Structure and Relative Potency of Several Karlotoxins from *Karlodinium veneficum*— Supporting Information

Ryan M. Van Wagoner,[a] Jonathan R. Deeds,[b] Avery O Tatters,[a] Allen R. Place[c] Carmelo R. Tomas,[a] and Jeffrey L.C. Wright[a,]*

[a]Center for Marine Science
University of North Carolina at Wilmington
5600 Marvin K Moss Lane, Wilmington, NC 28409, USA
[b]Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration, College Park, MD 20740, USA
[c]Institute for Marine and Environmental Technology
University of Maryland Center of Environmental Sciences
701 East Pratt Street, Suite 236, Baltimore, MD 21202, USA

[*]To whom correspondence should be addressed. Tel: 910-962-2397, E-mail: wrightj@uncw.edu

NMR spectra of KmTx 3 (3)

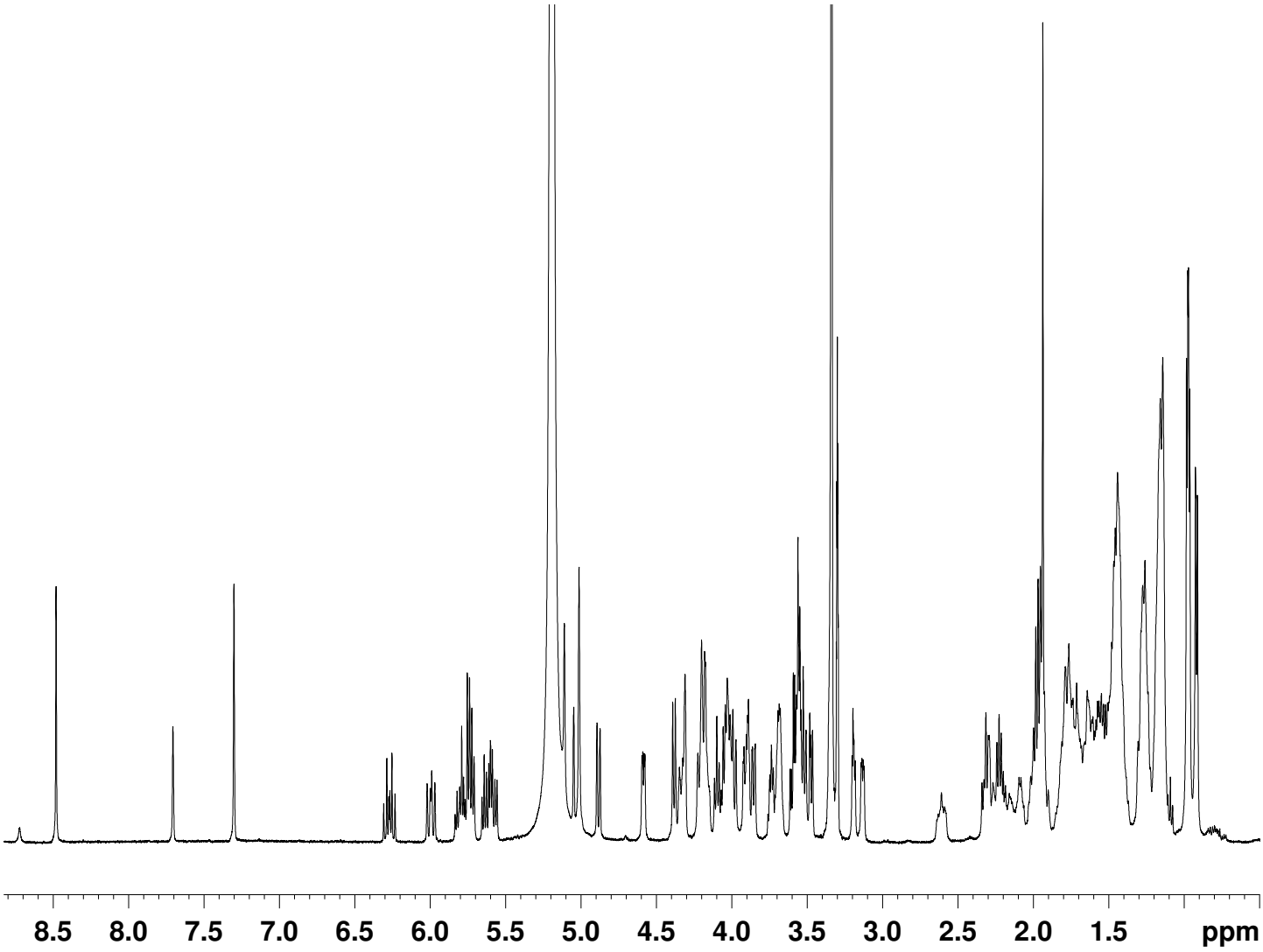


Figure S1. ^{1}H NMR spectrum of KmTx 3 (**3**).

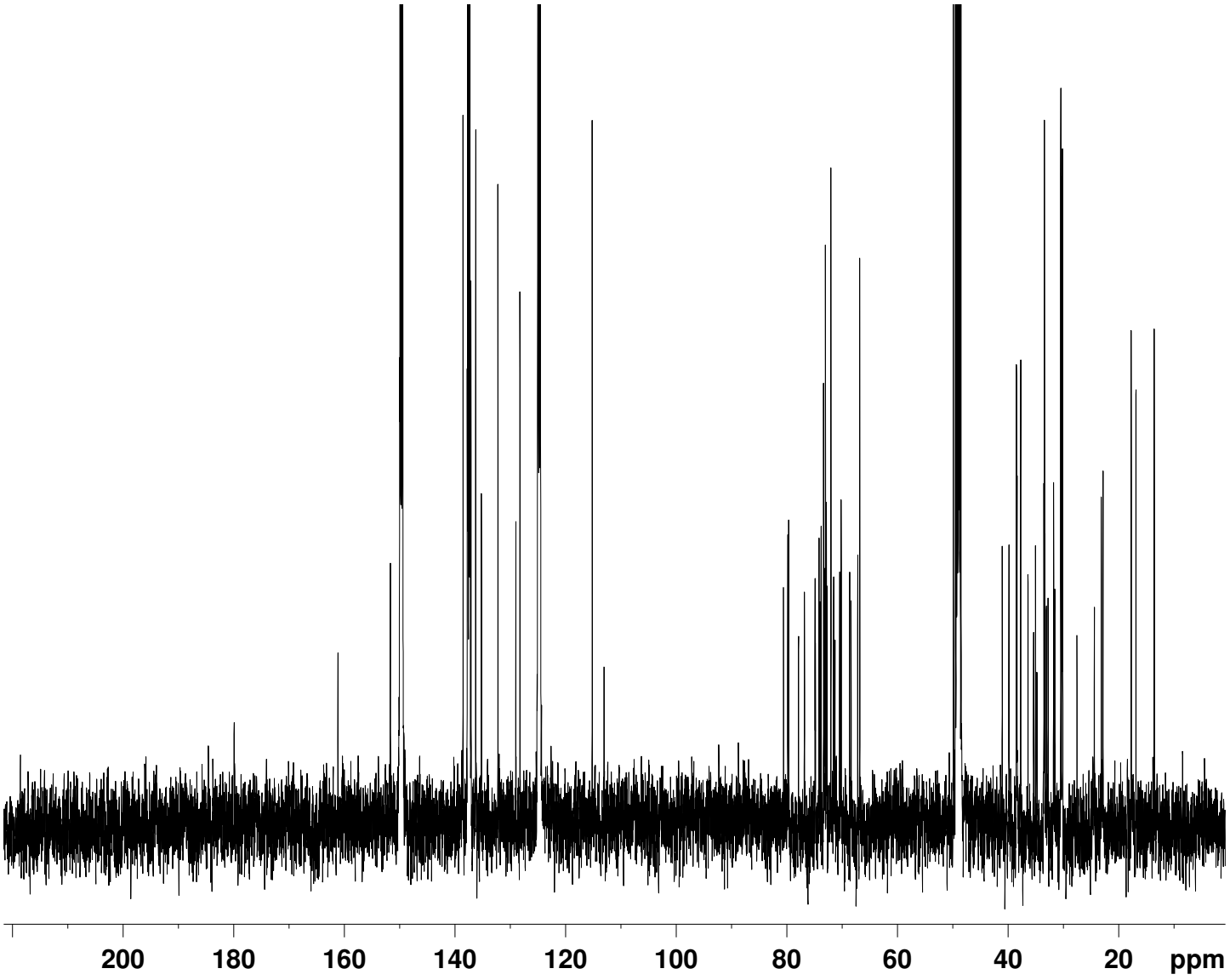


Figure S2. ^{13}C NMR spectrum of KmTx 3 (**3**).

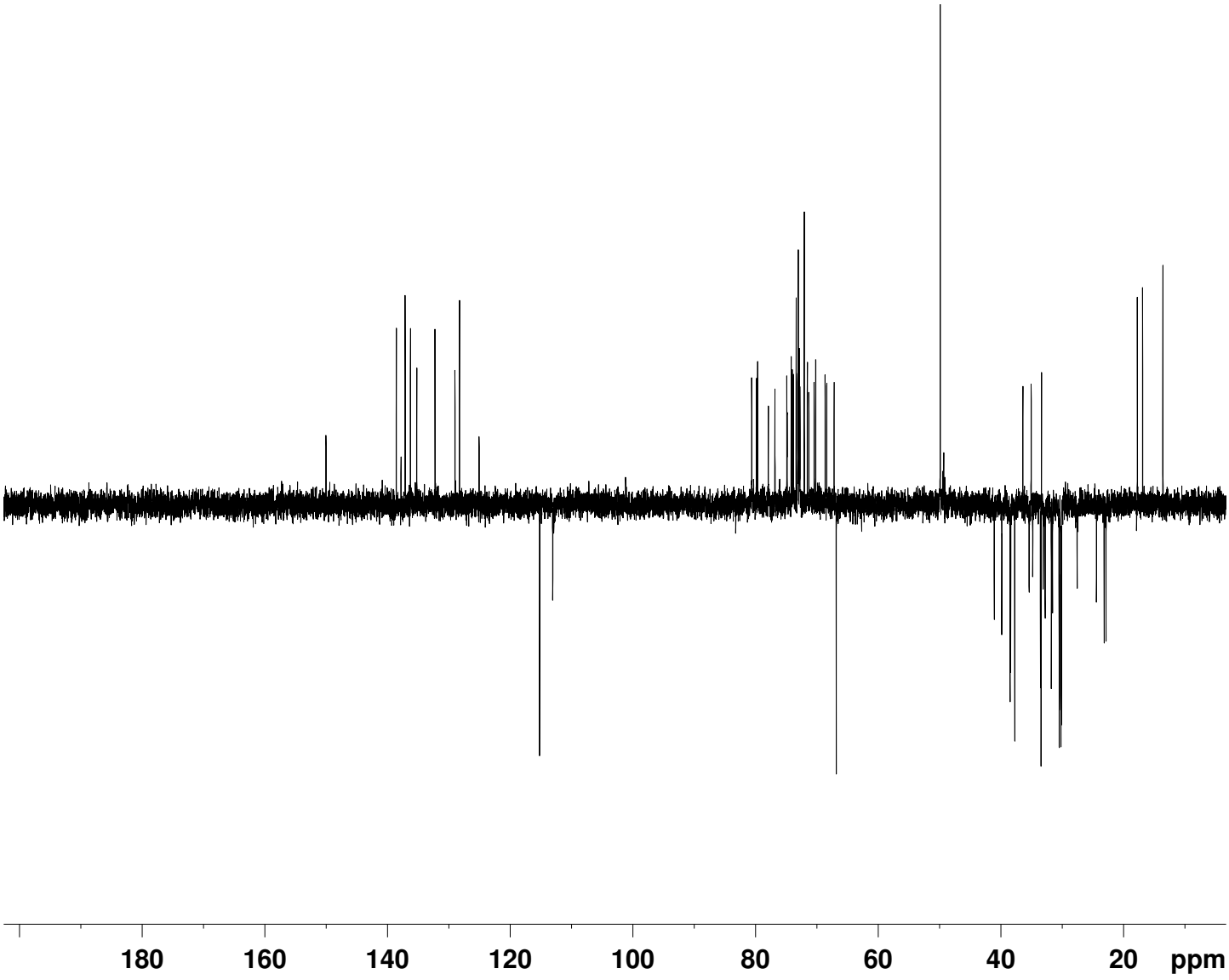


Figure S3. DEPT spectrum of KmTx 3 (**3**).

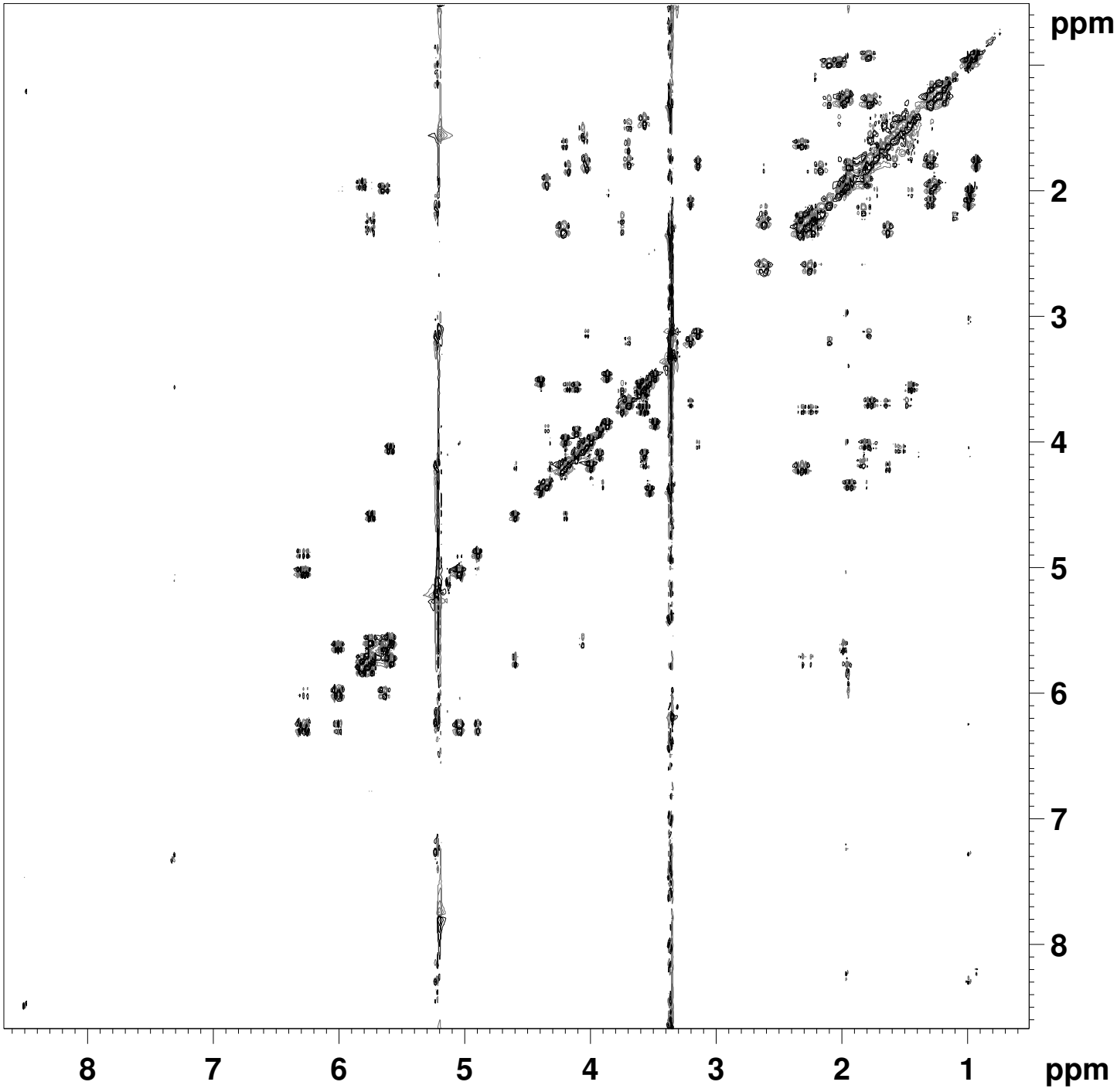


Figure S4. COSY spectrum of KmTx 3 (**3**).

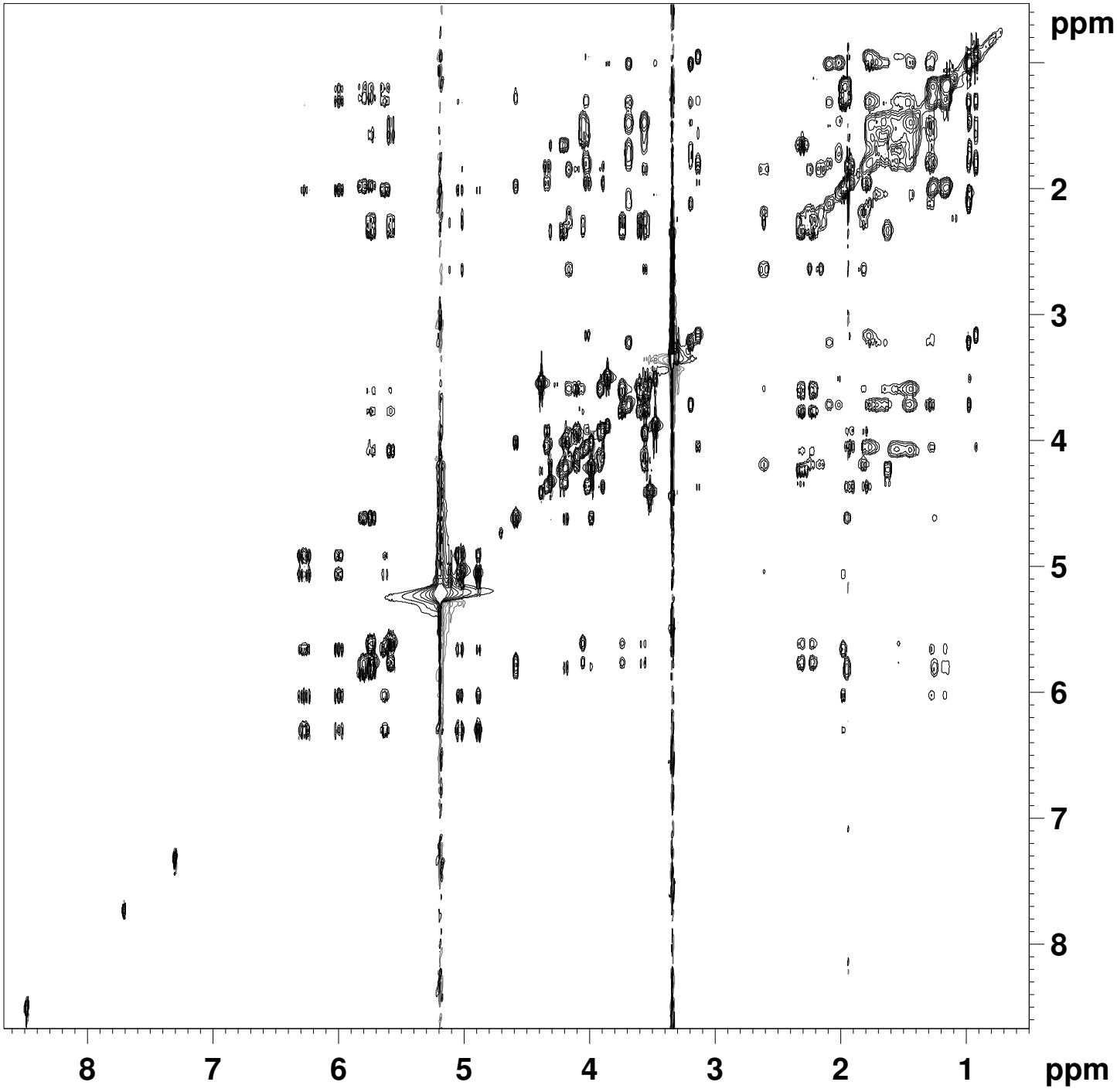


Figure S5. TOCSY spectrum of KmTx 3 (**3**).

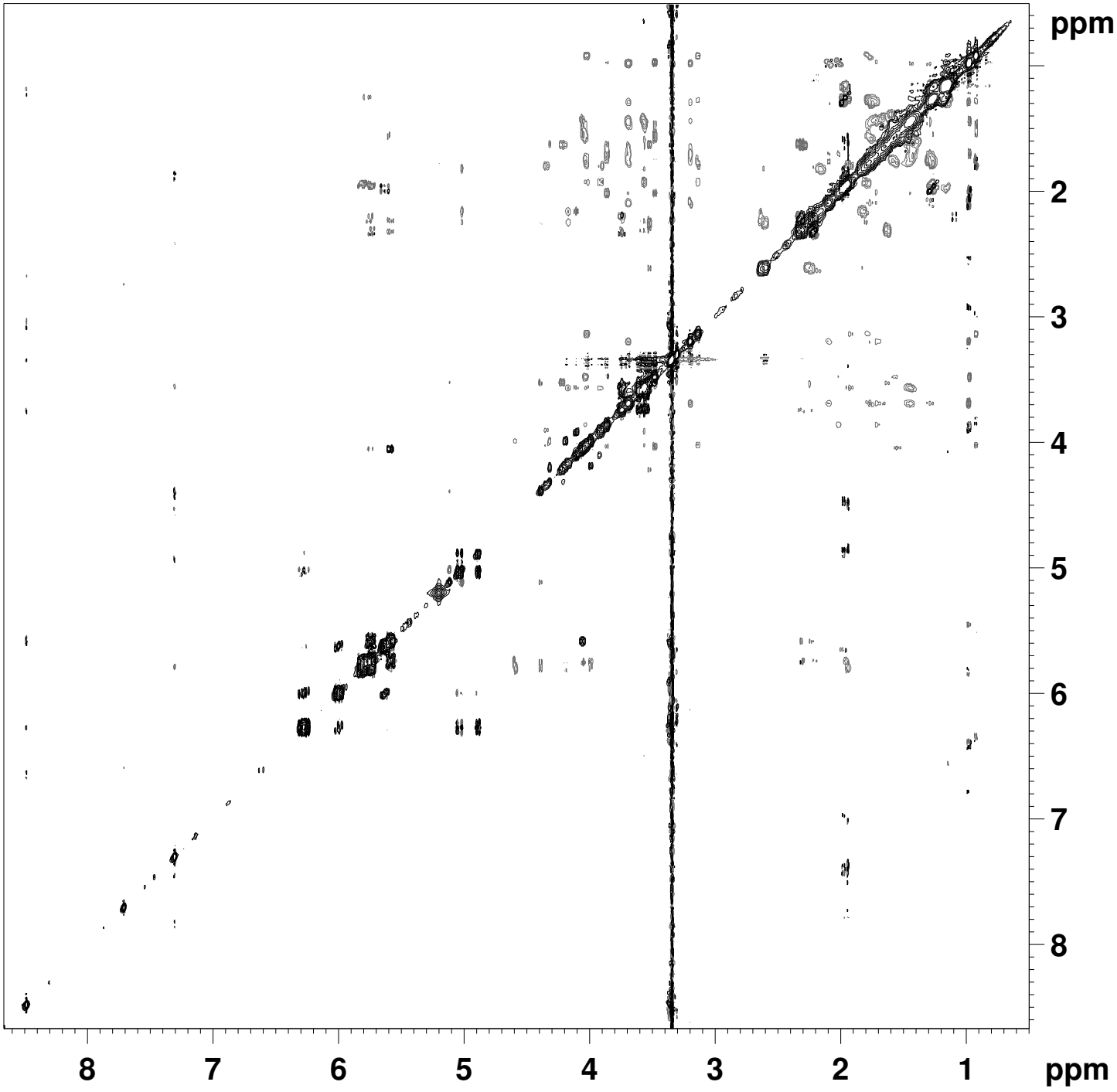


Figure S6. ROESY spectrum of KmTx 3 (**3**).

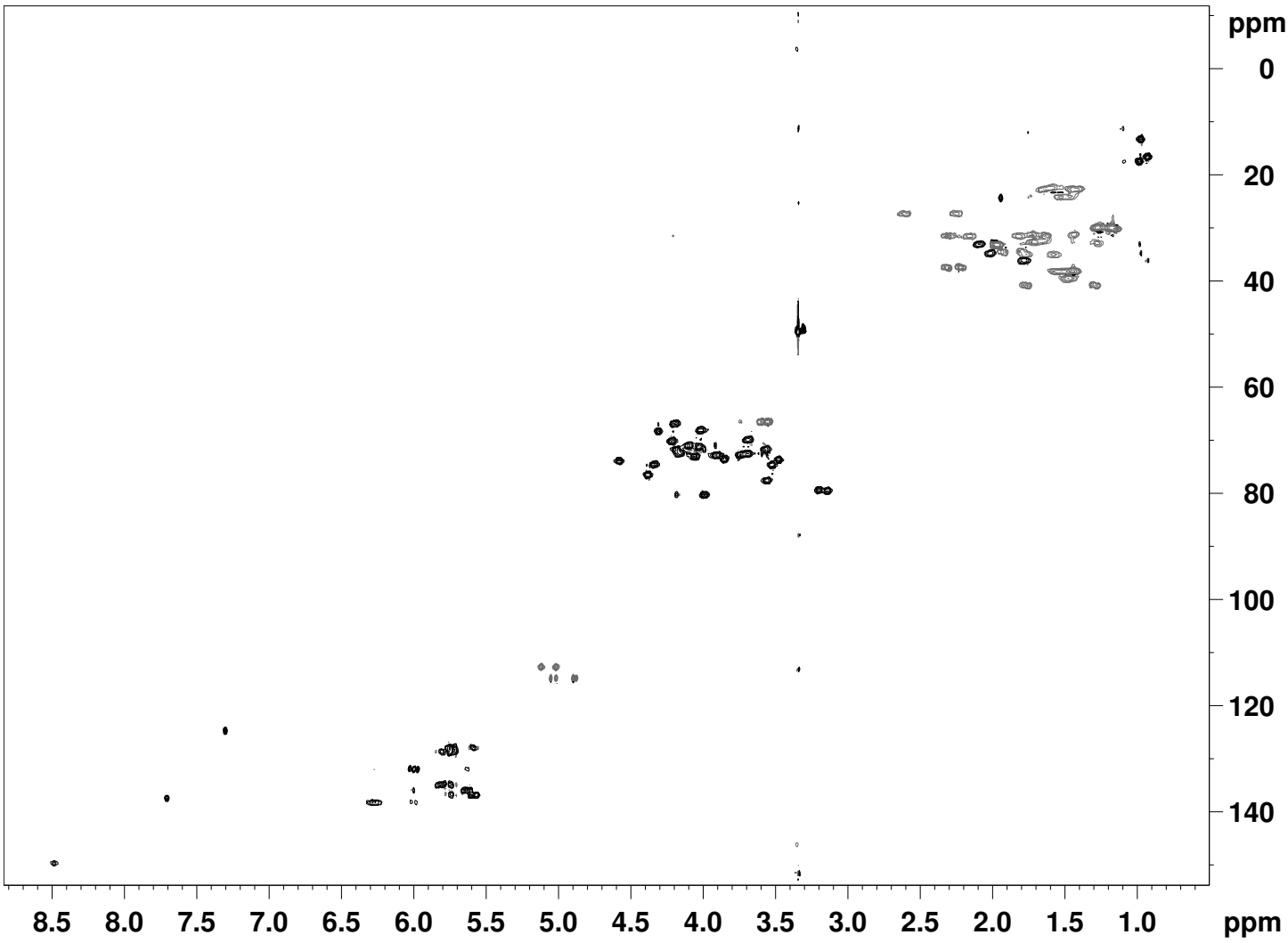


Figure S7. HSQC spectrum of KmTx 3 (**3**).

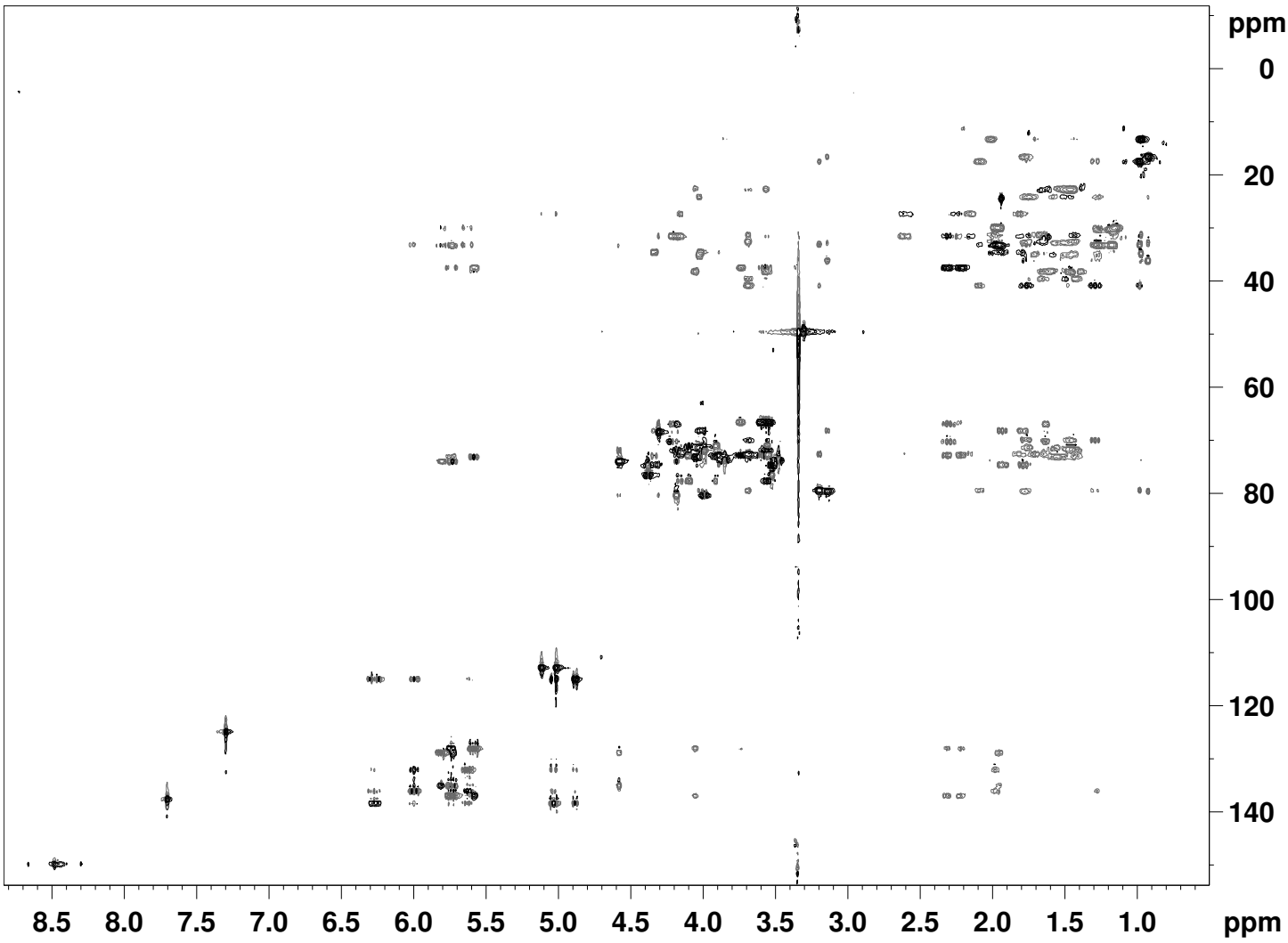


Figure S8. HSQC-TOCSY spectrum of KmTx 3 (**3**).

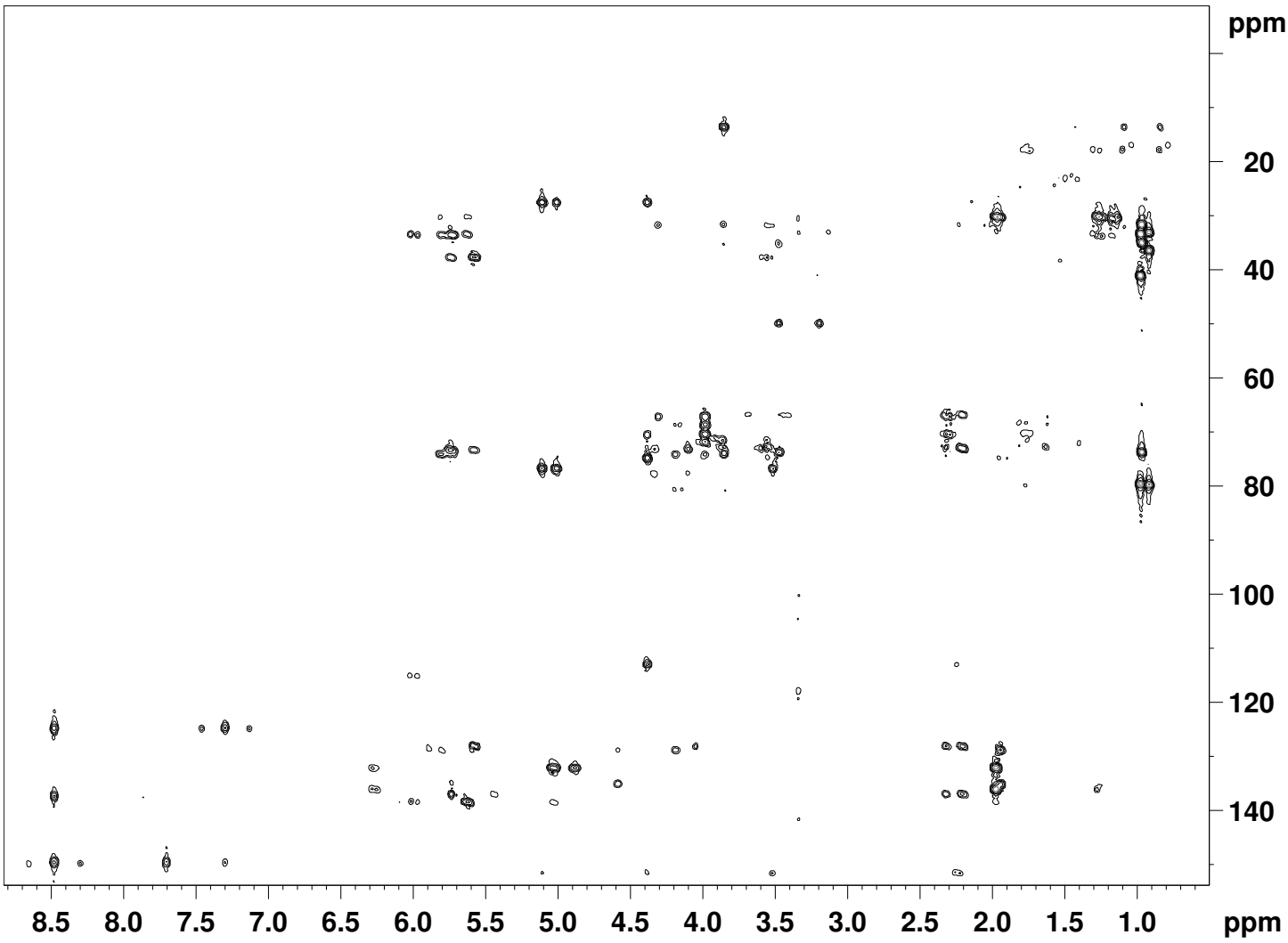


Figure S9. HMBC spectrum of KmTx 3 (**3**).

NMR spectra of 64-*E*-chloro-KmTx 3 (4)

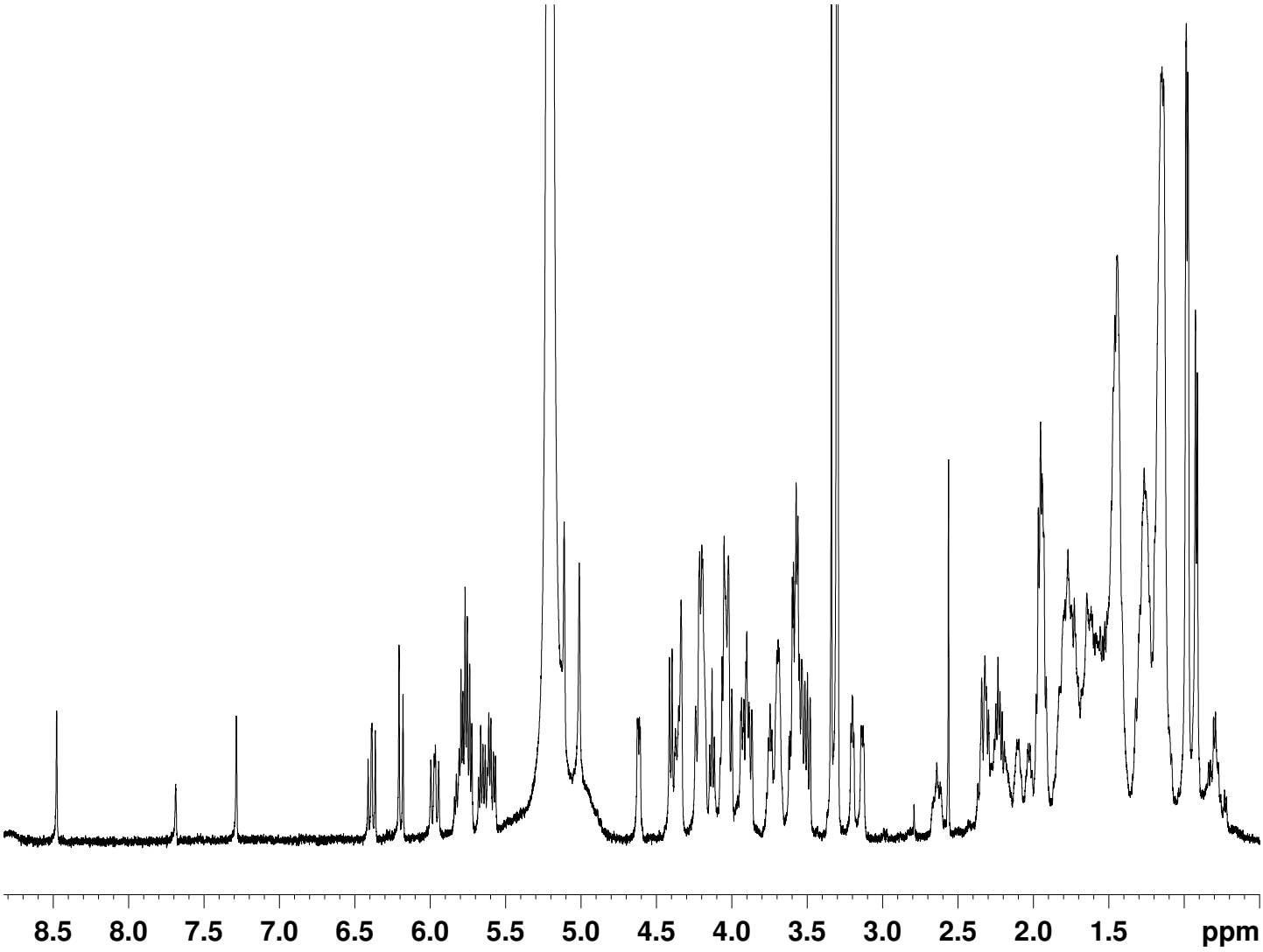


Figure S10. ^{1}H NMR spectrum of 64-*E*-chloro-KmTx 3 (**4**).

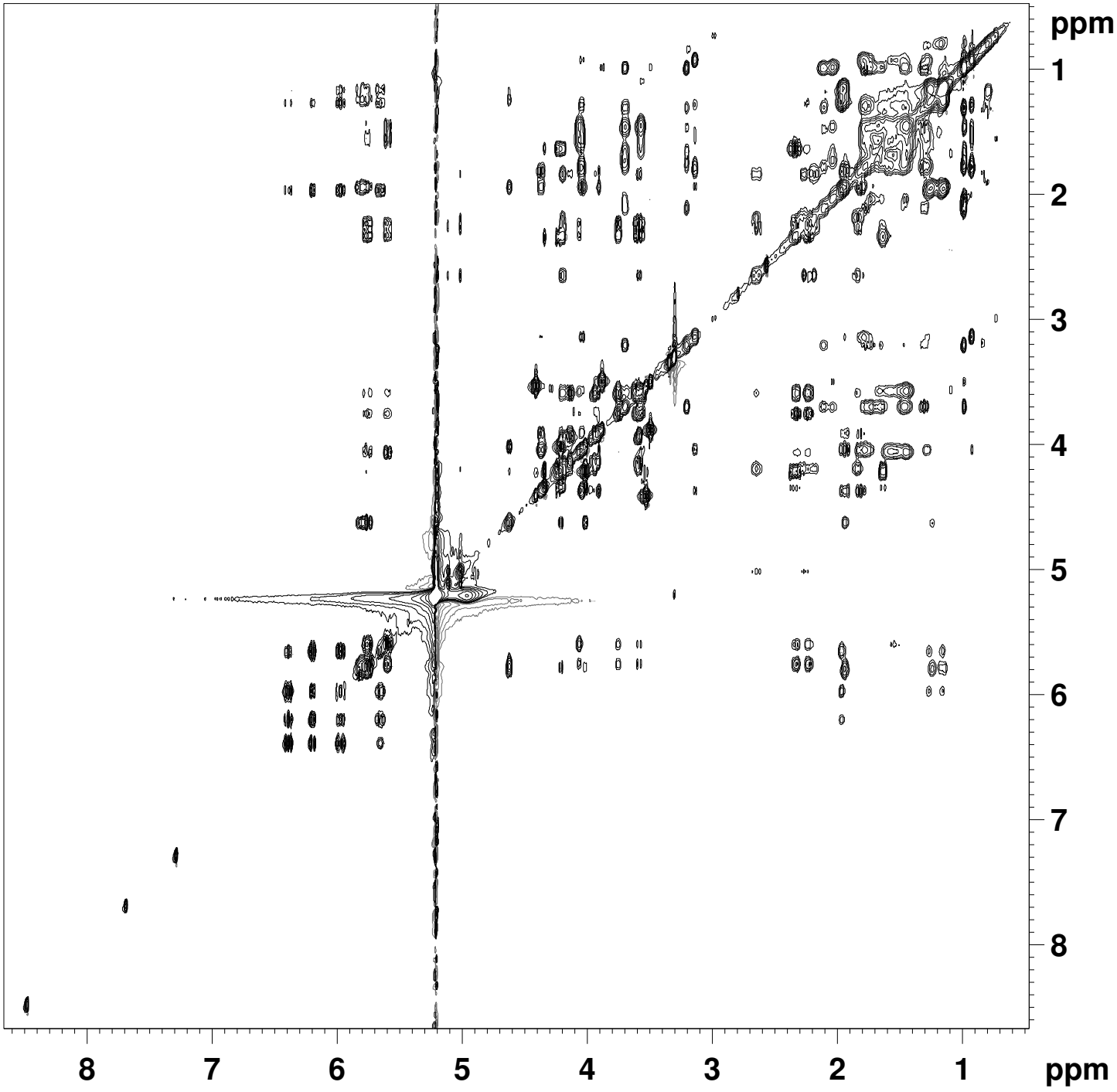


Figure S11. TOCSY spectrum of 64-*E*-chloro-KmTx 3 (**4**).

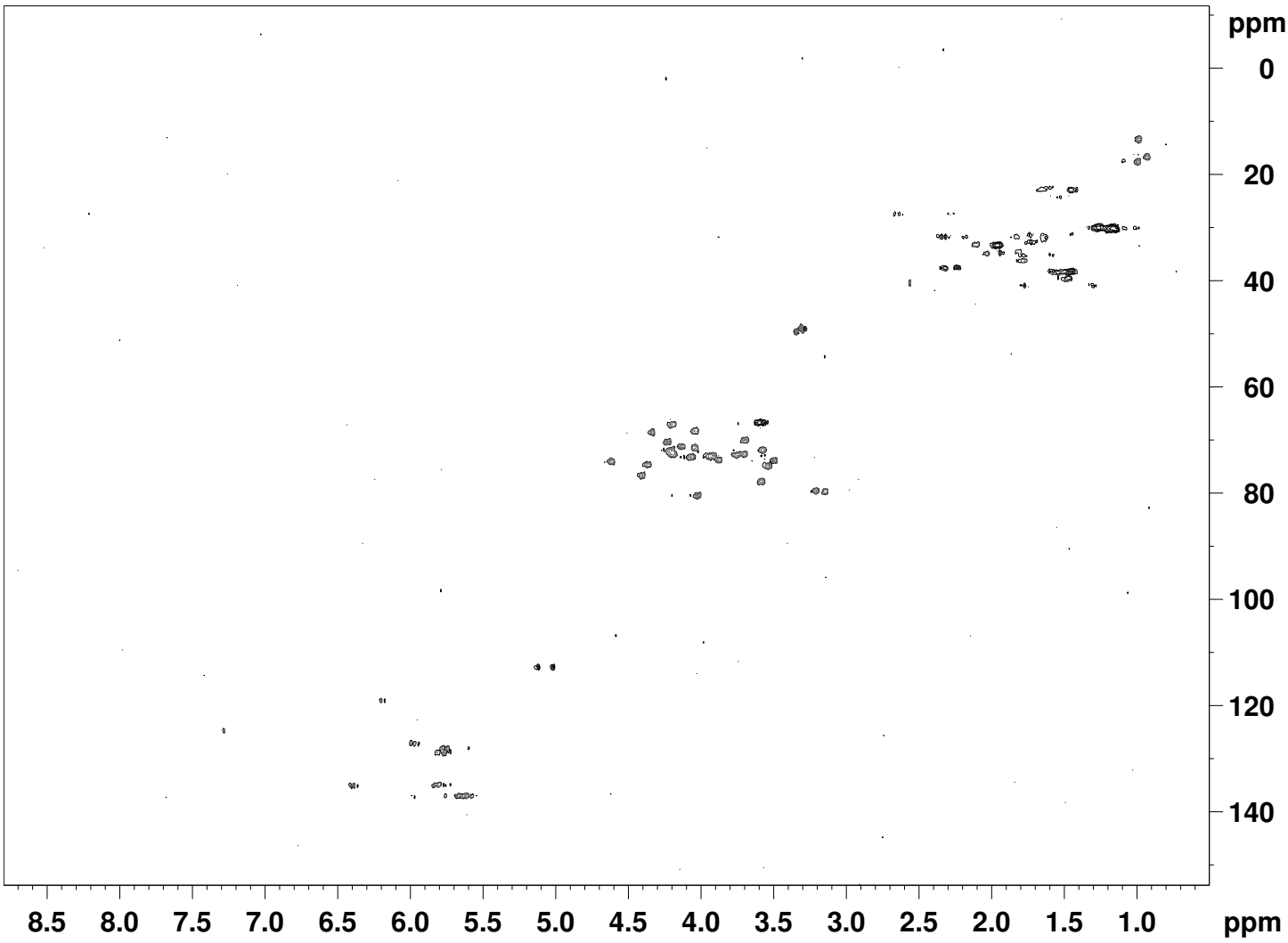


Figure S12. HSQC spectrum of 64-*E*-chloro-KmTx 3 (**4**).

NMR spectra of 10-*O*-sulfo-KmTx 3 (**5**)

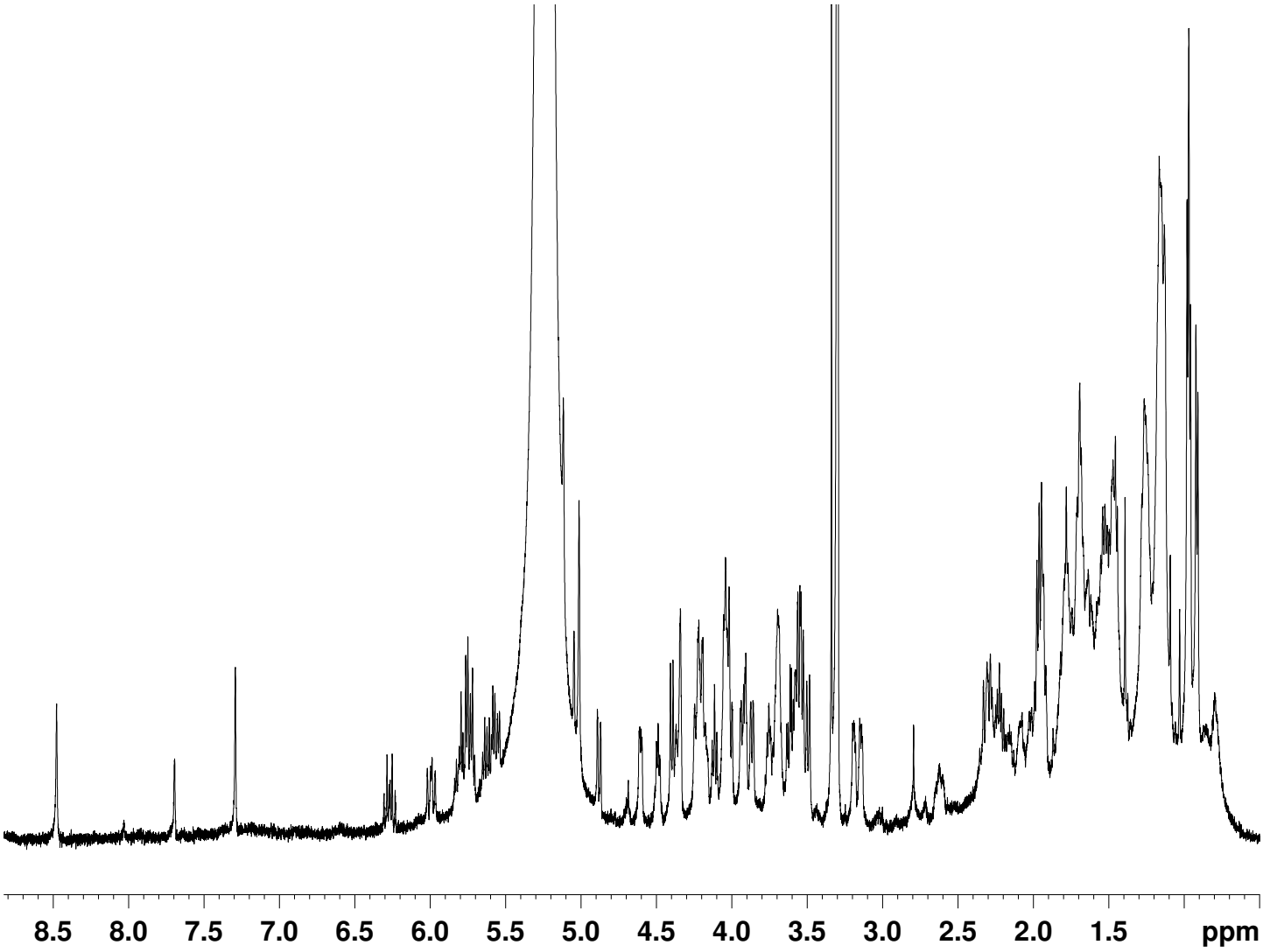


Figure S13. ^{1}H NMR spectrum of 10-*O*-sulfo-KmTx 3 (**5**).

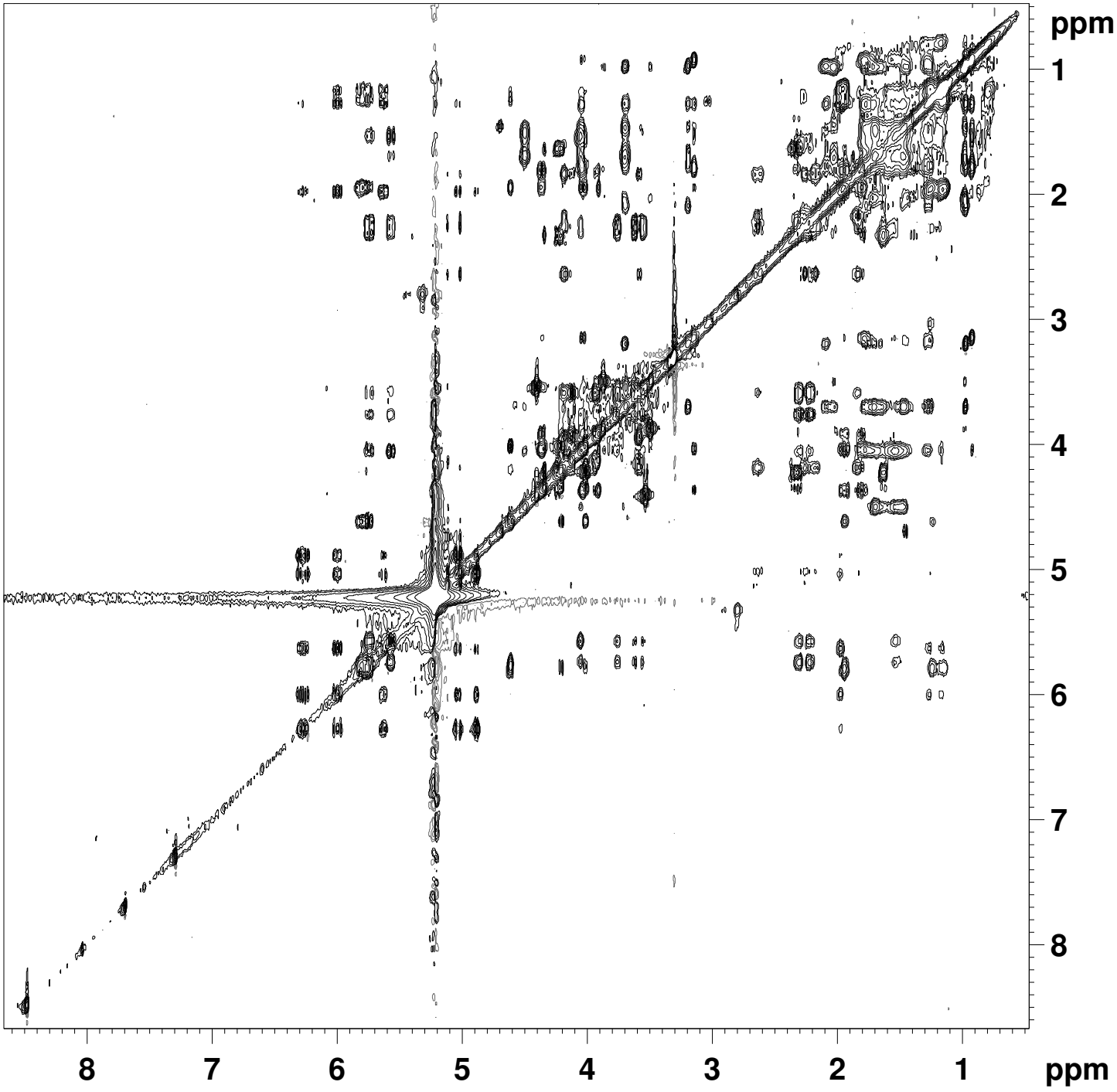


Figure S14. TOCSY spectrum of 10-*O*-sulfo-KmTx 3 (**5**).

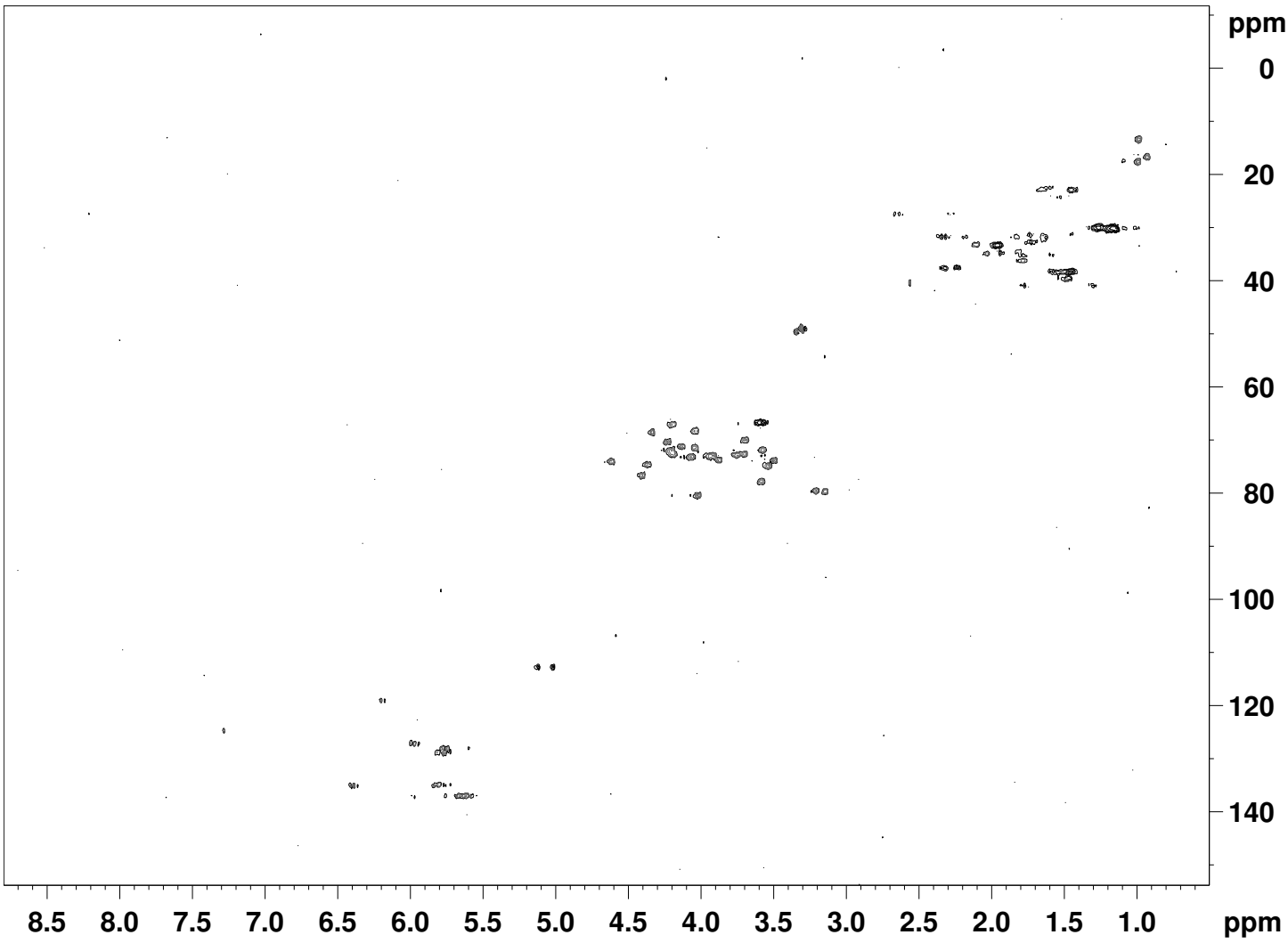


Figure S15. HSQC spectrum of 10-*O*-sulfo-KmTx 3 (**5**).

NMR spectra of 65-*E*-chloro-KmTx 1 (6)

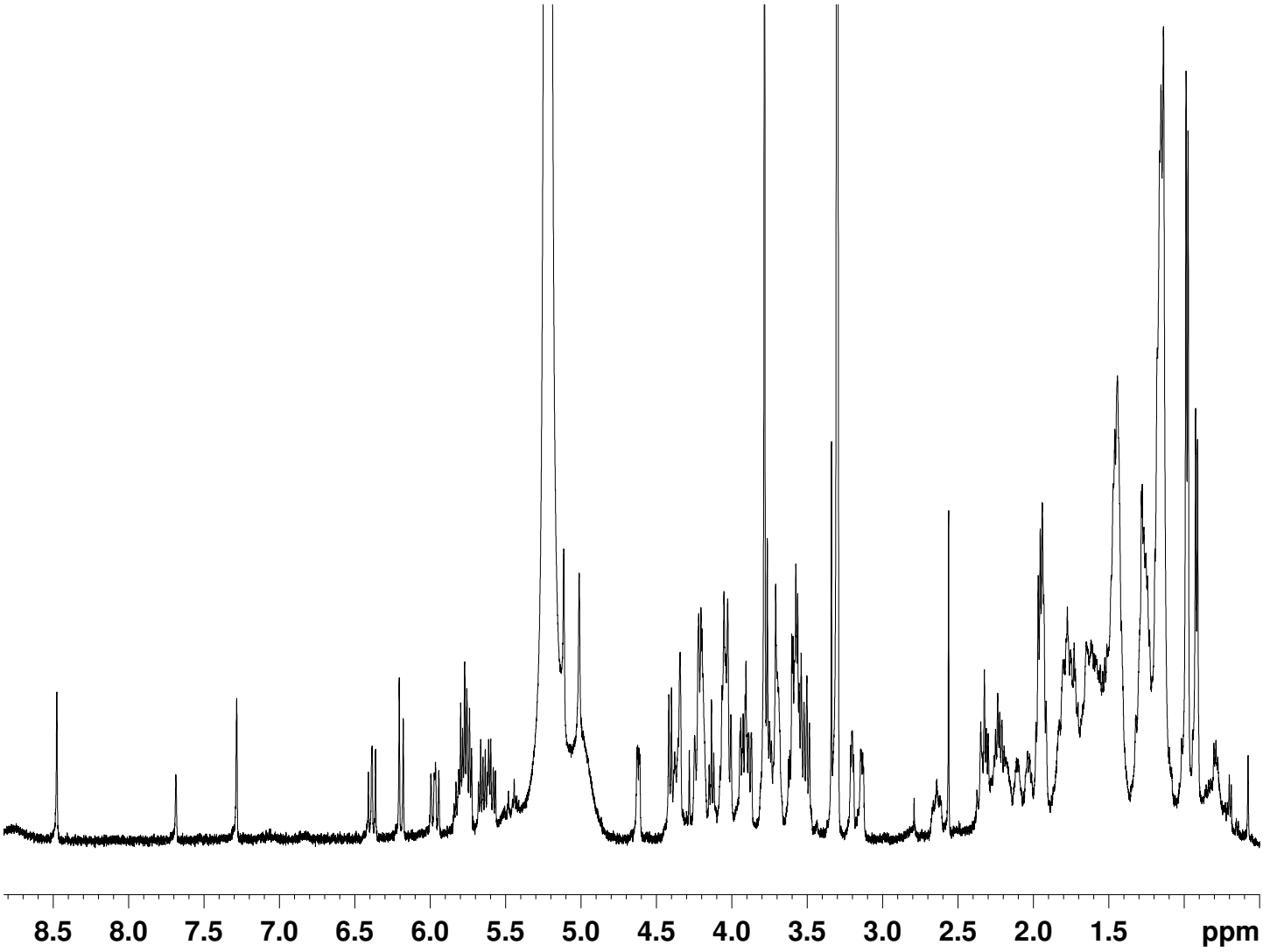


Figure S16. ^{1}H NMR spectrum of 65-*E*-chloro-KmTx 1 (**6**).

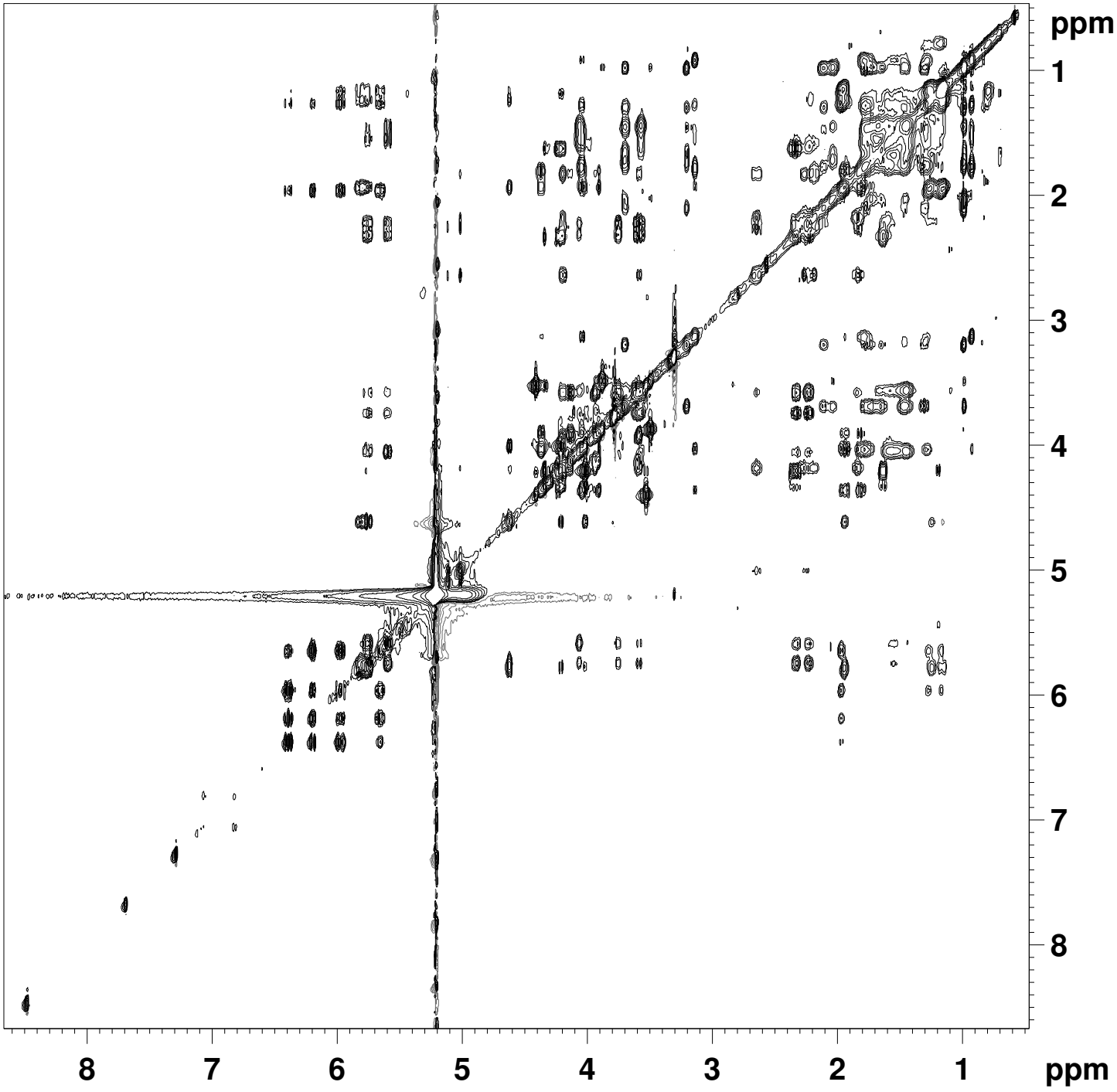


Figure S17. TOCSY spectrum of 65-*E*-chloro-KmTx 1 (**6**).

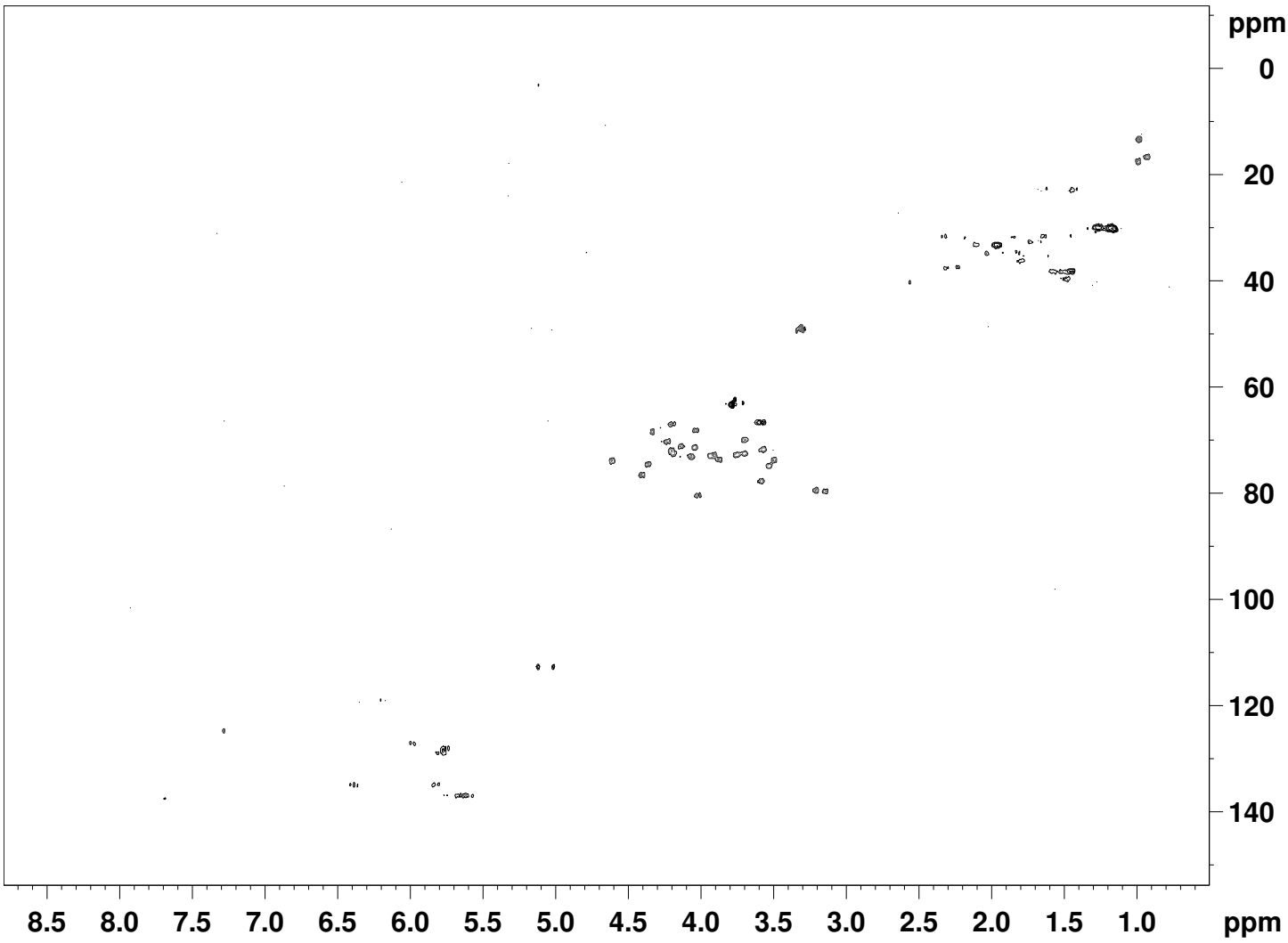


Figure S18. HSQC spectrum of 65-*E*-chloro-KmTx 1 (**6**).

NMR spectra of 10-*O*-sulfo-KmTx 1 (7)

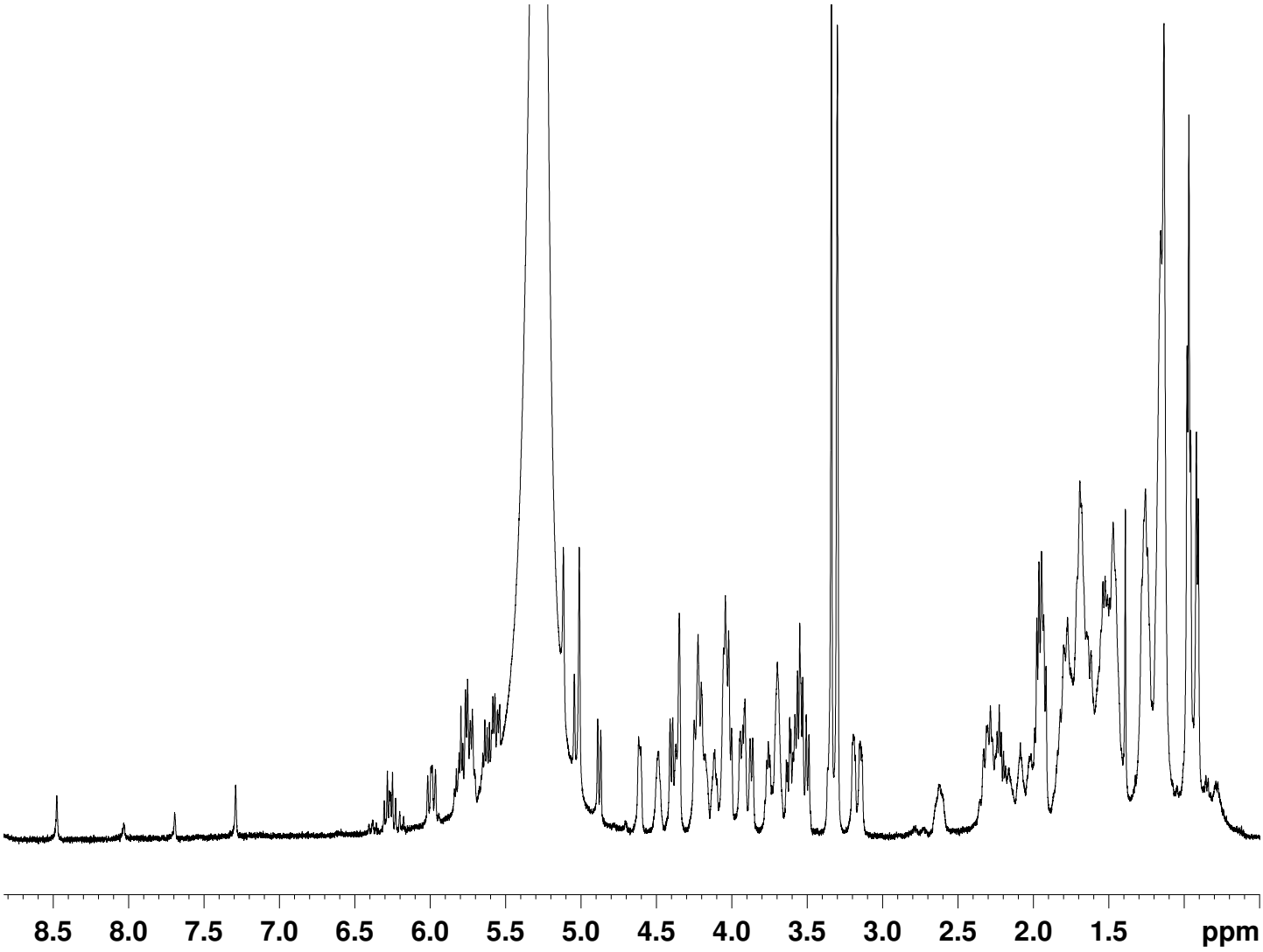


Figure S19. ^{1}H NMR spectrum of 10-*O*-sulfo-KmTx 1 (**7**).

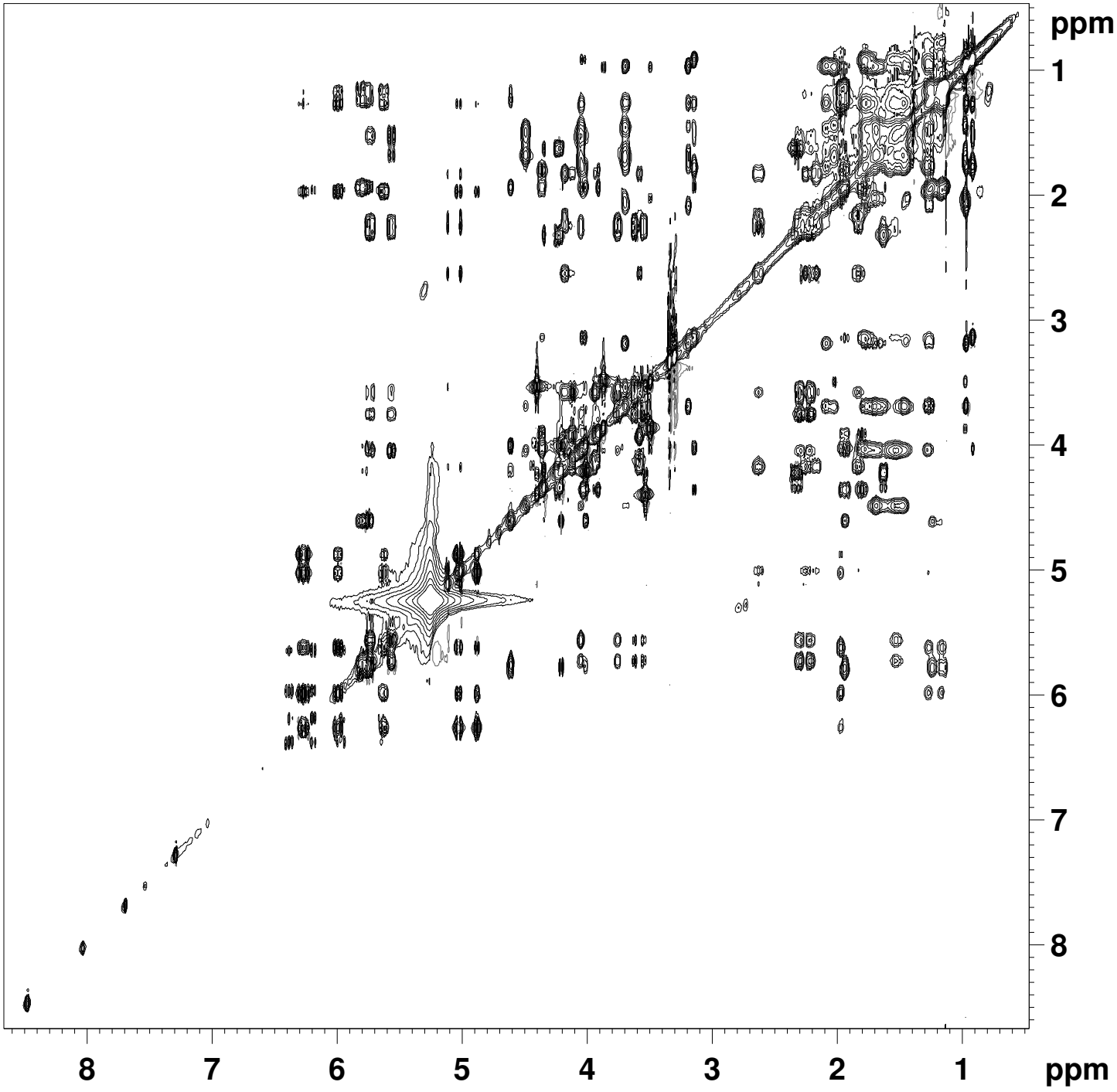


Figure S20. TOCSY spectrum of 10-*O*-sulfo-KmTx 1 (**7**).

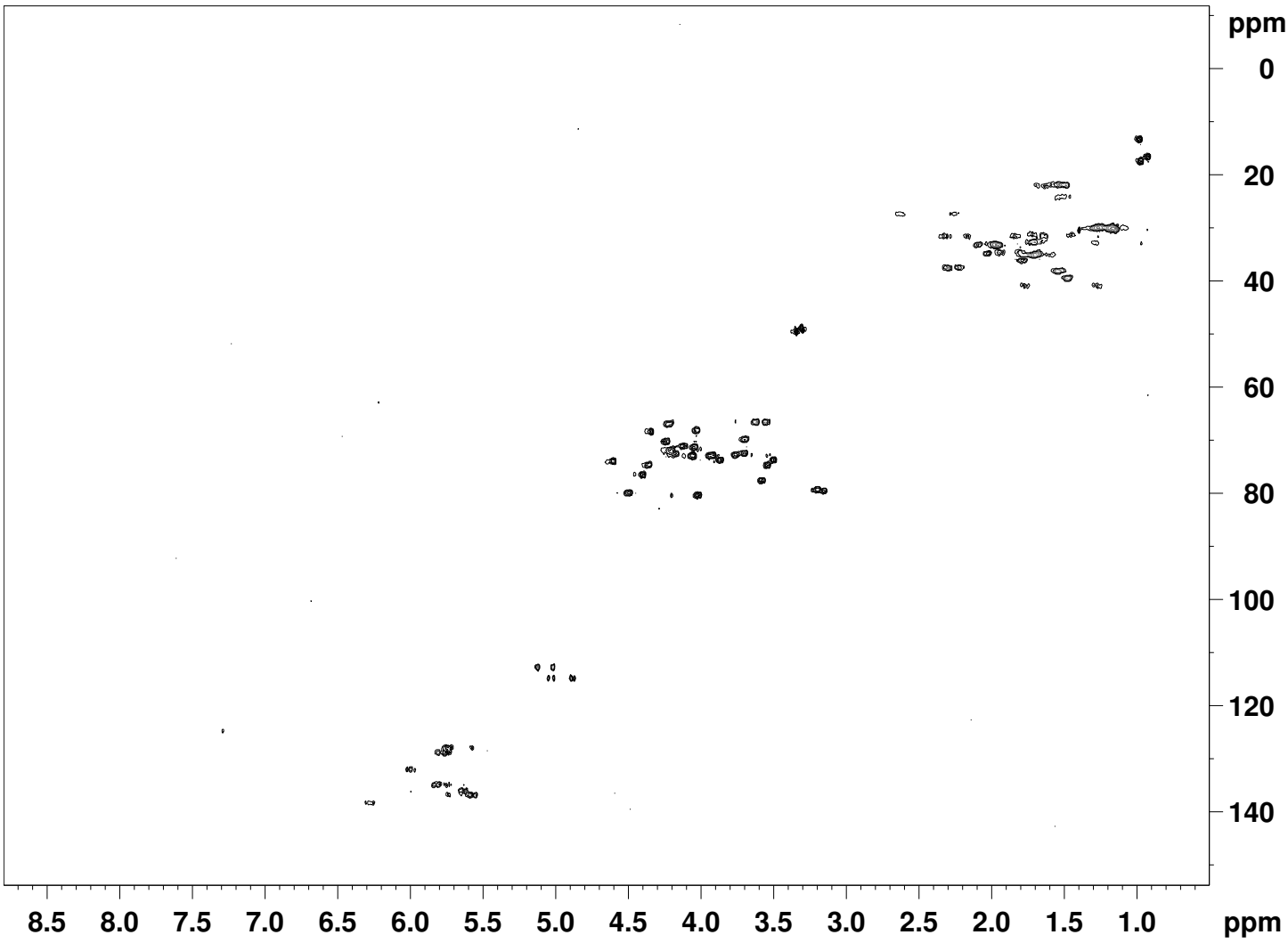


Figure S21. HSQC spectrum of 10-*O*-sulfo-KmTx 1 (**7**).

Mass spectral data for the karlotoxins

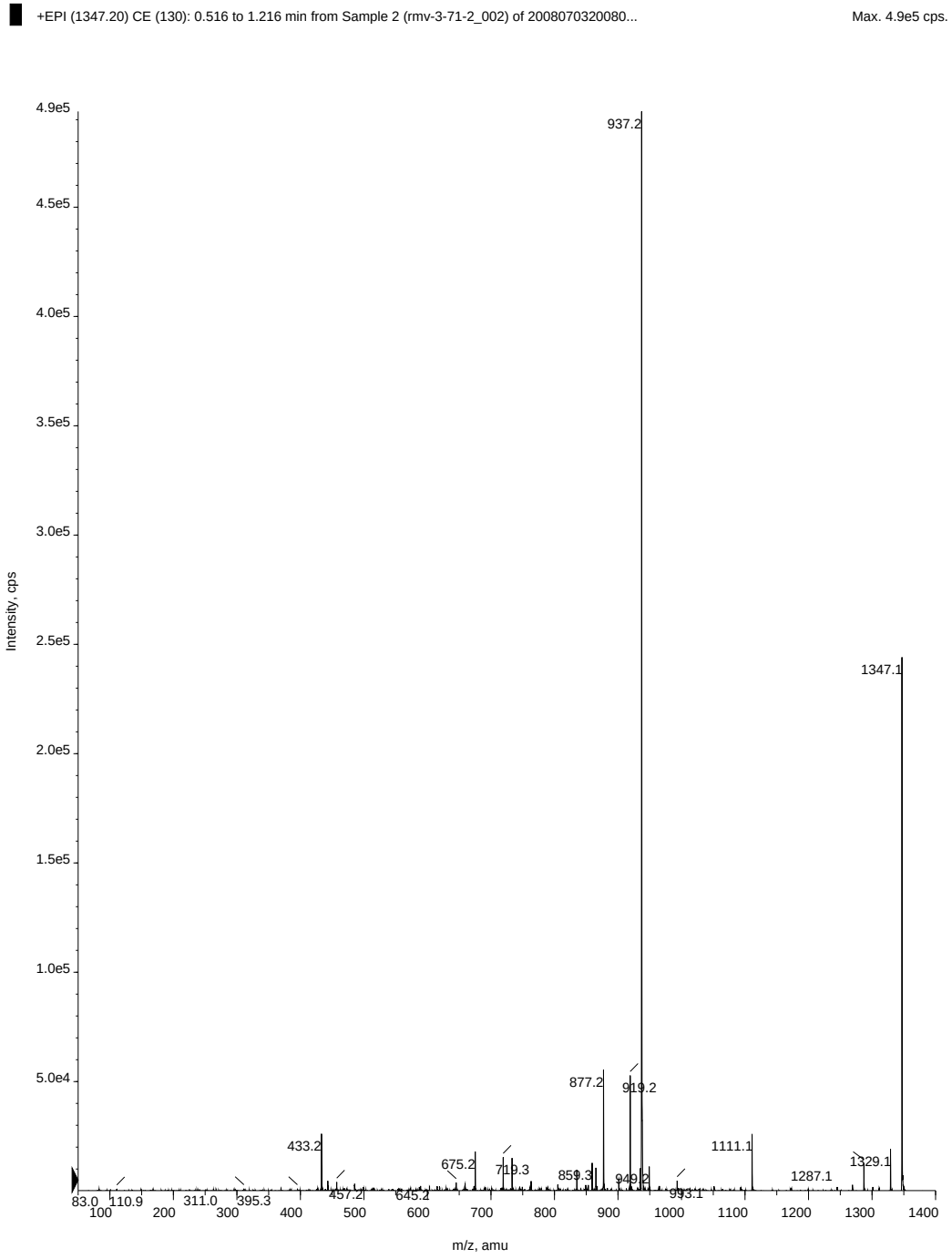


Figure S22. EPI mass spectrum for KmTx 3 (**3**).

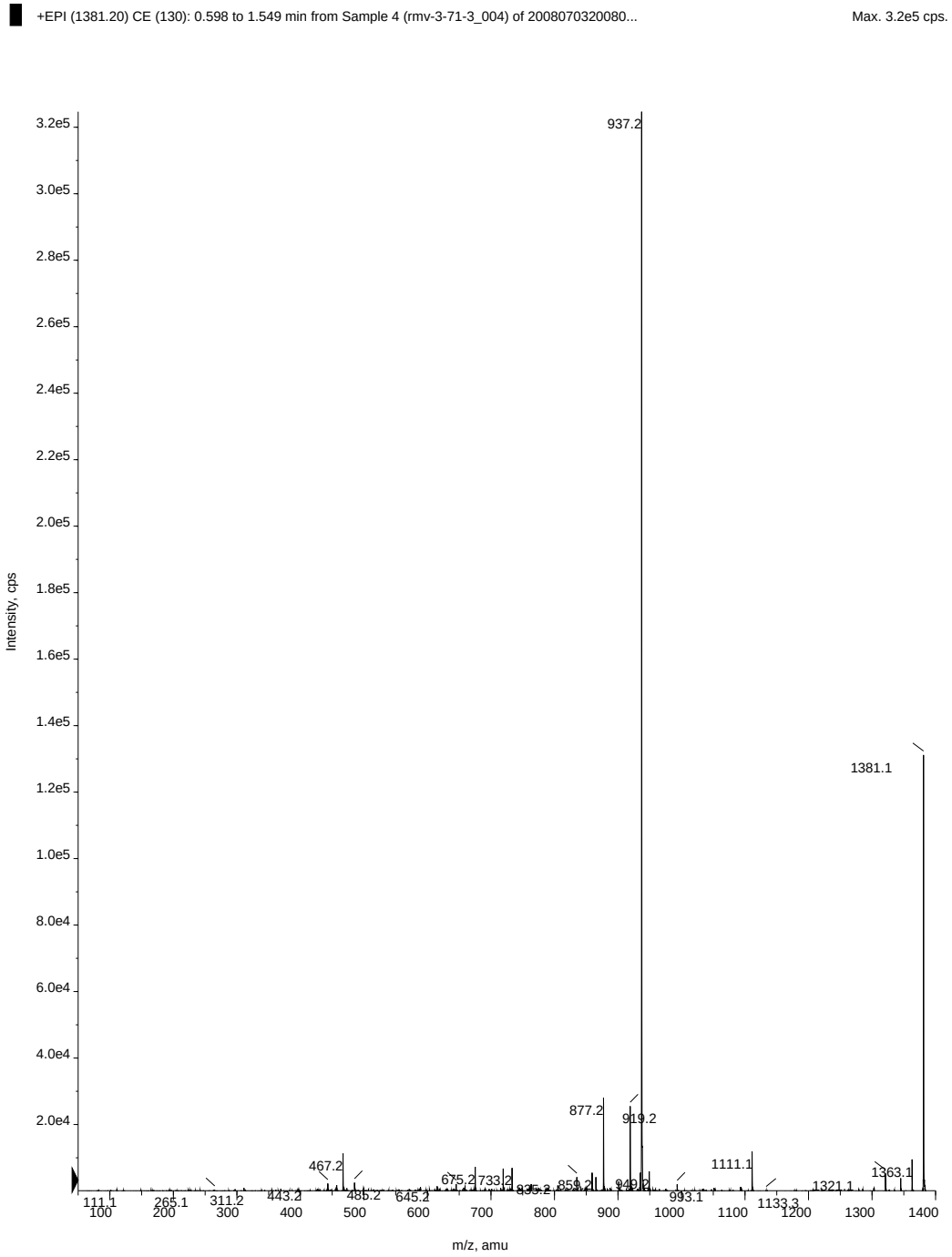


Figure S23. EPI mass spectrum for 64-*E*-chloro-KmTx 3 (**4**).

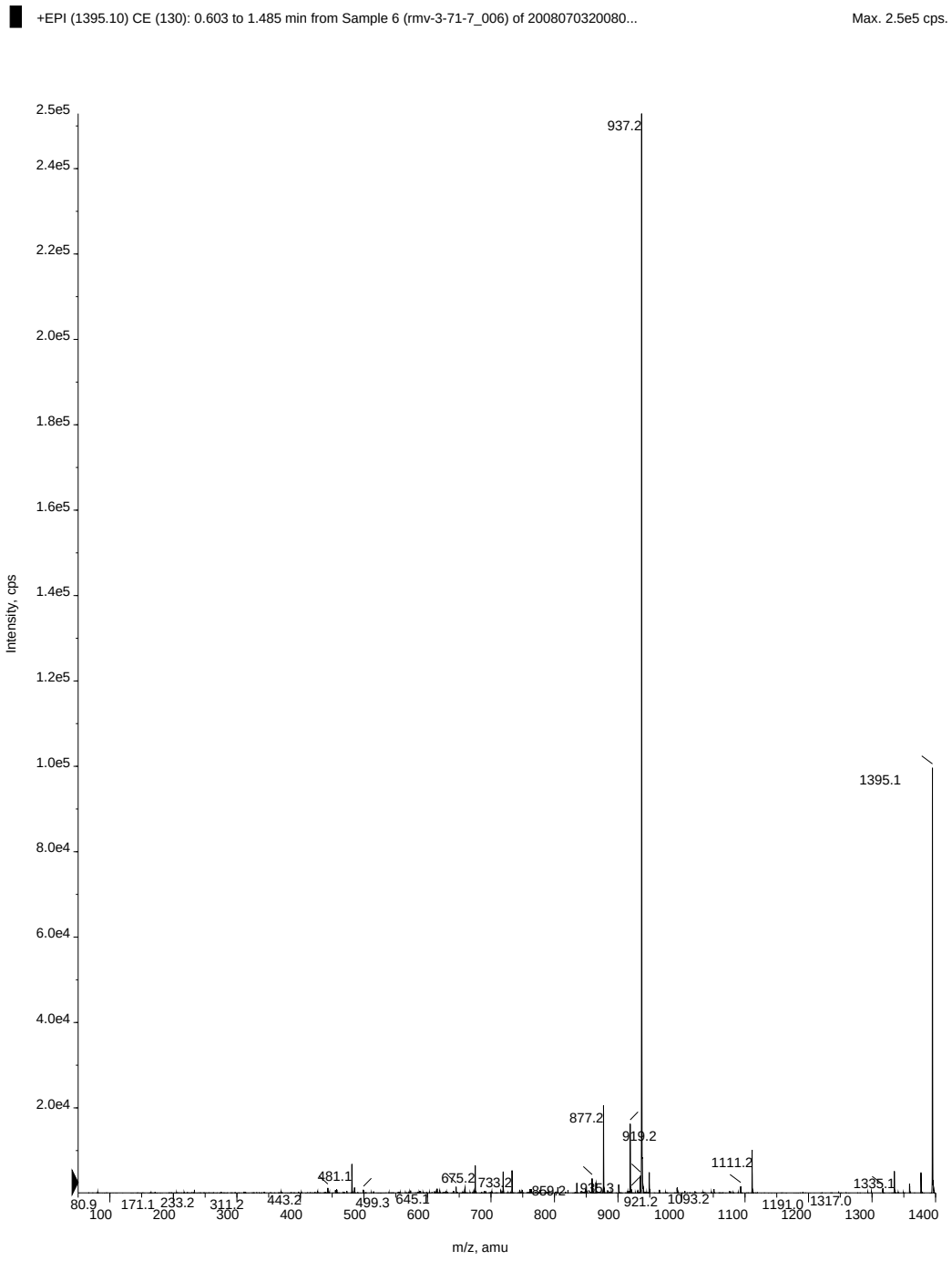


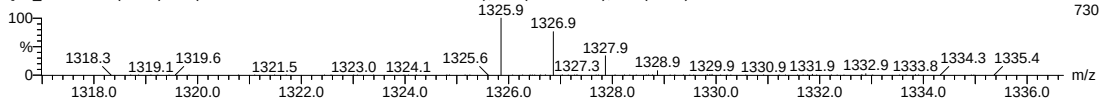
Figure S24. EPI mass spectrum for 65-*E*-chloro-KmTx 1 (**6**).

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
36 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-150 H: 0-200 O: 23-25

Ryan Van Wagoner, RMV3-71-2 Mass Spec Lab, SCS, University of Illinois QTOF
Qtof_19553a 28 (2.009) AM (Cen,3, 80.00, Ar,14000.0,716.46,0.70,LS 3); Sm (SG, 2x3.00); Cm (27:28) 1: TOF MS ES+
730



Minimum:				-1.5		
Maximum:		5.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1325.8534	1325.8561	-2.7	-2.0	6.5	0.2	C68 H125 O24

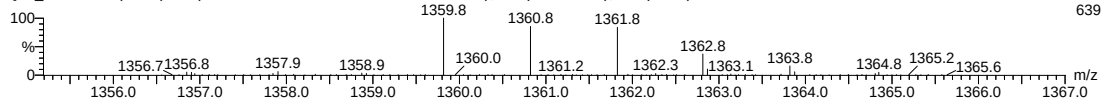
Figure S25. High-resolution mass spectrum for KmTx 3 (**3**).

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
75 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-150 H: 0-200 O: 23-25 Cl: 0-1

Ryan Van Wagoner, RMV3-71-3 Mass Spec Lab, SCS, University of Illinois QTOF
Qtof_19554a 29 (2.080) AM (Cen,3, 80.00, Ar,14000.0,716.46,0.70,LS 3); Sm (SG, 2x3.00); Cm (21:29) 1: TOF MS ES+
639



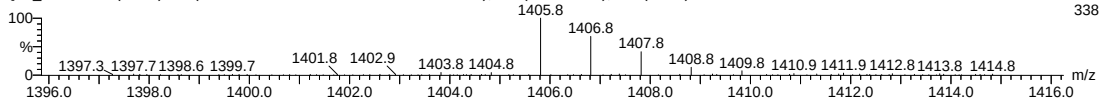
Minimum:				-1.5		
Maximum:		5.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1359.8165	1359.8171	-0.6	-0.4	6.5	7.0	C68 H124 O24 Cl
	1359.8040	12.5	9.2	11.5	74.4	C70 H119 O25

Figure S26. High-resolution mass spectrum for 64-*E*-chloro-KmTx 3 (**4**).

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
74 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-150 H: 0-200 O: 26-28 S: 0-1

Ryan Van Wagoner, RMV3-83-1 Mass Spec Lab, SCS, University of Illinois QTOF
Qtof_19557b 30 (2.130) AM (Cen,5, 80.00, Ar,14000.0,716.46,0.70,LS 3); Sm (SG, 2x5.00); Cm (30:35) 1: TOF MS ES+
338



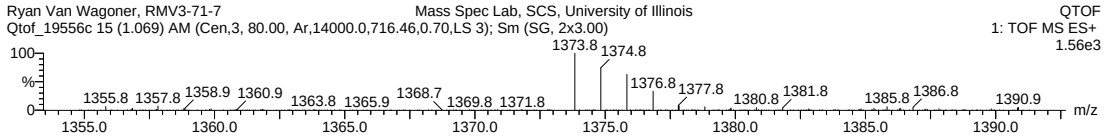
Minimum:				-1.5		
Maximum:		5.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1405.8108	1405.8095	1.3	0.9	11.5	3.5	C71 H121 O27
	1405.8129	-2.1	-1.5	6.5	2.0	C68 H125 O27 S

Figure S27. High-resolution mass spectrum for 10-*O*-sulfo-KmTx 3 (**5**).

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
76 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-150 H: 0-200 O: 23-25 Cl: 0-1



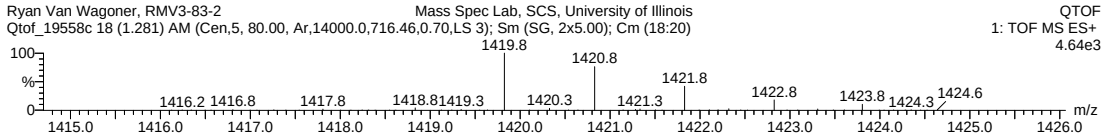
Minimum:				-1.5		
Maximum:		5.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1373.8344	1373.8328	1.6	1.2	6.5	2.8	C69 H126 O24 Cl

Figure S28. High-resolution mass spectrum for 65-*E*-chloro-KmTx 1 (**6**).

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
76 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-150 H: 0-200 O: 26-28 S: 0-1



Minimum:				-1.5		
Maximum:		5.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1419.8248	1419.8252	-0.4	-0.3	11.5	14.1	C72 H123 O27
	1419.8285	-3.7	-2.6	6.5	3.5	C69 H127 O27 S

Figure S29. High-resolution mass spectrum for 10-*O*-sulfo-KmTx 1 (**7**).

Hemolytic assays of the karlotoxins.

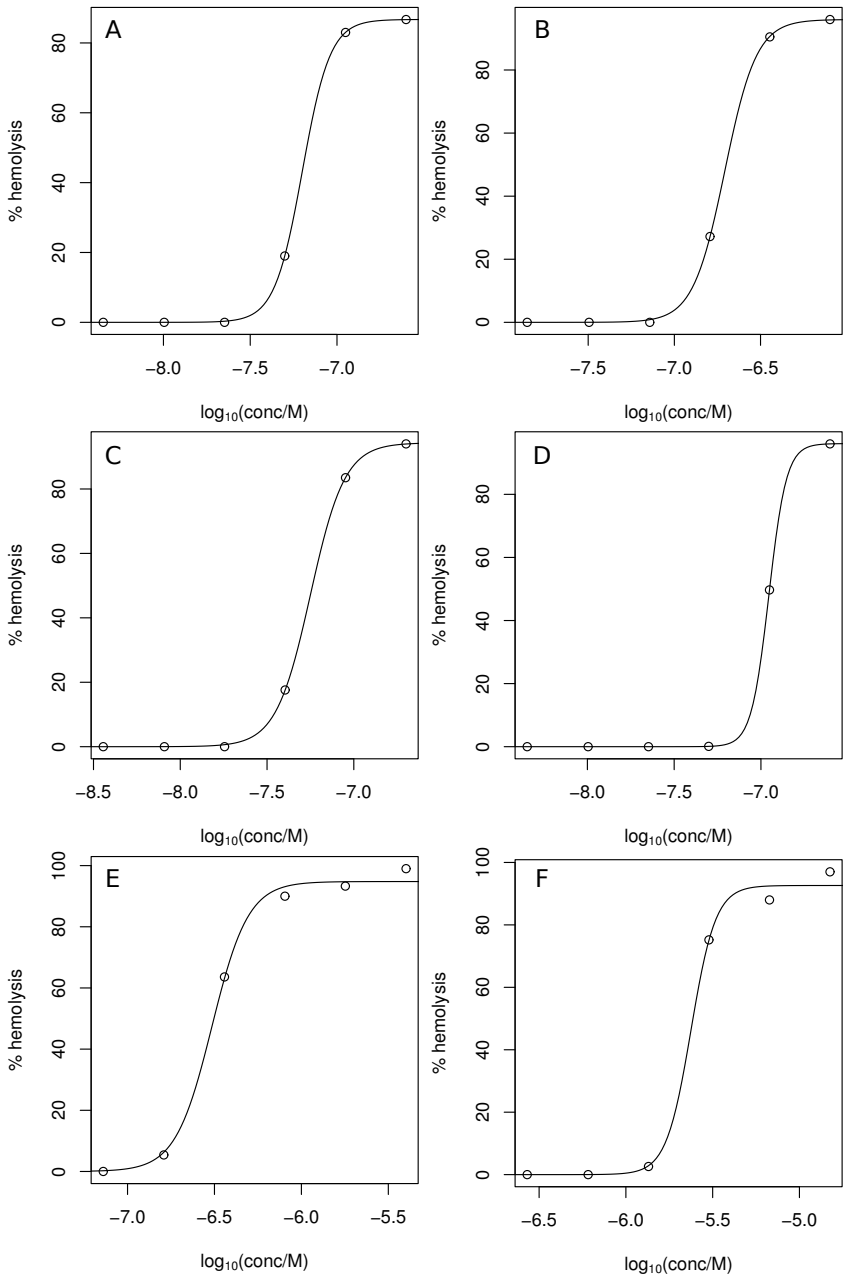


Figure S30. Hemolytic activity of the karlotoxins. Also shown are the fitted EC_{50} curves for each compound. Data points represent an average of three replicates. A- KmTx 1. B- KmTx 3. C- 65-*E*-chloro-KmTx 1. D- 64-*E*-chloro-KmTx 3. E- 10-*O*-sulfo-KmTx 1. F- 10-*O*-sulfo-KmTx 3.