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

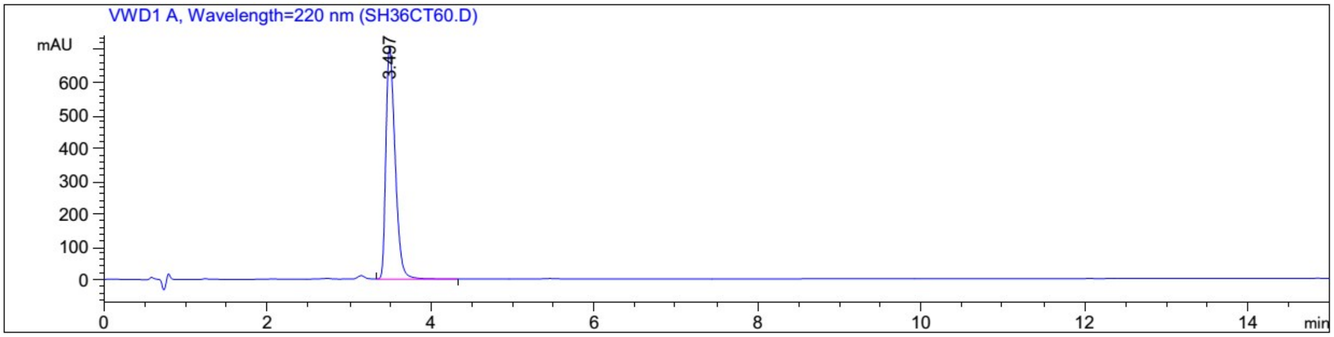
Formation of N^{α}-Terminal 2-Dialkyl Amino Oxazoles from Guanidinated Derivatives under Mild Conditions

S A. H. Abdel Monaim,[#,a,b] J. T. Mhlongo[#,a,b] A. Kumar,[a] A. El-Faham,[c,d] F. Albericio *[b,c,e] and B. G. de la Torre*[a]

[a] KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa
[b] Peptide Science Laboratory Institution, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4001, South Africa
[c] Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[d] Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Ibrahimia, 12321 Alexandria, Egypt
[e] Department of Organic Chemistry, CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, University of Barcelona, Barcelona 08028, Spain
#Both authors have contributed equally to the work
Corresponding Author
* E-mail: *Email: albericio@ukzn.ac.za, garciadelatorreb@ukzn.ac.za

Figure S1: HPLC of **NH_2-D-Phe-Gly-OH**:

HPLC condition: A linear gradient of 8–20% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 20°C.



B) Guanidination of the model sequence with HBTU or COMU:

Figure S2: HPLC chromatogram after guanidinylation with HBTU:

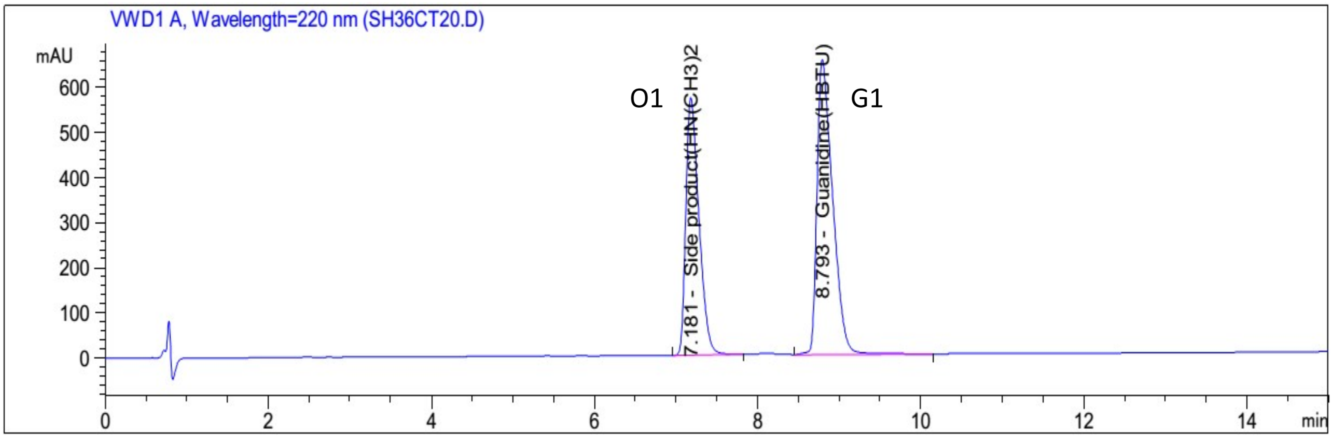


Figure S3: HR-MS spectra for of O1: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{14}H_{18}N_3O_3$ 276.3080; Found 276.1340

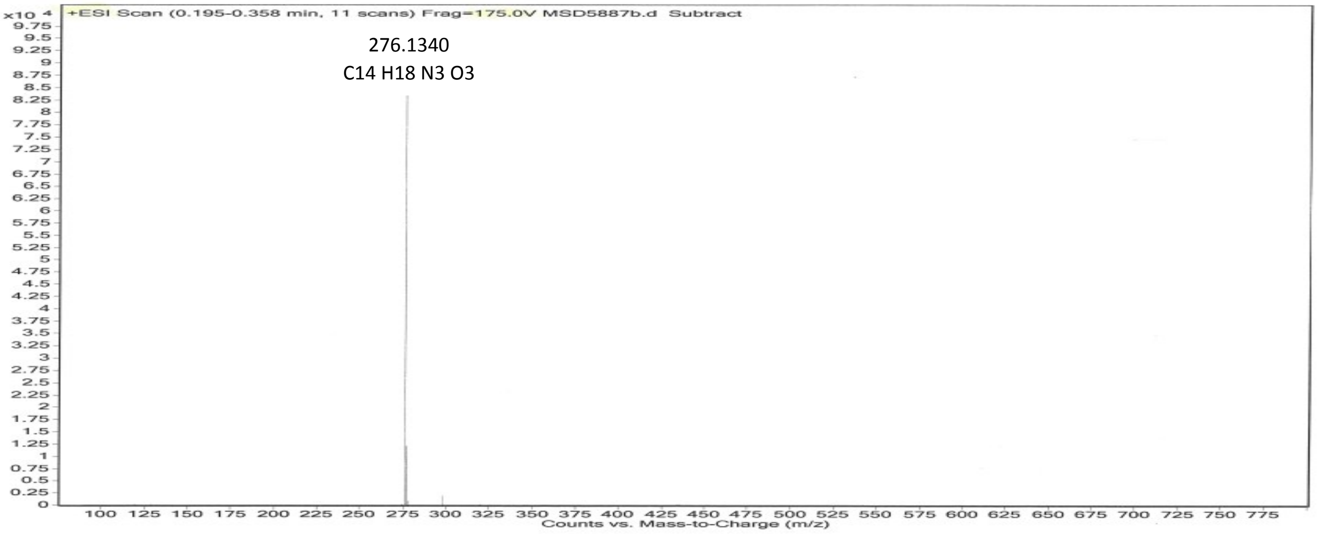


Figure S4: HR-MS spectra for of G1: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{25}N_4O_3$ 321.1848; Found 321.1918

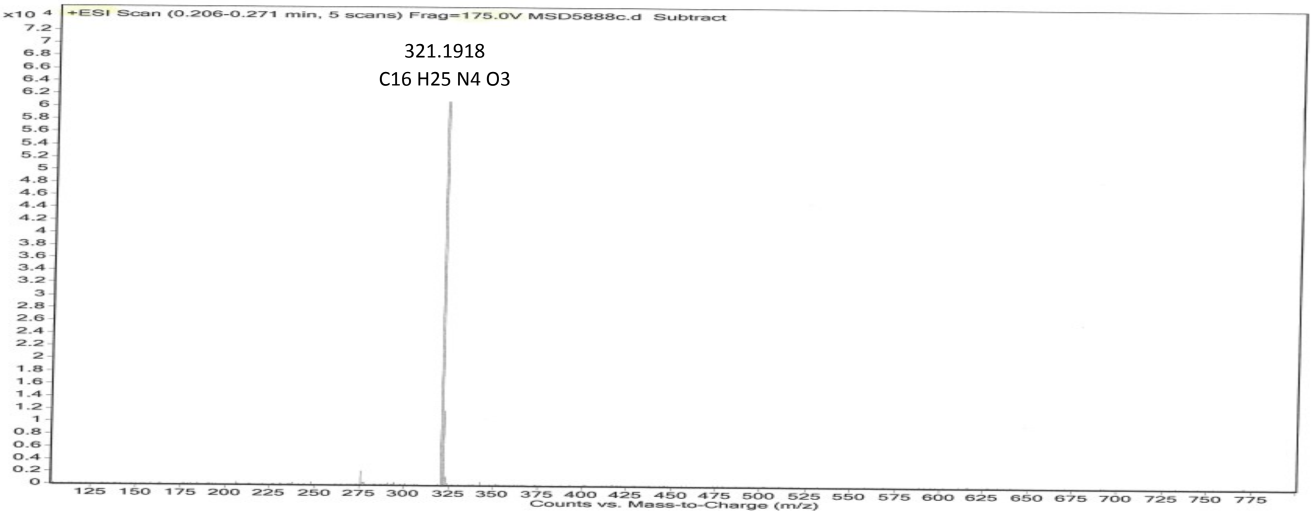


Figure S5: HPLC chromatogram after guanidinylation with COMU:

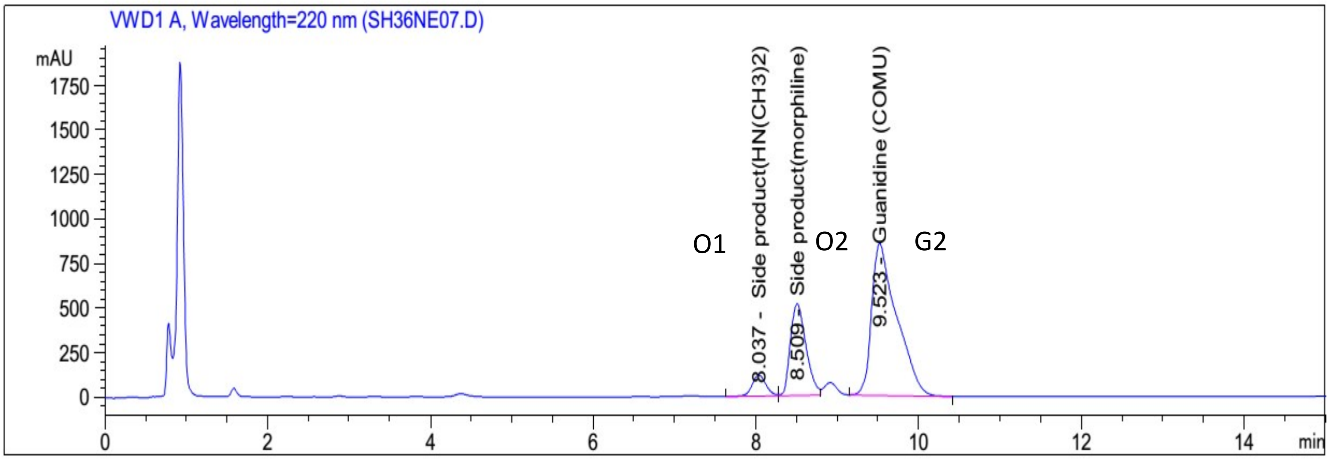


Figure S6: HR-MS spectra for of O2: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{20}N_3O_4$ 318.1376; Found 314.1446

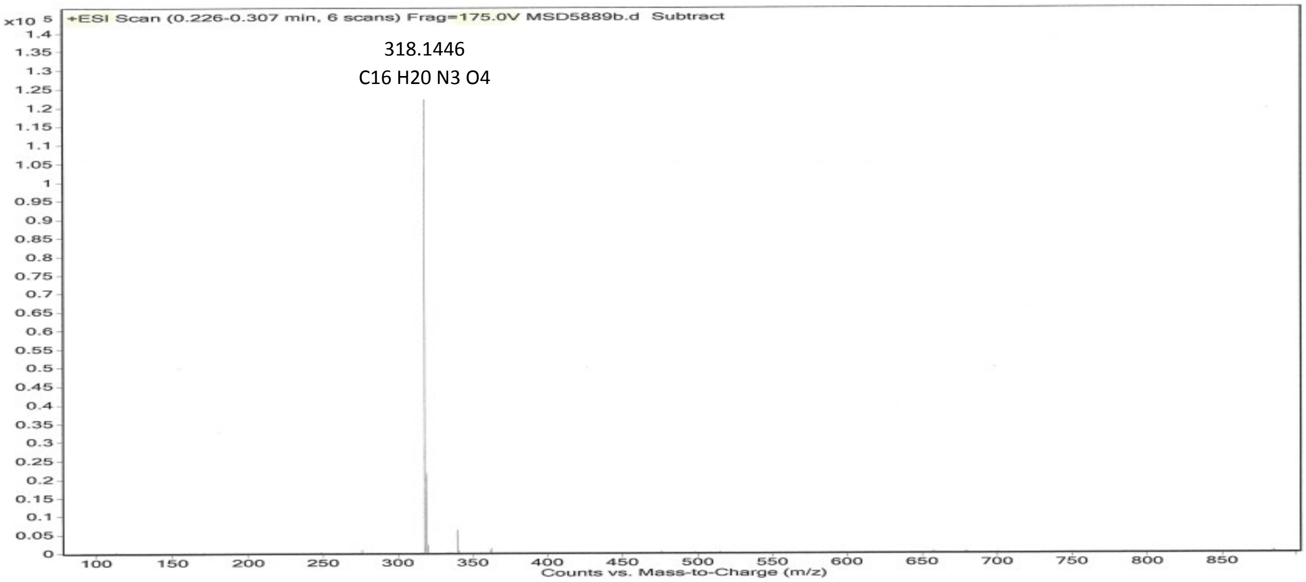


Figure S7: HR-MS spectra for of G2: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{18}H_{26}N_4O_4$ 363.1954; Found 363.2044

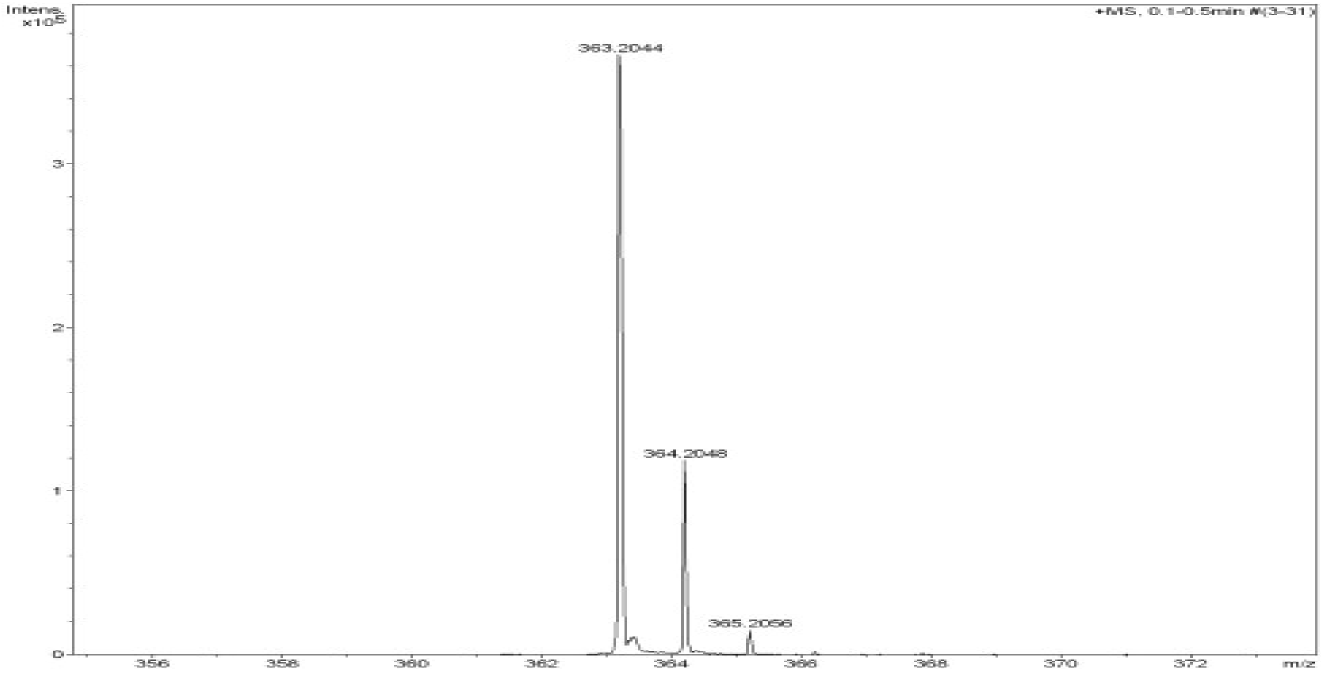


Figure S8: ^{1}HNMR spectra for H-D-Phe-Gly in D_2O

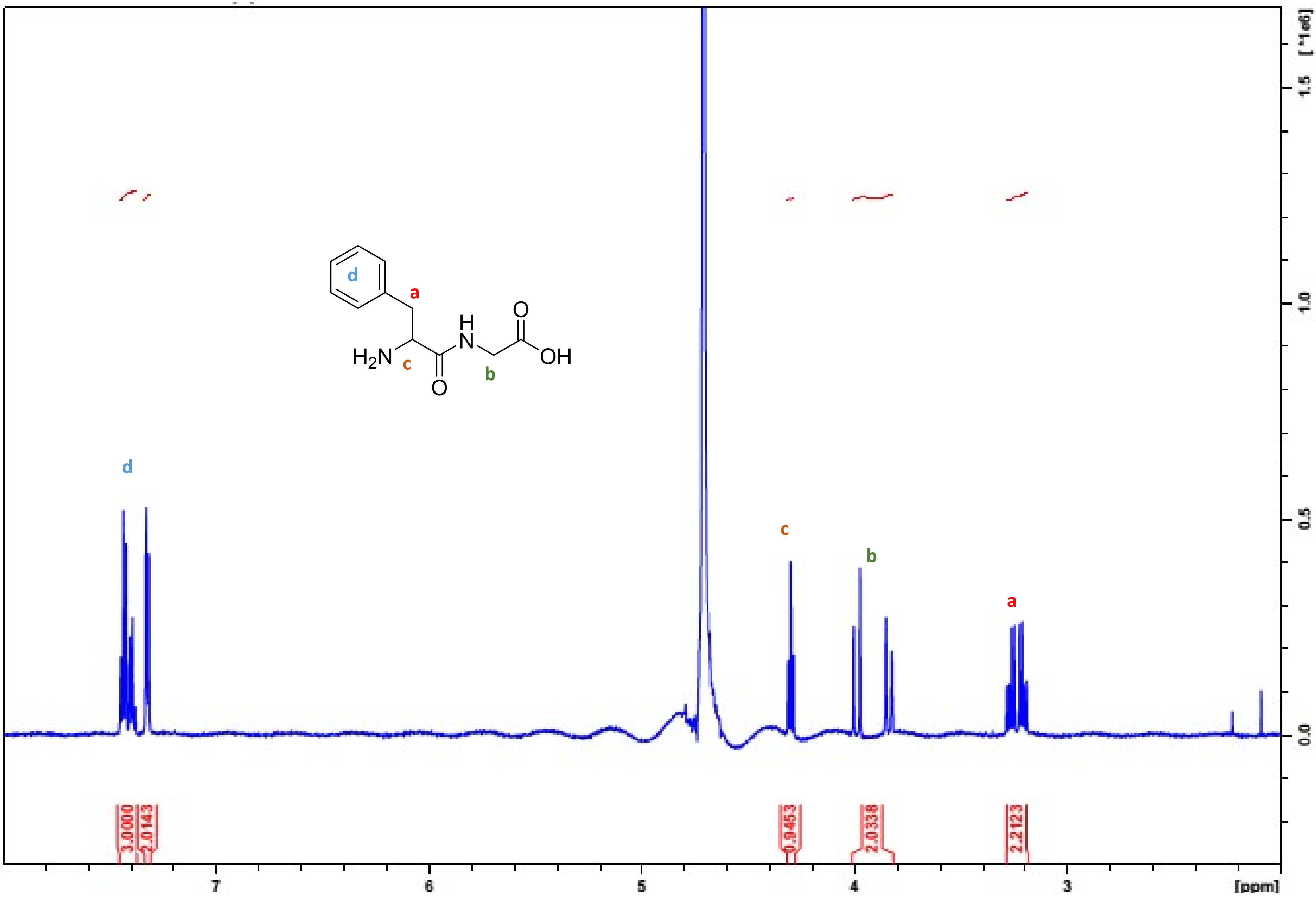


Figure S9: ¹HNMR spectra for G1 in D_2O

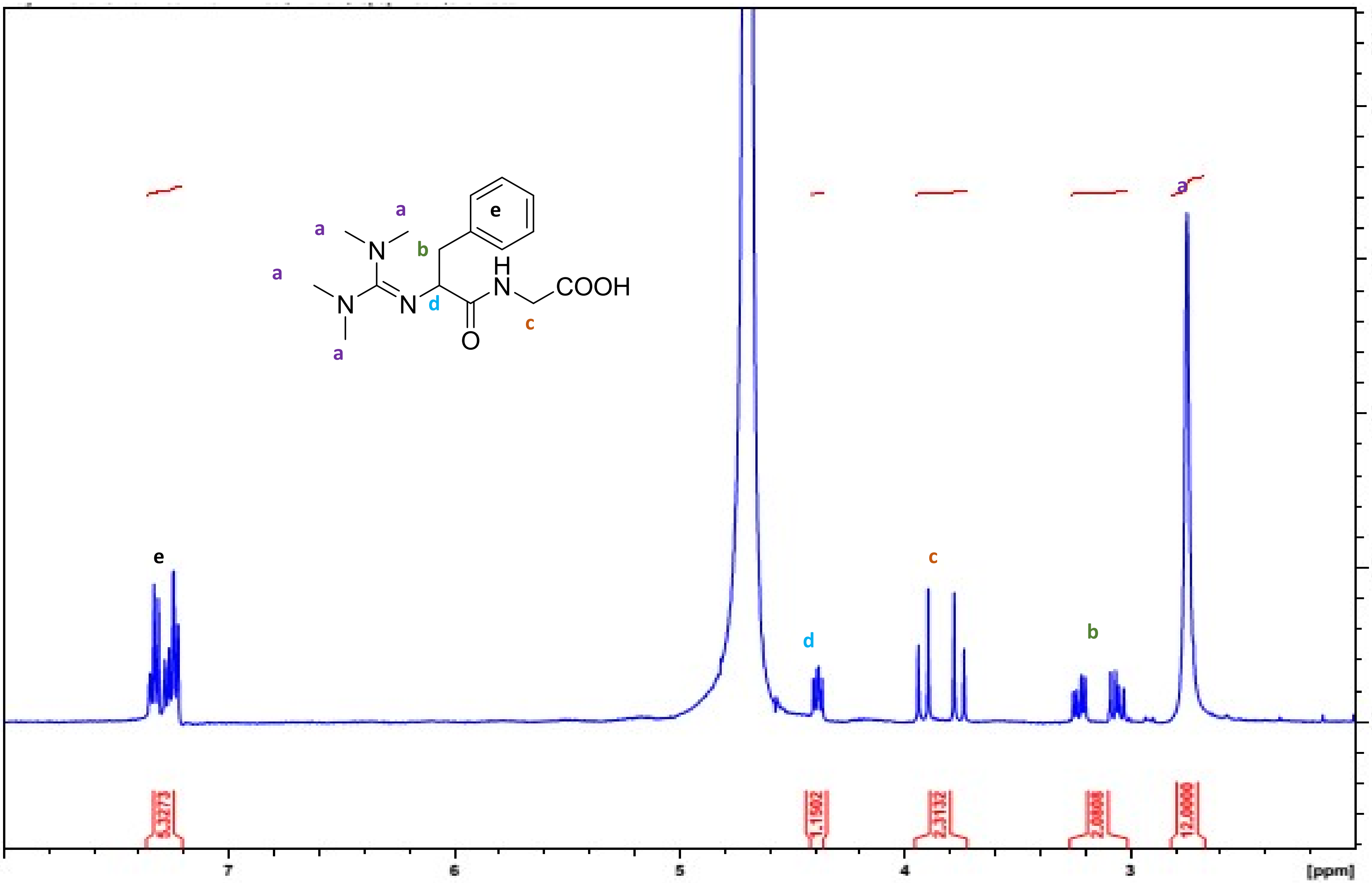


Figure S10: ^{1}HNMR spectra for G2 in D_2O

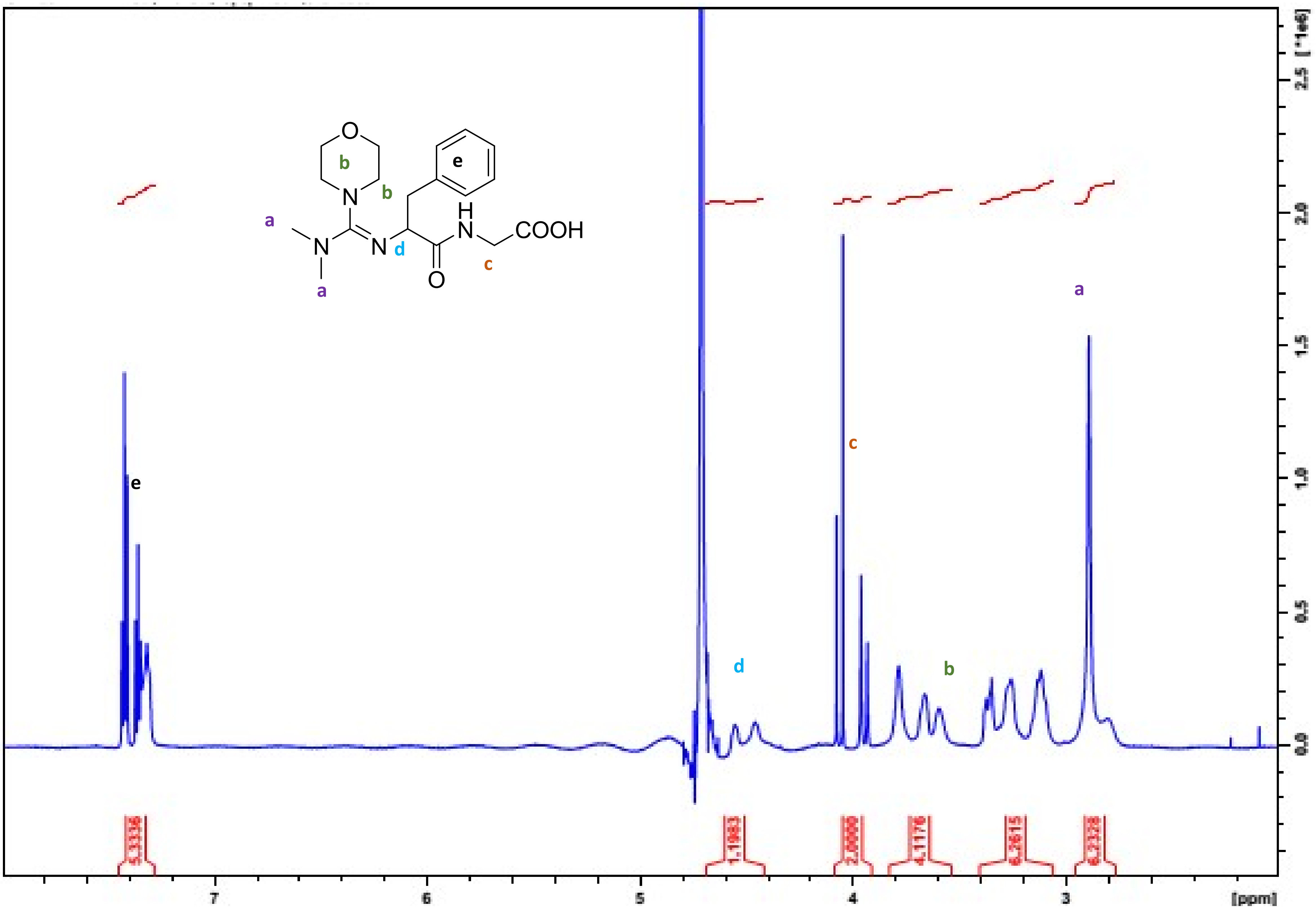


Figure S11: ^{1}HNMR spectra for O1 in D_2O

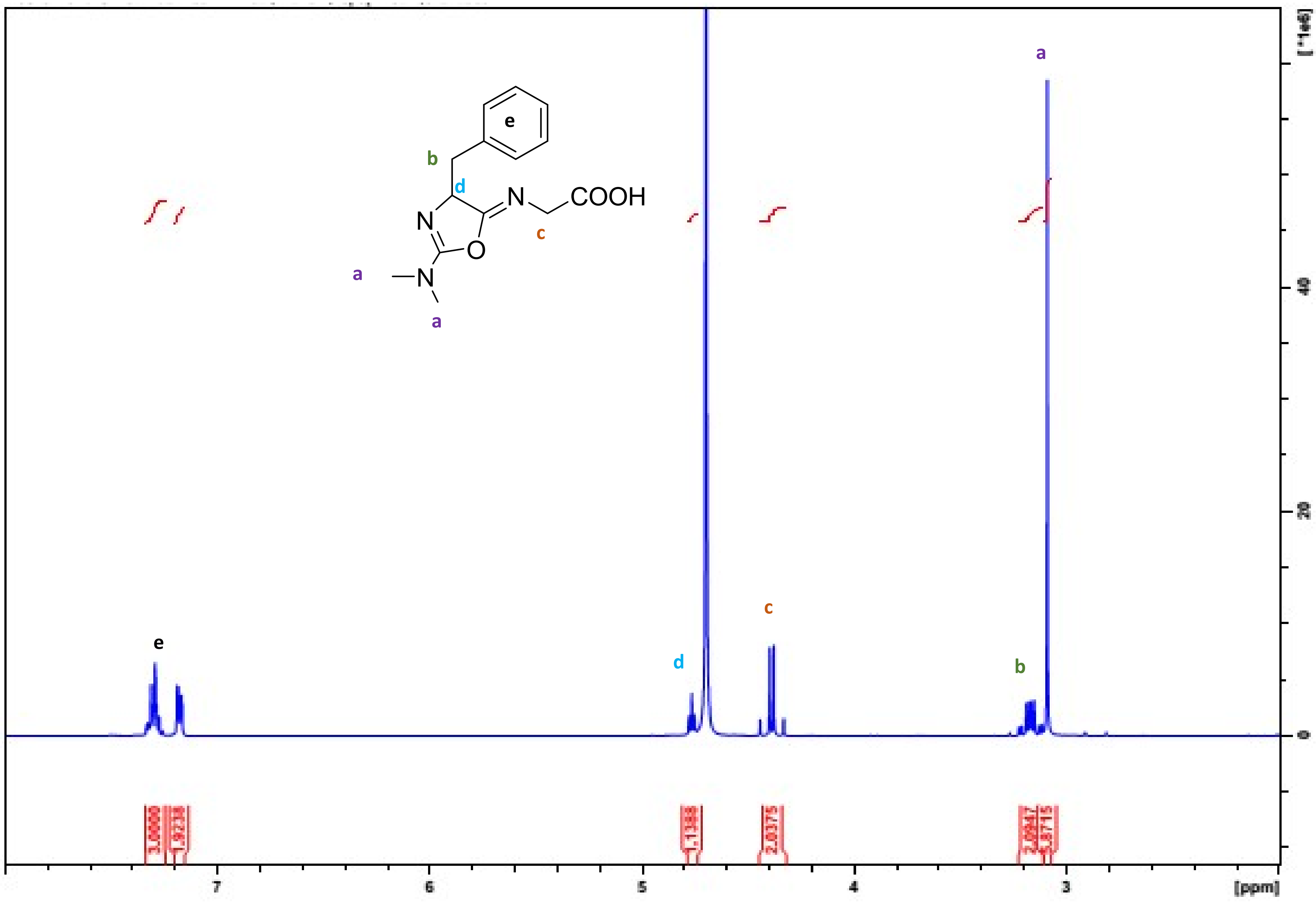


Figure S12: ^{1}HNMR spectra for O1 and O1B in D_2O

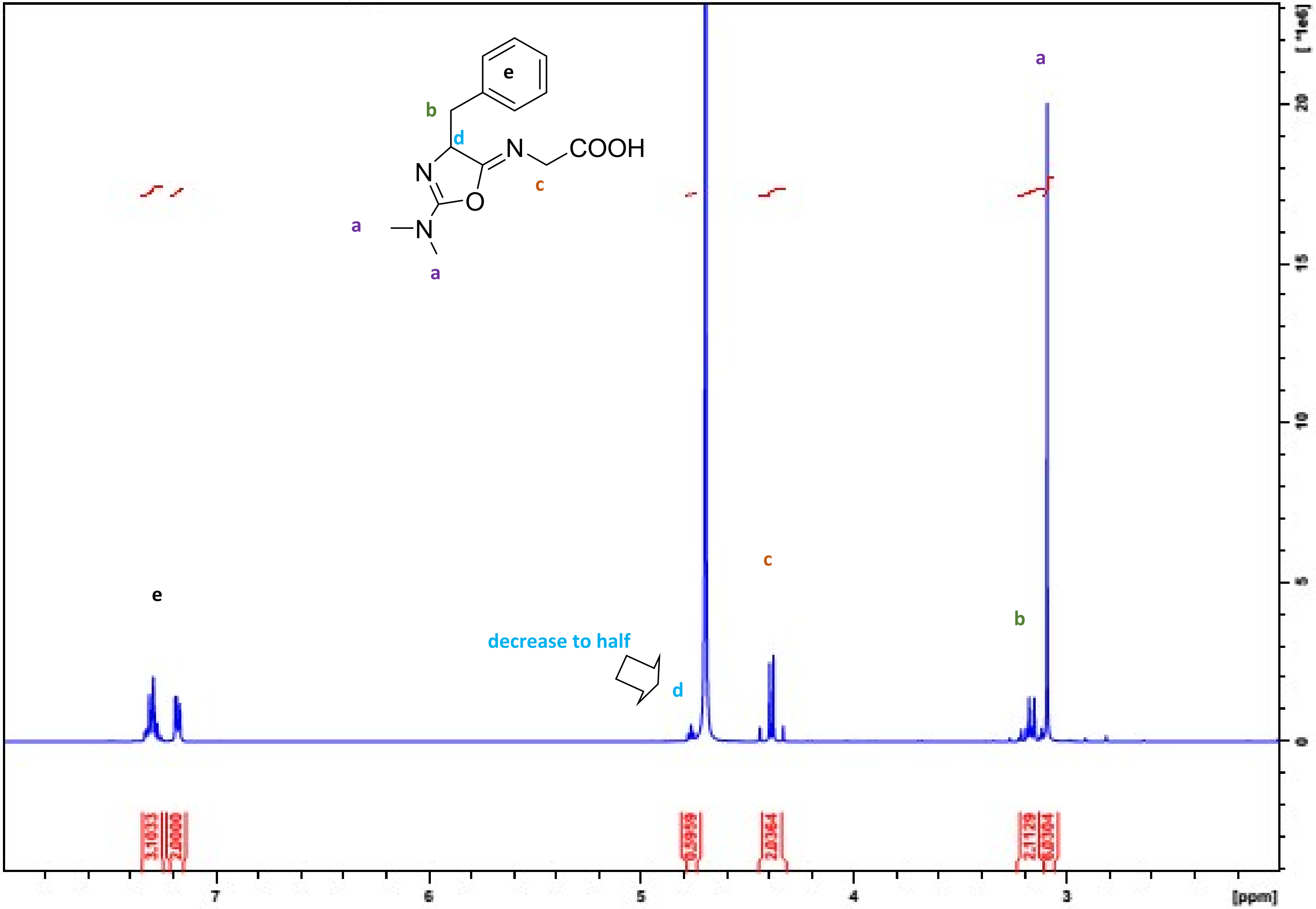


Figure S13: ^{13}CNMR spectra for O1 and O1B in D_2O

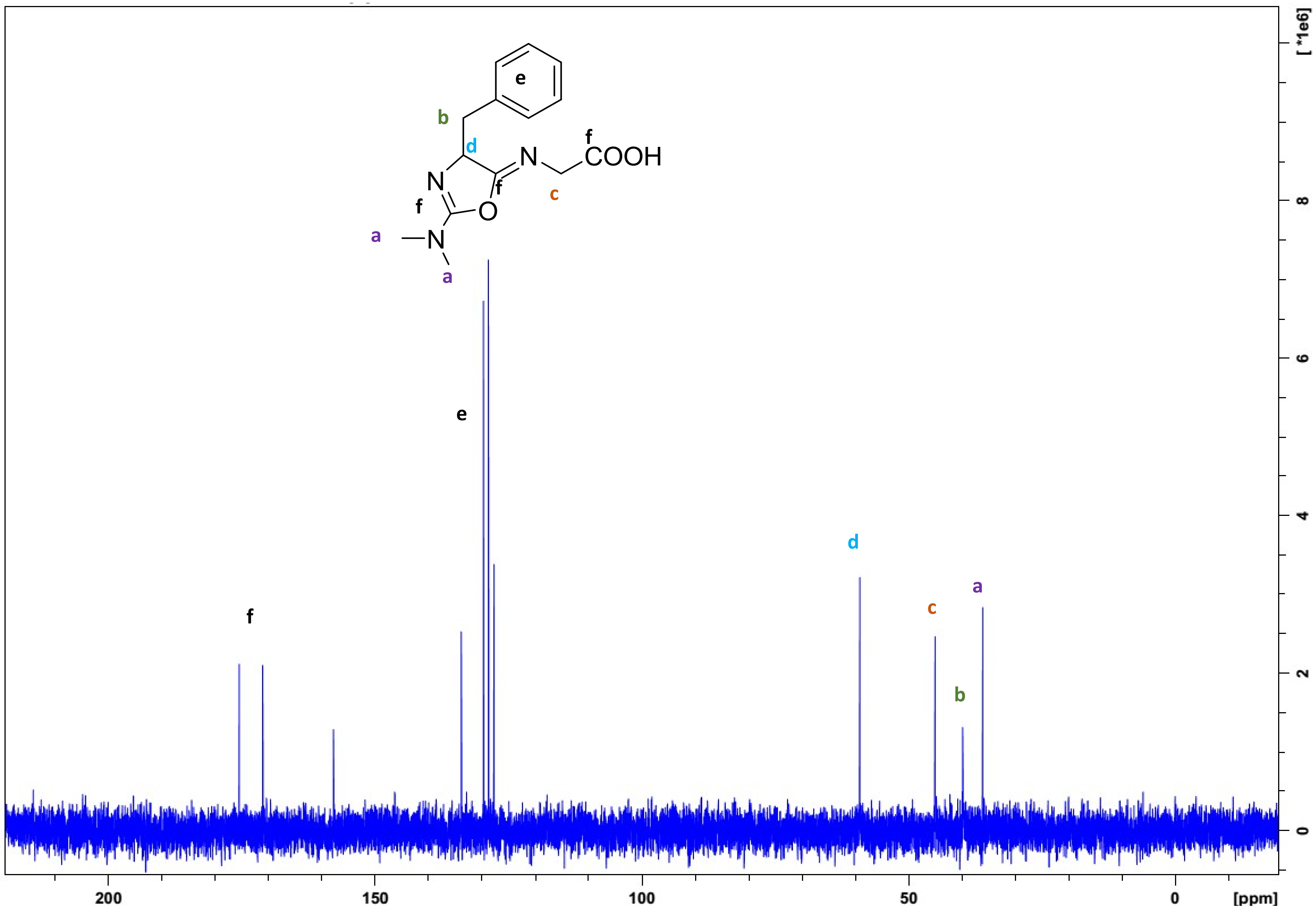


Figure S14: COSY spectra for O1 and O1B in D_2O

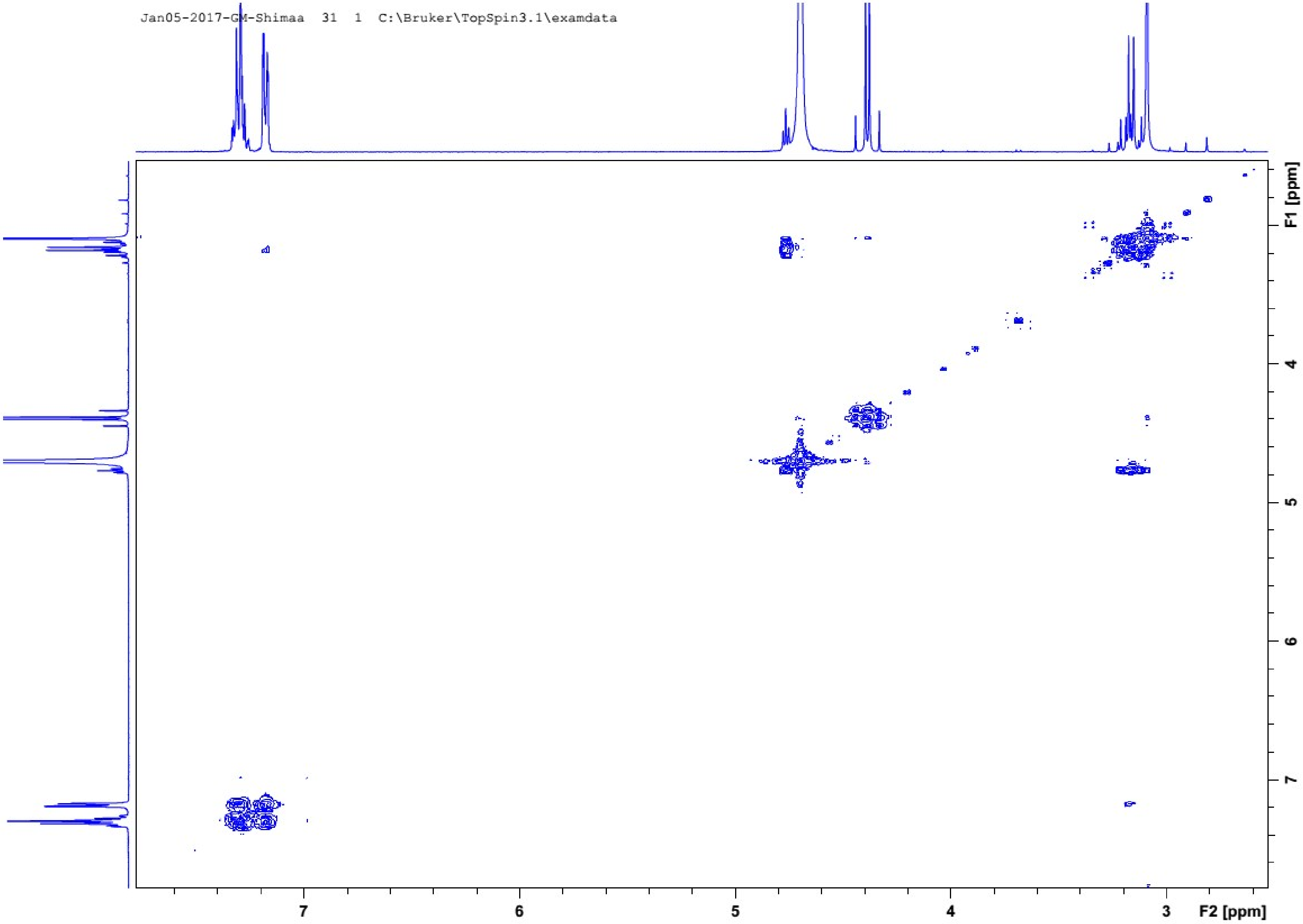


Figure S15: HMBC spectra for O1 and O1B in D_2O

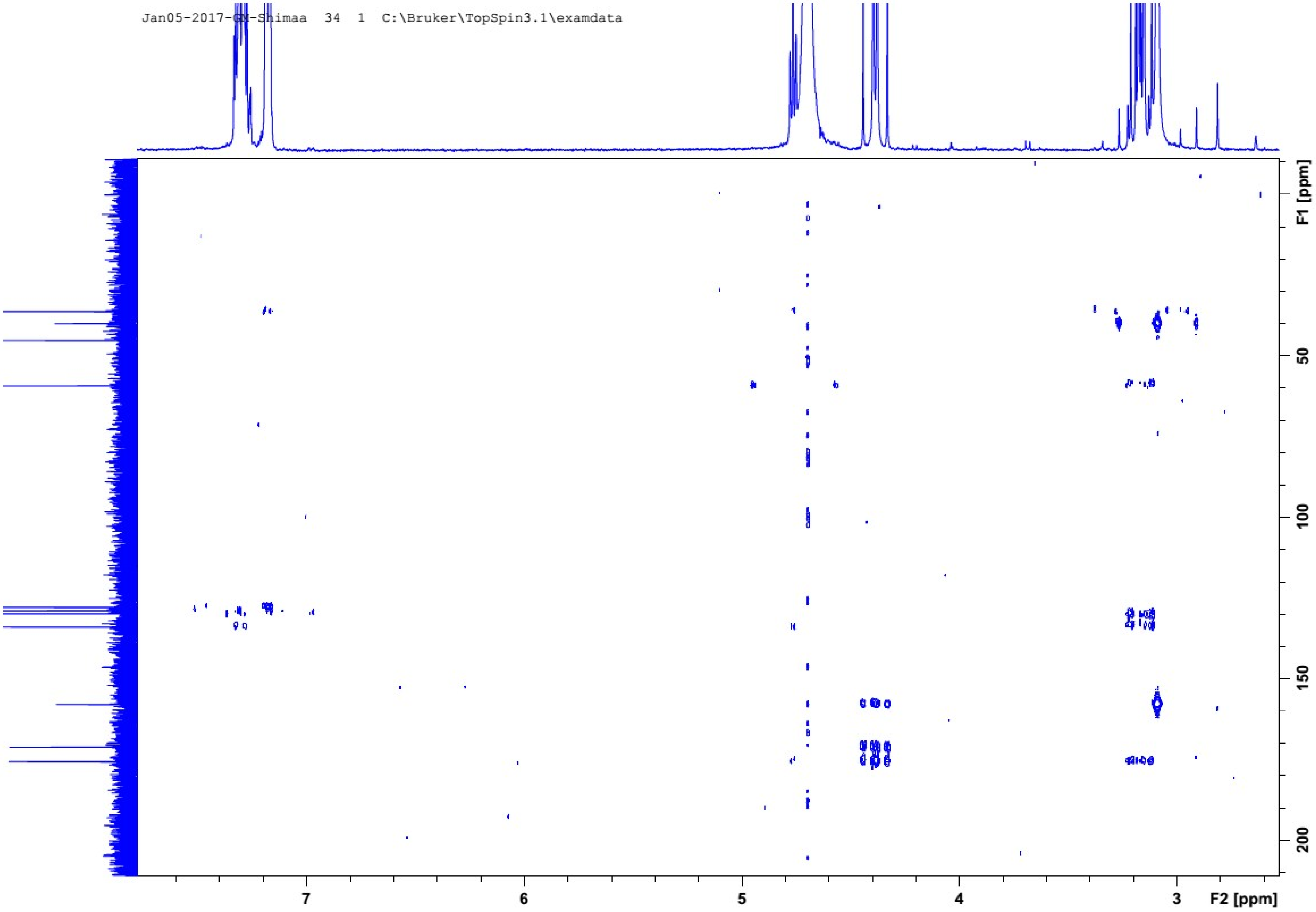


Figure S16: HSQC spectra for O1 and O1B in D_2O

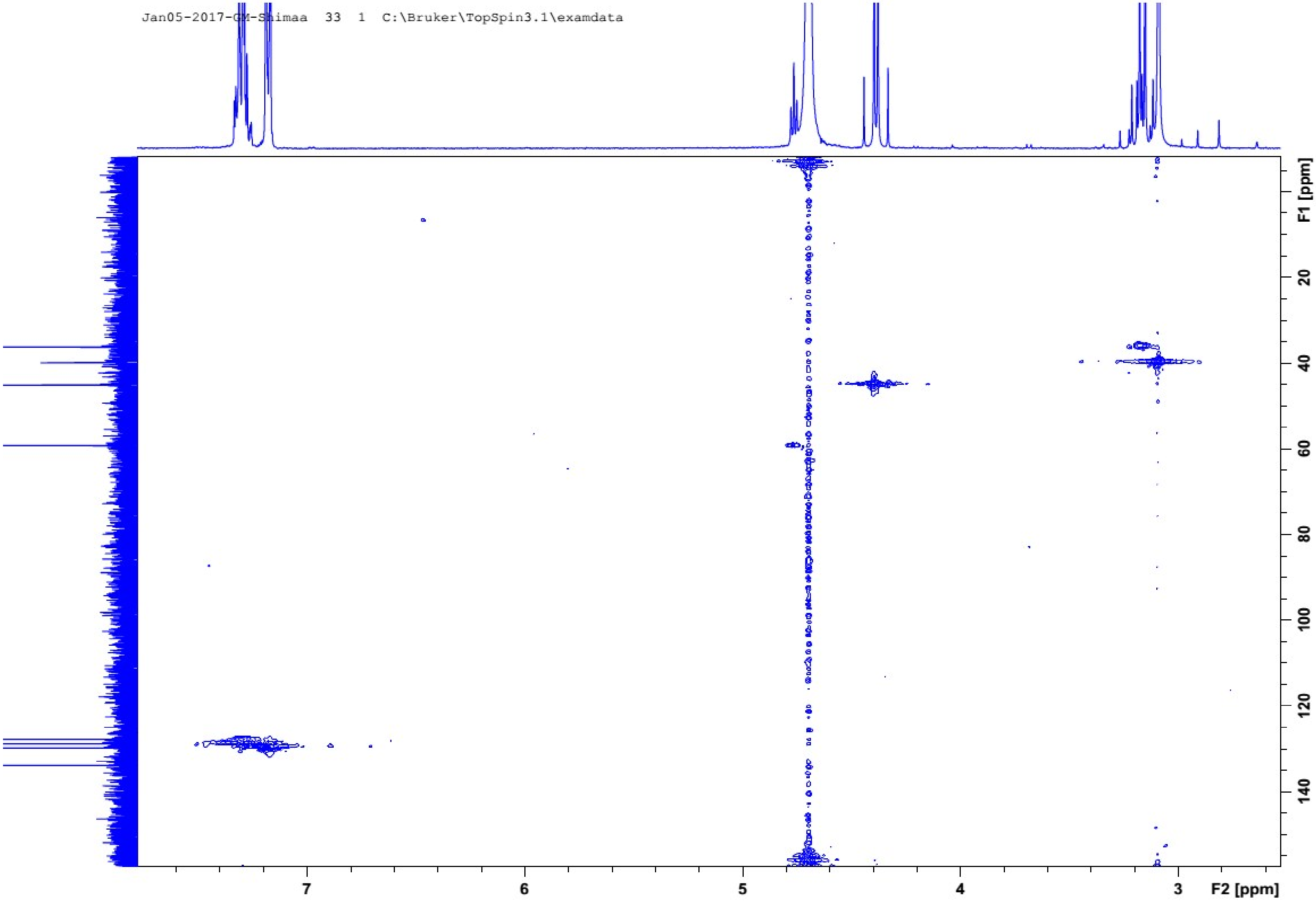


Figure S17: ^{1}HNMR spectra for O1B in D_2O

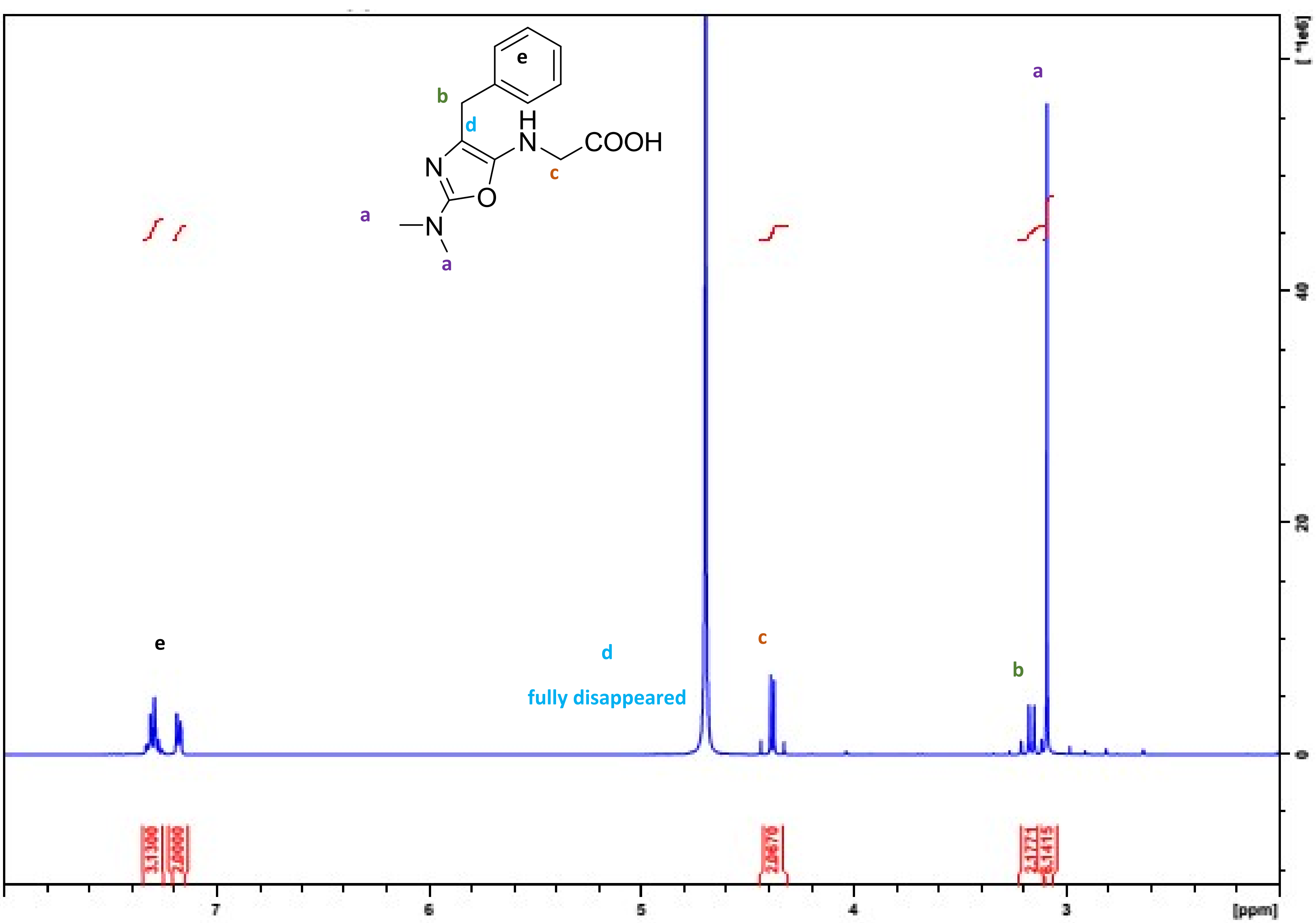


Figure S18: ^{13}CNMR spectra for O1B in D_2O

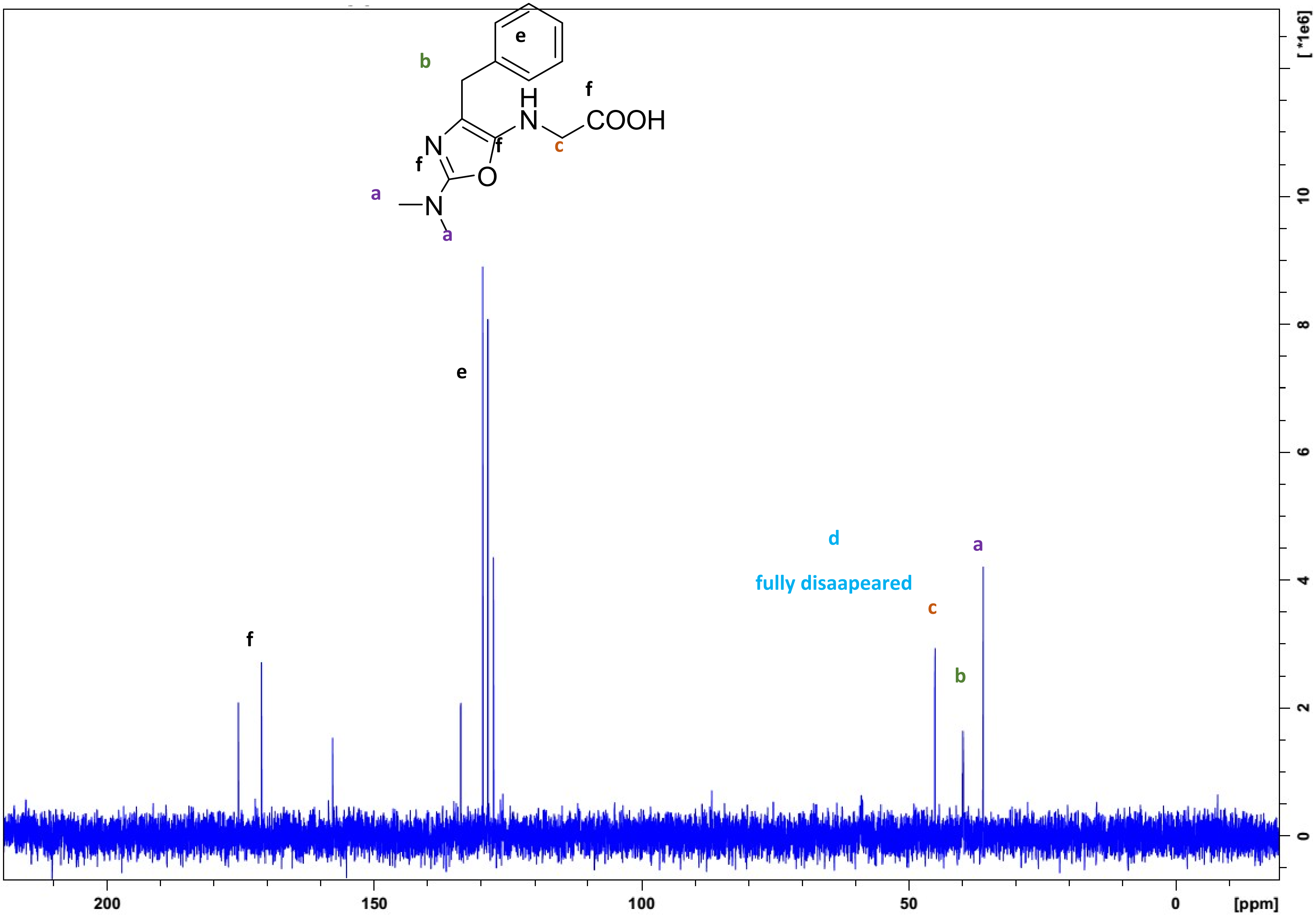


Figure S19: ^{1}HNMR spectra for O1 in MeOD-d4

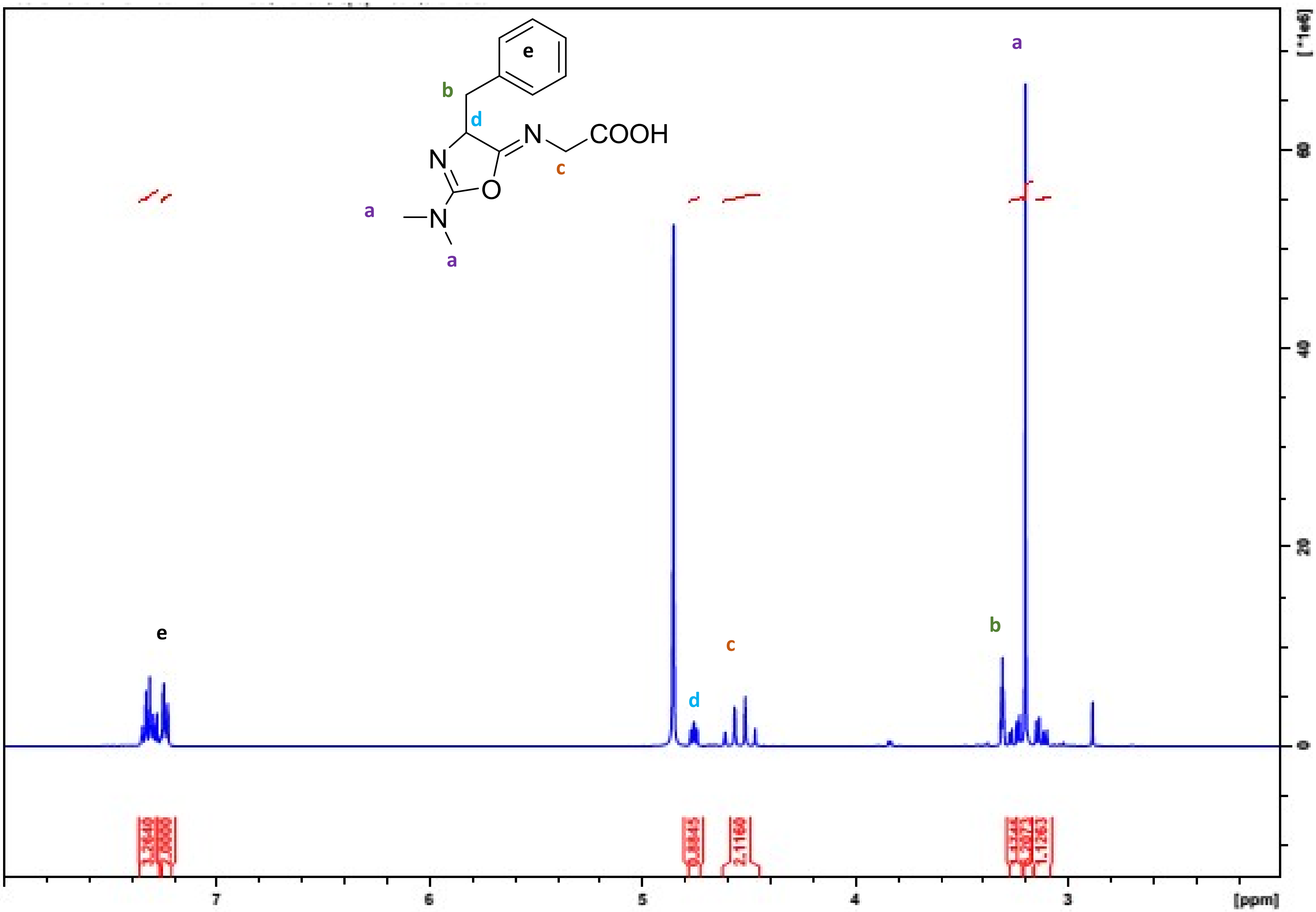


Figure S20: ^{1}HNMR spectra for O1B in MeOD-d4

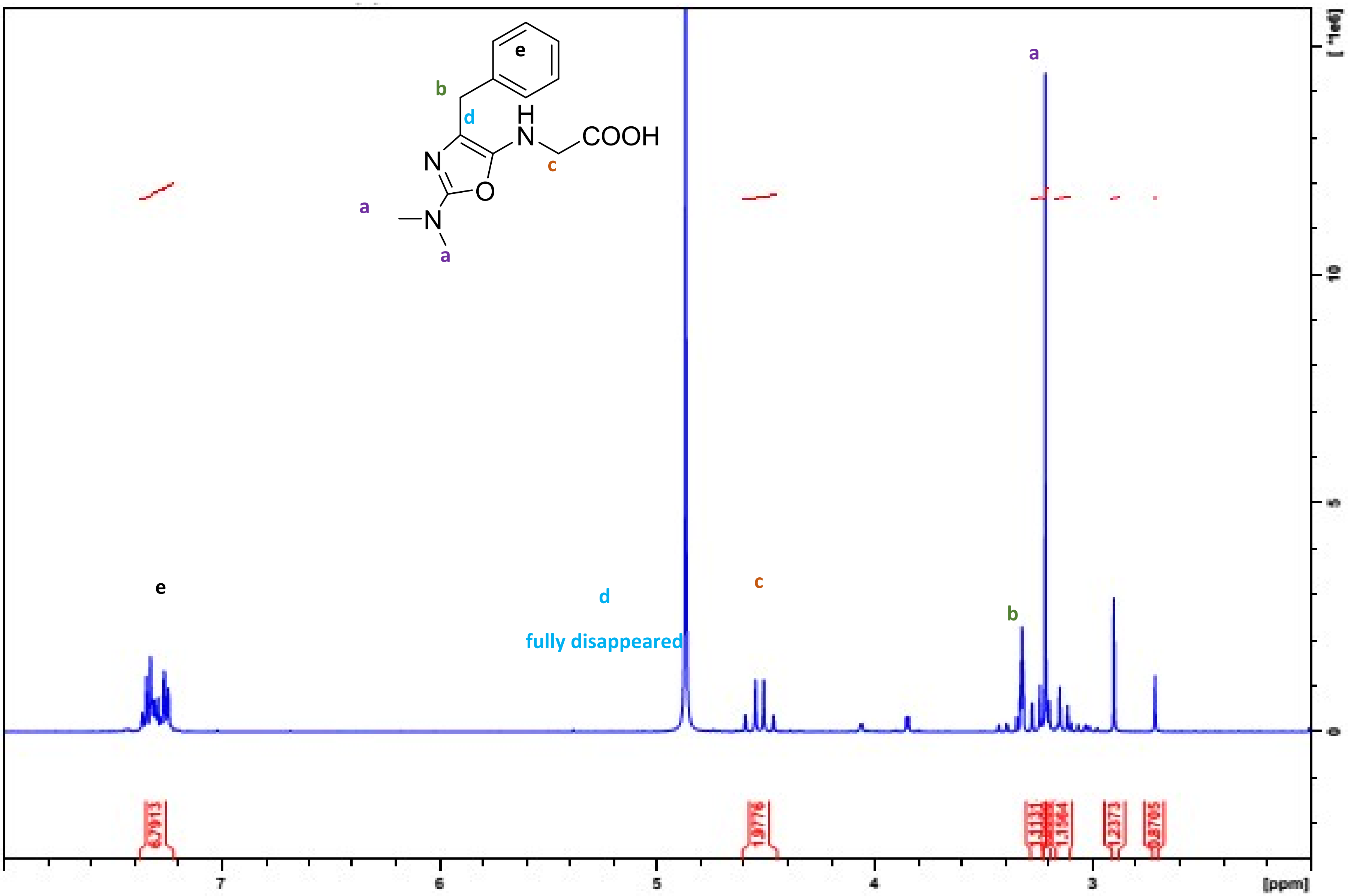


Figure S21: ^{1}HNMR spectra for O1 in DMSO-d6

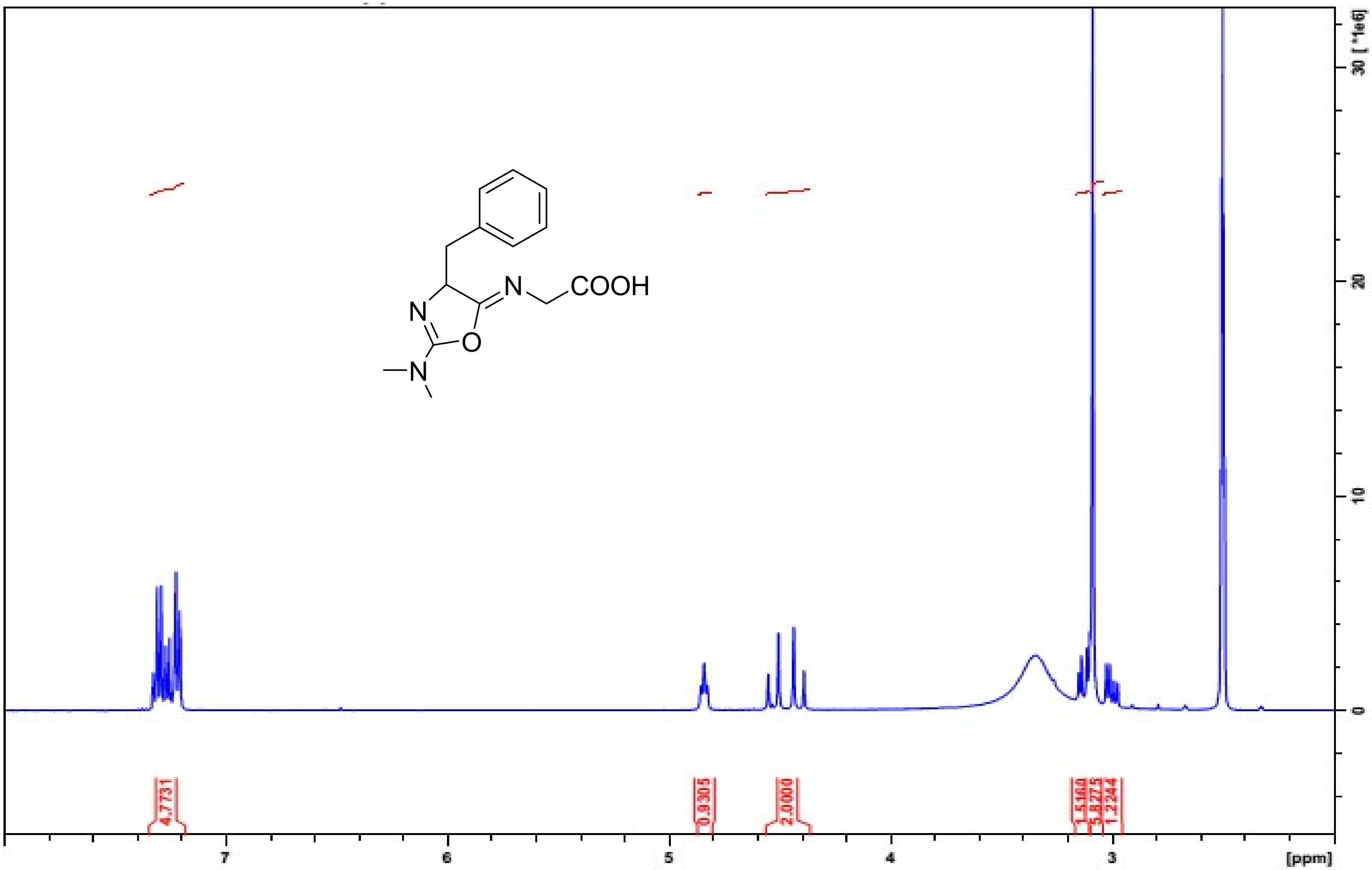


Figure S22: ^{1}HNMR spectra for O1 in DMSO-d6 after 7 days shows very slow conversion to O1A

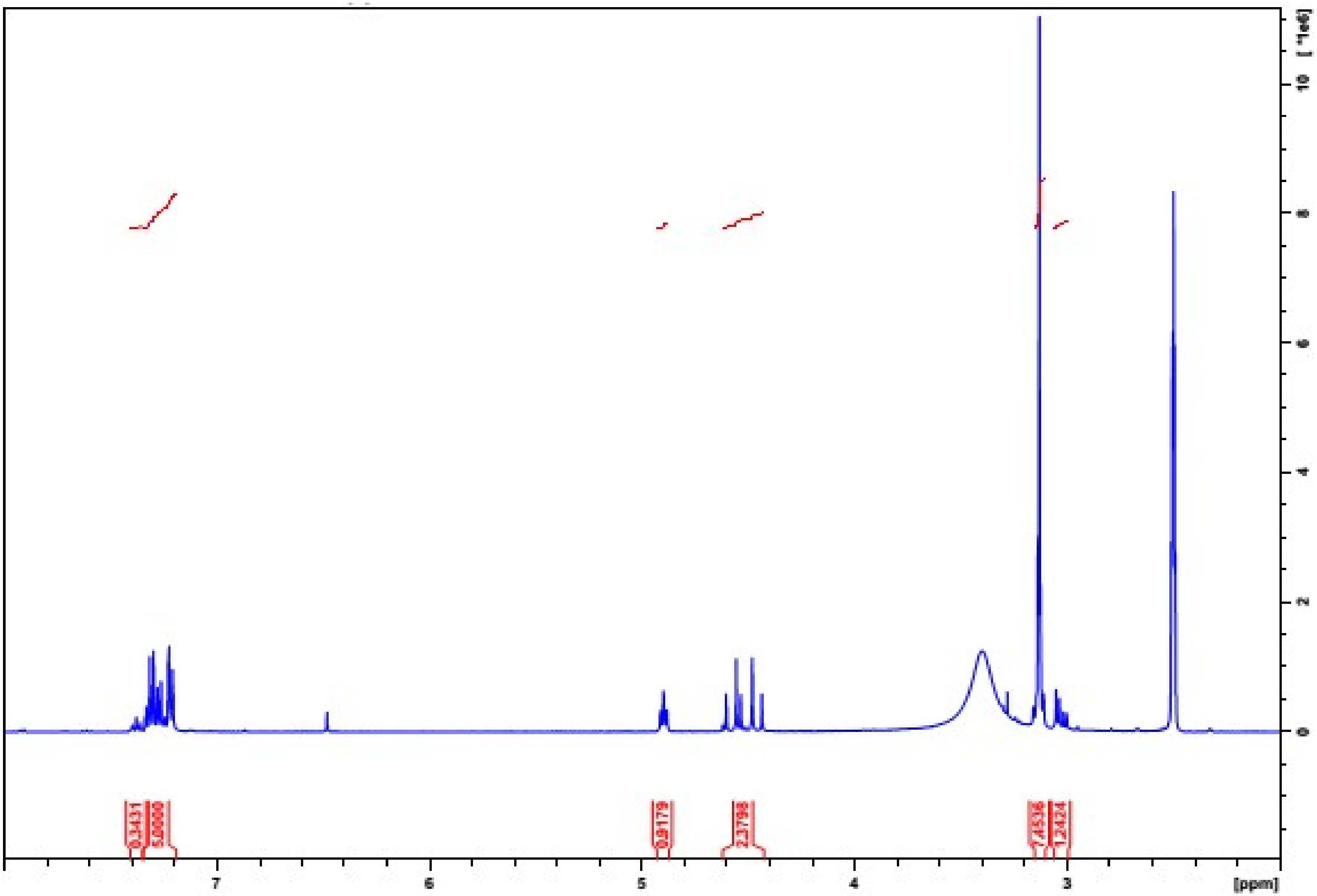


Figure S23: ^{1}HNMR spectra for O2

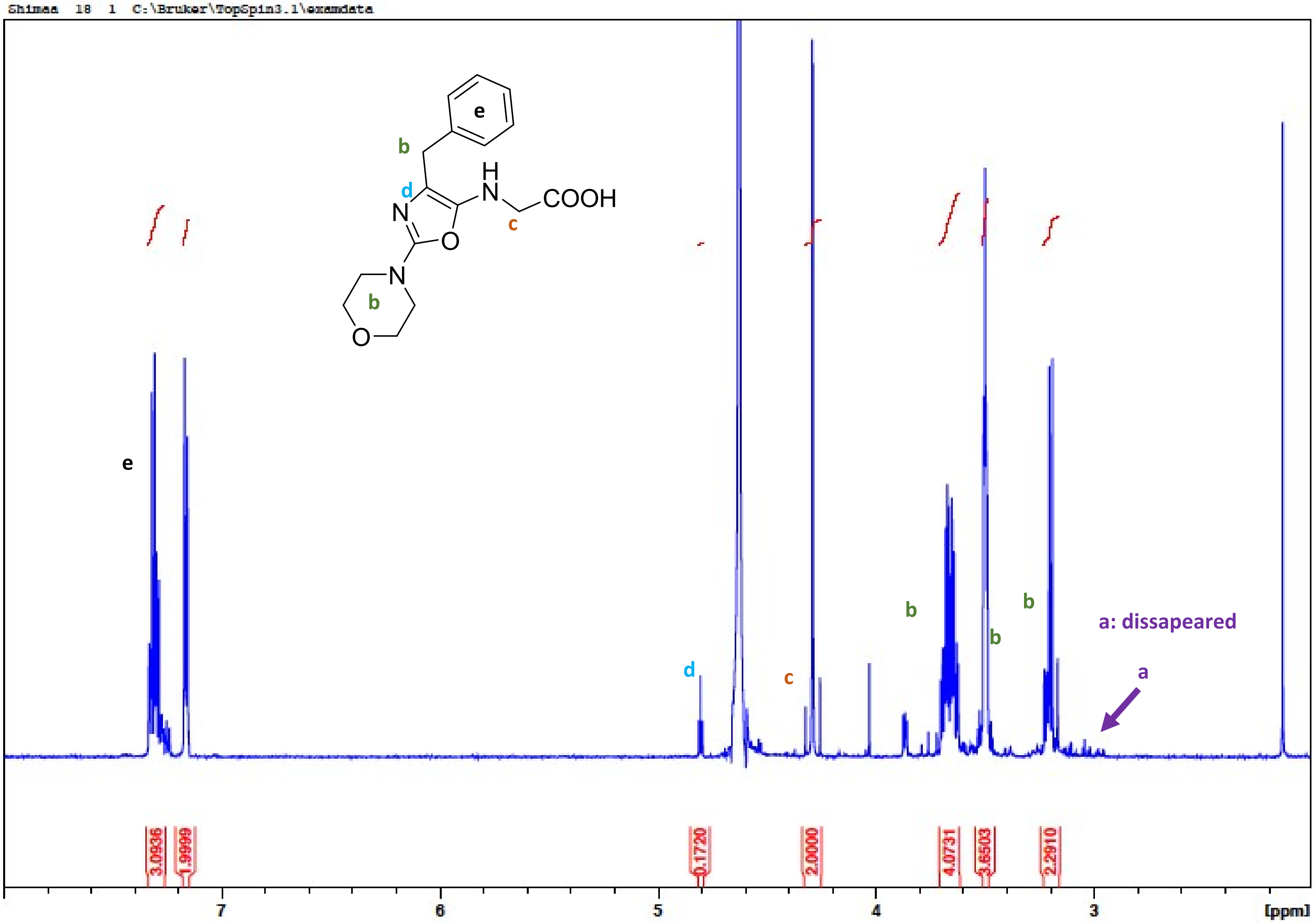


Figure S24: Pure HPLC of **O3**:

HPLC condition: A linear gradient of 5–50% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

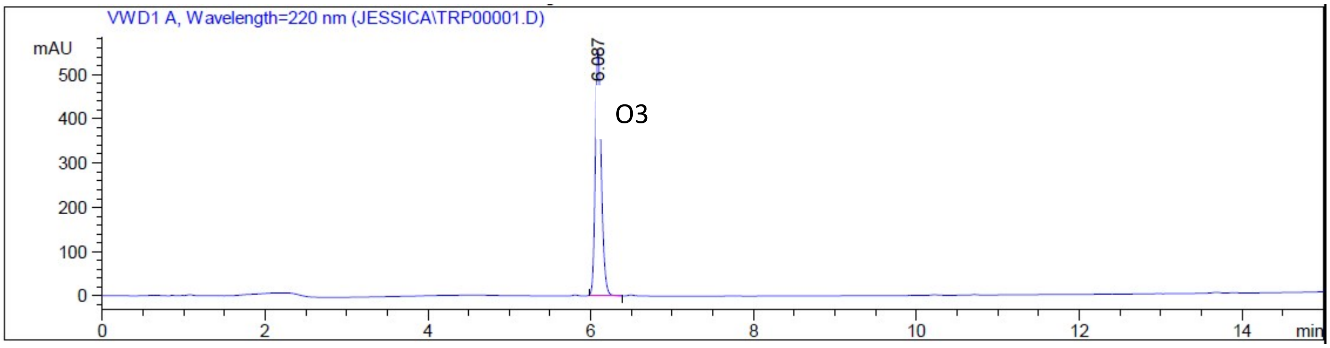


Figure S25: HR-MS spectra for of **O3**: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{20}N_5O_2$ 314.1611; Found 314.1648

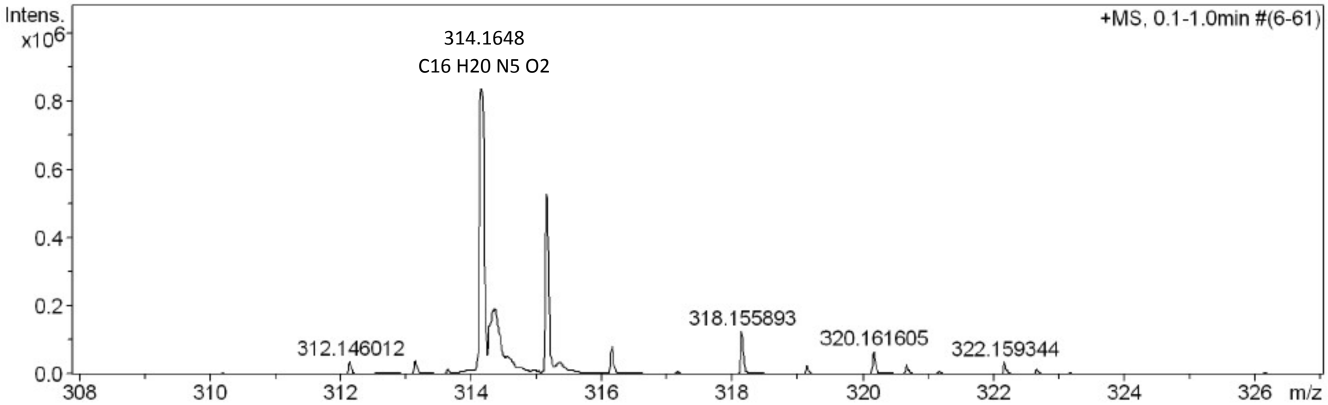
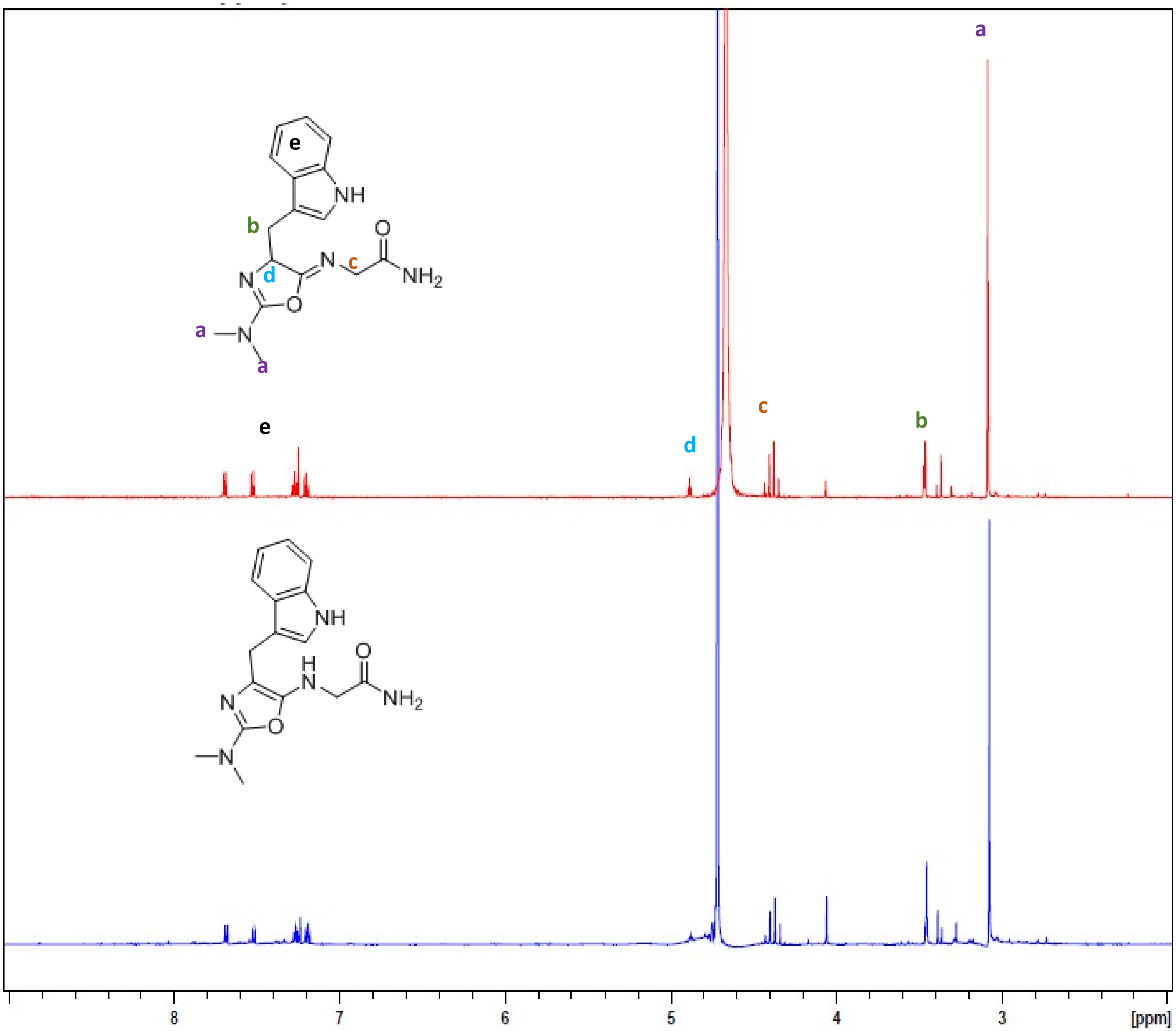


Figure S26: ^{1}HNMR spectra for **O3** in D_2O



Final result was confirmed by using Bruker AVANCE III 600 MHz instrument to confirm the full conversion of the triplet peak (d)

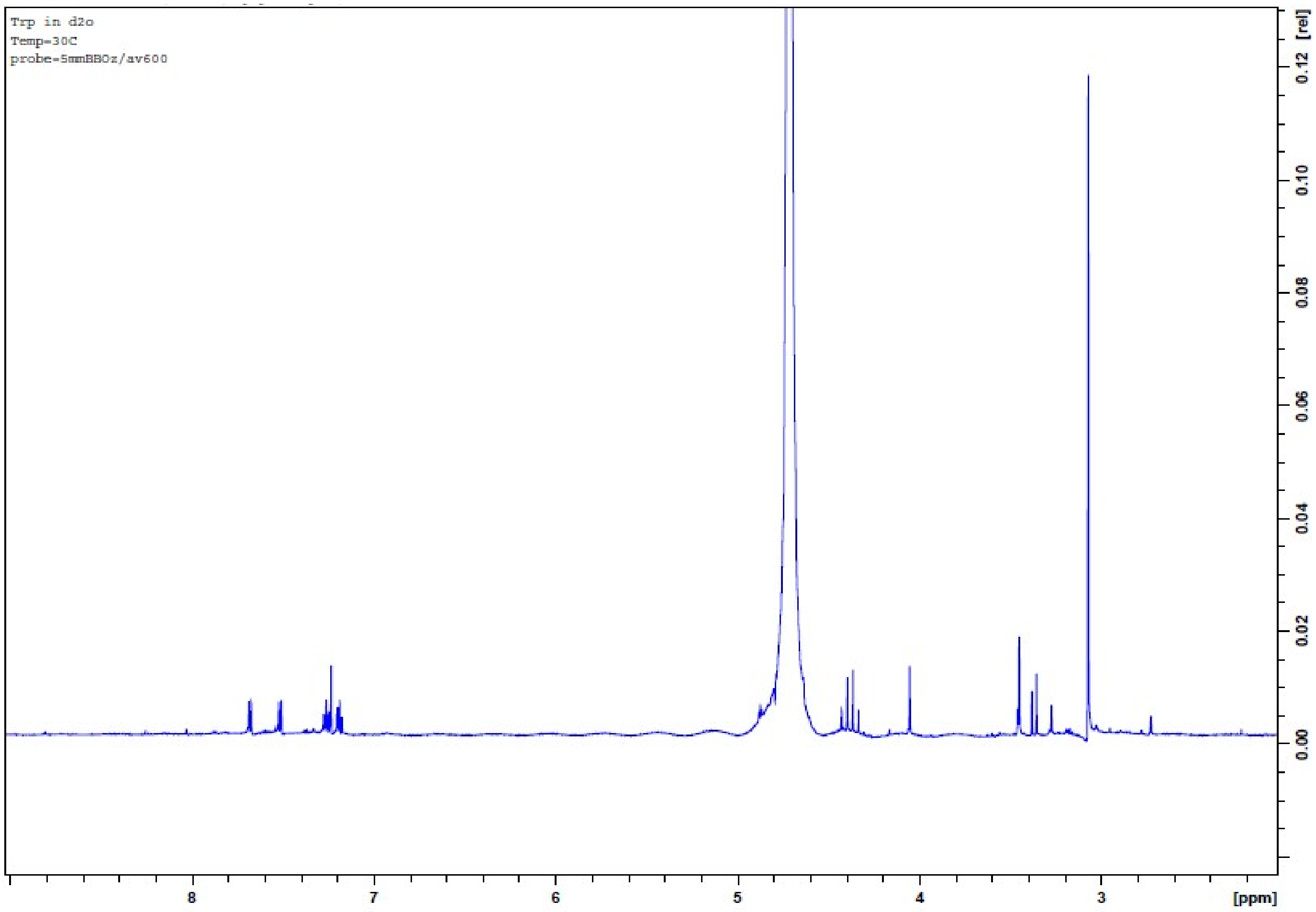

Trp in d2o
Temp=30C
probe=5mmBBOz/av600
[rel]
0.12
0.10
0.08
0.06
0.04
0.02
0.00
8
7
6
5
4
3
[ppm]

Figure S27: Pure HPLC of **O4**:

HPLC condition: A linear gradient of 0–30% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

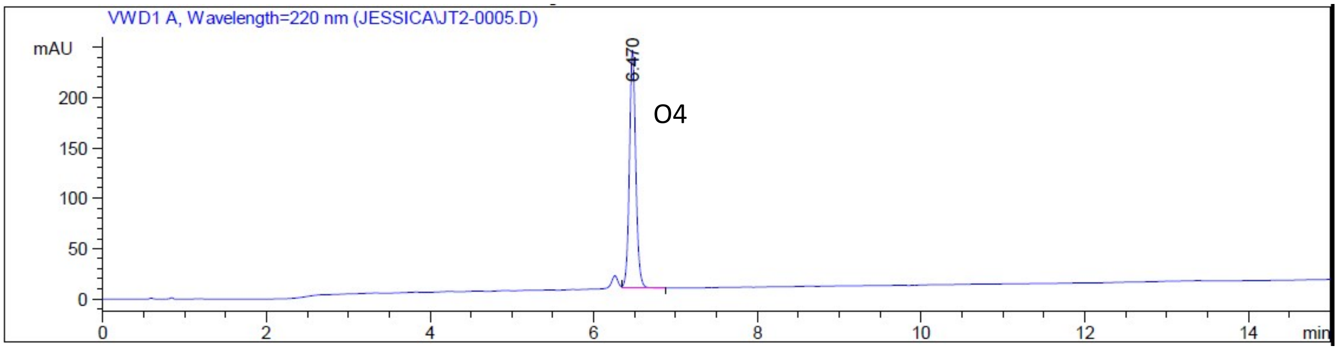


Figure S28: HR-MS spectra for of **O4**: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{14}H_{18}N_4O_3$ 291.3230; Found 292.1468

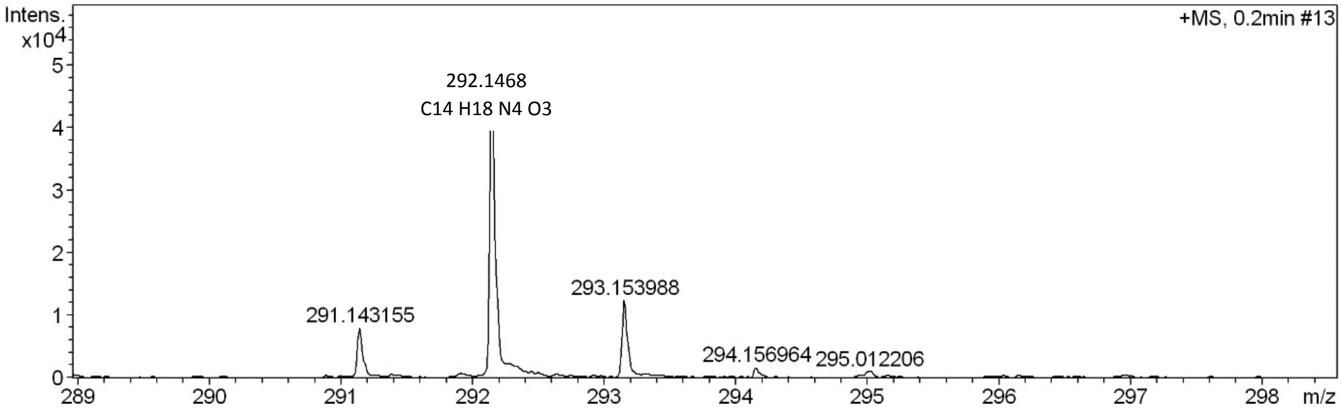


Figure S29: ^{1}HNMR spectra for **O4** in D_2O

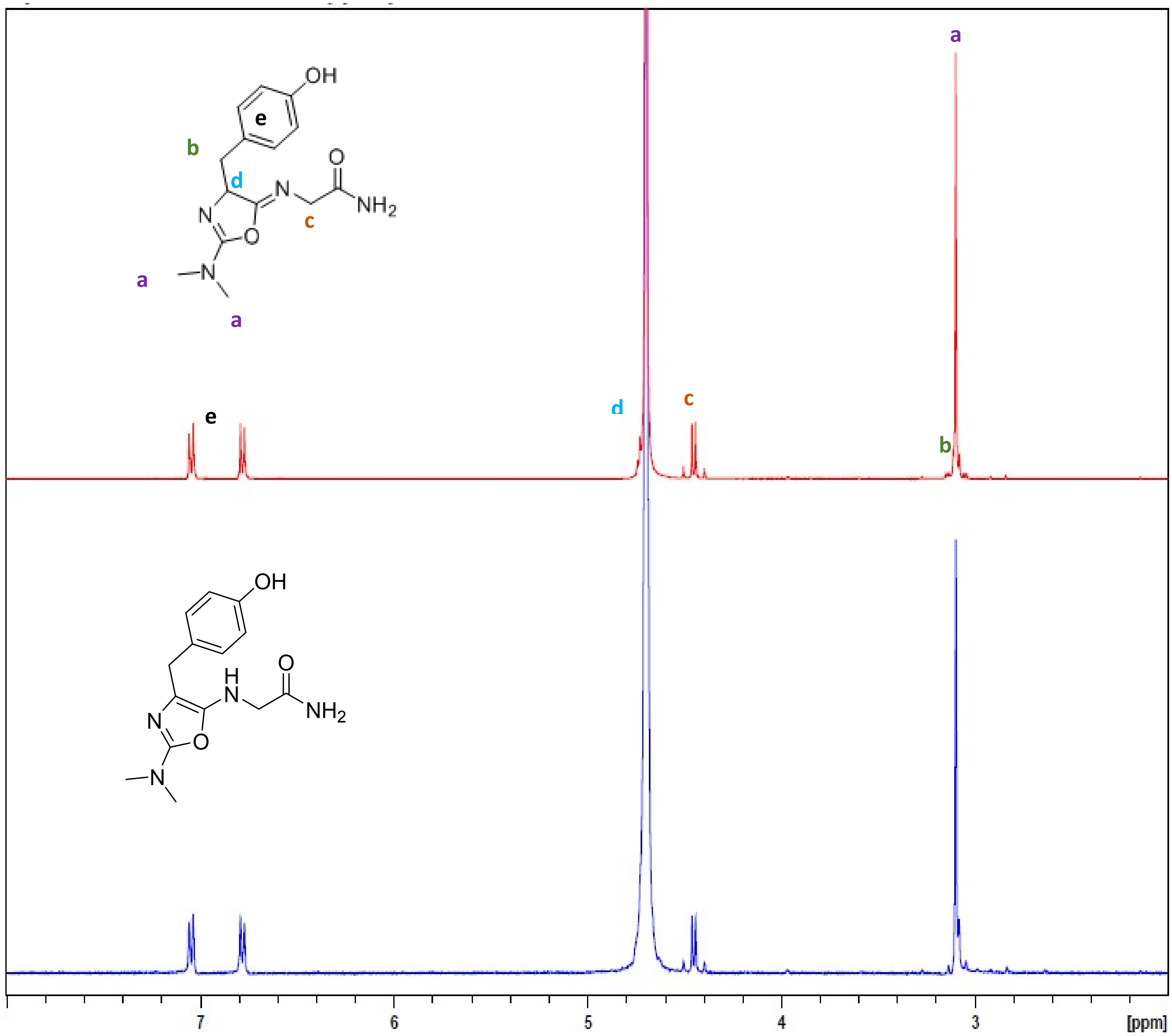


Figure S30: Pure HPLC of **O5:**

HPLC condition: A linear gradient of 0–5% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

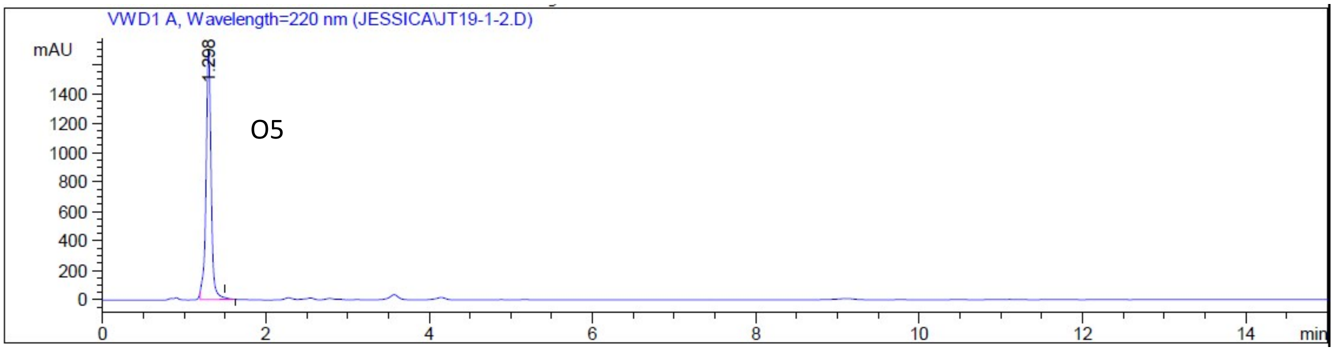


Figure S31: HR-MS spectra for of **O5**: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{11}H_{17}N_6O_2$ 265.1407; Found 265.1407

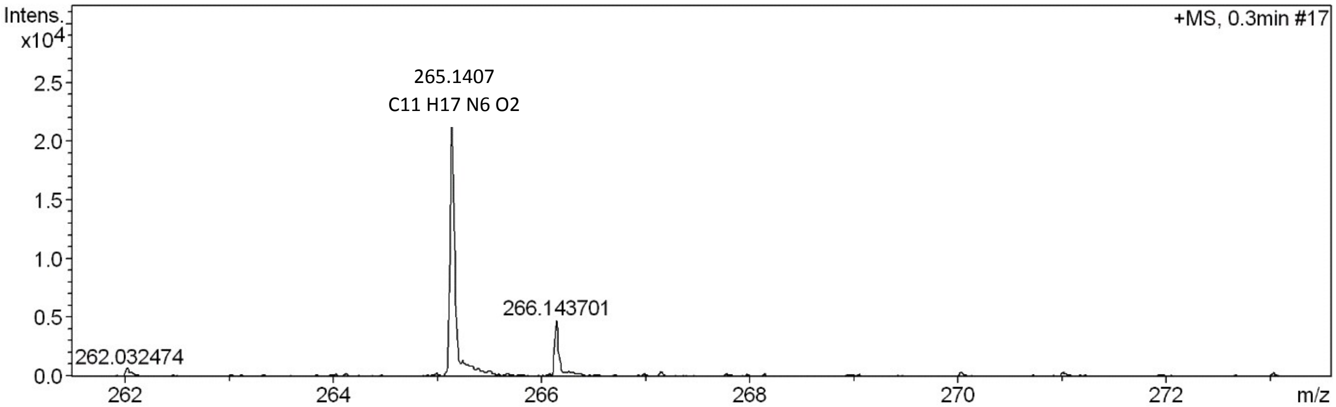
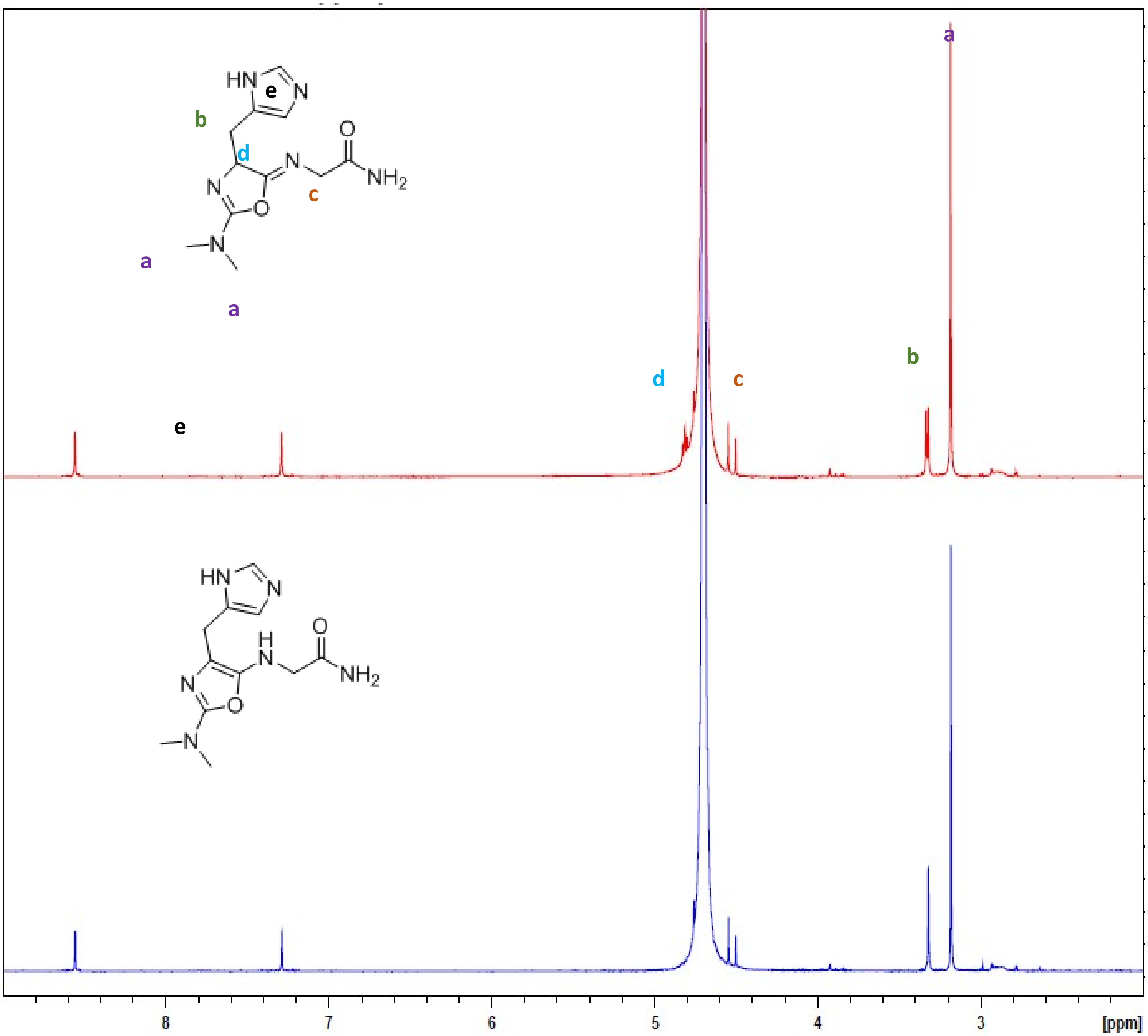


Figure S32: ^{1}HNMR spectra for O5 in D_2O



Final result was confirmed by using Bruker AVANCE III 600 MHz instrument to confirm the full conversion of the triplet peak (d)

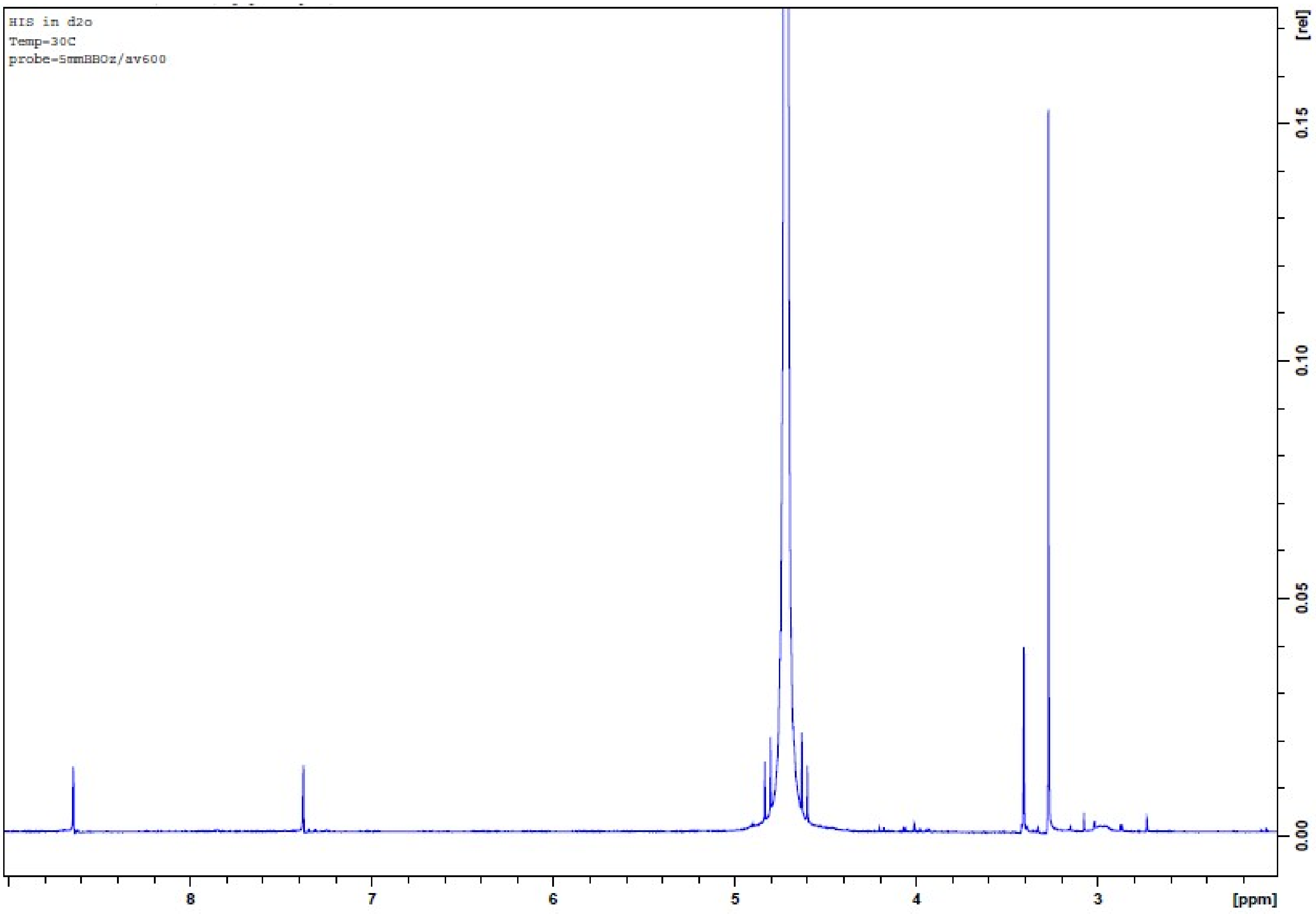

HIS in d2o
Temp=30C
probe=5mmBBOz/av600
[rel]
0.15
0.10
0.05
0.00
8
7
6
5
4
3
[ppm]

Figure S33: Pure HPLC of **O6**:

HPLC condition: A linear gradient of 0–0% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

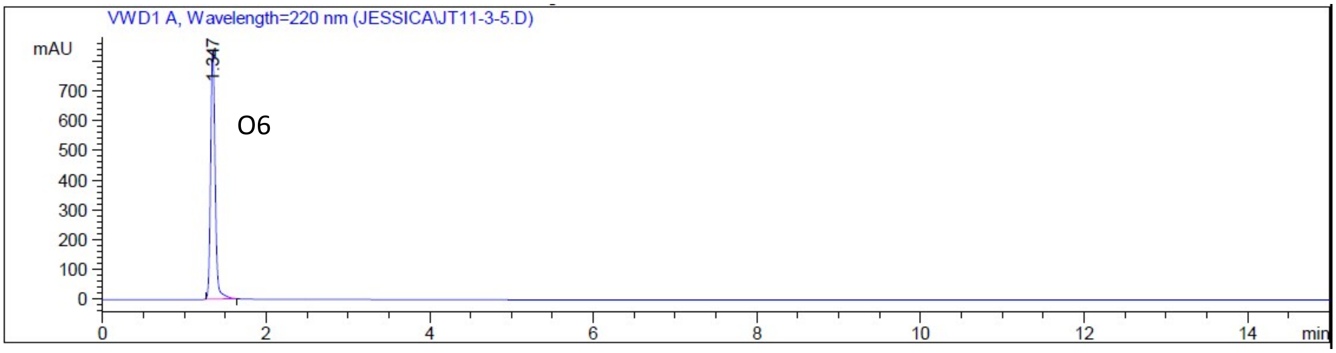


Figure S34: HR-MS spectra for of **O6**: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{11}H_{22}N_5O_2$ 256.1768; Found 256.1755

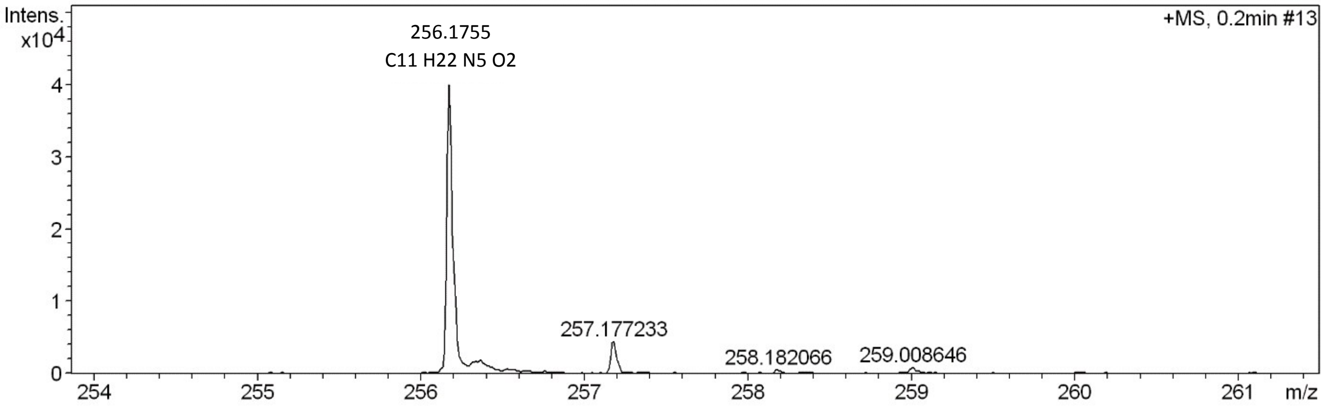


Figure S35: ^{1}HNMR spectra for O6 in D_2O

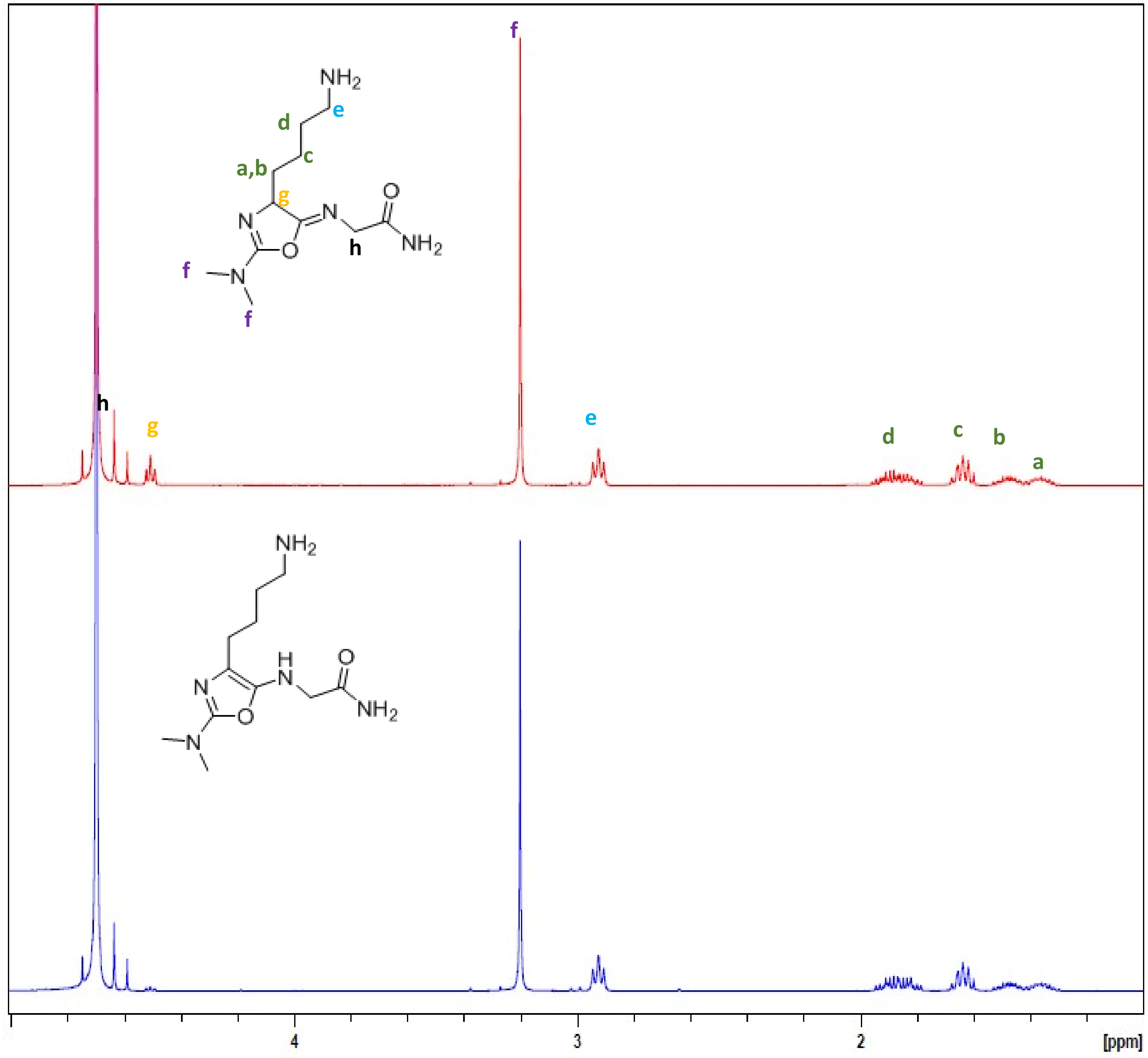


Figure S36: Pure HPLC of **O7**:

HPLC condition: A linear gradient of 0–50% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

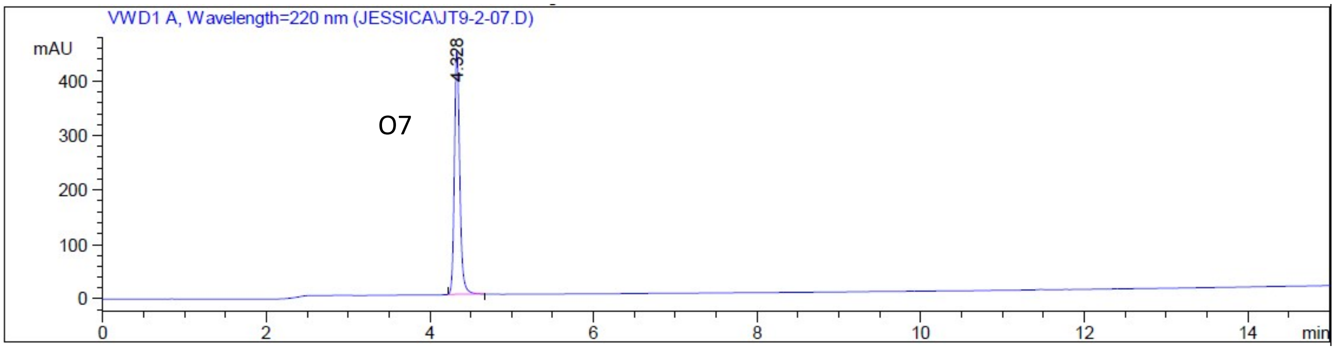


Figure S37: HR-MS spectra for of **O7**: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{10}H_{19}N_4O_2$ 227.1502; Found 227.1503

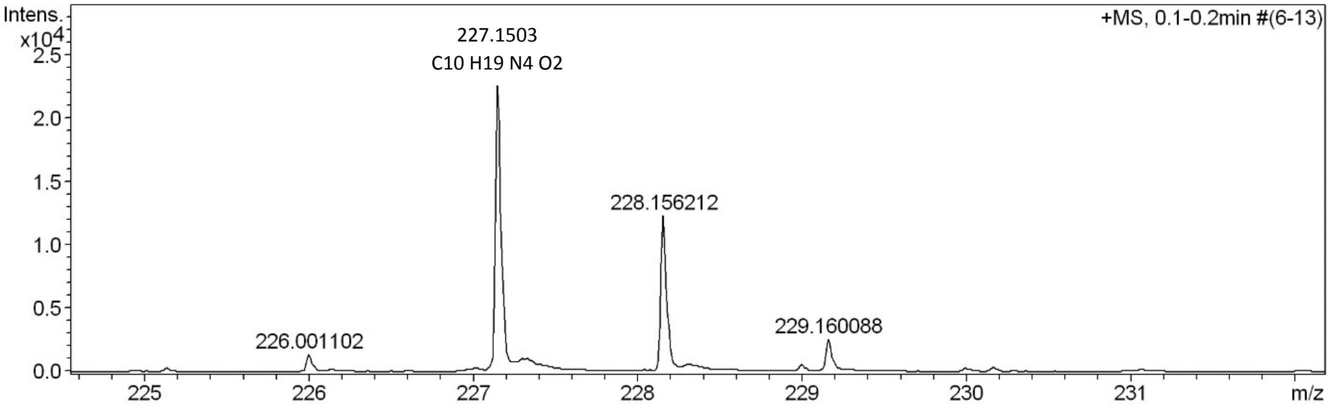


Figure S38: ^{1}HNMR spectra for O7 in D_2O

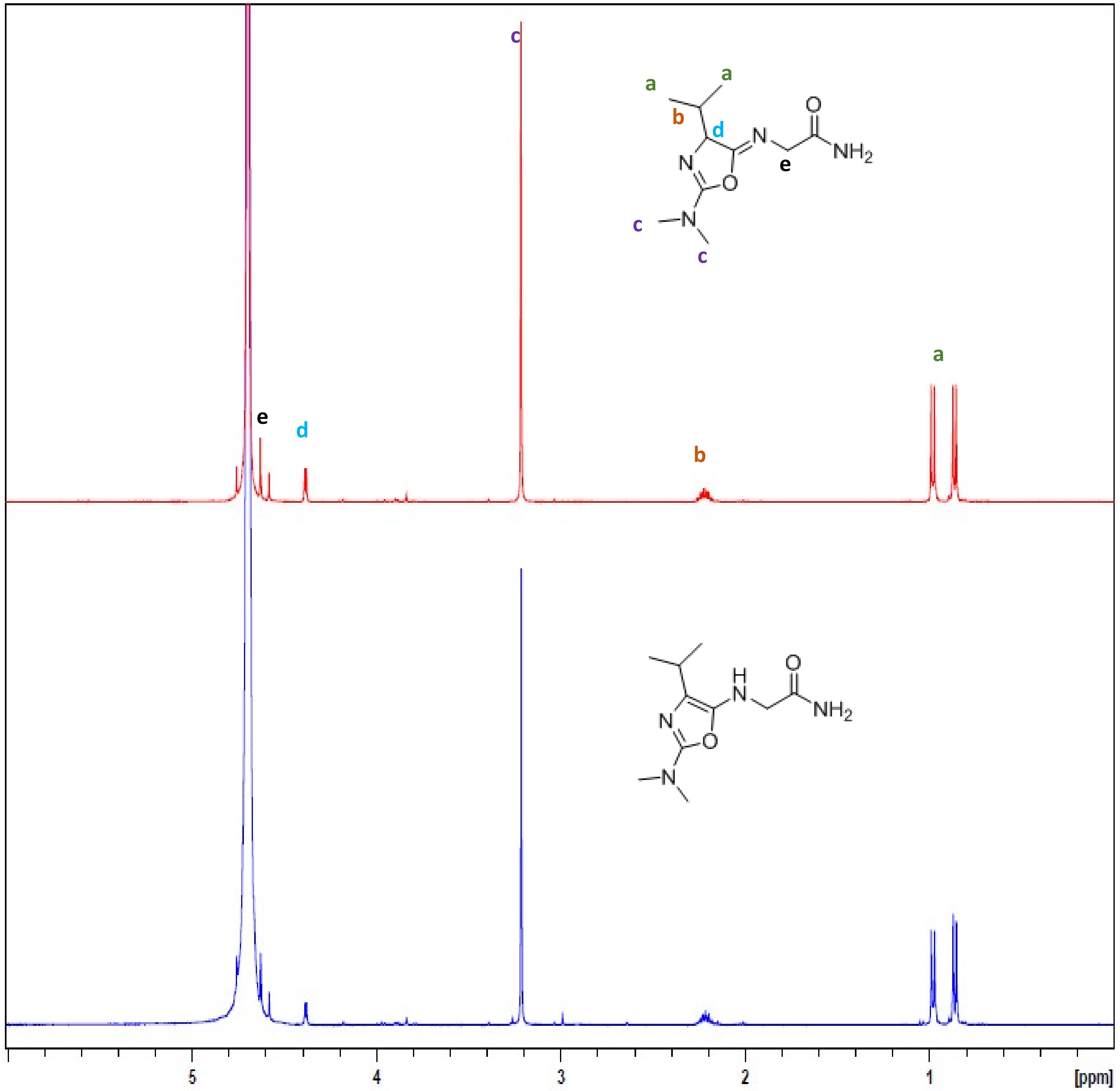


Figure S39: Pure HPLC of **O8:**

HPLC condition: A linear gradient of 0–30% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

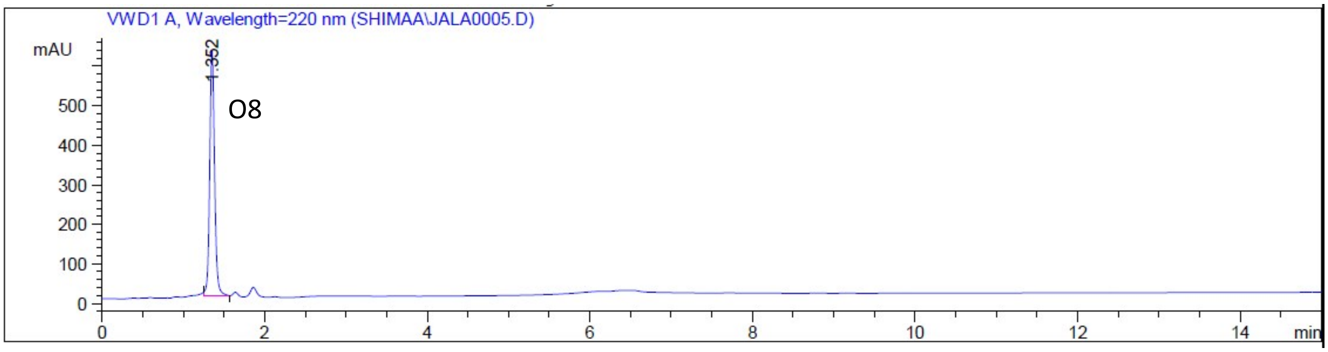


Figure S40: HR-MS spectra for of **O8**: HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_8H_{15}N_4O_2$ 199.1189; Found 199.1189

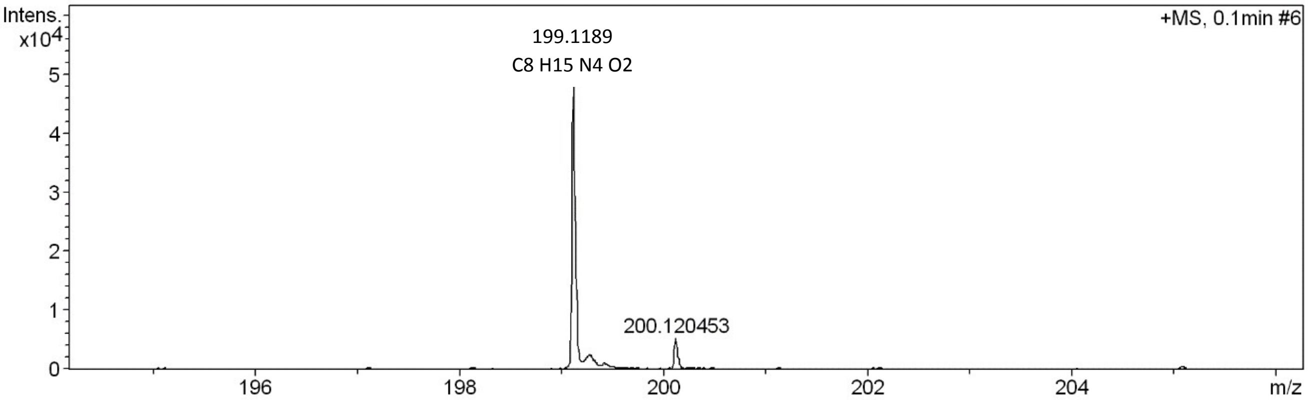


Figure S41: ^{1}HNMR spectra for **O10** in D_2O

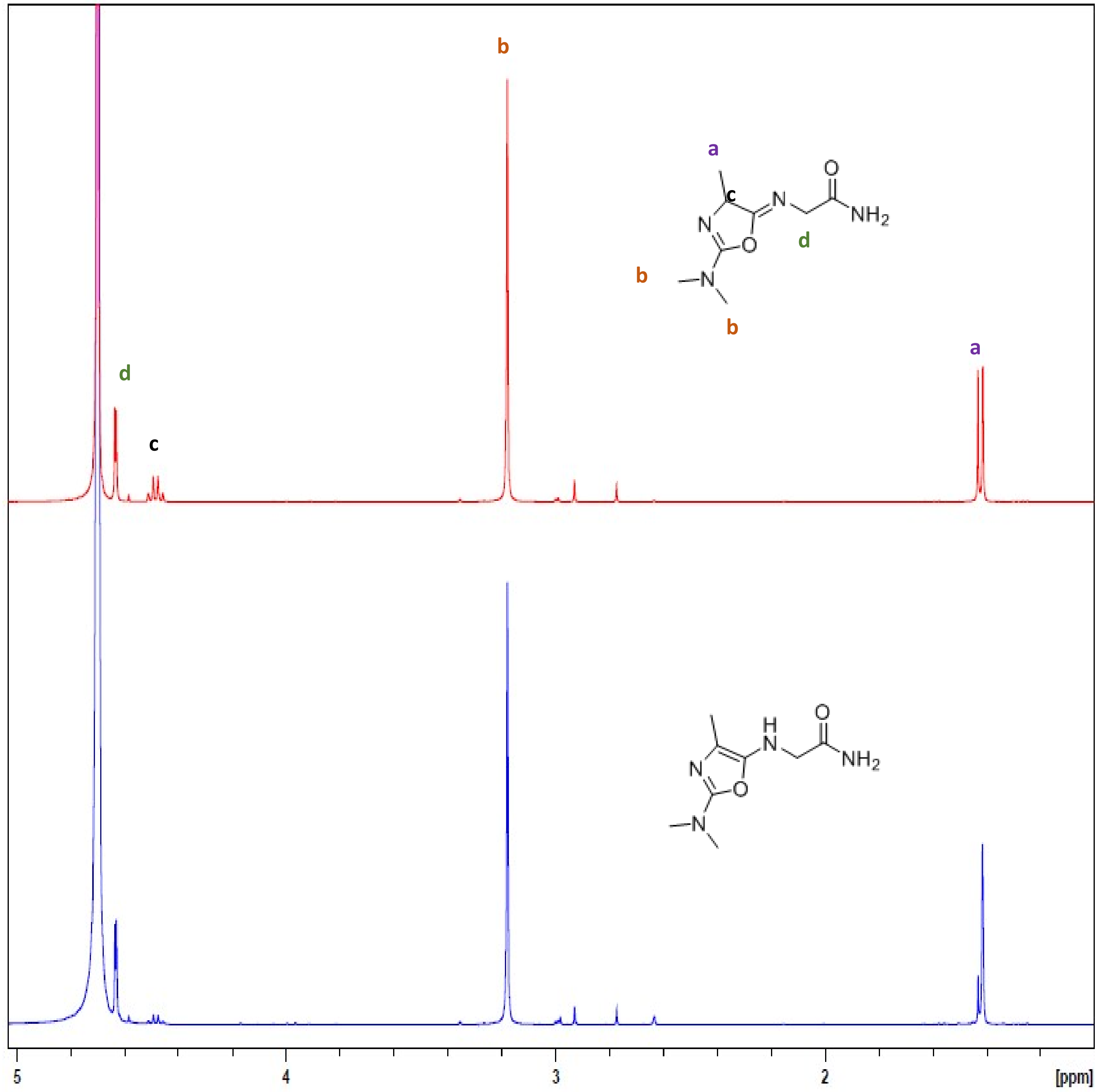


Figure S42: Pure HPLC of **O9**:

HPLC condition: A linear gradient of 5–95% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

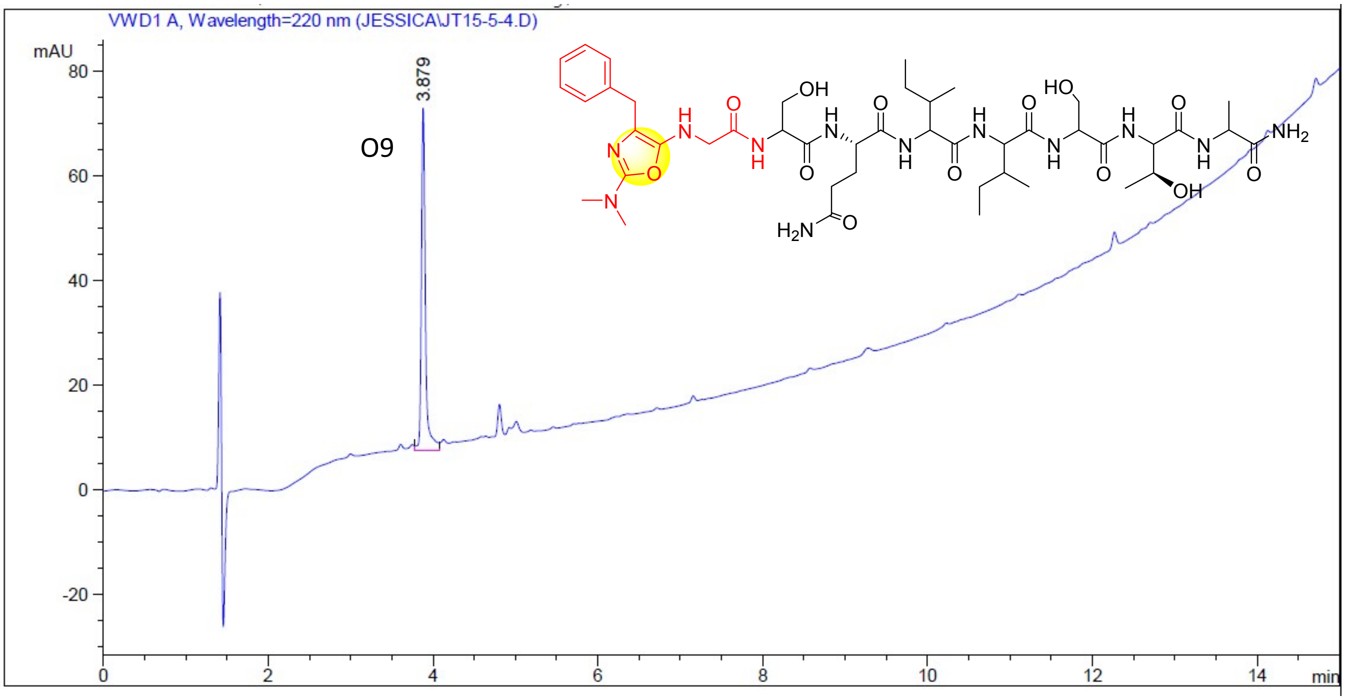


Figure S43: HR-MS spectra for of **O9**: HRMS (ESI-TOF) m/z: $[M + 2H]^+/2$ Calcd for $C_{44}H_{72}N_{12}O_{13}$ 975.1151; Found 488.3180

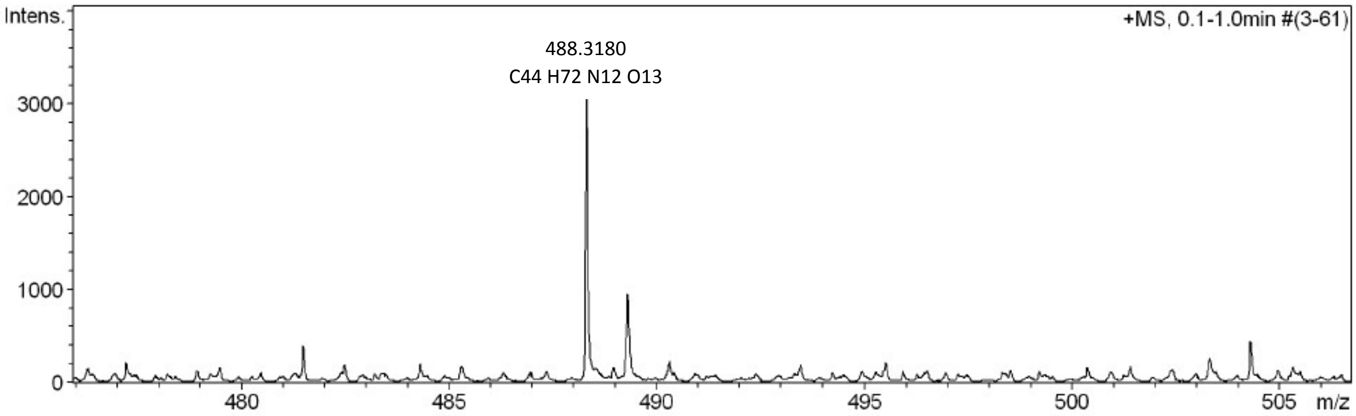


Figure S44: Crude HPLC of **G3 (tertraalkylamino-guanidino-Phe-Ala-NH$_2$)**:

HPLC condition: A linear gradient of 5–95% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

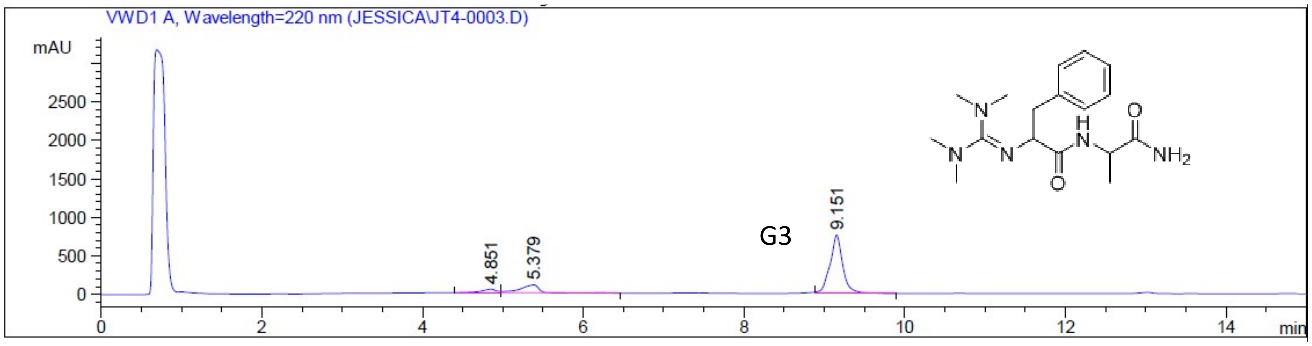


Figure S45: Pure HPLC of **G3 (dialkylamino-oxazolo-Phe-Ala-NH$_2$) No conversion was observed**:

HPLC condition: A linear gradient of 5–95% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

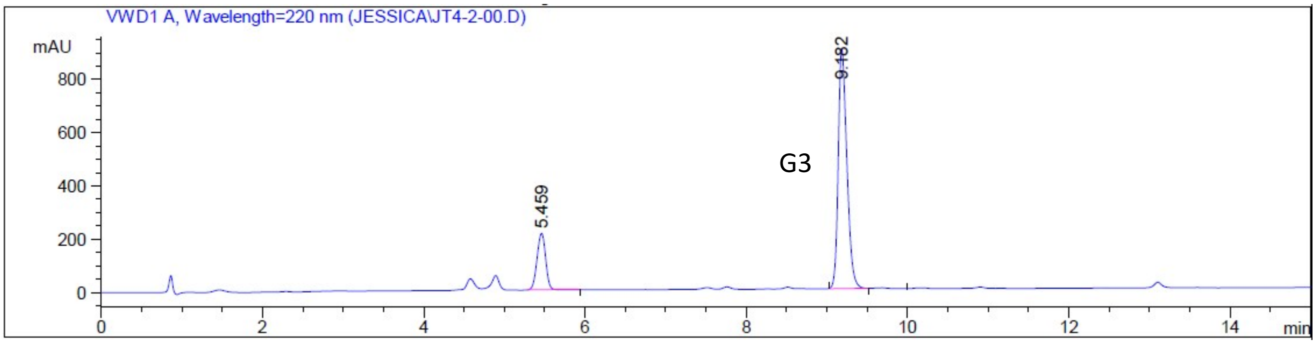


Figure S46: Crude HPLC of **G4 (tertraalkylamino-guanidino-Phe-Ser-NH$_2$)**:

HPLC condition: A linear gradient of 5–95% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

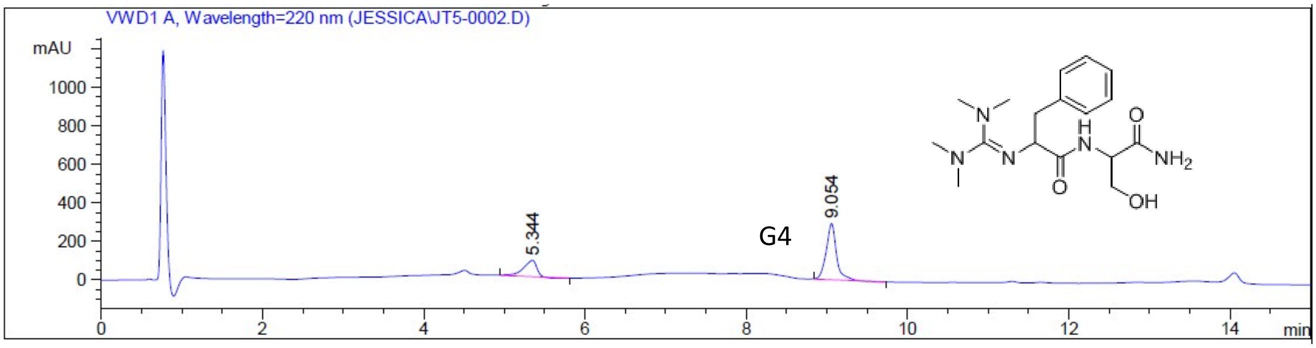


Figure S47: Pure HPLC of **G4 (dialkylamino-oxazolo-Phe-Ser-NH$_2$) No conversion was observed**:

HPLC condition: A linear gradient of 5–95% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

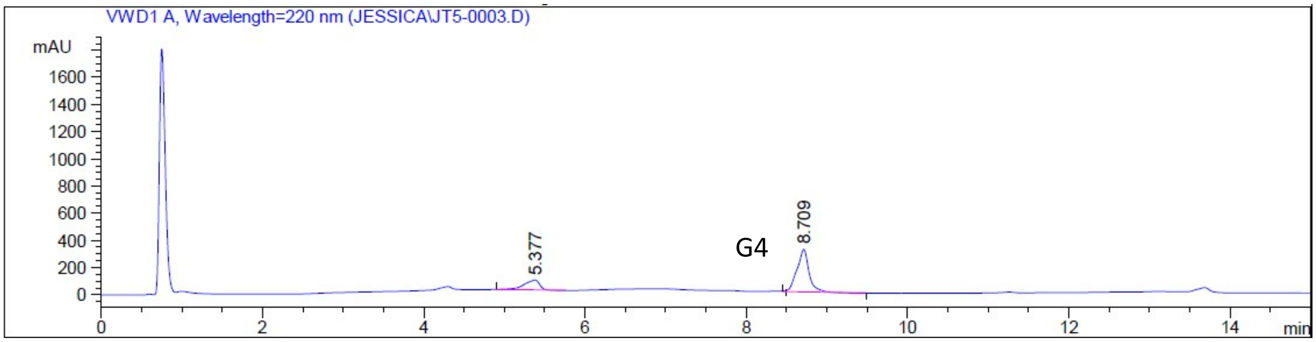


Figure S48: Crude HPLC of **G5 (tertraalkylamino-guanidino-Phe-Phe-NH$_2$)**:

HPLC condition: A linear gradient of 5–95% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.

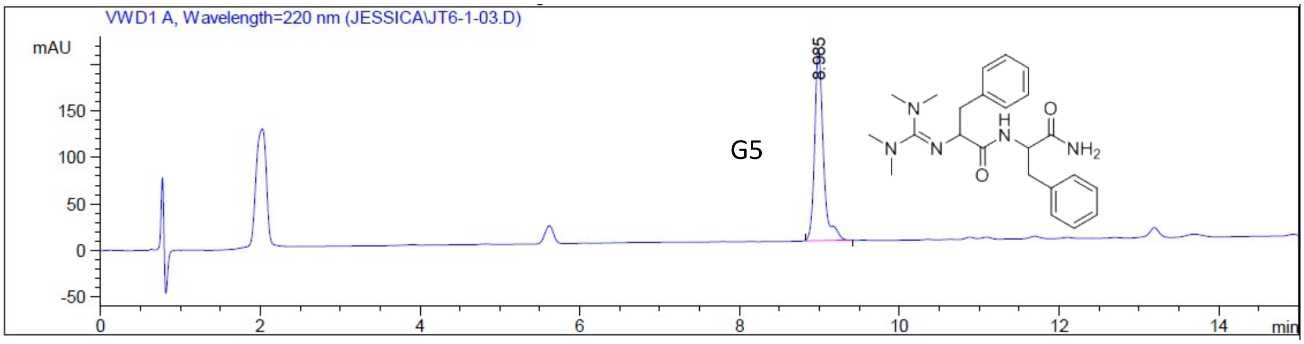


Figure S49: Pure HPLC of **G5 (dialkylamino-oxazolo-Phe-Phe-NH$_2$) No conversion was observed**:

HPLC condition: A linear gradient of 5–95% CH_3CN/H_2O and 0.1% TFA over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomenex C_{18} (3 µm, 4.6 × 50 mm) column at 30°C.