favourable.

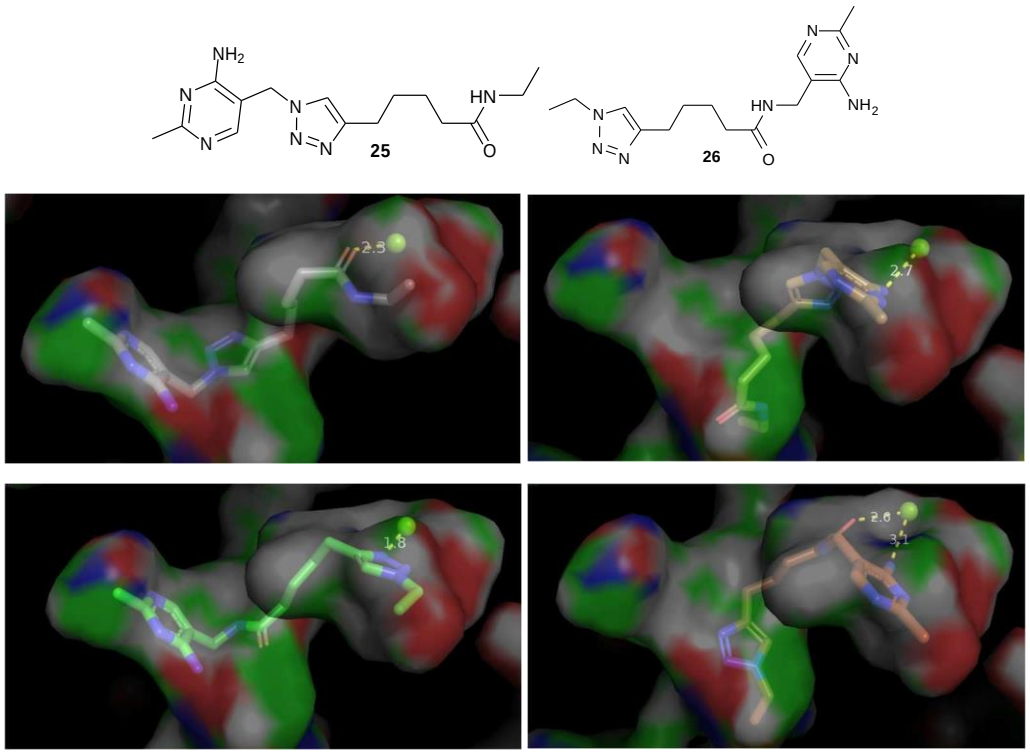


Figure S3. Predicted binding modes of 25 and 26 in TPP pocket of human PDH E1 with potential interactions with Mg^{2+}. Compounds **25** and **26** are **12c** with one or the other aminopyrimidine ring replaced by a methyl group. Docking of **25** with the aminopyrimidine ring in the aminopyrimidine pocket (top left, Goldscore 54.5) and in the pyrophosphate pocket (top right, Goldscore 34.5). Docking of **26** with aminopyrimidine in aminopyrimidine pocket (bottom left, Goldscore 60.7) and aminopyrimidine in pyrophosphate pocket (bottom right, Goldscore 35.9). The Goldscores suggest a strong preference for aminopyrimidine-containing compounds to adopt a pose with the aminopyrimidine ring in the aminopyrimidine binding pocket rather than in the pyrophosphate pocket.

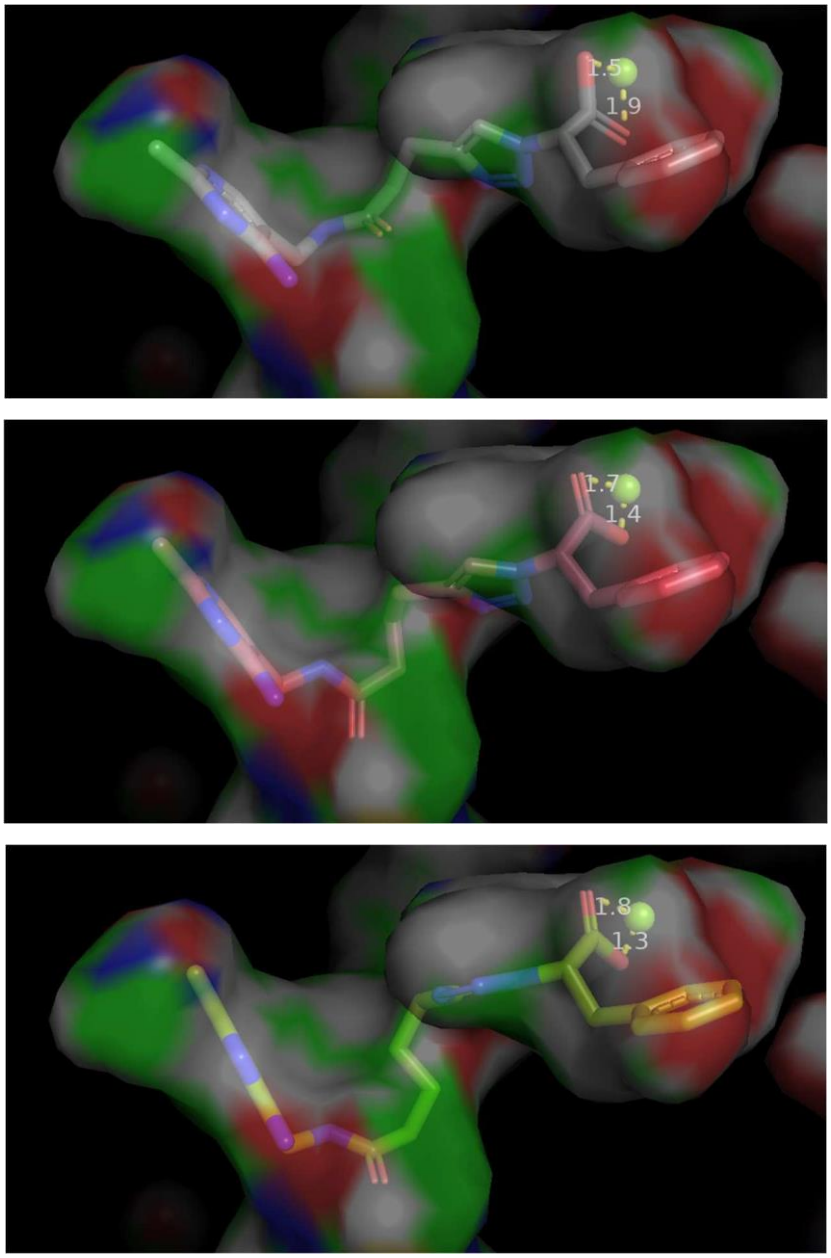


Figure S4. Predicted binding modes of carboxylates 18a-c in the TPP pocket of human PDH E1. All show the terminal carboxylate interacting with the Mg^{2+} while the terminal phenyl group extends deeper into the pyrophosphate pocket. **18a** (top), **18b** (middle) and **18c** (bottom). The Goldscores of **18a**, **18b** and **18c** are 44.3, 36.8 and 41.0 respectively.

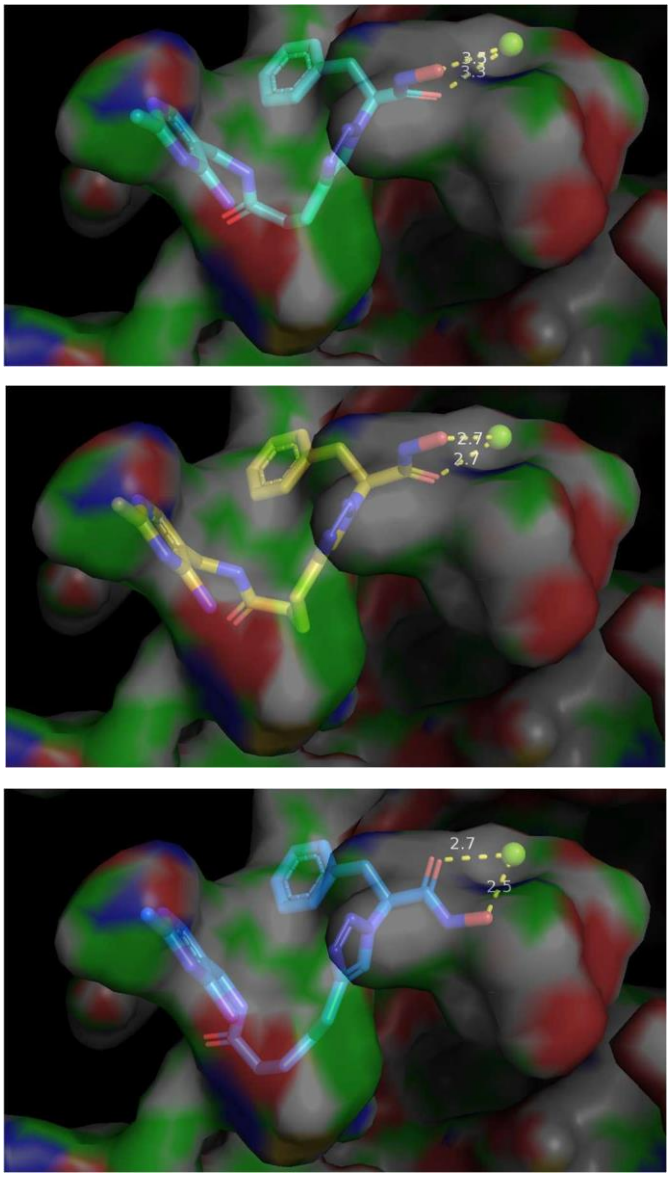


Figure S5. Predicted binding modes of hydroxamates 22a-c in the TPP pocket of human PDH E1. All show the terminal hydroxamate as a bidentate MBG interacting with the Mg^{2+} while the terminal phenyl group flips towards the central region (green). Regions of the active site cavity highlighted in green are lined with hydrophobic residues. TPP-dependent enzymes all have this hydrophobic central region that stabilises the catalytically active TPP ylide. These phenylalanine-derived ligands have the terminal phenyl group positioned close to this central region and thus stabilised by the hydrophobic interactions. The binding modes of **22a** (top) and **22b** (middle) are very similar and so their binding affinities are comparable (Table 1), although the slightly shorter interaction distance between the hydroxamate and Mg^{2+} with **22b** may explain why it is slightly more potent than **22a**. The alkyl linker in **22c** (bottom) seems to be too long so may have distorted the optimal binding mode, thus lowering the affinity. Goldscores of **22a**, **22b** and **22c** are 42.6, 36.2 and 39.2 respectively.

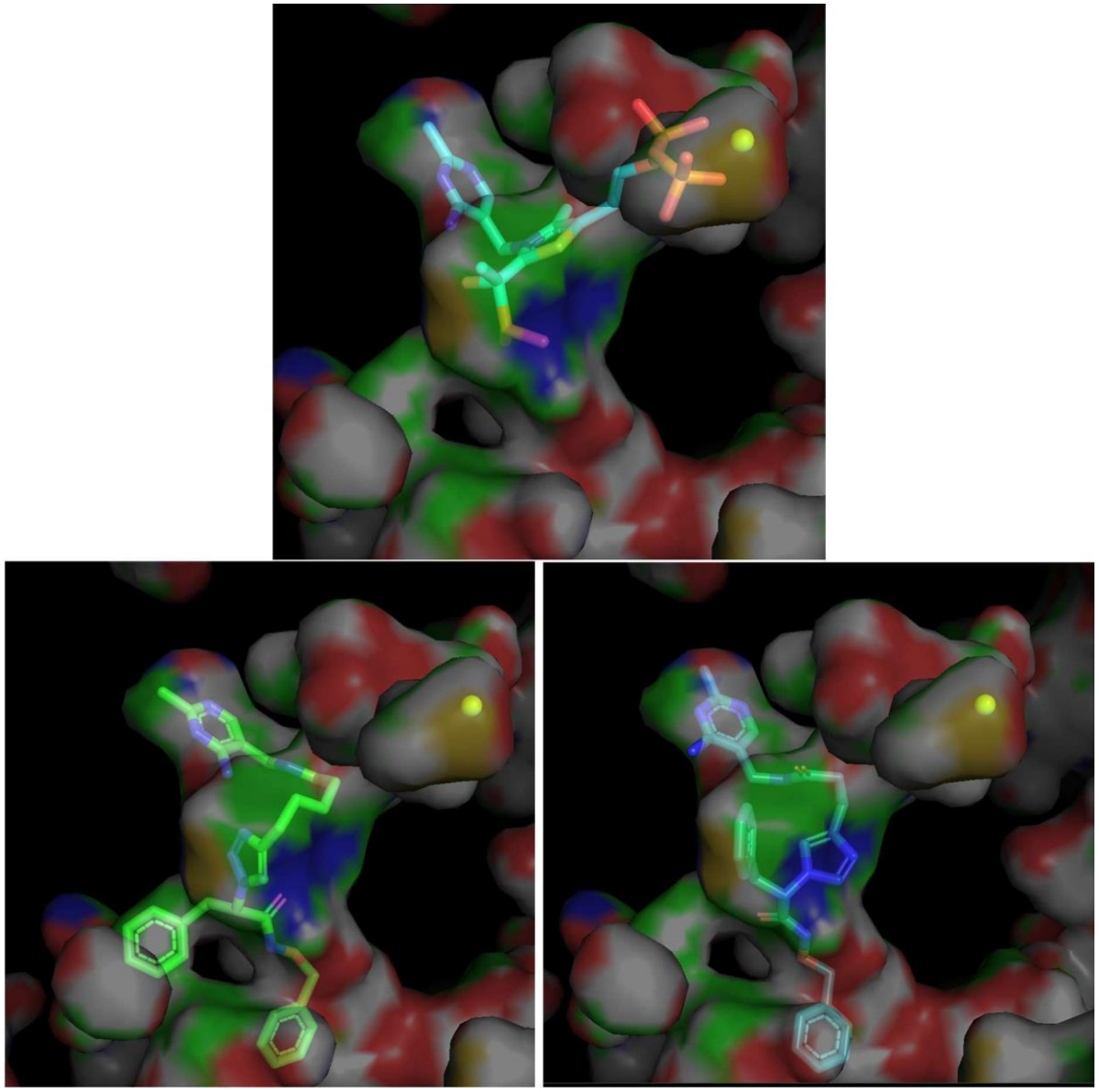


Figure S6. Predicted binding modes of *O*-benzyl hydroxamates 21a and 21c in the TPP pocket of human PDH E1. (Top) The crystal structure of TPP in complex with a pyruvate mimic in human PDH E1 (PDB entry 6CFO). This image helps to define the approximate location and the size of the pyruvate pocket. **(Bottom left)** Docking of **21c** into the active site of human PDH E1. The terminal phenyl group occupies a hydrophobic side-pocket off the entrance tunnel and the benzyloxygroup is in the entrance tunnel. **(Bottom right)** Docking of **21a** into the active site of human PDH E1. The benzyloxygroup is again in the entrance tunnel but the terminal phenyl group does not reach the side-pocket and instead occupies part of the pyruvate pocket. This may explain why **21c** is more potent than **21a**. Goldscores of **21a** and **21c** are 32.8 and 39.8 respectively.

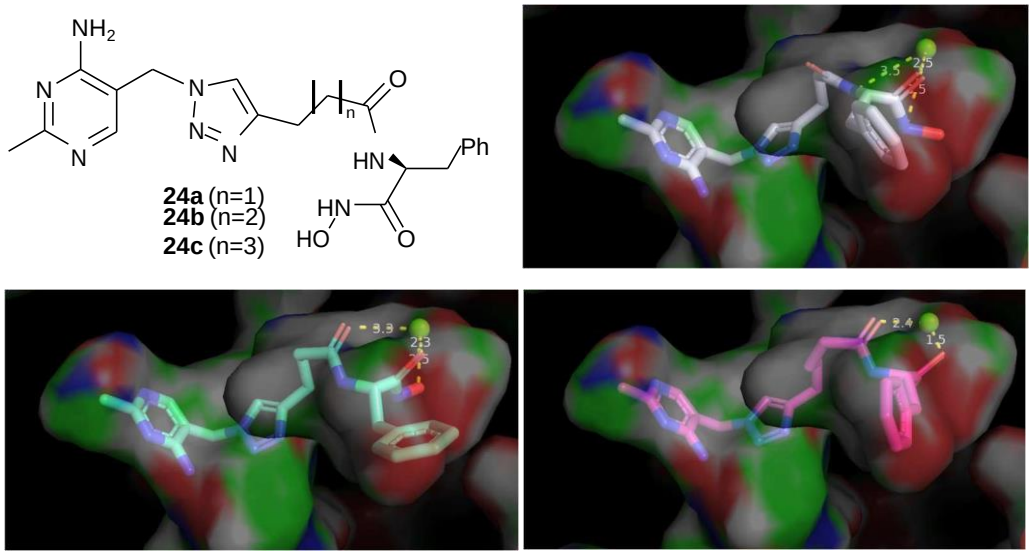


Figure S7. Predicted binding modes of 24a-c in TPP pocket of human PDH E1. 24a (top right), **24b** (bottom left) and **24c** (bottom right). [We thank one of the referees for suggesting we should dock these compounds.] All show the terminal amide and hydroxamate interacting with the Mg^{2+} while the terminal phenyl group extends deeper into the pyrophosphate pocket. Goldscores of **24a**, **24b** and **24c** are 60.2, 76.3 and 65.7 respectively. These scores suggest that **24a-c** would be well worth synthesising and testing but time did not allow this in the current project.

Calculation of cavities in human PDH E1

The calculation was perform by program Caver Analyser 1.0, using the Caver 3.0 algorithm, on PBD entry 6CFO. The minimum and maximum probe diameters were 1.5 and 3.0 Å. The two active sites (containing the two adducts of TPP with acetyl phosphinate) were the two largest cavities found and are shown in Figure S8.

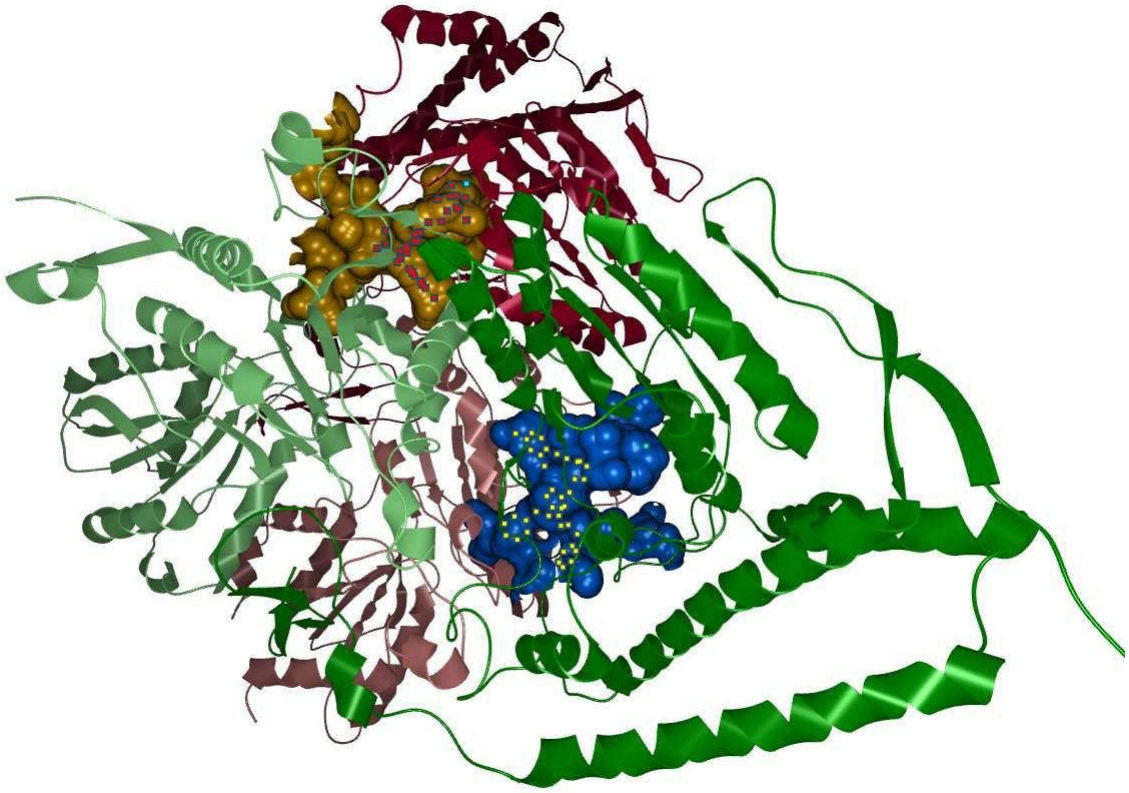


Figure S8. Overall structure of human PDH E1 showing the two active site cavities. The protein is an $\alpha_2\beta_2$ tetramer and each chain, in cartoon view, is coloured differently (β chains have the lighter colours). The active-site cavity in gold contains the adduct between TPP and inhibitor acetyl phosphinate (red dots, part of chain C in PDB entry 6CFO). The blue cavity (which contained the ligand in chain A in 6CFO) contains the docked structure of **21c** (yellow dots). The Mg^{2+} ions are cyan dots.

Computational calculation of molecular properties

Physicochemical properties of all key compounds in this work were calculated using MarvinSketch 21.2.

For compounds considered as drug-like and predicted to be orally bioavailable, the Lipinski's Rule of Five[8] is a well-known guideline since its first publication in 1997. However, since then many studies have further refined the guidelines and provided new recommendations[9] for drug design:

- Molecular weight (MW): ≤ 400
- Log P: ≤ 3-4
- HB donors (HBDs, i.e., no. of N-H and O-H bonds): ≤ 5
- HB acceptors (HBAs, i.e., no. of N and O atoms): ≤ 10

Ligand efficiency (LE) calculation: Binding free energy ΔG (in kcal per mole) / ligand's non-hydrogen (heavy) atoms. Preferred LE: > 0.3.[10]

Synthetic Procedures

General synthesis methods

Oxygen- and moisture-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Unless otherwise stated, all chemicals and reagents were purchased from commercial suppliers and used without further purification.

Reaction progress was monitored by analytical thin-layer chromatography (TLC). TLC was conducted using Merck glass plates with silica Kieselgel 60 F254 of thickness 0.25 mm and visualised under 254 nm UV lamp or potassium permanganate staining solution (with gentle heating).

Flash column chromatography was carried out in the indicated solvent system using prepacked silica gel cartridges for use on the Biotage Purification System.

All solvents were removed under reduced pressure using a Büchi rotary evaporator with dry ice traps.

Reverse-phase HPLC: 0-80% MeOH in water (with 0.1% formic acid).

All yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Compounds were characterised by, at minimum, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy and HRMS, unless otherwise stated.

Melting points of compounds were measured using a Reichert machine and are uncorrected.

^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$ or CD_3OD solution on a Bruker 400 MHz or 700 MHz spectrometer and chemical shifts were recorded in parts per million (ppm). ^{13}C NMR spectra were recorded on a Bruker 400 MHz or 700 MHz spectrometer. Resonances are described using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), qnt (quintet), sext (sextet), m (multiplet), br (broad), dd (doublet of doublets), *etc*. Coupling constants (*J*) are given in Hz and are rounded to the nearest 0.1 Hz. All NMR data were collected at 25 °C.

Mass spectra used electrospray ionisation (ESI).

Optical Rotations were measured on a Perkin Elmer Model 343 Polarimeter using a sodium lamp (λ = 589 nm, D-line). $[\alpha]_D$ values are reported at the stated temperature, with concentration in g/100 mL.

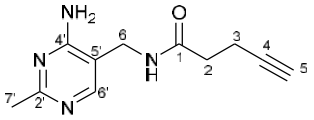
The synthesis and characterisation data for **8**[2], **9**[2], **16**[11] and **19**[12] have been described previously.

Experimental procedures – Synthesis

General procedure for preparation of 11a-c:

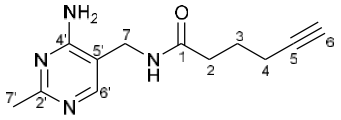
To a stirred solution of the alkynoic acid **10a-c** (1.3 equiv.) and DCC (3 equiv.) in dry DMF (15 mL, 0.2 M) under nitrogen at 0 °C was added DMAP (1.3 equiv.) and amine **9** (3 mmol, 1 equiv.). The resultant mixture was stirred at 40 °C for 2 days, diluted with DCM, filtered through cotton wool (to remove DCC/DCU), treated with aqueous phosphate buffer (pH 7), and extracted with DCM. The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography (10% MeOH in DCM) to yield amide **11a-c** as a solid.

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]pent-4-ynamide **11a**



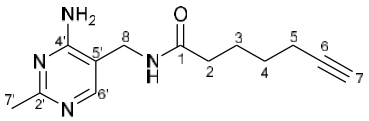
Prepared from 4-pentynoic acid. White solid (405 mg, 62%). **m.p.** 111-114 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.95 (s, 1H, H-6'), 4.24 (s, 2H, H-6), 2.42-2.52 (m, 4H, H-2 and H-3), 2.40 (s, 3H, H-7'), 2.28 (t, 1H, *J* = 2.5 Hz, H-5). **^{13}C NMR** (100 MHz, CD_3OD) δ 173.4 (C-1), 166.2 (C-2'), 162.1 (C-4'), 153.7 (C-6'), 111.3 (C-5'), 81.9 (C-4), 69.1 (C-5), 36.5 (C-2), 34.5 (C-6), 23.4 (C-7'), 14.2 (C-3). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{11}H_{14}N_4O$: 219.1240; found: 219.1255.

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]hex-5-ynamide **11b**



Prepared from 5-hexynoic acid. White solid (473 mg, 68%). **m.p.** 113-116 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.92 (s, 1H, H-6'), 4.23 (s, 2H, H-7), 2.40 (s, 3H, H-7'), 2.37 (t, 2H, *J* = 7.3 Hz, H-2), 2.17-2.26 (m, 3H, H-4 and H-6), 1.82 (qnt, 2H, *J* = 7.3 Hz, H-3). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.7 (C-1), 166.3 (C-2'), 162.1 (C-4'), 153.8 (C-6'), 111.4 (C-5'), 82.5 (C-5), 68.9 (C-6), 36.5 (C-7), 34.2 (C-2), 24.3 (C-3), 23.4 (C-7'), 17.1 (C-4). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{12}H_{16}N_4O$: 233.1397; found: 233.1400.

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]hept-6-ynamide **11c**

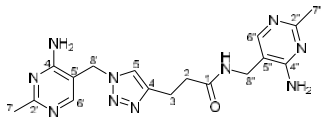


Prepared from 6-heptynoic acid. White solid (524 mg, 71%). **m.p.** 115-117 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.92 (s, 1H, H-6'), 4.23 (s, 2H, H-8), 2.40 (s, 3H, H-7'), 2.26 (t, 2H, *J* = 7.4 Hz, H-2), 2.18-2.22 (m, 3H, H-5 and H-7), 1.71-1.76 (m, 2H, H-3), 1.51-1.55 (m, 2H, H-4). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.2 (C-1), 166.3 (C-2'), 162.1 (C-4'), 153.8 (C-6'), 111.4 (C-5'), 83.1 (C-6), 68.3 (C-7), 36.5 (C-8), 34.9 (C-2), 27.6 (C-4), 24.5 (C-3), 23.4 (C-7'), 17.3 (C-5). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{13}H_{18}N_4O$: 247.1553; found: 247.1566.

General procedure for preparation of 12a-c:

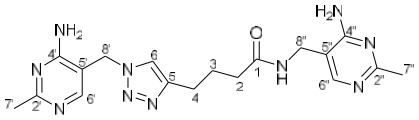
To a stirred solution of alkyne **11a-c** (0.2 mmol, 1 equiv.) and azide **8** (1 equiv.) in *t*-BuOH and water (2:1, 0.2 M) was added $CuSO_4 \cdot 5H_2O$ (0.03 equiv.) and sodium ascorbate (0.3 equiv.). The resultant mixture was stirred at 40 °C for 2 days. The reaction mixture was concentrated under reduced pressure, diluted in EtOAc, washed with 1 M K_2CO_3, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography (10-30% MeOH in DCM) to yield bis-pyrimidine **12a-c** as a solid.

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-3-{1-[(4-amino-2-methylpyrimidin-5-yl)methyl]-1H-1,2,3-triazol-4-yl}propanamide **12a**



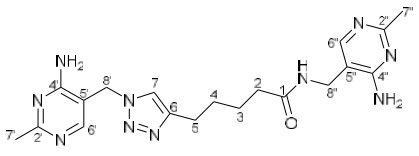
Prepared from **11a**. White solid (19 mg, 25%). **m.p.** 165-167 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 8.01 (s, 1H, H-6'), 7.87 (s, 1H, H-6"), 7.73 (s, 1H, H-5), 5.44 (s, 2H, H-8'), 4.18 (s, 2H, H-8"), 3.00 (t, 2H, *J* = 7.4 Hz, H-3), 2.59 (t, 2H, *J* = 7.4 Hz, H-2), 2.42 (s, 3H, H-7'), 2.40 (s, 3H, H-7"). **^{13}C NMR** (100 MHz, CD_3OD) δ 173.8 (C-1), 167.5 (C-2'), 166.3 (C-2"), 162.0 and 162.1 (C-4' and C-4"), 154.9 (C-6'), 153.8 (C-6"), 146.6 (C-4), 122.1 (C-5), 111.3 (C-5"), 108.5 (C-5'), 47.2 (C-8'), 36.5 (C-8"), 34.7 (C-2), 23.6 (C-7'), 23.4 (C-7"), 20.9 (C-3). **HRMS** (ESI) *m/z*: $[M+H^+]$ calculated for $C_{17}H_{22}N_{10}O$: 383.2051; found: 383.2065.

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-4-{1-[(4-amino-2-methylpyrimidin-5-yl)methyl]-1H-1,2,3-triazol-4-yl}butanamide **12b**



Prepared from **11b**. White solid (21 mg, 27%). **m.p.** 175-176 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 8.03 (s, 1H, H-6'), 7.91 (s, 1H, H-6"), 7.78 (s, 1H, H-6), 5.46 (s, 2H, H-8'), 4.20 (s, 2H, H-8"), 2.71 (t, 2H, *J* = 7.6 Hz, H-4), 2.41 (s, 3H, H-7'), 2.39 (s, 3H, H-7"), 2.28 (t, 2H, *J* = 7.2 Hz, H-2), 1.96 (qnt, 2H, *J* = 7.4 Hz, H-3). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.8 (C-1), 167.5 (C-2'), 166.3 (C-2"), 162.1 (C-4'), 162.0 (C-4"), 155.0 (C-6'), 153.8 (C-6"), 147.4 (C-5), 122.0 (C-6), 111.4 (C-5"), 108.5 (C-5'), 47.2 (C-8'), 36.5 (C-8"), 34.7 (C-2), 25.1 (C-3), 24.2 (C-4), 23.6 (C-7'), 23.4 (C-7"). **HRMS** (ESI) *m/z*: $[M+H^+]$ calculated for $C_{18}H_{24}N_{10}O$: 397.2207; found: 397.2219.

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-{1-[(4-amino-2-methylpyrimidin-5-yl)methyl]-1H-1,2,3-triazol-4-yl}pentanamide **12c**

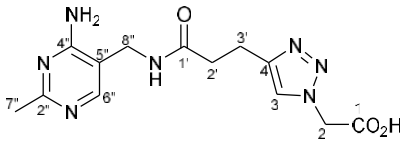


Prepared from **11c**. White solid (25 mg, 30%). **m.p.** 179-180 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 8.03 (s, 1H, H-6'), 7.91 (s, 1H, H-6"), 7.76 (s, 1H, H-7), 5.46 (s, 2H, H-8'), 4.21 (s, 2H, H-8"), 2.68-2.72 (m, 2H, H-5), 2.42 (s, 3H, H-7'), 2.39 (s, 3H, H-7"), 2.23-2.28 (m, 2H, H-2), 1.62-1.70 (m, 4H, H-3 and H-4). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.2 (C-1), 167.5 (C-2'), 166.3 (C-2"), 162.1 (C-4'), 162.0 (C-4"), 154.9 (C-6'), 153.8 (C-6"), 147.9 (C-6), 121.8 (C-7), 111.4 (C-5"), 108.5 (C-5'), 47.2 (C-8'), 36.5 (C-8"), 35.1 (C-2), 28.4 (C-3), 24.8 (C-5), 24.4 (C-4), 23.7 (C-7'), 23.6 (C-7"). HRMS (ESI) *m/z*: $[M+H^+]$ calculated for $C_{19}H_{26}N_{10}O$: 411.2364; found: 411.2359.

General procedure for preparation of 14a-c:

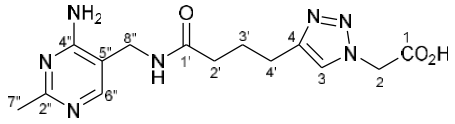
To a stirred solution of alkyne **11a-c** (0.2 mmol, 1 equiv.) and 2-azidoacetic acid **13** (1 equiv.) in DMF (0.2 M) was added $CuSO_4 \cdot 5H_2O$ (0.03 equiv.) and sodium ascorbate (0.3 equiv.). The resultant mixture was stirred at 40 °C for 8 h, diluted in sat. aq. $NaHCO_3$, and then washed with EtOAc. The aqueous phase was evaporated under reduced pressure, dissolved in a minimal amount of water, and purified by reverse-phase HPLC to yield carboxylic acid **14a-c** as a solid.

2-[4-(2-{[(4-Amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}ethyl)-1H-1,2,3-triazol-1-yl]acetic acid **14a**



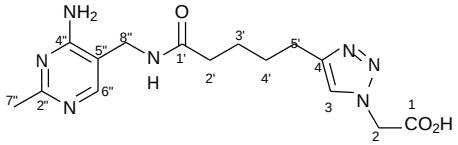
Prepared from **11a**. White solid (10 mg, 15%). **m.p.** 212-215 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.92 (s, 1H, H-6”), 7.74 (s, 1H, H-3), 5.18 (s, 2H, H-2), 4.21 (s, 2H, H-8”), 2.95 (t, 2H, *J* = 7.5 Hz, H-3’), 2.55 (t, 2H, *J* = 7.5 Hz, H-2’), 2.37 (s, 3H, H-7”). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.1 (C-1’), 170.2 (C-1), 166.7 (C-2”), 162.3 (C-4”), 153.5 (C-6”), 145.6 (C-4), 122.1 (C-3), 111.1 (C-5”), 55.0 (C-2), 36.6 (C-8”), 34.8 (C-2’), 23.5 (C-7”), 21.1 (C-3’). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{13}H_{17}N_7O_3$: 320.1466; found: 320.1477.

2-[4-(3-{[(4-Amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}propyl)-1H-1,2,3-triazol-1-yl]acetic acid **14b**



Prepared from **11b**. White solid (8 mg, 11%). **m.p.** 201-203 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.94 (s, 1H, H-6”), 7.71 (s, 1H, H-3), 5.17 (s, 2H, H-2), 4.22 (s, 2H, H-8”), 2.63-2.68 (m, 2H, H-4’), 2.39 (s, 3H, H-7”), 2.20-2.25 (m, 2H, H-2’), 1.91-1.96 (m, 2H, H-3’). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.7 (C-1’), 170.5 (C-1), 166.6 (C-2”), 162.4 (C-4”), 154.1 (C-6”), 145.9 (C-4), 122.0 (C-3), 111.4 (C-5”), 54.4 (C-2), 36.3 (C-8”), 34.3 (C-2’), 25.2 (C-3’), 24.2 (C-4’), 23.9 (C-7”). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{14}H_{19}N_7O_3$: 334.1622; found: 334.1635.

2-[4-(4-{[(4-Amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}butyl)-1H-1,2,3-triazol-1-yl]acetic acid **14c**

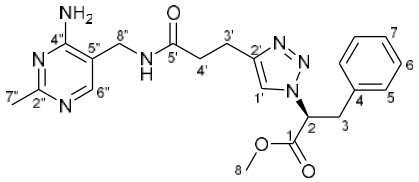


Prepared from **11c**. White solid (14 mg, 19%). **m.p.** 213-216 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.94 (s, 1H, H-6”), 7.72 (s, 1H, H-3), 5.16 (s, 2H, H-2), 4.25 (s, 2H, H-8”), 2.63-2.67 (m, 2H, H-5’), 2.38 (s, 3H, H-7”), 2.22-2.26 (m, 2H, H-2’), 1.60-1.65 (m, 4H, H-3’ and H-4’). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.9 (C-1’), 170.0 (C-1), 166.1 (C-2”), 162.5 (C-4”), 153.8 (C-6”), 146.2 (C-4), 122.3 (C-3), 111.3 (C-5”), 54.8 (C-2), 36.7 (C-8”), 35.3 (C-2’), 28.2 (C-3’), 24.4 and 24.7 (C-4’ and C-5’), 23.6 (C-7”). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{15}H_{21}N_7O_3$: 348.1779; found: 348.1785.

General procedure for preparation of 17a-c:

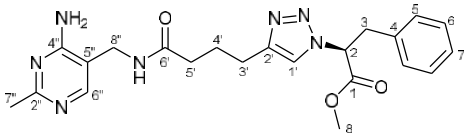
To a stirred solution of alkyne **11a-c** (0.3 mmol, 1 equiv.) and azide **16** (1.5 equiv.) in *t*-BuOH and water (2:1, 0.2 M) was added $CuSO_4 \cdot 5H_2O$ (0.03 equiv.) and sodium ascorbate (0.3 equiv.). The resultant mixture was stirred at 40 °C for 2 days. The reaction mixture was concentrated under reduced pressure, diluted in DCM, washed with aqueous phosphate buffer (pH 7), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography (10% MeOH in DCM) to yield ester **17a-c** as a solid.

Methyl (2S)-2-[4-(2-{[(4-amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}ethyl)-1H-1,2,3-triazol-1-yl]-3-phenylpropanoate **17a**



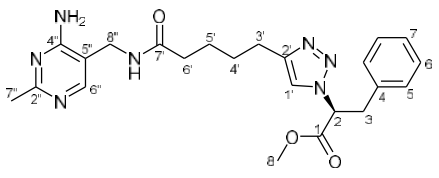
Prepared from **11a**. White solid (61 mg, 48%). **m.p.** 185-186 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.90 (s, 1H, H-6”), 7.75 (s, 1H, H-1’), 7.06-7.19 (m, 5H, H-5, H-6 and H-7), 5.65 (dd, 1H, *J* = 5.4 and 10.4 Hz, H-2), 4.20 (s, 2H, H-8”), 3.77 (s, 3H, H-8), 3.59 (dd, 1H, *J* = 5.4 and 14.2 Hz, H-3a), 3.43 (dd, 1H, *J* = 10.4 and 14.2 Hz, H-3b), 2.98 (t, 2H, *J* = 7.5 Hz, H-3’), 2.55 (t, 2H, *J* = 7.5 Hz, H-4’), 2.36 (s, 3H, H-7”). **^{13}C NMR** (100 MHz, CD_3OD) δ 173.8 (C-5’), 168.7 (C-1), 166.3 (C-2”), 162.1 (C-4”), 153.9 (C-6”), 145.9 (C-2’), 135.4 (C-4), 128.5, 128.1 and 126.8 (C-5, C-6 and C-7), 122.3 (C-1’), 111.4 (C-5”), 63.9 (C-2), 52.1 (C-8), 37.5 (C-3), 36.6 (C-8”), 34.7 (C-4’), 23.4 (C-7”), 20.8 (C-3’). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{21}H_{25}N_7O_3$: 424.2092; found: 424.2089. **$[\alpha]_D^{25}$** = -64.2° (c = 0.4, MeOH).

Methyl (2S)-2-[4-(3-{[(4-amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}propyl)-1H-1,2,3-triazol-1-yl]-3-phenylpropanoate **17b**



Prepared from **11b**. White solid (76 mg, 58%). **m.p.** 181-183 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.93 (s, 1H, H-6”), 7.76 (s, 1H, H-1’), 7.06-7.21 (m, 5H, H-5, H-6 and H-7), 5.67 (dd, 1H, *J* = 5.3 and 10.5 Hz, H-2), 4.23 (s, 2H, H-8”), 3.77 (s, 3H, H-8), 3.61 (dd, 1H, *J* = 5.3 and 14.1 Hz, H-3a), 3.46 (dd, 1H, *J* = 10.5 and 14.1 Hz, H-3b), 2.67 (t, 2H, *J* = 7.5 Hz, H-3’), 2.40 (s, 3H, H-7”), 2.22 (t, 2H, *J* = 7.5 Hz, H-5’), 1.91 (qnt, 2H, *J* = 7.5 Hz, H-4’). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.8 (C-6’), 168.7 (C-1), 166.3 (C-2”), 162.1 (C-4”), 153.9 (C-6”), 146.6 (C-2’), 135.5 (C-4), 128.6, 128.2, 126.8 (C-5, C-6 and C-7), 122.4 (C-1’), 111.5 (C-5”), 63.9 (C-2), 52.1 (C-8), 37.4 (C-3), 36.5 (C-8”), 34.5 (C-5’), 25.1 (C-4’), 24.1 (C-3’), 23.4 (C-7”). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{22}H_{27}N_7O_3$: 438.2248; found: 438.2258. **$[\alpha]_D^{25}$** = -55.4° (c = 0.35, MeOH).

Methyl (2S)-2-[4-(4-{[(4-amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}butyl)-1H-1,2,3-triazol-1-yl]-3-phenylpropanoate **17c**

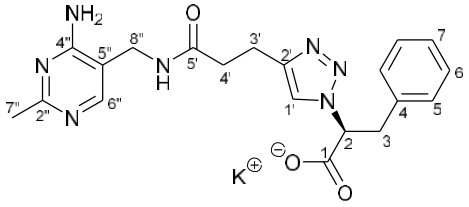


Prepared from **11c**. White solid (75 mg, 56%). **m.p.** 193-195 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.93 (s, 1H, H-6”), 7.74 (s, 1H, H-1’), 7.06-7.20 (m, 5H, H-5, H-6 and H-7), 5.67 (dd, 1H, *J* = 5.2 and 10.5 Hz, H-2), 4.23 (s, 2H, H-8”), 3.78 (s, 3H, H-8), 3.61 (dd, 1H, *J* = 5.2 and 14.2 Hz, H-3a), 3.47 (dd, 1H, *J* = 10.5 and 14.2 Hz, H-3b), 2.66 (t, 2H, *J* = 7.0 Hz, H-3’), 2.39 (s, 3H, H-7”), 2.24 (t, 2H, *J* = 7.0 Hz, H-6’), 1.57-1.64 (m, 4H, H-4’ and H-5’). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.2 (C-7’), 168.7 (C-1), 166.3 (C-2”), 162.1 (C-4”), 153.9 (C-6”), 147.1 (C-2’), 135.5 (C-4), 128.6, 128.1 and 126.8 (C-5, C-6 and C-7), 122.2 (C-1’), 111.1 (C-5”), 63.9 (C-2), 52.1 (C-8), 37.4 (C-3), 36.5 (C-8”), 35.1 (C-6’), 28.4 (C-5’), 24.6 (C-3’), 24.3 (C-4’), 23.4 (C-7”). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{23}H_{29}N_7O_3$: 452.2405; found: 452.2417. **$[\alpha]_D^{25}$** = -44.7° (c = 0.2, MeOH).

General procedure for preparation of 18a-c:

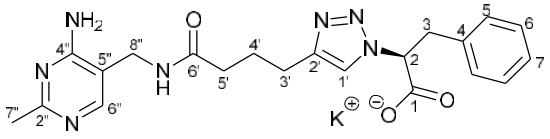
To a stirred solution of ester **17a-c** (0.1 mmol, 1 equiv.) in THF and water (1:1, 0.1 M) was added potassium hydroxide (1 equiv.). The reaction mixture was stirred at r.t. for 1-2 h and then evaporated under reduced pressure to yield carboxylate **18a-c** as a solid.

Potassium (2S)-2-[4-(2-{[(4-amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}ethyl)-1H-1,2,3-triazol-1-yl]-3-phenylpropanoate **18a**



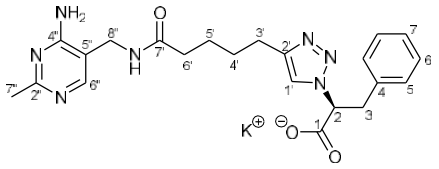
Prepared from **17a**. White solid (44 mg, 98%). **m.p.** 202-204 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.91 (s, 1H, H-6”), 7.76 (s, 1H, H-1’), 7.03-7.19 (m, 5H, H-5, H-6 and H-7), 5.28 (dd, 1H, *J* = 4.6 and 10.6 Hz, H-2), 4.20 (m, 2H, H-8”), 3.57 (dd, 1H, *J* = 4.6 and 14.2 Hz, H-3a), 3.32 (dd, 1H, *J* = 10.6 and 14.2 Hz, H-3b), 2.97 (t, 2H, *J* = 7.4 Hz, H-3’), 2.55 (t, 2H, *J* = 7.4 Hz, H-4’), 2.35 (s, 3H, H-7”). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.0 (C-5’), 173.1 (C-1), 166.3 (C-2”), 162.1 (C-4”), 153.9 (C-6”), 145.3 (C-2’), 137.4 (C-4), 128.4, 127.9, 126.2 (C-5, C-6 and C-7), 121.9 (C-1’), 111.4 (C-5”), 67.8 (C-2), 39.0 (C-3), 36.6 (C-8”), 35.0 (C-4’), 23.4 (C-7”), 21.1 (C-3’). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{20}H_{23}N_7O_3$: 410.1935; found: 410.1944. **$[\alpha]_D^{25}$** = -22.2° (c = 0.5, MeOH).

Potassium (2S)-2-[4-(3-{[(4-amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}propyl)-1H- 1,2,3-triazol-1-yl]-3-phenylpropanoate **18b**



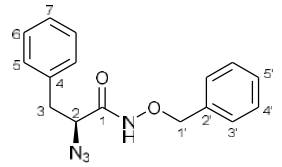
Prepared from **17b**. White solid (44 mg, 95%). **m.p.** 205-207 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.94 (s, 1H, H-6”), 7.72 (s, 1H, H-1’), 7.06-7.21 (m, 5H, H-5, H-6 and H-7), 5.29-5.33 (m, 1H, H-2), 4.23 (s, 2H, H-8”), 3.58-3.62 (m, 1H, H-3a), 3.36-3.39 (m, 1H, H-3b), 2.65-2.67 (m, 2H, H-3’), 2.40 (s, 3H, H-7”), 2.18-2.23 (m, 2H, H-5’), 1.91-1.96 (m, 2H, H-4’). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.9 (C-6’), 173.2 (C-1), 166.3 (C-2”), 162.1 (C-4”), 154.0 (C-6”), 145.8 (C-2’), 137.4 (C-4), 128.4, 127.9, 126.3 (C-5, C-6 and C-7), 122.1 (C-1’), 111.5 (C-5”), 67.7 (C-2), 38.8 (C-3), 36.5 (C-8”), 34.4 (C-5’), 25.1 (C-4’), 24.0 (C-3’), 23.4 (C-7”). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{21}H_{25}N_7O_3$: 424.2092; found: 424.2098. **$[\alpha]_D^{25}$** = -31.4° (c = 0.7, MeOH).

Potassium (2S)-2-[4-(4-{[(4-amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}butyl)-1H-1,2,3-triazol-1-yl]-3-phenylpropanoate **18c**



Prepared from **17c**. White solid (46 mg, 97%). **m.p.** 210-212 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.94 (s, 1H, H-6"), 7.73 (s, 1H, H-1'), 7.06-7.17 (m, 5H, H-5, H-6 and H-7), 5.30 (dd, 1H, *J* = 4.5 and 10.8 Hz, H-2), 4.22 (s, 2H, H-8"), 3.59 (dd, 1H, *J* = 4.5 and 14.3 Hz, H-3a), 3.38 (dd, 1H, *J* = 10.8 and 14.3 Hz, H-3b), 2.66 (t, 2H, *J* = 6.7 Hz, H-3'), 2.38 (s, 3H, H-7"), 2.23 (t, 2H, *J* = 6.9 Hz, H-6'), 1.59-1.66 (m, 4H, H-4' and H-5'). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.3 (C-7'), 173.2 (C-1), 166.3 (C-2"), 162.1 (C-4"), 153.9 (C-6"), 146.4 (C-2'), 137.4 (C-4), 128.4, 127.9, 126.2 (C-5, C-6 and C-7), 121.9 (C-1'), 111.5 (C-5"), 67.7 (C-2), 38.9 (C-3), 36.5 (C-8"), 35.1 (C-6'), 28.3 (C-5'), 24.7 (C-3'), 24.4 (C-4'), 23.4 (C-7"). **HRMS** (ESI) m/z: [M+H$^+$] calculated for $C_{22}H_{27}N_7O_3$: 438.2248; found: 438.2250. **$[\alpha]_D^{25}$** = -26.5° (c = 0.5, MeOH).

(2S)-2-Azido-N-(benzyloxy)-3-phenylpropanamide **20**

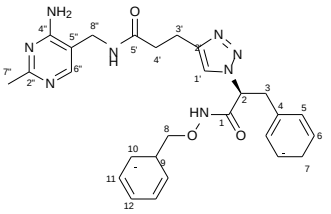


To a stirred solution of carboxylic acid **19** (573 mg, 3 mmol) in dry THF and dry DMF (7:1, 0.1 M) under nitrogen was added CDI (729 mg, 4.5 mmol) slowly. The resultant mixture was stirred at r.t. for 1 h and then treated with $NH_2OBn{\cdot}HCl$ (954 mg, 6 mmol). The reaction mixture was stirred at r.t. overnight, concentrated under reduced pressure, diluted in aqueous phosphate buffer (pH 7), and extracted with EtOAc. The organic phase was washed with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography (30% EtOAc in hexane) to yield *O*-Bn hydroxamate **20** as a colourless oil (639 mg, 72%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.69 (br, 1H, NH), 7.25-7.42 (m, 10H, H-5, H-6, H-7, H-3', H-4' and H-5'), 4.80 (s, 2H, H-1'), 4.04 (dd, 1H, *J* = 6.0 and 7.5 Hz, H-2), 3.25 (dd, 1H, J = 6.0 and 13.8 Hz, H-3a), 3.07 (dd, 1H, *J* = 7.5 and 13.8 Hz, H-3b). ^{13}C NMR (100 MHz, CD_3Cl) δ 166.4 (C-1), 135.7 (C-4), 134.7 (C-2'), 129.5, 129.4, 128.9, 128.7, 128.6, 127.3 (C-5, C-6, C-7, C-3', C-4' and C-5'), 78.4 (C-1'), 62.6 (C-2), 37.8 (C-3). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{16}H_{16}N_4O_2$: 297.1346; found: 297.1362. **$[\alpha]_D^{25}$** = +81.1° (c = 0.1, MeOH).

General procedure for preparation of 21a-c:

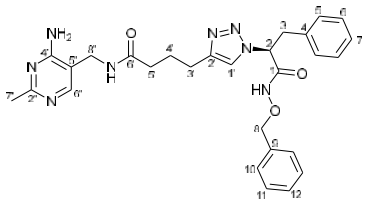
To a stirred solution of alkyne **11a-c** (0.4 mmol, 1 equiv.) and azide **20** (1.2 equiv.) in *t*-BuOH and water (2:1, 0.2 M) was added $CuSO_4{\cdot}5H_2O$ (0.03 equiv.) and sodium ascorbate (0.3 equiv.). The resultant mixture was stirred at 40 °C for 2-3 days. The reaction mixture was concentrated under reduced pressure, diluted in aqueous phosphate buffer (pH 7) (50 mL), and extracted with DCM (50 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography (10% MeOH in DCM) to yield *O*-Bn hydroxamate **21a-c** as a solid.

(2S)-2-[4-(2-{[(4-Amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}ethyl)-1H-1,2,3-triazol-1-yl]-N-(benzyloxy)-3-phenylpropanamide **21a**



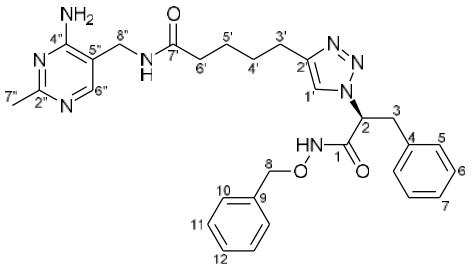
Prepared from **11a**. White solid (102 mg, 50%). **m.p.** 166-168 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.91 (s, 1H, H-1'), 7.88 (s, 1H, H-6"), 7.17-7.33 (m, 10H, H-5, H-6, H-7, H-10, H-11 and H-12), 5.27 (app. t, 1H, *J* = 7.9 Hz, H-2), 4.75 (d, 1H, *J* = 11.0 Hz, H-8a), 4.62 (d, 1H, *J* = 11.0 Hz, H-8b), 4.24 (d, 1H, *J* = 15.0 Hz, H-8"a), 4.19 (d, 1H, *J* = 15.0 Hz, H-8"b), 3.44 (dd, 1H, *J* = 8.5 and 13.5 Hz, H-3a), 3.28 (dd, 1H, *J* = 7.5 and 13.5 Hz, H-3b), 3.02 (t, 2H, *J* = 7.2 Hz, H-3'), 2.60 (t, 2H, *J* = 7.2 Hz, H-4'), 2.31 (s, 3H, H-7"). **^{13}C NMR** (100 MHz, CD_3OD) δ 173.8 (C-5'), 166.3 (C-2"), 164.7 (C-1), 162.1 (C-4"), 153.8 (C-6"), 146.2 (C-2'), 135.1 (C-4), 135.0 (C-9), 129.0, 128.9, 128.4, 128.3, 128.0, 127.0 (C-5, C-6, C-7, C-10, C-11 and C-12), 121.1 (C-1'), 111.4 (C-5"), 77.6 (C-8), 62.4 (C-2), 37.9 (C-3), 36.6 (C-8"), 34.7 (C-4'), 23.4 (C-7"), 20.9 (C-3'). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{27}H_{30}N_8O_3$: 515,2514; found: 515,2522. **$[\alpha]_D^{25}$** = +23.1° (c = 0.4, MeOH).

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-4-{1-[(1S)-1-[(benzyloxy)carbamoyl]-2-phenylethyl]-1H-1,2,3-triazol-4-yl}butanamide **21b**



Prepared from **11b**. White solid (110 mg, 52%). **m.p.** 169-171 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.92 (s, 2H, H-1' and H-6"), 7.18-7.33 (m, 10H, H-5, H-6, H-7, H-10, H-11 and H-12), 5.30 (app. t, 1H, *J* = 8.0 Hz, H-2), 4.76 (d, 1H, *J* = 11.1 Hz, H-8a), 4.63 (d, 1H, *J* = 11.1 Hz, H-8b), 4.23 (s, 2H, H-8"), 3.47 (dd, 1H, *J* = 8.2 and 13.6 Hz, H-3a), 3.33-3.38 (m, 1H, H-3b), 2.71 (t, 2H, *J* = 7.0 Hz, H-3'), 2.38 (s, 3H, H-7"), 2.26 (t, 2H, *J* = 7.0 Hz, H-5'), 1.96 (qnt, 2H, *J* = 7.0 Hz, H-4'). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.8 (C-6'), 166.3 (C-2"), 164.8 (C-1), 162.1 (C-4"), 153.8 (C-6"), 146.9 (C-2'), 135.1 (C-4), 135.0 (C-9), 129.0, 128.9, 128.4, 128.4, 128.0, 127.0 (C-5, C-6, C-7, C-10, C-11 and C-12), 121.2 (C-1'), 111.5 (C-5"), 77.6 (C-8), 62.4 (C-2), 37.8 (C-3), 36.5 (C-8"), 34.6 (C-5'), 25.1 (C-4'), 24.2 (C-3') 23.4 (C-7"). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{28}H_{32}N_8O_3$: 529.2670; found: 529.2679. **$[\alpha]_D^{25}$** = +18.0° (c = 0.6, MeOH).

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-{1-[(1S)-1-[(benzyloxy)carbamoyl]-2-phenylethyl]-1H-1,2,3-triazol-4-yl}pentanamide **21c**

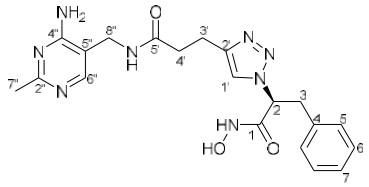


Prepared from **11c**. White solid (98 mg, 45%). **m.p.** 175-178 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.92 (s, 2H, H-1' and H-6"), 7.18-7.32 (m, 10H, H-5, H-6, H-7, H-10, H-11 and H-12), 5.29 (app. t, 1H, *J* = 8.1 Hz, H-2), 4.77 (d, 1H, *J* = 11.2 Hz, H-8a), 4.65 (d, 1H, *J* = 11.2 Hz, H-8b), 4.23 (s, 2H, H-8"), 3.48 (dd, 1H, *J* = 8.1 and 13.7 Hz, H-3a), 3.35-3.39 (m, 1H, H-3b), 2.70 (t, 2H, *J* = 6.6 Hz, H-3'), 2.38 (s, 3H, H-7"), 2.24-2.28 (m, 2H, H-6'), 1.63-1.67 (m, 4H, H-4'and H-5'). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.2 (C-7'), 166.3 (C-2"), 164.8 (C-1), 162.1 (C-4"), 153.8 (C-6"), 147.3 (C-2'), 135.3 (C-4), 135.2 (C-9), 129.0, 128.9, 128.3, 128.2, 128.0, 126.9 (C-5, C-6, C-7, C-10, C-11 and C-12), 121.0 (C-1'), 111.5 (C-5"), 77.4 (C-8), 62.4 (C-2), 37.8 (C-3), 36.5 (C-8"), 35.1 (C-6'), 28.4 (C-5'), 24.7 (C-3'), 24.5 (C-4'), 23.4 (C-7"). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{29}H_{34}N_8O_3$: 543.2827; found: 543.2855. **$[\alpha]_D^{25}$** = +37.9° (c = 0.5, MeOH).

General procedure for preparation of 22a-c:

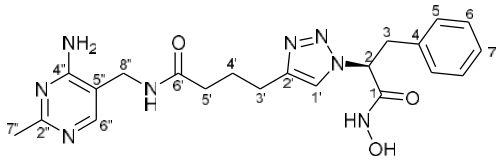
To a stirred solution of *O*-Bn hydroxamate **21a-c** (0.1 mmol, 1 equiv.) in dry DCM (0.1 M) under nitrogen at -78 °C was added BCl_3 (1 M in DCM) (5 equiv.) dropwise. The reaction mixture was stirred at r.t. overnight and the resultant white suspension concentrated under reduced pressure. The residue was purified by silica flash chromatography (15% MeOH in DCM) to yield hydroxamate **22a-c** as a solid.

(2S)-2-[4-(2-{[(4-Amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}ethyl)-1H-1,2,3-triazol-1-yl]-N-hydroxy-3-phenylpropanamide **22a**



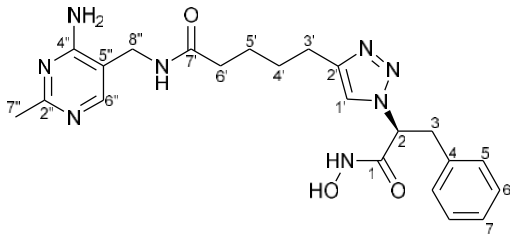
Prepared from **21a**. White solid (21 mg, 50%). **m.p.** 206-207 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.99 (s, 1H, H-1'), 7.88 (s, 1H, H-6"), 7.18-7.28 (m, 5H, H-5, H-6 and H-7), 5.35 (app. t, 1H, *J* = 7.7 Hz, H-2), 4.27 (d, 1H, *J* = 15.4 Hz, H-8"a), 4.17 (d, 1H, *J* = 15.4 Hz, H-8"b), 3.50 (dd, 1H, *J* = 7.9 and 13.6 Hz, H-3a), 3.38 (m, 1H, H-3b), 3.02 (t, 2H, *J* = 6.7 Hz, H-3'), 2.64 (t, 2H, *J* = 6.7 Hz, H-4'), 2.44 (s, 3H, H-7"). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.1 (C-5'), 167.4 (C-2"), 165.2 (C-1), 163.0 (C-4"), 146.3 (C-6"), 146.1 (C-2'), 135.2 (C-4), 128.7 (C-5), 128.3 (C-6), 126.9 (C-7), 121.3 (C-1'), 112.2 (C-5"), 62.5 (C-2), 37.8 (C-3), 36.1 (C-8"), 34.5 (C-4'), 21.4 (C-7"), 20.7 (C-3'). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{20}H_{24}N_8O_3$: 425.2044; found: 425.2050. **$[\alpha]_D^{25}$** = +53.8° (c = 0.1, MeOH).

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-4-{1-[(1S)-1-(hydroxycarbamoyl)-2-phenylethyl]-1H-1,2,3-triazol-4-yl}butanamide **22b**



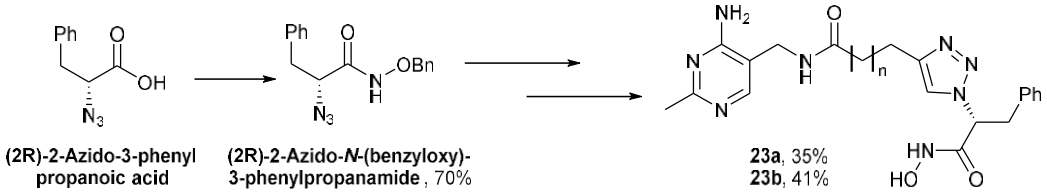
Prepared from **21b**. White solid (24 mg, 55%). **m.p.** 210-214 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 8.15 (s, 1H, H-6"), 8.04 (s, 1H, H-1'), 7.19-7.29 (m, 5H, H-5, H-6 and H-7), 5.43 (app. t, 1H, *J* = 8.2 Hz, H-2), 4.28 (s, 2H, H-8"), 3.54 (dd, 1H, *J* = 7.4 and 13.6 Hz, H-3a), 3.43 (dd, 1H, *J* = 8.6 and 13.6 Hz, H-3b), 2.76 (t, 2H, *J* = 7.3 Hz, H-3'), 2.56 (s, 3H, H-7"), 2.32 (t, 2H, *J* = 7.3 Hz, H-5'), 1.99 (qnt, 2H, *J* = 7.3 Hz, H-4'). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.2 (C-6'), 164.7 (C-2"), 164.0 (C-1), 161.1 (C-4"), 146.0 (C-2'), 141.2 (C-6"), 135.0 (C-4), 128.7 (C-5), 128.3 (C-6), 126.9 (C-7), 122.2 (C-1'), 112.7 (C-5"), 62.9 (C-2), 37.7 (C-3), 36.0 (C-8"), 34.3 (C-5'), 24.7 (C-4'), 23.8 (C-3'), 20.1 (C-7"). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{21}H_{26}N_8O_3$: 439.2201; found: 439.2220. **$[\alpha]_D^{25}$** = +56.5° (c = 0.2, MeOH).

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-{1-[(1S)-1-(hydroxycarbamoyl)-2-phenylethyl]-1H-1,2,3-triazol-4-yl}pentanamide **22c**

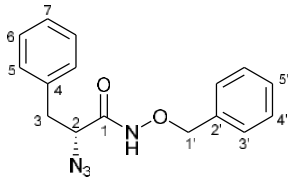


Prepared from **18c**. White solid (23 mg, 51%). **m.p.** 218-220 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.97 (s, 1H, H-1'), 7.93 (s, 1H, H-6"), 7.17-7.27 (m, 5H, H-5, H-6 and H-7), 5.35 (app. t, 1H, *J* = 7.9 Hz, H-2), 4.26 (d, 1H, *J* = 15.4 Hz, H-8"a), 4.21 (d, 1H, *J* = 15.4 Hz, H-8"b), 3.51 (dd, 1H, *J* = 7.6 and 13.7 Hz, H-3a), 3.39 (dd, 1H, *J* = 8.3 and 13.7 Hz, H-3b), 2.70 (t, 2H, *J* = 6.6 Hz H-3'), 2.39 (s, 3H, H-7"), 2.26 (t, 2H, *J* = 6.6 Hz, H-6'), 1.60-1.69 (m, 4H, H-4'and H-5'). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.3 (C-7'), 166.4 (C-2"), 165.9 (C-1), 162.2 (C-4"), 153.1 (C-6"), 147.3 (C-2'), 135.2 (C-4), 128.7 (C-5), 128.2 (C-6), 126.8 (C-7), 121.0 (C-1'), 111.6 (C-5"), 62.5 (C-2), 37.9 (C-3), 36.4 (C-8"), 35.1 (C-6'), 28.4 (C-5'), 24.8 (C-3'), 24.3 (C-4'), 23.2 (C-7"). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{22}H_{28}N_8O_3$: 453.2357; found: 453.2354. **$[\alpha]_D^{25}$** = +57.6° (c = 0.3, MeOH).

Preparation of hydroxamates 23a and 23b (enantiomers of 22a and 22b):



(2R)-2-Azido-N-(benzyloxy)-3-phenylpropanamide

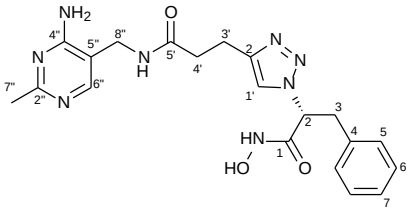


Prepared from (2R)-2-azido-3-phenylpropanoic acid[13] (1 mmol) by the same method as for synthesising **19**. Colourless oil (207 mg, 70%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.63 (br, 1H, NH), 7.24-7.42 (m, 10H, H-5, H-6, H-7, H-3', H-4' and H-5'), 4.80 (s, 2H, H-1'), 4.03 (dd, 1H, *J* = 6.1 and 7.7 Hz, H-2), 3.27 (dd, 1H, J = 6.1 and 13.8 Hz, H-3a), 3.08 (dd, 1H, *J* = 7.7 and 13.8 Hz, H-3b). **^{13}C NMR** (100 MHz, CD_3Cl) δ 166.6 (C-1), 135.8 (C-4), 134.6 (C-2'), 129.6, 129.4, 128.9, 128.7, 128.6, 127.2 (C-5, C-6, C-7, C-3', C-4' and C-5'), 78.7 (C-1'), 62.6 (C-2), 38.0 (C-3). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{16}H_{16}N_4O_2$: 297.1346; found: 297.1353. **$[\alpha]_D^{25}$** = -82.8° (c = 0.2, MeOH).

General procedure for preparation of 23a-b:

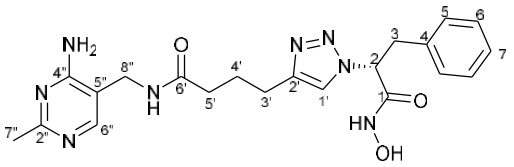
To a stirred solution of (2R)-2-azido-*N*-(benzyloxy)-3-phenylpropanamide (90 mg, 0.3 mmol) and alkyne **11a** (or **11b**) (0.3 mmol) in *t*-BuOH (1 mL) and water (0.5 mL, 0.2 M) was added $CuSO_4 \cdot 5H_2O$ (1.4 mg, 0.009 mmol) and sodium ascorbate (18 mg, 0.09 mmol). The resultant mixture was stirred at 40 °C for 3 days, concentrated under reduced pressure, diluted in aqueous phosphate buffer (pH 7) (10 mL), and extracted with DCM (3 x 30 mL). The combined organic phases were with dried over $MgSO_4$, filtered, and evaporated under reduced pressure to yield the ***O*-Bn hydroxamate** as a solid mixture, which was used in the next step without further purification. To a stirred solution of the resultant crude product in dry DCM (3 mL, 0.1 M) under nitrogen at -78 °C was added BCl_3 (1 M in DCM) (1.5 mL, 1.5 mmol.) dropwise. The reaction mixture was stirred at r.t. overnight and the resultant white suspension concentrated under reduced pressure. The residue was purified by silica flash chromatography (15% MeOH in DCM) to yield hydroxamate **23a** (or **23b**) as a solid.

(2R)-2-[4-(2-{[(4-Amino-2-methylpyrimidin-5-yl)methyl]carbamoyl}ethyl)-1H-1,2,3-triazol-1-yl]-N-hydroxy-3-phenylpropanamide **23a**



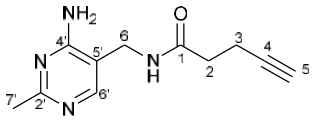
Prepared from **11a**. White solid (45 mg, 35% yield over two steps). **m.p.** 205-206 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 8.00 (s, 1H, H-1'), 7.87 (s, 1H, H-6"), 7.19-7.28 (m, 5H, H-5, H-6 and H-7), 5.36 (t, 1H, *J* = 7.8 Hz, H-2), 4.26 (d, 1H, *J* = 15.5 Hz, H-8"a), 4.17 (d, 1H, *J* = 15.5 Hz, H-8"b), 3.50 (dd, 1H, *J* = 7.8 and 13.6 Hz, H-3a), 3.34 (m, 1H, H-3b), 3.03 (t, 2H, *J* = 6.6 Hz, H-3'), 2.63 (t, 2H, *J* = 6.6 Hz, H-4'), 2.44 (s, 3H, H-7"). **^{13}C NMR** (100 MHz, CD_3OD) δ 174.3 (C-5'), 167.9 (C-2"), 165.1 (C-1), 163.0 (C-4"), 146.5 (C-6"), 146.0 (C-2'), 135.1 (C-4), 128.7 (C-5), 128.2 (C-6), 126.9 (C-7), 121.2 (C-1'), 112.3 (C-5"), 62.3 (C-2), 37.6 (C-3), 35.9 (C-8"), 34.4 (C-4'), 21.5 (C-7"), 20.6 (C-3'). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{20}H_{24}N_8O_3$: 425.2044; found: 425.2042. **$[\alpha]_D^{25}$** = -52.9° (c = 0.1, MeOH).

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-4-{1-[(1R)-1-(hydroxycarbamoyl)-2-phenylethyl]-1H-1,2,3-triazol-4-yl}butanamide **23b**

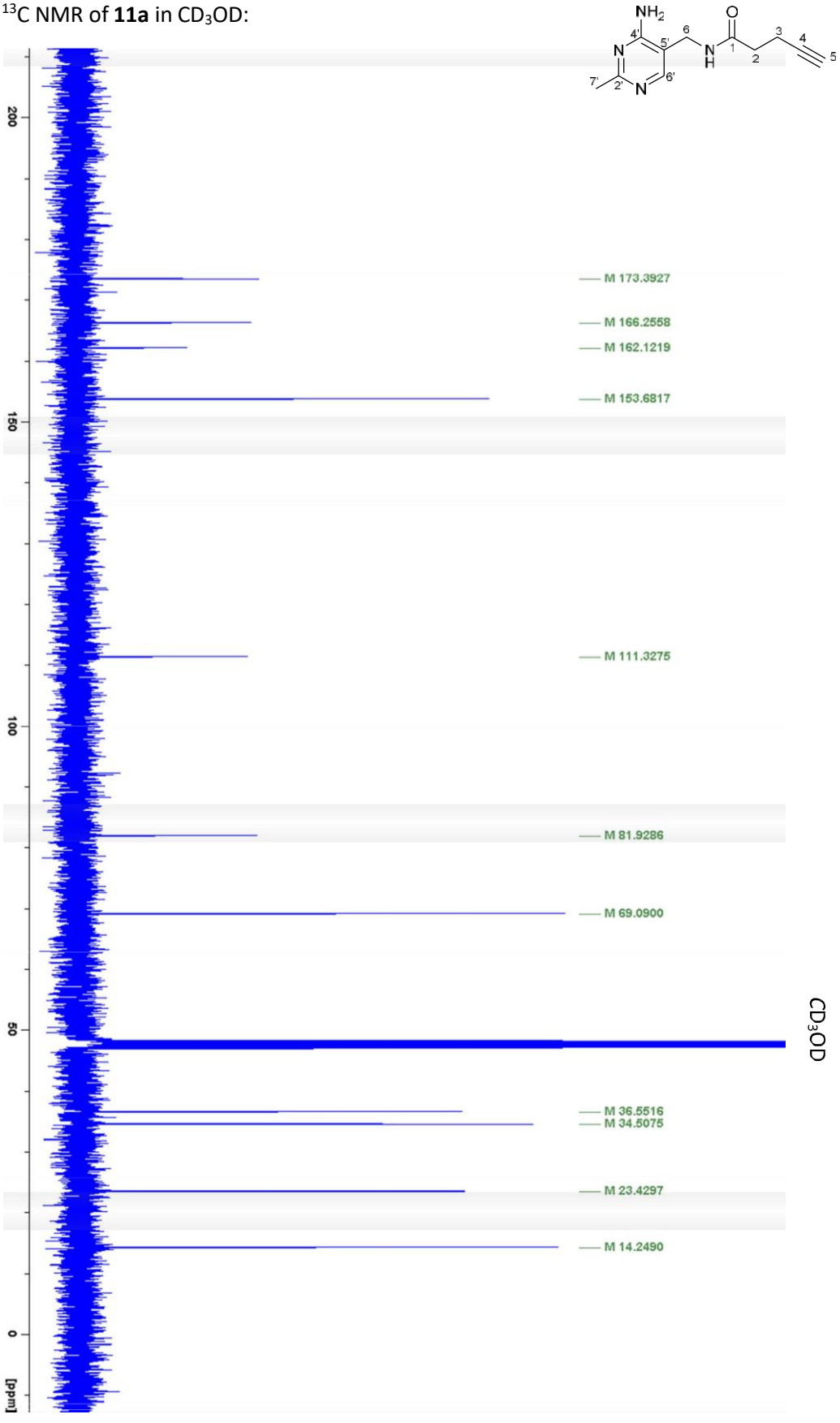


Prepared from **11b**. White solid (54 mg, 41% yield over two steps). White solid (26 mg, 60%). **m.p.** 212-214 °C. **^{1}H NMR** (400 MHz, CD_3OD) δ 8.15 (s, 1H, H-6"), 8.04 (s, 1H, H-1'), 7.17-7.27 (m, 5H, H-5, H-6 and H-7), 5.42 (dd, 1H, *J* = 7.5 and 8.3 Hz, H-2), 4.29 (s, 2H, H-8"), 3.56 (dd, 1H, *J* = 7.5 and 13.4 Hz, H-3a), 3.45 (dd, 1H, *J* = 8.3 and 13.4 Hz, H-3b), 2.75 (t, 2H, *J* = 7.3 Hz, H-3'), 2.55 (s, 3H, H-7"), 2.33 (t, 2H, *J* = 7.3 Hz, H-5'), 1.97 (qnt, 2H, *J* = 7.3 Hz, H-4'). **^{13}C NMR** (100 MHz, CD_3OD) δ 175.2 (C-6'), 165.1 (C-2"), 164.2 (C-1), 161.0 (C-4"), 146.5 (C-2'), 141.4 (C-6"), 135.1 (C-4), 128.9 (C-5), 128.2 (C-6), 127.0 (C-7), 122.1 (C-1'), 112.9 (C-5"), 63.0 (C-2), 37.7 (C-3), 36.0 (C-8"), 34.4 (C-5'), 24.8 (C-4'), 24.1 (C-3'), 20.0 (C-7"). **HRMS** (ESI) *m/z*: [M+H$^+$] calculated for $C_{21}H_{26}N_8O_3$: 439.2201; found: 439.2206. **$[\alpha]_D^{25}$** = -57.1° (c = 0.2, MeOH).

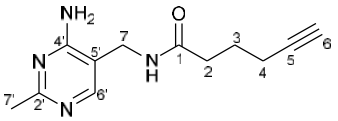
^{1}H NMR of **11a** in CD_3OD:

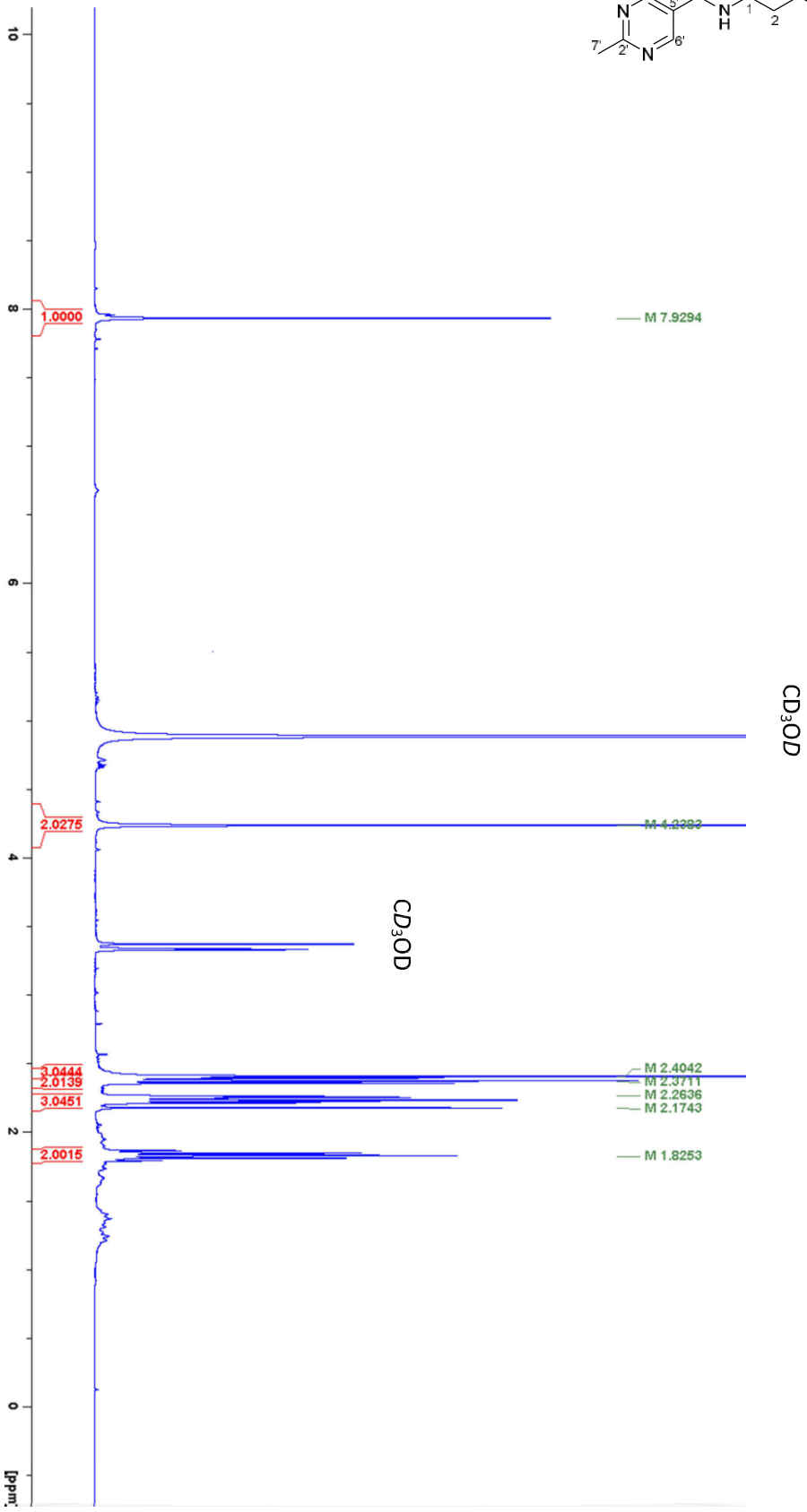


^{13}C NMR of **11a** in CD_3OD:

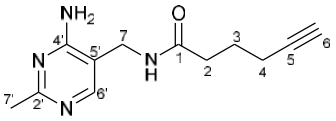


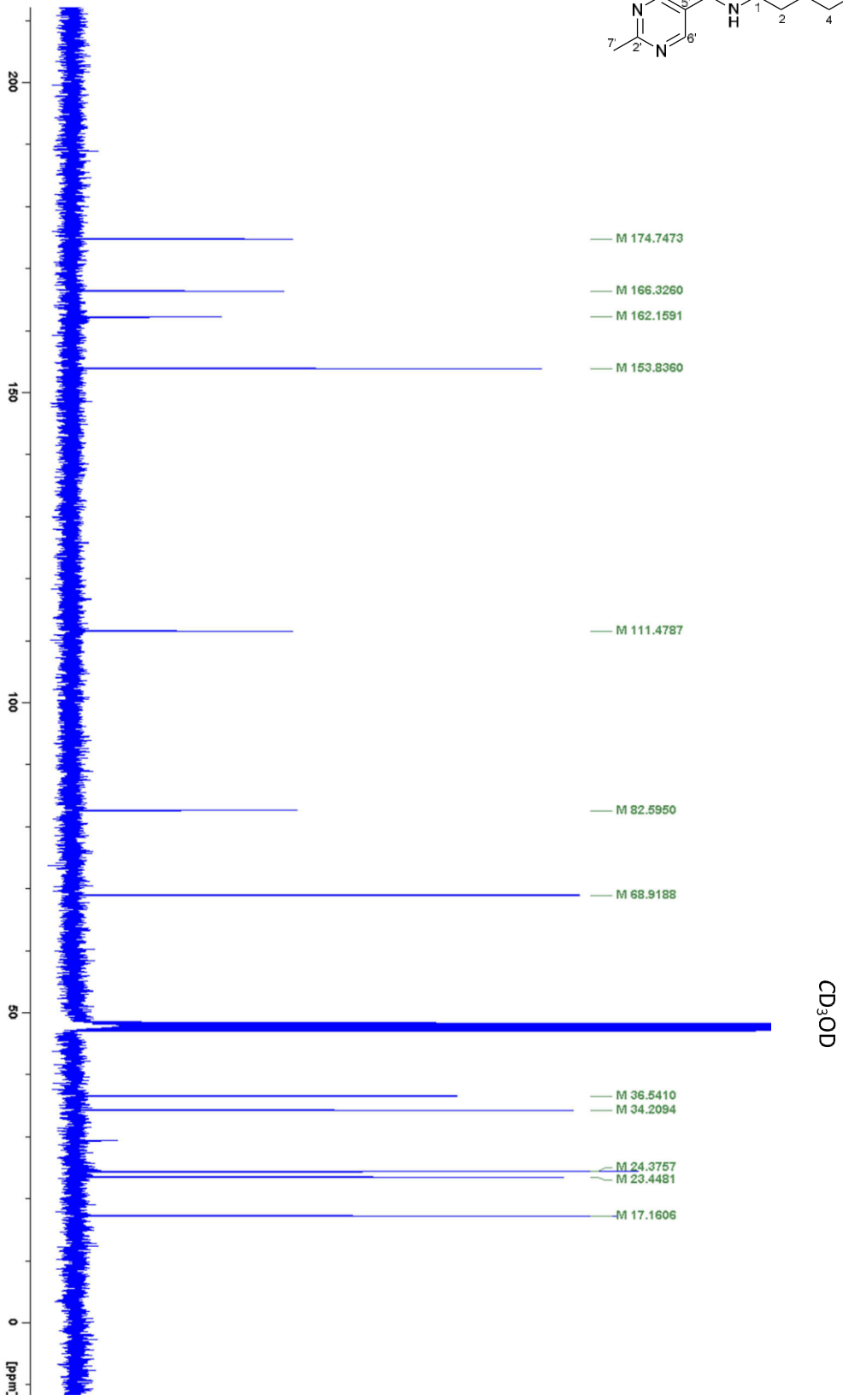
^{1}H NMR of **11b** in CD_3OD:



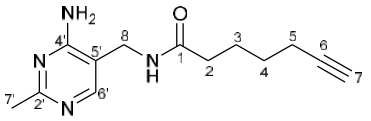


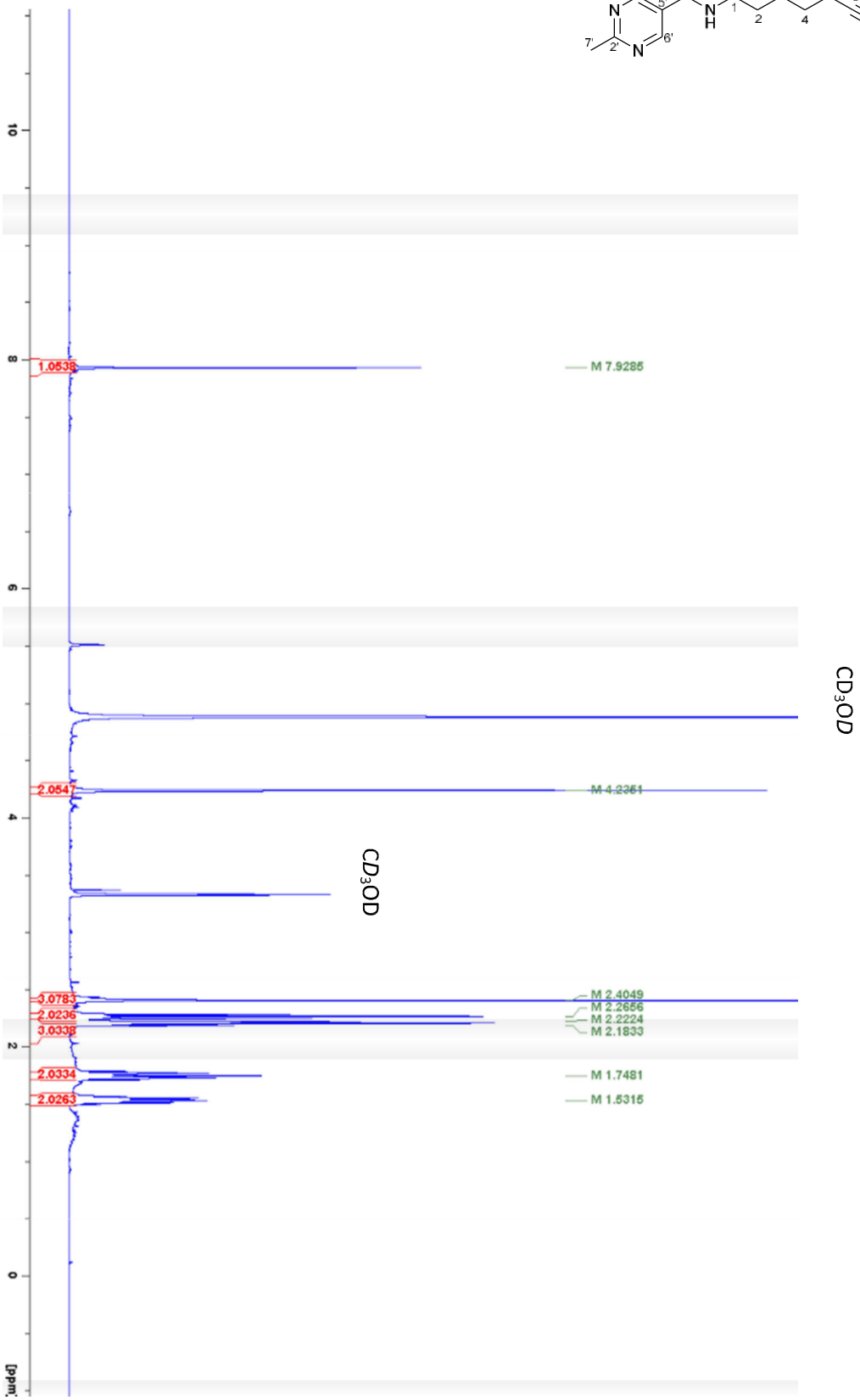
^{13}C NMR of **11b** in CD_3OD:



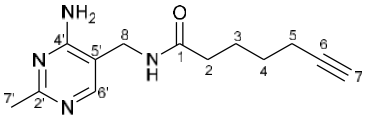


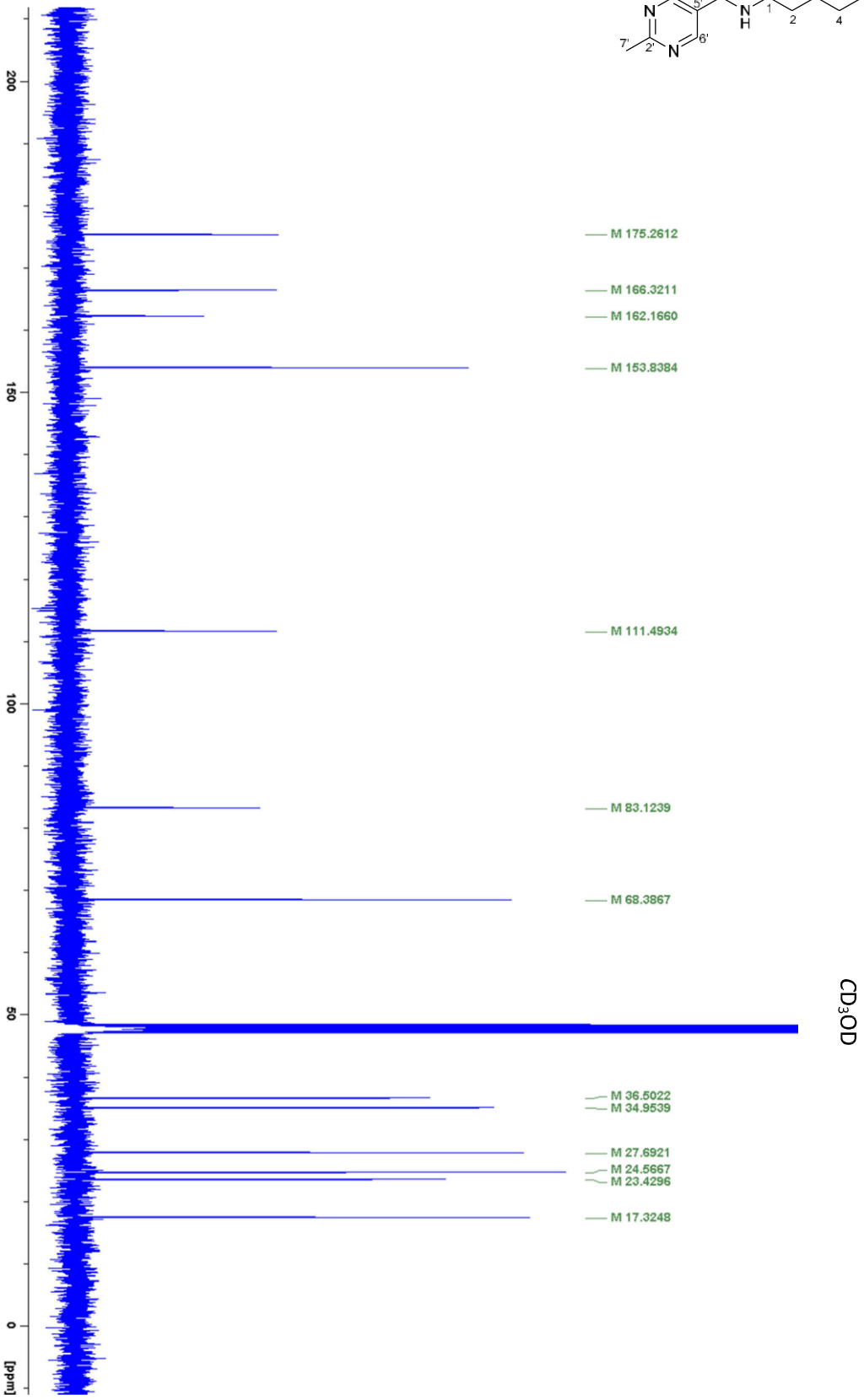
^{1}H NMR of **11c** in CD_3OD:



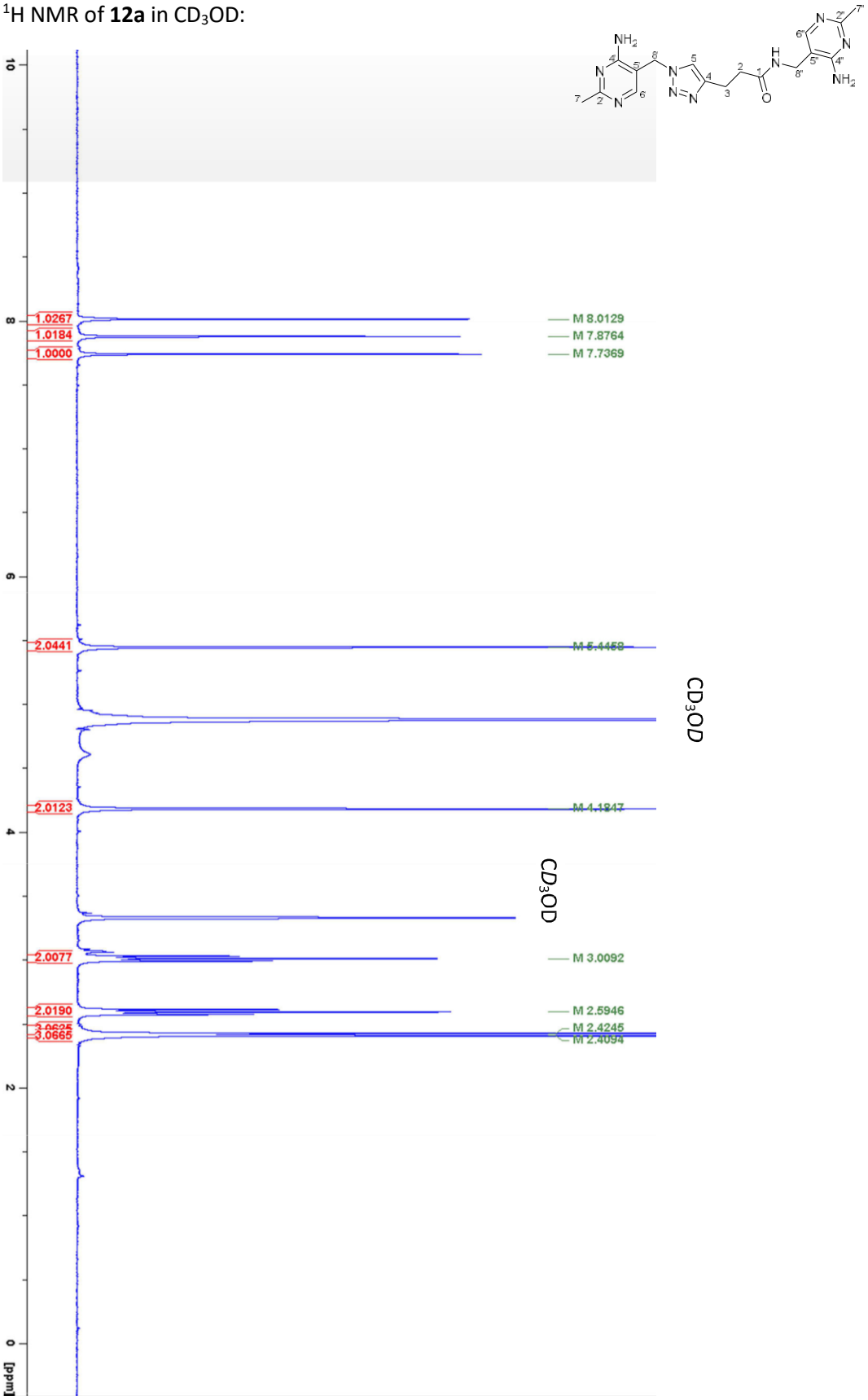


^{13}C NMR of **11c** in CD_3OD:

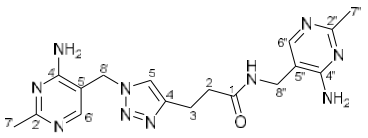


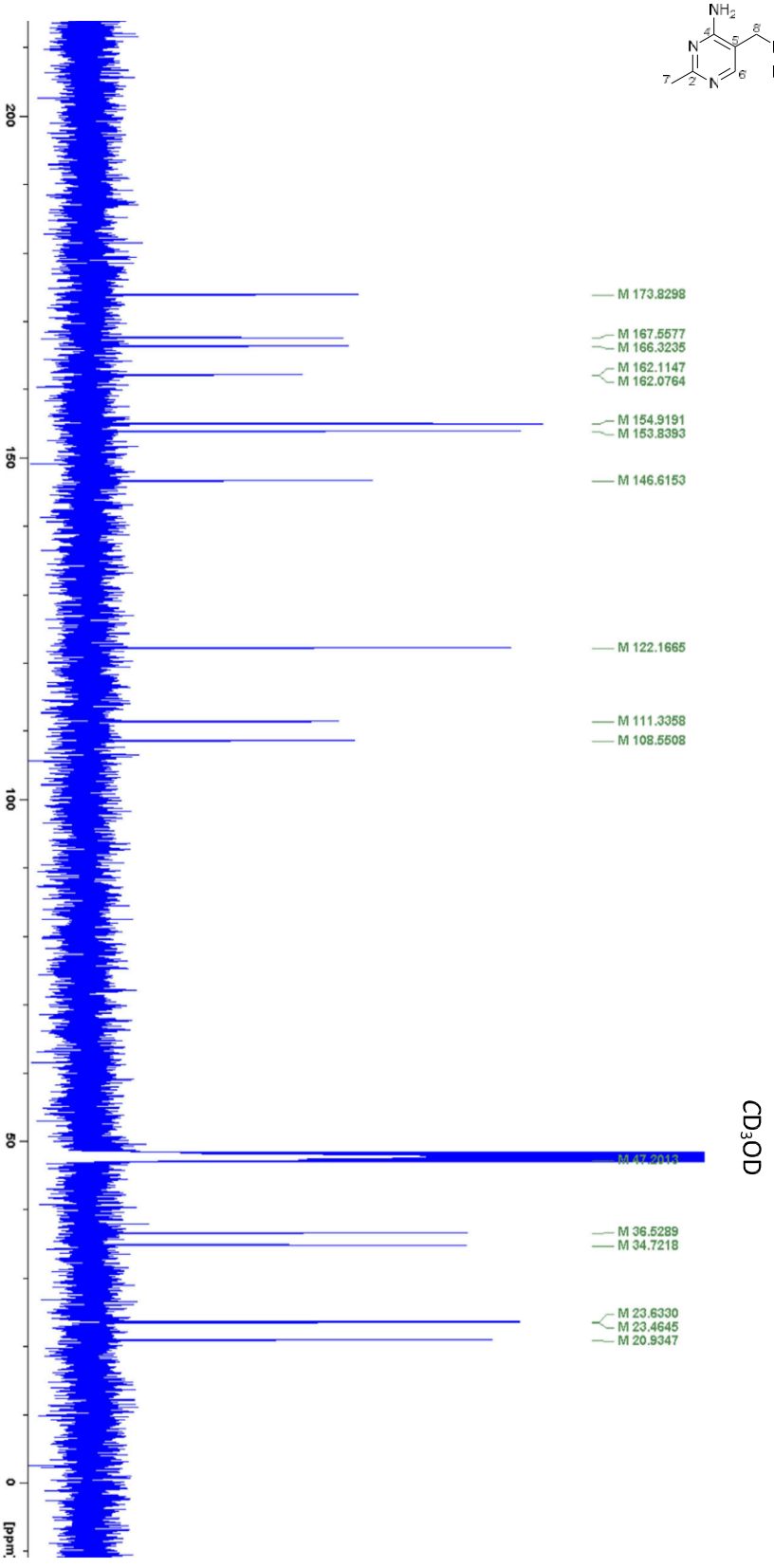


^{1}H NMR of **12a** in CD_3OD:

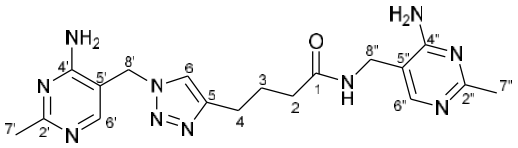


^{13}C NMR of **12a** in CD_3OD:

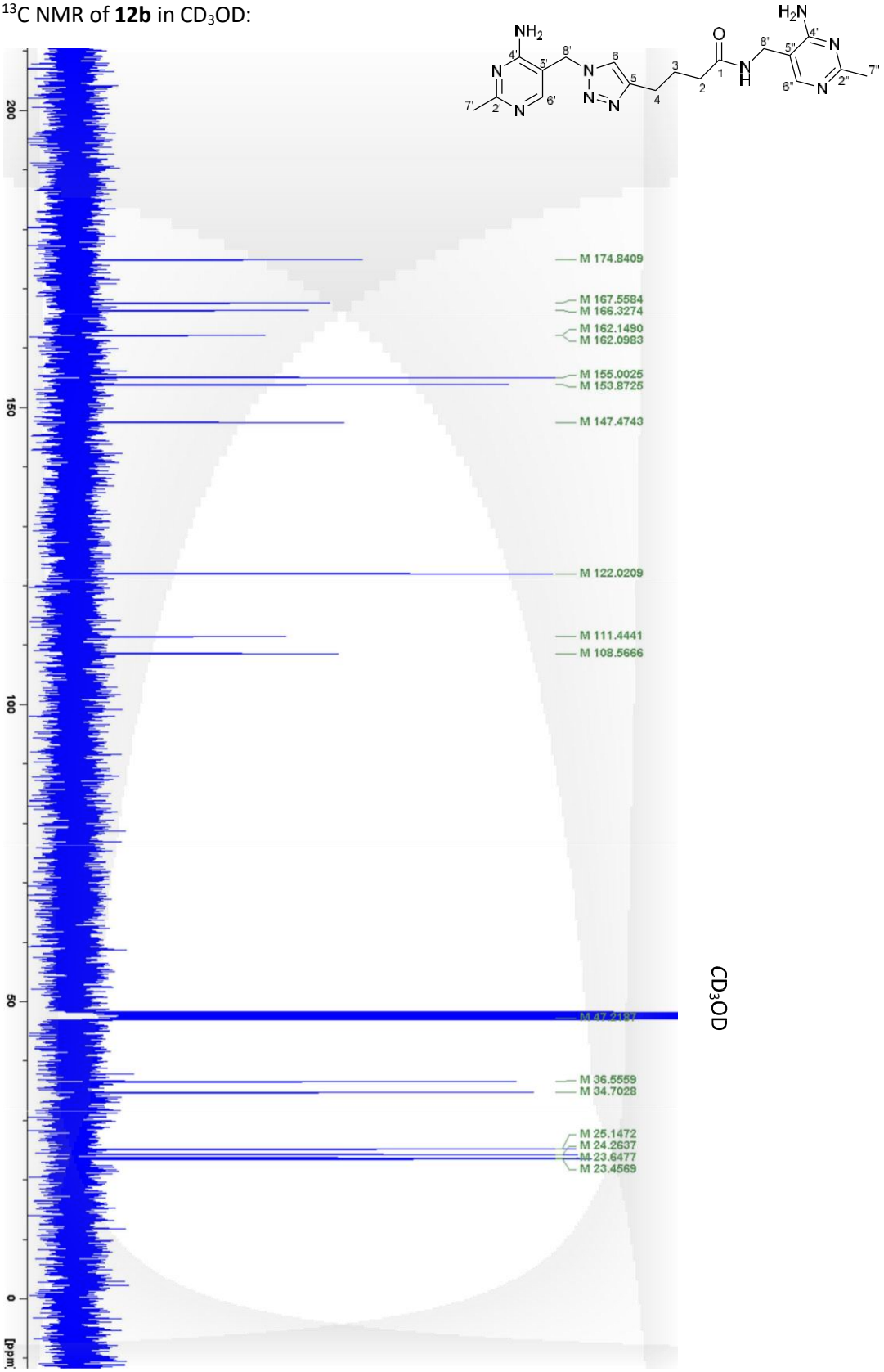




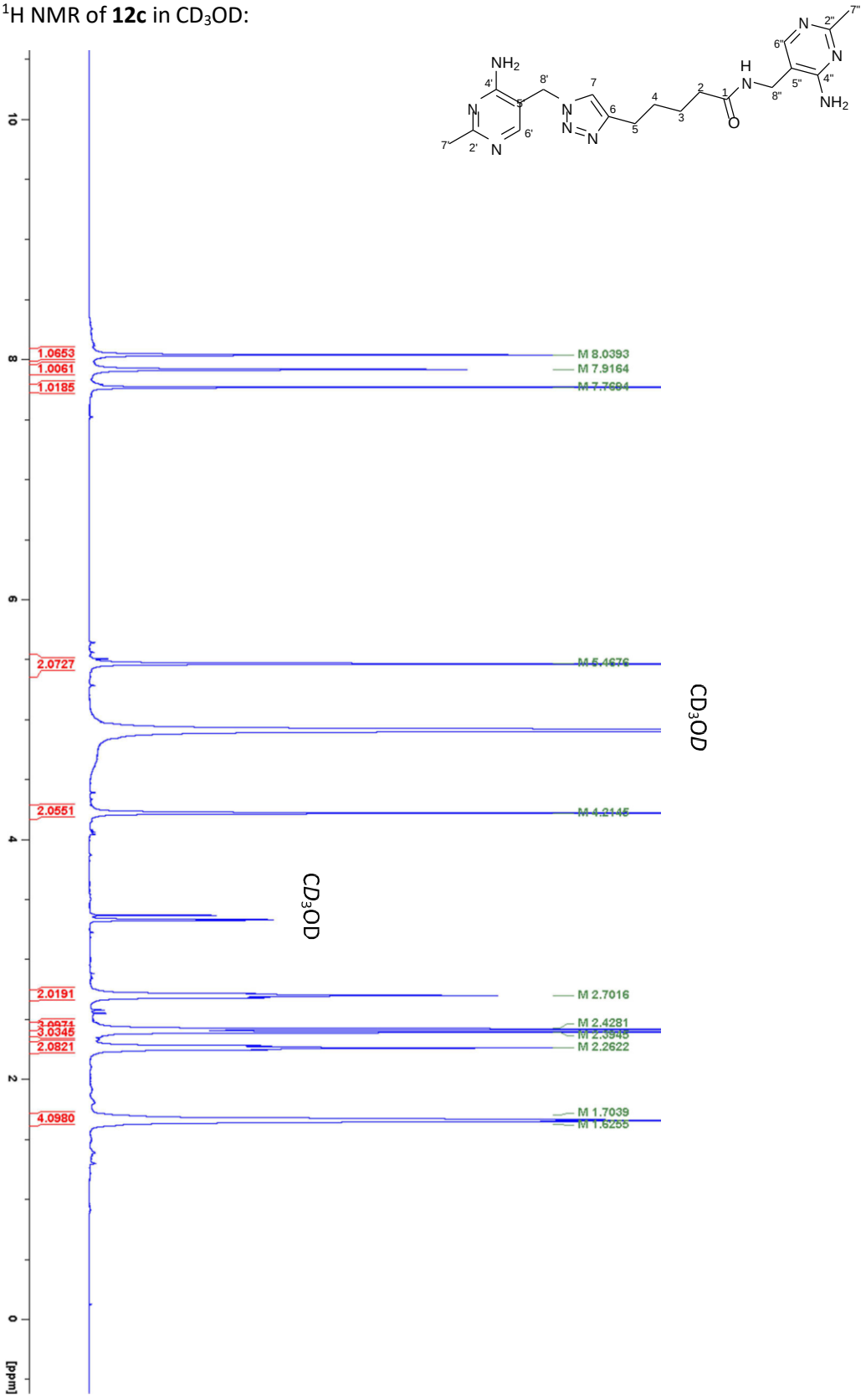
^{1}H NMR of **12b** in CD_3OD:



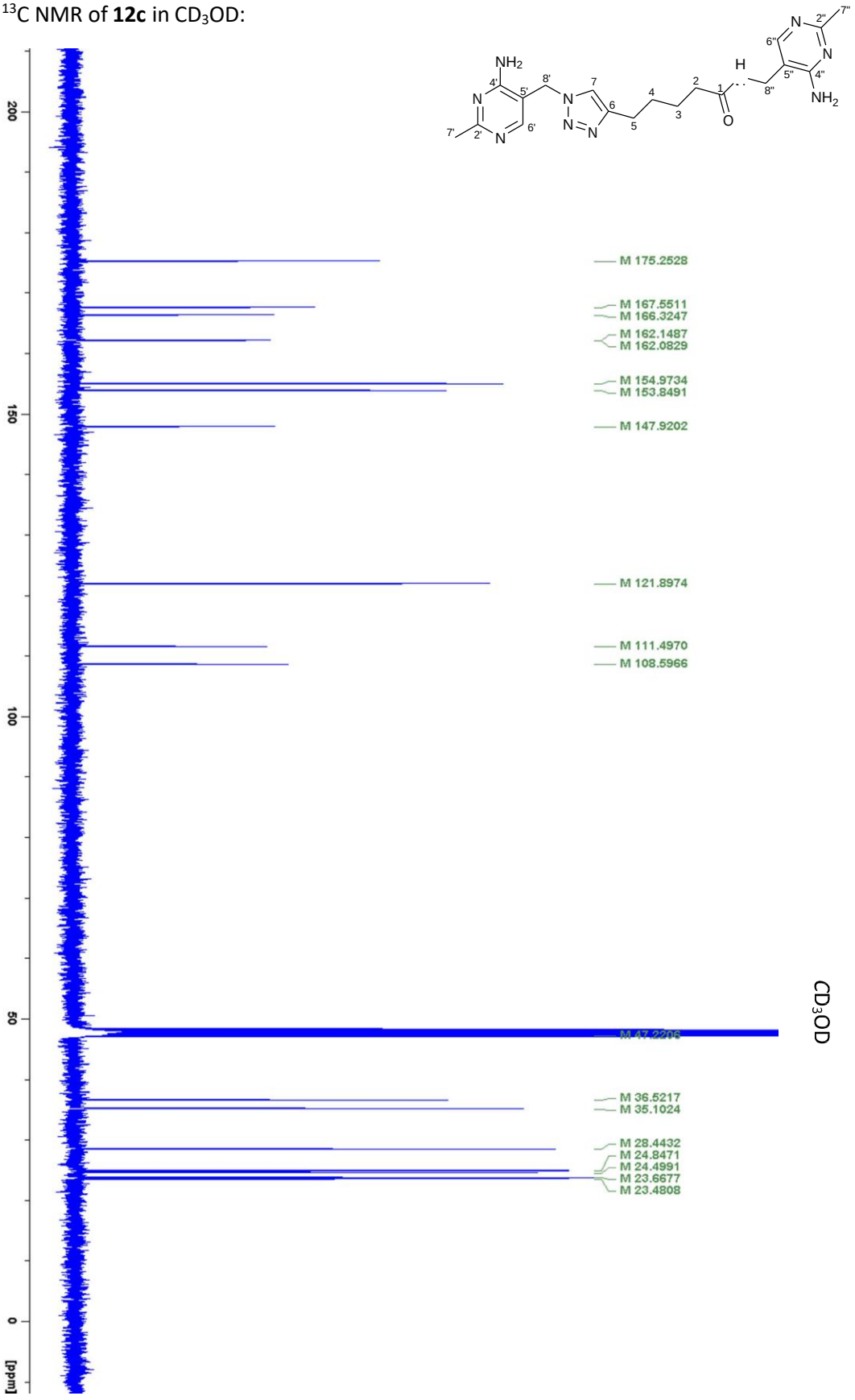
^{13}C NMR of **12b** in CD_3OD:



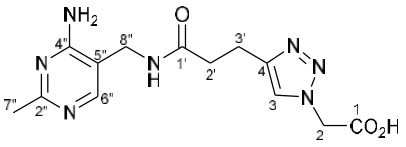
^{1}H NMR of **12c** in CD_3OD:

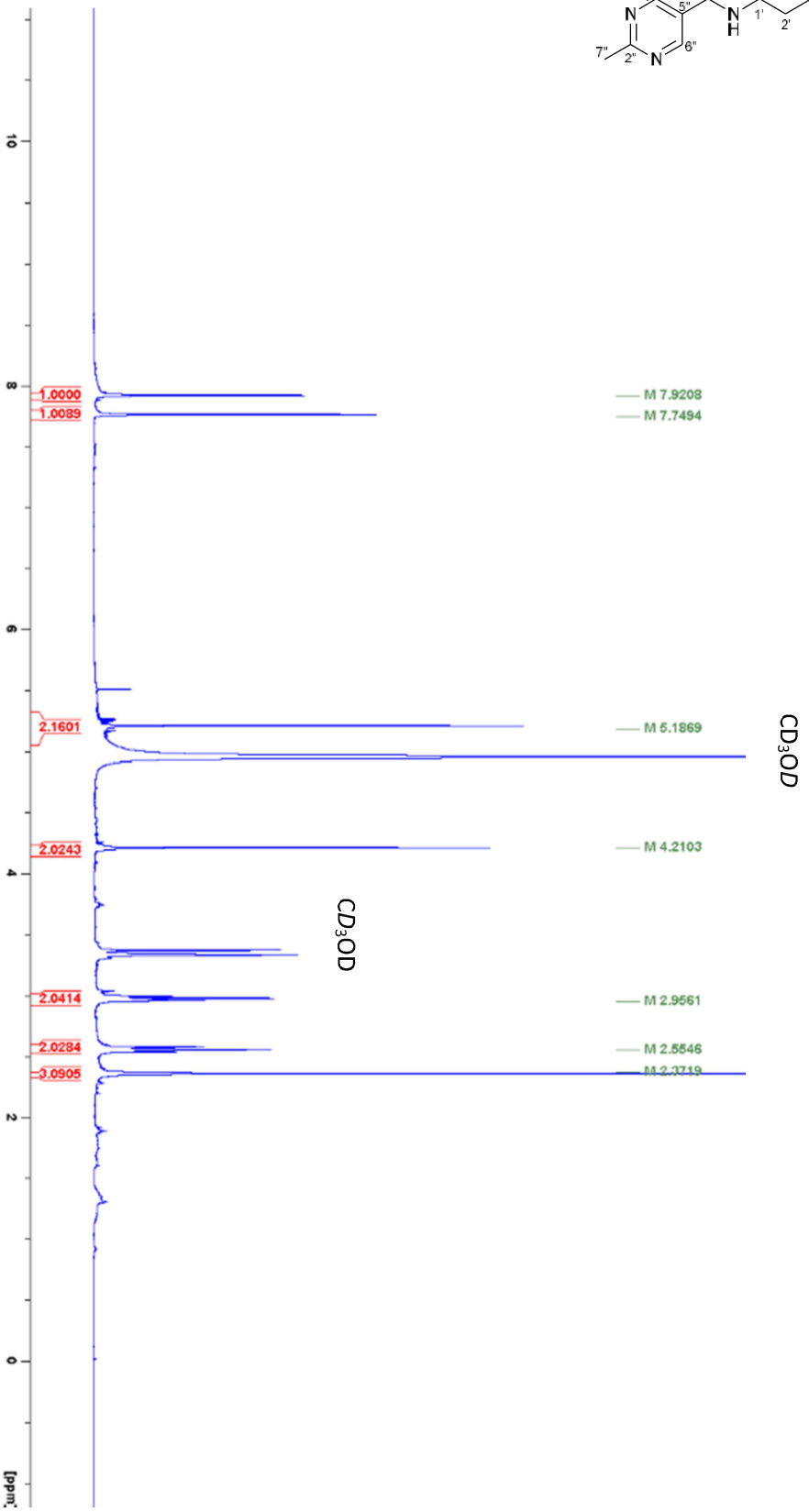


^{13}C NMR of **12c** in CD_3OD:

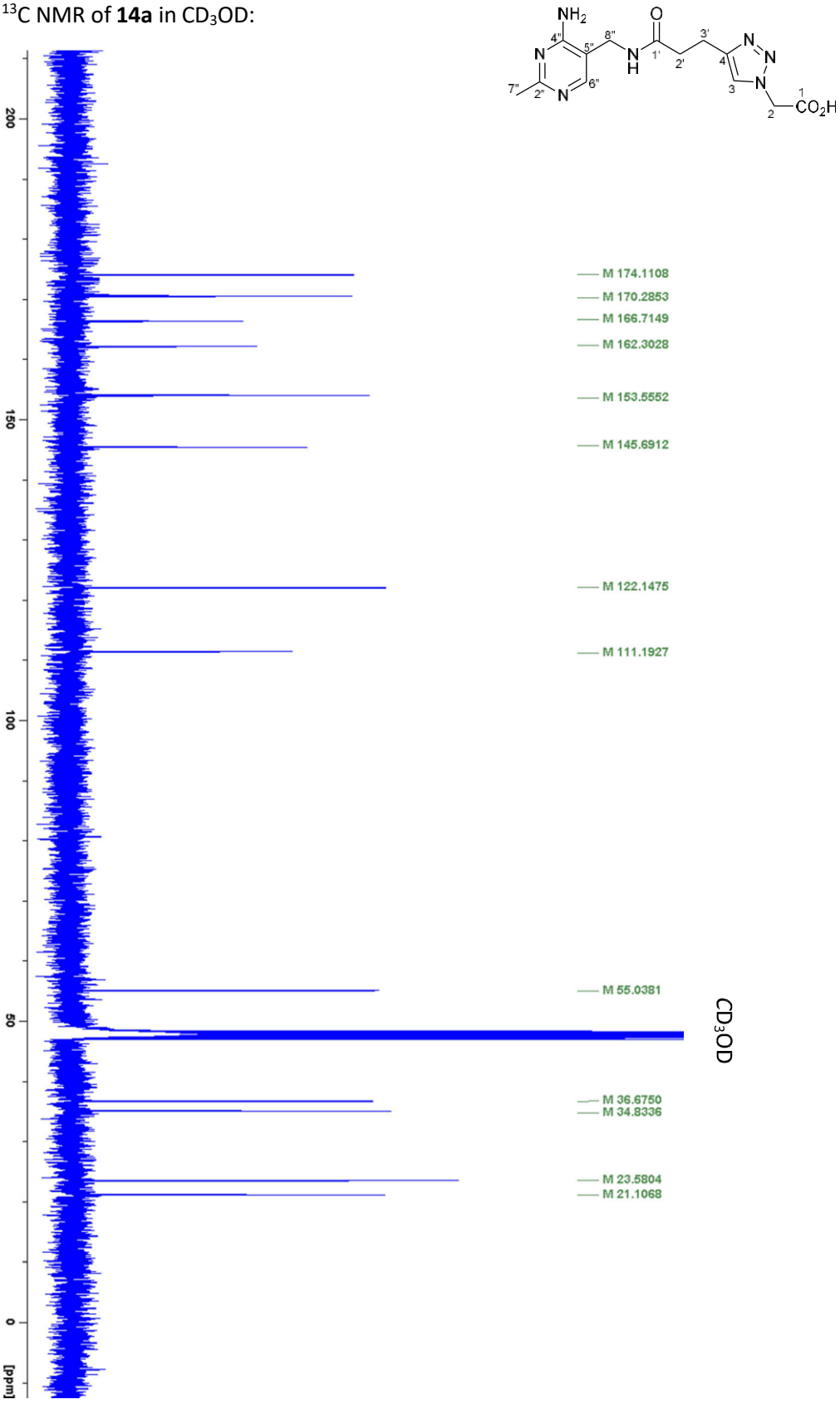


^{1}H NMR of **14a** in CD_3OD:

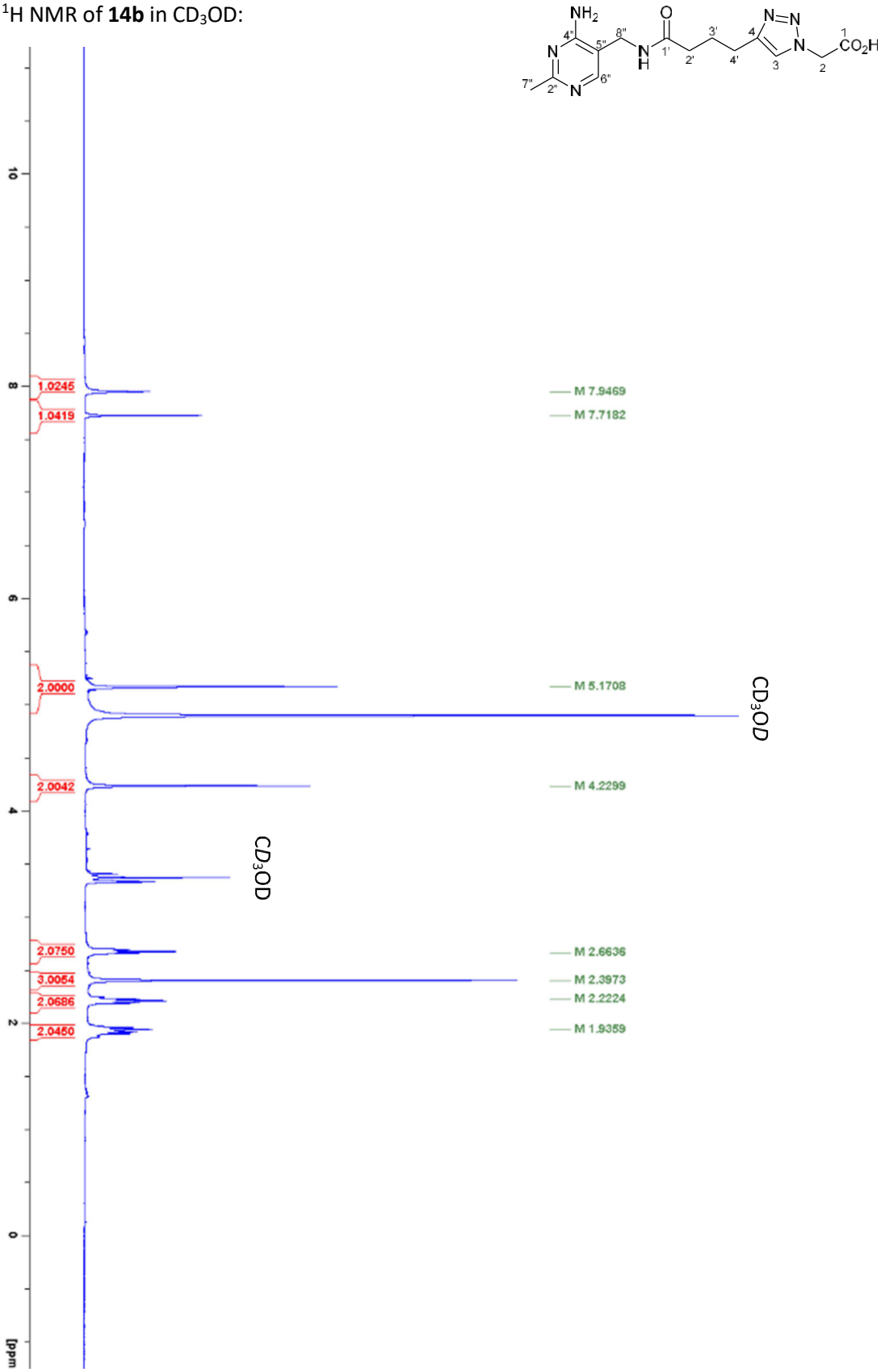




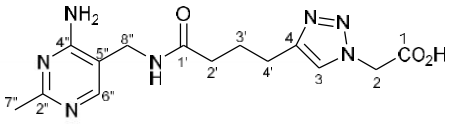
^{13}C NMR of **14a** in CD_3OD:

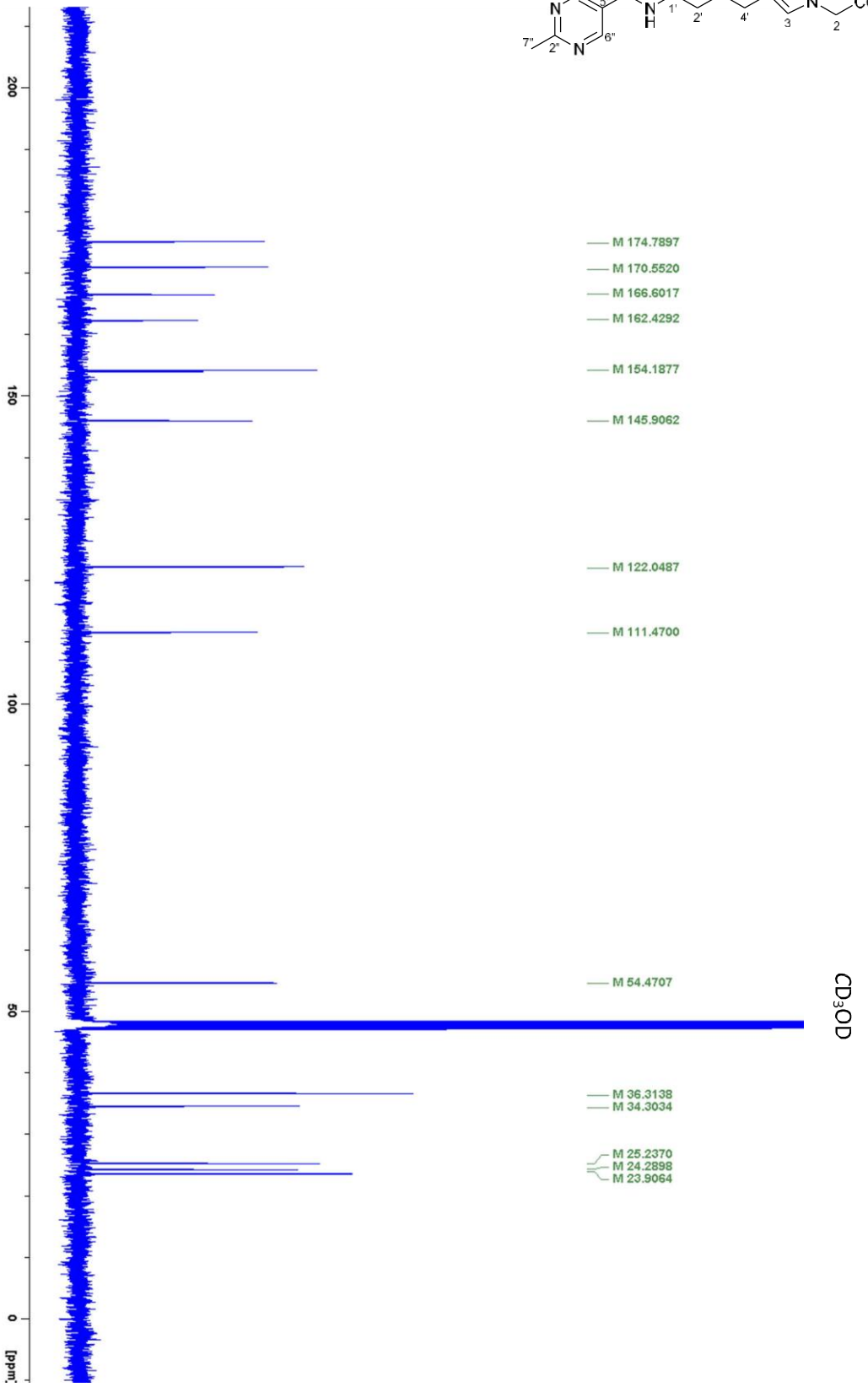


^{1}H NMR of **14b** in CD_3OD:

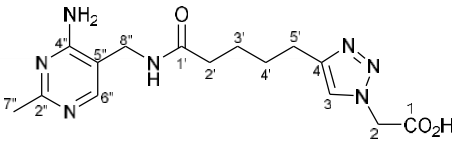


^{13}C NMR of **14a** in CD_3OD:

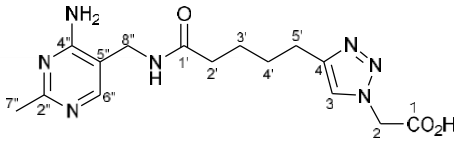


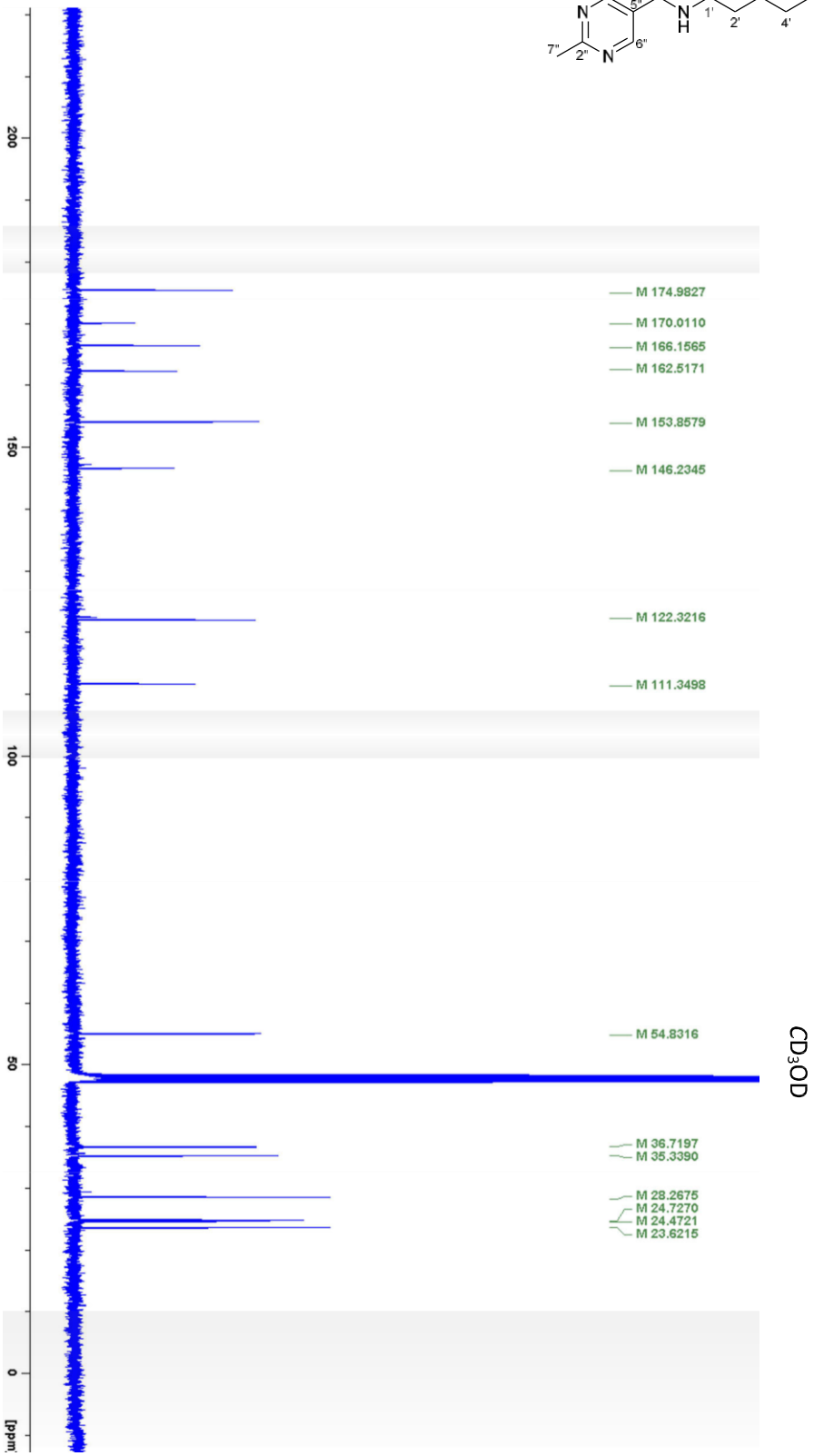


^{1}H NMR of **14c** in CD_3OD:

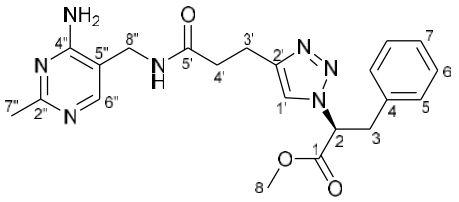


^{13}C NMR of **14c** in CD_3OD:

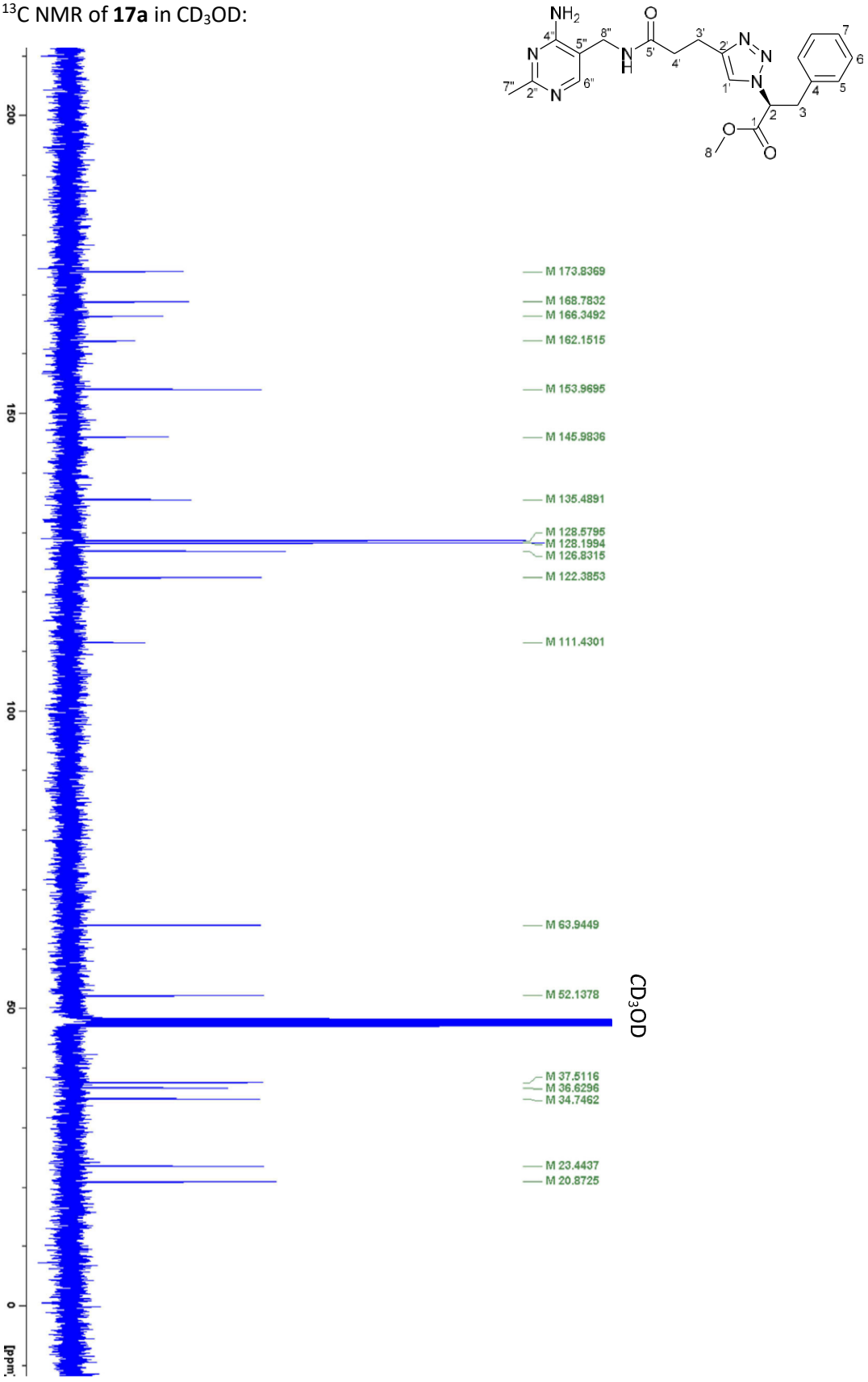




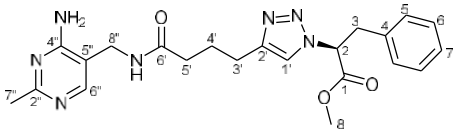
^{1}H NMR of **17a** in CD_3OD:



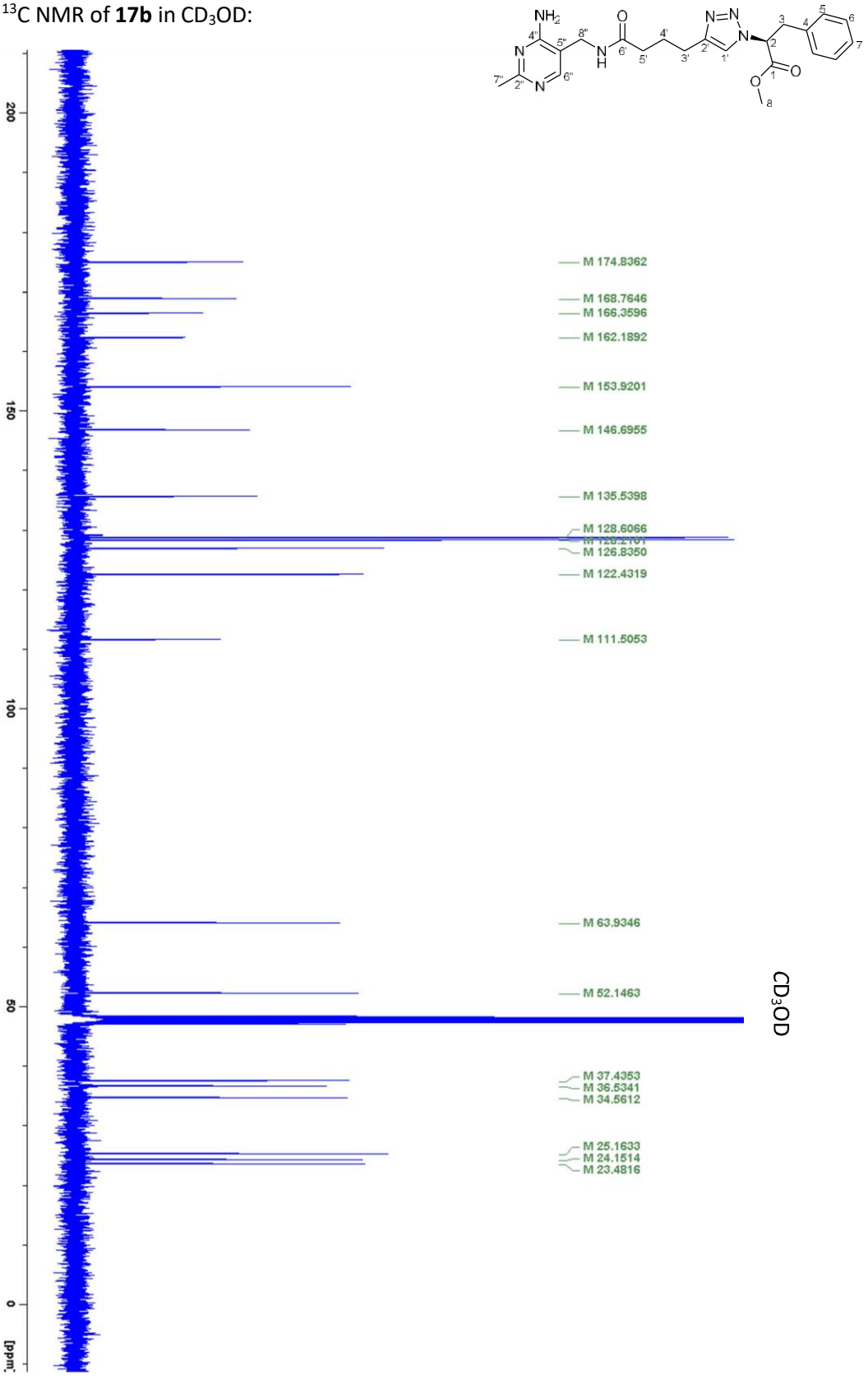
^{13}C NMR of **17a** in CD_3OD:



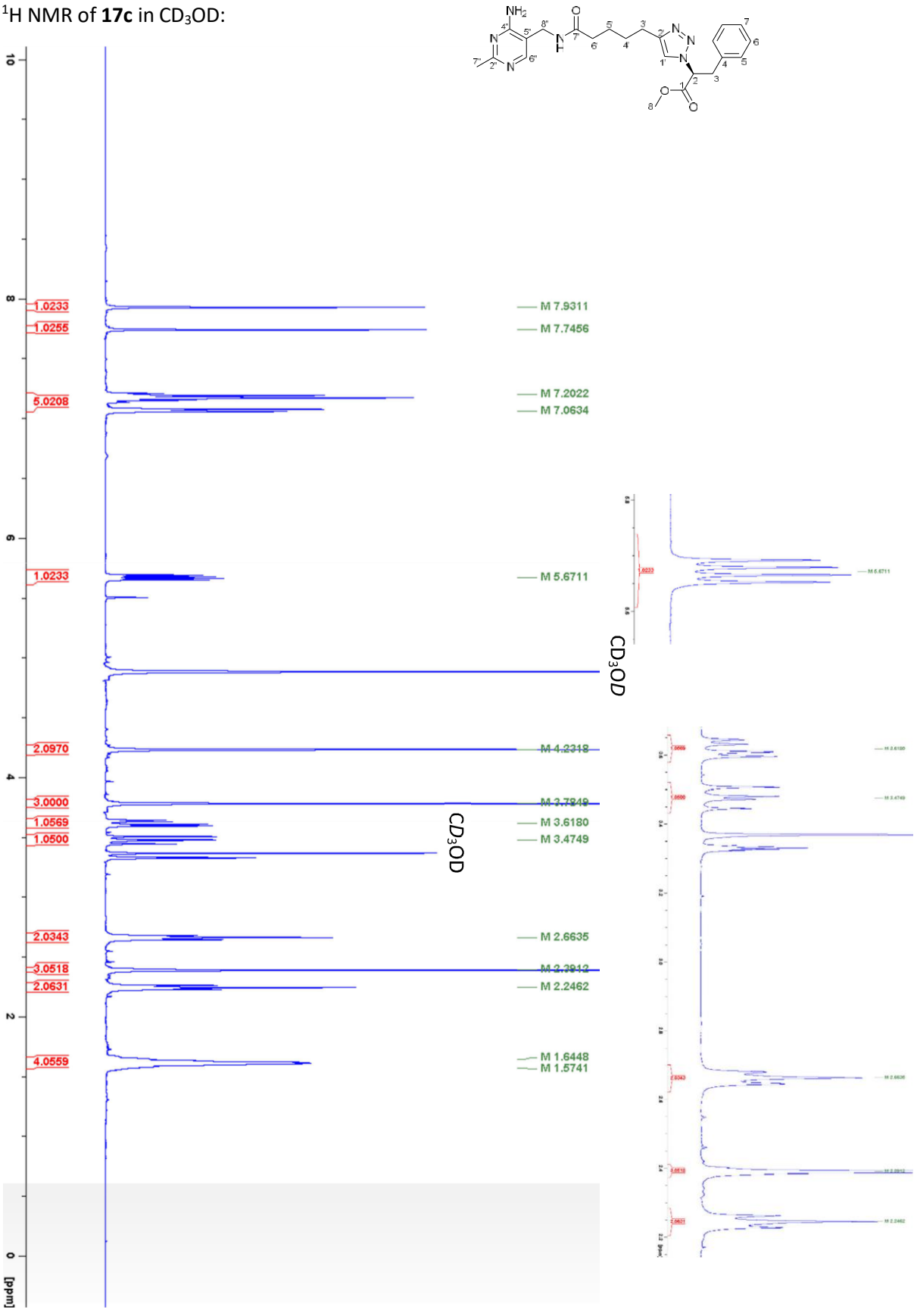
^{1}H NMR of **17b** in CD_3OD:



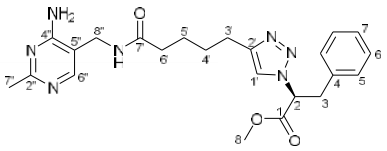
^{13}C NMR of **17b** in CD_3OD:

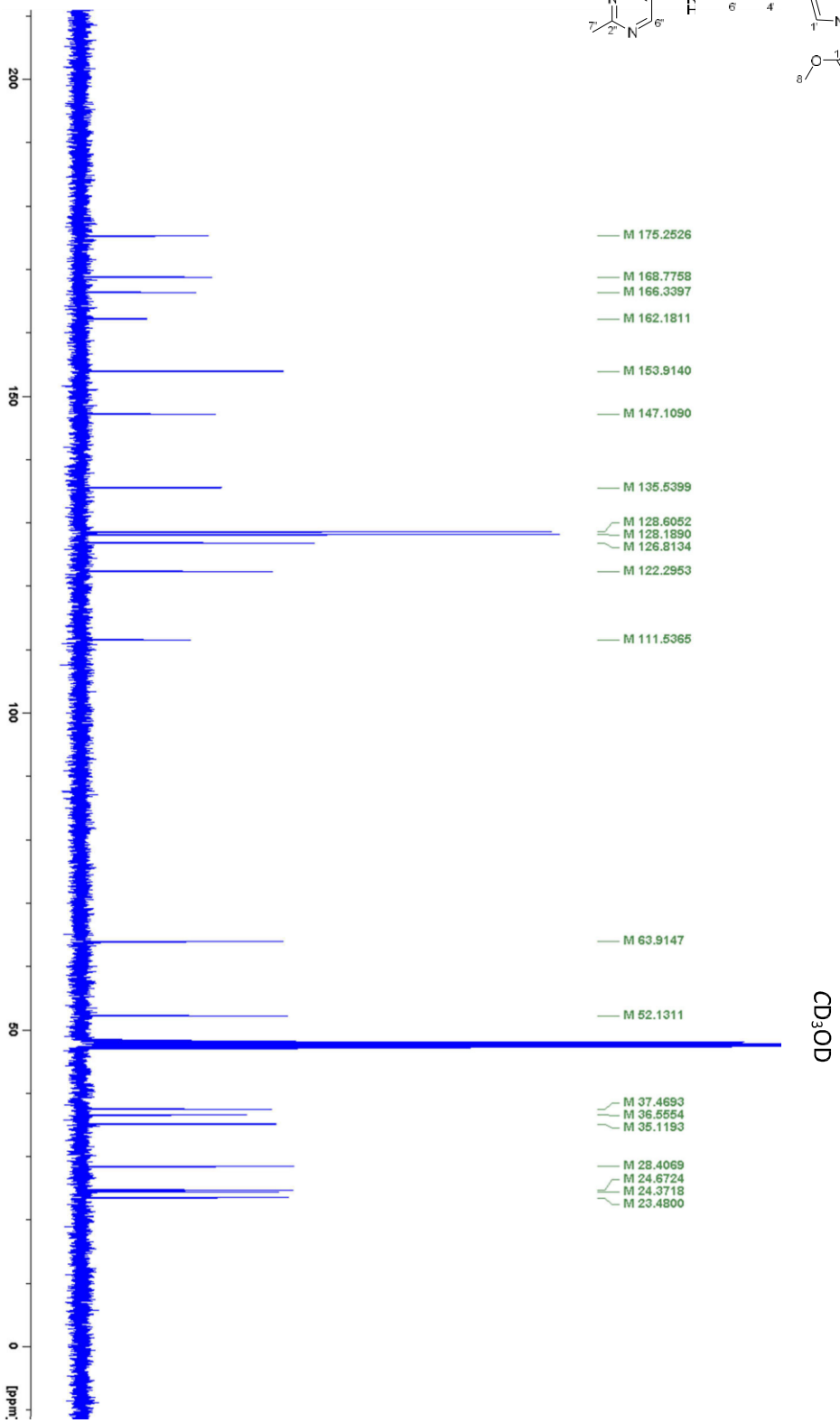


^{1}H NMR of **17c** in CD_3OD:

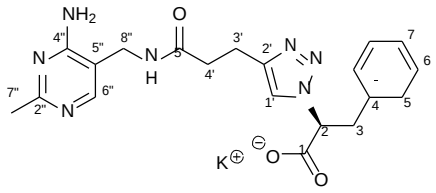


^{13}C NMR of **17c** in CD_3OD:

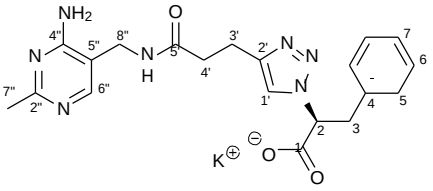


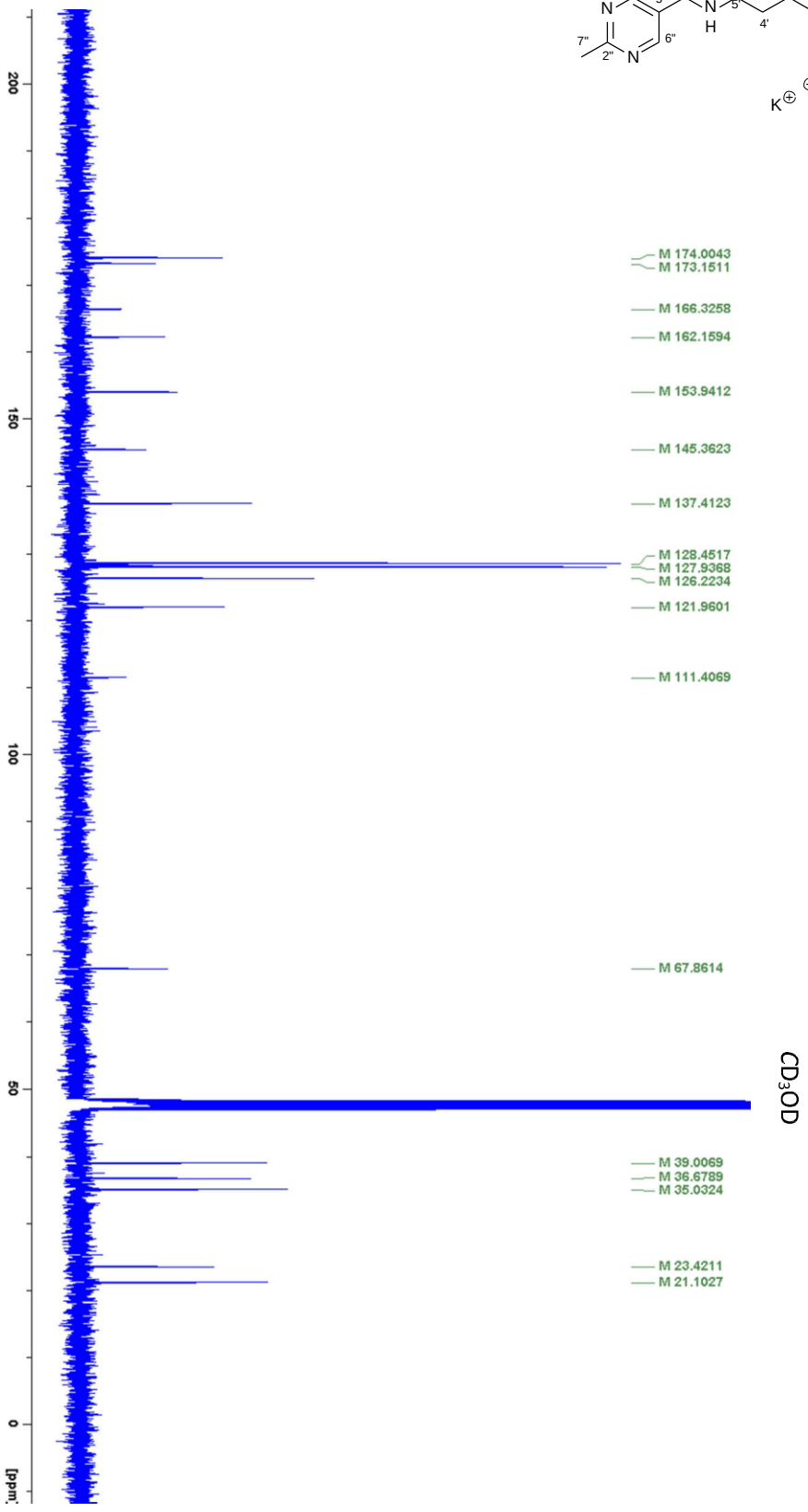


^{1}H NMR of **18a** in CD_3OD:

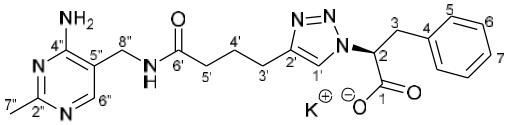


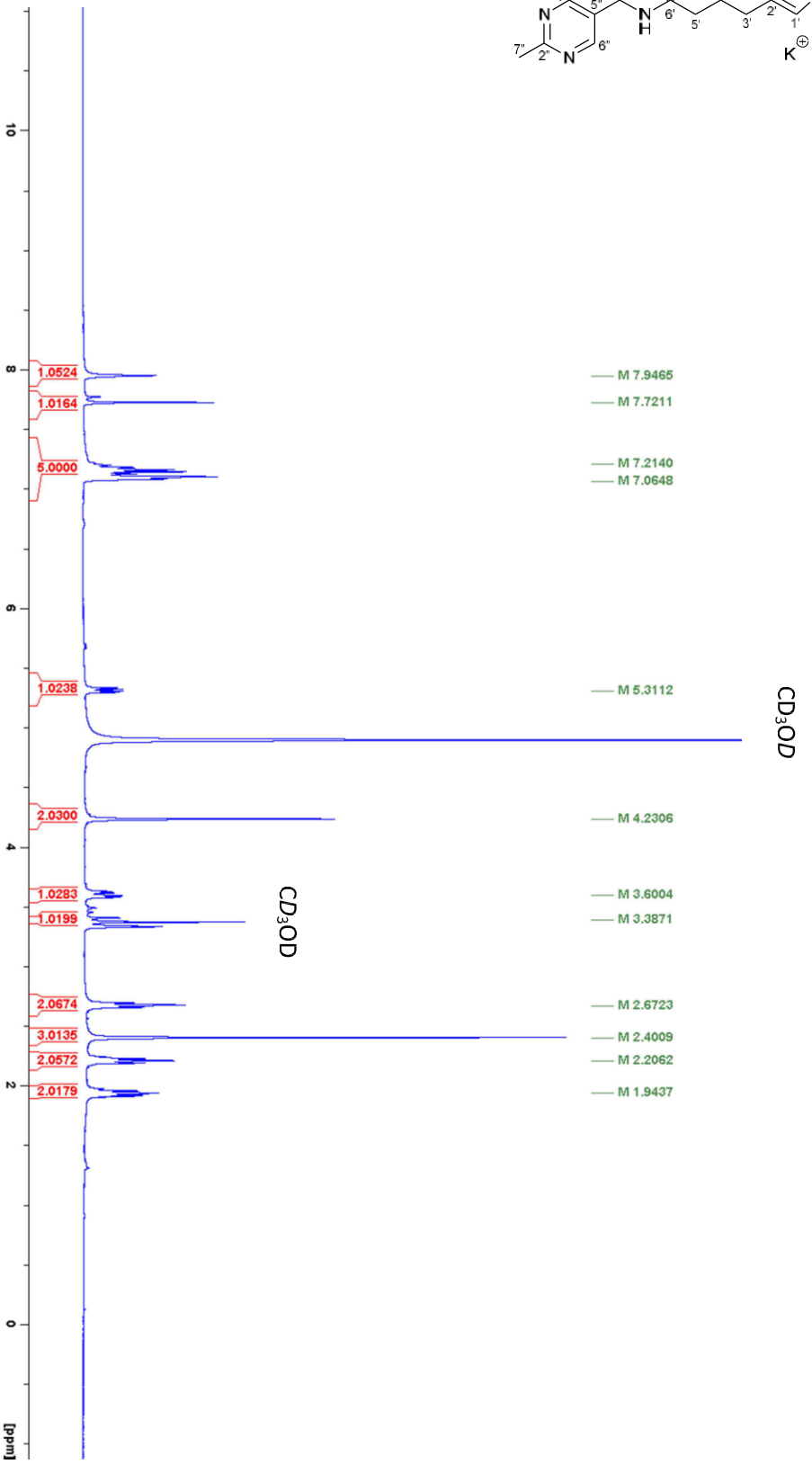
^{13}C NMR of **18a** in CD_3OD:



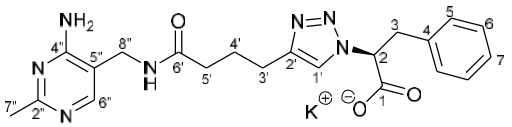


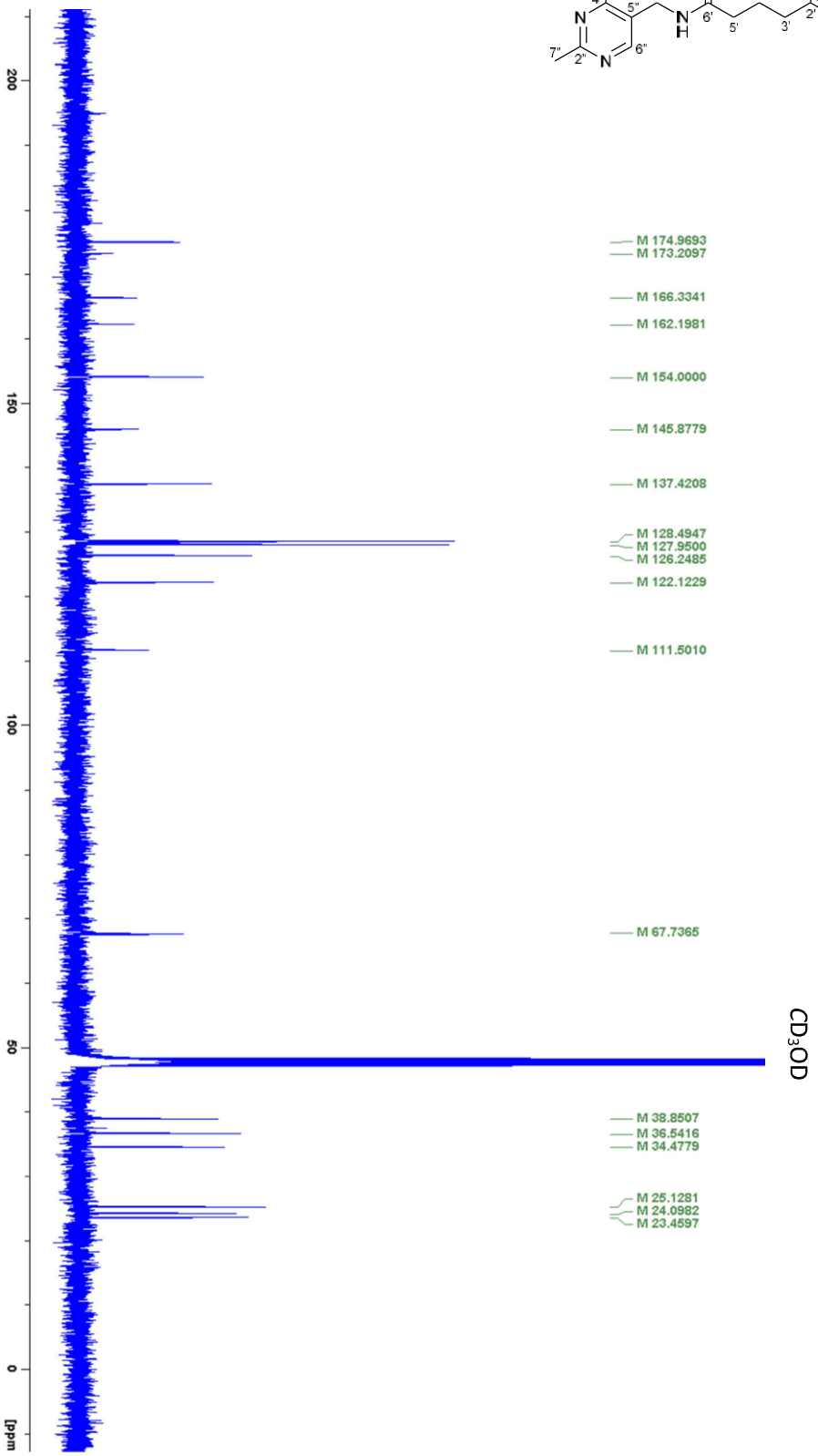
^{1}H NMR of **18b** in CD_3OD:



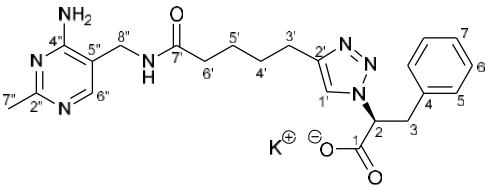


^{13}C NMR of **18b** in CD_3OD:

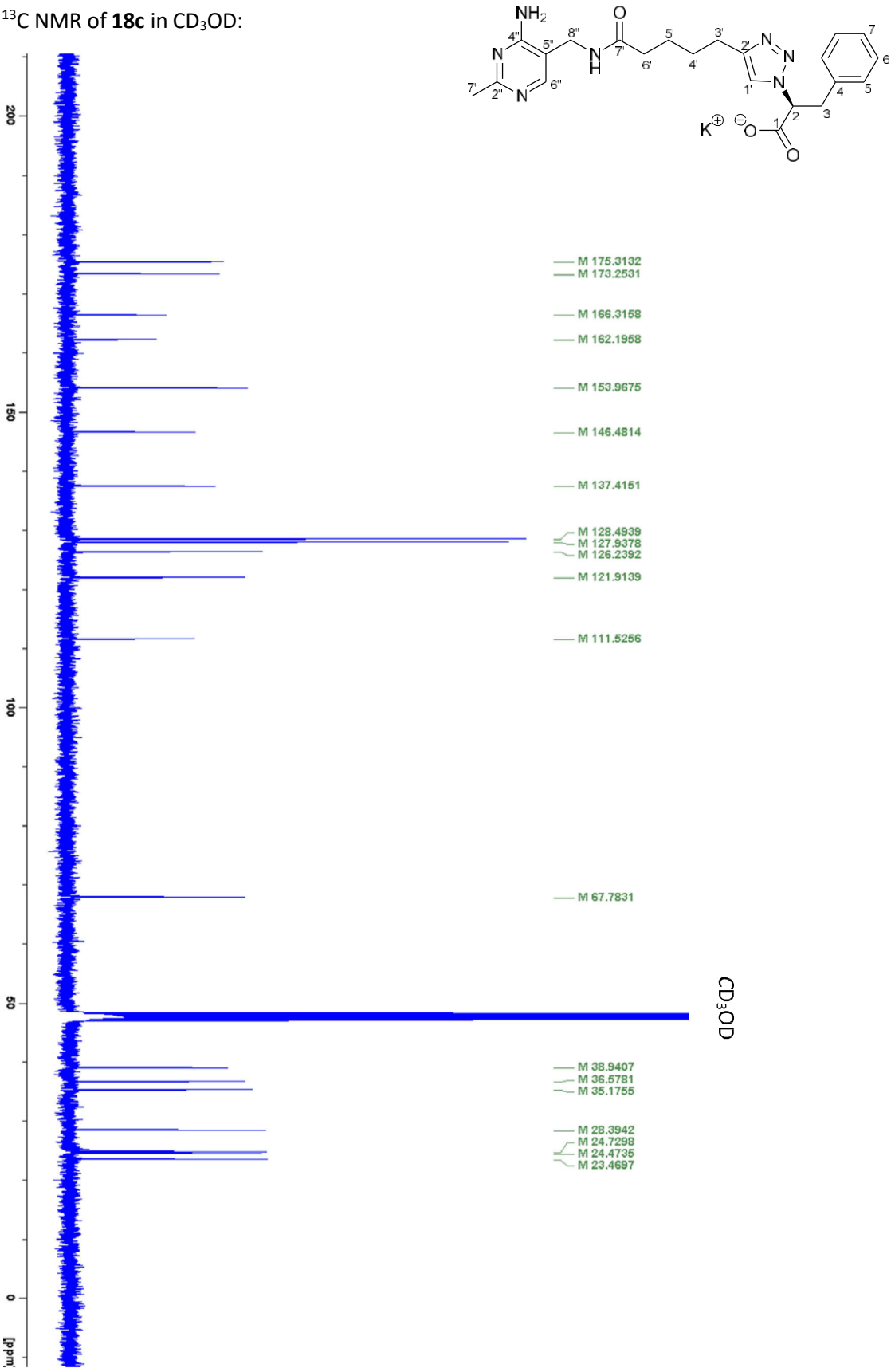




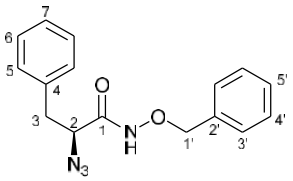
^{1}H NMR of **18c** in CD_3OD:

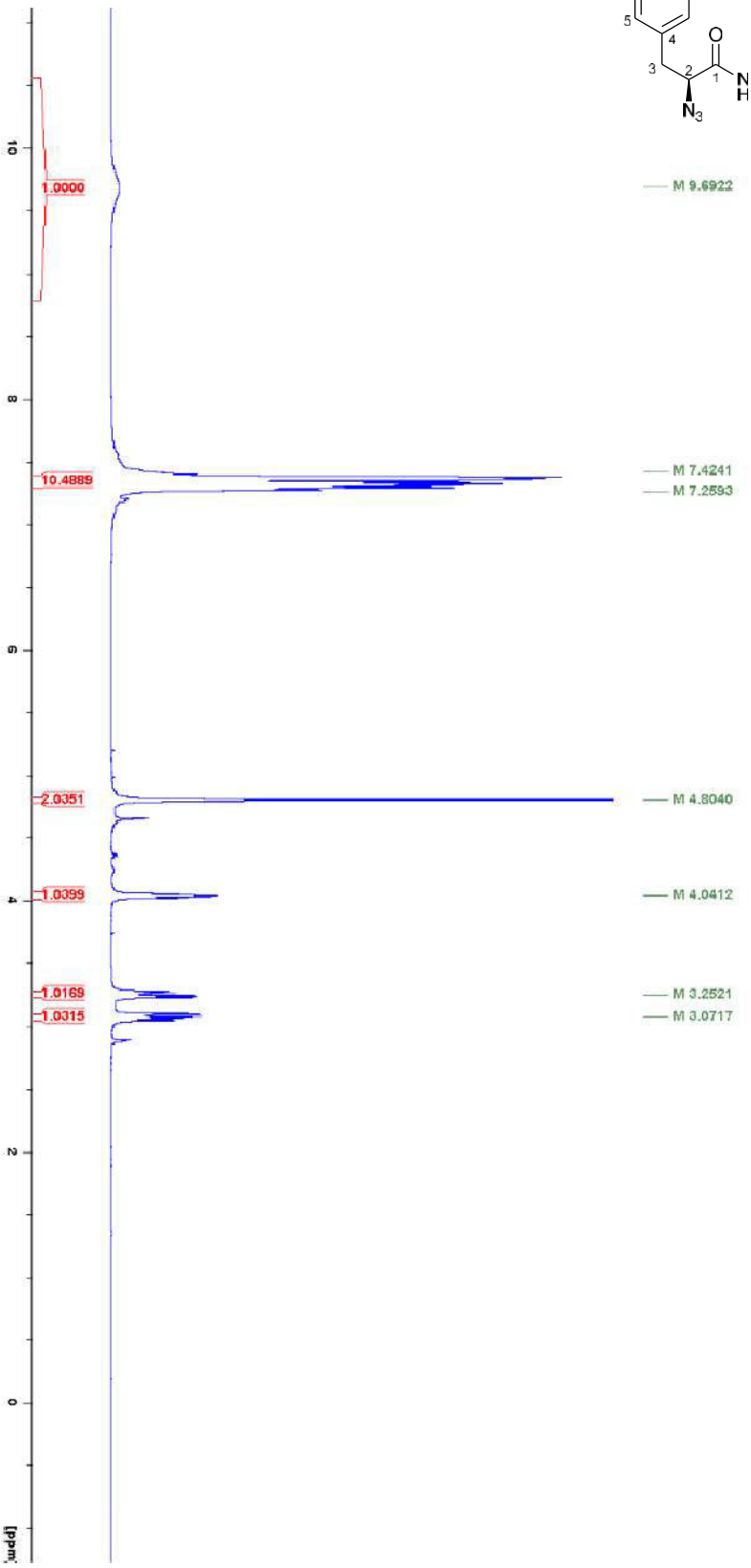


^{13}C NMR of **18c** in CD_3OD:

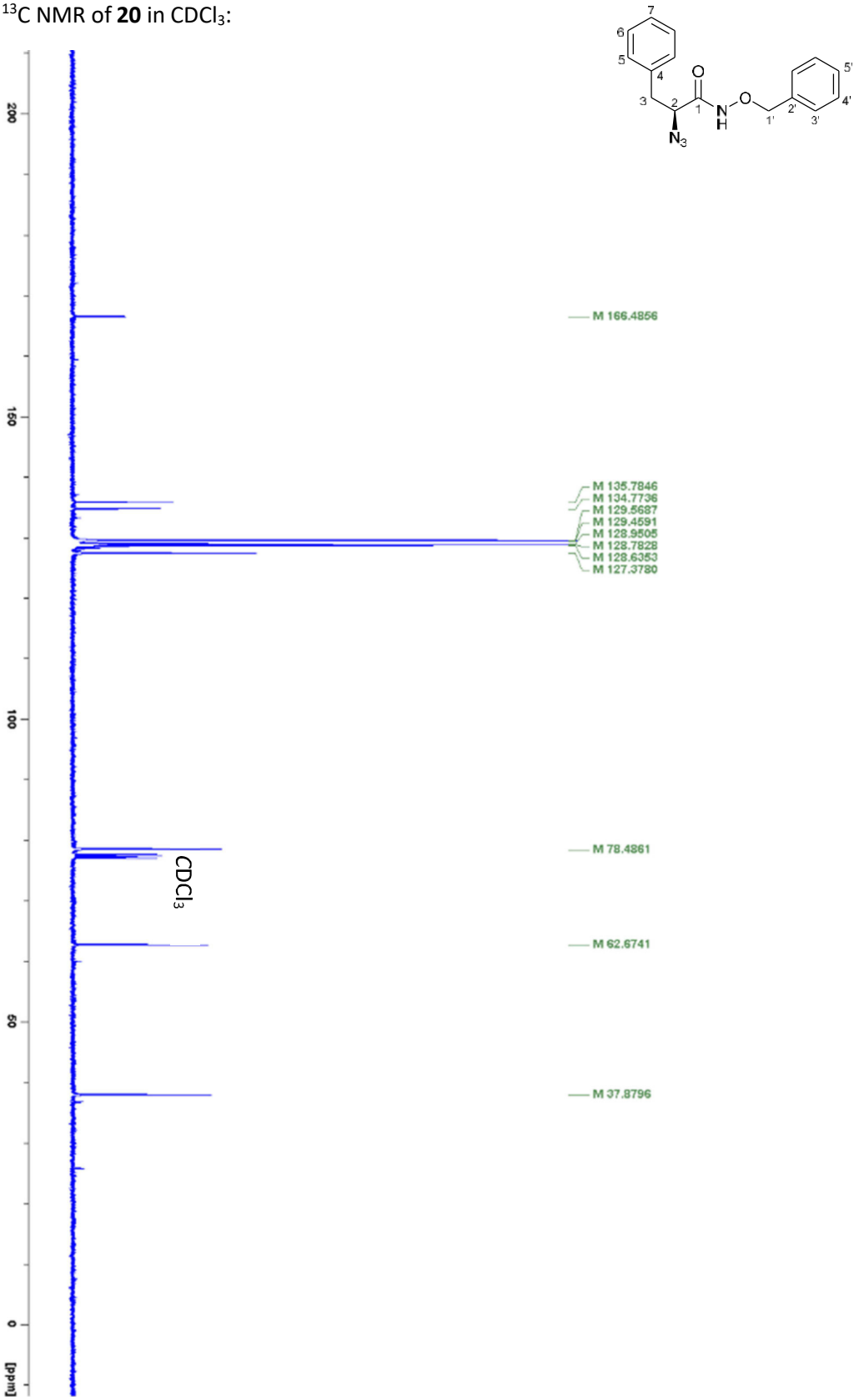


^{1}H NMR of **20** in $CDCl_3$:

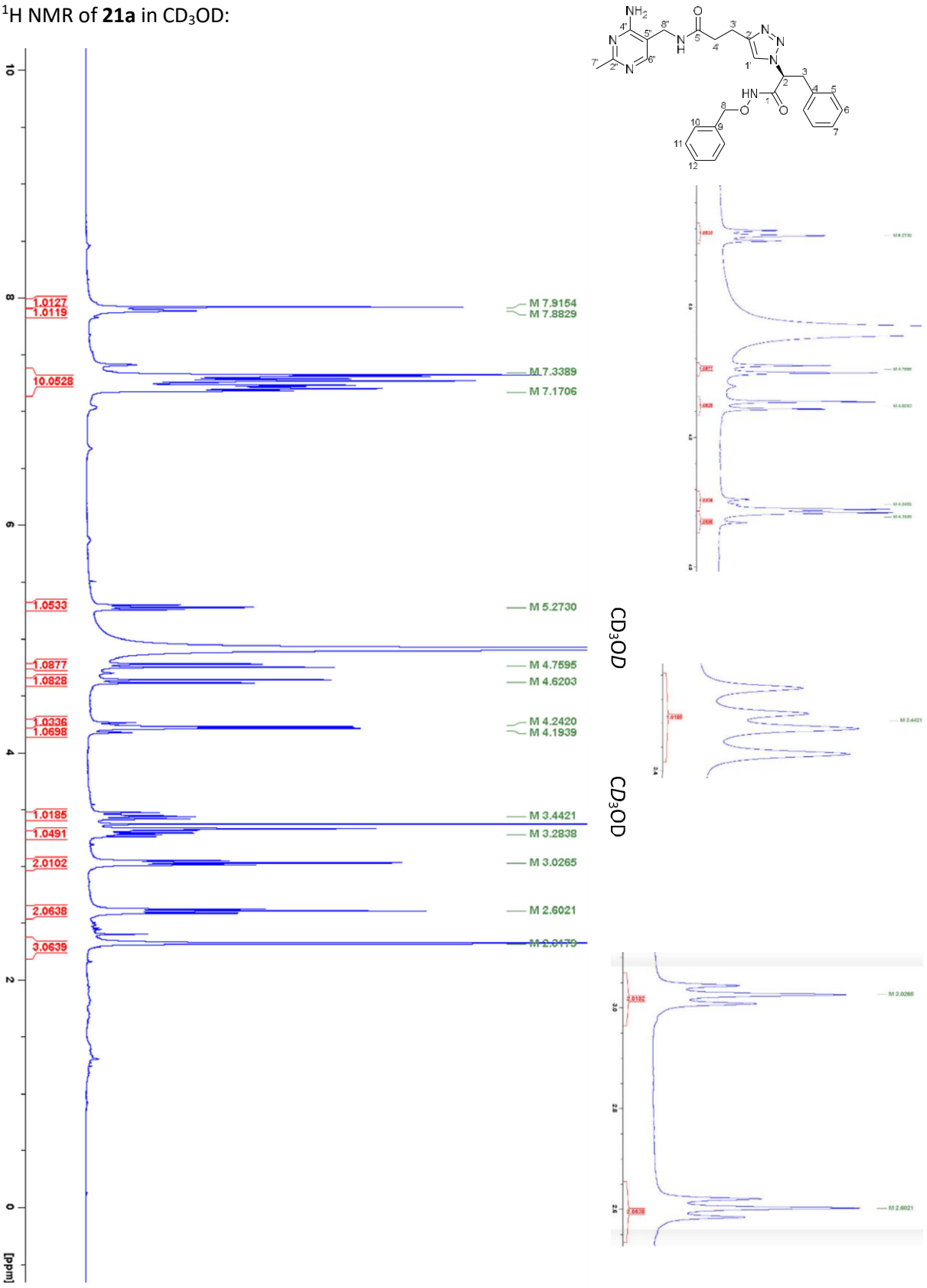




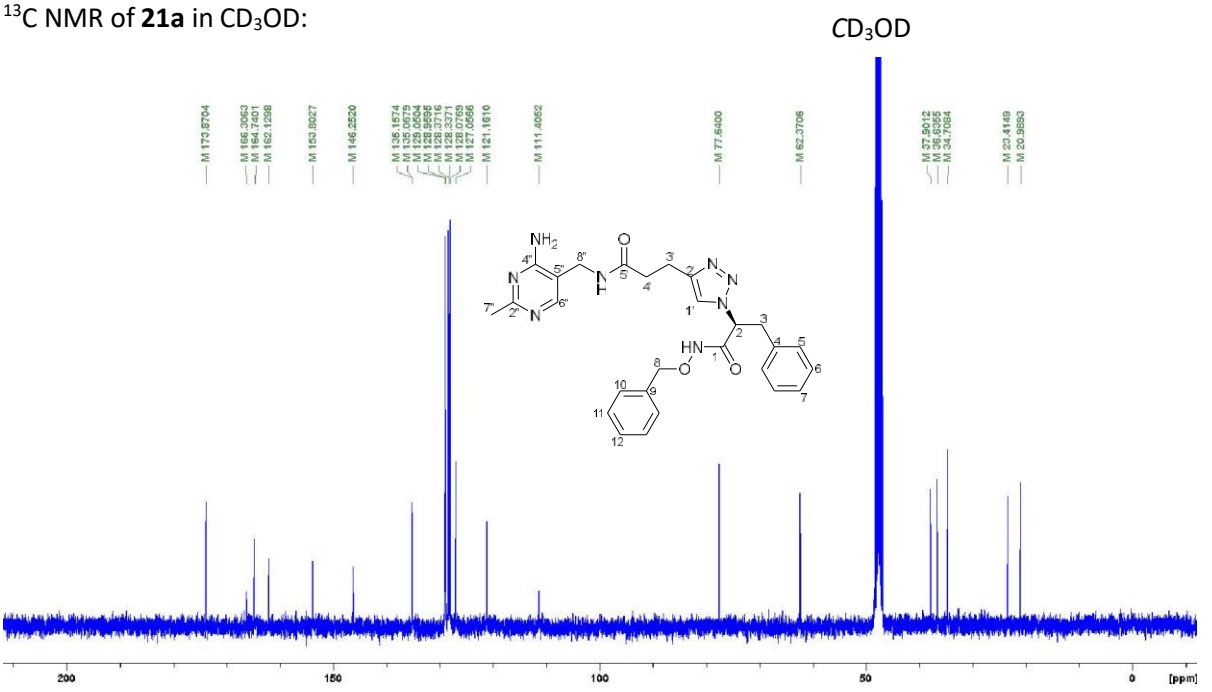
^{13}C NMR of **20** in $CDCl_3$:



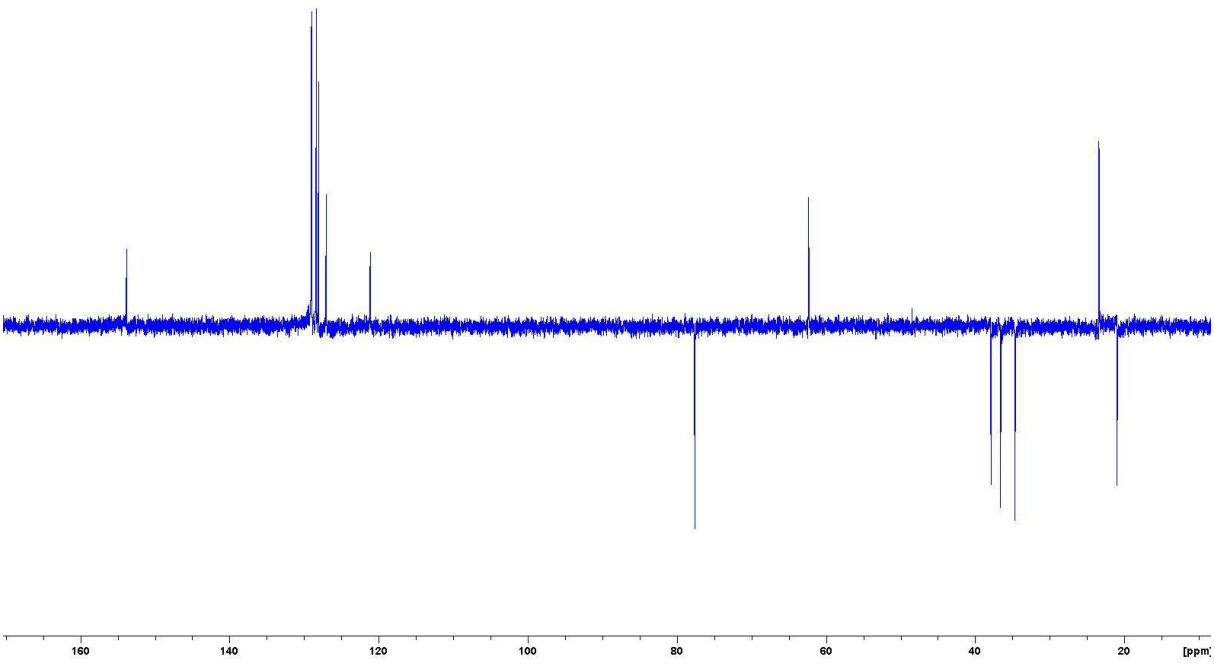
^{1}H NMR of **21a** in CD_3OD:



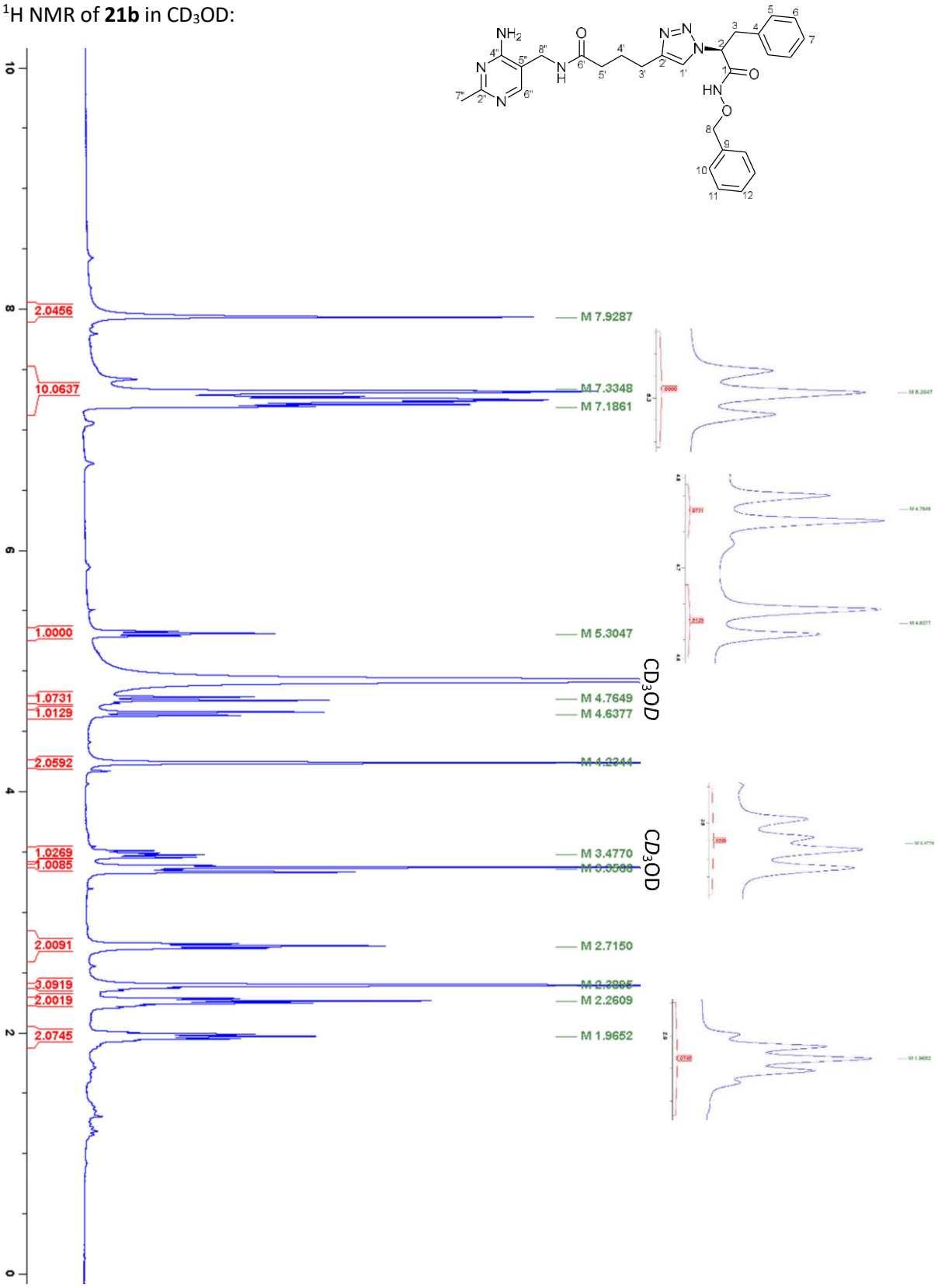
^{13}C NMR of **21a** in CD_3OD:



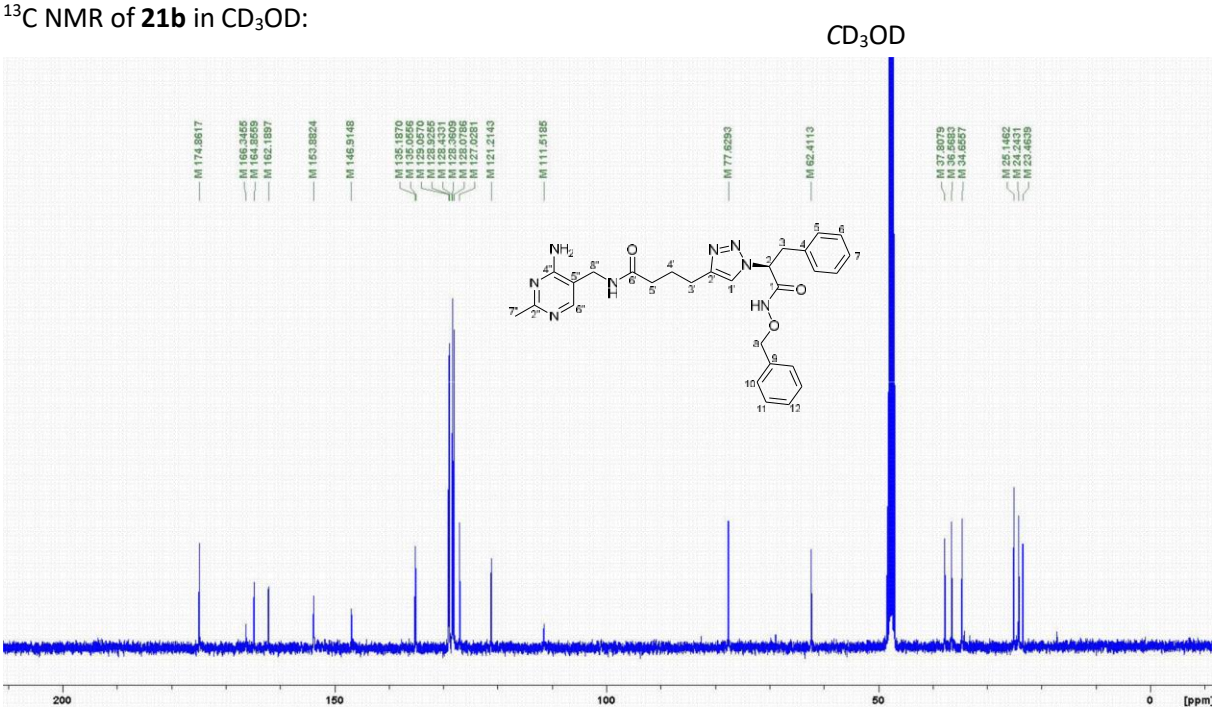
^{13}C DEPT-135 NMR of **21a** in CD_3OD:



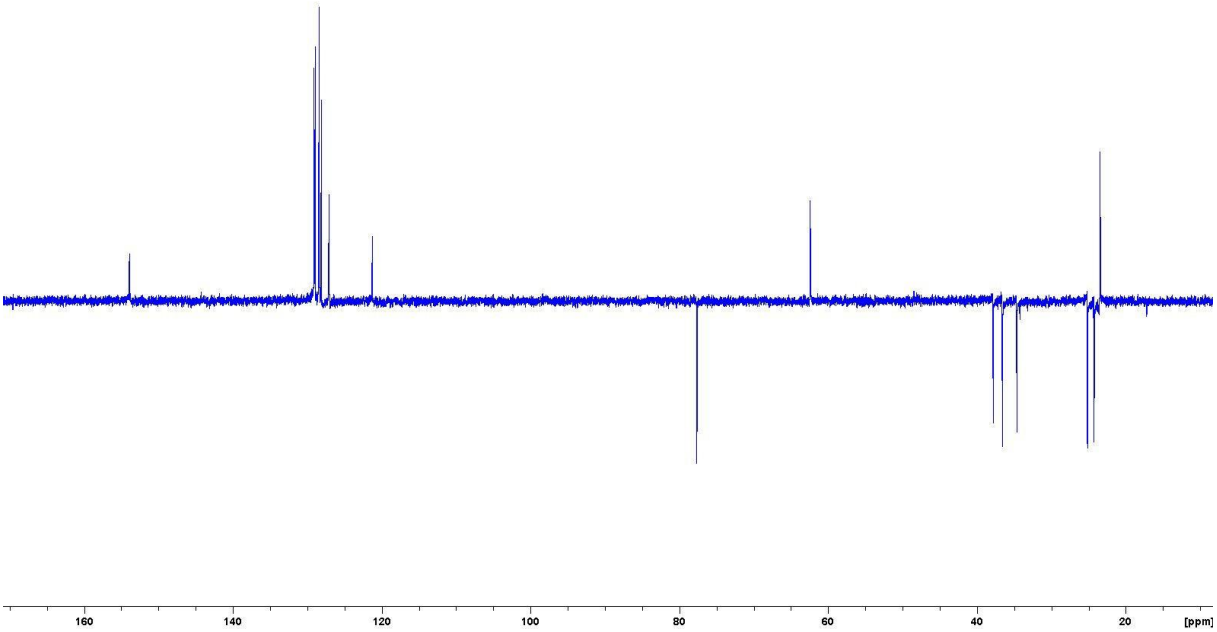
^{1}H NMR of **21b** in CD_3OD:



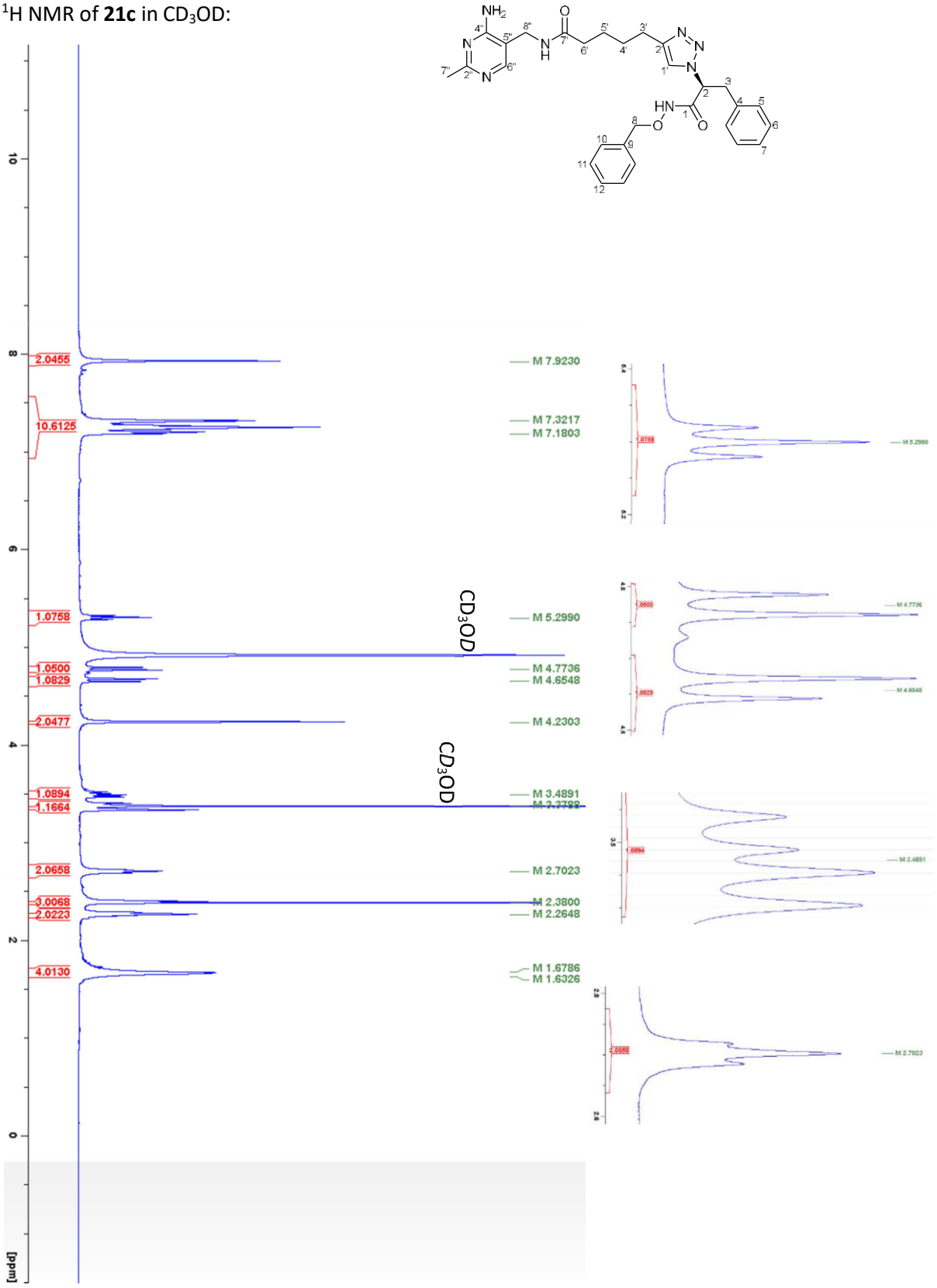
^{13}C NMR of **21b** in CD_3OD:



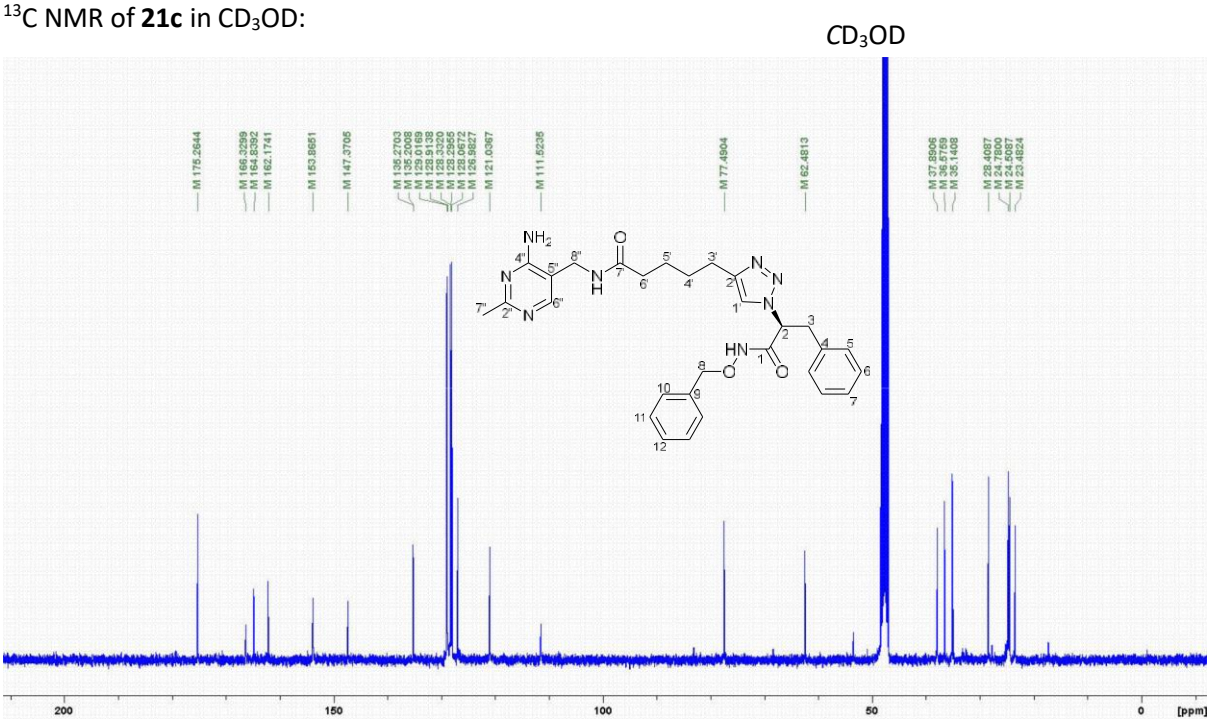
^{13}C DEPT-135 NMR of **21b** in CD_3OD:



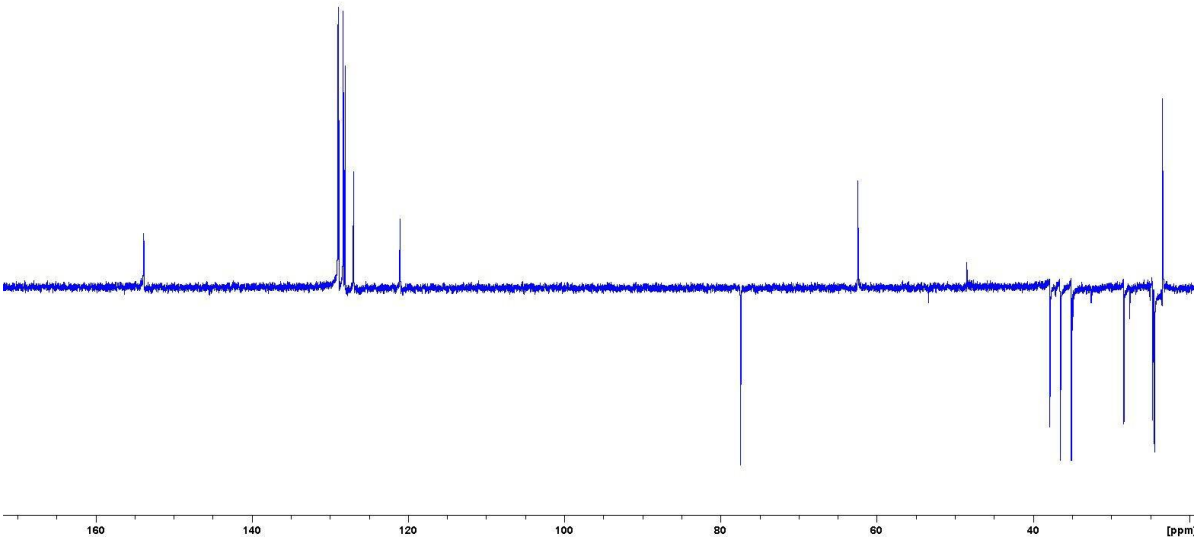
^{1}H NMR of **21c** in CD_3OD:



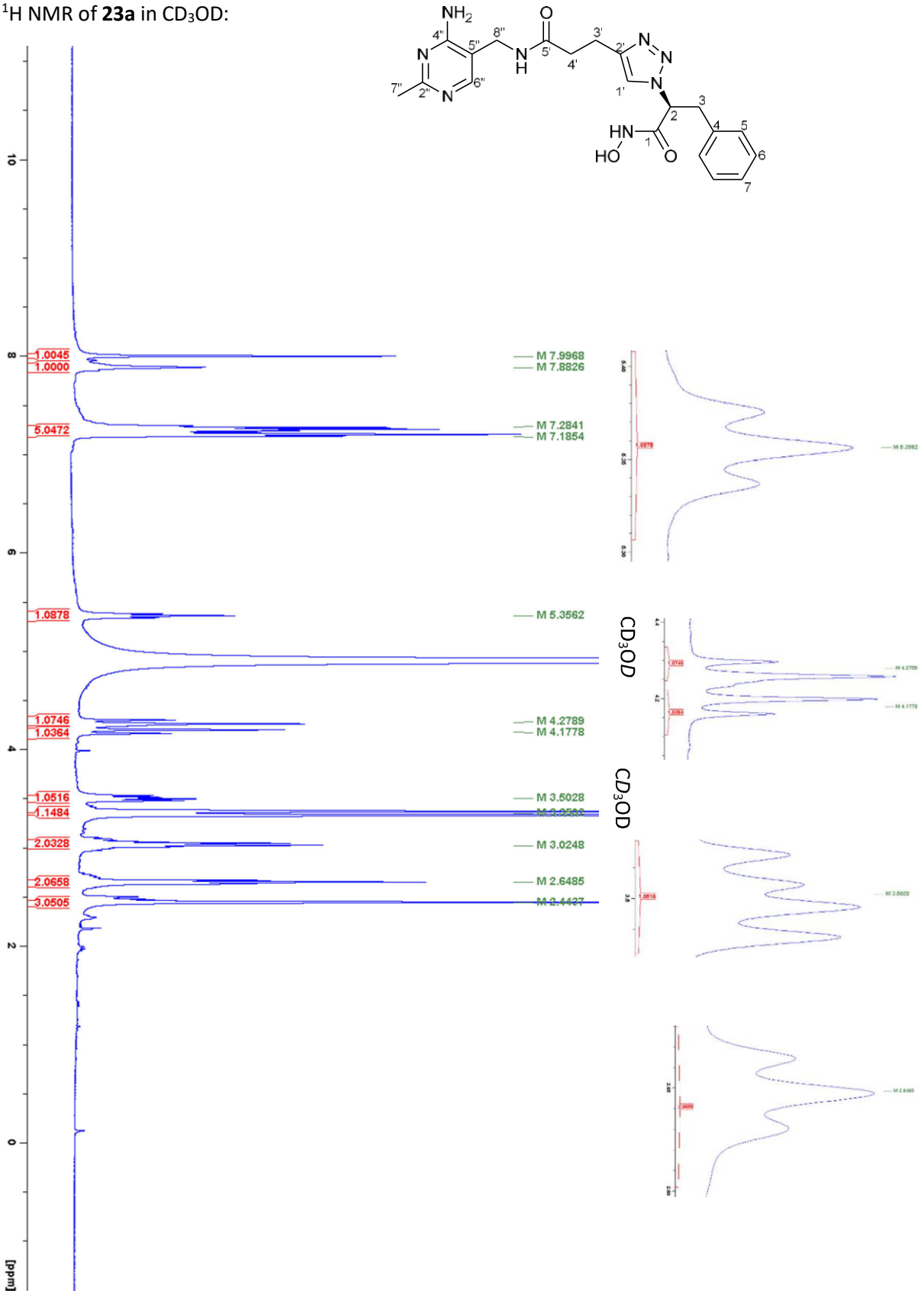
^{13}C NMR of **21c** in CD_3OD:



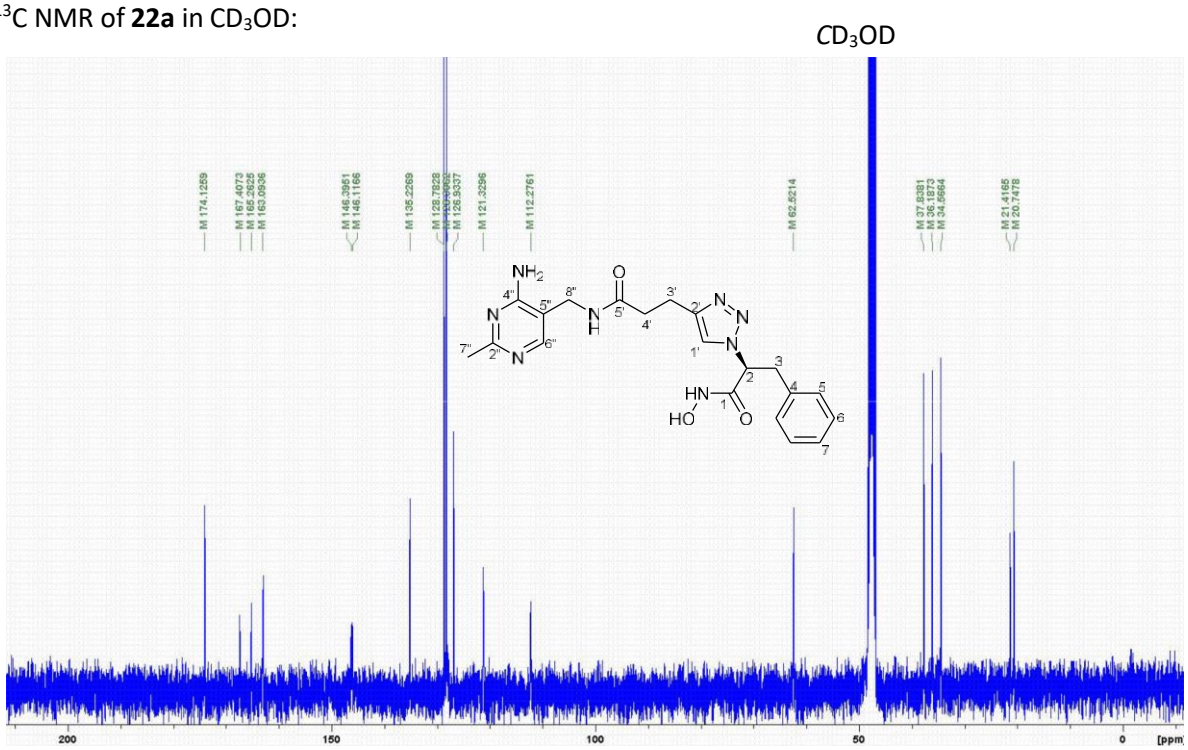
^{13}C DEPT-135 NMR of **21c** in CD_3OD:



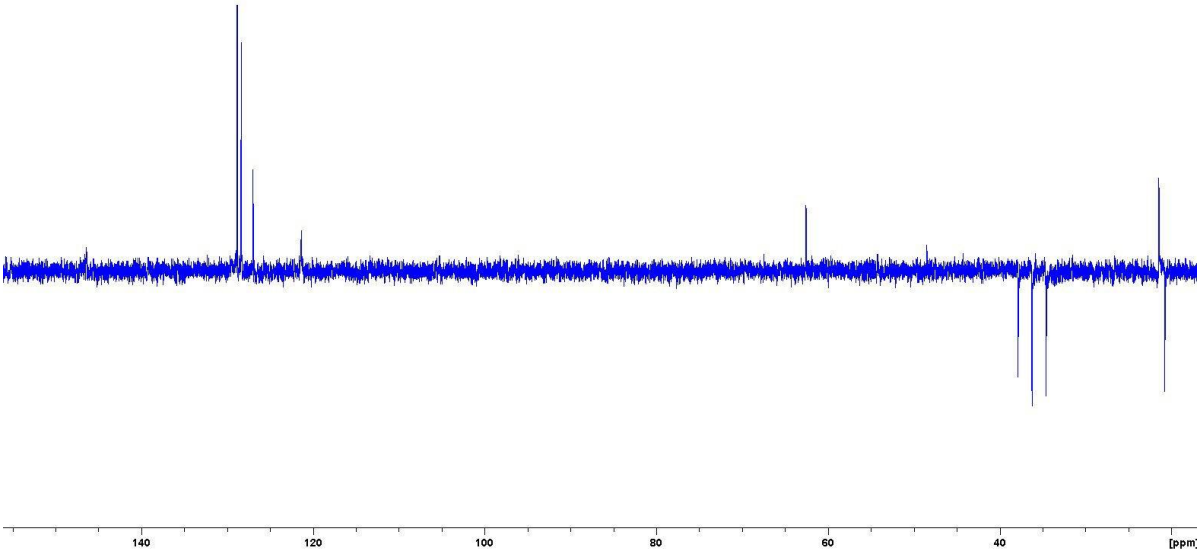
^{1}H NMR of **23a** in CD_3OD:



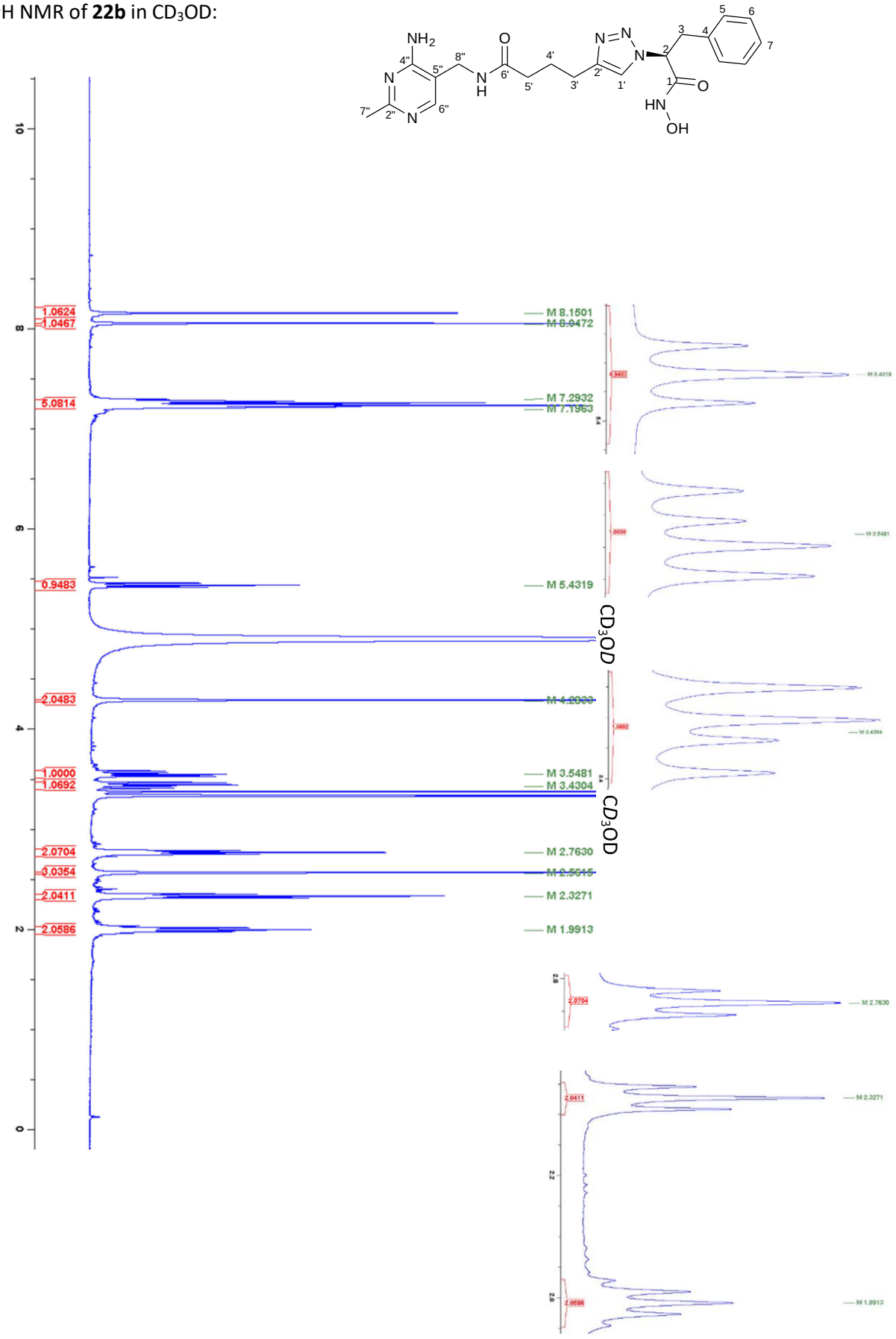
^{13}C NMR of **22a** in CD_3OD:



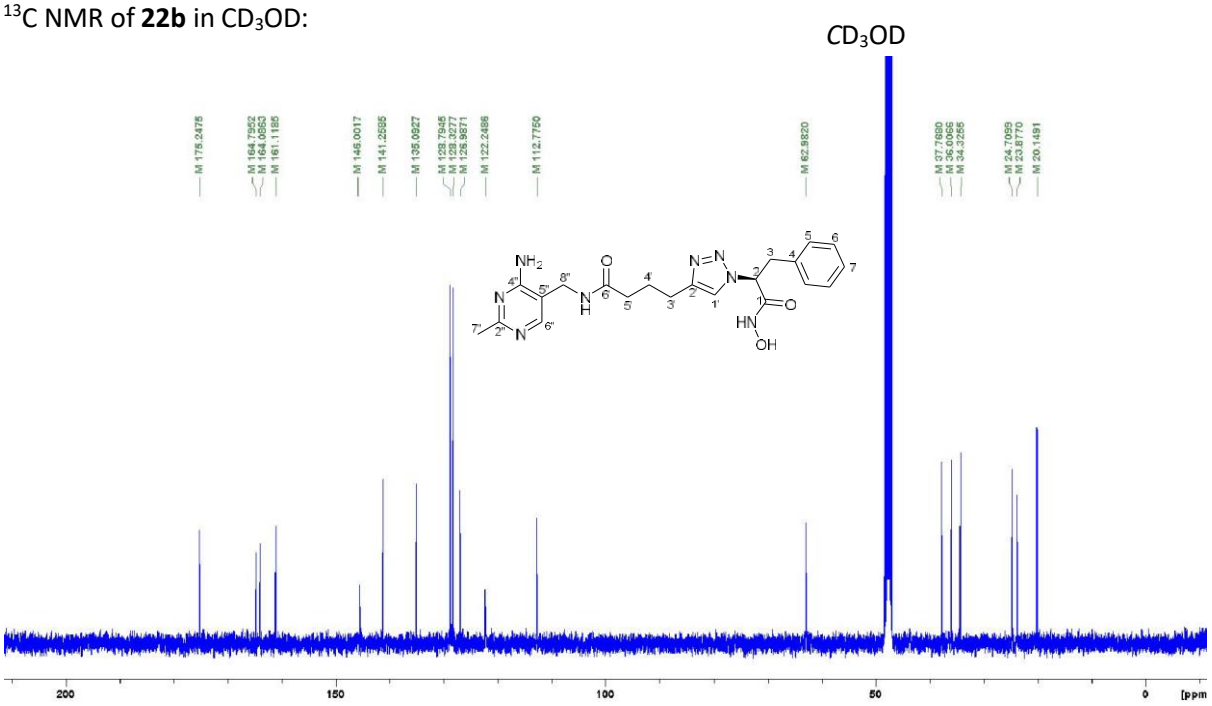
^{13}C DEPT-135 NMR of **22a** in CD_3OD:



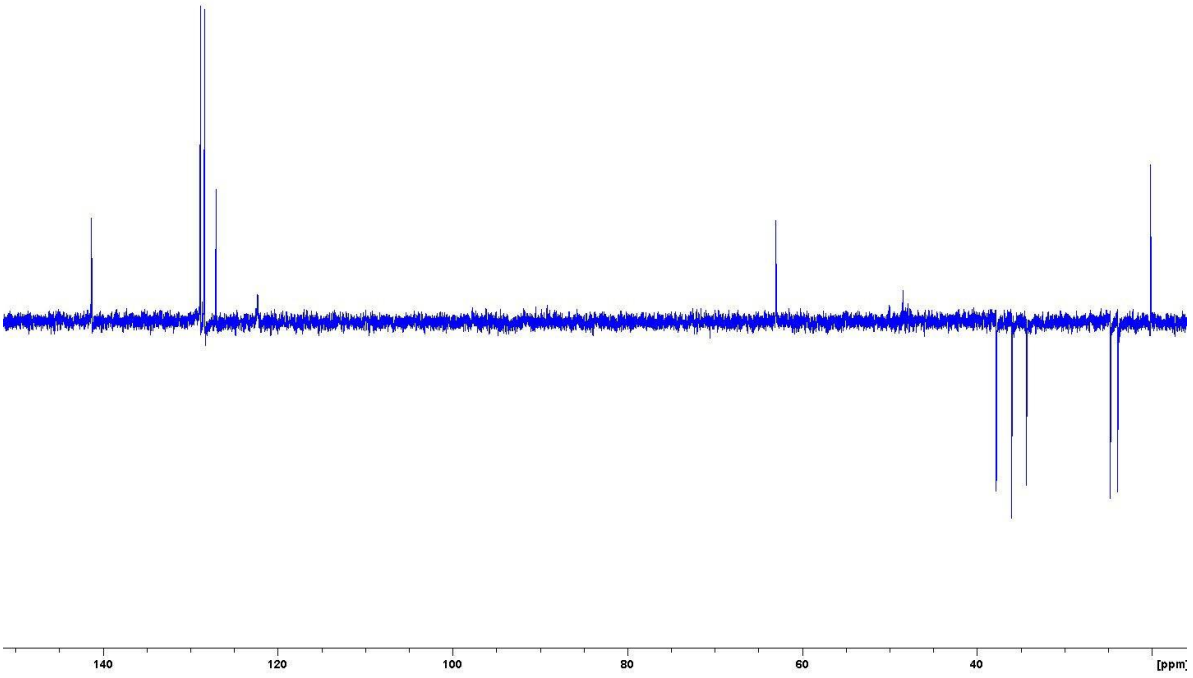
^{1}H NMR of **22b** in CD_3OD:



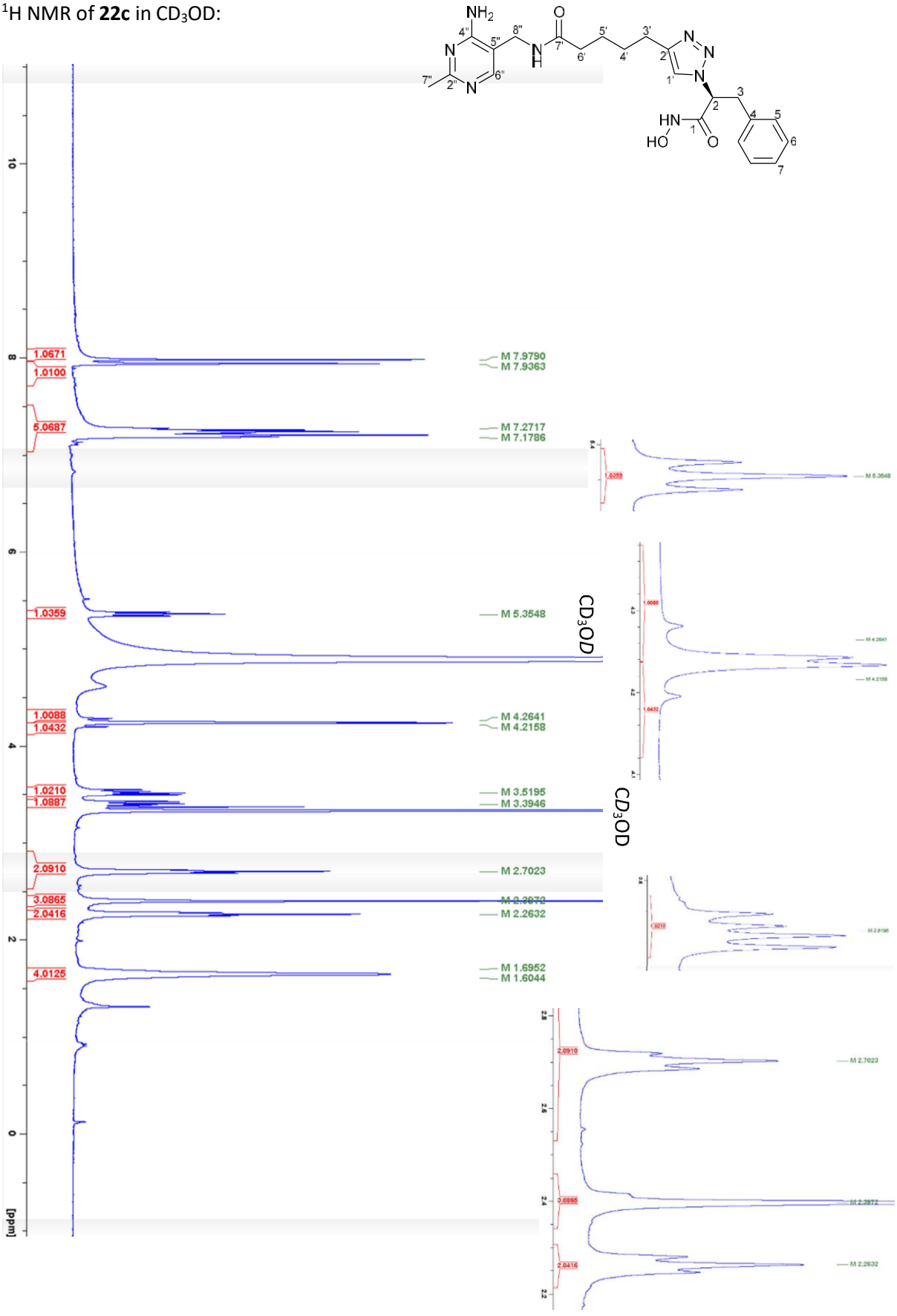
^{13}C NMR of **22b** in CD_3OD:



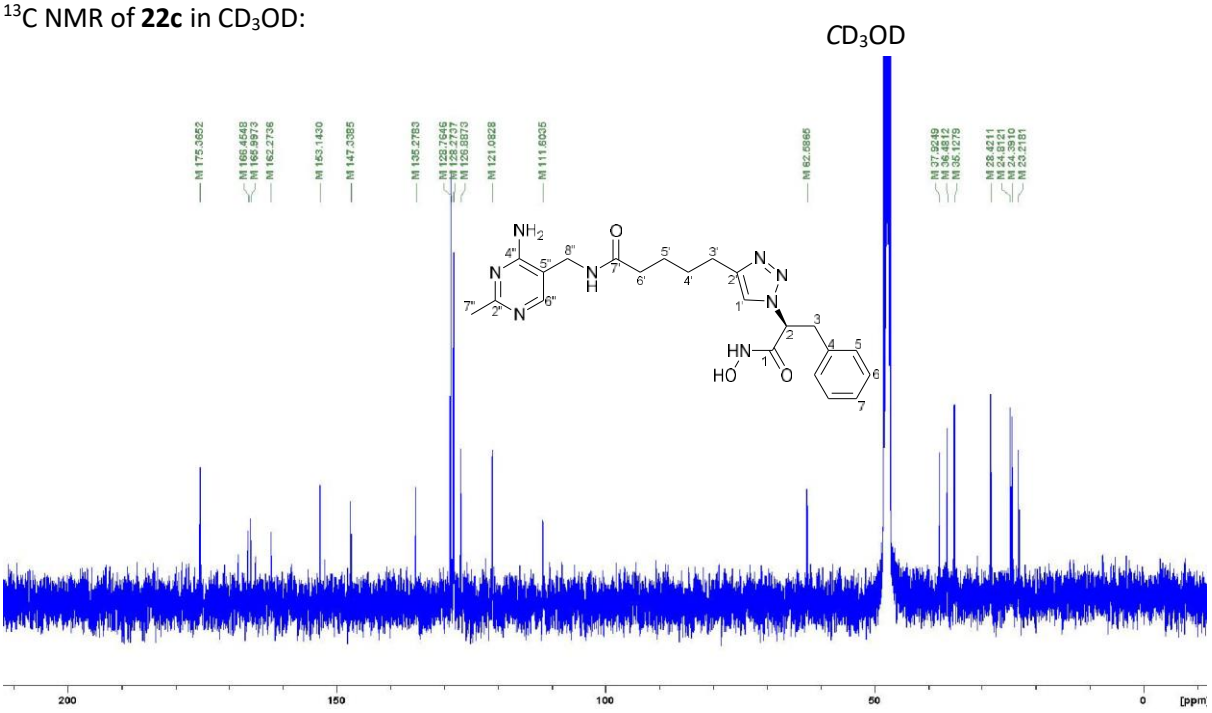
^{13}C DEPT-135 NMR of **22b** in CD_3OD:



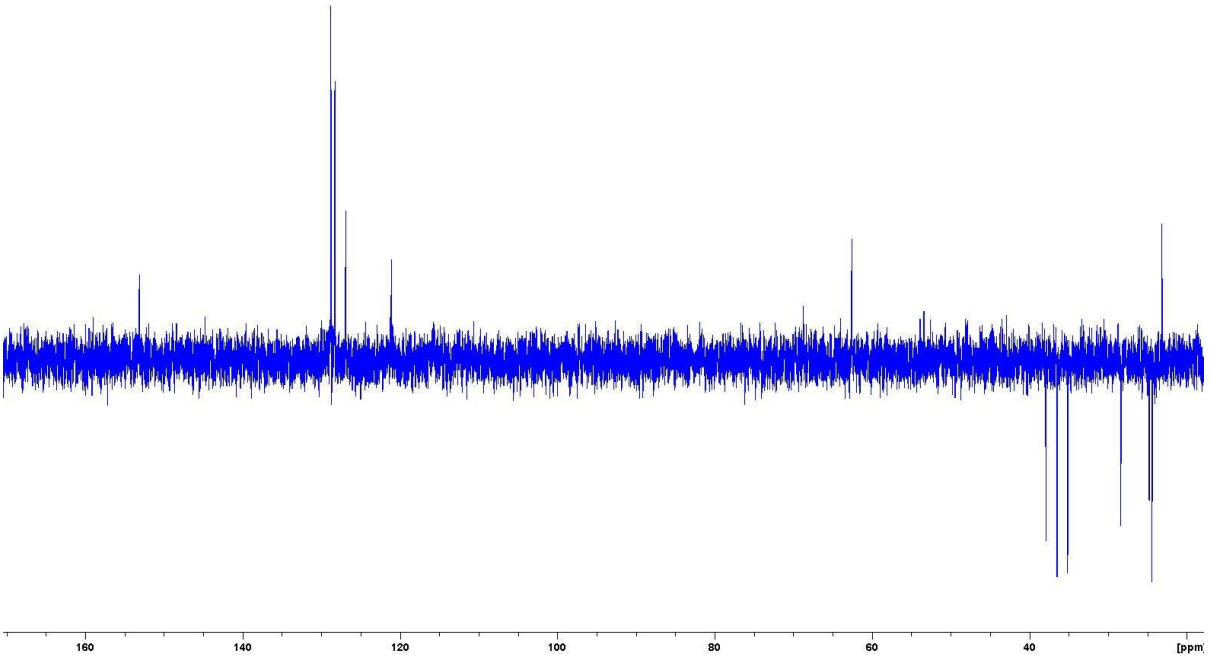
^{1}H NMR of **22c** in CD_3OD:



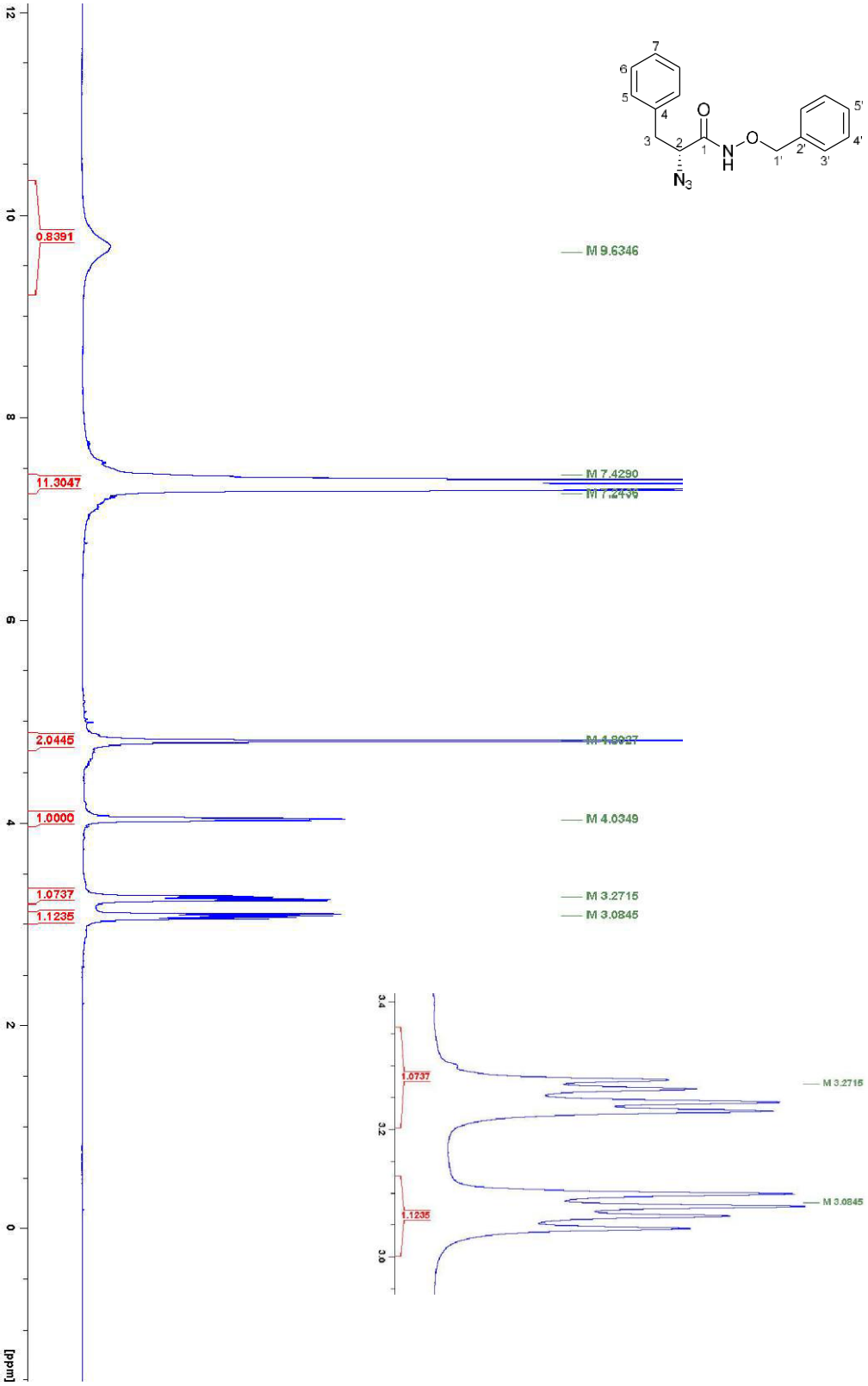
^{13}C NMR of **22c** in CD_3OD:



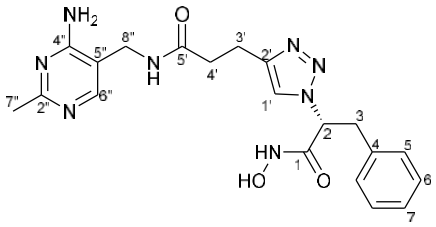
^{13}C DEPT-135 NMR of **22c** in CD_3OD:



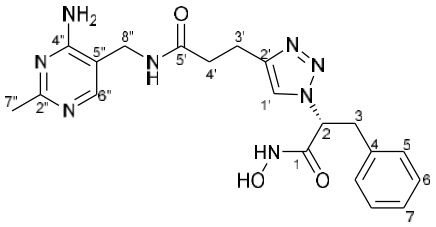
^{1}H NMR of (2R)-2-Azido-*N*-(benzyloxy)-3-phenylpropanamide in $CDCl_3$:

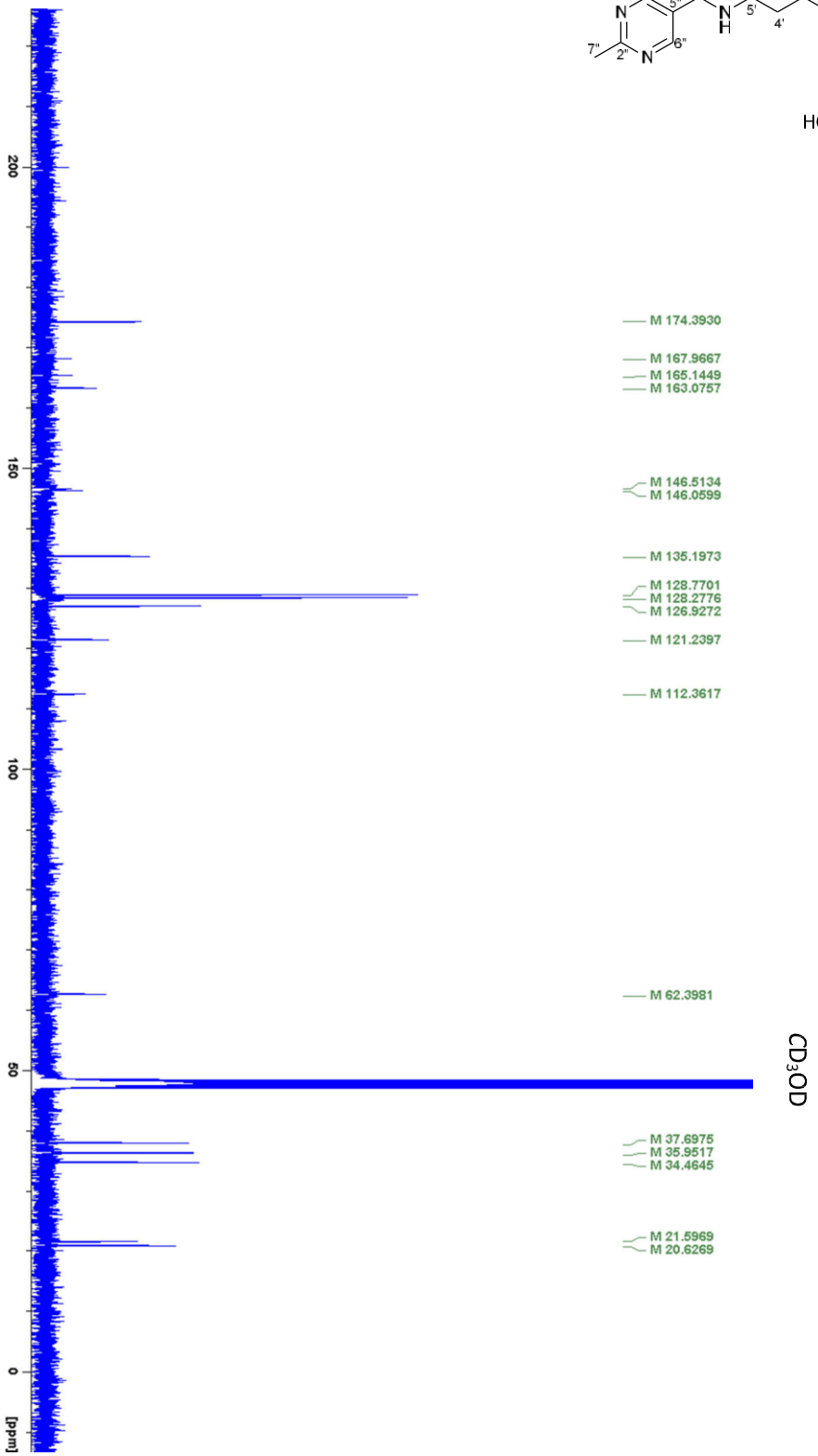


^{1}H NMR of **23a** in CD_3OD:

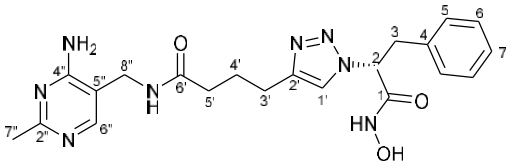


^{13}C NMR of **23a** in CD_3OD:

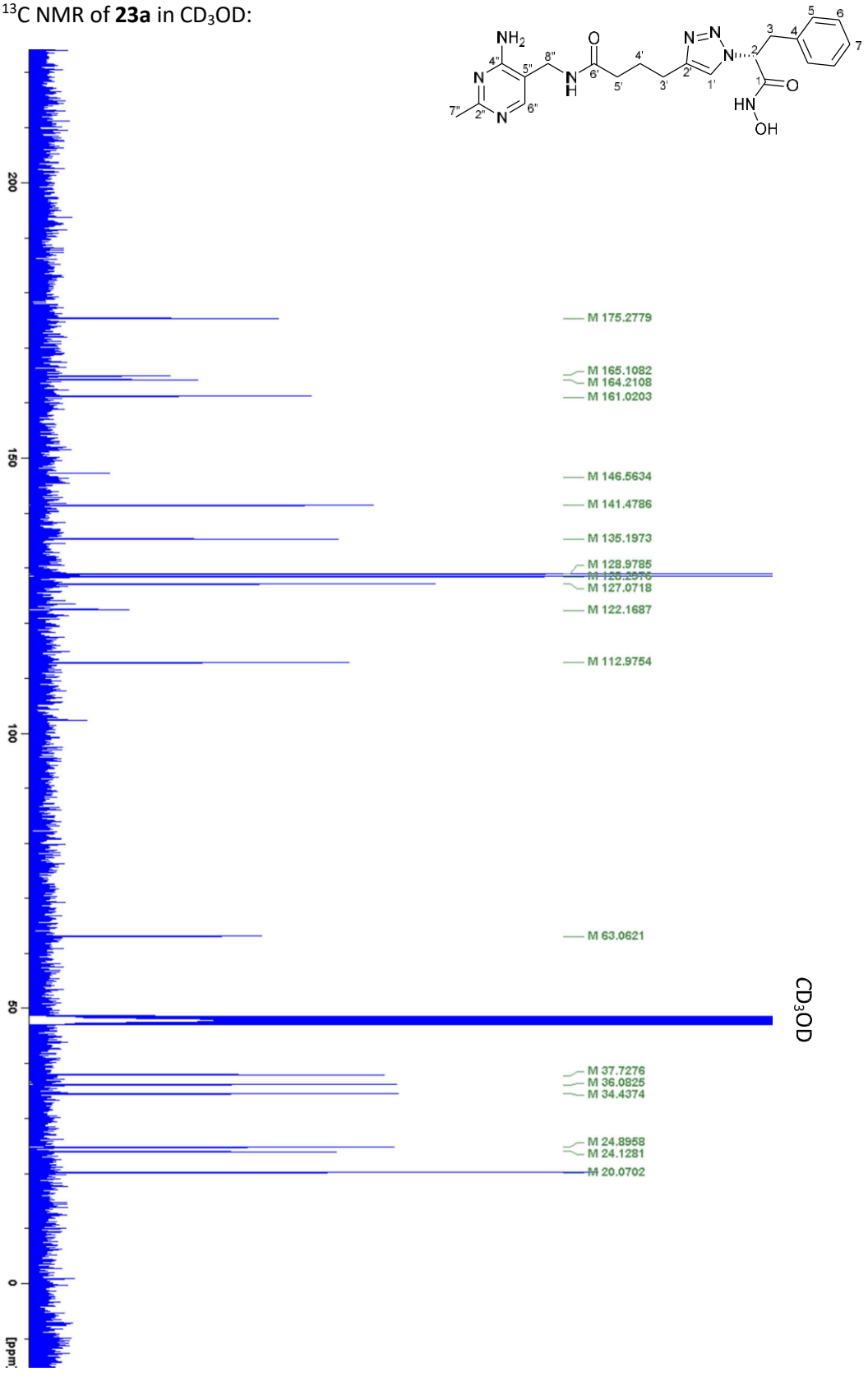




^{1}H NMR of **23b** in CD_3OD:



^{13}C NMR of **23a** in CD_3OD:



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