Problem Session

Oct 13, 2018

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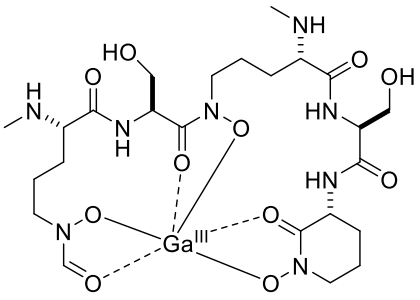
Please assign the signals of region A in Figure 1. ^{1}H–^{1}H DQF-COSY, ^{1}H–^{1}H ROESY, ^{1}H–^{13}C HSQC spectra, and an overlay of the ^{1}H–^{1}H TOCSY and ^{1}H–^{1}H ROESY spectra are available as “spectra.pdf” (pp. 9–13).



1

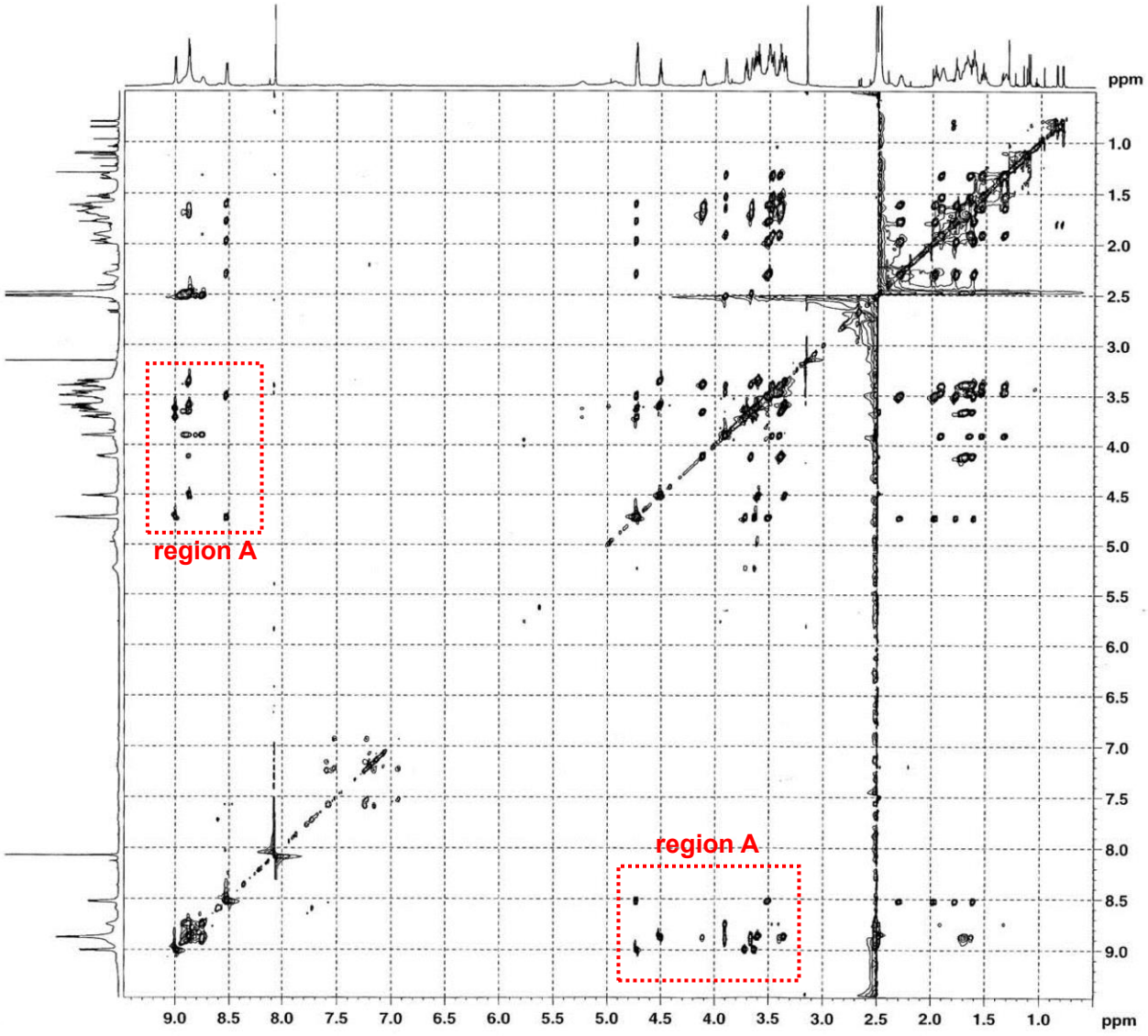


Figure 1. ^{1}H–^{1}H TOCSY spectrum of **1** in DMSO-d_6.

^{1}H chemical shift assignment of peptides using 2D NMR experiments

1. Introduction

^{1}H chemical shift assignment of peptides/proteins is important for

- Structure validation or determination of peptides
- Secondary or higher-order structure analysis of peptides/proteins
- Chemical shift perturbation experiment for analysis of intermolecular interaction

One-dimensional ^{1}H NMR spectrum is less meaningful in peptide or protein analysis due to the peak overlapping (Figure 1).

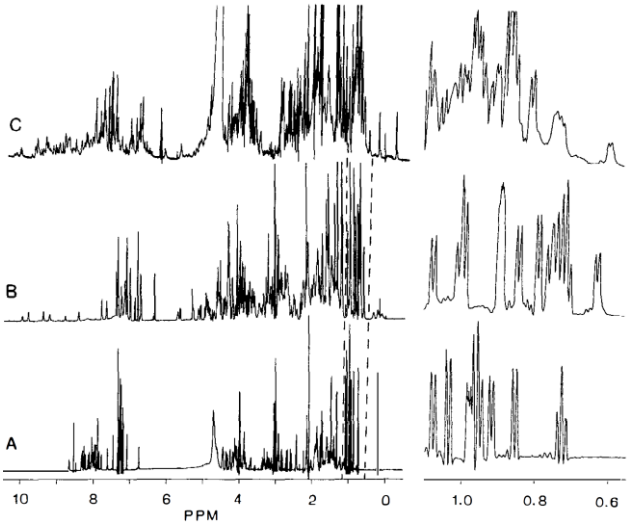


Figure 1. ^{1}H NMR spectra of (A) magainin 2 (23 residues) in H_2O/trifluoroethanol-d_3 (3:1), (B) basic pancreatic trypsin inhibitor (58 residues) in D_2O, and (C) staphylococcal nuclease (156 residues) in H_2O/D_2O (9:1). This figure was taken from ref 1.

2. Methods

2D NMR experiments for the analysis

homonuclear (^{1}H-^{1}H) correlation

through-bond correlation: DQF-COSY (correlation of J-coupled protons), TOCSY (correlation of all protons in a coupling network)

through-space correlation: NOESY, ROESY (spatial proximity)

heteronuclear (^{1}H-^{13}C for non-labeled peptides) correlation

HSQC, HMBC

Assignment

1. Classification of the spin system of each amino acid using COSY and TOCSY
2. Determination of the sequence of amino acids using NOESY, ROESY or HMBC
3. HSQC is also helpful to determine a pair of geminal protons and the proton connected to the characteristic carbon that has an isolated chemical shift

Classification of spin system (20-proteinogenic amino acids)[2] using COSY and TOCSY

unique: Gly/Ala/Val/Leu/Ile/Thr

AMX (CH^{α}, two CH^{β} signals form AMX spin system): Phe, Tyr, His, Trp, Asp, Asn, Cys, and Ser

Ser can be distinguished because of lower field $C^{\beta}H$ shifts

long side chain: Lys, Arg, Met, Glu, and Gln (Orn)

Met, Glu, and Gln can be distinguished because of lower field $C^{\gamma}H$ shifts

no NH: Pro

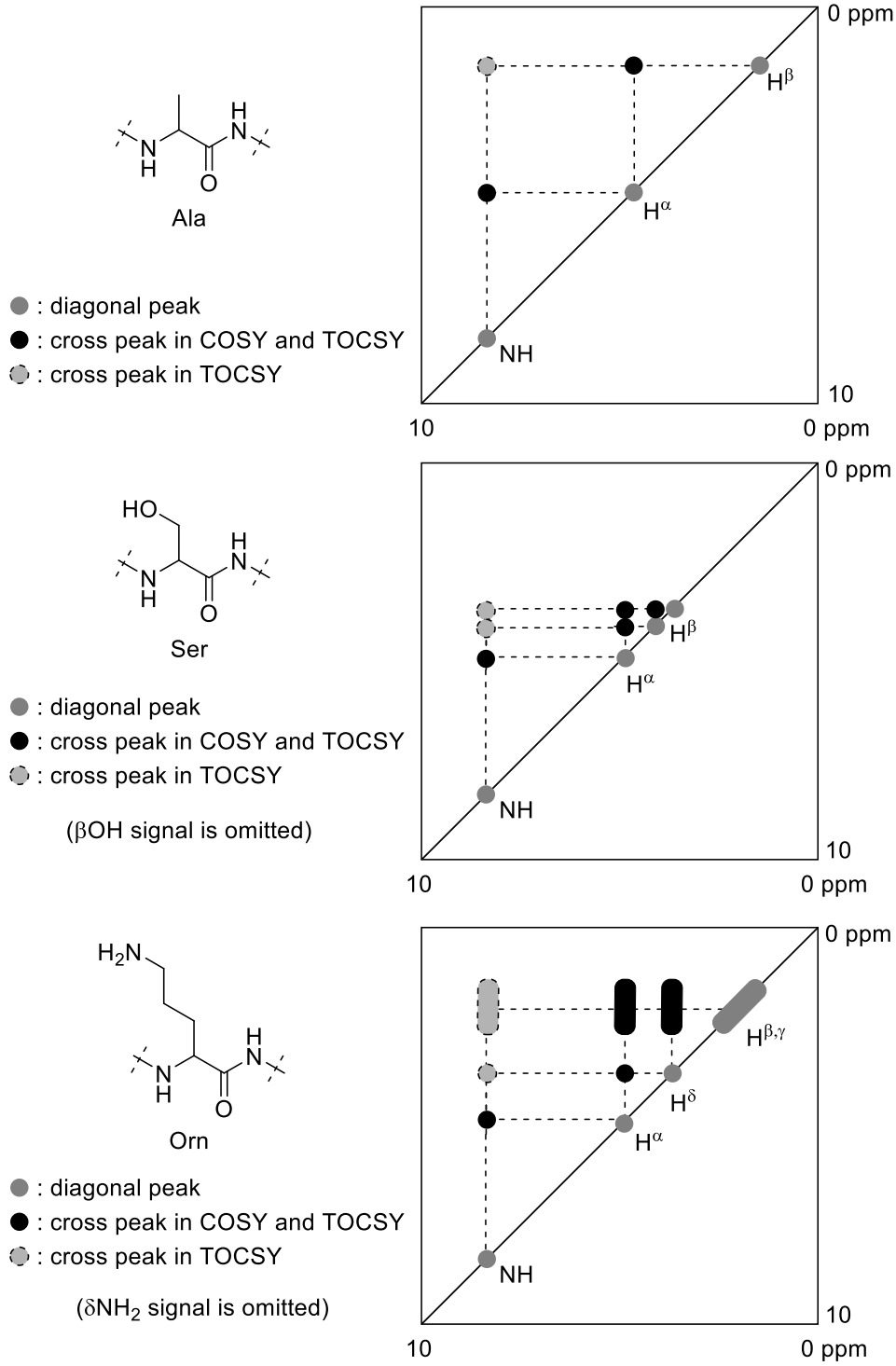


Figure 2. Schematic representations of the patterns of cross peaks in COSY and TOCSY spectra of alanine, serine, and ornithine in DMSO-d_6.

Determination of the amino-acid sequence (connectivity of each spin system) using NOESY/ROESY

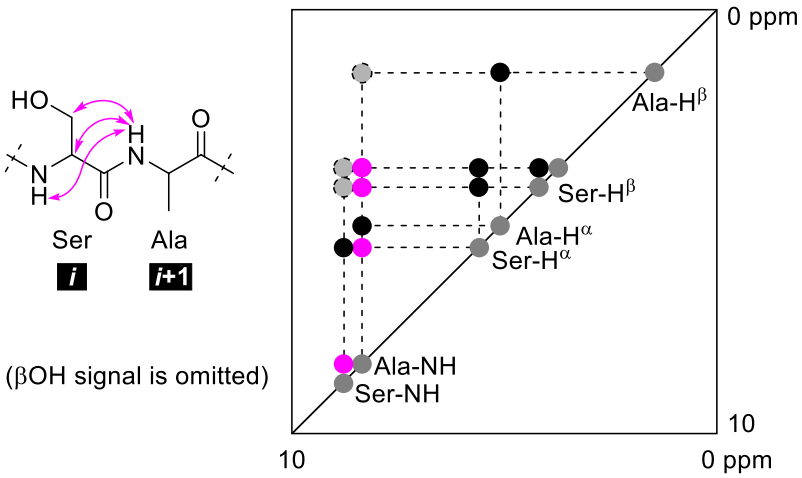
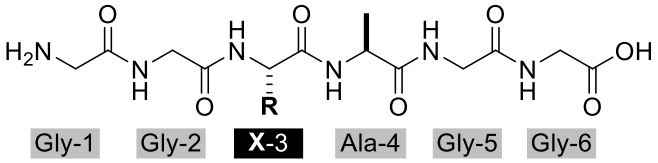


Figure 3. Schematic representations of the patterns of cross peaks in NOESY/ROESY spectra of Ala-Ser dipeptide unit in DMSO-d_6

Table 1. Random coil ^{1}H chemical shifts for the 20-proteinogenic amino acids[3]

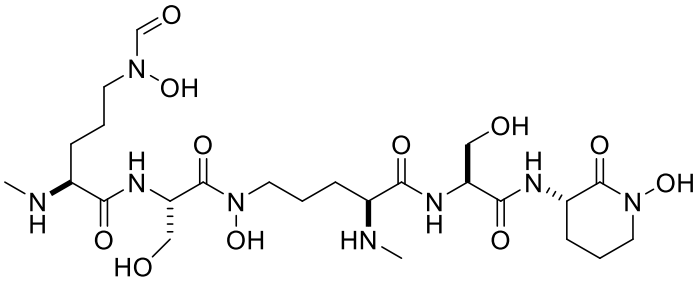


Gly-Gly-**X**-Ala-Gly-Gly in deutrated buffer (pH 5.1 50 mM phosphate, 1.0 M deutrated urea in D_2O)

residue **X**	NH	H^α	H^β	others
Ala	8.24	4.32	1.39	
Cys	8.32	4.55	2.93, 2.93	
Asp	8.34	4.64	2.72, 2.65	
Glu	8.42	4.35	2.06, 1.96	γCH_2 2.31
Phe	8.30	4.62	3.14, 3.04	Ar 7.28, 7.38, 7.32
Gly	8.33	3.96		
His	8.42	4.73	3.29, 3.16	Ar 8.58, 7.29
Ile	8.00	4.17	1.87	γCH_2 1.45, 1.16, γCH_3 0.91. δCH_3 0.86
Lys	8.29	4.32	1.84, 1.75	γCH_2 1.44, δCH_2 1.68, εCH_2 2.99, εNH_3^+, 7.81
Leu	8.16	4.34	1.62, 1.62	γCH 1.59, δCH_3 0.92, 0.87
Met	8.28	4.48	2.11, 2.01	γCH_2 2.60, 2.54, εCH_3 2.10
Asn	8.40	4.74	2.83, 2.75	γNH_2 7.59, 6.91
Pro	-	4.42	2.29, 1.94	γCH_2 2.02, δCH_2 3.63
Gln	8.32	4.34	2.12, 1.99	γCH_2 2.36, δNH_2 7.52, 6.85
Arg	8.23	4.34	1.86, 1.76	γCH_2 1.63, δCH_2 3.20, εNH 8.07
Ser	8.31	4.47	3.89, 3.87	
Thr	8.15	4.35	4.24	γCH_3 1.21
Val	8.03	4.12	2.08	γCH_3 0.94, 0.93
Trp	8.25	4.66	3.29, 3.27	Ar 7.27, 7.65, 7.18, 7.25, 7.50
Tyr	8.12	4.55	3.03, 2.98	Ar 7.14, 6.84

These basics of assignment can be applicable to the non-ribosomal and post-translationally modified peptides.

3. Turgichelin



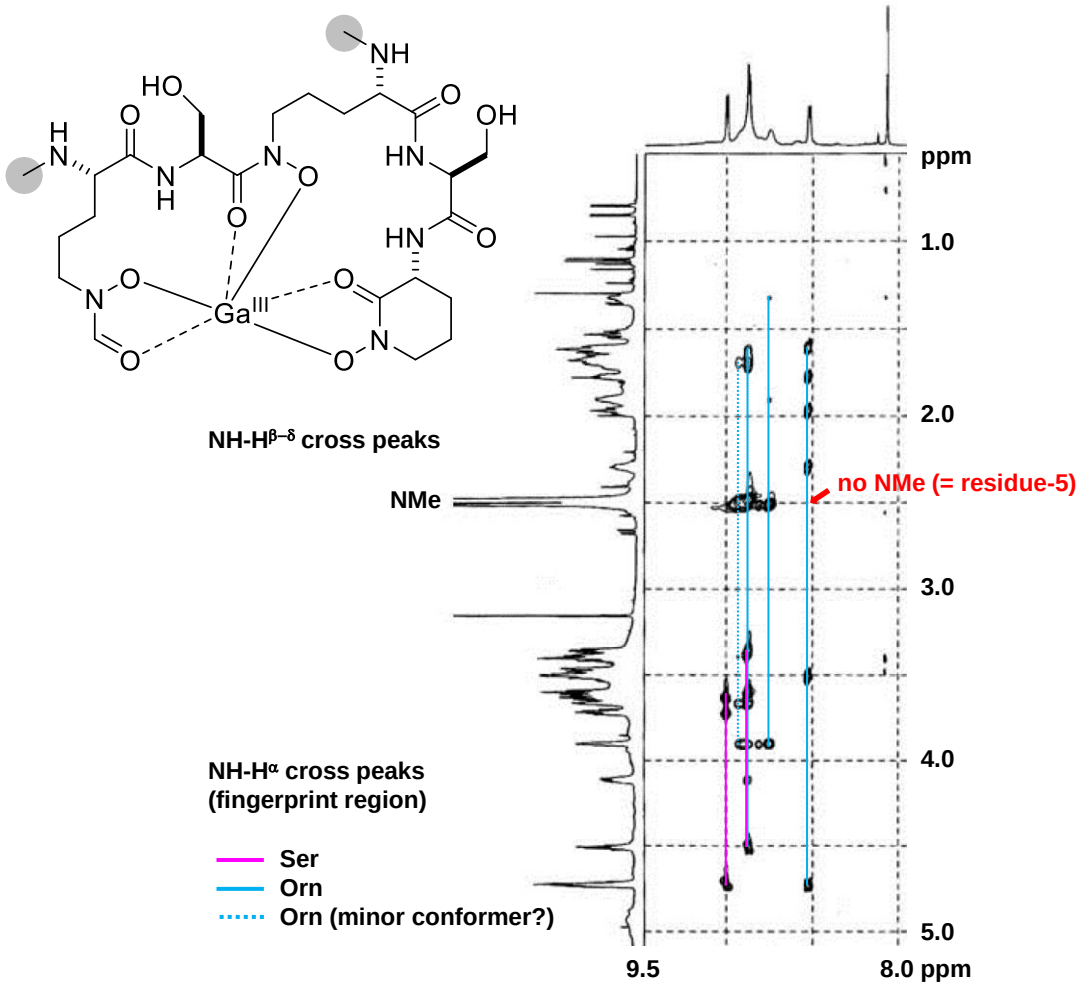
turgichelin (**1**)

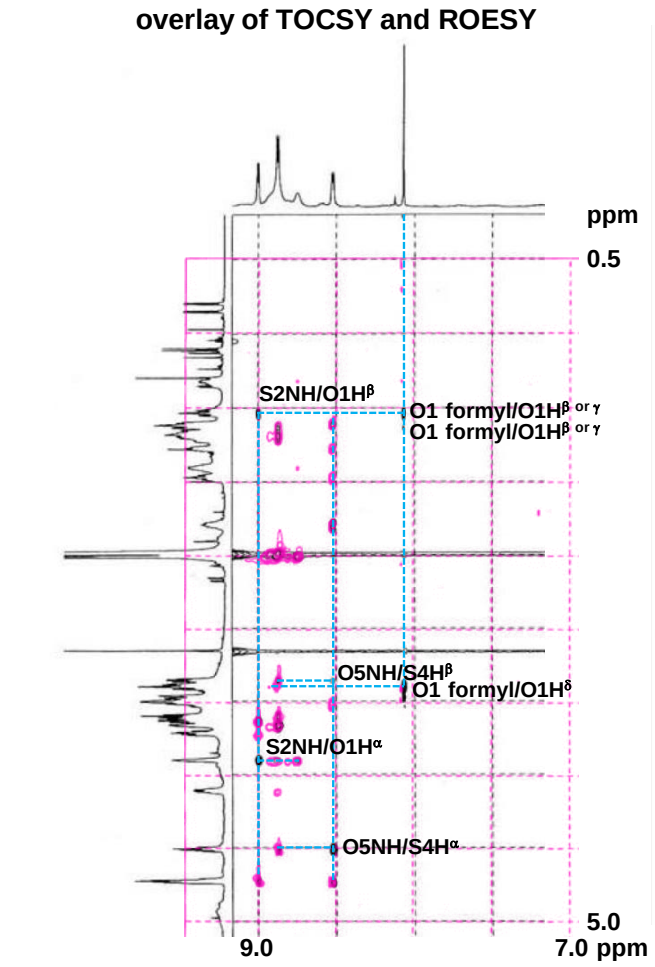
turgichelin: an oligomer of two Ser and three Orn derivatives
isolation: from *Streptomyces turgidiscabies* as a siderophore (natural product as an iron chelator produced by bacteria to uptake Fe ions for their growth)[4]
For structure determination and assignment of siderophore, gallium (III) is utilized (substitution of paramagnetic Fe(III) with diamagnetic Ga(III) for the NMR experiments).

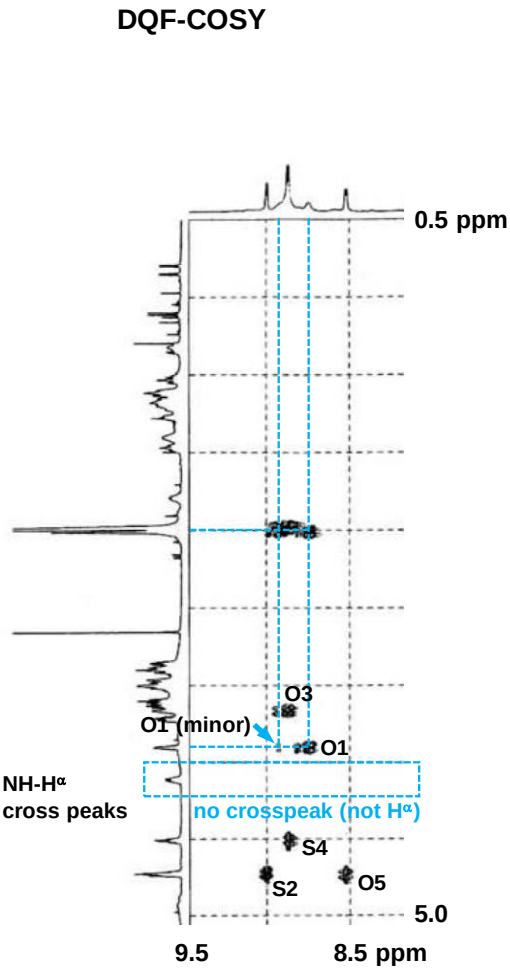
Step 1. classification of spin system using TOCSY spectrum

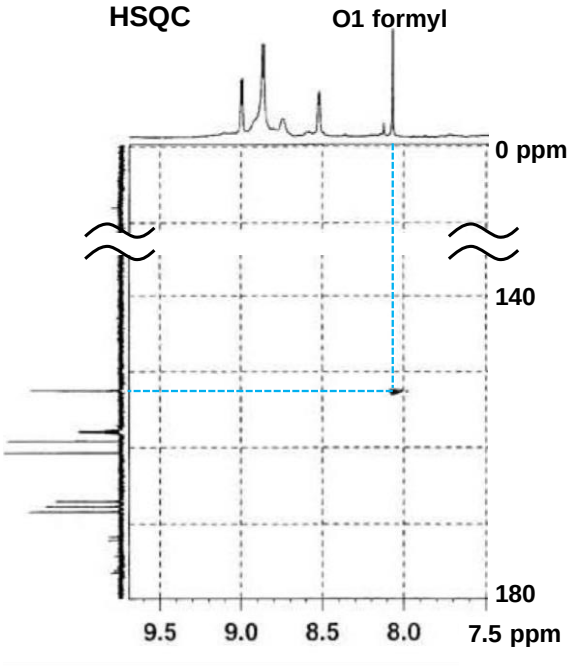
Step 2. differentiation of three Orn derivatives

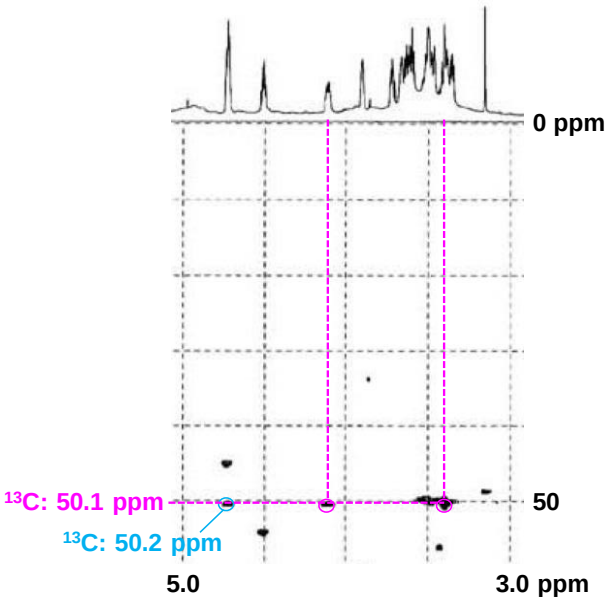
Step 3. determination of the amino-acid sequence




overlay of TOCSY and ROESY
ppm
0.5
S2NH/O1Hβ
O1 formyl/O1Hβ or γ
O1 formyl/O1Hβ or γ
O5NH/S4Hβ
O1 formyl/O1Hδ
S2NH/O1Hα
O5NH/S4Hα
5.0
9.0
7.0 ppm


DQF-COSY
0.5 ppm
O3
O1 (minor)
O1
NH-Hα
cross peaks
no crosspeak (not Hα)
S4
S2
O5
5.0
9.5
8.5 ppm


HSQC
O1 formyl
0 ppm
140
180
9.5
9.0
8.5
8.0
7.5 ppm


0 ppm
13C: 50.1 ppm
13C: 50.2 ppm
50
5.0
3.0 ppm

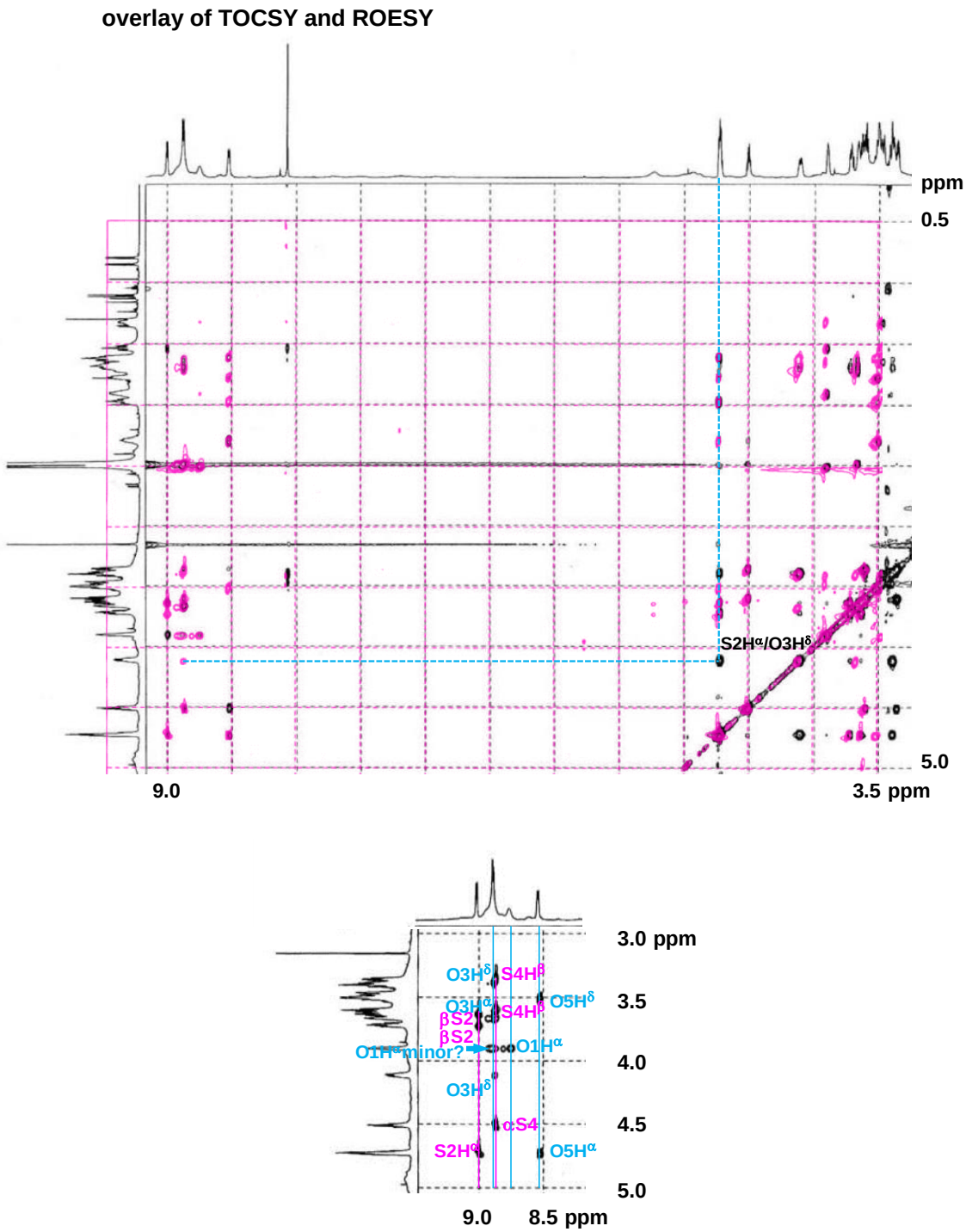


Figure 4. Assignment of TOCSY cross peaks of **1**.

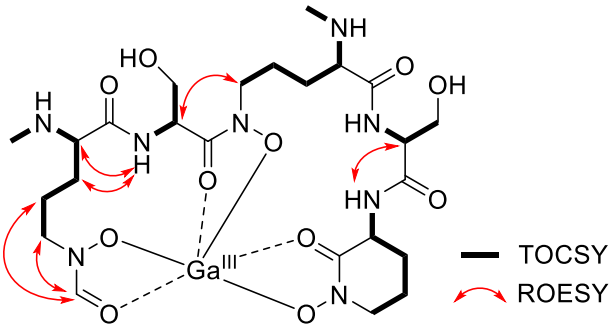


Figure 5. TOCSY correlations and key ROESY correlations of **1**.

Table 2. ^{1}H NMR chemical shifts of **1** (2.1 mM) in DMSO-d_6 at 30 °C (800 MHz)

residue	position	δ ppm
N-Me hfOrn-1	N*Me*	2.50
	N*H*	8.77
	H^{α}	3.90
	H^{β}	1.90
		1.53
	H^{γ}	1.63
		1.31
	H^{δ}	3.47
		3.40
	formyl	8.07
Ser-2	N*H*	8.99
	H^{α}	4.72
	H^{β}	3.71
		3.60
N-Me hOrn-3	N*Me*	2.47
	N*H*	8.92
	H^{α}	3.63
	H^{β}	1.71
		1.65
	H^{γ}	1.68
		1.59
	H^{δ}	4.10
		3.39

residue	position	δ ppm
Ser-4	N*H*	8.87
	H^{α}	4.50
	H^{β}	3.36
		3.59
cyclic hOrn-5	N*H*	8.52
	H^{α}	4.73
	H^{β}	1.96
		1.60
	H^{γ}	2.28
		1.77
	H^{δ}	3.50

References

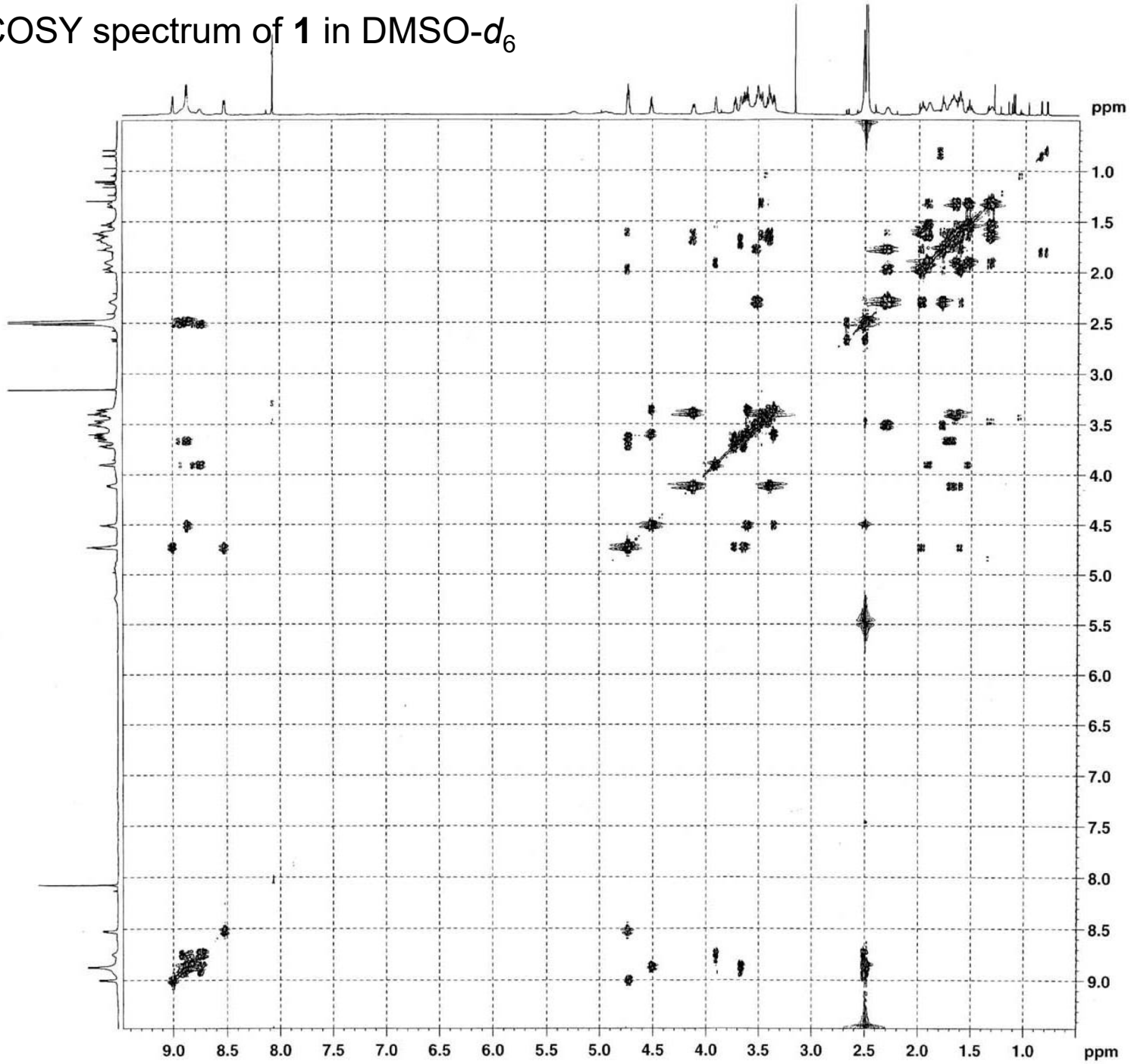
(1) Bax, A. *Annu. Rev. Biochem.* **1989**, *58*, 223-256.

(2) Jones, C.; Mulloy, B.; Thomas, A. H. Eds. Spectroscopic methods and analyses. *Methods in Molecular Biology* (1993).

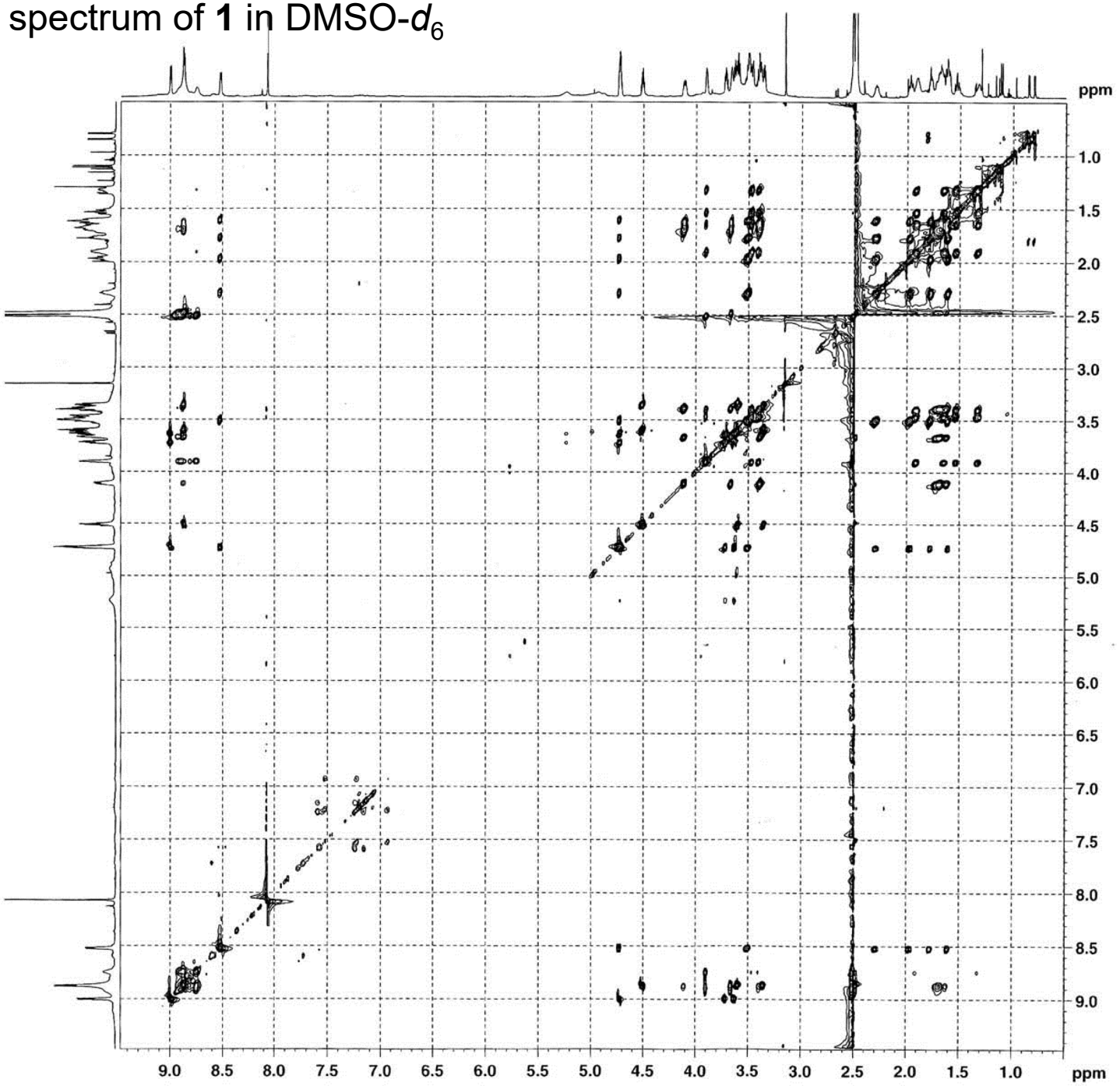
(3) Wishart, D. S. Bigam, C. G.; Holm, A.; Hodges, R. S.; Sykes, B. D. *J. Biomol. NMR* **1995**, *5*, 67-81.

(4) Kodani, S.; Bicz, J.; Song, L.; Deeth, R. J.; Ohnishi-Kameyama, M.; Yoshida, M.; Ochi, K.; Challis, G. L. *Org. Biomol. Chem.* **2013**, *11*, 4686-4694.

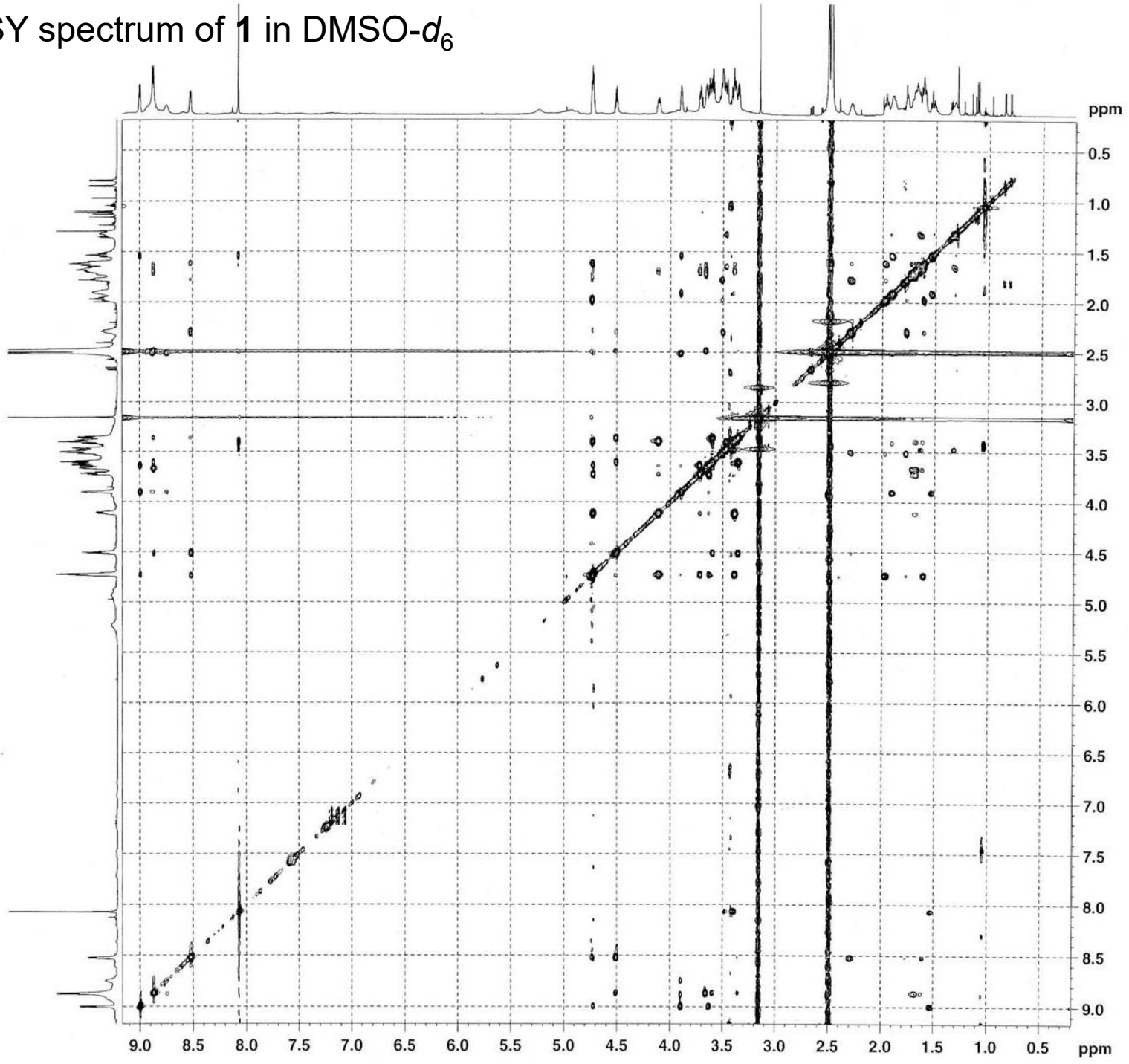
^{1}H-^{1}H DQF-COSY spectrum of **1** in DMSO-d_6



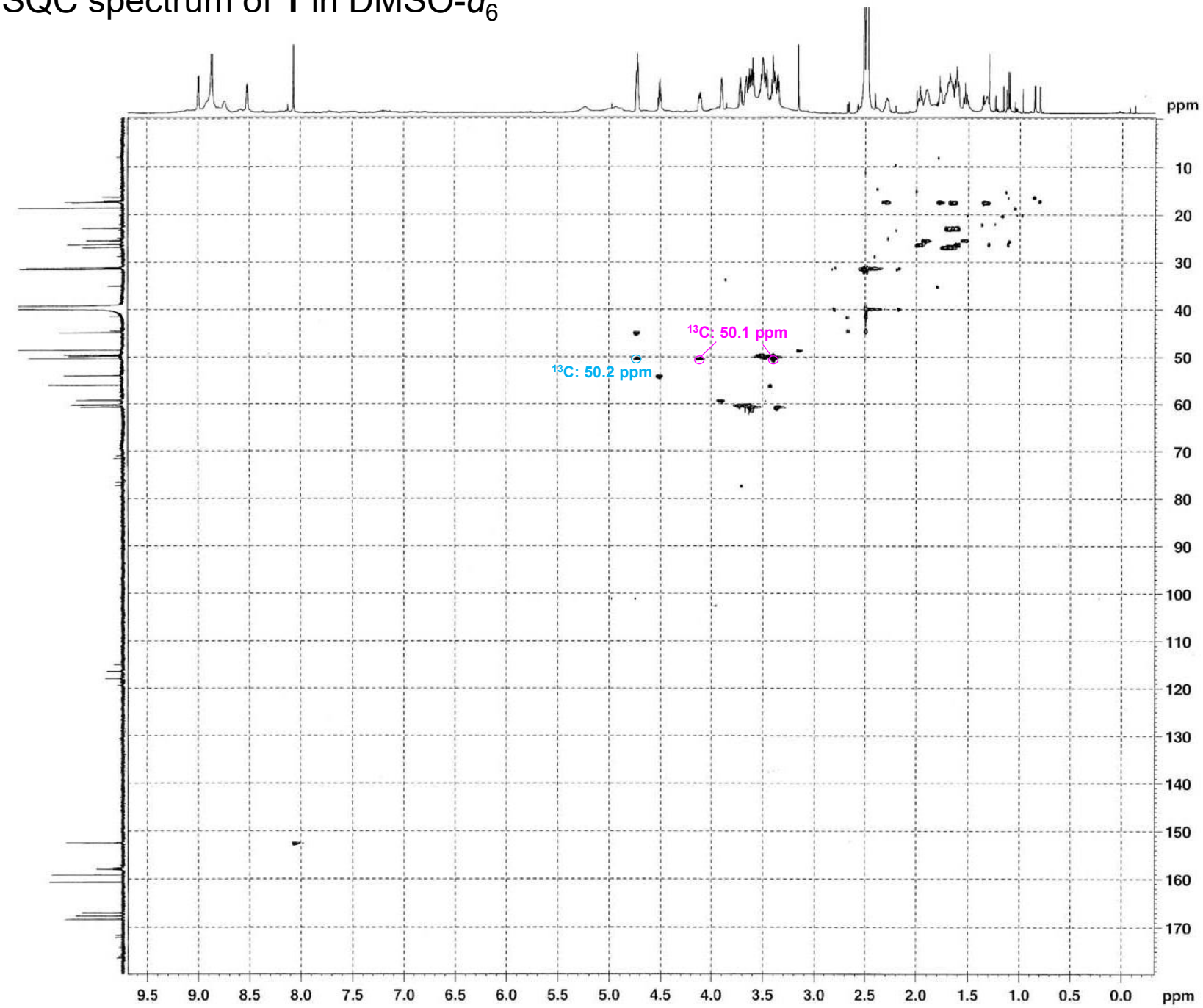
^{1}H-^{1}H TOCSY spectrum of **1** in DMSO-d_6



^{1}H-^{1}H ROESY spectrum of **1** in DMSO-d_6



^{1}H-^{13}C HSQC spectrum of **1** in DMSO-d_6



an overlay of the ^{1}H-^{1}H TOCSY spectrum (purple) and the ^{1}H-^{1}H ROESY spectrum (black)