Supporting Information

Dual agents: fungal macrocidins and synthetic analogues with herbicidal and antibiofilm activities

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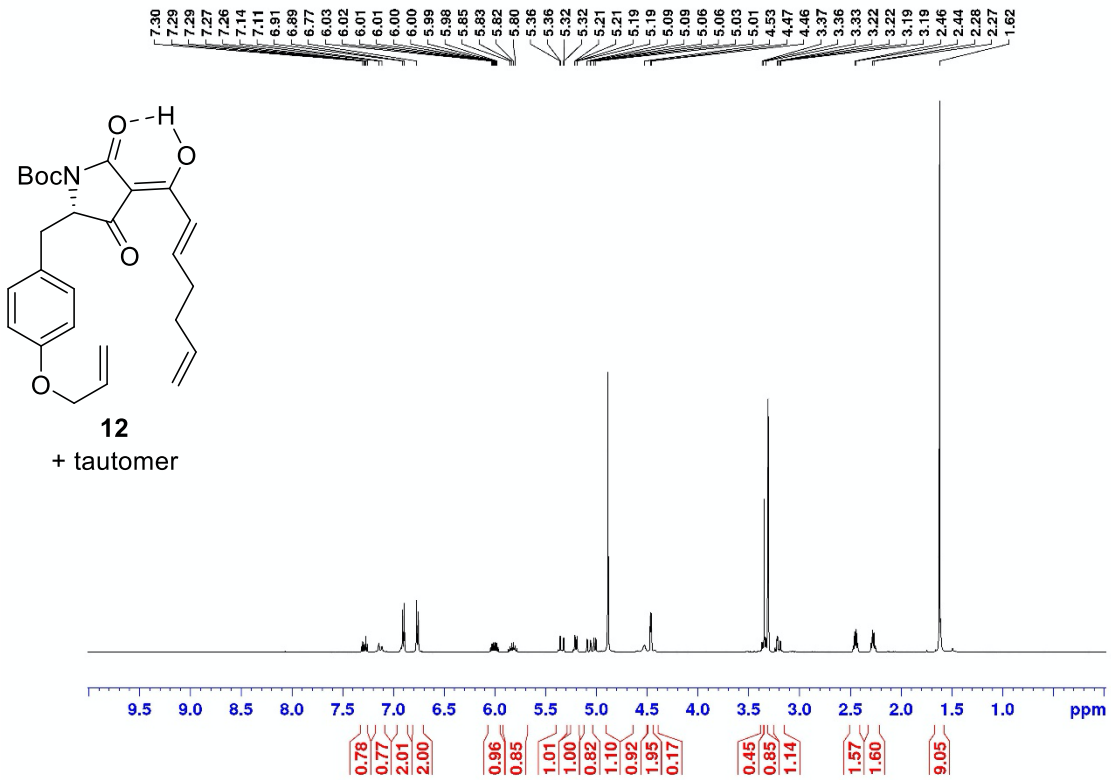

Figure S1. ^{1}H-NMR spectrum of compound **12** in CD_3OD.

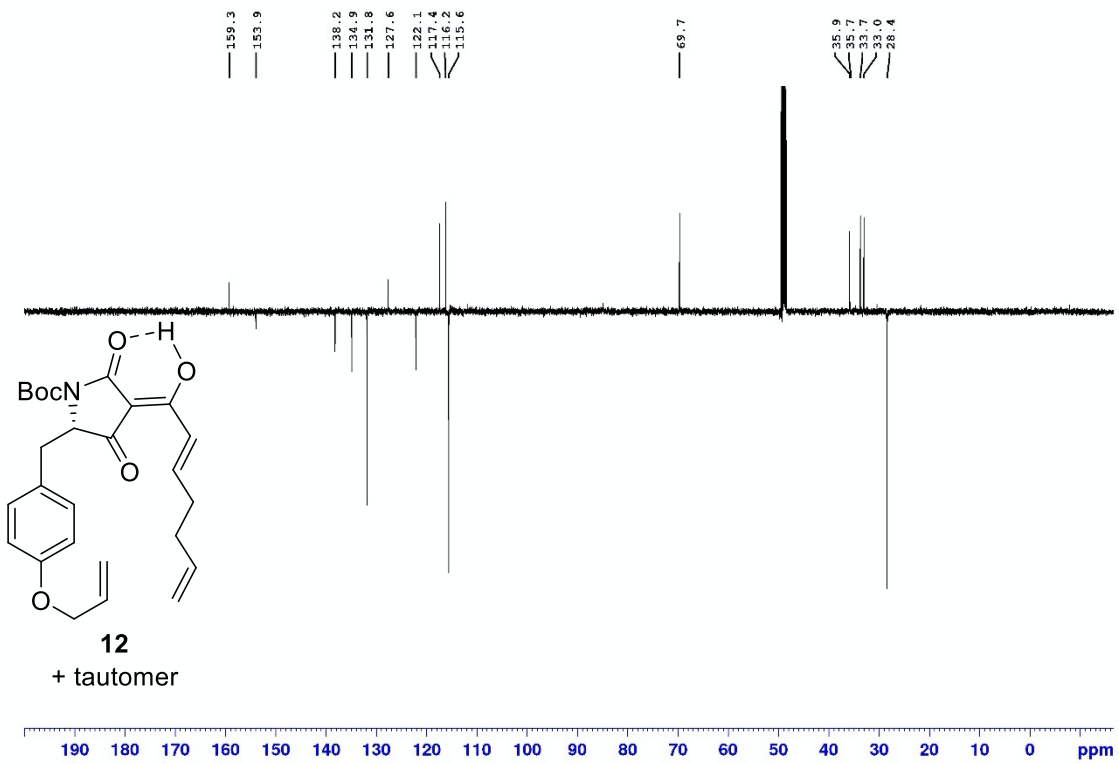

Figure S2. ^{13}C-NMR spectrum of compound **12** in CD_3OD.

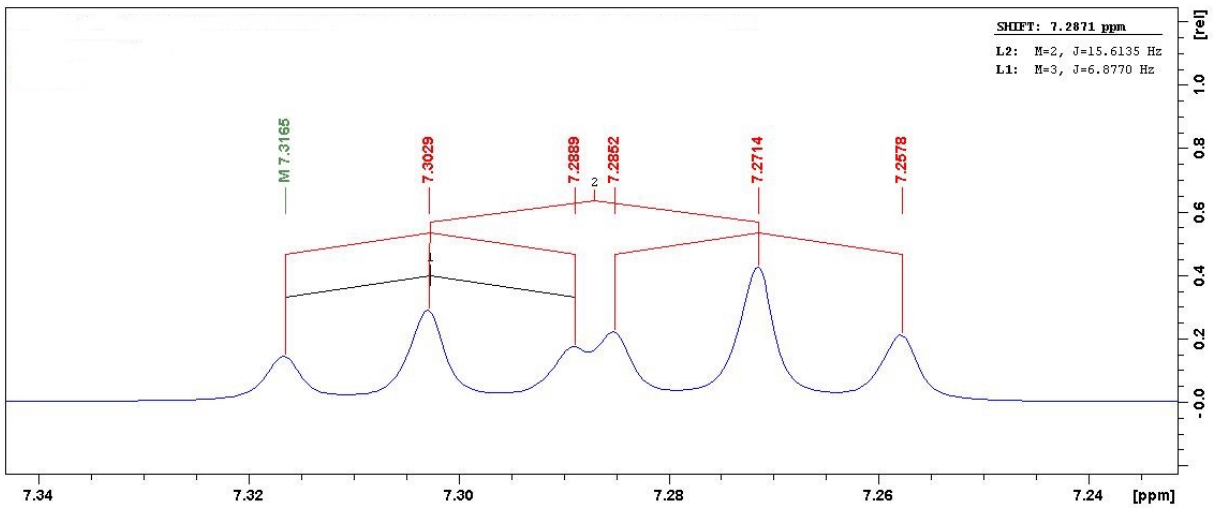

Figure S3. Part of ^{1}H-NMR spectrum of compound **12** in CD_3OD with defined multiplet and coupling constants (J =15.6, 6.9 Hz) that prove the (*E*)-configuration of the resulting double bond.

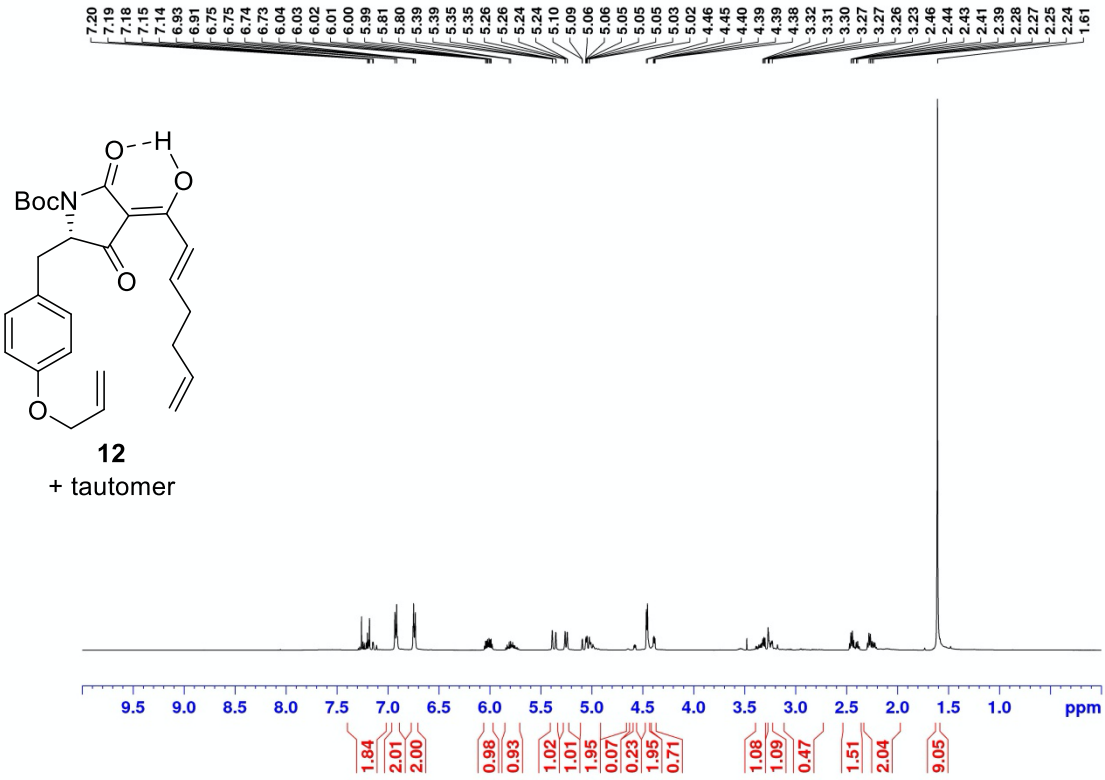

Figure S4. ^{1}H-NMR spectrum of compound **12** in $CDCl_3$.

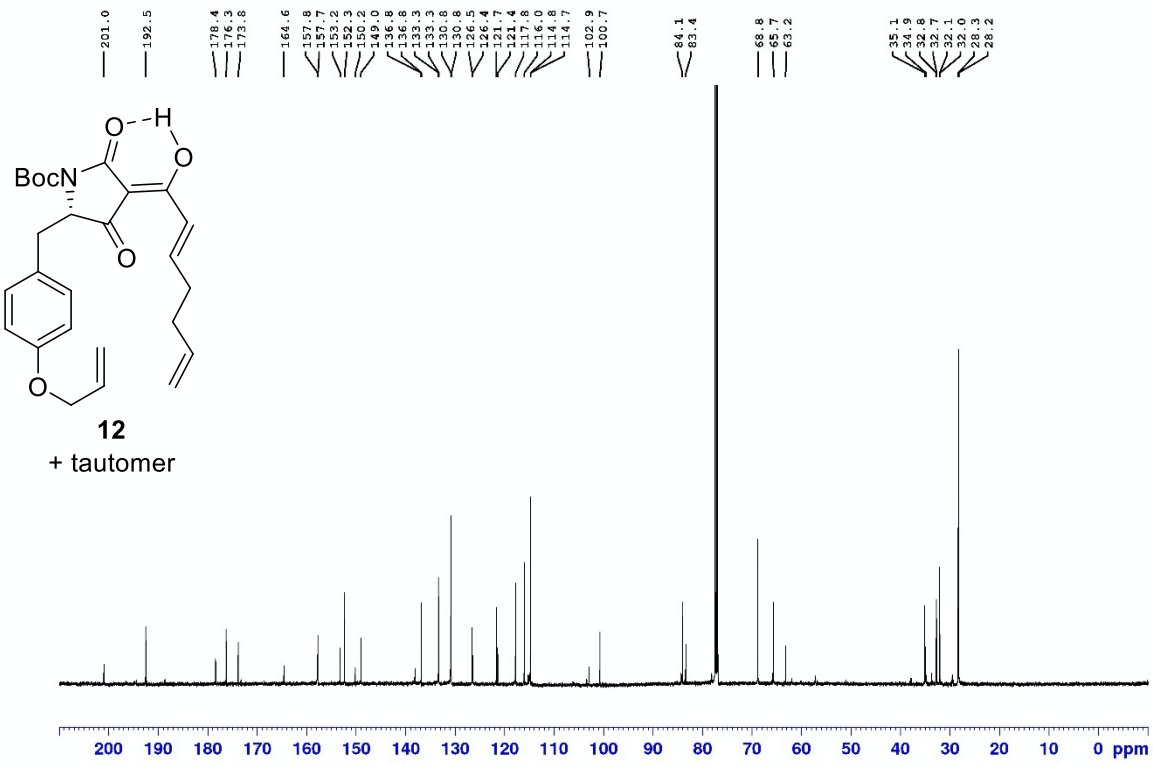

Figure S5. ^{13}C-NMR spectrum of compound **12** in $CDCl_3$.

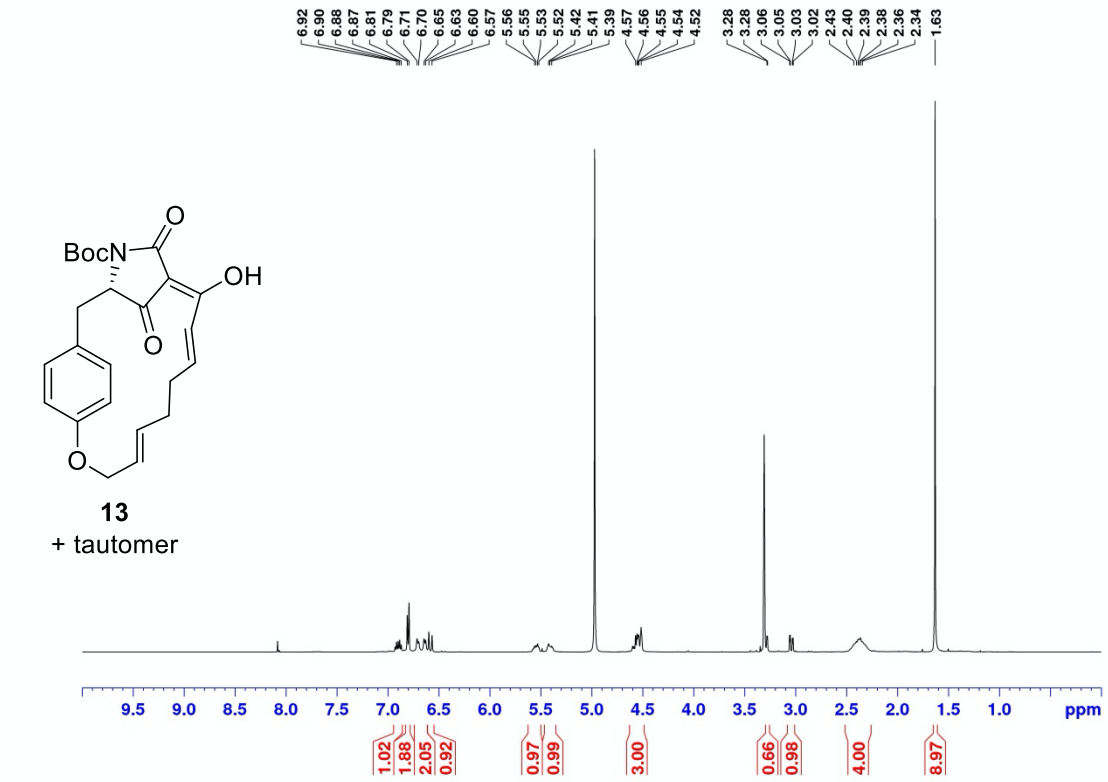

Figure S6. [1]H-NMR spectrum of compound **13** in CD_3OD.

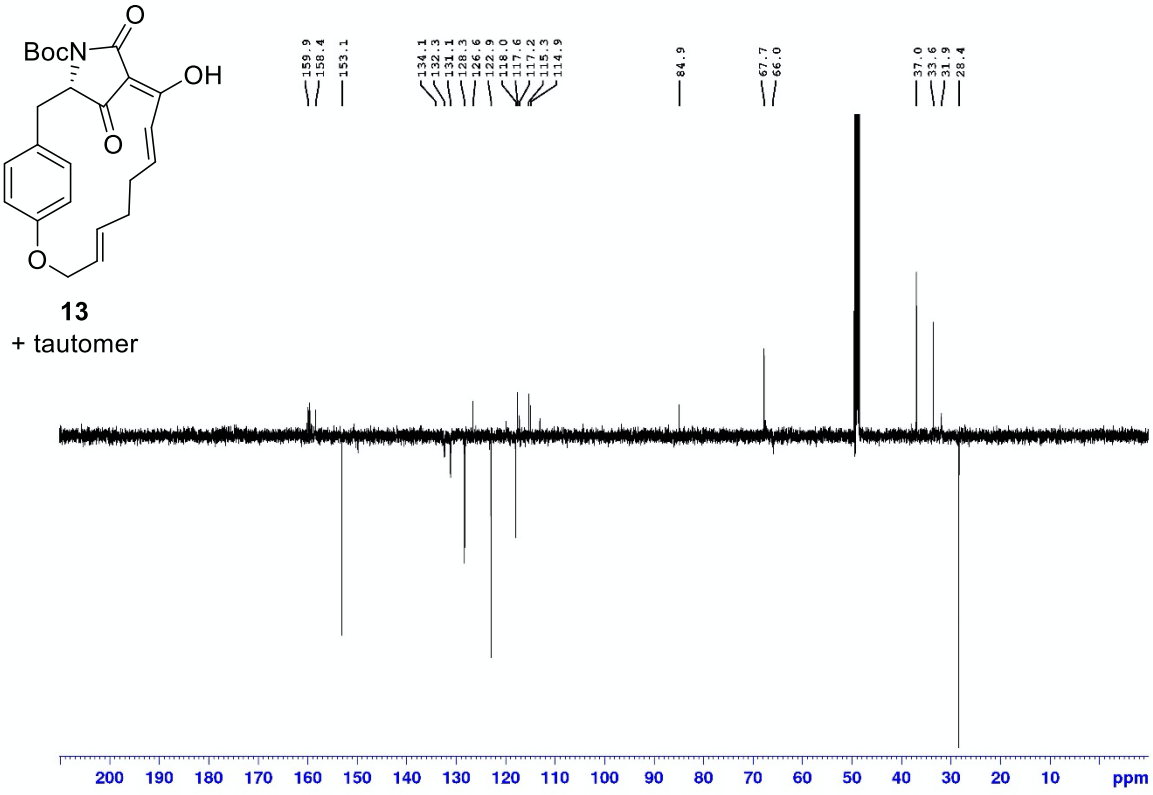

Figure S7. [13]C-NMR spectrum of compound **13** in CD_3OD.

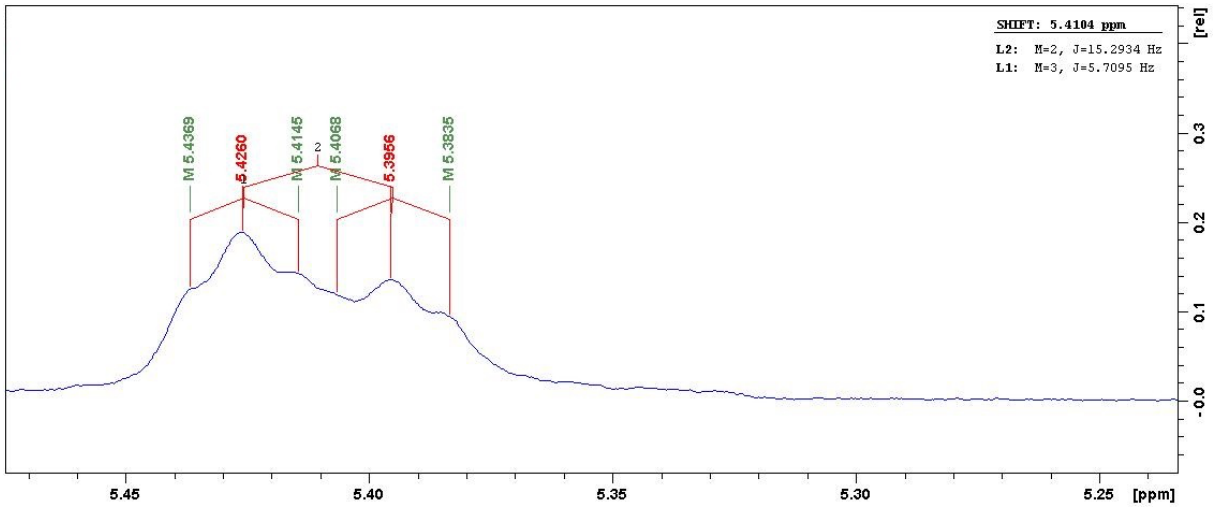

Figure S8. Part of ^{1}H-NMR spectrum of compound **13** in CD_3OD with defined multiplet and coupling constants (J =15.3, 5.7 Hz) that prove the (*E*)-configuration of the resulting isolated double bond.

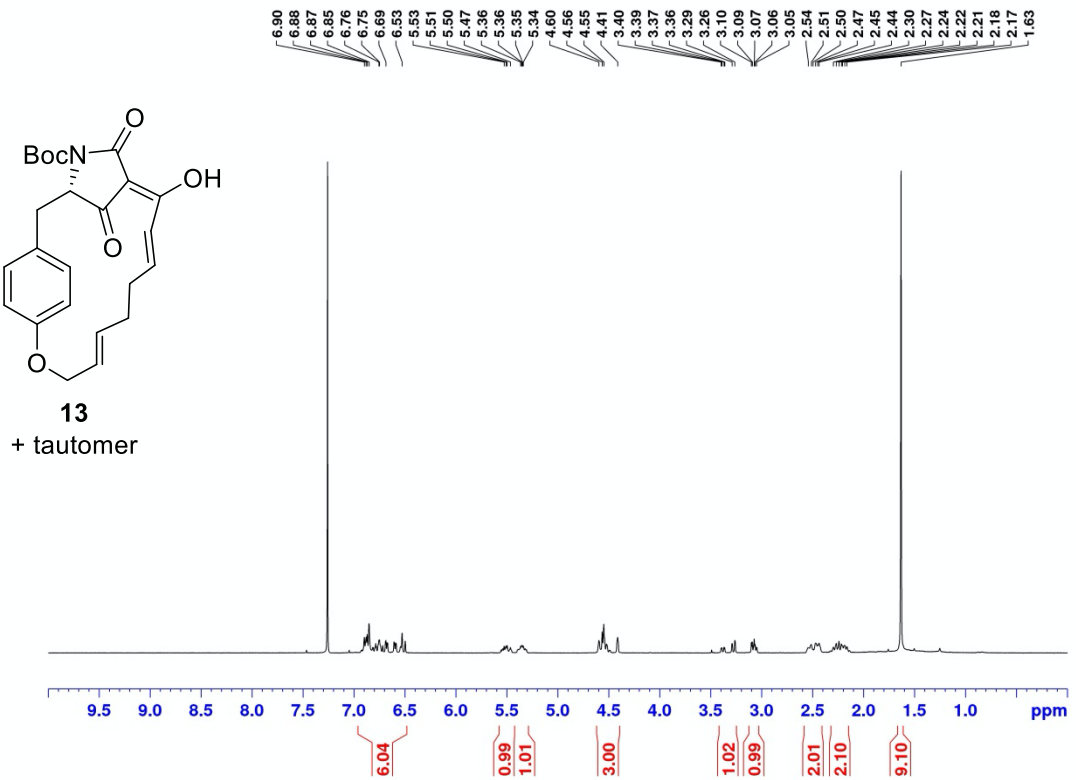

Figure S9. ^{1}H-NMR spectrum of compound **13** in $CDCl_3$.

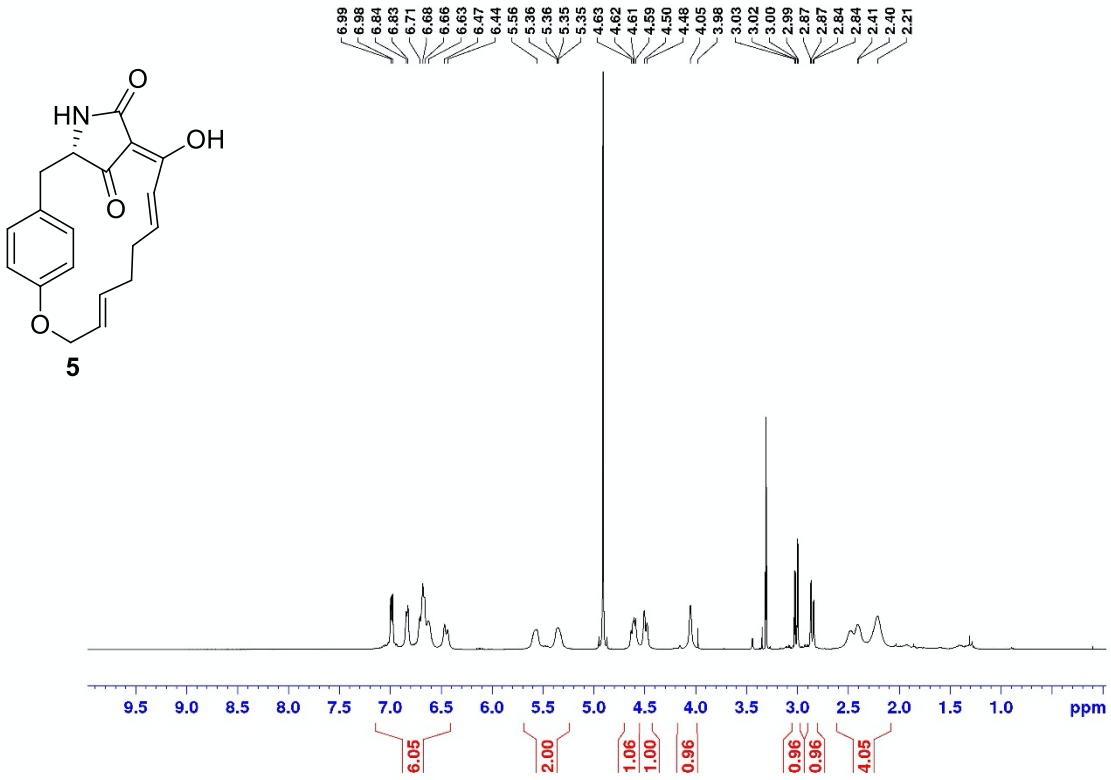

Figure S10. ^{1}H-NMR spectrum of compound **5** in CD_3OD.

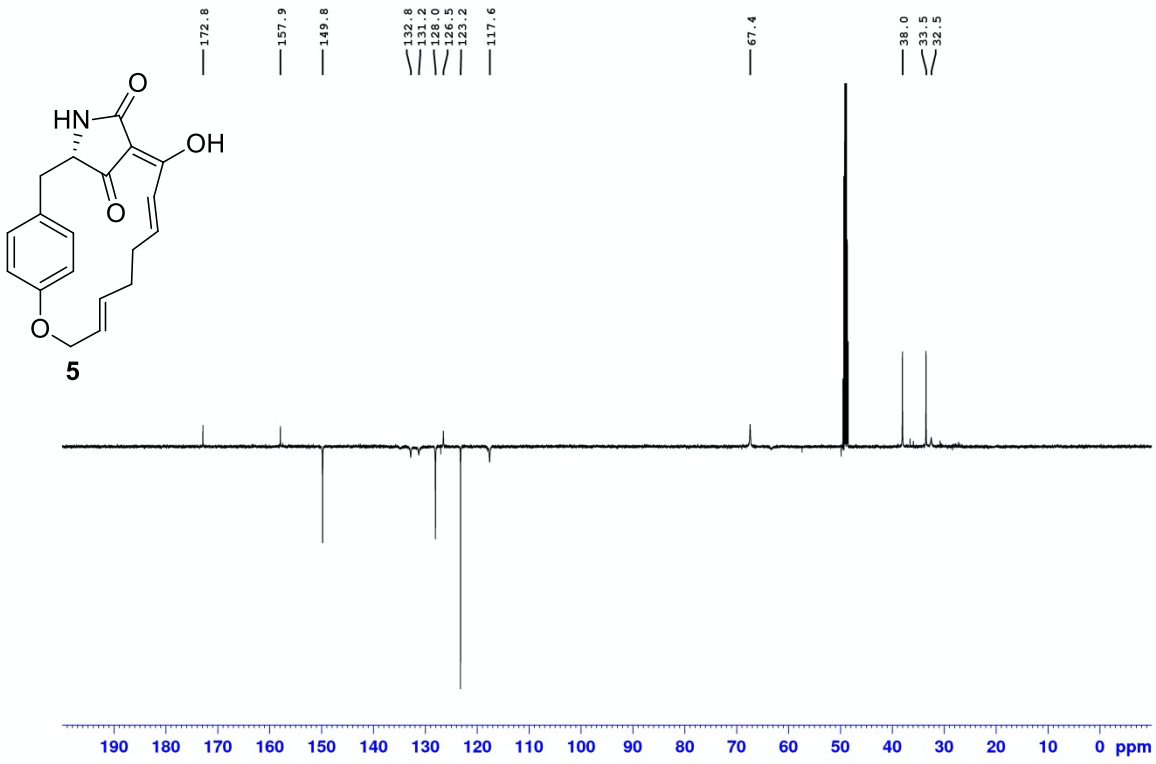

Figure S11. ^{13}C-NMR spectrum of compound **5** in CD_3OD.

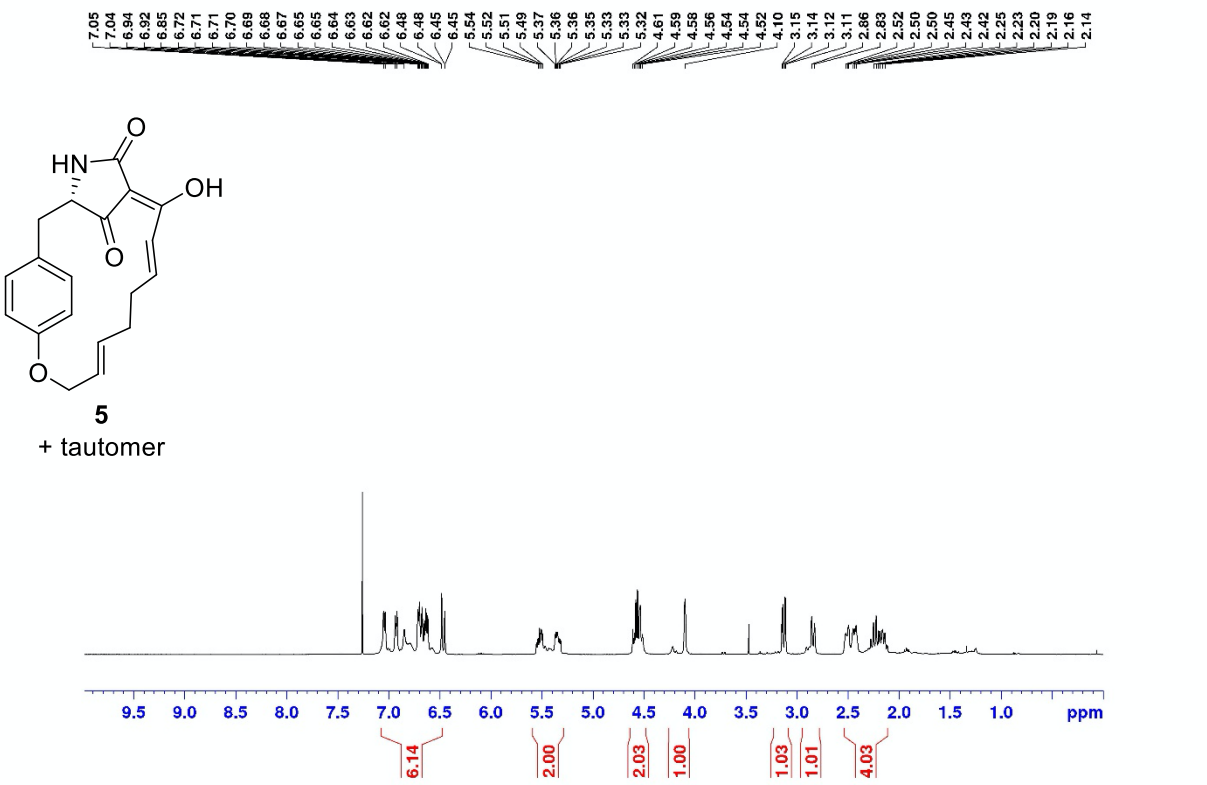

Figure S12. ^{1}H-NMR spectrum of compound **5** in $CDCl_3$.

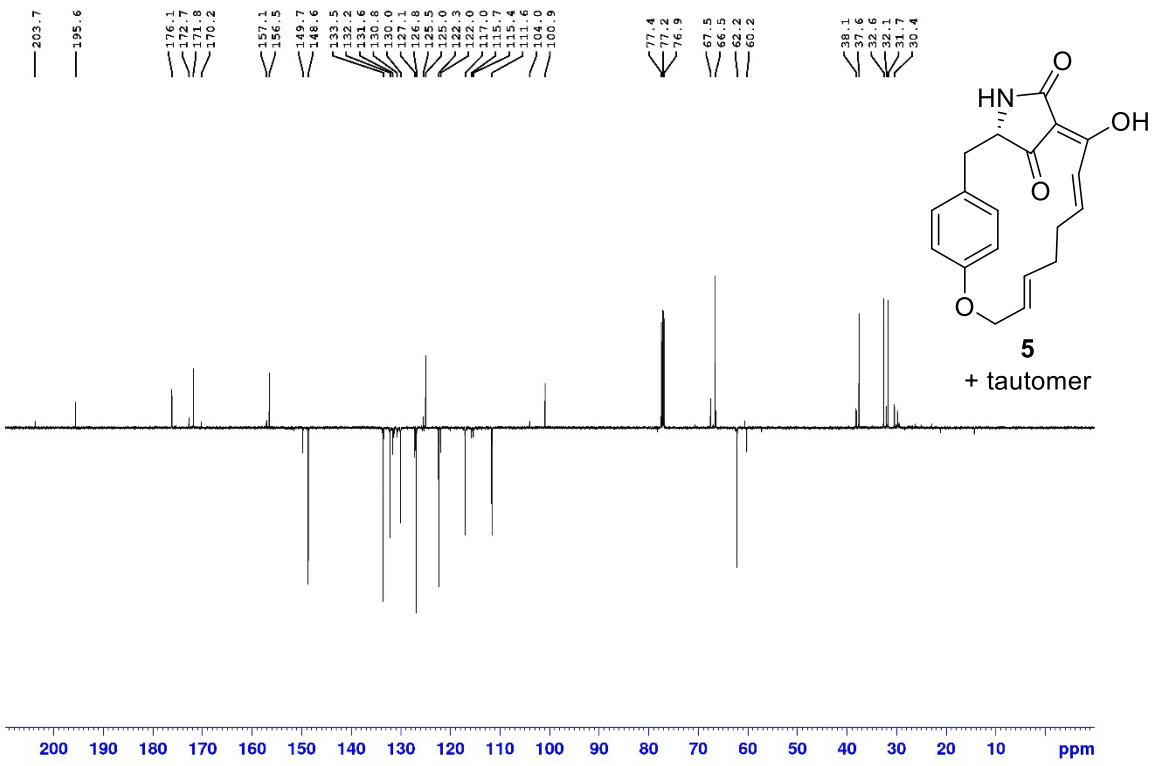

Figure S13. ^{13}C-NMR spectrum of compound **5** in $CDCl_3$.

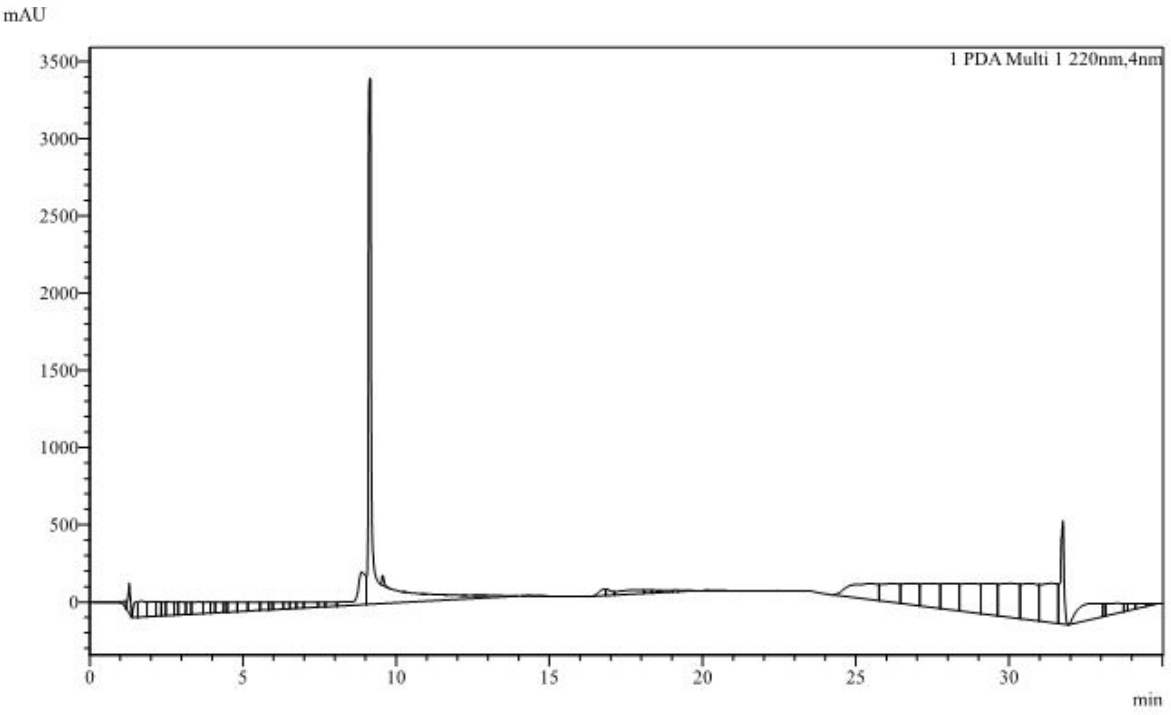

Figure S14. Chromatogram of compound **5**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

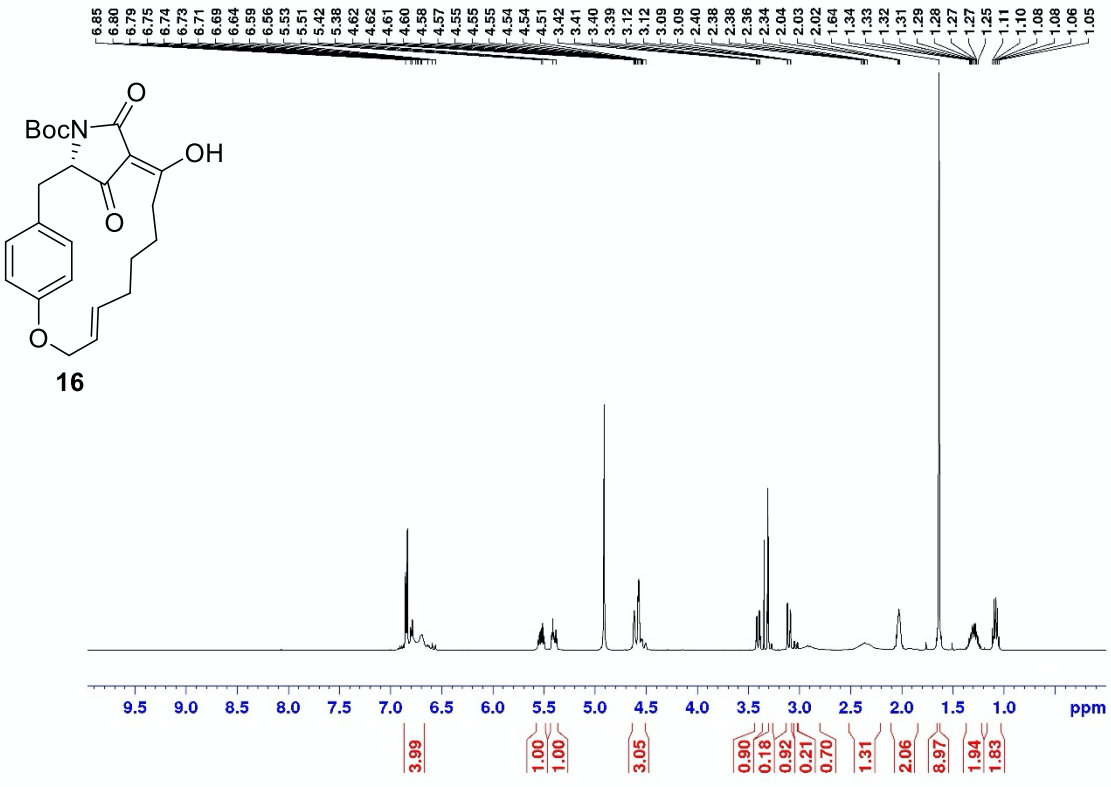

Figure S15. ^{1}H-NMR spectrum of compound **16** in CD_3OD.

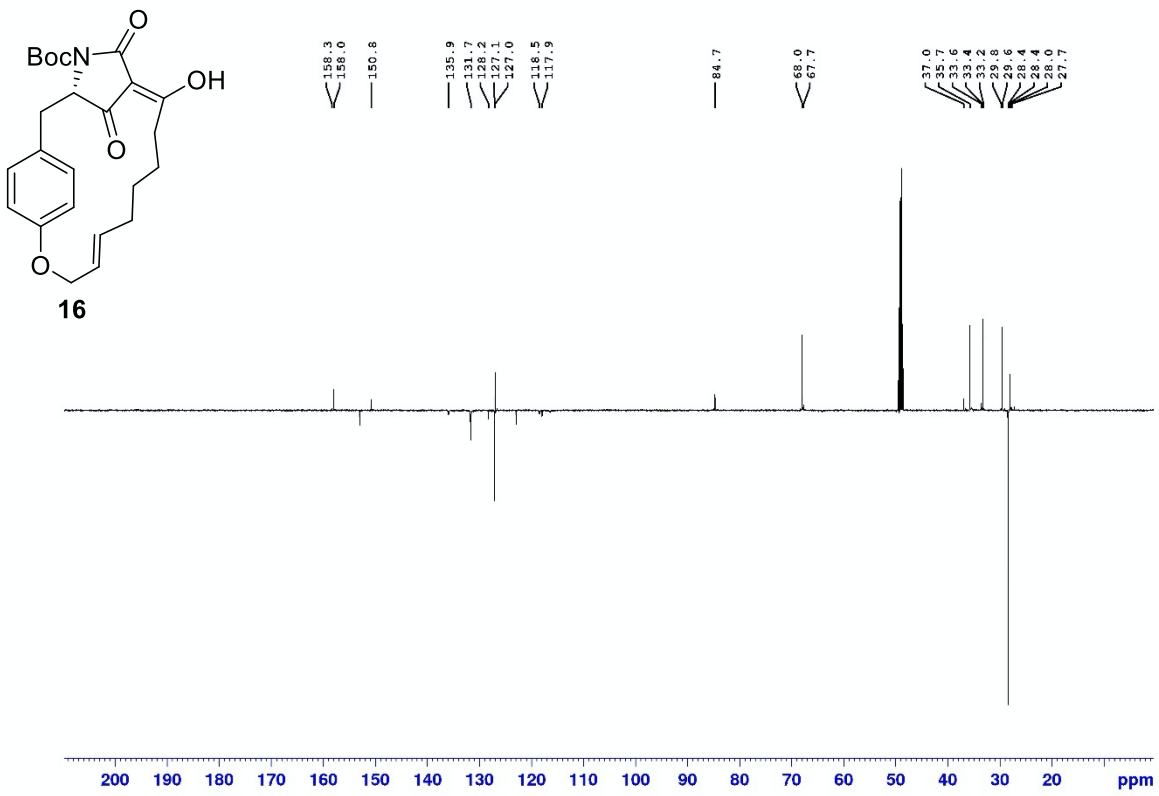

Figure S16. ^{13}C-NMR spectrum of compound **16** in CD_3OD.

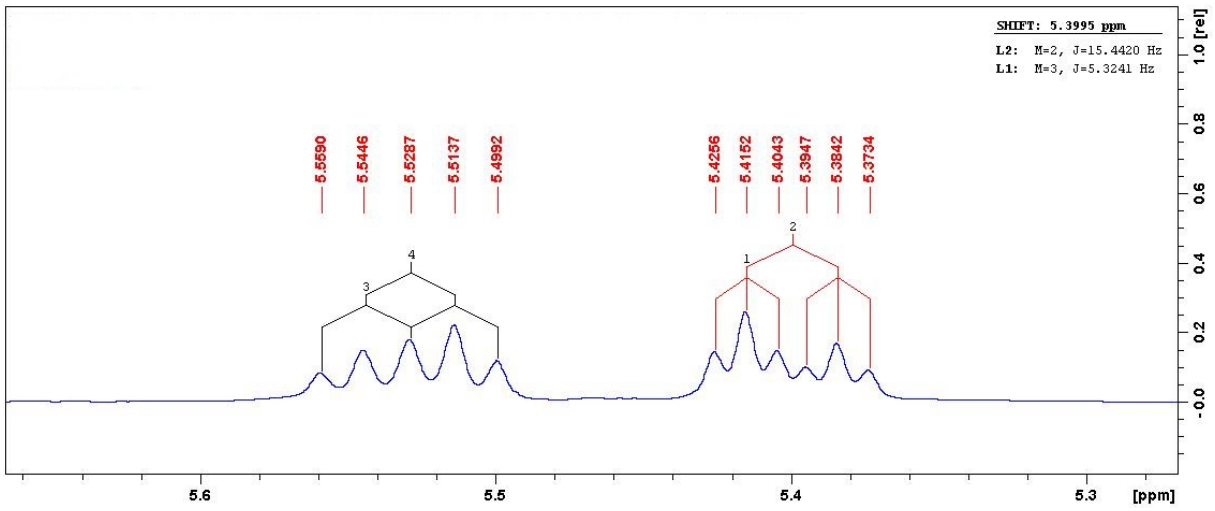

Figure S17. Part of ^{1}H-NMR spectrum of compound **16** in CD_3OD with defined multiplet and coupling constants (J =15.4, 5.3 Hz) that prove the (*E*)-configuration of double bond.

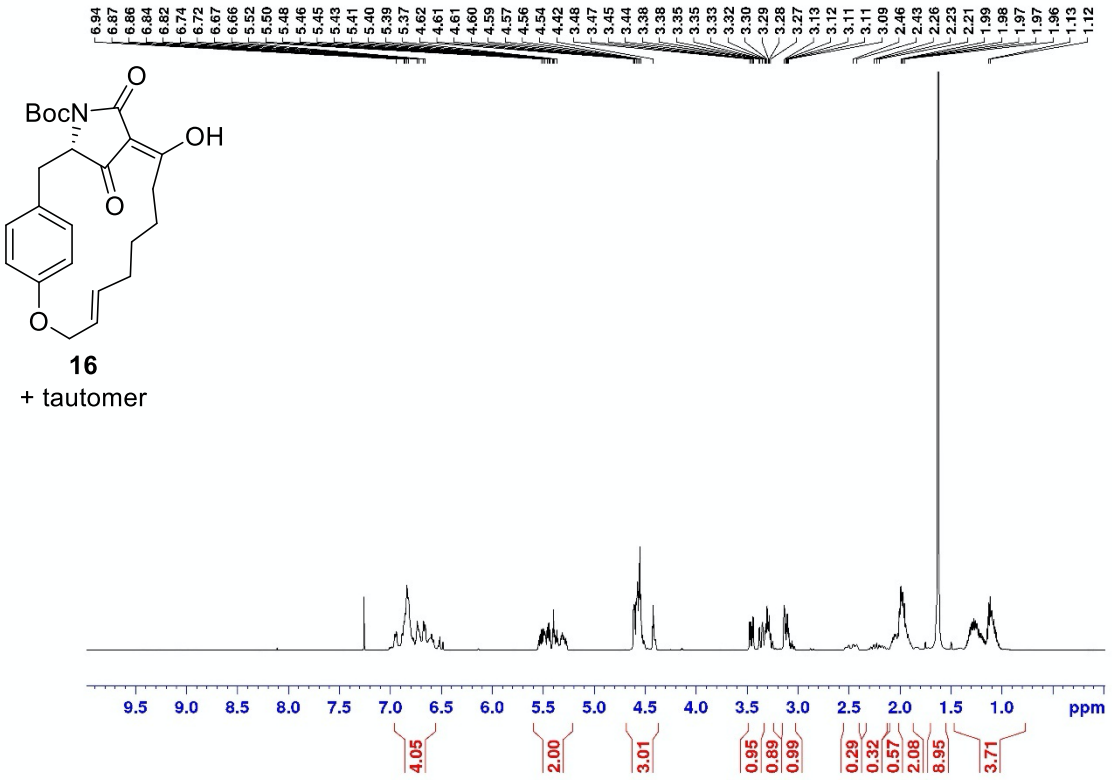

Figure S18. ^{1}H-NMR spectrum of compound **16** in $CDCl_3$.

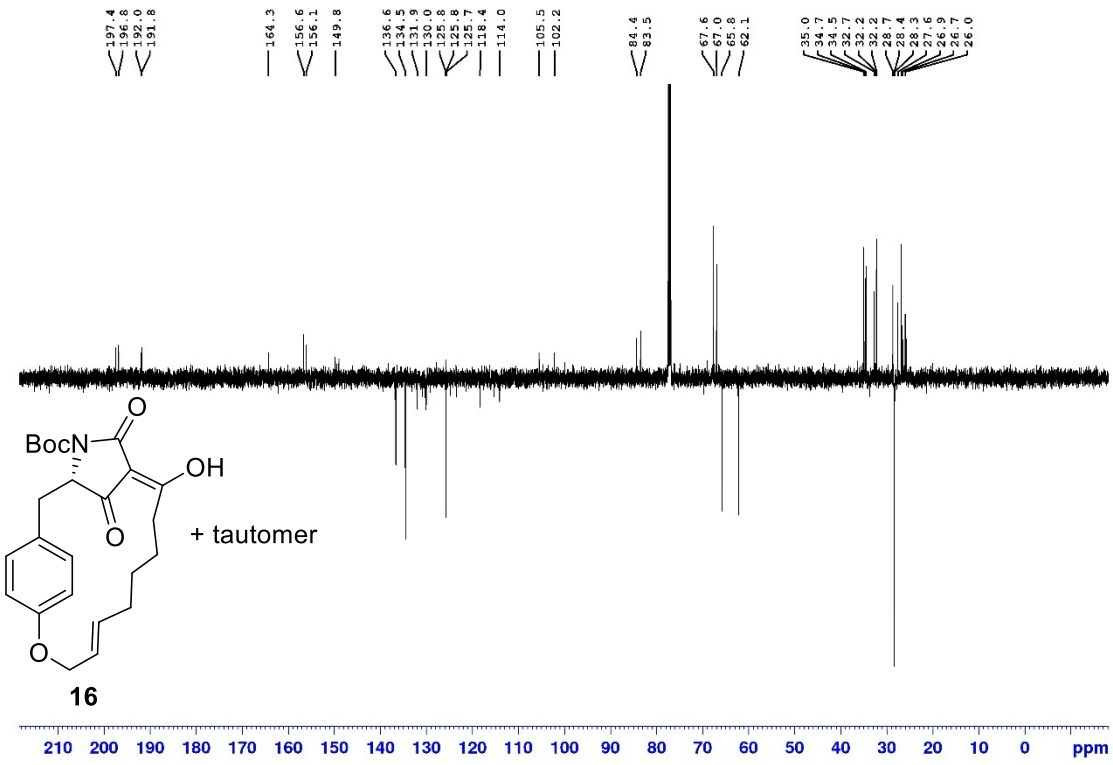

Figure S19. ^{1}H-NMR spectrum of compound **16** in $CDCl_3$.

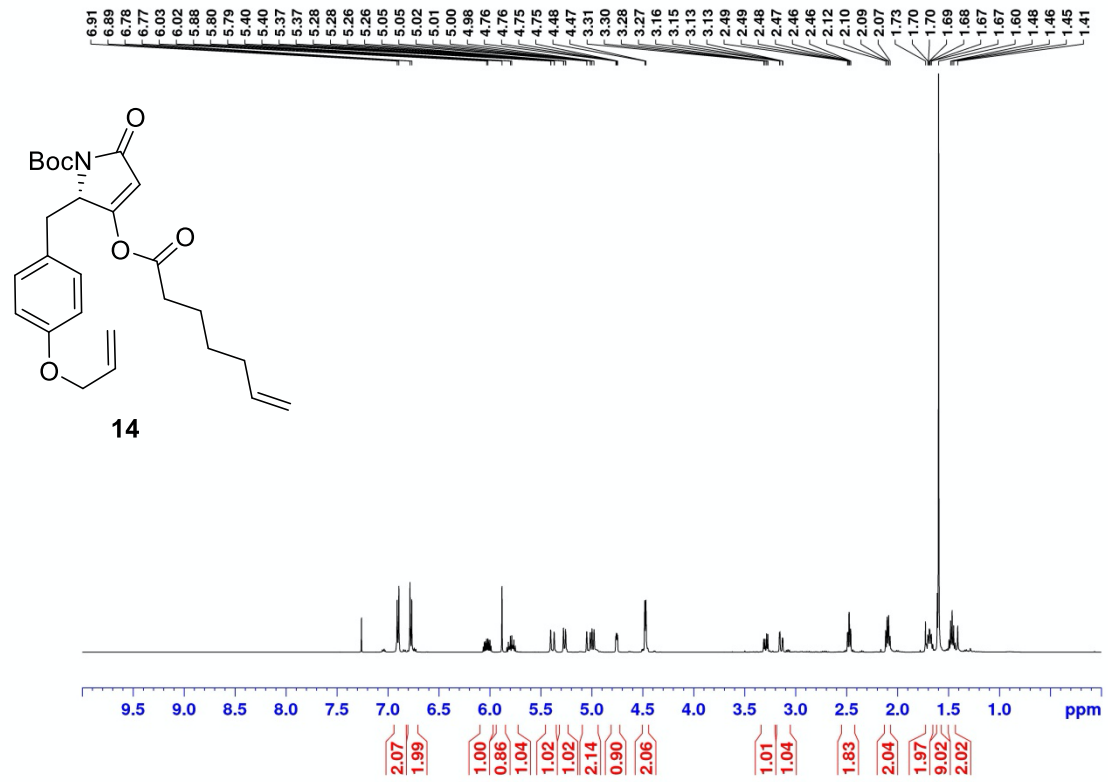

Figure S20. ^{1}H-NMR spectrum of compound **14** in $CDCl_3$.

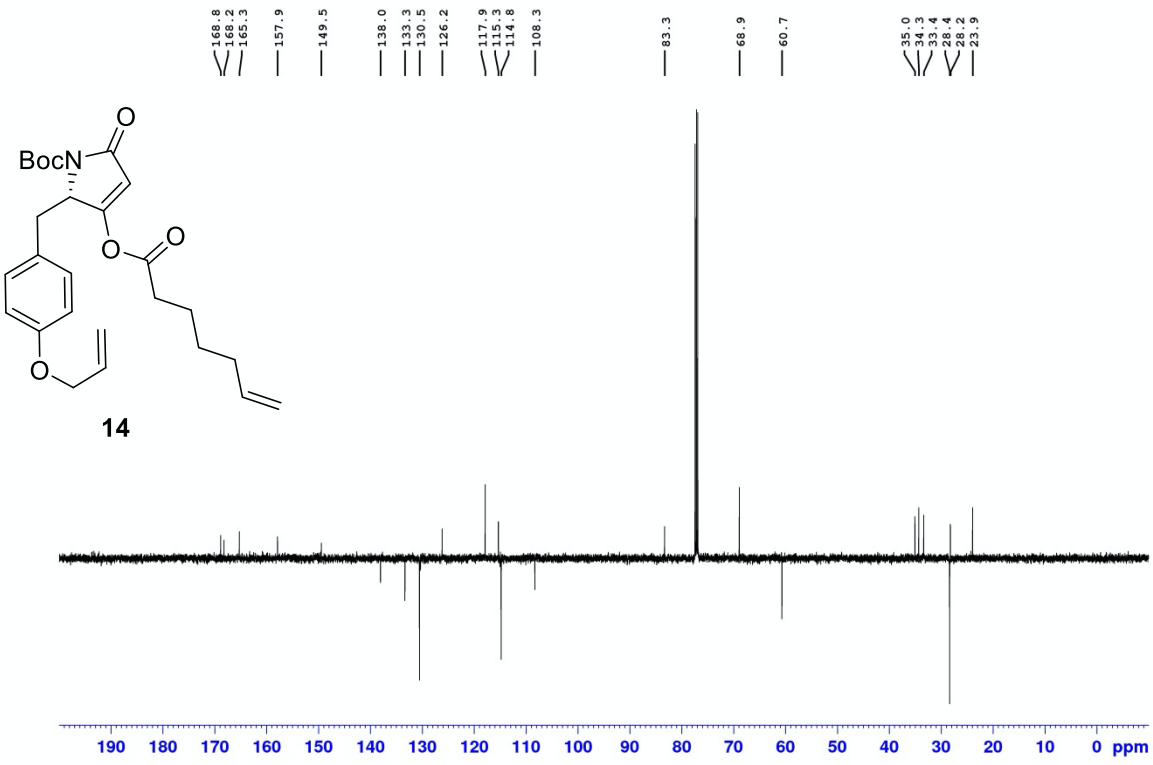

Figure S21. ^{13}C-NMR spectrum of compound **14** in $CDCl_3$.

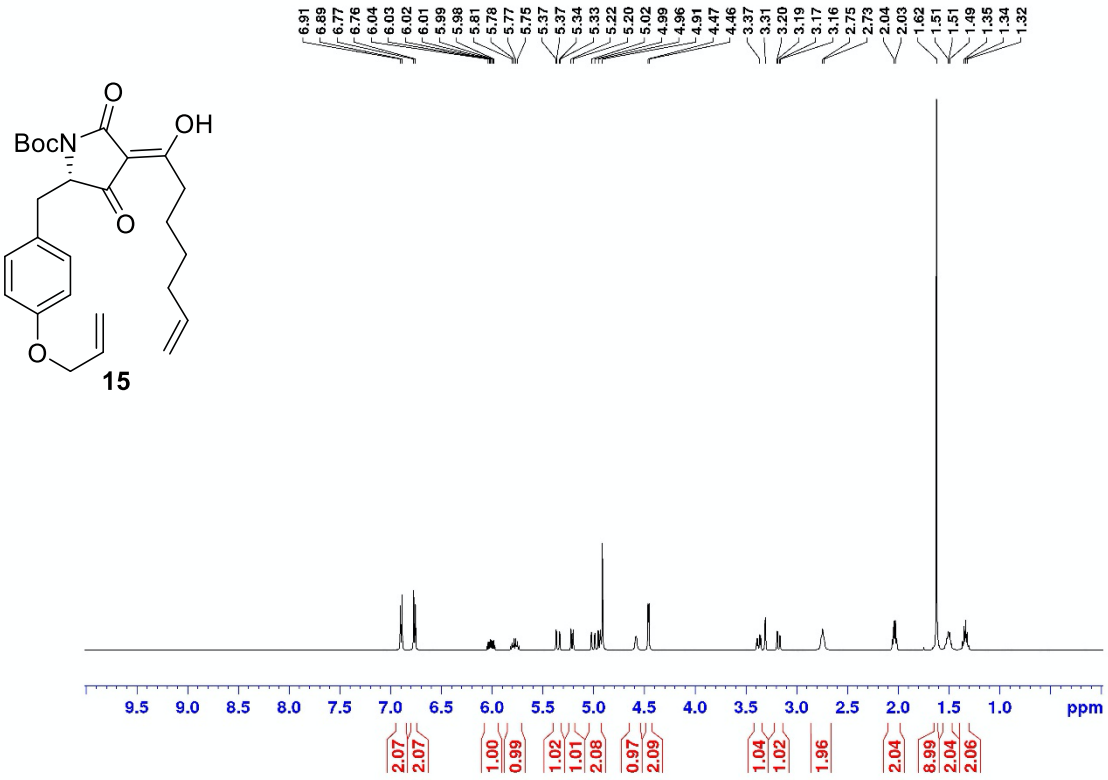

Figure S22. ^{1}H-NMR spectrum of compound **15** in CD_3OD.

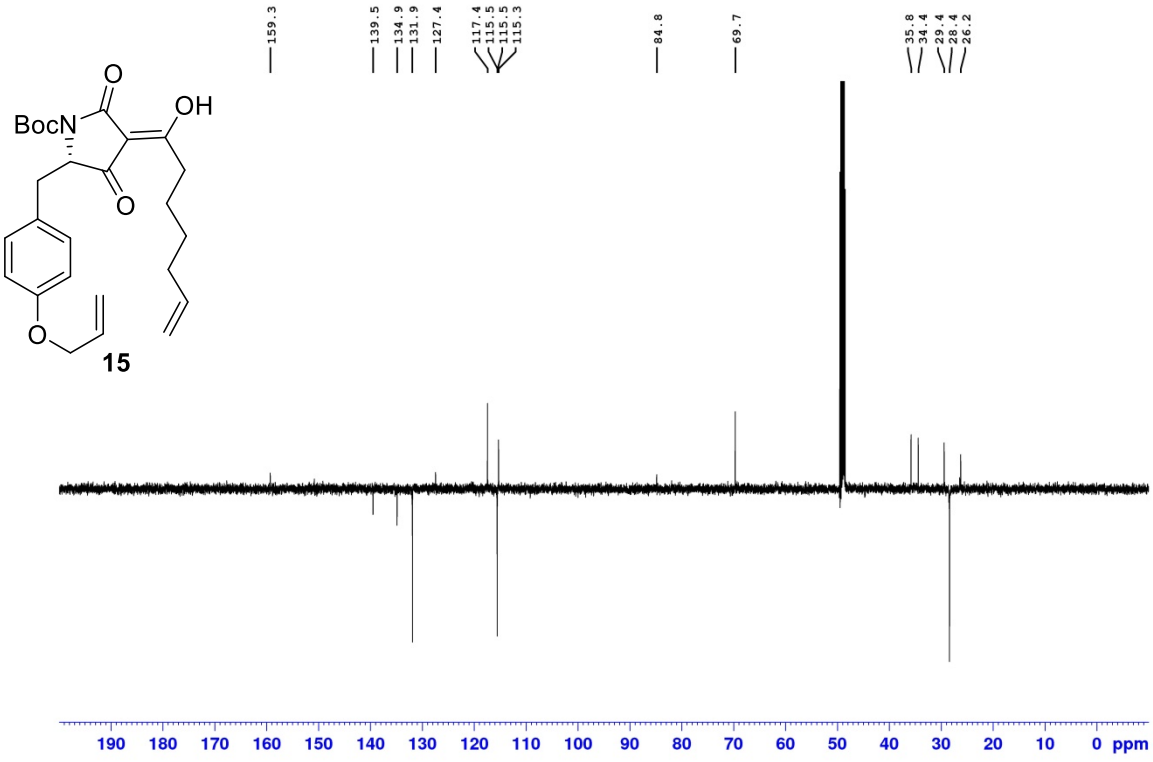

Figure S23. ^{13}C-NMR spectrum of compound **15** in CD_3OD.

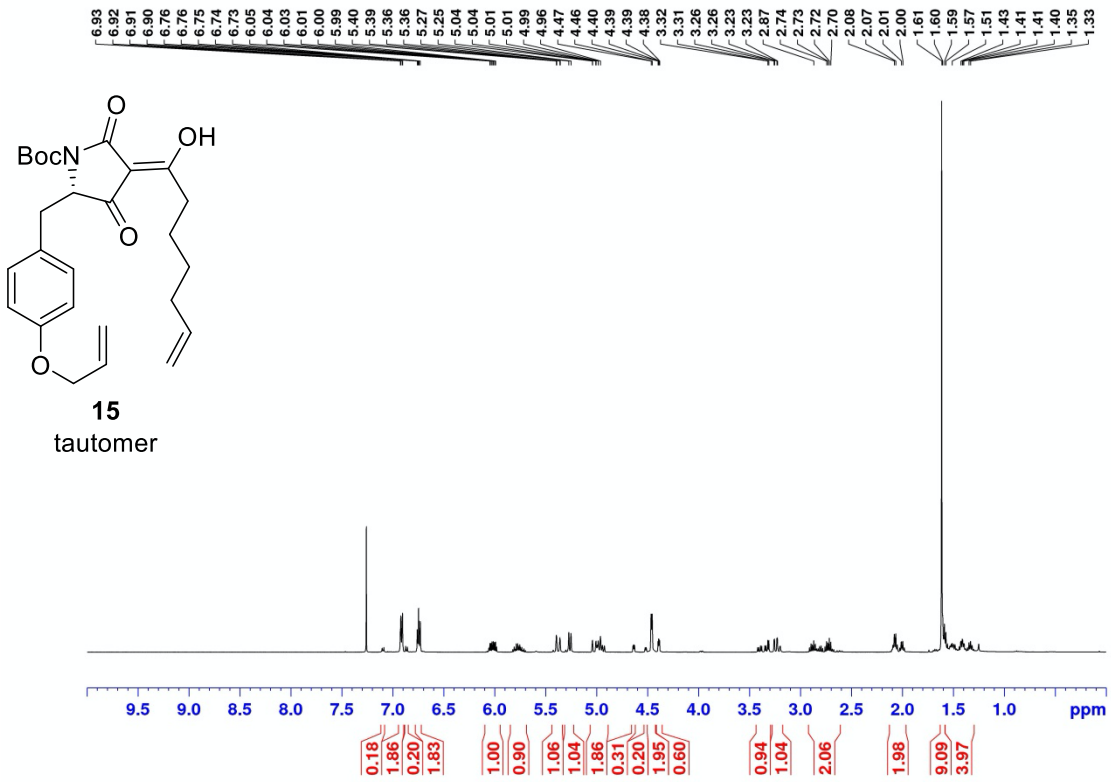

Figure S24. ^{1}H-NMR spectrum of compound **15** in $CDCl_3$.

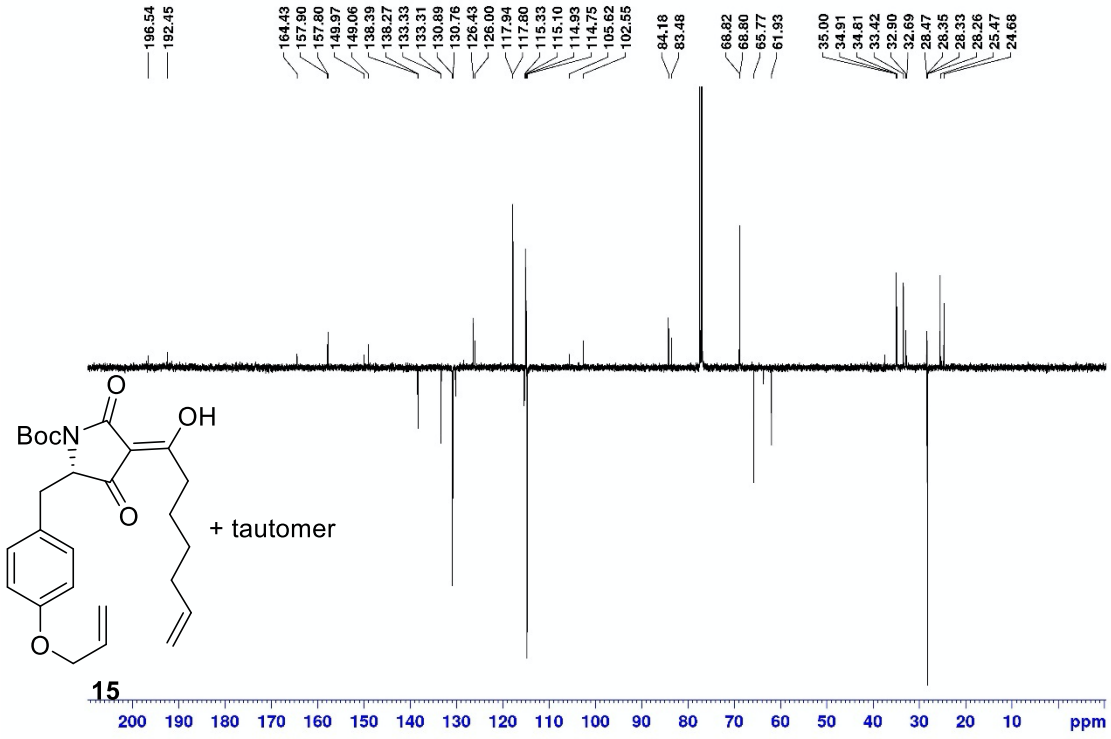

Figure S25. ^{13}C-NMR spectrum of compound **15** in $CDCl_3$.

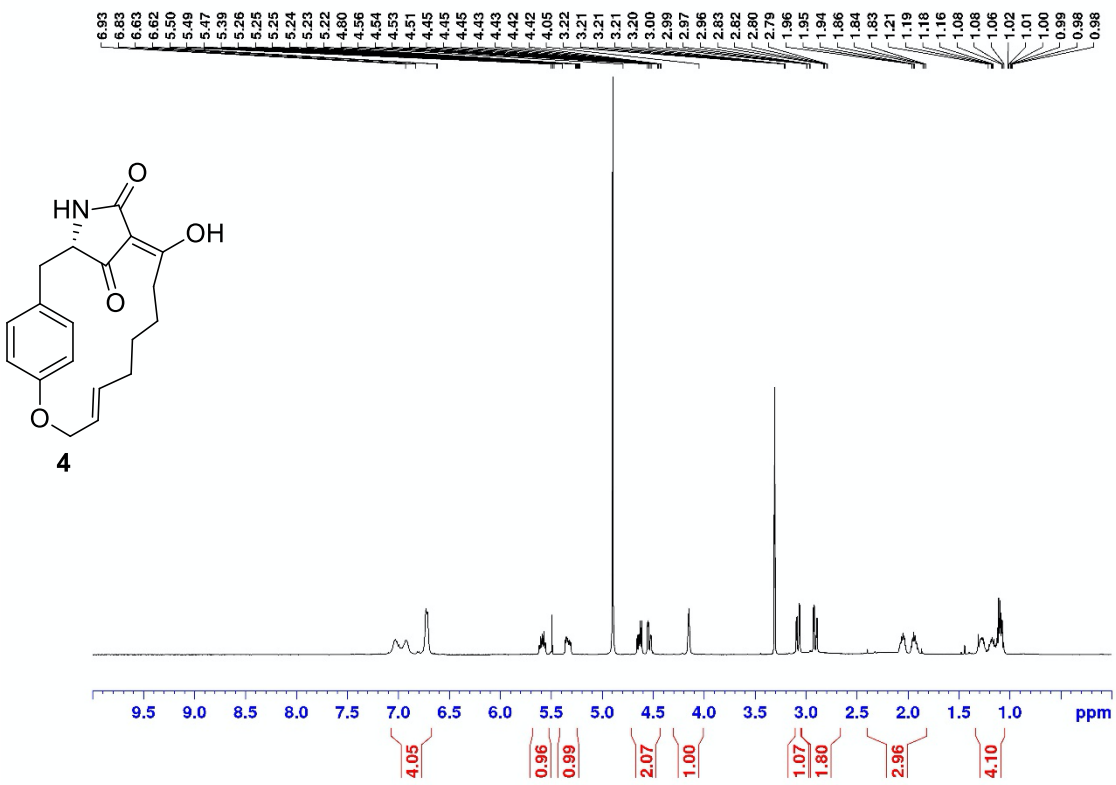

Figure S26. ^{1}H-NMR spectrum of compound **4** in CD_3OD.

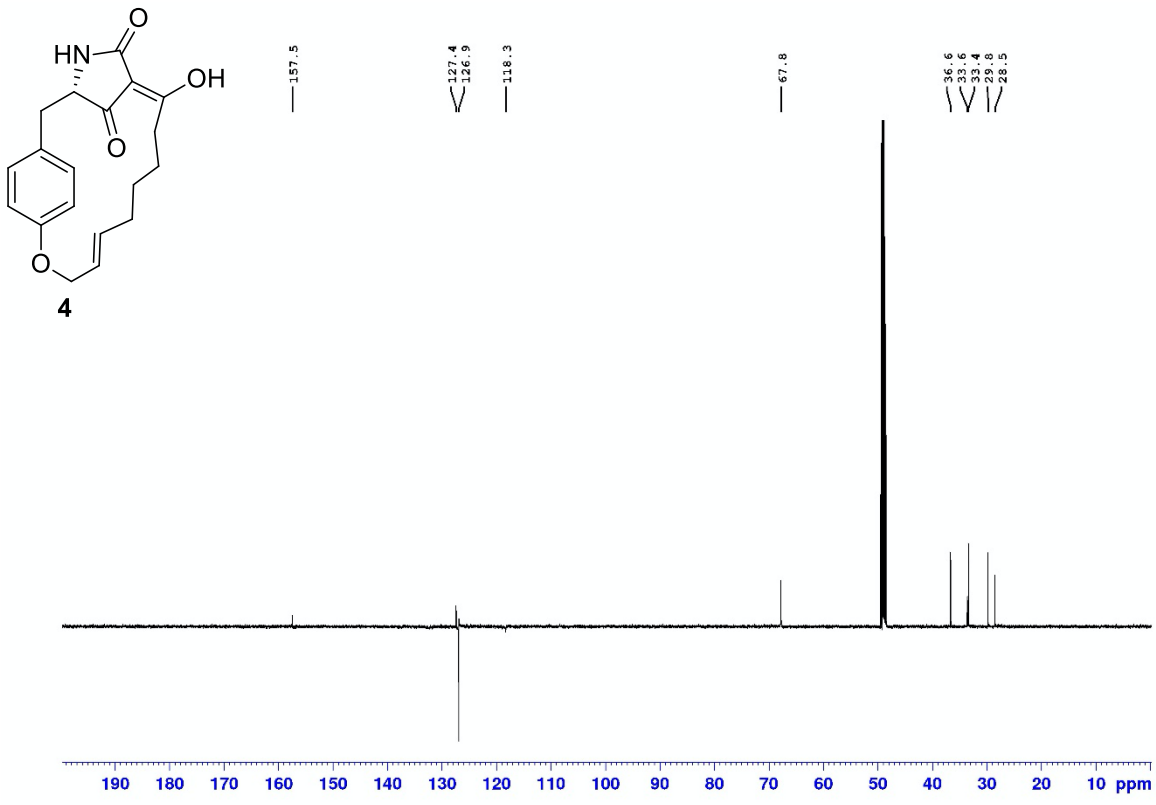

Figure S27. ^{13}C-NMR spectrum of compound **4** in CD_3OD.

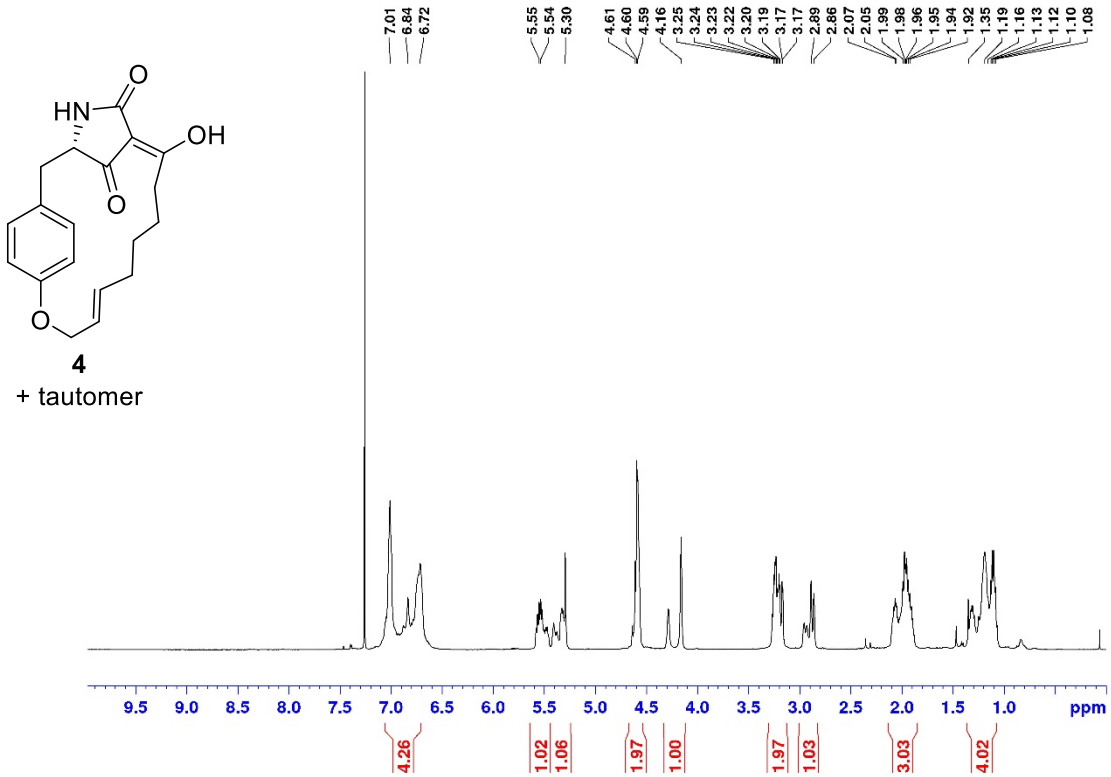

Figure S28. ^{1}H-NMR spectrum of compound **4** in $CDCl_3$.

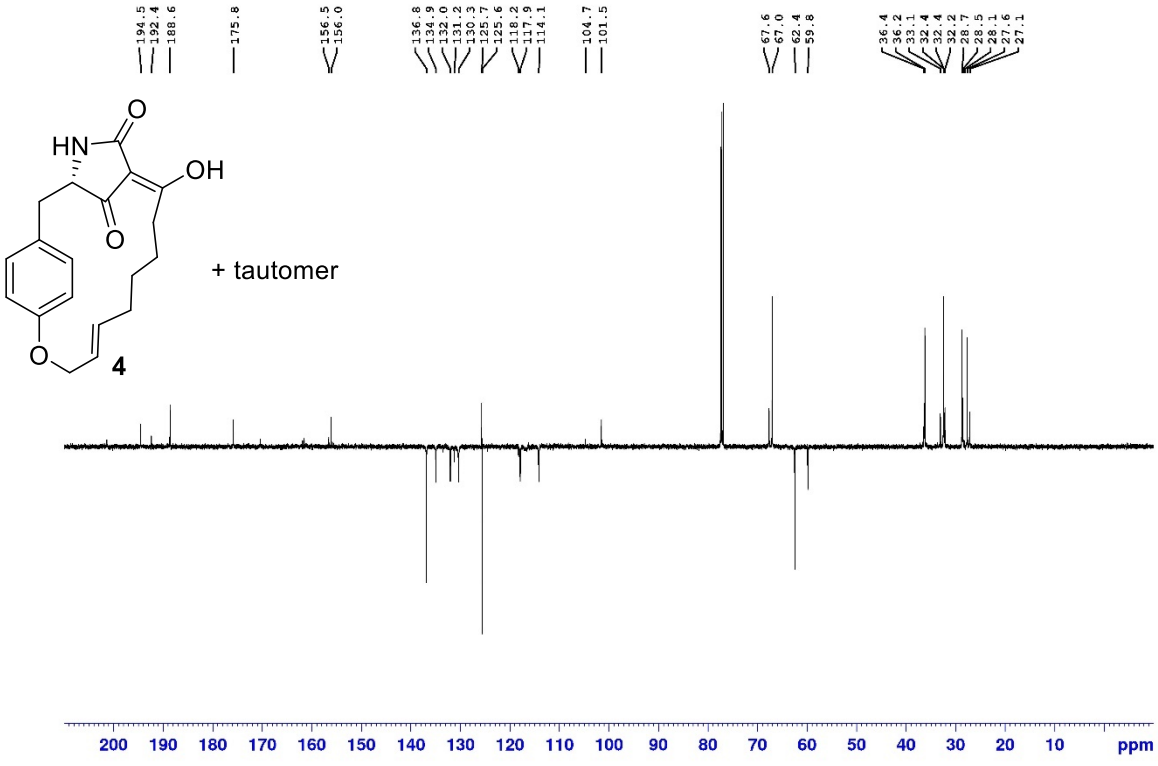

Figure S29. ^{13}C-NMR spectrum of compound **4** in $CDCl_3$.

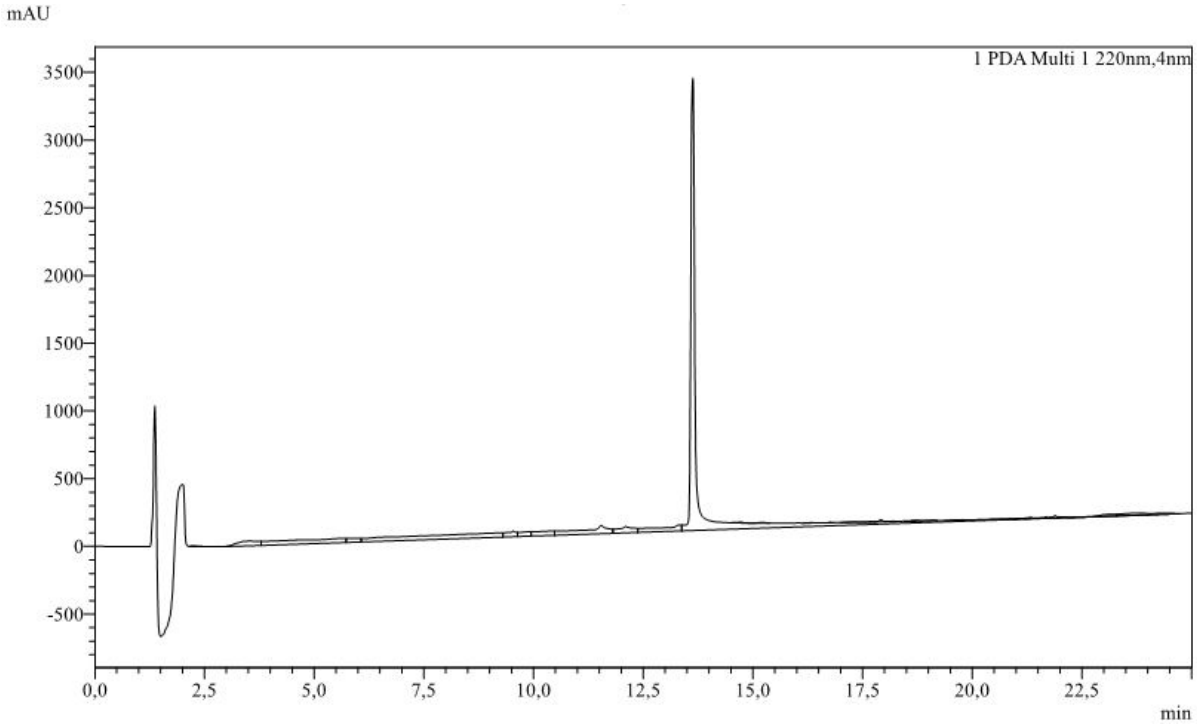

Figure S30. Chromatogram of compound **4**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 10% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

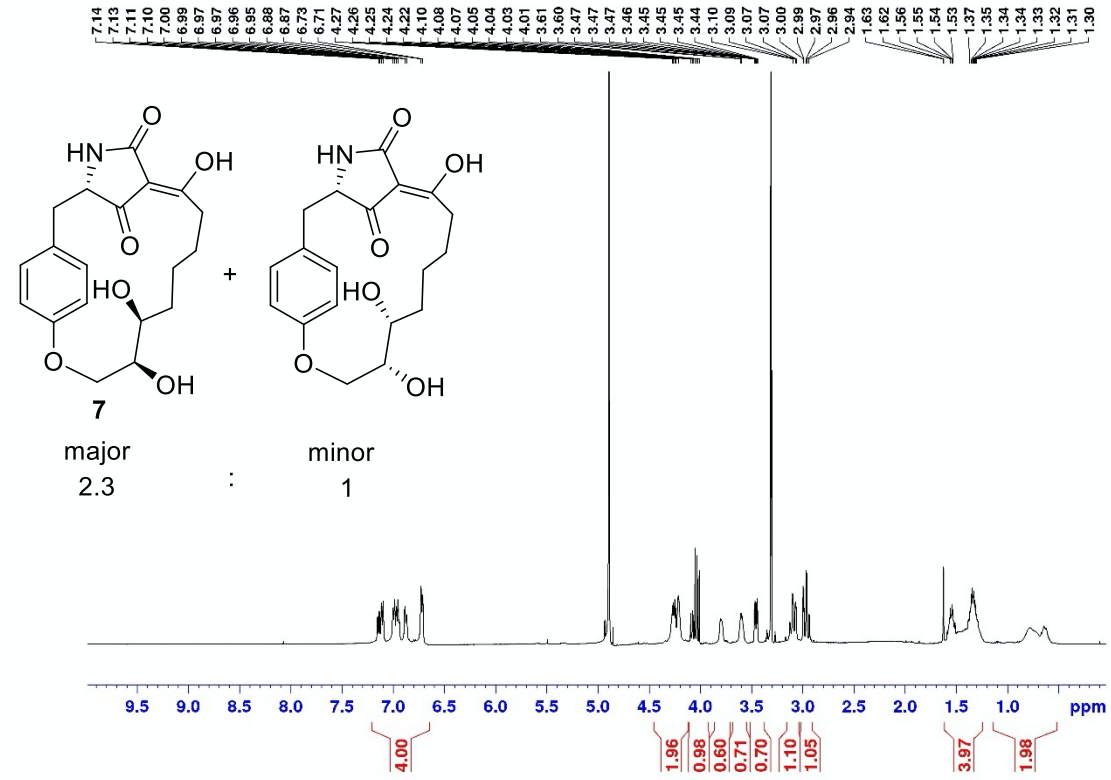

Figure S31. ^{1}H-NMR spectrum of compound **7** in CD_3OD.

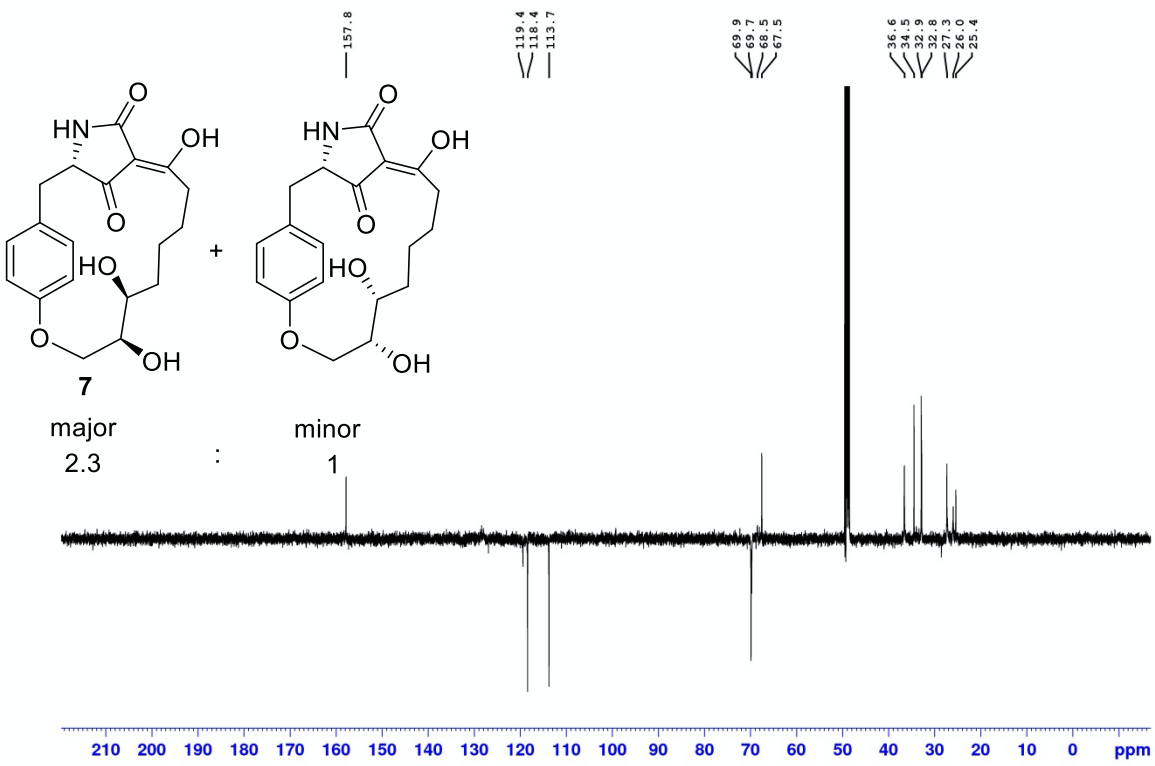

Figure S32. ^{13}C-NMR spectrum of compound **7** in CD_3OD.

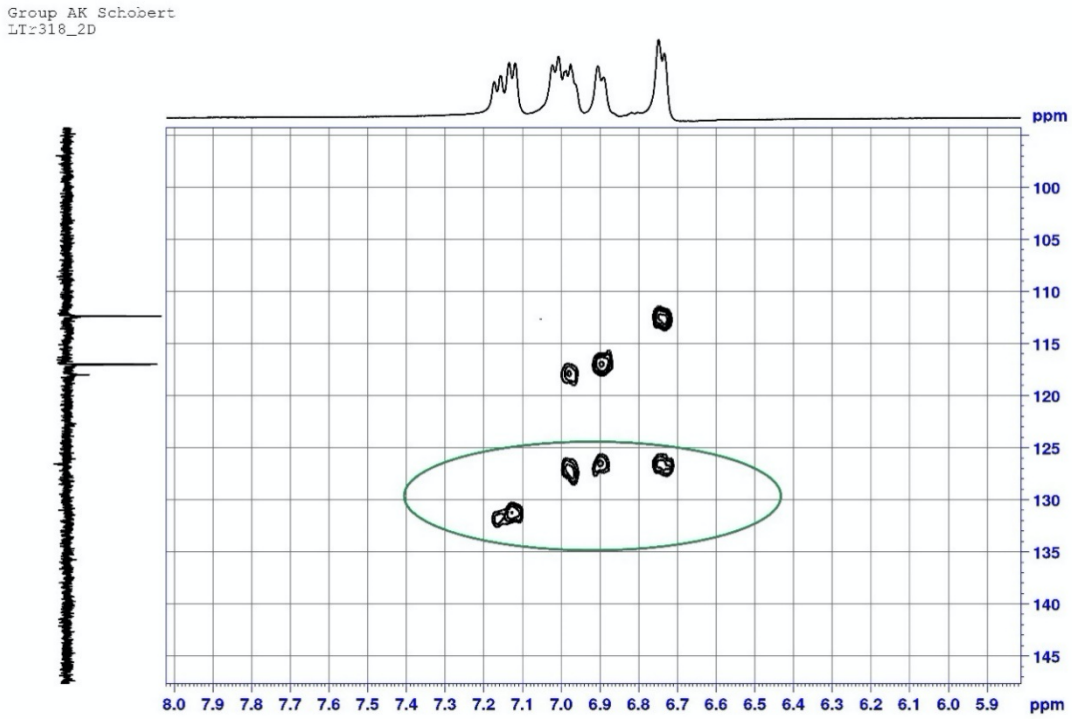

Figure S33. Part of HMBC-2D-NMR spectrum of compound **7** in CD_3OD, which was used for peak assignment.

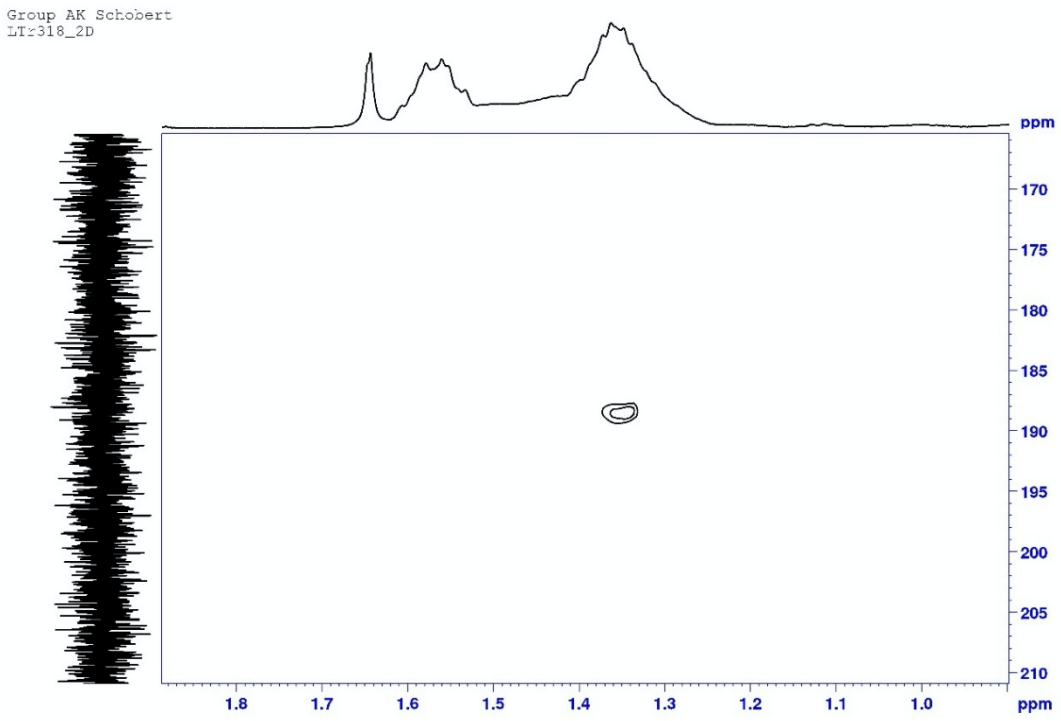

Figure S34. Part of HMBC-2D-NMR spectrum of compound **7** in CD_3OD, which was used for peak assignment.

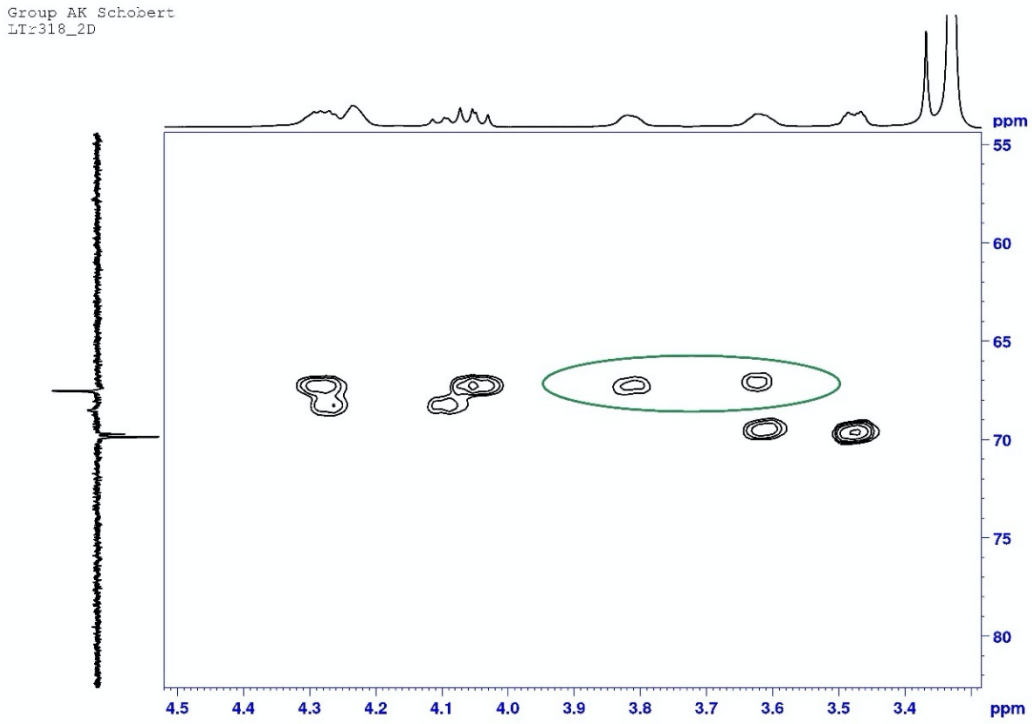

Figure S35. Part of HSQC-2D-NMR spectrum of compound **7** in CD_3OD, which was used for peak assignment.

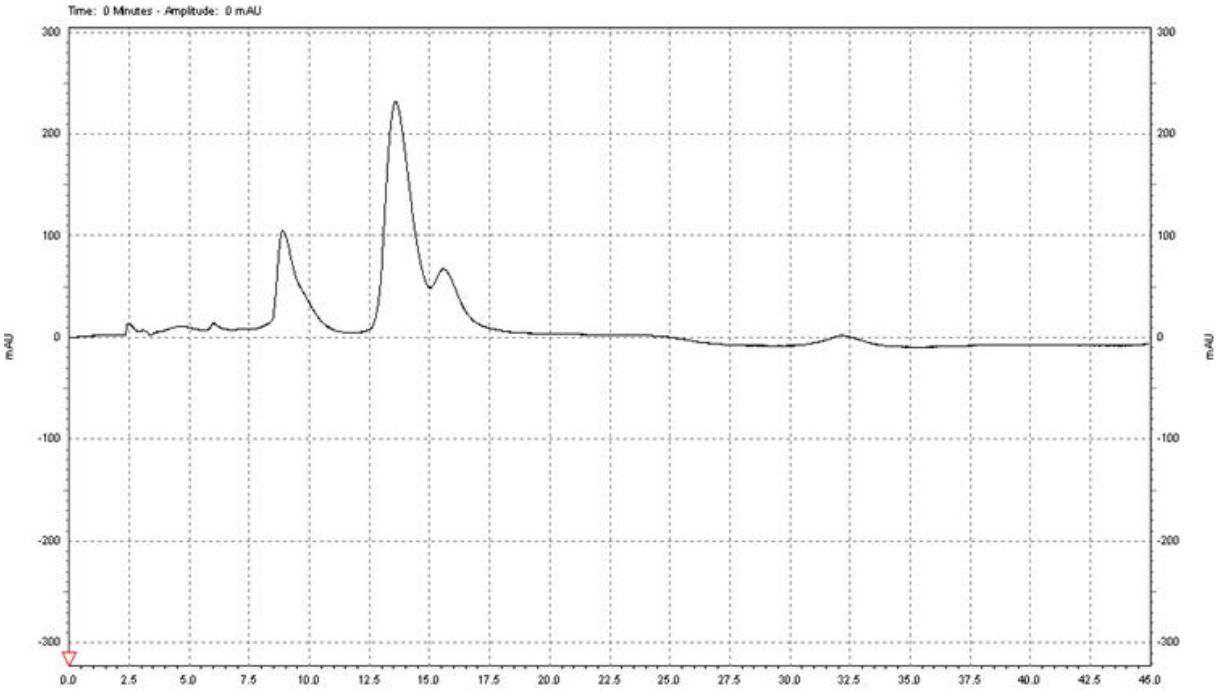

Figure S36. Chromatogram of compound **7**. HPLC: *Beckmann System Gold Programmable Solvent Modul 126,* Beckmann instruments diode array detection module 128, Phenomenex Lux® Amylose-1-HPLC column (100 × 4.6 mm). Method: 30% EtOH in hexanes → 40% EtOH in hexanes → 50% EtOH in hexanes → 60% EtOH in hexanes → 80% EtOH in hexanes, flow: 0.7 mL/min. A mixture of tautomers and diastereomers can be seen

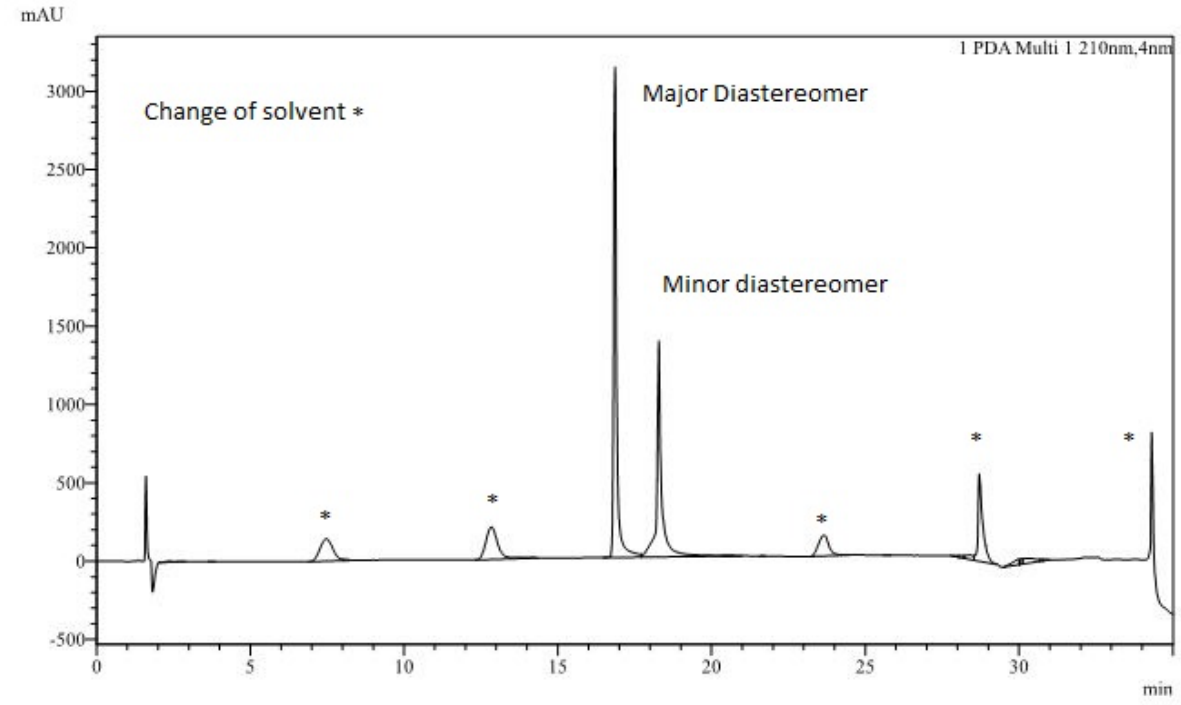

Figure S37. Chromatogram of compound **7**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 5% MeCN in H_2O + 0.1% HCOOH → 10% MeCN in H_2O + 0.1% HCOOH → 20% MeCN in H_2O + 0.1% HCOOH → 30% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH→ 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

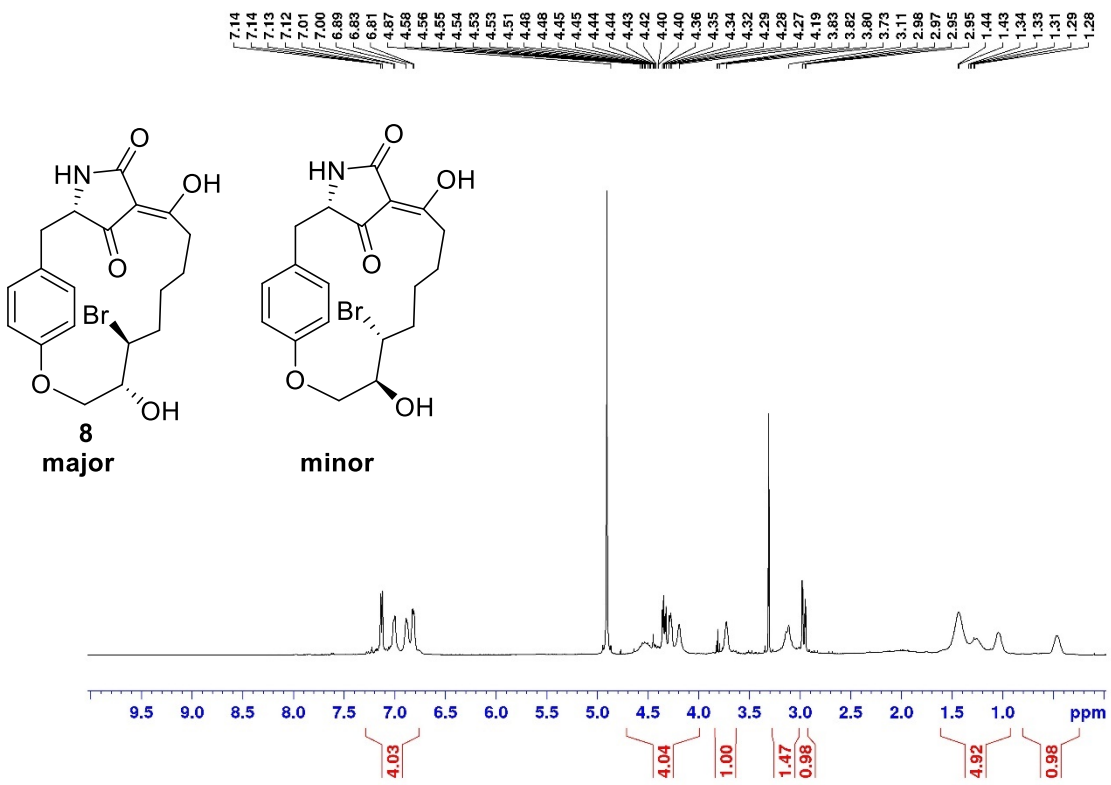

Figure S38. ^{1}H-NMR spectrum of compound **8** in CD_3OD.

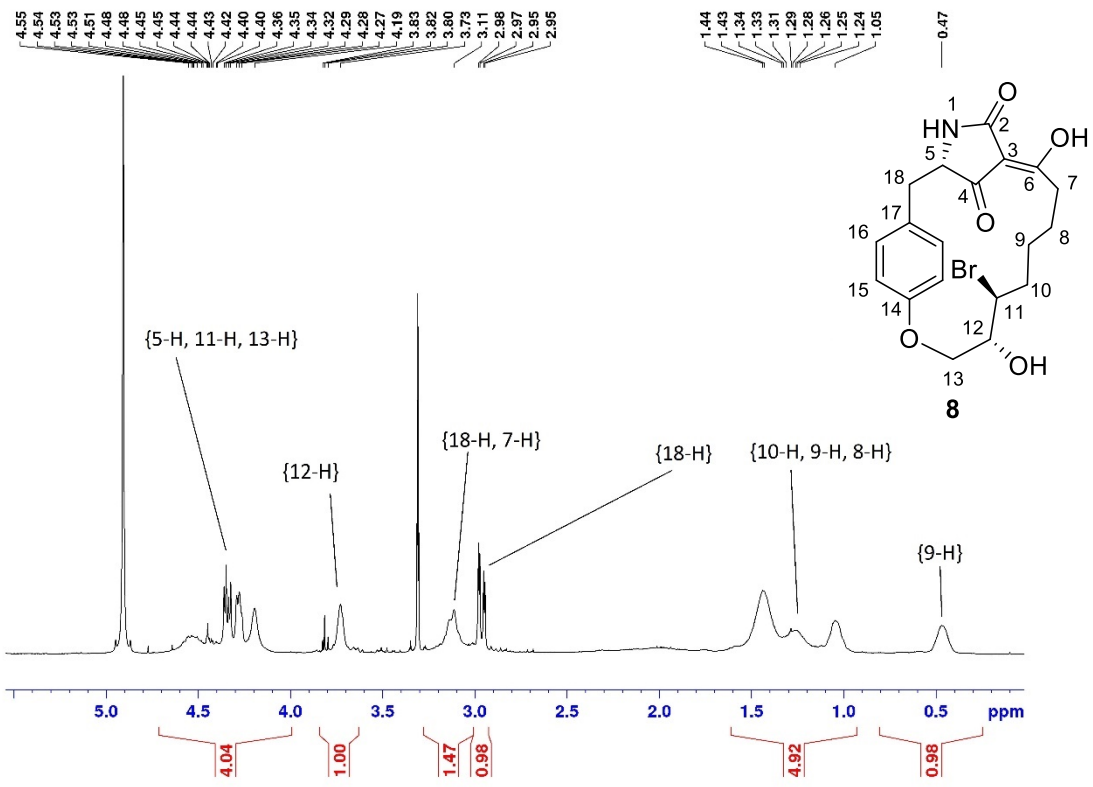

Figure S39. ^{1}H-NMR spectrum of compound **8** between 5.5 ppm and 0.0 ppm in CD_3OD with assignment of signals.

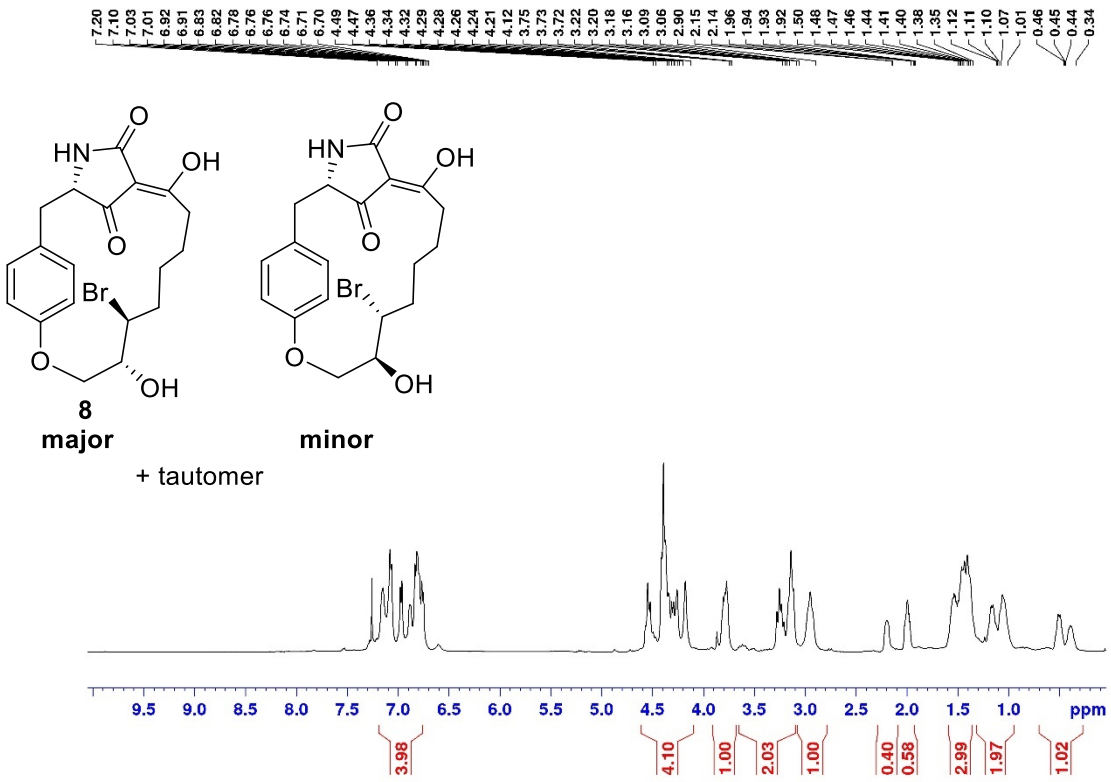

Figure S40. ^{1}H-NMR spectrum of compound **8** in $CDCl_3$.

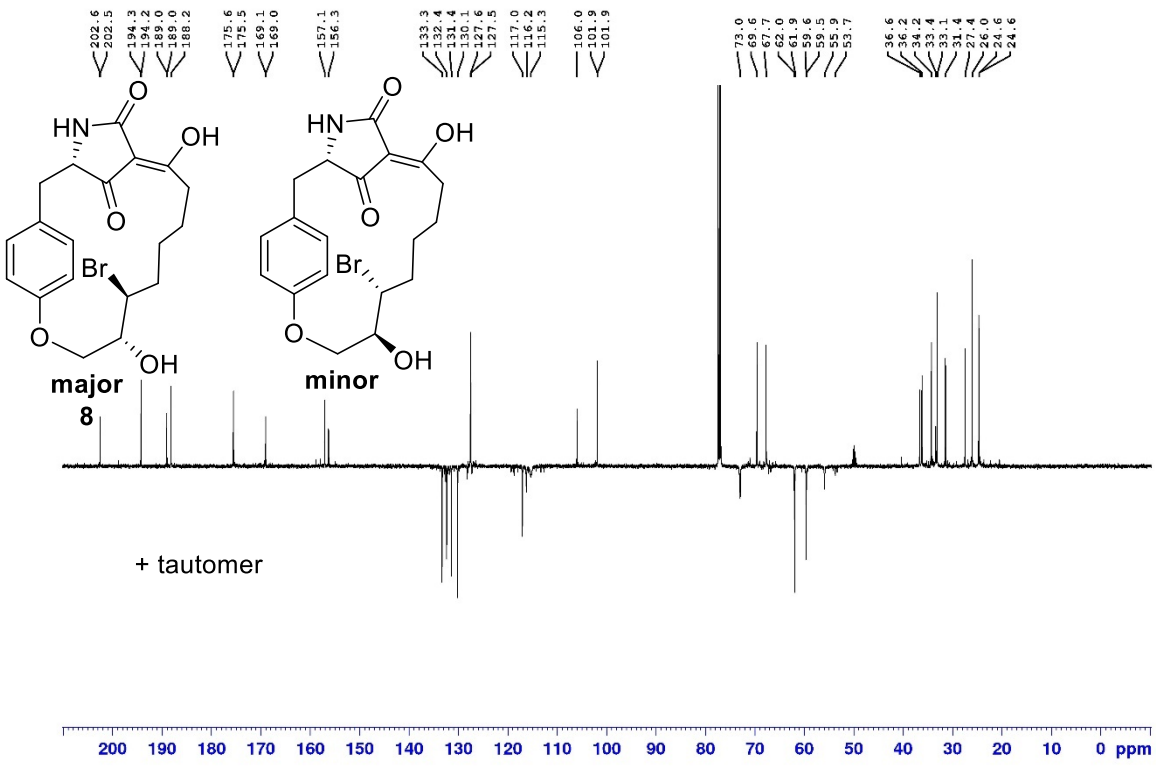

Figure S41. ^{13}C-NMR spectrum of compound **8** in $CDCl_3$.

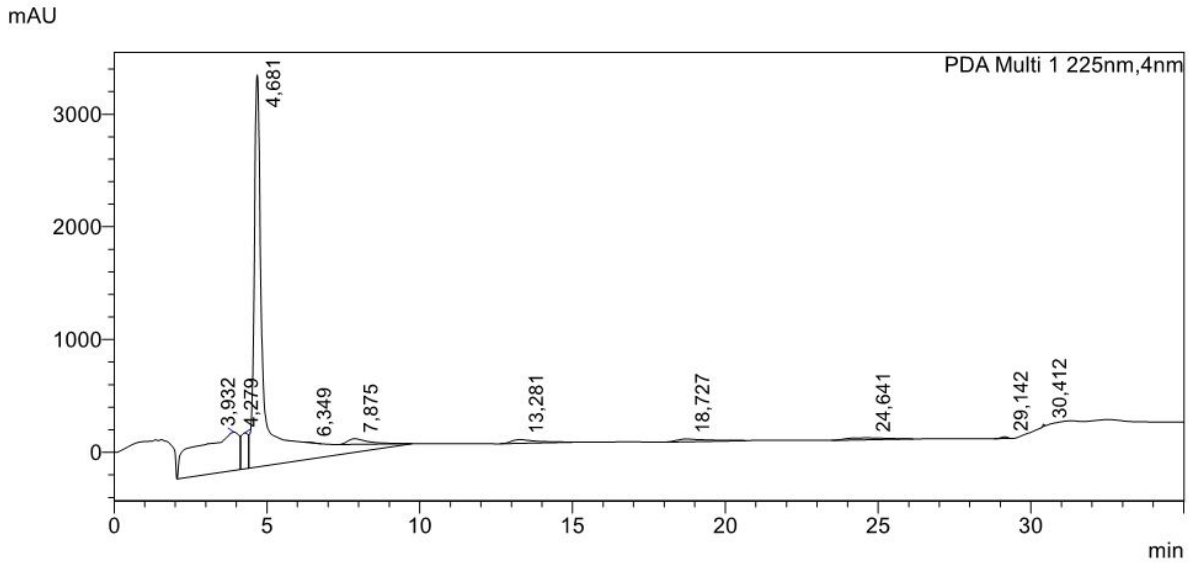

Figure S42. Chromatogram of compound **8**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 30% MeCN in H_2O + 0.1% HCOOH → 35% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 45% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH→ 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

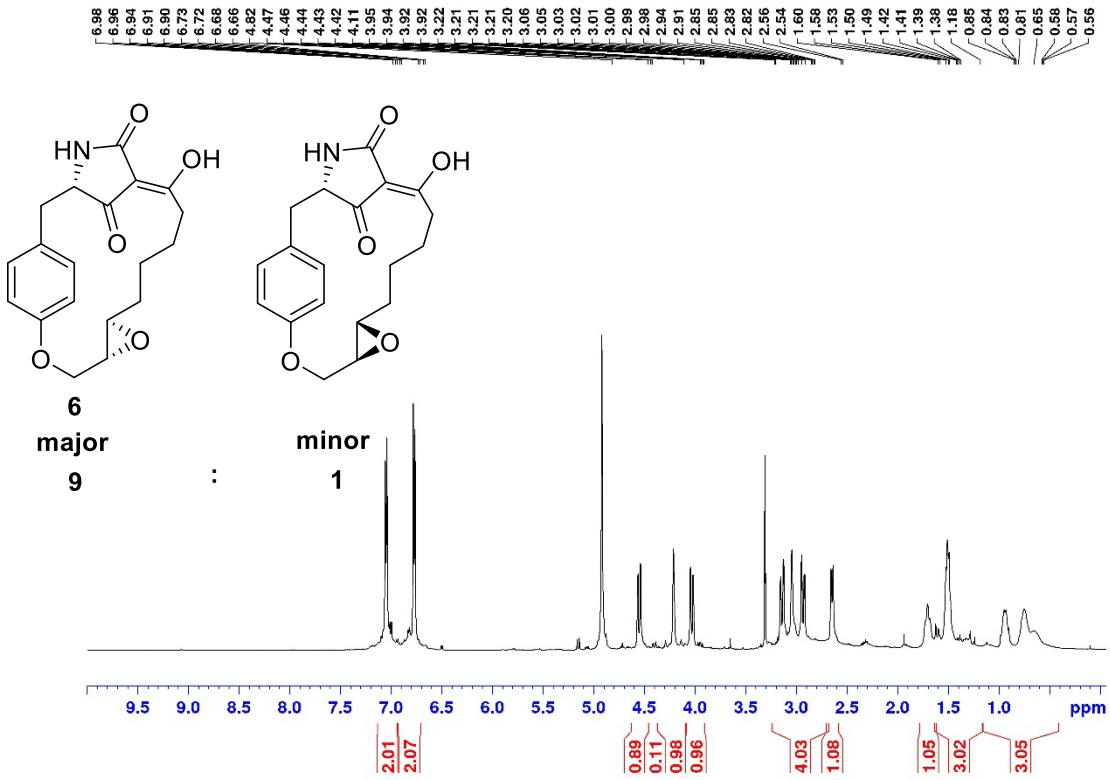

Figure S43. ^{1}H-NMR spectrum of compound **6** in CD_3OD.

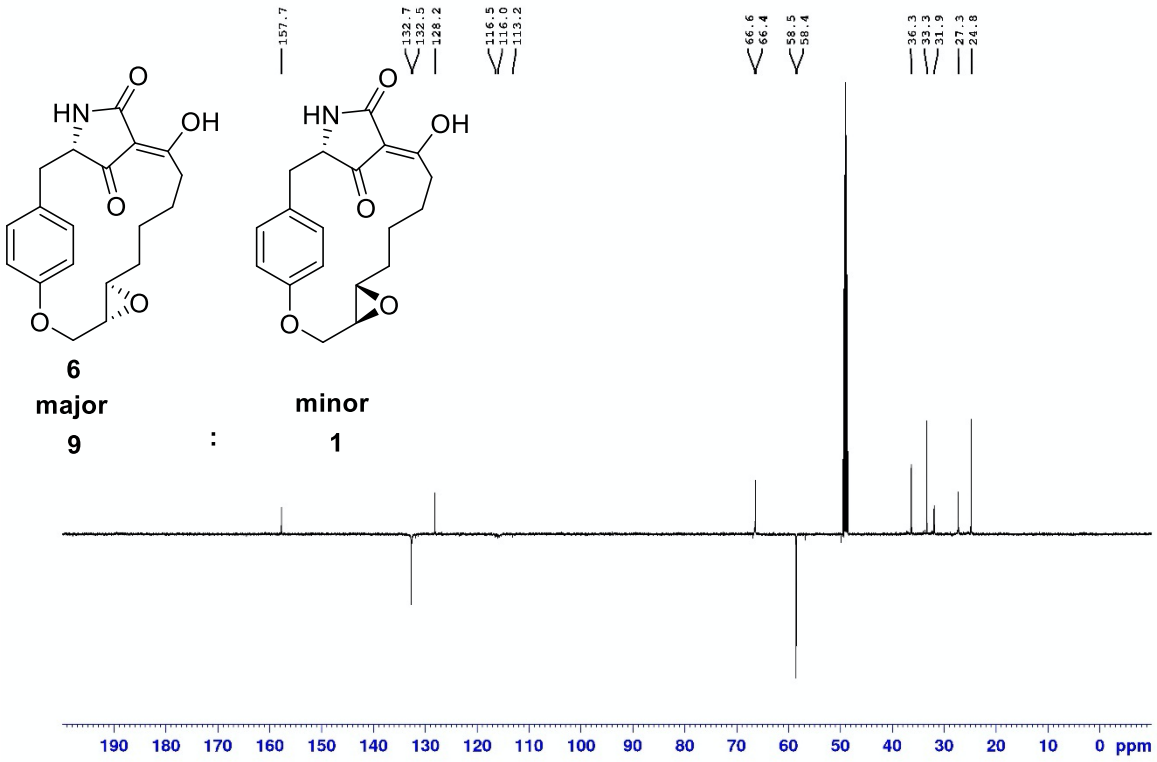

Figure S44. ^{13}C-NMR spectrum of compound **6** in CD_3OD.

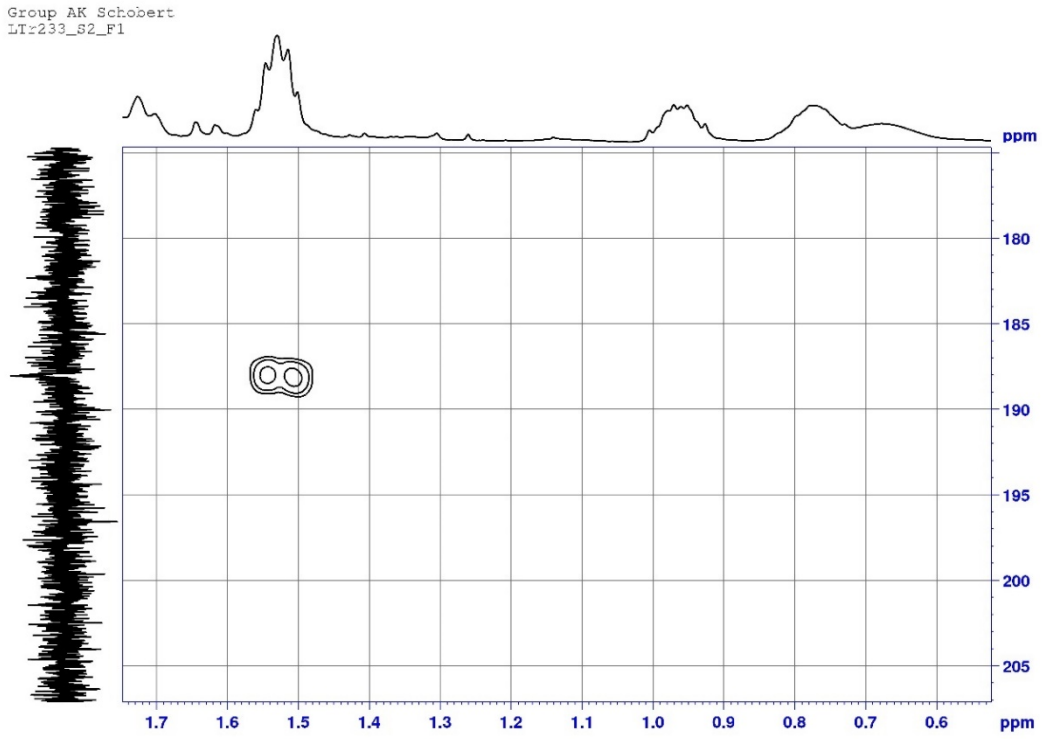

Figure S45. Part of HMBC-2D-NMR spectrum of compound **6** in CD_3OD, which was used for peak assignment.

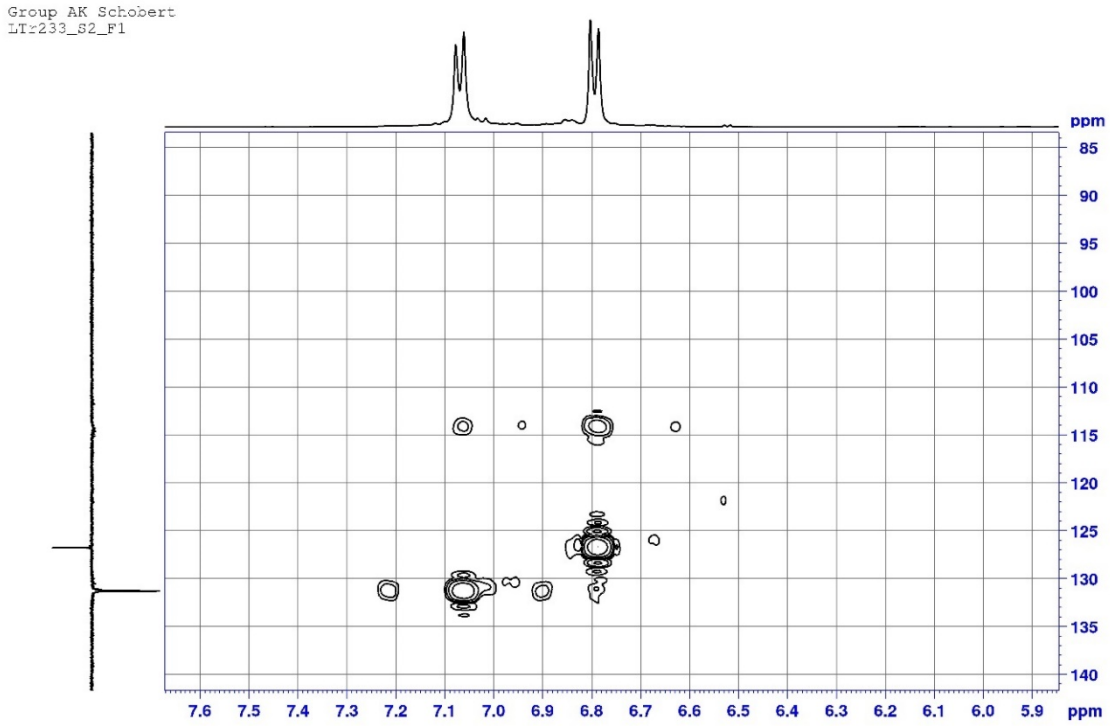

Figure S46. Part of HMBC-2D-NMR spectrum of compound **6** in CD_3OD, which was used for peak assignment.

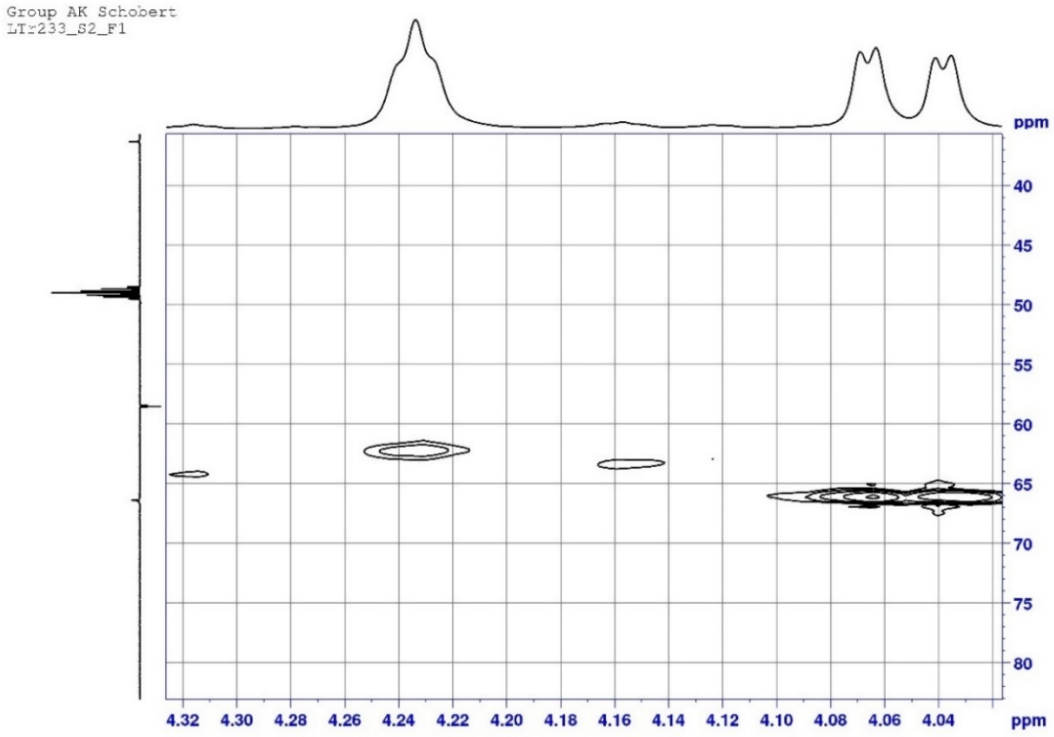

Figure S47. Part of HSQC-2D-NMR spectrum of compound **6** in CD_3OD, which was used for peak assignment.

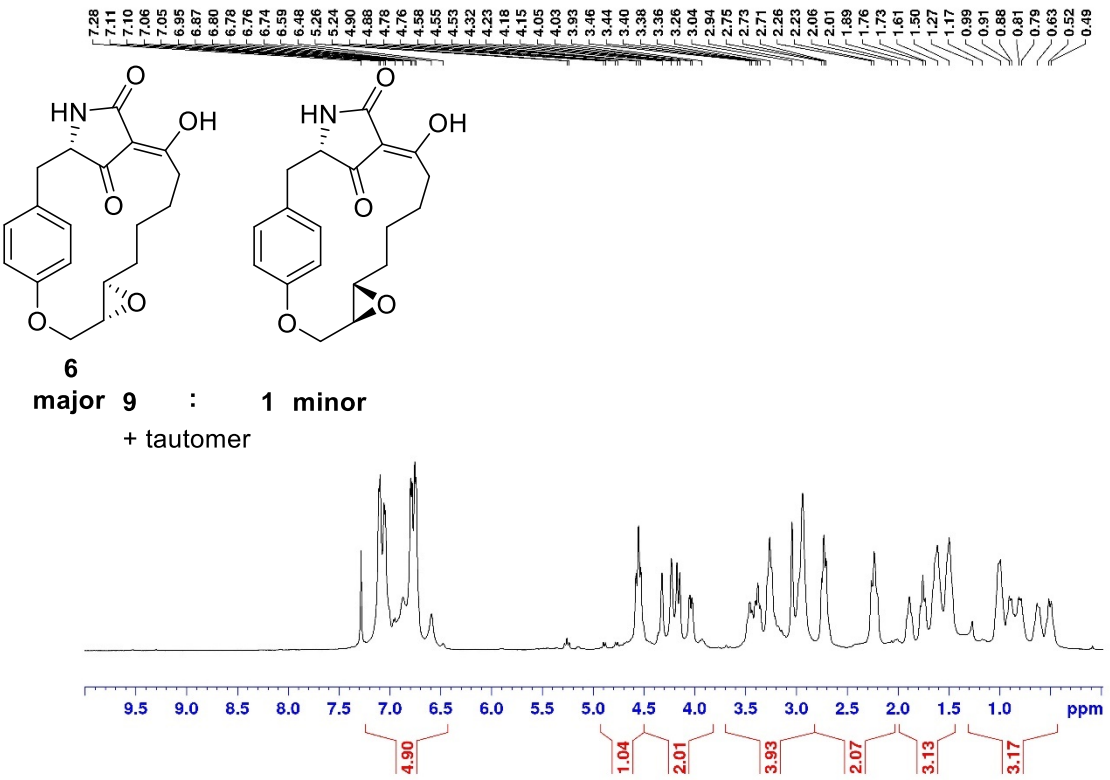

Figure S48. ^{1}H-NMR spectrum of compound **6** in $CDCl_3$.

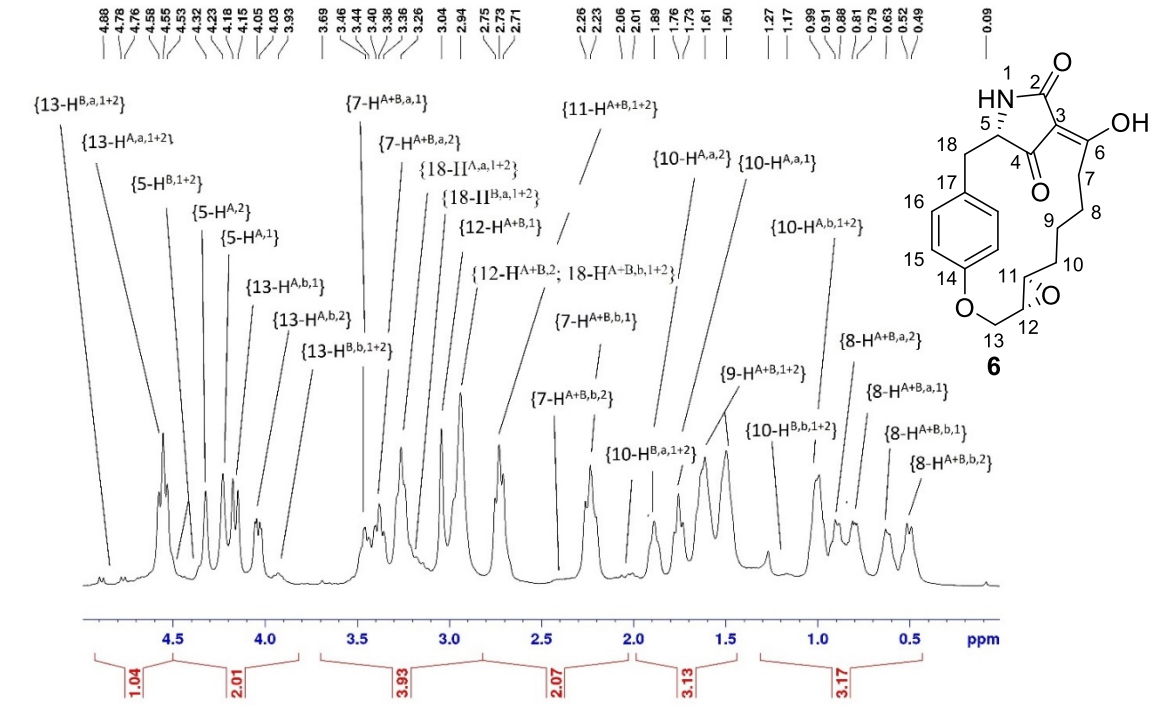

Figure S49. [1]H-NMR spectrum of compound **6** between 5.0 ppm and 0.0 ppm in $CDCl_3$ with assignment of signals. Signals of major diastereomer marked as A, signals of minor diastereomer marked as B; diastereotopic H-atoms indicated as a, b; signals of major tautomer marked as 1, signals of minor tautomer marked as 2.

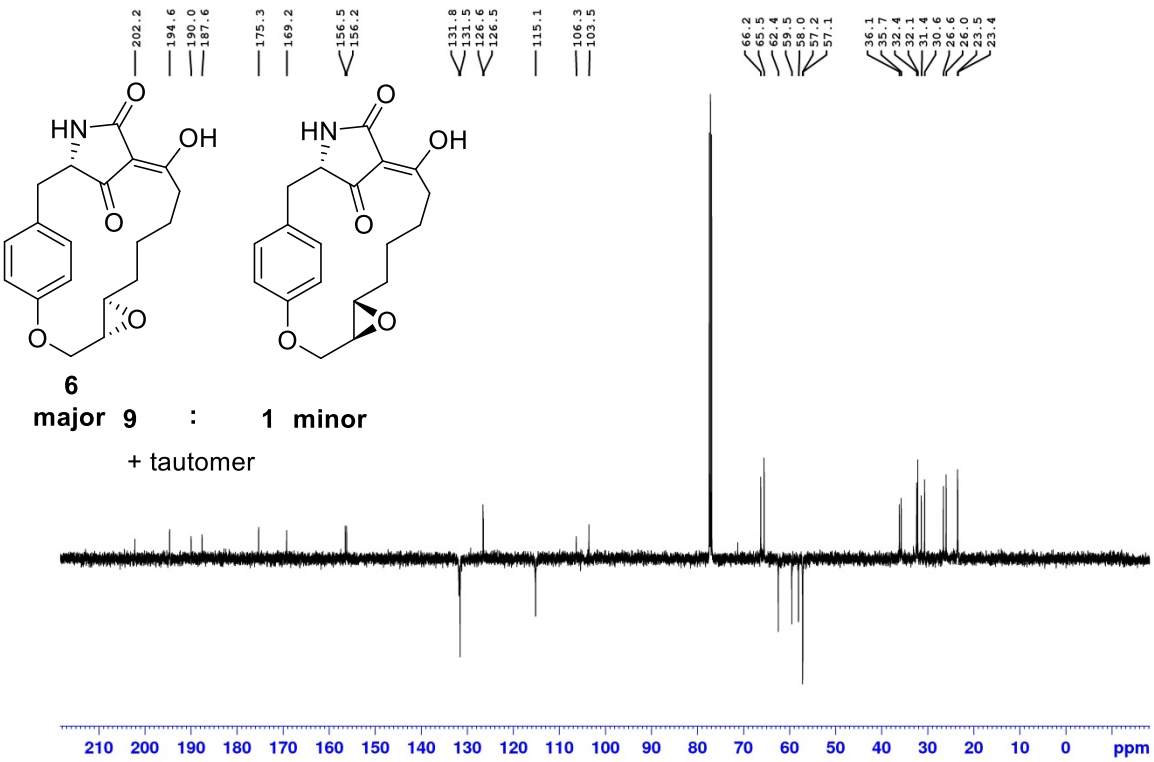

Figure S50. [13]C-NMR spectrum of compound **6** in $CDCl_3$.

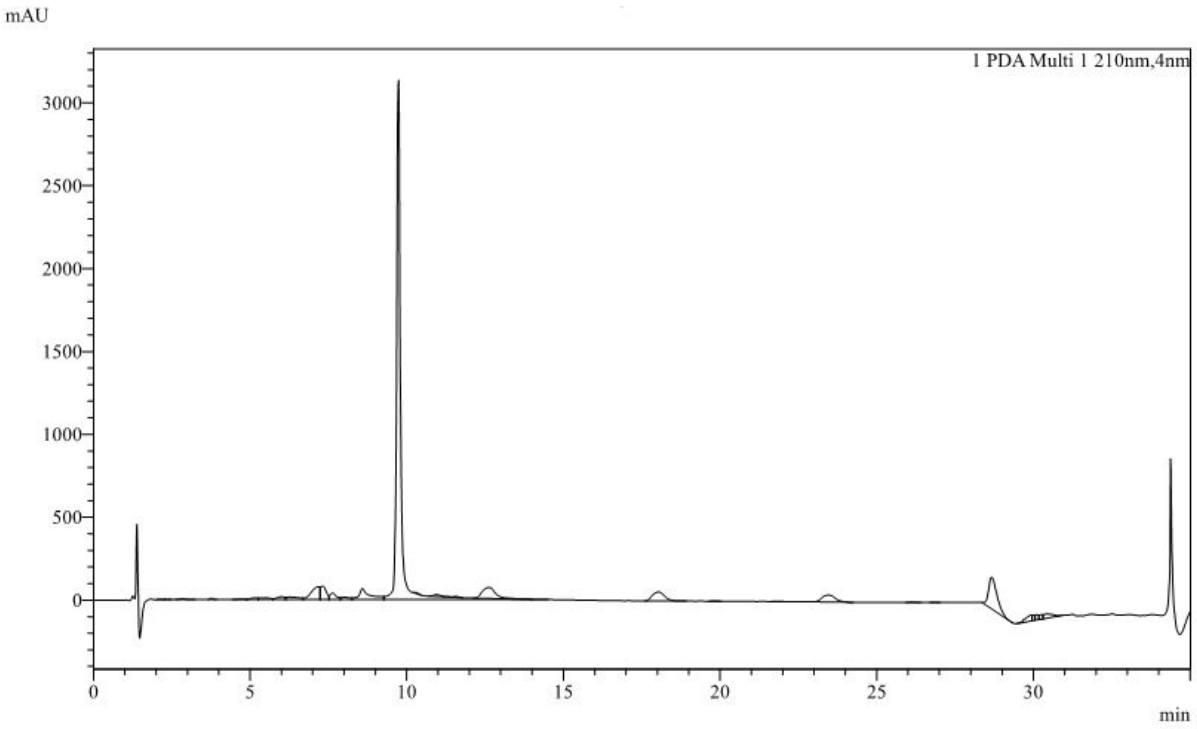

Figure S51. Chromatogram of compound **6**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 30% MeCN in H_2O + 0.1% HCOOH → 35% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 45% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH→ 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

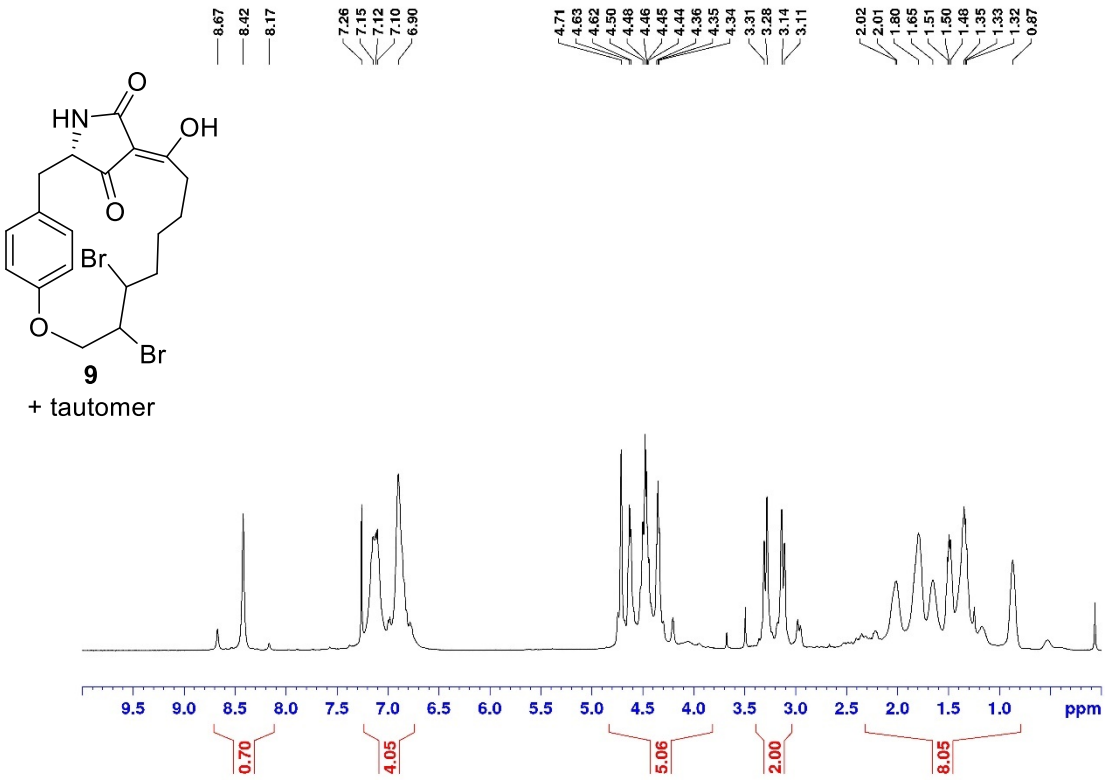

Figure S52. ^{1}H-NMR spectrum of compound **9** in $CDCl_3$.

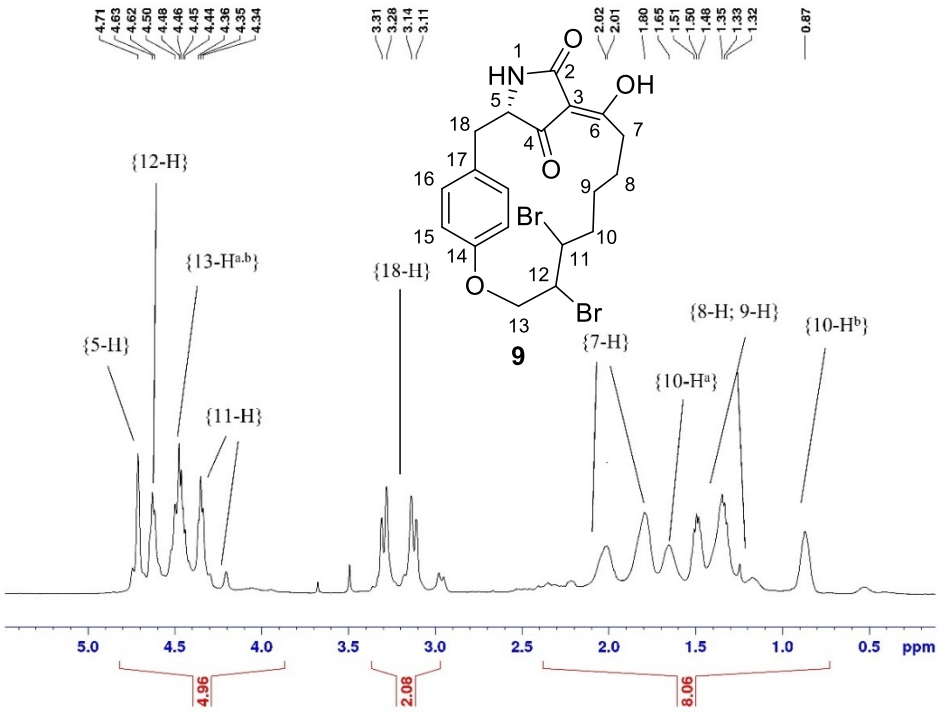

Figure S53. ^{1}H-NMR spectrum of compound **9** between 5.0 ppm and 0.0 ppm in $CDCl_3$ with assignment of signals. Signals of major diastereomer marked as A, signals of minor diastereomer marked as B; diastereotopic H-atoms indicated as a, b; signals of major tautomer marked as 1, signals of minor tautomer marked as 2.

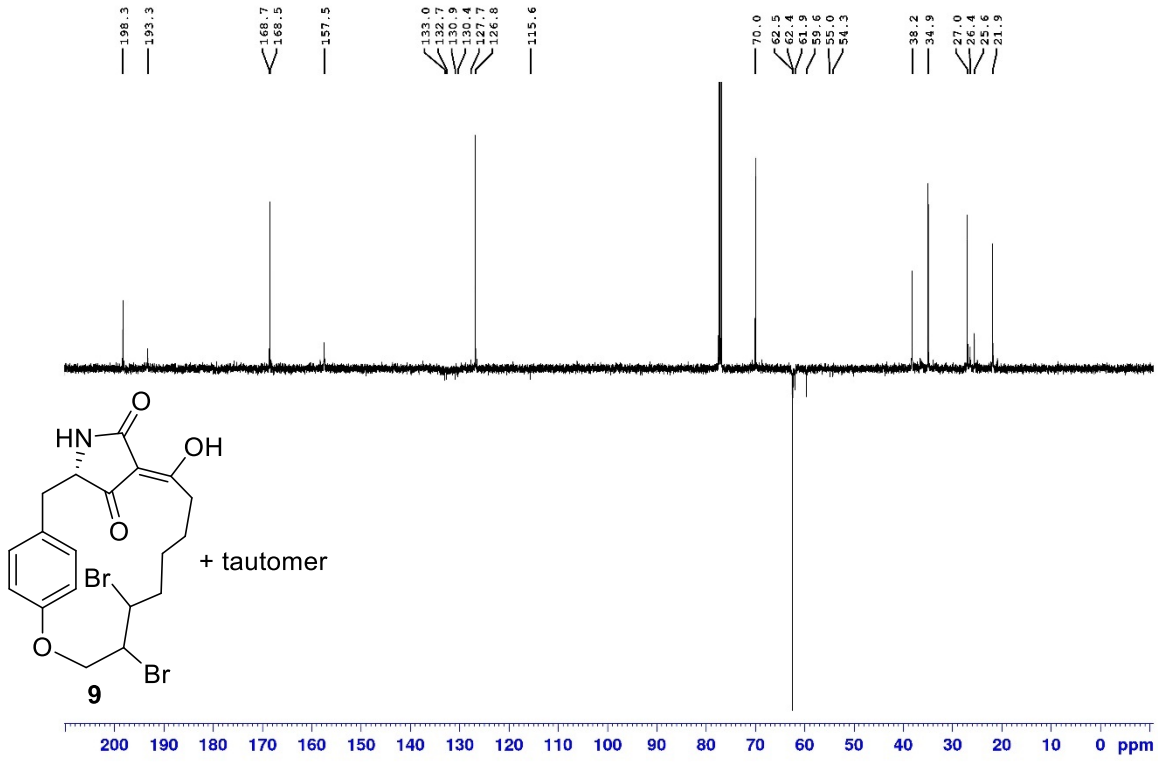

Figure S54. ^{13}C-NMR spectrum of compound **9** in $CDCl_3$.

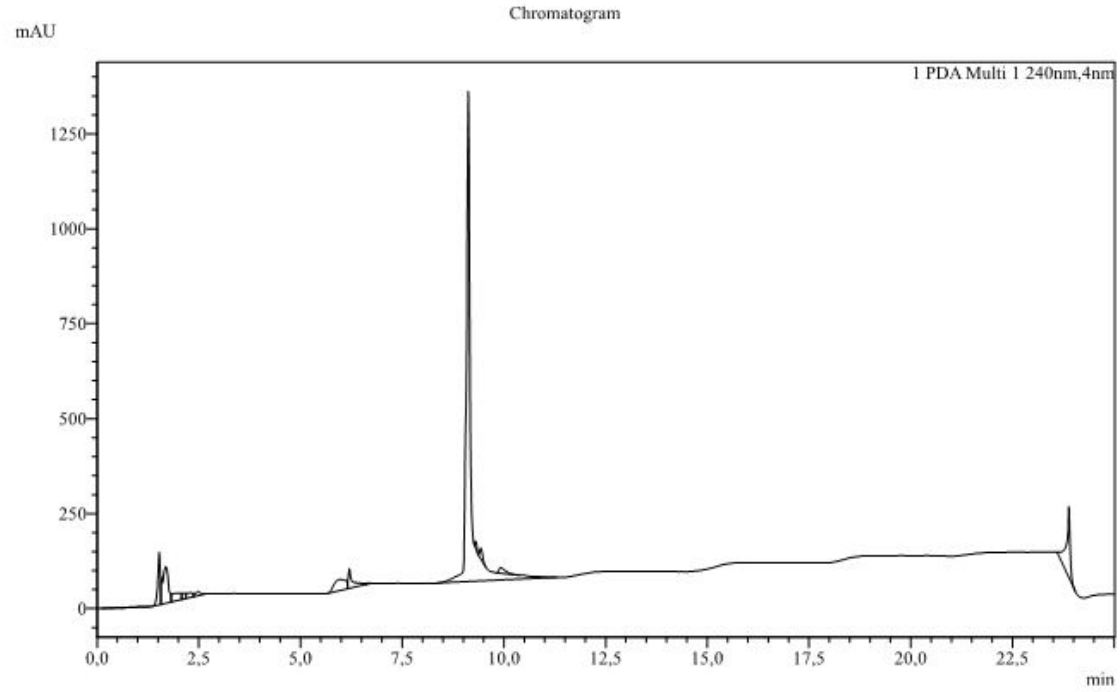

Figure S55. Chromatogram of compound **9**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 70% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 90% MeCN in H_2O + 0.1% HCOOH → 95% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

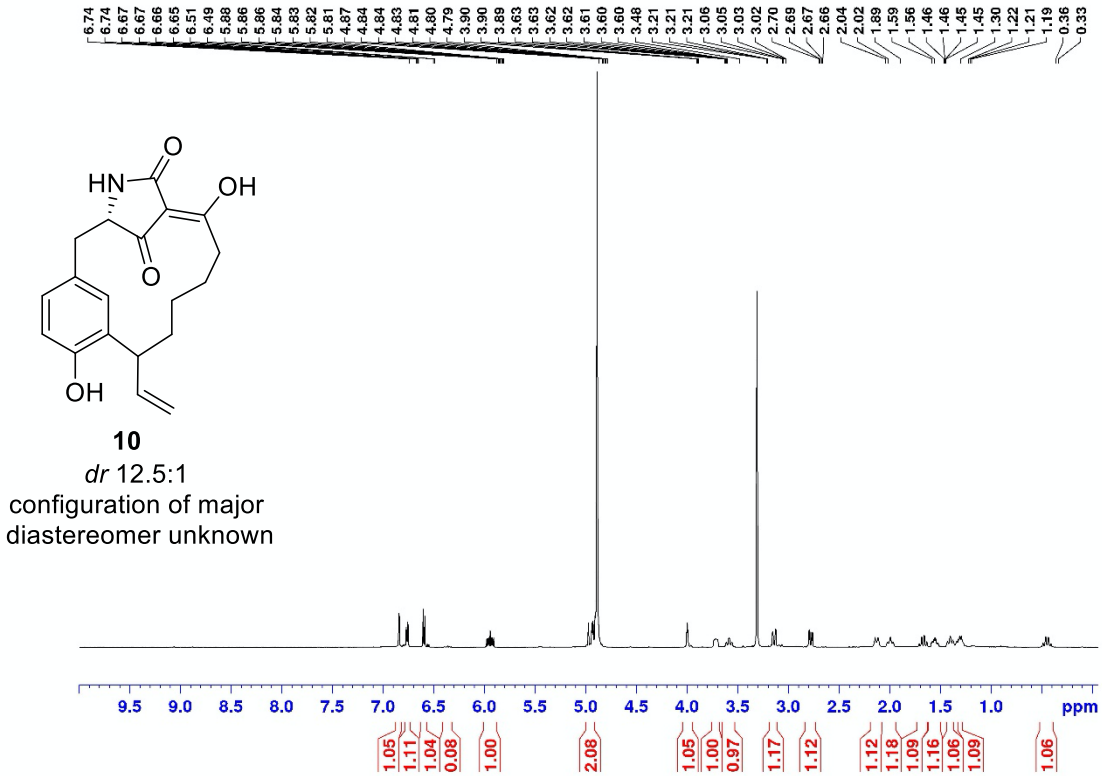

Figure S56. ^{1}H-NMR spectrum of compound **10** in CD_3OD.

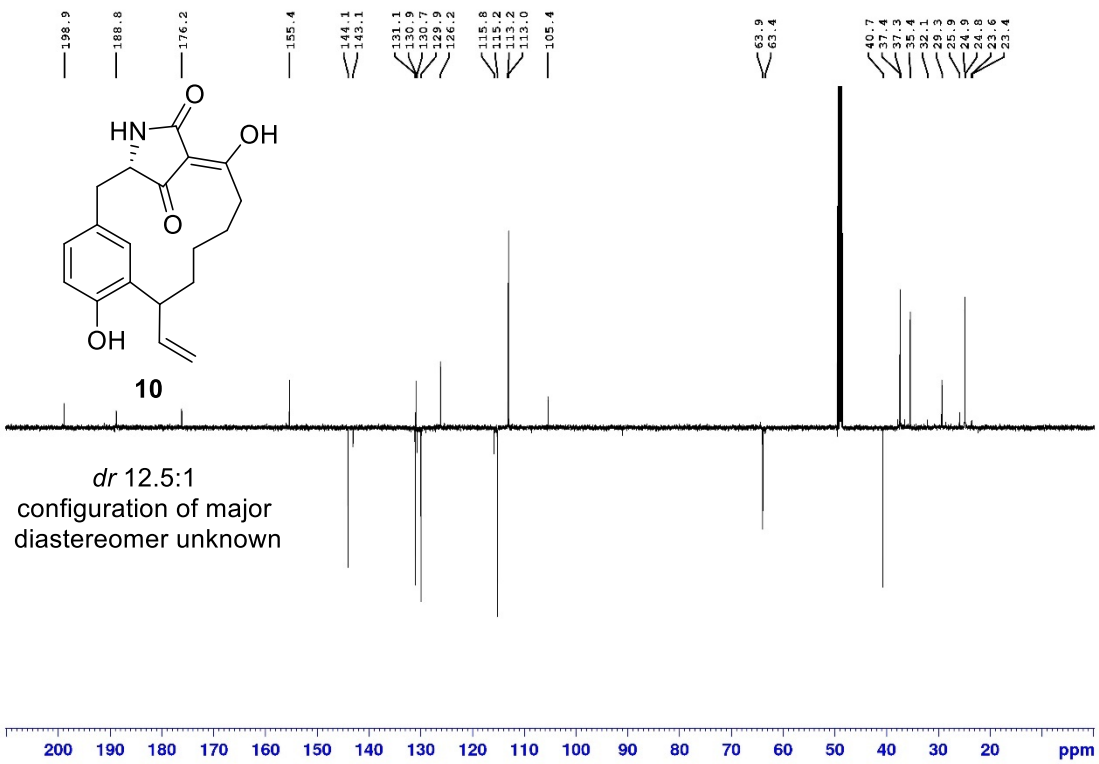

Figure S57. ^{13}C-NMR spectrum of compound **10** in CD_3OD.

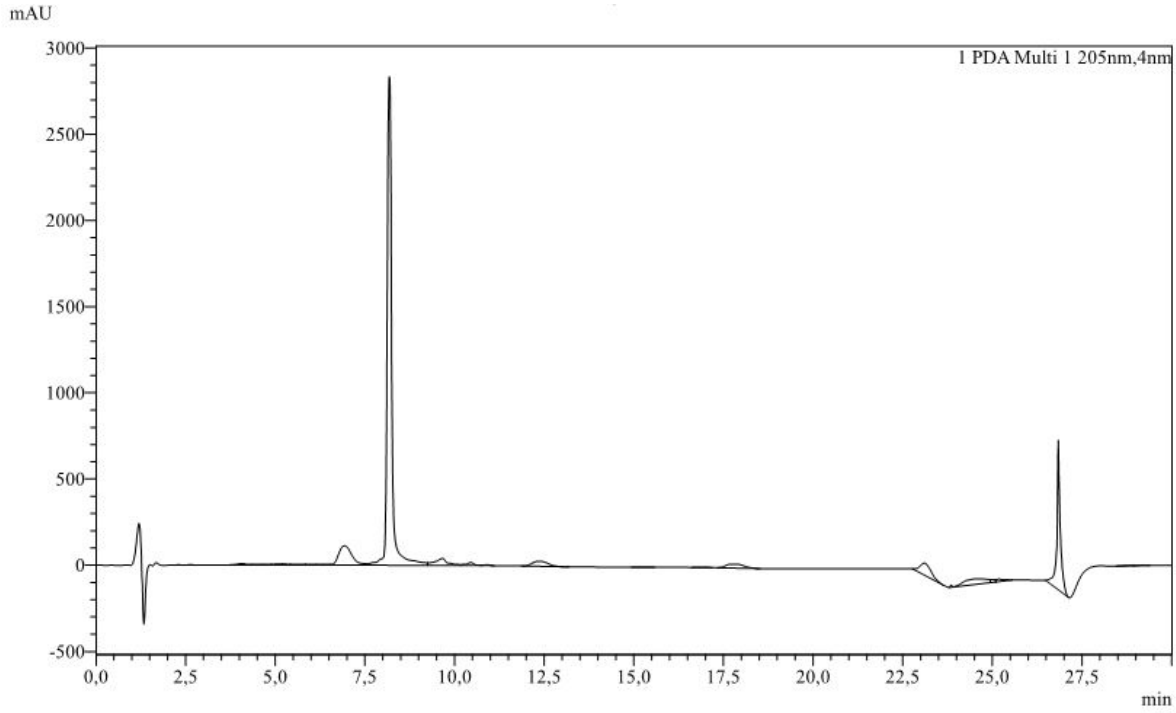

Figure S58. Chromatogram of compound **10**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 55% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

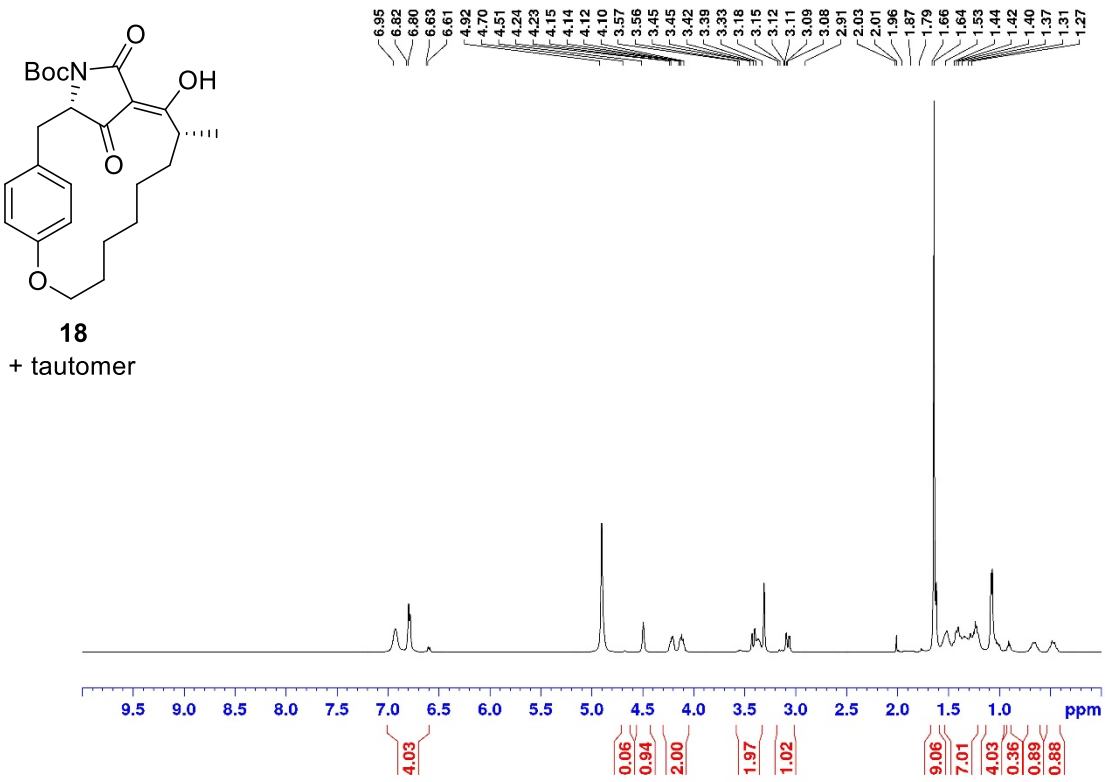

Figure S59. ^{1}H-NMR spectrum of compound **18** in CD_3OD.

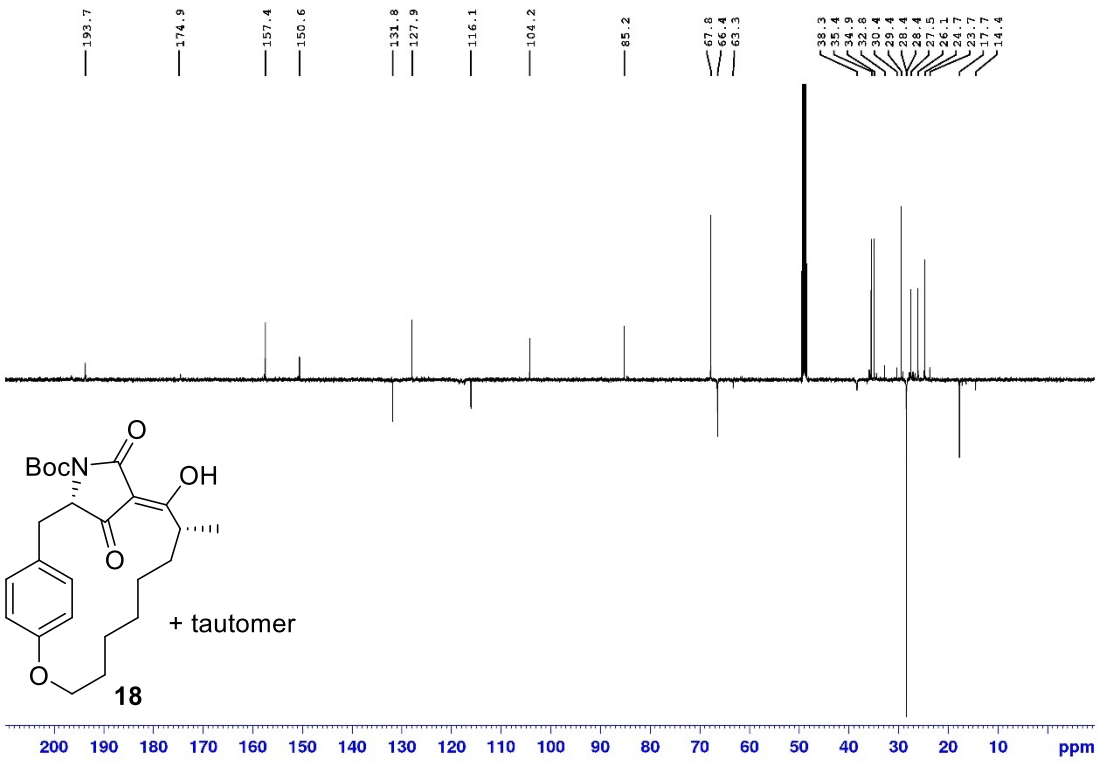

Figure S60. ^{13}C-NMR spectrum of compound **18** in CD_3OD.

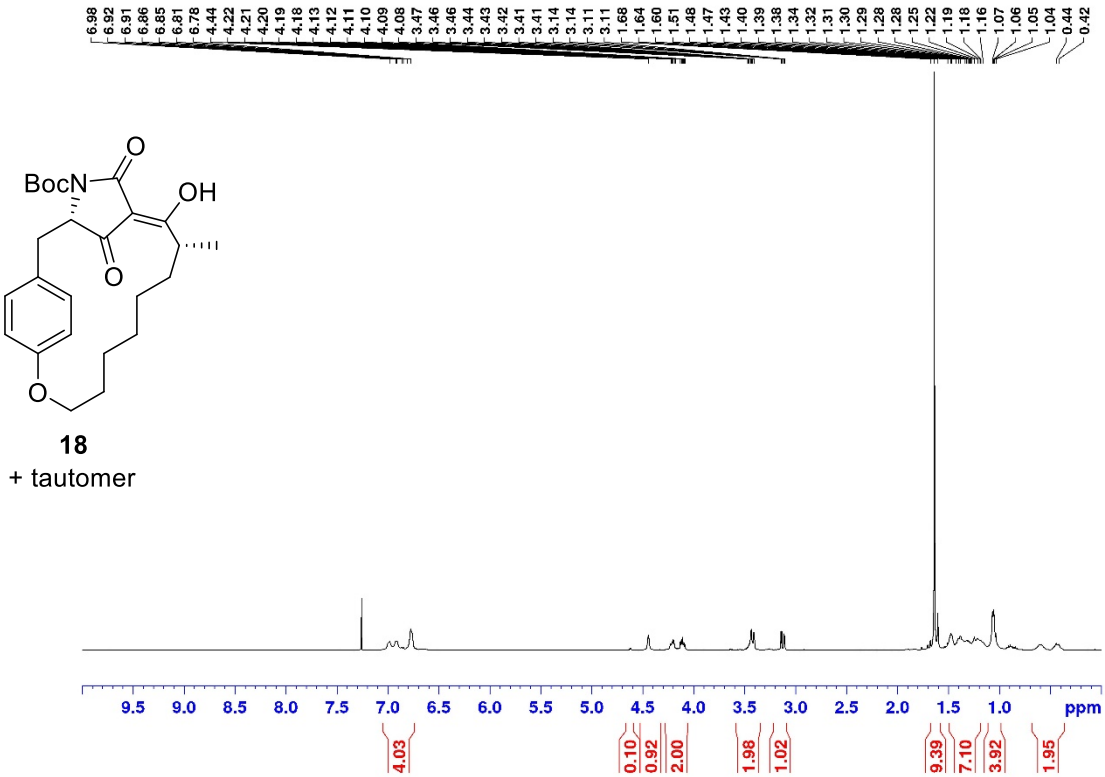

Figure S61. ^{1}H-NMR spectrum of compound **18** in $CDCl_3$.

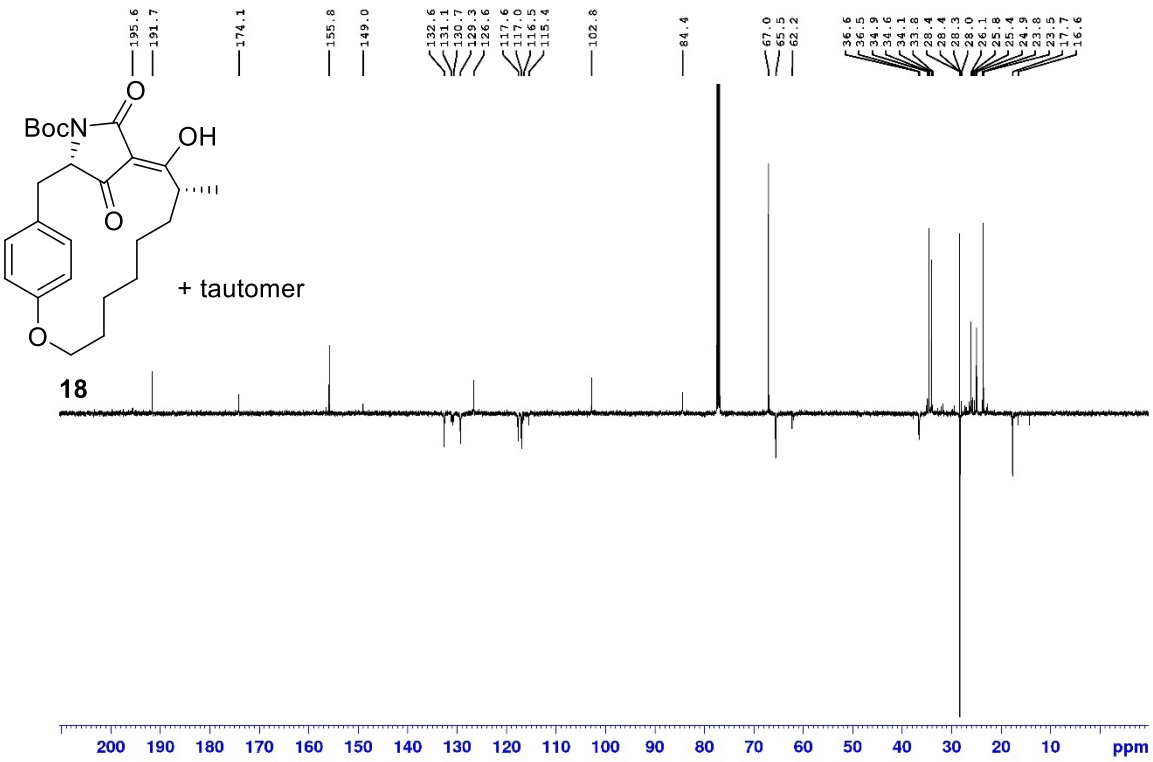

Figure S62. ^{13}C-NMR spectrum of compound **18** in $CDCl_3$.

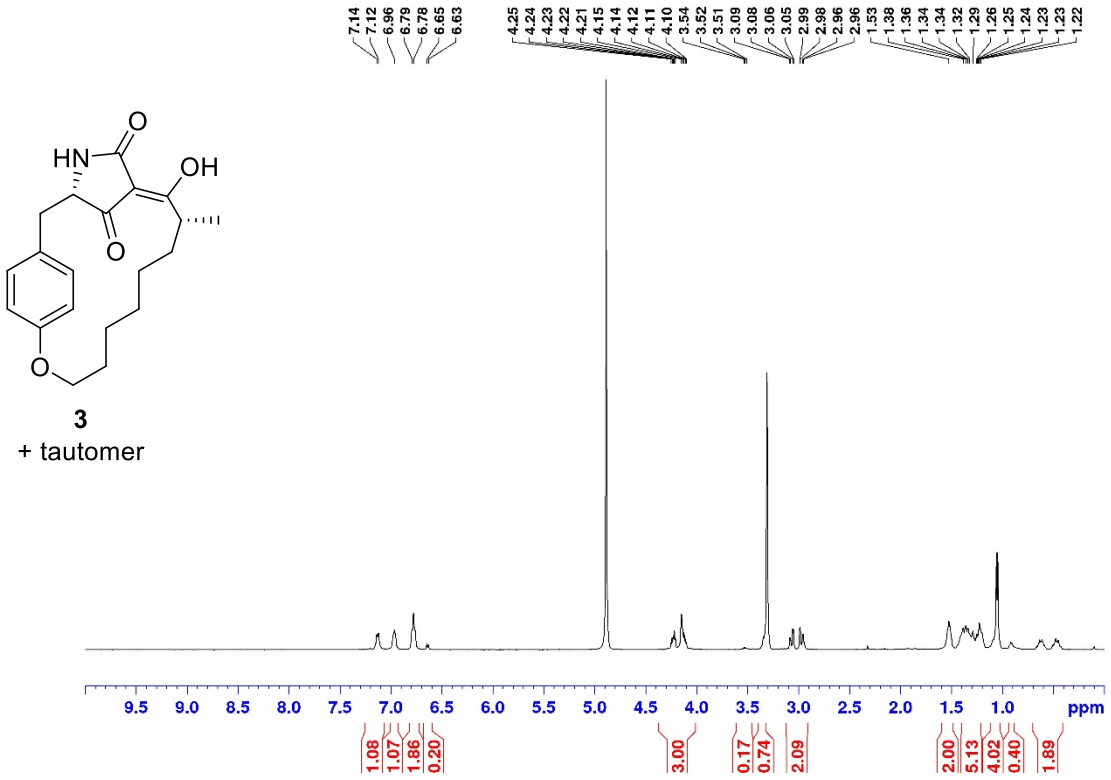

Figure S63. ^{1}H-NMR spectrum of compound **3** in CD_3OD.

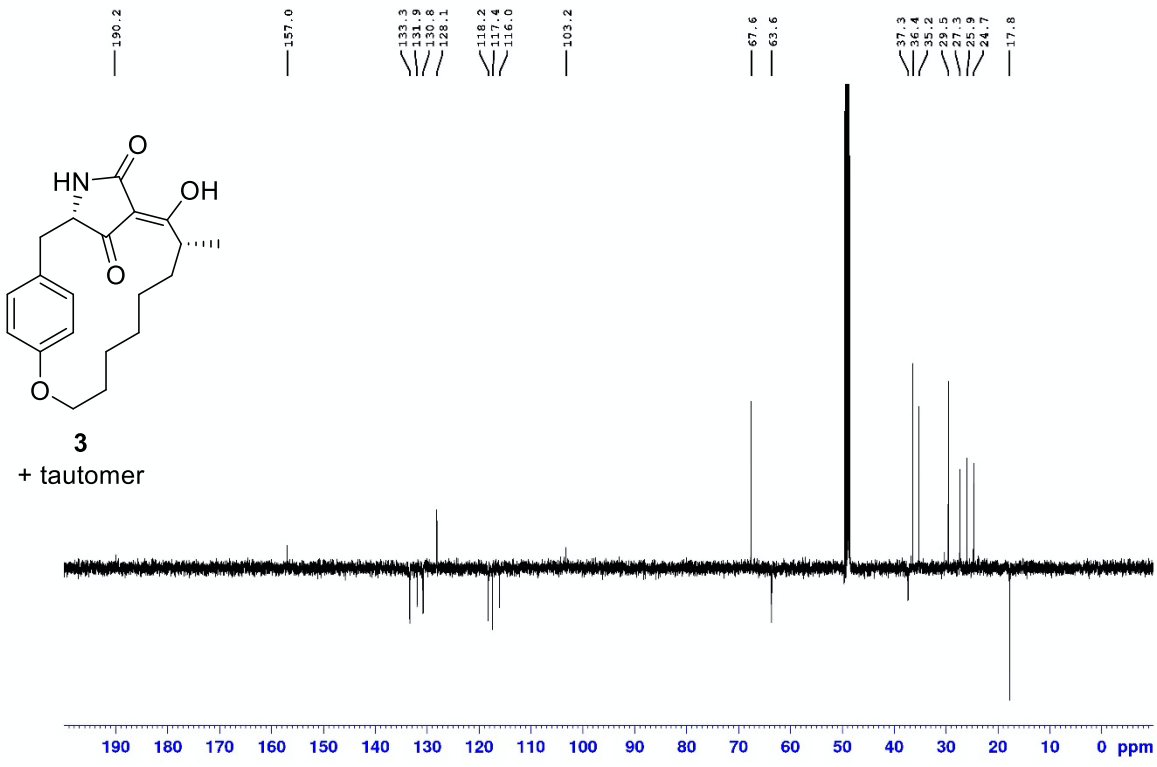

Figure S64. ^{13}C-NMR spectrum of compound **3** in CD_3OD.

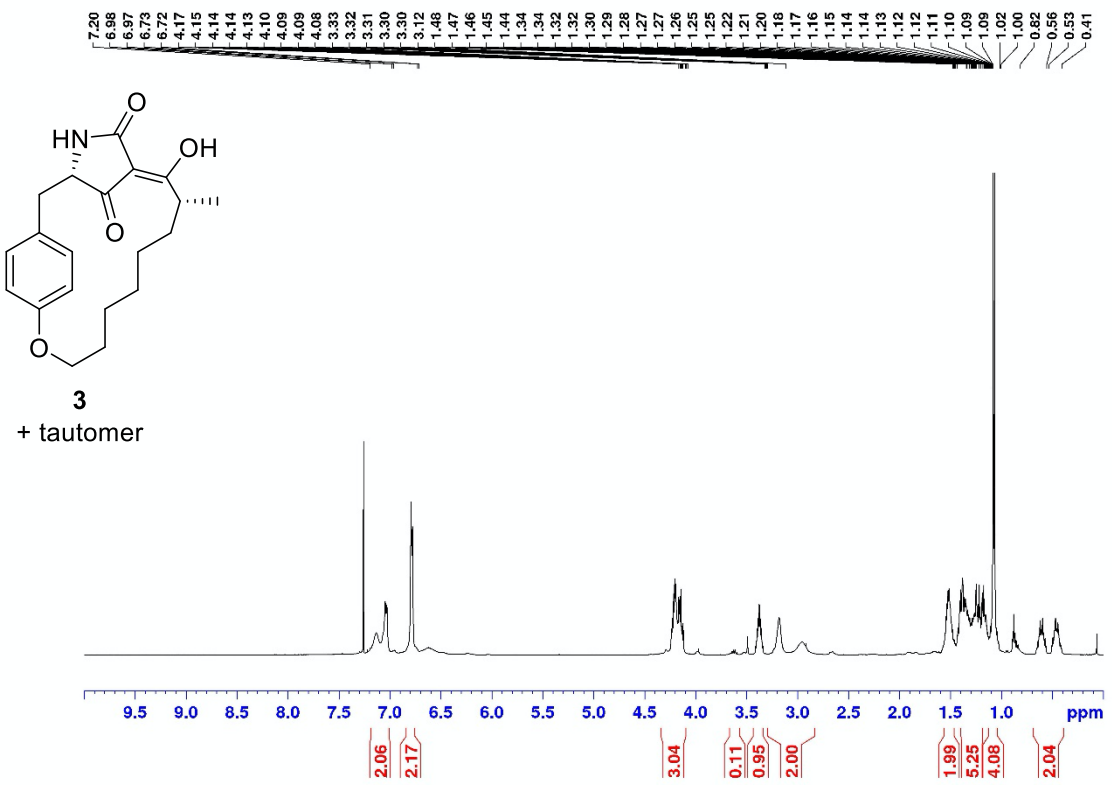

Figure S65. ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.

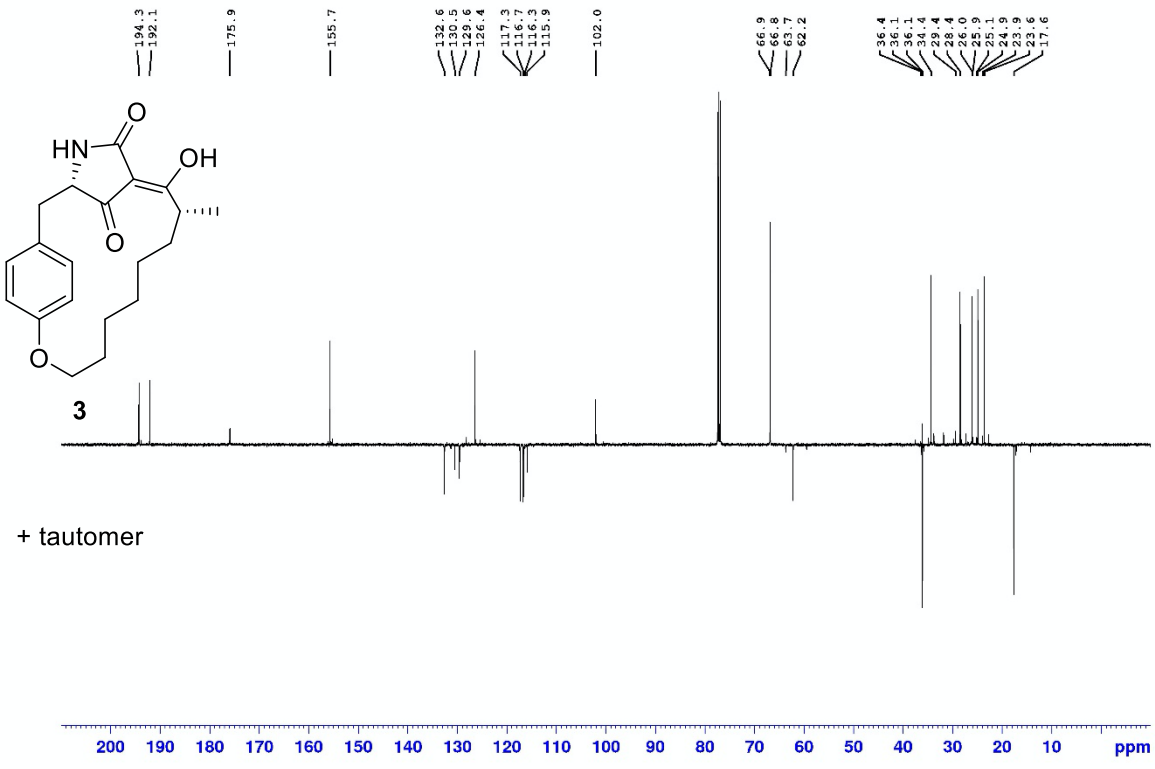

Figure S66. ^{13}C-NMR spectrum of compound **3** in $CDCl_3$.

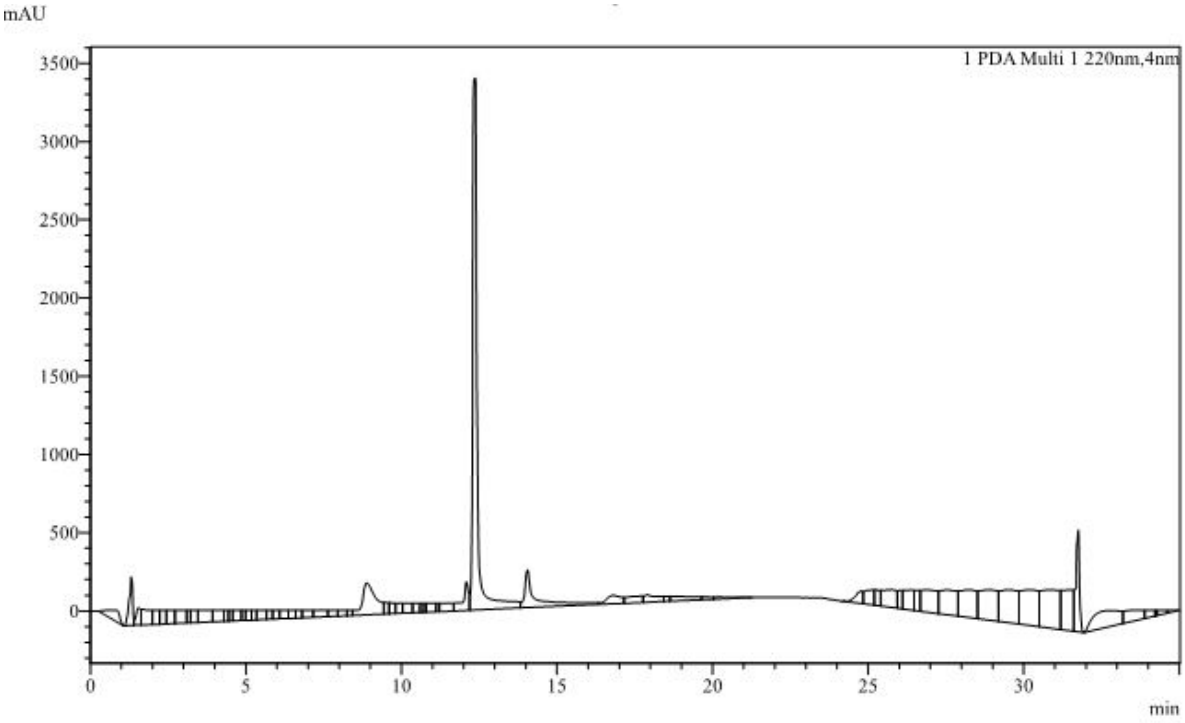

Figure S67. Chromatogram of compound **3**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

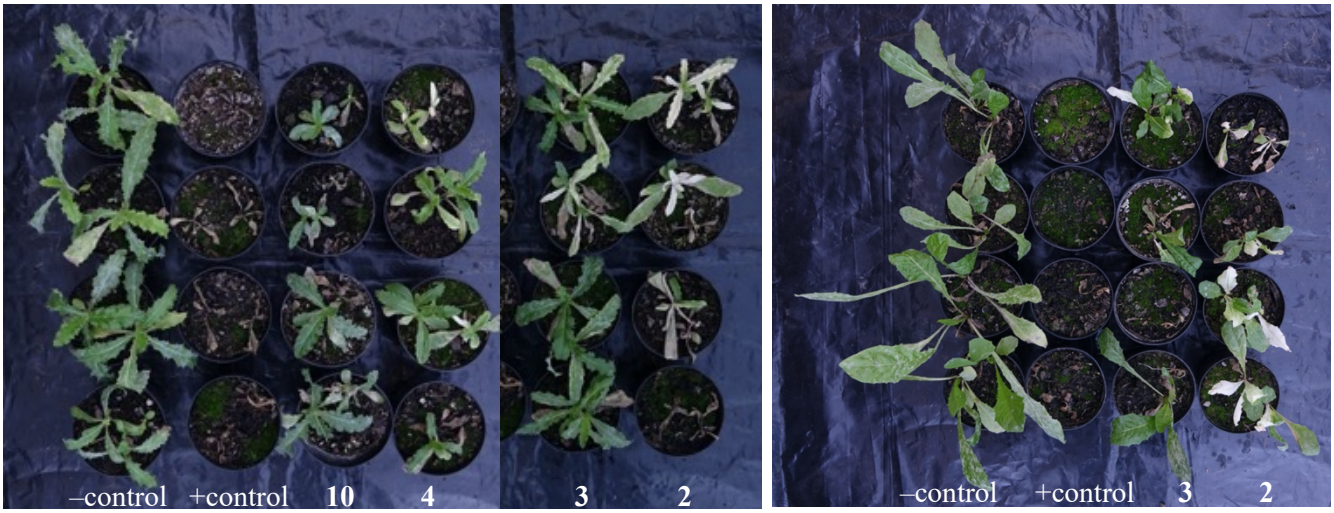

Figure S68: Thistles (left) and dandelions (right), 28 d after treatment with compounds **10** (150 mM), **4** (150 mM), **3** (100 mM) and **2** (100 mM). Negative control: isopropanol:water = 1:1 + 0.25% Tween20; positive control: commercial herbicide diflufenican (1.2 mM).

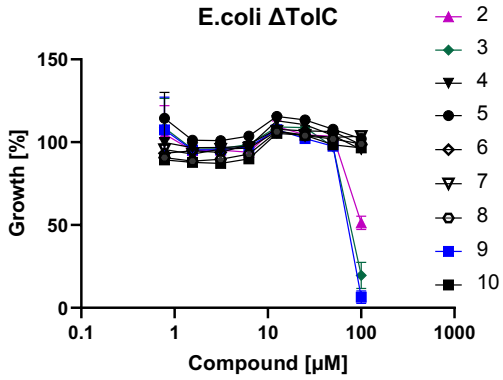

Figure S69. Growth inhibitory effects of various concentrations of macrocidin derivatives on *E. coli ΔTolC* cultures.

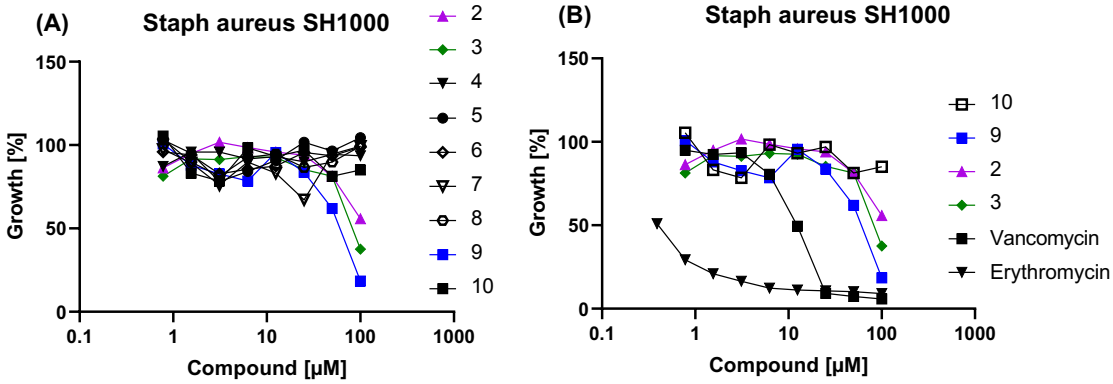

Figure S70. Growth inhibitory effects of various concentrations of (A) all macrocidinoids and of (B) the most active macrocidin derivatives **2**, **3** and **9**, and vancomycin and erythromycin on *S. aureus* (SH1000) cultures.

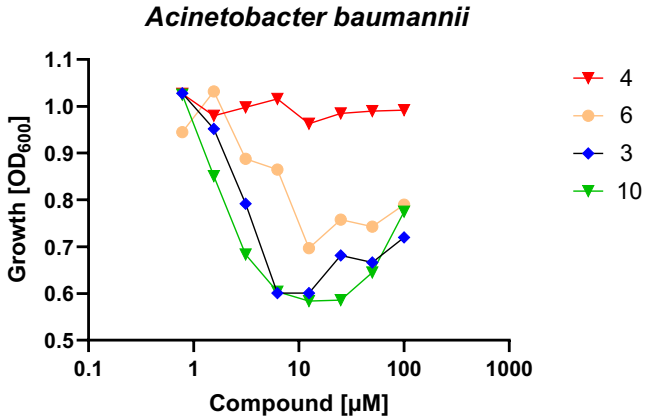

Figure S71. Growth inhibitory effects of various concentrations of selected macrocidin derivatives on *Acinetobacter baumannii* cultures.

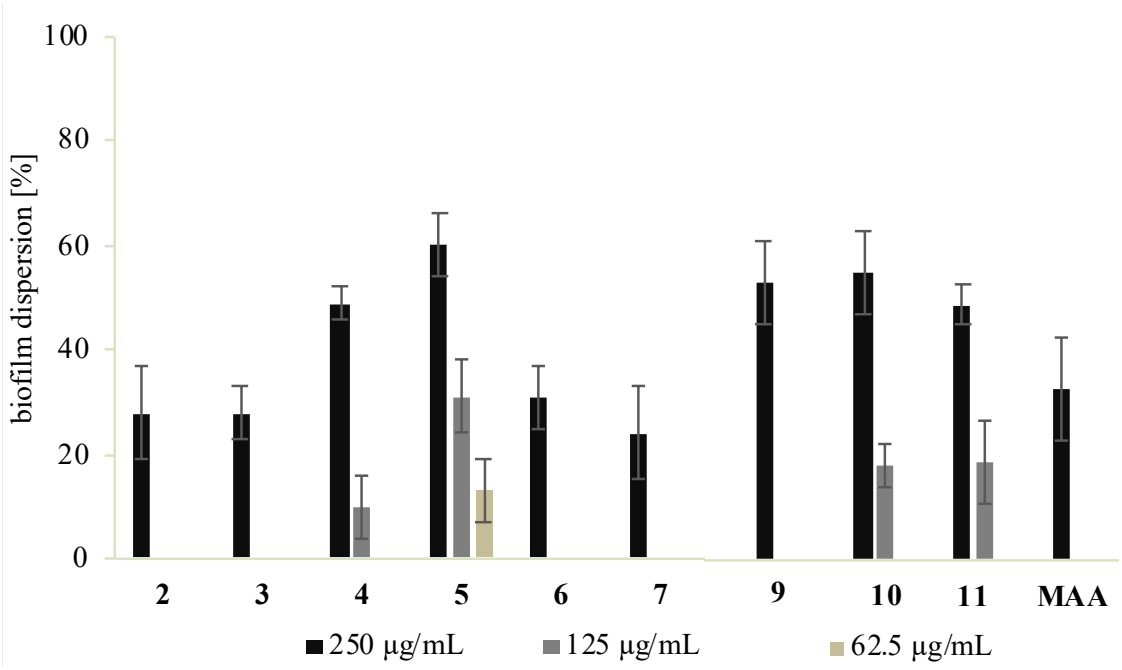

Figure S72. Dispersal effects on preformed biofilms of *C. albicans* by compounds **2-7** and **9-11** at various concentrations, error bars indicate SD.

Table S1. Antibacterial effects of compounds **2-10** on *E. coli* ΔTolC and *S. aureus*. Inhibitory concentrations IC_{50} [µM] as determined by the broth microdilution method [ref. 21 and section 4. Materials and methods].

Compound	***Escherichia coli ΔTolC*** [µM]	***Staphylococcus aureus SH1000*** [µM]
2	100	100 ± 20
3	82 ± 15	83 ± 20
4	inactive	inactive
5	inactive	inactive
6	inactive	inactive
7	inactive	inactive
8	inactive	inactive
9	75 ± 15	57 ± 20
10	inactive	inactive
Vancomycin		12 ± 2

Table S2. Effects of compounds **2-10** on the formation of *S. aureus* biofilms and their dispersive effects on preformed biofilms of *S. aureus* and *C. albicans*, SD values are shown in brackets.

		Biofilm inhibition / dispersion effects [%]								
compounds	**organisms**	**250 µg/mL**	**125 µg/mL**	**62.5 µg/mL**	**31.3 µg/mL**	**15.6 µg/mL**	**7.8 µg/mL**	**3.9 µg/mL**	**2 µg/mL**	**1.3 µg/mL**
	S. aureus	85 (±3)	84 (±3)	84 (±2)	82 (±1)	74 (±1)	69 (±2)	56 (±4)	30 (±5)	30 (±10)
2	**preformed *S. aureus***	82 (±4)	85 (±3)	82 (±3)	74 (±8)	49 (±9)	30 (±5)	/	/	/
	preformed *C. albicans*	28 (±9)	/	/	/	/	/	/	/	/
	S. aureus	83 (±1)	79 (±3)	80 (±3)	80 (±3)	81 (±2)	74 (±2)	53 (±6)	36 (±9)	34 (±9)
3	**preformed S. aureus**	73 (±4)	75 (±3)	70 (±6)	63±14	35 (±12)	16 (±11)	/	/	/
	preformed *C. albicans*	28 (±5)	/	/	/	/	/	/	/	/
	S. aureus	83 (±1)	85 (±1)	84 (±3)	77 (±4)	58 (±7)	31 (±10)	14 (±9)	13 (±7)	/
4	**preformed *S .aureus***	76 (±7)	68 (±12)	49 (±13)	14 (±10)	/	/	/	/	/
	preformed *C. albicans*	49 (±3)	10 (±6)	/	/	/	/	/	/	/
	S. aureus	82 (±3)	81 (±4)	77 (±3)	65 (±10)	35 (±10)	15 (±10)	/	/	/
5	**preformed *S. aureus***	77 (±8)	64 (±13)	36 (±12)	23 (±2)	/	/	/	/	/
	preformed *C. albicans*	60±6	31 (±7)	13 (±6)	/	/	/	/	/	/
	S. aureus	82 (±3)	56 (±3)	17 (±8)	/	/	/	/	/	/
6	**preformed *S. aureus***	25 (±11)	/	/	/	/	/	/	/	/
	preformed *C. albicans*	31 (±6)	/	/	/	/	/	/	/	/
	S. aureus	/	/	/	/	/	/	/	/	/
7	**preformed *S. aureus***	/	/	/	/	/	/	/	/	/
	preformed *C. albicans*	/	/	/	/	/	/	/	/	/
	S. aureus	76 (±10)	28 (±10)	/	/	/	/	/	/	/
8	**preformed *S. aureus***	/	/	/	/	/	/	/	/	/
	preformed *C. albicans*	53 (±8)	/	/	/	/	/	/	/	/
	S. aureus	79 (±4)	81 (±3)	79 (±3)	79 (±6)	82 (±3)	68 (±7)	42 (±7)	35 (±9)	24 (±10)
9	**preformed *S. aureus***	73 (±9)	80 (±4)	79 (±4)	80 (±4)	57 (±3)	12 (±10)	/	/	/
	preformed *C. albicans*	49 (±4)	19 (±8)	/	/	/	/	/	/	/
	S. aureus	82 (±3)	81 (±4)	79 (±3)	53 (±7)	33 (±8)	/	/	/	/
10	**preformed *S. aureus***	83 (±4)	79 (±4)	58 (±15)	33 (±12)	/	/	/	/	/
	preformed *C. albicans*	/	/	/	/	/	/	/	/	/
	S. aureus	83 (±3)	82 (±4)	82 (±5)	80 (±4)	81 (±4)	77 (±6)	40 (±9)	/	/
MAA	**preformed *S. aureus***	68 (±2)	59 (±12)	50 (±4)	58 (±8)	/	/	/	/	/
	preformed *C. albicans*	33 (±10)	/	/	/	/	/	/	/	/
(/) no activity										

Table S3. Inhibitory concentrations IC_{50} [µM] of compounds **2-10** when applied to KBV cervix carcinoma, 518A2 melanoma, HCT-116, HCT-116$^{p53-/-}$ knockout mutant colon carcinoma and EaHy hybrid endothelial cells. Compounds were tested in a range from 50 nM to 100 µM. Values are the means ± SD determined in four independent experiments and derived from dose-response curves after 72 h incubation using the MTT assay. [n.d. = not determined]

	IC_{50} values [µM]				
	518A2	**HCT-116^{wt}**	**HCT-116^{p53-}**	**EaHy**	**KbV**
2	>50	13.9±0.8	17.2±2	n.d.	31.3±3
3	n.d.	>50	>50	n.d.	>50
6	n.d.	>50	>50	>50	>50
7	>50	>50	>50	>50	>50
8	n.d.	>50	>50	>50	>50
9	n.d.	>50	>50	>50	>50
10	n.d.	>50	>50	>50	>50