Supporting information

Proline *N*-Oxide: Manipulation of the 3D Conformation of Linear Peptides

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author. It contains NMR assignments for each compounds, NMR-based thermal coefficient graphs, NMR and HRMS spectra and experimental details for all compounds.

Contents

1. General

Material and Methods

Fmoc-protected amino acids, CTC resin and HBTU were bought from GL-Biochem (Shanghai, China). HPLC–grade CH_3CN, MeOH and peptide synthesis-grade DMF, CH_2Cl_2, DIEA, TFA and all other reagents were purchased from Sigma-Aldrich (Germany). Analytical reversed-phase HPLC was performed on a C_{18} column (4.6 × 150 mm, 5 μm, YMC Triart, UK) with a LC-MS 2020A system (Shimadzu, Kyoto, Japan). Solvents A and B were 0.01% (v/v) formic acid in milli q water, and CH_3CN, respectively. Elution was achieved with linear 0-90% gradients of solvent B to A over 15 minutes at 1 ml/min flow rate, with UV detection at 200 nm. Preparative HPLC was performed with a C_8 column (150 × 21.2 mm, 10 μm, Hichrom) attached to a Shimadzu LC-8A instrument. Solvents A and B were 0.1% formic acid (v/v) in water and MeOH, respectively and a linear gradient of solvent B to A over 40 minutes, at 15 mL/min flow rate was applied. The UV detection wavelength was set at 200 nm. Purified fractions (>95%) by HPLC were pooled and lyophilized on a Virtis SP scientific Sentry 2.0. Purified peptides and conjugates were characterized with HRMS, on BrukermicrOTOF-Q II instrument operating at ambient temperatures and sample 1 ppm. DMSO-d6 was used as a solvent for sample preparation of NMR. ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and ROESY experiments were performed at ambient temperature on a AVANCE III 600 MHz NMR (Bruker, Germany). The ^{1}H NMR spectra were reordered with 600 MHz while ^{13}C NMR spectra were obtained with 150 MHz as the frequency. The chemical shifts are expressed in ppm downfield from TMS as the internal standard. To achieve better resolution in the NMR spectra, the water peak was suppressed in necessary cases.

General procedure for the synthesis of the tetrapeptides.

Peptides were manually synthesized on a 0.1 mmol scale on CTC resin (0.6 mmol/g). The resin was activated using 10% thionylchloride (v/v) in dry DCM, after which incorporation of the C-terminal amino acids was performed with a minimum of four equivalents of Fmoc-amino acid and six equivalents of DIEA in dry DCM for 3 hrs. Elongation of the peptide chains was achieved following standard Fmoc protocols. The coupling conditions were: 5-fold molar excess of Fmoc-amino acid and HBTU, double molar excess of DIEA in DMF. Deprotections

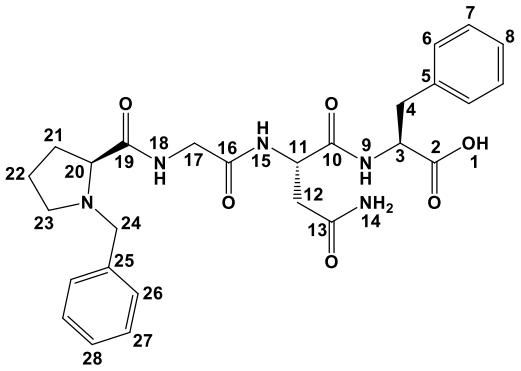
were achieved with 20% piperidine in DMF (3 x 10 min). Coupling of N-benzylproline with the same method. After chain assembly, total deprotection and cleavage was carried out with TFA/DCM (40:60) for one hour and the peptides were precipitated by adding chilled diethyl ether. After evaporation to dryness, the solid forms of the crude peptides were obtained. The crude peptides were purified via semi preparative HPLC and characterized using NMR and HRMS.

General procedure for the preparation of *N*-oxide tetrapeptides.

Crude peptide (0.1 mmol) and K_2CO_3 (2.5 equiv.) were dissolved in dry DCM (10 ml). m-CPBA (1.6 equiv.) was added to a cooled solution mixture to (-72 °C). The reaction was stirred for four hours under the same conditions and warmed up to room temperature over two hours. Finally, the solvent was evaporated and the product was purified by preparative HPLC and characterized with NMR and HRMS.

2. NMR and HRMS results

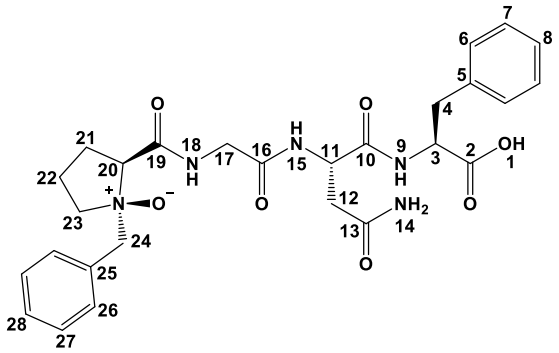
1a, (BzPGNF)



		Single conformer	
Residue	**Entry**	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
Phe	1	-	-
	2	-	173.50
	3	4.05	54.90
	4	2.90, 3.06	37.20
	5	-	138.80
	6	7.13	129.50
	7	7.16	127.50
	8	7.09	125.50
	9	7.48	-
Asn	10	-	169.70
	11	4.54	50.00
	12	2.34, 2.50	37.26
	13	-	171.40
	14	6.82, 7,42	-
	15	8.23	-
Gly	16	-	168.40
	17	3.72	41.55
	18	7.98	-
Nbp	19	-	173.50
	20	3.05	66.60
	21	1.74, 2.08	29.62
	22	1.68	23.30
	23	2.26, 2.85	52.80
	24	3.39, 3.88	59.20
	25	-	138.58
	26	7.39	128.90
	27	7.30	128.16
	28	7.23	126.90

HRMS (ESI+) *m/z* calcd. for $C_{27}H_{33}N_5O_6$: 524.2503; found [M+H] 524.2553

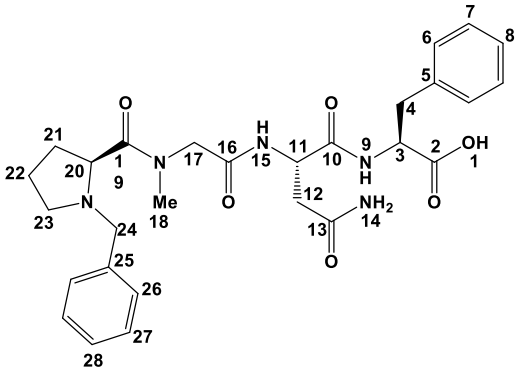
1b, [Bz(NO)PGNF]



Residue	Entry	Major conformation		Minor conformation	
		^{1}H Chemical shift (ppm)	^{13}C chemical shift (ppm)	^{1}H Chemical shift (ppm)	^{13}C chemical shift (ppm)
Phe	1	-	-	-	-
	2	-	172.60	-	179.68
	3	4.30	53.70	4.33	54.90
	4	2.91, 3.01	36.70	2.88, 3.04	37.26
	5	-	137.60	-	138.8
	6	7.18	128.10	7.13	129.50
	7	7.36	127.97	7.16	127.50
	8	7.39	129.01	7.09	125.50
	9	7.86	-	7.92	-
Asn	10	-	170.70	-	169.62
	11	4.50	50	4.60	50.00
	12	2.36, 2.41	36.80	2.34, 2.47	37.20
	13	-	171.56	-	171.44
	14	6.81, 7.48	-	6.89, 7.33	-
	15	8.55	-	8.19	-
Gly	16	-	168.77	-	168.40
	17	3.71	41.90	3.73	41.55
	18	10.78	-	8.02	-
Nbp	19	-	167.51	-	173.50
	20	3.96	74.30	2.83	66.60
	21	2.19, 2.24	26.00	1.66, 2.25	29.62
	22	1.85, 2.05	20.10	1.74, 2.08	23.30
	23	3.01, 3.46	66.05	2.22, 3.03	52.80
	24	4.54, 4.64	68.70	3.38, 3.89	59.20
	25	-	131.31	-	138.58
	26	7.58	132.48	7.39	128.90
	27	7.18	129.28	7.30	128.16
	28	7.16	126.27	7.23	126.90

HRMS (ESI+) *m/z* calcd. for $C_{27}H_{33}N_5O_7$: 540.2452; found [M+H] 540.2487

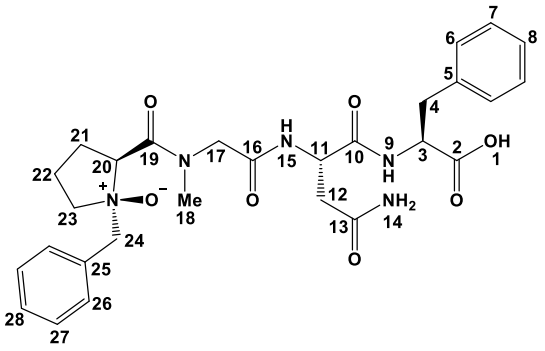
2a, (BzPMeGNF)



		Major conformation		Minor conformation	
Residue	**Entry**	**^{1}H Chemical shift (ppm)**	**^{13}C chemical shift (ppm)**	**^{1}H Chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
Phe	1	-	-	-	-
	2	-	172.65	-	172.65
	3	4.32	53.84	4.35	53.84
	4	2.91, 3.01	36.65	2.91, 3.01	36.65
	5	-	137.52	-	137.52
	6	7.19	126.89	7.19	126.90
	7	7.23	126.89	7.23	126.90
	8	7.28	128.80	7.28	128.80
	9	7.90	-	7.97	-
Asn	10	-	170.74	-	170.74
	11	4.57	49.45	4.61	49.66
	12	2.35, 2.48	37.11	2.35, 2.48	37.11
	13	-	171.28	-	171.28
	14	6.91, 7.32	-	6.91, 7.32	-
	15	8.12	-	8.27	-
Gly	16	-	168.02	-	168.16
	17	3.81, 3.99	50.48	3.96, 4.31	51.31
	18	2.95	35.76	2.71	3.20
Nbp	19	-	172.38	-	172.38
	20	3.58	63.40	3.41	64.60
	21	1.73, 2.09	27.73	1.68, 1.94	27.91
	22	1.72	22.40	1.72	22.40
	23	2.32, 2.87	51.95	2.24, 2.83	52.40
	24	3.41, 3.89	56.85	3.36, 3.80	57.32
	25	-	138.64	-	138.44
	26	7.30	128.11	7.30	128.11
	27	7.22	129.23	7.22	129.23
	28	7.16	123.38	7.16	123.38

HRMS (ESI+) *m/z* calcd. for $C_{28}H_{35}N_5O_6$: 538.2660; found [M+H] 538.2718

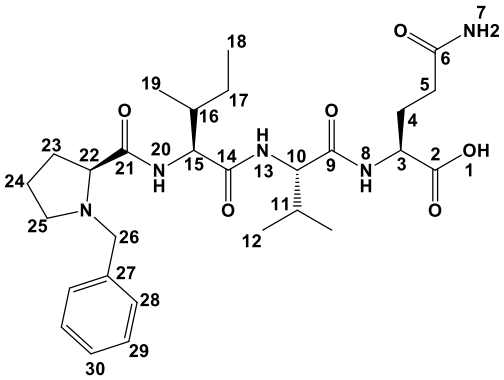
2b, [Bz(NO)PMeGNF]



		Major conformer	
Residue	**Entry**	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
Phe	1	-	-
	2	-	172.91
	3	4.28	54.20
	4	2.93	36.81
	5	-	137.93
	6	7.24	128.06
	7	7.21	129.60
	8	7.17	126.23
	9	7.79	-
	10	-	170.73
Asn	11	4.36	50.89
	12	2.31	37.71
	13	-	171.45
	14	6.71, 7.78	-
	15	8.99	-
	16	-	167.33
Gly	17	4.48	69.00
	18	2.72	36.60
	19	-	167.80
Nbp	20	4.76	68.98
	21	2.23	23.93
	22	2.07	20.10
	23	3.43	66.10
	24	4.48	51.50
	25	-	130.50
	26	7.68	132.70
	27	7.37	128.00
	28	7.42	129.44

HRMS (ESI+) *m/z* calcd. for $C_{28}H_{35}N_5O_7$: 554.2609; found [M+H] 554.2646

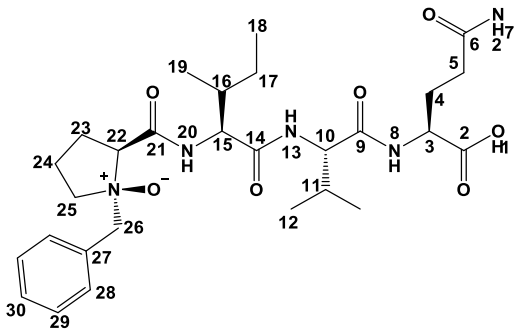
3a, (BzPIVQ)



Residue	Entry	Single conformer	
		^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)
Gln	1	-	-
	2	-	173.60
	3	4.12	51.90
	4	1.77, 1.92	27.03
	5	2.14	31.50
	6	-	173.07
	7	6.70, 7.22	-
	8	8.11	-
Val	9	-	170.60
	10	4.23	57.50
	11	1.96	30.60
	12	0.81, 0.82	18.00, 19.20
	13	8.03	-
Ile	14	-	170.90
	15	4.37	55.60
	16	1.75	37.60
	17	1.02, 1.42	24.16
	18	0.80	11.08
	19	0.83	15.42
	20	7.90	-
Nbp	21	-	172.80
	22	3.04	66.90
	23	1.69, 2.11	30.20
	24	1.62, 1.73	23.30
	25	2.27, 2.79	52.67
	26	3.34, 3.79	58.77
	27	-	138.47
	28	7.29	128.90
	29	7.37	128.10
	30	7.25	127.00

HRMS (ESI+) *m/z* calcd. for $C_{28}H_{43}N_5O_6$: 546.3286; found [M+H] 546.3333

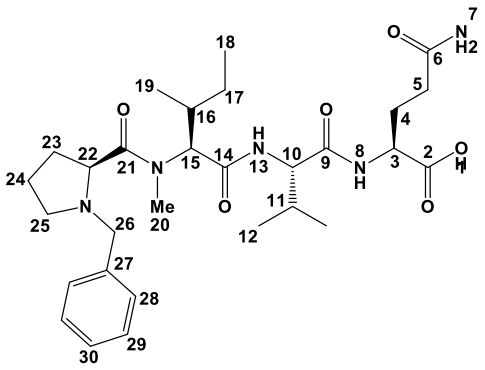
3b, [Bz(NO)PIVQ]



		Single conformer	
Residue	**Entry**	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
Gln	1	-	-
	2	-	170.30
	3	4.07	52.10
	4	1.76, 1.90	27.32
	5	2.11	31.60
	6	-	173.80
	7	6.7, 7.24	-
	8	8.00	-
Val	9	-	170.70
	10	4.23	57.60
	11	1.99	30.66
	12	0.81, 0.85	18.06, 19.20
	13	7.92	-
Ile	14	-	171.24
	15	4.35	56.60
	16	1.84	36.70
	17	1.09, 1.48	24.30
	18	0.84	11.30
	19	0.88	15.80
	20	12.10	-
Nbp	21	-	167.90
	22	3.70	73.06
	23	2.14, 2.23	26.80
	24	1.82, 2.02	19.60
	25	2.84, 3.42	65.40
	26	4.49, 4.55	67.71
	27	-	131.56
	28	7.51	132.30
	29	7.37	128.80
	30	7.37	127.80

HRMS (ESI+) *m/z* calcd. for $C_{28}H_{43}N_5O_7$: 562.3235; found [M+H] 562.3280

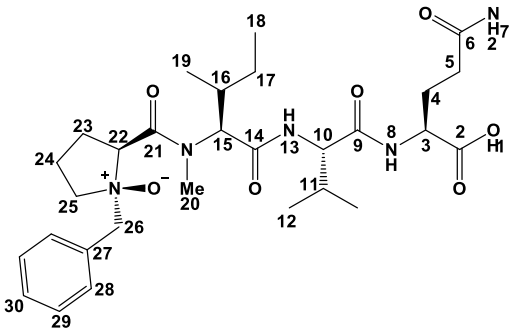
4a, (BzPMeIVQ)



		Major conformation		Minor conformation	
Residue	**Entry**	**^{1}H Chemical shift (ppm)**	**^{13}C chemical shift (ppm)**	**^{1}H Chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
Gln	1	-	-	-	-
	2	-	170.37	-	170.37
	3	4.17	51.90	4.19	57.10
	4	1.75, 1.97	27.26	1.74, 1.91	25.84
	5	2.11	31.38	2.11	31.35
	6	-	173.61	-	173.61
	7	6.79, 7.29	-	6.80, 7.31	-
	8	7.45	-	8.42	-
Val	9	-	170.71	-	170.32
	10	4.14	57.18	4.26	57.17
	11	1.93	30.82	1.83	30.53
	12	0.77, 0.85	19.15	0.55, 0.81	15.21
	13	8.08	-	8.91	-
Ile	14	-	169.29	-	169.29
	15	4.72	59.60	4.72	59.60
	16	1.95	31.12	1.78	32.61
	17	0.93, 1.12	24.60	0.58, 1.13	24.43
	18	0.82	10.10	0.76	17.51
	19	0.81	18.13	0.75	11.08
	20	2.87	29.87	2.71	28.70
Nbp	21	-	173.11	-	172.12
	22	3.58	62.75	3.83	62.60
	23	1.78, 2.13	27.22	1.59, 2.10	25.84
	24	1.75	22.60	1.85	22.33
	25	2.40, 2.93	51.81	2.54, 2.72	52.10
	26	3.55, 3.80	56.50	3.44, 3.46	57.71
	27	-	139.02	-	138.53
	28	7.28	128.02	7.28	128.27
	29	7.24	126.78	7.24	127.20
	30	7.30	128.50	7.35	129.38

HRMS (ESI+) *m/z* calcd. for $C_{29}H_{45}N_5O_6$: 560.3442; found [M+H] 560.3482

4b, [Bz(NO)PMeIVQ]



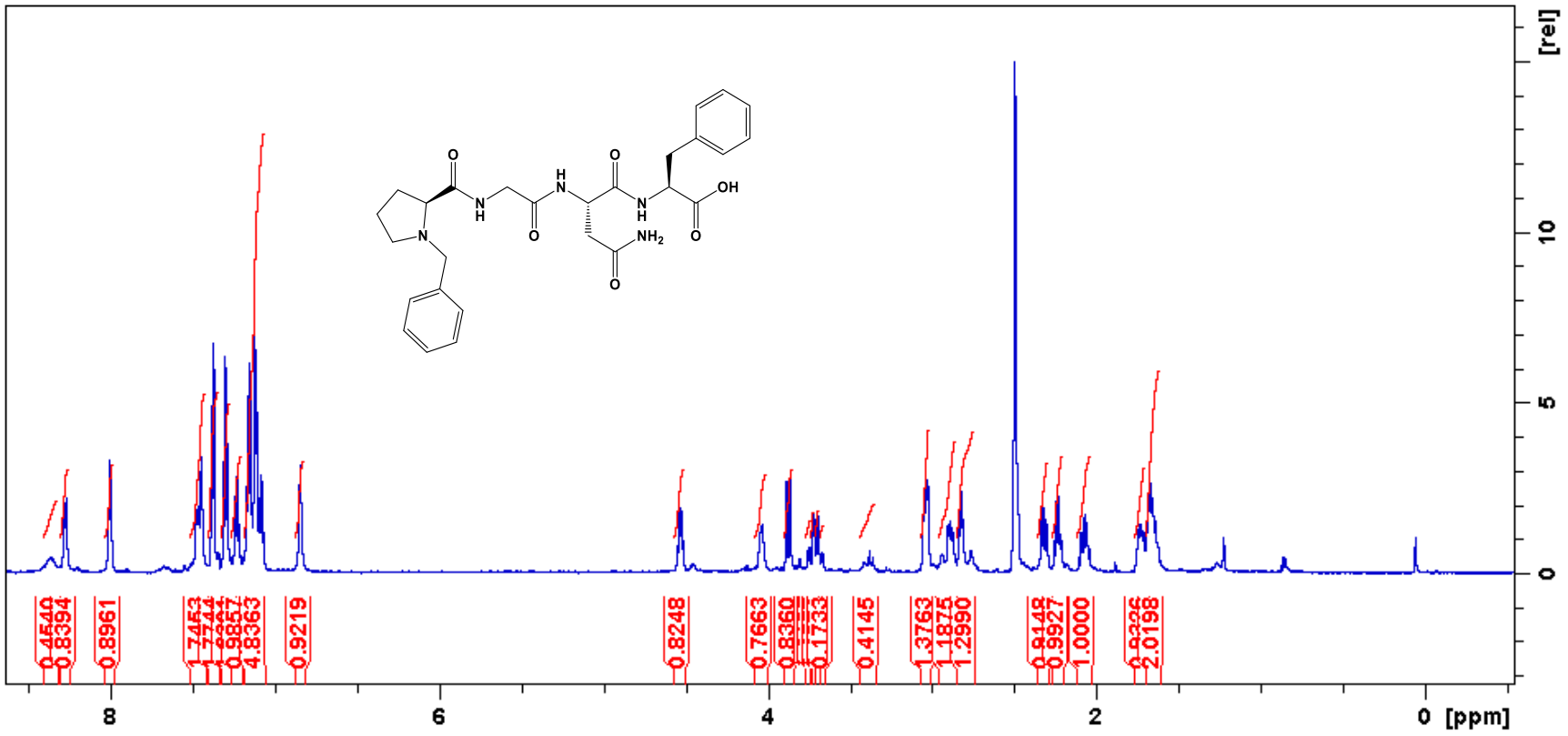
		Major conformation	
Residue	**Entry**	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
Gln	1	-	-
	2	-	173.67
	3	4.22	52.10
	4	1.88, 1.97	26.98
	5	2.21	23.80
	6	-	173.38
	7	6.82, 7.29	-
	8	8.00	-
Val	9	-	170.87
	10	4.03	58.40
	11	1.96	30.20
	12	0.80, 0.83	18.10, 19.10
	13	9.85	-
Ile	14	-	167.70
	15	3.15	63.80
	16	1.66	31.21
	17	0.28, 0.97	23.82
	18	0.69	11.53
	19	0.32	15.63
	20	2.48	28.76
Nbp	21	-	167.30
	22	3.70	68.70
	23	2.09, 2.22	19.75
	24	1.96, 2.21	23.80
	25	2.10, 2.20	31.26
	26	4.31, 4.47	69.44
	27	-	130.66
	28	7.82	132.95
	29	7.39	129.75
	30	7.43	128.41

HRMS (ESI+) *m/z* calcd. for $C_{29}H_{45}N_5O_7$: 576.3391; found [M+H] 576.3443

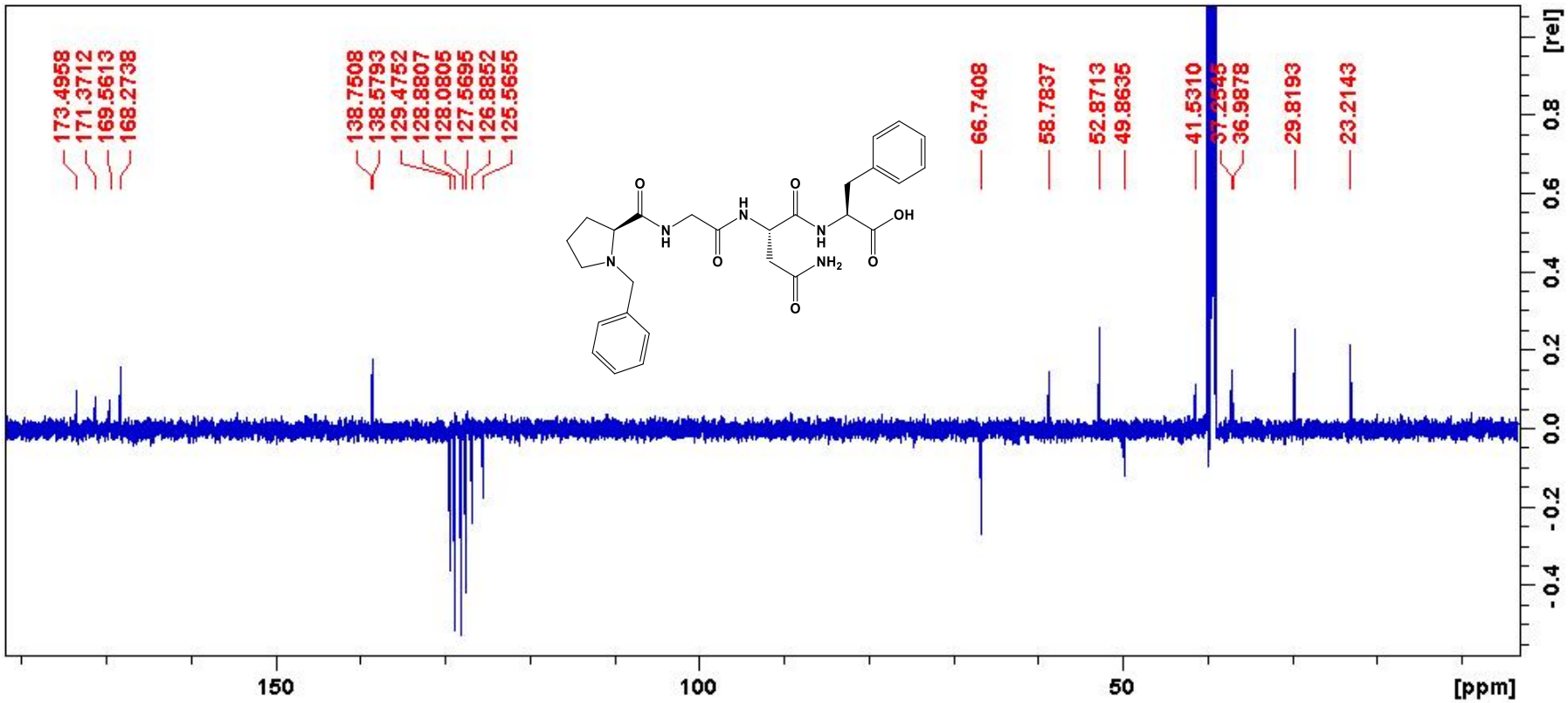
3. NMR Spectra

^{1}H NMR spectra of **1a,** (**BzPGNF**)

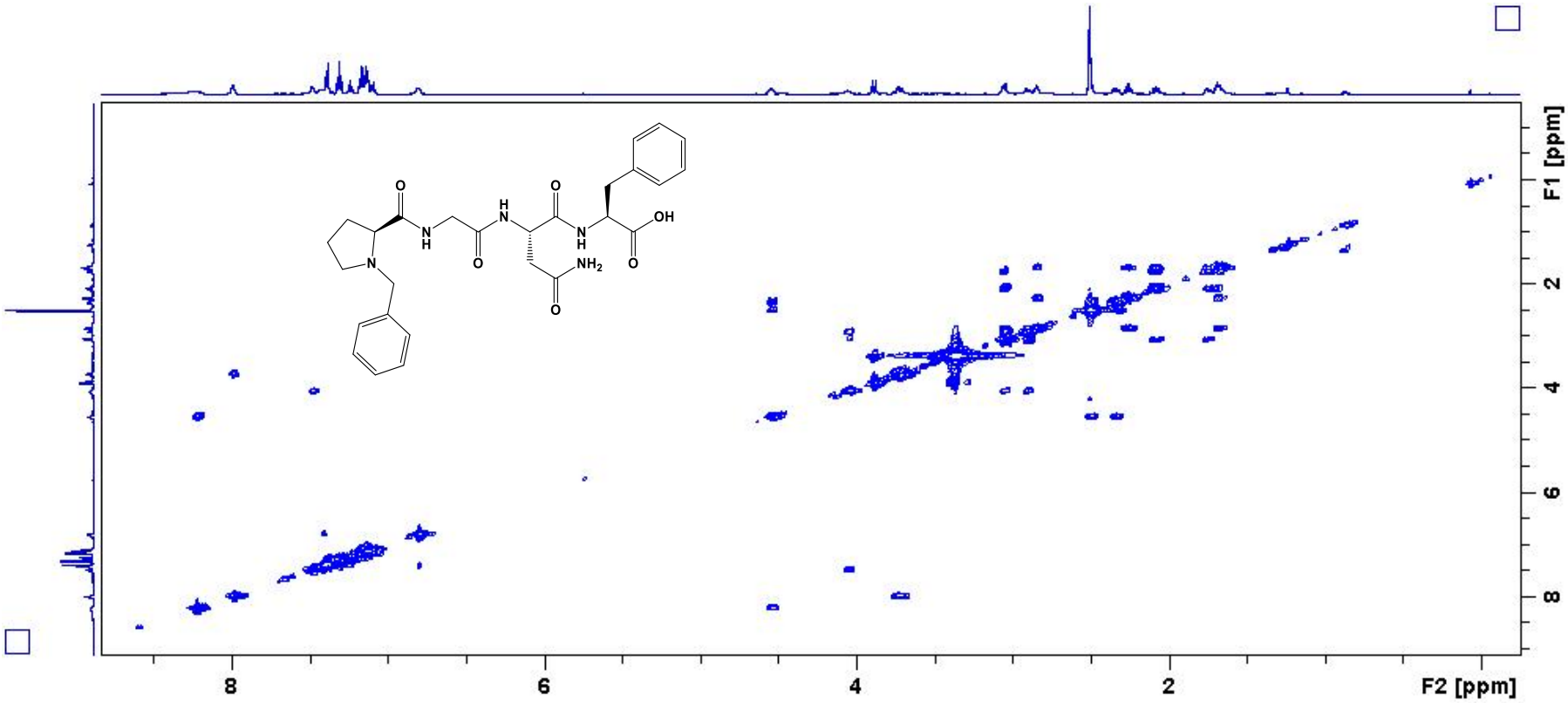
(Water peak was suppressed)



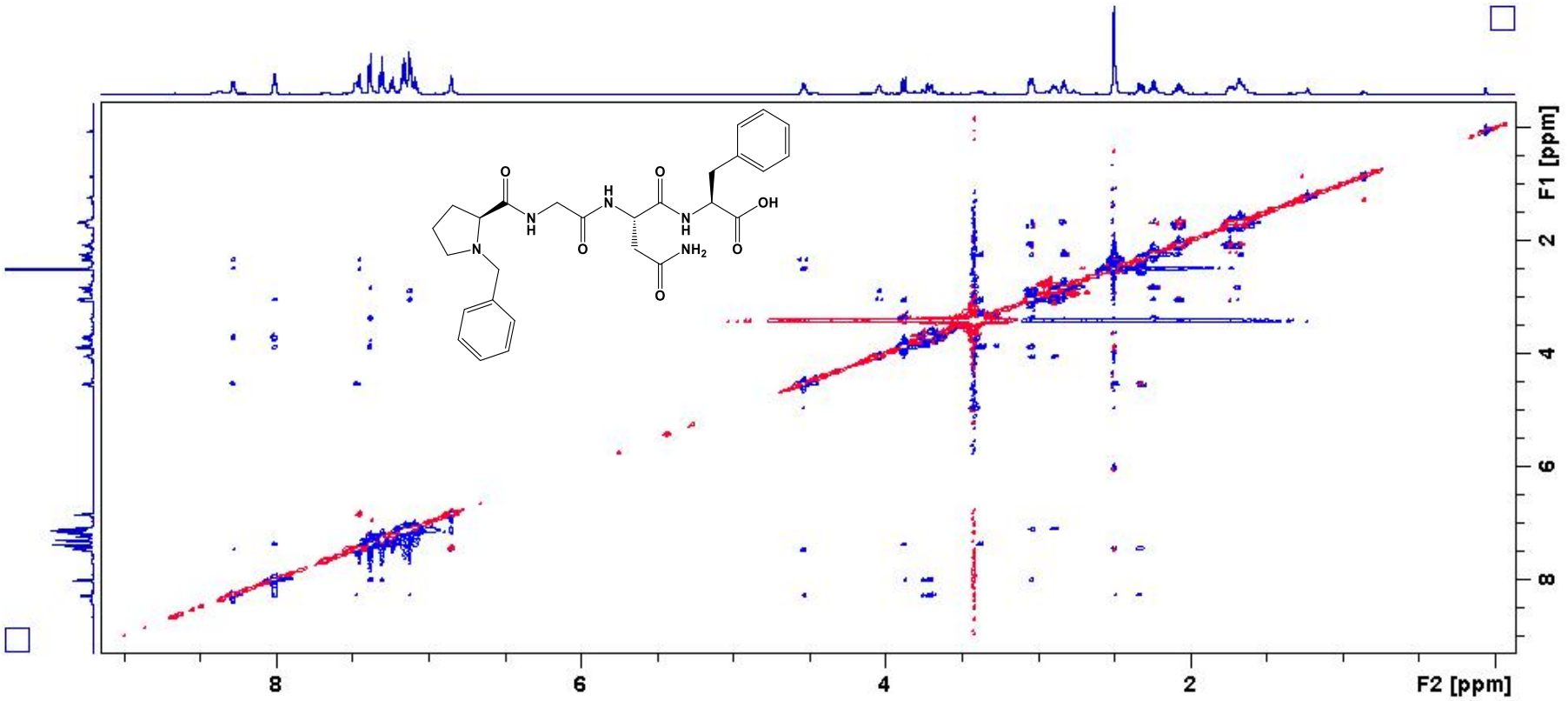
^{13}C NMR spectra of **1a,** (**BzPGNF**)



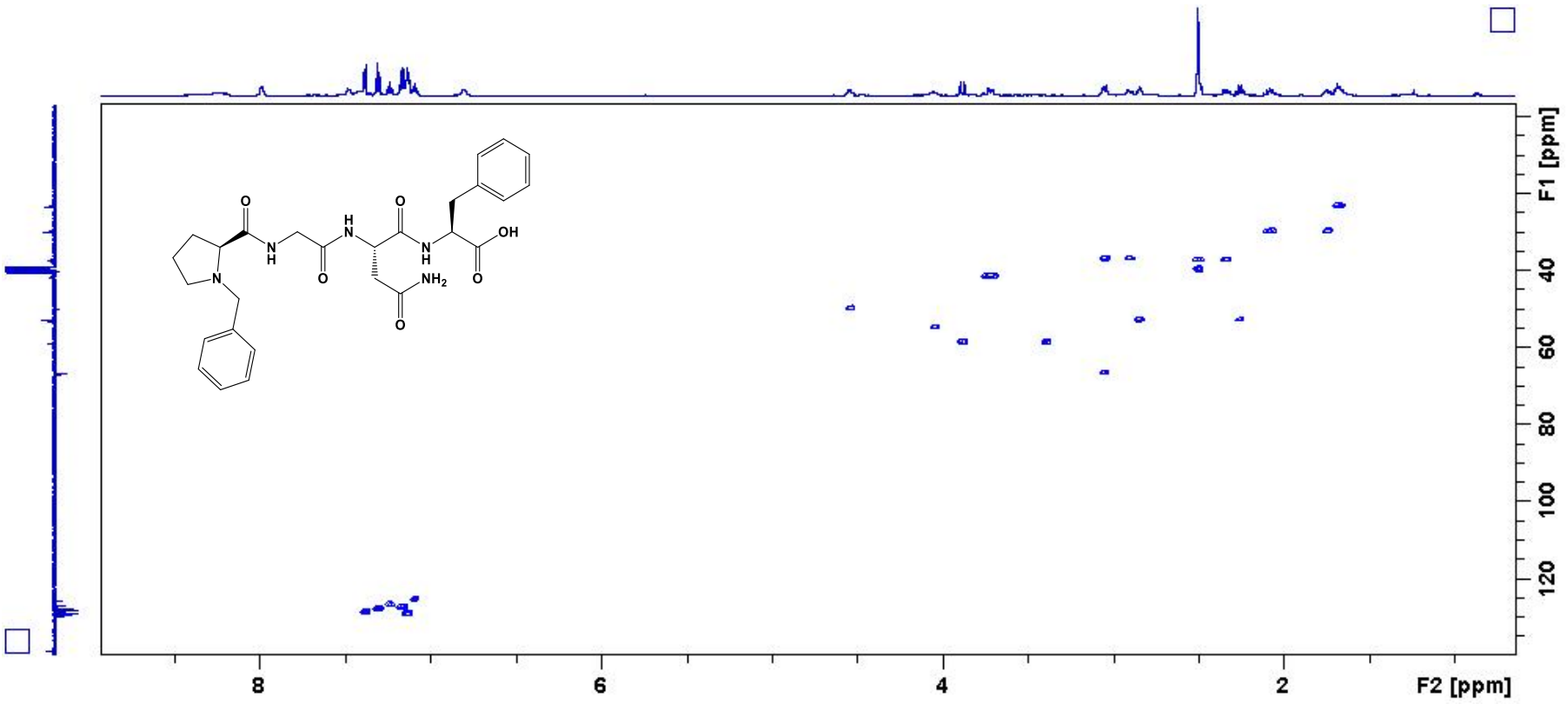
COSY of **1a, (BzPGNF)**



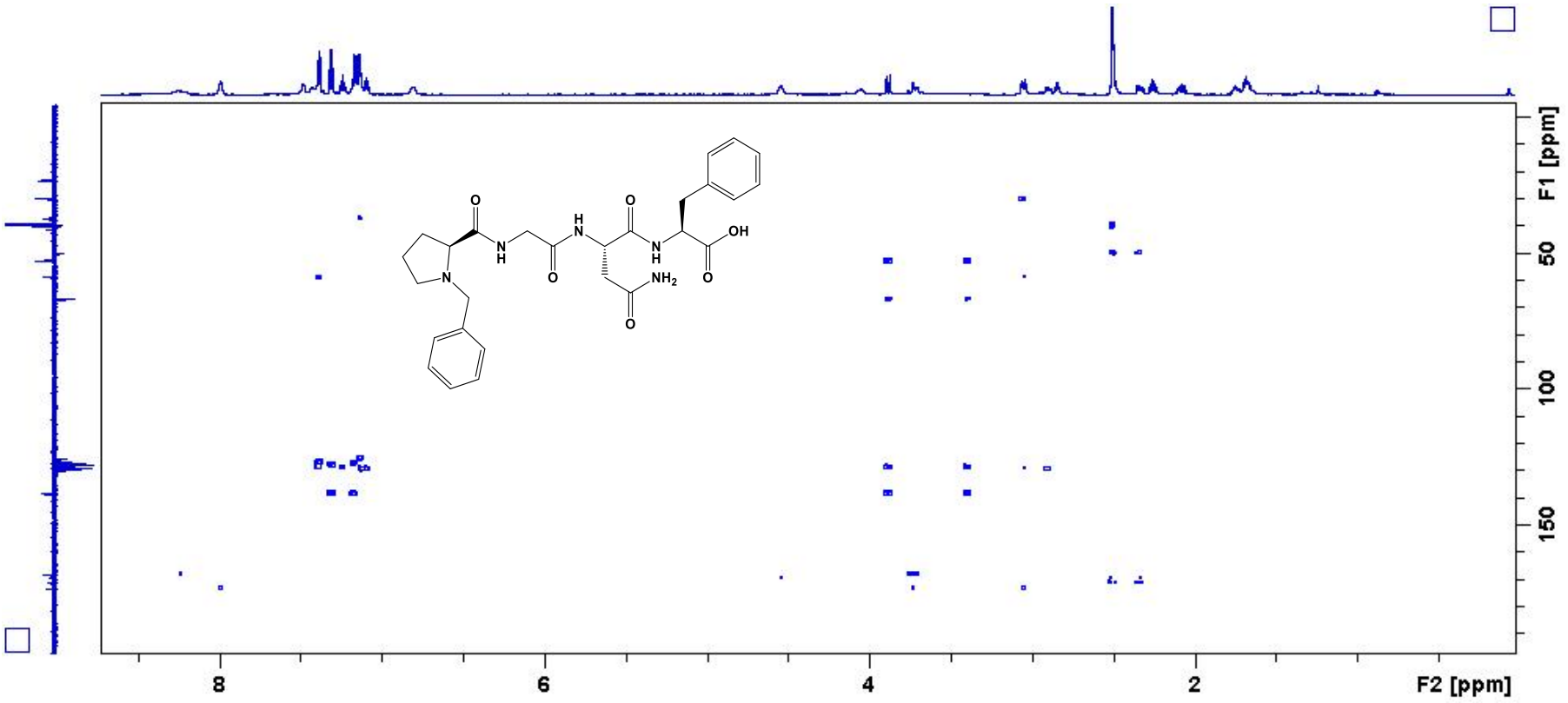
ROESY of **1a,** (**BzPGNF**)



HSQC of **1a, (BzPGNF)**

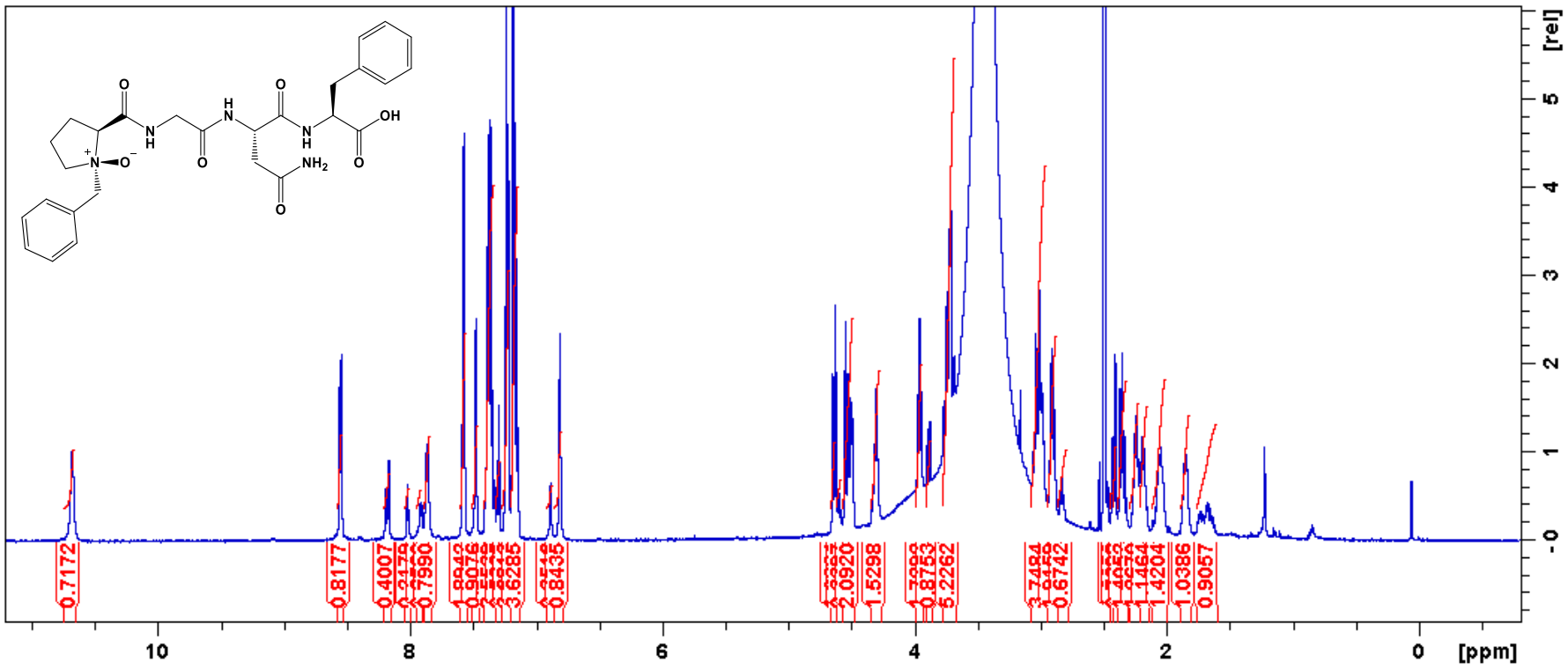


HMBC of **1a,** (**BzPGNF**)



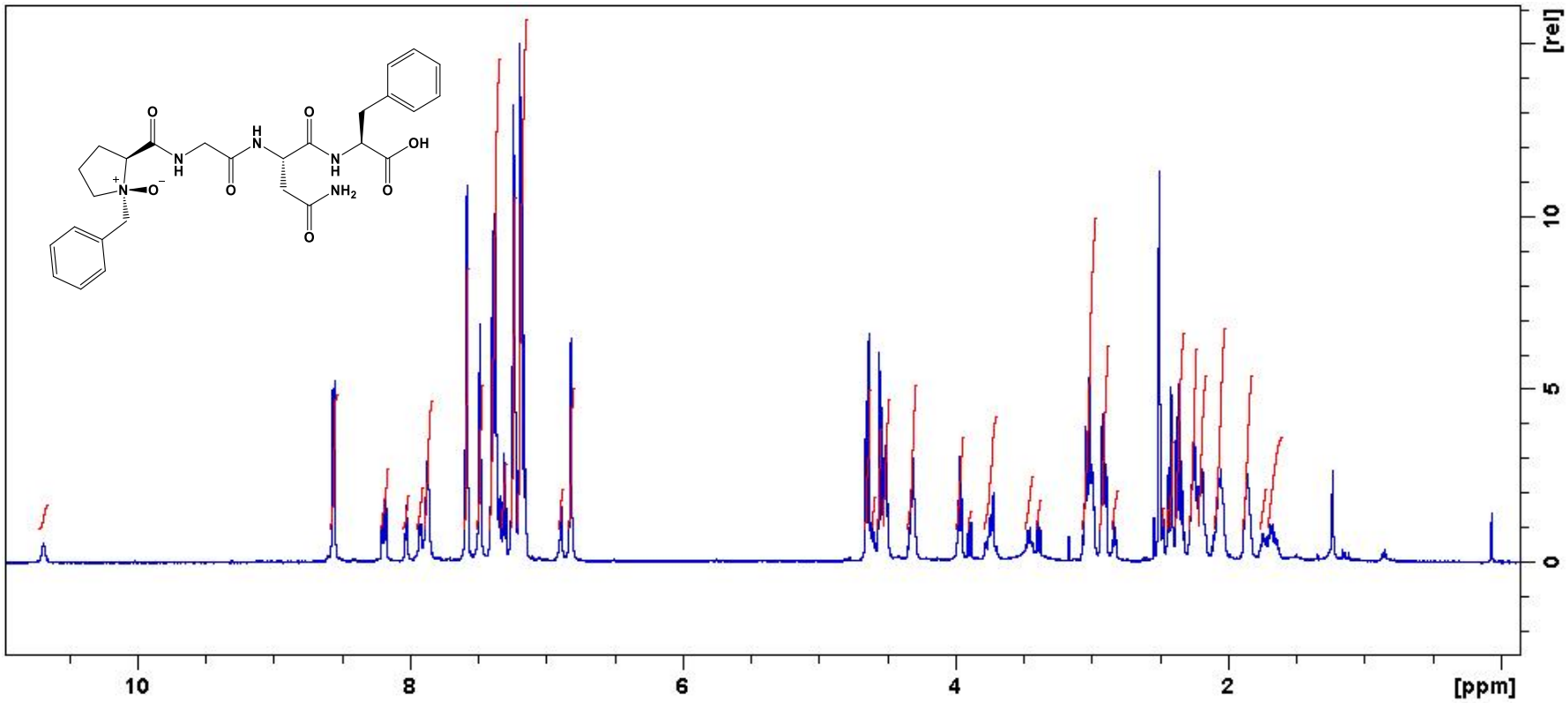
^{1}H NMR spectra of **1b,** [**Bz(NO)PGNF**]

(Water peak was not suppressed)

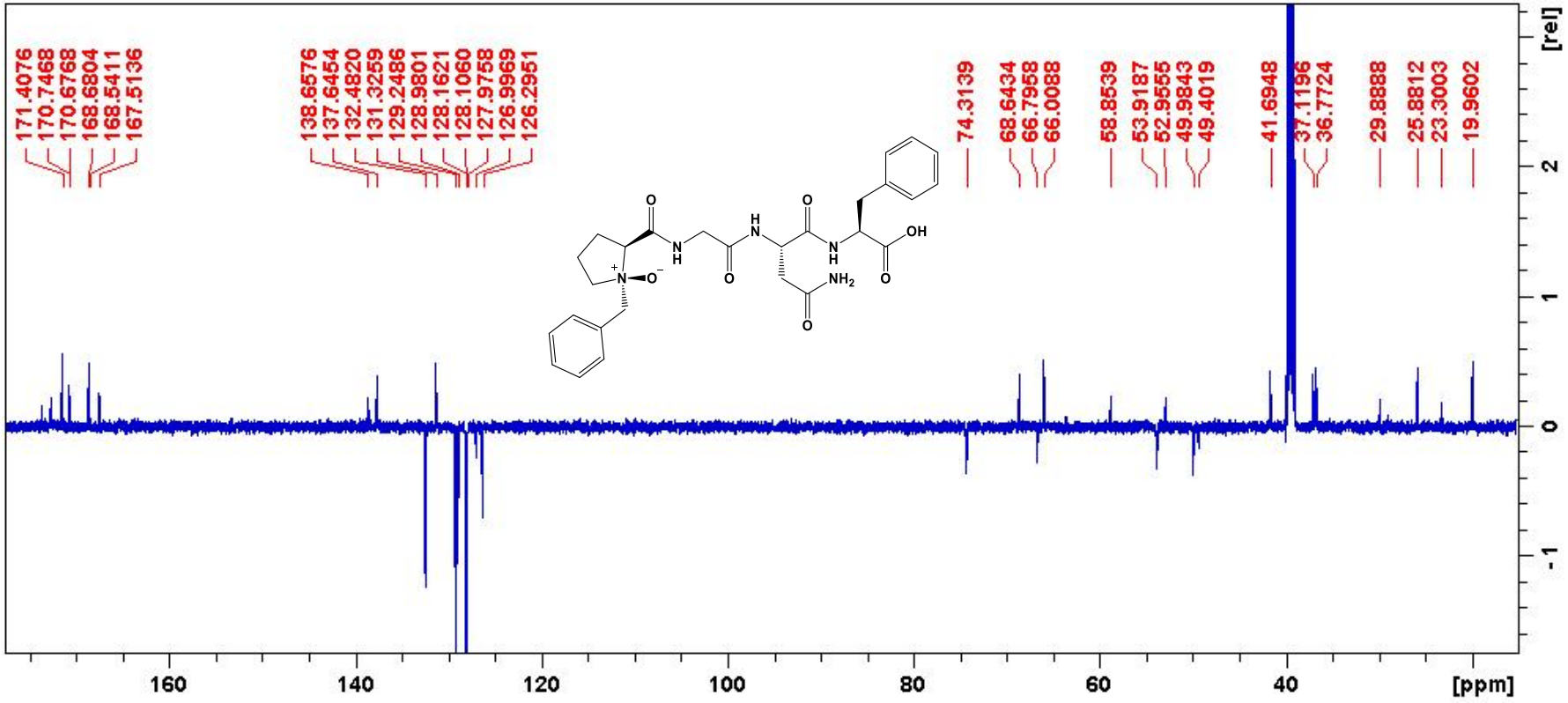


^{1}H NMR spectra of **1b,** [**Bz(NO)PGNF**]

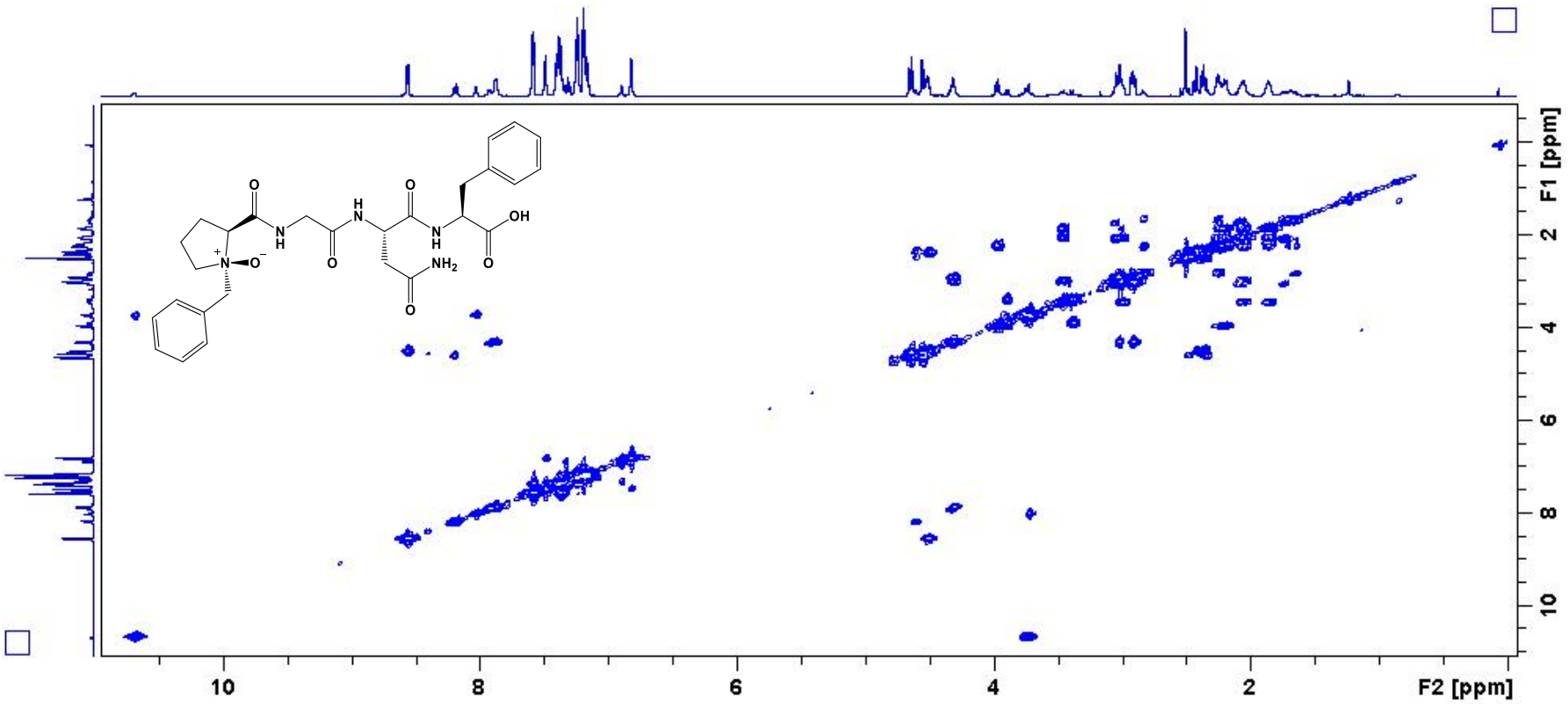
(Water peak was suppressed)



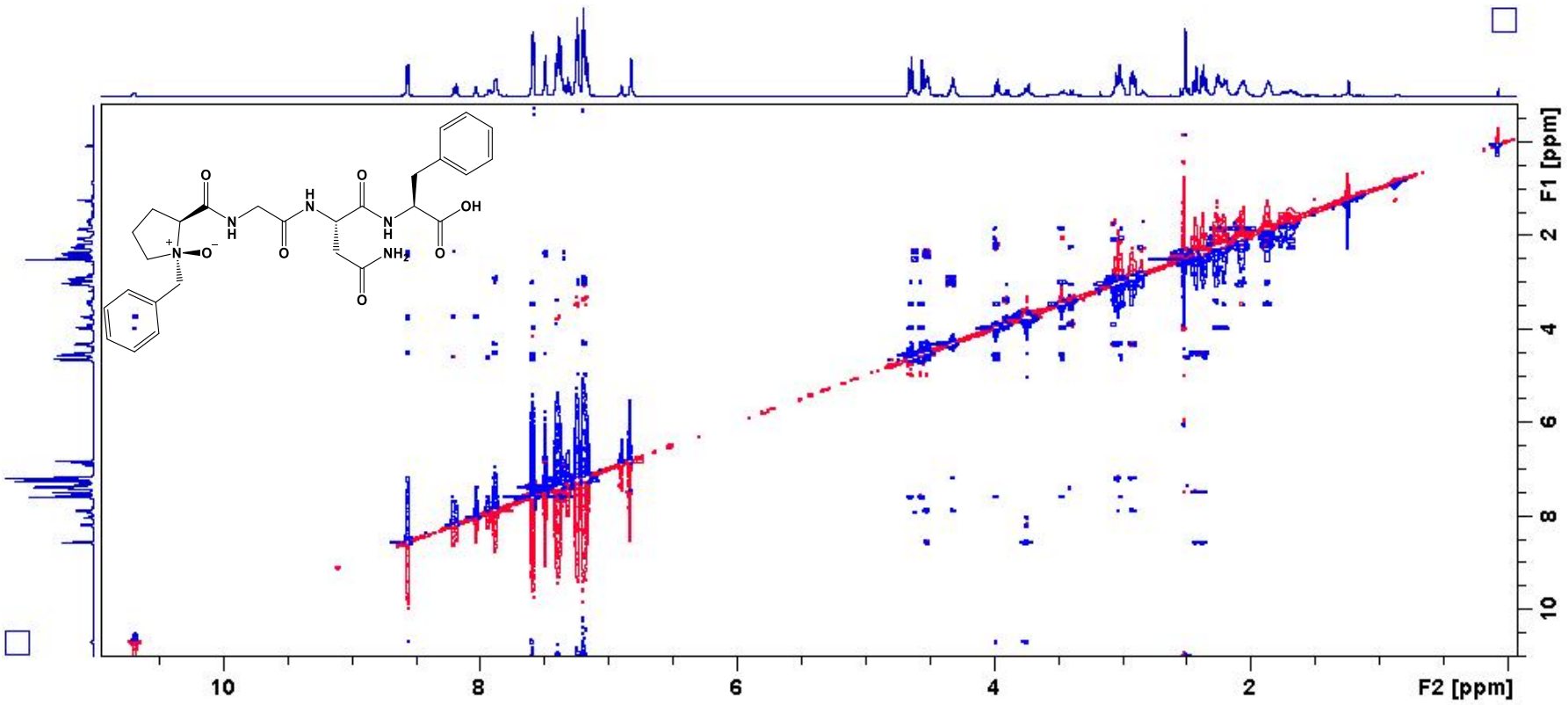
^{13}C NMR spectra of **1b,** [**Bz**(**NO**)**PGNF**]



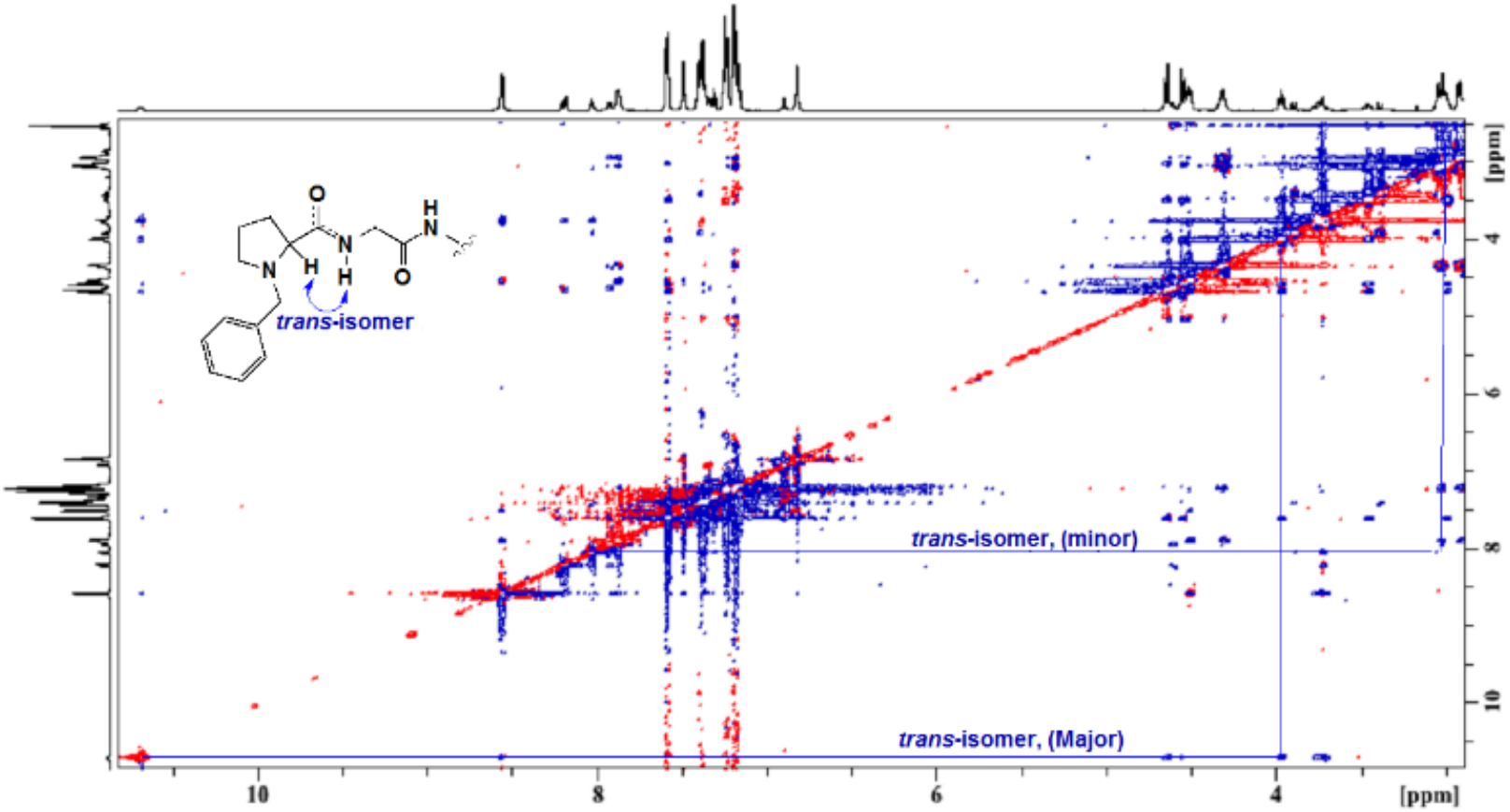
COSY of **1b,** [**Bz(NO)PGNF**]



ROESY of **1b,** [**Bz(NO)PGNF**]

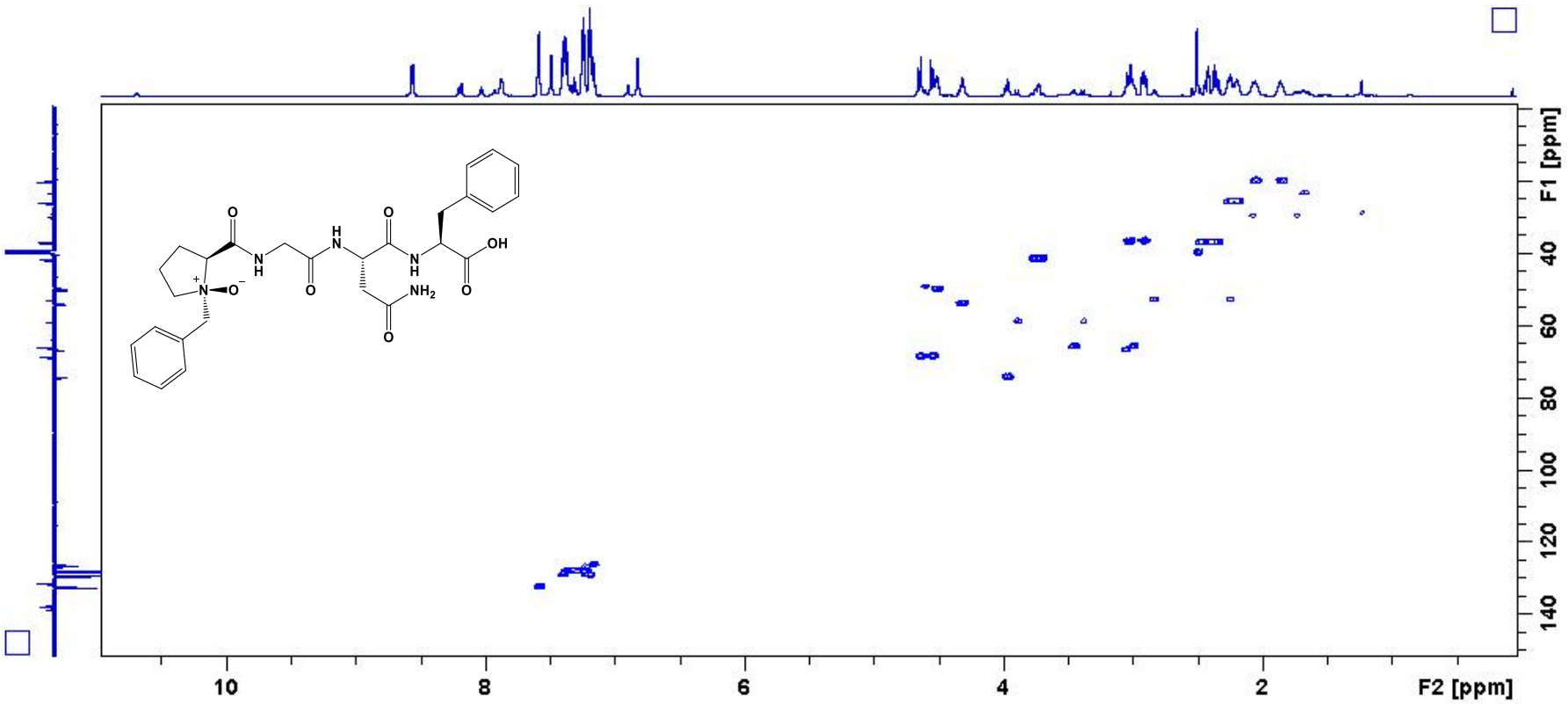


Detection of two different conformers in **1b,** [**Bz(NO)PGNF**]

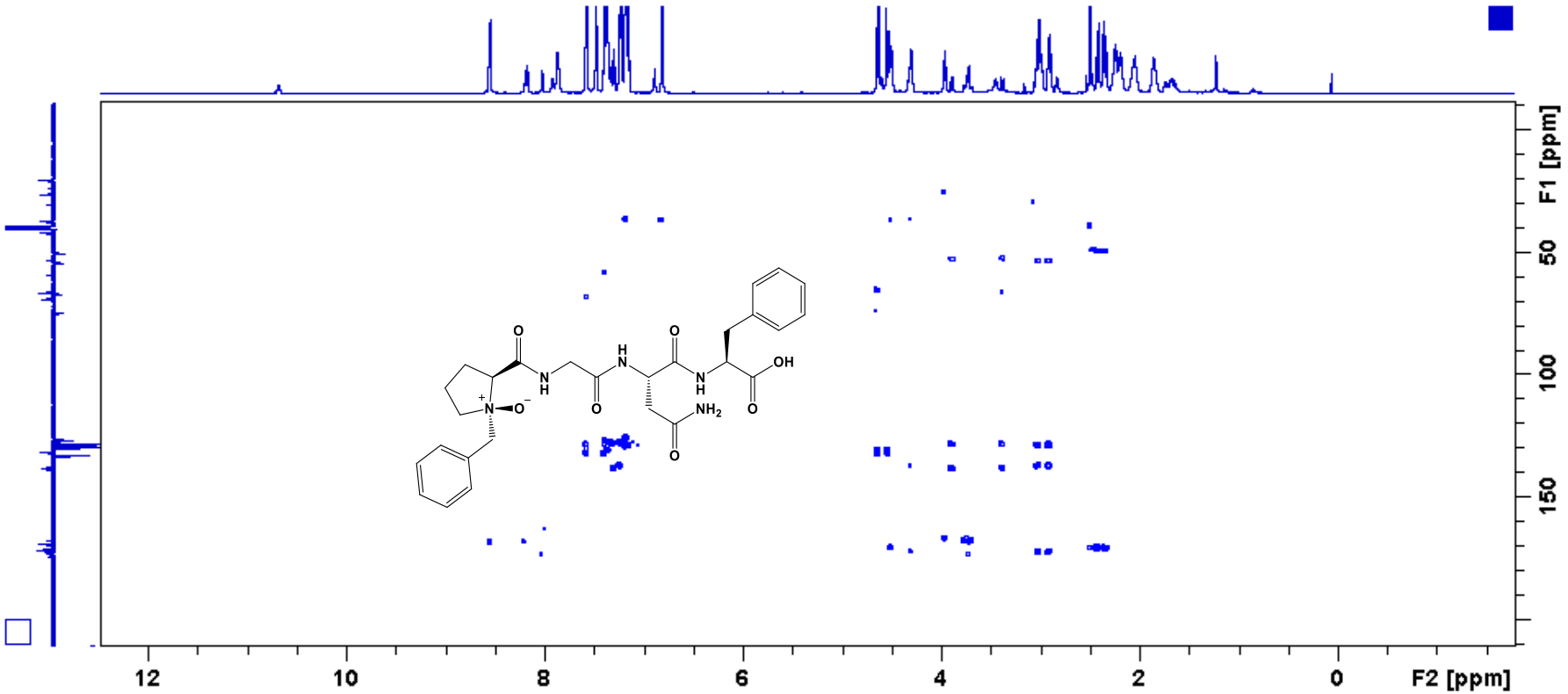


Detection of both *trans* conformers by usage of ROESY in **1b, [Bz(NO)PGNF]** peptide at room temperature. The water peak is suppressed. (Solvent: DMSO-d_6)

HSQC of **1b,** [**Bz(NO)PGNF**]

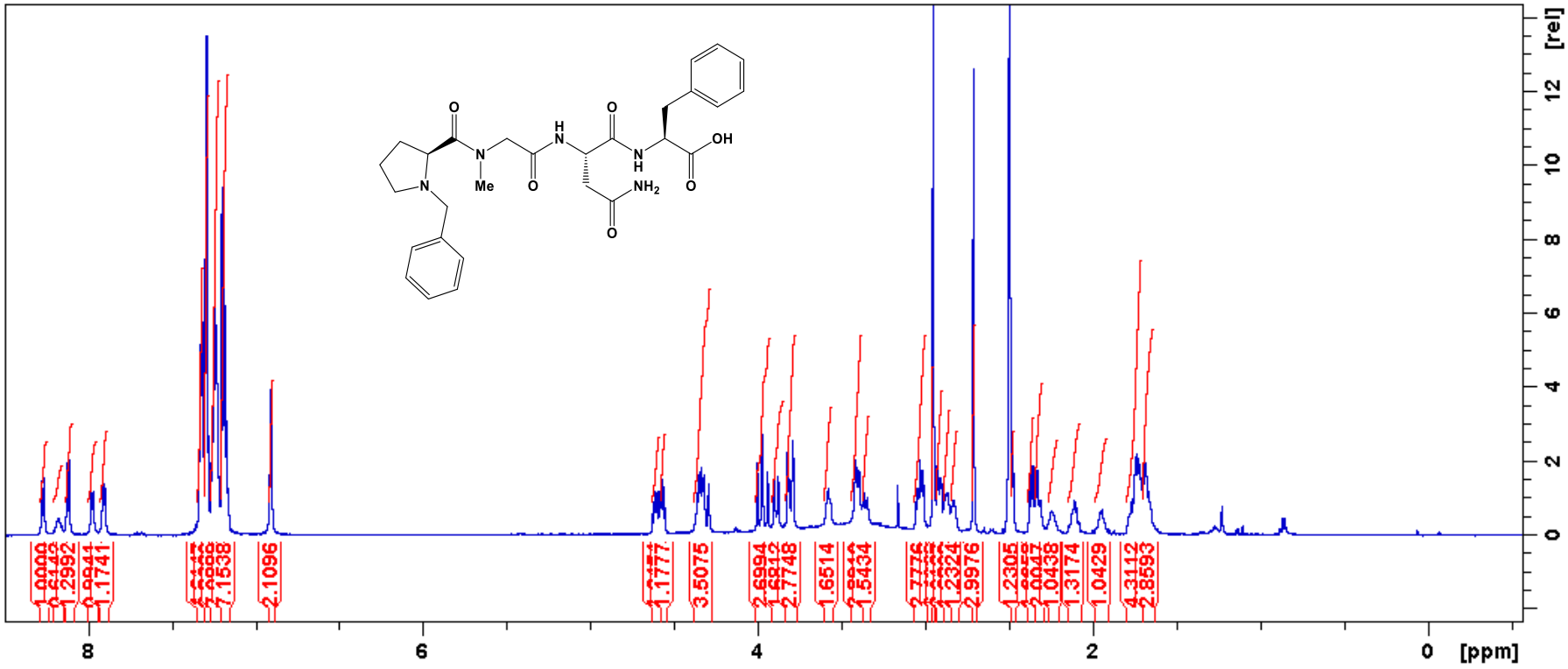


HMBC of **1b,** [**Bz(NO)PGNF**]

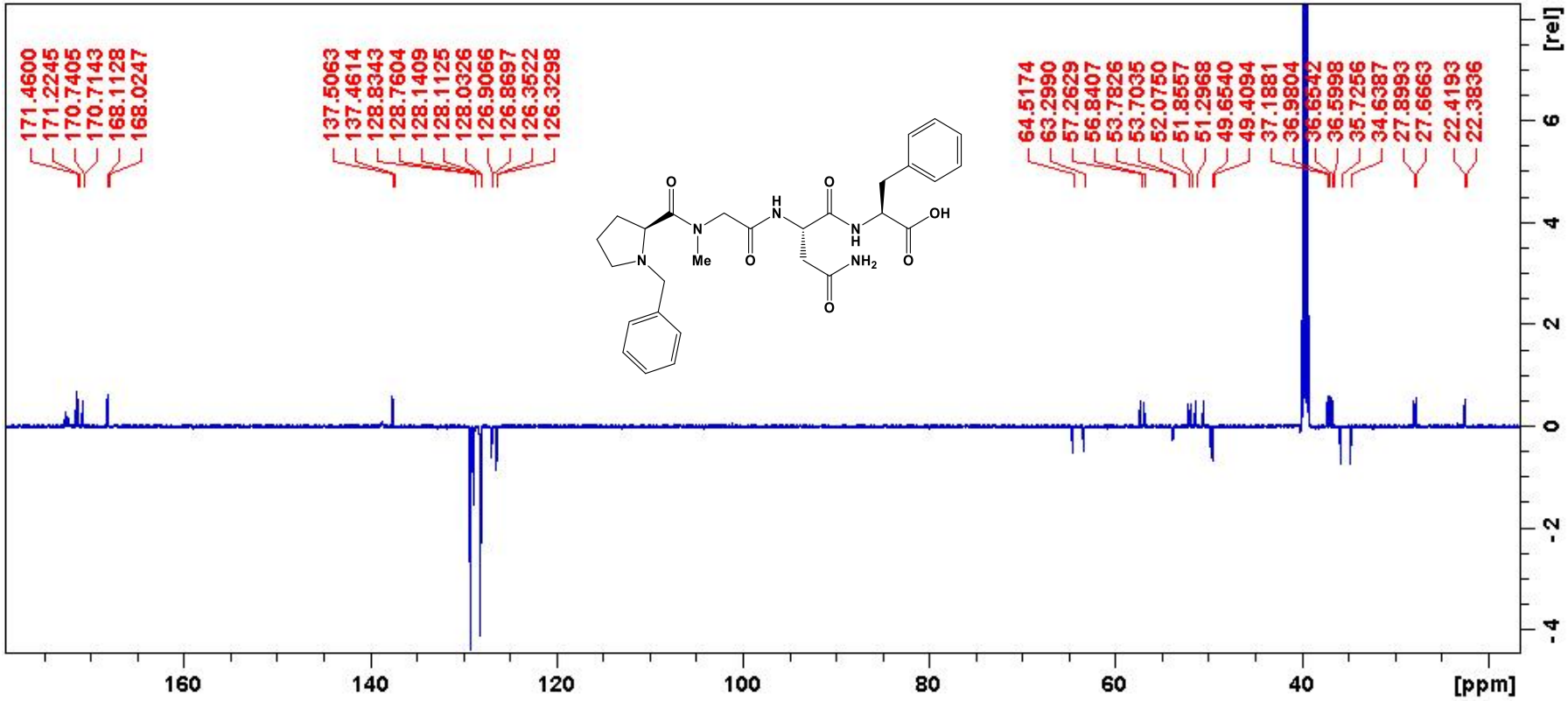


^{1}H NMR spectra of **2a,** (**BzPMeGNF**)

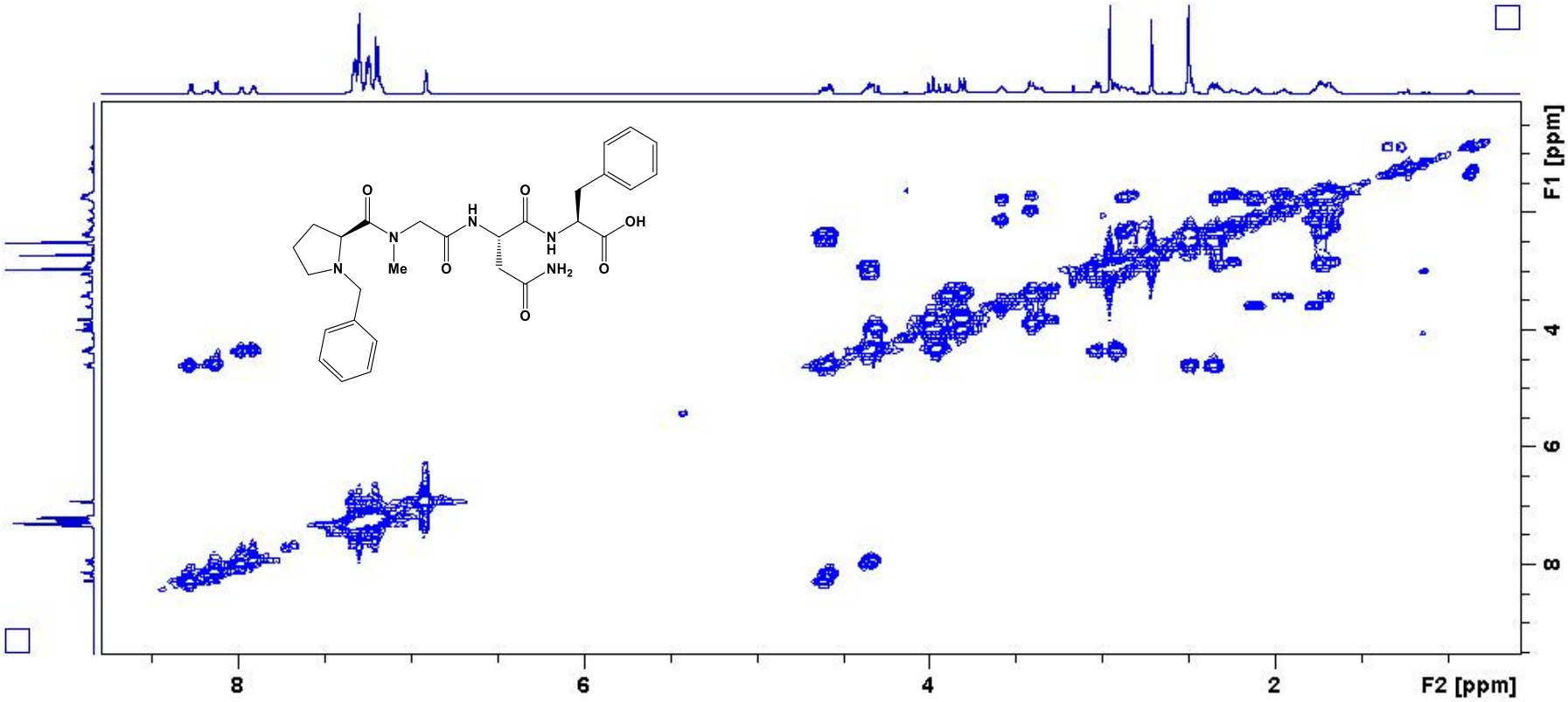
(Water peak was not suppressed)



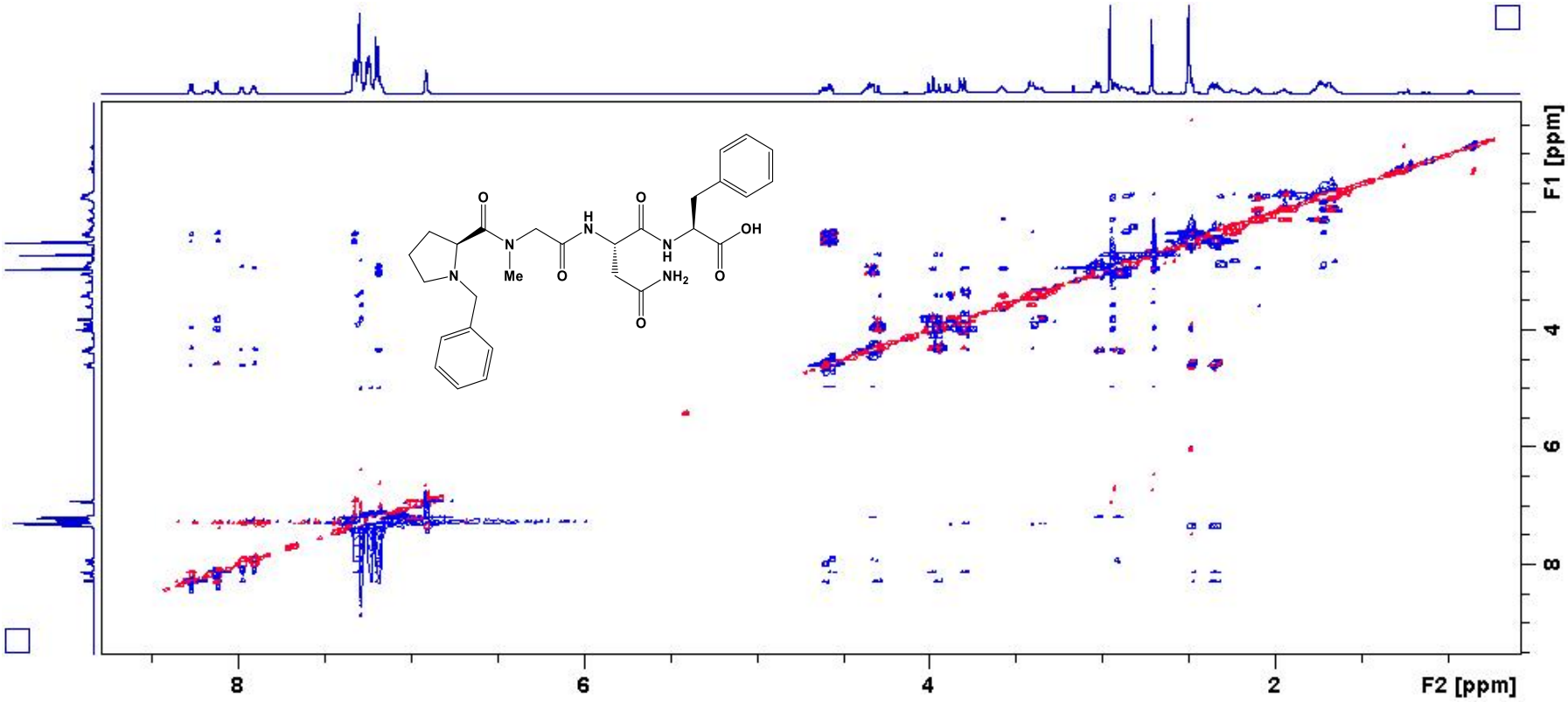
^{13}C NMR spectra of **2a,** (**BzPMeGNF**)



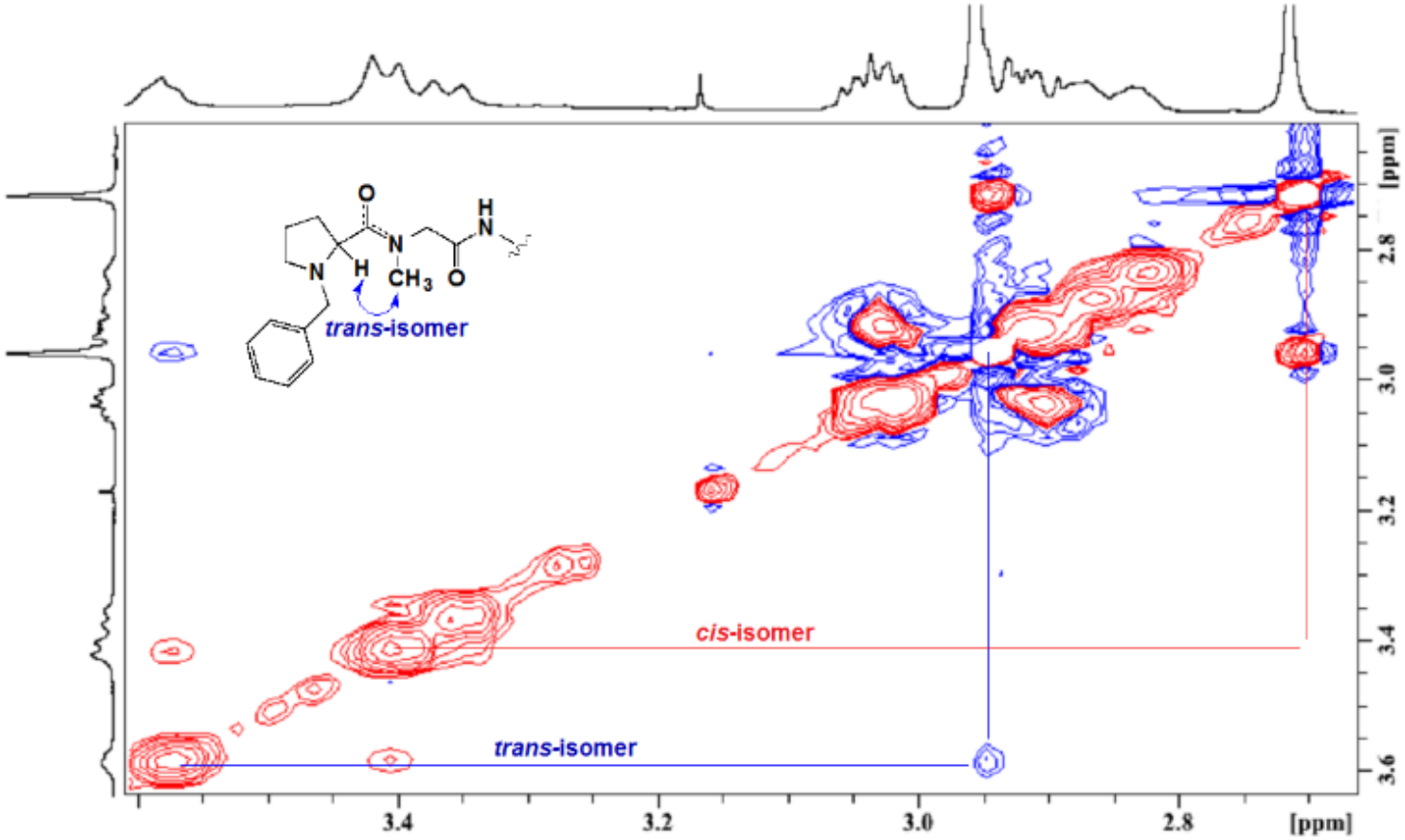
COSY of **2a, (BzPMeGNF)**



ROESY of **2a, (BzPMeGNF)**

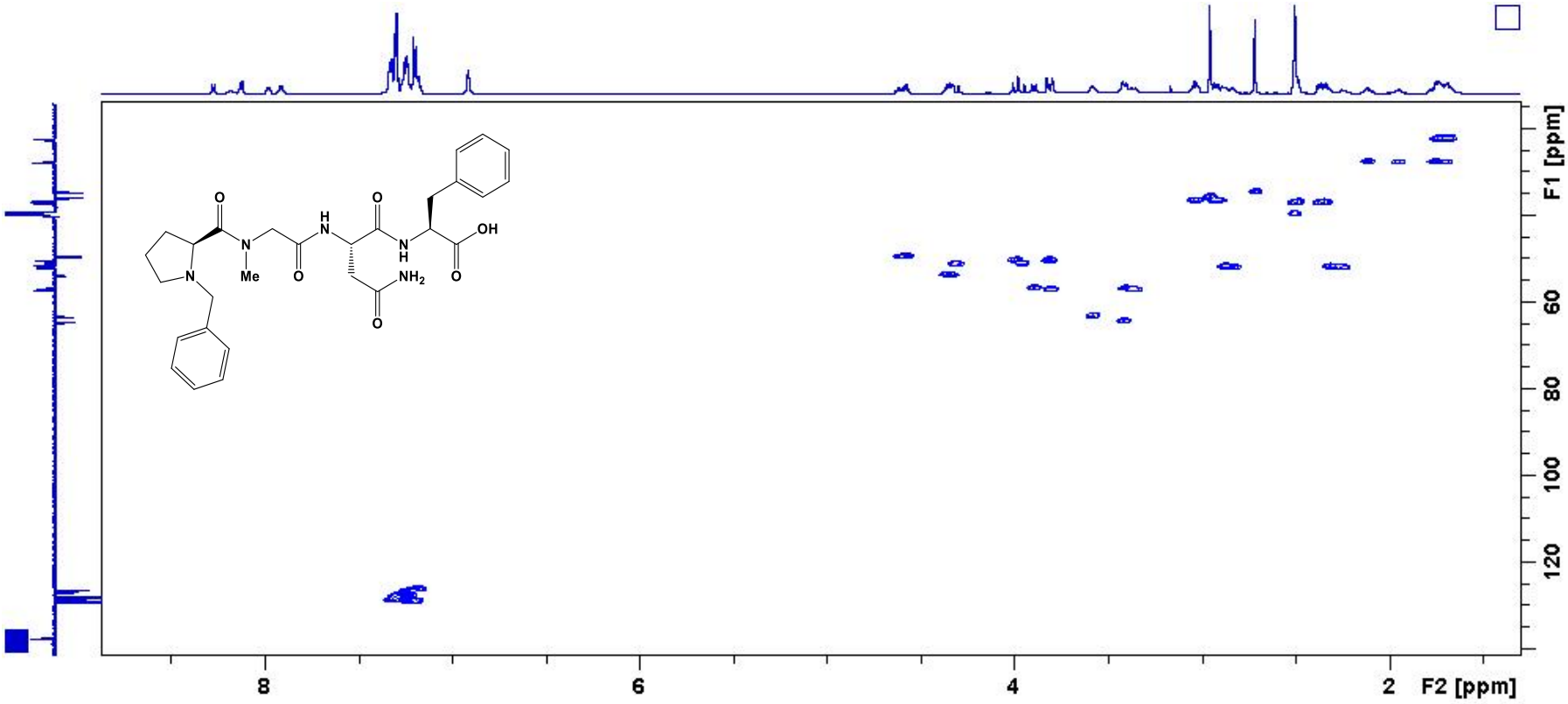


Detection of *cis*- and *trans*-isomers in **2a,** (**BzPMeGNF**)

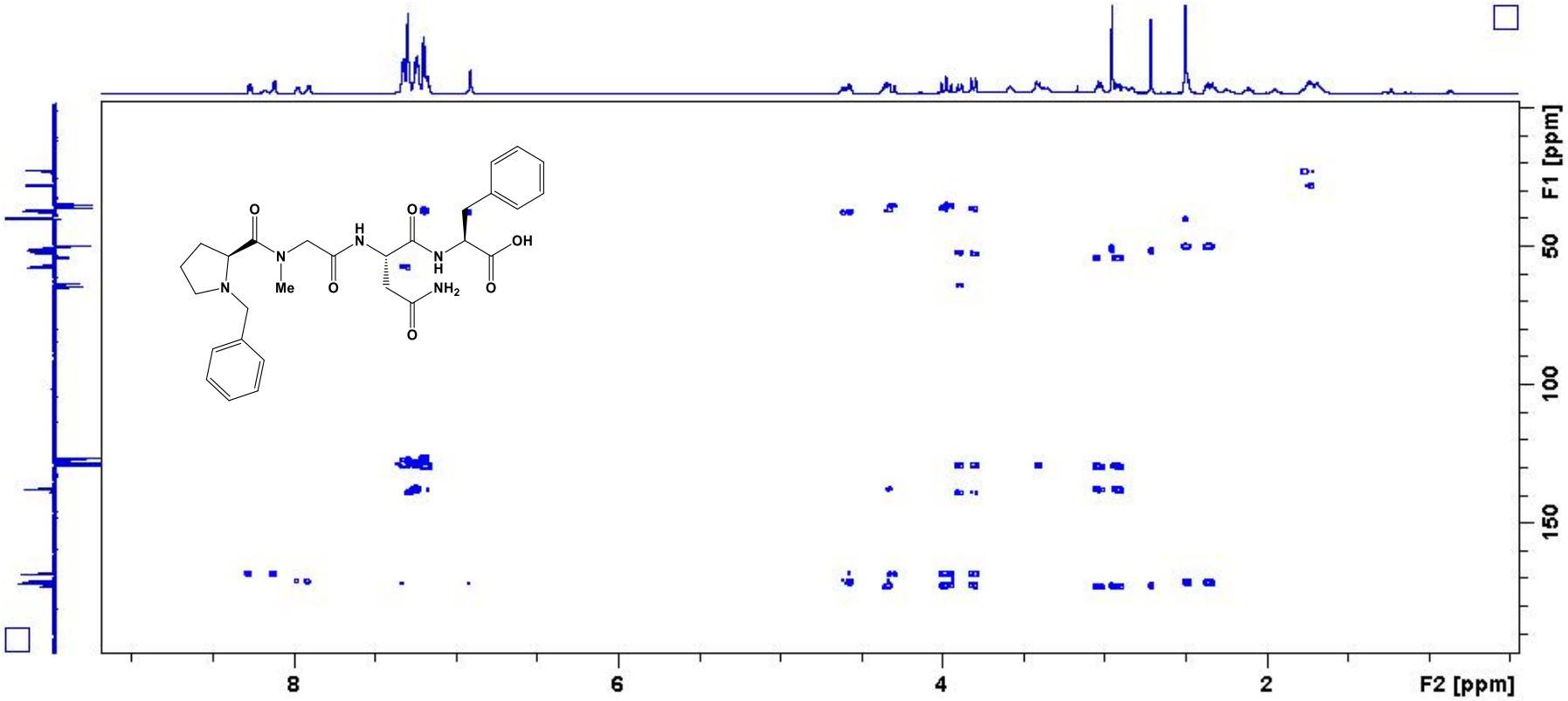


ROESY study for the detection of *cis*- and *trans*-isomers in **2a, (BzPMeGNF)** at room temperature. The water peak is suppressed. (Solvent: DMSO-d_6)

HSQC of **2a,** (**BzPMeGNF**)

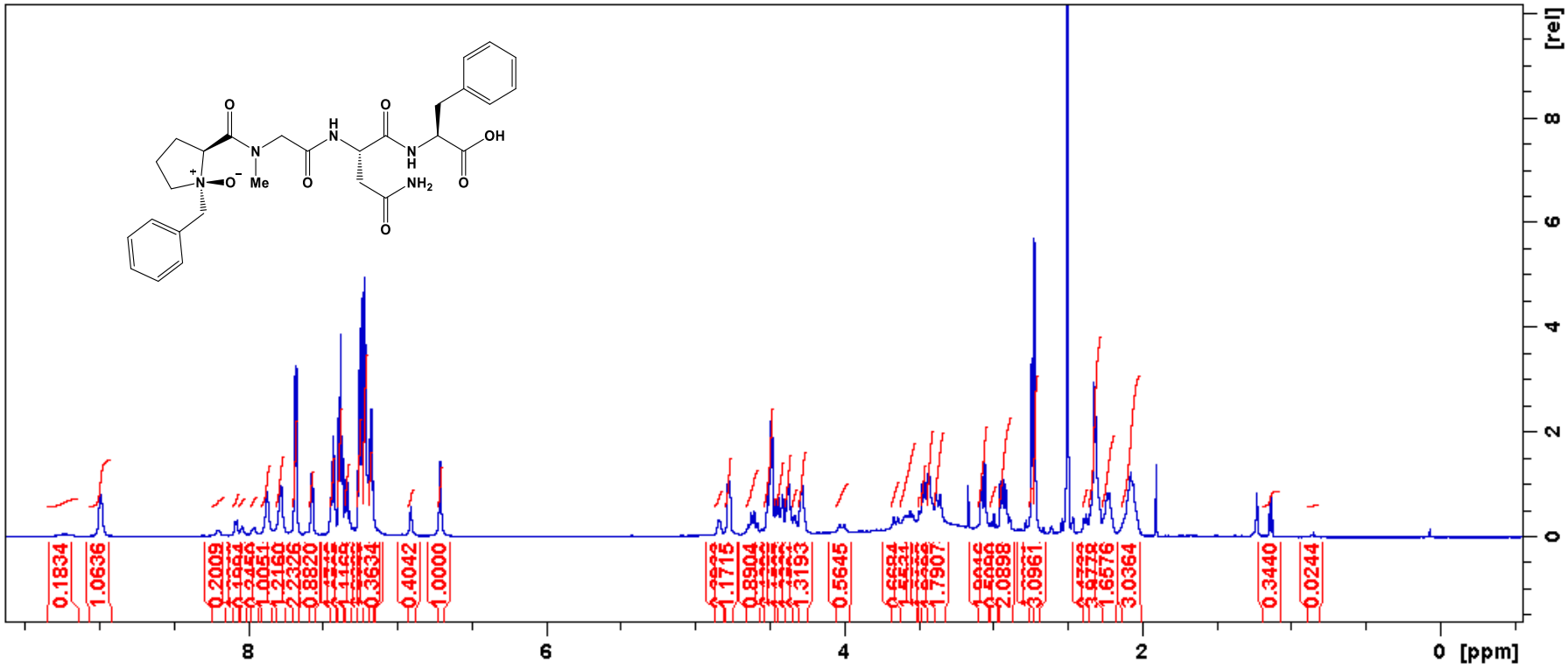


HMBC of **2a,** (**BzPMeGNF**)

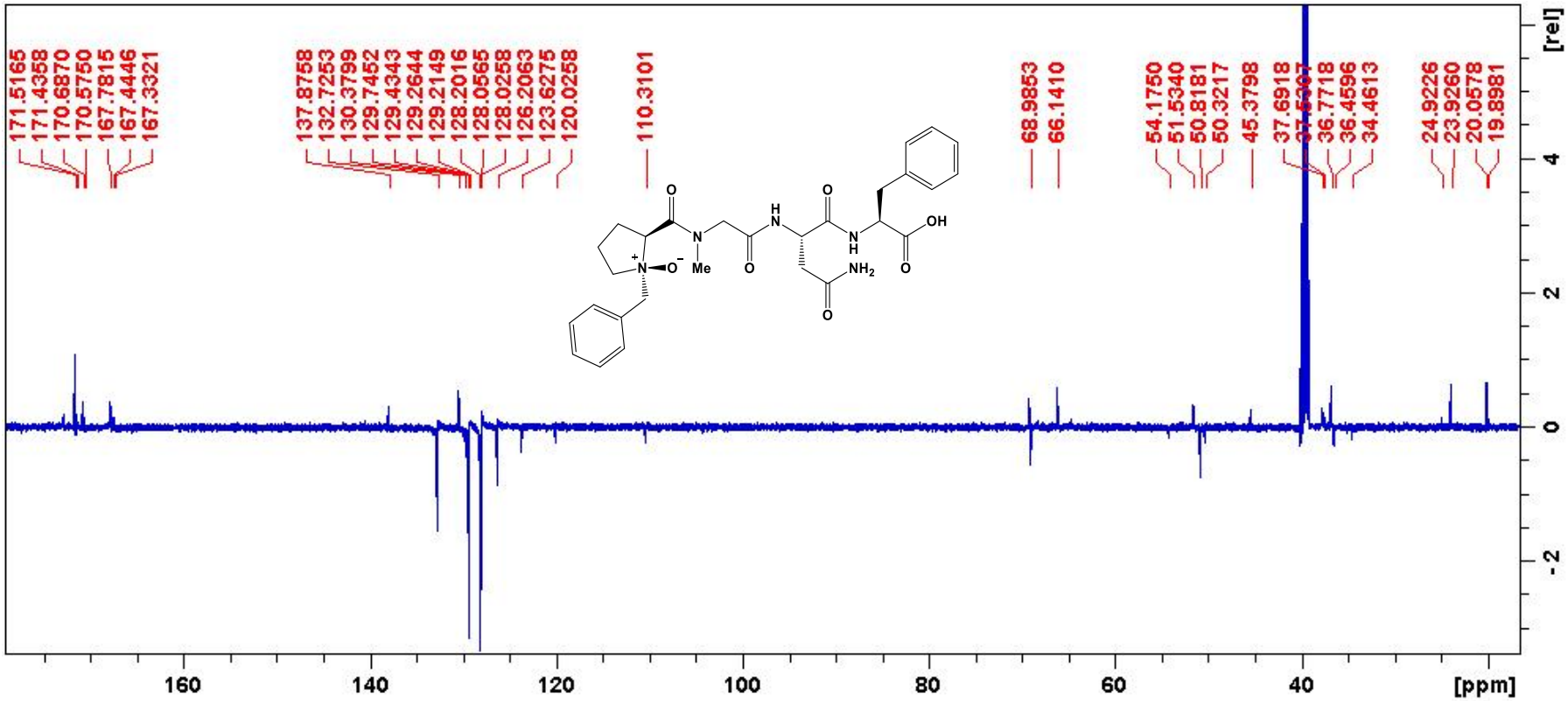


^{1}H NMR of **2b,** [**Bz(NO)PMeGNF**]

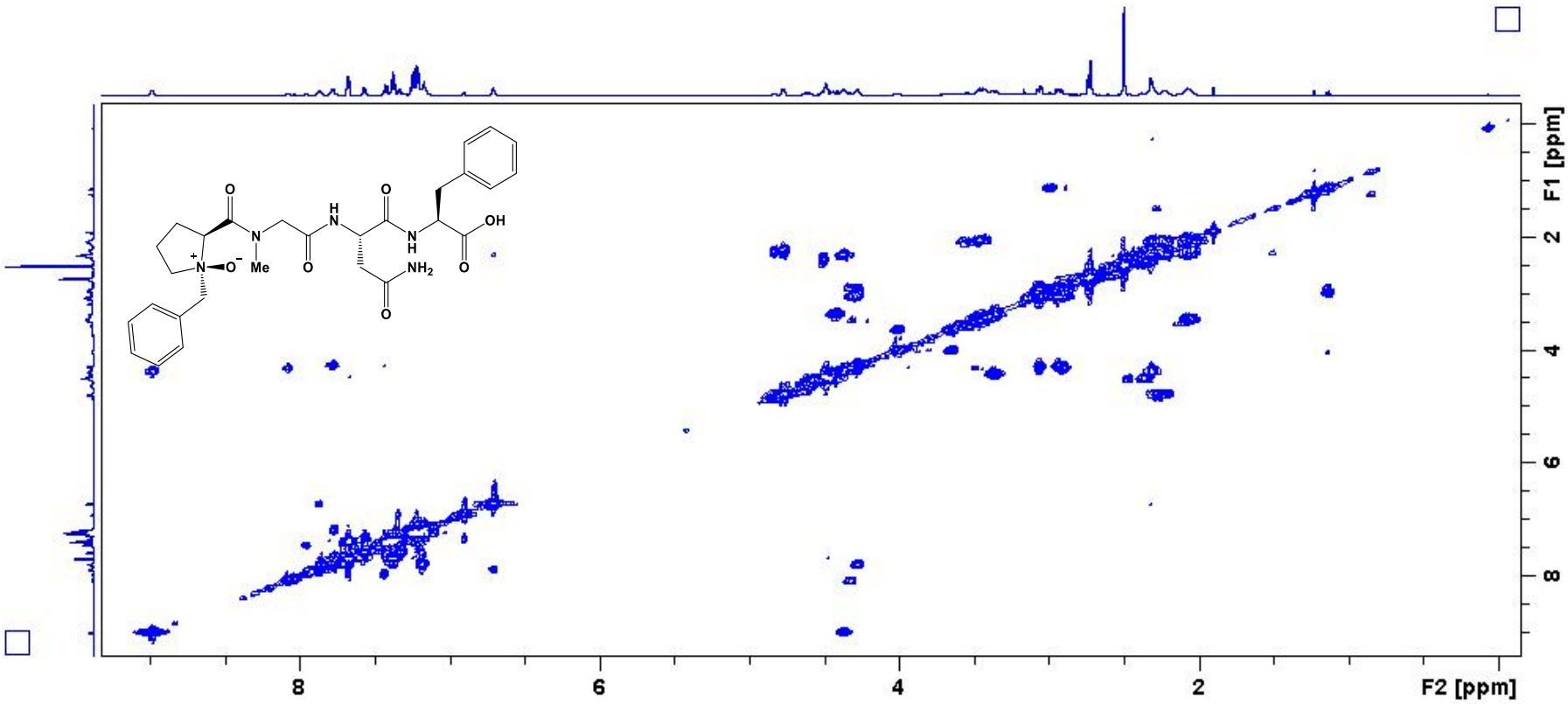
(Water peak was not suppressed)



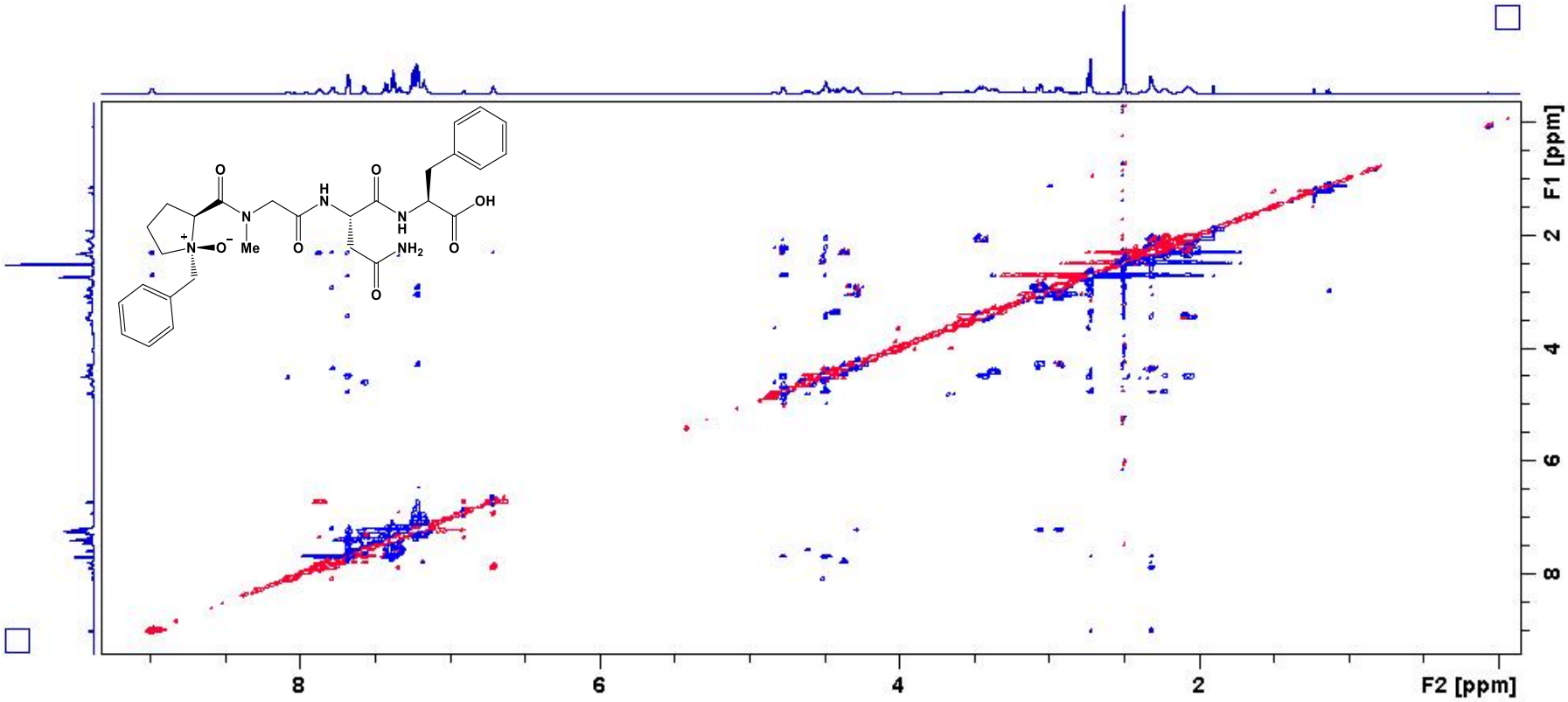
^{13}C NMR of **2b, [Bz(NO)PMeGNF]**



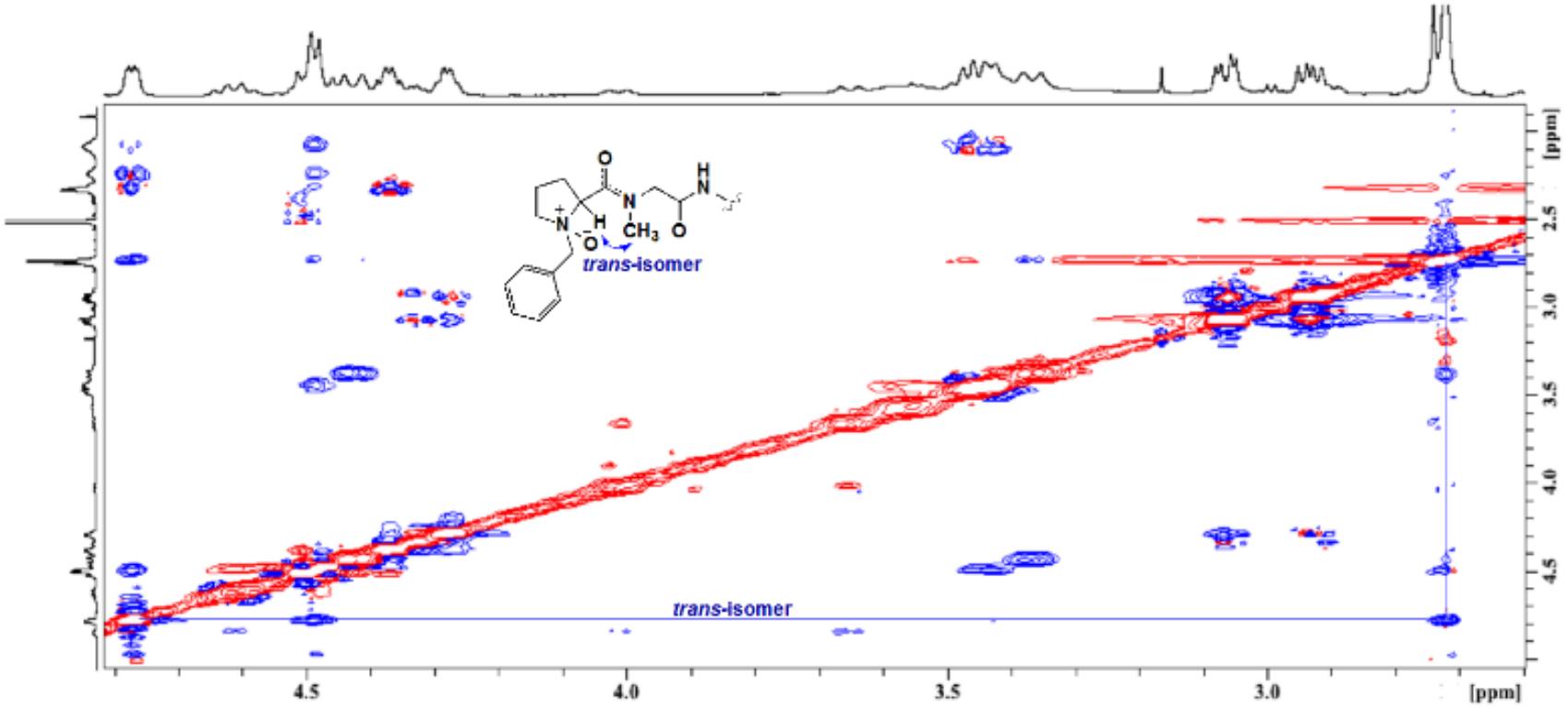
COSY of **2b,** [**Bz(NO)PMeGNF**]



ROESY of **2b,** [**Bz(NO)PMeGNF**]

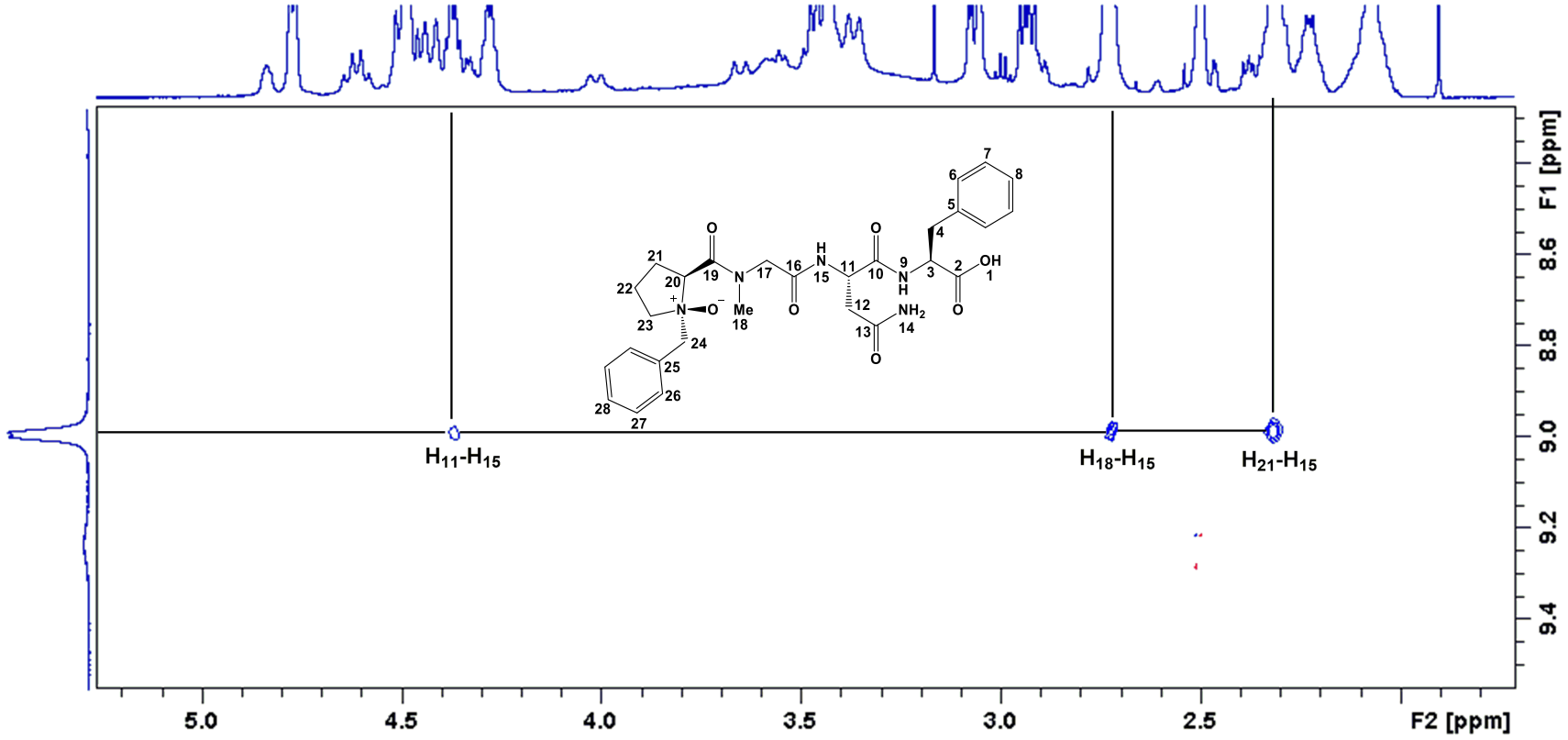


Detection of *trans*-isomers in **2b,** (**Bz(NO)PMeGNF**)

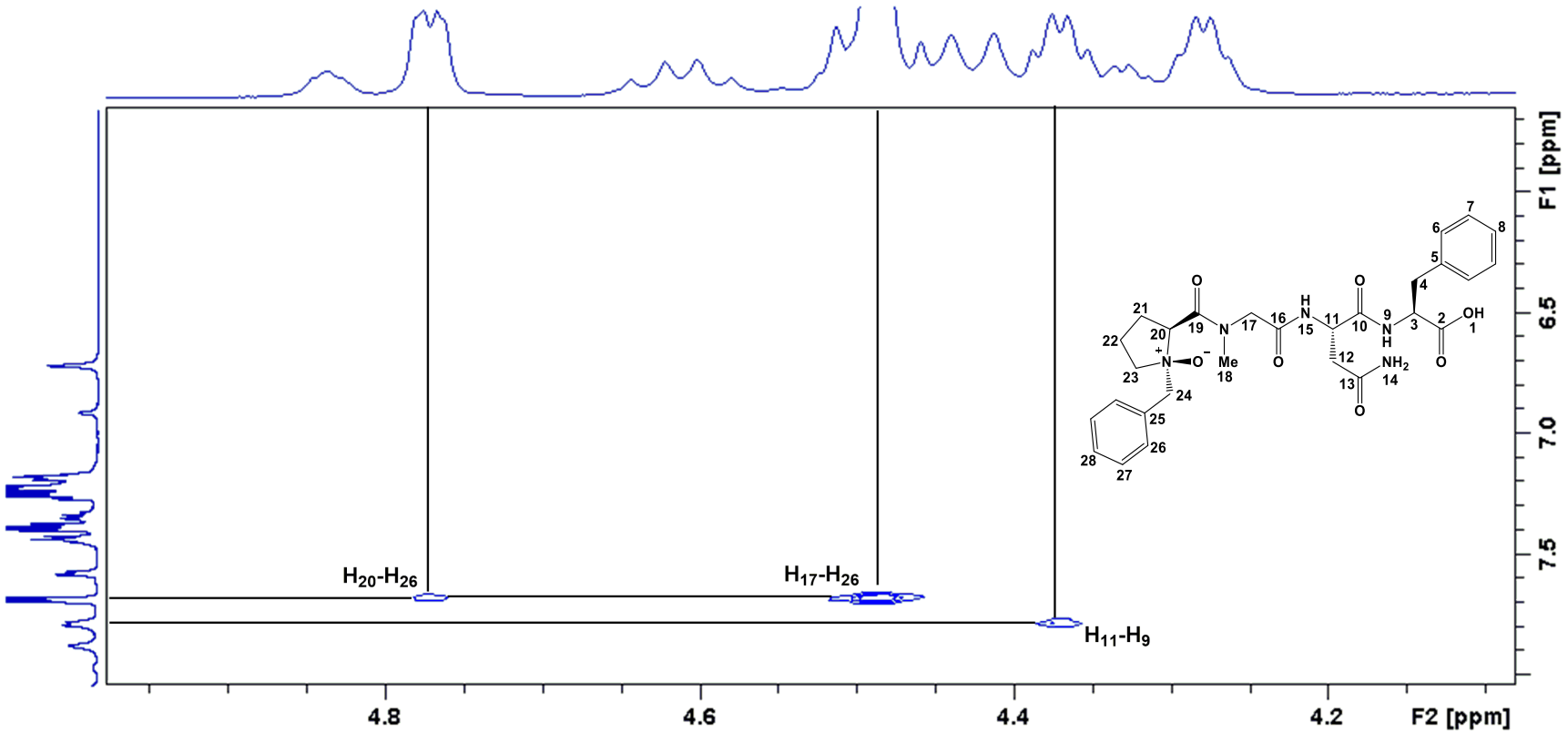


ROESY study for detection of *cis*- and *trans*-isomers in **2b, [Bz(NO)PMeGNF]** at room temperature. The water peak is suppressed. (Solvent: DMSO-d_6)

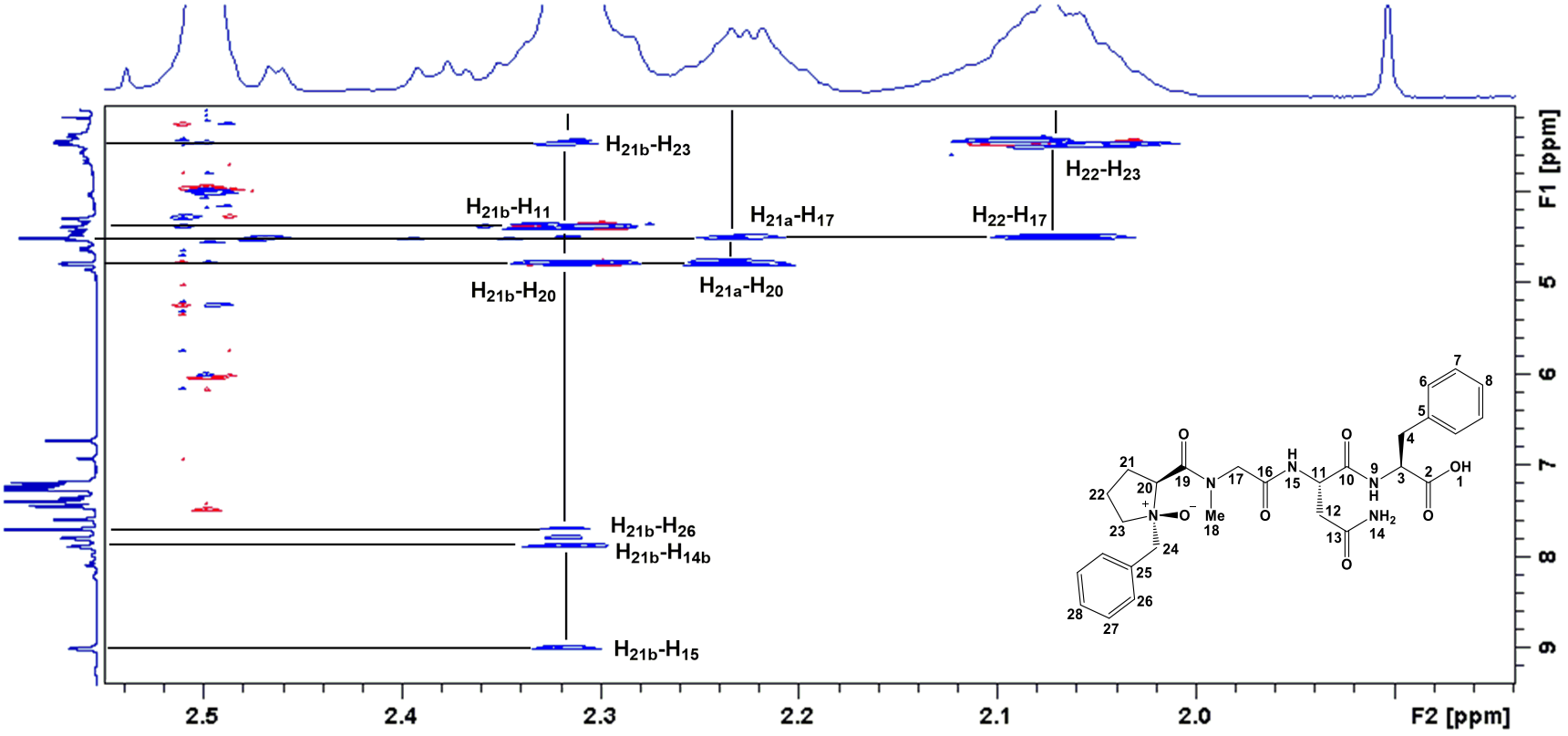
Expansion of observed NOE correlations of **2b,** [**Bz(NO)PMeGNF**]



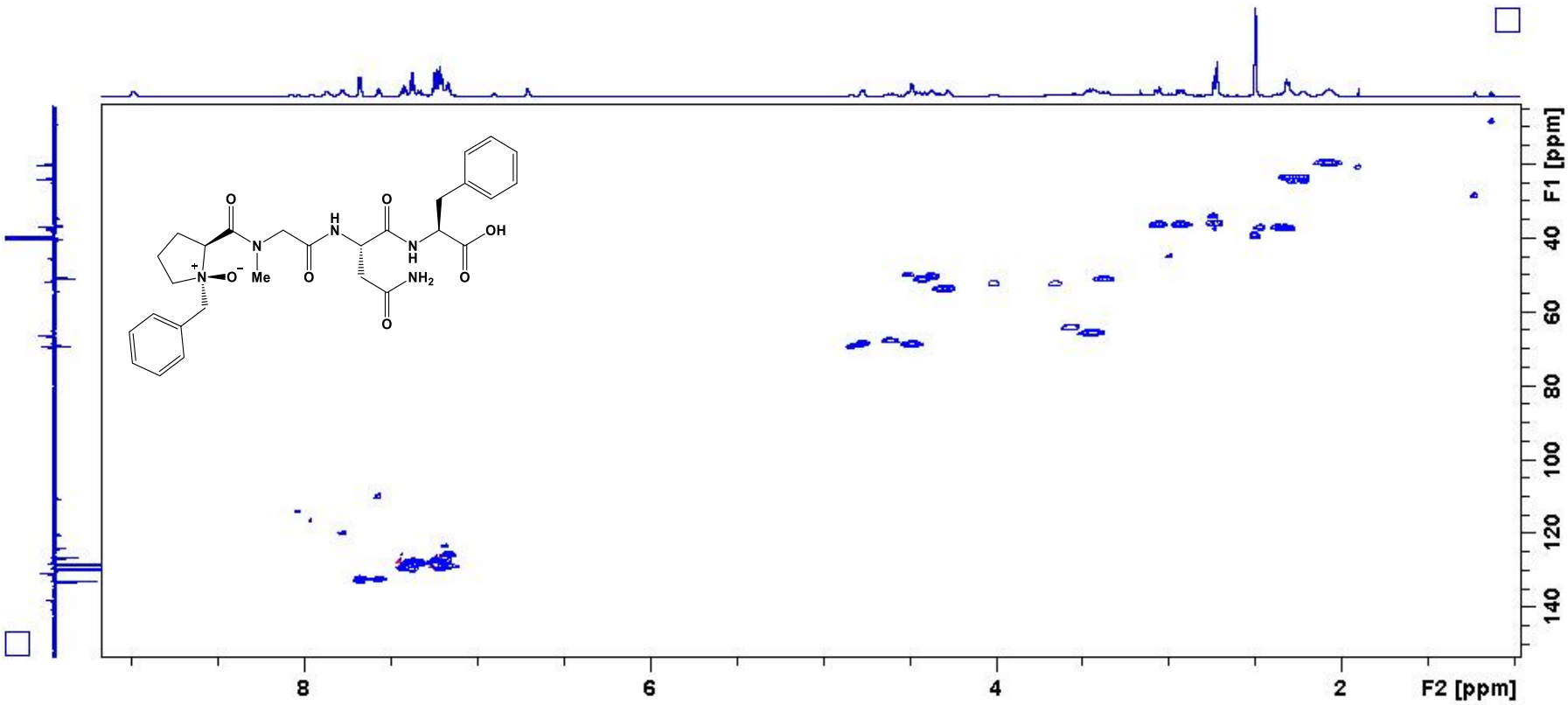
Expansion of observed NOE correlations of **2b,** [**Bz(NO)PMeGNF**]



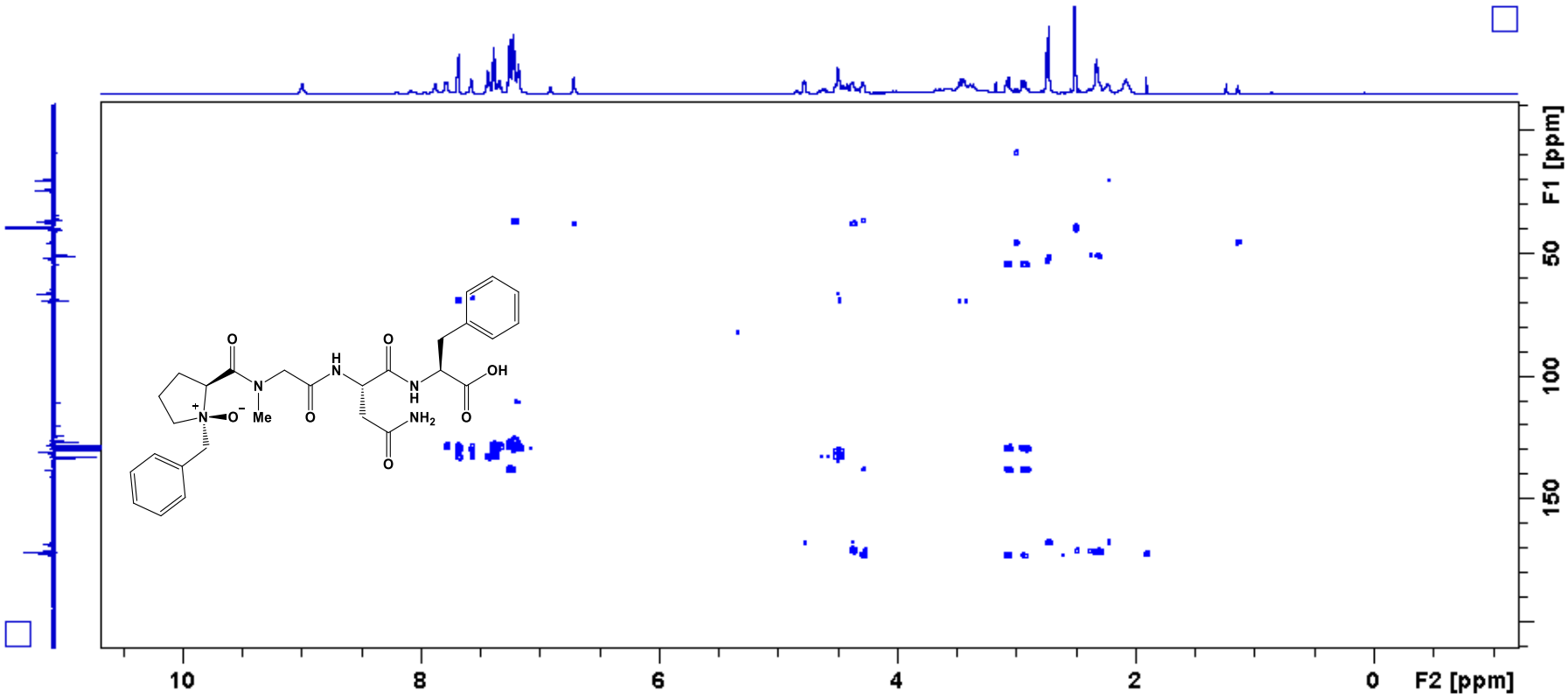
Expansion of observed NOE correlations of **2b,** [**Bz(NO)PMeGNF**]



HSQC of **2b,** [**Bz(NO)PMeGNF**]

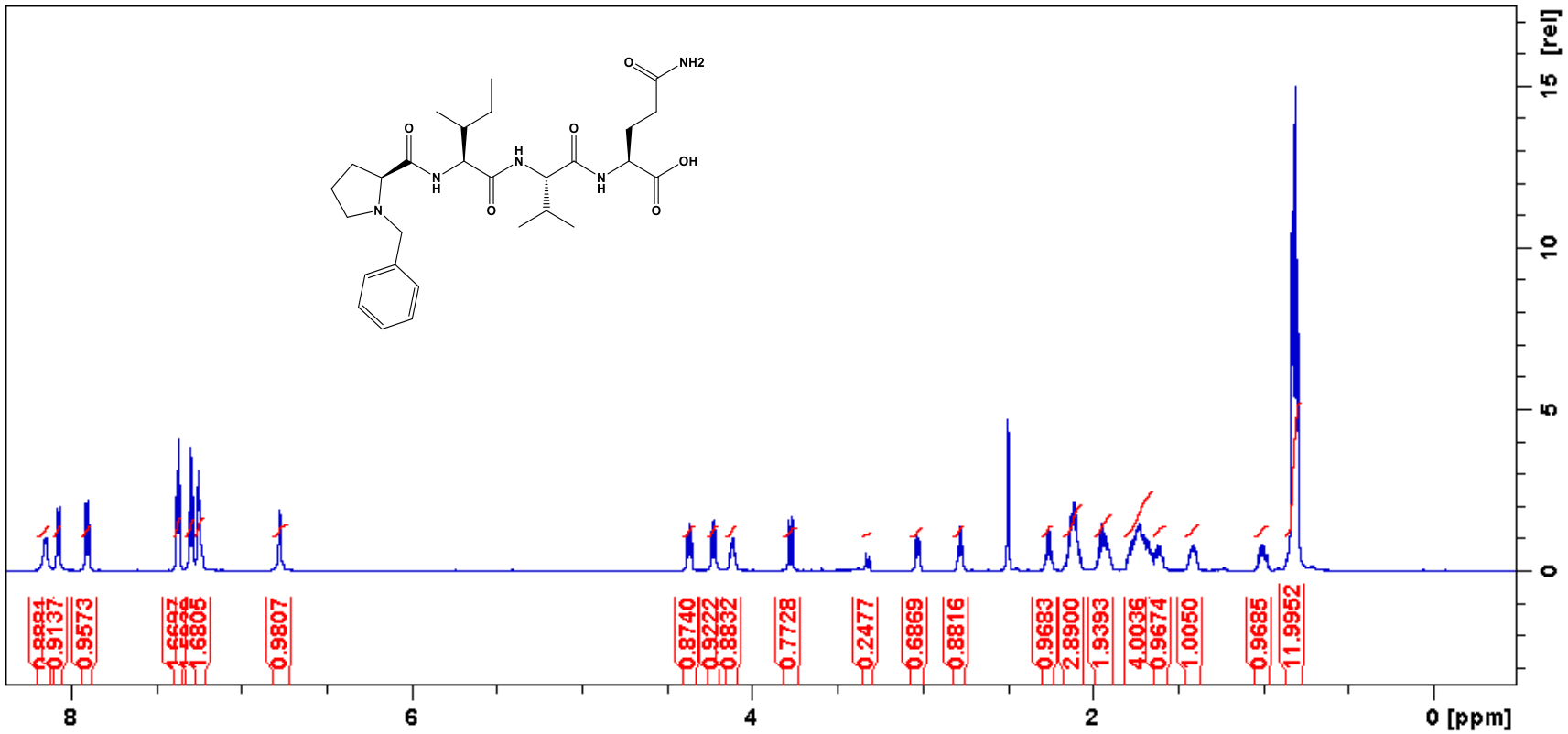


HMBC of **2b,** [**Bz(NO)PMeGNF**]

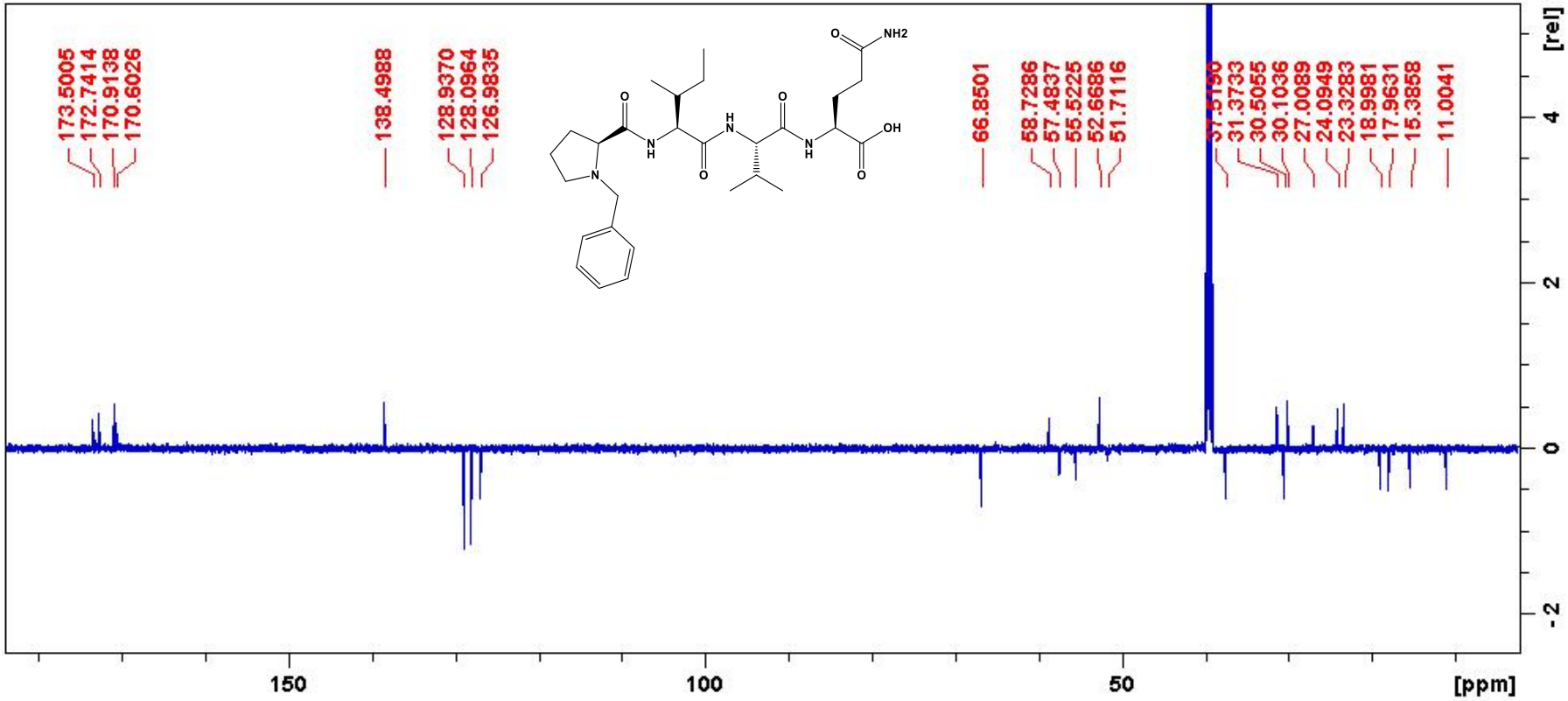


^{1}H NMR spectra of **3a,** (**BzPIVQ**)

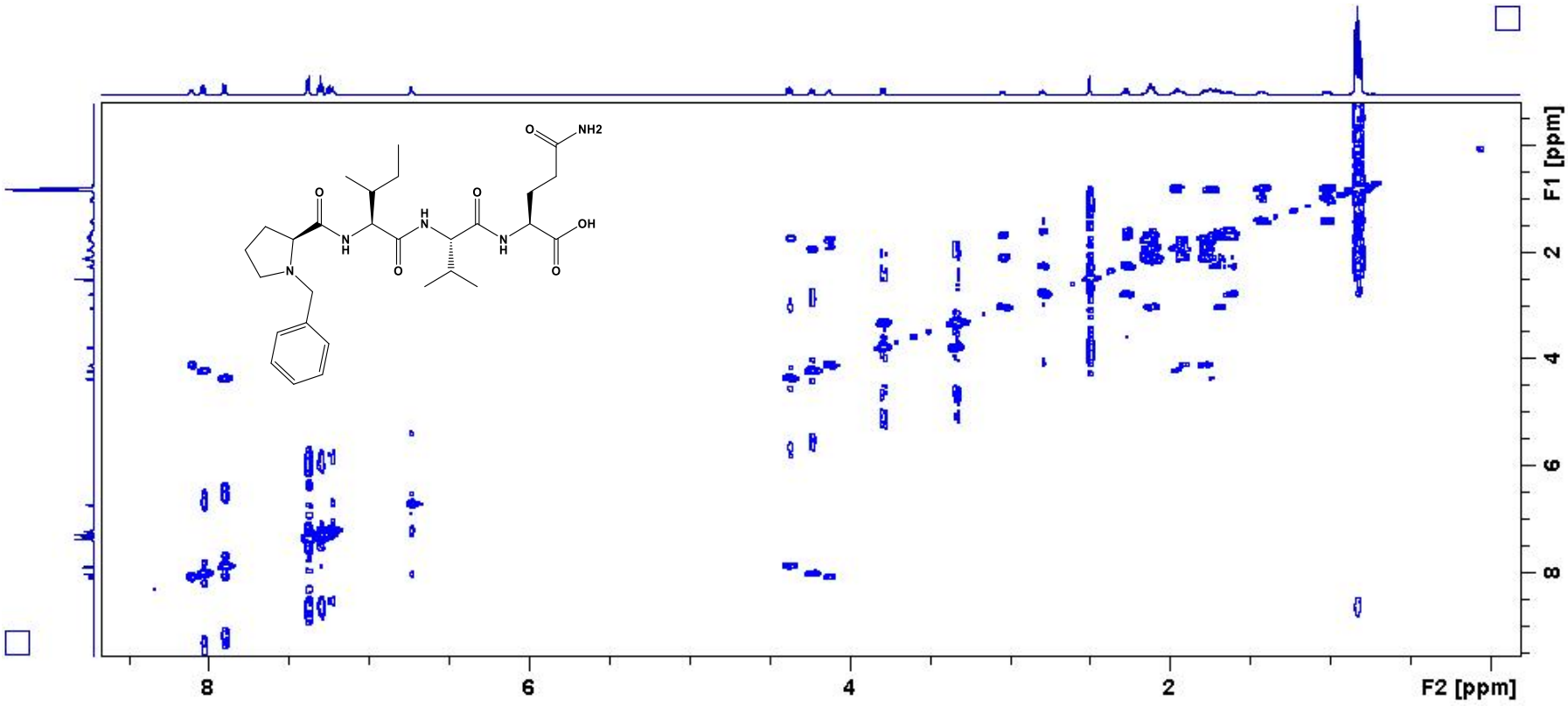
(Water peak was suppressed)



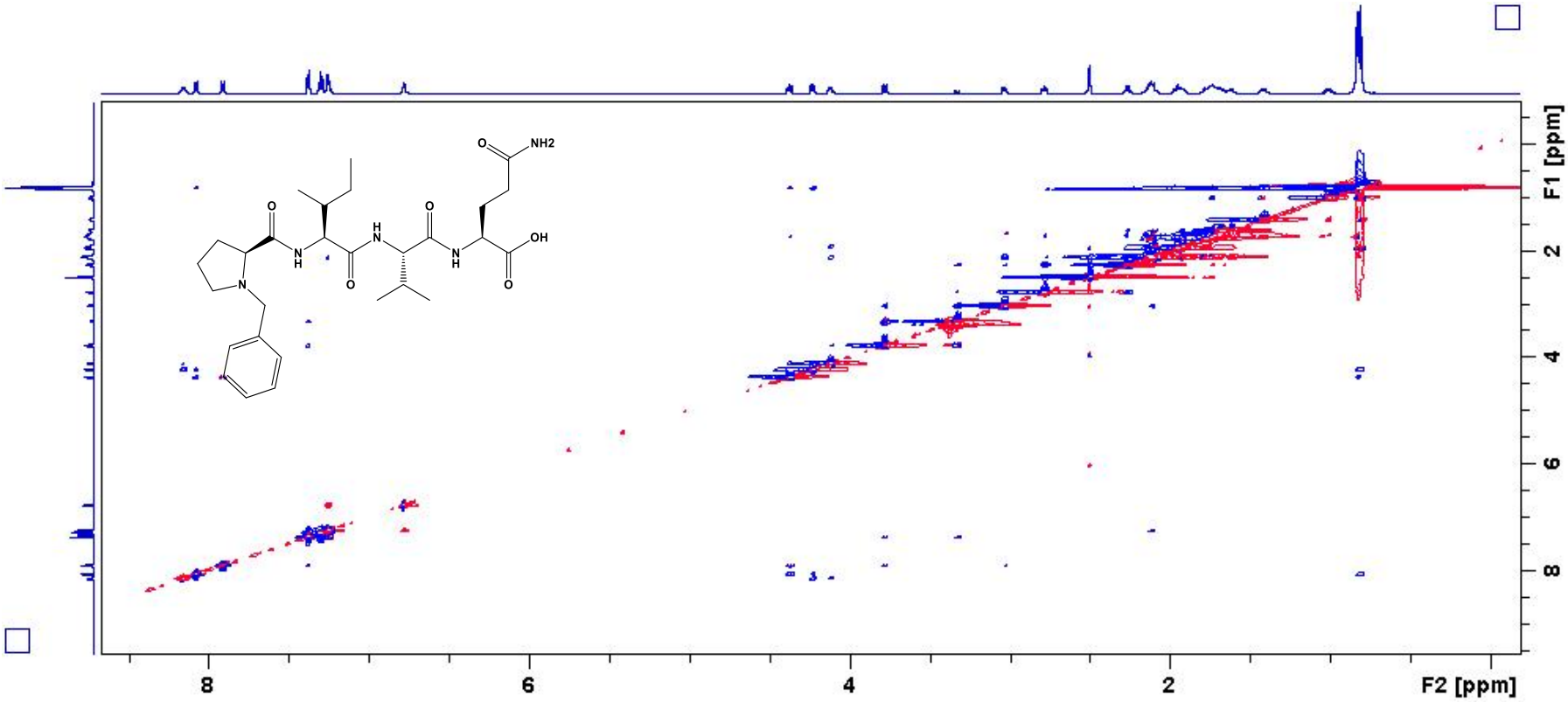
^{13}C NMR spectra of **3a,** (**BzPIVQ**)



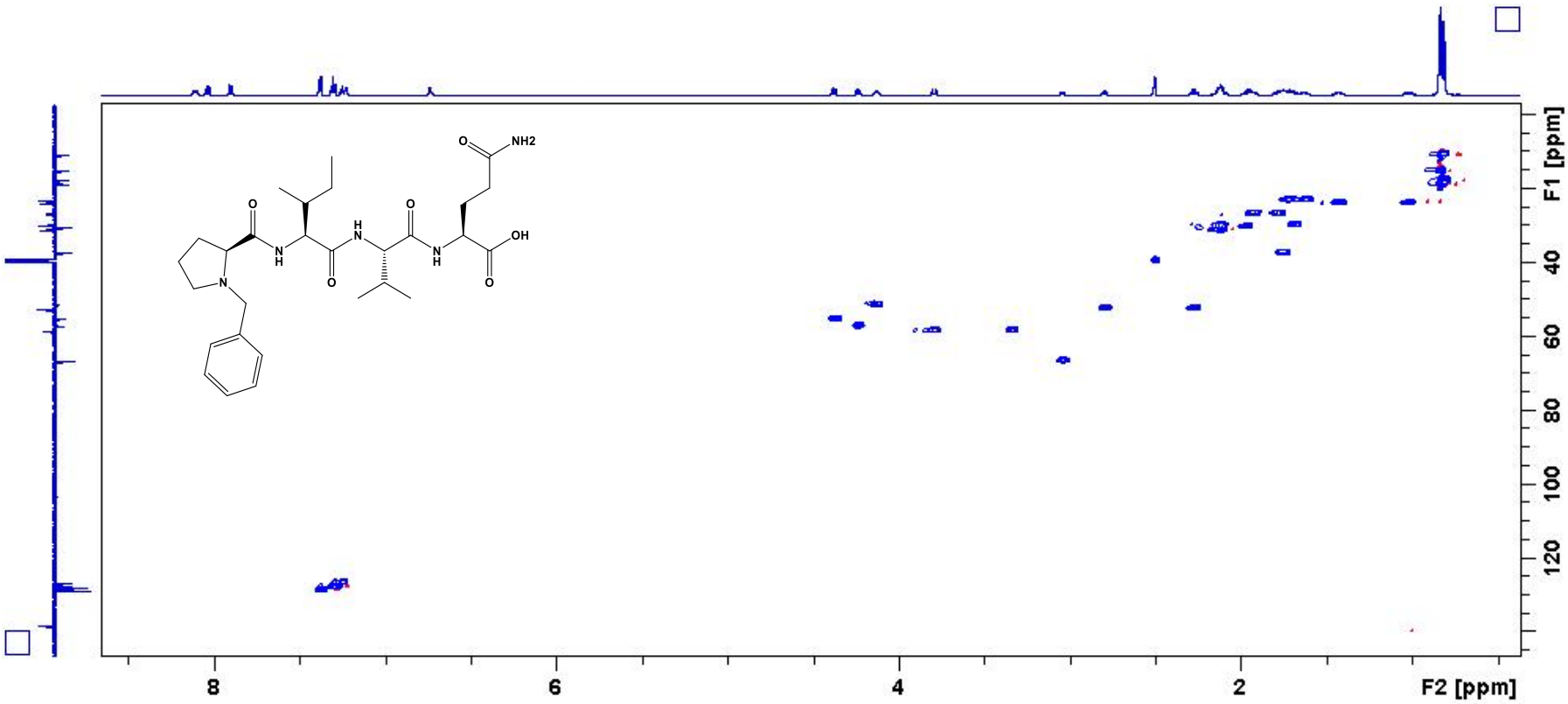
COSY of **3a,** (**BzPIVQ**)



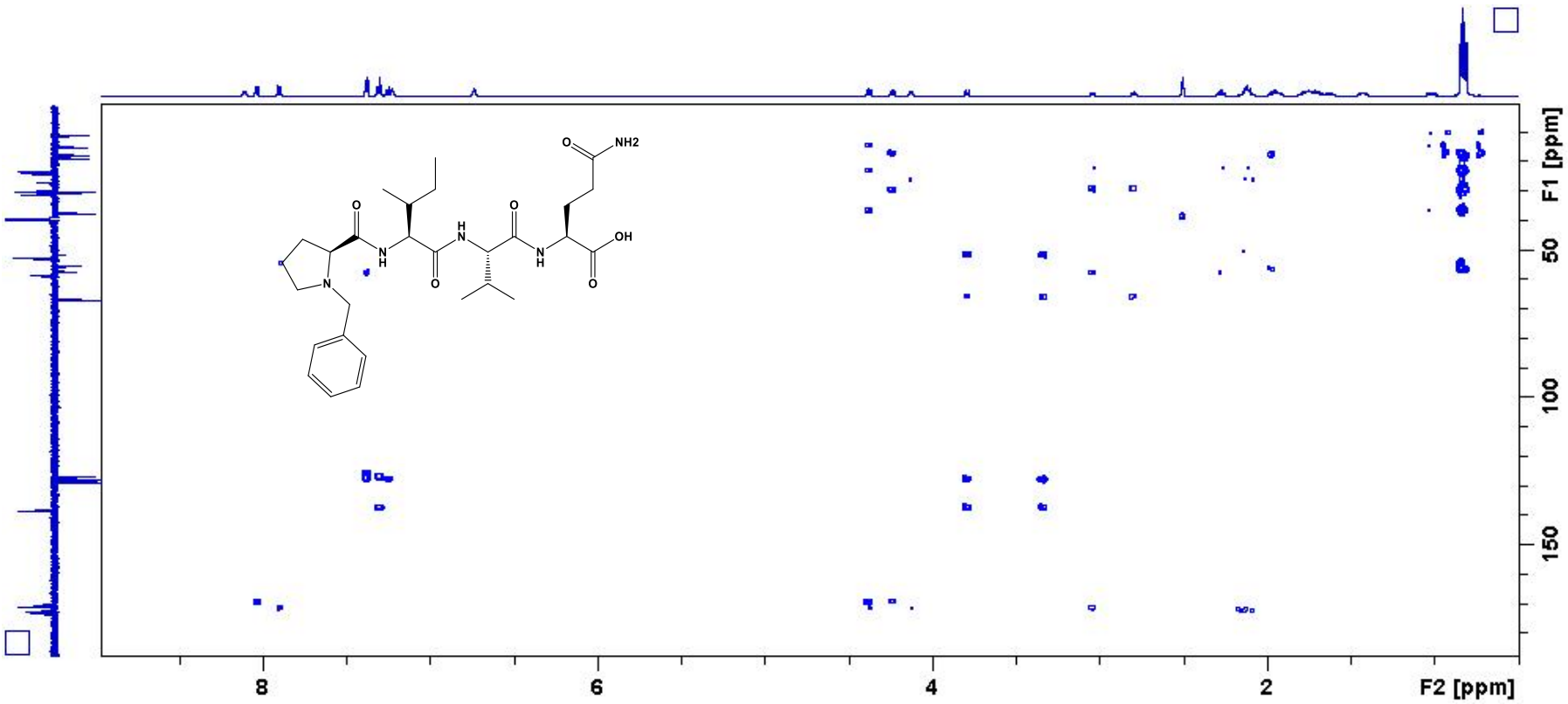
ROESY of **3a,** (**BzPIVQ**)



HSQC of **3a, (BzPIVQ)**

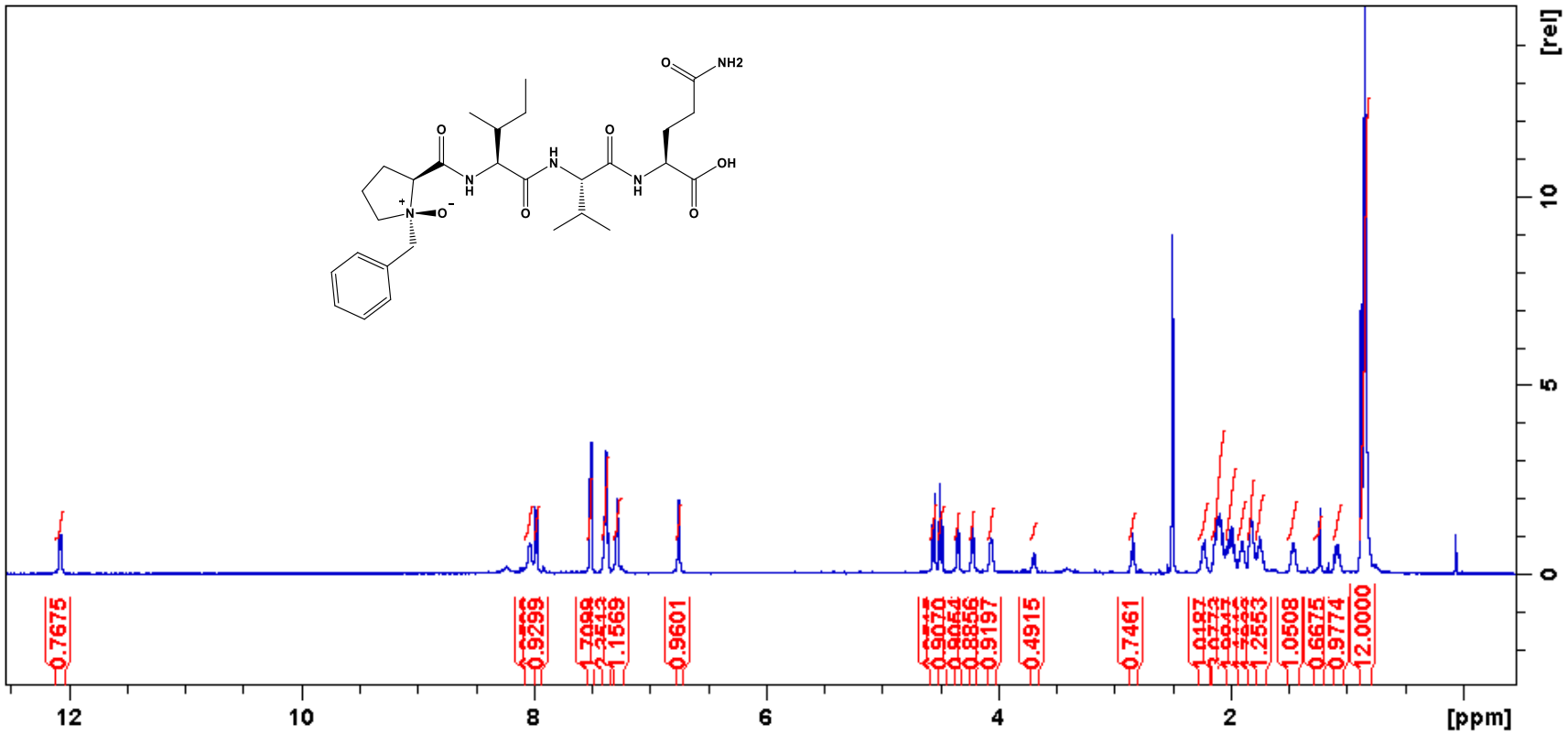


HMBC of **3a,** (**BzPIVQ**)

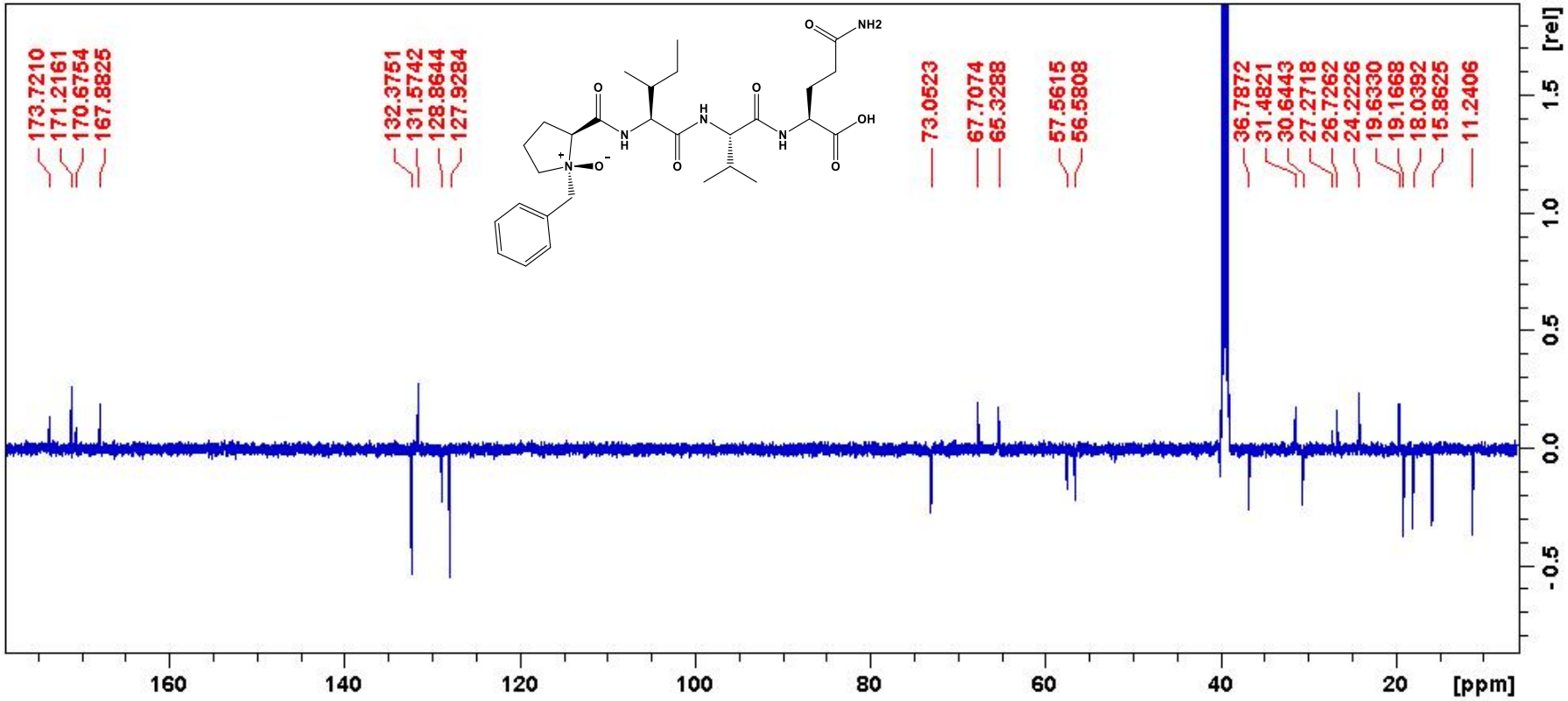


^{1}H NMR spectra of **3b, [Bz(NO)PIVQ]**

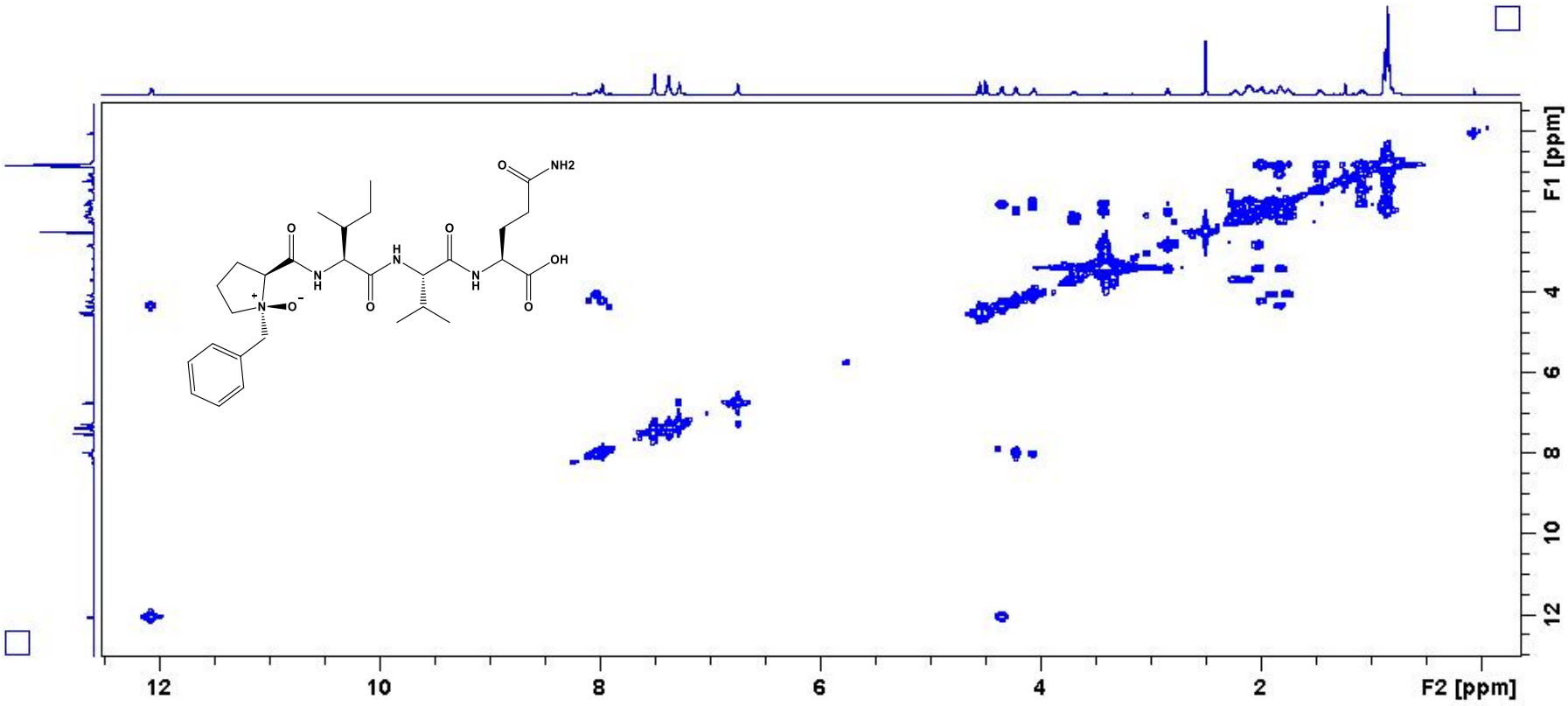
(Water peak was suppressed)



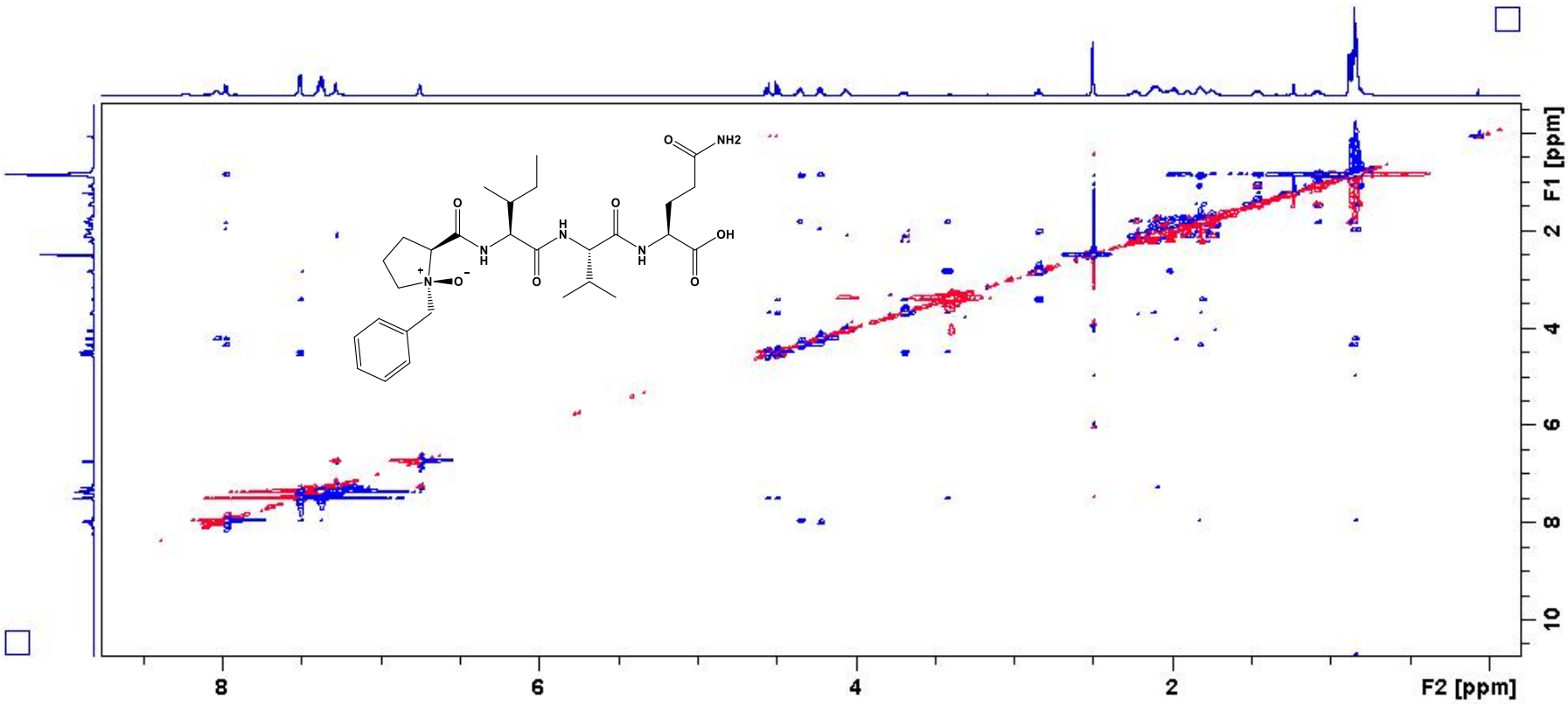
^{13}C NMR spectra of **3b,** [**Bz**(**NO**)**PIVQ**]



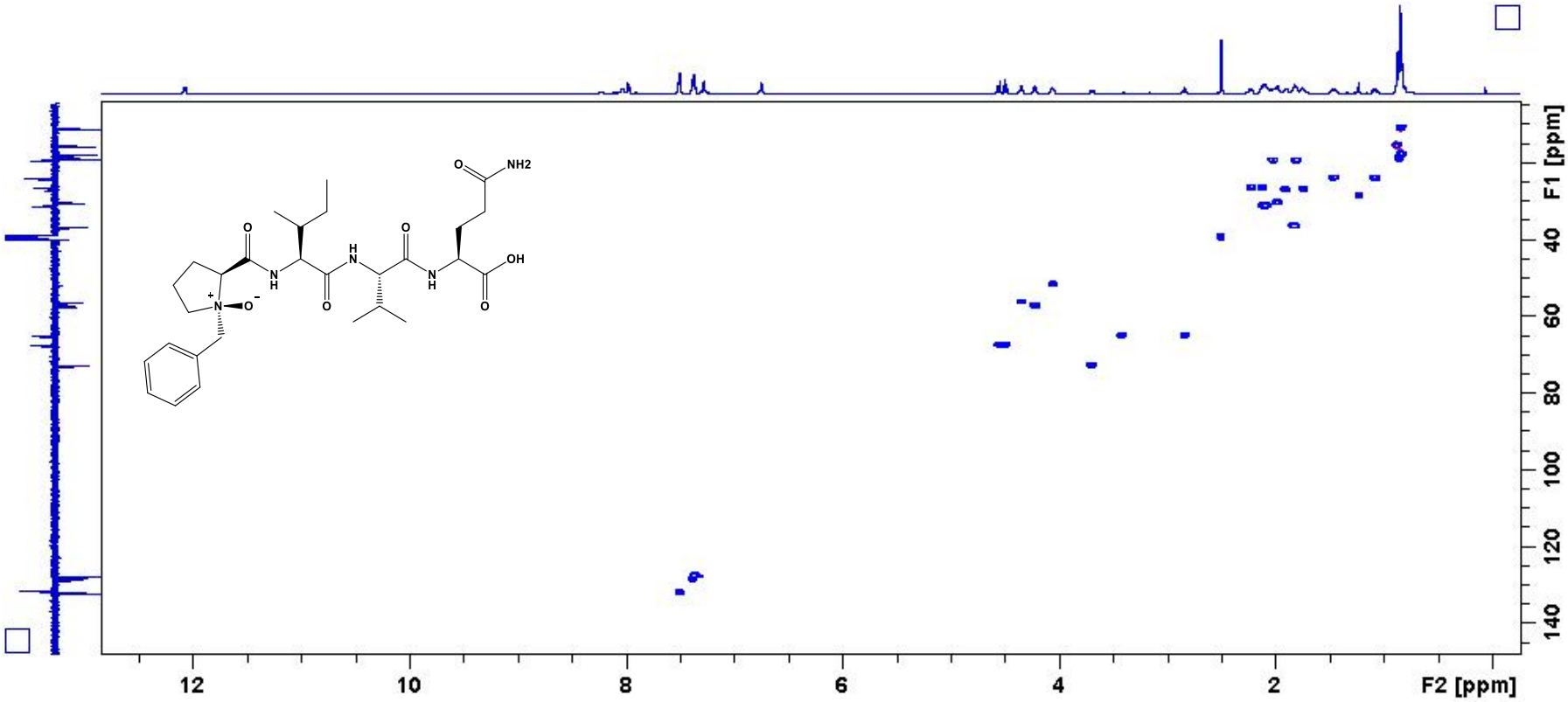
COSY of **3b,** [**Bz(NO)PIVQ**]



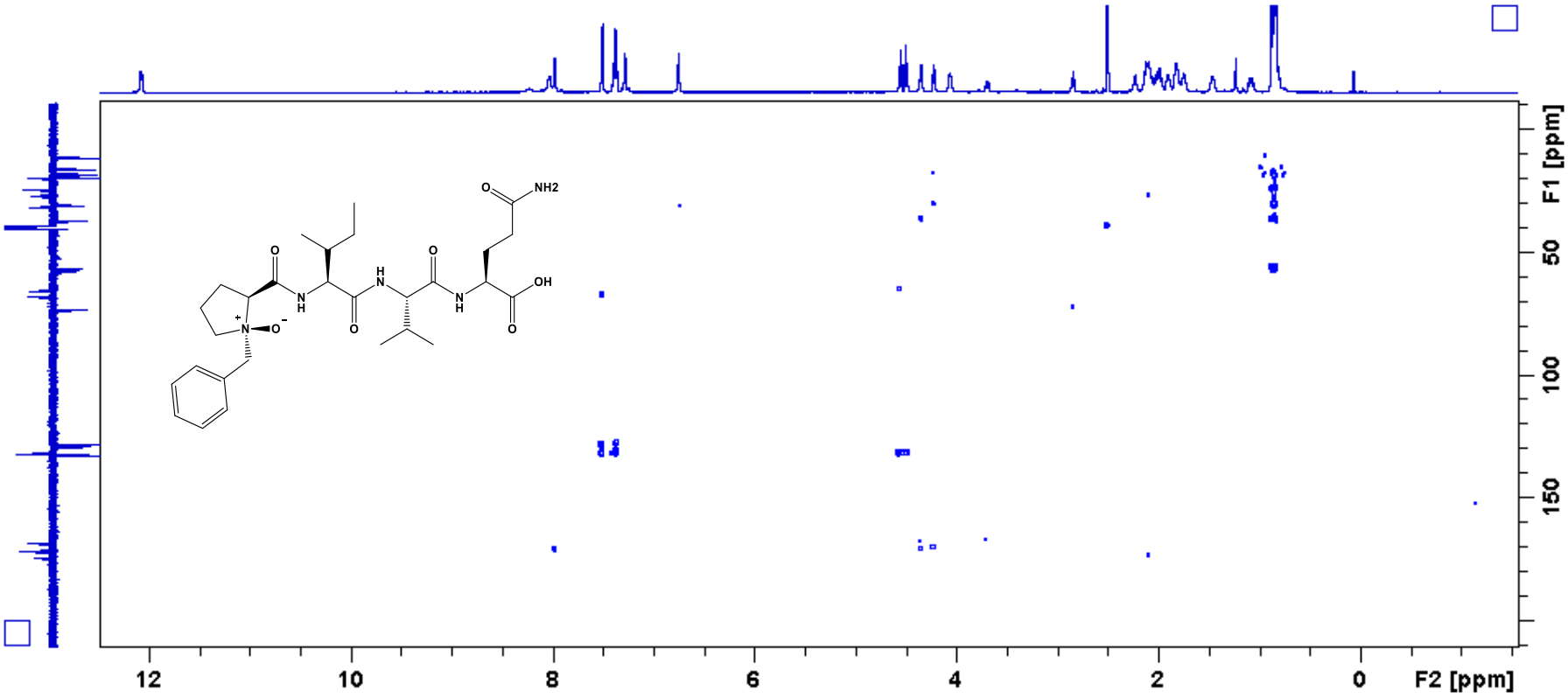
ROESY of **3b,** [**Bz(NO)PIVQ**]



HSQC of **3b, [Bz(NO)PIVQ]**

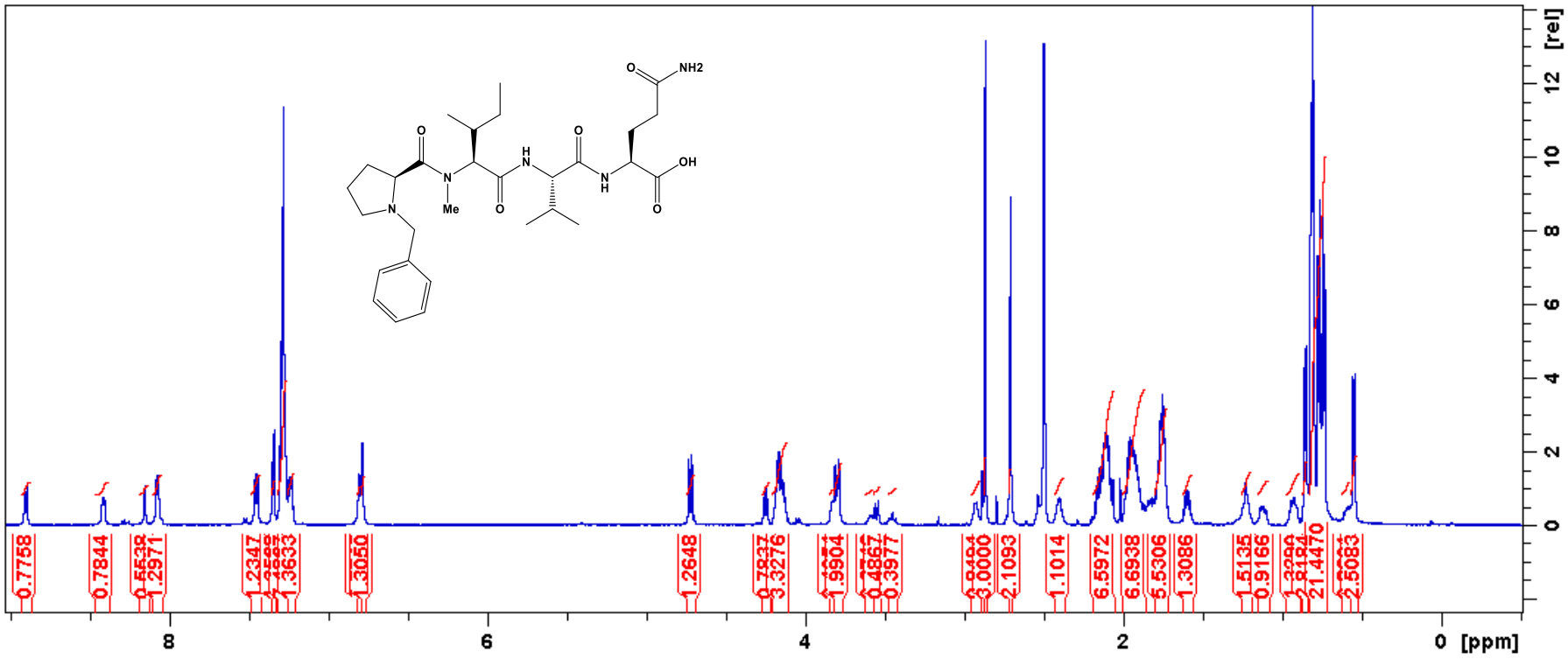


HMBC **of 3b, [Bz(NO)PIVQ]**

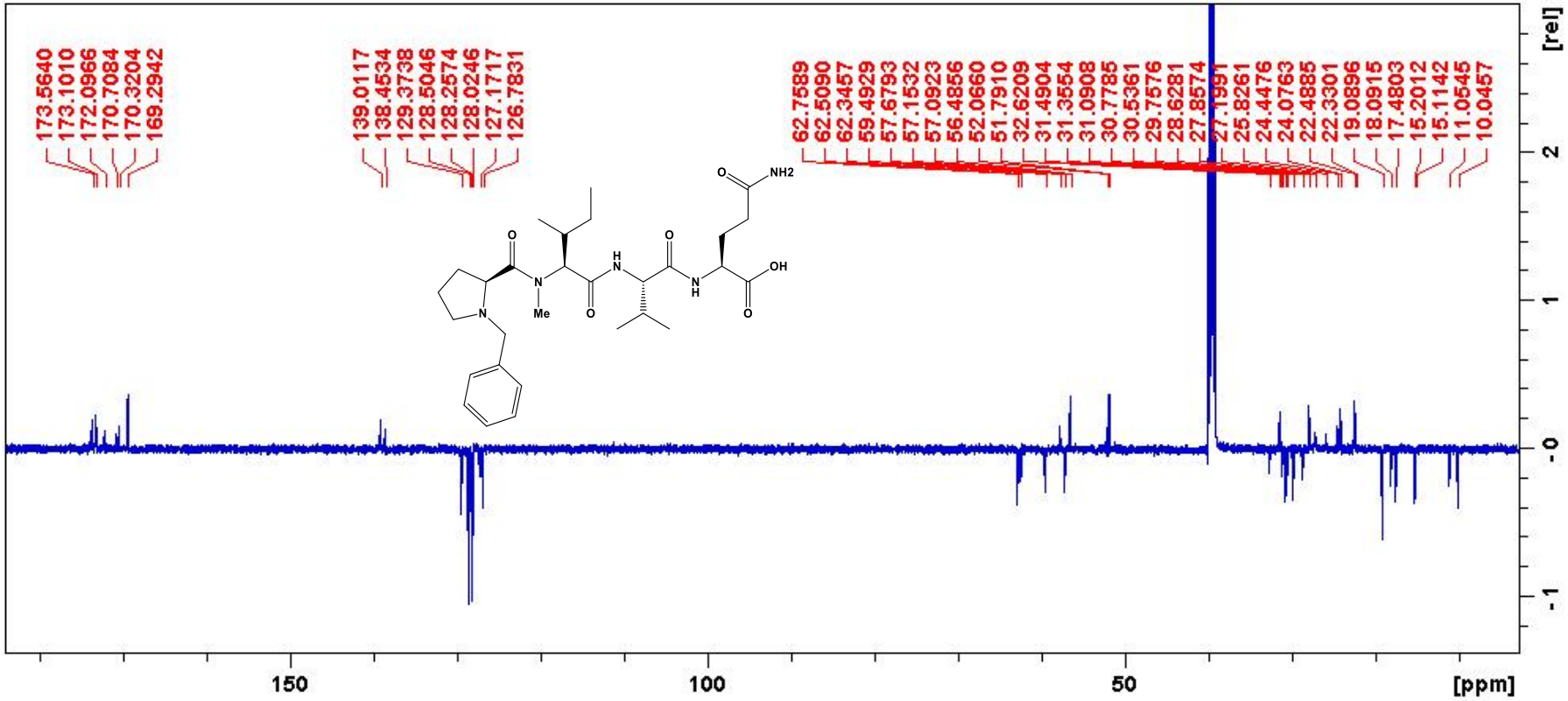


^{1}H NMR spectra of **4a, (BzPMeIVQ)**

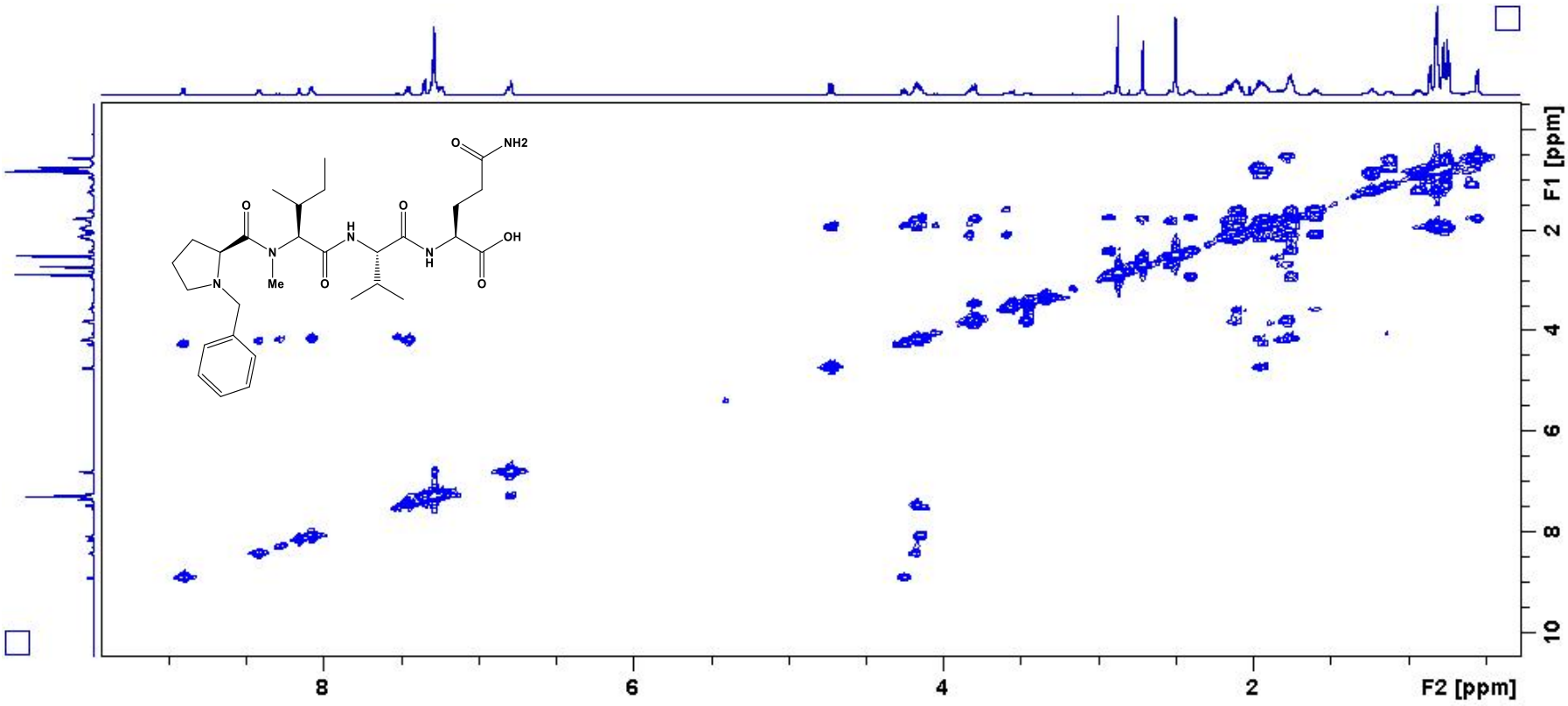
(Water peak was suppressed)



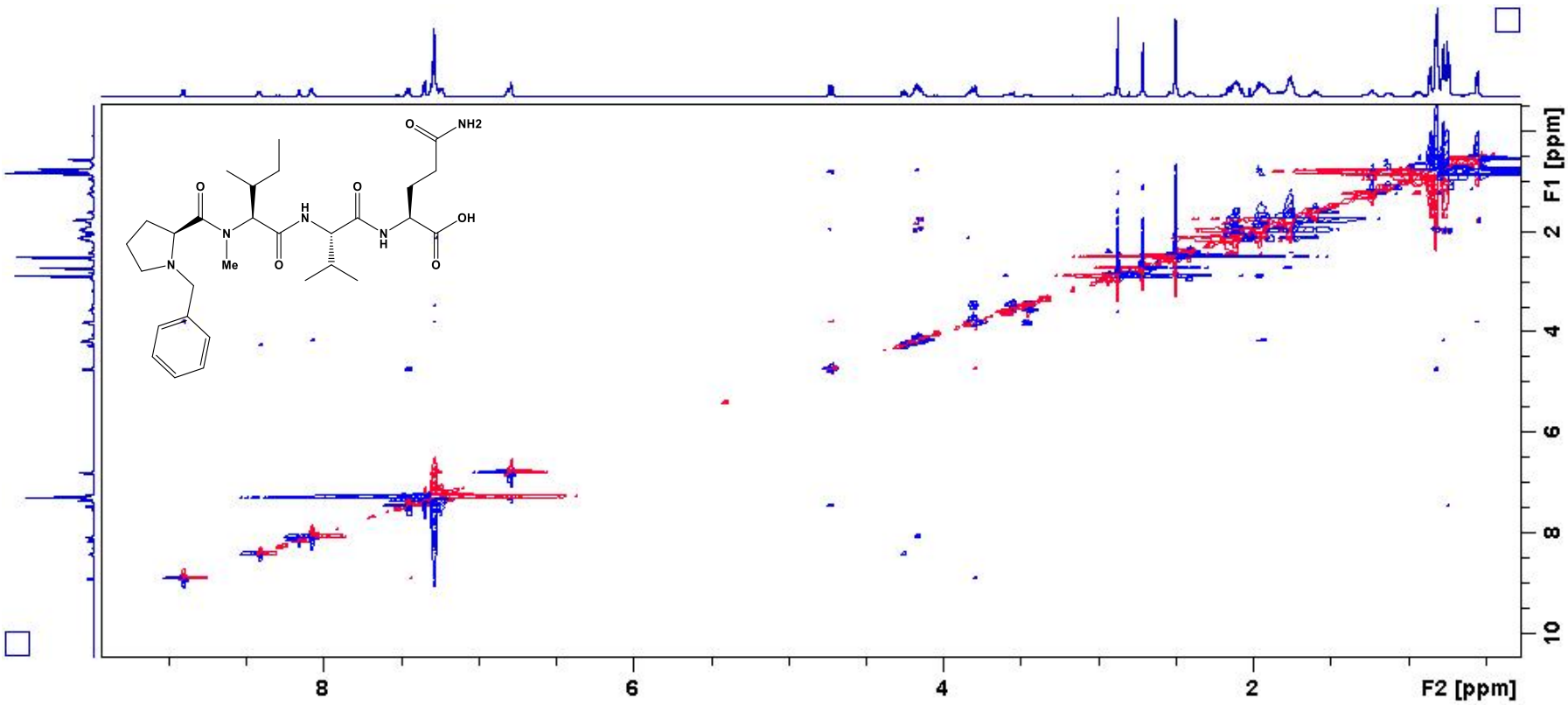
^{13}C NMR spectra of **4a,** (**BzPMeIVQ**)



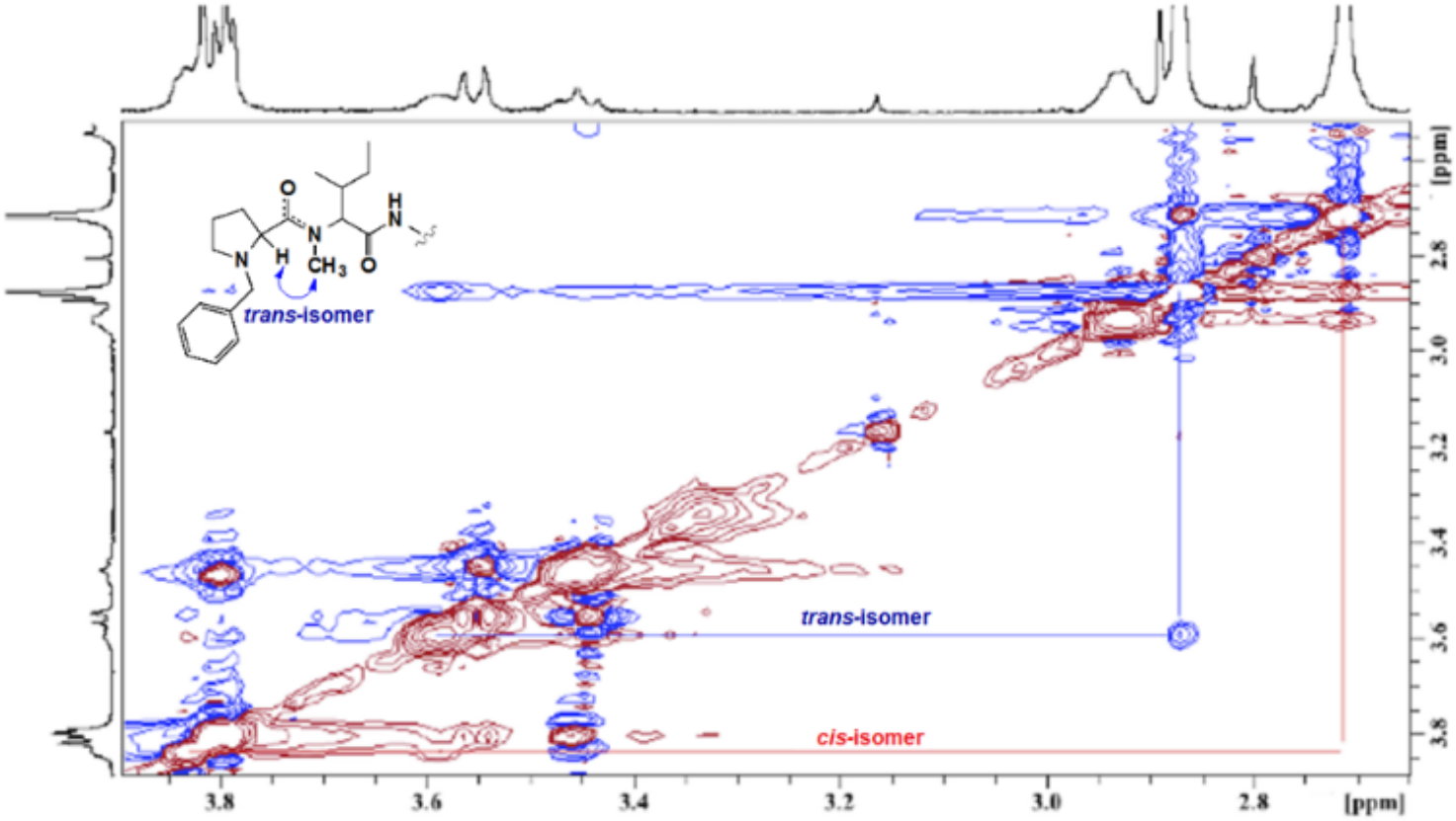
COSY of **4a, (BzPMeIVQ)**



ROESY of **4a, (BzPMeIVQ)**

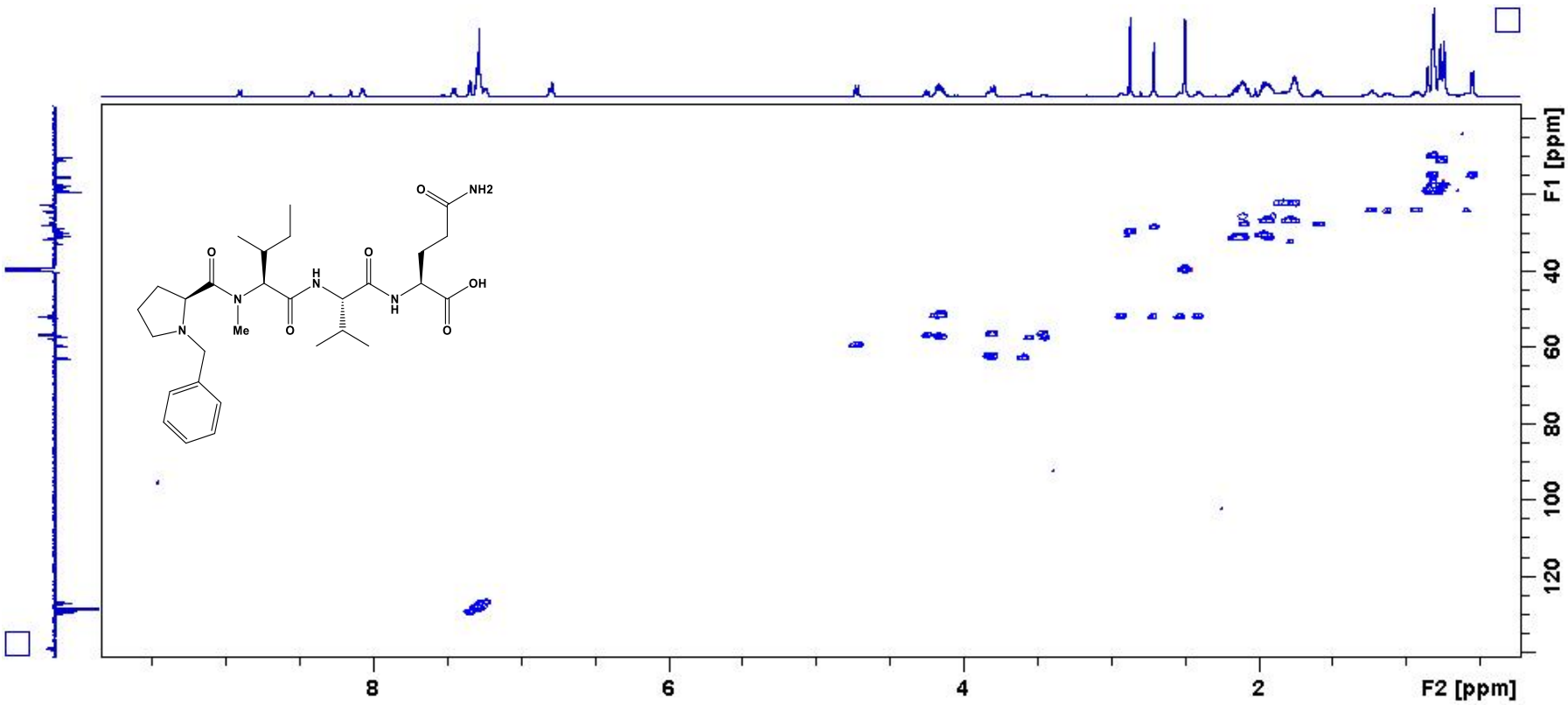


Detection of *cis*- and *trans*-isomers in **4a,** (**BzPMeIVQ**)

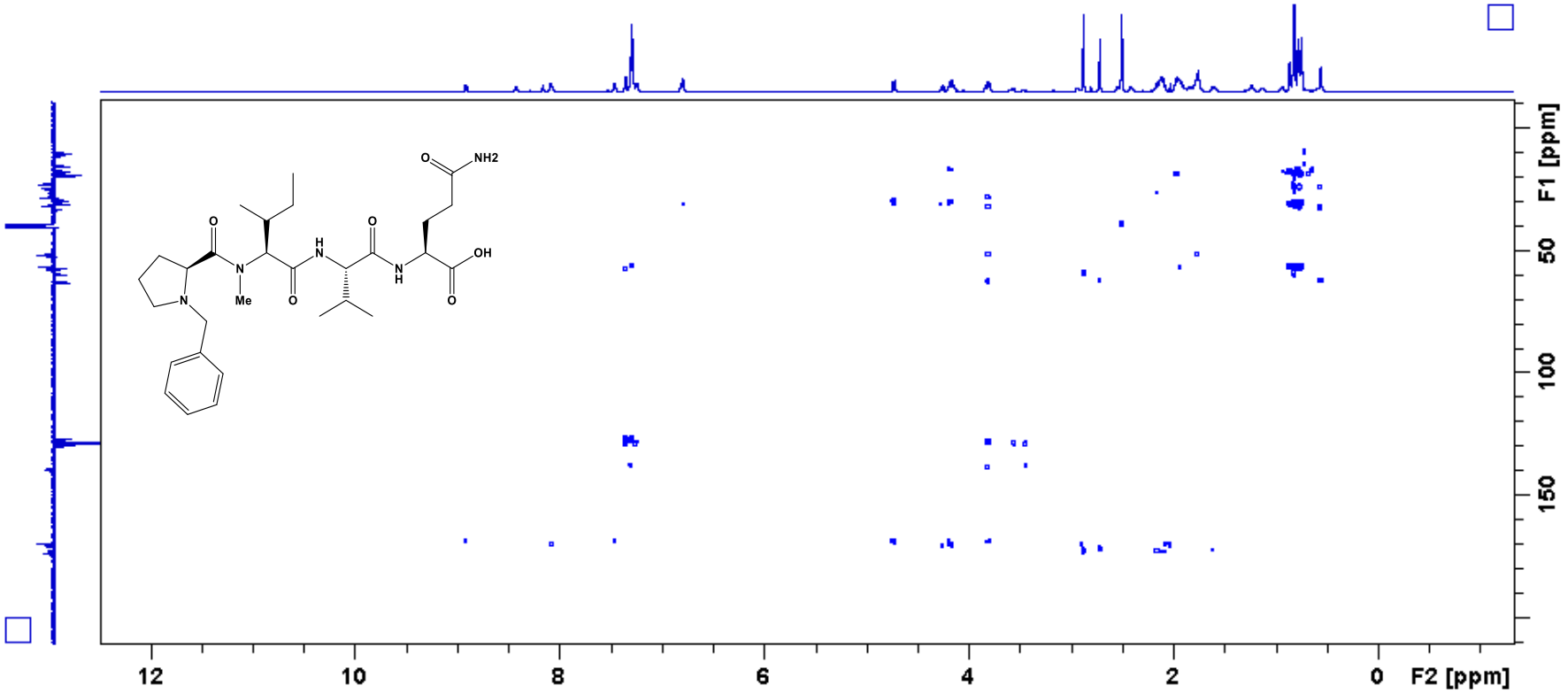


ROESY study for detection of *cis*- and *trans*-isomers in **4a, (BzPMeIVQ)** at room temperature. The water peak is suppressed (Solvent: DMSO-d_6)

HSQC of **4a, (BzPMeIVQ)**

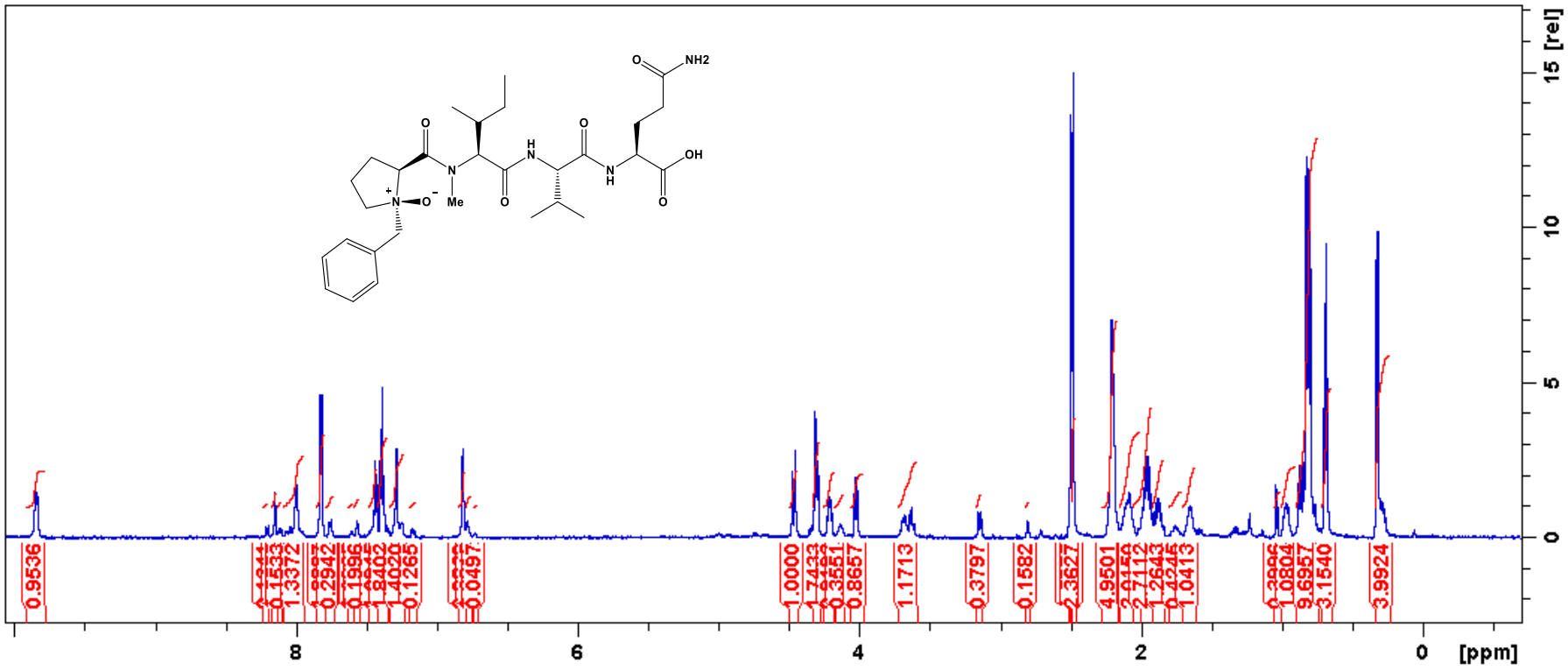


HMBC of **4a, (BzPMeIVQ)**

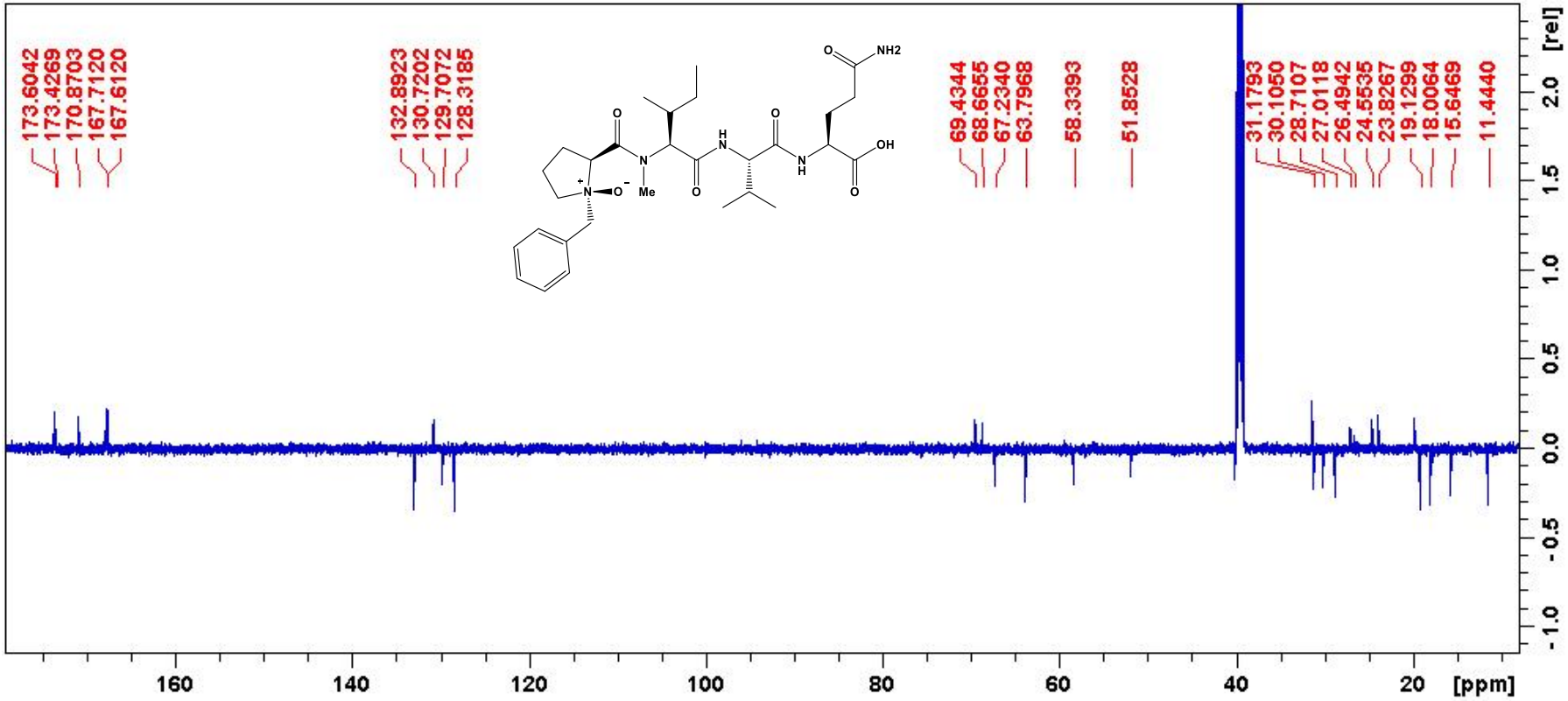


^{1}H NMR spectra of **4b, [Bz(NO)PMeIVQ]**

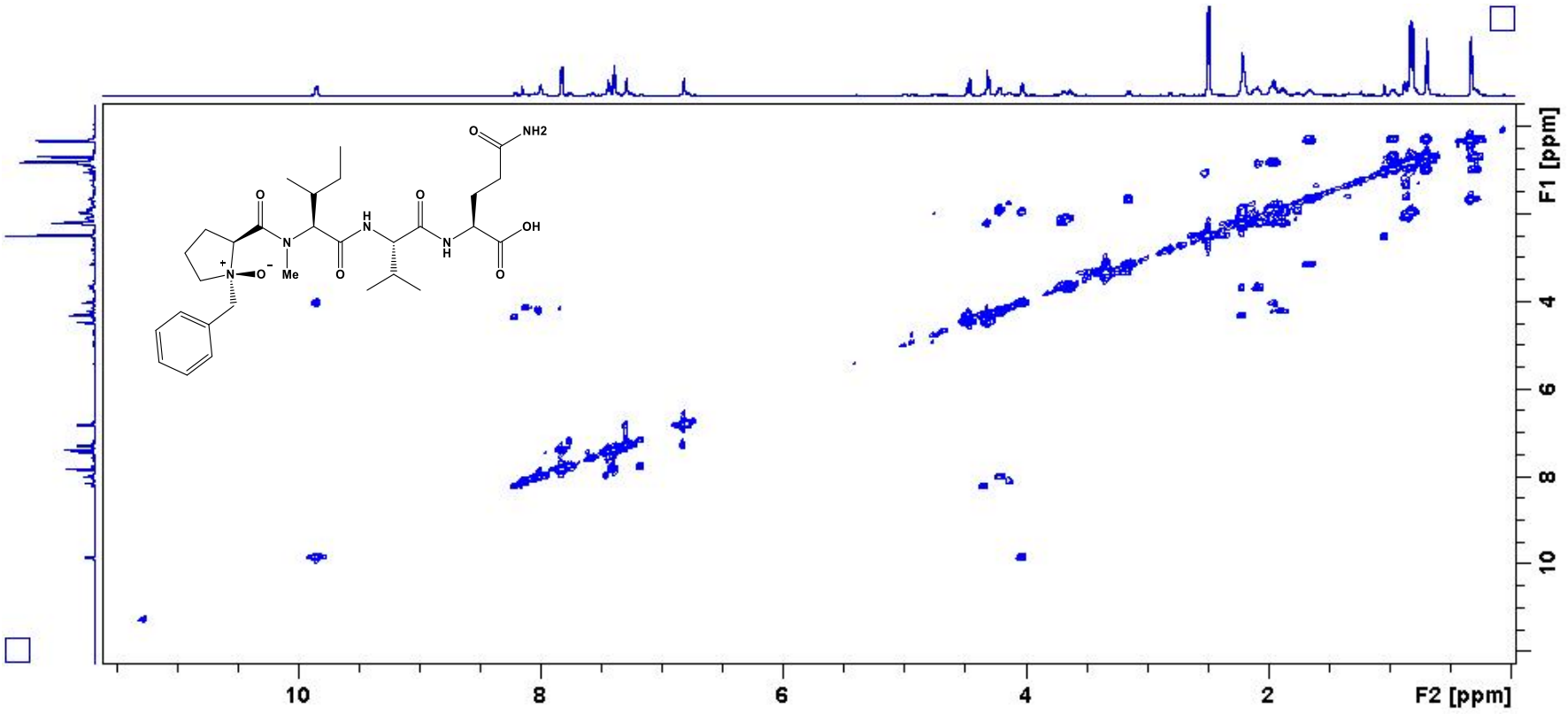
(Water peak was suppressed)



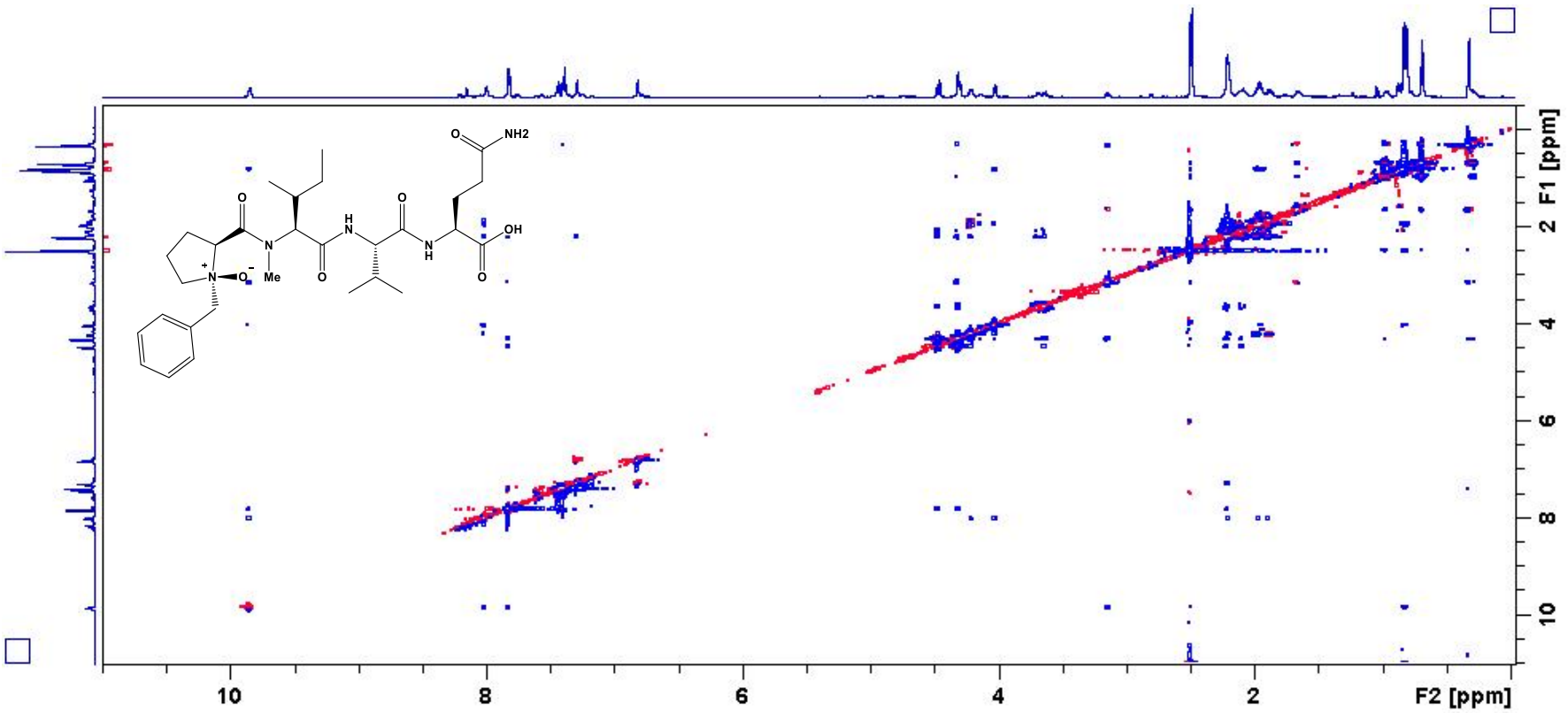
^{13}C NMR spectra of **4b,** [**Bz(NO)PMeIVQ**]



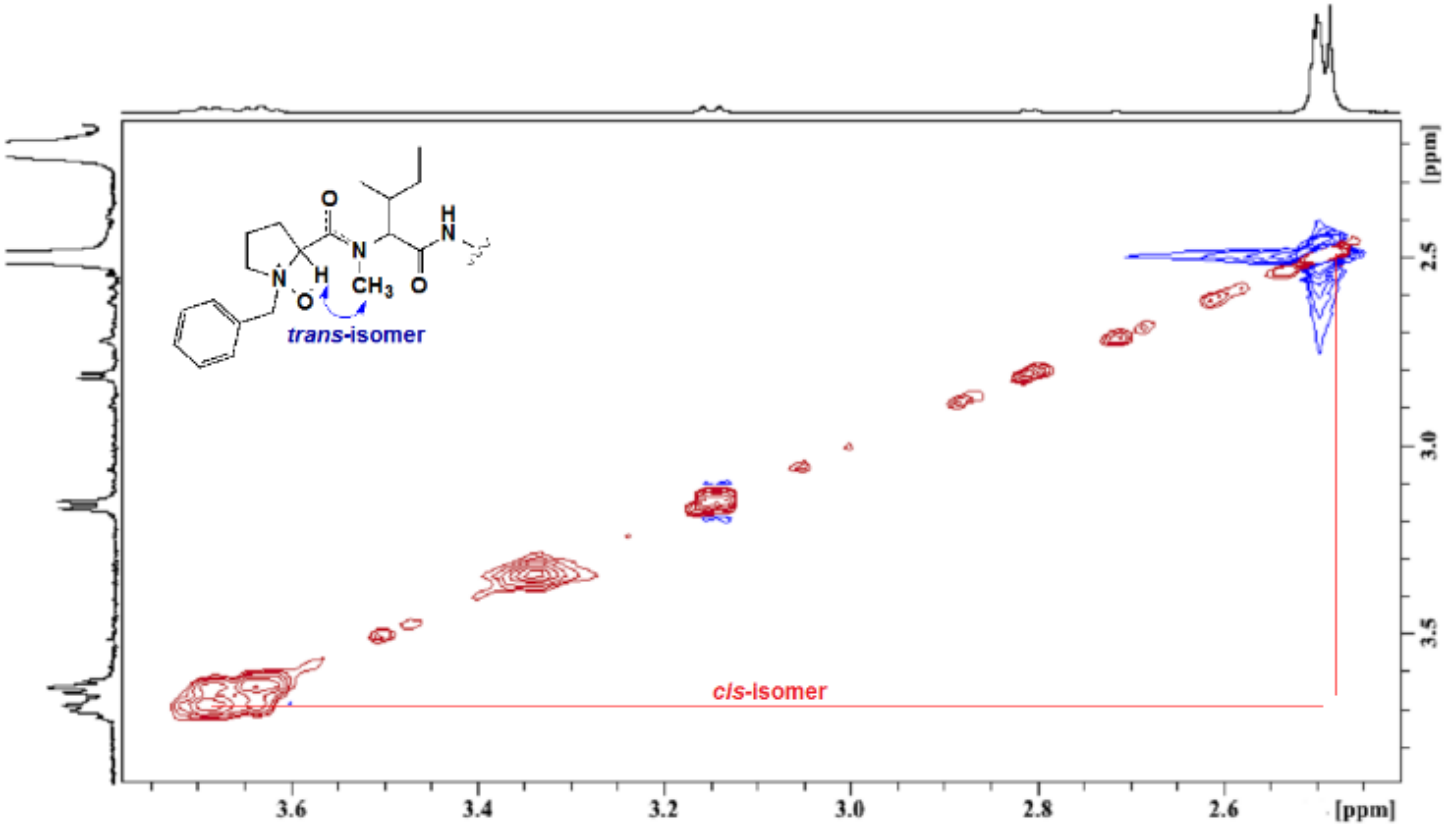
COSY of **4b,** [**Bz(NO)PMeIVQ**]



ROESY of **4b,** [**Bz(NO)PMeIVQ**]

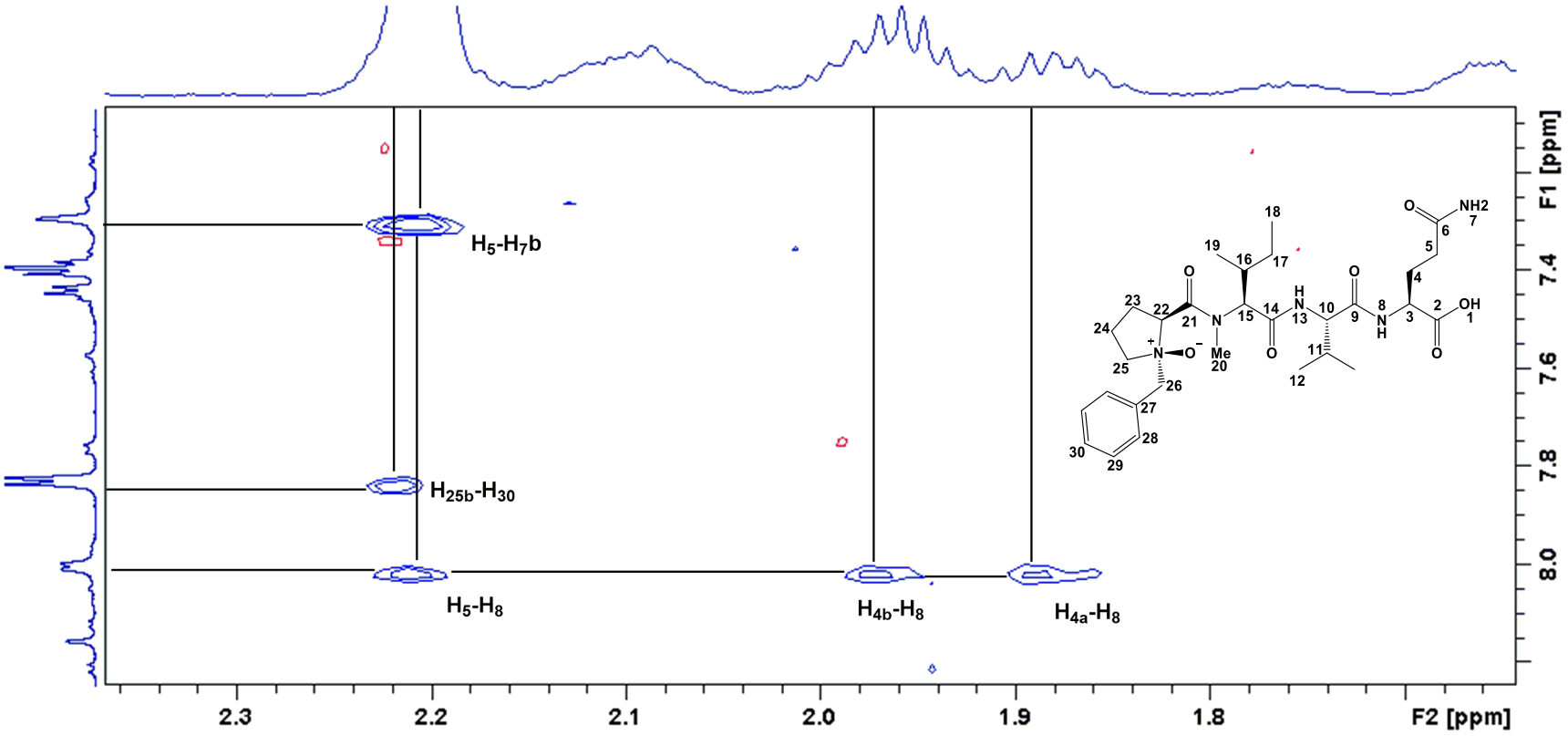


Detection of *cis*-isomers in **4b, (Bz(NO)PMeIVQ)**

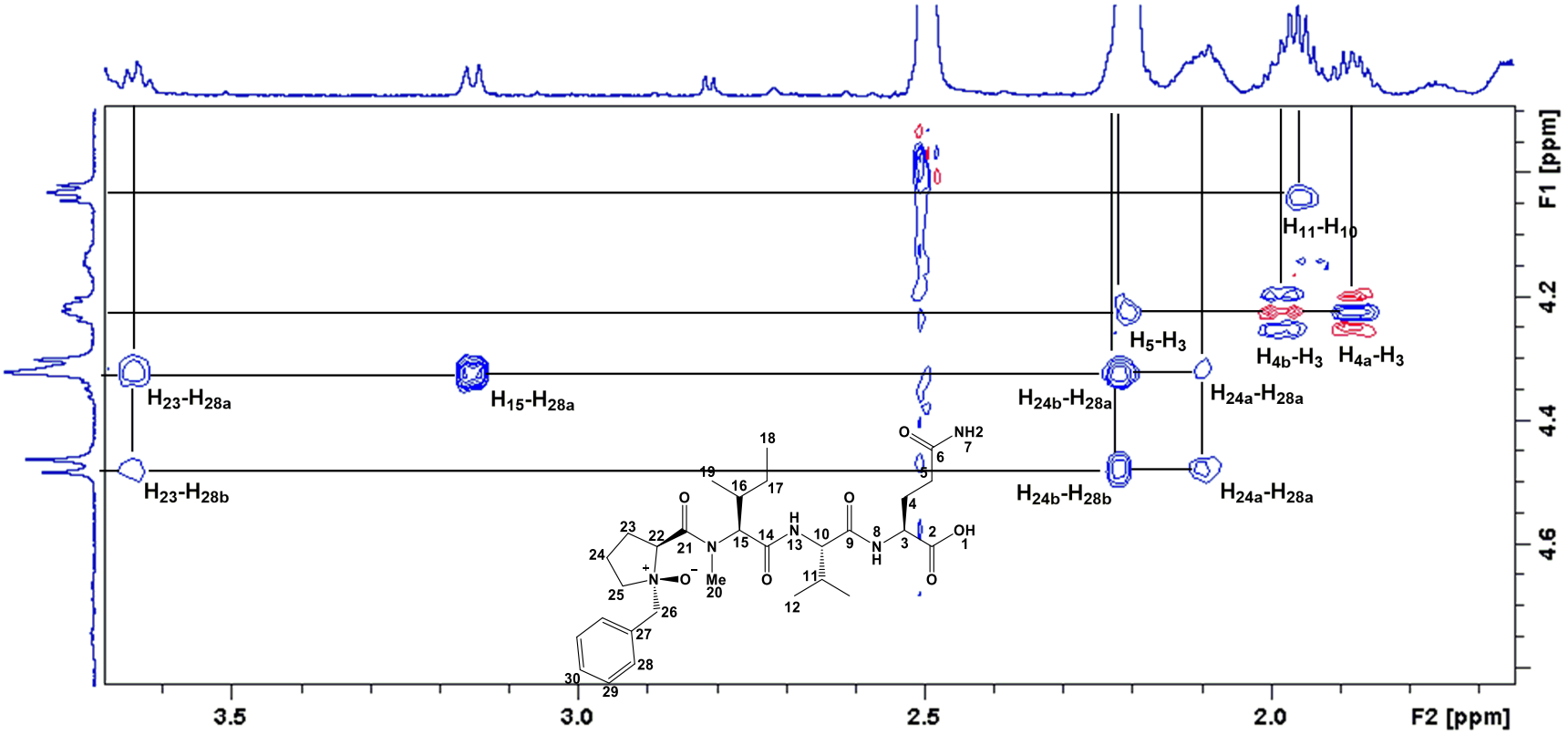


ROESY studies for the detection of *cis*- and *trans*-isomers in **4b, [Bz-P(NO)-MeI-V-Q]**. The absence of a NOE correlation between the *cis*-*N*-methyl group and the proline Hα, assisted to differentiate between the conformations. The water peak is suppressed. The spectra was obtained at room temperature. (Solvent: DMSO-d_6)

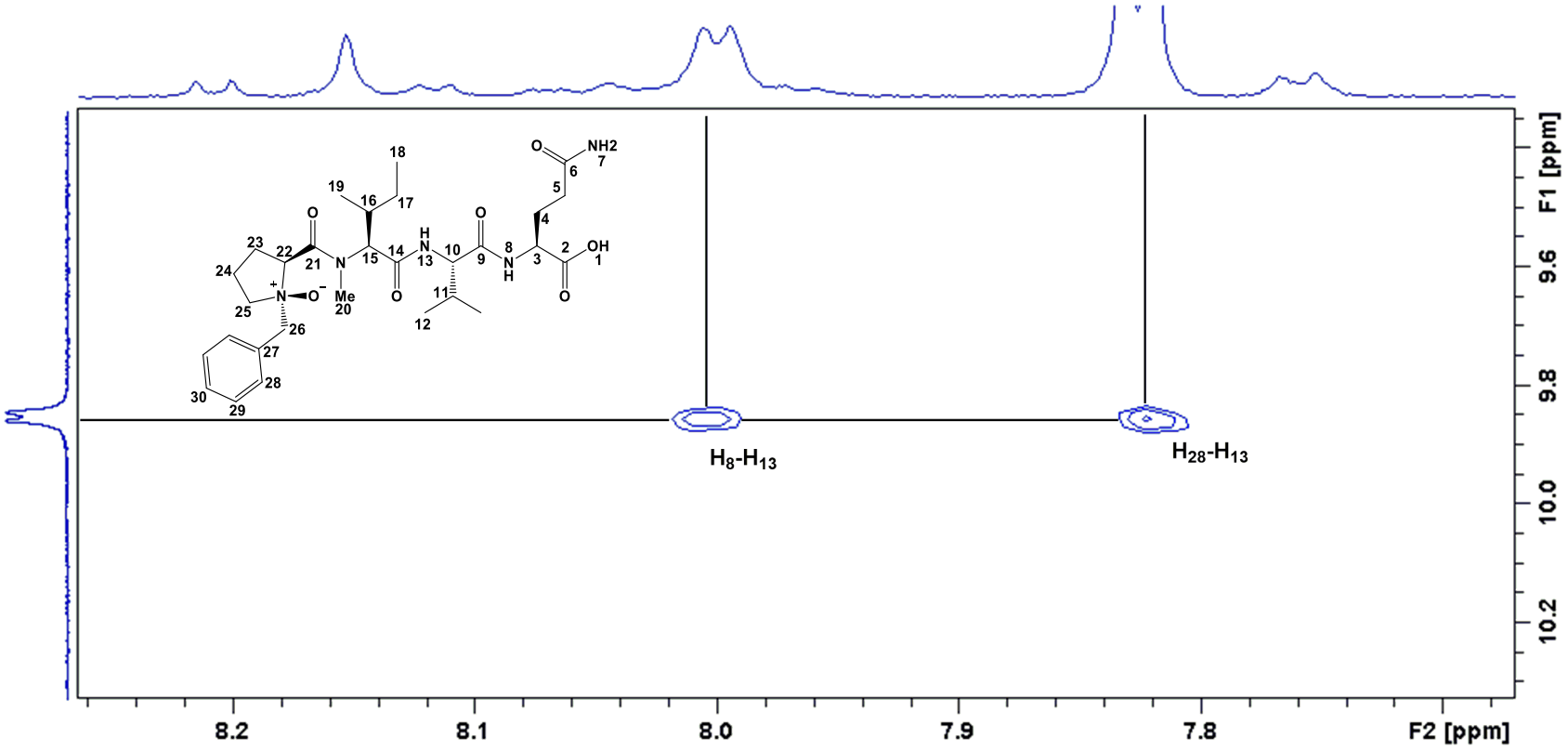
Expansion of observed NOE correlations of **4b,** [**Bz(NO)PMeIVQ**]



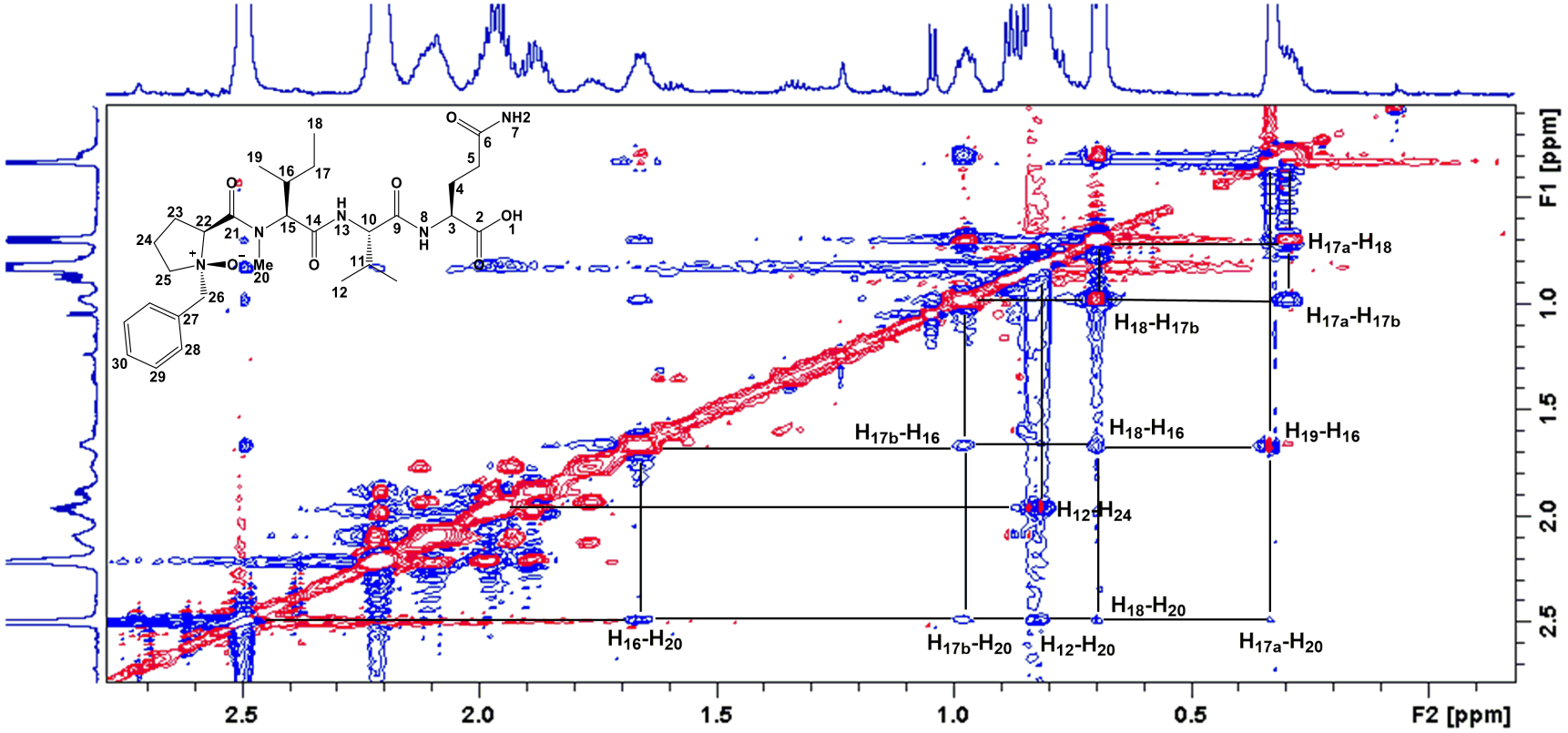
Expansion of observed NOE correlations of **4b,** [**Bz(NO)PMeIVQ**]



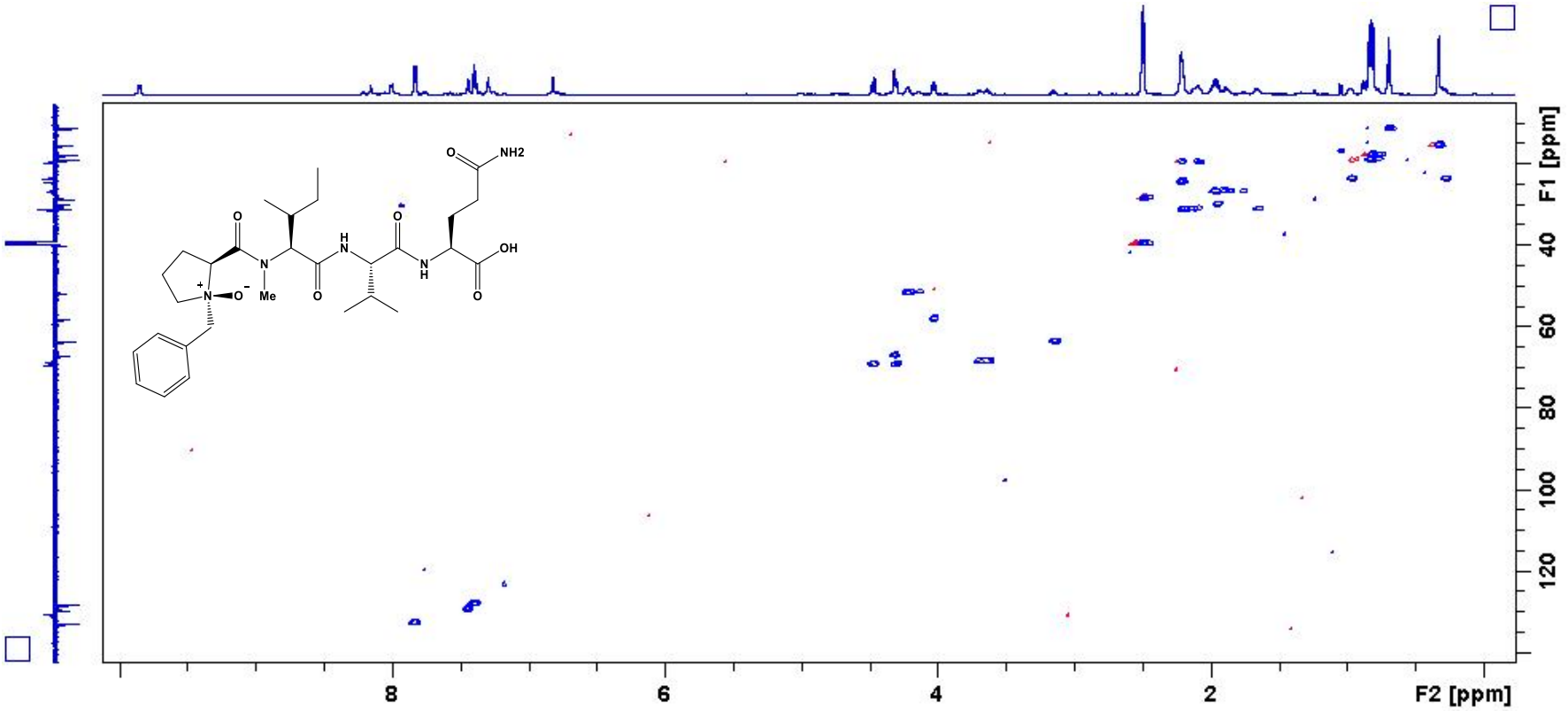
Expansion of observed NOE correlations of **4b,** [**Bz(NO)PMeIVQ**]



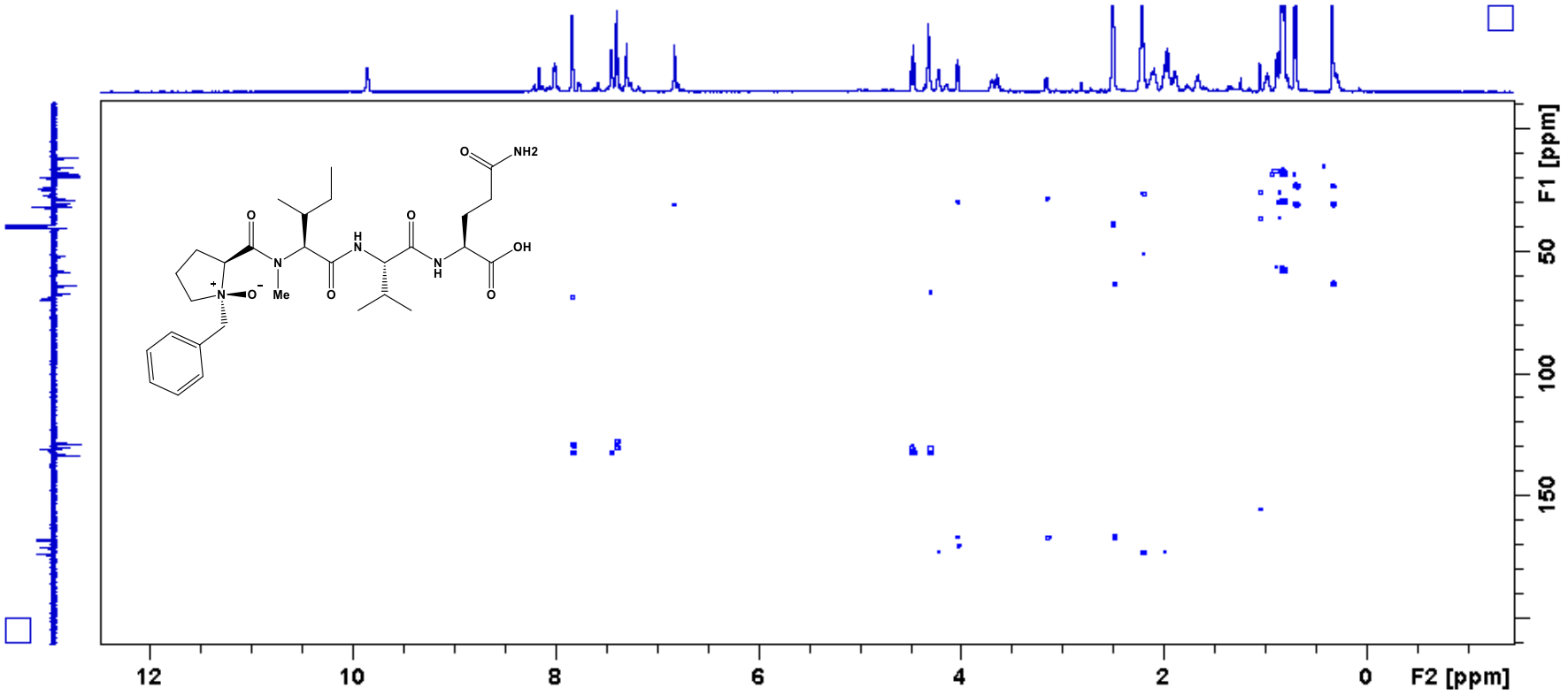
Expansion of observed NOE correlations of **4b,** [**Bz(NO)PMeIVQ**]



HSQC of **4b,** [**Bz(NO)PMeIVQ**]

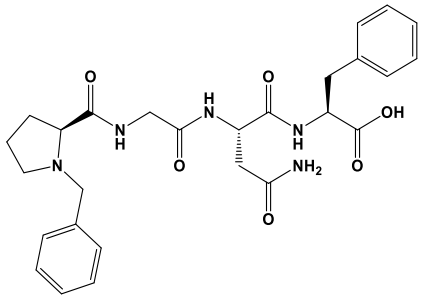


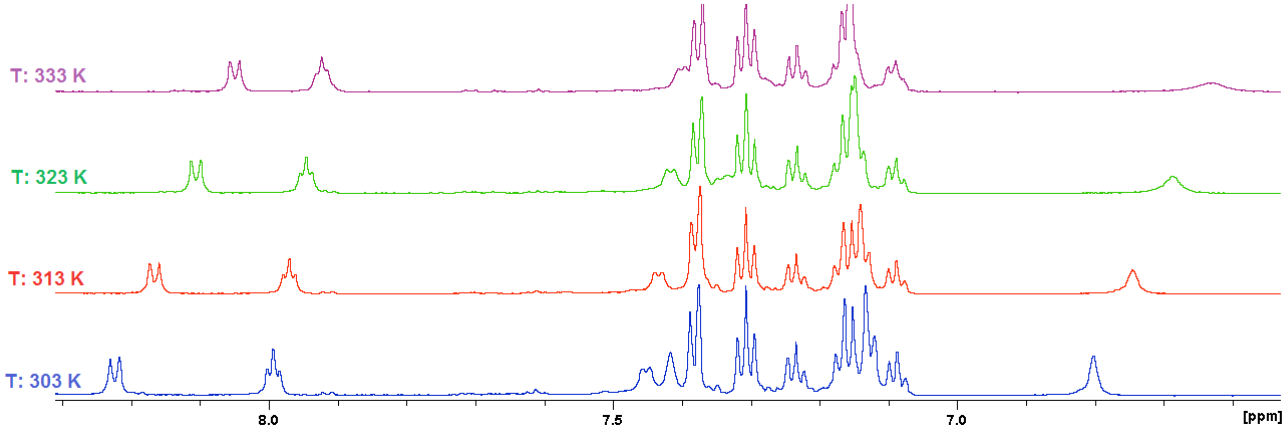
HMBC of **4b, [Bz(NO)PMeIVQ]**

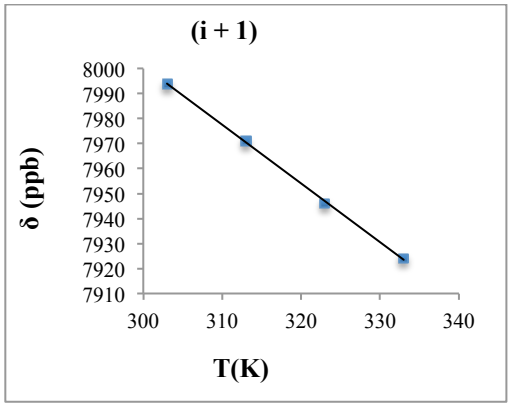


4. Thermal coefficient plots and NMR Spectra

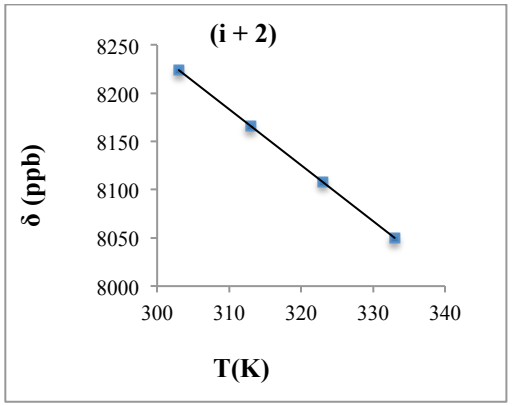
1a, (BzPGNF)



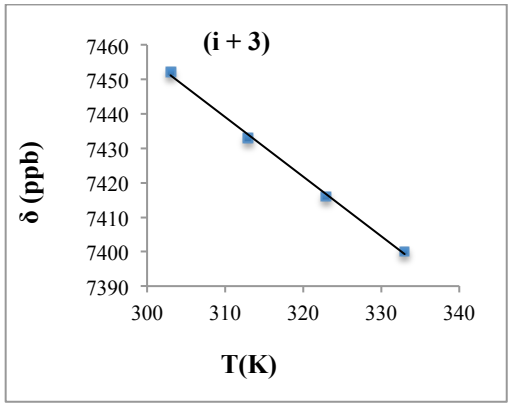




(1)



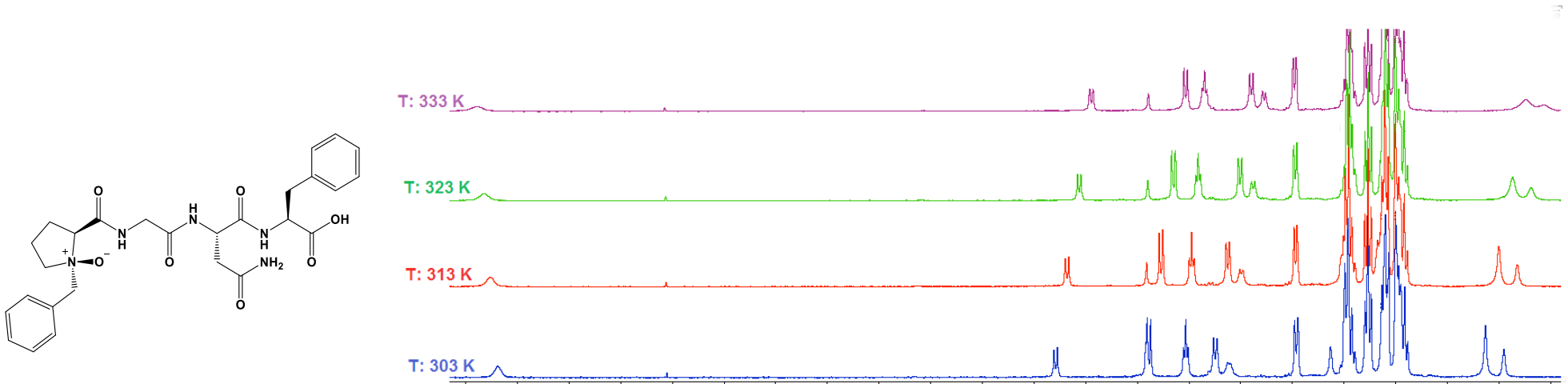
(2)



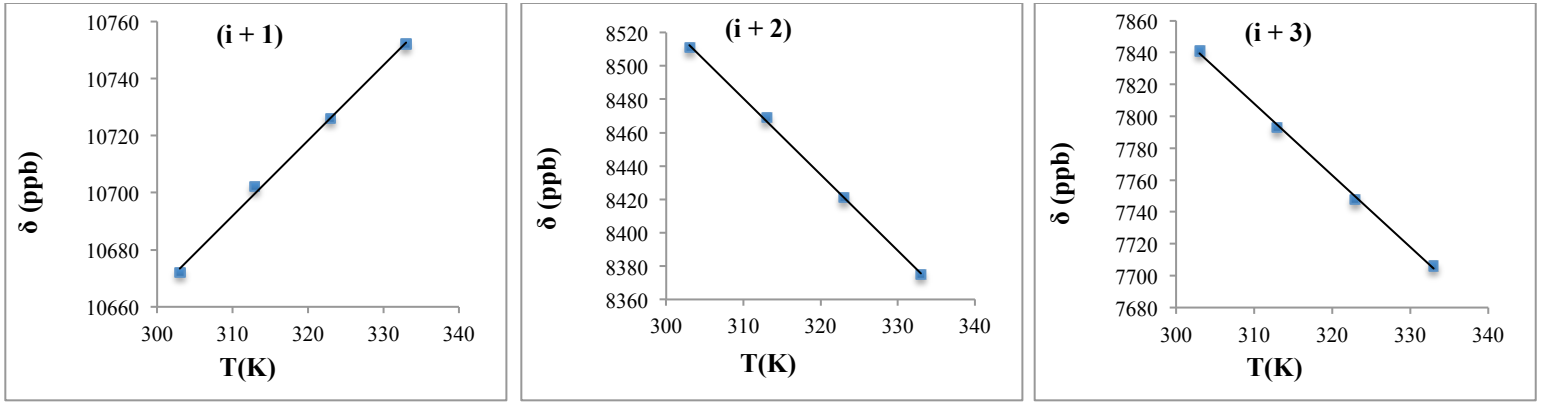
(3)

(1) HN-Gly (-Δδ/ΔT= 2.35 ppb/K, R^2 = 0.999), (2) HN-Asn (-Δδ/ΔT= 5.80 ppb/K, R^2 = 1.00), (3) HN-Phe (-Δδ/ΔT= 1.73 ppb/K, R^2 = 0.998)

1b, [Bz(NO)PGNF]

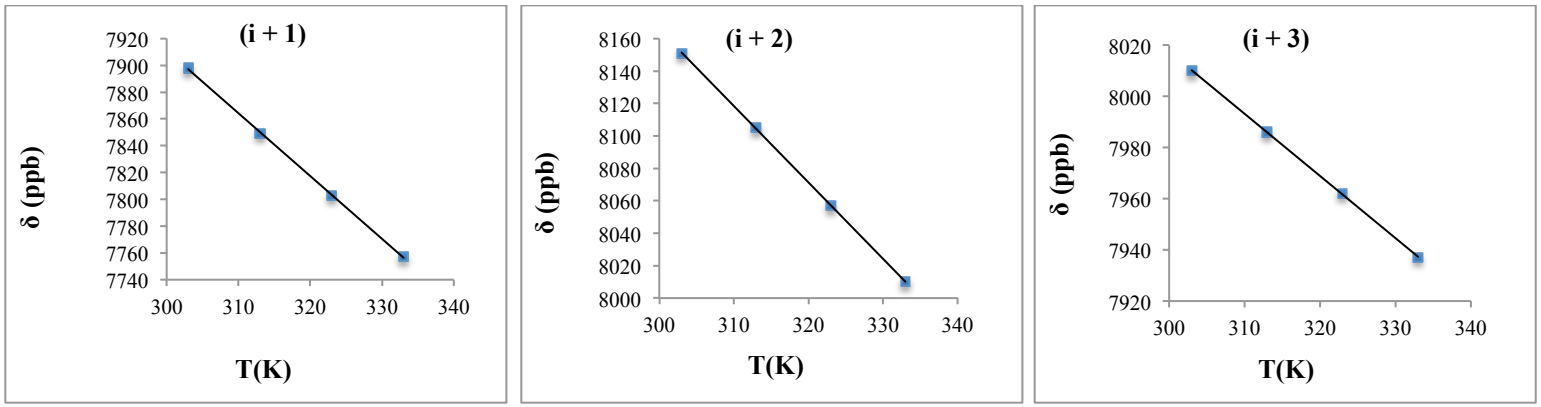


Temperature for this analysis was started from 303 K. Therefore, the major product in room temperature (about 293 K) is revealed as the minor from the first (blue) spectrum.



(1) **(2)** **(3)**

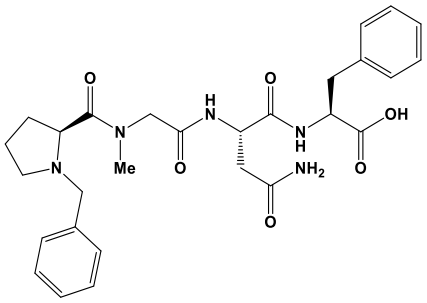
Major conformer at RT: (1) HN-Gly (-Δδ/ΔT= -2.64 ppb/K, R^2= 0.997), (2) HN-Asn (-Δδ/ΔT= 4.56 ppb/K, R^2= 0.999), (3) HN-Phe (-Δδ/ΔT= 4.50 ppb/K, R^2= 0.999)

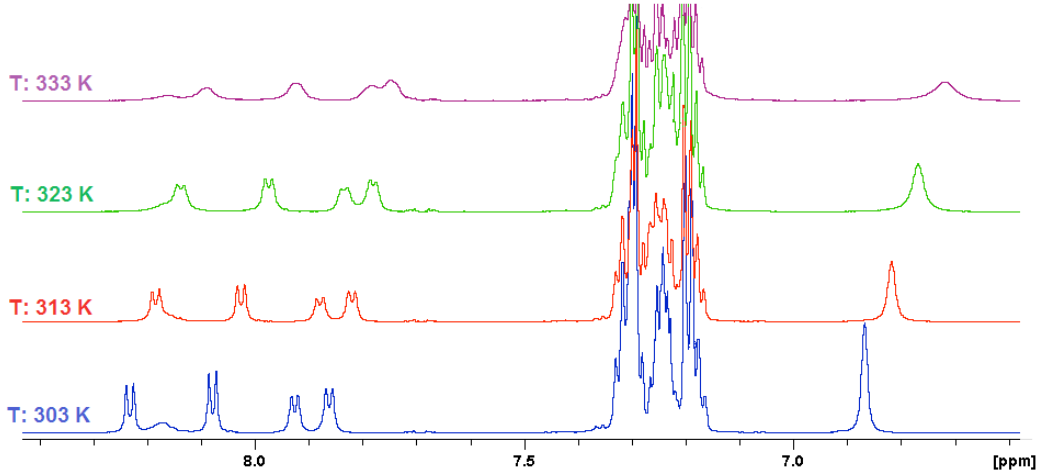


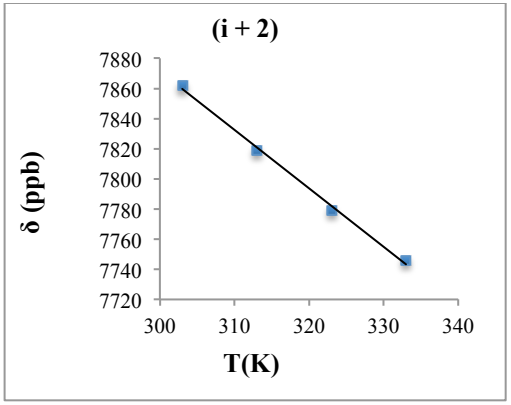
(1) **(2)** **(3)**

Minor conformer at RT: (1) HN-Gly (-Δδ/ΔT= 4.69 ppb/K, R^2= 0.999), (2) HN-Asn (-Δδ/ΔT= 4.71 ppb/K, R^2= 0.999), (3) HN-Phe (-Δδ/ΔT= 2.43 ppb/K, R^2= 0.999)

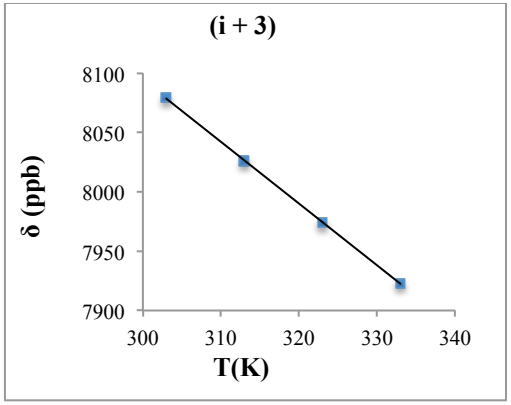
2a, (BzPMeGNF)





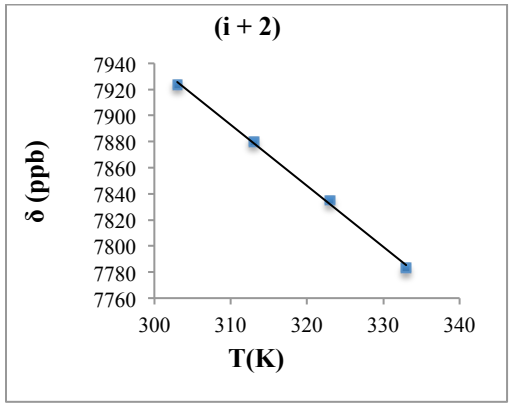


(1)

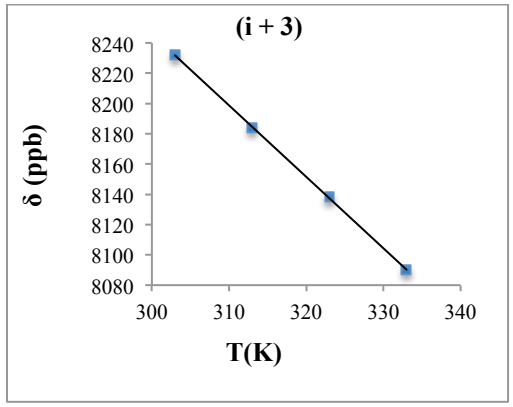


(2)

***Trans*-isomer (major):** (1) HN-Asn (-Δδ/ΔT= 3.88 ppb/K, R^2= 0.996), (2) HN-Phe (-Δδ/ΔT= 5.20 ppb/K, R^2= 0.999)



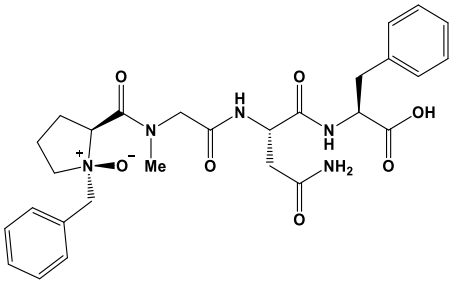
(1)

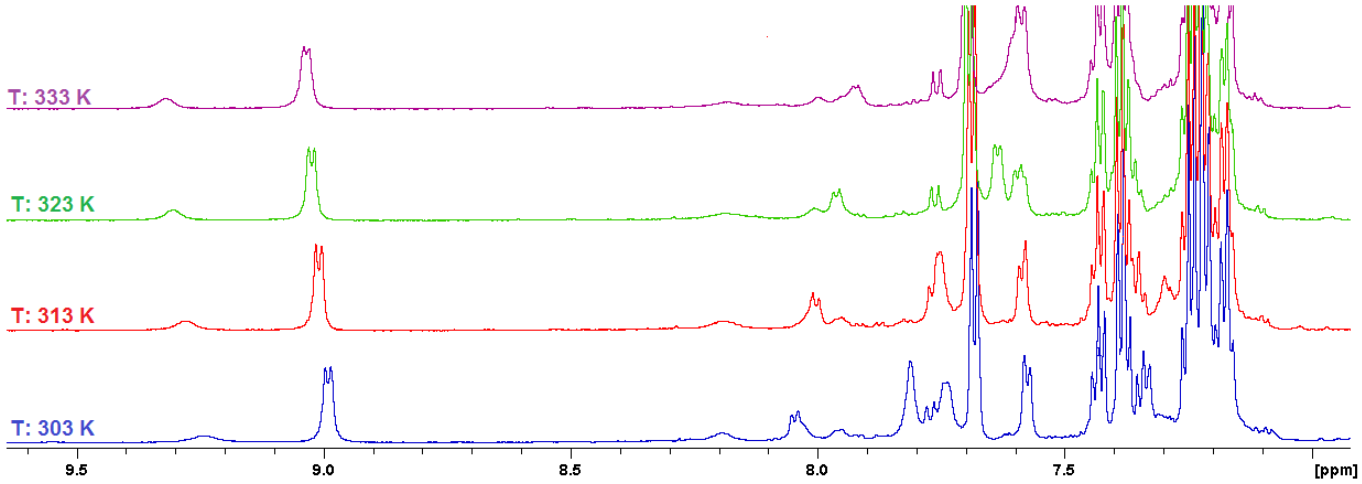


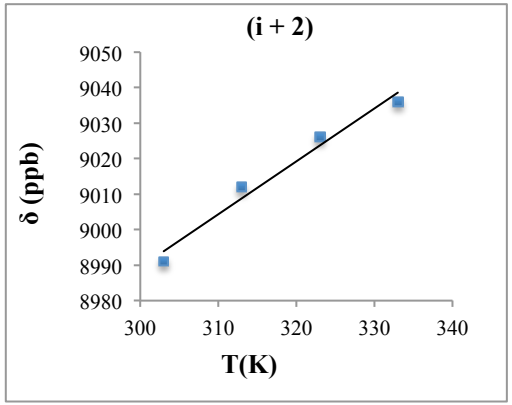
(2)

***Cis*-isomer (minor):** (1) HN-Asn (-Δδ/ΔT= 4.68 ppb/K, R^2= 0.998), (2) HN-Phe (-Δδ/ΔT= 4.72 ppb/K, R^2= 0.999)

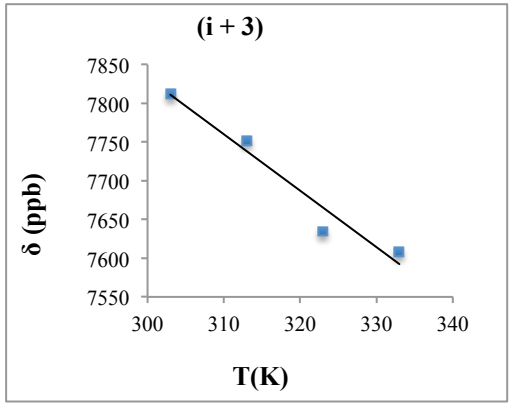
2b, [Bz(NO)PMeGNF]







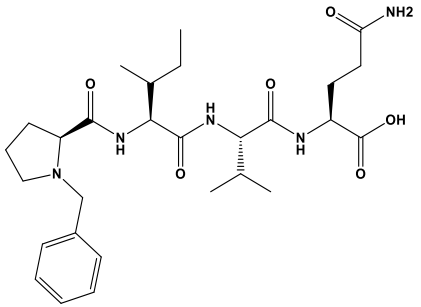
(1)

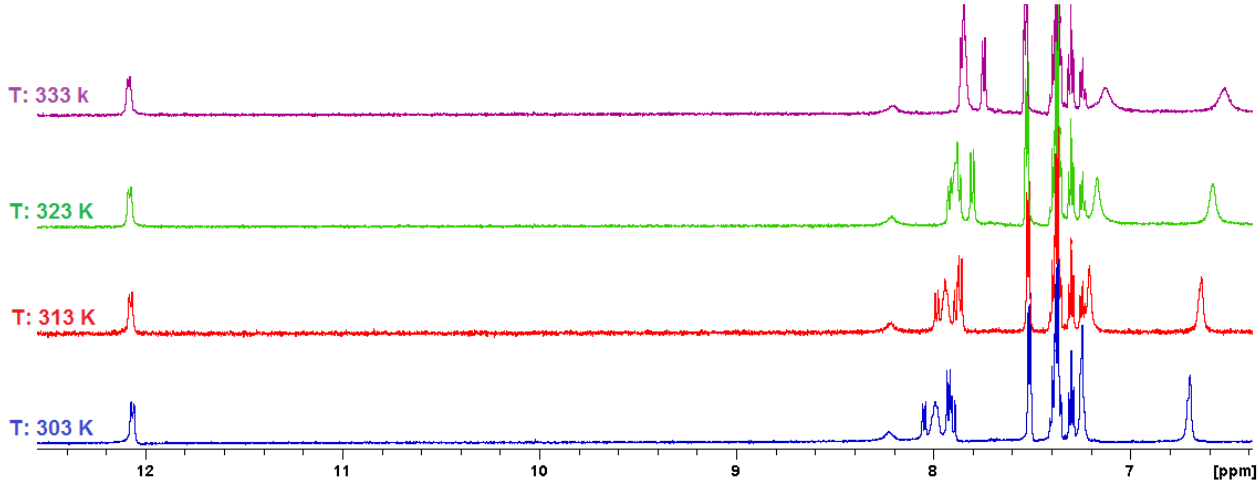


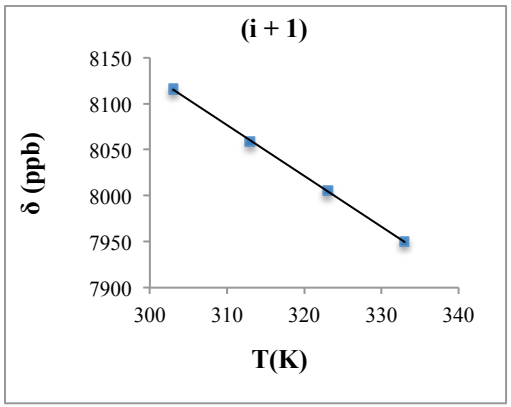
(2)

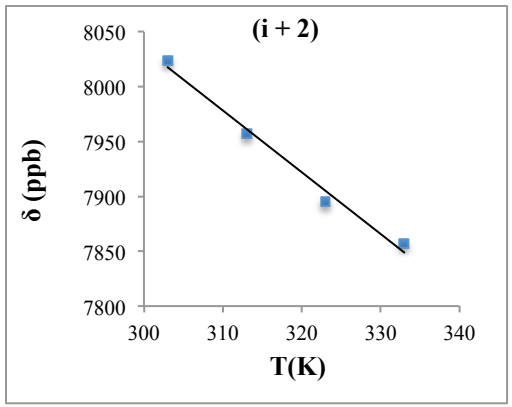
***Trans*-isomer (major):** (1) HN-Asn ($-\Delta\delta/\Delta T$= -1.49 ppb/K, R^2= 0.993), (2) HN-Phe ($-\Delta\delta/\Delta T$= 7.29 ppb/K, R^2= 0.951)

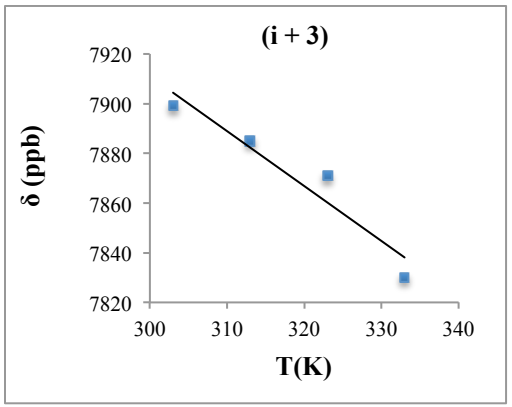
3a, (BzPIVQ)







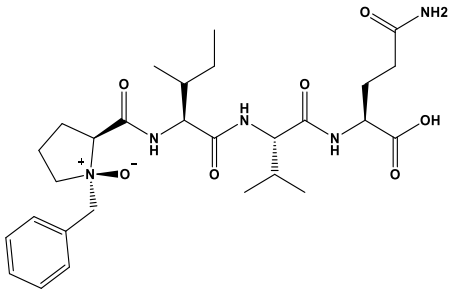


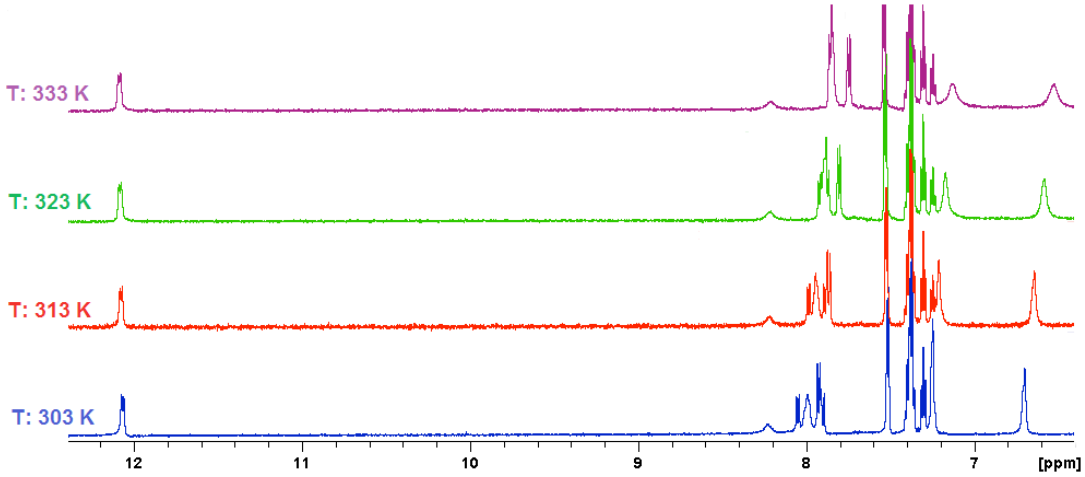


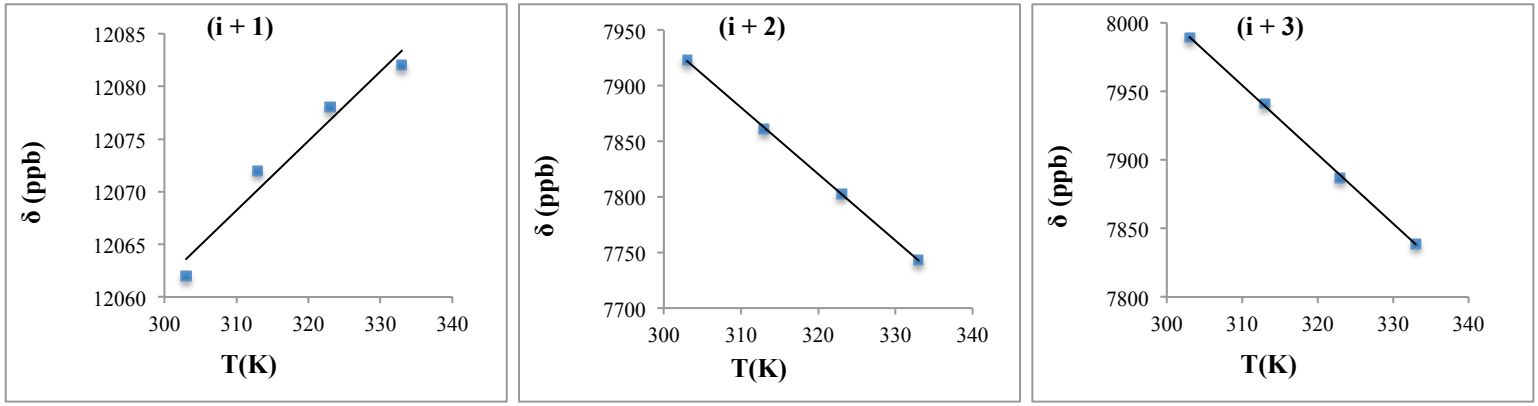
(1) **(2)** **(3)**

(1) HN-Ile (-Δδ/ΔT= 5.52 ppb/K, R^2= 0.999), (2) HN-Val (-Δδ/ΔT= 5.63 ppb/K, R^2= 0.985), (3) HN-Gln (-Δδ/ΔT= 2.21 ppb/K, R^2= 0.917)

3b, [Bz(NO)PIVQ]



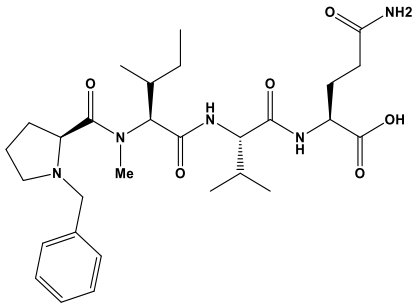


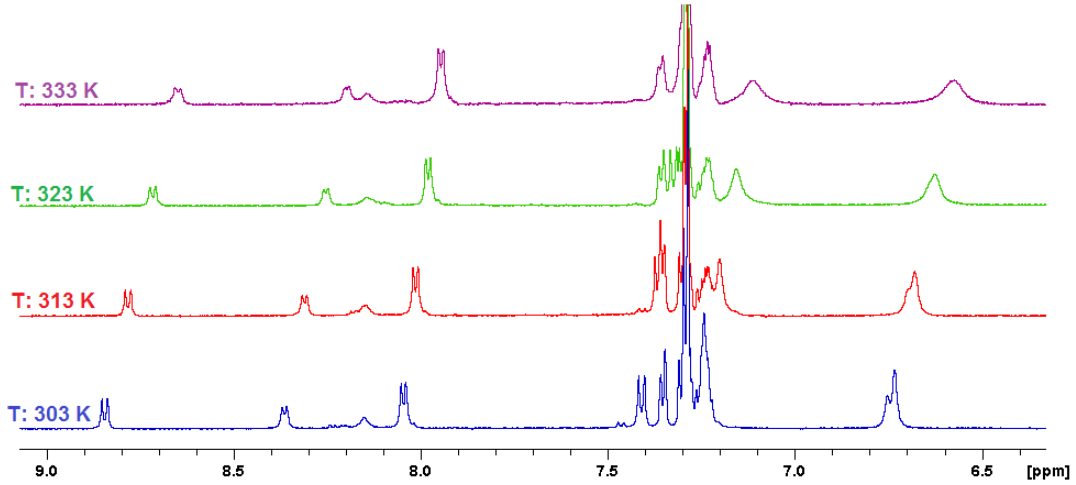


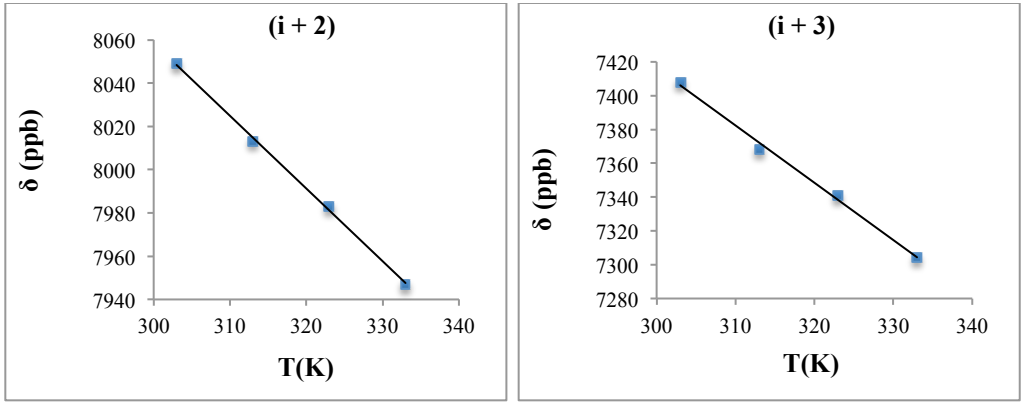
(1) **(2)** **(3)**

(1) HN-Ile (-Δδ/ΔT= -0.66 ppb/K, R^2= 0.959), (2) HN-Val (-Δδ/ΔT= 5.98 ppb/K, R^2= 0.999), (3) HN-Gln (-Δδ/ΔT= 5.04 ppb/K, R^2= 0.999)

4a, (BzPMeIVQ)

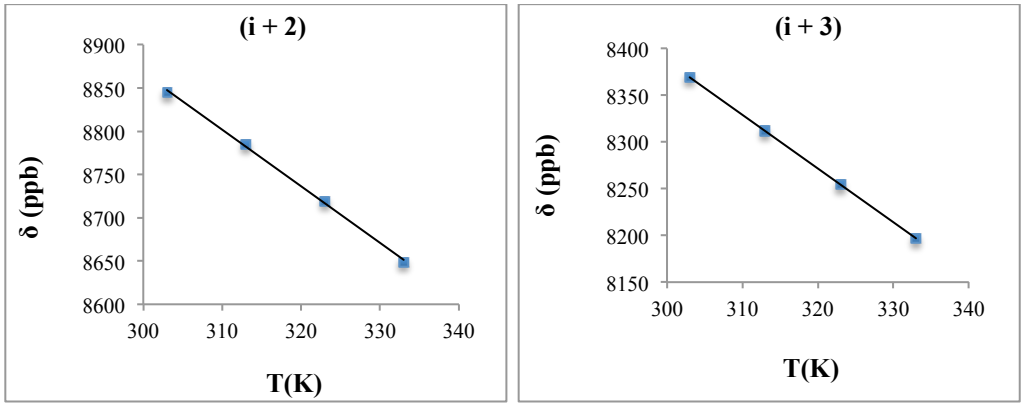

O
NH2
O
N
Me
O
H
N
O
N
H
OH
O
N


T: 333 K
T: 323 K
T: 313 K
T: 303 K
9.0
8.5
8.0
7.5
7.0
6.5
[ppm]



(**1**) (**2**)

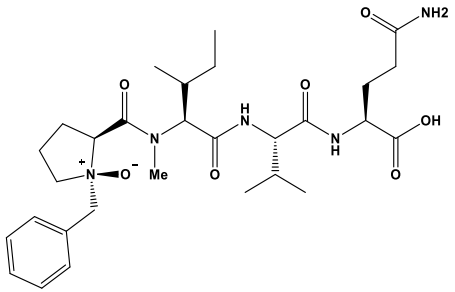
***Trans*-isomer (major):** (1) HN-Val (-Δδ/ΔT= 3.36 ppb/K, R^2= 0.959), (2) HN-Gln (-Δδ/ΔT= 3.39 ppb/K, R^2= 0.995)

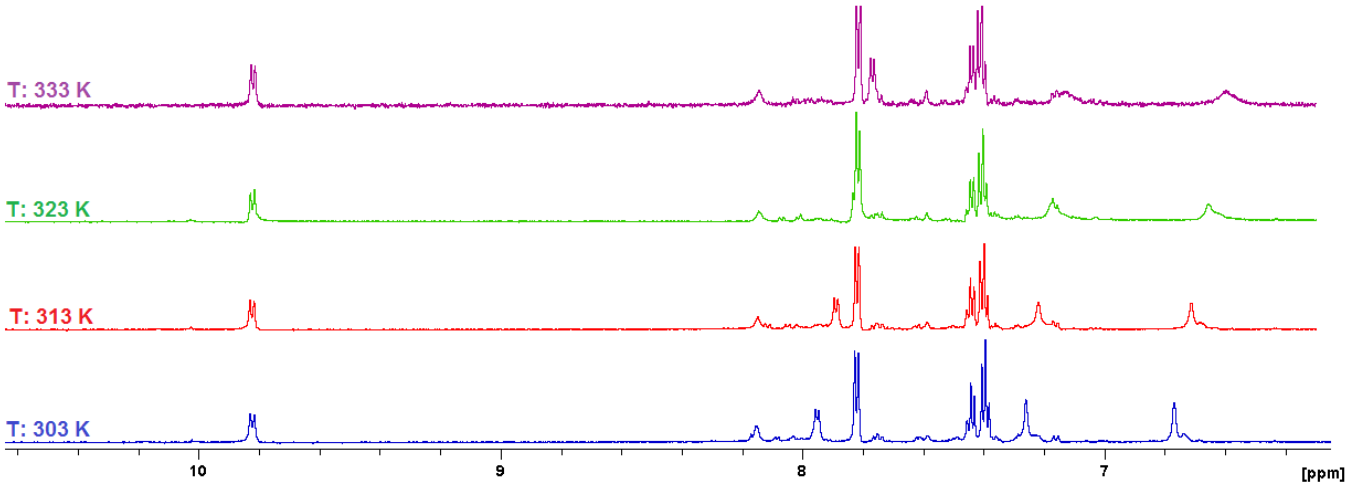


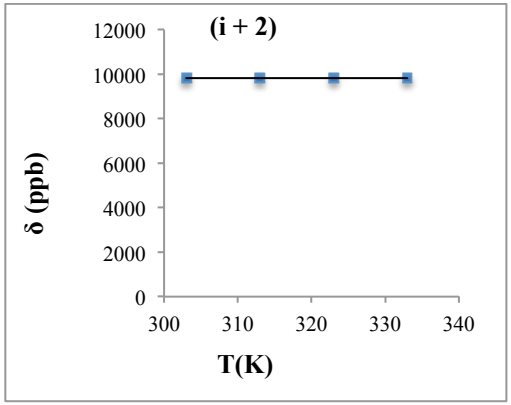
(**1**) (**2**)

***Cis*-isomer (minor):** (1) HN-Val (-Δδ/ΔT= 6.54 ppb/K, R^2= 0.998), (2) HN-Gln (-Δδ/ΔT= 5.74 ppb/K, R^2= 0.999)

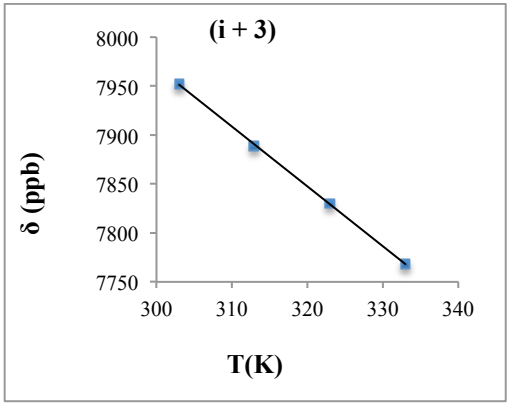
4b, [Bz(NO)PMeIVQ]







(1)



(2)

***Cis*-isomer (major):** (1) HN-Val (-Δδ/ΔT= 0 ppb/K, R2= N/A), (2) HN-Gln (-Δδ/ΔT= 6.11 ppb/K, R^2= 0.999)

5. HRMS spectra

Tune mix for calibration

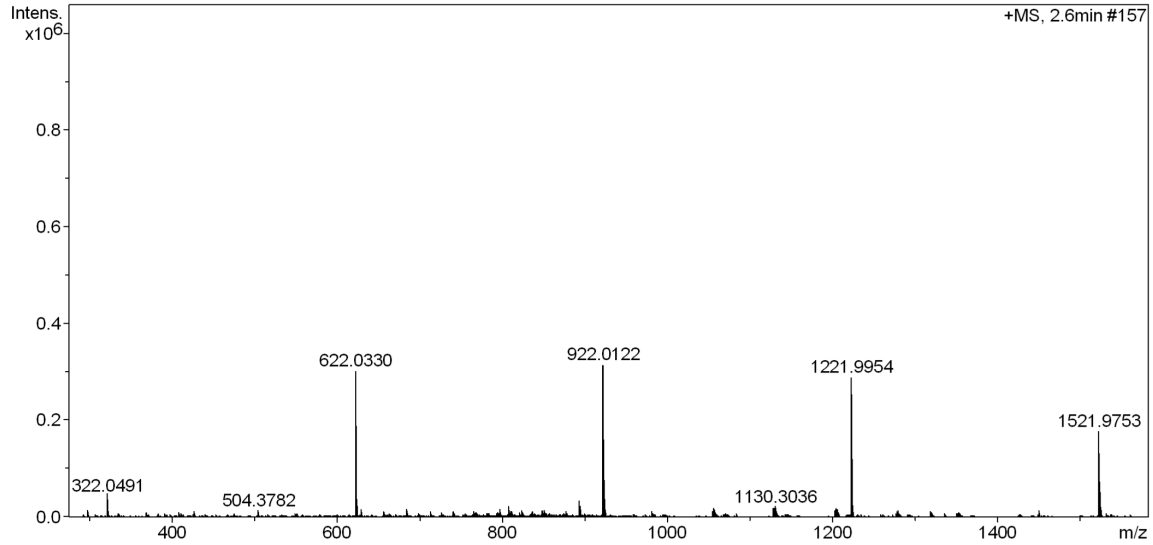
Display Report

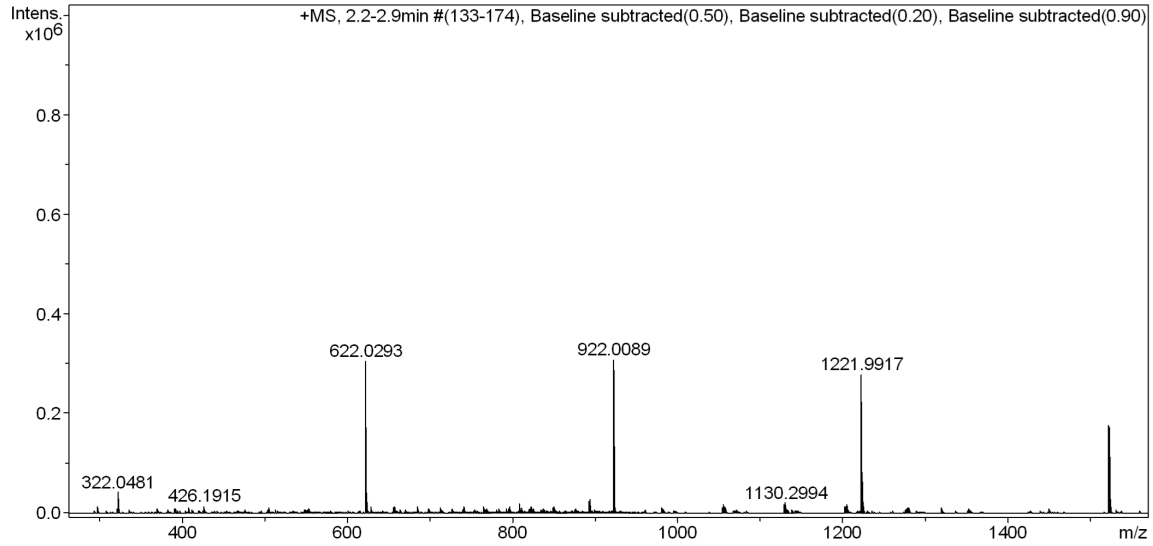
Analysis Info

Analysis Name	D:\Data\thavi\Majid 1A spec_1_01_210.d	Acquisition Date	11/29/2013 10:16:59 AM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	Majid 1A spec	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source





Bruker Compass DataAnalysis 4.0 printed: 11/29/2013 10:35:37 AM Page 1 of 1

1a, (BzPGNF)

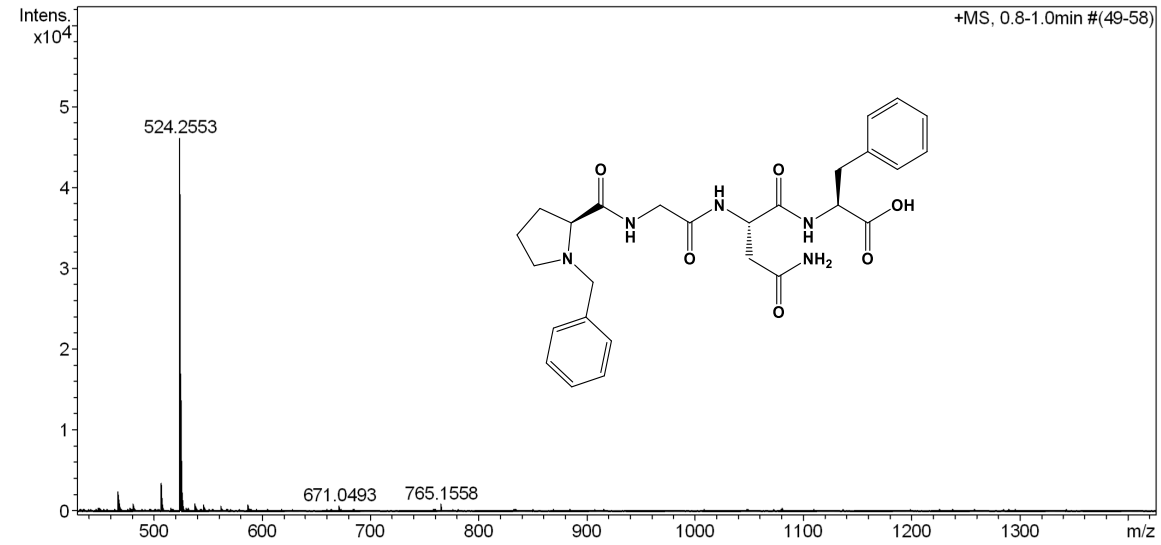
Display Report

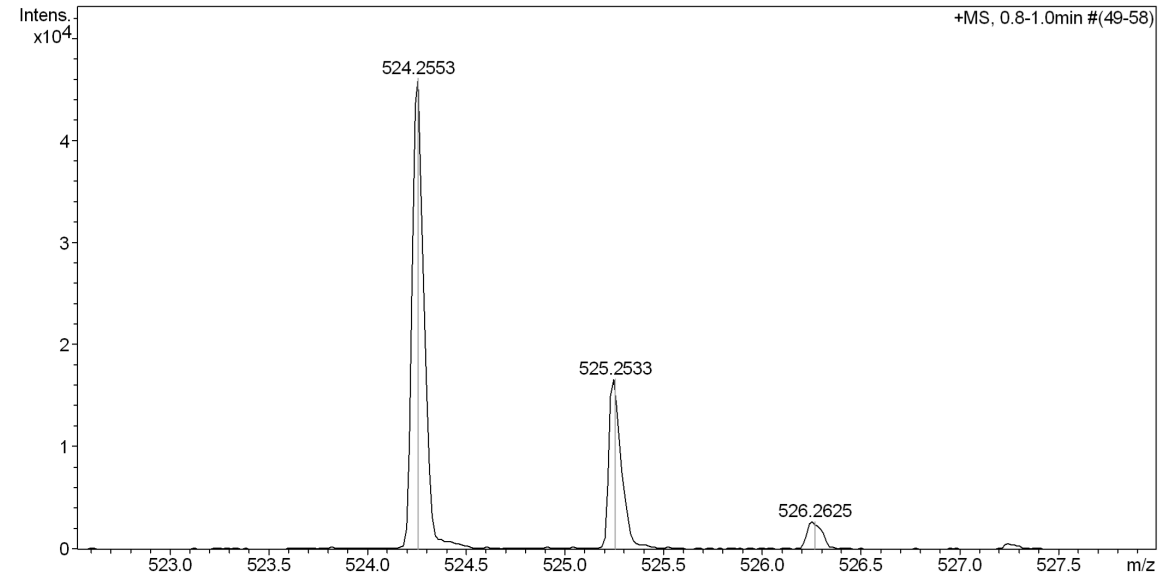
Analysis Info

Analysis Name	D:\Data\thavi\4 Oct Majid 3a_4_01_88.d	Acquisition Date	10/4/2013 11:17:49 AM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	4 Oct Majid 3a	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source





1b, [Bz(NO)PGNF]

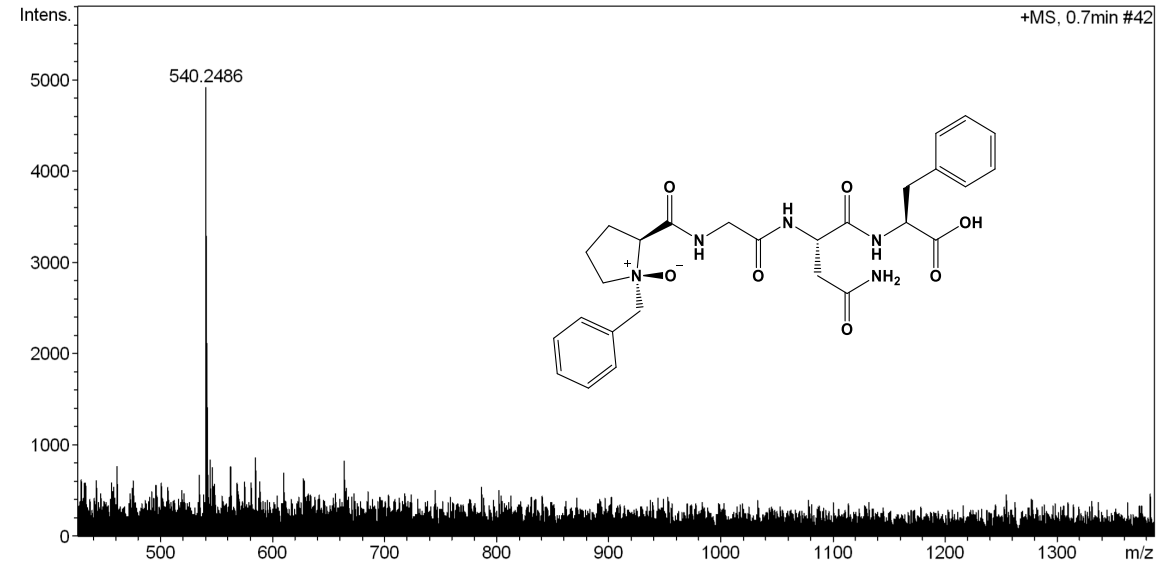
Display Report

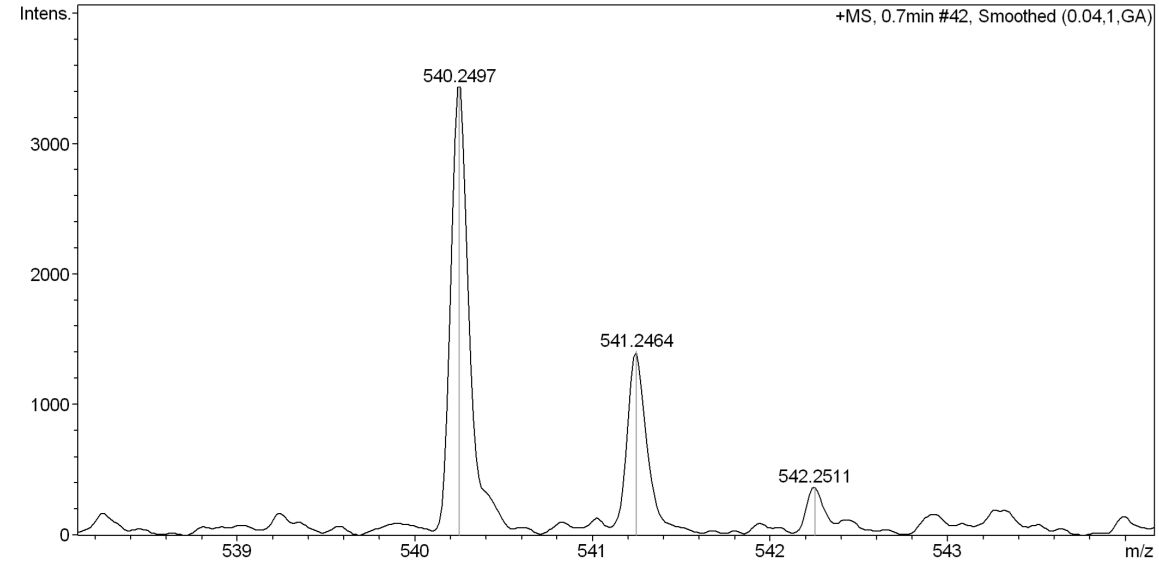
Analysis Info

Analysis Name	D:\Data\thavi\Majid 3b_7_01_66.d	Acquisition Date	9/24/2013 4:22:10 PM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	Majid 3b	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source





2a, (BzPMeGNF)

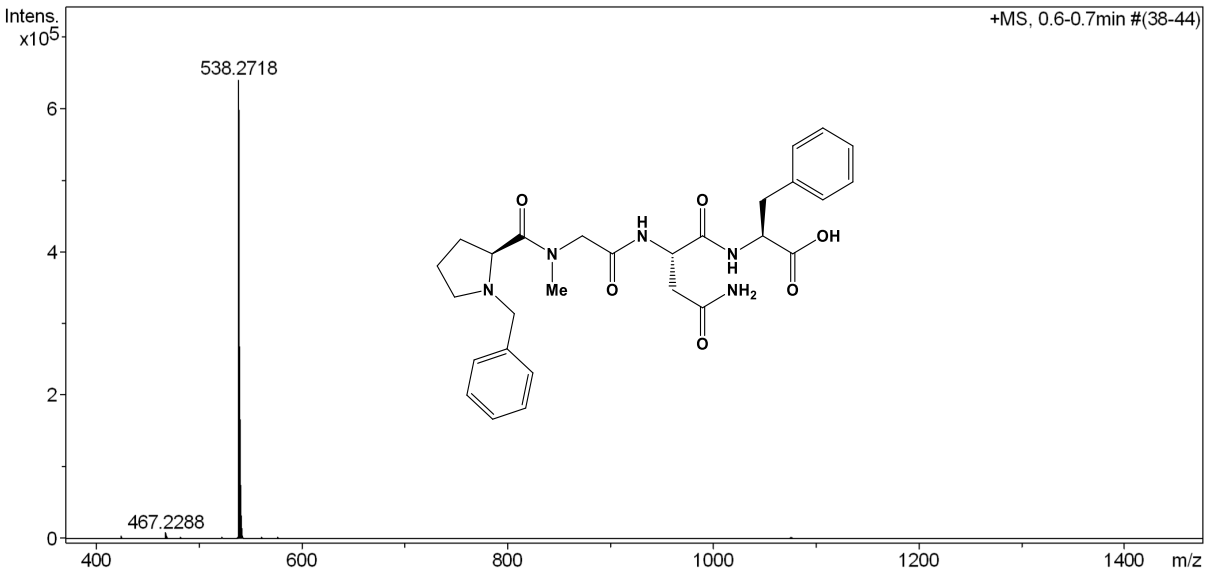
Display Report

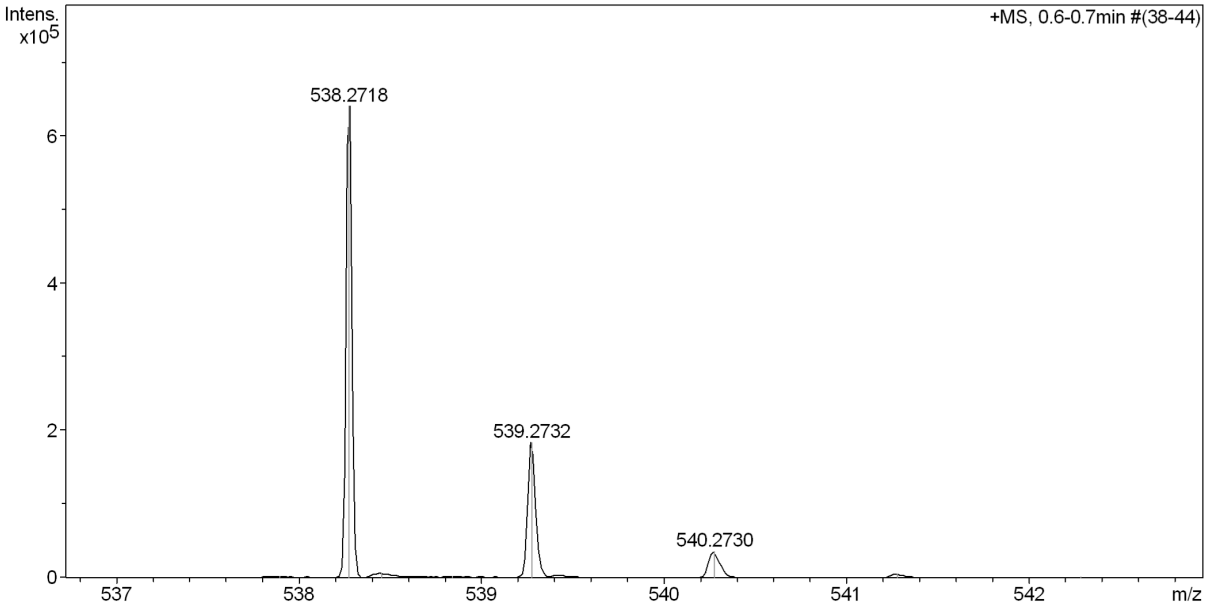
Analysis Info

Analysis Name	D:\Data\thavi\Majid 4a_8_01_68.d	Acquisition Date	9/24/2013 4:28:16 PM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	Majid 4a	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source





2b, [Bz(NO)PMeGNF]

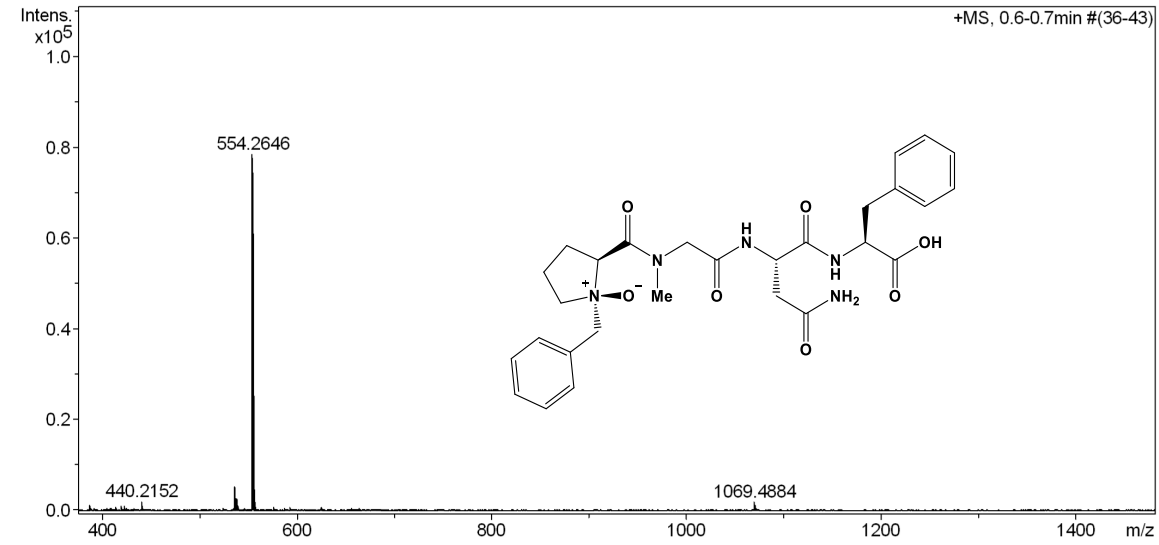
Display Report

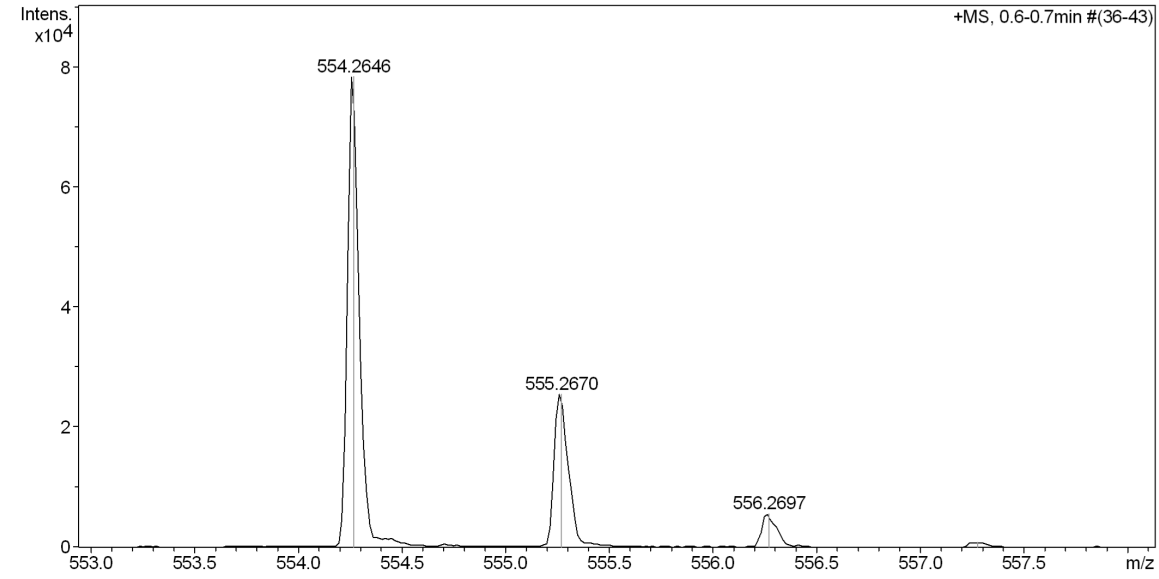
Analysis Info

Analysis Name	D:\Data\thavi\Majid 4b_9_01_70.d	Acquisition Date	9/24/2013 4:34:21 PM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	Majid 4b	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source





3a, (BzPIVQ)

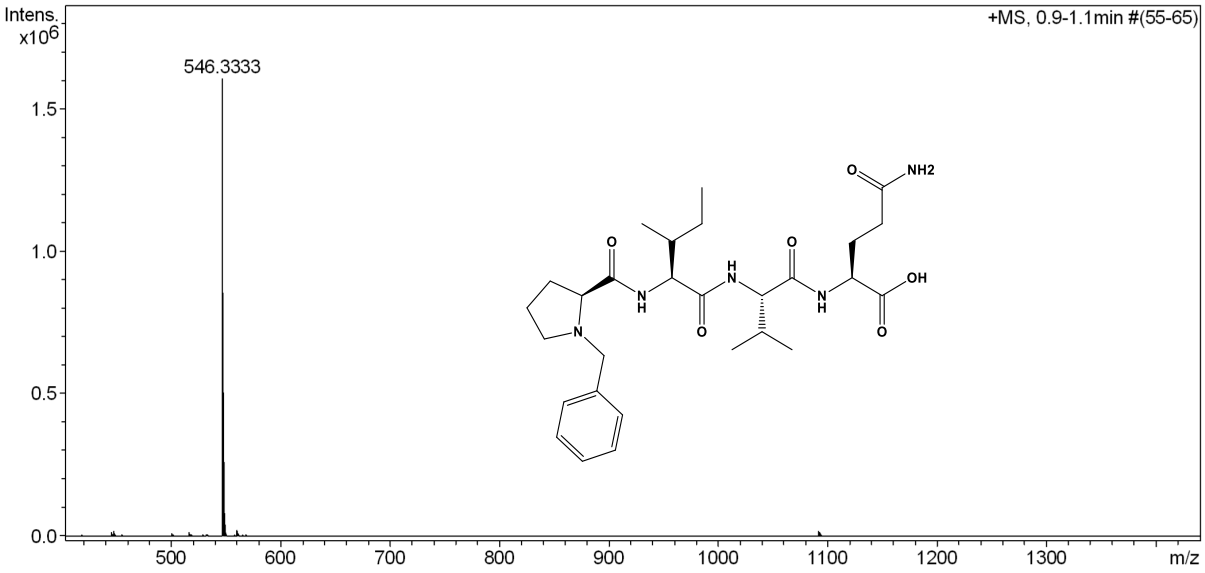
Display Report

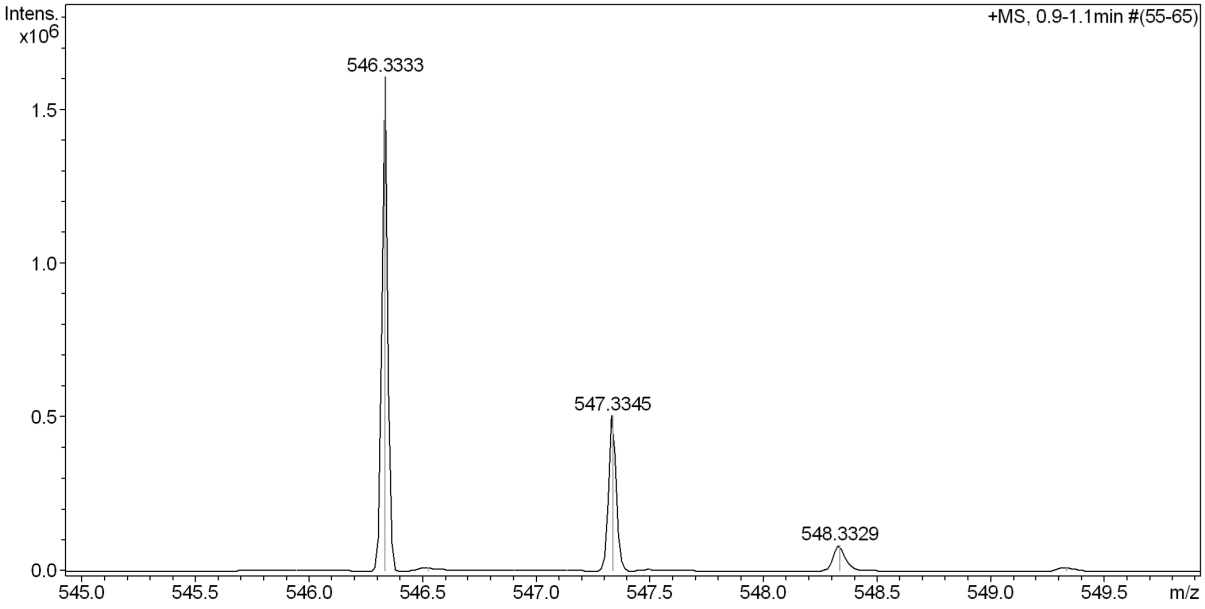
Analysis Info

Analysis Name	D:\Data\thavi\Majid 1a_2_01_48.d	Acquisition Date	9/24/2013 2:48:35 PM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	Majid 1a	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source





3b, [Bz(NO)PIVQ]

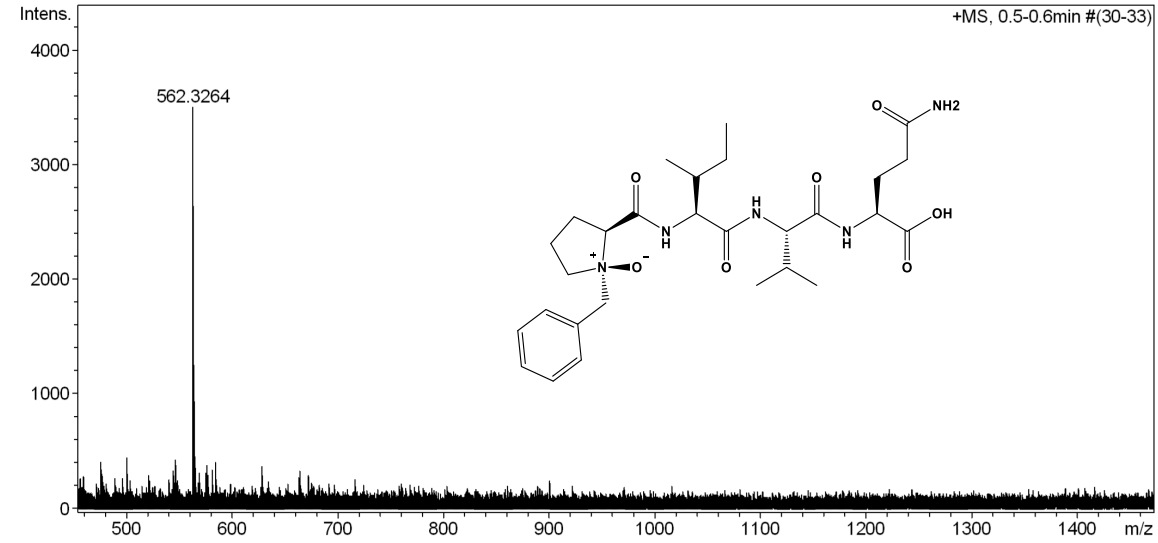
Display Report

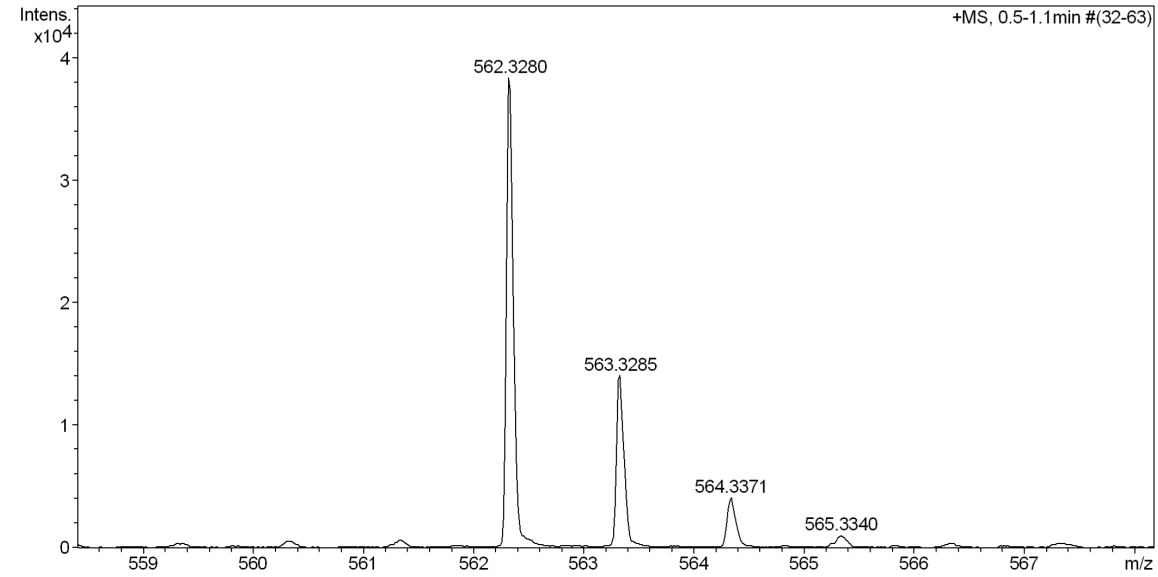
Analysis Info

Analysis Name	D:\Data\thavi\Majid 1B_3_01_64.d	Acquisition Date	9/24/2013 4:16:03 PM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	Majid 1B	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source





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4a, (BzPMeIVQ)

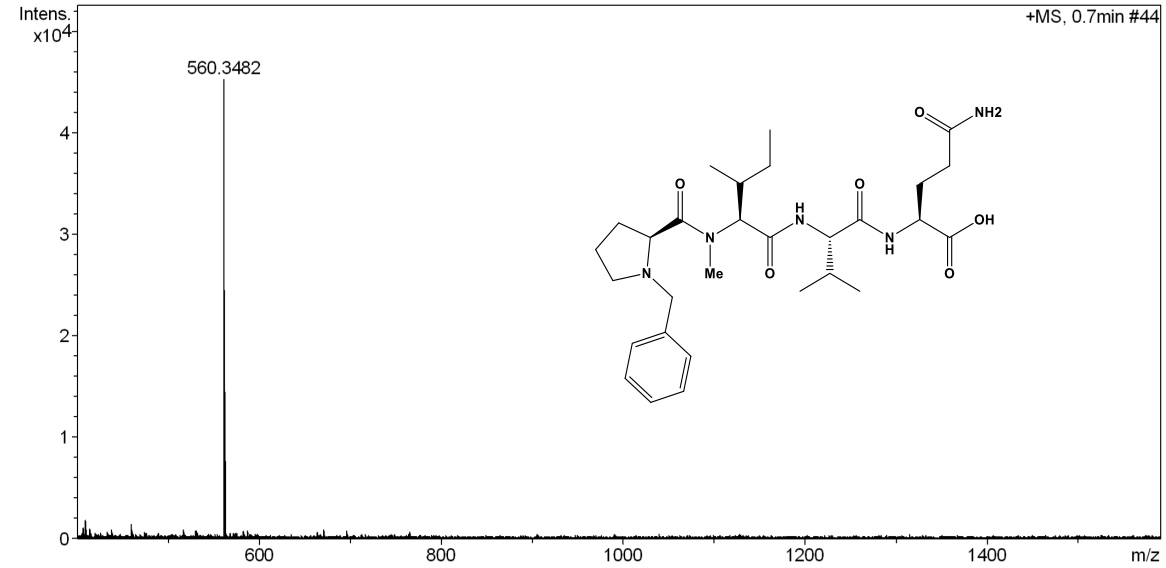
Display Report

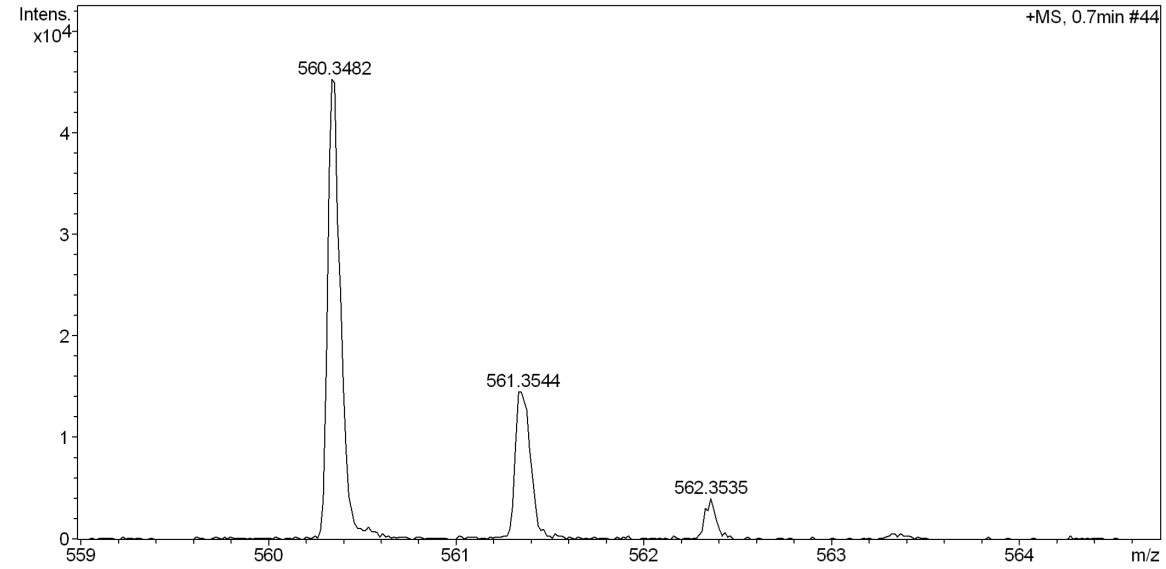
Analysis Info

Analysis Name	D:\Data\thavi\4 Oct Majid 2a_2_01_84.d	Acquisition Date	10/4/2013 11:05:43 AM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	4 Oct Majid 2a	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source





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4b, [Bz(NO)PMeIVQ]

Display Report

Analysis Info

Analysis Name	D:\Data\thavi\4 Oct Majid 2b_3_01_102.d	Acquisition Date	10/4/2013 12:00:06 PM
Method	test tunemix.m	Operator	BDAL@DE
Sample Name	4 Oct Majid 2b	Instrument	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source